

You are invited to the Biophysics Seminar by

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Tuesday, May 7th at 14:00

Physics Department, Seminar Room (3rd floor)

Pulsed-interleaved Acceptor Stimulated Emission Förster Resonance Energy Transfer

In Förster Resonance Energy Transfer (FRET), the fluorescence of the acceptor fluorophore is a direct reporter of the energy transfer process. Several events that affect the fluorescence of the acceptor fluorophore are relative to the moment of the donor fluorophore excitation. One interesting event is the spatio-temporal dependency of FRET due to the different energy transfer rate constants for different donor-acceptor distances, in a situation where there is an equilibrium distance distribution and where distances might change due to fast fluctuations. We devised a method that harnesses pulsed donor-excitation laser and acceptor stimulated-emission-laser in order to get FRET-driven acceptor fluorescence decays, resulting out of spontaneous emission, not necessarily after the donor-excitation moment, but rather at any given moment after that. Using this methodology, we will show how we diminish the effects of direct-acceptor excitation from fluorescence decays. We will show how our method aids in reducing background from acceptor-driven FRET-FLIM (Fluorescence Lifetime Imaging) images. Finally we will show how we can achieve higher accuracy in finding the end-to-end distance distribution as well as diffusion coefficient out of PASE-FRET experiments. We hope to use the methodology in order to solve a long-lasting theoretical question – are unfolded proteins necessarily random?