Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Supplementary Information

Fluorescence lifetime of Rhodamine B in aqueous solutions of polysaccharides and proteins as a function of viscosity and temperature

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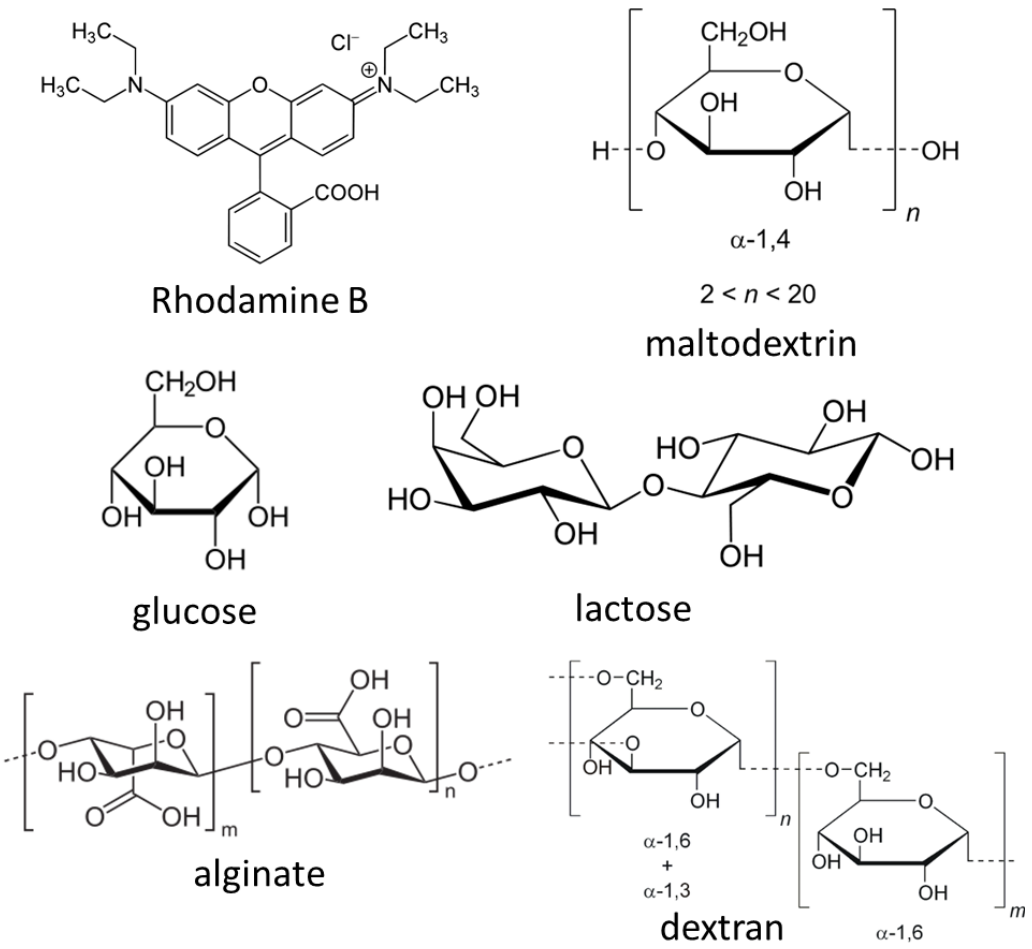


Figure S1. Chemical structure of the different compounds used in this study. WPI is not included as it is a mixture of primarily β-lactoglobulin, α-lactalbumin and bovine serum albumin. Their tertiary structure can be found in the Protein Data Bank (PDB).

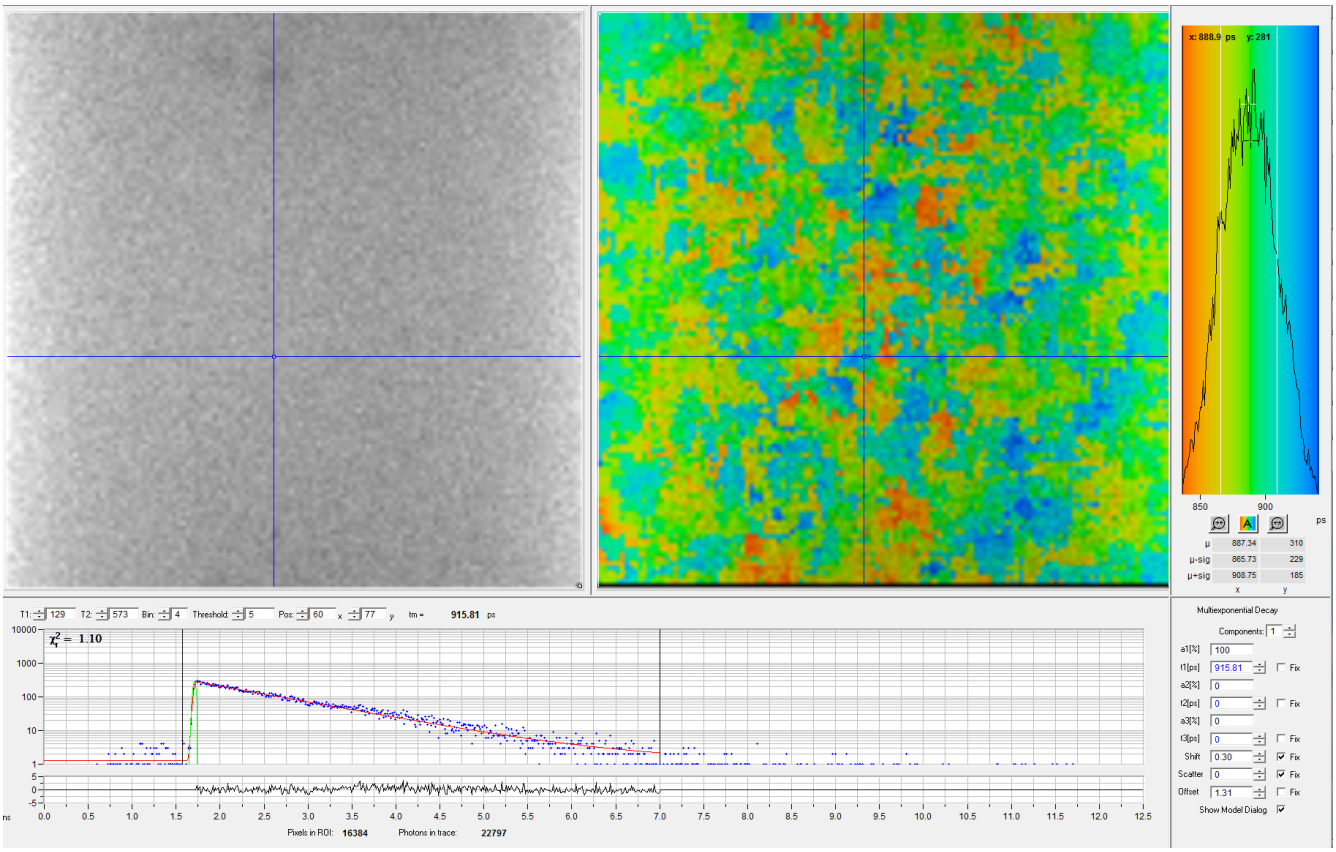


Figure S2. Example of a FLIM image using the Becker&Hickel software SPCImage 6. The upper part of the Figure shows (from left) the intensity image, the color-coded lifetime image and the lifetime histogram of a 10^{-5} M RhB in water at 45°C. The lower part of the Figure shows the fluorescence decay curve from a single pixel in the lifetime image as indicated by the blue crosshair. The green curve at the start of the decay curve is the instrument response function. The χ^2 value was close to 1, meaning that the curve was a good fit for a single lifetime component, as was selected in the right lower part of the Figure.

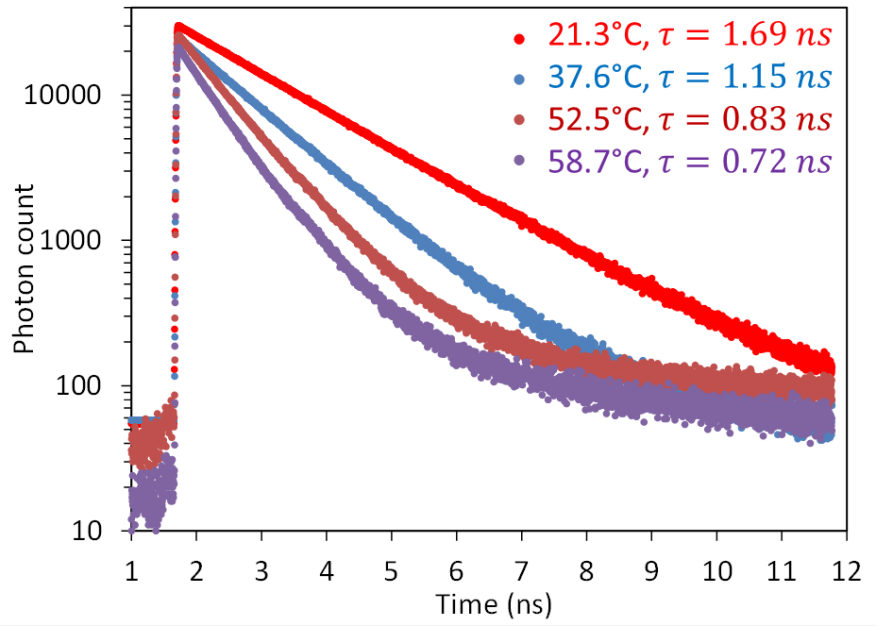


Figure S3. Fluorescence decays of RhB in water ($1.2 \cdot 10^{-5}$ M) at different temperatures. The calculated mono-exponential lifetimes calculated are given in the legend.

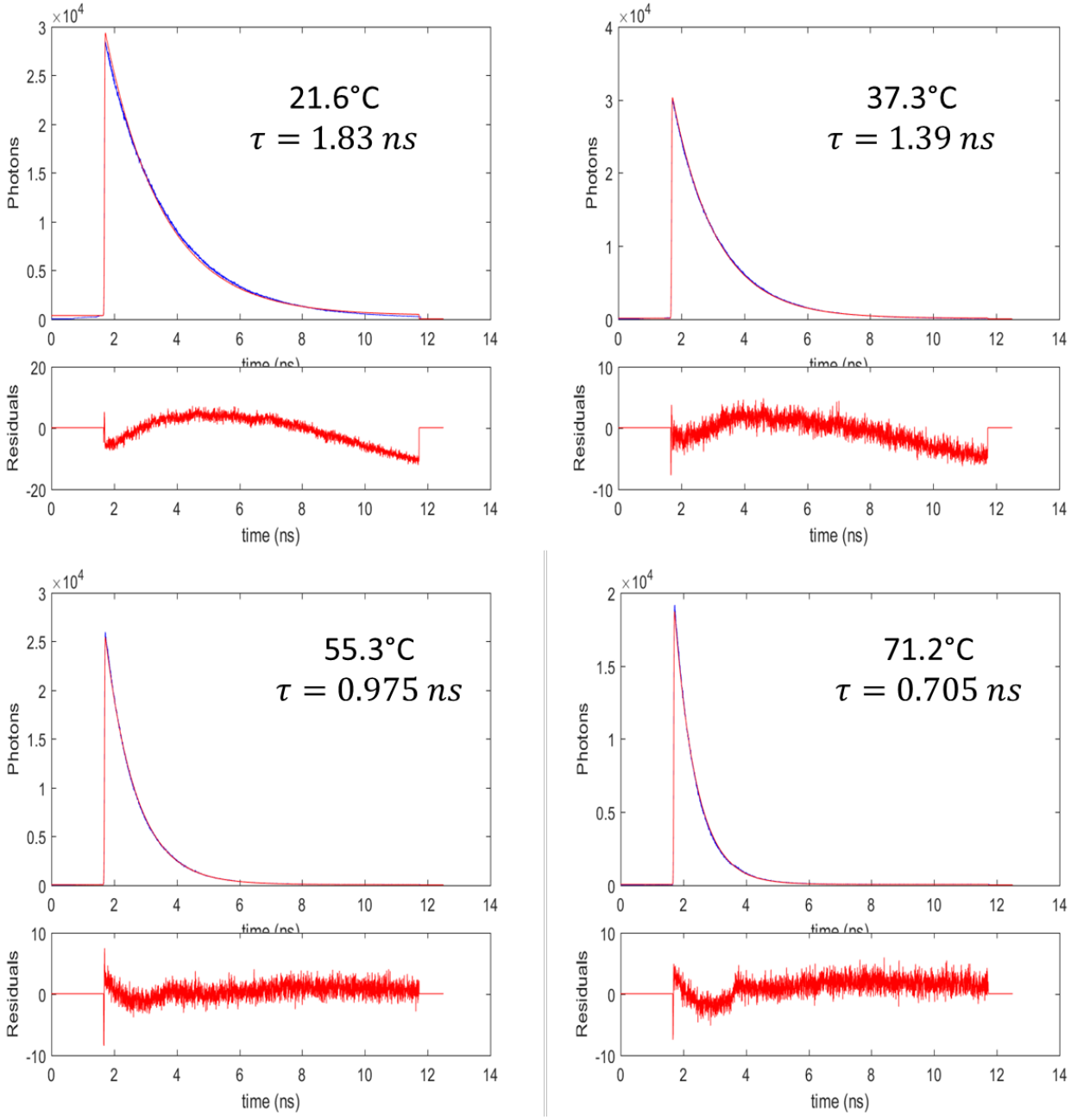


Figure S4. Examples of the mono-exponential fit quality of 4 of the exponential decays shown in Fig. 1(a) for a RhB solution in 20 wt% glucose.

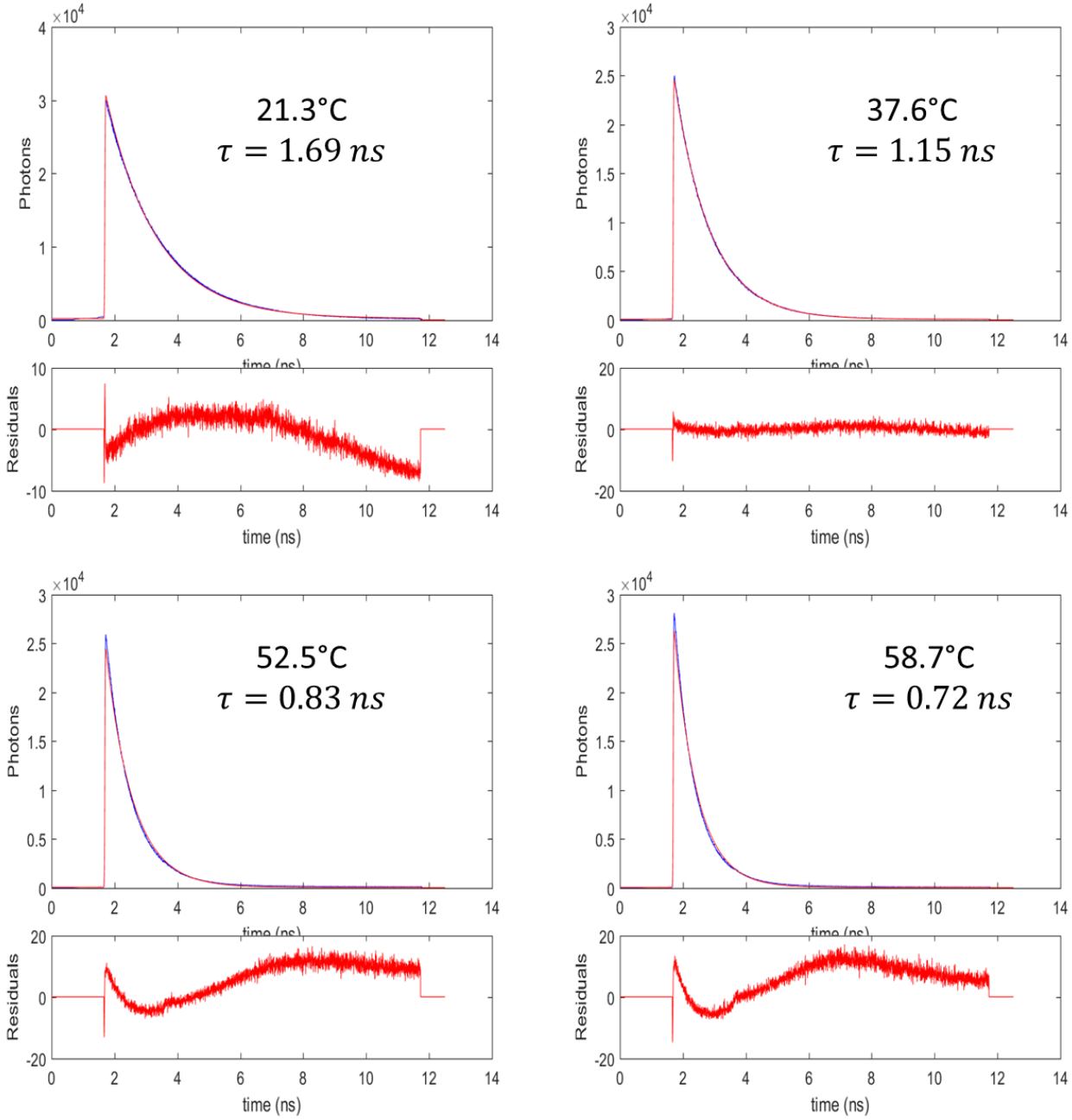
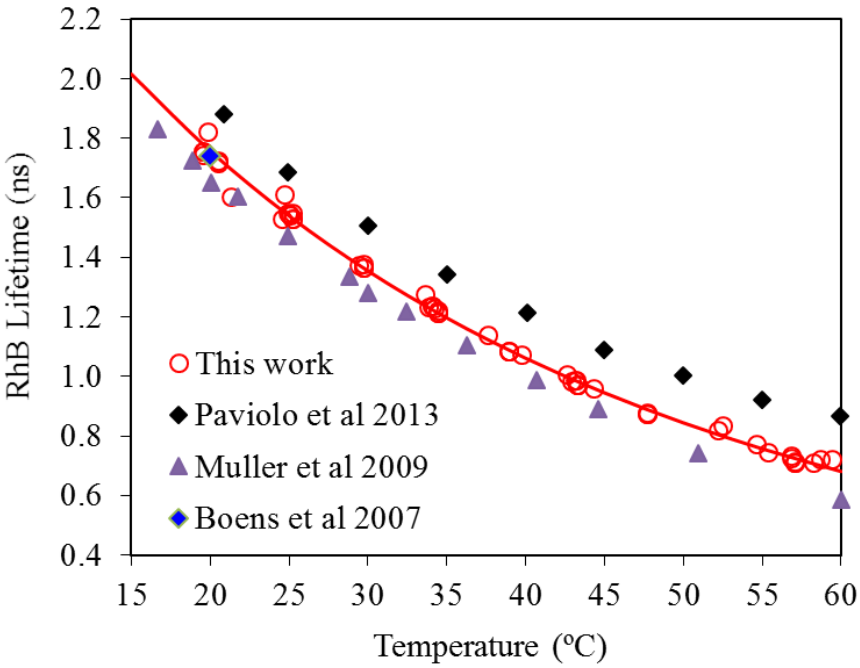
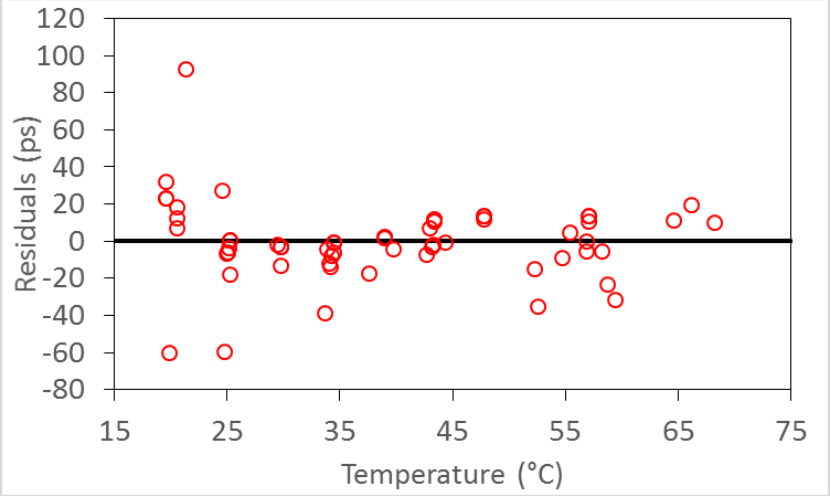


Figure S5. Examples of the mono-exponential fit quality of the data shown in Fig. S3 for a RhB solution in water.



(a)



(b)

Figure S6. (a) Temperature dependence of the RhB lifetime in water. Continuous line shows eq. 4 with E_a = 19.2 ± 0.5 (95%CI) kJ/mol. The τ values in this study where obtained at different RhB concentrations (10^{-4}-10^{-6} M), pH (5.6-12), heaters and objectives; all values agreeing well. Literature data from [1–3]. (b) Residuals of the Arrhenius fit for the data from this study shown in (a).

Effect of the refractive index

The lifetime of a fluorophore is known to be affected by changes of in solutions refractive index, using typically the Stricker-Berg equation, resulting that the reciprocal of the lifetime τ is proportional to the square of the refractive index *n*, e.g. $\tau^{-1} \alpha n^2$. Thus increases in *n* show a corresponding decrease in the lifetime, as observed for example for green fluorescent protein (GFP) in glycerol/water mixtures [5]. In solutions with solutes, the refractive index always increases at higher solute concentrations, typically in a linear manner for proteins [6] as well as for sugars [7]. For the highest solute concentrations used, about 30 wt% WPI and ~40 wt% polysaccharides, the refractive index is similar at ~1.39 [6,7], slightly higher to that of water, at 1.333. Therefore, it would be expected a decrease of ~8% in the lifetime for both cases, whereas it is observed an increase of ~40% for glucose or maltodextrins, and ~70% for WPI, both at 25°C.

Note that as the increase of *n* with the solute content is generally very, if not wholly, linear, then the increase of n^2 will be for practical effects also linear with the protein concentration, see Fig. S7 for the case of glucose. Hence, if corrections due to *n* were desired, it would simply modify slightly the linear slope observed in Fig. 2(a).

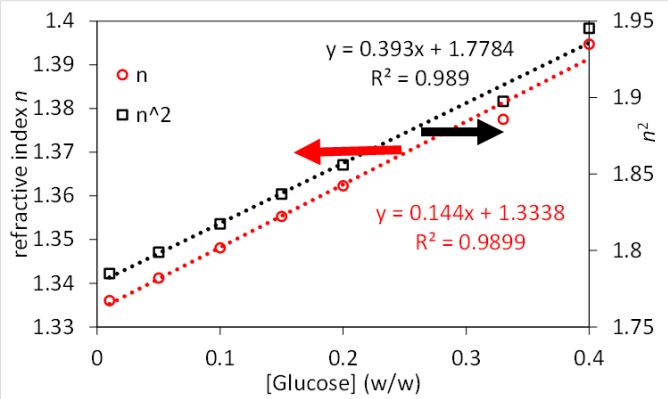


Figure S7. Apparent linear dependence of the refractive index *n* as well as n^2 for aqueous glucose solutions at 293.16 K, data reported by Tan and Huang [8].

Usually a good linear correlation between τ^{-1} and n^2, with a positive slope, is given as evidence of the role of the refractive index on the fluorescence lifetime [5]. In our system of study, a liner relationship

could also be considered, but with a negative slope, and there is no statistical difference using n instead of n^2 for the linear regression (check the R^2 values in Fig. S8). Further evidence of the small role of n in our study is evident from the WPI data in Fig. 7. Between 15-30 wt% WPI τ is constant yet n increases significantly.

It can be concluded that n has only a minor effect in the results presented in this study. Nevertheless, its role is unimportant in future thermometry/viscometry microscopy experiments meanwhile proper calibrations are performed, and data is not extrapolated to other solutes.

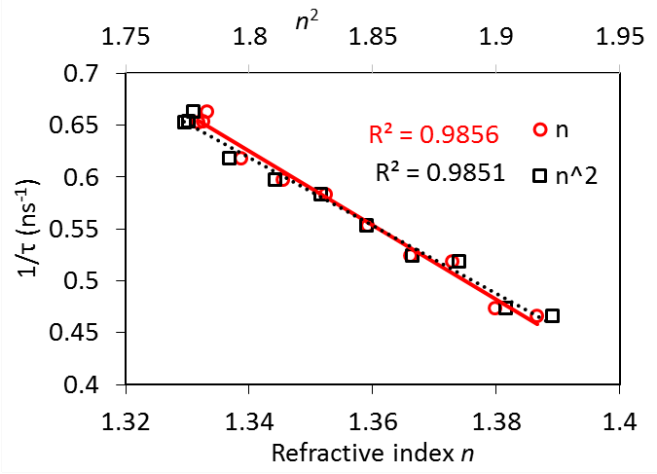


Figure S8. Correlation of the reciprocal lifetime of glucose solutions at 25°C against the refractive index n (black), or n^2 (red), at different solute concentrations.

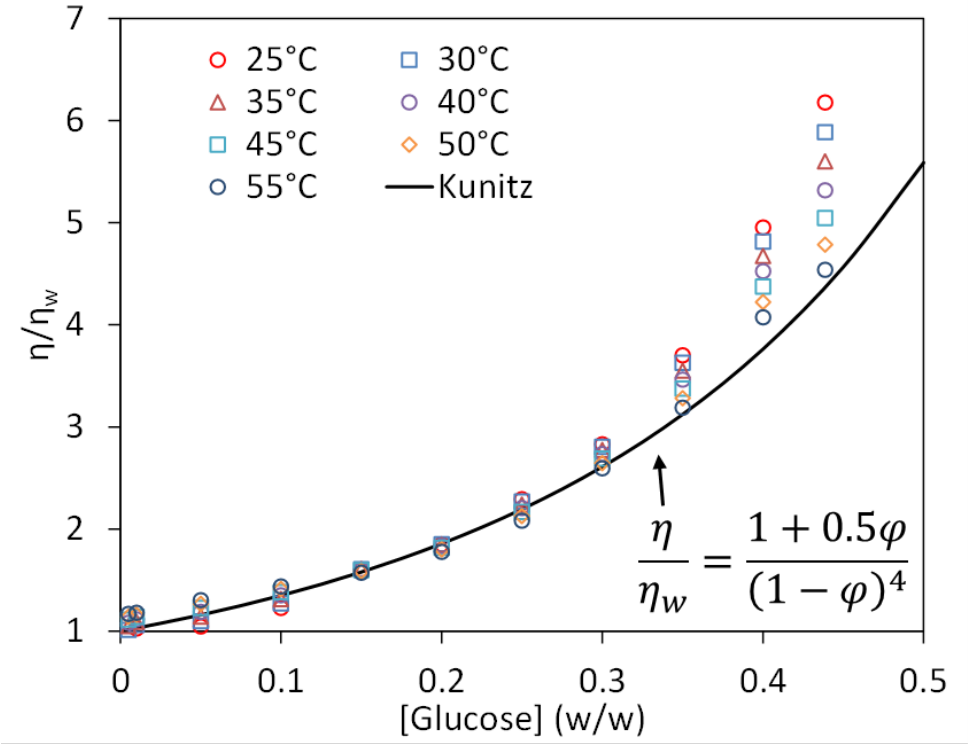


Figure S9. Normalized viscosity of glucose solutions, using the water viscosity at different temperatures, at different glucose concentrations. Continuous line is the Kunitz equation [4] with the volume fraction φ, which is calculated using the density of solid glucose, at 1.54 g/cm^3.

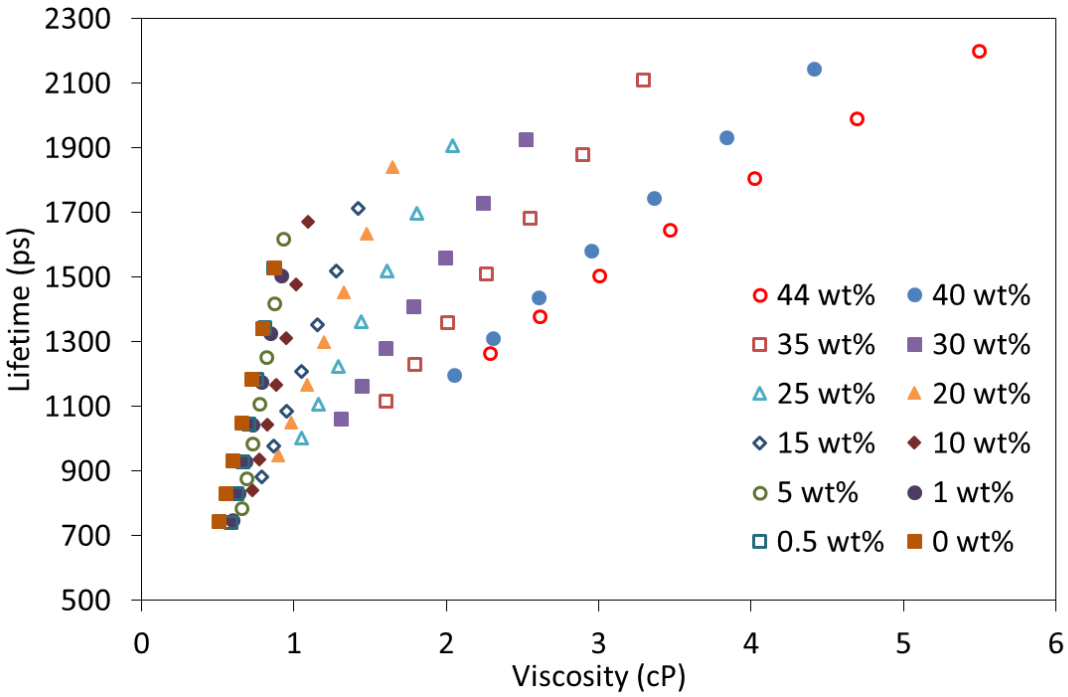
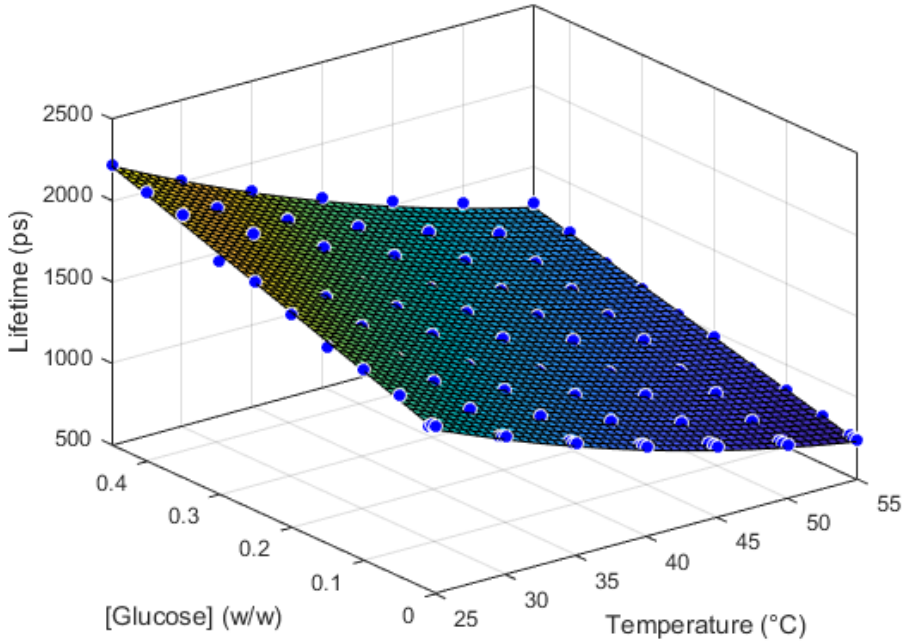
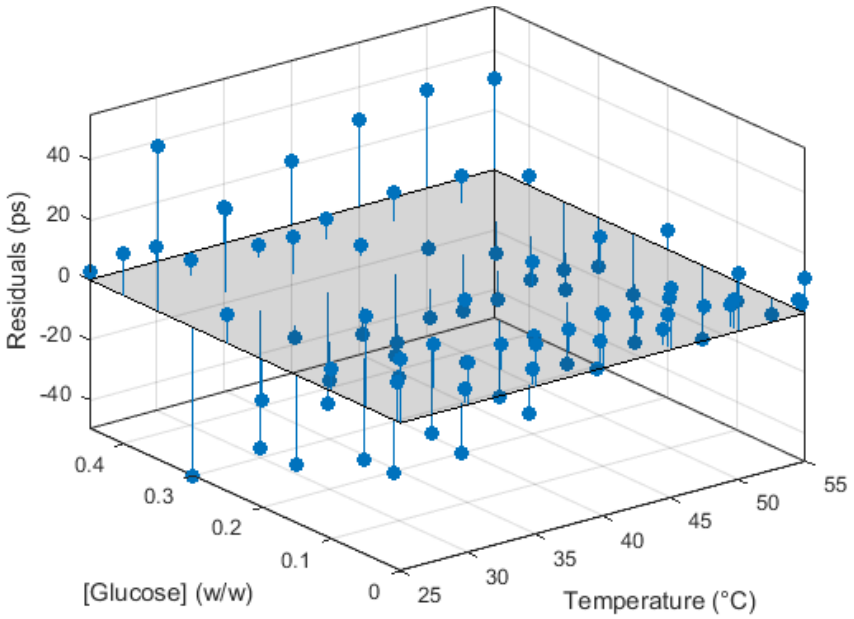


Figure S10. Lifetime against the solution viscosity in glucose solutions, showing no collapse of the data.



(a)



(b)

Figure S11. Example of polynomial regression that can be performed to describe empirically the dependence of the lifetime of glucose solutions at different concentrations and temperatures. The surface in (a) show the best fit polynomial: τ (ps) = 2702 + -57.65*T+ 1894*[G] + 0.3972*T^2 +

-13.39*[G]*T, with the units given in the figure. The root-mean square error (RMSE) of the regression is 18 ps.

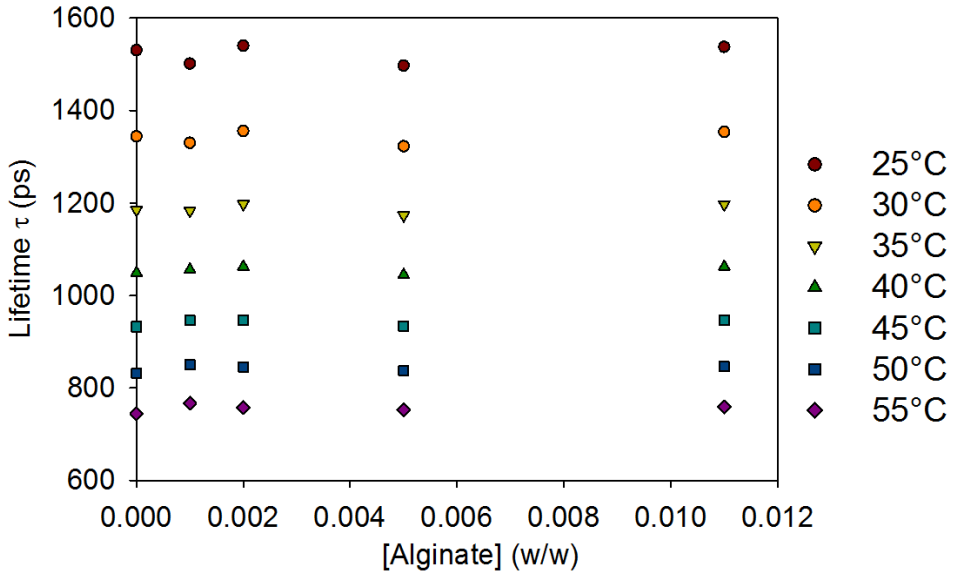


Figure S12. Lifetime of RhB at different concentrations of sodium alginate, MW ~100 kDa, and temperatures.

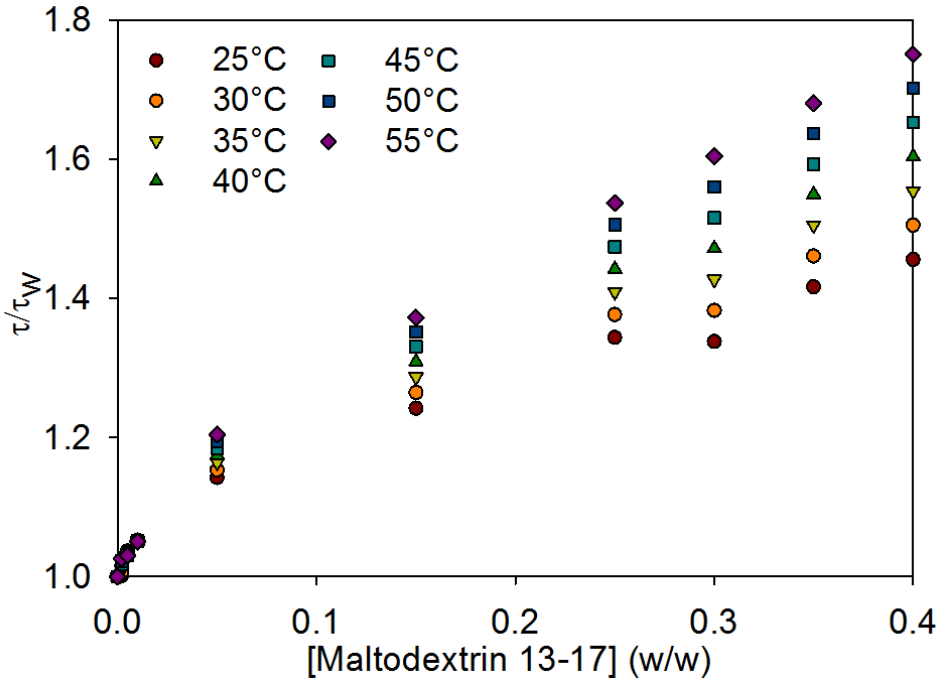


Figure S13. Normalized lifetime of RhB at different concentrations of maltodextrin 13-17 using the lifetime in water τ_w at different temperatures.

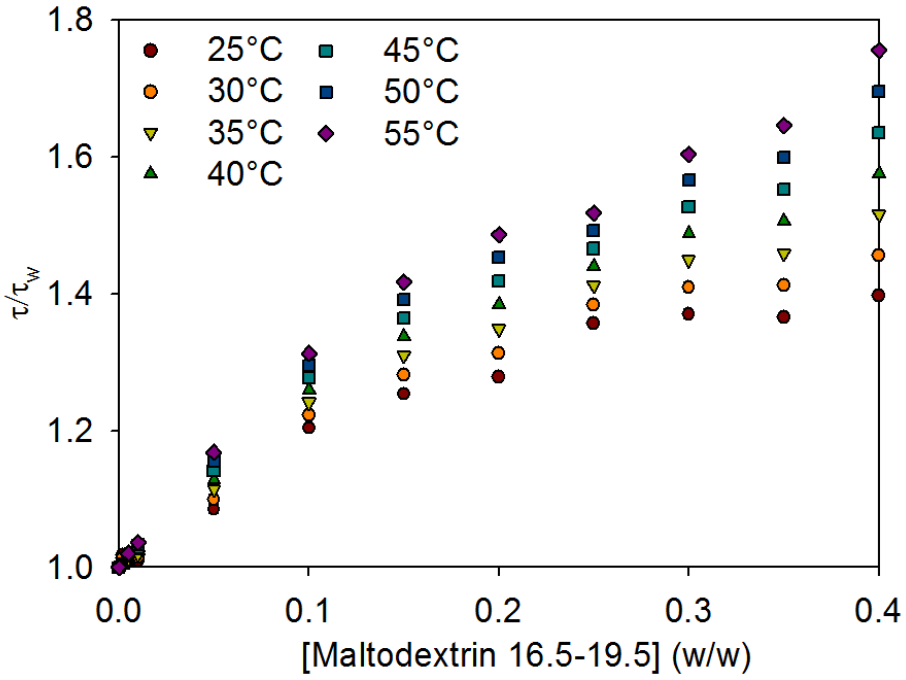


Figure S14. Normalized lifetime of RhB at different concentrations of maltodextrin 16.5-19.5 using the lifetime in water τ_w at different temperatures.

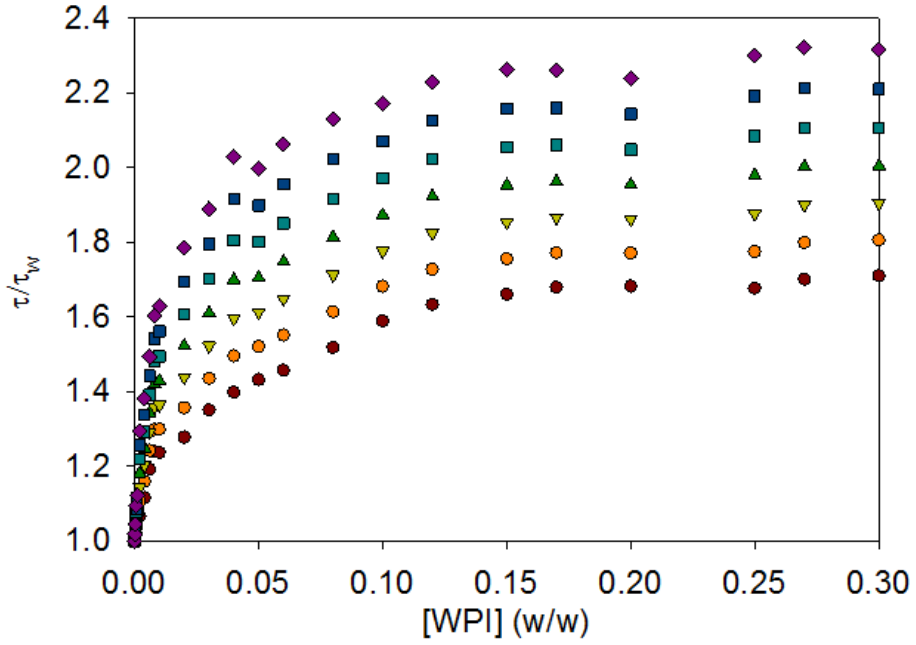


Figure S15. Normalized lifetime of RhB at different concentrations of WPI using the lifetime in water τ_w at different temperatures.

References

[1] N. Boens, W. Qin, N. Basarić, J. Hofkens, M. Ameloot, J. Pouget, J.-P. Lefèvre, B. Valeur, E. Gratton, M. vandeVen, N.D. Silva, Y. Engelborghs, K. Willaert, A. Sillen, G. Rumbles, D. Phillips, A.J.W.G. Visser, A. van Hoek, J.R. Lakowicz, H. Malak, I. Gryczynski, A.G. Szabo, D.T. Krajcarski, N. Tamai, A. Miura, Fluorescence Lifetime Standards for Time and Frequency Domain Fluorescence Spectroscopy, Anal. Chem. 79 (2007) 2137–2149. doi:10.1021/ac062160k.

[2] C.B. Muller, K. Weib, A. Loman, J. Enderlein, W. Richtering, Remote temperature measurements in femto-liter volumes using dual-focus-Fluorescence Correlation Spectroscopy, Lab. Chip. 9 (2009) 1248–1253. doi:10.1039/B807910B.

[3] C. PAVIOLO, A.H.A. CLAYTON, S.L. MCARTHUR, P.R. STODDART, Temperature measurement in the microscopic regime: a comparison between fluorescence lifetime- and

intensity-based methods, J. Microsc. 250 (2013) 179–188. doi:10.1111/jmi.12033.

[4] M. Kunitz, An empirical formula for the relation between viscosity of solution and volume of solute, J. Gen. Physiol. 9 (1926) 715–725.

[5] K. Suhling, J. Siegel, D. Phillips, P.M.W. French, S. Lévêque-Fort, S.E.D. Webb, D.M. Davis, Imaging the Environment of Green Fluorescent Protein, Biophys. J. 83 (2002) 3589–3595. doi:10.1016/S0006-3495(02)75359-9.

[6] R. BARER, S. TKACZYK, Refractive Index of Concentrated Protein Solutions, Nature. 173 (1954) 821–822. doi:10.1038/173821b0.

[7] W.M.B. Mat Yunus, A.B.A. Rahman, Appl. Opt. 27 (1988) 3341–3343.

[8] C.-Y. Tan, Y.-X. Huang, Dependence of Refractive Index on Concentration and Temperature in Electrolyte Solution, Polar Solution, Nonpolar Solution, and Protein Solution, J. Chem. Eng. Data. 60 (2015) 2827–2833. doi:10.1021/acs.jced.5b00018.