Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry
This journal is © The Royal Society of Chemistry 2012

Protein supramolecular complex formation by site-specific avidin-biotin interactions

Yutaro Mori,[1] Masahiro Goto,[1, 2] and Noriho Kamiya[1, 2] *

[1]Department of Applied Chemistry, Graduate School of Engineering, Kyushu University 744 Motooka, Fukuoka 819-0395, Japan.

[2]Center for Future Chemistry, Kyushu University 744 Motooka, Fukuoka 819-0395, Japan

*Corresponding author
E-mail: nori_kamiya@mail.cstm.kyushu-u.ac.jp; Tel: +81-(0)92-802-2807; Fax: +81-(0)92-802-2810

Contents

Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry
This journal is © The Royal Society of Chemistry 2012

Trihedral figure of the AP crystal structure (1ALK)

Molecular image of the wild-type AP with the N- and C- terminal residues highlighted and colored (blue and red) as CPK models. The images were produced with the Molecular Operating Environment (*MOE*, v 2009.10) software developed by the Chemical Computing Group Inc. (Montreal, Canada). The regions of residues 91-93 are circled in blue and residues 219-221 are circled in red.

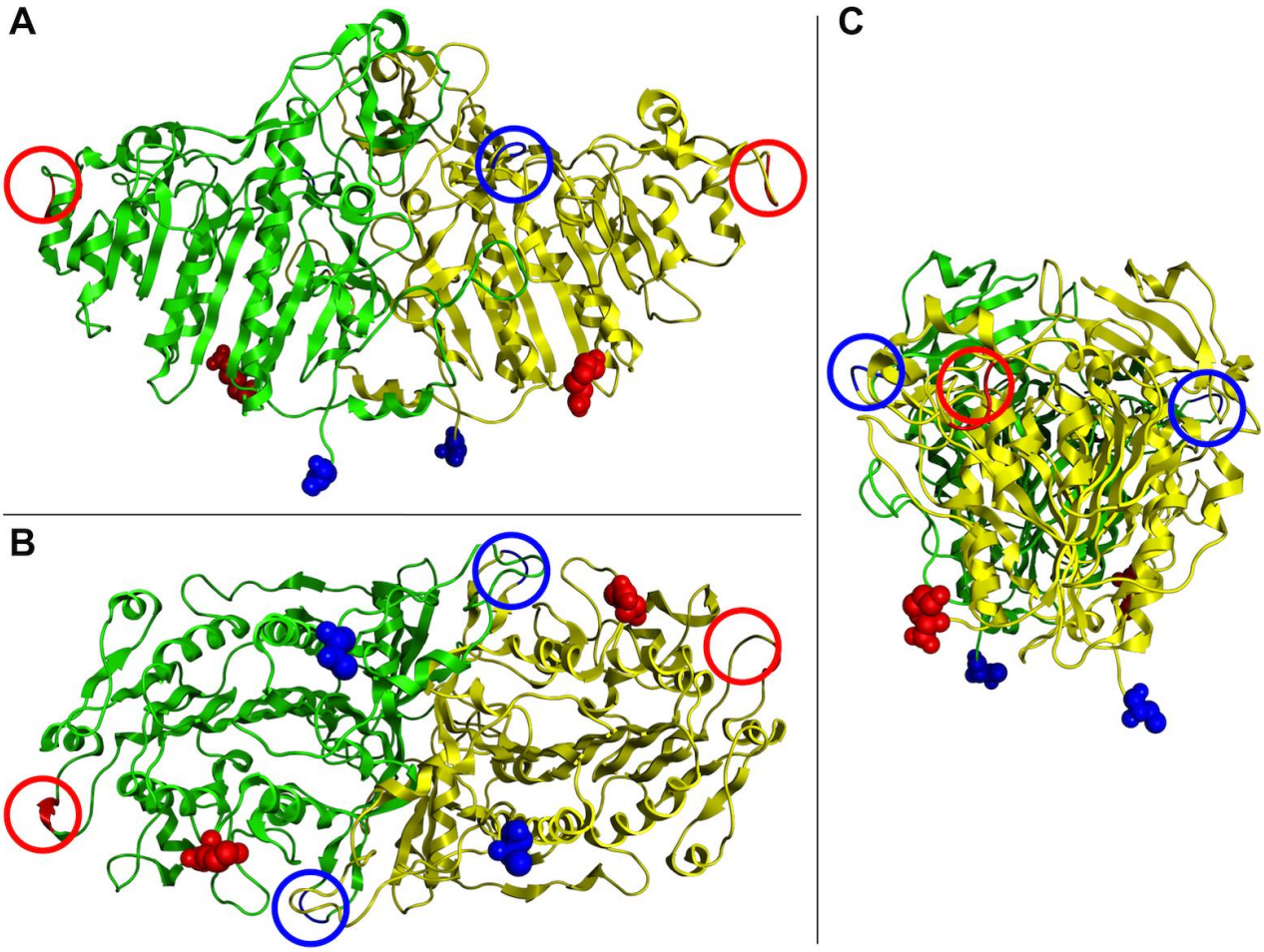

Fig. S1. A trihedral figure of the crystal structure (1ALK). (A) front, (B) plane, and (C) side view.

Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry
This journal is © The Royal Society of Chemistry 2012

RP-HPLC chromatograms

RP-HPLC purification (absorbance was monitored at 230 nm) was used and the collected fractions were lyophilized. Analytical conditions were as follows: column; Inertsil ODS-3 (GL Sciences, Inc., 4.6 × 250 mm), mobile phase; CH_3CN/H_2O = 10/90 (0 min) → 30/70 (30 min) → 80/20 (40 min). The synthesis of these biotinylation substrates was confirmed by RP-HPLC.

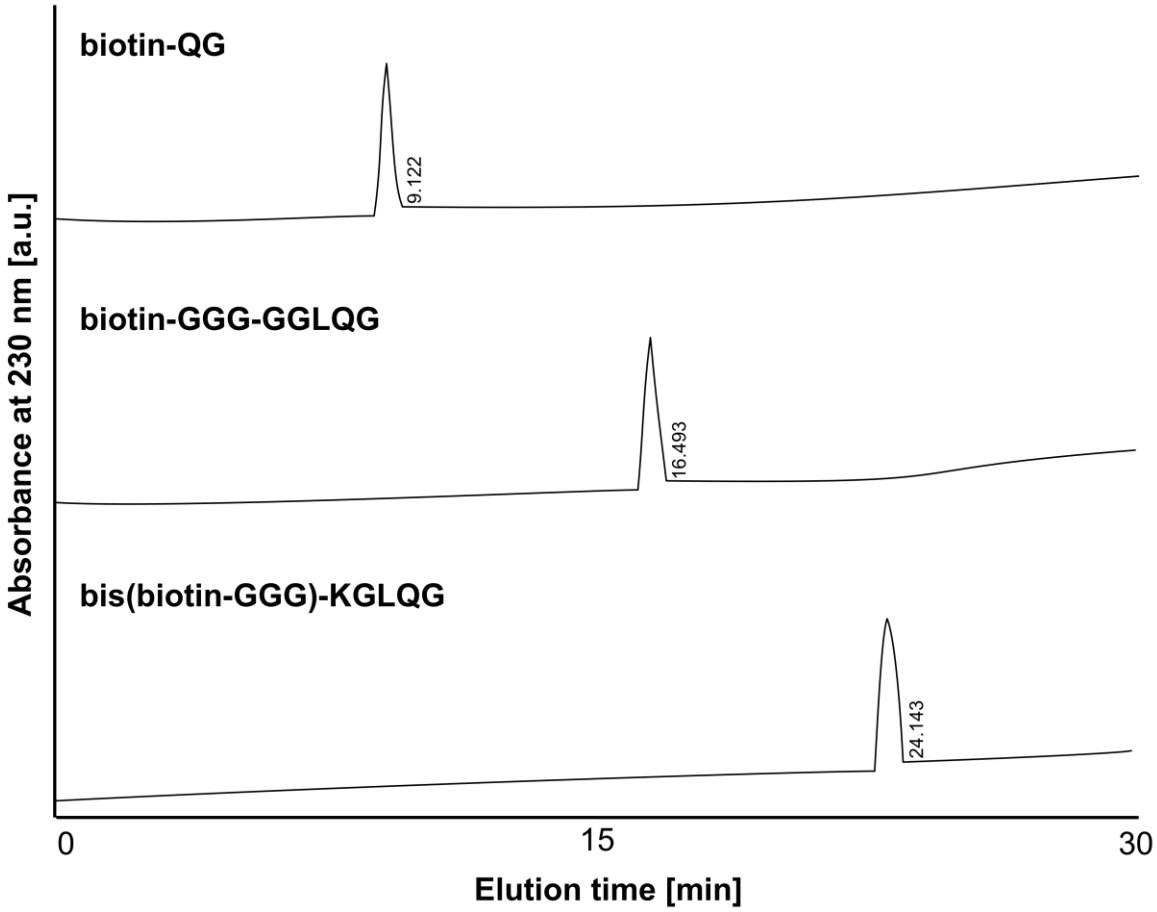

Fig. S2. RP-HPLC chromatograms of the purified biotinylation substrates.

Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry
This journal is © The Royal Society of Chemistry 2012

Immobilization of the biotinylated APs on the SA coated plate

Immobilization of wild-type AP and recombinant APs on an avidin coated plate (Nunc, Roskilde, Denmark) was carried out using aqueous stock solutions of a biotinylated wild-type AP or AP(219-221)-K with biotin-QG (1), biotin-GGG-GGLQG (2) and bis(biotin-GGG)-KGLQG (3). The relative activity of biotin-QG labeled AP(219-221)-K was defined as 100%.

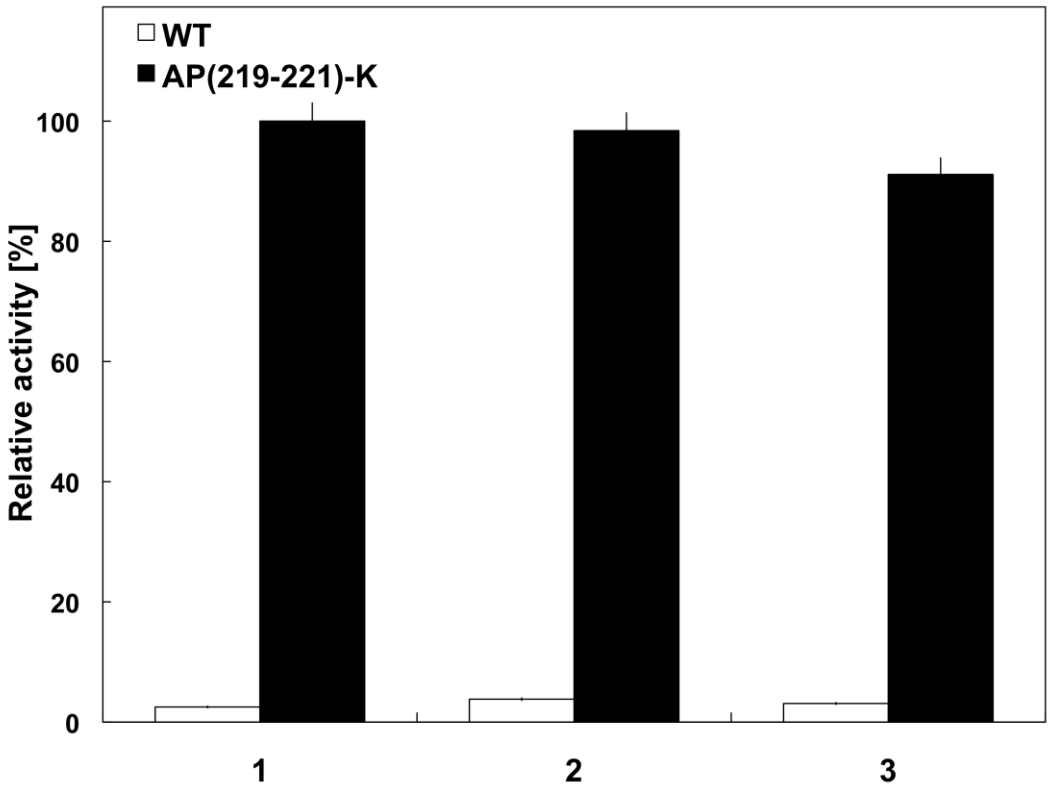

Fig. S3. The relative activity of biotinylated APs on an SA coated plate.

Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry
This journal is © The Royal Society of Chemistry 2012

DLS data of biotinylated APs

Dynamic light scattering data of self-assembled biotinylated AP with SA. Blue curves: biotinylated AP; purple, green and red curves: the ratio of concentration of SA to AP is 1/4, 1/2, 1 (for AP(219-221)-K(1) and AP(219-221)-K(2)) and 1/2, 1, 2 (for AP(219-221)-K(3)); the ratio of biotin binding-sites of SA to biotin groups of AP is 1/4, 1/2, and 1.

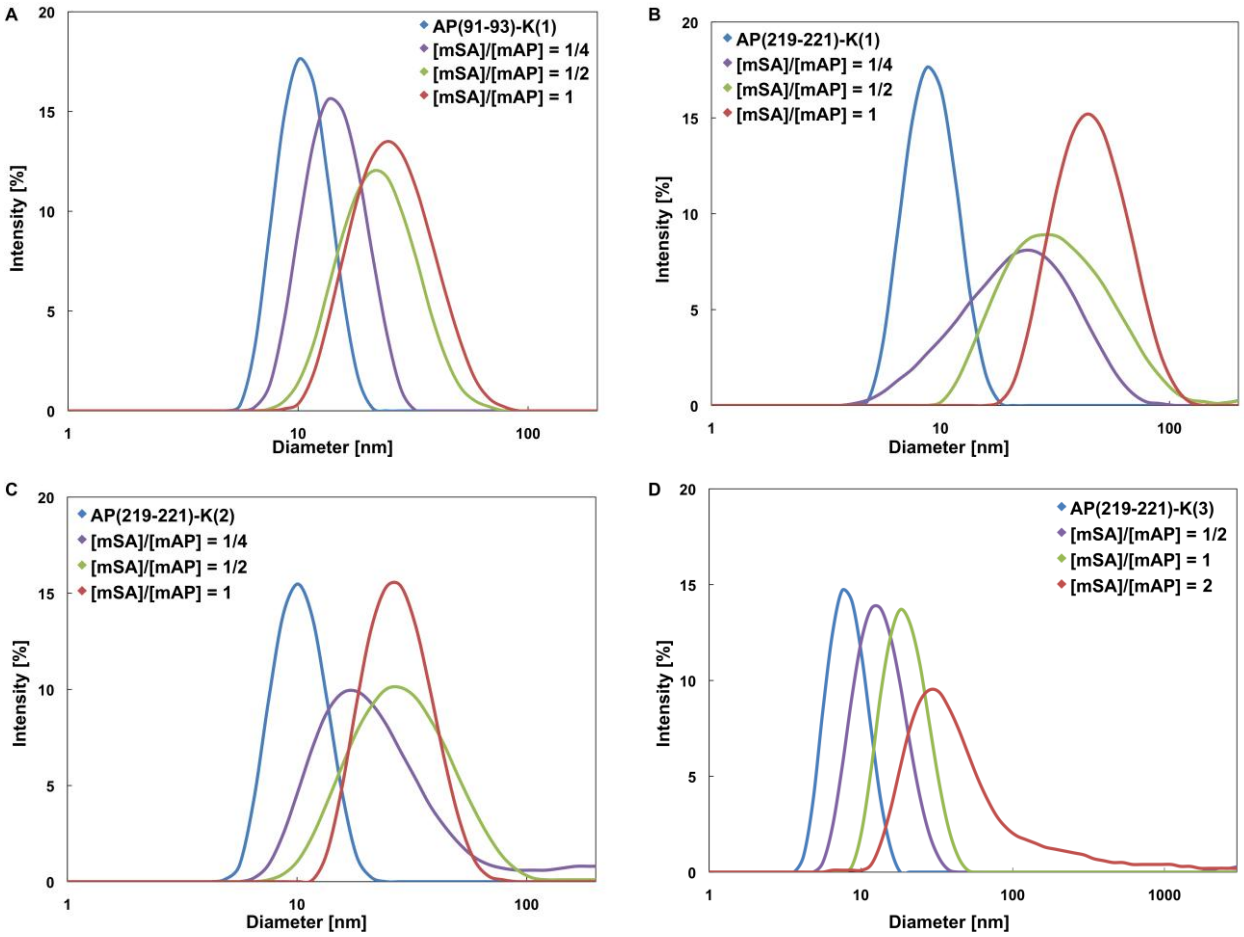

Fig. S4. DLS date of self-assembled biotinylated recombinant APs with SA. (A) AP(91-93)-K(1), (B) AP(219-221)-K(1), (C) AP(219-221)-K(2), (D) AP(219-221)-K(3).

Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry
This journal is © The Royal Society of Chemistry 2012

SEC chromatogram of the molecular markers and associated standard curve

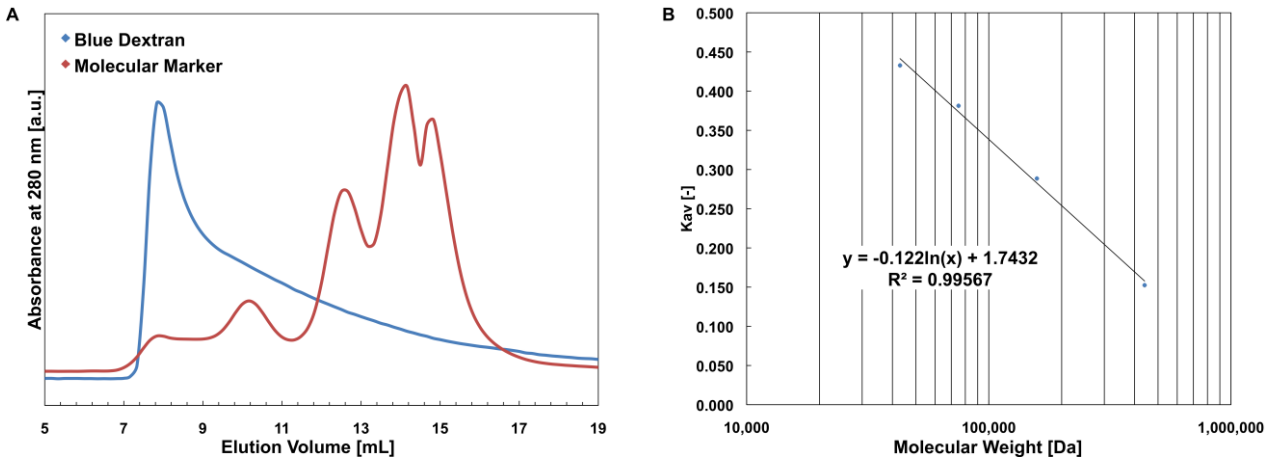

Fig. S5. Calibration of the molecular weight using the calibration results of the SEC column. (A) SEC chromatogram for the molecular weight markers and blue dextran eluting at the void volume. 1: Ferritin, 440 kDa; 2: Aldolase, 158 kDa; 3: Conalbumin, 75 kDa; 4: Ovalbumin, 43 kDa; (B) Standard curve for the molecular weight based on the results of SEC analysis of the molecular weight markers and blue dextran.

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

	Content	MW [Da]	V_E [ml]	K_{av}
1	Ovalbumin	43000	15.66	0.449050495
2	Conalbumin	75000	14.73	0.388118812
3	Aldolase	158000	13.32	0.295049505
4	Ferritin	440000	10.97	0.139933993
V_0	BlueDextran	2000000	8.85	-

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

V_c : Column Volume, 24 [ml].

Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry
This journal is © The Royal Society of Chemistry 2012

Effect of ionic strength on PSCs formation

Dynamic light scattering of self-assembled AP(219-221)-K(1) with 1 equiv. SA. Method 1: salts were added after self-assembly formation (blue); Method 2: salts were added before self-assembly formation (red).

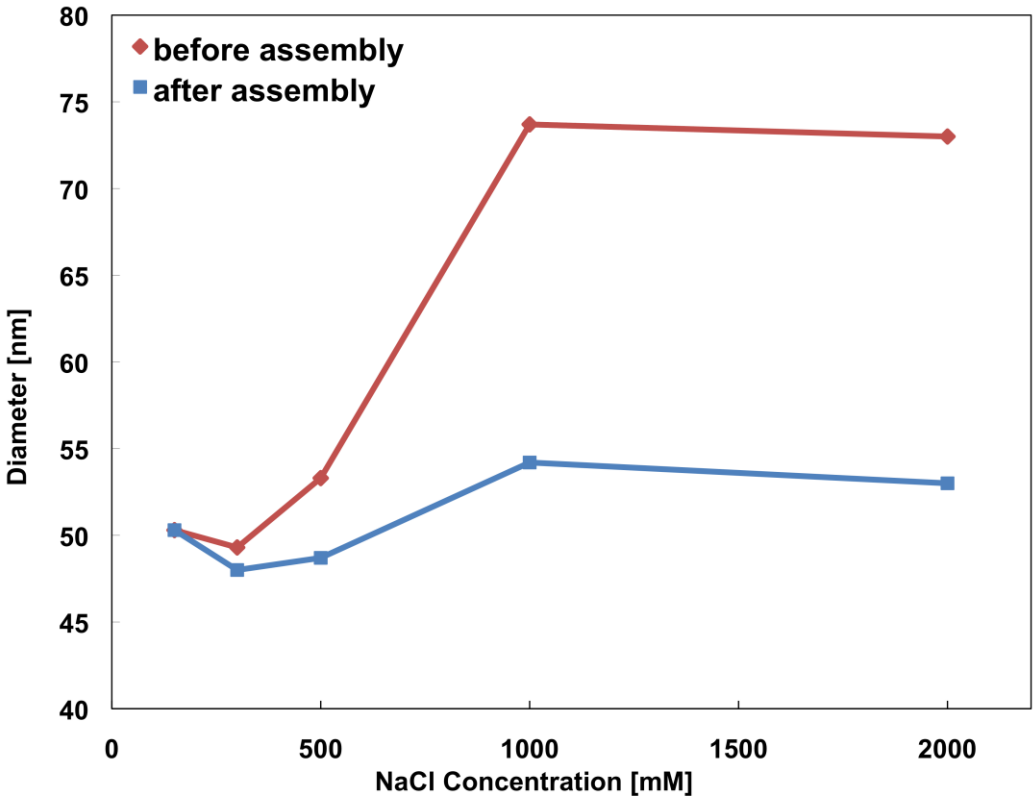

Fig. S6. DLS data of AP(219-221)-K(1) with SA. Salts were added after self-assembly formation (blue) or before self-assembly formation (red).

Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry
This journal is © The Royal Society of Chemistry 2012

Immunoassay by using PSCs

Comparison of the sensitivity of the AP supramolecular complex molecular biosensor with that of conventional indirect ELISAs using biotinylated antibodies for ovalbumin detection. Color development achieved by AP was measured at 410 nm on a microplate reader. The self-assembly complexes with AP(219-221)-K(1) were prepared.

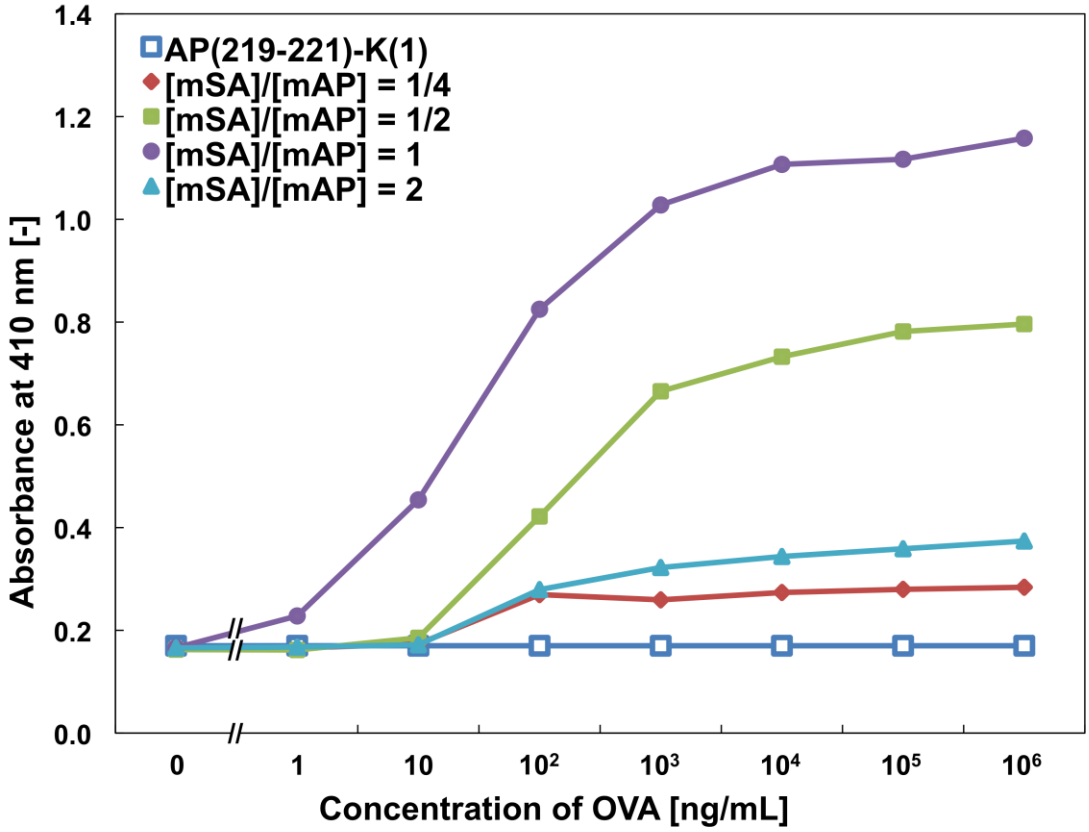

Fig. S7. Comparison of the sensitivity of AP supramolecular complex molecular biosensor with that of conventional indirect ELISAs using biotinylated antibodies for ovalbumin detection. The self-assembly complexes with AP(219-221)-K(1) were prepared. Blue curves: a biotinylated AP; purple, green, red and light blue curves: the ratio of concentration of SA to AP is 1/4, 1/2, 1, and 2.