

Seeing is believing: the modern approach to intracellular biochemistry

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The late biophysicist Mario Ageo (1915-1992), former Enrico Fermi's student, once stated that "Life is a coherent molecular system ruled by a program". The intrinsic coherency of life explicates in a complex interplay between temporal and spatial scales. Thus, an intriguing approach to biophysics targets the spatiotemporal description of cellular processes such as protein-protein or protein-DNA interactions, taking into account diffusion as well as molecular binding. This approach greatly relies upon the exquisite sensitivity of fluorescence microscopy combined with its high spatial and temporal resolution when applied to biological specimens.

In the first part of the talk, I will show how different techniques based on scanning fluorescence microscopy can be combined together to highlight the subtle interplay between protein dynamics and protein signaling/binding on cell membrane and between intracellular compartments.

Yet, the arsenal of fluorescent biosensors tailored to functional imaging of cells and theranostic applications is rapidly growing and benefits from recent developments in imaging techniques. In this context, I shall show how the rational tuning of the excited-state physicochemical properties may confer peculiar sensing and actuating capabilities to otherwise insensitive fluorescent dyes. For instance, rational design of the chemical structure transforms organic dyes into efficient biosensors of dielectric and viscosity properties with confocal spatial resolution at intracellular level. These biosensors were effectively applied to image physicochemical properties of intracellular organelles, shedding light on cell drug delivery mechanisms and chromatin compaction upon nuclear lamina misassembly in the Hutchinson-Guilford progeria syndrome, and membrane rigidification/fluidization upon ion-channel activation. Similarly, a single mutation in the primary sequence of otherwise photochemically-stable popular fluorescent protein variants relieves their intrinsic photoswitchable behaviour. Notably, photoswitchable (photochromic) fluorescent proteins (FPs) have become an invaluable tool for the optical labeling and tracking of living cells, organelles and intracellular molecules in a spatio-temporal manner. I shall describe their application to highlight the spatial organization of epigenetic modulators with relevant role in tumorigenesis.