Electronic Supplementary Material (ESI) for Journal of Materials Chemistry

Supporting Information

Ultra-Stable Hemoglobin-Poly(Acrylic Acid) Conjugates

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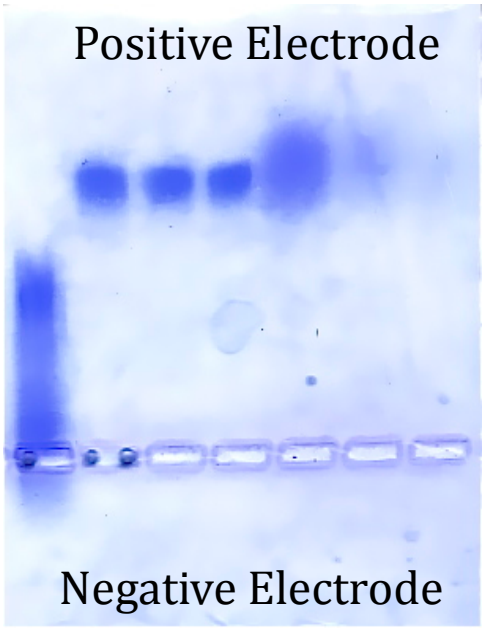


Lane # 1 2 3 4 5 6 7

Figure S1. Agarose gel of Hb (lane 1), Hb-PAA(100) samples synthesized at pH 6, 7, and 8 (lanes (104, respectively) and Hb-PAA(1000) samples synthesized pH 6, 7, and 8, (lanes 5-7, respectively) spotted at the center of the gel (0.5% agarose, 40 mM Tris acetate, pH 7). Hb, migrated towards the negative electrode, while all the three Hb-PAA(100) covalent conjugates moved towards the positive electrode. These lanes did not show any unreacted Hb.

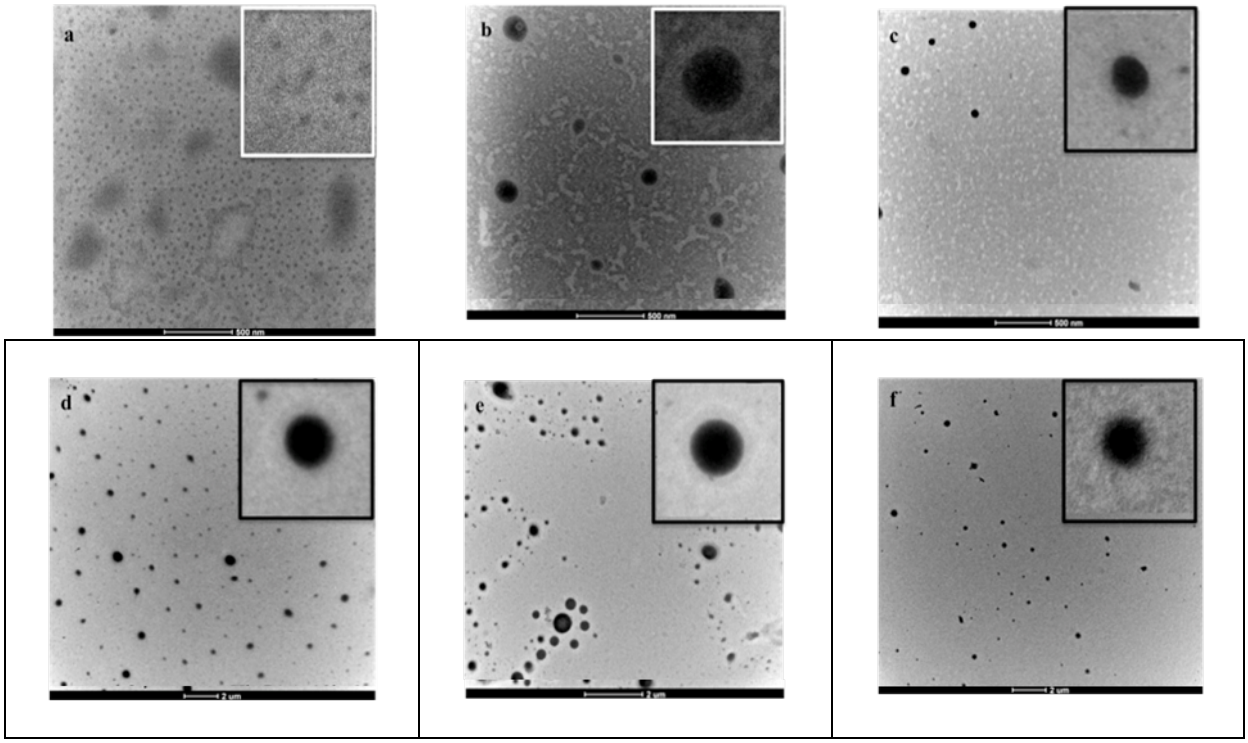


Figure S2. TEM images of Hb-PAA(100) and Hb-PAA(1000) samples synthesized at pH 6, 7 or 8 after ruthenium oxide staining: **a)** Hb-PAA(100)-6, **b)** Hb-PAA(100)-7, **c)** Hb-PAA(100)-8, **d)** Hb-PAA(1000)-6, **E)** Hb-PAA(1000)-7 and **F)** Hb-PAA(1000)-8. In some micrographs, the dark spots at the centers (insets) are clearly surrounded by a very light corona.

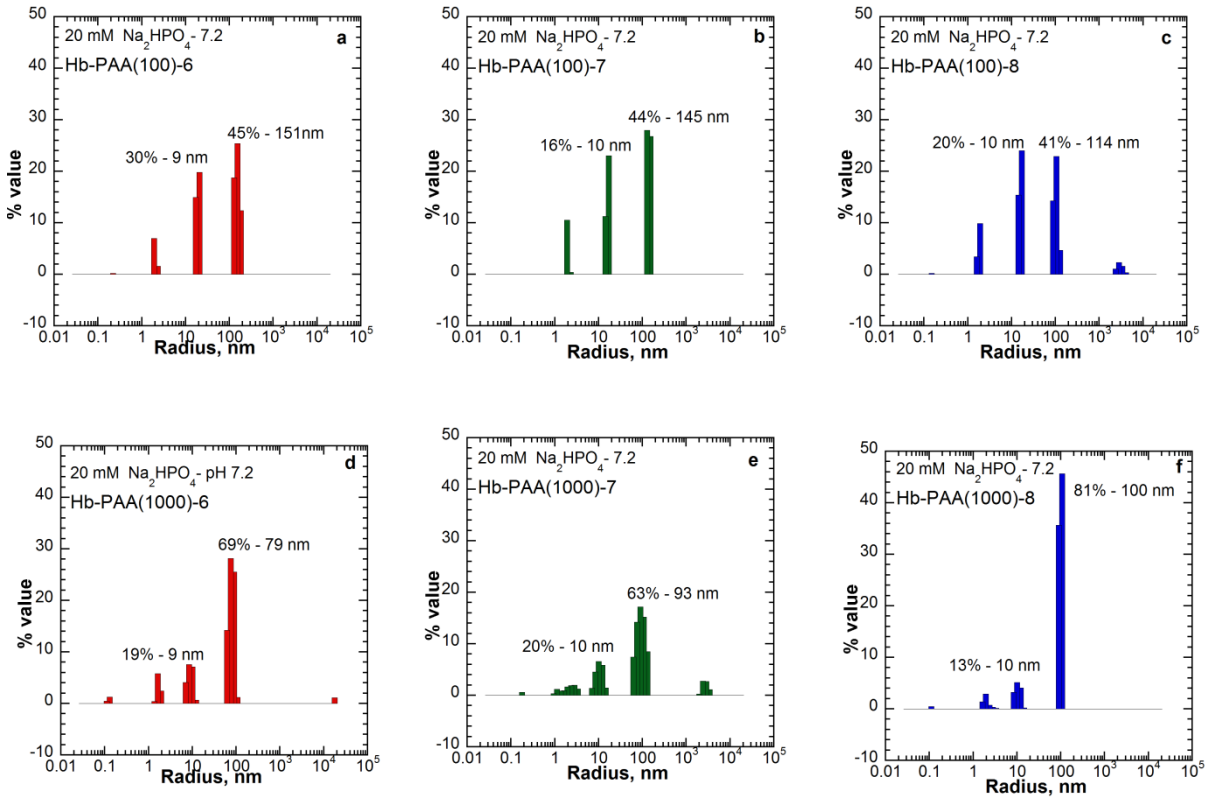


Figure S3. Dynamic light scattering plots for Hb-PAA(100) and Hb-PAA(1000) conjugates synthesized at pH 6, 7 and 8, respectively. All samples were in 20 mM Na_2HPO_4 buffer.

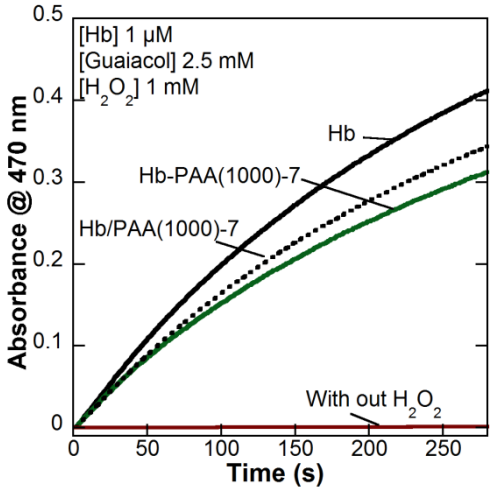


Figure S4. Representative kinetic traces showing the peroxidase-like activities of Hb-PAA(1000)-7 (green curve) in comparison to that of Hb (black curve) and physical mixture of Hb/PAA (black dotted line). There has been no reaction in the absence of H_2O_2 (red curve) (1 µM protein, 2.5 mM guaiacol and 1 mM H_2O_2 in 20 mM Na_2HPO_4 buffer, pH 7.2).

Table S1.Optimized Reaction Conditions for the synthesis of Hb-PAA(100)-6/7/8 and Hb-PAA(1000)-6/7/8 from Hb (10 µM, 500 µL, 50 nmol), PAA (270/2700 µL, 1 or 10 mM, 5 or 50 µmol, 1:100 or 1:1000 Hb to PAA mole ratio) and EDC (100 mM, 95.8 mg, 500 µmol) in sodium dibasic phosphate buffer.

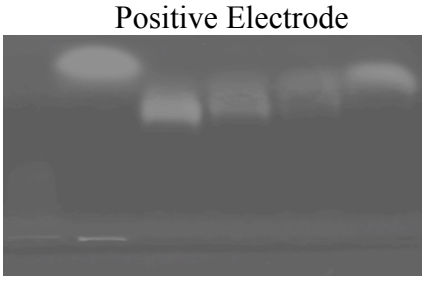
Sample	[PAA] (mM)	PAA Vol (µL)	Moles PAA	Buffer pH	Buffer Vol (µL)	DI Vol (µL)
Hb-PAA(100)-6	1	270	5 µmol	6	500	3730
Hb-PAA(100)-7	1	270	5 µmol	7	500	3730
Hb-PAA(100)-8	1	270	5 µmol	8	500	3730
Hb-PAA(1000)-6	10	2700	50 µmol	6	500	1300
Hb-PAA(1000)-7	10	2700	50 µmol	7	500	1300
Hb-PAA(1000)-8	10	2700	50 µmol	8	500	1300

Table S2. Optimized Reaction Conditions for Synthesis of Polyamine Cross-linked Bioconjugates from Hb-PAA(1000)-8 (5 µM, 2500 µL, 25 nmol), polyamine (en [2 M], teta [1 M] or tepa [1 M]) and EDC (100 mM, 95.8 mg, 500 µmol) in 20 mM Na_2HPO_4, pH 8

Sample	[en] (mM)	Moles en	[teta] (mM)	Moles teta	[tepa] (mM)	Moles tepa
Hb-PAA(1000)-8-5en	5	25 µmol	-	-	-	-
Hb-PAA(1000)-8-10en	10	50 µmol	-	-	-	-
Hb-PAA(1000)-8-20en	20	100 µmol	-	-	-	-
Hb-PAA(1000)-8-40en	40	200 µmol	-	-	-	-
Hb-PAA(1000)-8-60en	60	300 µmol	-	-	-	-
Hb-PAA(1000)-8-5teta	-	-	5	25 µmol	-	-
Hb-PAA(1000)-8-10teta	-	-	10	50 µmol	-	-
Hb-PAA(1000)-8-20teta	-	-	20	100 µmol	-	-
Hb-PAA(1000)-8-40teta	-	-	40	200 µmol	-	-
Hb-PAA(1000)-8-60teta	-	-	60	300 µmol	-	-
Hb-PAA(1000)-8-5tepa	-	-	-	-	5	25 µmol
Hb-PAA(1000)-8-10tepa	-	-	-	-	10	50 µmol
Hb-PAA(1000)-8-20tepa	-	-	-	-	20	100 µmol
Hb-PAA(1000)-8-40tepa	-	-	-	-	40	200 µmol
Hb-PAA(1000)-8-60tepa	-	-	-	-	60	300 µmol

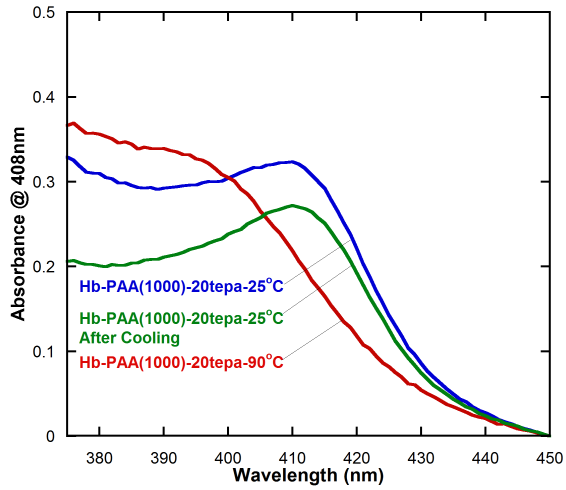
Table S3. Specific activities ($x10^{-3}$ μM/mg) of Hb-PAA nanoparticles measured at 25 ˚C, 20 mM phosphate buffer, pH 7.2.

Sample	Specific activity ($x10^{-3}$ μM/mg)
Hb	2. 5 ± 0.3
Hb/PAA(100)	2. 3 ± 0.07
Hb-PAA(100)- 6	2. 4 ± 0.5
Hb-PAA(100)- 7	2. 0 ± 0.2
Hb-PAA(100)- 8	1. 4 ± 0.07
Hb/PAA(1000)	2. 0 ± 0.07
Hb-PAA(1000)- 6	2. 7 ± 0.4
Hb-PAA(1000)- 7	2. 6 ± 0.07
Hb-PAA(1000)- 8	2. 4 ± 0.1

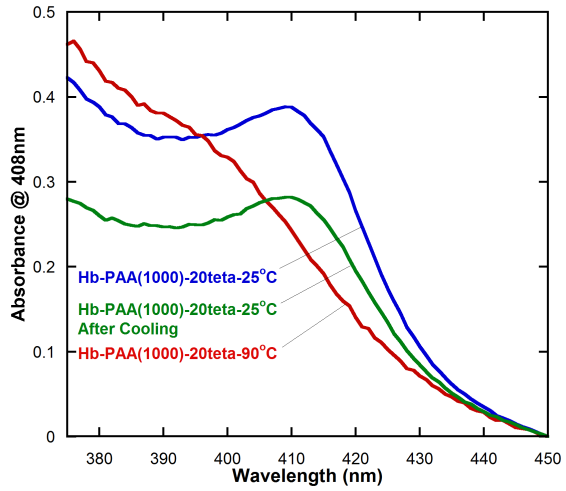


Lane #	1	2	3	4	5	6
EDC	-	+	+	+	+	+
teta (mM)		-	60	40	20	10

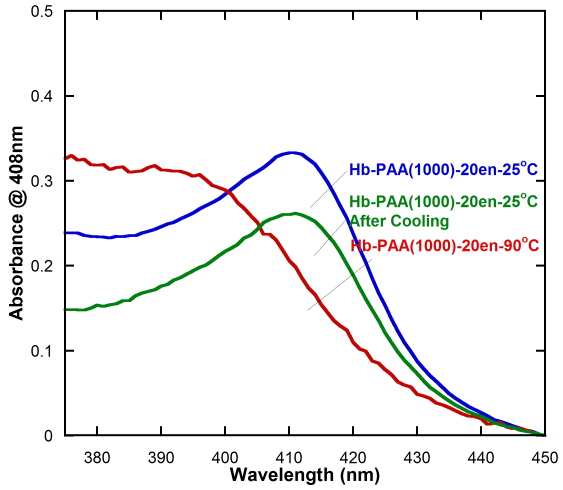
Figure S5. Agarose gel (0.5%) of Hb (lane 1), Hb-PAA(1000) (lane 2), and Hb-PAA(1000)-teta (lanes 3-6) synthesized in phosphate buffer, pH 8. The samples were spotted at the bottom of the gel (running buffer, 40 mM Tris acetate pH 7.2). While Hb moved as a streak (lane 1, faint band), Hb-PAA(1000)-8 (lane 2) and Hb-PAA(1000)-teta (lanes 3-6, crosslinked with 60, 40, 20 and 10 mM teta, respectively) showed single bands. Hb-PAA(1000)-teta crosslinked nanoparticles moved lesser toward the positive electrode than Hb-PAA(1000)-8 due to neutralization of the negative charge by the reaction with the amine.



a.



b.



c.

Figure S6 a-c. Absorbance spectra of Hb-PAA(1000)-8-polyamine conjugates before heat denaturation at room temperature (blue curve), during denaturation at 90 ˚C (red curve) and after cooling back to room temperature (green curve), panels as labeled. Thermal denaturation clearly shifted the Soret band to higher temperatures and upon cooling most of the Soret band intensity and position are recovered, which suggests a high degree of reversibility of the thermal denaturation of these samples. In contrast, Hb underwent near complete irreversible thermal denaturation, under the same conditions.