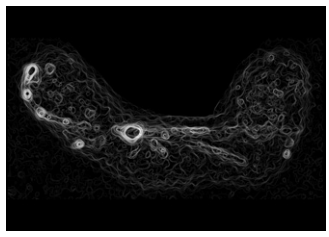
within F-actin, which may help explain the exquisite conservation of actin's sequence. We can show at better than 10 Å resolution that within the actin filament subdomain 2 can undergo significant structural alterations from an ordered position to complete disorder. We show that the DNase I-binding loop of actin can exist in multiple conformations, as can the N-terminal region of actin.Overall, these insights into structural polymorphism within protein polymers suggest an under-appreciated mechanism for evolutionary divergence.

20-Symp
Actin Filament Nucleation: Structure-Function Relationships
Malgorzata Boczkowska, Suk Namgoong, Grzegorz Rebowski, **Roberto Dominguez**.
University of Pennsylvania School of Medicine, Philadelphia, PA, USA.
The actin cytoskeleton is intimately involved in most cellular functions, including cell motility, endo/exocytosis and intracellular trafficking. These processes are characterized by rapid oscillations of actin polymerization/depolymerization under tight temporal and spatial regulation. Hundreds of G- and F-actin-binding proteins, along with signaling and scaffolding proteins regulate the assembly of actin networks. Among these proteins, *filament nucleators* play a critical role by determining the time and location for actin polymerization, as well as the specific structures of the actin networks that they generate. Eukaryotic cells and certain pathogens use filament nucleators to stabilize actin nuclei (small oligomers of 2-4 actin subunits), whose formation is rate-limiting. Known filament nucleators include the Arp2/3 complex and its large family of Nucleation Promoting Factors (NPFs), Formins, Spire, Cobl, Lmod, VopL/VopF and TARP. Structural and functional studies are beginning to shed light on the diverse mechanisms used by these molecules to stabilize actin nuclei. Thus, with the exception of Formins known filament nucleators use the WASP-Homology 2 domain (WH2 or W), a small and versatile actin-binding motif, for interaction with actin. A common architecture, found in Spire, Cobl and VopL/VopF, consists of tandem W domains that bind three to four actin subunits to form a nucleus. Structural considerations suggest that NPFs-Arp2/3 complex can also be viewed as a specialized form of tandem W-based nucleator. The nucleation activities of these proteins vary significantly, and the most effective nucleators are not necessarily those with the largest number of W domains. We show that the inter-W linkers play a critical role in determining the nucleation activities of filament nucleators and the structures of the actin nuclei that they generate. Furthermore, we present evidence that a previously neglected factor, oligomerization, is a major determinant of filament nucleation activity and nuclei structure.

21-Symp
Collective Action of Motor Proteins on Microtubules Regulates Large-Scale Forces in the Cell
Iva Tolic-Norrelykke.
Max Planck Inst Molec Bio/Genet, Dresden, Germany.
How do living cells deal with basic concepts of physics such as length and force? Cell interior is neatly yet dynamically organized through constant movements of organelles, which is to a large extent based on microtubules and motor proteins. Two concepts are emerging as key to the regulation of organelle movement: preferred disassembly of longer microtubules and preferred detachment of motors under high load. We have studied both experimentally and theoretically the role of these mechanisms in nuclear centering and nuclear oscillations in fission yeast. These universal concepts may be crucial for a variety of cell processes, including nuclear and mitotic spindle positioning, control of spindle length, and chromosome congression on the metaphase plate.



Platform A: Member-Organized Session: Biopolymer Dynamics in Cell-like Environment

22-Plat
Protein Structure, Stability and Folding in the Cell - in Silico Biophysical Approaches
Margaret S. Cheung.
University of Houston, Houston, TX, USA.
How the crowded environment inside a cell affects the structural conformation of a protein with aspherical shape is a vital question because the geometry of proteins and protein-protein complexes are far from globules in vivo. Here we address this question by combining computational and experimental studies of a spherical protein (i.e. apoflavodoxin), a football-shaped protein (i.e., Borrelia burgdorferi VlsE) and a dumbbell-shaped protein (i.e. calmodulin) under crowded, cell-like conditions. The results show that macromolecular crowding affects protein folding dynamics as well as an overall protein shape associated with changes in secondary structures. Our work demonstrates the malleability of ''native'' proteins and implies that crowding-induced shape changes may be important for protein function and malfunction in vivo.
*This research is supported by an award from the National Science Foundation (MCB 0919974).

23-Plat
Molecular Modeling of the Bacterial Cytoplasm
Adrian Elcock.
University of Iowa, Iowa City, IA, USA.
Advances in the fields of structural biology and quantitative proteomics mean that it is now possible to consider developing working molecular models of intracellular environments. This talk focuses on the construction of such a model for the bacterial cytoplasm, describes the use of Brownian dynamics simulations to model diffusion and association of macromolecules, and shows that calculations of protein stability in the model cytoplasm are in excellent agreement with those measured experimentally in vivo.

24-Plat
Enthalpic Vs. Entropic Effects of Crowded Cellular Environments
Michael Feig.
Michigan State University, East Lansing, MI, USA.
The role of crowded cellular environments on biomolecular energetics and dynamcis is often only considered from an entropic point of view in the form of excluded volume effects. Here, the enthalpic contribution of dense cellular environments is considered with two different models. 1) Dense cellular environments are modeled as reduced dielectric continua. 2) Biomolecular sampling in the presence of explicit protein crowders is explored with a new coarse-grained model.

25-Plat
Protein Diffusion and Macromolecular Crowding
Gary Pielak, Yaqiang Wang, Conggang Li.
University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.
Having recently quantified how crowding can affect equilibrium protein stability (1), we have turned our attention to diffusion. Our test molecule is the small globular protein, chymotrypsin inhibitor 2. Our crowding molecules are both the synthetic polymer polyvinylpyrrolidone (PVP) and several globular proteins. We assessed both translational and rotational diffusion by using nuclear magnetic resonance spectroscopy (2). Whereas crowding by PVP results in negative deviations from the Stokes laws, crowding by globular proteins leads to positive deviations. I will discuss our results in terms of what can be learned from in vitro, versus in cell (3) studies.
1. Charlton LM, et al. (2008) Macromolecular crowding effects on protein stability at the residue level. Journal of the American Chemical Society 130: 6826-6830.
2. Li C, Wang Y, Pielak GJ (2009) Translational and rotational diffusion of a small globular protein under crowded conditions. Journal of Physical Chemistry 113: in press.
3. Slade KM, Steele BL, Pielak GJ, Thompson NL (2009) Quantifying GFP diffusion in Escherichia coli by using continuous photobleaching with evanescent illumination. Journal of Physical Chemistry 113: 4837-4845.

26-Plat
Understanding How the Crowded Interior of Cells Stabilises DNA/DNA and DNA/RNA Hybrids - in Silico Predictions and in vitro Proof
Michael Raghunath[1], Karthik Harve[1], Ricky R. Lareu[2], Raj Rajagopalan[1].
[1]National University of Singapore, Singapore, Singapore, [2]University of Western Australia, Perth, Australia.
Amplification of DNA in vivo occurs in intracellular environments characterized by macromolecular crowding (MMC). In vitro Polymerase-chain-reaction (PCR), however, is non-crowded and requires thermal cycling to effect melting of DNA strands, primer-template hybridization and enzymatic primer extension. The temperature optima for primer annealing and extension are strikingly disparate which predicts primers to dissociate from the template during extension thereby compromising PCR efficiency. We hypothesised that MMC is not only important for the extension phase in vivo but also during PCR by stabilising nucleotide hybrids. Novel atomistic Molecular Dynamics simulations revealed that MMC stabilises hydrogen