Published in final edited form as:

Anal Biochem. 2010 July 1; 402(1): 105–106. doi:10.1016/j.ab.2010.03.026.

Förster distances for FRET between mCherry and other Visible Fluorescent Proteins

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Abstract

We present, for the red fluorescent protein mCherry acting as both FRET donor and acceptor, r_0 values with five important VFP variants as well as with itself. The pair EYFP-mCherry exhibits an r_0 of 5.66 nm, equaling or exceeding any combination of VFPs reported previously. Moreover, mCherry should be an excellent chromophore for homoFRET with r_0 of 5.10 nm for energy transfer between two mCherry moieties. Finally, mCherry exhibits higher r_0 values than does dsRed. These characteristics, combined with its rapid folding and excellent spectral properties, suggest that mCherry constitutes a valuable long-wavelength heteroFRET acceptor and probe for homoFRET experiments.

Keywords

dsRed; energy transfer; Förster; FRET; GFP; green fluorescent protein; homoFRET; mCherry; visible fluorescent protein; YFP

The Visible Fluorescent Proteins (VFP²) have revolutionized cell biology by allowing the cellular expression of intrinsically-fluorescent protein constructs. This has in turn allowed biophysical measurements on cellular proteins without ambiguities introduced by extrinsic labeling with antibodies or other ligands. A particularly important class of such measurements involves fluorescence resonant energy transfer (FRET) between two chromophores as originally described by Förster [1]. FRET measurements can demonstrate whether two different proteins associate within a cell (heteroFRET) or whether multiple copies of the same protein aggregate (homoFRET, see below). Protein constructs incorporating pairs of variously-colored VFPs are particularly convenient for such experiments. We therefore previously calculated and published the Förster critical distances (r_0) between pairs of common VFPs

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²Abbreviations used: DsRed, red fluorescent protein from *Discosoma*; EBFP, enhanced blue fluorescent protein (F64L/S65T/Y66H/Y145F); ECFP, enhanced cyan fluorescent protein (F64L/S65T/Y66W/N146I/M153T/V163A); EGFP, enhanced green fluorescent protein (F64L/S65T); EYFP, enhanced yellow fluorescent protein (S65G/V68L/S72A/T203Y); FRET, fluorescence resonant energy transfer; GFP, green fluorescent protein, VFP, visible fluorescent protein.

namely EBFP, ECFP, EGFP, EYFP and dsRed [2] and other investigators have extended such calculations [3].

Unfortunately, cellular studies involving the red fluorescent protein dsRed have encountered special difficulties including slow protein folding and spectroscopically different fluorescent forms. To address these problems Tsien and colleagues [4] have introduced a number of alternative red VFPs of which mCherry has been most broadly used. To facilitate experiments using mCherry in FRET combinations with other VFPs, we have now calculated the Förster r_0 for mCherry acting as both a fluorescence donor and acceptor in combination with the above VFPs. Moreover, because the absorption and fluorescence emission spectra of any chromophore overlap, FRET also occurs between like chromophores with eventual fluorescence being depolarized according to the number of prior transfers of excitation energy. This is the basis of the “homoFRET” method, a technique for identifying homotropic aggregation which has been applied recently to a number of VFP-protein constructs (see, for example, [5]). Hence, we have also obtained the r_0 value for homoFRET between two mCherry moieties.

Purified mCherry was prepared as described [6]. The absorption spectrum of the purified protein, at approximately 0.27 μM concentration in 40mM pH 7 potassium phosphate buffer was recorded from 325-700 nm using a Varian Cary 300 Bio UV-Visible Spectrophotometer. The fluorescence spectrum of the protein was measured in a Kontron SFM25 fluorometer with excitation at 475 nm. A molar absorptivity of 72,000 $\text{M}^{-1}\text{cm}^{-1}$ at 587 nm and a fluorescence quantum yield of 0.22 were assumed [4]. Other spectral data were taken from sources previously cited and calculations to obtain r_0 values were performed as described [2].

Table 1 shows of the results of these calculations. The r_0 value of 5.24 nm calculated for EGFP donor and mCherry acceptor agrees well with the 5.1 nm reported recently for transfer to mCherry by the UV-excited GFP variant E⁰GFP [7]. Moreover, several features stand out among our calculated values. The first of these is that the pair EYFP-mCherry exhibits an r_0 of 5.66 ± 0.11 nm equaling or exceeding the largest value previously reported, 5.64 ± 0.11 nm for EGFP-EYFP. The second feature of interest is that mCherry should be an excellent chromophore for homoFRET. The Förster distance of 5.10 nm for energy transfer between two mCherry moieties virtually equals the largest value reported previously, 5.11 nm for transfer between two EYFP. Finally, we note that mCherry, largely because of its high peak molar absorptivity, exhibits, with all VFP donors, higher r_0 values than does dsRed. These characteristics of mCherry, combined with its rapid folding and excellent spectral properties, suggest that this VFP should prove specially valuable as a long-wavelength heteroFRET acceptor and as a probe for homoFRET experiments.

This work was supported in part by NSF grant CHE-06282260 and NIH grant RR0231256 (BGB) and by Deutsche Forschungsgemeinschaft grant SFB613TPA5 (TS).

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TABLE 1

Förster distances for energy transfer between various combinations mCherry and other Visible Fluorescent Proteins

Other VFP	r_0 (mCherry as acceptor)	r_0 (mCherry as donor)
EBFP	3.21 ± 0.06^a	-
ECFP	4.51 ± 0.09	-
EGFP	5.24 ± 0.10	2.32 ± 0.05
EYFP	5.66 ± 0.11	2.99 ± 0.06
dsRed	5.15 ± 0.10	3.14 ± 0.06
mCherry	5.10 ± 0.10	5.10 ± 0.10

^a r_0 values are given in nm. Uncertainties indicated for these quantities are the estimated standard errors of r_0 as calculated by propagation of errors methods [2].