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.

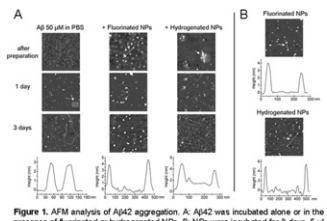

Figure 1. AFM analysis of Aβ42 aggregation. A: Aβ42 was incubated alone or in the presence of fluorinated or hydrogenated NPs. B: NPs were incubated for 3 days. 5 µl aliquots were spotted on freshly cleaved mica. The image size is 1 x 1 µm² and the z-range is 5 nm. Section analysis corresponding to the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