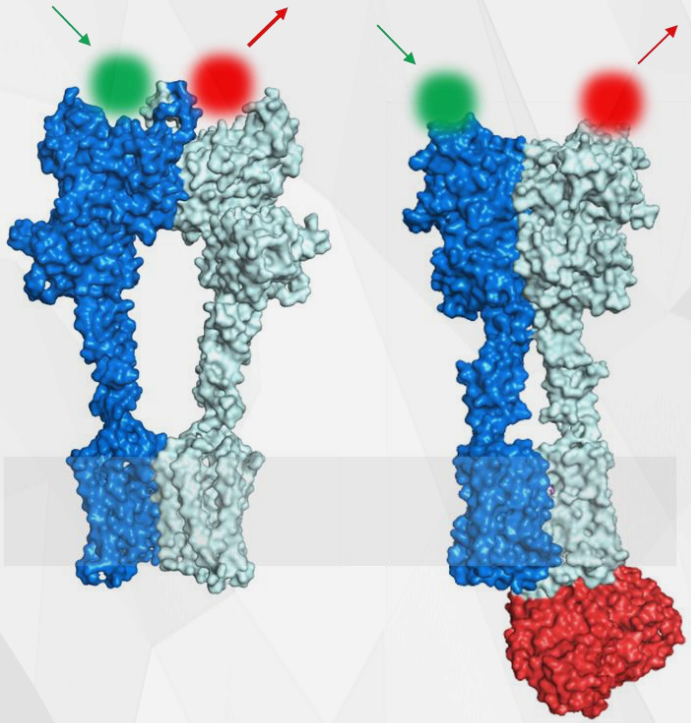
INTRODUCTION TO SINGLE MOLECULE FRET



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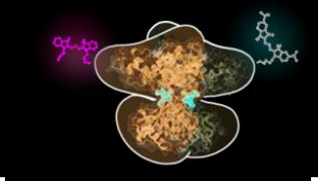
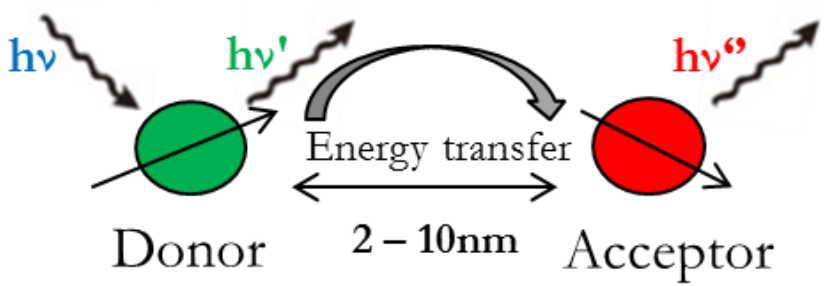
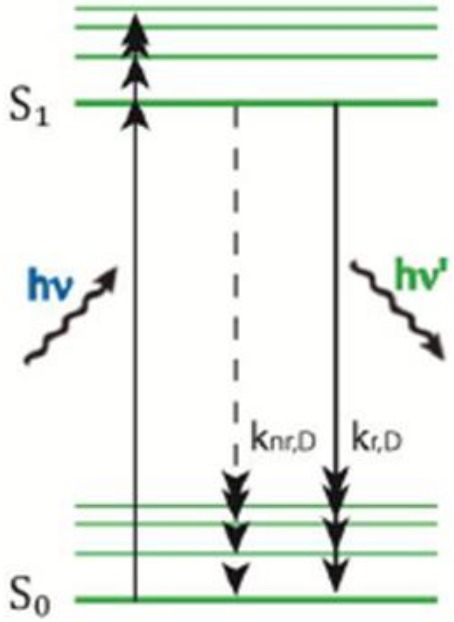
FRET: Förster Resonance Energy Transfer





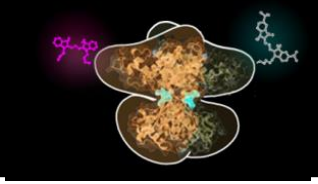
$$FRET(PR) = \frac{Red\ signal}{Green\ signal\ + Red\ signal}$$



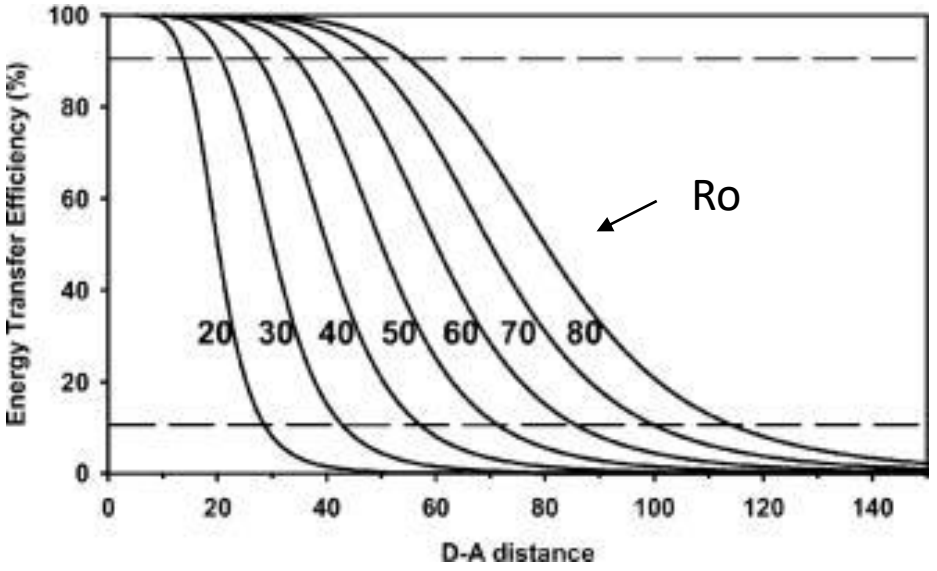
$$\tau_D = \frac{1}{k_{nr} + k_r}$$

- ✓ Informs on the distance separating two fluorescent dyes
- ✓ Ratiometric FRET efficiency (PR)
- ✓ $\tau_{D(A)}$ decreases when FRET increases

FRET: Förster Resonance Energy Transfer

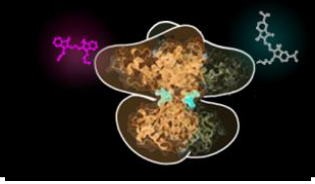


$$FRET = \frac{R_0^6}{R_0^6 + R^6}$$

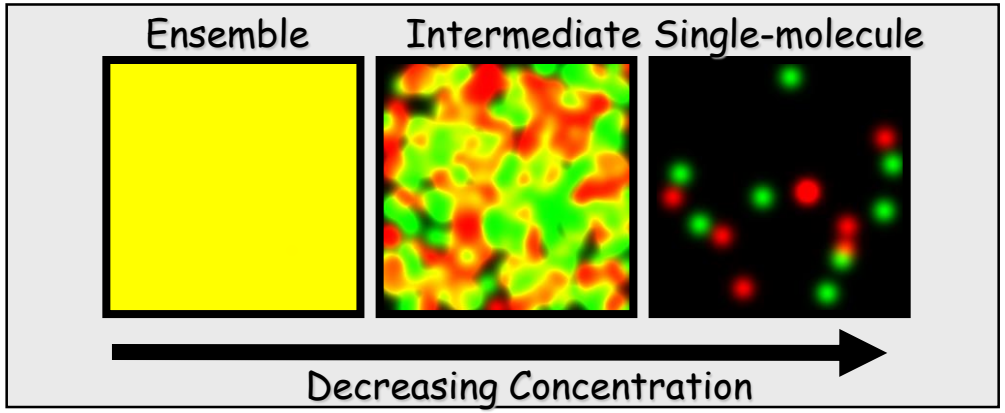


- ✓ FRET can be used as a ruler
- ✓ Very sensitive to distance changes around Ro
- ✓ An accurate FRET measurement can report on distance change, down to the Angstroms and nanosecond timescales

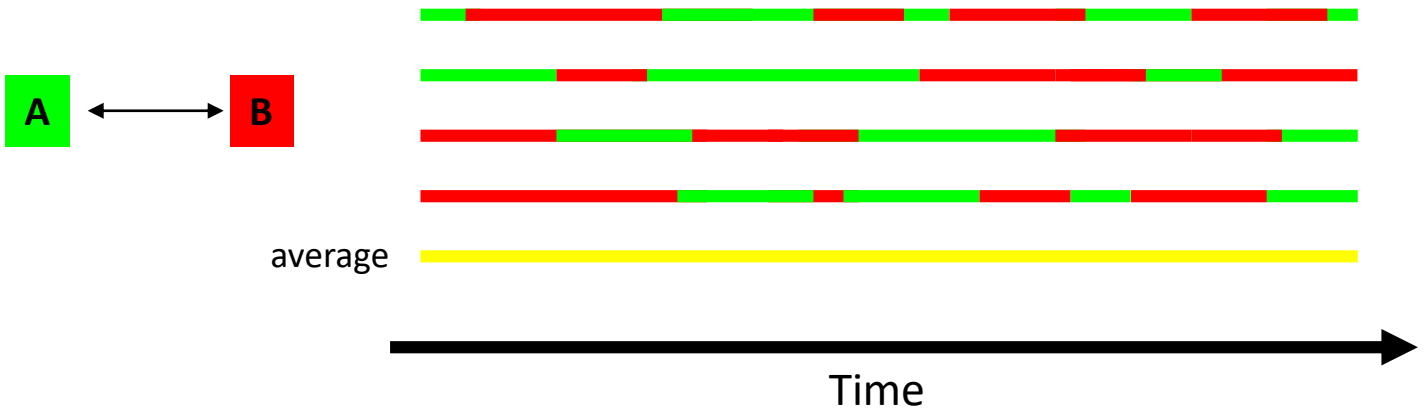
Why do we study single molecules ?



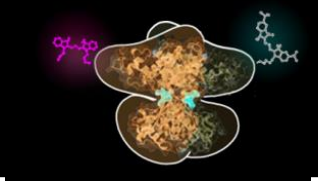
- Looking at ensemble of molecules generally leads to average values
- In particular, you cannot see static heterogeneities, i.e. the presence of subpopulations :

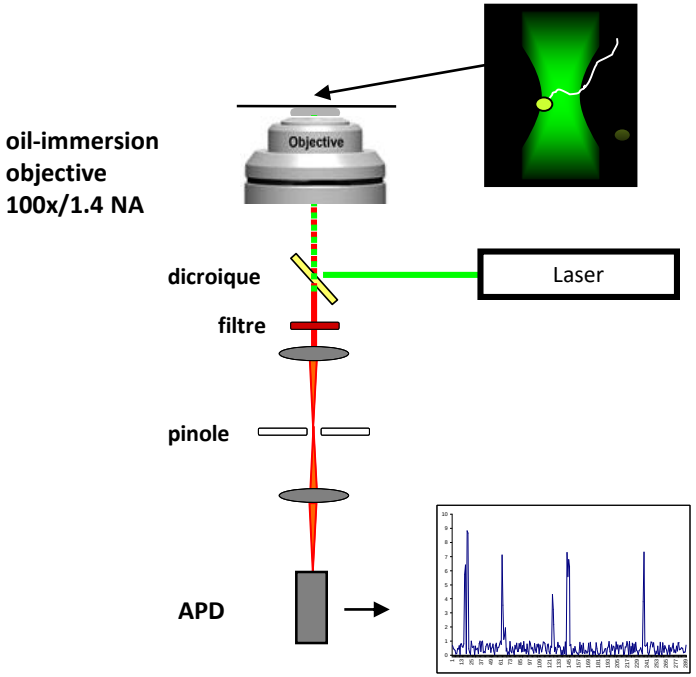


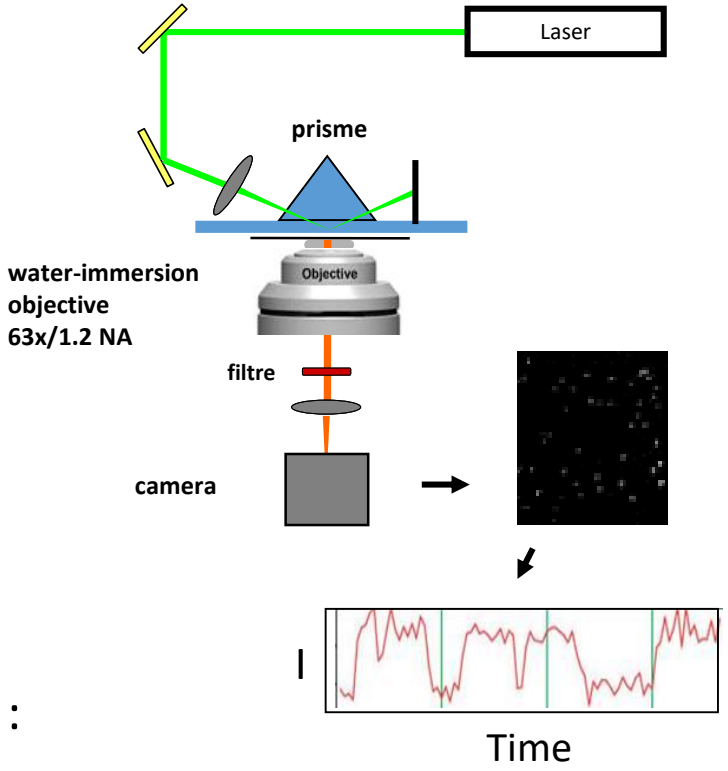
- Moreover, ensemble measurement do not allow to see the dynamics of unsynchronized molecules (dynamic heterogeneities)



Confocal and TIRF microscopies







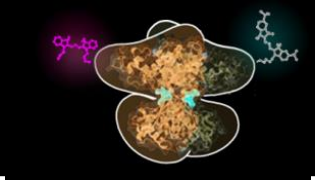
Confocal :

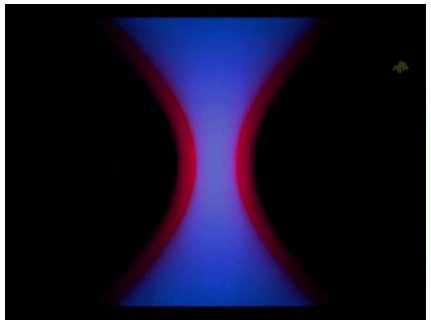
- immobilized or diffusing molecules in 3D
- 1 molecule at the time
- detection sub- populations
- High temporal resolution (µs-ns)

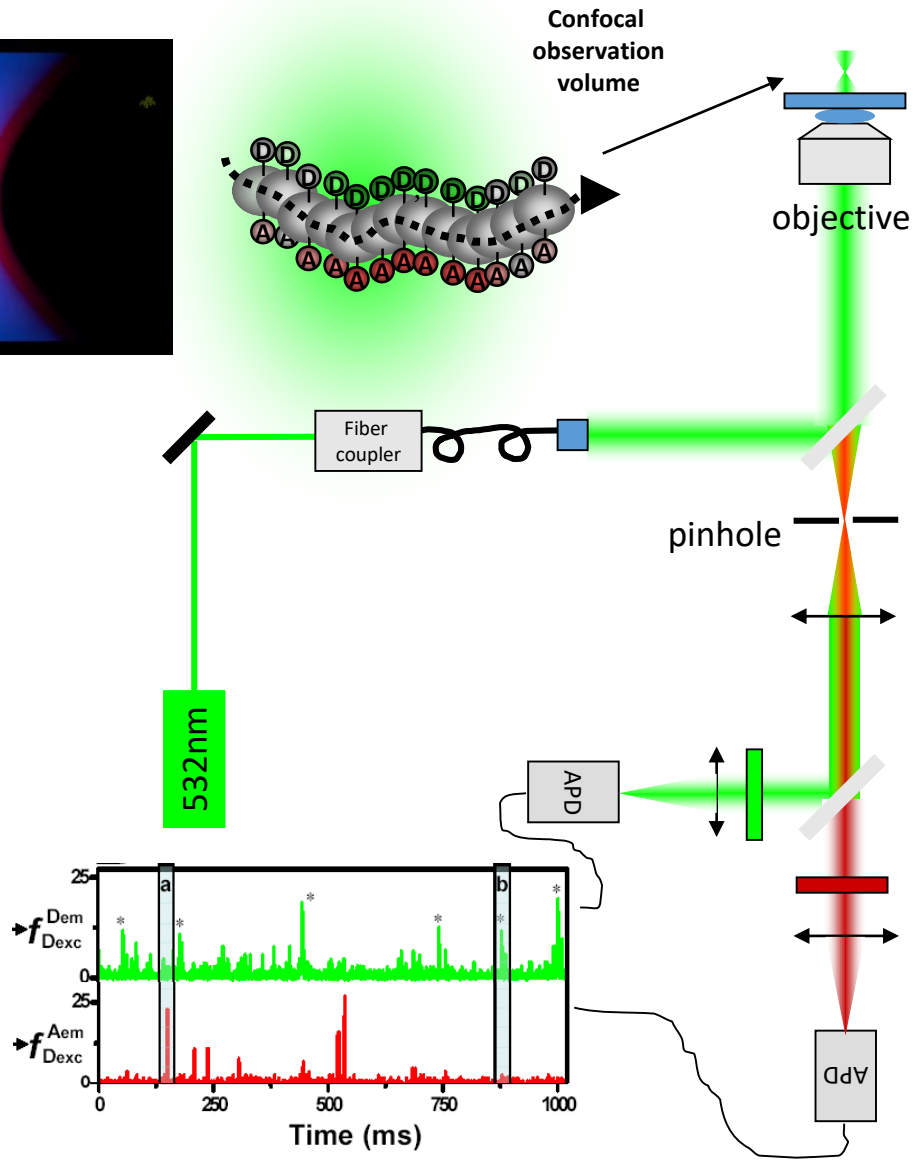
TIRF :

- immobilized or diffusing molecules in 2/3D
- dozens of molecules
- follow each molecules vs.time
- lower temporal resolution (ms)

smFRET on diffusing molecules

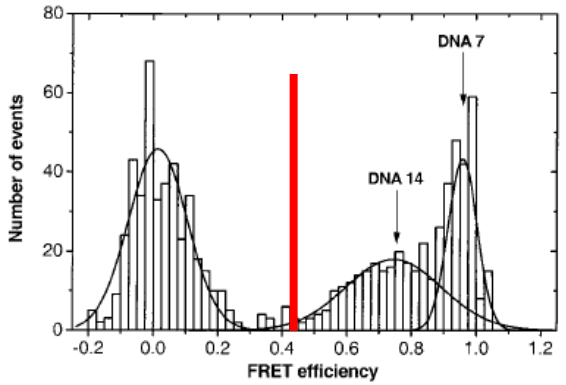






For each molecule :

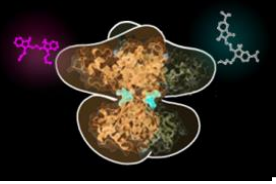
$$E_{PR} = \frac{F_{Aem}}{F_{Dem} + F_{Aem}}$$



→ resolve subpopulations

Deniz et al, *PNAS*, 1999

Single laser smFRET



- ⊕ Not necessary to have a high yield of labeling, or to know it exactly
- ⊕ Ability to recover subpopulations
- ⊕ On immobilized molecules, spFRET traces = f(time)

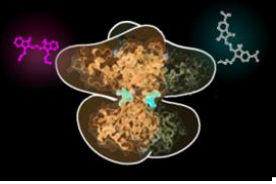
- ⊖ Acceptor photophysics : blinking, bleaching : D-only species
- ⊖ Sensitive to fluorescent contamination, appears as D-only
- ⊖ Unable to recover the correction factors needed to extract quantitative FRET efficiency, and thus distances

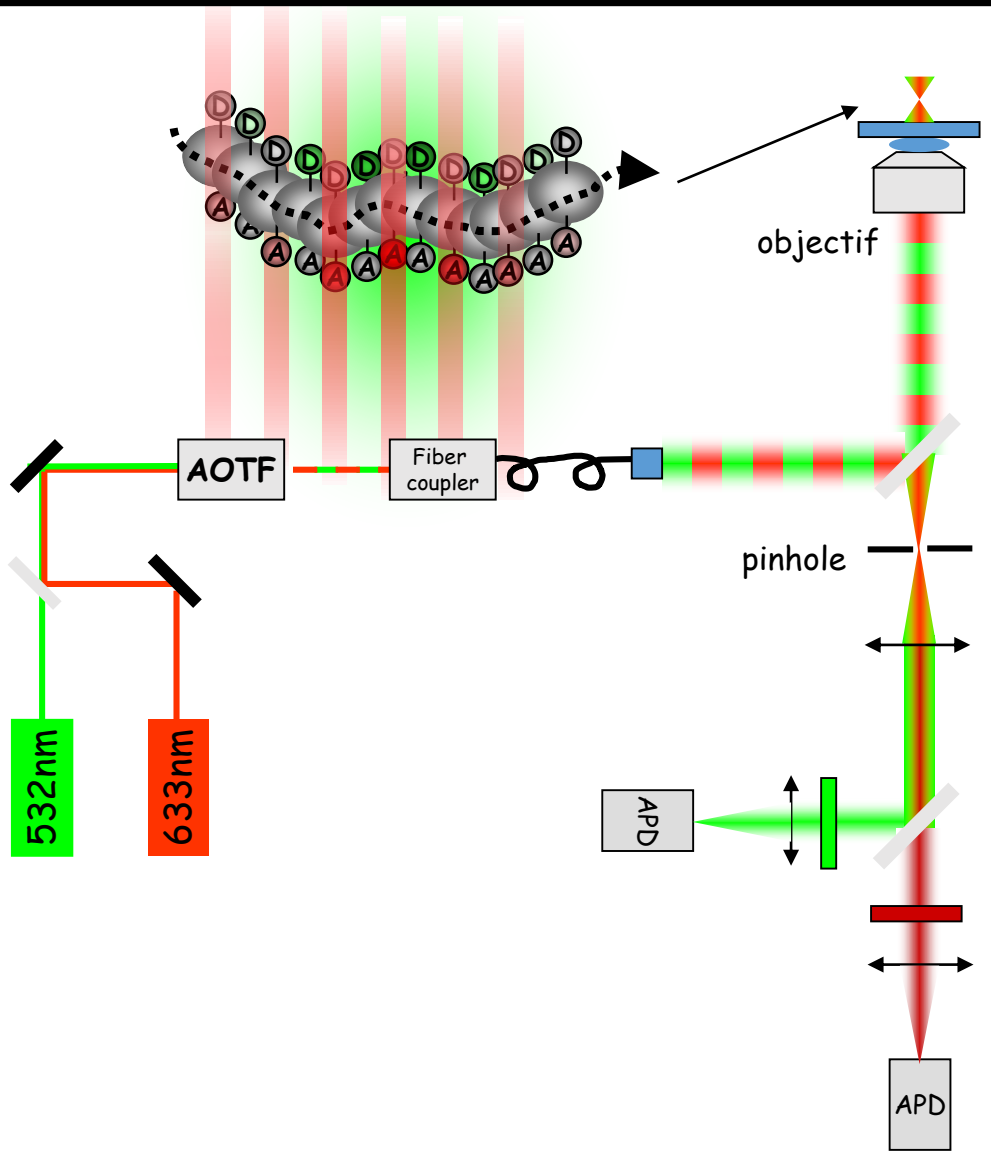
→ Addition of a second laser to directly probe the emission of the acceptor

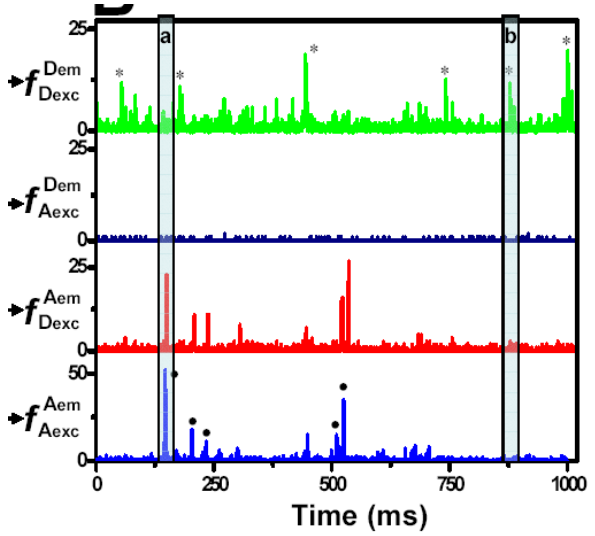
Alternating Laser EXcitation

Kapanidis et al, *PNAS*, 2004

Alternating laser excitation (ALEX) - smFRET

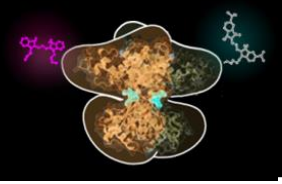






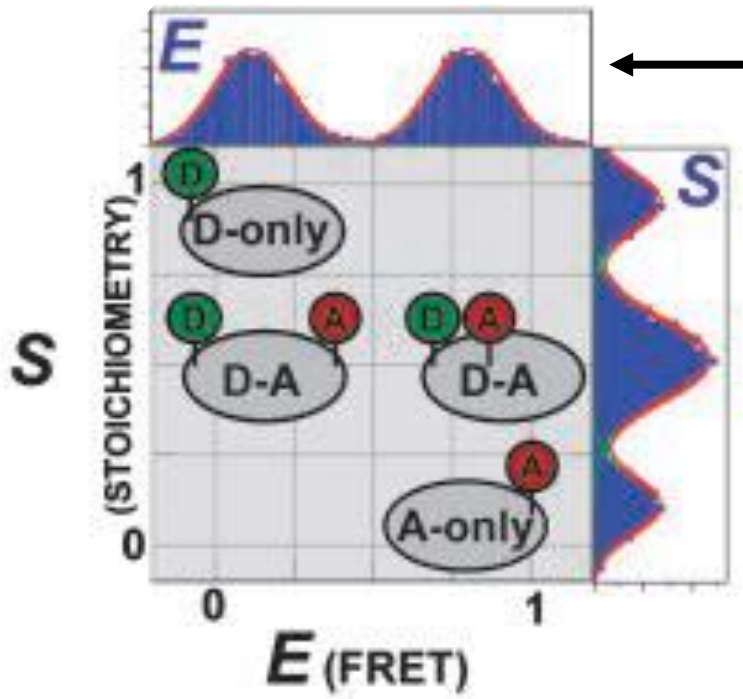
Kapanidis et al. *PNAS* 2004

Identifying the species with ALEX



For each molécule

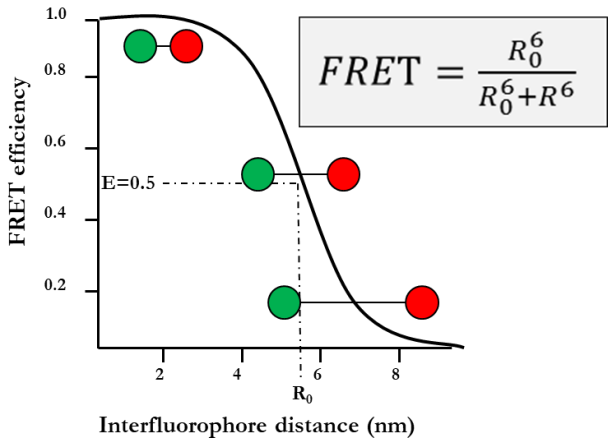
$$E_{PR} = \frac{F_{Dex,\,Aem}}{F_{Dex,\,Dem} + F_{Dex,\,Aem}} \qquad S = \frac{F_{Dex}}{F_{Dex} + F_{Aex}}$$

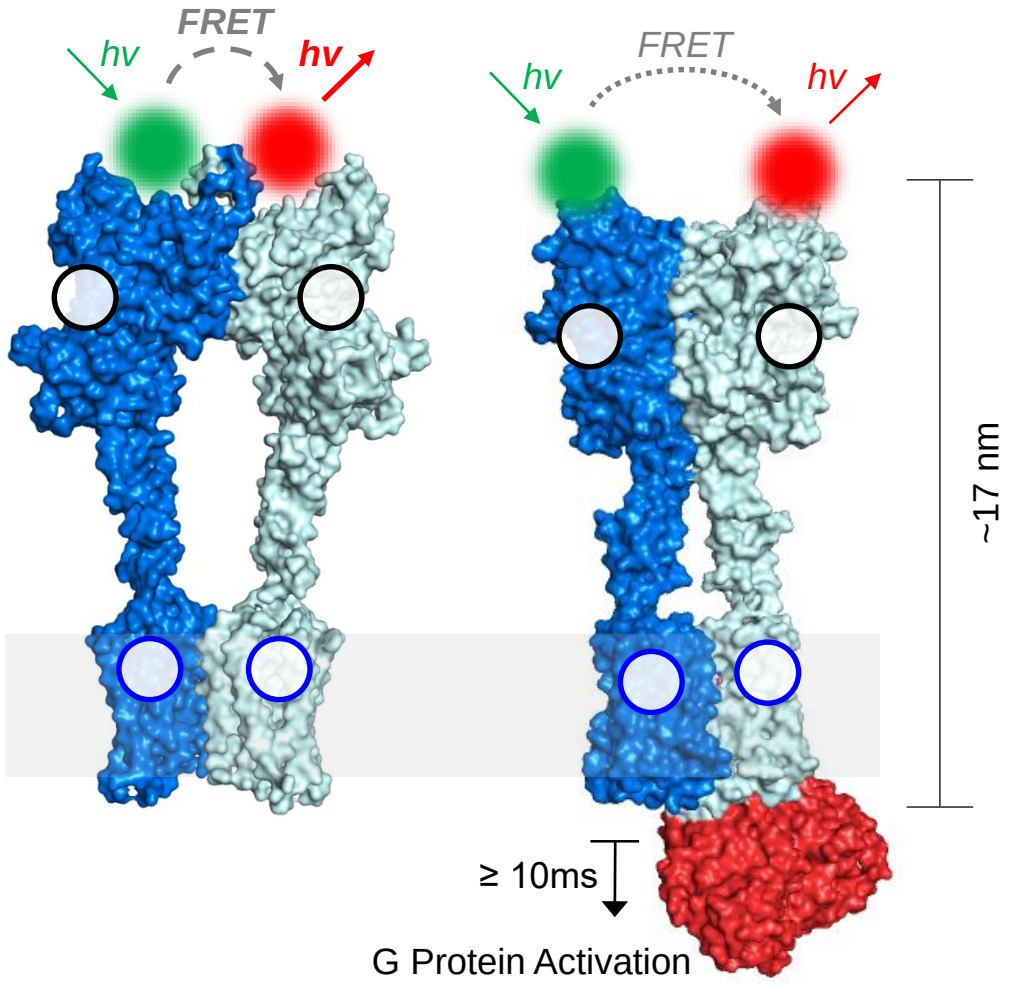


E_{PR} : distance between fluorophores

S : Presence of each fluorophores
→ Interaction
If D and A are on different molecules

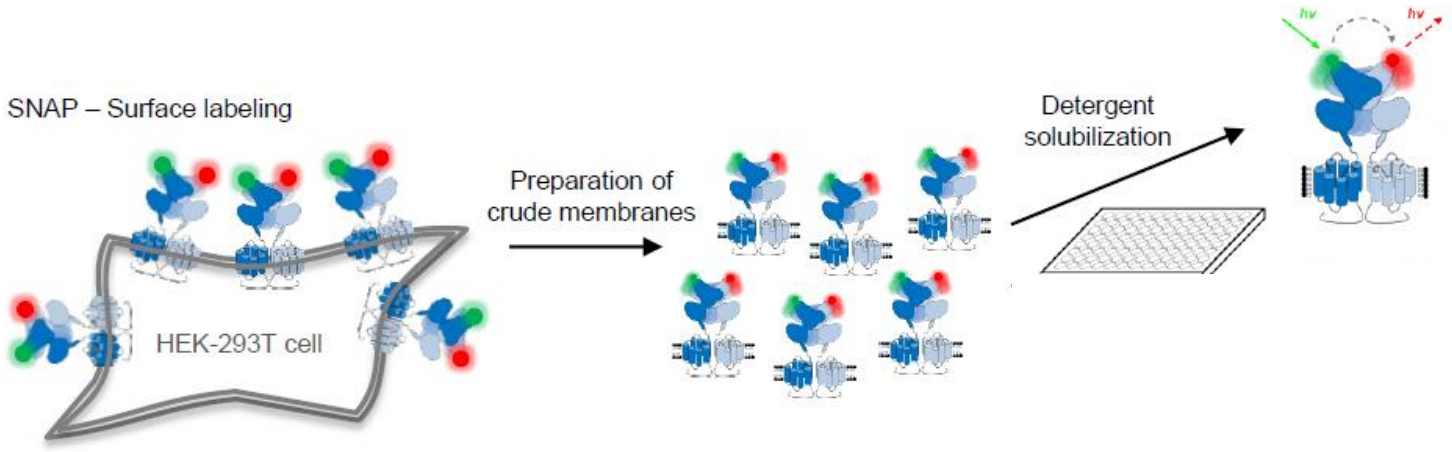
Structural dynamics of single metabotropic glutamate receptors



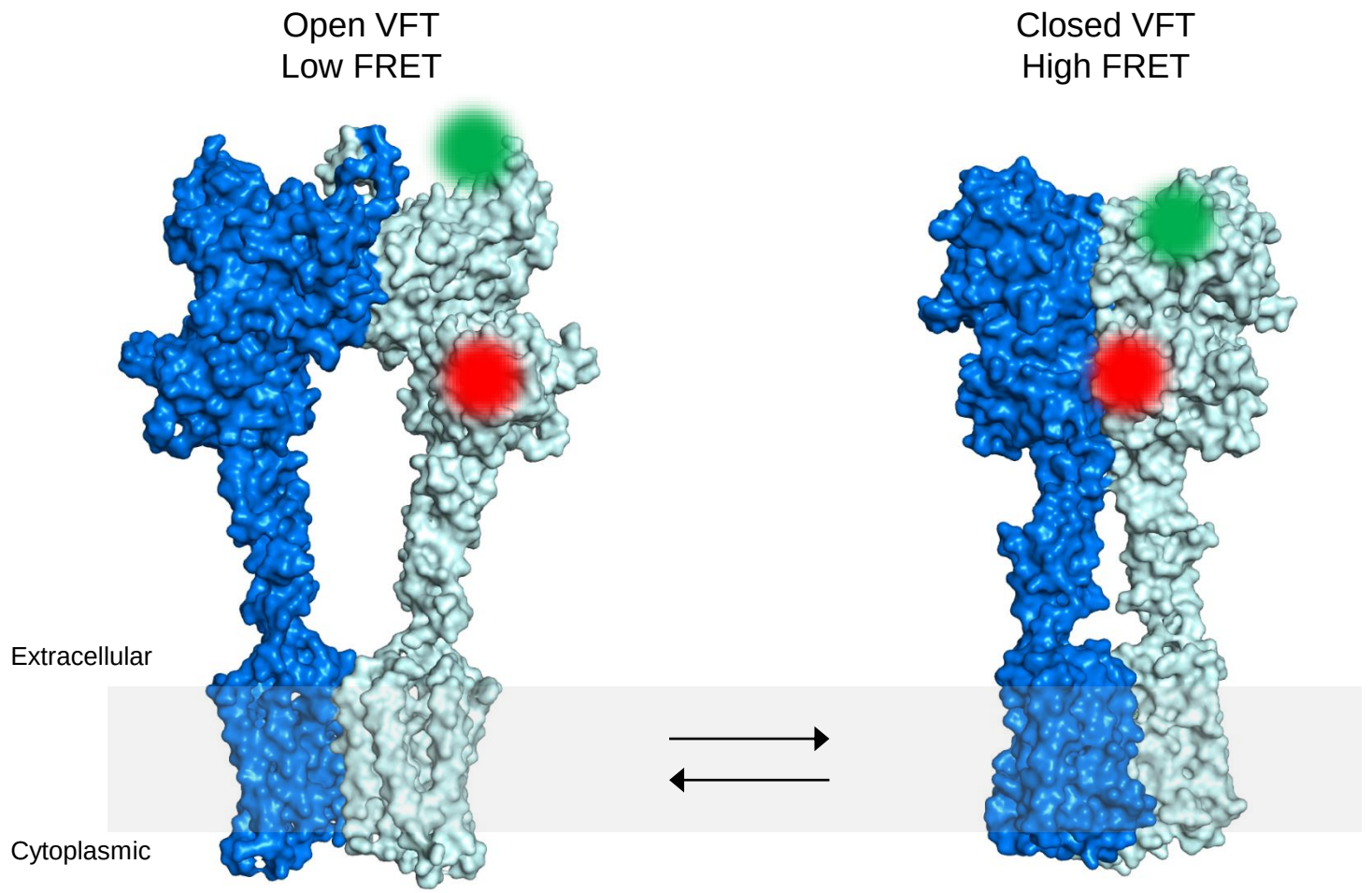


PDB 7EPA and 7E9G

Structural dynamics of single metabotropic glutamate receptors



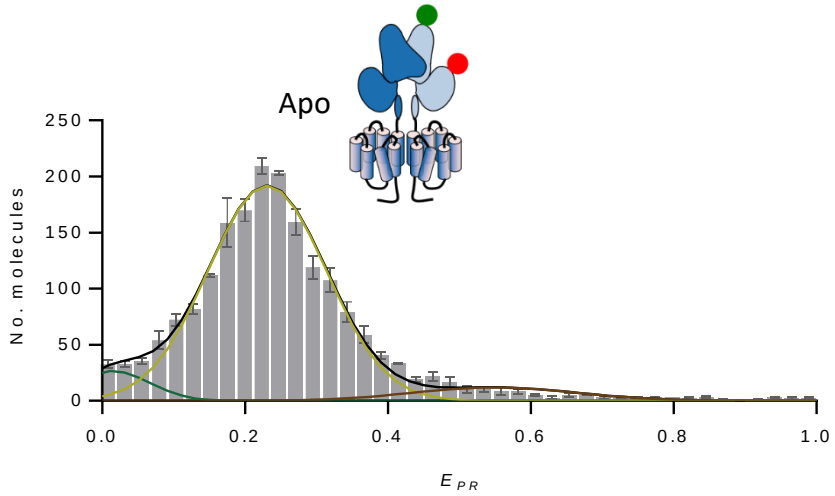
VFT Closure – Intrasubunit Sensor



PDB 7EPA and 7E9G

***Quast** et al., in preparation*

Agonist-dependend Allosteric Effect on VFT Closure



Unpublished data

***Quast** et al., in preparation*

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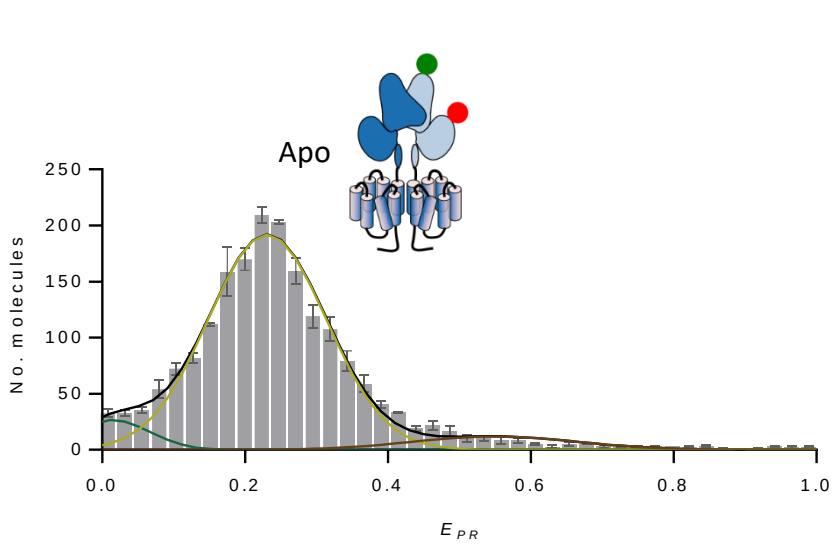

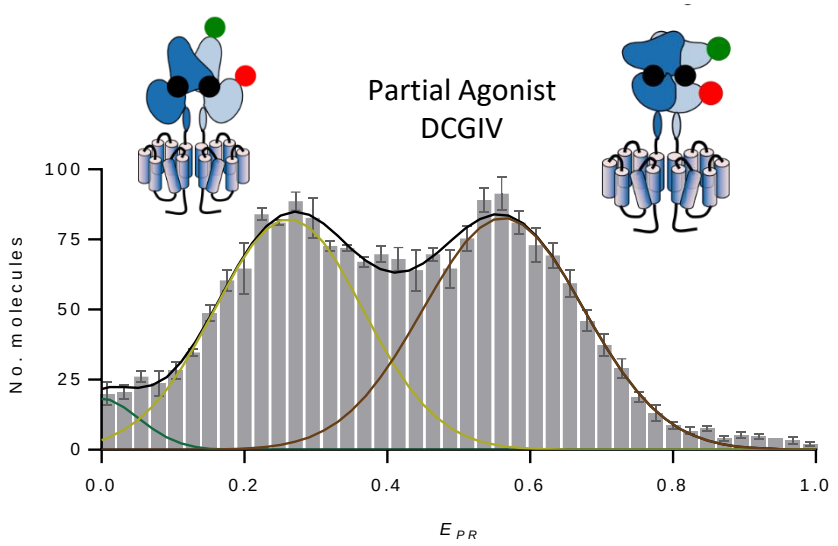

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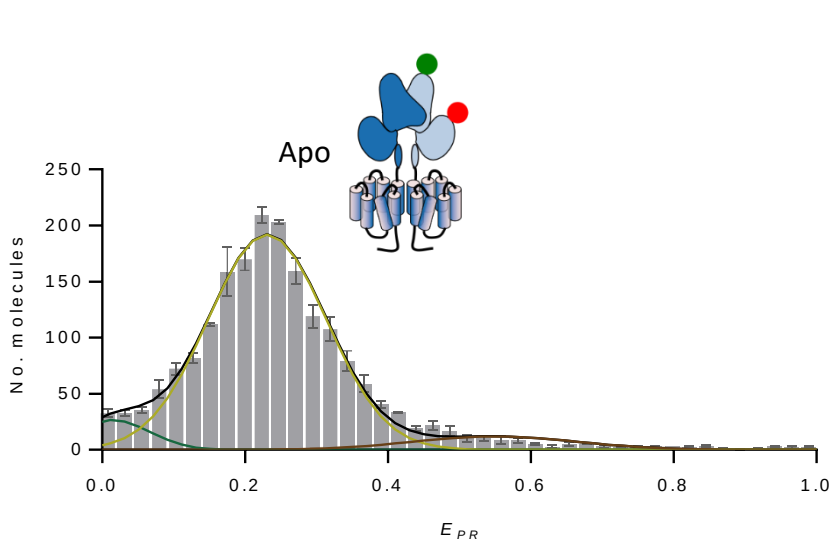

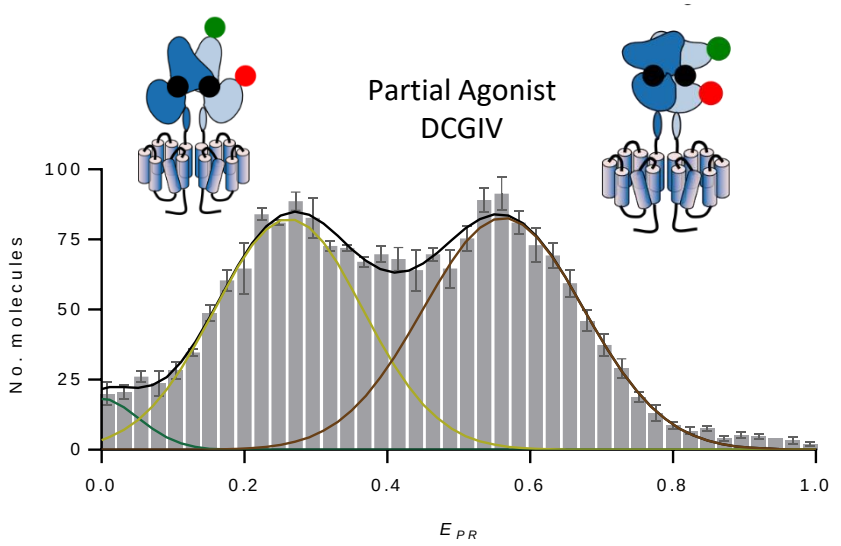

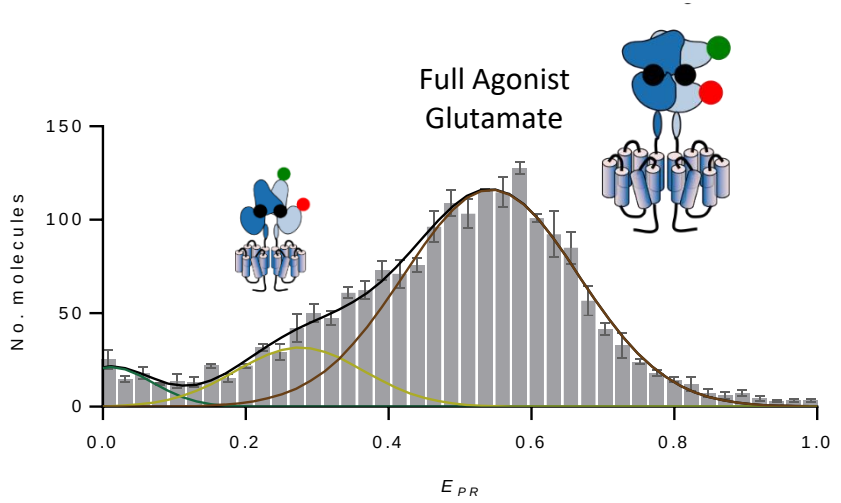

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