Symposium: RNA Assemblies and DNA Origami

132-Symp
Designing Synthetic Regulatory RNAs: New Tools for Temporal and Spatial Control in Biological Systems
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Advances in synthetic biology are transforming our ability to design and build synthetic biological systems. While progress has been made in the design of complex genetic circuits, capabilities for constructing large genetic systems currently surpass our ability to design such systems. This growing 'design gap' has highlighted the need to develop methods that support the generation of new functional biological components and scalable design strategies for complex genetic circuits that will lay the foundation for integrated biological devices and systems.
As examples of functional RNA molecules playing key roles in the behavior of natural biological systems have grown over the past decade, there has been growing interest in the design and implementation of synthetic counterparts. Researchers are taking advantage of the relative ease with which RNA molecules can be modeled and designed to engineer functional RNA molecules that act as diverse components including sensors, regulators, controllers (ligand-responsive RNA regulators), and scaffolds. These synthetic regulatory RNAs are providing new tools for temporal and spatial control in biological systems. I will describe recent advances in the design of RNA controllers and in addressing challenges in their implementation as user-programmed cellular control systems. In particular, I will discuss how the application of synthetic RNA controllers in biological pathways is leading to the elucidation of integrated systems design strategies and new capabilities for programming genetic systems.

133-Symp
Molecular Machinery from DNA: Synthetic Biology from the Bottom up
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DNA is a wonderful material for nanoscale construction, enabling the creation of synthetic biological systems from the bottom up. Its self-assembly can be programmed using information embedded in base sequence, and its hybridization or hydrolysis can be used as to provide energy for synthetic molecular machinery. With DNA it is possible to design and build three-dimensional scaffolds, to attach molecular components to them with sub-nanometre precision - and then to make them move. I shall describe our work on assembly pathways, on autonomous, biomimetic molecular motors powered by chemical fuels and the use of synthetic molecular machinery to control covalent chemical synthesis. I shall demonstrate bipedal motors whose operation depends on the coordination of the chemomechanical cycles of two separate catalytic centres and burnt bridges motors that can be programmed to navigate networks of tracks. I shall also discuss the use of kinesin motor proteins to power synthetic devices.

134-Symp
Structural Evolution of RNA Self-Assembly
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In nature, a stable RNA molecule takes advantage of an extensive network of tertiary interactions to fold and self-assemble into a functional three-dimensional (3D) structure. Recently, extensive sequence and structural analysis of known RNA 3D structures has revealed that most natural RNA tertiary interactions belong to a rather limited set of prevalent self-assembling modules, which correspond to well-defined structural conformers characterized by sets of conserved and semi-conserved nucleotides. As such, a natural stable RNA can be seen as a complex 3D network that is hierarchically built from prevalent modular 3D networks of smaller sizes. The underlying structural syntax behind modern day RNA architectures could be seen as an informational proto-language that likely emerged during the early evolutionary process of life on Earth. However, reasons behind the emergence of this particular proto-language are largely unknown. Using artificial self-assembling RNA systems, we isolated by in vitro evolution several novel self-assembling modules of RNA. By extensively characterizing their biochemical and biophysical properties, we were able to explore the genotype/phenotype landscape of both natural and in vitro selected self-assembling modules of RNA. Our investigation shed light on the evolution of modularity and self-assembly in RNA. It also provides valuable insights for the design and construction of RNA nanostructures of increasing complexity.

135-Symp
Dynamic DNA Origami-Based Nanoparticle Assemblies
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We used the DNA origami method [1] for the fabrication of functional self-assembled nanoscopic objects and materials [2]. In DNA origami, a virus-based 8 kilobase-long DNA single-strand is folded into shape with the help of ~ 200 synthetic oligonucleotides and the resulting DNA nanostructures can be designed to adopt any three-dimensional shape. By harvesting the potential to offer attachment sites with nanometer precision on these objects, we have realized complex assemblies of nano-components, including organic fluorophores as well as magnetic, fluorescent and plasmonic nanoparticles. Our nanoconstructs can exhibit striking optical properties such as strong optical activity in the visible range [3] and they can be tethered to surfaces and be operated by external stimuli. Currently, several methods to manipulate DNA nanoconstructs are investigated in our laboratory. For example, by switching the orientation of nanoparticle helices we were able to dynamically control the optical activity of the composite material. The observed circular dichroism signals are reversible and can be explained qualitatively and quantitatively with plasmonic dipol theory. In recent experiments, we were able to show that the optical response of chiral biomolecules can be transferred from the UV into the visible region in non-chiral plasmonic hotspots. Thus, sensitive detection of chiral bio-molecules may become feasible with this approach.
[1] Rothemund, P.W.K. Folding DNA to create nanoscale shapes and patterns. Nature 440, p207, 2006.
[2] Seeman, N.C. Nanomaterials based on DNA. Annu. Rev. Biochem. 79, p12.1, 2010.
[3] Kuzyk, A. et al. DNA-based self-assembly of chiral plasmonic nanostructures with tailored optical response. Nature 483, p311, 2012.

Platform: Optical Microscopy and Super Resolution Imaging I

136-Plat
3D Real-Time Orbital Tracking Microscopy in Zebra Fish Embryos
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Single particle tracking makes it possible to follow individual molecules and biomolecular complexes in living systems as they perform their biological function. This provides a wealth of information regarding where the molecules reside, how they are transported and with whom they interact.
There are several methods for performing 3D single-particle tracking. Our approach is to use orbital tracking with dual-plane detection. In this method, the laser is rotated about the particle of interest. When the particle moves from the center of the orbit, a modulation of the signal is detected and the new lateral position of the particle can be determined. Two confocal pinholes allow simultaneous detection of two planes slight above and below the focus of the laser to allow determination of the axial position of the particle. Using a feedback loop, the center of the orbit is repositioned on the particle of interest. The 3D orbital tracking microscope is controlled by an FPGA so that tracking can be performed in real time with nanometer resolution independent of any latency difficulties from the controlling computer. In addition, a wide-field setup has been mounted such that the surrounding environment is measured simultaneously.
Cells, and in particular neuron cells, are dependent on the proper functioning of mitochondria. Although much is known regarding the function of mitochondria in cell culture, few experiments have been performed in living organisms. With our advances in 3D particle tracking, we are now able to follow individual mitochondria as they are transported in neuronal cells in developing zebra fish embryos. From the single particle tracking data, we can quantify the motion of mitochondria, its velocity, the number of pauses, etc. Hence, it is now possible to follow the life cycle of mitochondria in a living organism.