

## SUPPLEMENTAL INFORMATION

## METHODS

**Materials** Acrylodan-labeled GGBP was provided by Ge *et al.*<sup>1</sup> The protein was suspended in 20 mM phosphate buffer, pH 7.5. Samples were centrifuge filtered with Amicon 5000 MWCO centrifuge filters four times for 30 minutes to remove any free dye or glucose in solution.

**Experimental** Time-correlated single photon counting measurements of polarised fluorescence were performed as previously described.<sup>2</sup> The pulsed-laser excitation used was 390 nm, and the fluorescence emission was observed at 510 nm. Temperature was maintained using a Quantum Northwest TLC150 Peltier controller. Photons were counted for 3–6 minutes to obtain transients with approximately 10000 peak counts.

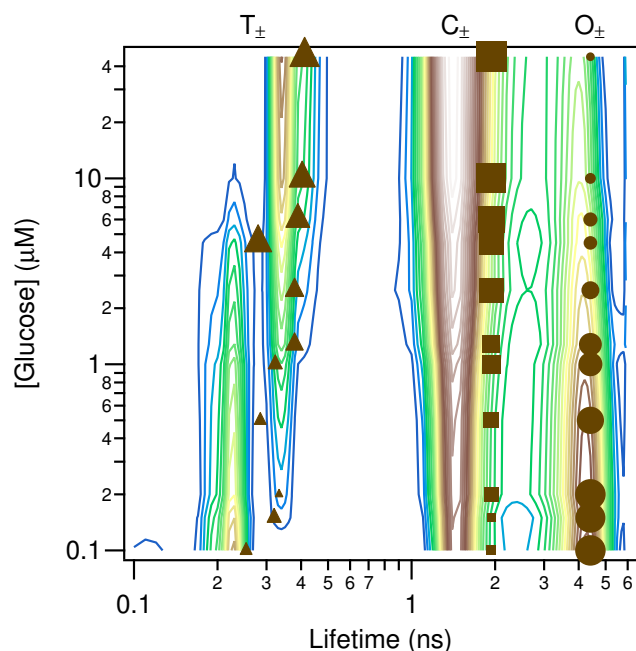


Figure SI-1: Contour plots representing the isotropic fluorescence lifetime distributions from global regularised TNNLS fitting of a glucose titration at 35°C. The solid symbols represent the parameters from a discrete 3-exponential global fit to the same data set, but also including the parallel and perpendicular fluorescence components. The size of the symbols represents the relative amplitude changes of the components. Two lifetime distributions ( $\sim 1.5$  and  $4.5$  ns) are nearly constant with glucose concentration, while a third lifetime shifts from  $\sim 250$  to  $400$  ps with increasing glucose.

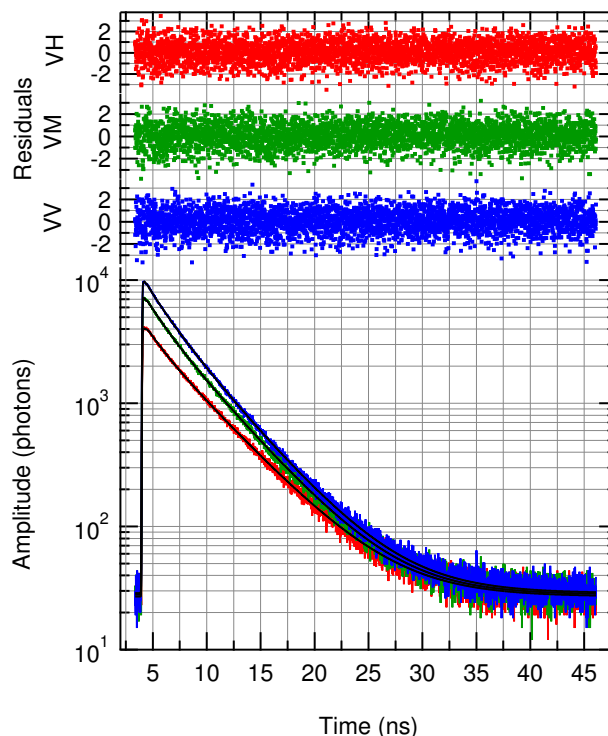


Figure SI-2: Simultaneous least-squares fitting to three polarizations ( $VM=54.7^\circ$ ,  $VV=0^\circ$ , and  $VH=90^\circ$  with respect to the excitation polarization) is performed to identify anisotropic decay components that correspond to the rotational diffusion of the dye and protein in solution. The residuals from the curve-fitting routine are shown above. The residuals are gaussian distributed about zero indicating a well-behaved fit to the data.

**Global regularised distribution fits** TCSPC fluorescence lifetime data is typically fit to a linear combination of one or more exponentials convoluted by the instrument response function. If a basis set of exponentials with regularly spaced decay constants is used, then the fitting procedure is reduced to solving a set of linear algebraic equations.<sup>3</sup> The number of free parameters in this case is very large and the solution to the fitting problem is not statistically unique and therefore not mathematically stable. Regularisation is a technique used to stabilize results by mathematically imposing prior knowledge to constrain the immense set of available solutions. The regulariser acts as a constraint that reduces the number of effective free parameters. The prior knowledge that is tacitly assumed in most regularisation procedures is piece-wise continuity of the solution in the fluorescence lifetime dimension as represented by a line. In the case of several possible conformations of GGBP this is not necessarily a valid assumption. However the population of a particu-

lar species should be piece-wise continuous with respect to a changing ligand concentration.

We have formulated a general global regularisation scheme which will incorporate prior knowledge of the evolution of a data set over a changing variable. This minimal model that assumes only continuity with respect to glucose concentration allows evaluation of possible models without choosing them *a-priori*. We fit eleven isotropic fluorescent decays (3456 points per decay) at seven temperatures using a continuity-in-glucose-concentration regulariser to estimate the fluorescence lifetimes and amplitudes in our data set. The strength of regularisation was estimated using the L-curve method.<sup>4</sup> The regularisation parameter value was then optimised using the F-test at  $P=0.5$  to compare the residuals from the final regularised fits with the unregularised fit.<sup>5</sup> In the final fits, the estimated number of effective free parameters was  $\sim 60$ . A typical result of the totally non-negative linear least squares (TNNLLS) procedure is shown in Fig. SI-1.

**Discrete Local Fits** Fluorescence lifetimes were also analysed using a convolute-and-compare, non-linear, least-squares technique.<sup>6</sup> The data sets at each temperature were fit with one, two, three, and four exponential terms. Three exponents was the highest statistically justifiable number of exponential terms and correlated well with the results of the minimal model regularised TNNLLS fits.

Analysis of fluorescence anisotropy decay was performed based on the results of the isotropic analysis.<sup>7</sup> A typical fit to the transients measured for the three polarisation angles for a single temperature and glucose concentration is shown in Fig. SI-2.

Based on the above results, magic-angle (isotropic), parallel, and perpendicular polarised fluorescence decay transients were simultaneously analysed for the eleven different glucose concentrations. Fluorescence decay rates were globally linked across all glucose concentrations and a third decay rate was allowed to vary with glucose concentration,  $g$ , according to  $A_M(t)$  in the following equation:

$$A_M(t) = A_0(f_1(g)e^{-k_1t} + f_2(g)e^{-k_2t} + f_3(g)e^{-k_3(g)t}) + C,$$

$$A_V(t) = A_M(t)(1 + 2r(t)),$$

$$A_H(t) = A_M(t)(1 - r(t)),$$

$$r(t) = \frac{\sum_{i=1}^n f_i(g)e^{-k_it} \sum_{j=1}^m r_{ij}(g)e^{-\kappa_jt}}{\sum_{i=1}^n f_i(g)e^{-k_it}}$$

The global results of a typical discrete fit are represented by the filled symbols in Fig. SI-3

With this technique, we were able to observe changes in the relative populations of the three decay

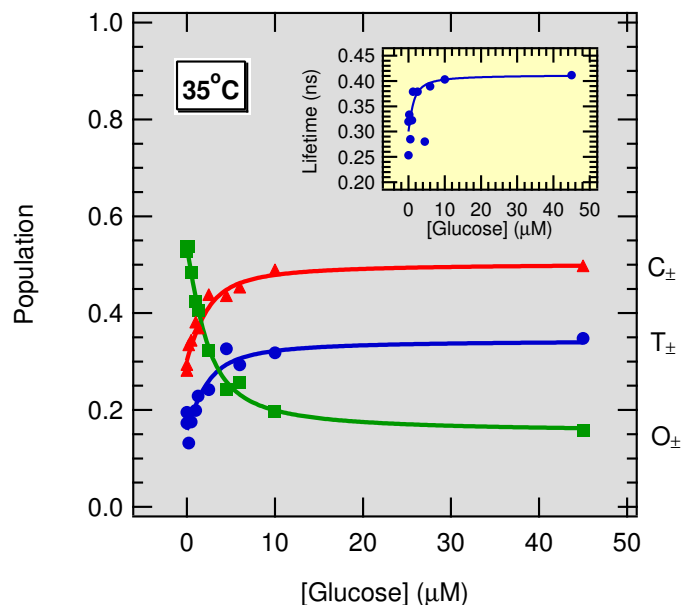


Figure SI-3: Fluorescence lifetime populations as a function of glucose concentration at 35°C resulting from global least-squares fitting. The inset shows the third lifetime, which is fit locally to each glucose concentration. This lifetime showed isotherm-type behavior with a binding constant of  $\sim 0.2 \mu\text{M}$ .

rates (i.e., the three spectroscopically distinct conformational states) as a function of glucose concentration. To verify this model, we also analyzed the data with global populations ( $f_i$ ) and local fluorescent rates ( $k_i$ ), as well as all of the permutations of local versus global rates and populations. All other models resulted in a significant increase in the reduced  $\chi^2$ .

The residuals for a typical global fit are shown in Fig. SI-4. The residuals are nearly Gaussian across the entire global data set, indicating a good fit to the data. The reduced  $\chi^2$  for each of the seven global fits was approximately 1.03–1.05. Error estimates for the parameters were determined from the diagonal elements of the covariance matrix. Seven temperatures from 5°C to 35°C were globally analysed with this methodology.

## REFERENCES

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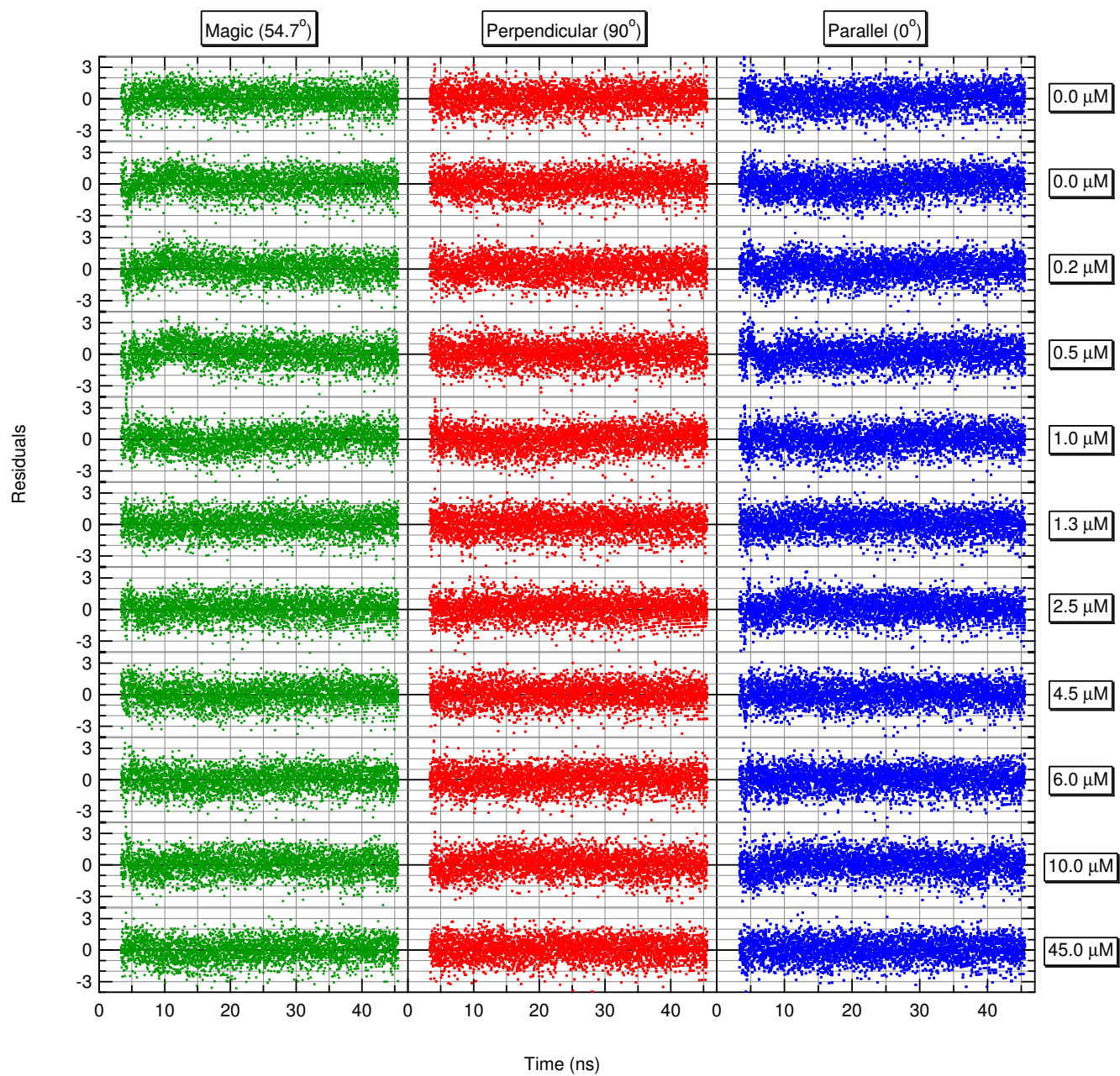


Figure SI-4: Residuals from global anisotropy analysis of 33 transients measured at 25°C.