Supporting Information

Hemoglobin-loaded ZIF-8 Nanoparticles Equipped with PEGylated Metal-phenolic Network Coatings: An Oxygen Carrier with Antioxidant and Stealth Properties

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Protocol SLS-Hb method

To quantify the Hb concentration in the Hb@ZIF-8 NPs, the SLS-Hb method was employed. Different dilutions of the Hb stock solution (used as a standard) and Hb@ZIF-8 NPs were prepared to obtain Hb concentration in the range 2 mg mL^{-1}. EDTA was used to dissasemble the NPs. Specifically, 10 µL from each diluted standard or Hb@ZIF-8-NPs were pipetted in triplicate into a 96-well plate (Nunclon™ Delta Surface), and 200 µL of SLS (0.6 mg mL^{-1} in MQ) was added to each well. After covering the plate with aluminum foil, it was placed on a shaker for 5 min at RT, and the Abs at 534 nm was measured using a plate reader (Tecan Spark, Tecan Group Ltd, Männedorf, Switzerland). The average Abs of each standard was plotted against its known concentration. Linear fitting was used to display a trendline, in which equation and R^2-value were calculated. Finally, the obtained equations were used to determine the concentration of Hb in the Hb@ZIF-8 NPs.

Labelling of BSA with FITC

Briefly, a FITC solution (1 mL, 10 mg mL^{-1} in DMSO) was added dropwise to a BSA solution (20 mL, 5 mg mL^{-1} in 50 mM $NaHCO_3$, pH 10) in the dark and left it incubating overnight at RT. During the next two days, the excess of FITC was removed by dialysis (MWCO 12.4 kDa) using MQ. The resulting solution was freeze dried and stored at -20 °C for future use.

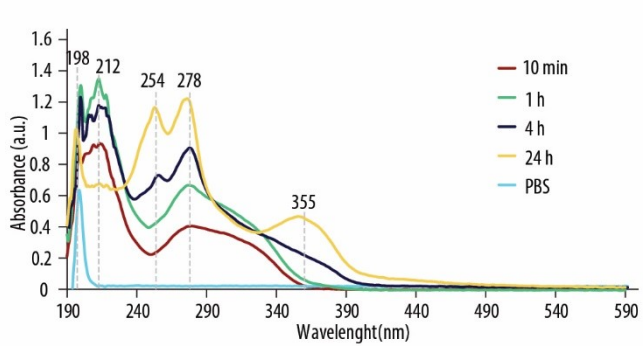

Figure S1. UV-vis spectra of PBS and a solution of TA in PBS following incubation at 37 °C for different time intervals (i.e., 10 min, 1, 4, and 24 h).

$FeCl_3$ (mg mL^{-1})	ζ-potential (mV)	Conductivity (mS cm^{-1})
0	-6.7 ± 0.1	0.0133 ± 0.0022
0.015	-11.3 ± 0.1	0.0222 ± 0.0139
0.030	-18.9 ± 7.7	0.0235 ± 0.0066
0.06	-19.9 ± 5.9	0.0194 ± 0.0003
0.10	-21.8 ± 4.0	0.0199 ± 0.0078
0.15	-23.3 ± 0.1	0.0234 ± 0.0129
0.20	-26.1 ± 3.5	0.0161 ± 0.0045

Table S1. ζ-potential and conductivity values of the $FeCl_3$ screening performed with Hb@ZIF-8/MPN NPs prepared with 0.4 mg mL^{-1} TA. Results are expressed as mean ± standard deviation from two independent samples.

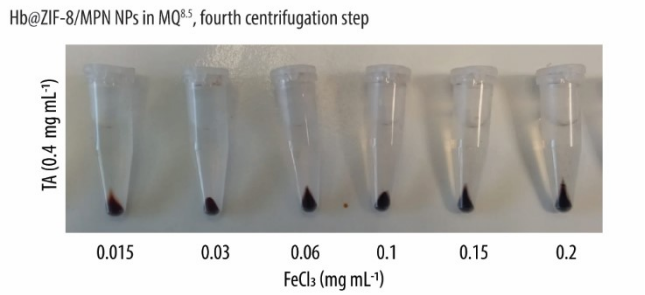

Figure S2. Photographic images after the fourth centrifugation step of Hb@ZIF-8/MPN NPs in MQ at pH 8.5 prepared during the $FeCl_3$ screening.

TA (mg mL^{-1})	ζ-potential (mV)	Conductivity (mS cm^{-1})
0	-6.7 ± 0.1	0.013 ± 0.002
0.1	-2.6 ± 1.5	0.013 ± 0.009
0.2	-1.4 ± 0.6	0.008 ± 0.003
0.4	-8.2 ± 2.7	0.010 ± 0.002
0.8	-20.7 ± 6.2	0.013 ± 0.003
1	-18.7 ± 2.4	0.013 ± 0.006
2	-19.4 ± 0.8	0.017 ± 0.005
4	-17.3 ± 0.6	0.011 ± 0.008

Table S2. ζ-potential and conductivity values of the TA screening performed with Hb@ZIF-8/MPN NPs prepared with 0.015 mg mL^{-1} $FeCl_3$. Results are expressed as mean ± standard deviation from two independent samples.

TA (mg mL^{-1})	ζ-potential (mV)	Conductivity (mS cm^{-1})
0.1	-19.6 ± 0.1	0.0010 ± 0.0060
0.2	-17.7 ± 0.1	0.0103 ± 0.0068
0.4	-26.4 ± 1.1	0.0128 ± 0.0045
0.8	-31.3 ± 0.4	0.0098 ± 0.0065
1	-32.4 ± 1.2	0.0159 ± 0.0014
2	-30.7 ± 2.1	0.0065 ± 0.0021
4	-29.8 ± 0.3	0.0161 ± 0.0003

Table S3. ζ-potential and conductivity values of the TA screening performed with Hb@ZIF-8/MPN NPs prepared with 0.15 mg mL^{-1} $FeCl_3$. Results are expressed as mean ± standard deviation from two independent samples.

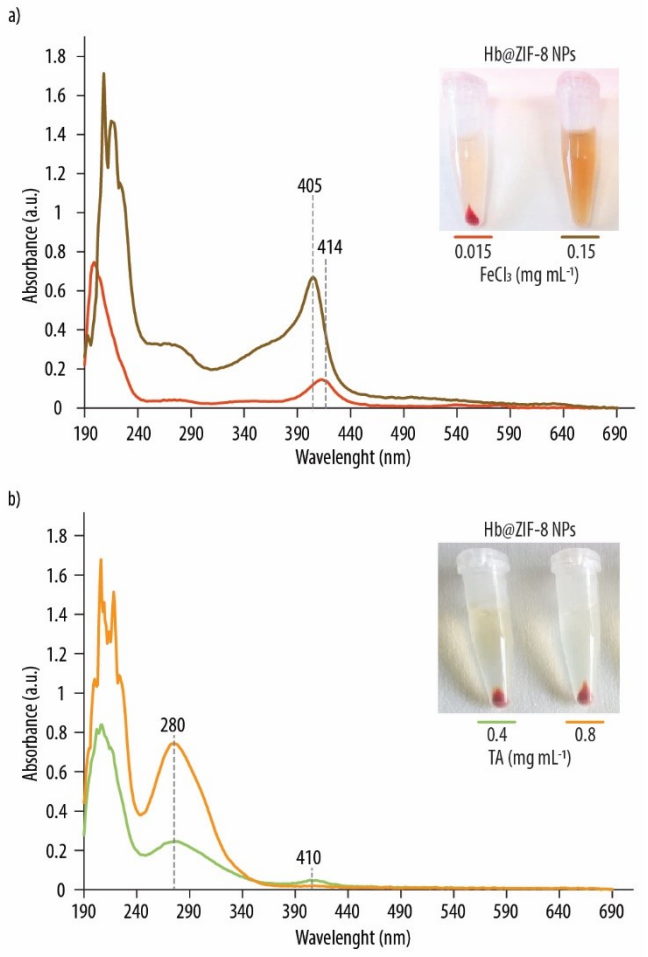

Figure S3. UV-vis spectra of the supernatants after centrifugation following incubation of Hb@ZIF-8 NPs in MQ at pH 8.5 with $FeCl_3$ (0.015 and 0.15 mg mL^{-1}) in the absence of tannic acid (TA). Photographic images showing the appearance of the Hb@ZIF-8 NPs after centrifugation following incubation with $FeCl_3$.

	ζ-potential (mV)	Conductivity (mS cm^{-1})
Hb@ZIF-8 NPs	-6.6 ± 0.7	0.025 ± 0.003
Hb@ZIF-8/0.2 MPN NPs	-13.8 ± 0.6	1.100 ± 0.099
Hb@ZIF-8/0.4 MPN NPs	-16.9 ± 0.1	1.075 ± 0.035
Hb@ZIF-8/0.8 MPN NPs	-23.2 ± 0.8	1.140 ± 0.071
Hb@ZIF-8/MPN/PLL/PEG NPs	-13.6 ± 0.2	1.060 ± 0.000

Table S4. ζ-potential and conductivity values of the different NPs following incubation in PBS for 1 h. Hb@ZIF-8 NPs were dissolved in MQ instead, due to their instability in PBS. Results are expressed as mean ± standard deviation from at least two independent samples.

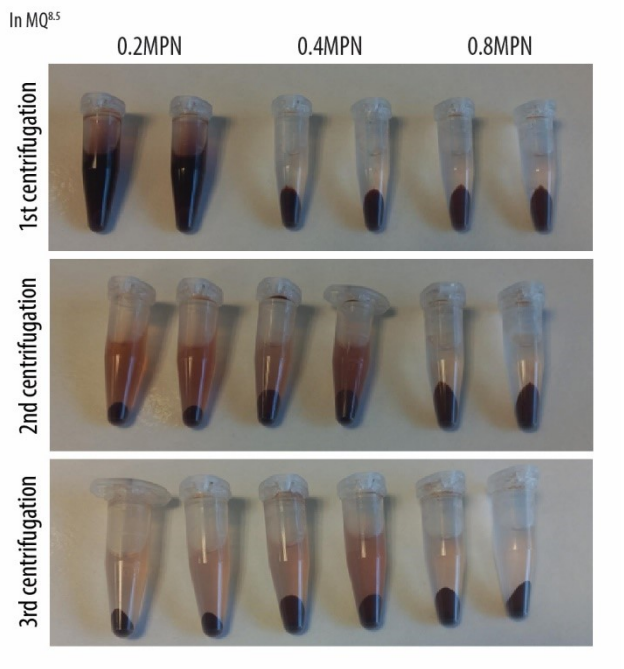

Figure S4. Photographic images of the up-scaled Hb@ZIF-8/MPN NPs in MQ at pH 8.5 in duplicates following each centrifugation step.

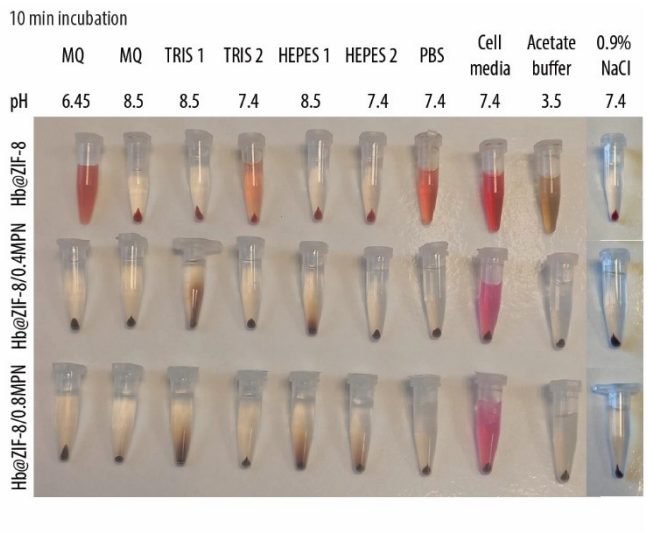

Figure S5. Photographic image of the Hb@ZIF-8 NPs, Hb@ZIF-8/0.4MPN NPs and Hb@ZIF-8/0.8MPN NPs after centrifugation following 10 min incubation at 4 °C in various solvents: milli-Q (MQ), MQ at pH 8.5, TRIS 1 (10 mM TRIS), TRIS 2 (10 mM TRIS and 150 mM NaCl), HEPES 1 (10 mM HEPES), HEPES 2 (25 mM HEPES), PBS, cell media, buffer acetate, and 0.9% NaCl.

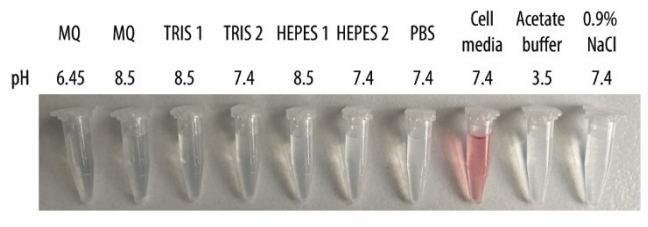

Figure S6. Photographic image of the solvents that were used to perform the stability experiments of the NPs: milli-Q (MQ), MQ at pH 8.5, TRIS 1 (10 mM TRIS), TRIS 2 (10 mM TRIS and 150 mM NaCl), HEPES 1 (10 mM HEPES), HEPES 2 (25 mM HEPES), PBS, cell media (DMEM supplemented with FBS (10% v/v) and penicillin/streptomycin (1% v/v, 10 000 U mL^{-1} and 10 µg mL^{-1}, respectively), acetate buffer (20 mM NaOAc), and 0.9% NaCl.

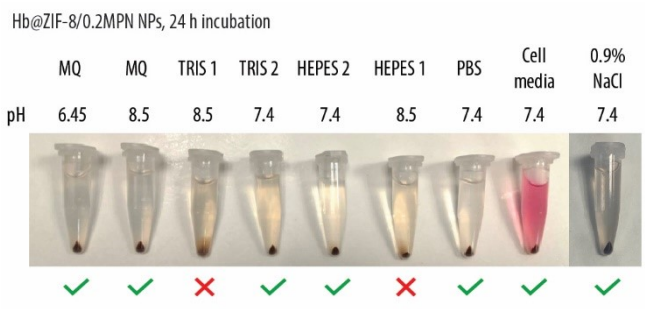

Figure S7. Photographic image of the Hb@ZIF-8/0.2MPN NPs after centrifugation following 24 h incubation at 4 °C in various solvents: milli-Q (MQ), MQ at pH 8.5, TRIS 1 (10 mM TRIS), TRIS 2 (10 mM TRIS and 150 mM NaCl), HEPES 1 (10 mM HEPES), HEPES 2 (25 mM HEPES), PBS, cell media, buffer acetate, and 0.9% NaCl.

PLL (mg mL^{-1})	ζ-potential (mV)	Conductivity (mS cm^{-1})
0	-26.3 ± 2.8	1.290 ± 0.410
0.25	-25.8 ± 2.1	1.265 ± 0.276
0.5	-17.3 ± 5.4	1.435 ± 0.148
1	10.2 ± 3.6	1.255 ± 0.219
2	17.2 ± 1.3	1.125 ± 0.035
4	18.3 ± 0.4	1.080 ± 0.014

Table S5. ζ-potential and conductivity values of the PLL screening performed with the Hb@ZIF-8/0.8 MPN NPs. Results are expressed as mean ± standard deviation from two independent samples.

PEG (mg mL^{-1})	ζ-potential (mV)	Conductivity (mS cm^{-1})
0	17.2 ± 1.3	1.125 ± 0.035
0.05	-0.8 ± 0.1	1.085 ± 0.035
0.1	-8.4 ± 3.5	1.110 ± 0.014
0.25	-9.9 ± 3.1	1.135 ± 0.078
0.5	-12.2 ± 0.6	1.150 ± 0.014
1	-13.5 ± 1.6	1.130 ± 0.014
2	-13.6 ± 0.3	1.120 ± 0.014
5	-14.4 ± 0.6	1.150 ± 0.028

Table S6. ζ-potential and conductivity values of the PEG screening performed with the Hb@ZIF-8/0.8MPN/PLL NPs. Results are expressed as mean ± standard deviation from two independent samples.

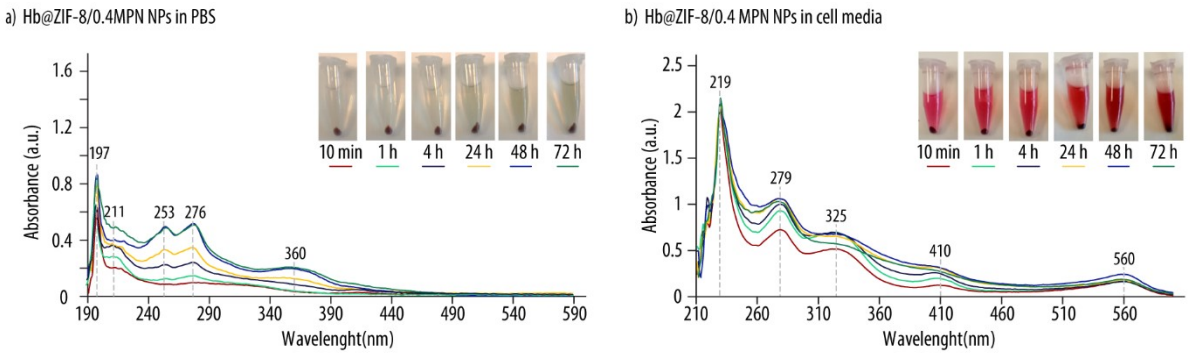

Figure S8. Stability of Hb@ZIF-8/0.4 MPN NPs at 37 °C in PBS or cell media. **a)** Photographic images of Hb@ZIF-8/0.4 MPN NPs after centrifugation following incubation at 37 °C for different periods of time (i.e., from 10 min to 72 h), along with UV-vis spectra of the corresponding SNs in PBS, and **b)** cell media.