The supporting information

Figure legend of the supporting information

Fig. S1. Identification of the sites of PEGylation. MALDI-TOF/TOF spectra are shown for tryptic digestion of α-globin (A), β-globin (B), PEGylated α-globin from Fig. 2b (C) and PEGylated β-globin from Fig. 2c (D), respectively. MS fragmentation is shown for the peptide at m/z 6178.3 Da in Fig. S1C (E) and the peptide at m/z 5957.3 Da in Fig. S1D (F).

Fig. S2. Size exclusion chromatography analysis of the PEGylated Hbs. HbA (a), [Propyl-PEG5K-Val-1(α)]$_2$-Hb (b), [Propyl-PEG5K-Val-1(β)]$_2$-Hb (c) and [Propyl-PEG5K-Val-1(α)]$_2$-αα-Hb (d) were loaded on two HR10/30 Superose 12 columns (1 × 31 cm^2) at the protein concentration of 31 μM in 100 μl loop. The columns were eluted by PBS buffer, pH 7.4 at a flow rate of 0.5 ml/min.

Fig. S3. $S_{20,W}$ of the modified Hbs a function of the protein concentration. Sedimentation velocity measurements of HbA (a), [Propyl-Val-1(α)]$_2$-Hb (b), [Propyl-PEG5K-Val-1(α)]$_2$-Hb (c), [Propyl-PEG5K-Val-1(β)]$_2$-Hb (d) and [Propyl-PEG5K-Val-1(α)]$_2$-αα-Hb (e) were conducted in a Beckman XL-I analytical ultracentrifuge in PBS buffer at pH 7.4, 25 °C and 55,000 rpm. Boundary movement was followed at 405 nm using the centrifuge's absorption optics.

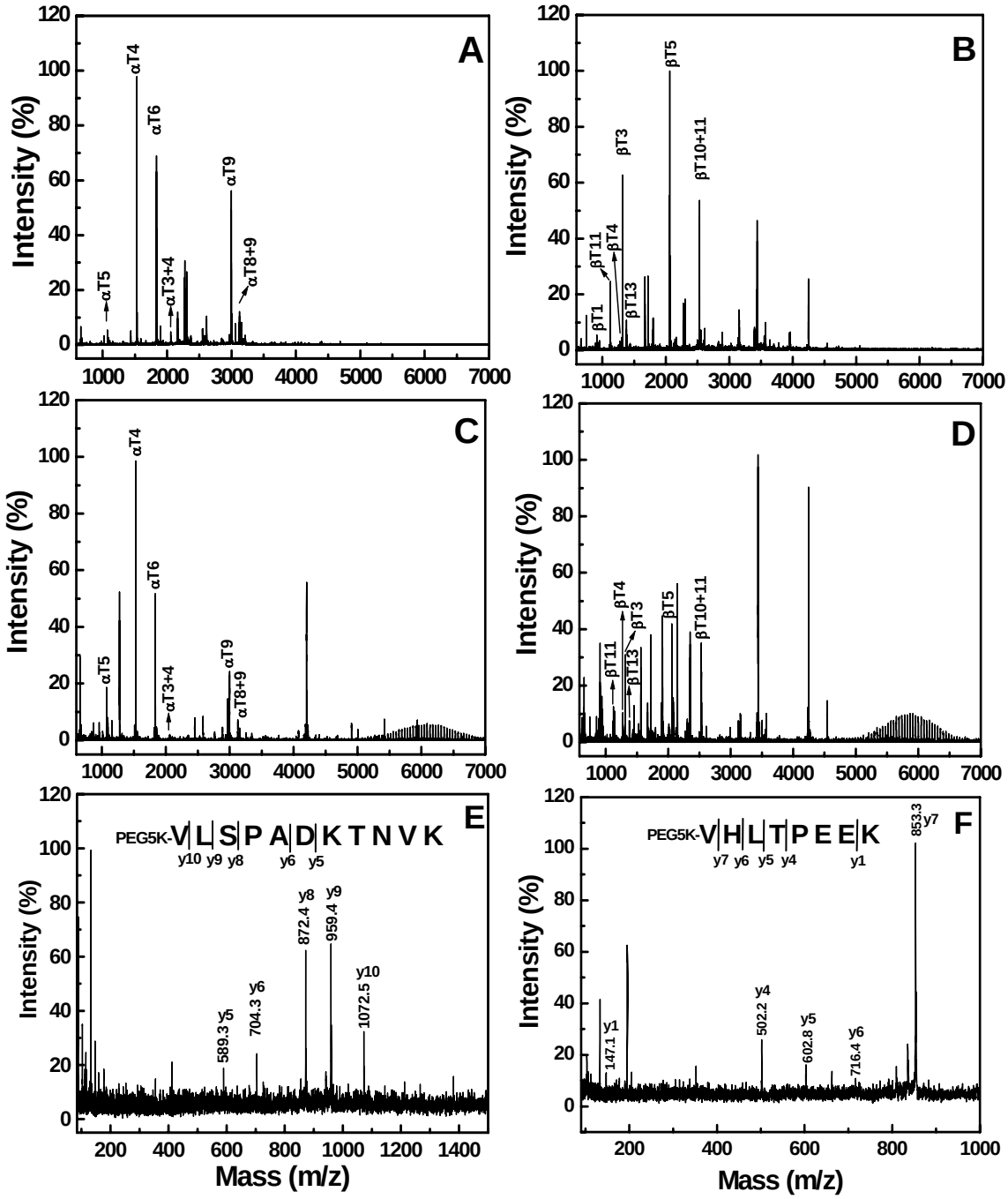
A
αT4
αT6
αT9
αT5
αT3+4
αT8+9
B
βT5
βT3
βT10+11
βT11
βT4
βT13
βT1
C
αT4
αT6
αT5
αT3+4
αT9
αT8+9
D
βT4
βT3
βT5
βT10+11
βT11
βT13
E
PEG5K-V L S P A D K T N V K
y10 y9 y8 y6 y5
y8
y9
872.4
959.4
y10
1072.5
y6
704.3
y5
589.3
F
PEG5K-V H L T P E E K
y7 y6 y5 y4 y1
853.3
y7
y4
502.2
y5
602.8
y6
716.4
y1
147.1
Intensity (%)
Mass (m/z)

Figure S1

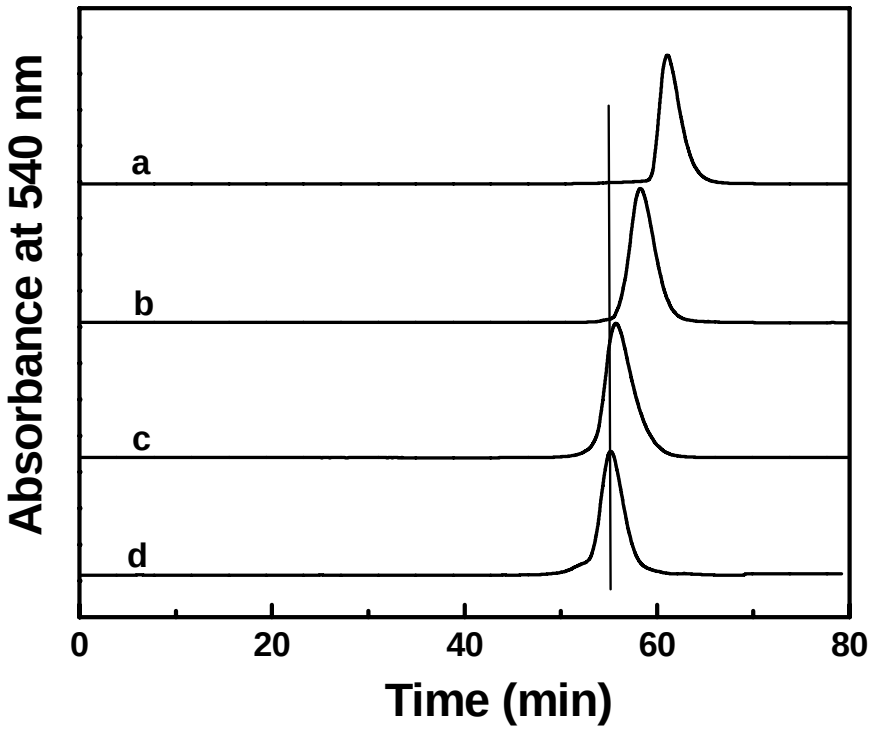

Figure S2

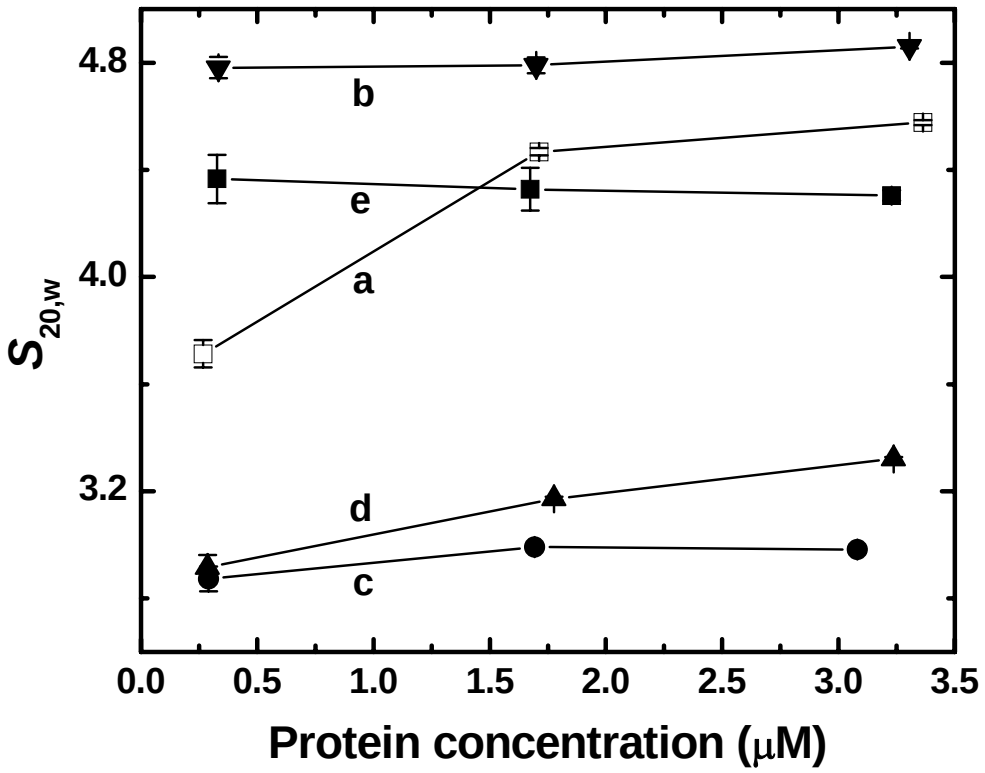
4.8
4.0
3.2
$S_{20,w}$
b
e
a
d
c
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Protein concentration (μM)

Figure S3