Glycocalyx Engineering

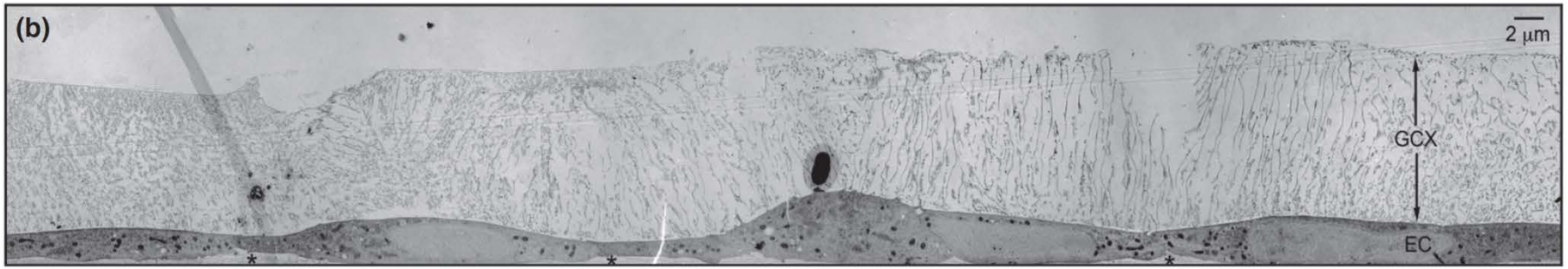
LS: Dec 16, 2017

Hiroaki Itoh

1-1. Glycocalyx

glycocalyx: a glycoprotein and glycolipid covering the membrane of endothelial cells, bacteria, and other cells.

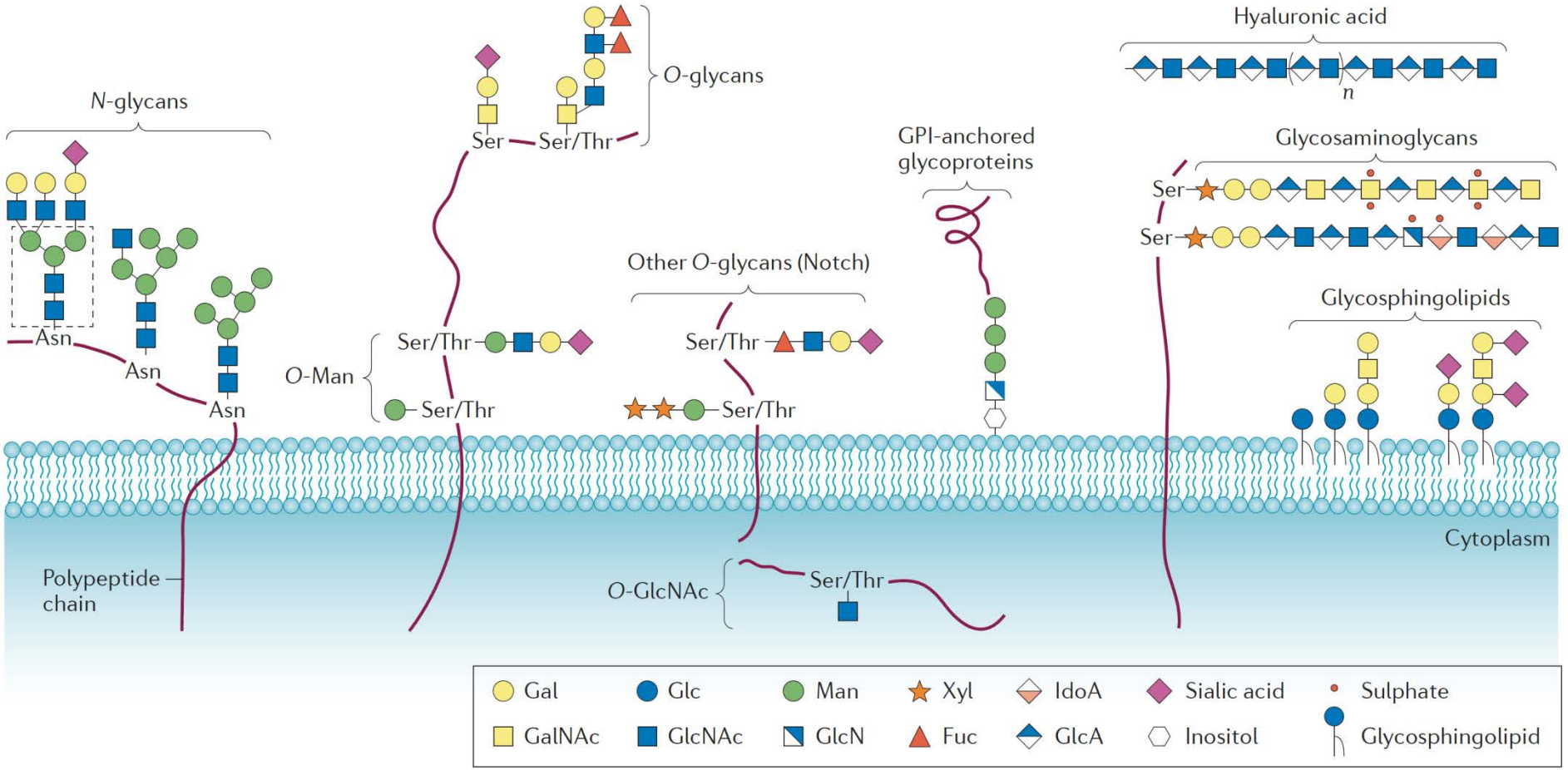
The term was introduced by Bennett, H. S. in 1963.[1)]
"*sweet husk*" (ancient Greeks used the word "sweet" for sugars)



Transmission electron micrographs of the glycocalyx (GCX) on cultured bovine aortic endothelial cells (EC)[2)]

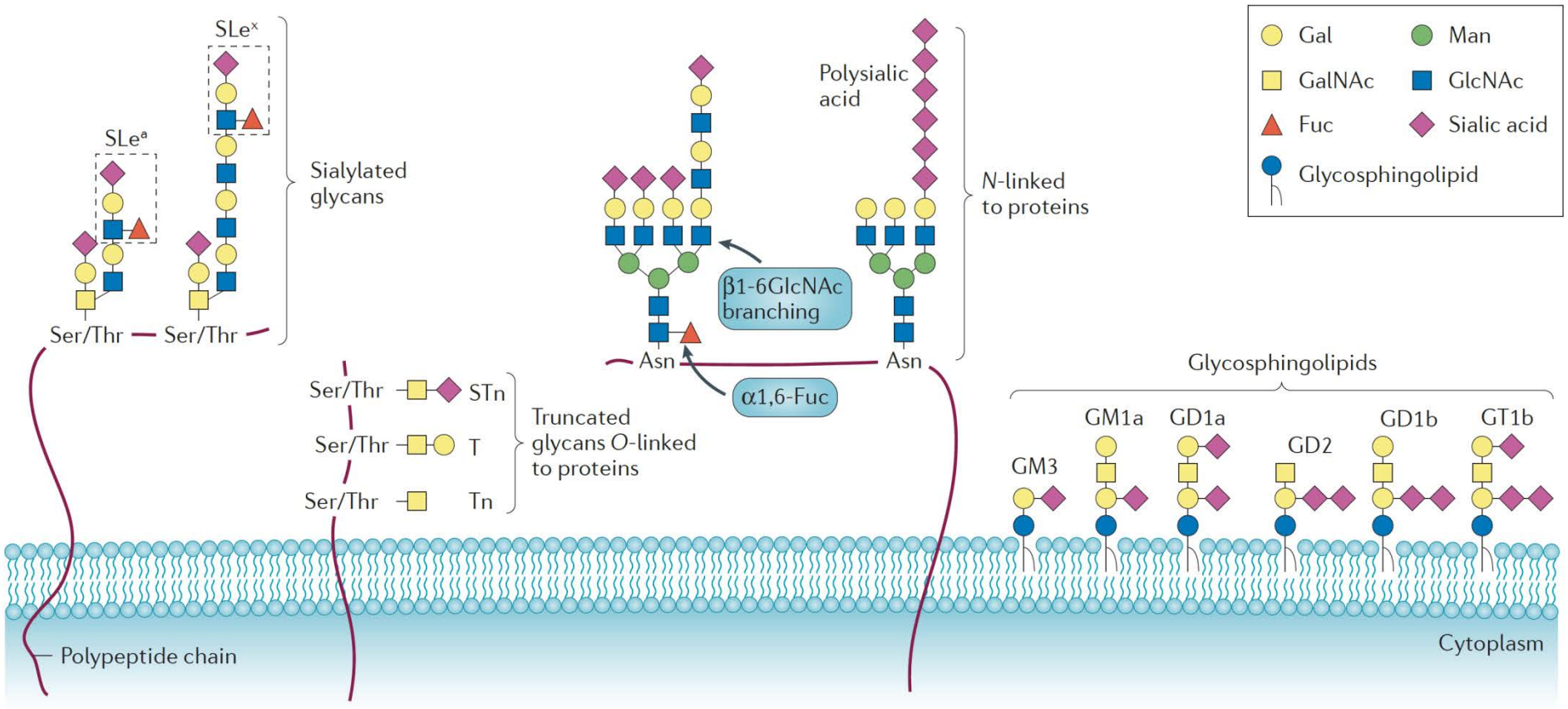
1) Bennett, H. S. *J. Histochem. Cytochem.* **1963**, *11*, 15. 2) Tarbell, J. M.; Cancel, L. M. *J. Intern. Med.* **2016**, *280*, 97.

1-2. Cell Surface Glycoconjugates



1) Pinho, S. S.; Reis, C. A. *Nat. Rev. Cancer* **2015**, *15*, 540.

1-3. Glycosylation Alteration in Cancer Cells



Several glycoprotein and origosaccharides are known to be overexpressed on cancer cells: e.g. mucin 1 (MUC1) , hyaluronic acid (HA)

1) Pinho, S. S.; Reis, C. A. *Nat. Rev. Cancer* **2015**, *15*, 540.

1-4. Issue

The composition of glycocalyx changes markedly cellular functions.

Understanding the biochemical functions of glycocalyx has been insufficient:
Why are broad changes in glycosylation and glycoprotein expression critical to cellular functions?
How do the changes of glycocalyx cause diseases?

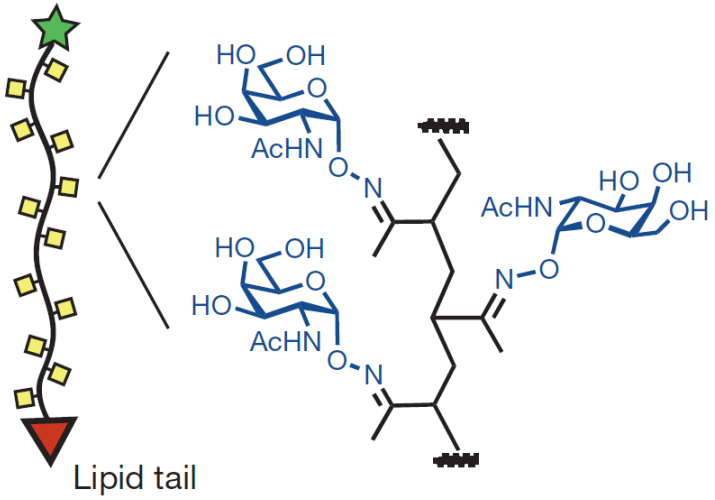
1-5. Glycocalyx Engineering

Chemical or enzymatic editing of glycocalyx: potentially applicable to understanding biological functions of glycocalyx and development of therapeutic methods

- **understanding precise functions of glycocalyx**

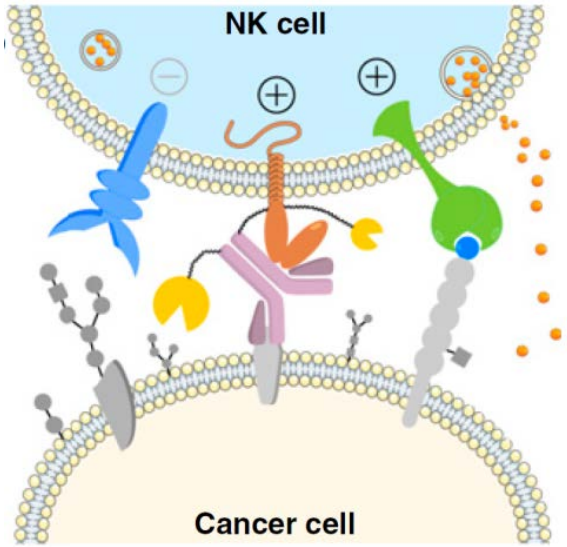
  bioorthogonal chemical reporters (Staudinger ligation)[1,2)]

  analysis by using glycoprotein mimetics[3)]



- **seeking new therapeutic methods[4)]**

  glycoprotein editing

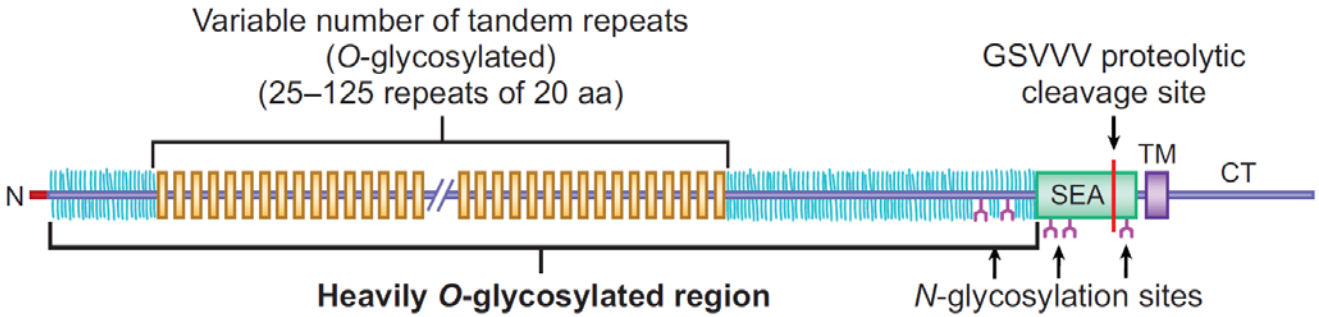


1) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007. 2) Prescher, J. A.; Dube, D. H.; Bertozzi, C. R. *Nature* **2004**, *430*, 873. 3) Paszek, M. J.; DuFort, C. C.; Rossier, O.; Bainer, R.; Mouw, J. K.; Godula, K.; Hudak, J. E.; Lakins, J. N.; Wijekoon, A. C.; Cassereau, L.; Rubashkin, M. G.; Magbanua, M. J.; Thorn, K. S.; Davidson, M. W.; Rugo, H. S.; Park, J. W.; Hammer, D. A.; Giannone, G.; Bertozzi, C. R.; Weaver, V. M. *Nature* **2014**, *511*, 319. 4) Xiao, H.; Woods, E. C.; Vukojicic, P.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 10304.

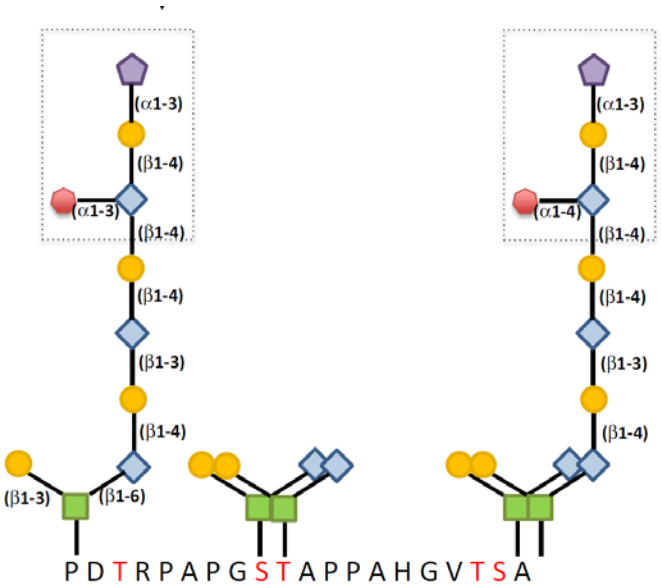
1-6. Mucin

mucin: a class of modular proteins characterized by the presence of a mucin domain (also called the PTS domain) rich in proline/serine/threonine amino acids.

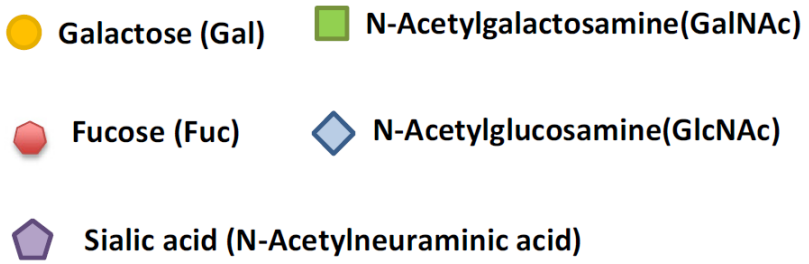
tandem repeat: PDTRPAPGSTAPPAHGVTSA



MUC1[1)]



2)

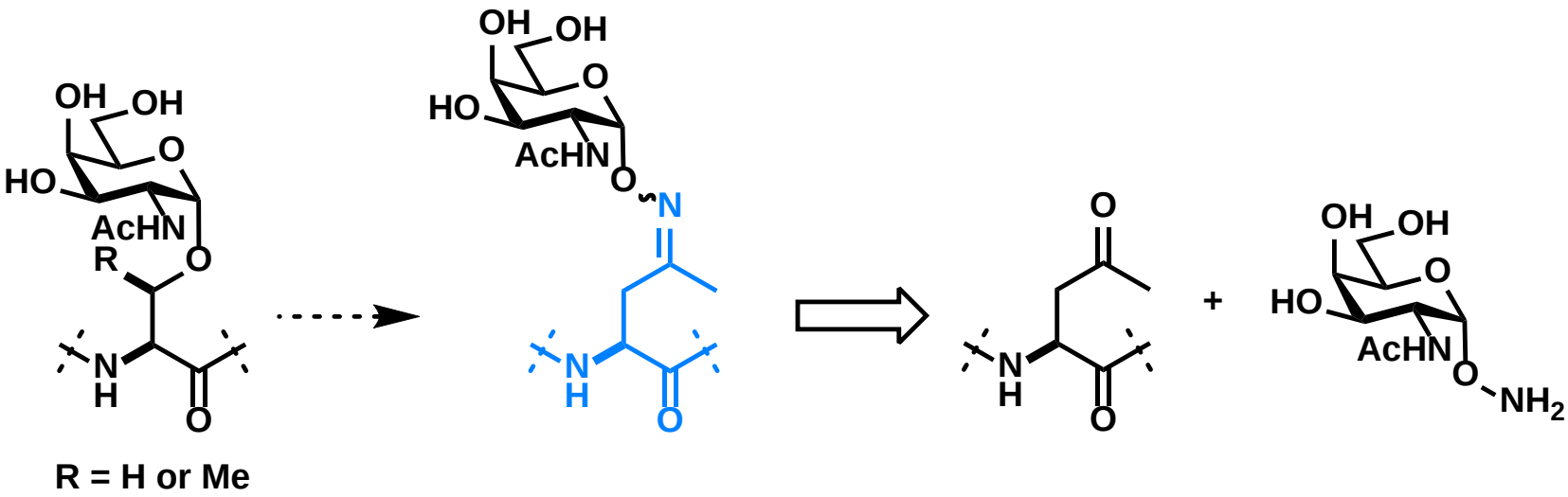


1) Hattrup, C. L.; Gendler, S. J. *Annu. Rev. Physiol.* **2008**, *70*, 431. 2) Nath, S.; Mukherjee, P. *Trends Mol. Med.* **2014**, *20*, 332.

1-7. Mucin Mimetics

To develop simple mimetics for mucin, oxime-linked peptide-sugar conjugates were designed and utilized.

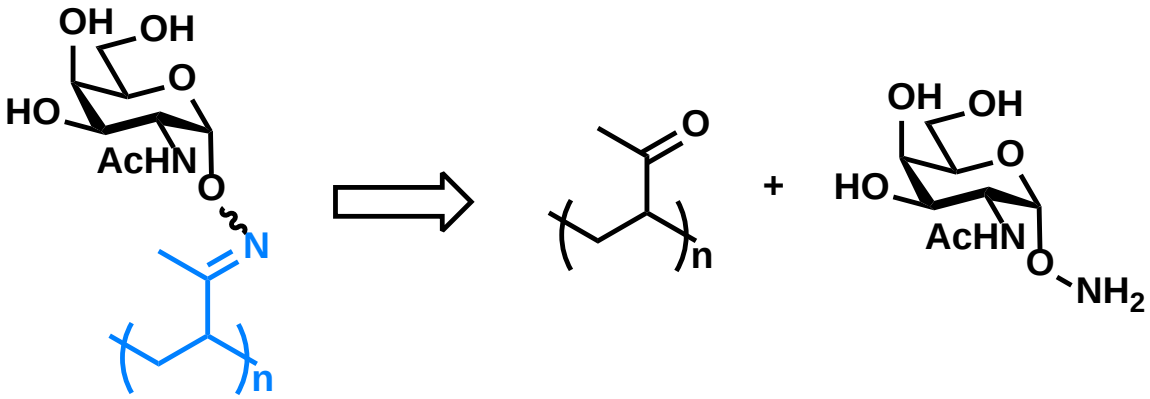
peptide scaffold[1,2)]
not applicable for larger molecule
(up to 132 residues)[2)]



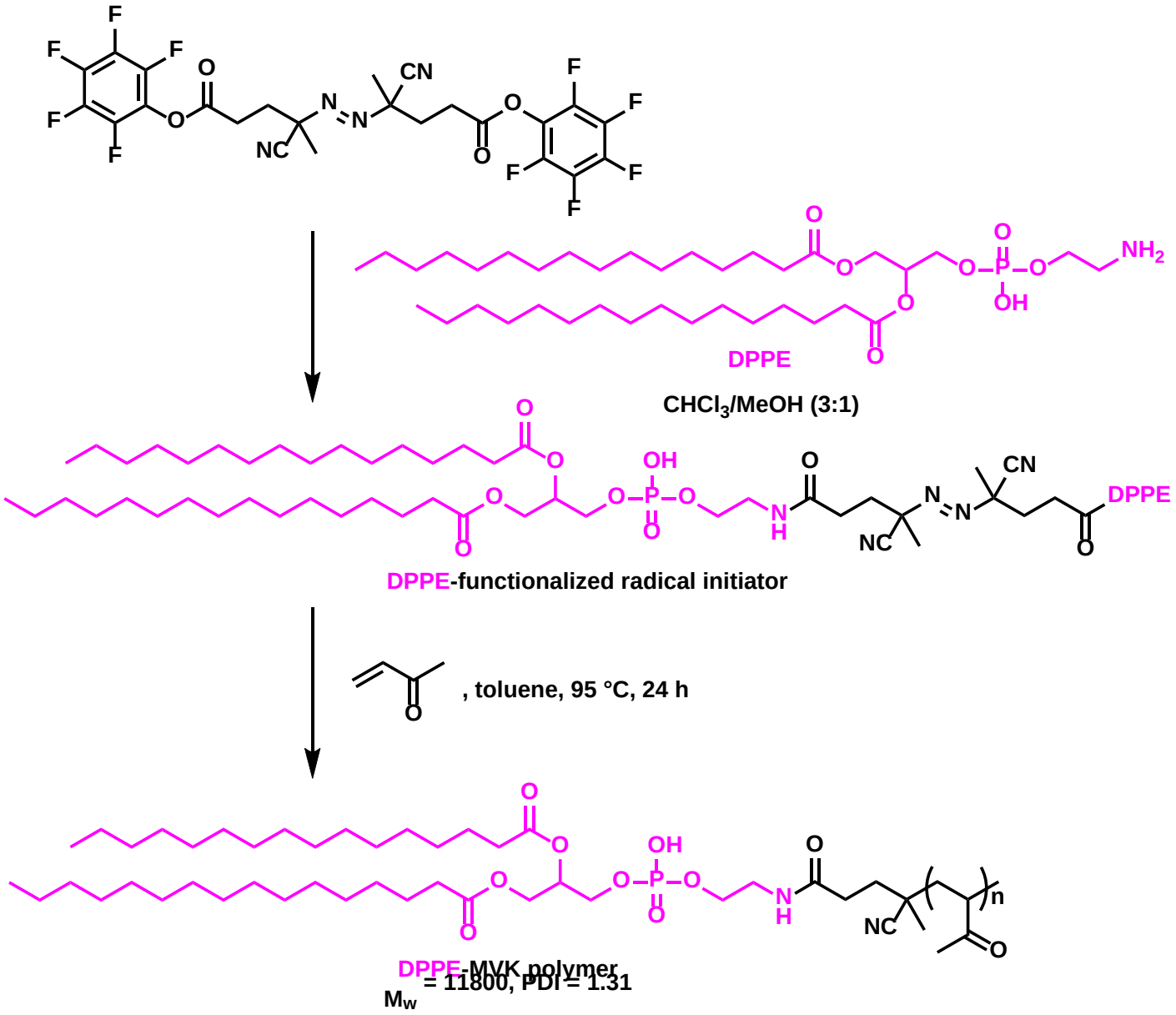
1) Marcaurelle, L. A.; Shin, Y.; Goon, S.; Bertozzi, C. R. *Org. Lett.* **2001**, *3*, 3691. 2) Macmillan, D.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 1355.

1-7. Mucin Mimetics

macromolecular scaffold

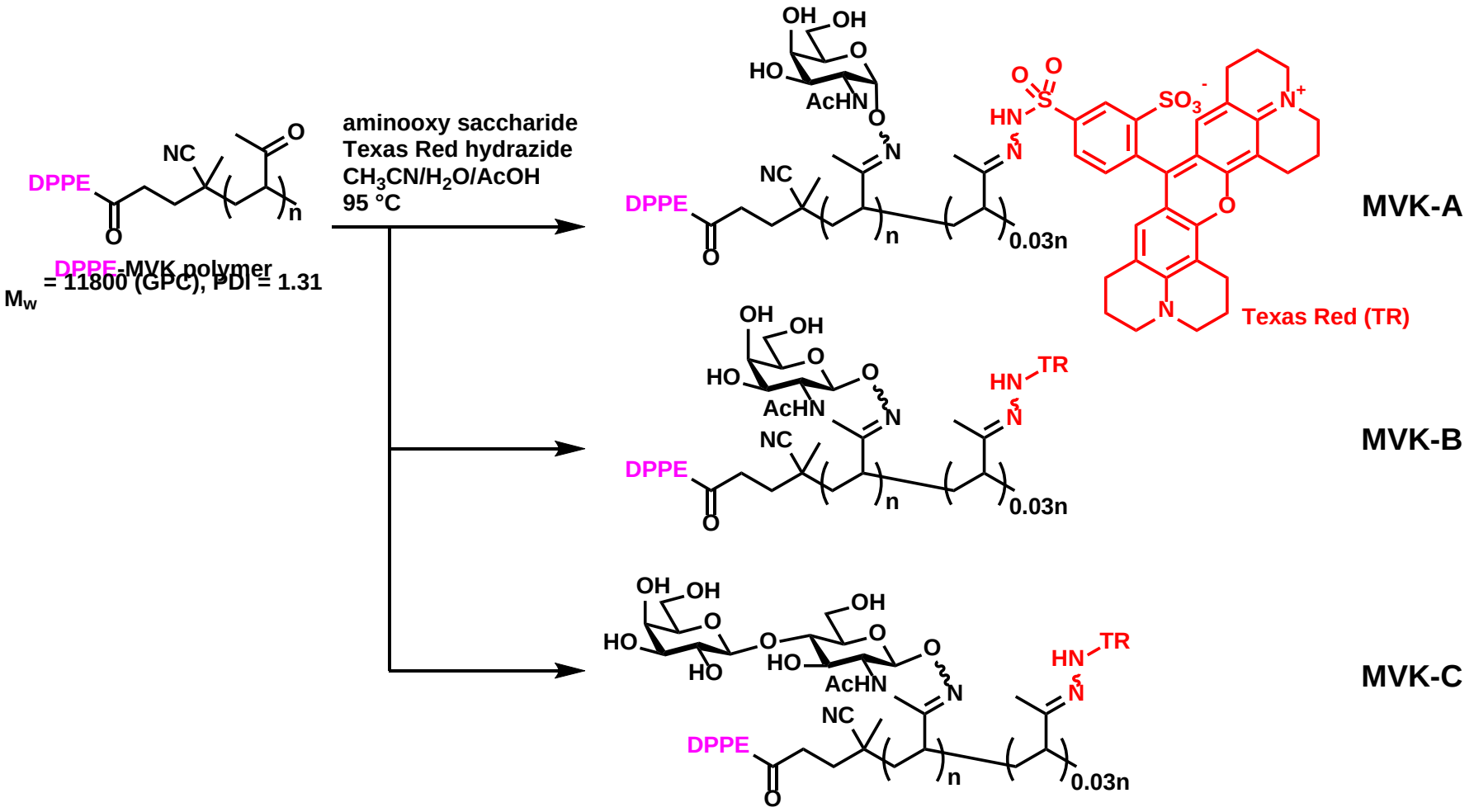


1-8. DPPE-Functionalized MVK Polymer



1) Rabuka, D.; Parthasarathy, R.; Lee, G. S.; Chen, X.; Groves, J. T.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2007**, *129*, 5462.

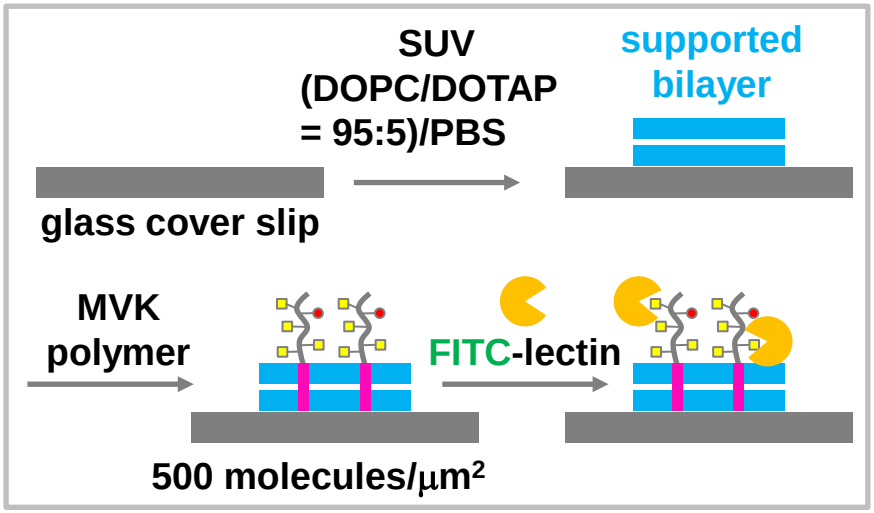
1-9. Radical Polymerization of MVK Polymer

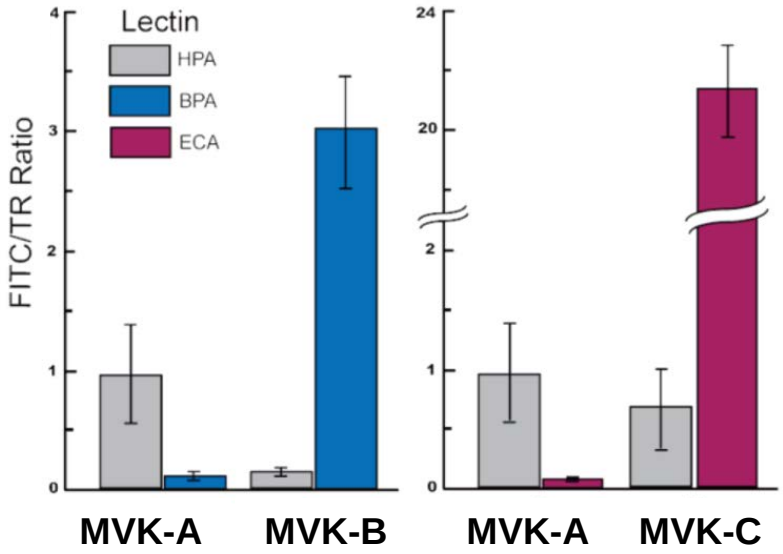


1) Rabuka, D.; Parthasarathy, R.; Lee, G. S.; Chen, X.; Groves, J. T.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2007**, *129*, 5462.

1-10. Binding of Lectins against MVK Polymers

polymers	lectins
MVK-A	HPA
	α-linked GalNAc selective
MVK-B	BPA
	β-linked GalNAc selective
MVK-C	ECA
	LacNAc selective



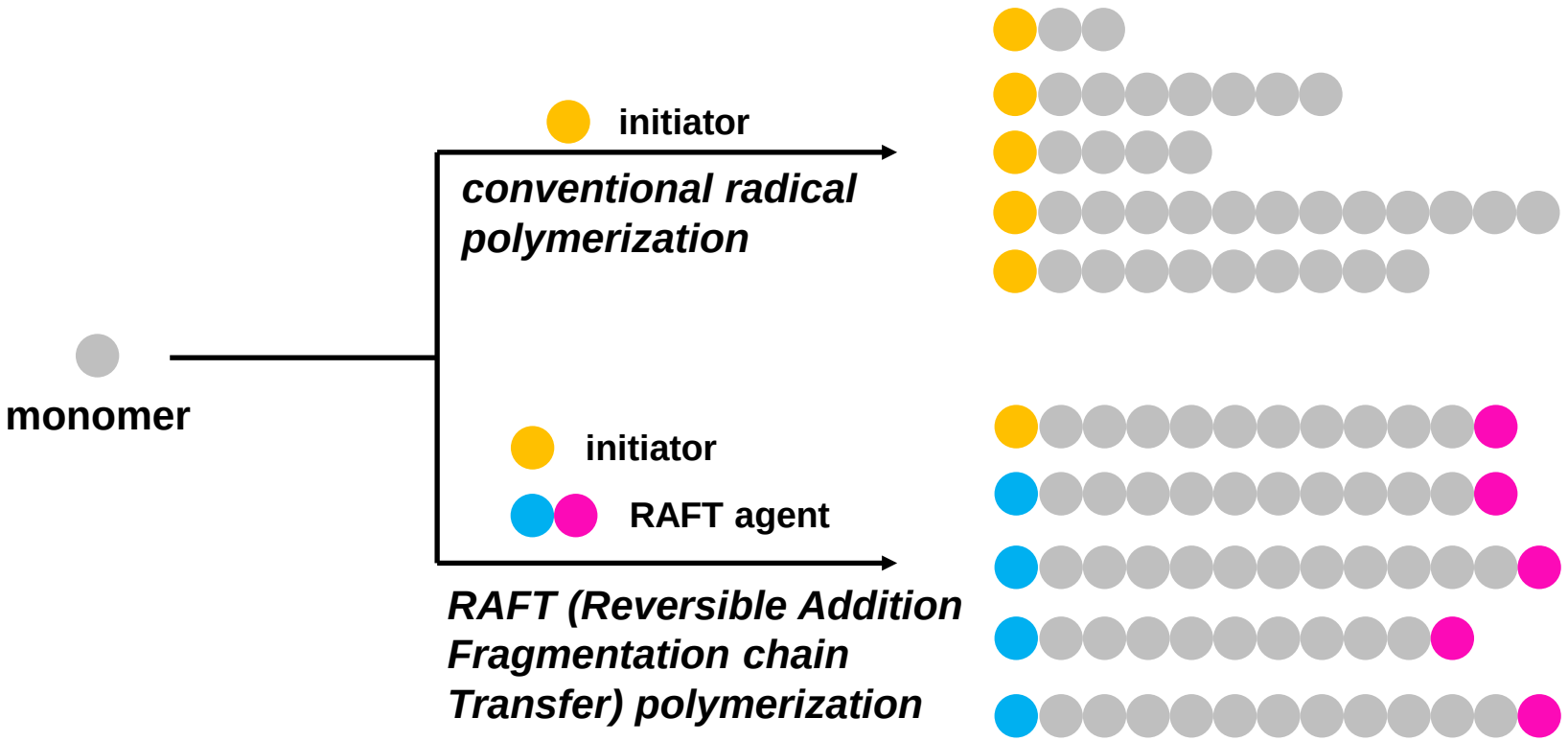


Lectins selectively recognized MVK polymer-conjugated saccharides.

1) Rabuka, D.; Parthasarathy, R.; Lee, G. S.; Chen, X.; Groves, J. T.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2007**, *129*, 5462.

1-11. Application of RAFT Polymerization

RAFT polymerization was discovered in 1998.[1)]

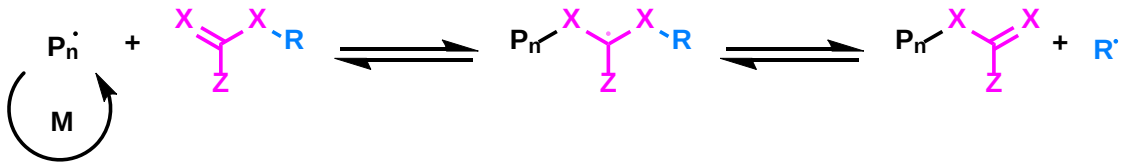


1) Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A. ; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1998**, *31*, 5559.

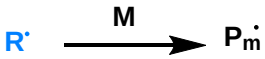
1-11. Application of RAFT Polymerization

1. initiation

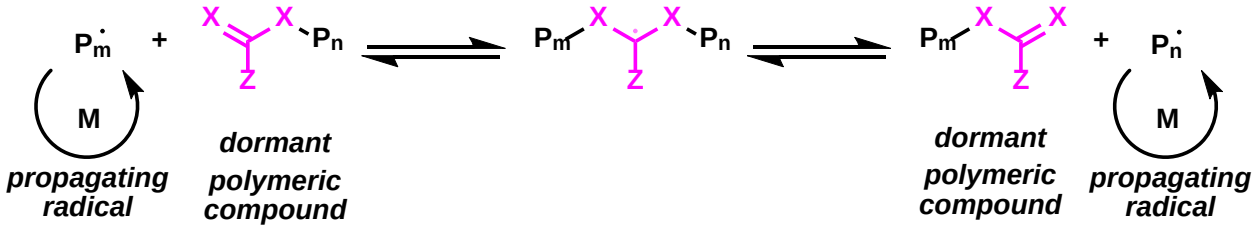
2. reversible chain transfer



3. reinitiation



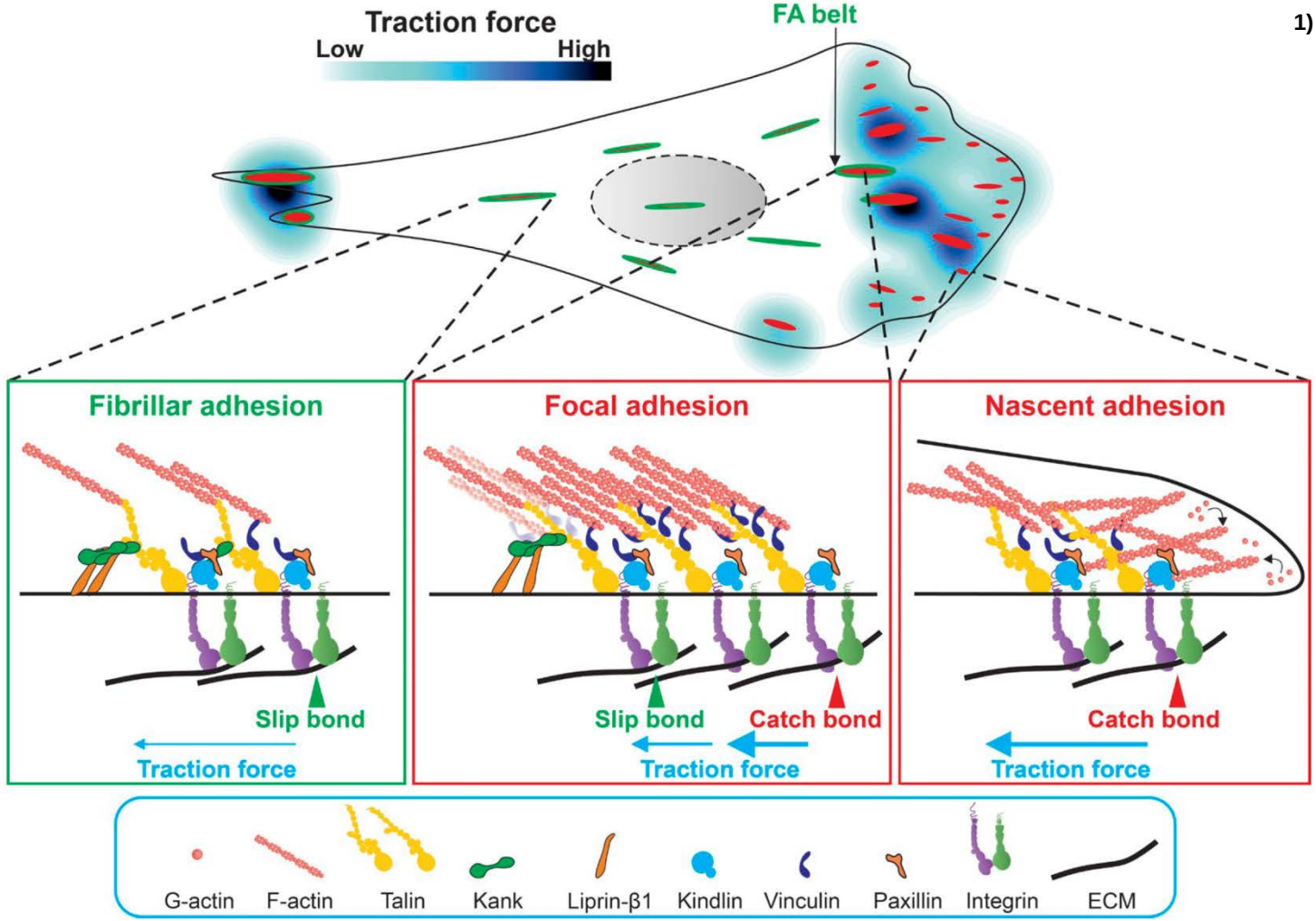
4. chain equilibration



5. termination

1) Moad, G.; Rizzardo, E.; Thang, S. H. *Polymer* **2008**, *49*, 1079.

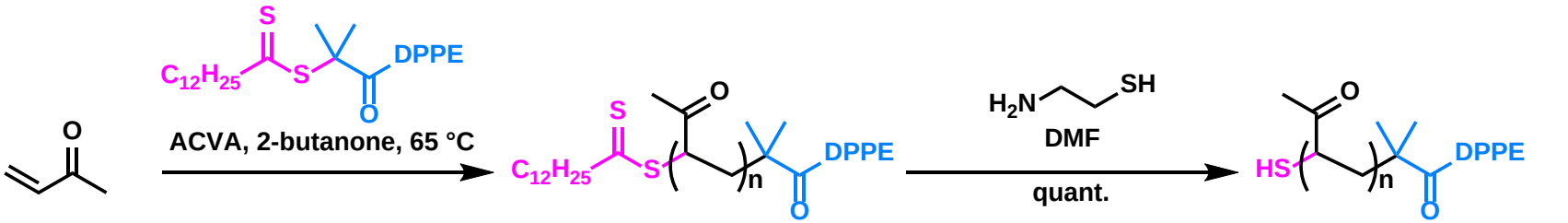
2-1. Adhesion Sites of Cells

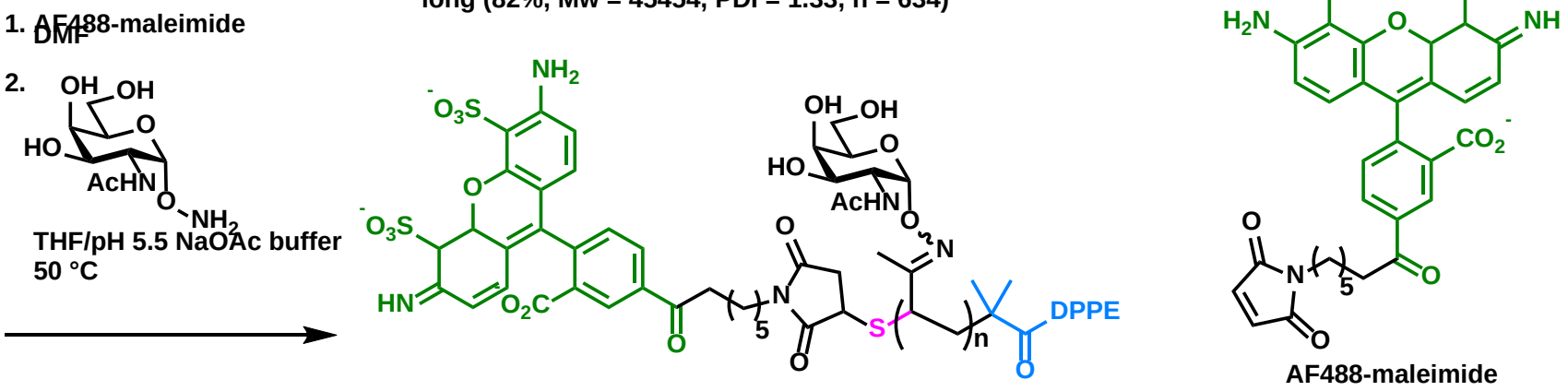


- **Integrins connect the ECM with intracellular actin cytoskeleton.**
- **Several observation indicated that integrin is involved with tumor cell survival, migration, invasion, and proliferation.[2)]**

1) Sun, Z.; Guo, S. S.; Fässler, R. *J. Cell Biol.* **2016**, *215*, 1. 2) Desgrosellier, J. S.; Cheresh, D. A. *Nat. Rev. Cancer* **2010**, *10*, 9.

2-2. RAFT Polymers for the Analysis



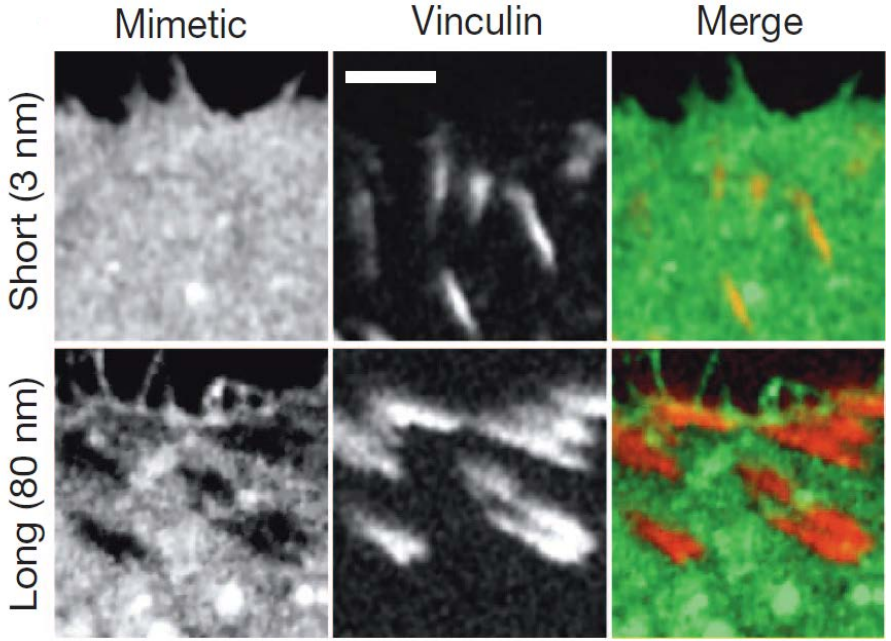


mutin mimetic	yield/%	GalNAc loading/%	Mw	AF488/polymer	estimated length/nm
short	70	92	8700	0.7	3
medium	76	90	64900	0.6	30
long	93	89	168900	0.5	80

chain length was estimated from MM2 force field-based molecular mechanics prediction, DLS, and TEM.

1) Paszek, M. J.; DuFort, C. C.; Rossier, O.; Bainer, R.; Mouw, J. K.; Godula, K.; Hudak, J. E.; Lakins, J. N.; Wijekoon, A. C.; Cassereau, L.; Rubashkin, M. G.; Magbanua, M. J.; Thorn, K. S.; Davidson, M. W.; Rugo, H. S.; Park, J. W.; Hammer, D. A.; Giannone, G.; Bertozzi, C. R.; Weaver, V. M. *Nature* **2014**, *511*, 319.

2-3. Integrin Clustering Driven by Glycopolymer



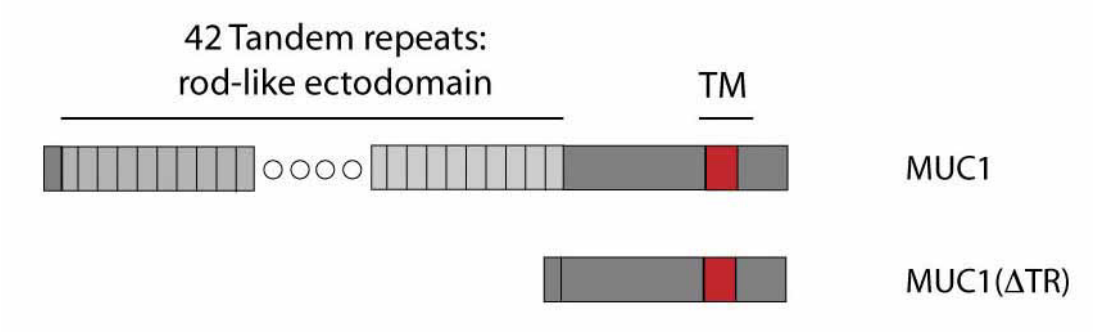
cells: glycopolymer (3 nm or 80 nm)-introduced non-malignant mammary epithelial cell (MCF-10A)
vinculin: adapter protein

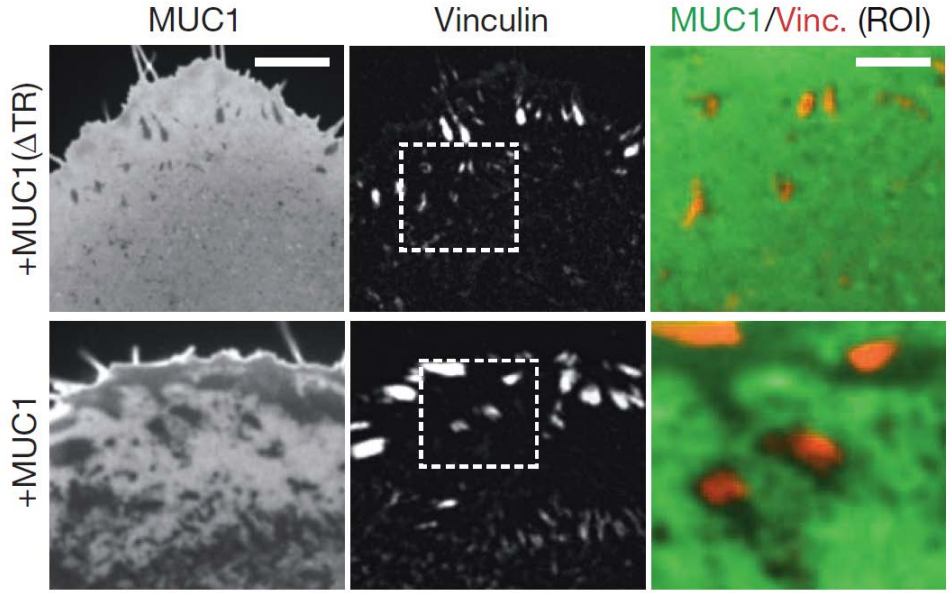
scale bar: 3 μm

Longer glycopolymer are excluded from the sites of integrin adhesion.

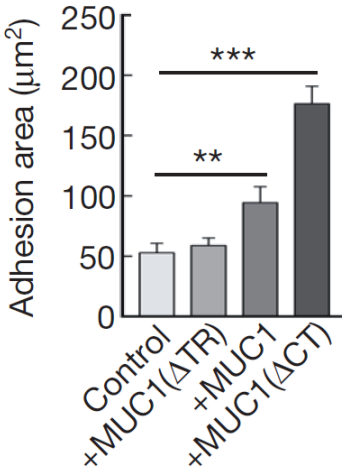
1) Paszek, M. J.; DuFort, C. C.; Rossier, O.; Bainer, R.; Mouw, J. K.; Godula, K.; Hudak, J. E.; Lakins, J. N.; Wijekoon, A. C.; Cassereau, L.; Rubashkin, M. G.; Magbanua, M. J.; Thorn, K. S.; Davidson, M. W.; Rugo, H. S.; Park, J. W.; Hammer, D. A.; Giannone, G.; Bertozzi, C. R.; Weaver, V. M. *Nature* **2014**, *511*, 319.

2-4. Integrin Clustering Driven by MUC1





scale bar: 3 μm [region of interest (ROI): 1.5 μm]

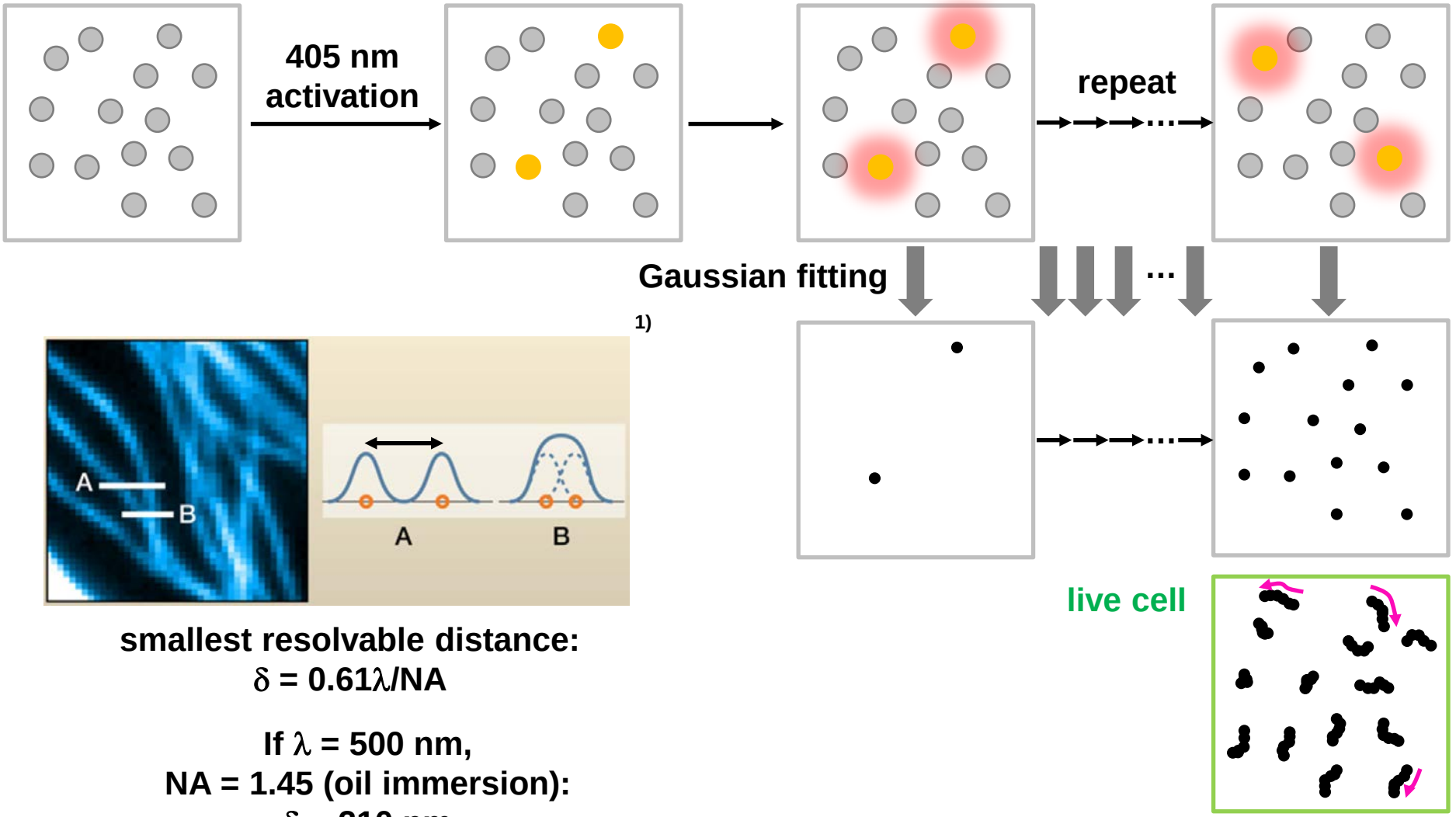


Full-length MUC1 enhanced the size of adhesion clusters/total adhesion area.

1) Paszek, M. J.; DuFort, C. C.; Rossier, O.; Bainer, R.; Mouw, J. K.; Godula, K.; Hudak, J. E.; Lakins, J. N.; Wijekoon, A. C.; Cassereau, L.; Rubashkin, M. G.; Magbanua, M. J.; Thorn, K. S.; Davidson, M. W.; Rugo, H. S.; Park, J. W.; Hammer, D. A.; Giannone, G.; Bertozzi, C. R.; Weaver, V. M. *Nature* **2014**, *511*, 319.

2-5. sptPALM

sptPALM: single particle tracking photoactivated localization microscopy

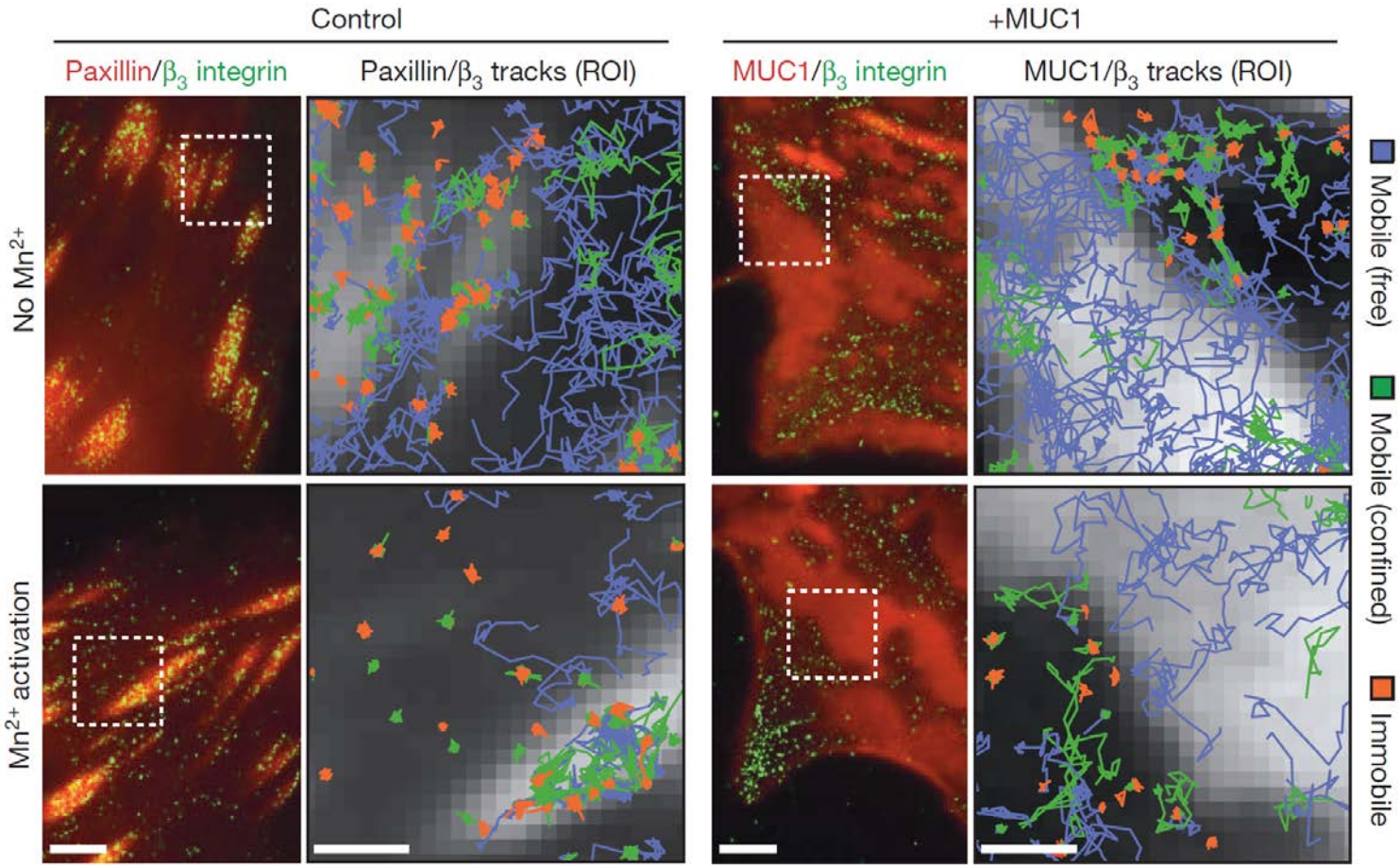


smallest resolvable distance:
$\delta = 0.61\lambda/NA$

If λ = 500 nm,
NA = 1.45 (oil immersion):
δ = 210 nm

1) Huang, B.; Babcock, H.; Zhuang, X. *Cell* **2010**, *143*, 1047.

2-6. Integrin Dynamics on Cell Surface

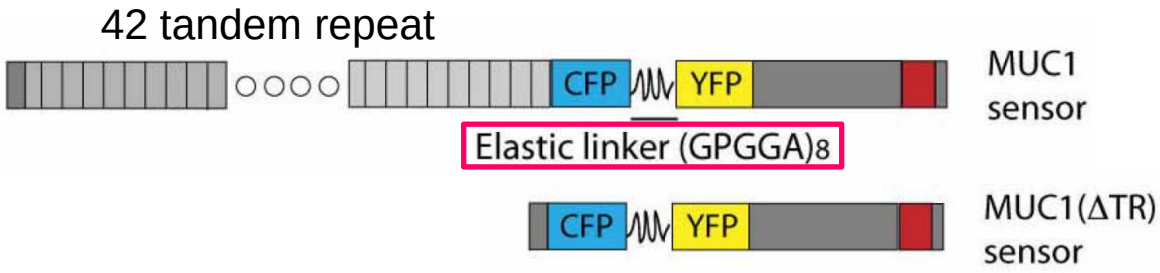


cells: mouse embryotic fibroblast (MEF) expressing paxillin-GFP or MUC1-GFP/mEOS2-fused β_3 integrin, scale bar: 3 μm

Immobilized integrin in MEF expressing high MUC1 was restricted to sites of adhesion.

1) Paszek, M. J.; DuFort, C. C.; Rossier, O.; Bainer, R.; Mouw, J. K.; Godula, K.; Hudak, J. E.; Lakins, J. N.; Wijekoon, A. C.; Cassereau, L.; Rubashkin, M. G.; Magbanua, M. J.; Thorn, K. S.; Davidson, M. W.; Rugo, H. S.; Park, J. W.; Hammer, D. A.; Giannone, G.; Bertozzi, C. R.; Weaver, V. M. *Nature* **2014**, *511*, 319.

2-7. Strained Glycoprotein by Integrin Adhesion

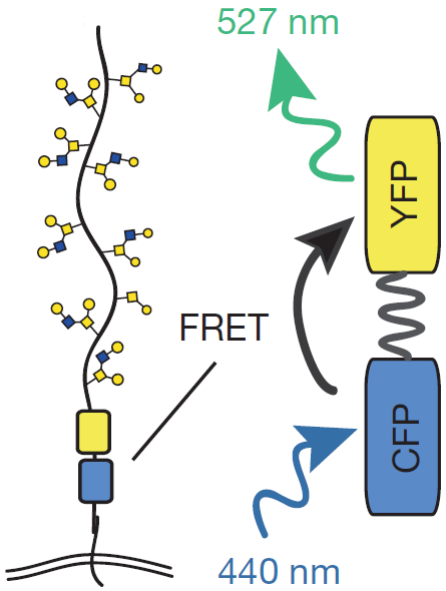


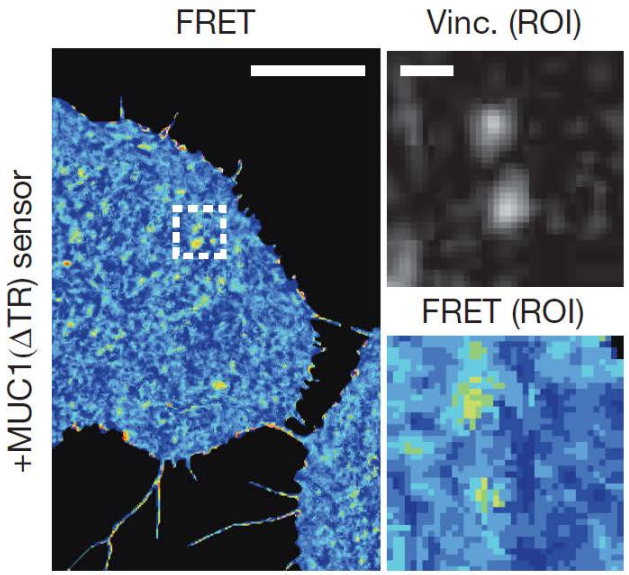
FRET efficiency/%
$= [1-(I_{pre}- B_{pre})/(I_{post} - B_{post})] \times 100$

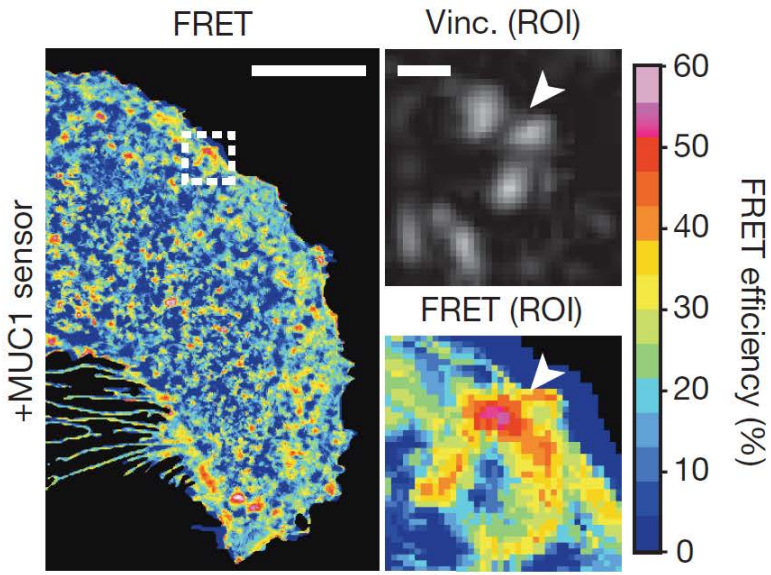
I_{pre}: CFP intensity before bleaching YFP
B_{pre} : CFP channel background before bleaching YFP
I_{post}: CFP intensity after bleaching YFP
B_{post} : CFP channel background after bleaching YFP

"MUC1-based strain gauge"

cells: MCF-10A







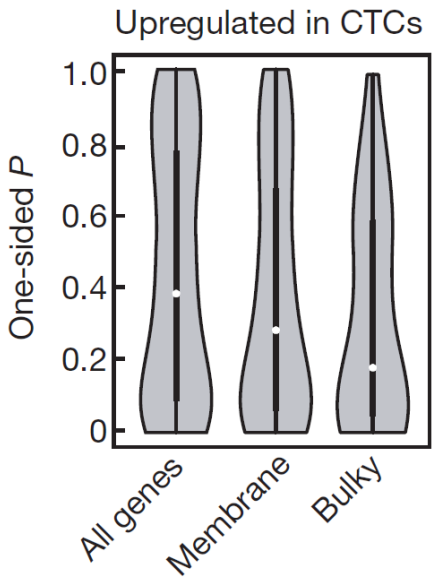
vinc.: vinculin

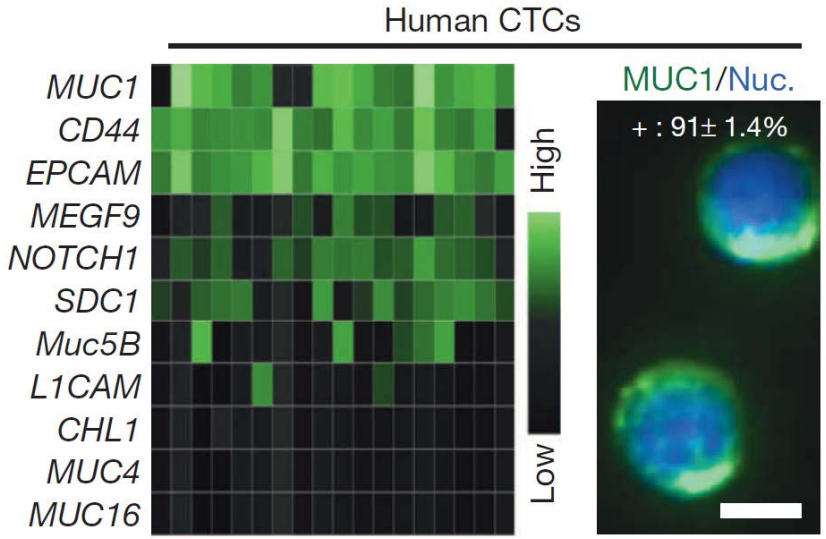
Highest FRET efficiency was observed in the sites of adhesive contact.

1) Paszek, M. J.; DuFort, C. C.; Rossier, O.; Bainer, R.; Mouw, J. K.; Godula, K.; Hudak, J. E.; Lakins, J. N.; Wijekoon, A. C.; Cassereau, L.; Rubashkin, M. G.; Magbanua, M. J.; Thorn, K. S.; Davidson, M. W.; Rugo, H. S.; Park, J. W.; Hammer, D. A.; Giannone, G.; Bertozzi, C. R.; Weaver, V. M. *Nature* **2014**, *511*, 319.

2-8. Bulky Glycoproteins in CTCs

Bulky glycoproteins
MUC12
MUC4
MUC16
DPCR1
MUC1
HAVCR1
CDH23
ROS1
STAB1
NRG2
GPR126
L1CAM
PRRT1
CHL1
DSCAM
SDC1
ROBO3

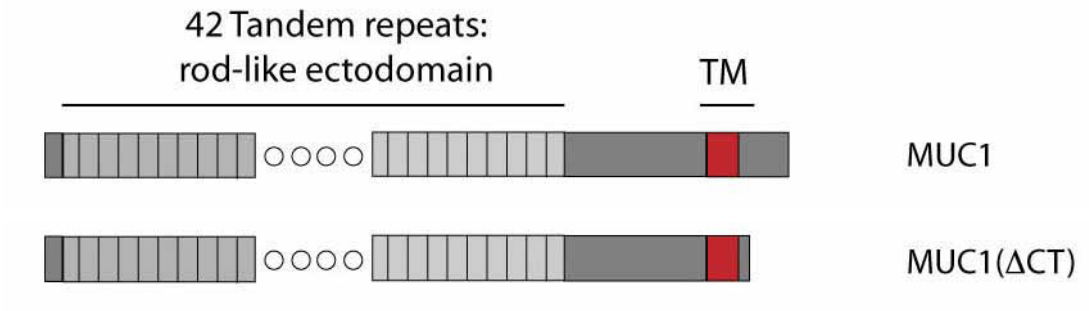




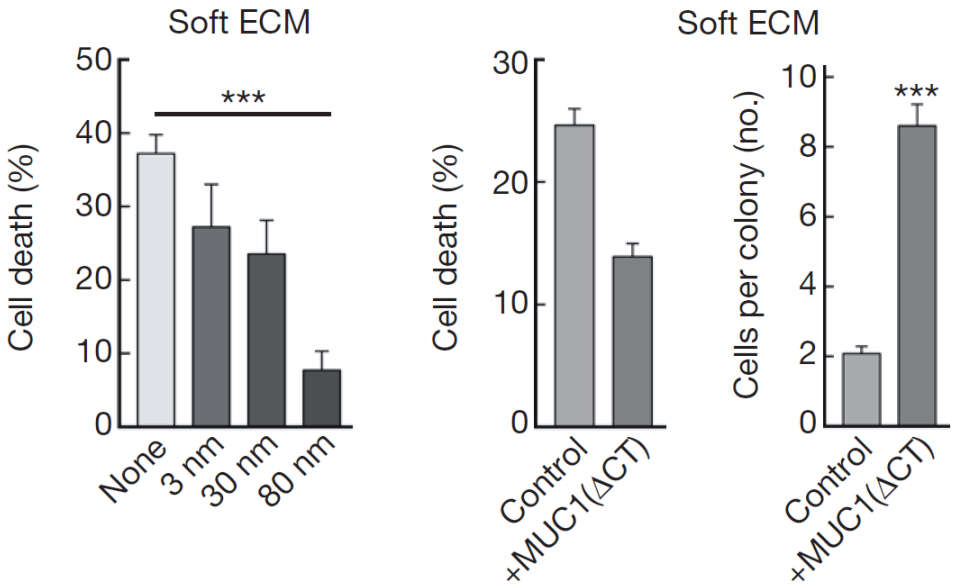
- **Bulky glycoproteins were highly expressed in patients with circulating tumor cells (CTCs).**
- **MUC1 was confirmed to be highly expressed in CTCs isolated from 18 breast cancer patients.**

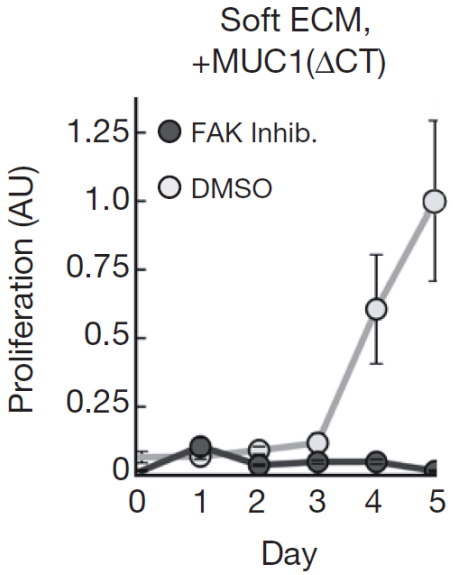
1) Paszek, M. J.; DuFort, C. C.; Rossier, O.; Bainer, R.; Mouw, J. K.; Godula, K.; Hudak, J. E.; Lakins, J. N.; Wijekoon, A. C.; Cassereau, L.; Rubashkin, M. G.; Magbanua, M. J.; Thorn, K. S.; Davidson, M. W.; Rugo, H. S.; Park, J. W.; Hammer, D. A.; Giannone, G.; Bertozzi, C. R.; Weaver, V. M. *Nature* **2014**, *511*, 319.

2-9. Bulky Glycoproteins Promote Survival of CTCs



cells: MCF-10A
soft ECM: soft polyacrylamide (Young's modulus E = 140 Pa) functionalized with fibronectin, mimicking soft sites of colonization (e.g. lung or brain)



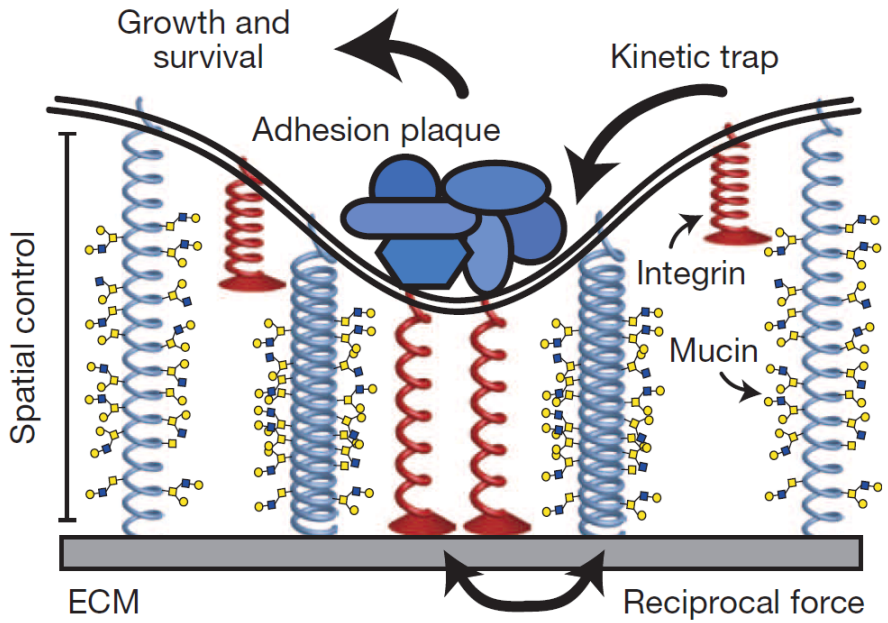


Cell death (MCF-10A) was reduced by long glycopolymer (80 nm) and MUC1 (ΔCT).

Growth and survival phenotype requires integrin signaling through FAK

1) Paszek, M. J.; DuFort, C. C.; Rossier, O.; Bainer, R.; Mouw, J. K.; Godula, K.; Hudak, J. E.; Lakins, J. N.; Wijekoon, A. C.; Cassereau, L.; Rubashkin, M. G.; Magbanua, M. J.; Thorn, K. S.; Davidson, M. W.; Rugo, H. S.; Park, J. W.; Hammer, D. A.; Giannone, G.; Bertozzi, C. R.; Weaver, V. M. *Nature* **2014**, *511*, 319.

2-10. Integrin-GCX-Mediated Mechanotransduction



Bulky glycocalyx component can physically influence receptor organization such as integrin clustering and the activity.

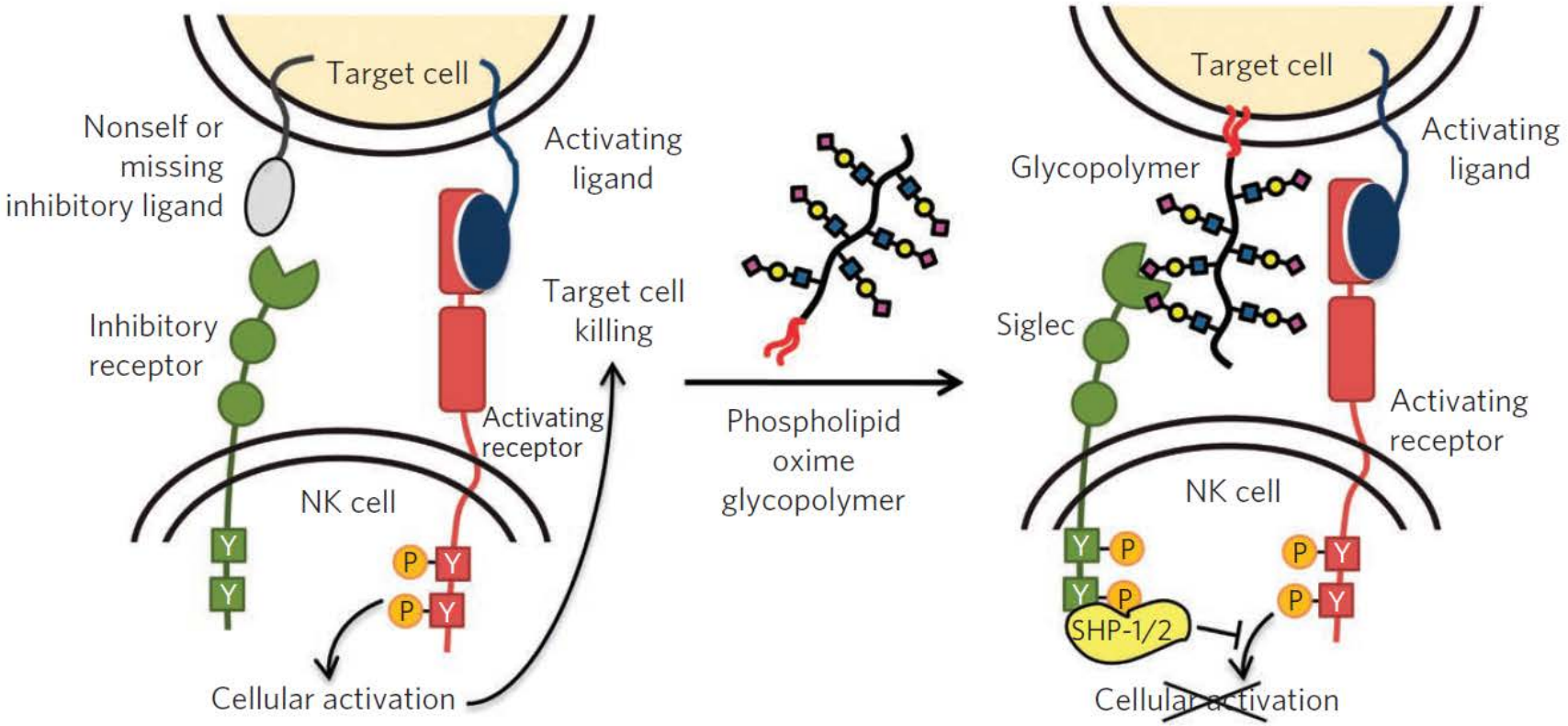
1) Paszek, M. J.; DuFort, C. C.; Rossier, O.; Bainer, R.; Mouw, J. K.; Godula, K.; Hudak, J. E.; Lakins, J. N.; Wijekoon, A. C.; Cassereau, L.; Rubashkin, M. G.; Magbanua, M. J.; Thorn, K. S.; Davidson, M. W.; Rugo, H. S.; Park, J. W.; Hammer, D. A.; Giannone, G.; Bertozzi, C. R.; Weaver, V. M. *Nature* **2014**, *511*, 319.

3-1. Siglec-Based Immunoevasion

Upregulation of sialic acid on the surface of cancer cells is known to lead to decresed immunogenicity and natural killer (NK) cell resistance

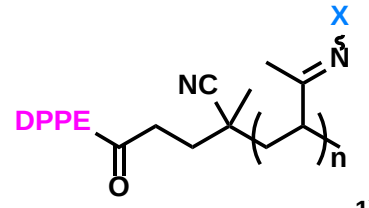
Molecular details between hypersialylation and immunoevasion remains to be solved.

concept: remodeling sialylation status of cancer cell surface by glycopolymer



1) Hudak, J. E.; Canham, S. M.; Bertozzi, C. R. *Nat. Chem. Biol.* **2014**, *10*, 69.

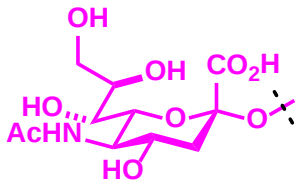
3-2. Origosaccharide Panel



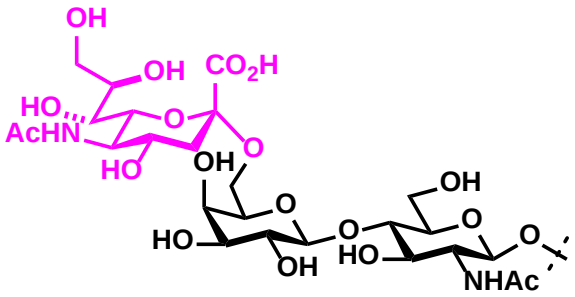
DPPE-MVK-oxime polymer[1)]
M_w = 26000, PDI = 2.89

Various aminooxy glycans were prepared by chemical/chemoenzymatic synthesis.[2)]

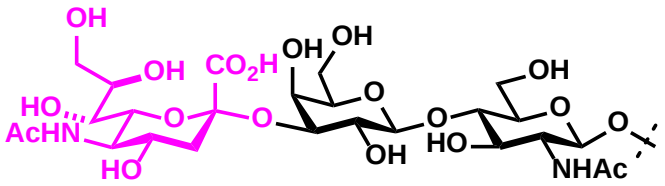
X =



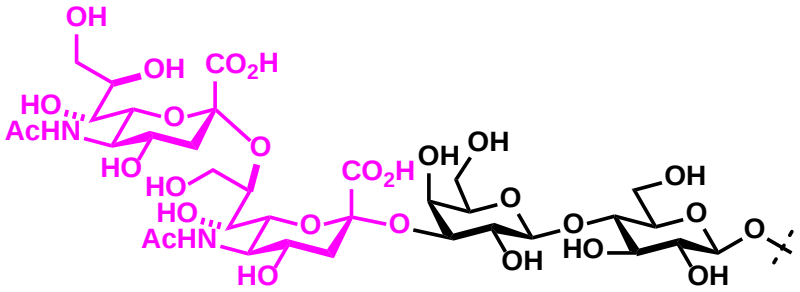
Sia



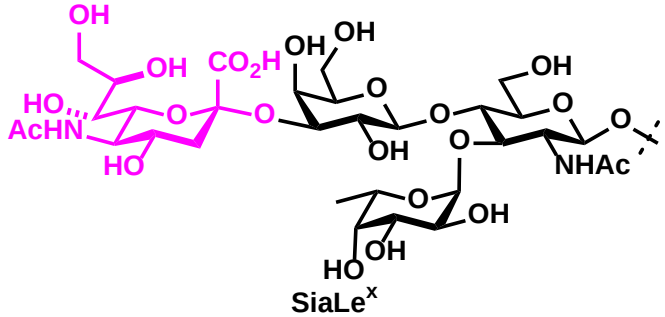
2,6-SiaLacNAc



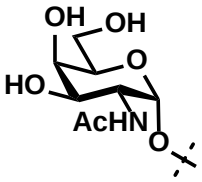
2,3-SiaLacNAc



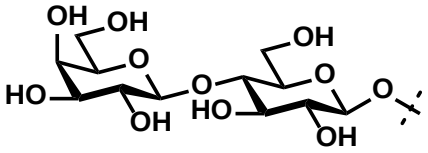
GD3



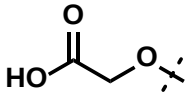
$SiaLe^x$



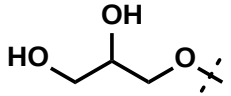
GalNAc



lactose



acetic acid

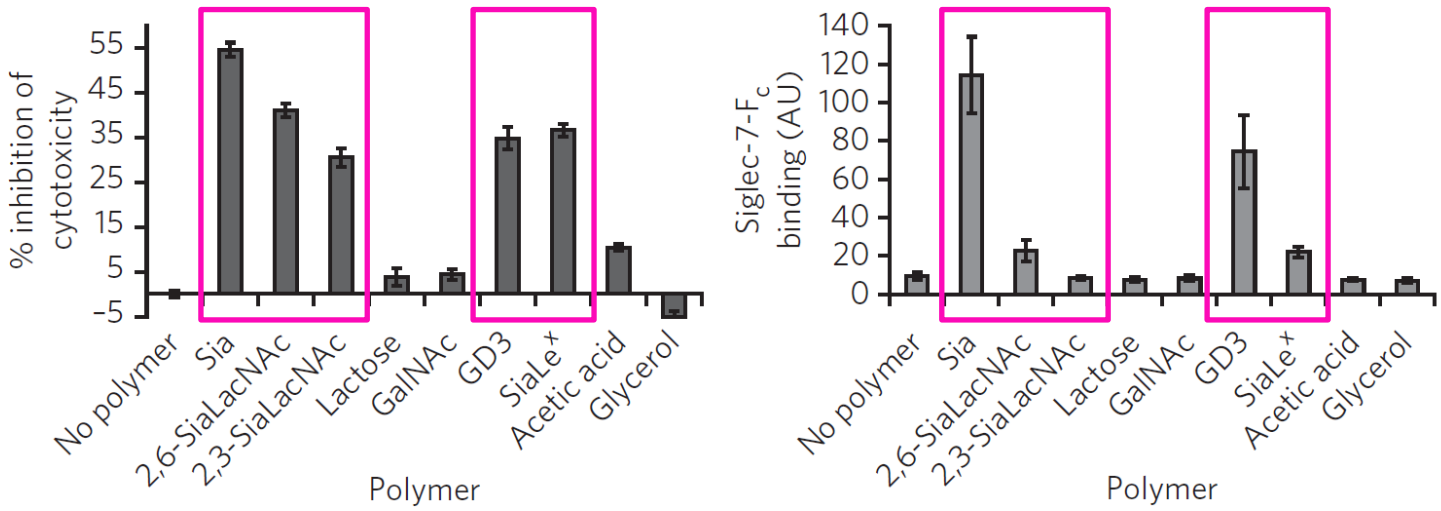


glycerol

1) Hudak, J. E.; Canham, S. M.; Bertozzi, C. R. *Nat. Chem. Biol.* **2014**, *10*, 69. 2) Hudak, J. E.; Yu, H. H.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2011**, *133*, 16127.

3-3. Protection Effect of Glycopolymer

: sialic acid-conjugated polymer

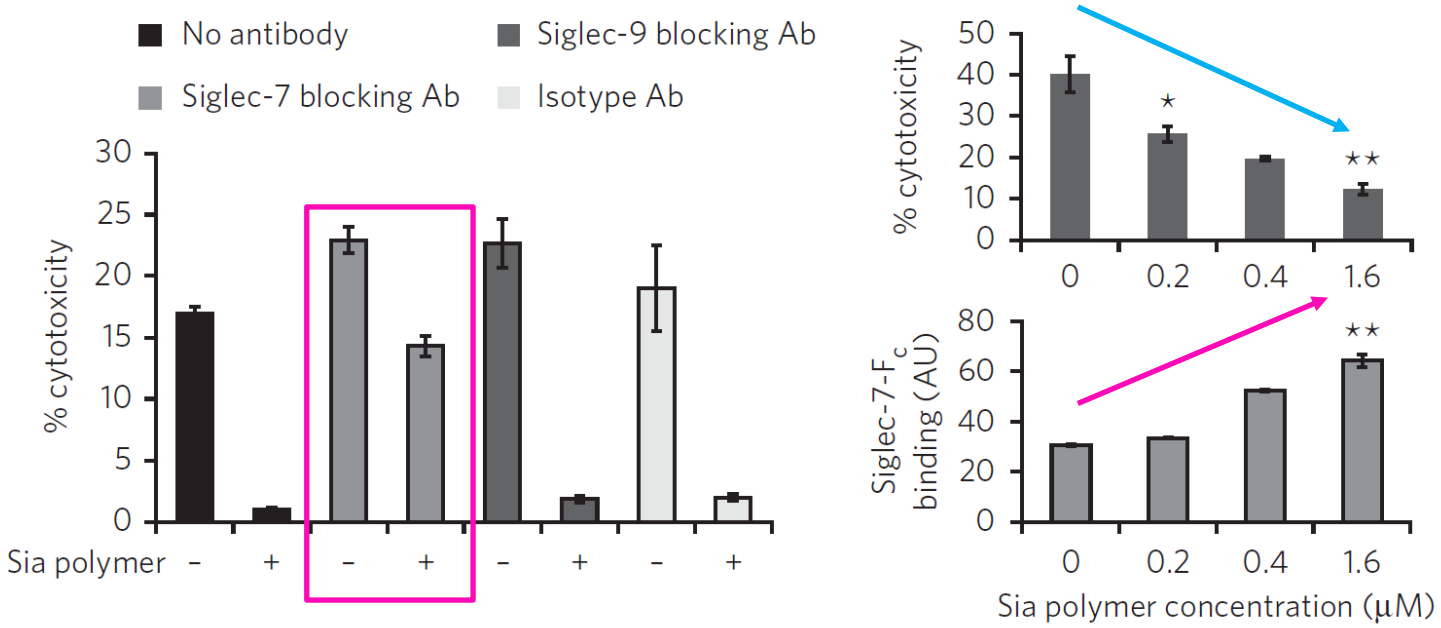


cells: Jurkat cells
polymer conc.: 1 μM
incubation: 4 h with purified NK cells (effector/target = 3:1)
Siglec-7-Fc: soluble siglec-7-Fc chimera protein (detected on flow cytometry, labelled by anti-human Fc-647)

Sia polymer exhibited the strongest protection against NK-mediated killing.

1) Hudak, J. E.; Canham, S. M.; Bertozzi, C. R. *Nat. Chem. Biol.* **2014**, *10*, 69.

3-4. Protection Effect of Glycopolymer

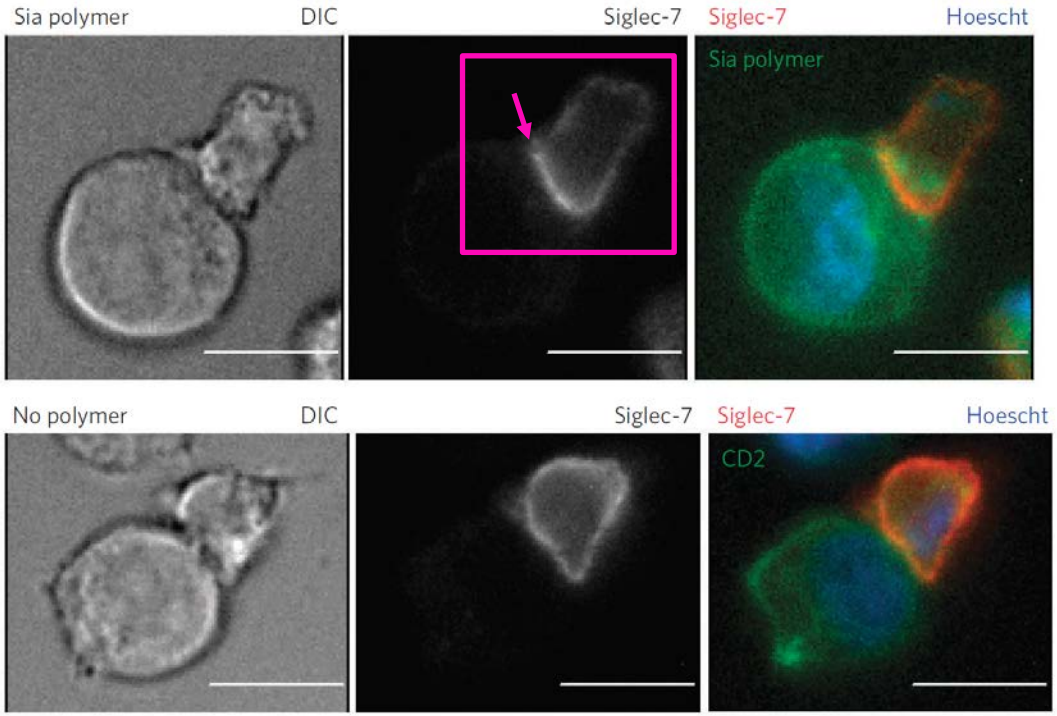


cells: Jurkat cells
incubation: 4 h with purified NK cells (effector/target = 4:1)

- **Protection effect was diminished by Siglec-7 blocking antibody.**
- **Protection was dependent on the Sia polymer density.**

1) Hudak, J. E.; Canham, S. M.; Bertozzi, C. R. *Nat. Chem. Biol.* **2014**, *10*, 69.

3-5. Recruiting Siglec-7 by Sia Polymer

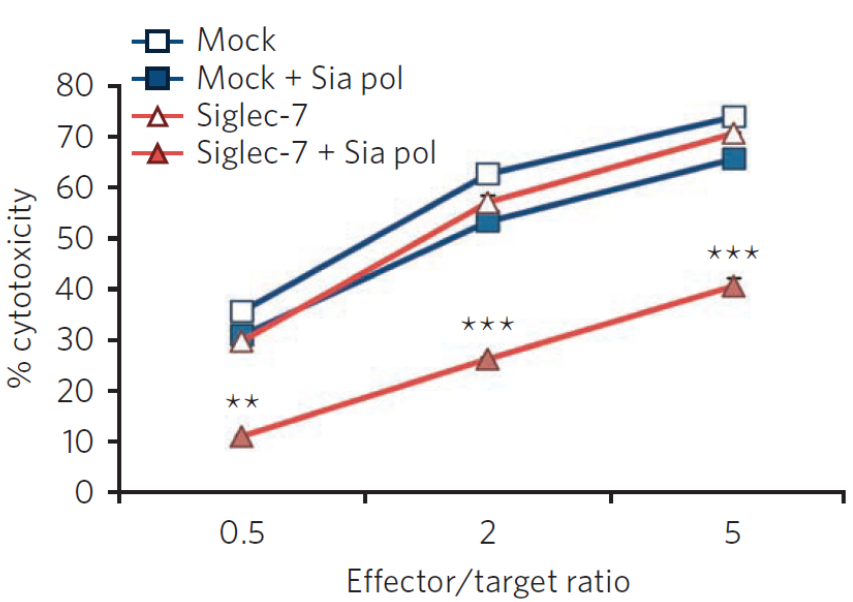


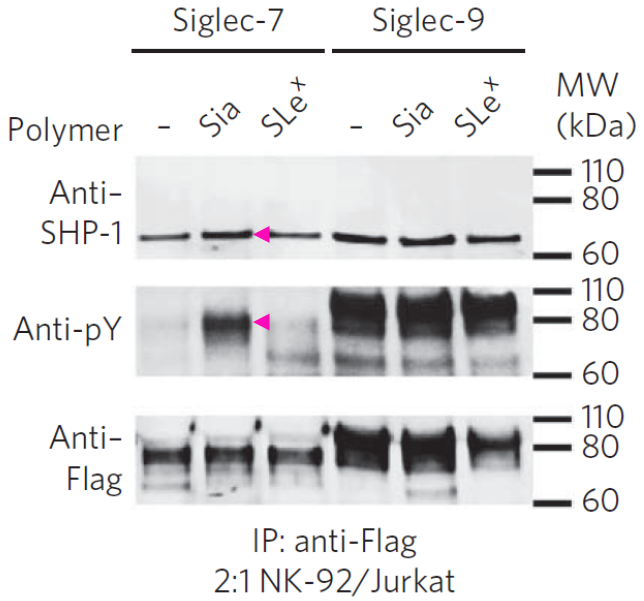
cells: Jurkat cells/NK cells, fixed and stained by antibodies
polymer: Alexa-Fluor 488-Sia polymers
scale bar: 10 μm

Recruiting Siglec-7 by Sia polymer was observed.

1) Hudak, J. E.; Canham, S. M.; Bertozzi, C. R. *Nat. Chem. Biol.* **2014**, *10*, 69.

3-6. Reduced NK Cell-Promoted Cytotoxicity





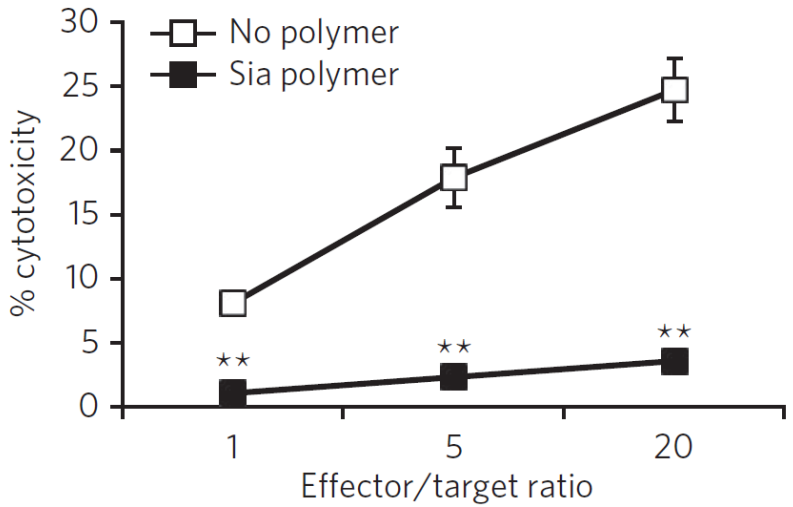
cells: Jurkat cells treated with Sia polymer/NK-92 cells overexpressing Flag-tagged Siglec-7
anti-pY: anti-phosphotyrosine antibody
NK-92 cells: highly cytotoxic human NK line exhibiting low expression of inhibitory receptors

NK-92 cells expressing Siglec-7 were susceptible to Sia polymer inhibition.

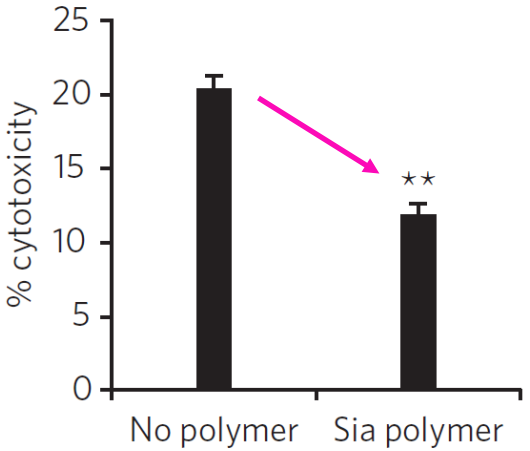
1) Hudak, J. E.; Canham, S. M.; Bertozzi, C. R. *Nat. Chem. Biol.* **2014**, *10*, 69.

3-7. Protection of Xenogeneic and Allogeneic Cells

CD34⁺ HSC from bone marrow



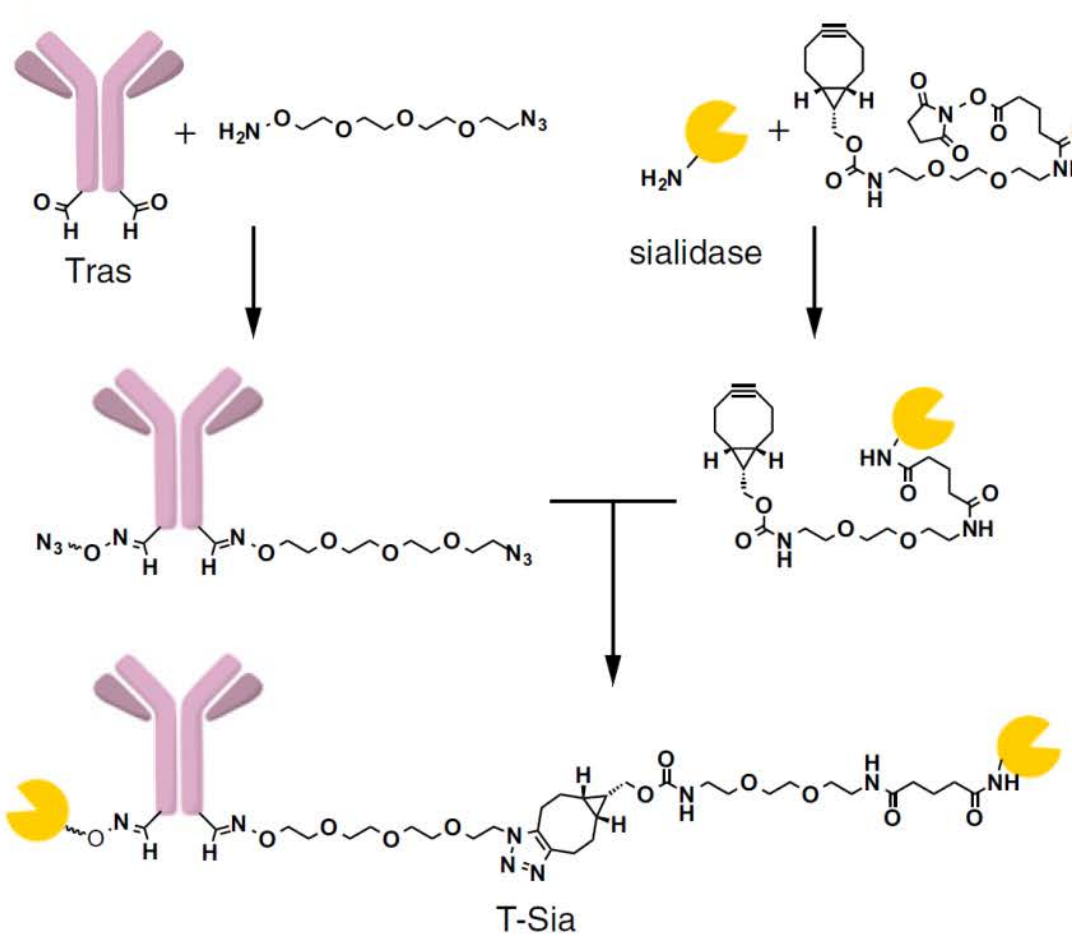
pig aortic epithelial cells

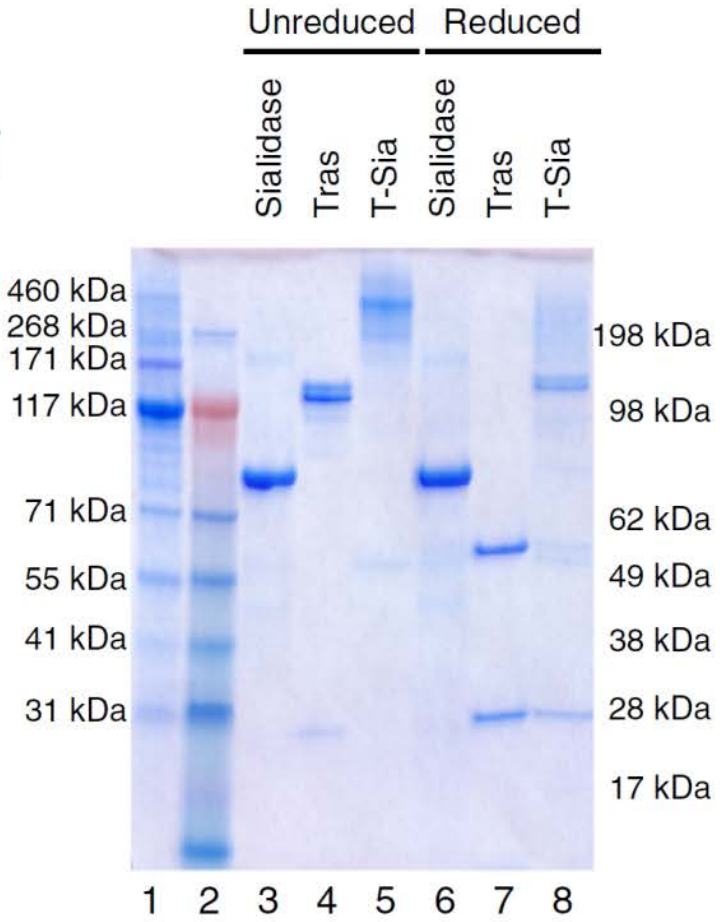


Addition of Sia polymer reduced NK-induced cytotoxicity against xenogenic porcine and allogenic hematopoietic stem cells.

1) Hudak, J. E.; Canham, S. M.; Bertozzi, C. R. *Nat. Chem. Biol.* **2014**, *10*, 69.

4-1. Synthesis of T-Sia for Glycocalyx Editing



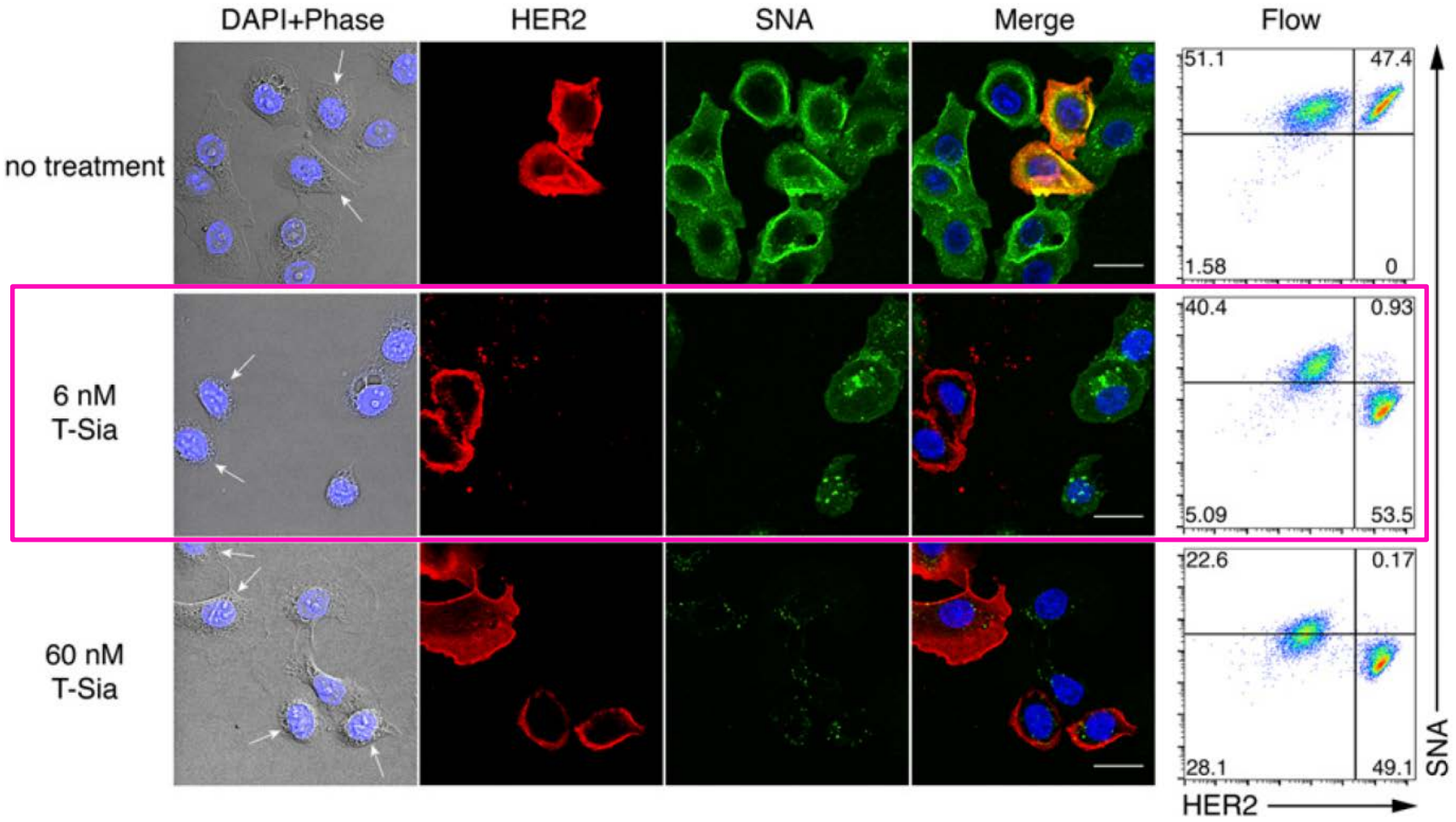


Tras-N_3: light chain 23 kDa/heavy chain 50 kDa
T-Sia: light chain 23 kDa/conjugated heavy chain 134 kDa

Sialidase-conjugated trastuzumab (Tras) was prepared for glycocalyx editing.

1) Xiao, H.; Woods, E. C.; Vukojicic, P.; Bertozzi, C. R. *Proc. Natl. Acad. Sci.* **2016**, *113*, 10304.

4-2. Glycocalyx Editing by T-Sia

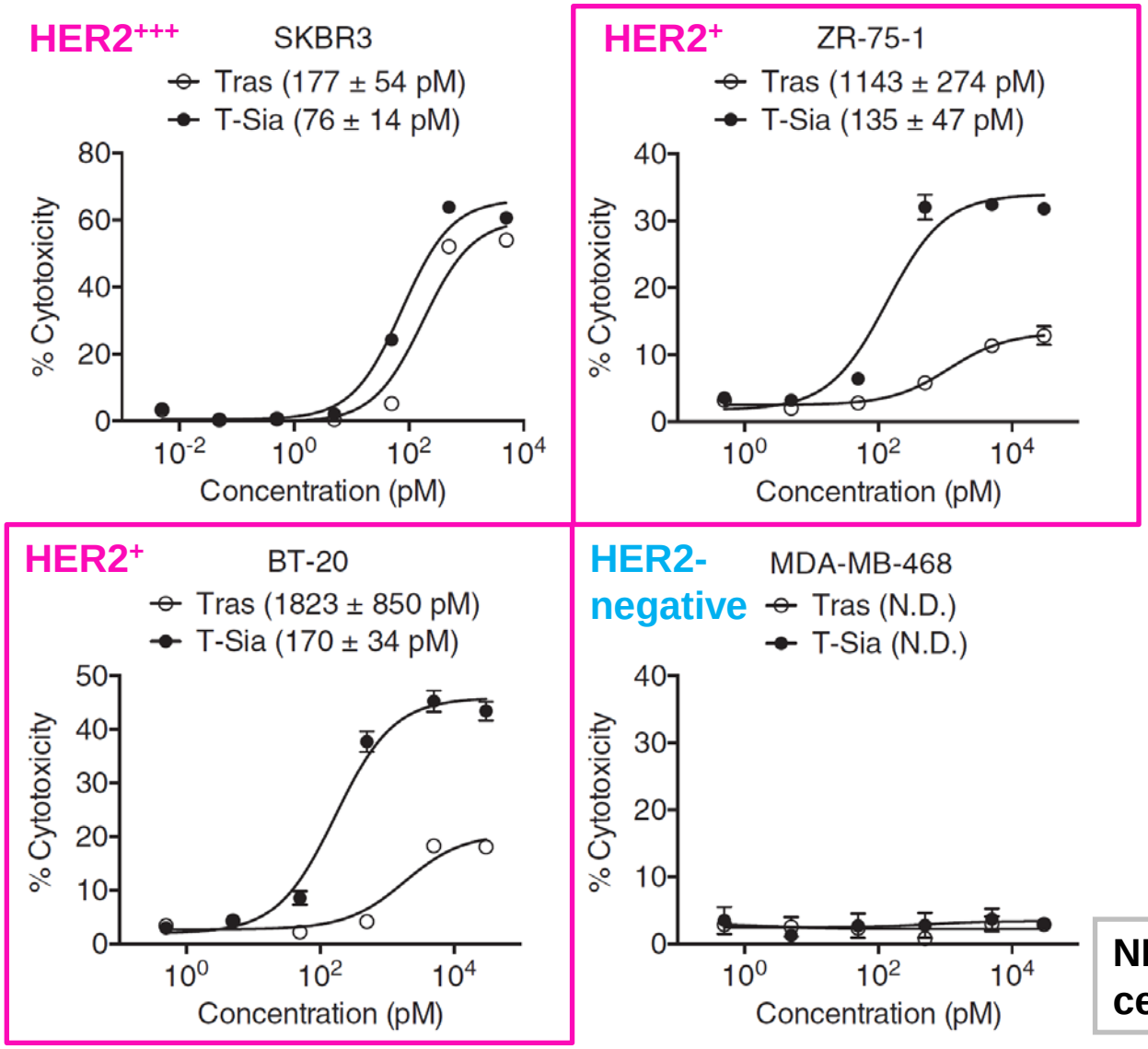


cells: coculturing SKBR3 (HER2^{+++})/MDA-MB-468 (HER2-negative)
staining: FITC-conjugated Sambucus nigra agglutinin (SNA, recognition of sialic acid)/Alexa Fluor 647-labeled anti-HER2 (HER2)

T-Sia (6 nM) selectively desialylated HER-2-positive cells.

1) Xiao, H.; Woods, E. C.; Vukojicic, P.; Bertozzi, C. R. *Proc. Natl. Acad. Sci.* **2016**, *113*, 10304.

4-3. T-Sia Enhances NK-Promoted Cytotoxicity

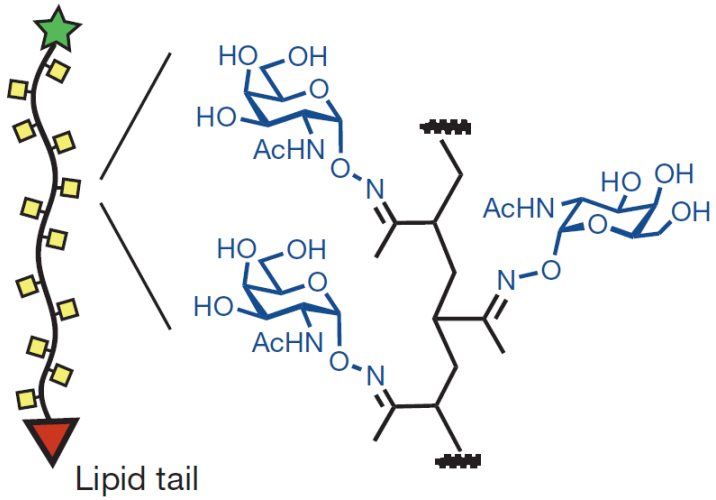


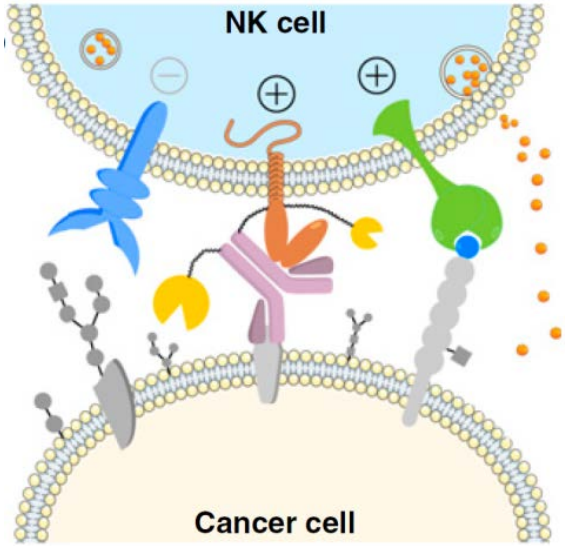
NK cells/target cells = 4:1

T-Sia was more potent than Tras alone in NK-mediated cell killing against the cell lines expressing lower levels of HER2.

1) Xiao, H.; Woods, E. C.; Vukojicic, P.; Bertozzi, C. R. *Proc. Natl. Acad. Sci.* **2016**, *113*, 10304.

5. Summary and Future Perspective





Understanding the functions of glycocalyx by using the glycomimetics was valuable in the context of glycobiology.

Seeking of the methods for editing glycocalyx on cancer cells potentially enables us to develop new cancer therapeutic methods.