ABSTRACT – KEYNOTE ADDRESS

Structural basis of antibacterial action of innate immune proteins and their applications as PROTEIN-ANTIBIOTICS

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Considering the alarming rise in the incidence of bacterial resistance to known antibiotics, there is a desperate need to develop bacterial resistance-free antibiotics. The proteins of the innate immune system provide the first line of defense against infecting microbes. These proteins recognize the conserved motifs that are present on the cell walls of bacteria. Thus the success of the innate immune system depends on the affinity of the proteins of innate immune system towards the bacterial cell wall molecules. The conserved motifs of microbial cell walls are called pathogen associated molecular patterns (PAMPs) that include the well known peptidoglycans (PGN) and lipopolysaccharides (LPS) of Gram-negative bacteria, PGN and lipoteichoic acid (LTA) of the Gram-positive bacteria and mycolic acid (MA) and other fatty acids of *Mycobacterium tuberculosis*. These PAMPs are classified into two groups: (i) those which contain glycan moieties such PGN, LPS, LTA etc. and (ii) those that are derivatives of fatty acids such as MA. Therefore, there should be two independent binding sites for the two different types of PAMPs. The PAMPs are specifically recognized by innate immunity molecules which are historically known as peptidoglycan recognition proteins (PGRPs).These proteins bind to PAMPs with significant affinities and neutralize the infecting pathogens through a variety of actions. There are four types of PGRPs in mammals including humans, PGRP-L (MW = 90kDa), PGRP-Iα and Iβ (MW = 45kDa) and PGRP-S (MW = 21kDa). PGRP-S represents the domain that has the binding site for PAMPs. The binding affinities of PGRP-S and structures of unbound and bound PGRP-S from various species showed that the protein from camel has considerably higher affinity than those of other animals including humans. The epidemiological data indicate that the camels have the lowest rates of infections. Structurally, PGRP-S from camel exists in the form of a dimer whereas the human protein acts as a monomer. There are only a few sequence differences in the proteins from two species which are responsible for dimerization of camel protein. As a result of dimerization, a deep binding cleft is formed in the camel protein whereas only a shallow cleft is present in the case of human monomeric protein. Because of dimerization, the potency of camel protein is much higher than the same protein from other species. Thus if camel protein is used or a suitably mutated human protein is prepared and used, the fight against bacterial infection will improve.

The mechanism of action of PGRP-S involves an effective sequestration of bacteria which results in the killing of bacteria. Since PGRP-S interacts with bacterial cell wall, the kinetics of bacterial cell death appears to be similar to those antibiotics which inhibit the biosynthesis of PGN. Due to this similarity, PGRP-S is suggested to be termed as **"protein antibiotics"** and since they bind to bacterial cell wall molecules the issues of side effects and resistance will not arise and if the potencies are high, the invading bacteria can be tackled rapidly.

ABSTRACT – PLENARY TALK

NMR Methodological Advances for Protein Research

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Nuclear Magnetic Resonance (NMR) spectroscopy has come a long way since its discovery seven decades ago and continues to evolve unabated with new applications emerging in many areas of biology and chemistry. Our group has been engaged for the past several years on enhancing the speed of protein structure determination, elucidation of equilibrium folding transitions and self-association pathways, characterization of intrinsically disordered proteins, on one hand and on obtaining high resolution spectra of complex organic mixtures which are involved in interactions with the target proteins, on the other. Encompassing the above, this talk will summarize the methodological advances from our laboratory that includes design of new pulse sequences, use of dual receivers, pure shift spectroscopy and Hadamard NMR.

ABSTRACT – INVITED LECTURES (IL)

IL-1

Peptides of Bromelain from Pineapple (*Ananascomosus*): Potent destabilizing agent of protein aggregates

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Diseases related to deposition of protein aggregates on vitalorgans of human are prominent nowadays because of our advanced knowledge of medical sciences. Prevalence of such diseases are on the rise for altered life style where effects of pollutants, oxidative stress, intake of processed fruits, psychological anxiety dominate. In the background of bestowing large number of medicinal properties of different parts of the pineapple plant, it is our ongoing interest to investigate the effects of these plant products on destabilization of protein aggregates. As a model, we selected human insulin as its deposition on the liver or at the site of application is a concern for diabetic patients. Aggregates of insulin were prepared as par published protocol. They were incubated with different forms of bromelain – the functional protein, inactive protein or the protein digested with proteases as par human digestive system. In all cases, bromelain destabilized preformed insulin aggregates and inhibited growth of aggregates from monomeric state of insulin. To have insight of the interaction, the des-octapeptide sequence of insulin which is believed to be the point of self-aggregation, was synthesised. Peptides of bromelain also dissociated the aggregate of this octa-peptide. The processes were followed by dynamic light scattering, sixe-exclusion HPLC, transmission electron microscopy, atomic force microscopy and FT-IR spectroscopy. Deposition of insulin and related cytotoxicity on Hep G2 cells were also prevented by the bromelain derived peptides. Based on these observations, the mechanism/s of dissociation of the insulin aggregates by the peptides has been proposed. Whether similar mechanisms are operating in the dissociation of other protein aggregates by specific peptides remain speculative at this stage [1].

[1] S. Das and D. Bhattacharyya (2017) J. Cell.Biochem. 1-17 (online version is available)

IL-2

Rational drug discovery of the human Superoxide Dismutase I (hSOD1) modulators: A potential therapeutic target for ALS

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SOD1 is an enzyme involved in detoxification, such as removal of charged oxygen molecules called superoxide radicals. The mutation in the gene causes amyotrophic lateral sclerosis (ALS). The SOD1 toxic gain-of-function is mainly due to the mutation in SOD1 gene; hence, protein gets aggregated. Since both aggregation propensity and protein stability strongly influences patient survival time after onset of symptoms, working on protein stability could be an important step to improve patient survival rate. Stabilization of the SOD1 dimer can increase the protein's thermostability and thus, preventing monomerization and inhibit aggregation. Hence, identifying and developing potential library compounds to stabilize the functional SOD1 dimerization are in need for the treatment of ALS.

The hSOD1 cDNA was cloned in to a pET vector, expressed in the *E.coli* bacterial system, and the His-tag hSOD1 protein was purified to homogeneity with >95% purity by using multiple chromatography techniques. The purified hSOD1 protein was concentrated to 9mg/ml concentration and used for protein crystallizations. hSOD1 was checked for its intact mass analysis and its purity by Mass spectrometry analysis. The X-ray diffraction data for the apo-form of hSOD1 and hSOD1-ligand complexes were collected on the beamline BM14 at European Synchrotron Radiation Facility (ESRF), Grenoble, France. The structures of an apo-form and protein-ligand complexes of hSOD1 refined to 1.9A resolution and their binding studies will be discussed.

IL-3

Application of chaperone assisted protein folding tool for the enhancement of production of Human Serum Albumin in Escherichia Coli

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Human serum albumin (HSA), one of the most demanded therapeutic proteins with immense biotechnological applications. The current source of HSA is human blood plasma, which is a limited and unsafe source. Thus, there exists an indispensable need to promote non-animal derived recombinant HSA (rHSA) production. Escherichia coli is one of the most convenient hosts whichhad contributed to the production of more than 30% of the FDA approved recombinant pharmaceuticals. It grows rapidly and reaches high cell density using inexpensive and simple substrates. E. coli derived recombinant products have more economic potential as fermentation processes are cheaper compared to the other expression hosts. The major bottleneck in exploiting E. coli as a host for a disulfide-rich multidomain protein is the formation of aggregates of overexpressed protein. The majority of the expressed HSA forms inclusion bodies (more than 90% of the total expressed rHSA) in the E. coli cytosol. Recovery of functional rHSA from inclusion bodies is not preferred because it is difficult to obtain a large multidomain disulfide bond rich protein like rHSA in its functional native form. Purification is tedious, time-consuming, laborious and expensive. Because of such limitations, the E. coli host system was neglected for rHSA production for the past few decades despite its numerous advantages.

Bacterial chaperonin GroEL binds with the non-native polypeptide substrates, prevents their aggregation, and assists in the correct folding of various bound polypeptides through the assistance of its co-chaperonin GroES and nucleotide ATP. It is very common fact that recombinant protein production in E.coli system is complicated through the formation of inclusion body, and preparation of folded protein from inclusion body is expensive and uncertain practice. Thus, keeping in mind of the ability of molecular chaperones to assist in the folding of nascent polypeptides in cells, attempts have been made to improve the production of recombinant human serum albumin, a heavily aggregation prone protein in Escherichia coli cytosol.

In the present work, we have exploited the capabilities of E. coli as a host for the enhanced functional production of rHSA. Parameters like intracellular environment, temperature, induction type, duration of induction, cell lysis conditions etc. which play an important role in enhancing the level of production of the desired protein in its native form in vivo have been optimized. We have demonstrated the effect of assistance of different types of exogenously employed chaperone systems on the functional expression of rHSA in the E. coli host system. Different aspects of cell growth parameters during the production of rHSA in presence and absence of molecular chaperones in E. coli have also been studied.

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IL-4

Inhibiting the aggregation of Superoxide Dismutase

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Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease which is characterized by the progressive death of motor neurons of the motor cortex, brainstem and spinal cord. Deposition

ofamyloid of superoxide dismutase (SOD1)is pathological hallmark of ALS. An effective strategy to combat ALS would be to use antioxidants to inhibit or modulate the aggregation of SOD1. Using various biophysical techniques, we demonstrated that quercetin and baicalein inhibited the *in vitro* fibrillation of SOD1. Seeding experiments suggest that these compoundsaffected the fibril elongation rate. Moreover, quercetin and baicalein also destabilized the existing fibrils by fragmenting them into shorter fibrils. Our observations suggest that these antioxidants may serve as potential therapeutic candidates in combating ALS.

IL-5

Structural insights into mode of activation of serine protease HtrA2 through a dual regulatory switch

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High-temperature requirement protease A2 (HtrA2), a multitasking serine protease that is involved in critical biological functions and diseases, such as apoptosis and cancer, is a potent therapeutic target. It promotes apoptosis through multiple pathways, complex mechanisms of which are yet to be delineated. Previous literature reports proposed an activation model that emphasizes relative intramolecular movements between C-terminal PDZ and protease domains has not been able to unambiguously demonstrate dynamics of its allosteric actions. Interestingly, HtrA2 exhibits an additional level of functional modulation through its unique N-terminus by binding and cleaving 'inhibitor of apoptosis proteins (IAPs).' This phenomenon emphasizes multiple activation mechanisms, which so far remain elusive. Using structure-guided design, conformational dynamics, binding kinetics and enzymology, we addressed this complex behaviour with respect to defining its global mode of regulation and activity. Our findings highlight importance of oligomerization, and intricate intermolecular PDZ-protease interaction in proper active-site formation. Our studies further demonstrate a novel N-terminal ligand-mediated triggering of an allosteric switch essential for transforming HtrA2 to a proteolytically competent state in a PDZ-independent yet synergistic activation process. Interaction with binding partners/ substrates occurs through a series of coordinated structural reorganizations at distal regulatory loops (L3, LD, L1), leading to a population shift towards the relaxed conformer. This precise concerted coordination among different domains might be physiologically relevant to enable tighter control upon HtrA2 activation for fostering its diverse cellular functions. Understanding this complex rheostatic dual switch mechanism offers an opportunity for targeting various disease conditions with tailored site-specific effector molecules.

IL-6

Structure, function and modulation of G Protein-Coupled Receptors

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G Protein-Coupled Receptors (GPCRs) represent the largest class of cell surface receptors in the human genome. GPCRs are involved in almost every cellular and physiological process in our body, and they constitute a major class of drug targets for a range of human disorders. We have utilized a phage display based synthetic antibody platform to generate high-affinity antibody fragments against GPCRs and their signaling effectors. Using these antibody fragments, we have deduced novel structural information on receptor-effector coupling, and corresponding signaling outcomes. We are also employing these antibodies in cellular context to image ligand induced GPCR activation and trafficking, as well as to rewire GPCR signaling with potential therapeutic implications.

IL—7

Graphene-based nanocomposite as novel scaffolds for the construction of high yield and robust enzymes

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Graphene-based nanocomposites are particularly useful nanostructured materials that show great

promise in field of biotechnology and biomedicine. Due to their high chemical, physical, electrical and mechanical stability and their ability to influence the microenvironment of enzymes, graphene-based nanocomposites are suitable for use in various applications, such as immobilization of enzymes. Graphene nanocomposite possess a large surface area with abundant functional groups, therefore it works as an ideal support for high yield immobilization of enzymes via physical adsorption or by covalent attachment. Currently we have developed some nanobiocatalysts of hydrolytic enzymes; β-galactosidase and lipases by employing some graphene nanocomposite as novel scaffolds. Graphene nanocomposite bound enzymes have been characterized by using SEM, TEM, spectrophotometry, spectrofluorimetry, Raman spectroscopy and atomic force microscopy etc. These nanocomposites showed very high efficiency of binding and loading of enzymes and the obtained nanobiocatalysts were found significantly highly stable against different kinds of chemical and physical denaturants. Bound enzyme retained remarkably very high catalytic activity on repeated uses and long time storage. Graphene nanocomposite immobilized galactosidase and lipase have demonstrated their high potential at large scale hydrolysis of lactose and the synthesis of flavor compunds, respectively.

IL-8

Structural and biochemical studies of *Mycobacterium tuberculosis* histidine biosynthesis pathway enzymes

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Mycobacterium tuberculosis (Mtb)makes its own Histidine (His) in 10 steps by employing 10 enzymes. *Mtb* deprived of even a single His pathway gene fails to grow/multiply. Moreover, the facts that His constitutes one of the building blocks of protein synthesis and that humans do not make His *de novo*, inhibition of the enzymes that are involved in its biosynthesis gleams a rational strategy for new anti-TB agents design. Primarily in the context of designing new anti-tuberculosis inhibitors through structure guided approach, structural and biochemical aspects enzymes of *Mtb* His pathway are being carried out in my laboratory. We have elucidatedhigh resolution crystal structures of HisB (imidazole glycerol phosphate dehydratase) and HisC (histidinol phosphate aminotransferase). HisB, which catalyses the conversion of imidazole glycerol phosphate to imidazoleacetol phosphate (IAP), is pseudo symmetric and is made up of a four-helix bundle sandwiched between two four-stranded mixed ß-sheets. The biological functional unit exhibits a quaternary assembly composed of 24 identical subunits with 432 molecular symmetry. HisC catalyses the seventh step, the conversion of IAP to L-histidinol phosphate. It contains of two domains, a PLP-binding domain withan α/ß/α topology and a C-terminal domain. Using structural and biochemical information, we have designed a few inhibitors against HisB.

IL-9

Mechanism of toroid formation around DNA by the Mismatch Sensor protein

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The DNA mismatch repair pathway serves to maintain the integrity of the genome through removal of errors that appear during genome replication. MutS is the primary mismatch sensor and forms an asymmetric dimer that encircles DNA and bends it to scan for mismatches. The mechanism utilized to load DNA into the central channel was not known and the origin of the force required to bend DNA was unclear. We show that, in the absence of DNA, MutS forms a symmetric dimer wherein a gap exists between the monomers through which DNA can enter the central tunnel. The comparison with structures of MutS-DNA complexes suggests that the mismatch scanning monomer (Bm) will have to move by nearly 50 Å to associate with the other monomer (Am). As a result, the N-terminal domains of both monomers will press onto DNA to bend it. The proposed mechanism of toroid formation evinces that the force required to bend DNA arises primarily due to the movement of the monomer Bm and hence, the MutS dimer acts like a pair of pliers to bend DNA. We also shed light on the allosteric mechanism that influences the expulsion of ATP from Am on DNA binding. Overall, this study provides mechanistic insight regarding the primary event in DNA Mismatch repair i.e. the assembly of the MutS-DNA complex.

IL-10

Evaluation of serum apolipoprotein E as a potential biomarker for pharmacological therapeutic efficacy monitoring in dopamine dictated disease spectrum of Schizophrenia and Parkinson's disease

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Parkinson's disease and schizophrenia are disease end points of dopaminergic deficit and hyperactivity, respectively in the mid brain. Accordingly, current medications aim to restore normal dopamine levels, overshooting of which results in adverse effects of psychosis and extra-pyramidal symptoms. There are currently no available laboratory tests to guide treatment decisions or help predict adverse side effects of the drugs. The possibility of using apolipoprotein E as a biomarker to monitor pharmacological intervention in dopamine dictated states of Parkinson's disease and schizophrenia for optimum therapy has been explored in this study. Naïve and treated, Parkinson's disease and schizophrenic patients were recruited from neurology and psychiatry clinics. Serum of research staff volunteers was collected as healthy controls. Serum concentrations of apolipoprotein E was estimated by ELISA. Apolipoprotein E levels are higher in Parkinson's disease patients as compared to schizophrenic samples (P<0.05). Also, post treatment apolipoprotein E levels in both disease states were on par with levels seen in healthy controls. In conclusion, inverse relation shown by apolipoprotein E concentration across the dopaminergic spectrum suggests that it can be pursued not only as a potential biomarker in schizophrenia and Parkinson's disease, but can also be an effective tool for clinicians to determine efficacy of drug based therapy.

IL-11

Acesulfame Potassium inhibits the formation of AGEs and glycation induced aggregation

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Sweeteners have replaced the natural sugars in the food and beverage industry because of many reasons such as hyperglycemia and cost. Saccharin, Sucralose, Aspartame and Acesulfame-K are the most commonly used sweeteners. In the present study, Acesulfame potassium was used to assess its glycating properties by established methods like fructosamine assay, determination of carbonyl content, protein aggregation and measurement of fluorescence. Amadori and Advanced Glycation End products (AGEs) are formed as a result of the interaction between carbonyl groups of reducing sugars and amino groups of proteins and other macromolecules during Glycation. The objective of this study was to investigate the influence of Acesulfame potassium on the formation of AGEs and protein oxidation in an in vitro model of glucose-mediated protein glycation. The results indicated that Acesulfame-K was found to have antiglycating potential as it caused decreased formation of Amadori products and AGEs. It was also observed that Acesulfame potassium prevented the glycation induced aggregation of BSA. This study is significant in understanding the probable role of artificial sweeteners in the process of glycation and the subsequent effect on macromolecular alteration.

IL-12

Turning ideas into drug candidates for Leishmaniasis

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Leishmaniasis one of the most neglected infectious diseases is caused by the bite of sand-fly of the genus *Phlebotomus*. The available drugs of the disease have several limitations and no vaccine against the parasite is available. Using integrated computational and biochemical approaches, we have identified novel drug target proteins (mainly enzymes) and designed inhibitor molecules against these targets. The identified

inhibitors are evaluated as potential drug candidates. Apart from drug development, the studies on various other cost effective approaches like vaccine and immunotherapeutics are also in process. We are trying to decipher the exact mechanism of NLRP3 inflammasome inhibition during *Leishmania donovani* infection. Moreover we are also trying to find out the possible interacting partner molecules from the host and/or parasite counterpart, which are causing the impairment of the NLRP3 inflammasome. These basic understandings of the immunosuppressive mechanism will help us to design novel immunotherapeutic molecules against the parasites.

IL-13

Arginase of *Helicobacter* gastric pathogens uses a unique set of non-catalytic residues for catalysis

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Helicobacter pylori arginase, a bimetallic enzyme is crucial for pathogenesis of the bacterium in human stomach. Despite conservation of the signature motifs in all arginases, the *Helicobacter pylori* homologue has a non-conserved motif (^{153}ESEEKAWQKLCSL^{165}), whose role was yet not known. We recently reported the significance of this motif in catalytic function, metal retention, structural stability of the protein. The sequence analysis also shows the existence of this motif with vital residues in the homologue of other *Helicobacter* gastric pathogens. However, the underlying mechanism for its importance in catalytic function was not clearly understood. Using *H. pylori* arginase, we show that the interactions of two non-conserved residues with Trp159 are indispensable for tertiary structural intactness through optimal positioning of the motif and thus for the catalytic function. The single and double mutants of these two residues not only enhanced the solvent accessibility and conformational flexibility of Trp159 in the holo protein, but also showed complete loss of catalytic function. An intact bimetallic center and unaltered substrate binding indicate that proper positioning of the motif by aromatic-aromatic contact is vital for the generation of a catalytically active conformation. We also identified the presence of these two residues exclusively in arginase of other *Helicobacter* gastric pathogens, which may have similar function. Our findings therefore highlight that arginase of all *Helicobacter* gastric pathogens utilizes a unique non-catalytic triad for catalysis, which could be exploited for therapeutics.

IL-14

How *Escherichia coli* RNase Z acts as a dual function ribonuclease

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Ribonuclease Z family of proteins are essential for the 3Œ§-end processing of tRNA precursors in all domains of lives, except in some eubacteria like *Escherichia coli*. Structure-function analysis of *E. coli* RNase Z identified it as very distinct member of the family, as this enzyme has both endo and exoribonuclease activity, whereas, all of its homologues act exclusively as endoribonuclease. Comparison of the x-ray structure of RNase Z of *E. coli* and *Bacillus subtilis* RNase Z, which lacks exoribonuclease activity, revealed that *E. coli* RNase Z has a narrower and more rigid channel downstream of the catalytic site. We hypothesized that this difference in the putative RNA exit channel might be responsible for the acquisition of exoribonuclease activity by RNase BN. Accordingly, we generated several mutant RNase Z proteins in which residues within a loop in this channel were converted to the corresponding residues present in *B. subtilis* RNase Z, thus widening the channel and increasing its flexibility. The resulting mutant RNase Z proteins had reduced or were essentially devoid of exoribonuclease activity *in vitro*. Substitution of one mutant *rnz* gene (P142G) for wild type *rnz* in the *E. coli* chromosome revealed that the exoribonuclease activity of *E. coli* RNase Z isnot required for maturation of phage T4 tRNA precursors, a known specific function of this RNase. On the other hand, removal of the exoribonuclease activity of *E. coli* RNase Z in a cell lacking other processing RNases leads to slower growth and affects maturation of multiple tRNA precursors. These findings help explain how RNase Z can act as both an exo- and an endoribonuclease and also demonstrate that its exoribonuclease activity is capable of functioning *in vivo*, thus widening the potential role of this enzyme in *E. coli*.

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IL-15

Understanding the role of steric hindrance in RNAi mechanism for nanoparticle mediated siRNA delivery

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RNAi is emerging as a promising technology for treatment of various diseases due to its ability to silence specific target genes. Till date, a number of nanoparticle based formulations have been reported for delivery of small interfering RNA (siRNA), with continuous modifications in the nanoparticle design for enhancing their efficiency. While majority of the design aspects are focused on avoiding or overcoming the endosomal entrapment, limited studies are available that address the role of interaction of nanoparticles with the RNA induced silencing complex (RISC) machinery, which is a crucial aspect deciding the outcome. Here, we systematically probe the effect of steric hindrance of nanoparticles on RISC interaction, by modulating two parameters, nanoparticle size and hardness.For studying the effect of size, polymeric nanoparticles (nanogels) of two different sizes (~90 nm and ~300nm) were used as model system, while gold nanoparticles were used as hard nanoparticles. As the surface chemistry is known to alter the interactions, gold nanoparticles were covered with the polymer shell, such that the overall size of the nanoparticle is comparable to the small nanogel. All the nanoparticles were functionalized by carboxylic acid, which was used to load siRNA by means of a cationic polymer, poly-å-lysine.The nanoparticles were thoroughly characterized by electron microscopy, DLS and AFM, while siRNA loading was confirmed by electrophoretic mobility shift assay (EMSA). An assay was developed for studying the extent of interaction of different nanoparticles *in vitro*. It makes use of one of the important component of RISC machinery, argonaute 2, which is responsible for the nuclease activity of RISC. The assay monitors the efficiency of the siRNA guide strand on the nanoparticle to bind to the target RNA. The results of the assay were also confirmed by evaluating the gene silencing efficiency of polo-like-kinase 1 (PLK1) siRNA loaded nanoparticles.

IL-16

Aβ, a risk factor for Parkinson's pathogenesis: Mechanisms and prevention

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Amyloid beta (Aβ) aggregation is generally associated with Alzheimer's onset. We have demonstrated that incubation of dopaminergic SH-SY5Y cells with an Aβ peptide fragment (an 11-mer composed of residues 25–35; Aβ (25–35)) results in elevated intracellular nitrosative stress and induces chemical mutation of protein disulfide isomerase (PDI), an endoplasmic reticulum-resident oxidoreductase chaperone. Furthermore, Aβ (25–35) provokes aggregation of both the minor and major biomarkers of Parkinson's disease, namely, synphilin-1 and α-synuclein, respectively. Importantly, fluorescence studies demonstrate that Aâ (25–35) triggers colocalization of these Parkinsonian biomarkers to form Lewy-body-like aggregates, a key and irreversible milestone in the neurometabolic cascade leading to Parkinson's disease. In addition, fluorescence assays also reveal direct, aggregation-seeding interactions between Aβ (25–35), PDI and α-synuclein, suggesting neuronal pathogenesis occurs via prion-type cross-transfectivity. These data indicate that the introduction of an Alzheimer's-associated biomarker in dopaminergic cells is proliferative, with the percolative effect exercised via dual, independent, Parkinson-pathogenic pathways, one stress-derived and the other prion-like. The results define a novel molecular roadmap for Parkinsonian transfectivity via an Alzheimeric burden and reveal the involvement of PDI in amyloid beta induced Parkinson's. We have also explored the ability of phytochemicals to intercept Aβ-driven Parkinson's pathogenesis via multiple mechanisms. Results from these studies will be discussed.

IL-17

Protein conformation, dynamics and aggregation: one molecule at a time

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Our laboratory has been investigating the mechanistic details of how a protein attains its functional three dimensional structure. We are also studying the conformational and other factors which contribute to the alteration of folding pathways leading to aggregation. The problems of protein mis-folding and aggregation, which have serious implications in a number of neurodegenerative diseases, are difficult to study. This is because; the folding and aggregation landscape is inherently heterogeneous, consisting of multiple pathways. Since the traditional biochemical and biophysical techniques require an optimum concentration of aggregated molecules for their detection, monitoring the early stages is difficult. Our lab has been using sensitive fluorescence methods, which can provide single molecule resolution, to address these problems. In this talk, we would discuss some of these data, which have been obtained using a number of relevant model proteins.

IL-18

Evolution-Structure-function relationship of GRO Chemokines

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Chemokines are a group of chemotactic cytokines that are involved in regulating leucocyte migration to the infected tissue. They do so by binding to glycosaminoglycans (GAGs) on the endothelial cell surface and thus activating the G-protein coupled receptors (GPCR) present on the leucocytes. Glycosaminoglycans (GAGs) such as heparan sulfate are highly negatively charged linear polysaccharides. They are ubiquitously expressed on cell surfaces, and mediate a wide variety of biological functions. Neutrophil activating chemokines (NACs) are one of the major classes of CXC chemokines recognized by their highly conserved "ELR" motif that recruit neutrophils at the site of infection. Growth related Oncogene (GRO) chemokines, subfamily NACs, consisting of three members CXCL1 (GRO-a), CXCL2 (GRO-b) and CXCL3 (GRO-g). A variety of protein NMR spectroscopy, biophysical and molecular evolution techniques were employed to delineate the promiscuity and GAG binding features of GRO chemokines. Evolutionary studies elucidated that these chemokines formed upon two rounds of tandem duplication events, and they possess species specific GAG binding surfaces. Further, the analysis also suggested that the GRO chemokines followed concerted evolution and the differential GAG binding surfaces are under positive selection. NMR studies with synthetic GAG molecules suggest that positioning of sulfates, chain length and backbone saccharide conformation etc, of GAGs significantly contributes to their binding to GRO proteins. Further, this study promises to aid in creation of chemokine and GAG based decoys to circumvent the endogenous GAG based chemokine recruitment in numerous infectious/ inflammatory diseases.

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IL-19

Computational drug discovery: An avenue for targeting the protein-protein interaction betweenHIV-1 integrase and LEDGF/p75

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Rapidly increasing emergence of drug resistance has become a wide-reaching challenge to combat latent viral infection in recent years. HIV-1 virus is a causative agent of AIDS, has cost millions of lives across the world in last two decades. As, the genome organization of HIV-1 virus consist of three essential viral enzymes Reverse transcriptase, Protease and Integrase; are essential in replication. Of these, HIV-1 integrase is unique responsible factor in catalyzing the integration of reverse transcribed viral cDNA into the host cell genome. Since, integrase activity takes help of both cellular and viral co-factors for accomplishing the integration process in gain of productive infection. Among them, the human lens epithelium-derived growth factor (LEDGF/p75) has been recognized as a pleasing binding partner of HIV-1 IN to facilitate the integration process during early stage of retroviral replication. Olden research reveals the protein-protein interaction of IN-LEDGF/p75 as an essential target for anti-HIV drug discovery. However, in spite of the enormous efforts in the developing potentially effective antiviral inhibitors next to IN-LEDGF/p75 couldn't diminish the viral infections. Consequently, novel therapeutics should be developed to slow down the viral infection of HIV and enhance the lifespan of the infected population. Getting a new potent therapeutics from laboratory to market takes time as well as cost. Hence, computational drug discovery has taken a step forward to design and develop a new anti-viral vaccine or drug within less time and less effort to impede the IN-LEDGF/p75 interaction. Herein, we postulate advanced computational drug discovery as a great outline towards the inhibition of HIV/AIDS in nearest future.

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IL-20

New Insights into cysteine biosynthetic pathway enzymes of *E. histolytica*

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Cysteine is crucial for survival of *E. histolytica* and *Trichomonas* and it is only thiol in *E. histolytica* acting as a cellular reducing agent. Serine – Cysteine biosynthetic pathway and sulfate activation pathways are crucial for survival of *E. histolytica*. 3-phospho glyceric acid (PGA), one the intermediate of glycolysis is converted to serine and then to cysteine by five different enzymes. Our lab has reported the structures of all serine/cysteine biosynthetic pathway enzymes from *E. histolytica* (Proteins, 2008; JBC, 2011; BBA, 2013, FEBS J, 2014), as well as the structures of some of their homologues from other organisms for comparative studies (Acta D, 2012; BBA, 2014). The *E. histolytica* Phosphoglycerate dehydrogenase (PGDH) converts PGA to phosphohydroxypyruvate (PHP) has Lys as catalytic residue, while in others catalytical triad Asp, His and Arg are catalytical residues. The crystal structures of PGDH in apo form, as well as in complexes with substrate (3-phosphoglyceric acid-PGA) and cofactor (NAD^+) to 2.45, 1.8 and 2.2 Å resolution has been determined. The cofactor-bound structure also shows a closed-cleft conformation, where the NAD^+ is bound to the nucleotide-binding domain and a formate ion occupies the substrate-binding site. Superposition of substrate- and cofactor-bound structures represents a snapshot of the enzyme in the active form, where C2 of the substrate and C4N of the cofactor are 2.2 Å away from each other, and the amino group of Lys 263 is close enough to the substrate to remove the proton from the hydroxyl group of PGA, indicating the role of Lys in the catalysis. Phosphoserine aminotransferase (PSAT) is a PLP, pyridoxal phosphate, dependent enzyme that catalyzes the second reversible step, where 3-phopshoglycerate is converted to serine. The crystal structure of PSAT in complex with PLP (cofactor) has been determined at 3.0 Å resolution and their mutants, Δ45 and Δ4_EhPSAT at 1.8 and 2.4 Å, respectively. While purifying *Eh*PSAT, it was observed the degradation of first 45 residues. The deletion of 45 residues (Δ45*Eh*PSAT) resulted in an inactive protein and structure showed drastic difference in dimeric arrangement, where two monomers slide and rotate with respective to each other by almost 180°.

Comparison of *Eh*PSAT and Δ45*Eh*PSAT structures indicated residues 11 to 14 are crucial for arrangement of active site wall. Deletion of these 4 residues (Δ4*Eh*PSAT) and first 15 residues (Δ15*Eh*PSAT) showed drastic decrease in the activity but Δ4*Eh*PSAT structure was similar to native structure indicating the importance of these four residues in active site architecture.

IL-21

Intramers for therapeutic intervention in protein misfolding diseases

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Huntington's disease (HD) is a posterchild of protein misfolding diseases. An abnormal expansion of the polyglutamine stretch at the N-terminus of the protein, huntingtin, results in its misfolding and aggregation. Aggregation of mutant huntingtin has been linked to disease progression and inhibition of protein aggregation is a valid therapeutic target [1,2]. Like other diseases of its class, only symptomatic cure is available for HD. Conventional osmolytes as well as antibodies have shown promise in inhibiting protein aggregation but their use is associated with several challenges. Nucleic acid aptamers provide a possible alternative as inhibitors of protein aggregation [3]. Aptamers are short single-stranded DNA/RNA sequences which adopt specific three-dimensional shapes and bind to their targets (which could be small molecules, proteins, viruses or even whole cells) with high affinity and specificity. Based on the specificity of interaction with their targets, aptamers have been referred to as 'chemical antibodies' and have found applications in target validation, analysis and diagnostics, targeted drug delivery, etc. This talk will present a novel application of aptamers, as inhibitors of protein aggregation. RNA aptamers were selected against mutant huntingtin by an iterative selection process. The selected aptamers were able to inhibit aggregation of mutant huntingtin *in vitro* and were able to attenuate pore formation in cells, which is a widely recognized route by which the oligomeric protein species induces oxidative stress and causes cell death [4]. The selected aptamers were expressed intracellularly ('intramers') and were able to inhibit protein aggregation inside the cell and improve cell survival. Irrespective of the protein involved, protein misfolding diseases follow a common theme of nucleation, growth and saturation phases [5]. Because of their non-immunogenic and non-toxic nature, aptamers present a viable therapeutic option as inhibitors of protein aggregation in this class of diseases.

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IL-22

Non Planar Peptide Bond?

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Peptide bond planarity is accessed in terms of the torsion angle ω, with ω = 180°and ω = 0°representing *trans* and *cis* peptide bond respectively. This is for an ideal situation with each angle around carbonyl carbon and amide nitrogen being 120°and each C^{α} atoms being in ideal sp^3 hybridization. Deviations in ω from 180°and 0°referred as distortions of individual peptide units from planarity due to pyramidalization of carbonyl carbon and amide nitrogen's have been discussed in terms of improper dihedral angles.

Analysis of high resolution pdb structures and molecular dynamics simulation of polypeptides in water for the bond angels around carbonyl carbon's and amide nitrogen's completely rules out the change in hybridization (sum the angles being ~360°) and hence pyramidalization of these atoms. There is only readjustment of bond angels. Likewise, the sum of angles around each C^{α} atoms was found to be ~

656.8°and the maximum increase in the angle N- C^{α}-C ($\Delta\tau$) is observed up to ~10°and decrease is found up to 5°. Thus, the peptide bond is planar and the deviations in ω arise due to the positions of $C^{\alpha's}$ around the C—N bond. Minimum deviations both in ω and in the angle N– C^{α} – C ($\Delta\tau$) are found in helical and compact structures

IL-23

Antibiotic resistance of novel aminoglycoside phosphotransferase and related enzymes

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Antibiotics resistance is a serious global public health problem. It is thus necessary to understand the cause of antibiotic resistance in detail at the molecular level. Hence, we studied the resistance mechanism of aminoglycoside and polymyxin group of antibiotics towards different pathogenic microorganisms. Bacterial resistance towards aminoglycoside group of antibiotics mainly occurs through the enzymatic modification by aminoglycoside phosphotransferases (APH), also known as aminoglycoside kinases (AKs). Purification, characterization of APH and enzymatic modification studies of different aminoglycoside antibiotics revealed a novel class of aminoglycoside phosphotransferase i.e. APH(5). Structure based virtual screening (SBVS) by targeting one of the AKs (4FEX) from *Acinetobacter baumannii* investigated ZINC71575479 as a most stable inhibitor. Biochemical analysis of virtually screened inhibitor ZINC71575479 by coupled spectrophotometric assay showed complete enzymatic inhibition of purified APH(5). The comparison between virtually screened inhibitor, ZINC71575479 with known inhibitor of APH, tyrphostin AG1478 have also been done using *in silico* methods. Molecular docking results revealed the efficient binding of ZINC71575479 to nucleotide triphosphate (NTP) binding site, which is a known drug target site of different AKs from *Mycobacterium tuberculosis, Acinetobacter baumannii, Enterococcus gallinarum* and *Escherichia coli.* Molecular dynamics (MD) simulations showed stable behavior of all docked complexes with notable conformational stability of salt bridges at NTP- binding site of different AKs. Similarly, synergistic effect of antimicrobial peptides (AMPs) along with different antibiotics has also been studied. Thus, these studies open avenues for the development of new antimicrobial agents for targeting diverse MDR strains.

IL-24

Multidisciplinary approach to understand carbapenem resistance mechanism in *A. baumannii* and assessment of alternate therapeutics

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Acinetobacter baumannii causes nosocomial infections and its prevalence in clinical setup has increased gradually. *A. baumannii* has emerged resistance against carbapenem (commonly prescribed drug), which is a significant health problem and responsible for high morbidity and mortality. We have identified the carbapenem resistance mechanism of the *Acinetobacter baumannii* using proteomics, biophysics and bioinformatics, microbiological and molecular biology techniques. Phenotyping, genotyping and quantitative proteomics studies concluded the presence of different carbapenem hydrolyzing β-lactamase (OXA-51, AmpC and NDM), efflux pumps and upregulation of metabolism in carbapenem resistant strain. Further, the bacterium also down regulates putative OmpW and other transporter that decreases the uptake of carbapenem. OXA-51 was cloned and purified. The recombinant OXA-51 has secondary structure that is very resistant to pH and temperature change. This plays a vital role in retaining function of this beta-lactamase under stress conditions. We have also signified the use of novel excitation at 305nm for monitoring the surface tryptophan of protein. Bioinformatics studies on the modeled beta-lactamase showed that carbapenem is effectively hydrolyzed by OXA-51 or NDM harboring *Acinetobacter*. We have also pointed out that old β-lactam such, as penicillin might be better antibiotic for NDM-harboring A. *baumannii.* We are also trying to find the suitable nano-based, herbal-based and *in-silco* design alternative medicine against carbapenem resistant strain of *A. baumannii*.

IL-25

Characterization of Weak Forces Involved in Protein–Ligand Binding

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Protein–ligand interactions are of fundamental importance for biological processes. A ligand binds to the protein through many weak, non-covalent interactions such as electrostatic, hydrogen bonds and van der Waals interactions. These week forces play an important role in ligand-protein binding. The binding strength of a ligand on the protein depends upon a precise fit to the surface-exposed amino acid residues. The understanding of molecular recognition processes of ligands and proteins requires a complete characterization of the binding energetic and correlation of thermodynamic data with interacting structures involved. A quantitative explanation of the forces that govern molecular associations needs to determine all thermodynamic parameters, including free energy change of binding (ΔG), enthalpy change (ΔH), and entropy change (ΔS) of binding. Since, ligand-protein interaction provides the fundamental knowledge for the development of structure-based molecular design strategies therefore; a close insight into the ligand-protein binding is of significant interest.

IL-26

Ras hyperactivation and actin polymerization in *Candida albicans*: An interesting connection

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Ras is a highly conserved small GTPase involved in a variety of physiological processes, in organisms ranging from lower eukaryotes to humans. Active Ras triggers multiple signaling events and defects in Ras signaling can lead to a vast number of defects including some types of developmental disorders and cancers. In the human pathogenic fungus *C. albicans* too Ras participates in a number of important signaling events, the most important of which is the cAMP-dependent PKA signaling that determines morphogenetic transformations and governs the virulence of the organism. Heightened Ras signaling in *C. albicans* via the PKA pathway can be achieved in multiple ways. For example, overexpression of Ras1 can increase cAMP dependent PKA signaling. Ras1 can also be constitutively activated via a G13V mutation that prevents its GTP-bound form from interacting with the GTPase activating protein (GAP) and returning to its inactive GDP-bound form. Similarly, Ras1 can be hyperactivated by depleting *C. albicans* cells of Hsp90, the inhibitory heat shock protein that modulates the interaction of Ras1 with adenylyl cyclase (Cyr1) and promotes its interaction with GAP. Depleting available levels of Hsp90 reduces interaction of Ras(GTP) with GAP, and helps the organism to rapidly transform from yeast to hyphal form for invasive colonization of the host.

We examined the difference in dynamics of Ras1 under conditions of overexpression versus constitutive activation or hyperactivation in *C. albicans* and analyzed its implications for the pathogen. Using fluorescence correlation spectroscopy (FCS), we demonstrate that overexpression of Ras1 is dynamically a very different event from Ras1 hyperactivation via either constitutive activation of Ras1 or depletion of Hsp90. We show that Ras hyperactivation results in significantly slower dynamics due to actin polymerization. In an unusual sterol-deficient hyperfilamentous GPI mutant of *C. albicans* too Ras hyperactivation is a result of Hsp90 downregulation and actin polymerization. Upon hyperactivation, Ras1 co-localizes with G-actin at the plasma membrane rather than with F-actin. Treatment with actin depolymerization agents substantially improve Ras1 dynamics in these and other strains that show Ras1 hyperactivation. Based on these results, we propose a model for Ras1 hyperactivation in *C. albicans* and present its implications for hyphal morphogenesis in this pathogenic fungus.

IL-27

Differential Expression Pattern of Proteins in Response to Elevated CO_2 and Low Nitrogen in Wheat

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Carbon (C) and nitrogen (N) are two essential elements that influence plant growth and development. The C and N metabolic pathways influence each other to affect gene expression, but little is known about which

genes are regulated by interaction between C and N or the mechanisms by which the pathways interact. In the present investigation, proteome analysis of N-efficient and N-inefficient wheat, grown under varied combinations of low-N, sufficient-N, ambient [CO_2], and elevated [CO_2] was carried out to identify proteins and the encoding genes of the interactions between C and N. Two-dimensional gel electrophoresis (2-DE) revealed 62 differentially expressed protein spots. These protein spots were identified by matrix-assisted laser desorption ionization-time of flight/time of flight mass spectrometry (MALDI-TOF/TOF). The identified proteins are related to various molecular processes including photosynthesis, energy metabolism, protein synthesis, transport and degradation, signal transduction, nitrogen metabolism and defense to oxidative, water and heat stresses.Nitrogen-efficient cultivar showed a higher potential of redox homeostasis, protein stability, osmoprotection and regulation of nitrogen levels. The identified differentially expressed proteins can pave the way for enhancing the multiple-stress tolerance in wheat and developing a better understanding of its mechanism.

IL-28

Role of epigenetics and Proteasomal pathway gene(s) in combating virus infection in plants

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Tomato leaf curl disease (ToLCD), caused by strains of Tomato leaf curl virus (ToLCV), is a major constraint to tomato production. To understand the molecular mechanism of virus tolerance, we studied the abundance of viral genomic replicative intermediate molecules and virus-derived siRNAs by host plant in a naturally tolerant (H-88-78-1) and susceptible (Punjab Chhuhara) cultivars at different days-post-infection. We showed that less abundance of viral replicative intermediate in tolerant cultivar may have a correlation with a relatively higher accumulation of virus-specific siRNAs. Further, a significant correlation was observed between altered methylation patterns in tolerant versus susceptible cultivars. These suggest that both viral DNA methylation and siRNA-mediated degradation play an important role in conferring tolerance against ToLCNDV. Further, we functionally characterized a 26S proteasomal subunit RPT4a (SlRPT4) gene, which was differentially expressed after ToLCNDV infection in tolerant cultivar. The study showed that SlRPT4 protein binds to promoter region of ToLCNDV genome thereby hindering the expression of virus genes which subsequently reduces viral replication and infection in tolerant cultivar. Transient overexpression of SlRPT4 resulted in activation of programmed cell death and antioxidant enzymes system. Overall, present study highlights non-proteolytic function of SlRPT4 and their participation in defense-pathway against virus infection in tomato.

IL-29

DHX9 Helicase suppress genome instability at non-B DNA structures sites in Human Cells

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Non-B DNA structures in the human genome have been implicated in stimulating genomic instability in human cells by inducing mainly double-strand breaks. Thus, it is of interest to determine the mechanism(s) involved in preventing non-B DNA structure induced strands break in cells. For this we used a mutation-reporter system containing non-B DNA structures to examine the effect of DHX9 activity on naturally occurring non B-DNA structures in human cells. We have also performed the next-generation sequencing on a HiSeq 2000 sequencing system to detect base variants that might be present in plasmids.We found that non B-DNA structures significantly increased mutagenesis in small-interfering siRNA-treated, DHX9-depleted cells, affecting mostly deletions. Moreover, DHX9 associated with non B-DNA in the context of supercoiled plasmids. To further investigate the role of DHX9 in the recognition/processing of non B-DNA, we performed binding assays in vitro and chromatin immunoprecipitation assays in cells. DHX9 recognized non B-DNA, as evidenced by it's binding to the Non B-DNA structure and enrichment at the non B-DNA region compared with a control region in human cells. The data suggest that DHX9 processnon B-DNA structures in vivo and support its role in the overall maintenance of genomic stability at sites of alternatively structured DNA.

IL-30

Alternate pathway to ascorbic acid induced inhibition of *Mycobacterium tuberculosis* growth

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Ascorbate has been demonstrated to interfere with the growth of *Mycobacterium tuberculosis*. It scavenges oxygen in the culture medium to induce dormancy of *M. tuberculosis*. It kills the mycobacteria by generating reactive oxygen intermediates via iron mediated Fenton reactions. In this study, we observed that ascorbate can inhibit *M. tuberculosis* isocitrate lyase (MtbICL) with an IC_{50} of 2.15 mÌ. MtbICL is an essential enzyme for the survival of *M. tuberculosis* under dormancy. We studied the effect of ascorbate on the growth of *M. tuberculosis* H37Rv metabolizing through citric acid cycle or glyoxylate cycle with glucose or acetate respectively as the sole carbon source. It was observed that 4 mM ascorbate inhibited ~89 % of the growth in glucose medium, which was confirmed to be mediated by Fenton reaction, as the inhibition was significantly lesser (61%) under low iron condition. On the other hand, in acetate medium, ~97% of the growth was inhibited and the inhibition was uninfluenced by the iron levels. 3-nitropropionate, a known inhibitor of MtbICL, was seen to cause significantly higher inhibition in the acetate medium than in the glucose medium; however it was indifferent to iron levels in either medium. Molecular docking and dynamic simulation studies confirmed stable binding of ascorbate to MtbICL leading to its inhibition. These observations suggest an additional pathway for ascorbate induced inhibition of *M. tuberculosis* through inhibition of glyoxylate cycle.

IL-31

Role of N-terminal of Dexras-1 in modulation of N-type Calcium channel, Cav2.2: Structure function relationship

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Dexras1 (Rasd1) and RHES (Ras Homologues Expressed in Striatum, Rasd2) are the monomeric G proteins, which are involved in many physiological processes and share 62% similarity. Both these monomeric Ras show about 35% similarities to other Ras proteins Human DEXRAS1/Dexras1 is a 30 kDa protein (281 amino acids, accession number AF498923) expressed in almost all tissue whereas Rhes is a protein with 266 amino acids and is primarily expressed in brain and its expression can be regulated by thyroid hormones (accession number BC013419). Both Dexras1 and Rhes have 3 prominent structural domains/ conserved regions: (1). 4 highly conserved GTP binding domains, (2). Effector loop - might possibly participate in protein-protein interactions with other signalling molecules and is necessary for full biological activity. (3). A CAAX sequence at end of C-terminal - a consensus site for isoprenylation (CVIS). All these conserved regions are thought to be important for activity of Dexras1. Using whole cell patch clamp recording, we had earlier shown that Dexras1 has similar signalling properties as Rhes in terms of modulation of Cav2.2 and both show pre-pulse facilitation and inhibition of Cav2.2.

The enormous range of activity (in terms of modulation of various pathways) that the two monomeric proteins, Dexras1 and Rhes show must be unique to some of its structural determinants/domains that might assist them in performing a wide range of functions. These monomeric Ras proteins have a unique C and N-Terminal. The C-Terminal has been shown to interact with proteins. In the present study we have investigated function(s) of its N-terminal that is/are still unknown using and supplemented data with bioinformatics based studies. Several mutant versions with modified N-terminus and chimeras of Dexras1 were prepared using cDNA and their activity was assessed via whole cell patch clamp recording. As the experimentally determined crystal structure of these proteins is not available, in the present study, with the help of bioinformatics tools, we have tried to develop a hypothesis about the unique 3D structure of these proteins.

The prevalent belief is that Dexras1 and Rhes alter the Gβg levels and along with Gα-i modulate this channel. Our results suggest that N-terminus of Dexras1 might possible interact with either the Cav2.2 or its supporting subunits like alpha2 delta b or beta 3 and alter the current and this model is supported by the mutant activity data where the two functions of pre-pulse facilitation and inhibition of channel are segregated.

Acknowledgement

Patch clamping and mutant study was done in the lab of Dr. Brett A Adams, USU, Logan, UT- USA.

ABSTRACT – ORAL AND POSTER PRESENTATIONS

(Arranged in alphabetical order of names of presenting author)

P-1

Effect of Gamma Rays on Vigna unguiculata and Detection of DNA Polymorphism through SSR Marker

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Vigna unguiculata (L.) Walp is an important pulse crop and a fodder with great ecological value. The effects of different gamma irradiation doses (100-400 Gy) on seed germination and seedling morphology were studied in V unguiculata and simple sequence repeat (SSR) markers were employed to identify the DNA polymorphism among mutants. Significant variations were recorded for seed germination, seeds per pod and number of pods per plant. The improved agronomic traits, such as seeds per pod and number of pods per plant, were recorded at 300Gy dose and 100Gy dose for seed germination. SSR analysis generated in total 153 scorable fragments, of which 64 (41.83%) were polymorphic. The percentage of polymorphism ranged from 14.29 to 83.33 with an average of 36.69%. Jaccard's coefficients of dissimilarity varied from 0.6785 to 1.000, reflecting the level of genetic variation among the mutants. The constructed dendrogram grouped the entities into seven clusters. Consequently, it was concluded that gamma rays irradiation of seeds generates a sufficient number of induced mutations and that SSR analysis offered a useful molecular marker for the identification of mutants.

P-2

Evaluation of Phytochemicalsas Prospective Inhibitors Touching Human Carbonic Anhydrase V

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Natural products play a substantial role in therapeutics. Polyphenols like curcuminoids, flavonols, flavonoids, epiandrins, coumarin, ellagic acid, resveratrol are being employed as potential inhibitors against human carbonic anhydrase VA (hCAVA). Some of these polyphenolic compounds act as good hCAVA inhibitors. We have examined polyphenolic content in citrus fruits (such as mandarins, oranges, grapefruit, and acid citrusfruits, namely bergamots, lemons), Garcinia cambogia etc. Lemon contains polyphenols likenaringin, hesperetin, and naringenin which are helpful in suppressing diet-induced obesitythrough up regulation of mRNA levels of all the enzymes that are involved in â-oxidation,such as acyl-CoA oxidase, peroxisome proliferator activated receptor (PPAR), FA synthasein liver and white adipose tissue. Further protein-phytochemical interactions were examined by fluorescence binding and isothermal titration calorimetry. CA enzyme hydration assay disclose that these compounds significantly inhibits the activity and values of dissociation constant lies in nanomolar range. Hence these natural foodstuffs might be used as potential inhibitors against CAV, which in turn helpin curing obesity that is foremost life style disordernowadays and directly-indirectly associated with other life style disease named cancer.

P-3

Molten Globule conformation of L94G mutant of horse Cytochrome-*c*

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A newly synthesized polypeptide chain rapidly folds into its unique biologically active structure, how does this occur is known as protein folding problem. There are several related questions to this problem. One of them is by what kinetic process or pathway does a protein fold, it is now acceptable that proteins fold *via* a number of different partially folded structured intermediates such as molten globule (MG) and pre-MG states. Understanding the structure of these states at the atomic level is often a challenge, as these states are observed mostly under extreme conditions of pH, temperature and chemical denaturants. Several other processes such as chemical modification, site directed mutagenesis (or point mutation) and cleavage of covalent bond of natural proteins often lead to MG and pre-MG states. The dynamic nature of the protein in these states makes them unsuitable for 3D structure determination. Most of the intermediate states studied so far have been characterized using techniques, circular dichroism, fluorescence, viscosity, dynamic light scattering measurements, dye binding, infrared techniques, etc. There is limited amount of structural data available on these intermediate states by nuclear magnetic resonance (NMR), if any, and hence the need to characterize these states at the molecular level. In horse cytochrome-*c*, a small 104- residue protein, we previously identified a mutant L94G which shows characteristics of MG state under native buffer condition at pH 6.0. We are in the process of characterizing this MG state at atomic level resolution by NMR using a $^{15}N^{13}C$ labeled protein. Our studies suggest that the MG state is remarkably different from the wild type protein.

P-4

Biophysical Characterization of Cytochrome *c* in the Presence of PEG 10,000

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The protein folding, an important process for protein to fulfill normal cellular functions, takes place in crowded environment. Due to presence of large amount of macromolecules, a significant fraction of intracellular space is not available to expanded macromolecule confirmation. Crowded environment results in excluded volume effect and opportunity for specific as well as nonspecific intermolecular interactions. In our work, we examined the changes on the structure and conformational stability of cytochrome *c* (cyt-*c*) in the presence of different concentrations (50-300 mg ml^{-1}) of polyethylene glycol (PEG) 10,000 at two different pH values. i.e. pH 6.0 and pH 2.0 at 25 °C using far-UV, near-UV and Soret CD, UV-visible absorbance spectroscopy, ANS (8-anilino-1-napthalenesulfonic acid) binding fluorescence and intrinsic fluorescence spectroscopy. We studied the effect of PEG on the acid induced state of cyt-*c*. Our result showed that PEG 10,000 refolds the acid-induced cyt-*c* to the molten globule (MG) like state at pH 2.0. In far-UV CD, no significant change has been observed in the secondary structure of cyt-*c* with different concentrations of PEG 10,000 at pH 6.0. Tertiary structure of cyt-*c* does not perturb in the presence of PEG 10,000 as observed by UV-visible absorbance spectroscopy, near-UV and Soret CD. We measured heat-induced denaturation of protein by monitoring changes in $??_{405}$ and $[\theta]_{222}$. A decrease in the thermal transition temperature (T_m) for cyt-*c* was observed in the presence of different concentration of PEG 10,000. PEG is reported to bind to the hydrophobic group of protein and it preferentially interacts with the denatured state of protein whereas excluded from the surface of native state. Unfolded state is favorable in the presence of PEG 10,000 than the native state. Hence, thermodynamic equilibrium between native and denatured state (N↔D) shifts towards the right i.e towards the denatured state in the presence of different concentration of PEG 10,0000.

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P-5

Molecular dynamic simulation analysis of novel mutations identified in PTENamong North Indian cervical cancerpatients

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Background: Somatic mutations are critical genetic anomaly accountable for underlying heterogeneity of the tumour cells. Currently, PTEN incongruity is potent and persuasive malfunctioning in varied human malignancies.

Aims and objectives: In this study we have identified different mutations in the exon region of PTEN genes. Functional consequences of these mutations were explored using *In-Silico* techniques.

Materials and Methods: Mutations were identified using Sanger's sequencing in a total of 102normal-tumour pairs. The findings were statistically correlated with the clinical parameters and the effect of non-synonymous mutations was studied with the help of software's such as PolyPhen, SIFT and Molecular Dynamics simulations.

Results: Out of five non-synonymous mutations identified MD simulation analysis showed that E150L exhibited highest deviation in crystal structure of PTEN.

Conclusion This study will provide valuable insight into structural and functional aspects of PETN's involvement in cancer.

P-6

Drug Repurposing Concept for FDA Approved Drugs against Anti-Alzheimer drug targets: Virtual Screening, Docking and Dynamics Study

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Alzheimer's disease (AD) is the leading cause of dementia, accounts for 60 to 80 percent cases. Two main factors called β-amyloid (Aβ) plaques and tangles are prime suspects in damaging and killing nerve cells. However, oxidative stress, the process which produces free radicals in cells, is believed to promote its progression to the extent that it may responsible for the cognitive and functional decline observed in AD. As of today there are few drugs in the market for treatment, but their cholinergic adverse effect, potentially distressing toxicity and limited targets in AD pathology limits their use. Therefore, it is crucial to find effective compounds to combat with AD. It is therefore needed to search for newer and effective drugs that could inhibit the increasing number of putative drug targets. In this work, drug repurposing concept was applied to identify potential FDA approved drugs against 13 potential drug targets of Alzheimer (1 *EQG*, 1*MX*, 1*PBQ*, 1*Q5K*, 1*QWC*, 1*UDT*, 2*FV5*, 3*BKL*, 3*G9N*, 3*QMO*, 4*BOP*, 4*DJU* and 4EY5) and these total 1985 FDA drugs were virtually screened using a structure-based approach. In our study, we used GOLD tool for virtual screening and applied rigid docking followed by induced fit docking algorithm in order to enhance the accuracy of docking and prioritizing drugs for repurposing. We further performed molecular dynamics simulation for 40*ns* to see the effect of top scorer compounds on the structural stability of all the five targets that indicates that inhibitors preferentially bind into the active site of the targets. To understand the system level process, we have considered these 13 proteins as the seed molecules from which we get direct and indirect protein-protein interactions too. However, these FDA-approved drugs, resulting in an urgent need to develop effective anti-Alzheimer inhibitors that display good safety profiles in a short duration.

P-7

Understanding the role of transcription factors associated with Pancreatic Cancer

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Pancreatic cancer kills more than 300,000 people every year. This makes it the seventh most common cause of cancer-related deaths worldwide. Its prognosis is very poor with a five-year survival rate about 5%. Pancreatic ductal adenocarcinoma (PDAC) is the most frequently observed type of pancreatic cancer which occurs due

to the progression of precursor lesions such as Pancreatic Intraepithelial Neoplasia (PanIN), Intraductal Papillary Neoplasm (IPMN) and Mucinous Cystic Neoplasm (MCN). Among these precursor lesions, PanIN is the most frequently detected. Genes directing the origin and differentiation of precursor cells have the tendency to transform the normal cells into tumor cells in response to activating or inactivating mutations. Therefore, we investigate the role of genes expressing transcription factors (TFs) involved in pancreas development and cell fate differentiation. Pancreas/duodenum homeobox protein 1 (PDX1), Pancreas transcription factor 1 subunit alpha (PTF1A), Nuclear receptor subfamily 5 group A member 2 (NR5A2), Hepatocyte nuclear factor 1-alpha (HNF1A) and Hepatocyte nuclear factor 1-beta (HNF1B) play critical role in the development and differentiation of pancreatic precursor cells. Mutation induced abnormalities in the regular function of these TFs cause abnormal cell growth and proliferation that leads to the formation of cancerous cells. Hence, we hypothesize that these TFs are highly susceptible for the origin of pancreatic cancer. Therefore, we propose that these TFs might be considered potential therapeutic targets against development of anticancer drugs.

P-8

Proteomic, metabolomic and hormonal aspects of polycystic ovarian syndrome: An insight into the systematic analysis of PCOS-associated biomarkers

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Polycystic ovary syndrome (PCOS) represents a multifactorial complex disorder due to rise of set of symptoms including oligo/anovulation, hyperandrogenism and polycystic ovaries leading to reproductive, hormonal and metabolic disturbances among the reproductive age women. The etiopathogenesis of PCOS remains uncertain despite the presence of numerous research studies devoted to this disease. A large number of affected proteins, hormones and metabolites have been characterized in PCOS, which interact within various metabolic pathways like protein metabolic process, energy metabolism, immune response, inflammation, oxidative stress, fibrinolysis and thrombosis. These biomolecules provide pivotal information about the altered metabolic pathways of this disease. Total of 3745 articles related to hormonal, proteomic and metabolomic approaches on PCOS were screened and 185 of them were further assessed in this study. The identified PCOS-associated biomolecule markers utilizing proteomic approaches should be considered as potential candidates in future researches with the goal to indicate a clear view on the molecular phenotypes of PCOS. This review aims to perform an extensive survey on the identified biomarkers in blood samples of PCOS-afflicted women among research studies conducted during 2011 to 2016 and sort them with respect to their association with proteomic, metabolomic and hormonal contributions as well as their functions. Therefore, it can be served as a reference for the pathology of PCOS and its relevance with its consequences such as cardiovascular and inflammatory disorders, insulin resistance, endometrial cancer (EC), ovarian cancer (OC) and hormonal and reproductive disturbances. It is intended to provide a general insight into the various biomolecules found to be associated with PCOS within recent years, as the etiology and a transparent view on molecular phenotype for this syndrome still remain controversial.

P-9

In-silico and *In-vitro* Evaluation Of GABAergic Effect of Synthesized 1,2,4-Triazine Derivatives as a Potential Candidate for Treatment of Epilepsy

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Epilepsy is a chronic neurological disorder characterized by the presence of recurrent seizure. There are large number of antiepileptic drugs are available for treatment of epilepsy, But unfortunately all these drugs are associated with certain side effects. Antiepileptic drugs exert their anti-seizure effects through different mechanism. The potentiation of GABAergic inhibitory neurotransmission represents a classical and well known anti-seizure effect. In the view of above prospects we have designed and synthesized a new series of 1,2,4-triazine derivatives as an

antiepileptic agents. In first step we have synthesized different substituted urea derivatives from different substituted anilines. In second step we have synthesized semicarbazide derivatives from different substituted ureas on treatment with hydrazine hydrate. In third step condensation of different substituted semicarbazides with isatin/5-chloroisatin in presence of glacial acetic acid, different substituted semicarbazones were prepared. These substituted semicarbazones were then cyclized in presence of aqueous sodium hydroxide(IM) yielded 1,2,4-triazine derivatives. The structures of all synthesized derivatives were confirmed by spectral data such as I.R., ^{1}HNMR and mass spectrometry. The antiepileptic activity of these derivatives were carried out both *In-silico* as well as *in -vitro*. The *In -silico* study comprises molecular docking study which measure the binding affinity of these derivatives on GABA receptors. Molecular docking was performed on developed homology model of $GABA_A$ receptor with the help of Schrodinger 2016 software using mestro 11 programme. In addition, the compounds having high docking score were also subjected to *In-vitro* GABA enzyme estimation. The *In-vitro* method of GABA enzyme estimation measured the whole brain GABA level. The results of molecular docking study showed that most of the compounds exhibited good docking scores on binding with homology model of GABA receptor. The results of *In-vitro* GABA enzyme estimation showed that oral treatment of compound increased the GABA level significantly compared to the control animals. On the basis of above findings we can say that synthesized triazine derivatives have GABAergic potential which can be usedas a potent antiepileptic agent in drug design and development.

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P-10

Multistage Unfolding Study of RRM1 domain of TDP-43

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The thermodynamic stable conformation of a protein is of paramount importance to perform the biological function. However, the capability of adapting the different dynamic behaviour due to physio-chemical modulation in native structure, display the distinct functional properties. Various and efficient computational methods have been evolved to capture dynamic behaviours of proteins in different physiological condition. Here, we determined the biophysical properties of TDP-43 (TAR DNA-binding protein), normally involved in mRNA splicing, translational regulation, and transport. However, mutations in human TDP-43 results in the formation of ubiquitinated inclusion bodies leading to the pathological neurodegenerative condition known as amyotrophic lateral sclerosis (ALS). We performed long-time (2μs) all-atoms molecular dynamics simulations to investigate the stability and unfolding of RRM1 domain of wild-type and mutant TDP-43. Results from free-energy landscape (FEL) analysis and time independent component analysis (tICA) showed the existence of multiple transition states ensemble as an intermediate and metastable state in mutant as compared to WT. The presence of these intermediate states may modulate the unfolding pathway and leads to misfolding and aggregation seen in ALS. These results suggested that the characterization of intermediate ensembles will provide better understanding of pathogenesis of ALS and could lead to therapeutic intervention.

P-11

Interaction of Transforming Growth Factor Beta 3 (TGFb3) With Its Receptors: A Biophysical Perspective

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Members of transforming growth factor beta (TGFb) family of proteins are important cytokines involved in many fundamental cellular processes including cell proliferation and differentiation, wound repair, apoptosis etc. There are three isoforms of TGFb, TGFb1, TGFb2 and TGFb3. TGFb3 shows strong tendency to aggregate making this protein very difficult to study at physiological pH. This study deals the interaction of TGFb3 with the soluble domain of its receptors, transforming growth factor b receptor type I (TRI) and transforming growth factor b receptor type II (TbRII). Since the proper orchestration of signal transduction depends upon a delicate and well-balanced set of interactions between the three proteins mentioned above forming a hetero-hexameric complex, an in-depth profiling of the binding mechanistic becomes necessary to have a proper grasp on understanding the underlying molecular processes. Binding studies are carried out between TGFb3 and (wild type) TbRII using native poly acrylamide gel electrophoresis (native PAGE) and isothermal titration calorimetry (ITC). The binding constant of the binary complex deduced from the thermodynamic characterization of the receptor-ligand interaction is in close agreement with what was previously reported. To obtain a comparative analysis of the binding affinity of TâRII mutants, ITC with TGFb3 was also performed. Formation of the hetero-hexameric ternary complex composed of two residues each of TâRII and TâRI with TGFb3 dimer was confirmed by Native PAGE analysis. More experiments are on the way to deduce the binding isotherm of the binary complex with TâRI titration giving rise to the ternary complex using ITC. Fluorescence correlation spectroscopic (FCS) studies have also been initiated for a more in-depth delineation of the dynamics of the receptor-ligand (TâR-TGFb3) assembly.

P-12

High throughouput screening (HTS) of CAMK4 to identify drug candidates using molecular docking approach

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Calcium/calmodulin-dependent protein kinase IV (CaMKIV) plays a key role in the regulation of calcium-dependent gene expression. CaMK1V is associated with several diseases including cerebral hypoxia and neurodegenerative disorders and is also over expressed in lung and hepatocellular carcinoma. As CAMKIV is a responsible factor for neurodegenerative diseases and varieties of cancer, it could be considered as a new target for the structure-based drug designing. Here, we used natural compounds to find potent inhibitors against CAMK4. In search of better and natural inhibitors of CAMKIV, here we made an attempt to elucidate the role of natural inhibitors to inhibit CAMKIV activity and its role in apoptosis. This study reports new observations on docking and the calculated binding affinity. The study was carried out using 12500 natural compounds from zinc databases. Virtual high throughput screening of 12500 compounds were being carried out using Auto dock vina, subsequently 40 best docked score were sorted out which have binding affinities in the range of -11.3 to -10.0Kcal/mol. Docking analyses revealed that ligands bind in the large hydrophobic cavity of the kinase domain of CAMK4 through several hydrophobic and hydrogen-bonded interactions. Among 40 compounds 35 compounds follow Lipinski rule of five. Further we carried out pharmacokinetics and toxicity of the drugs that follow Lipinski rule of five. Finally here we get five compounds which are being sorted out to understand the structure-function relationship in detail. MD simulation will be used for the top three compounds having docked score -11.3,-11.1,-11.0 to find the effective ligands, the result of which will be further validated experimentally by synthesizing compound.

P-13

Exploring conformational landscape in pregnane X receptorInsight into rational drug design

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Ligand-regulated pregnane X receptor (PXR), a member of the nuclear receptor superfamily, plays a central role in xenobiotic metabolism. Despite its critical role in drug metabolism, PXR activation can lead to adverse drug-drug interactions and early stage metabolism of drugs. Activated PXR can induce cancer drug resistance and enhance the onset of malignancy. Since promiscuity in ligand binding makes it difficult to develop competitive inhibitors targeting PXR ligand binding pocket (LBP), it is essential to identify allosteric sites for effective PXR antagonism. Here, molecular dynamics simulation studies of PXR's ligand binding domain (LBD), in monomeric and in complex with RXR and co-activator, were carried out to explore the conformational landscape of PXR LBD in apo and ligand-bound state. The study unraveled the existence of two different conformational states, namely 'expanded' and 'contracted', in apo PXR LBD.[1] Ligand binding events shifted this conformational equilibrium and locked the LBD in a single 'ligand-adaptable' conformational state. Ensemble-based computational solvent mapping identified a transiently open potential small molecule binding pocket between α5 and α8 helices, named 'α8 pocket', whose opening-closing mechanism directly correlated with the conformational shift in LBD. Presence of a small molecule NSC1014 at the allosteric α8 pocket disrupts the characteristic dynamics of PXR LBD. The molecular details provided here could guide new structural studies to understand PXR activation and antagonism. Though PXR in complex with RXR and co-activator exhibited the expanding/contracting motion, the degree of flexibility was hampered as a result of complex formation. Such reduced dynamics due to heterodimerization may result in a partial loss of promiscuity in the apo state of PXR-RXR-CoA complex. This aspect will be probed further by additional studies.

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P-14

Successful Refolding of Dengue Virus Specific Single Chain Variable Fragment Antibody (scFv)

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Dengue is one of the most prevalent human pathogen that annually infects around 50 million people worldwide. However, there is no effective vaccine or drugs for the treatment hence imposinga big challenge to human. It is already reported that the fusion (Fu) and BC loop of dengue envelope protein are highly conserved and critically responsible for viral genome receptor mediated endocytosis and its pathogenesis as well. Therefore, screening and development of high affinity monoclonal antibody or single chain variable fragment antibody specific to dengue Fu-BC could be effective inpreventing the receptor mediated endocytosis of the virus and thus inhibiting the viral growth inside the host cells. For these purposes, we retrieved short chain variable fragment antibody (scFv) sequences from PDB database (3IXY) and cloned it in three (pET-28b, pGEX-4T-1 and pMAL-p5X) different vectors which possess His, GST and Mal tag respectively. But, in all the cases of *E.coli* BL21 expression, the scFv proteins are undergone maximally in pellet rather than in solution. To regain protein in solution scFv is over-expressed in *E.coli*,yielding maximum fraction in inclusion bodies (IB). After worthy extraction and vigorous washing with TE(50/20) buffer, IB pellet was re-solubilized in guanidine hydrochloride and allowed for refolding with buffer containing 4.5 M urea, 0.55 M L-arginine–HCl, 100 mMTris-HCl, pH 8.1, 1 mM of reduced glutathione (GSH), and 0.1 mM of oxidized glutathione (GSSH) to a final concentration of 50 ug/ml. The refolding mixture was then extensively dialyzed against Tris-HCl buffer pH 8.1 and concentrated up to 0.5mg/ml, yielding in total 8 ml volume of soluble scFv antibody from 500ml bacterial super broth. The concentrated scFv was finally purified by size exclusion chromatography (FPLC) and confirmed its proper folding by ELISA and pull-down interaction assay with dengue Fu-BC antigenic protein.

P-15

Genome-wide identification of AP2/ERF protein family in *Oryzasativa* L. *Indica* and their drought induciblity

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Rice is become a staple food which grown in more than 100 countries. Indica and Japonica rice together shared 90% of the world market. Increase in world temperature results into erratic rain pattern which subsequently reduces the total production of rice. A transcription factor plays an important role to regulate many genes in different environmental conditions and pose suitable candidate for further study. The role of AP2 family transcription factors is critical among all the TFs due to their involvement in variety of developmentaland environmental conditions like floral and embryo development,abiotic andbiotic stresses. Although, AP2 is one of the well-studied families of transcription factor in Japonica but still no report is came for Indica genotype apart from their global share and commercial viability. In this study, we identified 169 proteins of AP2/ERF family in Oryza sativa L. ssp. Indica genotype which phylogenetically classified into 31 AP2, 135 ERF/DREB and 5 RAV three major groups during in-silico characterization. We not only identify 4 more novel proteinscompared to Japonicagenotype but also find one unique sugar transporter hybrid protein which was previously unidentified. Cis-regulatory sequence analysis of these protein genes shows 20 genes contains each of DRE, C-repeat elements (CRT) and ABRE- element in their upstream region. This can regulate in either way through ABA dependent or independent pathway during drought stress. Furthermore, the expression analysis in four different drought tolerant Indica verities under the drought condition was also performed to validate the result of meta-transcriptomic and protein database analysis.This identification, characterizationand expression analysis of Indica AP2/ERF protein genes will provide rich resources and opportunities to understand abiotic stress tolerance in crops. However, for the elucidation of biological roles and physiological functions of AP2/ERF family genes in Oryzasativa L. ssp. Indica will require further work.

P-16

Structural insight into the metal-ion mediated modulation of the catalytic function of *H.pylori* arginase

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H.pylori arginase (RocF), a well-characterized virulence factor acid-protection and pathogenesisin the human stomach is a bimetallic enzyme whose activity is dependent on either Co^{2+} or Mn^{2+}. It has been previously shown that the recombinant protein as well as the enzymatic activity of arginase from bacteria grown in presence of these two different metal ions show higher activity with Co^{2+} than Mn^{2+}. These observations suggest that Co^{2+} is the preferred metal ion for higher catalytic activity and the metal-induced active-site architecture of these two proteins could be different. To test this hypothesis, we performed a detailed investigation that includes inhibition kinetics, pH-dependent studies, steady-state and time-resolved tryptophan fluorescence measurements, anisotropy-decay kinetics and molecular dynamic (MD) simulations. In the absence of the crystal structure of Co^{2+}-protein, we generated model structures of these two holoproteins and performed extensive MD simulations at microsecond time-scale, which provided useful insights into the difference of conformational changes induced by the metal-ions at the active-site. The study also provides insight into the relative change of the positioning of the loop that contains the catalytic residues. These observations along with the time-resolved fluorescence studies using intrinsic tryptophan probe explain the basis for higher catalytic activity in the Co^{2+}-protein. Additionally, the active-site architecture of these two holoproteins was found to be different compared with that of the human homologue, thereby highlighting a possibility for the development of a new class of inhibitors which is specific to this pathogen.

P-17

Induction of apoptosis in cisplatin-resistant ovarian cancer cell line by pre-treatment with Vitamin C followed by combinatorial therapy

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Cisplatin Resistance is a major deterrent in the successful management of ovarian cancer. Combinatorial therapy has long been used for the treatment of recurrent ovarian cancer, but their effectiveness is limited due to side effects coupled with resistance. Vitamin C (VC) has the tremendous potential to selectively target cancer cells at concentrations that are pharmacologically relevant. We investigated the impact of combinatorial therapy [Cisplatin (CP) and Gemcitabine (GB)] on VC sensitized cisplatin-resistant (CaOV-3) ovarian cancer cells and compared with cisplatin-sensitive (PA-1) cells alongwith HEK cells (Human embryonic kidney). IC_{50} was achieved when PA-1 cells were sensitized for 24 h with 2mM VC followed by 24 h treatment with 4.5µM CP + 31.25nM GB and CaOV-3 cells were sensitized with double dose i.e. 4mM VC and 62.5nM GB, keeping the concentration of cisplatin constant, significant cytotoxicity i.e. 89.7 ± *2.8* was observed in PA-1 cells when compared to treatment with individual molecules, whereas, in CaOV-3 cells, this combination resulted in only 60.5 ± *6.5*% cytotoxicity. When CaOV-3 cells were given a higher dose i.e 4mM VC and 62.5nM GB but concentration of CP was kept constant, a significant enhancement of cytotoxicity i.e. 81.3 ± *2.4* was observed. Reduced anti-oxidants (GPx, GR, SOD, CAT assay), enhanced DNA fragmentation and RNS generation (NO assay), upregulated cell damage marker i.e. LDH, altered Ca^{2+} signaling, loss of mitochondrial membrane potential, enhanced Bax, PTEN expression and reduced Nrf-2, BRCA-1 and Bcl-2 expression was reported in PA-1 and CaOV-3 cells post combinatorial therapy, while there was no harm to normal HEK cells. Perturbation of redox balance resulted in irreversible oxidative damage causing unbearable metabolic insults in ovarian cancer cells. Interestingly, Vitamin C exhibits remarkable specificity and pro-oxidative potential for sensitizing the cisplatin resistant ovarian cancer cells thereby preferentially protecting the normal cells.

P-18

Synthesis and biological evaluation of sulfonamides bearing bis-thiosemicarbazones as potential antiprotozoal candidates

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Sulfonamides have been known for a widevariety of pharmacological properties which include antibacterial, antiviral, antidiabetic, antitumor, anticancer and many others. Not only this, sulfonamides and their metal complexes have often been used as catalysts in organic reactions. Moreover, thiosemicarbazones also are a versatile class of compounds which have been a subject of interest owing to their incredible range of pharmaceutical properties. An improved biological activity by synthesizing a ligand which has the properties of both sulfonamides and thiosemicarbazones followed by their complexation with metal ions was the basic strategy behind this synthesis. Preparation of mononuclear complexes of cobalt, nickel and zinc with sulfonamides containing bis-thiosemicarbazones having general formula $[M(L)Cl_2]$ is described. A total of 15 compounds were synthesized including 2 sulfonamides, 2 bis-thiosemicarbazones and their corresponding 8 transition metal complexes. The ligands or the two bis thiosemicarbazones were prepared by reacting a mixture of bis aldehyde (synthesized as one of the intermediates) and thiosemicarbazide in the ratio of 1:2 in ethanol. These ligands were then used for complexation with metal (II) ions to obtain bis thiosemicarbazone metal complexes.The complexes werecharacterized using various spectroscopic techniques (CHN analysis, FT-IR, UV-Visible, H-NMR and ES-mass spectra) which suggested an octahedral geometry around the central metal ion. This was further confirmed from their magnetic moment study. All the compounds were screened for their antiamoebic activity (HM1: IMSS strain of *E. Histolytica*). Compound15was the most active antiamoebic compound (IC_{50} = 0.24±0.01µM), when compared with the standard reference drug, metronidazole (IC_{50}= 1.8± 0.01µM). It was observed that the incorporation of metal ions into the ligand structures enhanced their biological activity to a large extent.

P-19

In silico prediction of active site of Pathogenesis Related-4 protein from *Cicer arietinum*displaying RNase and DNase activities

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Plants are endowed with innate immune system, which enables them to protect themselves from pest and pathogen. The participation of pathogenesis-related (PR) proteins is one of the most crucial events of inducible plant defense response. Herein, we report on the characterization of *Helicoverpa*-inducible class II PR-4 protein from chickpea named CaHaPR-4. Bioinformatics based analysis of CaHaPR-4 protein indicated the presence of a signal peptide, barwin domain but it lacks the chitin-binding site / hevein domain. The recombinant CaHaPR-4a is bestowed with RNase and bivalent ion dependent DNase activity. Further, *in silico* approaches were adopted to identify the exact RNA and DNA binding sites and established by ensuring interactions between mutated CaHaPR-4 with altered active site and ribonuclease inhibitor, 5'ADP and DNase inhibitor, 2-nitro-5-thiocyanobenzoic acid (NTCB) using 3D modeling and docking studies. This study confirms the existence of active site for RNA binding and also predicts the presence of DNA binding site which supports the RNase and DNase activity shown by PR-4 from Chickpea.

P-20

Proteomics of oral potentially malignant and malignant disorders: An update

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Oral squamous cell carcinoma (OSCC) represents the 6th most commonly diagnosed cancer with approximately 62% occurrence in developing countries. There is an imperative necessity to develop biomarkers to identify high risk individuals, improve detection of oral potentially malignant disorders (OPMD), predict disease outcome and develop strategy for therapy. Proteomics is now an established molecular profiling techniquethat may be employed for OSCC diagnosis and prognosis by comparing protein profiles of cancer cells, tissues, plasma and salivawith that of the normal ones. The protein profile useful for diagnosis of a specific cancer qualifies to be a reliable marker. The biomarkers of OSCC, so far identified with Surface-enhanced/matrix assisted laser desorption/ionization mass spectrometry, are calcium/phosphate-binding proteins, DNA/RNA-binding proteins, proteins that maintain cell architecture, signaling and carrier proteins, protease inhibitors, acute-phase proteins, redox mediators, auto antibodies and enzymes of key biological pathways. Cancer proteomics in India was pursued on gliomas and oral cancer. Two-dimensional gel electrophoresis-mass spectrometry based investigations and quantitative liquid chromatography–mass spectrometry methods were used on OSCC tissues and in the process, differentially expressed proteins were identified. Research is being conducted on different cohorts to resolve defined clinical queries and delineate histologically normal surgical margins from the likely tumor areas or OPMD. Specific proteins eliciting an autoantibody response in oral cancer have been identified using immune-proteomics. Secretomes of cell lines from head and neck cancers have been analyzed. Proteomic approach has been used to investigate normal oral mucosa and paired biopsies of OSCC and oral submucous fibrosis by quantitative proteomics. We provide a critical appraisalon the role of proteomics in oral potentially malignant and malignant disorders.

P-21

Variations in Climatic Factors and their Association with Dynamics of Dengue Virus Transmission

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From the past several decades, dengue fever has been considered as the most prevalent vector-borne

infectious disease by world health organization. It is caused by one or more of the four dengue virus (DENV) serotypes. The disease is known to be widespread mainly in tropical and subtropical geographical locations of the world. The virus that causes dengue fever is transmitted to humans through bites of infective female mosquito species *Aedesaegypti*and *Aedesalbopictus.* The ecology of the mosquitoand the transmission pattern of dengue virus depend on the climatic conditions of the local area under consideration. Climate change may be defined as long term alterations in weather conditions. Undesirable variations in intense weather events may lead to changes in health threat to human beings which may further augment existing health problems. Three components that play major role in dengue virus transmission are; vector, pathogen and the transmission environment which are further influenced by variations in climatic parameters such as temperature, rainfall, precipitation, humidity,wind, and sunshine. Sometimes the effect of climate change on dengue virus transmission may be direct or indirect. Direct effects include impact on survival and reproduction of the virus inside the vector and the indirect effects take account of the impacts on the habitat of mosquito vectors. The analysis on climatic parameters in several studies revealed that low temperature (20-29ºC) and high humidity (70-90%) during rainy season, favor dengue virus propagation by enhancing mosquito's survival ratethat further leads to outbreaks of dengue hemorrhagic fever in those areas. Therefore, early warnings based on climate predictions may help in surveillance and control of dengue fever.The analysis will also help in understanding the association of climatic factors and dengue epidemiology.

P-22

The prospective of ionic liquids for the inhibition of *Bacillus cereus* EMB20 producing β-lactamase and its cells

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The repeated use and misuse of antibiotics has created an alarming signal to combat emerging multidrug resistant microorganisms. β-lactamase production is the major reason for inefficiency of most of the antimicrobial therapies. Hence the search for novel and potent lactamase inhibitors remains in demand. Maline, a deep eutectic solvent (DES) was found to efficiently inhibit β-lactamase from an environmental strain of *B. cereus* EMB20 in a non-competitive manner. The structural insights of maline inhibition were further gained by far-UV CD and intrinsic fluorescence spectroscopy. A disrupted secondary as well as tertiary structure was found as a function of maline concentration. The effect of individual components of maline on lactamase inhibition showed that malonic acid was mainly responsible for inhibiting lactamase activity. Far-UV CD, intrinsic fluorescence and docking studies found that malonic acid led to major perturbations in the secondary and tertiary structure of the enzyme while H-bondingwith the active site residues. Further the antibacterial and cytotoxic studies also confirmed the potential of maline as a potent growth inhibitor of â-lactamase producing *Bacillus cereus* EMB20. The present study thus provides the potential of ILs in designing suitable non â-lactam based inhibitors for antibiotic resistant microorganisms.

P-23

Systems Studies Unveil Role of Il6 in Macrophage Polarization During *Leishmania Major* Infection

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Leishmaniasis, the second most neglected tropical disease after malaria, is cause by obligatory intracellular protozoan parasite from the genus *Leishmania.* The parasite resides within the macrophage and establishes the infection by interfering with the cytokine signaling. Interleukin 12 (IL12), Interferon gamma (IFN γ), Tumor necrosis factor alpha (TNFα) are the pro-inflammatory cytokines that activates macrophages and promotes killing of parasite whereas IL4, IL12 & Transforming growth Factor beta (TGFβ) are anti-inflammatory cytokines which can deactivate macrophage and promote parasite survival. Release of Interleukin 6 (IL6) during leishmania infection, is previously known to have pro-inflammatory actions but recently its anti-inflammatory action has also been reported with respect to other infectious disease. Apart from this, Toll like receptors (TLR2-TLR1/TLR6) plays crucial role here. Lipophosphoglycan (LPG), an abundant molecule of parasite cellular membrane activate TLR2 and causing the release of IL6 together IL1 beta and TNF alpha through the activation of

transcription factor NFkB. Macrophage itself expresses Glycoprotein 130 (GP130) and gp 80 (IL6 receptors), therefore, by using systems biology approach we are trying to establish link between TLR2-TLR1/TLR6 and IL6 signaling pathway in Leishmania infected macrophages in the *insilico* conditions, which would be later validated in wet lab conditions. Using computational tools, mathematical model of Anti-inflammatory (diseased) and Proinflammatory (healthy) state of macrophage is generated to show the effect of IL6 in polarization of macrophage during infection. The simulated model is use to establish crosstalk point of intervention which will further be validated in wet lab condition. Refinement of the model is an iterative process; requires repetitive action of *in silico* simulation as well as wet lab validation. Target identified from refined models is IL6-IL6R-gp 130 complex. Protein-Protein docking studies of this complex reveal crucial interfacial residues that will be further use to design peptide for synthetic device mediated anti-leishmanial therapy.

P-24

Plasmodium falciparum SUFs system as a promising drug target againstcerebral malaria

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The plastid of *Plasmodium falciparum* known as apicoplast, performs several metabolic functions having essential role in propagation and survival of parasites in their host. Apicoplast composed of several pathways of bacterial origin measured to be striking drug target. Among these, the sulfur mobilization (SUF) pathway of Fe-S cluster biogenesis one of the promising drug target. Functional mechanisms of the Plasmodium SUFs in the maintenance of apicoplast and survival of parasite have not been elucidated. Biochemical investigation of its components and inhibitors of *Plasmodium* SUFs provided thrust in plasmodium biology and drug discovery. Various reactions in the plastid require the assembly of [Fe–S] prosthetic groups on participating proteins as well as the reductant activity of ferredoxin that is converted from its apo-form by the assembly of [Fe–S] clusters inside the apicoplast. The [Fe–S] assembly pathway involving sulphur mobilising Suf proteins has been predicted to function in the apicoplast with one component (PfSufB) encoded by the plastid genome itself. We demonstrate the ATPase activity of recombinant *P. falciparum* nuclear encoded SufC and its localisation in the apicoplast. Further, an internal region of apicoplast SufB was used to detect PfSufB–PfSufC interaction in vitro; co-elution of SufB from parasite lysate with recombinant PfSufC on an affinity column also indicated an interaction of the two proteins. As a departure from bacterial SufB and similar to reported plant plastid SufB, apicoplast SufB exhibited ATPase activity, suggesting the evolution of specialized functions in the plastid counterparts. Our results provide experimental evidence for an active Suf pathway in the *Plasmodium* apicoplast. We further reported the characterization of two proteins, *Plasmodium falciparum* SufS (PfSufS) and PfSufE that mobilize sulfur in the first step of Fe-S cluster assembly and confirm their exclusive localization to the apicoplast. The cysteine desulfurase activity of PfSufS is greatly enhanced by PfSufE, and the PfSufS-PfSufE complex is detected in vivo. Structural modeling of the complex reveals proximal positioning of conserved cysteine residues of the two proteins that would allow sulfide transfer from the PLP (Pyridoxal phosphate) cofactor-bound active site of PfSufS. Sulfide release from the L-cysteine substrate catalyzed by PfSufS is inhibited by the PLP inhibitor D-cycloserine, which forms an adduct with PfSufS-bound PLP. D-Cycloserine is also inimical to parasite growth, with a 50% inhibitory concentration close to that reported for Mycobacterium tuberculosis, against which the drug is in clinical use. Our results establish the function of two proteins that mediate sulfur mobilization, the first step in the apicoplast SUF pathway, and provide a rationale for drug design based on inactivation of the PLP cofactor of PfSufS.

P-25

Anti-oxidant, Anti-inflammatory and *In silico* studies of newly synthesized Hydrazone Derivatives of Triazole

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NSAIDs (non-steroidal anti-inflammatory drugs) are used as curative measure in the treatment of both acute and chronic inflammatory diseases. The mode of action of NSAIDs is to inhibit both the forms of cyclo-oxygenase enzyme (COX) i.e. COX-1 and COX-2,

responsible for synthesizing mediators of inflammation such as prostaglandins and thromboxanes. Beside their curative property, NSAIDs long term use caused severe adverse effects such as gastrointestinal perforation, ulceration, bleeding and renal toxicity. Medicinal chemists are in search of newer and safer anti-inflammatory agents for treatment of various inflammatory diseases. So, our work comprises of synthesis of Triazole, a five membered heterocyclic compound and its derivatives. It has been found that Triazole nucleus constitute as a core structural component displaying versatile biological activities and has been incorporated into various clinical drugs categories, including anti-microbial, anti-inflammatory, analgesic, anti-epileptic, anti-viral, anti-anxiety, anti-depressant, anti-histaminic, anti-oxidant, anti-tubercular, anti-Parkinson2 s, anti-diabetic, anti-obesity and immune modulatory agents, etc. We have designed and synthesized a series of triazole derivatives and characterized using various spectroscopic techniques such as FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass Spectroscopy. All the newly prepared compounds (B1-B16) have been evaluated for their anti-inflammatory as well as antioxidant activities *in vitro* using DPPH, TRC and Griess Nitrite assay. Compounds B1, B5, B6, B9 and B13 showed significant free radical scavenging and anti-inflammatory activities. *In silico* studies performed on derivatives B1-B16, using AutoDock 4.2. Tool also confirmed the compounds B1, B5, B6, B9 and B13 showed strong interaction with the targeted interleukin receptors and possessing negatively highest binding energies as compared to the known drug. From our findings we can conclude our derivatives of triazole as potent anti-oxidant as well as anti-inflammatory agents.

P-26

Structural and Biochemical Characterization of 4′ Phosphopantetheinyl transferase of *Leishmania major* to Facilitate Therapeutic Application against Leishmaniasis

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Leishmaniasis impose devastating impacts on much of the world's population. The increasing prevalence of drug resistant parasites and the growing number of immuno-compromised individuals, particularly patients infected by HIV, are exacerbating the problem to the point that the need for novel, inexpensive drugs is greater now than ever. Fatty acid metabolism of *Leishmania* is one such candidate pathway, that has gained widespread attention due to its alteration in the drug resistant strains, directly associating fatty acid biosynthesis with virulence. The presence of Type II fatty acid synthesis (FAS) pathway in *Leishmania major* as suggested by genome sequencing has provided a wealth of potential novel drug targets. The first enzyme involved in the fatty acid biosynthesis pathway is 4′ phosphopantetheinyl transferase (LmPPT) which catalyzes the transfer of 4′-phosphopantetheine arm from Coenzyme A to Acyl carrier protein. Study of PPTs from other pathogen species viz *M. tuberculosis*, *P. aeroginosa* has underscored its importance in the survival and pathogenecity of the organism. Since, *L. major* genome encodes a single PPT, it can act as a potential drug target.

In attempt to characterize LmPPT structurally, biophysically and biochemically; CD and fluorescence Spectroscopy as well as Native-PAGE analysis were exploited. Additionally, our study involves virtual screening of small molecules from chemical libraries available from National Cancer Institute, USA, against LmPPT followed by *ex vivo* testing of best hits against *Leishmania donovani* promastigotes culture. We have also evaluated the hit molecules against LmPPT using *in vitro* enzyme kinetics and spectroscopic assays. In future we aim to take forward these inhibitors to animal models of Leishmaniasis.

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P-27

Design and synthesis of sulfonylurea derivatives as carbonic anhydrase II

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Selective carbonic anhydrase (CA) inhibitors have gained prominence owing to the implication of specific isoforms of CA in glaucoma, leukemia, cystic fibrosis, and epilepsy diseases. A novel class of sulfonylurea derivatives was synthesized from corresponding sulfonyl chlorides and amines. Compounds with different pendant moieties in the sulfonylurea derivatives show significant interactions with human carbonic anhydrase II (CAII). *In vitro* evaluation of the sulfonylurea derivatives revealed three compounds possessing admirable inhibitory activity against CAII. Compounds containing methyl (compound G2), isopropyl (compound G4) and o-tosyl (compound G5) groups displayed nano molar IC_{50}s for CAII. Fluorescence binding and cytotoxicity studies revealed that these compounds are showing excellent binding affinity to CAII and non toxic to cells. Molecular docking studies of compounds with CAII showed these compounds fit nicely in the active site of CAII. Molecular dynamics simulation studies of these compounds complexed with CAII also showed essential interactions which were maintained up to 50 ns of simulation. These results indicate the promising nature of the sulfonylurea scaffold towards CAII inhibition and opens scope of hit to-lead optimization for discovery of effective drugs against CAII-associated disorders.

P-28

Folding and unfolding studies of DNA Gyrase B subunit from *Salmonella*entericaserovarTyphi

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DNA gyrase is a type II topoisomerase, essential for survival of bacteria pathogens as it plays important role in maintaining the topological states of DNA during DNA transaction process such as replication, transcription and segregation. Its essential role in cell cycle maintenance makes it an ideal target due for the design of inhibitors against this enzyme. DNA gyrase is known to undergo dynamic conformations during its enzymatic cycles. Functionally, DNA gyrase introduces negative supercoils into the DNA using the energy from ATP hydrolysis. It is composed of two subunits GyrA and GyrB. GyrA subunit consist of N-terminal domain (GANTD, 59kDa) called as DNA breakage reunion domain and the C-terminal (GACTD, 33kDa domain). GANTD contains the active tyrosine residue that helps in formation of transient double strand break in DNA and GACTD that wraps the DNA around itself. GyrB subunit comprises of N-terminal domain (GBNTD, 43kDa) and C- terminal domain (GBCTD, 47kDa). GBNTD further divided into ATPase subdomain and transducer subdomain while the GBCTD contain TOPRIM subdomain participate in formation of active catalytic core of the enzyme. DNA gyrase catalytic cycle starts with the binding of hetero tetramer of $GyrA_2$-$GyrB_2$ subunit to the double-stranded Guide segment (G), which is then cleaved by active tyrosine residue of GANTD. Once the G segment is trapped, another DNA transfer segment (T) capture occurs in the upper cavity formed by the GyrB-ATPase subdomain referred to as N gate which forms the entry point for DNA. Once the DNA gate opens, the T segment passes through the DNA gate. After this, DNA gate closes and T segment exits thorough the C-gate (exit gate) formed by a coiled coil of GyrA and the G segment religation occurs. Upon ATP hydrolysis, DNA gets separated and enzyme starts its next cycle. Opening and closing of these gates is mediated by rigidity and plasticity of different domains of DNA gyrase. In an effort to understand the conformational stability of protein, we have studied the folding and

unfolding states of DNA gyrase B subunit as a whole and as individual domains. Chemical induced denaturation studies were carried out to calculate thermodynamics parameters in presence of GdmCl and urea. Changes in secondary and tertiary structures were monitored using spectroscopic techniques (circular dichroism and fluorescence spectroscopy). Our study shows that GdmCl is a more potent denaturant than urea. GBNTD found to be more than GBCTD and contributes to the overall stability of the full length protein. We also observed that folding and unfolding studies of gyrase B subunit is reversible for GdmCl- and urea-induced denaturation.

P-29

Understanding of an essential interaction between DnaG and DnaB *in* eubacteria

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The helicase-primase interaction is a critical event in DNA replication and is mediated by the helicase (DnaB) interaction domain within the primase (DnaG). To better understand the poor conservation of the DnaB binding domain of DnaG among the eubacteria, we determined the crystal structure of the Se-Met-labeled helicase binding domain of DnaG from *M. tuberculosis* (*Mt*DnaG-CTD) at 1.58 Å. The overall structure of *Mt*DnaG-CTD displayed two subdomains, the N-terminal glob region (GR) and the C-terminal helix hairpin region (HHR) connected with a small loop. Further, to study the helicase-primase interaction in *M. tuberculosis*, a complex was modeled using the *Mt*DnaG-CTD and *Mt*DnaB-NTD crystal structures. By using this model, a nonconserved hydrophobic residue Ile605 on helicase binding interface of DnaG-CTD was identified as a potential key residue. Mutation guided by molecular dynamics and biophysical studies validated our model. Biosensor binding studies show ten-fold higher binding affinity of *Mt*DnaB-NTD with native *Mt*DnaG-CTD (Kd-250 nM) to mutant Ile605Ala *Mt*DnaG-CTD (Kd-2.5 µM). Both *in silico* calculations and *in vitro* binding sensor experiments suggest the crucial role of the Ile605 (*Mt*DnaG) in the stabilization of the helicase-primase complex in *M.tuberculosis*. Investigation of specificity in the interaction of DnaB with non-cognate DnaGs wasstudied by the *in silico* complex model and the biophysical interaction of *Mt*DnaB-NTD with other DnaG-CTDs from *E. coli*, *H. pylori,* and *V. cholerae*. The complex model, together with mutagenesis and binding analysis, explain the role of loop region of HHR in the stability of DnaG-DnaB complex by aligning in the proper orientation for maximizing the binding affinity with species-specific helicases. Apart from hydrophobic interaction, electrostatic surface potentials also influence the species-specific DnaB-DnaG complex interactions.

P- 30

Evaluate the structure basis of TRAF 6 binding to TRAM, MAL, TRIF AND ATM

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TRAF6 (TNF receptor associated factor 6), an E3 ubiquitin ligase, is known to bind with adaptors of TLR (Toll like receptor) signaling pathways as well as radiation induced pathways.For the downstream activation of TLR signaling pathway, TRAF 6 activates K-63 linked polyubiquitination of both TAK 1 (Transforming growth factor beta-activated kinase 1) and TRAF 6 itself, which leads to activation of NF-κβ (nuclear factor κβ) pathway and MAP kinase pathway. On the other hand, TRAF 6 plays a crucial role in DNA damage inducedNF-κβ signaling.The interaction between TRAF 6 to ATM (Ataxia telangiectasia mutated) triggers Ubc 13 mediated K-63 linkedpolyubiquitination and cIAP 1 recruitment, resulting in IKK and NF-κβ activation in response to genotoxic stress.In the present study, we have aimed to determine the interaction between TRAF 6 with other adaptors like TRAM, MAL, TRIF and ATM by using homology modeling and molecular docking. So, binding affinity of TRAF 6 binding motif to the different adaptors ATM, TRIF (TIR-domain-containing adapter-inducing interferon-β), MAL (Myd88- adaptor like) and TRAM (TRIF related adaptor molecule) will provide a better understanding of the synergistic effect of radiation induced pathway and TLR signaling pathways.

P-31

Characterization of indigenous medicinal plants against dopamine beta-hydroxylase to combat hypertension and cardiac hypertrophy

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Dopamine β-hydroxylase catalyzes the synthesis of norepinephrine (NE) from dopamine. NE, the neurotransmitter released from sympathetic nerve endings, is the essential transmitter in the sympathetic nervous system (SNS) which leads to vasoconstriction. Moreover, hyper-activation of the sympathetic nervous system and increased amount of norepinephrine are responsible for the pathophysiology of hypertension (HTN) and cardiac hypertrophy (CH). If the concentration of NE is reduced, HTN and CH can be prevented. It has been demonstrated that inhibition of DBH can lead to vasodilatation which might be due to reduce the level of NE and thus can be targeted to prevent HTN and CH. Currently, most of the inhibitors being investigated against this target are mainly synthetic compounds with higher risk of non-specific activities. Medicinal plants have been historically proven their value as a source of many molecules with therapeutic potential, and nowadays still represent an important pool for the identification of novel drug leads. Thus, the present study was designed to screen and characterize indigenous medicinal plant extracts against dopamine beta-hydroxylase to identify naturally occurring inhibitors of the enzyme target. Different plant hydroalcoholic extracts like *Terminalia arjuna*, *Glycyrrhiza glabra, Punica granatum* and *Emblica officinalis* were screened against purified DBH from native sources and IC_{50} were evaluated. Interaction of extracts with DBH were further evaluated using an array of biochemical and biophysical methods [like fluorescence, Isothermal Titration Calorimetry (ITC), CD spectroscopy and Fourier-transform infrared spectroscopy (FTIR)]. We observed that some of the plant extracts inhibited DBH activity and IC_{50} were calculated to be in low microgram range. These extracts are evaluated for interaction with DBH to calculate binding affinity against DBH as well as change in secondary structure of DBH. The present study demonstrates that plant extract based lead molecules could be beneficial for the treatment and prevention of HTN and CH.

P-32

Synergetic impact of DOTS in pulmonary tuberculosis patients and Hepatoprotective action of *Allium sativum*

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In India tuberculosis (TB) patient's burden is very high. It can affect any age, caste or class but cases are mainly poor people and mostly men. Slum dwellers, tribal populations, prisoners and people already sick with compromised immune systems are over-represented among the cases, compared to their numbers in the population.

DOTS (directly observed treatment, short-course) strategy was approved by WHO, It has been a viable strategy to treat tuberculosis. The synergetic impact of anti-tubercular drugs (ATD) Isoniazid and Rifampicin are associated with severe hepatotoxicity; in Indian patients when contrasted with United State,which is 30% and 2% respectively. The present research is focused on pulmonary tuberculosis patient treatment under DOTS and hepatoprotective role of garlic.

Oxidative stress is the principal cause of hepatotoxicity in patients enrolled under DOTS treatment, which has been considered as the most important mechanism of hepato-toxicity and in severe case liver failure. Garlic or Allium sativum has many active ingredients in which one is Allicin, having sulphur. Allicin is athiosulfinate compound of Allium sativum reported for its antibacterial actions. Allicin breaks down to release a number of volatile compounds, including diallyltrisulfide (DAS) and diallyl-di-sulphide (DADS). There are some non-sulphur compounds are also found like flavonoids, steroid saponins, organoselenium and allixin.

These active compounds of garlic help in reduction of hepatotoxicity and also boosting body natural antioxidants (GSH, CAT & SOD) level and thus help in reduction of hepatotoxicity occurred due to DOTS and malnutrition in developing countries like India.

P-33

Biophysical and functional studies of prokaryotic cytokinesis protein FtsZ from Salmonella enterica serovar Typhi

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Cytokinesis in prokaryotes is a constriction process, where the cell division proteins localized to the site of division i.e. mid cell form divisome complex [1]. The divisome complex starts with the positioning of a filamentous ring structure FtsZ ring) in the middle of the cell [2]. The Z-ring is anchored to the membrane and stabilized by other cell division proteins like FtsZ, ZipA, FtsN, etc and form divisome complex [3]. FtsZ consists of two domains, N - and C terminal domain separated by long central core helix with self activating GTPase activity. To analyze the role of N-terminal and C terminal region in FtsZ polymerization, N-and C-truncated FtsZ constructs were generated to analyze polymerization and GTPase activity. We observed binding affinity of Km = 0.37mM for truncated and Km = 0.34mM for full length. By light scattering measurements, we observed that the truncated FtsZ has the average mean distribution of 131nm and 164 nm in presence of 0.5mM GTP and full length FtsZ has 80 nm (No GTP) and 125 nm (0.5mM GTP). We propose that N-and C-terminal random coil region might interfere in polymerization as observed in DLS studies. CD spectroscopy studies showed that both truncated and full length FtsZ has similar secondary structure elements. We also carried out *In silico* ligand screening using FDA approved drugs by pharmacophore model and identified twenty potential candidates against FtsZ.

The study shows that the N-terminal and C-terminal region of FtsZ does not contribute for GTP hydrolysis and polymerization rather they interfere. Hence, the role of the random coiled N-and C-terminal region is to interact with other cell division proteins like ZipA, FtsA and ZapA etc. We have also identified some potential ligands against FtsZ although *in vitro* studies are required to validate the studies.

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Spectroscopic studies of fungal lipase and eugenol interaction

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Extracellular lipases play a key role in microbial infections. Several human fungi secrete lipase to dissolve host membranes which aids them in tissue invasion. Investigations on molecules which can bind, inhibit or denature lipases but have no effect on host carrier proteins continues to be a challenging area of microbial pathogenesis. Eugenol has been reported to inhibit fungal lipase activity on solid medium. In this study we investigate interaction of eugenol with fungal lipase by UV- V is spectroscopy, fluorescence spectroscopy and circular dichroism techniques. The UV- Vis spectroscopy and fluorescence spectroscopy results reveal that there is a complex formation between lipase and eugenol. Binding sites and the binding constant of eugenol has been obtained. Further, binding of eugenol to lipase alters its conformation and causes change in secondary structure as indicated by the CD spectra. Results obtained suggest profound alteration of fungal lipase structure by eugenol, thus making it a potential phytotherapeutic for diminishing microbial virulence

P-35

An interaction of eugenol with fungal lipase: Eugenol as potential phytotherapeutic for diminishing microbial virulence

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Extracellular lipases play a key role in microbial infections. Several human fungi secrete lipase to dissolve host membranes, which aids them in tissue invasion. Investigations on molecules which can bind, inhibit or denature lipases but have no effect on host carrier proteins continues to be a challenging area of microbial pathogenesis. Eugenol has been reported to inhibit fungal lipase activity on solid medium. In this study we investigated the interaction of eugenol with fungal lipase by UV- Vis spectroscopy, fluorescence spectroscopy and circular dichroism techniques. The UV- Vis spectroscopy and fluorescence spectroscopy results revealed that there is a complex formation between lipase and eugenol. Binding sites and the binding constant of eugenol has been obtained. Further, binding of eugenol to lipase alters its conformation and causes change in secondary structure as indicated by the CD spectra. Results obtained suggested profound alteration of fungal lipase structure by eugenol, thus making it a potential phyto therapeutic for diminishing microbial virulence.

P-36

Sequence and structure analysis of inositol 1,4,5 Triphosphate 3-kinase A

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Inositol 1,4,5 triphosphate 3-kinase A belongs to the family Inositol phosphokiase (IPK) family. Inositol 1,4,5-trisphosphate 3-kinase A is highly expressed in the brain and neurons which regulates intracellular calcium levels by signaling through the inositol trisphosphate receptor. IP3KA is a 461- residue long polypeptide that is divided into two distinct domains: (a) Inositol phosphate kinase domain (245-455) and (b) Inositol polyphosphate multikinase domain (190 - 375), plus five modified residues (35, 55, 62, 137, 197) which is involve in the proper folding and function of enzyme. One domain is calmodulin binding and next is substrate binding. IP3KA is activated by calmodulin, and the annotation score is 5 out of 5 which provides a heuristic measure of the annotain content of a UniProtKB entry or proteome. The best structure found in PDBe entry for ITPKA is 1w2f based on coverage and structure quality that is Inositol (1,4,5)-triphosphate 3-kinase substituted with selenomethionine. One bound ligand of inositol 1,4,5 triphosphate 3-kinase A is Sulphate Ion ad one modified residue is Selenomethionine.20 disease which is related to inositol 1,4,5 triphosphate 3-kinase A, Oral Squamous cell carcinoma which is caused by down regulation of inositol 1,4,5 triphosphate 3-kinase A.

P-37

Recent Stratigies Against Leishmaniasis, Nanomedicine: Offering Hope

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Leishmaniasis, caused by protozoa *Leishmania* has been classified as major tropical disease by WHO. More than one million new cases are reported every year and 20,000 to 30,000 deaths occur annually throughout the world. Various immunological component acting against *Leishmania* are exploited by the *Leishmania* parasite for the persistence of the disease. Development of highly sensitive, rapid, non-invasive and less expensive diagnostic procedures had led to the early detection of the disease. The currently available chemotherapeutic drugs have the problem of toxicity, resistance and high cost. Due to these reasons researchers are compelled to search the new treatment strategies. In this review, we have thrown light on the pathogenesis of the disease, the current available as well as future possible arsenals particularly the current developments in the field of nanomedicine for the treatment of leishmaniasis. Nanoparticles due to its characteristics like small size, customized surface and improved solubility has potential to target and treat the several devastating diseases including leishmaniasis. More research in

future on nanomedicine seems to show satisfactory result in treating leishmaniais.

P-38

Targetting Cytochrome B5 Reductase 3 to counter hypertension

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Many health agencies have declared hypertension as a critical health issueas hypertension has become a global burden.Although, currently available therapeutics are effective in countering hypertension, but they are having associated complications *viz.* target organ damage including renal failure. Therefore, there is an urgent need for the alternate strategies to be discovered against hypertension. Here, we have targeted hsCYB5R3 enzyme as a therapeutic protein, since it is involved in altering the bioavailability of nitric oxide, a vasodilator involved in governing the vascular tone which is in turn; is an important aspect for hypertension treatment. The present research work included virtual screening of small molecule databases against the crystal structure of the enzyme to identify inhibitors and to obtain binding energies and inhibitory constants. Virtually screened inhibitors were tested *in vitro*in order to calculate IC_{50} values as a measure of inhibitory potential of these compounds against purified hsCYB5R3. Further, biophysical techniques including fluorescence and CD spectroscopy, were employed to obtain binding affinity, number of binding sites and effect on secondary structure, respectively. Potential binding and stoichiometric ratios for some of the inhibitors were observed, some of which were not hemolytic either. CD analysis also revealed a bit of effect over the secondary structure of the enzyme. In addition, enzyme kinetics analysis also gave K_m and V_{max}values to elucidate *in depth* mechanism of action of these compounds. Moreover, site directed mutagenesis has indicated some of key amino acid residues involved in catalysis. These findings have encouraged us to screen the inhibitors in *in vivo* studies.

P-39

Pyrimidine favored recognition by RRM1 of *Pf*SR1 protein involved in alternate splicing in *Plasmodium falciparum*

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Plasmodium falciparum causes most deadly kind of malaria in humans. The complexity of proteome of *P. falciparum,* which is quite diverse despite a genome size of merely 23 mega base pairs, encoding 5300 genes[1] is a big challenge to drug and vaccine development. This proteomic diversity is majorly due to the process of alternative splicing (AS). AS leads to thesynthesis of different mature mRNA isoforms and consequently, different translation products, from the same pre-mRNA transcript[2].

The *P. falciparum* Ser/Arg-rich protein 1(*Pf*SR1) is the first protein to be functionally characterized as an alternative splicing factor in any Apicomplexans. Here, we have performed astudy to understand themolecular basis of RNA recognition by *Pf*SR1 alternative splicing factor. We have determined its three-dimensional solution-structure in free and RNA bound state and used thermodynamic parameters to understand the specificity and affinity of RNA recognition by *Pf*SR1. This is the first structure of a binary protein-RNA complex from any Apicomplexans. Calorimetric studies suggest that RNA recognition motifs-1 (RRM1) of *Pf*SR1 is biased toward pyrimidine during RNA recognition.

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P-40

Biochemical Characterization of Holo-Acyl Carrier Protein Synthase of *Mycobacterium tuberculosis*

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Fatty acids play a vital role in the survival of several pathogens. These primary metabolites serve as important components of biological membranes. Most prokaryotes employ the type II Fatty Acid Synthase (FAS II) to synthesize fatty acids, while eukaryotes possess type I FAS. FAS I is a multidomain megasynthase complex, while FAS II has discrete monofunctional proteins. 4'-Phosphopantetheinyl transferase (PPTase), an enzyme necessary for the activation of the acyl carrier protein, an important component of FAS, is indispensible for the growth of most pathogens, and therefore has been regarded as a potential drug target. Type I (AcpS type) PPTases are involved in the synthesis of primary metabolites, while the type II PPTases, (Sfp type), participate in the synthesis of secondary metabolites. M. tuberculosis possesses both types of FAS, a feature unique to Corynebacteriaceae, Nocardiaceae and Mycobacteriaceae family, and both types of PPT[1]. Comparison of the AcpS from other bacterial sources, viz. *Bacillus subtilis, E. coli* etc, suggest that *M. tuberculosis* AcpS displays differences in interacting surface. Owing to these differences, the enzyme is unable to act on its cognate type-II ACP (AcpM)[1]. We therefore, intend to biochemically characterize *M. tuberculosis* AcpS.

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P-41

On the Early and Late Conformational Alterations in Cytochrome c upon Modification via Glycation

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Hyperglycaemia represents one common hallmark of diabetic complications and is characterized by increased levels of sugars and their metabolites. These sugars metabolites have a high tendency of covalently modifying proteins (specifically lysine and arginine residues) via a process termed as protein glycation (a non-enzymatic post-translational modification). Such modifications induce protein structural alterations, functional loss and even lead to aggregate/amyloids formation, and have been associated with several age related disorders and neurodegenerative diseases. In the present study, the effects of glycation by glyoxal and methylglyoxal on the early and late conformational alterations in Cyt c were studied. Spectroscopic (CD and UV-Visible) measurements revealed that Cyt c upon overnight incubation undergo certain conformational alterations and exposure of heme. These were accompanied with reduction of heme moiety (EPR study) and activation of peroxidase-like function of Cyt c, which is crucial for initiation of intrinsic apoptotic pathway. An extended incubation of Cyt c with these agents results in appearance of AGE-like fluorescence of the modified protein with significant alterations in the secondary structures. However, no amyloidogenic conversions were observed as suggested by TEM analyses. The study provides an insight to the role of glycating agents, which are elevated under diabetic conditions in inducing Cyt c release and apoptosis.

P-42

Evaluation of serum apolipoprotein E as a potential biomarker for pharmacological therapeutic efficacy monitoring in dopamine dictated disease spectrum of schizophrenia and Parkinson's disease

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Parkinson's disease and schizophrenia are disease end points of dopaminergic deficit and hyperactivity, respectively in the mid brain. Accordingly, current medications aim to restore normal dopamine levels, overshooting of which results in adverse effects of psychosis and extra-pyramidal symptoms. There are currently no available laboratory tests to guide treatment decisions or help predict adverse side effects of the drugs. The possibility of using apolipoprotein E as a biomarker to monitor pharmacological intervention in dopamine dictated states of Parkinson's disease and schizophrenia for optimum therapy has been explored in this study. Naïve and treated, Parkinson's disease and schizophrenic patients were recruited from neurology and psychiatry clinics. Serum of research staff volunteers was collected as healthy controls. Serum concentrations of apolipoprotein E was estimated by ELISA. Apolipoprotein E levels are higher in Parkinson's disease patients as compared to schizophrenic samples ($P<0.05$). Also, post treatment apolipoprotein E levels in both disease states were on par with levels seen in healthy controls. In conclusion, inverse relation shown by apolipoprotein E concentration across the dopaminergic spectrum suggests that it can be pursued not only as a potential biomarker in schizophrenia and Parkinson's disease, but can also be an effective tool for clinicians to determine efficacy of drug based therapy.

P-43

Oxidation of Prdx6 at Cys-47 induces changes in its Conformation and Oligomer state

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Peroxiredoxin 6 (Prdx6), a unique non-seleno mammalian peroxidase, has a bifunctional activities i.e GSH peroxidase at pH 7.4 and phospholipase A_2 (PLA_2) activities at pH 4.0. It is known that multiple factors regulate its bifunctional activities such as redox state, heterodimerization with other protein, post-translational modification and pH. Prdx6 use its thiol group of Cys47 to catalyze the reduction of reactive oxygen species. Simulation studies of reduced and oxidised Prdx6 revealed that there is no large pocket conformational change after its oxidation of thiol group at Cys47. Both the reduced and oxidized pockets volume averaged around 1500 Å3. But the in-vitro study indicates that there is profound change on the tertiary and secondary structure of Prdx6 after oxidation as evident from far-UV and near-UV CD studies. These studies are also supported by tryptophan and ANS fluorescence measurements. These changes in the conformation may induce weakening of its dimeric state leading to the formation of its unstable monomeric state as evident from DLS studies. The formation of monomeric state may facilitate the hetrodimerization of Prdx6 with GSH S-transferase π (πGST) which is required for its peroxidatic catalytic cycle.

P-44

Pyruvate Dehydrogenase Kinase as potential drug target: Structural and functional view

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Pyruvate Dehydrogenase Complex (PDC) consists of three components which take part in different steps in

the conversion of pyruvate to acetyl co-A. These components are pyruvate decarboxylase (E1), dihydrolipoyl acetyltransferase(E2) and dihydrolipoyl dehydrogenase (E3). PDC has to be tightly regulated to prevent metabolic diseases. Reduced PDC activity often leads to the increased glucose level in the liver resulting in progression of diabetes and decreases reactive oxygen level in the tumor cell, leading to cancer cell proliferation. Regulation of PDC is done by pyruvate dehydrogenase phosphatase (PDP) and PDK. PDC inactivation leads to dysregulation of glucose metabolism. As noted earlier any disruption in glucose metabolism leads to metabolic diseases like diabetes and cancer. PDK has gained more attention than PDP, due to its ability to inactivate PDC and ultimately being responsible for progression of metabolic diseases. Overall PDK overexpression leads to inactivation of PDC and promotes diseases like cancer, type 2 diabetes and obesity. Dichloroacetate (DCA), a well-known inhibitor of PDKs, leads to activation of PDCs and induces hypoglycemia in the already existing hyperglycemic condition. The retained activity of PDC is a possible goal for treating metabolic diseases. In conclusion, studying PDKs and targeting their inactivation by designing drug like DCA that target PDK and help in regaining PDC activity in tissue may result in a significant breakthrough in treating various metabolic diseases that occur due to elevated PDK levels.

P-45

Structural and Functional characterization of *Helicoverpa*-inducible Thioredoxin h from *Cicer arietinum*

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Thioredoxins are small and universal proteins, which are involved in the cell redox regulation. In plants, they participate in a broad range of biochemical processes like self-incompatibility, seed germination, pathogen & pest defense and oxidative stress tolerance. The h-type of thioredoxin (Trx-h) protein represents the largest Trx family and is a disulfide reductase characterized by a conserved di-cysteine active site. Pests and pathogens can induce the expression of Trx-h and it contributes to systemic acquired resistance (SAR). Herein, we characterized the Helicoverpa – inducible Trx h from an important legume, Cicer arietinum, CaHaTrx-h, which encodes for 113 amino acid protein and exhibits features of other known 'CGFS' type Trxs. Phylogenetic analysis indicates the presence of the characteristic motif "FLKVDVDE" and "VVDFTASWCGPCRFIAPIL" and it shows 73% sequence identity with AtTrx-h. The simulation of its modeled structure gives an idea of the flexible regions to accommodate an approaching protein target and facilitate their interaction. PR-5 (thaumatin) and Mannitol Dehydrogenase were nominated as potential targets and were found to share close interaction with CaHaTrx-h via disulfide bond reduction. The study is an effort in the direction of understanding stress-related mechanisms in crop plants to overcome losses in agricultural yield.

P-46

Mycobacterium tuberculosis stress induced proteins as drug targets against tuberculosis

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Mycobacterium tuberculosis (MTB) is the causative organism of the tuberculosis disease in human which kills millions of people in the world every year. The successful infection of the bacterium to the human lies in a compromise between the host macrophages and the bacterium. When the bacterium is phagocytised by the macrophages, the MTB from the phagocytes are transferred inside the lipid bodies where the bacterium accumulate lipids inside, especially triacylglycerol with the help triacylglycerol synthase 1 protein leading to drug resistance and proceeding towards a state of dormancy which last up to many years. During this latent period the bacterium decreases its molecular machinery of replication, transcription and reduced translation of proteins, but certain other genes which induce dormancy are transcribed. Our recent work on stress conditions in MTB infected macrophage THP1 cell line have shown that Mycobacterial protein Rv1179c having unknown function along with its interacting proteins Rv0046c, Rv0694,Rv1329c, Rv0668 and Rv2090 are over-expressed in stress condition. Functional analysis of such proteins over-expressed in

stress conditions which are characteristic features of MTB dormancy and developing more potent drugs against such proteins targets will be a promising strategy against drug resistant tuberculosis. Here we predicted the structures of the proteins and their corresponding active sites. Later in-silico structure based ligand screening will be performed to discover potential drugs against them. Then, the lead compounds will undergo pharmacophore modelling for better biding affinity and tested for lesser toxicity.

P-47

Leishmania donovani L-asparaginase: Role in Amphotericin B resistance and nitrogen metabolism

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Aspartate and glutamate metabolism has been proved to regulate many pathogenesis of many diseases as including leishmaniasis. In this regard, we had identified and proposed L-asparaginase (cytoplasmic isoform, LdAI) of *Leishmania donovani* as crucial metabolic target in nitrogen metabolism. In general, L-asparagianse I hydrolyzes both Asn/Gln to respective acids along with release of ammonia. Similar in sequence and structure to earlier proven L-asparaginases of *M. tuberculosis, S. typhi and H. pylori* while dissimilar to human L-asparaginase, the LdAI makes a promising drug target. While the Asp/Glu serve as metabolic precursors, the ammonia release could neutralize stress induced pH imbalances within the cell. In our study, we have cloned and purified LdAI followed and by screening of specific inhibitors, we have demonstrated the growth dependency of *L. donovani*on LdAI. This was preceded by validating in-vitro efficiency of inhibitors through biochemical/ biophysical approaches. LdAI interaction network analysis establishes its close connection with important metabolic enzymes. Further, we have shown its role in conferring early resistance to Amphotericin B, the front line anti-leishmaniasis drug in India. Proliferating parasites under drug pressure showed upregulation of LdAI. Taken together, our results conclusively show importance of such enzyme in metabolic homeostasis and in conferring AmB resistance.

P-48

Synthesis and Enzymatic Biotransformations of a New Series of 4-aryl-1, 4-Dihydropyridines for Production of its Pharmacologically Important Stereo Selective Enantiomers

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Dihydropyridines form the most important class of calcium antagonists as they serve broad range of pharmaceutical and therapeutic effects. Second generation dihydropyridines possess at least one chiral centre and the effects differ from one enantiomer to the other. One enantiomer is pharmacologically active and the other enantiomer of the drug is generally an antagonist, with lot of side effects. The present work describes the role of several microbial enzymes from the class of hydrolases such as lipases, esterases and proteases in the stereospecific synthesis of new series of antihypertensive and cardiovascular drugs from 4-aryl-1, 4-dihydropyridines. These enzymes have the activity to transform the substrate into one pure form of the enantiomer, which in turn reduces the dosage as well as reduces the side effects of the other undesired enantiomer present in the drug. Racemic mixtures when treated with the partially purified lipases and esterases from the culture broth of organisms grown in laboratory, resulted in enantiomeric excess of the desired product which are then resolved using HPLC (using Chiralcel ODH column). The wavelength so chosen is based on the substantial absorption by the compound possessing the best S/N ratio. This was first assayed with a mobile phase comprising of n-hexane and isopropanol in different proportions between 22 to 30^{o}C. The racemic analogues were resolved best at 353nm with a retention time of less than 10 minutes. The optical rotation values were measured using polarimeter. Commercially available purified enzymes such as ***Candida rugosa*** lipase (CRL) ***Candida antartica*** lipase (CAL) gave good results for enantioselecive transformation when compared with esterases and proteases. The optical yields and optical configurations are being investigated by HPLC where an enantiomeric access of 65-70% has been attained. However complete conversion of one isomer to the other desired one is still to be achieved.

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Prediction of Putative Protein Interaction Between Zika Virus and its Hosts Using Computational Technique

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Zika fever is an increasingly significant arbovirus disease, with 1.5 million people has been infected by Zika in Brazil, and also has spread to other countries i.e. South America, Central America, North America and the Caribbean, with over 3,500 cases of microcephaly reported between October 2015 and Jan 2016. Protein-protein interactions between the virus and its host are one avenue through which Zika can connect and exploit these host cellular pathways and processes. To be successful, Zika virus must manipulate host cell biological processes towards its own ends, while avoiding elimination by the immune system. Generally, prediction of protein-protein interaction between the virus's proteins and the host's proteins are quite crucial for the infection and the pathogenesis of the virus, which make them striking targets for the development of the therapeutics. From the study, we come up with the interactions which are very crucial for the virus infection propagation into. As there is a notable relationship between the Zika virus and the neurodevelopment abnormalities, still there is no specific system is underlying which impaired neurological development has not been determined. We encounter some of the interaction which is predicted from the Structure based Computational methodology adopted in our work, through which we can say that these are some interactions which causes the neuron disorders as the major problem associated with this viral infection. Zika virus alters the cellular processes through definite interactions with the host's protein interaction network. The networks generated provide a set of premise for more experimental study into the ZIKAV life cycle as well as probable therapeutic.

P-50

Anomalistic Implications of Protein Tyrosine Phosphatases: Need of Small Molecule Inhibitors Targeting PTPs in Human Diseases

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Protein tyrosine phosphatases (PTPs) are hydrolytic enzymes (EC 3. 1. 3. 48) that catalyses the hydrolysis of Phosphate esters via nucleophilic attack of phosphate by cysteine residues or coordinated metal ions from tyrosine residues of a target protein. The pervasive mechanism of cell signalling is served by phosphorylation on protein tyrosine residues during proliferation, migration and apoptosis. The tyrosine phosphorylation condition is regulated by the balanced and opposing action of two molecular switchers called protein tyrosine phosphatases (PTPs) and protein tyrosine kinases (PTKs). Anomalistic function of tyrosine phosphorylation is associated with pathological process like cancer, diabetes, obesity, and autoimmune disorders. Indeed, PTPs constitutes 107 large family of signalling enzymes can be a one of the causative agent in number of diseases offer ample of targets. Therefore, there is a need to develop highly potent inhibitors that are specific towards specific PTPs. This article summarizes recent progress made in last two decades for the development of different inhibitors to target PTPs. Here we discuss *in vivo* and *in vitro* inactivation of different PTPs by small molecules isolated from different sources.

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Electrospun Nanostructured Scaffold of Carbon Nanotubes and Hydroxyapatite Composite for Bone Tissue Engineering

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Large bone defects caused by trauma, tumor resection, deformity, and infections are increasing year by year, but the rare resources for autogenous bone grafts and allograft rejections make it difficult to treat all of these

deficiencies. In spite of high request in clinical medicine, nature's capability to self-organize the inorganic component with a preferred alignment in the bioorganic matrix is still not reproducible by synthetic techniques because of its complex nature. Therefore, in fields ranging from biology and chemistry to materials science and bioengineering a large developmental effort is essential in order to fabricate bone and dentin-like biocomposite materials, which may permit the ingrowth of hard tissues though improving mechanical properties with respect to the hard tissue regeneration.

In recent years, certain attention has been paid to biomimetic approaches, which allow us to mimic such natural bio-inorganic and bio-organic composite materials. The main idea in biomimetic methodologies is to control and fabricate the morphology and composition of developed biomaterials, in which the nano crystallites of inorganic compounds are spread with special orientation in the organic matrices due to its large potential in biomedical applications.

In the present work, we successfully mimicked electrospun bio-nanocomposite fibers on the basis of Poly Vinyl Alcohol (PVA) as matrix and Hydroxy Apatite (HA) nanoparticles with a highly anisotropic three-dimensional structure, microscopically the same as a substructure of bone. We have used two-step methodology that combines an in situ co-precipitation synthesis route with electrospinning process to prepare a unique type of biomimetic nanocomposite nanofibers of HA/PVA. The fibers produced by the electrospinning machine were in 100-200 nm .The result obtained from UTM analysis highlights the great tensile strength and young's modules of the nanofibers. A combination of structural, mechanical and biological properties of bone graft play a critical role in cell seeding, proliferation and new tissue formation in orthopaedic research. Nano-biomaterials should promote cell adhesion and be optimized for ECM production, mineralization and subsequent tissue regeneration. Hence, electrospun biomimetic HA/PVA/CNT nanofibers hold great potential for adhesion, proliferation and mineralization of osteoblasts and are favourable biocomposite scaffolds suitable for bone tissue redevelopment.

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Effect of macromolecular crowding on the structure and stability of apomyoglobin

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The effect of macromolecular crowding on the structure and stability of apomyoglobin was studied. Effect of ficoll 70 and dextran 70 on the structure and stability of apomyoglobin was investigated using absorption spectroscopy and circular dichroism spectroscopy. It was observed that the maximum absorption peak at 280 nm increases as well as it shows blue shift upon addition of ficoll 70 or dextran 70, whereas the far-UV CD spectra of apomyoglobin in the absence and presence ficoll 70 or dextran 70 appear to be overlapping. Thus the results revealed that ficoll70 as well as dextran 70 compacts the tertiary structure of apomyoglobin leaving the secondary structure intact. GdmCl-induced denaturation as well as urea-induced denaturation in the absence and presence of each crowder showed that both ficoll 70 as well as dextran 70 stabilizes apomyoglobin in terms of free energy change as well as the increase in midpoint of chemical denaturation. Thus, it can be concluded that the excluded volume effect is responsible for the stabilization of apomyoglobin. The stabilizing effect of dextran 70 is greater than that of ficoll 70 because dextran 70, owing to its rod like structure excludes greater volume than that of ficoll 70 and thus leads to greater stabilization of apomyoglobin under physiological conditions.

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The role of concentration and anion effect of imidazolium-based Ionic Liquids on the structure and stability of stem bromelain

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The broader scope of ILs in chemical sciences particularly in pharmaceutical, bioanalytical and many more applications is increasing day by day. Hitherto, a very less amount of research is available in the

depiction of conformational stability, activity, and thermal stability of enzymes in the presence of ILs. In this article, we have explored the influence of a series of members of imidazolium-based ionic liquids (ILs), 1-butyl-3- methylimidazolium chloride ([Bmim][Cl]), 1-butyl-3- methylimidazolium bromide ([Bmim][Br]) and 1-butyl-3-methylimidazolium iodide ([Bmim][I]) on the stability of stem bromelain (BM) by using UV-vis spectroscopy, fluorescence, thermal fluorescence, circular dichroism (CD) spectroscopy, and dynamic light scattering (DLS) measurements. We attempt to understand the effect of imidazolium-based ILs based on the variation of anion of the IL. The commendatory results obtained from the multi-spectroscopic approaches provided some guidance regarding the mechanism of interaction between BM and imidazolium-based ILs. Our results illustrate that interactions of ions of ILs with proteins are important for understanding the effects shown by them on proteins whether in stabilization or destabilization. From the above results, it can be stated that interaction of IL with protein is dependent on the interaction of anion present in the imidazolium-based ILs with the amino-acids present in the protein structure.

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P-54

Dissecting the Stability Attenuation Characteristics of Hen Egg Lysozyme upon Charge Transfer Complex Formation

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The structure-function-stability paradigm of proteins is highly governed by the pre-existence of repulsive and attractive non covalent interactions in proteins. Charge transfer interaction is one of those non-covalent electrostatic interactions that are formed between electron donor and the acceptor molecule via partial transfer of charge. Protonated imidazole moiety of histidine and indole ring of tryptophan forms an ideal charge transfer complex (CTC) pair at acidic pH. Several reports are available in literature regarding their molecular geometry and regulatory functional roles. However, studies representing their stability characteristics are limited till date. In order to unravel the pH dependent stability features of charge transfer complexes, we have performed extensive spectroscopic and calorimetric analysis on Hen Egg Lysozyme (HEL) upon addition of imidazole derivatives (imidazole, histidine and histamine) in the pH range 4 to 2 using CD, fluorescence, NMR and differential scanning calorimetry. Surprisingly, we have observed pH dependent changes in stability of HEL upon the formation of CTC. At pH values 4 and 3 all the imidazole derivatives destabilized HEL almost by a Tm of 5-7 ^{0}C, leaving pH 2 conformation intact. We thought this study report that, CTCs can attenuate the stability of proteins to a significant extent in a pH dependent manner, which in turn is correlated to the orientation of indole-imidazole pair with respect to the protein's side chain network. We strongly believe that our results are unique and are of immense help in deciphering the contributions of non-covalent interactions in biomolecular recognition and signalling processes.

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P-55

Doripenem induced increased efficacy of cefotaxime against CTX-M-15 producing bacterial strain: microbiological and biophysical views

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Enterobacteria producing CTX-M-15 type β-lactamase enzyme is categorised under plasmid encoded extended spectrum beta lactamases (ESBLs). It has the ability to hydrolyse third generation cephalosporins such as cefotaxime and ceftazidime (Rehman *et al*.,

2015), showing an elevated level of resistance against cefotaxime. The infections caused by multidrug resistant strains, especially CTX-M-15 producing strains are being treated with carbapenem group of â-lactams which are considered as last resort of antibiotics (Shaikh *et al.*, 2015). The objective of the study was to know if cefotaxime in combination with doripenem, (carbapemen antibiotic) at very low concentration, inhibits the CTX-M-15 producing bacterial strains. Clinical strain of *Enterobacter cloacae* was used to clone $bla_{CTX\text{-}M\text{-}15}$ in *E. coli* cells. The protein was then expressed and purified. Results showed that CTX-M-15 producing strains are susceptible to doripenem. Doripenem and CTX-M-15 binding was an endothermic and spontaneous process with the binding constants in the range of 10^2 - 10^4 M^{-1} as shown by fluorescence study. It led to change polarity around enzyme and drug molecules and induces conformation changes in CTX-M-15 as shown by UV and CD spectroscopic study. The catalytic efficiency of CTX-M-15 enzyme was reduced to about 15.86% when it was treated with doripenem, then with cefotaxime, as compared to the studies where enzyme's efficiency was increased by 33% when treated with cefotaxime alone (Maryam *et al.*, 2016). Hence presence of doripenem along with cefotaxime reduces enzyme's efficiency to hydrolyse cefotaxime by about 48%. FIC study showed that doripenem paired with cefotaxime showed synergistic effect against CTX-M-15 producing bacterial strain. The study concludes that doripenem at very low concentration of 25 nM, induces such a structural changes in CTX-M-15 which reduces enzyme's hydrolytic capability to hydrolyze cefotaxime. Hence the synergistic use of a carbapenem and cephalosporin drug plays significant role in inhibiting the efficiency of CTX-M-15 â-lactamase, reducing the resistance against the cephalosporin antibiotic.

P- 56

The Sugarcane defense protein SUGARWIN2 causes cell death in *Collectotrichum falcatum*, thus preventing "cancer" of sugarcane

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Plant pathogenic fungus *Colletotrichum falcatum*is responsible for a disease in sugarcane that is highly variable in nature. It causes the frequent breakdown of resistant sugarcane varieties. Sugarcane is an important agro industrial crop of the world. India being the largest consumer as well as the second largest producer of sugar, therefore, it requires sugarcane production on large scale. But diseases are the major concern for the sugarcane, responsible for its low yield. Among all the diseases, fungal disease named as red rot of sugarcane is the most threatening disease of sugarcane, rightly called as 'Cancer' of sugarcane. It causes severe loss in yield and quality of the sugarcane. The spread of the red rot can be prevented during the growing season by timely rogueing and burning of the affected clumps with utilization of the green leaves for cattle fodder. In no case ratoons of sugarcane should be kept in the red rot affected fields.

Plants respond to pathogens and insect attacks by inducing and accumulating a large set of defense-related proteins.Recombinant SUGARWIN2 has been demonstrated to modulate*C. falcatum* morphology by increasing vacuolization, points of fractures and a leak of intracellular material, leading to germling apoptosis.The spread of the red rot can be prevented during the growing season by timely rogueing and burning of the affected clumps with utilization of the green leaves for cattle fodder. In no case ratoons of sugarcane should be kept in the red rot affected fields. Attention should always be given to sanitation by digging out stubbles of di-seased canes and burning them with other trash in the field.

P- 57

Identification of novel multi-target inhibitors against bacterial peptidoglycan biosynthesis enzymes by structure-based virtual screening of antituberculosis agents from ChEMBL and FDA approved drugs.

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Tuberculosis is caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) that most often affect the lungs. According to WHO Global tuberculosis report 2015, TB remains one of the world's deadliest communicable

diseases. Multi-target drugs are currently being used extensively to treat both infectious and inherited diseases. Current therapeutic strategies for several diseases including *M. tuberculosis* infection have evolved from an initial single-target treatment to a multi-target one. A combination of anti-tubercular drugs targeting different mycobacterial proteins is more effective at suppressing bacterial growth. In this study, a high throughput virtual screening was performed to identify hits to the potential anti-tubercular multi drug targets: MurA, MurB, MurC, MurD, MurE, MurF, MurG and MurI from *M. tuberculosis* that is involved in peptidoglycan biosynthesis. In the virtual screening 56,400 compounds of ChEMBL anti-mycobacterial library and 1596 FDA approved drugs were docked and rescored, identified top 10 ranked compounds as anti-tubercular leads. New anti-tubercular therapies that include multi-target drugs may have higher efficacy than single-target drugs and provide a simpler regimen for anti-tubercular therapy.

P-58

Expressionand Purification of E1-protein of Chikungunya virus and generation of polyclonal antibody in BALB/c mice

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Chikungunya virus (CHIKV) is an emerging arbovirus that spread by primary vector*Aedesaegypti* and *Aedesalbopictus.* Chikungunya viral illness is generally characterized by high-grade fever, headache,rashes, conjunctivitis,vomiting, polyarthralgia, neurological failure etc.The CHIKV genome consists of a single-stranded, positive sense-RNA with 11.8 Kb in length.E1-CHIKV is class IItransmembrane glycoproteins responsible for fusion of virion to the host cell. In this study, we planned to elucidate expression, characterization and antigenic properties of E1-protein. We cloned E1 gene of CHIKV prokaryotic expression vector (pET28a), and expressed the protein in*E.coli* (BL21DE3 cells). Expressed protein was purified through Ni-NTA columnand was confirmed by SDS-PAGE and Western blotting. Polyclonal antibody was raised in BALB/c miceagainst E1-protein. This antibody can be be used as a biomarker in serodiagnosis test to identify the E1-CHIKV antigen present in patient's samples.

P-59

Electro-interaction mediated Lab on chip platform for detection of kidney malfunction

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Proteases and their inhibitors are among the most intensively studied protein–protein complexes. There are 30 structurally distinct proteinaceous inhibitor families that are able to block serine, cysteine, metallo- and aspartyl proteases with variable mechanisms. One such inhibitor, CysC belonging to class of cysteine protease inhibitors, is omnipresent in the body fluids and has tendency to be reabsorbed by a healthy functioning kidney.

Chronic kidney disease (CKD) is characterized by progressive damage of the renal parenchyma and the loss of functional nephrons, which ûnally lead to chronic renal failure. It is estimated that it effect 1 in every 10 adults in India. CKD is divided into 5 stages based on the severity of the disease which is determined by glomerlular filtration rate (GFR). Failure to curb the progression at initial stages leads to end-stage renal disease (ESRD) and/or development of CVD.

CysC is a novel marker for the diagnosis of CKD, which is particularly useful for detection in the early stages. It is a 60kd non glycosylated protein that has been statistically linked to evaluate the functioning of kidney. Patients with CKD release CysC in urine and thus increase in biomarker level is detectable at a much earlier stage of CKD. Thus a biosensor capable of monitoring CysC levels would be rapid, ultrasensitive and specific for CKD with an additional benefit of being handy.Here, we report design of nano-sensor platform for detection of early stage CKD. Screen printed multiwalled carbon nanotube (SPMWE) electrode immobilized with cysteine protease was developed as a working electrode. Specificity of capture molecules was employed to capture the CKD biomarker, CysC. Protein protein interaction mediated transduction of electrons by the modified SPMWE/papain was measured through voltammetric measurements.

P-60

Structural and functional analysis of human BMP-2 kinase

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Human BMP-2 kinase (BMP2K) is a ~130kDa single polypeptide chain belongs to the TGF-β superfamily. Certain clinical studies have indicated its essential role in tissue and skeletal development including WNT and Hedgehog cell signalling whereas overexpression of BMP2K levels have been linked to various congenital anomalies and pathologies involving the mesenchymal cells that differentiate into muscle, fat, cartilage, and bone. The molecules and conditions that influence BMP2 synthesis are also very diverse. Here, structural and functional analysis of this protein was carried out using various bioinformatics tools and web-servers. Three-dimensional coordinates of BMP-2K crystal structure were taken from Protein Data Bank (PDB ID: 4W9W) which was revealed by X-Ray Diffraction technique with resolution of 1.72 Å. The secondary structure contains 10 beta strands forming a structural feature of two finger-like double-stranded beta-sheets and a helical structure concentrated as a four-turn helix perpendicular to the beta-sheets. It contains two conserved domains namely, Protein kinase domain (aa. 51-316) and Protein inducible kinase at C-terminal (aa. 893-1161) which are responsible for the catalytic activity of protein. This kinase contains three motifs including BMP2 inducible protein kinase C-terminus, protein kinase domain and protein tyrosine kinase. Phylogenetic analysis was performed using the complete amino acid sequences and coding regions of BMP family of proteins which showed high sequence similarity. Our studies thus indicates that regulation of human BMP2K and its gene products by various synthetic and natural molecules can be a promising drug-target in preventing, ameliorating and correcting various disorders of the gastrointestinal, cardiovascular, skeletal systems including cancer.

Keywords: BMP2K, WNT signalling, overexpression, congenital, skeletal, cancer

P-61

A Natural Remedy: Plants extracts to curb Hypertension

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With the escalation in deaths by hypertension globally novel drugs with lesser side effects are required; hence healing by naturopathy is indispensable. The aim is to find out natural plant based inhibitors against peripheral dopamine beta hydroxylase (DBH) which is a pivotal enzyme involved in the conversion of dopamine to norepinephrine which leads to vasoconstriction and causes hypertension.

High throughput screening (via colorimetric assay against DBH) was applied to speed up the search of a novel inhibitor. Various plant extracts were screened. The extracts which showed inhibition were further characterized in search of bioactive components which exhibited inhibition against DBH. The ones which exhibited higher or equivalent inhibitory constant as compared to the known inhibitors like Nepicastat and Disulfiram were taken forward. Furthermore reversed phase HPLC based assay was performed to determine the IC_{50}. To validate the binding of leads and the enzyme, fluorescence spectroscopy was used. Fluorescence measurements were used to determine the K_D and number of binding sites in the enzyme. Hemo-toxic experiments of the identified leads were then performed to evaluate their hemolytic dose if any. Any compound or drug will first interact with the blood cells, especially red blood cells, when given *in vivo* and should not cause any toxicities against them. Interactions of the compounds can have any of the two fates – either they can cause hemolysis or they might have certain low dose requirement to be non-hemotoxic. For a compound to be a promising drug candidate it must be non-hemolytic even at a high dose far higher than its IC_{50}. In near future we aspire to test these compounds in animal models of hypertension to evaluate their therapeutic efficacies.

P-62

Identification of inhibitors of Aurora kinases for development of new anticancer agents

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Aurora kinases are a novel family of serine/threonine kinases which have been identified as key regulators of the mitotic cell division and also essential for accurate segregation of genomic material from parent to daughter cells. The elevated expression profile of all three members (Aurora kinase, A, B and C) of this family in variety of human cancers indicates their significant role in pathogenesis of cancer. Inhibition of Aurora kinase activity arrest the cancer cells in mitotic state and make them destined for programmed cell death. This makes Aurora kinase an attractive drug target for treatment of cancer. Each Aurora kinase has N-terminal regulatory domain and a C-terminal catalytic domain. The catalytic domain is highly conserved and shares more than 70% sequence homology among Crystal structures of inhibitor complexes clearly reveal that most of the available inhibitors of Aurora kinases target the ATP binding pocket.

Studies have indicated promising chemotypes for multi-targeting drug candidates as antitumor compounds based on heteroanthraquinone scaffolds. Two novel heteroanthraquinones C1 and C4 decreased the activity of Aurora A and B protein kinases *in vitro*. Additionally C4 was also seen to inhibit Aurora Kinase C. An in-depth *in silico* investigation was performed to determine the mechanisms of interaction and binding mode of the inhibitors with protein kinases. The available crystal structures of Aurora A and B were taken as the starting point. However, in the absence of a three dimensional structure, Aurora C was modeled using Aurora Kinase B as the template. Molecular docking of the two compounds was carried out with the three Aurora kinases A, B and C to in an effort to understand the role of interactions between inhibitor and protein which are responsible for differential inhibitory activity. Both compounds occupied the respective ATP binding site in Aurora kinase A/B and mainly stabilized by non-polar interactions with side chain of hydrophobic residues in the binding pocket. C4 made higher number of hydrogen bonds than C1 and had binding affinity indicating that it was a more potent inhibitor of Aurora kinase.

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P-63

Effect of Levofloxacin on the Surface Properties of Imidazolium Based Ionic Liquid

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Ionic liquids (IL) are the green volatile organic solvent with melting points below 100 ^{0}C. IL possesses number of attractive properties such as insignificant vapour pressure, non flammability, salvation abilities, low toxicity and antimicrobial properties. IL also enhances the solubility of drug therefore; it is uses as drug carrier. Herein, we investigating the effect of levofloxacin on the surface properties of ionic liquid, 1-Butyl-3- methylimidazolium chloride ([BMIM]Cl) have been investigated by surface tension method. The critical micelle concentration (CMC) as a function of levofloxacin concentrations at various temperatures was investigated. The CMC of [BMIM]Cl increases with the increasing concentration of levofloxacin as well as the temperature of the system. The interfacial parameters viz; maximum surface excess concentration (Γ_{max}), minimum area per molecule (A_{min}), and surface pressure at CMC (Π_{cmc}) were calculated. In addition, thermodynamic parameters of adsorption and micellization were evaluated by using surface tension data. The results indicated that the binding of [BMIM]Cl to levofloxacin is spontaneous and exothermic in nature. The process is entropy driven and hydrophobic interactions are the major driving forces.

P-64

In vitro Anticancer and Apoptotic Potential of Thymol on Human Cancer Cells

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Background: Thymol a phenolic monoterpene, has been reported to be an apoptotic inducer in HL-60 cell line through both caspase dependent and caspase independent pathway.

Objective: The aimed of this study was to evaluate Thymol as anticancer and apoptotic inducer against Human Cancer cells.

Methods: Thymol was tested against liver cancer cell line (HepG2), cervical cancer cell line (Siha) and normal cell line HEK293. Cytotoxic/antiproliferative effect of Thymol was evaluated through the MTT and NR assays. Apoptotic induction on the cancer cell lines were demonstrated by Western blot, Fluorescence microscopy, DNA fragmentation assay, Comet assay as well as FACS.

Results: The result of cytotoxicity assessment through MTT as well as NR assays established that Thymol was significantly ($p< 0.005$) effective on both the cancer cell lines (viz *SiHa* and *HepG2)*. Pro-apoptotic activity was evaluated through the western blot for P53, Bcl2, Bax, caspase 3 and caspase 9. Our result demonstrated that Thymol induced apoptosis through activation of p53, caspase 3, caspase 9 and Bax with decreased expression of Bcl-2. Further, it was confirmed through the nuclear staining dye (DAPI and PI), comet tail and DNA fragmentation pattern in agarose gel. The nuclei of treated cells showed altered nuclear morphology (nuclear condensation, nuclear blebbing and nuclear fragmentation) with comet tail pettern and DNA fragmentation in agarose gel.

Conclusions: This study suggested both the utility and importance of Thymol in cancer therapy as it demonstrated a strong pro-apoptotic behaviour against the multiple and important human cancer cells.

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P-65

TLR3 and RIG-I Agonist Adjuvant Enhances the Immunogenicity and Protective Efficacy of the recombinant E2 protein-based vaccines against Chikungunya virus

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Chikungunya virus (CHIKV) causes outbreaks of Chikungunya fever worldwide and represents an emerging pandemic threat. Vaccine development against CHIKV has proved challenging. Currently there is no approved vaccine or specific therapy for the disease. Double-stranded RNA (dsRNA), generated during the replicative cycle of many viruses, is sensed by receptors such as Toll-like receptor 3 (TLR3) and different members of the RIG-I-like receptor (RLR) family. The aim of the present study is to develop candidate vaccines employing TLR3 and RIG-I agonist adjuvant in combination with recombinant E2 protein, to confer protection against murine CHIKV.

E2 gene of CHIKV isolate of ECSA genotype was cloned in pET15b vector, expressed and purified (rE2p). Five to six weeks old female BALB/c mice were immunized intramuscularly with two doses of 30µg of vaccine formulations (poly I:C+5′-triphosphorylated RNA+rE2 protein), 2 weeks apart and divided in two groups. In the first group immunization led to the highest ELISA/neutralizing antibody (nAb) titers and the expression profiling of TLR, antiviral genes and cytokines in mice tissue revealed significant up regulation of TLR3, TRAF-6, IL-6, IL-4 genes. Second group of immunized mice was challenged 2 and 10 weeks after the second immunization. Pre-immunization of mice caused reduction of viral load in the serum and tissues (muscles and spleen) and offered 100% protection of animals. The protection was mediated by an increased induction of TLR3, IFN-β and antiviral genes in mice tissues. Re-stimulation with CHIKV, T cells activated with an expansion peak and elicited memory CD4+ and CD8+ T cells that produced IFN-γ, TNF-α and IL-2.

Finally our results demonstrate immunopotentiating effect of adjuvant poly I:C and 5′-triphosphorylated RNA on recombinant E2 protein,representing a promising vaccination strategy for CHIKV.

P-66

Isolation and purification of MIS12 homolog from Acorus calamus

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Acorus calamus (Sweet flag) is a plant of wetland which the aromatic leaves and rhizomes have been traditionally used medicinally and contains a wide variety of phytoconstituents having different medicinal properties. The active constituents such as phenyl propanoids, sesquiterpenes and monoterpenes as well as xanthone glycosides, flavones, lignans, steroids obtained from the plant has been for the prevention and treatment of several diseases ranges from neurological disorder to cancer. We for the first time isolate and purify proteins from *Acorus calamus* for their therapeutic uses. We crushed stem of *Acorus calamus* in homogenization buffer (50 mM Tris–HCl pH 8.0 with 500 mM NaCl). The homogenate was used for ammonium sulphate precipitation at different cut-off value. The 60% ammonium sulphate precipitate extensively dialyzed again 50 mM Tris–HCl pH 8.0. After dialysis the sample was subjected to anion-exchange chromatography on Hi Trap DEAE-FF. The elution profile shows three peaks but our desired protein was found in unbound region. The protein in unbound region was concentrated and load on gel-filtration column for further purification. The protein thus purified showed a sharp band at around 30 kDa on SDS-PAGE. The purified protein was MIS12 homolog when identified through MALDI-TOF.

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Osmolyte induced functional loss in an intrinsically disordered protein

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Osmolytes are small low molecular weight organic compounds known to promote protein folding, stabilize proteins, correct misfolding and prevent aggregation of proteins. Most of these observations were found involving osmolyte and well folded globular proteins. However, genome of all the organisms encode proteins which are natively unfolded and are involved in various important biological functions like cell signaling, cell cycle etc. This important group of proteins once considered as rare exceptions are known as intrinsically disordered proteins (IDPs). In the present study, we have investigated the effect of one of the important osmolyte found in most animal tissues on an intrinsically disordered protein using various spectroscopic techniques. Using far- and near-UV CD spectroscopy we observed that there occurs compaction in IDP without any structure creation in presence of the osmolyte. Further, we also found that there was loss of activity not because of aggregation but due to compaction as observed by light scattering and dye binding assays. As expected, there was a direct correlation between intrinsic disorder and activity. We conclude that the osmolyte induced structural alterations in IDP which leads to its compaction without taking aggregation pathway makes it functionally inactive.

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Identification and Biological Evaluation of Natural Compounds as CDK-6 inhibitor

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Protein Kinases play an important role in several cellular processes like cell growth, cell signaling development and metabolism. Expression of many

kinases like CDK, PFK, MARK, GSK3â, ILK, gets altered in many modern life style mediated disease like diabetes, cancer, neurodegenerative diseases. Cycline dependent kinase (CDK-6) is a cell cycle enzyme that regulate the cell cycle and cell metabolism. CDK-6 phosphorylate the glycolytic key enzymes like PKM, PFK2 and inhibit their activity which leads to inhibit the production of ROS and prevent the apoptosis. We report cloning, expression and purification of CDK-6 in the bacterial system followed by binding studies with selected natural compounds. The recombinant protein was purified by Ni–NTA chromatography from the supernatant, SDS-PAGE showed a band of 32-kDa, which was further confirmed by Western blot and MALDI-TOF/MS. Initially all the selected natural compounds were screened by molecular docking studies. Docking results were further confirmed and validated by fluorescence and isothermal titration calorimetry. Enzyme inhibition assay was carried out to ensure the inhibition of enzyme activity. The final selected inhibitors will be evaluated for mRNA level expression (qPCR) and protein levels expression (Western blot) of selected kinases using different cell lines.

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Thermoglobins, a novel model system for further insight into hemoglobin folding and stability

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In the last two decades, the field of hemoglobin has witnessed an increase in the number of new globins, ubiquitously identified in all kingdom of life. Novel globins are different from classical pentacoordinated hemoglobins and therefore, categorized based on their heme coordination chemistry as "hexacoordinated hemoglobins" and their structural fold as "truncated hemoglobins". Studies over the years have shaped a comprehensive picture of the structure-function relationship of classical hemoglobins. However, an understanding of molecular mechanisms that evolution has been employing to adapt to environmental temperatures of novel globins is partial. Several properties believed to be involved in the adaptation to different temperatures like amino acid composition, analysis of the loops and secondary structure, number of salt bridges and hydrogen bond, accessibility to hydrophobic surface, absence of a residual structure in the unfolded state, small cavity volume in the native state, low conformational stability and a low melting temperature. To gain some further insight into Hb folding and stability we have employed comparative *in silico* anlaysis, biophysical techniques and performed extensive site directed mutagenesis of key residues surrounding the heme pocket in globins from extremophile organism in relation to their mesophilic counterparts. Interesting observations were recorded for Hb from extremophile organism was found to be less stable relative to the mesophilic counterpart and exhibited irreversible thermal denaturation unlike other thermo globins investigated. Such findings and their implications will be presented.

P-70

Riboflavin kinase: structure and sequence similarity, activity in many living organisms, use of many tools for the prediction of its structural activities and homology modellig for structural activity

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Riboflavin kinase is an enzymatic protein that can catalyses a reaction. It can catalyses the reaction of ATP with riboflavin to form ADP and FMN.

The role of this enzyme is in alenylation reaction of FAD synthetase. A transferase enzyme namely riboflavin 5′-phosphotransferase plays an great role in the metabolism and the and therefore also known as flavokinases. Different tools like Interproscan , motif Finder, signal P, string, Impred, CDART, BLAST, superfamily and Smart. All these tools gives some important information regarding the protein of our interest that is riboflavin kinases in this case.

As this protein shows more than 40% of similarity, so after applying homology modeling the results are that there are 40 models and 1 template and it also includes the following properties like seq identity, oligostate, range and description of the ligands and its information and the value of the theoretical PI it also gives information on about the intermolecular interactions and the cys residues and some other related information. There is one motif present along the flavokinase position (5..129[1,4e-39]).Domain

present in this protein are PFO1687 (flavokinase), SM00904 (flavokinase- 2) and SSF082114 (riboflavin). This protein plays many important functions such as ATP binding , metal ion binding, FMN biosynthetic process of riboflavin biosynthetic process. This protein is mainly found in many organisms we can say that more than 4510 organism are there of having this particular protein.

It is helpful in the production of TNF – induced reactive oxygen species production with two co-factors Zn+ and Mg2+. Thios protein has the sequence similarity in between SGK1, RFK, ribK and Fmn. Further it can gave information on no. of amino acid, molecular weight, positively and negatively charged residues as well.

P-71

Structural and Functional Analysis of Sphingosine Kinase 1

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Human sphingosine kinase 1 (SPHK1) is a ~43KDa lipid mediator protein which plays a key role in the TNF-alpha signalling and the NF-kappa-B activation pathway by catalysing the phosphorylation of sphingosine to sphingosine-1-phosphate (S1P). Recent studies have found that overexpression of SPHK1 may lead to progression of various cancers.Crystal structure of Sphingosine kinase 1 resolved at 2.0 Å (PDB ID-3VZB) which has one conserved domain-Diacylglycerol kinase catalytic domain (aa. 12-159), classically plays a role in glycerophospholipid biosynthesis and phosphoinositide pathway. The secondary structure of Sphk1 comprises of nine α-helices, seventeen β-strands and a 3_{10}-helix.Sub-cellular localization studies suggested significant nuclear localization signal along with some cytoplasmic and plasma membrane localization. Phylogenetic analysis was done using the amino acid sequence and also of coding region of SPHK family of proteins which showed high sequence similarity. Sphk1 protein and gene products have an essential role in inflammatory, antiapoptotic and immune responses. Our studies thus elucidates that modulation of this protein and its gene products through synthetic and natural compounds can play a pivotal role as a potential drug target and in therapy of cancer- breast cancer, human thyroid cancer, cervical cancer including lipid metabolism disorders.

P-72

Determination of Structural Conformation and Stability of CBRLK2 protein purified from medicinal plant, *Oroxylum indicum*

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The ***Oroxylum indicum,*** a medicinal plant of immense medicinal importancehas been used for centuries as a traditional medicine in Asia in for the prevention and treatment of several diseases including tumors, diabetes and dysentery etc. The crude extracts exhibit a wide spectrum of pharmacological activities involving antimicrobial, anti-inflammatory, anti-arthritic, anticancer and anti-diabetic. We have isolated and purified Calmodulin binding receptor like cytoplasmic kinase 2 (CBRLK2) from the stems of *O. indicum.* The tissue was homogenized in homogenization buffer (50 mM Tris–HCl pH 8.0 with 500 mM NaCl) having protease inhibitors in 1mM concentration. After homogenization, polyvinyl pyrrolidone (PVP)was added to remove large phenolic compounds.The resultant crude extract was used for ammonium sulphate precipitation. The 60% ammonium sulphate precipitate was dialyzed extensively against 50 mM Tris–HCl pH 8.0 and subjected to weak anion-exchange chromatography on Hi Trap DEAE FF. The purity of protein was checked using SDS-PAGE and then identified by mass spectrometry (MALDI-TOF). By using far-UV CD data, secondary structure content was determined with the help of online K2D2 server, and found that CBRLK2 protein is mainly â-sheet containing (43% β-sheet and 10% α-helix). The stability of CBRLK2 was monitored thermally and isothermally using two probes (CD and Fluorescence spectroscopy).

P-73

Structural and Functional Analysis of Human Bruton Tyrosine-Protein Kinase

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Bruton's Tyrosine Kinase (BTK) is encoded by the *BTK* gene which belongs to the protein kinase

superfamily and Tyrosine kinase family. It consists of 659 amino acid residues with a molecular mass of 76,281 Da at pI 7.89. BTK plays an important role in B-lymphocyte development, differentiation and signalling. We have performed structural analysis to understand the mechanism of the protein function. This protein is composed of four domains:- Protein kinase-like (PK-like); PH domain-like; SH2 domain; SH3-domain and a single active site at 521 position. BTK has two regulatory tyrosine residues, Tyr-223 and Tyr-551, which participate in kinase activation. Structure analysis is suggesting that N-terminal lobe (residues 397–475) contains five strands of antiparallel â sheets (β1–β5) and one α helix (C helix). The C-terminal lobe (residues 479–659) contains a four-helix bundle (αD, αE, αF, and αH) flanked by a short antiparallel β sheet (β6, β8, and β9) and four additional helices (αI, αDE, αEF, and αHI). The N- and C-terminal lobes are connected by a linker region (residues 475–479) and form a cleft at the ATP binding site. It is involved X-linked agammaglobulinemia, a humoral immunodeficiency disease which results in developmental defects in the maturation pathway of B-cells. BTK plays crucial role through regulation of a broad group of cytokines and chemokines both at transcriptional and translational levels. BTK participates in signal transduction pathways initiated by the binding of a variety of extracellular ligands to their cell surface receptors.

P-74

Detection of diagnostic proteins and various biomarkers in the saliva for oral and systemic diseases: a Review

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Saliva based diagnosis for the detection of specific biomarkers has drawn significant attention since the sample extraction is simple, cost-effective, and precise along with its abundance of biomarkers, such as genetic material and proteins. Due to the diffusive properties of saliva, it has been referred to as "the mirror of the human body". Compared to blood, saliva contains a similar variety of DNA, RNA, proteins, metabolites, and microbiota that can be compiled into a multiplex of disease detection markers.

This review will update the clinician on recent advances in salivary biomarkers to diagnose autoimmune diseases, cardiovascular diseases, diabetes, hepatitis, HIV, oral cancer, caries and periodontal diseases. It is expected that the advent of sensitive and specific salivary diagnostic tools and the establishment of defined guidelines and results following rigorous testing will allow salivary diagnostics to be used as chair-side tests for several oral and systemic diseases in the near future.

P-75

Quantification of nano particle influencing stability of aggregation prone proteins

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Protein aggregation can be totally a factor of inconvenience in many *in vivo* and*in vitro* studies of proteins. It can also be a problem of concern in the biotechnology and pharmaceutical industries. Its effects can be lethal in patients who suffer from a variety of diseases involving protein aggregation e.g. amyloidoses, prion diseases and other protein deposition disorders. The menace of aggregation is intimately tied to folding and stability of proteins.

DHFR is an important enzyme involved in the nucleic acid synthesis. It helps in the conversion of dihydrofolate to tetrahydrofolate using NADPH as an electron donor. In this study we have chosen DHFR as our model protein to analyze the role of nano particles to comprehend the interaction of these nanoparticles with DHFR. This will also aid in monitoring the conformational change of the protein in presence of nano particles as well as the extent of in vivo folding of recombinant DHFR in *E.coli*.

This study is beneficial to increase the production of functional and soluble proteins in the cell extract. It can also be significant in finding therapeutic agents against many diseases that are caused due to protein aggregation. This study will also help in analyzing the stabilizing role of these nano particles on aggregation prone proteins that may help in the discovery of pharmacological chaperones that can be used in the healthcare sector.

P-76

Progression Dynamics of Zika Virus Infection using Temperature and Rainfall Dependent Mathematical Model

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In the family Flaviviridae, Zika virus is a recently emerged mosquito-borne Flavivirus, which hold the liability for causing several outbreaks globally. An important and challenging issue for health authorities of affected countries is to plan the strategies to fight against this virus in such a way that future outbreak casualty could be minimized. In this way, it is essential to understand the significant characteristics of Zika virus infection for the purpose of its control. The present study focuses on the temperature and rainfall dependent mathematical model for the progression dynamics of Zika virus infection. The model includes both the population of humans and mosquitoes. The model was used to estimate different parameters using the data obtained from Puerto Rico during 2015-16 that were responsible for the outbreak of ZIKV in this region.The calculated value of basic reproduction number R_0= 3.2869 at suitable values of parameters indicates the rapid spread of the infection in the human population. Disease free equilibrium for constructed model is identified which is locally asymptotically stable if R_0< 1. Sensitivity analysis suggests top three parameters for model which are mosquito death rate, mosquito biting rate and maturation rate of immature mosquitoes. Several numerical simulations are done to reflect the changes in progression dynamics of Zika virus infection in the human population.

P-77

Evaluation of Thienopyrimidine Based Chalcones as FASTK Inhibitor: Strategy to Combate Breast Cancer

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Breast cancer has become the second most death causing disease (after lung cancer) among women. Instead of advances in the treatment of breast cancer, survival rate of the patient's affected by this disease remains potentially low. Nowadays, researchers have diverted towards the usage of plant derived compounds as these compounds are non-toxic to the normal cells. Fas-activated Serine/Threonine Kinase (FASTK) belongs to serine-threonine kinase family which has been implicated in apoptotic evasion and hence resulting in the progression of cancers. Apoptotic evasion being one of the striking hallmark of cancer has turned out to be a new arena for the exploration of drugdiscovery. In our study, a series of novel thienopyrimidine basedchalcones have been synthesised and evaluated to modulate the FASTK mediated apoptotic evasion. Initial screening was achieved by binding studies and enzyme inhibition assay, which indicate strong binding affinity and enzyme inhibition (nM range) by three thienopyrimidine based chalconederivatives. Cell proliferation assessment of the selected compounds was further executed on MCF-7 and HEK-293 cells. Three compoundsdisplay an IC_{50}value of 20.22±1.50 µM, 6.52±0.82 µM and 8.20±0.61 µM, respectively. Moreover, we observed that these molecules induce apoptosis in MCF 7 cells and consequently inhibit cell migration and arrest cell cycle in G0/G1 phase presumably by inhibiting FASTK and reactive oxygen species (ROS) production. These compounds may be exploited as potential anticancer agents.

P-78

Interaction of Diclofenac with Human Hemoglobin: An Experimental and Computational Approach

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The interaction between Diclofenac (DIC) and human hemoglobin (Hb) have been investigated by using fluorescence, time-resolved fluorescence, UV-visible and circular dichroism (CD) spectroscopy and molecular docking method. The Föster resonance energy transfer (FRET) study was also carried out which provides the distance (r) between fluorophore of Hb and DIC molecule. The Fluorescence result suggested that DIC quenches the fluorescence of Hb through static quenching mechanism which was in good agreement with our UV-visible and time-resolved fluorescence spectroscopic results. The thermodynamic parameters such as ΔH, ΔS and ΔG were also calculated for DIC-Hb interaction. The negative value of ΔH and positive value of ΔS shows that hydrogen bond and electrostatic interaction played an important role in the complex formation between DIC and Hb. Also, the negative value of ΔG indicates the spontaneity in the interaction system. The molecular distance (r) of 4.02 nm between the donor (Hb) and accepter (DIC) was calculated by the Foster theory which again supports our fluorescence quenching result. CD spectroscopy result showed the DIC induced small changes in the secondary structure of Hb. Molecular docking result revealed that the DIC molecule binds with the Hb on α_1 chain.

P-79

Designing and Construction of FRET-based mercury nanosensor

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Biological systems require essential metal ions for biochemical reactions not only at cellular but also at molecular level. Metalloenzymes are proteins that contain metal ions as cofactors for various metabolic processes. Mercury is a non-essential heavy metal which when present in the body at relatively higher level can be toxic and prove to be fatal to the human health causing cancers and tumors. This metal ion is a major environmental and occupational pollutant negatively affecting the lungs, kidneys and nervous, digestive, neuromuscular and immune systems. Balance of metal homeostasis requires understanding the pathways involving cellular distribution, transport and uptake/efflux of metal ions. Methods developed so far to study their level are not able to address the sensitivity, selectivity and have limited spatial resolution. Subsequently higher resolution can be achieved by using fluorescence resonance energy transfer (FRET)-based nanosensors that exploit conformational changes in a metal-binding domain that binds the analyte-of-interest as a proxy for analyte levels. A genetically encoded FRET-based nanosensor using *merP* as a metal-binding receptor domain is sandwiched between a donor fluorophore, ECFP and an acceptor fluorophore, Venus at N- and C- terminus of the binding domain respectively. Binding of the mercury may either bring together the two fluorophores or move them away from each other resulting in FRET. The receptor domain is responsible for recognition of mercury ions. Mercury exposure introduces free radicals which lead to oxidative damage and may affect vital metabolic functions in the body. This sensor produces a fluorescent signal in response to the binding of a specific target metabolite i.e., mercury ions. This sensor will prove to be useful in detecting the physiological concentration of mercury ions in living cells and understanding mercury homeostasis.

P- 80

Unraveling the Binding Nature of Cationic Amphiphilic Drugs with Human Hemoglobin: a comparative study

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The binding nature of amphiphilic drugs viz. promethazine (PMT) and adiphenine (ADP) with human hemoglobin (Hb) was unraveled by fluorescence, absorbance, time resolved fluorescence, fluorescence resonance energy transfer (FRET) and circular dichroism (CD) spectral techniques in

combination with molecular docking and molecular dynamic simulation methods. The steady state fluorescence spectra indicated that both PMT and ADP quenches the fluorescence of Hb through static quenching mechanism which was further confirmed by time resolved fluorescence spectra and FRET results. The UV-Vis spectroscopy suggested the ground state complex formation. The thermodynamic data revealed that the binding of PMT with Hb are exothermic in nature involving hydrogen bonding and van der Waal's interaction while in case of ADP hydrophobic forces play's the major role and binding process is endothermic in nature. The CD and MD simulation results showed that the interaction of the amphiphilic drugs induced structural change in protein conformation. Molecular docking results suggested that PMT bound to Hb principally at subunit β_2 chain whereas ADP binds to Hb at subunit α_1 chain.

P-81

Effect of interdomain interactions on the stability of two closely homologous proteins hGBP-1 and -2

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Human guanylate binding protein-1 (hGBP1) is a multi-domain large GTPase and comprised of an N-terminal globular (G) domain and a C-terminal helical domain, which are linked by an intermediate region. It hydrolyzes GTP to both GDP and GMP with GMP being the major product. Previous data suggested that the isolated G-domain of full-length hGBP1 is stable to some extent without the nucleotide, but the protein is more stable in the presence of the helical domain. The present study shows that the thermodynamic stability of the full-length hGBP1 is higher than the sum of its individual domains, suggesting that interdomain interactions provide further stability to the full-length hGBP1. However, hGBP2, a close homolog of hGBP1, hydrolyzes GTP to GDP and GMP, where GDP is the major product. To investigate whether interdomain interaction of hGBP2 play similar role in the stability, we prepared truncated proteins and determined their thermodynamic stability. We found that the thermodynamic stability of the truncated protein that lacks the helical domain, is higher than that of the full-length hGBP2, suggesting that the presence of the helical domain reduces the stability of the full-length hGBP2. This finding is further supported by unfolding kinetics in the presence of the denaturant and differential scanning calorimetric studies. MD simulation studies as well as mutational analysis also provided insight into the interaction between the interdomain residues that are responsible for reducing the stability of the full-length protein. Thus, this study highlights how interdomain interactions affect the stability of the two proteins despite their high sequence identity.

P-82

Targeting Matrix Metalloproteinases (MMPs): A Therapeutics Approach for Cancer Metastasis

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Major challenges in cancer include the metastasis; the cancer progression, complexity of cellular interactions and cell signalling within tumor microenvironment due to the abnormal expression of oncogenes. Matrix metalloproteinases (MMPs), also known as matrixins, are the enzymes responsible for degrading the wall of extra cellular matrix during cancer progression. MMPs have many roles in normal health conditions but as the cancerous cells takes their place inside the body, this enzyme gets over expressed due to the up-regulation of their genes which in case degrades the major components of the extracellular matrix. The breakdown of the extracellular matrix (ECM) arise the spreading of the cancerous cells from one part of the body to another.

The search for an MMPs inhibitors with anticancer efficacy is a nearly three-decade endeavour. These inhibitors are yet to be found. There as ones for this failure include short comings in the chemistry of these compounds (including broad MMP sub-type selectivity, metabolic liability, and toxicity) as well as the emerging, and arguably extraordinary, complexity of MMP cell (and cancer) biology. In our project, we tried to search for a potential inhibitor(s) of MMPs, especially for MMP3, MMP7 and MMP9 through in-silico approach using various bioinformatics tools and servers. We performed structure based virtual

screening of naturally occurring compounds to select possible binding partners of MMPs and filter them through various pharmacokinetics parameters such Lipinski rule and ADMET properties. Here, finally we are able to find some suitable compounds which may be potent inhibitors of MMPs family for the development of anticancer drug. However the limitation of our study is the lack of experimental validation, so, we could not fully verify our results, but one could improve this deficiency in a further experimentation.

P-83

Characterization of a crucial enzyme in serine biosynthetic pathway from *Entamoeba histolytica*

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Phosphoserine phosphatase catalyzes the last step of the phosphorylated serine biosynthesis pathway, it hydrolyzes phospho-L-serine (PLS) to serine and phosphate. In *Entamoeba histolytica,* there is a missing link in serine anabolism, it possesses other enzymes of serine biosynthesis but PSP activity has not been reported yet. Here, first time we are reporting structure and kinetic characterization of the enzyme. *Entamoeba histolytica p*hosphoserine phosphatase is crystallized in $P6_122$ space group with two molecules in asymmetric unit. Individual molecule of protein adopts a classical dPGM like fold, where core of the structure comprises an $\alpha/b/\alpha$ sandwich. Active site is situated above the mixed b sheet, where His9 and His 144 are the catalytic residues. PSP activity was assayed by measuring the production of inorganic phosphate. We have also done activity in presence of different substrate just to confirm the substrate specificity of the enzyme. The Km for O-phospho-l-srine is 0.6mM. All known classical PSPs are Mg^{2+}dependent enzymes, and are inhibited up to 80% in the presence of Mn^{2+} and Ca^{2+}. But, EhPSP neither require Mg^{2+} ions for activity nor inhibited by Mn^{2+} and Ca^{2+}. Though the same enzyme has been annotated as phosphoglycerate mutase, a glycolytic enzyme. Mutase activity also has been checked that shows, it has residual mutase activity which confirms that it works more as a phosphatase rather than as a mutase. So, it makes EhPSP different from other known PSPs as structure is also completely different from human PSP, suggesting a potent drug target.

P-84

Culinary Spices and Herbs as Potential Source of Thermostable Superoxide Dismutase: A Therapeutically Significant Enzyme

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Traditionally, spices and herbs are termed as a plant parts that are used in food for culinary purpose owing to their unique aroma or flavor with or without nutritional value. Recently, spices and herbs have been identified as sources of various phytochemicals, majority being antioxidants indicating their role in defense by succumbing the effect of oxidative stress caused by free radicals. Production of superoxide radicals or anions occurs during metabolic processes like catalytic transformation of various molecules by enzymes. The excess of reactive species result in damage of proteins, cell lipids and DNA by oxidative action, resulting in loss of function and cell death, thereby linking few diseases with oxidative stress. Superoxide anions are strongly implicated in the development of numerous degenerative diseases, including Parkinson's disease, neurologicaldisorders, atherosclerosis, chronic and acute inflammatory conditions, stroke, heart attack, cancer and various age-related disorders. Antioxidants act as bodyguard to prevent cells against oxidative stress. Superoxide dismutase (SOD, EC 1. 15. 1. 1) is one of the most powerful internal antioxidant defenses and plays a critical role in reduction of oxidative stress thereby, various diseases. It has been proposed as a potential marker and a useful tool to predict metastatic potential of various cancers. Although, human cells are competent enough toprotect themselves against free radical damage, however, sometimes requirement of exogenous antioxidants arises to prevent oxidative damages due to insufficient supply of endogenous antiradicals. Therefore, sometimes antioxidant supplements are required to reduce this damage.Thus, consumption of food sources rich in antioxidants may help in prevention of oxidative stress. Various sources have been found to contain substantial amount of SOD viz; spices and other plant sources. SOD obtained from plant parts may be a potential candidate for targeting many diseases. To live longer and enjoy better health,

one of the most important ways is to increase SOD levels. Because of various benefits and importance of this enzyme the work has been undertaken to explore the potential of SOD enzyme. In the present work, five spices; *Zingiber officinale, Capsicum annuum, Myristica fragrans, Cuminum cyminum* and *Trigonella foenum-graecum* have been studied as potential source of SOD. The crude enzyme was partially purified by salt fractionation into three parts; 0-30%, 30-60% and 60-90% based on $(NH_4)_2SO_4$ saturation level; out of which the fraction showing highest specific activity was dialyzed and further subjected to next step of purification using anion-exchange chromatography with DEAE-cellulose as matrix. The purified samples were characterized on the basis of various biochemical and kinetic parameters viz; pH and temperature optimum values along with stability, incubation time, enzyme and substrate concentration, K_m, V_{max}, activation energy values, etc. The thermal studies were performed on SOD obtained from all sources and stability temperatures ranged from 50^0C to 80^0C. The results obtained suggest that the mentioned spices and herbs may act as potential source of thermally resistant SOD enzyme, an antioxidant, which further has many applications in various bio-industries. It may be summarized from the present study that common culinary spices and herbs possess SOD enzyme with high thermostability, thereby, retaining activity during cooking and digestion. However, further studies need to be undertaken in this direction to determine the potential of SOD enzyme from spices and herbsvia *in vivo* conditions.

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Serine Proteases and Inhibitors in Cancer

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Serine proteases, the largest human protease family, are found in many key developmental and physiological processes in the biological system. Protease signalling pathways are stringently controlled, and deregulation of proteolytic activity results in the degradation of extracellular matrix which plays a major role in cancer progression. The Type II transmembrane serine protease; hepsin, matriptase-2, TMPRSS4, and secreted serine protease; urokinase plasminogen activator (uPA), kallikreins, HtrA are closely related to cancer-associated proteases and also involved in perturbation of uPA plasminogen system, matrix metalloproteases (MMPs), upregulation of adhesion molecules like integrin family, activation of intracellular signalling cascade, inhibition of apoptosis pathway in various types of cancers which causes cell proliferation, invasion and metastasis. Serpin, an endogenous serine protease inhibitor regulates the homeostasis by maintaining a delicate balance with the serine protease and prevent the process of invasion and metastasis of cancer cells and thus inhibit tumour growth. This chapter focuses on the role of serine proteases and their inhibitors in different types of tumours associated with cancer prognostication and therapy.

P-86

Development of a FRET based nanosensor for measurement of vitamin B_1 levels in living cells

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Vitamin B_1 (thiamine) is an important dietary nutrient. Malnutrition of thiamine is a significant public health issue in most of the developing world. Most of the human population in developing countries relies on staple crops, rice, wheat and legumes, which are deficient in thiamine. Deficient levels of thiamine result in severe neurological disorders- the dry beri beri and wet beri beri. In extreme cases, dry beri beri leads to neuronal damage and wet beri beri modulates into permanent heart failure. Severe and acute deficiency of thiamine in alcoholics causes Wernicke's encephalopathy leading to dementia.

Monitoring of the vitamins is not easy since vitamins are highly compartmentalized in specific tissues or even sub-cellular organelles, which we cannot know by grinding up the whole organ. No currently available technology measures the flux of metabolites in a satisfactory manner. In present study, we have constructed a fluorescence resonance energy transfer (FRET) basednanosensor for living cells (FLIPT) to selectively monitor and measure the thiamine levels at *real time* in a *non-invasive* manner. We have utilized two genetically engineered fluorescent proteins and a substrate binding domain for thiamine to develop our nanosensor. An open to

close conformational dynamics of FLIPT in presence of thiamine measures the FRET ratio, which ultimately serves our aim of measuring vitamin B_1 levels in prokaryotic or eukaryotic living systems.

P-87

In-Vitro Model to Study Hepcidin Expression in Mammalian Cells: The Molecular Mechanism of Hepcidin Expression

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Hepcidin is a liver specific peptide hormone that regulates the iron uptake in human body. Expression of hepcidin is regulated by different iron regulatory proteins and physiological conditions of our body. Hereditary hemochromatosis factor E (HFE) and transferrin receptor 2 (TfR2) are the glycoproteins, involved in iron regulation. They act as sensors for the iron uptake, and regulate hepcidinexpression, but exact mechanism is unknown.This study was carried out to see the relationship between HFE and TfR2 inhepcidin regulation. To evaluate the expressional regulation of hepcidin, TRVb-1, TRVb-2, CHO, HuH7, HepG2 and HT-29 cell lines has been used, and the iron-uptakewas studied. With the help of q-PCR, mRNA expression of iron-regulatory genes was studied. It was found that expression of HFE, hepcidin and TfR2 is increased in the presence of iron. Western blot and flow-cytometry based studiesinferencethat activation of STAT3 and ERK1/2 is also increased, but in the presence of wild type HFE and TfR2. Increased expression of STAT3 and ERK1/2 correlated with their phosphorylation. Expression of IL-17 also found to be enhanced; it further confirms the activation of STAT3. Results suggested that in iron surplusconditions, HFE and TfR2 activateSTAT3, ERK1/2 and IL-17. Present study provides a non-redundant model to study iron homeostasis and characterization of the intracellular molecular mechanism of TfR2 and HFE roles in iron uptake.

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Crystal structure and active site regulation of a novel POP enzyme from *Deinococcus radiodurans* R1

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The prolyl oligopeptidase (POP) family of serine proteases represents a new class that differs from classical trypsin-subtilisin families in many aspects but mainly with length restricted up to 30 residues. This family includes prolyl oligopeptidase (POP), oligopeptidase B (OPB), dipeptidyl peptidase IV (DPP-IV) and acylpeptide hydrolase (APH) that are important targets of drug design. For instance, POP is involved in celiac sprue, DPP-IV in type 2 diabetes, OPB in trypanosomiasis and APH in cataract formation. Though POP enzymes adopt similar fold structure, they show different substrate specificities and regulatory mechanisms for substrate entry into the active site that was not fully understood. Here, we report the crystal structure of novel peptidase in POP family from an extremely radiation resistant bacterium *Deinococcus radiodurans* using x-ray diffraction studies at PX BL21, Indus-2, India. Crystal structure of this tetrameric enzyme (MW ~295 kDa) was solved at 2.3 Å and 1.7 Å resolution for wild type and mutant protein, respectively. The enzyme adopts two domain structure with N-terminal 7-bladed β-propeller and C-terminal α/β hydrolase domain. We also present the regulatory mode of enzyme by solving the structure in two forms i.e., active and inactive where a unique arginine disassembles Ser-His-Asp catalytic triad. Reassembly of active site is mediated by substrate binding that induces large conformational changes in both the domains. Certain structural adaptations are observed that minimizes the space and exclude the peptide length towards the C-terminal side. This results highlightens the importance of critical arginines in holding the free carboxylate of peptide substrates thus reports the novel substrate binding and regulatory mode in POP family. The presence of this enzyme in *D. radiodurans* may play a significant role in growth and survival of this bacterium as it requires high nutrient needs to protect the proteome against oxidative damages.

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Evaluation of Docking Programs to Predict the Binding Mode of Small Peptide Ligands

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Drugs interact with receptor targets and modify their function. However changes in receptor due to mutation in encoding gene cause the drug resistance. Most of the available drugs are simple organic compounds having relatively rigid conformation which increases the chances of resistance. This necessitates the requirement for finding the novel class of compounds which can provide potent and resilient drugs. Peptides could be such a competent class of compound which has inherent conformational flexibility that can adapt the limited changes in receptor. Hence peptides could provide drug candidates resilient to drug resistance. In addition, peptides are less toxic and have higher affinity for their targets. However, synthesis of peptides for chemical screening is time consuming and costly affair. Therefore in silico screening beforehand to shortlist the potential peptides for chemical screening will reduce the time and cost of finding peptide based lead compounds. Molecular docking is the more reliable method of in silico screening. Therefore, selection of docking program is the first and foremost criterion which has to be examined in order to achieve appropriate results. Currently, a comparative study on docking programs for peptide ligands is unavailable. We have evaluated three most commonly used docking programs for protein-peptide ligand docking to facilitate the choice of a relatively more reliable program and establish a benchmark. These programs include AutoDock 4.2, commonly used open source software, and two industry standard commercial docking programs, GOLD and Glide. Peptides consisting of 2-9 amino acids were taken from their respective protein complexes available in PDB. The ability of the three docking programs to reproduce the native crystal conformation was assessed. This study suggests that AutoDock 4.2 outperforms GLIDE and GOLD docking program in case of peptides as ligands.

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Identification of Actin binding proteins in Entamoebahistolyticaproteome using bioinformatics tools

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Entamoebahistolytica, an organism standing out distinctly in the phylogenetic tree is markedly different even within the parasite world. It causes amebic colitis and liver abscess, accounting for nearly 10000 deaths per year. Its virulence is attributed to a process known as phagocytosis. Aptly named, E. histolyticatrophozoites invade the host colon while degrading the extracellular matrix, killing and phagocytosing host cells. Actin, being the major cytoskeleton of the cell mediates the pathogenesis by its rapid assembly and disassembly and thus regulating the phagocytic cup formation. This dynamics is orchestrated by various actin binding proteins (ABP)s, which are grouped into few conserved families like Wiskott–Aldrich syndrome protein (WASP)-homology domain-2 (WH2), the actin-depolymerizing factor/cofilin (ADF/cofilin) domain, the gelsolin-homology domain, the calponin-homology (CH) domain, and the Myosin etc. The above stated groups are only some of the known domains but all in all around 162 distinct actin-binding proteins exist. But E.histolytica ABPs aren't well characterized or studied yet. Hence, we tried to fish out and study the various ABPs bioinformatically. Beginning the study by searching amoeba proteomic database we found 300 possible actin binding proteins in Entamoebahistolytica HM-1 IMSS strain. After filtering out kinases, heat shock proteins and few others, we got 167 proteins to look at. These were further studied through interproscan domain assignment; BLAST alignments etc. and we found 66 uncharacterized proteins, out of which 33 contained known domains that help in actin regulation. 101 proteins were characterized as known actin regulators. Further in depth analysis of these proteins need to be done.

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Antinutritional property of a protease inhibitor from C*yamopsis tetragonoloba*

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Legume grains contain significant amount of protein that is high in amounts of amino acids so consumed to meet the dietary requirements. Antinutritional factors reduce the activity of various useful component like proteases and reduce the available nutrient for absorption in the gut. Some anti-nutrients may for example Lectins ,tannins, saponins, amylase inhibitors, protease inhibitor have been shown to reduce availability of nutrients and cause growth inhibition.

We have characterized anti-nutritional factor from C*yamopsis tetragonoloba* that could inhibit the activity of serine protease trypsin and chymotrypsin but does not inhibit aspartate protease pepsin.

The protease activity was checked by using synthetic substrate BAPNA as well as natural proteins as casein and heamogobin. The protease inhibitor was purified from *Cyamopsis tetragonoloba* seeds to a homogeneity by ammonium sulfate precipitation and ion exchange chromatography on DEAE- sephadex column .It moved as a single band of 11 KDa under non reducing SDS PAGE . Protein was found to be glycosylated. The yield of the protein was found to be 0.02% only . About 10 ug of purified inhibitor inhibited 0.4 ug of trypsin and chymotrypsin completely. Purified inhibitor did not inhibit starch degradation by salivary amylase. Due to its trypsin inhibitory, chymotrypsin inhibitory properties, heat stability and a low molecular weight it appears to be a Bowman Birk type of inhibitor.

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Cloning, Overexpression, and Crystallization of interacting protein of Myosin 1B from*Entamoeba Histolytica*

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Entamoeba Histolytica, a protozoan parasite is a causative agent of amoebiasis, a global health threat responsible for an estimated 40-50 million cases of invasive colitis or liver abscess leading to approximately 100,000 deaths per year. Owing to its spread through contaminated food and water the incidence is very high in areas with poor sanitation conditions developed nation like India.The mechanism of pathogenesis of infection is an example of host-parasite interaction which involves attachment of trophozoite to the host cell surface followed by cytolysis, invasion of theparasite into the host cells and phagocytosis. Phagocytosis and motility have been shown to play a pivotal role in the establishment of infection. It was demonstratedthat phagocytosis and cell movementcontrols the cytoskeleton, and its regulators, including actins, myosins, kinases, phosphatase and Rho/Rac small GTPases.

Rho guanine nucleotide exchange factor(RhoGEF), the activator of Rho/Rac small GTPases, is also crucial in regulating biological processes where cytoskeletal reorganization involved, including chemotaxis, cytokinesis,tumorigenesis, and phagocytosis. The number of RhoGEFs in the *E. histolytica*and human genomes are also comparable. Sixty-twoRhoGEFs had identified in *E. histolytica*, allbelonging to the Dbl homology (DH) protein family,while 69 RhoGEFsare present in mammals.In the present study, thegeneencodes for EhRhoGEF (putative) protein.A domain of 980 bpwas taken for cloning. The domain sequence belongs to Dbl homology (DH) protein family.A DNA sequence which was encoded the RhoGEF extended domain region protein was synthesized and inserted intopET28b vector. The recombinant plasmid was overexpressed in BL21-DE3 *E.Coli*. After protein overexpression, therecombinant protein was purified by Ni-NTA affinity chromatography and Gel filtration chromatography. The GEFD purified protein confirmed by SDS-PAGE and used for crystallization by hanging drop-vapour diffusion methods.

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Development of ELISA for Medroxyprogesterone acetate using antigen plus bridge heterology

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Medroxyprogesterone acetate (MPA) is a synthetic progestin which is used as long-acting hormonal

contraceptive and as anabolic steroid. MPA is also used as fattening agent in animals for increasing their body mass. The use of excessive MPA poses adverse effect on humans. Therefore, measurement of MPA is essential for monitoring its level in treated patients as well as contaminated food and environment. The present research is on developing heterologous ELISA for MPA to improve sensitivity and specificity. Antigen heterology was used in enzyme conjugate whereas bridge heterology was used in immunogen. Enzyme HRP was conjugated to MPA-3-CMO and other 59 steroid derivatives by using NHS mediated carbodiimide reaction. On the other hand immunogens having different length spacers between MPA and BSA were prepared by using NHS mediated carbodiimide reaction. Binding studies were performed using MPA-3-CMO-BSA- antibody, MPA-3-CMO-EDA-BSA-antibody and MPA-3-CMO-U-BSA antibody with 59 enzyme conjugates. There were 177 combinations. Among these, MPA-3-CMO-BSA antibody has shown binding with 11 enzyme conjugate but good displacement with four enzyme conjugate(P-11-HS-HRP, 17α-OH P-3-CMO-HRP, 17α, 20β-DHP-3- CMO-HRP and DHEA-7-CMO-HRP). Whereas MPA-3-CMO-EDA-BSA has shown binding with 3 enzyme conjugate and only one (Nandrolone-17-HS-HRP) enzyme conjugate has shown good displacement. In comparison to this, MPA-3-CMO-U-BSA has shown both binding and good displacement with only Nandrolone-17-HS-HRP. These six assays that have shown displacement have been further compared for sensitivity and specificity. Among the six combinations, MPA-3-CMO-BSA and P-11-HS-HRP gave the best results of its analytical variables where sensitivity and ED_{50} was 0.07ng/mL and 1.05ng/mL respectively. The assay has shown less than 0.025% cross- reaction with 51 analogous steroids. This assay combination was further evaluated for recovery and precision. The intra- and inter-assay coefficient of variation was <9.55% and the percent recoveries range from 95.83% to 100.81%. Thus, the use of antigen heterologous enzyme conjugate has increased the sensitivity of the assay but introduction of spacers in immunogen lead to compromising with both sensitivity and specificity.

Recovery of bioactive protein from inclusion bodies using mild solubilisation agent

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Insoluble and **inactive** protein aggregates known as inclusion bodies are the major bottleneck in the recovery of bioactive recombinant proteins from *Escherichia coli*. Strong denaturants such as high concentrations of urea and guanidine hydrochloride offer **good solubility** over a wide range of IBs but result in **poor recovery** of bioactive protein. Recent studies showing the presence of native-like secondary structure in inclusion bodies have led to the development of mild solubilization agents like organic solvents, alkaline pH and low concentration of denaturants. These mild solubilization agents offer 5-6 times better recovery of bioactive protein from inclusion bodies than aforementioned strong denaturants.

In this study, we demonstrated the solubilization potential oftrifluoroethanol (TFE),an organic solvent, in nine inclusion body proteins. Different concentrations of TFE with or without low concentration of denaturant were screened to arrive at an optimal ratio. A mixture of 30% TFE with 3M Urea performed the best at solubilizing maximum amounts of protein. Taking human growth hormone (hGH) as a model protein, mode of action of TFE against strong denaturants was investigated using fluorescent spectroscopy and circular dichroism. The results from these techniques suggested the disruption of tertiary structure and stabilization of secondary structure of protein. Furthermore, the cell number based activity assays indicated the presence of fully functional and bioactive protein recovered from TFE solubilized hGH inclusion bodies. We concluded that TFE could be used as a mild solubilization agent to recover maximum amount of bioactive protein from inclusion body proteins.

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Phylogeny and homologous recombination analysis of complete genome sequences of Chikungunya virus

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Chikungunya virus (CHIKV) is the arthropod mediated re-emerging infection of global importance. The outbreaks recent caused by CHIKV have effected public health in wider geographical regions. CHIKV is transmitted to humans mainly by *Aedes aegypti* and *Aedes albopictus* mosquitoes. The clinical features of CHIKV illness in humans is often characterized by sudden onset of fever, headache, fatigue, nausea, vomiting, rash, myalgia and severe arthralgia. The genome of CHIKV consists of a linear, positive-sense, single-stranded RNA of approximately 11.8 kb. The genome contains two open reading frames of about 7424bp (non-structural genes) and 3747bp (structural genes) in length. The complete genome based phylogenetic analyses and recombination of CHIKV have rarely been reported in literature. The present study was thus conceptualized to carry out the phylogenetic analysis on 321 complete genome sequences of CHIKV. The two ORFs were aligned using Bioedit and the tree was generated by MEGA 6 software. The tree was found to cluster in the 3 phylogroups of genotypes namely West African (group I), Asian (group II) and ESCA (East South Central African genotypes (group III). The recombination analysis using the RDP4 software revealed some potential recombinant sites. Hence, this data revealed that currently available phylogeny of CHIKV and homologous recombination do contribute to the evolutionary significance. Further, comprehensive investigations using Bayesian and Network methods will provide insight into the evolutionary trajectories of this re-emerging viral pathogen.

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A Novel Method for Identification of Hemoglobin Variants by Modified Sample Preparation combined with Hemoglobin variant database

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Around 7% of the global population carries an abnormal hemoglobin gene. Over 330,000 infants are born annually with hemoglobinopathies and it is the major cause of morbidity and mortality in early childhood. The treatments rely heavily on the diagnosis of hemoglobin variants. The routine/ conventional techniques used for the identification of mutation in hemoglobin variants have their own limitations like co-migration of variants in electrophoresis and co-elution in HPLC. The WHO (2002) report on Genomics and Health has emphasized on the development of precise molecular techniques for screening of hemoglobin disorders. A sensitive, robust and reproducible method was thus developed to identify single substitution mutations in the hemoglobin disorders from sequence of the entire globin chains. The method was MS compatible and dealt with certain limitations like difficulty in getting complete sequence coverage. Different methods like using organic solvent, digestion with a different proteases and combining results, treating the digestion mixture with 10% acetonitrile prior to incubation and combining the separation power of LC coupled with MALDI MS/MS were tried for standardization and optimization of protocol. Finally we optimized a method using an organic solvent and heat denaturation step prior to digestion resulting in 100% sequence coverage in the β chains and 95% sequence coverage in the α chains. A hemoglobin variant database was created to specify the search and reduce the search time. All the mutations were thus identified using a non-targeted approach. This method could be used in future for regular screening of any single mutation in hemoglobin variants.

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Mycobacterium Tuberculosis: Genome Analysis to Therapeutics Study

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Tuberculosis (TB) is one of the leading causes of infectious disease mortality in the world, with approximately 3 *million* deaths per year. Every second someone in the world becomes newly infected with the causative agent of TB. Although TB was thought to be under control till three decades ago, its re-emergence has raised serious alarms all over the world. One of the major causes of re-emergence of TB has been attributed to the development of drug resistance in M.tb to all the commonly used M.tb drugs i.e. *Isoniazid, Rifampicin, Ethambutol and Streptomycin.* Therefore, a highly effective TB drug(s) has to be developed which can reduce the treatment duration, kill bacilli that might otherwise reactivate later in life and must show activity against multi drug resistant-TB strains. Sequencing the complete genome of M.tb has revolutionized the approach to develop new anti-TB drugs. Structure characterization of microbial enzymes that belong to metabolic pathways is very important for the structure–based drug design since some of these cell-essential proteins may be present in the bacterial genome, but absent in humans. Bacterial cell wall, commonly referred to a Peptidoglycan (PG) layer, is an extensively cross-linked polymer, an integral component for the viability of bacteria, as disruption of cell wall synthesis results in immediate cell lysis, and thus, killing of bacteria. Chemically, PG is a complex heteropolymer composed of long glycan chains that are cross-linked through the short peptide chains. The assembly of the peptide moiety of the PG monomer unit is carried out by the successive addition of *L – alanine*, *D-glutamicacid*, *a diaminopimelic acid (meso-DAP or L-lysine)* and *D-alanine-D-alanine* to *UDP-N-acetylmuramic acid(UNAM)*. These reactions are catalyzed by four *ADP*- forming enzymes, known as the *Mur* ligases (*Mur C*, *D*, *E* and *F*) and these enzymes catalyse similar reactions and shows high structural similarity. As all these *Mur* ligases are essential for the bacterial cell viability and attractive targets for antibacterial chemotherapy. Since all four Mur ligases bind to a substrate which has a large common structural element (the *UDP- Nacetylmuramoyl* group), the potential to develop a single broad-spectrum inhibitor for all the four enzymes seems highly likely. The need for developing a multi-target inhibitor has also been emphasized by various researchers. This approach could also reduce the possible emergence of bacterial resistance, since it is less likely that genetic mutations would occur at multiple targets. Increasing use of *System Biology* approach including gene circuit modeling, gene circuit switching, infringe multiple pathways and bioinformatics techniques would help to achieve this goal.

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Characterization of Weak Forces Involved in Protein–Ligand Binding

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Protein–ligand interactions are of fundamental importance for biological processes. A ligand binds to the protein through many weak, non-covalent interactions such as electrostatic, hydrogen bonds and van der Waals interactions. These week forces play an important role in ligand-protein binding. The binding strength of a ligand on the protein depends upon a precise fit to the surface-exposed amino acid residues. The understanding of molecular recognition processes of ligands and proteins requires a complete characterization of the binding energetic and correlation of thermodynamic data with interacting structures involved. A quantitative explanation of the forces that govern molecular associations needs to determine all thermodynamic parameters, including free energy change of binding (ΔG), enthalpy change (ΔH), and entropy change (ΔS) of binding. Since, ligand-protein interaction provides the fundamental knowledge for the development of structure-based molecular design strategies therefore; a close insight into the ligand-protein binding is of significant interest.

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Defense inducers with potential to protect *Brassica* crops from necrotrophic fungal pathogens

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The productivity of *Brassica* crops is adversely affected by several fungal pathogens among which most destructive is *Alternaria brassicae* and *Alternaria brassicicola*, a necrotrophic fungal pathogen, which is responsible for causing Alternaria blight disease. In India this disease has been reported to cause 30-70% yield losses at different locations. Plant Breeders have so far been unsuceesful in development of disease resistant lines due to lack of source of resistant germplasm within *Brasssica* genus. At present the disease is largely controlled with the application of fungicides which, however, are biohazardous and ecounfriendly. In recent years increasing interest has been shown in the science of chemoinformatics which uses computer and information technologies to design novel defense inducers which could induce *de novo* defense in plants without being toxic to environment, farmers or consumers. Exogenous use of low molecular weight molecules mimicking the action of key defense related hormones such as Jasmonic acid (JA), Salicylic acid (SA) and phytoalexins holds immense application in triggering systemic acquired or induced systemic response against wide range of pathogens. JA mediated signaling pathway plays significant role in development of defense against necrotrophic fungal pathogens. In the present study a chemoinformatics based approaches was utilized for discovery of novel defense inducers which could induce JA mediated defense to prevent necrotrophic mode of colonization by *Alternaria brassicae*. Few mimicking compounds which were more efficient than naturally occurring JA were identified through virtual screening and molecular dynamics simulation studies. Besides, novel phytoalexins were also identified which having ability to bind and block some of the pathogenic proteins of *Alternaria brassicicola*. These molecule(s) can also be used as defense inducers for induction of *de novo* resistance against Alternaria blight to boost the agricultural productivity for securing food and nutritional security of the rapidly growing world population only after further validation through field trials.

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P-100

Protein Misfolding and Human Diseases

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For a protein to function appropriately, it must first achieve its proper conformation and location within the crowded environment inside the cell. The ability of proteins to fold into complex three-dimensional shapes is truly amazing. Given the difficulty of the reaction it is perhaps surprising that many proteins in vivo are unable to fold correctly. These misfolded proteins are generally recognized by the cell's quality control machinery and dealt with through degradation. Multiple chaperone systems are required to fold proteins correctly. Incorrect folding and clumping together of proteins is being recognized as the cause for a growing number of age-related diseases, including Alzheimer's and Parkinson's disease as well as other neurodegenerative disorders. Despite the differences, an emerging paradigm suggests that the

cellular effects of protein misfolding provide a common framework that may contribute to the elucidation of the cell pathology and guide intervention and treatment strategies of many genetic and age-dependent diseases. In this study we present an overview of protein misfolding and examine recent data which is beginning to reveal the mechanisms by which protein aggregates are toxic to cells.

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Recognition of L-Pyroglutamate by crystal structure complex with pyro-glutamyl peptidase I reveals substrate binding mechanism

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Pyrrolidone-carboxylate peptidase (PCP) is an enzyme that catalyses removal of unusual amino acid i.e. pyro-glutamate from N-terminus of peptides and proteins. Two types of enzyme exists in nature, PCP-I and PCP-II. PCPII involves in regulation of variety of hormones such as TRH, LH-RH etc. in eukaryotes by cleaving N terminal blocked peptides which is not cleaved by other conventional exo-peptidases. PCPI is distributed widely among prokaryotes to eukaryotes. In Bacteria, it helps in detoxification of toxins and nutrient assimilation of N terminally blocked proteins and peptides. It also has many commercial and industrial applications such as protein sequencing, biotransformation etc. None of the PCPII structure available to date, although PCPI structure has solved from few organisms but unable to get substrate bound structure. So it is interesting to know how unusual amino acid recognize by active site of enzyme.

We cloned PCP coding DNA sequence under the T7 promoter from *Dienococcusradiodurans R1* (PCPdr), protein was expressed in Rosetta pLysS expression host and protein was purified from supernatant of the lysate through Ni-immobilized metal affinity chromatography. It exists as biological tetrameras identified by gel filtration. The enzyme showed maximum activity at pH 6.5 to 7.5 range and 30^0C temperature.PCPdrcrystallized in I 121 space group with unit cell 118.16, 46.88, 88.88, 90 120.83, 90 at resolution 1.8Å. We solved this structure through molecular replacement. PCPdr refined with 22% R free and 18% R work. PCPdrfound in closed form of structure that is different from open form of other thermophilic bacteria available in PDB database. We also solved PCPdr structure with substrate product (L-pyroglutamate)atresolution 1.55Å. PCPdr withsubstrate product complex structural enlighten mechanism of substrate recognition and product formation.

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Effect of tumor derived factor on Natural Killer cells

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Natural Killer (NK) cells have natural capacity to kill target cell. They do not require prior sensitization with an antigen and is capable of dealing with broad range of virus infected cells. NK cell surface receptor repertoire enables them to distinguish between normal healthy cell and abnormal cells Thus, NK cells play important role in host defence against tumor cells as well as virus infected cell.[1]. However, traditional NK based immunotherapy protocols have shown limited efficacy, possibly due to tumor escape mechanism due to NK cell inhibition [2]. The present study involves the investigation of tumor induced modulation of NK receptor expression. Our study aims to analyse the effect of co-culturing NK cell resistant tumor cell line, P815 with Natural Killer (NK) cells on expression of NK cells receptors profile using flow cytometry. It was found that tumor cells altered the expression of NK receptors that inhibit their function, including Ly49A and Ly49C. Later, we found that theprotein derived from P815 cells may be responsible for the alteration in NK profile. Our findings suggests that protein derived from P815 cells may responsible for tumor cells

escape from NK cell cytolytic activity in mice strain C57BL6, thereby favours tumor progression. In view of this immunosuppressive effect, a new approach might be developed to interruptinhibition of potentially efficient anti-tumor NK cells.

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Suppression of thermal aggregation of a moonlighting protein, Peroxiredoxin6 by Polyamines

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Peroxiredoxin6 (Prdx6) is a ubiquitously expressed bifunctional enzyme with glutathione peroxidase (maximal activity @ pH 7.0) and calcium independent phospholipase A2 activity (maximal activity @ pH 4.0). This pH dependent mutually exclusive dual functioning allows Prdx6 to prevent oxidative stress and maintain phospholipid homeostasis within cells. Interestingly, this moonlighting protein at low pH is quite stable at higher temperatures while at cytosolic pH, it tends to aggregate. In fact, we are reporting for the first time here that Prdx6 starts aggregating at 37 degree Celsius, indicating presence of some stabilizer in the cells which might be responsible for preventing Prdx6 thermal aggregation at physiological temperature. Polyamines are one such cellular small weight co-solutes that have been well-established as protein aggregationmodulators.However, they have been reported to show contrasting effects of stabilizer and destabilizer on different proteins. In the present study we found that in the presence of 50mM mammalian polyamines (putrescine, spermidine and spermine), aggregation of Prdx6 was suppressed. On the basis of the obtained data a mechanism of thermal aggregation of BSA in the presence of polyamines has beenproposed. It is assumed that polyamines under study stabilize the native form of proteinwith a subsequent decrease in the aggregation rate and aggregation onset time. These results implythat polyamines could be the cellular moieties that prevent Prdx6 aggregation within cell. Also, they could be used as molecular additivesfor long-term storage and transportation ofheat-labile Prdx6.

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Analysis of the variations in amino acid sequence of Hsp100 proteins in diverse genotypes of rice (*Oryza sativa* L.)

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Most Hsps act as molecular chaperones which serve to ensure proper assembly and folding of cell proteins under stressful regimes, particularly under high temperature stress conditions. ClpB/Hsp100 proteins show strong heat stress inducibility. Genetic studies in bacteria, yeast and plants have shown that knockout mutation of this protein makes the cells sensitive to heat stress indicating that Hsp100 proteins perform crucial role(s) in survival of the organism under heat stress conditions. ClpB/Hsp100 proteins consist of three distinct regions namely NBD1, NBD2 and long middle domain (M-domain) which separates the two NBDs (with flanking N- and C-terminii). In plants, ClpB/Hsp100 proteins are classified as ClpB-C (cytoplasmic), ClpB-M (mitochondrial) and ClpB-P (chloroplastic) based on their organellar localization. Considering the crucial role of ClpB-C/Hsp100 proteins in governing heat tolerance phenotype, we have interest to score the naturally existing genetic variation(s) in this protein in rice plant. Rice is the staple food crop for large population. Different rice cultivars respond differentially to heat stress. The genome sequences of large numbers of cultivated and wild rice types are available in Genebank (Rice SNP-SEEK databasehttp://snp-seek.irri.org; Gramene database-http://www.gramene.org). From these databases, we have accessed Hsp100 nucleotide and protein sequences of 3000 diverse rice varieties coming from the International Rice Genebank Collection Information System and 10 wild rice varieties. We have noted selective variations in various domains of the ClpB-C/OsHsp100 proteins. These variations possibly cause changes in instability index, aliphatic index, GRAVY and other parameters of protein stability and structure. We wish to showcase these variations and highlight the possible effects of these variations onto the structure, stability and functional attributes of rice Hsp100s.

P-105

Miltefosine resistance reversal in *Leishmania major* by a synthetic peptide

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Cutaneous leishmaniasis is a neglected tropical disease that is endemic in India. Amongst the various available drugs, miltefosine is the only orallyadministereddrug, but its efficiency is decreasing day by day due to the development of resistance by the parasites. This resistance is due to less accumulation of drug inside the parasite, i.e. *Leishmania major*, either by less uptake of drug due to decrease in the activity of P4ATPase-CDC50 complex or by increased efflux of the drug by P-glycoprotein (Pgp). Our study aims at finding parasite specific motifs of these proteins against which small therapeutic peptides can be designed. These peptides would allosterically modulate the activity of mentioned proteins so that the maximum amount of drug (miltefosine) is retained inside the parasite. We have used *insilico* approach through phylogenetic analysis and Statistical coupling analysis to find conserved residues. 3D structures of these proteins were predicted and validated. *Leishmania major* specific motifs in Pgp were identified and peptides were designed against them. The protein-peptide docking shows good binding energy as well as specific binding to the motifs. These small peptides would help in reducing miltefosine resistance thereby designing novel therapeutic approaches for drug resistance tropical pathogens.

P-106

Bone Morphogenetic Proteins in Dentistry

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Bone is unique of all the tissues in the vertebrate organism. When injured, it heals by formation of new bone. Grafting with autogenous bone, guided bone regeneration (GBR), distraction osteogenesis and tissue engineering have been developed to perform intraoral bone augmentation in dentistry. However these procedures have some limitations. In order to overcome some of these limitations, research has been driven towards the use of bioactive molecules to induce local bone formation. A variety of growth factors (GFs) including bone morphogenetic protein (BMP), platelet-derived growth factor (PDGF), and peptides of the parathyroid hormone (PTH) have been tested for local bone regeneration.

Bone morphogenetic proteins (BMPs) are a group of osteoinductive proteins obtained from non-mineralized bone matrix, they are capable of stimulating the differentiation of pluripotent mesenchymal cells to osteoprogenitor cells. They have become a likely treatment option, given their action on regeneration and remodelling of bone lesions and increasing the bone response around alloplastic materials. It may be feasible in the near future for BMP's to replace autologous and allogenic bone grafts.The use of BMP is not only focused on osteogenic regeneration. There are a variety of studiesinvestigating other properties such as periodontal or dental regeneration from the conservative viewpoint. This poster highlights the role of the BMP in bone,periodontal and dental regeneration.

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Distant Leu418 mutation compromises the activity of Mycobacterium tuberculosis isocitrate lyase by modulating its structural flexibility and interaction dynamics

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Mycobacterium tuberculosis Isocitrate lyase (ICL), a potential anti-tubercular drug target, catalyzes the first step of the glyoxylate shunt. It catalyzes the cleavage of isocitrate to succinate and glyoxylate. The aim of this research was to explore the structural alterations induced by L418A point mutation that caused the loss of enzyme activity. In-depth structural analyses were carried out for understanding the influence of L418A mutation using techniques, viz. molecular dynamics, principal component analysis, time-dependent secondary structure, residue interaction network and molecular docking. Since L418A mutation site is structurally far from the active site, it cannot influence the binding of the substrate directly. Our results showed that collective motions, residual mobility, and

flexibility of the enzyme increased upon mutation. The mutated residue changed the global conformational dynamics of the system along with the residue-residue interaction network, leading to a loss of the enzyme activity. The docking results suggest that L418A mutation influenced the binding interactions of the substrate with several residues in the active site of MtbICL. This study provides information on the structural dynamics of MtbICL and highlights the importance of residue level interactions in the protein. Thus, our results may provide significant guidance to the scientific community engaged in designing potent inhibitors targeting MtbICL.

P-108

Proton Transfer Mechanisms, Dipole Moment Indicators and Qm Calculations on Trends in Changes of Proton NMR Chemical Shifts

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It is well known that the water accessible surfaces on protein molecules play a crucial role in determining the protein functions. The metabolic pathways also seem to be affected by the way proton transfer occurs in various situations and water is invariably the predominant constituent in bio fluids. And the proton transfer in water medium is part of the role of proton in protein functions. This aspect of proton transfer has been studied by Proton NMR spectroscopy from the early days. When the computational facilities currently permit study of mutations of huge bio molecules by molecular modelling software and the quantum chemical structure reactivity correlations, the details of the proton transfer which can be studied by Quantum Chemical methods of earlier days still remain unexplored. The computation of proton chemical shifts and the trends in the variations of these NMR shifts as can be viewed by theoretically plotted spectra have a lot to indicate what one observes experimentally in the various contexts. Hence, these kind of trends on the basis of Quantum Chemical calculations would be illustrated; which, even without citing examples from actual biological contexts, can enable the experienced biologists the key inferences for devising experiments.

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Folding mechanism and thermodynamic stability of monomeric GroEL

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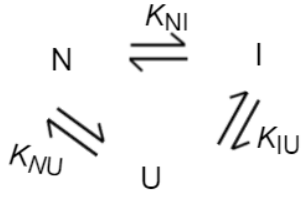
The molecular chaperone GroEL is a tetradecameric protein having identical subunits of 57 kDa. The elucidation of thermodynamic stability parameters for the native GroEL is not feasible as it undergoes irreversible unfolding process. But it is important to quantify the stability parameters for the highly stable GroEL protein. Native GroEL denaturation with urea and dilution in buffer leads to formation of a folded monomeric GroEL. The monomeric nature of this protein was verified by size-exclusion chromatography and native PAGE. Being a properly folded and reversibly refoldable, monomeric GroEL is amenable for the study of protein-denaturant interactions and thermodynamic stability by unfolding transition methods. We present the equilibrium unfolding of monomeric GroEL as studied by urea and heat mediated unfolding processes. The urea mediated unfolding shows two transitions and a single transition in the heat mediated unfolding process. In the case of thermal unfolding, some residual structure unfolds at a higher temperature (70-75ºC). The process of folding/unfolding is reversible in both cases. Analysis of folding/unfolding data provides a measure of various thermodynamic parameters of the monomeric GroEL. The thermodynamic stability parameter in terms of ($\ddot{A}G_{NU}^{H_2O}$) is similar with all the probes (CD and Intrinsic Fluorescence Spectroscopy) used for equilibrium unfolding studies. The proposed mechanism of folding of monomeric GroEL is



It shows that the highly stable monomeric GroEL joining via non-covalent interaction leads to formation of highly stable GroEL protein.

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P-110

Biological synthesis and characterization of Gold nanoparticles of entomopathogenic fungi

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The field of nanotechnology favors the use of various biological units instead of toxic chemicals for the production and stabilization of nanoparticles. Among the many possible bio resources, biologically active products from fungi and yeast represent excellent scaffolds for Nanoparticle production because of their fast growing ability and ease of maintenance. In the current investigation, an environmentally sustainable protocol for the synthesis of gold nanoparticles from entomopathogenic fungi was performed. Twenty two entomopathogenic fungal strains were isolated from insect breeding grounds in Lucknow region, purified and maintained under laboratory conditions. These fungal strains were given accession numbers from IB-01 to IB-22. They were screened for biosynthesis of gold nanoparticles. The synthesized nanoparticles were characterized by UV-VIS spectrophotometry, their size and morphology was confirmed by Scanning Electron Microscopy (SEM). The largest size of nanoparticles was observed in strain no IB-13, IB-19 and IB-20 (22-34nm). Such fungal strains can be subjected to various potential applications in the fields of genomics, immune response enhancement, biosensors, chemical chemistry, control of microorganism and detection and targeted delivery of drugs.

P-111

Middle East Respiratory Syndrome-Coronavirus (MERS-CoV): from Genome to Vaccine

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Middle East respiratory syndrome (MERS) is a viral respiratory disease caused by a novel Coronavirus (MERS CoV) that was first identified in Saudi Arabia in 2012. MERS-CoV is a zoonotic virus that is transmitted from animals to humans. The origins of the virus are not fully understood but, according to the analysis of different virus genomes, it is believed that it originated in bats and was transmitted to camels sometime in the distant past. The disease outbreak caused by Middle East respiratory syndrome Coronavirus (MERS-CoV) that started in the Middle East. To date, WHO has notified 2090 laboratory-confirmed cases from 27 countries, and 730 deaths, mostly in Saudi Arabia. Other cases were reported from Middle East, North Africa, Europe, USA and Asia. The virus has reported an overall case fatality rate 35%. Despite great efforts, licensed vaccines or therapeutics against MERS-CoV are still unavailable. The MERS-CoV spike (S) protein is an important viral antigen which is known to mediate host-receptor binding and virus entry. The protein also induces robust humoral and cell-mediated responses in humans during infection. There has been currently no vaccine is available against MERS-CoV infection to protect human or animals. However researchers are working to develop potential vaccine, based on various platforms. Development of DNA vaccine can be a promising platform against MERS-CoV since it has shown high efficacy in clinical trial phase-1.

P-112

Biophysical and molecular dynamics insight into the interaction of Gallic acid with model transport protein, bovine serum albumin

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The present study reports a comprehensive energetic and conformational aspects of the binding of Gallic acid (GA), a naturally occurring plant phenolic acid mainly used as antioxidant, anti-carcinogenic, antifungal and anti-inflammatory with a model transport protein, bovine serum albumin (BSA) by using isothermal titration calorimetric (ITC) in combination with fluorescence, circular dichroism (CD) and molecular modeling studies. ITC revealed that the binding of GA to BSA is an enthalpy-driven process and electrostatic interactions played a vital role along with hydrogen bond and van der Waals force. The fluorescence result indicates that GA quenches BSA in a static manner. Secondary structure alteration and effect of temperature on native BSA in presence of GA was studied by CD which showed an increase in á-helicity of BSA and the protein is stabilized against thermal unfolding upon binding with GA. In addition, the molecular docking and molecular dynamics (MD) simulation studies show that GA interact with site I of BSA with docking score of -27.636 kJ·mol^{-1} and forms two hydrogen bonds with R256 and 10 hydrophobic contacts with nearby residues. The effect of GA on different parameters, namely root mean square deviation (RMSD), radius of gyration (R_g), root mean square fluctuation (RMSF), and solvent accessible surface area (SASA) has also been studied. This work provides a useful experimental strategy for studying the interaction of GA with BSA, helping to understand the thermodynamics and mechanism of drug binding.

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P-113

Glycation induced transition of collagen from native to aggregated state

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Most proteins have an intrinsic potential of forming aggregates under pathological conditions in variety of diseases ranging from neurodegenerative diseases to diabetes. In more recent times, a lot of importance is given to glycation which has been suggested to play role in long-term complications. Advanced glycation end products (AGEs) formed after Maillard reaction is currently most sought out reason for the pathogenesis of several diseases and hence are the objective of numerous investigations. The purpose of this study was to monitor and characterize the oligomeric aggregates and AGEs of human collagen on addition of glyoxal using ultraviolet, fluorescence, circular dichroism spectroscopy, docking studies, ITC, and microscopy. Collagen was incubated for varying time periods with different concentrations of glyoxal. Molten globule, AGEs and aggregates of collagen were observed at day 6, 18 and 21, respectively. The obtained AGEs were characterized with respect to the extent of side chain modifications forming the Schiff base, the carboxy methyl lysine, and carbonyl content and non-tryptophan fluorescence with emission at 400 and 440 nm, respectively. SEM and TEM confirmed the oligomeric nature of aggregates.

This study focuses on the glyoxal induced aggregates and AGEs formation of collagen, which is one of the most abundant proteins in human body, thus implying its importance. Here we propose that high concentration of glyoxal for extended time results in the formation of harmful aggregates and AGEs of collagen with maximum alteration at 40 mM followed by 20 and 5 mM glyoxal. Glycation results in complex structural changes in collagen and primary damage is caused by aggregation resulting in loss of its native structure. Thus an insight into the mechanistic aspects resulting in the formation of AGEs and aggregates will be useful in many diseases.

P-114

Conformational modulations of a multi-domain protein during the early stages of aggregation

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Switching from the functional form of a particular protein to its non-functional aggregated form has been shown to be the prime cause for a diverse range of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington disease etc. While aggregation is a very complicated process, is made even more complex by the presence of extremely congested cellular interior. Therefore, it is very crucial to perceive in details the mechanism of aggregation where unfolding/misfolding and/or conformational rearrangement plays a critical role. Present work deals with the study of domain movement during the early stages of aggregation of a multi-domain protein BSA (bovine serum albumin) by measuring the energy transfer between the FRET pair tryptophan (Donor; TRP-214) and IAEDANS (Acceptor), labeled at Cys-34 of domain I. Moreover, solvation response of BADAN labeled BSA was also explored to study the behaviors of domain I during the early stages of aggregation. Both the above sets of experiments were performed in dilute media (i.e. buffer only) as well as in presence of 100 and 200 g/L of different macromolecular crowding agents.

In absence of the crowding agents, the energy transfer efficiency at the very early time points was seen to be decreased slightly. However, a steady increase in the efficiency was observed thereafter throughout the course of aggregation, implying initial increase and then decrease in the inter-domain distance. Solvation time of domain I of BSA-BADAN in buffer showed an initial increase followed by steady decrease as a function of the incubation time. Moreover, similar behaviour in the average solvation time of domain I was also observed when the aggregation was carried out in presence of crowding agents. Thus while the increase in FRET efficiency suggests the domains as represented by the FRET pair coming closer, that is, the protein structure becoming more compact as aggregation proceeds, however the decrease in the solvent correlation times imply that even under increased complex formation/ oligomerization, domain I of BSA retains a high degree of flexibility thereby providing insights into how the individual monomer units adjust under such conditions.

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Stability and regulation of Ligand Binding in GsuHbt

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Hemoglobin and myoglobin, that transport and store gaseous molecules, are widely known proteins. Initially it was believed that Hbs as a family of proteins only belonged to mammals and vertebrates. However, the field of hemoglobins has witnessed a revolution and re-evaluation in the last decade. New classes of hemoglobin have been discovered infact, hemoglobins are now known to be ubiquitous and are found in all kingdom of life, that differ from traditional hemoglobins in their primary amino acids sequence structure, fold and probably function as well. Hbs are not totally unknown to algae. Recently, light induced globins in *C. eugametos* were reported (Couture et al) and we had discovered one such globin in *C. reinhardtii* in 2008 and annotated the same (NCBI ID EU095254). These mesophilic algal Hbs (from *C. eugametos* and *C. reinhardtii*), with interesting structural and ligand binding properties. It is expected that an understanding of the properties of extremophilic globins in relation to their mesophilic equivalents would provide a platform for comparative investigation for greater insights into Hbs by using various biophysical tools like Uv-Vis, Flu, CD, FTIR, DSC, Stopped flow and Laser flash photolysis. It would also provide an insight into the factors that dictate stability in Hbs. Such knowledge would then provide scope for engineering stability in other globins and most importantly hemoglobin based artificial blood substitutes. With such objectives, Hb from the thermoacidophilic alga *G. sulphuraria*, named GsuHbt, was characterized and compared with its mutant form, structurally aligned with other known Hbs, Interesting properties were revealed; the Hbs were found to be hexacoordinated and truncated providing novel evolutionary perspective. Thus, the Hb from *G. sulphuraria* thus presenting a novel model system for further insight into Hb folding and stability.

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Identification of Major Redox Modulated Proteins from Brassica juncea Seedlings, and demonstration of differential sensitivity of RuBisCO large and small subunit towards oxidative stress

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The activity of proteins is also regulated by PTMs (post translational modifications) including the thiol–disulphide exchange, a redox modulation. Studiesto analyze reactive oxygen species (ROS), particularly, hydrogen peroxide (H_2O_2) induced changes in the geneexpression are reported in good number, but attempts to detect H_2O_2modified proteins are comparatively very few. Here, an effort was made to identify proteins undergoing thiol–disulphide exchange in Brassicajuncea seedlings crude protein extract using 2D Redox SDS PAGE techniqueafter H_2O_2(10 mM) treatment for30 min. In all, 17 spots showed redox response of which 11 were subjected to MS analysis resulting in the identification 13 proteins. These thirteen redox responsive proteins included 6 Cruciferin subunits, 3 RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase) large subunits, one NLI [Nuclear LIM (Lin11, Isl-1 & Mec-3 domains)] interacting protein phosphatase and Myrosinase. Redox modification ofRuBisCO large subunit was further confirmed by western blotting. The small subunit of RuBisCO was not redox responsive to H_2O_2. All the targets of H_2O_2except NLI interacting protein (which containstwo cysteines) showed oxidation sensitive cysteines byin silico analysis. Interactome analysis was done using STRING (Search Tool for the Retrievel of Interacting Genes/Proteins) database. Interestingly, the interactome of myrosinaseand cruciferin indicated that they may have additionalrole(s) beside their well-known functions in the abiotic stress response and seedlingdevelopment respectively. Cruciferinshowed interactions with stress associated proteinslike 2-cys peroxiredoxin & defensing-like protein 192. Similarly, myrosinase showed interactions with nitrilaseand cytochrome p450 indicating involvement in nitrogenmetabolism and/or hormone biosynthesis. This simpleapproachcan be used to detect and validate major stress mediated redoxchanges in other plants.

P-117

Unraveling the folding pathway of human Fas-activated serine/threonine kinase by urea-induced denaturation

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Fas-activated serine/threonine kinase (FASTK) is a mitochondrial protein which binds to RNA and known to be a component of cytosolic RNA granules. FASTK is generally activated during Fas-mediated apoptosis by phosphorylating a nuclear RNA-binding protein TIA-1, and thus considered as a modulator of apoptosis. For the biophysical characterization of FASTK, cloning, expression and purification was performed. The equilibrium unfolding and conformational stability of the FASTK protein was studied in the presence of urea. The folding and unfolding transitions were monitored with the help of circular dichroism, intrinsic ûuorescence and UV absorption spectroscopy. Analysis of transition curves revealed that the folding of FASTK is a two-state process. Transition curve, the plot of fluorescence (F_{340}), CD signal at 222nm (θ_{222}) and difference absorption coefficient (ε_{278}) versus the molar concentration of urea revealed that the urea induced denaturation is follows a classical two-state process with the midpoint (C_m) value at 3.50±0.1M. To estimate the protein stability, denaturation curves were further analyzed for Gibbs free energy change in the absence of urea (ΔG_D^0) associated with the equilibrium of denaturation exists between native state "! denatured states. This study will be helpful to understand the insight of structural stability of FASTK.

P-118

Role of Proteomics in Dentistry

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Proteins are polypeptides, which are the building blocks of all living beings, and are the main components of the physiological metabolic pathways of cells. All the structural and functional aspects of the body are carried out by protein molecules. These proteins not only play a role in physiological condition of the cell but also in altered manner during pathologic conditions. These altered proteins in diseased conditions are called as biomarkers. Several such biomarkers have been identified in oral diseases. Human oral cavity contains hard and soft tissues and various biofluids including saliva and crevicular fluid. Human saliva contains proteins that can be a revealing tool for disease detection and surveillance of oral health. The discovery of salivary protein markers for human disease detection, in particular for oral cancer, Sjogren's syndrome and many other oral diseases were identified by complete analysis and documentation of the proteomic contents in human whole and ductal saliva. Recent progress in tissue isolation, protein separation, quantification, sequence analysis and structural interaction using proteomic techniques offers great promise for understanding the change in oral physiology and pathology. This paper discusses the role of proteomics in odontogenesis and altered proteins identified in various dental and oral diseases. The knowledge about the role of proteomics in dentistry and the importance of proteomic studies in early diagnosis and prognostic part of oral diseases helps in application of precise and successful treatment.

P-119

Insight into the binding mechanism of cholesterol and their derivative with Mce4A protein

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Tuberculosis is one of the deadliest diseases among the world.*Mycobacterium tuberculosis*is a causative agent of this deadly disease. Mce4A, a protein encoded by *mce4A* gene of *mce4* operon of *M. tuberculosis*helps in cell invasion and long term survival of the bacilli through cholesterol utilization of host thus it could be a drug target of the tuberculosis. In this study, in order to search for potential inhibitors against this protein, isothermal titration calorimetry measurements were performed with the cholesterol and their selected derivatives at pH 6.0 and 25 ± 0.1 °C. ITC has emerged as an excellent technique to characterize the ligand-protein interactions.It gives direct measurement of heat (q) either released or absorbed in molecular binding during gradual titration. The ITC provides the thermodynamic parameters, the enthalpy (—H^0), the reaction stoichiometry (*N*), the binding constant (*K*), and hence the entropy changes (—S^0) in a single experiment. According to this study, all ligands were binds to Mce4A. All these interactions were spontaneous under these conditions. On the whole, all ligand-protein interactions were entropy-driven. This study may further open a new avenue in designing of a novel therapeutic molecules against tuberculosis.

P-120

Characterization and biophysical analysis of Acyl-Co-A binding proteins of *Leishmania major*

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Acyl-Co-A-binding proteins (ACBP's) are ~10kDa proteins, ubiquitously present in all living systems. They are conserved from prokaryotes to eukaryotes and participate in the acyl-Co-A transport and its

intracellular pool formation. ACBP's are also involved in membrane biosynthesis, regulation of gene expression and enzyme activities related to the lipid metabolism. They play a key role in lipid metabolism and bind to medium and long chain acyl-Co-A esters with high specificities and affinities. ACBP gene has been shown to be essential in the blood stream form of *Trypanosoma brucei*, which is closely related to *Leishmania*. Based on genome sequence, there are six acyl-Co-A binding proteins/domains in *L. major*, of which three are free standing proteins, with unknown function. As the amastigote form of *Leishmania* derives its fatty acids from its host, these proteins might have importance in parasite survival. Therefore, we have structurally and functionally characterized the free standing ACBP's of *Leishmania major* using biochemical and biophysical techniques.

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P-121

Protective role of flavonoids on antioxidant enzymes in alloxan induced diabetic mice: A therapeutic approach

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Diabetes is characterized by chronic hyperglycemia leading to various vascular implications accelerated atheroschlerosis, retinopathy, neuropathy and rheumatoid arthritis. Oxidative stress also play central role in diabetes associated complications including increased free radical formation and decreased antioxidant potential. The present study focuses on protective effect of some flavonoids against glycation induced changes in superoxide dismutase (SOD) and catalase. The mice were chemically induced with diabetes using alloxan and animals were sacrificied after 21 days post treatment with experimental drug. Our finding suggests that administration of rutin, quercetin and naringin caused significant reduction in fasting glucose levels. The activity of SOD and catalase were found elevated when treated with flavonoids while untreated group exhibited elevated lipid peroxidation, protein carbonylation and DNA damage. Among the flavonoids investigated the order of specific inhibitory activity was quercetin>rutin>naringin. The decrease in carbonylated proteins and increase in SOD and catalase activity clearly indicate these flavonoids have remarkable potential to restrict the glycation reactions.

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Effect of Molecular and Macromolecular Crowding on Lysozyme

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Intracellular environment is highly crowded due to the restricted amount of free water and presence of different macromolecular crowders like DNA, RNA, protein, nucleic acid, and polysaccharides etc. These crowders show excluded volume effects inside the cell. To understand the structure and stability of protein inside the cell, it is necessary to understand excluded volume effects of a crowder. Here, we report lysozyme as our protein of interest and Dextran 70 as the macromolecular crowder and glucose (an osmolyte) as molecular crowder. In this study, both the crowder are used separetly and in mixture. The structural and thermal stability of the protein in absence and presence of mixture of glucose and dextran is measured by using UV-Vis spectroscopy, circular dichroism, and fluorescence spectroscopy at acidic pH. Our experimental observation suggests no significant amount of perturbation towards the secondary and tertiary structure of the protein but we observed increase of thermal stability under similar conditions. On the other hand, in presence of a combination of crowders, we identify the increase of net thermal stability follows additive property with respect to individual mixture. We can conclude that protein folding in lysozyme under molecular as well as macromolecular crowding is a two state phenomenon which is cooperative in nature

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Role of naturally occurring small molecules in transthyretin-induced familial amyloid cardiomyopathy

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Transthyretin (TTR), a homotetrameric protein responsible for the transport of thyroid hormones and retinol binding protein. TTR is present in serum and cerebrospinal fluid synthesized by liver and choroid plexus in brain. It functions to transport thyroxine and retinol-binding protein. Mutations in TTR disrupt its tetramer and form aggregates or amyloids. The TTR aggregates are known to be internalized specifically by cardiomyocytes and neuronal cells and deposited in the extracellular matrix (ECM). This results in the development of Familial amyloid cardiomyopathy (FAC) or Familial Amyloid Polyneuropathy (FAP) respectively. Some small molecule drugs have already been discovered to have the potential to inhibit TTR aggregation. These include NSAIDS, polyphenols, and herbal drugs etc. However, their use for the therapeutic intervention has been challenged due to (i) high toxicity, practicability to reach target organ, and (ii) low effectiveness. It may be noted that most strategies developed so far has been confined to suppressing TTR aggregates present in blood but not the aggregates deposited in the ECM. Therefore, it is important to develop agents that can suppress TTR aggregation both in blood and tissues. It has been reported that macrophages and fibroblast cells internalize protein aggregates and help them to undergo lysosomal-mediated degradation. Since, macrophages and fibroblast cells are part of the ECM wherein misfolded mutant TTR is deposited, therefore, we intend to screen certain small molecule chaperones that could increase internalization of such mutant protein so as to minimize the aggregate induced cytotoxicity. Thus, these small molecule chaperones will serve as promising candidate for the therapeutic intervention of FAC or FAP.

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QSAR studies on amino-pyrimidineinhibitorsof mutant EGFR

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Aseries of amino-pyrimidine derivatives with their known activity against EGFR mutant inhibitors were taken to derive correlation between biological activity and various descriptors so as to generate a quantitative structure-activity relationship model.We have generated several 2D and 3D QSAR models using different statistical methods: partial least square regression (PLSR), multiple linear regression (MLR) and principal component regression (PCR) methods via stepwise forward-backward, simulated annealing and genetic algorithm variable selection methods.Among various models, we obtained best model with multiple linear regression (MLR) via stepwise forward-backward variable selection method. 2D and 3D QSAR model showed correlation coefficient r^2 (>0.80), predicted correlation coefficient predicted r^2 (>0.60), and cross-correlation coefficient q^2 (>0.60) which implied that the model can be considered stable and accurate. Additionally, high value of F-test provided additional support that the developed model is robust.QSAR analysis revealed the importance of descriptorswhich showedthat chemical group variations significantly influencesits biological activity. 2D-QSAR studies demonstrated that increasing number of electronegative atom, number of N atom separated from Oby 4 bonds, number of H-bond donorand presence of hydrophobic groups would enhance the inhibitory activity of compounds.In addition, 3D-QSAR studies showed that near R1 positionless electronegative group is preferred while near R2 position presence of more bulky groups, electronegative group as well as hydrophobicity is favourable.Thus, the generated model could explain variation in biological activity and can be used to predict inhibitory activity of the new amino-pyrimidine based compounds.

P-125

An analysis approach to identify critical residue involve in Neuroserpin inhibition and polymerization

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Serpin include diverse group of homologous proteins that carefully regulate various physiological processes by targeting serine proteases such as blood clotting, complement activation, apoptosis, fibrinolysis and inflammatory processes. Neuroserpin is a serpin that is expressed mainly in the nervous system and inhibits the serine proteases tPA (tissue plasminogen activator), a reaction that is directly responsible for causing pathological states such as autosomal dominant dementia, epilepsy and seizures. The native fold of neuroserpin is composed of a five stranded β-sheet A, nine α-helices and a mobile helical reactive centre loop (RCL). F helix and β sheet A of neuroserpin move during inhibition, however the nature of this movement is not clearly understood. F helix residues interact with the strand of â sheet A are important in neuroserpin polymerization and inhibition mechanism. *In silico* analysis revealed the importance of interaction pattern between these conserved residues. We prepared a series of cysteine variants of residue involved in interactions between helix F and strands of â sheet A. Secondary structure change of WT NS and NS mutant was observed by Far UV CD spectra. Far-UV CD spectra of both mutant show an increase in á-helical content as compared to WT NS suggesting that these mutation do significantly alter NS secondry structure. A rise in surface hydrophobicity was observed in NS variant due to binding of Bis-ANS to hydrophobic patches. Intrinsic fluorescence analysis of these variants showed change in the tertiary structure of variant N182C which showed a reduction in fluorescence intensity, while W154C on F helix showed significant reduction in fluorescence intensity due to tryptophan removal. Inhibition assay indicated a significant effect of turn as on inhibition and polymerization of neuroserpin.

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In-silico and *in-vitro study* of thymoquinone against lung cancer cells

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Cancer is characterized by the development of abnormal cells and spread/affect to other part of the body. Thymoquinone is a well known to have shown anti-cancer activity in various studies.

Thymoquinone a compound isolated from *Nigella sativa oil*, was able to suppress a range of carcinomas Many potential targets which thymoquinone regulates for its anticancer activities have been identified including p53, p73, STAT3, NF-κB, PPAR-γ and reactive oxygen species (ROS). Herein, we have identified a crucial target TOP1 and CDK1 which played a vital role in cell cycle. *Insilico* have aided to much extent in exploring the inhibition and binding properties of drugs. So we have explored some over expressed protein in tumor cells which are established and recognized. Docking results of thymoquinone against TOP1 and CDK1 have shown potential binding free energy and strong hydrogen bonding. These results motivates us to do *invitro* studies of TOP1-thymoquinone complex. specific *invitro* methods can validiate the in silico results. that helps to claim thymoquinone as potential pharmacophore model for clinical studies.

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Proteometabolomic analyses revealed cultivar-specific acquisition of phytochemicals and nutrients in sweetpotato

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Sweetpotato has long been acknowledged as a significant contributor of global caloric needs, which

continues to be of remarkable economic value, and ranked seventh in terms of annual global production. The adaptability of sweetpotato to an extensive range of agro-ecological conditions besides minimal growth requirements, makes it a favorable crop of great commercial importance. Despite its agronomic merit, the knowledge of nutrient fluxes and phytochemicals in sweetpotato is still fragmentary. To explicate the molecular basis for nutritional diversity, and to exploit the natural genetic differences in sweetpotato, a comprehensive physiochemical and proteomics analyses were performed using two contrasting ecotypes, an orange-fleshed sweetpotato (OFSP) and a white-fleshed sweetpotato (WFSP). While carbohydrate, reducing sugar and total phenolic contents were found to be higher in cv. WFSP, augmented level of total protein, flavonoids, anthocyanins, and carotenoids was observed in OFSP. We aimed to develop proteometabolic profiles of both the cultivars to understand the role of proteins as well as metabolites for nutritional diversity and availability of phytochemicals. Comparative proteomic analysis by 1-DE coupled with mass spectrometry led to the identification of 1541 and 1201 proteins in cv. OFSP and WFSP, respectively, which might play a key role for their functional diversity leading to differential nutrient acquisition. The proteomic analysis further revealed cultivar-specific accumulation of proteins, besides evolutionarily conserved proteins. Metabolome profiling exhibited 148 and 126 metabolites in cv. OFSP and WFSP, respectively. Quantitative proteomic analysis using 2-DE revealed differential expression of 68 proteins in both cultivars, whereas 105 proteins were exclusive to cv. OFSP and 65 proteins to WFSP. Altogether, these results give new insights into molecular basis for differential nutrient and phytochemical availability in tuber crops in particular and plants in general.

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Identifiction and Characterisation of Moonlight Protein from Periplaneta spp.

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Periplaneta spp. are notorious pests with nocturnal habit. They have inherent capability ofadapting to complex environments and can survive in most unhygienic environments. A study has been undertaken to analysetheir ability to withstand the environment based on their brain and hemolymph proteins. It was foundproteins isolated from brain and hemolymph were antibacterial. Two proteins were effective in their antibacterial activity and were found tocontrol multi drug resistant species of S.aureus (MRSA) and Streptococcus spp. (MRSS). These proteins were Identified and characterised using LC-MS/MS and can be categorised as moonlight proteins.

P-129

Molecular modeling, virtual screening and molecular dynamics studies of antimalarial drug targets

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Malaria continues to be a major parasitic disease affecting a large population in tropical and subtropical countries. Despite a substantial progress towards control and elimination of malaria, it continues to have a devastating impact on people's health and livelihoods.The existing drugs are riddled with inadequate implications of cost, safety, drug availability, administration, and resistance. Therefore, there is an imperativeneed to design and develop new and improved low cost drugs with minimal side effects. In the present study, structure based drug design has been performed on two less explored target proteins of malaria, such as, earlytranscribed membrane protein 8 (ETRAMP8) and sodium dependent phosphate transporter (PiT).3D structureprediction of the above proteins of *Plasmodium falciparum* was carried outusing homology modelling and threading methodologies. The overall quality of the model, stereochemical values and non-bonded interactions were evaluated using Procheck and Errat and reliable models developed. The binding site of each protein was predicted using Sitemap. High Throughput Virtual Screening of natural biogenic compound library in ZINC was performed for each of the above proteins and detailed molecular interactions evaluated using Schrodinger software.The binding affinity of the compounds was further studied using "Extra Precision" (XP) algorithm of Glide Docking. The binding affinity was calculated using MMGBSA. The interaction studies using molecular docking and MMGBSA revealed appreciable docking scores and ΔGbind. The stability of the best ligand-protein complexes was further studied using molecular

dynamics simulations. ZINC35464278 for *Pf*ETRAMP8 and ZINC02137523 for *Pf*PiT, constitute the lead compounds that were identified from the above study.Our data generates evidence that the screened compounds indicate a potential binding to the target which can be further validated using cell based assays. Also the analysis of interactions of these compounds can be exploited for better and efficient design of novel drugs against the said targets.

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P-130

Generation of Human LINE1 retrotransposon encoded ORF2p antibody and its detection in human tissues

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Retrotransposons are sequences which move from one place of the genome to another place using RNA as an intermediate and the process is called retrotransposition. The Long Interspersed Element 1 (LINE1 or L1), a type non-LTR retrotransposon is responsible for almost half of the human DNA in mass. It is still actively jumping in different parts of normal human brain and in certain types of cancer. An active L1 is 6 kb in length contains two open reading frames designated as L1-ORF1p and L1-ORF2p. The L1-ORF1p encodes a 40 kDa protein with single stranded nucleic acid binding activities where as ORF2p encodes a 150 kDa protein with demonstrated reverse transcriptase (RT) and endonuclease (EN) activities. Both proteins are critical for the process of retrotransposition. Recently we made the antibody of ORF1pand detected significant ORF1pexpression in different parts of normal human brain and oral cancer samples. Our data suggest that L1 retrotransposon might bevery active in those tissues tested, although we don't know whether L1-ORF2p is also expressing in those tissues.Very little is known regarding humanL1-ORF2p as this protein is notoriously difficult to express. The L1-ORF2p has three distinct domains and N-terminal EN, central RT and C- terminal CCHC type of DNA binding domains. No commercial antibody of L1-ORF2p is available in the market. By employing bio-informatic approaches we have selected few fragmentsfrom human L1ORF2p, cloned in bacterialexpression vector and checked its expression. Among all fragments, only two ORF2p fragments showed significant expression which we are purifying in homogeneity in order to raise antibody against that fragments. The ORF2p antibody if generate will be a valuable reagent to study the biology of human L1 retrotransposon.

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Monitoring kinetics of CAVA aggregation during Guanidine Hydrochloride induced unfolding

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Carbonic anhydrase VA (CAVA) is a mitochondrial enzyme belonging to the á-family of CAs, and plays central role in the metabolism, and are directly associated with obesity and other metabolic disorders. Here, we tried to understand the folding mechanism of CAVA using guanidine hydrochloride (GdnHCl)-induced denaturation at pH 8.0 and 25 °C. The conformational stability was measured from the GdnHCl-induced denaturation study of CAVA monitored by circular dichroism (CD), fluorescence measurement and dynamic light scattering. CD data indicates that CAVA undergoes a transition from β-sheet to α-helix on addition of GdnHCl. The CD spectra of CAVA were recorded at 1, 30 and 90 mins, respectively for 0.5 to 2.5 M GdnHCl. The aggregation of CAVA in different concentrations of GdnHCl with the time was increased but at 2.5 M GdnHCl CAVA immediately undergoes aggregation. The kinetic of CAVA aggregation was measured at 0.5 and 1.0 M GdnHCl for 2 hours. The hydrodynamic properties of CAVA at different concentrations of GdnHCl were also determined. The fluorescent dye 1-anilinonaphthalene-8-sulfonic acid (ANS) binds with these aggregates. Our study suggests that the exposed hydrophobic surface

and/or the disruption of the structural features protecting a β-sheet protein might be the major reason (s) for the high aggregation propensity of non-native intermediate conformation of CAVA.

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Insight into differential GTPase activity between interferon-gamma induced Human Guanylate Binding Protein-1 and -2

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Interferon-gamma inducible large GTPases, human guanylate binding protein -1 and -2 (hGBP-1 and -2) have a unique characteristic to hydrolyze GTP into both GDP and GMP, where GMP and GDP are the predominate products in hGBP-1 and hGBP-2, respectively. Previous studies showed that the helical domain of hGBP-1 is important for tetramerization, which is crucial for both enhanced GMP formation and antiviral activity. The present study aims to understand the significant difference of GMP formation in these two proteins despite high sequence identity (~78%). Unlike hGBP-1, hGBP-2 forms ~80% tetramer, suggesting that this tetramer is not capable of forming higher GMP. To understand it further, we prepared chimeraswhere the helical domain alone and the helical domain plus the intermediate region of hGBP-2 were swapped with that of hGBP-1. These chimeric proteins were defective in tetramerization but showed GMP formation similar to wild-type hGBP-2, indicating that both tetramer and helical domain of hGBP-2 are not essential for GMP formation. Additionally, these data suggest the importance of helical domain in tetramerization. This is consistent with the results of truncated hGBP-2^{1-307}, where the helical domain is deleted. Altogether, these observations suggest that the lower GMP formation in hGBP-2 is mainly because of its globular domain, which could be due to differences in the residues primarily in the guanine-cap region, since for hGBP-1 these are involved in the nucleotide movement required for second phosphate cleavage. We also provide evidence for hGBP-2 tetramer in mammalian cells, which may have a biological significance. We using a series of truncated proteins also identified the sites important for dimerization and tetramerization.

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In silico Structural and dynamical characterization of Probable arabinosyl transferase B (EmbB) mutations in Mycobacterium tuberculosis

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Tuberculosis is one of the most deadly diseases in the world because of its persistent nature.India carries a burden of 2.2 million cases of TB out of a global incidence of 9.6 million as estimated by WHO in 2015.Multidrug-resistant strains of Mycobacterium tuberculosis is aserious threat for controlling and prevention of tuberculosis (TB) and this grim situation has thrown a challenge to the scientific community to understand the drug resistance mechanisms in case of M. tuberculosis.

Arabinosyltransferase B (EmbB) protein, a member of Arabinosyl transferase family (consists of embB, embA, embC proteins) is responsible for the polymerization of arabinose into the arabinan of arabinogalactan and lipoarabinomannan. EmbB is apotential target of ethambutol (ETB). Studies conducted by various researchers have manifested that point mutations within the EMB resistance determiningregion (ERDR) of the protein cause the drug resistance and thus the failure of treatment. But abetter understanding of themechanism of resistance to this antibiotic; help assess the real value of mutations considered to be potential diagnostic markers of drug resistance against EMB and MDR-TB.There are a few reports from isolated studies about mutations in the gene. However, structural and dynamical characterization of the mutations is in backlog because of the unavailability of three dimensional structure of the EmbB protein. This study focuses onmodeling of the three dimensional structure of the protein andto understand the cause and effect of the point mutations (M306V, M306L, M306I, L413P, F330V, D328Y, and a double mutation M306I&R507G) on the conformational and dynamical background.

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A Single Troptophan(W) Residue Modulates the Stability, Packing and Folding of Kunitz (STI) Family of Inhibitors

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β-trefoil fold, consisting of a six stranded β-barrel capped at one end by a lid comprising of another six â-strands,is one of the most important folds among proteins. Important classes of proteins like Interleukins (ILs), Fibroblast Growth Factors (FGFs), Kunitz (STI) family of inhibitors etc. belong to this fold. Their core is packed by hydrophobicresidues contributed by the 6 stranded β-barrel and three β-hairpins that make essential contacts with each other and keep the protein in 'topologically minimal frustrated state'. A complete database analysis of the core residues of the β-trefoil fold proteins presented here identified a conserved tryptophan (W91) residue in the Kunitz (STI) family of inhibitors that projects from the lid and interacts with the bottom layer residues of the barrel. This kind of interactions is unique in Kunitz (STI) family because no other families of β-trefoil fold have such a shear sized residue at the barrel lid junction; suggesting its possible importance in packing and stability. We took WCI as a representative of this family and prepared four cavity creating mutants W91F ,W91M, W91I & W91A. CD experiments show that the secondary structure of the mutants remain indistinguishable with the wild type. Crystal structures of the mutants W91F-WCI, W91M &W91A also show the same feature. However, slight readjustments of the side chains around the site of mutation have been observed so as to minimize the cavity created due to mutation. Comparative stability of these mutants, estimated using heat denaturation CD spectroscopy, indicates that stability of the mutants inversely correlates with the size of the cavity inside the core. Interestingly, although we mutated at the core, mutants show varying susceptibility against tryptic digestion that grossly follow their instability determined by CD. Our findings suggest that theW91 residue plays an important role in determining the stability and packing of the core of WCI. To get further insight in to the folding pathway, Molecular dynamics (MD) simulations were run with a set of total six proteins, including wild type WCI (WT) & five mutants namely W91F, W91M, W91A, W91Hand W91I. Among all of them the last two was generated using in-silico modelling. Our results suggest that truly this W91 residue plays a determining role in stability and folding pathway of Kunitz (STI) family. The mutants are less stable and more susceptible to quicker unfolding at higher temperatures compared to the wild type WCI. These effects are most pronounced for the smallest mutants namely W91H and W91A, indicating more is the cavity created by mutation atW91 position more the proteins becomes unstable.

P-135

Biological protein aggregates as potential micro-particulate catalysts

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Recombinant proteins expressed in bacterial systems often accumulate inside the cytoplasm of the host in the form of insoluble, mostly inactive, aggregates known as inclusion bodies (IBs). Only after the extensive downstream processing is an active form of such proteins achieved, mostly with moderate to low refolding yields. It would be interesting and at the same time fast, efficient and cost effective, if otherwise inactive inclusion bodies of a protein, could be used as such to perform a function. Here we describe the use of IBs of an immunoglobulin domain containing protein, modified at its N-terminal, as a potential aldolase enzyme. The primary structural features of the protein coupled with the micro-particulate nature of its inclusion body form have been investigated in stereoselective heterogeneous aminocatalysis. In the world of asymmetric catalysis, heterogeneous bio-catalysts capable of offering good efficiency and stereoselectivity under environmentally benign conditions, coupled with stability and recyclability, are of great importance. The present study explores the application of inclusion bodies, generated in a bacterial system, as a particulate bio-catalyst for asymmetric transformations.

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Impact of macromolecular crowding on the thermodynamic stability of proteins: A size-dependent approach

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The intracellular environment, where protein folds and performs various functions, differs from the dilute buffer solutions often used during *in vitro* experiments. These dilute buffer solutions have been typically assumed to represent the *in vivo* scenario. However, a major difference lies between the idealized (diluted) conditions and the environment present within cells. The existence of plethora of different macromolecules (overall concentration of 50 to 400 mg ml^{-1}) including proteins, nucleic acids, ribosomes and carbohydrates makes the intracellular milieu extremely crowded by occupying around 10-40% of the total cellular volume restrictingthe space available to each molecule, and such a cellular condition is known as macromolecular crowding. It is, thus,essential to determine how different degrees of macromolecular crowdingalter the biophysical properties of proteins. Therefore, to investigate the effect of macromolecular crowding on the thermodynamic stability of hen egg white lysozyme and apo α-lactalbumin (α-LA), thermal denaturation experimentsof both the proteins were performed in the presence of multiple concentrations of ficoll 70, dextran 70 and 40 (synthetic polymers with different sizes, shapes and composition) at different pH values. It has been observed that with increasing concentration of each crowder, there was an increase in the stabilization of both the proteins which is entropic in nature and the extent of stabilization depends upon the shape and size of the crowder at all the pH values. The stabilization effect was relatively more in the case of dextranthan ficoll. Moreover, the small sized and rod shaped dextran 40 resulted in more stabilization of both the proteins than dextran 70 and ficoll 70 due to its low average molecular mass and large number of molecules leading to highest packing and hence more volume exclusion. Thus, the order of stabilization of proteins wasas follows: Dextran 40 > Dextran 70 >Ficoll 70.

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The structural basis of urea-induced protein unfolding of Integrin-linked kinase

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Integrin-linked kinase (ILK) is an evolutionarily conserved adhesion protein, involved in verities of physiological functions. It is a well known serine-threonine protein kinase that binds to β1 and β3 cytoplasmic domains of integrins and regulates integrin-mediated signal transduction to initiate actin rearrangement, cell proliferation, migration, polarisation, angiogenesis and apoptosis. The overexpression of ILK was reported in various cancer cells where it shows an unique properties as an adaptor or the dysregulation of its kinase activity. To measure the stability parameters of ILK, we carried out urea-induced denaturation at pH 7.4 and 25 °C using three different probes, namely, far-UV CD, near-UV absorption and tryptophan fluorescence. Coincidence of normalized transition curves of all optical properties suggests that unfolding/refolding of ILK is a two-state and reversible process. Analysis of these denaturation curves gave values of 3.90 ± 0.23 kcal mol^{-1}, 5.36 ± 0.24 M, and 0.72 ± 0.08 kcal mol^{-1} M^{-1} for —G_DÚ, (Gibbs free energy change in the absence of urea), Cm (molar urea concentration at the midpoint of denaturation curve), and m (=—ΔGD/—[urea]), respectively. We further performed molecular dynamics simulation of ILK to see the dynamics of protein structure in the presence of different urea concentrations. An excellent agreement was observed between *in silico* and *in vitro* studies.

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Insilico identification of antimalarials against apicoplast

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Apicoplast discovery offers a new direction in the development of novel anti-malarial compounds. Known apicoplast inhibitors prevent the apicoplast development and lead to delayed death type phenotypes. Employing state-of-the-art machine learning techniques, we developed a computational model to predict apicoplast inhibitors. For the study high-throughput chemical screening data (AID-504850), (AID-504848) from PubChem BioAssay database was used. The performance of the models was assessed on various types of binary fingerprints and molecular descriptors. We observed 73.7% sensitivity and 84% specificity along with 81.4% accuracy rate only on 41 PubChem on 48 hrs datasets (AID-504850). Similarly, an accuracy rate of 75.8% was observed for 96 hrs dataset (AID-504848). Furthermore, molecular signatures analysis suggested that compound with at least one heteroatom containing hexagonal ring would most likely belong to the antimalarial category as compared to simple aliphatic compounds. Active compounds preferred a high molecular weight and XlogP but low average value of the topological polar surface area and number of the rotable bonds in comparison to inactive compounds. We also observed that aromatic compounds with oxygen and chlorine atoms are preferred in inhibitors class as compared to sulphur. Present investigation revealed about the important molecular properties along with some preferred structural patterns in inhibitors class which would help us in designing the novel anti-malarial compounds. Based on this study, we developed freely available software to screen large chemical libraries.

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Deciphering teratogenic role of WNT/FZD signalling in humans using *In-silico* approach

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WNT/FZD signalling pathways play significant role in embryonic development, organogenesis, cell fate determination, body axis patterning. This pathway has been proved as a major ground for a variety of congenital and non-congenital diseases. Secretary proteins WNTs show selective binding to cysteine rich domain (CRD) of seven transmembrane proteins called FZDs, and respective WNT-FZD complex wield functional selectivity in various downstream signalling pathways. Binding of WNT ligand and FZD receptor is a complex process in which 19 WNT's and 10 FZD's are involved. Due to this complicacy, there is lack of structural and interactive knowledge of WNT-FZD. *In silico* study may prove beneficial to sum up the data regarding their interactions and their role in development and various diseases. In the present work, molecular models of hWNT3a, hFZD1, hWNT5a, hFZD2, hFZD3 and hFZD6 proteins were constructed by using Modeller9.1 software and their structural complexes were studied by using Hex standalone software. The protein-protein interaction and the amino acids involved in binding of the WNT-FZD complex were examined. Molecular dynamic simulation studies of all the WNT/FZD complexes for 20ns with 25ps timeframe were performed by using Gromacs5.1 software. The present study generated a well defined complex of WNT and FZD proteins and its simulation results indicate the close interactions between WNT and FZD. The energy trajectory of simulation well supports the simulated complex. The trajectory of energy with respect to time due to RMSDs and heavy atoms differed from other contributing parameters and needed further computational time for achieving ideal results. The result has given a good platform for further investigations for deriving putative drug binding sites of WNT/FZD complex and suitable the pharmacophore for ligand search and drug development which will be helpful in treating numerous diseases.

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Fas-activated serine/threonine kinase: Structure and function

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Fas-activated serine/threonine kinase (FASTK) is a mitochondrial phosphoprotein having ability to bind with RNA and is known to be a component of cytosolic RNA granules. Structurally, FASTK contains two conserved kinase domains, subdomain-1 (277–345aa) and subdomain-2 (353–444aa) and a putative RNA-binding domain (RAP domain: 477–535aa) at its C terminal end. FASTK contributes to several biological functions i.e. phosphorylating diûerent substrates, mitochondrial stress sensing, regulation of alternative splicing and is considered as a modulator of apoptosis. We found a strong association of FASTK with TIA-1 which plays significant role in the regulation of alternative splicing by many pre-mRNA. FASTK is involved in diverse human diseases including astrocytoma, asthma and various auto immune diseases including type I diabetes, multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus. Here, we provide comprehensive information on FASTK with a special attention to structure, function, interactions and role in human diseases. The present study will be helpful to understand the structural and functional aspect of FASTK which could establish FASTK as a potential therapeutic agent.

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Virtual high-throughput screening for potential Inhibitors of Microtubule affinity-regulating kinase- 4

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Microtubule affinity regulating kinase 4 (MARK4) is an attractive drug target for the treatment of several diseases such as cancer and Alzheimer's disease, because overexpression of this kinase causes the microtubules to destabilize and the tau proteins to aggregate, thus affecting the normal structure and functioning of the protein and instability of the cell. Hence, inhibition of MARK4 would thus be an appealing therapeutic option in order to treat these disorders. Although, various inhibitors of MARK4 are previously reported showed appreciable affinity and selectivity, but novel chemical compounds with improved pharmacological properties are needed for the development of safe and potent MARK4 inhibitor(s).

Here, in this study, we performed virtual high-throughput screening (vHTS) of natural compounds of ZINC database and NCI diversity Subset-II against MARK4 to find the potent inhibitors of MARK4 and the observations made here in our study may extend an assuring platform for developing novel competitive inhibitors of MARK4.

The three dimensional model of MARK4 (PDB ID: 5ES1) was used for virtual screening against a ZINC database of natural compounds and NCI diversity Subset-II, which contain ~20000 and 1889 compounds respectively. Based on the binding affinities and docking scores, top fifty ligands were selected, showing lowest energy scores which reveal higher binding affinity towards the binding pocket of MARK4. These compounds were further filtered out based on the Lipinski rule of five and hydrogen bonds (HB) analysis to check the specificity towards the binding pocket of MARK4. Furthermore, in-silico pharmacokinetic assays were also carried out to predict the ADME properties, toxicity and carcinogenicity of the compounds. Finally, based on consensus scoring values, critical interactions with active site residues, and predicted activity values, 10 drug-like compounds are proposed as possible potent inhibitors of MARK4.

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Role of Calcium ion in Modulating the functional activity of Sin3, a co-repressor

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Sin3, a global transcription regulator, acts as a molecular scaffold for complex assembly and also as a molecular adapter bridging HDAC (histone deacetylase complex) with an astonishingly large and

diverse group of DNA binding transcription factors and chromatin-binding proteins to bring regulation of genes. Interaction with large group of transcription factors is possible, due to the fact that the Sin3 exhibits conformation flexibility and structural heterogeneity due to presence of six different conserved domains of Sin3 that include the four imperfect repeats of paired amphipathic helices (PAH 1–4), histone deacetylase interaction domain (HID) and highly conserved region (HCR). It has been also known that many of the divalent cations such as Ca^{2+} are involved in the transcription regulation of various genes by modulating the chromatin structure or acting as a cofactor for various transcription factor and nucleoprotein. In an effort to investigate if calcium helps to regulate the structure and function of Sin3, we carried out a few preliminary studies that suggest that (i) Ca^{2+} binds to the PAH domains and this resulted in the increase in the overall steady state level of Sin3 expressed in *S. cereviseae*. (ii) Apparently, Sin3 was found to render functionless due to calcium binding. (iii) HDAC fails to bind to the Sin3 under calcium treatment conditions. The results revealed insights that calcium might help to regulate the function of Sin3. Further studies will be focused on to study mechanism of regulation of functional activity of hSin3B in presence of Calcium ions and understanding the pathway of regulation of genes involved in Sin3 mediated mitochondrial dysfunctioning.

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Determination of the 3-D structure of Porcine Epidemic Diarrhoea Virus (PEDV) viral proteinases and their application in anti-PEDV drug discovery

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The study undertaken aims to determine the 3-D structure of Porcine Epidemic Diarrhoea Virus (PEDV) 3C-like proteinase (3CL) via X-ray Crystallography and find an inhibitor to aid in PEDV drug development. PEDV is a coronavirus that causes severe diarrhoea and a high mortality rate in suckling piglets. There have been significant outbreaks worldwide leading to financial and emotional losses, in 2013 there were several alarming PEDV outbreaks reported in North America. Traditional antiviral measures such as vaccinations have not shown sufficient effectiveness in protecting piglets against PEDV infection, thus the development of specific anti-PEDV drugs has become paramount. PEDV 3CL is critically involved in viral replication and polypeptide processing, and therefore inhibiting its enzymatic activity can halt viral infection at an early stage and prevent PEDV's subsequent spread. To achieve the goal of inhibitor development we will introduce the gene encoding PEDV 3CL into a suitable host for overexpression. We will then purify the overexpressed PEDV 3CL using affinity chromatography, size exclusion, and ion exchange methods. This will be followed by crystallisation, 3-D structure determination, and the development of an in vitro assay for PEDV 3CL activity. Finally, we hope to identify potential PEDV 3CL inhibitors by testing the activity of PEDV 3CL against virtually discovered inhibitors (in silico screening) using the developed in vitro assay. Our working hypothesis is that a structure-based inhibitor design will potentially lead us to the development of highly specific and effective PEDV treatment for infected pigs.

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Effect of 1-butyl-3-methylimidazolium based Ionic Liquids on heme retention of Myoglobin

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Solvent systems are quite essential in storage of proteins: natural or synthesized, and hence are of a great significance industrially. In recent years, room temperature ionic liquids (RTILs) have emerged as fairly competent organic "green" solvent systems against the traditional solvents due to their coveted properties such as non-volatility, non-inflammability, outstanding solvating potential, thermal stability and their tunable properties by suitable choices of cations and anions, along with recyclability. Substantial bodies of research have been established to understand the applicability of these RTILs to the protein storage [1]. The phenomena of macromolecular crowding can help us in formation of a better of such a competent system since the responsible crowding agents are well known to cause an indirect stabilization of the folded state at their low volume fraction, which arises from an

entropic destabilization of the unfolded polypeptide because of compaction due to excluded volume effects [2]. Myoglobin, an intensively studied protein aiding in oxygen binding was chosen for our study. A previous study done from our group has revealed that the crowding agents have significant effects on the heme retention of myoglobin by providing stability to the protein [3]. The current work deals with heme retention of myoglobin in a multitude of Imidazolium based RTIL solvent systems assisted by macromolecular crowding agents. We probed the thermal stability of myoglobin with the normalized soret band absorbance [A_{409}/A_{360}] [3] as the signature to get insights about the heme environment, and protein structure and stability.

The RTILs were found to decrease the capability of myoglobin to retain heme by destabilizing it. The different anionic head of the RTILs were also a significant determinant of protein stability. The osmolyte increased the heme retention while the macromolecular crowding agent showed quite an interesting result which brought up more avenues to be explored in such a diversely occupied but potentially useful solvent system.

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Elucidating the Molecular Interactions of the Lipoate Protein Ligase B in the Endogenous Lipoic Acid Synthesis

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Lipoic acid serves as an important co-factor for key metabolic enzymes such as pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase, branched-chain 2-oxoacid dehydrogenase, acetoin dehydrogenase and glycine cleavage complex H protein[1]. The enzymes involved in lipoylation pathway have gained enough attention lately, owing to their association with pathogenicity. Lipoic acid metabolism has been targeted for drug therapy in *Mycobacterium tuberculosis* and *Plasmodium falciparum*[2]. It has been shown to be essential for the survival and virulence of *Plasmodium falciparum* and *Listeria monocytogenes.* In *Leishmania*, fatty acids are an important energy source in the amastigote stage. Moreover, one of the substrates (Glycine cleavage complex) of the lipoic acid pathway has been associated with lesion formation in *Leishmania major*[3]. In view of the importance of lipoic acid, we propose this study, to understand the interactions of this enzyme with its cognate substrates using NMR, in *Leishmania* and *E. coli*. As of now, there is no information available pertaining to the interactions of this enzyme in any microorganism. Our study helps to map the key amino acid residues involved in interaction.

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Crystal structure and biochemical characterization of a novel prolidase from *Deinococcus radiodurans* R1

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Xaa-Pro iminopeptide bonds in polypeptides are resistant to cleavage by common peptidases. Xaa-Pro

peptidases (XPP) are the binuclear peptidases that hydrolyse Xaa-Pro iminopeptide bond with a trans-proline at the second position of the peptide substrate. XPPs specific towards dipeptides are called prolidases (XPDs, Peptidase-Q; EC 3.4.13.9), while those that prefer longer oligopeptides are called aminopeptidases P (APP, EC 3.4.11.9). Mutations in *Mycobacterium tuberculosis* XPD confer low-level resistance to the antibiotics bedaquiline and clofazimine. XPDs are important commercially in the food and dairy industries for improving taste and texture of the food.Though XPPs are strictly conserved in bacterial and archaeal species, the structural and sequence features that distinguish between prolidases and aminopeptidases P are not always clear. Here, we report 1.4 Å high resolution crystal structure of a novel XPP from *Deinococcus radiodurans* (XPPdr) which is solved by single wavelength anomalous dispersion (SAD) method. XPPdr forms a novel dimeric structure via unique dimer stabilization loops of N-terminal domains such that their C-terminal domains are placed far apart from each other. This novel dimerization is also the consequence of a different orientation of N-terminal domain in XPPdr monomer than those in other known prolidases. The enzymatic assays show that it is a prolidase with broad substrate specificity. Our structural, mutational, and molecular dynamics simulation analyses show that the conserved Arg46 of N-terminal domain is important for the dipeptide selectivity. Our BLAST search found XPPdr orthologs with conserved sequence motifs which correspond to unique structural features of XPPdr, thus identify a new subfamily of bacterial prolidases.

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Green synthesis of maghemite nanoparticles and their application as an adsorbent for the removalof selected heavy metals (Pb, Cd) from fly ash

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Adsorption based removal of heavy metals by iron oxide nanoparticles is widely popular due to its low cost, efficiency and simplicity. This study investigated the applicability of maghemite (γ-Fe_2O_3) nanoparticles for the selective removal of toxic heavy metals from the fly ash prepared wastewater. Maghemite nanoparticles synthesized by Tridax plant extract by sonochemical process and further characterized by instruments like HRTEM, X-ray diffraction XRD, PSA, FESEM-EDS, UV-VIS, FTIR and VSM. Size of maghemite nanoparticles were 40-60 nm by HRTEM. XRD, FTIR and Raman confirmed the material as maghemite and its crystalline nature. SEM-EDS revealed the spherical shape of the particles with size varying from 40-70 nm. TEM and SEM both reveals the aggregation nature of the synthesized magnetic nanoparticles. While the EDS spectra showed the peak for Fe and O along with C and Na. Na is present as an impurity, due to improper washing and C from the plant extract. The magnetization was 1.258 emu analyzed by VSM. Here 20% fly ash solution was used as a source of heavy metals and after every regular time interval an aliquot of sample was collected and analyzed by ICP-OES. Pb was removed up to 85% within two hours only and at 24 hours it was removed up to 96%. While Cd concentration reached below the ND value i.e. 0.01 ppm after two hours.

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Stabilization and counteraction of denaturing effect of urea on structure and stability of Human carbonic anhydrase II (HCAII) by mammalian kidney osmolytes

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In mammalian kidney cells, the urine concentrating mechanism of urea is very high which may denature many kidney proteins. Cells of the renal medulla are exposed under normal physiological conditions to widely fluctuating extracellular solute concentrations, and respond to hypertonic stress by accumulating the organic osmolytes. The mechanism of adaptation in the inner medulla depends on the accumulation of high concentration of certain low molecular weight organic substances, called osmoprotectants. Osmolytes are known to counteract the deleterious effects of urea on structure, stability and function of proteins at 2:1 molar ratio of urea to osmolytes. The kidney protein human carbonic anhydrase II (HCAII) was taken to see the deleterious effect of urea. The thermodynamic stability ($?G^0_{N\leftrightarrow D}$, the Gibbs free energy change in absence of GdmCl associated with the equilibrium, native (N) state $\leftrightarrow$ denatured (D) state) was measured from the GdmCl-induced denaturation curves in the presence of different concentrations of urea and each kidney osmolyte individually and in combination. It was observed that glycine betaine and myo-inositol provide perfect counteraction at 2:1 molar ratio of urea to osmolyte, i.e., denaturing effect of 2 M urea is completely neutralized by 1 M of glycine betaine or myo-inositol, and sorbitol fails to refold urea denatured proteins.

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Onion genomic resource for onion breeding: A genomics and bioinformatics driven resource

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Usually, onion (*Allium cepa* L.) regarded as a crop having an antediluvian coexistence with humans, is by far one of the most challenging plant species to be worked on, especially with respect to delineating its genomic information. It is considered as a plant of immense culinary and medicinal importance. However, there are very limited genomic ventures that have so far been established that could shed light on some of the most captivating aspects of the onion genome. Onion Genomic Resource (OGR), with *three-tier architecture*, is the first of its kind, comprehensive web-resource/database, built in MySQL database and PHP. It houses information of assembly of 20,204 publicly available onion expressed sequence tags (ESTs), available 20,755 assembled transcripts and 249,987 unigenes from *Allium cepa* transcriptome shotgun assembly (TSA) along with their annotations and functional significance. A total of 1915 SSRs from Onion ESTs and 123,282 SSRs from Onion TSA data have been catalogued in OGR. Also, 135,424 SNPs and 11,891 Indels identified from Onion TSA data as well as 15 and 13 SNPs and Indels identified, respectively from Onion ESTs have been put in database. The resource also contains information of gene annotations, linked with KEGG pathways and seven previously reported and one predicted onion miRNAs with their associated targets, which range from cytoplasmic globular proteins to membrane ion channels. More additionally, gene prediction was carried out for the unannotated sequences, of which few were observed to harbor coding regions for novel protein coding genes and transcripts that so far have not yet been identified. The OGR can be useful for onion molecular breeders as well as a valuable tool for confirmation of predicted ORF once the whole genome of onion is sequenced.

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The Crowding Agent Induces Intermediate Conformation in the Heme Protein under Physiological Conditions.

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Proteins function as workhorses in a small factory i.e. in a living cell. Their significance lies in their position for chemical reactions as catalysts, but also acts as a communication means to facilitate the cell to work together with its surroundings via secreted proteins and as structural elements in the cytoskeleton. It is a fact that the entirety concentration of macromolecules within cells is so high that a considerable fraction of the volume is physically unavailable and hence, occupied to extra molecules. Gradually the macromolecular crowding has been gaining renown in recent years as it acts as a sword with double-edge on protein stability and folding, i.e., showing assorted results of having both stabilizing and destabilizing effects on protein folding, structure, stability and function. We studied the effects at different concentrations of polyethylene glycol (PEG-10) on the structure ofheme protein i.e., myoglobin. The tertiary structure was found to be perturbed in the presence of polyethylene glycol, however there was insignificant change in the secondary structure of the proteinanalyzed by far-UV circular dichroism (CD) and Fourier transform infrared (FTIR). It was observed that the PEG 10 induces intermediate conformation in Mb, which holds secondary (α-helical) structure and is compact sufficiently to keep solvent bared hydrophobic clusters in concert, so endow with stronger affinity for ANS (8-anilino-1-naphthalenesulfonic acid) to bind.In addition, isothermal titration calorimetry (ITC) showed strong binding between myoglobin and polyethylene glycol, at the physiological conditions. We hypothesize that polyethylene glycol induces molten globule conformation in myoglobin by interacting with heme group of myoglobin.

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Dual binding mode of antithyroid drug methimazole to mammalian heme peroxidases - structural determination of the lactoperoxidase-methimazole complex at 1.97 Å resolution

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Lactoperoxidase (LPO, EC 1.11.1.7) is a member of the mammalian heme peroxidase family which also includes thyroid peroxidase (TPO). These two enzymes have a sequence homology of 76%. The structure of LPO is known but not that of TPO. In order to determine the mode of binding of antithyroid drugs to thyroid peroxidase, we have determined the crystal structure of LPO complexed with an antithyroid drug, methimazole (MMZ) at 1.97 Å resolution. LPO was isolated from caprine colostrum, purified to homogeneity and crystallized with 20% poly(ethylene glycol)-3350. Crystals of LPO were soaked in a reservoir solution containing MMZ. The structure determination showed the presence of two crystallographically independent molecules in the asymmetric unit. Both molecules contained one molecule of MMZ, but with different orientations. MMZ was held tightly between the heme moiety on one side and the hydrophobic parts of the side chains of Arg255, Glu258, and Leu262 on the opposite side. The back of the cleft contained the side chains of Gln105 and His109 which also interacted with MMZ. In both orientations, MMZ had identical buried areas and formed a similar number of interactions. It appears that the molecules of MMZ can enter the substrate-binding channel of LPO in two opposite orientations. But once they reach the distal heme pocket, their orientations are frozen due to equally tight packing of MMZ in both orientations. This is a novel example of an inhibitor binding to an enzyme with two orientations at the same site with nearly equal occupancies.

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Isolation, purification and characterization of proteins from seeds of *Datura stramonium*

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The worldwide problem of emerging antibiotic resistance has created a need to explore alternative approaches of treatment. Our ability to effectively treat disease is dependent on the development of new pharmaceuticals, and one potential source of novel drugs is traditional medicine. One such approach is based on evaluating herbal compounds for their activity against pathogens causing infections. Antimicrobial compounds present in different plants are active against a large spectrum of Infectious bacterial strains.

The present study was conducted to investigate the antimicrobial property of proteins present in seeds of *Datura stramonium*. The isolated and extracted proteinsample was subjected to Dialysis in which all the salt was removed and then purified using Ion-exchange chromatography to obtain acidic and basic proteins which were also subjected to sodium dodecyl sulphate polyacrylamide gel Electrophoresis (SDS-PAGE) to visualize their different molecular weight. Antibacterial activities of both acidic and basic proteins were determined by the microbiological technique using paper –discs –diffusion method against clinical bacterial isolates namely *E. coli*, *Pseudomonas*and *Klebsiella*. More antimicrobial activity was observed in basic fraction as compared with acidicone.Tissue culturing of medicinal plants is widely used to produce active compounds for herbal and pharmaceutical industries.This work can be very useful in the production of antimicrobial protein by using plant tissue culture technique.

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Synthesis of $AgInS_2$ and $AgInS_2$/ZnS Quantum dots: A Spectroscopic study of interaction and Effect on Enzymatic Activity of Lysozyme.

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Herein, the high quality luminescent $AgInS_2$ (AIS) based AgInS2@ZnS core/shell quantum dots (QDs) have been prepared in presence of glutathione (GSH) as a stabilizer. The interaction of these QDs with lysozyme was comprehensively investigated. The bio-interaction of water soluble quantum dots with lysozyme (Ly) was investigated with spectroscopic techniques such as absorbance, static fluorescence and synchronous ûuorescence spectroscopy. Circular dichroism (CD) spectroscopy was used to analyze the conformational changes in lysozyme during the formation of complexes. Experimental results show that QDs enhances the enzymatic activity of lysozyme in a dose-dependent manner. It was concluded that core only QDs were found to bind poorly to lysozyme, but produced much enhanced enzymatic activity compared to core-shell QDs. It is also attributed that the hydrophobic and electrostatic forces are responsible for the QD-lysozyme interaction. In summary, comprehensive characterization of stability of lysozyme-bound QDs is a necessary step in their potential use as intracellular delivery vectors and imaging agents.