Supporting Information

OaAEP1-mediated PNA-protein conjugation enables erasable imaging of membrane protein

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Materials

All DNA oligonucleotides were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). *E. coil* BL21 (DE3) and XL1-Blue cells were purchased from TransGen Biotech Co., Ltd (Beijing, China). All other reagents were purchased from Sangon Biotech Co., Ltd. (Shanghai, China) or Sigma-Aldrich (USA). HEK293 cell was purchased from KeyGEN Biotech Co., Ltd. (Nanjing, Jiangsu).

DNA oligonucleotides were dissolved in 1×PBS (1×PB, 300 mM NaCl, pH 7.4); 1×TAE Mg^{2+} (40 mM Tris, 40 mM Acetic acid, 1 mM EDTA, 12.5 mM Mg^{2+}, pH 8.5) or 1×TE-Mg^{2+} buffer (5 mM Tris, 10 mM EDTA, 16 mM Mg^{2+}) and stored at -20 °C for use.

Instrument

Atomic force microscopy (AFM) micrographs were obtained on a Multimode 8 (Bruker, Germany). Gel electrophoresis was conducted using a DYCZ-24DN electrophoresis cell (LIUYI, Beijing, China) and gel imaging system (Clinx Science Instrument, GenoSens 2000, Shanghai, China). Flow cytometry was used to evaluated the expression of EGFP (BD FACSVerse, USA). Confocal fluorescence images of cells were acquired with an Olympus FV3000 confocal laser scanning fluorescence microscope (Olympus, Japan). Mass spectrometry was conducted using a Waters Xevo G2-QTOF (Waters, USA)

Protein engineering

ELP is abbreviated for elastin-like polypeptides. X module and Doc are from type III cohesin-dockerin-X module domain complex of Ruminococcus flavefaciens. GB1 is the B1 domain of streptococcal protein G. *Oa*AEP1 (C247A), abbreviated as *Oa*AEP1, is the cysteine 247 to alanine mutation of asparaginyl endoproteases 1 from *oldenlandia affinis*. All other plasmids were purchased from Genscript Inc. Genes encoding for target proteins, such as GL-ELP20, GL-GXD, and GL-protein G were constructed in expression vector pET-28a by standard molecular biology techniques.

All the plasmids were transformed in *E. coli* BL21 (DE3) for overexpression. The bacteria cultured in LB medium were induced by the addition of 1 mM IPTG (final concentration) overnight at 16 °C. Then, cells were collected by centrifugation at 8000 rpm for 10 min, and resuspended and lysed by high-pressure homogenization. After centrifuged at 18000 rpm for 15 min, the supernatants were loaded onto the Co^{2+} affinity chromatography column. The recombinant proteins were washed with washing buffer (20 mM Tris, 400 mM NaCl, 2 mM imidazole, pH 7.4) and subsequently eluted in elution buffer (20 mM Tris, 400 mM NaCl, 250 mM imidazole, pH 7.4). Finally, the purified proteins were transferred in buffer without imidazole (50 mM Tris, 100 mM NaCl, pH 7.4) through ultrafiltration and can be stored at -80°C after the addition of 20% glycerol (final concentration). The expression and purification of *Oa*AEP1 can be found in literature[1,2].

POI and DNA ligation by *Oa*AEP1

HS-DNA (100 μM, 50 μL) was first reacted with a 20-fold excess of Sulfo-SMCC in 1× PBS (pH 7) for two hours. Excess SMCC was removed by Amicon, 3 kDa cutoff filters. Next, SMCC modified DNA was conjugated to peptide GGNGL. DNA-GGNGL (50 μM, 10 μL) and GL-protein G (20 μM, 10 μL) were mixed with the addition of 1 μM *Oa*AEP1 at 25 °C for 2 hours. The reaction was stopped in SDS loading buffer and characterized by SDS-PAGE gel. Only a small amount of ligated products were observed (Figure S1).

Hybridization efficiency of PNA/DNA and DNA/DNA

DNA capture strand (1 μM) was mixed with PNA-2aa, or a DNA strand with the same sequence, in 1:1 ratio. Then heated to 95 °C with 2 min and cooled to 10 °C for 20 min. Native 12% PAGE analysis of the hybridization product (Figure S2).

POI and PNA ligation by *Oa*AEP1

PNA-2aa/PNA-6aa (100 μM, 10 μL) and GL-POI (20 μM, 10 μL) were mixed

with the addition of 1 μM *Oa*AEP1. Different reaction conditions including spacer length, reaction time, pH and temperature were employed. The reaction was stopped in SDS loading buffer and the product was characterized by SDS-PAGE, high performance liquid chromatography (HPLC) and MALDI mass-spectrometry (Figure S4, S5 and S6). The catalytic efficiency for each reaction was calculated based on the band intensity of the reactant/product using the software ImageJ.

Analysis of POI-PNA conjugates by HPLC

The reaction product (100 μL) was mixed with 200 μL of Solvent A (0.1% trifluoroacetic in 100% acetonitrile) and isolated by HPLC. The HPLC analyses were carried out using an Agilent 1100 HPLC system with a DAD UV-vis detector. Chromatographic separation was carried out using a Sepax Bio-C18 column (4.6×250 mm, 5 μm, 300 Å). Analytical conditions: the mobile phase was a linear gradient of 0.1% trifluoroacetic in 100% water (solvent B) in 0.1% trifluoroacetic in 100% acetonitrile (solvent A), both adjusted to pH 7 with trifluoroacetic, at a flow rate of 1 mL/min. The following gradient was used: 0 min, 15% A; 0-20 min, 15-40% A; 20.1 min 100% A.

Synthesis of DNA origami

A triangular DNA origami (Figure S4) was annealed, referring to Yan's protocol[4]. Scaffold M13mp18 DNA (5 nM) was mixed with staple strands and PNA-2aa/PNA-6aa in a 1:5 ratio. The DNA origami was assembled in 1×TAE-Mg^{2+} buffer (40 mM Tris, 40 mM Acetic acid, 1 mM EDTA, 12.5 mM Mg^{2+}) by cooling down from 85°C to 4°C. 100 kDa MWCO Amicon filters were used to remove the excess staple and PNA stands.

The design of DNA origami bundle was show in Figure S5. Scaffold p7560 DNA (10 nM) was mixed with staple strands, capture strands of PNA-6aa and Cy5 in a 1:5:5 ratio. The DNA origami was assembled in 1×TE-Mg^{2+} buffer (5 mM Tris, 10 mM EDTA, 16 mM Mg^{2+}), and annealed with 36 h program by cooling down from

85°C to 15°C with a -0.05°C/min sloop. 100 kDa MWCO Amicon filters were used to remove the excess staple and capture stands.

Characterization of DNA origami

The self-assembled products were subjected to electrophoresis on 1% agarose gel (1×TAE-Mg^{2+}buffer, 5 μL GelRed) at 50 V for two and a half hours in an ice bath, and visualized by UV light. The DNA origami nanostructures were then characterized by atomic force microscope (AFM) (Figure S6).

PNA-protein conjugation on DNA origami

PNA modified DNA origami triangles (20 nM) and GL-POI (200 nM) were mixed with the addition of 0.5 μM *Oa*AEP1. Different reaction conditions, including spacer length, pH and temperature were employed. The reaction was stopped in 1×TAE-Mg^{2+} buffer and characterized by atomic force microscope (AFM) (Figure S7, S8 and S10).

To acquire AFM image, DNA origami or DNA origami-POI complex (1 nM, 3 μL) diluted with TAE buffer containing 3 mM $NiCl_2$ was added onto the surface of the freshly uncovered mica and adsorbed for 3 min. Then, 30 μL TAE buffer was added onto the mica surface. Under the liquid phase model of the AFM, triangle origami or origami-POI complex was imaged.

Characterization of the PNA/DNA hybridization efficiency with different pH

DNA capture strand (1 μM) was mixed with PNA-6aa under different pH in a 1:1 ratio. The hybridization products were then subjected to electrophoresis on 12% PAGE (Figure S9). The hybridization efficiency for each reaction was calculated based on the band intensity of the reactant/product using the software BandScan.

Plasmids design and expression of GL-EGFR-EGFP on HEK293 cell surface

A plasmid for GL-EGFR-EGFP was cloned into the EGFP-N1 vector by Gibson

assembly using a homemade mixture. To locate the fusion protein onto cell membrane, a signal peptide sequence was added in front. The 24-mer signal peptide is inserted before EGFR, and will be cleaved off during biosynthesis. The DNA sequence of GL and a linker peptide KGG is inserted between the C-terminal end of the signal peptide and the N-terminal end of EGFR (Figure S11). After expression and cleavage of the signal peptide, the mature protein with N terminal GL-KGG tag should be revealed.

The EGFP-N1 vector was restricted by Hind III and BamH I. The DNA fragments of signal peptide and EGFP with GL peptide were amplified by PCR and purified by agarose gel. Each of the three pieces contained a 24 bp homology to its neighboring piece, and was assembled together through Gibson assembly. Plasmid construction was verified by gene sequencing (Figure S12).

Human embryonic kidney cells (HEK 293) were grown as monolayers at 37 °C, 5% CO_2, and 95% humidity if not stated otherwise. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) from Thermo Fischer Scientific with additional 2 mM L-glutamine, and penicillin / streptomycin (10,000 units/ mL), G418 (50 μg/mL) and 10 % FBS. Plating cells were then washed with PBS and detached with 1 mL 0.25% trypsin / 0.02% EDTA solution for 1 min at 37°C. Cells were centrifuged and cultured in fresh medium.

To transiently transfected cell line carrying G418 inducible GL-EGFR-EGFP, HEK293 cells were transfected with the vector at a 3:1 ratio using Lipofectamine 3000 (Thermo Fisher Scientific) according to the manufacturer's instructions. 6-8 hours after, 5 ml DMEM with 10% serum was added, and the cells were incubated for 48 hours.

Characterization of EGFR expression in HEK293 cell

To characterize the expression of EGFP by flow cytometry, the transiently transfected cells were seeded on the six-cell culture plates and cultured for 48 h. Cells were then digested with trypsin and washed three times with PBS. Data was acquired on FACSverse (BD Biosciences) and quantified by FlowJo-V10 software (Figure S13A and S13B). To characterize the expression of EGFP by Western Blotting, the transiently transfected cells were lysed by a total protein extraction kit, and separated by SDS-PAGE. The protein bands were transferred to fluoride membrane (PVDF) by Western Semi-dry Transfer, and blocked with 5% (w/v) BSA in TBST buffer for 2 hours at room temperature. The corresponding primary antibodies were added and incubated overnight at 4°C. After washing for three times with TBST buffer, the membrane was incubated with DyLight 800 modified secondary antibody for 1 hour at room temperature. The band was obtained by ODYSSEY CLX System (Figure S13C).

Stability analysis of DNA origami bundle at 10%FBS

DNA origami bundle (10 nM, 5 μL) was incubated with 10% FBS at 37°C for 0 h, 1 h, and 2 h, respectively. Its structural integrity was analysed by electrophoresis (Figure S14).

Fluorescence microscopic analysis

The transiently transfected cells (about 1.6×10^6) were treated with or without *Oa*AEP1 (1 μM, 50 μL) and PNA (50 nM, 200 μL) for ligation. After washing with PBS, we hybridized the PNA strand with its complementary, fluorescently labelled DNA bundle origami (10 nM, 10 μL for 15 min). After washing with PBS to get rid of excess strands, the cell was stained with Hoechst 33342, and imaged under a fluorescence microscope in two different channels (EGFP: λ_{ex} = 490 ± 20 nm, λ_{em} > 520 nm; Cy5: λ_{ex} = 600 ± 50 nm, λ_{em} = 650 ± 30 nm).

To explore factors affecting the imaging of cell membrane proteins, transfected cells were then incubated with two different concentrations of DNA origami bundle (10 nM and 20 nM) for 15 min, or with 10 nM DNA origami bundle for three different periods (15 min, 30 min and 1h). The fluorescence intensity in CYSM images did not show obvious difference, suggesting the high hybridization efficiency of PNA and DNA (Figure S15 and S16).

Subsequently, toehold-mediated strand displacement of the Cy5-bundle was carried out with 200 nM, 20 μL fuel strand for 10 min in DMEM. Transfected cells were then imaged under fluorescent microscopy in three different channels (Hoechst 33342: λ_{ex} = 350 ± 50 nm, λ_{em} = 460 ± 50 nm; EGFP: λ_{ex} = 490 ± 20 nm, λ_{em} > 520 nm; Cy5: λ_{ex} = 600 ± 50 nm, λ_{em} = 650 ± 30 nm).

Result and Discussion

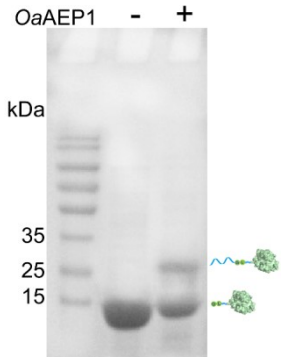

Figure S1. SDS-PAGE analysis of protein G (MW 13.2 kDa) before and after ligation with DNA by *Oa*AEP1. The expected molecular weight of the ligation product is 17.5 kDa.

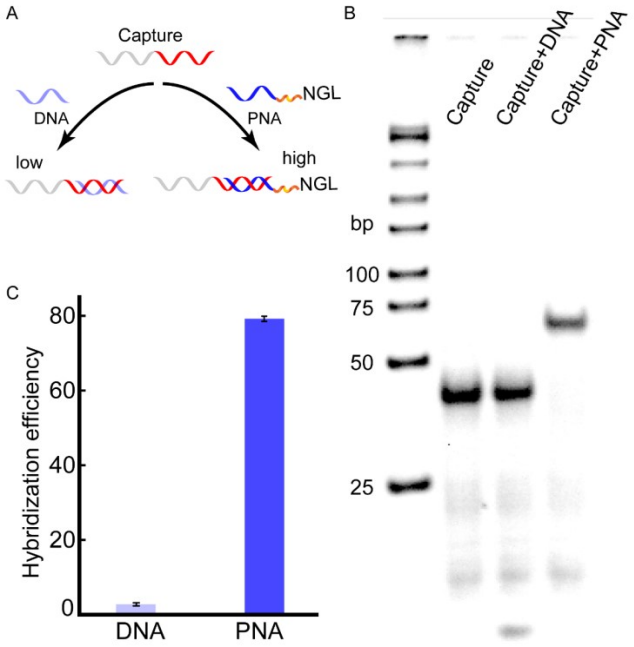

Figure S2. Comparison of hybridization efficiency between PNA/DNA and DNA/DNA. (A) Schematic illustration of the hybridization process. DNA capture strand (1 μM) was mixed with PNA-2aa, or a DNA strand with the same sequence, in a 1:1 ratio. (B) Native 12% PAGE analysis of the hybridization product. (C) Quantitative analysis of the hybridization efficiency based on the gel band intensity using the software BandScan. PNA showed a much higher hybridization efficiency to the capture strand than its DNA counterpart.

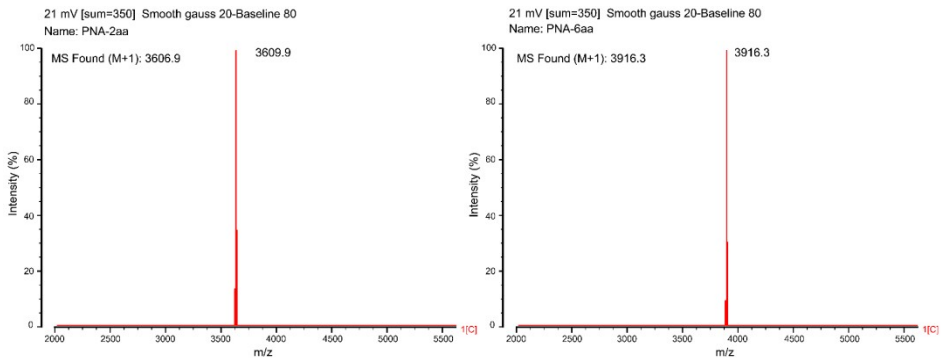

Figure S3. MALDI-MS analysis of PNA-2aa and PNA-6aa.

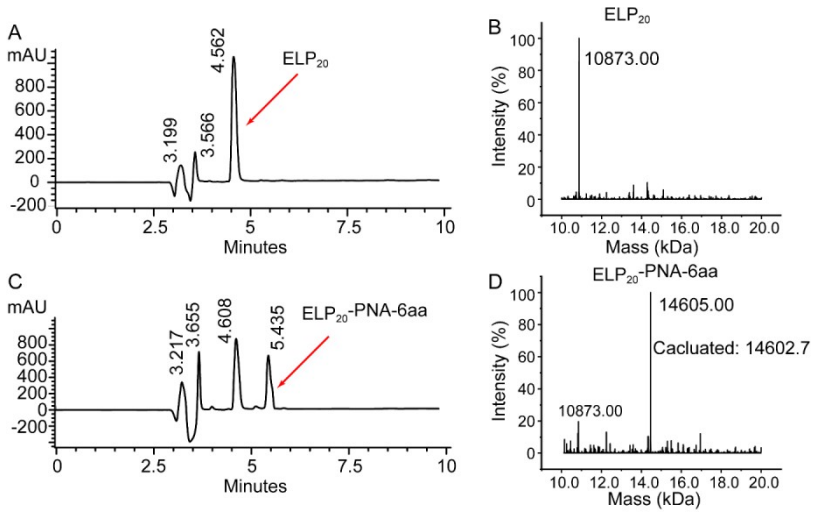

Figure S4. HPLC chromatography (A) and MALDI-MS (B) analysis of ELP_{20}. HPLC chromatography (C) and MALDI-MS (D) analysis of ELP_{20}-PNA-6aa.

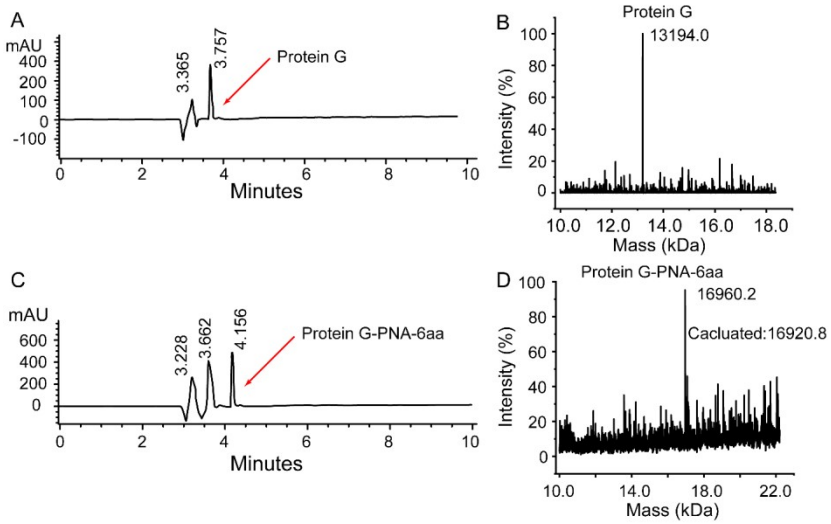

Figure S5. HPLC chromatography (A) and MALDI-MS (B) analysis of protein G. HPLC chromatography (C) and MALDI-MS (D) analysis of protein G-PNA-6aa.

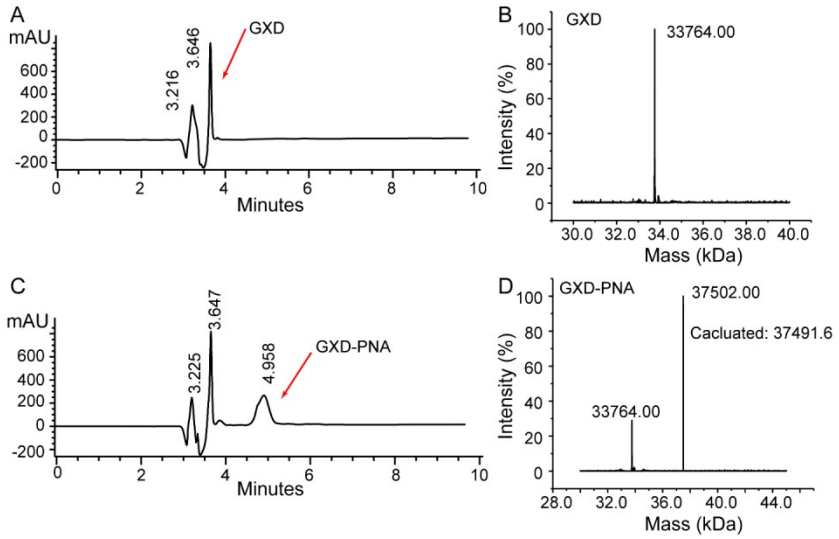

Figure S6. HPLC chromatography (A) and MALDI-MS (B) analysis of GXD. HPLC chromatography (C) and MALDI-MS (D) analysis of GXD-PNA-6aa.

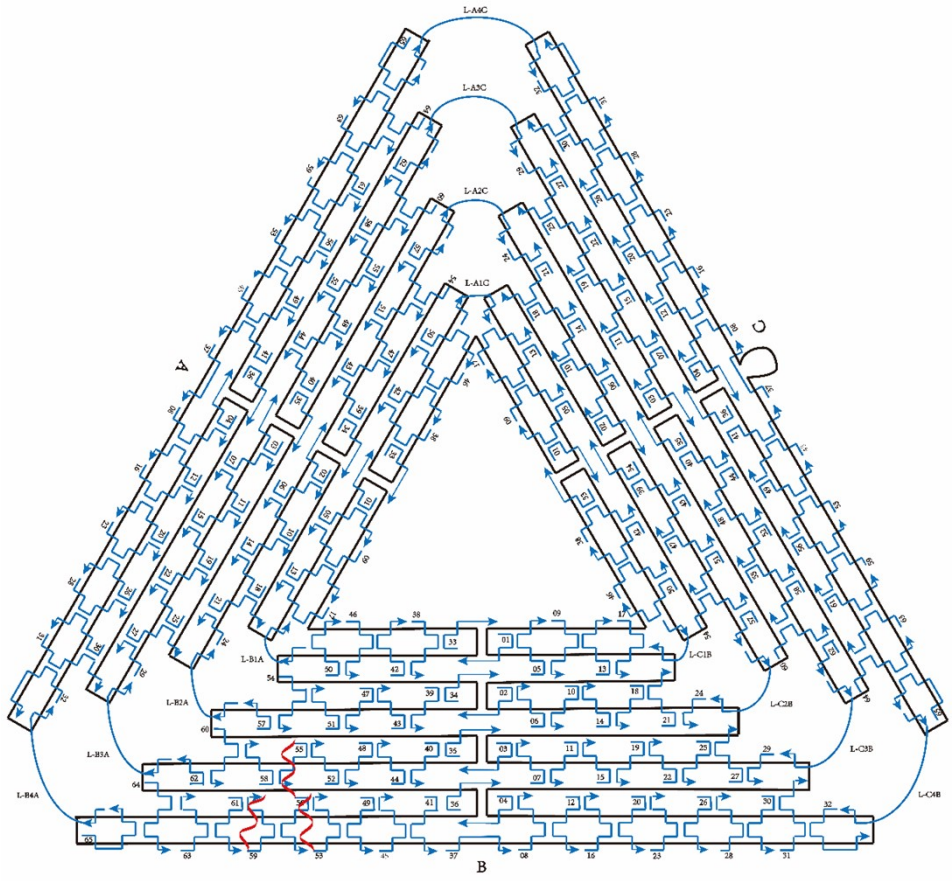

Figure S7. Design of the triangular DNA origami. Highlighted in red are capture strands that hybridize with PNA.

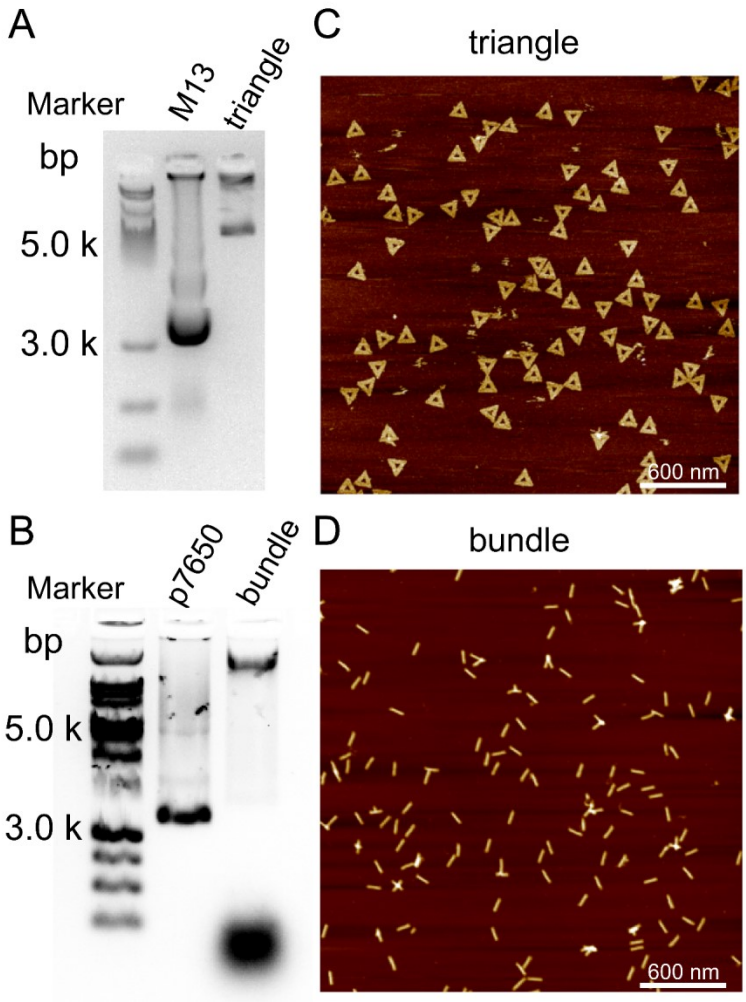

Figure S8. Characterization of DNA origami nanostructures. 1% agarose gel analysis of DNA origami triangle (A) and bundle (B). AFM image of DNA origami triangle(C) and bundle (D). All scale bars: 600 nm.

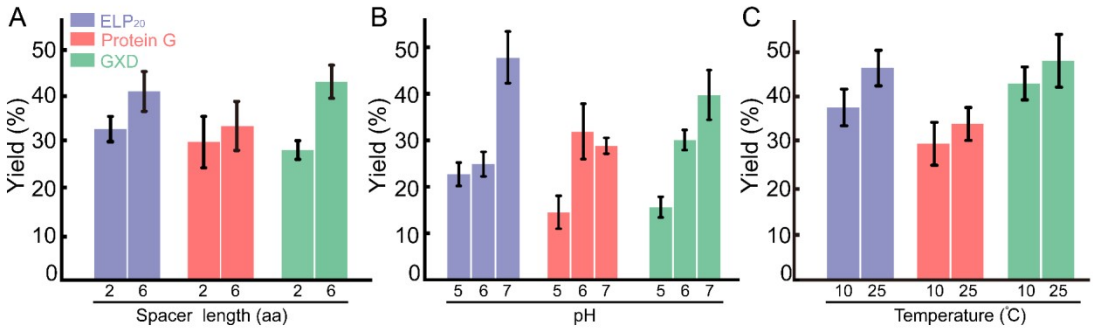

Figure S9. Factors including spacer length (A), pH (B), and temperature (C) affecting OaAEP1-catalyzed ligation yield on DNA origami. Statistical analysis is derived from calculations of 100 origami nanostructures.

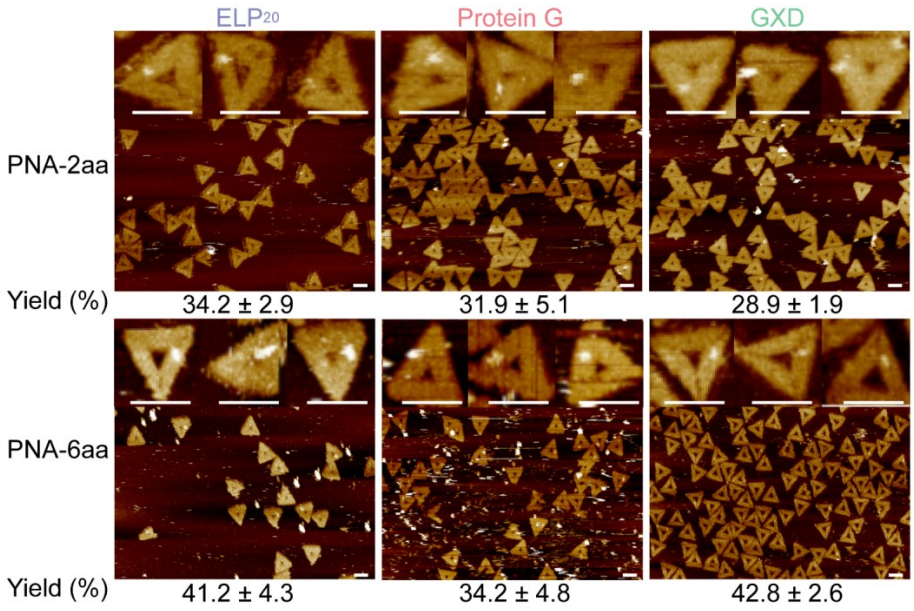

Figure S10. AFM images of site-specific organization of ELP_{20}, protein G, and GXD through *Oa*AEP1 catalyzed ligation under different spacer lengths. All scale bars: 100 nm.

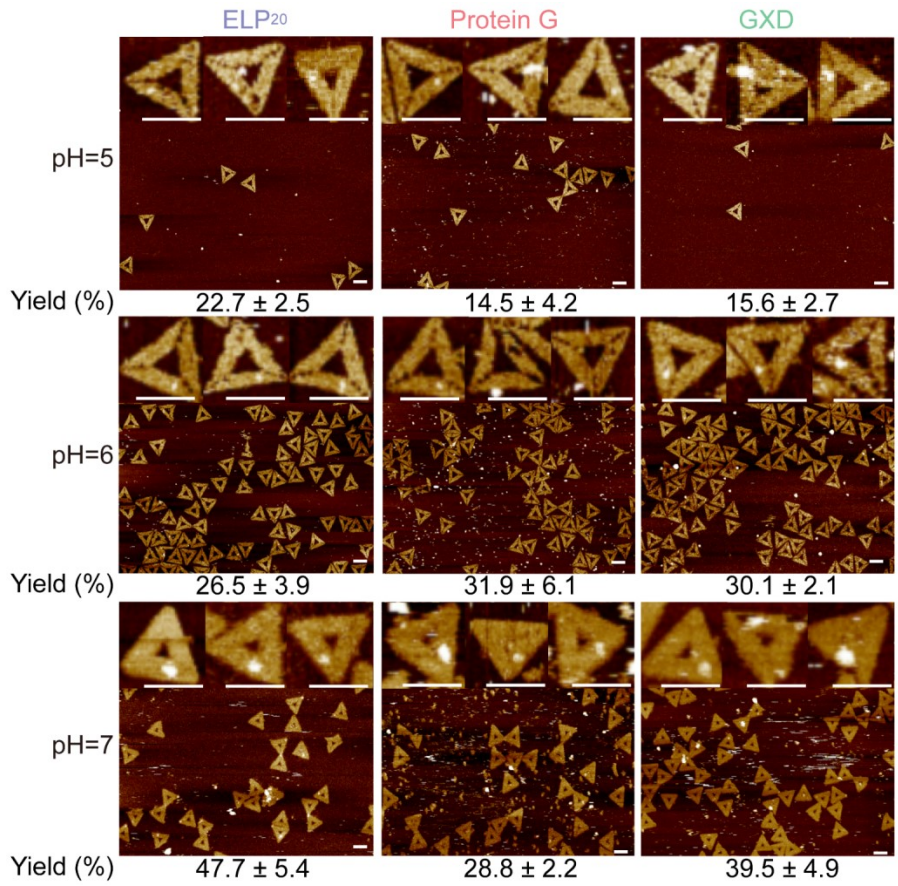

Figure S11. AFM images of site-specific organization of ELP_{20}, protein G, and GXD through *Oa*AEP1 catalyzed ligation under different pH. All scale bars: 100 nm.

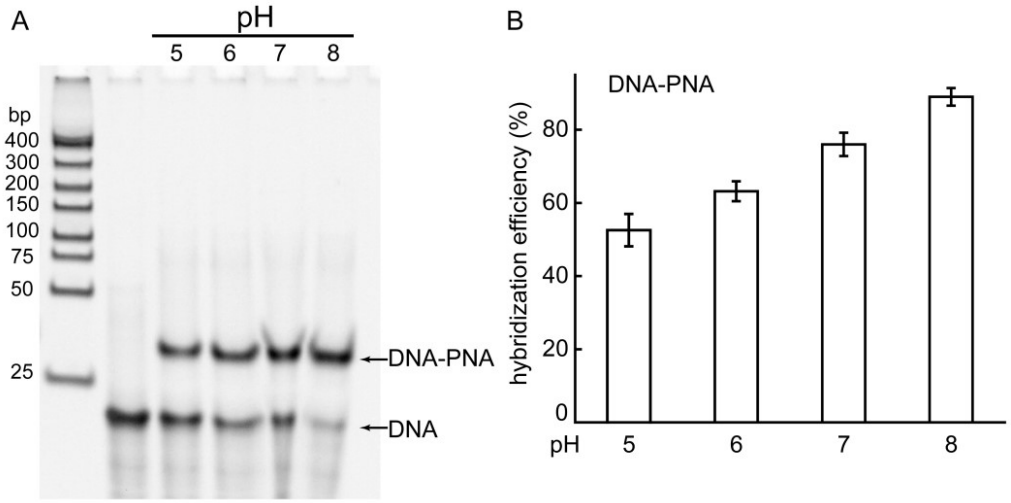

Figure S12. The PNA/DNA hybridization efficiency with different pH. (A) Native 12% PAGE analysis of the hybridization reactions. (B) Gel quantitative analysis of the hybridization efficiency based on the band intensity.

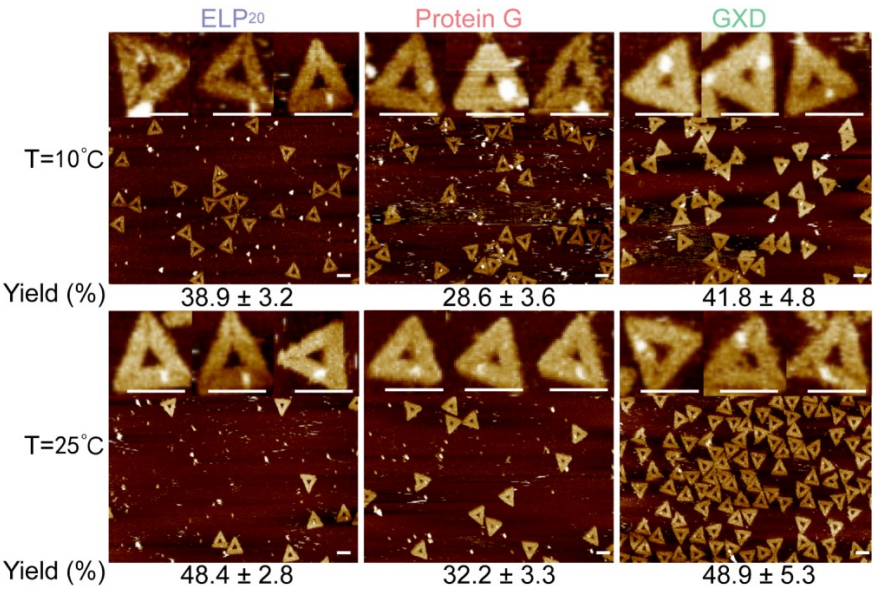

Figure S13. AFM images of site-specific organization of ELP_{20}, protein G, and GXD through *Oa*AEP1 catalyzed ligation under different temperatures. All scale bars: 100 nm.

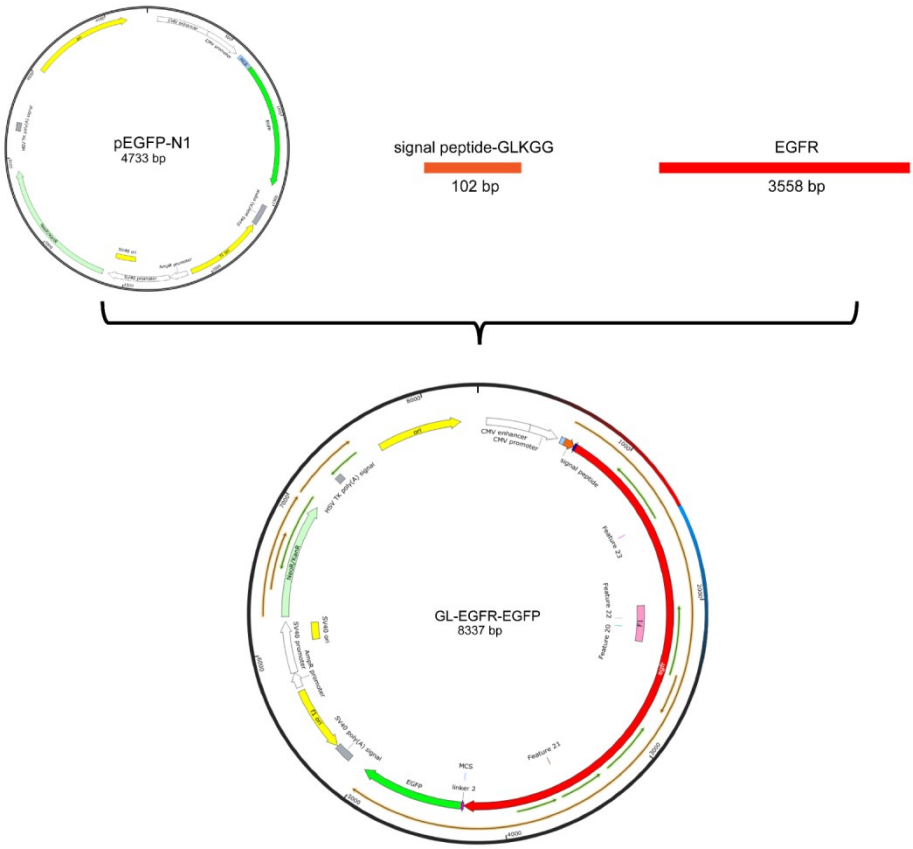

Figure S14. Vector map of GL-EGFR-EGFP assembled by Gibson assembly. A 24-mer signal peptide is inserted before EGFR, and will be cleaved off during biosynthesis. The DNA sequence of GL and a linker KGG is inserted between the C-

terminal end of the signal peptide and the N-terminal end of EGFR. Thus, after biosynthesis and cleavage of the signal peptide, the mature protein with N-terminal GL-tag is revealed. The image was made by Snap Gene Viewer 3.0.

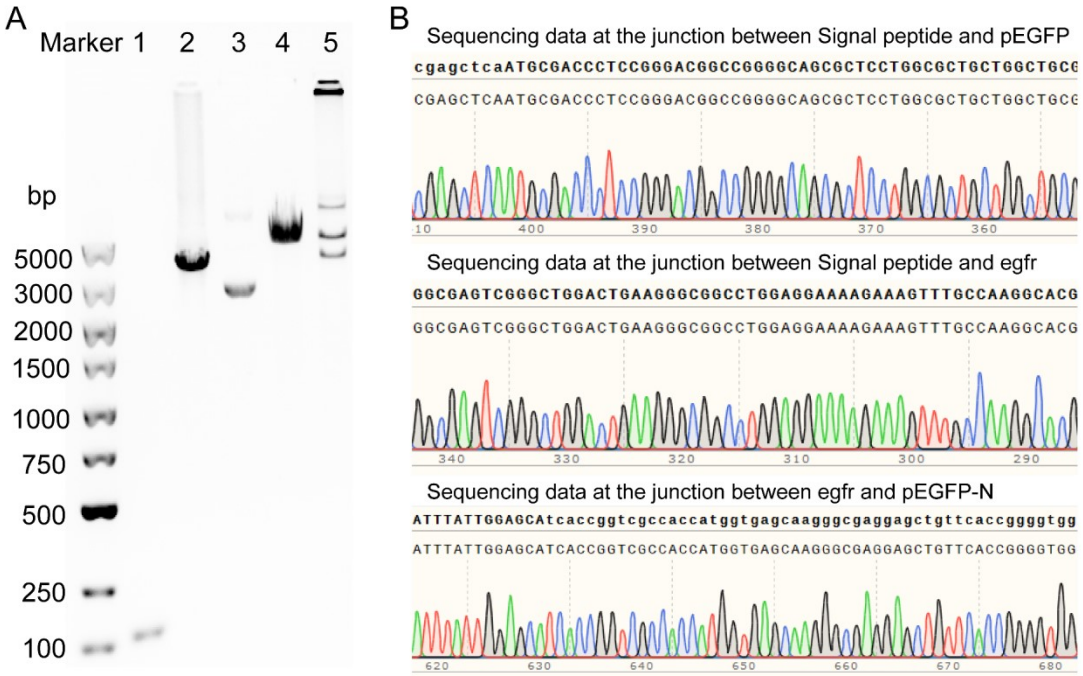

Figure S15. Characterization of GL-EGFR-EGFP plasmid. (A) Agarose gel analysis of DNA fragments for Gibson assemby. Lane 1: fragment for signal peptide with GL-KGG (102 bp); lane 2: fragment for EGFR (3558 bp); lane 3: circular plasmid pEGFP-N1 (4733 bp); lane 4: linearized plasmid pEGFP-N1 (4694 bp) after endonuclease digestion; lane 5: product after Gibson assembly (8377 bp). (B) DNA sequencing maps at the junctions of each fragment.

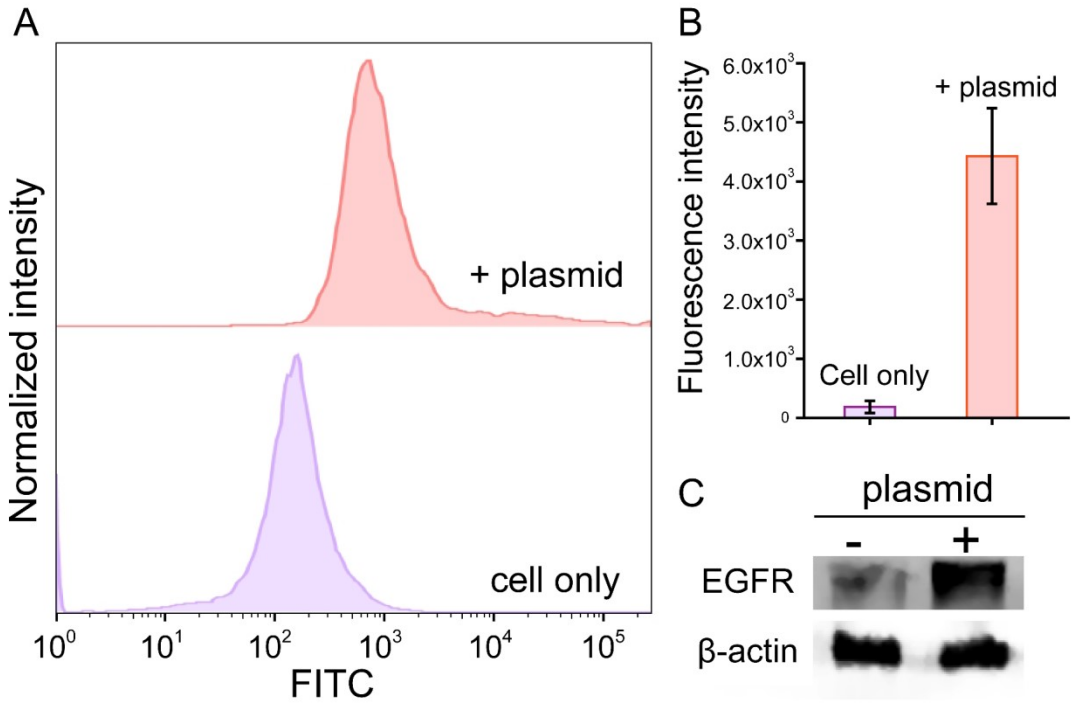

Figure S16. Characterization of transfection of GL-EGFR-EGFP plasmid into HEK293 cells. (A) Flow cytometry analysis of the transiently transfected cells. (B) Quantitative analysis of the fluorescence signals in (A). (C) Western blotting data of EGFR expression levels before and after plasmid transfection.

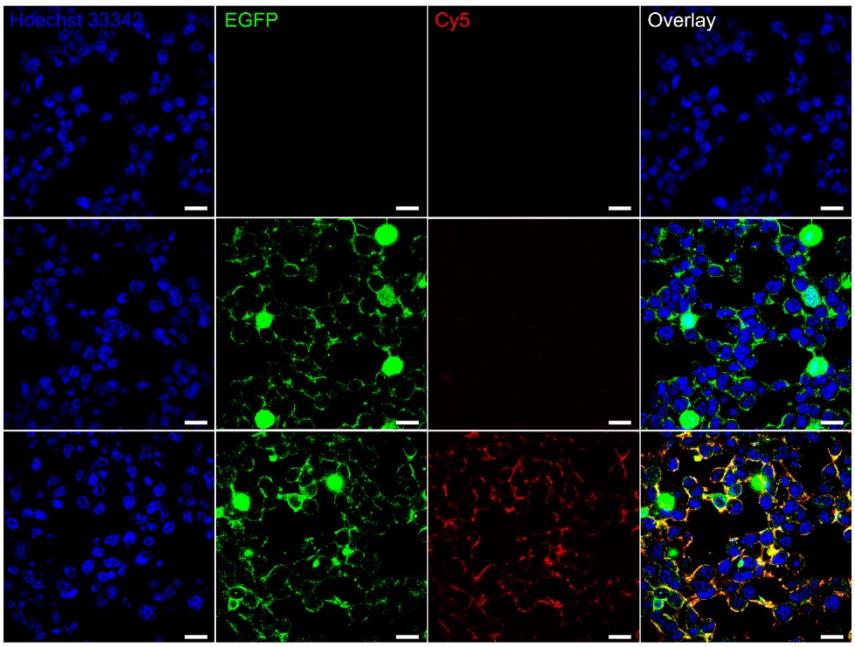

Figure S17. Fluorescent imaging of cell membrane proteins. CYSM images of EGFR on the HEK293 cell surface without the transfected GL-EGFR-EGFP plasmid (top).

At 48 h after transfection, HEK293 cells were incubated without (middle) or with (bottom) *Oa*AEP1. Fused EGFP was used as an internal control. Scale bars represent 20 μm.

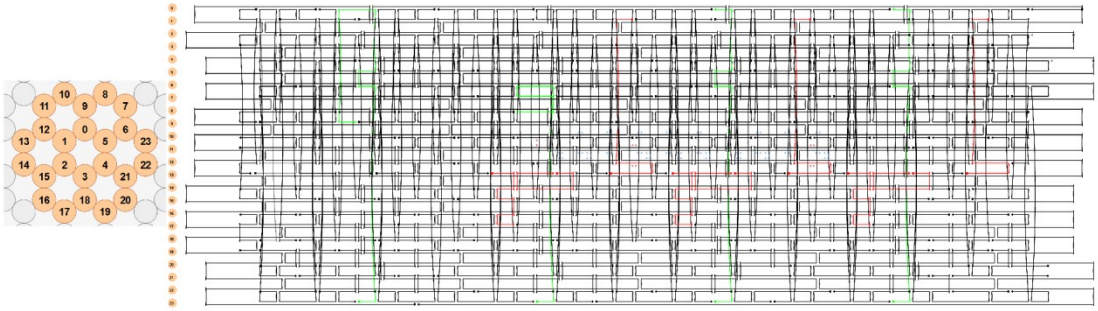

Figure S18. Design of the bundle DNA origami. Highlighted in red are capture strands that hybridize with PNA. Highlighted in green are capture strands that hybridized with Cy5-DNA.

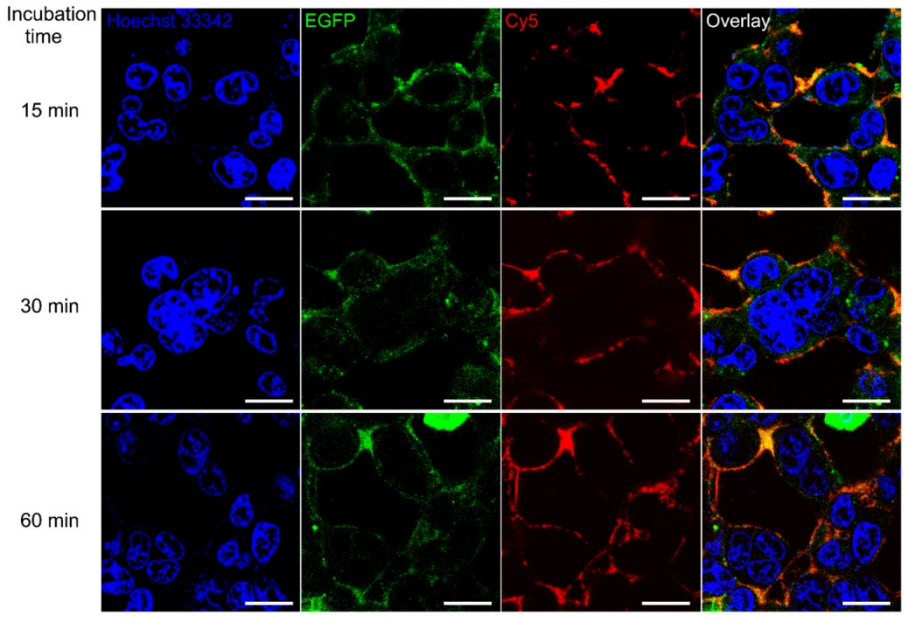

Figure S19. Fluorescent imaging of EGFR on live HEK293 cells, incubating with 10 nM Cy5-modified DNA origami bundle for different periods. All the experiments were repeated three times under similar conditions. Scale bars represent 25 μm.

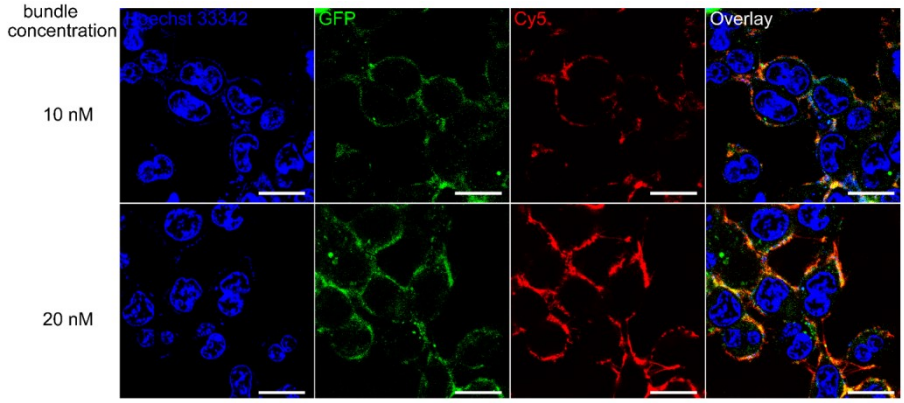

Figure S20. Fluorescent imaging of EGFR on live HEK293 cells, incubating with different concentrations of Cy5-modified DNA origami bundle for 15 min. All the experiments were repeated three times under similar conditions. Scale bars represent 25 μm.

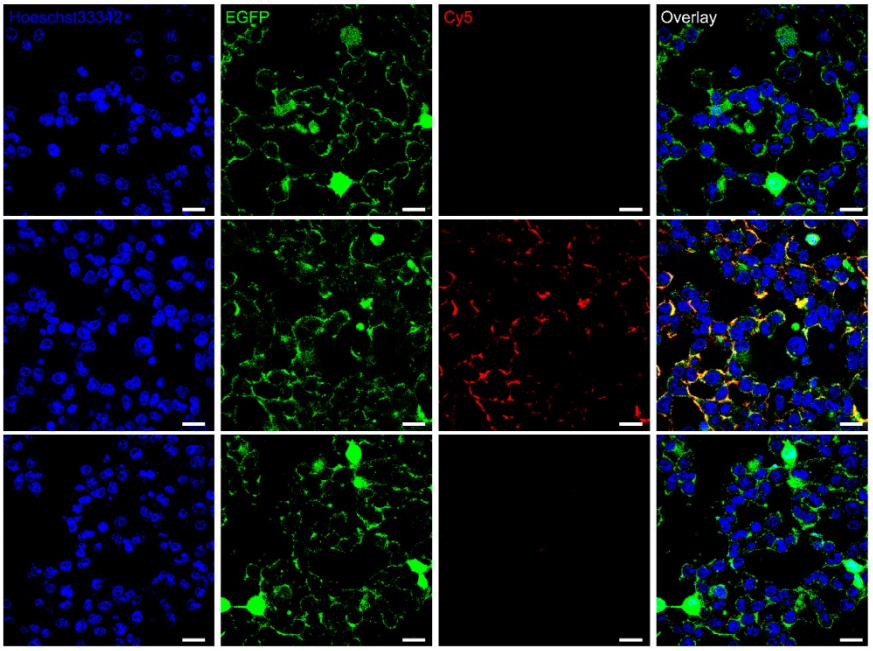

Figure S21. Erasable fluorescent imaging of cell membrane protein EGFR on HEK293 cells. CYSM images of EGFR on HEK293 cell surface in different conditions: after ligation of PNA (top); after incubation of Cy5-bundle (middle); and after strand displacement (bottom). Scale bars represent 20 μm.

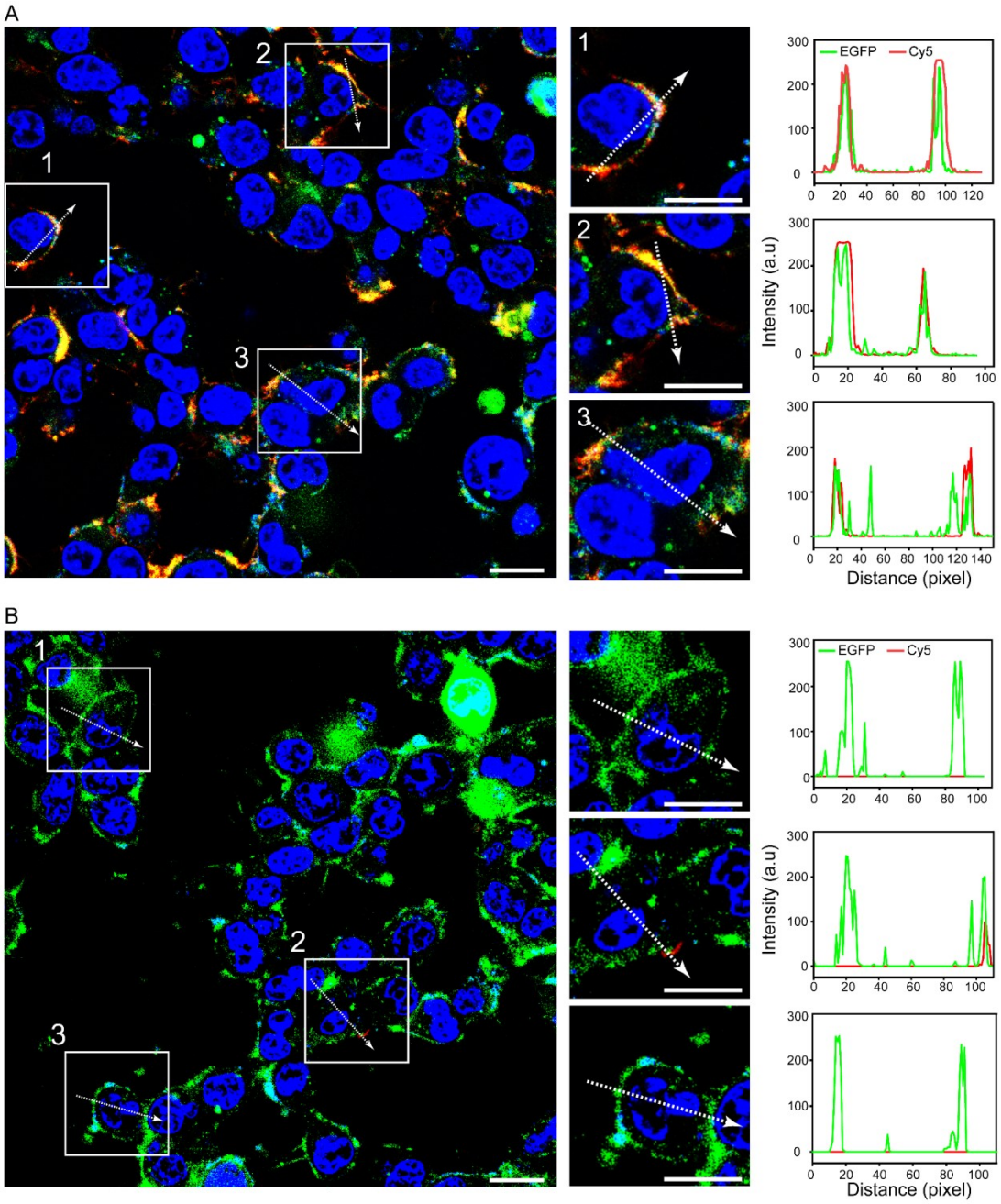

Figure S22. (A) Wide-view fluorescence images of HEK293 cells expressing GL-EGFR-EGFP labeled with Cy5, and representative fluorescence intensity profiles (along the dashed lines in the cell images). (B) Fluorescence images after the removal of Cy5-labeled probe by strand displacement, and representative fluorescence intensity profiles (along the dashed lines in the cell images). Scale bar: 25 μm.

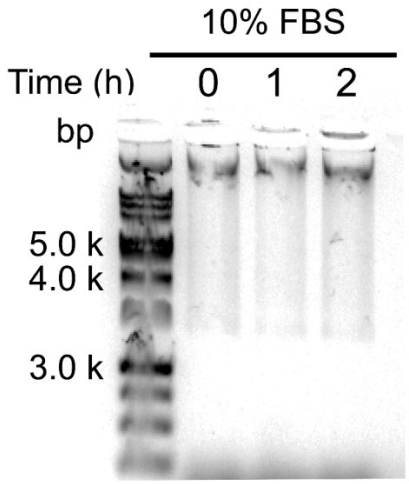

Figure S23. Gel analysis of DNA origami bundle with pretreament in 10% FBS for different incubation time. The nanostructures maintained the structural integrity.

Table S1. PNA sequences used in this study. Each PNA strand contains a linker (2- or 6-residue) followed by an NGL tripeptide at the C terminus.

Name	Sequence (N→C)
PNA-2aa	ACTAGTACGCTC-**GlyGlyAsnGlyLeu**
PNA-6aa	ACTAGTACGCTC-**GlyGlySerGlyGlySerAsnGlyLeu**

Table S2. Amino acid sequence of each POI. Each POI contains a GL dipeptide at the N terminus.

Name	Sequence (N→C)
ELP	MGLHHHHHHGSVPGEGVPGVGVPGVGVPGVGVPGVGVPG AGVPGAGVPGGGVPGGGVPGEGVPGEGVPGVGVPGVGVPG VGVPGVGVPGAGVPGAGVPGGGVPGGGVPGEGRSC
Protein G	MGLHHHHHHPVSTTYKLVINGKTLKGETTTEAVDAATAEK VFKQYANDNGVDGEWTYDDATKTFTVTE
GB1-X module-Doc (GXD)	MGLHHHHHHGSMDTYKLILNGKTLKGETTTEAVDAATAEK VFKQYANDNGVDGEWTYDDATKTFTVTERSGGNTVTSAV KTQYVEIESVDGFYFNTEDKFDTAQIKKAVLHTVYNEGYTG

	DDGVAVVLREYESEPVDITAELTFGDATPANTYKAVENKFD YEIPVYYNNATLKDAEGNDATVTVYIGLKGDTDLNNIVDGR DATATLTYYAATSTDGKDATTVALSPSTLVGGNPESVYDDF SAFLSDVKVDAGKELTRFAKKAERLIDGRDASSILTFYTKSS VDQYKDMAANEPNKLWDIVTGDARS
GL-EGFP	MGLHHHHHHMVSKGEELFTGVVPILVELDGDVNGHKFSVS GEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCF SRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAE VKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYI MADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPV LLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMD ELYK

Table S3. Sequences used as capture strands in DNA origami triangle.

Name	Sequence (5'→3')
triangle-capture-1	GAGCGTACTAGTCTTTTTACCAGTCAGGACGTTGG AACGGTGTACAGACCGAAACAAA
triangle -capture-2	GAGCGTACTAGTCTTTTTCCAAGCGCAGGCGCATA GGCTGGCAGAACTGGCTCATTAT
triangle -capture-3	GAGCGTACTAGTCTTTTTACCTTATGCGATTTTATG ACCTTCATCAAGAGCATCTTTG

Table S4. Quantitative analysis of the reaction yields of POI-PNA conjugation under various conditions.

	Yield (%)						
	Spacer length (aa) (2 h, pH 7, 25 ℃)		pH (PNA-6aa, 2 h, 25 ℃)			Temp (℃) (PNA-6aa, 2 h, pH 7)	
	2	6	5	6	7	10	25
ELP_{20}	34.2±2.9	41.2±4.3	22.7± 2.5	26.5± 3.9	47.7± 5.4	38.9±3.2	48.4±2.8

Protein G	33.2±5.1	34.2±4.8	14.5± 4.2	31.9± 6.1	28.8± 2.2	28.6±3.6	32.2±3.3
GXD	28.9±1.9	42.8±2.6	15.6± 2.7	30.1± 2.1	39.5± 4.9	41.8±4.8	48.9±5.3

Table S5. DNA sequences used as capture strands in DNA origami bundle and fuel strand. Highlighted in red are capture strands that hybridize with PNA. Highlighted in green are capture strands that hybridized with Cy5-DNA.

Name	Sequence (5'→3')
bundle-P-capture-1	GAGCGTACTAGTTTTTACCTTATATTGGGCCGAGA AATCAACGTAAGAACC
bundle-P-capture-2	GAGCGTACTAGTTTGCATAACTTAAAGGCCGCTGA GTAGTAAGCGATTTTAAGAAC
bundle-P-capture-3	GAGCGTACTAGTTTTCGCCCACCACCAGAGATTCA CAAACAACCTAGAACC
bundle-P-capture-4	GAGCGTACTAGTTTCCAGCATTCACCACCCCCTCA GCCGGAACTGCCATC
bundle-P-capture-5	GAGCGTACTAGTTTAAACGCAATTAAGACTCCTCC TCAGAGCGACAGGAGGTTGGG
bundle-P-capture-6	GAGCGTACTAGTTTTACCAATTGAGAATCGTAATA AGAGAATAACAGGGAG
bundle-P-capture-7	GAGCGTACTAGTTTTTTAACAACAAAGCCAGCGTT ATACACCGGTAAATAA
bundle-P-capture-8	GAGCGTACTAGTTTTTGCTTTATTATTCATTTCTAC CAGTATGCCAACATGTAAGA
bundle-P-capture-9	GAGCGTACTAGTTTACAATTGCAACAGGAAATTTA CATTGGCGGATGAGGA
bundle-C-capture-1	CACCGGCTTGAAGTGCCGTTAGGGCGACTTCGCTA CGCCAGAGTGACAATGTCCCGGCACCG
bundle-C-capture-2	CACCGGCTTGAAGTGCCGTTTTTGGGGCGAGAGGC TTTTGCCGATAAAAACCAAATCAAAGGAATTACG
bundle-C-capture-3	CACCGGCTTGAAGTGCCGTTTTGACGGCTGAAACG GTTTTGCCACCCT
bundle-C-capture-4	CACCGGCTTGAAGTGCCGTTTAGGGCGCCAAGAAT TTCATCCATTTGC
Cy5-probe	Cy5-CGGCACTTCAAGCCGGTGTAATGGTC
Fuel strand	GACCATTACACCGGCTTGAAGTGCCG

Table S6. Amino acid sequences and corresponding DNA sequences of functional peptides in the construction of GL-EGFR-EGFP plasmid.

Name	Sequence (N→C or 5'→3')
Signal peptide	MRPSGTAGAALLALLAALCPASRA
DNA sequence of signal peptide	ATG CGA CCC TCC GGG ACG GCC GGG GCA GCG CTC CTG GCG CTG CTG GCT GCG CTC TGC CCG GCG AGT CGG GCT
GL and linker peptide	GLKGG
DNA sequence of GL and linker peptide	GGC CTG AAG GGC GGC

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