UMD Department of Chemistry & Biochemistry
Spring 2021 Seminar Series
Friday, March 26, 2021
3:00 p.m. Remote Via Zoom

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Hetero-FRET Sensors in Vitro

Living cells are crowded with macromolecules and organelles, which are believed to influence a wide range of biochemical processes and pathological conditions of eukaryotic cells. A deeper understanding of this correlation can lead to breakthroughs in finding treatments to diseases. Förster resonance energy transfer (FRET) is considered as a molecular ruler that is able to quantify protein-protein interactions and structural conformation in a range of biomolecules in both controlled environments and in living cells. Currently, a series of genetically encoded fluorescent protein constructs have been designed to quantify both the macromolecular crowding and ionic strength using FRET. It is capable of transferring energy non-radiatively from an excited donor to an acceptor. This transfer is done without the donor emitting a photon and the acceptor absorbing a photon. Researchers have seen how increasingly beneficial FRET is when it comes to accurate spatial measurements and detecting biological organizations. The focus is to examine the effects of the differing donor (mCerulean3 and mTurquoise2.1) on the biosensors' sensitivity to either macromolecular crowding or ionic strength with mCitrine acting as the FRET acceptor. These FRET pairs are connected via a flexible hinge that is designed to report changes in the environment surrounding the biosensor. In one family of FRET sensors, the efficiency of energy transfer increases as macromolecular crowding increases; that is, the donor and acceptor come in close proximity. In another family of FRET sensors, energy transfer decreases due to electrostatic screening between charged α-helices in the hinge, thereby leading to an increase in the donor-acceptor distance between the donor and acceptor. There are many analysis tools used to calculate the FRET efficiency of the FRET probes for either macromolecular crowding or ionic strength. The analysis tools we specifically used are fluorescence lifetime measurements, fluorescence correlation spectroscopy, and time-resolved polarized fluorescence anisotropy. We compared the energy transfer efficiencies of the donors, mCerulean3 and mTurquoise2.1 with the mCitrine acceptor. These results will inform future design of engineered protein-based biosensors.