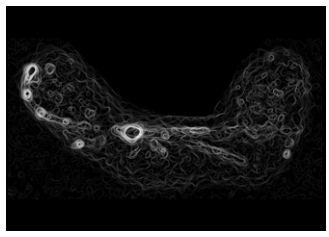
within F-actin, which may help explain the exquisite conservation of actin's sequence. We can show at better than 10 Å resolution that within the actin filament subdomain 2 can undergo significant structural alterations from an ordered position to complete disorder. We show that the DNase I-binding loop 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

bonds between complementary nucleotides. Real-time PCR under MMC confirmed that melting-temperatures of complementary DNA-DNA and DNA-RNA hybrids increased by up to 8°C with high specificity and high duplex preservation after extension (71% vs 37% non-crowded). MMC enhanced DNA hybrid helicity, and drove specificity of duplex formation preferring matching versus mismatched sequences, including hair-pin-forming DNA single strands.

27-Plat
Simulations of Protein Aggregation in the Cellular Milieu
Joan-Emma Shea.
UCSB, Santa Barbara, CA, USA.
A number of diseases, known as amyloid diseases, are associated with pathological protein folding. Incorrectly or partially folded peptides or proteins can self-assemble into a variety of neurotoxic aggregate species, ranging from small soluble oligomers to amyloid fibrils. I will introduce a novel off-lattice coarse-grained peptide model that can be used to simulate the aggregation process from monomers to fibrils. The effects of beta-sheet propensity and of surfaces on the morphology of the aggregates will be discussed.

28-Plat
Protein Structure, Stability and Folding in the Cell - *in vitro* Biophysical Approaches
Pernilla Wittung-Stafshede.
Umeå univeristy, Umeå, Sweden.
Folding processes of simple proteins have been studied for years in test tubes. However, many proteins in the cells are more complex: some bind cofactors and others interact with other polypeptides in order to form their functional units. To obtain mechanistic information of the folding reactions of such proteins, we combine protein engineering in strategic model systems (e.g., a/b flavodoxin, b-barrel azurin, all-a VlsE) with a range of biophysical methods (e.g., circular dichroism, stopped-flow mixing, calorimetry). To take a step closer to the *in vivo* scenario, we assess how the crowdedness of the cell milieu affects protein biophysical parameters using synthetic macromolecular crowding agents (e.g., Ficoll, dextran) that take up significant volume but do not interact with the targets or have interfering spectroscopic signals. We have found that in the presence of macromolecular crowding *in vitro*, proteins can fold faster, become more thermodynamically stable and, surprisingly, the folded forms may change in terms of both secondary structure content and overall shape. Our discoveries imply that Nature may use excluded volume effects as a tool to tune protein biophysical parameters, and thereby function, *in vivo*.

29-Plat
Atomistic Simulations of Macromolecular Crowding
Huan-Xiang Zhou.
Florida State University, Tallahassee, FL, USA.
Protein folding, binding, and aggregation are known to be affected by macromolecular crowding [1], which is an integral part of intracellular environments. Realistic modeling of intracellular crowding requires computer simulations. In direct simulations of test proteins mixed with crowders, it has only been practical to represent the proteins at a coarse-grained level. Our recently developed ''postprocessing'' approach has made it possible to represent test proteins at the atomic level [2, 3]. In this approach, the motions of a test protein and those of the crowders are followed in two separate simulations. The effects of crowding are then modeled by calculating $\Delta\mu$, the crowding-induced change in the chemical potential of the test protein. For a repulsive type of protein-crowder interactions, $\Delta\mu$ is related to the fraction, *f*, of allowed placements of the test protein into a box of crowders. For spherical crowders, two methods have been devised to calculate *f*. The first is an efficient implementation of Widom's insertion method. The second is a theoretical prediction, which uses the volume, surface area, and linear size defined on a ''crowder-exclusion'' surface. These methods have been applied to study crowding effects on protein folding, binding, and internal dynamics. We have also tested the postprocessing approach against direct simulations of folding-unfolding and open-to-closed transitions in the presence of crowders. To calculate *f* for atomistic crowders, we have just devised an algorithm based on fast Fourier transform. These computational tools establish a solid foundation for realistic modeling of intracellular environments.
[1] H.-X. Zhou, G. Rivas, and A. P. Minton, Annu Rev Biophys **37**, 375 (2008).
[2] S. Qin and H.-X. Zhou, Biophys J **97**, 12 (2009).
[3] J. Batra, K. Xu, S. Qin, and H.-X. Zhou, Biophys J **97**, 906 (2009).

Platform B: Cardiac Muscle I

30-Plat
Sted Based Super-Resolution Imaging of Transverse Tubules in Ventricular Myocytes
Eva Wagner[1], Marcel Lauterbach[2], Volker Westphal[2], Brian Hagen[3], W.J. Lederer[3], Stefan W. Hell[2], **Stephan E. Lehnart[1]**.
[1]University Medical Center, Goettingen, Germany, [2]Max Planck Institute for Biophysical Chemistry, Goettingen, Germany, [3]University of Maryland Biotechnology Institute, Baltimore, MD, USA.
Transverse tubules (TTs) are specialized membrane invaginations in mammalian cardiomyocytes (CMs) that facilitate excitation-contraction coupling. TTs are reported to be about 300 nm in diameter (or less) by electron microscopy precluding structural characterization by conventional light microscopy (widefield or confocal). However, investigation of TTs and the associated signaling domains like ryanodine receptor containing junctions or caveoli in live cells is important towards understanding of dynamic subcellular signaling processes. To overcome the limitations in conventional cell imaging, we have examined TTs using *Stimulated Emission Depletion* (STED) super-resolution microscopy. The sarcolemmal and TT membranes were stained with lipophilic fluorescent dyes (di-8-ANNEPS). We used isolated CMs under quiescent conditions and confocal images were acquired and compared to STED images using the same optical path, microscope and sample. The apparent diameter of the TTs was determined both by confocal and STED imaging in live heart cells: fitting a Gaussian function to the fluorescence signal distribution yielded the following full width at half maximum (mean ± SEM): 271 ± 4 nm (confocal) versus 224 ± 5 nm (STED). Accordingly, STED-determined TT diameters were significantly smaller by 47 ± 6 nm (n=111; $P<0.05$) than those measured by confocal imaging. While TT sections from confocal images typically showed a 1-peak intensity distribution devoid of structural information, the STED intensity distribution showed a 2-peak pattern consistent with morphometric identification of tubular cross-sections. Thus, STED provides real-time data of tubule structures in living cells. How TTs are related to other subcellular structures will be presented. These findings suggest that STED microscopy may improve our quantitative understanding of complex subcellular structures in heart cells and enable study of their dynamic reorganization in normal and diseased states.

31-Plat
Impact of Loss of T-Tubules on Myocardial Contractile Force and Kinetics
Cecilia Ferrantini[1], Raffaele Coppini[1], Guo Liang Wang[2], Mei Luo Zhang[2], Ewout de Vries[2], Chiara Tesi[1], Corrado Poggesi[1], Henk Ter Keurs[2].
[1]University of Florence, Florence, Italy, [2]University of Calgary, Calgary, AB, Canada.
Action potential driven Ca^{2+} currents via the transverse tubular membrane synchronously trigger Ca^{2+}release from the SR close to the myofibril rapidly activating contraction. Loss of T-tubules has been reported in disease including heart failure, but the effect of detubulation on muscle mechanics has never been investigated.
We dissected thin (50-200μm) right ventricular and left atrial trabeculae from adult rats and recorded force and sarcomere length. To achieve acute detubulation, we exposed trabeculae to formamide 1.5M for 20 minutes. Return to normo-osmotic solution increases cell-volume suddenly and disconnects T-tubules from the sarcolemma as was directly verified using di-8-anepps staining and confocal microscopy.
In seven ventricular trabeculae, detubulation prolonged the twitch, i.e. time to peak force (by 31±9%) and time to 50% relaxation (by 46±14%) at 1Hz. Detubulation reduced the inotropic effect of increased stimulus-rate (by 29±7%). Maximal twitch force after post-rest potentiation was unchanged. Detubulation increased the fraction of Ca^{2+} recirculating to the SR (by 17 ± 5%) measured by the decay of potentiation, suggesting an increased SERCA vs. NCX activity. None of these effects was seen in four formamide-treated atrial trabeculae, which constitutively lack T-tubules in rodents.
T-tubular disruption from the membrane implies loss of 80% L-Type I_{Ca} and 60% I_{NCX}. Mathematical modeling shows that in myocytes with EC-Coupling via T tubules and SR alone the loss of the aforementioned currents is not sufficient to explain the differences between control and detubulated trabeculae. Such differences can be predicted assuming that EC-coupling is maintained by a fast Ca^{2+} rise near the sarcolemma but now followed by, Ca2+-diffusion mediated, propagated Ca^{2+} induced SR-Ca^{2+} release toward the core. Enhanced Ca^{2+}–wave spread and recruitment of all myofibril layers can contribute to maintain maximal contractile force in the absence of T-tubules.