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Optimization of Cyan Fluorescent Protein Fluorescence for Förster Resonance Energy Transfer

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The development of multi-colored variants of the Aequorea victorea green fluorescent protein has enabled quantification of intracellular processes by Förster resonance energy transfer (FRET). FRET between two fluorophores is typically observed over distances less than 10 nm and is steeply dependent on the distance separating the FRET pair and the relative orientation of the fluorophores[1]. Thus, FRET is sufficiently sensitive to be used for detecting changes in protein conformations or protein-protein interactions. However, the sensitive nature of FRET also presents technical challenges to its measurement, especially when applied to complicated experimental systems that are not well suited for detecting FRET. This is particularly true of FRET between two fluorescent proteins. The molecular size of fluorescent proteins occupies a great portion of the Förster distance, where half-maximal FRET occurs, resulting in FRET efficiencies below 30%. In addition, the excitation and emission spectra of fluorescent proteins are very broad and highly overlapping. These properties greatly complicate detection of FRET between fluorescent proteins.

The most widely used donor fluorescent proteins in FRET reactions is ECFP. Despite its widespread use, ECFP suffers from a number of additional drawbacks, including a two-component fluorescence lifetime, dim fluorescence, and a weakly dimeric nature. Using, site-directed mutagenesis, the fluorescence properties of ECFP were improved. This new variant, mCerulean, fits to a single component fluorescence lifetime, is over two-fold brighter than ECFP, and is a true monomer. Since numerous fluorescent proteins have been developed for use in FRET, a useful comparison of available fluorescent protein FRET partners would provide a basis for selecting the most suitable partners for any particular FRET experiment. Therefore, a series of conjoined FRET donor:acceptor fluorescent protein pairs were constructed and the FRET efficiency of each of these conjoined pairs was calculated from time-resolved fluorescence lifetime measurements. FRET between mCerulean and monomeric Venus fluorescent proteins were found to be the most efficient FRET pairing. Förster distances for each pairing were also calculated using reference spectra from purified recombinant protein (Figure 1). The Förster distance of this pair was ten percent greater than that of the more commonly used ECFP:EYFP pairing. Finally, utilization of the mCerulean:mVenus optimized pairing provided a great enhancement to the signal-to-noise ratio for FRET measurements taken using live cell microscopy.

References

[1] T. Förster, Annalen der Physik 2 (1948) 55.

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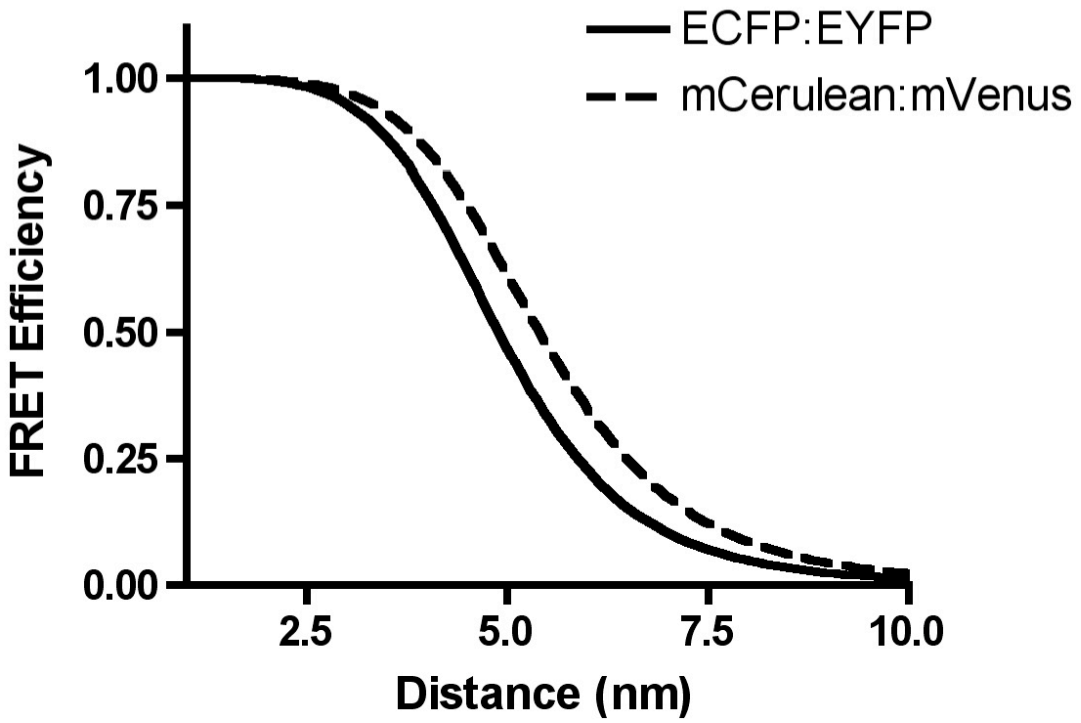

Figure 1: Theoretical plot of FRET efficiency vs. distance (nm). Theoretical plots of ECFP:EYFP FRET efficiency (solid line) and mCerulean:mVenus (dashed line) were calculated using the Förster equation.

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