IMAGING DIELECTRIC PERMITTIVITY AND LOCAL ORDER IN LIVING CELLS BY A BIOCONJUGABLE GFP CHROMOPHORE ANALOG

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Fluorescent sensors of polarity and viscosity at nanoscale are particularly interesting for high-resolution microscopy imaging of living cells as these physicochemical properties modulate many cellular processes [1-3]. Ideally, polarity/viscosity probes should fulfill these requirements: a) optical responses (intensity, wavelength-shift, lifetime) predictably related to the environmental polarity or viscosity changes, b) strong brightness for high-sensitivity detection, c) easy conjugation to biomolecules. Conventional probes sense local polarity as expressed by orientation polarizability, which depends in a complicated way on both local static dielectric constant $\varepsilon$ and refractive index [4]. Here, we describe for the first time a visible-absorbing/emitting fluorescent probe, structurally similar to the GFP chromophore, which efficiently reports on sole $\varepsilon$ with good accuracy both in vitro and in living cells. Notably, we found that Generalized Polarization (GP), a classical parameter for ratiometric imaging in cell microscopy [1], shows a linear dependence upon the logarithm of $\varepsilon$, thus making the probe an effective indicator of local $\varepsilon$ through GP measurements. Our probe is suitable for bioconjugation, and its derivatives can report on local polarity of micelles, LUVs, and protein surfaces in vitro. By confocal microscopy we obtained spatially resolved $\varepsilon$ maps for many subcellular compartments, such as endoplasmic reticulum, nuclear envelope, and plasma membrane in cultured CHO cells. From a photophysical point of view, we also demonstrated that this probe behaves as a molecular rotor, allowing for the measurement of environmental fluidity (viscosity) through lifetime. Accordingly, we determined maps of local membrane fluidity in living cells at physiological and non-physiological conditions by Fluorescence Lifetime Imaging (FLIM) in conventional and “phasor” mode [5].