single-molecule fluorescence resonance energy transfer II

(6) homo-FRET

michael börsch

25/02/2005

Energy transfer between identical fluorophores

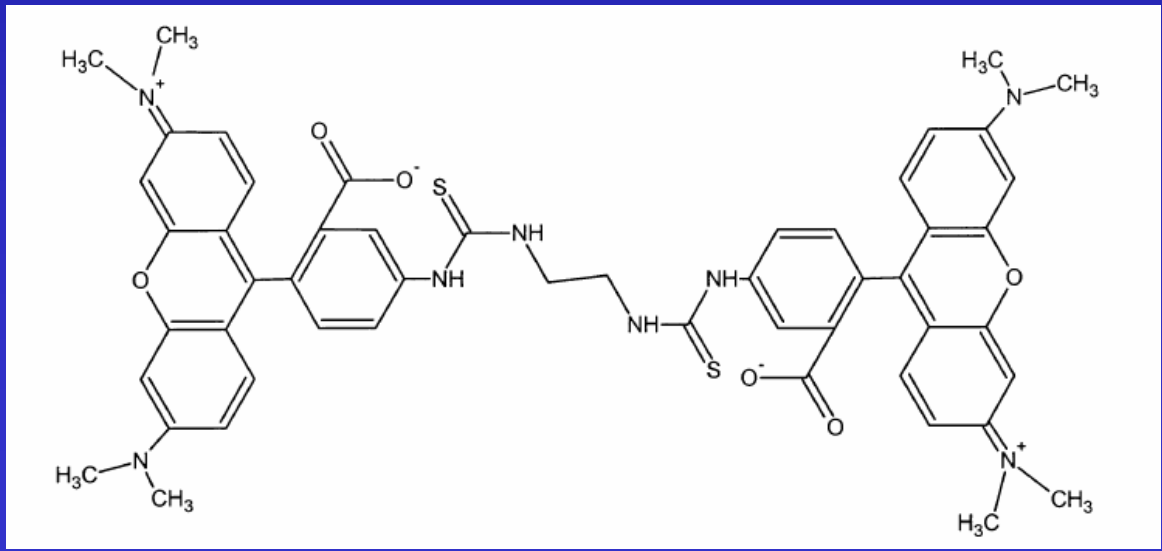
Examples

1. artificial homo-dimers
2. light harvesting complexes

Energy transfer between two identical fluorophores

Examples

1a. artificial homo-dimers:
two crosslinked TMR-fluorophores "$TRITC_2$"

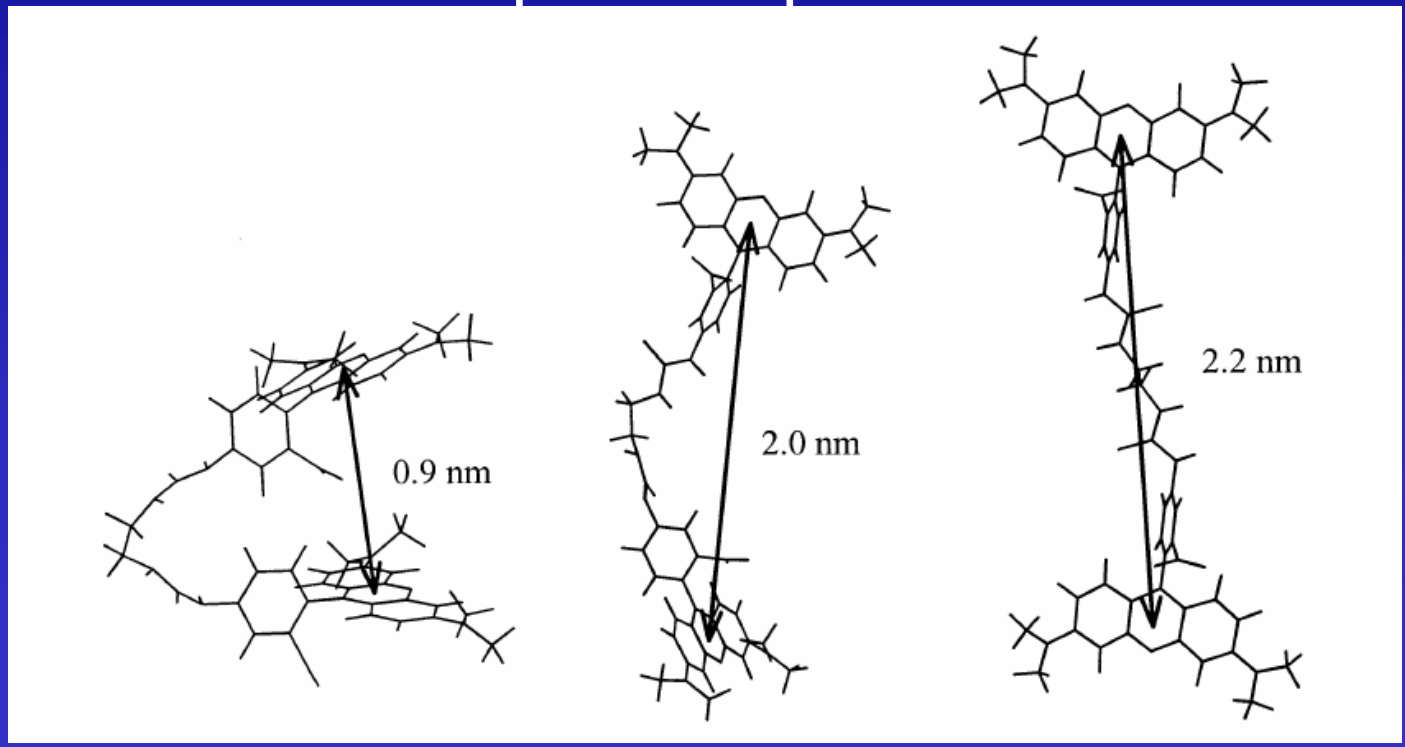


Jordi Hernando,*,† Martijn van der Schaaf,† Erik M. H. P. van Dijk,† Markus Sauer,‡ María F. García-Parajó,† and Niek F. van Hulst†

J. Phys. Chem. A **2003,** *107,* 43−52

Energy transfer between two identical fluorophores

two TMR-fluorophores: possible distances



strong ↔ weak coupling

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Energy transfer between two identical fluorophores

two TMR-fluorophores ("$TRITC_2$"): absorption spectra

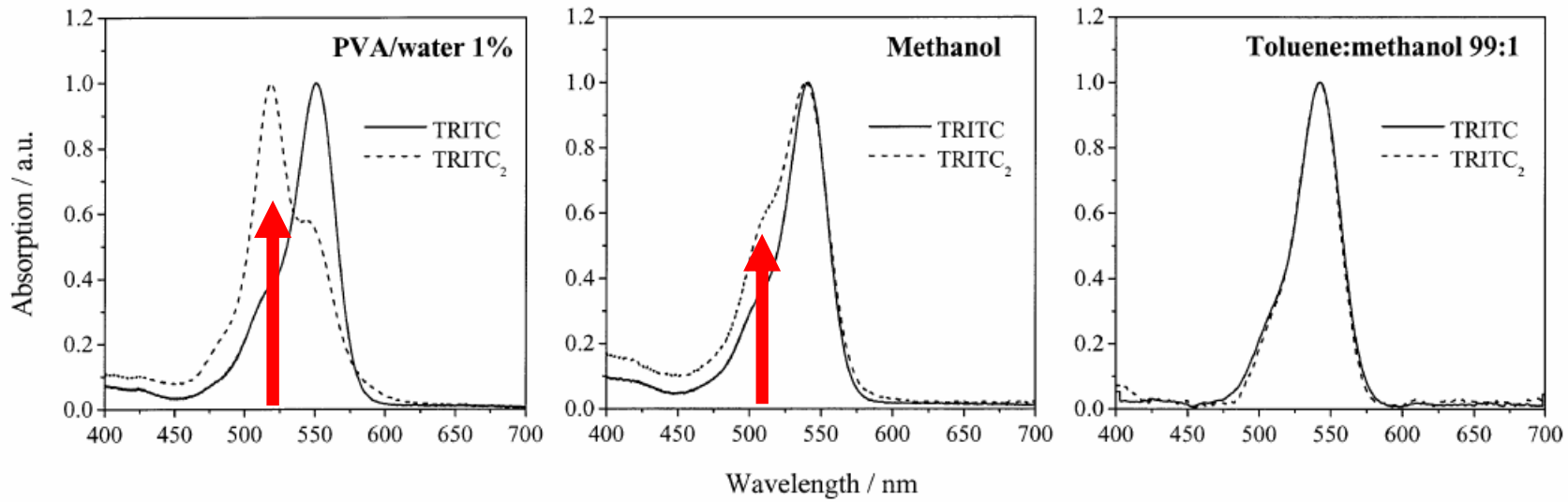

Figure 2. Absorption spectra of TRITC and $TRITC_2$ in 1 wt % PVA/water, methanol, and 99:1 toluene/methanol.

solvent dependent conformations of the dimer

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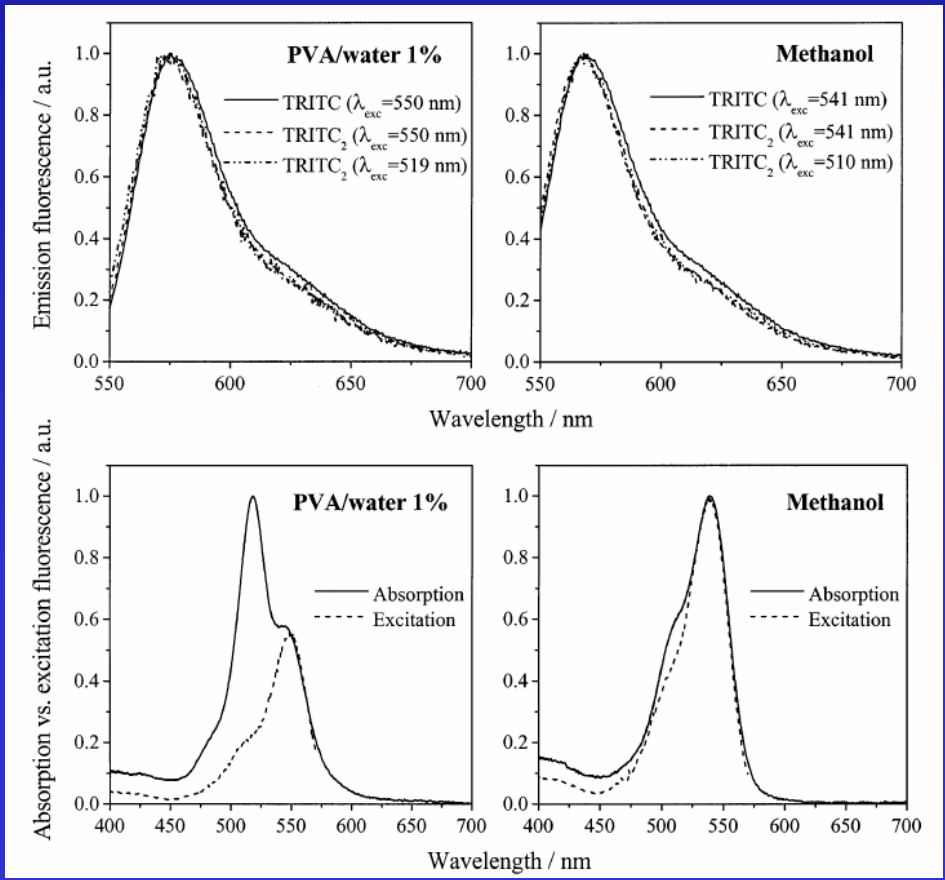
Energy transfer between two identical fluorophores

two TMR-fluorophores ("$TRITC_2$"): fluorescence shows only one spectrum



excitation spectra → a second population with $\Phi_{FI}=0$ in water

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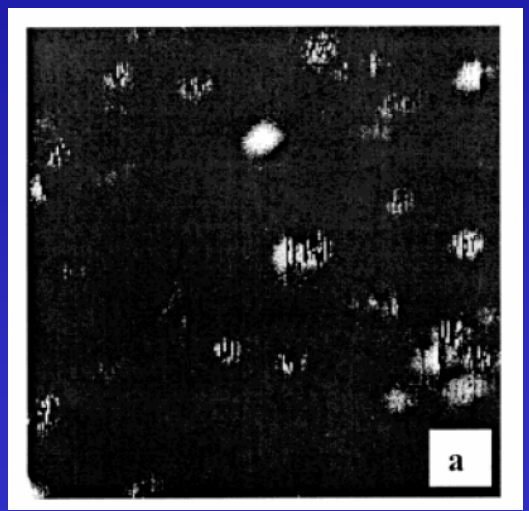
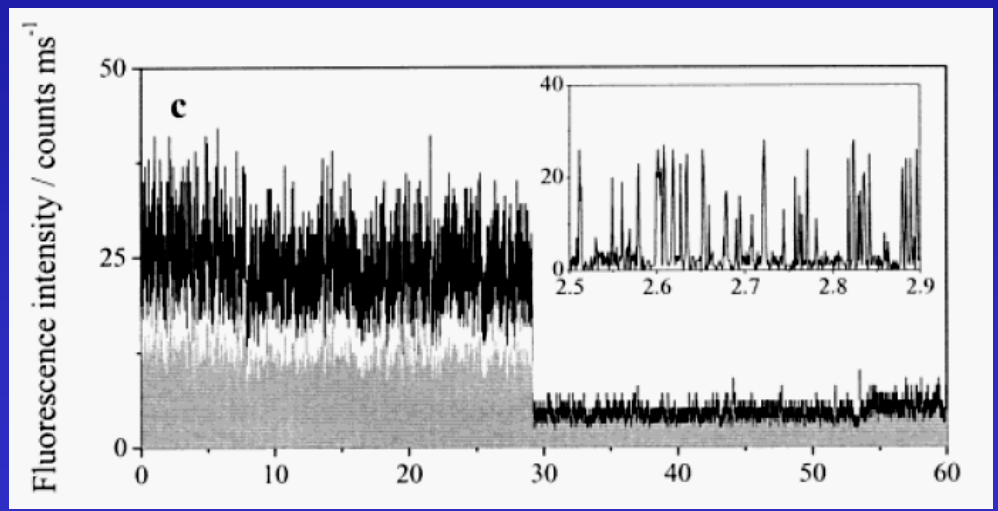
Energy transfer between two identical fluorophores

"TRITC": single monomer images





black: total fluorescence intensity
gray: two orthogonal polarizations
→ one-step photobleaching, constant intensity

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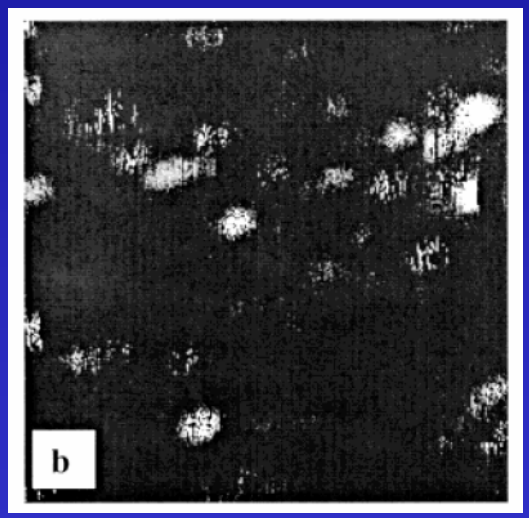
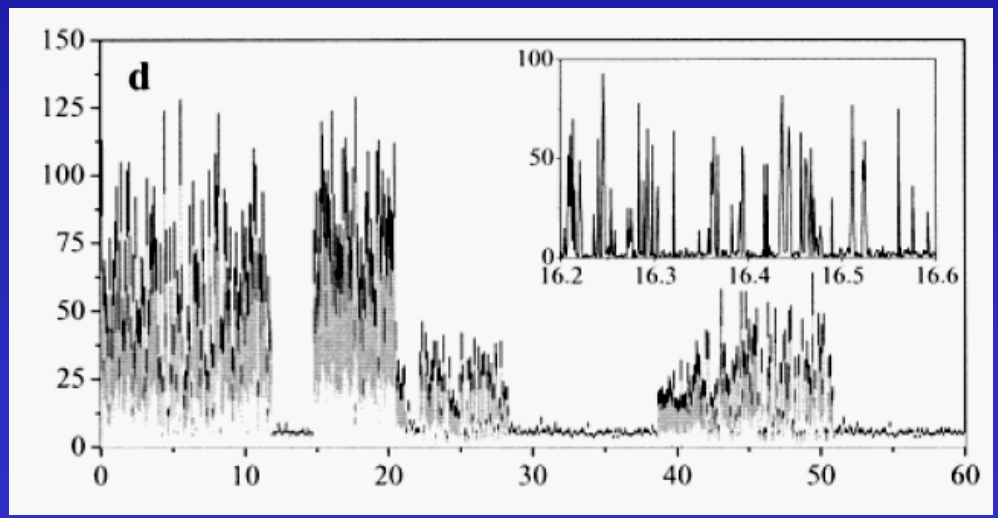
Energy transfer between two identical fluorophores

"$TRITC_2$": single-**dimer** images





black: total fluorescence intensity
gray: two orthogonal polarizations
→ two intensity levels

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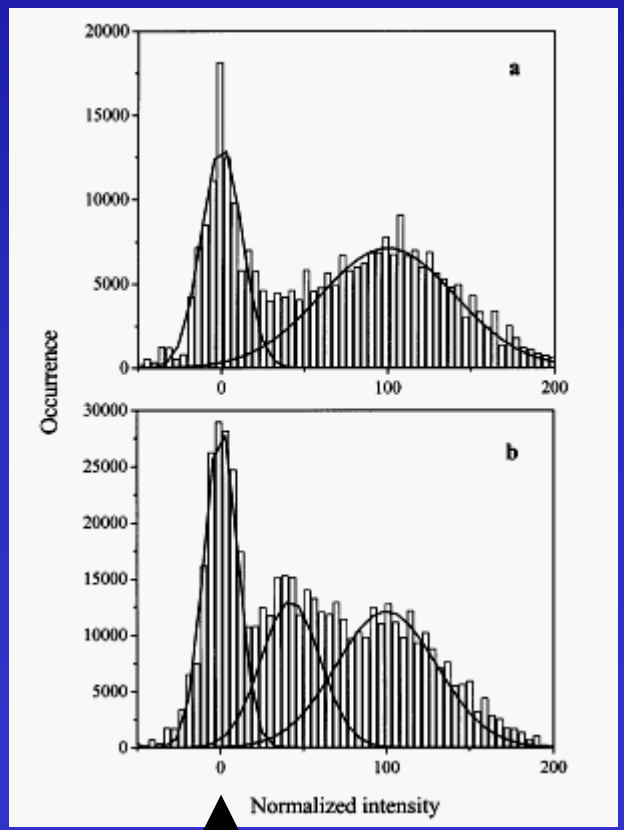
Energy transfer between two identical fluorophores

TRITC and $TRITC_2$: intensity distributions



background level

the monomor exhibits only one intensity level,

the dimer shows two distinct intensity levels I_1 and I_2.

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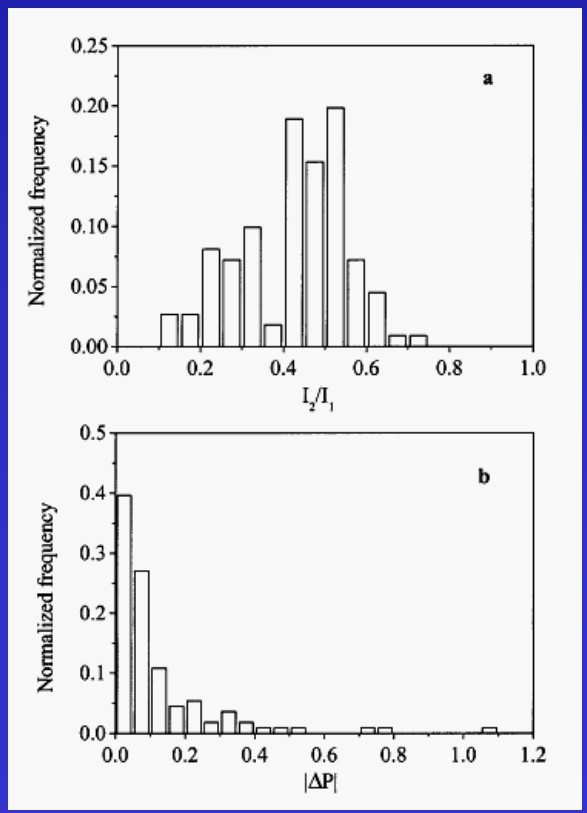
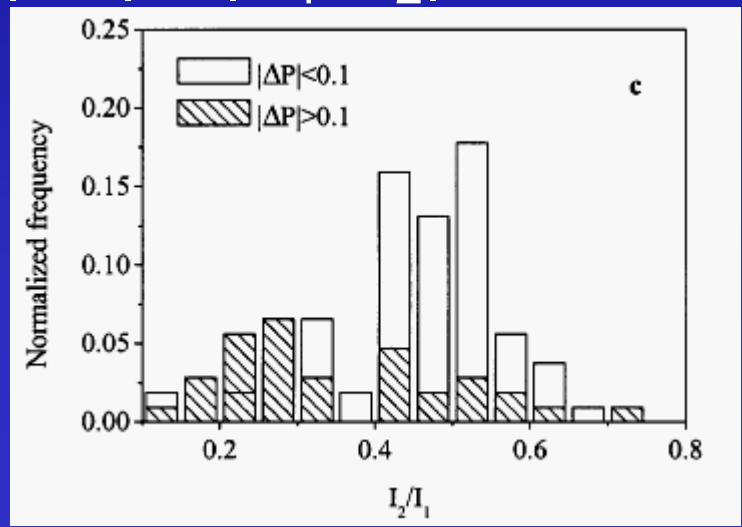
Energy transfer between two identical fluorophores

TRITC$_2$: intensity ratio I_2/I_1 and differences in linear polarization $|\Delta P| = |P_1 - P_2|$





small $|\Delta p|$ changes correspond to I_2/I_1=0.5
i.e. parallel transition dipoles in the dimer and the remaining monomer after bleaching

$$P = (I_s - I_p)/(I_s + I_p)$$

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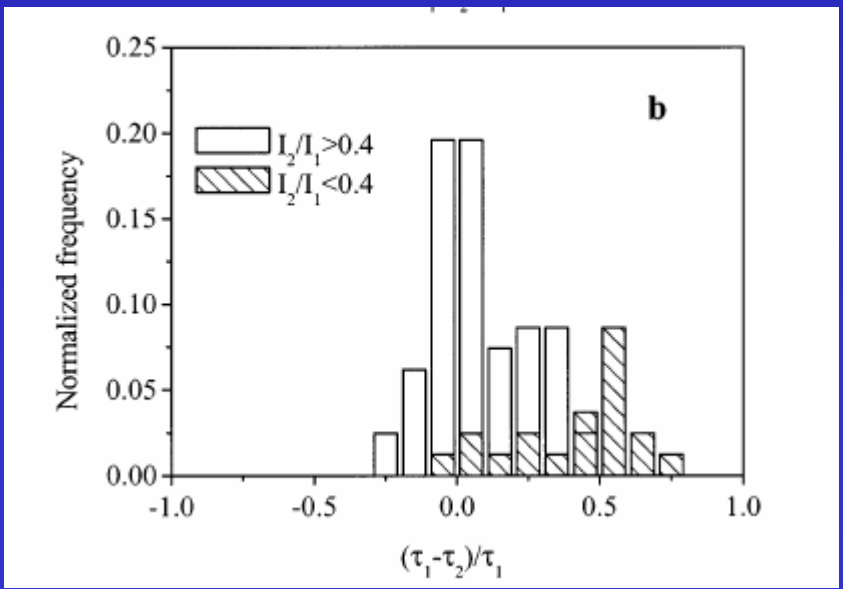
Energy transfer between two identical fluorophores

$TRITC_2$: two lifetimes (3.2 ns and 2.1 ns) and correlation with intensity levels:

3.2 ns ↔ higher intensity level



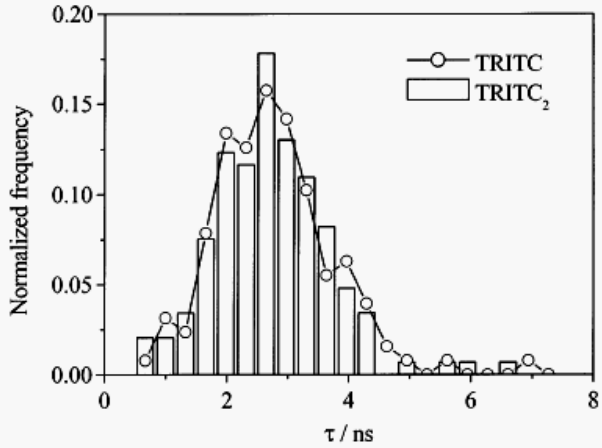


Figure 8. Distributions of fluorescence lifetimes for 127 TRITC and 146 $TRITC_2$ molecules (λ_{exc} = 550 nm, power = 4.5 kW/cm²).

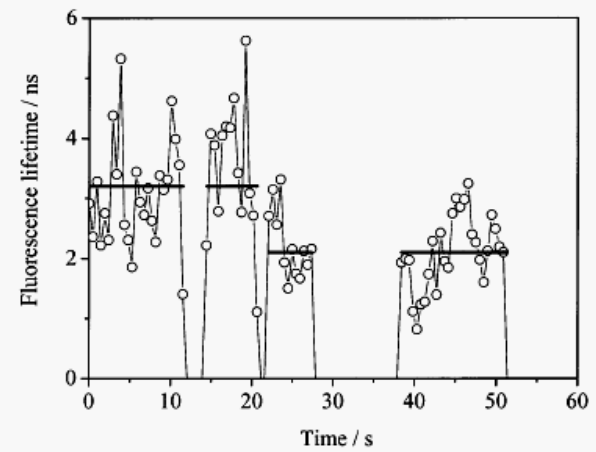


Figure 9. Fluorescence lifetime trajectory corresponding to the $TRITC_2$

Energy transfer between two identical fluorophores

photophysics of $TRITC_2$:

- strong coupling: non-fluorescent H-type dimer, concentration independent
- weak coupling: similar excitation and fluorescence spectra as monomer
- dimer emits more photons than monomer ($6.5*10^5$ vs. $3.4*10^5$), shows two intensity levels (i.e. photobleaching of one dye before the second)
- small polarization changes Δp after photobleaching correlate with a 50% loss in intensity: this indicates parallel transition dipole moments in the dimer
- large polarization changes Δp after photobleaching correlate with $I_2/I_1 \neq 0.5$ i.e. non-parallel dipoles, as both dyes are not excited / detected with the same efficiency
- unimodal and similar lifetime distribution for monomer and dimer
- lifetime jumps: shorter lifetime for dimmer level indicates a remaining and absorbing chromophore after bleaching

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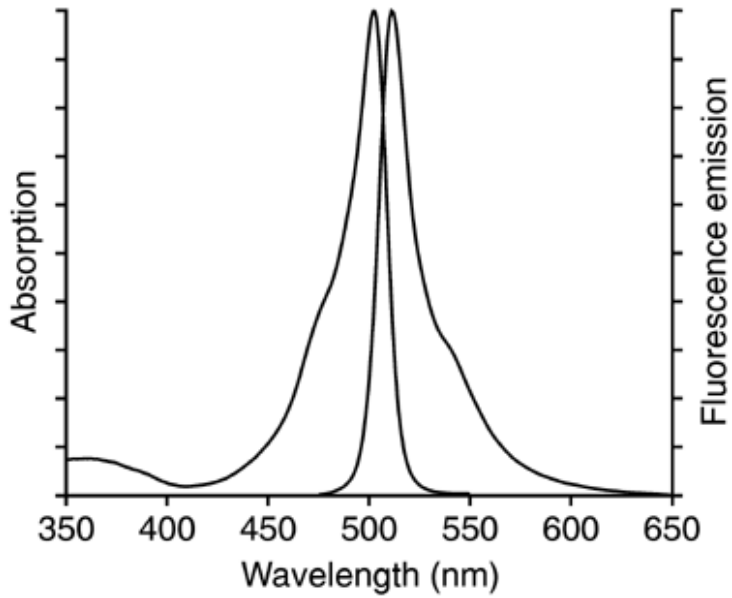
Energy transfer between two identical fluorophores

Examples

1b. artificial homo-dimers in PAI 1 (plasminogen activator inhibitor type 1)

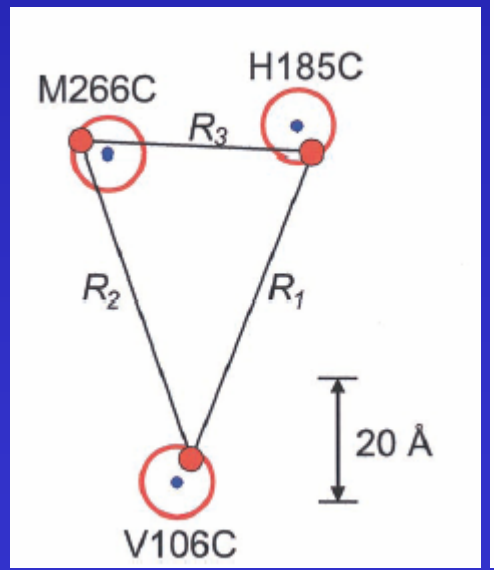


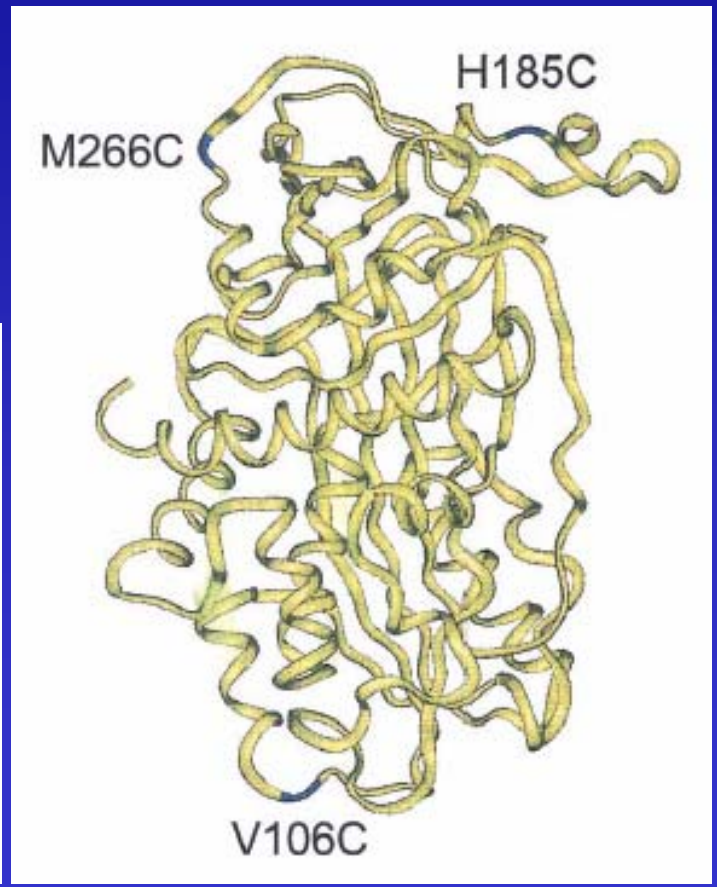
with two Bodipy-FL fluorophores

Energy transfer between two identical fluorophores

PAI1

- three cysteines
- mono- and dual-labeled with SBDY
- timeresolved anisotropy measurements



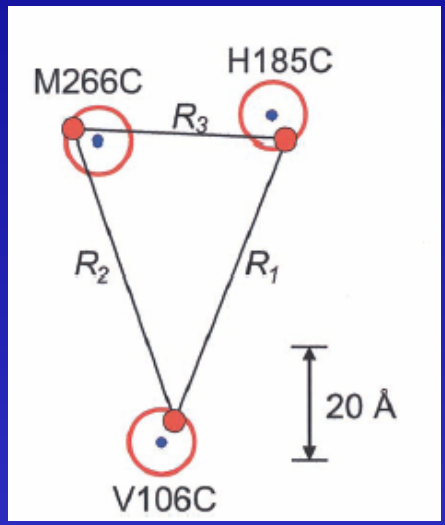


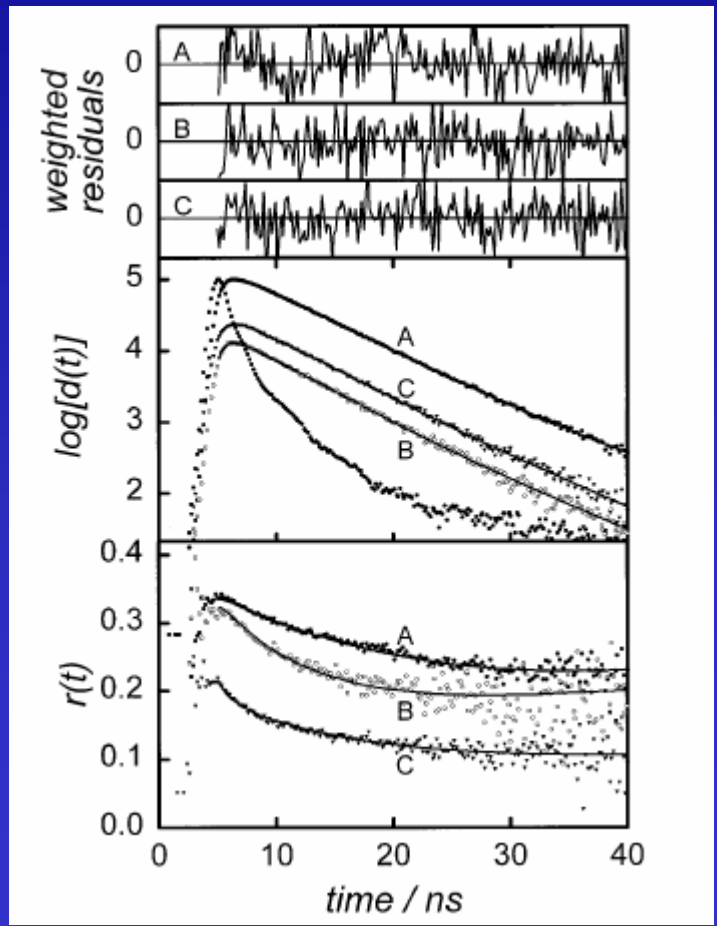
Fredrik Bergström*†, Peter Hägglöf*‡, Jan Karolin†, Tor Ny‡, and Lennart B.-Å. Johansson†§
PNAS | October 26, 1999 | vol. 96 | no. 22 | 12477–12481

Energy transfer between two identical fluorophores

PAI1

- three cysteines
- A: single cys 185
- B: single cys 266
- C: double cys 185-266





- timeresolved anisotropy measurements
  → faster decay for double cys185-266

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Energy transfer between two identical fluorophores

rate ω for donor-donor energy migration and fluorophore distance R:

$$\omega = \frac{3}{2}\langle\kappa^2\rangle\frac{1}{\tau}\left\{\frac{R_0}{R}\right\}^6$$

Mutant	r_s	$r(0)$	ϕ/ns	ρ_0	S_J	S_δ	ω/ns^{-1}	$\langle\kappa^2\rangle$	R/Å	R_C/Å
V106C	0.303	0.332	12 ± 1		0.80 ± 0.02					
H185C	0.306	0.343	12 ± 1		0.78 ± 0.02					
M266C	0.264	0.329	7 ± 1.5		0.69 ± 0.01					
V106C-H185C	0.272	0.340		0.81		0.17	0.6 ± 0.1	1.5	54 ± 4	60.9
H185C-M266C	0.167	0.329		−0.26		−0.36	2.1 ± 0.3	0.6	38 ± 3	30.8
M266C-V106C	0.22	0.333		0.40		0.06	0.4 ± 0.1	1.3	55 ± 3	55.1

The fluorescence steady-state anisotropy, the initial anisotropy, and the rotational correlation time are denoted by r_s, $r(0)$, and ϕ, respectively. R and R_c denote the distance between the center of mass of the BODIPY groups and the C_α-atoms of the mutated amino acids obtained from the x-ray structure, respectively. The local order of the BODIPY moieties ($j = 1$ and 2) is described by the order parameter S_j, and S_δ accounts for the mutual projection of the symmetry axes of the local orientational distributions. ρ_0 is the maximum contribution to the fluorescence anisotropy from secondary excited donors. The rate of DDEM is ω, and $\langle\kappa^2\rangle$ accounts for the angular part of dipole–dipole coupling. Further explanation to the notation is given in the text (see *Theoretical Model*) and elsewhere (3, 14). The results represent average values obtained from two to three experiments, using independently prepared samples.

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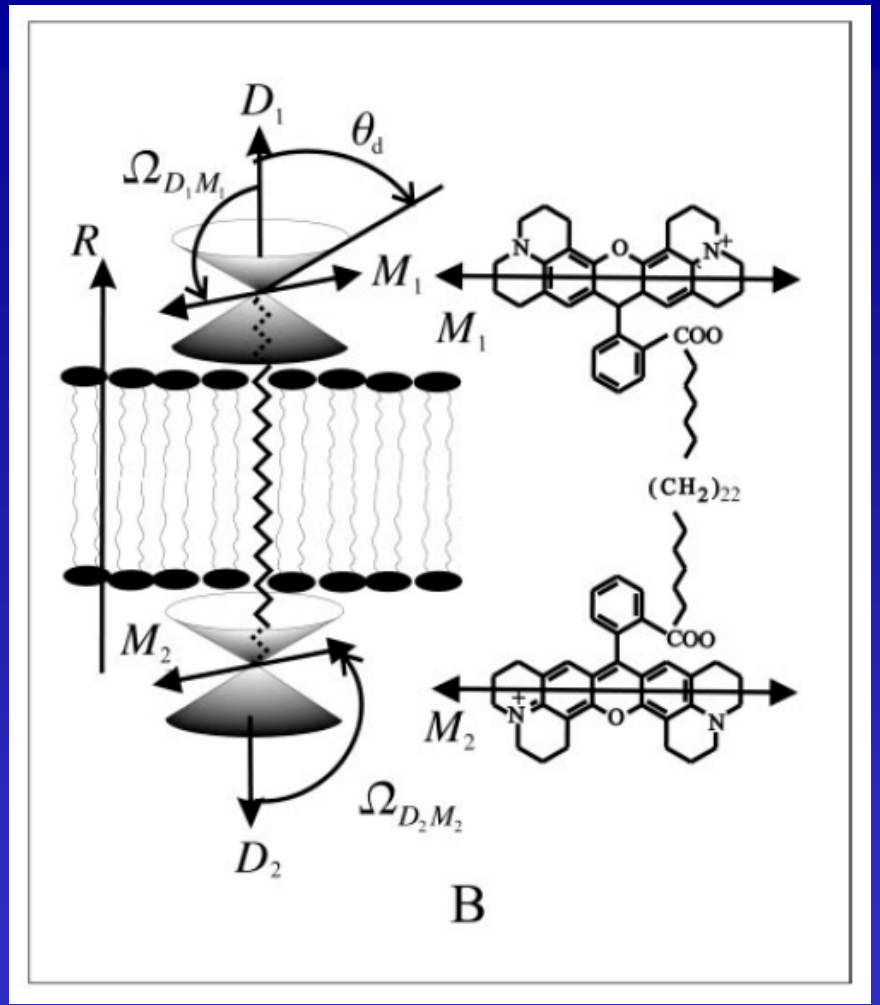
Energy transfer between two identical fluorophores



Phys. Chem. Chem. Phys., 2000, **2**, 2795–2801

$r_s=R_s$: steady-state anisotropy, τ_c=rotational correlation time, D_r=rot. Diffusion constant, Λ=coupling strength, κ^2=mean square angular dependence of the dipole-dipole coupling

System	T/K	r_s	$r(0)$	D_r/ns^{-1}	$\tau_{c,\,theor}$/ns	$\tau_{c,\,exp}$/ns
Rh101C$_{18}$	293	0.22	0.30 ± 0.02	1.9 ± 0.3	12.0 ± 2.0	7.8 ± 1.5

System	T/K	R_s	$r(0)$	Λ/ns^{-1}	$\langle\kappa^2\rangle$	R/Å	R_{ref}/Å
Rh101C$_{32}$Rh101	293	0.15	0.28 ± 0.01	5.5 ± 1.5	0.50 ± 0.01	36.5 ± 1.0	38[b]

Energy transfer between more than two identical fluorophores

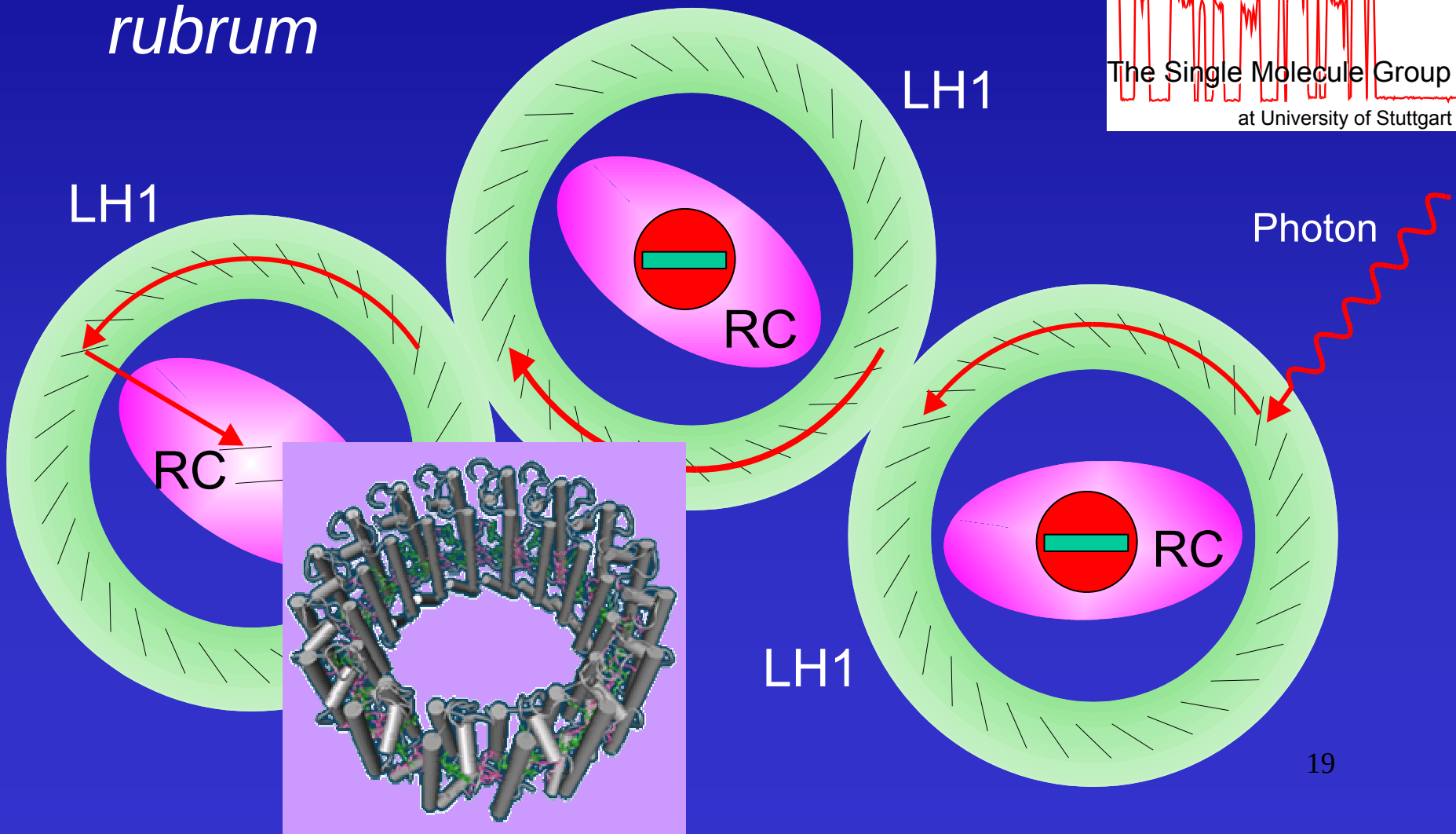
Examples

1. artificial homo-dimers

2. light harvesting complex LH1

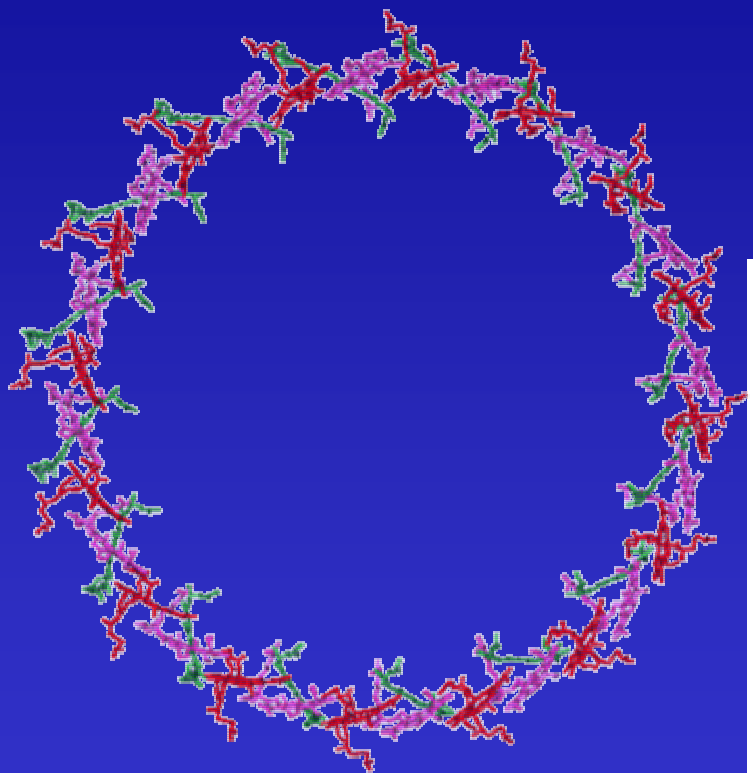
analysis of a multichromophor

the Photosynthetic Unit of *Rhodospirillum rubrum*

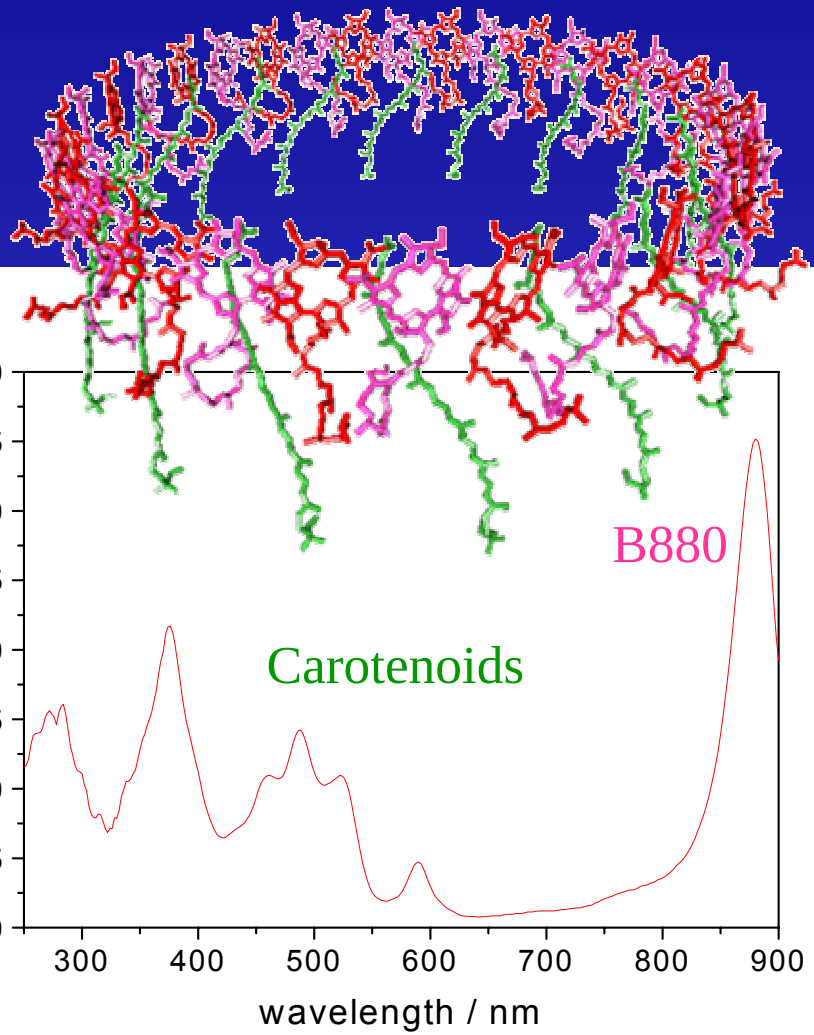


The Single Molecule Group
at University of Stuttgart

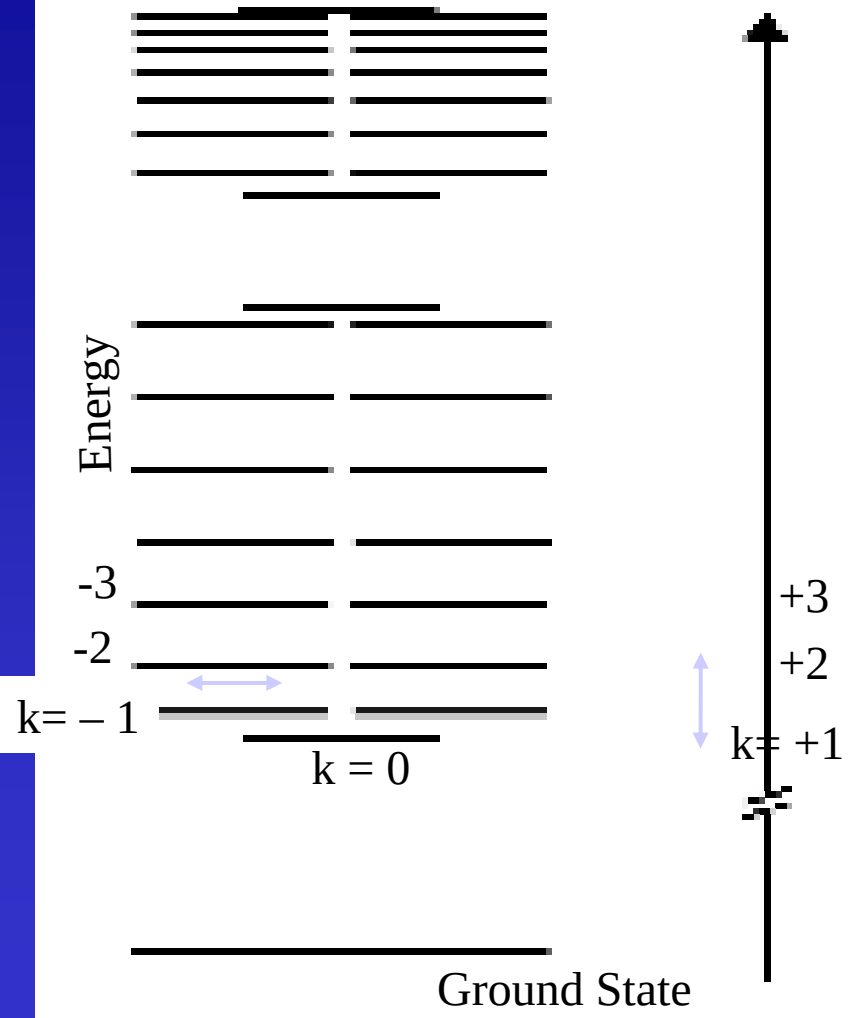
The LH1 structure contains 32 chlorophylls



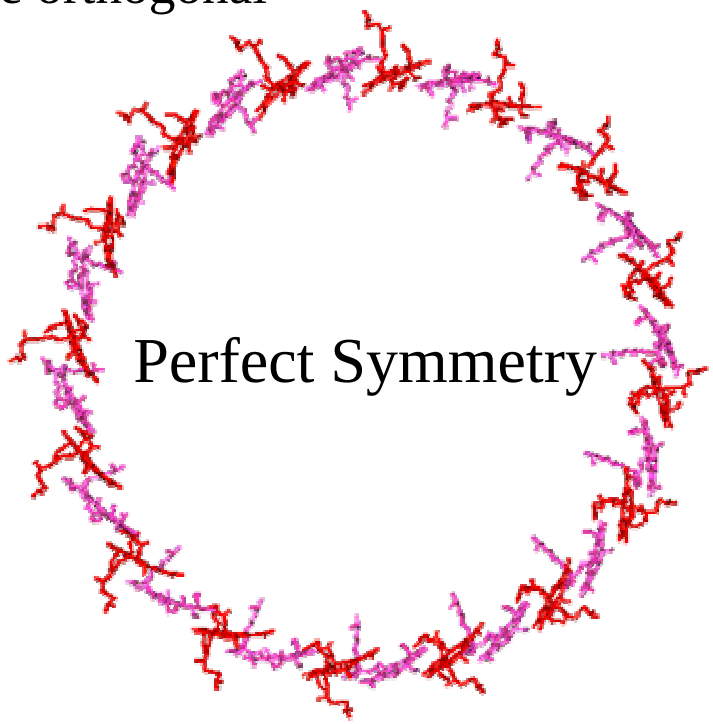
view without proteins



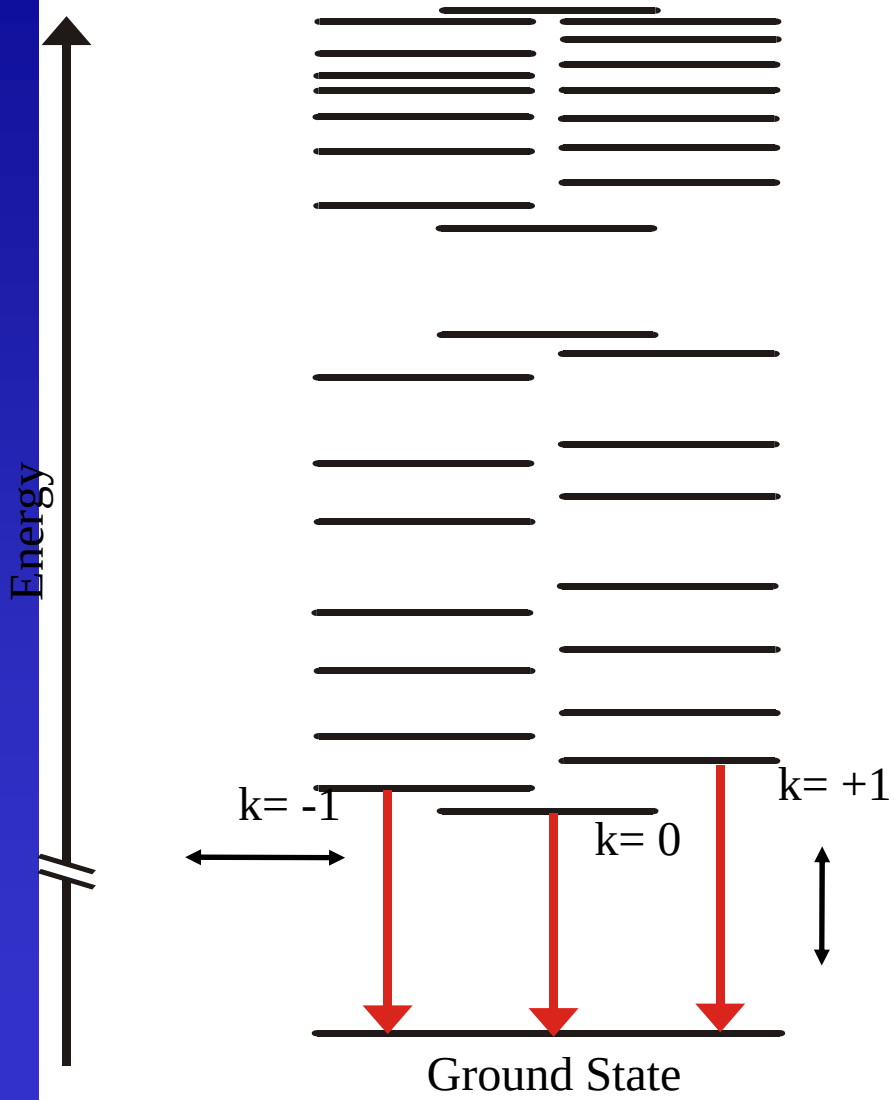
electronic structure of LH1 (I)



- k=+1 and k= –1 carry almost all oscillator strength
- dipole moments of k= +1 and k= – 1 are orthogonal



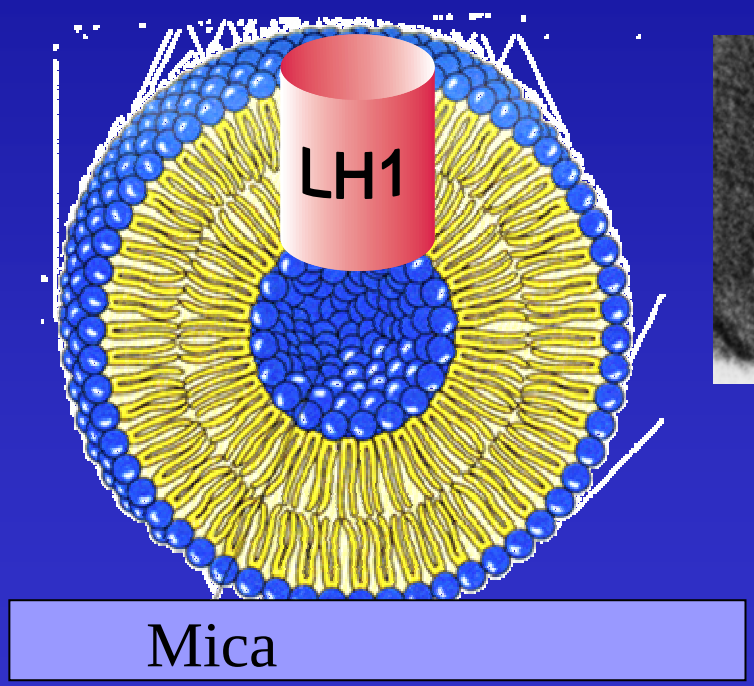
electronic structure of LH1 (II)



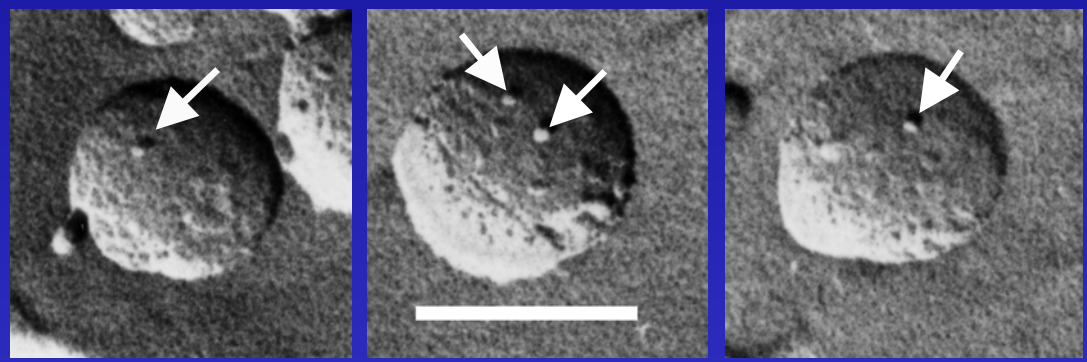
- k=0 gets oscillator strength
- **dipole moments of k=+1 and k= –1 are orthogonal**
- degeneracy is lifted

Disordered Ring

LH1 embedded in a lipid vesicle



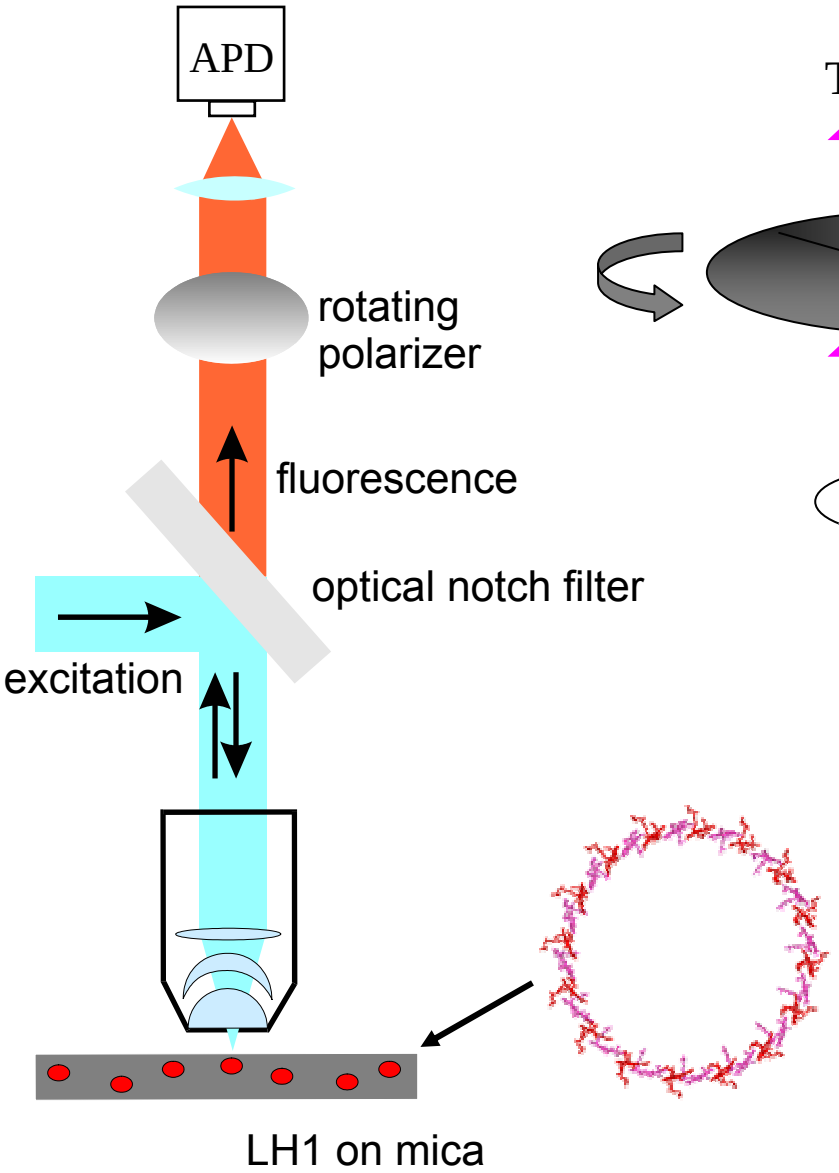
Freeze-fracture electron micrographs of LH1:

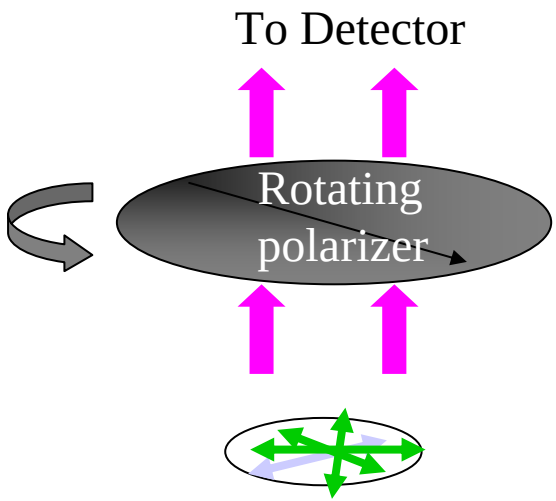


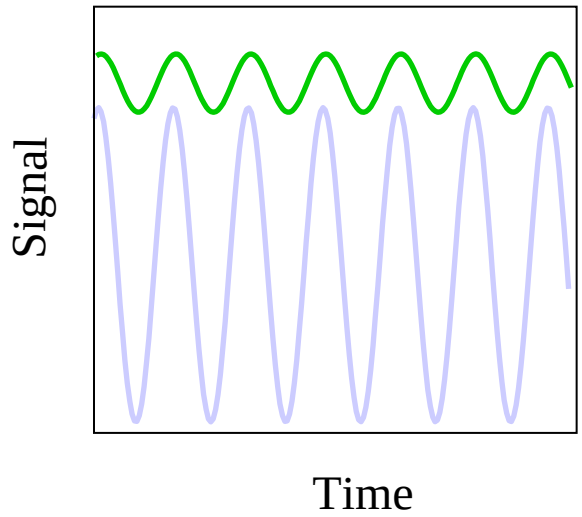
200nm

Molar ratio
(LH1 / Phospholipid): 1:400

polarization of LH1@ 2K





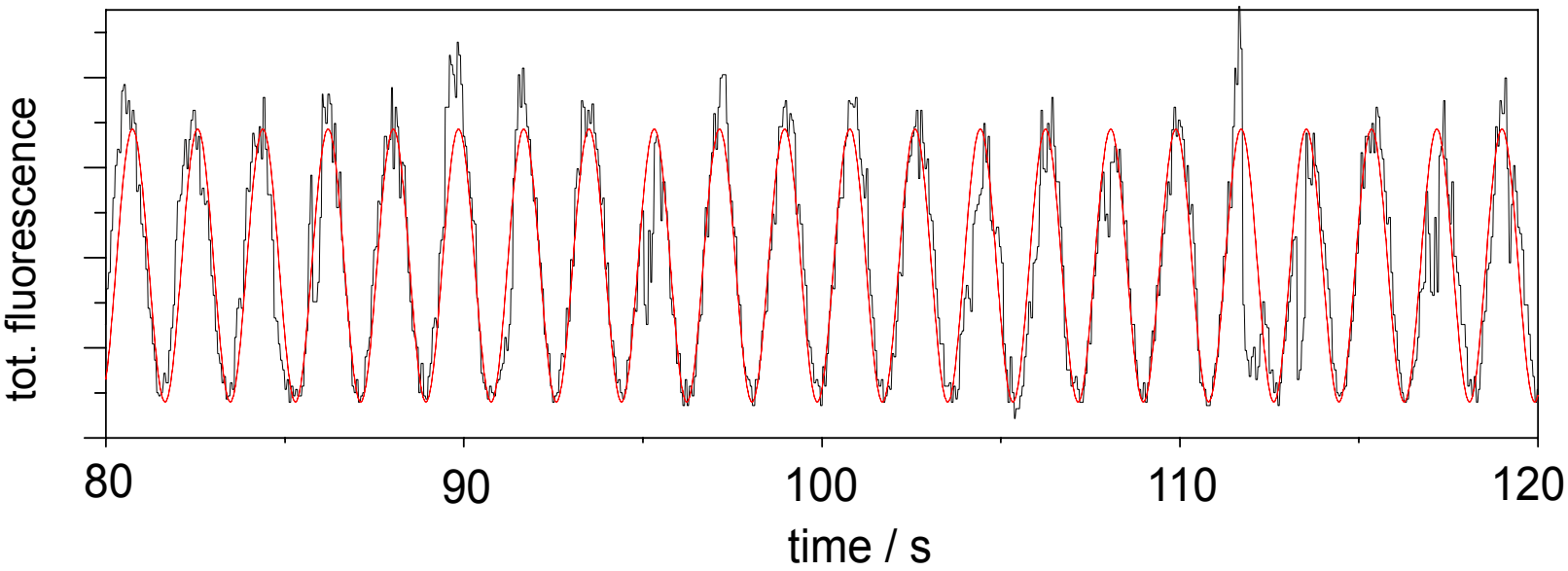


one emitter

or

many emitters ?

polarization of LH1@ 2K

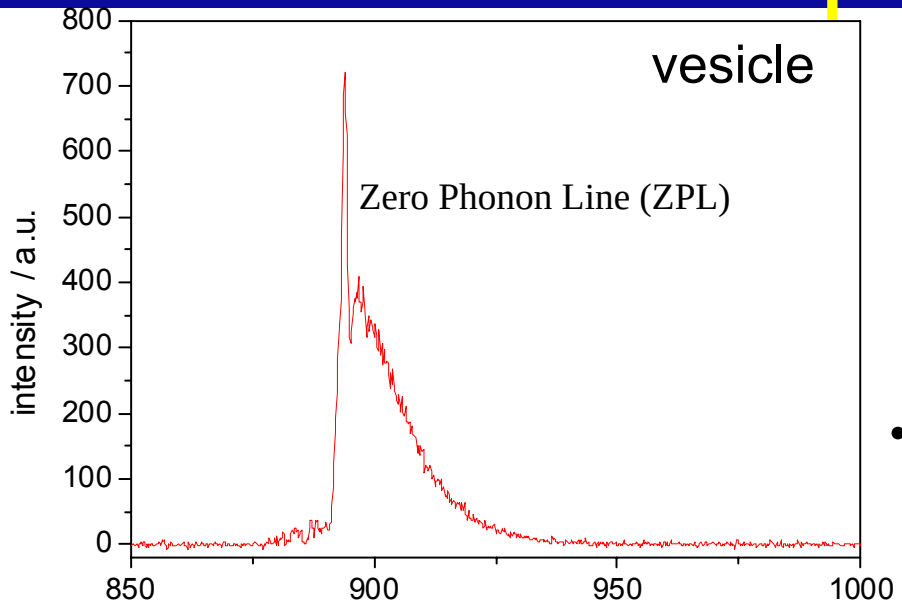


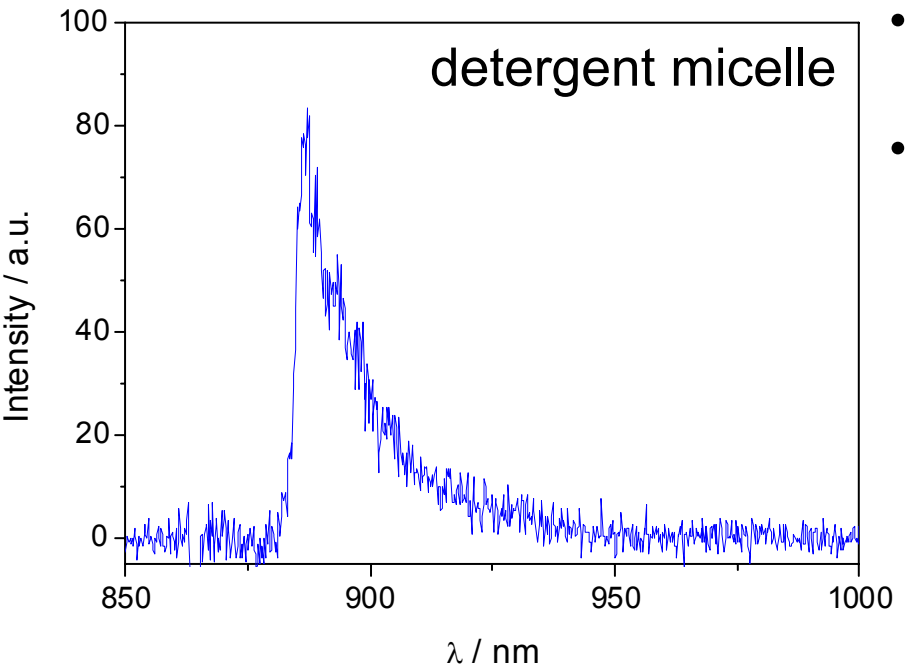
Fluorescence is linearly polarized.

➡ Only one emitting state (k=0).

➡ Only one LH1 complex in focus.

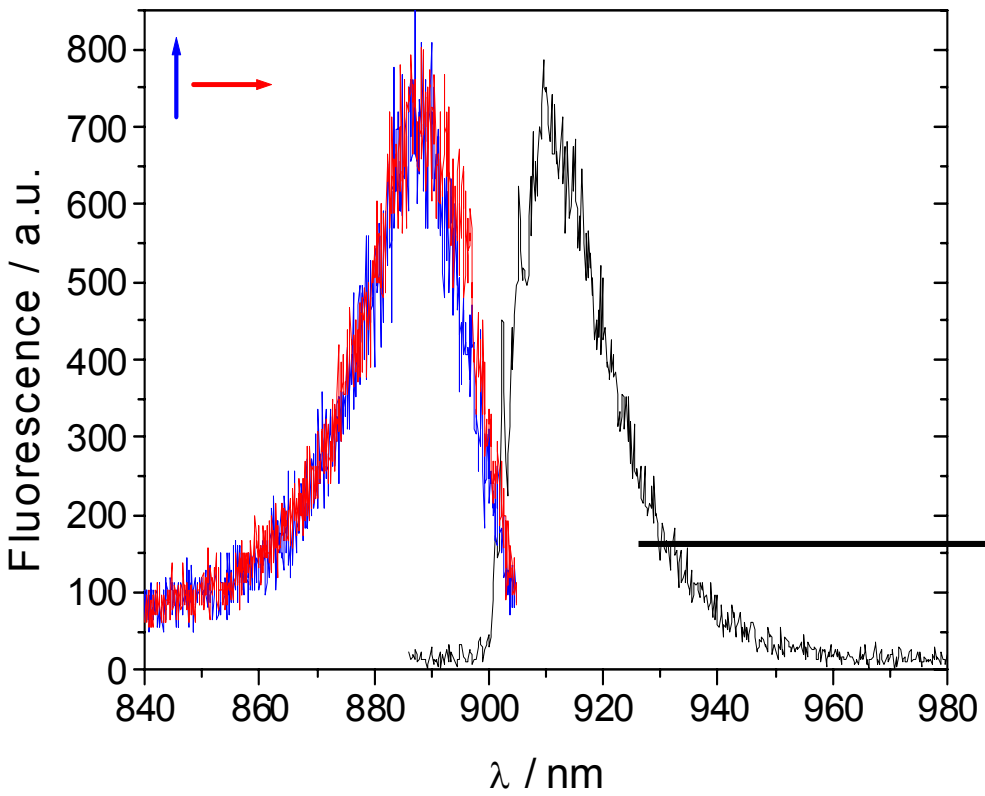
fluorescence spectra of LH1 @ 2K





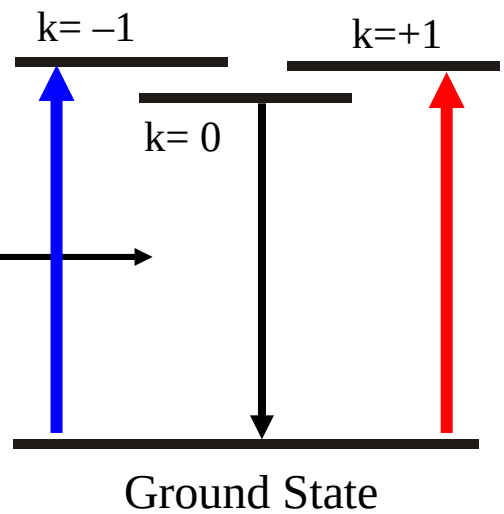
- only lowest transition (k=0) is visible in emission
- strong electron-phonon coupling
- spectra of LH1 in membranes are structured
  due to weaker spectral diffusion

fluorescence excitation spectra @ 2K of LH1 in vesicles

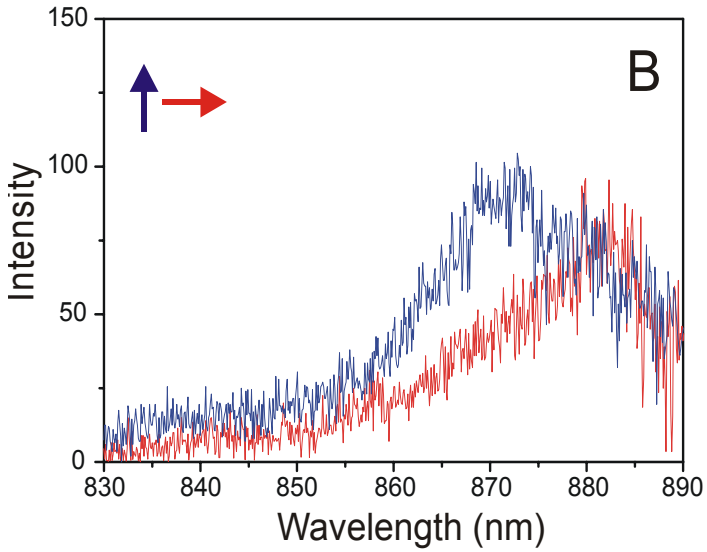


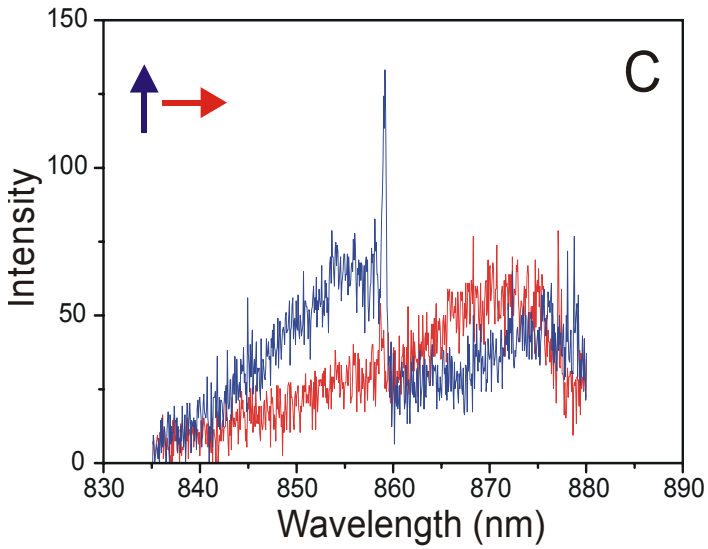
most of the complexes do not show any splitting

→ nearly circular symmetry



fluorescence excitation spectra

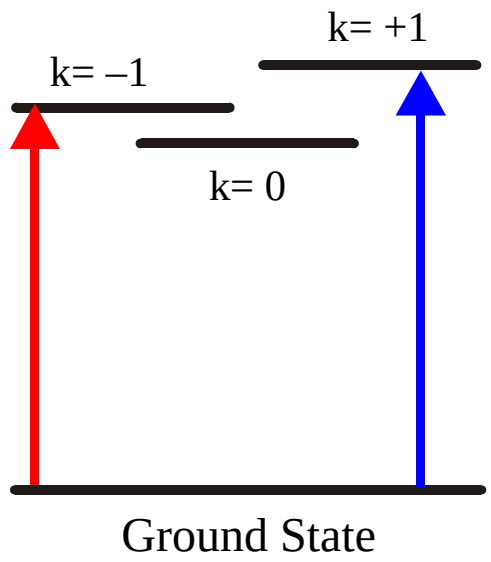




LH1 in detergent

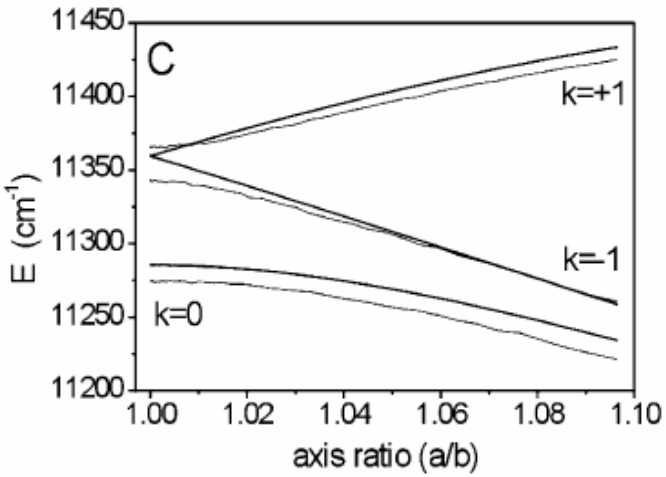
- most of complexes show splitting of k= ±1 transitions

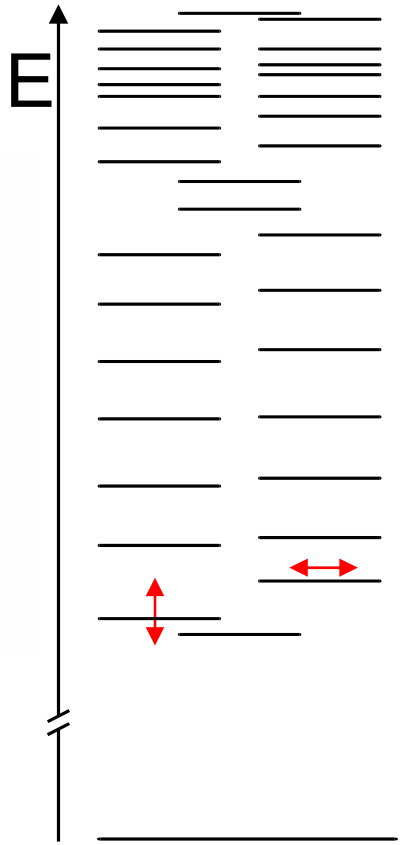
→ B880 ring is deformed



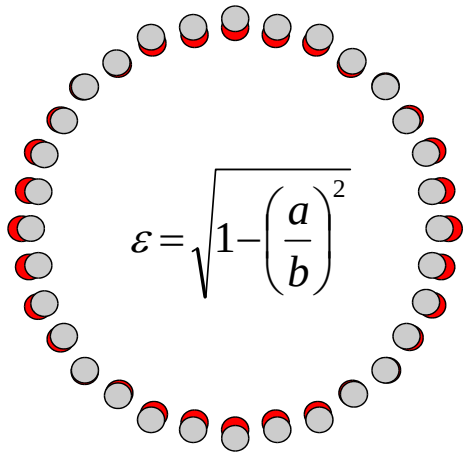
energy level diagram

calculated:





elliptical deformation:
eccentricity: ε = 0.3



splitting of k = ± 1

minor shifting of oscillator strength to higher lying states

conclusions on LH1

• First single molecule measurements on light-harvesting complexes in a native environment.

• Fluorescence excitation spectroscopy proves nearly undisturbed circular organization of the LH1 complexes in membranes.

• LH1 complexes solubilized with detergent show a wide distribution of elliptically deformations.

→ The membrane environment is crucial for the structural integrity of the LH1 complex.

U. Gerken, F. Jelezko, C. Tietz, J. Wrachtrup. R. Ghosh, Uni Stuttgart

Biochemistry **2003,** *42,* 10354−10360

Energy transfer between identical fluorophores

next :

1. artificial homo-dimers

2. light harvesting complexes

3. dendrimers

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