and binding studies using real time kinetics and surface plasmon resonance of these proteins have shown several features that are important for signaling roles of IGF and SPX-40. The detailed study of various structural characterstics of LF and LP have revealed their new functions and have helped in the understanding of the mechanism of their action. IGF is a signaling component secreted during proliferative phase and its structure showed many novel features pertaining to structure and function relationship. We have also studied the structures of C-terminal halves of LF and their various complexes with tight binders of C-lobe and NSAIDs. It indicates a new role of lactoferrin C-lobe as a protector of side effects of NSAIDs. The structural studies of PGRP have revealed that this protein existed in a tetrameric form with four structurally identical molecules giving rise to a potent and versatile binding site.

1279-Pos Board B49

Design of Protein Sequences which Fold into Secondary Structures using an Explicit Solvent Model

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The folding of proteins into helices and beta sheets has been investigated for the past five decades. To date, there has not been a thorough understanding of the physics behind this. Theoretical predictions by Zimm and Bragg give us some information but many studies have revealed contradicting results. For example short chain polyalanine, which according to Zimm and Bragg should form a random coil forms an unusually stable helix in solution and some peptides which have a low propensity for helix formation according to the theory tend to form stable helices. In the current work we seek the helix and beta sheet forming properties of individual short peptides using a simplistic model with explicit solvent through Monte Carlo simulations. The protein back bone and side chains are represented as either hard spheres (hydrophobic) or Jagla particles (hydrophilic) in a Jagla solvent. The helix and beta sheet forming tendencies are studied while varying the chain length, backbone sequence, size of the side group and the strength of the interactions.

1280-Pos Board B50

Is the Amino Acid Dipeptide a Suitable Model for Investigating Structural Preferences in the Unfolded State?

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In view of many observations of local order within unfolded peptides and proteins, intrinsic conformational propensities of individual amino acid residues have been warranted for an understanding of how the conformational distributions in unfolded proteins affect protein folding. Various and sundry experimental and computational techniques have produced conflicting conformational sampling distributions even when similar unfolded model systems were employed. One of the most utilized model systems have been the amino acid dipeptides because the classical definition of the unfolded state was supported by the Ramachandran plot of the alanine dipeptide, which exhibited a nearly homogeneous sampling of sterically allowed conformations. Many studies on amino acid dipeptides have indicated that conformational preferences vary between different amino acids. In order to quantify these differences, we measured the amide I' band profiles of dipeptides in water using infrared absorption, vibrational circular dichroism, and Raman spectroscopy. A conformational distribution model was utilized in order to reproduce all experimental data, which was further constrained by 3J(NHNα) and 1J(C'Cα) coupling constants. For alanine, our results suggest that it samples much less PPII-like conformations in a dipeptide than in GAG. Our experimental results were confirmed by results from Molecular Dynamics simulations. A first analysis of data obtained for other dipeptides also suggest reduced PPII fractions. We tentatively assign this to different hydration shells of blocked and unblocked peptides.

1281-Pos Board B51

Conservation of Complex Knotting and Slipknotting Patterns in Proteins

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The last decade has shown that there are an increasing numbers of known proteins that contain linear open knots or slipknots in their native folded structure. In general, knots in proteins are orders of magnitude less frequent than would be expected for random polymers with similar length, compactness, and flexibility. Explaining why they are so rare is an intriguing question. While analyzing all available protein structures for the presence of knots and slipknots we detected a surprising conservation of complex knotting patterns within and between several protein families despite their large sequence divergence. Since protein folding pathways leading to knotted native protein structures are slower and less efficient than those leading to unknotted proteins with similar size and sequence, the strict conservation of the knotting pattern in some families of proteins indicates an important physiological role of knots and slipknots in these proteins. Although little is known about the functional role of knots, recent studies have demonstrated a protein-stabilizing ability of knots and slipknots. In the slipknots studied here, some of the conserved knotting patterns occur in transmembrane domains of proteins, suggesting that slipknots may stabilize these domains against forces acting during their translocation through protein lined membrane pores.

Protein Aggregates I

1282-Pos Board B52

Is the Prevention for Alzheimer's Already in Your Medicine Cabinet?

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Alzheimer's disease (AD) is characterized by the excessive production of amyloid protein and deposition in senile plaques, which are mainly composed of 1-40 amyloid β-proteins (Aβ40). Various natural mutations of Alzheimer's β-amyloid have been shown to promote an early onset of AD. Recent studies have shown that the Wild Type mutation (Aβ22-35) enhances neurotoxicity of 40-residue Aβ(1-40). Our interest is to use this shorter (14 residue) fragment of the Aβ. Silymarin, a mixture of flavonolignans extracted from the seeds, fruits, and leaves of Milk Thistle, has long been used for the treatment of hepatic disorders, most commonly for liver diseases. Many studies have investigated the inhibitory effects of various flavonoids on Aβ aggregation and neurotoxicity. As a result, silymarin, being a mixture of flavonolignane diastereomers and having already been proven safe for human consumption, might be capable of having a preventative effect against the Aβ-dependent phenotypes of AD.

To identify silymarin as a potential therapeutic agent for the treatment of Alzheimer's disease, various Attenuated Total Reflection Infrared Spectroscopy, ATR-IR,and Ultraviolet Visible Spectroscopy, UV-Vis, assays will be developed to identify and rank whether or not the mixture of flavonolignane diastereomers could inhibit aggregation of Aβ. To carry out this test, Aβ will be incubated with the test compound silymarin at a controlled temperature for a set amount of hours followed by ultrafiltration in order to separate the monomeric Aβ from its aggregates. Aliquots of the ultrafiltrate will be analyzed for monomeric Aβ.

1283-Pos Board B53

Myosin Storage Myopathy Mutations Cause Age Dependent Muscle Degeneration and Cardiac Dysfunction in a Drosophila Model

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Myosin storage myopathy (MSM) is a rare congenital disorder caused by missense mutations in the beta cardiac MHC rod and characterized by subsarcolemmal accumulation of beta cardiac MHC that has a hyaline appearance. These mutations map near to or within the assembly competence domain known to be crucial to filament assembly. We hypothesize that mutations interrupt assembly of coiled-coil rod dimers or thick filaments causing aggregation. We have made a *Drosophila* model for MSM whichcan serve as a powerful model for mechanistic investigations. This *in vivo* model makes it possible to examine interactions between wild-type and mutant full-length myosins, as the majority of mutant alleles are dominant. We introduced the R1845W, L1793P or the E1883K mutation into a *Drosophila* myosin heavy chain transgene and expressed it in the indirect flight/jump muscles. Our studies show a severe reduction in the flight and jump ability of the transgenic flies ($p<0.0001$) in both homozygotes and heterozygotes, with an age-dependent loss of muscle function. Electron and confocal microscopy of the indirect flight muscles of transgenic lines show myofibrillar disarray with large areas of granular/ filamentous inclusions similar to hyaline bodies found in affected humans. In addition, heterozygotes of at least two mutants show restrictive cardiomyopathy phenotypes with arrhythmia that mirrors cardiomyopathy reported in human

subjects. We plan to examine the biophysical defects by studying *in vitro* filament forming ability of the mutant myosin to determine if defective filament formation or instability of the myosin filaments is the basis of MSM. Our study will be an important step in exploring the mechanistic basis of MSM, and identify potential therapeutic approaches by over-expressing myosin chaperones or the autophagic response.

1284-Pos Board B54

Modulating HIV Infection by Controlling the Kinetics of SEVI Fibrillization

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Despite the rapid progress of the AIDS pandemic, HIV is a surprisingly weak pathogen in vitro. The large difference between in vitro and in vivo infection rates suggests that cofactors absent in vitro but essential for the natural transmission of the virus may be responsible for this discrepancy. A recently identified peptide in human semen, $PAP_{248-286}$, has emerged as a candidate for the missing cofactor as it dramatically enhances the infectivity of HIV by up to five orders of magnitude. The $PAP_{248-286}$ peptide fragment has been shown to only induce its synergistic effect with HIV infection when in the form of amyloid fibers. Amyloid formation by $PAP_{248-286}$ into the active SEVI form is a slow process during which it is susceptible to being degraded and inactivated. Therefore, initiators of this fibrillization process would be an indirect cause of the increase in viral infectivity. For this reason, we have searched for possible inhibitors and enhancers of SEVI amyloid formation including metals, lipids, other amyloids, and polyphenolic inhibitors. The effects of the metals are metal specific, with some enhancing kinetics, while others either inhibit or have little effect. High resolution structures of $PAP_{248-286}$ and the green tea extract compound epigallocatechin gallate (EGCG) show binding to the monomer subunit through the lysine side-chains and inhibiting fiber growth, which could prove as an effective preventative measure for HIV infection. In contrast, amyloidogenic fibers produced by *E. coli* are seen to strongly enhance the kinetics of SEVI formation and HIV infectivity, indicating that bacterial infection could enhance the probability of HIV transmission. This phenomenon appears to be quite general and could be an important seeding mechanism for other amyloid proteins.

1) Biophysical. Journal (2009), 97(9), 2474-2483.
2) Biochemica et Biophysica Acta, Biomembranes (2011), 1808(4), 1161-1169.

1285-Pos Board B55

A Statistical Mechanical Approach to Protein Aggregation

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We develop a theory of aggregation using statistical mechanical methods. An example of a complicated aggregation system with several levels of structures is peptide/protein self-assembly. The problem of protein aggregation is important for the understanding and treatment of neurodegenerative diseases and also for the development of bio-macromolecules as new materials. We write the effective Hamiltonian in terms of interaction energies between protein monomers, protein and solvent, as well as between protein filaments. The grand partition function can be expressed in terms of a Zimm-Bragg-like transfer matrix, which is calculated exactly and all thermodynamic properties can be obtained. We start with a two-state treatment that can be easily generalized to three or more states using a Potts model, for which the exactly solvable feature of the model remains. We focus on $n \times N$ ladder systems, corresponding to the ordered structures observed in some real fibrils. We have obtained results on nucleation processes and phase diagrams, in which a protein property such as the aggregate concentration is expressed as a function of the initial protein concentration and inter-protein or interfacial interaction energies. We have applied our methods to Aβ(1-40) and Curli fibrils and obtained results in good agreement with experiments.

1286-Pos Board B56

Fusion Proteins are Able to Form Amyloid Structure

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As shown previously, practically every protein can form amyloid structures in appropriate conditions. It is of great interest to investigate fusion proteins because they are used to produce target proteins. This study is focused on amyloid formation by fusion proteins with thioredoxin and artificial proteins. Aggregation of fusion thioredoxin-albebetin proteins was investigated by overnight incubation at 37°C. This process was monitored by light scattering, fluorescence, and electrophoresis. Properties of the aggregates were determined by far UV CD, electron microscopy, and X-rays diffraction. Trp fluorescence was used to observe changes specifically in thioredoxin. Kinetics of aggregate formation and urea equilibrium unfolding were monitored by Trp fluorescence. Amyloid-like properties of the fusion proteins were revealed using thioflavin T binding and X-rays diffraction. The latter gave 4.5 and 11 Å reflexes typical of the cross-beta structure. Unchanged Trp fluorescence indicated that thioredoxin retained its structure and was not involved in amyloid formation. This fact was also confirmed by urea unfolding. The mode of Trp fluorescence changes was evidence for unchanged thioredoxin properties before and after incubation of fusion proteins at 37°C, as well as in its free state. This means that amyloids were formed by albebetin alone. It should be stressed that unbound albebetin mutants form amyloid structures at 45°C, but when in fusion proteins, the event occurs as early as at 37°C. Such a behavior suggests that in fusion proteins the albebetin structure was destabilized, which might facilitate the amyloid formation. Destabilization of a target protein structure might influence its yield.This work was supported in part by RFBR 09-04-01348, RAS MCB Program.

1287-Pos Board B57

Oxidative Modification of Alpha-Synuclein Modifies its Cytotoxicity

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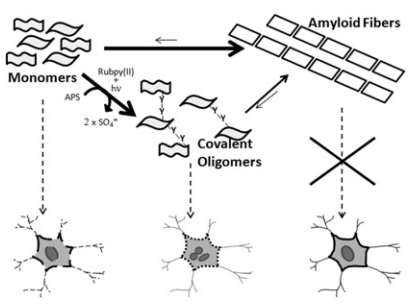
Parkinson's disease is a progressive neurodegenerative disorder, histologically defined by intracellular aggregates of proteins and lipids, associated with selective loss of dopaminergic neurons. The protein alpha-Synuclein (aSyn) is the most abundant component in these aggregates and has been identified as a key player in a series of neurodegenerative diseases. Early intermediates are thought to be the main ''culprits'', in combination with oxidative stress and lipid oxidation. Nevertheless, a comprehensive description of the relationship between protein aggregation and selective neuronal death is still missing. Photo-tunable oxidative modifications of aSyn were achieved using a sensitizer-dependent radical mechanism to generate stable covalent oligomers by specific crosslinking of Tyr residues. Different species were isolated and characterized by a complementary set of techniques, such as spectroscopy, electrochemistry and biochemical characterization that demonstrated the presence of diTyrosine crosslinkings. This led to reduced aggregation *in vitro*, possibly stabilizing more toxic species or avoiding its neutralization into amyloid fibers. Furthermore, modified covalent oligomer showed increased toxicity upon exposure of differentiated SH-SY5Y cells.



These results indicate that oxidative modifications seem to alter the conformation of aSyn and its tendency to aggregate, presumably impairing aSyn functions and promoting the development of its neuropathologies.

1288-Pos Board B58

Role of (htt^{NT}) α-Helix Formation in Huntingtin N-Terminal Fragment Aggregation

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The N-terminal 17 amino acid sequence in huntingtin (htt^{NT}) plays a crucial role in the aggregation of htt N-terminal fragment peptides (htt NTFs). In the current mechanistic model, htt^{NT} segments pack into α-helical bundles to form oligomers that create high local concentrations of appended polyglutamine (polyQ) segments, favoring nucleation of polyQ amyloid. Consistent