and troponin T) indicates that mutant cardiomyocytes like cells also exhibited a progressive disorganization of their contractile apparatus. This cellular model describes for the first time a complex morphological pathology caused by the dysfunction of an ion channel. Finally, the study of current proton leak in physiological conditions provides helpful understanding on its pathological impact.

**Platform: Molecular Dynamics I**

82-Plat

All-Atom Simulation and Coarse-Grained Analysis of the Rigor Actomyosin System

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Interactions of actin filaments with myosin motor proteins are important for a variety of cellular functions. The myosin mechanochemical cycle is a complicated sequence of steps including ATP hydrolysis by myosin, binding and unbinding of myosin with actin, and phosphate release from myosin during the force-producing “power stroke.” No high-resolution crystal structure of acto-myosin exists, however low resolution data from cryo-EM has allowed a characterization of the rigor state (ATP free) of actomyosin at an atomic level of detail. Here we present the first simulation study at an all-atom level of detail of the myosin II S1 domain in the rigor state interacting with a fully periodic actin filament. Additionally, we study myosin II in the rigor and post-rigor states in the absence of actin. Through a combination of all-atom level and coarse-grained (CG) analysis, we are able to identify effects of myosin binding on the actin filament, the effects on myosin dynamics of being bound to actin, and differences in the collective motions between simulated myosin states. CG level analysis allows further characterization of the influence of myosin binding on the definitions of domains which exhibit collective motion for the actin monomers in the periodic filament, and the identification of CG domains of myosin which interact strongly with actin domains based on a heterogeneous elastic network model analysis.

83-Plat

The Common Functional Dynamics of Molecular Motor and Switch Proteins

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Understanding how protein-ligand binding can promote distinct conformational states, with different affinities for binding partners, is key to understanding the structural basis of protein efficacy. Here we study eight molecular motor and G-protein families that undergo GTP or ATP associated conformational changes to regulate important cell processes, including signal transduction and intracellular transport. Employing comparative structure analysis, accelerated molecular dynamics, and Brownian dynamics simulations we unveil the pervasive similarity of functionally associated dynamical fluctuations. Different families were observed to have variable inactive but common active nucleotide binding site configurations. Activating conformational changes that reconfigure analogous nucleotide binding site residues were also observed in nucleotide free molecular dynamics simulations. This result suggests that this common flexibility is an intrinsic feature of these families. In addition, conformational changes at the nucleotide binding site in all families were observed to accompany the concerted rearrangements of distinct family specific sub-domains. These sub-domains range from 16-202 residues in length and are joined to common core structural elements at topologically equivalent sites. Moreover, structural changes, correlated with those at the nucleotide binding site, were found to alter the geometry, dynamics and electrostatic field properties of these sites. Furthermore, Brownian dynamics simulations reveal that for kinesin, ras, rab, and rho families these electrostatic differences can modulate the kinetics of protein-protein association events. In summary, our accumulated results indicate that similar activating conformational changes link nucleotide binding to distal topologically equivalent sub-domains that in turn play a role in modulating distinct protein-protein interactions. We speculate that this fundamental mechanism operates in all motor and switch proteins. These results have implications for allosteric drug development and future protein engineering efforts on these systems.

Images and animations related to this work can be found at: http://thegrantlab.org/

84-Plat

Structural Ensembles of Intrinsically Disordered FG-Nucleoporins Depend on Force Field

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Intrinsically disordered proteins (IDPs) fulfill important biological roles including cell signaling, cell cycle regulation, and rubber-like elasticity. IDPs pose a tremendous challenge both to traditional structural determination methods as well as their theoretical description via molecular dynamics (MD) simulations: it is difficult to obtain sufficient data to determine the ensembles of structurally heterogeneous systems. Furthermore, because established MD force fields have been developed primarily to study folded proteins, it is not clear how accurately these force fields are able to describe disordered states.

Here, we performed microsecond-timescale MD simulations using four recently-developed force fields: Amber ff99SB*-ILDN, Amber ff03w, CHARMM22*, and CHARMM36. We studied a set of FG-nucleoporin peptides with sequences derived from yeast Nsp1p. FG-nucleoporins are IDPs responsible for the high selectivity of the nuclear pore complex (NPC). They form a mesh-like structure in the central region of the NPC that controls the passage of macromolecules into and out of the nucleus. FG-nucleoporins are a prototypic example of the biological role of protein disorder, and beyond their particular function are key model systems for disordered proteins.

Overall trends, such as temperature-induced unfolding and differences in compactness between cohesive and extended coil domains of Nsp1p, are described reasonably well by all force fields. However, we find marked differences in the extent of hydrogen bonding and secondary structure preferences. The average chain dimensions with the CHARMM force fields are more than 20% larger than the Amber force fields. Taken together, our results strongly suggest that disordered states are particularly sensitive to force field choice. As a next step, we will therefore compare to experimental measurements of both chain dimensions and secondary structure to determine which force field provides the most accurate description.

85-Plat

Molecular Mechanism of Proflavine Intercalation: Evidence for Drug-Induced Minimum Base-Stacking Penalty Pathway

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DNA intercalation[1] is associated with anti-cancer therapeutics and therefore the process is of enormous interest. Molecular mechanism of DNA intercalation has remained elusive. The previous attempts to understand the molecular mechanism of intercalation[2] indicated that role of DNA structural changes, and origin of multi-step nature[3] of the intercalation process using a de-intercalation pathway. A successful intercalation event, which require transition from several possible out-side bound states to a unique intercalated state, is not achieved. Here we used an appropriate configurational restraint to witness the process of a direct intercalation mechanism of proflavine. This study answers a long-standing question that intercalation indeed occurs through a DNA-induced cavity formation mechanism rather than through natural fluctuation of the DNA[4]. This study shows that in fact intercalation of proflavine proceeds through a minimum base-stacking penalty pathway through minor groove edge of the DNA even though the barrier through major groove is smaller. The reason for such a peculiar observation lies in the more stable minor groove-bound state, which forms fast, however, but depletes slowly due to higher barrier to intercalate. The origin of the higher barrier through minor groove originates from the desolvation energy of the DNA and entropy. The barrier for intercalation through the major groove-bound state, in the absence of desolvation, is entropic in nature. The study also shows, using a simple kinetic scheme that while intercalation happens through minor groove, de-intercalation would likely happen through the major groove having a cyclic intercalation/de-intercalation pathway.[4] [1] L. S. Lerman, J. Mol. Biol. 3 (1961). [2] A. Mukherjee et al., J. Am. Chem. Soc. 130 (2008). [3] M. Wilhelm et al., J. Am. Chem. Soc. 134 (2012). [4] W. D. Sasikala, and A. Mukherjee, J. Phys. Chem. B (submitted).
approximated by Hooke’s law, it is not clear if that is still valid for large bending angles. The experimental evidence is controversial. We explore the strong bending regime of the double helix using a model that represents the solvent implicitly, which allows for greater efficiency. First, we are able to reproduce results of Strauss and Maher. Next, we compare the energetics of weakly and strongly bent DNA. We find that Hooke’s law is violated for strongly bent DNA and discuss the energetic contribution that may be responsible for the effect.

87-Plat

Mechanisms for Efficient TRNA Translocation through the Ribosome
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After peptide bond formation the transfer RNAs (tRNAs) bound to the ribosome translocate by more than 7 nm to adjacent binding sites, accompanied by large-scale conformational motions of the ribosome. Combining cryo-EM reconstructions of translocation intermediates (Fischer, Nature 2010) with high resolution crystal structures, we obtained 13 near-atomic resolution structures. The quality of these structures was validated using recent crystal structures and subsequently all-atom molecular dynamics simulations of the fully solvated 70S ribosome were carried out for each of the 13 intermediate states, totaling 1.5 µs. The obtained dynamics within the intermediate states allowed us to estimate transition rates between states for motions of the L1-stalk, tRNAs and intersubunit rotations. These rates revealed rapid motions of the L1-stalk and the small subunit on sub-microsecond timescales, whereas the tRNA motions were seen to be rate-limiting for most transitions. By calculating the free energy of interaction between L1-stalk and tRNA, we obtained molecular forces revealing that the L1-stalk is actively pulling the tRNA from P to E site, thereby overcoming barriers for the tRNA motion. Further, ribosomal proteins L5 and L16 guide the tRNAs by ‘sliding’ and ‘stepping’ mechanisms involving key protein-tRNA contacts, explaining how tRNA binding affinity is kept sufficiently constant to allow rapid translocation despite large-scale displacements.

88-Plat

Coarse-Grained Computational Characterization of RNA Nanocube Flexibility Correlates with Experiments
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The emerging field of RNA-based nanotechnology can benefit from the development of new computational methods capable of helping in the design and characterization of nano-scale particles, leading to the development of qualitatively new structures and novel therapeutics. We have approached the computer-aided design process by creating a pipeline of tools, starting with a database of n-way junctions and kissing-loops called RNAJunction. This database provides building blocks for our programs, such as NanoTiler and RNA2D3D, which use them to design 3D models of RNA nanostructures. First, the building blocks are treated as rigid objects. Then, just as the natural RNA is shaped (deformed) by the larger structural contexts, our programs allow for deformations to be applied in order to produce fully assembled models. To assess the realistic limits of these deformations, we consider flexibility data available as alternative structures in databases as well as results of Molecular Dynamics (MD) simulations at the atomic resolution level and coarse-grained computational methods. Here we present an example of the modeling process including RNA flexibility information for three nanocube model variants and a novel application of a coarse-grained Anisotropic Network Model (ANM) to the RNA nanostructure characterization. The predictions of different efficiency of assembly for three nanocube variants, based on the exploratory modeling, were confirmed in in vitro experiments. The ANM simulations showed that the dynamics of the full nanostructure has to be considered in order to explain the differences between the size of the initial static models and that of the experimentally measured nanoparticles, thus bringing the computational and the experimental results into agreement. The ANM simulations also offered an additional insight into the assembly yields and the difference in the melting temperatures of the cube variants.

89-Plat

Conformational Transitions of Nucleic Acids under External Forces: Computer Simulations and a Stochastic Theory for their Kinetics
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I will present molecular dynamics simulations of several examples of conformational transitions that nucleic acids and their complexes undergo upon the application of external forces and/or torques: (1) DNA supercoil relaxation by topoisomerases, (2) the condensation of DNA by dendrimers and, (3) RNA unfolding.
Then I will showcase the use of the formalism of stochastic path integrals to deduce the kinetics of these transitions, from simulation trajectories or experimental single molecule recordings of the transition, under other conditions that those that are actually simulated or recorded.

Platform: Cardiac Muscle I

90-Plat

Using FRET to Characterize the Actomyosin Complex in Cardiac Muscle
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Eliciting actomyosin interactions within cardiac muscle is key to understanding molecular mechanisms of force generation in the heart. Precise myosin-actin interactions throughout the power stroke are still unclear and study of actomyosin within functional muscle systems is required [1]. We exploit the nanometre precision of Förster resonance energy transfer (FRET) to study the actomyosin complex in healthy and diseased cardiac muscle, using mouse papillary muscle. The distance between the essential light chain (ELC)-AlexaFluor488 (labelled at a single cysteine in position 180 of a modified ELC exchanged into the fibre [2]) and Actin-AlexaFluor594-Phallolidin is evaluated by the acceptor-photobleaching method. The hypertrophic cardiomyopathy-causing actin mutation, E99K [3], was also studied in terms of the ELC-Actin distance, and compared with wild-type results. The mean FRET efficiencies evaluated for wild-type and E99K relaxed-state fibres were 15.1% and 15.0% respectively (p>0.05), corresponding to ELC-Actin distances of 87.6Å and 87.2Å. Rigor-state FRET efficiencies were approximately 10% lower than in the relaxed-state, corresponding to distances around 20Å shorter. Our preliminary results suggest: i) E99K actin-mutation does not affect the acto-myosin structures in terms of FRET efficiencies evaluated; ii) ELC-Actin distance in cardiac fibres is within the FRET range; iii) ELC-Actin distance in relaxed cardiac fibres is shorter than rigor-state distances.

In conclusion, FRET is viable for studying nanometre distances in intact cardiac tissue and provides a new perspective into the study of cardiac contraction. Furthermore, in contrast to skeletal fibres [4], the ELC-Actin distance in rigor cardiac fibres is within the range for FRET, indicating that cardiac and skeletal muscle may possess differing cross-bridge conformations.


91-Plat

Length Dependence of Force Generation is Controlled by Phosphorylation of cTnI at Serines 23/24
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The steepness of the Frank-Starling relationship is modulated by numerous physiological factors including beta-adrenergic stimulation, which steepens the relationship. This arises in part from increased myofibrillar length dependence of force and power by PKA, a downstream signaling molecule of the beta-adrenergic system. Since PKA has multiple myofibrillar substrates including titin, myosin binding protein-C (MyBP-C), and cardiac troponin I (cTnI), we sought to define if phosphorylation of one of these molecules was sufficient to control length-tension relationships. We focused on cTnI since (i) we previously observed a relationship between cTnI phosphorylation and the steepness of ventricular function curves in rat working hearts, (ii) 2D-DIGE indicated a distribution of cTnI phosphorylation states consistent with our previous observation of two populations of length-tension relationships (one shallow the