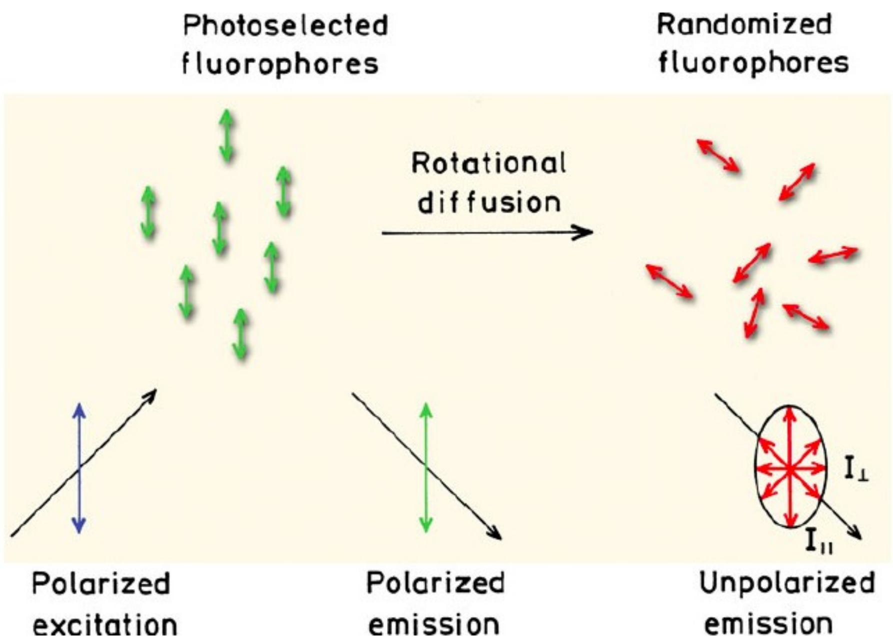
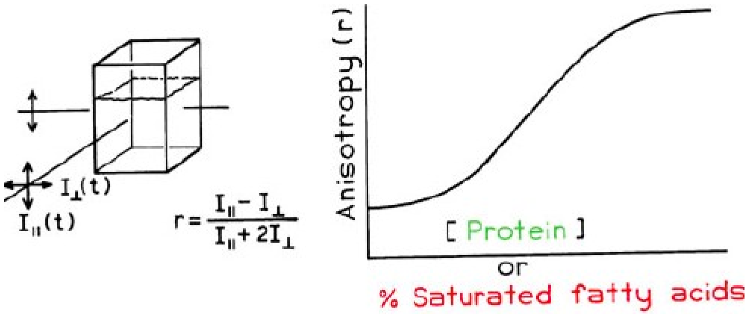
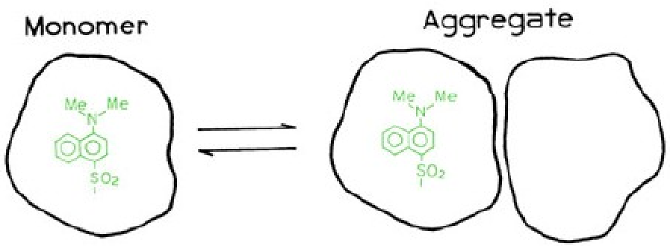
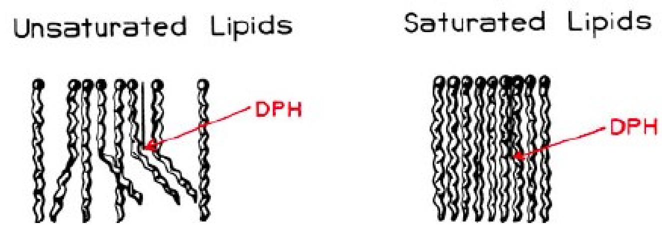
Anisotropy and Photoselection



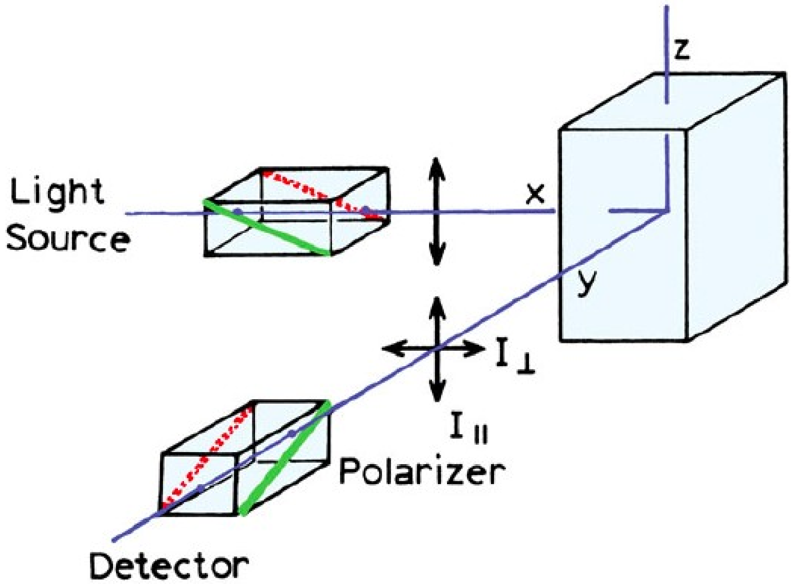


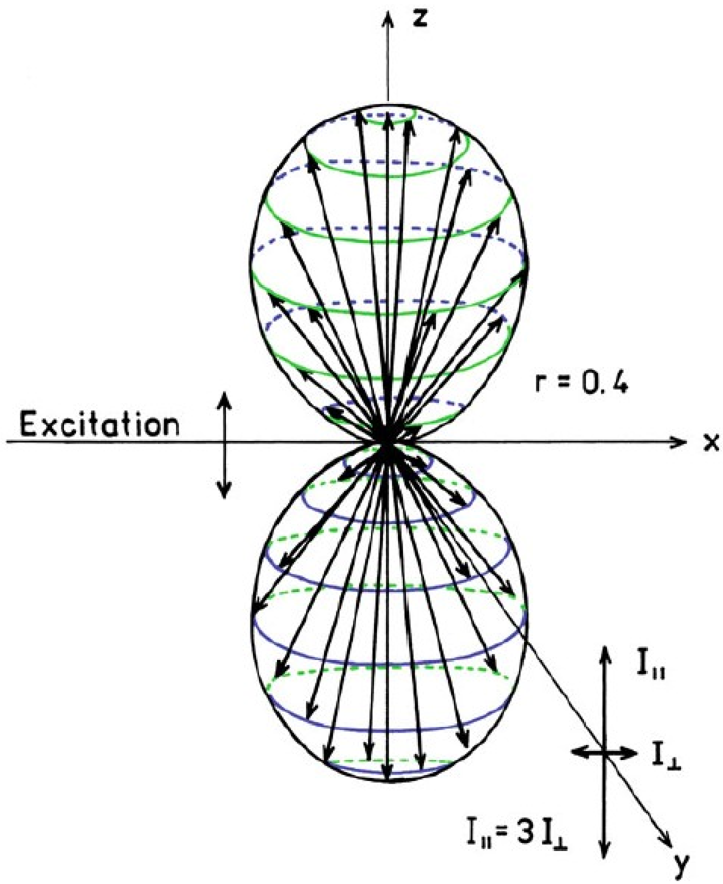




Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Anisotropy and Photoselection





Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Anisotropy and Magic Angle

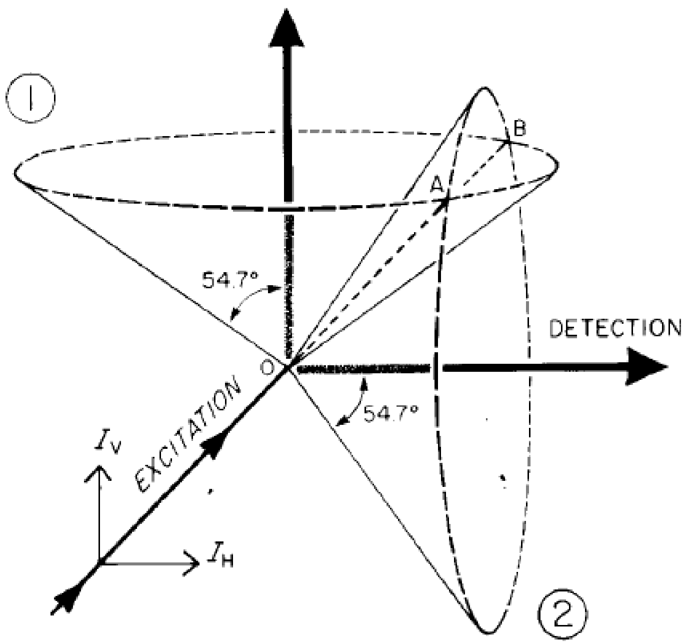


FIG. 15. "Magic" angle cones. Viewing the emission originating from O along the lines BO or AO eliminates the polarization related artifacts for any kind of excitation. See text for details.

Magic Angle conditions

$I_{total} = I_x + I_y + I_z = I_x + 2\ I_y$

$I_{viewed} = I_x \cos^2\theta + I_y \cos^2(90° - \theta)$

I_{viewed} will be proportional to I_{total} if

$[\cos^2(90° - \theta)]/(\cos^2\theta) = \tan^2\theta = 2$

then $\theta = 54.7°$

Badea and Brand, Meth Enzymol 61, 378 (1979)

Anisotropy and Fluorescence

Interactions (binding) and addition laws

$F_{total} = F_V + 2\ F_H$

$F_{total} = \sum f_i = \sum \varphi_i\, a_i = \sum \varphi_i\, \varepsilon_i\, c_i\, l$

$FR_{total} = \sum f_i\, r_i = \sum \varphi_i\, a_i\, r_i$ leads to the non-addition law:

$R_{total} \neq \sum r_i$ (except under special circumstances)

Molecular Processes & Dynamics

Time Scales and Techniques

1 ns — 1 µs — 1 ms — 1 s — Time scale

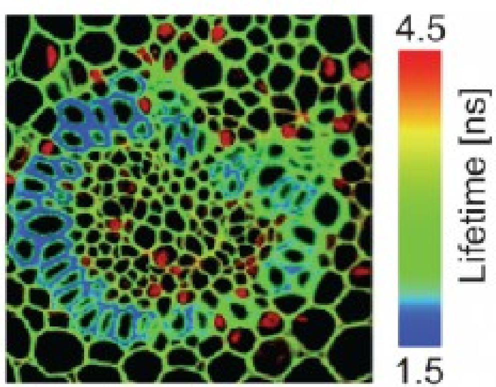
Local environment

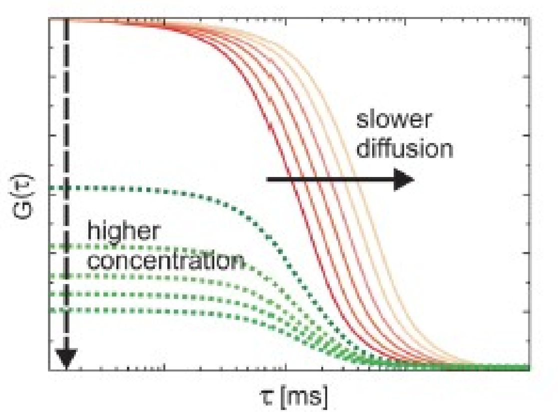
Translational diffusion

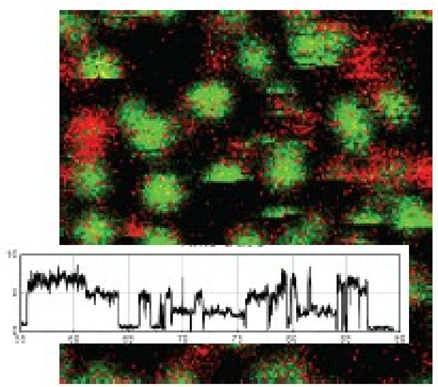
Rotational diffusion

Binding

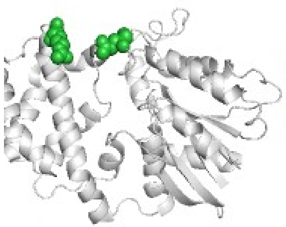
Conformation change



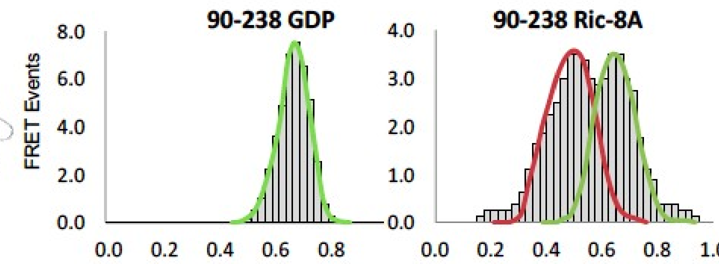




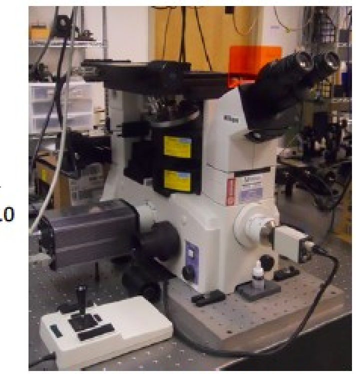
BioSpectroscopy Lab UM



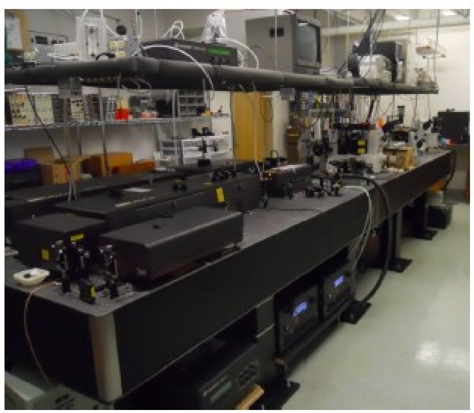
G Protein Dynamics (NIH)
Sprang & Ross, UM



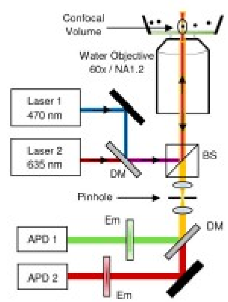
Labe Black, UM



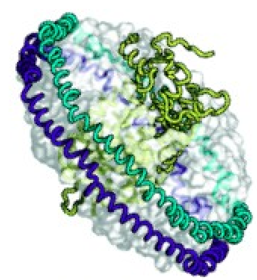
Fluorescence Microscope



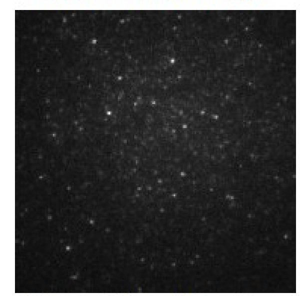
Ultra-Fast Lasers



SM-FRET setup
Chelle Terwilliger, UM

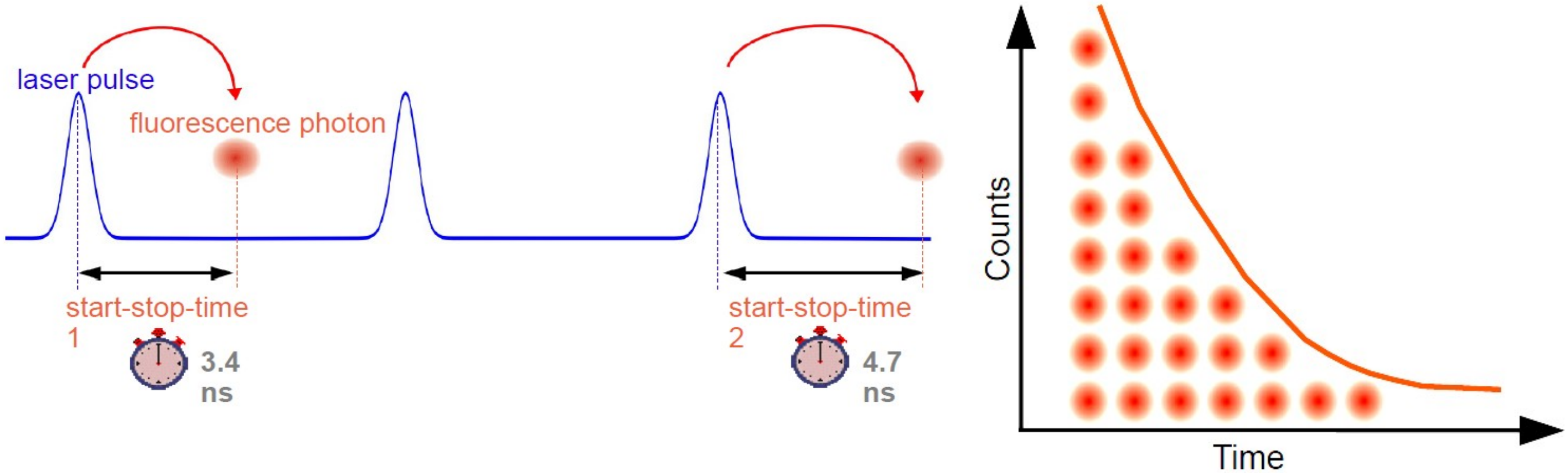


Nanodisc Project (NSF)
Ross & Bowler, UM



Surface–tethered Nanodiscs
Ayesha Sharmin, UM

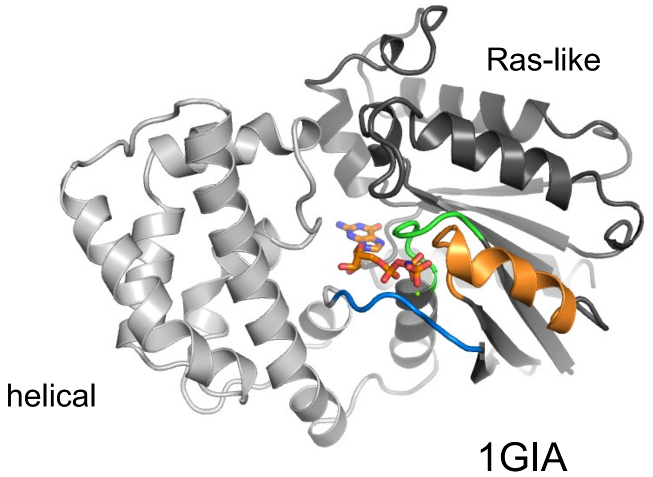
What is Time-Correlated Single-Photon Counting?

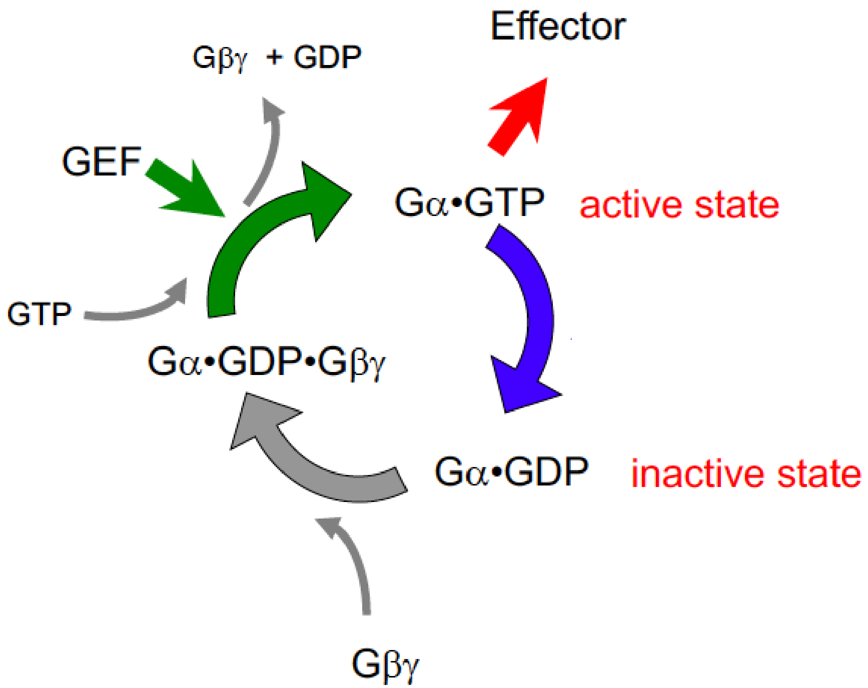


PicoQuant, Inc.

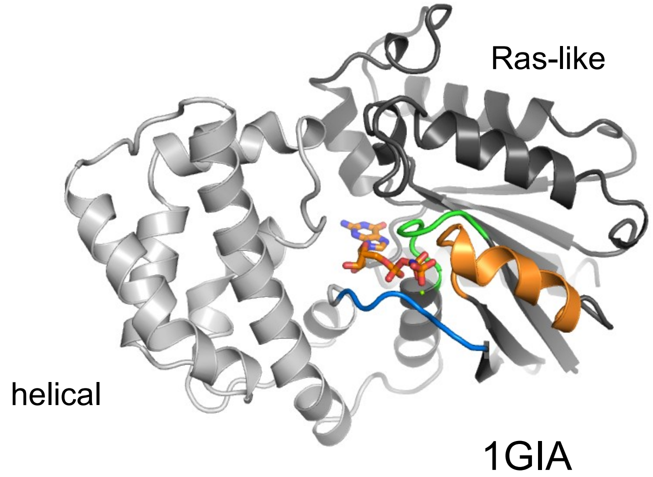
Spectroscopy

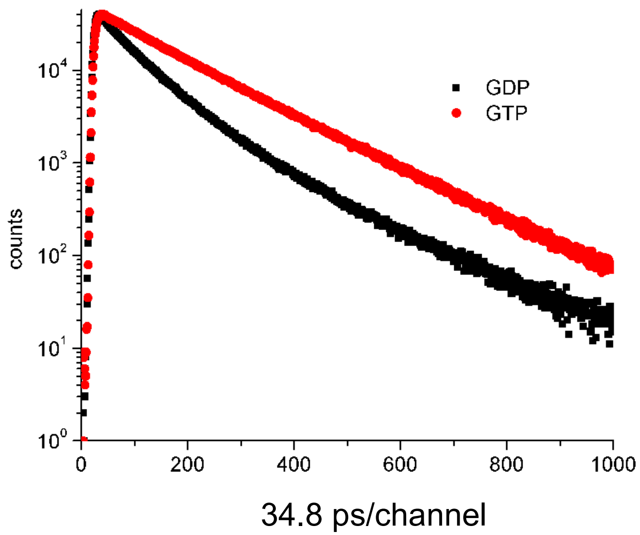
membrane receptor-dependent G-protein regulation





Probing Conformational States
Fluorescence Intensity Decay





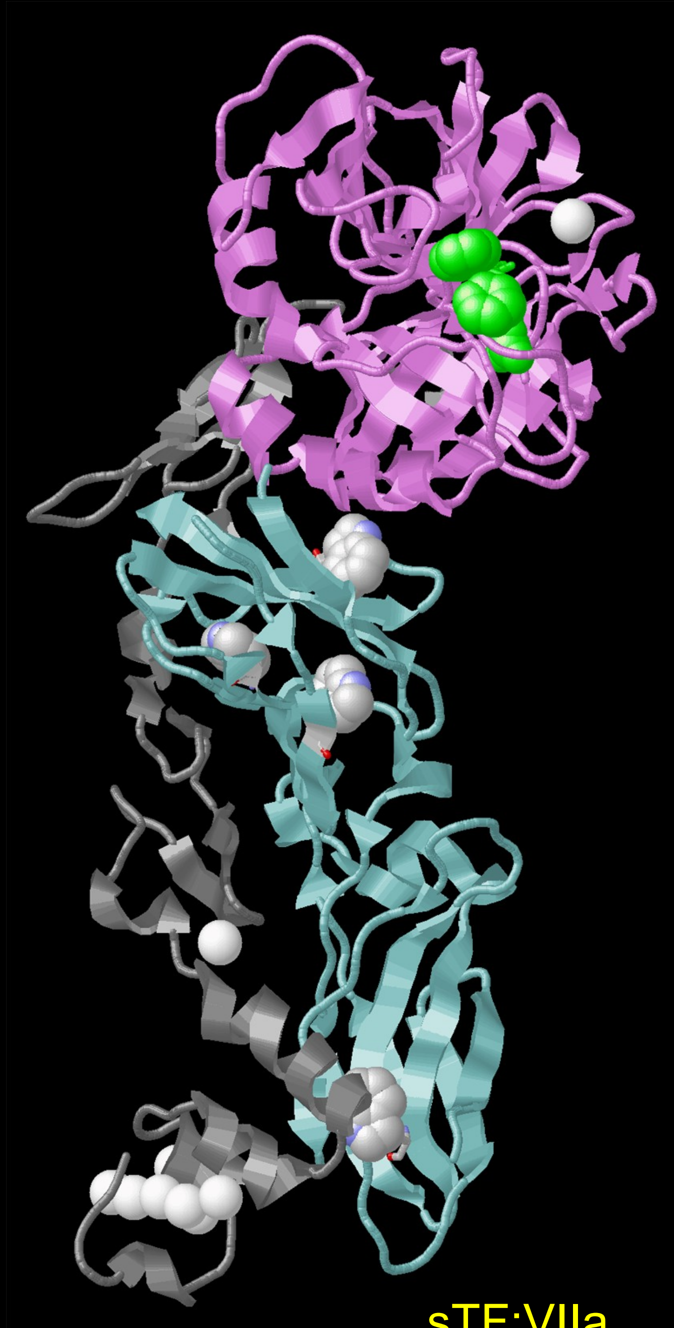
GTPγs-BODIPY bound state: $\langle\tau\rangle = 5.0$ ns

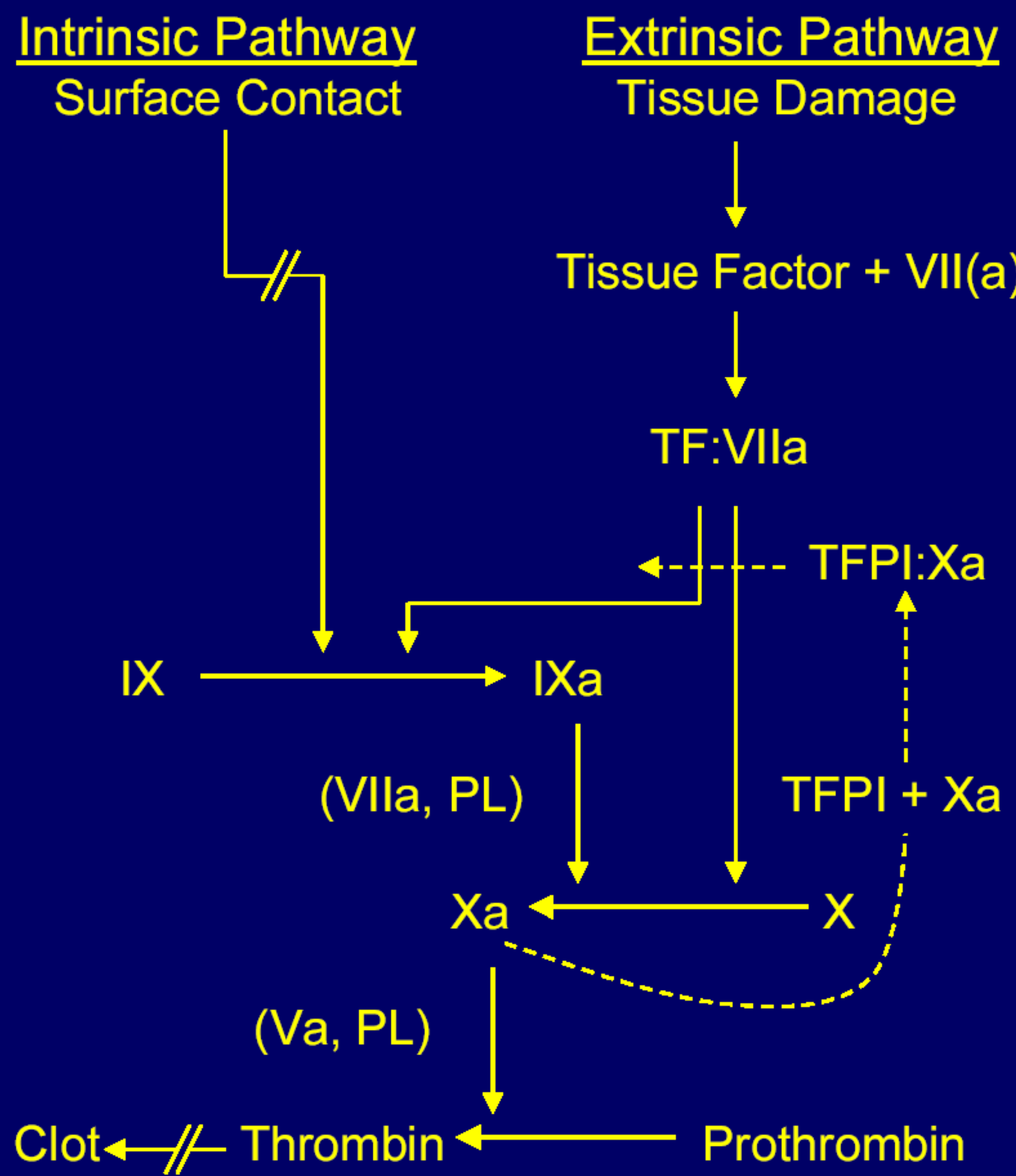
GDP-BODIPY bound state: $\langle\tau\rangle = 3.1$ ns

$$I(t) = \Sigma\, \alpha_i \exp(-t/\tau_i)$$

$$\langle\tau\rangle = \Sigma\, \alpha_i\, \tau_i^2 / \Sigma\, \alpha_i\, \tau_i$$

Celestine Thomas, Sandy Ross

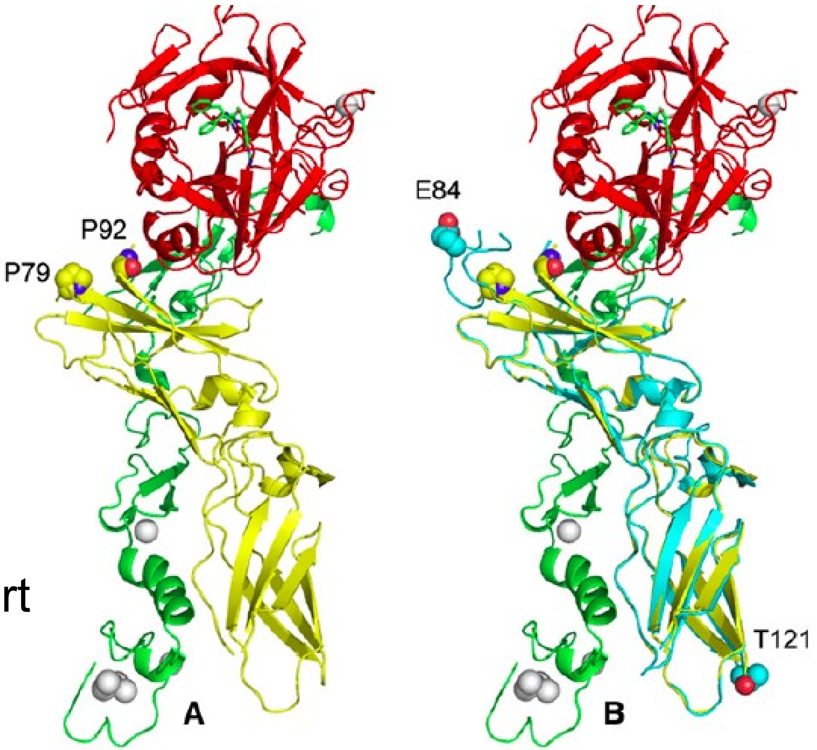

sTF:VIIa


Intrinsic Pathway
Surface Contact
Extrinsic Pathway
Tissue Damage
Tissue Factor + VII(a)
TF:VIIa
TFPI:Xa
IX
IXa
(VIIa, PL)
TFPI + Xa
Xa
X
(Va, PL)
Clot
Thrombin
Prothrombin

Probing Conformational States
Anisotropy Decay

$r(t) = \Sigma\, \beta_j \exp(-t\,/\,\phi_j)$

$j = 1$ to 5, $\Sigma\, \beta_j = r_0$



In proteins, with fluorescence probes attached, for example to cys, analysis of the anisotropy decay usually yields two ϕ_j terms. The long correlation time reflects global motion. The short correlation time reflects segmental motion(s).

in sTF alone, ϕ_{long} ~ 19 ns and ϕ_{short} ~ 1-2 ns.

The amount of segmental motion decreases on formation of the complex with factor VIIa.

Minnazo, A, et al, (2009) Biophys J 96, 681

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Fluorescence Correlation Spectroscopy (FCS)

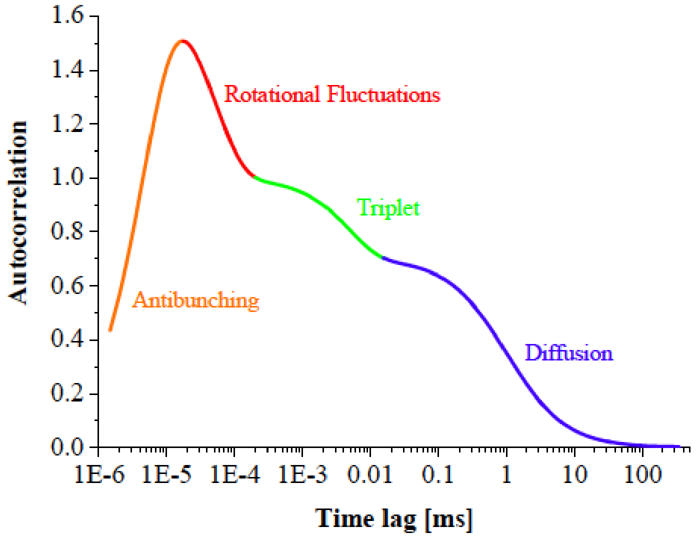
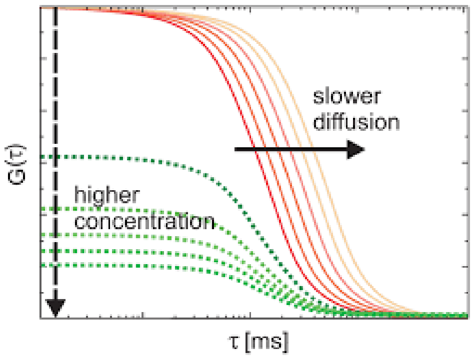


Figure 11: Timescales of various processes monitored by autocorrelation analysis

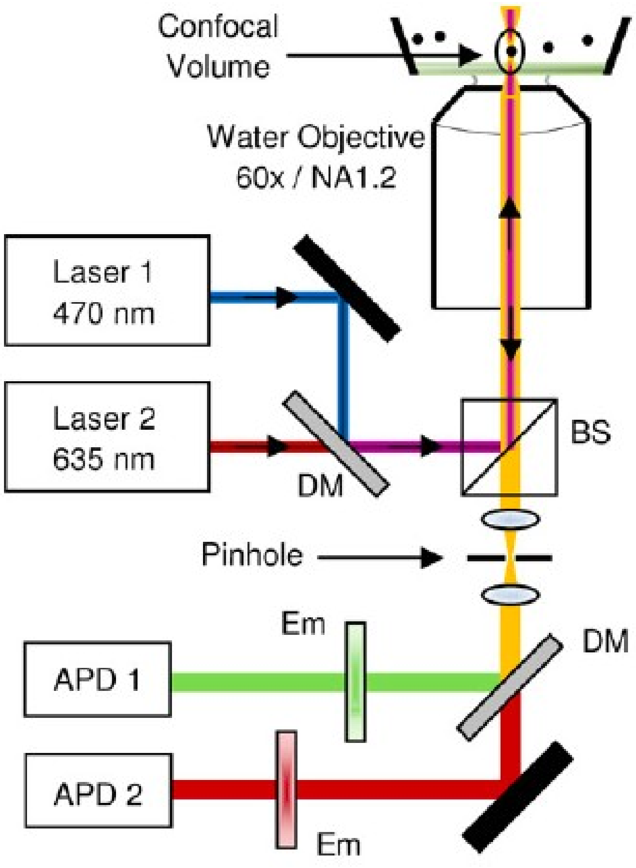


$$D_r = \frac{k_B T}{8\pi\eta {R_H}^3}$$

$$D_t = \frac{k_B T}{6\pi\eta R_H}$$

$$D_r = D_t \frac{3}{4{R_H}^2}$$

Optics to Observe Freely Diffusing Molecules



BCRL New Instrumentation

Zeiss 880 CLSM
PicoQuant FLIM/FCS

PicoQuant
MicroTime 200

NSF MRI CHE- CHE-1531520
Award Amount: $ 323k

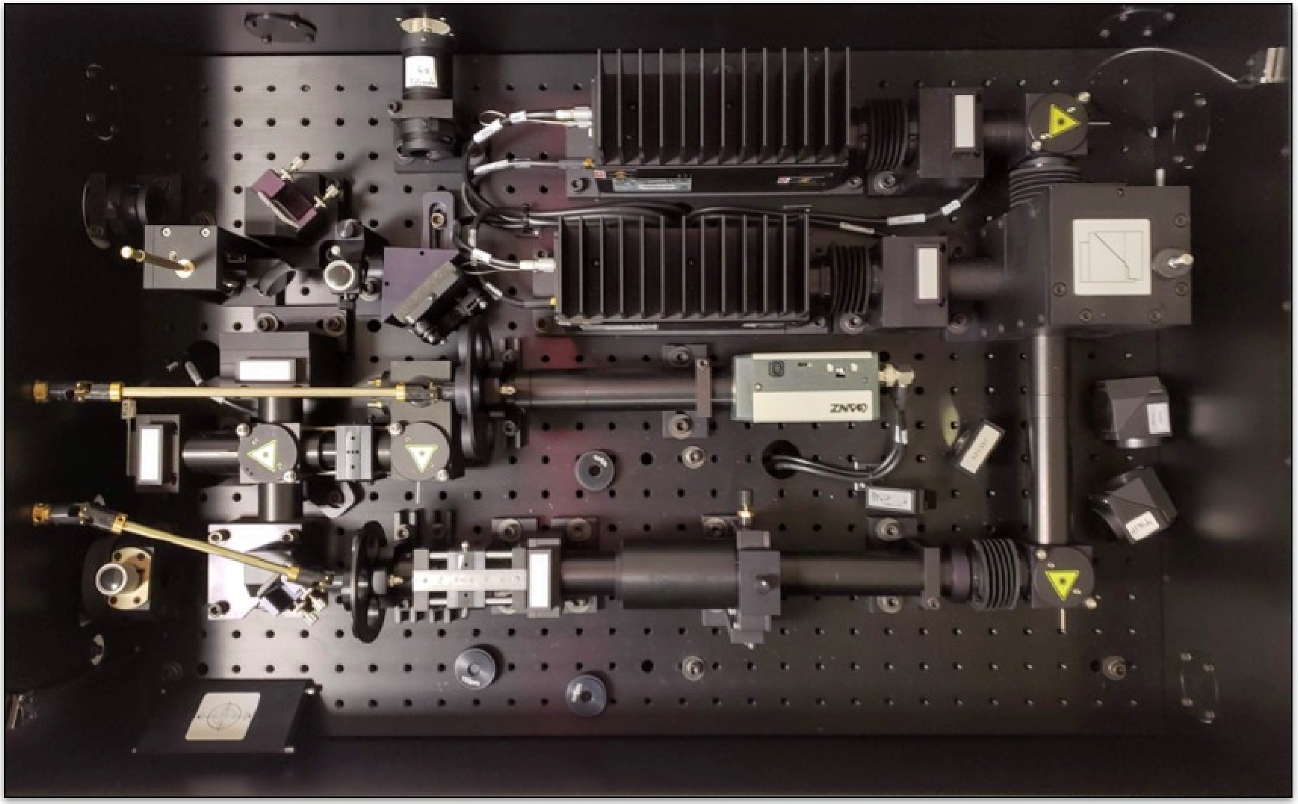
NIH S10 OD021806
Award Amount: $ 555k

Murdock Charitable Trust
Award Amount: $191k

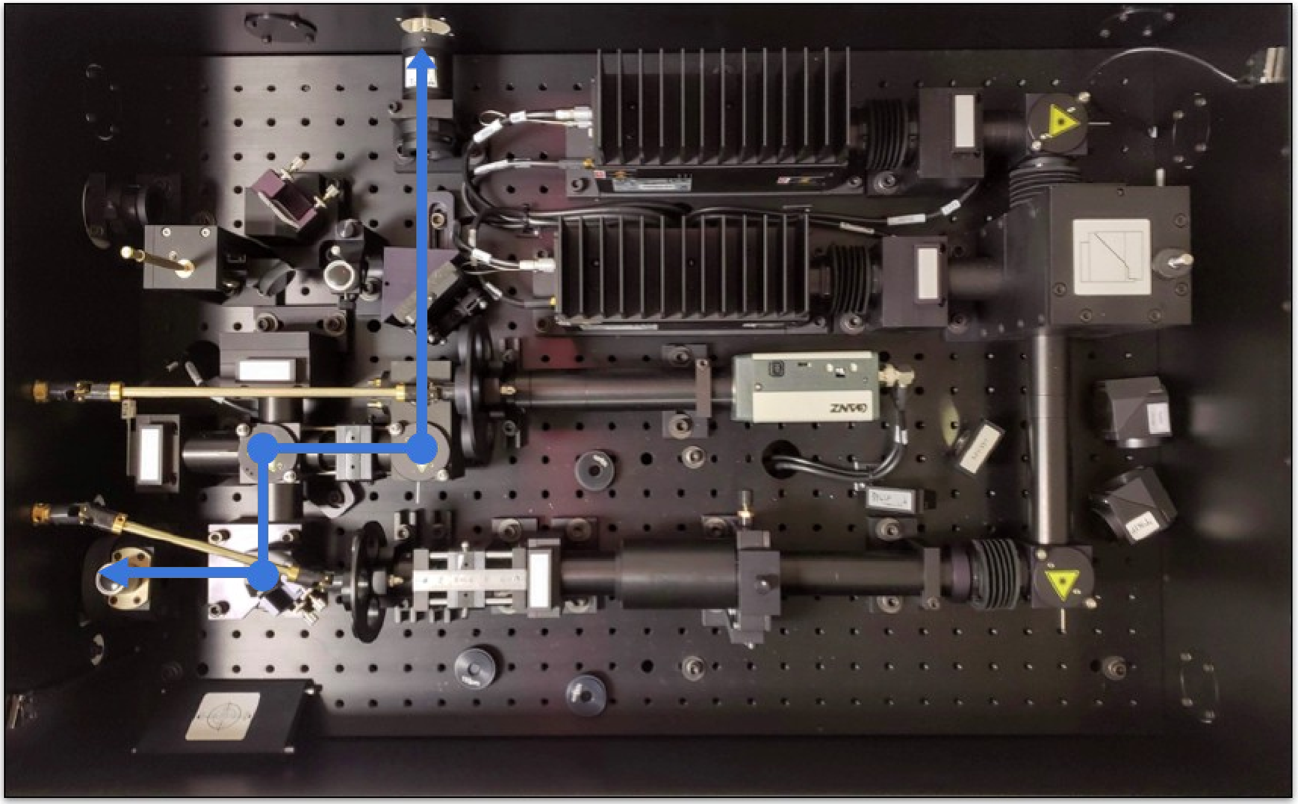
UM VPRCS: $127K

MicroTime 200

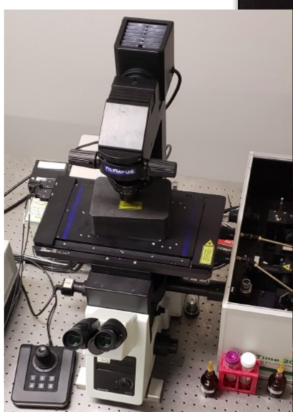
MicroTime 200

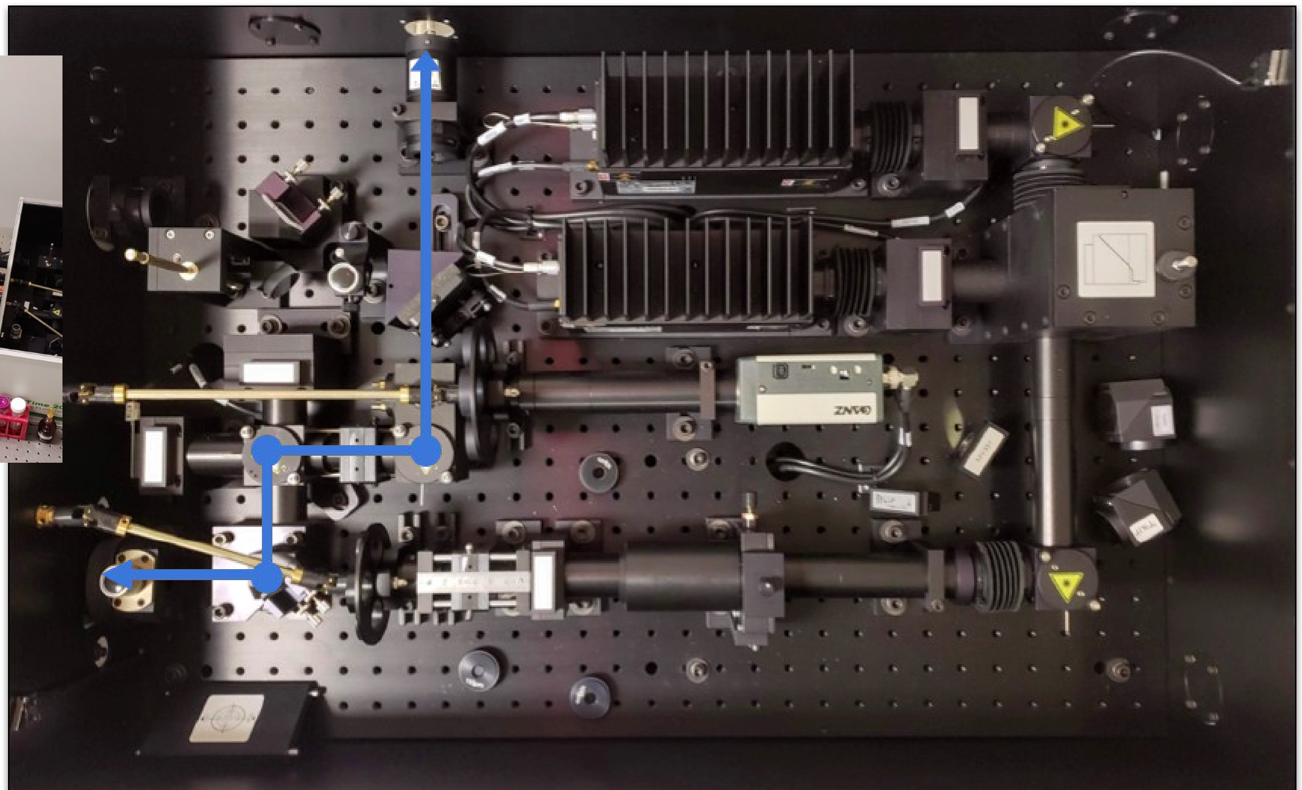


MicroTime 200

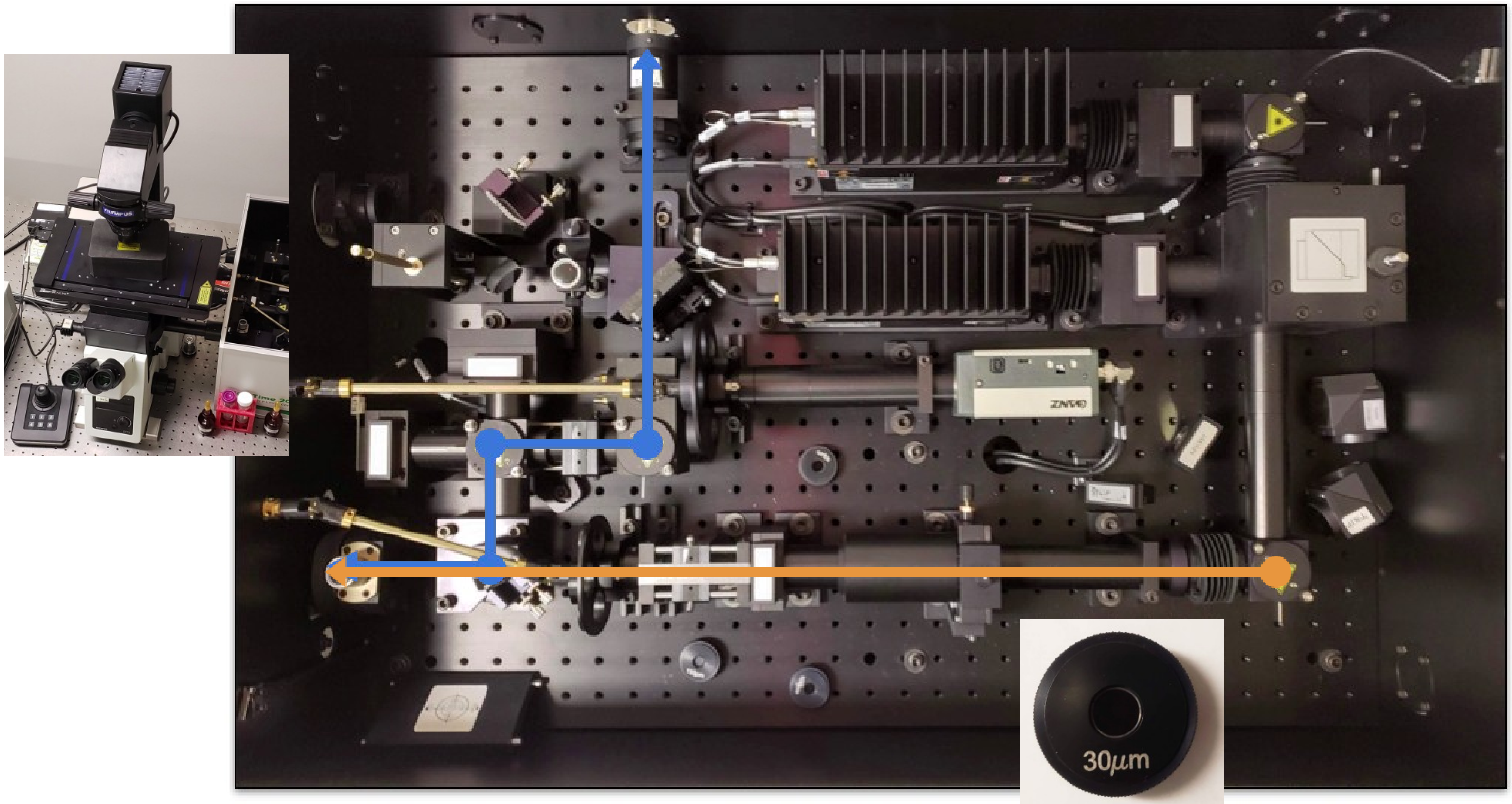


MicroTime 200

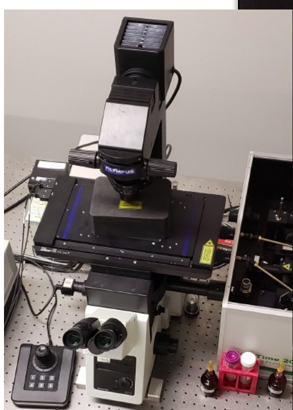


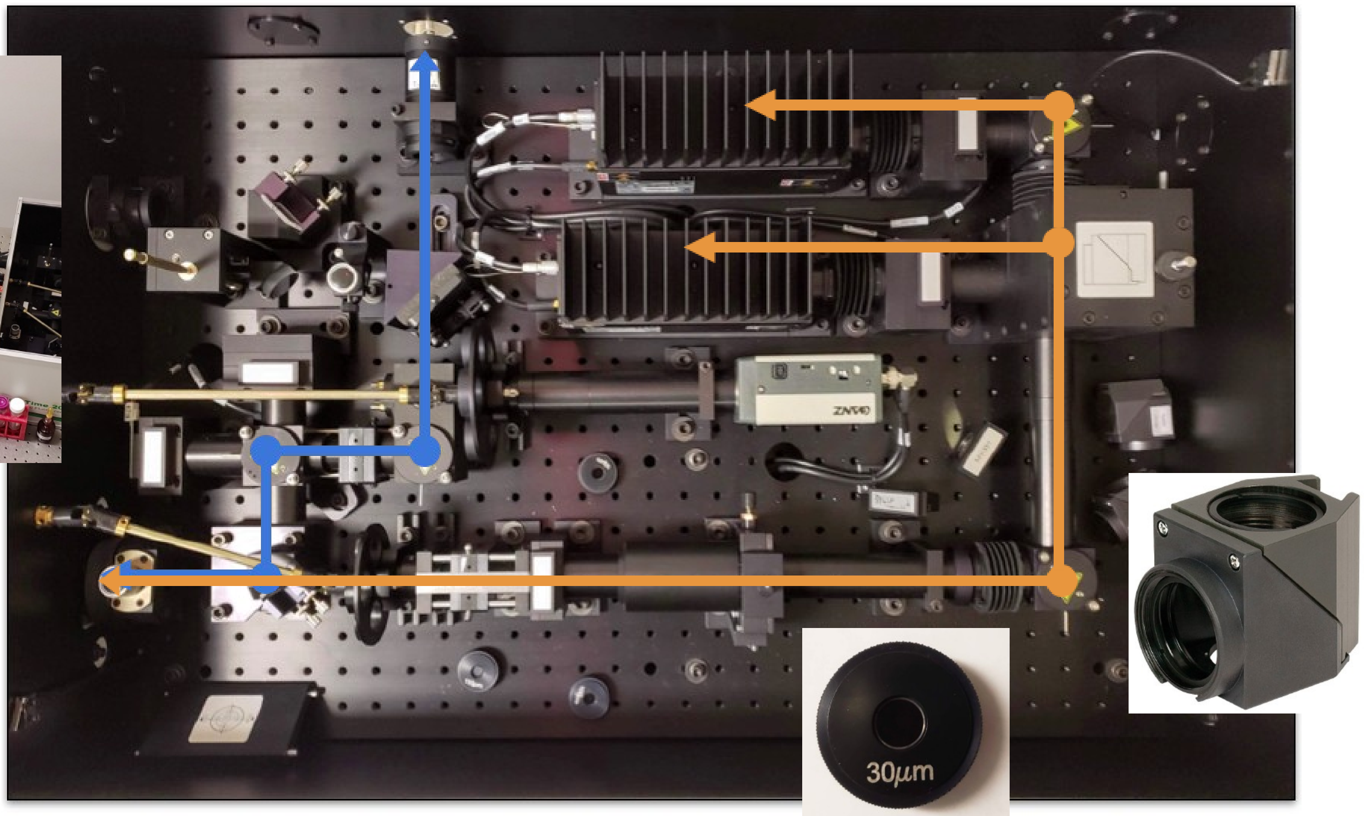


MicroTime 200



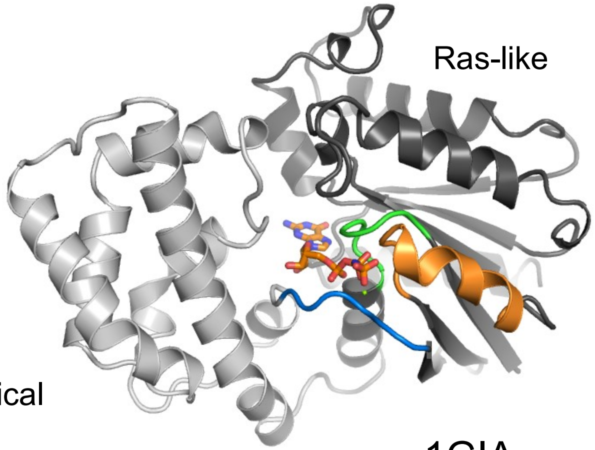
MicroTime 200



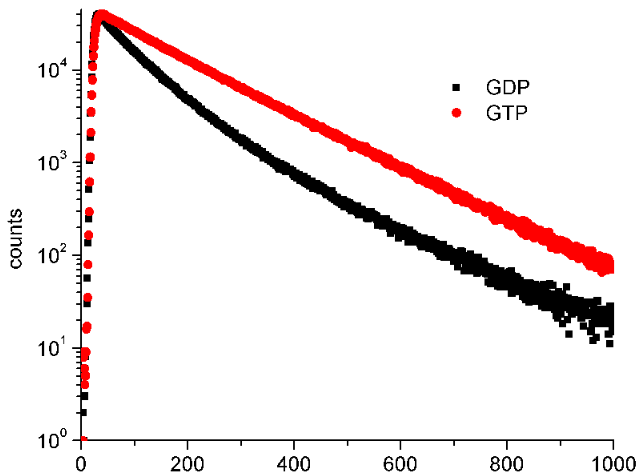


Spectroscopy

Probing Conformational States
Fluorescence Intensity Decay



1GIA



34.8 ps/channel

GTPγs-BODIPY bound state: $\langle\tau\rangle$ = 5.0 ns

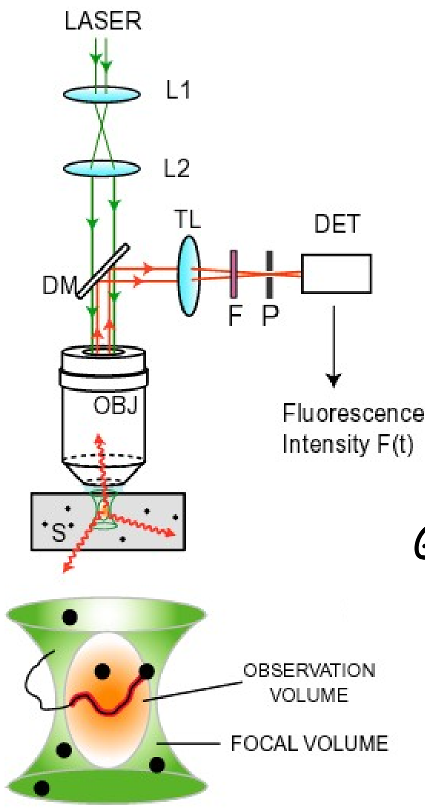
GDP-BODIPY bound state: $\langle\tau\rangle$ = 3.1 ns

$$I(t) = \Sigma\, \alpha_i \exp(-t/\tau_i)$$

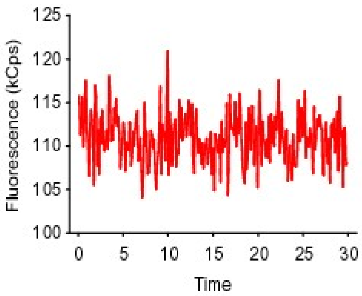
$$\langle\tau\rangle = \Sigma\, \alpha_i\, \tau_i^2 / \Sigma\, \alpha_i\, \tau_i$$

Celestine Thomas, Sandy Ross

Spectroscopy



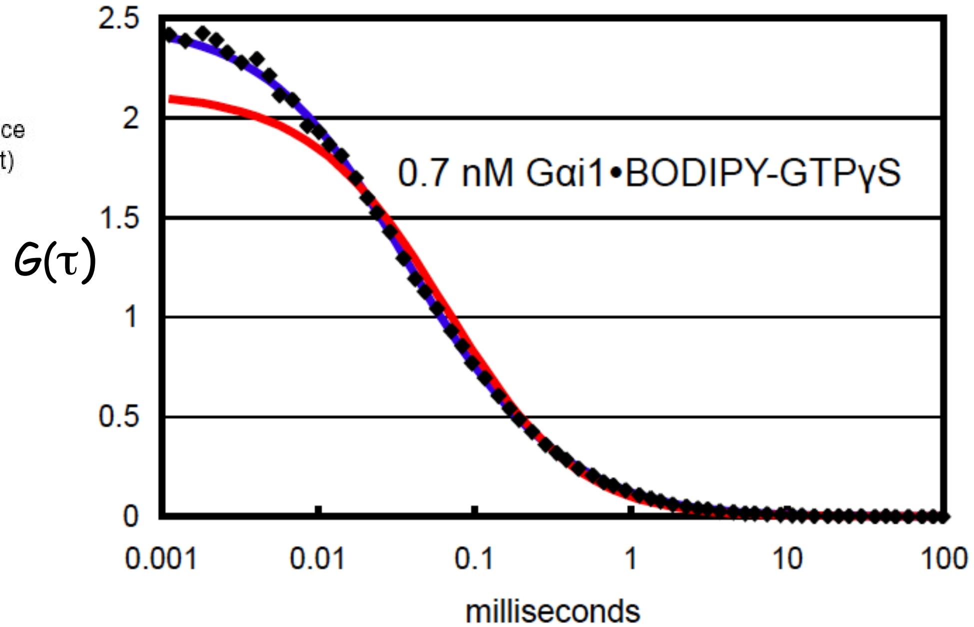
Petra Schwille



$\tau_D = r_o^2/4D$

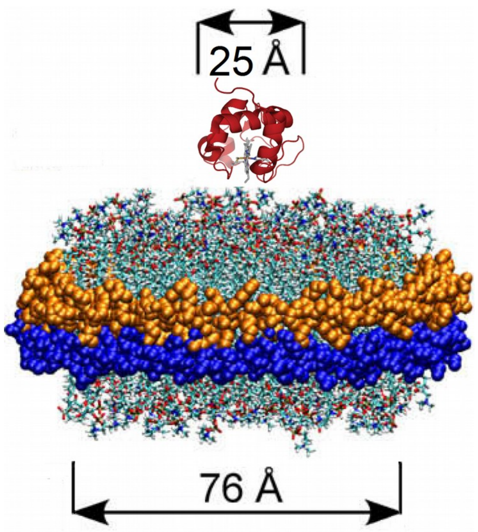
$D = kT/6\pi\eta R_h$

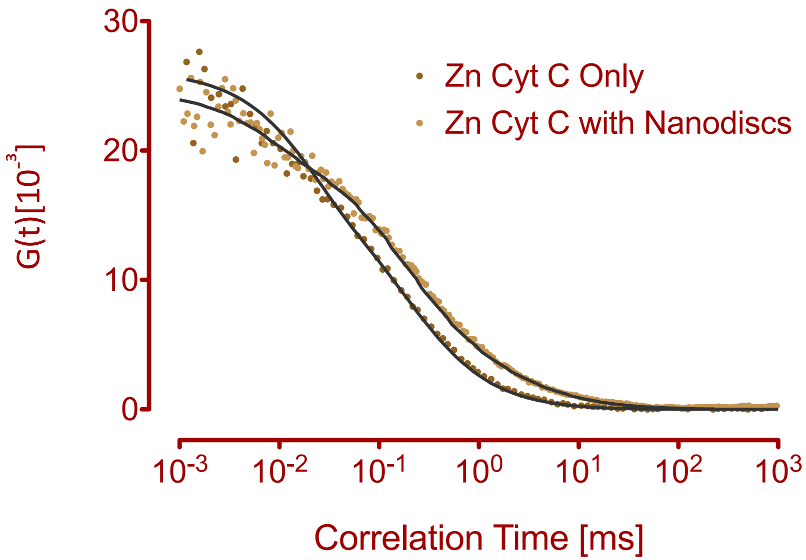
$G(0) = 1/\langle N \rangle$



Celestine Thomas, Sandy Ross

Harmen's MicroTime project





Harmen's MicroTime project

FCS

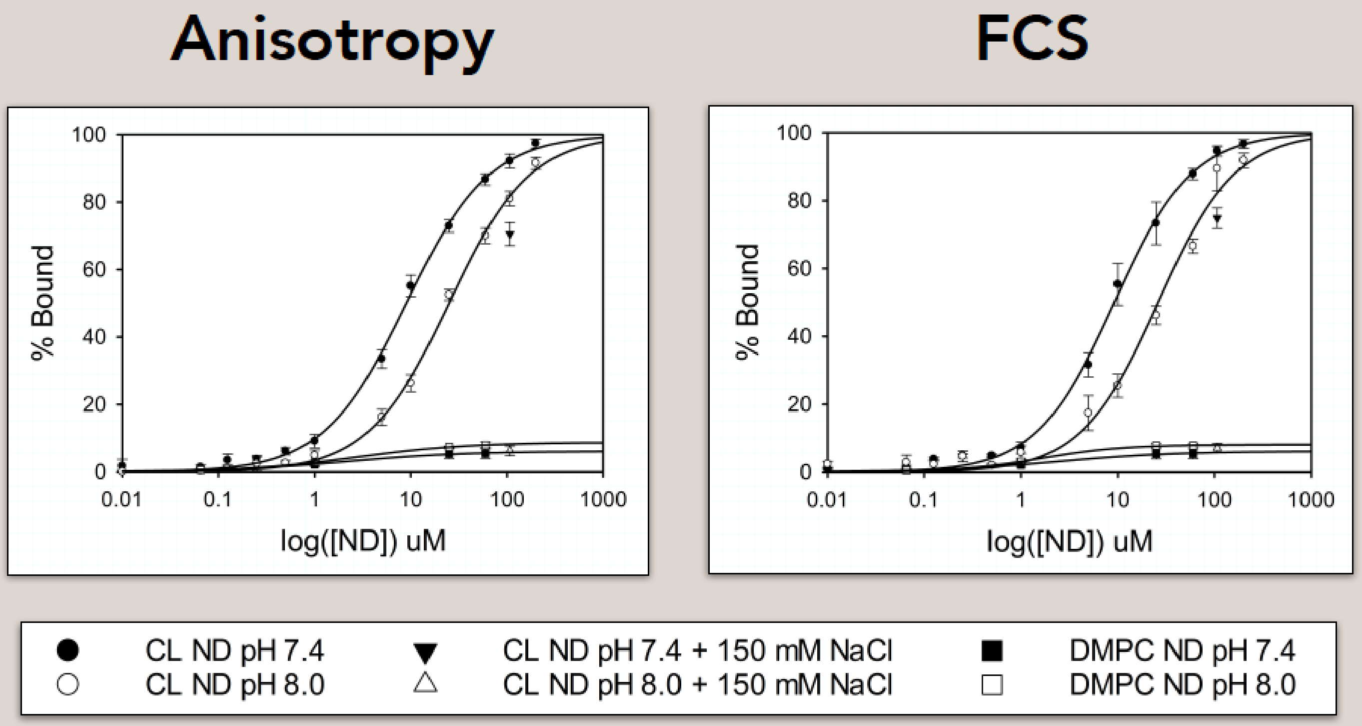
Variant	τ_D (μs)	τ_T (μs)	D (μm²/s)	R_H (nm)
Monomer	0.061 ± 0.010	12 ± 9.1	125 ± 21	1.80 ± 0.25
Monomer & CL ND	0.480 ± 0.031	2.7 ± 1.9	33 ± 2.6	6.83 ± 0.51
Dimer	0.083 ± 0.008	25 ± 5.2	92 ± 8.2	2.39 ± 0.19
Dimer & CL ND	0.440 ± 0.23	2.7 ± 2.1	22 ± 2.4	10.05 ± 0.9

Anisotropy

Variant	*Intensity-Weighted Lifetime (ns)*	*Correlation time (ns)*	R_H (nm)
Monomer	3.27 ± 0.31	6.37 ± 0.16	1.83 ± 0.05
Dimer	4.55 ± 0.04	12.7 ± 0.17	2.31 ± 0.04

Spectroscopy

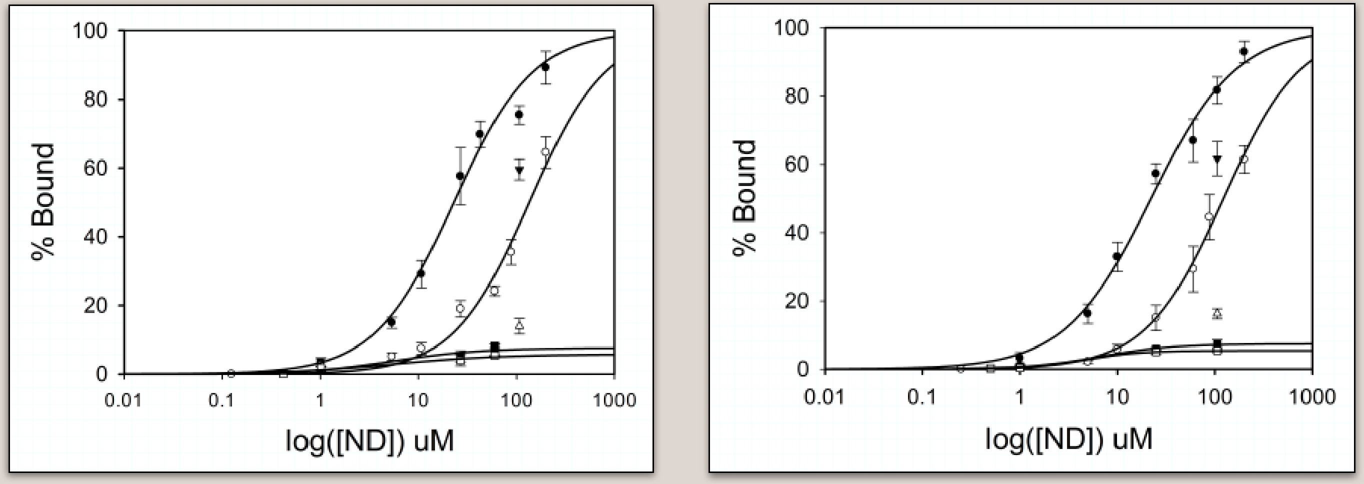
Monomer Binding to a Nanodisc



Dimer Binding to a Nanodisc

Anisotropy

FCS

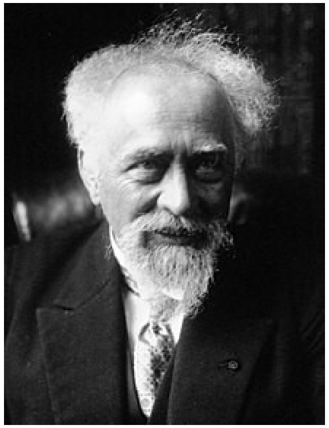


● CL ND pH 7.4
○ CL ND pH 8.0
▼ CL ND pH 7.4 + 150 mM NaCl
△ CL ND pH 8.0 + 150 mM NaCl
■ DMPC ND pH 7.4
□ DMPC ND pH 8.0

Kinetic Binding Data from Fluorescent Experiments

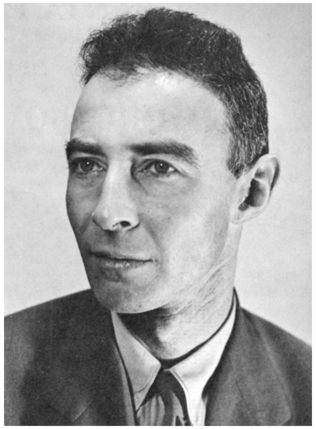
		pH 7.4		pH 8.0	
		K_d (μM)	$-\Delta G$ (kcal/mol)	K_d (μM)	$-\Delta G$ (kcal/mol)
Monomer	**Anisotropy**	9.0 ± 0.2	11.5	25.0 ± 0.6	9.0
	FCS	9.2 ± 0.3	11.4	26.1 ± 1.3	8.9
Dimer	**Anisotropy**	23.0 ± 1.5	9.2	134.5 ± 7.1	4.9
	FCS	22.1 ± 1.2	9.5	122.8 ± 6.0	5.2

Spectroscopy



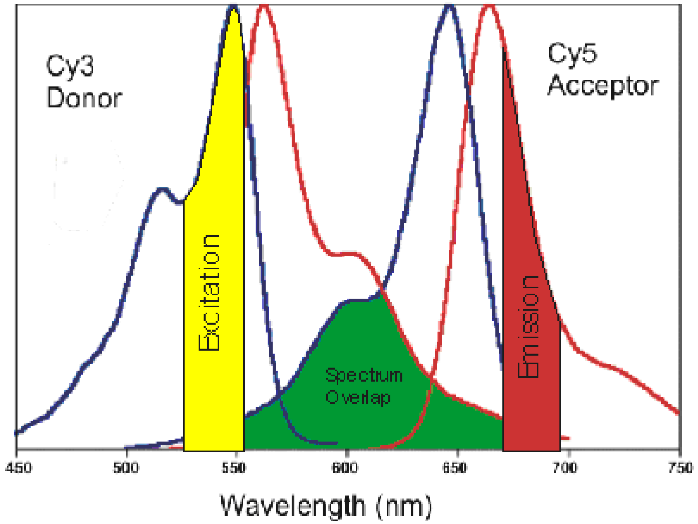
Jean B. Perrin
1870-1942

Francis Perrin
1901-1992

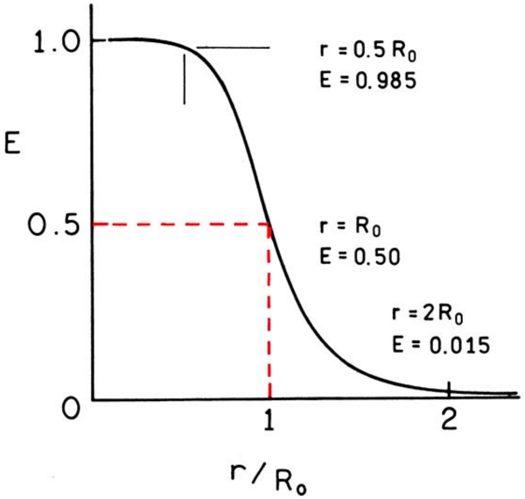


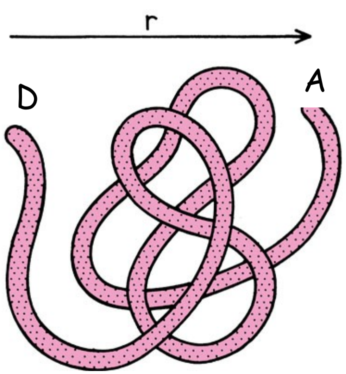
J. Robert Oppenheimer
1904-1967

Theodor Förster
1910-1974



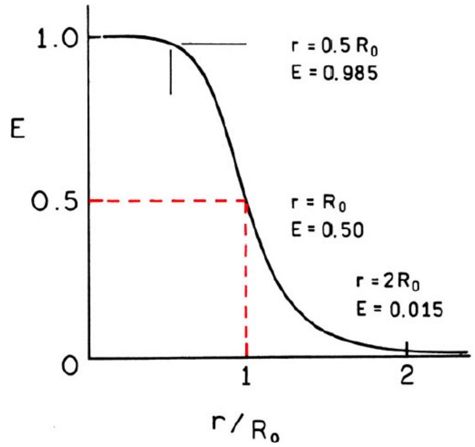
Held, BioTek.com, 2005

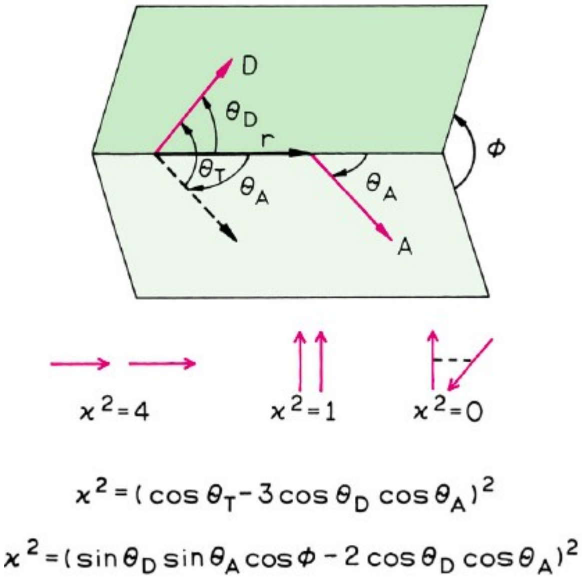


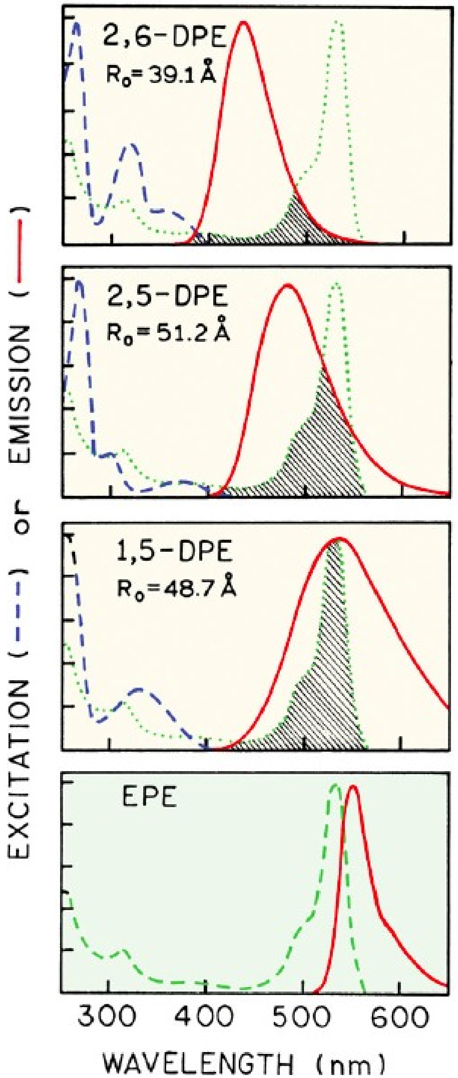


Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Resonance Energy Transfer



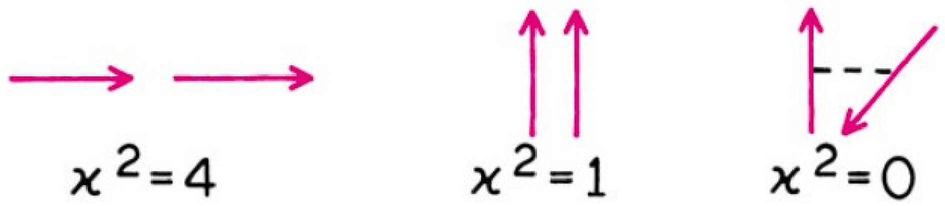




Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Resonance Energy Transfer

The orientation factor, κ^2



The orientation factor, κ^2 can be calculated from the projections of the 9 combinations of donor and acceptor axes (draw projection of donor axes on acceptor axes):

1 orientation where $\kappa^2 = 4$

2 orientations where $\kappa^2 = 1$

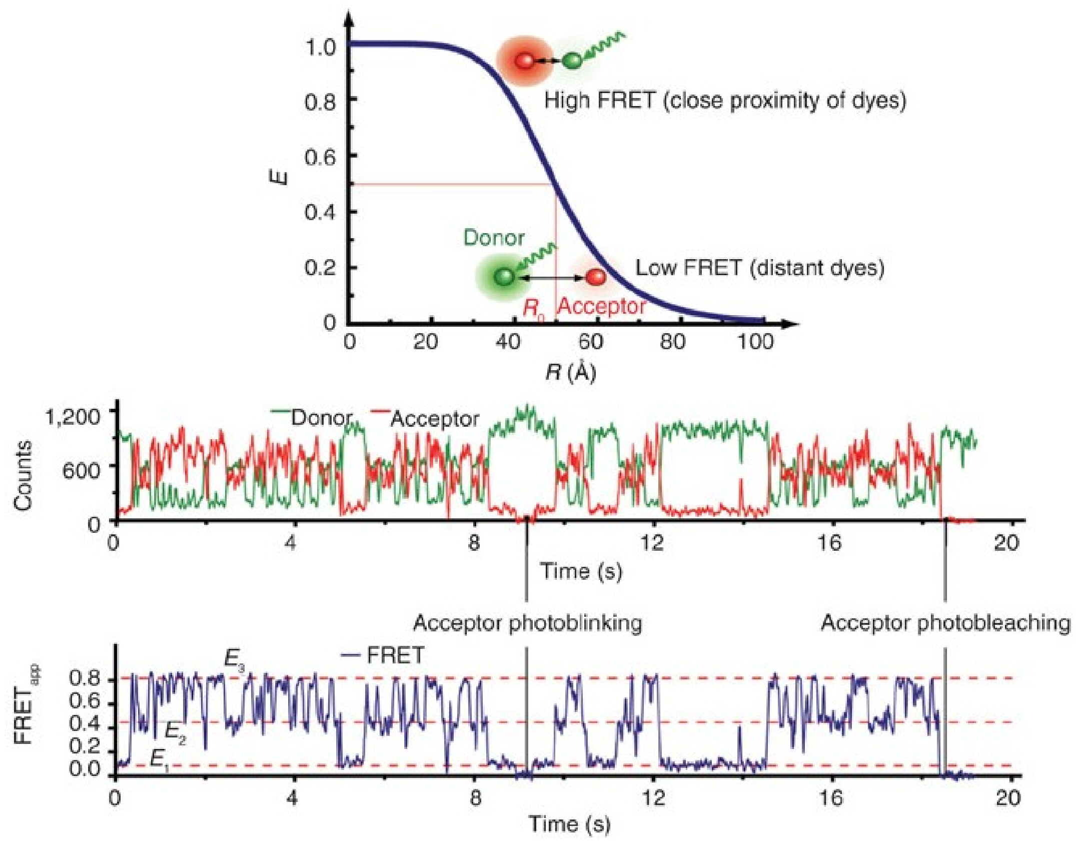
6 orientations where $k^2 = 0$

3 of the 9 combinations contribute to FRET: $\sum\kappa^2 = (1 \times 4) + (2 \times 1) = 6$

6 of 9 total combinations do not contribute to FRET: $\sum\kappa^2 = 0$

So the average κ^2 for all combinations is 6/9 = 2/3

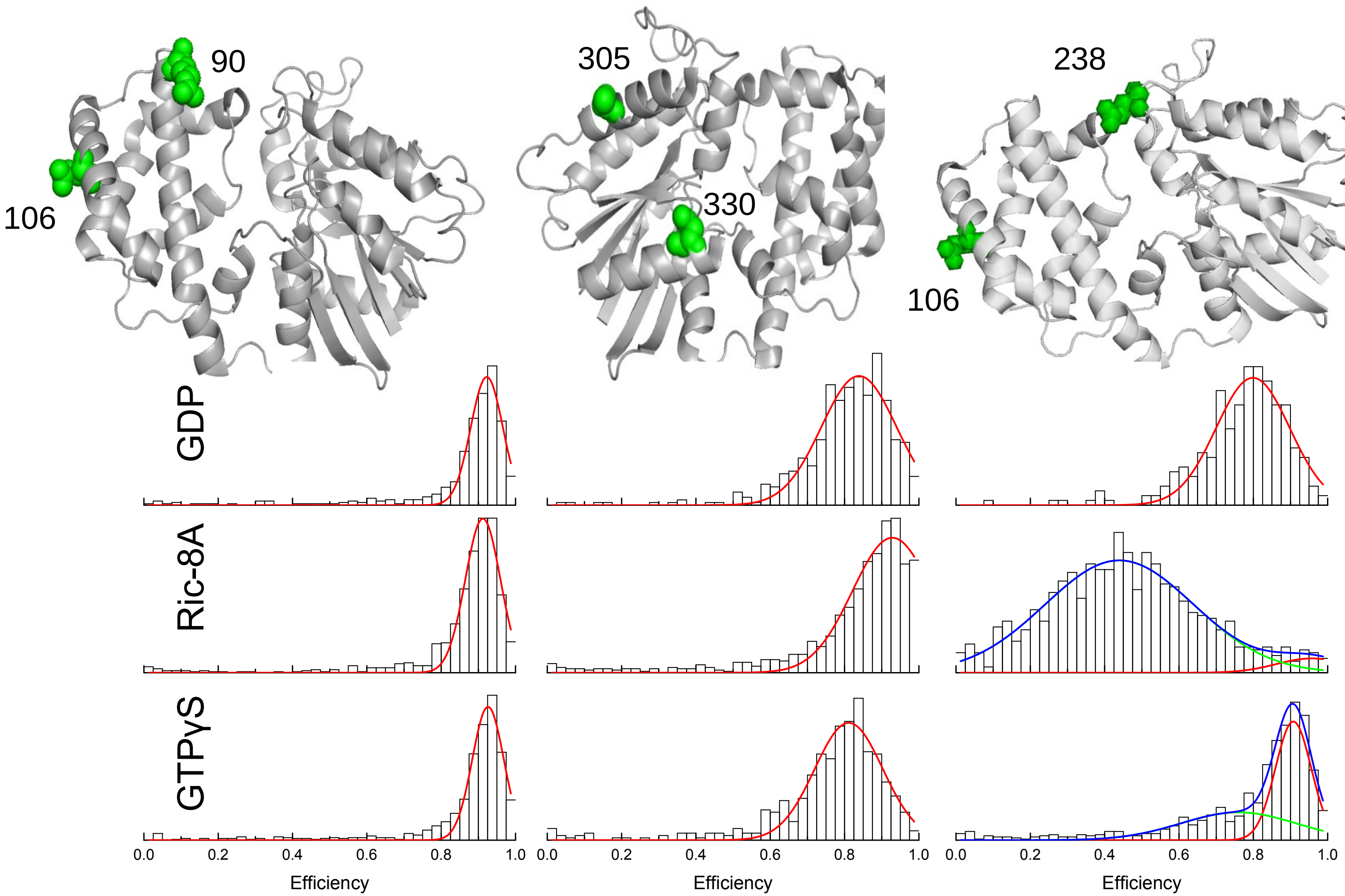
FRET in freely-diffusing single molecules



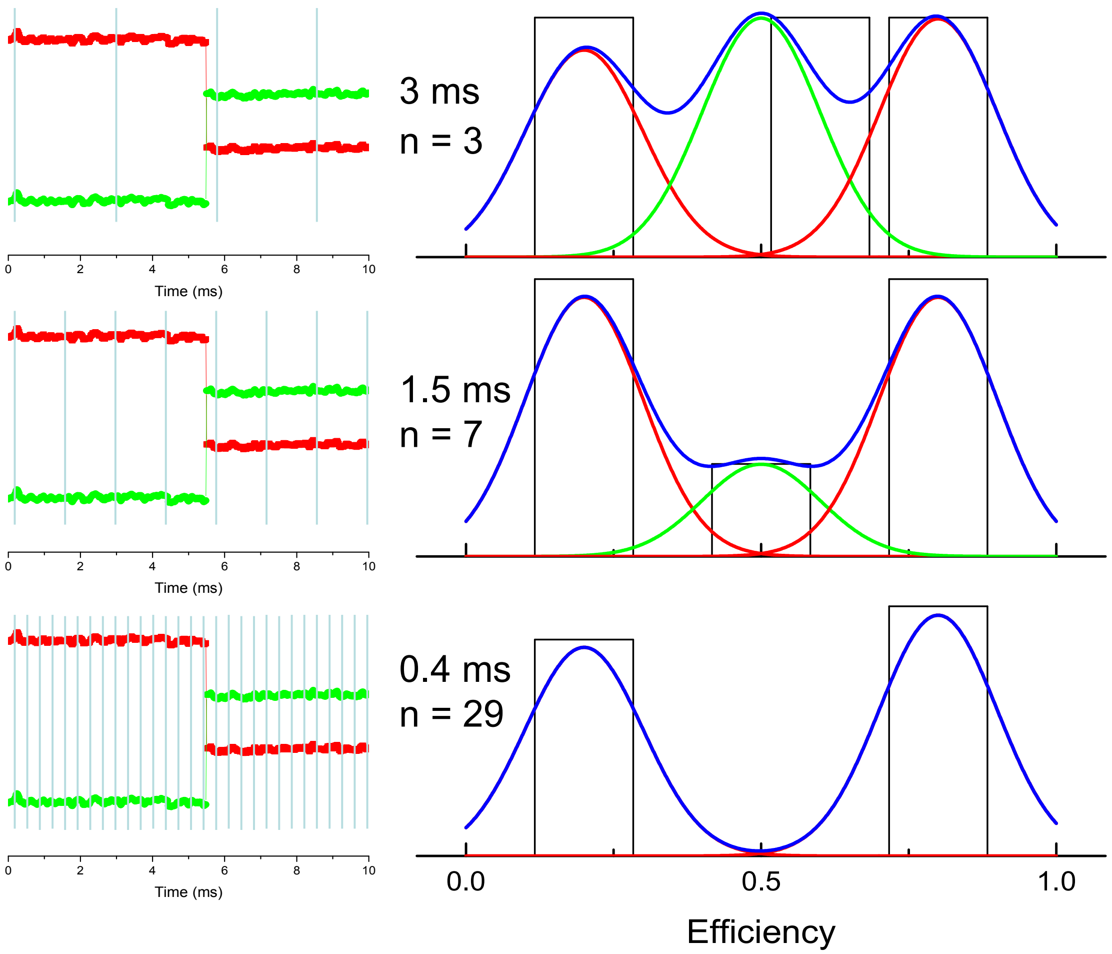
Roy, Hohng & Ha, *A practical guide to single-molecule FRET* Nature Methods (2008)

Spectroscopy

1 ms binning and 25 (minimum) photons per event



3-Gaussian Model for Two-State Conformational Equilibria

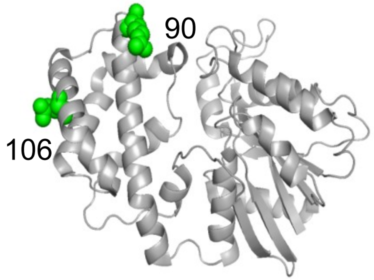


Gopich, Szabo J. Phys. Chem. B 111:12925 (2007)

Spectroscopy

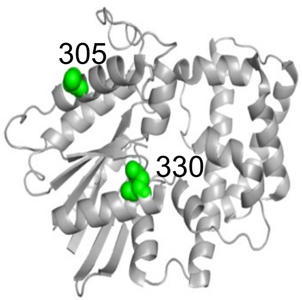
Global fitting of bin-time-dependent FRET: 0.5 – 5 ms

Helical-Helical



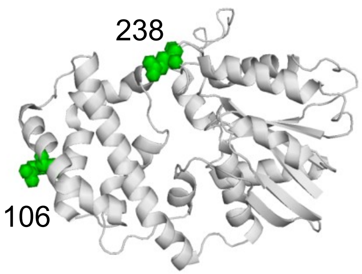
90-106	GDP	Ric-8A	GTP
k_{12} (ms^{-1})	1.1 ± 0.8	1.1 ± 0.7	1.0 ± 0.6
k_{21}(ms^{-1})	0.5 ± 0.2	0.4 ± 0.1	0.3 ± 0.2
T_T (ms)	0.6 ± 0.4	0.7 ± 0.4	0.8 ± 0.5

Ras-Ras



305-330	GDP	Ric-8A	GTP
k_{12} (ms^{-1})	1.3 ± 0.8	3.4 ± 2.5	1.0 ± 0.5
k_{21}(ms^{-1})	0.4 ± 0.1	0.8 ± 0.1	0.3 ± 0.1
T_T (ms)	0.6 ± 0.3	0.2 ± 0.1	0.8 ± 0.5

Helical-Ras



106-238	GDP	Ric-8A	GTP
k_{12} (ms^{-1})	0.05 ± 0.04	0.01 ± 0.01	0.02 ± 0.01
k_{21}(ms^{-1})	0.01 ± 0.01	0.05 ± 0.03	0.01 ± 0.01
T_T (ms)	17 ± 14	17 ± 11	30 ± 20

Labe Black, Celestine Thomas

Linda’s 880 project – FLIM/FRET

FLIM-FRET between CFP/YFP-tagged NMDA receptor subunits