Supporting Information

Hemoglobin-loaded ZIF-8 nanoparticles functionalized with human serum albumin as stealth, stable, and biocompatible oxygen carriers

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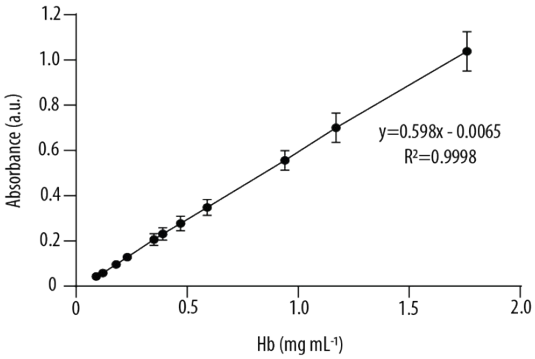

Figure S1. Standard curve for Hb stock, including the obtained linear formula and R^2 value. Dilution series for Hb were prepared and UV-Visible absorbance was measured at 413 nm and plotted against the Hb concentration.

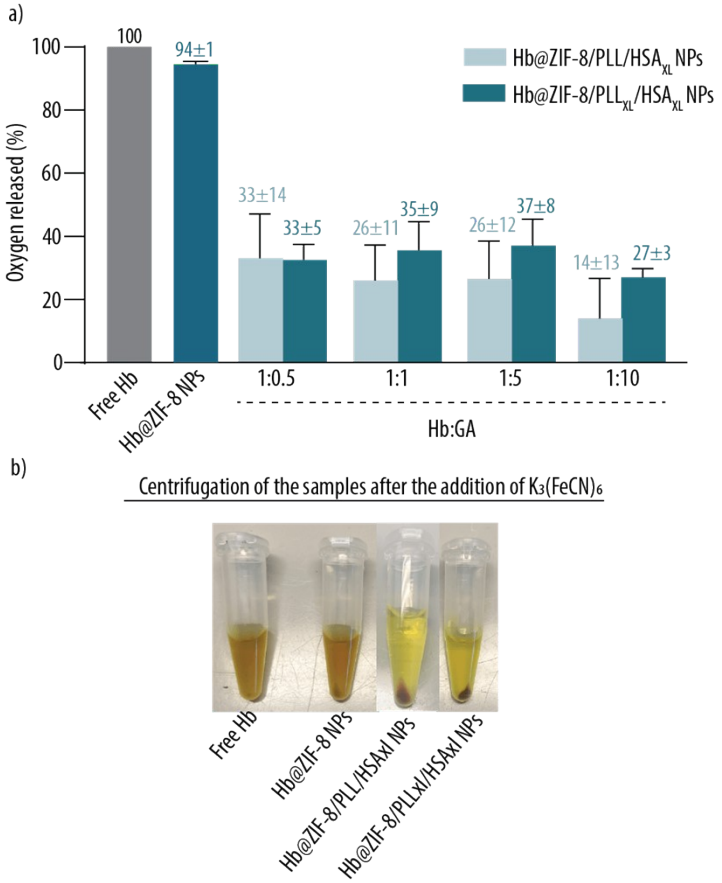

Figure S2. a) Oxygen released from free Hb, Hb-loaded ZIF-8 NPs (Hb@ZIF-8 NPs), and poly-L-lysine (PLL)- and human serum albumin (HSA)-coated Hb@ZIF-8 NPs crosslinked with glutaraldehyde (GA) at different Hb:GA molar ratios. The NPs are denoted as Hb@ZIF-8/PLL/HSA$_{XL}$ and Hb@ZIF-8/PLL$_{XL}$/HSA$_{XL}$ NPs, where the subscript 'XL' indicates the crosslinking with GA. The data has been normalized to the oxygen released from free Hb at equivalent concentrations**. b)** Photographic images of free Hb, Hb@ZIF-8 NPs and Hb@ZIF-8/PLL$_{XL}$/HSA$_{XL}$ NPs after the addition of $K_3[Fe(CN)_6]$ reagent. As seen by the pellet formed after centrifugation, Hb@ZIF-8/PLL/HSA$_{XL}$ and Hb@ZIF-8/PLL$_{XL}$/HSA$_{XL}$ NPs remain intact upon $K_3[Fe(CN)_6]$ addition, unlike free Hb and Hb@ZIF-8 NPs, which disassemble and rapidly convert to metHb. This indicates that direct comparison of oxygen release between free Hb, Hb@ZIF-8 NPs and Hb@ZIF-8/PLL/HSA$_{XL}$ or Hb@ZIF-8/PLL$_{XL}$/HSA$_{XL}$ NPs can be misleading.

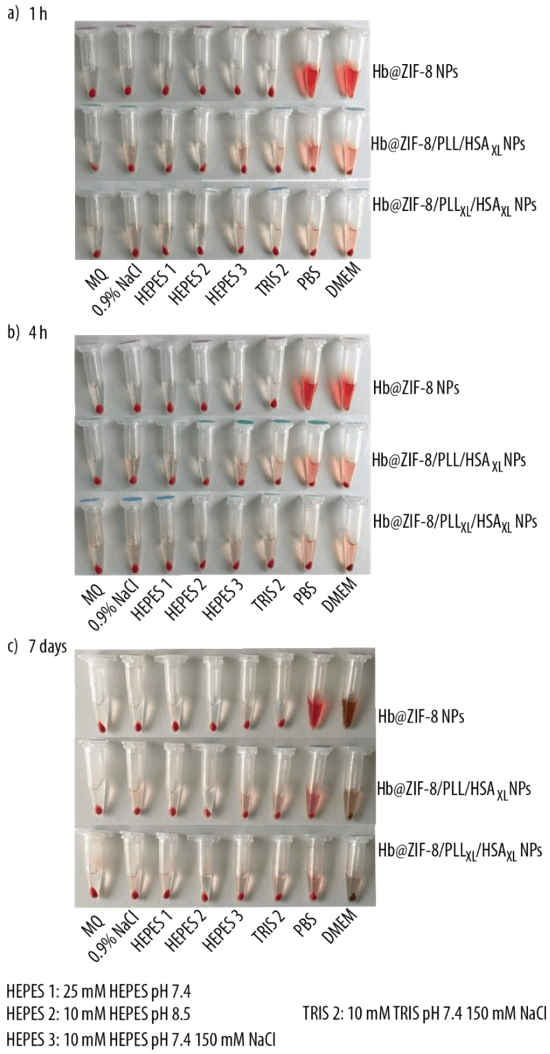

Figure S3. Photographic images of Hb-loaded ZIF-8 NPs (Hb@ZIF-8 NPs), and poly-*L*-lysine (PLL)- and human serum albumin (HSA)-coated Hb@ZIF-8 NPs incubated in Mili-Q water (MQ), saline (0.9% NaCl), 4-(2-hydroxyethyl)piperazine-1-ethane-sulfonic acid (HEPES), tris(hydroxymethyl)aminomethane (TRIS), phosphate buffered saline (PBS) and Dulbecco's Modified Eagle Medium (DMEM) for a) 1 h, b) 4 h and c) 7 days. The NPs are denoted as Hb@ZIF-8/PLL/HSA_{XL} and Hb@ZIF-8/PLL_{XL}/HSA_{XL} NPs, where the subscript 'XL' indicates the crosslinking with glutaraldehyde.

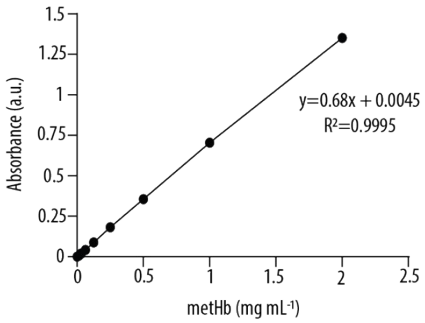

Figure S4. Standard curve for metHb, including the obtained linear formula and R^2 value. Dilution series for metHb were prepared and UV-Visible absorbance was measured at 404 nm and plotted against the metHb concentration.

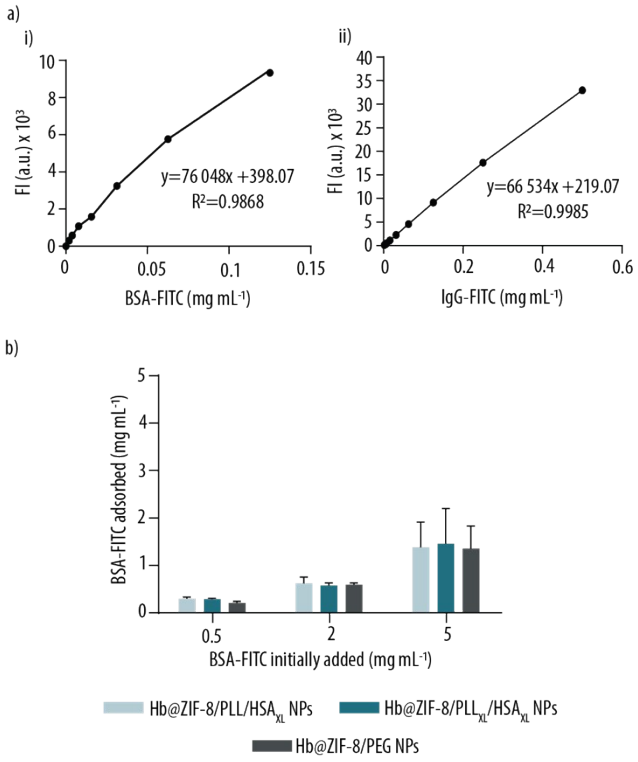

Figure S5. a) Standard curve for Fluorescein 5(6) – Isothiocyanate-labelled i) bovine serum albumin (BSA-FITC) and ii) Immunoglobin G (IgG-FITC), including the obtained linear formula and R^2 value. Dilution series for BSA-FITC and IgG-FITC were prepared, and fluorescence intensity (FI) was measured at an excitation wavelength of 493 nm and an emission wavelength of 516 nm and plotted against the BSA-FITC and IgG-FITC concentration, respectively. b) Concentration of BSA-FITC adsorbed onto 2 mg mL^{-1} Hb-loaded ZIF-8 NPs (Hb@ZIF-8 NPs) coated with poly-*L*-lysine (PLL)- and human serum albumin (HSA) (i.e., Hb@ZIF-8/PLL/HSA_{XL} and Hb@ZIF-8/PLL_{XL}/HSA_{XL} NPs), where the subscript 'XL' indicates the crosslinking with glutaraldehyde, and polyethylene glycol-coated Hb@ZIF-8 NPs (Hb@ZIF-8/PEG NPs). The NPs were prior incubated with increasing concentrations (i.e., 0.5, 2 and 5 mg mL^{-1}) of BSA-FITC.

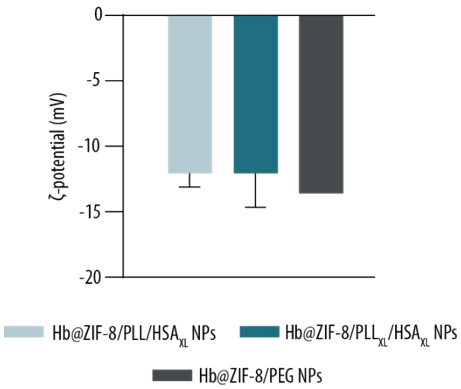

Figure S6. Zeta (ζ)-potential measurements of Hb-loaded ZIF-8 NPs (Hb@ZIF-8 NPs) coated with poly-*L*-lysine (PLL)- and human serum albumin (HSA) (i.e., Hb@ZIF-8/PLL/HSA_{XL} and Hb@ZIF-8/PLL_{XL}/HSA_{XL} NPs), where the subscript 'XL' indicates the crosslinking with glutaraldehyde, and polyethylene glycol-coated Hb@ZIF-8 NPs (Hb@ZIF-8/PEG NPs). Prior the measurement, the NPs were resuspended in 10 mM HEPES pH 7.4 150 mM NaCl and diluted 10× in MQ.

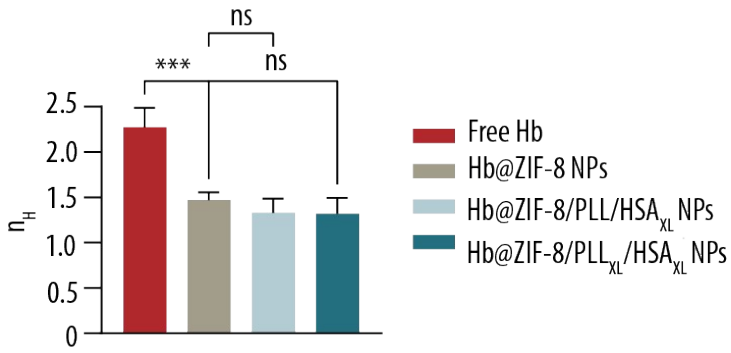

Figure S7. Hill coefficient (n_H) values of free Hb, bare Hb-loaded ZIF-8 NPs (Hb@ZIF-8 NPs) and poly-L-lysine (PLL)- and human serum albumin (HSA)-coated Hb@ZIF-8 NPs, denoted as Hb@ZIF-8/PLL/HSA$_{XL}$ and Hb@ZIF-8/PLL$_{XL}$/HSA$_{XL}$ NPs, where the subscript 'XL' indicates glutaraldehyde crosslinking following PLL and/or HSA deposition. Statistical significance: ns = not significant; ***$p < 0.001$.