Designing Synthetic DNA Walkers

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Intracellular protein motors have evolved to perform specific tasks critical to the function of cells such as intracellular trafficking and cell division. For example, kinesin and dynein motors transport intracellular cargos to required destinations within a cell, because large intracellular components such as vesicles and mitochondria are too big to diffuse to their destinations. The motor proteins achieve their progressive walking along microtubules by converting the energy from adenosine triphosphate (ATP) hydrolysis into mechanical motion. During the course of ATP binding, hydrolysis and product release, the proteins undergo a series of conformational change, thereby making discrete 8-nm center-of-mass steps and travelling over 1 μm. Inspired by remarkable molecular machineries of protein motors, synthetic analogues have been developed including self-assembled DNA walkers that can make stepwise movements on RNA/DNA substrates or can function as programmable assembly lines. The DNA walker dynamics is usually achieved through a series of reactions, including hybridization, enzymatic cleavage, and strand displacement. However, the kinetics of their stepping reactions is not well understood, and thus, it is unclear how to design them.

In our research, we focus on (i) demonstrating all-synthetic DNA walkers that can transport inorganic cargos along non-DNA tracks autonomously and (ii) studying how design parameters affect the kinetics and processivity of the DNA walkers. Our approach is to use RNA-cleaving DNA enzymes as a model walker, carrying CdS nanocrystal cargos along single-walled carbon nanotube tracks. Similar to the protein motors, DNA motors extract chemical energy from RNA molecules decorated on the nanotubes and use that energy to fuel autonomous, processive walking through a series of conformational changes along the one-dimensional track. Initially, a nanoparticle-capped DNA enzyme strand is bound to an RNA fuel strand through base-pairing of the upper and lower recognition arms on the nanotube track. The enzyme core cleaves the prearranged part of the fuel strand into two fragments in the presence of divalent metal cations. After cleavage reaction, the upper RNA fragment is displaced by the next unbound RNA strand. The lower recognition arm subsequently displaces from the initial RNA and migrates to the next RNA strand, completing a single turnover event. Repeated completion of the single turnover reaction propels autonomous, processive, unidirectional movement of the DNA walker.

To probe the motor operation at the single-molecule level, we have developed a new two-color optical microscopy, termed single-particle/single-tube spectroscopy. Here we use optical properties of the nanoparticle cargo and carbon nanotube track, which fluoresce in the visible and near-infrared spectra, respectively. We monitor the nanoparticle emission against the fluorescent, immobilized nanotube track over time. The spectroscopic measurements provide rich information for motor kinetics, which we theoretically model within the framework of single-molecule kinetics. From the combined experimental and theoretical studies, we elucidate several key parameters that govern the kinetics and processivity of DNA enzyme-based walkers. These parameters include the catalytic core type and structure of DNA enzymes, lengths of the upper and lower recognition arms, and environmental factors such as the type and concentration of divalent metal cations. A better understanding of kinetics and design parameters enables us to

demonstrate the unprecedented walker movement near 5 μm at a speed of ~1 nm/s. We also provide a set of design guidelines to design guidelines to construct highly processive, autonomous DNA walker systems and to regulate their translocation kinetics, which should facilitate the development of functional DNA walkers. This presentation will include our benchmark study of DNA walkers against protein motors.