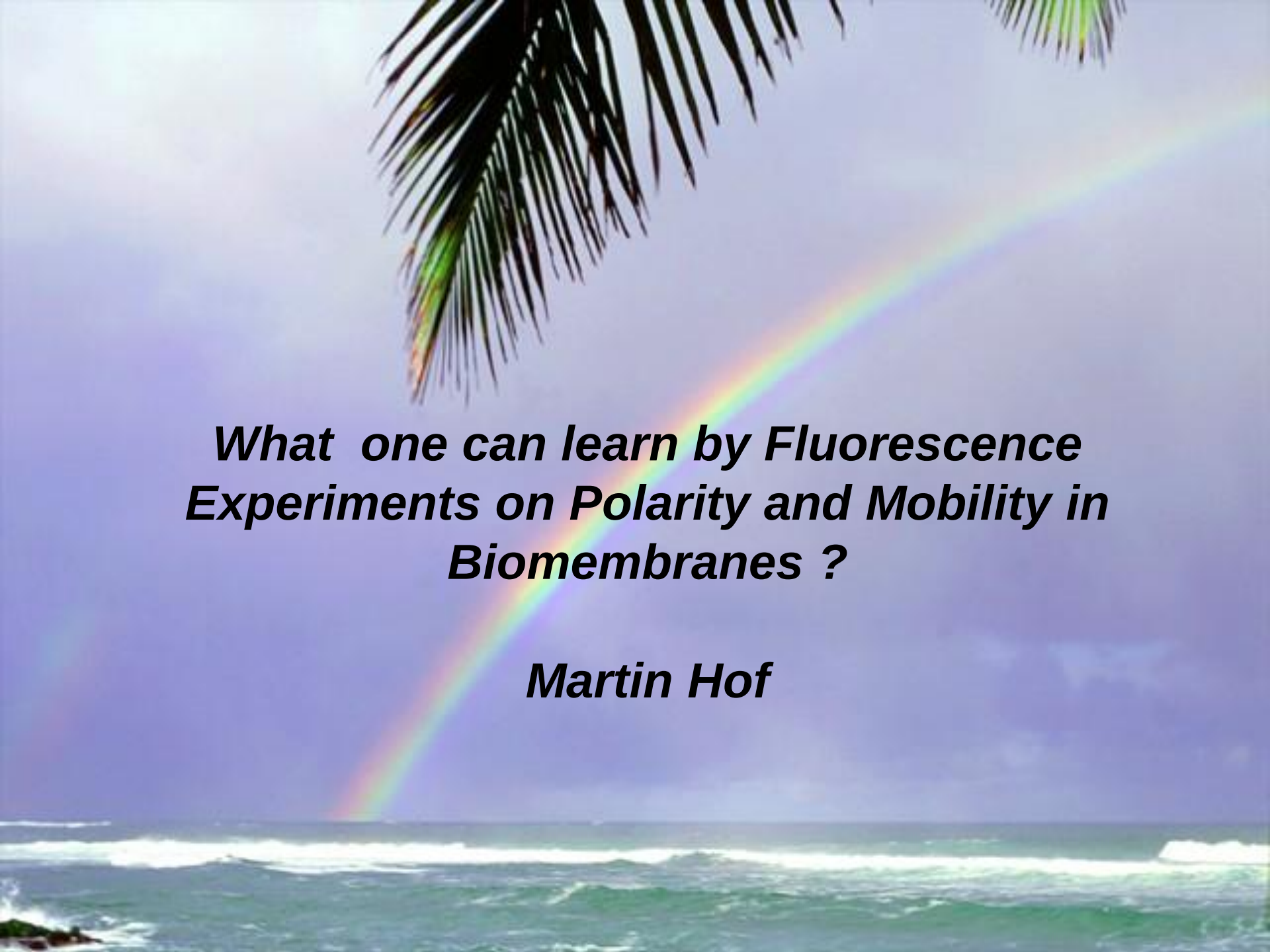
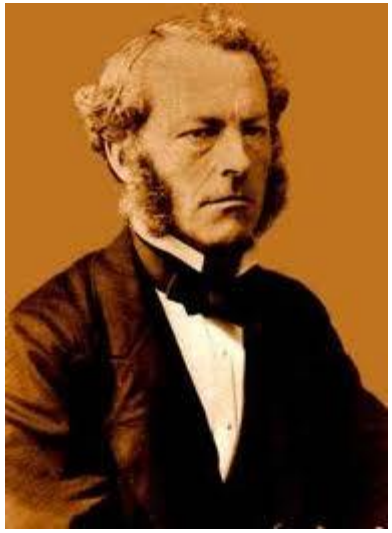


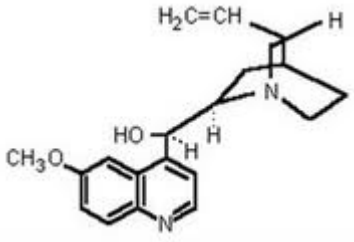
What one can learn by Fluorescence Experiments on Polarity and Mobility in Biomembranes ?

Martin Hof

What is fluorescence?

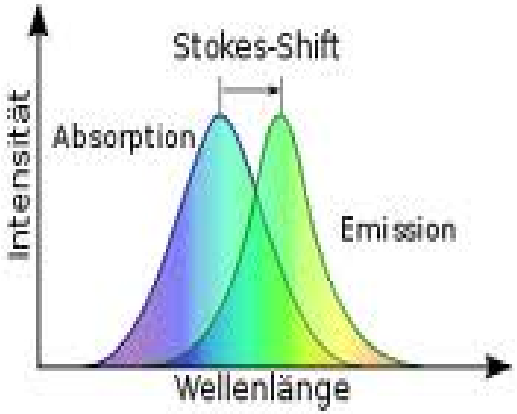


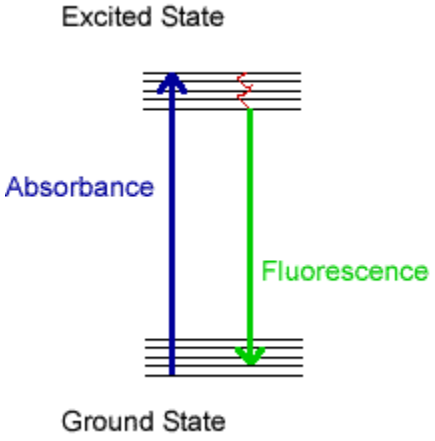
1852
Sir George Gabriel Stokes



Quinine in water

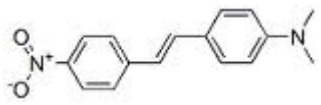
Fluorescence is the emission of light by a substance that has absorbed light of a different (lower) wavelength





Why fluorescence for probing polarity?

• it provides information on the molecular environment of the fluorescent dye. Specifically, fluorescence of a dye is dependent on the polarity of the environment.

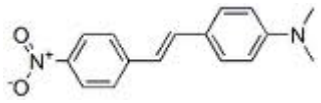


Dissolved in

a) Cyclohexane (unpolar)
b) Diethylether (medium polar)
c) Ethylacetat (polar)

Why fluorescence for probing polarity?

• it provides information on the molecular environment of the fluorescent dye. Specifically, fluorescence of a dye is dependent on the polarity of the environment.



Dissolved in

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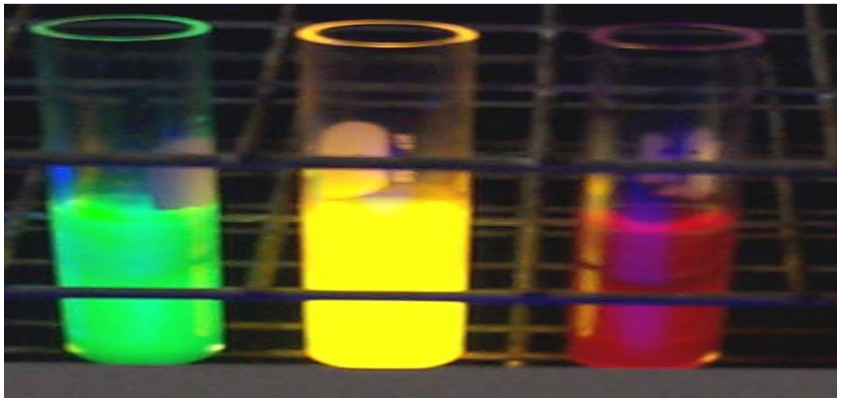
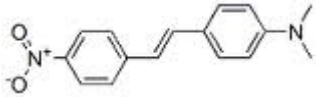
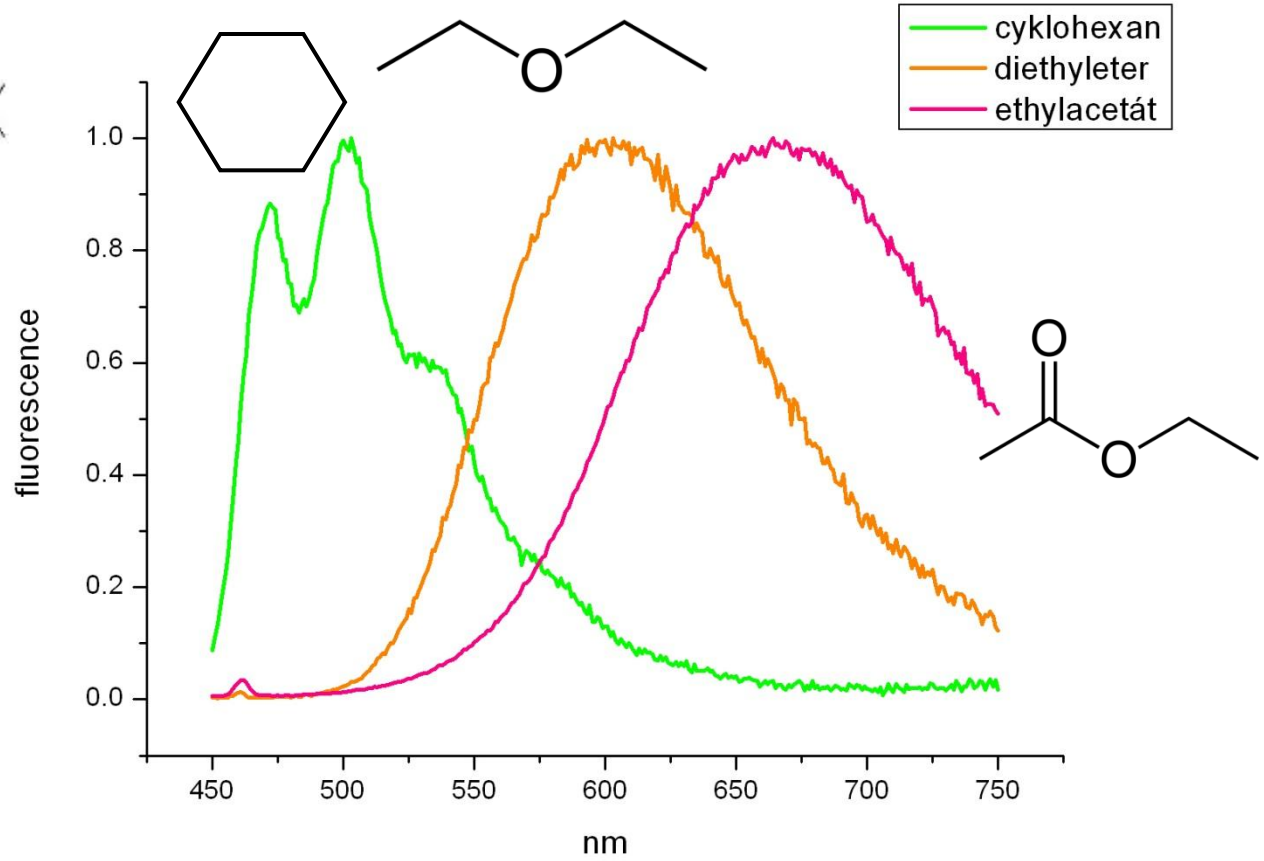


Emission spectra gets red-shifted by increase of solvent polarity

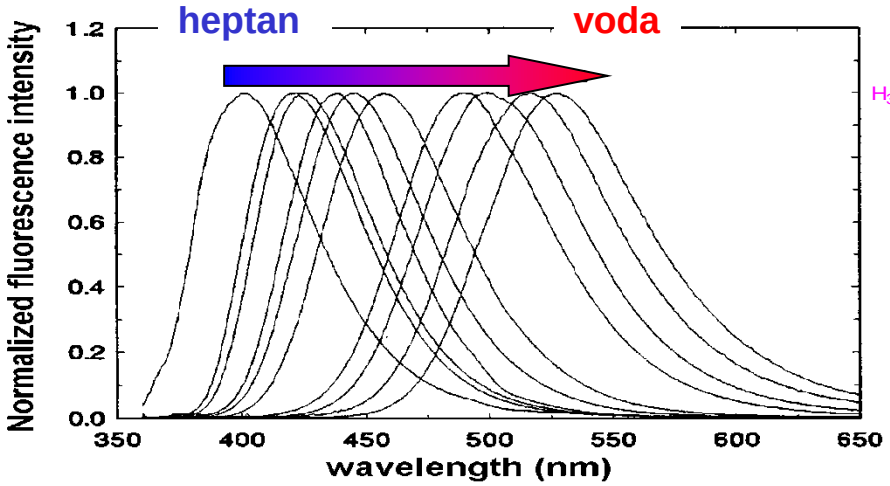




Fluorescence provides information on the molecular environment of a fluorescent dye.

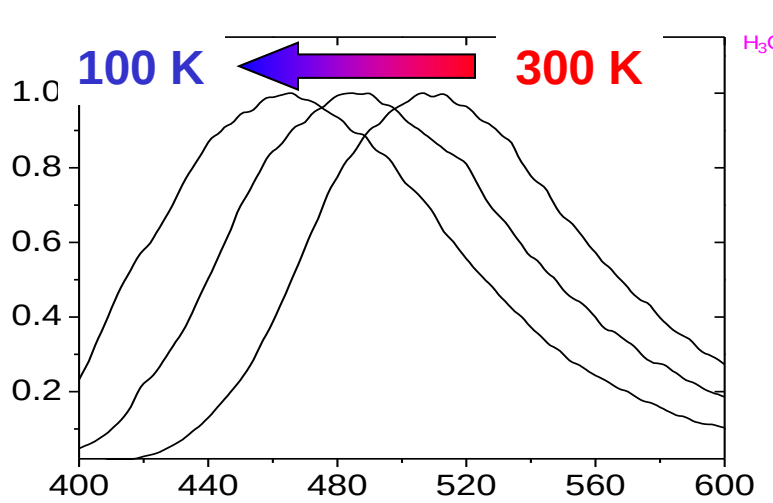
- A. Specifically, **fluorescence** of a dye is dependent on the polarity of the environment.

"Red-shift due to increase in polarity"



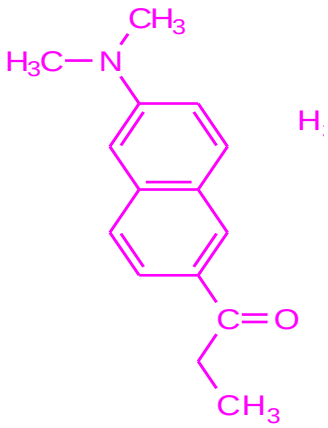
- B. **Fluorescence** of a dye can give information on the viscosity of the dye's environment

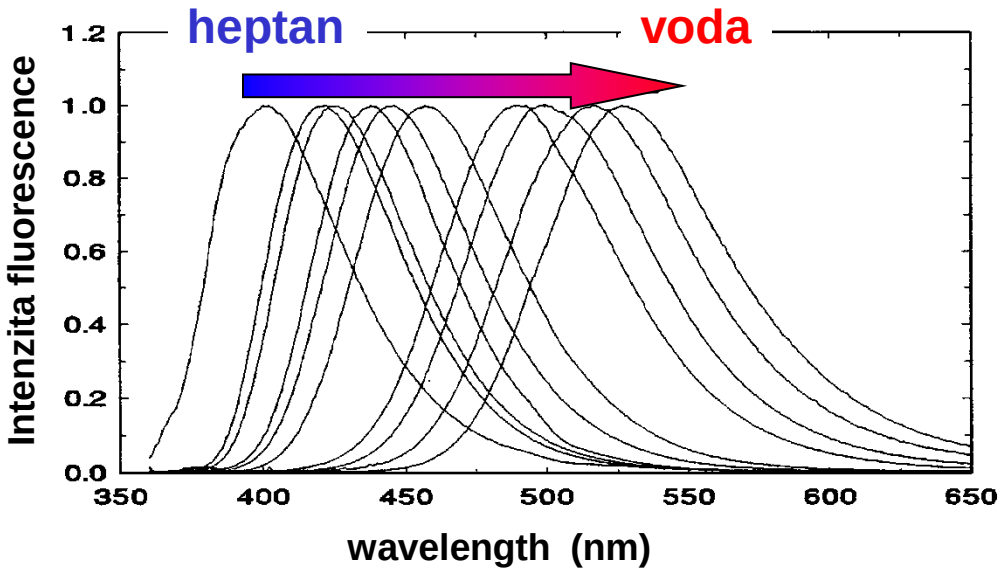
"Blue-shift due to increase in viscosity"



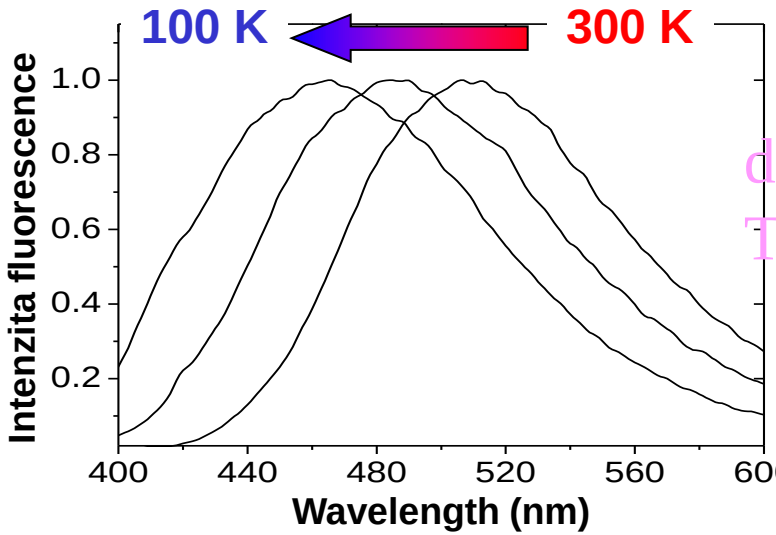
dissolved in THF

Increase of solvent polarity leads to red-shift





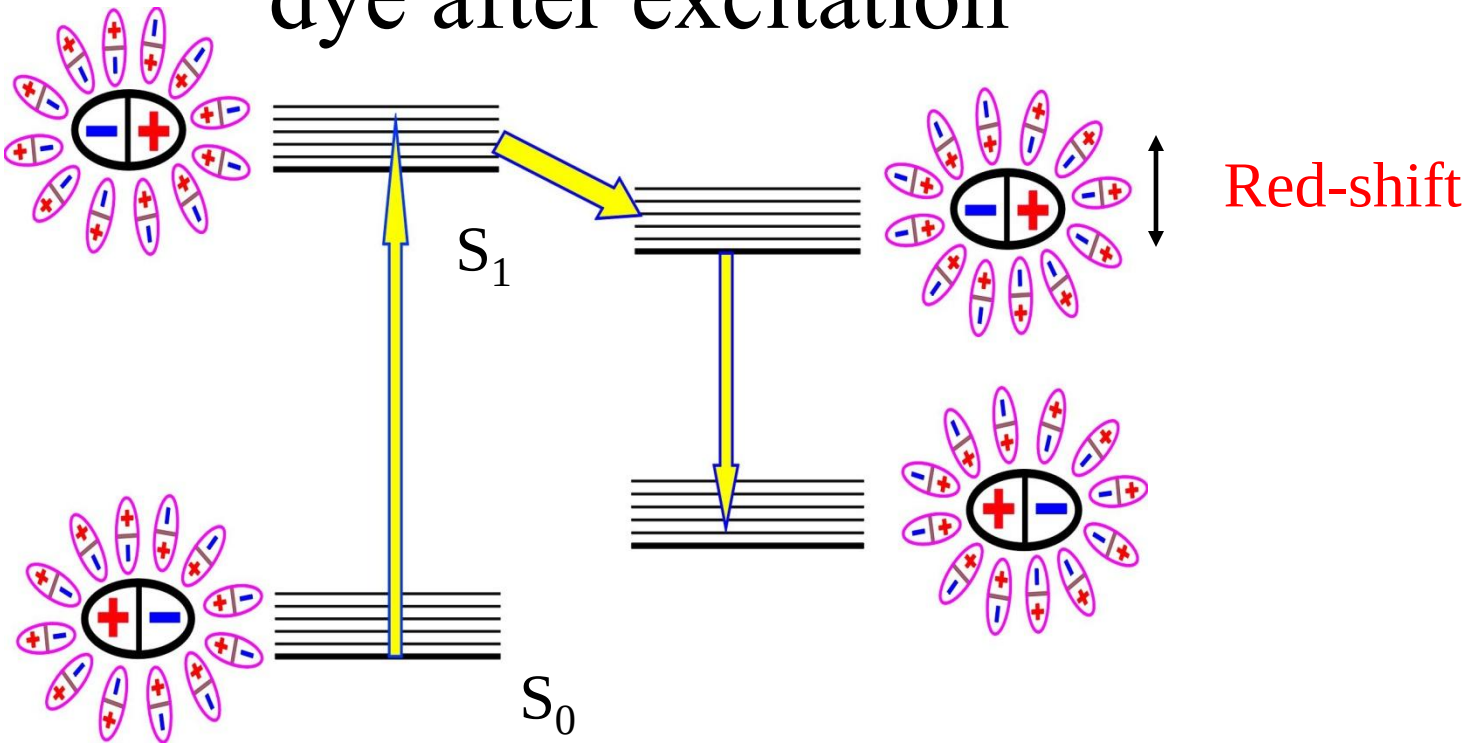
Increase of viscosity leads to blue-shift



dissolved in THF

Red and blue-shifts are solvent effects and are based on the solvent relaxation process

What is **solvent relaxation**: "photophysics of dye after excitation"

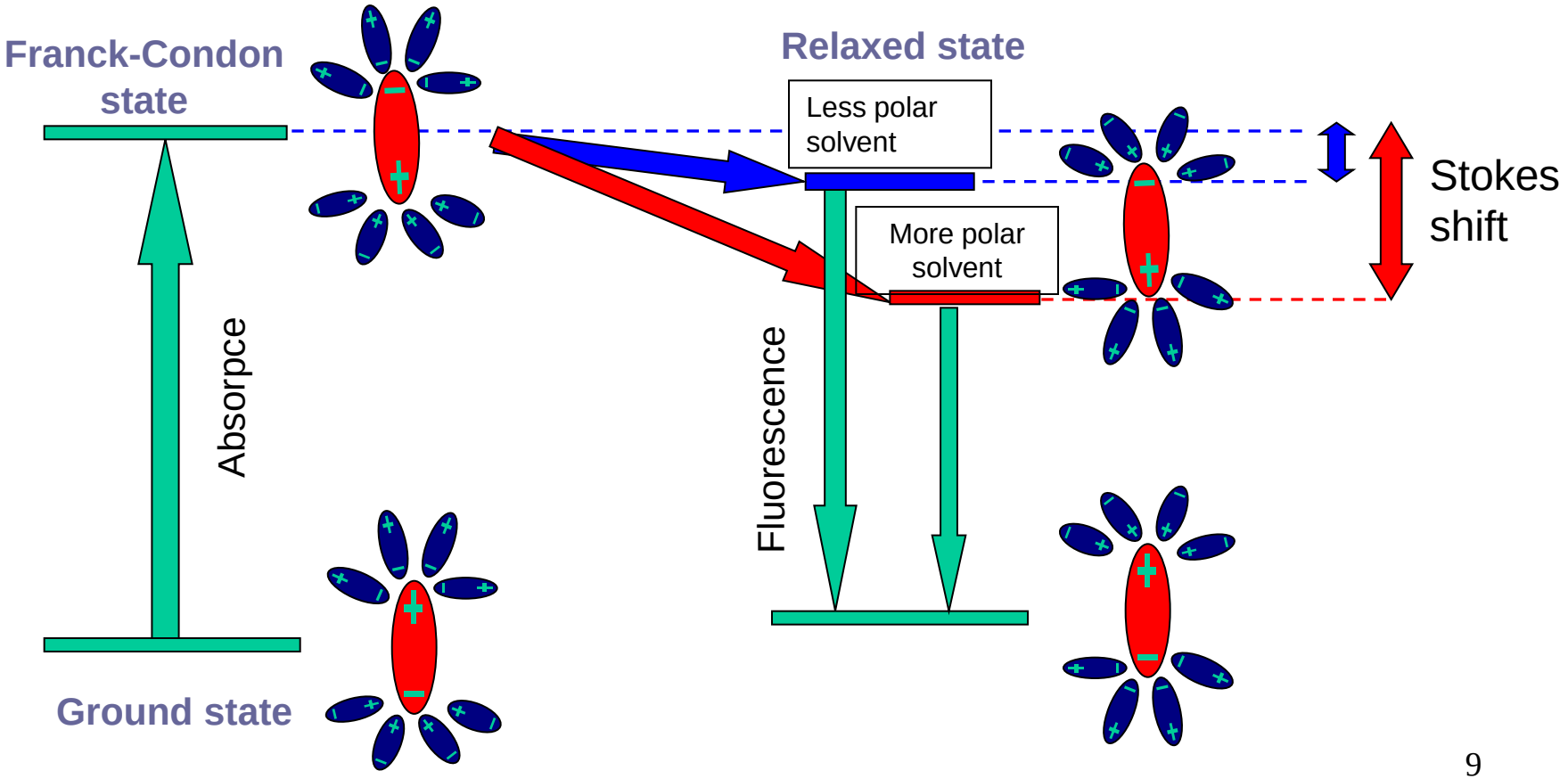


Dye excitation leads to a instantaneous change in the dye's dipole moment → dipoles of the solvent molecules have to react to this non-equilibrium situation and start to reorient →this reorientation leads to stronger dipole-dipole interactions and decreases the energy of the system (relaxation) →red-shift

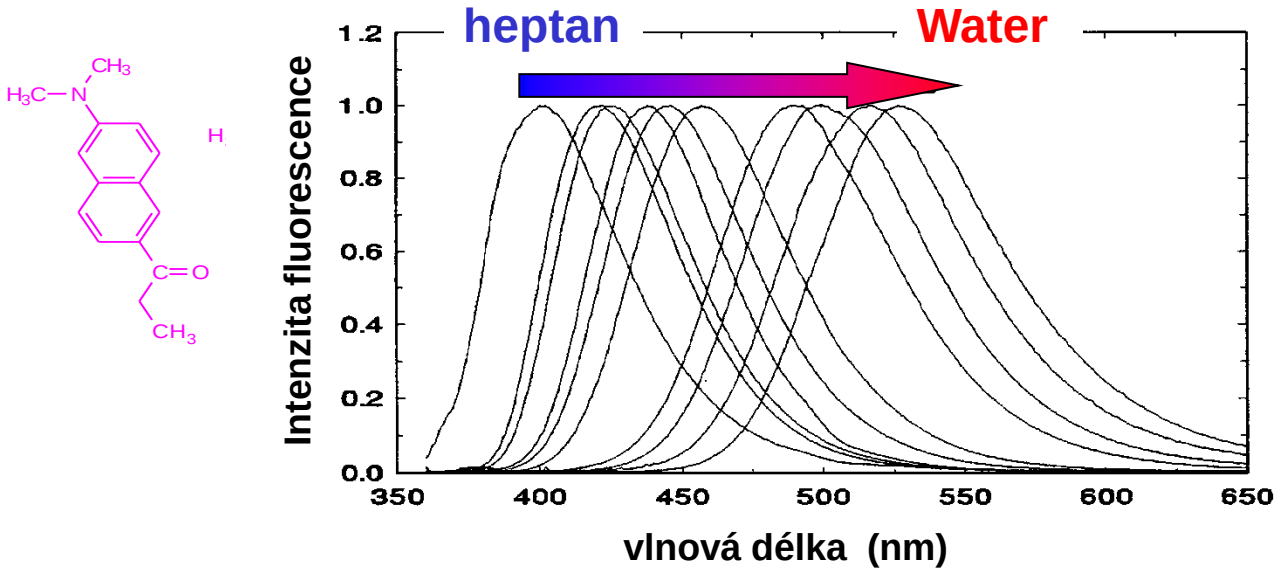
Red-shifts in steady-state fluorescence spectra

Solvent relaxation is faster than fluorescence

Jablonski diagram:

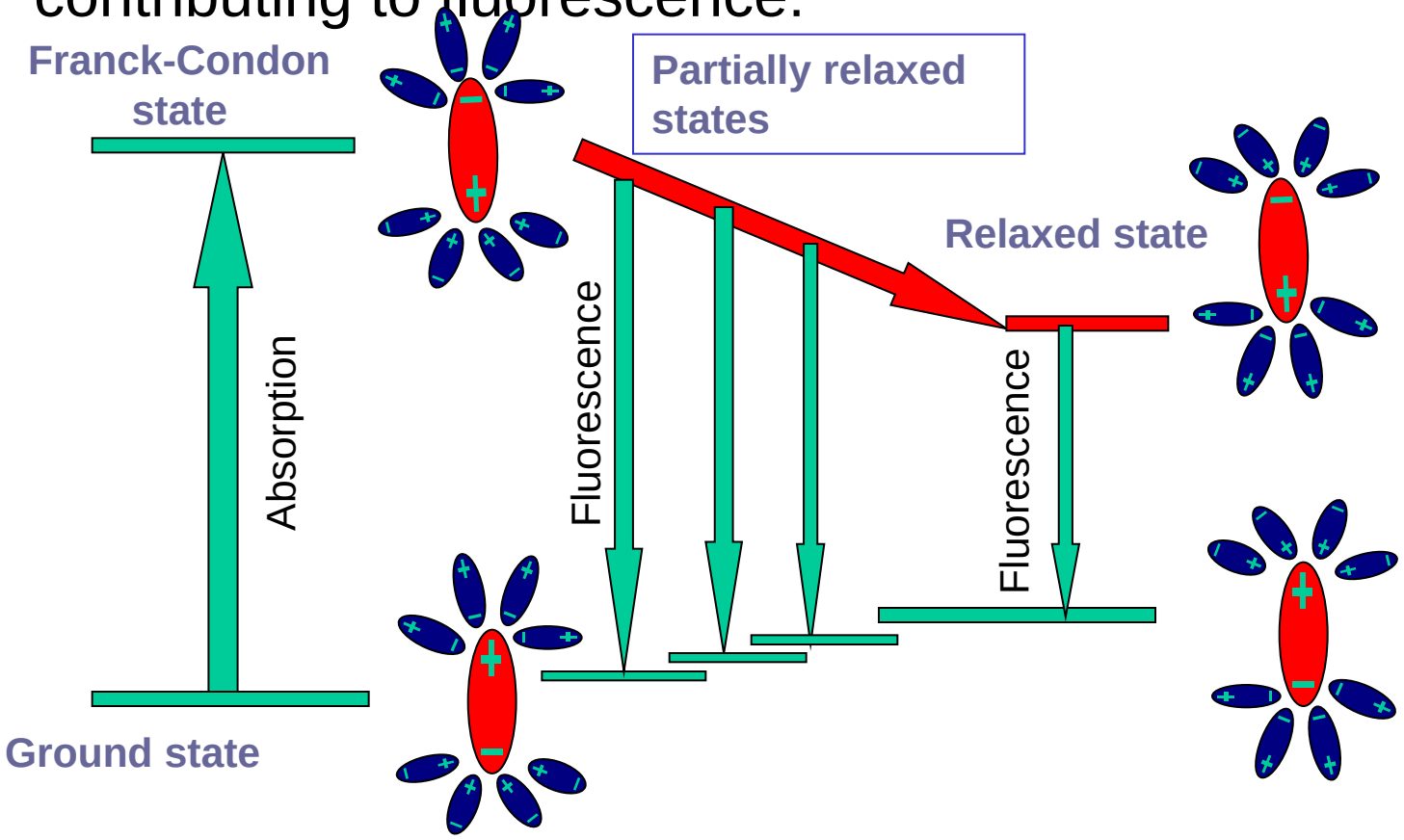


Solvent relaxation is faster than fluorescence: increase of polarity of solvent leads to stronger dipole-dipole interactions and thus to a decrease of the energy of the relaxed state. Almost all dye molecules are fluorescing from this state, thus increased solvent polarity leads to red-shift

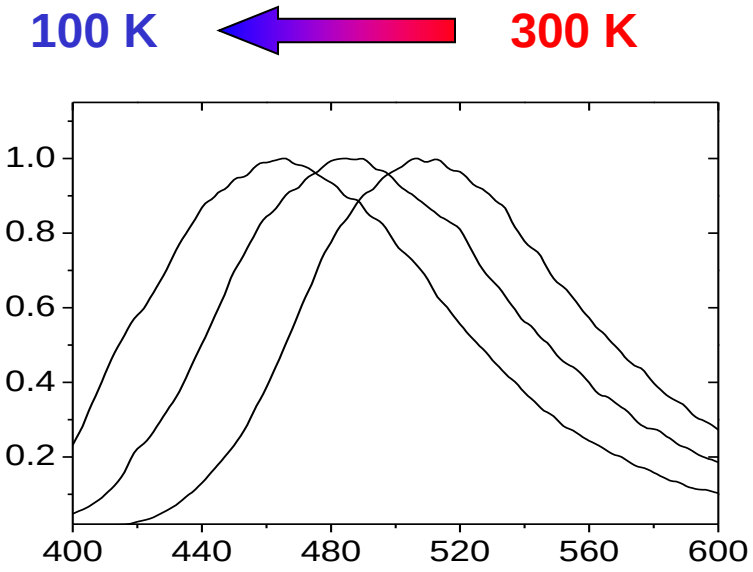


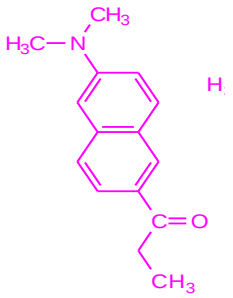
Blue-shifts in steady-state fluorescence spectra

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



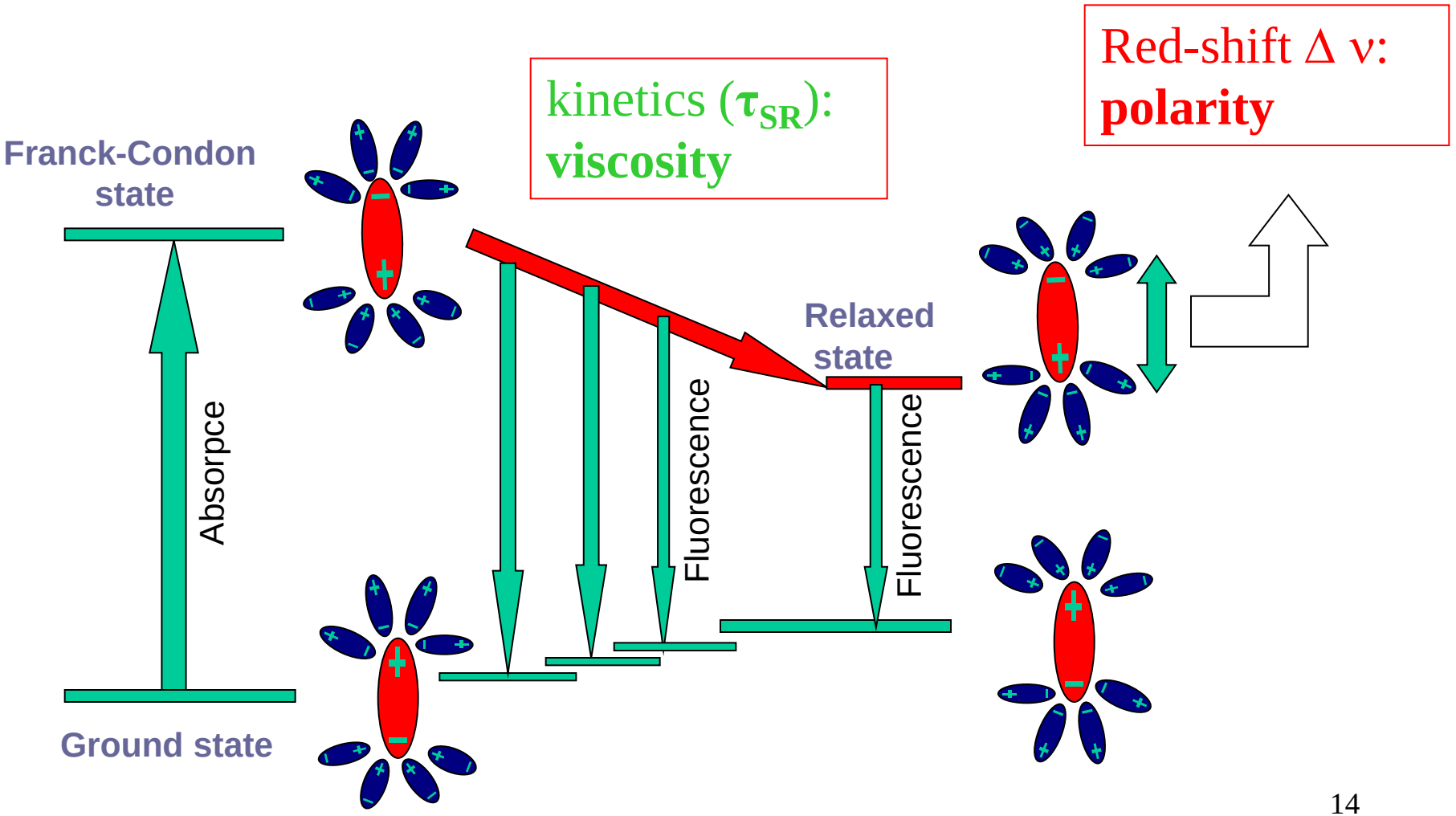


in THF

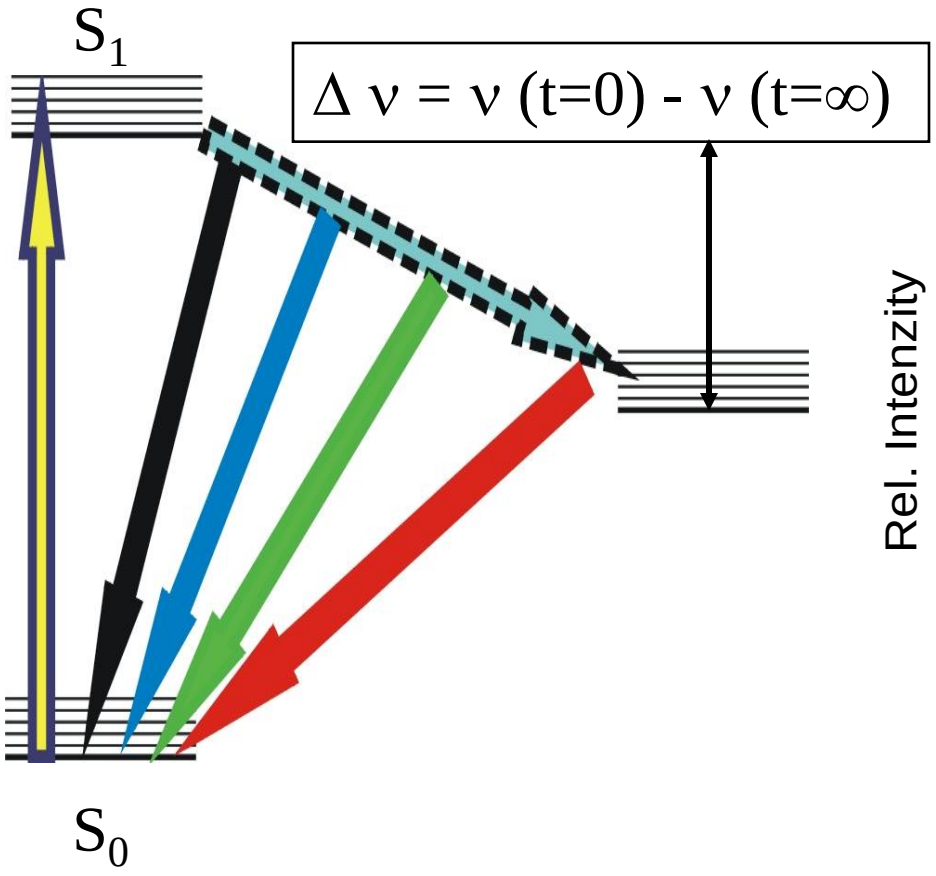
Qualitative connection between fluorescence emission of a dye and polarity/viscosity of the dye's molecular environemt

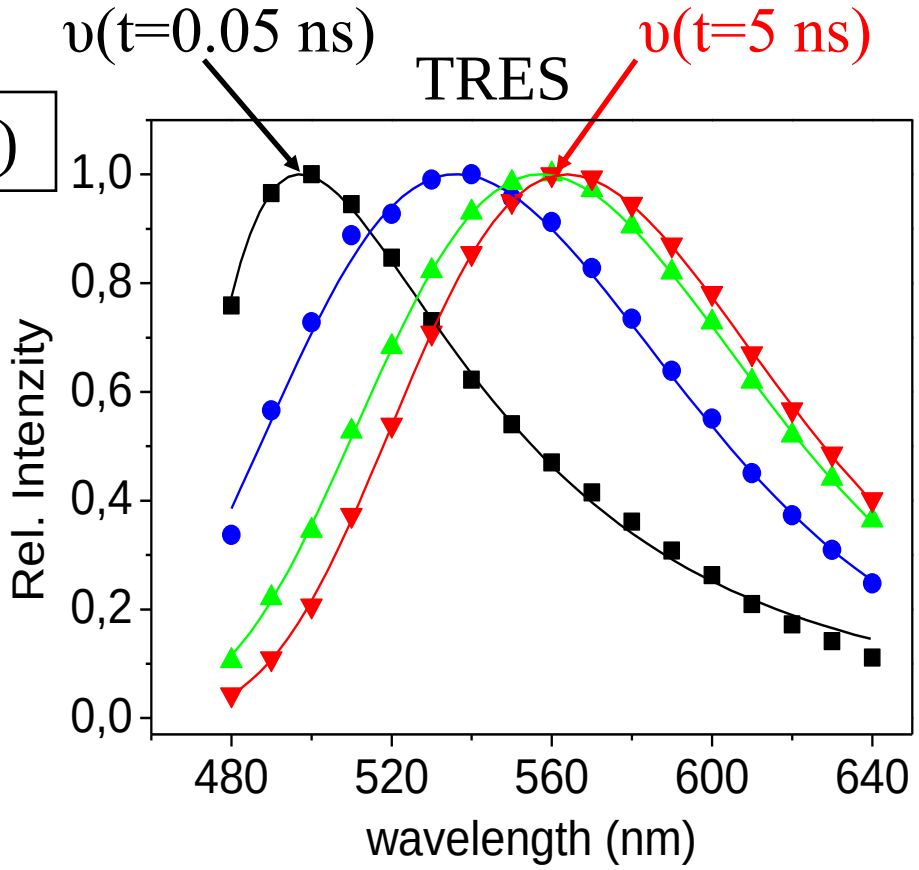
Quantitative?

Quantitative monitoring the solvent relaxation process: Time-resolved fluorescence spectroscopy

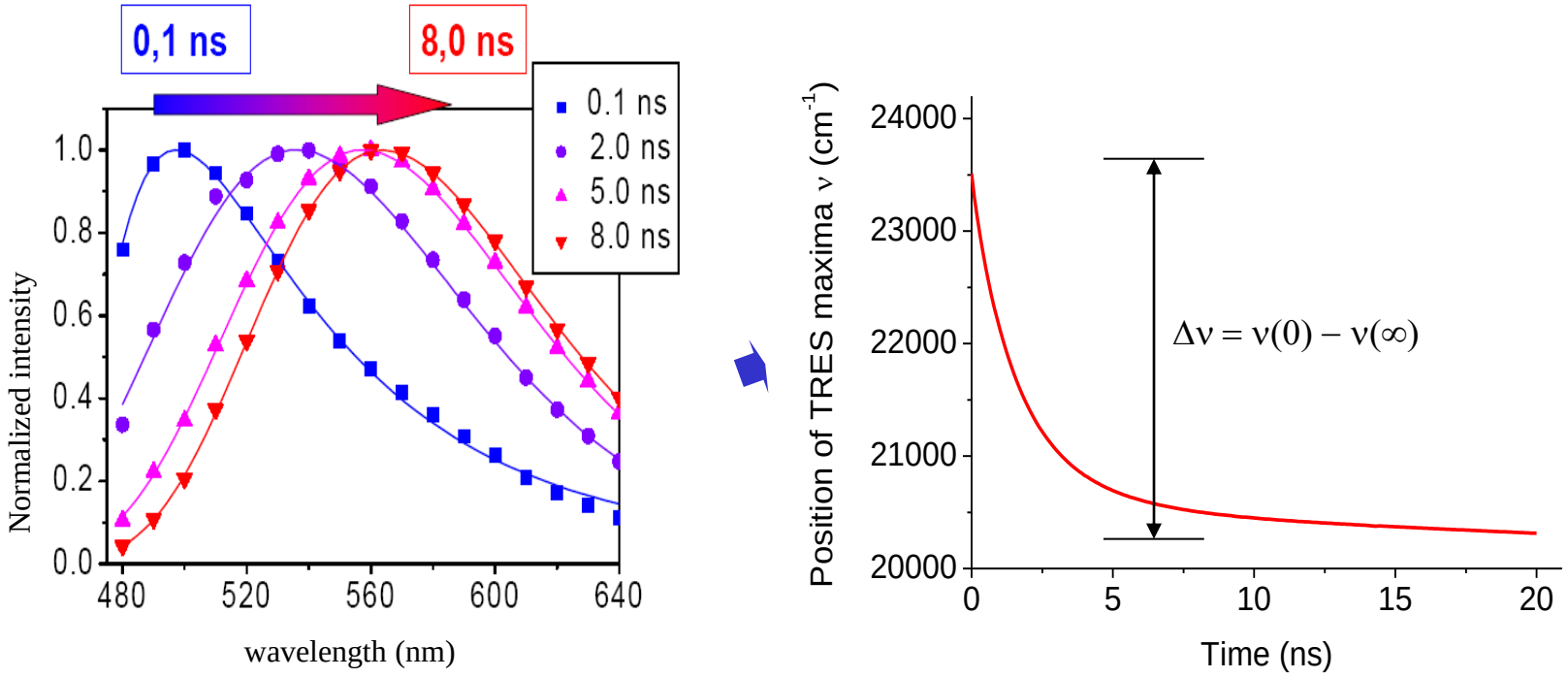


SR is monitored by "time-resolved fluorescence emission spectra"



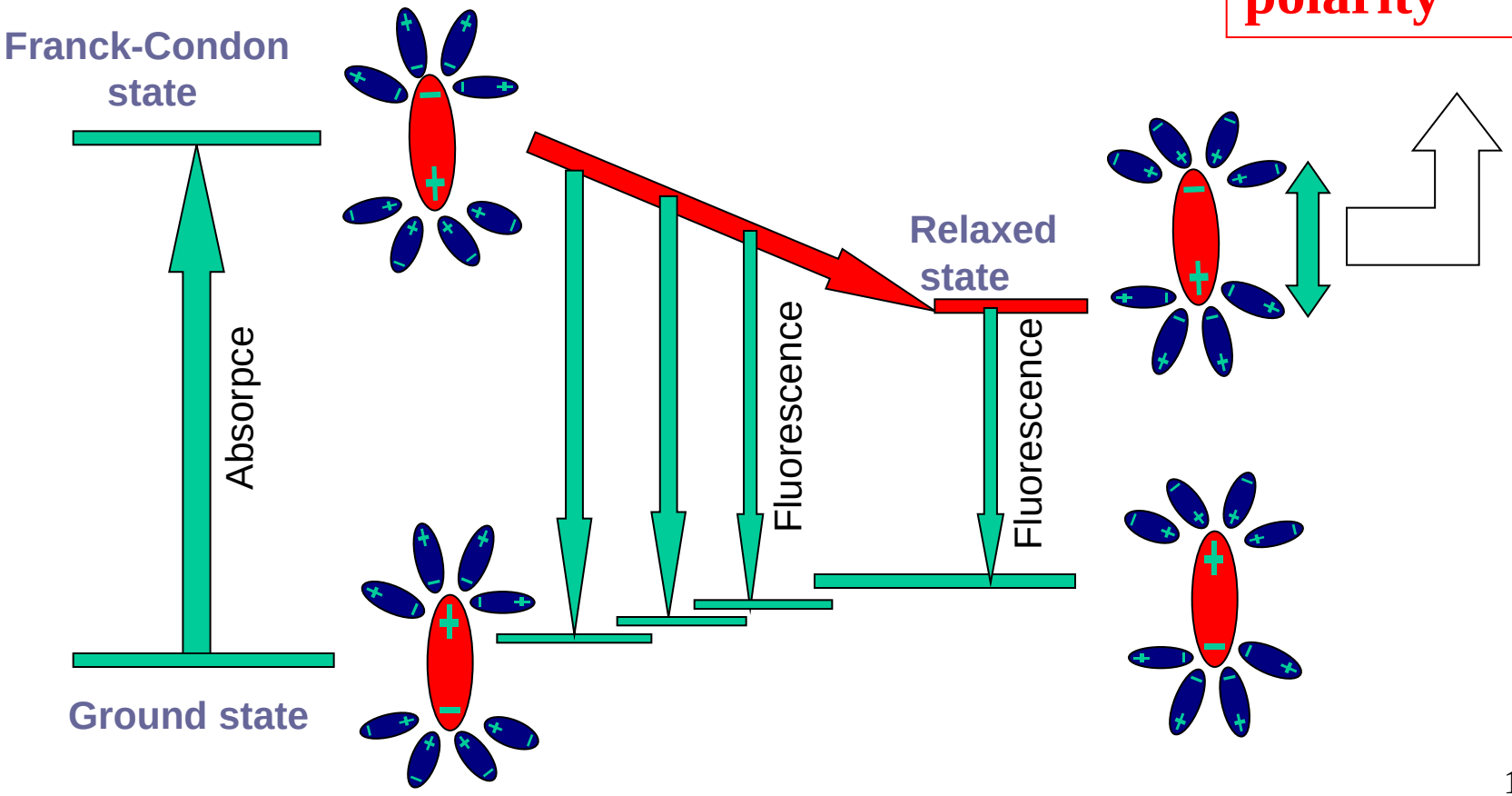


Time-dependent Stokes shift Δ ν

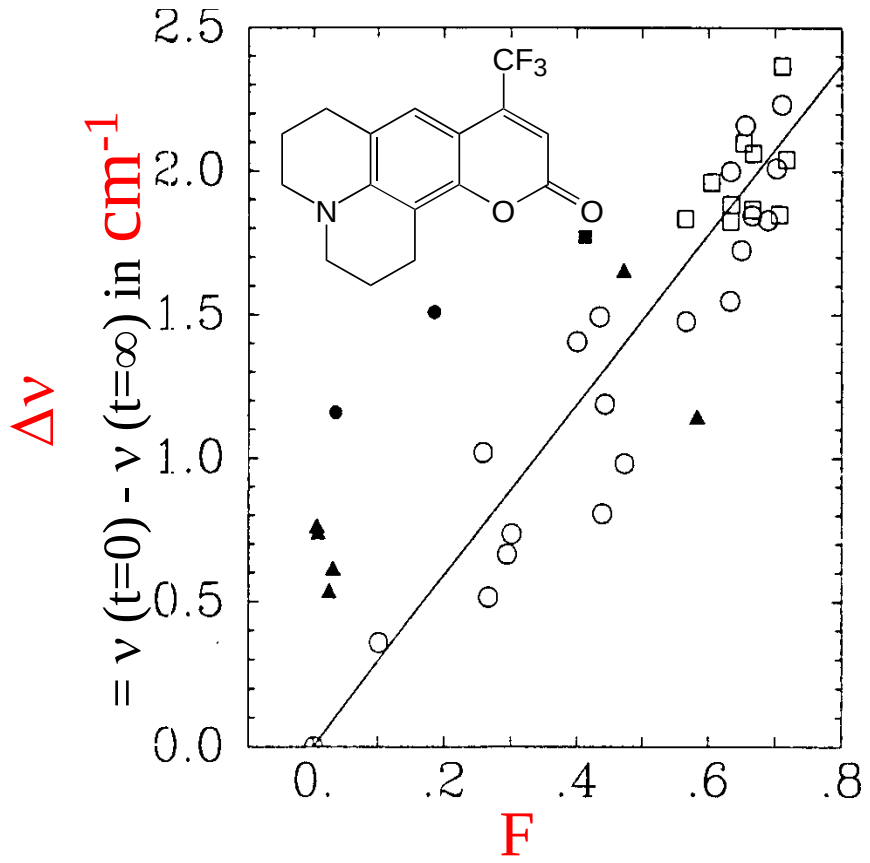


Quantitative monitoring the solvent relaxation process: Time-resolved fluorescence spectroscopy

Red-shift Δ ν: **polarity**



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:

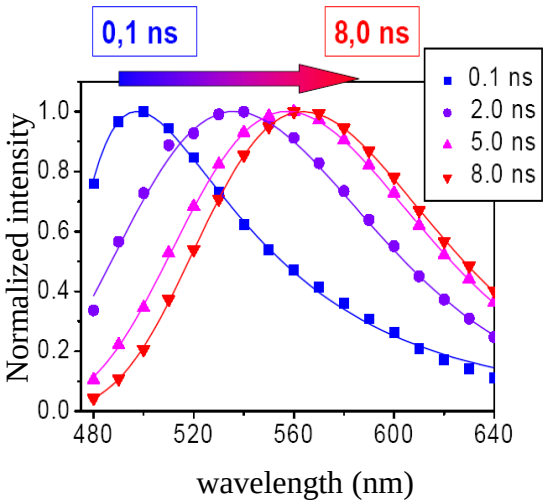
C_1OH: F = 0.71; Δ ν = 2370 cm^{-1}

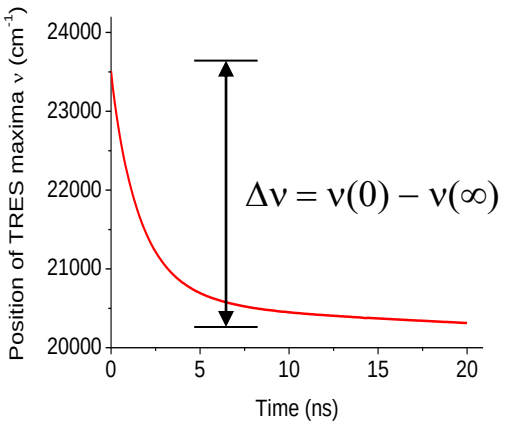
C_5OH: F = 0.57; Δ ν = 1830 cm^{-1}

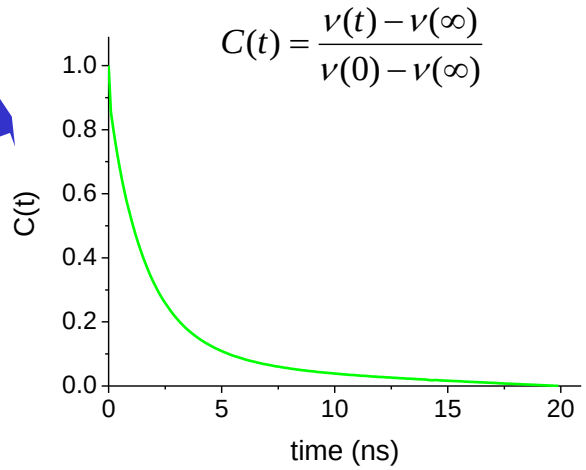
Horng et al., J Phys Chem 1995 99:17311

Dependence of SR kinetics on the solvent

Kinetics: Normalisation of Stokes shift υ(t): C(t)=(υ(t)-υ(∞))/ Δυ

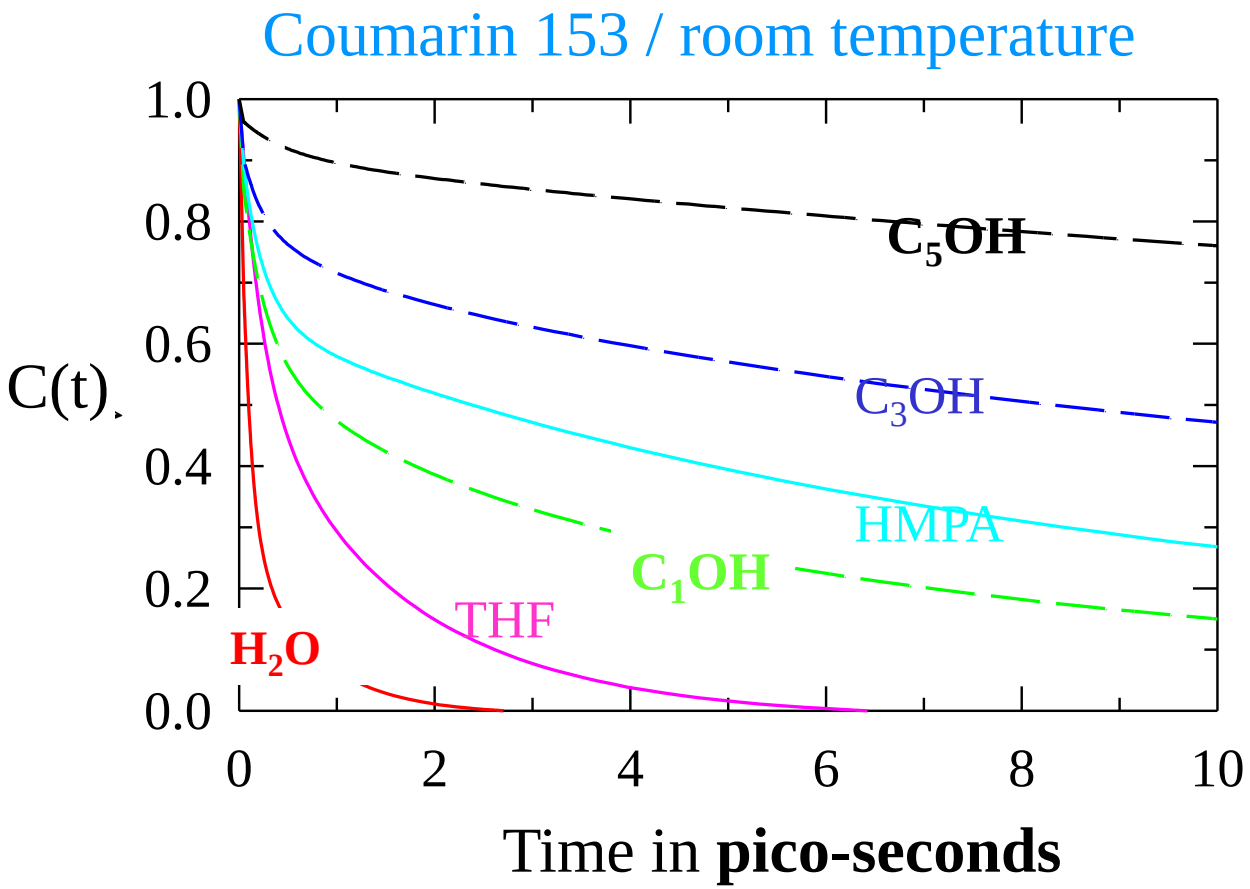






Dependence of SR kinetics on the solvent

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



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

0.3 "bulk water"

0.9

5

10

26

103

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

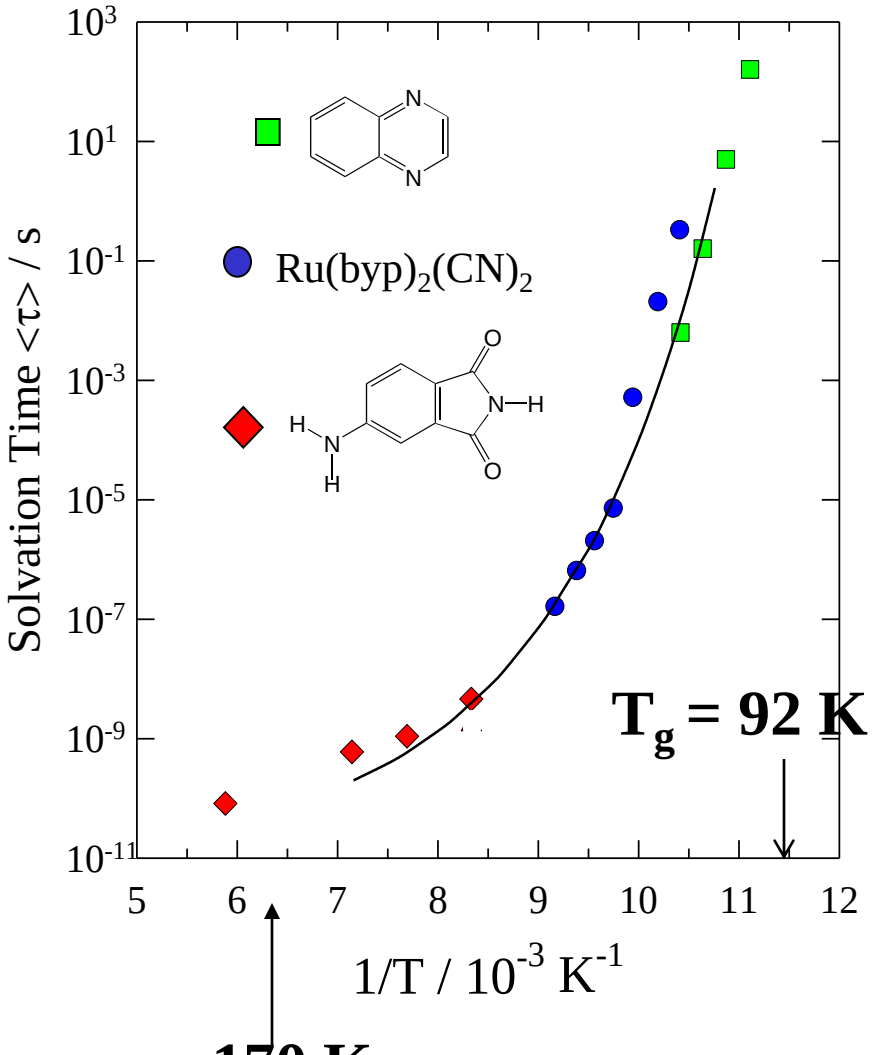
Kinetics of the SR is related to the viscosity of the microenvironment

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 of the dye

What can we learn by Fluorescence Solvent Experiments on Polarity and Mobility in Biomembranes ?

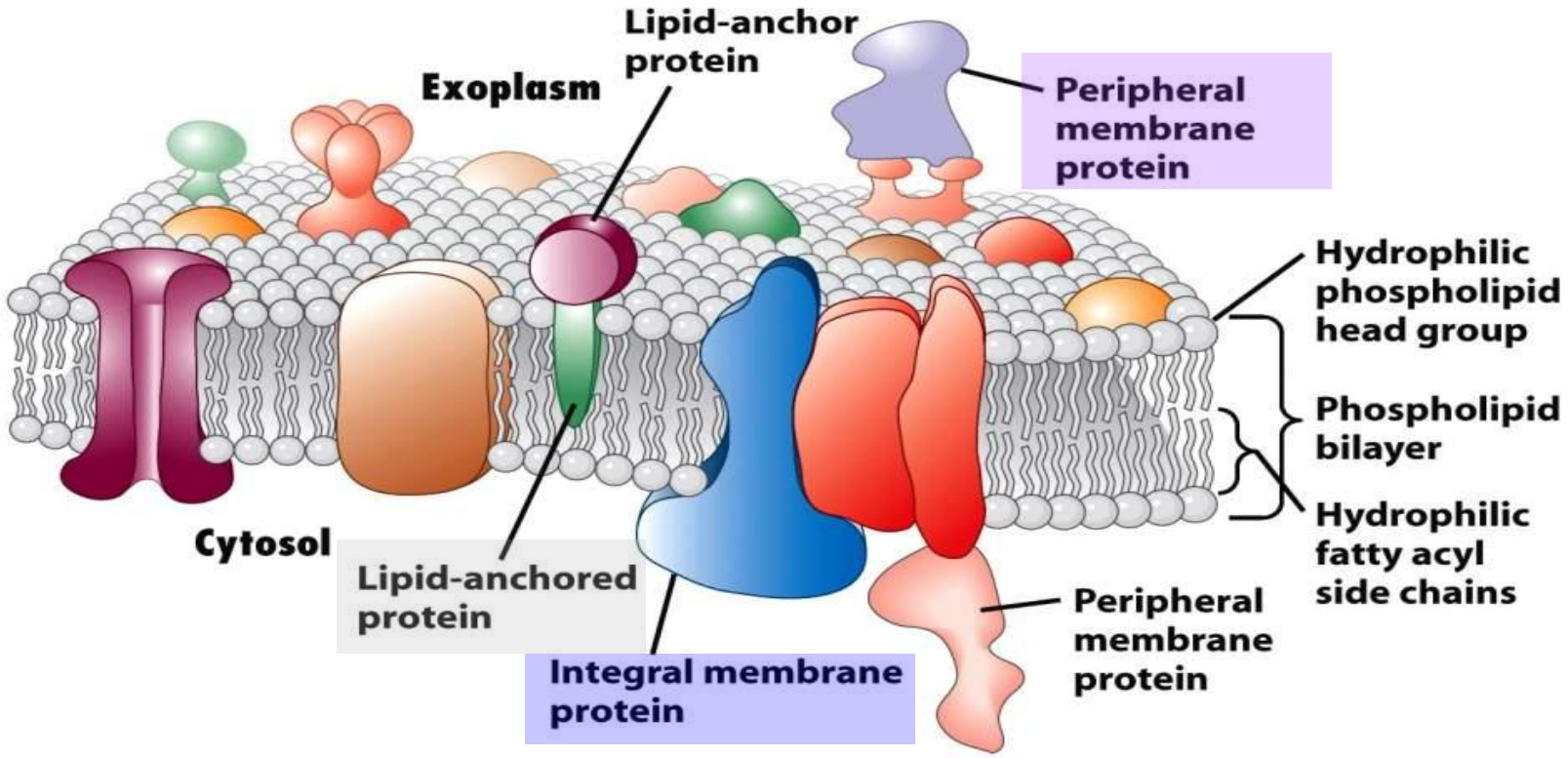
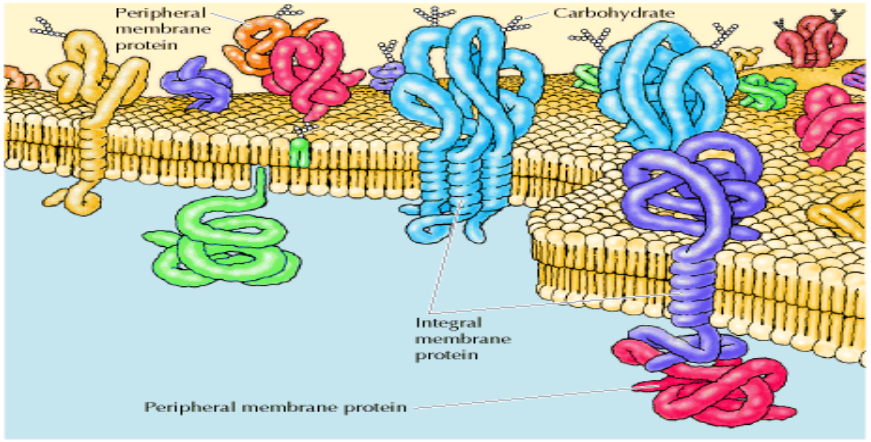


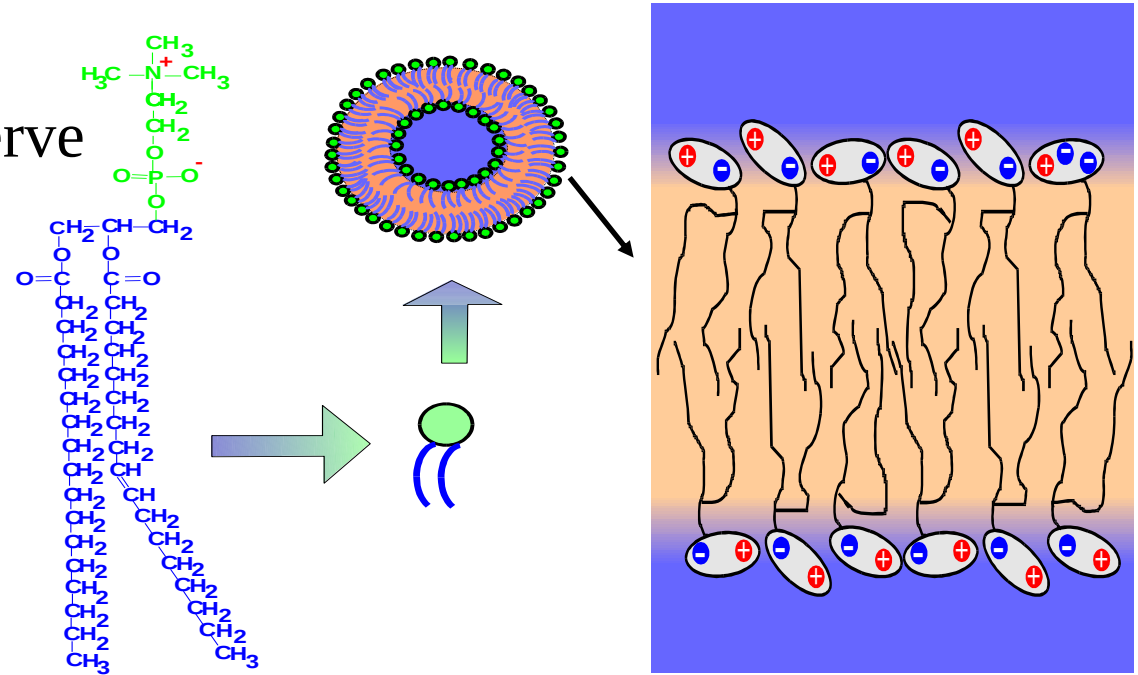
Figure 10-1
Molecular Cell Biology, Sixth Edition

The "Fluid Mosaic" Model of a cell membrane and unilamellar vesicles as their model system

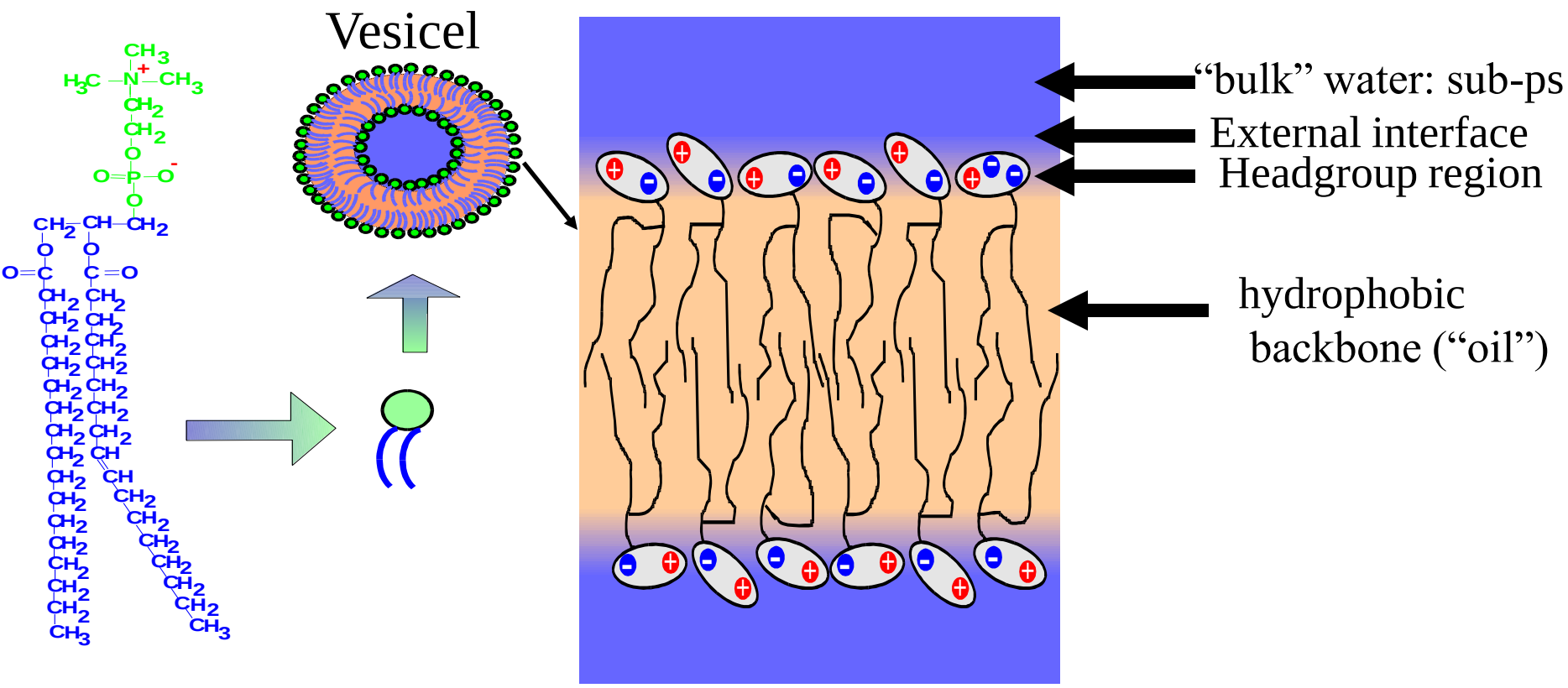
- The cell membrane is a two-dimensional mosaic, the structure of which is given by phospolipids forming a phospolipid bilayers



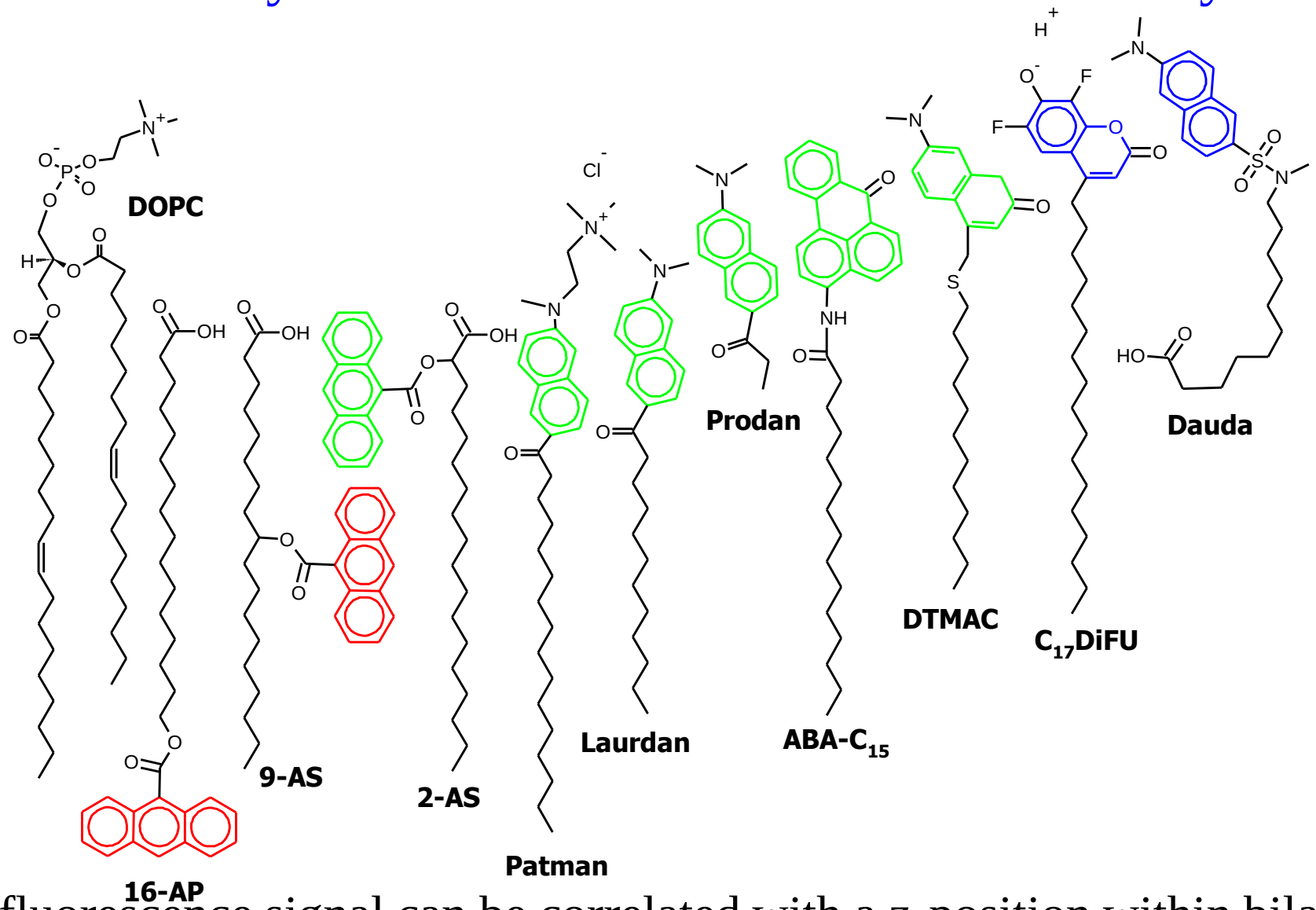
- Unilamellar vesicles serve as a model system



How does hydration and mobility change from the water phase towards the “oil” phase?



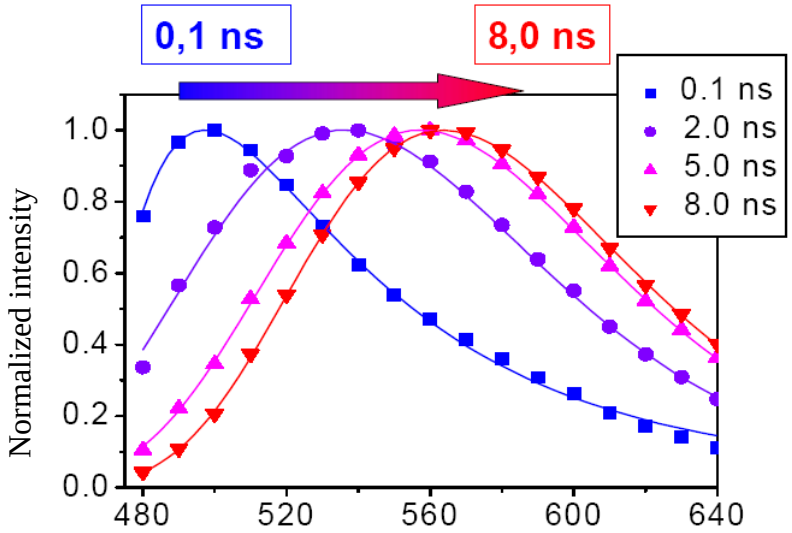
Fluorescent dyes are defined localized within the bilayer

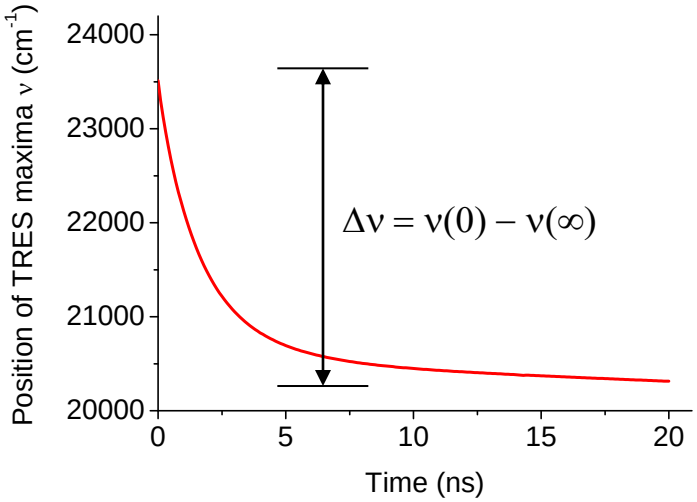


⇒fluorescence signal can be correlated with a z-position within bilayer

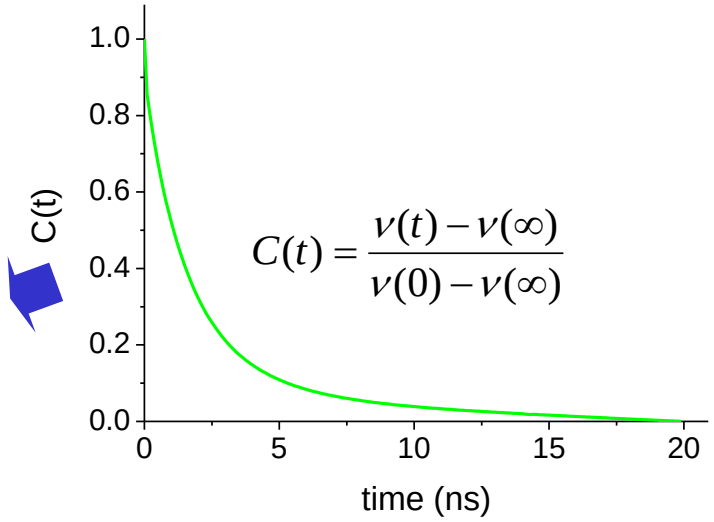
backbone headgroup external interface

Information obtained from TRES





SR parameters	neat solvents	Within bilayer
$\Delta\nu = \nu(0) - \nu(\infty)$	solvent polarity	amount of bound water molecules & its mobility
$\tau = \int_0^\infty C(t)\,dt$	viscosity	



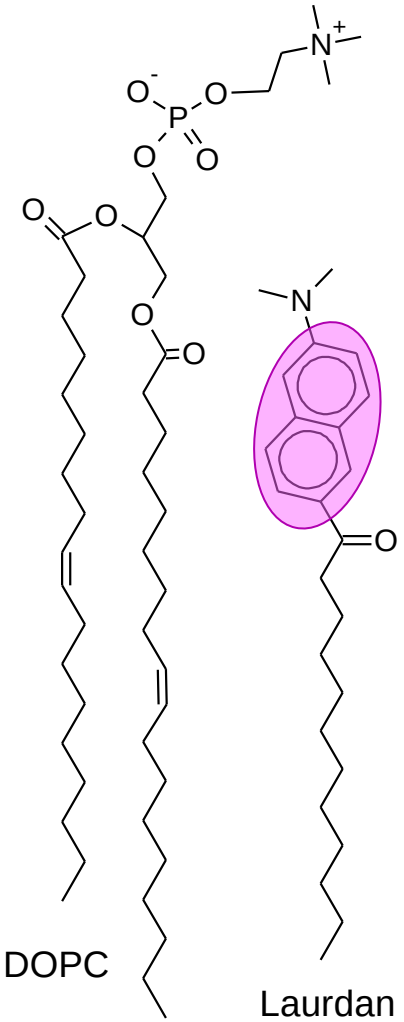
Ions in model lipid membranes:

Do ions with same charge interact differently?

K^+ versus Na^+

In order to get atomistic understanding also Cs^+

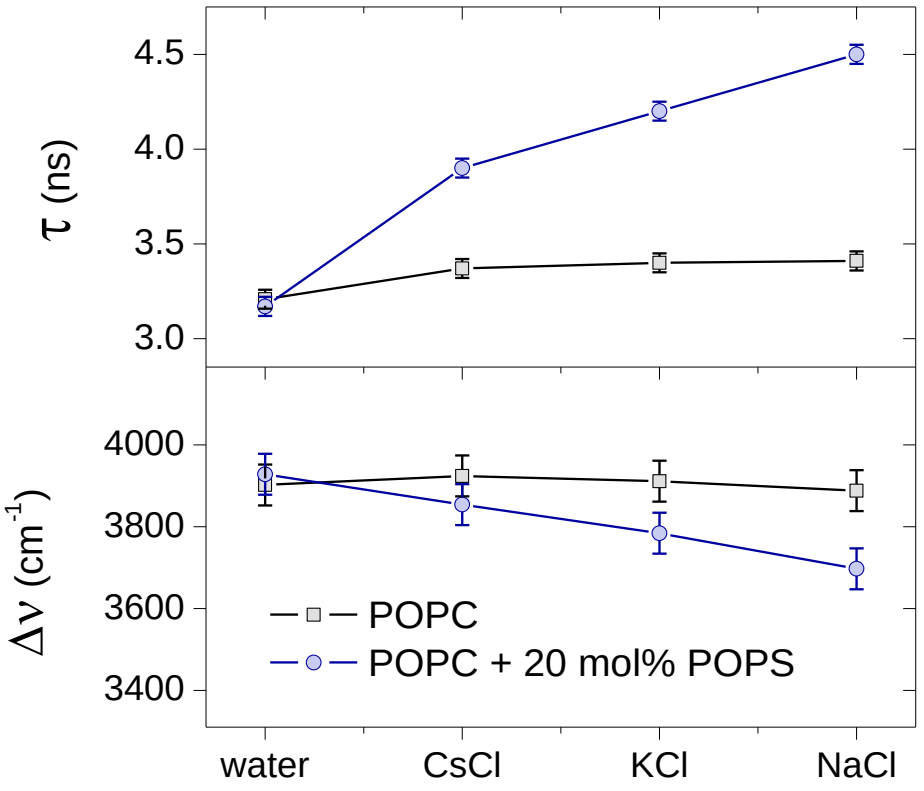
Laurdan TRES:
How does hydration and mobility of the sn_1 acyl-group change by addition of different cations ?

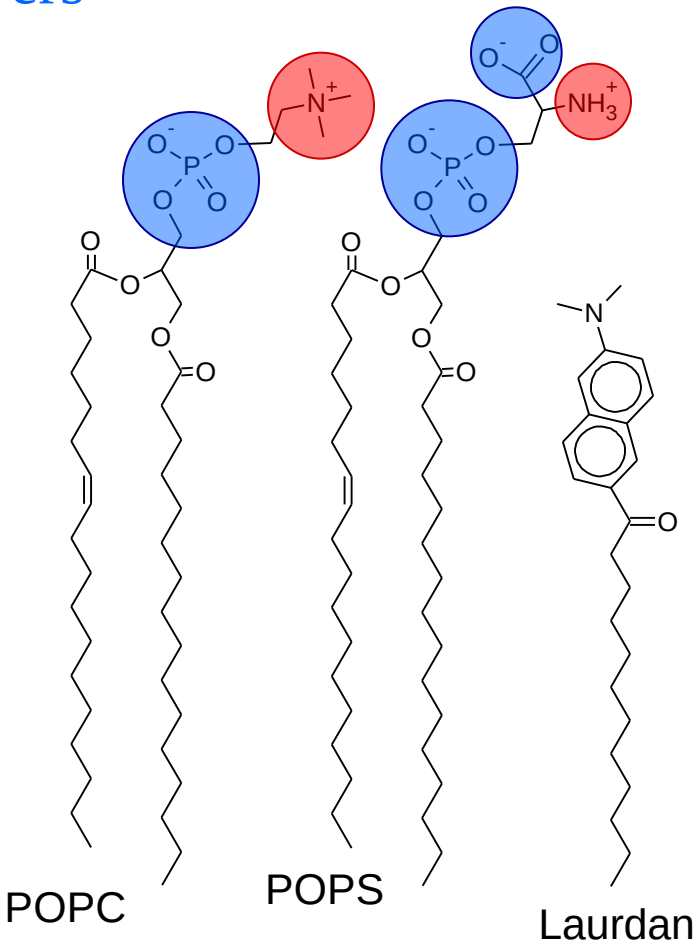


Solvent relaxation experiments

a) Weak cation packing effects in neutral bilayers; no ion specificity

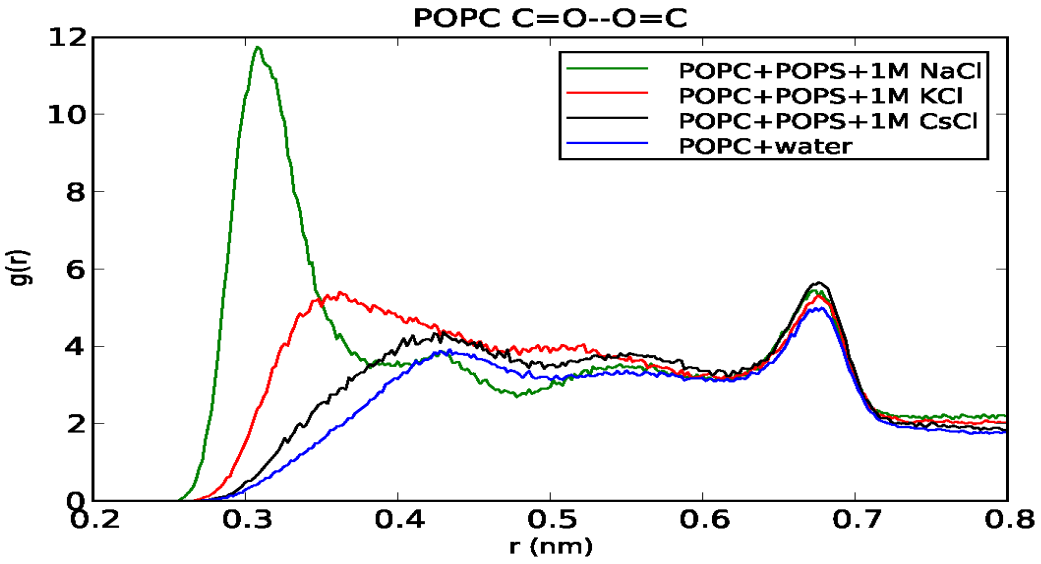
b) Specific cation effects in (negatively charged) Phosphatidyl-Serine containing bilayers

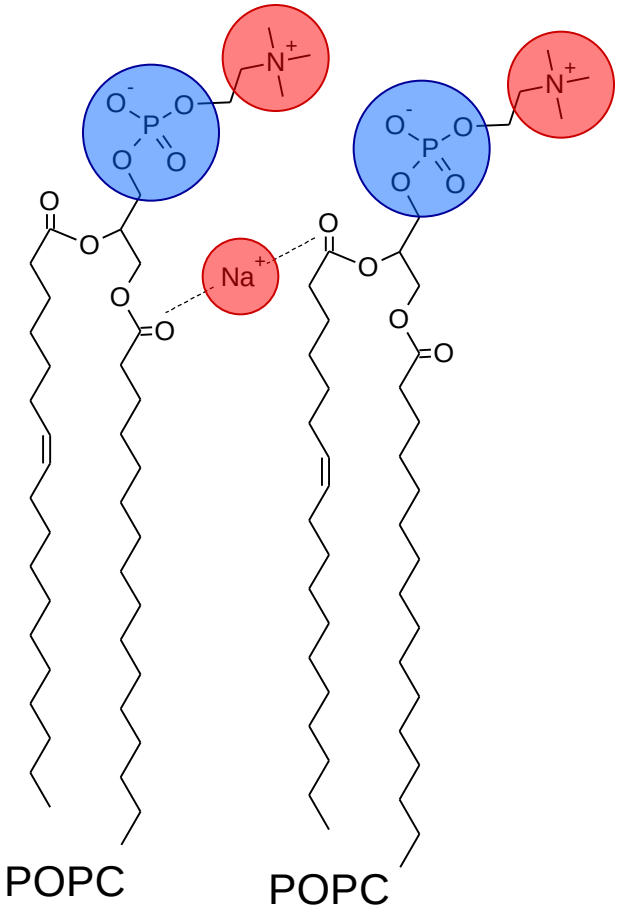




Na^+ is dehydrating and packing the glycerol level more than Cs^+ and K^+

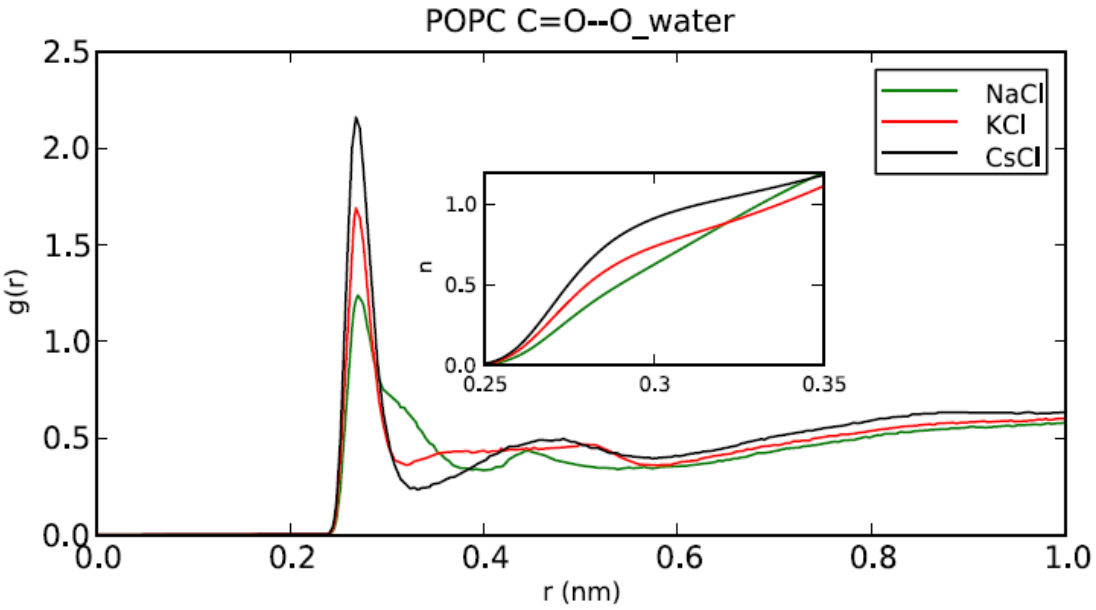
MD simulations: Na^+ is bridging the carbonyls and thus packing the glycerol level more than the other cations

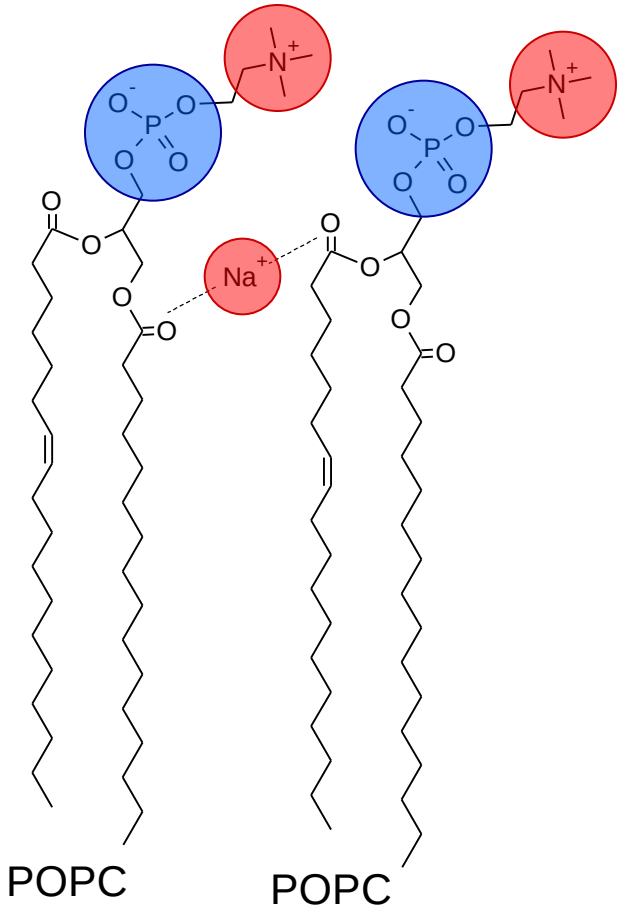




Bridging effect is much stronger for Na^+ than for the other cations in POPC/POPS bilayers

MD simulations: Na^+ is bridging the carbonyls and thus dehydrating glycerol level more than the other cations





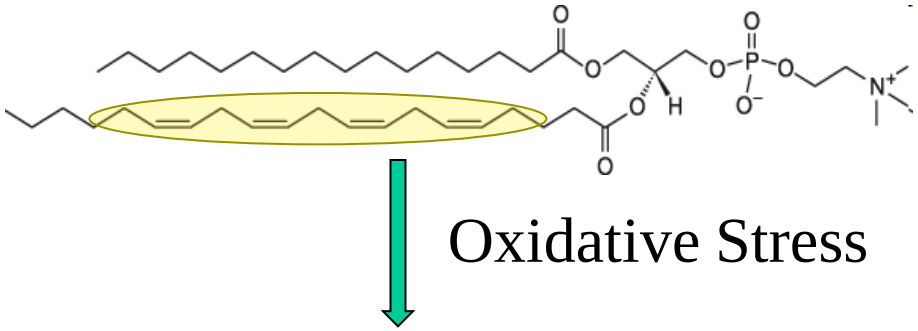
Please note the analogy to the $\Delta\nu$ values determined for this system!

Summary to strong ion effects observed by solvent relaxation experiments and explained by MD simulations

- ✓ Cations strongly influence probed hydration and mobility at the glycerol level when PS is present
- ✓ Small cations are attracted by negative charge; but then bridge the carbonyl groups leading to increased packing and decreased hydration. As larger the cation as smaller the bridging tendency.
- ✓ There is a strong difference between Na^+ and K^+

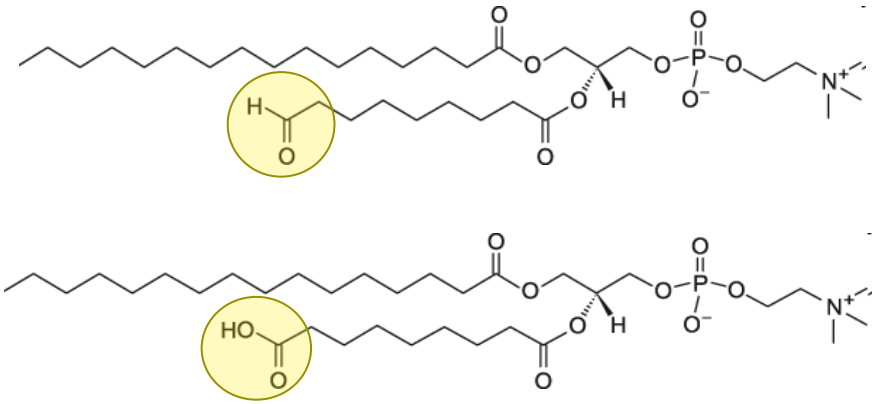
J Phys Chem A 2009 113 7235; J Phys Chem B 2010 114 9504 + submitted

B. “Truncated” oxidized phospolipids in lipid membranes

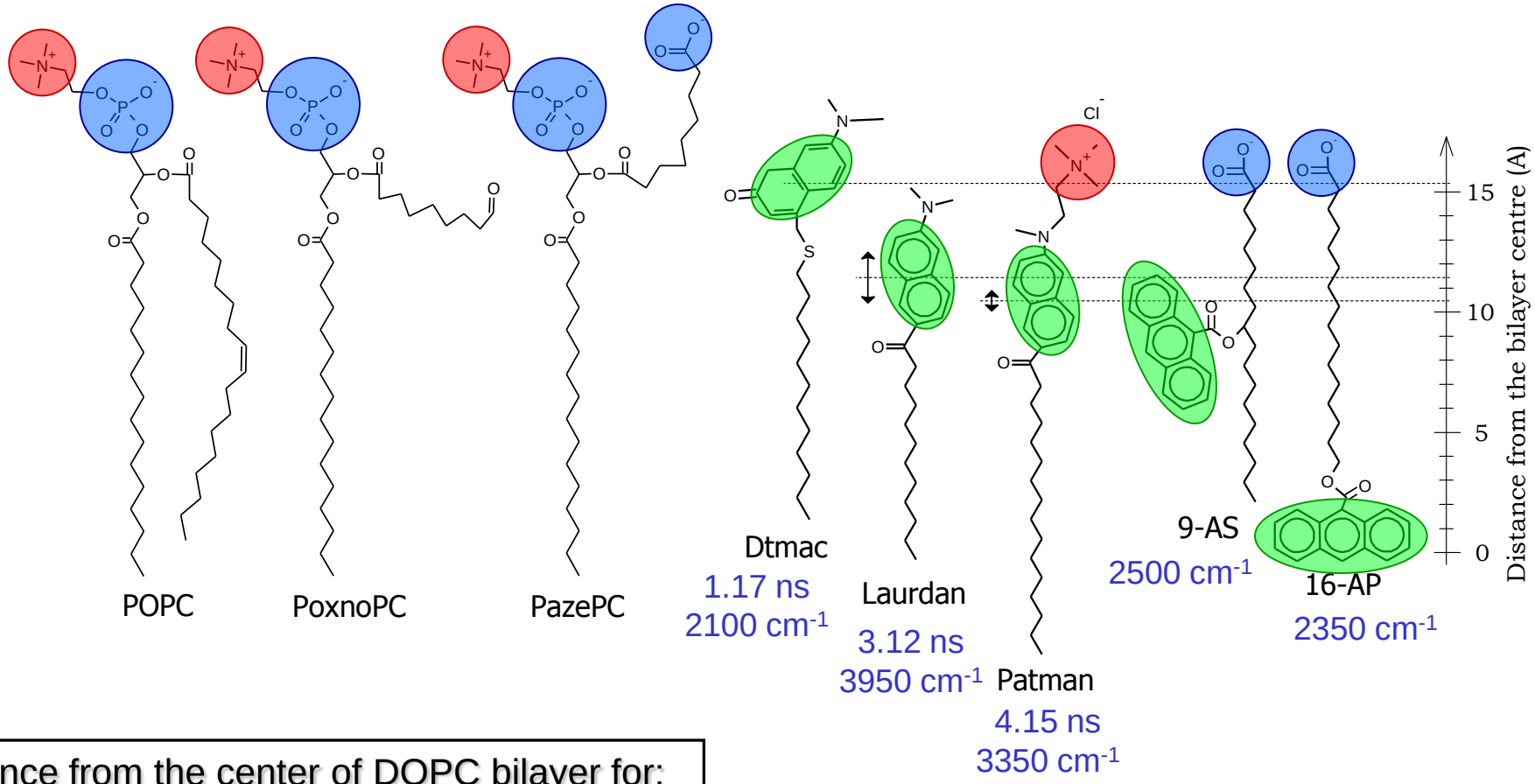


Oxidative Stress

Series of products, physiological relevance do have e.g.:



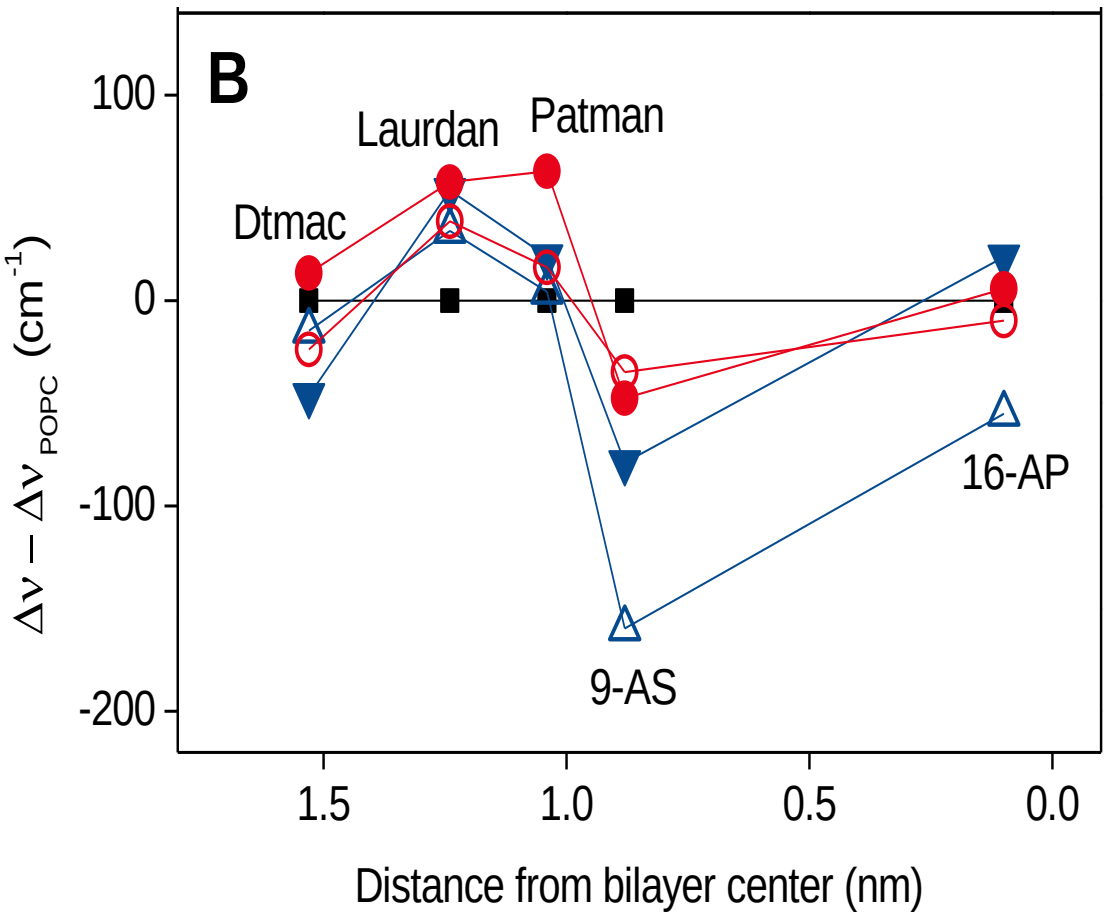
Do those truncated lipids (oxPL) change Hydration and mobility profiles?



Distance from the center of DOPC bilayer for:

- Patman – **10.4 A**
- Laurdan – **11.4 A**
- DTMAC – **15.3 A**

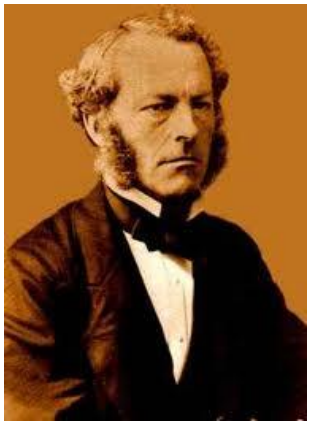
Relative changes in Δ ν (hydration) induced by incorporation of oxPL



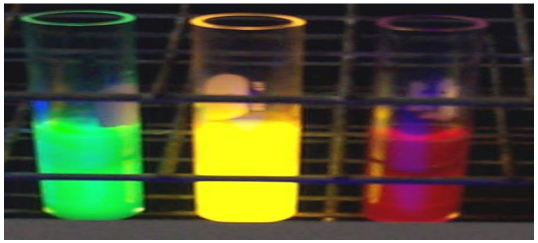
•Sinusoidal modification of hydration profile:

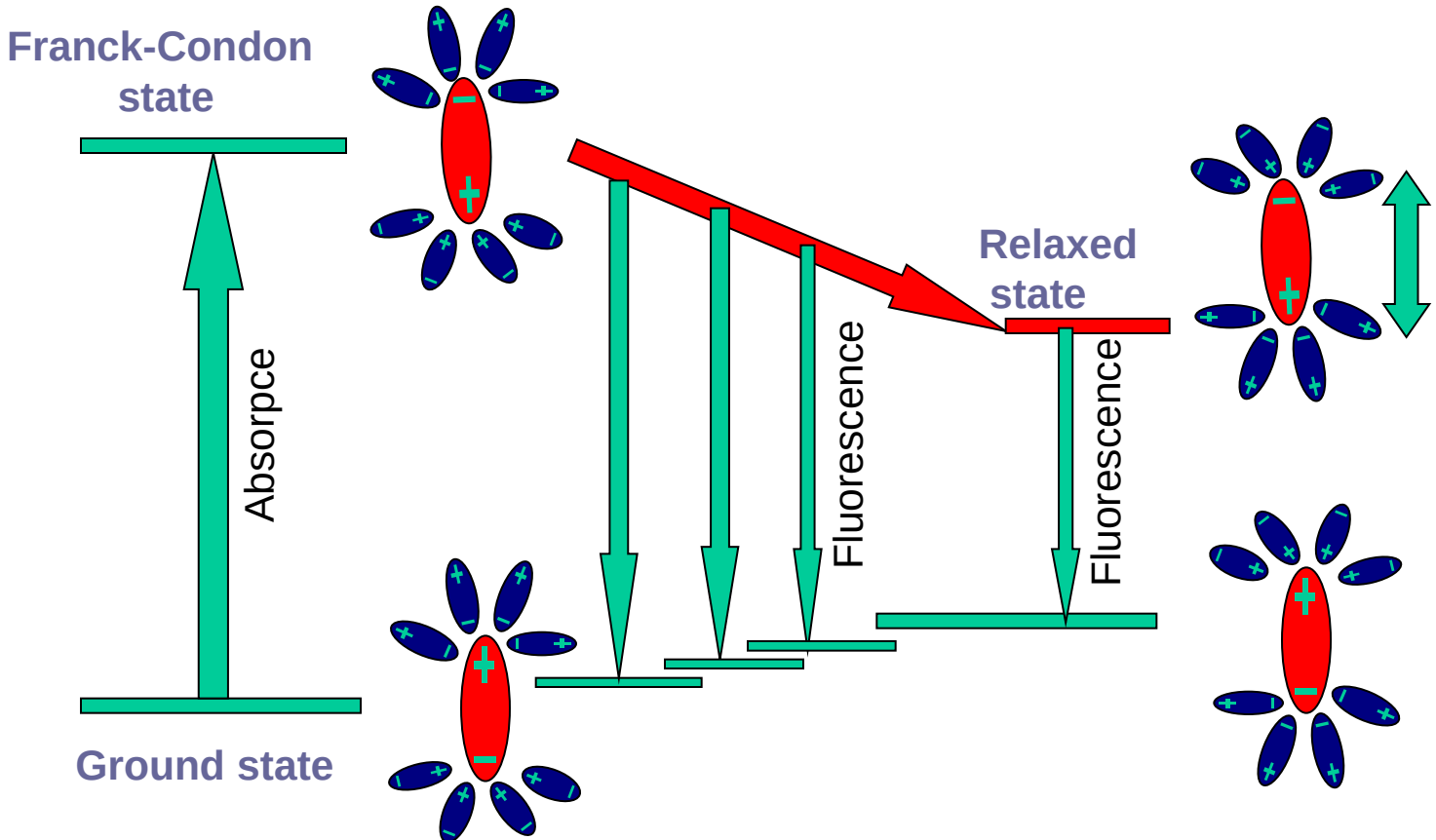
•Phosphate-groups become less hydrated
•Acyl-groups become more hydrated
•Backbone becomes less hydrated

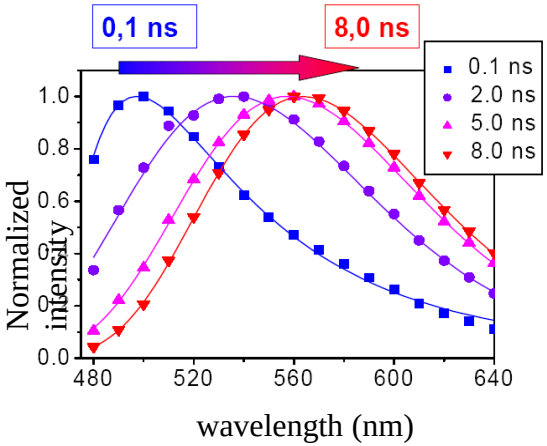
Beranova, Langmuir 2010 26, 6140, *Volinsky, Biophys J 2011 101, 1376*

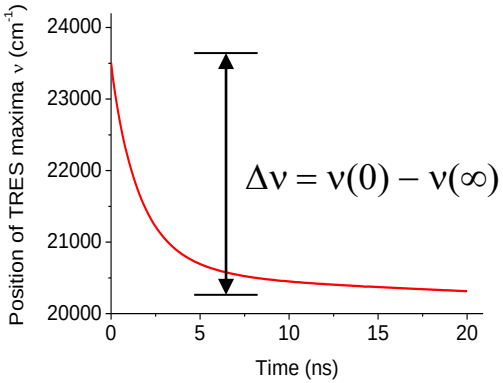


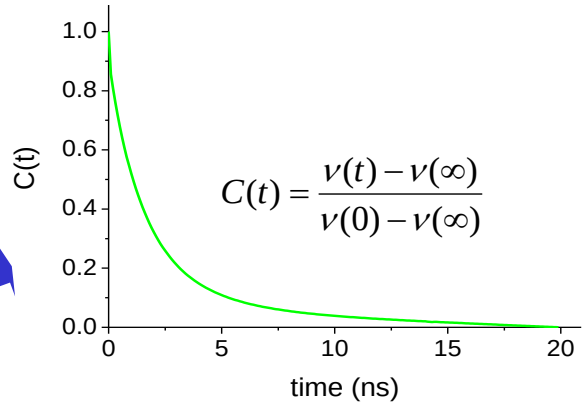

CANADA DRY
CANADA DRY



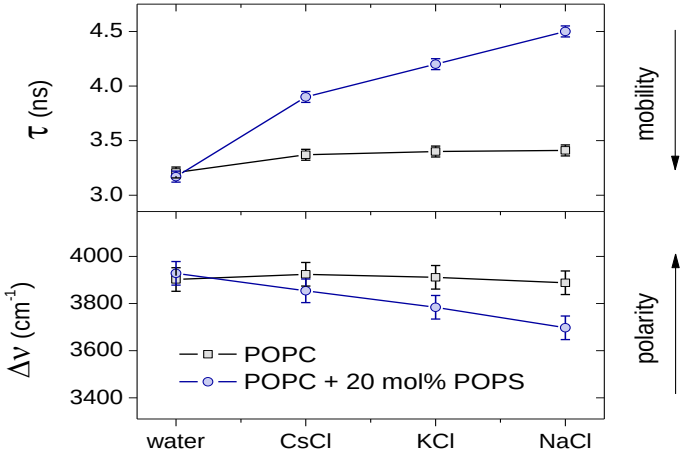

Franck-Condon state
Absorpce
Ground state
Fluorescence
Fluorescence
Relaxed state

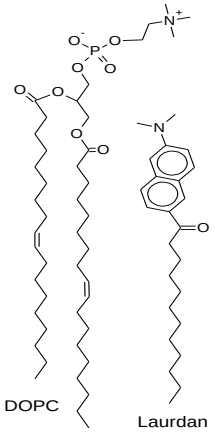

0,1 ns
8,0 ns
0.1 ns
2.0 ns
5.0 ns
8.0 ns
Normalized intensity
wavelength (nm)

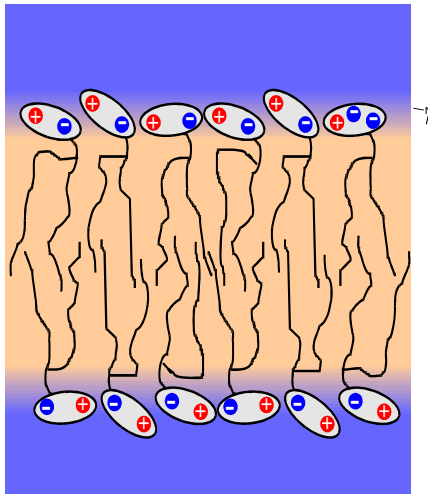

Position of TRES maxima ν (cm-1)
Δν = ν(0) − ν(∞)
Time (ns)

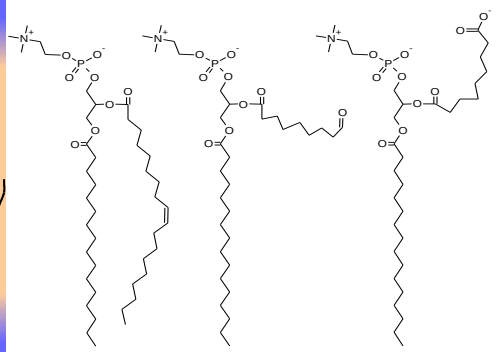

C(t)
$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)}$
time (ns)

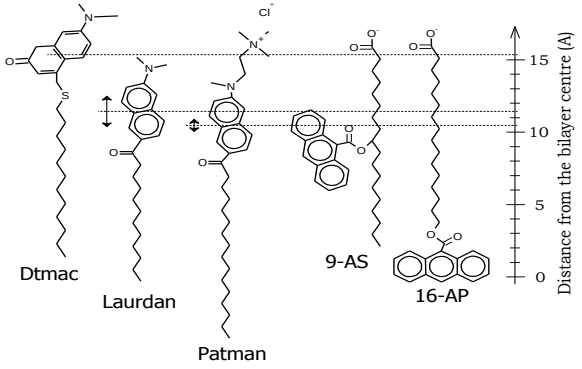
K+
versus
Na+


τ (ns)
Δν (cm-1)
mobility
polarity
POPC
POPC + 20 mol% POPS
water
CsCl
KCl
NaCl


DOPC
Laurdan




POPC
PoxnoPC
PazePC


Dtmac
Laurdan
Patman
9-AS
16-AP
Distance from the bilayer centre (A)

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