TIME-RESOLVED ANISOTROPY MEASUREMENTS ON FLUORESCENTLY TAGGED PROTEINS: EFFECT OF EGFP ROTATION

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Protein interactions are implied in a high number of biological processes. In order to understand them, there is a great need to be able to detect interactions inside a living cell. This is widely done by using the FRET (*Förster Resonance Energy Transfer*) phenomenon. A particular and less commonly exploited case is homo-FRET where energy transfer occurs between similar fluorophores. This is notably used for membrane protein interactions or molecular clustering studies [1, 2].
This phenomenon leads to a fast fluorescence depolarization that can be measured by fluorescence anisotropy analysis. However, depolarization can be due to other processes and in particular fluorophore rotational diffusion. In many studies, the fluorophore used is the EGFP. Due to its size, it is most of the time assumed that the rotation is too slow during the time window of fluorescence anisotropy decay measurements (typically ~ 12.5 ns) to have any effect on decays. Indeed, EGFP rotation correlation time has been determined to be around 23 ns [1].
However, using specific EGFP constructs that bind to the cell plasma membrane, we could measure significant anisotropy decays in absence of homo-FRET. Furthermore, we observed variations in anisotropy decays depending on the orientation of the cell membrane upon polarization of excitation light. This effect could also be measured on EGFP-fused proteins localized also on cell membrane, confirming that it is present in experimental conditions commonly used for protein interaction studies.

These observations indicate that EGFP rotational diffusion should be taken into account for homo-FRET measurements when using EGFP-tagged proteins.

REFERENCES:

[1] Gautier I., Tramier M., Durieux C., Coppey J., Pansu R. B., Nicolas J-C., Kemnitz K., Coppey-Moisan M. "Homo-FRET Microscopy in Living Cells to Measure Monomer-Dimer Transition of GFP-Tagged Proteins." *Biophysical Journal,* June (80): 3000–3008. (2001)

[2] Sharma P., Varma R., Sarasij R.C. Ira, Gousset K., Krishnamoorthy G., Rao M., Mayor1 S. "Nanoscale Organization of Multiple GPI-Anchored Proteins in Living Cell Membranes." *Cell*, Vol. 116, 577–589, Feb 20, (2004)