**Time-Resolved Laser Spectroscopy of FRET-based Environmental Biosensors**

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The interior of eukaryotic cells can influence intermolecular interactions and their respective dynamics through localized heterogeneities in macromolecular crowding and ionic strength. For example, macromolecular crowding affects transport, biochemical reaction kinetics, protein-protein interactions, and protein folding, whereas compartmentalized ionic strength can impact enzymatic activities and protein-protein interactions as well as cellular osmosis. Here, I highlight our recent work on two newly developed families of fluorescent protein-based biosensors in which we use time-resolved laser-induced fluorescence to study these proteins in well-defined solutions that mimic biologically relevant conditions of crowding or ionic strength. The structure-dependent sensitivity of these sensors to changes in environmental crowding or ionic strength is determined using Förster resonance energy transfer (FRET, a molecular ruler) efficiency and the donor-acceptor distance. The biosensors are comprised of a donor (typically, a cyan fluorescent protein) and an acceptor (a yellow fluorescent protein) that are linked by a region that is designed to respond to changes in either crowding or ionic strength by varying the linker’s amino acid sequence and electrostatic charge, and its length as well as flexibility. In paired experiments, the enzymatically cleaved counterparts of these biosensors, which are incapable of FRET, were used as controls. The observed time-resolved fluorescence was also used to determine the corresponding Gibbs free energy associated with the structural conformational equilibrium of the biosensors in response to environmental changes. Using these results as a benchmark, we also developed complementary time-resolved fluorescence depolarization and fluorescence correlation spectroscopy methodologies of donor-linker-acceptor constructs for FRET analysis and protein-protein interactions, applicable for both *in vivo* and *in vitro* studies. Importantly, our solution-based studies provide the foundation for future studies in which these genetically encoded sensors will be used to map localized dynamics of compartmentalized intracellular crowding or ionic strength under different pathophysiological conditions such as cancer cells in tumor-like model systems.