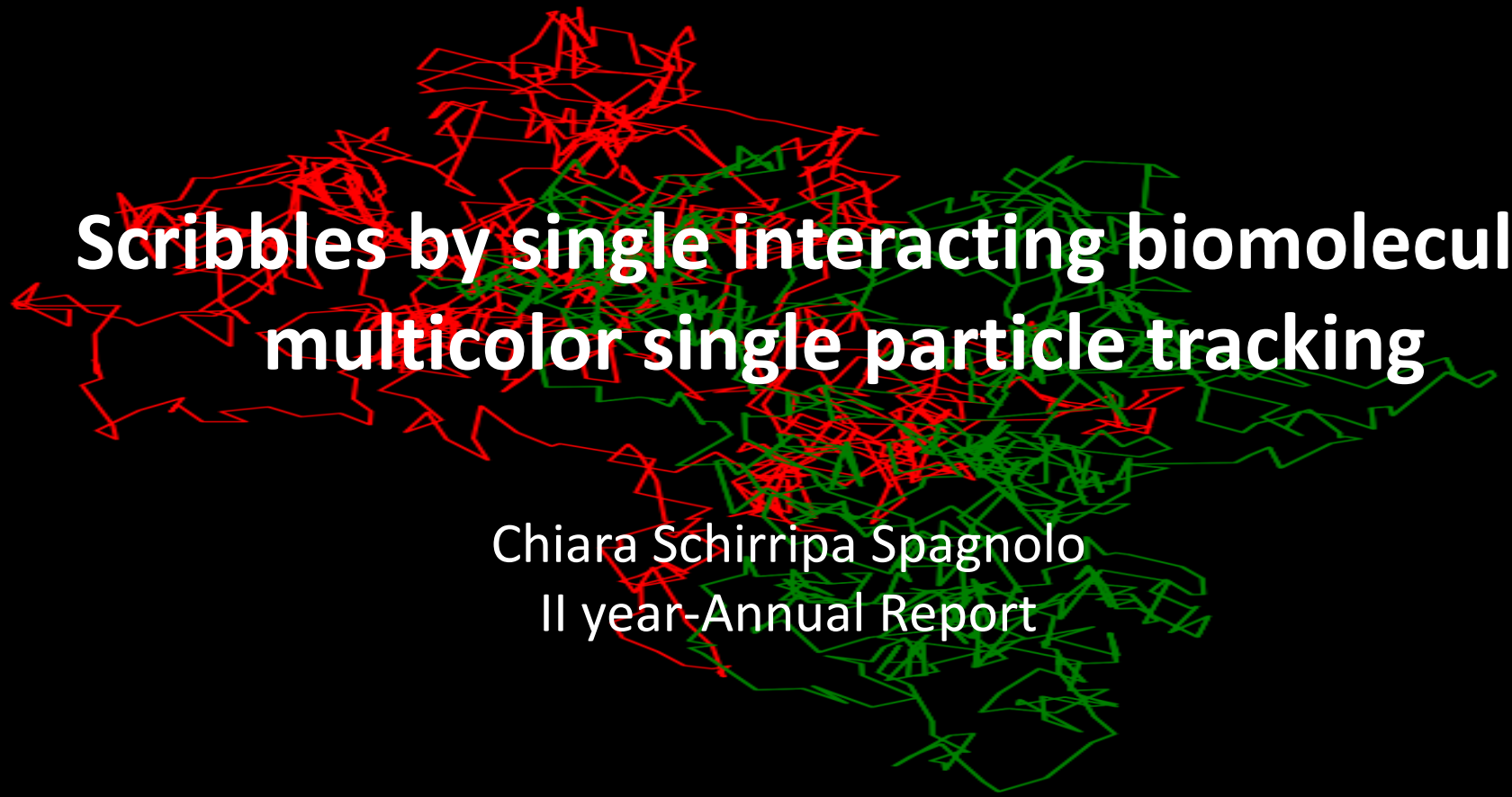


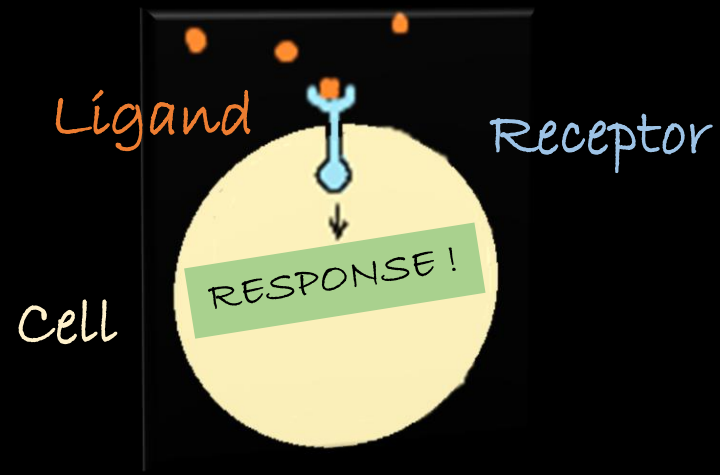
Scribbles by single interacting biomolecules: multicolor single particle tracking

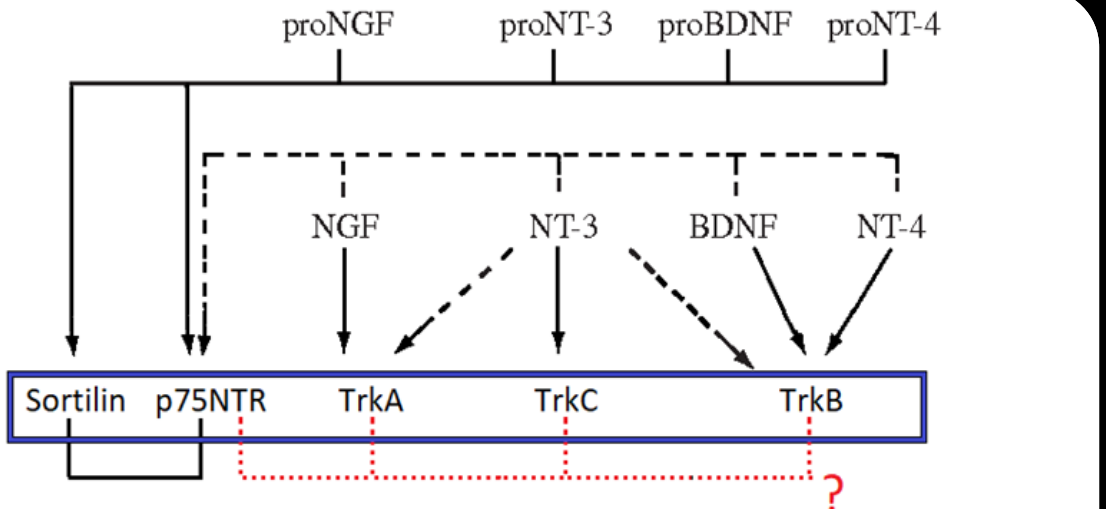
Chiara Schirripa Spagnolo

II year-Annual Report

THE STARTING QUESTIONS

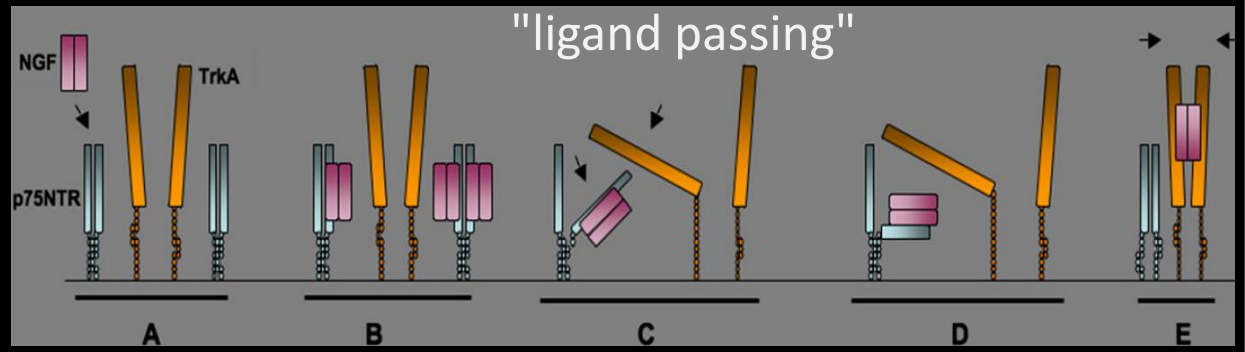
Neurotrophic signalling

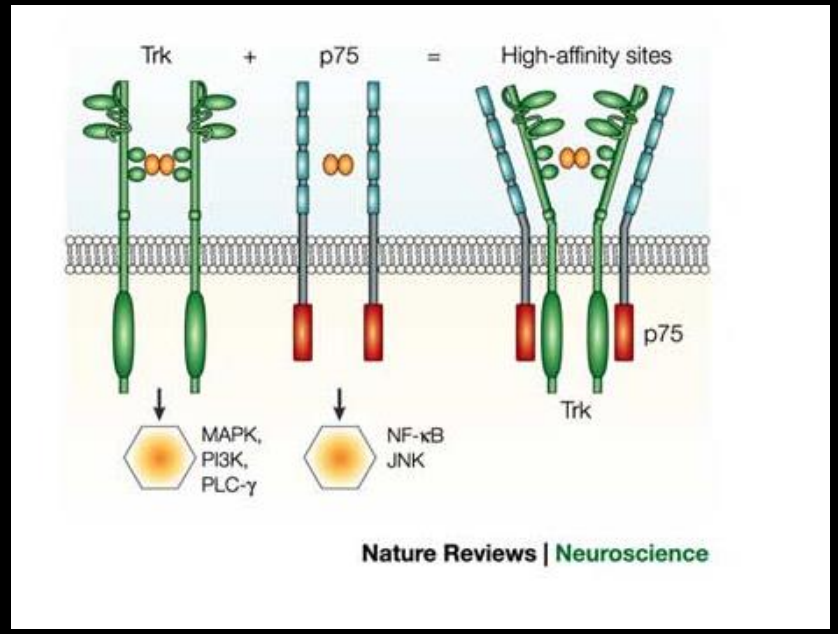




Survival / Differentiation / Proliferation / Synaptic plasticity / Apoptosis / Cell death

TrkA - p75 receptors interaction



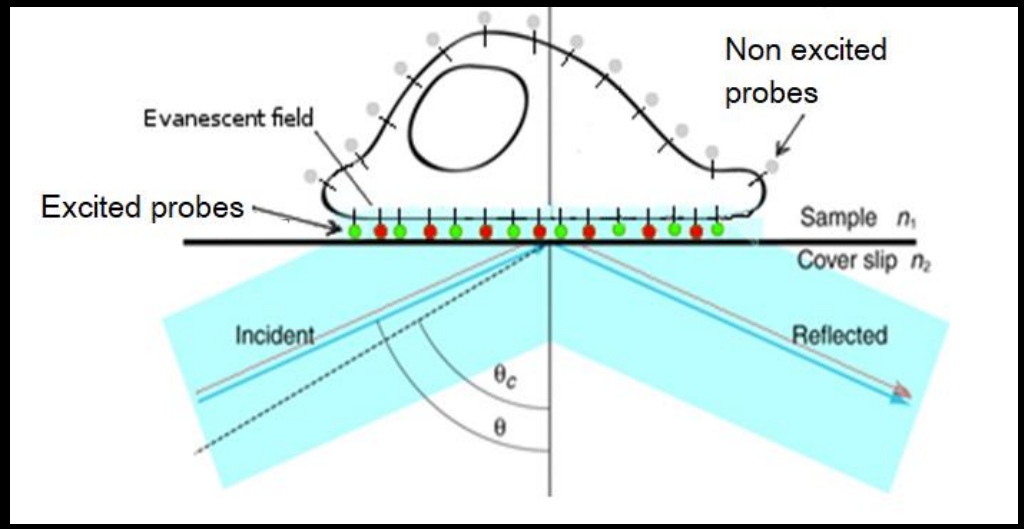


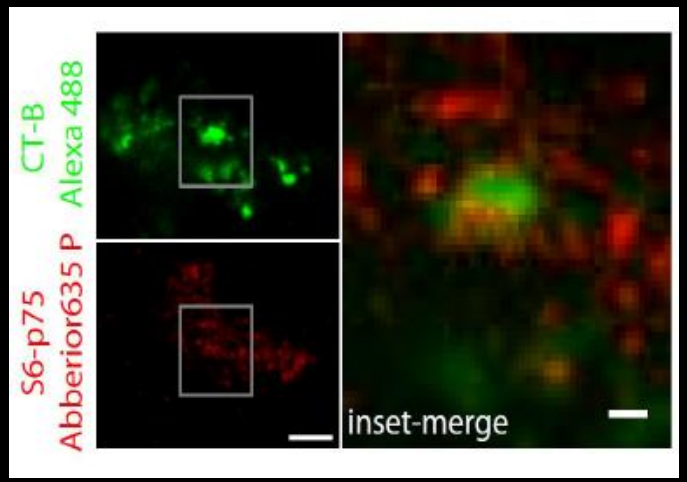
TWO-COLOUR TIRF MICROSCOPY

Laser combiner (488 and 635 nm)

Wide field epifluorescence microscope equipped with TIRF module

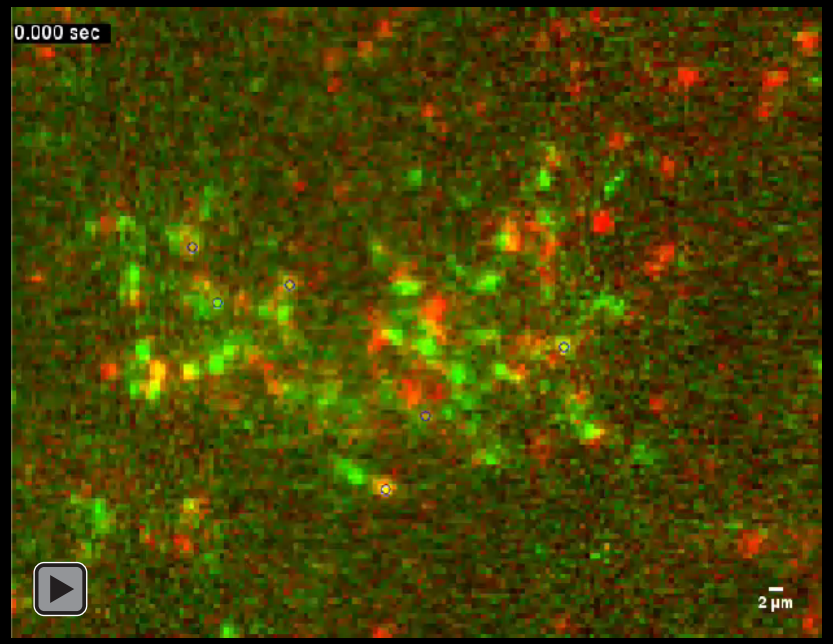
Optomask Dual View EMCCD Camera



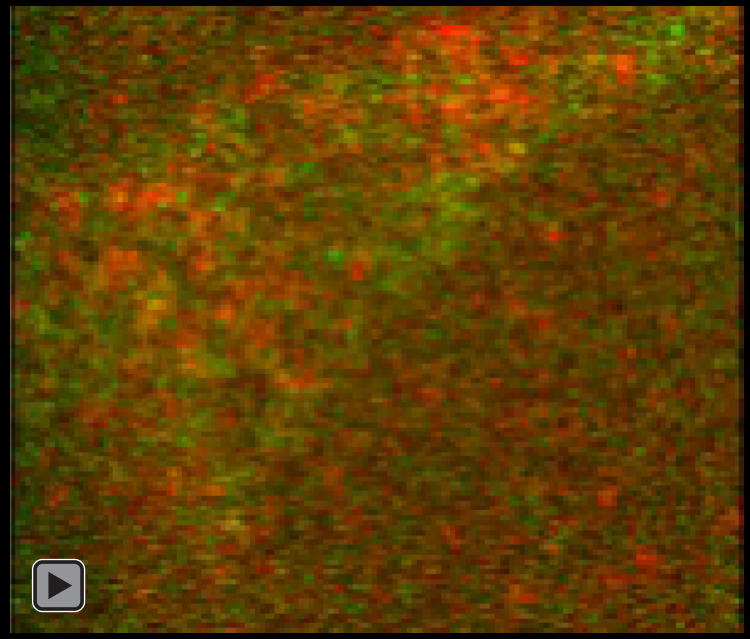


Marchetti et al. "Fast-diffusing p75NTR monomers support apoptosis and growth cone collapse by neurotrophin ligands." *Proceedings of the National Academy of Sciences* (2019)

TrkA

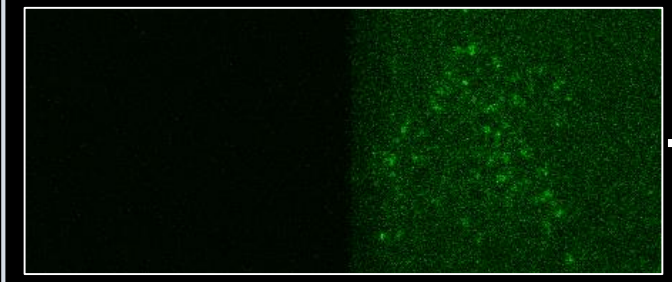


p75^{NTR}



REDESIGN OF THE SETUP

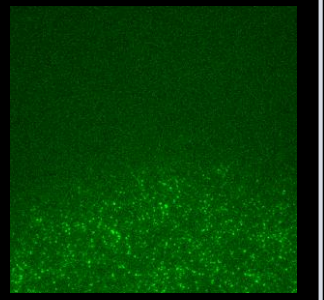
The struggle against the "WillCo-dish"



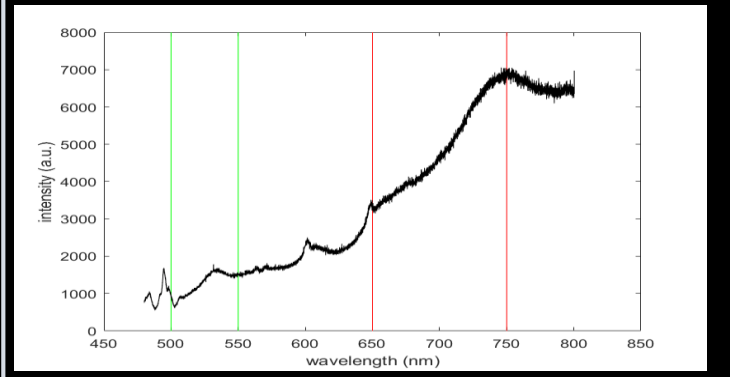
635 nm excitation

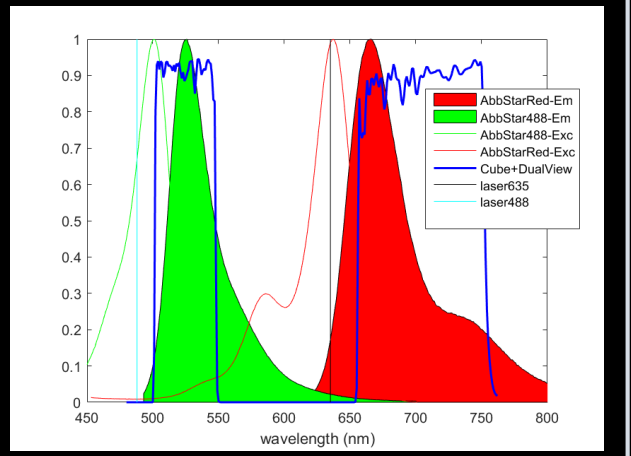
635 and 488 nm excitation

Quartz



No fluorescence and correct refraction index?
"To the best of my knowledge, such cover does not exist on the globe" (TIRF-labs)





REDESIGN OF THE SETUP

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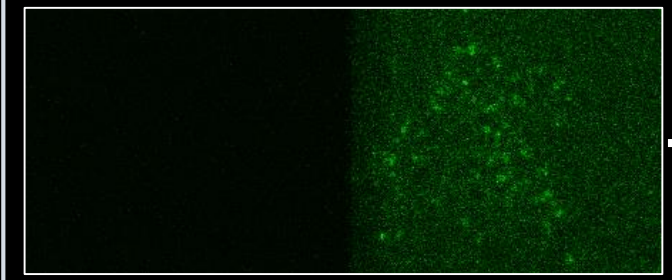
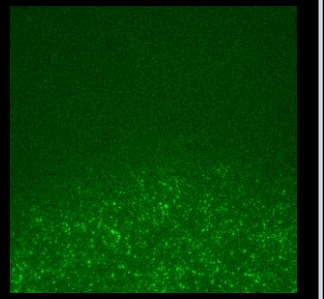


635 nm excitation

635 and 488 nm excitation

Quartz



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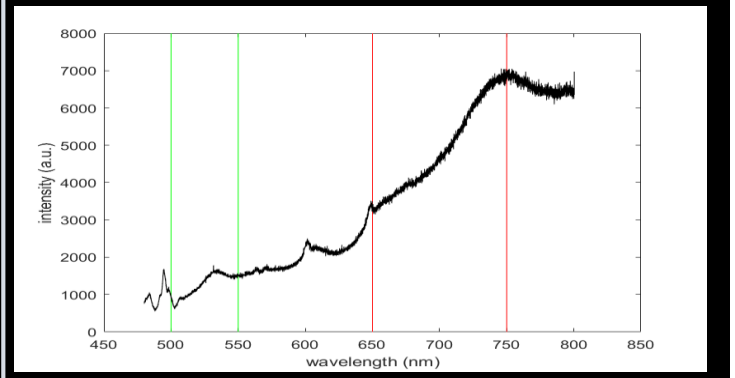
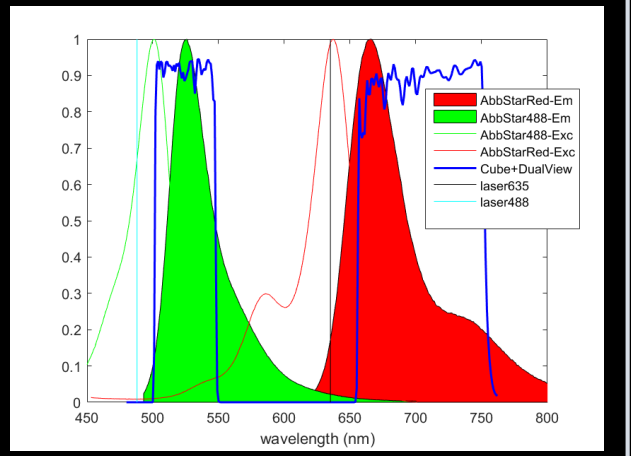
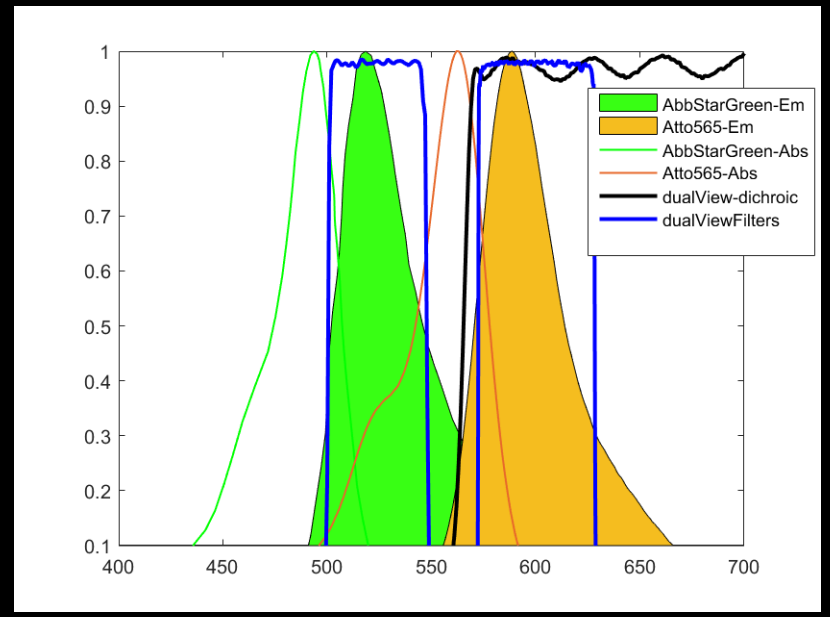
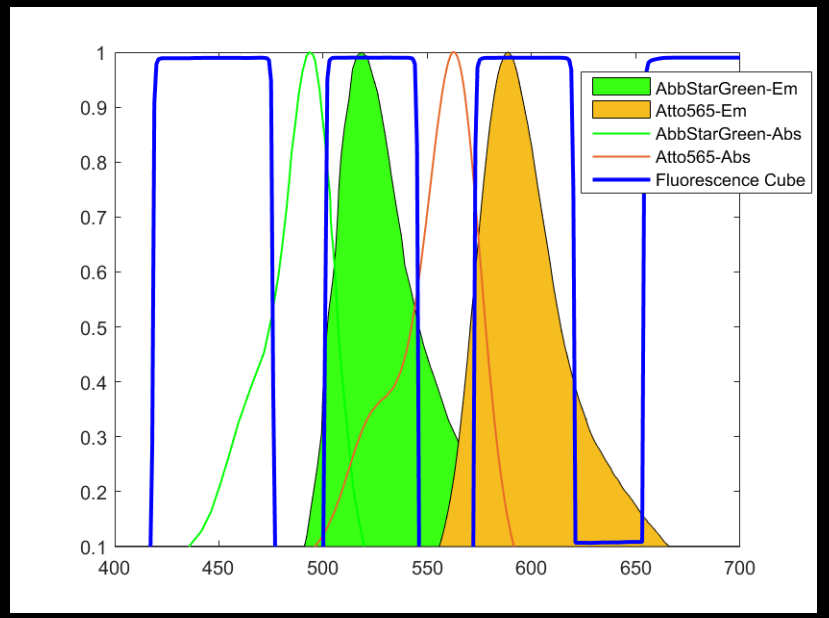
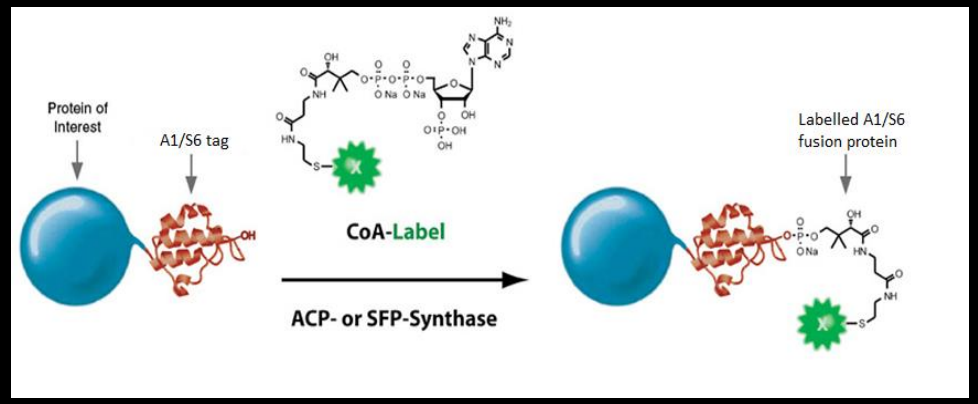


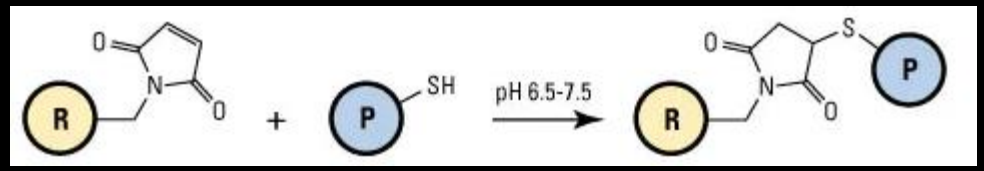






COENZYME A CONJUGATION OF THE NEW FLUOROPHORES





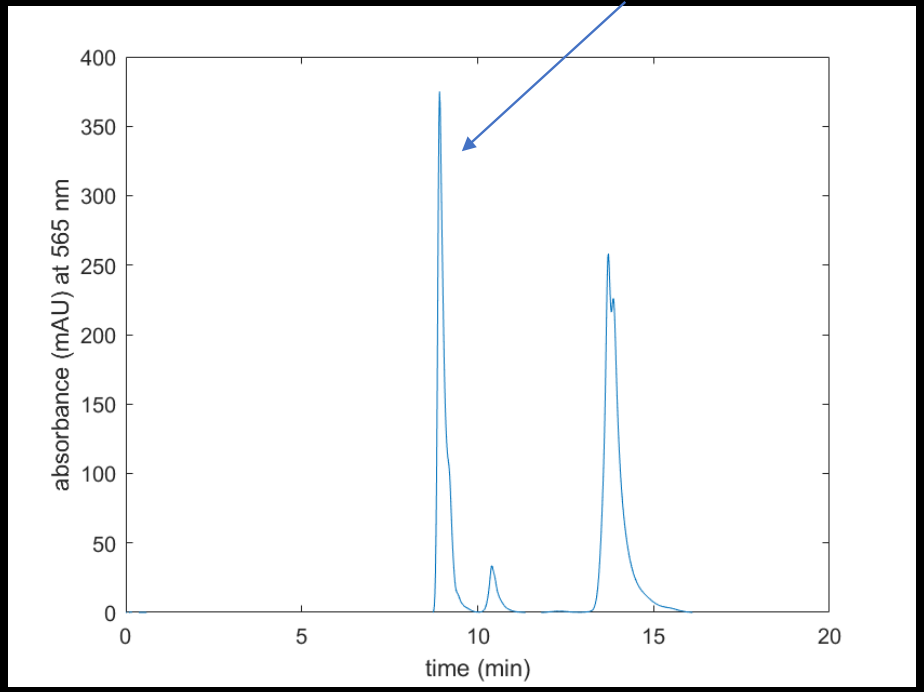
Maleimide fluorophore

Coenzyme-A (thiol)

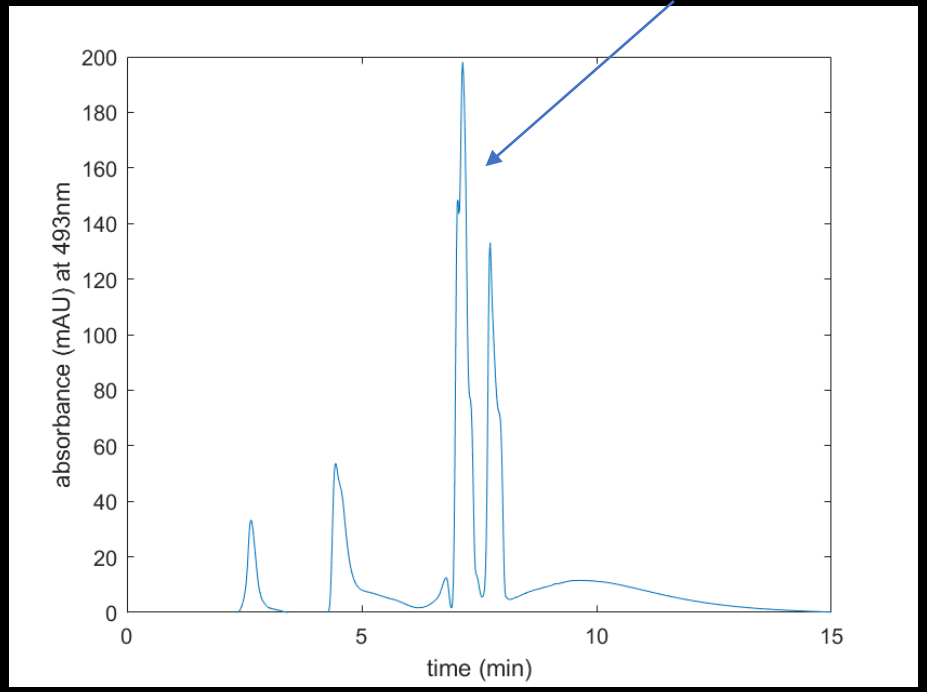
CoA-fluorophore

Purification by HPLC

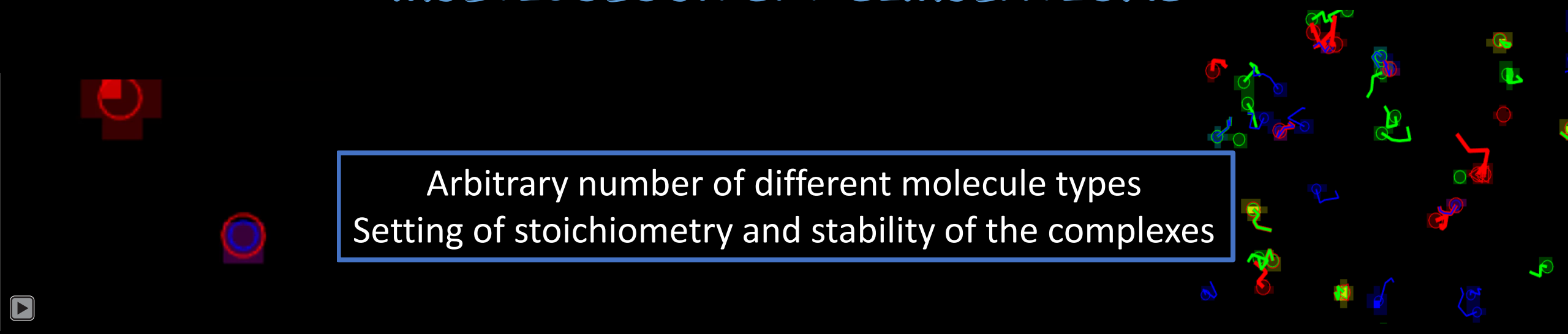
CoA-atto565



CoA-abberior star green

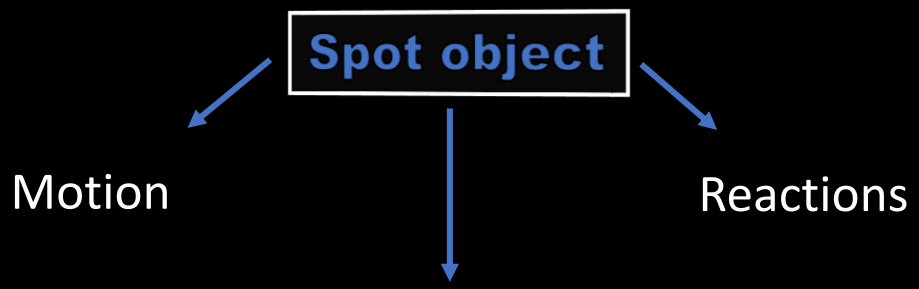


MULTICOLOUR SPT SIMULATIONS

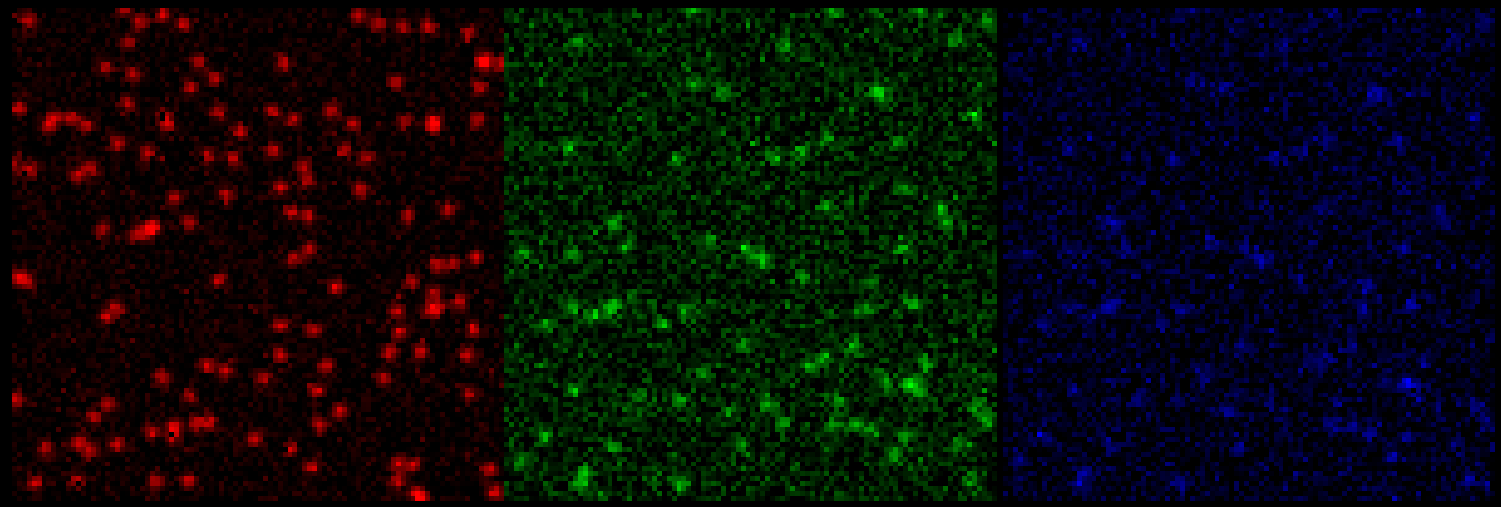


Arbitrary number of different molecule types
Setting of stoichiometry and stability of the complexes

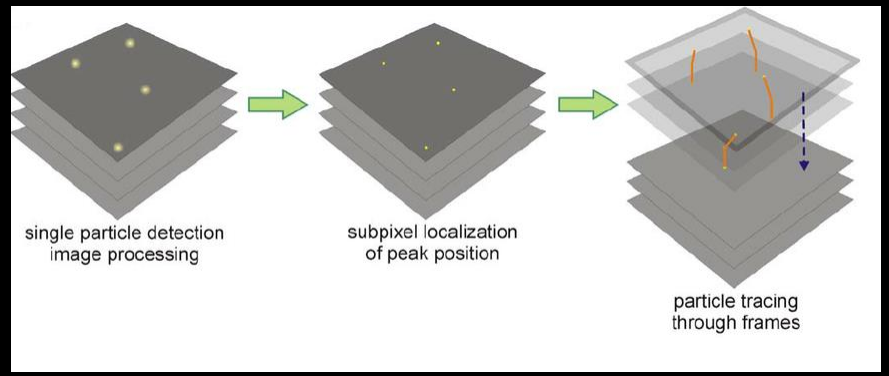
OOP in Python



Movie simulations (different S/N, PSF, ...)

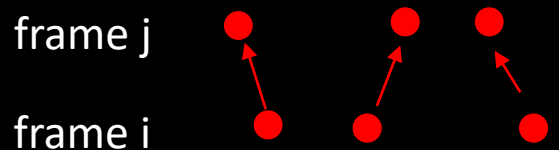


TRACKING SOFTWARE

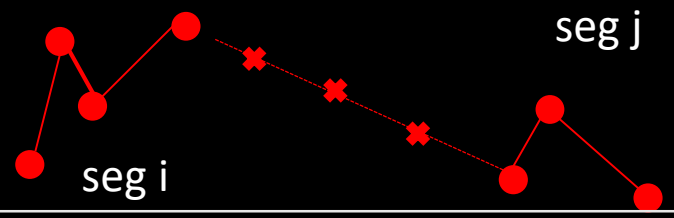


"Utrack" implementation

1. Linking between consecutive frames

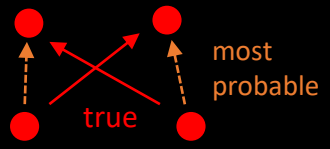


2. Gap closure between segments and merge and split

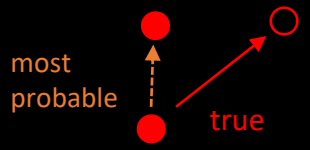


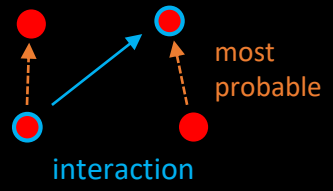
The enemies of tracking

Crossing tracks

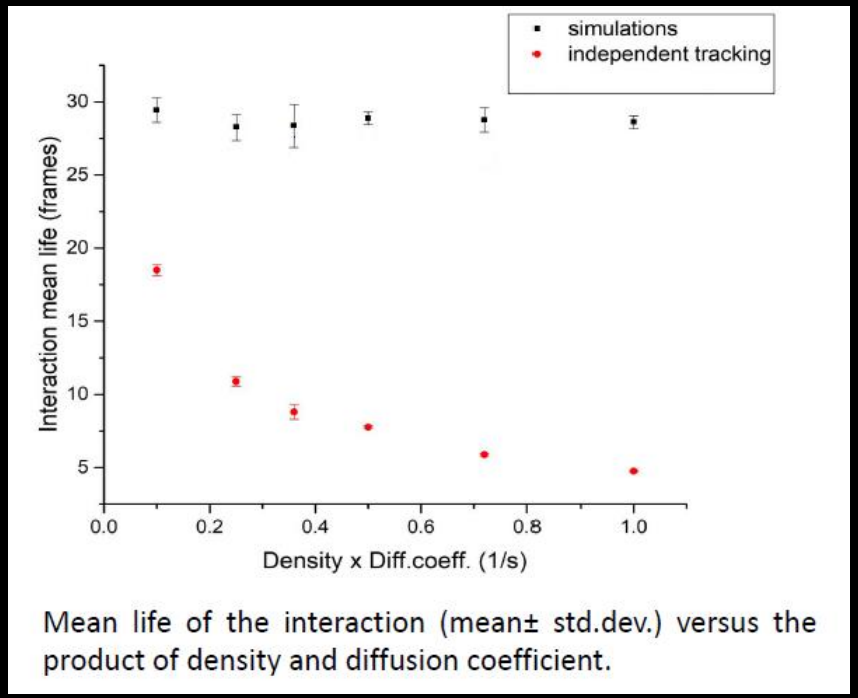


Missed detections





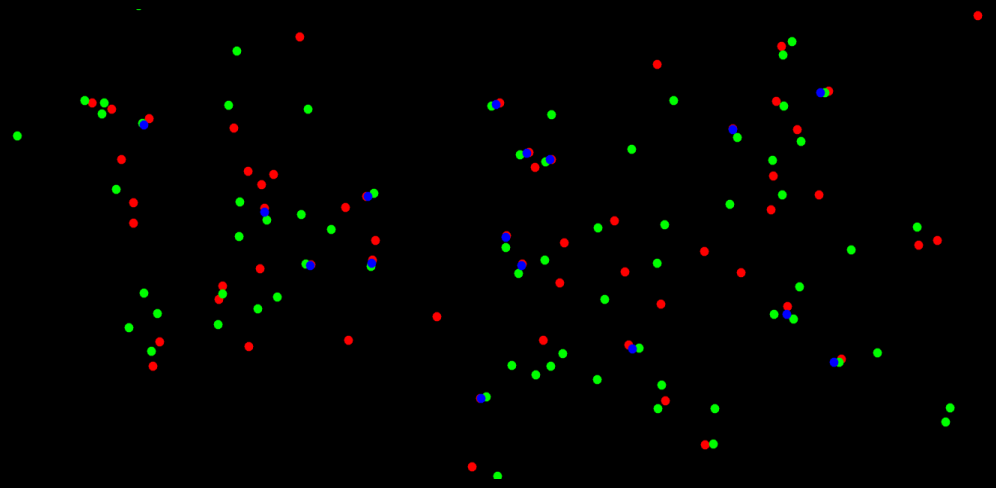
Interaction broken with most probable linkings!



Mean life of the interaction (mean± std.dev.) versus the product of density and diffusion coefficient.

MESSAGE PASSING PARALLEL LINKING

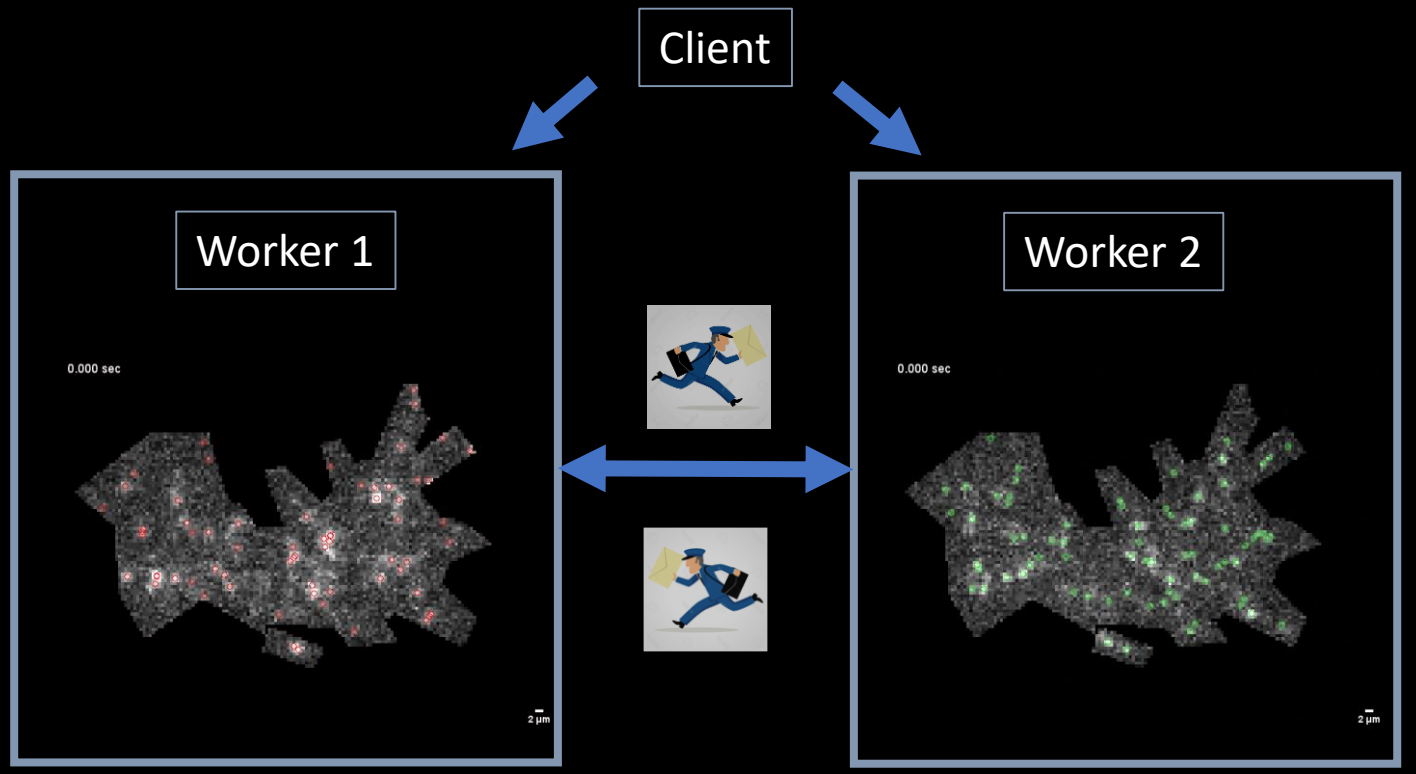
Search for colocalizations



Exhaustive Searcher with custom distance:

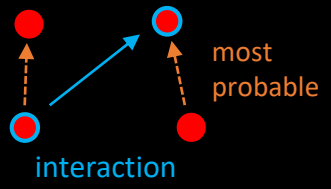
$$d = \frac{(x_G - x_R)^2 + (y_G - y_R)^2}{2.54^2(\sigma_{G_x}{}^2 + \sigma_{G_y}{}^2 + \sigma_{R_x}{}^2 + \sigma_{R_y}{}^2)} < 1$$

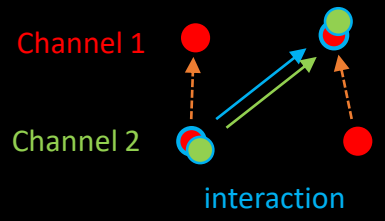
MatLab SPMD: single program multiple data

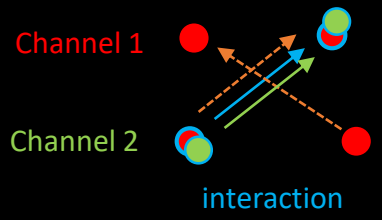


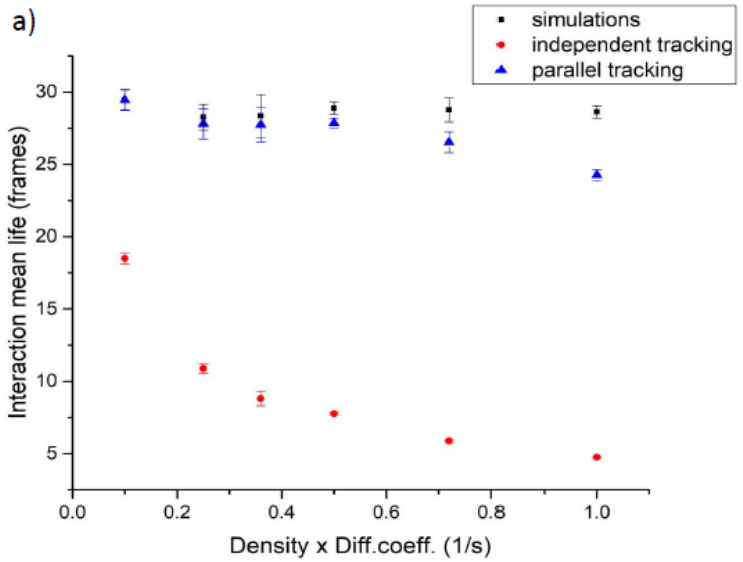
LabSendReceive:
explicit managment of communication

MESSAGE PASSING PARALLEL TRACKING

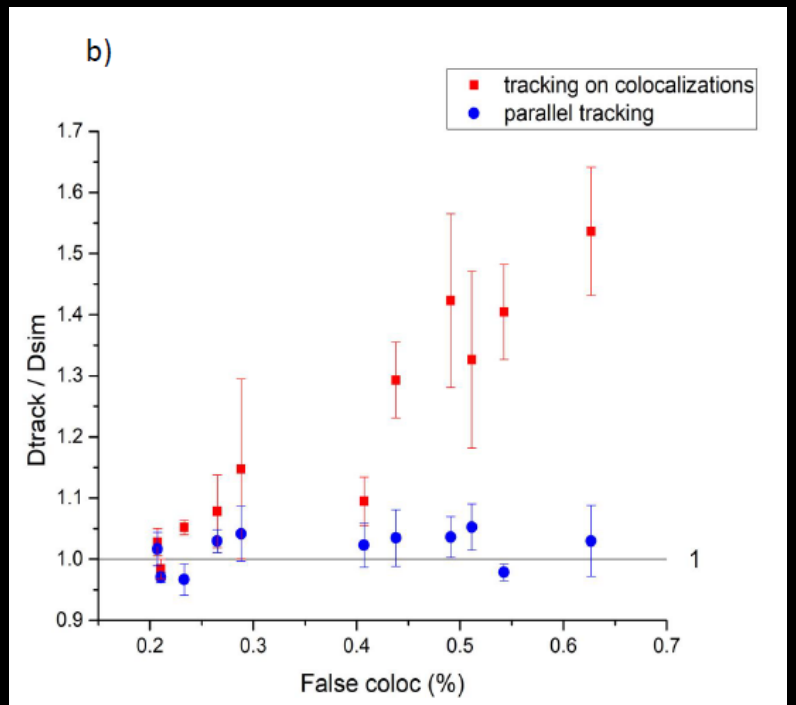






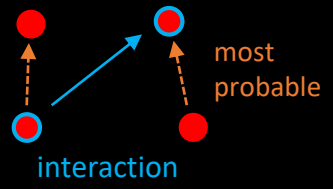


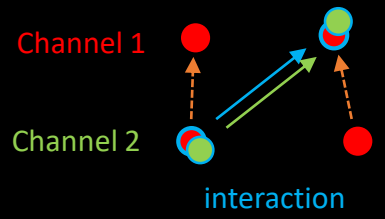
Mean life of the interaction (mean± std.dev.) versus the product of density and diffusion coefficient.

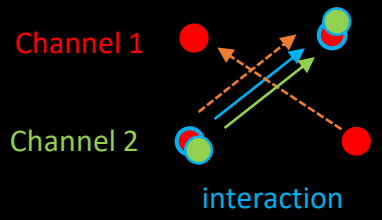


Ratio of the diffusion coefficient estimate from tracking and that resulting from simulations (mean± std.dev.) versus percentage of false colocalization.
Localization uncertainty varied form 20 to 50 nm; other simulations parameters varied to have different percentage of true colocalizations.

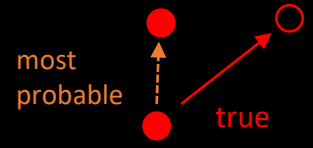
MESSAGE PASSING PARALLEL TRACKING

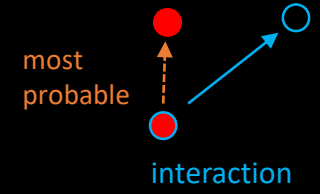


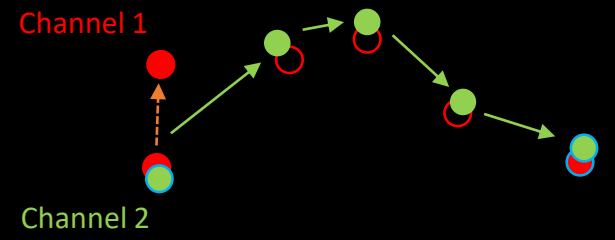


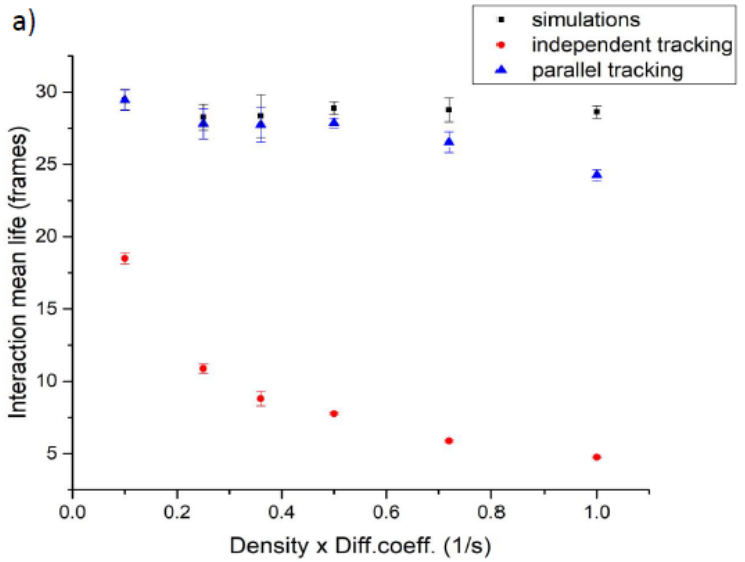


Correction of missed detections









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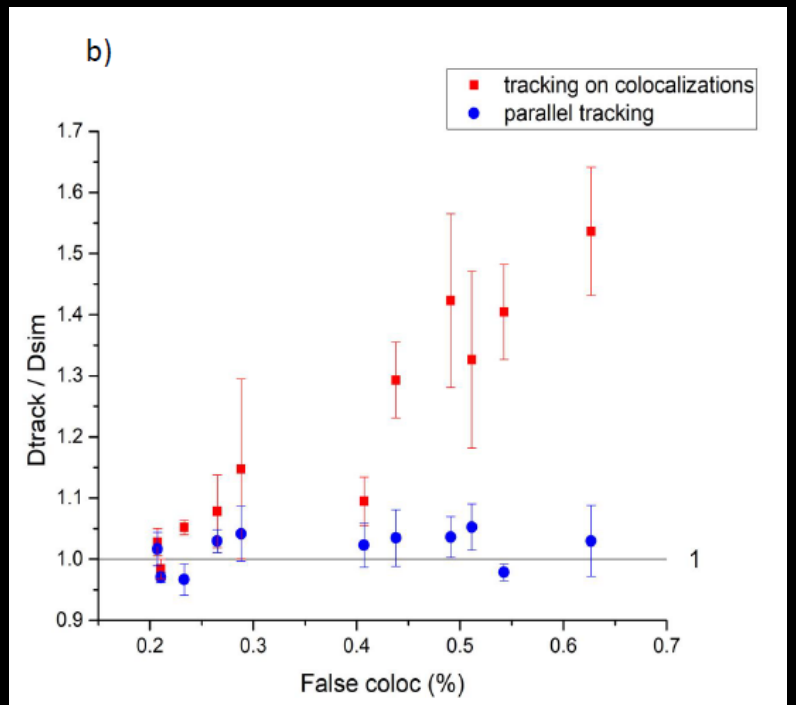

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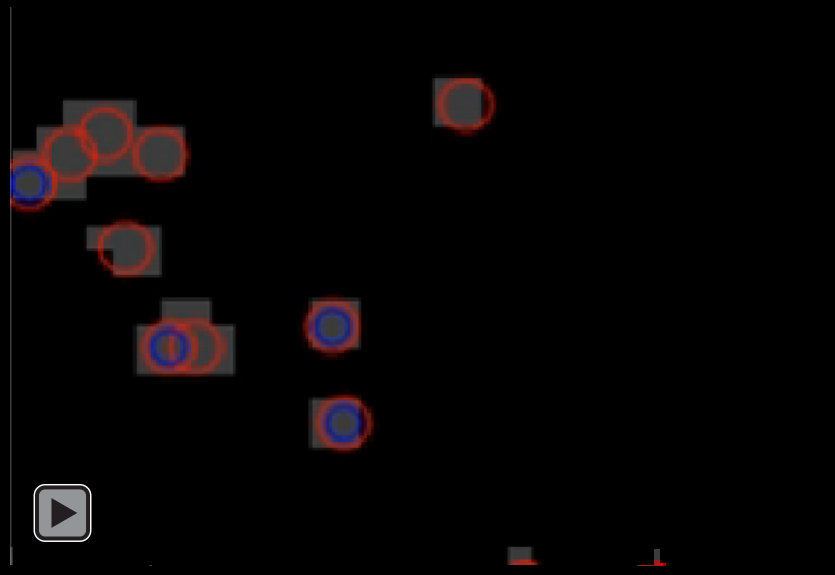
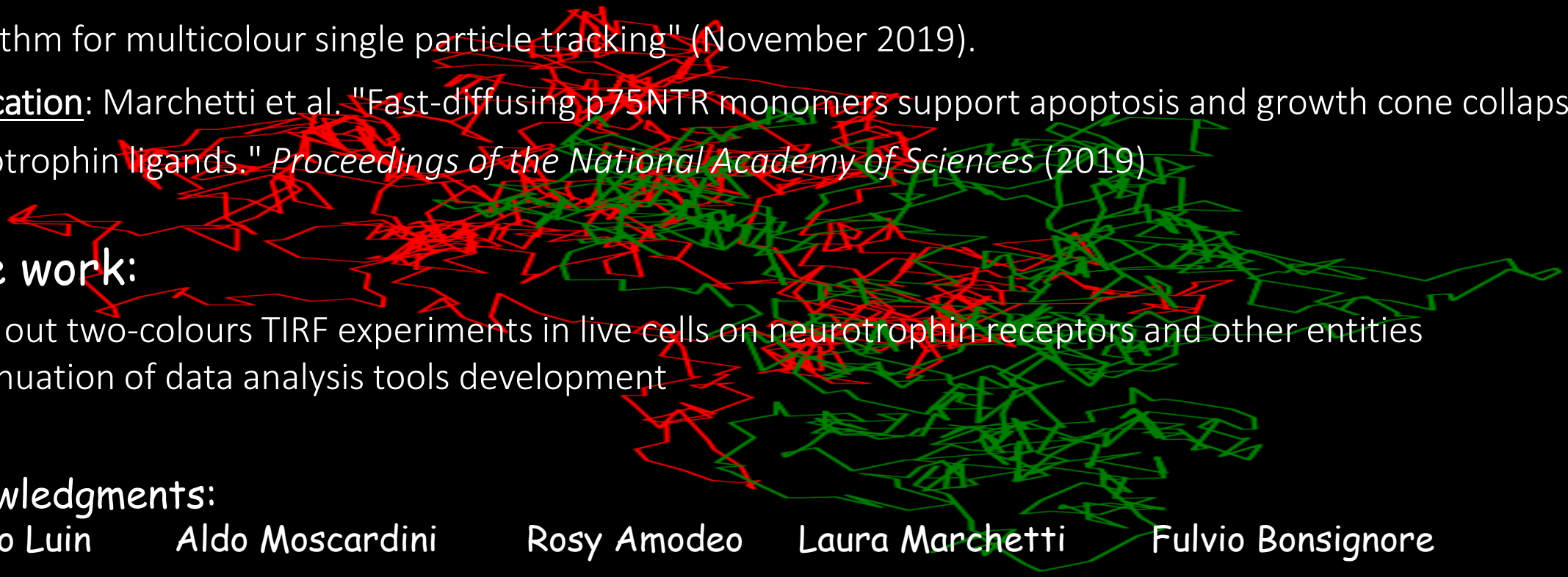


Other activities:

- NEST Meeting - Highlights in Nanoscience. 10 – 11 giugno 2019. **Poster presentation** : “Dynamics and interactions of neutrophin receptors investigated by single-molecule tracking”.
- Seminar cycle- Biophysical Sciences (**exam** on 25/10/19).
- **Scuola** di calcolo scientifico con MATLAB 2019. Corso avanzato. Strumenti e tecniche Matlab per il calcolo parallelo, l’apprendimento automatico e l’analisi massiva dei dati. Palermo, 29 luglio – 2 agosto 2019.
- **Oral presentation** at SPAOM19 - Spanish & Portuguese Advanced Optical Microscopy: "Message passing paralle algorithm for multicolour single particle tracking" (November 2019).
- **Publication**: Marchetti et al. "Fast-diffusing p75NTR monomers support apoptosis and growth cone collapse by neurotrophin ligands." *Proceedings of the National Academy of Sciences* (2019)

Future work:

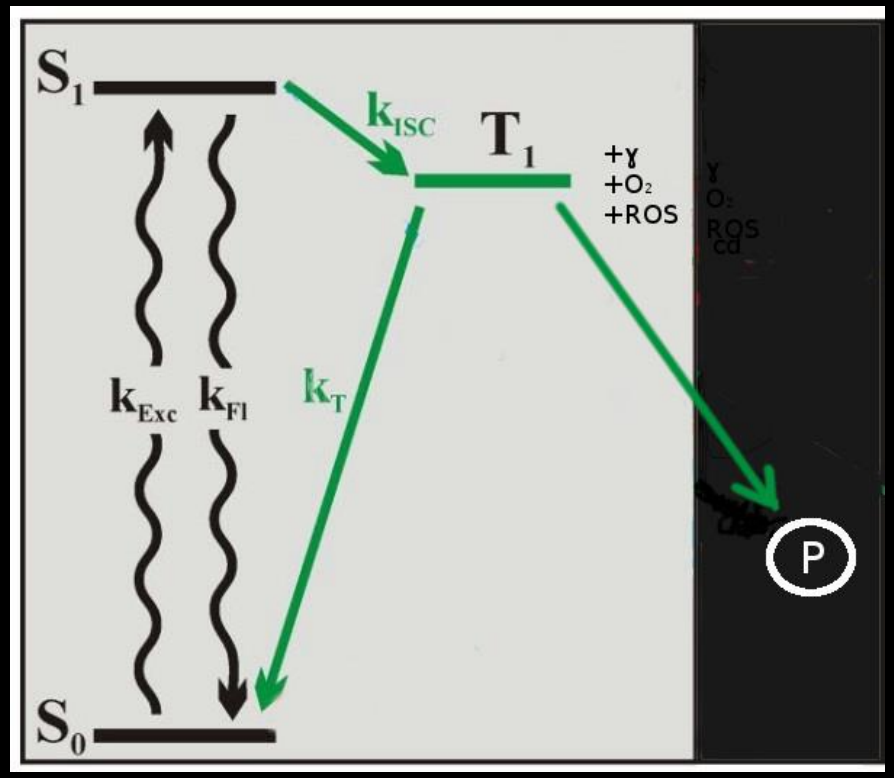
- Carry out two-colours TIRF experiments in live cells on neurotrophin receptors and other entities
- Continuation of data analysis tools development



Acknowledgments:

Stefano Luin Aldo Moscardini Rosy Amodeo Laura Marchetti Fulvio Bonsignore

Fenomeno di photobleaching



Sistema ROXS (reducing and oxidizing system)

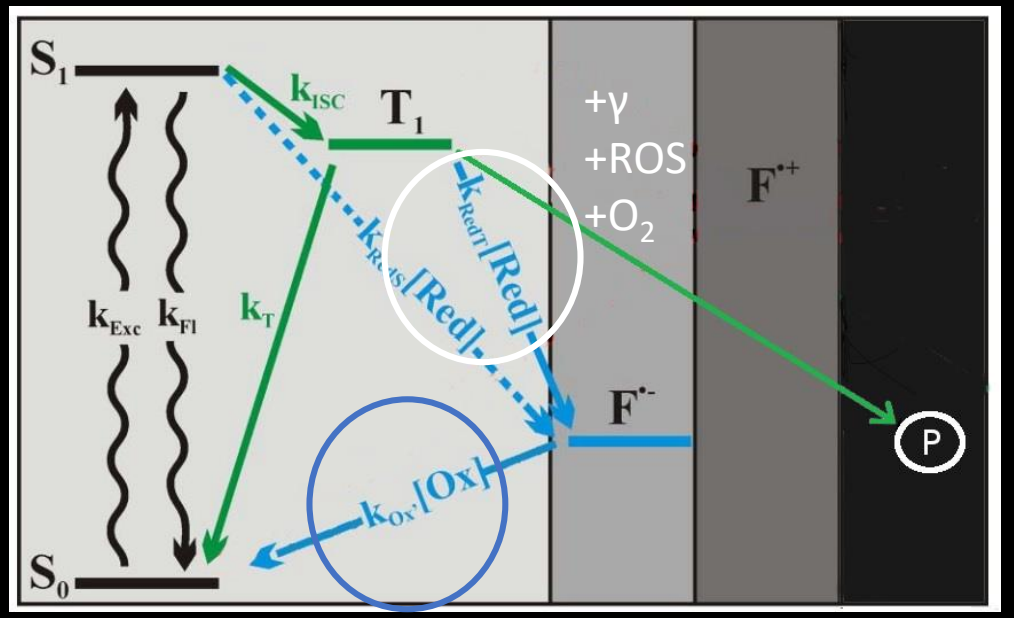
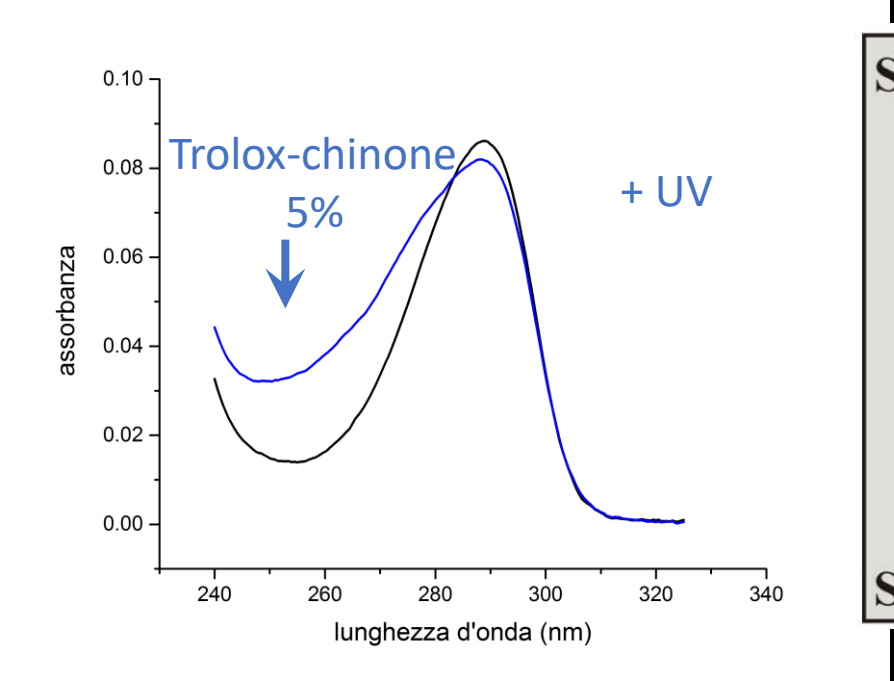


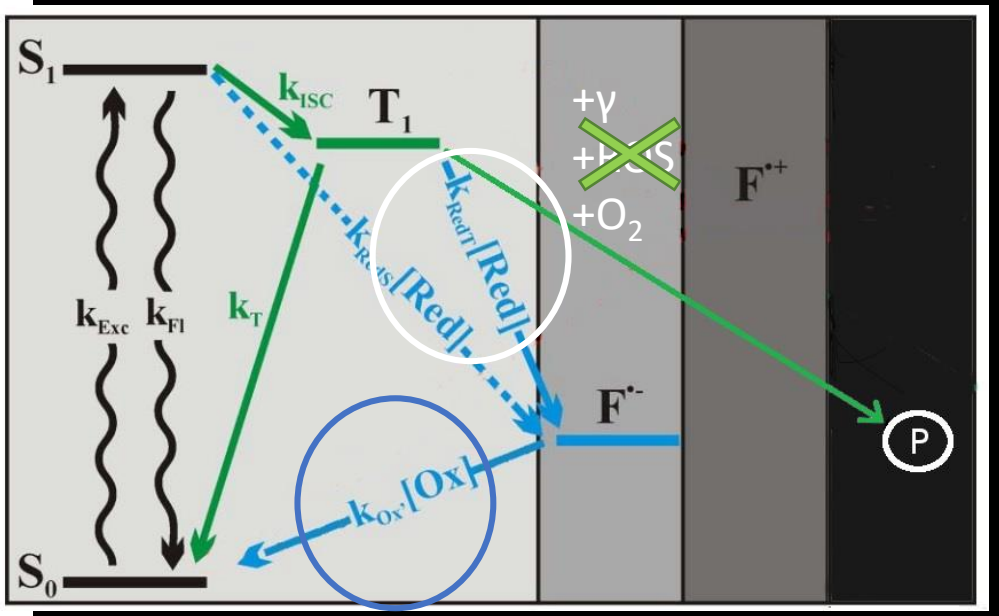
Figure modificate da Vogelsang et al, *Proceedings of the National Academy of Sciences* (2009)

Fenomeno di photobleaching

Trolox (1mM)



Sistema ROXS (reducing and oxidizing system)



n-propyl gallato (NPG, 5 µM)