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

Supplementary Information for

Ultrafast Fluorescence Depolarisation in Green Fluorescence Protein Tandem Dimers as Hydrophobic Environment Sensitive Probes

Alejandro Sanchez-Pedreno Jimenez[1,2,4], Henry L. Puhl III[3], Steven S. Vogel[3*], and Youngchan Kim[1,2,4*]

[1]Leverhulme Quantum Biology Doctoral Training Centre, University of Surrey, Guildford GU2 7XH, UK.
[2]Department of Microbial Sciences, School of Biosciences, University of Surrey, Guilford GU2 7XH, UK.
[3]Laboratory of Biophotonics and Quantum Biology, NIAAA, NIH, Bethesda, USA.
[4]Advanced Technology Institute, University of Surrey, Guildford GU2 7XH, UK.

*Corresponding authors. Emails: stevevog@mail.nih.gov; youngchan.kim@surrey.ac.uk

This file includes:

Figures S1-5

Equation S1

Table S1

Instrument response function

To measure the intrument reponse funtion we used ludox as a scattering sample at the same wavelength of excitation Figure S1. Each curve was fit to a Gaussian fucntion to parametrise the instrument response function (IRF) of the TCSPC system. The full widtIh half maximum (FWHS) of the IRF lies in the range FWHMS = 83.62 ± 1.14 ps.

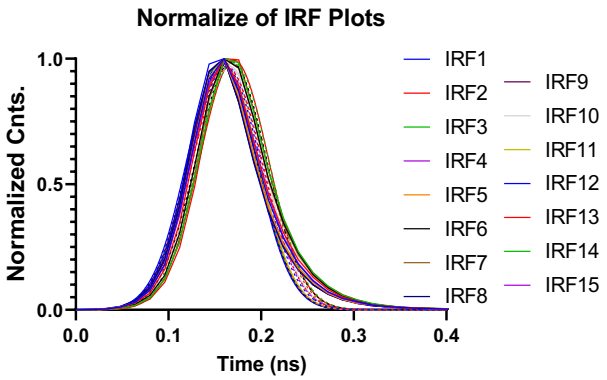

Figure S1. Instrument response function. Ludox was used as a scattering sample. The curves represent individual measurements.

Fluorescein tail fitting for G-Factor calibration

G-Factor was calculated by tail fitting of fluorescein decay curves using equation S1.

$$G = \frac{\int VV(t)dt}{\int VH(t)dt} \qquad \text{[Equation S1]}$$

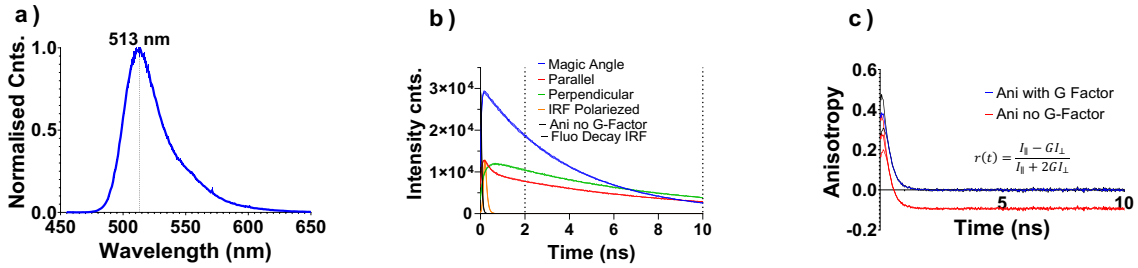

Figure S2. Instrument calibration using Fluorescein 0.5 μM. a) Steady state fluorescence spectra. b) TCSPC Blue is magic angle red is parallel green is perpendicular (IRF in orange), c) Anisotropy of fluorescein with (blue) and without (red) G-Factor correction.

Individual decay curves

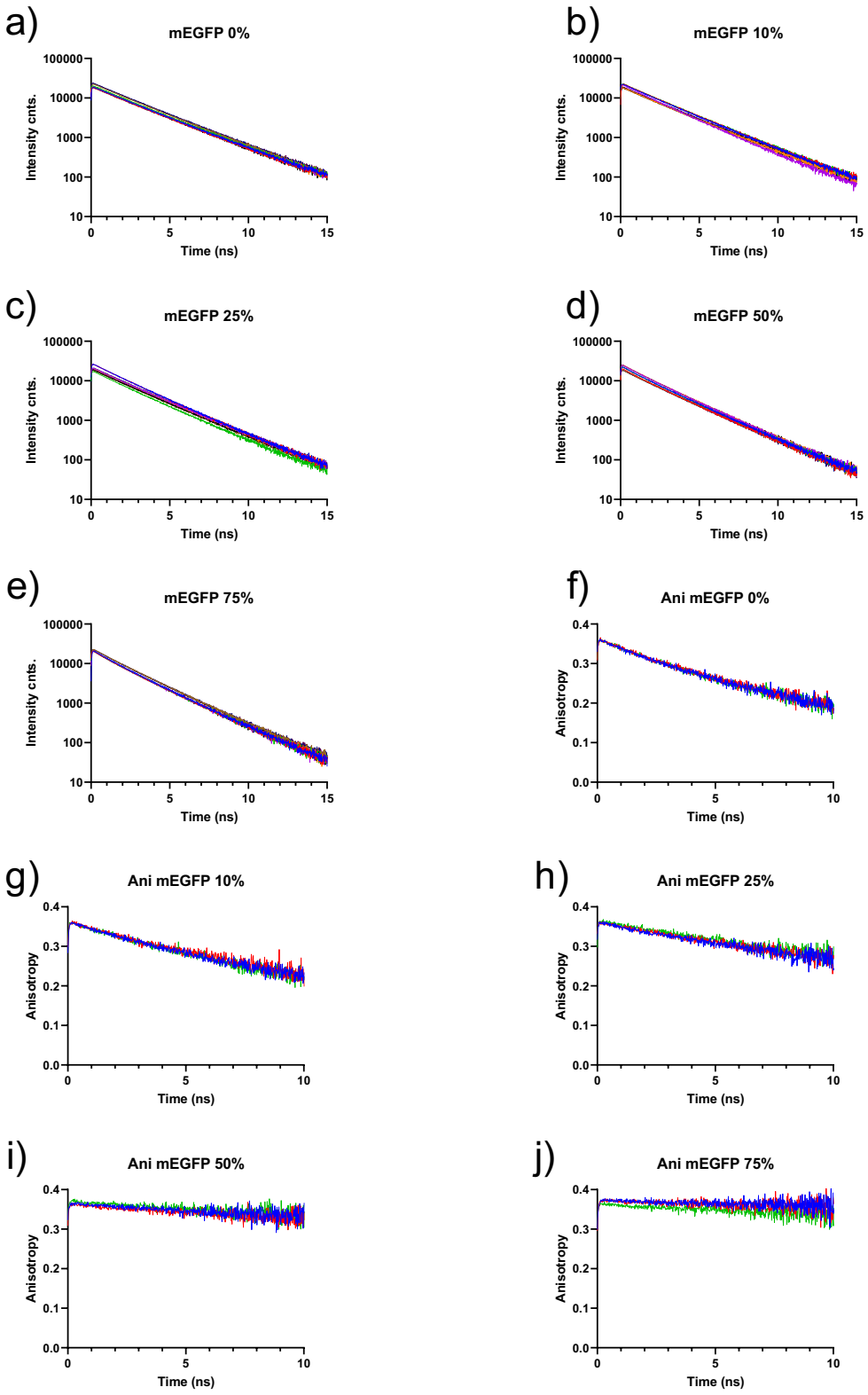

Figure S3. Time resolved fluorescence decay curves of 1 µM mEGFP in PBS in a range of glycerol concentrations. TCSPC decay curves (a-e). Anisotropy curves (f-j). Each decay curve represents the average of 3 technical replicates.

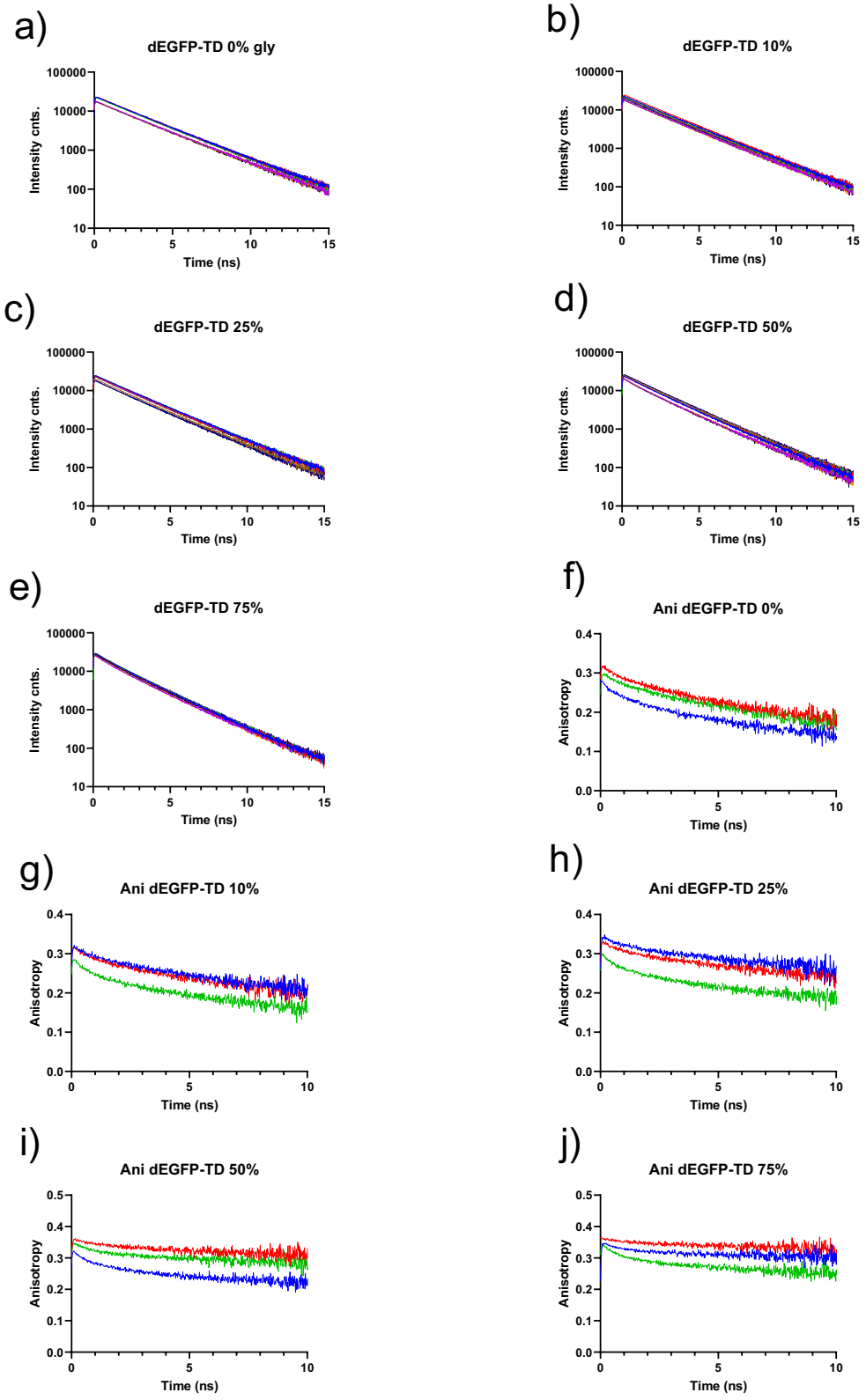

Figure S4. Time-resolved fluorescence decay curves of 1 µM dEGFP-TD in PBS in a range of glycerol concentrations. TCSPC decay curves (a-e). Anisotropy curves (f-j). Each decay curve represents the average of 3 technical replicates.

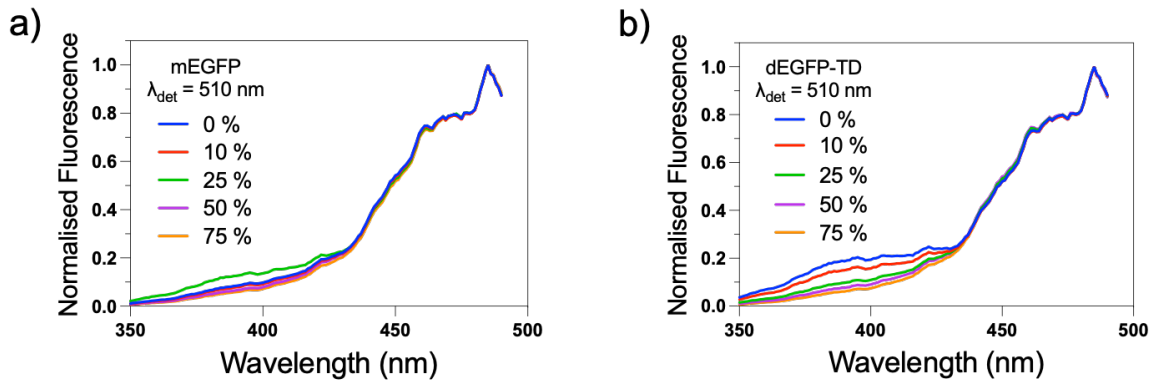

Figure S5. The normalised excitation spectra of a) mEGFP and b) dEGFP-TD at a range of glycerol/PBS mixtures. The fluorescence intensity was measured at 510 nm.

Table S1 The initial anisotropy of mEGFP and dEGFP-TD, which is the value of the anisotropy at t=0 after short pulse excitation. The glycerol percentages are given by volume.

% Glycerol in PBS	Limiting anisotropy (r) ±SD	
	mEGFP	dEGFP-TD
0%	0.37±0.00	0.30±0.02
10%	0.37±0.00	0.31±0.02
25%	0.37±0.01	0.33±0.02
50%	0.37±0.01	0.35±0.02
75%	0.38±0.01	0.36±0.01