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Celebrating differences: A single-molecule approach to DNA nanotechnology

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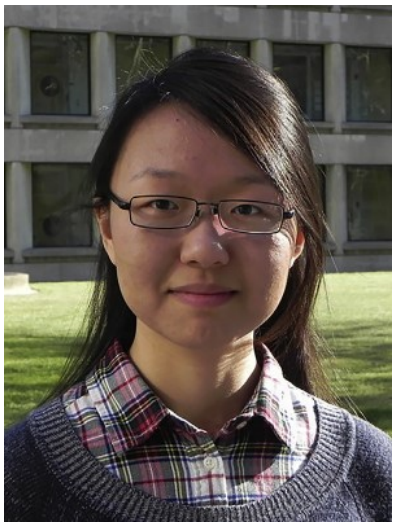

Wei Jia Crystal Chen is a Natural Sciences and Engineering Research Council of Canada postgraduate scholar in the Schlau-Cohen lab in the Department of Chemistry at the Massachusetts Institute of Technology. Her research currently focuses on using a bio-inspired framework and spectroscopic techniques to understand the fundamental principles behind energy transport under physiological conditions. She received her HBSc from the University of Toronto, where she focused on theoretical chemical physics.

One of the remarkable properties of natural photosynthetic machinery is that remarkably high quantum efficiency can be achieved with a relatively scant suite of components:[1] the basic schema can be described as a collection of chromophores, the workhorses of light harvesting, held in precise positions by a protein scaffold. Yet, from simple chemical building blocks emerge sophisticated photophysical behavior, which is still being dissected.

Since its first experimental demonstration,[2] single-molecule fluorescence spectroscopy has been regularly used on a variety of photosynthetic systems to discern complex heterogeneous and asynchronous photophysical behaviors, particularly those that emerge as a direct result of scaffold conditions. Although many recent advances have been made in understanding how scaffolds can control photophysics, experimental difficulties in systematically manipulating protein scaffolds have so far made it difficult to generalize the kind of constraints that the scaffold can impose on chromophores in order to optimize the capture and transport of energy. This presents a notable gap of knowledge in the fundamental understanding of light harvesting because the ability of photosynthetic devices to use the scaffold itself to diversify pathways across multiple timescales, especially under ambient conditions, is responsible for the function of both natural and bio-inspired devices.[3,4] Therefore, the combination of single-molecule spectroscopy with recent advances in DNA nanotechnology has been an exciting step toward a bottom-up approach of understanding the fundamental structural-functional relationship of photosynthetic devices.

In this issue of *Chem*, my colleagues and I employ novel phosphoramidite chemistry to incorporate doubly linked chromophores, Cy3, onto DNA scaffolds of varying complexity and use single-molecule confocal spectroscopy to look at the differences in fluorescence lifetime distribution of Cy3 that arise when its scaffold is changed from a simple duplex DNA to more rigid double-crossover (DX) DNA tiles[5] (Figure 1A). Single-molecule spectroscopy is the prime tool for investigating heterogeneity in any system, especially those under physiological conditions, where thermal noise and ensemble averaging often obfuscate energetic and/or conformational subpopulations.

In our case, where an ensemble-averaged technique recovered the same fluorescence lifetime for Cy3 regardless of how the chromophore was scaffolded, under a microscope we discovered that the added rigidity of the DX tile constrained the chromophore such that the electronic excited states displayed a ~4-fold decrease in the lifetime heterogeneity in comparison with that scaffolded by the duplex (Figure 1C). In other words, an increase in the rigidity of the scaffold decreases the photophysical heterogeneity of the associated chromophore. By a combination of spectroscopy and simulation, we found that the decrease in photophysical heterogeneity is most likely a result of the difference in rigidity, and therefore the accessibility of the scaffolded chromophore, given that the added rigidity restricts DNA kinking (Figure 1D). This result suggests a general schema for controlling exciton properties by tuning the scaffold, or bath, which oftentimes is more experimentally tenable.

We were surprised to discover that, although added rigidity reduces the

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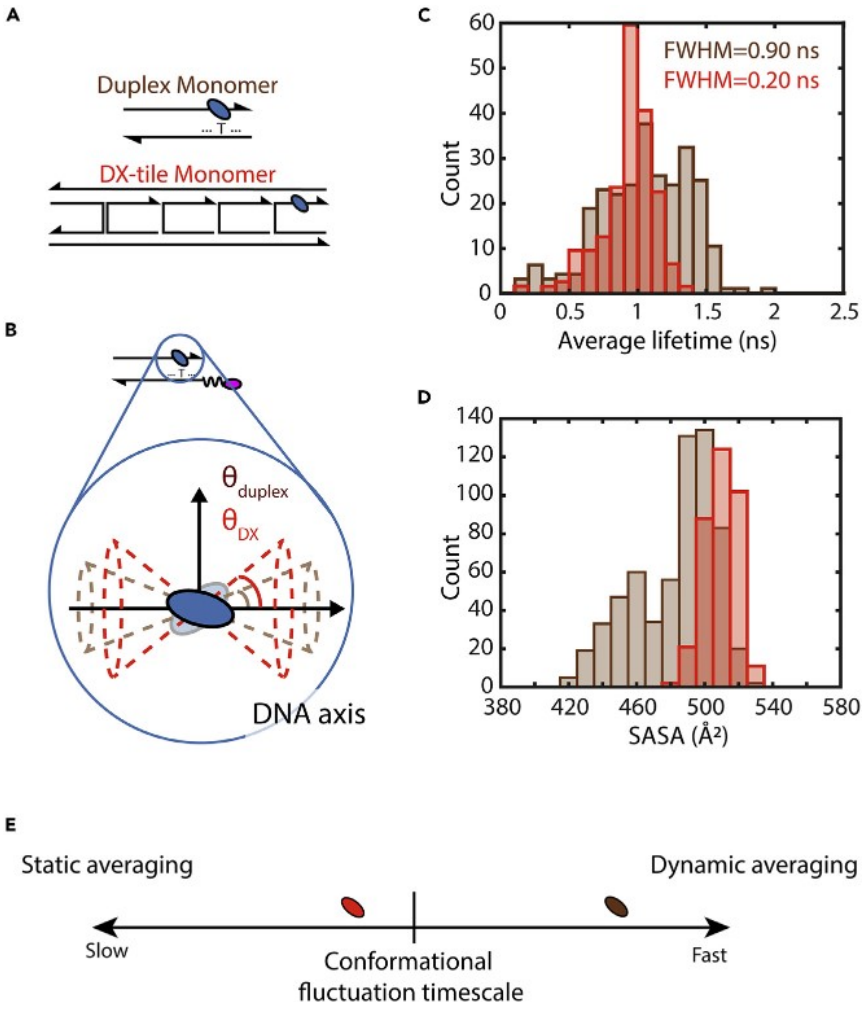

Figure 1. Effects of rigidity differences on properties of duplex DNA and DX DNA tiles scaffolds

Differences in heterogeneity in fluorescence lifetime (C) and solvent accessible surface area (D) of monomeric Cy3 are conferred by rigidity differences between duplex DNA or by DX DNA tiles scaffolds (A). Such differences in rigidity also cause differences in the range (B) and timescale (E) of molecular reorientation, the latter of which directly controls energy-transfer efficiency.

heterogeneity of the excited-state lifetimes, this same structural attribute slows down the depolarization time of the chromophore (Figure 1B). This discovery led to our realization that a much more nuanced interpretation of energy transfer than the typical assumptions employed in fluorescence resonance energy-transfer experiments (dynamic averaging; Figure 1E) is required when one deals with structural fluctuations that occur on timescales similar to that of energy transfer. Namely, each possible position of the chromophore must be considered as a distinct static state, leading to a range of possible energy-transfer efficiencies that must all be accounted for (static averaging; Figure 1E).

Single-molecule spectroscopy is still a relatively new field with many exciting possible directions to pursue. In combination with DNA nanotechnology, it is now possible to break down otherwise complex, interdependent energy-transduction networks to methodically interrogate each heterogeneous component of the system. This will hopefully spur similar types of inquiries in other likewise complex systems, such as quantum information devices and other condensed-phase supramolecular constructs. Being a PhD student under Prof. Schlau-Cohen has exposed me to these exciting new spectroscopic techniques and, perhaps more importantly, really tightened my method of scientific investigation. Our collaborators in the Bathe and Häner labs always have new and interesting DNA designs to share with me. I continue to be awed every day by the sheer versatility and creativity of DNA nanotechnology. The Willard group has shown me just how important numerical and molecular modeling is to the experimental sciences, especially as the field moves toward grappling with the fundamental principles that underlie increasingly more massive, noisier assemblies. It has been such a pleasure to work with a group of scientists with incredibly varied backgrounds and expertise. As science in general becomes increasingly interdisciplinary, I feel extremely lucky to be able to take part in this exercise as a graduate student and am now only more eager to answer those complicated and nuanced questions a single molecule at a time.

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