Estimations of turnover rates in Single-Molecule microscopy

September-December 2025
CADMO

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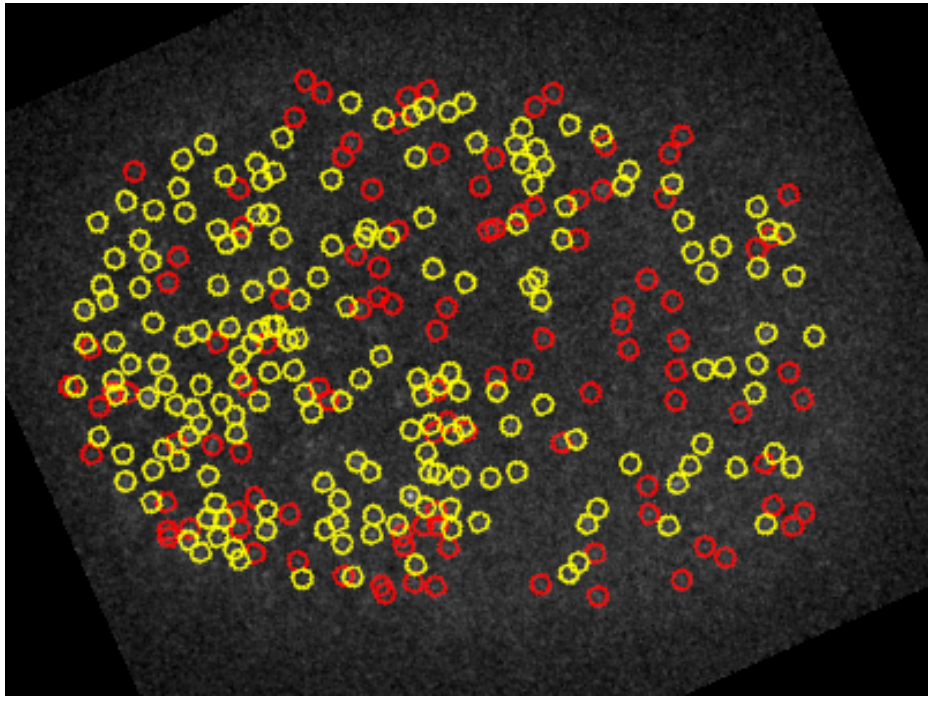
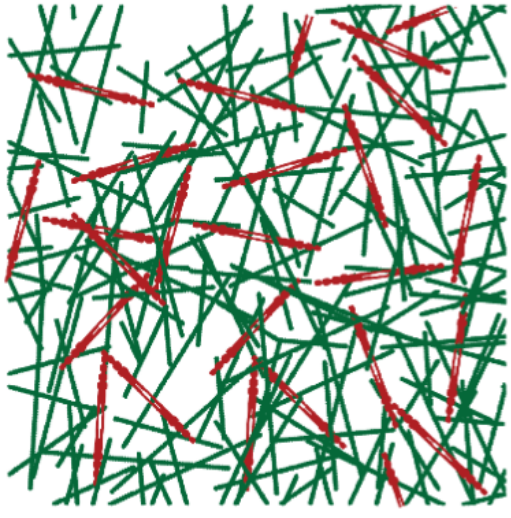
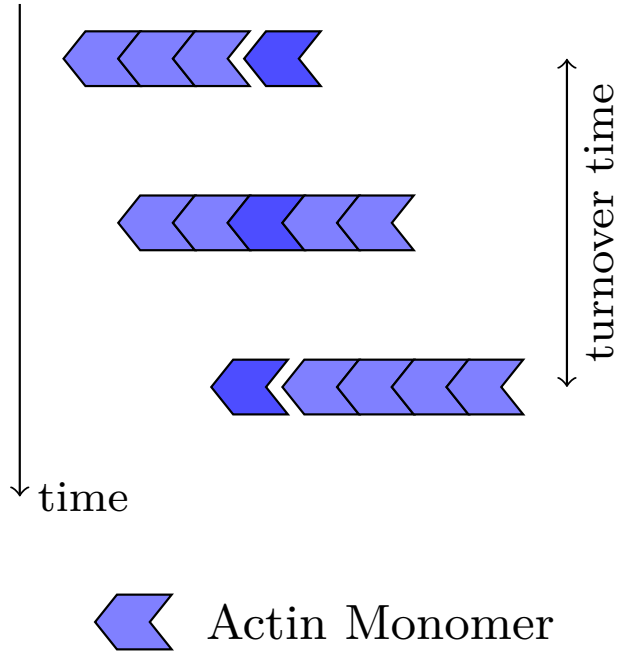


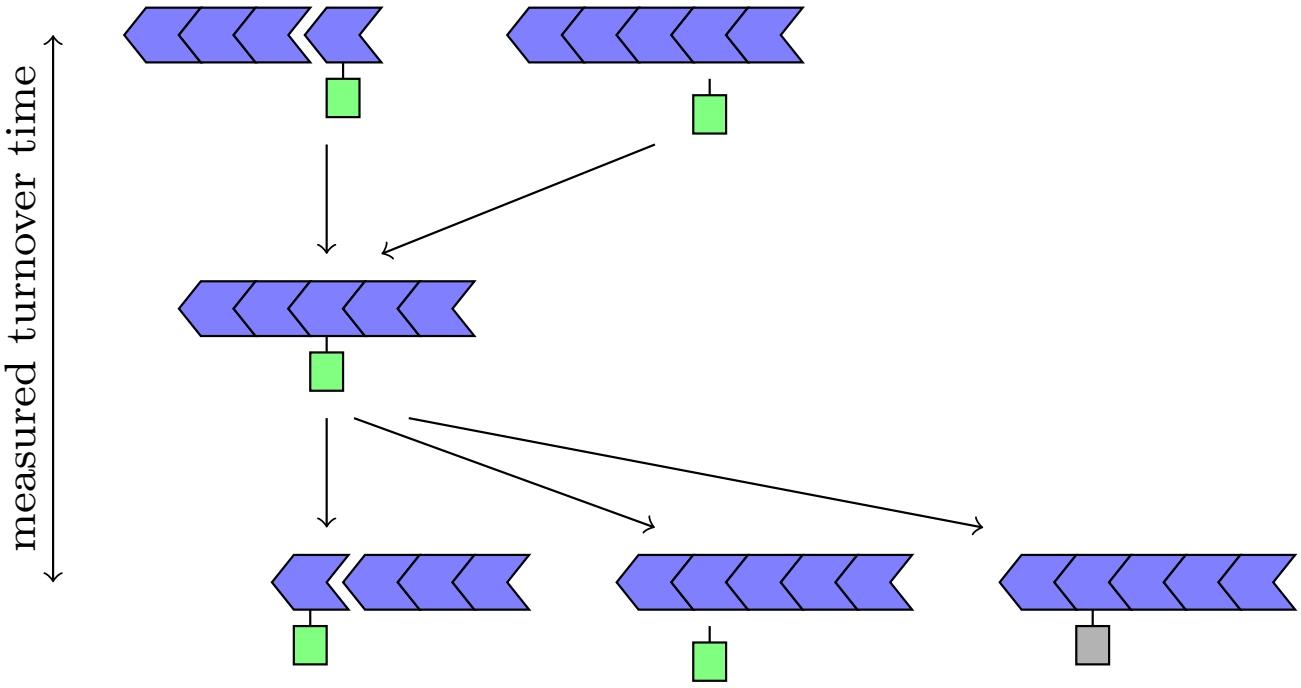
Actin network and turnover





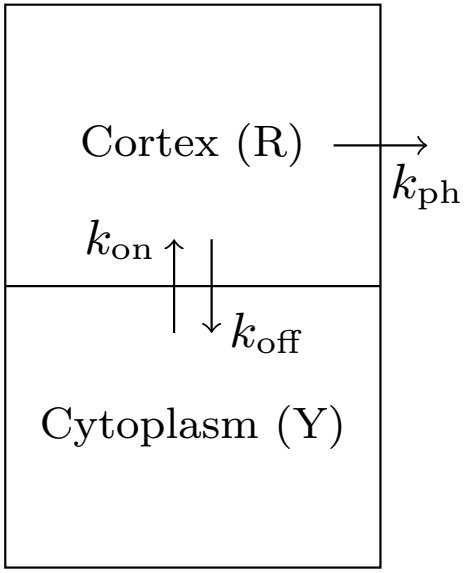
The turnover time is not always the same, it depends on the length of the polymer, the speed of depolymerisation...

The turnover time we can measure



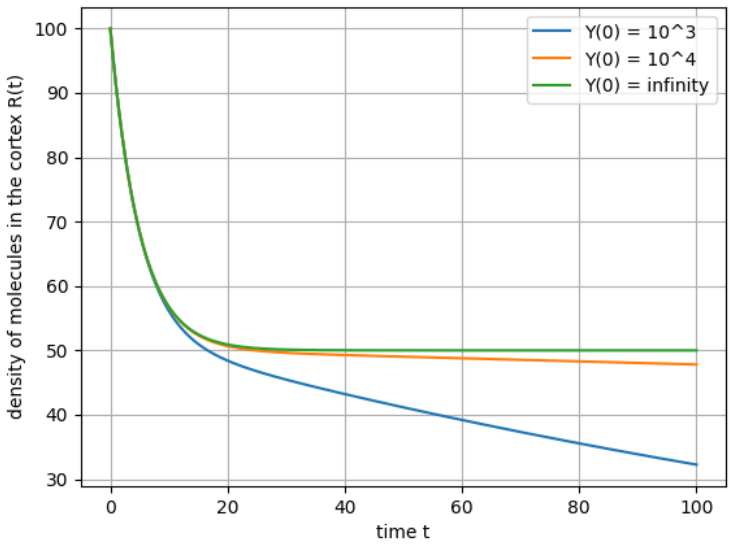
In these slides, the ABP used is always Utrophin : :GFP (except for the data from Marjolaine).

The 2-boxes model for the dynamic of ABP

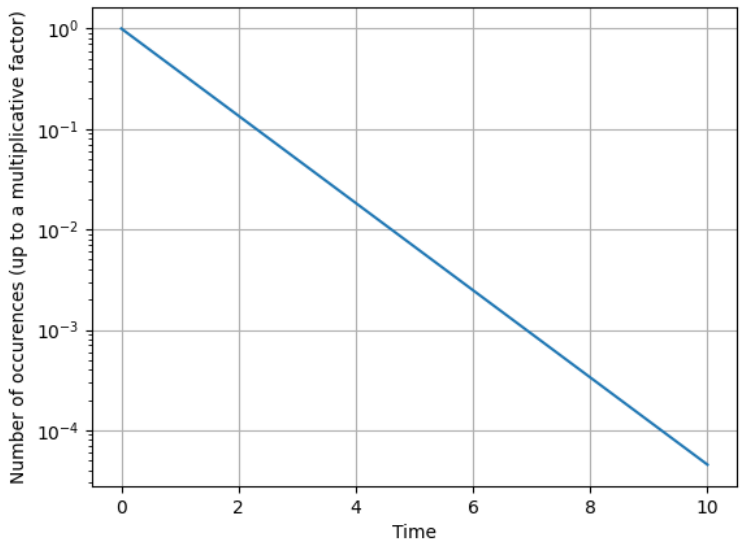


$$R'(t) = k_{\text{on}}Y(t) - (k_{\text{off}} + k_{\text{ph}})R(t),$$
$$Y'(t) = k_{\text{off}}R(t) - k_{\text{on}}Y(t).$$

Here the turnover time follows an exponential distribution with mean $1/(k_{\text{off}} + k_{\text{ph}}) =: 1/k$.

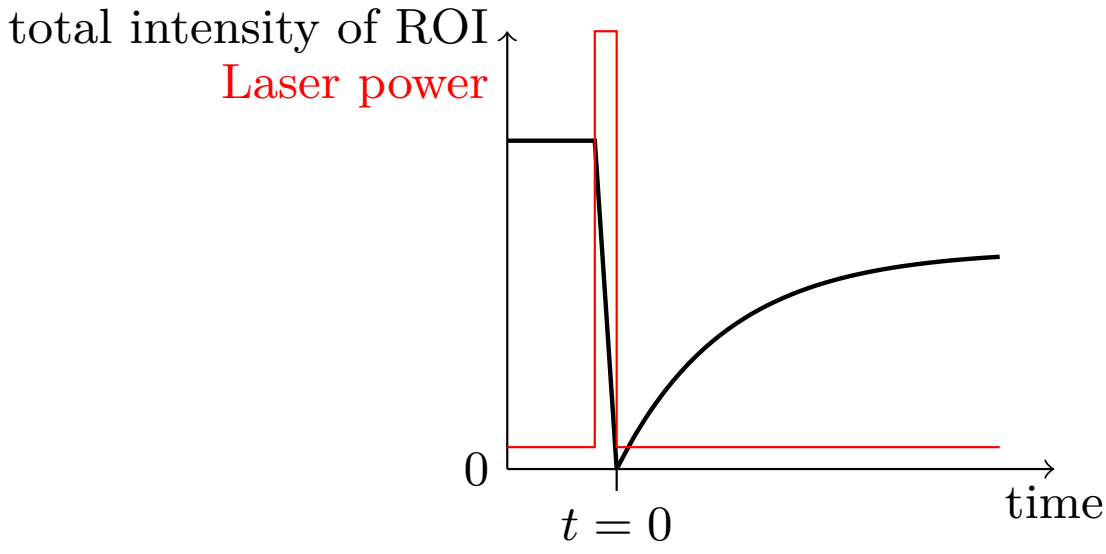


Number of detected molecules



Proportion of tracks of each length

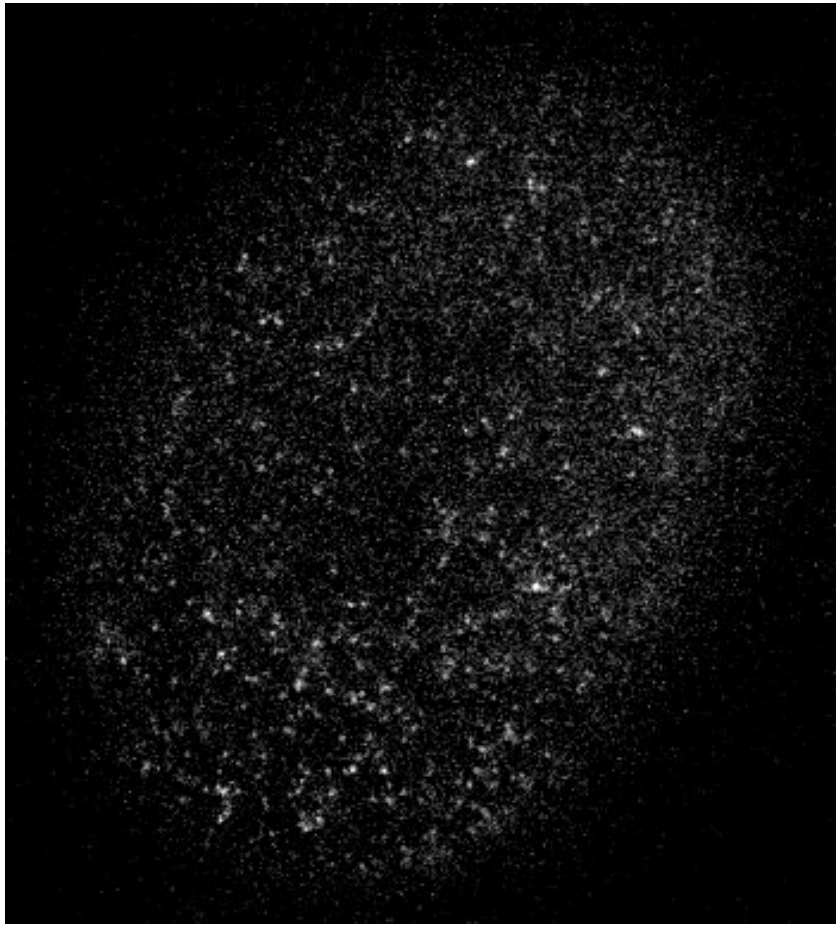
Baseline : Estimation of k by FRAP



For $t > 0$ the intensity of the ROI follows

$$I(t) = A(1 - \exp(-kt)).$$

Single-Molecule Microscopy



Photobleach until the fluorescence is so low, that we can distinguish single fluorescent proteins.

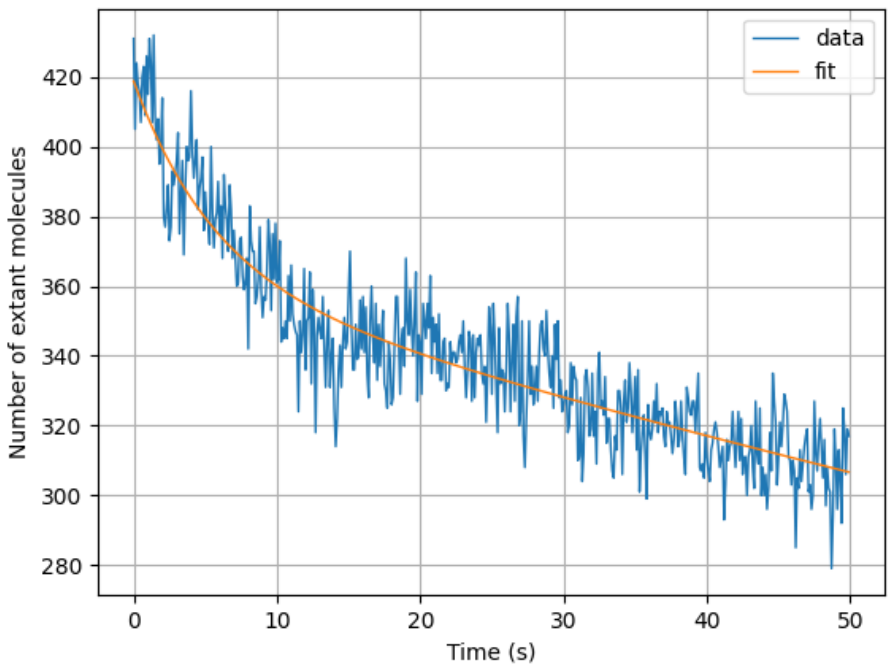
Estimation of k_{off} and k_{ph} by smPRESS

If the system is at the equilibirum and we turn the laser on at $t = 0$, we have an explicit solution for the 2-boxes model

$$R(t) = R(0)\left(\frac{k_{\text{ph}} + r_2}{r_2 - r_1}e^{r_1 t} + \frac{k_{\text{ph}} + r_1}{r_1 - r_2}e^{r_2 t}\right),$$

with

$$k_{\text{on}} = \frac{r_1 r_2}{k_{\text{ph}}}, \qquad k_{\text{off}} = -(r_1 + r_2) - (k_{\text{on}} + k_{\text{ph}}).$$



Estimation of $k_{\text{off}} + k_{\text{ph}}$ by Tracking (ideal scenario)

The slope corresponds to $-k_{\text{off}}$:

$$f(t) \propto \exp(-k_{\text{off}} t).$$

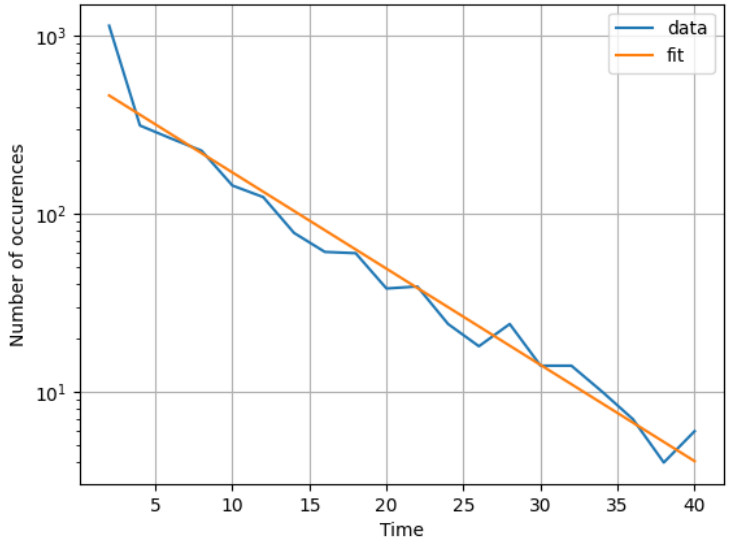
We can also use

$$k^{\text{mean}} = \frac{1}{\text{mean residence time}}.$$

If $\tau_{\text{frame}} k_{\text{off}} \sim 1$, it is better to use

$$k^{\text{discr}} = -\frac{1}{\tau_{\text{frame}}} \log(1 - \tau_{\text{frame}} k^{\text{mean}})$$

to compensate for the discretisation.

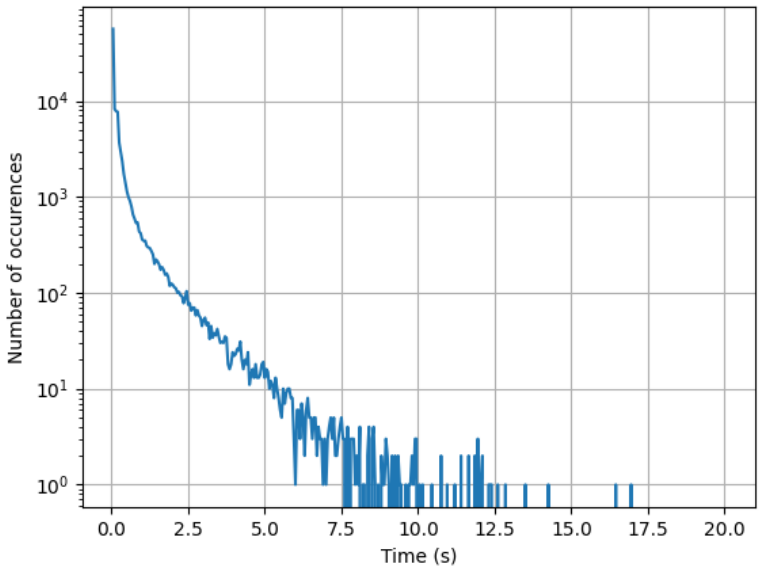


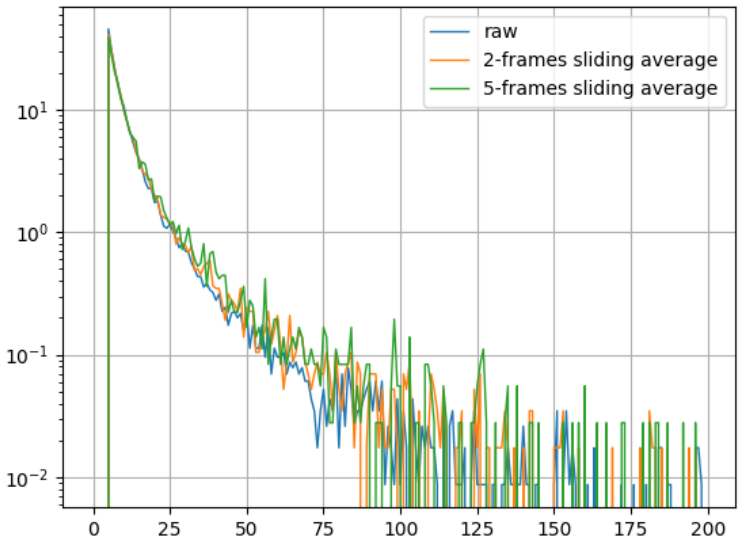
Histogram f of the residence times of the tracks.

Are the different methods coherent ?

The estimation by tracking is not coherent with the other two methods. Why ?

Estimation of $k_{\text{off}} + k_{\text{ph}}$ by tracking



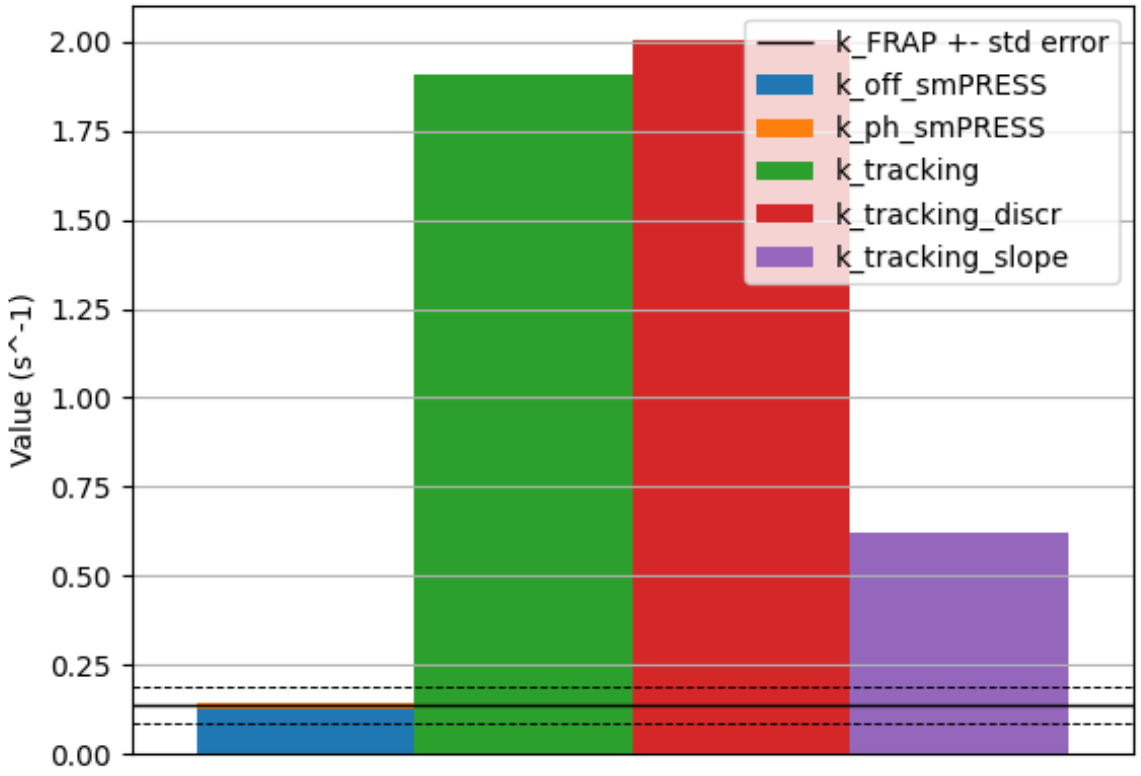


Possible explanations :

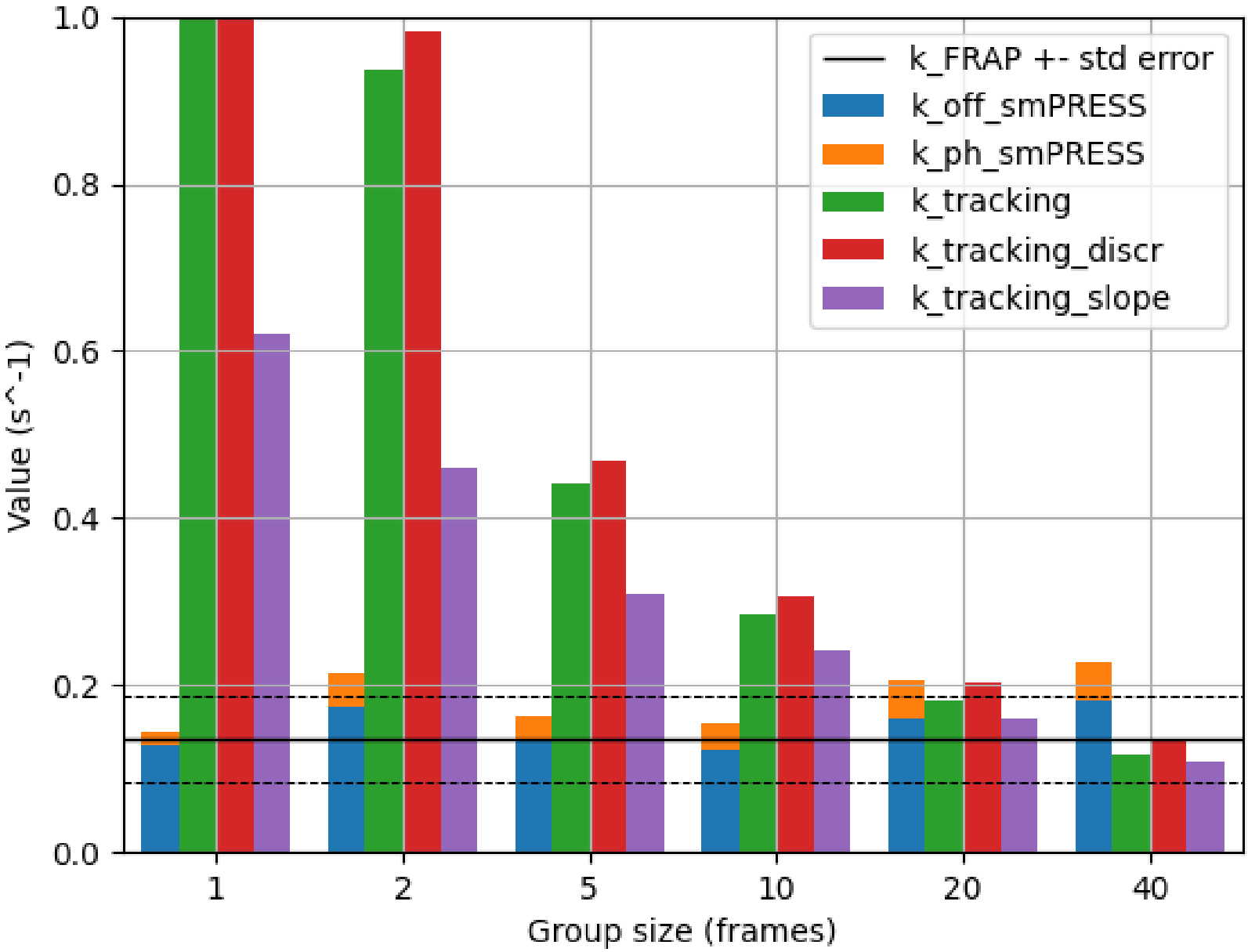
- There are actually two populations.
- It is noise coming from the measurements, the tracking algorithm...

(More on that later)

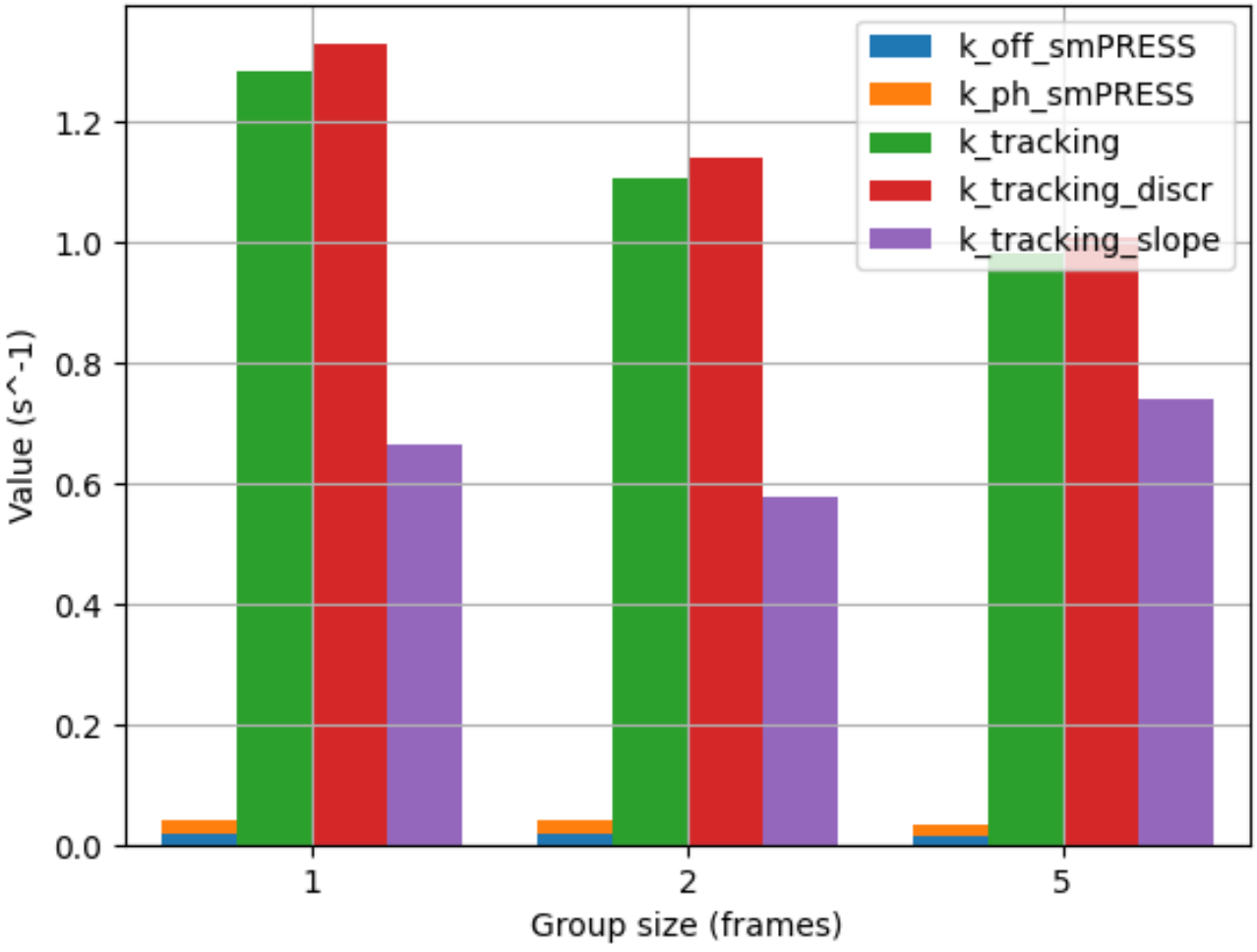
Are the different methods coherent ?



Are the different methods coherent ? Averaging over several frames

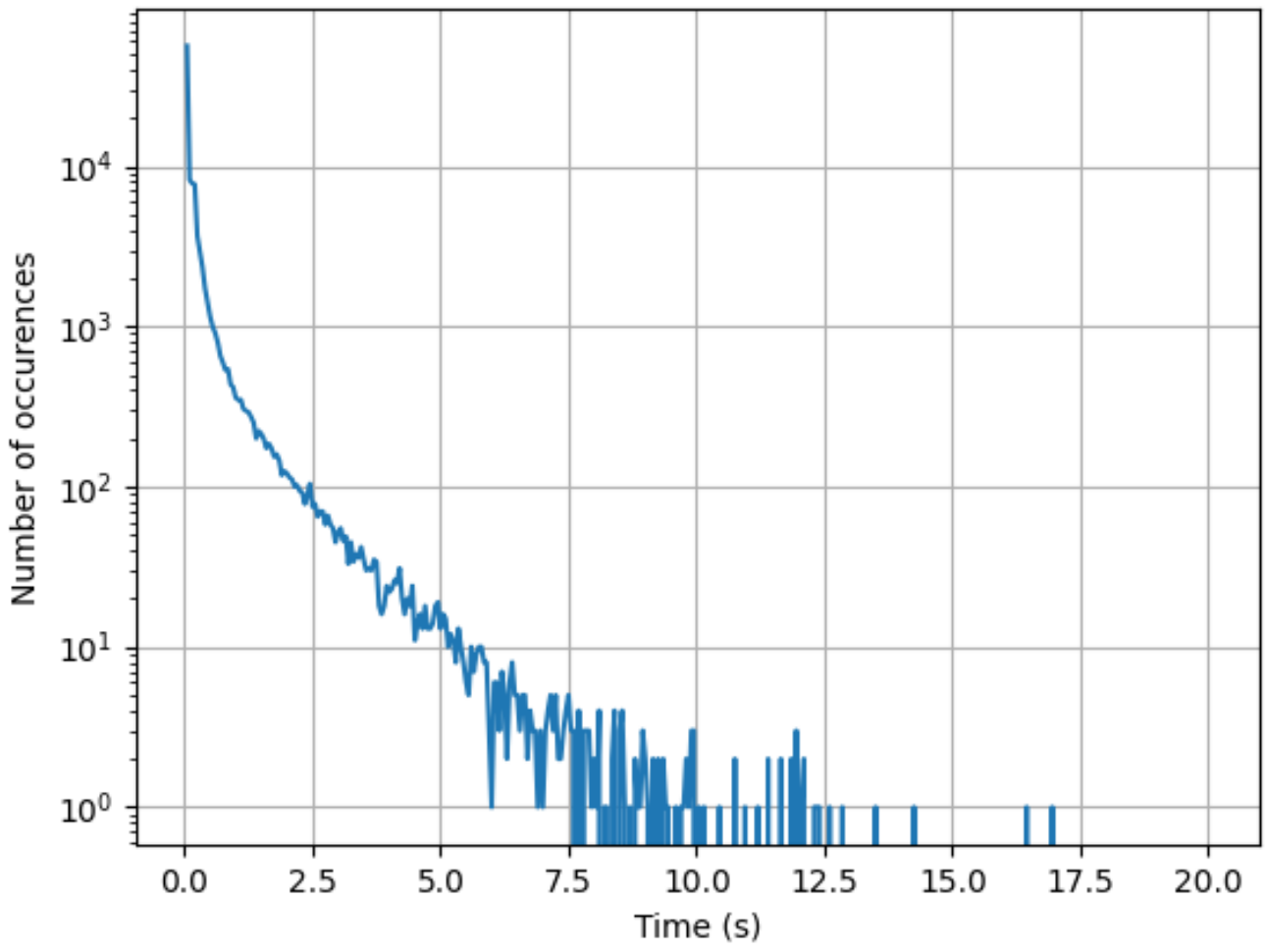


Are the different methods coherent ? Data from Marjolaine

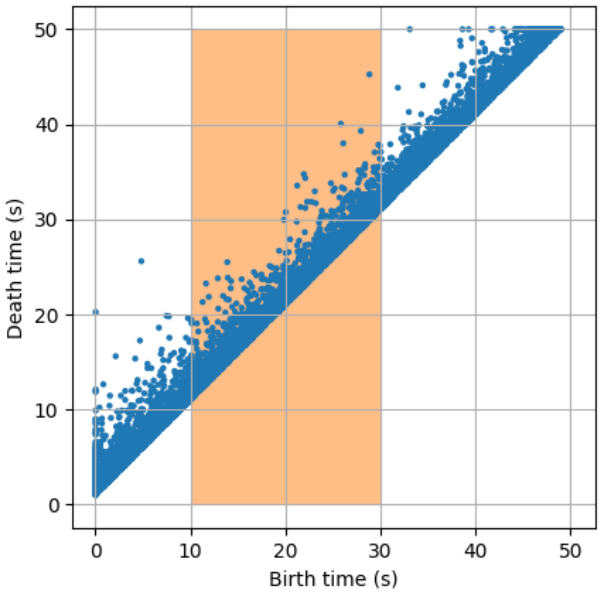


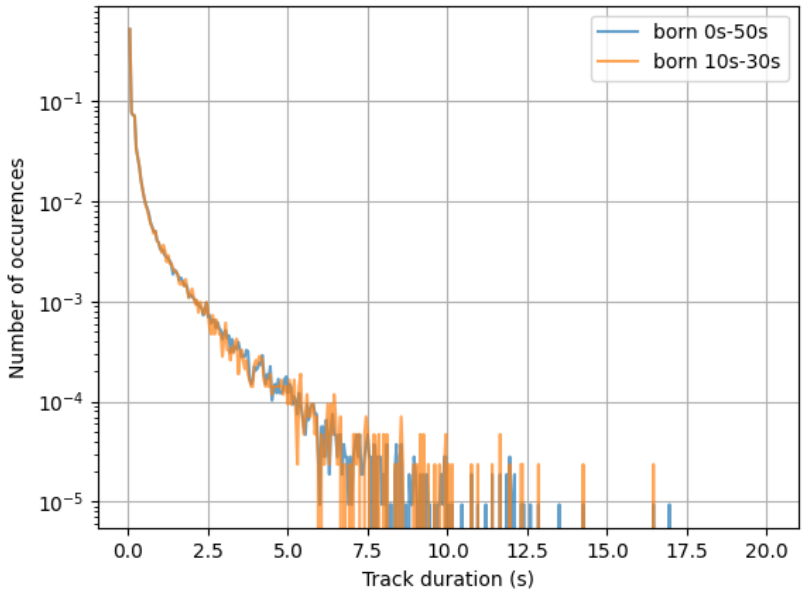
Marjolaine tracks formins, which move ballistically.

Where does the deviation from the model come from ?



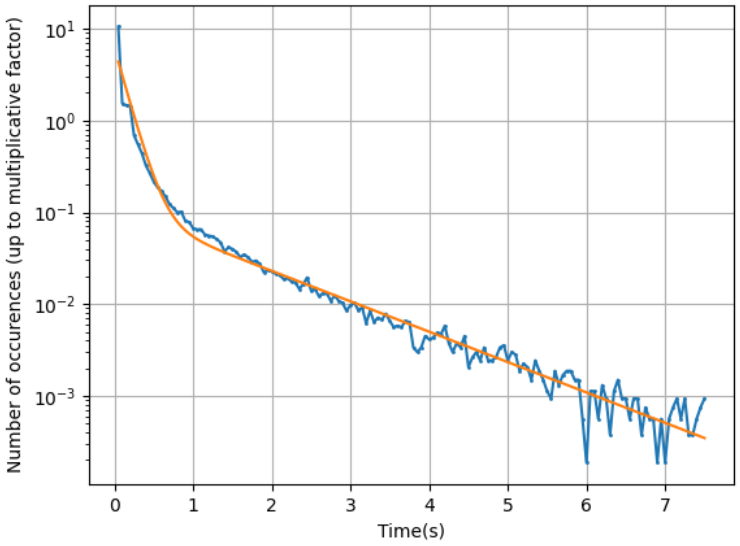
No bias from the beginning and end of the tracks

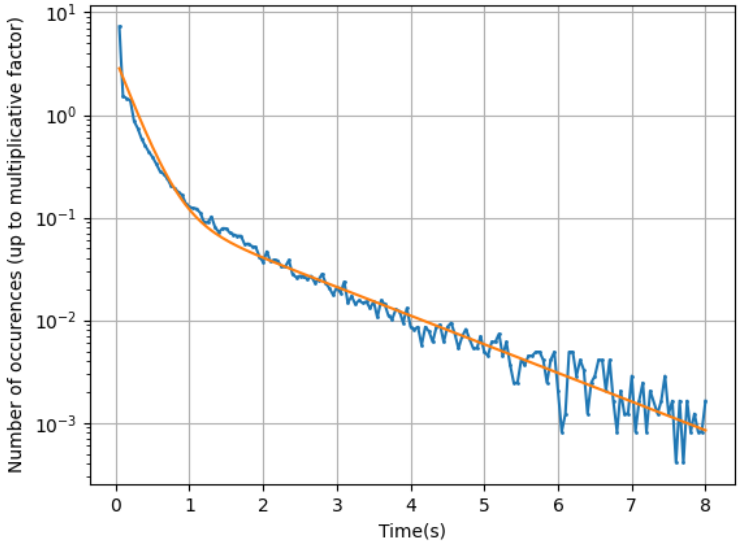


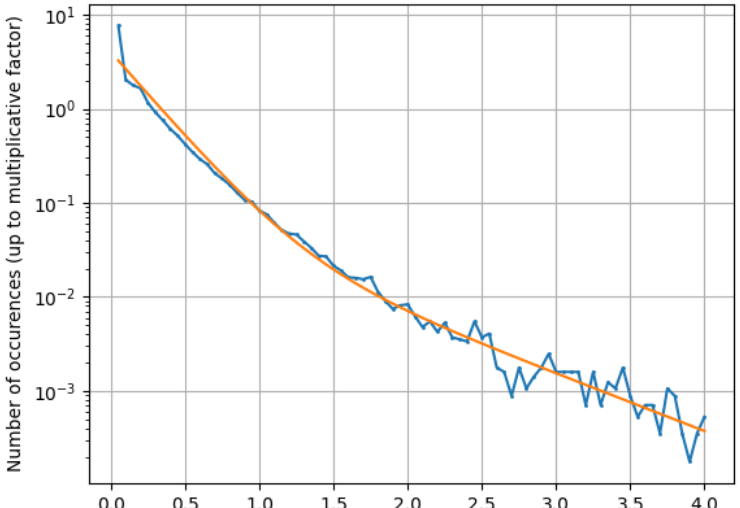


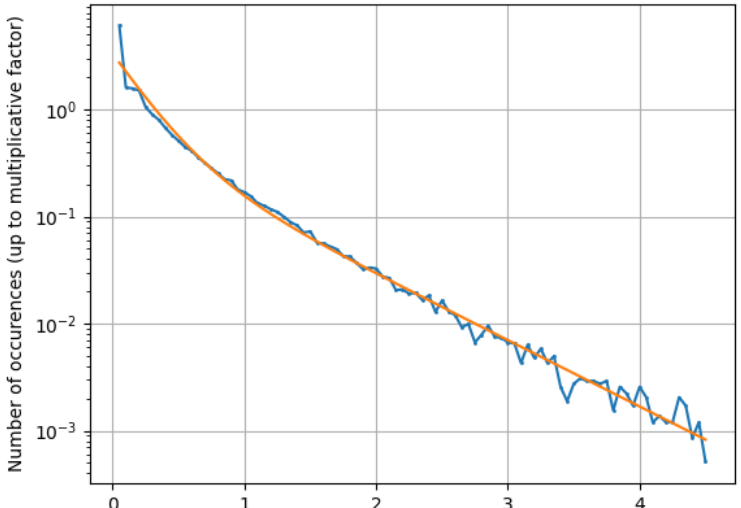
Mixture of two exponential populations

$$f(t) \propto p_1 k_1 e^{-k_1 t} + p_2 k_2 e^{-k_2 t}, \qquad p_1 + p_2 = 1.$$

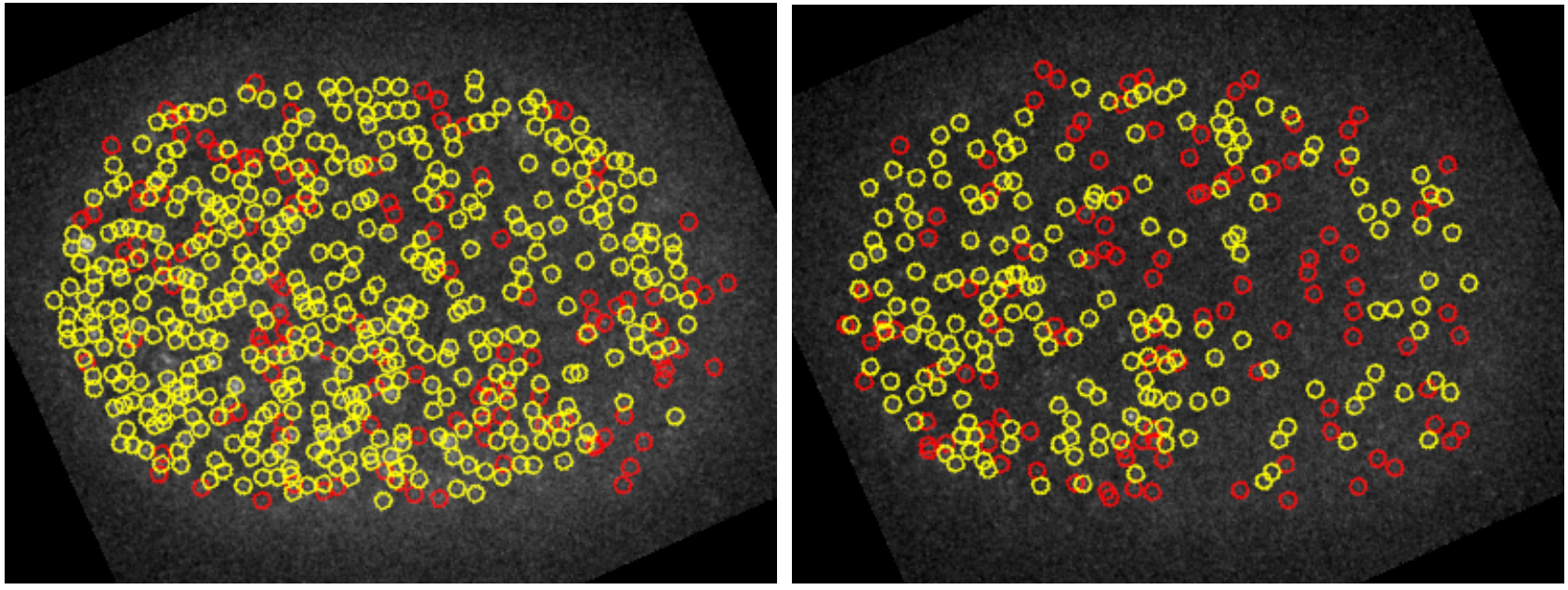








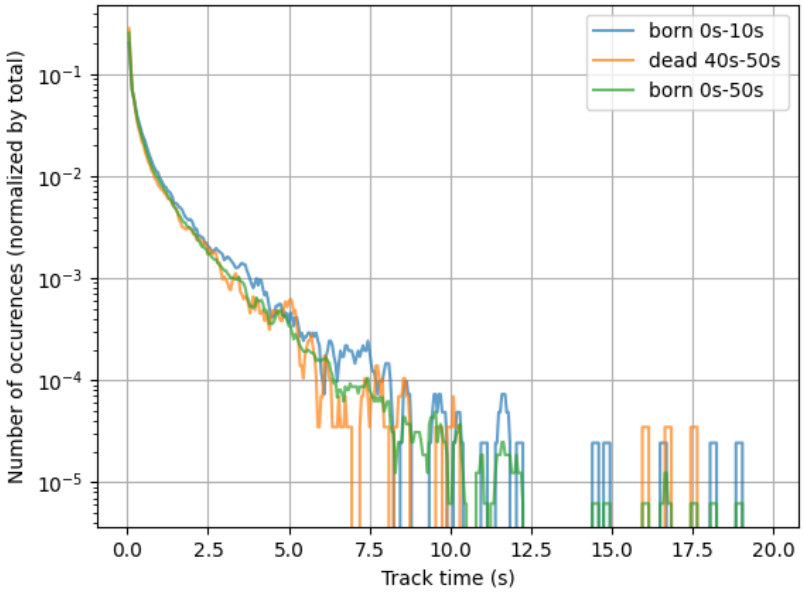
The two populations do not have different locations in the cell

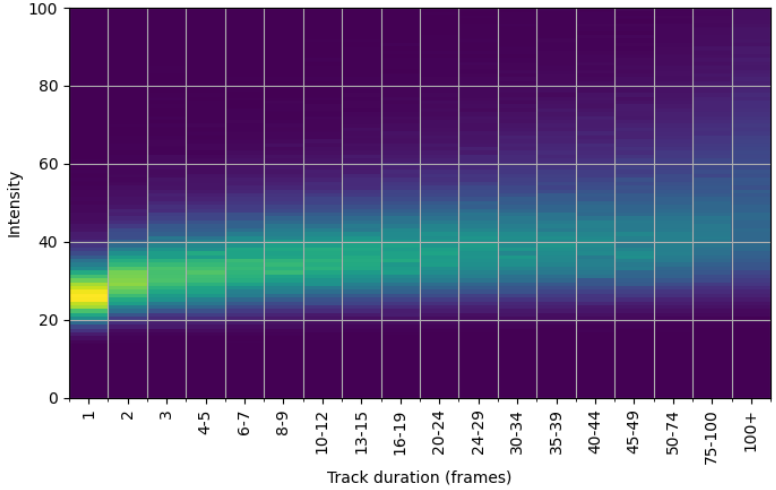


○ : Long tracks

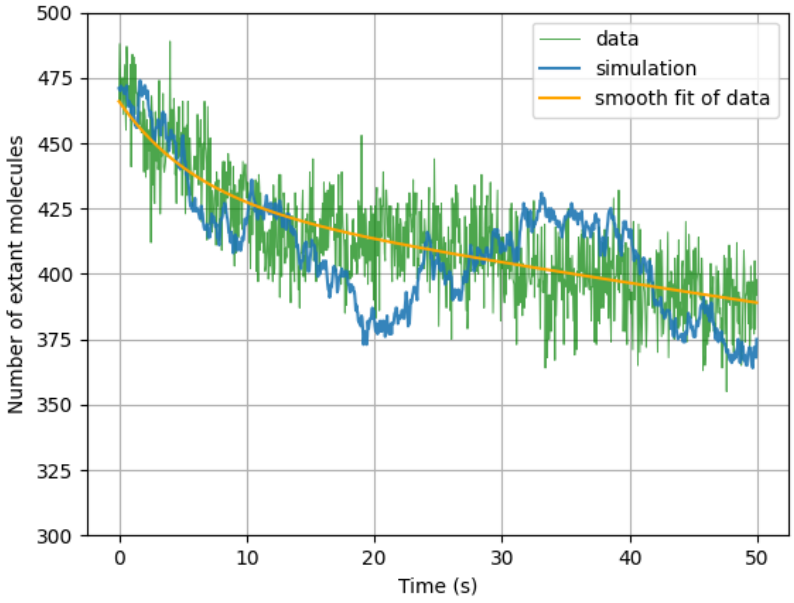
○ : Shorts tracks

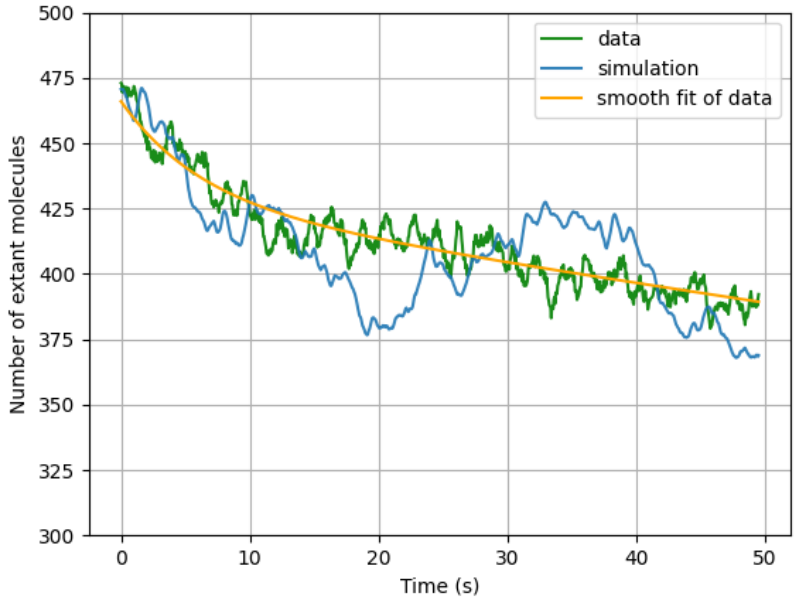
Track length is not affected by the density of measurement, but affects intensity



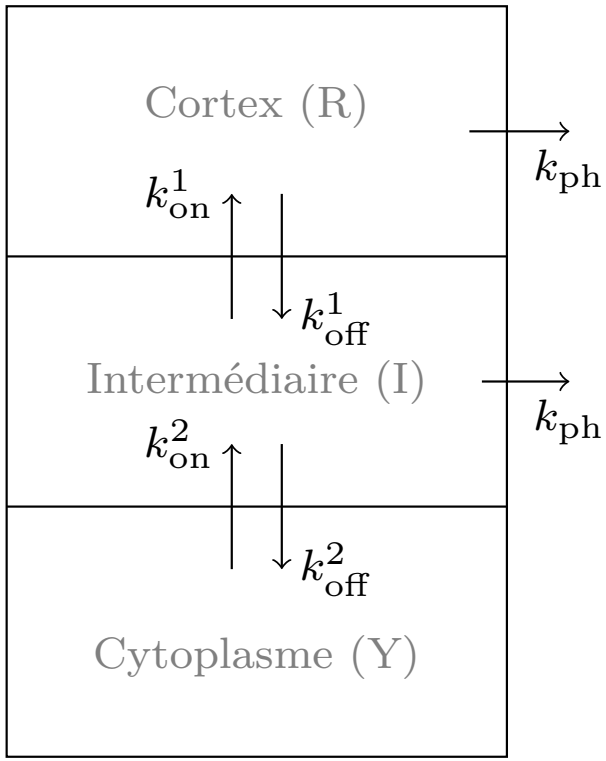


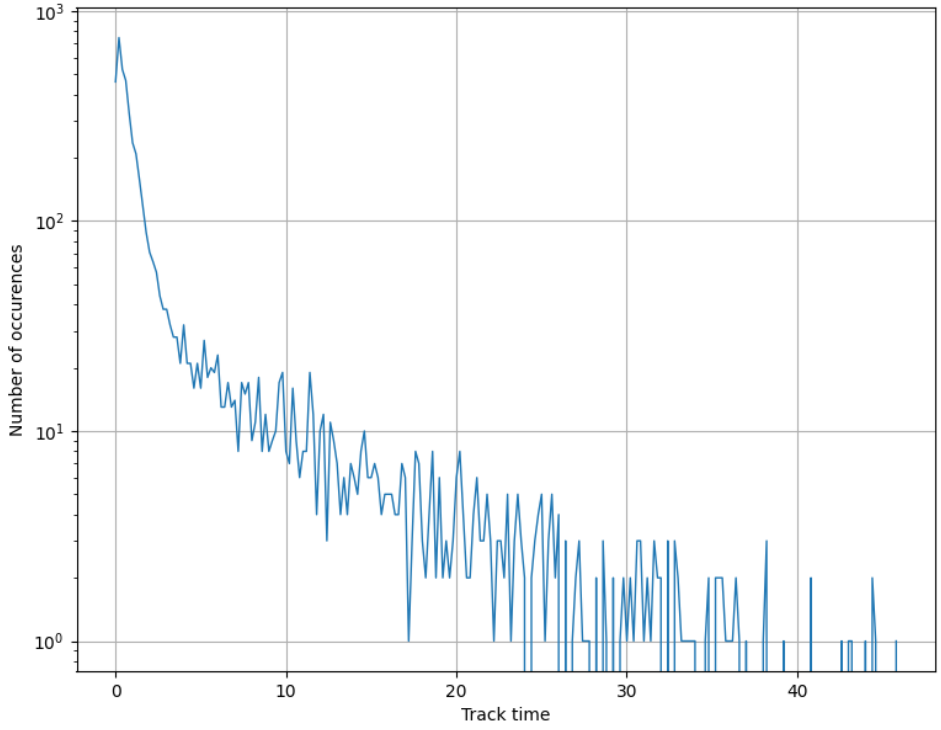
Simulations of 2-box model's fluctuations are higher than data's fluctuations





3 boxes model





Histogram of the track lengths

However, the model has too many parameters and I was not able to extract relevant informations about the parameters by fitting the curve.

Conclusion and perspectives

- We measured the turnover rates of ABP with three methods.
- The smPRESS estimate of the turnover rate is much more robust than the tracking estimate.
- We found a way to make the three estimations (FRAP, smPRESS, tracking) of the turnover coincide in the case of sub-diffusive molecules $\rightarrow$ By averaging the movies over sufficiently long periods of time.

- How to make the tracking estimate coincide with smPRESS for mobile molecules, such as formins ?
- Are there really two populations ? What do the two populations correspond to ? Are they really two distinct types of molecules or can the behavior be explained by e.g. the 3-boxes models ?
- How to explain that the fluctuations are higher in the simulation than in the data ?