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

Martin Hof
What is fluorescence?

1852
Sir George Gabriel Stokes

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

Quinine in water
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?

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Dissolved in
a) Cyclohexane (unpolar)
b) Diethylether (medium polar)
c) Ethylacetat (polar)
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”
Increase of solvent polarity leads to red-shift.

Increase of viscosity leads to blue-shift.

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 an 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:

Franck-Condon state

Absorbed

Ground state

Relaxed state

Less polar solvent

More polar solvent

Stokes shift

Absorption

Fluorescence
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 environment

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

kinetics ($\tau_{SR}$): polarity

Red-shift $\Delta \nu$: polarity

Franck-Condon state

Absorption

Ground state

Relaxed state

Absorption

Fluorescence

Fluorescence

Fluorescence
SR is monitored by “time-resolved fluorescence emission spectra”

\[ \Delta \nu = \nu(t=0) - \nu(t=\infty) \]
Time-dependent Stokes shift $\Delta \nu$

- **Normalized intensity**
  - Wavelength (nm)
  - Time (ns)
  - Position of TRES maxima $
u$ (cm$^{-1}$)

- **Position of TRES maxima** $\nu = \nu(0) - \nu(\infty)$

- **Graphs**
  - Different time points: 0.1 ns, 2.0 ns, 5.0 ns, 8.0 ns
  - Graph showing normalized intensity vs. wavelength (nm)
  - Graph showing position of TRES maxima vs. time (ns)
Quantitative monitoring the solvent relaxation process: Time-resolved fluorescence spectroscopy

Red-shift $\Delta \nu$: polarity
Time-dependent Stokes shift $\Delta \nu$ gives directly information about the micro-polarity

- $\Delta \nu$ is directly proportional to the polarity function $F$

- example:
  - $C_1OH$: $F = 0.71$; $\Delta \nu = 2370 \text{ cm}^{-1}$
  - $C_5OH$: $F = 0.57$; $\Delta \nu = 1830 \text{ cm}^{-1}$

$$\Delta \nu = (\varepsilon_s - 1) / (\varepsilon_s + 2) - (n^2 - 1) / (n^2 + 2)$$

Dependence of SR kinetics on the solvent

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

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

$$\Delta \nu = \nu(0) - \nu(\infty)$$

Normalized intensity vs. wavelength (nm)

Normalized intensity vs. time (ns)

Position of TRES maxima vs. time (ns)
Dependence of SR kinetics on the solvent

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

Summarised from contributions by M. Maroncelli (1993-1997)
Kinetics of the SR is related to the viscosity of the microenvironment.

\[ \frac{1}{T} \sim 10^{-3} \text{K}^{-1} \]

- \( \tau_{\text{Phosp}} = 0.25 \text{ s} \)
- \( \tau_{\text{CT}} = 4 \mu \text{s} \)
- \( \tau_{\text{Fluor}} = 20 \text{ ns} \)

- \( T_g = 92 \text{ K} \)

Dyes in THF 90-170 K

Probed by:
- \( T_1 \rightarrow T_0 \) Phosphorescence
- Charge-Transfer Emission
- \( S_1 \rightarrow S_0 \) Fluorescence

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?
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 phospholipids forming a phospholipid bilayers

- Unilamellar vesicles serve as a model system
How does hydration and mobility change from the water phase towards the “oil” phase?

Vesicle

“bulk” water: sub-ps
External interface
Headgroup region
hydrophobic backbone (“oil”)

Vesicle
Fluorescent dyes are defined localized within the bilayer. The fluorescence signal can be correlated with a z-position within bilayer backbone, headgroup, and external interface.
Information obtained from TRES

\[ \Delta \nu = \nu(0) - \nu(\infty) \]

\[ \tau = \int_{0}^{\infty} C(t) \, dt \]

\[ C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)} \]
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?
a) Weak cation packing effects in neutral bilayers; no ion specificity

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

\[ \Delta \nu \text{ (cm}^{-1}) \]

\[ \tau \text{ (ns)} \]

\[ \text{POPC, POPC + 20 mol% POPS} \]

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 $\text{Na}^+$ and $\text{K}^+$.
B. “Truncated” oxidized phospholipids 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 Å
- Laurdan – 11.4 Å
- DTMAC – 15.3 Å
Relative changes in $\Delta \nu$ (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

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Franck-Condon state

Absorption

Ground state

Relaxed state

Fluorescence

Fluorescence
$\Delta \nu = \nu(0) - \nu(\infty)$

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

$\tau$ (ns)

$\Delta \nu$ (cm$^{-1}$)

K$^+$ versus Na$^+$

POPC

POPC + 20 mol% POPS

DOPC

Laurdan

16-AP

9-AS

PoxnoPC

Patman

PazePC

Dtmac

Distance from the bilayer centre (Å)
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