Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.


# Supporting information

## Azobenzene Photoisomerization Probes Cell Membrane Viscosity

*Arianna Magni,[†,‡] Gaia Bondelli[†,‡], Giuseppe M. Paternò,[‡] Samim Sardar,[‡] Valentina Sesti,[§,‡] Cosimo D'Andrea,[†,‡] Chiara Bertarelli,[§,‡] Guglielmo Lanzani*[†,‡]*

[†] Dipartimento di Fisica, Politecnico di Milano, Piazza L. da Vinci 32, 20133 Milano, Italy

[‡] Center for Nano Science and Technology, Istituto Italiano di Tecnologia, Via Pascoli 70, 20133, Milano, Italy

[§] Dipartimento di Chimica, Materiali e Ingegneria Chimica 'Giulio Natta', Politecnico di Milano, Piazza L. da Vinci 32, 20133 Milano, Italy

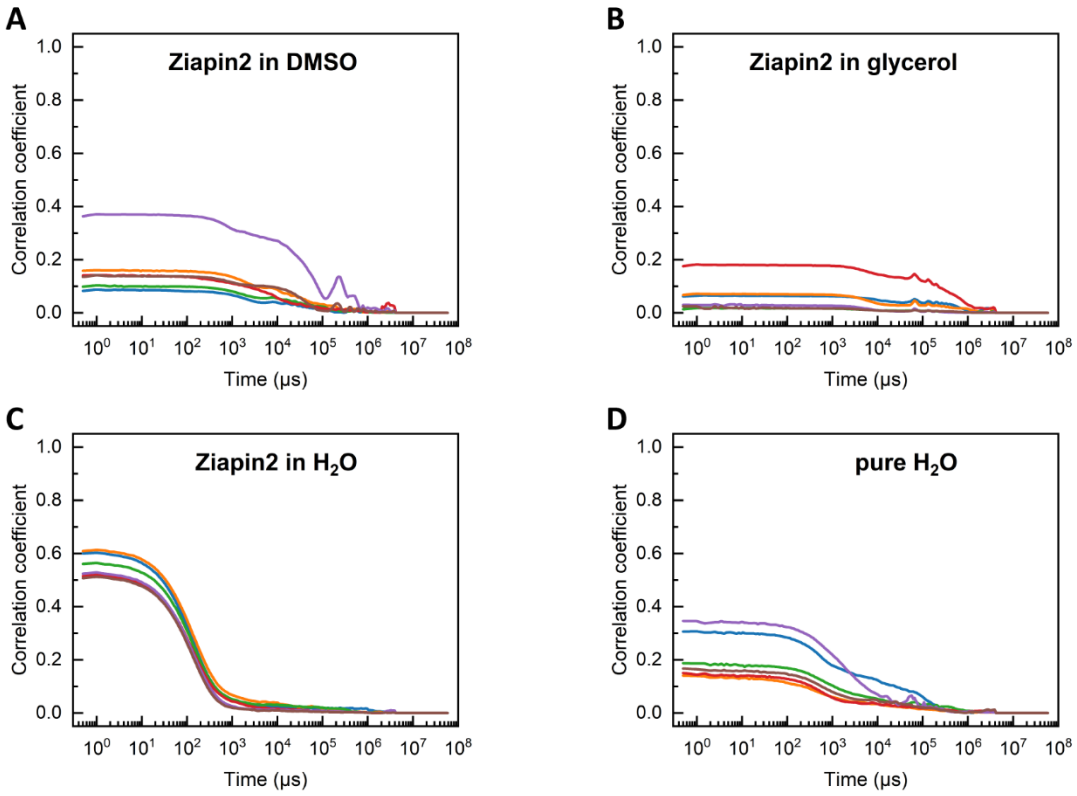


**Figure S1.** Dynamic light scattering (DLS) measurements of ZIAPIN2 in different solvents. The plots show the correlograms obtained for samples of ZIAPIN2 dissolved in (A) DMSO, (B) glycerol, (C) water and (D) pure water. The low correlation coefficient and the noisy curves in panels A, B and D indicate that scattering is low in those samples. Moreover, the few scattering events occurring in these samples are not related to particles homogeneous in size. On the other hand, when ZIAPIN2 is dissolved in water (C) the correlation curves have a smoother shape indicating the presence of scattering particle of almost homogeneous size. Indeed, being ZIAPIN2 amphiphilic, the molecules tend to form micelles, whose size depends on the ZIAPIN2 chemical properties.

Measurements are obtained using a Malvern Instruments Zetasizer Nano ZS. Each sample has been scanned six times. Before each scan, the sample was stabilized for 2 mins at 20 °C.

**Table S1.** Mean size values and polydispersity index (PdI) for Ziapin2 in different solvents. Values are expressed as the means of six repetitions ± SD. The width of the fitted Gaussian size distribution is displayed as the PdI. The dispersity values, which reflect the size distribution, ranges from 0 (monodispersed) to 1 (polydispersed).

| Sample | Size (nm) | PdI |
|---|---|---|
| ZIAPIN2 10µM in DMSO | 478 ± 640 | 0.95 ± 0.13 |
| ZIAPIN2 10µM in $H_2O$ | 345 ± 102 | 0.32 ± 0.05 |
| ZIAPIN2 10µM in glycerol | 504 ± 783 | 0.78 ± 0.27 |
| $H_2O$ | 419 ± 205 | 0.71 ± 0.13 |

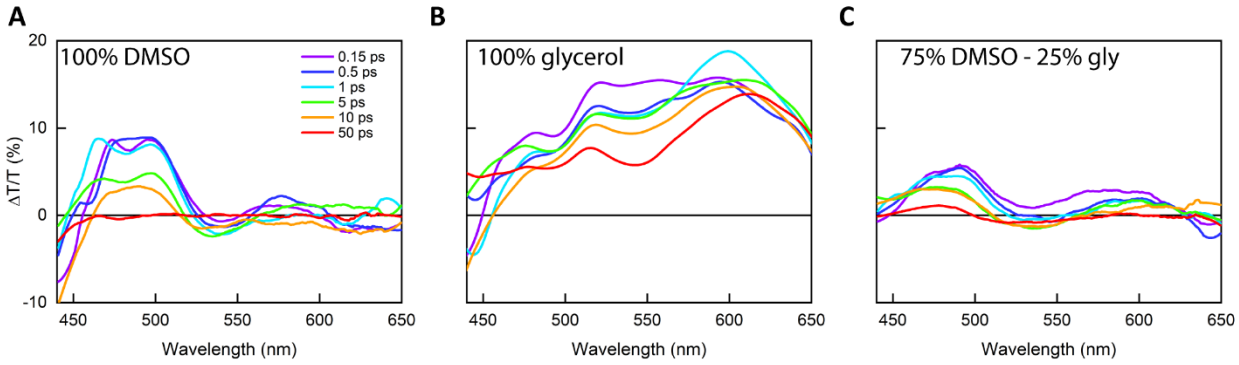


**Figure S2.** Differential transient transmittance spectra of ZIAPIN2 in (A) DMSO, (B) glycerol and (C) a mixture of the two (75% DMSO, 25% glycerol). Observing the spectra of panel (A) we can distinguish three different regions. Specifically, the spectral band between 460 nm and 500 nm shows a positive ΔT/T, due to the ground state bleaching (GSB) of the *trans* isomer of the molecule: after the excitation, part of the molecules is temporary stored in the excited state while others undergo isomerization. The spectral region between 520 nm and 560 nm exhibits a negative differential transmission as it corresponds to the *cis* form absorption band of ZIAPIN2. Finally, the 560–620 nm region has positive ΔT/T. This last spectral region corresponds to the PL peak of the fluorophore and hence positive values of differential transmission are attributed to stimulated emission (SE). The transient absorption spectra of ZIAPIN2 in glycerol (B) is larger than zero in the whole spectral range under investigation. In this case the signal has a different shape from the previous one, with the peak of the curves shifted around 600 nm, which represents the SE band peak of the molecule in a viscous media. The GSB signal is reduced with respect to the SE due to the absence of a long-living *cis* isomer and a favored radiative relaxation. In the case of intermediate viscosity (C), the SE band displays an increase in amplitude while the band corresponding to the *cis* isomer absorption decreases in amplitude, due to the reduced isomerization quantum yield with respect to (A).

These measurements were performed using a Ti:Sapphire laser with 2 mJ output energy, 1 kHz repetition rate, a pulse width of 100 fs and a central wavelength of 800 nm. Samples were pumped with 490 nm light generated using a visible optical parameter amplifier (OPA). Pump pulses were focused on a 200 µm spot (diameter), keeping a power of 100 µW. The white-light probe pulse was generated with a sapphire plate. The sample was contained in a quartz cuvette, so that the optical path length was 1 mm. The transmitted probe signal was collected by an optical multichannel amplifier (OMA).

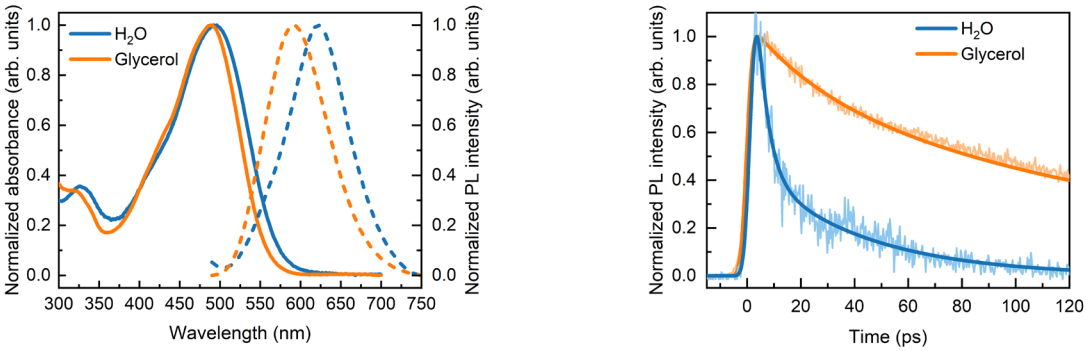


**Figure S3.** Absorption (solid lines) and emission (dashed lines) spectra of ZIAPIN2 dissolved in water and glycerol (left). PL kinetics in the spectral region between 550-590 nm for ZIAPIN2 in water and glycerol (right).

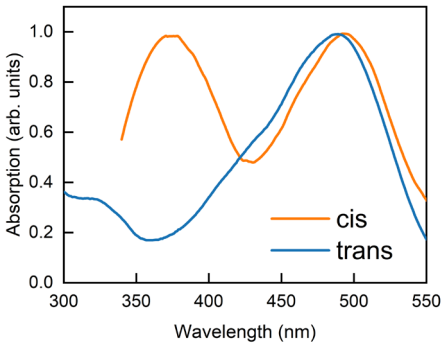


**Figure S4.** Absorption spectra of the two isomer of ZIAPIN2 in DMSO. The *trans* isomer spectrum is the same as figure 1, while the spectrum of the *cis* isomer comes from [1].

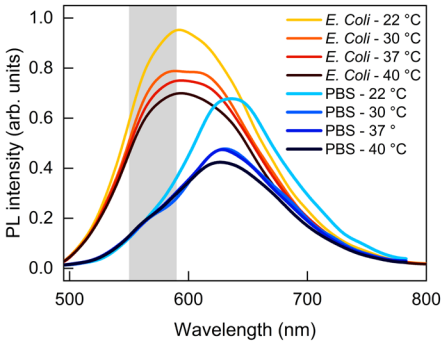


**Figure S5.** Fluorescence spectra of ZIAPIN2 in *E. Coli* suspensions and PBS at different temperatures, integrated over the first 130 ps of the PL decay. As ZIAPIN2 fluorescence is strongly influenced by the medium, there is a significant spectral shift. In a water-like environment the PL peak is moved to 620 nm due to the formation of aggregates.[118] The huge spectral shift indicates that the molecule has a strong affinity for the bacteria membrane. Moreover, the fluorescence quantum yield decreases with temperature, being isomerization favored. The gray rectangle highlights the spectral region (550-590 nm) where the PL dynamics have been considered for our viscometer. Here the PL contribution of ZIAPIN2 molecules in water is minor.

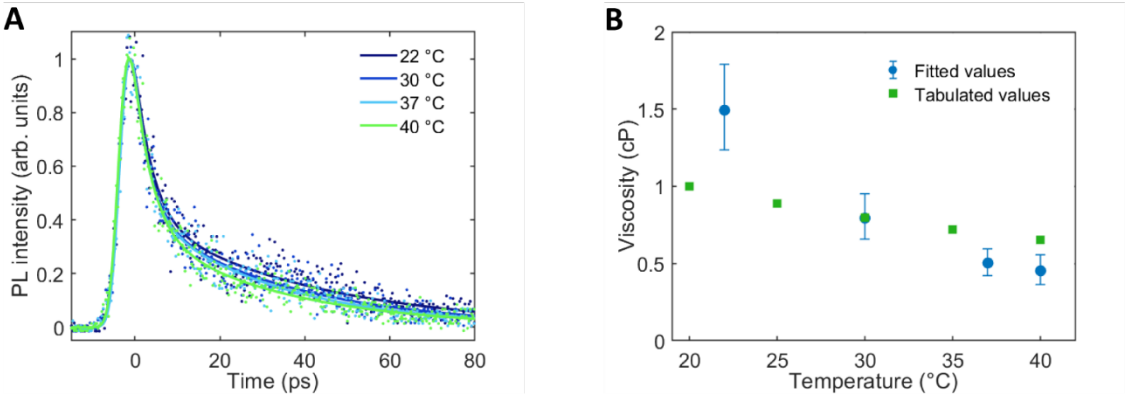


**Figure S6.** (A) PL dynamics of ZIAPIN2 in PBS buffer at different temperatures. The PL decays are in the region 550 - 590 nm. (B) Estimated viscosity of PBS buffer at different temperatures and tabulated values. The viscosity estimation is achieved using the ZIAPIN2 – viscometer. The error bars were obtained as reported in the main text.

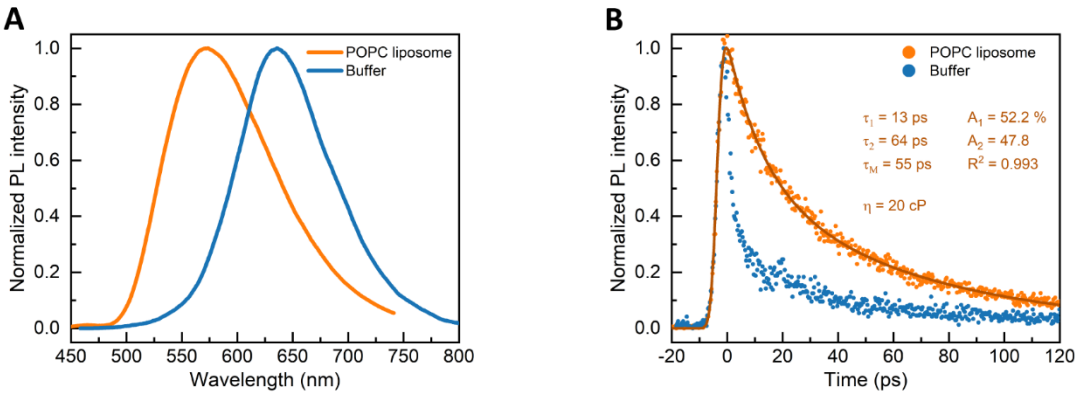


**Figure S7.** (A) PL spectra of ZIAPIN2 in POPC liposomes and in their aqueous buffer and (B) PL decays are in the region 550 - 590 nm. The shift in the spectra and the difference in the PL kinetics indicate the interaction of ZIAPIN2 with the artificial lipid vesicles. The fitted PL lifetime in the liposome suspension allow to estimate the viscosity of the lipid bilayer around 20 cP.

POPC was purchased from Avanti Polar Lipids and used without further purification. Liposomes were prepared by extrusion following the experimental protocol suggested by the supplier. Briefly, a stock solution of lipids in chloroform/methanol (1:1) was dried out under high vacuum via rotary evaporation (40 ° C, 150 rpm), and the resulting lipid film was kept overnight at −20 °C. The film was then hydrated with a buffer solution (10 mM Tris, 100 mM NaCl, pH 7.4) and subjected to freeze−thaw cycles by alternately placing the flask in liquid nitrogen and in a water bath (60 °C). The lipid dispersion was then extruded at least 17 times through a polycarbonate membrane with a pore size of 100 nm to yield small unilamellar vesicles. Finally, the obtained dispersion was diluted to a concentration of 25 mg/mL and kept at 4 °C. ZIAPIN2 intercalation was performed by simply adding the molecule solution (25 µM) to the liposome suspension.

**Table S2.** Fitting parameters of TRPL decay dynamics of ZIAPIN2 in DMSO and glycerol mixtures in the spectral range 550-590 nm. The decay kinetics have been fitted with a biexponential model, whose two components amplitudes and lifetimes are here reported. The mean fluorescence lifetime was calculated as in equation (4). The second column reports the viscosity calculated as in equation (5).

| | **Viscosity (cP)** | $\tau_1$ **(ps)** | $A_1$ **(%)** | $\tau_2$ **(ps)** | $A_2$ **(%)** | $\tau_M$ **(ps)** | $R^2$ |
|---|---|---|---|---|---|---|---|
| **Glycerol 0%** | 2 | 7 | 93.0 | 56 | 7.0 | 25 | 0.990 |
| **Glycerol 25%** | 7.8 | 10 | 84.9 | 74 | 15.1 | 46 | 0.985 |
| **Glycerol 50%** | 35.0 | 18 | 63.4 | 98 | 36.6 | 79 | 0.987 |
| **Glycerol 75%** | 180.4 | 30 | 51.3 | 164 | 48.7 | 142 | 0.984 |
| **Glycerol 100%** | 1078.2 | 28 | 26.6 | 176 | 73.4 | 168 | 0.993 |

**Table S3.** Fitting parameters of TRPL decay dynamics of ZIAPIN2 in DMSO at different temperatures in the spectral range 550-590 nm. The decay kinetics have been fitted with a biexponential model, whose two components amplitudes and lifetimes are here reported. The mean fluorescence lifetime was calculated as in equation (4). The second column reports viscosity values taken from ref. [3].

| **Temperature (°C)** | **Tabulated viscosity (cP)** | $\tau_1$ **(ps)** | $A_1$ **(%)** | $\tau_2$ **(ps)** | $A_2$ **(%)** | $\tau_M$ **(ps)** | $R^2$ |
|---|---|---|---|---|---|---|---|
| **30** | 1.81 | 7 | 92.5 | 51 | 7.5 | 24 | 0.989 |
| **40** | 1.51 | 6 | 93.1 | 49 | 6.9 | 23 | 0.989 |
| **50** | 1.29 | 6 | 91.0 | 48 | 9.0 | 24 | 0.989 |

**Table S4.** Fitting parameters of TRPL decay dynamics of ZIAPIN2 in glycerol at different temperatures in the spectral range 550-590 nm. The decay kinetics have been fitted with a biexponential model, whose two components amplitudes and lifetimes are here reported. The mean fluorescence lifetime was calculated as in equation (4). The last column reports viscosity values taken from ref. [4].

| **Temperature (°C)** | $\tau_1$ **(ps)** | $A_1$ **(%)** | $\tau_2$ **(ps)** | $A_2$ **(%)** | $\tau_M$ **(ps)** | $R^2$ | **Tabulated viscosity (cP)** |
|---|---|---|---|---|---|---|---|
| **24** | 25 | 11.8 | 192 | 88.2 | 189 | 0.994 | 987.6 |
| **30** | 23 | 19.4 | 163 | 80.6 | 158 | 0.995 | 612 |
| **40** | 25 | 35.1 | 138 | 64.9 | 128 | 0.997 | 284 |
| **50** | 22 | 46.7 | 111 | 53.3 | 98 | 0.998 | 142 |

**Table S5.** Fitting parameters of TRPL decay dynamics of ZIAPIN2 in four different solvents in the spectral range 550-590 nm. The decay kinetics have been fitted with a biexponential model, whose two components amplitudes and lifetimes are here reported. The mean fluorescence lifetime was calculated as in equation (4).

Polyethylene glycol 8000 (PEG8000) is dispersed in water.

| | $\tau_1$ **(ps)** | $A_1$ **(%)** | $\tau_2$ **(ps)** | $A_2$ **(%)** | $\tau_M$ **(ps)** | $R^2$ | **Tabulated viscosity**[2,5] **(cP)** |
|---|---|---|---|---|---|---|---|
| **Ethylene glycol** | 16 | 58.8 | 89 | 41.2 | 74 | 0.988 | 18.3 |
| **Acetonitrile** | 3 | 98.4 | 90 | 1.6 | 34 | 0.991 | 0.35 |
| **THF** | 5 | 93.2 | 50 | 6.8 | 24 | 0.990 | 0.52 |
| **1-butanol** | 7 | 83.7 | 47 | 16.3 | 30 | 0.993 | 2.96 |
| **PEG8000 – w0.5** | 14 | 34.7 | 97 | 65.3 | 91 | 0.997 | 325.5 |
| **PEG8000 – w0.33** | 24 | 27.4 | 122 | 72.6 | 115 | 0.996 | 50.9 |

**Table S6.** Fitting parameters of TRPL decay dynamics of ZIAPIN2 in *E .Coli* suspension at different temperatures in the spectral range 550-590 nm. The decay kinetics have been fitted with a biexponential model, whose two components amplitudes and lifetimes are here reported. The mean fluorescence lifetime is calculated as in equation (4). The viscosity has been estimated according to the viscosity calibration curve hereby proposed.

| **Temperature (°C)** | $\tau_1$ **(ps)** | $A_1$ **(%)** | $\tau_2$ **(ps)** | $A_2$ **(%)** | $\tau_M$ **(ps)** | $R^2$ | **Viscosity (cP)** |
|---|---|---|---|---|---|---|---|
| **22** | 7 | 78.2 | 70 | 21.8 | 53 | 0.986 | 14.1 |
| **30** | 7 | 79.1 | 68 | 20.9 | 51 | 0.986 | 11.9 |
| **37** | 7 | 79.6 | 64 | 20.4 | 47 | 0.985 | 9.6 |
| **40** | 6 | 78.3 | 60 | 21.7 | 45 | 0.983 | 8.2 |

# References


1. Paternò, G. M. *et al.* Membrane Environment Enables Ultrafast Isomerization of Amphiphilic Azobenzene. *Adv. Sci.* **7**, 1903241 (2020).
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