

NOVEL POLARITY-SENSITIVE PROBES FOR LIVE CELL IMAGING

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The detection of environmental polarity and/or binding processes at nanoscale level, with high spatiotemporal resolution, is essential to the understanding of subtle cellular processes. In this perspective, solvatochromic compounds are very interesting as fluorescent probes for microscope imaging [1]. Indeed, the fluorescence emission of these molecules can be highly enhanced upon polarity variations at nanoscale such those induced by protein binding at their contact surface [1,2]; this allows for a fine detection of molecular recognition events even at the single-molecule level.

Here we report on the development of a toolbox of solvatochromic coumarins [3], endowed with push-pull electronic groups stable in biological environments, and characterized by excellent fluorescence quantum yields (up to 0.95), high molar-extinction coefficients (up to 46,000 M⁻¹cm⁻¹), and large Stokes shifts. These molecules display marked solvatochromism: they are virtually non emissive in water, but intensely fluorescent in less polar media (up to 780-fold fluorescence enhancement). Depending on the push-pull groups, the brightness change takes place in a narrow range of solvent polarity (owing to the loss of H-bonding water molecules) or in a more gradual fashion. In some cases the solvent polarity modulates also the emission wavelength and the fluorescence lifetime, allowing for multi-parameter imaging of biological specimens.

These compounds were tested in cultured cells, showing unchanged photophysical properties and high biocompatibility [3]. Colocalization experiments highlighted that our coumarins, in non-bioconjugated form, stain selectively subcellular organelles where they find a lipophilic environment capable to elicit fluorescence emission [3]. One of these compounds has also been tested as ratiometric/lifetime indicator of nanoenvironment polarity at intracellular level. Finally, the efficiency of our solvatochromic probes in reporting on biomolecular binding processes has been thoroughly tested [4]. Coumarin-labeled streptavidin showed a significant fluorescence enhancement upon binding with biotinylated-BSA *in vitro*, thus providing clear evidence of protein-protein interaction [4]. Preliminary experiments in living cells indicated that these coumarins allowed also for protein binding detection *in vivo*. We believe that these probes will expand the range of fluorescence imaging applications in living specimen and we are currently active in the engineering of further compounds better tailored to specific intracellular sensing.

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