3268-Plat
Mechanism of Selective Urea Permeation through a Bacterial Urea Transporter
Elena J. Levin, Matthias Quick, Ming Zhou.
Columbia University, New York, NY, USA.
In addition to its function as an intermediate in nitrogen metabolism, the small molecule urea plays an important role in the homeostasis of osmolarity and fluid volume in many organisms, including mammals, which concentrate urea in the kidney to produce the osmotic gradient necessary for water re-absorption. Because urea is highly polar and consequently poorly permeable through lipid bilayers, specialized urea transporters have evolved to increase its rate of diffusion across cell membranes. To better understand how urea transporters achieve the rapid and selective transport of urea, we have solved the 2.3 Å structure of a urea transporter from the bacterium Desulfovibrio vulgaris (dvUT), which has significant homology to mammalian urea transporters. The dvUT fold contains two homologous domains related by a two-fold pseudosymmetry axis perpendicular to the plane of the membrane. Each protomer contains a continuous membrane-spanning pore, suggesting that the protein operates by a channel-like rather than transporter-like mechanism. The constricted selectivity filter at the center of pore can accommodate dehydrated urea molecules passing in single file. Urea is stabilized by backbone and side chain oxygen atoms that provide continuous coordination as it progresses through the filter, and by well-positioned α-helix dipoles. We are now using a variety of functional assays to probe the physical and chemical interactions involved in the transport mechanism, as well as interactions between dvUT and various high-affinity UT blockers.

Platform BE: Protein Conformation

3269-Plat
Switching Amyloid β-Peptides Oligomerization and Cytotoxicity with Nanoparticles
Ana M. Saraiva[1,2], Isabel Cardoso[3], Maria João Saraiva[3], M. Carmo Pereira[2], Manuel A.N. Coelho[2], Helmuth Moehwald[1], Gerald Brezesinski[1].
[1]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, [2]LEPAE, Dept. of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal, [3]Molecular Neurobiology Unit, Instituto de Biologia Molecular e Celular, Porto, Portugal.
The amyloid-β peptide (Aβ) plays a central role in the mechanism of Alzheimer's disease (AD), being the main constituent of the plaque deposits found in AD brains. Aβ amyloid formation is due to a conformational switching to a β-enriched structure. However, the relevance of the plaques to the pathogenesis is unclear and evidences indicate that Aβ toxicity is mediated by soluble oligomers. Our strategy to inhibit aggregation involves re-conversion of Aβ conformation by adsorption to nanoparticles (NPs). We have shown that fluorinated NPs induce α-helical rich structures on Aβ and inhibit fibrillogenesis, whereas their hydrogenated analogues lead to aggregation (Figure 1). In order to test the influence of the zeta potential of NPs on the peptide structure, NPs without fluorine were synthesized by sulfonation and sulfation of polystyrene, leading to microgels and latexes. Studies about the conformational behaviour and oligomerization/cytotoxicity of Aβ in the presence of polymeric nanostructures were performed. Cytotoxicity assays confirmed our hypothesis that the conformational conversion of Aβ has an antiapoptotic activity, increasing the viability of cells treated with oligomeric species. The proper balance between hydrophilic moieties and hydrophobic chains seems to be an essential feature of effective NPs.

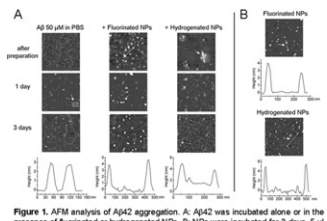

Figure 1. AFM analysis of Aβ42 aggregation. A: Aβ42 was incubated alone or in the presence of fluorinated or hydrogenated NPs. B: NPs were incubated for 3 days. 5 µl aliquots were spotted on freshly cleaved mica. The image size is 1 x 1 µm² and the z-range is 5 nm. Section analysis corresponding to the dotted lines indicated by white arrows is shown.

3270-Plat
α-Lactalbumin, Engineered to be Non-Native and Inactive, Kills Tumor Cells When in Complex with Oleic Acid: A New Biological Function Resulting from Partial Unfolding
Jenny Pettersson-Kastberg[1], Ann-Kristin Mossberg[1], Maria Trulsson[1], Yeon Joong Yong[2,1], Soyoung Min[2], Yoongho Lim[3], John E. O'Brien[2], Catharina Svanborg[1,4], **K. Hun Mok**[2,1].
[1]Lund University, Lund, Sweden, [2]Trinity College Dublin, Dublin, Ireland, [3]Konkuk University, Seoul, Korea, Republic of, [4]Singapore Immunology Network (SIgN), Biomedical Sciences Institutes, A*STAR, Singapore, Singapore.
HAMLET (human alpha-lactalbumin made lethal to tumor cells) is a tumoricidal complex consisting of partially unfolded protein and fatty acid, and was first identified in casein fractions of human breast milk. The complex can be produced from its pure components through a modified chromatographic procedure where pre-applied oleic acid binds with partially-unfolded α-lactalbumin on the stationary phase *in situ*. Because native α-lactalbumin itself cannot trigger cell death, HAMLET's remarkable tumor-selective cytotoxicity has been strongly correlated with the conformational change of the protein upon forming the complex, but whether a recovery to the native state subsequently occurs upon entering the tumor cell is yet unclear. To this end, we utilize a recombinant variant of human α-lactalbumin in which all eight cysteine residues are substituted for alanines (rHLA$^{all\text{-}Ala}$), rendering the protein non-native and biologically inactive under all conditions. The HAMLET analogue formed from the complex of rHLA$^{all\text{-}Ala}$ and oleic acid (rHLA$^{all\text{-}Ala}$-OA) exhibited equivalent strong tumoricidal activity against lymphoma and carcinoma cell lines, and was shown to accumulate within the nuclei of tumor cells, thus reproducing the cellular trafficking pattern of HAMLET. In contrast, the fatty acid-free rHLA$^{all\text{-}Ala}$ protein associated with the tumor cell surface but was not internalized and lacked any cytotoxic activity. Structurally, whereas HAMLET exhibited some residual native character in terms of NMR chemical shift dispersion, rHLA$^{all\text{-}Ala}$-OA showed significant differences to HAMLET, and in fact was found to be devoid of any tertiary packing. The results identify α-lactalbumin as a protein with strikingly different functions in the native and partially unfolded states. We posit that partial unfolding offers another significant route of functional diversification for proteins within the cell.

3271-Plat
Neutron Spin Echo Studies of Dynamics in Hemoglobin and Myoglobin
Jyotsana Lal[1], Peter Fouquet[2], Marco Maccarini[2], Lee Makowski[1].
[1]Argonne National Laboratory, Argonne, IL, USA, [2]Institut Laue-Langevin, Grenoble, France.
Neutron spin-echo (NSE) spectroscopy was used to study structural fluctuations that occur in hemoglobin (Hb) and myoglobin (Mb) in solution. Using NSE data to very high momentum transfer, q (~ 0.62 Å-1), the internal dynamics of these proteins were characterized at the level of the dynamical pair correlation function and self-correlation function in the time range of several picoseconds to a few nanoseconds. Comparison of data from the two homologous proteins collected at different temperatures and protein concentrations was used to assess the contributions to the data made by translational and rotational diffusion and internal modes of motion. The temperature dependence of the decay times can be attributed to changes in viscosity and temperature of the solvent as predicted by the Stokes-Einstein relationship. This is true for contributions from both diffusion and internal modes of motion indicating an intimate relationship between the internal dynamics of the proteins and the viscosity of the solvent. Viscosity change associated with protein concentration can account for changes in diffusion observed at different concentrations, but is apparently not the only factor involved in the changes in internal dynamics observed with change in protein concentration. Comparison of data from Hb and Mb at low q indicate an unexpectedly rapid motion of the hemoglobin αβ-dimers relative to one another. These observations are consistent with the notion that movements of structural elements along paths of intrinsically low free energy - as may form during evolution to expedite conformational changes between different functional states - are a major factor in determining the dynamic behavior of proteins in solution.

3272-Plat
Temperature Dependence of the Amide I Frequency as a Probe of Solvent Accessibility of the Protein Backbone
Dana M. Alessi, **Sean M. Decatur**.
Oberlin College, Oberlin, OH, USA.
Infrared (IR) spectroscopy of proteins often focuses on the relationship between the amide I band and polypeptide secondary structure; IR spectra has been used to probe backbone conformation on a wide range of samples (solution, films, gels, and solids), at a broad range of time scales (from ns to days), and as a function of a variety of perturbations (solvent, ionic strength, presence of lipid membrane). Often ignored is the solvent dependence of the amide I mode. Solvent-backbone hydrogen bonding can have a large effect on the observed amide I frequency (1). In this work, we demonstrate that the temperature dependence of amide I bands can be used to determine the solvent exposure of the peptide backbone. We have compared the IR spectra as a function of temperature in a number of different protein systems, including small, alanine-based peptides that form alpha-helices in solution; short peptides that aggregate to form fibrous, beta-sheet rich fibrous structures; short, dynamic peptides lacking regular secondary structure; membrane-embedded peptides; and globular proteins. When backbone groups are buried from solvent, the amide I band frequencies

are independent of temperature (in the range of 5°C to 75°C). By contrast, backbone groups exposed to (and hydrogen-bonded with) solvent water show a temperature dependence similar to that observed in solvated model compounds (2). When this approach is combined with specific isotope-labeling (3), site-specific information about solvent accessibility can be obtained from the variable-temperature IR spectra.

(1) A. Starzyk, W. Barber-Armstrong, M. Sridharan and S. M. Decatur, Biochemistry, 2005, 44, 369.
(2) K. E. Amunson and J. Kubelka J. Phys. Chem. B, 2007, 111, 9993.
(3) S. M. Decatur, Acc. Chem. Res., 2006, 39, 169.

3273-Plat

A Hinge Region Cis-Proline in Bovine Pancreatic RNase a Acts as a Conformational Gatekeeper for C-terminal Domain Swapping

Katherine H. Miller[1], Susan Marqusee[1], Jessica Karr[2].
[1]University of California at Berkeley, Berkeley, CA, USA, [2]Amgen Scholars Program, University of California at Berkeley, Berkeley, CA, USA.

Structural studies have implicated proline as an important residue for domain swapping and oligomerization due to its increased frequency in hinge regions preceding swapped arms. Proline is unique in its ability to populate both cis and trans peptide bond conformations; we hypothesized that this property may allow proline to act as a conformational gatekeeper, regulating interconversion between monomer and domain-swapped dimer. The hinge region of RNase A contains a proline at residue 114 that adopts a cis conformation in the monomer and extends to a trans conformation in the C-terminal domain-swapped dimer. We find that substitution of P114 with residues that strongly prefer a trans peptide bond (Ala, Gly) results in significant population (~20%) of the C-terminal domain-swapped dimer under near-physiological conditions (pH 8.0, 37°C). This is in stark contrast to dimerization of wild-type RNase A, which requires incubation under extreme conditions such as lyophilization from acetic acid or elevated temperature. Our results suggest isomerization at P114 may facilitate population of a partially unfolded intermediate along the reaction trajectory of RNase A domain swapping, and provide support for a more general role for proline isomerization as a conformational gatekeeper in domain swapping and oligomerization.

3274-Plat

The Importance of Protein-Protein Interactions on the pH-Induced Conformational Changes of Bovine Serum Albumin: A Small Angle X-Ray Scattering Study

Leandro R.S. Barbosa[1], Maria Grazia Ortore[2], Francesco Spinozzi[2], Paolo Mariani[2], Sigrid Bernstorff[3], Rosangela Itri[1].
[1]Institute of Physics of University of Sao Paulo, Sao Paulo, Brazil, [2]Dipartimento SAIFET, Sezione Scienze Fisiche, Università Politecnica delle Marche and CNISM, Ancona, Italy, [3]National Laboratory of Synchrotron Light, Elettra, trieste, Italy.

The combined effects of concentration and pH on the conformational states of Bovine Serum Albumin, BSA, are investigated by Small Angle X-Ray Scattering. Serum Albumins, at physiological conditions, are found at concentrations around 35-45 mg/ml (42 mg/ml in the case of humans). In the current work, BSA at 10, 25 and 50 mg/ml, and pH values ranging from 2.0 to 9.0 have been studied. Data were analyzed by means of the Global Fitting procedure, being the protein form factor calculated from Human Serum Albumin, HSA crystallographic structure and the interference function described considering repulsive and attractive interaction potentials within a Random Phase Approximation. SAXS data show that BSA maintains its native state from pH 4.0 up to 9.0 at all investigated concentrations. A pH-dependence of the absolute net protein charge is demonstrated and the charge number per BSA is quantified to 10(2), 8(1), 13(2), 20(2), 26(2) for pHs 4.0, 5.4, 7.0, 8.0 and 9.0, respectively. The attractive potential diminishes as BSA concentration increases. The coexistence of monomers and dimers is observed at 50 mg/ml and pH 5.4, near the BSA isoelectric point. Samples at pH 2.0 show a different behavior, as BSA overall shape changes as a function of concentration. At 10 mg/ml, BSA is partially unfolded and a strong repulsive protein-protein interaction occurs due to the high amount of exposed charge. At 25 and 50 mg/ml, BSA has some refolding and a molten-globule state probably takes place. As a conclusion, the present work confirms that the protein concentration plays an important role on the pH-unfolded BSA state, due to a delicate compromise between interaction forces and crowding effects. This work was recently accepted for publication in the Biophysical Journal.

3275-Plat

The Mechanical Properties of PCNA: Implications for the Loading and Function of Sliding Clamps

Joshua L. Adelman[1], John D. Chodera[2], I-Feng W. Kuo[3], Thomas F. Miller III,[4], Daniel Barsky[3].
[1]University of Pittsburgh, Pittsburgh, PA, USA, [2]California Institute for Quantitative Biosciences (QB3), Berkeley, CA, USA, [3]Lawrence Livermore National Laboratory, Livermore, CA, USA, [4]California Institute of Technology, Pasadena, CA, USA.

Sliding clamps are toroidal proteins that encircle DNA and act as mobile platforms on which components of the DNA replication and repair machinery bind. While trimeric sliding clamps assemble as stable planar rings, they must be splayed open at one of the subunit-subunit interfaces in order to thread duplex DNA into their central pore. The opening process is driven by the ATP-dependent clamp loader, RFC, whose clamp-interacting sites form a right-handed spiral. Previous molecular dynamics (MD) studies suggested that when PCNA opens, it preferentially adopts a right-handed spiral to match the spiral of the clamp loader. We present evidence from considerably longer MD simulations that PCNA does not have a preference for forming a spiral structure with a particular handedness, although conformations that match the helical pitch of RFC were observed. The strong correspondence between all-atom simulations of PCNA and a coarse-grained elastic model suggest the behavior of the open clamp is primarily due to elastic deformation governed by the topology of the clamp domains. The simple elastic model further allows us to quantitatively describe the energetic cost of deforming PCNA to allow mating with the clamp loader or strand passage once an interface has been disrupted. A picture of PCNA emerges of a protein of considerable flexibility once opened, which is mechanically compliant in the clamp opening process.

3276-Plat

Coordination between N- and C-terminal Kinetics of Hsp90 Investigated by SmFRET

Thorsten Hugel, Christoph Ratzke, Moritz Mickler, Martin Hessling, Johannes Buchner.
TU München, Munich, Germany.

Hsp90 is a molecular chaperone required for the activation of a large amount of client proteins and survival of the cell during heat shock. It consists of two monomeric chains with dimerization interfaces at the C and N-terminal end[1]. Its chaperone function is dependent on ATP binding and hydrolysis as well as N- terminal and C-terminal dimerization[2].
Up to now mainly the N-terminal dimerization kinetics of Hsp90 has been investigated[3], while the C-terminal interface was assumed to be closed for many minutes, because of its low equilibrium binding constant. We developed a fluorescent based single molecule assay, which allows to investigate C-terminal dimerization independent of N-terminal kinetics. Surprisingly, we find C-terminal dissociation / association kinetics on the timescale of seconds. In addition, this kinetics is nucleotide dependent although the nucleotide binding pocket is far away in the N-terminal domain. Therefore, we conclude that there is coordination through the complete Hsp90 monomer. These findings are confirmed by well defined N-terminal mutations[4].

[1] Ali M.M. et al. Nature (2006) 440, 1013-1017.
[2] Wegele H., L. Müller, J. Buchner; Rev Physiol Biochem Pharmacol (2004) 151, 1-44.
[3] Mickler M., M. Hessling, C. Ratzke, J. Buchner, T. Hugel, NSMB, 16, 281 (2009)
[4] Ratzke C., M. Mickler, J. Buchner, T. Hugel, Manuscript in prep.