Supporting Information

Entropy-Driven Supramolecular Ring-Opening Polymerization of a Cyclic Hemoglobin Monomer for Constructing a Hemoglobin–PEG Alternating Polymer with Structural Regularity

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1. DLS measurements for CMs and XLSPs

The particle size distribution histograms of CMs and XLSPs determined by DLS measurement are shown in **Figure S1**. Using a larger PEG tended to show a larger particle size. The results are presented in **Tables 1** and **3** in the main text.

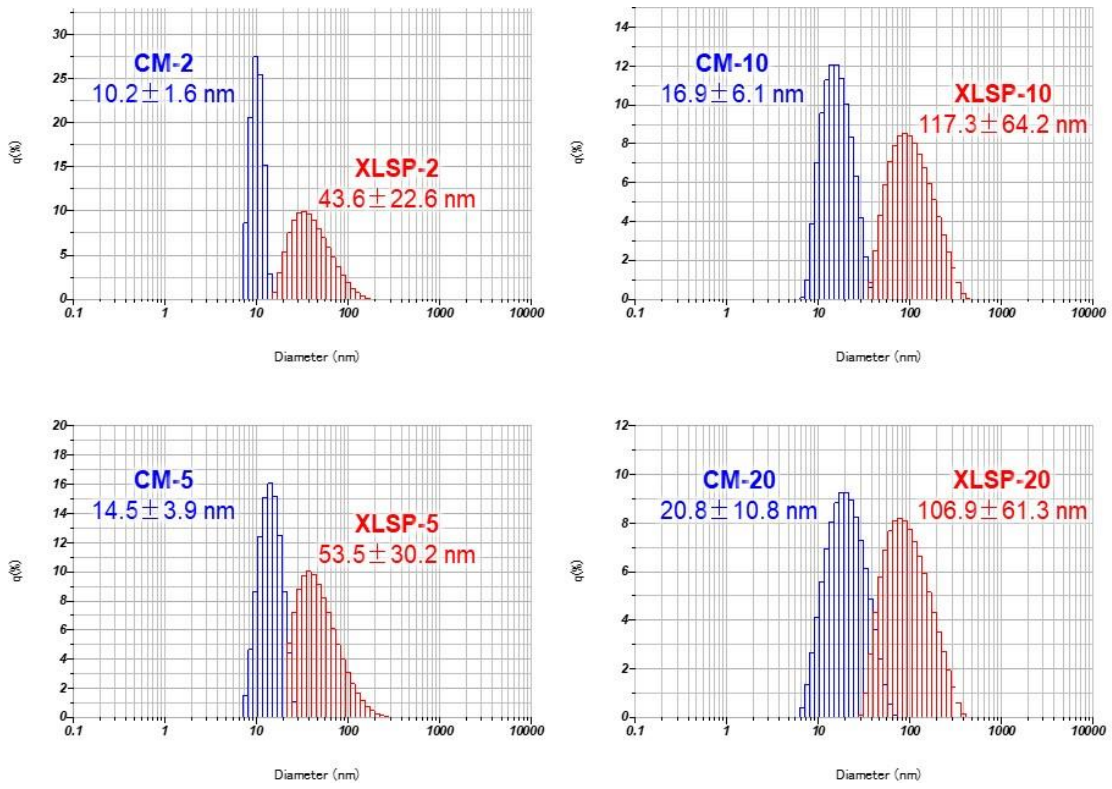

Figure S1. Particle size distributions of CMs and XLSPs at [Hb] = 0.010 mM in PBS (pH 7.4).

2. SDS-PAGE analysis of XLCM-20 synthesized in a diluted condition

CM-20 was reacted with 2.0 equimolar DBBF in PBS at the further diluted monomer concentration of $[M]_0$ = 0.010 mM to produce XLCM-20 while avoiding production of XLSP-20 (**Figure 4D**). SDS-PAGE analysis of XLCM-20 shown in **Figure S2** revealed that CM-20 consist of free α subunits and a ring-closed PEGylated β−β subunit (β−β−ringPEG: band ***f***, which is identical to that in **Figure 5**). Bands ascribed to the polymeric β components (poly(β−β−PEG); band ***g*** in **Figure 5**) were not observed in **Figure S2**, indicating no polymerization but the selective production of XLCM-20 by fixing the ring-closed structure.

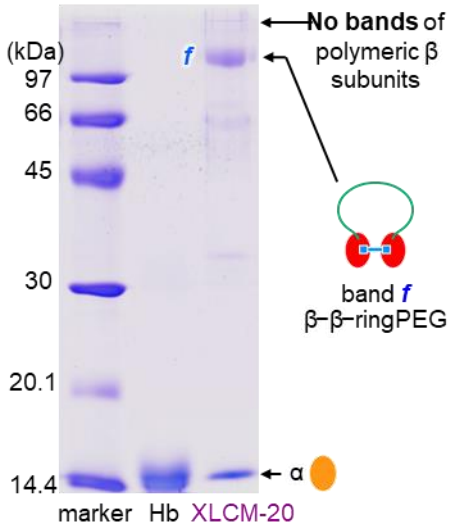

Figure S2. SDS-PAGE analysis of XLCM-20 synthesized in a diluted condition ($[M]_0$ = 0.010 mM).

3. Estimation of intra-molecular distance between PEG terminated αβ dimers from effective molarity

Local concentration of the chain end in the proximity of the other chain end of the same molecule is expressed as an effective concentration.[1] Seen from the one terminal αβ dimer of a CM molecule, the average volume in which the other terminal αβ dimer exists (V_1) is expressed as **Eq. S1.**, where d (m) is the mean intra-molecular end-to-end distance of αβ dimers at PEG terminals (**Figure S3**).

$$V_1 = \frac{4\pi d^3}{3} \quad (\mathrm{m}^3) \qquad \cdots \text{(S1)}$$

The number of CM molecules at $[M]_0$ (mM) can be described as $N_A \times [M]_0 \times 10^{-3}$ in 1 L (10^{-3} m^3) solution, where N_A is Avogadro's number. A CM molecule contains two αβ dimers (**Figure S3**). The average volume of solution per αβ dimer (V_2) is described as **Eq. S2.**

$$V_2 = \frac{1}{2N_A[M]_0} \quad (\mathrm{m}^3) \qquad \cdots \text{(S2)}$$

When $[M]_0 = [M]_{crit}$, V_2 would be identical to V_1. In other words, the concentration of an inter-molecularly separated αβ dimer is equivalent to effective concentration of intra-molecularly PEG-bound αβ dimer. Therefore, d can be described as **Eq. S3**.

$$d = \sqrt[3]{\frac{3}{8\pi N_A [\mathrm{M}]_{crit}}} \ (\mathrm{m}) \qquad \cdots \text{(S3)}$$

Actually, d should be approximated as mean end-to-end distances of PEG linker in CM by neglecting the excluded volumes of Hb molecules. Substituting the apparent $[M]_{crit}$ for **Eq. S3**, The mean intra-molecular end-to-end distances of CM-2, -5, -10, and -20 are estimated respectively as 61, 84, 117, and 147 Å.

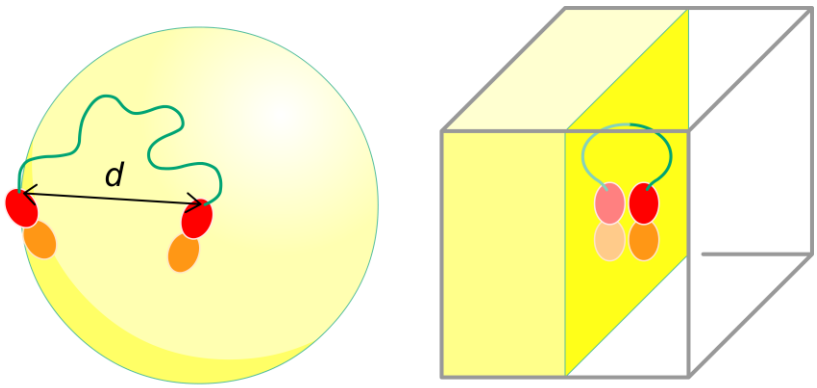

Figure S3. Schematic illustration showing the average volume (V_1) in which the other terminal αβ dimer is present around the terminal αβ dimer (left), and the average volume of solution (V_2) per αβ dimer at CM concentration of $[M]_0$ (right).

4. Estimation of root-mean-squared end-to-end distance of free PEG chain

Reportedly, the root-mean-squared end-to-end distance (r_{rms}) of a free PEG chain can be estimated theoretically as presented in **Eq. S4**.[2,3]

$$r_{rms}^2 = C_n n l^2 \quad \cdots \text{(S4)}$$

In this equation, n represents the number of atoms, l denotes the skeletal bond length, and C_n stand for the experimentally determined characteristic ratio unique to the polymer species, influenced by stiffness, bond angle, and rotational barriers of the polymer chain.[2] The values of l and C_n are reported for long PEG as 1.46 Å and 4.1,[3] so that r_{rms} of 2, 5, 10, and 20 kDa PEG linkers (2.0, 4.4, 10.5, and 20.8 kDa in actual M_n) are estimated respectively as 35, 51, 79, and 111 Å.

5. Empirical PEG molecular weight dependence of maximal $[M]_0$, $[M]_{eq}$, and DP

The higher $[M]_0$ is, the higher the degree of polymerization (DP) becomes.[4] However, a limitation in solubility exists for each CM because of the giant molecular masses of Hb molecules and PEG linkers. Following experiment was conducted to ascertain the maximally concentrated $[M]_0$ of each CM, represented as $[M]_{max}$.

Each CM solution was concentrated to the greatest extent possible (maximally) by repeating the 60 min ultrafiltration twice using a centrifugal filter (Amicon Ultra-0.5 mL, 10 kDa cutoff, 14,000 × g; Merck Millipore Ltd., Merck KGaA, U.S.A.). Every maximally concentrated CM solution was red-black sticky pastes. Maximally concentrated CM solution of 10 μL was picked up using a micropipette equipped with a capillary piston (Microman M25; Gilson Inc., Wisconsin, U.S.A.). Then, it was dissolved in 5 mL of dissolution of a kit (Hemoglobin B-test Wako; Wako Pure Chemical Industries Ltd.). Furthermore, the concentration of Hb unit was determined from absorption spectra. The measurements were conducted three times. A maximally concentrated native Hb solution was also prepared for comparison using the same procedure. As a result, the $[M]_{max}$ showed the clear exponential decrease concomitantly with the increasing the molecular weight of the PEG linker (**Figure S4**).

As shown in our earlier report,[4] DP can be estimated roughly as $[M]_0 / [M]_{eq}$ as follows, where $[M]_0$ signifies the initial monomer concentration and $[M]_{eq}$ stands for the concentration in the equilibrium state of S-ROP. The ratio of the monomer reacted (P) is defined as **Eq. S5**.

$$P = \frac{[M]_0 - [M]_{eq}}{[M]_0} \qquad \boxtimes \text{(S5)}$$

The number average degree of polymerization (DP), defined using P, is converted to the ratio of $[M]_0$ to $[M]_e$ as **Eq. S6**.[5]

$$\mathrm{DP} = \frac{1}{1-P} = \frac{[\mathrm{M}]_0}{[\mathrm{M}]_{\mathrm{eq}}} \qquad \boxtimes \text{ (S6)}$$

The value of DP is proportional to both $[M]_0$ and the reciprocal $[M]_{eq}$ ($1/[M]_{eq}$). The value of $1/[M]_{eq}$ is equal to the equilibrium constant K of polymerization and depolymerization equilibrium for S-ROP.[3] The value of $1/[M]_{eq}$ obtained in this work (calculated from the value shown in **Figure 7**) showed a quadratic increase with the molecular weight of the PEG linker (**Figure S5**).

From the product of equations of regression curves of **Figures S4** and **S5**, DP for maximally concentrated CM (DP_{max} at $[M]_0 = [M]_{max}$) can be estimated empirically as shown in **Eq. S7**.

$$\mathrm{DP}_{max} \approx (8.54e^{-0.047x}) \times (0.0186x^2 + 0.2290x) \quad \cdots \text{ (S7)}$$

In this equation, x stands for a molecular weight of PEG linker. The estimated DP_{max} is shown for the molecular weight of PEG linker (**Figure S6**). By extrapolating this relation above 20 kDa, the estimated DP_{max} becomes highest ($DP_{max} = 51$) when using a 37 kDa PEG linker.

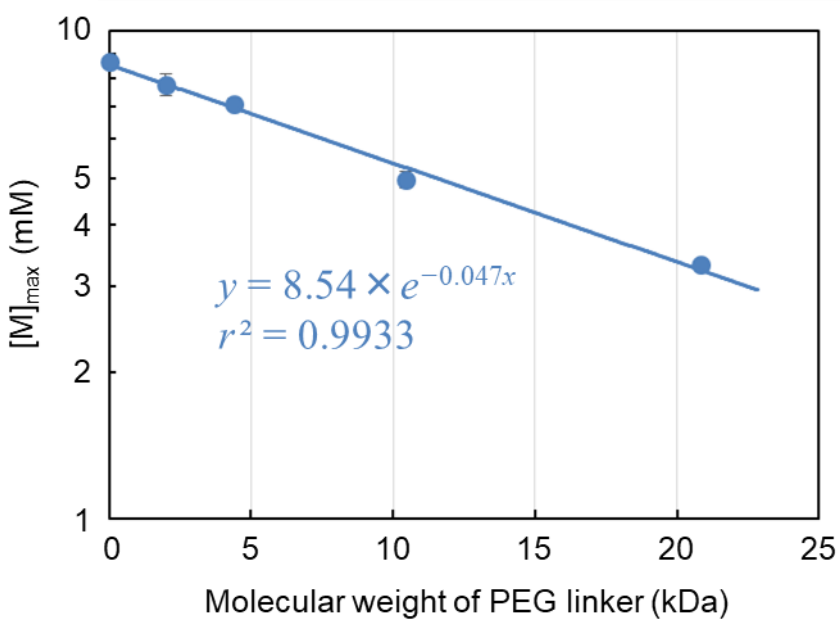

Figure S4. Relation between $[M]_{max}$ and molecular weight of PEG linker, which is approximated by exponential curve fittings. The error bars represents the standard deviations (n = 3).

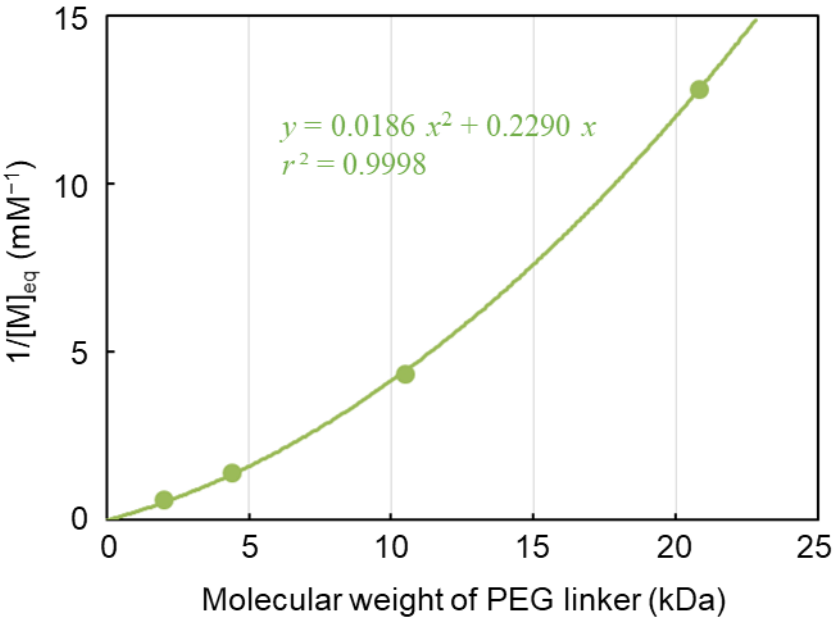

Figure S5. Relation between reciprocal $[M]_{eq}$ and molecular weight of PEG linker.

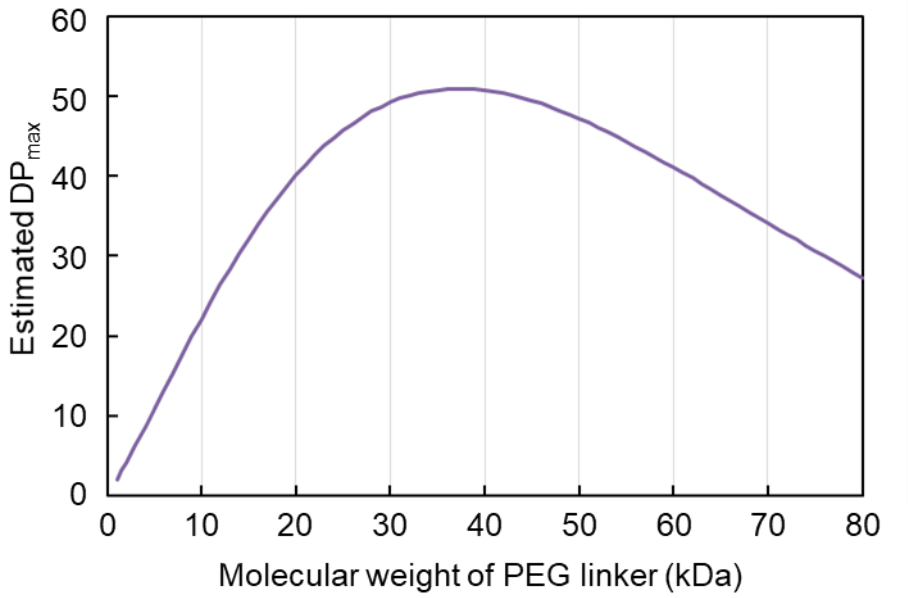

Figure S6. Relation between estimated DP_{max} and molecular weight of PEG linker.

References

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