Symposium 11: DNA Nanomachines in Vitro and Inside Living Cells

2114-Symp
Self-Assembly of DNA into Nanoscale Three-Dimensional Shapes
William Shih.
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I will present a general method for solving a key challenge for nanotechnology: programmable self-assembly of complex, three-dimensional nanostructures. Previously, scaffolded DNA origami had been used to build arbitrary flat shapes 100 nm in diameter and almost twice the mass of a ribosome. We have succeeded in building custom three-dimensional structures that can be conceived as stacks of nearly flat layers of DNA. Successful extension from two-dimensions to three-dimensions in this way depended critically on calibration of folding conditions. We also have explored how targeted insertions and deletions of base pairs can cause our DNA bundles to develop twist of either handedness or to curve. The degree of curvature could be quantitatively controlled, and a radius of curvature as tight as 6 nanometers was achieved. This general capability for building complex, three-dimensional nanostructures will pave the way for the manufacture of sophisticated devices bearing features on the nanometer scale.

2115-Symp
The I-Switch: a DNA Nanomachine that Maps Spatiotemporal pH Changes in Living Systems
Yamuna Krishnan.
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Thus far, directed DNA assembly has relied on Watson-Crick base pairing, and this has been a powerful and preferred approach in structural DNA nanotechnology.[1] We have been interested in developing non-Watson-Crick based building blocks to make functional assemblies in structural DNA nanotechnology.[2] I will describe how one can use a four-stranded DNA motif called the i-tetraplex[3] to build a pH triggered conformational switch. We demonstrate the first intracellular application of DNA nanoswitches by mapping spatiotemporal pH changes associated with endosome maturation in living cells.[5] I will also describe our recent developments of this system that improve the temporal resolution, tune pH sensitivity to desirable pH regimes suited to measuring pH in various cellular compartments.
Figure 1. DNA nanomachine maps spatiotemporal pH changes in living cell endosome maturation.
References
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2116-Symp
Single Molecule Cut and Paste by DNA-Hybridization and AFM-Positioning
Hermann Gaub.
Ludwig Maximilians Univ, Muenchen, Germany.
Molecule by molecule assembly of functional units promises a wide range of new applications in different fields of nanotechnology. In this lecture a new method for the bottom-up assembly of biomolecular structures is introduced, which combines the precision of the atomic force microscope with the selectivity of DNA hybridization. Functional units coupled to DNA oligomers were picked up from a depot using a complementary DNA strand bound to an AFM tip. These units were transferred to and deposited on a target area. Each of these cut and paste events were characterized by single molecule force spectroscopy. Using this technique basic geometrical structures were assembled from units with different functions. The precision of the assembly and the accuracy of the quantification by force spectroscopy were confirmed by single molecule fluorescence microscopy using TIRF excitation. We demonstrated the reproducibility and robustness of this new technique through the transport and deposition of more than 5000 units without significant loss in transfer efficiency. This technology was furthermore used to write ligand pattern for the assisted self assembly of nanoparticles. Pattern of DNA-Hybrids with different length and composition were furthermore employed as force sensors in a parallel label free format to sense various analytes like peptides and transcription factors. Aptamer sequences were employed for small molecule detection in such force based differential assays.
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2117-Symp
Designer DNA Architectures for Nanobiotechnology
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Naturally existing biological systems, from the simplest unicellular diatom to the most sophisticated organ such as human brain, are functional self-assembled architectures. Scientists have long been dreaming about building artificial nanostructures that can mimic such elegance in nature. Structural DNA nanotechnology, which uses DNA as blueprint and building material to organize matter with nanometer precision, represents an appealing solution to this challenge. Based on the knowledge of helical DNA structure and Watson-Crick base pairing rules, we are now able to construct DNA nanoarchitectures with a large variety of geometries, topologies and periodicities with considerably high yields. Modified by functional groups, those DNA nanostructures can serve as scaffolds to control the positioning of other molecular species, which opens opportunities to study inter-molecular synergies, such as protein-protein interactions, as well as to build artificial multi-component nano-machines. In this talk, I will introduce the principle of DNA self-assembly, describe our recent progress in designing and implementing designer DNA architectures for directed self-assembly, biosensing and molecular robotics and discuss some potential applications of structural DNA nanotechnology.

Symposium 12: Target Structure-Guided Drug Design

2118-Symp
Histone Acetylation: Inhibition, Regulation, and Mimicry
Philip Cole.
Johns Hopkins Univ, Baltimore, MD, USA.
Histone acetyltransferases (HATs) catalyze the targeted acetylation of lysine residues in histones and other proteins. Through reversible protein acetylation, they modulate gene expression, cell growth, and development. Among the HATs, the paralogs p300 and CBP appear to have major roles in many pathways related to metabolism, immune regulation, cardiac development, and cancer. We have taken a design approach to generate synthetic HAT inhibitors with selectivity against p300/CBP and are applying these compounds in mechanistic analyses. In addition, we have found that autoacetylation of p300 and its yeast structural homolog Rtt109 can contribute to the regulation of these enzymes. Finally, we have recently developed a chemical approach to install an acetyl-Lys mimic into proteins at sites of acetylation and will describe this new method.

2119-Symp
Chemical Genetic Approaches for Dissecting Signaling Cascades
Kavita Shah.
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Our laboratory focuses on the development of chemically based tools to dissect signaling pathways in cancer and Alzheimer's disease. Recently we developed a chemical genetic approach for specific activation or inhibition of G Proteins. G proteins are a large family of proteins comprising approximately 0.5% of mammalian genomes. To date, there exists a lack of small-molecule modulators that could contribute to their functional study. We used H-Ras to develop a system that answers this need. Small molecules that allow for the highly specific inhibition or activation of the engineered G protein were developed. The rational design preserved binding of the natural substrates to the G protein, and the mutations were functionally innocuous in a cellular context. This tool can be used for isolating specific G protein effectors. We demonstrate the feasibility of this approach by identifying Nol1 as a putative novel effector of H-Ras. Nol1 is overexpressed in a variety of tumors, including lung adenocarcinoma, prostate adenocarcinoma, breast cancer, oral carcinoma, follicular lymphoma, and human gliomas, and this overexpression is correlated with poor prognosis and shorter patient survival. Identification of Nol1 as a downstream effector of Ras might thus suggest a novel mechanism by which Ras may influence malignancy. Finally, to ensure the transferability of this approach to other G proteins

of interest, we demonstrated a straightforward transfer to Rap1B. As understanding the precise functions of closely related family members is a current frontier in Ras research, this specific control over the activity of a given member is of particular interest. More importantly, successful sensitization of a different G protein to the compounds controlling the activity of the previously engineered H-Ras demonstrates the potential breadth of application of this approach.

2120-Symp
Protein Structure Prediction by Golbal Optimization and its Applications
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One of the fundamental goals of modern sciences is to understand the nature of life, and deciphering the protein structure and its working mechanism lies at the very heart of this agenda. Due to the tremendous success of many genome projects, the number of available protein sequences reached over 5.3 million as of 2007, but less than 1% of these protein structures are known. Reliable and accurate protein structure prediction using only the sequence information is greatly in demand, but it remains as an unsolved problem even after many years of efforts. We intend to establish a successful protein modeling method that is solely based on direct application of principles excluding human interference in modeling steps. This should be contrasted to the common conception in the field that human expertise accumulated by many years of protein modeling is the most important asset for accurate protein structure prediction. In this talk we will discuss recent progresses of our efforts in protein structure prediction. It appears that our newly proposed method, which is based on the direct and rigorous optimization of relevant score functions, can provide significantly improvement for 3D modeling of proteins in the category of High-Accuracy Template-Based Modeling. Applications of highly accurate proteins 3D models to various biological systems will be discussed.

2121-Symp
Finding Small Molecule Ligands for Protein-Protein Interactions and Other "undruggable" Targets
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The central tenant of chemical biology and small-molecule drug discovery is that biology can be manipulated using small, organic compounds. Nevertheless, the known drugs act on only ~1% of the proteome, and the realm of undrugged targets is vast. Protein complexes occupy much of this realm, yet are widely considered ''undruggable'' or, at best, ''challenging.'' Thus, there is an opportunity to greatly expand the range of chemical tools and drugs if we can identify which protein-protein interactions are most amenable to small-molecule interference, and what small-molecule discovery approaches are most likely to yield potent and selective modulators. This presentation will describe some of the outstanding issues and promising advances in tackling protein-protein interactions. For example, we note that many protein interfaces are structurally adaptive, and therefore could have low-energy conformations that are amenable to binding small ligands. Additionally, many enzymes are allosterically regulated by protein complexation, and these protein-protein interfaces are also targets for unconventional enzyme inhibitors.

Platform AC: Cardiac Electrophysiology

2122-Plat
Diverse Effects of a Familial Atrial Fibrillation (FAF)-Related KCNE2 Mutation, R27C, on Cardiac Voltage-Gated Potassium (Kv) Channels
Yu-Hong Wang, Min Jiang, Mei Zhang, Gea-Ny Tseng.
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Background: KCNE2 (E2) is expressed in human heart and can potentially associate with all major cardiac Kv channels to modulate their current amplitude and/or gating kinetics. An E2-R27C mutation was identified in fAF patients, and shown to have a gain-of-function effect on E2/(KCN)Q1 channel complex. However, it is not clear whether/how E2-R27C affects E2 modulation of other cardiac Kv channels, and the biophysical nature of its gain-of-function phenotype when associated with Q1. **Methods**: We express E2-WT or E2-R27C with partner Kv channel α-subunits (E2:α-subunit = 3:1), and record currents using TEVC. **Results**: Coexpressing E2-WT with Kv4.3 (pore-forming subunit of I_{to} channels) reduces peak current amplitude and induces a depolarizing shift in $V_{0.5}$ of inactivation (from −45+5 to −37+5 mV). Relative to E2-WT/Kv4.3, E2-R27C reduces the current-suppressing effect and shifts $V_{0.5}$ of inactivation in the hyperpolarizing direction (to −41+5 mV). Relative to E2-WT/hERG (pore-forming subunit of I_{Kr} channels), E2-R27C induces a modest current suppressing effect along with a hyperpolarizing shift in $V_{0.5}$ of activation (from 10+4 to −8+1 mV). Relative to E2-WT/Q1 (pore-forming subunit of I_{Ks} channels), E2-R27C markedly increases the estimated fully-available current amplitude and induces a hyperpolarizing shift in $V_{0.5}$ of activation (from −7+2 to to −41+5 mV). **Conclusion**: E2-R27C affects how E2 modulates the current amplitude and voltage-dependence of gating of Kv4.3 and hERG channels. The net results can be gain-of-function or loss-of-function, depending on the resting membrane potential (RMP, depolarizing RMP exacerbates Kv4.3 inactivation by E2-R27C) and action potential plateau voltage (APPV, loss of APPV favors currents through E2-R27C/hERG channels). E2-R27C exerts a strong gain-of-function effect on E2/Q1 channels by 2 mechanisms: increasing the fully-available current amplitude and shifting the voltage range of activation in the hyperpolarizing direction.

2123-Plat
Simulation of the Impact of Elevated Cytosolic Na+ on Ca2+ Handling, Mitochondrial Energetics and Cellular Electrophysiology in Guinea Pig Myocytes
Lufang Zhou, An-Chi Wei, Ting Liu, Sonia Cortassa, Raimond Winslow, Brian O'Rourke.
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Chronic heart failure is one of the leading causes of morbidity and mortality in the United States. One of the classical strategies for treating heart failure is to inhibit sarcolemmal Na+/K+-ATPase (NKA). Blocking NKA can result in dramatic elevation of [Na+]i, increasing the sarcoplasmic reticulum (SR) Ca2+ load by acting on the plasmalemmal Na+/Ca2+ exchanger (NCX). Whether and how change of [Na+]i affects mitochondrial Ca2+ dynamics and energetics is still under investigation. Since intracellular Na+ is regulated by a complex system involving multiple ions, channels, exchangers and membrane potentials, unraveling its effect on cell physiology and function requires an integrative view of cardiomyocyte physiology. In the present study we developed a mathematical model of cardiomyocyte that incorporates mitochondrial energetics, ion channels and exchangers, and E-C coupling. Using this model, we simulated the effect of elevated cytosolic Na+ on Ca2+ handling, mitochondrial energetics and reactive oxygen species (ROS) generation. Model simulations show that inhibition of NKA (50%) dramatically increased [Na+] in both the cytosol and mitochondria, which consequently caused Ca2+ overload in the cytoplasm during increased workload. Elevated Na+ also decreased ATP concentration and increased mitochondrial ROS production. Concomitant inhibition of mitochondrial Na+/Ca2+ exchanger (mNCE) ameliorated these effects by attenuating cellular Ca2+ overload and increasing [Ca2+]m. Furthermore, inhibiting mNCE also prevented the [ATP]i drop and decreased ROS production. The findings indicate that increasing cytosolic Na+ has an adverse effect on mitochondrial energetics that can be attenuated by simultaneous inhibition of mNCE.

2124-Plat
Biexcitability and Early Afterdepolarization-Mediated Cardiac Arrhythmias
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Under normal conditions in ventricular tissue, both planar wave propagation and spiral wave reentry are mediated by Na current (I_{Na})-mediated depolarization. Under diseased conditions in which repolarization reserve is reduced, however, secondary depolarizations can occur in the plateau or repolarizing phase of the action potential (AP) due to reactivation of the L-type calcium current ($I_{Ca,L}$), known as early afterdepolarizations (EADs). Under these conditions, we observed a novel behavior in which both I_{Na}-mediated spiral wave reentry and $I_{Ca,L}$-mediated spiral wave reentry coexisted in the same homogeneous tissue. I_{Na}-mediated spiral waves were similar to those observed under normal condition, with high rotation frequency (~10 Hz) and nearly full repolarization between beats. $I_{Ca,L}$-mediated spiral waves, however, rotated much slower (2-3 Hz) with membrane voltage remaining above −40 mV, at which I_{Na} is inactivated. We call this novel property of an excitable medium ***biexcitability***. In heterogeneous tissue with transmural AP gradients, pause-induced EADs initiated $I_{Ca,L}$-mediated rotors from the M-cell region. The resulting arrhythmia was characterized by co-existing $I_{Ca,L}$- and I_{Na}-mediated wavefronts, with a frequency and electrocardiographic appearance resembling Torsades de Pointes. The arrhythmia either terminated spontaneously or degenerated to ventricular fibrillation. We propose biexcitability as a novel mechanism of Torsades de Pointes in long QT syndromes.

2125-Plat
B-Type Natriuretic Peptide (BNP) Prolongs Action Potential Duration through Suppressing Transient Outward Potassium Current in Rat Hearts
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