BIOPHYSICS

# Background FRET

**Models are presented of background fluorescence resonance energy transfer (FRET) for non-interacting membrane proteins.**

Physical interactions between proteins can be studied using FRET, in which there is a nonradiative transfer of energy between a donor and an acceptor fluorophore with overlapping spectra, when labeled proteins come very near each other. But proteins are typically dynamic and can come close purely by chance. Such nonspecific proximity will produce a background FRET signal.

It is well appreciated that this so-called proximity FRET signal can be a problem, particularly for membrane proteins. Being confined to a two-dimensional plane, these proteins are much more likely to come into contact by chance than proteins diffusing in three-dimensional space.

There have been previous models of the proximity FRET signal for membrane proteins; they now form the basis for a study by Kalina Hristova and colleagues at Johns Hopkins University in which the authors refine and extend the predictions made by previous simulations. Hristova and colleagues report simulations of proximity FRET not only between monomeric membrane proteins but also between proteins that form oligomers of varying stoichiometry (dimers, trimers and tetramers) as well as for proteins that exist in an equilibrium between a monomeric and a dimeric state. Their simulations predict that proximity FRET depends on acceptor concentrations in these more complex cases, too, and that the signal decreases with increasing oligomer size.

Turning to experimental measurements, the researchers prepared plasma-membrane vesicles from cell lines expressing proteins of interest fused to fluorescent proteins and monitored the FRET quantitatively in hundreds of these vesicles. By comparing the resulting signal to that of known amounts of fluorescent proteins in solution, they determined donor and acceptor concentrations in the vesicles as well as FRET efficiency.

Hristova and colleagues then used this system to monitor proximity FRET between non-interacting monomeric membrane proteins. But a truly non-interacting pair is not trivial to define. The researchers therefore identified protein pairs that match their theoretical predictions for non-interactors and that show low and uniform FRET signal relative to that of interacting pairs. This characterization should help other researchers studying mammalian membrane proteins to account for nonspecific FRET signals.

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RESEARCH PAPERS
King, C. *et al.* The FRET signatures of noninteracting proteins in membranes: simulations and experiments. *Biophys. J.* **106**, 1309–1317 (2014).