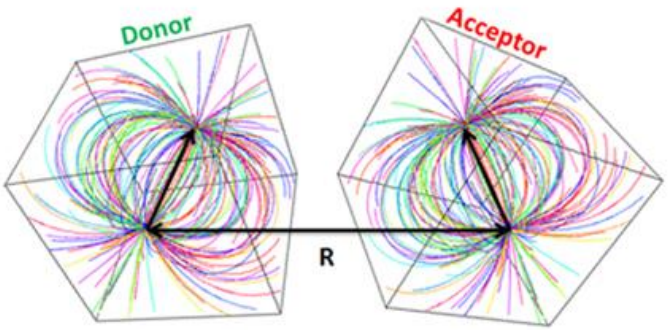
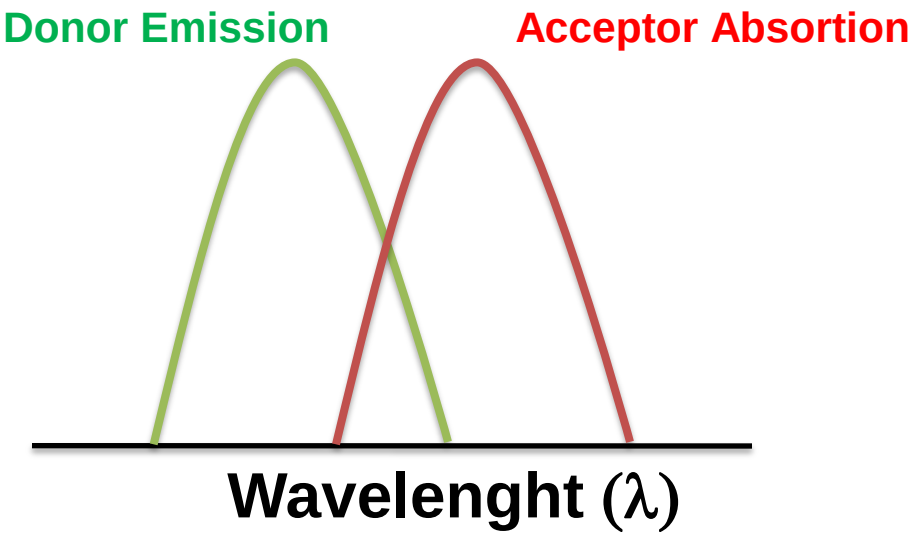
FRET, FLIM and FRAP

Sergi Padilla-Parra

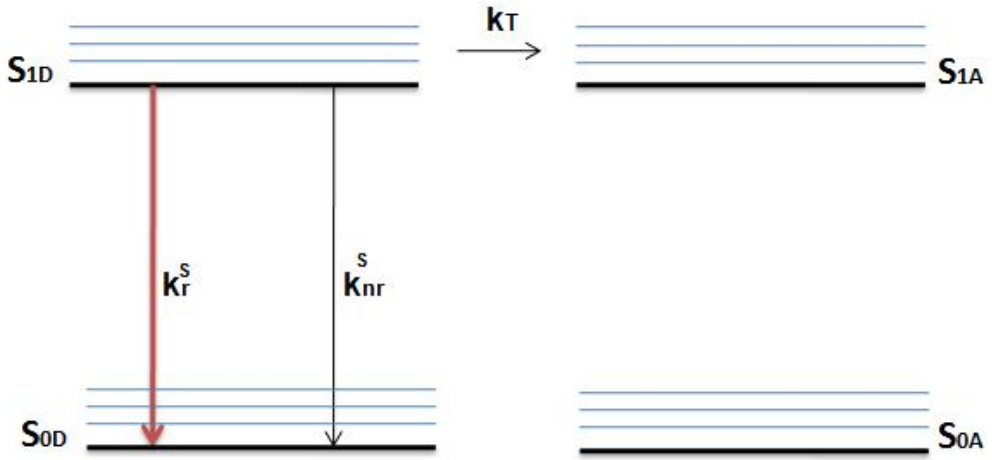
20th of September 2013

Introduction: What is FRET?



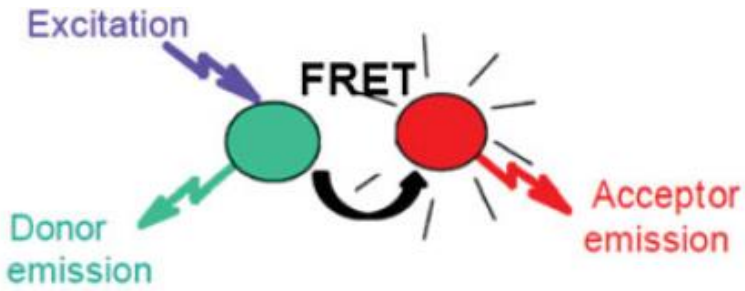


Introduction: How do we measure FRET by FLIM?

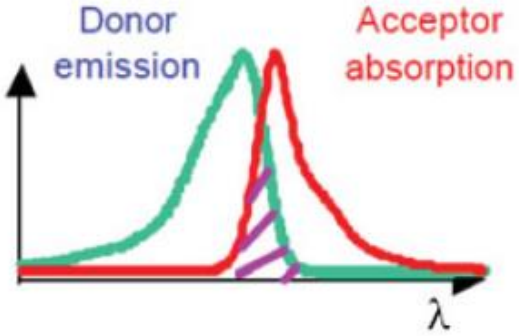


$$\tau_D = \frac{1}{k_r + k_{nr}}$$

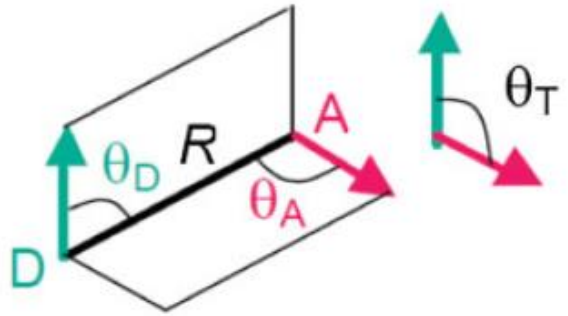
$$\tau_F = \frac{1}{k_r + k_{nr} + k_T}$$



$$E = \frac{R_0^6}{R_0^6 + R^6} \quad with \quad R_0 = \left[\frac{9000\,(ln10)\cdot\Phi_D\cdot\kappa^2\cdot J(\nu)}{128\,\pi^5\cdot N_A\cdot n^4}\right]^{\frac{1}{6}} \quad (in\ Å)$$



$$J = \int F_D(\lambda)\,\varepsilon_A(\lambda)\lambda^4 d\lambda \,/\, \int F_D(\lambda)$$



$$\kappa = \cos\theta_T - 3\cos\theta_A\cos\theta_D$$

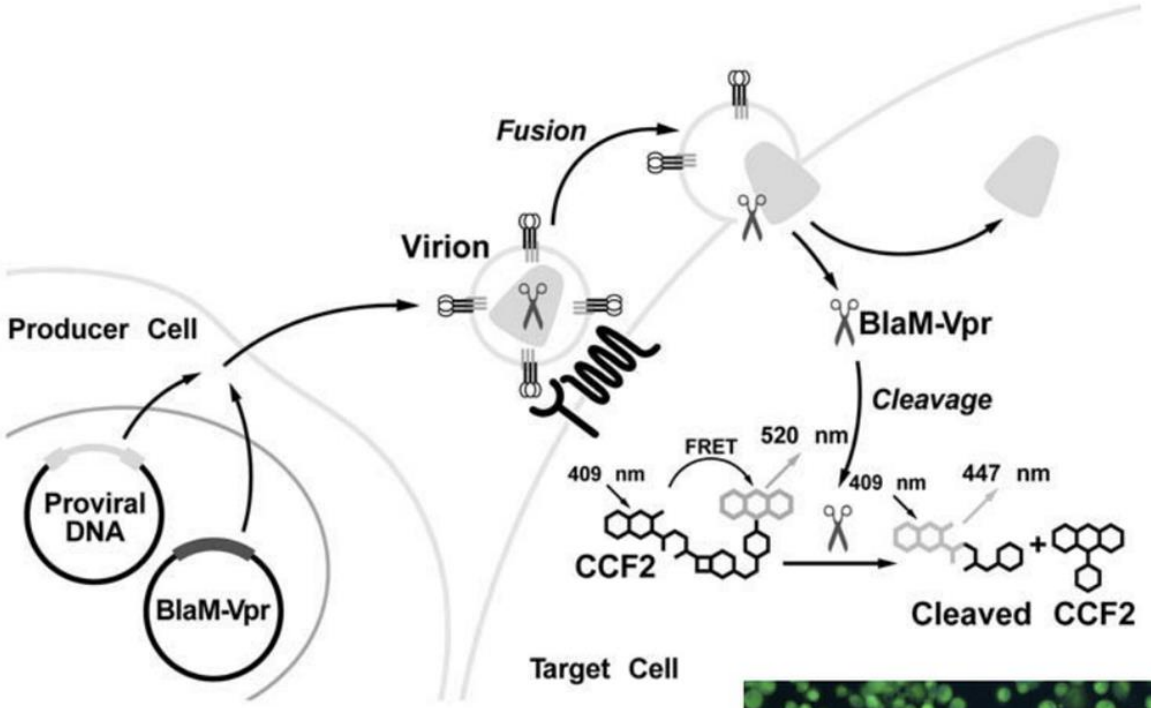
$$0 < \kappa^2 < 4$$

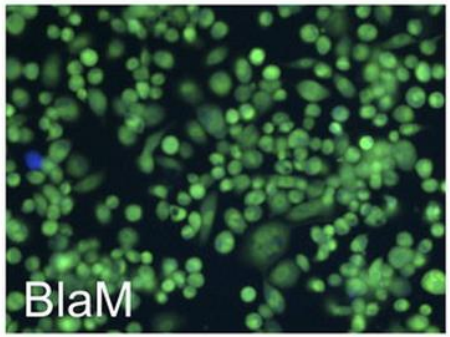
Random orientation $\kappa^2 = 2/3$

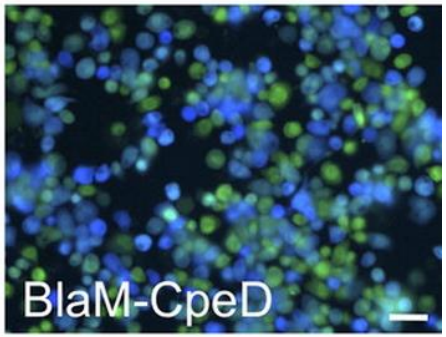
Introduction: FRET techniques

Approaches	Material	Acquisition procedures	Acquisition time	Data analysis	Quantitative results	Applications
Ratiometric FRET	Wide field, confocal or spinning disk microscope	Simultaneous acquisition	Seconds to tens of ms	Ratio imaging	Only for known donor/acceptor stoichiometry	FRET biosensors
3-cube FRET	Wide field, confocal or spinning disk microscope	Sequential acquisition and bleed through controls	Seconds	Image calculation	Semi quantitative Apparent FRET efficiency	Quantitative FRET biosensors Qualitative prot-prot interaction
FLIM by single photon counting	Confocal microscope with TCSPC module	Single photon by single photon	Few minutes	Pixel by pixel fit	FRET efficiency and fraction of donor in interaction	Quantitative FRET biosensors Quantitative prot-prot interaction
FLIM by time gated or frequency domain	Time-gated or fast-modulated intensifier on wide field or spinning disk microscope	Sequential stack of time-gated or phase-shift images	Minute to seconds	Fit or direct analysis	FRET efficiency and fraction of donor in interaction	Quantitative FRET biosensors Quantitative prot-prot interaction

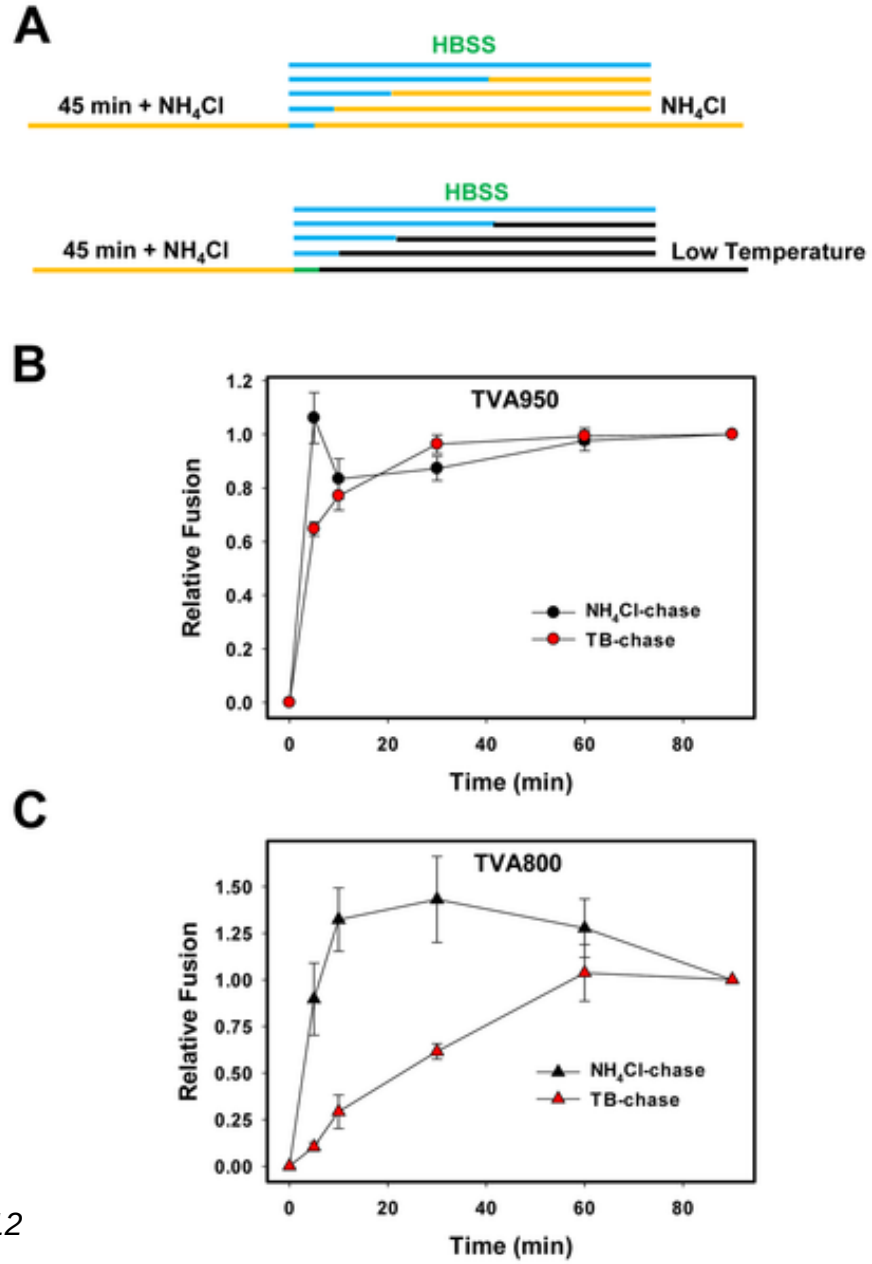
BlaM assay to detect HIV fusion





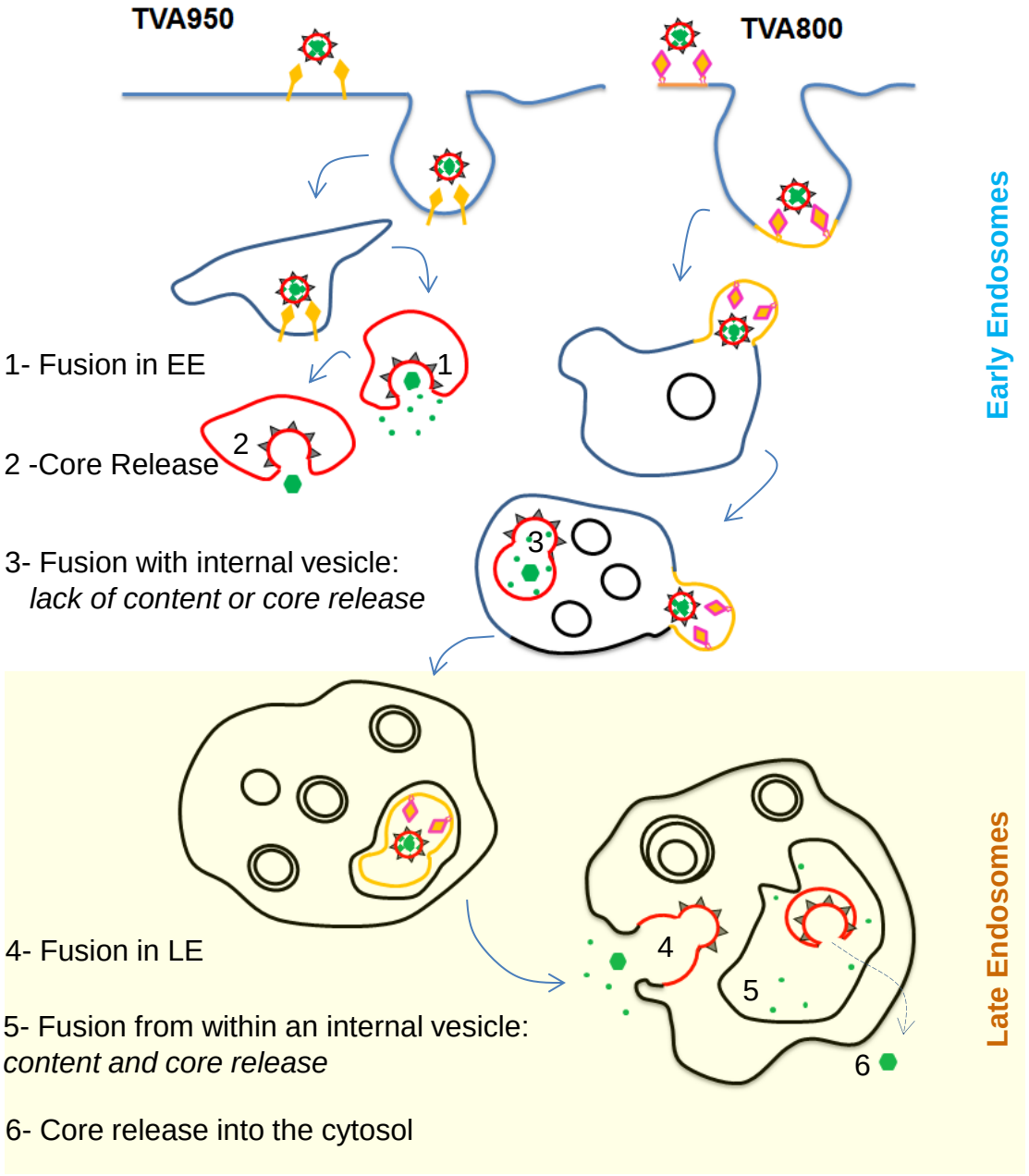


BlaM

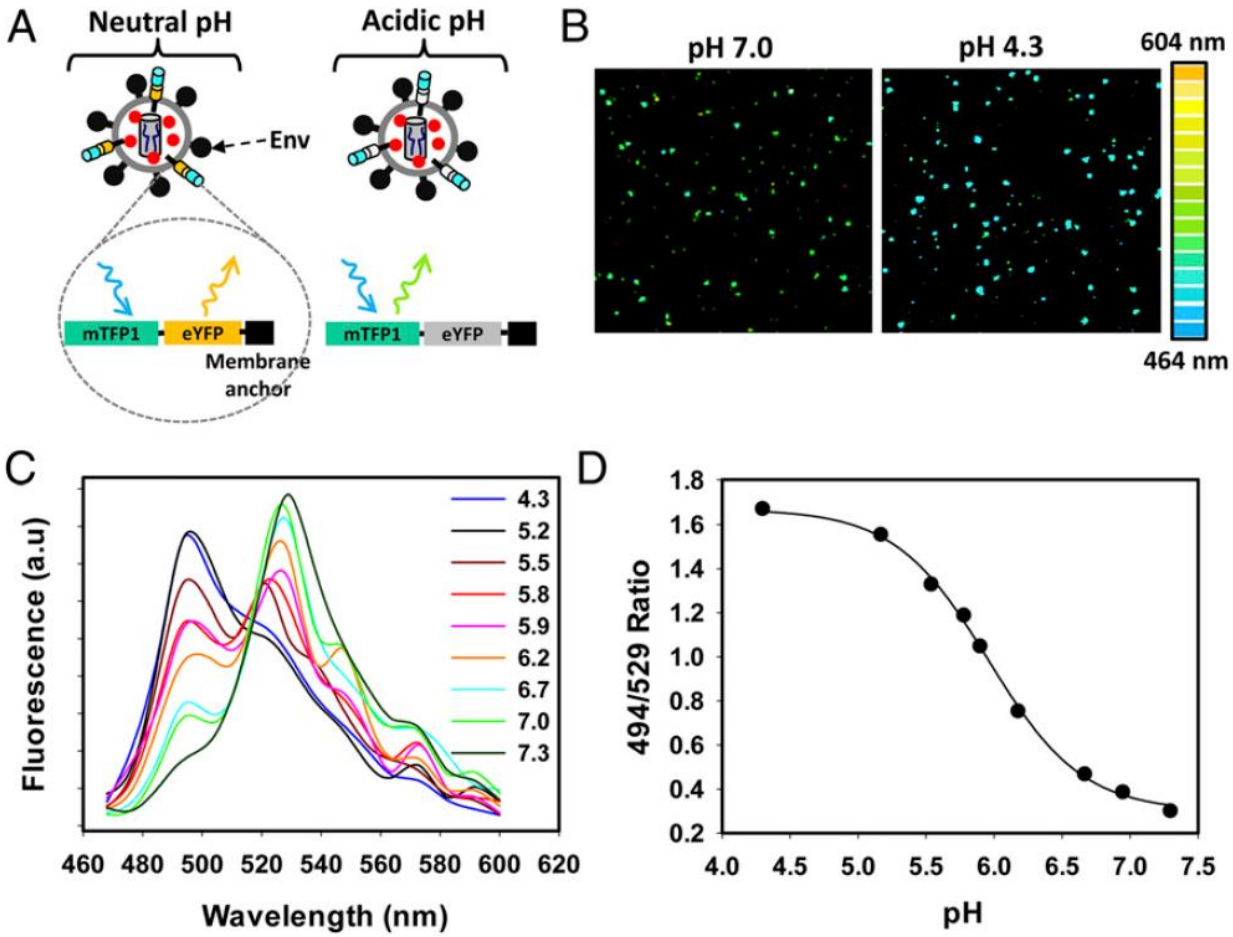


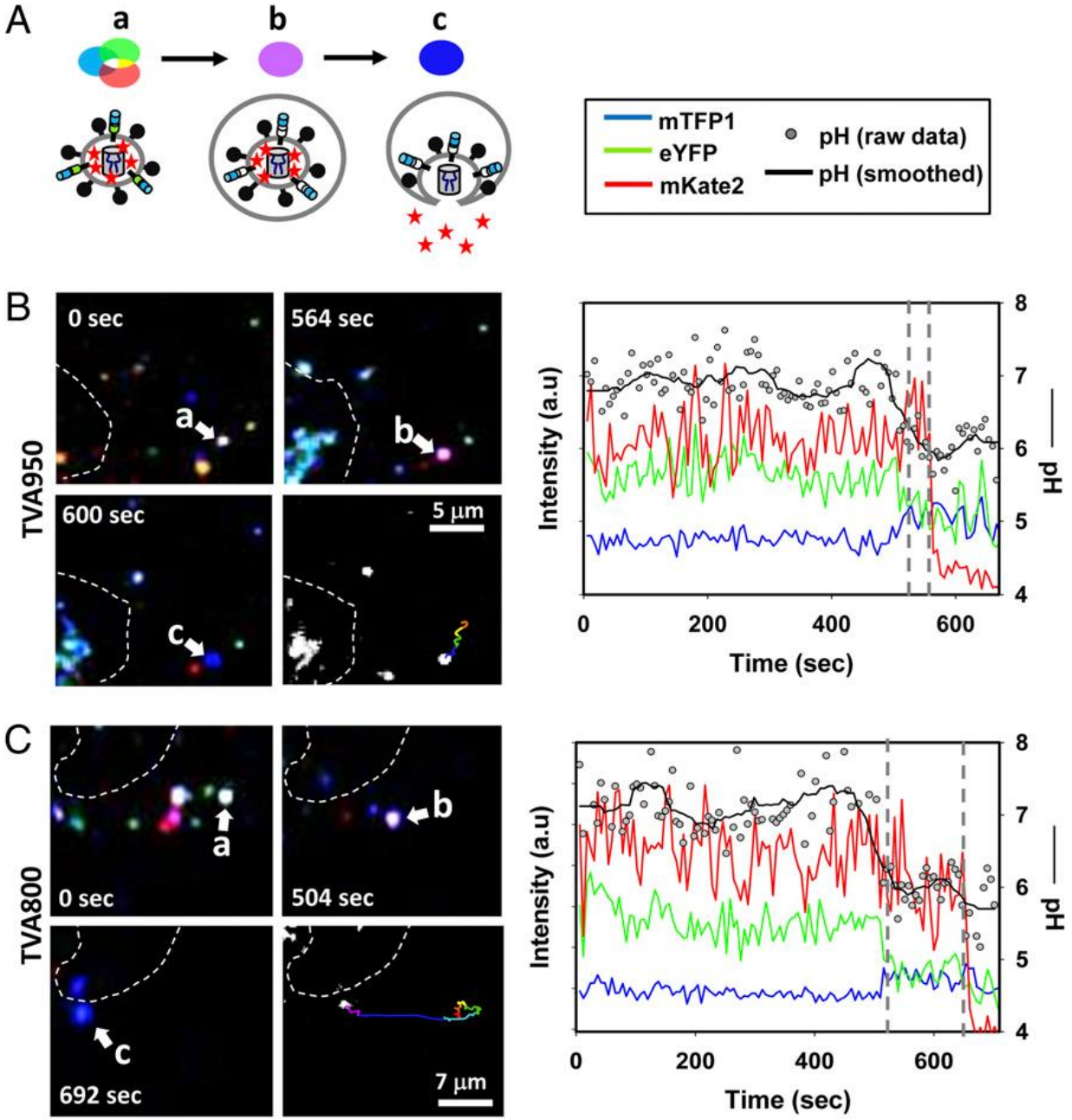
Padilla-Parra et al., Plos Path.. 2012

Model



Early endosome
Late endosome
DiD
Core
GFP
TVA950
TVA800


A
Neutral pH
Acidic pH
Env
mTFP1
eYFP
Membrane anchor
mTFP1
eYFP
B
pH 7.0
pH 4.3
604 nm
464 nm
C
Fluorescence (a.u)
4.3
5.2
5.5
5.8
5.9
6.2
6.7
7.0
7.3
460 480 500 520 540 560 580 600 620
Wavelength (nm)
D
494/529 Ratio
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5
pH



Padilla-Parra et al., PNAS. 2012

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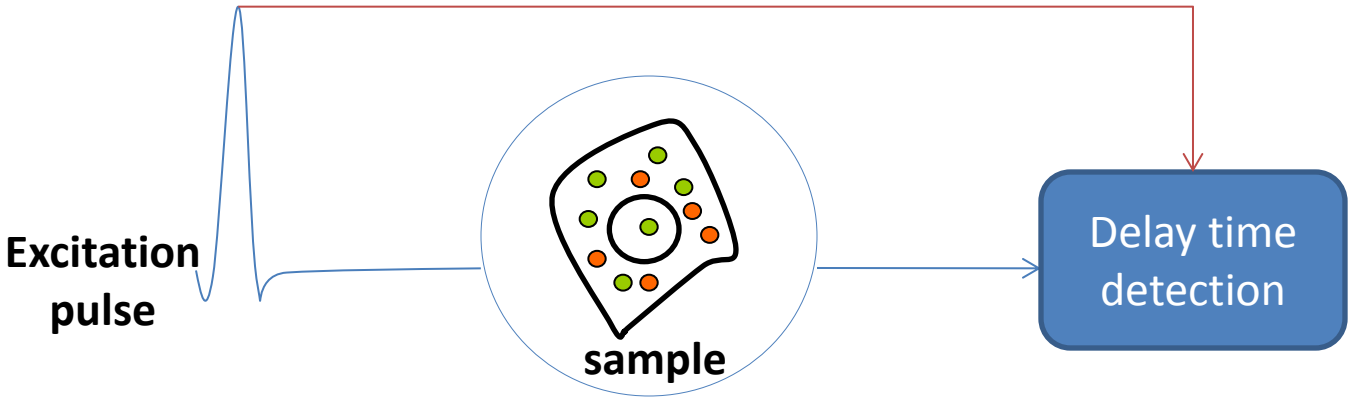
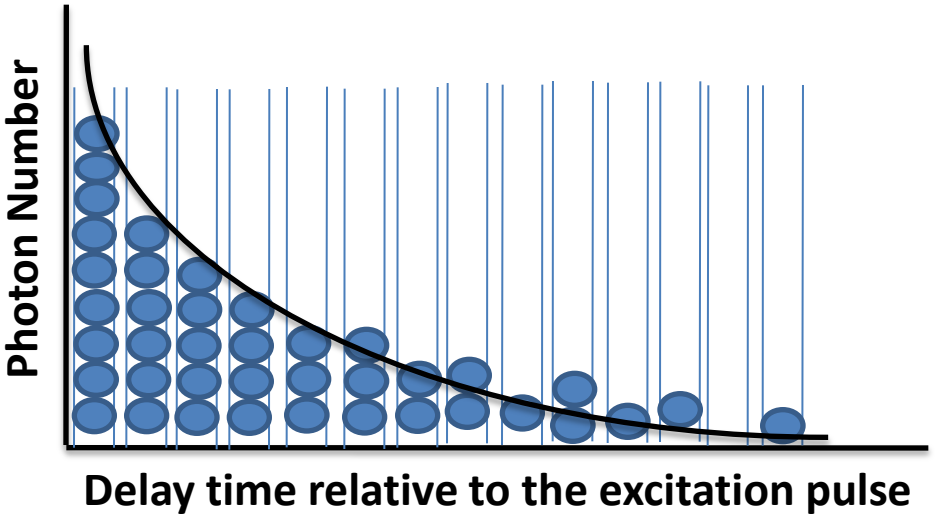
Introduction: FRET by FLIM Advantages

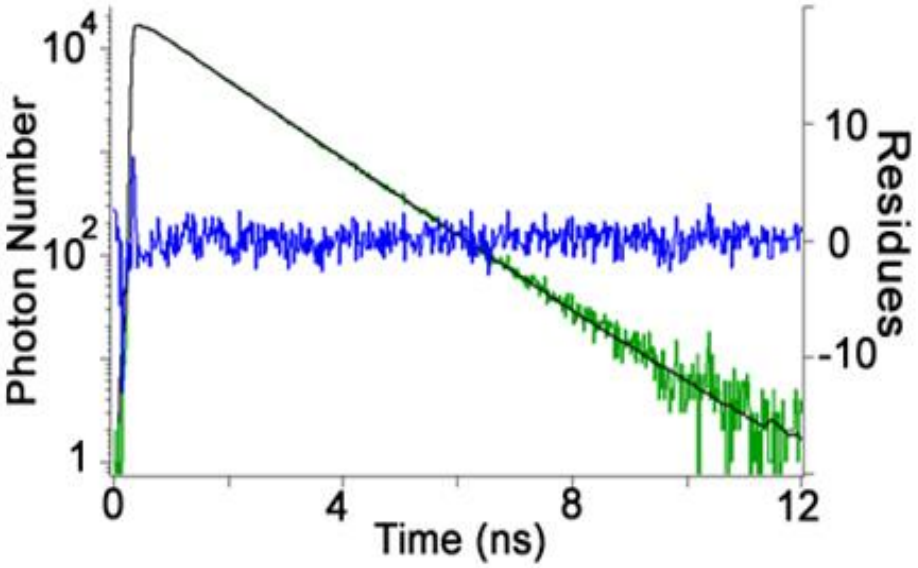
- The **lifetime** is an spectroscopical property which does not depend on the optical path or the fluorophore concentration
- High Precision
- Quantification of protein interactions in live cells with high accuracy

Instrumentation: Counting Photons

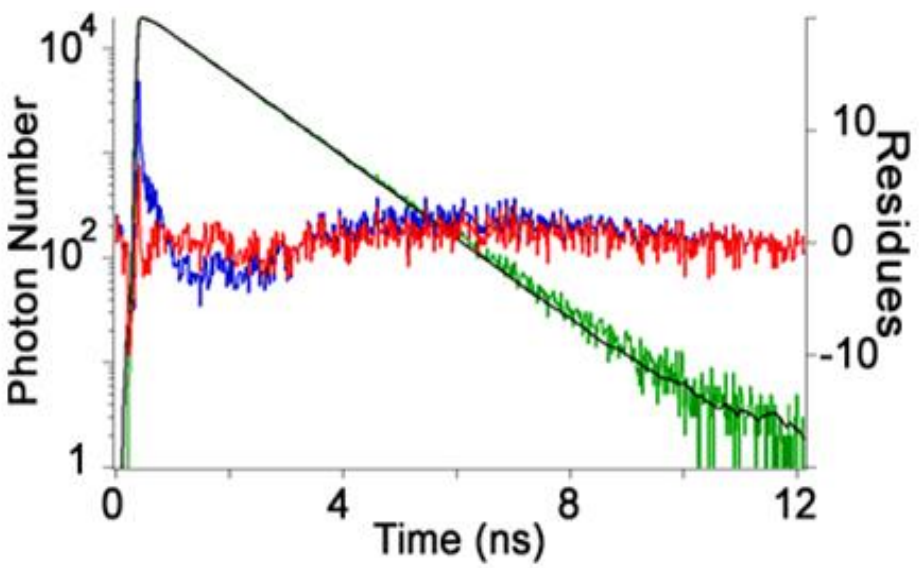




Data Analysis: single and multi lifetime donors



Homogeneous population



Heterogeneous population

Padilla-Parra et al., Biophys J 2008

Data analysis: Fit approach Advantages

- High precision finding mechanistic parameters of the system under investigation

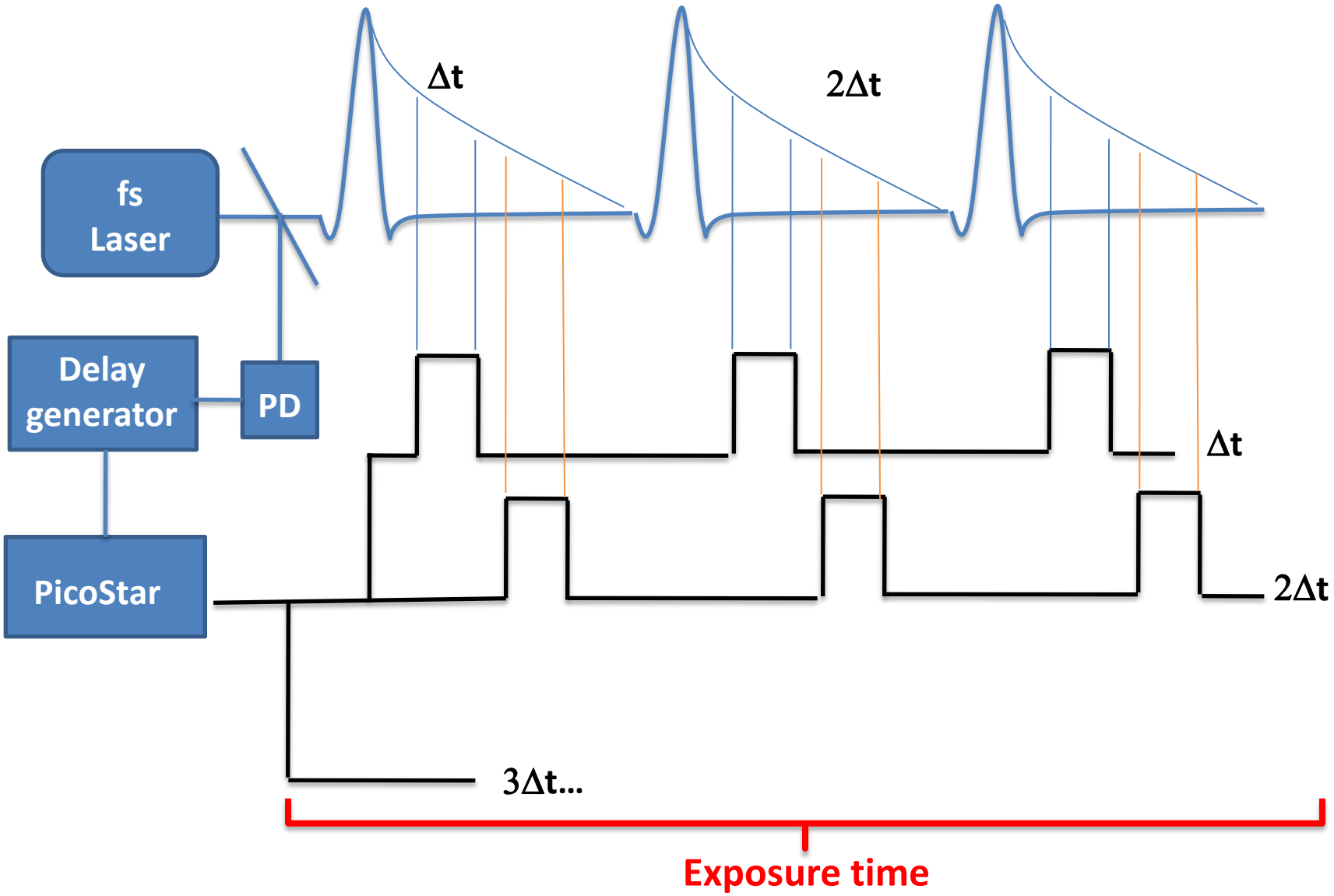
- Quantitative approach adapted to data coming from in vivo samples

Data analysis: Fit approach Drawbacks

- High expertise needed to compare between two fits using different models

- Long acquisition times not adapted for fast events (protein interaction kinetics in live cells)

Instrumentation: Sequential acquisition



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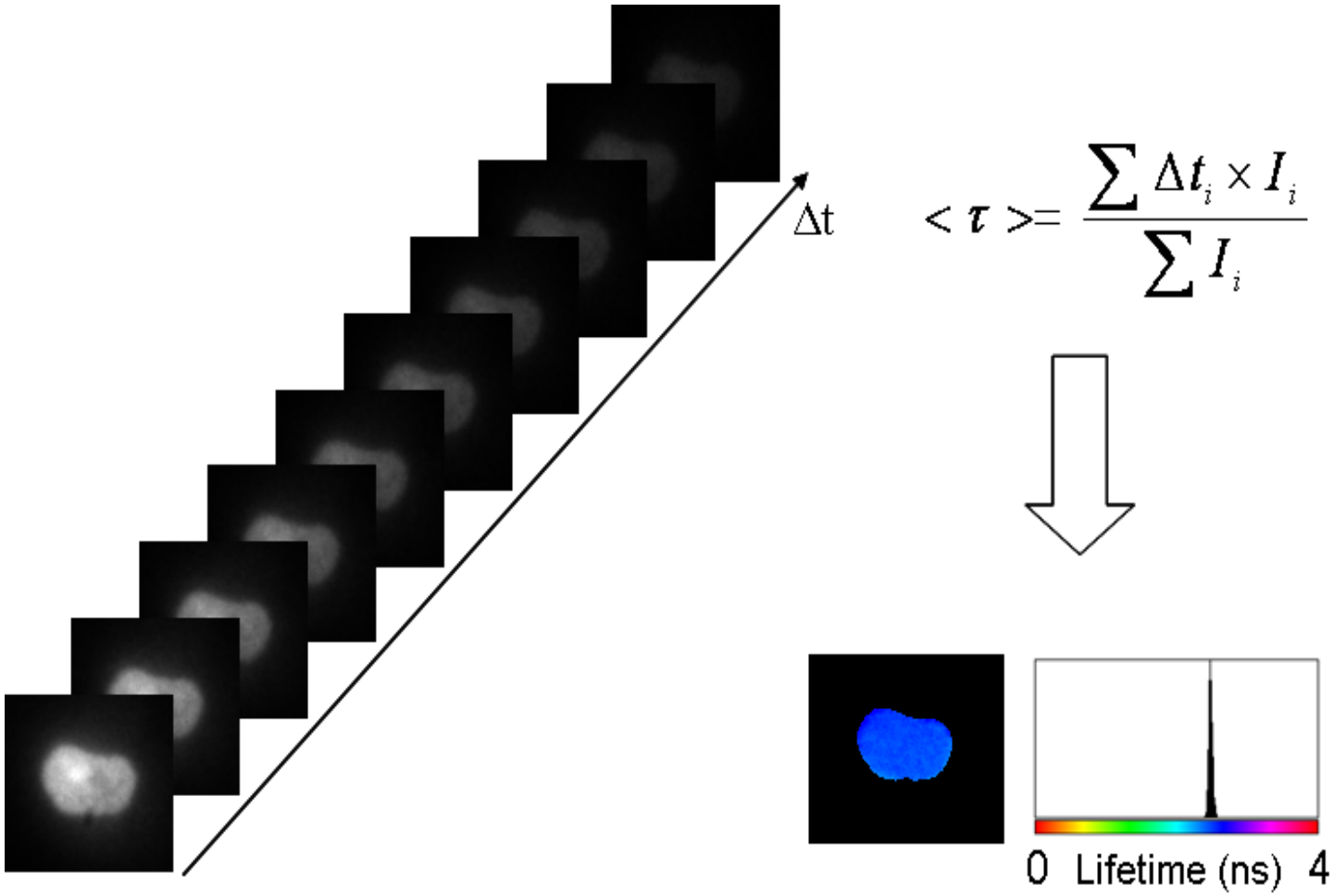

Data analysis II: Non-Fitting approaches

- Minimal fraction of interacting donor: mfD
  (Padilla-Parra et al., 2008)
- The Phasor Plot approach
  (Digman et al., 2008)

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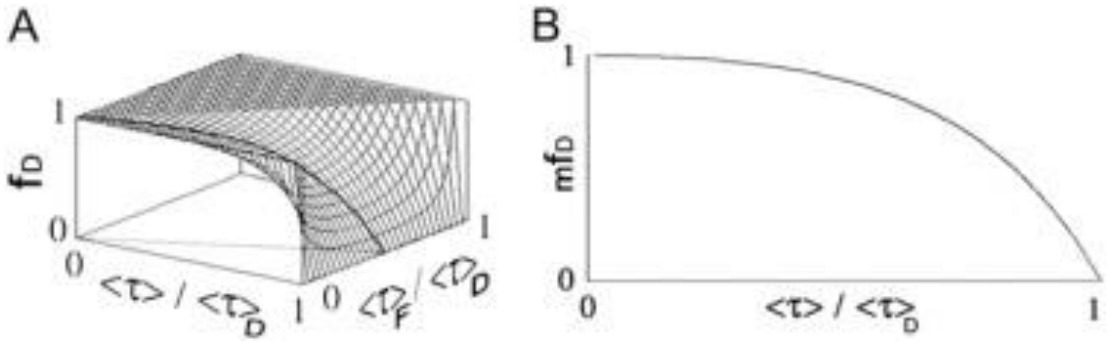
Data analysis II: mf_D

1. $$< \tau >= \int t * i(t) dt \Big/ \int i(t)\, dt$$

2. $$i(t) = f_D \cdot e^{-t/\tau_F} + (1 - f_D) \cdot e^{-t/\tau_D}$$



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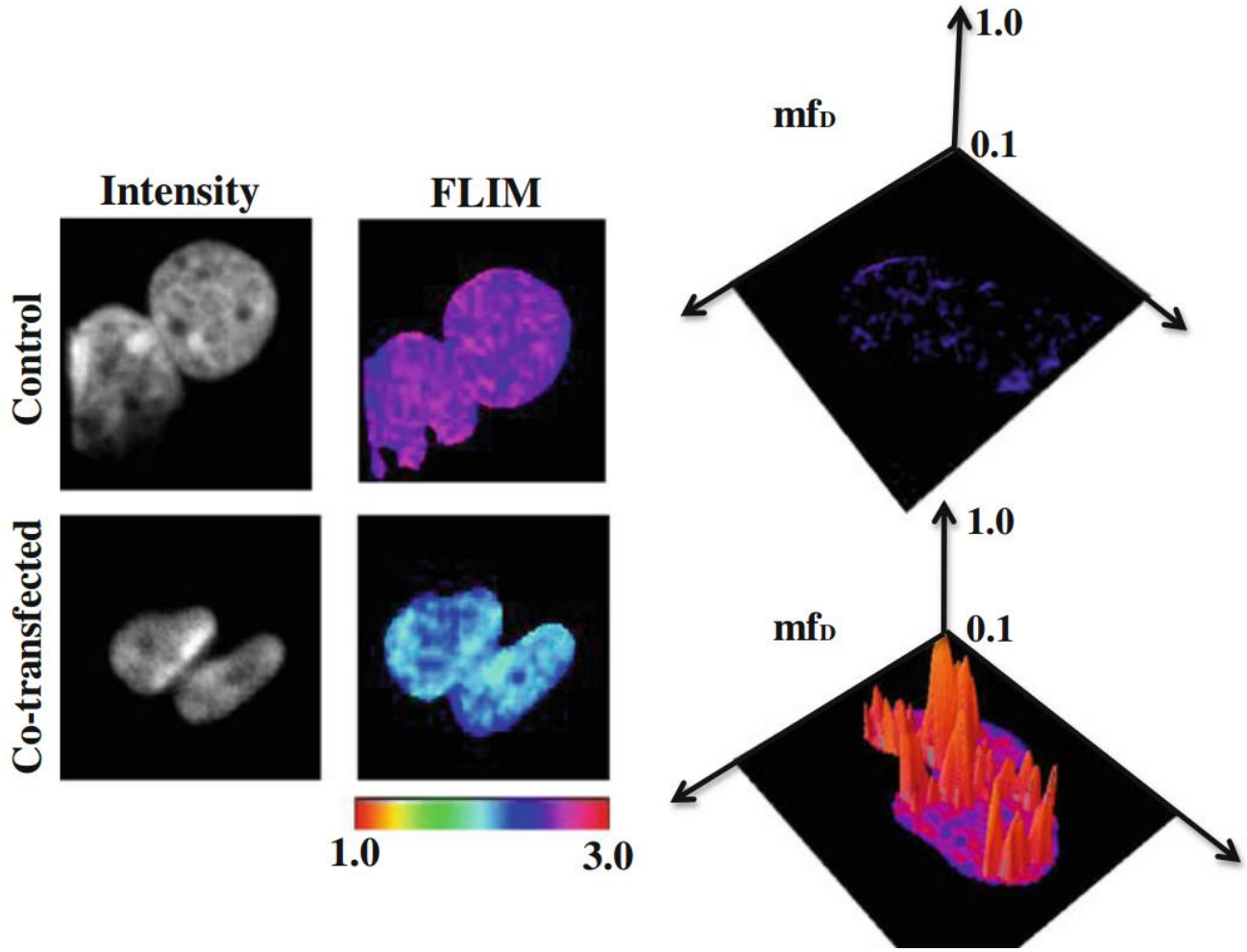
3. $$mfD=[1-(<\tau>/\tau_D)]\Big/[(<\tau>/2\cdot\tau_D)-1]^2$$

Histone H4 acetylaltion



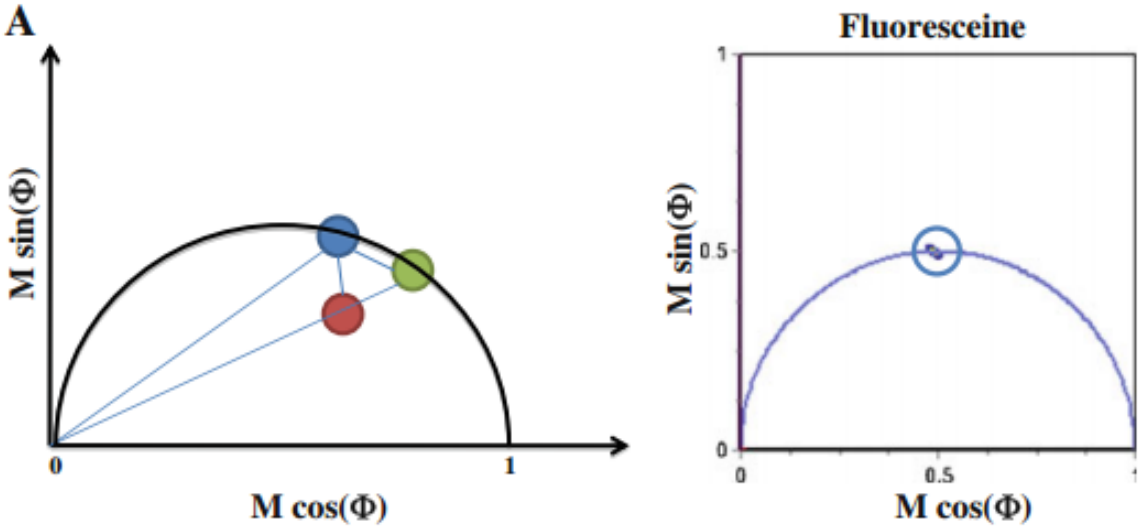
Data analysis II: Non-Fitting approaches

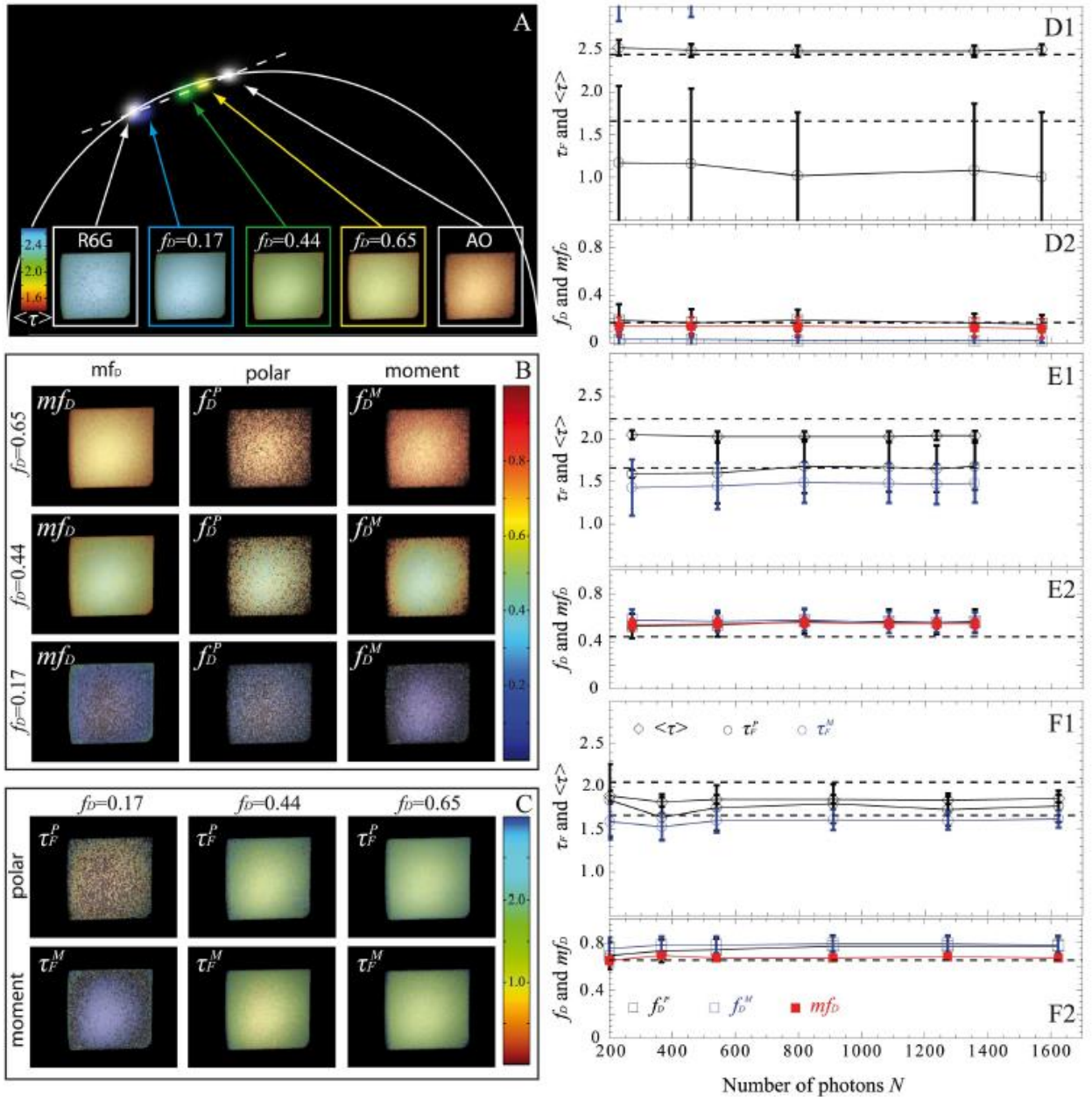
- Minimal fraction of interacting donor: mfD
  (Padilla-Parra et al., 2008)
- **The Phasor Plot approach**
  (Digman et al., 2008)

Data analysis II: Phasor Plot (TD)

$$g_{i,j}(w) = \int I_{i,j}(t)\cos(wt)dt / \int I_{i,j}(t)dt$$

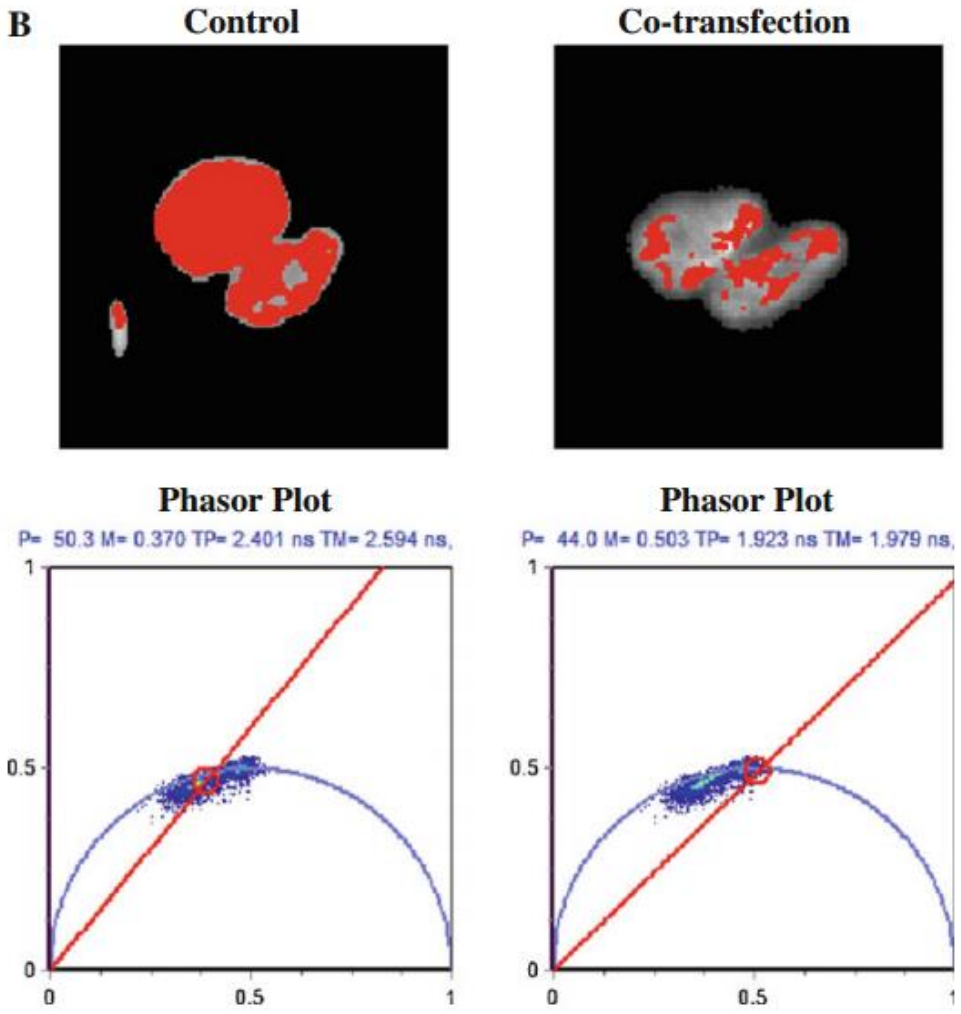
$$s_{i,j}(w) = \int I_{i,j}(t)(\sin)(wt)dt / \int I_{i,j}(t)dt$$



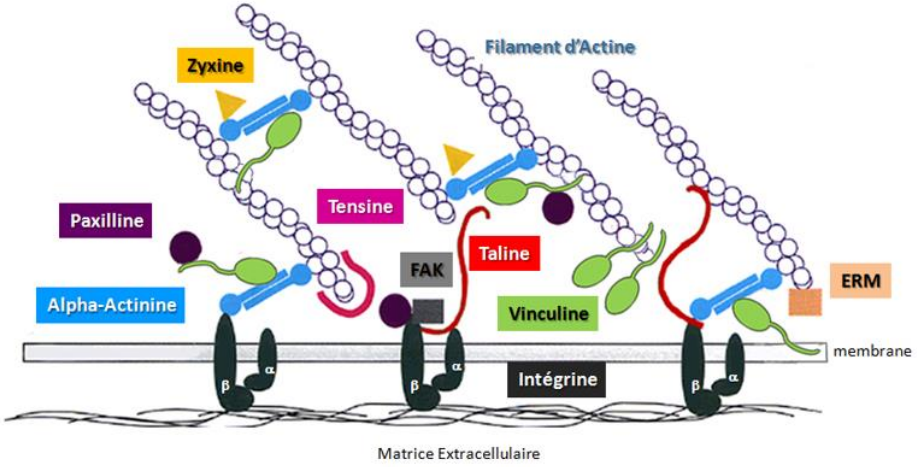


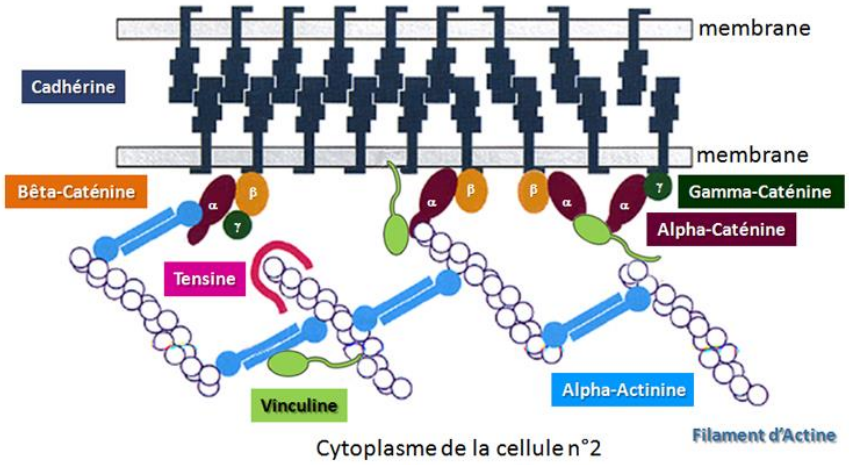
Leray, Padilla-Parra et al., Plos One. 2013

Data analysis II: Phasor Plot (TD)



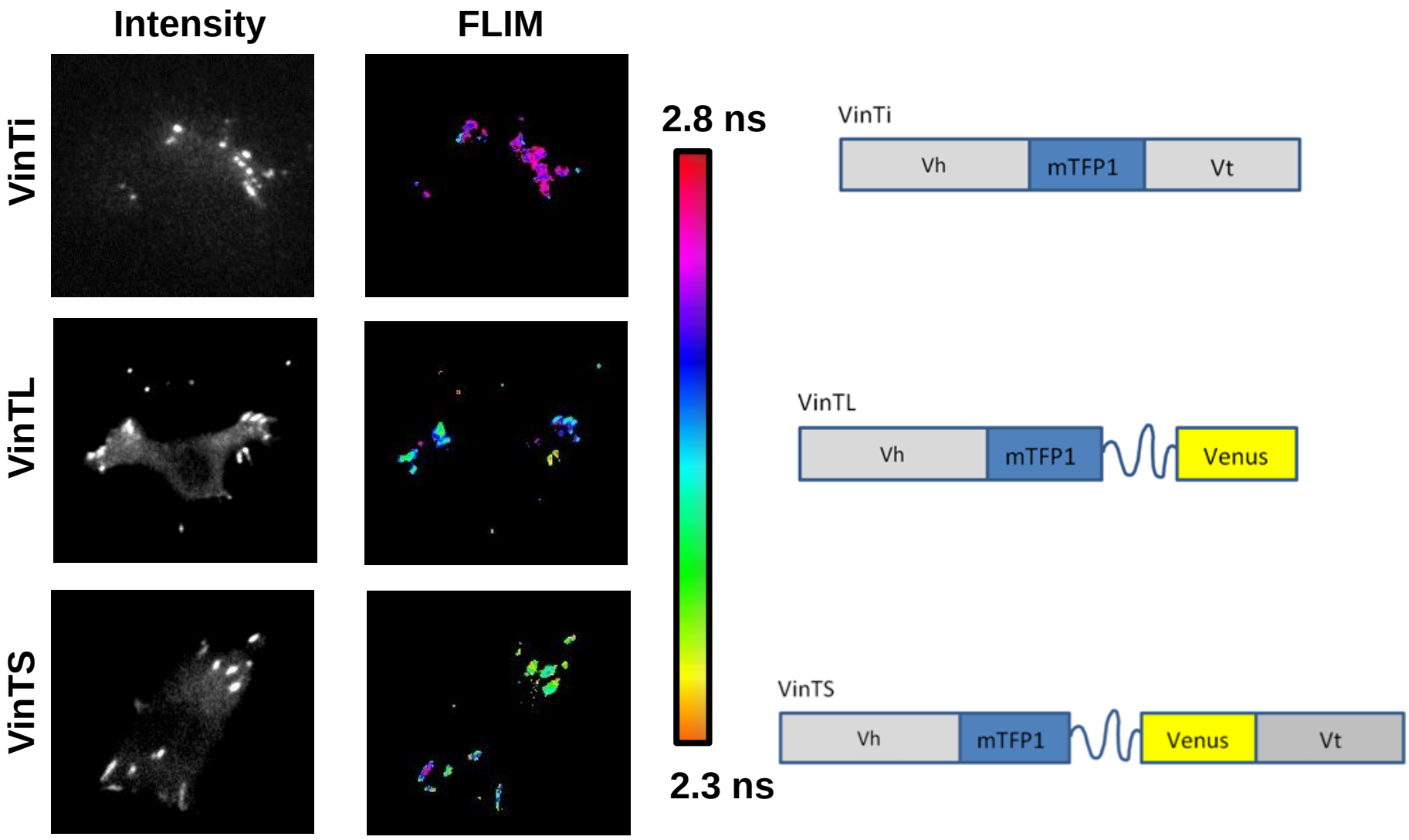
Mechanical tensions in FA and Cell-Cell junctions





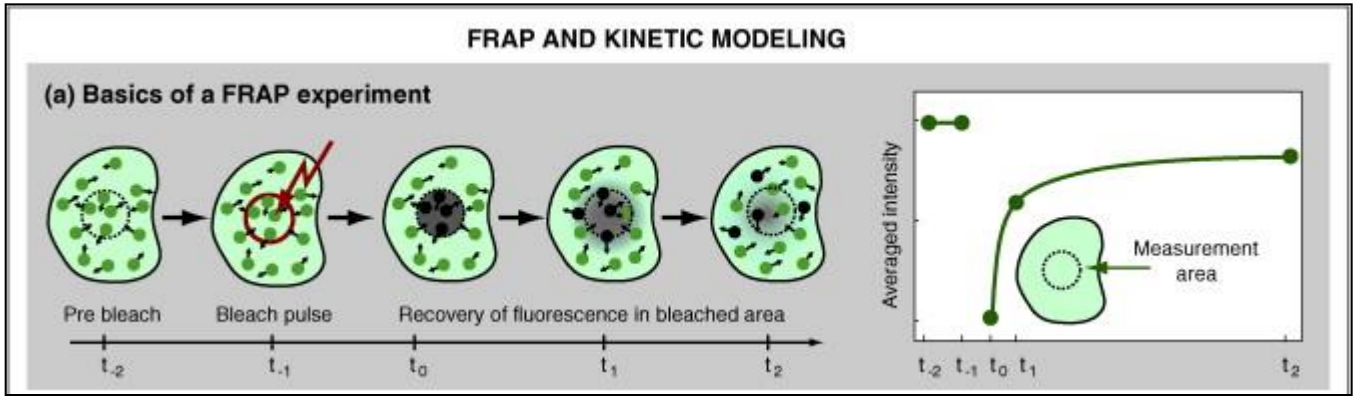
VinTS

Direct mean lifetime calculation in focal adhesions

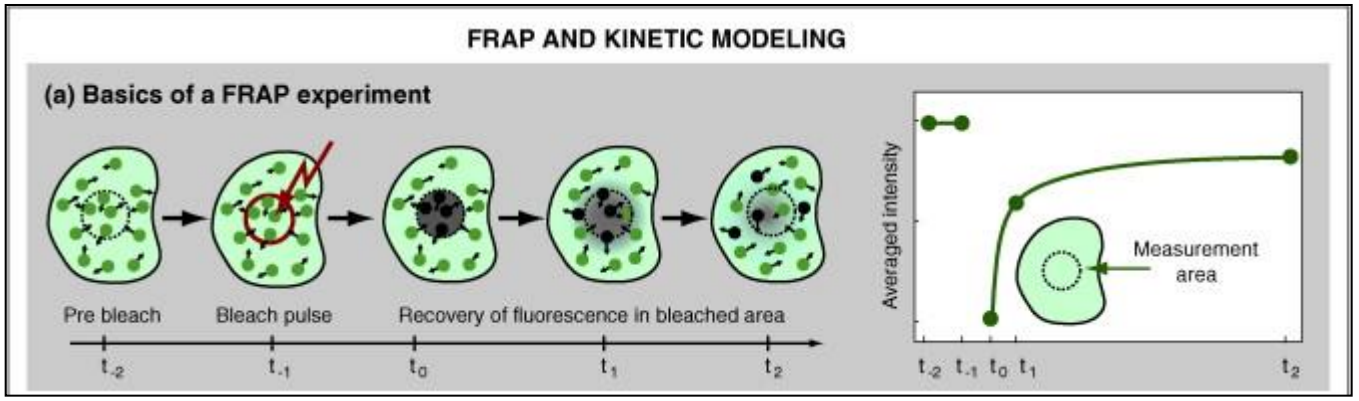


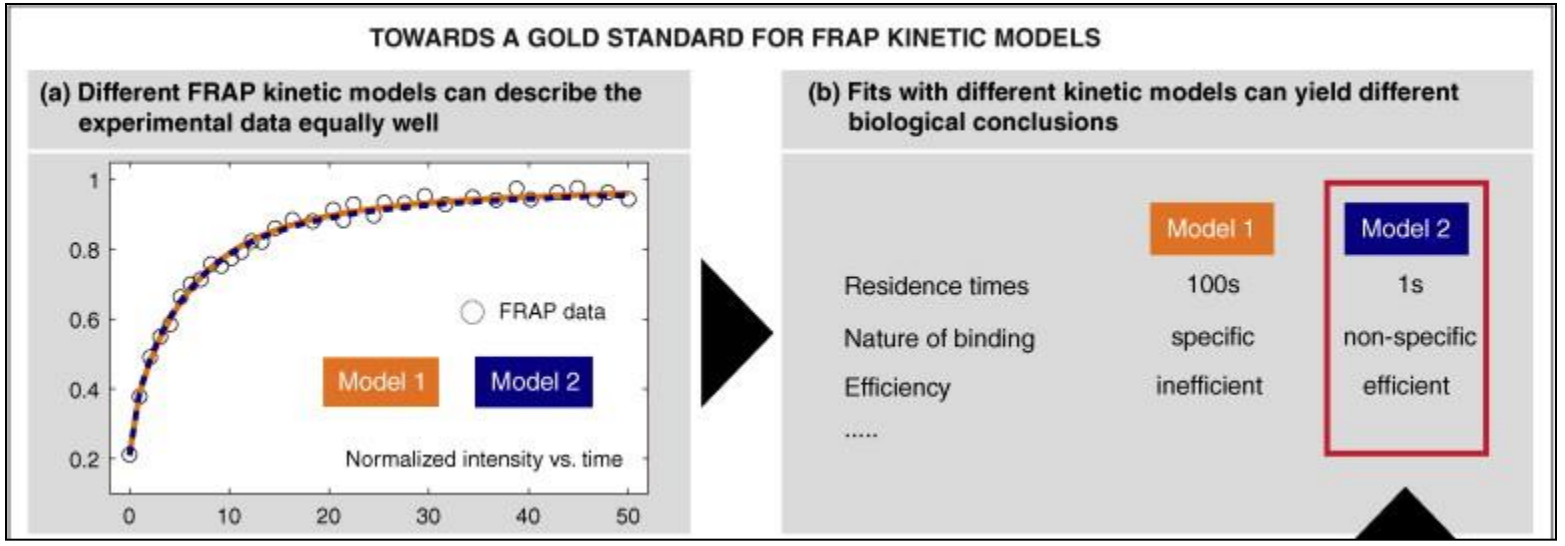
Unpublished data

Introduction: FRAP

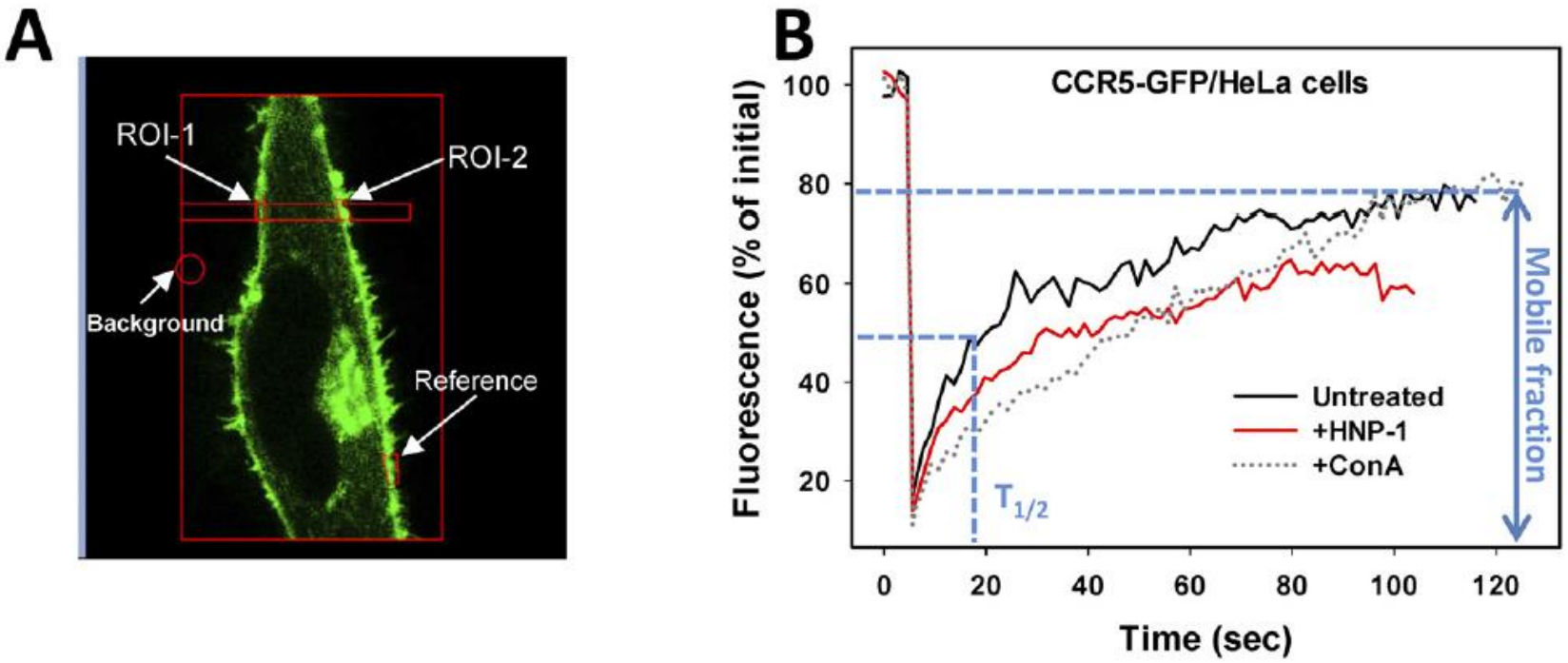


Introduction: FRAP





FRAP


A
ROI-1
ROI-2
Background
Reference
B
CCR5-GFP/HeLa cells
Fluorescence (% of initial)
100
80
60
40
20
0
20
40
60
80
100
120
Time (sec)
Untreated
+HNP-1
+ConA
$T_{1/2}$
Mobile fraction

Thanks!