

single-molecule
fluorescence resonance
energy transfer II

(7) multichromophore-FRET

michael börsch

11/03/2005

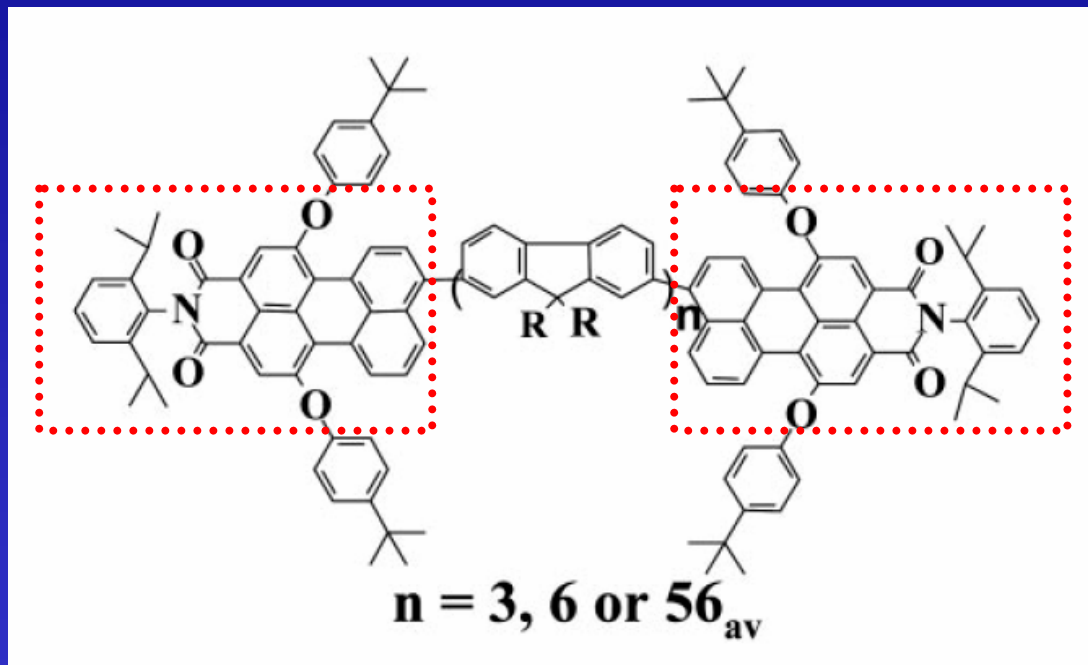
Energy transfer between identical fluorophores

Examples

1. artificial homo-dimers
2. light harvesting complexes
3. dendrimers
4. dsRED

Energy transfer between two identical fluorophores

Bis-Peryleneimide
with different distances,
fluorene as a rigid spacer
(3, 6, poly-fluorene)



Revealing competitive Förster-type resonance energy-transfer pathways in single bichromophoric molecules

Johan Hofkens^{*,†}, Mircea Cotlet^{*}, Tom Vosch^{*}, Philip Tinnefeld[‡], Kenneth D. Weston^{‡§}, Christophe Ego[¶], Andrew Grimsdale[¶], Klaus Müllen[¶], David Beljonne[¶], Jean Luc Brédas^{¶*}, Sven Jørgens^{*}, Gerd Schweitzer^{*}, Markus Sauer[‡], and Frans De Schryver^{*,†}

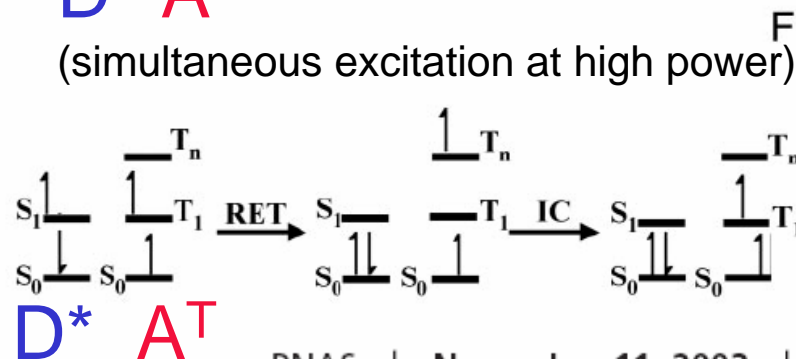
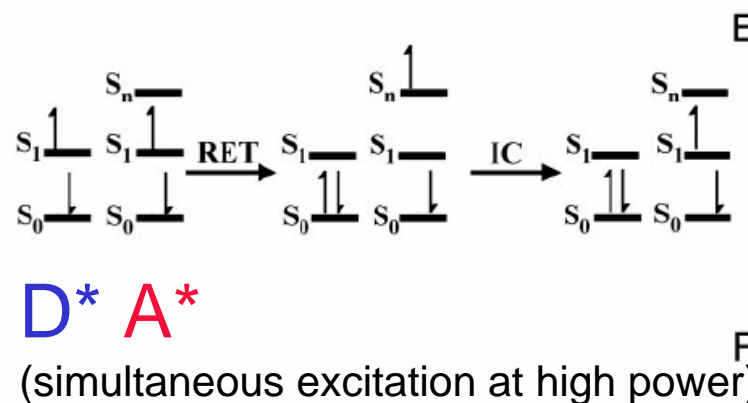
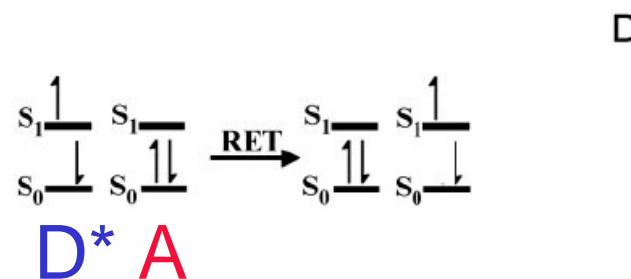
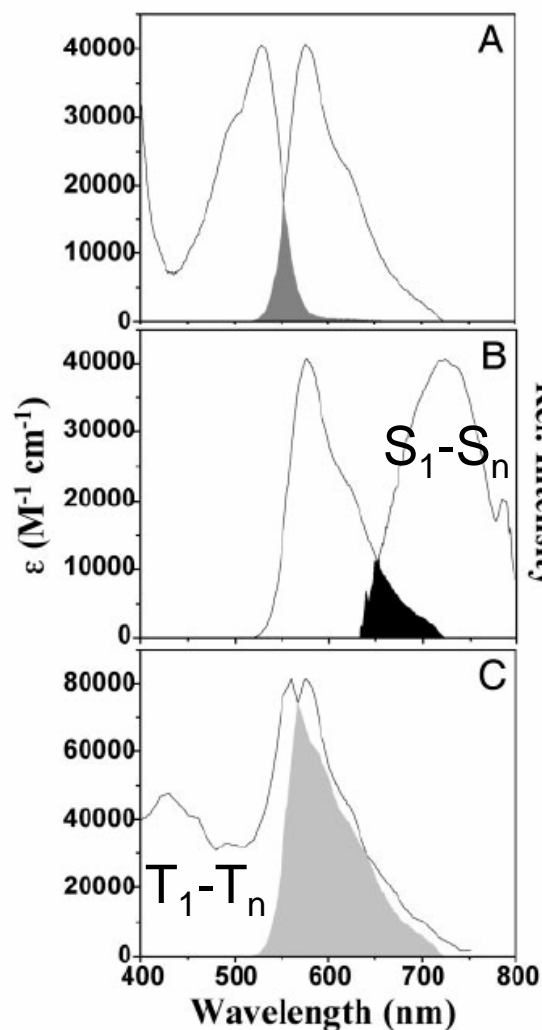
Energy transfer between two identical fluorophores

Förster-types of ET:

1. energy hopping

2. singlet-singlet annihilation

3. singlet-triplet annihilation



Energy transfer between two identical fluorophores

energy hopping: dark-gray
 S-S annihilation: black
 S-T annihilation: gray

structures and radii (with $\kappa^2 = 4$!)

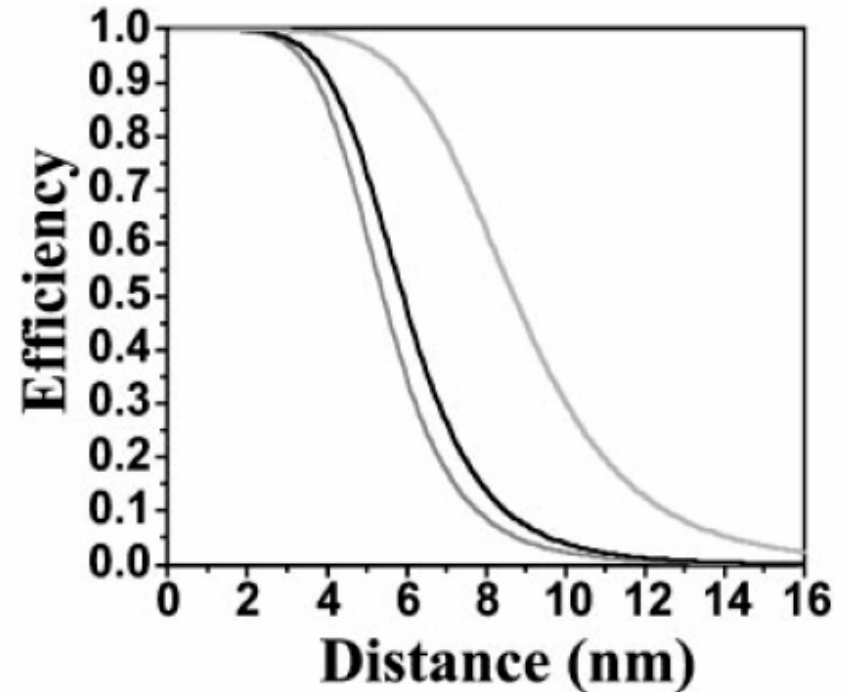
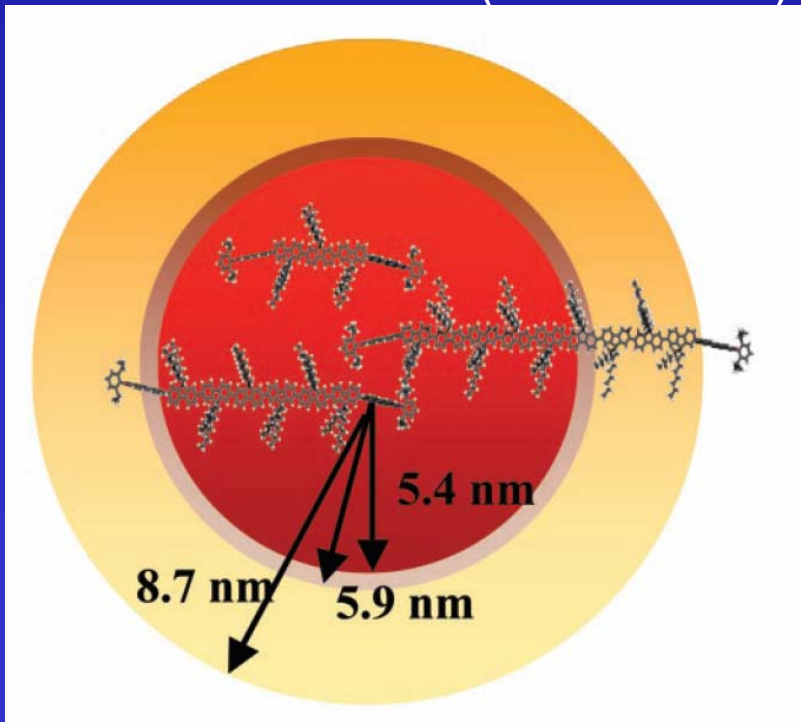


Table 1. Förster radii

	Experimental results, nm	Theoretical results, nm
Hopping	5.4	4.8
Singlet annihilation	5.9	6.0
Triplet annihilation	8.7	6.5

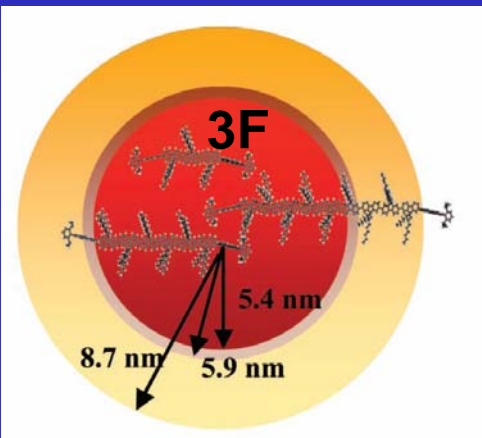
Energy transfer between two identical fluorophores

energy hopping: Förster radius 4.8nm (theor.) to 5.4nm (experimental)

efficient transfer for the three fluorene spacer 3F (3.4 nm),
not with hexamer 6F (5.9 nm)

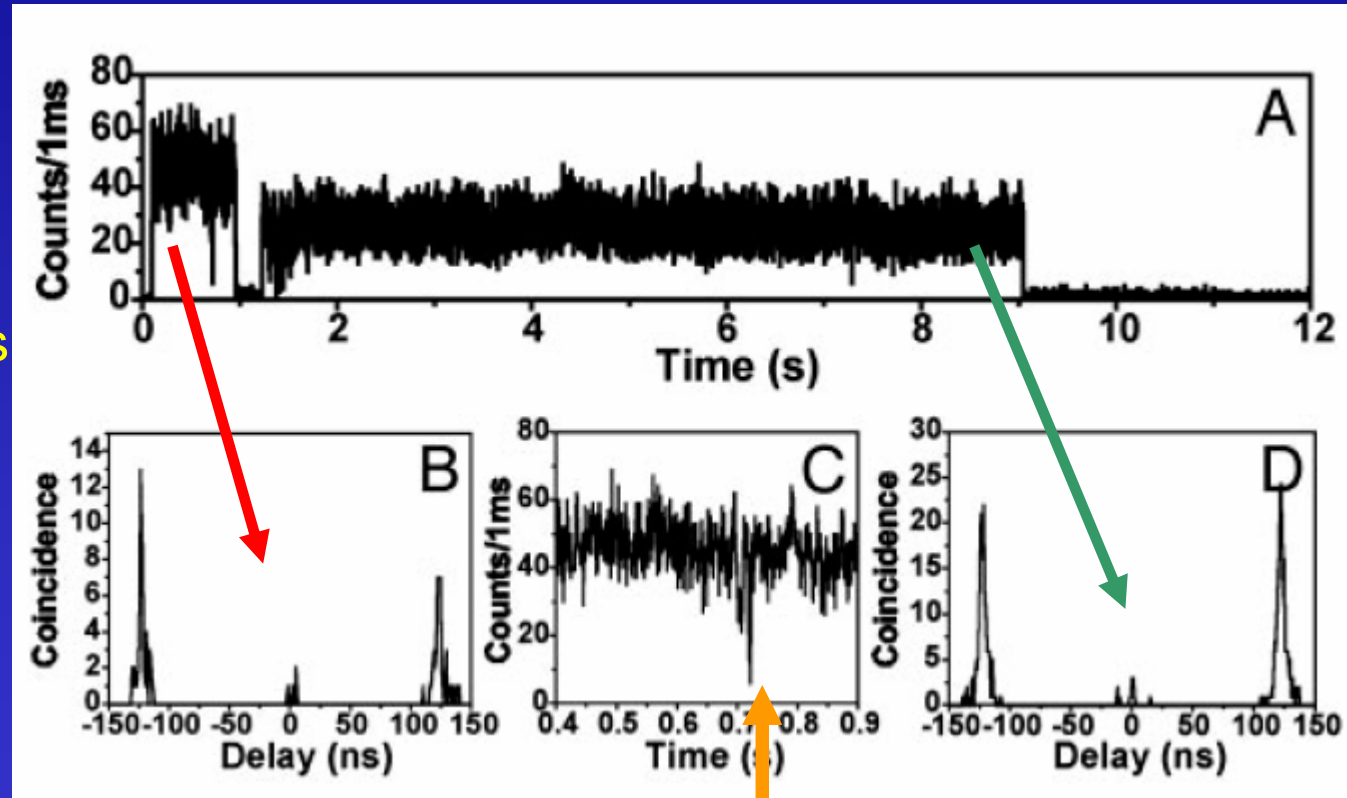
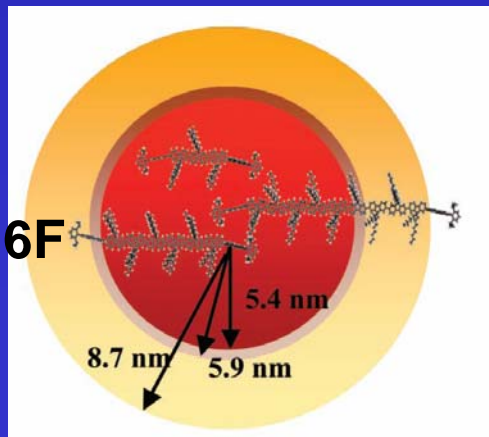
not with poly-fluorene (average distance ~ 42 nm):
distribution of chain lengths results in expected distances
from 6nm to 300nm.

25% of 72 molecules show one-emitter state in the
antibunching experiments.



Energy transfer between two identical fluorophores

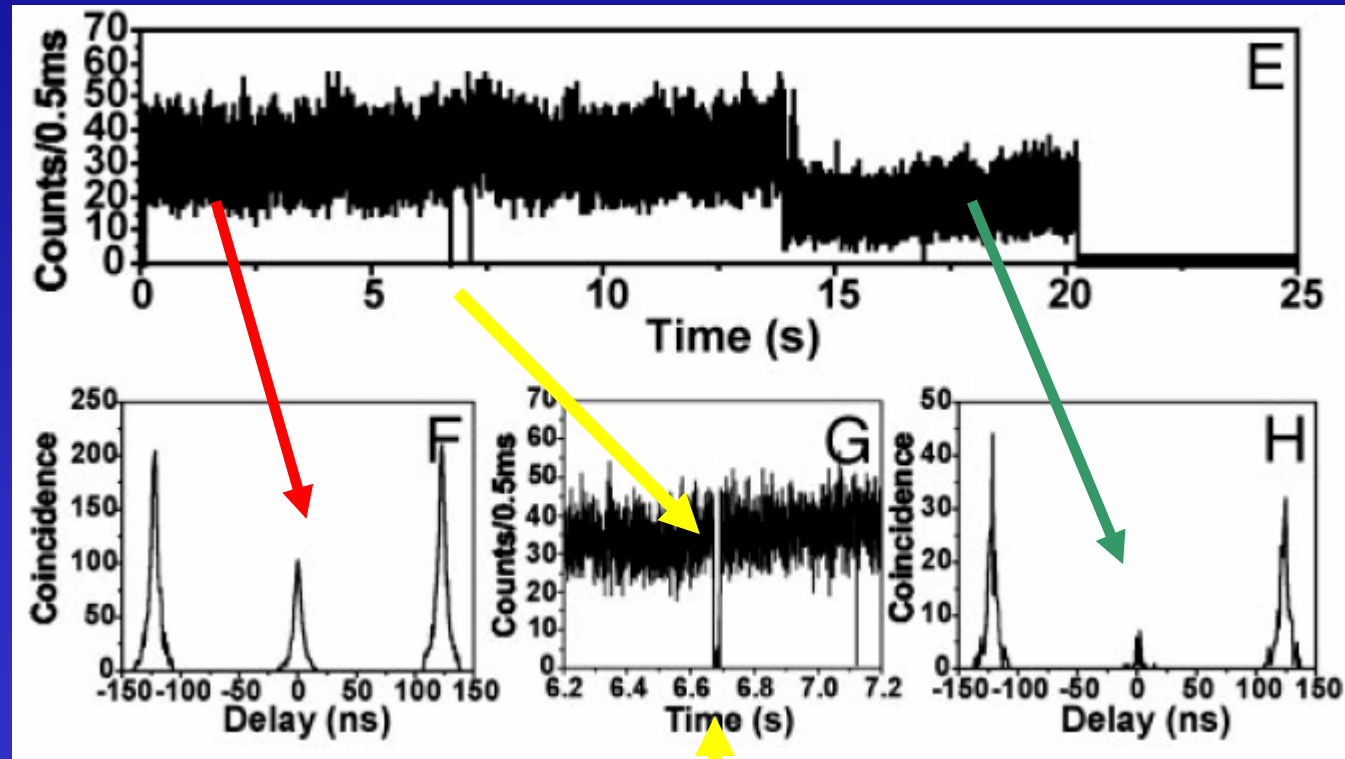
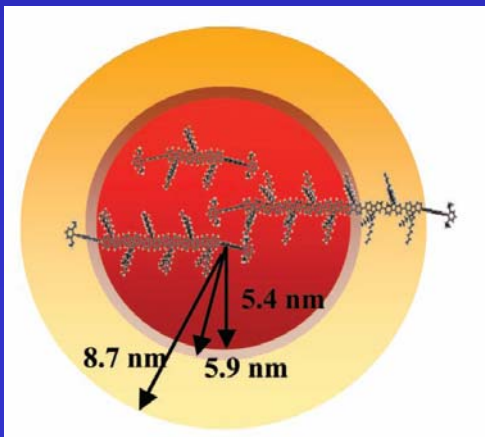
S-S annihilation
with 6F spacer:
two stable intensity
states,
coincidence analysis
(antibunching) reveals
a single emitter (B)
before bleaching.



collective on/off (C) is explained by
by S-S annihilation

Energy transfer between two identical fluorophores

polymer spacer
(mean ~ 42 nm):
two intensity states,
coincidence reveals
two independent
emitters (F),
after bleaching one
remains (H)



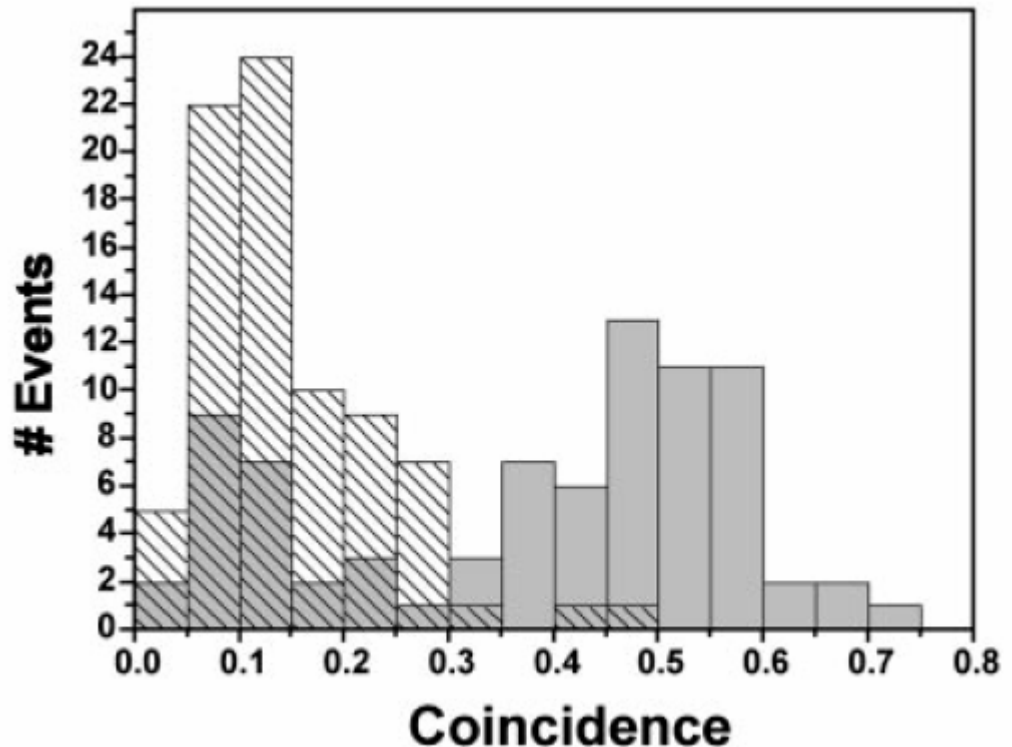
singlet-triplet annihilation is revealed by
collective on/off (G)

Energy transfer between two identical fluorophores

polymer spacer
(mean ~ 42 nm)

two classes of distances in the **coincidence ratio**:
long chain results in 0.5 value,
i.e. two independent emitters;
short chain result in 0.1 value
i.e. one emitter (F)

collective on/off in the
two-emitter conformation is
explained by S-T annihilation
as one fluorophore is in the
dark triplet state



gray: high intensity level
dashed: low intensity level

Energy transfer between several fluorophores

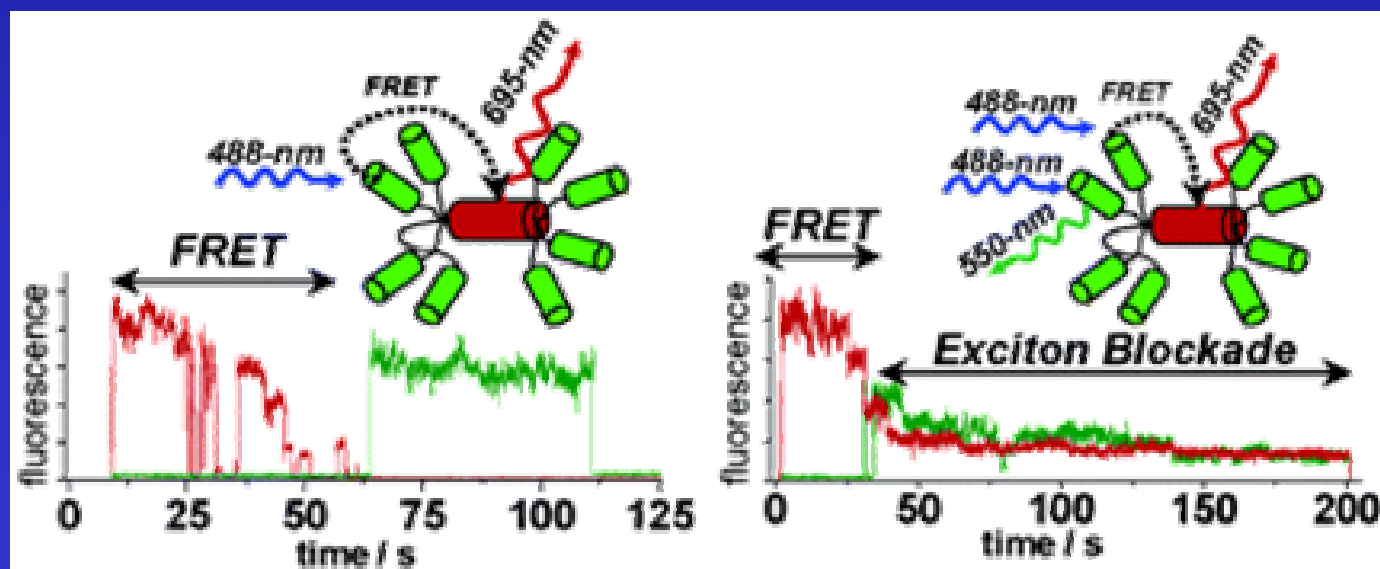
Examples

1. artificial homo-dimers
2. light harvesting complexes
3. dendrimers

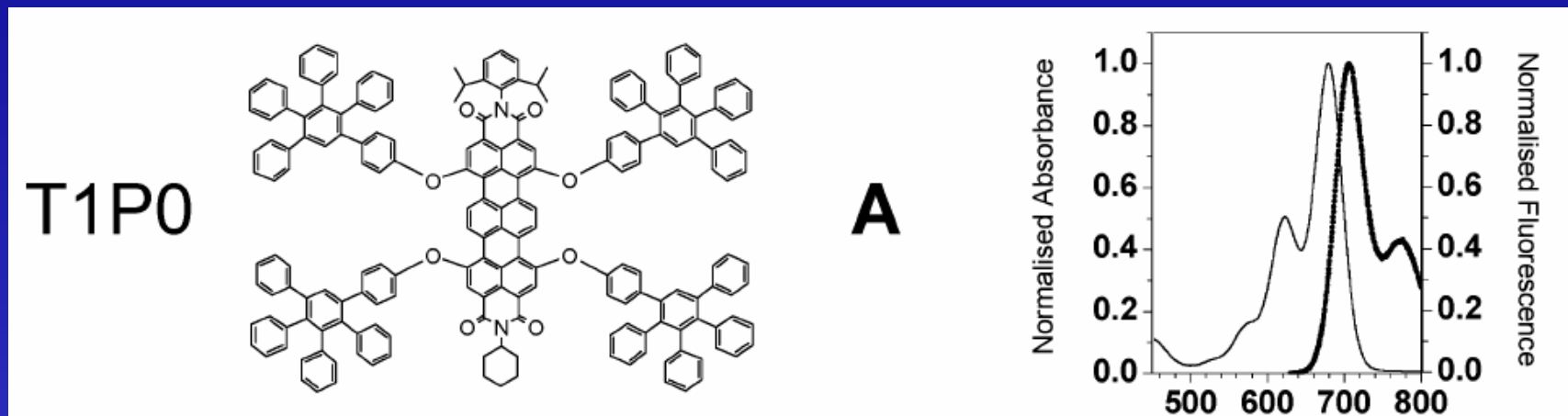
Energy transfer between several fluorophores

Intramolecular Directional Förster Resonance Energy Transfer at the Single-Molecule Level in a Dendritic System

Mircea Cotlet,[†] Roel Gronheid,[†] Satoshi Habuchi,[†] Alina Stefan,[†] Arianna Barbafina,[†] Klaus Müllen,[§] Johan Hofkens,^{*,†} and Frans C. De Schryver^{*,†}

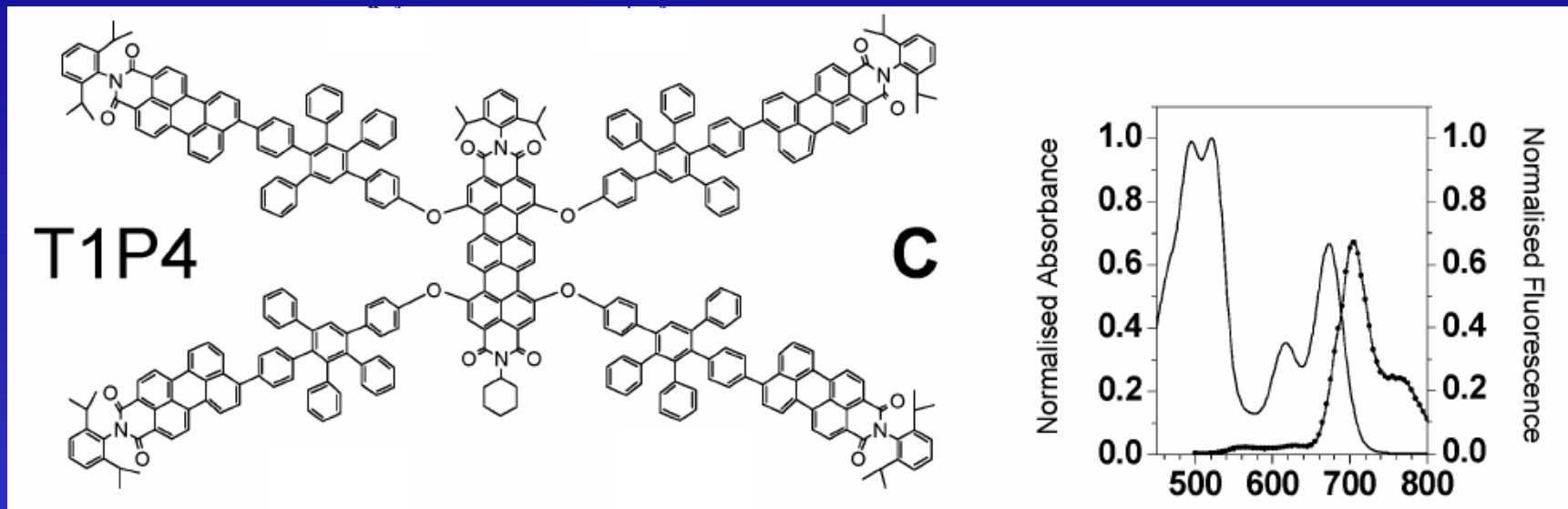


Energy transfer between several fluorophores



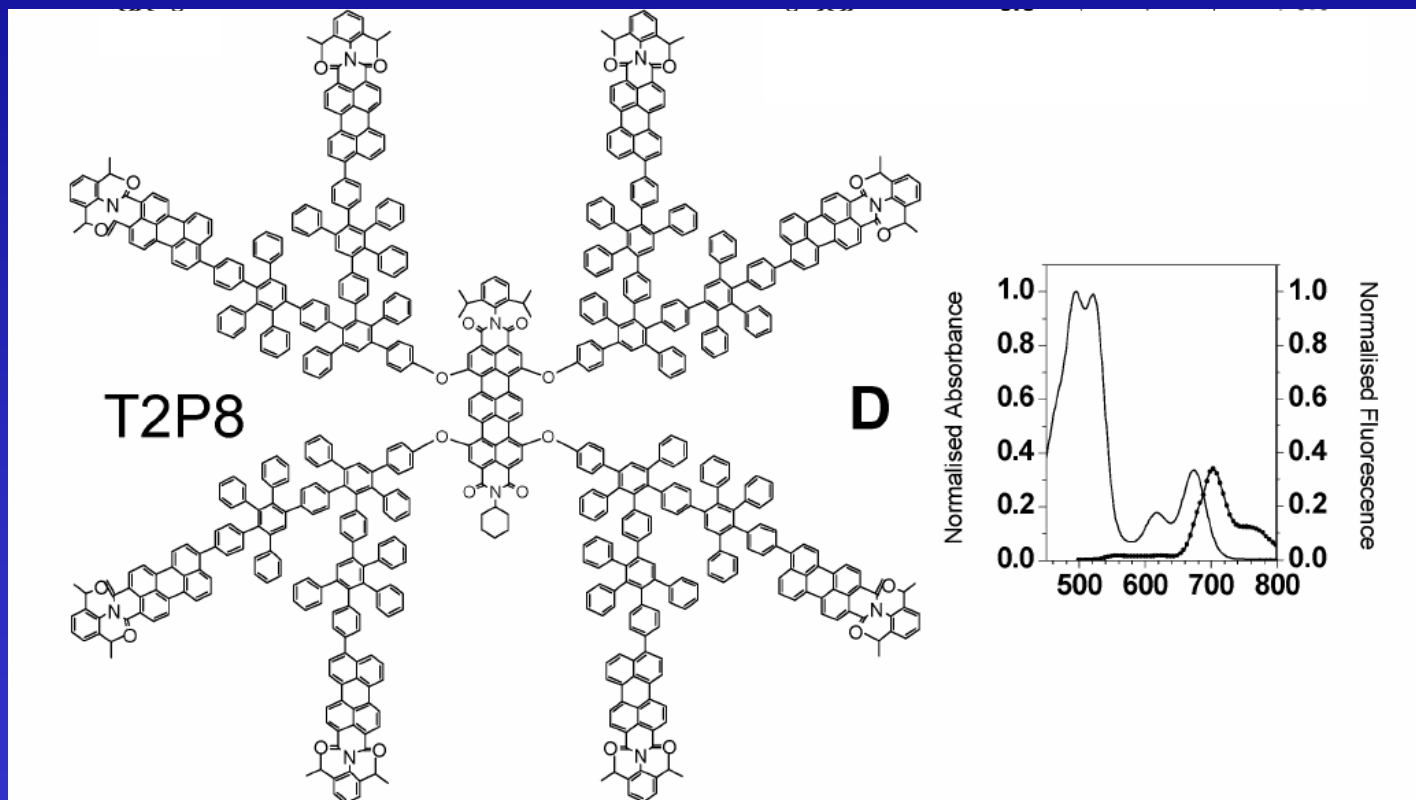
FRET acceptor **terryleneimide**
first generation dendrimer

Energy transfer between several fluorophores



FRET acceptor **terryleneimide**
first generation dendrimer
4 FRET donors **peryleneimide**

Energy transfer between several fluorophores



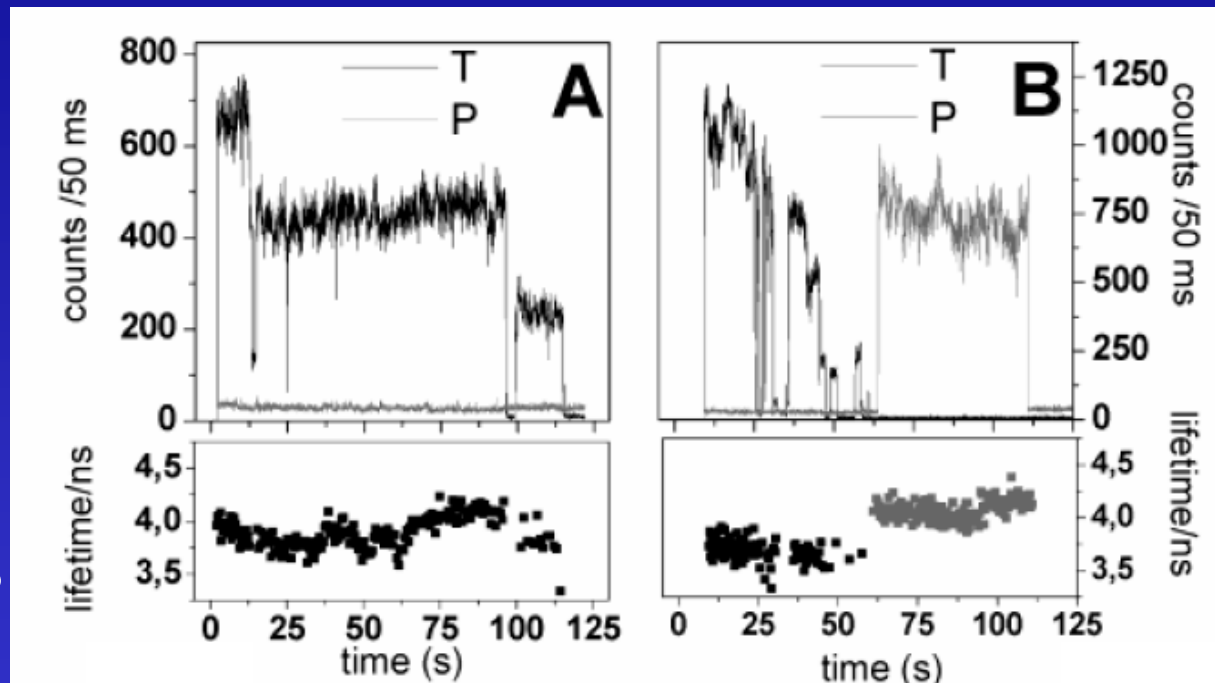
FRET acceptor **teryleneimide**
second generation dendrimer
8 FRET donors peryleneimide

Energy transfer between several fluorophores

T1P4 in polymer
(Zeonex)

T: FRET acceptor
(black)

P: 4 FRET donors
(gray)



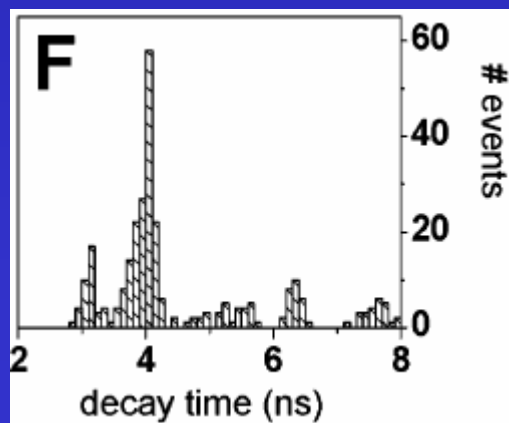
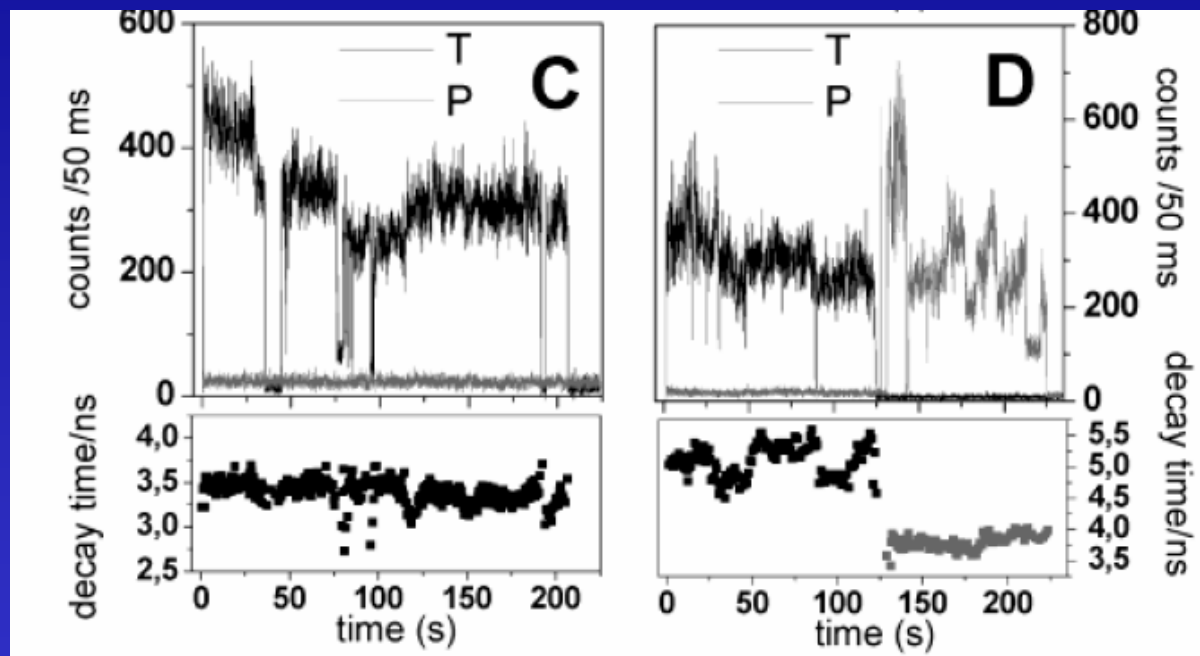
efficient FRET efficiencies,
up to 4 intensity levels of FRET acceptor,
acceptor bleaching in (B)

Energy transfer between several fluorophores

T2P8 in polymer
(Zeonex)

T: FRET acceptor
(black)

P: 8 FRET donors
(gray)



efficient FRET efficiencies for all donors

multiple acceptor lifetimes (direct excitation) are due to steric crowding and distinct twisting of terrylene

Energy transfer between several fluorophores

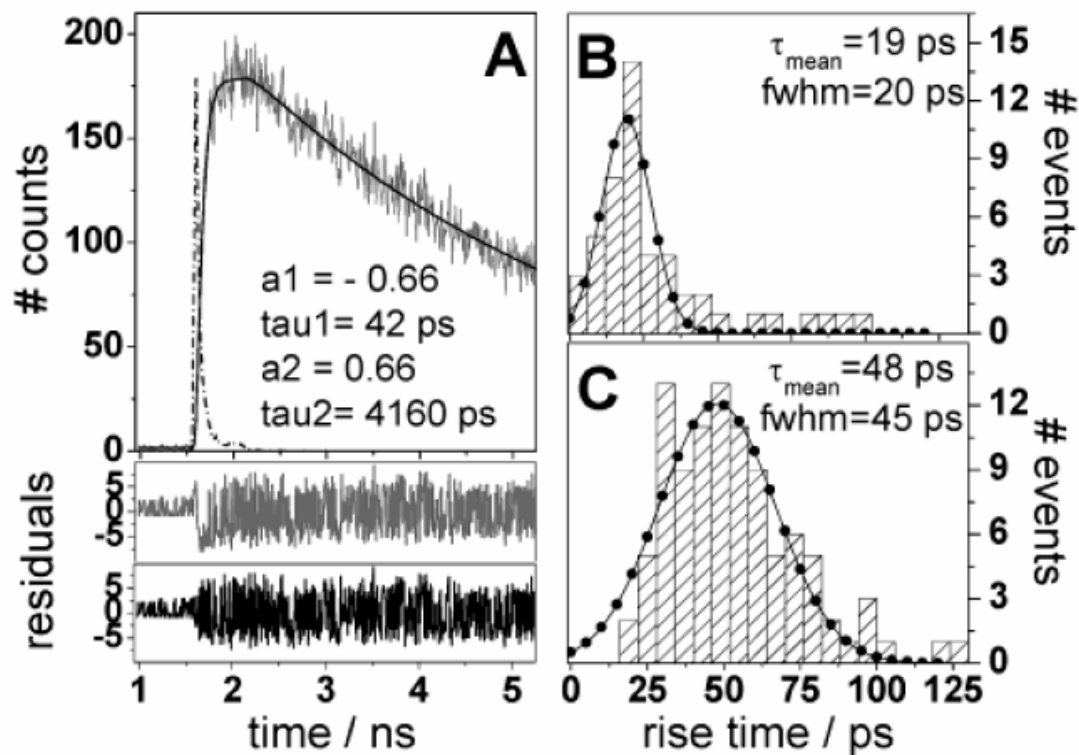


Figure 4. (A) Upper panel: fluorescence decay histogram (gray line) recorded with an MCP-PMT detector and accounting for the acceptor emission of a single T2P8 molecule in Zeonex upon selective 488 nm excitation of the donors. The instrumental response function of the system

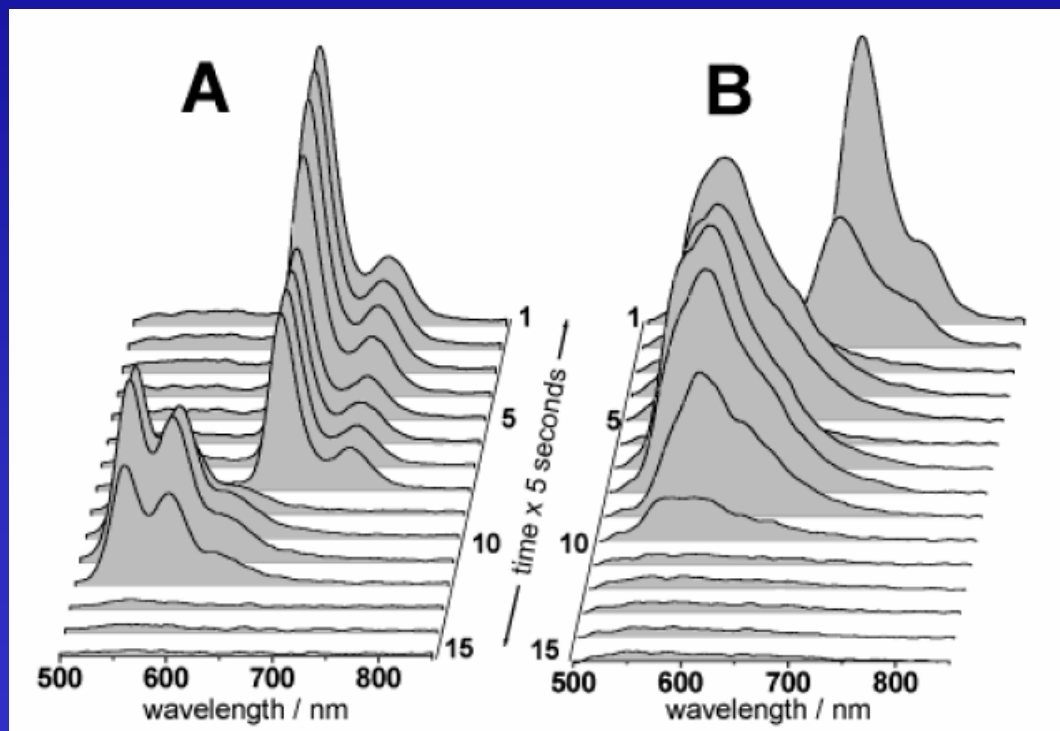
rise time
distributions of
FRET acceptor

(B) T1P4

(C) T2P8

broad range of different
rates, i.e. distances and/
or orientations of the
FRET donors

Energy transfer between several fluorophores



fluorescence spectra of single
(A) T1P4
(B) T2P8
before / after acceptor bleaching

Energy transfer between several fluorophores

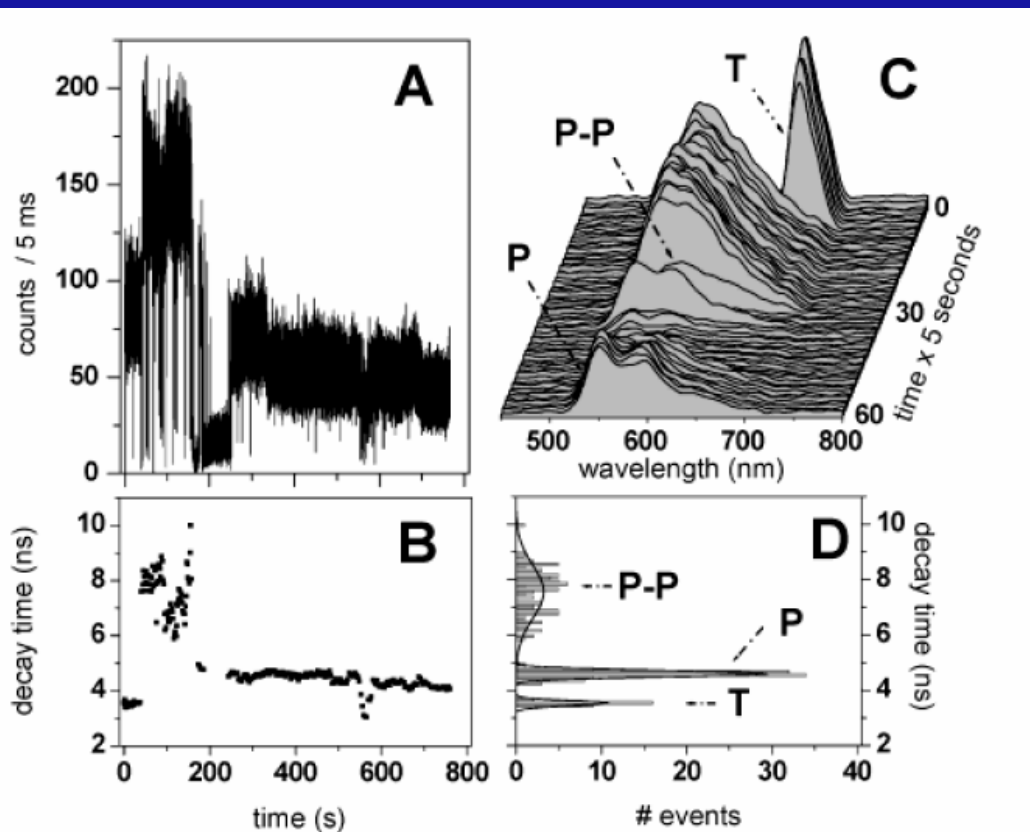


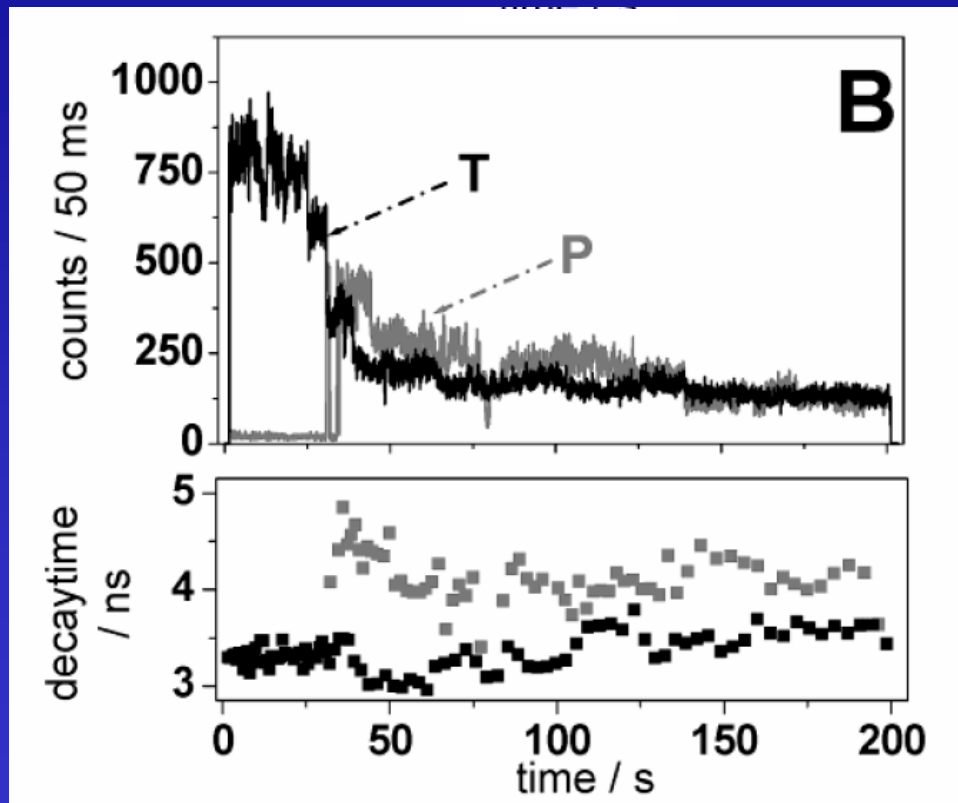
Figure 6. Time course of the fluorescence intensity (A), of the fluorescence decay time (B), and of the fluorescence spectrum (C) recorded simultaneously from a single T2P8 molecule immobilized in Zeonex upon 488 nm excitation. Arrows in graph C indicate bleaching of the T, P-P, and P excited species, respectively. (D) Histogram of the fluorescence decay time data from panel B.

T: FRET acceptor
P: FRET donors

P-P: excited dimer
red-shifted, long lifetimes,
fluctuations due to confor-
mational dynamics

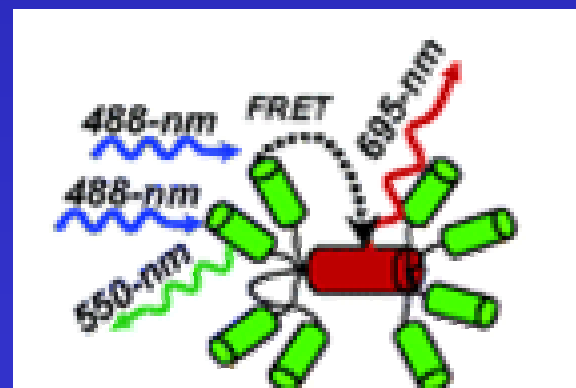
further bleaching results in
structured monomer emission
(P spectra)

Energy transfer between several fluorophores



ps-pulsed excitation: donor and acceptor lifetime fluctuations, S-S annihilation (donors) possible

Exciton blockade in T2P8 at high excitation power
T (black) acceptor
P (gray) donors



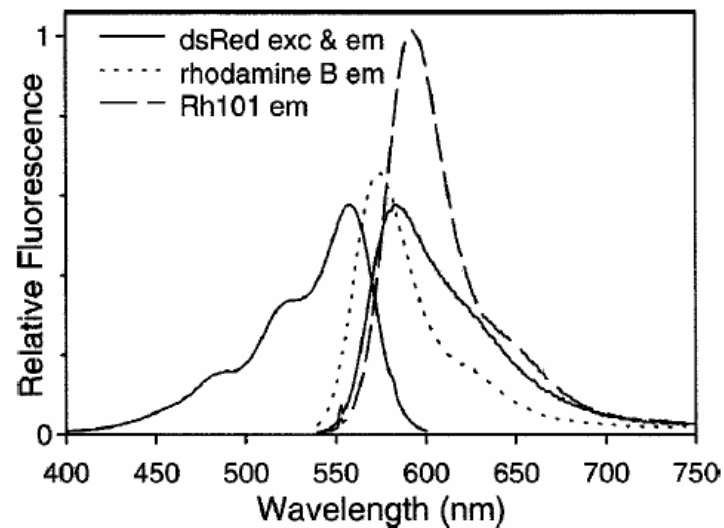
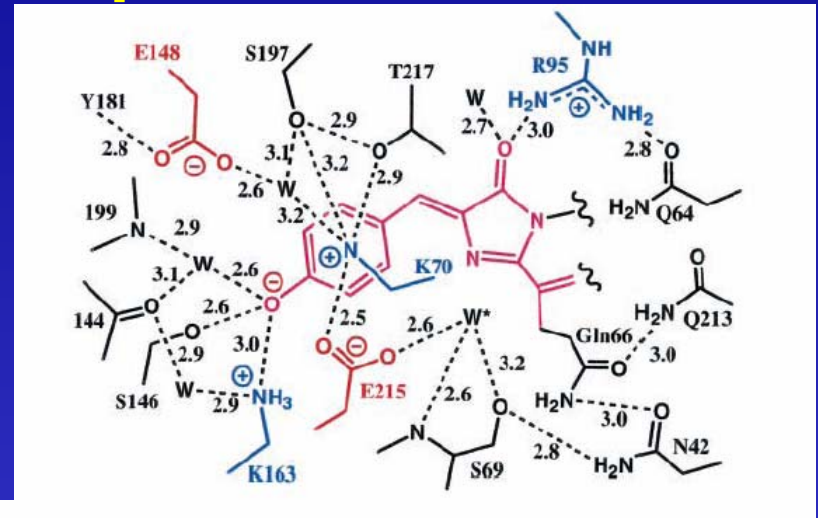
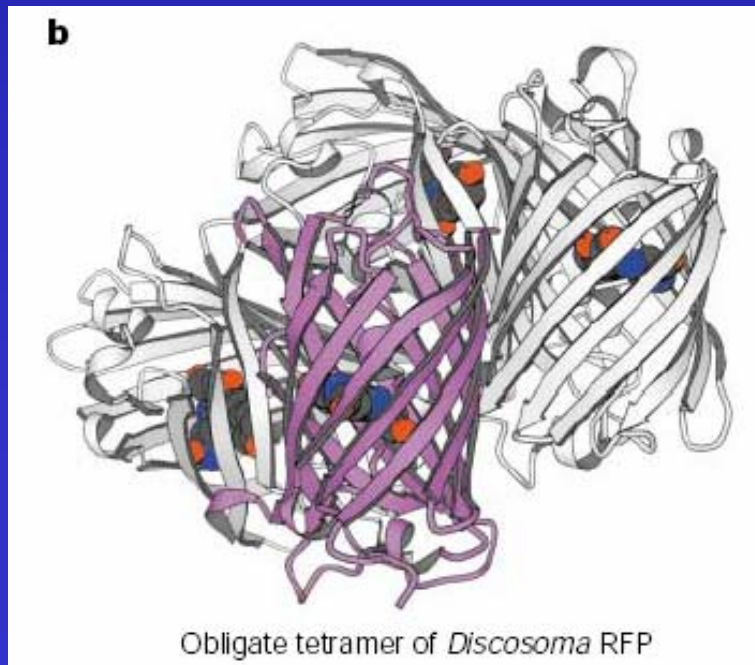
Energy transfer between several fluorophores

Examples

1. artificial homo-dimers
2. light harvesting complexes
3. dendrimers
4. dsRED

Energy transfer between four identical fluorophores

dsRED: 28 kDa,
autofluorescent protein
tetrameric



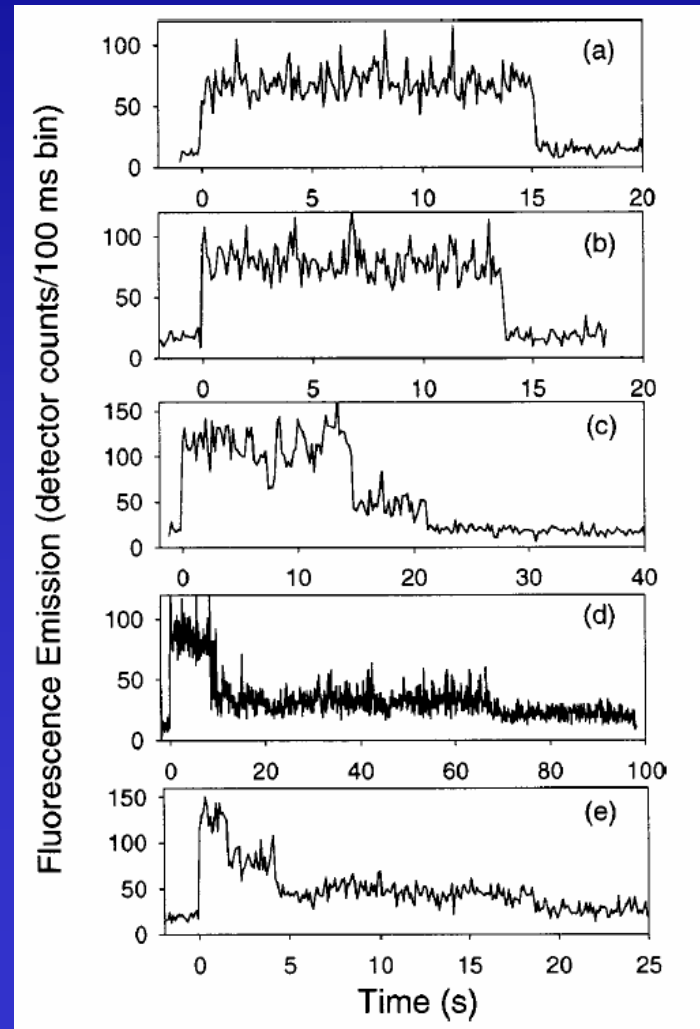
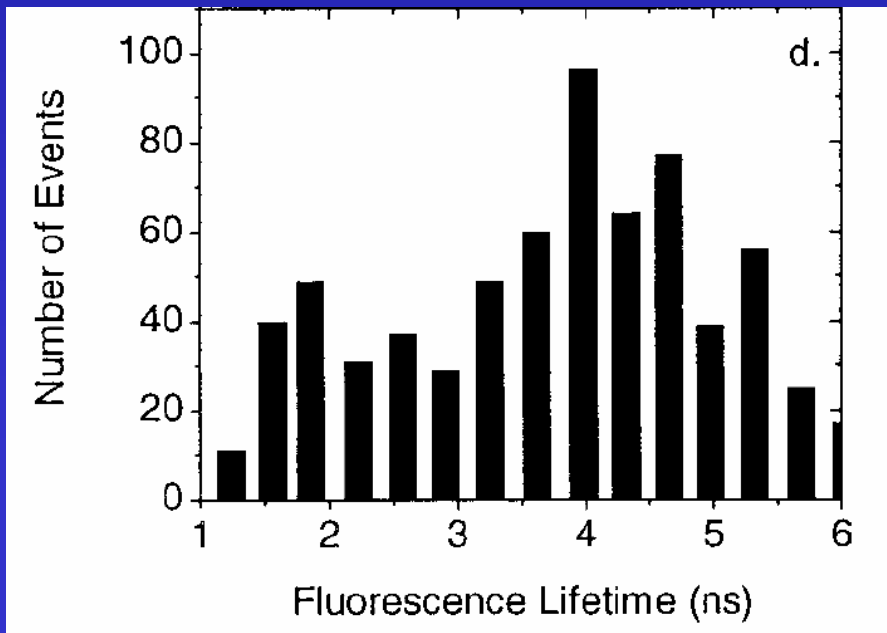
Energy transfer between four identical fluorophores

dsRED: single-molecule fluorescence trajectories

[Moerner, JCP, 2002]

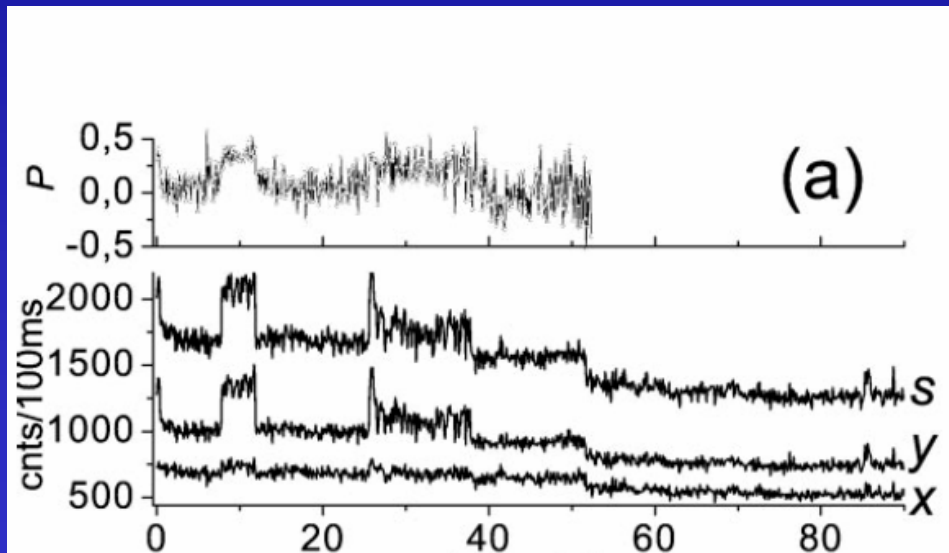
single-molecule lifetimes

[Bowen, Woodbury, Photochem. Photobiol. 2003]

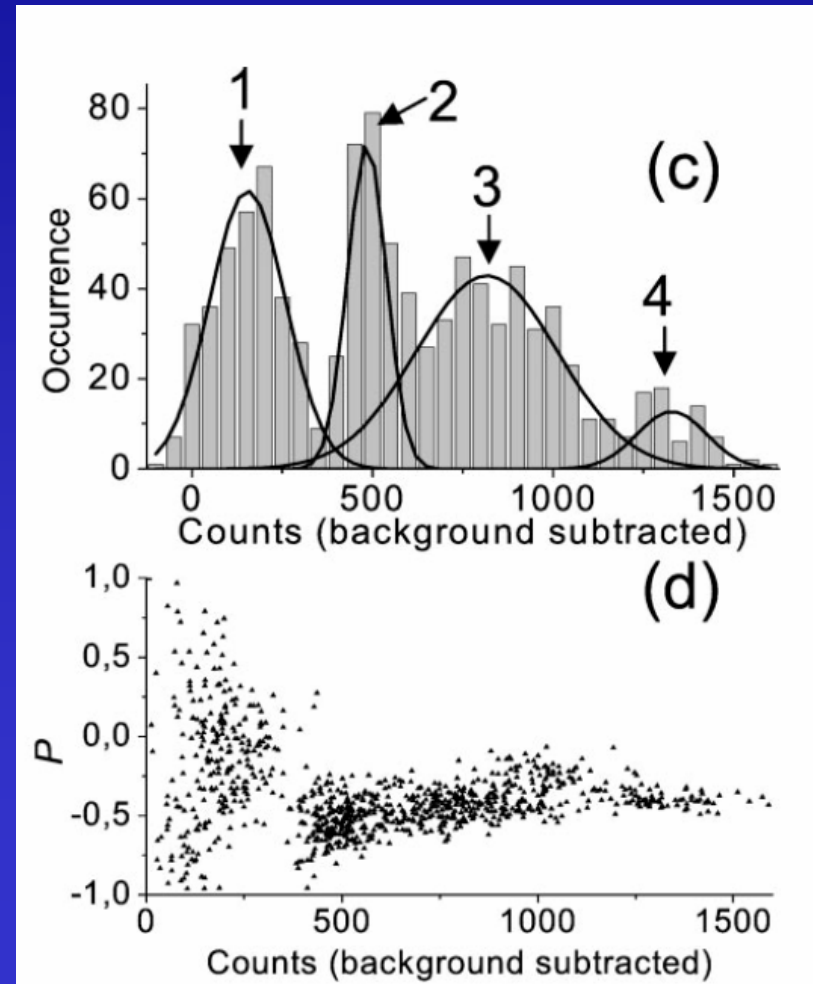


Energy transfer between four identical fluorophores

dsRED anisotropy changes

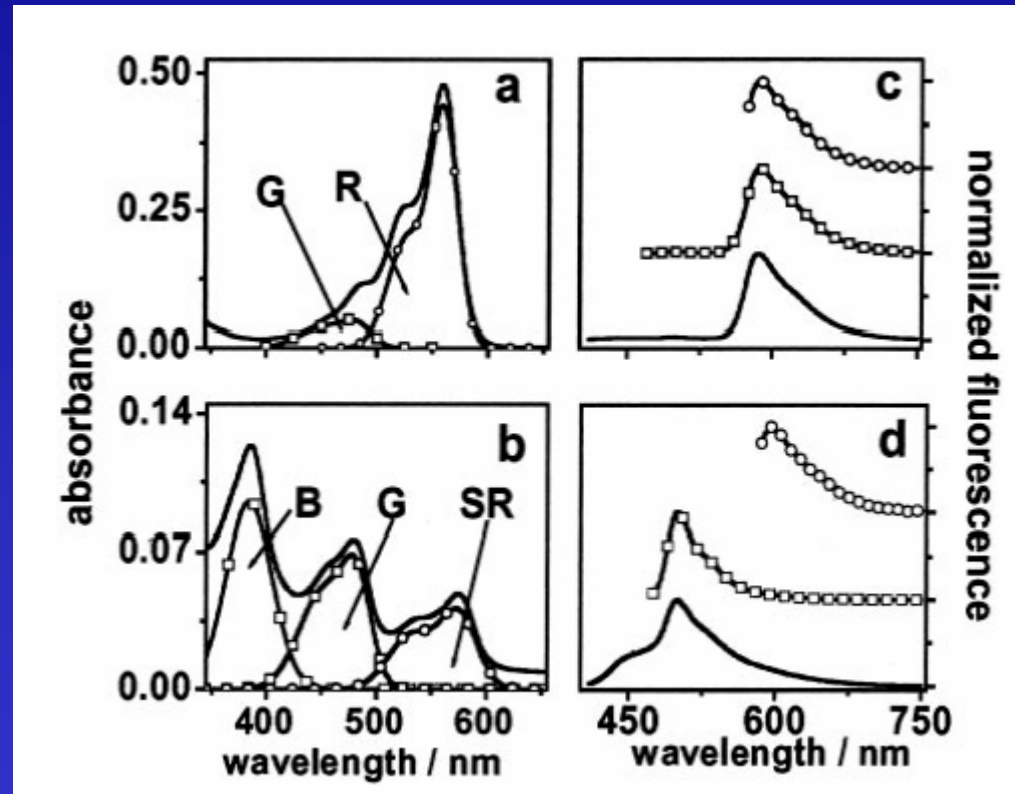
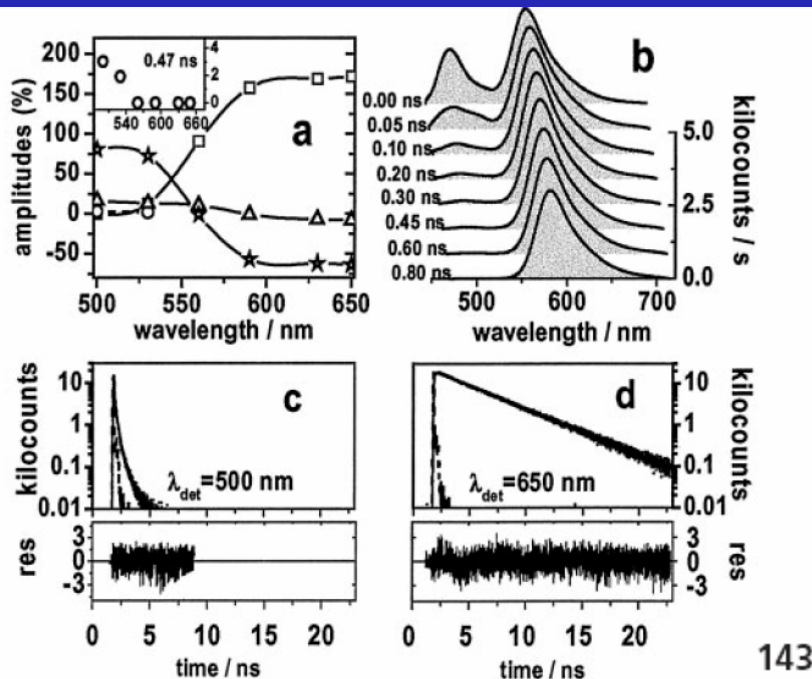


four intensity levels: level 4, 3, and 2 with 'constant' anisotropy, level 1 fluctuates



Energy transfer between four non-identical fluorophores

immature dsRED with
'green' fluorescent components
(G) and red (R) component,
time resolved FRET (bulk)



Energy transfer between four non-identical fluorophores

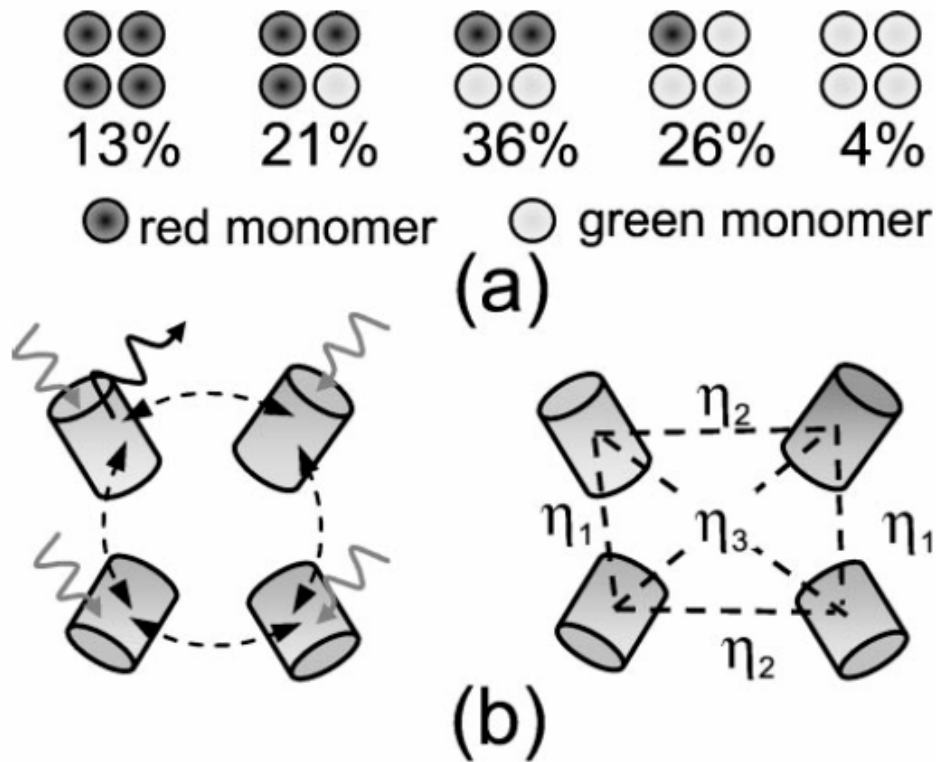


Fig. 6. (a) Distribution of red and green species within the DsRed, as derived from single-molecule data. (b) The cartoon illustrates the mechanism of fluorescence in DsRed. For simplicity, only red species are considered. (Left) Energy transfer occurs between all chromophores, and emission results with equal probability in time from any of the four chromophores. (Right) A

collective on/off blinking:

one 'trapping' dark monomer and efficient energy transfer within the tetramer

Energy transfer between four identical fluorophores

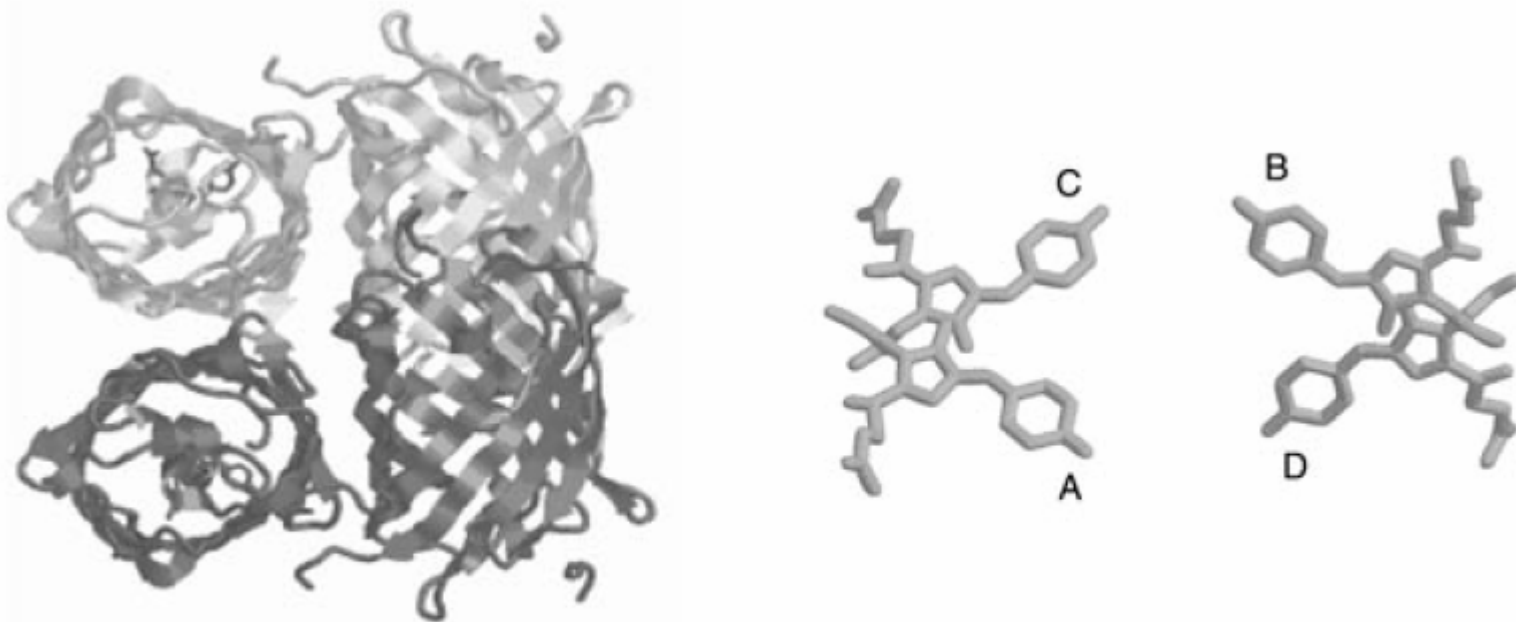


Figure 1. Schematic structure of DsRed. Ribbon diagram (left) and relative orientation of the four chromophores (right). Distance between the monomer pairs: A–B and C–D is 2.2 nm, A–C and B–D is 3.8 nm and A–D and B–C is 4.3 nm. The angular orientations between the chromophore pairs are: 21° between A–B and C–D, 47° between A–C and B–D and 41° between A–D and B–C.

Energy transfer between four identical fluorophores

Photon Antibunching Proves Emission from a Single Subunit in the Autofluorescent Protein DsRed

Gabriel Sánchez-Mosteiro, Majolein Koopman, Erik M. H. P. van Dijk, Jordi Hernando, Niek F. van Hulst, and María F. García-Parajó*^[a]

interphoton time histogram

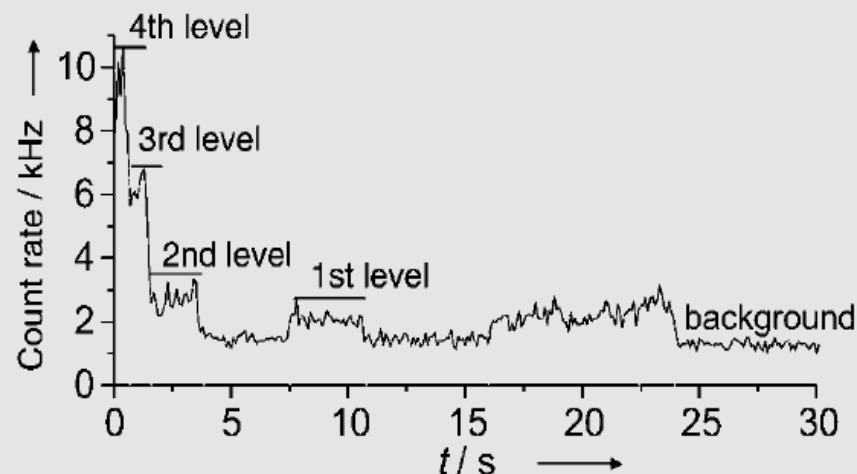
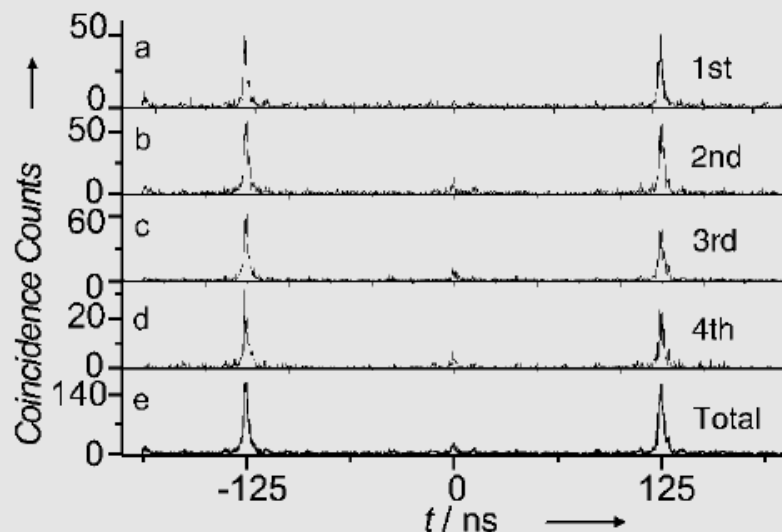


Table 1. N_0/N_L ratios as expected for four independent emitters and the N_0/N_L as derived from the photon-arrival-time histograms on DsRed

Number of absorbers	N_0/N_L (independent emitters)	N_0/N_L (exptl.)
1	0	0.10 ± 0.03
2	0.5	0.14 ± 0.03
3	0.67	0.16 ± 0.04
4	0.75	0.19 ± 0.07

Energy transfer between three and more distinct fluorophores

1. three-color FRET
2. photonic wires