Electronic Supplementary Material (ESI) for Chemical Communications.

A Modular Zinc Finger Adaptor Accelerates Covalent Linkage of Proteins at Specific Locations on DNA Nanoscaffolds

Eiji Nakata, Huyen Dinh, Tien Anh Ngo, Masayuki Saimura and Takashi Morii*

Institute of Advanced Energy, Kyoto University, Uji, Kyoto 611-0011, Japan
Fax: (+81) 774-38-3585
E-mail: t-morii@iae.kyoto-u.ac.jp

Supporting Information

Table S1. Equilibrium dissociation constants (K_D) for the complexes of ZF-SNAP with oligo DNA.

	oligo DNA	K_D (nM)
ZF-SNAP	ODN-zif	63 ± 18
	ODN-non	> 500

Table S2. Total numbers and yields of DNA origami bound by ZF-SNAP-EGFP observed by AFM imaging analyses.

DNA origami	Conditions	Numbers of the expected structure (N_{total})	Numbers and yields of the modified DNA origami	
			at the expected position ($N_{expected\ posi}$) [yield %]	at the unexpected position ($N_{unexpected\ posi}$) [yield %]
I-4zifBG	a	333	332 [100%]	43 [3%]
I-4zif	a	334	161 [48%]	153 [11%]
I-4nonBG	a	342	332 [97%]	29 [2%]
I-4zifBG	b	385	306 [79%]	20 [2%]
I-4nonBG	b	270	59 [22%]	10 [1%]
5-1mixBG[*1]	b	187	448 [60%]	14 [8%]
I-4zifBG	c	159	131 [82%]	13 [2%]
I-4nonBG	c	214	18 [8%]	18 [2%]
4-1zifBG	d	107	392 [92%]	4 [4%]
I-2zifBG	d	84	81 [96%]	14 [4%]
I-4zifBG	d	268	263 [98%]	30 [3%]
I-4zif	d	177	91 [51%]	75 [11%]

a : [DNA origami] = 5 nM, [ZF-SNAP-EGFP] = 100 nM with 30 min incubation at ambient temperature.

b : [DNA origami] = 5 nM, [ZF-SNAP-EGFP] = 20 nM with 30 min incubation at ambient temperature.

c : [DNA origami] = 5 nM, [ZF-SNAP-EGFP] = 50 nM, [ODN-zif] = 500 nM with 30 min incubation at ambient temperature.

d : [DNA origami] = 5 nM, [ZF-SNAP-EGFP] = 300 nM with 120 min incubation at ambient temperature.

[*1] In the case of 5-1mixBG, the expected position and the unexpected position indicated zifBG and nonBG, respectively.

Figure S1. A possible molecular model for the complex of ZF-SNAP and ODN-zif-BG based on the crystal structure of the complex between zif268 and DNA (PDB ID: 1ZAA) and the complex of BG and SNAP-tag (PDB ID: 3KZY). The molecular model was constructed by using Discovery Studio (version 3.1, Accelrys Inc.).

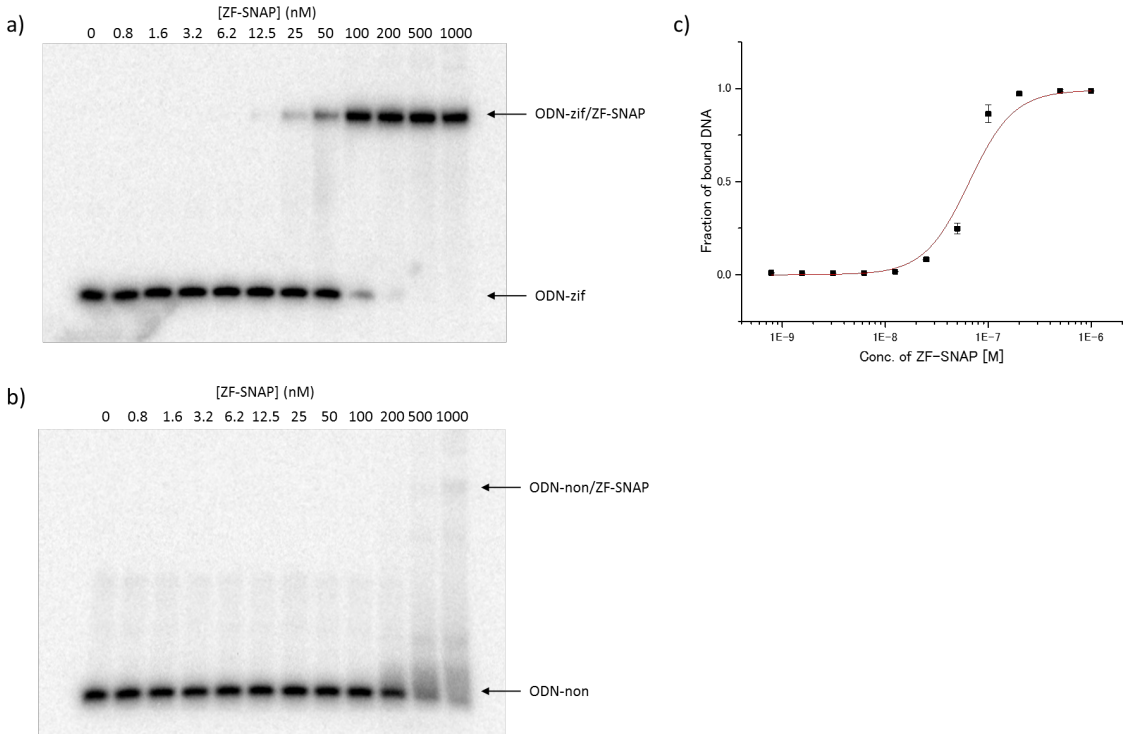


Figure S2. Autoradiograms show the electrophoretic mobility shift titration of ZF-SNAP to (a) ODN-zif or (b) ODN-non in a buffer containing 40 mM Tris-HCl, 20 mM acetic acid, 12.5 mM $MgCl_2$, 1 mM DTT, 1 μM $ZnCl_2$, 0.02 % Tween 20, and 1 μg/ml BSA, pH 8.0, at ambient temperature. Arrows denote ODN and ODN/ZF-SNAP complexes, respectively. (c) A semilogarithmic plot shows the fractions of 5'-^{32}P-labeled ODN-zif bound to ZF-SNAP.

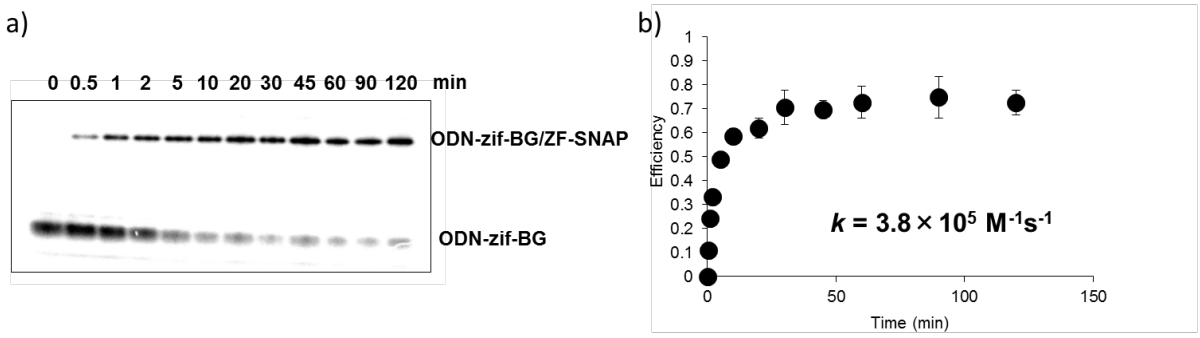


Figure S3. (a) Denaturing PAGE (15%) analysis of the crosslinking reactions of 5'-^{32}P-end-labeled ODN-zif-BG (0.5 nM) with ZF-SNAP (10 nM). (b) A time-course profile for the crosslinking reaction of ODN-zif-BG and ZF-SNAP to obtain the rate constant (***k***).

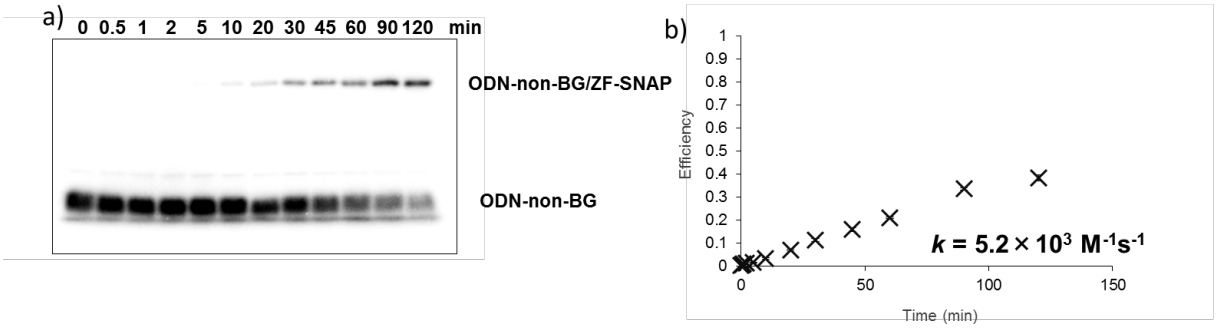


Figure S4. (a) Denaturing PAGE (15%) analysis of the cross-linking reactions of 5'-^{32}P-end-labeled ODN-non-BG (0.5 nM) with ZF-SNAP (10 nM). (b) A time-course profile for the crosslinking reaction of ODN-non-BG and ZF-SNAP to obtain the rate constant (***k***).

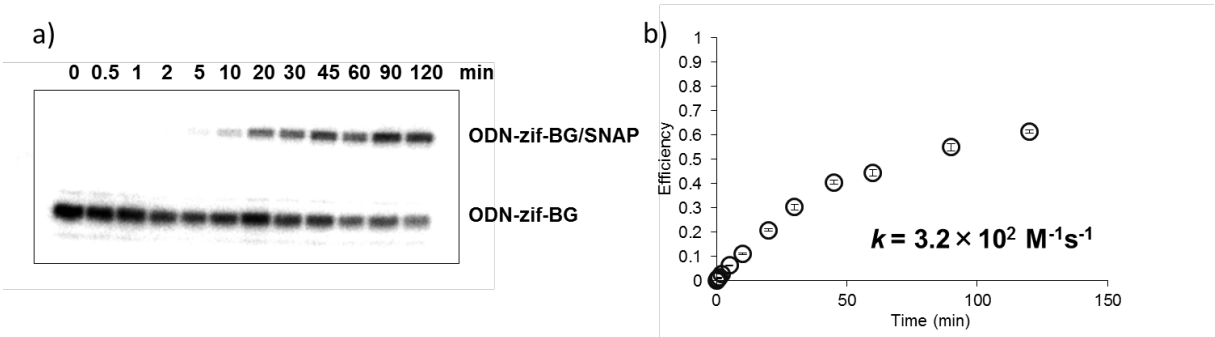


Figure S5. (a) Denaturing PAGE (15%) analysis of the cross-linking reactions of 5'-^{32}P-end-labeled ODN-zif-BG (5 nM) and non-labeled ODN-zif-BG (1 μM) with SNAP (10 μM). (b) A time-course profile for the crosslinking reaction of ODN-zif-BG and SNAP to obtain the rate constant (***k***).

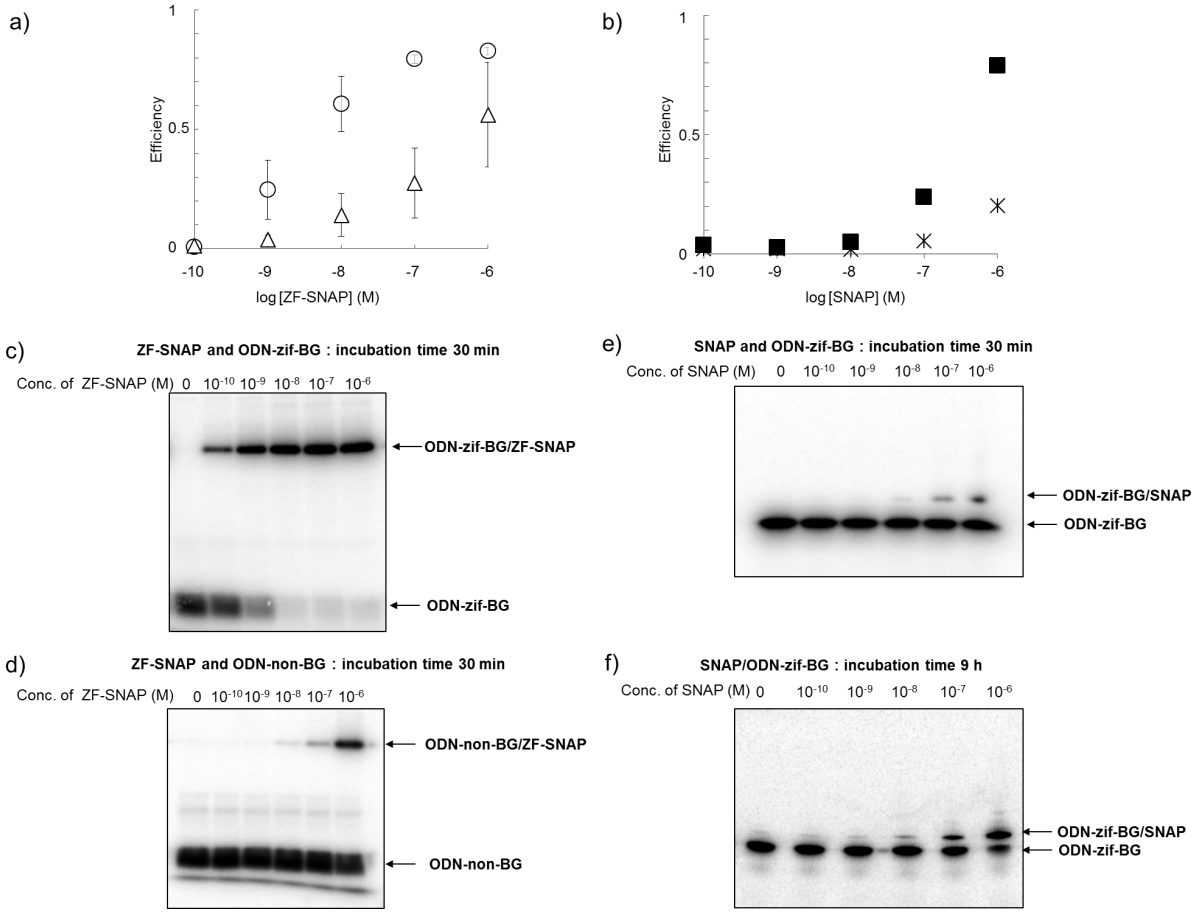


Figure S6. a) Semilogarithmic plots show the fractions of 5'-^{32}P-end-labeled ODN-zif-BG (open circles) or 5'-^{32}P-end-labeled ODN-non-BG (open triangles) covalently linked to ZF-SNAP after 30 min incubation.
b) Semilogarithmic plots show the fractions of 5'-^{32}P-end-labeled ODN-zif-BG covalently linked to SNAP after 30 min (filled squares) or 9 h (asterisks) incubation.
Denaturing PAGE (15%) analyses of the covalent-linkage formation of c) 5'-^{32}P-end-labeled ODN-zif-BG (0.5 nM) or d) 5'-^{32}P-end-labeled ODN-non-BG (0.5 nM) with various concentrations of ZF-SNAP (0.1 nM to 1 μM) after 30 min incubation. Denaturing PAGE (15%) analyses of covalent linking formation of 5'-^{32}P-end-labeled ODN-zif-BG (0.5 nM) with various concentrations of SNAP (0.1 nM to 1 μM) after e) 30 min or f) 9 h incubation.

The crosslinking yields for the reaction of ODN-zif-BG and ODN-non-BG with various concentrations of ZF-SNAP were evaluated after 30 min of incubation at ambient temperature. Crosslinking products of ODN-zif-BG and ODN-non-BG were obtained at yields of 60% and 14%, respectively, when using 10 nM ZF-SNAP. When 1 μM ZF-SNAP was used, the yield of the nonspecific crosslinking product with ODN-non-BG reached 56%. Under the same conditions, the product between SNAP (1 μM) and ODN-zif-BG was obtained at a 20% yield. These results confirmed the efficient and rapid formation of a covalent linkage between ODN-zif-BG and the ZF-SNAP adaptor.

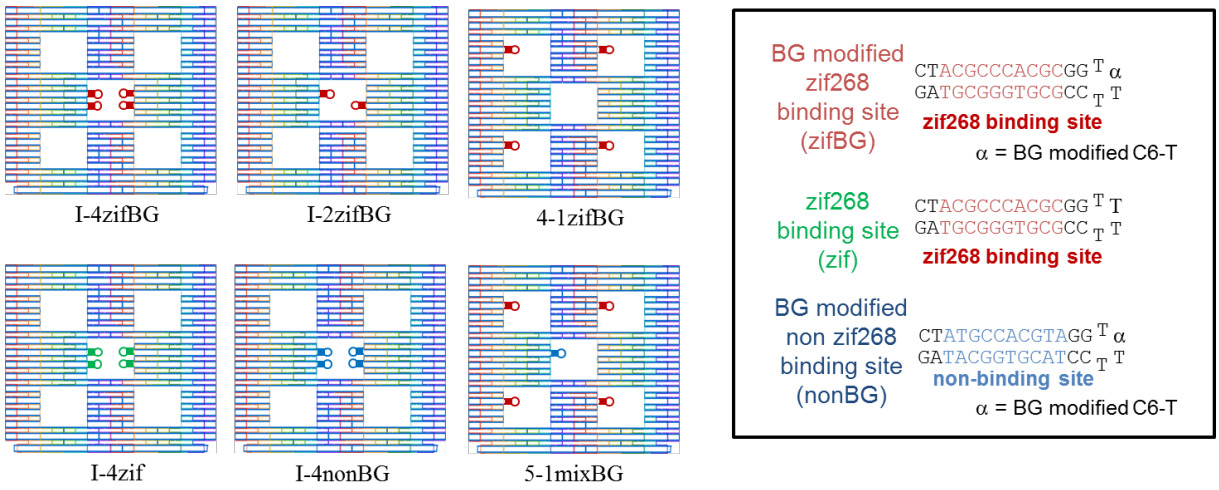


Figure S7. Illustrations show the structures of addressable DNA nanoscaffolds synthesized by the DNA origami method used in this study. The stem region in red denotes the binding site of zif268 with a BG-modification at the loop region (zifBG). The stem region in green denote the binding site of zif268 without the BG-modification at the loop region (green : zif).The stem region in blue denotes the nonspecific binding site of zif268 with a BG-modification at the loop region (nonBG).

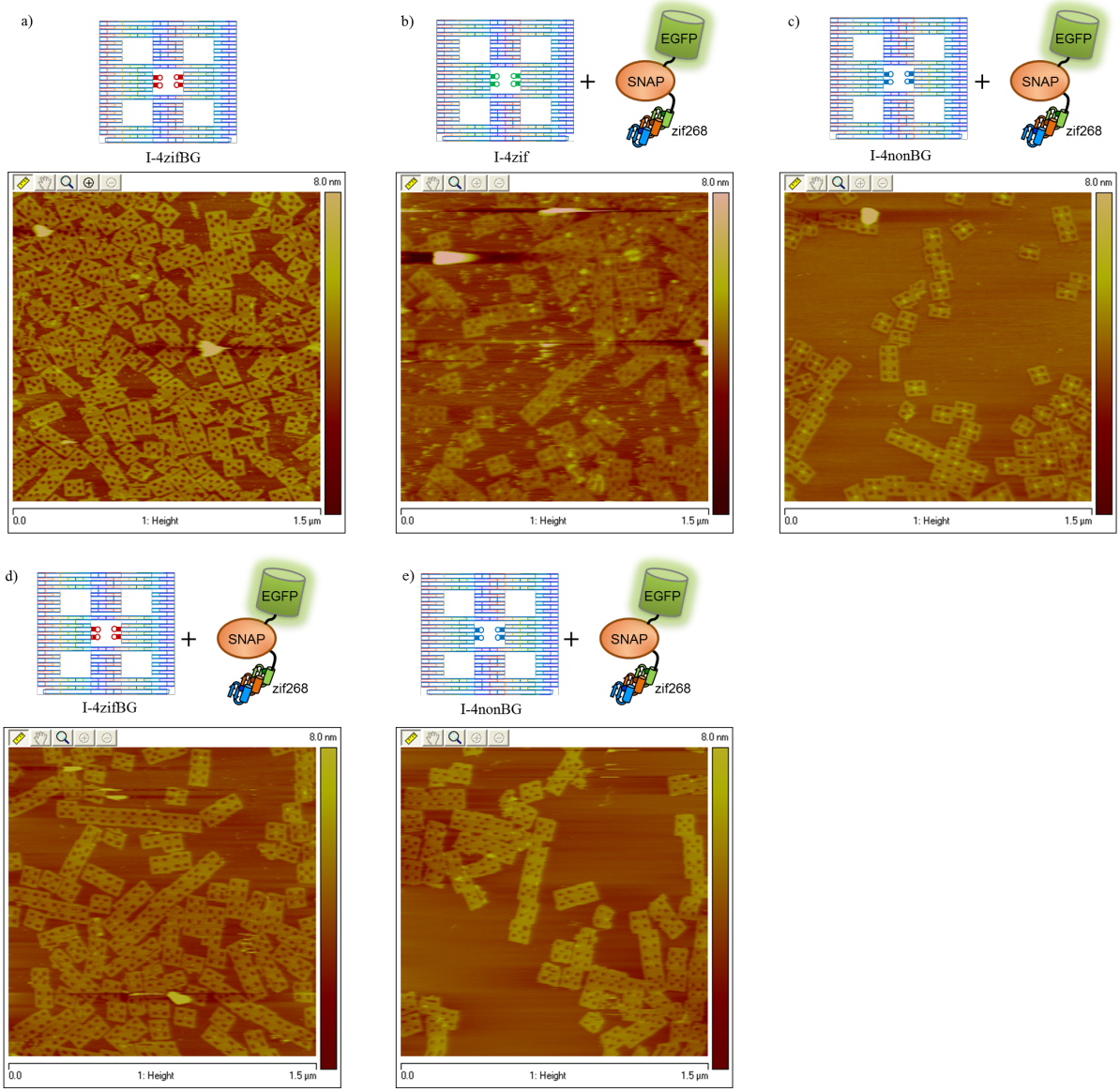


Figure S8. AFM images of (a) I-4zifBG (5 nM) only, (b) I-4zif (5 nM) modified with ZF-SNAP-EGFP (100 nM) (condition a), (c) I-4nonBG (5 nM) modified with ZF-SNAP-EGFP (100 nM) (condition a), (d) I-4zifBG (5 nM) modified with ZF-SNAP-EGFP (20 nM) (condition b), and (e) I-4nonBG (5 nM) modified with zif-SNAP-EGFP(20 nM) (condition b). All the samples were incubated at ambient temperature for 30 min. AFM images were taken by MultiMode^{TM} microscope.

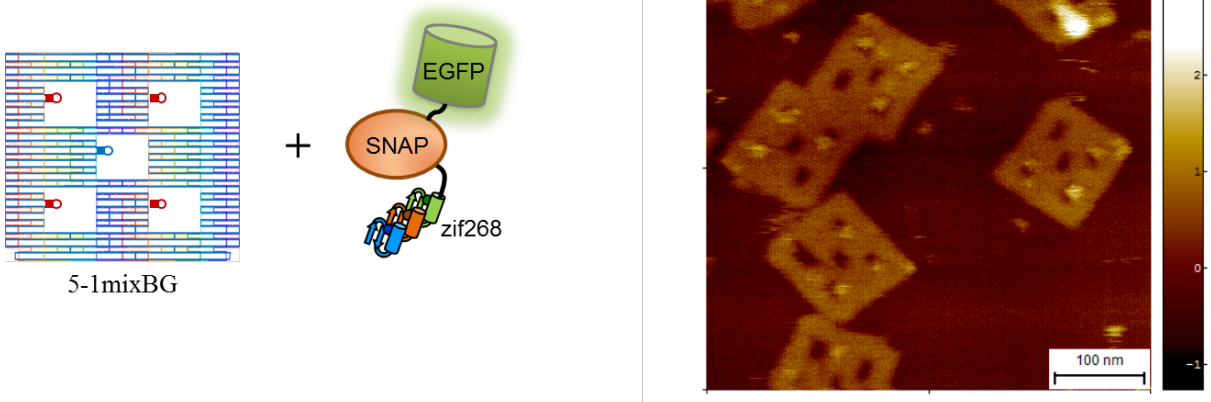


Figure S9. AFM images of ZF-SNAP-EGFP (20 nM) modified (a) 5-1mixBG (5 nM) (condition b). Samples were incubated for 30 min at ambient temperature. AFM images were taken by Nano Live Vision.

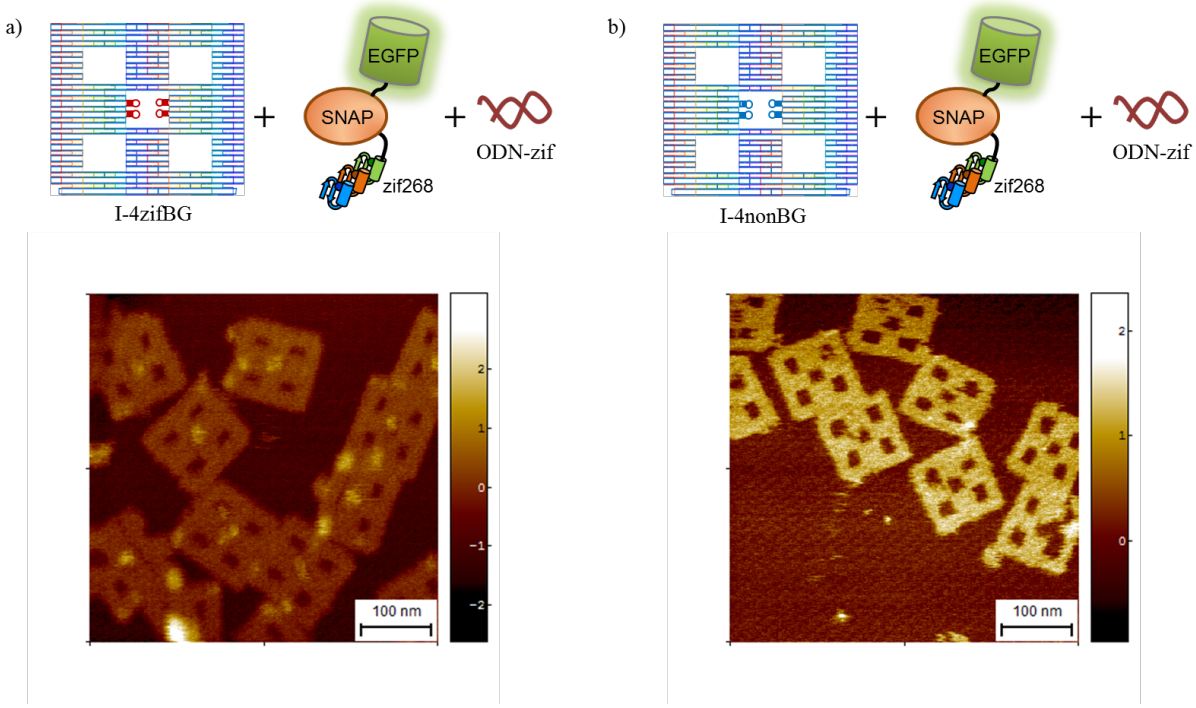


Figure S10. AFM images of ZF-SNAP-EGFP (50 nM) modified (a) I-4zifBG (5 nM) and (b) I-4nonBG (5 nM) in the presence of competitor ODN-zif (500 nM) (condition c). Samples were incubated for 30 min at ambient temperature. AFM images were taken by Nano Live Vision.

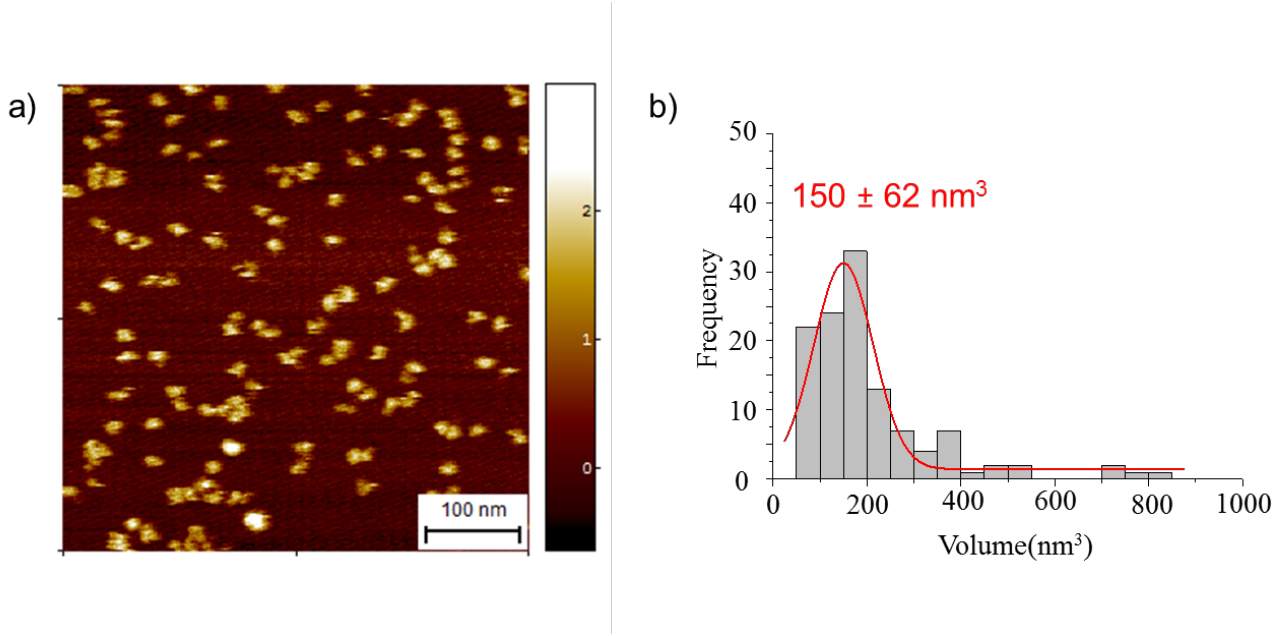


Figure S11. (a) An AFM image and (b) frequency distribution of molecular volumes of ZF-SNAP-EGFP (n = 119). AFM image were taken by Nano Live Vision.

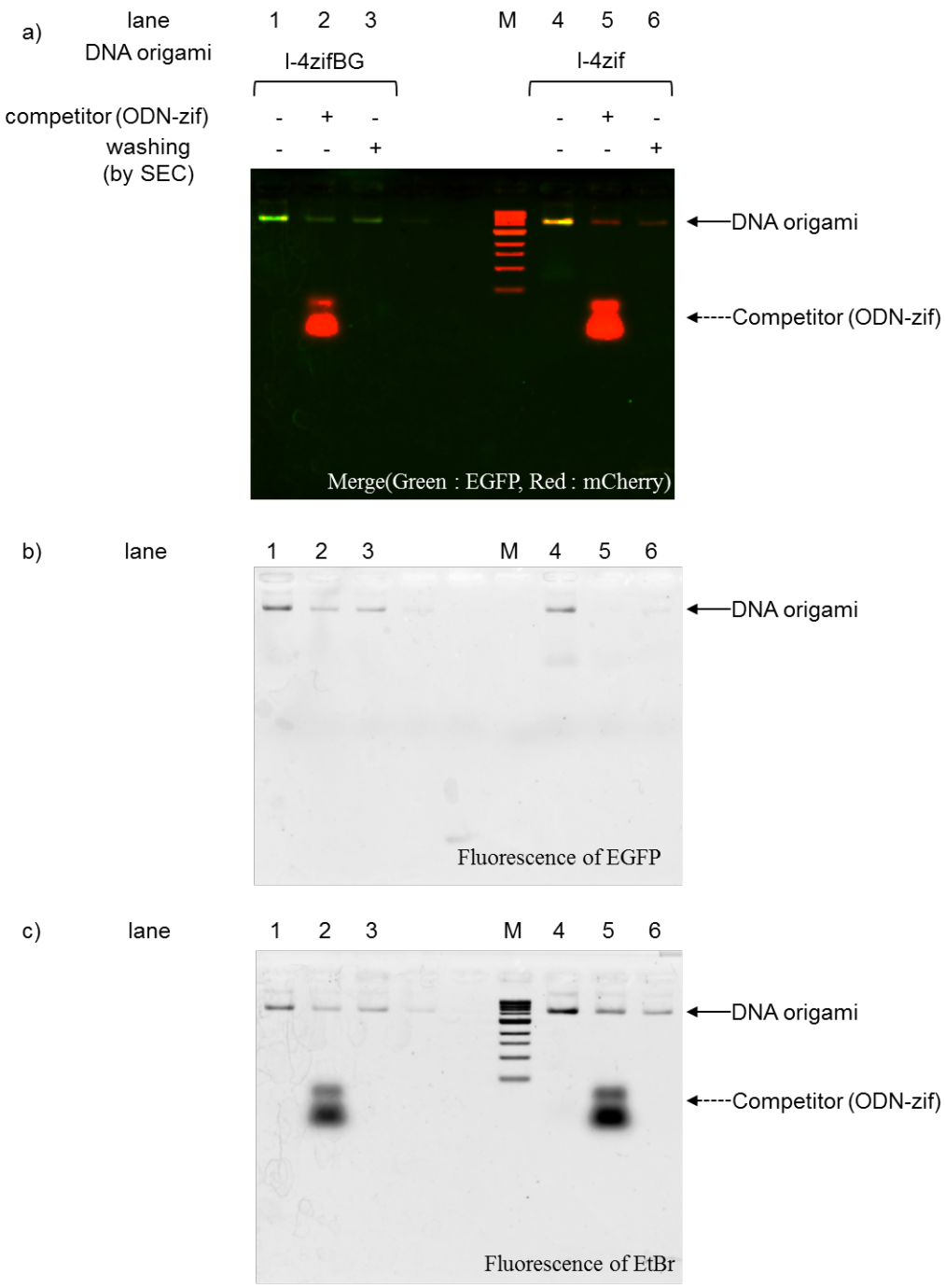


Figure S12. Agarose gel electrophoretic analysis of the binding stability of ZF-SNAP-EGFP (100 nM) on DNA origami (I-4zifBG or I-4zif, 8 nM) visualized by (a) EGFP (green) and ethidium bromide (EtBr) (red), by (b) EGFP, or by (c) EtBr fluorescence upon 120 min incubation at ambient temperature.

M: 1 kb DNA ladder; lanes 1-3: I-4zifBG; lanes 4-6: I-4zif, lanes 1, 2, 4 and 5: assembly of I-4zifBG/ZF-SNAP-EGFP or I-4zif/ZF-SNAP-EGFP loaded without (lanes 1 and 4) or with (lanes 2 and 5) an addition of excess amount of ODN-zif (1 μM) as a competitor; lanes 3 and 6: DNA origami after purified with size-exclusion gel chromatography (SEC). Solid arrows denote DNA origami, and dashed arrows denote ODN-zif.

Stability of a covalently linked protein assembly on DNA nanoscaffold was evaluated by agarose gel electrophoretic analysis (Figure S12). DNA origami I-4zifBG and I-4zif were incubated with ZF-SNAP-EGFP, then analyzed by agarose gel electrophoresis. Fluorescent emission derived from EGFP were observed at the bands corresponding I-4zifBG and I-4zif, respectively, in lanes 1 and 4. Upon addition of a competitor (ODN-zif) to remove noncovalently interacting ZF-SNAP-EGFP, fluorescence emission derived from EGFP on I-4zif was diminished (lane 5), while that on I-4zifBG remained intact (lane 2). Based on the quantitation of fluorescence intensity (see *Supporting Table for Figure S12*), the specific binding site of I-4zifBG was occupied by ZF-SNAP-EGFP in 84% yield (lane 2), which was consistent with the yield (88 %) obtained from the volume analysis of AFM images (see *formula S1 for Figure S12*). Because the fluorescence emission observed on DNA origami in the absence of ODN-zif without the SEC treatment (lane 1) was far greater than those in the presence of ODN-zif (lane 2) or with the SEC treatment (lane 3), both covalently and noncovalently bound ZF-SNAP-EGFP would contribute to the fluorescence emission in the absence of ODN-zif (lanes 1 and 4). After washing the protein-DNA origami assembly with SEC (lanes 3 and 6), the binding sites of I-4zifBG was occupied in 81% yield, that is, the non-specifically bound ZF-SNAP-EGFP was removed by this procedure. On the other hand, ZF-SNAP-EGFP was retained on I-4zif in a lower yield (31%) after SEC. These results strongly indicated that the covalently linked protein assembly of the DNA nanoscaffold was effectively purified by SEC without losing the bound protein on the DNA nanoscaffold.

Supporting Table for Figure S12. The calculated value determined from the fluorescent imaging.

Lane No.	1	2	3	4	5	6
DNA origami	I-4zifBG			I-4zif		
Amount of ZF-SNAP-EGFP [fmol]	460 [a]	135 [a]	149 [a]	310 [a]	n.d. [d]	30 [a]
Amount of DNA origami [fmol] (Amount of the binding sites)	40 [b] (160)	40 [b] (160)	46 [c] (184)	40 [b] (160)	40 [b] (160)	24 [c] (96)
Occupancy of binding sites (%) [f]	n.d. [e]	84%	81%	n.d. [e]	n.d. [d]	31%

a : determined from the fluorescence emission of EGFP
b : based on the experimental condition
c : determined from the fluorescence emission of EtBr
d : not determined because of a low fluorescent intensity
e : not determined because of the contribution of nonspecifically bound ZF-SNAP-EGFP.
f : determined by the following formula:
[Occupancy of binding sites] = [amount of ZF-SNAP-EGFP] / [amount of binding site on DNA origami] ×100

Formula S1 for Figure S12 :

$$[\text{Occupancy of binding sites}] = 100 \times \{(4 \times 0.6 + 3 \times 0.4) / 4\} \times 0.98 = 88\ (\%)$$

Based on the volume analysis data (Figure 3d), occupancy of 60% for the four binding sites and 40% for the three binding sites in 98 % yield of DNA origami were used to calculate the occupancy of binding sites.

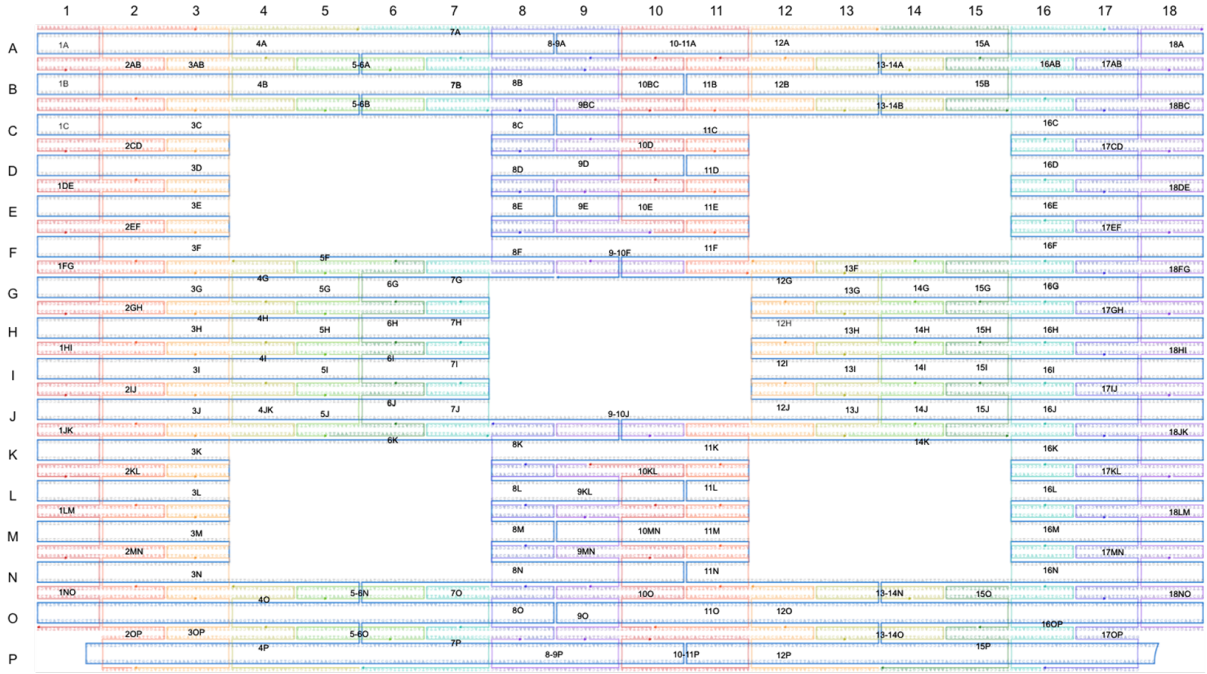


Figure S13. An illustration showing the shape and addresses of the DNA origami scaffold used in this study.

Table S3. The staple strands including zif268 binding site with or without BG modified dT (indicated as @) or GCN4 binding site used for the DNA origami. The zif268 or non-zif268 sequences on the staple strands were colored in red or blue, respectively. The positions such as 7H corresponded the address shown in **Figure S13**.

Oligo name	Sequence (from 5' to 3')
7H-zifBG	AACAGCTG TTTCTTTTCACCAGTG CTACGCCCACGCGG T@TT CCGCGTGGGCGTAG AATTGTTA
7I-zifBG	TCCGCTCA GCTGTTTCCTGTGTGA CTACGCCCACGCGG T@TT CCGCGTGGGCGTAG CTGTTGGG
12H-zifBG	AAGGCCGG CTACGCCCACGCGG T@TT CCGCGTGGGCGTAG AAAGACACCACGGAAT CATATAAA
12I-zifBG	CAGAGCCA CTACGCCCACGCGG T@TT CCGCGTGGGCGTAG AAACGTCACCAATGAA CCATTAGC

Oligo name	Sequence (from 5' to 3')
3D-zifBG	CCGAGTAA CTACGCCCACGCGG T@TT CCGCGTGGGCGTAG GGAGCTAAACAGGAGG CGTTAGAA
11D-zifBG	CAAGAAAA CTACGCCCACGCGG T@TT CCGCGTGGGCGTAG AAAAGAAGATGATGAATTTCAATT
3L-zifBG	ACGGTAAT CTACGCCCACGCGG T@TT CCGCGTGGGCGTAG ATGCCGGAGAGGGTAGTCTAGCTG
11L-zifBG	TATACCAGATGCGATTTTAAGAAC CTACGCCCACGCGG T@TT CCGCGTGGGCGTAG CATTGTGA

Oligo name	Sequence (from 5' to 3')
7H-nonBG	AACAGCTG TTTCTTTTCACCAGTG CTATGCCACGTAGG T@TT CCTACGTGGCATAG AATTGTTA
7I-nonBG	TCCGCTCA GCTGTTTCCTGTGTGA CTATGCCACGTAGG T@TT CCTACGTGGCATAG CTGTTGGG
12H-nonBG	AAGGCCGG CTATGCCACGTAGG T@TT CCTACGTGGCATAG AAAGACACCACGGAAT CATATAAA
12I-nonBG	CAGAGCCA CTATGCCACGTAGG T@TT CCTACGTGGCATAG AAACGTCACCAATGAA CCATTAGC

Oligo name	Sequence (from 5' to 3')
7H-zif	AACAGCTG TTTCTTTTCACCAGTG CTACGCCCACGCGG TTTT CCGCGTGGGCGTAG AATTGTTA
7I-zif	TCCGCTCA GCTGTTTCCTGTGTGA CTACGCCCACGCGG TTTT CCGCGTGGGCGTAG CTGTTGGG
12H-zif	AAGGCCGG CTACGCCCACGCGG TTTT CCGCGTGGGCGTAG AAAGACACCACGGAAT CATATAAA
12I-zif	CAGAGCCA CTACGCCCACGCGG TTTT CCGCGTGGGCGTAG AAACGTCACCAATGAA CCATTAGC

Materials and Methods

1. Materials

The single-stranded M13mp18, restriction enzymes (*Nde*I and *Hind*III), pSNAP-tag (T7)-2 Vector (N9181S), BG-GLA-NHS (S9151S), SNAP-tag Purified Protein (P9312S) were purchased from New England Biolabs. Purified DNA origami staple strands, oligonucleotide primers and all other modified oligonucleotides were obtained from Sigma-Aldrich (St. Louis, MO) or Gene Design Inc. (Osaka, Japan). *E. coli* BL21 (DE3) pLysS competent cells were purchased from Invitrogen (Carlsbad, CA). Mini Elute Gel Extraction Kit was prepared from QIAGEN (Tokyo, Japan). HiTrap SP XL cation exchange column (5 ml), HisTrap HP column (5 ml), and Sephacryl S-400 were from GE Healthcare Japan Inc. (Tokyo, Japan). PrimeSTAR HS DNA polymerase, T4 DNA ligase, and *E. coli* DH5α competent cells were obtained from TaKaRa Bio Inc. (Shiga, Japan). *E. coli* Rossetta (DE3) pLysS competent cells was obtained from Merck millipore (Darmstadt, Germany). Phosphate buffer (PB) was prepared from 20 mM Na_2HPO_4 and 20 mM NaH_2PO_4. DNA origami buffer (pH 8.0) contained 40 mM Tris-HCl, 20 mM acetic acid, and 12.5 mM $MgCl_2$.

2. Preparation of the ZF-SNAP derivatives

2-1. Construction of vector for the ZF-SNAP (pET-30a-ZF-SNAP)

SNAP-tag in pSNAP-tag (T7)-2 vector was amplified by PCR using the following primer pairs. The PCR products were run on a 1% agarose gel (TAE) and were purified by Mini Elute Gel Extraction Kit. The PCR products and pET-30a encoding zif268-mCer[S1] were digested with *Eco*RI and *Hind*III and were purified in the same manner, separately. These products were incubated with T4-DNA-ligase. The mixture was transformed into *E. coli* DH5α competent cells for amplification. And then the purified and sequence checked vector encoding ZF-SNAP (termed as *pET-30a-ZF-SNAP*) was transformed into *E. coli* BL21(DE3)pLysS competent cells.

primer	from 5' to 3'
F_EcoRI_SNAP	TAATAA GAATTCGGCG GCTCCGGCGG CTCCGACAAA GATTGCGAAA
R_SNAP_HindIII	TTATTAAAGC TTTTAATGAT GGTGATGATG ATGGTGATGA TGGTGGGTAC CATTAACCTC GAGCCCGGGG

2-2. Construction of vector for the ZF-SNAP-EGFP (pET-30a-ZF-SNAP-EGFP)

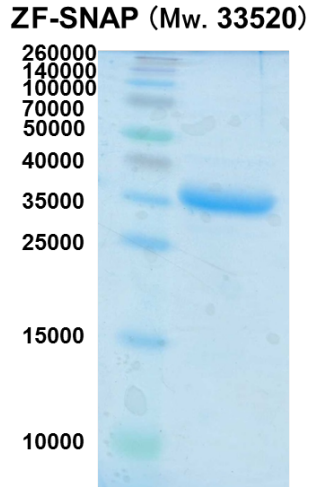
The plasmid encoding EGFP was amplified by PCR using the following primer pairs. The PCR products were run on a 1% agarose gel (TAE) and were purified by Mini Elute Gel Extraction Kit. The PCR products and *pET-30a-ZF-SNAP* were digested with *Kpn*I and *Hind*III and were purified in the same manner, separately. These products were incubated with T4-DNA-ligase. The mixture was transformed into *E. coli*

DH5α competent cells for amplification. And then the purified and sequence checked vector encoding ZF-SNAP (termed as *pET-30a-ZF-SNAP-EGFP*) was transformed into *E. coli* BL21(DE3)pLysS competent cells.

primer	from 5' to 3'
F_KpnI-EGFP	TAATTAGGTACCGGTG GTTCCGGAGG ATCTGGTGGA TC
R_EGFP_HindIII	TAAAATAAGC TTTCAGTGGT GGTGGTGGTG GTGCTC

2-3. Overexpression and purification of ZF-SNAP

The transformed cells were grown at 37°C until OD_{600} reached 0.5, and protein expression was induced with 1 mM IPTG for 24 h at 25 °C. The soluble fraction of the cell lysate containing ZF-SNAP was loaded to HisTrap HP column under following buffer conditions (pH 8.0, 50 mM phosphate buffer containing 200 mM NaCl, 1 mM DTT) and eluted by imidazole gradient. The main fractions containing ZF-SNAP were loaded to HiTrap SP HP column under following conditions (pH 7.0, 50 mM phosphate buffer containing 1 mM DTT) and eluted by NaCl gradient. The purified ZF-SNAP was dialyzed by using 50 mM phosphate buffer (pH 7.0) containing 1 mM DTT, 50 μM $ZnCl_2$, 50% glycerol and stocked at -20°C. The purity of ZF-SNAP was checked by SDS-PAGE. The major band in SDS-PAGE (see below) corresponds to the calculated molecular weight of ZF-SNAP (33,520) with purity over 95%. An amino acid sequence of ZF-SNAP is shown below.

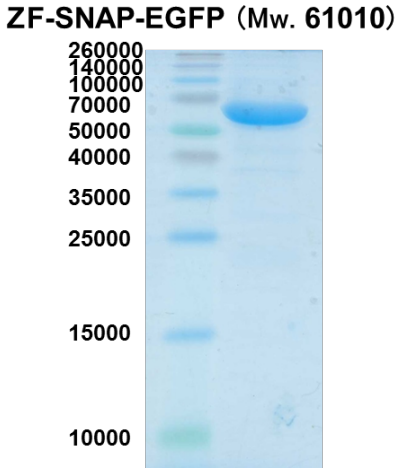



```
1     MMCKTGEKRP YACPVESCDR RFSRSDELTR HIRIHTGQKP FQCRICMRNF
51    SRSDHLTTHI RTHTGEKPFA CDICGRKFAR SDERKRHTKI HTGEKEFGGS
101   GGSDKDCEMK RTTLDSPLGK LELSGCEQGL HEIKLLGKGT SAADAVEVPA
151   PAAVLGGPEP LMQATAWLNA YFHQPEAIEE FPVPALHHPV FQQESFTRQV
201   LWKLLKVVKF GEVISYQQLA ALAGNPAATA AVKTALSGNP VPILIPCHRV
251   VSSSGAVGGY EGGLAVKEWL LAHEGHRLGK PGLGPAGGSP GLEVNGTHHH
301   HHHHHHH
```

An amino acid sequence of ZF-SNAP.

2-4. Overexpression and purification of ZF-SNAP-EGFP

The transformed cells were grown at 37°C until OD_{600} reached 0.5, and protein expression was induced with 1 mM IPTG for 24 h at 25 °C. The soluble fraction of the cell lysate containing ZF-SNAP-EGFP was loaded to HisTrap HP column under following buffer conditions (pH 7.0, 50 mM phosphate buffer containing 200 mM NaCl, 1 mM DTT) and eluted by imidazole gradient. The main fractions containing ZF-SNAP-EGFP were loaded to HiTrap SP HP column under following conditions (pH 7.0, 20 mM phosphate buffer containing 1 mM DTT) and eluted by NaCl gradient. The main fractions containing ZF-SNAP-EGFP was loaded to HiTrap Q HP column under following condition (pH 8.0 20 mM Tris-HCl buffer containing 1 mM DTT) and eluted by NaCl gradient. The purified ZF-SNAP-EGFP were dialyzed by using 50 mM phosphate buffer (pH 7.0) containing 1 mM DTT, 50 μM $ZnCl_2$, 50% glycerol and stocked at -20°C. The purity of ZF-SNAP was checked by SDS-PAGE. The major band in SDS-PAGE (see below) corresponds to the calculated molecular weight of ZF-SNAP-EGFP (61,010) with purity over 95%. An amino acid sequence of ZF-SNAP-EGFP is shown below.




```
1     MMCKTGEKRP YACPVESCDR RFSRSDELTR HIRIHTGQKP FQCRICMRNF
51     SRSDHLTTHI RTHTGEKPFA CDICGRKFAR SDERKRHTKI HTGEKEFGGS
101    GGSDKDCEMK RTTLDSPLGK LELSGCEQGL HEIKLLGKGT SAADAVEVPA
151    PAAVLGGPEP LMQATAWLNA YFHQPEAIEE FPVPALHHPV FQQESFTRQV
201    LWKLLKVVKF GEVISYQQLA ALAGNPAATA AVKTALSGNP VPILIPCHRV
251    VSSSGAVGGY EGGLAVKEWL LAHEGHRLGK PGLGPAGGSP GLEVNGTGGS
301    GGSGGSMVSK GEELFTGVVP ILVELDGDVN GHKFSVSGEG EGDATYGKLT
351    LKFICTTGKL PVPWPTLVTT LTYGVQCFSR YPDHMKQHDF FKSAMPEGYV
401    QERTIFFKDD GNYKTRAEVK FEGDTLVNRI ELKGIDFKED GNILGHKLEY
451    NYNSHNVYIM ADKQKNGIKV NFKIRHNIED GSVQLADHYQ QNTPIGDGPV
501    LLPDNHYLST QSALSKDPNE KRDHMVLLEF VTAAGITLGM DELYKAAALE
551    HHHHHH
```

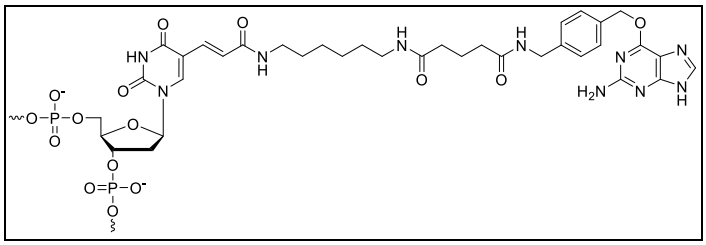
An amino acid sequence of ZF-SNAP-EGFP.

3. *Preparation of ^{32}P-end-labeled oligo DNA and the analysis of covalent-linkage formation between ^{32}P-end-labeled oligo DNA and ZF-SNAP by denaturing gel shift assay*

Oligo DNAs (ODN-zif-BG or ODN-non-BG) with following nucleotide sequences were used to study the covalent-linkage formation. Oligo DNAs were ^{32}P-end-labeled as previously described.[S1] In a typical experiment, ^{32}P-ODN-zif-BG (0.5 nM) was incubated with ZF-SNAP (10 nM) in a buffer containing 40 mM Tris-HCl, 20 mM acetic acid, 12.5 mM $MgCl_2$, 1 mM DTT, 1 μM $ZnCl_2$, 0.002 % Tween20, 0.1 mg/ml BSA, 100 nM calf thymus DNA,pH 8.0 at 25 °C and aliquots taken at defined incubation times were quenched by addition of an SDS buffer. The aliquots were analyzed by SDS-PAGE and the intensities of the bands on the gel were analyzed by using Storm 860 Molecular imager (Amersham). The kinetic data were fitted to a reaction model assuming first-order kinetics, and then the second-order rate constant were determined.

ODN-zif-BG 5'- CGCGTATAACGCCCACGCGGT@TTCCGCGTGGGCGTTATACGC -3'
ODN-non-BG 5'-CGCGTATAATGCCACGTAGGT@TTCCTACGTGGCATTATACGC-3'

@ = BG modified C6-T



An oligo DNA (ODN-zif) with the following sequence was used as a competitor.

ODN-zif : 5'-CTTTTTTGCGTGGGCGTTTTTTTAAACGCCCACGCAAAAAA-3'

4. *Preparation of the DNA origami with five cavities*

The sample solution (50 μL) containing M13mp18 single-stranded DNA (New England Biolabs, 10 nM), staple DNA strands (5 equiv, 50 nM, newly prepared staple DNA strands were shown in Table S3 and the rest of staple DNA strands were reported previously[S2]) in DNA origami buffer (40 mM Tris-HCl, 20 mM acetic acid, 12.5 mM $MgCl_2$, pH 8.0) was heated at 95 °C for 1 min, annealed at 53 °C for 30 min, and kept at 4 °C by using a thermal cycler. The sample was purified by using a size exclusion chromatography (400 μL of Sephacryl S-400, GE Healthcare) in Ultrafree-MC-DV (Millipore).

5. *Preparation of DNA origami treated by ZF-SNAP-EGFP*

Each of the DNA origami was incubated with ZF-SNAP-EGFP at conditions as shown in the caption of Figure and Table S2. A mixture of ZF-SNAP-EGFP conjugated DNA origami was subjected to a size-exclusion chromatography to remove the excess amount of unbound ZF-SNAP-EGFP, then the fractions containing DNA origami were utilized for AFM analysis.

6. AFM imaging

The sample was deposited on freshly cleaved mica (5 mmϕ or 1.5 mmϕ) surface and adsorbed for 5 min at room temperature and then washed three times with the DNA origami buffer. The sample was scanned in tapping mode using a MultiMode^{TM} microscope (Bruker) equipped with a Nanoscope V controller and a silicon nitride cantilever (Olympus BL-AC40TS-C2) or a fast-scanning AFM system (Nano Live Vision, RIBM Co. Ltd., Tsukuba, Japan) with a silicon nitride cantilever (Olympus BL-AC10DS-A2).

7. Statistical analysis of AFM images

At least two independent preparations of each sample were analyzed by AFM and several images were acquired from different regions of the mica surface. Total number of DNA origami corresponds to the number of DNA origami with the expected rectangular shape possessing five cavities observed by AFM. The specific and non-specific binding of ZF-SNAP-EGFP were counted for only ZF-SNAP-EGFP bound to the perfectly folded DNA origami. The results were analyzed as follows and summarized in Table S2.

I-4zifBG, I-4zif, I-2zifBG or I-4nonBG:

The yield of ZF-SNAP-EGFP-DNA origami conjugation ($P_{zf\text{-}origami}$) was calculated as the percentage of attached structures, i.e. the modified DNA origami bearing proteins at the expected position (position I of the 5-wells frame DNA origami) ($N_{expected\ posi}$), over the amount of well-formed DNA origami structures (N_{total}):

$$P_{zf\text{-}origami} = (N_{expected\ posi} / N_{total}) \times 100$$

On the other hand, the yield of ZF-SNAP-EGFP which existed at the unexpected position ($P_{\mathrm{nonspecific}}$) was also calculated as the percentage of attached structures, i.e. the number of wells (position II-V of the 5-wells frame DNA origami) on which existed ZF-SNAP-EGFP nonspecifically ($N_{unexpected\ posi}$), over the total number of wells (position II-V of the 5-wells frame DNA origami) of well-formed DNA origami structure ($4N_{total}$):

$$P_{nonspecific} = (N_{unexpected\ posi} / 4N_{total}) \times 100$$

4-1zifBG or 5-1mixBG:

The yield of ZF-SNAP-EGFP-DNA origami conjugation ($P_{zf\text{-}origami}$) was calculated as the percentage of attached structures, i.e. the modified DNA origami bearing the proteins at the expected position (position II-V of the 5-wells frame DNA origami) ($N_{expected\ posi}$), over the amount of well-formed DNA origami structures ($4N_{total}$):

$$P_{zf\text{-}origami} = (N_{expected\ posi} / 4N_{total}) \times 100$$

On the other hand, the yield of ZF-SNAP-EGFP which existed at the unexpected position ($P_{\mathrm{nonspecific}}$) was also calculated as the percentage of attached structures, i.e. the number of wells (position I of the 5-wells frame DNA origami) on which existed ZF-SNAP-EGFP nonspecifically ($N_{unexpected\ posi}$), over the amount of well-formed DNA origami structures (N_{total}):

$$P_{nonspecific} = (N_{unexpected\ posi} / N_{total}) \times 100$$

Note : In the case of 5-1mixBG, the expected position and the unexpected position indicated zifBG (position II-V) and nonBG (position I), respectively.

8. Volume analysis of AFM images

In the case of volume analysis, the AFM images taken by a fast-scanning AFM system (Nano Live Vision, RIBM Co. Ltd., Tsukuba, Japan) with a silicon nitride cantilever (Olympus BL-AC10DS-A2) were used. The volume of ZF-SNAP-EGFP immobilized on DNA origami were analyzed by using SPIP^{TM} software (ver. 6.2.8, Image Metrology) and Z material volume value defined as the volume of all pixels inside the shape's contour with a Z value was used. The volume data were displayed as a histogram plot and each fraction were analyzed by means of non-linear curve fit on a software (Origin, ver. 9.1).

9. Agarose gel electrophoresis analysis of the protein-modified DNA origami

The samples of DNA origami modified with adaptor fused fluorescent proteins were directly applied for agarose gel electrophoretic analysis without further purification. The samples were run on a 1% agarose gel containing 45 mM Tris, 45 mM boronic acid. After recording fluorescence images by using Molecular Imager FX pro (BioRad) under EGFP-mode, the gel was stained with ethidium bromide (EtBr) and images were taken by using Molecular Imager FX pro (BioRad) under EtBr-mode. The nucleotide sequences of oligo DNA with the zif268 binding site (ODN-zif) was as follows:

ODN-zif: 5'-CTTTTTTGCGTGGGCGTTTTTTTAAACGCCCACGCAAAAAA-3'

Reference

S1. Morii, T; Saimei, Y; Okagami, M.; Makino, K. and Sugiura, Y. *J. Am. Chem. Soc.,* **1997**, *119*, 3649-3655.
S2. E. Nakata, F. F. Lew, C. Uwatoko, S. Kiyonaka, Y. Mori, Y. Katsuda, M. Endo, H. Sugiyama, T. Morii, *Angew. Chem. Int. Ed*. **2012,** *51*, 2421-2424.