Electronic Supplementary Material (ESI) for RSC Advances.

Dextran-based Thermo-Responsive Hemoglobin-Polymer Conjugates with Oxygen-Carrying Capacity

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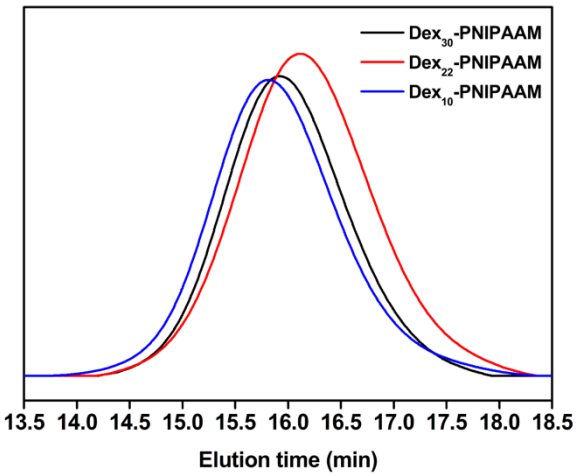

Fig. S1 GPC chromatograms of PNIPAAm grafted chains isolated by hydrolysis of Dex-Cl_{10}-g-PNIPAAm, Dex-Cl_{22}-g-PNIPAAm and Dex-Cl_{30}-g-PNIPAAm.

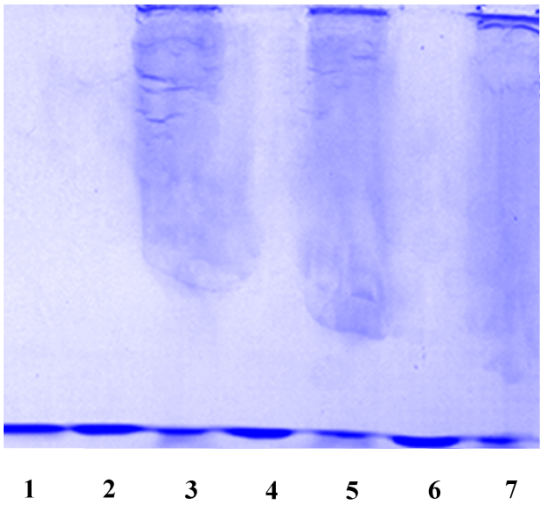

Fig. S2 SDS-PAGE of Hb (lane 1), physical blends of Hb and HOOC-Dex-Cl_x-g-PNIPAAm (lane 2, 4, and 6, corresponding to x=10, 22, and 30 respectively) and Hb-HOOC-Dex-Cl_x-g-PNIPAAm covalent conjugates (lane 3, 5, and 7, corresponding to x=10, 22, and 30 respectively).

SDS-PAGE

A conventional Tris-glycine SDS-PAGE procedure was performed as described previously[1]. Briefly, samples were dissolved in PBS (10 mM, pH 7.4) making sure to contain 0.25 mg/mL Hb, and then boiled for 10 min in the presence of SDS as denaturant and beta-mercaptoethanol as reducing agent. The 4 % and 7 % polyacrylamide gels containing SDS were prepared as stacking gel and separating gel, respectively. The samples were loaded into the gel and electrophoresis was carried out at 50 mV in an ice bath. The gels were directly stained by using Coomassie blue for 2 h and then destained with acetic acid (10%, v/v) overnight.

As shown in Fig. S1, there exist distinct bands at the bottom of the separating gel in lane 1, 2, 4, 6, corresponding to free Hb and

physical blends of Hb and HOOC-Dex-Cl_x-g-PNIPAAm respectively. Hemoglobin was dissociated into its four subunits after denaturation of its secondary and tertiary structures by SDS and mercaptoethanol, resulting in a relative small molecular weight (~15 kD)[2]. While the bands located at the border of the stacking and separating gel in lane 3, 5, 7 were ascribed to the hemoglobin molecules which were covalently linked to polymers (Mn (^{1}H NMR) > 200 kD). The electrophoretic mobility of Hb had dropped too low to migrate in the 7% separating gel, indicating that the Hb molecules were covalently conjugated to the copolymers of dextran grafted with poly(NIPAAM). Besides, at the same position with free Hb (lane 1) appeared a weaker band in lane 3, lane 5, or lane 7, presumably attributed to the uncoupled subunits which were dissociated from the Hb-polymer conjugates in the presence of SDS.

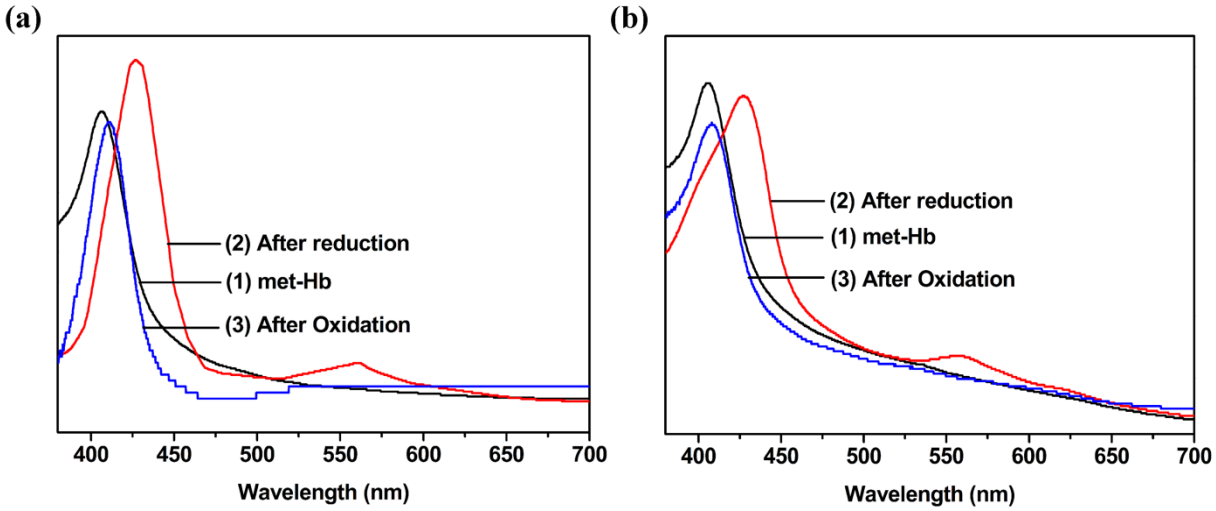

Fig. S3 Redox activity of Hb-Dex-Cl_{22}-g-PNIPAAm (a) and Hb-Dex-Cl_{30}-g-PNIPAAm (b) indicated by the shift of Soret bands from state 1 to 3.

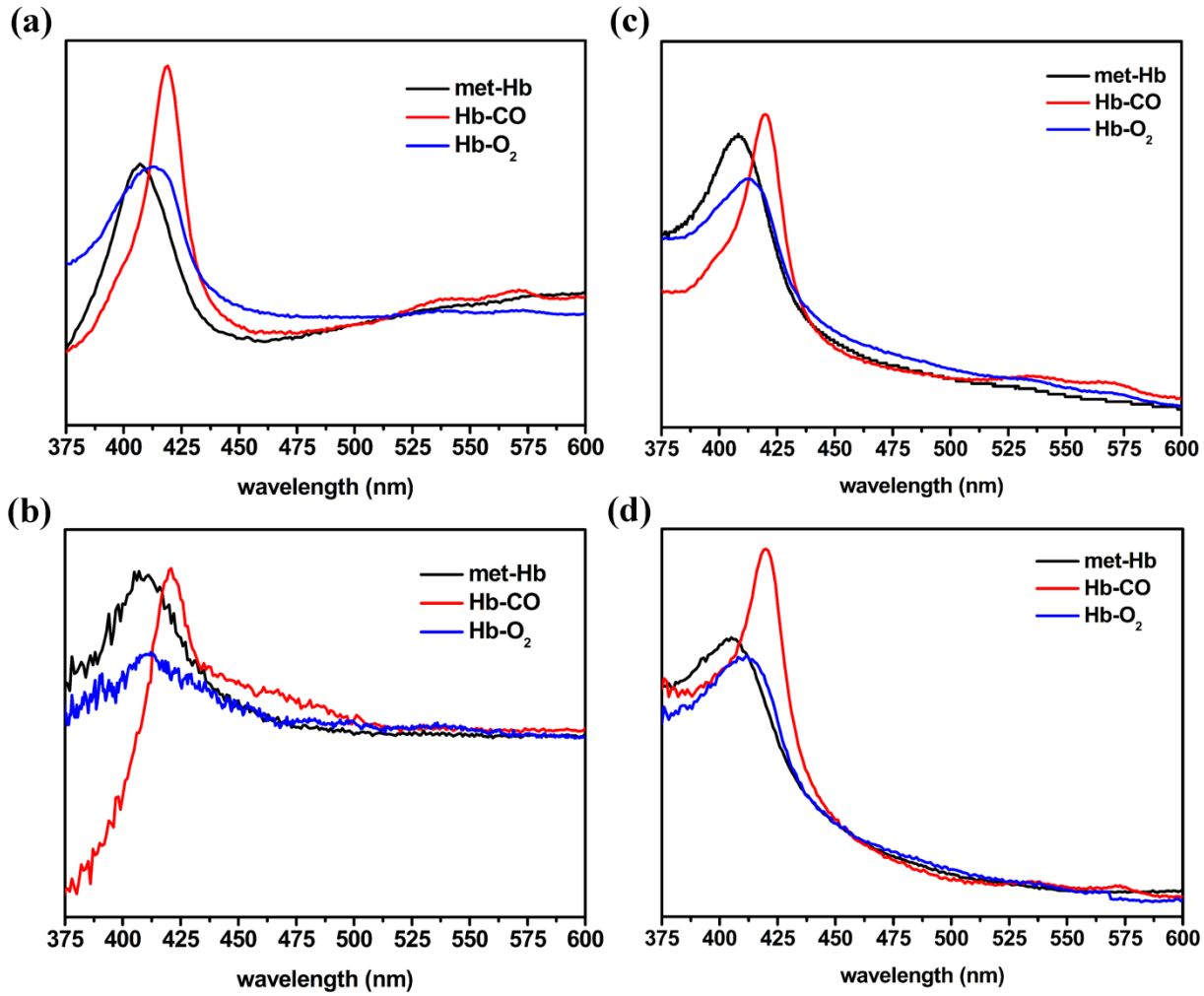

Fig. S4 UV–vis spectra of Hb-Dex-Cl_{10}-g-PNIPAAm at 25 °C (a) and 37 °C (b), Hb-Dex-Cl_{30}-g-PNIPAAm at 25 °C (c) and 37 °C (d) under various atmospheres

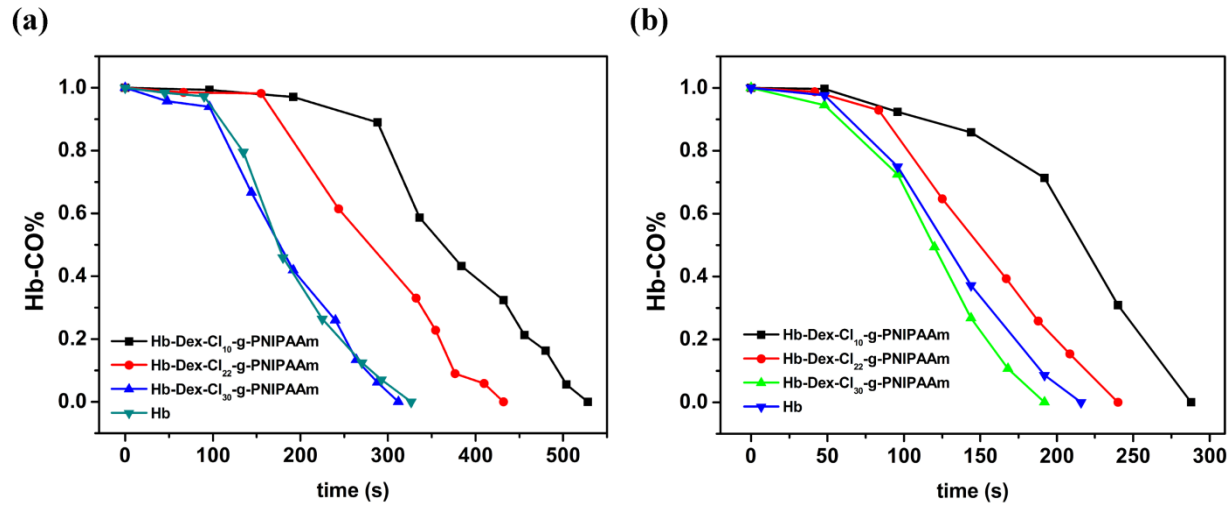

Fig. S5 The kinetic data on oxygen binding for the native Hb and three Hb-Dex-Cl_x-g-PNIPAAm conjugates with different DS at 25 °C (a) and 37 °C (b).

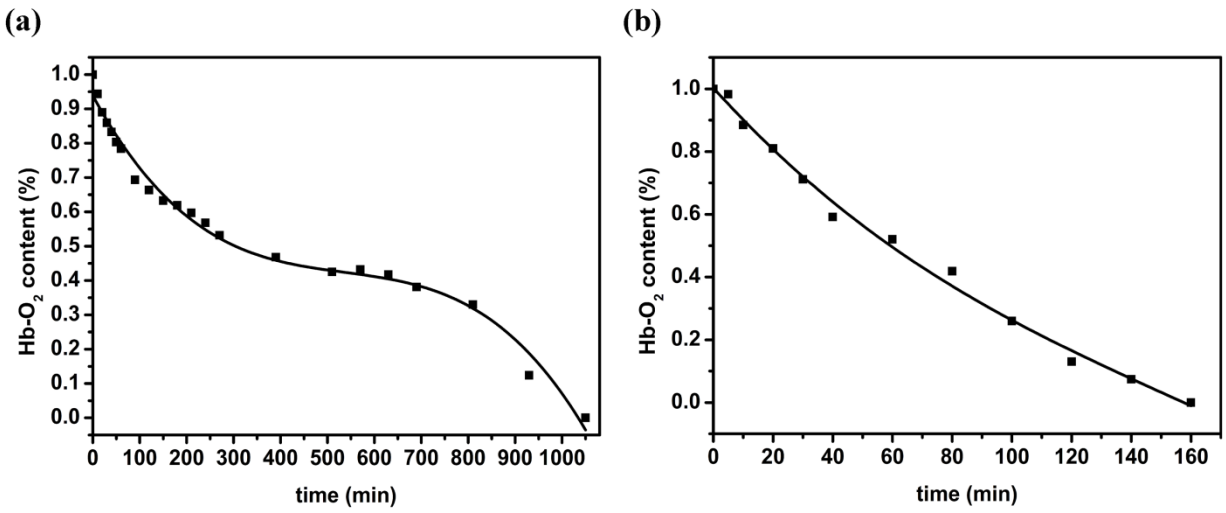

Fig. S6 Stability tests of native O_2-Hb at 25 ^oC (a) and 37 ^oC (b).

References:

1. D. Li, H. Chen, S. Wang, Z. Wu and J. L. Brash, *Acta Biomaterialia*, 2011, **7**, 954-958.

2. B. Li, G. Chen, F. B. Meng, T. H. Li, J. Yue, X. B. Jing and Y. B. Huang, *Polym. Chem.*, 2012, **3**, 2421-2429.