1.3.21 Biosensors for functional imaging of cells and diagnostic applications at the nanoscale

Fluorescence offers the unique opportunity to combine high detection sensitivity (down to single molecule detection) and spatial imaging resolution, with an optical signal responsive to nanoscale changes of environmental physicochemical properties. Here, we shall show how the rational design of the chemical structure transforms organic dyes into efficient biosensors of dielectric and viscosity properties with confocal spatial resolution (200 nm) at intracellular level. These environmental biosensors were effectively applied to image physicochemical properties of intracellular organelles, shedding light on several biomedically-relevant phenomena including drug-delivery mechanisms and chromatin compaction upon nuclear-lamina misassembly in the Hutchinson-Guilford progeria syndrome.

Intracellular polarity and viscosity play regulative roles irrespective of the specific nature of any biochemical reaction. Accordingly, intensive research has been recently devoted to polarity/viscosity fluorescent sensors for high-resolution fluorescent microscopy. Usually, polarity affects the energy of emission, whereas viscosity effectively modulates the fluorescent lifetime.

By a combined computational and experimental approach, we developed fluorescent "molecular rotors" able to monitor independently intracellular polarity and viscosity [1]. These probes provided quantitative measurements of polarity and viscosity of the endosomal membrane. Real time monitoring of polarity and viscosity allowed for validating engineered drug delivery peptides able to disrupt the lipid bilayer once internalized, thus providing a novel assay of drug delivery efficacy (Fig. 1).

As an alternative approach, we focused on a peculiar fluorescent "molecular rotor", whose excited state is unaffected by local polarity [2]. According to a strategy previously established by us, we followed the lifetime-viscosity dependence by the phasor approach to fluorescence lifetime imaging, a fit-free graphical method based on the frequency-domain analysis of the fluorescence decay. Our probe highlighted that cells characterized by a genetically-encoded misassembly of lamin A, as observed in Hutchinson-Gilford progeria syndrome (HGPS) [2], are associated with a reduced nucleoplasm viscosity (Fig. 2), supporting a strict correlation between HGPS and chromatin compaction and regulation.

Complementary to these approaches, a coumarine derivative was demonstrated to possess two concomitantly emitting excited states with different energies, breaking the so-called Kasha's rule. On account of its unique photophysics, this probe was applied to cell imaging to report both the dielectric constant and the water content of subcellular regions.

In conclusion, on account of the exquisite sensitivity of fluorescence, we developed a toolbox of intracellular sensors to monitor polarity and viscosity of nano-environments, in view of diagnostic applications.

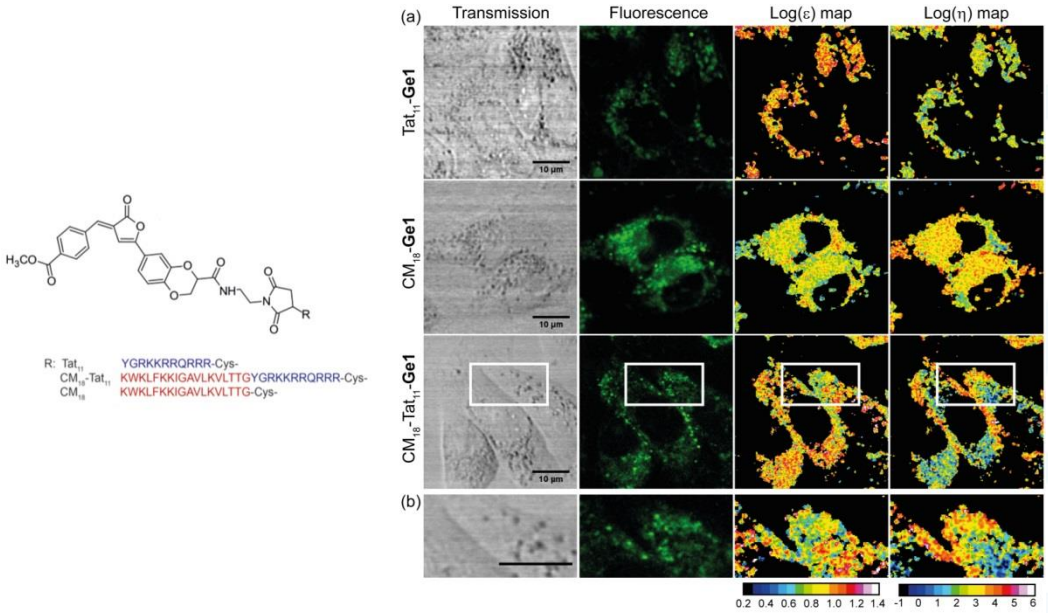

Figure 1. Left panel: molecular structure of the dual polarity (dielectric constant,) / viscosity () fluorescent sensor Ge1 conjugated to the membrane-active peptides Tat_{11} and/or CM_{18}. Right panel: administration of conjugated Ge1 to Hamster Ovary Cells. (a) Transmission, Fluorescence, Log(ε) and Log(η) maps of cells treated with CM_{18}-Ge1 (upper panels), CM_{18}-Tat_{11}-Ge1 (medium panels) and Tat_{11}-Ge1 (lower panels). (b) Zoomed region (white squares in panel a) of CM_{18}-Tat_{11}-Ge1 treated cells. Polarity and viscosity data clearly show that mono-substituted Tat_{11}-Ge1 and CM_{18}-Ge1 remain entrapped inside the endosomal membranes; conversely, the fusion construct CM_{18}-Tat_{11}-Ge1 disrupt the lipid bilayer, as witnessed by the much lower viscosity and higher polarity, and allows for cargo release to the cytoplasm.

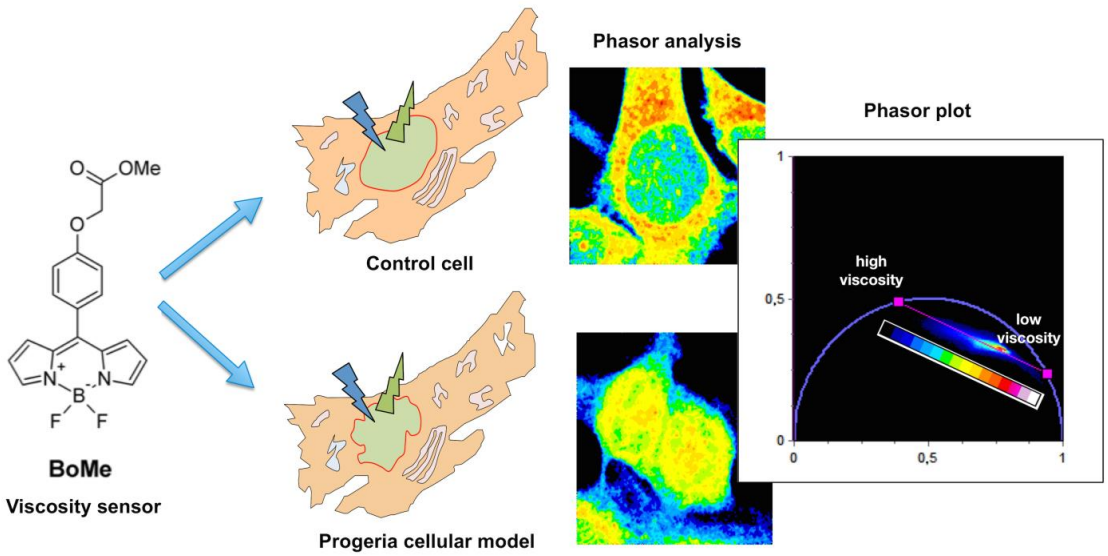

Figure 2. Left panel: molecular structure of the polarity-independent viscosity-dependent fluorescent sensor BoMe. Middle panels: BoMe was administered to both health and HGPS-affected human osteosarcoma cells 2 (U2OS), which displayed different structure of nuclear lamina (round and smooth: healthy cells; irregular and blebbed: HGPS cells). BoMe binds to chromatin and reports on the local viscosity. Right panel: phasor plot of frequency-domain lifetime response of BoMe: different positions on the phasor plot indicate different viscosity values, and are color codified according to cell images in the middle panel. Color distribution clearly shows lower chromatin viscosity for HGPS cells.

References

[1] M. Koenig, B. Storti, R. Bizzarri, D.M. Guldi, G. Brancato, G. Bottari, *A fluorescent molecular rotor showing vapochromism, aggregation-induced emission, and environmental sensing in living cells*, J. Mater. Chem. C **4**, 3018 (2016).

[2] G. Ferri, L. Nucara, T. Biver, A. Battisti, G. Signore, R. Bizzarri, *Organization of inner cellular components as reported by a viscosity-sensitive fluorescent Bodipy probe suitable for phasor approach to FLIM*, Biophys. Chem. **208**, 17 (2016).