Supporting Information

Dual-functional co-crystal of streptavidin and ssDNA: electrostatic assembly with positively charged peptide tags

Ayasa Nagatani,[a] Kosuke Minamihata,[a,*] Motoyasu Adachi,[b,c] Rie Wakabayashi,[a] Masahiro Goto,[a,d] Noriho Kamiya,[a,d]

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

[b]Institute for Quantum Life Science, National Institutes for Quantum Science and Technology, 4-9-1 Anagawa, Inage, Chiba 263-8555, Japan.

[c]Department of Quantum Life Science, Graduate School of Science, Chiba University, Chiba, 263-8522, Japan.

[d]Division of Biotechnology, Center for Future Chemistry, Kyushu University, 744 Motooka, Fukuoka 819-0395, Japan.

*E-mail: minamihata.kosuke@kyudai.jp

Material and Methods

Expression of SAs with positive charged peptides (SA-R_4Y and SA-R_6Y)

The expression vectors of SAs were transformed into E. coli BL21 Star (DE3) by heat shock and seeded on lysogeny broth (LB) agar plates containing 100 μg/mL of ampicillin sodium. A single colony was picked up and used to inoculate LB medium (5 mL). The suspension was precultured at 37 °C and 160 rpm overnight. The cell suspensions were then poured into LB medium (500 mL) containing the same antibiotic in a baffled Erlenmeyer flask and cultured at 37 °C and 180 rpm until optical density at 600 nm reached around 1.0. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was added final concentration 0.5 mM to induce protein expression, and culturing was continued at 37 °C for 5 h with shaking at 180 rpm. Cells were harvested by centrifugation at 5,000×g for 20 min and kept at −30 °C until purification. The cells were suspended in 30 mL of the Wash Buffer 1 (10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, pH 8.0). Lysozyme (20 mg), 60 units of Cryonase™ Cold-active Nuclease (Takara Bio Inc., Japan) and 1 M $MgCl_2$ (120 μL) were added to the cell suspension. After incubation at 4 °C for 1 h, the cells were completely lysed by sonication for 10 min of cooling between each sonication. The insoluble materials containing the inclusion body of SAs were separated by centrifugation at 5,000×g for 10 min at 4 °C. The precipitates were resuspended in 30 mL of Wash Buffer 2 (30 mM Tris-HCl, 2 mM EDTA, 0.1% Triton-X100, pH 8.0) using a pipette. The inclusion

body was centrifuged again at 5,000×g for 10 min at 4 °C to remove supernatant. This washing process was repeated at least three times to obtain the inclusion body of SAs. The same washing process was repeated using the Wash Buffer 1 to completely remove surfactant. Finally, the inclusion body was dissolved in 6 M guanidinium hydrochloride (1.2 mL, pH 1.5) and centrifuged at 20,000×g for 10 min at 4°C. The supernatant containing denatured SAs was gradually added dropwise into vigorously stirred Tris-buffered saline (TBS) (100 mL, 25 mM Tris-HCl, 150 mM NaCl, pH 7.4) on ice to induce refolding. The TBS solutions were then stirred gently for 3 h at 4 °C. The precipitates were removed by centrifugation at 15,000×g for 10 min at 4 °C. Finely ground ammonium sulfate (49.2 g) was added to the solution with gently stirring to precipitate the refolded SAs. The precipitates were collected by centrifugation at 15,000×g for 10 min at 4 °C. SAs were dissolved in Milli-Q water (20 mL) and then subjected to cation-exchange chromatography. SAs were purified using a HiTrap SP FF column, 5 mL (GE Healthcare Life Sciences) equilibrated with 10 mM Tris-HCl (pH 7.4). Unbound proteins were washed out with the same buffer (five column volumes). The SAs were eluted using a salt gradient of 1 M NaCl in 10 mM Tris-HCl (pH 7.4). The fractions containing SAs were collected and concentrated using an ultrafiltration membrane (30 kDa MWCO). After, these SAs were simply buffer-exchanged into 10 mM Tris-HCl (pH 8.0), concentrated using an ultrafiltration membrane (30 kDa MWCO) and then stored at −25 °C.

Preparation of crystals of SAs with charged peptides

Equimolar amounts of SA-R_nY and ssDNA were mixed together in 10 mM Tris-HCl (pH 8.0). The reaction mixtures (10 μL each) were set up in the wells of a 96 well plate and the surface was covered with paraffin oil to prevent evaporation. Each set of conditions was run in five individual wells. After setting up the reaction mixtures, the plates were left at 25 °C overnight, and then each well was observed with a microscope (BZ-X800, Keyence, Japan). SA-R_nY and ssDNA were mixed in 10 mM Tris-HCl (pH 8.0) containing an appropriate amount of NaCl.

Structure determination

The co-crystals for structure determination of SA complex were soaked into mother liquor containing 40% w/v polyethylene glycol 400. The cryoprotected crystal was flash-cooled under nitrogen cryo stream. Diffraction data were collected at 100 K on beamline NW12A at KEK-PF (Tsukuba, Japan). The wavelength used was 1.0 Å. Data were indexed, integrated and scaled using the HKL2000 (HKL Research Inc.) or XDS program suite[1] for A_9 and C_9 co-crystals, respectively. The initial protein models were obtained by molecular replacement with a monomer search model (PDB entry 6LNG, chain A) using the program Phaser in the CCP4 Suite[2,3]. The model was built using COOT[4]. The

structure was refined using the program PHENIX[5]. The data and refinement statistics are listed in Table S1. Ramachandran plot values were analyzed with Rampage[6]. Images in Fig. 3 and Fig.S5 were prepared with PyMOL[7].

Modification of co-crystals of ssDNAs

Co-crystals were prepared from a mixture of 10 μM SA-R_4Y and 10 μM ssDNA in 10 mM Tris-HCl (pH 8.0). After crystallization, 10 μM Fluorescein labeled ssDNA (FL-ssDNA) or TAMRA-labeled ssDNA (Fasmac) in 10 mM Tris-HCl (pH 8.0) was then added to the co-crystals and incubated for 1 h at 25 °C. The FL-ssDNA solution was removed and the concentration of remaining FL-ssDNA in the solution was estimated by measuring UV absorbance at 494 nm with extinction coefficient of 68,000 M^{-1} cm^{-1}. The SA crystals modified with FL-ssDNA were immersed in 10 mM Tris-HCl (pH 8.0) and observed with a confocal laser microscope (LSM710, Carl Zeiss Co. Ltd., Germany). For the CLSM observations of the modified SA crystals, a 488-nm laser was used for co-crystal modified with FL-ssDNA with appropriate emission filter sets.

Modification of Co-crystals of SAs

Co-crystals were prepared from a mixture of 10 μM SA-R_4Y and 10 μM A_{20} in 10 mM Tris-HCl (pH 8.0). After crystallization, 10 μM biotin-4-fluorescein (B4F, Sigma Aldrich) or Fluorescein in 10 mM Tris-HCl (pH 8.0) was then added to the Co-crystals and incubated for 1 h at 25 °C. The B4F solution was removed and the concentration of remaining B4F in the solution was estimated by measuring UV absorbance at 494 nm with extinction coefficient of 68,000 M^{-1} cm^{-1}. The SA crystals modified with B4F were immersed in 10 mM Tris-HCl (pH 8.0) and observed with a confocal laser microscope (LSM710, Carl Zeiss Co. Ltd., Germany). For the CLSM observations of the modified SA crystals, a 488-nm laser was used for co-crystal modified with B4F with appropriate emission filter sets.

Amino acid sequence

SA-R_4Y
MAEAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRYVLTGRY DSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARINTQWL LTSGTTEANAWKSTLVGHDTFTKVKPSAASRRRRY

SA-R_6Y
MAEAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRYVLTGRY DSAPATDGSGTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARINTQWL LTSGTTEANAWKSTLVGHDTFTKVKPSAASRRRRRRY

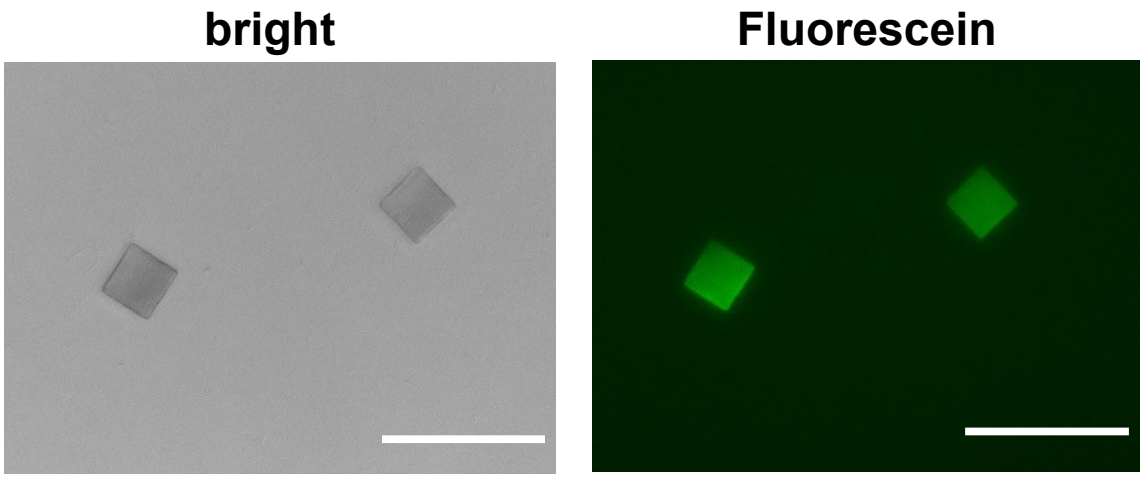


Fig. S1 Microscopic image of the co-crystal of FL-T_8 and SA-R_6Y. Scale bars: 100 μm.

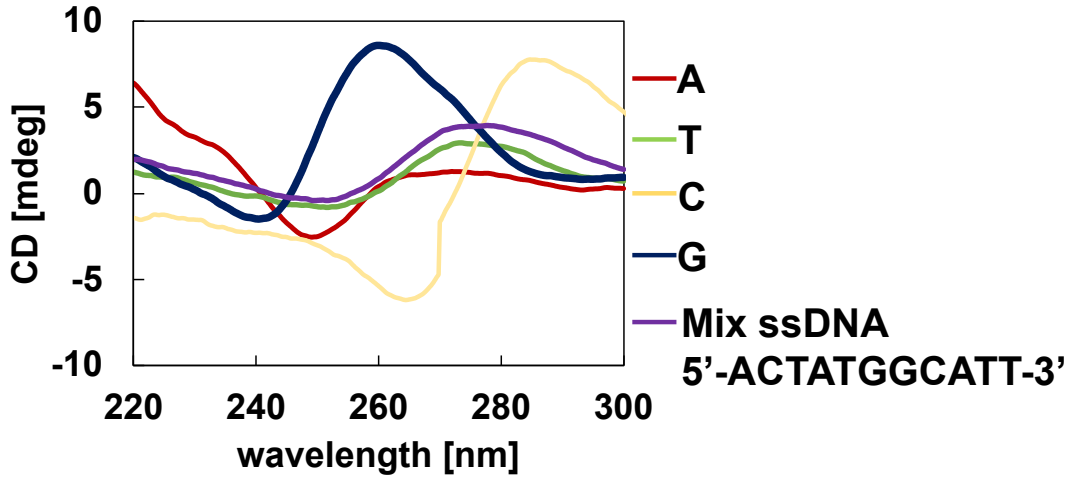


Fig. S2 The circular dichroism (CD) spectra of each ssDNA.

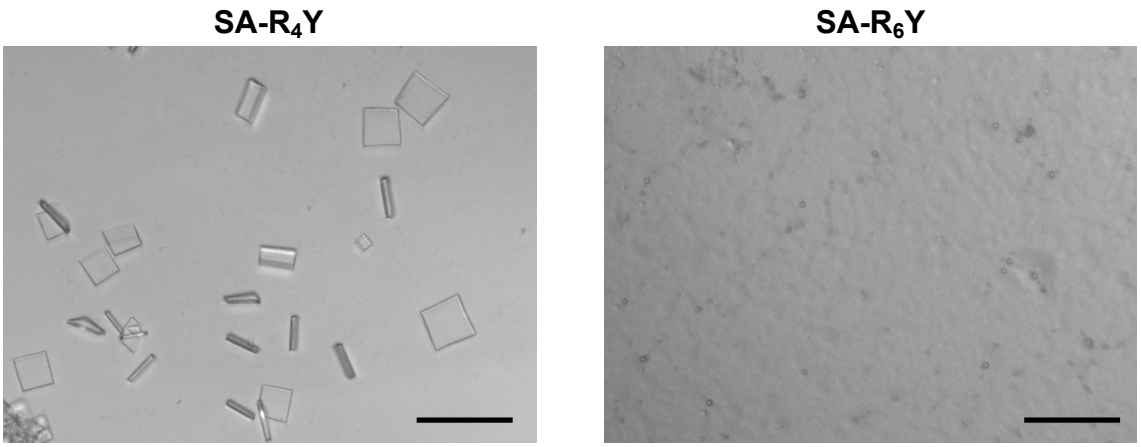


Fig. S3 Microscopic image of the co-crystal with SA-R_nY and T_{40}. Scale bars: 100 μm.

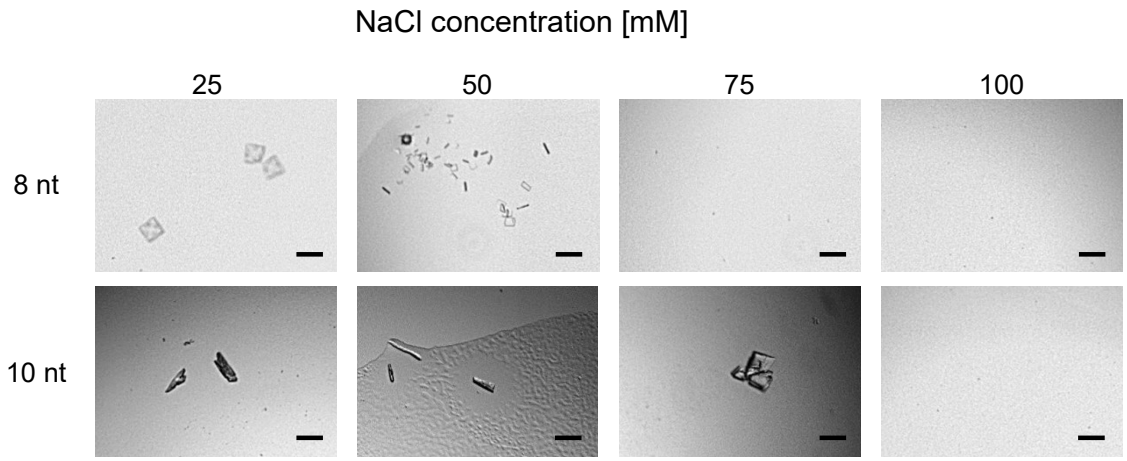


Fig. S4 Microscopic image of co-crystals of SA-R_6Y with T_8 or T_{10} under conditions with different NaCl concentration. Scale bars: 100 μm.

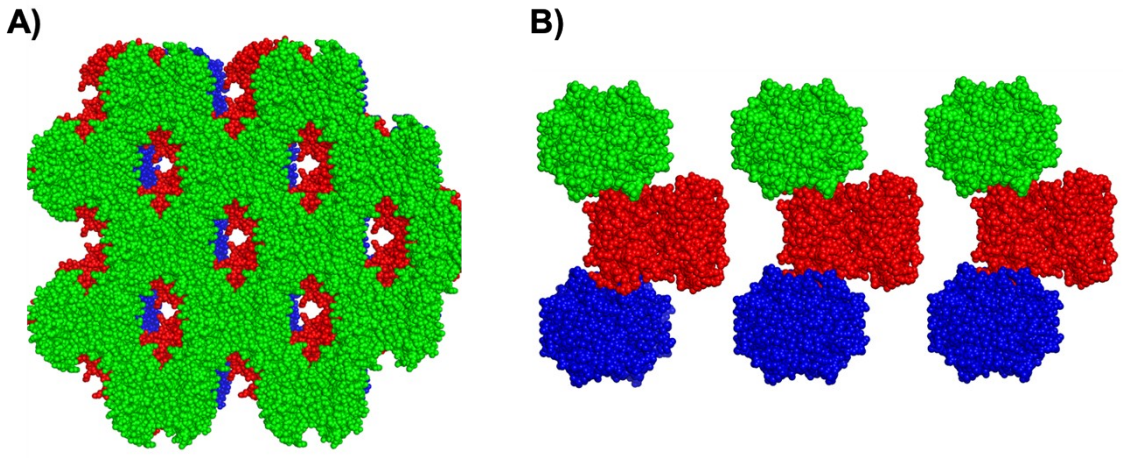


Fig. S5 Crystal structure of SA-R_6Y/C_9. A) The packing of each layer of SAs showing the layers. B) 3D packing of SAs showing the repeating unit of three layers in different colors. Enlarged view of the layer thickness.

Table S1 Data collection and refinement statistics (**Molecular replacement**)

	SA-R_6Y with A_9	SA-R_6Y with C_9
Data collection		
Space group	*P*1	*C*2
Cell dimensions		
a, *b*, *c* (Å)	46.084, 58.006, 57.791	80.937, 80.986, 71.316
□ *α*, *β*, *γ* (°)	90.65, 107.54, 98.84	90.00, 91.97, 90.00
Resolution (Å)	37.66-2.60(2.69-2.60)*	46.54-2.50(2.56-2.50)*
R_{merge}	0.140(0.514)	0.165(0.889)
$I/\sigma I$	8.8(2.3)	7.6(1.9)
Completeness (%)	97.5(97.3)	98.9(63.3)
Redundancy	1.9(1.9)	3.7(3.6)
Refinement		
Resolution (Å)	37.66-2.60	46.54-2.50
No. reflections	16697	17327
$R_{work/}$ R_{free}	0.198/0.282	0.234/0.346
No. atoms		
Protein	3620**	3620**
Water	164	56
B-factors		
Protein	28.7	29.6
Water	28.3	27.1
R.m.s deviations		
Bond lengths (Å)	0.009	0.008
Bond angles (°)	1.160	1.144
Ramachandran plot		
favoured region (%)	89.8	85.0
allowed region (%)	8.5	10.4
outlier region (%)	1.7	4.6
PDBID	8ZR1	8ZR2

One crystal was used for the structural analysis.

*Highest resolution shell is shown in parenthesis.

**Four molecules of SA are included in asymmetric unit of the crystal lattice.

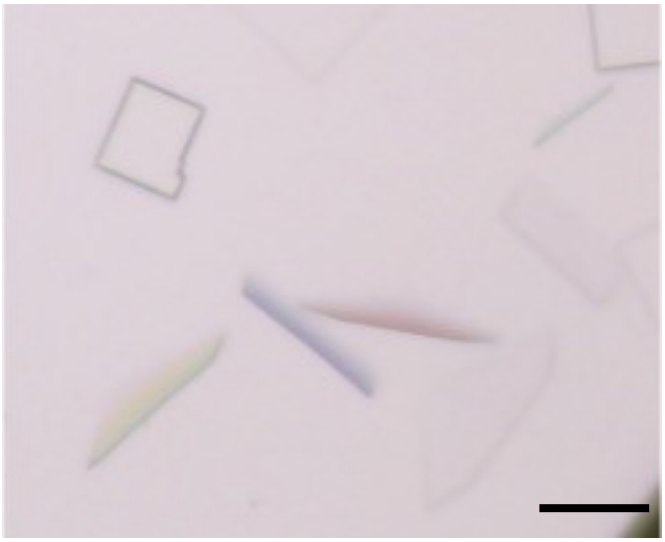
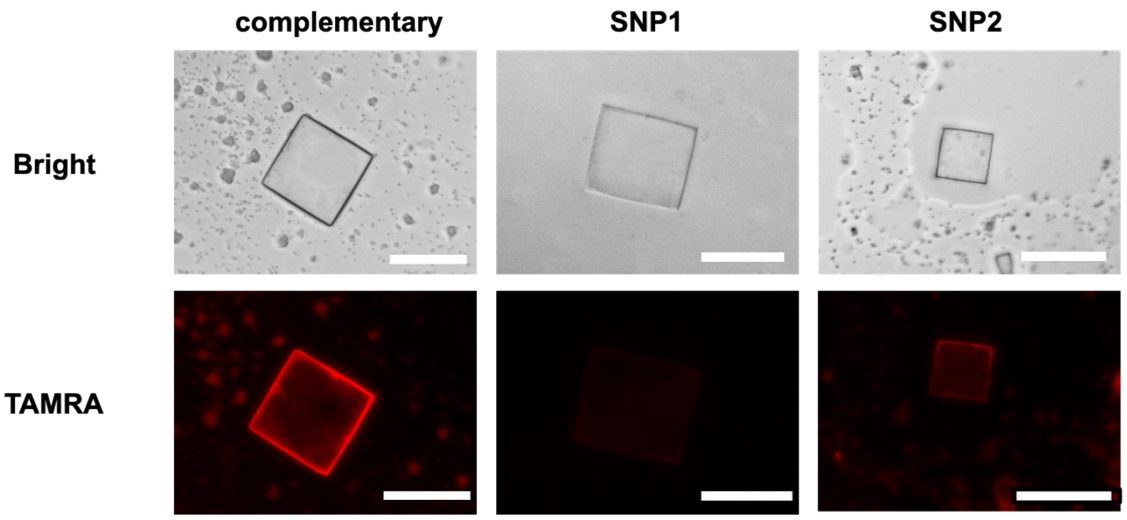


Fig. S6 Microscopic image of the co-crystals. Co-crystals showing structural colors were observed when illuminated. Scale bar is 100 μm.



name	sequence
TAMRA-labelled ssDNA	CGGCATGTCTATTTTGTA
Complementary	TACAAAATAGACATGCCG
SNP1	TAAAAAATAGACATGACG
SNP2	TACAAAATAAACATGCCG

Fig. S7 Fluorescence microscopy images of co-crystals formed with complementary, SNP1 or SNP2 treated with TAMRA-labelled ssDNA (yellow markers indicate the locations where mutations were introduced). Scale bars: 100 μm.

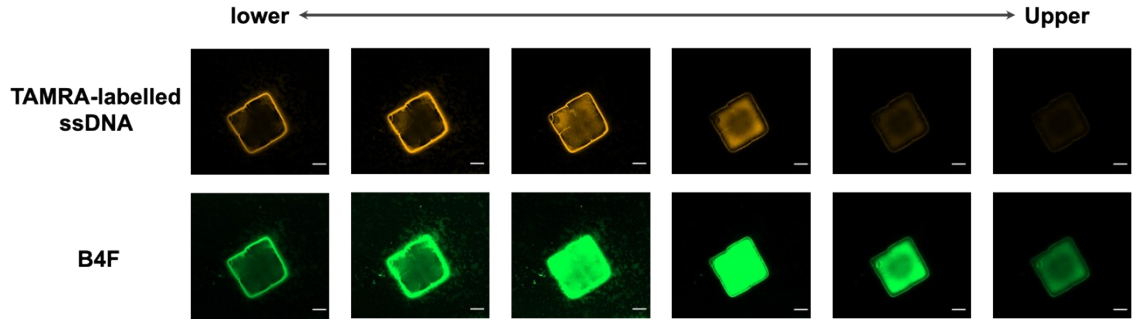


Fig. S8 Cross-sectional images of co-crystals modified with TAMRA-ssDNA and B4F. Scale bars: 20 μm.

References

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