Symposium 11: DNA Nanomachines in Vitro and Inside Living Cells

2114-Symp
Self-Assembly of DNA into Nanoscale Three-Dimensional Shapes
William Shih.
Harvard Med Sch, Boston, MA, USA.
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.
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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.
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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