+ activity in myocytes of intact heart. Rat hearts were loaded with fluo-4AM on the stage of a confocal microscope and rapid pacing protocols were used to study intracellular Ca2+ cycling during INa prolongation. A low concentration of ATX II (1nM) was used to increase QT duration of the ECG, mimicking the LQT3 phenotype. Prolongation of INa increased the duration of basal Ca2+ transients and vulnerability to the development of cellular Ca2+ alternans induced by rapid pacing. Ca2+ waves developed during exposure to ATX II that increased in incidence and decreased in coupling interval following rapid pacing, suggesting that triggered activity was the result of Ca2+ overload and ensuing delayed afterdepolarizations (DADs). This triggered activity was probably responsible for the arrhythmias that were triggered during rapid pacing in the presence of ATX II. Our data demonstrate that the slowly inactivating INa induced by low concentrations of ATX II

1. is responsible for QT prolongation that pharmacologically mimics genetically-induced LQT3;
2. causes a prolongation of intracellular Ca2+ transients which contributes to the development of Ca2+ alternans;
3. causes intracellular Ca2+ overload and resulting Ca2+ waves, DADs and triggered activity; and
4. causes pacing-induced ventricular tachycardias by triggered activity and/or reentrant excitation.

We conclude that induction of late INa causes profound disturbances in Ca2+ cycling which may contribute to arrhythmogenesis in addition to its direct effects on INa and the cardiac action potential.

Platform BC: Protein Assemblies

2640-Plat Clathrin Triskelia Self Assemble into Fullerene Cages Permitted by the Head-to-Tail Exclusion Rule

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Originally coined for carbon cages, the word "fullerene" describes a closed, convex cage with 3-connected vertices and with hexagonal and pentagonal faces, necessarily 12 of the latter (Kroto et al., Nature *318*, 162–163, 1985). Carbon atoms self assemble into large fullerene cages that obey the isolated-pentagon rule (IPR), the smallest of which is buckminsterfullerene with n=60 vertices. Clathrin triskelia self assemble into small fullerene cages as well as large ones. The small ones have adjacent pentagons, including at least one with n=28 and two with n=36 vertices.

To explain which cages could self assemble, we proposed a "head-to-tail exclusion rule", the strong form of which permits self assembly of all IPR cages ($n>60$), buckminsterfullerene (n=60), and just 15 of the 5769 mathematically possible, small ($n\leq 60$), non-IPR cages (Schein et al., Biophy J, in press). A weak form of this rule permits 99 additional small, non-IPR cages.

Cheng et al. (JMB *365*, 892–899, 2007) recently reported four new clathrin fullerene cage structures, identified here as three that obey the strong form and one that obeys the weak form. The new tally is thus six of the 15 that obey the strong form, one of the 99 that obey the weak form, and none of the remaining 5655 that obey neither. The recent clathrin cage structures thus confirm the predictions of the head-to-tail rule and favor the strong form, though not exclusively. All carbon cages reported to date obey the strong form.

2641-Plat Protein-Protein Interfaces: Amino Acids Bias for Heterocomplexes and Homodimers

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Protein-protein interactions are involved in a variety of biological processes at the molecular level. In particular, protein-protein interactions play a crucial role in such an intriguing process as protein folding. A nonredundant set of protein-protein interfaces from PDB was used for comprehensive analysis by a rigorous nonparametric method based on Voronoy-Delaunay tessellation. Full residue contact matrixes were constructed separately for homodimers and heterocomplexes at residue and atomic levels. The distributions obtained for contact areas and distances admit interpretation in terms of the model suggesting coexistence of stochastic and specific interresidue contacts. Based on the scheme of occasionally intersecting circles, we derived formula which in principle permit one to separate the contributions from casual and specific contacts. We calculated pairing preference indexes, and found that in homodimers they are largest for contacts Cys-Cys and those between residues with opposite charges. Rather small indexes are characteristic of contacts Gly-Pro and Met-Met. A similar pattern is observed for heterocomplexes. At the same time, in comparison with homodimers, we detect a higher preference index for contacts Cys-Cys and Gly-Gly but not for Met-Met. The basic difference between heterocomplexes and homodimers consists in enrichment of contacts between same residues in the latter case, even for contacts of similar charges. It may be explained by symmetric transformations in homodimers. We identified pairs of residues responsible for specific recognition and those that come in touch by chance. The biophysical nature of interactions in both cases is discussed.

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2642-Plat Revealing Physical Basis For Ruggedness Of Protein-protein Energy Landscapes: Interaction Cutoff Effect

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The concept of the energy landscape is important for better understanding of protein-protein interactions and for designing adequate docking procedures [1,2]. The intermolecular landscape has a rugged terrain that impedes search procedures. Its inherent rugged-

ness is related to the conformational characteristics of the molecules and to the form of the potential function - more rugged for short-range potentials and less rugged for "soft," typically long-range potentials. The landscape ruggedness is further substantially exacerbated by truncation of the potentials [3]. This additional ruggedness appears below certain critical interaction ranges that depend on the form of the potential. The theoretical model describing the cutoff effect on the landscape ruggedness is confirmed by the energy calculation on a dataset of protein-protein complexes. The analysis also showed that smoothed cutoffs decrease the effective range of potentials and thus increase the ruggedness of the protein-protein landscape. A connection between the ruggedness and intermolecular forces measured by chemical force microscopy is discussed. Revealing physical basis for ruggedness in terms of the energy landscape is important for better understanding of macromolecular binding.

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2643-Plat Monomolecular Fibrin Sheets: a New Form of Fibrin Polymer

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Fibrin is well known to polymerize into fibers. Such fibers stabilize clots that limit bleeding from wounds and cause life threatening thrombi. We have found that fibrin also readily assembles into monomolecular sheets or films. These appear by fluorescence light microscopy, scanning and transmission electron microscopy and atomic force microscopy to be single molecule thick, freely suspended, continuous elastic membranes that extend for hundreds of microns. Sheets have been observed to polymerize at hundreds of square microns/sec along surfaces and across 40 um gaps, with an apparent first order rate constant many orders of magnitude higher than that observed for fibers. Moreover, we observe transitions from sheets to fibers via combinations of folding and rounding into large fibers, as well as tearing and rolling up into smaller branched or anastomosing fiber networks. Thus fibrin sheets may represent an unrecognized kinetic intermediate in the assembly of fibers, explaining how fibers are observed across large gaps, but free fiber ends are almost never observed. How the sheets themselves are able to polymerize so rapidly across large gaps without support, how the fibrin subunits are aligned within the growing sheet, the role of thrombin activity in the transition from sheet to fiber, and the role sheets may play in the clotting process, are exciting new areas of investigation.

2644-Plat Structural Models Of Abeta Channels And Their Blockade By Synthetic Peptides

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Assembly of Abeta peptides into oligomers has been implicated Alzheimer's disease. To better understand molecular details of these processes, we have developed a series of models of both soluble and membrane-bound oligomers. In most of our models, the C-terminus third of six peptides assemble into an antiparallel six-stranded beta-barrel that forms a hydrophobic core. This abstract focuses on the membrane-bound assemblies, especially those that form channels, and the mechanisms by which the channels may be inhibited by heavy metals and synthetic peptides. Microscopy studies have revealed Abeta assemblies in membrane with diameters up to 16 nm. These are much too large to be formed by a single hexamer, but could be formed by an assembly of hexamers. We propose that in initial stages, hexamers reside on the surface of the membrane with the axis of the beta-barrel parallel to the membrane. A pore can form between hexamers; e.g., between six hexamers in our models. Initial channel may form when only the first two-thirds of one peptide per hexamer spans the trans leaflet of the bilayer. The cis entrance of these putative channels is formed by residues 8–14 of two other peptides per hexamer. We have modeled how these channels can be inhibited or blocked by synthetic hepta-peptides with sequences analogous to these segments; i.e. to His13 and His 14 plus five adjacent residues. The presence of a ring of H13 and H14 side chains in the pore also explains why heavy metals, such as zinc, block the channels. This relative dynamic stage may be followed by more complete insertion of the hexamer through the transmembrane region, with the axes of the beta-barrels perpendicular to the membrane.

2645-Plat Anatomy and Dynamics of a Supramolecular Membrane Protein Cluster

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Most plasmalemmal proteins organize in submicrometer-sized clusters whose architecture and dynamics are still enigmatic. With syntaxin 1 as an example, we applied a combination of far-field optical nanoscopy, biochemistry, fluorescence recovery after photobleaching (FRAP) analysis, and simulations to show that clustering can be explained by self-organization based on simple physical principles. On average, the syntaxin clusters exhibit a diameter of 50 to 60 nanometers and contain 75 densely crowded syntaxins that dynamically exchange with freely diffusing molecules. Self-association depends on weak homophilic protein-protein interactions. Simulations suggest that clustering immobilizes and conformationally constrains the molecules. Moreover, a balance between self-

association and crowdinginduced steric repulsions is sufficient to explain both the size and dynamics of syntaxin clusters and likely of many oligomerizing membrane proteins that form supramolecular structures.

2646-Plat Synchrotron X-ray Diffraction Study of Neurofilament Networks Interaction under Osmotic Pressure

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Neurofilaments (NFs), a major constituent of nerve cell axons, assemble from three subunit proteins of low, medium and high molecular weight to form a 10 nm diameter rod with radiating unstructured sidearms. The sidearm interactions result in NF physical hydrogel with the NF long axis running parallel to the axon. The NF network imparts mechanical stability to the axon and acts as a scaffold for microtubules. Here, we reassemble NFs in vitro from varying ratios of the subunit proteins, purified from bovine spinal cord. At high protein concentration, the NFs form a nematic hydrogel network with a well-defined interfilament spacing as measured by synchrotron small angle x-ray scattering (SAXS). The phase diagram demonstrating the transition between the nematic and isotropic gel phases in relation to varying side-arm grafting density has been mapped out [1]. Using the SAXS-osmotic pressure technique reported previously for microtubules [2] we directly probe the polyampholyte brush interactions between NF sidearms. We present data on the interfilament spacing at different osmotic pressure and at different salt and sidearm concentrations which begin to reveal the non-trivial electrostatic nature of the interfilament interactions within the NF hydrogel.

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2647-Plat Structure of the Plexin-B1 Effector Domain in Complex with the small GTPase Rac1 derived from NMR restraints: Characterization of the interaction surface by NMR, molecular modeling and thermodynamic measurements

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Plexin-B1 functions in axon guidance and cell motility. It is the first transmembrane receptor that directly binds to small GTPases. We recently determined the NMR solution structure of a monomeric form of the Rho GTPase binding domain (RBD) of human plexin-B1. Remarkably, three different Rho family GTPases, Rac1, Rnd1 and RhoD, bind to this central cytoplasmic region, which was shown to adopt a ubiquitin-like fold. The same surface of this structure is involved in the interaction with the three GTPases as shown by Nuclear Magnetic Resonance (NMR) surface mapping experiments, suggesting a common role for this domain as a GTPase effector. In order to determine the details of the interaction, NMR restraint-driven docking was used to calculate the structure of the Rac1 - RBD plexin-B1 complex employing Haddock2.0. It should be noted RBD was target 31 - the first NMR target - in a recent CAPRI challenge aimed at a blind prediction of the complex structure. Molecular modeling is used to suggest differences between GTPases that bind to the plexin-B1 RBD (Rac1, Rnd1 and RhoD) and those that do not interact (incl. Cdc42 and RhoA). The results are compared to mutagenesis data on the GTPases in a thermodynamic analysis to confirm our understanding of the protein-protein interaction surface and its specificity. The complex with Rac1 also shows that the GTPase binding site partially overlaps with a receptor dimerization interface and suggests a mechanism for destabilization of a dimeric receptor by GTPase binding.

Symposium 20: ABC Transporters: Molecular Structures and Mechanisms

2648-Symp Maltose Transporter Cycle

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The maltose transporter from E. coli is an ATP-binding cassette transporter that mediates the uptake of maltose and longer chain maltodextrins. After crossing the outer membrane, maltodextrins are bound with high affinity by a periplasmic maltose binding protein MalE and delivered to a membrane-bound complex of transmembrane membrane proteins MalF and MalG and two copies of an ATPase, MalK. If ATP hydrolysis is blocked by mutation of a conserved glutamate following the Walker B motif of MalK, addition of ATP to the transporter traps a high affinity complex MalEFGK2 that represents a catalytic intermediate in the transporter cycle. Both the biochemical analysis and the high resolution structure of this intermediate are consistent with a concerted mechanism for transport in which the interaction between maltose-bound MalE and ATP-bound MalFGK2 triggers a global conformational change that results in simultaneous activation of ATP hydrolysis and transfer of maltose from MalE to a sugar binding site lying deep within a cleft between MalF and MalG. The high resolution structure of this EFGK2 intermediate will be discussed, together with the results of biochemical analyses that suggest the structure represents an intermediate of the translocation cycle.