Monitoring of dynamic processes : An easy and reliable way to perform single molecule FRET and FCS measurements

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Abstract

Single Molecule studies and – more specifically – single molecule FRET methodologies have become a standard tool for studying dynamic structural changes in proteins and nucleic acids. These types of measurements can reveal dynamic events on time scales covering several orders of magnitude from ~ns to several seconds. This allows studying e.g., chain dynamics, binding, folding, allosteric events, oligomerization and aggregation. The power of these methodologies is highlighted by the study of Intrinsically Disordered Proteins (IDPs) whose biological relevance has been increasingly studied over the recent years.

In this workshop we will showcase how easy it is for new users to perform single molecule measurements on two model systems:

a) doubly labeled freely diffusing short oligonucleotides and

b) immobilized Cy5 molecules

We will demonstrate how easily these measurements can be performed and how all necessary correction parameters are automatically determined requiring no interaction from the user by employing methodologies benchmarked by the scientific community. We will also show how the variable PSF feature can be used in such measurements to fine-tune the observation window of freely diffusing biomolecules.