# 4P099 Site-Specific Ultrafast Dynamics in Single Lipid Vesicle: Picosecond Fluorescence Microscopy

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## Introduction:

Biomembranes are complex systems that contain lipid molecules of various chain-lengths and phase-transition temperatures. Mixed lipids form different microdomains (viz. disordered liquid-phase, ordered liquid-phase, gel-phase etc.) inside biomembranes and makes the microenvironment heterogeneous.[1,2] Thus, characterizing these domains in terms of their size, shape, biochemical properties, dynamic-evolution etc. are important to understand the structure-function relationship of these systems. Several intensity-based fluorescence imaging revealed the coexistence of lipid microdomains inside a single lipid vesicle.[1] However, recent time-resolved studies have shown that lifetime imaging is advantageous over the intensity imaging and is capable of unfolding the site-specific dynamic information inside single lipid vesicle.[2]

The study of picosecond solvation dynamics (dynamic Stokes shifts) and fluorescence resonance energy transfer (FRET) in complex systems are important to understand the role of hydration dynamics and molecular interactions in biochemical functions of various biological systems. Although solvation dynamics and FRET studies are common in bulk solutions,[3] relevant studies for the heterogeneous dynamics inside complex biological system like biomembranes are limited.

In the present study, we employed picosecond fluorescence imaging technique to probe the site-specific picosecond solvation dynamics and FRET inside a single mixed-lipid vesicle. At room temperature, coexistence of lipid-phases inside a single multi-lamellar vesicle (MLV) enabled us to monitor the lipid-phase dependent solvation dynamics. Another purpose of this study is to introduce a popular and well-studied solvatochromic fluorophore, coumarin-153 (C-153), as a probe in fluorescence imaging studies for the first time. This fluorophore is advantageous over commonly used lipid stains because it can be excited with one-photon in the UV-region without noticeable photo-degradation. The site-specific FRET study was performed using C-153 as donor and rhodamine 6G (R6G) as accepter.

## Experimental:

Mixed MLVs were prepared following standard procedure by mixing 1:1 mole ratio of DPPC and DOPC lipids.[4] Lipid solutions in chloroform with dyes (C-153 and/or R6G) was dried under vacuum to form lipid films and was subsequently hydrated with distilled water by mild vortexing followed by overnight aging. The vesicle solution was poured onto a glass-bottom dish and allowed to equilibrate before measurements. Steady-state fluorescence spectra were recorded using a spectrofluorometer (Horiba Jobin Yvon, Fluorolog-3). Time-resolved fluorescence measurements were carried out by the time-correlated single photon counting (TCSPC) method using PCI-based TCSPC board (Becker & Hickl, SPC-140). Dye molecules were photoexcited by the second harmonic (420 nm) of a Ti:sapphire oscillator (Coherent, Mira-900F) using an inverted confocal microscope (Nikon, TE2000-U) equipped with 100× objective (N.A. 1.3). Fluorescence photons were detected with a photon-counting avalanche photodiode (id Quantique, id-100-20). Wavelength selection was done by using band-pass filters. Time resolution of the setup was ~70 ps. The images were 128×128 pixels with 256 time channels. Times per channel were 32.6 ps or 97 ps. Images were recorded using scanning XY-stage (Sigma Koki, BIOS-412T).

## Results and Discussions:

The fluorescence lifetime characteristics of a solvatochromic probe, which shows slow solvation dynamics in a medium, are the appearance of fast decay at blue-edge and rise followed by slow decay at the red-edge of its fluorescence spectrum. Figure 1a shows the time-integrated intensity image and fig. 1b shows the corresponding first-moment lifetime image of single MLV recorded at blue-edge (~450nm) of C-153 spectrum. To check the lifetime variation clearly, we plotted single-point decays at two regions-of-interest (R1, R2) in fig. 1c, along with the first-moment lifetime distribution. It is nicely seen from lifetime distribution that there are two discrete lipid-phases. Fitting the raw decays with multi-exponential, we see average lifetimes vary from 550 ps to 825 ps (fig. 1c). To confirm the coexistence of lipid-phases with different solvation times, lifetime image was recorded also at ~650nm (red-edge) with an idea that different lipid-phases would show different rise-times. At two regions-of-interest in the first-moment lifetime image, single-point decays (fig. 1d) show two distinct lipid-phases with different rise-times of 875 ps and 1100 ps. Thus, we conclude that different lipid-phases show solvation dynamics with time constants of ~900 ps and ~1100 ps. We assign the slow solvation time of ~1100 ps for gel-phase and ~900 ps for liquid-phase, as it is found in earlier studies that gel-phase is more rigid and structured than liquid-phase.[1-3] Such differences in rigidity of the local environments are expected to influence the observed solvation dynamics inside lipid bi-layers, mainly through the motion of buried water molecules. Thus, we see slower solvation dynamics inside gel-phase than in liquid-phase.

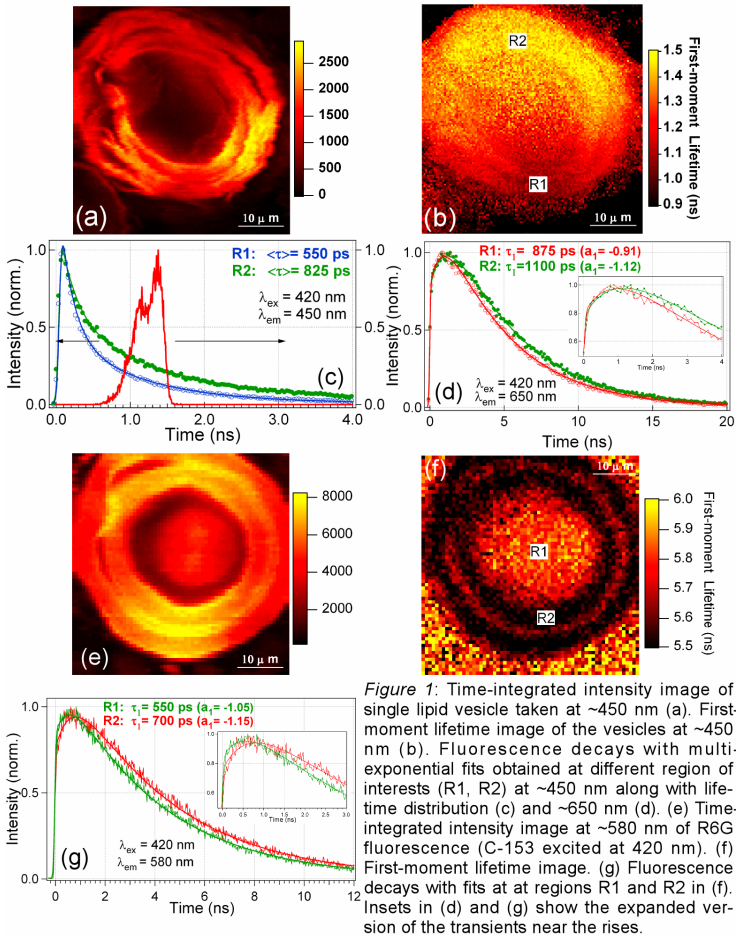


*Figure 1*: Time-integrated intensity image of single lipid vesicle taken at ~450 nm (a). First-moment lifetime image of the vesicles at ~450 nm (b). Fluorescence decays with multi-exponential fits obtained at different region of interests (R1, R2) at ~450 nm along with life-time distribution (c) and ~650 nm (d). (e) Time-integrated intensity image at ~580 nm of R6G fluorescence (C-153 excited at 420 nm). (f) First-moment lifetime image. (g) Fluorescence decays with fits at at regions R1 and R2 in (f). Insets in (d) and (g) show the expanded version of the transients near the rises.

In a separate experiment, we have also conducted the site-specific picosecond FRET study using C-153 and R6G to unravel the dynamics of heterogeneous intermolecular interactions inside a single MLV.[3b] Figure 1e shows the time-integrated image taken at ~580 nm (near the accepter fluorescence peak) and fig. 1f constructs the corresponding first-moment lifetime image. The fluorescence transients at two regions-of-interests (R1 and R2 in fig. 1f) show distinct accepter rise-times (550 ps and 700 ps) inside the single MLV (fig. 1g). Thus, heterogeneous FRET occurs inside a single MLV. In conclusion, our present lifetime imaging study shows that site-specific picosecond solvation dynamics and FRET are efficient to unravel the dynamic environmental heterogeneity inside a mixed lipid vesicle.

## References:


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(4) http://www.avantilipids.com/PreparationOfLiposomes.html.