Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Binding or aggregation? Hazards of interpretation in studies of molecular recognition by porphyrins in water

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General Experimental

Materials: Tetrakis(4-sulphonatophenyl)porphyrin (Tetraphenylporphine tetrasulfonate, TPPS) was purchased from Aldrich and was used without further purification. Glucose was obtained from Alfa Aesar and Glycerol from Acros. The ultra-pure water used throughout the experiments was purified with a Milli-Q A10 filtration system. All other reagents were of analytical grade and were used without further purification.

Measurements: UV-visible and CD spectra were recorded at 25 °C on JASCO V-560 or V-550 spectrophotometer and a JASCO J-820 spectropolarimeter, respectively, both of which were equipped with an ETC505T temperature controller. Fluorescence spectra were measured on a JASCO FP-6500 instrument. Fluorescence lifetimes were determined by the time-correlated single-photon-counting method on a Hamamatsu FL920S instrument equipped with a pulsed H_2 light source. ^{1}H NMR spectra were recorded on Varian 400 MHz at 25 °C. Isothermal titration calorimetry (ITC) was conducted on a MicroCal iTC200, with measurements recorded at 25 °C. The particle size distributions of TPPS were measured at 25 ºC by monitoring scattered light using a Malvern Instruments Zetasizer Nano dynamic light-scattering (DLS) instrument.

Sample Preparation: Solutions of D-glucose used were all allowed to equilibrate overnight prior to use, unless stated otherwise. TPPS solutions were prepared from dilution of a 1.0 mM stock solution in Millipore filtered water, unless otherwise stated.

UV-visible absorbance titration of glucose into TPPS in water

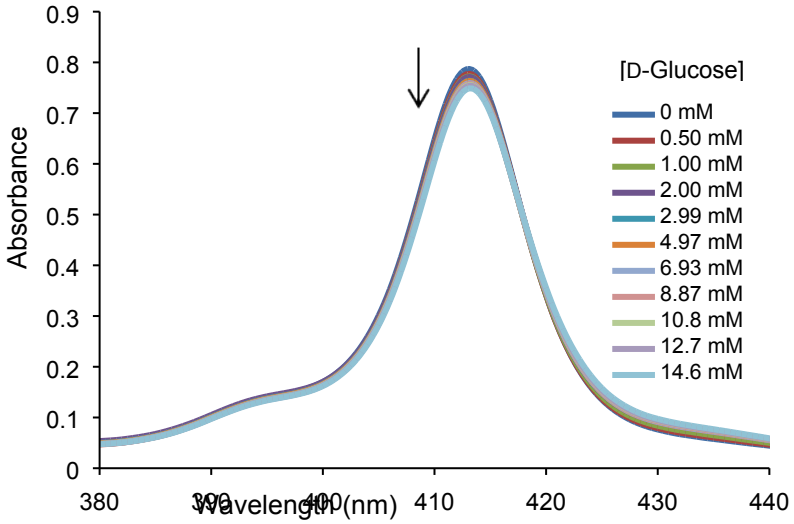

Fig. S1. Expansion of the Soret band region of the spectrum (λ_{max} 413 nm) for a titration of TPPS (6.15 μM in water) with D-glucose (0 – 14.6 mM) at constant TPPS concentration.

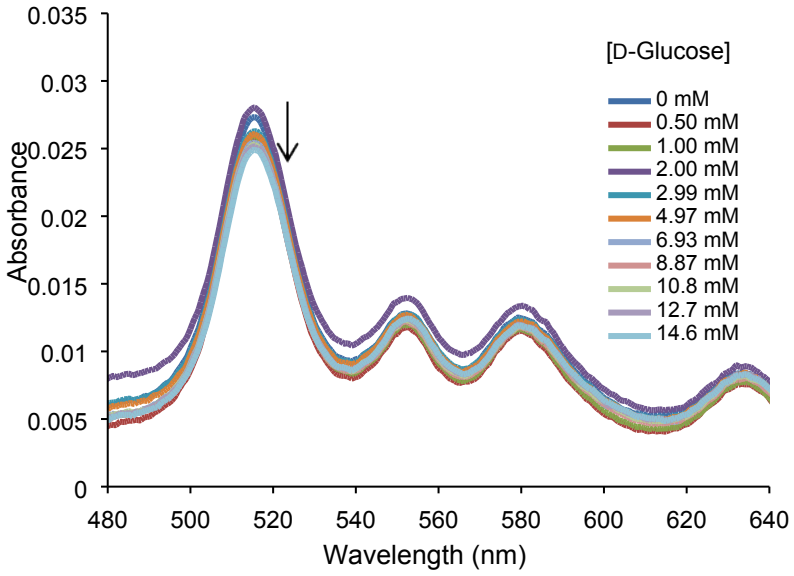

Fig. S2. Expansion of the Q band region of the spectrum for the titration referred to in Fig. S1.

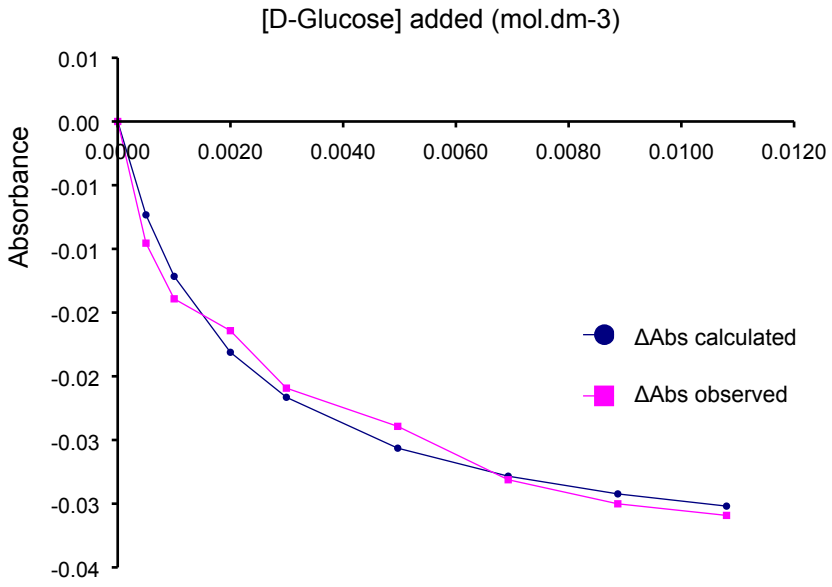

Fig. S3. The fitting of the absorbance data from Fig. S1 (413 nm, Soret band) to a 1:1 binding model. Apparent K_a = 5300 M^{-1}

UV-visible absorbance dilution study of TPPS in D_2O

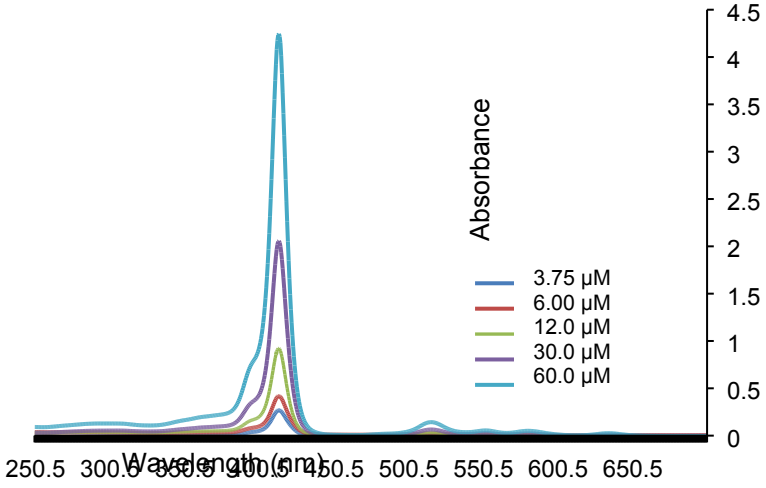

Fig. S4. UV-visible spectra for TPPS in water-methanol 95:5 at varying concentrations. Path length = 1 cm. For a plot of absorbance against concentration see Fig. 2.

^{1}H NMR dilution study of TPPS in D_2O

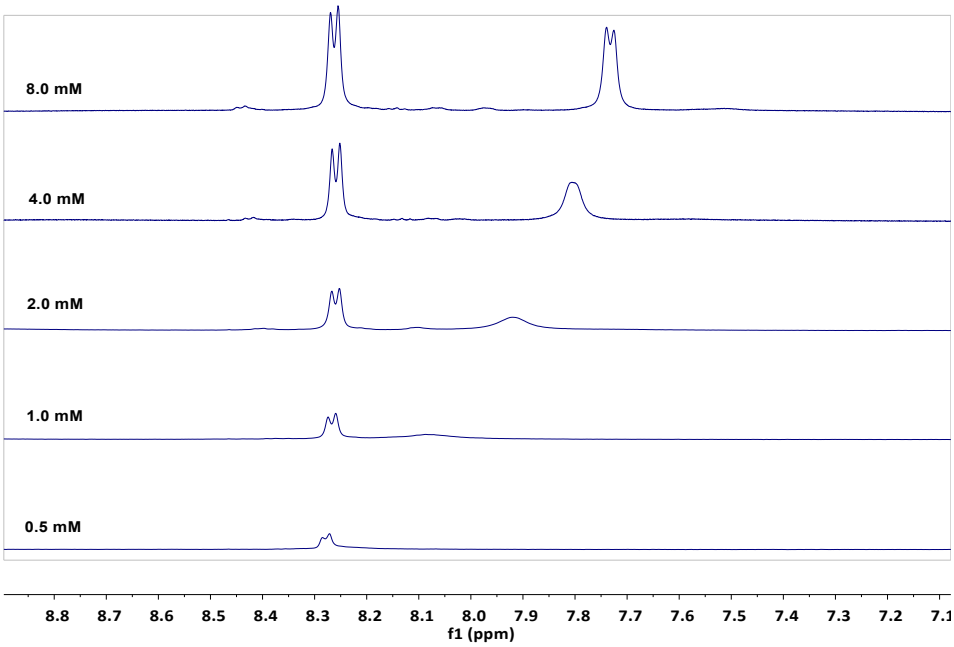

Fig. S5. Partial ^{1}H NMR spectra of TPPS **1** at different concentrations (8.0 mM diluted to 0.5 mM) in D_2O at 298 K. This experiment serves as a control for the titration of TPPS into glucose (Fig. 3a). The same ^{1}H NMR changes are observed in the presence and absence of glucose. They are therefore ascribed to changes in TPPS aggregation state and not to an interaction with carbohydrate.

Isothermal titration calorimetry of glucose added to TPPS in water

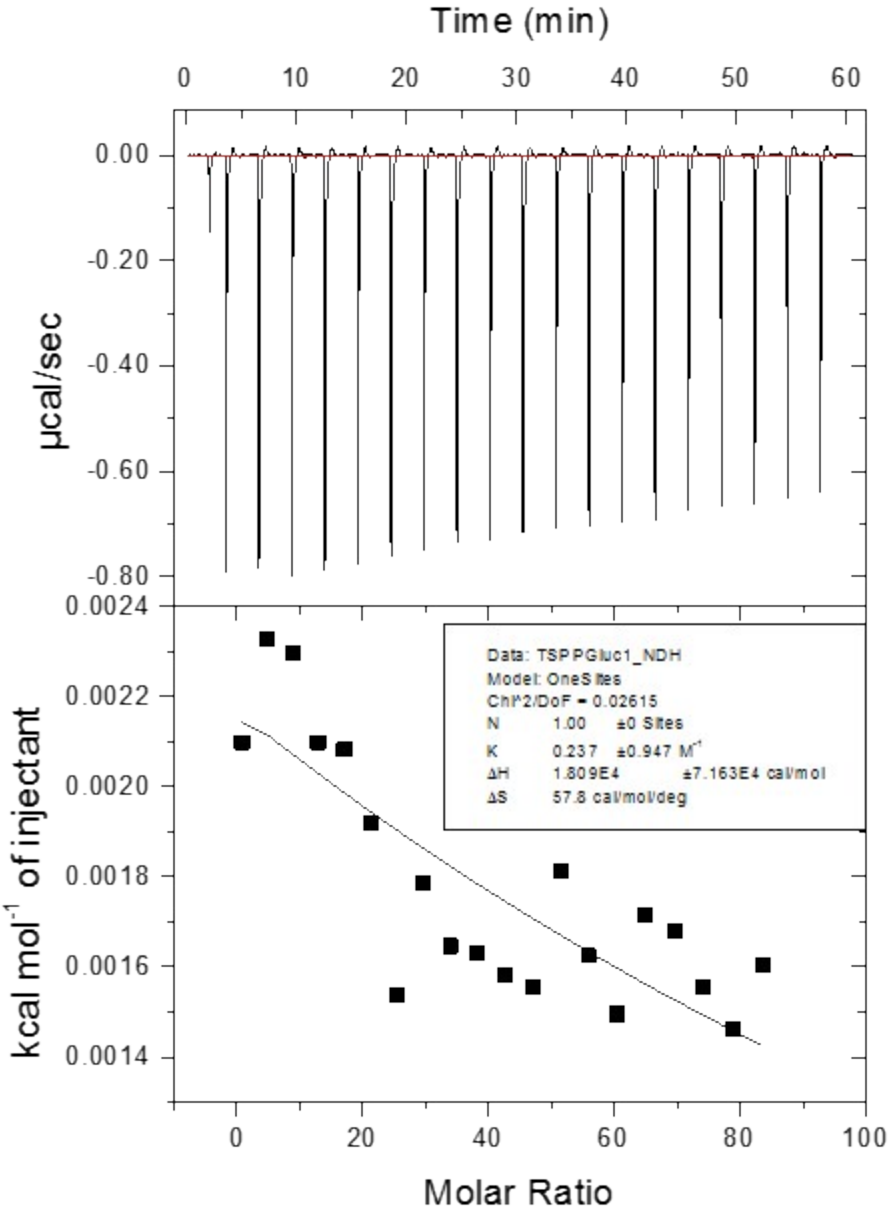

Fig. S6. Output from the ITC experiment for the addition of D-glucose (100 mM) to TPPS **1** (0.5 mM) in water. The raw data (top panel) is closely similar to the control titration with no TPPS present, implying that the heat evolved is mainly due to dilution of glucose. The corrected output (lower panel) shows no sign of saturation as expected for complex formation.

Circular dichroism titration of glucose into TPPS in water

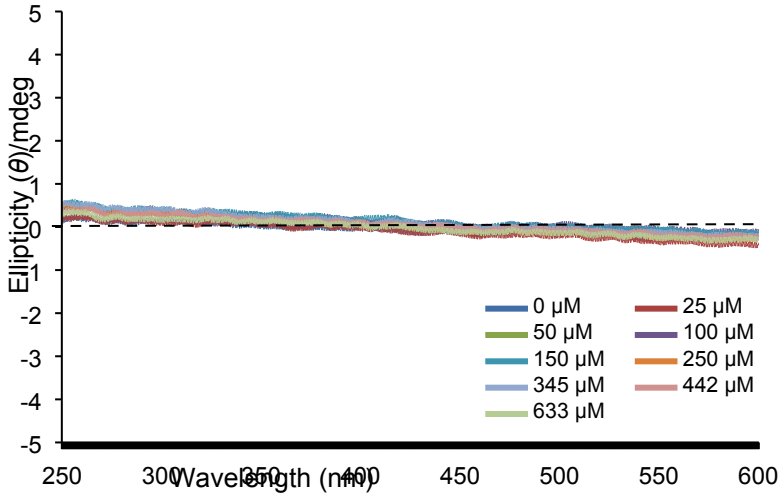

Fig. S7. The CD spectra for the addition of D-glucose (100 mM) to TPPS **1** (6.15 μM) in water-methanol (95:5). The absence of any significant signal argues against a close association between chiral carbohydrate and achiral chromophore.

Fluorescence dilution study of TPPS

As discussed in the main text, the fluorescence dilution study (Fig. 4) showed that the emission spectrum of TPPS is concentration-dependent between 0.05 and 6 μM, showing increased emission at λ_{em} 605 nm as [TPPS] decreases. Fluorescence lifetime experiments indicated that this was due to the presence of two species emitting at 605 nm. The species with the shorter fluorescence lifetime increased in abundance as the concentration decreased (Table S1). The presence of mutiple species implies multiple aggregation states, showing that TPPS is not monomeric across this concentration range.

Table S1. Fluorescence lifetimes (τ_n, nanoseconds) and relative abundances (A_n) for TPPS **1** at different concentrations in water-methanol, 95:5). The lifetimes were determined by single-photon-counting method in water:methanol (95:5) at room temperature.

Concentration (μM)	λ_{em}/nm	n[a]	τ_1	A_1	τ_2	A_2	χ^2
6.0	605	2	1.9	0.75	10.3	0.25	1.07
	640	1	10.5				1.09
	700	1	10.2				1.06
0.01	605	2	1.8	0.94	10.1	0.06	1.05
	640	1	10.3				1.04
	700	1	10.1				1.11

[a] Number of components.

Time-dependent phenomena

UV-visible spectra of TPPS

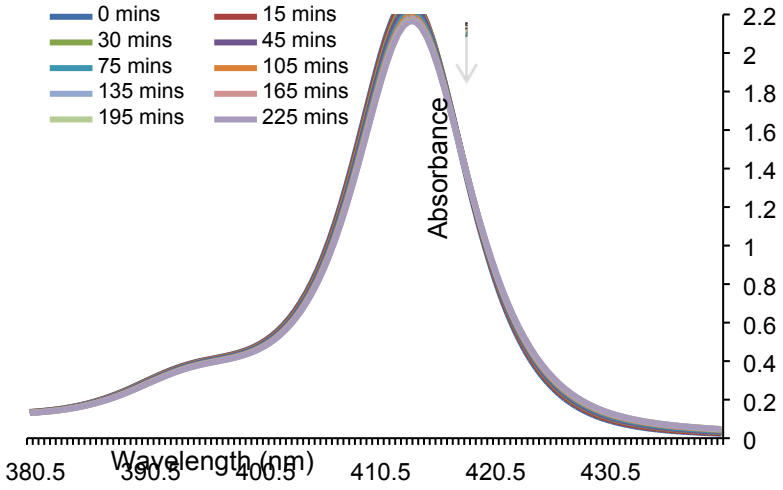

Fig. S8. Variation with time of the UV-visible spectrum (Soret band region) of TPPS (6.0 μM) in water. Spectra were acquired between 0 and 225 min after preparation of the sample by dilution from 1 mM aqueous solution.

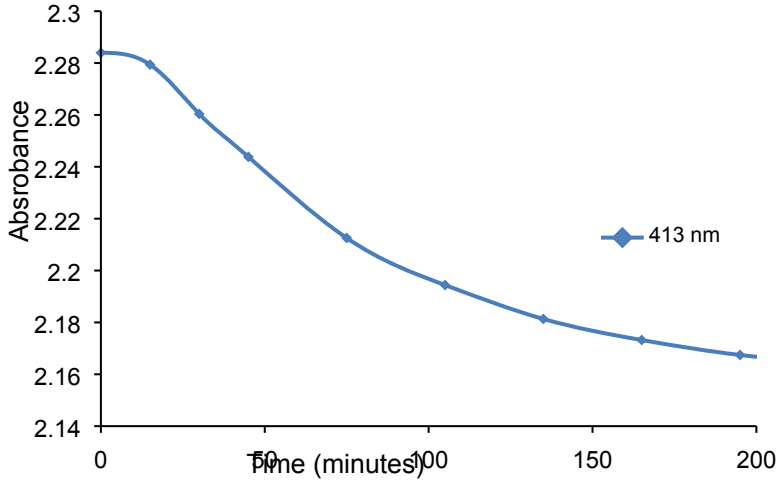

Fig. S9. Absorbance values from Fig. S8 at 413 nm plotted vs. time.

Fluorescence lifetime data for TPPS + glucose and glycerol.

Table S2. Fluorescence lifetimes (τ_n, nanoseconds) and relative abundances (A_n) for TPPS (6.0 μM) in water, and TPPS (6.0 μM) + glucose or glycerol (600 μM) in water at 0 h and 24 h. The lifetimes were determined by single-photon-counting method in water at room temperature. [a] Number of components.

Solution	Time (h)	λ_{em}/nm	n[a]	τ_1	A_1	τ_2	A_2	χ^2
TPPS	0	605	2	1.8	0.49	9.9	0.51	1.19
	24	605	2	1.9	0.72	10.1	0.28	1.03
TPPS + Glucose	0	605	2	2.0	0.49	10.2	0.51	1.05
	24	605	2	1.9	0.84	10.1	0.16	1.07
TPPS + Glycerol	0	605	2	1.9	0.63	10.2	0.37	1.04
	24	605	2	1.9	0.91	10.2	0.09	1.08

[a] Number of components.

Dynamic light scattering data for TPPS and TPPS + glucose.

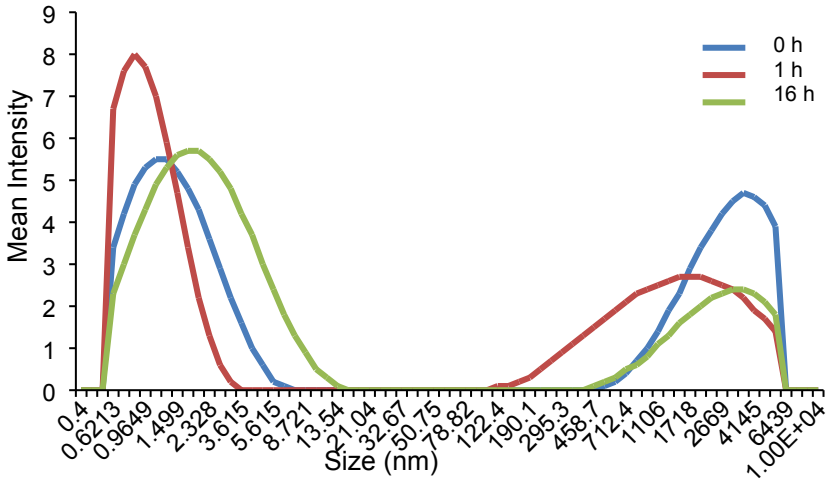

Fig. S10. Size distribution and mean intensities for the dynamic light scattering study of TPPS **1** (6.0 μM) in water at 25 ˚C at 0, 1 and 16 h after sample preparation.

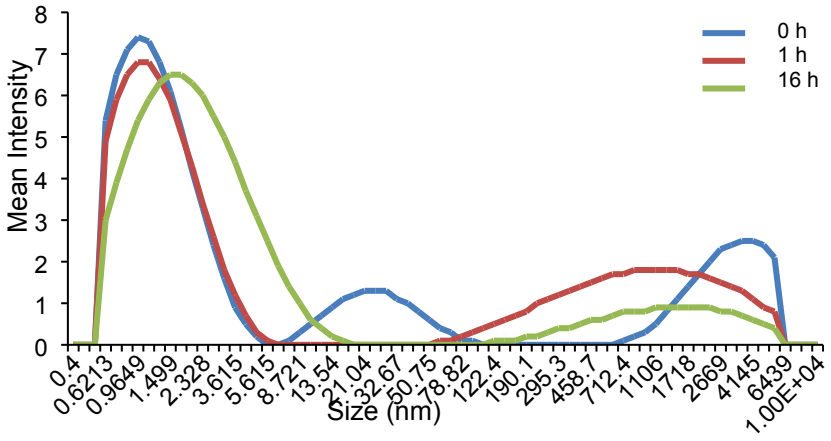

Fig. S11. Size distribution and mean intensities for the dynamic light scattering study of TPPS **1** (6.0 μM) + glucose (600 μM) in water at 25 ˚C at 0, 1 and 16 h after sample preparation.

Table S3. DLS-derived average particle sizes and relative abundances for TPPS and TPPS + D-glucose, 0, 1 and 16 h after sample preparation. Measurements were made at 25 °C.

Time (h)	TPPS in Water (6 μM)		TPPS in Water (6 μM) + D-Glucose (600 μM)	
	Average Size (nm)	Relative Abundance (%)	Average Size (nm)	Relative Abundance (%)
0	1.63 3011	56 44	1.39 27.8 3148	65 13 22
1	1.12 1832	55 45	1.46 1415	63 37
16	2.74 1361	74 26	3.21 1742	84 16