Lasso Grafting

Literature #1

2023/1/26

B4 寺田周平

Contents

Macrocyclic peptides

表1　医薬品群の比較

	低分子医薬品	中分子医薬品	高分子医薬品
形態	有機小分子	特殊環状ペプチド	抗体
分子量	500以下	1000～3000	150,000以上
標的特異性	低い	高い	きわめて高い
毒性・副作用	多い	少ない	少ない
経口投与	可能	一部可能	不可能
細胞膜透過性	高い	低い～高い	なし
製造コスト	低い	低い～普通	高い
免疫原性	低い	低い	普通～高い
標的結合面積	狭い	普通～広い	広い
PPI* 阻害	一部可能	可能	可能
生体内安定性	低い～普通	普通～高い	きわめて高い

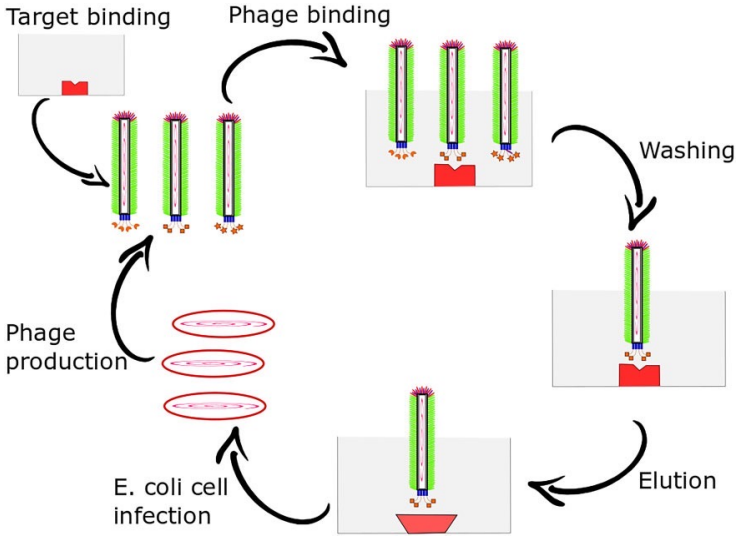
*PPI：タンパク質間相互作用.

- ✓Small molecules and macromolecules may have some problems for pharmaceutical use.
- ✓Macrocyclic peptides has stability in vivo by virtue of its cyclic structure and noncanonical amino acid they composed of.

Miura, T., *et al. Seikagaku*. **2021** *93*(3), 349-358.

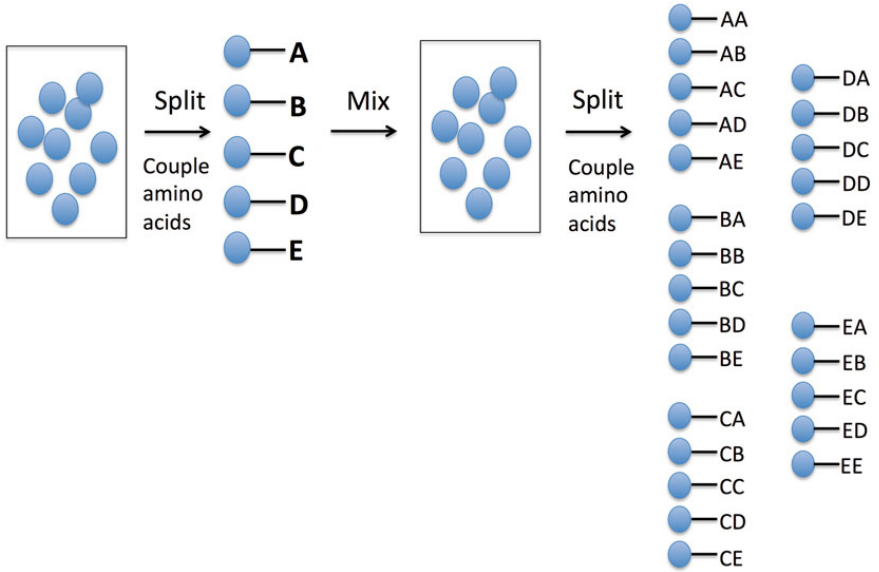
The platforms for selecting peptide binding drug targets

Phage display



- In vivo translational synthesis
  Small library size (~10^9)
  Toxic peptides cannot be handled because of the use of E. coli.

OBOC (one-bead-one-compound) display



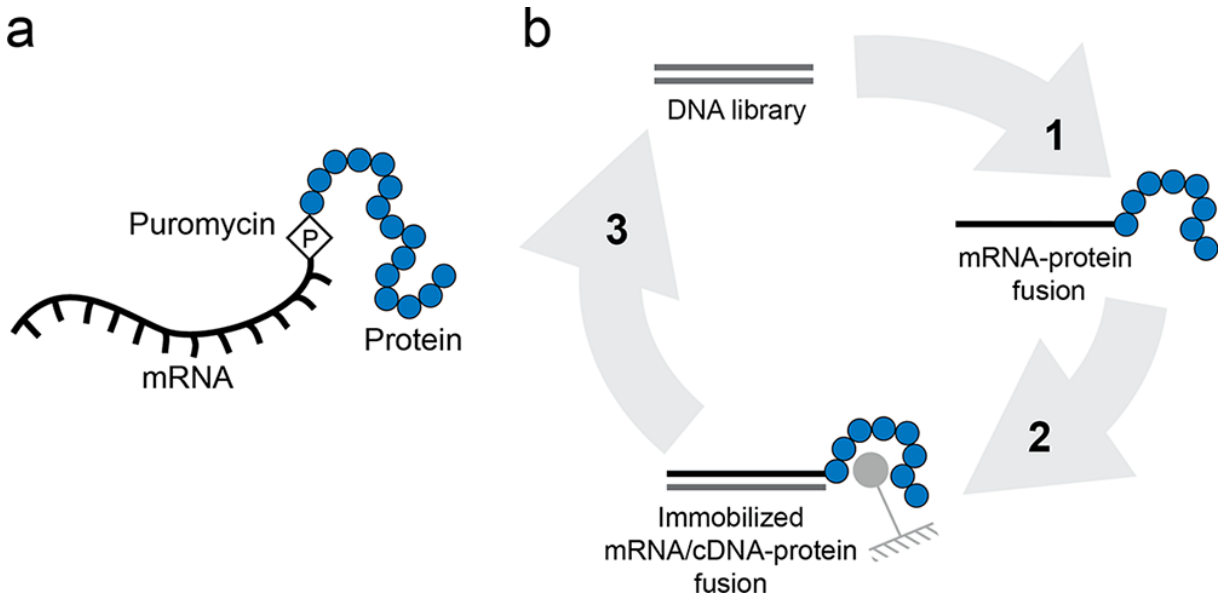
- Chemical synthesis
  Small library size(~10^7)

➢ Simplicity of operation and the size of the library that can be created are important.

Zambrano-Mila, M. S. et al, *Ther. Innov. Regul. Sci.*, *54*(2), 308-317. (2020)

Bononi, F. C., *et al. Peptide Libraries.* **2015**, 223-237.

mRNA display

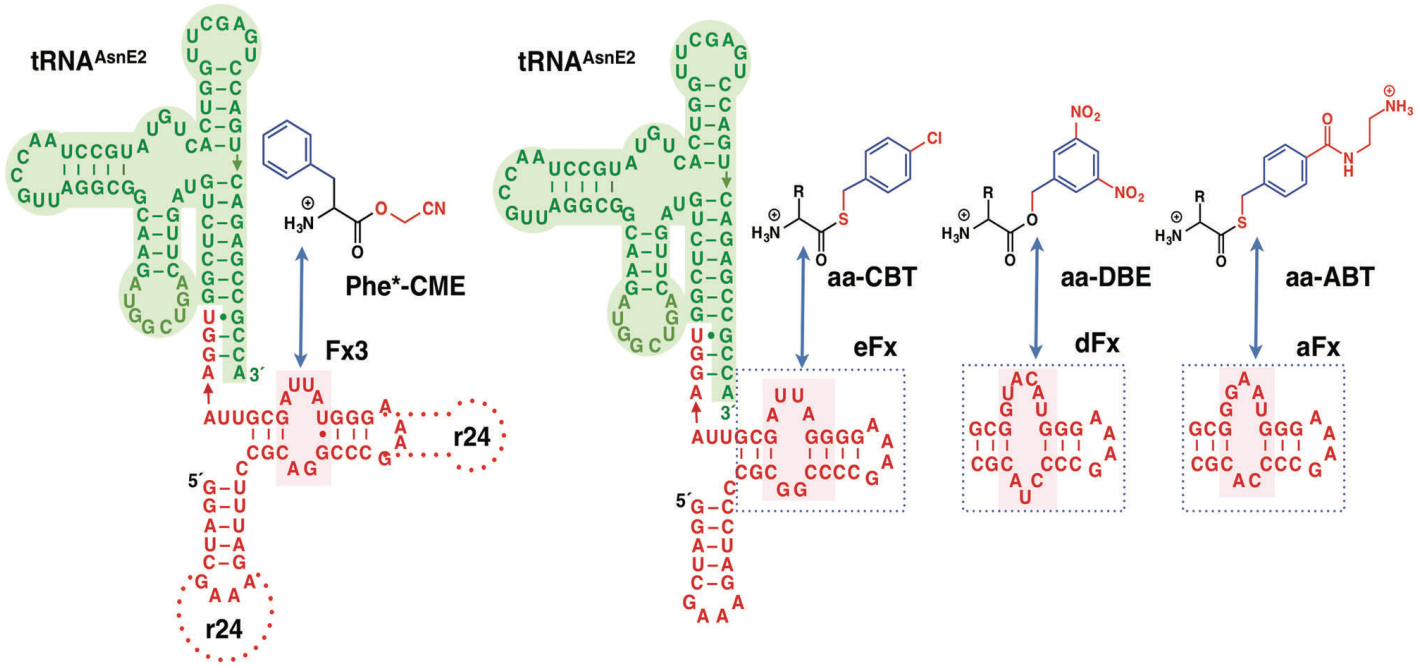


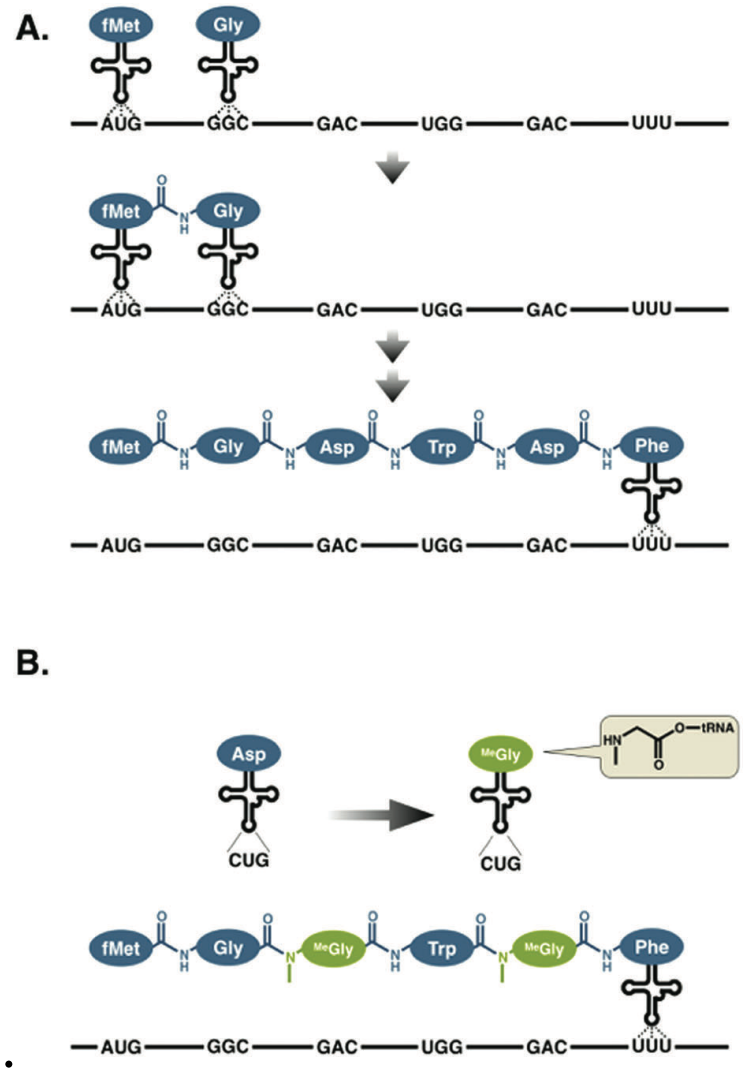
Procedure

1. A DNA library is transcribed → mRNA modified with puromycin → Translated to generate the mRNA–protein fusion.
2. The fusions undergo an experiment-specific selection step.
3. Reverse transcribed into cDNA → Amplified via PCR → Next cycle

Newton, M. S., *et al. ACS synthetic biology*, *9*(2), **2019**, 181-190.

Flexible In vitro Translation (FIT) and genetic code reprogramming



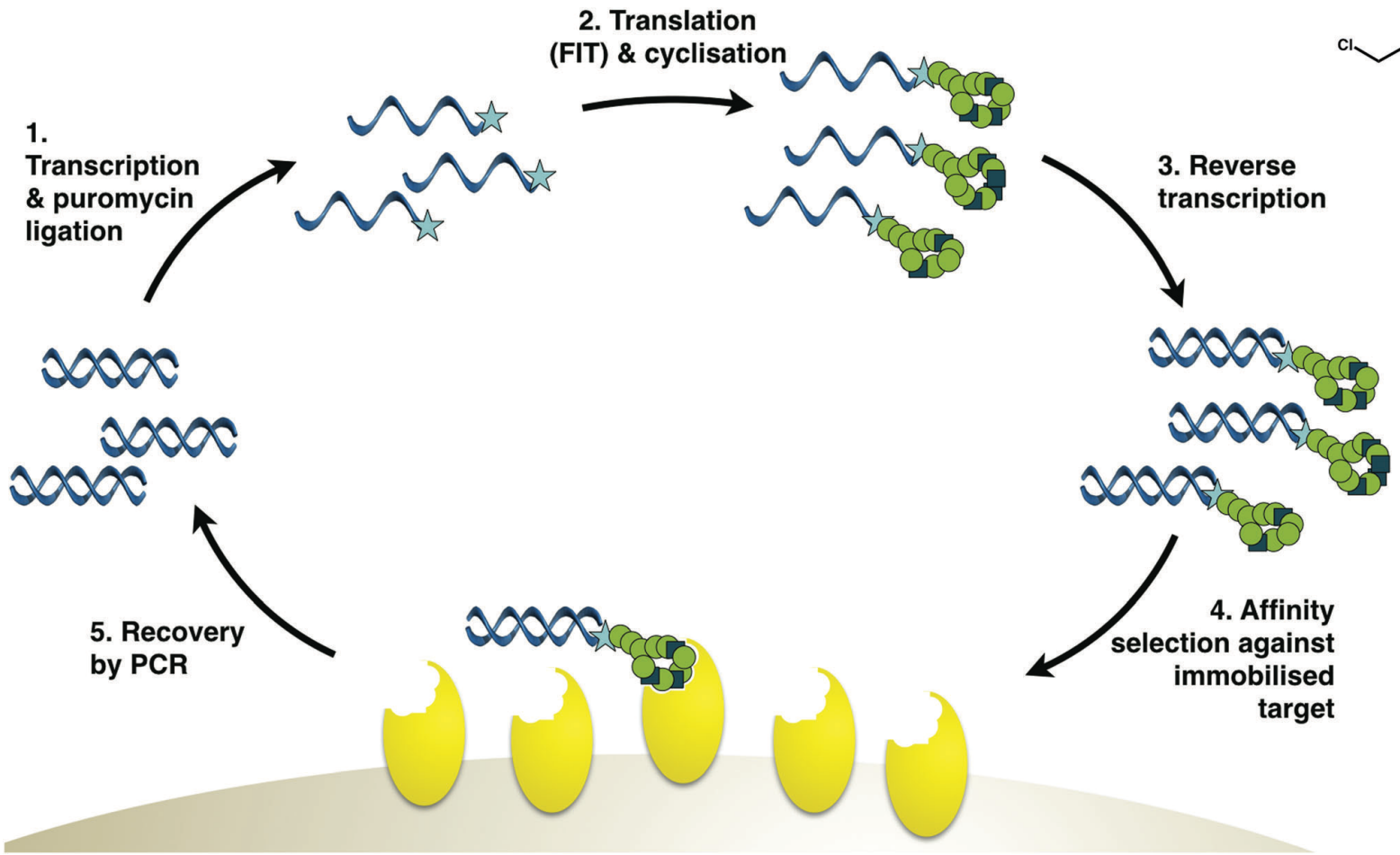


- ✓Flexizyme can incorporate non-canonical amino acids into tRNA.
- ✓The reconstituted translation system assigns non-normal amino acid-tRNA complexes to codons, allowing for the synthesis of non-natural peptides.

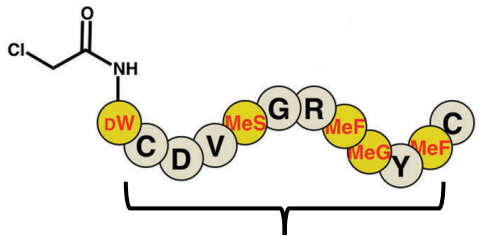
Passioura, T., *et al*. *Chem. Commun.*, **2017**, *53*(12), 1931-1940.

RaPID system

RaPID : Random non-standard Peptide Integrated Discovery



e.g.)

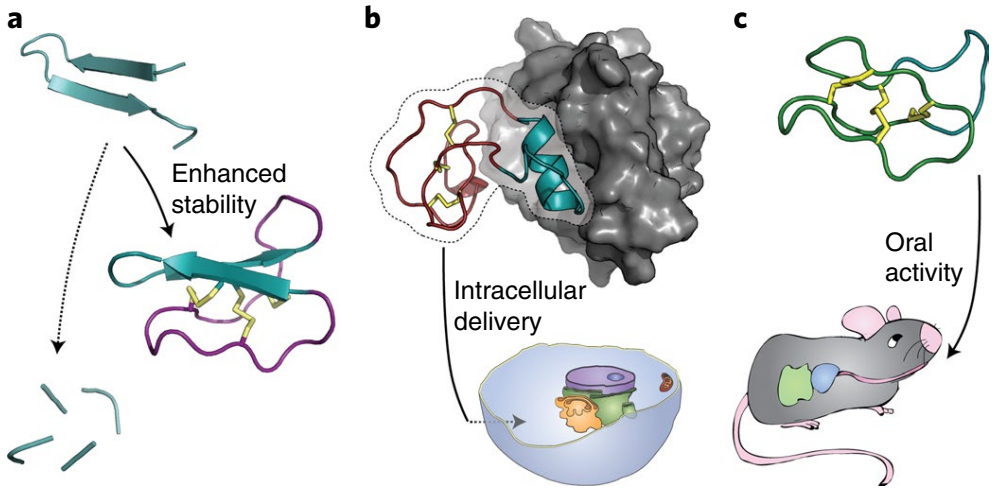


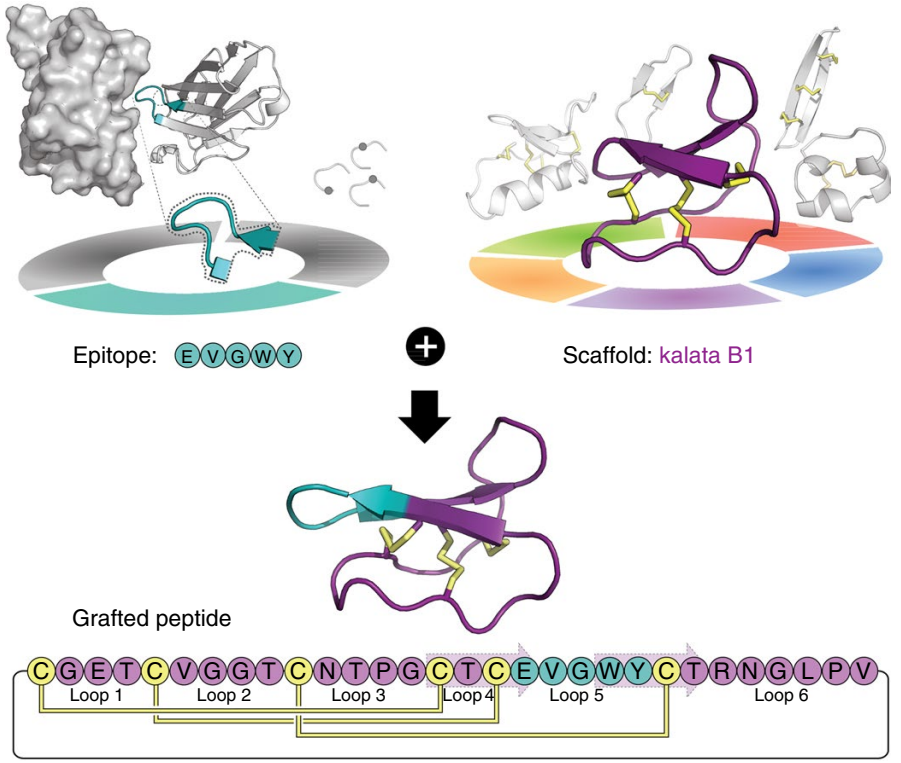
Random sequence

✓ RaPID system makes it possible to find out target-selective cyclic peptide.

Passioura, T., *et al*. *Chem. Commun.*, **2017**, *53*(12), 1931-1940.

Peptide grafting approach





- ✓ Peptide grafting can create new functional molecules that combine the functions of peptides and scaffold proteins.
- ✓ Grafting synthetic de novo identified peptides into an independently folded domain of a natural scaffold would often result in the misfolding and inactivation of both entities.

Wang, C. K., *et al. Nat. Chem. Biol.* **2018**, *14*(5), 417-427.

Macrocyclic peptides discovered by RaPID

Peptide name	Binding protein (target)	Original RaPID peptide	Sequence used for grafting
PB1m6[1]	PlxnB1	Ac-wRPRVARWTGQIIYC	WRPRVARWTGQIIY
PB1m6A9[2]	PlxnB1	Ac-wRPYIERWTGRLIVC	WRPYIERWTGRLIV
PB1m7[1]	PlxnB1	Ac-wNSNVLSWQTYSWYC	(C)NSNVLSWQTYSWY(C)
aMD4[3]	MET	Ac-yRQFNRRTHEVWNLDC	YRQFNRRTHEVWNLD
aMD5[3]	MET	Ac-yWYYAWDQTYKAFPC	YWYYAWDQTYKAFP

By convention, RaPID peptides are cyclized via the *N*-chloroacetyl group of the initiator amino acid and the sulfhydryl group of the terminal Cys. The small letter indicates *D*-amino acids.

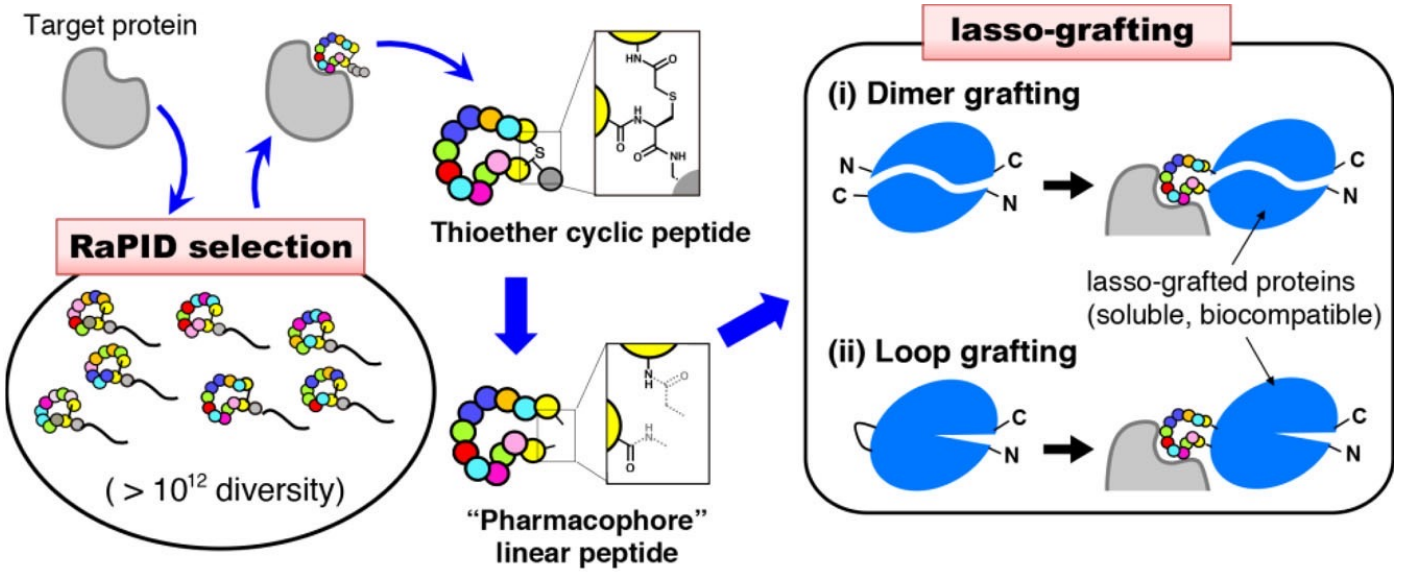
PlxnB1; transmembrane receptors for Semaphorins in neural tissues.
MET; hepatocyte growth factor receptor.

✓ From previous studies, the above binding peptides were selected.

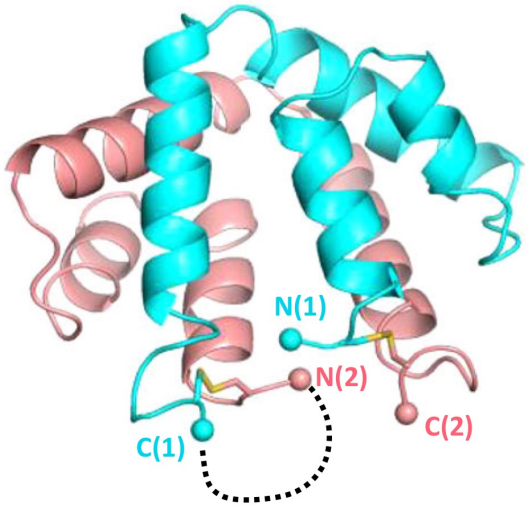
1) Matsunaga, Y., *et al. Cell Chem. Biol.*, **2016**, *23*(11), 1341-1350.
2) Bashiruddin, N. K., *et al. PNAS.* **2020**, 117(49), 31070-31077.
3) Ito, K., *et al. Nat. Commun.* **2015**, *6*(1), 6373.

Contents

LassoGraft Technology®



(i) Dimer grafting on uteroglobin (UG)



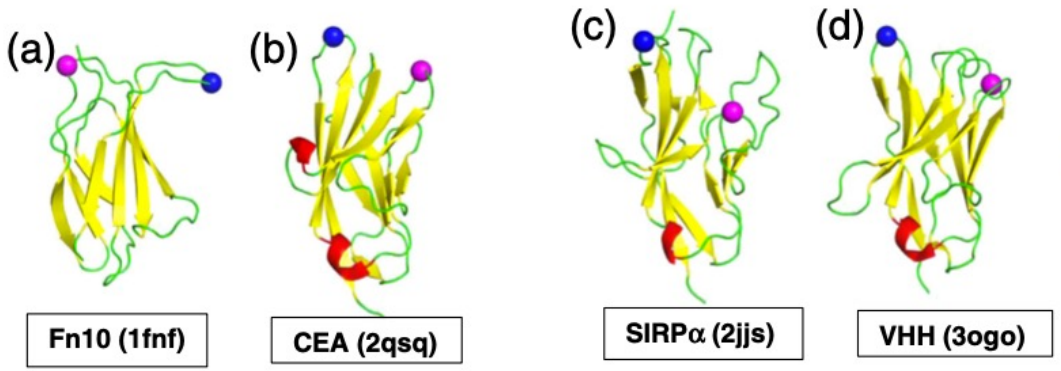
- ✓ RaPID-derived pharmacophore sequences can be readily implanted into surface-exposed loops on recombinant proteins (LassoGraft Technology®)
- ✓ Binding affinity of the parental peptide was not lost by dimer grafting.

Mihara, E., *et al. Nat. Commun.* **2021,** 12, 1543.

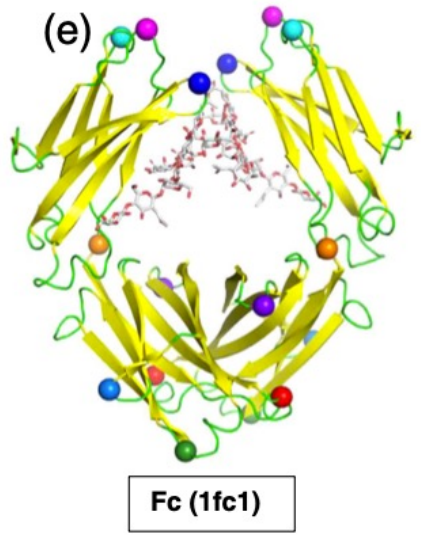
Loop grafting on various proteins

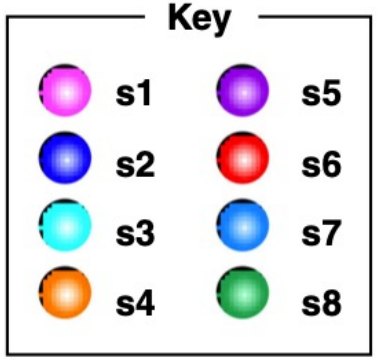
(ii) loop grafting on proteins

- β-sandwich fold

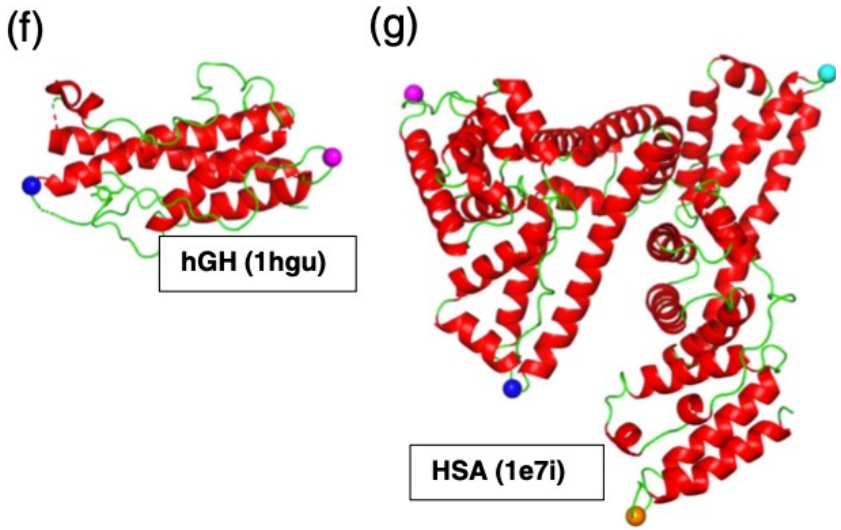


- β-hairpin

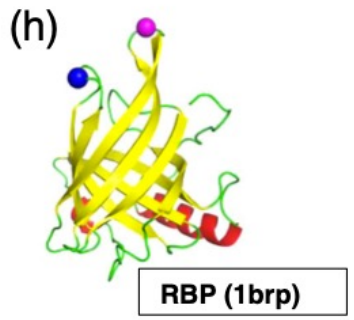




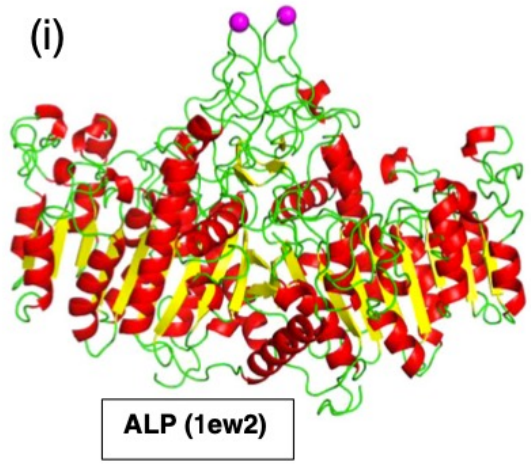
- all-α



- β-barrel

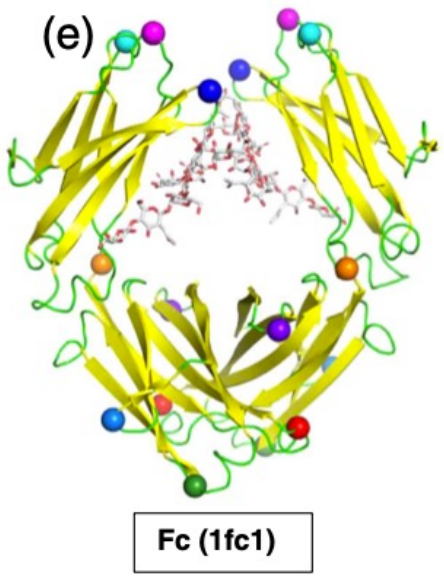


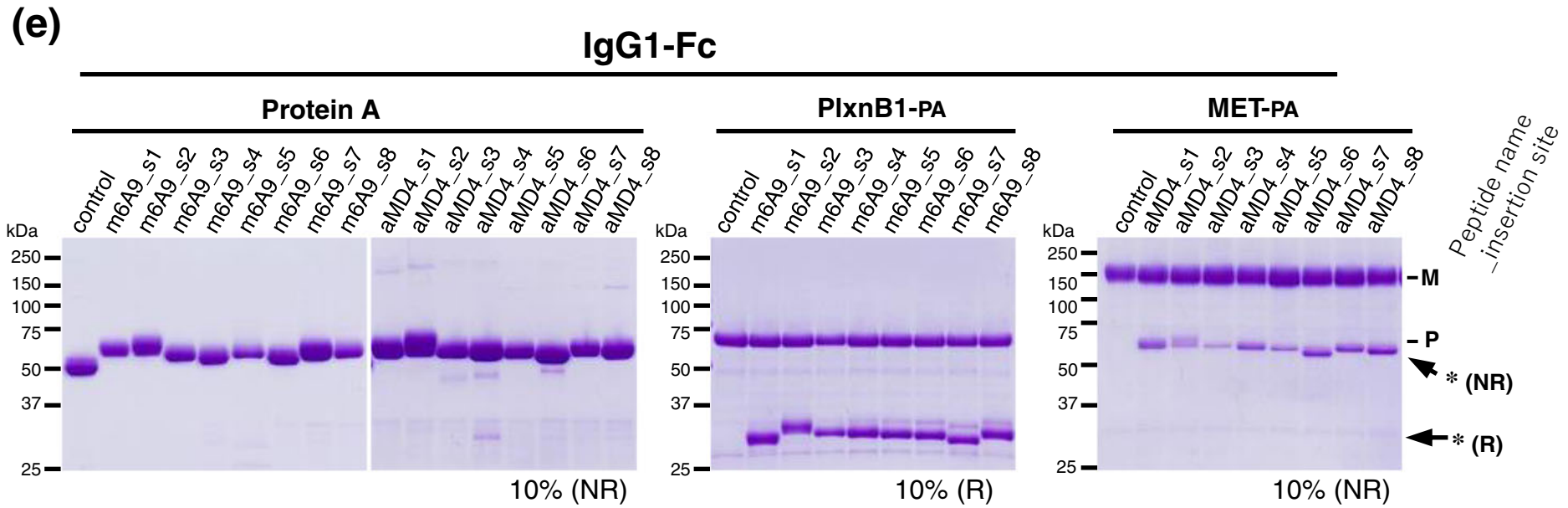
- α/β



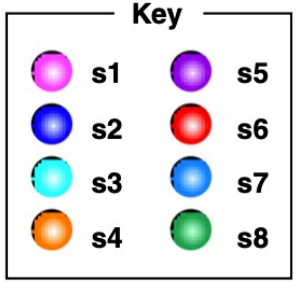
Mihara, E., *et al. Nat. Commun.* **2021,** 12, 1543.

Loop grafting on various proteins





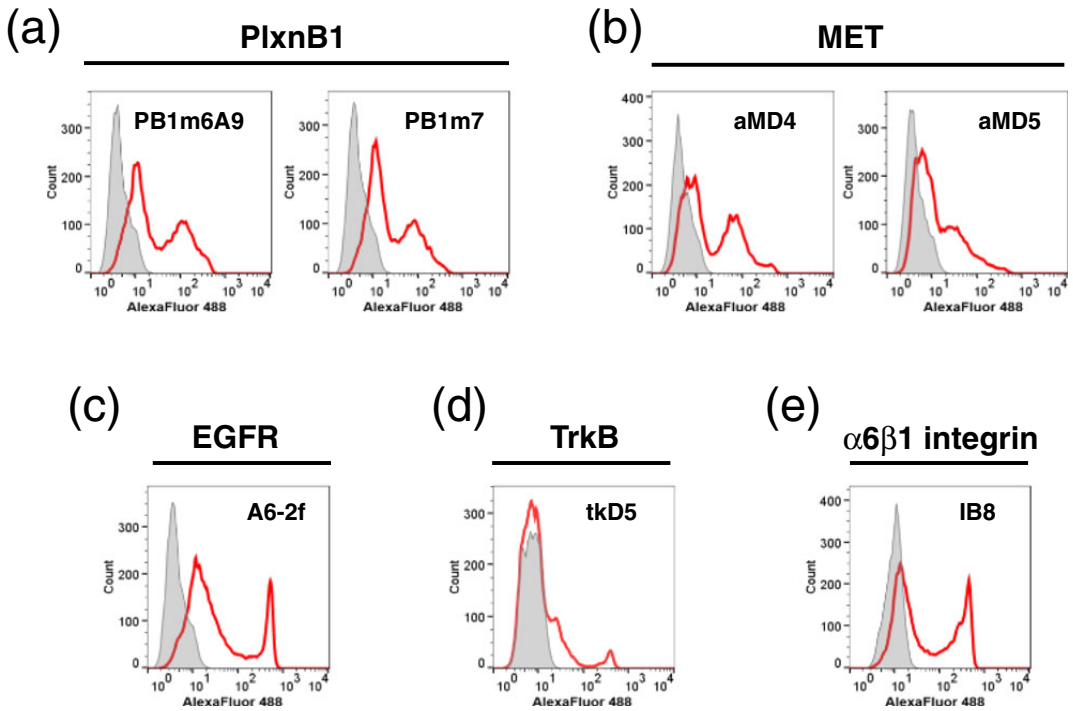
M: MET, P: PlxnB1, *: grafted protein
(NR): non-reducing condition, (R): reducing condition



✓ Lasso-grafted scaffold proteins retains the binding ability to the proteins on which lasso-grafting peptide targets.

Mihara, E., *et al. Nat. Commun.* **2021,** 12, 1543.

Lasso-grafting on Fc domain



analyte	kinetic constants		
	*k*on ($\mu M^{-1}s^{-1}$)	*k*off (s^{-1})	K_D (nM)
PB1m6 (free peptide)	3.46	0.012	3.5
PB1m6 (grafted on UG)	0.091	0.0041	45.8
PB1m6A9 (free peptide)	7.59	0.0021	0.28
PB1m6A9 (grafted on UG)	0.17	0.0014	8.26
PB1m6A9 (grafted on Fc)*	18.0	0.0004	0.023
PB1m7 (free peptide)	0.038	0.010	275
PB1m7 (grafted on UG)	0.0158	0.051	3250
PB1m7 (grafted on Fc)*	0.896	0.055	61.4
aMD4 (free peptide)	1.4	0.003	2.4
aMD4 (grafted on UG)	0.044	0.004	91.6
aMD4 (grafted on Fc)*	0.156	0.0007	4.5
aMD5 (free peptide)	4.8	0.011	2.3
aMD5 (grafted on UG)	0.088	0.019	211

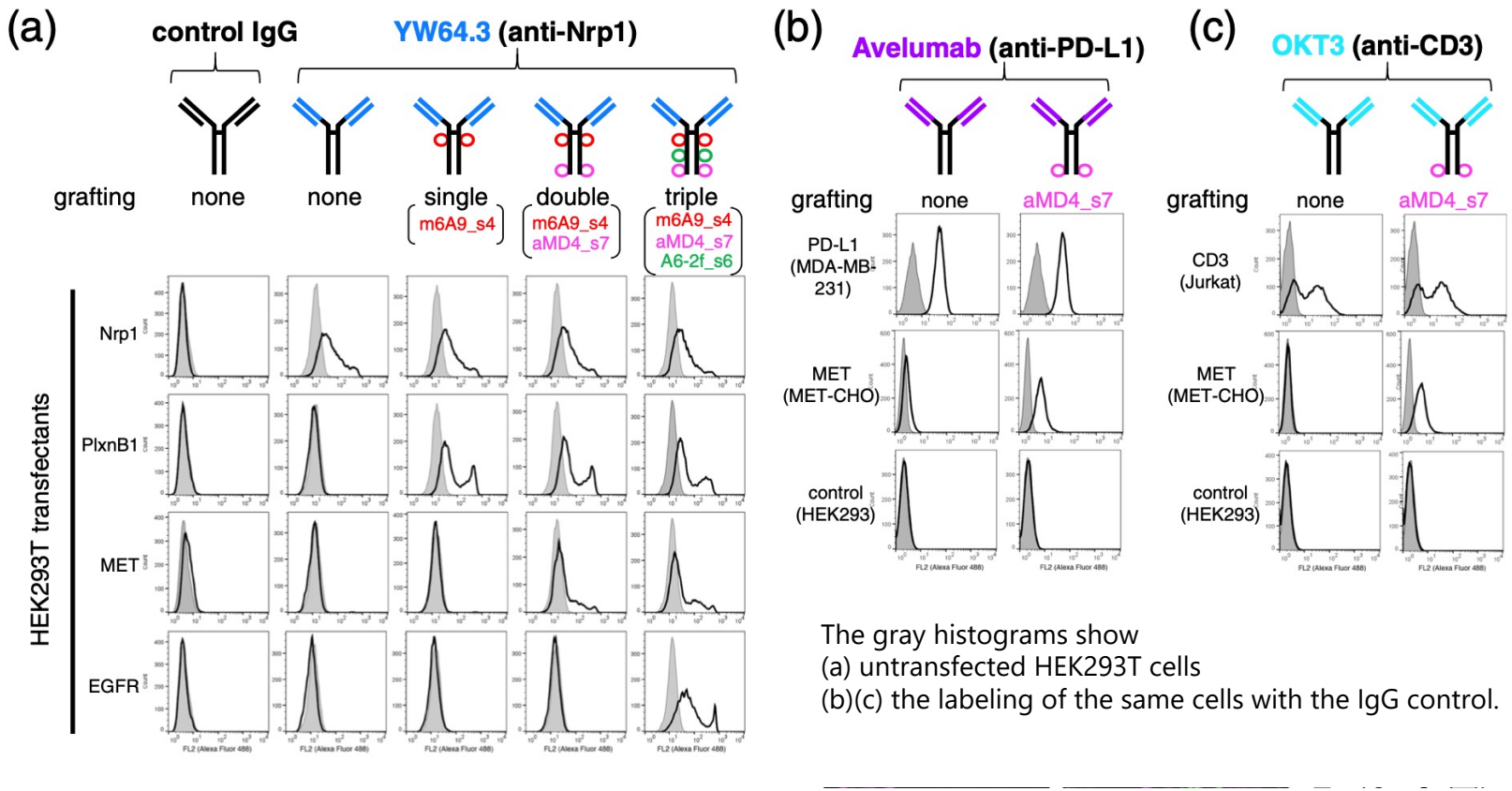
(determined using SPR)

✓ Peptide-grafted Fc protein binding to cell surface target receptors.

✓ The RaPID peptides for many different targets can be readily grafted into various protein scaffolds.

Mihara, E., *et al. Nat. Commun.* **2021,** 12, 1543.

Generating multi-specific antibody by Lasso-Grafting



The gray histograms show
(a) untransfected HEK293T cells
(b)(c) the labeling of the same cells with the IgG control.

- ✓ By grafting peptides into the Fc domain, multispecificity antibodies were created. <"Addbody®">
- ✓ Using this method, Addbody-mediated heterotypic cell engagement was achieved.

Mihara, E., *et al. Nat. Commun.* **2021,** 12, 1543.

Contents

MET targeting therapeutics

- A tyrosine kinase-type receptor
- Dimerizes and phosphorylates its intracellular domain upon activation by the ligand.
- Met-HGF is centrally involved in morphogenesis during development, wound repair and organ homoeostasis
- rHGF has been shown to have therapeutic efficiency.

  Short half-life

  Poor BBB permeability

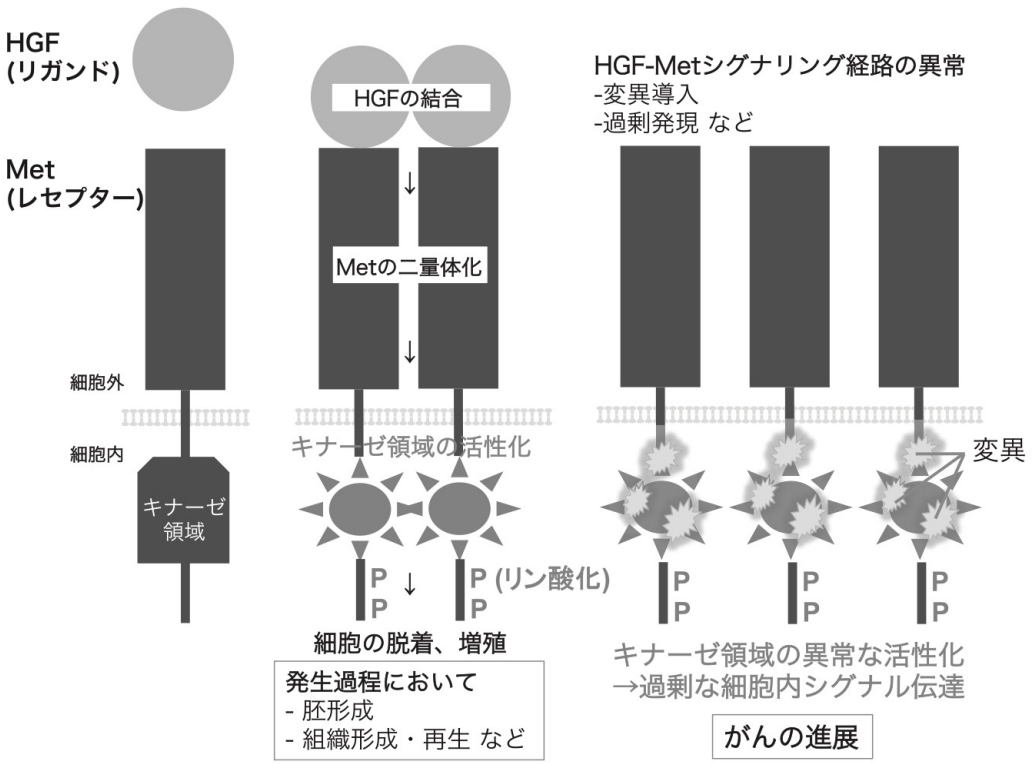
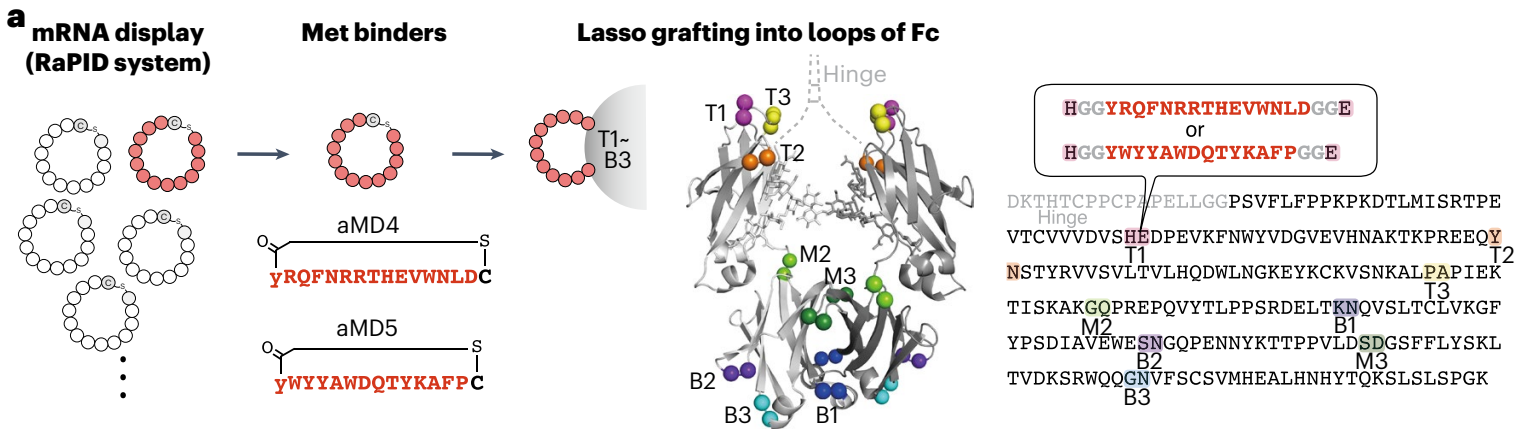
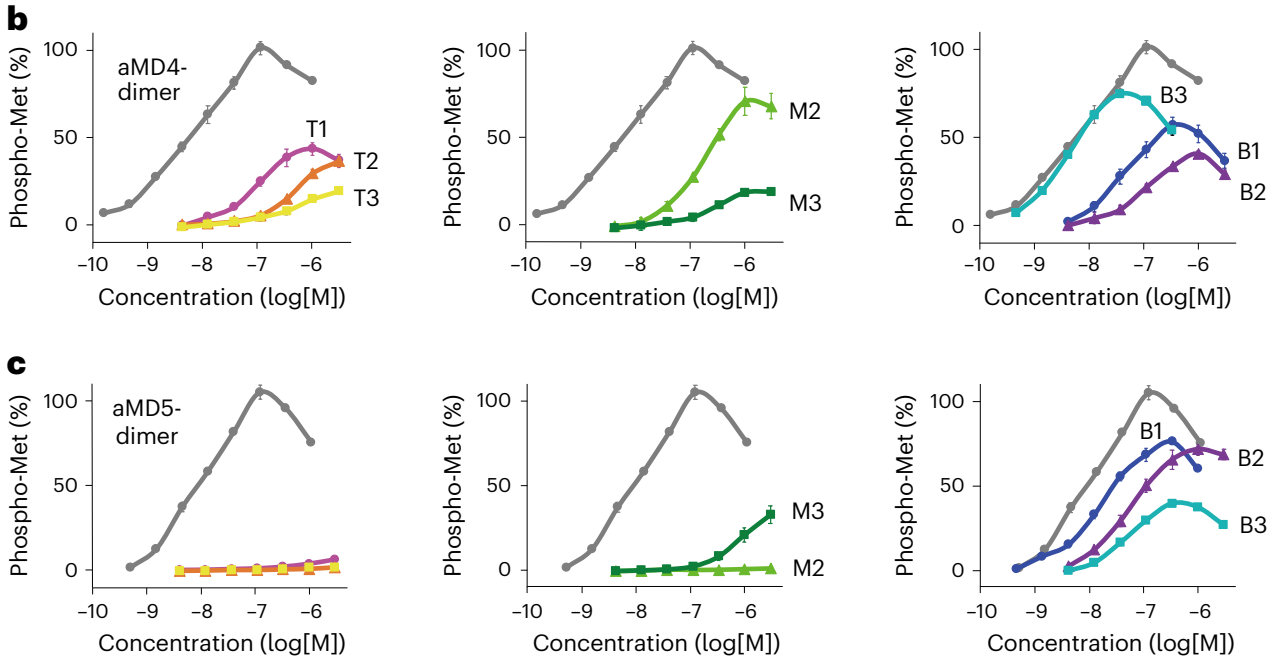


図 1　HGF-Met シグナリング経路の概念図

海津正賢, & 高木淳一. (2016). HGF-Met シグナル伝達の構造的基盤. *日本血栓止血学会誌*, 27(1), 77-84.

Agonist activity of peptide grafted Fc by graft position

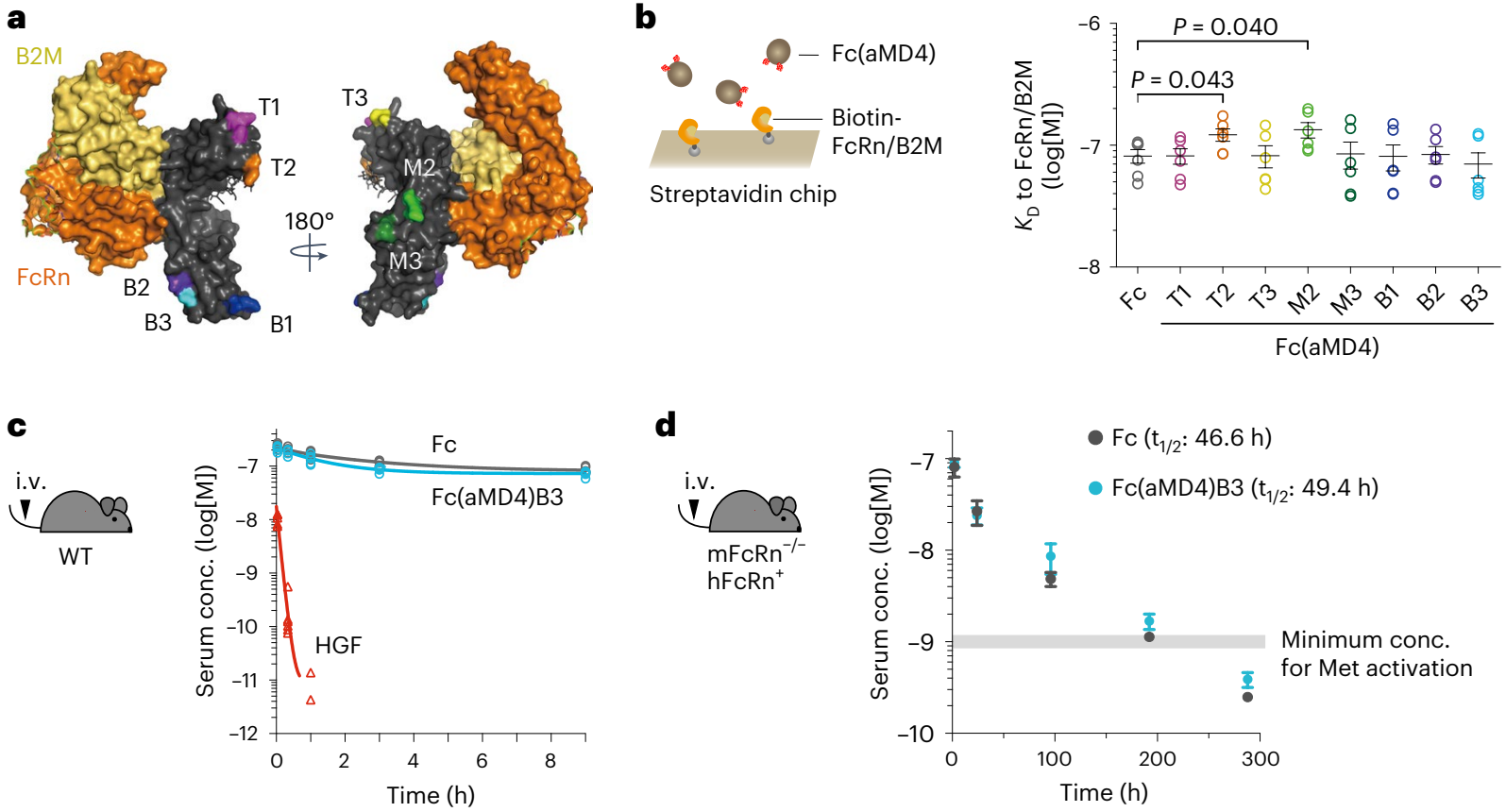




- ✓ B3 was the optimal site for aMD4
- ✓ B1 was the optimal site for aMD5.

Sakai, K., *et al.* *Nat. Biomed. Eng.* **2022**, 1-13.

Peptide grafted Fc has a long blood half-life

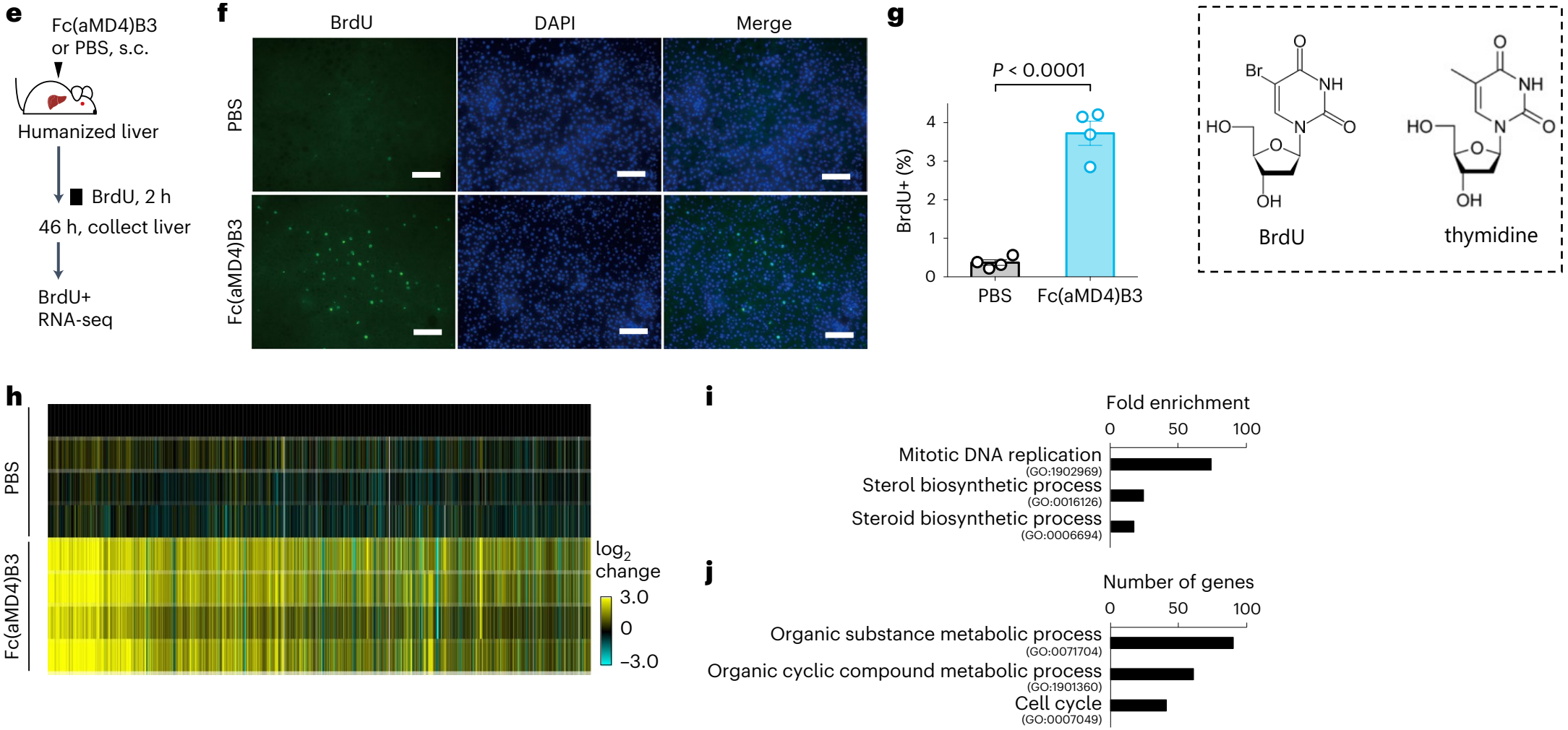


- ✓ Lasso-grafting into most loops did not affect the affinity of Fc to FcRn.
- ✓ Fc(aMD4)B3 had half-life comparable to that of control Fc.
- ✓ The serum concentration of Fc(aMD4)B3 remained above 1 nM, the minimum concentration for activating Met

FcRn:血漿中から細胞内に取り込まれたIgG抗体を血漿中に戻しリソソームでの分解を防ぐ、リサイクルの働きを持つ。

Sakai, K., *et al. Nat. Biomed. Eng.* **2022**, 1-13.

Fc(aMD4)B3 acts as an agonist of HGF.

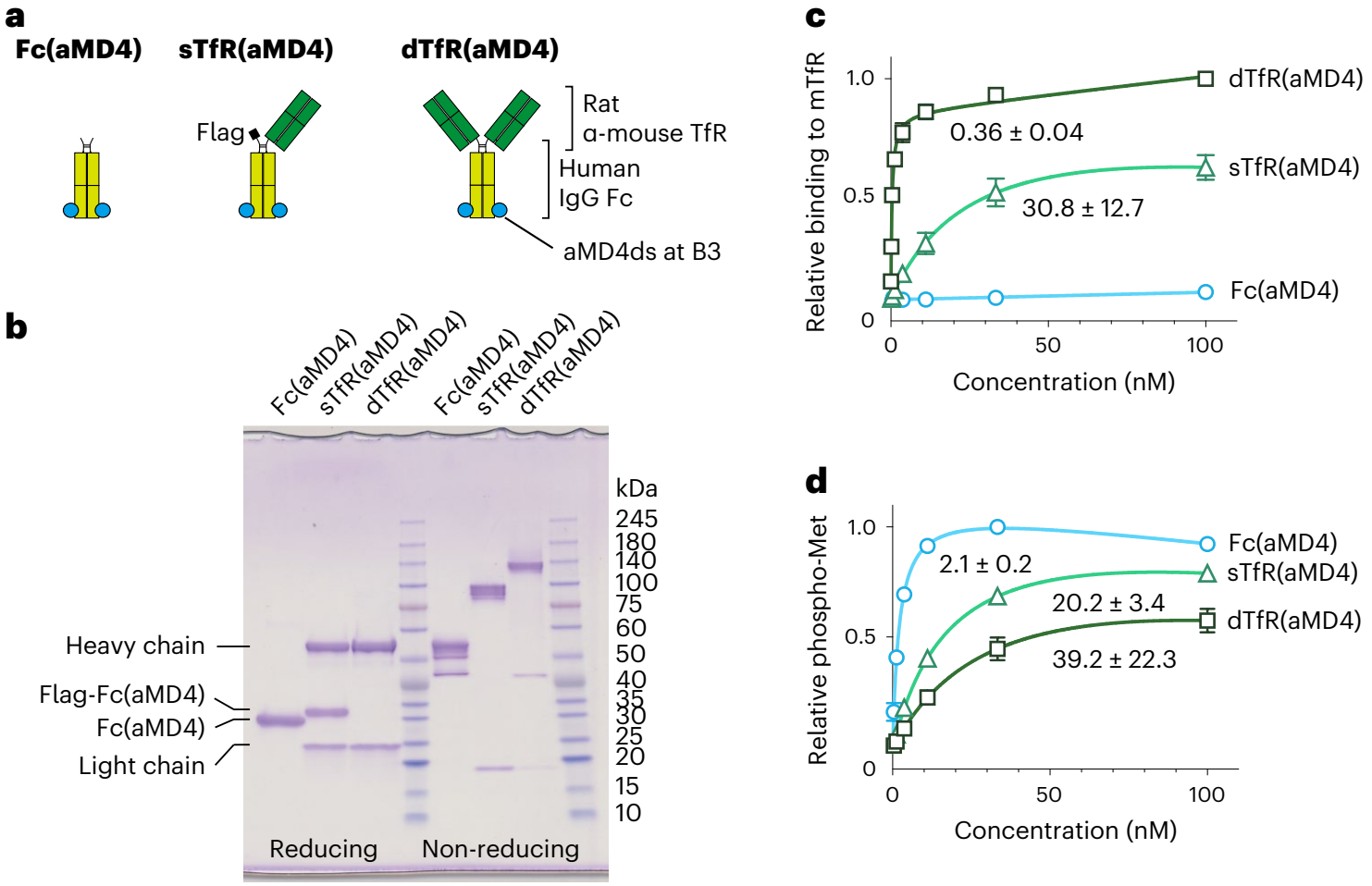


✓ Fc(aMD4)B3 significantly increased replicative DNA synthesis of hepatocytes.

→ Lasso-grafting generated a Met-activating Fc that is bioactive in vivo, with a markedly improved half-life that is comparable to that of unmodified Fc.

Sakai, K., *et al.* *Nat. Biomed. Eng.* **2022**, 1-13.

MET agonist with BBB penetration

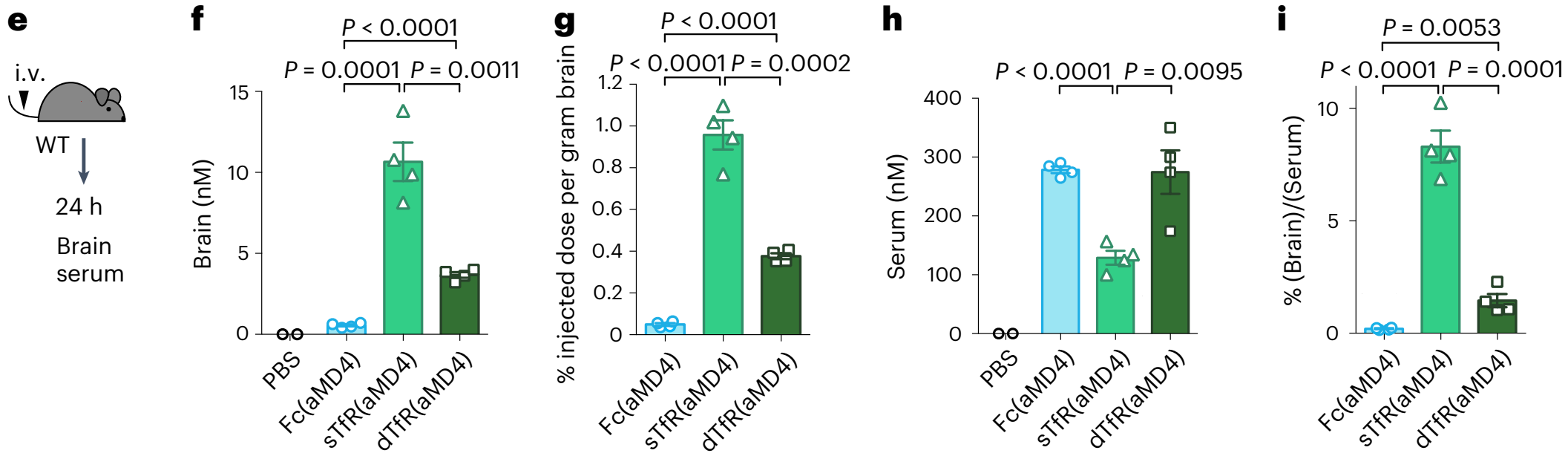


- ✓ Via lasso-grafting, Fab affinity was largely preserved.
- ✓ Via lasso-grafting, Met activity was reduced. (due to the balky Fab)

TfR: トランスフェリン受容体。受容体介在性トランスサイトーシスによりBBBを介した脳内ドラッグデリバリーを行うことができる。

Sakai, K., *et al.* *Nat. Biomed. Eng.* **2022**, 1-13.

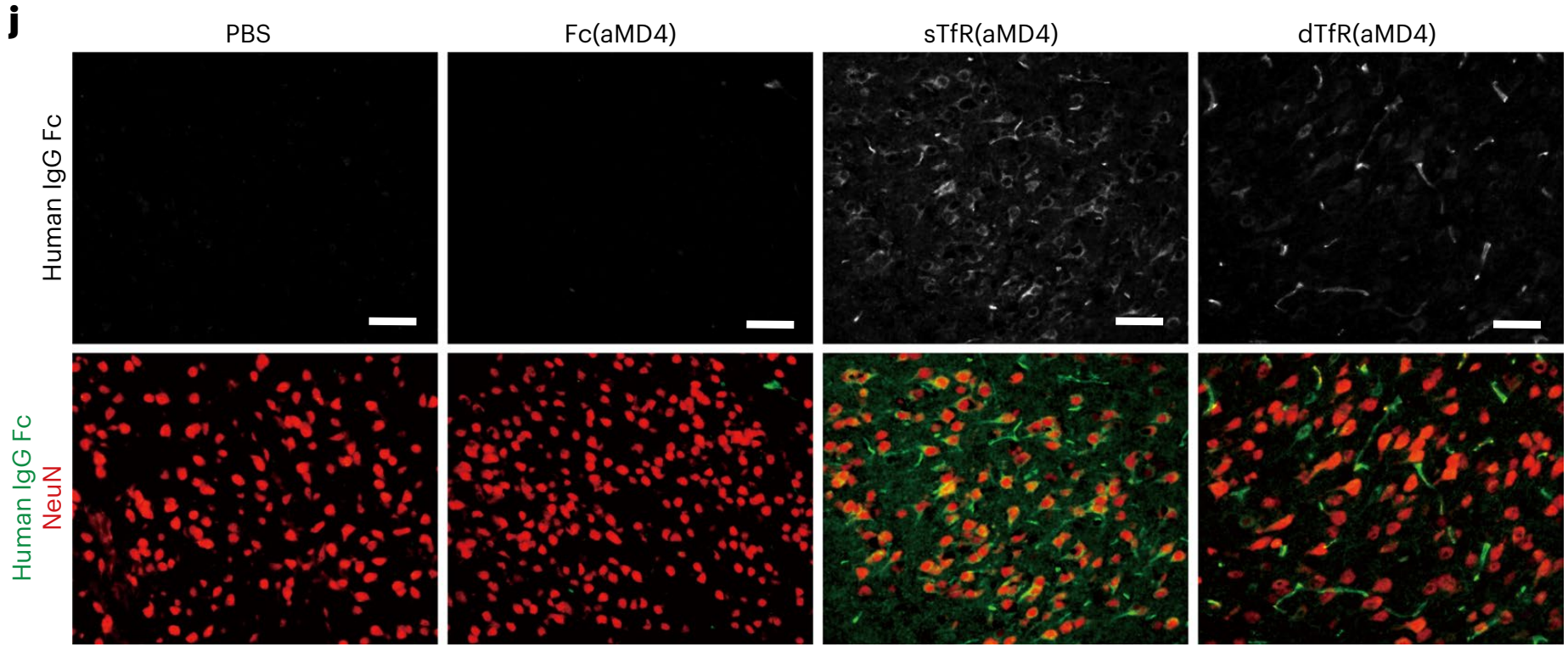
MET agonist with BBB penetration



✓sTfR(aMD4) showed the highest percentage injected dose per gram brain, brain-to-serum ratio, as well as the highest brain concentration .

Sakai, K., *et al.* *Nat. Biomed. Eng.* **2022**, 1-13.

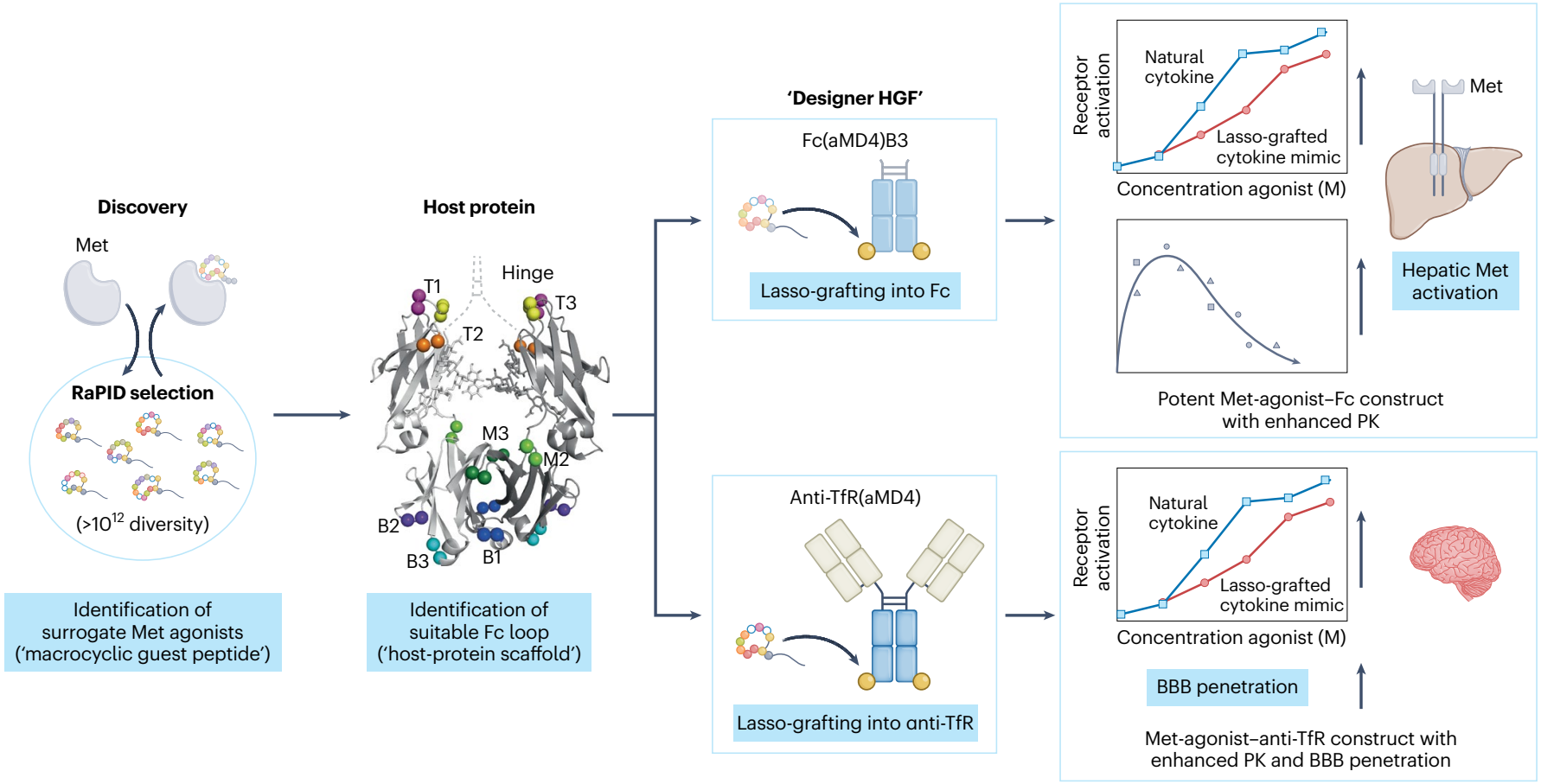
IHC using Fc(aMD4), sTfR(aMD4), dTfR(aMD4)



✓sTfR(aMD4) showed prominent parenchymal staining co-localized with NeuN, GFAP and Iba1, indicating its distribution to neuron, astrocytes and microglia, respectively.

Sakai, K., *et al.* *Nat. Biomed. Eng.* **2022**, 1-13.

Short summary



✓Binding to FcRn resulted in a longer half-life.

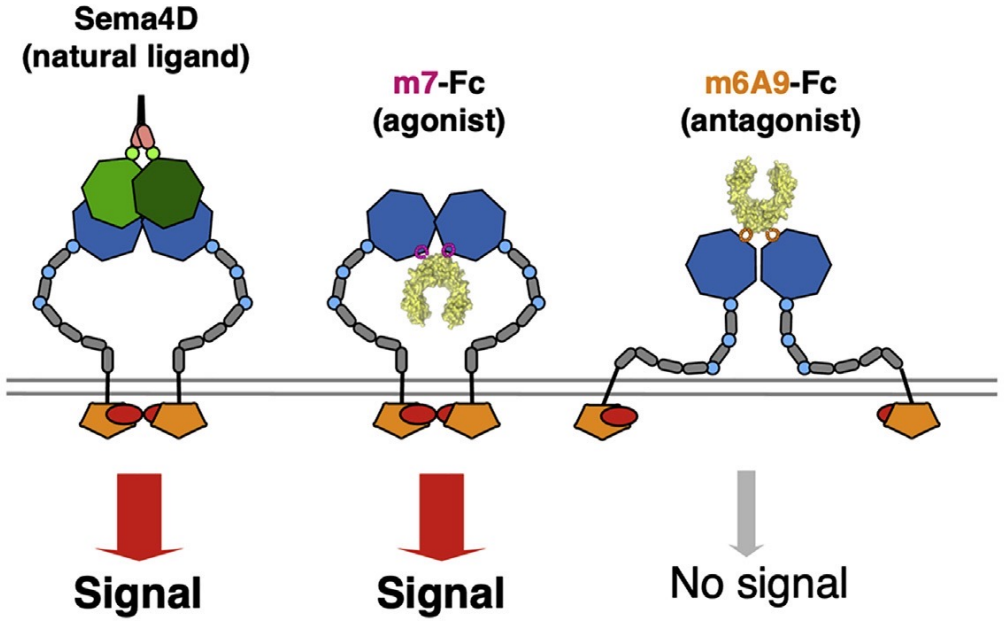
✓BBB permeability was improved by incorporating TfR.

→Lasso grafting on Fc fragments enhanced pharmacokinetics.

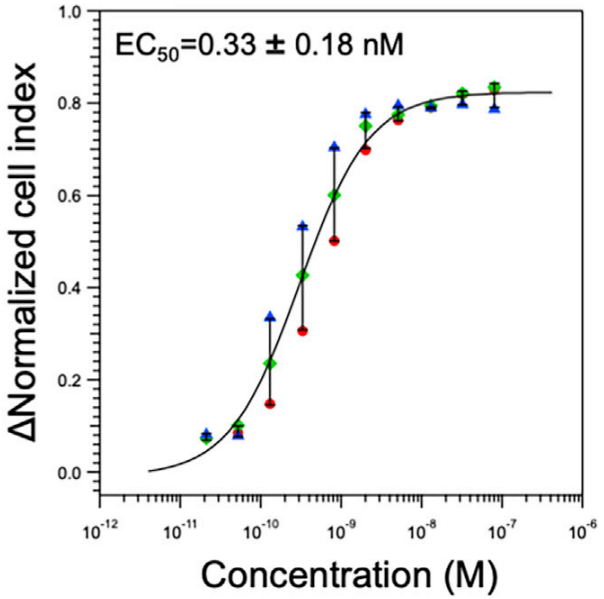
Kapurniotu, A., *et al. Nat. Biomed. Eng.* **2022**, 1-3.

Contents

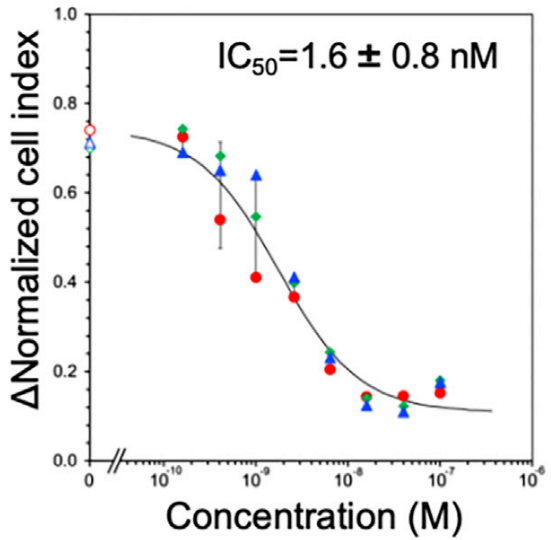
Antibody w/o Fab; Mirabody® acts as a receptor agonist



Fc(m7)B2
agonist



Fc(m6A9)B3
antagonist



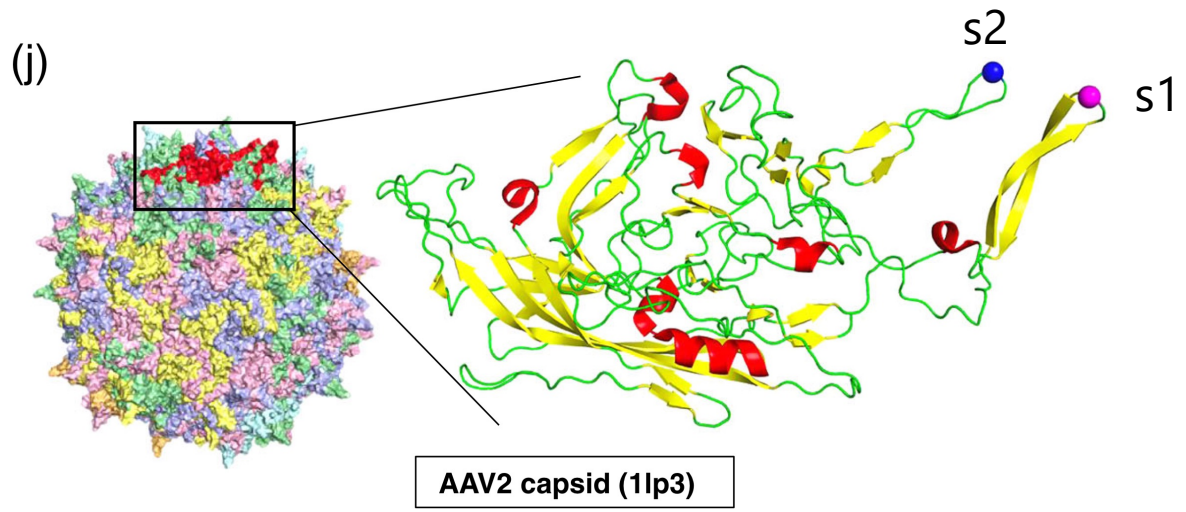
- ✓Mirabody® acted as the agonist toward PlxnB1.
- ✓m7-Fc activates receptor dimerization and subsequent signaling.
- ✓m6A9-Fc acted as antagonist toward PlxnB1 and inhibited Sema4D.
- ✓It could potentially be biotherapeutics due to the long plasma half-life.

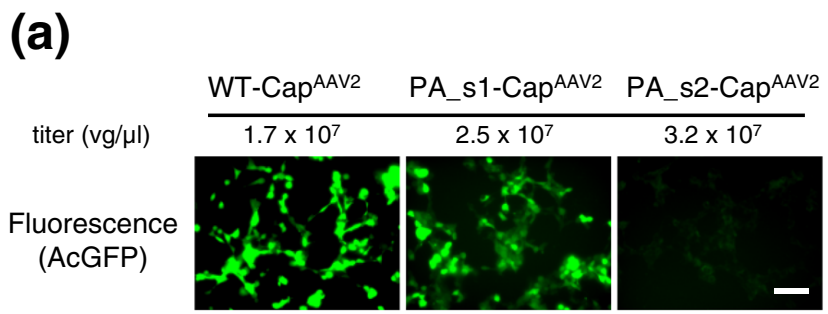
N Sugano-Nakamura. *et al. Nat. Commun.* **2021**, 12, 1543.

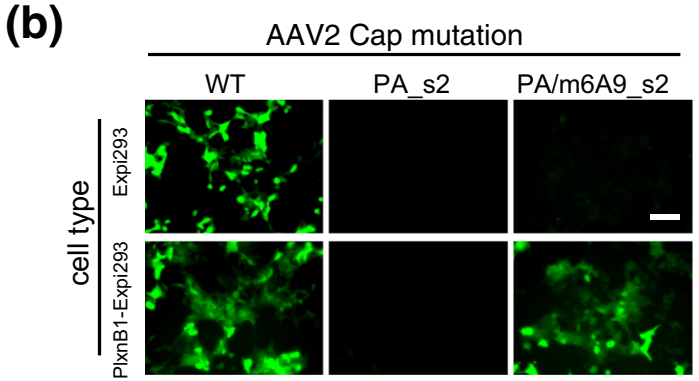
Lasso-grafting on AAV capsid

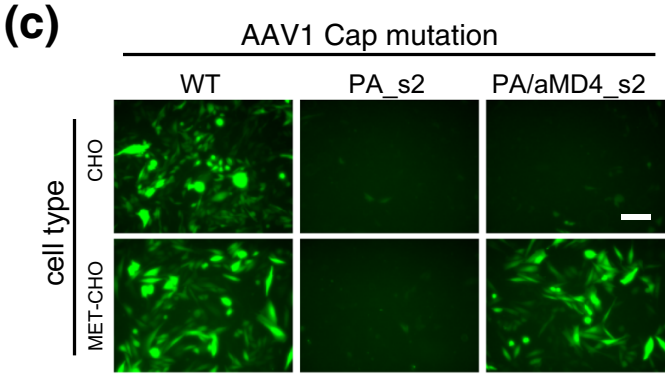
AAV (adeno-associated virus)

- Gene-delivery vehicles
- Broad tropism (Binds to receptors expressed on the surface of many cells)
- Capsid engineering has been used to impart tissue specificity.





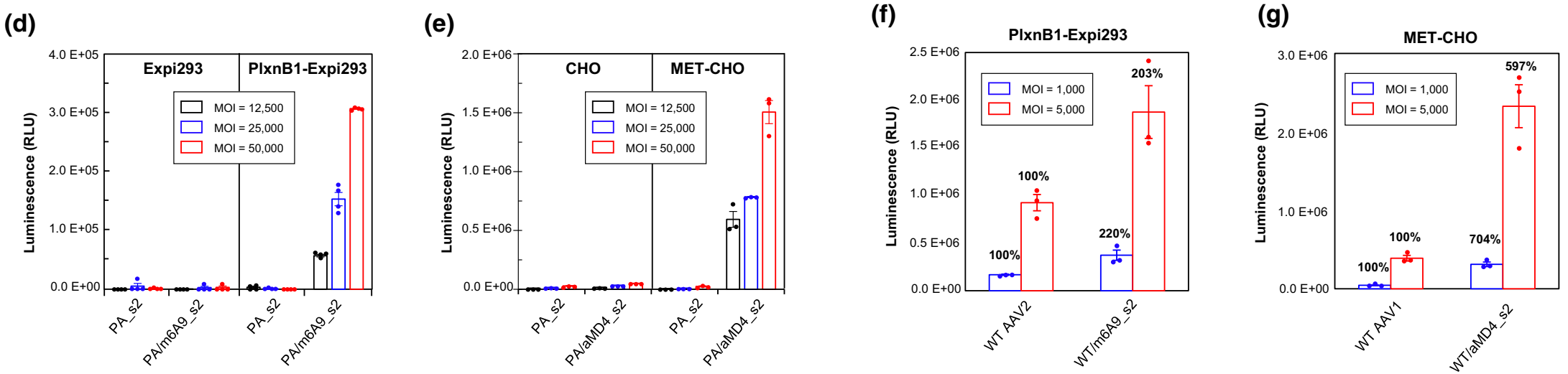




✓ By grafting on S2 site, lasso-grafted AAV acquired cell-specific localization.

Mihara, E., *et al. Nat. Commun.* **2021,** 12, 1543.

Lasso-grafting on AAV capsid



- ✓ Only viruses expressing a peptide that binds specifically to the receptor could perform gene transfer to cells expressing the receptor protein.
- ✓ Gene transfer efficiency of mutant viruses is higher than that of wild-type viruses.

MOI: Multiplicity of infectionの略で細胞１個あたりのウイルスの数

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Contents

Summary

RaPID system

- ✓Rapid screening of macrocyclic peptides

Lasso-Grafting

- ✓Functionalization of macrocyclic peptides in vivo
- ✓Functionalization of host proteins
- ✓Generation of multispecific antibodies (Addbody®)
- ✓Improved bioavailability and BBB permeability
- ✓Generation of receptor agonist/antagonist (Mirabody®)