Single Molecule RNA Biology: Of Intracellular MicroRNAs and Molecular Nanorobots

by

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Abstract:

Nature and Nanotechnology likewise employ nanoscale machines that self-assemble into structures of complex architecture and functionality. Fluorescence microscopy offers a non-invasive tool to probe and ultimately dissect and control these nanoassemblies in real-time. In particular, single molecule fluorescence resonance energy transfer (smFRET) allows us to measure distances at the 2-8 nm scale, whereas complementary super-resolution localization techniques based on Gaussian fitting of imaged point spread functions (PSFs) easily measure distances in the 10 nm and longer range. Here, I will describe a method for the intracellular single molecule, high-resolution localization and counting (iSHiRLoC) of microRNAs (miRNAs). Microinjected, singly-fluorophore labeled, functional miRNAs were tracked within diffusing particles. Observed mobility and mRNA dependent assembly changes suggest the existence of two kinetically distinct assembly processes, revealing the dynamic nature of an important gene regulatory pathway. In addition, I will describe how we have utilized super-resolution fluorescence microscopy to monitor the walk of molecular spider nanorobots on tracks defined by programmable DNA scaffolds called origami.

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