The presence of an abnormally expanded polyglutamine (polyGln) sequence in huntingtin protein ultimately results in beta-sheet-rich fibrillar aggregates, a hallmark of Huntington's disease. Current challenges are to map out the polyGln aggregation pathway by identifying the various precursor structures and establish their pathological roles. We are using time-resolved small-angle neutron scattering (SANS) to probe the aggregates formed by peptides having the protein context of huntingtin exon 1 (HD protein) and with varying polyGln lengths. SANS is a particularly useful technique for following structural changes on the nanometer length-scale in solution. From the time-resolved scattering data, we obtain snapshots of the polyGln structures as the kinetics reaction ensues, which yields quantitative information on the size and shape of precursors and the internal structure of the resulting fibrils. Measured changes in the radius of gyration and mass per length illustrate multiple growth regimes with a transition from early aggregates to fibril elongation and association. Our SANS results on mature polyGln fibrils are consistent with the Perutz beta-helix structural model. This research is providing new insights into the pathway of polyGln aggregation and should later assist in determining the role that precursors play in neuronal toxicity.

1130-Plat
Solid State NMR Studies Of Structural And Motional Complexity In Amyloid-Like Fibrils Of The Peptide GNNQQNY
Patrick C.A. van der Wel[1,2], Józef R. Lewandowski[2,3], Robert G. Griffin[2].
[1]University of Pittsburgh, Pittsburgh, PA, USA, [2]Massachusetts Institute of Technology, Cambridge, MA, USA, [3]Université de Lyon, CNRS/ENS Lyon/UCB-Lyon, Lyon, France.
Solid state NMR allows the site-specific characterization of structure and dynamics in a variety of immobilized biomolecules, and thus allows a unique structural view on amyloid fibrils. These fibrils are common to various human disorders and appear to share a number of characteristic features, both in terms of their structure and formation. In the hope of delineating the biophysical details of the fibrillization process and the fibrils themselves, various groups have focused on the experimental and theoretical study of small peptide fragments of amyloid-forming proteins. One prominent system is the $GNNQQNY_{7\text{-}13}$ fragment of the yeast prion protein Sup35p, since it was found to form not just amyloid-like fibrils, but also seemingly amyloid-like microcrystals. X-ray diffraction based structures from the latter have inspired numerous theoretical analyses and generalizations regarding the biophysics and structures of amyloid fibrils.
We have instead applied biological solid state NMR methods to characterize the GNNQQNY fibrillar aggregates. Magic angle spinning (MAS) solid state NMR was used for various structural measurements, aimed at both the intramolecular as well as intermolecular structural motifs of the fibrils (as well as the crystalline aggregates). Our studies have revealed a remarkable complexity in these fibrils, despite the relatively small size of the peptide building blocks. This is in marked contrast with the rigid and homogeneous nature of the crystalline structures, as revealed by X-ray crystallography and solid state NMR. These observations provide further insights into the structure of the fibrils of this peptide model system and should also be of importance as input to numerous theoretical studies that rely on the crystal structure data.

1131-Plat
Computational Study of Assembly and Toxicity Inhibition of Amyloid Beta-Protein and Its Arctic Mutant
Brigita Urbanc[1], Gal Bitan[2], Luis Cruz[1], Alfonso Lam[3], David Teplow[2].
[1]Drexel University, Philadelphia, PA, USA, [2]University of California, Los Angeles, Los Angeles, CA, USA, [3]Boston University, Boston, MA, USA.
Amyloid b-protein (Ab) exists in two main alloforms, Ab40 and Ab42, of which Ab42 is linked particularly strongly to Alzheimer's disease (AD). Prior computational work demonstrated that the ab initio discrete molecular dynamics approach with an intermediate-resolution protein model captures biologically relevant differences between Ab40 and Ab42 folding and oligomerization. In the present work we apply the same approach to explore the relationship between the structure and toxicity. Assuming that Ab42 oligomers are more toxic than oligomers formed by Ab40, our structural analysis indicates that the solvent accessible surface area (SASA) in the N-terminal region of Ab42 oligomers is significantly higher than that of Ab40 oligomers. We then investigate effects of the C-terminal fragment (CTF), which was shown to attenuate Ab42 oligomer toxicity in a cell culture, on Ab42 oligomerization. Our results indicate that CTFs associate with Ab42 to form heterooligomers, consistent with quasielastic light scattering data. We show that the presence of CTFs significantly reduces SASA in the N- terminal region of Ab42 compared to the same region in Ab42 oligomers formed in the absence of CTFs.
We further explore folding and oligomer formation of the Arctic mutants, [E22G]Ab40 and [E22G]Ab42, associated with a familial form of AD. Our results demonstrate that the substitution E22G disrupts the folding structure and oligomerization pathways of both Arctic mutants and results in increased SASA at the N-terminus of the Arctic Ab40 mutant. These findings suggest that Ab oligomer neurotoxicity might be directly or indirectly associated with the degree of solvent exposure of the N-terminal region of Ab.

1132-Plat
Discrete Molecular Dynamics simulations on hexameric amyloid-β (1-40) and (1-42) models
Sijung Yun, H. Robert Guy.
National Cancer Institute, Bethesda, MD, USA.
Aggregation of amyloid-β peptides (Aβ) may play a pivotal role in neurotoxicity of Alzheimer's disease. Two major alloforms of Aβ are the 40-residue long Aβ40 and the 42-residue long Aβ42. Though Aβ42 has only two more residues at the end of C-terminus, Aβ40 and Aβ42 show different characteristics in early aggregation: Aβ40 aggregates to exist from monomers up to tetramers while Aβ42 exists from monomers to hexamers, dodecamers, or even octadecamers. However, the molecular mechanism of the different aggregation between Aβ40 and Aβ42 is not clearly understood since their oligomeric structures are not available from experiments due to their meta-stable characteristics. Here, we simulated nine hexameric Aβ40 and nine hexameric Aβ42 models with Discrete Molecular Dynamics (Discrete MD). The hydrophobic core of these models is a six-stranded β-barrel formed by residues 30-40 that has three-fold symmetry about its axis. This core is shielded from water by residues 1-28. The models differ by the relative positions of the core β strands, and whether the other segments surrounding the core contain α helices or β-strands. The potential energy of Aβ40 measured by Miyazawa-Jernigan interaction matrix were considerably lower than the potential energy of Aβ42 in all of 18 models tested, probably because more hydrophobic residues are exposed to water in theAβ42 models. In two of nine models of Aβ42, dangling hydrophobic β-strands emerged on the surface. This implies that the association of these hexamers may be possible, which could lead to the formation of larger assemblies.

Platform X: Exploring the Unfolded State of Peptide & Proteins

1133-Plat
Concentration Dependent Instability of ß-sheet aggregates of Ac-(AAKA) 4NH2 in solution
Thomas J. Measey, Melinda Bendon, **Reinhard Schweitzer-Stenner**.
Drexel University, Philadelphia, PA, USA.
The amphiphatic polyalanine peptide, Ac-(AAKA)4-NH2, has recently been shown to aggregate into a hydrogel at high chloride concentration or alkaline pH. It forms soluble ß-sheet type aggregates at neutral pH and centimolar concentrations. In order to further characterize the transition from the monomeric to the aggregated state, we measured the far UV-ECD spectrum at different concentrations between 35 - 700 μM. At very low concentration (i.e. 0.05 mg/mL or 35 μM) the observed spectrum is indicative of a stable mixture of right handed α-helical and ß-strand (sheet) conformations. At higher concentrations (> 1 mM) we observed a spectrum reflecting a very stable ß-sheet aggregate. However, at concentrations between 70 - 700 μM the peptide shows a very strange, and totally unexpected, behavior. Upon dissolving it in solution, a statistical coil-like mixture comprising polyproline II (PPII), α-helical and ß-sheet-like conformations is formed. Subsequently, most of the ß-sheet fraction decays into a conformation which exhibits a PPII-type ECD spectrum on a time scale of 104-105 s. The kinetics of the process follows a power law at low concentrations and becomes mono-exponential at higher concentration. Generally, the relaxation slows down with increasing peptide concentration until the ß-sheet becomes stable on the time scale of our experiments (<105 s). We hypothesize that aggregation involves multiple steps with the formation of a rather unstable ß-sheet as the first step. The second step involves the formation of stable fibers. This step competes with the formation of collagen like coil-coil state, rather, which is known to exhibit a PPII-like ECD spectrum.

1134-Plat
Characterization of the Disordered Regulatory Domain from Calcineurin
Trevor P. Creamer, Veronique M. Chellgren, Anne E. Jensen, Terrence E. Lester.
University of Kentucky, Lexington, KY, USA.
Calcineurin (CaN) is a highly-conserved, ubiquitous Ser/Thr phosphotase that plays vital roles in memory development and retention, cardiac growth, and

immune system activation. Alterations in the regulation of CaN can lead to disorders such as Down syndrome-related mental retardation and cardiac hypertrophy. CaN is also the target for the immunosuppressant drugs FK506 and cyclosporin A. The regulation of CaN function is not well understood at the molecular level. CaN is inactive until bound by calmodulin (CaM). CaM binds at a site towards the N-terminus of a 95 residue regulatory domain in CaN. This regulatory domain is believed to be disordered. The binding of CaM to CaN causes an autoinhibitory domain located C-terminal to the regulatory domain to be ejected from CaN's active site. We hypothesize that the CaN regulatory domain undergoes a folding transition upon CaM binding, and that this folding provides the driving force for pulling the autoinhibitory domain from the active site. We have made a fragment of CaN that consists of the regulatory domain, autoinhibitory domain and a short C-terminal domain. We will present data from CD spectroscopy, fluorescence, NMR and analytical ultracentrifugation experiments that indicate this fragment is largely disordered in the absence of CaM, and gains structure when CaM binds.

1135-Plat
Bioinformatic Analysis of the Role of Intrinsic Disorder in Multiple Specificity
Christopher J. Oldfield, Vladimir N. Uversky, A. Keith Dunker.
School of Informatics, Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA.
Several lines of evidence suggest that intrinsically disordered proteins (IDPs) are a common mechanism used by nature to mediate protein-protein interactions. IDPs lack a stable three-dimensional structure under physiological conditions and many such proteins have been characterized by several biophysical methods. Additionally, IDPs are estimated to be abundant within various proteomes, particularly eukaryotes, and carry out a variety of molecular functions without the prerequisite of a specific, stable structure. It is thought that IDPs can facilitate protein interactions through an ability to mediate binding diversity, where one of the proposed mechanisms for this is multiple specificity - i.e. recognition of multiple molecular partners through use of the same binding residues - through contextual folding of IDPs.
In previous work, two contrasting examples of proteins with multiple binding specificity were examined, 14-3-3ζ and p53, which exemplify the potential of intrinsic disorder for mediating protein interactions. 14-3-3ζ has a structured domain with a single binging pocket that is responsible for the binding of various protein partners through interaction with sequence divergent intrinsically disordered segments in these partners. In contrast, the disordered termini of p53 contain discrete regions that are each involved in many interactions with different protein partners, where these interactions carry out and regulate p53 function. The common theme in both of these examples is structural variability in the bound state that is enabled by intrinsic disorder in one of the partners in the unbound state.
In current work, the previous analysis is expanded to many other examples of proteins that interact with multiple partners using a common binding site. These data support the conjecture that intrinsic disorder enables binding to multiple partners and provides detailed information about induced fit in structured regions.

1136-Plat
High-throughput Characterization of Intrinsically Disordered Proteins from the Protein Structure Initiative
Derrick E. Johnson, Megan D. Sickmeier, Jingwei Meng, Tanguy Le Gall, Vladimir Uversky, A. Keith Dunker.
Indiana University School of Medicine, Indianapolis, IN, USA.
The identification of intrinsically disordered proteins (IDPs) among the targets that fail to form satisfactory crystal structures in the Protein Structure Initiative represent a key to reducing the costs and time for determining three-dimensional structures of proteins. To help in this endeavor, several Protein Structure Initiative Centers were asked to send samples of both crystallizable proteins and proteins that failed to crystallize. Initially, the abundance of intrinsic disorder in these proteins was evaluated via computational analysis using Predictors of Natural Disordered Regions (PONDR®) and the potential cleavage sites and corresponding fragments were determined. Then, the target proteins were analyzed for intrinsic disorder by their resistance to limited proteolysis. The rates of tryptic digestion of sample target proteins were compared to those of myoglobin, apomyoglobin and α-casein as standards of ordered, partially disordered and completely disordered proteins, respectively. Results from these digestion experiments generally correlated with the results of disorder predictions. At the next stage, the protein samples were subjected to both far-UV and near-UV circular dichroism (CD) analysis. For most of the samples, a good agreement between CD data, predictions of disorder and the rates of limited tryptic digestion was established. Most samples corresponded to proteins that were predicted to be ordered had slower digestion rates and showed a good amount of ordered structure as determined by near- and far-UV CD analysis. On the contrary, predicted to be disordered proteins were digested fast and possessed spectral features characteristic of IDPs. Further experimentation is being performed on a smaller subset of these samples in order to obtain more detailed information about the ordered/disordered nature of the proteins.

1137-Plat
How Does Charge Content Modulate Conformational Equilibria of Intrinsically Disordered Proteins? An Illustration Using Protamines
Albert Mao, Andreas Vitalis, Rohit V. Pappu.
Washington University in St. Louis, St. Louis, MO, USA.
Intrinsically disordered proteins (IDPs) adopt heterogeneous ensembles of conformations at equilibrium under physiological conditions. Just as the structure of a folded protein determines its function, the conformational ensemble of an IDP governs its interactions with binding partners. We seek quantitative descriptions of conformational equilibria anchored in polymer physics concepts that capture the richness of IDP phase diagrams. Recent studies by our lab showed that archetypal polar homopolymer IDPs favor collapsed ensembles in water despite the absence of hydrophobes, a counterintuitive result that even held for polypeptide backbones alone. We now turn our attention to highly charged peptides, which constitute a different archetype of IDP. We simulated a variety of protamines - a class of arginine-rich IDPs involved in the condensation of nuclear chromatin during spermatogenesis - in aqueous 125 mM salt solutions in order to elucidate the influence of charge content on conformational equilibria. The simulations were performed with ABSINTH, a Monte Carlo engine that employs our recently-developed implicit solvation model. We find that protamines with high charge asymmetry are similar in their adoption of extended bent-rod conformations, a result in agreement with theoretical predictions. Sequences with identical charge asymmetry but different charge composition exhibited similar characteristics in terms of overall size measures such as radius of gyration. However, local properties such as alpha helix propensity remained strongly dependent on the particular sequence. These findings point towards a possible engineering principle for IDP sequence design: general size requirements set the charge asymmetry, while local conformational specifications govern the particular sequence. This principle is consistent with the evolutionary pattern of protamines: while sequences exhibit hypervariability across species, arginine content is highly conserved.
Funded by grant MCB 0718924 from the National Science Foundation.

1138-Plat
Characterization of the Unfolded State Under Native Conditions: A Missing Piece of the Protein Folding Puzzle
Daniel P. Raleigh, Bing Shan, Wenli Meng, Jae-Hyun Cho, Humeyra Taskent.
State University of New York, Stony Brook, NY, USA.
The nature of the denatured state ensemble is controversial owing in large part to the difficulty of characterizing the structure and energetics of denatured state interactions. Denatured states can be populated under a variety of extreme conditions but the state which is most relevant for protein folding and engineering is the denatured state ensemble which is populated in the absence of denaturant under native conditions. Unfortunately this state is usually experimentally inaccessible. We reported detailed characterized of the denatured state populated under native conditions for two α-β proteins, the N-terminal domain of the ribosomal protein L9 (NTL9) and the C-terminal domain of the same protein (CTL9) , as well as for a rapid folding all helical structure the villin headpiece helical subdomain, HP-36. Conditions have been found where the native and denatured states of CTL9 are both populated in the absence of denaturant and 1H, 15N and 13C NMR was used to define the conformation propensities of the denatured state. For NTL9 the thermodynamic linkage between proton binding and protein stability was used to characterize denatured state electrostatic interactions. Peptide models were exploited to characterize the denatured state of HP-36. In all three cases, the denatured states contains significant structure. The impact of this preformed structure on the kinetics and mechanism of protein folding is discussed.

1139-Plat
Distribution of Conformations Sampled by the Central Amino Acid Residue in GXG Peptides Inferred from Amide 1′ Band Profiles and NMR Scalar Coupling Constants
Andrew Hagarman[1], Thomas Measey[1], Daniel Matthieu[2], Harald Schwalbe[2], Reinhard Schweitzer-Stenner[1].
[1]Drexel University, Philadelphia, PA, USA, [2]Johann Wolfgang Goethe Universität, Frankfurt, Germany.