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.
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[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.