Supporting Information

1. Experimental Section

1.1 Chemicals:

CB[8], CB[6] and CB[7] were purchased from New South Innovation Pty Ltd, Sydney. Bovine-liver Catalase (CAT), β-Lactoglobulin from bovine milk (β-LG), Insulin from bovine and 3-Aminopropyltriethoxy-silane (APS) were purchased from Sigma-Aldrich. Glucose oxidase (GOX, EC 1.1.3.4 from Aspergillus niger) was purchased from TOYOBO. Glutathione S-Transferase (GST) protein from Schistosoma japonicum was expressed and purified in E.coli by Biology Department of Tsinghua University. Bovine-heart hemoglobin (Hb), bovine serum albumin (BSA) and other chemicals were obtained from Beijing Chemicals Company. Lysozyme (LYZ) was obtained from Amresco. In our work, the used proteins were selected for their special functionality as well as their ready availability. Bovine-heart hemoglobin (Hb) was selected for its optical absorption around 406 nm which served as a detection handle. Catalase (CAT) was selected for its catalytic activity. Glutathione S-Transferase (GST) was selected as another model protein to demonstrate the generality of this method. All the chemicals involved in our experiments were of analytical grade. The preparation of buffer is described as below. Deionized water was further purified with RF ultrapure water system.

The pI and size of the used proteins were showed in Table S1. All the protein data was obtained from RCSB Protein Data Bank.

Table S1 the details of proteins

Protein	pI	Size (nm)
Hb	6.9	6.5×7.8×10.9
CAT	5.4	8.3×14.1×23.0
GST	6.0	11.5×11.5×7.8
GOX	4.2	6.6×6.7×21.5
BSA	7.8	21.6×4.5×14.2
LYZ	11	5.7×6.1×3.1
β-LG	5.2	5.6×8.2×6.7
Insulin	5.3	8.2×8.2×3.4

1.2 Instrumentation:

SEM experiments were performed with SSX-550，Shimadzu. UV-vis spectrum was obtained using a PerkinElmer Lambda 35 spectrometer. FTIR spectra were collected with an IFS-66v/S FTIR spectrometer (Bruker) in vacuum at around 1 to 2 mbar of pressure. QCM experiment was measured by CHI440A, CH Instrument, Inc. ITC experiments were performed using a VP-ITC from Microcal. The thicknesses of the multilayers were measured with surface profilometry (AMBIOS XP-1).

1.3 Preparation of buffer:

PBS buffers were prepared from di-potassium phosphate and potassium di-phosphate, with the pH adjusted with potassium hydroxide or phosphoric acid, respectively. The buffer utilized in this

experiment is 5 mM potassium phosphate, pH 7.0. It is used for the preparation of protein solution, hydrogen peroxide solution and etc..

1.4 *Pretreatment of quartz plates/sheet glasses: clean and modification with aminos*

First of all, the quartz plates or sheet glasses were immersed in piranha solution consisted with H_2SO_4/H_2O_2 (30%) mixture (3:1, v/v) for about 24 h in room temperature or boiled in the piranha solution for 1 h. Secondly, the quartz plates or sheet glasses were cleaned in deionized water and ethanol sequentially by ultrasonic, and by carefully drying with a stream of N_2 in between. At the third step, the quartz plates or sheet glasses were incubated for 12 h in the APS solution in methanol whose mass fraction was 3%-4%. Following this treatment, the surfaces were thoroughly rinsed with ultrapure water and absolute ethanol, and dried with high-purity nitrogen. After these series of pretreatment, the surface of quartz plates or sheet glasses contained plenty of amino-group.

1.5 *Preparation of LBL construction for UV-VIS*

After the pretreatment of quartz plates which contains a lot of amino-group, they were incubated for 2 h in the 0.1 mM CB[8] and Hb in sequence, flushed with buffer and carefully dried with a stream of N_2 in between. Therefore, the protein monolayer was immobilized on the quartz plates. After that, the quartz paltes were incubated for 10min in CB[8] and for 20min in Hb solutions sequentially. After every step, buffer was utilized to remove the physical absorption and a stream of N_2 was used to dry the surface.

1.6 *Preparation for QCM*

For quartz crystal microbalance (QCM) measurement, gold-coated QCM resonator electrode whose geometric area was about 0.196 cm^2 and the fundamental frequency was 8 MHz, were cleaned with a piranha solution, and then washed in pure water and ethanol successively. The cleaned gold electrode was immersed in 0.1 mM CB[8] solutions for 8 h to form a monolayer[1], and was then incubated in the aqueous Hb buffer solution for 1 h. The procedure to make $(CB[8]/Hb)_n$ films on the QCM crystal surface was the same as that on quartz plates. After each adsorption step, the gold resonator electrode was washed with water, dried in N_2 stream, and then measured by QCM.

1.7 *Preparation of multi-protein layers*

After modification with aminos and CB[8], the quatz plate was incubated in buffer solution of Hb first, and then aqueous solution of CB[8], and third in buffer solution of CAT. Clean and drying process was performed as previously described.

1.8 *Preparation for FTIR*

Four pieces of CaF_2 (called A, B, C and D)were pre-cleaned by deionized water, ethanol and acetone in turn, and then put into the oven to dehydrate at 60℃ for about 30 min. A was immersed in the PEI (1 mg/mL) solution for 2 h. Then $(CB[8]/Hb)_7$ was constructed. B was coated by a drop of Hb solution, and C by CB[8]. D was blank. They were dried in 38℃ for about 6 h before the tests.

1.9 *Testing the activity of CAT:*

In our case, based on the Beer-Lambert Law, UV/Vis spectrometer was used to monitor the evolution of the concentration of H_2O_2. Concretely, quartz substrates coated with one, two, three or four CB[8]/CAT layer were exposed to 3ml aqueous solution of H_2O_2 (0.05 M), respectively. After 20 min exposure, the quartz substrates were removed from the reaction medium and the concentration of residual H_2O_2 in solutions were checked by UV/Vis, respectively. Since the absorption intensity in the range 230-250 nm is proportional to the concentration of hydrogen peroxide (J. Biol. Chem. 1951, 195, 133), the evolution the concentration of H_2O_2 in solution can be determined by using UV/Vis.

1.10 *Isothermal Titration Calorimetr(ITC) experiment*

Titration experiments were carried out in 10 mM sodium phosphate buffer (pH 7.0) at 25℃ on a VP–ITC calorimeter from Microcal, Inc. In a typical experiment, CB[8] was in the sample cell at a concentration of 0.1 mM, into what a solution of Hb was injected from the injection syringe at a concentration of 0.0625 mM. 28 consecutive injections of 5μL with at least a 420 s interval between injections was performed. Heats of dilution were tested as references and were too small to be noticed. All solutions were degassed prior to titration. The data were analyzed using Origin 7.0 software and was fitted using the one set of sites model in Origin software.

1.11 *Dynamic Light Scattering (DLS) experiment*

Dynamic light scattering for determination of protein aggregation size was measured using the Malvern Zetasizer nano series nano-zs90. The pristine protein was detected under the fresh prepared condition. The samples adding with CB[6], CB[7], or CB[8] were detected after 5 min in order to form a related bigger aggregation.

2. Figures

2.1 Adding CB[8] into aqueous solution of Hb induced aggregates and the aggregates re-dissolved in response to CB[8]'s competing guest MV.

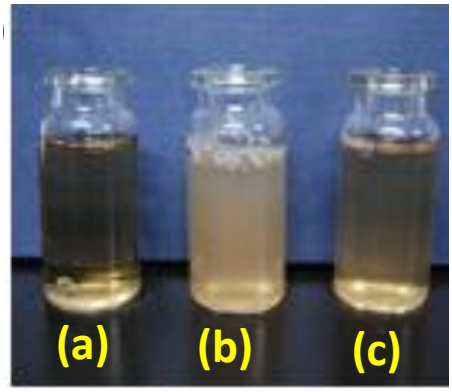

Figure S1. (a) aqueouse Hb solution, (b) adding CB[8] into aqueous solution of Hb induced aggregates and (c) the aggregates re-dissolved in response to CB[8]'s competing guest MV.

2.2 SEM images of CB[8]/Hb aggregates after addition excessive MV. Almost all aggregates are dissolved**.**

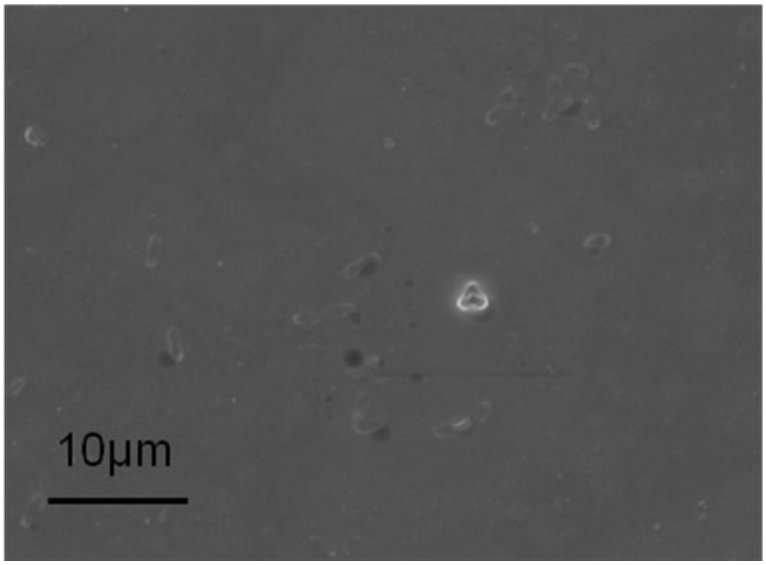

Figure S2. SEM images of CB[8]/Hb aggregates after addition excessive MV.

2.3 CB[8] also induced aggregation of catalase (CAT) and Glutathione S-Transferase Agents (GST). The aggregations were largely re-dispersed by adding CB[8]'s guest MV into the mixture.

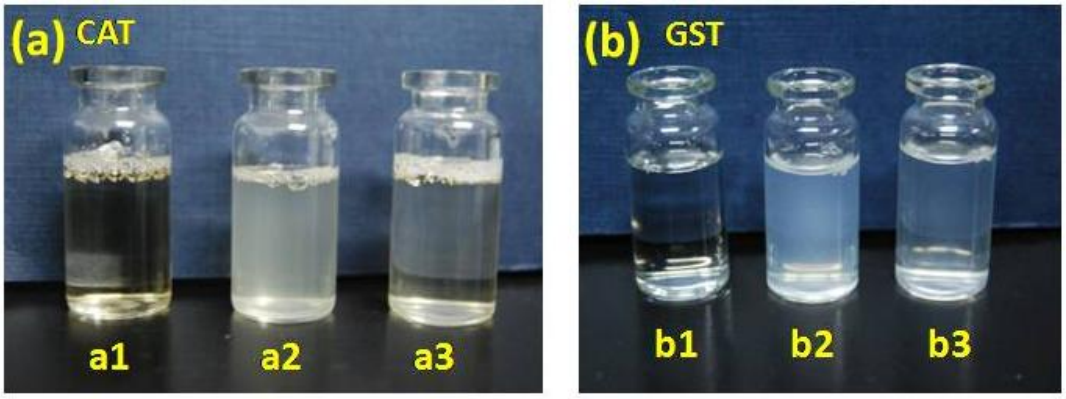

Figure S3. Clear aqueous solutions of protein (n1) get colloid after adding aqueous CB[8] solution (n2). Adding aqueous MV solution leads to a much clearer solution (n3). (n=a or b, a: CAT; b: GST)

2.4 The DLS of different protein with CB[6], CB[7] and CB[8]

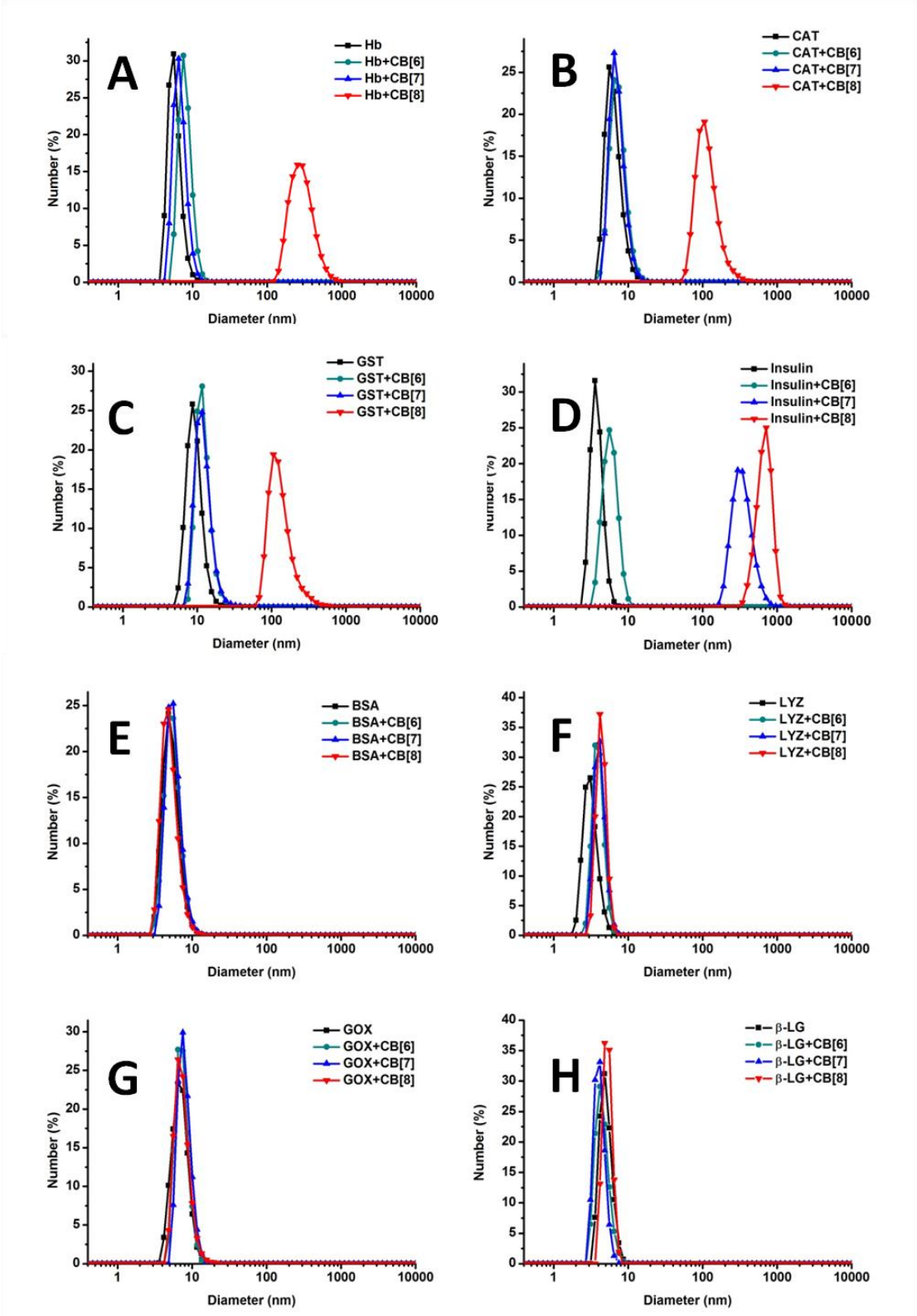

Figure S4. The DLS of (A) Hb, (B) CAT, (C) GST, (D) Insulin (E) BSA, (F) LYZ, (F) GOX and (G) β-LG adding with CB[6] (Dark cyan Line), CB[7] (Blue Line) and CB[8] (Red Line). The Black Line is the DLS measurement of the pristine protein.

2.5 The DLS of BSA and Hb with pH changed which displayed that the pH could not influence the aggregating process.

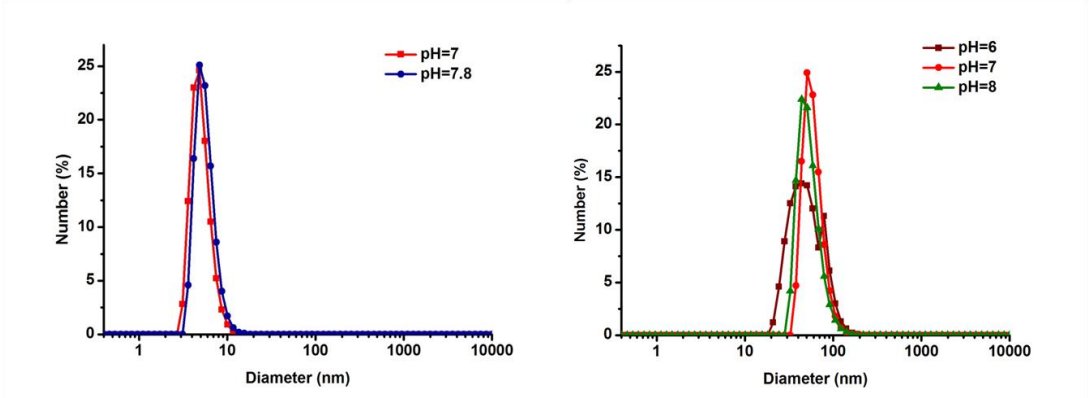

Figure S5. The DLS of (A) BSA adding CB[8] in pH = 7 (Red Line) and pH = pI = 7.8 (Blue Line), (B) Hb adding CB[8] in pH = 6 (Brown Line) and pH = 8 (Green Line).

2.6 The frequency shifts of (CB[8]/Hb)n, detected by QCM.

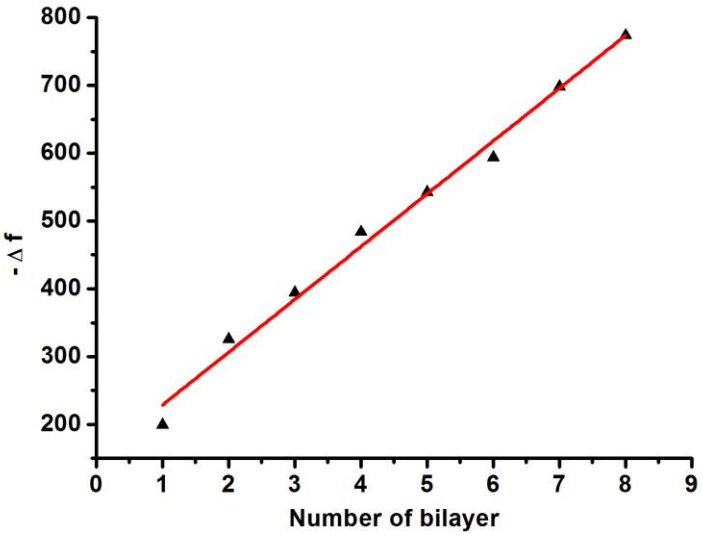

Figure S6. Frequency shifts with number of bilayers, detected by QCM. The red line is a guide to eyes.

2.7 Thicknesses of the multilayer

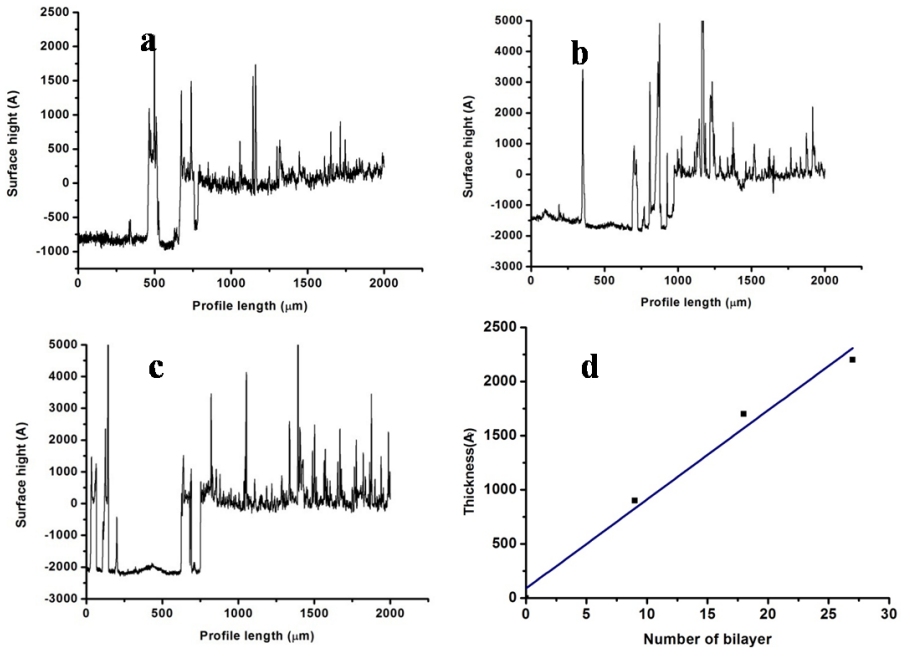

Figure S7. The stylus profiler of Si-APS-(CB[8]/Hb)$_n$. (a) n=9, (b) n=18, (c) n=27. d) The almost linear relationship between the thicknesses and the number of the bilayers. The measurements were carried out after removal of the material with the tip at a smaller scan size with a high force applied.

2.8 Multilayers with binary proteins.

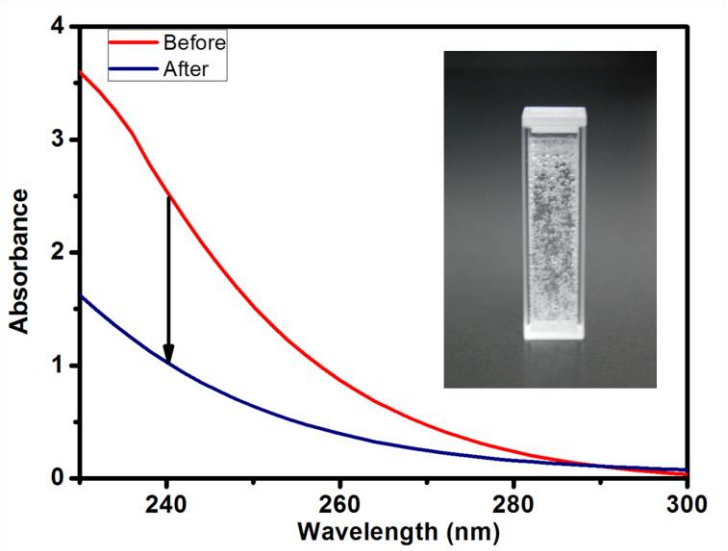

Figure S8. The absorbance of H_2O_2 decreases significantly after 20 min's reaction, with the multilayers of binary protein (CB[8]/Hb/CB[8]/CAT)$_n$ in the solution. The inset shows that bubbles generated vigorously with the multilayers in the aqueous H_2O_2 soluiton.

Acknowledgement

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