Tools for Molecular Dynamics study applied to transcription

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Keywords: molecular dynamics, FCS, FRET, FLIM, SPT, single molecule, subdiffusion, anomaly coefficient, P-TEFb.

Abstract

The study of transcription factors (TFs) target search is important to understand how genetic expression is regulated in cells. The information on TF molecular dynamics leads to a better understanding of how transcriptional activity is controlled. In our lab, we are focusing on P-TEFb complex (positive transcription elongation factor) which plays an essential role in the regulation of transcription by interacting with RNA polymerase II (RNA Pol II) in eukaryotic cells. We developed different microscopy strategies to measure dynamics and molecular interactions in live cells (based on FLIM/FRET and FCS). FRET (Förster resonance energy transfer) measurements enable us to characterize molecular interactions with a great efficiency, but their acquisitions are limited in time (requiring up to few minutes). FCS (Fluorescence Correlation Spectroscopy) techniques have a smaller temporal scale (microsecond to few seconds) but measurements are limited spatially by the focal volume of the laser beam. SPT (single particule tracking) seems to be a good compromise to analyse spatio-temporal dynamics of TFs up to 10um²/s, but this technique is blind for faster molecules. We propose to combine these techniques in order to take profit of their complementarity. We will present our recent results on P-TEFb and RNA Pol II complexes, and how we construe dynamics information coming from different measurement techniques. Also, we present our new microscopy module 'StellarScan' specially design to perform CLSM (confocal laser scanning microscopy), TIRFM/HiLo (Total Internal Reflection Fluorescence Microscopy / Highly inclined and laminated optical sheet), LSFM (light sheet fluorescence microscopy). 'StellarScan' module is ready to plug to any fluorescence microscope and provides multimodal settings for FCS/SPT PALM or FLIM-FRET measurements for example, making it a perfect tool for our molecular dynamics studies.