system for this study. Our results suggest that dipolar interactions play a role in aggregation, and thus more likely to play a role for aggregating, less-hydrophobic peptides.

1. Role of Backbone Dipole Interactions in the Formation of Secondary and Supersecondary Structures of Proteins; Sai J. Ganesan and S. Matysiak; Journal of Chemical Theory and Computation 2014 10 (6), 2569-2576

77-Plat

Combining Neutron Reflectivity and Hydrogen Deuterium Exchange Mass Spectrometry to Resolve Structural details of Membrane Associated Proteins

Michael S. Kent[1], Bulent Akgun[2], Hirsh Nanda[3], Gregory F. Pirrone[4], John Engen[4].
[1]8622, Sandia National Labs, Albuquerque, NM, USA, [2]Chemistry, Bogazici University, Bebek, Turkey, [3]National Institute of Standards and Technology, Gaithersburg, MD, USA, [4]Northeastern University, Boston, MA, USA.

While X-ray crystallography and NMR are available to determine the structure of folded proteins in solution, few methods can resolve structural details for membrane-associated proteins. Recently neutron reflection (NR) has emerged as a useful method to derive structural information for membrane-associate proteins. However, while NR provides the overall residue distribution normal to the membrane, information pertaining to specific residues is not available from this method. Toward addressing this deficiency we combined NR with hydrogen-deuterium exchange mass spectrometry (HDX-MS). HDX-MS has proven useful in studying protein complexes and binding of drug molecules to proteins in solution. We combined these methods to study the structure of membrane-associated HIV Nef. Nef is an HIV-1 accessory proteins and an essential factor in AIDS progression. Nef exists in both membrane-associated and cytosolic fractions. Membrane-association is achieved by an N-terminal myristoylation essential for the virus in vivo as well as a cluster of basic residues within the N-terminal arm. NR revealed that upon insertion of the myristate and residues from the N-terminal arm, Nef transitions from a closed to open conformation that positions the core domain 70 Å from the lipid headgroups.1 Deuterium exchange of HIV-1 Nef was analyzed in solution and also when membrane-associated. Significant differences in deuterium uptake between the solution form and lipid-associated state reveal the disposition of a flexible loop and the oligomeric state upon binding. Hundreds of proteins are known to be lipidated and many are potential targets for therapeutic intervention. The present approach will be useful to resolve the membrane-bound conformations of these proteins, and to inform on the effects of protein-protein interaction at the membrane and disruption of said interactions with pharmacological agents.

Akgun, B. et al, Structure 21, 1822, 2013.

78-Plat

Effect of Molecular Crowding on the Structure and Dynamics of Human Apo and Holo Transferrin using 2D-IR Correlation Spectroscopy

Sherif Abbas[1,2], Feride Severcan[1], Parvez I. Haris[3].
[1]Biological Sciences, Middle East Technical University, 06800, Ankara, Turkey, [2]Physics, Ain Shams University, Cairo, Egypt, [3]School of Allied Health Sciences, De Montfort University, The Gateway, Leicester, United Kingdom.

Human serum transferrin is an iron transport glycoprotein that is involved in the regulation and balance of iron content in blood plasma and cells. Little is known about the effect of molecular crowding on the structure, dynamics and aggregation of transferrin. Therefore, we investigated secondary structure, thermal denaturation, aggregation and hydrogen-deuterium (H/2H) exchange of apo and holo transferrin in the presence and absence of the molecular crowding agent dextran using Fourier transform infrared (FTIR) spectroscopy. The data obtained was analysed using second-derivative, deconvolution and two-dimensional correlation infrared (2D-IR) spectroscopy. Protein samples were prepared in phosphate buffer solutions (H2O and 2H2O; pH 7.4) at a concentrations of 20 and 50mg/ml. For the crowded environment condition, the same conditions were used but dextran was also included at a concentration of 200 mg/ml. No molecular crowding-induced changes in the secondary structure of transferrin was detected. However, the H/D exchange of apo and holo transferrin in dextran solution was significantly reduced suggesting a more rigid, solvent inaccessible structure induced by molecular crowding. The thermal study of transferrin in the range 25-95 oC was analysed using the synchronous 2D-IR correlation. The 2D-IR correlation spectra of holo transferrin in presence and absence of dextran revealed different negative peaks. In the absence of dextran, the loss of α-helical structure (1656cm-1) is associated with an increase in the intensity of a band at 1674cm-1. However, in the presence of dextran, the unfolding of the α-helical structure (1656cm-1) is accompanied by the formation of intermolecular β-sheet structure (1616cm-1 and 1690cm-1). Similar crowding effects were also observed for apo transferrin providing direct evidence of the effect of dextran, as molecular crowding agent, on the thermal aggregation characteristics and dynamics of transferrin.

79-Plat

Structural insight into the Phosphoinositide-Regulated Cellular Dynamics of Alpha-Actinin

Andrea Ghisleni[1], Euripides De Almeida Ribeiro[2], Nikos Pinotsis[2], Mark R. Holt[1], Pauline Bennett[1], Kristina Djinovic-Carugo[2], Mathias Gautel[1].
[1]Randall Division for Cell and Molecular Biophysics and Cardiovascular Division, King's College London, London, United Kingdom, [2]Department of Structural and Computational Biology, University of Vienna, Max F. Perutz Laboratories, Vienna, Austria.

Alpha-actinin2 is a fundamental component of striated muscle sarcomeres. It localizes to the Z-disk, the macromolecular assembly that connects two adjacent sarcomeres where it provides mechanical stability during contraction but also plays a pivotal role during Z-disk assembly. A regulatory role by phosphatidylinositol-bis-phosphate (PiP2) has been proposed for all 4 alpha-actinin isoforms but not validated at structural level. The X-ray structure of full-length alpha-actinin2 suggests the existence of two different conformational states. In the ground state, alpha-actinin exists in an intramolecular auto-inhibited conformation, where the C-terminal EF-hands bind a titin-like pseudoligand at the connection between rod and actin-binding domains. PiP2 acts as a potential trigger for the transition to an open state capable of titin-binding. We hypothesize this mechanism plays a key role during Z-disk maturation, with the open-state allowing interaction with key binding partners. Super-resolution and correlative electron microscopy of mutant-transfected Neonatal Rat Cardiomyocytes (NRC) shed new light on the internal organization of the Z-disk and the regulatory mechanism of alpha-actinin ligand binding. Combining fluorescence confocal microscopy and live-cell imaging in NRC, we identified a crucial role for PiP2, which regulates the intracellular dynamics of alpha-actinin as seen in Fluorescence Recovery After Photobleaching experiments with a PiP2-binding deficient mutant. A second alpha-actinin mutant, in constitutively open-conformation, impaired overall sarcomere assembly by reduced intracellular dynamics, suggesting that PiP2 might not be limited to a positive regulation, but is also essential for the dynamic equilibrium of the protein.

80-Plat

Determining How Many Ionic Interactions are Needed for the High Stability of Single Alpha Helical (SAH) Domains

Marcin D. Wolny, Matthew R. Batchelor, Peter J. Knight, Emanuele Paci, **Michelle Peckham**.
University of Leeds, Leeds, United Kingdom.

Stable single alpha helical (SAH) domains are found in ~0.2% of proteins and are rich in charged amino acids; E,R and K. Ionic interactions between oppositely charged amino acids (either i, i+3 and/or i, i+4) are responsible for their high stability. We recently showed that SAH domains can act as 'constant force springs (Wolny et al., J. Biol. Chem. 2014). Thus they do not simply act as a 'spacer' between two protein domains, but can actively respond to force.

It is still unclear what the minimal requirements are for a peptide to form a SAH domain. To test this, we used modelling and have characterised 96 residue constructs that vary in the number of charged interactions (E-R or E-K) from 1 every 7 residues (repeating 7mer pattern of (AAEAAKA)n or (AAEAARA)n to 3 every 7 residues ((AEEEKKK)n or (AEEERRR)n) to determine how many is 'enough' to stabilize a long (96 residue) alpha helix. 96 residue (AAEAAKA)n or (AAEAARA)n peptides tended to aggregate, and were only soluble in very low salt, when they were found to be highly helical, but unstable. Thus, sparsely placed charged residues (1 pair per heptad) are not sufficient to keep polyalanine stable in higher salt concentrations. 96 residue (AEEEKKK)n peptides were highly stable and helical (>90%) and melted non-cooperatively as expected for SAH domains. Surprisingly, 96 residue (AEEERRR)n peptides tended to aggregate, were only soluble at pH<3.5, and appeared to be in a monomer-dimer equilibrium. SAH domains normally contain mixtures of both K and R residues. These data suggest that K residues are required for solubility and monomeric behavior at neutral pH. Modeling helps to explain these data and further clarify the minimal requirements for a SAH domain.