

Tuning Far-Red Fluorescent Proteins: Insights from mPlum's Dynamic Stokes Shift

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In recent years, great efforts have been spent on the development of far-red fluorescent proteins (RFPs) for whole-body imaging applications^{1,2}. It turned out difficult to reach an emission in the window of 650-900 nm, where main tissue absorbers (water, lipids, and hemoglobin) are nearly transparent and, at the same time, preserve high quantum yields. A deeper understanding of the photophysics of the chromophore, in vacuum and in its protein environment, and the relation between wavelength regulation and radiationless decay channels is necessary to estimate the limit and remaining potential of the engineering process.

Most red-shifted RFPs, like mPlum feature an extended Stokes shift. Time-resolved fluorescence experiments³ on mPlum show that this shift of the emission maximum to longer wavelengths builds up during the excited-state lifetime. It was suggested that solvent reorganization around the chromophore in the excited state, specifically around the acylimine region, was responsible for this effect. The structural changes that occur during the relaxation of the excited-state are unknown.

The aim of the theoretical study presented here is to elucidate the microscopic process of the mPlum dynamic Stokes shift considering the isolated chromophore and the whole protein. The chromophore in the gas phase has a radiationless decay pathway similar to that of green-fluorescent protein (GFP) analogs. However, we discovered new features that are specific for RFP chromophores and were not observed in GFPs. The effect of the protein environment is tested using QM/MM calculations together with MD simulations. This allows us to describe a plausible mechanism for the observed dynamic Stokes shift, which may open new possibilities for the rational design of improved RFPs.

¹Subach FV, Verkhusha VV (2012) *Chem. Rev.* 112:4308-4327.

²Shcherbakova DM, Subach OM, Verkhusha VV (2012) *Ang. Chem. Int. Ed.* 51:10724-10738.

³Abbyad P, Childs W, Shi X, Boxer SG (2007) *PNAS* 104:20189-20194.