New Optical probes and Imaging Strategy for Biology and Biomedicine

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The arsenal of fluorescent probes tailored to functional imaging of cells is rapidly growing and benefits from recent developments in imaging strategies. Fluorescent sensors of polarity and viscosity at nanoscale are particularly interesting for high-resolution microscopy imaging and diagnostics 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 ε and refractive index [4]. Here, I shall review our activity in the field of environmental fluorescent probes for intracellular use. In particular, we shall focus on a visible-absorbing/emitting fluorescent probe, structurally similar to the GFP chromophore, which efficiently reports on ε with good accuracy both in vitro and in living cells [1]. As for living cells, using confocal microscopy we obtained spatially resolved ε maps for many subcellular compartments, such as endoplasmic reticulum, nuclear envelope, and plasma membrane. From a photophysical point of view, we demonstrated that this probe behaves also as a molecular rotor, allowing for the measurement of the fluidity of local environments through lifetime. Accordingly, we determined maps of local membrane fluidity in living cells at physiological and non-physiological conditions by Fluorescence Lifetime Imaging (FLIM) both in conventional and "phasor" mode [5]. Additionally, we shall discuss on simpler viscosity-sensitive probes that allow for monitoring local viscosity in plasma membrane, lysosomes, mitochondria [6], and chromatin [7]. Finally, I shall discuss the use of reversibly photoswitchable (photochromic) fluorescent proteins, which have become an invaluable tool for the optical labeling and tracking of living cells, organelles and intracellular molecules in a spatio-temporal manner [8]. We recently showed that synthetic chromophores isolated from *Aequorea Victoria* (AFPs) present a reversible *cis-trans* photoisomerization mechanism similar to that reported for photochromic FPs from *Anthozoa* or other organisms [9]. Owing to the relevance of AFPs for cell biology, a photochromic "toolbox" constituted by several AFPs is highly desirable. The known photochromic AFP mutants, however, are far from being optimized, since they need to absorb ~10^6 photons to carry out the on-off photoconversion [10], and their photochromic behaviour was often reported to be accompanied by other undesirable effects (e.g. irreversible photoconversion [11]). Here I shall describe new photochromic AFPs whose reversible photoswitching occurs between the native bright and a dark state at low illumination power, owing to a very efficient *cis-trans* photoisomerization (~10^3 photons for switching) [12, 13]. Most remarkably, the optical bistability of these AFPs derives from a single mutation in the primary sequence of otherwise photochemically stable popular AFP variants. The significance of these mutants for high-resolution cell imaging will be shown by means of super-resolution imaging schemes such as Stochastic Optical Fluorescence Imaging (SOFI) and PhotoActivation Localization Microscopy (PALM) applied to unveil the intracellular organization of key proteins at nanoscale.

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