# Probe Dependent Solvation Dynamics Study in a Microscopically Immiscible Dimethyl Sulfoxide-Glycerol Binary Solvent

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## I.    Experimental Section:

The steady state absorption spectra and fluorescence spectra were recorded in Perkin-Elmer Lambda-750 spectrophotometer and Perkin-Elmer LS 55 spectrofluorimeter, respectively. Picosecond lifetimes were measured in a time-correlated single-photon counting (TCSPC) spectrometer (Edinburgh, OB920). We used 375 nm, 405 nm and 445 nm laser diodes to excite nearly at the absorption peak of C480, C153 and C343. Fluorescence was collected at right angle by using MCP photomultiplier (Hamamatsu R3809U-50). Excitation lamp profile was obtained from a scatterer sample (dilute Ludox solution) and used in the deconvolution fitting function, which was found to be ~70 ps (IRF) for all laser diodes. Data were fitted by using F900 decay analysis software. All experiments were done at room temperature (23 $^{0}$C). Viscosities of binary solvents were measured by using Ubbelohde viscometer. Viscosity of DMSO/WT ($X_{DMSO}$=0.33) mixture and DMSO/GLY ($X_{DMSO}$=0.89) mixture were found to be 3.7 cP at room temperature.

Rotational anisotropy decay was measured by changing the emission polarizer at regular intervals to parallel and perpendicular direction with respect to the excitation polarizer. Anisotropy correlation function r(t) was calculated from the decay of parallel intensity ($I_{parallel}$) and perpendicular intensity ($I_{perpendicular}$) using the following equation,

$$r(t) = \frac{I_{parallel}(t) - G \times I_{perpendicular}(t)}{I_{parallel}(t) + G \times 2I_{perpendicular}(t)} \qquad (S1)$$

*G* value was obtained from the calibration of the setup using a dye whose rotational relaxation is very fast (i.e., C480 in methanol). The details of this technique has been described elsewhere.[1]

Fluorescence correlation spectroscopy (FCS) measurements were carried by using a confocal setup (Zeiss LSM780) with an inverted optical microscope. We used oil immersion objective (63× 1.4 NA) and 405 nm diode laser (for C480), 458 nm argon laser (for C153 and C343) as excitation sources. Very dilute coumarin solution (~50 μL) was placed on a coverslip on top of the objective. The fluorescence light from the sample was passed through a dichroic mirror and appropriate long pass filter to remove the excitation light before it was focused into a 70 μm pinhole. Finally, after passing through the pinhole, fluorescence signals were collected by single photon sensitive photo diodes. Detected photons were time-correlated within a single photon counting card which generates the auto-correlation function, G(τ) by using following algorithm.[2-3]

$$G(\tau) = \frac{<\delta F(0)\delta F(\tau)>}{<F>^2} \qquad (S2)$$

Where $<F>$ is the average intensity and $\delta F(\tau)$ is the fluctuation of intensity over the average intensity at delay $\tau$. If a particle diffuses through a 3D elliptical excitation volume with dimensions $\omega_{xy}$ (transverse) and $\omega_z$ (longitudinal), the correlation function can be expressed as,[2-3]

$$G_D(\tau) = \frac{1-T+T\exp(-\tau/\tau_{tr})}{N(1-T)}\left[1+\frac{\tau}{\tau_D}\right]^{-1}\left[1+\frac{\tau}{\omega^2\tau_D}\right]^{-1/2} \qquad (S3)$$

$T$ is the fraction of molecule in triplet state and $\tau_{tr}$ is the lifetime in triplet state. $N$ is the number of molecules in the confocal volume. $\omega(=\omega_z/\omega_{xy})$ is the structure parameter of the excitation volume. Structure parameter was found to be ~5, which was obtained from the calibration of excitation volume using Rhodamine 6G dye in water with known diffusion coefficient ($D_t$=426 $\mu m^2$ $s^{-1}$).[4] The estimated transverse radius ($\omega_{xy}$) ~ 292 nm was used to determine translational coefficient of coumarine dyes in DMSO/WT solvent mixture from the following relation.[2-3]

$$D_t = \frac{w_{xy}^2}{4\tau_D} \qquad (S4)$$

From the calculated value of $D_t$, one can obtain the hydrodynamics radius ($r_h$) of the diffusing particle from equation no S4 (Stokes-Einstein equation),[2-3]

$$r_h = \frac{k_B T}{6\pi\eta_0 D_t} \qquad (S5)$$

**Figure Captions**

**Figure S1**. Time-resolved emission spectra (TRES) of (A) C153 ($\lambda_{ex}$=405 nm), (B) C343 ($\lambda_{ex}$=445 nm) in DMSO/Water mixture ($\eta$~3.7 cP, $X_{DMSO}$=0.33) at different times (indicated within the figure).

**Figure S2.** Time-resolved emission spectra (TRES) of (A) C153 ($\lambda_{ex}$=405 nm), (B) C343 ($\lambda_{ex}$=445 nm) in DMSO/Glycerol ($\eta$~3.7 cP, $X_{DMSO}$=0.89) mixture at different times (indicated within the figure).

**Figure S3.** Decay of solvent correlation function C(t) of C153 ($\lambda_{ex}$=405 nm), C480 ($\lambda_{ex}$=375 nm) and C343 ($\lambda_{ex}$=445 nm) in (A) neat Glycerol ($\eta$~1412 cP) and (B) neat Isopropanol ($\eta$~2 cP). Solid lines represent the best fits.

**Figure S4.** Decay of rotational correlation function r(t) of C153 ($\lambda_{ex}$=405 nm), C480 ($\lambda_{ex}$=375 nm) and C343 ($\lambda_{ex}$=445 nm) in neat DMSO. Emissions were collected at 20 nm blue end of their respective steady state emission peaks.

**Figure S5**. FCS traces of C153 (A), C480 (B) and C343 (C) in DMSO/Water mixture ($\eta$~3.7 cP, $X_{DMSO}$=0.33). Solid line represents the best fit.
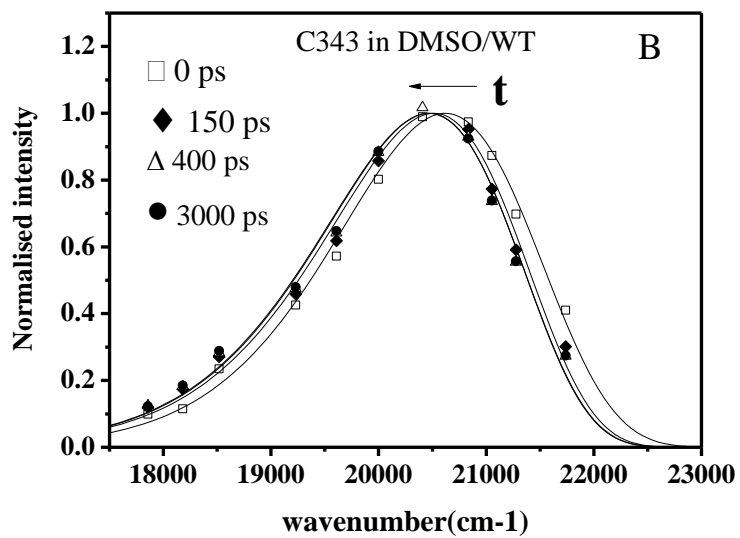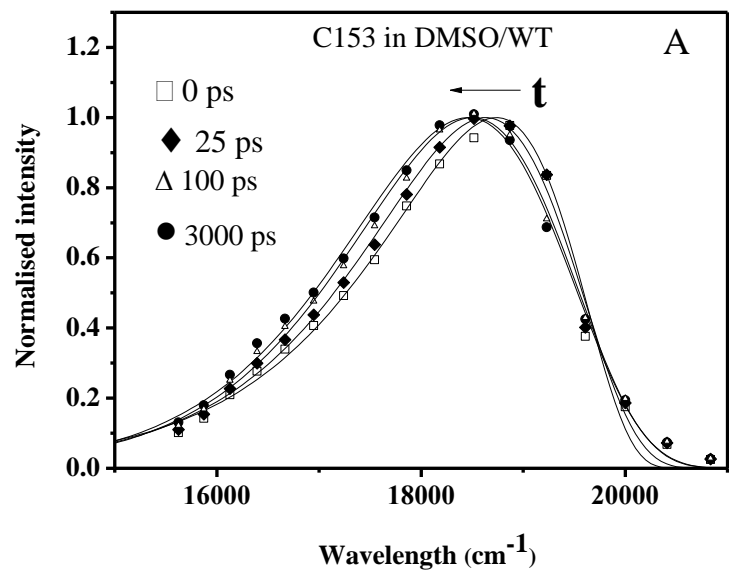
C153 in DMSO/WT

A

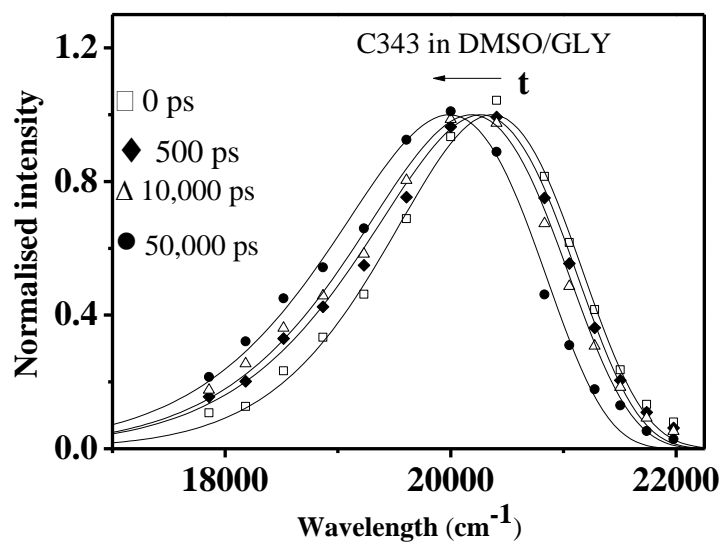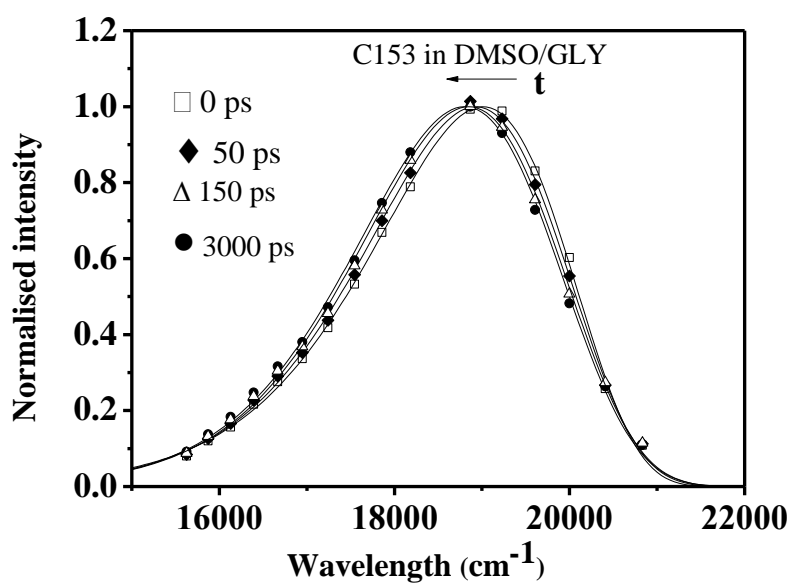□ 0 ps
◆ 25 ps
△ 100 ps
● 3000 ps

Normalised intensity

Wavelength (cm$^{-1}$)



C343 in DMSO/WT

B

□ 0 ps
◆ 150 ps
△ 400 ps
● 3000 ps

Normalised intensity

wavenumber(cm-1)
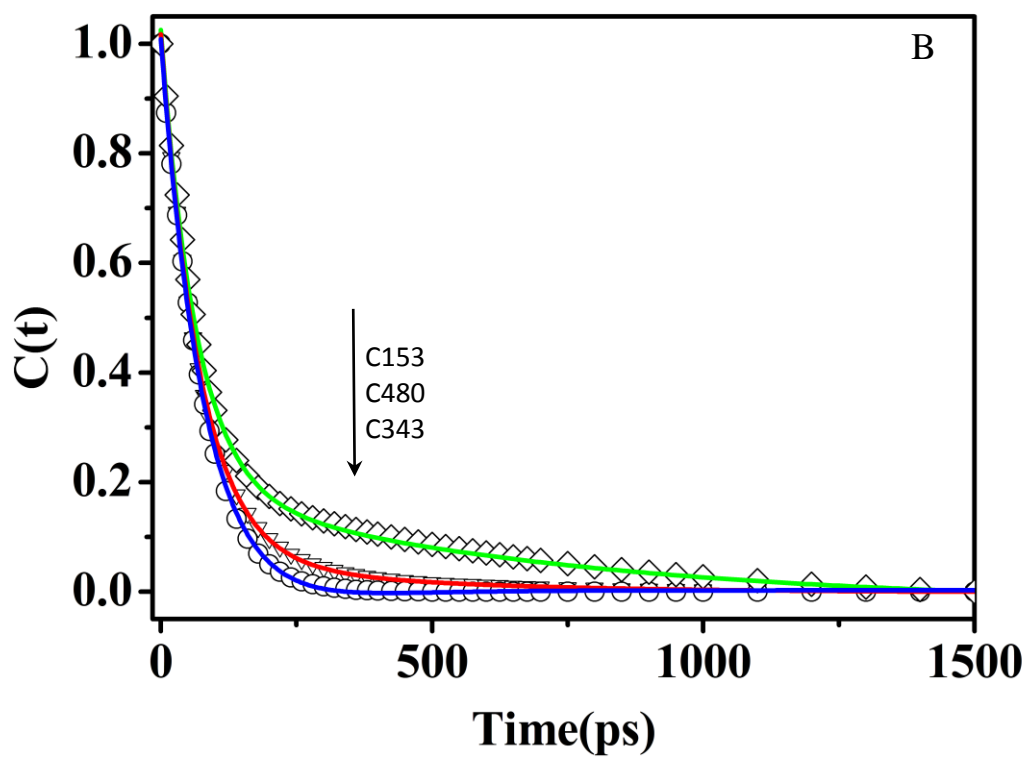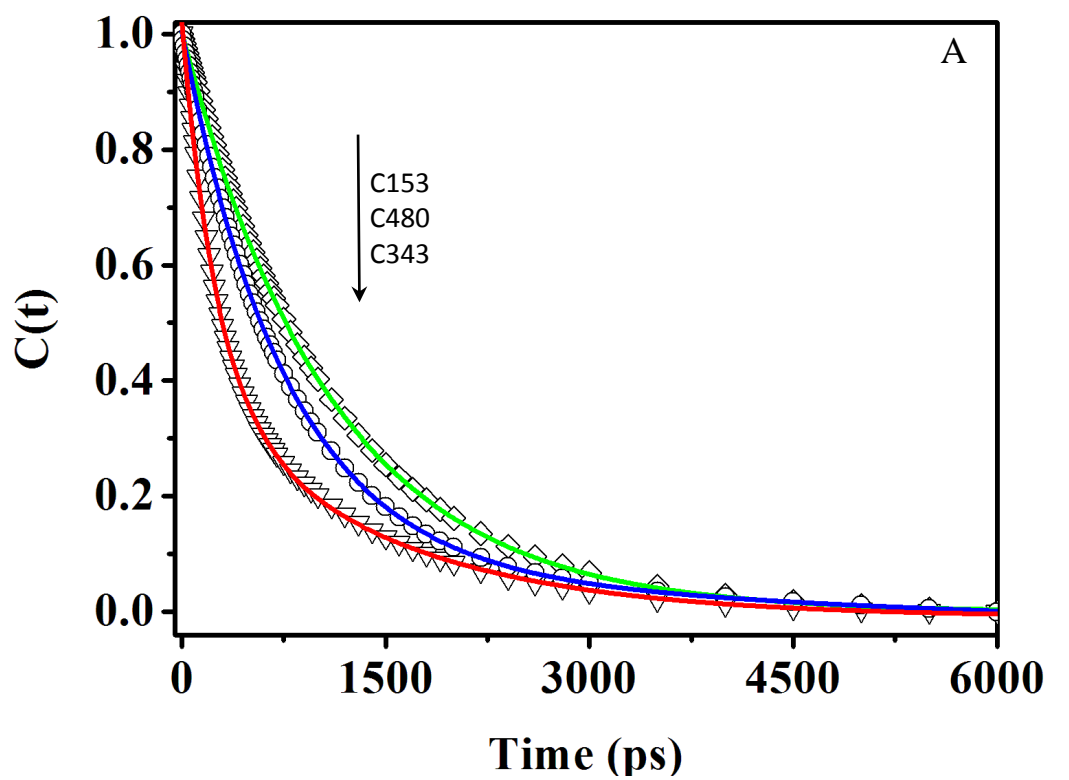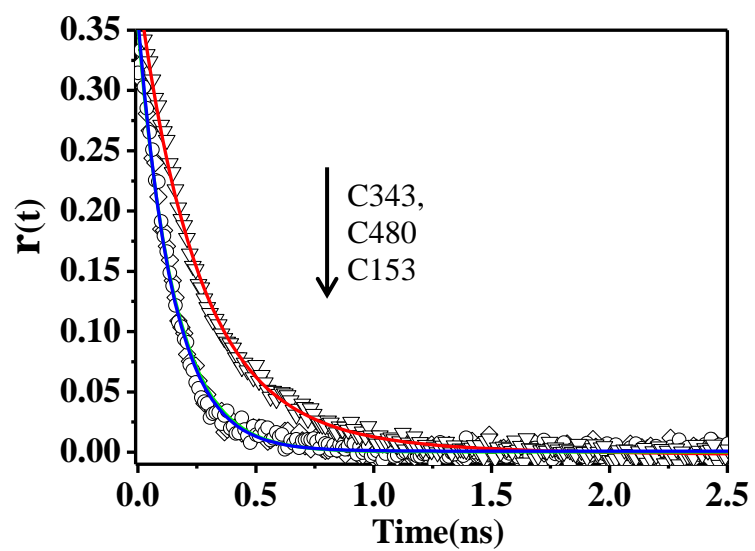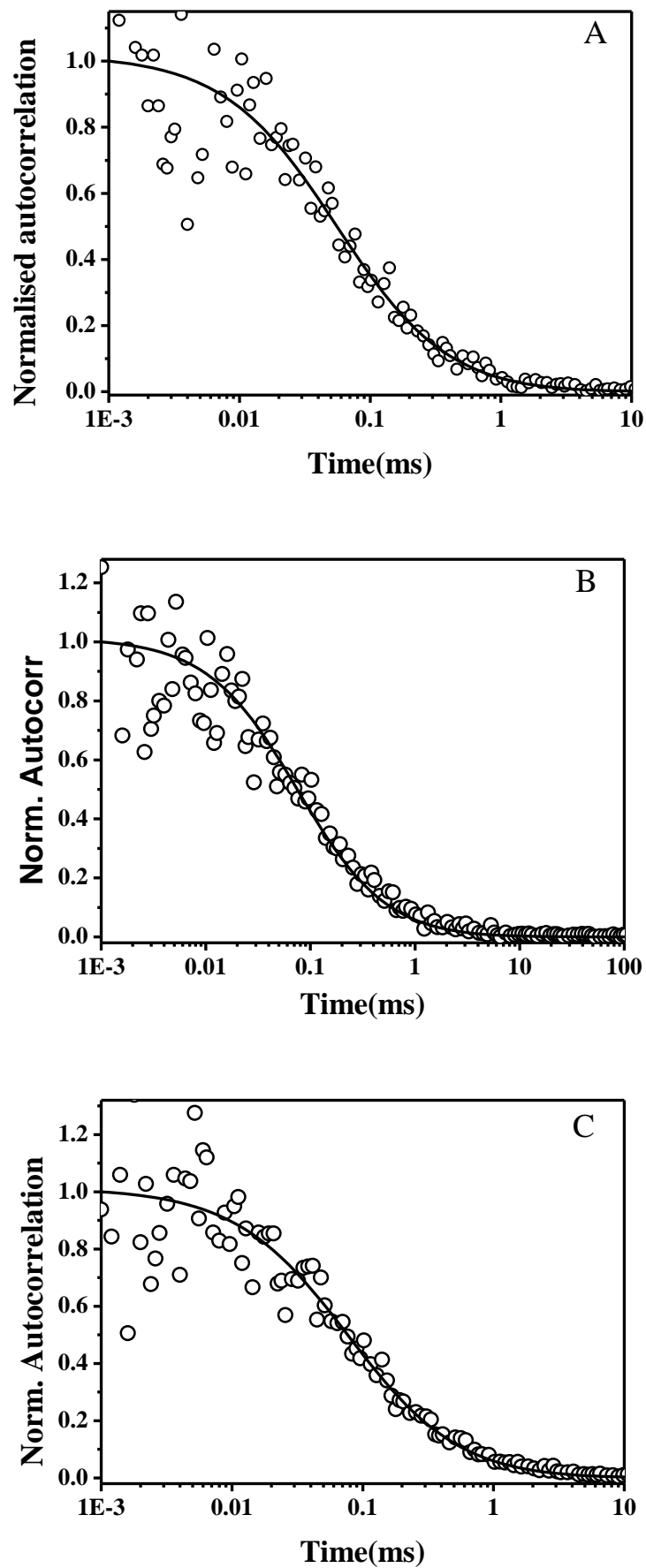
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4. Petrasek, Z.; Schwille, P. Precise Measurement of Diffusion Coefficients using Scanning Fluorescence Correlation Spectroscopy. *Biophys. J.* **2008**, *94*, 1437-1448.