aggregation phenomena. Amorphous aggregation of the γ-crystallins in the eye lens causes a widespread disease of aging, cataract. We combined simulations and experiments to study the mechanism of aggregation of two γD-crystallin mutants, the congenital cataract mutation W42R and the mimic of age-related oxidative damage W42Q. The two mutants had highly similar properties. We found that formation of an internal disulfide bond was necessary and sufficient for aggregation under physiological conditions *in vitro*. Two-chain all-atom Monte Carlo simulations predicted that one non-native disulfide in particular, between Cys32 and Cys41, was likely to stabilize an unfolding intermediate prone to specific intermolecular interactions. Mass spectrometry detected this internal bond in aggregates formed under physiological conditions *in vitro*; mutagenesis experiments confirmed that it is needed for aggregation; its formation is also consistent with a recent *in vivo* proteomic study. Mining our simulation results linked formation of this disulfide to specific conformational changes: extrusion of the N-terminal β-hairpin and shortening of the linker between it and the domain core. Specific binding between the extruded hairpin and a distal β-sheet in an intermolecular chain reaction similar to domain swapping is the most probable mechanism of aggregate propagation.

825-Plat
Biophysical and Evolutionary Aspects of Domain Swapping in the Forkhead Domain of Human FoxP Proteins

Exequiel Medina[1], Javiera Reyes[2], Pablo Villalobos[1], Elizabeth A. Komives[3], Jorge Babul[1], **Cesar A. Ramirez-Sarmiento**[2].
[1]Departamento de Biologia, Facultad de Ciencias, Universidad de Chile, Santiago, Chile, [2]Institute for Biological and Medical Engineering, Pontificia Universidad Catolica de Chile, Santiago, Chile, [3]Department of Chemistry and Biochemistry, University of California San Diego, San Diego, CA, USA.

Increasing evidence has shown that the forkhead DNA-binding domain of human FoxP proteins forms domain-swapped dimers. During domain swapping, identical segments are exchanged between two subunits, thus leading to an intertwined oligomer where interactions of the native monomer are reconstituted intermolecularly. Domain swapping in FoxP enables formation of long-range chromosomal interactions of high relevance in gene transcription, with several disease-causing mutations related to impairment of this process.

Commonly, extensive protein unfolding and refolding is required to enable domain swapping, thus taking from months to years to occur under physiological conditions. However, such mechanism impedes a conciliation of the occurrence of domain swapping of FoxP in a biological context.

We biophysically characterized the forkhead domain of FoxP1, showing that its monomer-dimer equilibrium is reached within hours and its folding follows a three-state mechanism where protein unfolding is preceded by a native-like monomeric intermediate, in contrast to the canonical two-state mechanism of most domain swapping proteins. Hydrogen-deuterium exchange mass spectrometry (HDXMS) experiments showed that dimerization leads to reduced flexibility of the hinge loop connecting the exchanged elements, whereas loosened interactions in β-strands 1-3 are observed where the intermediate is highly populated.

Phylogenetic analysis shows that FoxP and monomeric FoxO proteins diverge from a common ancestor that retains a conserved hinge-loop motif for all monomeric Fox family members (FPYF), whereas the common ancestor of FoxPs shows a Pro-Ala substitution experimentally described to enable partial or complete domain swapping in this subfamily. HDXMS reveals that this mutation in FoxP1 leads to a monomer species with reduced flexibility in regions distal from the hinge loop. Moreover, characterization of FoxP2 and FoxP4, with >72% sequence identity, suggests that localized sequence substitutions lead to important changes in stability of the domain swapped conformation.
Funding: FONDECYT 11140601 & 1130510.

826-Plat
Understanding Protein Domain-Swapping in the Cystatin-Monellin Family of Proteins

Nahren Manuel Mascarenhas, **Shachi Gosavi**.
National Centre for Biological Sciences-TIFR, Bangalore, India.

Domain swapping is the process by which two or more identical proteins exchange structural elements or ‘‘domains.’’ Monellin and stefin-B (a cystatin) have very similar structures but entirely different functions. They also have very different domain-swapping propensities. We show using structure-based models (SBMs) and MD simulations, that these propensities can be determined by the position and the chemistry of functional residues in the proteins. Thus, domain-swapping, often a first step in disease-causing aggregation, can be a by-product of the need to conserve function in the protein. We also show that SBMs and MD simulations can be used to predict mutations which introduce domain-swapping into a non-domain-swapping protein (monellin).

827-Plat
Using High Pressure NMR to Study Folding Cooperativity and Kinetics of Protein L9

Yi Zhang[1], Soichiro Kitazawa[1], Ivan Peran[2], Natalie Stenzoski[2], Scott McCallum[1], Daniel Raleigh[2], Catherine Royer[1].
[1]Rensselaer Polytechnic Institute, Troy, NY, USA, [2]Stony Brook University, Stony Brook, NY, USA.

The ribosomal protein L9 is comprised of two globular domains, the N-terminal (NTL9) and C-terminal (CTL9) domains, connected by a rigid linker. The folding properties of the two domains have been investigated extensively with a variety of denaturants, including urea, GdnHCl, temperature, and pH. Both NTL9 and CTL9 have exhibited cooperative folding in a two-state manner. Pressure perturbation, as a relatively new denaturing factor, has been proved to unfold proteins locally by targeting their packing defects, and to slow protein folding due to the positive activation volume of folding. Conventional NMR spectroscopy is too slow to characterize the folding transition state of most single domain proteins. When combined with NMR spectroscopy, which provides residue-specific resolution, high pressure (HP) could reveal sequence based information regarding protein folding cooperativity and conformational dynamics. Hence we applied HP-NMR to NTL9, CTL9 and their variants, seeking to gain more insights on their folding mechanism. To study their folding equilibria, ^{1}H-^{15}N HSQC spectra were recorded as a function of pressure, and the intensity of each amide proton resonance was analyzed as a function of pressure. We combined HP with ^{15}N ZZ-exchange NMR experiments to determine residue-specific folding and unfolding rate constants. Our thermodynamic and kinetic results reveal that NTL9 folding is very close to two-state, while small deviations from two-state behavior were observed for CTL9. The volumetric properties of these domains indicate that most of the solvent excluded voids in the hydrophobic cores of the folded structures are formed in their respective transition states.

828-Plat
Intracellular Modulation of Protein Folding Stability Probed by a Novel Folding Reporter

David Gnutt, Jonas Ahlers, Benedikt König, Simon Ebbinghaus.
Physical Chemistry II, Ruhr University Bochum, Bochum, Germany.

The cell is a heterogeneous and highly crowded environment in which biomolecules evolved to fold and function. Still, protein folding is predominantly studied in vitro, under conditions which cannot mimic the complexity of the cellular environment. Thus, the role of the cellular environment in modulating protein folding remains elusive. So far, only a few proteins were studied inside the cellular environment revealing a protein specific effect on stability. Utilizing a combination of subsequent fast laser-induced temperature jumps and fast fluorescence microscopy, we simultaneously study thermodynamics and kinetics of a novel folding reporter based on a truncated SOD1 variant. Our reporter is a strictly monomeric two-state folder which was mutated at different sites to study the effect of site-specific mutations on the intracellular folding modulation. We show that most mutants are destabilized by the intracellular environment which can be explained by nonspecific interactions with the environment. Although the cellular environment is destabilizing at basal conditions, we show that an osmotic stress induced increase of intracellular crowding can stabilize the folded state. Thus, intracellular stability depends both on the properties of the cellular environment and the protein’s sequence. Our results help to rationalize the effects of the intracellular environment on protein folding and stability.

829-Plat
Modulating SOD1 Folding Landscapes with Targeted Molecular Binders

David N. Bunck, Beatriz Atsavapranee, Katrine Museth, James Heath.
Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, USA.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by the deterioration of motor neurons that abates essential biological functions and exhibits survival times of 3 - 5 years after diagnosis. One driver of this disease derives from inherited mutations to the protein superoxide dismutase 1 (SOD1), which hinder proper folding and result in the accumulation of toxic aggregates. We identified cyclic peptides that target precise epitopes on SOD1 through an emerging screening platform that furnishes high-affinity binders against regions of a protein independent of secondary or tertiary structure. Binding these epitopes both stabilizes the native state and accelerates folding. In this context, these small peptides function as molecular chaperones and mitigate the impact of deleterious mutations to SOD1. They also display the traditional benefits of small molecules, such as straightforward chemical modifications and long-term stability. Overall, this method provides a route to rationally perturb the energy landscape of any protein through noncovalent binding, making it useful in fundamental studies of protein folding as well as designing therapeutics for misfolding diseases.