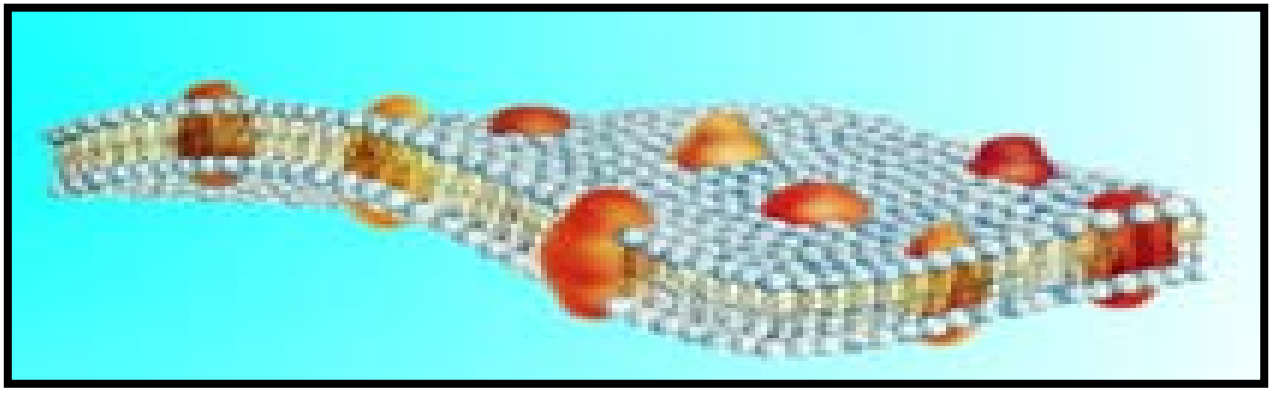
THE SOLVENT RELAXATION TECHNIQUE: APPLICATION IN STUDIES OF BIOMOLECULES



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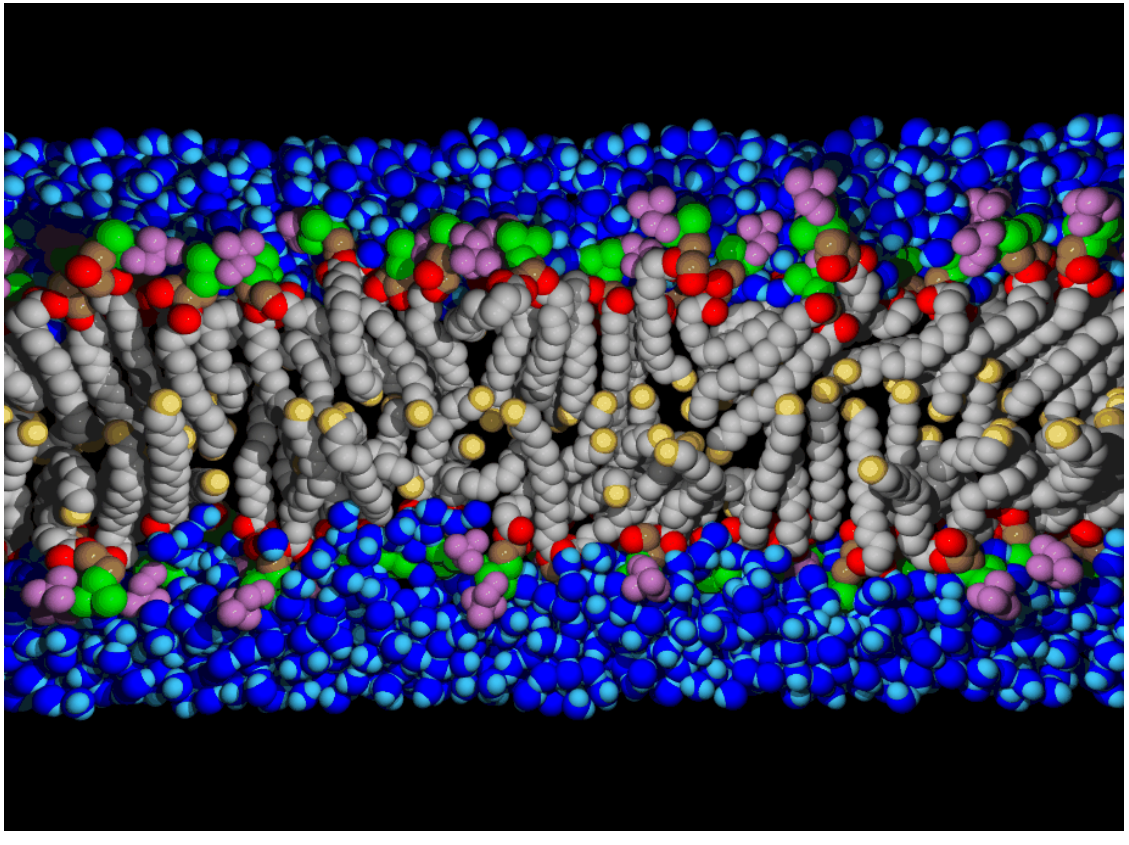
ttp://www.jh-inst.cas.cz/~fluorescence/solvent%20relaxation.htm

THE SOLVENT RELAXATION TECHNIQUE: APPLICATION IN STUDIES OF BIOMOLECULES

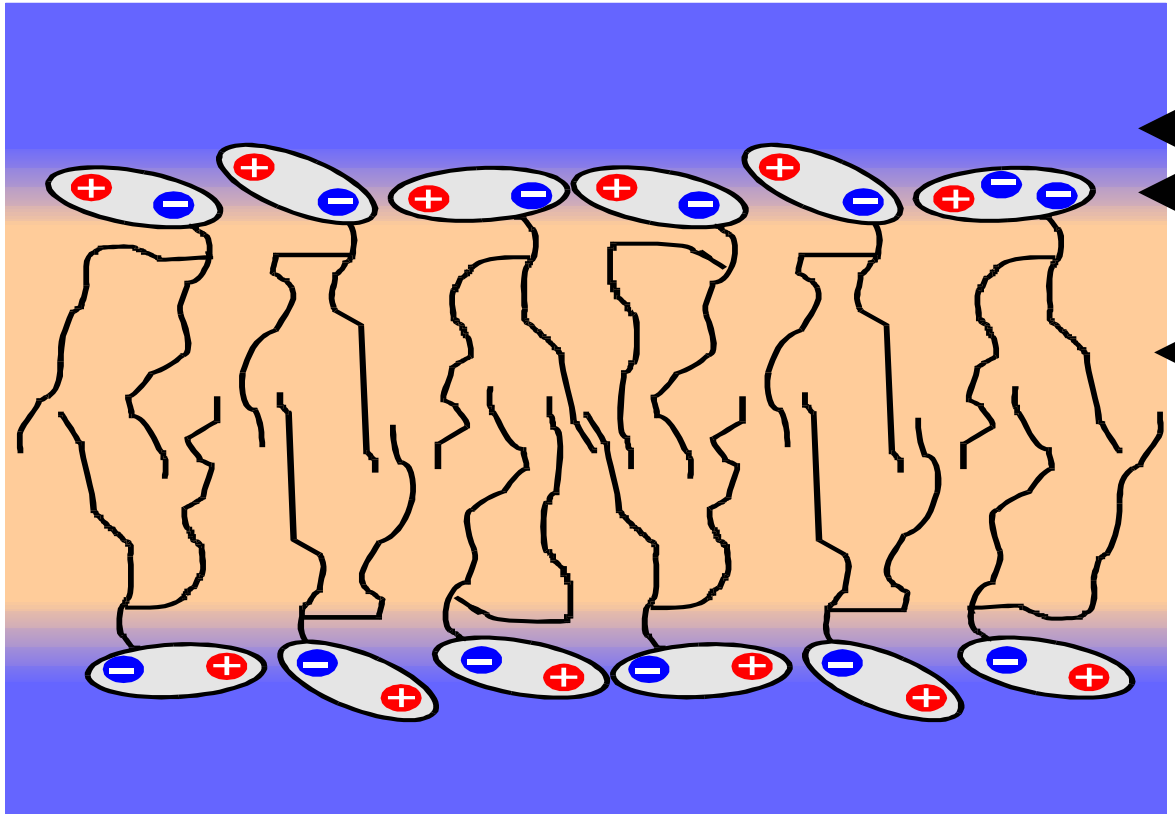
- Motivation for investigating solvent relaxation in lipid bilayers
- Principles of solvent relaxation
- Solvent relaxation in lipid bilayers
- Solvent relaxation in DNA and proteins

Motivation for investigating solvent relaxation in phospolipid bilayers



Overall Aims: 1) Characterisation of water associated with lipids
2) Connecting this "biological water" with functional properties

Solvent relaxation in phospholipid-bilayers by micro-enviroment of a defined located dye



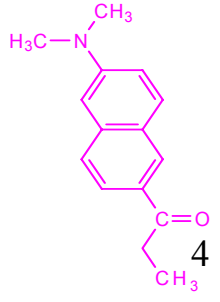
Dye is defined located in

external interface

headgroup region

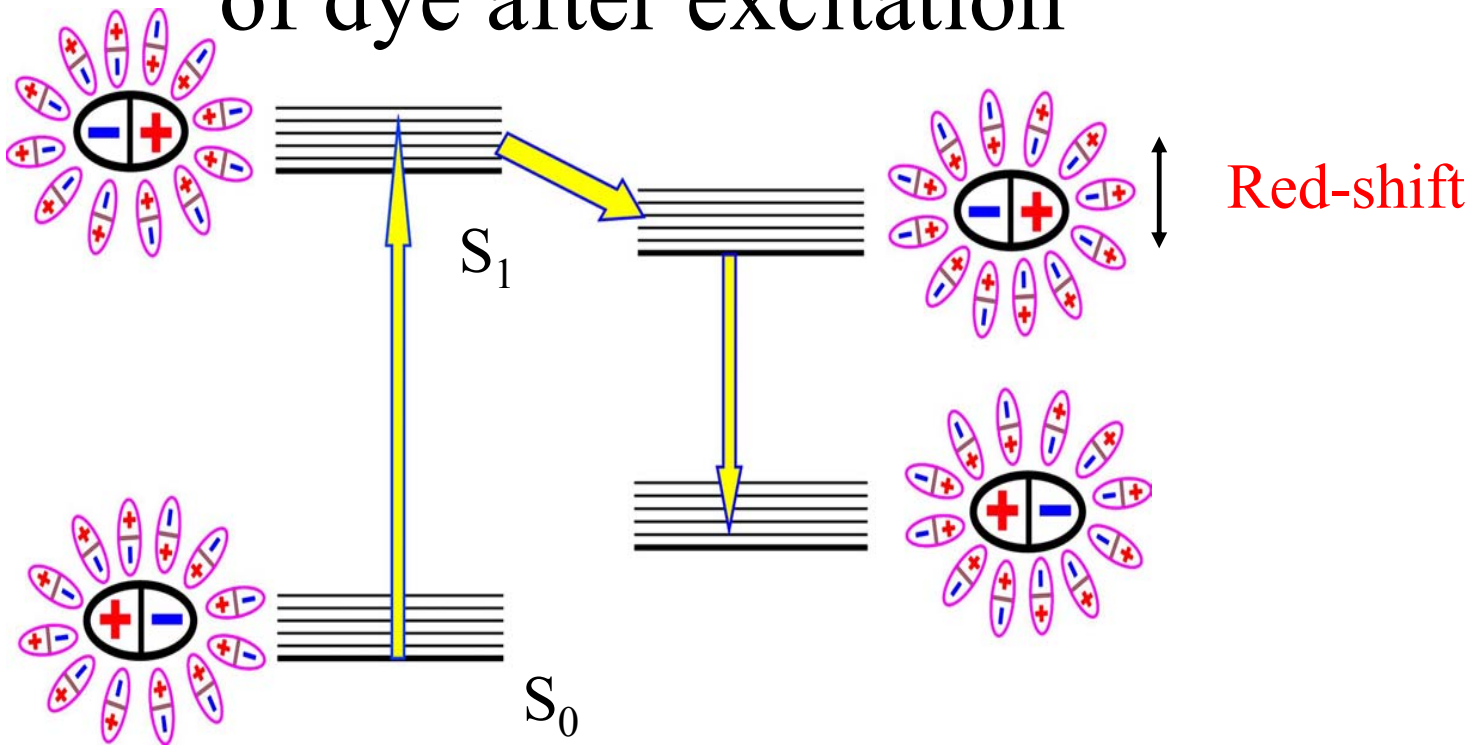
hydrophobic backbone

Dye is usually an aromatic molecule, one example: Proda



⇒ **Definition of "water structure" along the z-axis**

Principles of solvent relaxation: "photophysics of dye after excitation"

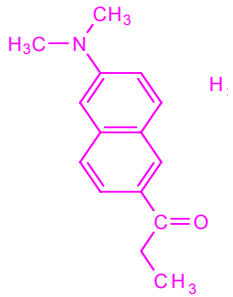


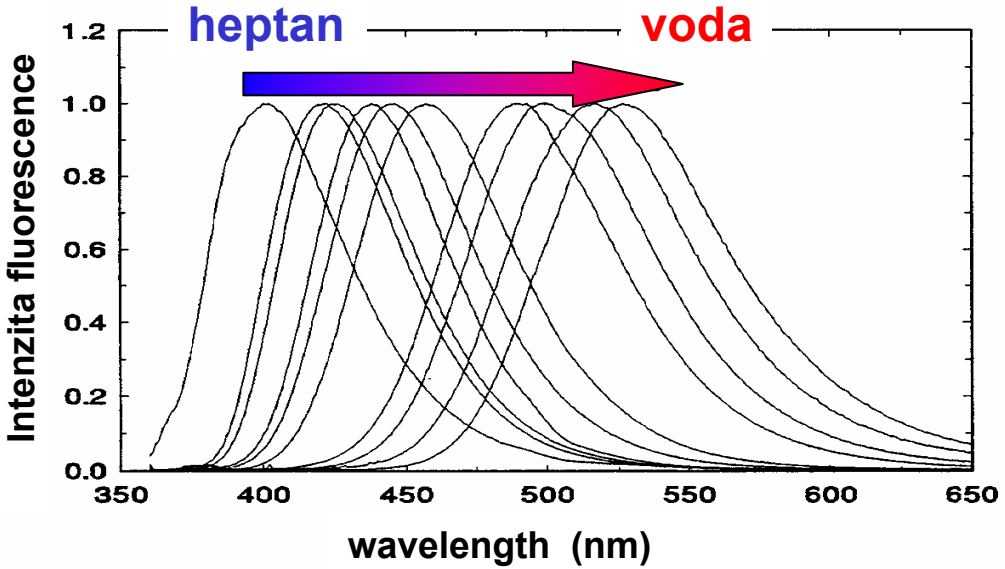
Dye excitation leads to a instantaneous change in the dye's dipole moment → dipoles of the solvent molecules have to react to this non-equilibrium situation and start to reorient →this reorientation leads to stronger dipole-dipole interactions and decreases the energy of the system (relaxation) →red-shift

Consequence of SR: Red and blue-shifts in steady-state fluorescence spectra

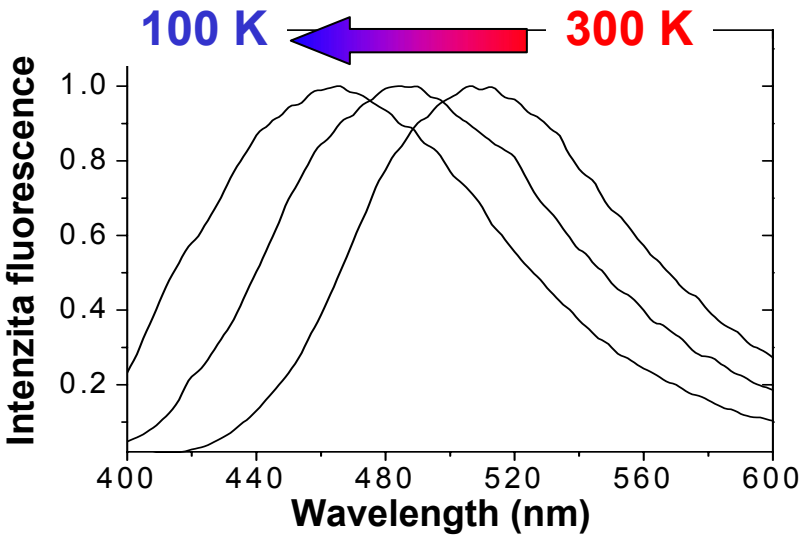
Increase of solvent polarity leads to red-shift

Prodan





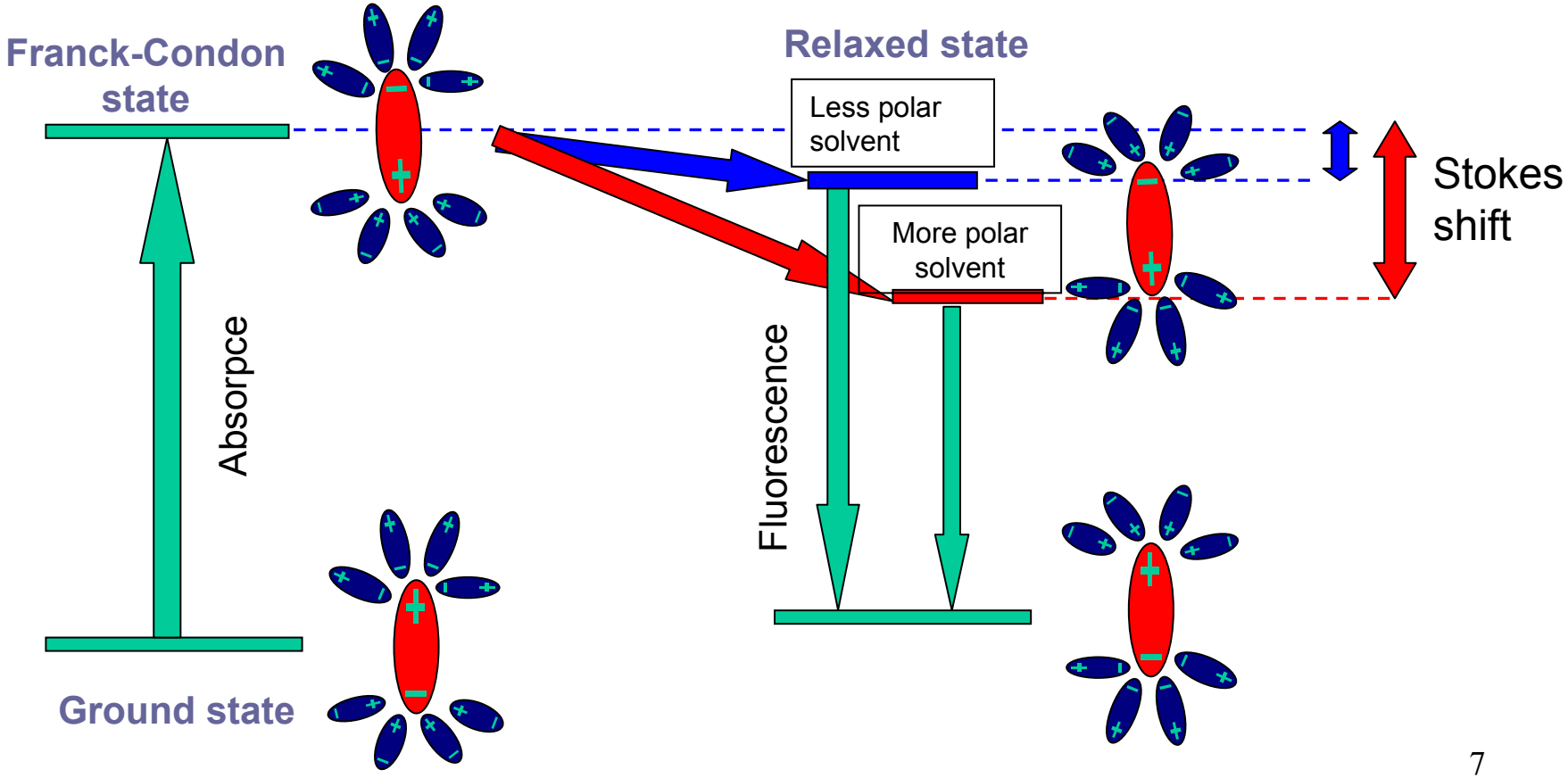
Increase of viscosity leads to blue-shift



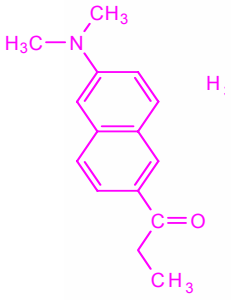
Red-shifts in steady-state fluorescence spectra

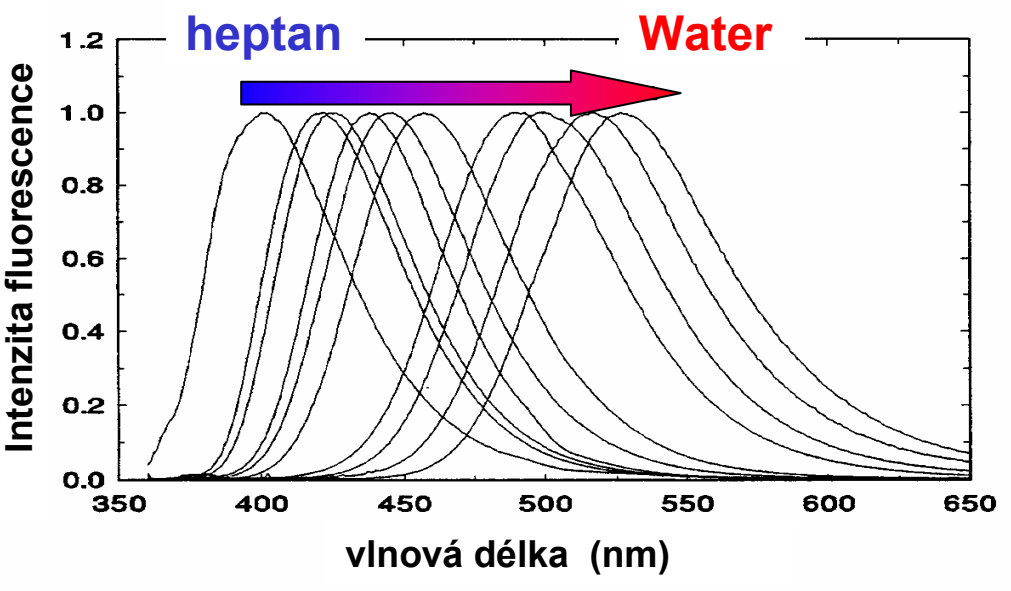
Solvent relaxation is faster than fluorescence

Jablonski diagram:



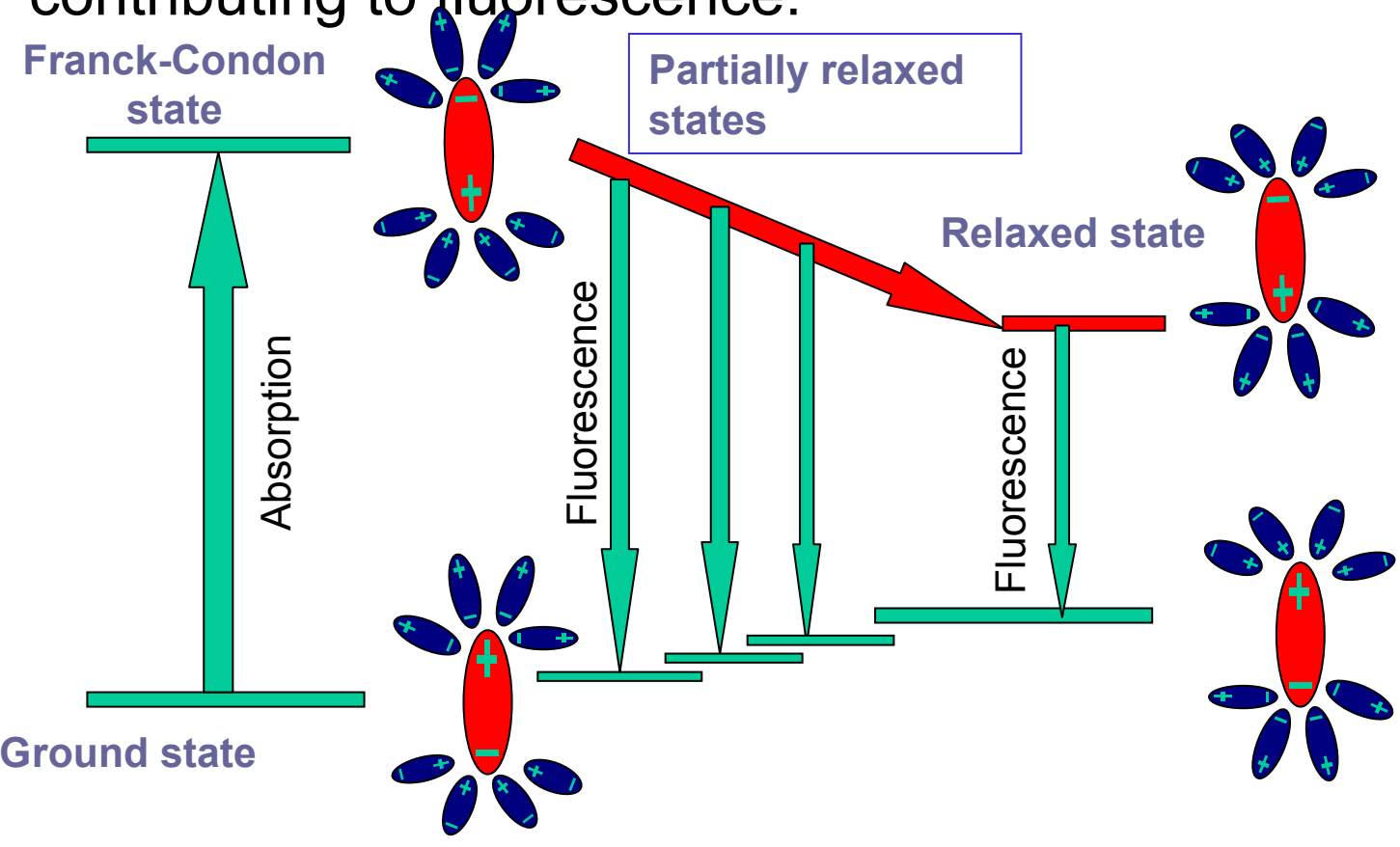
Solvent relaxation is faster than fluorescence: increase of polarity of solvent leads to stronger dipole-dipole interactions and thus to a decrease of the energy of the relaxed state. Almost all dye molecules are fluorescing from this state, thus increased solvent polarity leads to red-shift



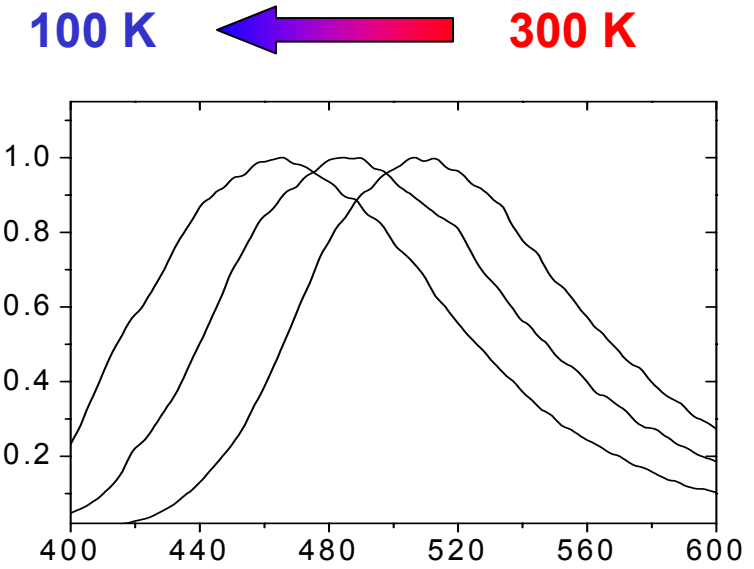


Blue-shifts in steady-state fluorescence spectra

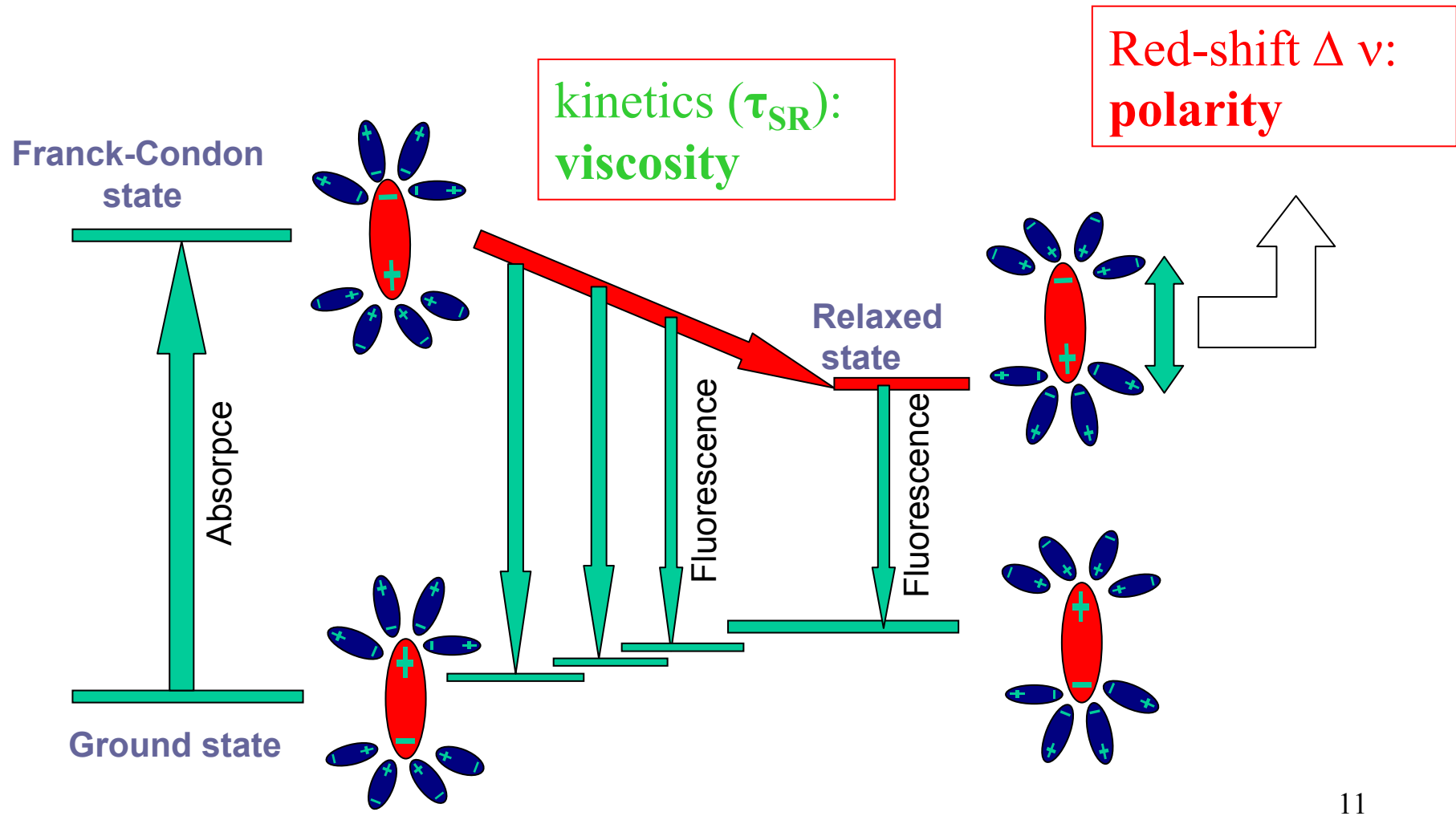
Increasing viscosity slows down the SR process. If then the SR occurs on the same time scale as the fluorescence (nanoseconds) → non-relaxed states are significantly contributing to fluorescence:



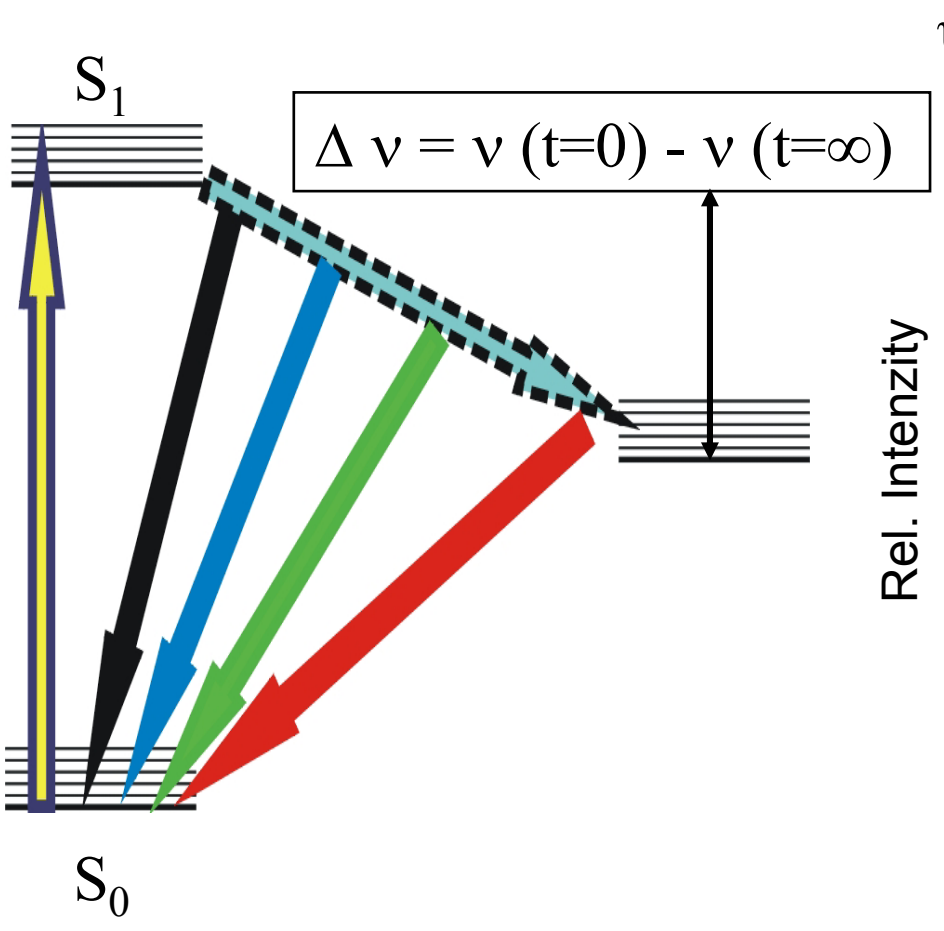
Solvent relaxation is on the same time scale than fluorescence: increase of viscosity leads to increasing fluorescence contributions of non-relaxed states and thus to an increasing blue-shift

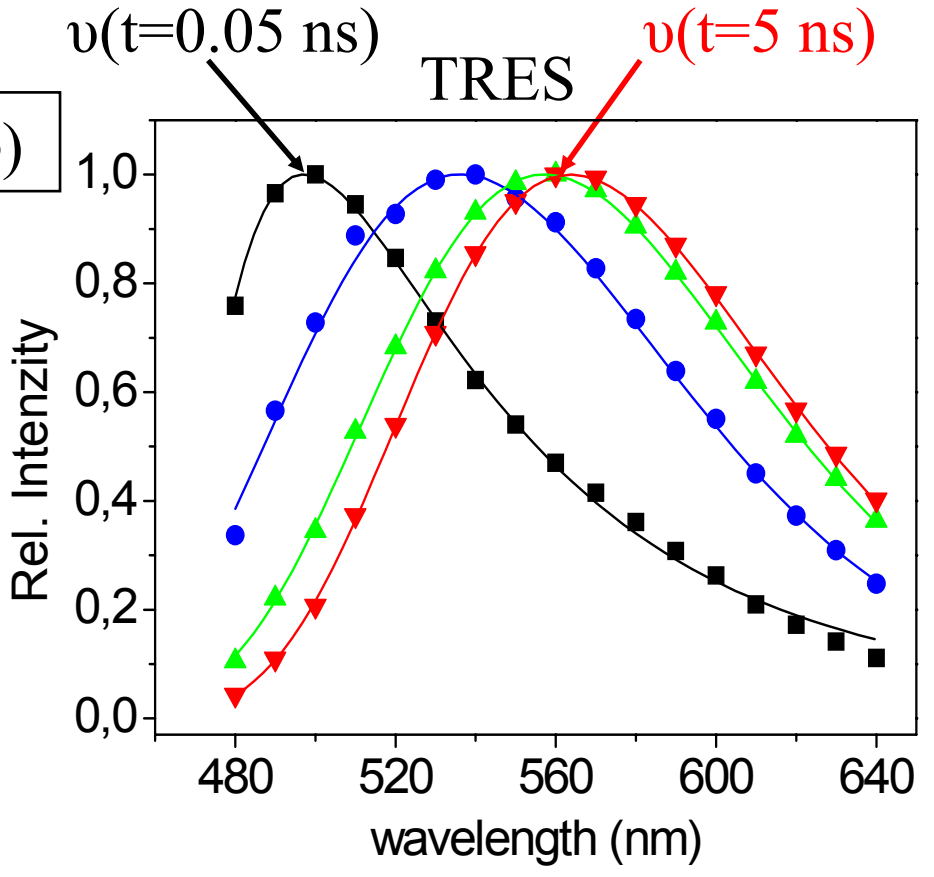


Time-resolved fluorescence spectroscopy: monitoring the solvent relaxation process



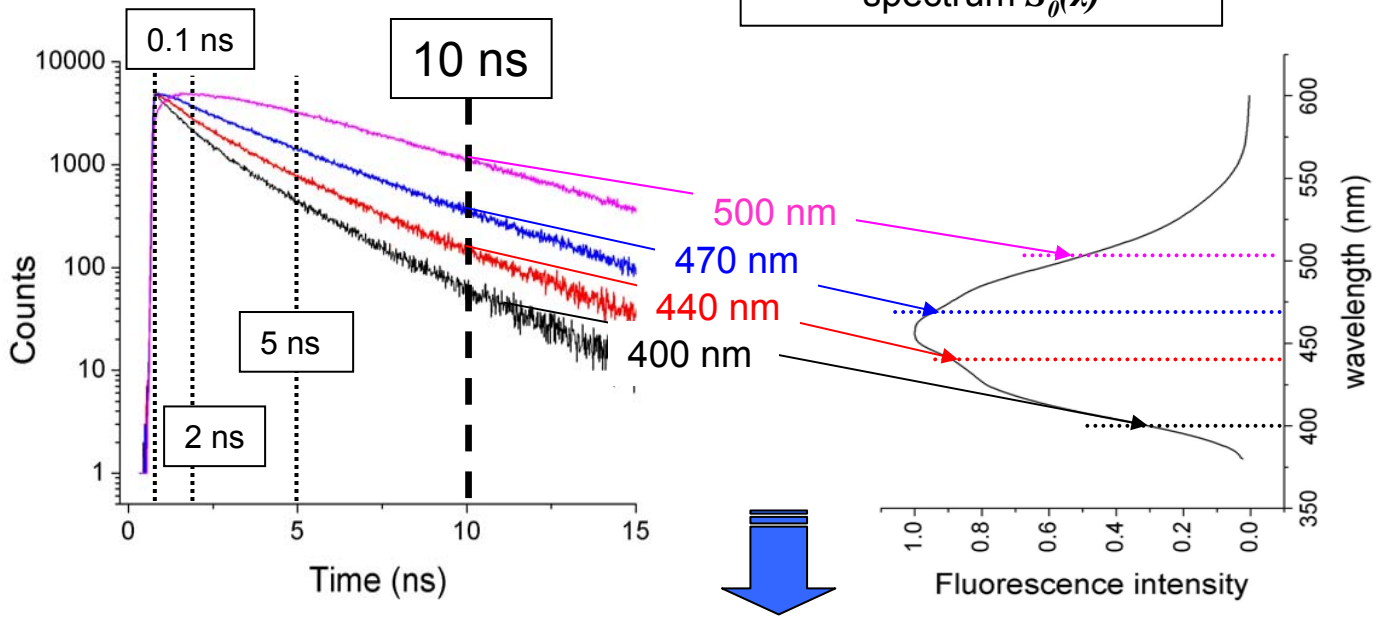
SR is monitored by "time-resolved fluorescence emission spectra"

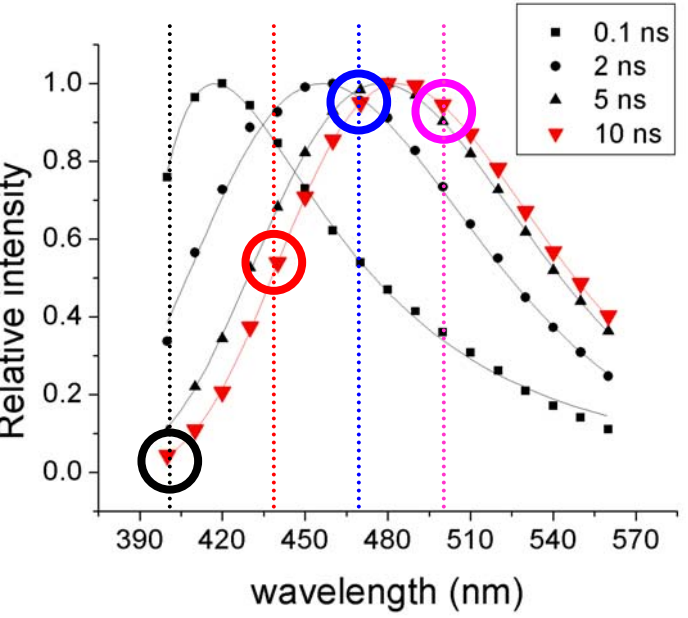




Fluorescence decays $D(t, \lambda)$

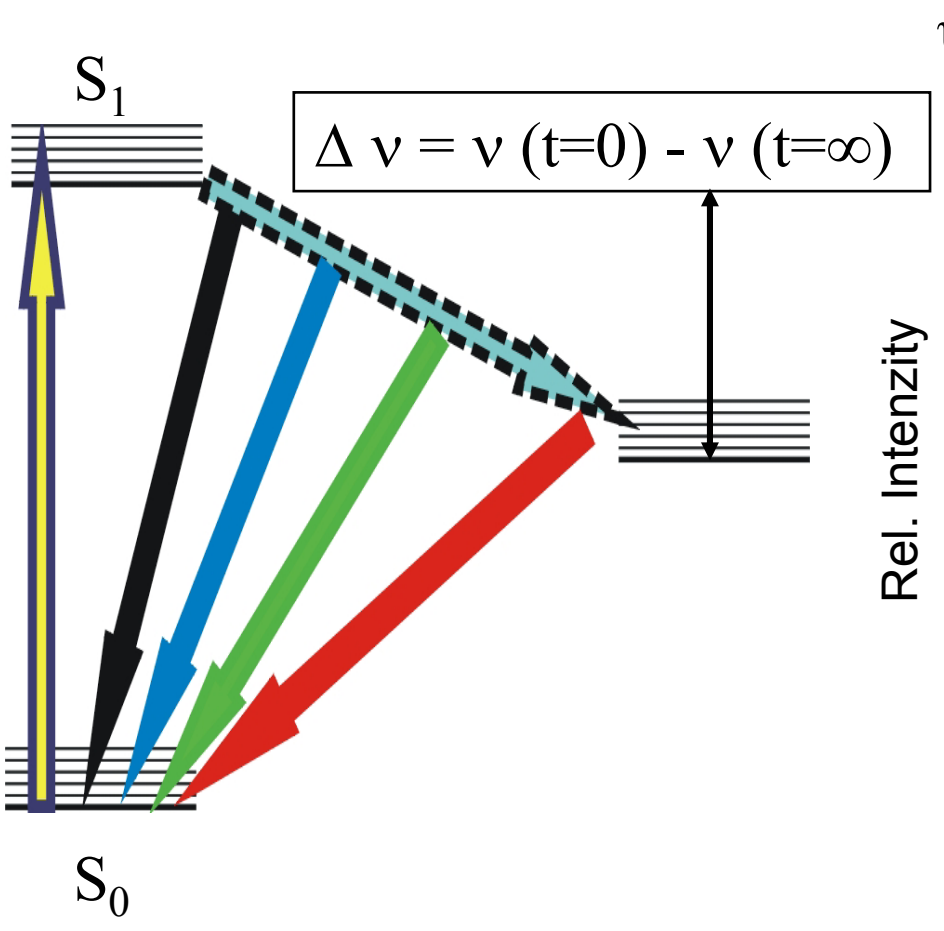
Steady-state fluorescence spectrum $S_0(\lambda)$

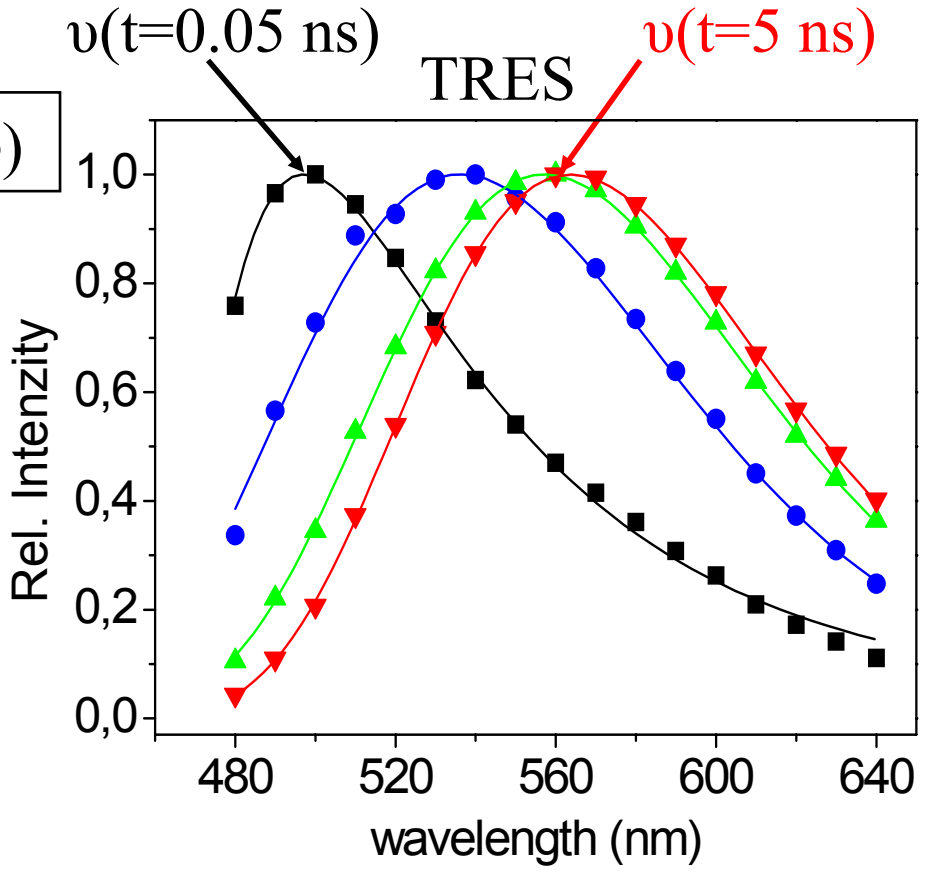




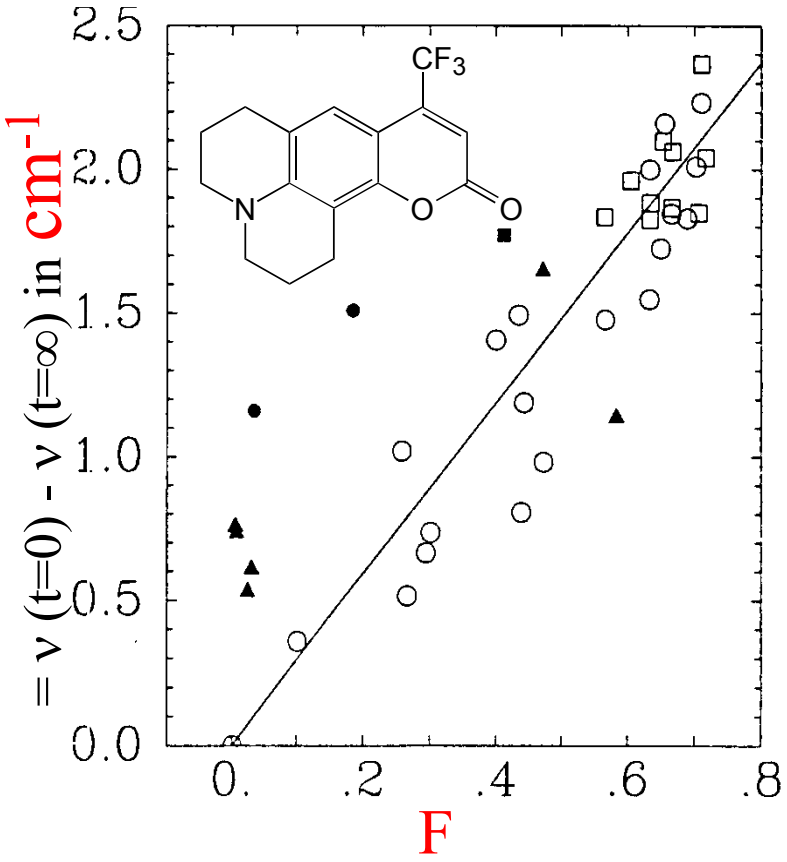
$$S(\lambda,t) = \frac{D(t,\lambda) \times S_0(\lambda)}{\int_0^\infty D(t,\lambda)dt}$$

SR is monitored by "time-resolved fluorescence emission spectra"





Time-dependent Stokes shift Δ ν gives directly information about the micro-polarity



= $[(\varepsilon_s-1)/(\varepsilon_s+2)] - [(n^2-1)/(n^2+2)]$

- Δ ν is directly proportional to the polarity function F

- example:

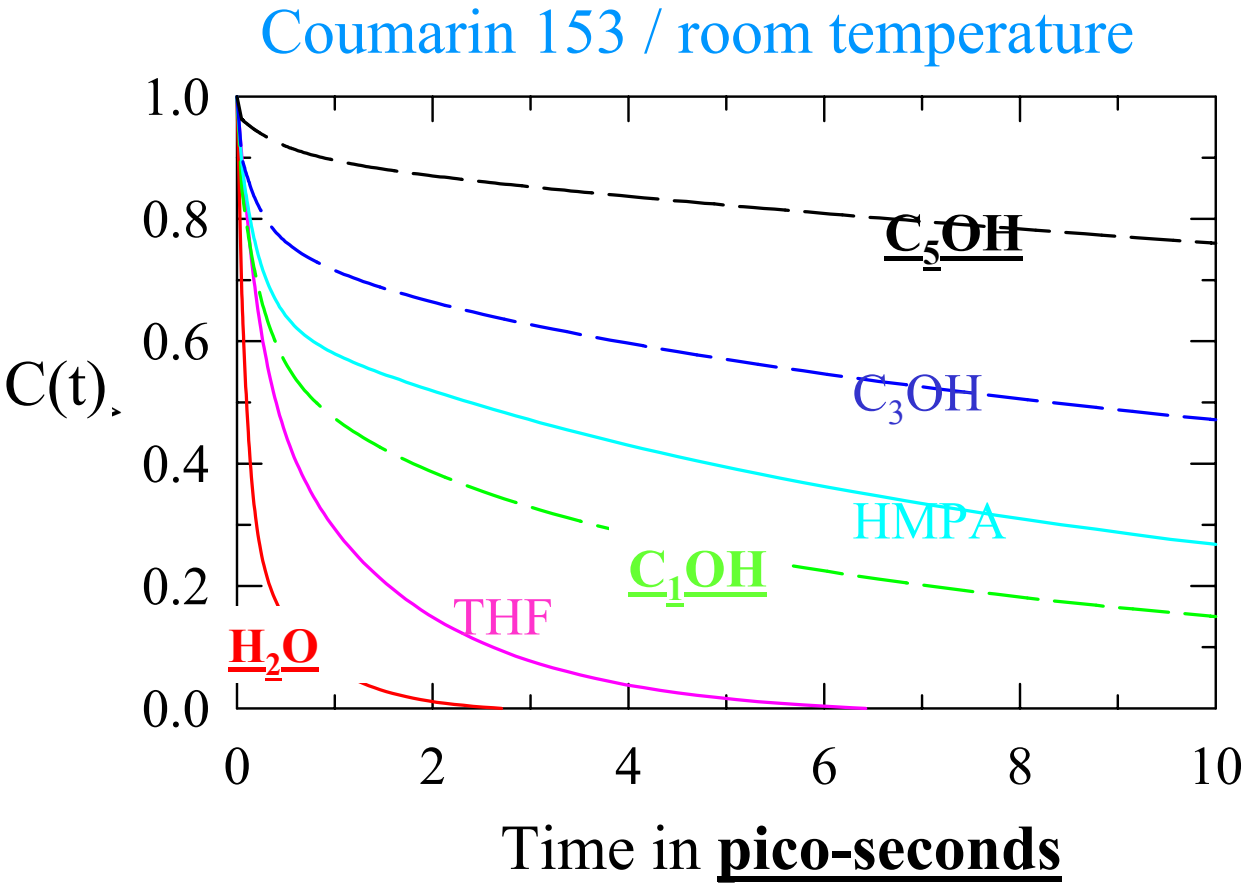
C_1OH: F = 0.71; Δ ν = 2370 cm

C_5OH: F = 0.57; Δ ν = 1830 cm

Horng et al., J Phys Chem 1995,99:1731

Dependence of SR kinetics on the solvent

Kinetics: Normalisation of Stokes shift υ(t): $C(t)=(\upsilon(t)-\upsilon(\infty))/\Delta\upsilon$



$\tau_{SR(average)}$: /ps

0.3 "bulk water"

0.9

5

10

26

103

ummarised from contributions by M. Maroncelli (1993-1997)

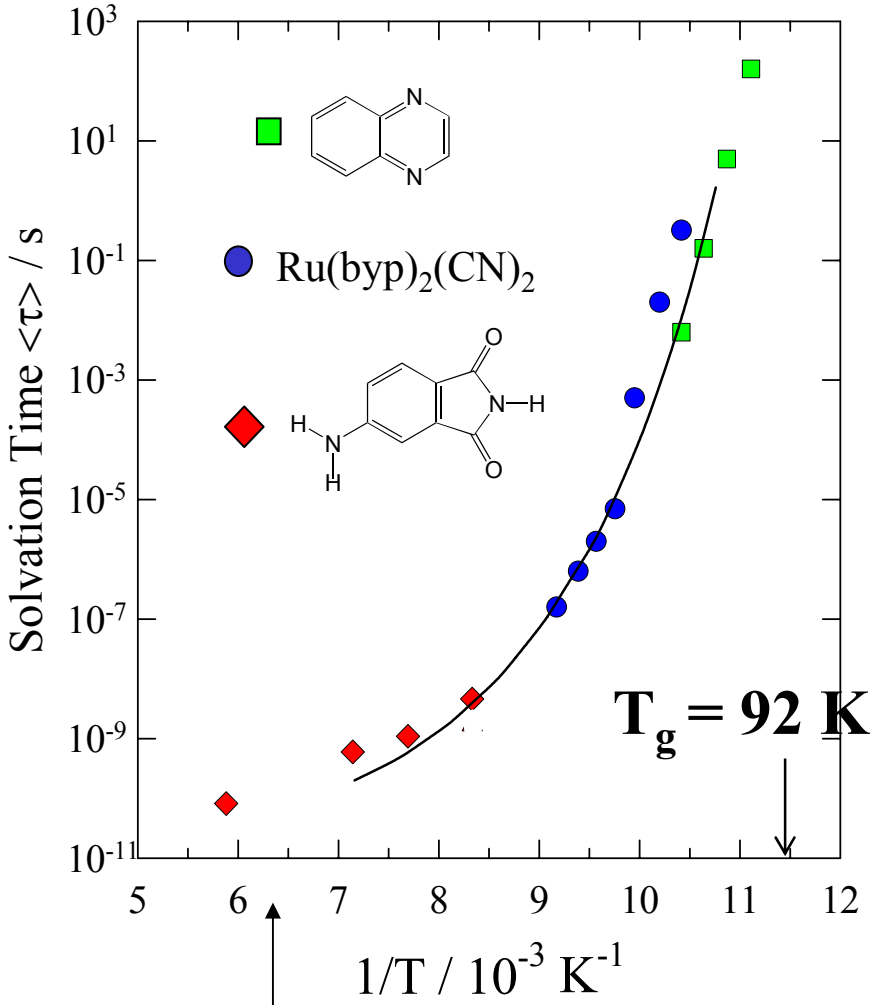
Kinetics of the SR is related to the viscosity of the microenvironment

dyes in THF 90-170 K

τ_{Phosp} = 0.25 s

τ_{CT} = 4 µs

τ_{Fluor} = 20 ns



Probed by $T_1 \rightarrow T_0$ Phosphoreszenz

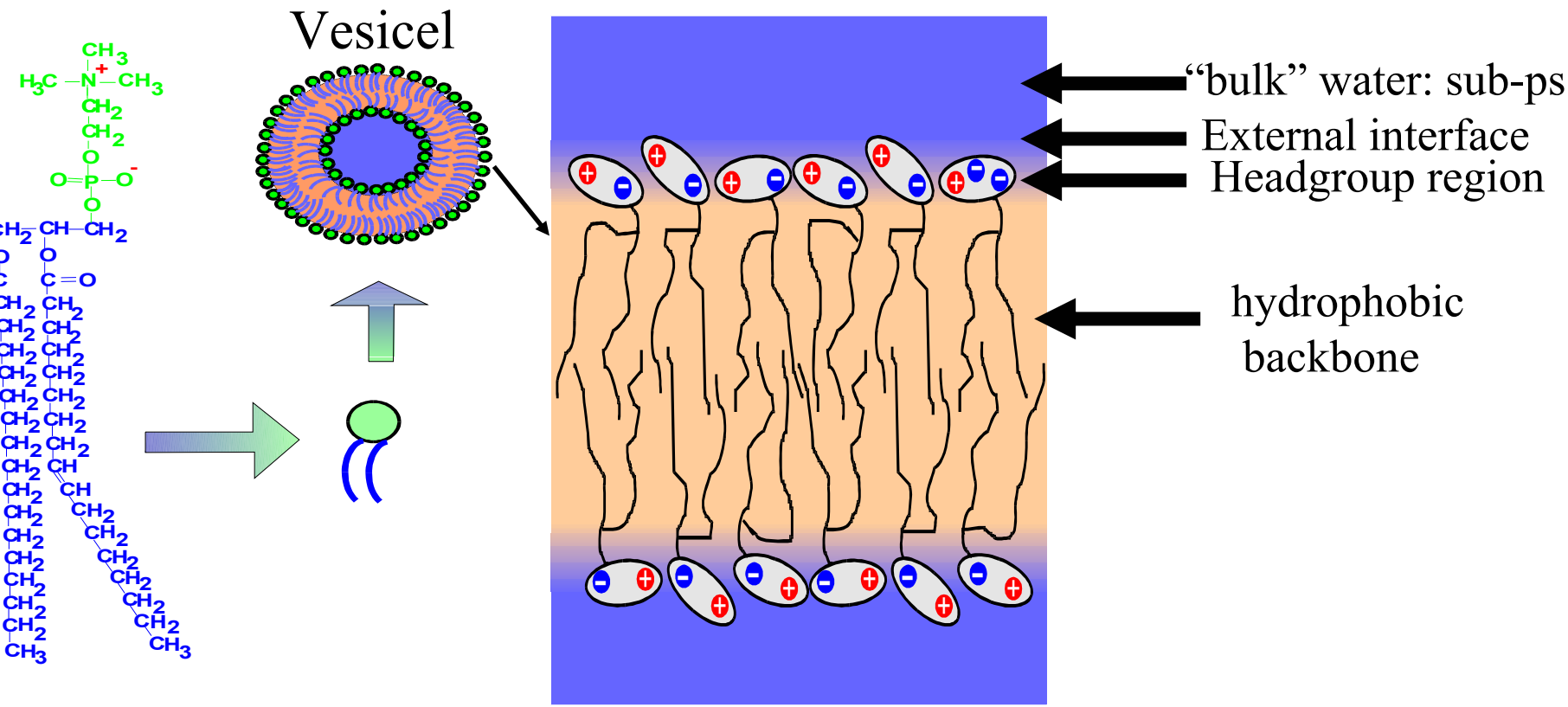
Probed by Charge-Transfer Emission

Probed by $S_1 \rightarrow S_0$ Fluoreszenz

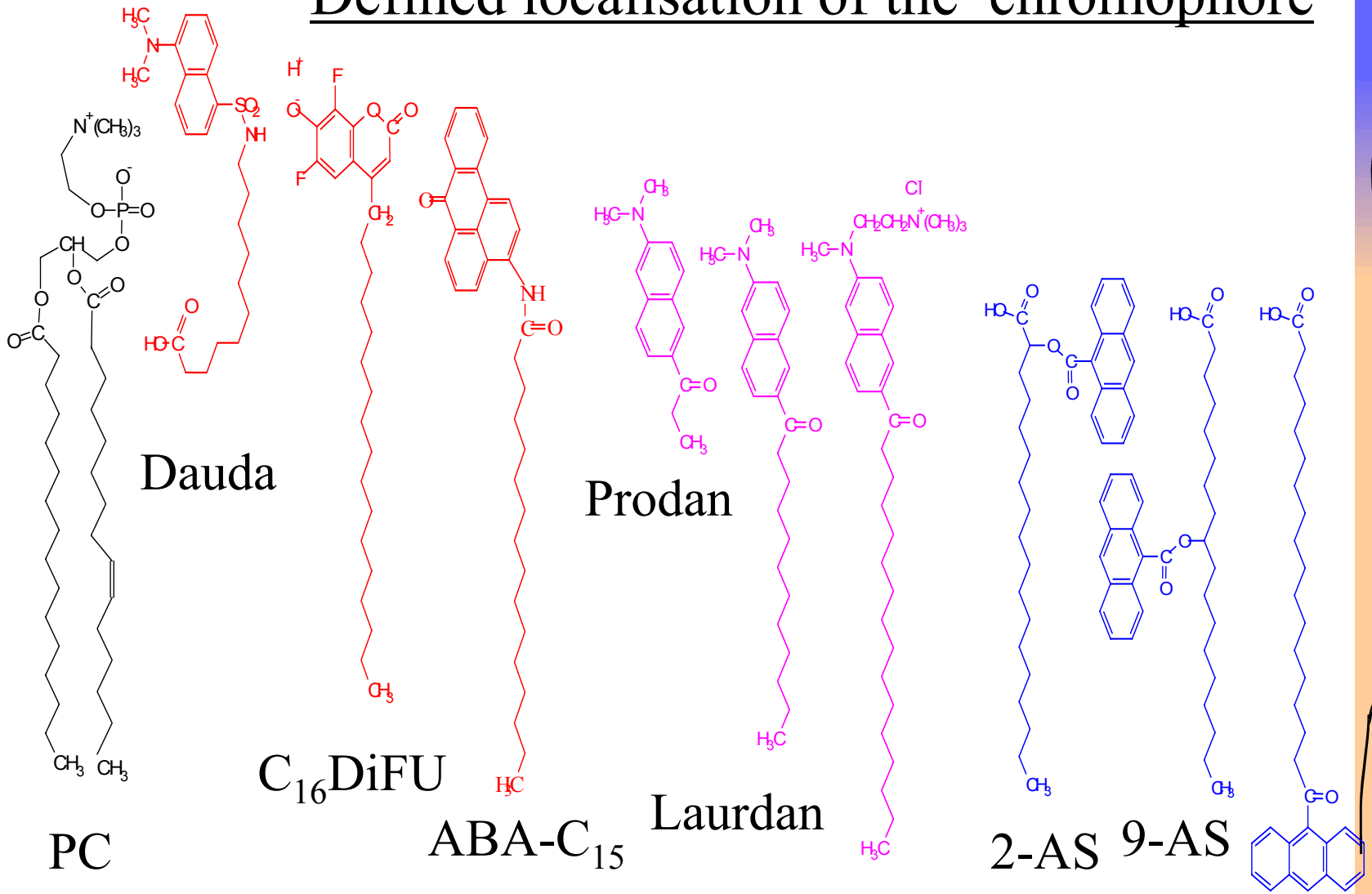
R. Richert et al. Chem. Phys. Lett. 1994

Characterisation of SR by time-resolved fluorescence emission spectra (TRES) gives directly information on viscosity (kinetics) and polarity ($\Delta \nu$) of the probed micro-environment

Solvent relaxation in phospholipid-bilayers

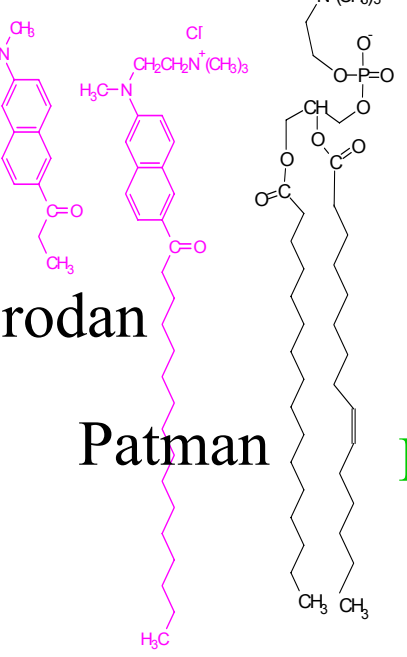


Defined localisation of the chromophore



→ micro-viscosities and -polarities in all domains

Headgroup labels (PC/fluid bilayer)



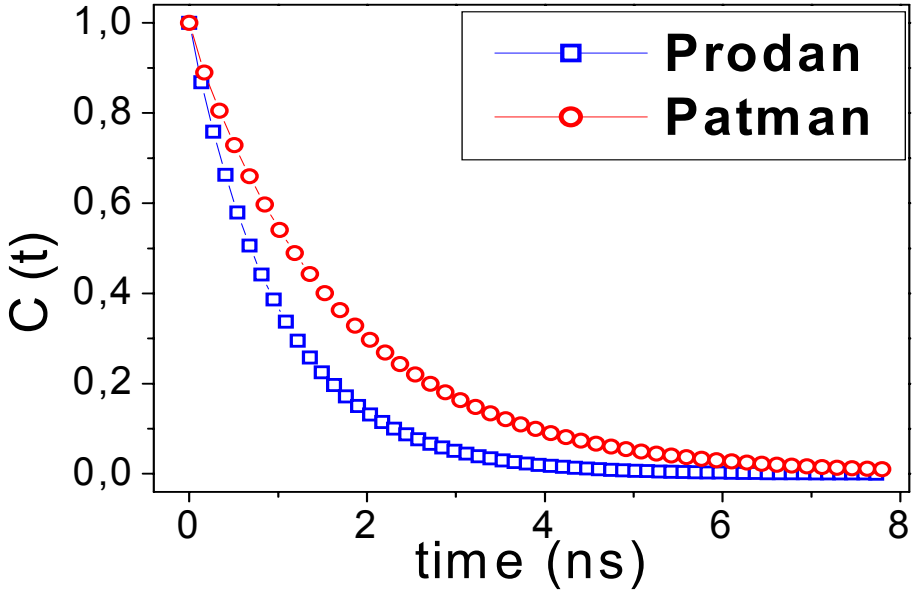
rodan

Patman

A) $\Delta\upsilon$: 3750 cm^{-1} (Prodan); 3000 cm^{-1} (Patman)

$\Rightarrow$Prodan probes larger polarity

B) Kinetics: $C(t)=(\upsilon(t)-\upsilon(\infty))/\Delta\upsilon$

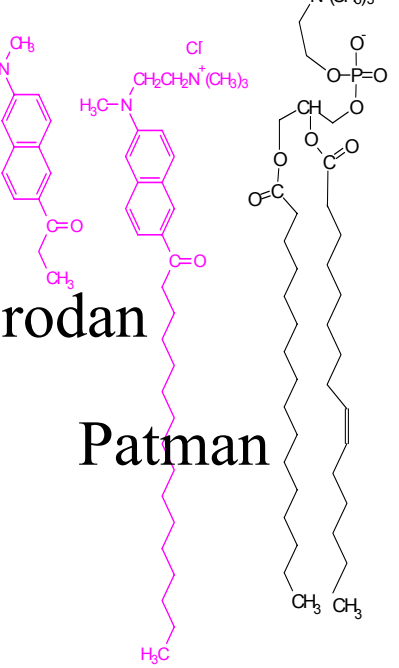


τ_{SR}: 1.0 ns (Prodan/PC)

1.7 ns (Patman/PC)

$\Rightarrow$ Prodan probes lower "micro-viscosity"

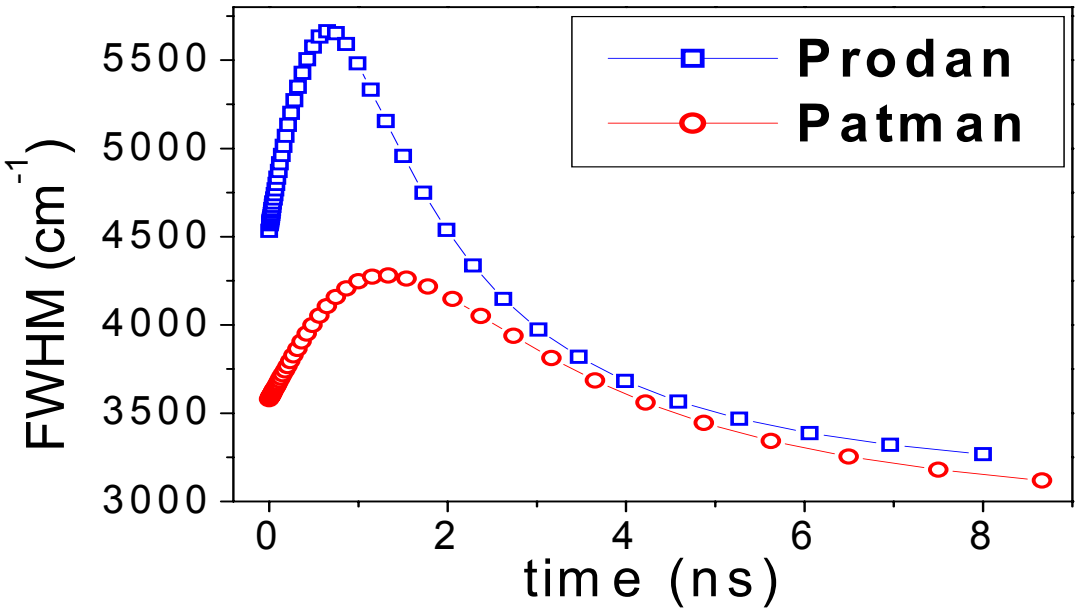
Headgroup labels (PC/fluid bilayer)



rodan

Patman

C) time-evolution of halfwidths (FWHM) of TRES



and "t=0" estimation [Maroncelli 95]

⇓

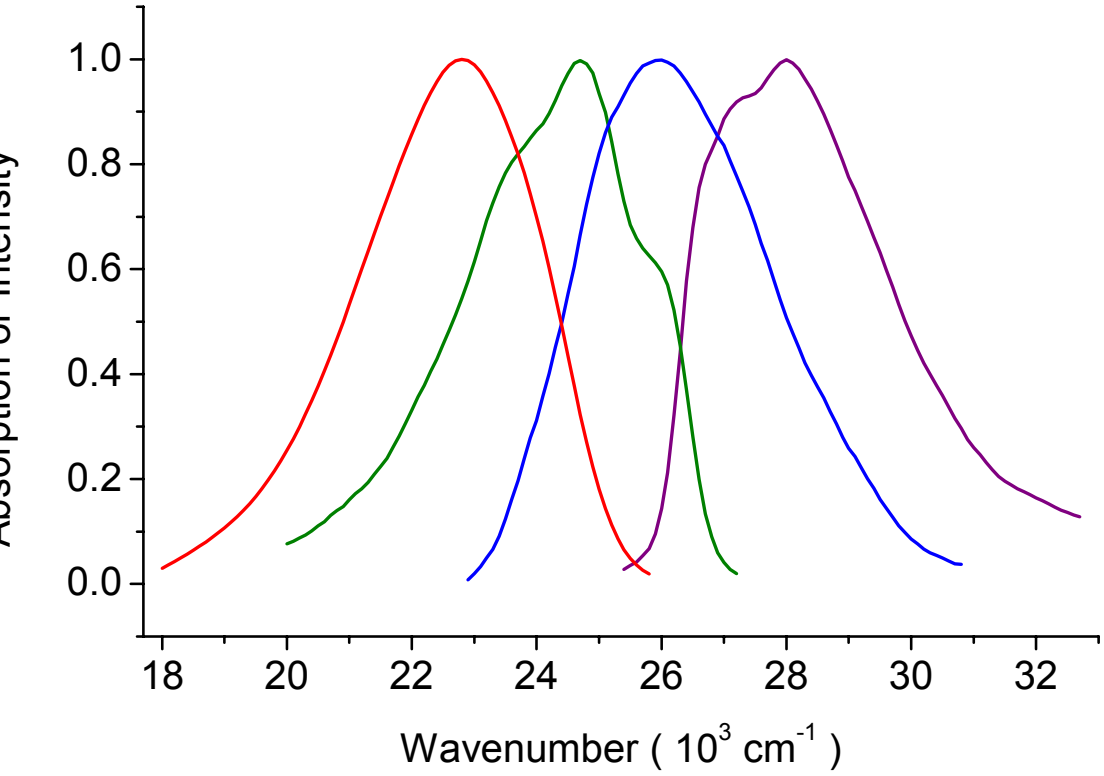
experiment with a 50 ps time-resolution governs 95 % of total SR

⇒ Both dyes are "seeing" no "bulk water". SR is the reorientation of the hydrated functional groups of the lipid

Time-zero estimation

Measurements:

1. Emission and absorption spectra of the dye in non-polar solvent (hexan,...)
2. Absorption spectrum of the dye in the polar system of interest (liposomes,...)



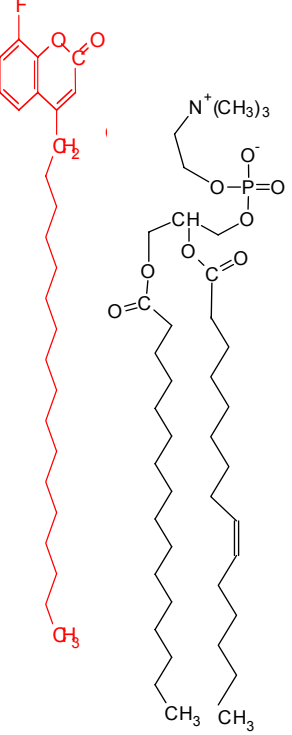
Spectra of **DTMAC** 4-[(n-dodecylthio)methyl]-7-(N,N-dimethylamino)coumarin

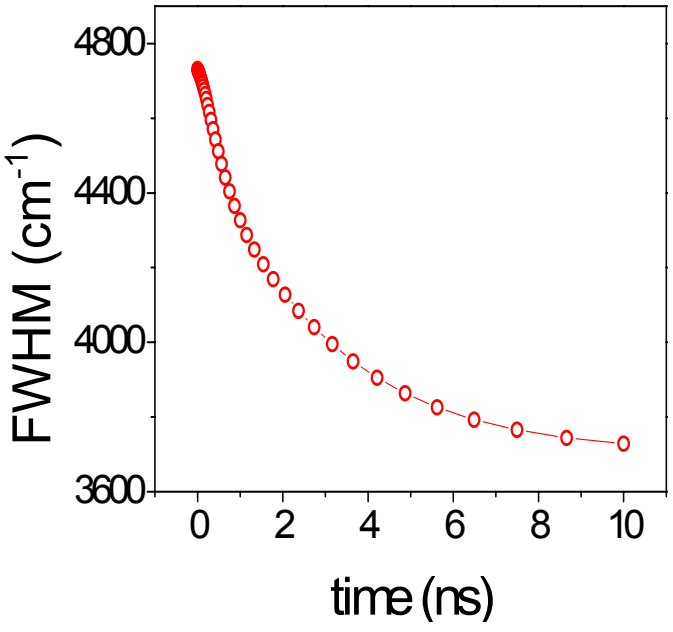
Data treatment:

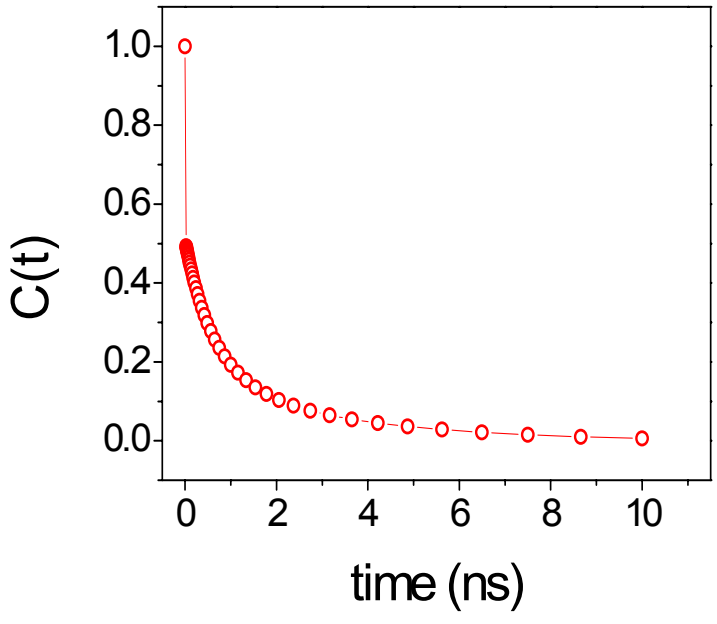
3. Calculation of the so called lineshape functions f(v), g(v) from the non-polar reference spectra
4. Finding shift distribution p(δ) by fitting convolution of p(δ) and g(v) with polar absorption spectrum Ap(v)
5. Calculation of time-zero spectrum using f(v), g(v), p(δ)

See e.g. J. Sykora et al. Chem. Phys. Lipids 2005, 135,213

External interface: C_{16}DiFU (PC/fluid bilayer)





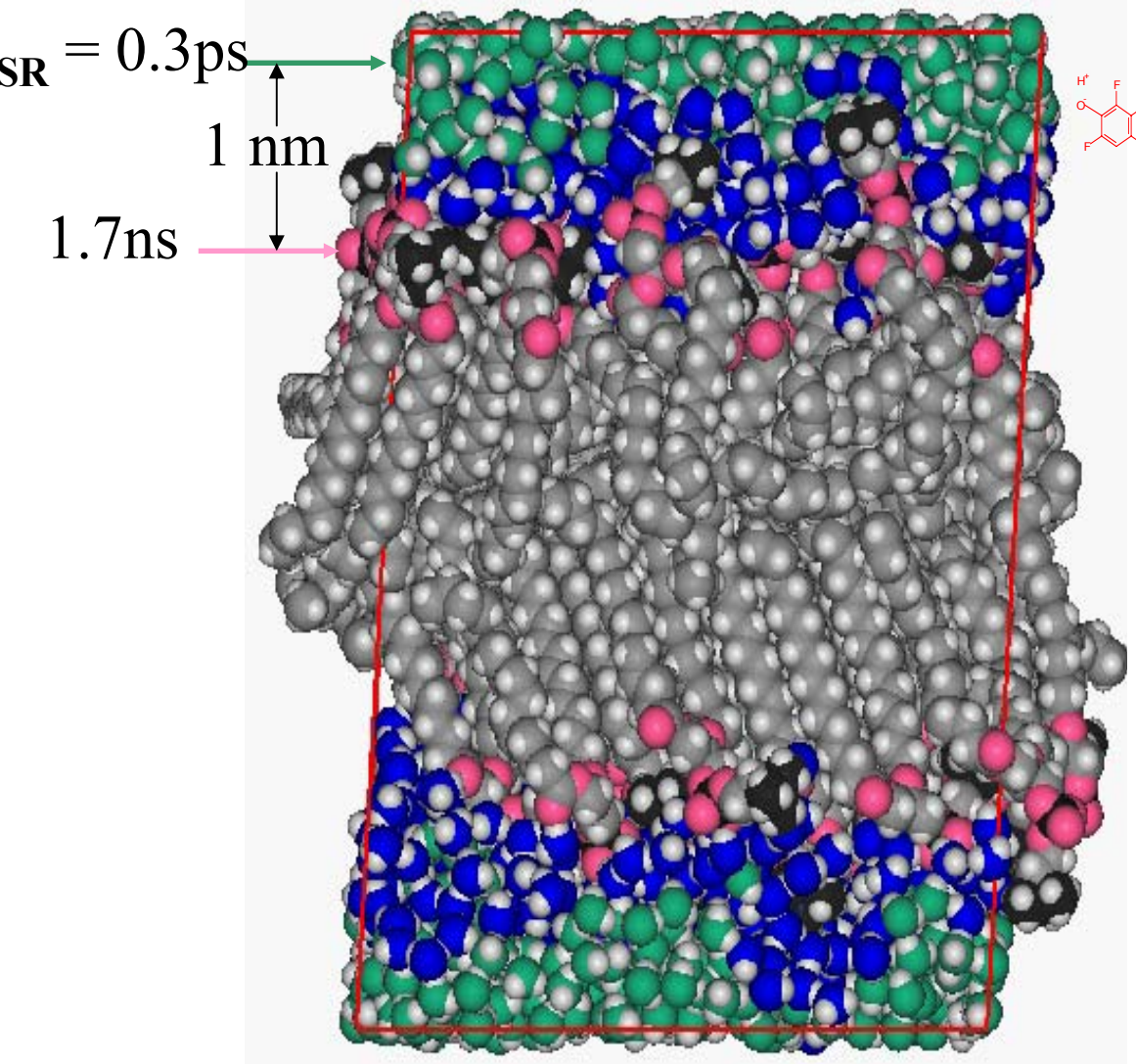


50 % of SR probed within the external interface < 50 ps

But also 50 % of the SR characterised by 2 nanoseconds components (0.5 ns and 2.6 ns)

⇒ microenvironment of the dye is relaxing in within a time scale from $10^{-13(-14)}$ s to 10^{-8} s !!

External interface: C_{16}DiFU



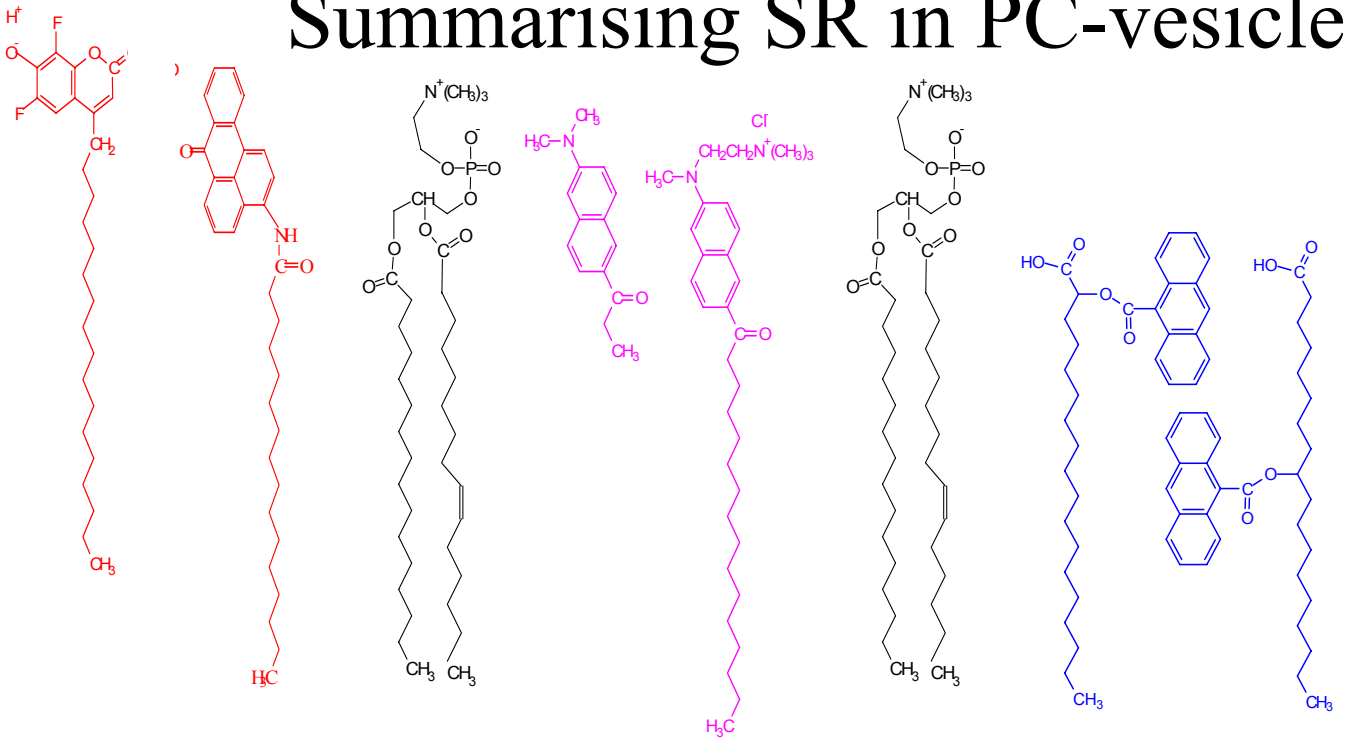
napshot of a DPPC-
layer (D. Tobias)

Time-scale of SR:
$10^{-13(-14)}$ s to 10^{-8} s

J. Nagle, BBA 2000:

"Most of the difficulty in obtaining quantitative structur for the biologically relevant fluid phase is due to the intrinsic presence of fluctuations. "

Summarising SR in PC-vesicles



ykora, Kapusta,
dler, Hof 2002
angmuir **18** 571

ν	1700	3100	3750	3000	2750	2100	cm^{-1}
SR(average)	n.d	0.5	1.0	1.7	2.1	3.4	ns
% SR (50 ps)	50	85	95	95	95	75	

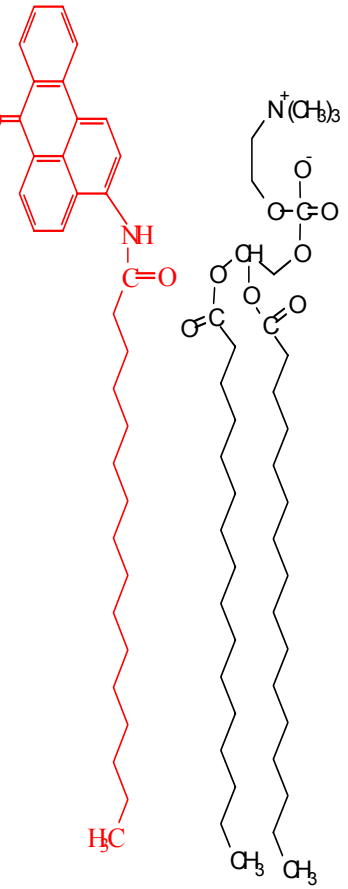
Deeper localisation means probing lower polarity and higher "vicosity"

Significant part within the external interface < 50 ps; partially "bulk" water

Headgroup labels: "pure" ns SR: bound water to charged and polar groups

Backbone: SR slows down with depth of location: water diffusion

Application 1: Phase transition and SR: ABA-C_{15}



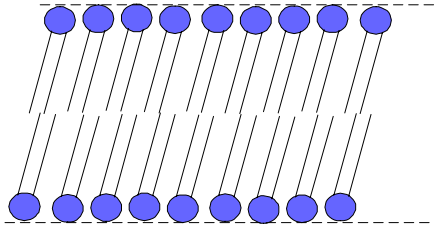
- $\tau_{SR(average)}$ in PC-SUV : 0.5 ns

$\Rightarrow$ lower "viscosity" than probed by Prodan (1.0 ns)

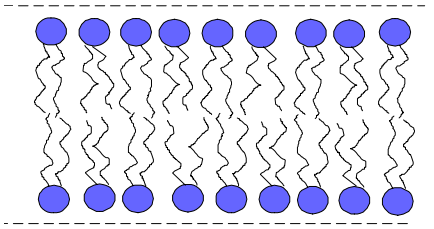
$\Rightarrow$ Compared to Prodan location towards the external interface

Sykora Mudogo Hutterer Nepraš Kapusta Fidler Hof, 2002 *Langmuir* **18** 9276

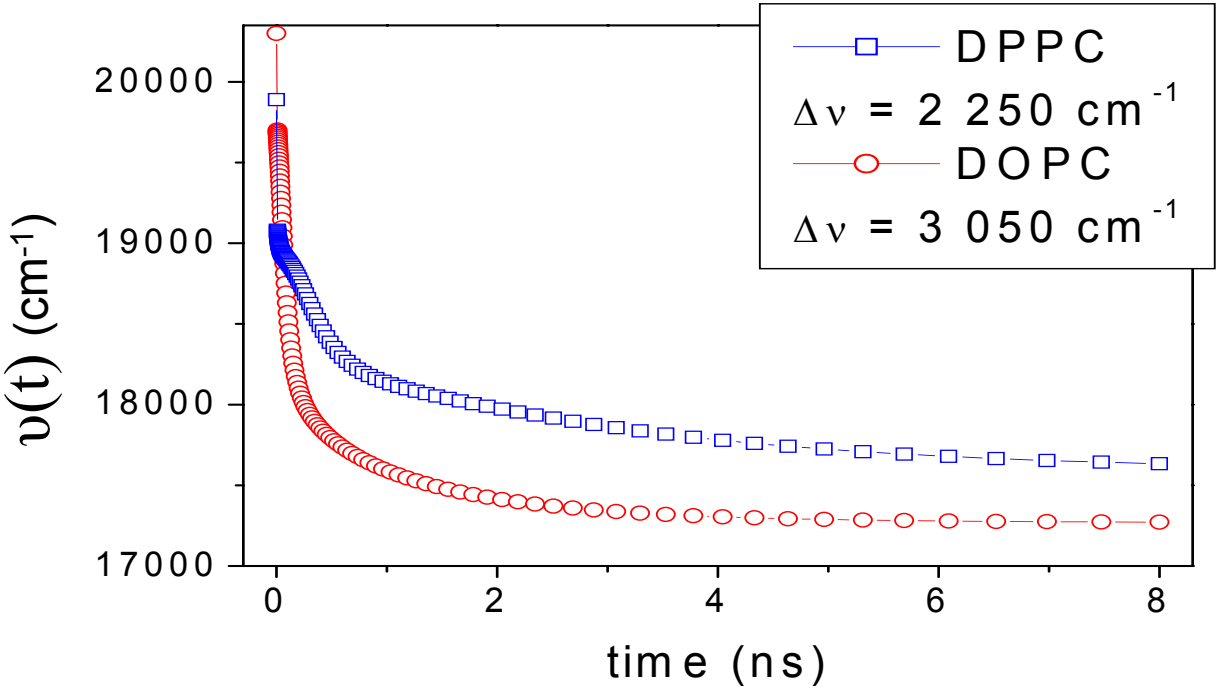
ABA-C_{15}: SR in rigid ("gel") versus fluid bilayers



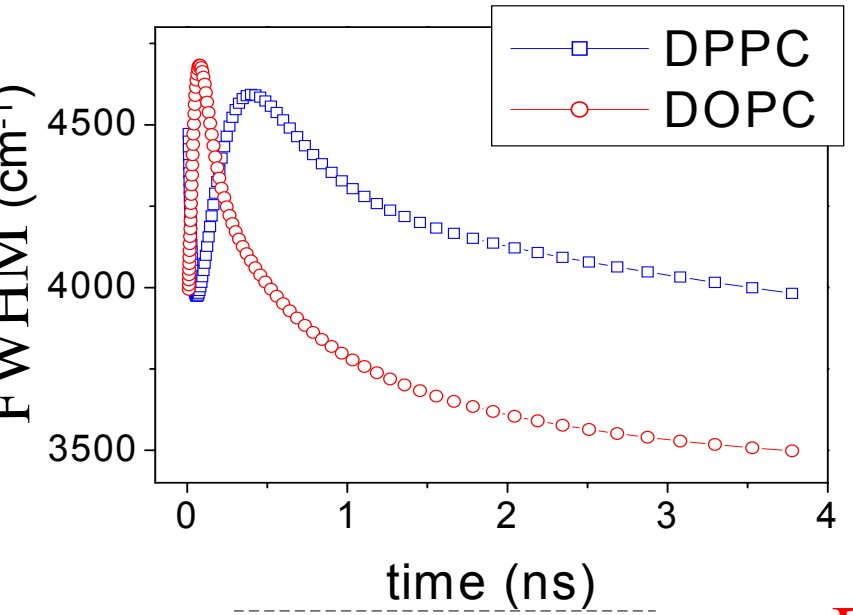
DPPC at
Room T

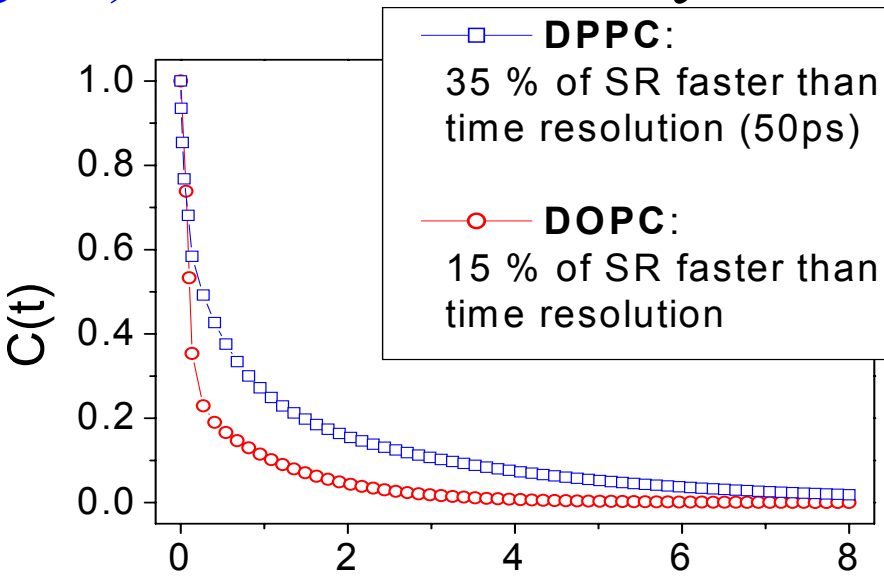


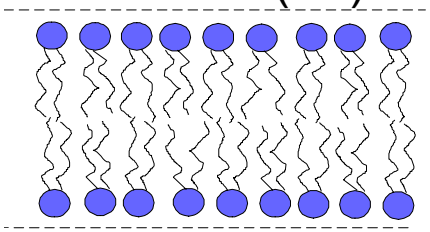
DOPC at
Room T



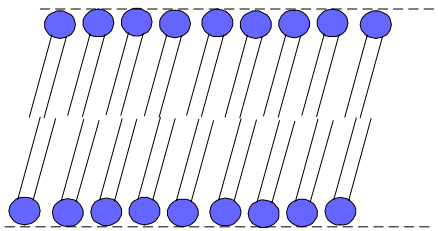
ABA-C_{15}: SR in rigid ("gel") versus fluid bilayers







DOPC: large Δυ, one localisation; $\tau_{SR(average)}$: 0.4 ns; ultrafast contribution < 15 %



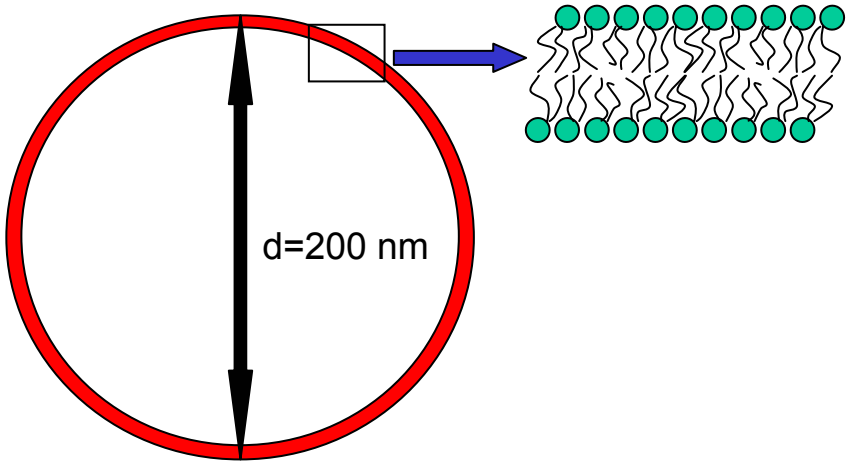
DPPC: small Δυ, two localisation; $\tau_{SR(average)}$: 1.0 ns; ultrafast contribution > 35 %

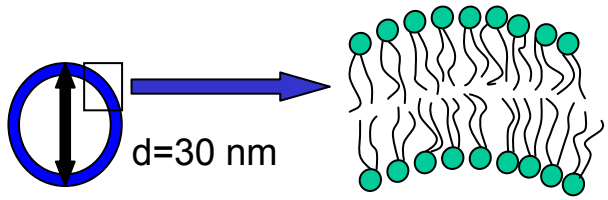
⇒ gel-phase: part probes bulk water; part more rigid and less polar headgroup

Application II: membrane curvature and headgroup hydration

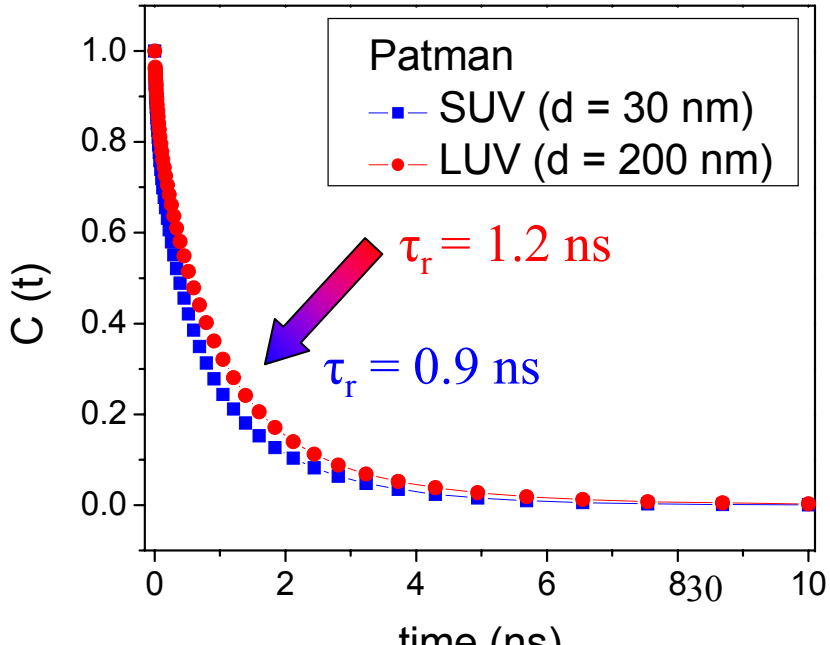
Large unilamellar vesicles (LUV) = low curvature

Small unilamellar vesicles (SUV) = high curvature





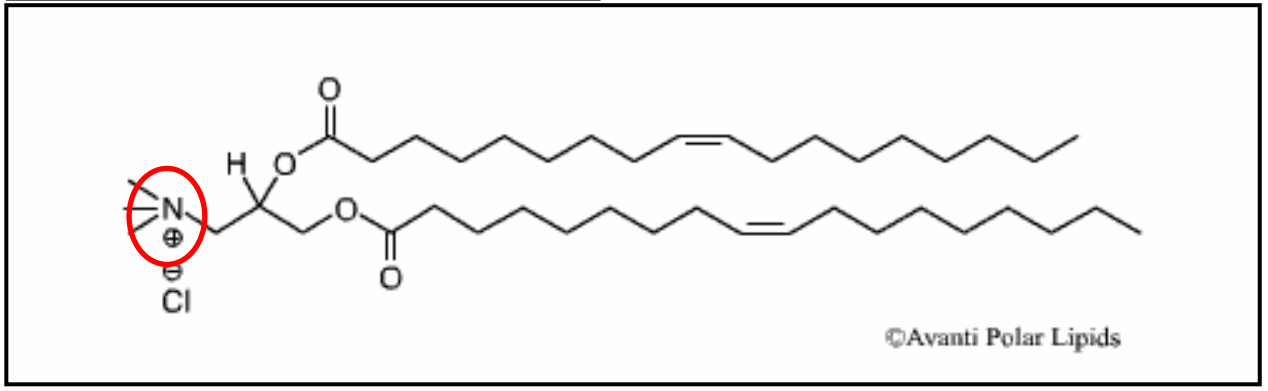
- degree of hydration remains *constant*
- the SR becomes *faster* with *increasing* curvature
- this effect is less and *less apparent* approaching the interface



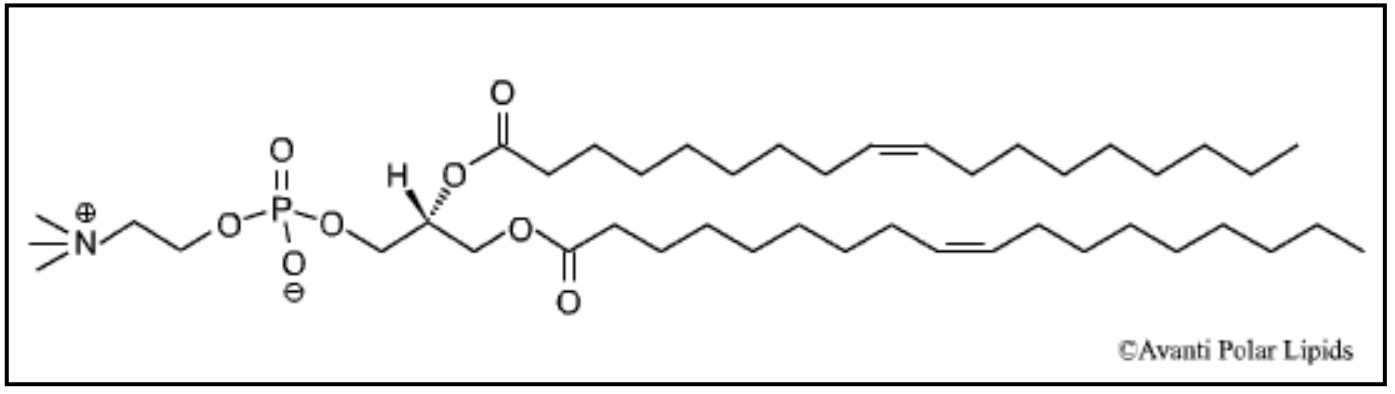
Application III: Effect of DOTAP on headgroup hydration and mobility

cationic lipid - **DOTAP**

cationic lipid used in transfection protols

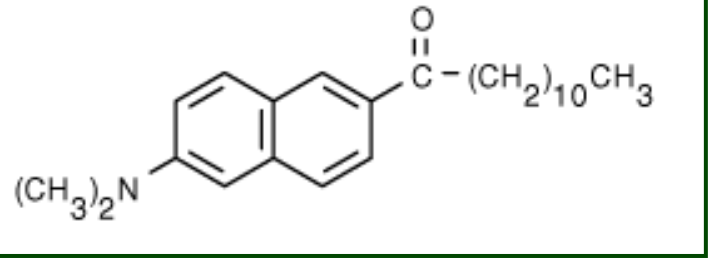


neutral lipid - **DOPC**

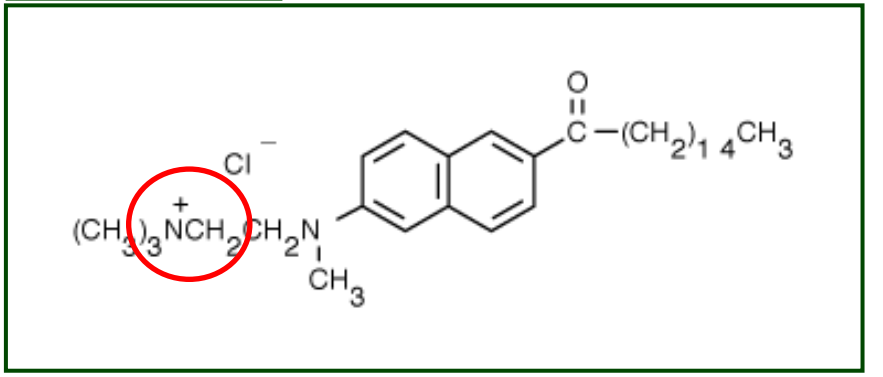


Fluorescent probes ...

Laurdan

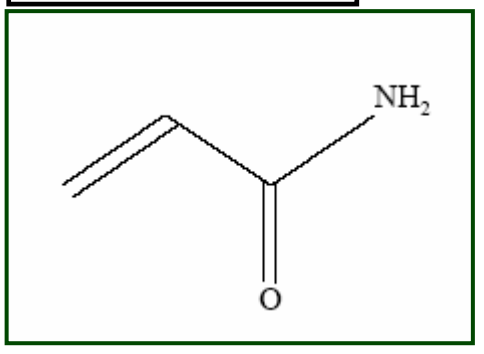


Patman

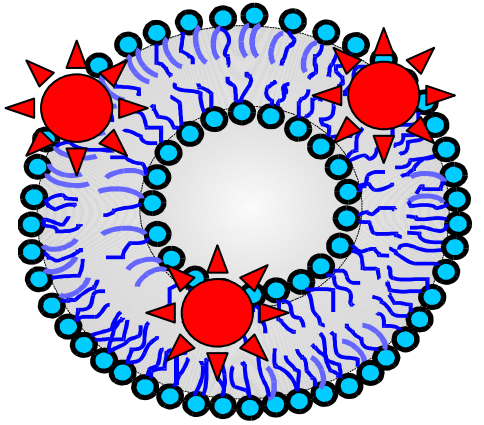


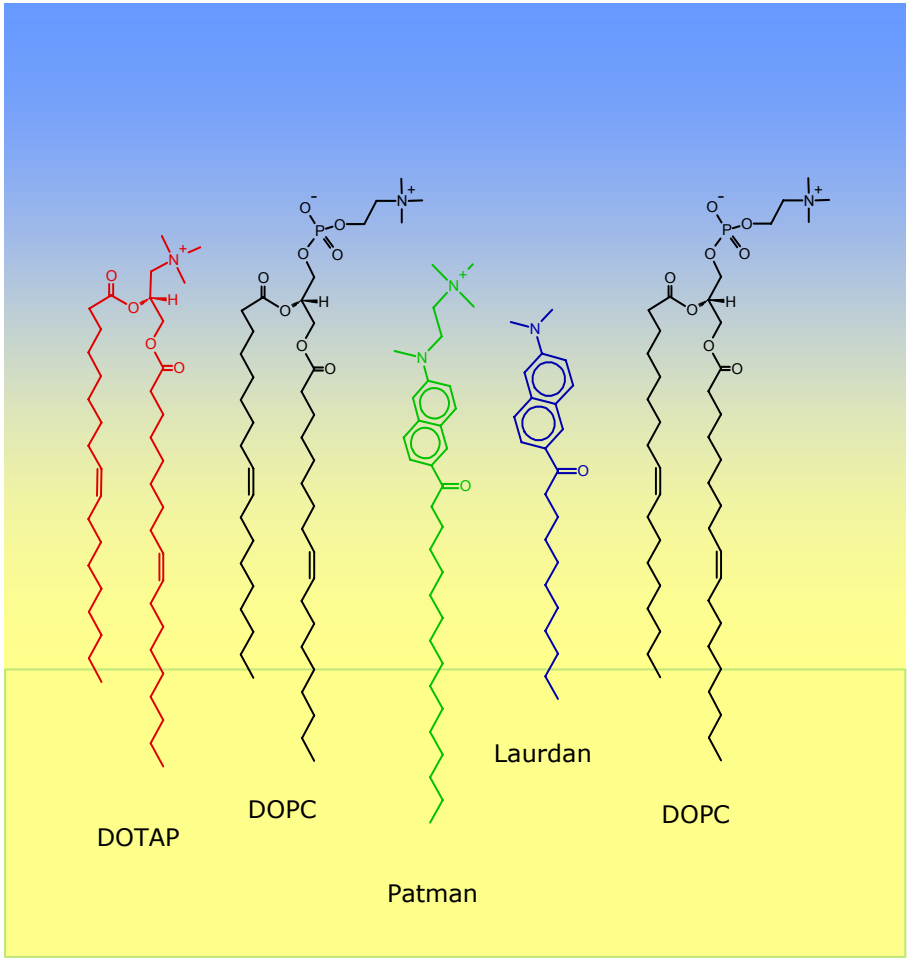
Quencher ...

Acrylamide



Where are the dyes localized?



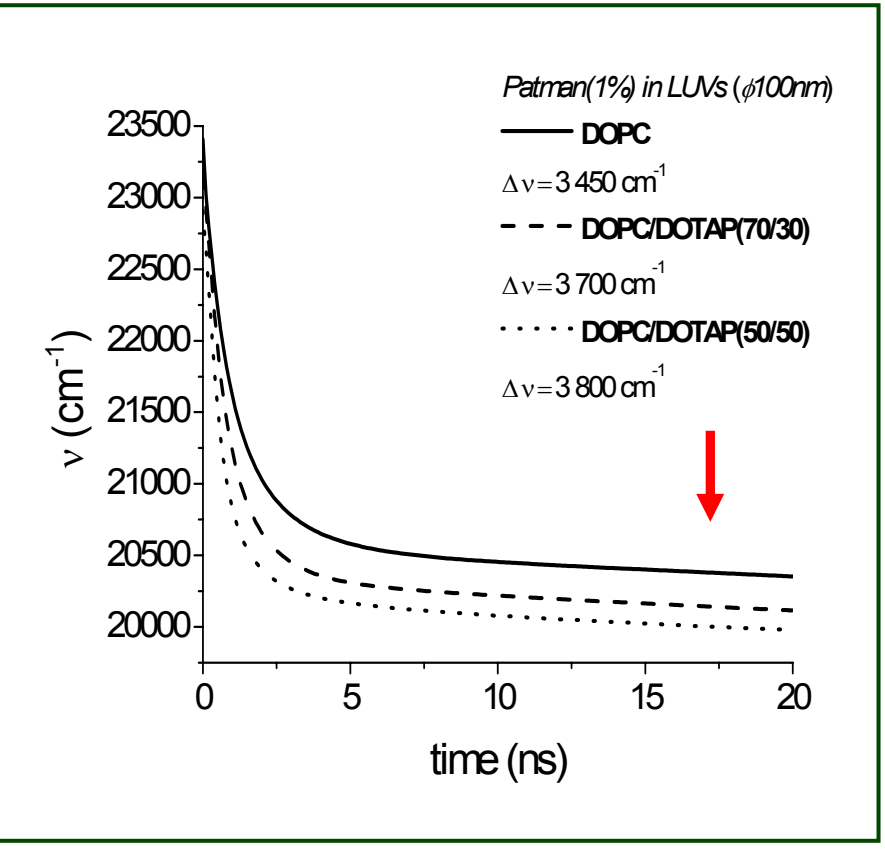


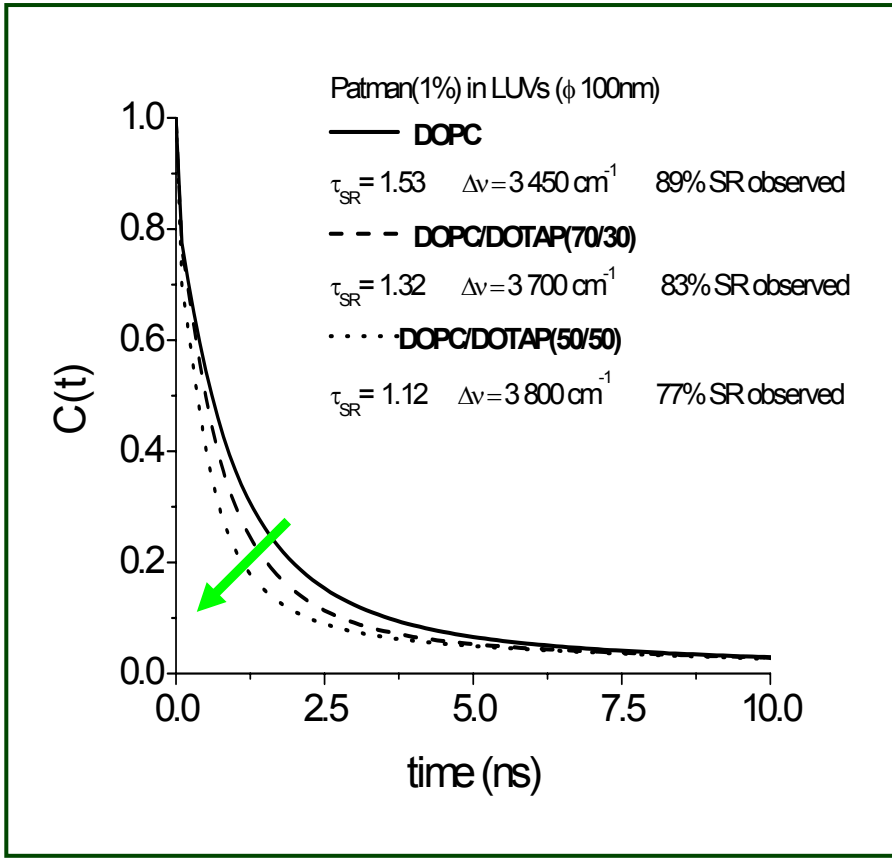
Distance from the center of DOPC bilayer for:

- Patman – **10.45 A**
- Laurdan – **11.35 A**

What information does Patman provide us?

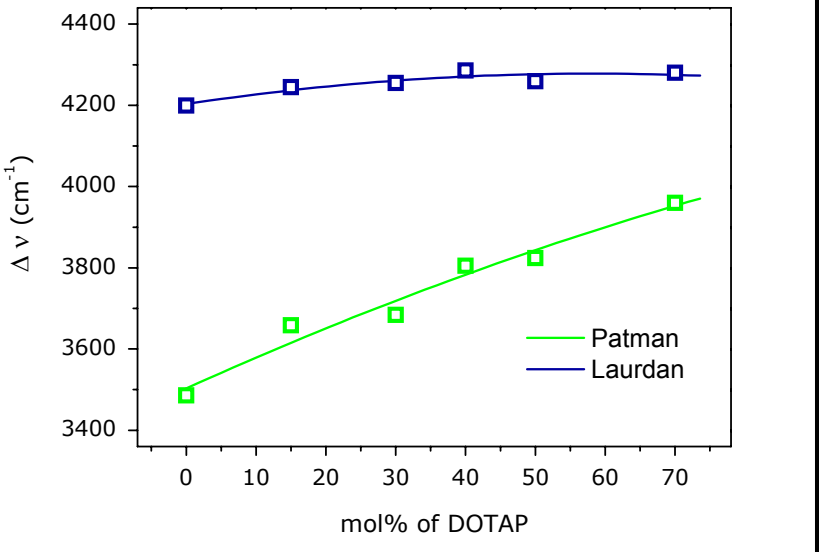
mol% of DOTAP ↑ ⇒ τ ↓

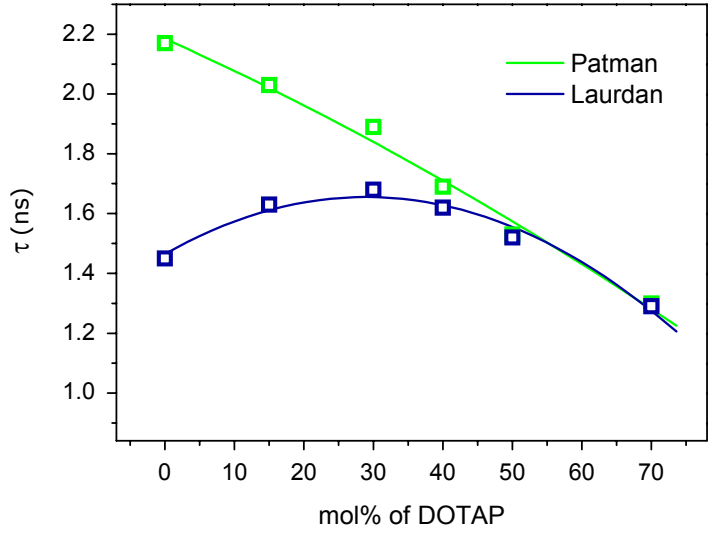


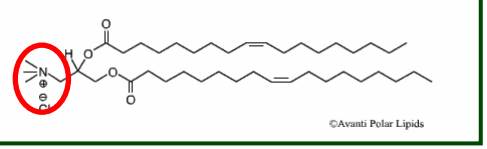


mol% of DOTAP ↑ ⇒ Δν ↑

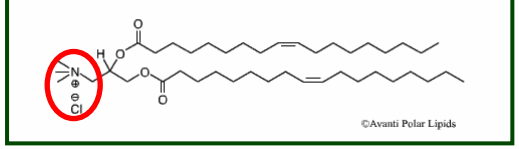
Solvent relaxation measurements at 10°C

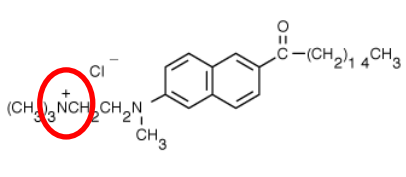






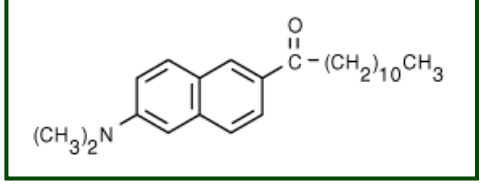
Acrylamide quenching



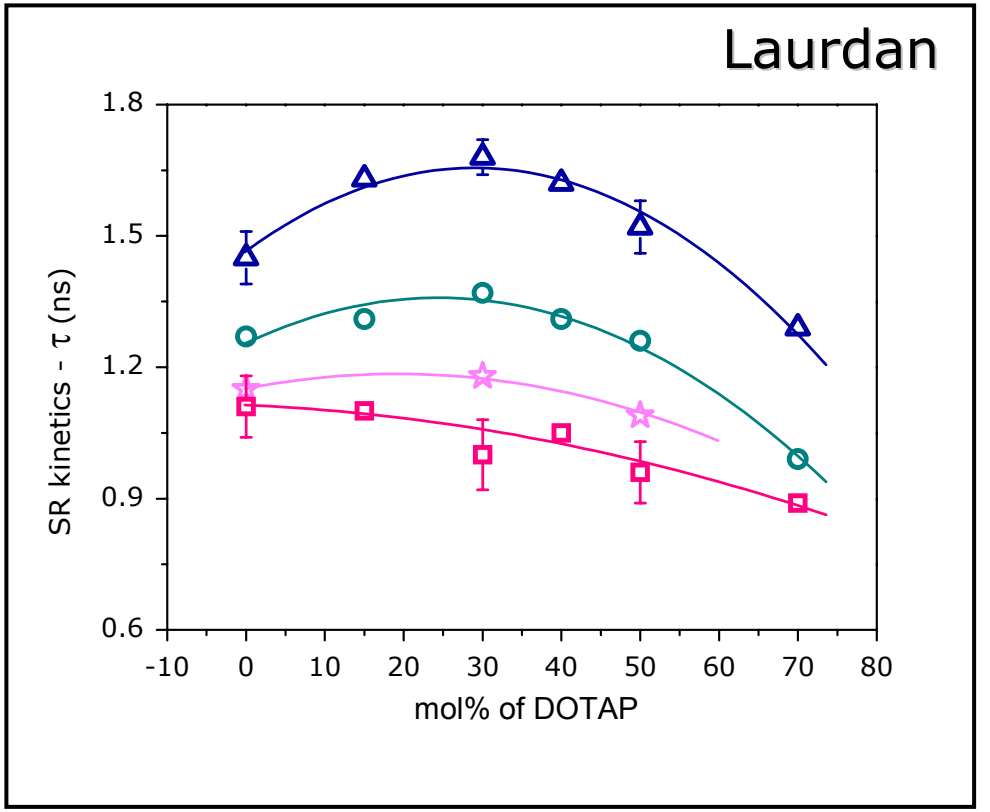


DOTAP	0%	30%	50%
Patman	0.275	0.315	0.320
Laurdan	0.340	0.347	0.350

Stern-Volmer constants in M^{-1}

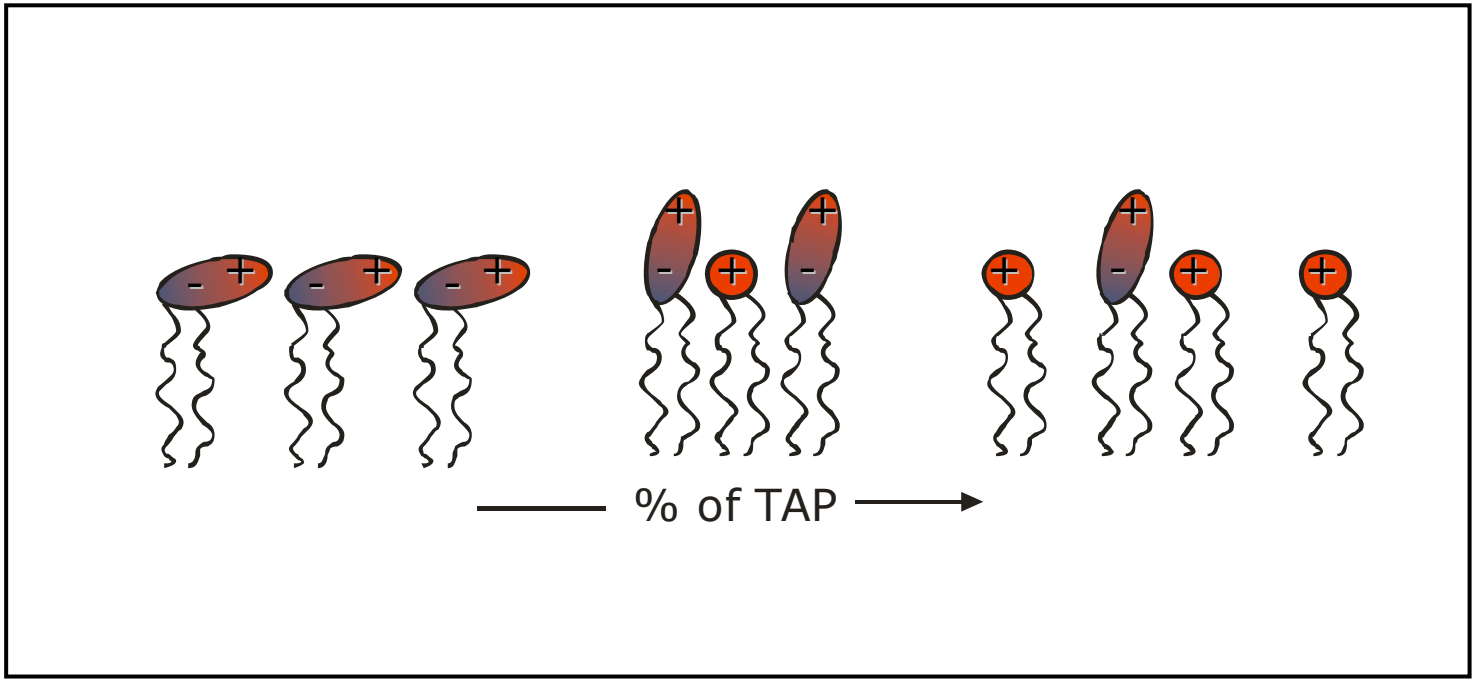


Solvent relaxation kinetics for different temperatures



10.0°C
15.0°C
17.5°C
20.0°C

Model proposed by Gurtovenko[*] ...



[*] Gurtovenko A.A., Patra M., Karttunen M., Vattulainen I. (2004) Biophys. J. 86, 3461-3472

Conclusions on DOTAP experiments

- ✓ Positively charged dye is not good any more for measuring reorganization of solvent molecules in the membranes containing positively charged lipids
- ✓ At 30mol% of DOTAP, there exists the maximum of bilayer packing!!!
- ✓ There is the significant decrease of microviscosity from 30 to 70 mol% of DOTAP in the lipid bilayer

Published in Langmuir 2006: Jurkiewicz, Olzynska, Langner, Hof

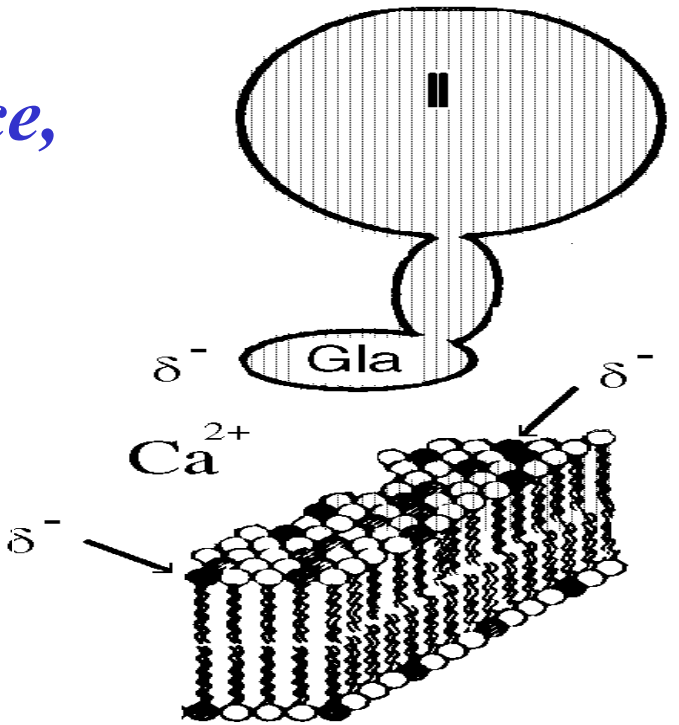
Protein binding A: prothrombin to negatively charged membranes

Key step in blood coagulation:

Prothrombin $\xrightarrow[Ca^{2+}, \text{lipids}]{\text{Va, Xa,}}$ Thrombin

ps-Trp-fluorescence,
TIRFM,
FCS

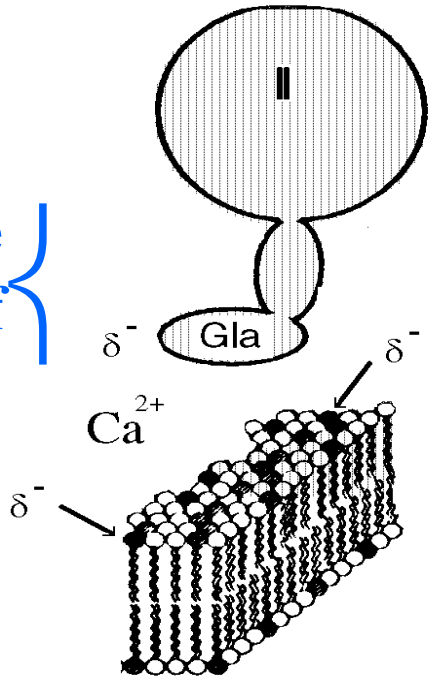
DPH-anisotropy ?
Pyrene-excimer ?

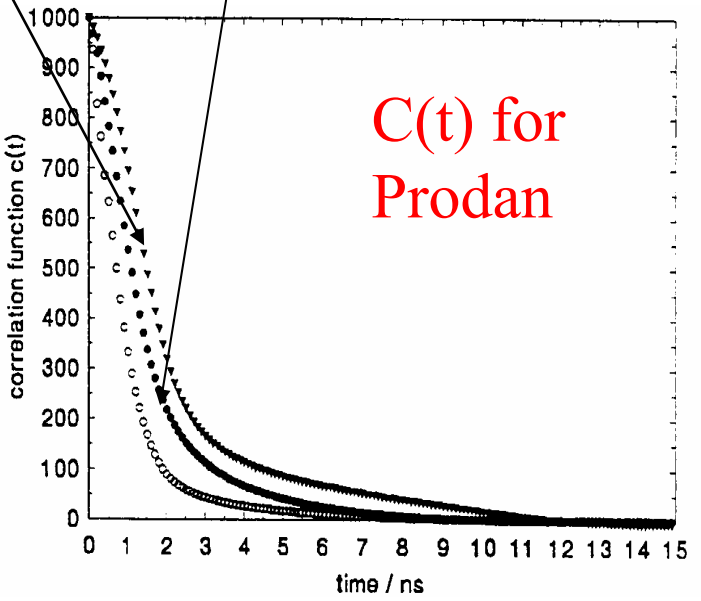


Both methods fail in detecting Prothrombin Fragment 1 binding

Application: Prothrombin and its fragment 1 binding to vesicles : Patman versus Prodan

ragment 1 onsists of the N-terminus of rothrombin





SR detects the protein binding (DPH, Pyrene not)

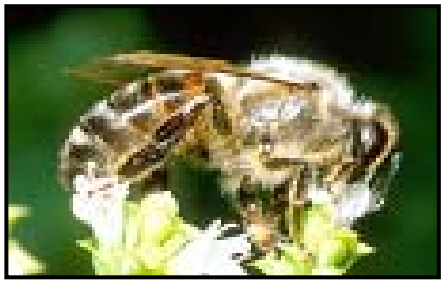
Prodan differentiates between both proteins⇒ non-fragment 1 part contributes

effect is larger for Prodan than for Patman ⇒ peripheral binding

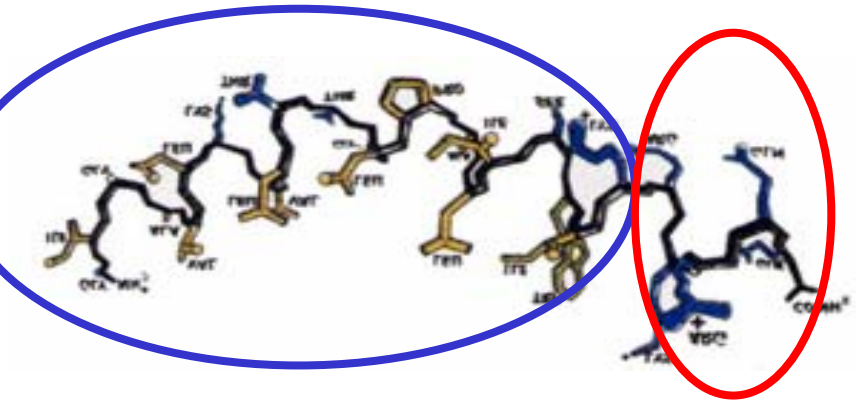
Dependence of lipid-composition on protein binding: Hutterer et al. BBA 1998,1414, 155

Protein binding B: Toxins vs. antibacterial peptides

Toxin
Melittin



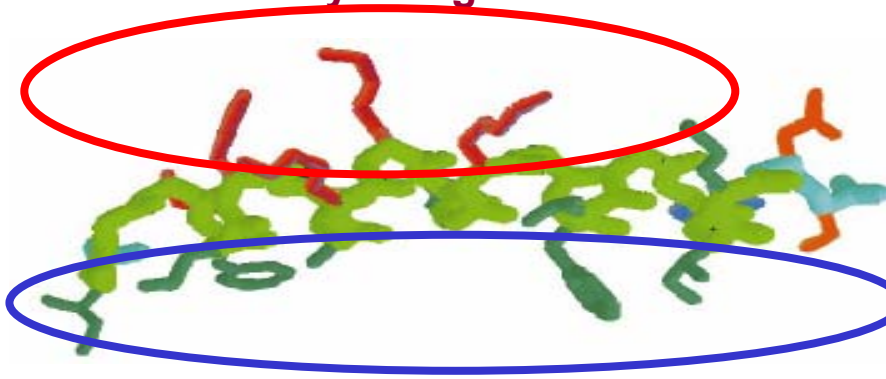
Antibacterial peptide
Magainin 2



Hydrophobic residues

Positively charged residues

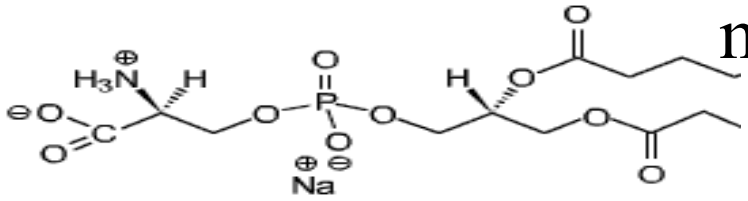
Positively charge residues

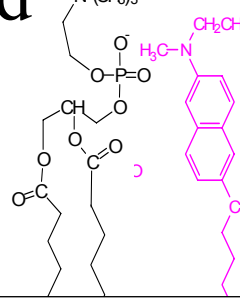


Hydrophobic residues

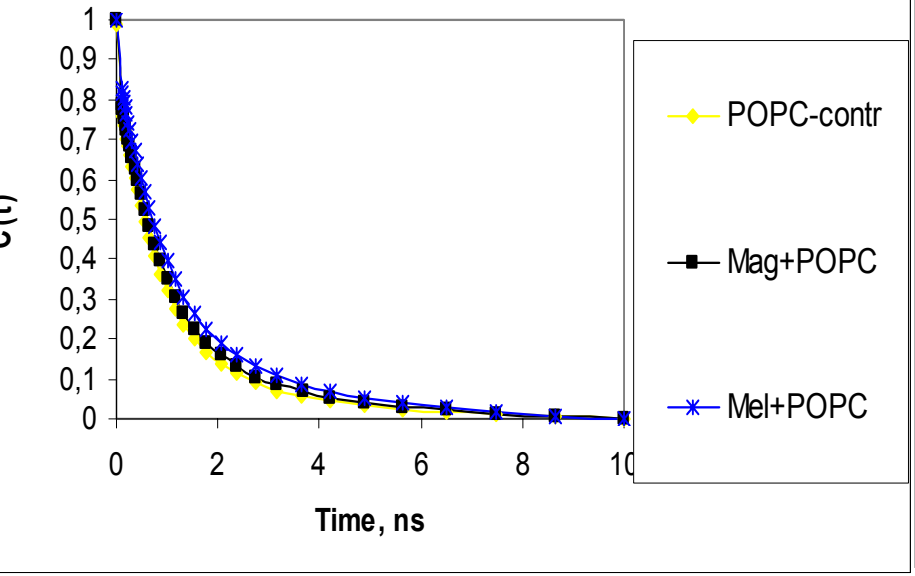
T. Shevnis et al. Eur J Biochem 2003 270 4478

Effect of PS content in the binding of magainin and melittin

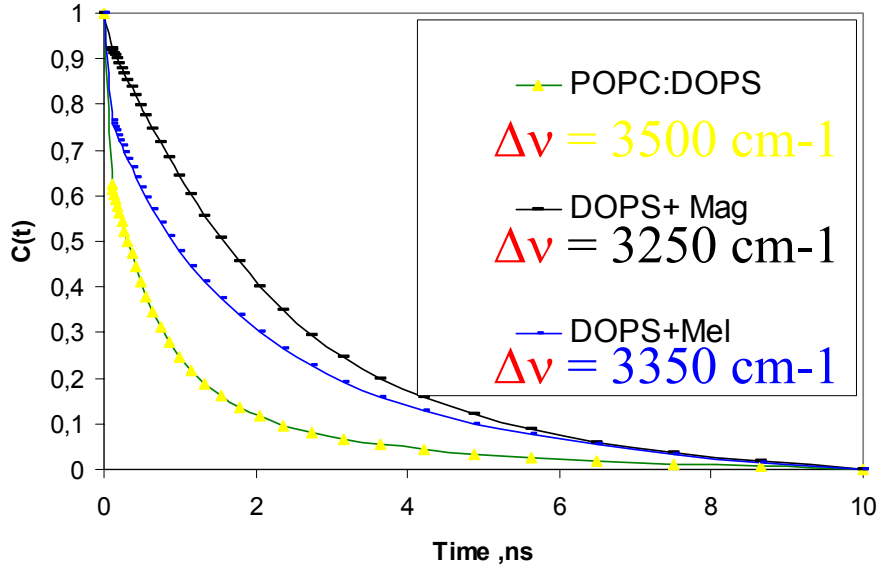




Correlat. Funct.- POPC + Patman



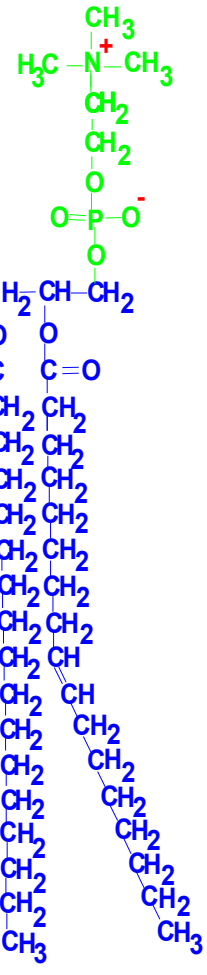
Patman+DOPS:POPC - Correl. funct



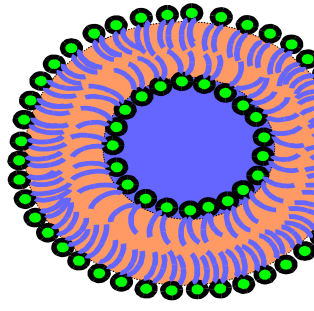
lo effect on Δν, only melittin has a small ffect on SR kinetics of Patman
⇒Melittin peripheral binding; Magainin nly unspecific binding

Decreased hydration and strong effect on mobililty of glycerol region. ⇒ proteins have direct interaction with glycerol-region; effect stronger for magainin than for melittin.

SR probed by membrane labels is influenced by:



- Lipid-composition:
  - variation of headgroups as well of alkyl chains
  - dietherlipids
  - cholesterol
  - DOTAP, PEGylated lipids
  - addition of detergents→Transferosomes
    (Rieber et al. BBA in press)



- temperature
- phase transition⇒relocalisation of chromophore
- membrane curvature
- addition of ethanol
- Addition of Ca^{2+} to negatively charged membranes

- Protein binding
  - prothrombin and its fragment 1
  - antimicrobial peptides

Advantages of SR for probing membranes

- direct information on *micro-viscosity and -polarity*
- in *defined positions within the bilayer*
- information on the *headgroup region (hydration and mobility)*

Literature on SR in biomembranes:

Recent Review: Jurkiewicz et al. 2005 J Fluor 15(6) 883-894

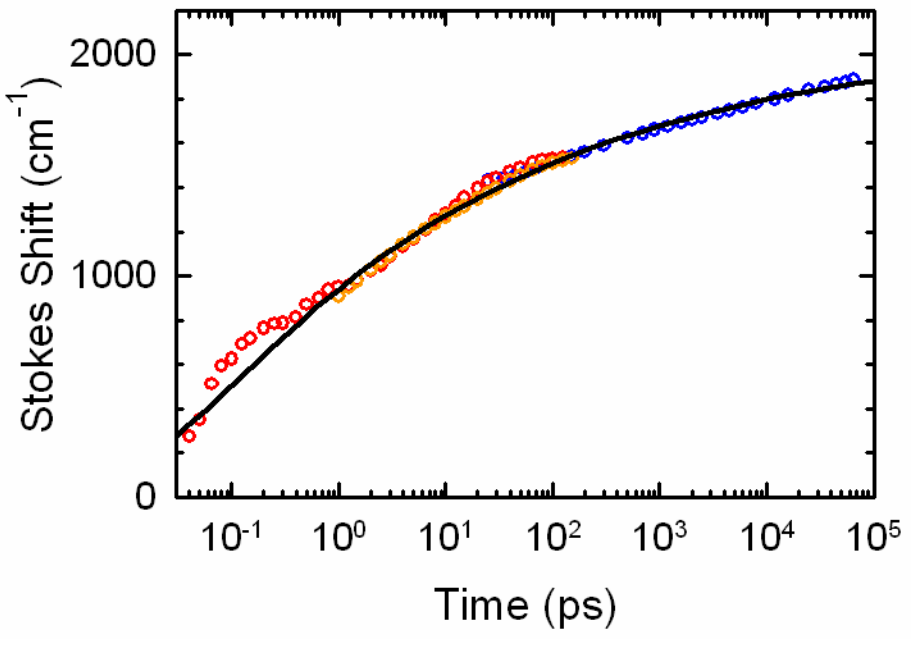
Recent recommended publication: Jurkiewicz et al. 2006 Langmuir, *in press*

or

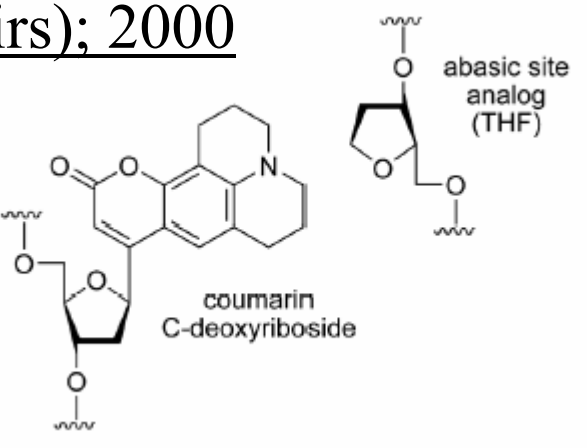
http://www.jh-inst.cas.cz/~fluorescence/solvent%20relaxation.htm

Overview on contributions on solvent relaxation in "biomolecules" using dyes with defined location within the anisotropic system

A. Biomembranes: Hof: first "good" publication 1996
B. DNA: M. Berg (USA) first publication 1998



Hybridisized Oligonucleotide (2×17 base pairs); 2000



JACS 2005: Power-law solvation dynamics in DNA over six decades in time

Overview on contributions on solvent relaxation in "biomolecules"

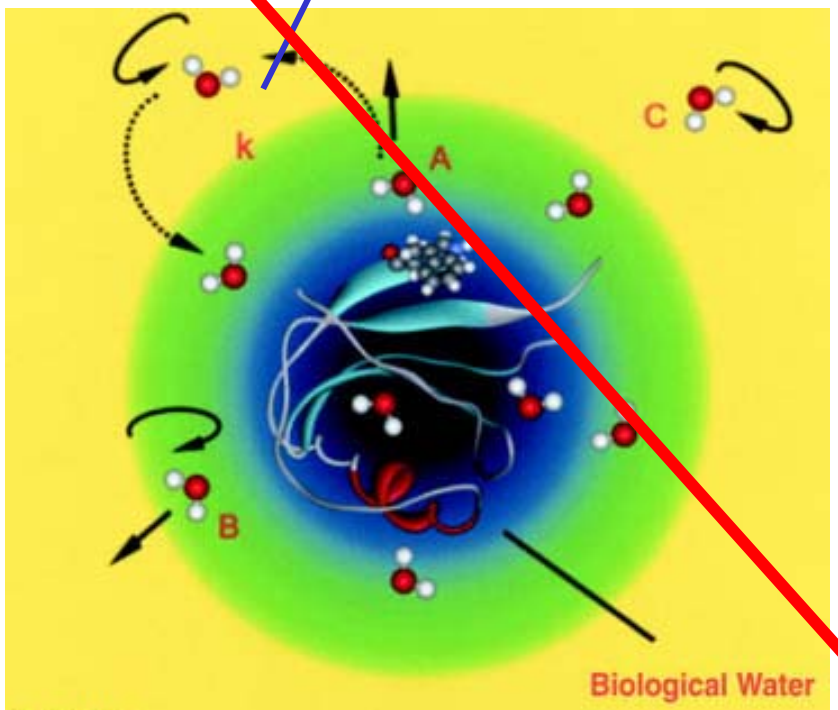
sing dyes with defined location within the anisotropic system

A. Biomembranes: Hof 1996-now

B. DNA: M. Berg (USA) 1998- now

C. A. Zewail (USA) 2001-now

s studies on exposed Trp (υ(∞) at 200ps)
xchange of individual water molecules
s responsible for a τ_{SR} of 50 ps!



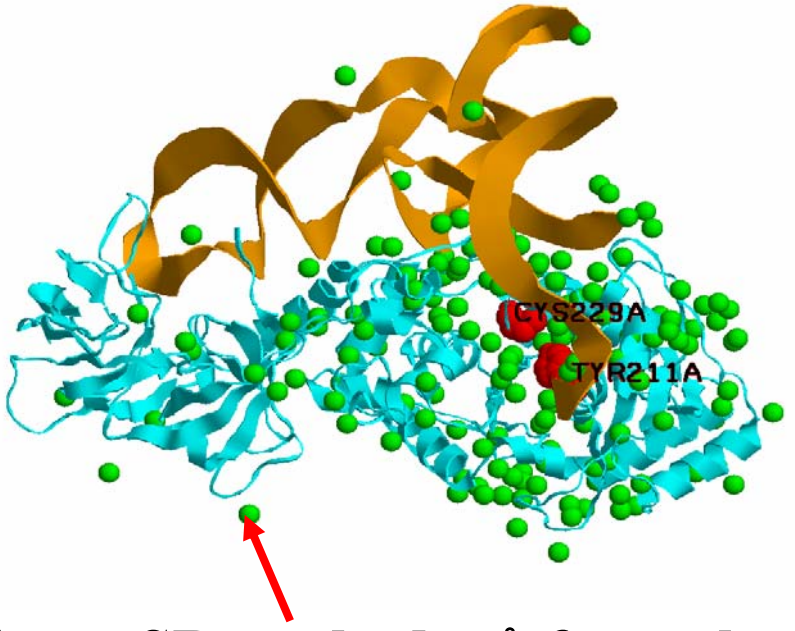
C. B. Halle (Sweden)

PNAS 2005: **Molecular origin of time-dependent fluorescence shifts in proteins**:

"Because of its collective nature (Maroncelli!), the TD Stokes-shift is insensitive to the motion of individual water molecules. Even for solvent exposed dye (5 ps) the TDSS shows the collective conformational protein dynamics"

Solvent relaxation in proteins

Protein: glutaminyl t-RNA Synthetase



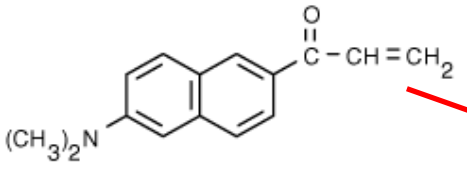
- **Binds to Glutamine (Gln) & t-RNA**
- **t-RNA carries Gln for protein synthesis**

A. SR probed 3 Å from the surface of the protein at the active site of an enzyme: 85% faster than 40 ps and 0.6 ns (15%)

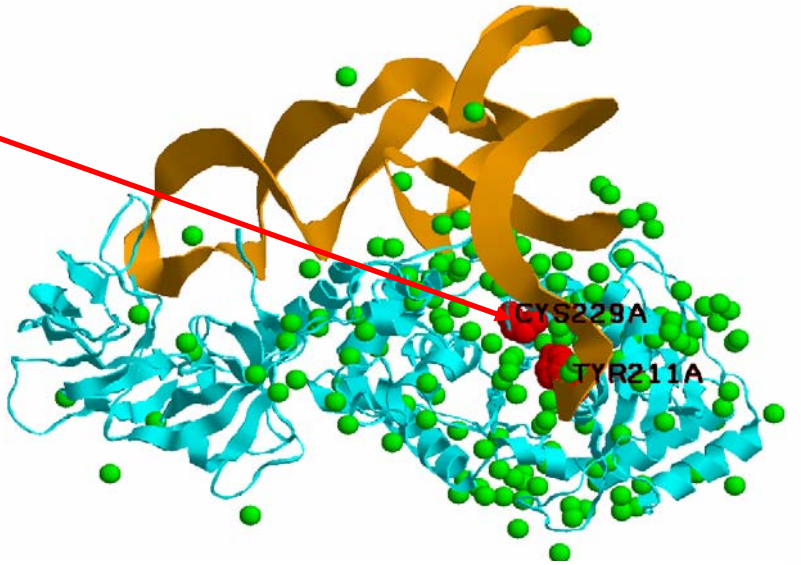
→ similar to external interface of bilayers

K. Bhattacharyya: J. Phys. Chem B.106 (2002) 10741

3. SR at the active site of the enzyme (glutaminyl t-RNA Synthetase)



-SH (C229, red) at a depth ~7 Å from surface, may be labeled with acrylodan



Prodan at active side of free GlnRS → 0.4 ns (45%) & 2 ns

→ Slow ns dynamics: Preorganization of water at active site

K. Bhattacharyya: Biochemistry 44 (2005) 8940

Solvation Dynamics of GlnRS + Substrates

- Free GlnRS → 400 ps (45%) & 2000 ps
- GlnRS + Gln → 750 ps (35%) & 2000 ps
- GlnRS + t-RNA → 450 ps (25%) & 2500 ps
- GlnRS + Gln + t-RNA → 800 ps (80%) & 3250 ps

❖Solvation Dynamics **further decreased by binding of substrates**

K. Bhattacharyya: Biochemistry 44 (2005) 8940

Acknowledgement

Mgr Agnieszka Olzynska, Dr Teresa Kral, Mgr. Jana Humpolickova, Mgr. Jan Sykora, Mgr. Ales Benda, Mgr. Martin Benes, Mgr Piotr Jurkewicz, Mgr. Tanya Sheynis, Mgr Adam Miszta, Veronika Fagulova (Prague; Czech R.)

Marc Maroncelli (Penn-State; USA) for providing the "ultrafast SR" slides

Kankan Bhattacharyya (Kolkata; India) for providing the "SR in proteins" slides

Mark Berg (South Carolina, USA) for providing the "SR in DNA" slide