Physical Chemistry Seminar

Friday, August 24, 2018 | **2:00 pm** | **Room 9341 Chemistry Building**

"Nanoscale confinement strategies for tether-free characterization of single-molecule heterogeneity (Or, how to wrangle single molecules)"

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Host: Prof. Randy Goldsmith

The ultimate goal of single-molecule techniques is to profile population-level or time-dependent heterogeneity in a system of interest by simply monitoring individual particles in a near-native environment. Confining a single molecule within an observation volume for long enough to detect a small, noisy signal can be challenging, especially in situations where tethering particles in place may restrict throughput or perturb the sample. Here, I will discuss two unique approaches to "wrangling" single molecules in free solution, and show that concurrent multi-parametric readout of the states of those molecules can be used to classify their nature and behaviors. First, I will present a nanopore-based genotyping platform that can discriminate among highly similar DNA sequences based upon detection of < 100 molecules. After restriction digestion, DNA is electrophoretically fingerprinted by monitoring the depth and duration of current blockades as each fragment passes through the nanopore. This technique is adaptable for detection of DNA variations that range from insertions and deletions of thousands of base pairs down to single nucleotide polymorphisms and indels, demonstrated here using benign and pathogenic variants of *Mycobacterium tuberculosis* and *Streptococcus aureus*. Second, I will present recent work using an Anti-Brownian Electrokinetic (ABEL) trap to directly observe an endogenous light-activated photoprotective mechanism in a photosynthetic antenna protein assembly called the phycobilisome. Using fast electrophoretic feedback to maintain the position of the phycobilisome within a confocal sensing region, we can directly monitor spectroscopic changes in the native fluorescence of this antenna complex. Surprisingly, we observe multiple quenched states of energy transfer, which directly inform the possible quenching sites and mechanisms that likely confer photoprotection. Because both of these techniques utilitize electrophoretic forces to position single molecules, they rely only on the native charge of the analyte to achieve tether-free nanoscale confinement, providing versatile platforms for addressing both applied and basic scientific questions.