Tools for Molecular Dynamics study applied to transcription

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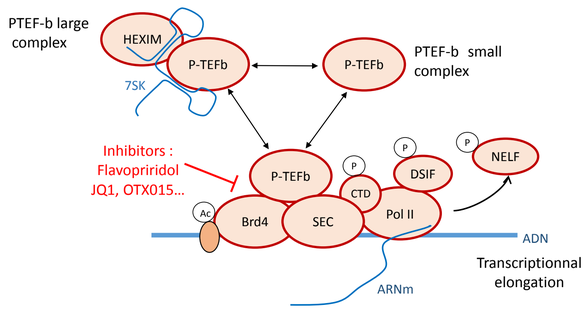
Abstract: The study of transcription factors (TFs) target search is important to understand how genetic expression is regulated in cells. The information on TF molecular dynamics leads to a better understanding of how transcriptional activity is controlled. We are focusing on P-TEFb complex (positive 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. **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**. Also, **we present our new microscopy module 'StellarScan'** specially designed 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.

Biological context : transcriptional pause release

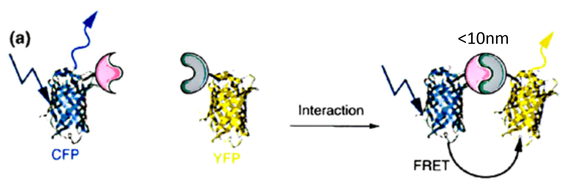


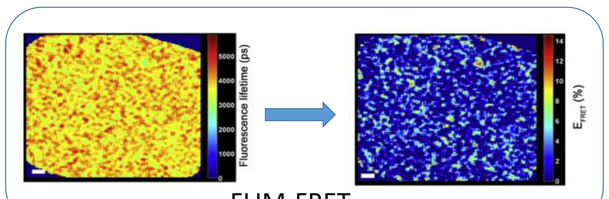
Furlan et al. 2018

Transcription pause is a key mecanism controlling the expression of most genes.

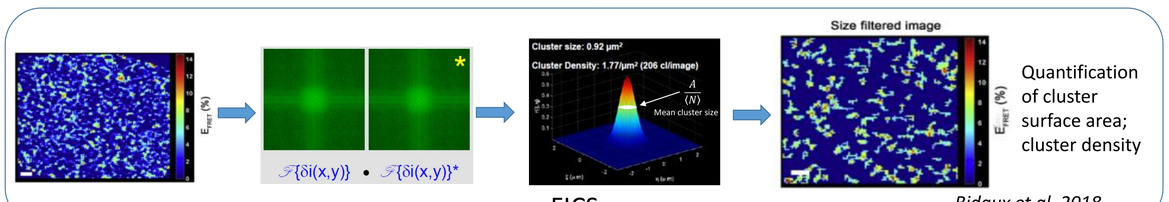
How P-TEFb is recruited on chromatin to release transcriptional pause?
Is dynamics of P-TEFb a regulator of transcription ?

Interactions between CT1 and RPB1 in FRET by FICS show a cluster organization





FLIM-FRET



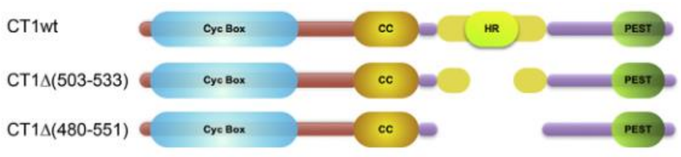
FICS

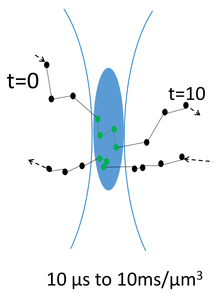
Quantification of cluster surface area; cluster density

Bidaux et al. 2018

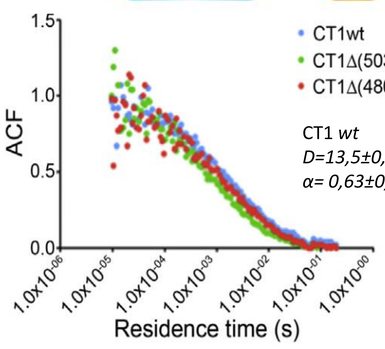
Interactions between CT1 mutants and RPB1 occur in a more diffuse area (2,03 and 2,43-fold increase) than between CT1*wt* and RPB1 : lower affinity of CT1 mutants with RPB1.

FCS demonstrates that HR domain does not constrain CT1 diffusion





10 µs to 10ms/µm³



CT1 *wt*
D=13,5±0,43 µm²/s
α= 0,63±0,02

N number of particles

τ_D characteristic residence time of the fluorophore

$\alpha < 1$ anomalous diffusion

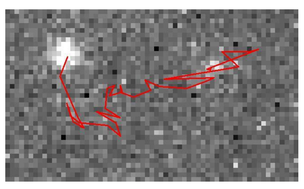
***D* diffusion coefficient** = $^{w_{xy}{}^2}/_{4\tau_D}$

$$G(\tau) = \frac{1}{\langle N \rangle} \frac{1}{(1+(\tau/\tau_D)^{\alpha})(1+a^{-2}(\tau/\tau_D)^{\alpha})^{1/2}} + G(\infty)$$

Bidaux et al. 2018

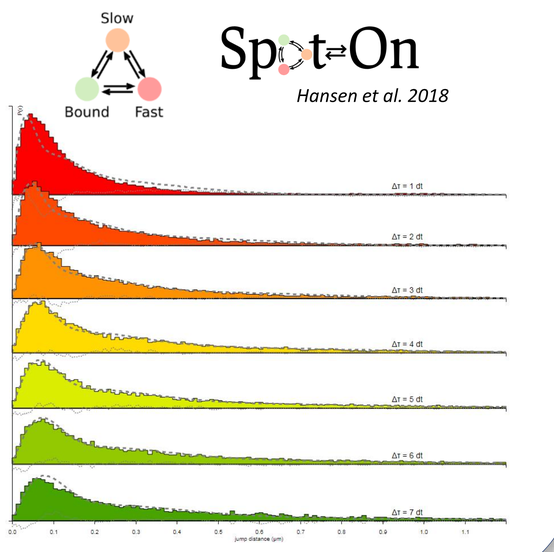
CT1*wt* and its mutants displayed similar diffusion properties (residence time and anomality coefficient). That highlights that HR domain does not constrain CT1 diffusion, although it is specifically involved in CT1-RPB1 interaction.

Single Particle Tracking (SPT) to complete spatial information about CT1 and RPB1 diffusion

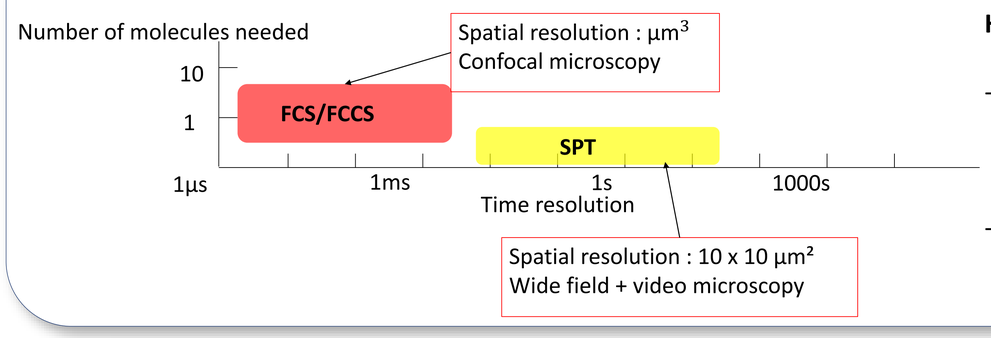


Subpopulation analysis of CT1*wt*

- D_{bound} : 0.036 ± 0.0005
- D_{slow} : 0.379 ± 0.0039
- D_{fast} : 2.692 ± 0.0129
- F_{bound} : 0.188 ± 0.0013
- F_{slow} : 0.337
- F_{fast} : 0.474 ± 0.0000
- I_2 error: 0.00017886
- σ (µm) 0.010 ± 0.0005
- AIC: -181,264.80, BIC: -181,217.08

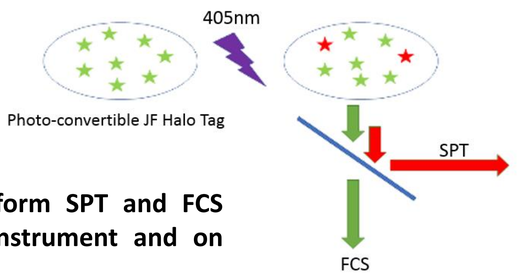


For fast and 3D diffusing transcription factors, FCS and SPT measurements do not lead to the same conclusion… why ?



Hypothesis :

- **FCS and SPT have different temporal scales, and according to their respective sensitivity they under- or over-estimate a specific subpopulation**
- **Different measuring instruments or fitting model can lead to a bias in analysis**



Objectives :

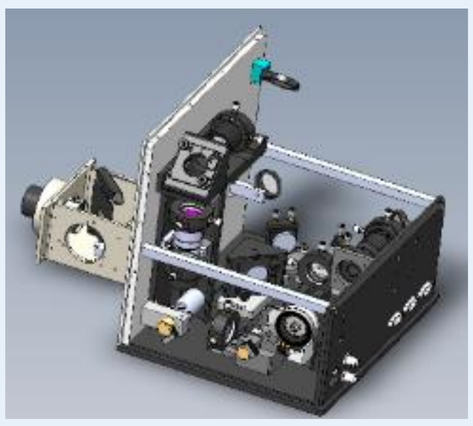
We want to perform SPT and FCS with the same instrument and on the same sample

Development of StellarScan multimodal microscopy module + light sheet microscopy for SPT measurements

Multifonction microscopy module to combine FCS/SPT : StellarScan

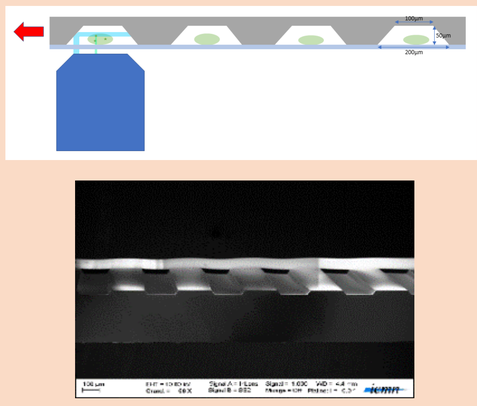
Creation of an innovative multiscan microscopy/spectrosopy module

- HiLo/TIRF
  - SPT PALM
- CLSM (confocal)
  - FCS
  - FLIM FRET
- Light Sheet Fluorescent Microscope
  - SPT PALM

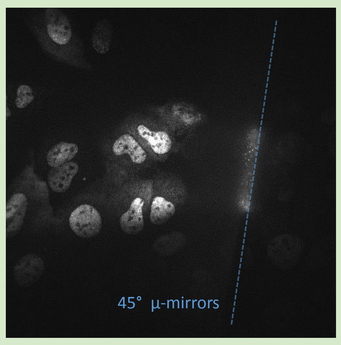


Characterization in progress…

To improve SNR of SPT images : development of 45° µ-mirrors for LSFM



Light sheet microscopy : 1st tests With StellarScan module



How PTEF-b complexes diffuse and find their targets remains a key question in the transcription field. We try to understand why apparent diffusion coefficients differ with different temporal scale, and if there is a link with the two PTEF-b forms (small and large complexes).