Electronic Supplementary Information

Enhanced catalytic activity in organic solvents using molecularly dispersed haemoglobin-polymer surfactant constructs

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Supporting Methods

All chemicals were purchased from Sigma-Aldrich and used without purification unless stated.

Synthesis of [C-Hb][S]

The cationization of haemoglobin was achieved *via* carbodiimide-mediated activation of the surface accessible amino acid carboxylate groups. In a typical procedure, a haemoglobin solution (5 mg mL^{-1}) was centrifuged and dialyzed (Visking dialysis tubing 12-14000 Da MWCO) extensively against distilled water to remove any impurities before surface bioconjugation. Subsequently, 5 mL of a *N,N'*-Dimethyl-1,3-propanediamine (DMPA, 1.6 M) solution was added drop-wise to 5 mL of haemoglobin (2 mg mL^{-1}) with continuous stirring. The coupling reaction was initiated by the addition of 200 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The reaction mixture was maintained at pH 6 with controlled additions of 0.2 M of HCl for 6 h. The resultant mixture was centrifuged, filtered (Millex Millipore 0.22 micron) and dialyzed (Visking dialysis tubing 12-14000 Da MWCO) against distilled water for 48 h to obtain a stable solution of DMPA-cationized haemoglobin (**C**-Hb). Cationized Hb/polymer-surfactant conjugates ([**C**-Hb][**S**]) were prepared by mixing 10 mL of **C**-Hb (0.5 mg mL^{-1}) with 25 mg of neat poly(ethylene glycol) 4-nonylphenyl 3-sulfopropyl ether, potassium salt (**S**) and the solution was stirred for 24 h. Any resulting precipitate was removed using centrifugation and the supernatant dialyzed (Visking dialysis tubing 12-14000 Da MWCO) against distilled water for 24 h to remove any unbound polymer surfactant molecules. The aqueous [**C**-Hb][**S**] conjugate solution was lyophilized for 48 h to produce a light brown low-density powder, which melted to produce a viscous translucent dark brown liquid after annealing at 30°C.

[C-Hb][S] Characterization

[**C**-Hb][S] nanoconstructs were studied using several laboratory techniques under both aqueous and non-aqueous conditions. Dynamic light scattering experiments (Malvern Nano-Z) were performed on 0.5 mg mL^{-1} aqueous solutions of polymer surfactant **S**, Hb, **C**-Hb, [**C**-Hb][**S**] conjugates, and on [**C**-Hb][**S**] dispersed in ethanol, isopropanol and acetonitrile. Zeta potential measurements (Malvern Nano-Z) were performed on 1.0 mg mL^{-1} aqueous solutions of Hb and **C**-Hb. MALDI-TOF mass spectroscopy (Applied Biosystems, 4700 Proteomics analyzer) was performed on Hb and **C**-Hb and the change in mass was used to determine the cationization efficiency. UV-Vis (Perkin Elmer Lambda 25; quartz cell with a path length of 10 mm) and circular dichroism (Jasco J-815 CD spectrometer; quartz cell with a path length of 1 mm) spectroscopy experiments were performed on aqueous solutions of Hb, **C**-Hb, [**C**-Hb][**S**], and on [**C**-Hb][**S**] dispersed in ethanol, isopropanol and acetonitrile with an integration time of 1 s. The CD spectrum from each sample was deconvoluted and the level of secondary structure elucidated using Dichrowed on-line circular dichroism analysis software with neural network K2D program.[1] UV-Vis spectroscopy was also performed on hemin dispersed in ethanol (0.01 mg mL^{-1}), isopropanol (0.03 mg mL^{-1}) and acetonitrile (0.05 mg mL^{-1}).

Enzyme Kinetics

To perform assays in aqueous or organic environments, stock solutions of [**C**-Hb][**S**] (3.0 μM) and *o*-phenylenediamine (OPD, 16 mM) were prepared by dissolving required quantities in distilled water, acetonitrile, ethanol or isopropanol. Since lyophilized Hb is not soluble in any of the chosen organic solvents, stock solutions of native Hb (3.0 μM) were prepared in distilled water and then injected into each organic solvent/substrate assay mixture. Typical assay mixtures consisted of 1.5 mL of solvent, 200 μL of OPD (4 to 16 mM) and 100 μL of hydrogen peroxide (300 mM) and reactions were triggered by injection of 200 μL of native Hb or [**C**-Hb][**S**] solutions. The initial rate of reaction was obtained by measuring the increase in the absorbance of the product (phenazine) at 426 nm (ε = 29000 $M^{-1}cm^{-1}$) over two-minute. Each assay was performed three times and the average initial rate values were used to determine the catalytic turnover rates (k_{cat}) and Michaelis constant (K_m) with using the Michaelis-Menten equation.

Supporting Figures and Tables

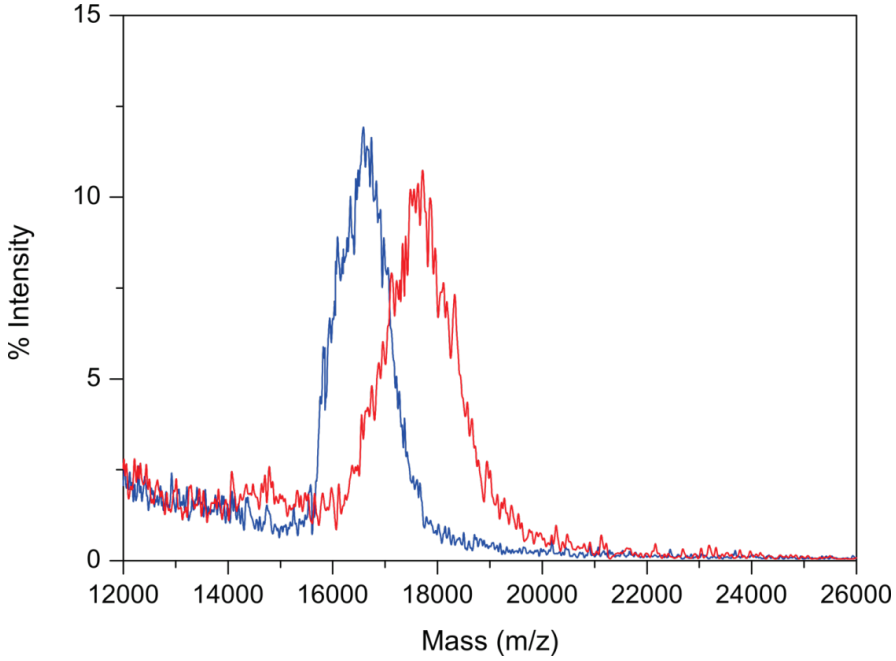

Fig. S1. MALDI-TOF mass spectra of native Hb monomer (blue) and **C**-Hb monomer (red) showing the increase in the average molecular weight of the subunit from 16587 to 17709 Da, which equated to approximately 11 DMPA molecules per subunit.

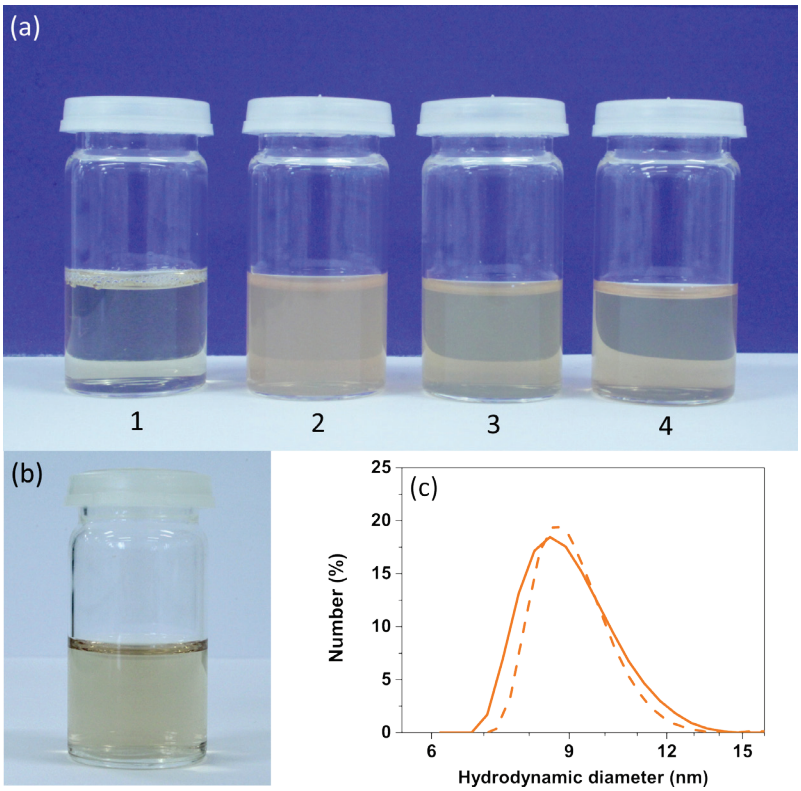

Fig. S2. (**a**) Native Hb in water (1), and after addition of aqueous Hb to acetonitrile (2), ethanol (3) and isopropanol (4). (**b**) [**C**-Hb][**S**] conjugate dispersion in acetonitrile after six months. (**c**) DLS particle size distributions of [**C**-Hb][**S**] in acetonitrile after 1 day (orange line) and six months (dashed orange line).

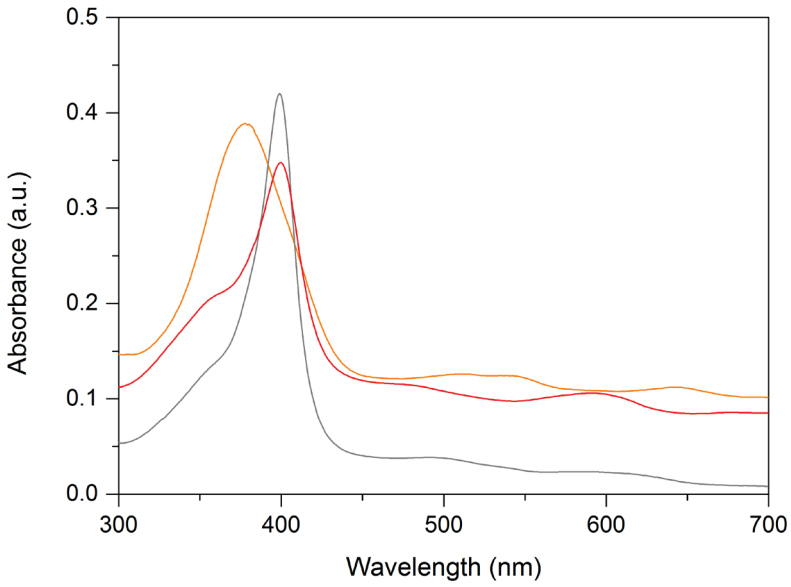

Fig. S3. UV-Vis spectra from hemin dispersed in acetonitrile (orange), ethanol (grey) and isopropanol (red) at 25°C.

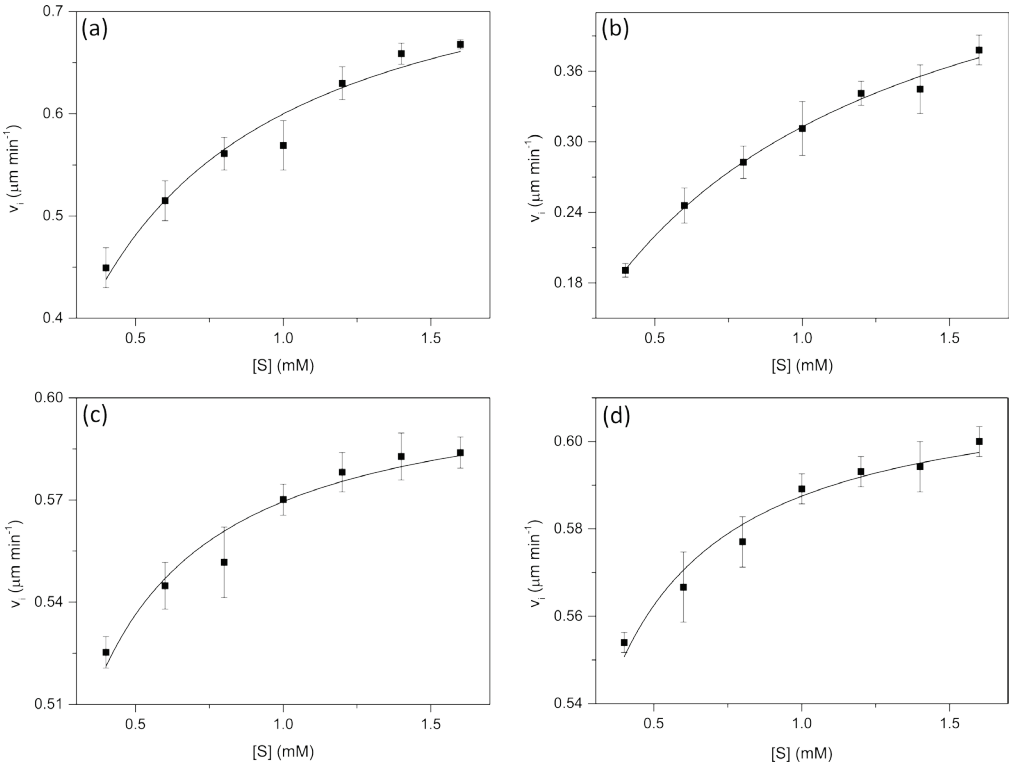

Fig. S4. Initial rate as a function of *o*-phenylenediamine concentration for Hb (0.3 μM) in (**a**) water, (**b**) acetonitrile, (**c**) ethanol and (**d**) isopropanol at 25°C and a H_2O_2 concentration of 15 mM. Solid lines show the fits using the Michaelis-Menten equation.

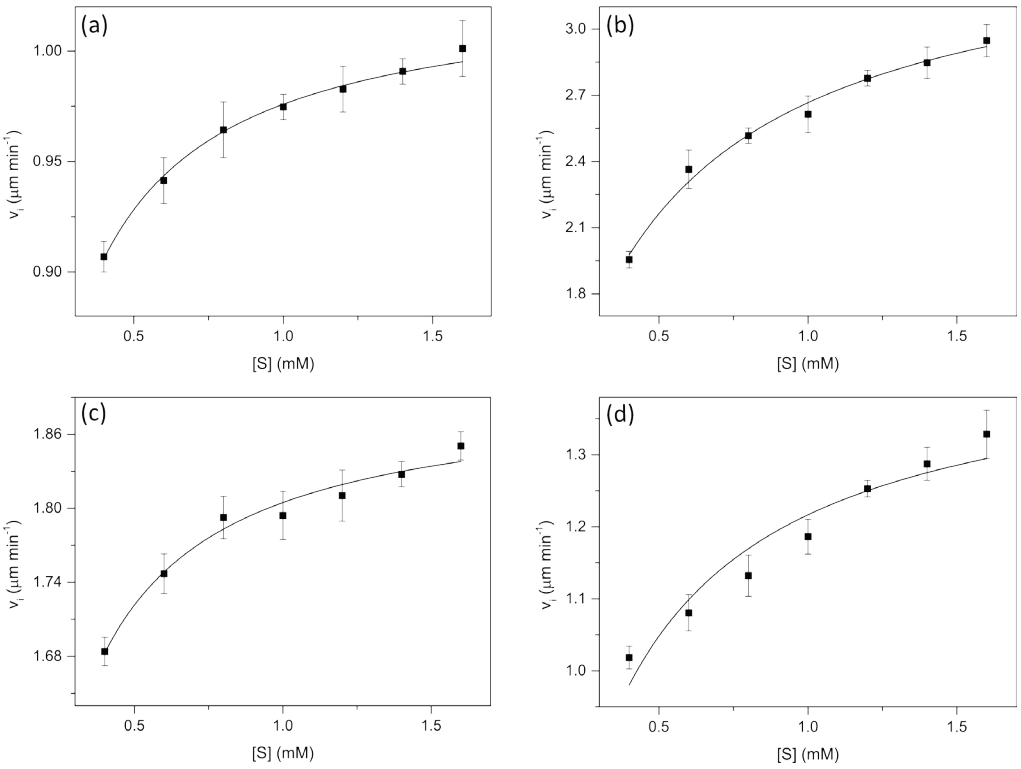

Fig. S5. Initial rate as a function of *o*-phenylenediamine concentration for [**C**-Hb][**S**] (0.3 μM) in (**a**) water, (**b**) acetonitrile, (**c**) ethanol and (**d**) isopropanol at 25°C and a H_2O_2 concentration of 15 mM. Solid lines show the fits using the Michaelis-Menten equation.

Table S1. Kinetic parameters resulting from Michaelis-Menten analyses of native Hb and [**C**-Hb][**S**] in water and organic solvents at a hydrogen peroxide concentration of 15 mM and an *o*-phenylenediamine (OPD) concentration range of 0.4 - 1.6 mM . The assay mixture contained 15% and 5% water w/v for native Hb and [**C**-Hb][**S**] conjugates, respectively.

Solvent	Dielectric Constant	Native Haemoglobin		[**C**-Hb][**S**] Conjugate	
		k_{cat} (min^{-1})	K_m (mM)	k_{cat} (min^{-1})	K_m (mM)
isopropanol	18.2	0.205 ± 0.001	0.043 ± 0.002	0.48 ± 0.01	0.19 ± 0.03
ethanol	24.3	0.202 ± 0.001	0.066 ± 0.006	0.631 ± 0.003	0.049 ± 0.004
acetonitrile	36.6	0.186 ± 0.003	0.77 ± 0.02	1.16 ± 0.02	0.30 ± 0.02
water	80.0	0.270 ± 0.005	0.34 ± 0.03	0.344 ± 0.001	0.056 ± 0.002

References

1. (a) L. Whitmore and B. A. Wallace. *Biopolymers*., 2008, **89**, 392; (b) L. Whitmore and B. A. Wallace. *Nucleic Acids Res*., 2004, **32**, W668.