PIFE used in the study of single intrinsically disordered proteins in vitro and heterochromatin proteins bio-condensation in vivo

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Abstract:

There are various approaches to gain insights at the macromolecular level from fluorescence-based assays, some of which depend on the structure of the fluorophores and photophysical effects they exhibit stemming from their structure. The effect of excited-state *cis-trans* isomerization occurring in fluorophores that act as molecular rotors in excited-state can be used if the de-excitation of one isomer exhibits high fluorescence quantum yield while the other exhibits a lower fluorescence quantum yield. In photoisomerization-related fluorescence enhancement, or PIFE, the potential steric obstruction of isomerization and its effect on fluorescence is used for reporting local structural changes. The combination of the PIFE effect with bright fluorescent dyes allows performing such measurements at the single-molecule level(*1–4*) or in fluorescence imaging.

In my talk, I will provide the fundamentals of PIFE, its uses in general, and in particular for studying differing questions:

(i) Do intrinsically disordered proteins (IDPs), exhibit only rapid structural transitions between unstable states, or do they also exhibit slow structural dynamics rendering them not well-folded proteins, but also not fully polymer-like ones(*4–6*). In this part I will summarize single-molecule PIFE measurements *in vitro* using the organic dye, sulfo-Cy3, to site-specifically tag residues of the Parkinson's disease-related protein α-synuclein. The results of this part support the larger project of showing that the free monomer α-synuclein exhibits different well-defined conformational states, some of which are stable enough to act as promoters of function.

(ii) How can the phase of heterochromatin protein (HP) condensates be discerned experimentally from cellular fluorescence imaging? Here, I will summarize how can fluorescence lifetime imaging of standard fluorescent proteins can be useful, via PIFE, to sense local densities within dense phases, such as ones occurring in HP biocondensates. Using this approach, I will show that a liquid phase cannot explain HP biocondensates before differentiation in mouse embryonic stem cells, however after initial differentiation, this changes towards a liquid phase.

References:

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