The Third International Symposium on

Protein Folding and Dynamics

at NCBS, Bengaluru

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Michael Woodside University of Alberta, Canada
Tae-Young Yoon KAIST, South Korea

8th -11th November, 2016
NCBS, Bengaluru

Registration deadline
July 1, 2016

The symposium will consist of talks by students and postdoctoral fellows, discussions led by faculty members but driven by students, poster sessions, as well as talks by leaders in the field of Protein Folding and Dynamics.

For more information and to apply visit
https://events.ncbs.res.in/event/third-international-symposium-protein-folding-and-dynamics

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Daily Schedule

THIRD INTERNATIONAL PROTEIN FOLDING SYMPOSIUM

Tuesday, November 8, 2016

08:00 – 08:45	**Registration**
08:45 – 09:00	**Welcome by Jayant B. Udgaonkar and C. Robert Matthews**

Session 1 – Functional impact of Protein Folding

Chairpersons: Yvonne Chan and Tyler Harmon

09:00 – 09:05	**Patricia Clark** *Ignite talk – What can protein function tell us about folding mechanisms, and vice versa?*
09:05 – 09:25	**Kathryn Geiger** *Trainee talk - Functional instability in Transcription Activator – Like Effectors (TALEs)*
09:25 – 09:45	**Kapil Dave** *Trainee talk - Effect of fluorescent tags on an enzyme's (PGK) stability*
09:45 – 10:05	**Luke Wheeler** *Trainee talk - Tracing the evolution of peptide binding specificity in the S100 protein family using phage display and deep sequencing*
10:05 – 10:30	**Open discussion**
10:30 – 11:00	**Tea / Coffee Break**

Session 2 – Intersecting theory and techniques

Chairpersons: Yvonne Chan and Tyler Harmon

11:00 – 11:20	**Rohit Pappu** *Tutorial talk – Computational methods to studying protein folding*
11:20 – 11:25	**Lisa Lapidus** *Ignite talk– Getting to a Predictive Theory of Protein Folding*

11:25 – 11:45	**Dipak B. Sanap** *Trainee talk - Interpretation of hydrogen exchange mass spectrometry data using molecular dynamics simulations*
11:45 – 12:05	**Emily Guinn** *Trainee talk - Using single molecule chemo-mechanical unfolding to probe the effect of environmental conditions on protein folding*
12:05 – 12:30	**Abani Bhuyan** *PI talk - Internal friction in protein – what is it?*
12:30 – 13:00	**Open discussion**
13:00 – 14:30	**Lunch Break**

Session 3 – Protein Dynamics

Chairpersons: Yvonne Chan and Tyler Harmon

14:30 – 14:35	**Cathy Royer** *Ignite talk – Pressure for Protein Dynamics: How and Why?*
14:35 – 14:55	**Sureshbabu Nagarajan** *Trainee talk - Dynamics of amyloid beta peptide early aggregation events*
14:55 – 15:15	**Gautam Basu** *PI talk - Direct observation of concerted backbone – side chain dynamics in short linear peptides*
15:15 – 15:35	**Ishita Sengupta** *Trainee Talk -Salt-binding induced oligomerization of the mouse prion protein monitored by real time NMR*
15:35–16:00	**Open Discussion**
16:00 – 16:30	**Tea / Coffee Break**
16:00 – 19:00	**Poster Session – 1**
19:00 – 20:30	**Dinner**

Daily Schedule

THIRD INTERNATIONAL PROTEIN FOLDING SYMPOSIUM

Wednesday November 9, 2016

Session 4 – Cooperativity

Chairpersons: Emily Guinn and Katie Geiger-Schuller

09:00 – 09:05	**Doug Barrick** *Ignite talk – Ising models and protein folding*
09:05 – 09:25	**Kelly Jenkins** *Trainee talk - Exploring folding cooperativity of a repeat protein using high pressure fluorescence and NMR*
09:25 – 09:45	**Santosh Jha** *PI talk - Alternatively packed, near – native states of a multi – domain protein*
09:45 – 10:05	**Tyler Harmon** *Trainee talk - Charge Patterned Sequences Form Helical Structures Through Charge Neutralization*
10:05 – 10:30	**Open discussion**
10:30 – 11:00	**Tea / Coffee Break**

Session 5 – Folding Pathways

Chairpersons: Emily Guinn and Katie Geiger-Schuller

11:00 – 11:20	**Sagar Kathuria** *Tutorial talk– Microfluidic turbulent mixers, time resolved SAXS and folding intermediates of CheY*
11:20 – 11:25	**Tobin Sosnick** *Ignite talk – Alternative methods for characterizing the folding transition state*
11:25 – 11:45	**Krishnananda Chattopadhyay** *PI talk - Subtle changes in the charge distribution at a protein surface can attenuate the competition between early conformational fluctuations and oligomerization*

11:45 – 12:05	**Sri Rama Koti Ainavarapu** *PI talk - Structural and mechanistic insights into the copper – modulated unfolding pathways of azurin*
12:05 – 12:30	**Ranabir Das** *PI talk - Observing a late folding intermediate of ubiquitin at atomic resolution by NMR*
12:30 – 13:00	**Open discussion**
13:00 – 14:30	**Lunch Break**

Session 6 – Consequences of Sequence

Chairpersons: Emily Guinn and Katie Geiger-Schuller

14:30 – 14:35	**Mike Harms** *Ignite Talk – High order epistasis in protein evolution*
14:35 – 14:55	**Yvonne Chan** *Trainee talk - Sequence and structure impose limits on the fitness landscape across three TIM barrel orthologs*
14:55 – 15:15	**Josh Riback** *Trainee talk - Hydrophobicity modulates IDR collapse and stress granule formation*
15:15–15:35	**Nilesh Aghera** *Trainee Talk-Homodimeric E.coli Toxin CcdB (Controller of Cell Division or Death B Protein) Folds via Parallel Pathways*
15:35 – 16:00	**Open discussion**
16:00 – 16:30	**Tea / Coffee Break**
16:00 – 19:00	**Poster Session – 2**
19:00 – 20:30	**Dinner**

Daily Schedule

THIRD INTERNATIONAL PROTEIN FOLDING SYMPOSIUM

Thursday November 10, 2016

Session 7 – The Unfolded State

Chairperson: Gautam Basu

09:00 – 09:30	**Rohit Pappu** *Unfolded states under folding conditions*
09:30 – 10:00	**Satoshi Takahashi** *Dynamics of protein folding studied by single molecule fluorescence measurements at microsecond resolution*
10:00 – 10:30	**Govardhan Reddy** *Protein collapse and folding*
10:30 – 11:00	**Tea / Coffee Break**

Session 8 - Folding Mechanisms - I

Chairperson: Abani K. Bhuyan

11:00 –11:30	**Tobin Sosnick** *The dimensions of the denatured state ensemble (DSE) and the degree to which water is a good solvent*
11:30 -12:00	**Michael Harms** *Physical origins and evolutionary effects of high-order epistasis in genotype-phenotype maps*
12:00 – 12:30	**Gerhard Hummer** *Native and nonnative interactions in protein folding*
12:30 –13:00	**Shachi Gosavi** *Understanding the effect of non-folding factors on the folding landscapes of proteins*
13:00 – 14:15	**Lunch Break**

Daily Schedule

THIRD INTERNATIONAL PROTEIN FOLDING SYMPOSIUM

Special Sponsor Talk (GE)

14:15-14:25 — ***Dev Chandran***
Continuous Chromatography

Session 9 – Membrane Protein Folding

Chairperson: Ravindra Venkatramani

14:30 –15:00 — **Tae-Young Yoon**
Mapping the energy landscape for second-stage folding of a single membrane protein

15:00 – 15:30 — **Paula Booth**
Biological self-assembly – membrane lipids and protein folding

15:30 – 16:00 — **R. Mahalakshmi**
Balancing protein folding and aggregation by interface residues

16:00 – 16:30 — **Tea / Coffee Break**

Session 10 – Folding in the Cell

Chairperson: Raghavan Varadarajan

16:30 –17:00 — **Jane Clarke**
Protein folding –on and off the ribosome

17:00 –17:30 — **Patricia Clark**
Kinetic effects on protein folding in vivo

17:30 – 18:00 — **Lila M. Gierasch**
The allosteric landscape of Hsp70 chaperones—molecular machines that help proteins fold and stay folded

18:00 –18:30 — **Hideki Taguchi**
Conversion of a chaperonin GroEL-independent protein into an obligate substrate, and vice versa

19:00 – 20:30 — **Dinner**

Daily Schedule

THIRD INTERNATIONAL PROTEIN FOLDING SYMPOSIUM

Friday November 11, 2016

Session 11 – Folding Mechanisms II

Chairperson: Santosh K Jha

09:00 – 09:30	**Thomas Kiefhaber** *The dry molten globule and its role in protein folding and function*
09:30 – 10:00	**Catherine Royer** *Pressure-based mapping of protein conformational landscapes*
10:00 – 10:30	**Douglas Barrick** *Folding of repeat and globular consensus proteins*
10:30 – 11:00	**Tea / Coffee Break**

Session 12 – Folding and Misfolding

Chairperson: Krishnananda Chattopadhyay

11:00 – 11:30	**Michael Woodside** *Effects of an anti-prion pharmacological chaperone on the folding dynamics of single PrP molecules*
11:30 - 12:00	**Sheena Radford** *The challenges and opportunities of understanding protein folding and protein misfolding in health and disease*
12:00 – 12:30	**Lisa Lapidus** *Monomer dynamics control the first steps of aggregation and folding*
12:30 – 13:00	**Jooyoung Lee** *Protein structure prediction/determination by global optimization*
13:00 – 14:30	**Lunch Break**

Daily Schedule

THIRD INTERNATIONAL PROTEIN FOLDING SYMPOSIUM

Session 13 - Misfolding and aggregation

Chairperson: Kanchan Garai

14:30 – 15:00	**Sudipta Maiti** *A reaction coordinate for amyloid beta misfolding*
15:00 – 15:30	**Yuji Goto** *Revisiting supersaturation as a factor determining amyloid fibrillation*
15:30 – 16:00	**Samrat Mukhopadhyay** *Water in amyloidogenic intrinsically disordered proteins*
16:00 – 16:30	**Tea / Coffee Break**

Session 14 – Native-State Dynamics

Chairperson: Sri Rama Koti Ainavarapu

16:30 – 17:00	**Tahei Tahara** *Microsecond protein dynamics revealed by two-dimensional fluorescence lifetime correlation spectroscopy*
17:00 – 17:30	**Gilad Haran** Every molecule is special*: enzyme molecules in action*
17:30 – 18:00	**Athi Naganathan** *A general mechanism for mutation-induced destabilization and modulation of allosteric coupling in proteins*
18:00 – 18:30	**Hagen Hofmann** *Observation of nanometer fluctuations in a multifunctional protein complex*
18:30 – 18:45	**Conclusion**
19:00 – 20:30	**Dinner**

ABSTRACTS OF TALKS

Lectures

THIRD INTERNATIONAL PROTEIN FOLDING SYMPOSIUM

What can protein function tell us about folding mechanism, and vice versa?

Patricia Clark

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Functional instability in Transcription Activator-Like Effectors (TALEs)

Kathryn Geiger-Schuller, *Jaba Mitra*[2], *Taekjip Ha*[1,3], *Doug Barrick*[1]

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Transcription activator-like effector proteins (TALEs) contain large numbers of repeats that bind double stranded DNA, wrapping around DNA to form a continuous superhelix. Since unbound TALEs retain superhelical structure, it seems likely that DNA binding requires a significantstructural distortion or partial unfolding. We have used nearest-neighbor "Ising" analysis of consensus TALE (cTALE) repeat unfolding to quantify intrinsic folding free energies, coupling energies between repeats, and the free energy distribution of partly unfolded states. We find a moderate level of cooperativity for cTALEs (stabilizing interfaces combined with unstable repeats), at values intermediate between those of TPR and ankyrin repeat proteins (low and high cooperativity, respectively). Using parameters from the Ising analysis, we have analyzed the distribution of partly folded states as a function of cTALE length. We findthat partly unfolded states with one or more unfolded repeats are energetically accessible.

To quantify the DNA binding of cTALES, and to probe the dependence of DNA binding dynamics on accessibility to partially folded states, single molecule Total Internal Reflection (smTIRF) experiments were performed. Immobilized Cy3-labelled cTALEs incubated with Cy5-labelled dsDNA show an average FRET efficiency of ~0.45. When immobilized protein is incubated with DNA in 200 mM KCl, many on and off events occur in single traces. Long movies were collected for cTALEs incubated with increasing DNA concentrations. FRET of single molecules was fit using Hidden Markov modeling analysis, and cumulative distribution functions (CDF) for on and off dwell times of many molecules were generated. CDFs for on and off events are well fitted by double exponentials, suggesting a complicated kinetic binding process. The fast on phase is DNA concentration dependent, while the slow phase is not DNA concentration dependent, suggesting a fast bimolecular binding event (5.9×10^8 $nM^{-1}*sec^{-1}$) and a unimolecular isomerization (0.15 sec^{-1}). The near-diffusion limited association indicates that association is not limited by the cTALE superhelical structure, and is consistent with rearrangement to a binding-competent (perhaps partly unfolded) state. It is possible that the subsequent slow isomerization involves locking the superhelix onto the DNA. Neither the fast nor slow off phase shows a DNA concentration dependence (1.2 sec^{-1} and 0.13 sec^{-1}), suggesting an isomerization in the bound state as well. Progress on binding kinetics of entropy enhancing variants will be discussed to explore how partly folded states affect DNA binding kinetics.

Environmental Fluctuations and Stochastic Resonance in Protein Folding

Kapil Dave K., *Aram Davtyan*[2], *Garegin A. Papoian*[3], *Martin Gruebele*[4,5], *Max Platkov*[6]

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Stochastic Resonance is the process in which a weak sub-threshold signal is amplified by addition of optimum noise. It is commonly observed in macroscopic biological phenomenon but here we investigated if protein folding is a subject to stochastic resonance. Human cell surface antigen protein VlsE; a two state folder was FRET labelled in order to report on its folding/unfolding. The protein was subjected to periodic thermal modulation below its melting temperature. On top of the periodic thermal modulation, controlled artificial noise was added to monitor the effect of noise on protein unfolding reactions. The phase shift in the donor and acceptor signal as a function of driving frequency revealed the reaction rates. We observed a maximum in the signal as a function of thermal noise confirming that addition of artificial noise was in fact capable of driving the unfolding reaction for the protein. Simulation of a small model-protein, and our experimental result both show clear evidence that correlated noise is a physically and chemically plausible mechanism by which cells could modulate biomolecular dynamics during threshold processes such as signaling.

Tracing the evolution of peptide binding specificity in the S100 protein family using phage display and deep sequencing

Luke Wheeler, *Hiranmayi Duvvuri, Michael J. Harms*

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Substitutions that alter the binding specificity of proteins are essential for the evolution of new biological functions. To directly study this process, we are tracing changes in the total set of partners recognized by proteins over evolutionary time through a combination of ancestral sequence reconstruction, high-throughput phage display, and rigorous biophysical characterization. We are using two members of the S100 protein family, S100A5 and S100A6, as a model of the evolution of specificity. These proteins bind short peptide regions of interaction-partners in a calcium dependent manner, with each protein having distinct biological targets. To determine how this peptide recognition specificity changed over time, we are deploying a quantitative, high-throughput phage display approach that combines four strategies: 1) use of a competitor peptide to target the relevant binding site on the protein; 2) careful experimental and computational treatment of library composition and sampling bias; 3) quantitative, global analysis of binding using a partition function; and 4) incorporation of clustering and machine-learning to identify important determinants of binding specificity. We have used this approach to characterize peptide binding by human S100A5. Machine-learning analyses indicate that binding peptides are distinguished from non-binders by higher rigidity and distinct side chain patterns. We are currently extending the approach to other S100s, including the last common ancestor of all S100A5 and S100A6 proteins. These experiments will allow us to directly measure how binding specificity has changed evolutionarily and aid identification of new biological targets for these proteins.

Lectures

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Computational methods to studying protein folding

Rohit Pappu

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Lectures

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Getting to a Predictive Theory of Protein Folding

Lisa Lapidus

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Interpretation of hydrogen exchange mass spectrometry data using molecular dynamics simulations

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Proteins are flexible entities and undergo conformational changes in solution due to motions ranging from transient local fluctuations to global unfolding. Hydrogen deuterium exchange (HDX) has been a ubiquitously used technique to probe these motions. Generally, HDX has two outcomes: 1) peptide level resolution in terms of observed rates and 2) amplitude (# of amide hydrogens exchanged with deuterium in a peptide fragment).We used all atom molecular dynamics simulations to get atomic insight into HDX data. We calculate mechanical force at atomic level associated with amide bonds and depth to understand the relationship of these atomic forces with HDX data. We find that no single structural property alone can best explain HDX amplitudes. Forces alone give a fairly good picture of amplitude but not the best. The forces in combination with depth best represent HDX amplitudes. Presence of mild concentration of Guanidinium Hydrochloride (~1M) is not significant to affect the network of atomic forces. Although coordination numbers show Guanidinium ions stick to specific parts of the protein, in simulated time, forces do not explain any relationship between HDX rates and timescale (reciprocal of observed rates). Therefore, the exact role of mild denaturant remains unclear. We found few residues showed an open state; consistent with a recent study.

Using single molecule chemo-mechanical unfolding to probe the effect of environmental conditions on protein folding

Emily Guinn, *Bharat Jagannathan, Susan Marqusee*

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In vivo, proteins function in a complex environment where they are subject to stresses like solutes, temperature and strain which can modulate the protein's energy landscape. Perturbing these conditions allows one to explore how proteins respond to changes in environment. This also helps to characterize protein energy landscapes because perturbant effects are related to the structure and energetics of the different protein states along the energy landscape. The effect of perturbants on protein stability is related to the structure of the native and denatured state, while the effect of perturbants on protein kinetics is related to the folding pathway. We have developed a technique called chemo-mechanical unfolding where we combine force and chemical denaturant using optical tweezers. We use chemo-mechanical unfolding as well as temperature and point mutations to explore the denatured state and the parallel pathways proteins fold through.

Lectures

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Internal friction in protein – what is it?

Abani K. Bhuyan

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Internal friction in macromolecules is one of the curious phenomena that control conformational changes and reaction rates. The phenomenon has been known since early studies of polymer viscoelasticity, but its definite meaning and manifestations in protein dynamics appear eclipsed by a large number of studies during the past 20 years that have invoked internal friction to model kinetics and dynamics of the protein folding reaction. Without referring to protein folding, we simply hold that dispersion interactions and London-van der Waals forces between nonbonded atoms are major contributors to internal friction. Root-mean-square volume fluctuations (δV_{RMS}) in a glycerol-smothered protein can be used as a proxy of internal friction. We ansatz that internal friction is related to nonbonded interactions by $f(n) = \sum_{i=0}^{j} f_i n^i$, where the variable n is the extent of nonbonded interactions with f_i coefficients.

Lectures

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Pressure for Protein Dynamics : How and Why?

Cathy Royer

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Characterization of amyloid Aβ oligomers by extrinsic fluorescence probe DCVJ

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Misfolded and intrinsically unfolded proteins are more prone to aggregation in the form of oligomers, protofibrils and fibrils. Oligomers and fibrils are toxic to neuronal cells in vitro, forms ion channels, and disturb the integrity of the synthetic membrane lipids and rafts. Although probes such as thioflavin-T/S and Congo Red binds more specifically to amyloid fibrils, there is a pressing need to identify probes that bind at the early stage of aggregation and reports on the mechanism of their aggregation. Here we show that molecular rotor 9-(dicyano-vinyl)julolidine (DCVJ) binds monomers as well as early aggregates and reports on the change in dynamics as well as oligomeric shape and packing. The binding of DCVJ to $A\beta_{40}$ and $A\beta_{42}$ increases the fluorescence intensity with time around 505 nm, and for the $A\beta_{40}$ there appears a second excimer peak at 575 nm. Further the DCVJ fluorescence at 505nm increases 5-fold greater for $A\beta_{42}$ compared to $A\beta_{40,}$ which suggests the oligomers are more tightly packed in $A\beta_{42}$ than in $A\beta_{40}$. The comparison of DCVJ kinetics profile at 505nm and 575 nm for $A\beta_{40}$ and two mutants show differences that reflect the size and shape of the corresponding oligomers obtained from AFM. The results of this study shows that DCVJ not only binds early aggregates, but also provides valuable information regarding oligomer packing, morphology and mechanism of its formation.

Direct observation of concerted backbone–side chain dynamics in short linear peptides

*Rubin Dasgupta, [1]Sushobhan Chowdhary, [2]Himal K.Ganguly,[1] Samir K. Pal,[2] **Gautam Basu***

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Protein dynamics, characterized by constant fluctuations around the equilibrium structure and dictated by the global free energy surface, greatly influencing its function. Molecular simulations can capture protein dynamics in atomistic detail. Experimental signatures of protein dynamics include B-factors in x-ray crystallography or order parameters in NMR. Protein dynamics results from highly correlated backbone–sidechain motions, as exemplified in protein structure database analyses where strong correlation is observed between populations of sidechain rotamers and the corresponding backbone dihedral angles. However, direct experimental observation of correlated backbone-sidechain dynamics is difficult to observe since that calls for independent observations of backbone and sidechain motions. We have studied a series of tri-peptides with a central Pro residue flanked by Trp at the C-termini(Ac-Xaa-Pro-Trp-NH_2). NMR showed that the cis-conformation of the peptides are stabilized by CH···π interactions, between α-H of Xaa and the side chain of Trp. Taking advantage of the slow backbone cis/trans isomerization kinetics and substantially enhanced cis-content of Xaa-Pro moieties in non-aqueous solvents containing LiCl, we have performed time-resolved fluorescence studies on the Trp residue in the peptides following solvent jump from TFE/LiCl to aqueous buffer. The time resolved fluorescence data fitted well with a three-component model where, upon solvent jump, the relative populations of each component ascribed to Trp rotamers g+ ($\chi1 = +60°$), g- ($\chi1 = -60°$) and t ($\chi1 = 180°$), varied exponentially with a rate constant compatible with NMR-determined cis/trans isomerization rates. The exponential decrease of g+ and t rotamers, known to be associated with the cis-isomer by NMR, and the concomitant exponential increase of the g- rotamer, known to be associated with the trans-isomer by NMR, with a time constant that matches the backbone cis/trans isomerization kinetics, is a direct experimental evidence of concerted nature of backbone–sidechain dynamics. The results also confirm the origin of multiple Trp lifetimes as arising from ground-state conformational heterogeneity ($\chi1$) and the peptide system provide an excellent framework to understand mechanisms governing Trp lifetimes associated with CH···π interactions.

Salt-binding induced oligomerization of the mouse prion protein monitored by real time NMR

Ishita Sengupta, Suhas H. Bhate, Ranabir Das and Jayant B. Udgaonkar

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The prion protein forms β-rich soluble oligomers in vitro at pH 4 in the presence of physiological concentrations of salt. In the absence of salt, oligomerization and misfolding does not take place in an experimentally tractable timescale. While it is well established that a lowering of pH facilitates misfolding and oligomerization of the prion protein, the role of salt remains poorly understood. Here, solution-state NMR was used to probe perturbations in the structure of the monomeric mouse prion protein (moPrP) immediately upon the addition of salt prior to the commencement of the oligomerization reaction. The weak binding of salt at multiple sites dispersed all over the monomeric protein causes an overall non-specific perturbation, albeit weakly of structure throughout the monomeric protein. The only significant perturbation occurs in the loop between helix 2 and helix 3 in and around the partially buried salt-bridge between residues K193 and E195. This perturbation, presumably because of the screening of this key electrostatic interaction, is the earliest detectable change in the monomer before any major conformational change occurs, and appears to constitute the trigger for the commencement of misfolding and oligomerization. Subsequently, the kinetics of monomer loss, due to salt-induced low pH oligomerization of moPrP was monitored at the individual residue level. The oligomerization reaction was found to be rate-limited by association and not conformational change, with an overall reaction order of 2.6, averaged across residues. Not surprisingly, salt accelerated the kinetics of oligomerization, albeit in a non-specific manner, by electrostatic screening of the highly charged monomers at acidic pH. Together, these results allowed a demarcation of the specific and non-specific effects of salt on prion protein misfolding and oligomerization.

Lectures

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Ising models and protein folding

Douglas Barrick

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Probing the cooperativity and stability of a repeat protein PP32 and cavity mutants using high pressure fluorescence and NMR

Kelly Jenkins, Martin Fossat[1,2], Thuy Dao[3], Yi Zhang[1], Zackery White[1], Nyashe Clark[4], Doug Barrick[5] and Catherine A. Royer[1]

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How the primary sequences of proteins define folding cooperativity and free energy landscapes is poorly understood. We examined the cooperativity determinants of PP32, a leucine rich repeat protein with a linear topology which exhibits increasing stability from the N- to the C-terminus. We made mutations in PP32 that created cavities at different locations in the protein. The effect of these cavities on PP32 folding landscapes was probed by high pressure fluorescence and NMR. Pressure denaturation offers an alternative approach to studying protein folding because its effects are due to void volume present in the protein's folded state, rather than the surface area of the unfolded state, leading to a more local effect and a rougher energy landscape with more intermediate states being populated than are observed with chemical denaturants. High pressure NMR studies of PP32 cavity mutants provide equilibrium unfolding data at the residue specific level. These results were used to constrain coarse grained structure-based simulations yielding pseudo free energy profiles and structural characterization of the ensemble at several pressures and temperatures. Pressure-jump (p-jump) studies yielded activation volumes for folding and unfolding, which are defined by the molar volume of the transition state relative to the folded and unfolded states.This high resolution structural and energetic characterization of PP32 cavity containing mutants reveal key features of the PP32 sequence which define its folding cooperativity.

Keywords: High pressure, cooperativity, fluorescence kinetics, LRR proteins

Alternatively packed, near-native states of a multi-domain protein

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Crystal structures of almost all proteins show a static image of the native state in which the hydrophobic side-chain residues are tightly packed inside the protein core, analogous to the crystals of small organic molecules, in order to maximize the strength of the van der Waals (vdW) interactions. It has been difficult to quantify the degree of side-chain conformational heterogeneity in the native state due to the dynamic fluctuations away from the crystallographic structure. In addition, the role and energetic contribution of vdW interactions relative to the hydrophobic effect in stabilizing the native state of proteins is also not clear.

In this study, we show using a battery of high-resolution spectroscopic probes, including fluorescence resonance energy transfer, dynamic fluorescence quenching, red-edge excitation shift and near-and far-UV circular dichroism, that the native-state state ensemble of a multi-domain protein, human serum albumin, contains a significant population of an intermediate state with an alternative side-chain packing arrangement. We show that the intermediate state is slightly expanded than the native-state, has a disrupted inter-domain side-chain packing interactions and the sole tryptophan residue is buried in the hydrophobic environment instead of partially exposed to the solvent. However, it has native-like secondary structure and has a dry core. The sequential population of this native-like intermediatestate during equilibrium unfolding allows us to dissect the energetic contribution of inter-domain side-chain packing interactions in protein stability.

Charge Patterned Sequences Form Helical Structures Through Charge Neutralization

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Many proteins with repeating blocks of oppositely charged residues form long single alpha helices (SAHs).The consensus sequence is a block of four Glu residues followed by a block of four Lys or Arg residues, $(Glu_4(Lys/Arg)_4)_n$. The current working hypothesis is that SAHs are stabilized by i:i+4 salt bridges between opposite charges in consecutive helical turns.We test the merits of this hypothesis to understand the sequence-encoded preference for SAHs and the logic behind the failure of certain atomistic simulations in anticipating the preference for stable SAHs.

In simulations with fixed charges the favorable free energy of solvation of charged residues and the associated loss of sidechain entropy hinders the formation of SAHs. We proposed that alterations to charge states induced by sequence context might play an important role in stabilizing SAHs.We tested this hypothesis using a $(Glu_4Lys_4)_n$ repeat protein and a simulation strategy that permits the substitution of charged residues with neutralized protonated or deprotonated variants of Glu / Lys. Our results predict that stable SAH structures derive from the neutralization of approximately half the Glu residues. These findings explain experimental observations and also provide a coherent rationale for the failure of simulations based on fixed charge models. Large-scale sequence analysis reveals that naturally occurring sequences often include “defects” in charge patterns such as Gln or Ala substitutions. This sequence-encoded incorporation of uncharged residues combined with neutralization of charged residues might tilt the balance toward alpha helical conformations.

Our results highlight the need for developing robust methodologies for constant pH simulationsthat can be applied to sequences with high charge contents. They also highlight the need for generalizing bioinformatics predictors to account for sequence-encoded charge regulation that might influence disorder predictions for sequences with high fractions of charged residues.

Microfluidic turbulent mixers, time resolved SAXS and folding intermediates of CheY

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The process of folding a polypeptide chain from an unstructured random coil in denaturant to a functional 3 dimensional form is a rapid process that is perhaps biased very early on during the initial steps, even as the solvent conditions are transitioning from favoring denaturation to favoring folding. Access to kinetics in the microseconds to milliseconds time scale by multiple spectroscopic probes is crucial to interpreting these initial steps of folding. Time resolved small angle X-ray scattering (trSAXS) is a powerful technique for studying 3 dimensional shapes of proteins as they transition from unfolded to native structures.By interfacing microfluidic devices with powerful x-ray sources we can access timescales in the sub-hundred microseconds range. Structural details determined using trSAXS of the kinetic refolding process of CheY reveal a burst phase in the sub 50-microsecond time-scale, that is perhaps structured around a local-in-sequence cluster of hydrophobic residues.

Lectures

THIRD INTERNATIONAL PROTEIN FOLDING SYMPOSIUM

Alternative methods for characterizing the folding transition state

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Subtle changes in the charge distribution at a protein surface can attenuate the competition between early conformational fluctuations and oligomerization

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Although the primary function of electron transfer is essentially identical for cytochrome c (cytc), its secondary function differs depending on whether its origin is from mammals or from lower organism. The secondary function of cytc involves its interaction with membrane, peroxidase activity and initiation of apoptosis. We report here a detailed experimental and computational study, which aims to understand at molecular level the difference in secondary functions of different cytc proteins. The conformational landscape of cytc has been found to be heterogeneous, consisting of an equilibrium involving compact and an extended conformers, and oligomeric species. Since the determination of relative populations of these conformers is difficult by ensemble measurements, we have used fluorescence correlation spectroscopy (FCS), which can monitor the diffusional and conformational dynamics of a biomolecule with single molecule resolution. These populations are found to vary depending on multiple factors, including protein sources, the presence of membrane or denaturing agents, and their concentrations. The complex interplay between the conformational distribution and oligomerization plays crucial roles in the variation of the pre-apoptotic regulations of cytc observed in different sources. Finally, computational studies reveal that the variation of the charge distribution at the surface and the charge reversal sites may be the key determinants of the conformational stability of cytc.

Structural and mechanistic insights into the copper-modulated unfolding pathways of azurin

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Metalloproteins make up one-third of all known proteins. Characterization of protein energy landscape of metalloproteins is challenging because of the subtle changes imparted by metal binding. Here we present copper induced changes in the mechanical unfolding pathways of azurin through a joint experimental-computational study. Single-molecule force spectroscopy experiments reveal an intermediate along the unfolding pathway of apo-azurin in ~50% of population. Steered molecular dynamics simulations attribute the native and intermediate unfolding transition states (TSs) to the rupture of interactions between the pairs of β-strands, 2B-8 and 4-7, respectively. We show that the binding to copper does not change the first TS of azurin and its mechanical properties, but influences the second TS. The rupture of the 4-7 β-strand pair is delayed in holo-azurin because of constraints imposed by the copper coordination sphere and occurs after the second TS. Our experimental and computational approach extracts unprecedented details along the unfolding landscape for apo- and holo-azurins.

Observing a late folding intermediate of Ubiquitin at atomic resolution by NMR

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The study of intermediates in the protein folding pathway provides a wealth of information about the energy landscape. The intermediates also frequently initiate pathogenic fibril formations. While observing the intermediates is difficult due to their transient nature, extreme conditions can partially unfold the proteins and provide a glimpse of the intermediate states. Here, we observe the high resolution structure of a hydrophobic core mutant of Ubiquitin at an extreme acidic pH by nuclear magnetic resonance (NMR) spectroscopy. In the structure, the native secondary and tertiary structure is conserved for a major part of the protein. However, a long loop between the beta strands 3 and 5 is partially unfolded. The altered structure is supported by fluorescence data and the difference in free energies between the native state and the intermediate is reflected in the denaturant induced melting curves. The unfolded region includes amino acids that are critical for interaction with cofactors as well as for assembly of poly-Ubiquitin chains. The structure at acidic pH resembles a late folding intermediate of Ubiquitin and indicates that upon stabilization of the protein's core, the long loop converges on the core in the final step of the folding process.

Lectures

THIRD INTERNATIONAL PROTEIN FOLDING SYMPOSIUM

High order epistasis in protein evolution

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Sequence and structure impose a specific fitness landscape across three phylogenetically divergent thermophilic TIM barrel proteins

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TIM barrel proteins are encoded by highly divergent sequences, but fold to highly conserved $(\beta\alpha)_8$ structures with varying stabilities and functions. The indole-3-glycerol phosphate synthase (IGPS) TIM barrelprotein, required for tryptophan biosynthesis in bacteria, archaea and yeast, was used to investigate how sequence and structure are correlated with fitness in a competition growth assay. IGPS from the *S. Solfataricus* (SsIGPS, archaea), *T. maritima* (TmIGPS, bacteria), and *T. thermophiles* (TtIGPS, bacteria) thermophiles were subjected to the EMPIRIC assay to assess the change in fitness of yeast transformed with these genes following saturating mutagenesis on the 8 β-stands and the preceding -loops, known to be important for protein stability. We explored the fitness landscapes of these three orthologous TIM barrel proteins to answer two important questions at the intersection of biophysics and evolutionary biology: what are the salient features of a TIM barrel fitness landscape, and whether the fitness landscapes of phylogenetically-divergent proteins are correlated. The fact that the thermophilic proteins we chose for this study were not well adapted to the mesophilic host environment was fortuitous, providing an opportunity to identify beneficial mutations that increase the fitness under sub-optimal growth conditions. Surprisingly, mutations in the αβ-loops distal from the active site increase the fitness of all 3 IPGS orthologs, implying a common allosteric effect. In general, despite the low sequence identity (<35%) of the wildtype (WT) sequences, the fitness landscapes of the three IGPS proteinsare correlated. While part of this result can be explained by the effects of side chain physical chemistry on protein folding and thus fitness, the correlation between fitness landscapes at structurally equivalent positions, irrespective of the WT amino acid, suggests that the structure of the TIM barrel fold imposes a specific fitness landscape. To our knowledge, the conservation of a fitness landscape in sequence and structure space across evolutionary time has not been previously observed.

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Measuring the (good) solvent quality of disordered proteins using a single SAXS measurement.

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Proteins often adopt non-native geometries during their synthesis, folding, transport, and turnover and a significant fraction of the proteome is intrinsically disordered. Here we report the conformational properties of a panel of disordered proteins having sequence compositions typical of folded proteins. Small angle X-ray scattering (SAXS) finds that their conformational ensembles are highly expanded in water. An analysis of the SAXS curves finds that the Flory exponent, which describes the collapse propensity of a chain, is $\nu \sim 0.53$. This value is between the ideal self-avoiding random walk ($\nu = 0.59$) and the theta solvent limit where intra-chain attraction exactly counterbalances excluded volume terms in an otherwise random walk ($\nu = ½$). The addition of moderate amounts of denaturants results in very mild expansion. Simulations generate a molecular form factor applicable to IDPs that can be used to determine the Rg and ν from a single SAXS measurement. These findings along with recent re-evaluation of fluorescence resonance energy transfer (FRET) studies largely resolves the ongoing SAXS/ FRET controversy concerning the behavior of disorder proteins.

Homodimeric *E. coli* Toxin CcdB (Controller of Cell Division or Death B Protein) Folds *via* Parallel Pathways

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The existence of parallel pathways in the folding of proteins seems intuitive, yet remains controversial. We explore the folding kinetics of the homodimeric *E. coli* toxin CcdB using multiple optical probes and approaches. Kinetic studies performed as a function of protein and denaturant concentrations demonstrate that the folding of CcdB is a four-state process. The two intermediates populated during folding are present on parallel pathways. Both form by rapid association of the monomers in a diffusion limited manner and appear to be largely unstructured, as they are silent to the optical probes employed in the current study. The existence of parallel pathways is supported by the insensitivity of the amplitudes of the refolding kinetic phases to the different probes used in the study. More importantly, interrupted refolding studies and ligand binding studies clearly demonstrate that the native state forms in a bi-exponential manner, implying the presence of at least two pathways. Our studies indicate that the CcdA antitoxin binds only to the folded CcdB dimer and not to any earlier folding intermediates. Thus, despite being part of the same operon, the antitoxin does not appear to modulate the folding pathway of the toxin encoded by the downstream cistron. This study highlights the utility of ligand binding in distinguishing between sequential and parallel pathways in protein folding studies, while also providing insights into molecular interactions during folding in Type II TA systems.

Unfolded states under folding conditions

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This talk will present new insights from recent experiments, simulations, and theories regarding protein unfolded states under folding conditions. The emerging integrative picture from a combination of FRET, SAXS, NMR, and computational analysis suggests that the statistical properties of unfolded states under folding conditions are consistent with flexible polymers in theta solvents. This has broad implications for interactions in crowded milieus, for folding-unfolding transitions, and for tilting the balance toward heterogeneous ensembles as is the case with intrinsically disordered proteins (IDPs). These implications will also be discussed with special emphasis on IDPs that undergo multisite phosphorylation.

Dynamics of protein folding studied by single molecule fluorescence measurements at microsecond resolution

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Single molecule fluorescence spectroscopy (SMFS) is a powerful technique for the investigation of the heterogeneity and dynamics of proteins in the unfolded state [1]. However, the time resolution of the conventional method of SMFS is limited to a few milliseconds. We developed a line-confocal microscope, which enables us to obtain the time resolution of 20 microsecond and the observation time of a few milliseconds in the FRET efficiency time series from single proteins [2].

We first investigated the B domain of protein A (BdpA) by doubly labeling the protein with donor and acceptor fluorophores [3]. We obtained traces having high and low FRET efficiencies, assignable to the native and unfolded states, respectively. The distribution assigned to the unfolded state was broad and split into two components near the unfolding midpoint, suggesting the presence of substates. The distribution assigned to the native state showed a gradual shift as the changes in the denaturant concentration, demonstrating a partial swelling of the native state.

We next investigated the unfolding transition of ubiquitin [4]. We established that the covalent coating of the cell surface by MPC polymer is important for the reproducibility of the single molecule data. At 0 M concentration of urea, the FRET distribution assignable to the native state was observed, whose width was narrow and comparable to the theoretical width estimated from the average photon numbers. In contrast, the FRET distributions at 4 and 8 M urea were assignable to the denatured state,and were obviously broader than the theoretical width. Furthermore, the traces assigned to the unfolded state mostly showed constant efficiencies but at different values during the observation time of a few milliseconds. Accordingly, the data suggested that the denatured ubiquitin are heterogeneous and that the each component of the unfolded state possess the lifetime longer than several milliseconds.

We are currently trying several new approaches to increase the time resolution of the FRET efficiency measurements and to combine the solution-mixing device for the line confocal optics. The line confocal detection of SMFS will become a powerful tool to understand the mechanism of protein folding and other dynamics as an experimental counterpart of molecular dynamics calculations.

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Protein collapse and folding

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A fundamental question in protein folding is whether the coil to globule collapse transition occurs during the initial stages of folding (burst-phase) or simultaneously with the protein folding transition. Single molecule fluorescence resonance energy transfer (FRET) and small angle X-ray scattering (SAXS) experiments disagree on whether Protein L collapse transition occurs during the burst-phase of folding. We study Protein L folding using a coarse-grained model and molecular dynamics simulations.The collapse transition in Protein L is found to be concomitant with the folding transition. In the burst-phase of folding, we find that FRETexperiments overestimate radius of gyration (Rg) of the protein due to the application of Gaussian polymer chain end-to-end distribution to extract Rg from the FRET efficiency. The actual decrease in Rg is close to the statistical uncertainties of the Rg data measured from SAXS experiments, which suggest no compaction, leading to a disagreement with the FRETexperiments.

The dimensions of denatured state ensemble (DSE) and the degree to which water is a good solvent

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With the goal of stimulating discussions on issues where opinions may differ within the expert audience,

I will cover three topics:

1. The dimensions of denatured state ensemble (DSE) and the degree to which water is a good solvent.

2. Properties of the transition state ensemble (TSE) including the level of diversity and native-like character.

3. Recent progress in cooperative de novo folding simulations to the native state ensemble (NSE) conducted using a single processor

Physical origins and evolutionary effects of high-order epistasis in genotype-phenotype maps

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A key goal in molecular evolution is to understand why one evolutionary trajectory is taken rather than others. One important determinant of these outcomes is epistasis, where the effect of mutation depends on the presence or absence of other mutations. While pairwise epistasis has been studied extensively, much less is known about high-order epistasis between three or more mutations. If present, high-order interactions could lead to a profound memory effect in evolution, with early mutations strongly shaping evolutionary outcomes. To investigate high-order epistasis, we analyzed a series of experimental genotype-phenotype maps using a robust statistical model. We found extensive high-order epistasis, with statistically-significant interactions between up to six mutations. Computationally removing these interactions dramatically altered outcomes of evolutionary simulations in these maps, revealing that high-order epistasis does indeed shape evolutionary outcomes. We next investigated the origins of this epistasis. Using simple protein lattice models, we then showed that the observed patterns of high-order epistasis arise naturally from the ensemble nature of biomolecular systems. From these results, we propose that high-order epistasis is the rule rather than the exception in molecular evolution, and that this is a natural consequence of the physical properties of biomolecular systems. This implies that, in general, the effect of mutations will be different if they occur early or late in evolution, and that early evolutionary events strongly constrain future ones.

Native and nonnative interactions in protein folding

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The mechanism of a reaction is contained in the dynamic transition pathways between the reactant and product states. The theoretical framework of transition path theory allows us to describe reaction mechanisms in quantitative terms. Within this framework and on the basis of the atomistic molecular dynamics (MD) simulations reported by the group of D.E.Shaw, we can address long-standing questions concerning the mechanism of protein folding [1,2]. For the relatively fast-folding proteins amenable to simulation, we can assess whether protein folding is primarily a build-up of native structure, as assumed in native-centric theoretical and simulation models; or if non-native interactions play an important role, e.g., by stabilizing intermediate structures along the folding pathway, as deduced from earlier simulations. By comparing phi-values calculated directly from the MD trajectories, we can also assess whether the simulations capture the folding mechanism correctly.

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Understanding the effect of non-folding factors on the folding landscapes of proteins.

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My group is interested in how potentially "non-folding" residues (active/binding residues, localization or secretion sequences, etc) modulate the folding landscapes of proteins. Using a few examples, I will show how computational protein folding can be used both to understand folding free energy landscapes and to detect regions of the protein that alter these landscapes. We have also shown that such non-folding regions often aid the function of the protein either directly or indirectly. Finally, our studies have shown that such functional regions modulate folding in a variety of ways which include increased or reduced folding free energy barriers, increased population of folding intermediates and altered folding routes.

Mapping the energy landscape for second-stage folding of a single membrane protein

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Membrane proteins are designed to fold and function in a lipid membrane, yet folding experiments within a native membrane environment are challenging to design. I will talk about our recent efforts where we have applied single-molecule force spectroscopy to study of helical membrane protein folding under native-like bicelle conditions. Applying force using magnetic tweezers, a transmembrane helix protein, *Escherichia coli* rhomboid protease GlpG, is unfolded in a highly cooperative manner, largely unraveling as one physical unit in response to mechanical tension above 25 pN. Considerable hysteresis is observed, with refolding occurring only at forces below 5 pN. Characterizing the energy landscape reveals only modest thermodynamic stability (DG = 6.5 kBT) but a large unfolding barrier (21.3 kBT) that can maintain the protein in a folded state for long periods of time (t1/2 ~3.5 h). The observed energy landscape may have evolved to limit the existence of troublesome partially unfolded states and impart rigidity to the structure.

Biological self-assembly – membrane lipids and protein folding

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Integral membrane proteins adopt diverse structures with different stabilities, dynamics and oligomeric states. It is unknown how much of their folding is dictated by the amino acid sequence and how much by the membrane environment. Successful *in vitro* approaches have been devised to fold helical membrane proteins, in which the mechanical properties of the lipid bilayer modulate the folding processes. This enables new methods to be developed to probe folding mechanisms.

Balancing protein folding and aggregation by interface residues

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The lipid bilayer interface is a complex environment that bridges the hydrophilic solvated exterior with the hydrophobic lipid core. The unique amphipathic chemical milieu presented at the bilayer interface demands equally complex amino acids to be positioned here, in the integral membrane protein. Aromatic amino acids, particularly tryptophan, are usually abundant at the interface and are believed to contribute significantly to the folding and stability of membrane proteins. Surprisingly, however, our estimates of the water-to-interface partitioning free energy reveal that at the interface, tryptophan is thermodynamically destabilizing. Similarly, the thermodynamic stabilization of the thiol side chain is considerably high, despite which cysteine is one of the least abundant amino acids at the bilayer interface. In this talk, I will present a detailed experimental investigation of these anomalies using two transmembrane beta-barrel proteins as model systems. We find that a fine-tuned balance between protein folding and protein aggregation dictates the choice of residues lining the bilayer interface.

Protein folding – on and off the ribosome

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Most eukaryotic proteins contain more than one independently folding domain. Since the local concentration of domains is high, how do natural proteins avoid misfolding? Our single molecule studies of the folding and misfolding of multidomain proteins suggest that the early folding events of multidomain proteins are much more intricate than previously thought. The single molecule data suggest that ~50% of the total population form transient non-native species.

Since co-translational folding is likely to be important to prevent misfolding particularly for multidomain proteins, we are now investigating the folding of multidomain proteins on stalled ribosomes. We conclude that individual domains can fold close to the ribosome, certainly before the following domain has been translated and that this will prevent inter-domain misfolding.

Kinetic effects on protein folding in vivo

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The vast majority of what we know about protein folding has been gleaned from equilibrium studies of the folding of small proteins that unfold and refold reversibly from chemical denaturants such as urea and guanidine hydrochloride. However, many proteins cannot unfold and refold reversibly. Instead, once diluted out of denaturant, these proteins tend to misfold and aggregate. For these proteins, the specific route taken to traverse the folding energy landscape can determine whether the protein will fold to its native state or not. Intriguingly, many of these proteins fold to high yield in vivo, despite the more challenging environment for folding (higher temperature and increased macromolecular crowding). These results have inspired our investigation of specific mechanisms in the cell that can reduce the conformational ensemble of partially folded proteins to favor those intermediates that have a high likelihood of forming the native protein structure. We have found that during translation (synthesis) of an aggregation-prone protein by the ribosome, N-terminal portions of the nascent protein can adopt conformations that increase the yield of native protein, versus the refolding of free, full length protein upon dilution from denaturant. Moreover, the local rate of translation can affect the populations of these co-translational folding intermediates. An analysis of protein coding sequences from 76 fully sequenced genomes indicates that local translation rate may play a role in the folding mechanism for a significant fraction of newly-synthesized proteins.

The allosteric landscape of Hsp70 chaperones—molecular machines that help proteins fold and stay folded

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Members of the Hsp70 family of molecular chaperones perform a wide array of cellular functions under non-stress and stress conditions. These functions range from several that involve interactions with nascent chains–facilitation of folding and avoidance of aggregation, assembly of specific complexes, shepherding polypeptides across membranes, and acting as decision points for protein degradation. Under stress conditions, they protect the proteome from misfolding and aggregation, and partner with disaggregases to break up aggregates. The essential mechanism of nucleotide-modulated substrate binding/release cycles and partnering with J-protein and nucleotide exchange factor co-chaperones is conserved among all Hsp70s. Then how do Hsp70s achieve their functional diversity? Sequence conservation among Hsp70s is relatively high, but nonetheless they fall in sub-families. We are seeking to understand the structural origins of Hsp70 allosteric function well enough that we can correlate sequence variations between Hsp70s with their functional diversity. This talk will describe our findings to date, including the striking observation that the propensity for docking of the nucleotide-binding and substrate-binding domains varies substantially between the bacterial Hsp70, DnaK, and the eukaryotic inducible cytoplasmic Hsp70, HspA1. We are dissecting the key residue contributions to allosteric interfaces that may account for this altered allosteric landscape. Building on our earlier analysis ofthe *E. coli* Hsp70, DnaK (Zhuravleva et al., *Cell* 151: 1296 [2012]), we have identified sites that tune the allosteric landscape in Hsp70s and postulate that these sites represent evolutionary specialization in this ubiquitous chaperone family. In addition, we are dissecting the role of the interdomain linker in coupling the energetics of the interdomain interfaces while also allowing conformational sampling. Lastly, we will describe recent efforts to elucidate the mode of binding of DnaK to a model substrate.

Conversion of a chaperonin GroEL-independent protein into an obligate substrate, and vice versa.

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Chaperones assist protein folding by preventing unproductive protein aggregation in the cell. In Escherichia coli, chaperonin GroEL/GroES (GroE) is the only indispensable chaperone and is absolutely required for the de novo folding of atleast ~60 proteins. We previously found that several orthologs of the obligate GroEL substrates in Urea plasma urea lyticum, which lacks the groEL gene in the genome, are E. coli GroEL-independent folders, despite their significant sequence identities.Here, we investigated the key features that define the GroEL-dependency. Chimera or random mutagenesis analyses revealed that independent multiple point mutations, and even single mutations, were sufficient to confer GroE-dependence on the UreaplasmaMetK. Strikingly, the GroEL-dependency was well correlated with the propensity to form protein aggregates during folding. In addition, we are pursuing the opposite direction, that is, the direction from GroEL-dependent to GroEL-independent folder. The results reveal the delicate balance between GroEL-dependence and independence.

The dry molten globule state and its role in protein folding and function

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A dry molten globule (DMG) state has been observed as a transient intermediate in protein unfolding and as an alternative native state for several proteins. The DMG state has a solvent inaccessible core but shows increased side-chain flexibility and reduced strength of side-chain interactions compared to the native state. Triplet-triplet energy transfer experiments discovered two native states in the villin headpiece subdomain (HP35). Analysing the effect of temperature, pressure and amino acid replacements on the equilibrium between the two native states yielded information on the energetics, dynamics and structural properties of the free energy landscape for native-state dynamics in HP35. The results indicate an equilibrium between a locked native state with low-amplitude motions and a more flexible, unlocked native state, from which local and most likely also global unfolding reactions occur. The properties of the unlocked native state are compatible with a compact DMG, whereas the transition state for interconverting between the two native state represents an expanded DMG. The structural properties of the two native states and their role in protein folding and function will be discussed.

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Pressure-based mapping of protein conformational landscapes

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A major challenge in protein science is obtaining detailed structural, dynamic and thermodynamic descriptions of protein conformational ensembles. One very useful approach towards this goal is to use pressure perturbation, combined with state-of-the-art experimental and computational methods. Pressure perturbs protein stability because the less structured conformations of proteins are also those that occupy the smallest molar volume. This is because the more structured forms ofproteins harbor internal solvent-excluded voids that are eliminated upon disruption of the structure. Hence, unlike the effects if denaturants, which are based on changes in surface area of the unfolded form, pressure effects arise due to very specific properties of the folded forms of proteins. Pressure acts locally on protein folded structures. Because of this mechanism, intermediate states are much more likely to be observed in pressure perturbation experiments. Coupling pressure with structural approaches such as multi-dimensional NMR, provides information about the structure of the ensemble at nearly every residue. These structural constraints can be used to computationally generate structural models of the ensemble. Another advantage of pressure is that it considerably slows folding relaxation rates due to positive activation volumes for folding. This allows for residue specific folding kinetics using different NMR approaches. Both the equilibrium and kinetic studies reveal complexities in folding that could not be observed using alternate approaches.

Folding of repeat and globular consensus proteins

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Linear repeat proteins provide an excellent framework for studying protein stability and cooperativity. Although the interactions stabilizing repeat proteins are the same as those stabilizing globular proteins, repeat proteins have rough translational symmetry over 20-50 residues, reducing structural heterogeneity on longer length-scales. Moreover, the linear architecture of repeat proteins permits the removal and addition of the repeated structural element, facilitating nearest-neighbor (1D-Ising) of folding, which provides a unique thermodynamic measure of cooperativity in folding. However, the repeat units of naturally occurring repeat proteins show significant sequence variation from, complicating analysis.

To eliminate this sequence variation and to improve the accuracy of nearest-neighbor analysis, we and other labs have found that highly stabilized proteins with identical repeats can be designed, using consensus information from multiple sequence alignments. Recent successes with this approach will be presented, along with generalities and variations in underlying cooperativity parameters. In addition, Ising-analysis will be presented for a series of rosetta-designed helical repeat proteins that bear no sequence or structural similarity to naturally occurring proteins.

Recently, we asked whether we could achieve similar success using consensus-based information to engineer globular proteins. Starting with the homeodomain fold, we created a highly thermostabilized consensus variant that adopts the homeodomain fold, folds extremely fast, and to our surprise, binds cognate DNA sequences with nearly 100-fold greater affinity than the Drosophila engrailed homeodomain. Very recently, we have had success with other folds, including the α-helical spectrin domain and a mixed β/α and β-sheet proteins. Results from these studies, along with implications for protein evolution and design, will be discussed.

Effects of an anti-prion pharmacological chaperone on the folding dynamics of single PrP molecules

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Many diseases involve the aggregation of misfolded proteins. In some cases,as with the prion disorders and prion-like diseases (e.g. Alzheimer's,Parkinson's, ALS), misfolded proteins can propagate by converting natively folded molecules into more misfolded molecules. Small-molecule pharmacological chaperones that act to prevent misfolding and conversion have been sought as therapeutics for several protein misfolding diseases, but their development has proven challenging, in part because their mechanism of action often remains unclear. We studied Fe-TMPyP, a tetrapyrrole that binds to the prion protein PrP and inhibits misfolding, using optical tweezers to unfold single PrP molecules with and without Fe-TMPyP present and thereby examine the effects of this ligand on PrP folding at the single-molecule level. We found that ligand binding to the native structure increased the unfolding force significantly and altered the transition state for unfolding, making it more brittle and raising the barrier height. Fe-TMPyP also bound the unfolded state, delaying native refolding and thereby allowing metastable misfolded states to form. Furthermore, looking at the folding of two PrP molecules linked in tandem we found that Fe-TMPyP binding blocked the formation of a thermodynamically stable misfolded state by interfering with intermolecular interactions, acting in a manner similar to that of some molecular chaperones. This anti-prion ligand thus promotes native folding by stabilising the native state while also suppressing interactions that drive aggregation.

The challenges and opportunities of understanding protein folding and protein misfolding in health and disease

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The last decade has seen astounding increases in our knowledge of how proteins fold, both *in vitro* and in living cells. Based on experiments with ever-increasing levels of sophistication, combined with increases in the powers of simulation and computational methods, we can now monitor folding transitions on rapid timescales in all atom detail, watch proteins folding as they first emerge from the ribosome, and witness the conformational fluctuations of individual protein molecules as they search for their native conformations on rugged energy landscapes. It is also clear that proteins can misfold, sometimes frequently during folding. If left unchecked by molecular chaperones, misfolding can result in disastrous consequences for the cell.

Understanding how different proteins assemble into the ordered, insoluble aggregates associated with amyloid disease is a formidable challenge. Whilst it is generally accepted that protein misfolding is required for the formation of amyloid fibrils, the point at which the folding and aggregation free energy landscapes diverge, and how or why non-native states of proteins are able to aggregate aberrantly remain obscure. Even more challenging is the identification of early aggregation-prone monomers and oligomeric species and their structural characterisation, since such species are aggregation-prone, short-lived and rapidly equilibrating. In this seminar I will describe how we have been using different biochemical and biophysical methods to reveal insights into the mechanism(s) by which normally soluble proteins convert into amyloidogenic conformations, how bimolecular collisions between non-native proteins can result in very different outcomes of assembly and how we have used small molecules to modulate the aggregation process.

Monomer dynamics control the first steps of aggregation and folding

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An important aspect of protein folding is understanding how folding competes with aggregation, which leads to diseases such as Parkinson's and Alzheimer's. The complexity and dynamics of unfolded protein ensembles may be the ultimate speed limit of folding and play a crucial role in aggregation. In my lab over the past several years we have investigated the reconfiguration dynamics of unfolded proteins by measuring the rate of intramolecular diffusion, the rate one part of the chain diffuses relative to another. We have measured diffusion coefficients ranging over three orders of magnitude and observed that aggregation-prone sequences tend to fall in the middle of this range. In this talk, I shall present our experiments on alpha-synuclein, the Alzheimer's peptide and various prion sequences. We correlated intramolecular diffusion of the disordered protein with solution conditions that promote aggregation. Finally, we have begun measurements on small molecule aggregation inhibitors and found that some can prevent aggregation by shifting intramolecular diffusion out of the dangerous middle range.

Protein structure prediction/determination by global optimization

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First, I will discuss our recent progresses on the protein structure prediction using the methodology of global optimization as illustrated in the CASP11 competition held in 2014. We will demonstrate that this method can be applied to difficult MR (molecular replacement) targets to determine X-ray crystallography structures of proteins and protein complexes, which could not be solved using conventional MR methods. We will also discuss the possible application of our method to the high throughput NMR structure determination of large proteins (over 20 kDa) and membrane proteins.

A reaction coordinate for amyloid beta misfolding

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Misfolding, like folding, is a highly multidimensional process. However, it may be possible to identify spatial coordinate(s) (i.e. specific intra-protein distance parameters) which largely describe the overall process, and manifest/control the main kinetic events. We study misfolding and aggregation of the Amyloid-β peptide, a phenomenon associated with Alzheimer's disease. Using a combination of fluorescence and solid state NMR spectroscopy, we identify a few atomic level reaction coordinates which capture the essence of the transitions between the initial monomeric to the final fibrillar state, through identifiable intermediates. Significantly, the evolution of these coordinates closely mirror the evolution of function of this toxic peptide.They provide us with a handle to monitor and potentially disrupt the progression towards toxicity.

Revisiting supersaturation as a factor determining amyloid fibrillation

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Amyloid fibrils involved in various diseases are formed by a nucleation-growth mechanism, similar to the crystallization of solutes from solution. To study amyloid fibrils, we developed several types of unique techniques. First, to visualize amyloid fibrils, we combined total internal reflection fluorescence microscopy (TIRFM) with amyloid-specific thioflavin T fluorescence (1). With this approach, we succeeded in observing the growth of amyloid fibrils in real-time at a single fibrillar level for various amyloidogenic proteins including Aβ. Second, we showed that ultrasonication is one of the best means of accelerating amyloid nucleation and thus the formation of fibrils (2, 3). By combining a water bath-type ultrasonicator and a microplate reader, we constructed a HANdai Amyloid Burst Inducer (HANABI), which enables a high-throughput analysis of ultrasonication-forced amyloid formation of proteins (4). Third, calorimetry, one of the most powerful methods used to study the thermodynamic properties of globular proteins, has not played a significant role in understanding protein aggregation. We succeeded with β2-microglobulin in direct heat measurements of the formation of amyloid fibrils using isothermal titration calorimeter (5). Our results with various approaches indicate that the solutions of denatured proteins are often supersaturated above the solubility limit and ultrasonic agitations release the supersaturation effectively, excluding solvated monomers to form fibrils. We suggest that amyloid fibrils and amorphous aggregates are similar to the crystals and glasses of solutes, respectively, and supersaturation is required to form crystal-like amyloid fibrils. We propose a general view of how the structures of protein and peptide precipitates vary from single crystals to amyloid fibrils and amorphous aggregates, in which "solubility" and "supersaturation" play critical roles (3, 6, 7).

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Water in amyloidogenic intrinsically disordered proteins

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Water molecules residing in the hydration layer at the protein-water interface of globular proteins exhibit unique dielectric and dynamical characteristics that are fundamentally different from bulk water. These dynamically restrained water molecules in the hydration layer of proteins are termed as "Biological Water" and play critical role in the folding,enzyme catalysis, protein-DNA and protein-protein interactions and aggregation. Protein misfolding and aggregation resulting in amyloid formation are associated with a variety of debilitating human disease such as Alzheimer's, Parkinson's and transmissible prion diseases, systemic, amyloidosis and cancer. My laboratory is involved in addressing some important structural, dynamical, nanoscopic and mechanistic issues of an emerging class of amyloid-forming proteins known as Intrinsically Disordered Proteins (IDPs). IDPs challenge the tenets of traditional sequence-structure-function paradigm and are associated with important functions and diseases. Using a diverse array of biophysical and biochemical techniques, we have previously shown how these proteins undergo a disorder-to-order transition during amyloid formation. Recently, using femtosecond and picosecond time-resolved fluorescence spectroscopy,we have embarked upon studies aimed at unraveling the crucial role of hydration dynamics in the conformational transition and aggregation of disease-related IDPs. I will discuss our recent ultrafast spectroscopic results that illuminate the intriguing behavior of water molecules in IDPs and reveal a novel mechanistic aspect of aberrant protein aggregation.

Microsecond protein dynamics revealed by two-dimensional fluorescence lifetime correlation spectroscopy

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Conformational heterogeneity and structural fluctuation are essential molecular properties of biomacromolecules. Protein folding is a central problem for which these properties are extensively studied, and the single-molecule fluorescence resonance energy transfer (smFRET) technique is an indispensable tool for such studies. Recently, the microsecond dynamics of protein folding have attracted much interest because of a number of reasons. For instance, the 'speed limit' of protein folding is supposed to be ~1 μs and it closely relates to the roughness of the folding free energy landscape as well as to the internal friction of the polypeptide chain in the unfolded state. Furthermore, microsecond structural fluctuations in the native and intermediate states are highly relevant to protein functions as well as protein-protein interactions. In addition, direct comparison with molecular dynamics simulation with experimental observation is possible for the dynamics on the microsecond time scale. Therefore, experimental elucidation of the microsecond conformational dynamics of proteins is critically important for obtaining a comprehensive understanding of the folding processes of proteins. Although several pioneering attempts have been made to detect submicro- to microsecond dynamics with smFRET techniques, it is still very challenging to detect transient states with a microsecond time-resolution.

Recently, we have developed a new single-molecule technique, i.e. two-dimensional fluorescence lifetime correlation (2D-FLCS) spectroscopy[1],by combining fluorescence correlation spectroscopy (FCS) and time-resolved fluorescence measurements, which has enabled us to investigate the microsecond structural dynamics of proteins. In this presentation, we will report on the application of 2D-FLCS to the study on the conformational dynamics of cytochrome *c* [2] as well as BdpA [3], and discuss the relevant energy landscape of the protein folding.

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Every molecule is special: enzyme molecules in action

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The catalytic mechanisms of complex biological machines, such as enzymes, may involve a combination of chemical steps and conformational transitions. The latter are often times hidden to traditional biochemical investigations, but can be exposed by single-molecule experiments.

I will first discuss an intriguing feature of enzymes when probed on the single molecule level, namely static and dynamic disorder: different molecules catalyze at different rates, and even the same molecule can switch its rate during activity. An attempt to understand this phenomenon using single-molecule spectroscopy has led us to discover strong correlations between conformational dynamics at different sites on the same enzyme molecule (1).

Single-molecule FRET is an ideal tool to probe the conformational dynamics accompanying enzyme catalysis. We have used single-molecule FRET to constrain mechanistic models for the activity of QSOX, a sophisticated catalyst of disulfide bond formation with two active sites (2).

Most recently, we developed a novel photon-by-photon analysis method that facilitates studying conformational dynamics even on the microsecond time scale, and applied our new method to the domain closure of the enzyme adenylate kinase. Surprisingly, we find that the bound enzyme opens and closes its domains much faster than the unbound enzyme, and two orders of magnitudes faster than the turnover rate of the enzyme! This exciting finding, which radically deviates from previous observations on adenylate kinase, suggests that the domain closure of this enzyme occurs multiple times before the right conformation is found for the chemical step to occur (3).

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A general mechanism for mutation-induced destabilization and modulation ofallosteric coupling in proteins

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Mutations in the hydrophobic interior of proteins are generally thought to weaken the interactions only in their immediate neighborhood. This forms the basis of protein-engineering based studies of folding mechanism and function. However, mutational work on diverse proteins has shown that distant residues are thermodynamically coupled, with the network of interactions within the protein acting as signal conduits, thus raising an intriguing paradox. Are mutational effects localized and if no, is there a general rule for the extent of percolation and on the functional form of this propagation? We explore these questions from multiple perspectives in the current work. Perturbation analysis of interaction networks within proteins and microsecond-long molecular dynamics simulations of several aliphatic mutants of ubiquitin reveal strong evidence for distinct alteration of distal residue-residue communication networks. We find that mutational effects consistently propagate into the second shell of the altered site (even up to15–20 Å) in proportion to the perturbation magnitude and dissipates exponentially with a decay distance-constant of ~4–5 Å. We also report evidence for this phenomenon from published experimental NMR data that strikingly resemble predictions from network theory and MD simulations. Reformulating these observations onto a statistical mechanical model, we reproduce the stability changes of 375 mutations from 19 single-domain proteins. Our work thus reveals a robust energy dissipation-cum-signaling mechanism in the interaction network within proteins, quantifies the partitioning of destabilization energetics around the mutation neighborhood and presents a simple theoretical framework for modeling the allosteric effects of point mutations.

Observation of nanometer fluctuations in a multifunctional protein complex

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Dynamics and flexibility are essential for protein function but are often difficult to probe and quantify. Ultrafast three-color single-molecule FRET allows us to probe flexibility in a folded complex of two intrinsically disordered proteins on previously inaccessible time- and length scales. Despite its high stability, the complex fluctuates on sub-microsecond timescales with nanometer amplitudes. Three-color photon correlations combined with molecular simulations reveal a lack of long-range collectivity in these dynamics, suggesting that rapidly exchanging interactions stabilize the complex. Our results show that high affinity and large-scale flexibility in protein complexes are not mutually exclusive but rather constitute a design principle to enable multifunctionality at the expense of a rigid three-dimensional structure.

ABSTRACTS OF POSTERS

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5. Alok Kumar Panda, Indian Institute of Technology Bhubaneswar
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Pathogenic mutations within the palindromic region of the prion protein induce structure therein and accelerate the formation

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The intrinsically disordered N-terminal region (NTR) of the prion protein is known to play an important role in its misfolding and aggregation leading to prion diseases. To understand how the unstructured NTR modulates misfolding of the structured C-terminal domain (CTD), the effects of two pathogenic mutations, G113V and A116V in the palindromic region of the NTR, on the misfolding and oligomerization of the mouse prion protein (moPrP) were studied. Although they had no effect on the structure, stability or dynamics of moPrP under native conditions, both mutations significantly accelerated both misfolding and oligomerization under misfolding conditions. Unlike in the case of wt moPrP, for which misfolding and oligomerization appeared to occur concurrently, oligomerization was shown to precede misfolding in the case of both G113V and A116V moPrP. The temporal sequence of misfolding events in the unstructured NTR and in the structured CTD of G113V and A116V moPrP was delineated by kinetic hydrogen deuterium exchange-mass spectrometry experiments. Sequence segment 89-132 from the NTR became structured, albeit weakly, during the oligomerization of both G113V and A116V moPrP. Importantly, it was seen that this occurs prior to structural change in the CTD, wherein the unfolding of helix 1 appeared to occur concurrently with the conformational conversion of helix 2 and helix 3.

Graded Structural Polymorphism in a Bacterial Thermosensor Protein

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Thermosensing is critical for the expression of virulence genes in pathogenic bacteria that infect warm-blooded hosts. Proteins of the Hha-family, conserved among enterobacteriaceae, have been implicated in dynamically regulating the expression of a large number of genes upon temperature shifts. However, there is little mechanistic evidence at the molecular level as to how changes in temperature are transduced into structural changes and hence the functional outcome. In this study, we delineate the conformational behavior of Cnu, a putative molecular thermosensor, employing diverse spectroscopic, calorimetric and hydrodynamic measurements. We find that Cnu displays probe-dependent unfolding in equilibrium, graded increase in structural fluctuations and temperature-dependent swelling of the dimensions of its native ensemble within the physiological range of temperatures, features that are indicative of a highly malleable native ensemble. Site-specific fluorescence experiments in combination with coarse-grained and all-atom MD simulations reveal that the fourth helix of Cnu acts as a unique thermosensing module displaying varying degree of order and orientation in response to temperature modulations. Our combined experimental-computational study unravels the folding-functional landscape of a natural thermosensor protein, highlights how functional constraints regulate specific folding-mechanistic behaviors and the design principles orchestrating the signal transduction roles of the Hha protein family.

Conformational Conversion of the Prion Protein in the Presence of Anionic Detergents and Lipids

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Prion diseases belong to a unique class of fatal neurodegenerative disorders that include transmissible spongiform encephalopathies and are manifested as infectious, genetic and sporadic diseases. The key step in the pathogenesis is the misfolding and aggregation of an endogenous GPI (glycosylphosphatidylinositol) anchored protein, termed as the prion protein (PrP). The cellular α-rich PrP is converted into a misfolded (protease-resistant) β-rich isoform. Lipid membranes are hypothesized to play a key role in the conversion to the pathogenic isoform. Our previous results have shown that in the presence of a membrane mimetic anionic detergent such as SDS (sodium dodecyl sulfate), PrP converts into two types of oligomers. Highly ordered large β -oligomers represent benign off-pathway intermediates that lack the ability to mature into amyloid fibrils. On the contrary, predominantly unstructured, small oligomers are capable of switching to ordered amyloids that are toxic to mammalian cells. These amyloids exhibited remarkable amyloid-polymorphism possessing varied nanoscale morphology. We are currently investigating the role of negatively charged lipid membranes in the prion conversion using an array of biochemical and biophysical techniques. Our preliminary data showing the synthetic lipid-mediated α→β conversion of PrP will also be presented.

Formation of inclusion bodies poses a major hurdle in obtaining recombinant protein from *E. coli*.

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Urea and guanidine hydrochloride have routinely been used as solubilization agents for inclusion bodies, but many a times result in poor recovery of bioactive protein. Mild solubilization has been shown to be an effective method for high throughput recovery of bioactive protein from bacterial inclusion bodies and also result in improved yield as compared to conventional solubilization methods. High pH buffers, detergents and organic solvents have been successfully used as mild solubilization agents. Here we show recovery of bioactive human growth hormone from bacterial inclusion bodies using organic solvents like n-propanol, β-mercaptoethanol and trifluoroethanol as solubilization agents. These organic solvents act as effective yet mild solubilization agents by denaturing the tertiary structure and preserving secondary structure of inclusion body proteins. We also show that organic solvents can be used to solubilize inclusion bodies of a number of different proteins. This also encourages screening different organic solvents for solubilization of inclusion body proteins.

Role of C-terminal Extension on the Structure and Chaperone Function of Mycobacterium tuberculosis Hsp16.3

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Hsp16.3 is a major immunodominant antigen of *M. tuberculosis*. Its molecular chaperone function is important for the growth and survival of this pathogen in macrophages. The importance of N-terminal region on the structure and chaperone function of Hsp16.3 is well understood. But, the role of C-terminal region (CTR) on its structure and function is still unclear. Therefore, we cloned, over-expressed and purified wild-type ($Hsp16.3_{1-144}$/Hsp16.3WT) and seven C-terminal truncated mutants of Hsp16.3. Partially truncated C-terminal extension (CTE) mutants ($Hsp16.3_{1-143}$/Hsp16.3ΔC1 and $Hsp16.3_{1-142}$/Hsp16.3ΔC2) have structure and chaperone function similar to wild-type protein; whereas, deletion of three residues from CTE ($Hsp16.3_{1-141}$/Hsp16.3ΔC3) triggers the perturbation in the tertiary structure, dissociation of oligomeric assembly (dodecamer to nonamer/trimer), enhancement in subunit dynamics (~1.5 folds) and improvement in the chaperone function of Hsp16.3. Interestingly, these structural modulations (except oligomeric dissociation) and chaperoning strength reaches its apex upon truncation of the entire CTE ($Hsp16.3_{1-140}$/Hsp16.3ΔC4). Further deletions ($Hsp16.3_{1-136}$/Hsp16.3ΔC8 and $Hsp16.3_{1-132}$/Hsp16.3ΔC12) increases only the degree of oligomeric dissociation of Hsp16.3 which is completed (trimer only) upon the truncation of entire CTR ($Hsp16.3_{1-128}$/Hsp16.3ΔC16). Overall, our study suggests a “new structural element” in the CTR i.e. C-terminal extension which plays an important role in oligomerization, subunit exchange dynamics and chaperone function of Hsp16.3.

Small Molecule Based Investigation on Different Stages of Alpha Synuclein Aggregation

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Parkinson's disease (PD) is one of the most common neurodegenerative diseases, affecting 1–2% of the population older than 65 years and 4–5% of those aged over 85 years. PD is a movement disorder, associated with different motor and nonmotor syndromes, arising from degeneration of dopaminergic neurons of substantia nigra. The hallmark of PD is the observation of intracellular protein aggregates, Lewy Bodies (LB). Alpha synuclein is one of the major components of this LB. The heterogeneity of aggregation process and the presence of large number of triggering mechanisms results in the difficulty to devise therapeutic strategies against these toxic inclusions formation. There have been continuing efforts to search for small molecules against alpha synuclein aggregation.

With the help of fluorescence correlation spectroscopy (FCS), confocal imaging, FRAP and other biophysical experimentswe have studied the early and late events of alpha synuclein aggregation. Subsequently, we have explored the possibilities of identifying possible small molecules, which would prevent early oligomer formation. For the FCS experiments, we have labeled a single cysteine mutant of alpha synuclein (G132C) using Alexa488 Maleimide. Our FCS data have shown significant changes in the conformation of the protein in the presence of an inhibiting small molecule. To obtain additional insights into these molecules; we have studied their effects inside two mammalian model cell lines, namely HeLa and SH-SY5Y. For this purpose, we have transiently transfected the cells using a tetra-cysteine tagged alpha synuclein, following which the cells have been stained using Flash-EDT2 dye for fluorescence imaging and FRAP studies. The results show that the electrostatic differences between the protein and the small molecules contribute significantly towards the ability of the small molecules to modulate the early and late stages of aggregation.

Biochemical and Structural Role of 104EWGWS108: An Insert Sequence in Plasmodium spp. Enolase

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Plasmodium falciparum enolase (Pfeno) has a unique pentapeptide insert, ^{104}EWGWS^{108} that is conserved in apicomplexans and plants and absent in host enolases. To examine the functional role of this insert, a deletion variant was made which resulted in poor affinity for cofactor Mg^{2+}, a ~100 fold decrease in k_{cat}/K_m and dissociation of the native dimeric form into monomers (Vora *et al.* (2009), Arch. Biochem Biophys 485, 128–138). To assess the importance of individual residues, site-directed mutagenesis was employed and the following variants were generated – S108G, S108A, S108T, W105,107A, W105,107K, W105A:W107E and KAGAG. Perturbing S108 to Gly/Ala led to a decreased enzymatic activity and affinity for cofactor Mg^{2+}, but the native dimeric form was retained. However, substituting the Trp residues with Ala/Lys not only drastically reduced the enzymatic activity, but also led to monomerization of rPfeno. Expressing the Trp variants as GST tagged proteins, led to dimerization of the enzyme. Such a stabilization of the subunit-subunit interface interactions via GST induced dimerization of rPfeno led to partial restoration of enzyme activity. Thus, the effects of these mutations reveal the importance of the insert in maintaining the active state as well as the three dimensional structure of *Plasmodium falciparum* enolase.

Development of peptide based therapeutics for Alzheimer's disease

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The pathological hallmark of the Alzheimer's Disease (AD) is the presence of amyloid β (Aβ) peptide aggregates in the Brain. The current therapeutics available for AD are known to mask the symptoms of the disease temporarily. Newer and more effective drugs thus need to be developed to cure AD. Small peptides are fast emerging as possible therapeutic molecules for AD. These peptides known as β-sheet breaker peptides (BSBPs) bind to aggregates of full length Aβ peptide and inhibit the formation of fibrils. Currently, peptide-based therapeutics suffer from multiple problems and are not yet approved for clinical trials. One of the major limitation is its poor proteolytic stability and its delivery. Novel strategies are therefore required to improve the therapeutic efficacy and delivery of peptides.

We have synthesized a series of novel peptides using solid phase peptide synthesis method and fmoc chemistry. Screening of the synthesized peptides were carried out to identify the peptides with higher efficiency to disrupt the fibril formation for Aβ 1- 42 peptide. This was performed by studying the kinetics aggregation of Aβ 1-42 peptide in presence or absence of synthesized therapeutic peptides. The kinetics were quantified using various method like Thioflavin-T fluorescence intensity, congo red binding & turbidity assay. Aggregation of Aβ 1-42 in presence of therapeutic peptides showed a lower rate of aggregation and the amount of fibril formation was also decreased significantly. Further, when the internal structures of amyloid fibrils of Aβ 1-42 peptide were probed using circular dichroism spectroscopy, it showed a complete loss of β-sheet content of fibrils on co-incubation with therapeutic peptides.

The results indicate that these peptides possess promising characteristic akin to BSBP.

Interface aromatics demarcate membrane protein folding and aggregation

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Polypeptide self-assembly into a folded, bioactive conformation occurs through controlled and rapid formation of thermodynamically favorable native contacts. Non-native interactions induce frustrated folding kinetics, causing protein misfolding and aggregation. This results in amyloidogenesis and several debilitating neurodegenerative diseases. It is known that interface residues act as anchoring elements in membrane proteins. Hence, we investigated the importance of aromatic amino acids in defining membrane protein folding, stability and aggregation kinetics using the outer membrane β-barrel Ail (Attachment invasion locus protein) from *Yersiniapestis*. Ail shows rapid unfolding and irreversible thermal denaturation coupled with protein aggregation. We demonstrate that the stability and aggregation propensity of Ail is controlled by the nature and position of interface aromatics (Y>F>W>A). Further, aggregation kinetics of Ail involves irreversible "seeding" by non-native unfolding intermediates. The aggregates show distinct amyloid like morphology. Overall, we propose a mechanism for irreversible association in transmembrane β-barrels and similarities and differences in the aggregation pathways. Most interestingly, our studies identify a sizeable contribution of the kinetic component in membrane protein stability. We find an evolutionary importance for different amino acids to maintain optimal folding and stability.

Examining the Mechanical Properties of Apo- and Copper-bound Azurins using Single-Molecule Force Spectroscopy and Steered Molecular Dynamics

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The metal-ligand bonds in metalloproteins are of special interest for their partial covalent character and redox properties. How metal ions influence the stability and dynamics of proteins is remains to be understood. Here, we used force spectroscopy and steered molecular dynamics (SMD) to study the effect of copper on the mechanical stability of azurin which is a periplasmic protein which undergoes forced-unfolding during translocation from cytoplasm to periplasm. The unraveling of both apo and holo azurin show two distinct force peaks with a contour length of ~6 nm between peaks. Holo azurin requires ~10pN higher force than apo form. SMD indicated that the unraveling is always initiated from the C-terminus by rupture of β-strands 7-8 and 2B-8. Both techniques show that 1st transition state (TS) is identical in both forms of azurin. The 2nd TS is distinct in two forms due to additional constraint by copper coordination, which is confirmed by the simulations. SMD also revealed a subtle shift in the position of the force peak of holo form upon varying stiffness of the copper coordination sphere. Solvent accessibility of the native disulfide bond of azurin in the X-ray structure and in MD simulation showed that only ~15% of its surface area is accessible to the external aqueous solvent. Indeed, we have found from our pulling experiments that the native disulfide bond is inaccessible and resistant to chemical reduction by DTT and β-mercapto-ethanol. However, the disulfide bond gets exposed after the initial rupture of the native state and undergoes mechano-chemical reduction in SMFS experiments.

Microsolvation assisted membrane insertion of Bcl-xl C-terminal via partial unfolding and refolding

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Bcl-xl, a protein of Bcl-2 family remains distributed over cytosol and mitochondrial membrane maintaining a balance between apoptosis and survival of cell. Translocation to membrane is essential for its biological functions (i.e. to antagonize pro-apoptotic proteins of Bcl2 family) which is known to be initiated by insertion of C-terminal into the membrane. This tail is understood from experiment to be predominantly helical in the aqueous condition, binding as a pseudo inhibitor to the protein itself, but gets released from confinement when it approaches to membrane. This work, hereby reports the energetic of the events associated with insertion of the helical tail into an all-atom membrane, which reveals an unfolding-refolding cycle. The peptide is most stable in fully inserted state where hydrophobic residues interact with hydrophobic tail of lipid and terminal charged residues are flanked to the charged head groups. Potential of mean force in 2D reveals that the peptide gets absorbed in a wide range of conformations at membrane-water interface followed by vertical insertion. Molecular dynamics further brings the insight that the peptide insertion associates an encapsulation of thin water layer around it throughout the course of insertion which plausibly motivates the protein to refold once the insertion is complete.

Dynamics of the growth of ds-DNA toroid

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A significant gap exists between experimental and theoretical work on ds-DNA folding and toroid formation regarding the length and the number of DNA molecules involved in a single toroid. While experimental systems use toroids with ~6 to 32 DNA molecules of ~3 kbp in a single toroid, till date simulations have only studied toroid formation by one, often shorter DNA molecule. To bridge this gap we performed Brownian Dynamics simulations to investigate the nucleation followed by growth of DNA toroids. A coarse grained bead-spring model of DNA has been used without any explicit charges. The simulation methods were validated by calculating the Flory exponents of non-stiff polymers in good and bad solvents. Firstly mechanisms of toroid formation were investigated for DNA chains of length ~2560 bp by allowing DNA pre-equilibrated in a good solvent to collapse in a bad solvent. Afterwards mechanisms of toroid growth were investigated by attaching non-toroidal structures, also formed in bad solvent conditions like spindles or free chains on formed toroids. Facile collapse of non toroidal DNA onto DNA toroids was observed. The different pathways followed by this collapse and the structure of the resulting toroid has been investigated and validated with experimental data.

Investigating the Structural Impact of S311C Mutation in DRD2 Receptor by Molecular Dynamics & Docking Studies

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Dopamine receptors (DR) are neuronal cell surface proteins that mediate the action of neurotransmitter dopamine in brain. Dopamine receptor D2 (DRD2) is a major therapeutic target for schizophrenia and Parkinson's disease. The third inter cellular loop (ICL3) in DRD2 is essential for coupling G proteins and several signaling scaffold proteins. We have examined the deleterious effect of serine to cysteine mutation at position 311 (S311C) in the ICL3 region that is genetically well associated with diseases like schizophrenia and alcoholism. An in silico structure modeling approach was employed to determine the wild type (WT) and mutant protein structures of DRD2. Atomistic molecular dynamics (MD) simulations were performed to provide insights into essential dynamics of the modeled structures. To provide information on intra-molecular arrangement of the structures, a comprehensive residue interactions network was studied. Besides, protein-ligand and protein-protein docking protocols were exercised to compare the interactions of natural agonist (dopamine) and scaffold proteins - Gαi/o and NEB2. We observed a marginal effect of the mutation in dopamine binding mechanism throughout the trajectories. However, we noticed a significant structural alteration of the mutant receptor which affects Gαi/o and NEB2 binding that can be causal for malfunctioning in cAMP-dependent signaling and Ca+ homeostasis.

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Dissecting the mechanisms of liquid to solid phase transition associated with neurodegenerative diseases

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FUS/TLS is a prion-like protein that contains intrinsically disordered domains and is associated with neurodegenerative disease. We recently showed that intracellular FUS/TLS compartments form under various cellular conditions and that these compartments exhibit liquid-like properties *in vivo* and *in vitro*. "Aging" experiments revealed that FUS/TLS liquid droplets undergo a phase transition to a solid-like state which is accelerated by disease mutations (Patel et al., 2015). We discovered that concentrating proteins by phase separation comes with the trade-off that can also promote protein aggregation. Solid-like aggregates of prion-like proteins are a hallmark of many aging-associated diseases. Aberrant phase transitions might be one trigger causing aging-associated diseases. However, the molecular mechanisms underlying this aberrant phase transition and the strategies cells have developed to sustain the function of these aggregation-prone proteins remain largely enigmatic. Here, we present recent advances we made in understanding the mechanisms cells might have developed to prevent the liquid-solid phase transitions, by using a wide range of biochemical, biophysical and cell biology techniques. We find that electrolytes, small compounds and protein interactors affect the liquid-liquid, as well as liquid-solid transitions. Insights gained from studying liquid-solid phase transition might help us developing drugs targeted to treat age-associated diseases.

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Effect of Silver Nanoparticles on the Structure, Stability and Molecular Chaperone Functionof Mycobacterium leprae HSP18: An Alternative Clue for the Treatment of Leprosy

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Nanoparticles (especially AgNPs) recently have been used to combat the cell permeability issues which are often encountered by different drugs in mycobacterial species. Moreover, it has been demonstrated that AgNPs control the growth of mycobacterial species. But, whether AgNPs influence the growth of non-culturable M. leprae (causative agent of leprosy) is unclear. Additionally, whether AgNPs affect the functionality of antigens that are important for the survival of this pathogen is still unknown. Here, we have taken an attempt to understand the effect of AgNPs on the structure and chaperone function of 18-kDa antigen, actually a small heat shock protein (HSP18), which plays a vital role in the virulence and survival of M. leprae. First, we prepared citrate capped AgNPs and then characterized this nanoparticle using scanning electron microscopy. Flurometric analysis revealed that HSP18 interacts with AgNPs with sub-micromolar binding affinity. "HSP18-AgNPs" interaction results dissociation in oligomeric assembly, decrease in thermodynamic stability as well as perturbation in secondary and tertiary structure of HSP18. In vitro aggregation and thermal inactivation assays clearly revealed that this interaction decreases the chaperone function of HSP18. Our in vitro structure-function studies provide a clue for using AgNPs for the effective treatment of leprosy.

A contact-based topological quantity and its relationships with the folding kinetics of non-two-state proteins and two-state proteins

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Understanding the protein folding mechanism from its primary sequence into its unique three-dimensional shape has been a long-standing problem in biology. It has been reported that there exists high correlation between the folding rate constant (k_f) of non-two-state protein and the number of non-local contact clusters (N_c) obtained from the residue-residue contact map. This study suggests the importance of the arrangement of substructures (α-helices, β-hairpins, and so on) for the folding kinetics in the non-two-state proteins[1,2]. However, the corresponding correlations for the two-state proteins were shown to be less significant.

We constructed the final non-redundant dataset of two-state (76) and non-two-state proteins (46) based on the complete experimental data and available databases in protein folding rates. We utilized this dataset to reinvestigate the relationship between (log k_f) and (log N_c) for both non-two-state and two-state proteins by introducing the new definition of non-local contact cluster. Our data has revealed statistically significant correlation for both non-two-state and two-state proteins. However, the optimized parameters such as sequence separation (L), steepness parameter (α), cutoff distance (d_{cut}) and contact strength (R) were different between the two-state and non-two-state protein. Long sequence separation, short-distance contact and high contact strength controls the folding of non-two-state proteins, while relatively short sequence separation, long-distance contact and very high contact strength controls the folding of two-state proteins. These results will be discussed in terms of the molecular mechanisms of folding of non-two-state proteins as well as two-state proteins.

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Evidence for Anti-parallel Beta Sheets in Aβ Oligomers from Atomistic Modeling and Simulations

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Alzheimer's disease (AD), a neurodegenerative disorder, is characterized by the presence of extracellular deposits of plaque and intracellular neurofibrillary tangles, of which the major components are the fibril form of amyloid beta peptide [1, 2]. Recent studies have shown that although plaques are composed of fibrils, small oligomeric assemblies of the peptide generated during the early stages of peptide assembly are the major toxic species [2]. However, structural details of the oligomer species remain inaccessible to commonly used molecular structure determination techniques. Here, we examine the oligomer structure in solution using atomistic molecular dynamics (MD) simulations. We constructed atomistic fully solvated models of oligomers of different sizes ranging from trimer to dodecamer, based on the structure of monomer unit from a fibril structural model (PDB ID: 2M4J). The models were sampled over time scale ranging from 40-500 ns and structural markers obtained from IR, Raman and solid state NMR experiments [3] were followed to examine the oligomeric state. Based on average non-bonded interaction energy per monomer, the tetramer and pentamer species were found to be the most stable species over the time scale of simulations. We find that oligomer structures larger than octamer are unstable in solution and break down into shorter oligomer components within 40 ns. We observed spontaneous formation of anti-parallel beta sheets for pentamer and hexamer fragments in solutions. The anti-parallel beta sheets show inter-residue interactions (D23-K28 salt-bridge, F19-L34 contact, E22-I31 contact) which are consistent with emerging experimental data on oligomers [2, 3, 4, 5, 6].

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Mechanism of amyloid-like protofibrils formation under near-native conditions

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Protein fibrillation is a generic property of proteins and the cause of a number of severe human diseases. As evidenced by several reports, the link between the process of fibril formation and disease rests in the protofibrils and soluble oligomers forming transiently during the process. Therefore, the formation of stable oligomers under physiological conditions and their characterization are important and would help their detection in biological specimens. In this work, we report that human serum albumin (HSA) converts into amyloid-like protofibrils on mild stirring (230 RPM) of the protein in 60 mM sodium-phosphate buffer of pH 7.4 at 37 °C. Contrary to reported mechanism of HSA aggregation, we have shown that HSA aggregation under near-native condition is a nucleation-dependent process that can be seeded by aggregated HSA acting as nuclei. On the basis of a comparativeaggregation and stability studies of natural fatted-HSA and experimental fatty acid free HSA, we conclude that conformational/stability alteration of the HSA due to ligand removal is the main cause of the aggregation. Slow stirring, which does not significantly alter stability of the protein speed up the fibrillation process by known mechanisms such as increasing the air-water interface and breaking up large complexes and increasing the collision of reactive complexes with each other.

The role of residues forming the B/C barrel in conformational transitions of Plasminogen Activator Inhibitor-1

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The serine proteinase inhibitor (serpin) superfamily of proteins belongs to a small subset of proteins that exist naturally in energetically less stable state(s). Many members of this superfamily are inhibitory proteins controlling proteolytic cascades. The core serpin fold is composed of three β-sheets (A-C) and an exposed mobile reactive center loop (RCL) connecting β-strands s5A of A-sheet and s1C of C-sheet. The RCL upon cleavage by a protease inserts in the central A-sheet of the protein thereby translocating the covalently attached protease by 70 Å to the opposite pole of the molecule. In the process, the protease is irreversibly inactivated and the serpin is transformed into its thermodynamically more stable alternative fold. Of the three β–sheets, the A-sheet is labile while the B- and C-sheets interact to form a stable β–barrel (B/C barrel) which is reported as an early forming structure in serpin folding. The RCL insertion in the A-sheet is also known to occur in the absence of a protease, a state referred to as latent form, but results in a non-functional serpin. In the latency transition and in polymer formation, in addition to the conformational change in the RCL, the β–strand s1C detaches from the C-sheet (or the B/C barrel) and adopts a loop like structure. Interactions in the B/C barrel in stabilizing the RCL via s1C is important for understanding folding of serpins to its functional metastable state or its other structural form(s). Of the known serpins, human Plasminogen Activator Inhibitor-1 (PAI1) spontaneously undergoes latency transition with a $t_{1/2}$ of about 2 hrs in plasma. Thus, PAI1 allows monitoring all the various conformations in serpins. We selected 22 residues of the B/C barrel in PAI-1 and made alanine substitutions at these positions to create single mutants. The role of each of these residues in the structure and function of PAI1 as a protease inhibitor will be presented in this poster.

Selective Monitoring of Domain III of HSA: Ensemble Average and Single Molecular Level Study with a Newly Synthesized Fluorescent Marker

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Mapping of the unfolding behaviour of domain III of human serum albumin (HSA) was carried out selectively using a newly synthesized coumarin based solvatochromic fluorescence marker *p*-nitrophenyl coumarin ester (NPCE) as specific tyrosine marker. Tyrosine-411 residing in domain III of HSA has been tagged selectively with NPCE. The chemical denaturation of the protein has been studied by means of steady state, time resolved spectroscopic techniques and Förster resonance energy transfer (FRET). Microsecond conformational dynamics of domain III of HSA associated with the breathing motion of the protein has also been studied by means of fluorescence fluctuation generating from the quenching of the probe by electron rich amino acids using fluorescence correlation spectroscopy (FCS). Overall results indicate that the unfolding of domain III occurs in a stepwise fashion including two intermediate steps whereas the overall protein is monotonous with respect to increasing guanidine concentration and completes at around 6(M) guanidine concentration.

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Modulation of β-hairpin peptide self-assembly: canonical β-hairpin reprogrammed as a hydrolase mimic

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The building blocks of protein structure are defined stereochemically and have remained frozen to only poly-L alphabet. The D-enantiomer has been explored to diversify the shapes of proteins stereochemically and in the design of the novel folds[1-4]. Illustrating the scope, we describe design of a hydrolase mimic over sixteen L- and D-a-amino-acid residues. The stepwise design of the molecular fold followed by sequence optimization for substrate binding and catalytic activity was followed. The fold is designed by exploiting stereochemical approach to bend the poly-L b-hairpin, which is prone to aggregation, to promote a monomolecular fold as an enzyme model. The sequences were inverse-designed to provide stability to the fold and the residues were reshuffled in the substrate binding site, catalytic active site and in the stereochemical bent region to assess the fold stability, substrate binding and catalytic activity. The designed peptide variants are proven to bind *p*-nitrophenyl phosphate using fluorescence studies and hydrolyze *p*-nitrophenyl acetate using UV-based enzyme kinetic assays. The study thus highlights the role of stereochemistry in design of novel folds with desired function.

Keywords: Protein design, Protein stereochemistry, Hydrolase enzyme, Enzyme models, b-hairpin aggregation

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Rational elicitation of cold sensitive phenotypes

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Cold sensitive (cs) mutants behave like loss-of-function mutants at temperatures below the restrictive temperature but display wild type phenotypes at higher temperatures and are useful tools to probe gene function *in vivo*. Cold-sensitive phenotypes have helped understand macromolecular assembly and biological phenomena, yet few attempts have been made to understand the basis of cold sensitivity or to elicit it by design. We report a method for rational design of cold-sensitive phenotypes. The method involves generation of partial loss-of-function mutants, at either buried or functional sites, coupled with selective over-expression strategies. The only essential input is amino acid sequence, though available structural information can also be used. The method has been used to elicit cold-sensitive mutants of a variety of proteins, both monomeric and dimeric, and in multiple organisms, namely *E. coli*, *S. cerevisiae* and *Drosophila melanogaster*. We have also carried out additional characterization using quantitative RT-PCR, protein thermal stability assays and biolayer interferometry to support our proposed mechanism for elicitation of cs phenotypes. This simple, yet effective technique of inducing cold sensitivity eliminates the need for complex mutations and provides a plausible molecular mechanism for eliciting cold sensitive phenotypes.

Torsional Dynamics of Polypeptide Chains in the Ramachandran Dihedral Space

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The torsional dynamics of a polypeptide chain in the Ramachandran ϕ-ψ dihedral angle space dictates the course of protein folding, misfolding and aggregation and is linked to the internal friction. However, the direct observation and unambiguous assignment of this inherent dynamics in chemically denatured proteins are challenging due to various experimental limitations. Intrinsically disordered proteins (IDPs) such as α-Synuclein, are excellent systems to study the torsional dynamics since their inherent conformational fluctuations are independent of any extraneous factor. In order to directly map the backbone torsional mobility, we created a number of single-tryptophan variants encompassing the entire chain. We then utilized picosecond time-resolved fluorescence depolarization measurements that allowed us to discern the site-specific torsional relaxation at a low protein concentration under physiological condition. Our results show the presence of a characteristic relaxation time of ~1.4 ns. We are now evaluating if different fluorescence labels at Cys variants can be used to recapitulate the torsional dynamics and the effect of dye linker on the correlation time. Using a long-lifetime probe, we are aiming at measuring the viscosity dependence of the correlation time that will allow us to quantify the component of internal friction arising due to the dihedral relaxation.

Investigation and Characterization of Intrinsically Disordered Proteins in Plants

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Intrinsically disordered proteins (IDPs), are a special class of proteins which lack stable unique 3-dimensional (3D) tertiary structure. Due to their binding promiscuity and plasticity, IDPs challenge the classic structure-function paradigm. These are found to play major roles in various stress conditions, diseases, regulation and signaling in both plants and animals. Their molecular basis of protein function and dynamics is not well understood. However, IDPs have a broad impact in many areas of plant biology which has attracted great attention in past few years.

Our aim is to investigate and characterize the IDPs in *Pongamia pinnata* (which has been used for biodiesel production for several years) by numerous biophysical techniques such as fluorescence, Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR). This study will achieve identification of novel IDPs and understanding their dynamics and functional role in plants. Another part of my work includes understanding the functional dynamics of Dehydrin 1(a member of Late Embryogenesis Abundant protein family) and its mutants from *Zea mays*.

Initial studies on the characterization of novel IDPs from *Pongamia pinnata* and interesting results on dynamics of DHN 1 will be presented.

Interpretation of hydrogen exchange mass spectrometry data using molecular dynamics simulations

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Proteins are flexible entities and undergo conformational changes in solution due to motions ranging from transient local fluctuations to global unfolding. Hydrogen deuterium exchange (HDX) has been a ubiquitously used technique to probe these motions. Generally, HDX has two outcomes: 1) peptide level resolution in terms of observed rates and 2) amplitude (# of amide hydrogens exchanged with deuterium in a peptide fragment).We used all atom molecular dynamics simulations to get atomic insight into HDX data. We calculate mechanical force at atomic level associated with amide bonds and depth to understand the relationship of these atomic forces with HDX data. We find that no single structural property alone can best explain HDX amplitudes. Forces alone give a fairly good picture of amplitude but not the best. The forces in combination with depth best represent HDX amplitudes. Presence of mild concentration of Guanidinium Hydrochloride (~1M) is not significant to affect the network of atomic forces. Although coordination numbers show Guanidinium ions stick to specific parts of the protein, in simulated time, forces do not explain any relationship between HDX rates and timescale (reciprocal of observed rates). Therefore, the exact role of mild denaturant remains unclear. We found few residues showed an open state; consistent with a recent study.

Structural and functional insights into ANTAR-like proteins using computational approaches

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RNA play diverse cellular roles by interacting with proteins having dedicated RNA-binding domains (RBD). Recently discovered, bacterial RBD called ANTAR (AmiR-NasR Transcriptional Antitermination Regulator) specifically recognizes and stabilizes the target mRNA conformation, thus modulating the downstream gene expression. Typically, the ANTAR domain occur along with signal-sensing domain suggesting signal dependent gene control.

Classical bacterial ANTAR domains form a three-helical structure showing high sequence conservation. However, recent studies have identified a family of GTPases that contain a structurally similar classical ANTAR domain having less sequence conservation. These GTPases with "ANTAR-like" domains occur in a subset of bacteria (eg. RbgA from *Bacillus subtilis)* and are also conserved across eukaryotes (eg. Lsg1, Mtg1 in *Saccharomyces cerevisiae*), but with additional N and C-terminal extensions in eukaryotes. These G-proteins are implicated in ribosome assembly thus universally making them important players in translation. How these proteins recognize the ribosome, its modulation by GTP, the role of "ANTAR-like" domain and N, C-terminal extensions remains unstudied.

We are attempting to use different structural-bioinformatic approaches such as threading and *ab-initio* modeling methods along with MD-simulations to obtain confident model structures. Thus these computational analyses will help us gain structural insights and understand the probable functions of "ANTAR-like" domain or proteins.

Using single molecule chemo-mechanical unfolding to probe the effect of environmental conditions on protein folding

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In vivo, proteins function in a complex environment where they are subject to stresses like solutes, temperature and strain which can modulate the protein's energy landscape. Perturbing these conditions allows one to explore how proteins respond to changes in environment. This also helps to characterize protein energy landscapes because perturbant effects are related to the structure and energetics of the different protein states along the energy landscape. The effect of perturbants on protein stability is related to the structure of the native and denatured state, while the effect of perturbants on protein kinetics is related to the folding pathway. We have developed a technique called chemo-mechanical unfolding where we combine force and chemical denaturant using optical tweezers. We use chemo-mechanical unfolding as well as temperature and point mutations to explore the denatured state and the parallel pathways proteins fold through.

Use of designed sequences in protein structure recognition

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It is common knowledge that the protein sequence space is increasing at a disproportionately faster pace in comparison to the structure space. Improvements in next generation sequencing methods has further widened this gap. Knowledge of the structure of a protein is crucial in characterizing its biochemical function, making use of information on other members of the same fold. However, the protein structure space is riddled with gaps and there exists a dearth of proteins which serve as evolutionary linkers between distantly related protein families. This limits the association of remotely related families through sequence searches hence curtailing attempts at relating a protein family of unknown structure with a remote homolog of known structure. In this work we used a search space consisting of natural protein sequences augmented with artificial protein-like sequences, which were designed to serve as bridges that link distantly related natural protein families. For our study we adopted an iterative sequence search method which exploited the use of designed sequences as linkers and identified relatives however distant, of the queried sequences. This we hoped would serve as a significant start point in structure prediction through fold assignment for protein families with yet unknown structure. We identified the number of protein domain families associated with a known structure in literature and identified the number of correct fold assignments made by our approach. We observed that in 99% of the cases we were successful. Further, the extension of our approach to protein domain families of yet unknown structure resulted in the robust fold assignment for over 3000 protein domain families. Detailed analysis of a few cases revealed blocks of residues conserved between the sequence and structure domains, which could be implicated in function. One of the prospective applications of this study is to decrease the limitations in the routine search algorithms imposed by the paucity of structural neighbors for making reliable fold associations.

Characterization of conformational heterogeneity of tau by intact mass spectrometry: Implication on disorder and fibrillation

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Tau is an intrinsically disordered protein which organizes itself into structured fibrils in several protein misfolding diseases including Alzheimer's. Intact mass spectrometry based study was carried out with the largest and smallest isoforms of tau to get deeper insight into the effects of pH, ionic strength, temperature and solvent polarity on the structure of the protein. For the first time we report that at physiological pH, three distinct conformations of the protein, *viz* unfolded, intermediate, and compact exist. An increase in pH showed an increase in the compact population, reaching maxima near isoelectric point of the protein, whereas acidic pH shifts the conformation completely towards unfolded population. Compaction of structure correlated with the enhanced fibrillation at alkaline pH. Similarly on increasing ionic strength, tau showed compaction due to charge neutralization, followed by unfolding of the less-structured population due to electrostatic repulsion. An increase in temperature showed slight compaction possibly due to increased hydrophobic interaction. Decrease in solvent polarity unwinds the protein, stabilizing further unfolded states. The study shows both lack of hydrophobicity and high net charge at physiological pH is responsible for the unfolded nature of tau and factors driving the protein towards more compact population might trigger its fibrillation.

Multiparameter approach based-method for Integral Membrane Protein from Prokaryotic Inclusion bodies

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Most membrane proteins are not naturally expressed at high levels in cell membranes.Over expressed in heterologous host systems such as *E. coli* yields better results. However, the protein poorly inserts into host membranes, which can lead to the formation of insoluble, inactive aggregates known as inclusion bodies. In order to obtain useable protein for structural and functional studies, these inclusion bodies must first be solubilized using chaotropic agents. In the current study Cloning and expression of *pgtE* gene, encoding a polypeptide of 297 amino acids, was carried out to solve the 3D structure of OmpT. The *S. typhipgtE* gene was amplified from genomic DNA using appropriate primers and cloned into pET28a (+) and pET30b (+) vector. The *S. typhi* OmpT (*pgtE* gene) was expressed under the control of T7 promoter. *S .typhi* OmpT inclusion bodies were solubilized using Chatropic agents by One-Step denaturation and Two-Step denaturation method under various pH, Detergents, reducing agents and low salt concentration conditions. The solubilisation of OmpT was confirmed by calculating aggregation index (AI). Further Refolding attempts by screening pH, Detergents, Temperature and *Insilco* Mutational & Molecular Dynamics studies on Aggregation Prone Regions (APR) are in progress. These observations will be presented.

Thioflavin-T reduces the structural heterogeneity in α-synuclein fibrils

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The transition of natively unfolded monomeric α-synuclein protein into β-sheet rich oligomers and fibrils is associated with various neurodegenerative (ND) diseases. Numerous disease phenotypes, associated with different types of aggregates made of the same protein show the existence of different strains/polymorphs. These polymorphs exhibit different physical and chemical properties and show different cytotoxic effects. Structural and functional characterization of these polymorphs can help to understand the association of different polymorphs with various pathological phenotypes. Small molecules can be used to redirect the aggregation reaction to form desired polymorphs. Here the co-existence of two structurally and morphologically different α-synuclein fibrils have been shown. The addition of Thioflavin T, during the aggregation process, enhances the aggregation rate of α-synuclein, stabilizes straight fibrils and reduces the extent of fibril polymorphism.

Negatively Charged Detergents and Lipopolysaccharides Promote the Curli Amyloidogenesis

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Amyloids are ordered protein aggregates that are associated with a diverse range of neurodegenerative diseases. Functional amyloids are a class of amyloids that are used by the organisms for various physiological functions. Curli, a functional amyloid expressed on the cell surface of enteric bacteria like E. coli, is involved in cell-cell adhesion, colonization, biofilm formation etc. Unlike the traditional amyloids, curli formation is stringently regulated by the cellular machinery. The curli fibres are composed of a major subunit CsgA and a minor subunit CsgB that are secreted as intrinsically disordered proteins. Previous studies have indicated that the membrane-associated CsgB nucleates the polymerization of CsgA on the cell surface. However, the mechanism by which bacteria allow the aggregation of curli subunits without disrupting the membrane integrity remains elusive. By using an array of spectroscopic and biochemical techniques, we have investigated the mechanism of CsgB aggregation induced by anionic detergents and lipopolysaccharides. The aggregation of CsgB also alters the course of CsgA polymerization. The molecular basis of the lipopolysaccharides-CsgB-CsgA interaction and its potential role in the curli biogenesis will also be presented.

Investigating the role of allostery in protein assembly/disassembly.

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Protein-protein interactions give rise to either structural or dynamics change at a site away from interfaces. These changes are functionally relevant and would either associate or dissociate the proteins under question. We are trying to understand the role of allostery in formation of multiprotein assembly. The ongoing project deals with CcdB-CcdA, a type II toxin-antitoxin system in E. coli. The balance between toxin (CcdB) and antitoxin (CcdA) proteins is essential for normal cell growth otherwise CcdB protein can hinder gyrase functioning. The CcdA and gyrase binding sites on CcdB are away from each other. But when CcdB is embraced by gyrase, only a part of binding site remains accessible for CcdA to interact and CcdA can displace gyrase off CcdB. This interesting observation has been proposed to be the result of allosteric changes happening due to binding of a part of CcdA to CcdB-gyrase complex. Using Normal Mode Analysis, we have observed change in dynamics between CcdB in free form, CcdA bound and gyrase bound forms hinting towards possibility of little difference in the conformational space accessible to the CcdB molecule. Using Perturbation Response Scanning method, residues of CcdB known to be involved in CcdA binding were perturbed and changes were observed at sites involved in gyrase binding. All these observations give an idea about how binding of one protein to already existing protein complex can cause dissociation of proteins.

Charge Transfer Bands from Charged Amino Acids Provide Distinctive UV and Visible Spectroscopic Markers for Tracking Protein Unfolding and Dynamics

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The electronic absorption spectra of the protein folds is primarily characterized over the ultraviolet region (180-300 nm) with specific absorption features attributed to peptide bonds (~ 190 nm) and aromatic amino acids (~270 nm). Here we report the distinctive absorption of a synthetic 67 residue protein (α_3C) devoid of aromatic amino-acids in the 250-500 nm region. Molecular Dynamics simulations of α_3C reveal inter-residue interactions between lysine amino acids mediated by water and lysine-glutamate salt bridges. TDDFT calculations on individual and clusters of amino acid fragments show that the unique spectral signatures beyond 250 nm arise from charge transfer transitions involving both, the amino (NH_3^+)/carboxylate (COO^-) headgroups of lysine/glutamate and the peptide backbone. The long tail of the experimental absorption spectra from 300-500 nm is attributed to the sensitivity of the charge transfer transitions to the amino acid conformation, interaction between charged amino acids, and their solvation environment. Indeed, the charge transfer band is shown to be sensitive to thermal destabilization of α_3C. Our studies introduce a novel spectral window (300-500 nm) for optical probes of protein structure and dynamics.

Probing misfolding kinetics at the monomeric level during protein aggregation

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Protein aggregation is often synonymous with beta-sheet formation. As a result, ensemble probes tracking overall secondary structural changes like Circular dichroism and Thioflavin T binding assays have been commonly employed to study protein aggregation kinetics. However, to probe the exact sequence of events which culminate in such drastic conformational changes is not trivial. Since both intra and intermolecular effects are operational in protein aggregation, a major challenge is to tease apart their contributions to the overall process. Here, using mouse prion protein as a model system, we attempt to specifically probe the *intramolecular* changes that occur in the monomer during oligomerization. Briefly, the kinetics of co-aggregation of a Trp-less mutant and several carefully designed single Trp-single Cys mutants (spanning the various secondary structural elements) of the protein were measured using steady-state FRET. While most probes traditionally used to follow protein aggregation output an average rate at which the protein aggregates (and/or changes conformation), our data suggest that intramolecular conformational changes take place with variable kinetics, allowing the elucidation of the steps through which misfolding proceeds.

Measuring the (good) solvent quality of disordered proteins using a single SAXS measurement.

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Proteins often adopt non-native geometries during their synthesis, folding, transport, and turnover and a significant fraction of the proteome is intrinsically disordered. Here we report the conformational properties of a panel of disordered proteins having sequence compositions typical of folded proteins. Small angle X-ray scattering (SAXS) finds that their conformational ensembles are highly expanded in water. An analysis of the SAXS curves finds that the Flory exponent, which describes the collapse propensity of a chain, is $\nu \sim 0.53$. This value is between the ideal self-avoiding random walk ($\nu = 0.59$) and the theta solvent limit where intra-chain attraction exactly counterbalances excluded volume terms in an otherwise random walk ($\nu = ½$). The addition of moderate amounts of denaturants results in very mild expansion. Simulations generate a molecular form factor applicable to IDPs that can be used to determine the Rg and ν from a single SAXS measurement. These findings along with recent re-evaluation of fluorescence resonance energy transfer (FRET) studies largely resolves the ongoing SAXS/ FRET controversy concerning the behavior of disorder proteins.

Environmental Fluctuations and Stochastic Resonance in Protein Folding

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Stochastic Resonance is the process in which a weak sub-threshold signal is amplified by addition of optimum noise. It is commonly observed in macroscopic biological phenomenon but here we investigated if protein folding is a subject to stochastic resonance. Human cell surface antigen protein VlsE; a two state folder was FRET labelled in order to report on its folding/unfolding. The protein was subjected to periodic thermal modulation below its melting temperature. On top of the periodic thermal modulation, controlled artificial noise was added to monitor the effect of noise on protein unfolding reactions. The phase shift in the donor and acceptor signal as a function of driving frequency revealed the reaction rates. We observed a maximum in the signal as a function of thermal noise confirming that addition of artificial noise was in fact capable of driving the unfolding reaction for the protein. Simulation of a small model-protein, and our experimental result both show clear evidence that correlated noise is a physically and chemically plausible mechanism by which cells could modulate biomolecular dynamics during threshold processes such as signaling.

A Tale of Two Amyloidogenic Proteins: Tau and α-Synuclein

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Tau and α-synuclein (α-Syn) are intrinsically disordered proteins (IDPs) which are localized in the intracellular compartment and exhibit an astonishing conformational plasticity under physiological conditions. Growing evidences suggest that tau and α-Syn interact and form amyloids in a pathologically relevant manner, which results in several neurological ailments like Parkinson's Disease, Alzheimer's Disease, Down syndrome, Multiple System Atrophy, etc. However, the molecular and structural determinants of tau-α-Syn interactions are not yet clearly understood. We are investigating the *de novo* molecular interactions between these two proteins using multiparametric fluorescence techniques. The aggregation of tau and α-Syn under identical solution conditions were monitored separately using Thioflavin T as an amyloid marker. We observed that α-Syn forms amyloids via a nucleation-dependent polymerization mechanism whereas tau did not aggregate in the absence of heparin. Upon addition of monomeric α-Syn, tau readily aggregates with a short lag phase. We speculate that the aggregation is primarily driven by electrostatic interactions between tau and α-Syn and subsequent formation of obligatory oligomers that mature into amyloid fibrils. Additionally, the structural insights into α-Syn aggregates at a residue specific level will be discussed.

Functional instability in Transcription Activator-Like Effectors (TALEs)

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Transcription activator-like effector proteins (TALEs) contain large numbers of repeats that bind double stranded DNA, wrapping around DNA to form a continuous superhelix. Since unbound TALEs retain superhelical structure, it seems likely that DNA binding requires a significantstructural distortion or partial unfolding. We have used nearest-neighbor "Ising" analysis of consensus TALE (cTALE) repeat unfolding to quantify intrinsic folding free energies, coupling energies between repeats, and the free energy distribution of partly unfolded states. We find a moderate level of cooperativity for cTALEs (stabilizing interfaces combined with unstable repeats), at values intermediate between those of TPR and ankyrin repeat proteins (low and high cooperativity, respectively). Using parameters from the Ising analysis, we have analyzed the distribution of partly folded states as a function of cTALE length. We findthat partly unfolded states with one or more unfolded repeats are energetically accessible.

To quantify the DNA binding of cTALES, and to probe the dependence of DNA binding dynamics on accessibility to partially folded states, single molecule Total Internal Reflection (smTIRF) experiments were performed. Immobilized Cy3-labelled cTALEs incubated with Cy5-labelled dsDNA show an average FRET efficiency of ~0.45. When immobilized protein is incubated with DNA in 200 mM KCl, many on and off events occur in single traces. Long movies were collected for cTALEs incubated with increasing DNA concentrations. FRET of single molecules was fit using Hidden Markov modeling analysis, and cumulative distribution functions (CDF) for on and off dwell times of many molecules were generated. CDFs for on and off events are well fitted by double exponentials, suggesting a complicated kinetic binding process. The fast on phase is DNA concentration dependent, while the slow phase is not DNA concentration dependent, suggesting a fast bimolecular binding event (5.9×10^8 $nM^{-1}*sec^{-1}$) and a unimolecular isomerization (0.15 sec^{-1}). The near-diffusion limited association indicates that association is not limited by the cTALE superhelical structure, and is consistent with rearrangement to a binding-competent (perhaps partly unfolded) state. It is possible that the subsequent slow isomerization involves locking the superhelix onto the DNA. Neither the fast nor slow off phase shows a DNA concentration dependence (1.2 sec^{-1} and 0.13 sec^{-1}), suggesting an isomerization in the bound state as well. Progress on binding kinetics of entropy enhancing variants will be discussed to explore how partly folded states affect DNA binding kinetics.

Probing the cooperativity and stability of a repeat protein PP32 and cavity mutants using high pressure fluorescence and NMR

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How the primary sequences of proteins define folding cooperativity and free energy landscapes is poorly understood. We examined the cooperativity determinants of PP32, a leucine rich repeat protein with a linear topology which exhibits increasing stability from the N- to the C-terminus. We made mutations in PP32 that created cavities at different locations in the protein. The effect of these cavities on PP32 folding landscapes was probed by high pressure fluorescence and NMR. Pressure denaturation offers an alternative approach to studying protein folding because its effects are due to void volume present in the protein's folded state, rather than the surface area of the unfolded state, leading to a more local effect and a rougher energy landscape with more intermediate states being populated than are observed with chemical denaturants. High pressure NMR studies of PP32 cavity mutants provide equilibrium unfolding data at the residue specific level. These results were used to constrain coarse grained structure-based simulations yielding pseudo free energy profiles and structural characterization of the ensemble at several pressures and temperatures. Pressure-jump (p-jump) studies yielded activation volumes for folding and unfolding, which are defined by the molar volume of the transition state relative to the folded and unfolded states.This high resolution structural and energetic characterization of PP32 cavity containing mutants reveal key features of the PP32 sequence which define its folding cooperativity.

Keywords: High pressure, cooperativity, fluorescence kinetics, LRR proteins

Do Peptidyl-Prolyl Isomerases modify protein aggregation in a Drosophila model of VAP-B/ALS8 associated Amyotrophic Lateral Sclerosis?

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Amyotrophic lateral sclerosis (ALS), also called "Lou Gehrig's disease" (Cleveland and Rothstein, 2001; Tarasiuk et al., 2012) is a progressive, lethal neurodegenerative disease characterized by loss of motor neurons leading to gradual paralysis and death of the patient within 2-5 years post diagnosis. The disease can occur sporadically; or in 5-10% of the patients, the disease occurs due to inheritance of a mutation. A missense mutation (P56S) in the VAMP Associated Protein gene [VAPB/ALS8], a highly conserved gene is one such locus (Nishimura et al., 2004). *Drosophila* models of ALS8 have been generated that show that VAP-P58S aggregates and recruits wildtype protein to these aggregates, eliciting a dominant negative effect (Chai et al., 2008; Ratnaparkhi et al., 2008; Tsuda et al., 2008).

We find that this proline residue at the 56^{th} position is present in the *cis* conformation in human VAPB. This leads to the possibility that any change of a *cis* peptide bond to a *trans* peptide bond may be the central feature in ALS8 (P58S) aggregation. Aggregation of VAPB in neurons has been proposed to be either a cause or a consequence of mechanistic changes in the cell that lead to neuronal cell death (Lev et al., 2008; Ratnaparkhi et al., 2008; Tsuda et al., 2008). We thus wish to explore the importance of having a *cis* conformation at the proline bond in the folding of VAP. Further, we attempt to identify the Peptidyl prolyl isomerase(s) in the cell that are responsible for aiding VAP fold to its correct conformation and modify aggregation kinetics of the protein. This study may provide insight into the mechanism and progression of the ALS8 in humans.

Tracing the evolution of peptide binding specificity in the S100 protein family using phage display and deep sequencing

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Substitutions that alter the binding specificity of proteins are essential for the evolution of new biological functions. To directly study this process, we are tracing changes in the total set of partners recognized by proteins over evolutionary time through a combination of ancestral sequence reconstruction, high-throughput phage display, and rigorous biophysical characterization. We are using two members of the S100 protein family, S100A5 and S100A6, as a model of the evolution of specificity. These proteins bind short peptide regions of interaction-partners in a calcium dependent manner, with each protein having distinct biological targets. To determine how this peptide recognition specificity changed over time, we are deploying a quantitative, high-throughput phage display approach that combines four strategies: 1) use of a competitor peptide to target the relevant binding site on the protein; 2) careful experimental and computational treatment of library composition and sampling bias; 3) quantitative, global analysis of binding using a partition function; and 4) incorporation of clustering and machine-learning to identify important determinants of binding specificity. We have used this approach to characterize peptide binding by human S100A5. Machine-learning analyses indicate that binding peptides are distinguished from non-binders by higher rigidity and distinct side chain patterns. We are currently extending the approach to other S100s, including the last common ancestor of all S100A5 and S100A6 proteins. These experiments will allow us to directly measure how binding specificity has changed evolutionarily and aid identification of new biological targets for these proteins.

A Consensus View of the Factors Favoring Protein Folding: Grappling with Free Energies of Folding via Molecular Dynamics Simulations

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The factors that favour protein folding have been discussed in literature extensively. Hydrophobicity, intramolecular main chain hydrogen bonds and van der Waals interactions are often implicated as favouring folding and loss of main chain and side chain entropy as disfavouring folding. A clear quantification of these factors applied to a large number of systems for developing a consensus view however is lacking. Such an insight would be useful for protein engineering as well as furthering basic science.

Free energies of folding can be arrived at through calorimetry. Parsing these energies into factors favoring or disfavoring folding is not currently amenable to experiment. Thus the question of which factor contributes how much remains answered only via intelligent guesses.

In this study, we seek to address the issue of developing quantitative estimates of the factors involved in protein folding via a large number of microsecond molecular dynamics simulations. We aim to develop a consensus view of the folding energetics and examine the various stabilizing and destabilizing components over the course of the early part of the folding pathway.

Subtle conformational changes between TLR3 TIR WT and A795P mutant revealed by molecular dynamic simulations and residue network analysis

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Toll-like receptors (TLRs), homologues of *Drosophila* Toll, sense pathogen-associated molecular patterns from a variety of pathogens and play a key role in the innate immune system. The TLRs contain N-terminal LRR repeats, a single transmembrane domain and a conserved C-terminal TIR domain. In addition to the receptors, there also exist five TIR domain-containing adaptor molecules. Ligand binding to the receptor leads to its dimerisation and subsequent recruitment of adaptor proteins. This results in a signalling cascade and production of pro-inflammatory cytokines and interferons. The Pro residue in the BB loop of the TIR domains is highly conserved and mutation of this Pro to His abrogates dimerisation. TLR-3 senses viral double stranded RNA and is localised at the endosome. However, TLR-3 is different from other TLRs in two aspects. Firstly, it contains a conserved Alanine (795A) instead of Proline in the BB loop of its TIR domain. Secondly, while all other TLRs utilize the MyD88 adaptor protein, TLR-3 binds directly to the TRIF adaptor protein. A mutation of Ala 795 to Pro 795 led MyD88 to be recruited to TLR-3 instead of TRIF. Currently, the structural basis of receptor TIR-adaptor TIR interactions is not well understood due to the transient nature of these interactions. In this work, using long scale molecular dynamic simulations and residue network analysis, we have attempted to investigate the subtle conformational changes induced by the mutation. The observations made from these analyses will contribute towards obtaining a better picture of the interactions between the TLR receptors and adaptor proteins.

Inhibition Of Formation Of Alpha-Synuclein Amyloid Fibrils And Their Dissolution By Triphala An Ayurvedic Preparation

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Neurodegenerative diseases such as Alzheimer, Parkinson, Huntington, etc. represent a significant and increasing threat to human health and well-being. Parkinson's disease (PD) belongs to the group of diseases known as Lewy body diseases (LBDs) which also includes PD with dementia (PDD) and dementia with Lewy bodies (DLB). The pathological characteristic of these diseases is progressive accumulation of the presynaptic protein alpha-synuclein (alpha-syn) and therefore they are often referred to as synucleinopathies. Various point mutations in the gene encoding alpha-syn (SNCA) namely A30P, A53T, E46K, etc. result in familial parkinson's disease. The oligomerization and fibrillation of alpha-syn and its mutants are central processes in the pathogenesis of synucleinopathies and hence represent significant therapeutic target. We have studied the effect of several ayurvedic preparations such as Ashwagandha, Triphala, etc. on the process of fibrillation of alpha-syn and its mutants. The fibrillation process was monitored by ThioflavinT (ThT)-binding assay and the structural changes were monitored by different spectroscopic and microscopic techniques. We observed that the fibrillation process was completely suppressed in the presence of Triphala. We also studied the effect of several ayurvedic preparations on the stability of pre-formed fibrils of alpha-syn and its mutants. We observed a disruption of the cross beta-sheet fibrils in the presence of Triphala. In conclusion, our results indicate that Triphala is a potential candidate for prevention as well as cure for synucleinopathies.

Design and characterization of novel trimeric cyclic permutants of HIV-1 Env

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HIV-1 Env is a trimeric glycoprotein synthesized as single chain gp160 precursor and expressed on the virion surface after being cleaved into gp120 and gp41 subunits, which are bound to each other in a non-covalent fashion. Previously, it has been shown that properly cleaved trimeric Env immunogens show better binding specificity to neutralizing antibodies by presenting the relevant epitopes in the right conformation and simultaneously occluding non neutralizing epitopes. Owing to the labile interaction of gp120 and gp41, the cleavage site has been mutated previously but these trimers failed to take up a native like structure. Recently, a 3.5 Å crystal structure of SOSIP gp140 from subtype A BG505 strain of HIV-1 has been reported. It provides important insights about the relative orientation and interactions of gp120 and gp41 subunits. The structure reveals that the gp41 C-terminus is in very close proximity (~8Å) to the N-terminus of gp120 from an adjacent subunit. We have designed a cyclic permutant of gp140 from JRFL and BG505 strain where the gp41 C terminus is now connected to the gp120 N-terminus with a short linker. This novel connectivity results in preservation of the native gp41 N-terminus along with a much shorter linker length than in conventional gp140. This may promote trimer folding and stabilization because of the resulting decreased magnitude of conformational entropy change during folding. The structure also reveals that the gp120 C-terminus is close to the trimer axis, and due to cyclic permutation, this becomes the new C-terminus of gp140. To further stabilize the trimeric form, we have attached a foldon trimerization domain at the C terminus. The protein has been expressed and purified from mammalian cells. The protein exists primarily as a trimer in solution as assessed by SEC-MALS. It shows better binding to broadly neutralizing antibody b12 when compared to b6, a non neutralizing antibody. Further biophysical characterization of the protein is in progress.

Significant heterogeneityand slow transition of the denatured ubiquitin detected by single molecule fluorescence spectroscopy

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We investigated the folding of ubiquitin at the single-molecule level using line confocal microscope at the temporal resolution of 100 μs. We obtained the time series of sm-FRET efficiency from ubiquitin labeled with two different dyes. At low concentrations of denaturant, we obtained the traces having the high FRET efficiency corresponding to the native state. At high concentrations of denaturant, the traces possessed the low FRET efficiency corresponding to the denatured state. We calculated the shot noise-limited probability density functions of the sm-FRET efficiency distribution for the native and denatured states, and compared them with the histograms of the observed FRET efficiency. While the observed peak for the native state is comparable to the noise width, the denatured peak is significantly broader than the noise width. Contrary to the previous concept of the denatured proteins, which fluctuate randomly without any structures, our data suggested that the denatured ubiquitin are heterogeneous and that the heterogeneous components possess the lifetime longer than several milliseconds. We inferred that the heterogeneity of the denatured state was caused by the local structural dynamics around the labeled fluorophores, and that the slow transitions revealed by sm-FRET measurements might be the common properties of the denatured proteins.

Effect of pH on the structure, function and stability of MARK4

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Microtubule affinity regulatory kinases 4 belongs to the family AMP-activated protein kinase, and is known to phosphorylates microtubule associated proteins at specific sites (serine in KXGS motifs) in the microtubule-binding repeats. Here, we have cloned, expressed and purified two variants of MARK4 [the kinase domain (MARK4-F2), and kinase domain along with 59 N-terminal residues (MARK4-F1)] and compared their stability at varying pH range. Structural and functional changes were observed by incubating both forms of MARK4 in buffers of different pH. We measured the secondary structure of MARK4 using circular dichroism and tertiary structure by measuring intrinsic fluorescence and absorbance properties along with the size of proteins by dynamic light scattering. We observed that at extremes of pH (below pH 3.5 and above pH 9.0), MARK4 is quite stable. However, a remarkable aggregate formation was observed at intermediate pH (between pH 3.5 and 9.0). To further validate this results, we have modelled both forms of MARK4 and performed molecular dynamics simulation for 15 ns. The spectroscopic observations are in excellent agreement with the findings of molecular dynamics simulation. Now the question is, what brings MARK4 stability at physiological pH? In order to solve this puzzle, we have cloned, expressed, and purified UBA-domain along with catalytic domain of MARK4. We found pronounced structure at different pH by using different spectroscopic techniques, supported by the activity assay. This variant of MARK4 was stable at physiological pH, indicates that UBA domain may provide stability to the protein and thus function at physiological pH conditions.

Amino Acid Impact Factor

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Conventionally intra and inter-protein amino acid interactions are studied using symmetric correlation matrices that can be formulated from sequence, structure or dynamics data. However, the asymmetric nature of amino acid interactions is not evident from such analysis. Here we introduce a new definition, called the amino acid impact factor to highlight the effect an amino acid mutation on others. We also illustrate this impact graphically with a network analysis. In the example networks we present, the effect of distal mutations becomes intuitive, and we hope Amino Acid Impact serves the purpose ofweighing the consequences of mutations in a protein.

References:

C. K. Sruthi, M. K. Prakash, Amino Acid Impact Factor (submitted)

Toward the molecular mechanism by which vitamin C inhibits amyloid formation and remodels mature amyloid fibrils: possible therapeutic interventions

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Insulin amyloid fibrillation is associated with type II diabetes and its inhibition is believed to be a promising strategy for the treatment of this amyloidosis. Here in, we have shown that vitamin C inhibits the aggregation and fibril formation of insulin in a dose-dependent manner at reasonable concentrations by combining biophysical characterization, computational modelling and cell toxicity measurements. This finding is supported by Thioflavin T assay, where the rapid evolution of insulin nucleation and elongation processes is slowed when vitamin C is present. Secondary structural changes of insulin were monitored by circular dichroism (CD) spectroscopy which inferred that less beta sheet formation in presence of vitamin C. Dynamic light scattering (DLS) and Transmission electron microscopy (TEM) images also confirmed the reduction of size of insulin aggregate in presence of vitamin C. Furthermore, Vitamin C was also found to prevent amyloid induced neurotoxicity on human neuronal cell line, SHY-5Y cells. Importantly, it was also found that vitamin C remodel preformed amyloids to less toxic amorphous aggregates with less cross beta sheet content. Moreover mode of interaction of vitamin C with insulin was monitored by molecular docking. This work would pave the way for rational designing of Vitamin C based molecules that may have therapeutic potential for treatment of debilitating amyloid associated diseases.

Design of halophilic mutants by predictive approaches

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Halophiles thrive in very high salt concentrations, requiring a vast array of sequence, structural and physiological adjustments. To understand the molecular basis of halo-adaptation of proteins, nucleotide sequence, primary amino acid sequence and the 3D protein structure of halophilic bacterial species were analyzed. Present study utilizes a novel two-step hybrid approach (machine learning and analytic hierarchy process-AHP) to derive halostabilizing mutations. Halostabilizing protein attributes have been ranked and a model has been generated for predicting multiple mutations leading to halostability. Various machine learning approaches were tested on 64 codon, 29 amino acid and 20 structural feature parameters. The ranking was developed on a final dataset of 100 pairs of halophilic and non-halophilic proteins. Statistical analysis reveals that k-nearest neighbour (k=10) and artificial neural network classifies halophilic/non-halophilic protein with 91.67% accuracy, respectively. Results also show that the trend of GAC (Asp) codon increases in halophilic proteins. Asp and Thr followed by acidic amino acid content was found to be increasing in halophilic proteins. The accuracy obtained by AHP was 90.0%. Further, the generated model will be validated by halostabilizing mutations using various spectroscopic methods and molecular dynamic simulation in *Bacillus subtilis* lipase to monitor dynamic changes.

Keywords: Halophilic proteins, codon, amino acid, mutagenesis, structure, stability, folding and dynamics

Characterizing the Transition State Ensemble and Reaction Coordinate during Mechanical Protein Unfolding using Steered Molecular Dynamics Simulations

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Despite the availability of computational resources to simulate a large number of independent protein unfolding simulations and to capture high (atomistic) resolution structures of protein along the unfolding pathway, the transition state and the reaction coordinate of the unfolding event remain poorly characterized. In this study we present a framework to characterize the structural changes occurring in proteins as they unfold under the action of an external force in Steered Molecular Dynamics (SMD) simulations. We propose possible strategies to identify the reaction coordinate connecting the transition state to the ground state.We show that a judicious choice of the reaction coordinate (rather than the conventional N-C terminal distance) can enable unambiguous identification and characterization of the transition state. The concept of a transition state ensemble (TSE) in the context of these simulations and its characterization using Principal Component Analysis of the simulation data is introduced. We applied the analysis to SMD simulations of structurally homologous ubiquitin family proteins (ubiquitin, SUMO1 and SUMO2) to probe the structural basis for the differences in the stability of these proteins.

Function Can Modulate the Mechanism of Domain Swapping

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Domain-swapping is a mechanism in which two protein chains fold by mutually exchanging their respective domains. One would expect protein possessing similar native fold to exhibit similar folding and domain swapping propensities. The cysteine protease inhibitor, stefin-B and sweet-tasting protein, monellin, present a unique case. Despite having similar structures (β1α1β2β3β4β5) the two differ in their folding mechanism and domain-swapping propensity. Using all-atom structure-based models and molecular dynamics simulations the folding of monellin and stefin-B is investigated to understand these differences. Results from simulations indicate that monellin folds in a cooperative manner while that of stefin-B populates an intermediate. In stefin-B, the backbone dihedral of VAL48 (involved in protease binding) was found to shift from a disallowed region of the Ramachandran map when folded as a monomer to an allowed region in the domain-swapped structure. Upon encoding this effect in stefin-B a new intermediate (β3β4β5) is formed which mimics one of the swapped region. We conclude that the function of monellin which is spread over the protein can accommodate cooperative folding while the more localized functional residues (in N-terminal, loop1 and loop2) as exhibited in stefin-B promote the formation of a folding-intermediate which in turn can lead to domain swapping and aggregation.

A General Mechanism for Mutation-Induced Destabilization and Modulation of Allosteric Coupling in Proteins

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Mutations in the hydrophobic interior of proteins are generally thought to weaken the interactions in their immediate neighborhood. However, network theory predicts such perturbations to influence even distal residue-residue communication networks. Analysis of microsecond-long molecular dynamics simulations of several aliphatic mutants of ubiquitin reveals that mutational effects consistently propagate into the second shell of the altered site (even up to 15-20 Å) in proportion to the perturbation magnitude and dissipates exponentially with a decay distance-constant of ~5 Å. We also report evidence for this phenomenon from published experimental data. Reformulating these observations onto a statistical mechanical model, we reproduce the stability changes of 375 mutations from 19 single-domain proteins. Our work thus reveals a robust energy dissipation-cum-signaling mechanism in the interaction networks within proteins, quantifies the partitioning of destabilization energetics around the mutation neighborhood and presents a simple theoretical framework for modeling the allosteric effects of point mutations.

Creation of depth dependent substitution matrices

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The 20 naturally occurring amino acids have different environmental preferences of where they are likely to occur in protein structures. Environments in a protein can be classified by their proximity to solvent, a measure called as residue depth. Since the frequencies of amino acids are different at various depth levels, the substitution frequencies should vary according to depth. To quantify the substitution rates, we built depth dependent substitution matrices. The dataset used for creation of the matrices consisted of high quality 3696 non redundant pairwise protein structural alignments. One of the applications of these matrices is to look at the tolerance of mutations at different environments. Using these substitution scores the prediction of deleterious mutations were done on 3344 mutations in T4 lysozyme and CcdB. The accuracy of the technique in terms of MCC (Matthews Correlation Coefficient) is 0.41, comparable to other state of art methods. Depth dependent substitution matrices could also help in improving structure-sequence alignment for protein 3D structure modeling.

Engineering domain swapping in monellin

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Domain swapping is a mode of protein self-association where dimers or higher order protein oligomers are formed from monomeric species by secondary or tertiary structural element exchange. Experiments and simulations have shown that the information from only the monomer structure and energetics can help predict the structure of domain swapped dimers and the mechanism of their formation. Structure-based molecular dynamic simulations of stefin-B and single chain monellin, two proteins with same topology (β1-α1-β2-β3-β4-β5) but very different domain swapping propensities show that domain swapping is a consequence of the constraints imposed by function on protein structure, which is captured very well in the folding simulations of these two proteins in the form of a folding intermediate (mimicking the β3- β4- β5 swapped domain of dimer) which can directly lead to domain swapping in case of stefin-B, but not monellin. It follows from this result that if similar dihedral frustration is introduced into monellin, it will increase its domain-swapping propensity.

I show experimental evidence confirming the predictions from the above study. Preliminary results from size-exclusion chromatography and multi-angle light scattering show that replacing the Gly-Phe linker of scMN by a QVVAG linker converts the protein from a monomer to a dimer, with no observable monomeric fraction. Dependence of the refolding rates of QVVAG-scMN on protein concentration further substantiates the claim that this protein exists as a dimer. In the QVNAG variant of scMN, roughly 20% of the protein exists as a monomer. We are currently crystallizing the QVVAG-scMN to deduce a high-resolution structure to prove that the experimentally observed dimer is indeed a domain-swapped dimer.

Early events in assimilation of GNNQQNY peptide: From segregation to aggregation

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Amyloid fibrils are aberrantly associated with numerous pathological neurodegenerative diseases. These deposits are formed via oligomeric intermediates, which are plausible cytotoxic entities in these diseases. Thus, complete understanding of the pathway particularly from segregated dispersed monomers to oligomerization, becomes imperative for rational therapeutic design. In this perspective, we have explored the kinetics of aggregate formation of fibril forming GNNQQNY peptide fragment from the N-terminal prion determining domain of yeast protein SUP35. Using all atom conventional atomistic MD simulations, we have validated the experimentally observed nucleated conformational conversion mechanism and gained additional insights into the factors governing the process. Results suggest that although at any temperature/concentration, beta sheet formation follows peptide assimilation; however its propagation is more consistent at lower concentration. Increasing concentration leads to unfavourable backbone-water interactions resulting in formation of clump of collapsed disordered peptides. Kinetic profile demonstrates that aggregation phenomenon is impeded by different barriers in the early and later oligomerization steps. Principal component analyses of 26 different properties were employed to delve into the cause of its existence. Compared to the latter, the initial barrier can be easily surpassed by higher temperature and concentration, representative of different barrier heights for structured and unstructured aggregates. Hydration layer water molecules around larger aggregates are slightly structured and exhibits moderately slow dynamics compared to early small oligomers. Different metastable oligomers with both parallel and antiparallel configurations were identified during both the lags. Hydrogen bonding interactions were observed to be the principal determinant of aggregate formation. The growth of these aggregates proceeds via bimolecular association with condensation as the predominant assembly pathway.

Polyphenols modulates the aggregation pathway of ALS linked human Cu/Zn Superoxide Dismutase

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that affects motor neurons. In vivo studies have shown that ALS is associated with aggregation of various forms of Cu/Zn Superoxide Dismutase (SOD1). Evidences suggest that SOD1 containing pathological inclusions in ALS exhibit amyloid like properties. Unfortunately, effective therapeutics against this disease has not yet been developed. In the present study, we observed the DTT induced fibrillation of SOD1 in the presence of various polyphenols (like curcumin, quercetin etc.). Using various biophysical techniques, we have demonstrated that curcumin and quercetin inhibit the fibrillation of SOD1. Further, there is a significant increase in SOD1 mediated toxicity in the regime of prefibrillar and fibrillar aggregates which is not evident in polyphenol containing samples. Our results show that polyphenols interacts strongly with these prefibrillar and fibrillar aggregates. Hence, these polyphenols can act as potential candidates to combat the pathogenic misfolded SOD1 species found in ALS patients and may pave the way in designing novel therapeutic agents. We have tried to shed some light on the mechanism of inhibition of fibrillation by these polyphenols

Isolated globular domain of human guanylate binding protein-2 is more stable than its full length protein

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Small GTPases are single domain proteins that are unstable in the absence of the nucleotide either GTP or GDP. However, large GTPases which are multi-domain proteins have evolved in a manner that they are stable even in the absence of the nucleotides. Human guanylate binding protein-1 (hGBP1) is such kind of large GTPase which is comprised of an N-terminal globular (G) domain, C-terminal helical domain which is linked by an intermediate region. It hydrolyzes GTP to both GDP and GMP with the GMP being the major product. Data from our laboratory suggest that the isolated G-domain in full length hGBP1 is stable to some extent without the nucleotide, but the protein is more stable in the presence of both the helical domain and the intermediate region. Thermodynamic stability of the full-length protein is found to be higher than the sum of its individual domains, suggesting that interdomain interactions provide further stability to the full length protein. However, hGBP2, a homolog of hGBP1, hydrolyses GTP to GDP and GMP, where GDP is the major product. To investigate whether in hGBP2 interdomain interaction play similar role in the stability of the protein, we prepared truncated proteins and determined their thermodynamic stability. We found that the free energy of the truncated protein lacking the helical domain is higher than that of the full-length protein, suggesting that the interaction of the helical domain reduces the stability of the full-length protein. Thermal denaturation studies of the isolated domains showed two-state transition, whereas the full length protein showed three states transition, indicating that domains are unfolding individually. This finding is supported further by unfolding kinetics, where the protein lacking the helical domain unfolds slower than that of the full-length protein. Structural analysis shows the presence of the positively charged clusters of both the G-domain and the helical-domain are in vicinity of each other. This could be the reason for the decreased stability of the full-length protein compared to its isolated G domain with intermediate region.

Modulation of the dynamics of CcdB by Gyrase and CcdA: insights into the mechanism of CcdA action

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CcdB is a toxin of the type-II TA system in *E. coli*, which kills the cell by inhibiting its target protein, DNA Gyrase. Normally, the toxicity of the CcdB is neutralized by its cognate anti-toxin CcdA. CcdA binds to free CcdB and also facilitates active dissociation of the CcdB-Gyrase complex. The molecular mechanism of the action of CcdA is yet to be understood. To obtain insights into the process, native state dynamics of CcdB has been investigated using HX-MS in both the absence and presence of either CcdA or Gyrase. The HX process of CcdB in the absence of any binding partner has three phases, where the two faster phases arise due to local unfolding, and the slowest phase arises due to global unfolding. Binding of CcdA and Gyrase causes increased protection in CcdB against HX and hinders its global unfolding. Interestingly, many segments of CcdB show decreased protection against HX upon binding to CcdA. These segments also show decreased protection upon Gyrase binding, although they don't interact with the Gyrase molecule. These results suggest that the modulation of the dynamics of CcdB by Gyrase may enable the action of CcdA by facilitating its binding to CcdB.

Evidence of dry molten globule like state in a multidomain protein

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Hydrophobic effects are believed as a major contributor in the stability of functional structure of proteins and a little is known about the significance and contribution of van der Waals (vdW) packing interactions in protein stability. In this study we have used various spectroscopic techniques viz., fluorescence resonance energy transfer, dynamic fluorescence quenching, red-edge excitation shift, and near- and far-UV circular dichroism to probe a dry molten globule (DMG) like state during pH induced structural changes in a multidomain protein. We provide evidence for presence of DMG like state in two of the three domains of a multidomain protein having disrupted side chain packing with dry core. Furthermore, we demonstrate the significance of vdW packing interactions in the stability of protein by quantitatively estimating the energetic contribution of vdW packing interactions in protein stability.

Dynamics and flexibility of Skp1 govern binding and recognition of Fbox proteins in SCF complex

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Ubiquitin mediated proteosomal system plays a central role in protein degradation machinery. In proteasomal system, E1-activating enzyme, E2-conjugating enzyme, and E3 ligase enzyme work in cascade to targate the substrate for ubiquitination. SCF (Skp1-Cullin-Fbox) complex is one of the E3 enzymes that ubiquitinates the cell cycle regulatory proteins. SCF complex is formed by substrate binding protein with F-box domain (Skp2), adapter protein Skp1, central scaffold Cullin, and the ring box protein Rbx. Skp1 protein recognizes the Skp2 protein and presents it to the rest of the SCF complex by binding to N-terminal domain of Cullin. NMR is the very usefull technique to calculate structure and dynamics of protein at atomic level resolution. NOESY-HSQC experiments performed for structure calculation and T1 and T2 relaxation experiments for dynamics calculation. Sequence specific backbone and side chain assignment was completed and residue specific secondary structure as well as dynamic nature of Skp1 protein from chemical shift was obtained from NMR assignment. Additionally we also performed three molecular dynamic simulations (each 200 ns long) of Skp1 protein using Gromacs 4.6 package. The assignments reported here will further our understanding of the standalone Skp1 structure and will pave way for the future characterization of protein structure-dynamics and will assist in ascertaining mode of interaction of Skp1 with various F-box proteins.

Observing a late folding intermediate of Ubiquitin

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The study of intermediates in the protein folding pathway provides a wealth of information about the energy landscape. The intermediates also frequently initiate pathogenic fibril formations. While observing the intermediates is difficult due to their transient nature, extreme conditions can partially unfold the proteins and provide a glimpse of the intermediate states. Here, we observe the high resolution structure of a hydrophobic core mutant of Ubiquitin at an extreme acidic pH by Nuclear Magnetic Resonance (NMR) spectroscopy. In the structure, the native secondary and tertiary structure is conserved for a major part of the protein. However, a long loop between the beta strands partially unfolded. The altered structure is supported by fluorescence data and the difference in free energies between the native state and the intermediate is reflected in the denaturant induced melting curves. The unfolded region includes amino acids that are critical for interaction with co-factors as well as for assembly of poly-Ubiquitin chains. The structure at acidic pH resembles a late folding intermediate of Ubiquitin and indicates that upon stabilization of the protein's core, the long loop converges on the core in the final step of the folding process.

Elucidating the conformational dynamics and anti-pathogenic activity of bovine lactoferrin–nanoparticle conjugates

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Delineating the complex molecular recognition in proteins can help to reveal the folding mechanism and conformational dynamics. Bovine lactoferrin (BLf) is a monomeric, bilobed protein principally isolated from secretory fluids such as milk, saliva, and tears. It has antipathogenic and immunomodulatory properties which solely depend on its unusual structure. It comprises of all types of secondary structure including a rare 3_{10} helix. Thus it can be used as a model protein to study the conformational dynamics. Studying protein-nanoparticle interactions can have far-reaching applications in terms of how a nanoparticle may behave inside biological systems. Moreover, the rough surface of nanoparticles is known to promote the conformational changes in interacting proteins. Therefore, in the present study, we aim to explore how the binding of Bovine Lactoferrin (300 ug/ml) with different concentration of silver nanoparticles (AgNPs) can alter its conformation, subsequently the anti-pathogenic activity. For the biophysical studies, UV-visible spectroscopy, fluorescence spectroscopy, circular dichroism (C.D.) spectroscopy and zeta potential studies have been employed. The binding of BLF over AgNPs is reflected by the surface Plasmon resonance peak shift of AgNPs conjugates from 396 nm to 388 nm. Also, the surface charge of BLF (-13.1 mV) neutralizing the surface potential of AgNPs (-36.8 mV) to -12.8 mV inferring that the charged residues of BLF are non-covalently interact with AgNPs. CD spectroscopy and intrinsic fluorescence data revealed that higher concentration of AgNPs (40 ug/ml) is disrupting the conformation and stability of BLF, however, the conformation is preserved in 20 ug/ml AgNPs. Therefore 20 ug/ml AgNPs was used for BLF-AgNPs conjugation. The antimicrobial investigation of BLF-AgNPs conjugates was found to be higher in contrast with BLF and AgNPs alone.

Alternatively packed native like intermediate as observed in a urea induced unfolding transition of a multi domain protein

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The folding funnel pictures folding of proteins into their native confirmations via different pathways in the energy landscape model of protein folding. The native state is believed to be assisted with minimum free energy. But very little is known about the equilibrium conformations of the native state itself. For instance, if the native state represents a single state or it is an equilibrium mixture of native state and many different native-like intermediates?

Herein, we present evidence for alternatively packed native like intermediate in the chemical denaturant induced unfolding transitions of a multi domain protein, Human Serum Albumin. The intermediate was characterised by different tools of fluorescence and CD spectroscopy and seemed to possess the characteristics of a dry molten globule where the core is de-solvated and side chain packings are disrupted.

Stabilization Induced Reduction in Cooperativity of Protein Folding

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Highly cooperative folding of small globular proteins (two state folder) imposed a view that cooperativity is the inherent property of protein molecules and one invariant transition state separates the native state from the unfolded state. However, energy landscape theory predicts that there are many pathways available to protein for folding and they are sensitive to solvent conditions. Evidences suggest that folding is not always a barrier limiting reaction; it could be highly non cooperative like one state downhill folding. In the current study we observed that native state of PI3K SH3 undergoes large cooperative unfolding suggesting that native and unfolded states are separated by a large free energy barrier. Reduction in cooperativity observed under strong stabilizing condition suggests that smooth free energy barrier becomes highly rugged and lead to non-cooperative unfolding. Our study suggest that, change in stability of protein lead to modulation of cooperativity of unfolding and resulting in change in free energy landscape from a barrier limited (highly cooperative) to a barrier less (non cooperative).

Biophysical Characterization of Aspartyl-tRNA synthetase from the eukaryotic human pathogen Entamoeba histolytica

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Entamoeba histolytica is a food and water borne pathogen abundant in the tropical areas affecting the large intestines of mammals, mostly humans. Aminoacyl-tRNA synthetases are central enzymes in protein translation, providing the charged tRNAs for the appropriate synthesis of peptide chains. The crystal structure of *Entamoeba histolytica* Aspartyl-tRNA synthetase (Ehis-AspRS) has been solved. The presence of a single tryptophan at position 507, makes it an attractive system for biophysical studies. The objective of this study is to elucidate the structure–function relationship of Ehis-AspRS and to establish it as a probable drug target. Ehis-AspRS follows a three state transition in presence of chemical denaturants, and the intermediate is not a molten globule. However, it is very stable under varying pH as some residual structural remains are present even above pH 12. Our preliminary observation suggests that Ehis-AspRS unfolds differently in presence of different denaturants. Ligand binding studies using fluorescence quenching technique and isothermal titration calorimetry (ITC) revealed that Ehis-AspRS has probably undergone conformational changes upon binding to its cognate amino acid (L-Aspartate) that increases its affinity for ATP.

Mechanistic Switching in Protein Aggregation: Nanoscale Diversity of Amyloids Derived from Melanosomal Protein

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The consensus that amyloid fibrils also play beneficial roles in various organisms, ranging from bacteria to humans, has evolved in recent years. This emerging class of amyloids is denoted as functional amyloids. One such functional amyloid is formed by the melanosomal protein Pmel17, which is a melanocyte specific transmembrane glycoprotein that plays an essential role in melanosome maturation and its amyloid fibrils acts as a template for melanin deposition in skin and eyes. Pmel17 comprises an intrinsically disordered polypeptide fragment namely, the repeat (RPT) domain, which forms the amyloid core that promotes the melanin biosynthesis. Previous studies have reported that the RPT domain aggregates under mildly acidic condition. However, the molecular mechanism of amyloid formation as well as the organization of individual protein molecules within the supramolecular amyloids still remains elusive. We have used an array of spectroscopic and imaging techniques involving fluorescence, circular dichroism, light scattering and atomic force microscopy to elucidate the mechanistic details of pH-dependent aggregation of the RPT domain. Our results suggest that a mechanistic switch in the aggregation pathway occurs from an isodesmic to a nucleation-dependent polymerization as a function of pH which leads to the genesis of diverse nanoscopic dendritic and straight fibrils.

Interactions of Conformationally Distinct Amyloid-β Oligomers with Prion and Tau Proteins

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A pathological characteristic of Alzheimer's disease is the extracellular fibrillar deposits of amyloid-β (Aβ) peptide known as amyloid plaques and intra-neuronal deposits of hyper-phosphorylated tau known as neurofibrillary tangles in the brain. Soluble oligomers of Aβ are recognized as key intermediates that cause synaptic dysfunction and neurotoxicity. Also, it has been conjectured that soluble Aβ oligomers induce tau hyper-phosphorylation. However, the mechanism of oligomers-induced neurotoxicity as well as Aβ-mediated tau hyper-phosphorylation remains elusive. Our efforts are directed towards understanding the mechanistic details of Aβ-induced tau pathology using a host of biochemical and biophysical tools. Additionally, recent evidences suggest that the cellular prion protein (PrP) acts as one of the receptors for soluble Aβ oligomers and this binding event is proposed to inhibit long term potentiation. Therefore, we are also investigating the binding affinities and the binding sites of two distinct conformational oligomers of Aβ namely, prefibrillar- and fibrillar oligomers recognized by conformation-dependent antibodies, with PrP by using an array of biophysical techniques.

Role of the active site lysine (K33) in ATP binding and activation of CDK1

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Cyclin dependent kinases (CDKs) are essential serine-threonine kinases controlling cell cycle transition and progression through different phases. CDKs have long been known to be regulated by activatory and inhibitory phosphorylations, which majorly impact kinase activity of the Cyclin-CDK complex. In contrast, while acetylation of CDKs (CDK2/CDK5/CDK9) has been reported, little is known about the physiological significance of this acetylation. In this study, we use atomistic molecular dynamics simulations to assess the effect of point mutations and acetylation of a key conserved active site lysine (K33) on ATP binding and complexation with CyclinB. Our studies reveal key switching interactions between K33, the incoming ATP, and conserved acidic residues (D146, E51) which are disrupted by the mutations. Our studies provide a molecular basis to understand the biochemical characterization of both K-R and K-Q mutants which indicate that acetylation is key in mediating the activation cycle of CDK1 and impinges on CDK1-CyclinB complex formation. Given the fundamental property of cells to divide and proliferate, our findings provide novel insights into CDK regulation and hence cell cycle progression by acetylation-deacetylation cycles.

Zika virus: Learning its disordered side of proteome

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Over the last few decades, concepts of protein intrinsic disorder have been implicated in different biological processes. Recent studies have suggested that intrinsically disordered proteins (IDPs) provide structural plasticity and functional diversity to viral proteins that are involved in rapid replication and immune evasion in host cells. In case of Zika virus, the roles of protein intrinsic disorder in mechanisms of pathogenesis are not completely understood. In this study, we have analysed the prevalence of intrinsic disorder in Zika virus proteome (strain MR 766). Our analyses revealed that Zika virus polyprotein is enriched with intrinsically disordered protein regions (IDPRs) and this finding is consistent with previous reports on the involvement of IDPs in shell formation and virulence of the Flaviviridae family. We found abundant IDPRs in Capsid, NS2B, NS3, NS4A, and NS5 proteins that are involved in mature particle formation and replication.

Structural and mechanistic insights into human splicing factor SF3b complex derived using an integrative structure modeling approach

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Spliceosome is a highly complex snRNP machinery and performs pre-mRNA splicing in eukaryotes. SF3b is a multi-protein complex and as part of U2 snRNP or U11/U12 di-snRNP in the dynamic spliceosome machinery recognizes the branch-point adenosine of pre-mRNA. Although a cryo-EM density map exists for the seven protein human SF3b complex, the structure and relative spatial arrangement of the SF3b components is not known. Hence, using an integrative approach, involving cryo-EM density maps, structural and other experimental data tailored with effective computational techniques, a pseudo-atomic model for the closed form of SF3b complex was deciphered. Thus, for the first time the model provides structural information for five proteins (SF3b10, SF3b155, SF3b145, SF3b130 and SF3b14b) and localization information for four proteins (SF3b10, SF3b145, SF3b130 and SF3b14b). In addition, it was found that SF3b is a "fuzzy complex" with highly flexible components and multiplicity of folds. An existing U11/U12 di-snRNP cryo-EM density map further allowed the modeling of an open form of SF3b complex. This open form provided insights on the mechanistic features involved in the transition between closed and open forms pivoted by a hinge region in the SF3b155 protein that also harbors cancer causing mutations. Finally, the open form guided model of the 5′ end of U12 snRNA which also comprises the branch point duplex region with pre-mRNA shows that the architecture of SF3b acts as a scaffold for U12 snRNA: pre-mRNA branch point duplex formation with potential implications for branch point adenosine recognition fidelity.

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Structural characterization of intermediates formed during folding of mouse prion protein using FRET

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Protein self packing into functional folded state or toxic aggregates is dictated by its energy landscape which is encoded in its primary sequence. Misfolding of cellular prion protein is known to cause several neurodegenerative diseases collectively known as transmissible spongiform encephalopathies (TSEs). Several folding studies of prion protein have implicated folding intermediates that might act as branch point between folding and aggregation pathways. However there is no structural information for these intermediates. The structural mechanism of conversion of helical monomer to beta sheet rich aggregates was elucidated by using hydrogen exchange mass spectrometry studies. We are currently studying the folding and unfolding of mouse prion protein using FRET. The single tryptophan residue at position 144 is used as donor fluorophore and a thionitrobenzoate attached to free cysteine at position 199 as acceptor. Using FRET as a probe distance changes as a function of folding or unfolding time will be monitored. This study will help in structurally characterizing the folding intermediates and also correlating sequence of events that occur during aggregation to sequence of events that occur during folding transition.

Interaction of α-synuclein with membranes: Role of lipid properties

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Aggregation of the protein α-synuclein (ASN) is associated with neurodegeneration in Parkinson's disease. A proposed function of ASN is regulation of synaptic vesicles in the brain. ASN binds strongly to anionic lipid vesicles in vitro and undergoes a transition from intrinsically-disordered to partially α-helical. Membrane properties such as lipid molecular shape, lipid phase, and curvature (size) will likely modulate the interaction of ASN with lipid vesicles but systematic studies have not been reported. Membrane bilayer properties affects the aggregation process (speed/mechanism) of ASN as both inhibition and promotion of ASN amyloid formation in the presence of vesicles have been reported. To separate the individual effects of lipid shape, charge and curvature, we used far-UV circular dichroism and fluorescence to probe ASN secondary structure and aggregation propensity upon interacting with bilayers of varying lipid composition (combinations of DOPG, DOPS, DOPC, DOPE and DOPA). All experiments were performed at 37° C, pH 6.5 and in the absence of salts. We observed that upon comparing ASN interactions with liposomes of equimolar mixtures of DOPS:DOPE and DOPG:DOPE, there is a higher number of lipids bound per ASN and more induced α-helix in ASN for DOPS-containing vesicles. Moreover, pure DOPS and DOPG vesicles accelerate ASN aggregation (in a process that depends non-linearly on the fraction-bound ASN) whereas such effect is lacking in DOPE-containing vesicles.

Chemical Environment Induced Cytoplasmic Phase Transitions Affect Cellular Proteostasis

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Natural environments such as chemical compositions of surroundings have profound role in shaping the evolutionary paths of organisms. Especially small molecules - which can diffuse across cell barriers - are potent effectors of cellular response and fitness on shorter and longer time scales respectively. Optimal proteostasis is crucial for cells fitness which is evident by the fact that maintenance of proteostasis is one of the most energetically costly processes in cell. Here we investigate potential impact of chemical environments on the proteostasis of *Saccharomyces cerevisiae.* We find that chemical chaperones i.e. small molecules that assist protein folding [1, 2] rescue the effect of inhibitors which inhibit protein folding. Interestingly, chemical chaperones also revert reduced diffusivity of cytoplasm by inhibitors as monitored by μNS probe [3]. From current data, we establish a role of cytoplasmic phase transitions [4] in maintenance of cytoplasmic proteostasis in cases of changes in chemical environments. Collectively we find that chemical chaperones assist cytoplasmic protein folding by improving fluidity of cytoplasm. We also find that physico-chemical properties of small molecules such as polarizability and hydrophobicity are important in guiding these effects while deeper investigation of underlying mechanistic details is still under progress.

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Rapid epitope mapping for neutralizing antibodies and other entry inhibitors of HIV-1

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The HIV-1 envelope glycoprotein (Env), plays a critical role in the initial events of viral infection. An effective HIV/AIDS vaccine is expected to elicit high titres of broadly neutralizing antibodies (bNAbs) against Env spikes. Identifying epitopes recognized by these bNAbs will inform immunogen design. The primary objective of this work is to develop a method for rapid epitope mapping of bNAbs and other entry inhibitors of HIV-1. Using high-throughput PCR based site-directed mutagenesis strategies, a site-saturation mutagenesis library of the env gene is being made in the context of full-length proviral DNA. Information obtained from crystal structures of the HIV-1 Env, as well as neutralization sensitivity data, was used to select 14 residues in the primary receptor CD4-binding site, which were randomized by our site-saturation mutagenesis approach. A library of single-site mutant viruses was made, and used to infect TZM-bl cells in the presence and absence of a bNAb. Viruses capable of productive infection are being isolated, and deep sequencing carried out on the env gene of virus pools before and after infection. Mutations that make the virus resistant to a bNAb are likely to be epitopic residues. In principle, this approach could be used for epitope mapping of any viral protein.

Role of Intermediates Populated in the Folding Pathway of the Prion Protein in Initiating Prion Aggregation

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The identification and structural characterization of the conformation of the prion protein, from which misfolding and aggregation initiate, has been a long-standing challenge. In this study, mutagenesis in the hydrophobic core of the globular domain of the mouse prion protein is observed to stabilize a monomeric, on-pathway, unfolding intermediate conformation, which populates significantly at equilibrium. The rate of misfolding of the protein to cytotoxic β-structured oligomers exhibiting partial proteinase-K resistance is found to correlate well with the extent to which the intermediate populates and is limited by dimerization of this intermediate suggesting that it is the direct monomeric precursor initiating misfolding and oligomerization. Structural and energetic characterization of the intermediate by native-state hydrogen exchange monitored by mass spectrometry indicates that the intermediate is a partially unfolded form of the native state of the protein which forms under misfolding-prone solvent conditions. Kinetic folding studies in the micro-second regime carried out at different experimental conditions indicates that the protein folds through multiple pathways at low pH while at higher pH, it folds predominantly via a single pathway with the intermediate being obligatory to the folding of the protein.

Clustering of STIM1 on the Endoplasmic Reticulum membrane

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Polarization spectroscopy coupled to Laser Scanning confocal microscopy has been established as a technique to study clustering of proteins. In cases, where protein of interest tagged with a fluorophore are in close proximity, homo-FRET (Forster's Resonance Energy Transfer) can occur. Using homo-FRET, we observed the clustering of STIM1. STIM is a single pass Endoplasmic Reticulum resident membrane protein. It has emerged as the central protein for sustained calcium signalling, especially in T-lymphocytes. It. Clustering of STIM1 is a prerequisite for its activation. Previous work has indicated a role for its trans-membrane region in the clustering of STIM. Here, we systematically study the role of the TM helix in activation of STIM.

Characterization of heterogeneity in the early stages of folding of Single chain monellin

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Single chain monellin (MNEI) is a variant of sweet plant protein monellin consisting of 95 amino acid residues. It has one of the 10 most common protein folds, "beta grasp fold", where the sole helix is packed against beta sheet consisting of 5 beta strands. The thermodynamics and kinetics of (un)folding of MNEI has been extensively characterized in the past decade. As reported earlier, the folding of this protein spans five distinct kinetic phases. However, we don't yet understand that structurally what is happening in each of the kinetics phases. In this study we are investigating the sequence of structural events accompanying the folding of MNEI by using Fluorescence Resonance Energy Transfer as the major methodology. For this purpose, we have monitored the folding kinetics of FRET pairs spanning different structural parts (such as helix, beta sheet, etc) of the protein using time resolved fluorescence measurements. The equilibrium unfolding data suggests that structural changes occur in a combination of continuous and cooperative manner. Interestingly, the equilibrium unfolding of helix is quite distinct from rest of the structure. The double kinetics trFRET data suggests that all the molecules do not collapse within few milliseconds of refolding process, a significant fraction of molecules still remain unfolded like even after 100 ms of refolding. This suggests that collapse is a barrier limited process. This observation has been made across different distances spanning different structural parts of the protein.

Sequence and Complexation Dependent Variation of the Flexibility and Stability of Ubiquitin Family Proteins

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Ubiquitin and small ubiquitin-like modifiers (SUMOs) are post-translational molecular tags, regulating diverse cellular processes in eukaryotes. Here, we provide a basis to understand how nature harnesses molecular descriptors to modulate the diversity of macromolecular interactions for ubiquitin-like proteins. We hypothesize that the flexibility and stability of ubiquitin family proteins is modulated by changes in protein sequence and complexation. To validate our hypothesis, we develop computational measures of protein stiffness, based on Elastic Network Models (ENM) and Principal Component Analysis of Molecular Dynamics Simulation (MD-PCA) trajectories. Further, we carried out steered molecular dynamics simulations of ubiquitin family proteins to examine the structural origin of differences in their unfolding forces. Our results show ubiquitin to be stiffer and more stable than SUMO proteins in free form. Complexation with protein-partners which attach ubiquitin/SUMO to target proteins, represent tagged targets, and recognize tagged protein cargo,increase or decrease intrinsic protein stiffness in a context specific manner. Changes in sequence and complexation both tune protein stiffness through global rearrangements of both protein side-chain packing and the protein backbone. We discuss the different modes of decomposing protein stiffness and its relevance towards understanding the function of ubiquitin family proteins.

High Temperature Unfolding Simulations of Peanut Agglutinin (PNA)

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Protein folding is an aspect that holds paramount importance in structural biology. The current scenario involves the incorporation of in-silico methods like molecular dynamics (MD) simulations in unveiling these events at the atomistic level. In this work, we aim at understanding the unfolding pathways of the homo-tetrameric legume lectin, Peanut Agglutinin (PNA). The monomer of this protein has more than 15 β-strands forming three β-sheets contributing to a stable and compact structure. It has been known that the unfolding of such proteins is comparatively more challenging than α-helical proteins. Here, atomistic MD simulations in total of around 1μs with explicit solvent have been performed to study the unfolding of PNA monomer at high temperature using the protein denaturants, urea and guanidium chloride.

Our analysis of the simulation trajectories shows gradual loss in both secondary and tertiary structure of the protein. However interestingly, at the same temperature, the extent and pattern of unfolding differs for both the denaturants. Additionally, the unfolding percentage has increased at higher temperature. This work sheds some light on the unfolding phenomenon including identification of structural regions that initiate the unfolding in Peanut agglutinin monomer.

"Interfacial water molecules" assisted the "quasi degenerate" binding mode of TN16 with Tubulin: A revelation from molecular dynamics

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The α and β tubulin proteins are the two main constructing subunit of microtubule polymers[1] which acts like a governing element of cell division process and hence it is a promising target for anticancer drugs by the drug developers[2]. TN16 is one of the promising inhibitors of tubulin that alters the conformation of tubulin dimer and interrupts the normal assembly/disassembly cycle of the subunits into the microtubules. The TN16 is reported to primarily bind at α-β interface along with some experimental hints of heterogeneity in binding modes[3]. TN16 is found to take various ways of binding possibilities within the same pocket and the present work explores the mechanism of which gets elucidated only when the structurally packed interfacial water molecules are explicitly considered in standard binding energy calculation (MMGBSA). With the help of QM calculations, this work underscored the importance of such consideration of the network of interfacial water molecules which is necessary to reveal the extra beneficial effect in binding energy stabilization due to multiple quasi degenerate states[4].This tiny molecular tricks could add up an extra rate of interest during the drug development strategy.

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Small Amplitude Afm : Understanding Mechanical Unfolding Of Proteins Via Direct Linear Measurements

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Atomic Force Microscopy (AFM) is widely used to study the Protein folding problem. Many researchers use commercially available AFMs which are operated on resonance frequency of the cantilever. In this, large on-resonance amplitude provides enough restoring force to overcome jump-in instability. However, the cantilever dynamics becomes non-linear and it is difficult to quantify the forces from measured quantities such as amplitude and phase. We have built an AFM which is operated off-resonance with operation amplitudes as small as 1Å. In this case the force field can be linearized over the amplitude of the tip. This allows the use of a simple relationship between observed dynamic quantities such as phase and amplitude with stiffness and damping. In order to detect the amplitude of the order of an angstrom, we have used fiber-based interferometer instead of the conventional laser-deflection-based detection system in commercial AFMs. We present our preliminary data on Force Spectroscopy experiments used to study the mechanical unfolding of single protein molecules (I27 protein) pulled using the novel technique. The method enables us to locally measure the protein stiffness and damping by the use of linear visco-elastic models. This technique can therefore help us understand the visco-elastic behaviour of a single protein molecule during its unfolding. The methodology developed may lead to the revelation of the dynamics of protein folding.

Characterization of refolding intermediates of a multidomain protein using mass spectrometric tools

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Understanding the protein folding pathway, its mechanism and the role of folding intermediates has always been a fundamental area in the field of biological research.Various approaches have been applied to increase our understanding of the intermediates and the transition states of proteins from their unfolded to the near-native or native states. Multi domain proteins comprise a major percentage of the total cellular proteome and little is known about their folding pathways.

In this study we have optimized a protocol to study the refolding event of a model multidomain protein, malate synthase G (MSG) at the molecular level and tried to answer some fundamental questions regarding complexity of protein folding. We have applied limited proteolysis approach with LC-MS/MS to get a molecular level resolution of these intermediates. The different refolding intermediates of MSG were captured and subjected to limited proteolysis using trypsin. The peptides thus generated were separated on a C18 column using nano LC and subjected to ESI-MS. With the use of softwares; HyStar 3.2, Data Analysis, Bio Tools and Pattern Lab we identified certain regions on the protein that are differentially sensitive to enzymatic cleavage, region that constitutes the folding nucleus and characterized the transition states at the molecular level. Using a simple approach along with MS we have tried to get a better understanding of the dynamic conformation of their folding intermediates and map the refolding pathway of a large protein. Our results demonstrate the utility of mass spectrometry for characterization of protein folding intermediates of large multidomain proteins that are a challenge to study.

Functionally Relevant Specific Packing Can Determine Protein Folding Routes

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Functional residues can modulate the folding mechanisms of proteins. In some proteins, mutations to such residues can radically change the primary folding route. Is it possible then to learn more about the functional regions of a protein by investigating just its choice of folding route? The folding and the function of the protein *Escherichia coli* ribonuclease H (ecoRNase-H) have been extensively studied and its folding route is known to near-residue resolution. Here, we computationally study the folding of ecoRNase-H using molecular dynamics simulations of structure-based models of increasing complexity. The differences between a model that correctly predicts the experimentally determined folding route and a simpler model that does not can be attributed to a set of six aromatic residues clustered together in a region of the protein called CORE. This clustering, which we term "specific" packing, drives CORE to fold early and determines the folding route. Both the residues involved in specific packing and their packing are largely conserved across E.coli-like RNase-Hs from diverse species. Residue conservation is usually implicated in function. Here, the identified residues either are known to bind substrate in ecoRNase-H or pack against the substrate in the homologous human RNase-H where a substrate-bound crystal structure exists. Thus, the folding mechanism of ecoRNase-H is a byproduct of functional demands upon its sequence. Using our observations on specific packing, we suggest mutations to an engineered HIV RNase-H to make its function better. Our results show that understanding folding route choice in proteins can provide unexpected insights into their function.

The direct and simultaneous measurement of local stiffness and damping in a single unfolding protein

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Many biological processes are dynamic in nature. Understanding the mechanical properties of bio-molecules involved in such processes is very vital for studying the fundamental biological phenomenon like gene transcription, adhesion properties in cells, mechanical stability of protein etc. Mechanical single molecule techniques offer exciting possibilities for investigating protein folding and stability in native environments at sub-nanometer resolutions. Recent developments in Atomic force microscopy enable us to go beyond the ensemble average and measure the behavior of individual molecules. Here, we present the visco-elastic properties of membrane protein Bacteriorhodopsin extracted from the thermal cantilever motion during force measurements.

Our aim is to get the thermal deflections of the cantilever tip as a function of time when the protein Bacteriorhodopsin attached to the tip unfolds in a typical force curve measurement. We investigated the thermal deflections using Power spectral density (PSD) function. The PSD gives information about stiffness and damping of the biomolecule. Results show that stiffness and damping of the biomolecule changes in a single unfolding event.

These information and approach are set to provide possibilities for further studies regarding protein folding as well as in resolving structure-dynamic relationships of complex bio-molecular systems.

Temperature Dependent Protein Malleability Probed by Single-Molecule Force Spectroscopy and Fluorescence Spectroscopy

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Increase in temperature decreases the stiffness of proteins and makes them more malleable as observed by single-molecule force spectroscopy (SMFS) experiments. However, the mechanism by which the temperature softening happens still remains to be understood. Here we use fluorescence spectroscopy, near UV CD and SMFS to probe the native and transition states of a ubiquitin-like protein, small ubiquitin-related modifier (SUMO2), to get molecular insights into the protein dynamics and mechanical resistance. Initial SMFS measurements indicated a decrease in mechanical resistance which intrigued us to look at the interactions which take over at higher temperatures. Tryptophan at the core of the protein (F64W) was used as a fluorescent probe. Fluorescence intensity measurements and quenching showed that the environment around tryptophan is nonpolar in the temperature range 5-55 ^{0}C. However, the anisotropy experiments showed that the amplitude of segmental motion around tryptophan increased a lot indicating that the local rotationally restricted environment of tryptophan at 5 ^{0}C becomes 'dry molten globule-like' and flexible with increase in temperature. The near UV CD reported the tertiary structure around the tryptophan to be restricted and achiral at 5 ^{0}C which is eventually lost as we increase temperature. This adds to the fact the tryptophan moves more freely in the 'melted' core at higher temperature hence losing its achirality. The flexible core around the tryptophan explains the reduction in the stiffness of SUMO2 as measured by SMFS.

Insights into the conformational propensities of peanut agglutinin in presence of different chaotropic agents

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Folding of a protein into an intricate three-dimensional structure is the most important aspect among different biological self-assembly. Studies on protein folding and misfolding are therefore crucial in understanding the key stages involved in the process. For multimeric proteins, intersubunit interactions play an important role in addition to the intrasubunit interactions for stabilization of its three-dimensional structure. Peanut agglutinin (PNA) is one such tetrameric protein isolated from *Arachishypogaea*. In the present study, we aimed to find out the key structural intermediates that populate when PNA is exposed to various stress conditions like guanidine hydrochloride (GdnHCl), trifluoroethanol (TFE), detergents (SDS and CTAB), hydronium ions and temperature. High temperature and GdnHCl concentration depict a three step unfolding pathway involving an intermediate. The anionic detergent SDS and TFE have been found to induce an a-helical predominant transient PNA population. Interestingly, the cationic detergent CTAB is more robust, and induces molten globule population at very low concentrations. Additionally, the protein is found to retain its secondary structure over a wide pH range. The data, altogether, indicate that the protein is significantly stable against stress conditions. However, each denaturant disrupts its structure in a different way depending upon denaturant functional groups that interacts with accessible side chains of the protein.

Water in Amyloidogenic Intrinsically Disordered Proteins

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The relationship between water and proteins is well-known and is believed to play a pivotal role during the process of protein folding and aggregation. The intricate balance between chain-chain and chain-solvent interaction underlies one of the major physical driving forces for amyloid formation. However, the role of water in amyloid formation remains elusive. The fact that many pathological amyloids are often formed by intrinsically disordered proteins (IDPs) underscores the importance of IDPs in amyloid biology. Using femtosecond and picosecond time-resolved spectroscopy, we have elucidated the water dynamics during the amyloid transition of a collapsed amyloidogenic IDP, namely bovine k-casein. Our femtosecond results indicate that the dynamics of "biological water" gets dampened and the contribution of the bulk water decreases significantly as a result of conformational sequestration during amyloid formation. Additionally, an increase in the ultraslow solvation component revealed the ordering of (trapped) interstitial water. Our findings suggest that water undergoes a profound reorganization, with chain desolvation and entrapping of water taking place in a concerted manner during amyloid assembly. Our results on the site-specific solvation dynamics of α-synuclein will also be presented.

Computational prediction of mutant phenotypes

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Understanding how mutations affect protein activity and organismal fitness is a major challenge. Available saturation mutagenesis data for 1664 single-site mutants of the 101 residue E. coli cytotoxin, CcdB was analysed to investigate the effects of substitutions on mutant phenotypes. Active-site residues could be distinguished from buried ones, based on their differential tolerance to aliphatic and charged amino acid substitutions. At non active-site exposed positions, almost all substitutions were tolerated. Among aromatic (F, H, Y, W) and charged (D, E, K, R) categories, phenotype was observed to be differentially dependent on residue size. Remarkably similar results were observed for two other small proteins, PDZ domain (PSD95pdz3) (McLaughlin et al, 2012) and IgG-binding domain of protein G (GB1) (Olson et al, 2014). Mutational sensitivity data obtained with CcdB were used to design a model for predicting functional effects of mutations. The performance of the model was evaluated using mutational data for PDZ and GB1 proteins. Results compared favourably with those of two widely used computational predictors, SNAP2 and SuSPect as well as to the ProTherm database. These analyses enhance our understanding of how mutations affect phenotype, as well as the ability to predict fitness effects of point mutations.

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Impact of turn propensity on the folding rates of Z34C protein: Implications for helix-turn-helix folding

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The rate-limiting step for the folding of the alpha-helical hairpin from a disulfide-modified and truncated DNA binding domain, Z34C, involves β-turn region, 20DPNL23. This reverse turn is part of the transition state in the folding process for Z34C and influences its folding rates. Molecular dynamics simulations were performed on the peptide corresponding to this turn region and two of its alanine mutants-D20A and P21A, and turnformation was studied using GROMOS54A7 force field. We found that this region has a turn propensity of its own and the highest turn propensity is observed for the wild-type, which is in agreement with CD and temperature-dependent IR spectroscopy measurements done by Du and Gai in 2006 (Biochemistry 2006, 45, 13131-13139). The turns sampled were largely native, with respect to the NMR structure, and of all the interactions present between the residues, we found that the interactions between the sidechain of D20 and the backbone of N22/L23 were predominantly stabilizing the turn conformations here. The alanine-mutants showed a lesser propensity for turn formation due to enthalpic factors and thus this turn is more enthalpy driven. We also found that a slight unfavorable change in ΔG for turn conformation causes a drastic change in the folding rate.

Decoding the folding mechanism of the human mitochondrial membrane protein VDAC2

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Transmembrane β-barrel proteins are precisely folded into biological membranes through complex assembly machineries and associated chaperones. Membrane proteins also retain the ability of unassisted folding in artificial lipidic systems (Jorg H. Kleinschmidt. *Biochim. Biophys. Acta* 2015, 81898), and the study of this process can provide useful insight on the folding energy landscape of β-barrels. The energetics of this folding process, and the presence of non-native interactions during folding, can be readily mapped using non-perturbing tryptophan probes as reporters. Using strategically positioned lipid-facing interface tryptophans, we have studied the folding pathway of the human mitochondrial outer membrane barrel VDAC2. Human voltage-dependent anion channel isoform 2 (hVDAC2) is a 19-stranded transmembrane β-barrel responsible for metabolite flux, maintaining homeostasis, and control apoptosis. It is one of the most important proteins for cell survival, and therefore serves as an interesting model system to address β-barrel folding pathways in eukaryotic systems. We find an overall C- to N-directionality in the hVDAC2 barrel formation, with strand-wise contribution of selected segments in the folding and stability of the barrel scaffold. Our study offers one of the first insights on the folding of a human transmembrane barrel.

Design and Characterization of scaffold based HIV-1 gp120 b12-epitope antigens

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Envelope Glycoprotein gp120 is critical to HIV viral fusion and binds to CD4 receptors on helper T-cells. Broadly neutralizing antibody (BNAb) b12 binds to the CD4 binding site (CD4bs) on gp120 thus blocking viral entry into the cell. Immunogens which elicit BNAb type of response are promising vaccine candidates. To develop such immunogens we designed a computational protocol to isolate and graft only the epitope region onto suitable scaffolds. 23 epitope-scaffold models were synthesized and expressed in-vivo and their binding with antibody b12 characterized via yeast surface display. To improve binding we created random mutant libraries of selected epitope-scaffolds and screened them for presence of enhanced binders using FACS. Some epitope-scaffolds were purified and their binding to antibody b12 characterized using SPR. We compare performance of similar epitope-scaffold based antigen made using other methods with ours and present our finding in this work

Determining the roles of helix α3 and conserved N-terminal residues in structure, function, stability and dynamics of FKBP22

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FKBP22 and the related peptidyl-prolyl *cis-trans* isomerases are mostly homodimeric and possess a V-shaped conformation. Their monomers carry successively an N-terminal domain (NTD), a long α-helix (helix α3), and a C-terminal domain, CTD. While NTDs are responsible for their dimerization, CTDs bind both substrates and inhibitors. To delineate the roles of helix α3 and some conserved NTD residues (such as Y15, D44, etc.) in FKBP22-like proteins, we investigated a recombinant FKBP22 (rFKBP22) and its two NTD mutants (e.g. Y15A and D44A) and three helix α3 mutants, I65P, V72P, and A82P. The results show that mutants are not only different from each other but also differ from rFKBP22 in structure, activity and stability. Conversely, they bound inhibitors normally. Unlike rFKBP22 and NTD mutants, other mutants were unfolded by a non-two state mechanism. Of the mutants, I65P showed maximum structural and functional loss. We also noticed an anomalous domain movement in I65P as well as the collapse of its V-shaped structure. Interestingly, Y15A exists as monomers in solution. A reduced number of non-covalent bonds at the dimeric interface of Y15A seemed to be responsible for its monomerization. Collectively, the helix α3 and the conserved NTD residues are critical for the FKBP22-like proteins.

Biophysical characterization of C26A and N47C mutants of Azurin

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Azurin, a bacterial copper-binding protein with an intramolecular disulphide bond, unfolds via an intermediate upon mechanical unfolding. To get insight into its complex mechanical unravelling process, and the role of native disulphide bond and the copper-binding site on the mechanical properties, we designed two mutants of azurin, N47C and C26A. Wild-type azurin gives a characteristic 628 nm band in the absorption spectra due to LMCT from S (cys, sigma donor) to Cu(II). However, the absorption profile of the C26A mutant has a weak 628 nm band and it is absent in the N47C mutant. These results suggest that holo C26A has two different Cu bound populations. C26A has the structured fluorescence emission of Trp similar to WT azurin with a broad shoulder around 340 nm whereas N47C has unstructured red-shifted fluorescence emission. C26A has a mixed population of water exposed and hydrophobic core. Cu binding property of these two populations is different, giving different bands in the absorption profile. Thermal denaturation reveals that the disulfide bond has a distinct role towards thermal stability of the protein. Fluorescence decay kinetics at 340 nm clearly shows Cu binding of N47C mutant whereas decay kinetics at 320 nm for C26A mutant shows Cu binding is ambiguous. Anisotropy measurement showed that N47C has a short rotational correlation time for Trp rotation, when measured at 340 nm, which is in accordance with the red-shifted fluorescence emission. For C26A, the short correlation time is long with very small amplitude when measured at 320 nm. This indicates the property of the population with compact core.

Effect of metal ion co-factor on the conformational landscape and the aggregation profile of Cu/Zn Superoxide dismutase

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Amyotrophic lateral sclerosis (ALS), also known as LouGehrig's disease, is a specific neurological disorder, which involves the death of motor neurons (1). Although the cause of this disease is unknown, aggregation of copper-zinc superoxide dismutase (SOD1) is considered to be one of the prime factor responsible for the disease pathology (2). A thorough understanding of the folding land scape pertaining to SOD1 native structure attainment and its intrinsic propensity towards misfolding is essential to identify suitable pharmaceutical leads against ALS. Since SOD1 is a metalloenzyme and Cu and Zn are responsible for the catalytic activity and structural stability, it is important to understand the role of metals in the context of stability and aggregation propensity of the metalloenzyme. Here we have probed into the folding landscape of SOD1 by studying the unfolding and aggregation of WT SOD1, Apo SOD1 and metal specific mutants in aqueous solution to identify the structural and conformational characteristics of SOD1 responsible for protein folding aberrations. Steady state fluorescence spectroscopy, circular dichroism spectroscopy (CD), Fourier transform infrared spectroscopy (FTIR), aggregation assay and dynamic light scattering (DLS) have been deployed for studying these secondary structural organization coupled with the intrinsic organizational bias and the aggregation profiles of SOD1 and its mutants. Our experiments have revealed the secondary organizational flip in SOD1 on disruption of the global structure with the chaotropes and the concurrent non-native local architectural signatures.

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Energy Account for BAX Activation

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Bax is one of the pro-survival Bcl-2 family proteins that instigate the cell death events by formation of deadly oligomers upon receiving specific signaling of acute cell stresses. But oligomeric construction needs to be initiated by activation of Bax (by BH3 only proteins) and eventual major conformational changes in the first place. Among many theories regarding BAX activation [1], some have suggested transient binding of activator BH3 proteins to the hydrophobic BC groove (α2-α5) initiates activation, whereas some have voted for the rear pocket (α1-α6) BH3 binding. However, C-terminal (α9) exclusion from the BC groove is the first major conformational change regardless of the activation pathway. In this work, we have tried to understand the energetic preference among the suggested pathways using MD simulation. We have found two distinct minima inside the BC groove with two different compactness of the C-terminal for a wild-type BAX, but in bulk water extended helices are more favorable. Later, comparison of this wild-type free energy landscape with the one having a BH3 only protein in the rear pocket of BAX, has provided useful insight into this debate. Moreover, atomistic MD simulation have explored the dynamical consequences of rear pocket BAX binding towards its activation.

Folding during unfolding of SH3 domain of PI3K

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The study of non-native interactions has become important, since their role in protein folding is not very clear. It is generally considered that only native contacts are formed during transition from unfolded to native state. But many interactions, other than those present in the native state, can be generated in the folding pathway, which might have relevance to their biological function. The PI3K SH3 domain was believed to be a two state folder, but recent studies have shown the presence of equilibrium as well as kinetic folding intermediates. The protein is known to encounter a transient non native burial of Trp53, the sole Trp in the protein, which is otherwise present in the solvent exposed surface, within dead time of mixing (8 ms). Here, we have studied kinetic unfolding of SH3 domain of PI3K in sub-millisecond time domain to capture the formation of the non native interaction, which could not be characterized earlier due to limitations in the dead time achieved by stopped flow mixer. Here, we see an inverse dependence of apparent unfolding rate constants on denaturant concentration, which being a characteristic of folding reaction, gives a clear indication, that it is a folding like event happening during unfolding of the protein. The non native interactions were further characterized by mutational analysis.

Effect of chirality on the helicity studied via the energy landscape of short (d, l)-alanine peptides

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The homochirality of natural amino acids facilitates the formation of regular secondary structures such as α-helices and β-sheets. Here, we study the relationship between chirality and backbone structure for short alanine peptides up to hexa-alanine. The most stable stereoisomers are identified through global optimisation. Further, the energy landscape, a database of connected low-energy local minima and transition points, is constructed for various neutral and zwitterionic stereoisomers of hexa-alanine. Three order parameters for partial helicity are applied and metric disconnectivity graphs are presentedwith "partial helicity" as a metric. We then apply the Zimm-Bragg model to derive average partial helicities for Ace-(L-Ala)6-NHMe (LLLLLL), Ace-(D-Ala-L-Ala)3-NHMe (DLDLDL), and Ace-(D-Ala)3-(L-Ala)3-NHMe (DDDLLL) from the database of local minima and compare with previous experimental and theoretical studies.

Coupled dynamics in Protein and Solvent

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Protein structure and function are attributed to its interaction with the surrounding solvent, water. However, not much attention has been paid to the dynamics of these interactions. In this project we examine the interdependence of protein-solvent dynamics by doing molecular dynamics simulation of myoglobin in water and checking the direction of information flow between time-series of variables representing the properties of protein and solvent using the information theoretic tool Transfer Entropy.

Investigation of domain interaction in Apolipoprotein E

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Apolipoprotein E plays a key role in lipid transport and cholesterol homeostatis in our body. One of the isoforms of Apolipoproteins, ApoE4 is a major risk in Alzheimer's disease (AD). The three isoforms of Apolipoproteins, ApoE2, ApoE3 and ApoE4 differ in 112 and 158 amino acid residues. ApoE4 has Arginine at both 112 and 158 positions, ApoE3 has Cystine at 112 position and Arginine at 158 position and ApoE2 has Cystines at both 112 and 158 positions.

ApoE has two folded functional domains, a 22 kD amino termional domain and a 10kD carboxy terminal domain. N-terminal domain contains four helix bundle of amphipathic α helices and C terminal contains one amphipathic α helix. N terminal domain is involved in binding with LDL receptor and C terminal domain is involved in lipoprotein binding and self association to form tetrameric ApoE in solution.

Domain interaction is possible between N terminal domain and C terminal domain by non covalent interactions. Domain interaction can have an effect in the difference of stability and functions between ApoE3 and ApoE4. In our current study we have investigated domain interaction in these two ApoE isoforms by chemical denaturation by urea and Guanidium Hydrochloride and refolding and thermal unfolding and refolding of different mutants of ApoE3 and ApoE4 by circular dichroism and fluorescence. Fluorescence study shows domain interaction in ApoE4 is greater than ApoE3 but the difference in domain interaction between ApoE3 and ApoE4 is very less. Thermal unfolding study using CD and fluorescence shows presence of an intermediate state in N terminal region of ApoE4 that may contribute to the isoform specific effects of ApoE in AD.

Study of the perturbation of the mouse prion protein by NMR

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Proper folded structure of a protein is crucial to carry its biological function(s) and hence maintain living systems. It is well known that several proteins upon misfolding cause fatal diseases. Prion is one such protein which upon aggregation causes fatal neurodegenerative diseases like Creutzfeldt-Jakob disease (CJD), Fatal Familial Insomnia (FFI), Kuru etc. which do not have any cure. In order to discover theraputic targets it is important to study how the conversion of the monomeric protein to oligomer(s) happens.

The mouse prion protein is alpha-helical at pH 4 in absence of salt. However upon addition of 150mM NaCl at pH 4 and 37 °C it oligomerizes to a beta-sheet rich structure. I have studied the effect of salt and temperature variations on the native structure using Nuclear Magnetic Resonance (NMR) spectroscopy as a probe. In the future I aim to study the effects on the dynamics of the prion protein of different non-pathogenic and pathogenic mutations.

Protein dynamics as a function of ionic strength, calcium ion concentration and temperature: Calmodulin as a first step

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Calmodulin (CaM) protein plays a very crucial role in the calcium signaling inside the eukaryotic cell structure [1] as it can also bind to other proteins/targets and facilitate various activities inside the cell [2, 3]. The response of CaM protein as a function of ionic strength, calcium ion removal and temperature at physiological pH condition is investigated using classical Molecular dynamics (MD) simulations. Changing the ionic strength and temperature came out to be two of the possible routes for observing a conformation change in the protein. In the present study, as the calcium ions are removed from the protein, the protein is observed to acquire more flexibility. This flexibility is observed to be more prominent at a higher ionic strength. At a lower ionic strength of 150 mM with all the four calcium ions intact, the N and C-lobes are observed to come close to a distance of 30 Å starting from an initial separation distance of 48 Å. This conformation change is observed to take place around 50 ns in a simulation of 100 ns. As a second parameter, temperature is observed to play a key role in the conformation change of the protein. Earlier these kind of studies have been performed experimentally using fluorescence measurements as in [4]. The calcium bound form of CaM is observed to undergo a reversible conformation change in the range 295-301 K at calcium ion concentration 150 mM. The transition temperature was observed to depend on the calcium ion concentration of the protein. Leap-dynamics approach was also used earlier to study the temperature dependent conformation change of CaM [5]. We perform MD simulations of 100 ns each for the temperature range 300-500 K on the apo form of CaM, 3CLN and 1CFD. A remarkable dependence of the temperature is observed on the overall dynamics of the protein as reported in our earlier study [6, 7]. 1CFD shows a much flexible linker as compared to 3CLN whereas the overall dynamics of the lobes mainly N-lobe is observed to be more in later case [8].

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Partially Native Intermediates Mediate Misfolding in SOD1 Studied by Single-Molecule Force Spectroscopy

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Prion-like misfolding of superoxide dismutase 1 (SOD1), a homodimeric cytosolic antioxidant protecting against damage from superoxide, is associated with the fatal neurodegenerative disorder amyotrophic lateral sclerosis (ALS), but the mechanism of misfolding remains unclear. We examined SOD1 folding at the single-molecule level, using optical tweezers to unfold and refold SOD1 under tension. Focusing on the reduced, un-metallated form of the protein (apo-SOD1^{SH}), which is the least stable form and thus thought to be most relevant for misfolding, we measured force-extension curves (FECs) of the unfolding and refolding of isolated monomers. FECs revealed multiple intermediate states, showing that the folding is much more complex than expected. By relating the contour-length changes observed in the FECs to structural features of apo-SOD1, we propose possible native folding pathways for the apo-SOD1^{SH} monomer wherein β-strands peel off sequentially. We also identified a stable core of the protein which always unfolds last and refolds first. Notably, FECs occasionally revealed length changes that did not match the value expected for native folding, suggesting misfolding. Comparing the sequence of intermediates observed during native folding and misfolding, we found that misfolding branched off from the native folding at different stages after formation of the stable core.

Characterization of amyloid Aβ oligomers by extrinsic fluorescence probe DCVJ

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Misfolded and intrinsically unfolded proteins are more prone to aggregation in the form of oligomers, protofibrils and fibrils. Oligomers and fibrils are toxic to neuronal cells in vitro, forms ion channels, and disturb the integrity of the synthetic membrane lipids and rafts. Although probes such as thioflavin-T/S and Congo Red binds more specifically to amyloid fibrils, there is a pressing need to identify probes that bind at the early stage of aggregation and reports on the mechanism of their aggregation. Here we show that molecular rotor 9-(dicyano-vinyl)julolidine (DCVJ) binds monomers as well as early aggregates and reports on the change in dynamics as well as oligomeric shape and packing. The binding of DCVJ to $A\beta_{40}$ and $A\beta_{42}$ increases the fluorescence intensity with time around 505 nm, and for the $A\beta_{40}$ there appears a second excimer peak at 575 nm. Further the DCVJ fluorescence at 505nm increases 5-fold greater for $A\beta_{42}$ compared to $A\beta_{40,}$ which suggests the oligomers are more tightly packed in $A\beta_{42}$ than in $A\beta_{40}$. The comparison of DCVJ kinetics profile at 505nm and 575 nm for $A\beta_{40}$ and two mutants show differences that reflect the size and shape of the corresponding oligomers obtained from AFM. The results of this study shows that DCVJ not only binds early aggregates, but also provides valuable information regarding oligomer packing, morphology and mechanism of its formation.

How the terminal region of a multi-domain protein dictates its folding, function and stability?

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Maltodextrin glucosidase (MalZ) is an *E.coli* cytosolic protein which hydrolyses short malto oligosaccharides from the reducing end releasing glucose and maltose. It is an aggregation prone protein, and forms inclusion body during recombinant expression, and provides very poor quantity of spontaneous refolding. However, molecular chaperone GroEL/GroES assists in its refolding process, and yields about 50% of refolding in vitro and significant enhancement of folding yield happens when co-expressed with recombinant MalZ. The N-terminal region of this enzyme appears to be a unique domain as seen in sequence comparison studies with other amylases as well as through homology modelling. The sequence and homology model analysis show a probability of disorder in the N-Terminal region of MalZ. The crystal structure of this enzyme has been recently solved, which indicates that the N-terminal region of the enzyme (Met1– Phe131) might be unstructured or flexible.

To understand the role of the N-terminal region of MalZ in its folding, enzymatic activity, and overall stability, a truncated version (Ala111-His616) of MalZ was created. The truncated version failed to fold into an active enzyme both in *E.coli* cytosol and *in vitro*, even with the assistance of chaperonins GroEL and GroES. Furthermore, the refolding effort of N-truncated MalZ in the presence of isolated N-terminal domain didn't succeed. Our studies suggest that while the structural rigidity or orientation of the N-terminal region of the MalZ protein may not be essential for its stability and function, but the said domain is likely to play an important role in the formation of the native structure of the protein when present as an integral part of the protein.

Solubility of TMRAβ42 fibrils is controlled by the equilibrium between the accessible conformations of the monomeric peptide

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Amyloids are one-dimensional aggregates of protein involved in multiple diseases e.g., Alzheimer's Diseases (AD), Parkinson's Diseases (PD) etc. Deposition of these aggregates is the pathological hallmark of these diseases and it keep accumulating over time due to its insoluble nature in native condition. Measured *in-vitro* solubility of these aggregates lies in micro molar range which contradicts with *in-vivo* pico molar solubility. We have measured solubility of TMRAβ42 aggregate starting from fibrillar species in native buffer and it showed minimal solubility even at low nM concentrations. These aggregates can be dissolved in chemical denaturant like Urea and GdnCl in a concentration dependent manner. The solubility data can be fit using a two-state folding-unfolding model allowing us to measure free energy difference between the conformations in soluble phase (random coil) and insoluble phase (beta-structure). Measurement of hydrodynamic radii using Fluorescence correlation Spectroscopy (FCS) revealed a similar free energy changes for transition from native to denatured conformations for the monomeric TMRAβ42. We hypothesize that dissolution of fibrils is controlled by the equilibrium of the conformations of the monomeric peptide between the soluble and the fibrillar phases.

Charge Patterned Sequences Form Helical Structures Through Charge Neutralization

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Many proteins with repeating blocks of oppositely charged residues form long single alpha helices (SAHs).The consensus sequence is a block of four Glu residues followed by a block of four Lys or Arg residues, $(Glu_4(Lys/Arg)_4)_n$. The current working hypothesis is that SAHs are stabilized by i:i+4 salt bridges between opposite charges in consecutive helical turns.We test the merits of this hypothesis to understand the sequence-encoded preference for SAHs and the logic behind the failure of certain atomistic simulations in anticipating the preference for stable SAHs.

In simulations with fixed charges the favorable free energy of solvation of charged residues and the associated loss of sidechain entropy hinders the formation of SAHs. We proposed that alterations to charge states induced by sequence context might play an important role in stabilizing SAHs.We tested this hypothesis using a $(Glu_4Lys_4)_n$ repeat protein and a simulation strategy that permits the substitution of charged residues with neutralized protonated or deprotonated variants of Glu / Lys. Our results predict that stable SAH structures derive from the neutralization of approximately half the Glu residues. These findings explain experimental observations and also provide a coherent rationale for the failure of simulations based on fixed charge models. Large-scale sequence analysis reveals that naturally occurring sequences often include “defects” in charge patterns such as Gln or Ala substitutions. This sequence-encoded incorporation of uncharged residues combined with neutralization of charged residues might tilt the balance toward alpha helical conformations.

Our results highlight the need for developing robust methodologies for constant pH simulationsthat can be applied to sequences with high charge contents. They also highlight the need for generalizing bioinformatics predictors to account for sequence-encoded charge regulation that might influence disorder predictions for sequences with high fractions of charged residues.

Influenza Immunogen Design on a Nanoparticle Platform

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Influenza virus is a human pathogen leading to frequent respiratory illness and current influenza vaccines need to be annually updated due to the ever evolving nature of the virus resulting in escape variants. Hemagglutinin (HA), a surface glycoprotein on Influenza virus has always been an attractive target for vaccine design. We recently designed a 'headless' HA stem immunogen pH1HA10-foldon, where Foldon is the trimerization domain based on corresponding sequence from H1N1 A/California/04/2009 (PDB-3LZG). Further in a recent study, Ferritin nanoparticles which self-assemble to 24-mers were engineered to display repetitive arrays of full-length HA and a HA mini stem that induced a strong immune response (Kanekiyo et al, 2013). We therefore engineered a fusion of pH1HA10-Foldon with Ferritin as the antigen display platform.This pH1HA10-Foldon fused to Ferritin construct was biophysically characterized. Since Ferritin can self-assemble itself into a 24-mer, we also removed the Foldon trimerization domain to generate pH1HA10 fused to Ferritin which was further characterized.

Multiple Intermediates in the Folding Pathway of Rabbit Prion Protein Studied by Single-Molecule Force Spectroscopy

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Prion protein (PrP) is one of the most extensively studied proteins in regards to misfolding and aggregation. It has long been known that different species of animals have different susceptibility to prion disease, but the factors that protect the resistant species that could help develop treatments both for prion diseases and prion-like diseases remain unclear. Hence, to explore the folding of individual PrP molecules from different species with different levels of disease susceptibility, we applied single-molecule force spectroscopy to observe directly the folding pathway of the Rabbit prion protein, RbPrP (less susceptible) to compare it with Syrian Hamster PrP (ShPrP, more susceptible) which has already been studied in detail at the single molecule level. By measuring force-extension curves (FECs) of single RbPrP molecules held under tension in a high-resolution optical trap, we found that RbPrP has multiple intermediate states unlike ShPrP which shows only two state unfolding. By relating the contour-length changes observed in the FECs to structural features of RbPrP, we propose possible native folding pathways for the RbPrP where all the α-helices unfold separately along with the loops and the β-strand. We observed similar intermediates by analyzing the refolding FECs and there were no dissimilarities between the unfolding and refolding pathways.

Investigation of multi-domain protein folding through characterization of folding/unfolding intermediates

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Multi domain proteins, despite of their large size and loosely-dependent/independent folding units manage to attain their native form with high accuracy. Recent studies have shown that Malate synthase G (MSG), a large, single chain multi-domain protein (82kDa), folds reversibly from GdmCl unfolded state and indicate presence of equilibrium intermediates[1]. Same study also shows that the protein refolds via formation of native like intermediates which slowly rearranges to native conformation[1].

As all previous studies have been conducted with GdmCl as denaturant, we further explore the mechanism by urea as denaturant to exclude the ionic effects of GdmCl. Equilibrium studies with urea give clear indication of three state transition curve and hence presence of stable intermediates. The manual refolding kinetics studies indicate double phase of refolding with curvature in both refolding arms. The point of inflexion at low urea concentration probably hints towards off-pathway intermediate which further needs to be confirmed.

The manual unfolding studies show two distinct intersecting chevron arms with different m_u corresponding to two phases of unfolding. The result might be due to heterogeneity in the native form or a consequence of unfolding of independent units of same protein.

Reference:

1. Dahiya V., Chaudhuri T. K. (2013). Biochemistry 52, 4517-30

Prion protein and calcium levels in cells

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The conformational dynamics of prion protein appears to be responsible for the causation of a number of fatal neurodegenerative disorders. It is believed that the normal cellular form of prion protein, which is membrane tethered and appears to be cyto-protective in nature, makes a transition to a conformationally altered, pathogenic form which is aggregation-prone. However, the precise nature of this transition, the physiological functions of the normal cellular form, and the molecular mechanism utilized by the pathogenic form for neurodegeneration are still not clear. There are indications that regulation of calcium levels in cells might be a key aspect of prion protein biology. The presence of different forms of prion protein, monomeric or aggregated, has been shown to affect calcium levels in cells. Further, the prion protein has been shown to interact with several proteins at cell surface that are linked with calcium signaling in cells, and the recombinant form of prion protein has been shown to form calcium selective ion-channels on artificial membranes, therefore suggesting that the prion protein could influence calcium levels in cells using multiple modes. However at this stage, it is not completely clear as to which mode of calcium transit (ion-channel or signaling) is preferred by which form of prion protein (monomeric or aggregated), and why. Using the mouse prion protein as a model system, we have explored such interactions of prion protein at cell surface and their impact on cellular calcium levels.

Effect of functional residues on folding of Ubiquitin

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Ubiquitin (Ub), a highly conserved protein has been studied extensively both experimentally and computationally. The current study is based on the observation that Ub folds through different pathways when simulated using 4.5 Å cutoff and CSU contact maps. Analysis of both the contact maps shows that CSU map has long range contacts made by residues His68 and Arg72 which are absent from the 4.5 Å map. In order to dissect out the role of these contacts a folding motif was constructed. The motif folds through same pathway, irrespective of the contact map used. Comparison of motif contact map with Ub led to the conclusion that contacts made by His68 and Arg72 are unique to Ub. These residues are known to be important for binding of Ub to its partners. The study concludes by speculating that these residues form binding pockets/cavities which cause them to have long range contacts.

Sequence and structure impose a specific fitness landscape across three phylogenetically divergent thermophilic TIM barrel proteins

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TIM barrel proteins are encoded by highly divergent sequences, but fold to highly conserved $(\beta\alpha)_8$ structures with varying stabilities and functions. The indole-3-glycerol phosphate synthase (IGPS) TIM barrelprotein, required for tryptophan biosynthesis in bacteria, archaea and yeast, was used to investigate how sequence and structure are correlated with fitness in a competition growth assay. IGPS from the *S. solfataricus* (SsIGPS, archaea), *T. maritima* (TmIGPS, bacteria), and *T. thermophiles* (TtIGPS, bacteria) thermophiles were subjected to the EMPIRIC assay to assess the change in fitness of yeast transformed with these genes following saturating mutagenesis on the 8 β-stands and the preceding -loops, known to be important for protein stability. We explored the fitness landscapes of these three orthologous TIM barrel proteins to answer two important questions at the intersection of biophysics and evolutionary biology: what are the salient features of a TIM barrel fitness landscape, and whether the fitness landscapes of phylogenetically-divergent proteins are correlated. The fact that the thermophilic proteins we chose for this study were not well adapted to the mesophilic host environment was fortuitous, providing an opportunity to identify beneficial mutations that increase the fitness under sub-optimal growth conditions. Surprisingly, mutations in the αβ-loops distal from the active site increase the fitness of all 3 IPGS orthologs, implying a common allosteric effect. In general, despite the low sequence identity (<35%) of the wildtype (WT) sequences, the fitness landscapes of the three IGPS proteinsare correlated. While part of this result can be explained by the effects of side chain physical chemistry on protein folding and thus fitness, the correlation between fitness landscapes at structurally equivalent positions, irrespective of the WT amino acid, suggests that the structure of the TIM barrel fold imposes a specific fitness landscape. To our knowledge, the conservation of a fitness landscape in sequence and structure space across evolutionary time has not been previously observed.

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