Supporting information

Table S1. Functional proteins prepared in this study

Name	Abbreviation	Amino acid sequence of the C-terminal regions of the recombinant protein
K-tagged CD(Tfu0901)	CD-CK	-YPYDVPDYA-GGGS-MRHKGS-HHHHHH
K-tagged EG(Tfu0901)	EG-CK	
K-tagged CBM	CBM-CK	

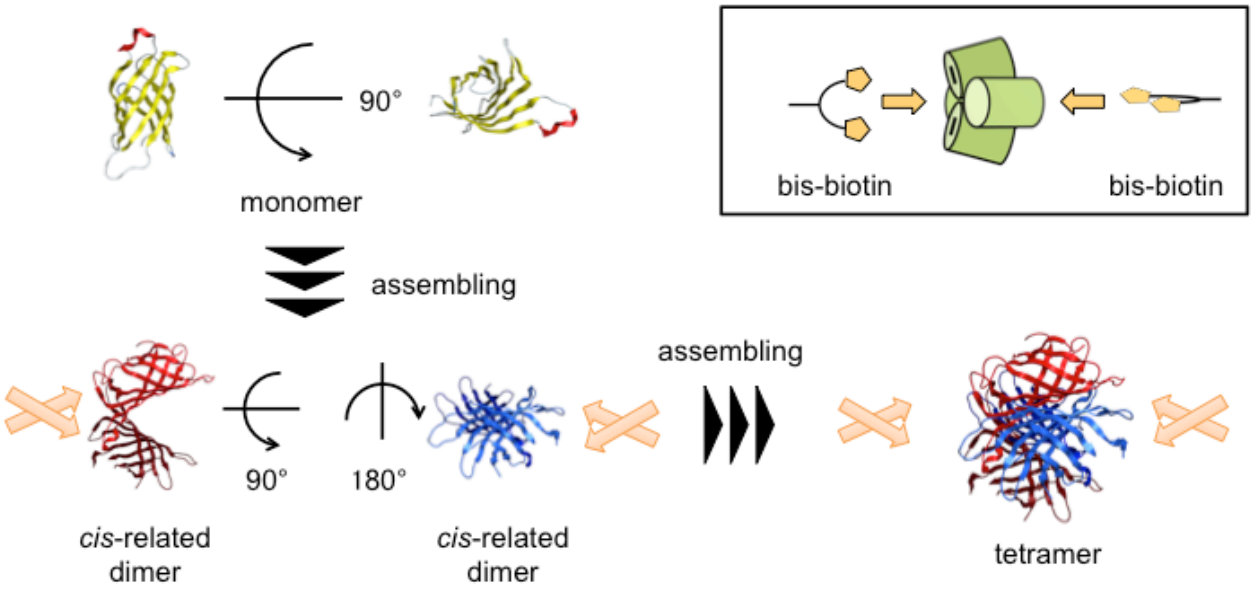

Fig. S1 SA forms a tetramer in its quaternary structure; the two monomer units (yellow) form a dimer (red and blue) with two biotin-binding sites facing the same direction, and the two dimer units are assembled into a tetramer facing the opposite direction at the right angles for the dimers. Based on its structure, using bis-biotin promotes the formation of one-dimensional structure.

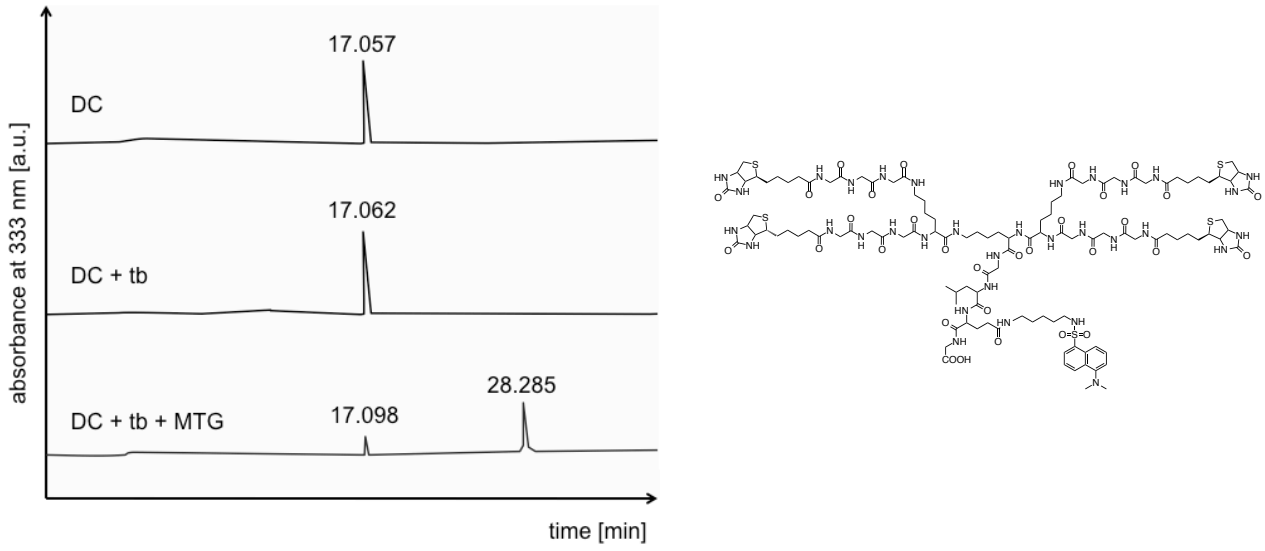

Fig. S2 The reactivity of Q-biotin$_4$ was examined with dansylcadaverine. Q-biotin$_4$ (20 μM) and MTG (0.1 U/mL) were added to an aqueous solution dansylcadaverine (1 μM in 10 mM phosphate buffer, pH 5.8) at 37 °C. After the enzymatic cross-linking reaction with MTG, the samples were analyzed with RP-HPLC (absorbance was monitored at 333 nm derived from dansylcadaverine). _The analytical conditions were used as follows: column; Inertsil ODS-3 (GL Sciences, Inc., 4.6 × 250 mm), mobile phase; CH_3CN–H_2O = 10:90 (0 min) → 50:50 (40 min). The dansylcadaverine was eluted at 17.1 min. After the MTG reaction, the new peak derived from the conjugates was appeared at 28.3 mL. DC; dansylcadaverine, tb; Q-biotin$_4$. (left). MALDI-Tof MS; m/z: calculated for $C_{114}H_{177}N_{33}O_{31}S_5Na$ ([M+Na]+) 2689.1, found 2689.4. (right) the structure of the conjugate consisting of dansylcadaverine and Q-biotin$_4$.

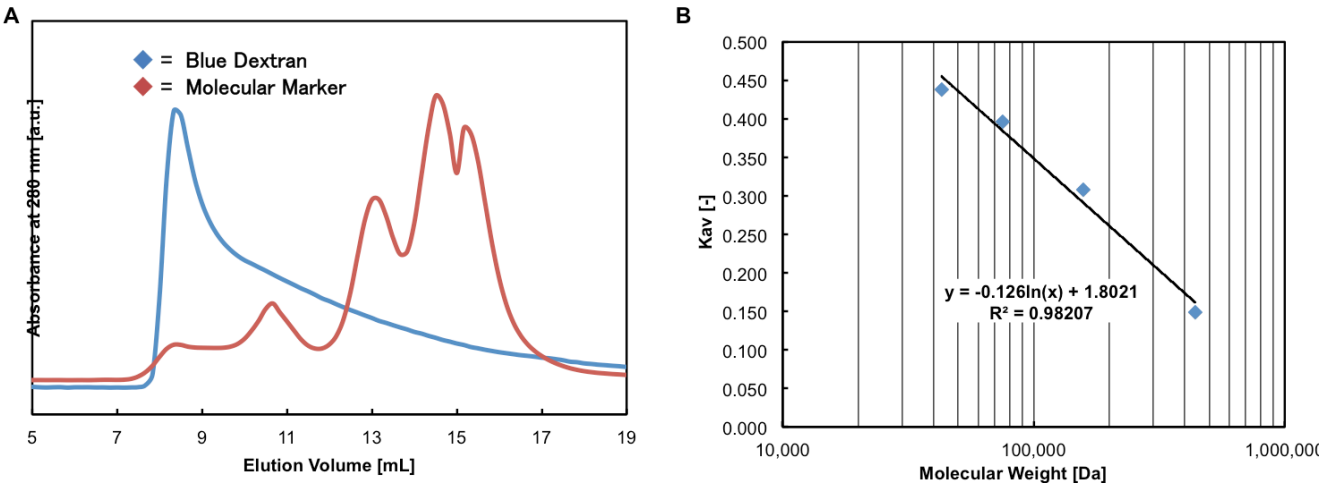

Fig. S3 Calibration of the molecular weight using the calibration results of the SEC column. (A) SEC chromatogram for the molecular weight markers and blue dextran eluting at the void volume. 1: Ferritin, 440 kDa; 2: Aldolase, 158 kDa; 3: Conalbumin, 75 kDa; 4: Ovalbumin, 43 kDa; (B) Standard curve for the molecular weight based on the results of SEC analysis of the molecular weight markers and blue dextran.

Table S2. SEC data regarding the molecular weight marker. (Content names, molecular weights, elution volumes, and K_{av}, the value was calculated from eq.1.)

	Content	MW [Da]	V_E [ml]	K_{av}
1	Ovalbumin	43000	15.20	0.438
2	Conalbumin	75000	14.55	0.397
3	Aldolase	158000	13.16	0.308
4	Ferritin	440000	10.67	0.149
V_0	BlueDextran	2000000	8.33	-

$$K_{av} = (V_E - V_0)/(V_C - V_0) \quad \ldots\ldots(\text{eq.1})$$

V_c : Column Volume, 24 [ml].

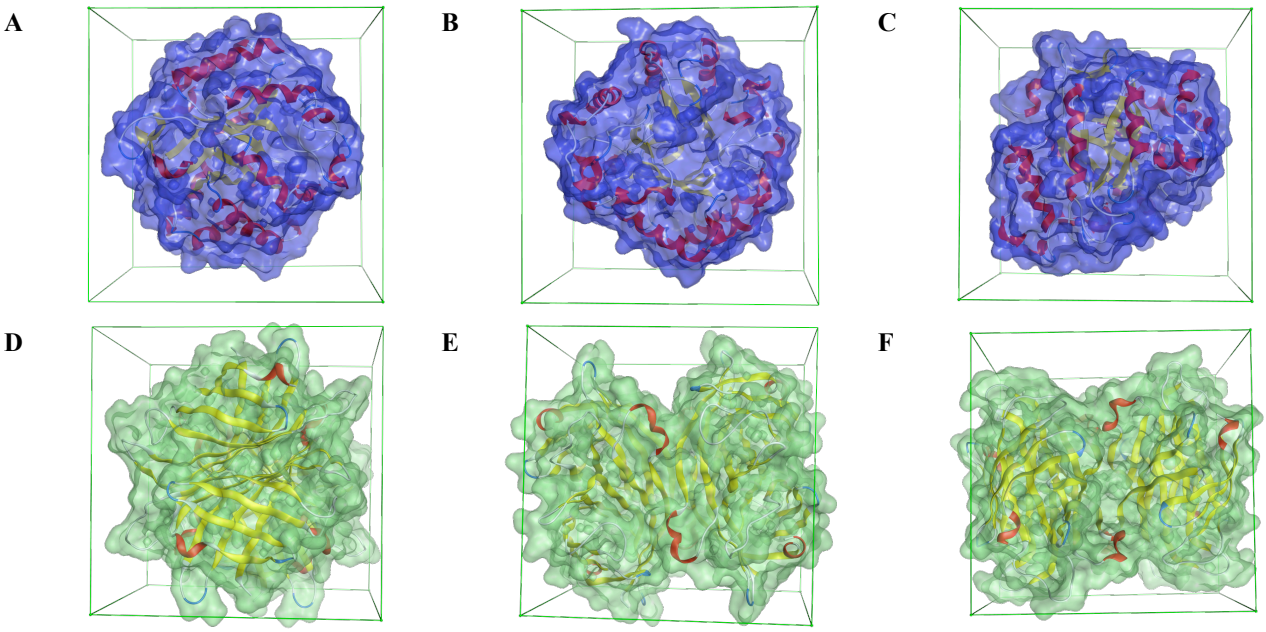

Fig. S4 The approximate size of CD (PDB: 2CKS) and SA (PDB: 1STP) with a molecular visualization software Molecular Operating Environment (Chemical Computing Group, Quebec, *Canada*). The image The size of green cube was Width 5 nm× Depth 5 nm× Height 5 nm. A front (A), side (B) and plane view (C) of CD. A front (E), side (F) and plane view (G) of SA. The surfaces of proteins were blue (CD) and green (SA).

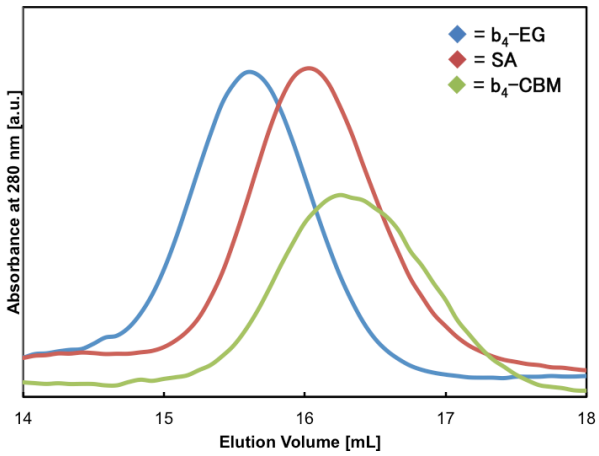

Fig. S5 The characterization of free protein units by size-exclusion chromatography. The proteins, b_4-EG (blue), SA (red) and b_4-CBM (green), were eluted at 15.3 mL, 16.0 mL and 16.3 mL, respectively.