Peptide Nucleic Acid (PNA)

23/10/26 Literature Seminar

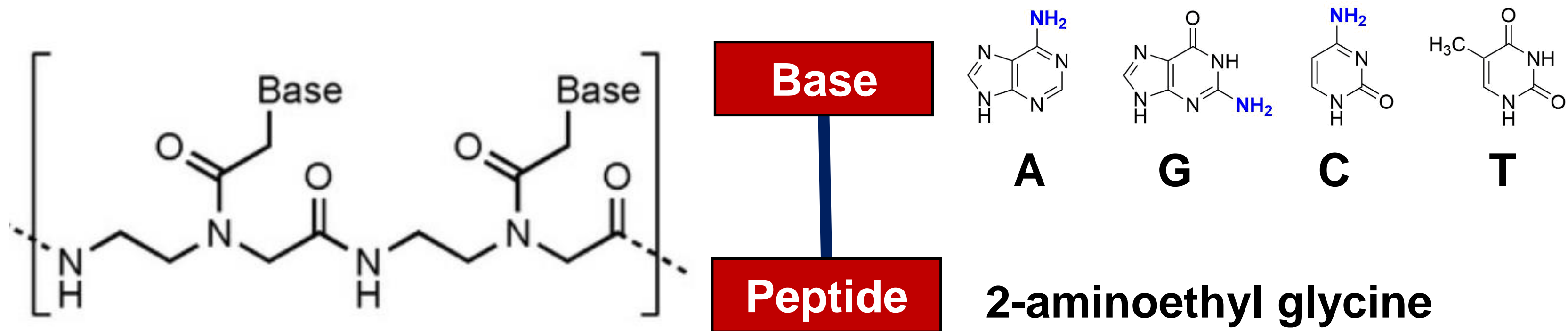
Mayu Onoda

What is PNA?

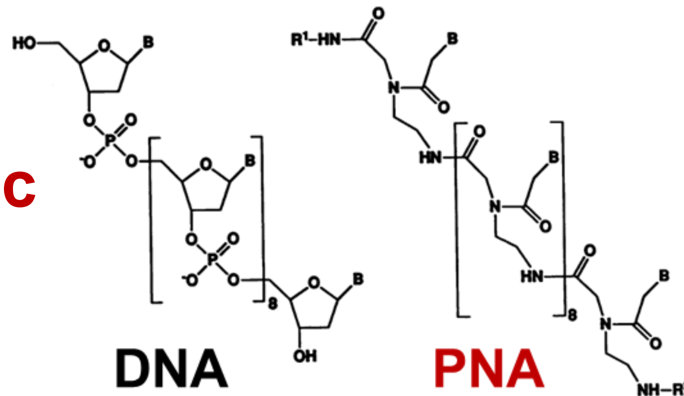
What is PNA?

PNA (= Peptide Nucleic Acid)

…「Peptide」+「Base」



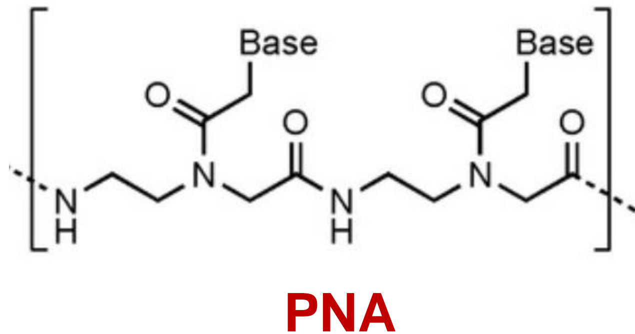
DNA mimic



Peter E. Nielsen *et al. Science*, **1991**, *254*, 1497

Le, Bao T. *et al. Molecular Therapy - Nucleic Acids*, **2018**, *14*, 142

What is PNA?



PNA

Invasion

- Probe
- Gene Expression Regulation

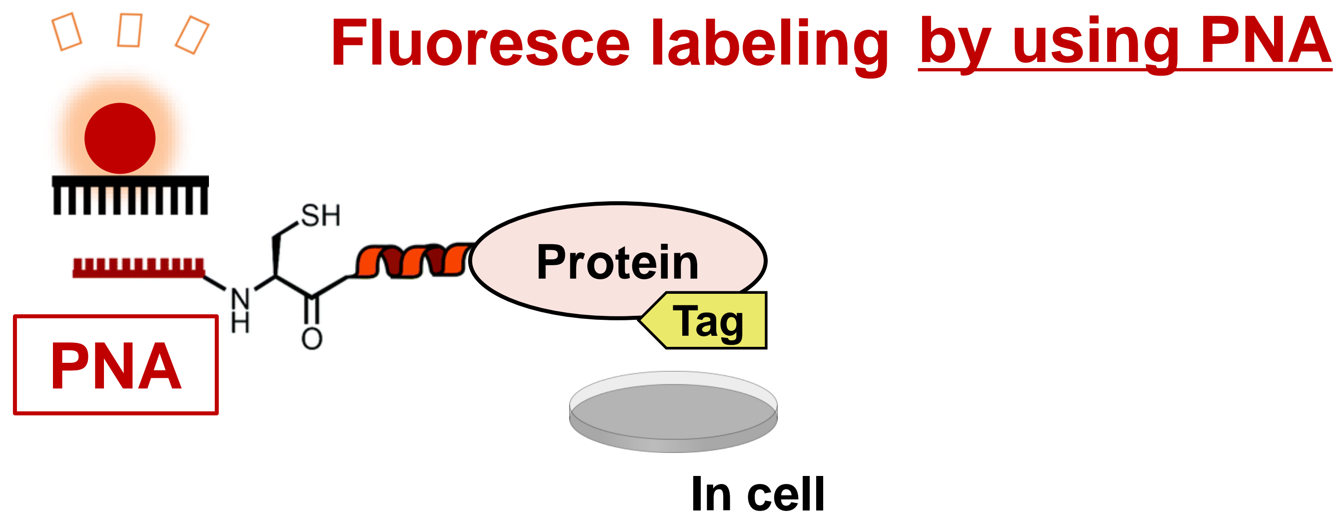
Target

✔ Electrically neutral
✔ Resistant to nuclease / protease

✘ Low cell membrane permeability

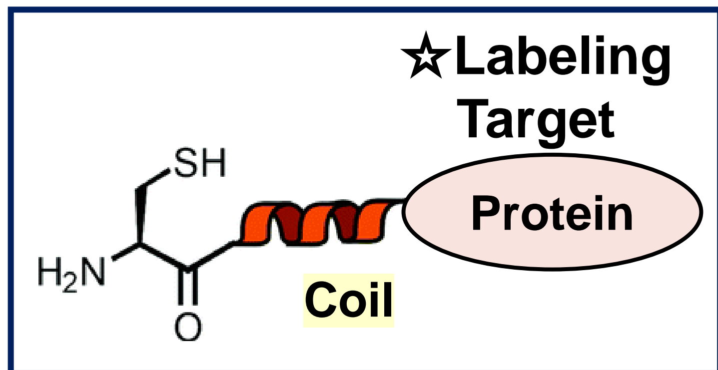
Jacques, S et al. *Current Opinion in Chemical Biology*, **2019**, *52*,112

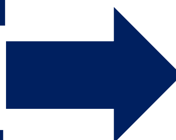
PNA application in Chemical Biology

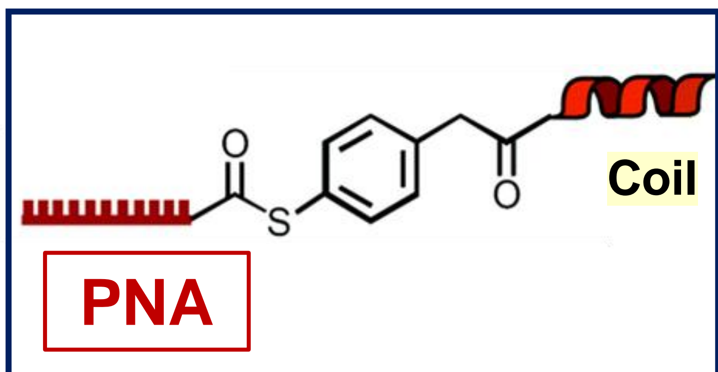


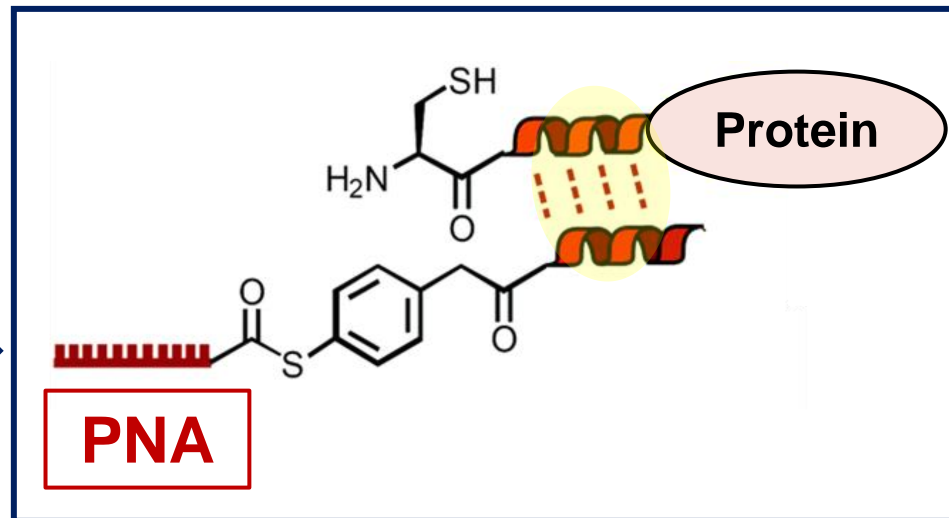
Ulrike Reinhardt. et al. *Bioconjugate Chemistry*, **2015,** *26*, 10
Gavins, G.C., et al. *Nat. Chem.*, **2021,** *13*, 15
Georgina, C. G, et al. *RSC Chem. Biol.*, **2021**, 2, 1291

PNA application



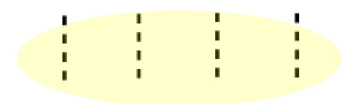






Already reported
"Peptide Coil-Coil Interaction"

Gradišar, H. and Jerala, R, **2011**, *J. Peptide Sci., 17*, 100



Gavins, G.C., et al. *Nat. Chem.*, **2021**, *13*, 15.
Georgina, C. G, et al. *RSC Chem. Biol.*, **2021**, 2, 1291

PNA application

Within 2min

SH

H_2N

O

Protein

O

O

S

PNA

Native Chemical Linkage

Fluorescence

DNA

SH

N H

O

Protein

PNA

<PNA – Coil structure>

O H N—DKD- PNA$_n$ -KR-$CONH_2$

O N=N N

O S

$_5$

O

O

H N

O

$_2$

O

H N

Peptide —$CONH_2$

Coil

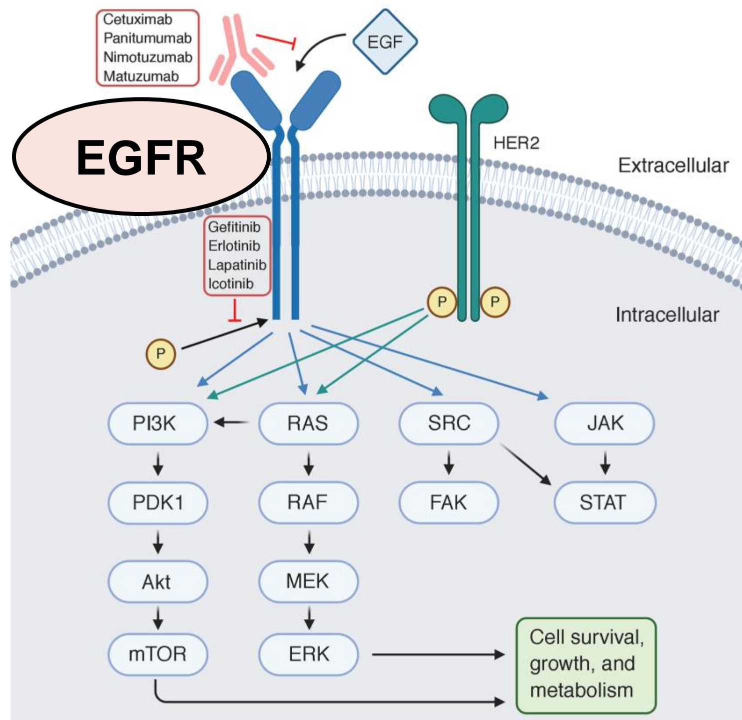
Gavins, G.C., et al. *Nat. Chem.*, **2021,** *13*, 15.

Georgina, C. G, et al. *RSC Chem. Biol.*, **2021**, 2, 1291

Fluoresce labeling
Target Protein

Gavins, G.C., *Nat. Chem.*, **2021,** *13*, 15.
Georgina, C. G, et al. *RSC Chem. Biol.*, **2021**, 2, 1291

EGFR (Epidermal Growth Factor Receptor)

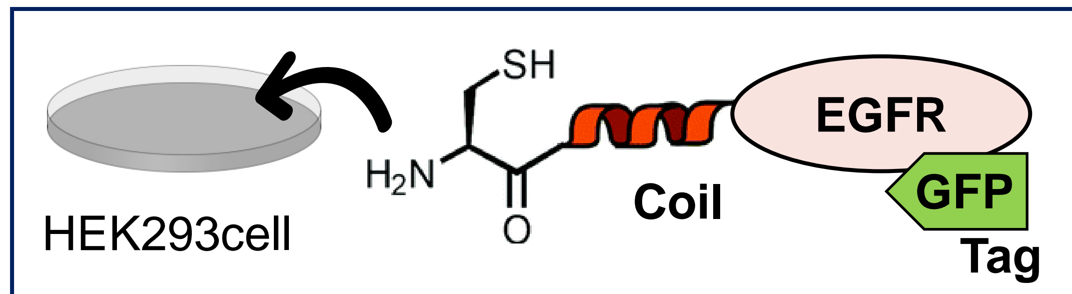


Maron Steven B., *et al. Frontiers in Oncology*, **2020**, *10*

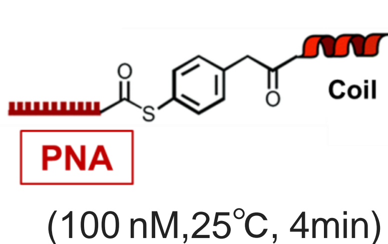
- Cell membrane protein
- Ligand-activated (EGF ligand)
- Related to signaling pathways, cell proliferation, differentiation and survival

PNA application

【Protocol】



EGFR expression in HEK293 cell



(100 nM,25℃, 4min)

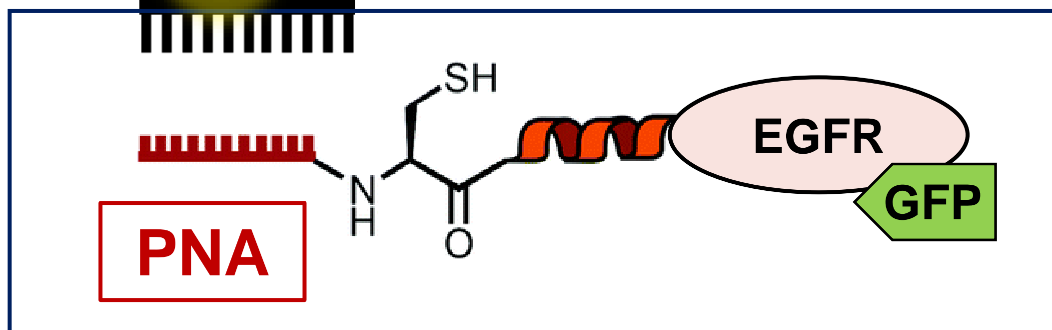
Coil-Coil

Native Chemical Ligation

Atto565 / Cy3

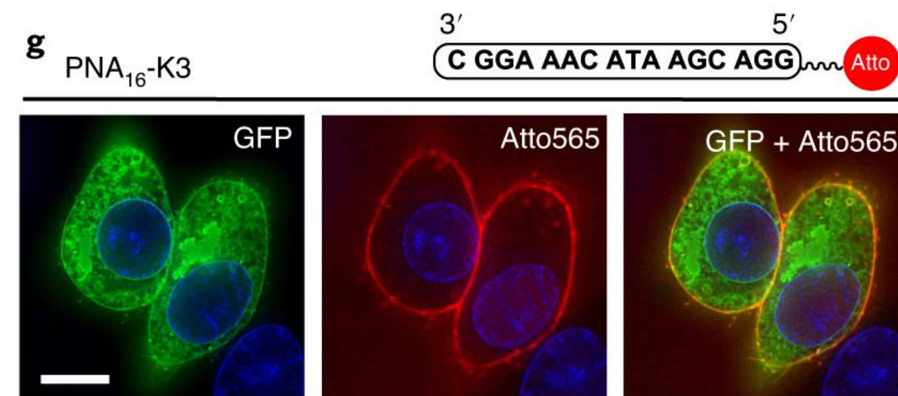
(200 nM, 25℃, 5min)

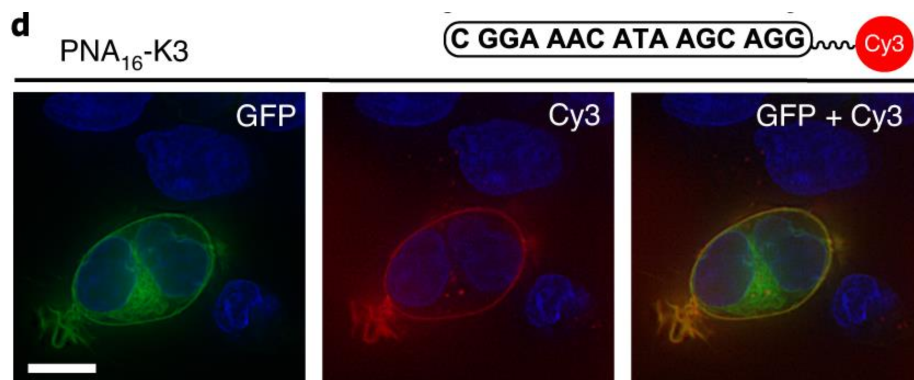
PNA-EGFR Fluorescent labeling



Gavins, G.C., et al. *Nat. Chem.*, **2021**, *13*, 15.

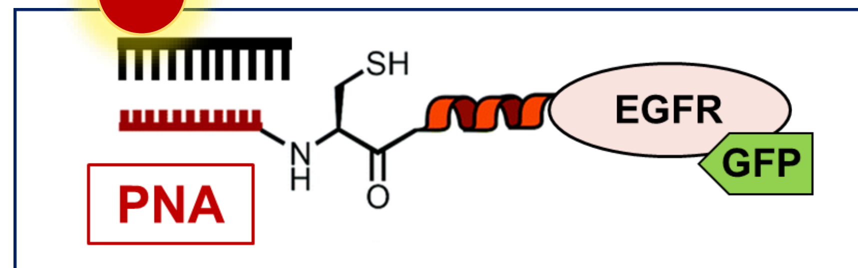
PNA application

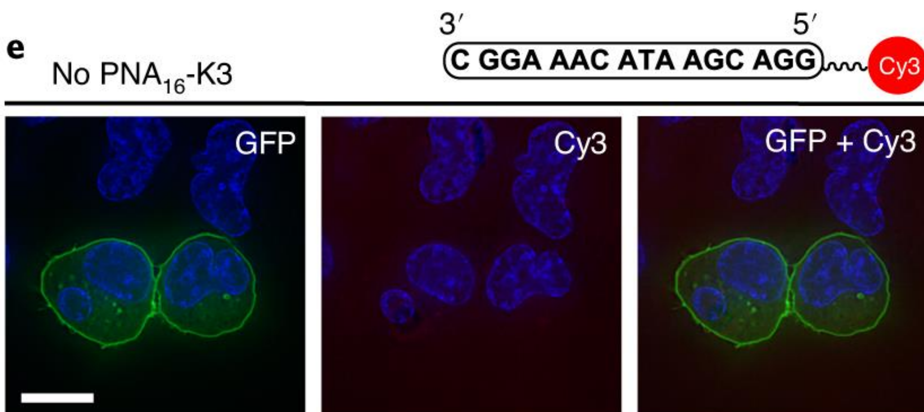




Atto565 or Cy3

✔Specific labeling on cell membrane





No PNA (Negative control)

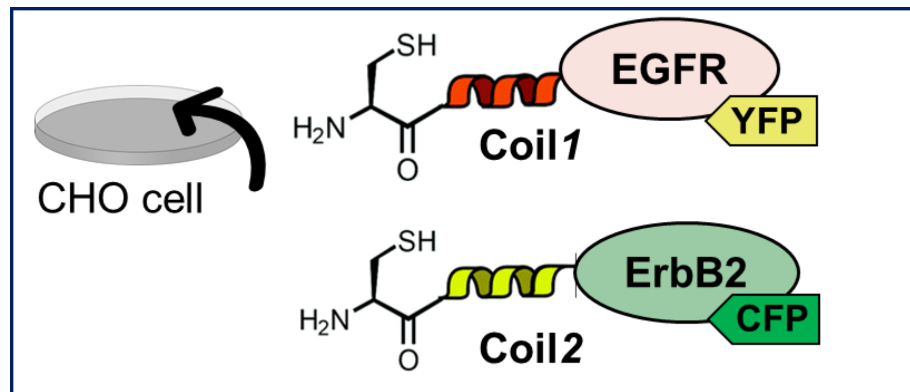
✔No labeling

a, Schematic structures of the Cys-E3-EGFR-eGFP construct and the PNA-tagging reagents **PNA_{11}-K3** and **PNA_{16}-K3** used for labelling. **b–f**, After staining of nuclei with Hoechst 33342 (shown in blue), transiently transfected cells were treated with **PNA_{11}-K3** (**b**,**f**) or **PNA_{16}-K3** (**d**,**g**). For control, PNA tagging was omitted in **c** and **e**. Subsequently, cells were incubated with TMR-labelled PNA-12mer (**b**,**c**), Cy3-labelled DNA-16mer (**d**,**e**), Cy3-labelled DNA-12mer (**f**) or Atto565-labelled DNA-16mer (**g**) single strands. Conditions for PNA transfer: (1) 0.1 mM TCEP in PBS (pH 7.0), 2 min, 25 ° C; (2) 100 nM donor **PNA_n-K3** in PBS buffer (pH 7.0), 4 min, 25 ° C. Conditions for hybridization: 200 nM TMR-/Cy3-/Atto565-labelled PNA/DNA strands in PBS (pH 7.0), 5 min, 25 ° C. Scale bars, 10 µm. All experiments were repeated three times independently with similar results.

Gavins, G.C., et al. *Nat. Chem.*, **2021**, *13*, 15.

PNA application

[Coil*1*-EGFR]
[Coil2-*ErbB2*]
expression in CHO cell

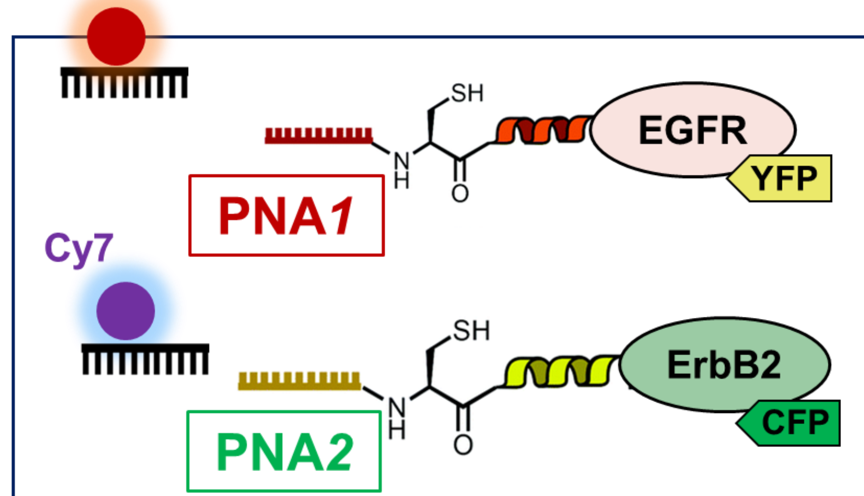


PNA*1* / PNA2
(100 nM, 4min)

Coil-Coil

Native Chemical Ligation

PNA1-EGFR
PNA2-ErbB2
Fluorescent labeling



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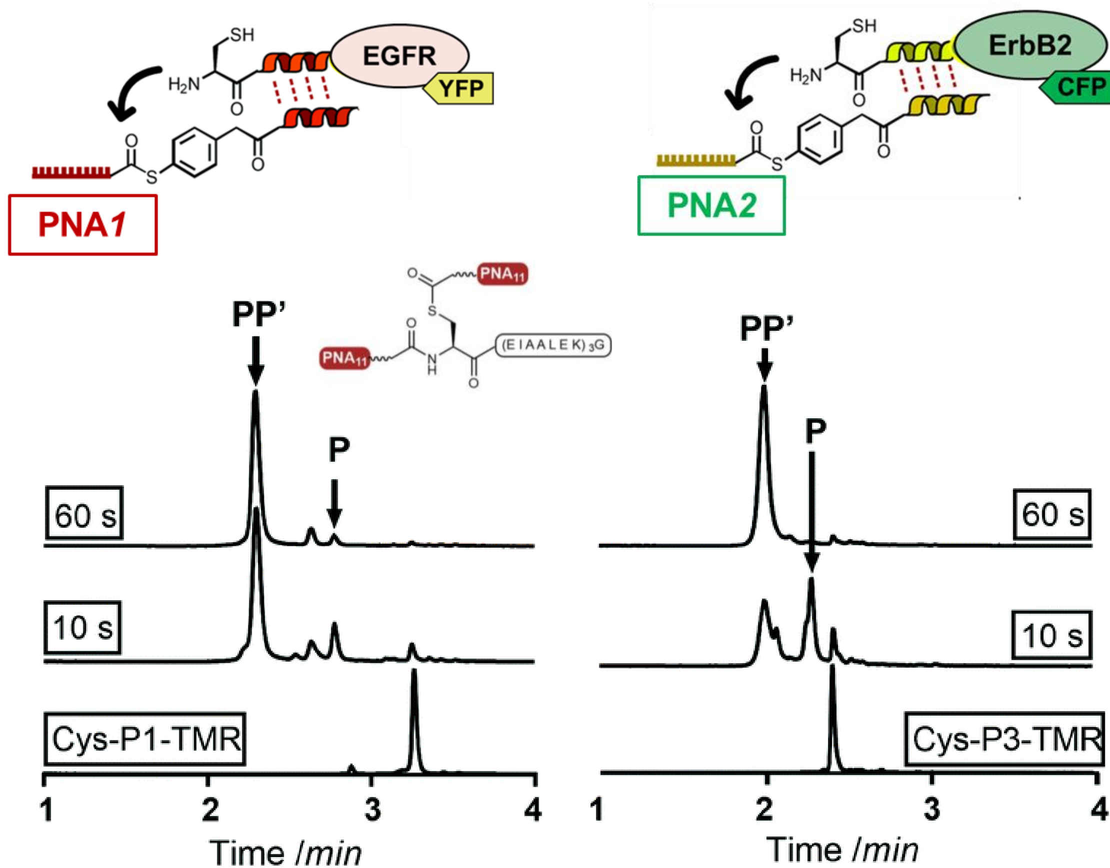
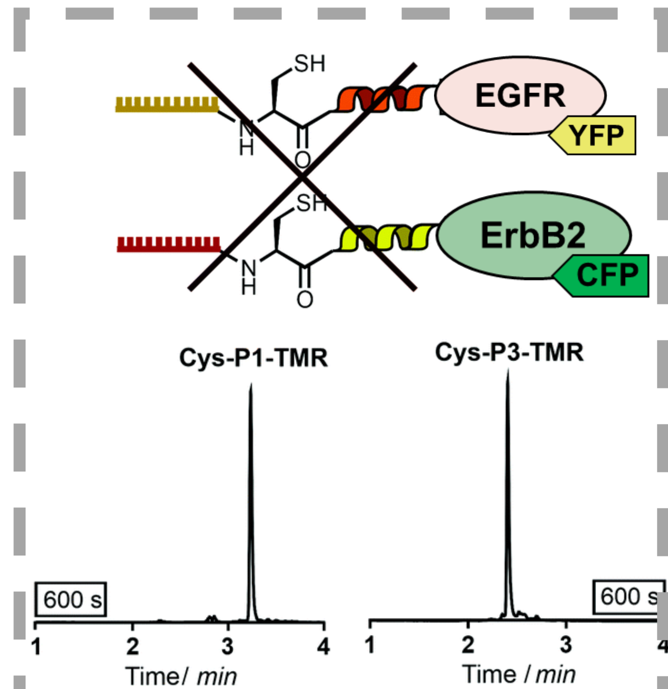
HPLC analysis





✔ Mismatch reactions do not occur.

(B) matched and (C) mismatched coiled coil peptides. Conditions: 200 nM Cys-P1-TMR or Cys-P3-TMR, 1200 nM PNA1-P2 or PNA3-P4 in 200 nM phosphate, 1 mM TCEP, 0.1% CHAPS, pH 7.2, 30 ° C. (D) One-pot reactions of a Cys-P1-TMR/Cys-P3-C343/PNA1-P2/PNA3-P4-mixture (red traces) overlaid with Fl-UPLC traces for reactions involving Cys-P1-TMR + PNA1-P2 or Cys-P3-C343 + PNA3-P4 in separate pots (black traces). Detector settings: TMR, Ex 550 nm, Em 580 nm; C343, Ex 420 nm, Em 500 nm.

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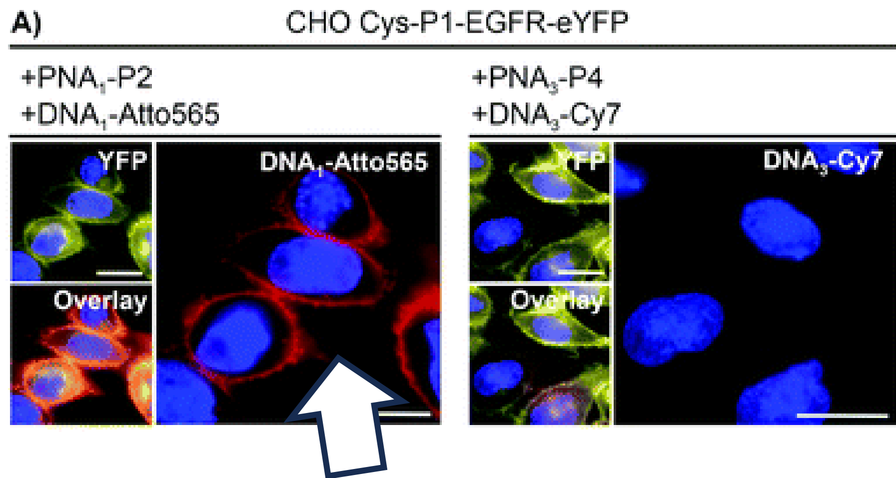
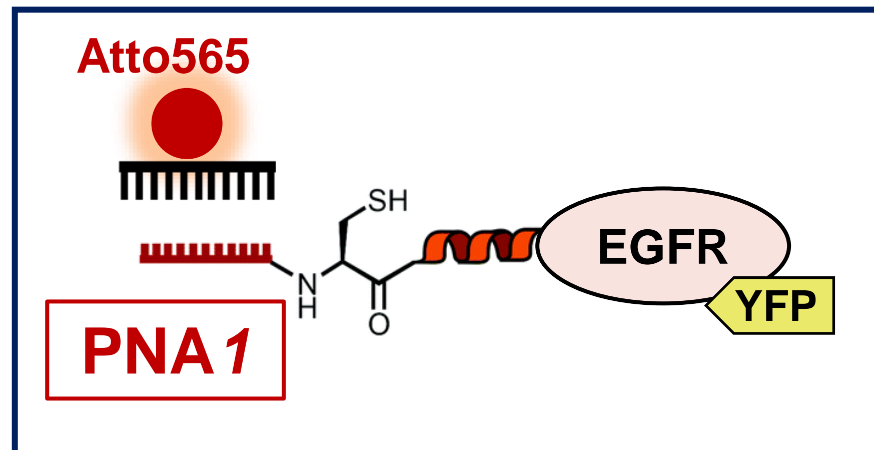
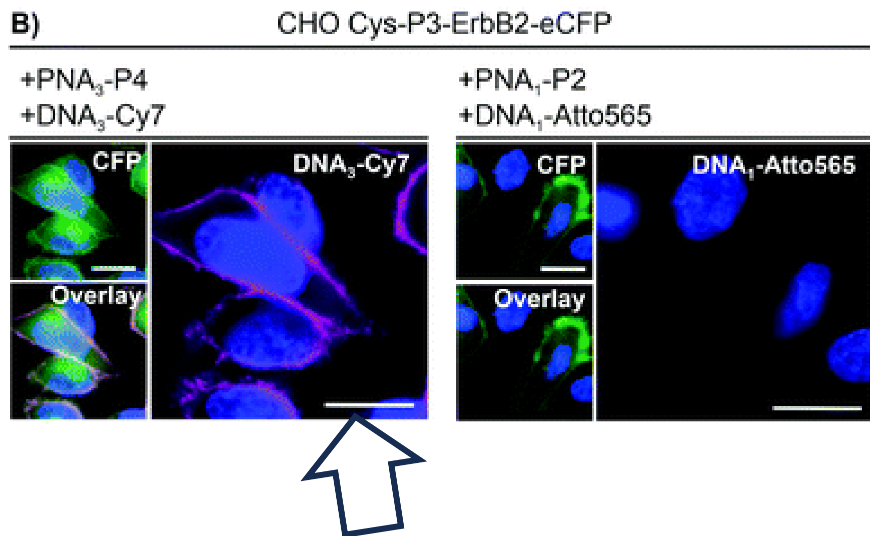
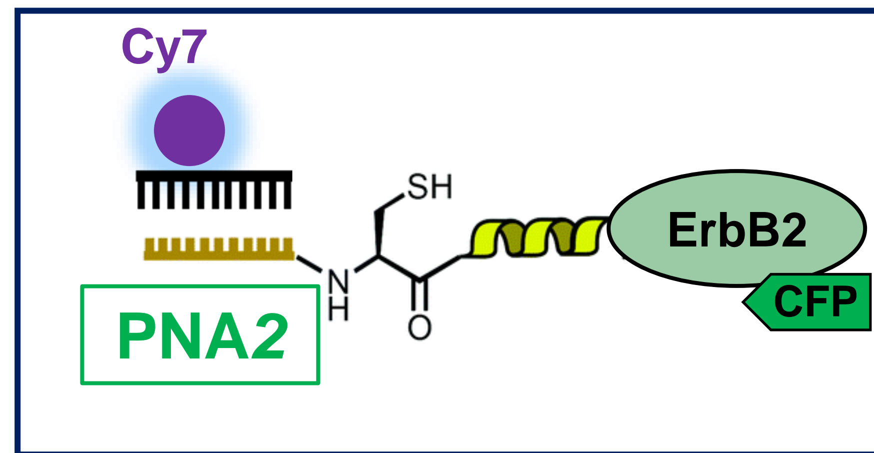
PNA application









Dual color live cell labeling. After nuclear staining with Hoechst33342 (blue), (A) Cys-P1-EGFR-eYFP or (B) Cys-P3-ErbB2-eCFP expressing CHO cells were treated with matched (left) or mismatched (right) PNA labeling reagents/fluorophore-DNA. Conditions: 4 min with 100 nM PNA1-P2 (EGFR) or/and PNA3-P4 (ErbB2) in DPBS, washing with HBSS, 4 min hybridization with 200 nM Atto565-DNA1 (EGFR) or Cy7-DNA3 (ErbB2). (C) Simultaneous labeling of Cys-P1-EGFR-eYFP/Cys-P3-EGFR-eCFP cells. Scale bar = 10 μm.

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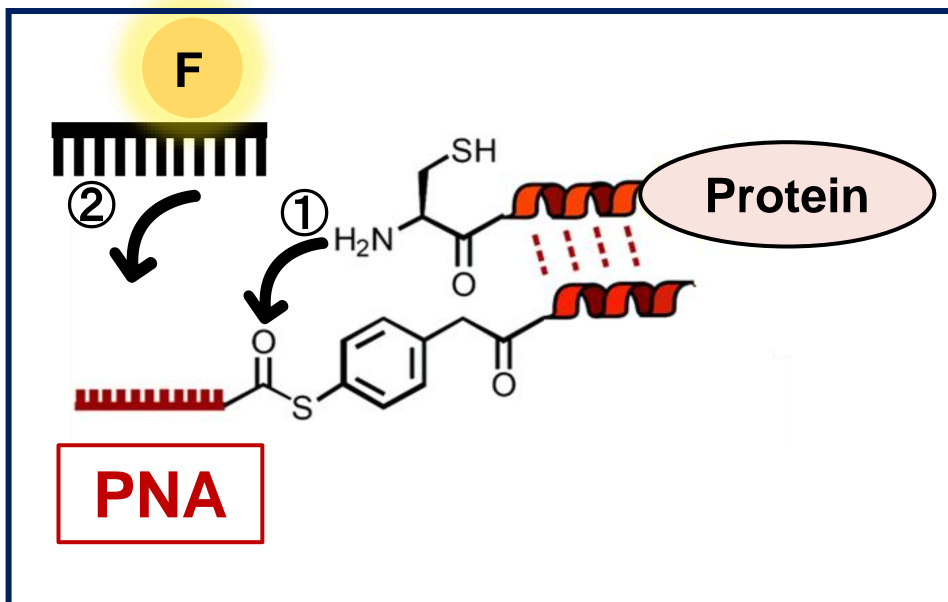
PNA application



What's the merit?

F
①
SH
H_2N
O
Protein
O
S
O

Already reported.

F
S
NH_2
Protein
TAMRA-Cys-E3-hY$_2$R-eYFP
unstimulated
stimulated
recycled

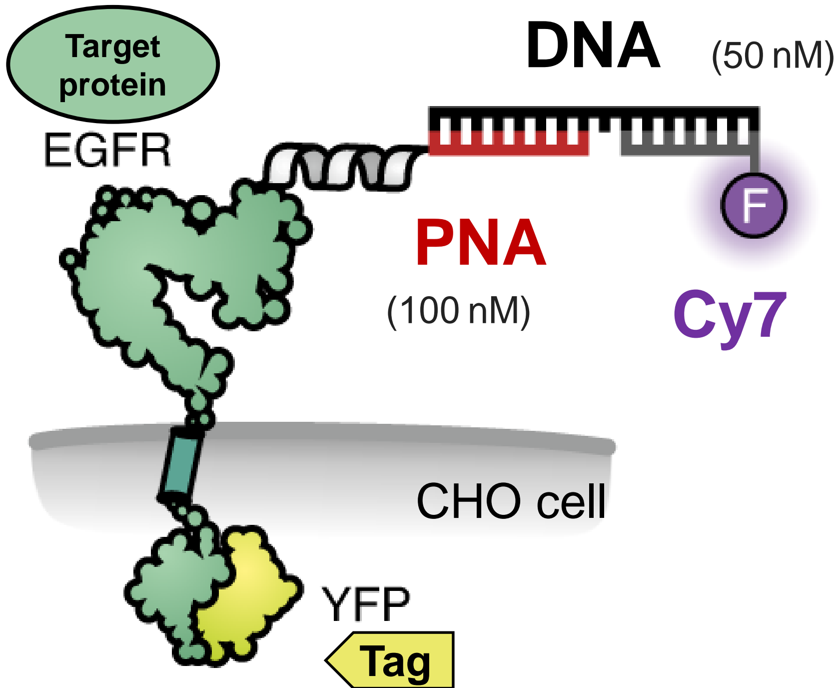
Ulrike Reinhardt. et al. *Bioconjugate Chemistry*, **2015,** *26*, 10

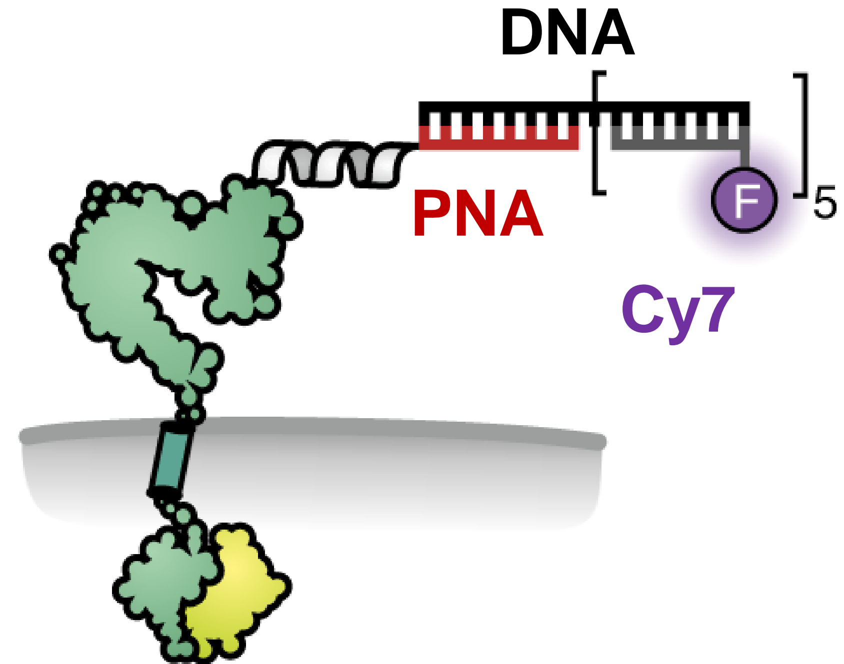
Gavins, G.C., et al. *Nat. Chem.*, **2021,** *13*, 15.

Georgina, C. G, et al. *RSC Chem. Biol.*, **2021**, 2, 1291

<PNA merit ①>
Increasing of fluorescence intensity

Gavins, G.C., et al. *Nat. Chem.*, **2021**, *13*, 15.





33

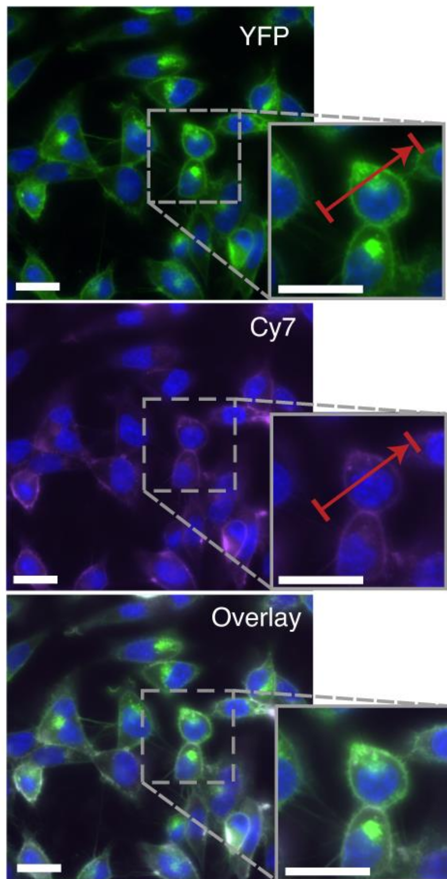
GCG TCA TCC AGA GTC CTA CTG GTA AGT GGT GTC
cgc agt agg tct cag GAC CAT TCA CCA CAG
15 **15**

105

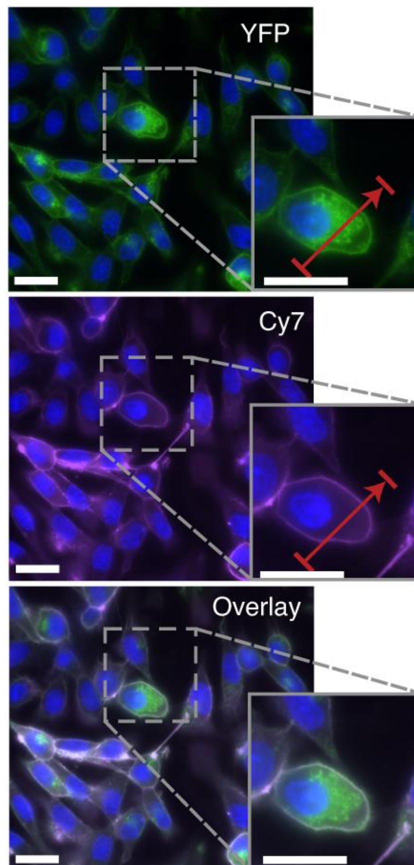
GCG TCA TCC AGA GTC (CTA CTG GTA AGT GGT GTC
cgc agt agg tct cag (GAC CAT TCA CCA CAG)5
15 **15**

PNA application

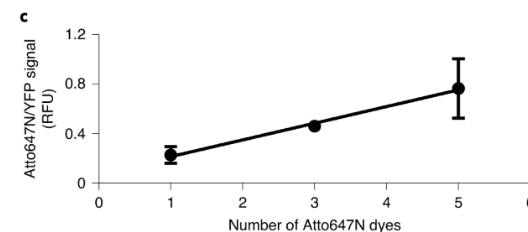
Cy7



5 × Cy7



✔ **Fluorescence intensity increase**

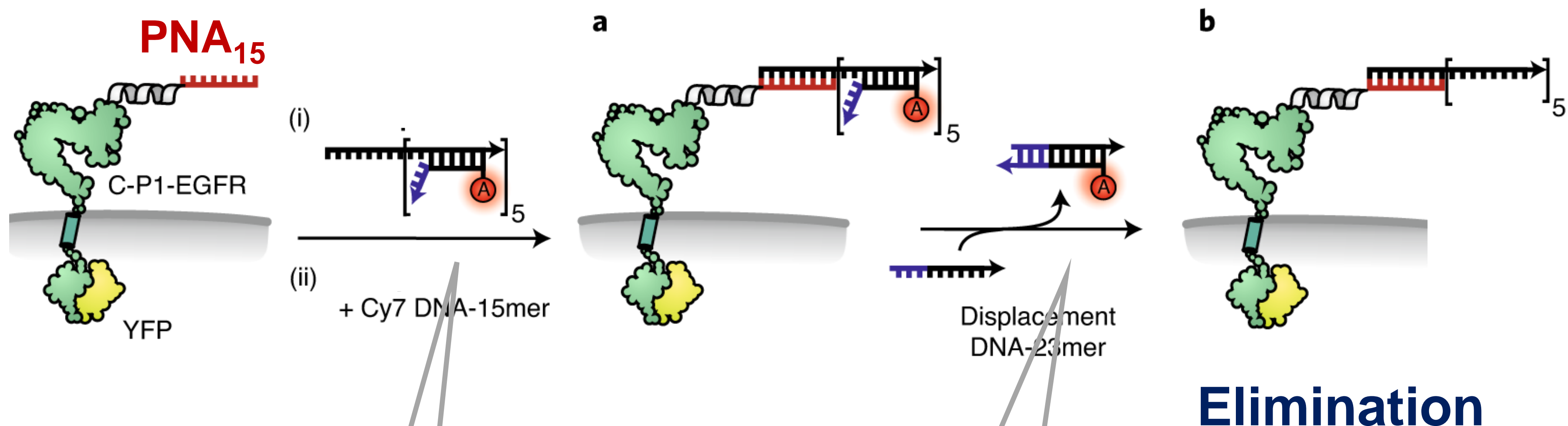


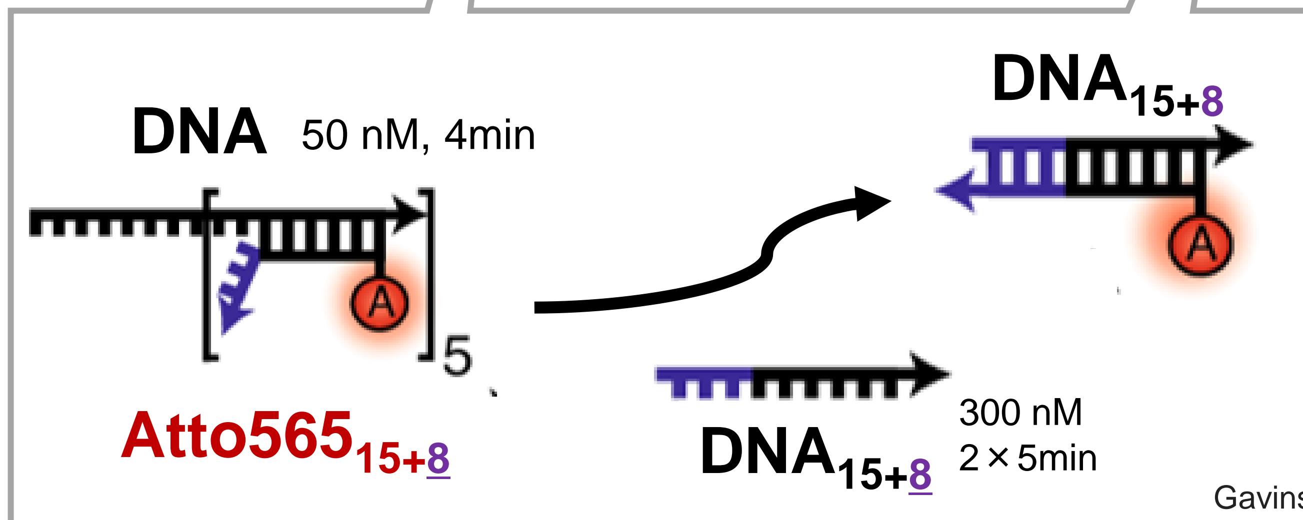
a, Labelling of CHO cells stably expressing Cys-P1-EGFR-eYFP by PNA tagging and subsequent hybridization with a single Cy7-DNA (Complex I, adaptor DNA 33mer with one Cy7-15mer, left) or five Cy7-DNA strands (Complex II, adaptor DNA 105mer with five Cy7-15mers, right). Red arrows indicate the lines on which line intensity profiles in Fig. 4b are measured. PNA transfer: 100 nM donor PNA15-P2 in HBSS buffer, 4 min, 25 ° C. Hybridization: 50 nM DNA complex in HBSS-BB, 4 min, 25 ° C. The brightness of images depicting 1 × Cy7 labelling was digitally increased relative to 5 × Cy7 for clarity. For images showing 1 × Cy7 and 5 × Cy7 labelling with equal brightness settings, see Supplementary Fig. 9-6. Scale bars, 20 µm. Experiments were repeated three times independently with similar results.

c, Dependence of staining intensity on the number of Atto647N dyes in complexes used for staining (DNA-105mer + 1, 3 or 5 equiv. of Atto647N-DNA-15mer; Complexes IV, V and VI, respectively). After labelling as described in **a**, cells were detached, fixed and analysed by flow cytometry to determine the ratio of mean Atto647N and YFP fluorescence intensity values, after gating was applied (Supplementary Fig. 12-1). Data are presented as the mean ± SD of $n = 3$ independent experiments.

Gavins, G.C., et al. *Nat. Chem.*, **2021,** *13*, 15.

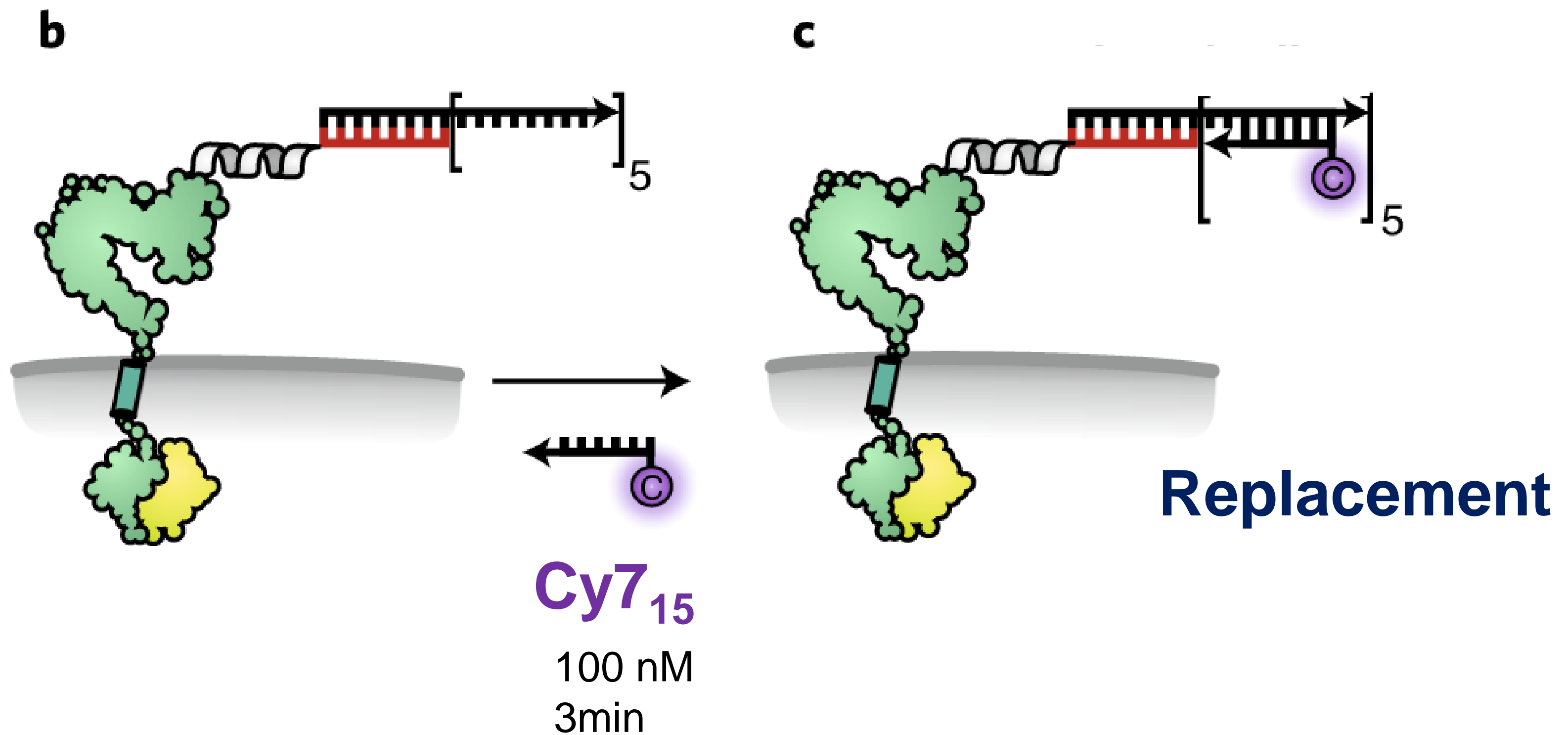
<PNA merit ②>
Fluorescence Elimination / Replacement





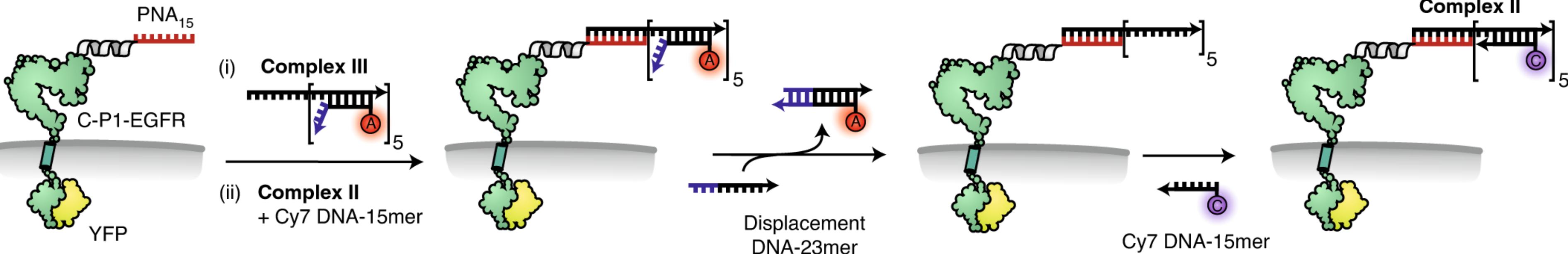
Gavins, G.C., et al. *Nat. Chem.*, **2021**, *13*, 15.

<PNA merit ②>
Fluorescence elimination / replacement



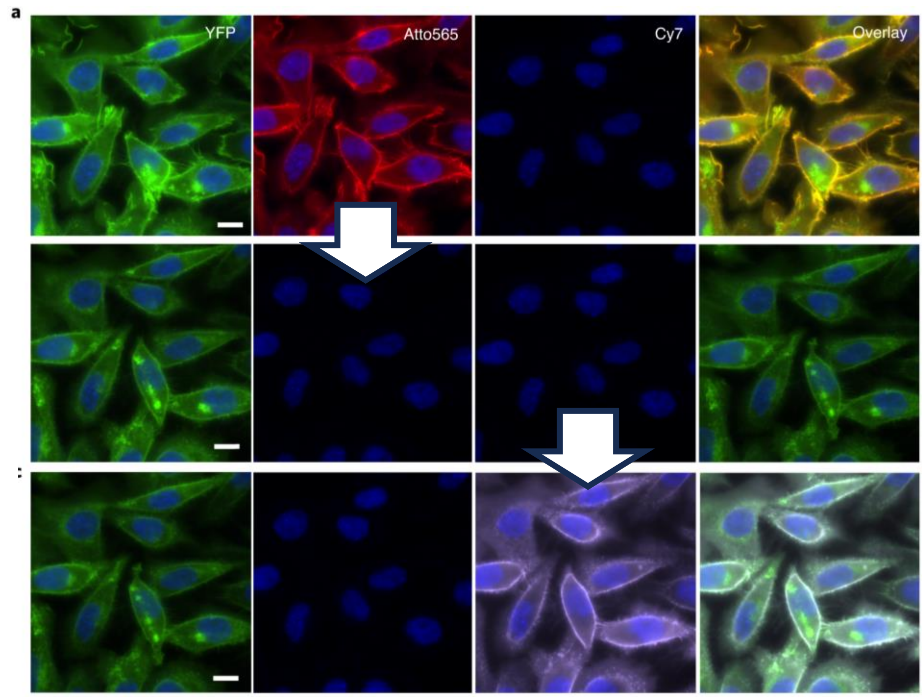
Gavins, G.C., et al. *Nat. Chem.*, **2021,** *13*, 15.

PNA application



✔Elimination

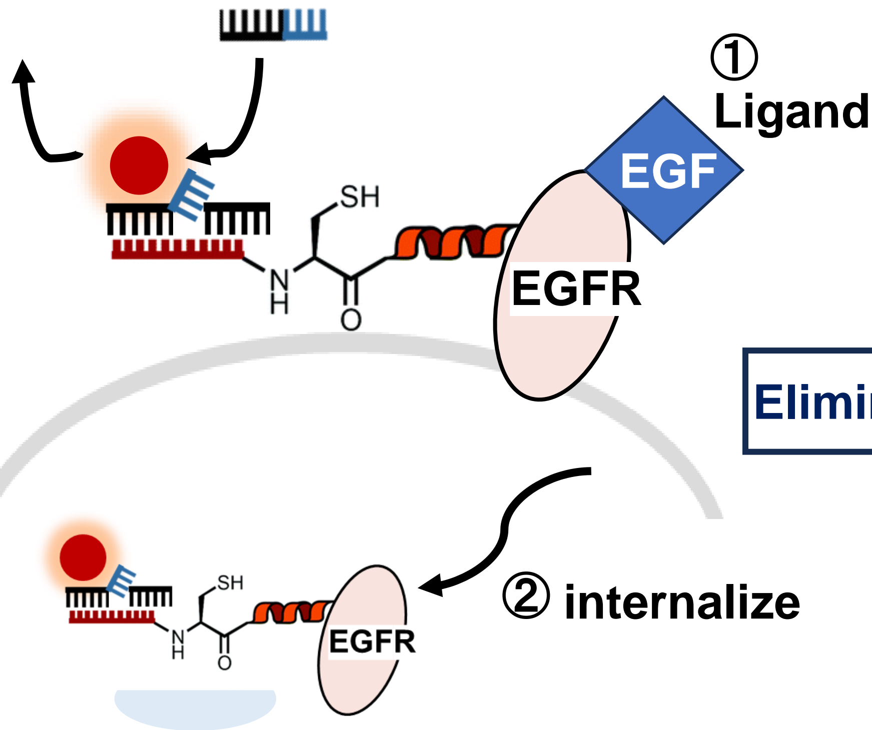
✔Replacement



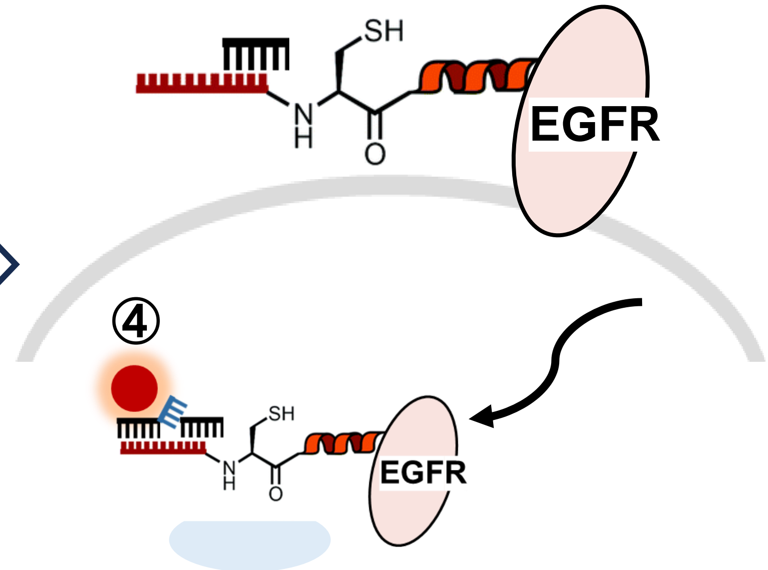
a–c, Wide-field images of reversible labelling without EGF stimulation. a, After staining nuclei with Hoechst 33342 in HBSS-BB, serum-starved Cys-P1-EGFR-eYFP cells were treated with PNA15-P2 in HBSS for 4 min. Cells were incubated with 50 nM Complex III (adaptor DNA-105mer with five Atto565-DNA-23mers containing a toehold for strand displacement) in HBSS-BB for 4 min. Subsequently, 50 nM Complex II (adaptor DNA-105mer carrying five Cy7 DNA15mer strands) and 50 nM Cy7 DNA-15mer were added for 4 min. b, Toehold-mediated strand displacement of the ATTO565-23mer DNA was carried out with 300 nM displacement DNA-23mer for 2 × 5 min in HBSS at 30 ° C. The brightness of the Atto565 images in rows a and b was digitally increased to give ‘overexposed’ images, which illustrate that Atto565 labelling was completely removed in b. c, 100 nM Cy7 DNA-15mer was hybridized for 3 min to illustrate restaining.

Gavins, G.C., et al. *Nat. Chem.*, **2021,** *13*, 15.

PNA application



Elimination



Label only internalized proteins
Separate labeling possible

Gavins, G.C., et al. *Nat. Chem.*, **2021,** *13*, 15.

PNA application

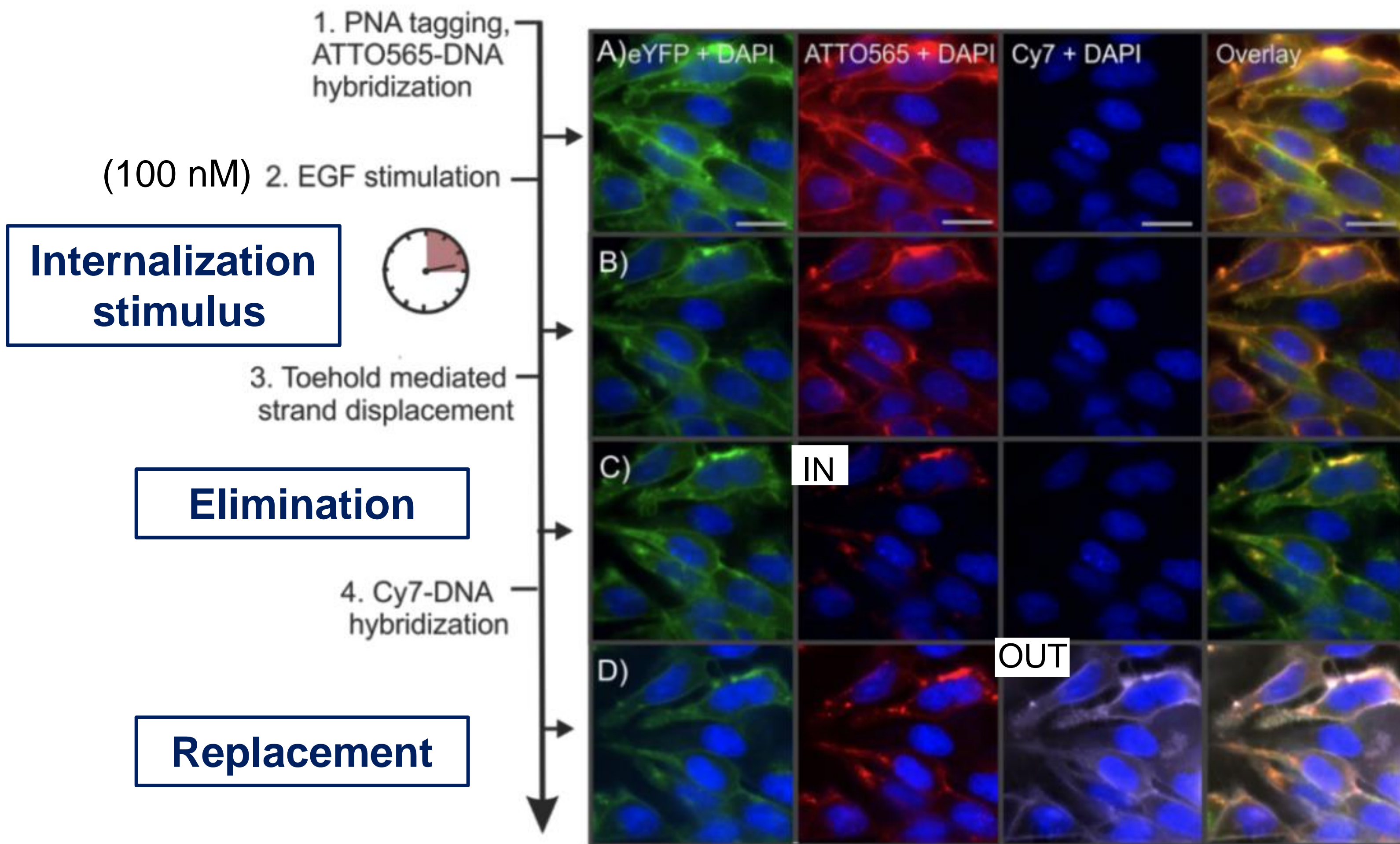
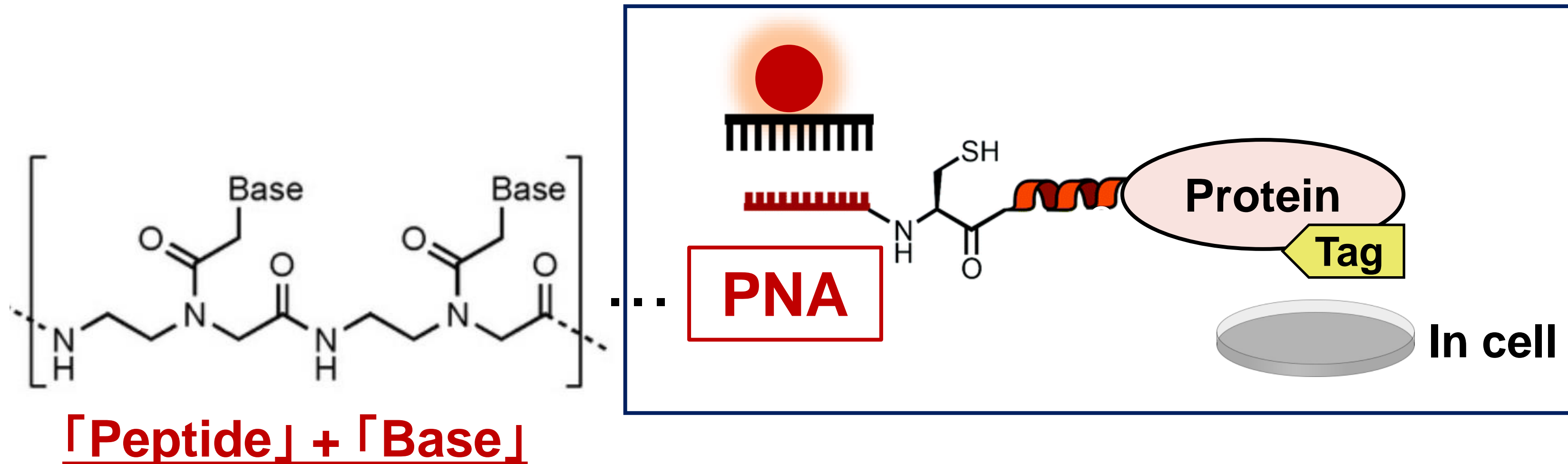


Figure 9-7 Widefield fluorescence microscope characterization of PNA-tag enabled reversible fluorescence labelling of Cys-P1-EGFR-eYFP on serum starved CHO cells, stimulated with EGF. After staining of nuclei with Hoechst 33342 in HBSS-BB, cells were treated with PNA15-P2 in HBSS, 4 min. A) Cells were incubated with 50 nM Complex III; adaptor DNA-105mer with five Atto565-DNA-23mers; in HBSS-BB for 4 min. B) 100 nM EGF stimulation for 15 mins C) Toehold mediated strand displacement of Atto565-23mer DNA with 300 nM displacement DNA-23mer and 100 nM EGF for 2 x 5 min in HBSS at 30° C. D) 100 nM Cy7-DNA-15mer, 3 min. E) The same experiment was carried out without addition of PNA or DNA.BB: Blocking Buffer: 0.1mg/mL salmon sperm DNA, 0.2% BSA, 1x ProLong™ Live Antifade in HBSS. Excitation times: Cy7: 500 ms, ATTO565: 50 ms YFP: 50 ms, Hoechst 33342:10 ms. Filter settings: Hoechst 33342) λ_{ex} = 350 ± 50 nm, λ_{em} = 460 ± 50 nm; YFP) λ_{ex} = 500 ± 24 nm, λ_{em} 545 ± 40 nm, Cy7) λ_{ex} = 710 ± 75 nm, λ_{em} 810 ± 90 nm. Scale bar= 20 µm. Experiments were repeated 3 times independently with similar results.

✔Separate intracellular and extracellular detection

Gavins, G.C., et al. *Nat. Chem.*, **2021,** *13*, 15.

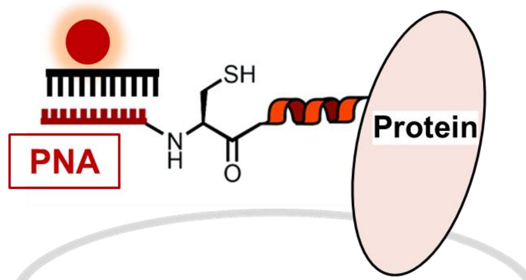
Fluorescent labeling



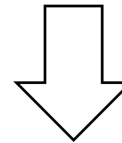
✔ Separate labeling of two proteins

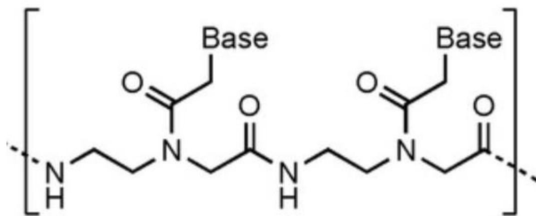
fluorescence
✔ Increasing
✔ Elimination
✔ Replacement

Short summary



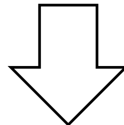
Target:
Cell membrane protein





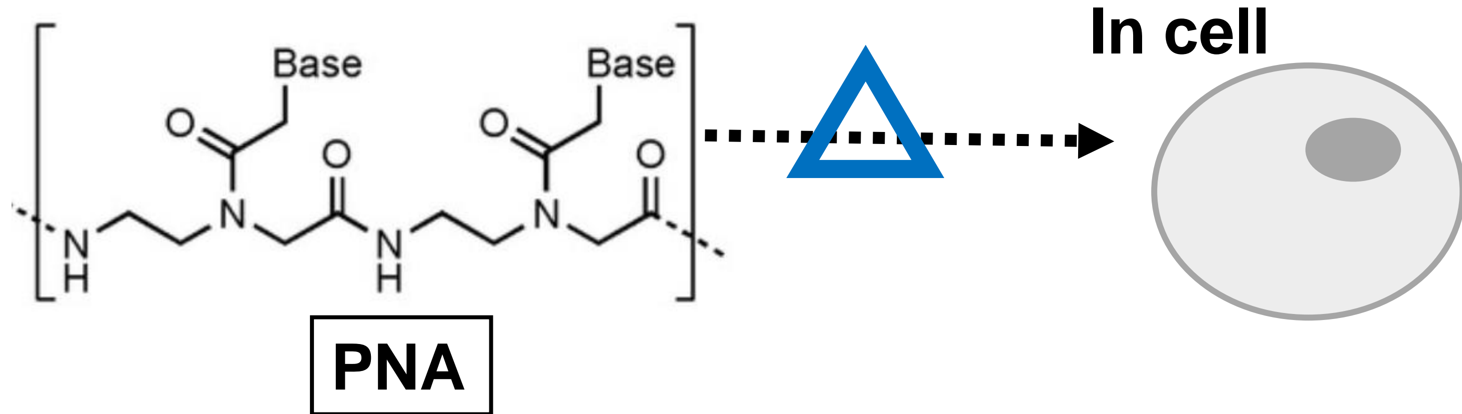
PNA : Low cell membrane permeability is not much of a problem.

In cell PNA application
(Example: Gene expression regulation)



Improvement of cell membrane permeability is needed.

Improvement of PNA cell membrane permeability



Improvement of PNA cell membrane permeability

CPP (=cell-penetrating peptide) strategy

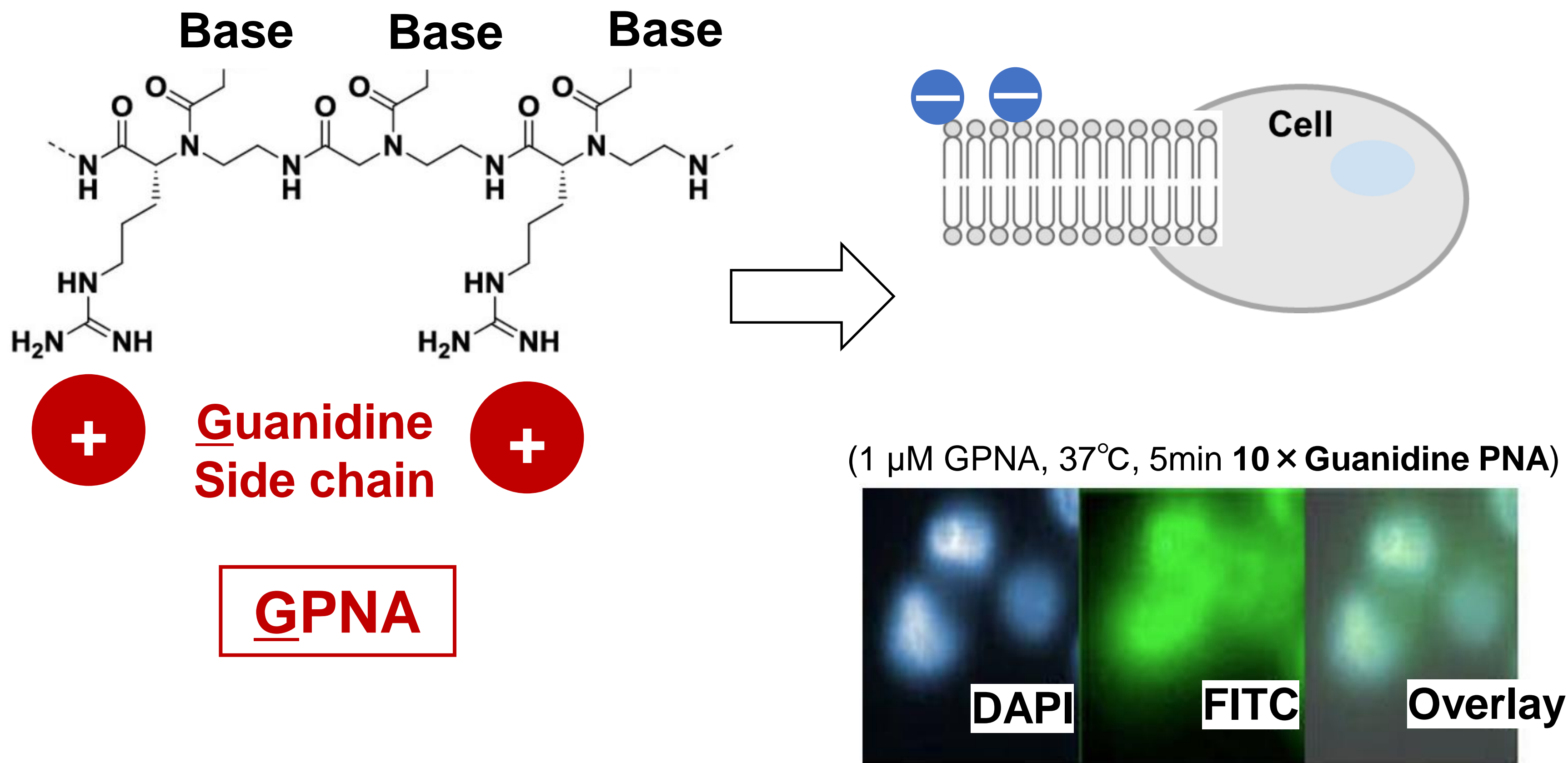


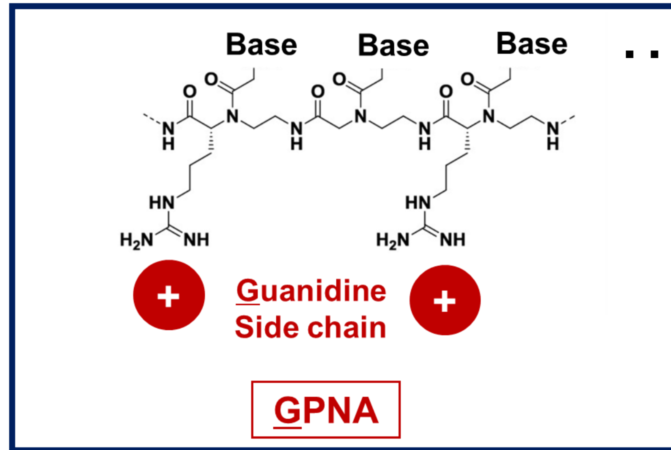
Figure S4. Fluorescent images of HCT 116 cells following: (i) incubation with 1 μM GPNA for 5min at 37oC, (ii) thoroughly washed with PBS, (iii) fixed with 4% paraformadehyde for 30min at room temperature, (iv) permeabilized with 1% Triton-X for 30min, (v) incubate with 1uM DAPI for 30min, (vi) thoroughly washed and mounted on the microscope slide. A: image taken with DAPI channel, B: image taken with FITC channel (emission from fluorescein covalently linked to GPNA), and C: an overlay between A and B.

Peng, Z. et al. *J. Am. Chem. Soc.*, **2003**, 125, 6878

Anca, D. A, et al. *J. Am. Chem. Soc.* **2006**, *128*, 16104

Improvement of low cell membrane permeability

Gene Expression Regulation (E-cadherin)

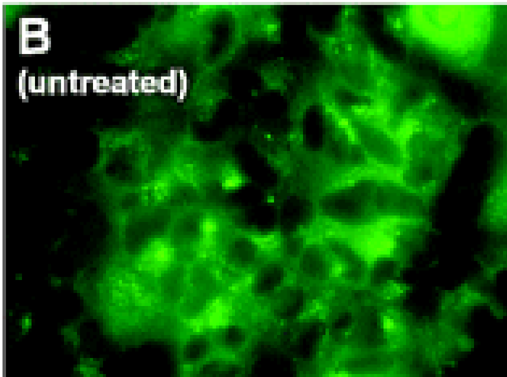


GTGGCTGCAGCCAGGT

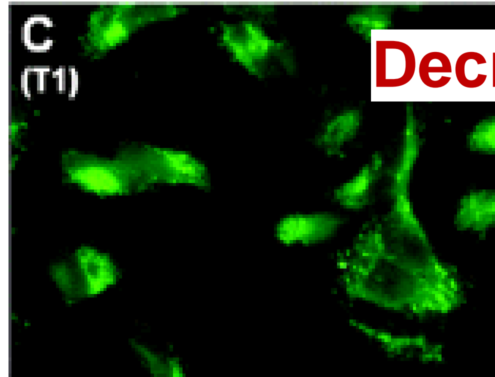
(Complementary **E-cadherin** transcription start site)

E-cadherin localization in A549 cells

GPNA (-) , 72 h



GPNA 10 μM, 72 h



Decreased protein levels.

Figure 2 E-Cadherin immunofluorescent-staining of the untreated (B) and treated cells (C−H). A549 cells were treated with 10 μM of GPNA for 72 h and then stained with E-cadherin primary antibody and counterstained with FITC-labeled secondary antibody. The identity of each oligomer is indicated in the parentheses.

Anca, D. A, et al. *J. Am. Chem. Soc.* **2006**, *128*, 16104

Improvement of low cell membrane permeability

Base

Guanidine

VS

Base

Poly Arg xR

B

PNA-Conjugate

GPNA

% Cell Death

4R 6R 8R 10R

(10 μM, 24 h)

C

P16-8R

P16

% Cell Death

Concentration (μM)

(x μM, 48 h)

Poly Arg 8R

Guanidine

D

EthD-1 (red, dead cells)

calcein (green, live cells)

E

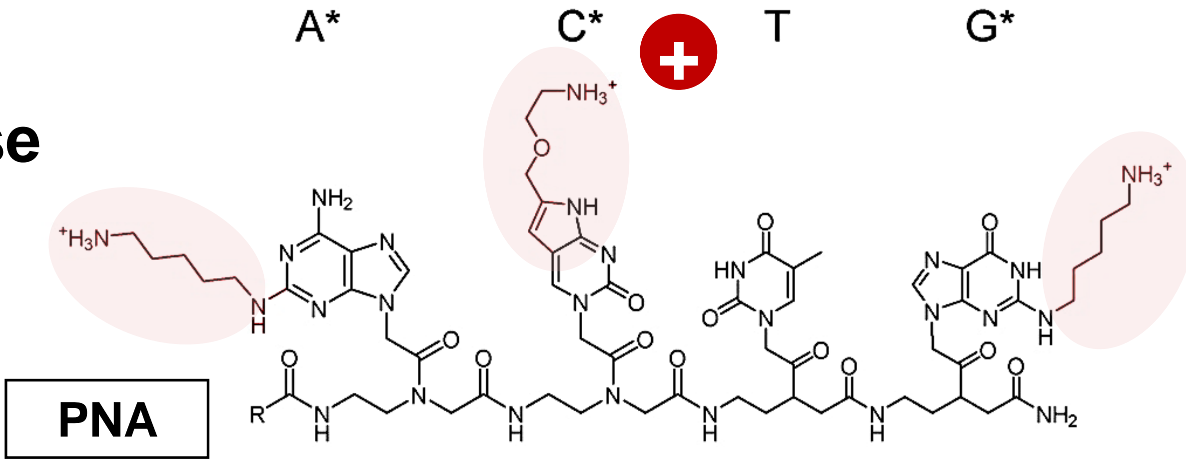
✔ Guanidine side chain ⇒ Low toxicity

Figure 7 Cytotoxic effects of PNA conjugates and GPNAs. (A) The sequence of PNA conjugates and GPNAs. The configuration of arginine residues in PNA conjugates is D; bold letters denote GPNA units. (B) The effects of oligomer size on cytotoxicity. Nearly confluent (80−90%) A549 cells were treated with 10 μM of the indicated PNA conjugates and GPNAs for 24 h and then stained with EthD-1 (dead cells) and calcein (live cells). (C) Exponentially growing A549 cells were treated with various concentrations of 16-mer PNA conjugate (**P16-8R**) and GPNA (**P16**) for 48 h and then stained with EthD-1 and calcein. Representative fluorescent images of cells treated with 15 μM of **P16-8R** (D) and **P16** (E) for 48 h and stained with EthD-1 (red, dead cells) and calcein (green, live cells).

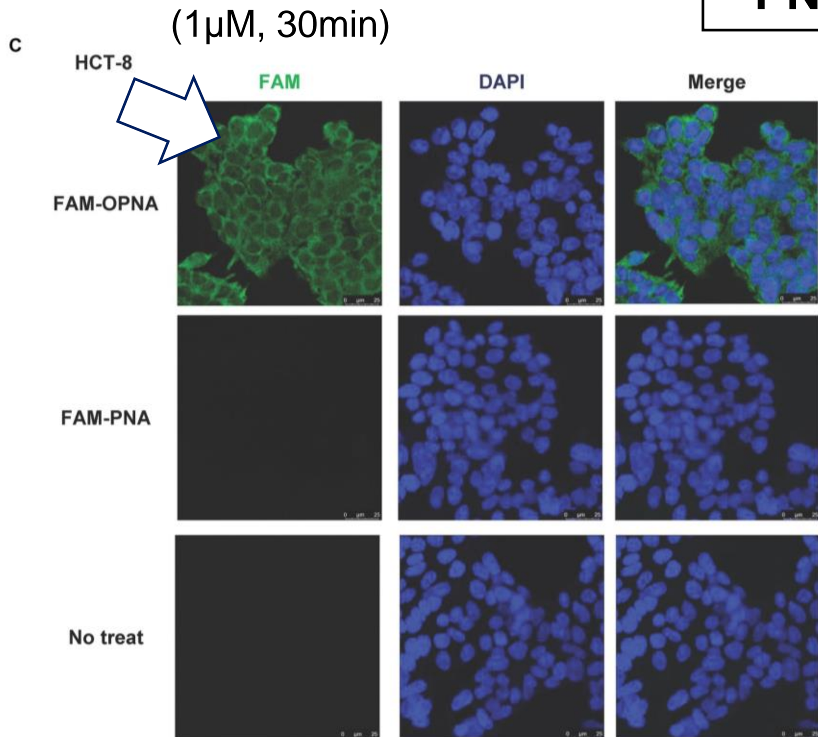
Anca, D. A, et al. *J. Am. Chem. Soc.* **2006**, *128*, 16104

Improvement of low cell membrane permeability

Cationic motifs on base



(1μM, 30min)



(C) Cell permeability of FAM-OPNA in HCT-8 cells. HCT-8 cells were seeded in an 8-well cell culture slide and incubated overnight. FAM-OPNA was treated at a concentration of 1 μM for 30 min, and nuclei were stained with DAPI for 20 min. Images were obtained by a confocal laser microscope.

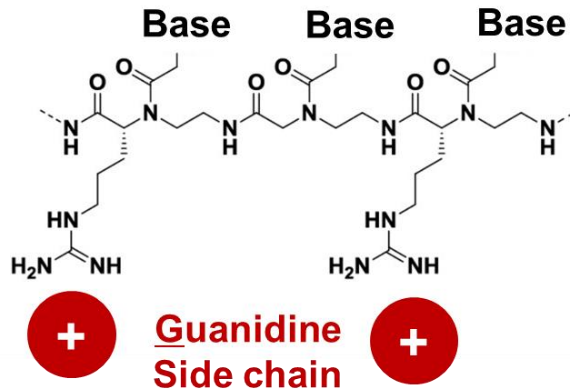
High cell membrane permeability

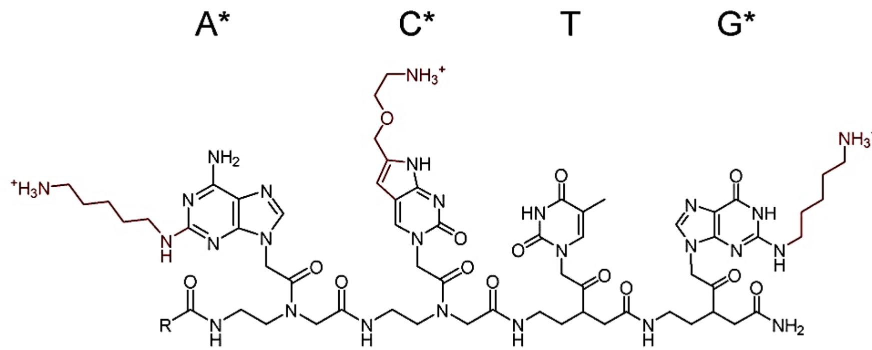
Park Soree, et al. *Frontiers in Microbiology*, **2023**, *14*

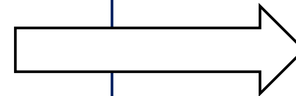
Tightly & selectively binds to pre-mRNA.
➡Regulate splicing
https://www.olipass.com/front/eng/competitive/technology.do

Summary

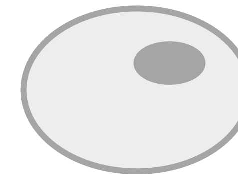
Improvement of cell membrane permeability

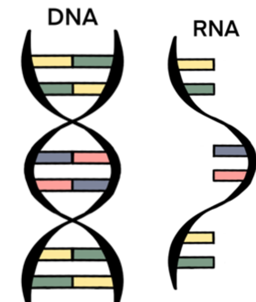






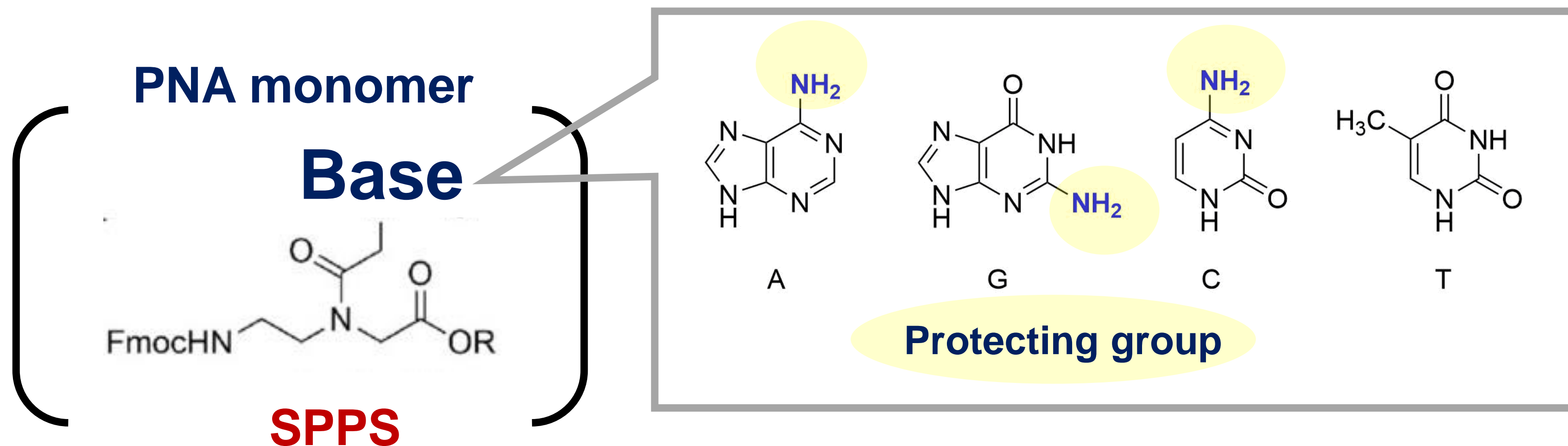
In cell



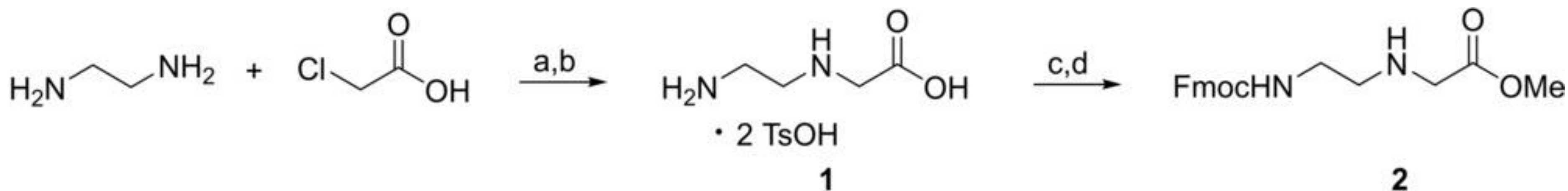


Appendix

PNA synthesis



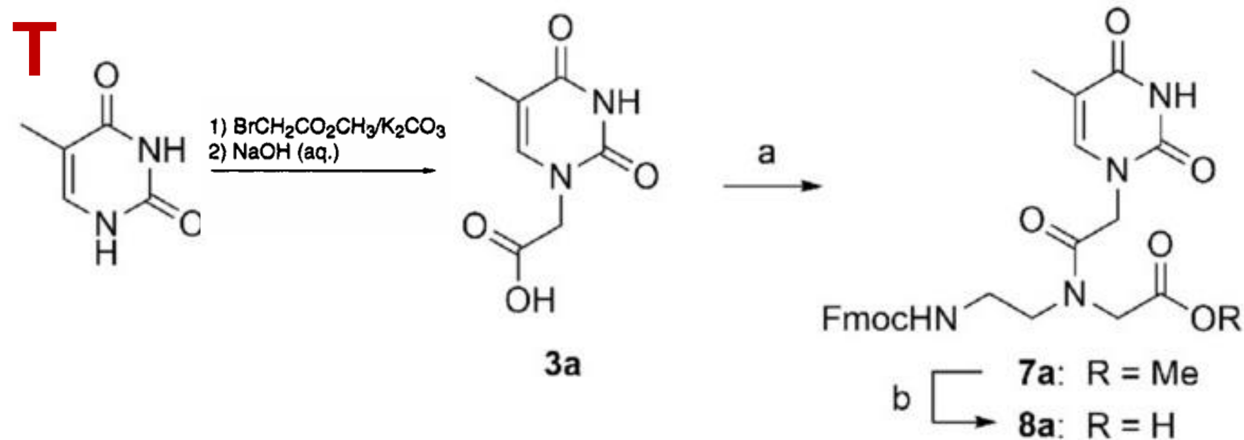
Skeleton synthesis of PNA monomer



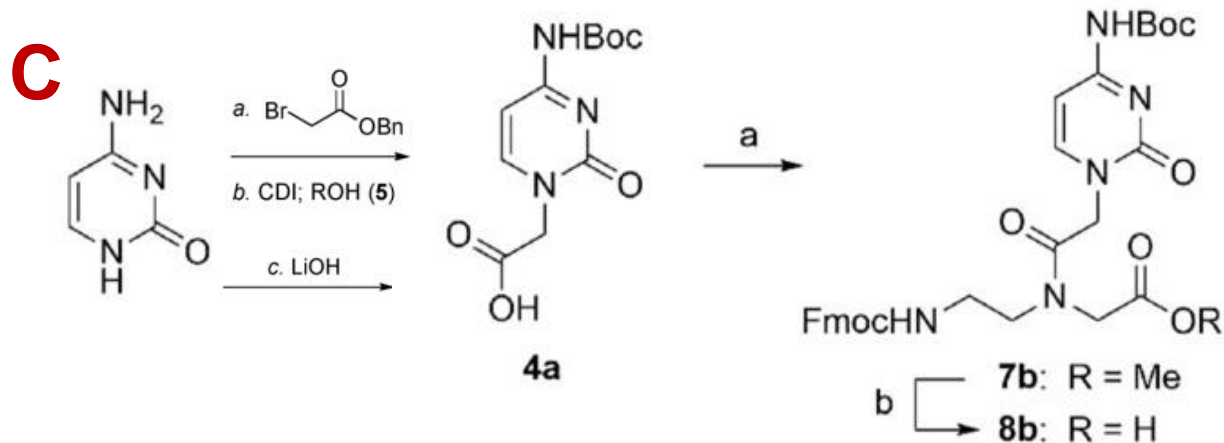
Synthesis of Fmoc-protected PNA backbone intermediate **2**:
(a) 4 ° C → room temp., 12 h, neat (56 %);
(b) PTSA, toluene, reflux with Dean-Stark setup, 3 h (99 %);
(c) MeOH−toluene (1 : 1), reflux at 110 ° C, 12 h (94 %);
(d) Fmoc−OSu, DIPEA, THF, 0 ° C → room temp., 3 h (96 %).

A. Y. Shaikh. *et al*. *Eur. J. Org. Chem.* **2021**, 2792.

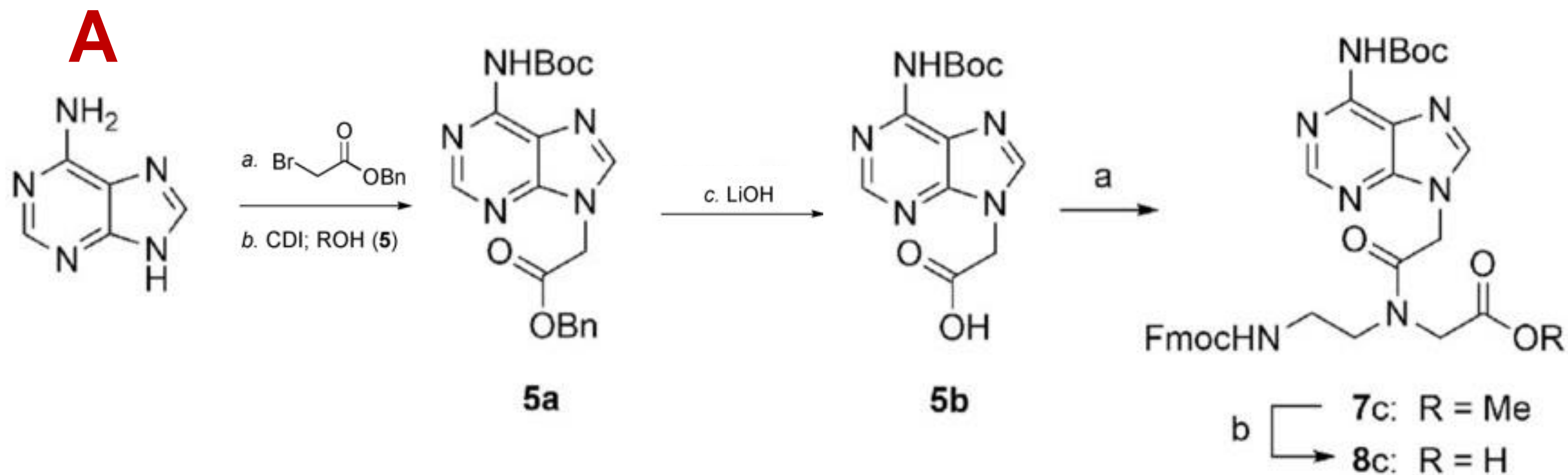
PNA synthesis

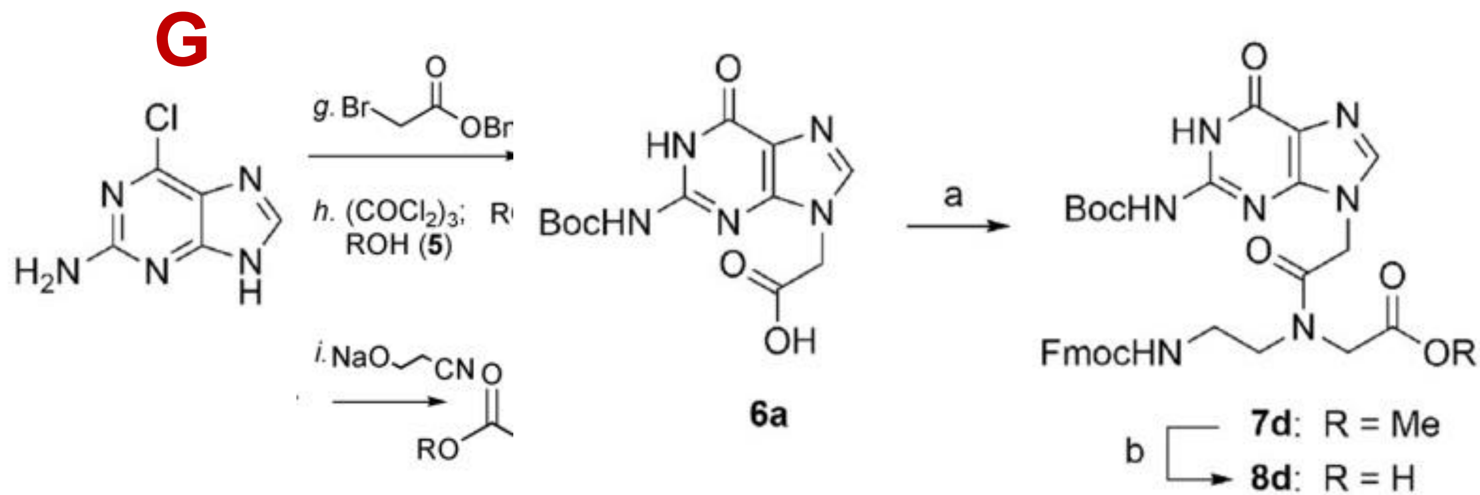


Fmoc-TGACT-NH2



Synthesis of Fmoc/Boc-protected PNA monomers
(a) **2**, TSTU, DMF, room temp., 12 h (to give **7 a**, 91 %; **7 b**, 87 %; **7 c**, 83 %; or **7 d**, 75 %);
(b) LiOH, and then Fmoc−OSu, THF−H_2O, pH 8, room temp., 3 h (to give **8 a**, 90 %; **8 b**, 92 %; **8 c**, 90 %; or **8 d**, 90 %).


A
NH2
N
N
N
N
H
a. Br OBn
O
b. CDI; ROH (5)
NHBoc
OBn
5a
c. LiOH
OH
5b
a
FmocHN
OR
7c: R = Me
b
8c: R = H


G
Cl
H2N
g. Br OBn
h. (COCl2)3; ROH (5)
i. NaO CN
RO
HN
BocHN
OH
6a
a
FmocHN
OR
7d: R = Me
b
8d: R = H

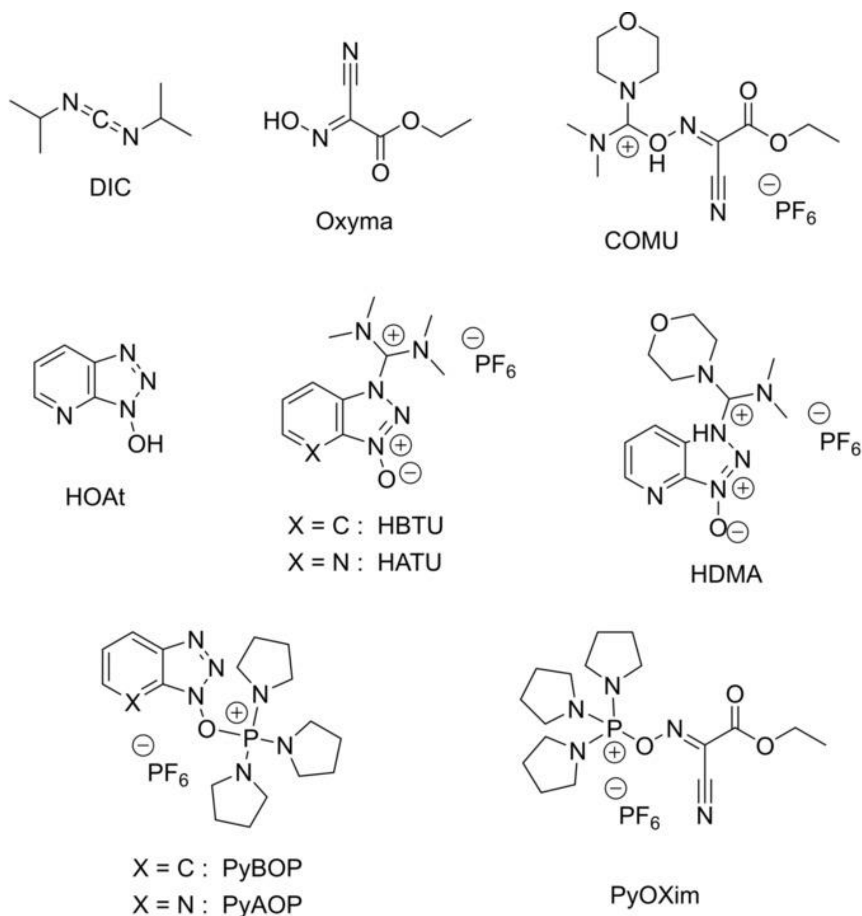
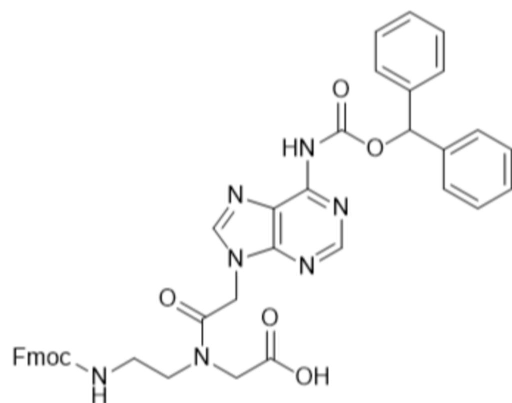


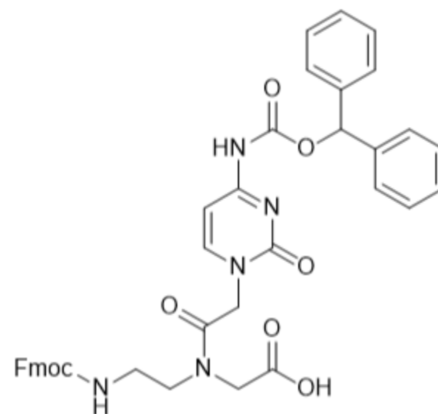
Table 1. Screening of coupling conditions during SPS of FmocHN–TGCAT–NH_2 (entries 1–17) and FmocHN–CTCATACTCT–NH_2 (entries 18–20).

Entry	Activator	Base	HPLC Purity [260 nm][a]
	5.0 eq Monomer, 5.0 eq Activator, 5.0 eq Base, 30 min, RT		
1	DIC/Oxyma		53.3
2	DIC/HOAt		50.5
3	HBTU	DIPEA	72.9
4	HATU	DIPEA	65.9
5	PyBOP	DIPEA	64.9
6	PyAOP	DIPEA	76.0
7	PyOxim	DIPEA	79.8
8	COMU	DIPEA	63.0
9	HDMA	DIPEA	72.7
	5.0 eq Monomer, 5.0 eq Activator, 5.0 eq Base, 15 min, RT		
10	HBTU	DIPEA	74.2
11	HATU	DIPEA	68.6
12	PyOxim	DIPEA	80.6
13	HDMA	DIPEA	69.3
	4.0 eq Monomer, 4.0 eq Activator, 4.0 eq Base, 10 min, RT		
14	HBTU	DIPEA	72.8
15	HATU	DIPEA	68.6
16	PyOxim	DIPEA	80.6
17	HDMA	DIPEA	70.3
	4.0 eq Monomer, 4.0 eq Activator, 4.0 eq Base, 10 min, RT		
18	HATU	DIPEA	33.8 (14%)[b]
19	PyOxim	DIPEA	67.5 (54%)[b]
20	PyOxim at 40 °C	DIPEA	59.3

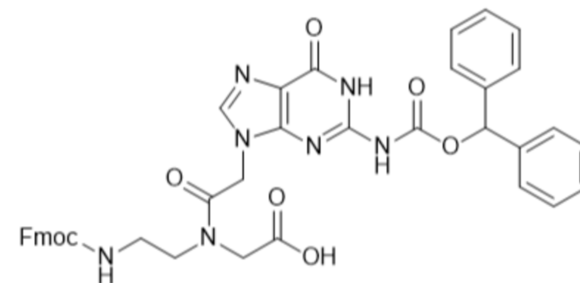
[a] HPLC-based purity of crude PNA products measured at 260 nm (close to absorption max. of both PNA and Fmoc). [b] Isolated yield after purification by preparative HPLC.



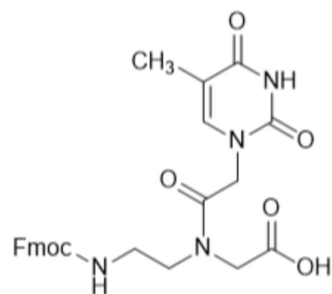
Fmoc-PNA-A(Bhoc)-OH
[D5931]



Fmoc-PNA-C(Bhoc)-OH
[D5932]



Fmoc-PNA-G(Bhoc)-OH
[D5933]



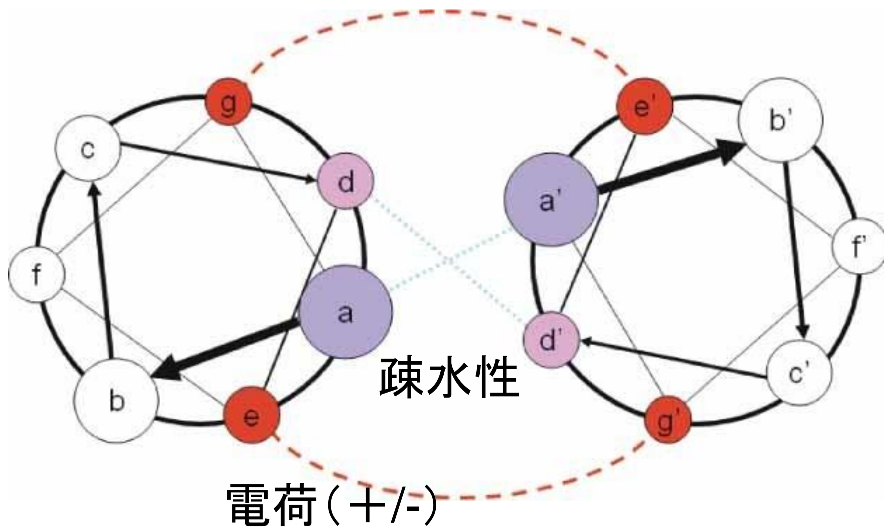
Fmoc-PNA-T-OH
[D5934]

Coil-Coil

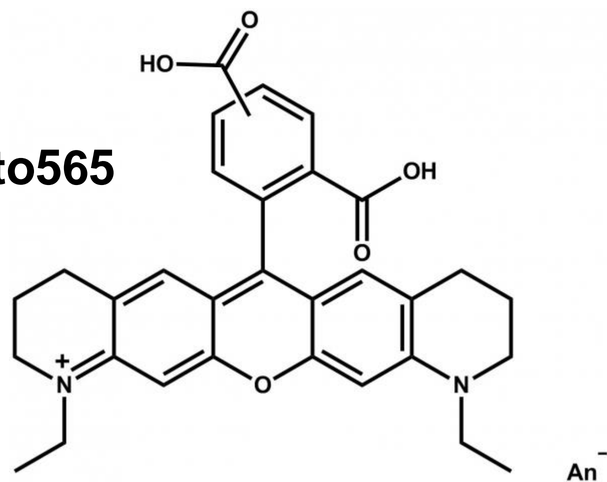
Table 1. Sequences of designed orthogonal peptides which form parallel coiled-coils P1-P2, P3-P4, P5-P6 and P7-P8

	Sequence[a]						Hydrophobic pattern at positions **a**[b]	Electrostatic pattern of heptads[c]
	SPED	gabc**L**ef	gabc**L**ef	gabc**L**ef	gabc**L**e**Y**	**G**		
P1	SPED	EIQALEE	ENAQLEQ	ENAALEE	EIAQLEY	G	I N N I	EEEE
P2	SPED	KIAQLKE	KNAALKE	KNQQLKE	KIQALKY	G	I N N I	KKKK
P3	SPED	EIQQLEE	EIAQLEQ	KNAALKE	KNQALKY	G	I I N N	EEKK
P4	SPED	KIAQLKQ	KIQALKQ	ENQQLEE	ENAALEY	G	I I N N	KKEE
P5	SPED	ENAALEE	KIAQLKQ	KNAALKE	EIQALEY	G	N I N I	EKKE
P6	SPED	KNAALKE	EIQALEE	ENQALEE	KIAQLKY	G	N I N I	KEEK
P7	SPED	EIQALEE	KNAQLKQ	EIAALEE	KNQALKY	G	I N I N	EKEK
P8	SPED	KIAQLKE	ENQQLEQ	KIQALKE	ENAALEY	G	I N I N	KEKE

[a] The sequences are written in the one-letter amino acid code. Asp residues (N) at positions **a** are bolded and underlined. Charged residues of glutamic acid (E) and lysine (K) are bolded.
[b] N residue is inserted twice in peptide, at the equivalent **a** positions of the same peptide pair.
[c] At positions **g** and **e** of each heptad repeat either acidic E or basic K is inserted.



https://onlinelibrary.wiley.com/doi/10.1002/psc.1331



Excitation 545～575 nm

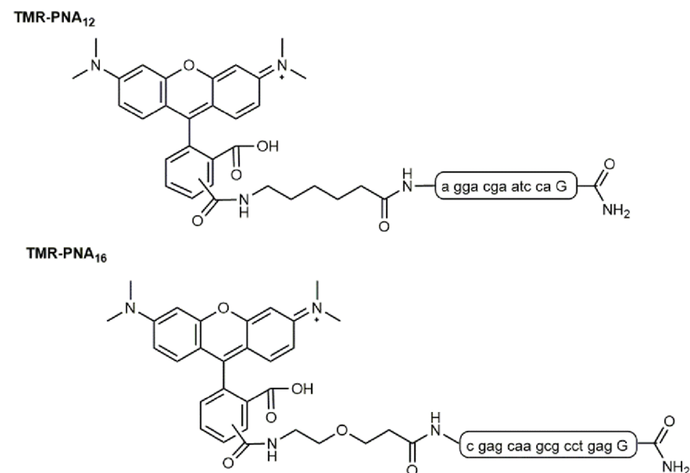
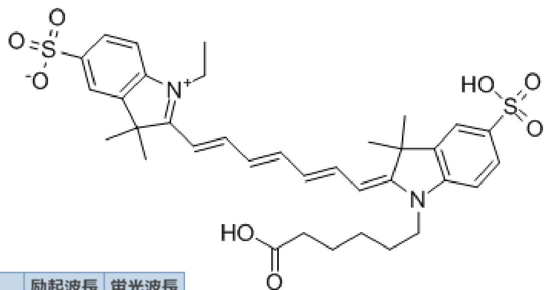


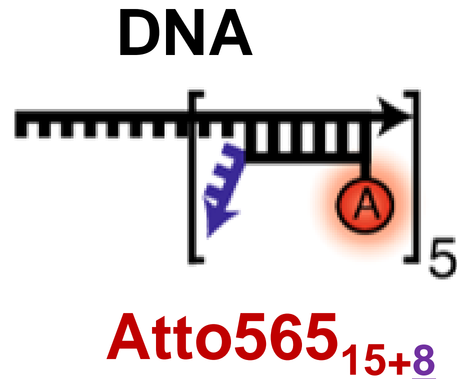
Figure 6-1 **Stuctures of 5,6 -Carboxytetramethylrhodamine (TMR) conjugated PNA used for hybridisation with PNA-tagged Cys-E3-EGFR-eGFP** in Figure 1 of the main article (TAMRA-PNA_{12}) and Supplementary Chapter 9.1.3 (TAMRA-PNA_{16}).

Cy7



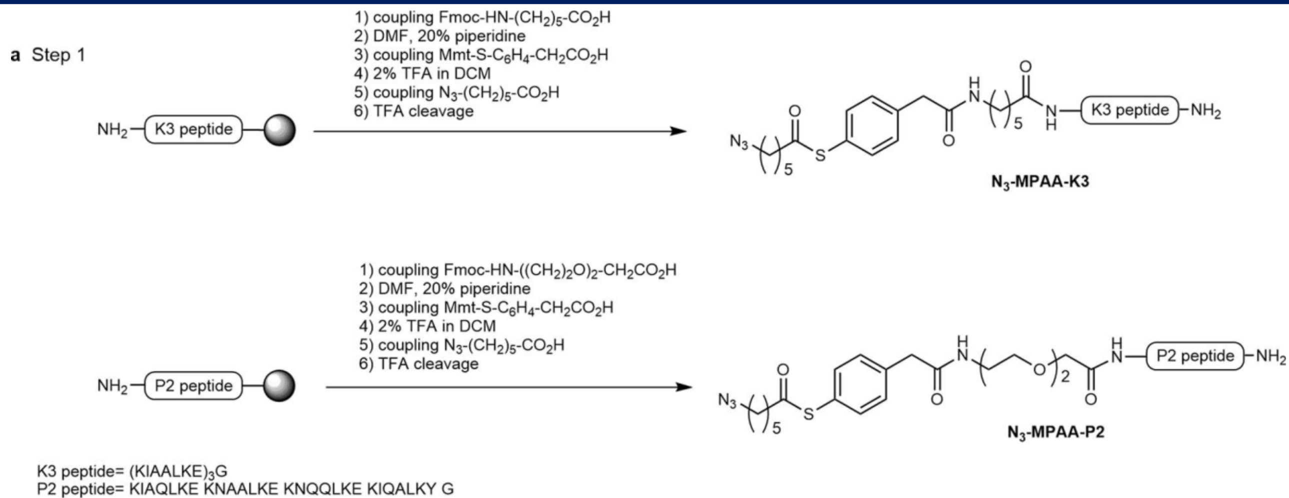
品名	励起波長	蛍光波長
Cy3 carboxylic acid	555 nm	570 nm
Cy5 carboxylic acid	646 nm	662 nm
Cy5.5 carboxylic acid	673 nm	707 nm
Cy7 carboxylic acid	750 nm	788 nm
Cy7.5 carboxylic acid	788 nm	808 nm
Sulfo-Cy5_carboxylic_acid	646 nm	662 nm

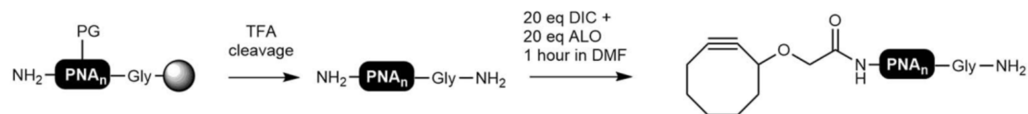
DNA comlex

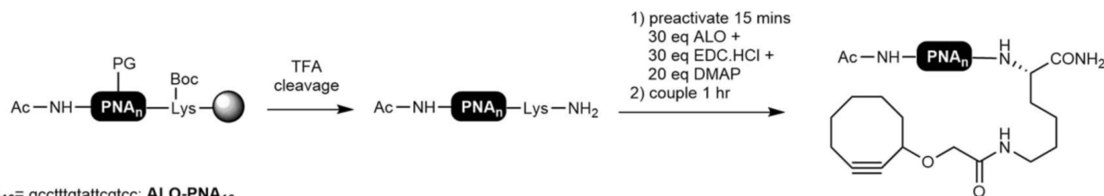


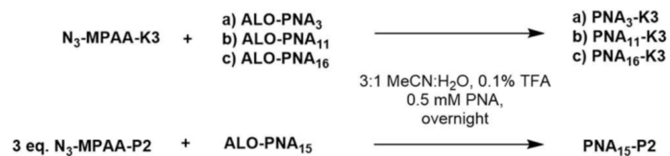
Atto565$_{15+8}$

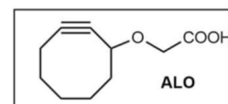
DNA complexes were hybridized by premixing to a final complex concentration of 10 μM in Hank's balanced salt solution (HBSS), heating to 70° C and cooling to room temperature over 30 min.


a Step 1
1) coupling Fmoc-HN-$(CH_2)_5$-CO_2H
2) DMF, 20% piperidine
3) coupling Mmt-S-C_6H_4-CH_2CO_2H
4) 2% TFA in DCM
5) coupling N_3-$(CH_2)_5$-CO_2H
6) TFA cleavage
NH_2 K3 peptide
N_3-MPAA-K3
1) coupling Fmoc-HN-$((CH_2)_2O)_2$-CH_2CO_2H
2) DMF, 20% piperidine
3) coupling Mmt-S-C_6H_4-CH_2CO_2H
4) 2% TFA in DCM
5) coupling N_3-$(CH_2)_5$-CO_2H
6) TFA cleavage
NH_2 P2 peptide
N_3-MPAA-P2
K3 peptide= $(KIAALKE)_3G$
P2 peptide= KIAQLKE KNAALKE KNQQLKE KIQALKY G

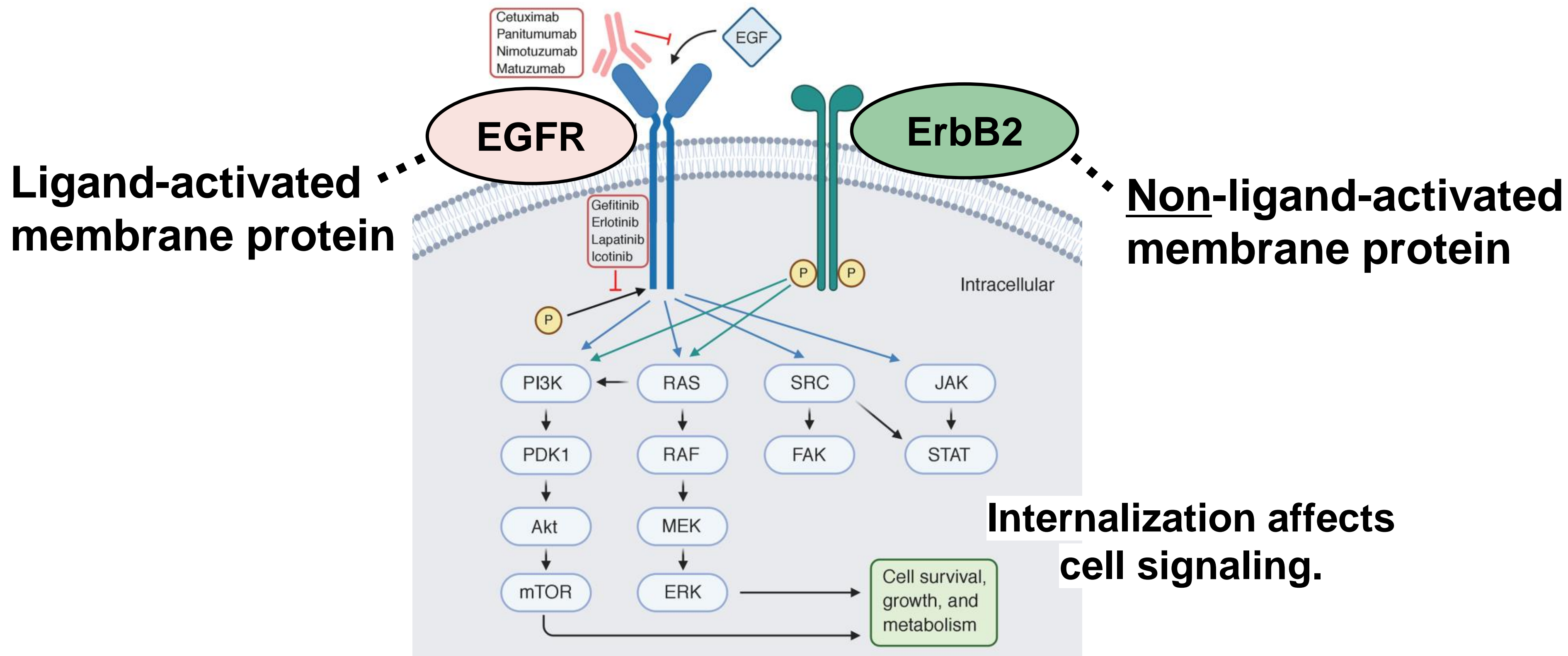

b Step 2
PG
NH_2 PNA_n Gly
TFA cleavage
NH_2 PNA_n Gly NH_2
20 eq DIC +
20 eq ALO
1 hour in DMF
PNA_3= ttt; ALO-PNA_3
PNA_{11}= tgtattcgtcc; ALO-PNA_{11}


PG
Boc
Ac NH PNA_n Lys
TFA cleavage
Ac NH PNA_n Lys NH_2
1) preactivate 15 mins
30 eq ALO +
30 eq EDC.HCl +
20 eq DMAP
2) couple 1 hr
$CONH_2$
PNA_{16}= gcctttgtattcgtcc; ALO-PNA_{16}
PNA_{15}= DDRgactctggatgacgcR; ALO-PNA_{15}


c Step 3
N_3-MPAA-K3 +
a) ALO-PNA_3
b) ALO-PNA_{11}
c) ALO-PNA_{16}
a) PNA_3-K3
b) PNA_{11}-K3
c) PNA_{16}-K3
3:1 MeCN:H_2O, 0.1% TFA
0.5 mM PNA,
overnight
3 eq. N_3-MPAA-P2 + ALO-PNA_{15}
PNA_{15}-P2


COOH
ALO

PNA application



Georgina, C. G, et al. *RSC Chem. Biol.*, **2021**, 2, 1291

Frontiers | Targeting EGFR in Esophagogastric Cancer (frontiersin.org)

PNA application

GA (Geldanamycin) :
Hsp90 inhibitor
Destabilize protein

Ligand stimulation
Promotes internalization

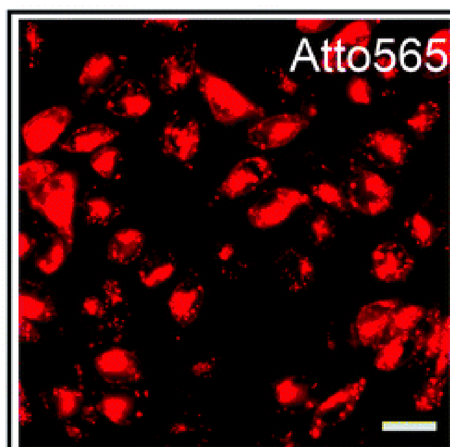
(-) 20 min

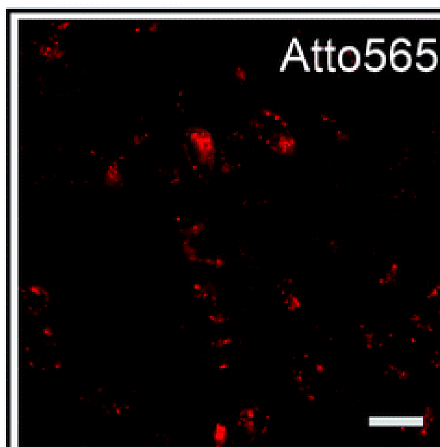
EGFR

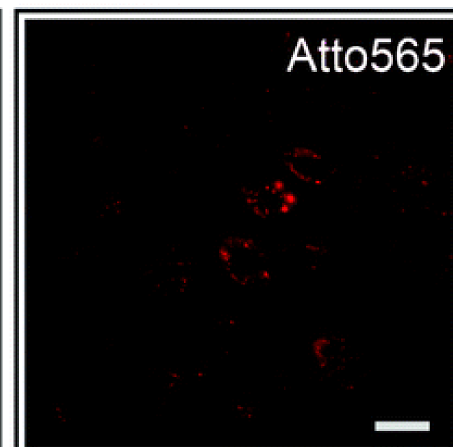
Ligand-activated membrane protein

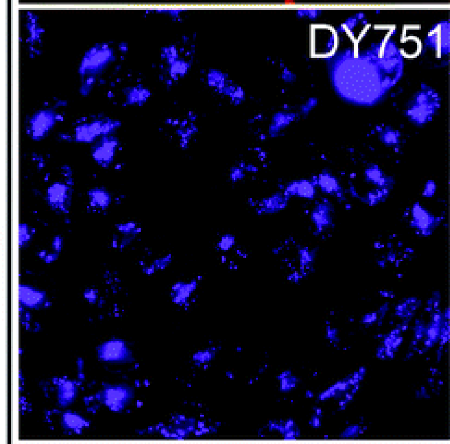
ErbB2

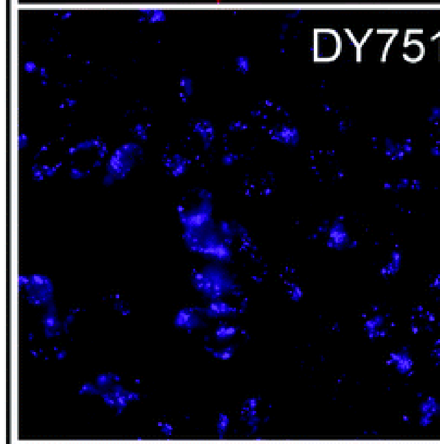
Non-ligand-activated membrane protein

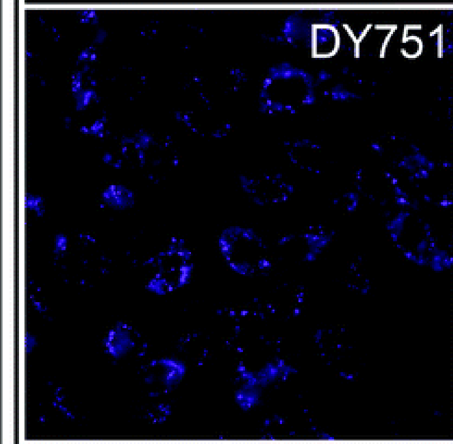












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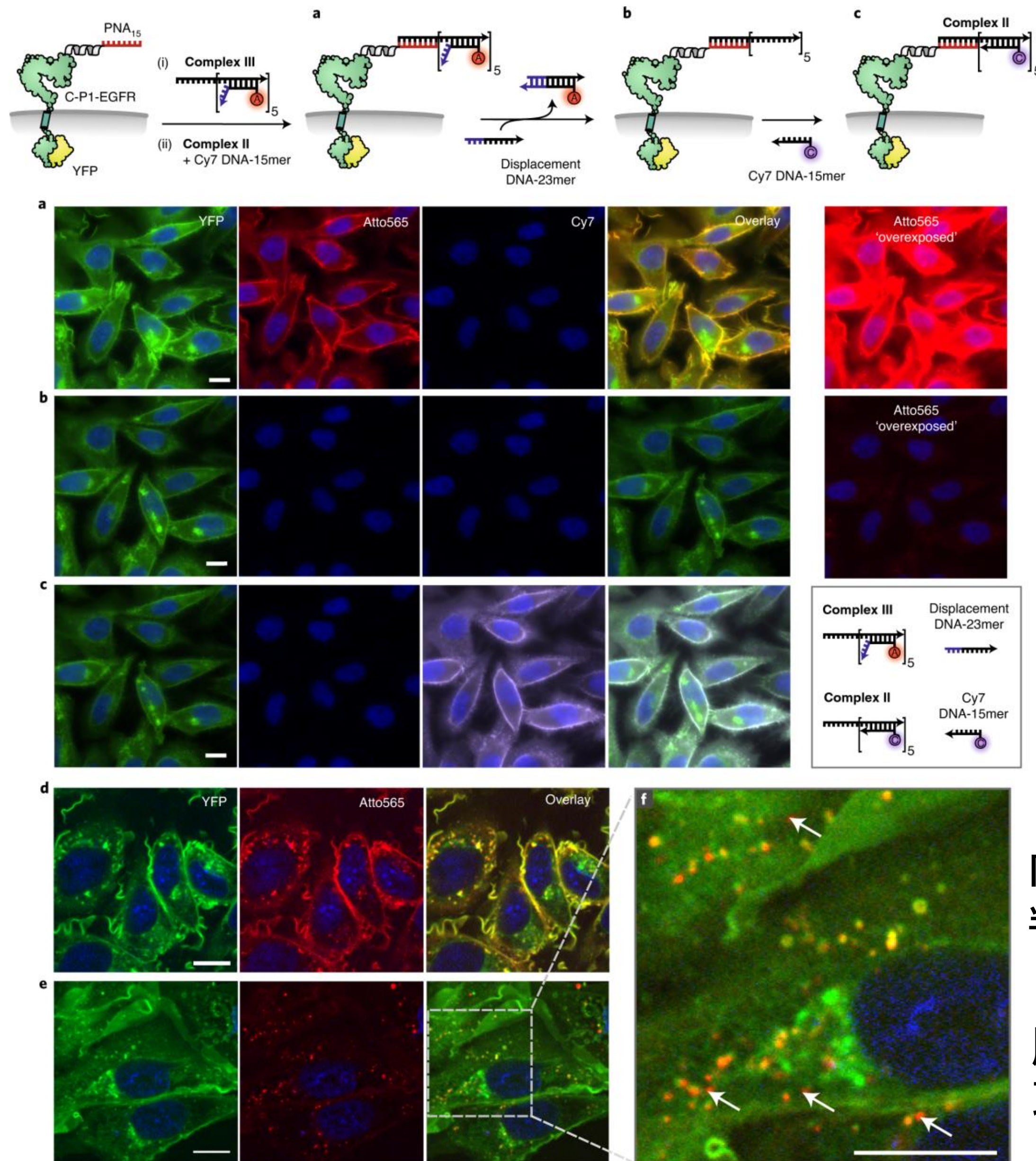


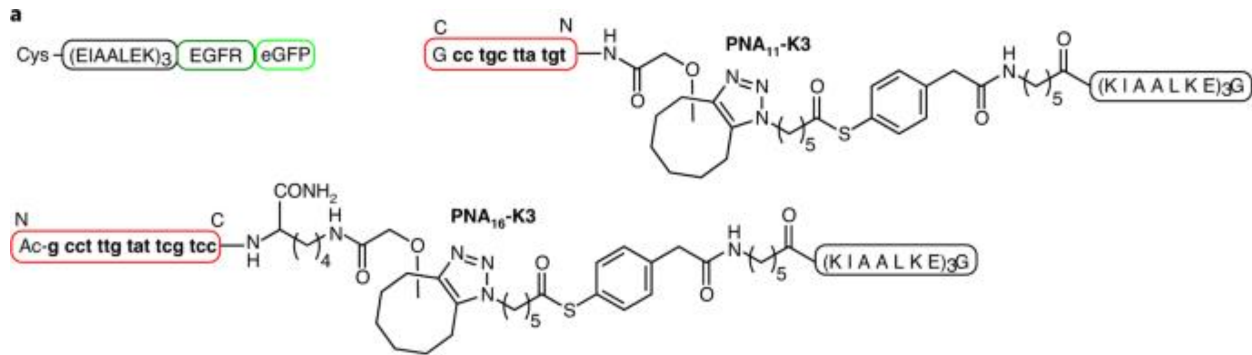
EGF刺激

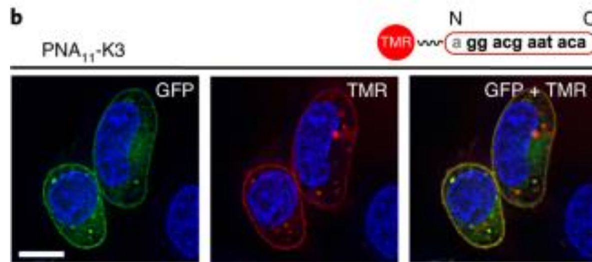
Elimination

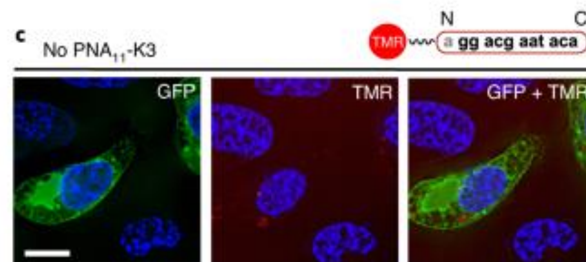
内在化と膜残留の
判別ができない

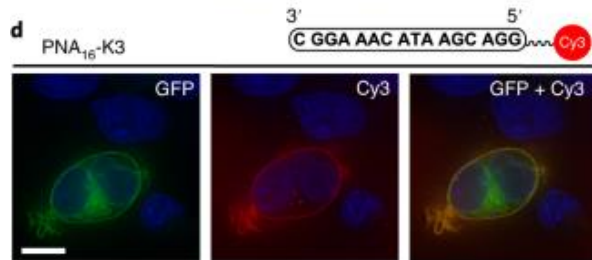
膜上に残ったものが
取り除かれた

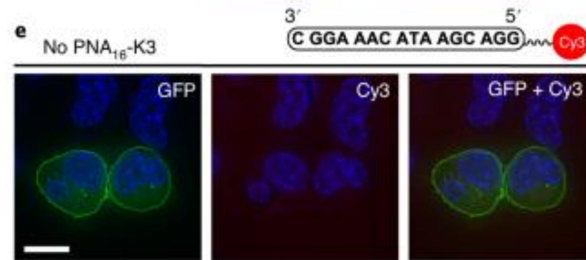
PNA11mer, TMRでの検出

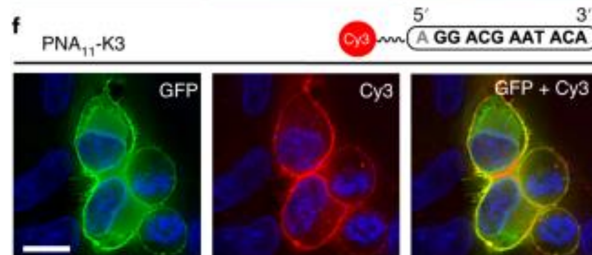


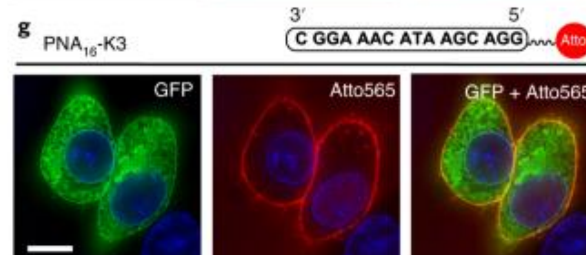




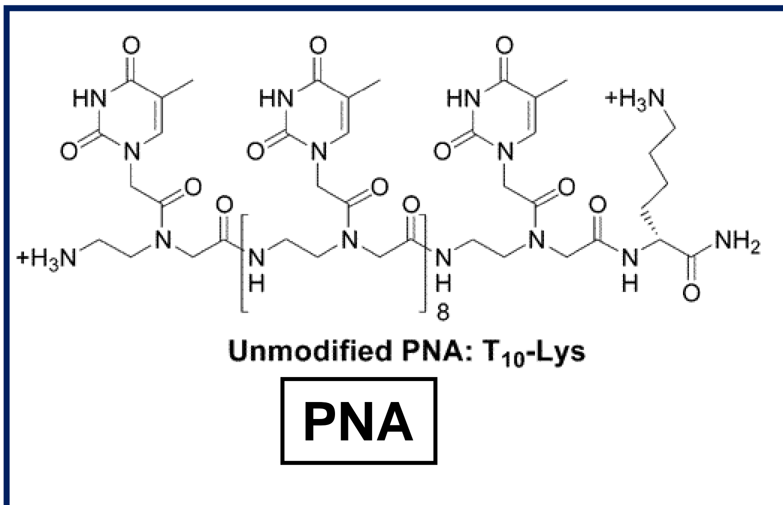


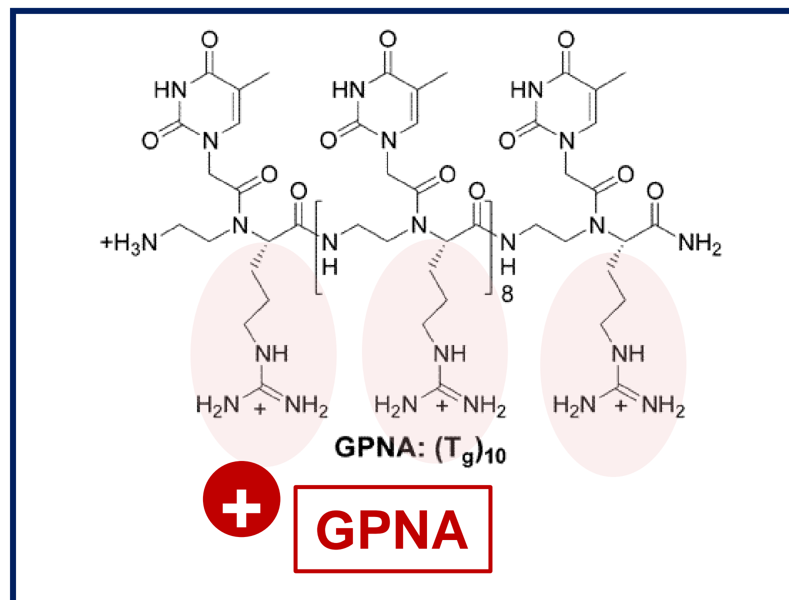




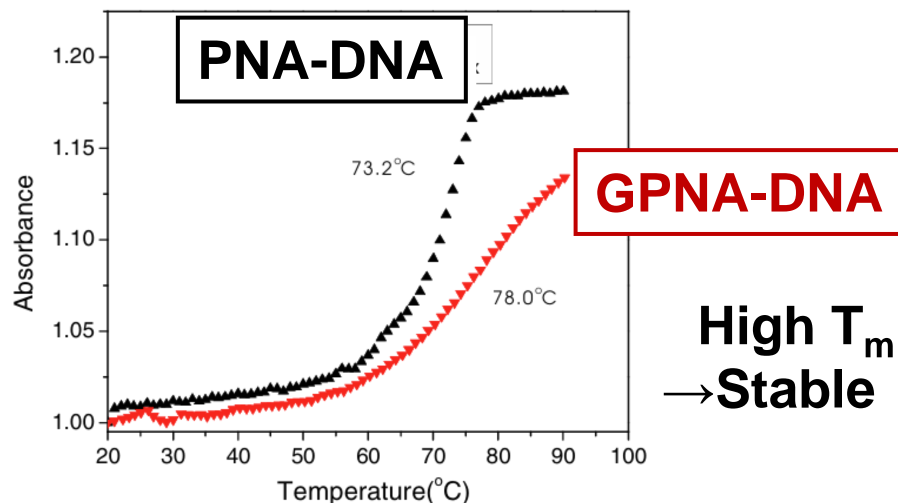


Improvement of PNA cell membrane permeability





Tm (℃) = Melting temperature

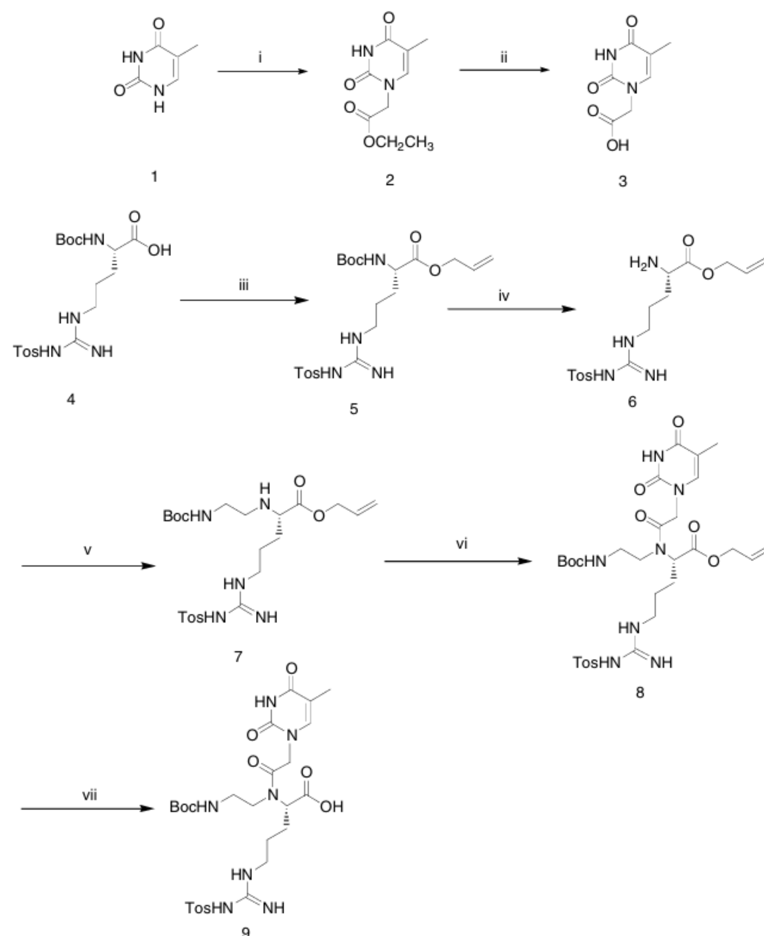


High T_m
→Stable

Figure S1. Melting curves of GPNA-DNA duplex and PNA2-DNA triplex. The strand concentrations of DNA, PNA, and GPNA were 2μM, 4μM, and 2μM, respectively. Buffer contained 10mM NaPi (pH 7.0) and 20mM NaCl. The samples were first pre-annealed by incubating at 90Û&IRU PLQ IROORZHGE¥ gradual-cooling to room temperature. UV-absorption was monitored at 260nm from 20 to 90Û&DW WKH rate of 0.5Û&SHUPLQXWH

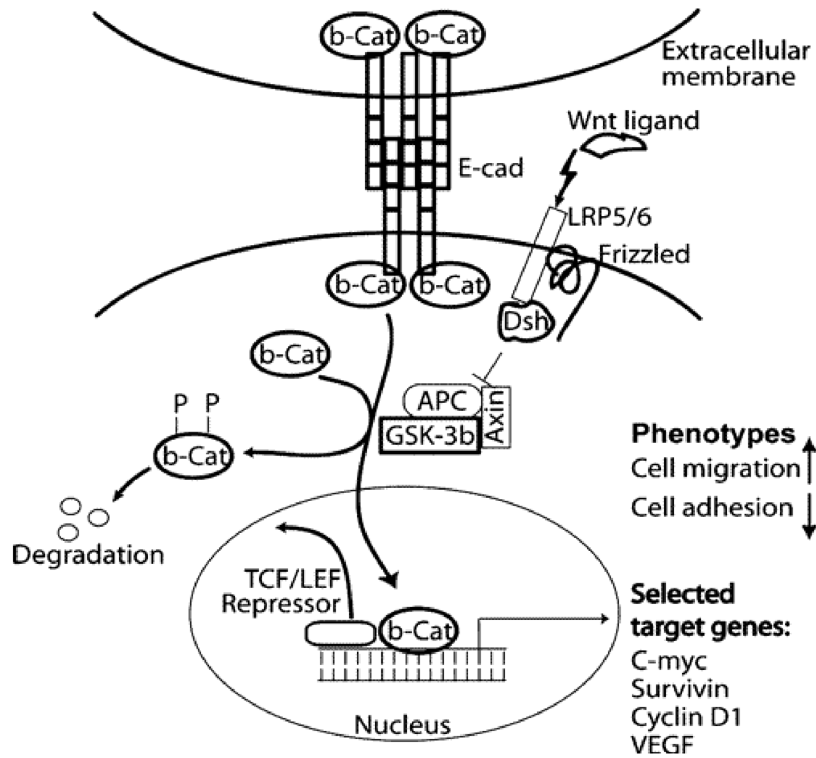
Novel Binding and Efficient Cellular Uptake of Guanidine-Based Peptide Nucleic Acids (GPNA) | Journal of the American Chemical Society (acs.or

Supporting Information



Scheme S1. Synthesis of GPNA monomers *N*-(2-Boc-aminoethyl)-N-(thymin-yl-1-acetyl)- arginine. *Reaction conditions*: (i) $BrCH_2COOCH_2CH_3$, K_2CO_3/DMF, room temperature, 24 hours; (ii) NaOH/H_2O, room temperature, 2 hours; then adjusted pH to 4 by 4M HCl at 0°C; (iii) Allyl bromide, Na_2CO_3/DMF, 35 °C, 24 hours; (iv) TFA/CH_2Cl_2, 0°C, 30min; (v) *t*-Butoxycarbonyl aminoacetaldehyde/MeOH, 0°C, 30min, then addition of $NaBH_3CN$, 16hour; (vi) Thymine-1-acetic acid **3,** DCC, DhbtOH/DMF, room temperature, 16hours; (vii) $Pd(PPh_3)_4$, morpholine/THF, room temperature, 30min.

E-Cadherin



Cell adhesion molecule

PNA structure

SH

N
H

O

Protein

PNA_{15}

gac tct gga tga cgc

Base

Base

O

O

O

O

N

N

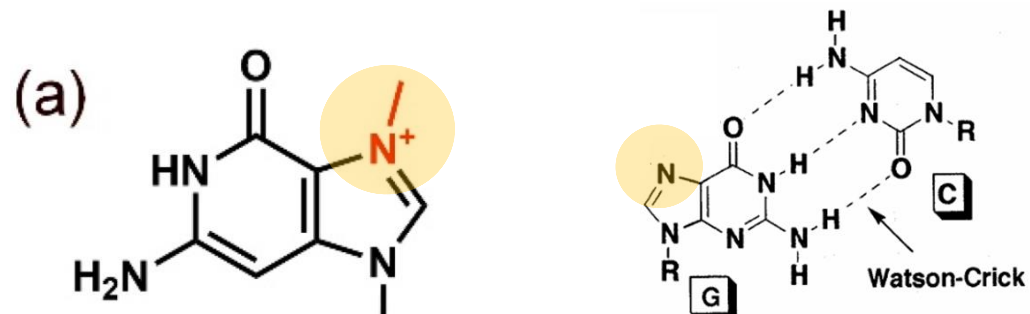
Asp Asp Arg

N
H

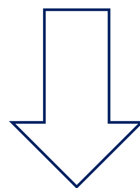
N
H

Arg Lys

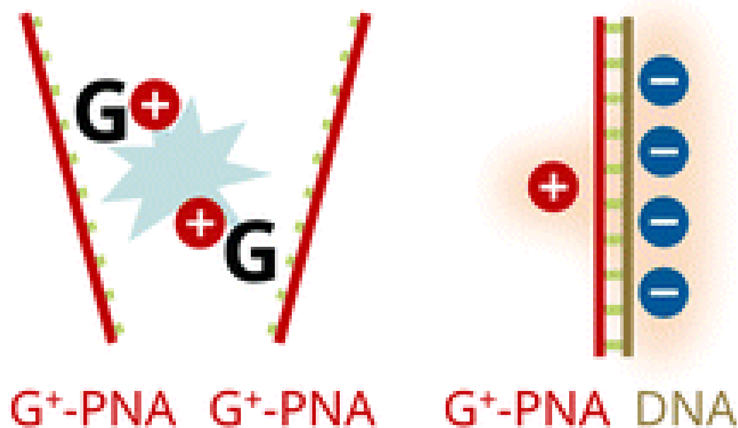
Add cationic motif



<Strategy> Guanine+ (= G +)



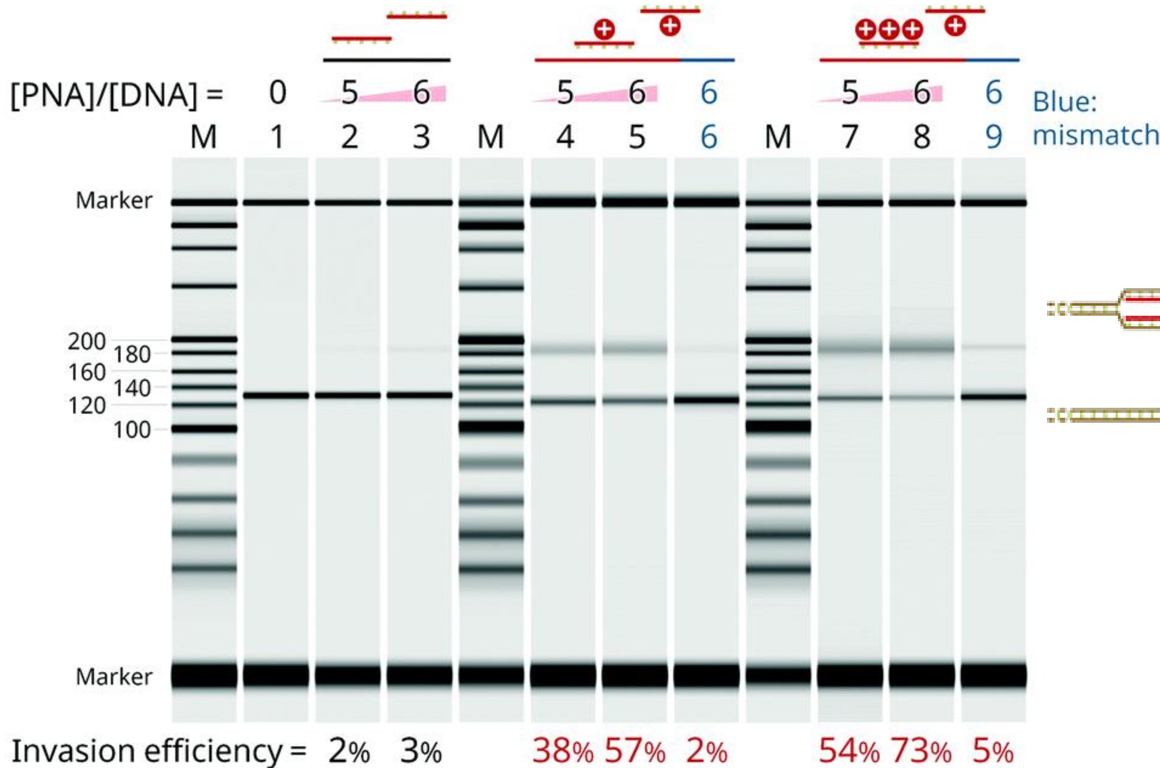
<Hypothesis> Ideal double-stand is formed?

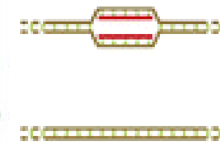


Cationic guanine: positively charged nucleobase with improved DNA affinity inhibits self-duplex formation - Chemical Communications (RSC Publishing)

Add cationic motif

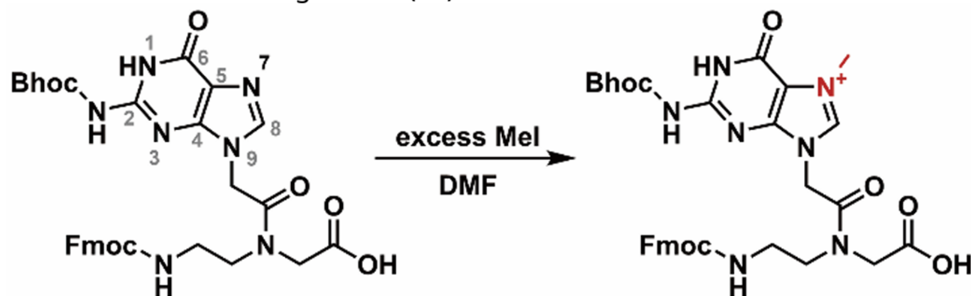
❑ Electrophoretic mobility shift assay





✔ Increased invasion efficiency.
✔ Mismatch sequences are not recognized.

Synthesis of Fmoc cationic guanine (G^+) PNA monomer



Scheme S1. Synthetic procedure of cationic guanine PNA monomer.

100 mg (135 μmol) of commercially available Fmoc-PNA(G)-OH was dissolved in 1 mL of dehydrated DMF under argon atmosphere, and 168 μL (2.70 mmol, 20 eq.) of iodomethane was added dropwisely. The reaction mixture was stirred at room temperature for 7.5 h and slowly turned yellow. After reaction, *ca*. 30 mL of diethyl ether was poured into the reaction mixture, and the solvent was kept at 4 °C overnight. The supernatant was carefully removed by decantation, and orange transparent oil was obtained. Followed by addition of 1 mL of water, yellowish-white precipitate was obtained by sonication and scratching with spatula. The precipitate was collected by centrif-ugation and washed with water and diethyl ether to give the white solid, Yield 101 mg (98%). The product was identified by ESI-TOF MS and ^{1}H NMR.

ESI-TOF MS (m/z): calcd $[M]^+$ 756.2776, found 756.3106

Table S1. Sequences of PNAs employed in this study.

Name	Sequence of 10-mer PNA (N to C)†
PNA-Fw	KGTTACTGATGKK
PNA^+-Fw	$\text{KGTTACT}\overset{+}{\text{G}}\text{ATGKK}$
PNA^{3+}-Fw	$\text{K}\overset{+}{\text{G}}\text{TTACT}\overset{+}{\text{G}}\text{AT}\overset{+}{\text{G}}\text{KK}$
pcPNA-Fw	$\text{KGU}_s\text{U}_s\text{DCU}_s\text{GDU}_s\text{GKK}$
$pcPNA^+$-Fw	$\text{KGU}_s\text{U}_s\text{DCU}_s\overset{+}{\text{G}}\text{DU}_s\text{GKK}$
$pcPNA^{3+}$-Fw	$\text{K}\overset{+}{\text{G}}\text{U}_s\text{U}_s\text{DCU}_s\overset{+}{\text{G}}\text{DU}_s\overset{+}{\text{G}}\text{KK}$
PNA-Rev	KCATCAGTAACKK
PNA^+-Rev	$\text{KCATCA}\overset{+}{\text{G}}\text{TAACKK}$
pcPNA-Rev	$\text{KCDU}_s\text{CDGU}_s\text{DDCKK}$
$pcPNA^+$-Rev	$\text{KCDU}_s\text{CD}\overset{+}{\text{G}}\text{U}_s\text{DDCKK}$

† K = lysine; G^+ = cationic guanine; D = 2,6-diaminopurine; U_s = 2-thiouracil.

Cationic guanine: positively charged nucleobase with improved DNA affinity inhibits self-duplex formation - Chemical Communications (RSC Publishing)

How PNA works?

❑ dsDNA

PNA
PNA'

Base
Base
O
O
O
O
N
N
N
H
N
H

Application

- Gene editing
- DNA labeling

❑ ssDNA / mRNA

PNA

Application

- Regulation of transcription and translation