of the 40 bp in the cruciform state was estimated using Mfold. The predicted secondary structure indicates that the 40 bp in the cruciform form an imperfect hairpin. Deletion of these 40 base pairs from the 5kb DNA molecule in which they were embedded abolished the fluctuations. Once the cruciform DNA structure is formed, the rate of extrusion and resorption of the 40 bp cruciform structure is directly and reversibly controlled by varying ΔLK or the applied stretching force. The measured equilibrium lifetime of the cruciform structure is 16 ± 2 seconds, and in low salt lifetimes follow single-exponential distributions. In higher salt conditions cruciform extrusion is a two-step kinetic process, as addition of 150 mM Nacl or 5 mM $Mg^{2+}$ led to the stabilization of intermediate states lying between the native and cruciform states of the DNA. Mutations in the loop did not affect the kinetics of cruciform formation/destruction. However, mutations in the stem dramatically reduced the lifetime of the cruciform structure or completely abolished the fluctuations. A mutation which destabilized the stem region while nevertheless preserving overall cruciform extrusion was shown to yield an equilibrium lifetime of 6 ± 1 seconds.

**Wednesday, February 6, 2008**

**Symposium 18: Damaged Proteins-Structural and Biological Consequences**

## 2593-Symp Proteome Dynamics - Parameterising Protein Turnover At A Global Level

Robert Beynon

*University of Liverpool, Liverpool, United Kingdom.*

An important, if overlooked, aspect of proteome characterization is the definition of the intracellular stability of individual members of the proteome, data that inform systems models and network dynamics, and which allow us to define mechanisms that regulate protein turnover. Global determination of protein stability in the cell is, coupled with absolute quantification of protein abundance defines one half of the protein turnover cycle, with protein synthesis (itself a product of mRNA abundance and translational activity) as the other. Indeed, the failure to fully define the parameters of this cycle may be the most compelling reason for the lack of a strict correlation between the abundance of a protein and its cognate transcript. Metabolic incorporation of stable isotope labeled amino acids, coupled with high resolution separation and mass spectrometry, permit accurate measurement of intracellular stability on a global scale, from which we can infer relationships between intracellular stability and function, and identify factors that dictate the rate at which proteins are sequestered into the degradative apparatus. In this presentation, I will discuss approaches to acquisition of large data sets of protein stability data, particularly in respect of isolated single cells but also in intact animals. I also will describe a novel approach to absolute quantification that is extendible to entire proteome. Finally, I will discuss some recent data that address structural determinants of protein stability.

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## 2594-Symp The Molecular Basis of Alzheimer's and other Protein Misfolding Diseases

Louise C. Serpell

*University Sussex, Falmer, Brighton, United Kingdom.*

Many unrelated proteins and peptides assemble to form amyloid fibrils that accumulate in the tissues in the misfolding diseases. These diseases include Alzheimerâ™s disease, Diabetes type 2 and the spongiform encepthalopathies. Although the precursor proteins share no similarities in native structure or primary sequence, the fibrils that are formed are rich in beta-sheet structure and share a common core conformation known as the cross-beta structure. Understanding the process of abnormal protein assembly is central to understanding pathology of the misfolding diseases. Small, oligomeric assemblies of amyloidogenic peptides may play a key role in cell toxicity and tissue degeneration. Recent advances in the structure determination of the amyloid core structure have lead to a clearer picture of the internal architecture of the amyloid fibril. Relating this structure of the mature fibril to the structure of the toxic oligomer is the next important step in understanding the disease process.

## 2595-Symp The Proteasomes Regulatory ATPases Stimulate Protein Degradation by Using A "Key-in-a-lock" Mechanism to Open the Gate in the 20S Particle

David Smith[1], Yifan Cheng[2], Julius Rabl[1], Shih-Chung Chang[1], Soyeon Park[1], Dan Finley[1], Alfred Goldberg[1]

[1] *Harvard Medical School, Boston, MA, USA,*
[2] *University of California-San Francisco, San Francisco, CA, USA.*

Protein degradation in all cells is an ATP-dependent process. In the eukaryotic 26S proteasome, the 20S core particle degrades proteins while associated with the six ATPases (RPT1-6) in the base of the 19S regulatory particle, and in archaea, the 20S functions with the homologous PAN complex, a homo-hexameric ATPase. These ATPases unfold protein substrates and catalyze the translocation of the unfolded polypeptides into the 20S particle. They are also members of the AAA family but contain a conserved C-terminal HbYX motif. We recently showed that upon binding of ATP to PAN this motif binds to intersubunit pockets in the 20S's outer alpha-ring, linking the ATPases to the 20S and trigger opening of a gated channel for substrate entry. This gate in the 20S's alpha-ring is formed by the N-termini of the alpha-subunits excludes substrates when closed. Eight-residue peptides corresponding to PAN's C-terminus (and containing the HbYX motif) can compete with PAN for binding to the 20S particle and by themselves cause gate opening. Therefore the proteasomal ATPases stimulate proteolysis by using their C-termini like a "key-in-a-lock" to catalyze substrate entry. To understand the mechanism of gate opening, we used Cryo-Electron Microscopy, to show that binding of this C-terminal ATPase domain to the 20S induces a rotation in the proteasomes' alpha-subunits, positioning a critical pro17 reverse-turn loop into a position that stabilizes the open-gate conformation. Mutations of these residues in yeast revealed that the more complex eukaryotic

26S proteasome uses the same "Key-in-a-lock mechanism", but the C-termini of these six different Rpt ATPases' play distinct roles, some participate in complex formation while others cause gate-opening. This novel "key-in-a-lock" mechanism for gate opening contributes to the energy-dependence of intracellular protein degradation.

## 2596-Symp CryoEM Studies of Small Heat-Shock Proteins

Phoebe Stewart

*Vanderbilt Univ, Nashville, TN, USA.*

Small heat shock proteins (sHsps) are a family of chaperones that bind unfolded proteins and prevent irreversible aggregation. sHsp's share a conserved central alpha-crystallin domain, ~90 amino acid long, with variable N- and C-termini. The polydispersity of human alpha-crystallin and Hsp27 have precluded their high resolution structural determination. Hsp16.5 from *Methanococcus jannaschii* forms a symmetrical assembly and has been studied by x-ray crystallography, cryoelectron microscopy (cryoEM) and site-directed spin labeling EPR (SDSL-EPR). CryoEM and SDSL-EPR studies of several engineered Hsp16.5 variants have revealed how modifying the N-terminal region can modulate the oligomer size as well as its oligomeric state (monodisperse and symmetrical or polydisperse). CryoEM results of Hsp16.5 wild-type, as well as engineered variants, complexed with a model substrate will be presented. The Hsp16.5 variants include a truncated form (Hsp16.5TR) that lacks the N-terminal 33 residues of wt Hsp16.5 and a form (Hsp16.5-P1) that has a proline-rich 14aa peptide from Hsp27 inserted between the N-terminal region and the alpha-crystallin domain. We use a thermodynamically unstable mutant of T4 lysozyme (T4L) as the model substrate. Our results on the Hsp16.5 wt/T4L complex indicate that substrate binding can trigger significant changes in the quaternary structure of the oligomer. Our results from the Hsp16.5 variants indicate particular regions of Hsp16.5 that are likely to be involved in substrate binding. In addition a cryoEM structure of Hsp16.5-R107G will be presented, which offers a clue to the impaired functionality of the human disease related R120G mutation in alpha-crystallin. Our structural studies indicate that the adaptable quaternary structure of sHsps is related to their ability to bind substrate.

### Symposium 19: Allostery and Dynamics in Protein Function

## 2597-Symp Defining Native Protein Ensembles Using X-ray Crystallography

Mark Sales, Ho-Leung Ng, James S. Fraser, P. Therese Lang, Nathaniel Echols, Tom Alber

*University California, Berkeley, Berkeley, CA, USA.*

Although proteins populate large structural ensembles, protein X-ray diffraction data are traditionally interpreted using a single dominant model. We developed two methods – Ringer and tau – that use direct electron-density sampling to measure structural polymorphism. Ringer analysis around dihedral angles of high-resolution structures suggests that in addition to side chains already built in multiple conformations, 5–15% of residues populate "missing" unmodeled rotamers. The side-chain tau value affords a complementary, model-independent metric of rotamer ensembles. The tau value is correlated to the Shannon entropy, connecting for the first time a crystallographic measurement with a thermodynamic quantity, the residual entropy of each residue. These results show that crystalline proteins are more polymorphic than current crystallographic models. Examples of polymorphism in allosteric switching, ligand binding and enzymatic catalysis will be discussed. The structural and functional implications of this polymorphism depend critically on the degree of conformational coupling. Independent conformers would contribute residual entropy to the native state, while simultaneous structural changes can provide a mechanism for signaling. Overall, our analyses using Ringer and tau indicate that far from providing a static picture of proteins, X-ray crystallographic data reveal populations of alternate structures that reflect the dynamics essential for folding, binding, catalysis and regulation.

## 2598-Symp Conformational Waves in Receptors

Anthony Auerbach

*SUNY Buffalo, Buffalo, NY, USA.*

Acetylcholine receptor-channels (AChRs) are large (~300 kD, five subunits) allosteric membrane proteins that switch between C(losed) and O(pen) conformations. We use Φ, a parameter derived from the forward (f) and backward (b) rate constants of a reaction, to provide temporal information about the moving parts of the AChR in C↔O 'gating'. Φ is the slope of a linear fit to a plot of log f vs log Keq (=f/b) for a family of mutations of a single residue. First, we develop a Markov model of the transition region (TR) and show that Φ reflects the relative time in the TR that the perturbed residue switches, in an all-or-none fashion, from a C-like to an O-like structure. Second, we describe the map of Φ-values for >300 different AChR mutant constructs. There is, approximately, a coarse-grained and decreasing gradient in Φ between the allosteric (transmitter-binding) sites and the catalytic site (the 'gate'). Third, we use this map and theory to *calculate* the shape of the micro-barriers of the TR, which turns out to be nearly flat. Finally, we use the 'speed-limit' for channel-opening to estimate the rates of the microscopic transitions between the intermediates states that link stable C with stable O. AChR gating is, approximately, a brownian conformational 'wave' in which nm-sized domains move back-and-forth (on ~50 ns timescales) to connect the stable ground states of the reaction.

## 2599-Symp Endopeptidases that cleave specific proteins

James Wells

*University of California, San Francisco, San Francisco, CA, USA.*

Caspases are thiol endopeptidases that cleave specific proteins after aspartic acid residues and drive apoptosis or inflammation upon zymogen activation. Despite considerable interest in developing