New Tools to Study and Perturb the Glycocalyx

Glycobiology 2015

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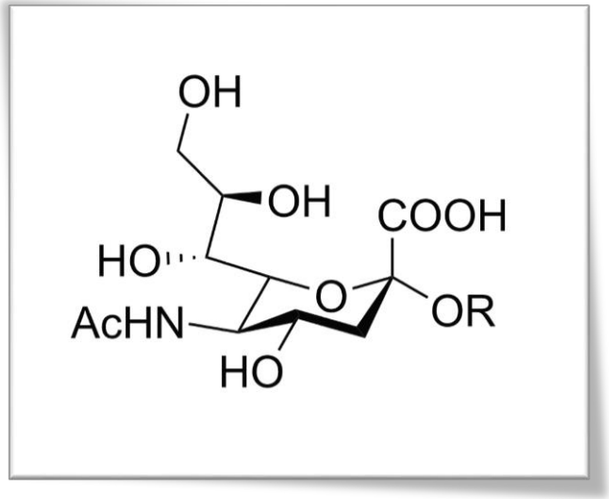
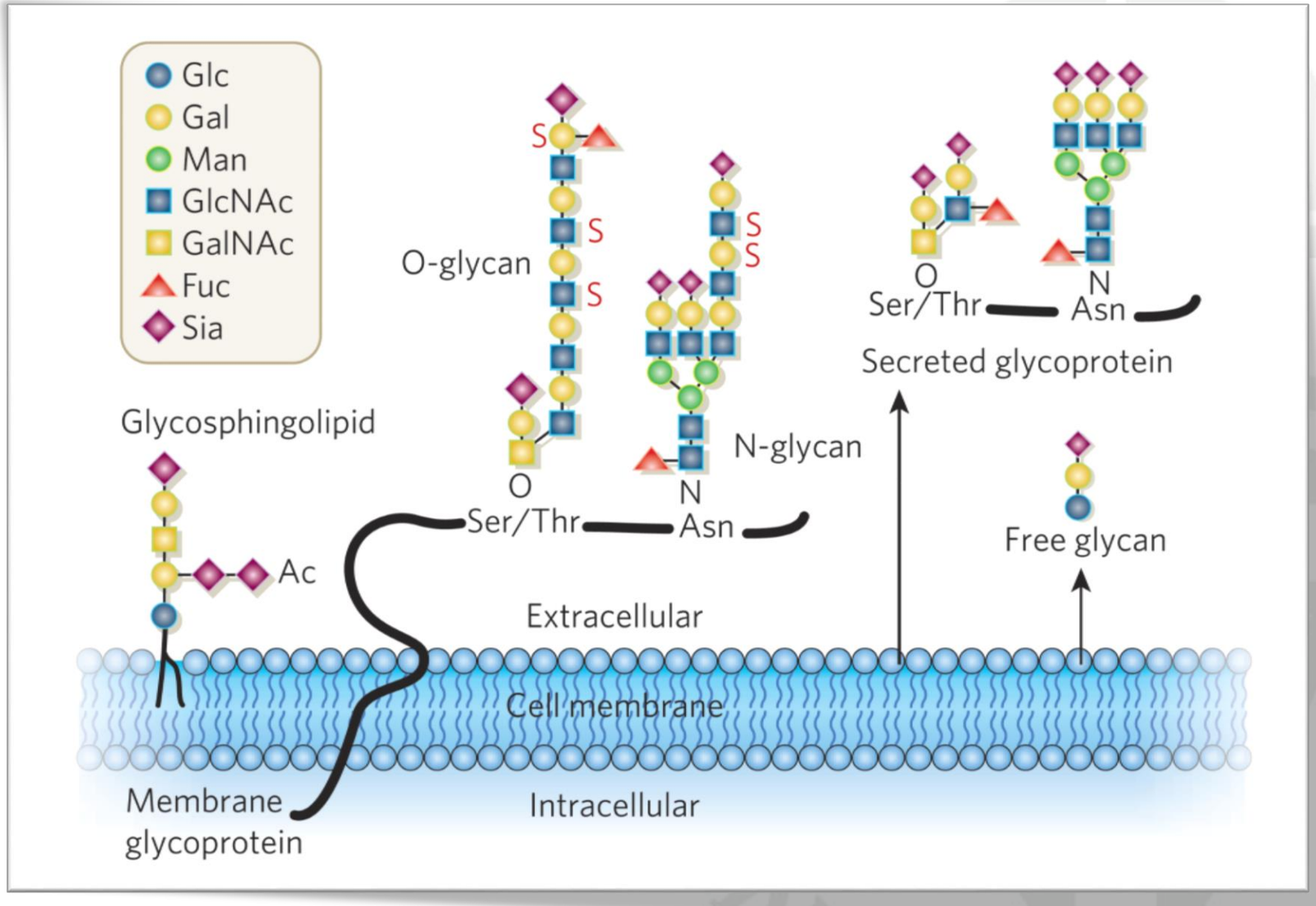
Sialic Acid Structure and Function



- Sialic acids: a family of over of >50 2-keto-3-deoxynononic acids

- Found on the termini of glycan chains

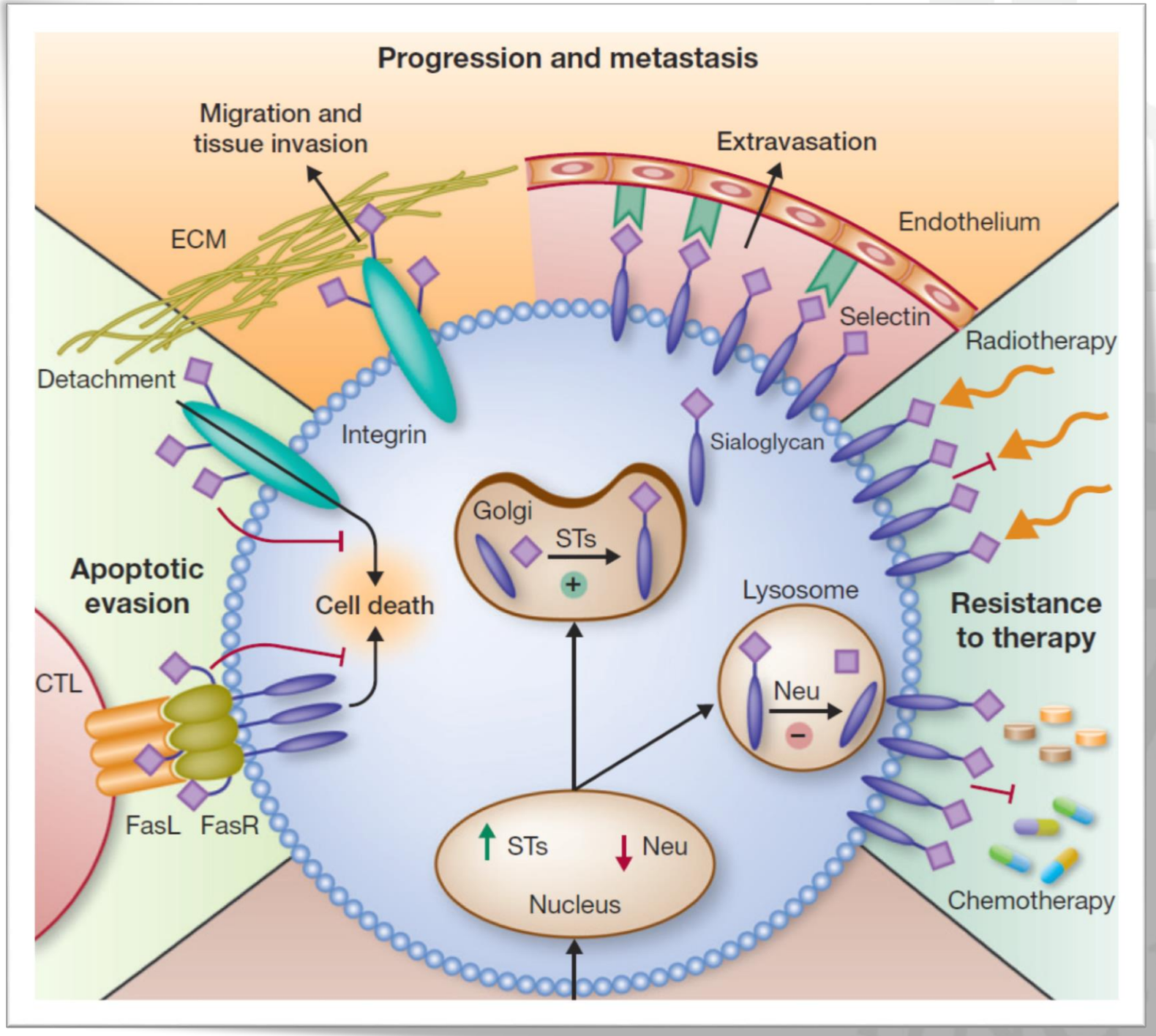
- Play an important role in the immune response



Sialic acid sweetens a tumors life

Cancer cells are oversialylated

- Immune Evasion
- Resistance to Apoptosis
- Migration and Metastasis
- Resistance to Therapy
- Poor Prognosis



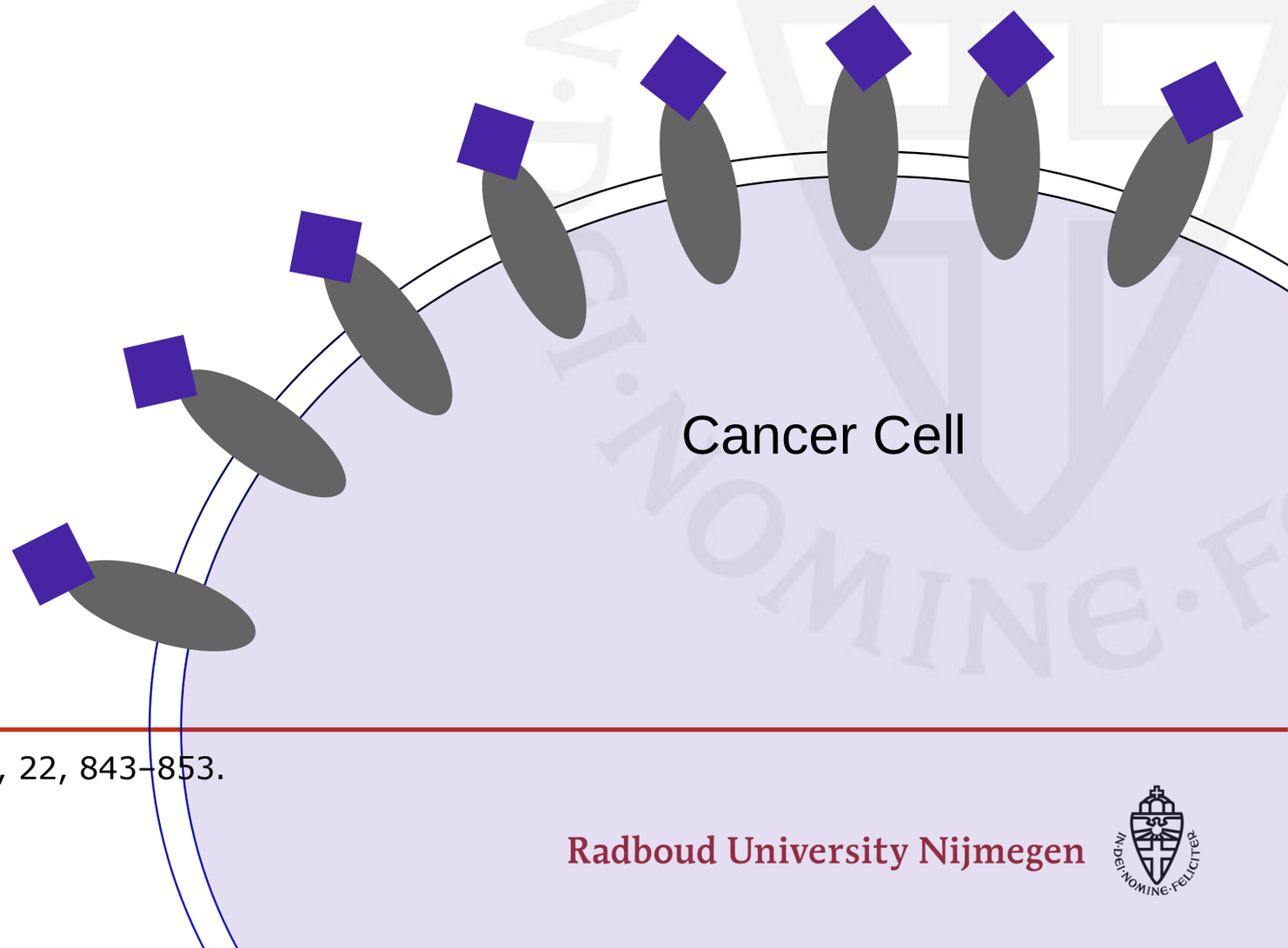
Büll C, Stoel M.A., den Brok M.H, Adema G.J., *Cancer. Res,* **2014**, *74*, 3199-3204.
Büll C, den Brok MH, Adema G.J., *Biochim. Biophys. Act. Rev. Cancer.* **2014**, *1846*, 238-246.

Reducing Oversialylation in Cancer

Sialidase treatment:

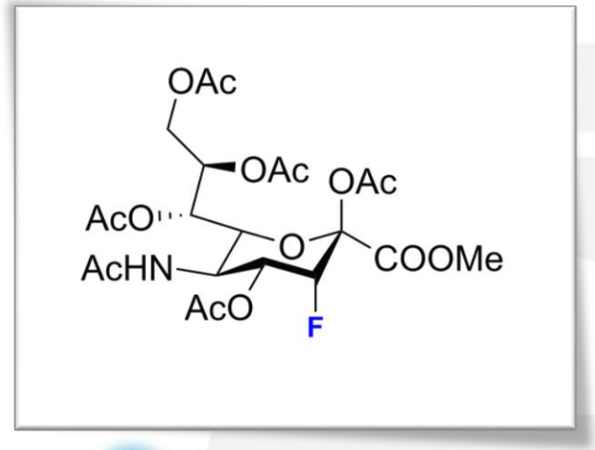
- Promising results
- Often contaminated
- Short lived effect
- Difficult to study effect on immune response

Removal

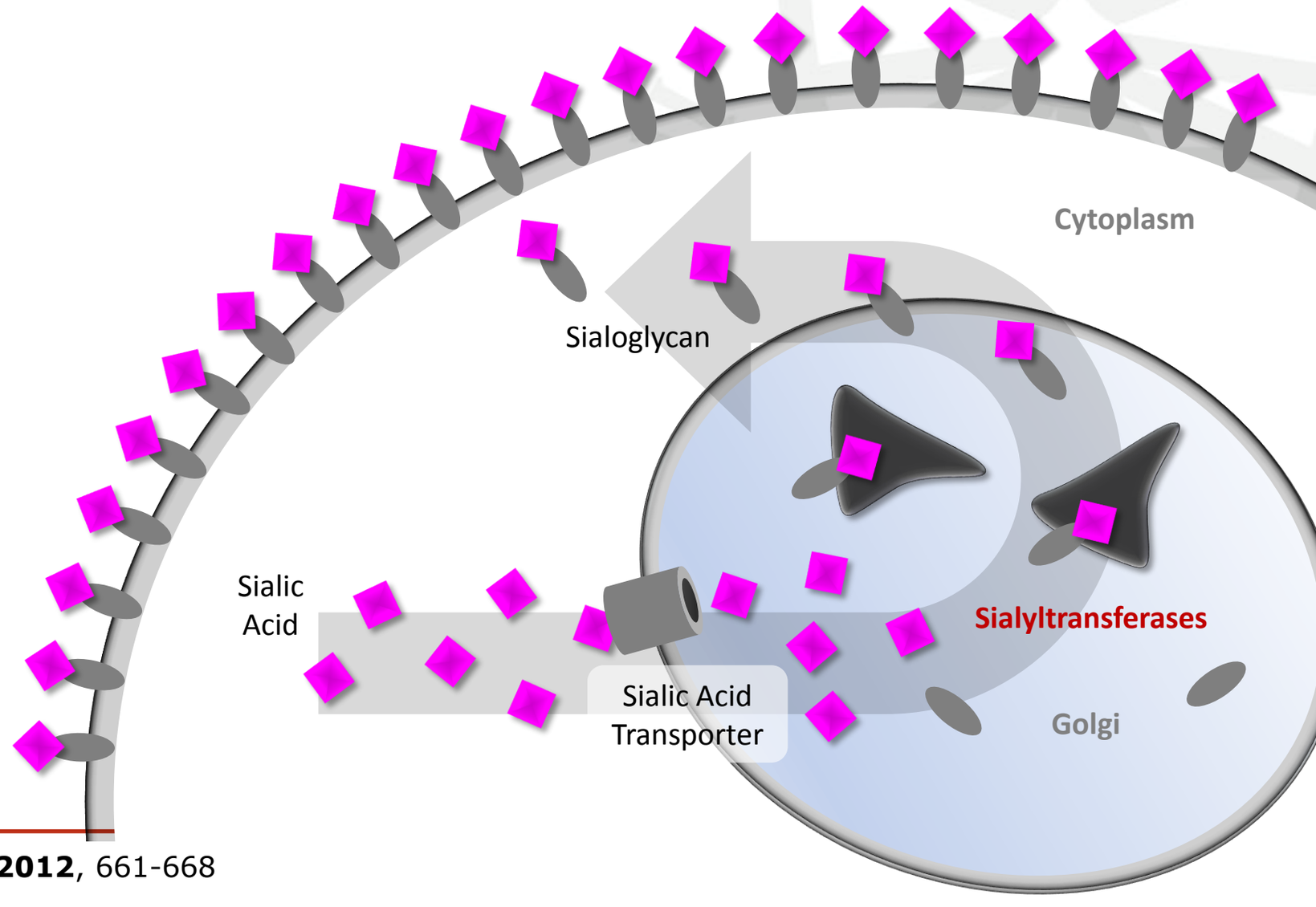


Currie G.A., Bagshawe K.D., *British Journal of Cancer* **1968**, 22, 843–853.
Sanford, B.H. *Transplantation*, **1967**.

Reducing Oversialylation in Cancer

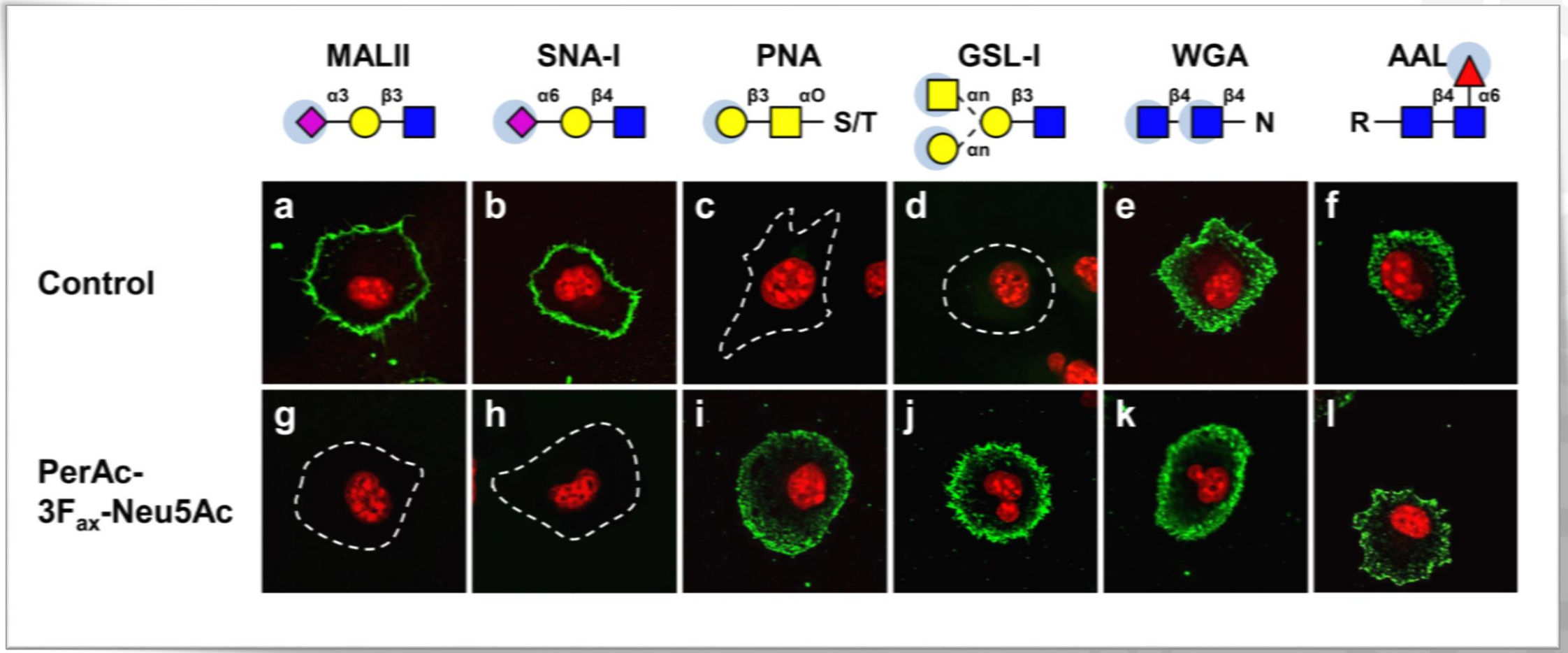


P-3F$_{ax}$-Neu5Ac



Rillahan C.D., Paulson J.C., *et.al.*, *Nat. Chem.Biol.* **2012**, 661-668

Inhibition of sialylation slows tumor growth and metastasis



- Cell culture with 64μM concentration of inhibitor leads to almost complete desialylation
- Cell viability is not affected

Büll C, Boltje T.J., Adema G.J. *et.al.*, *ACS Nano.* **2015**, *9,* 733-745.
Büll C, Boltje T.J., Adema G.J. *et.al.*, *Mol. Cancer Ther.* **2013**, *12,* 1935-1946.

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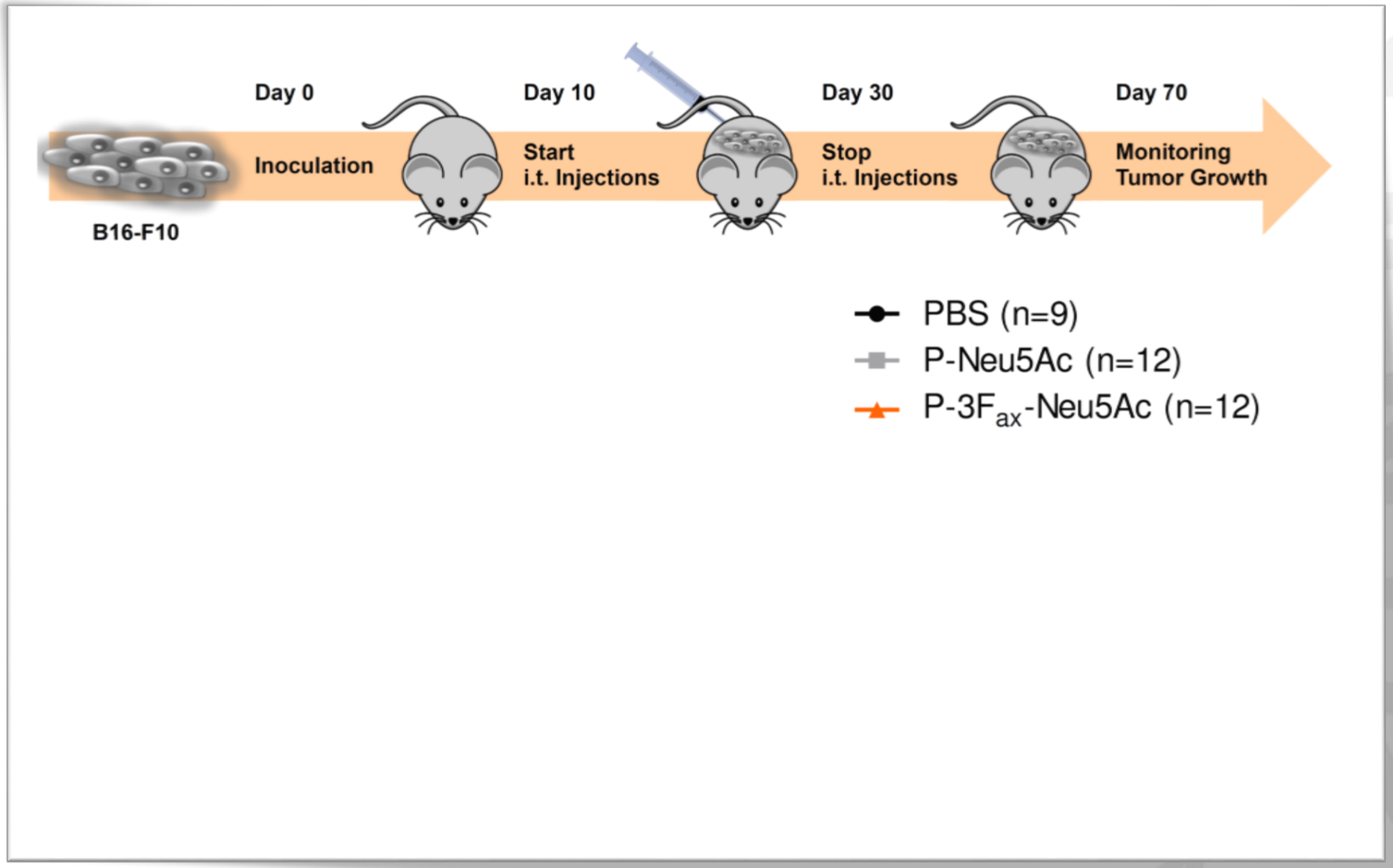

- Control groups treated with PBS, or sialic acid died after 40 days
- 60% of the treated group survived

Büll C, Boltje T.J., Adema G.J. *et.al.*, *ACS Nano.* **2015**, *9,* 733-745.
Büll C, Boltje T.J., Adema G.J. *et.al.*, *Mol. Cancer Ther.* **2013**, *12,* 1935-1946.

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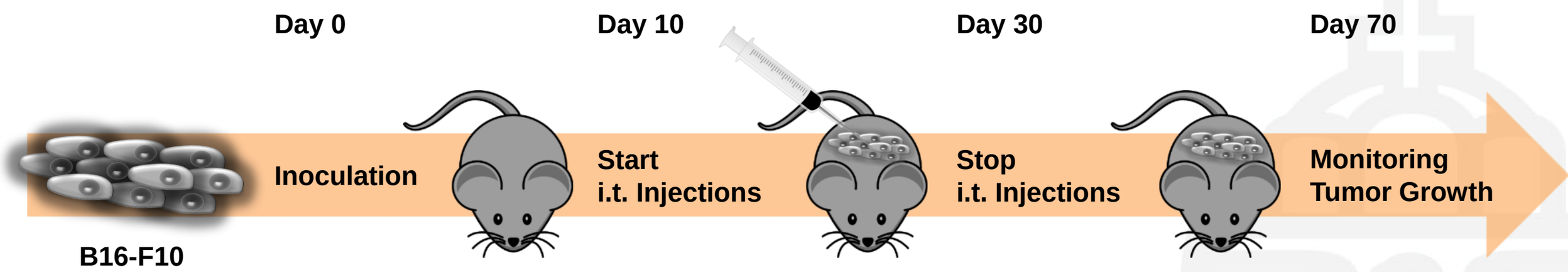

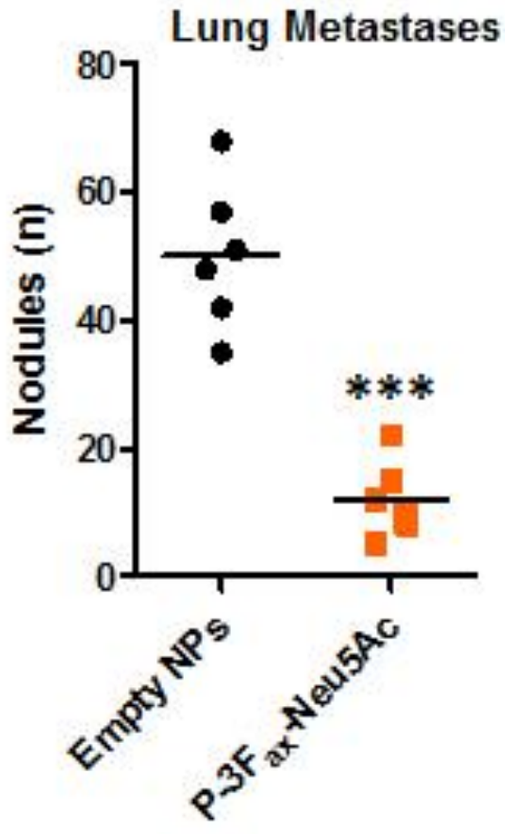

Mechanism?

Büll C, Boltje T.J., Adema G.J. *et.al.*, *ACS Nano.* **2015**, *9,* 733-745.
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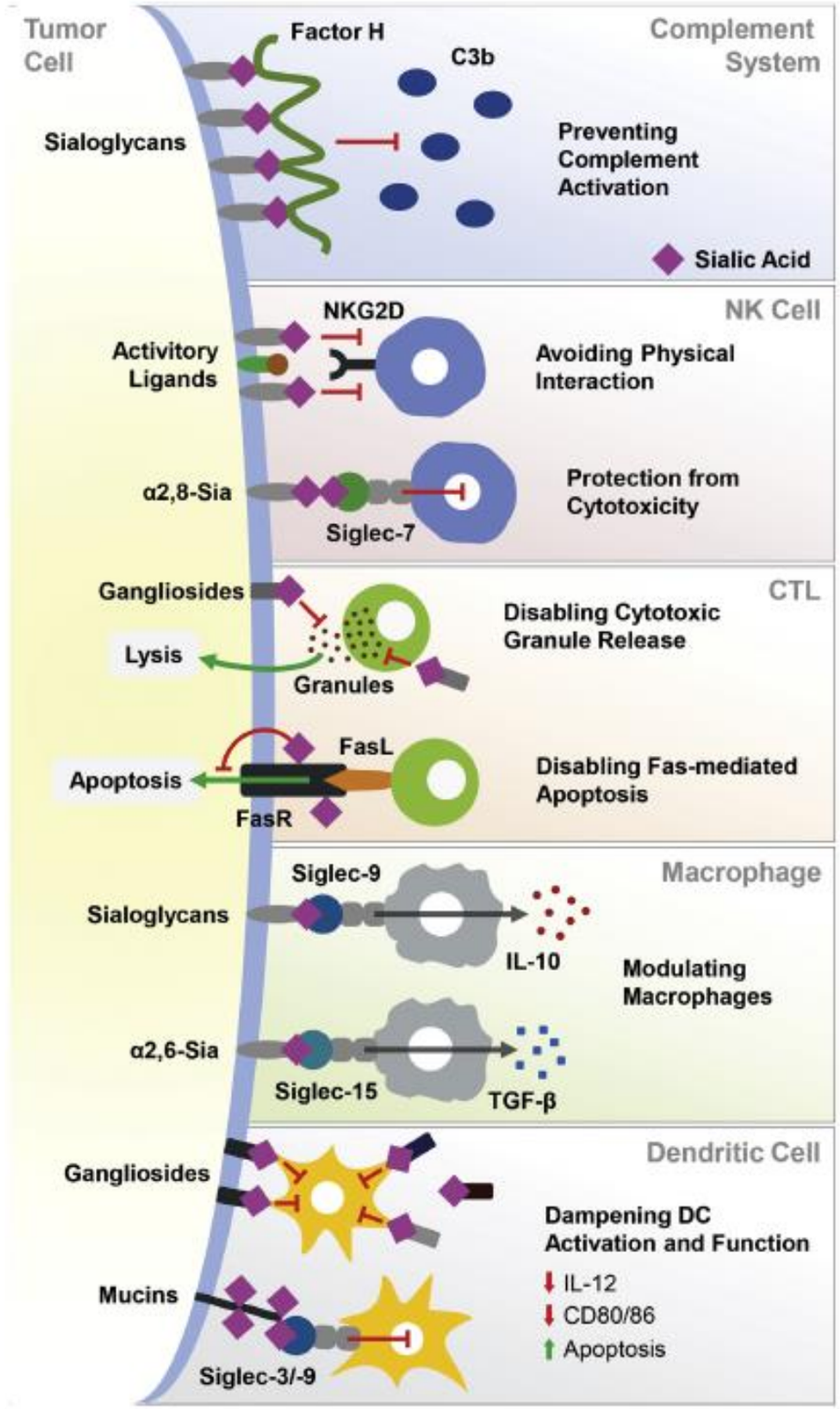
p<0.05 *, p<0.01 **, p<0.001 ***

Sialic acid: an important immune response regulator

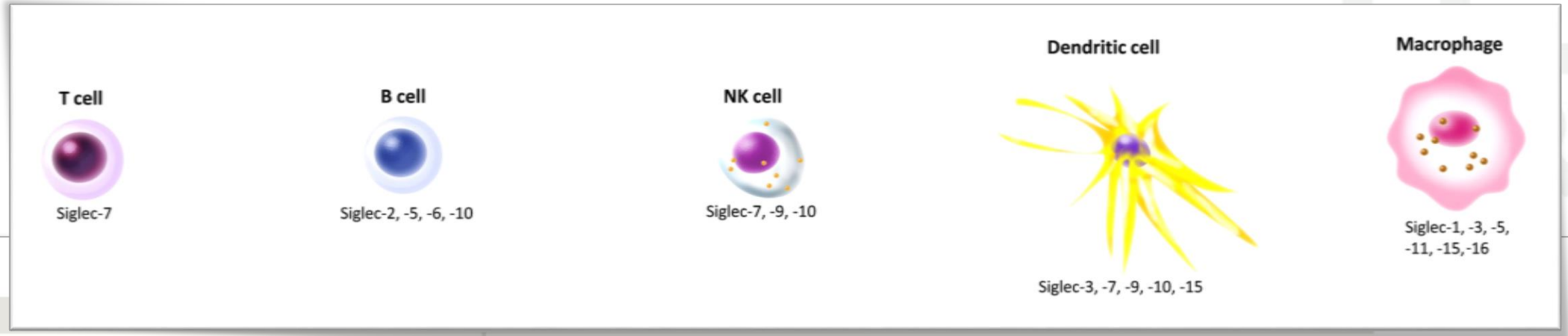
- Biophysical properties: high negative charge, dense highly hydrated layer
- Attracts complement inhibiting Factor-H
- Actively bound by immunosuppressive SIGLEC receptors on immune cells



Büll C, den Brok MH, Adema G.J., *Biochim. Biophys. Act. Rev. Cancer.* **2014**, *1846*, 238-246.

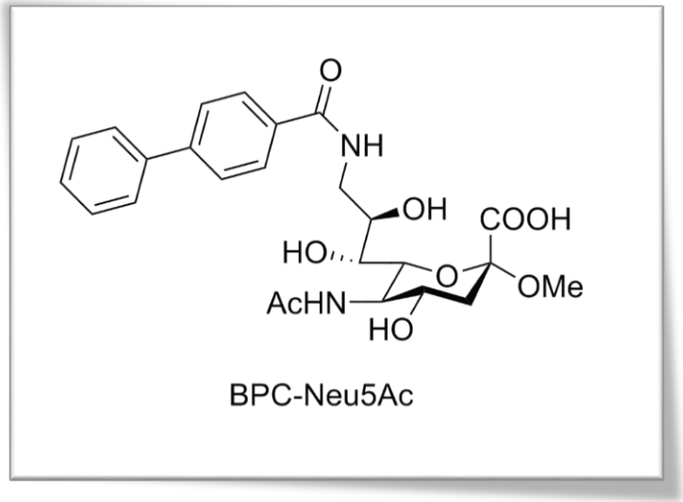
SIGLEC structure and expression

- Sialic acids inhibit complement activation
- Protective biophysical properties, hydration, negative charge etc.
- Bind a dedicated set of immuno suppressive SIGLEC receptors on immune cells



Jandus C., Simon H-U., von Gunten S., *Biochem. Pharmacol.* **2011**, *82*, 323-332
Büll C, den Brok MH, Adema G.J., *Biochim. Biophys. Act. Rev. Cancer.* **2014**, *1846*, 238-246.
Macauley M.S., Crocker P.R., Paulson J.C., Nat. Rev. Immunol. **2014**, *14*, 653-666

Targeting specific SIGLECs



BPC-Neu5Ac

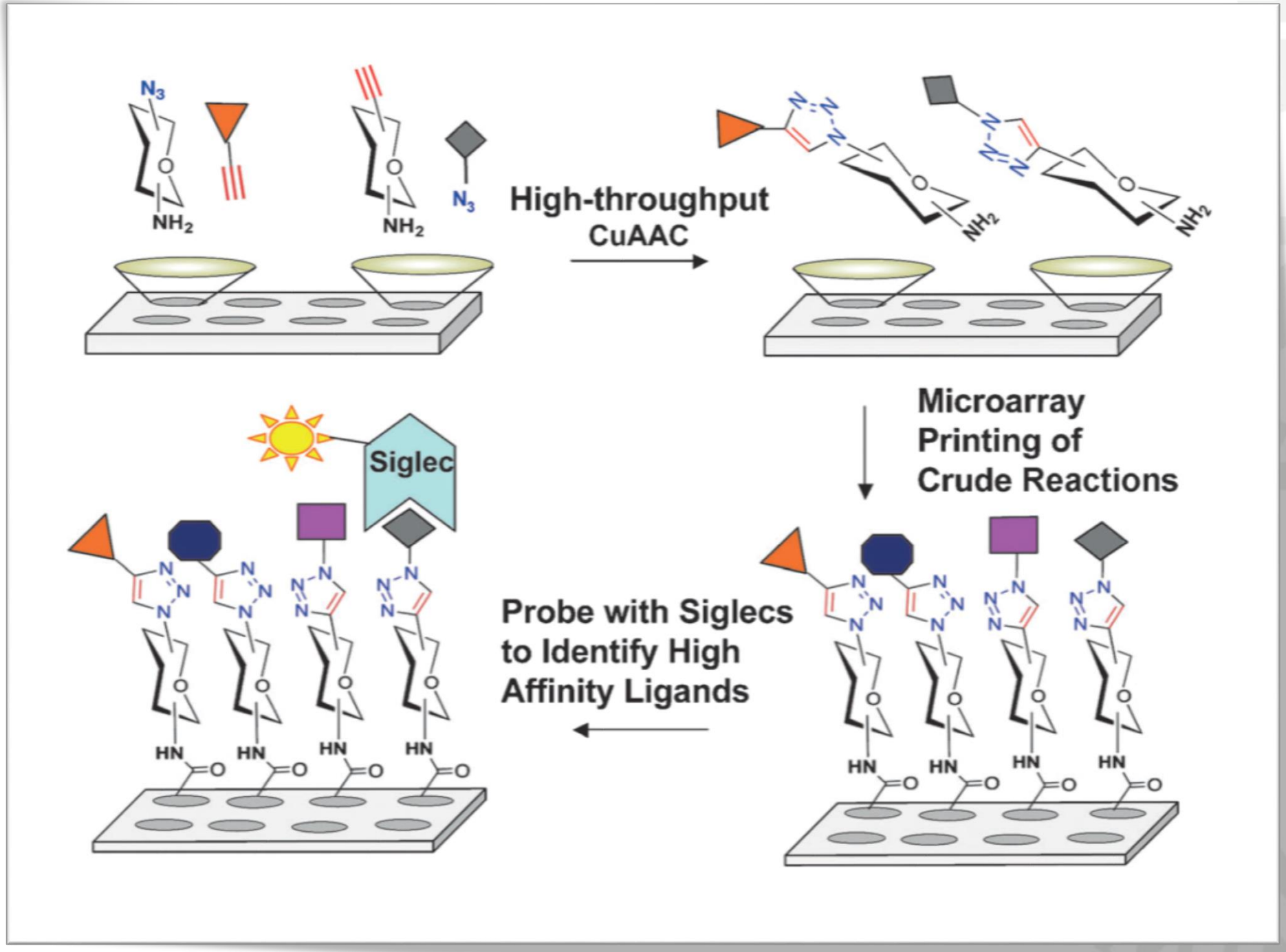
	hCD22	mCD22
	$IC_{50}\mu M$	$IC_{50}\mu M$
Me-Neu5Ac	1,400	4,689
BPC-Neu5Ac	4	1,220

Kelm, Nitschke and co-workers

- Modified sialic acids can bind SIGLECs with high affinity
- Modifications can be used to achieve selective binding
- Modifications can be attached at various positions (C-5, C-9 etc).

Kelm S., Gerlach J., Brossmer R., Danzer C.P., Nitschke L., *J. Exp. Med.*, **2002**, *195*, 1207-1213.

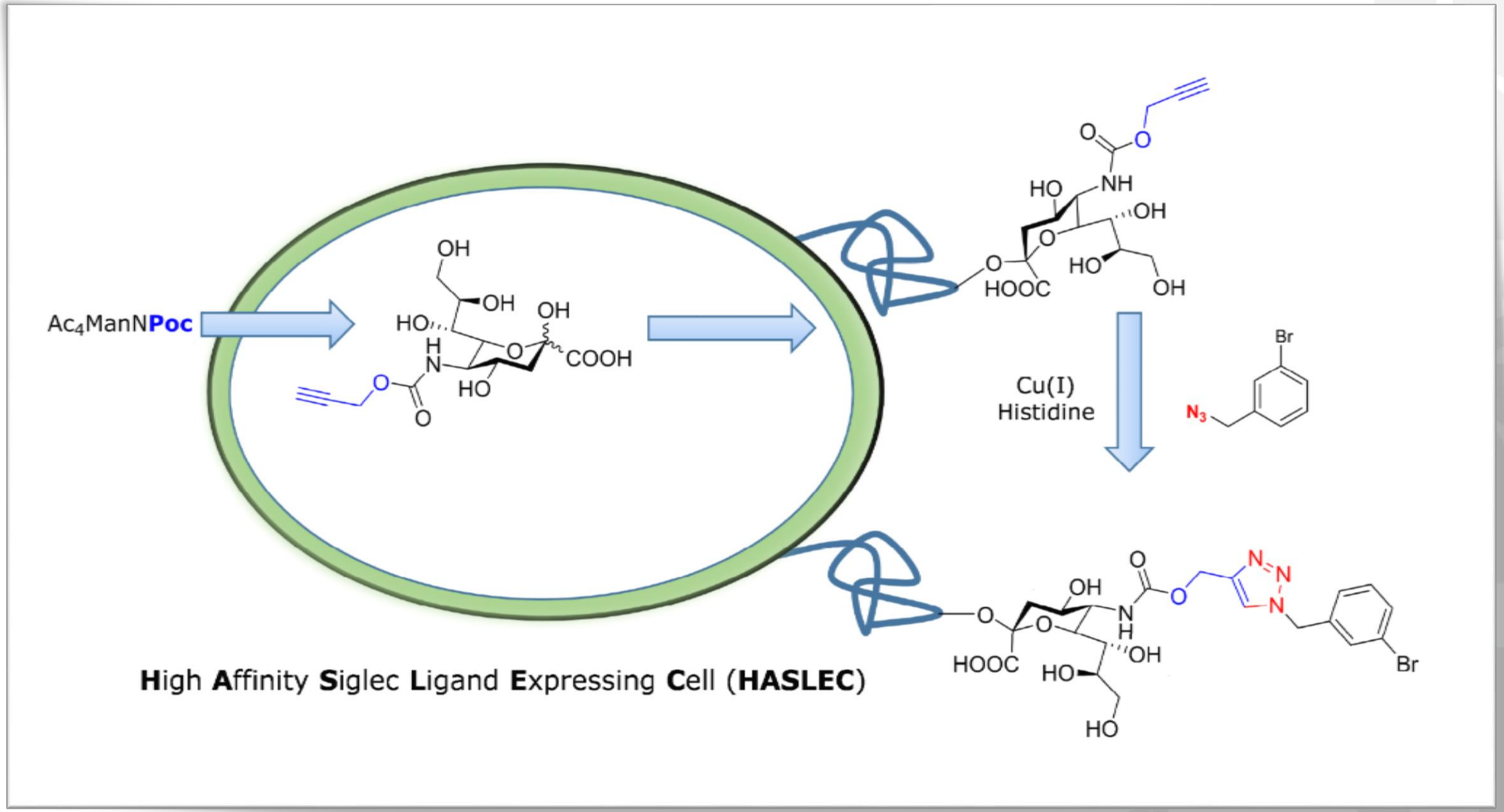
Targeting specific SIGLECs



High throughput modification and screening *via* click chemistry

Rillahan C.D., Schwartz E., McBride R., Fokin V.V., Paulson J.C., *Angew. Chem. Int. Ed.*, **2012**, *51*, 11014-11018.

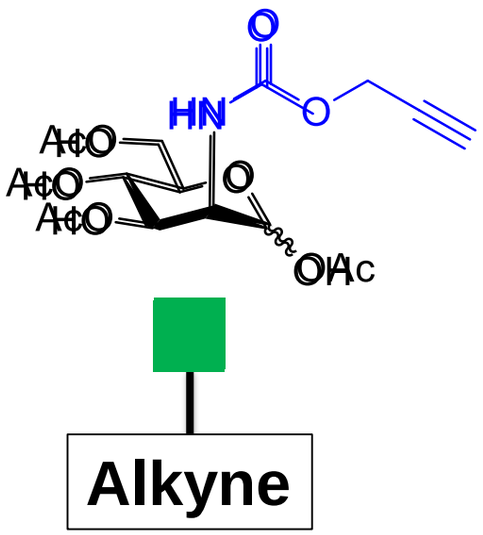
High Affinity SIGLEC Ligand Expressing Cells HASLECs

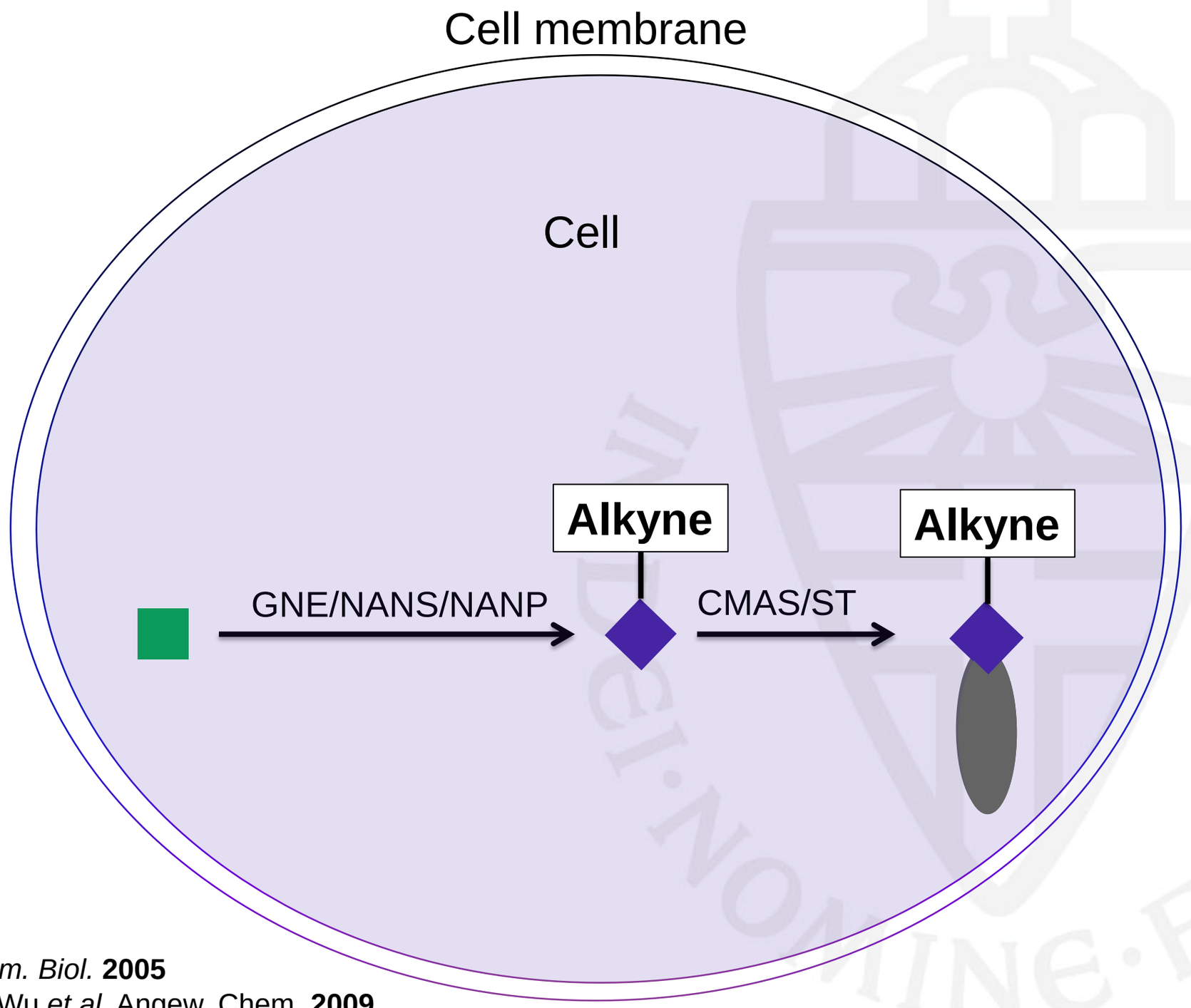


- On cell synthesis to create High Affinity SIGLEC Ligand Expressing Cells HASLECs
- Natural context of sialic acid expression
- Enables to study the effect of binding on signaling

Heise T., Büll C., Adema G.J., Boltje T.J. *et.al. ACS. Chem. Biol. ASAP*

Metabolic labeling of sialic acids and sialoglycans

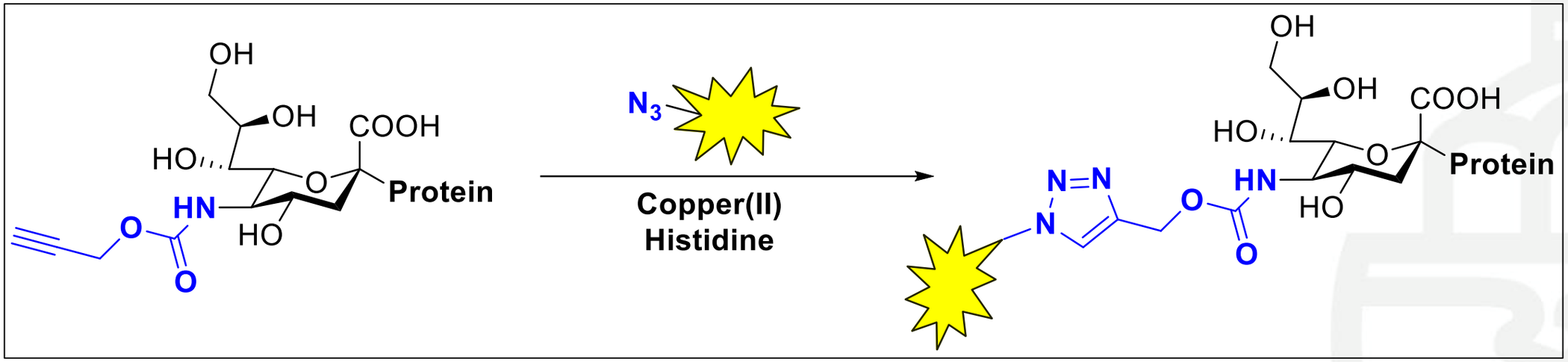




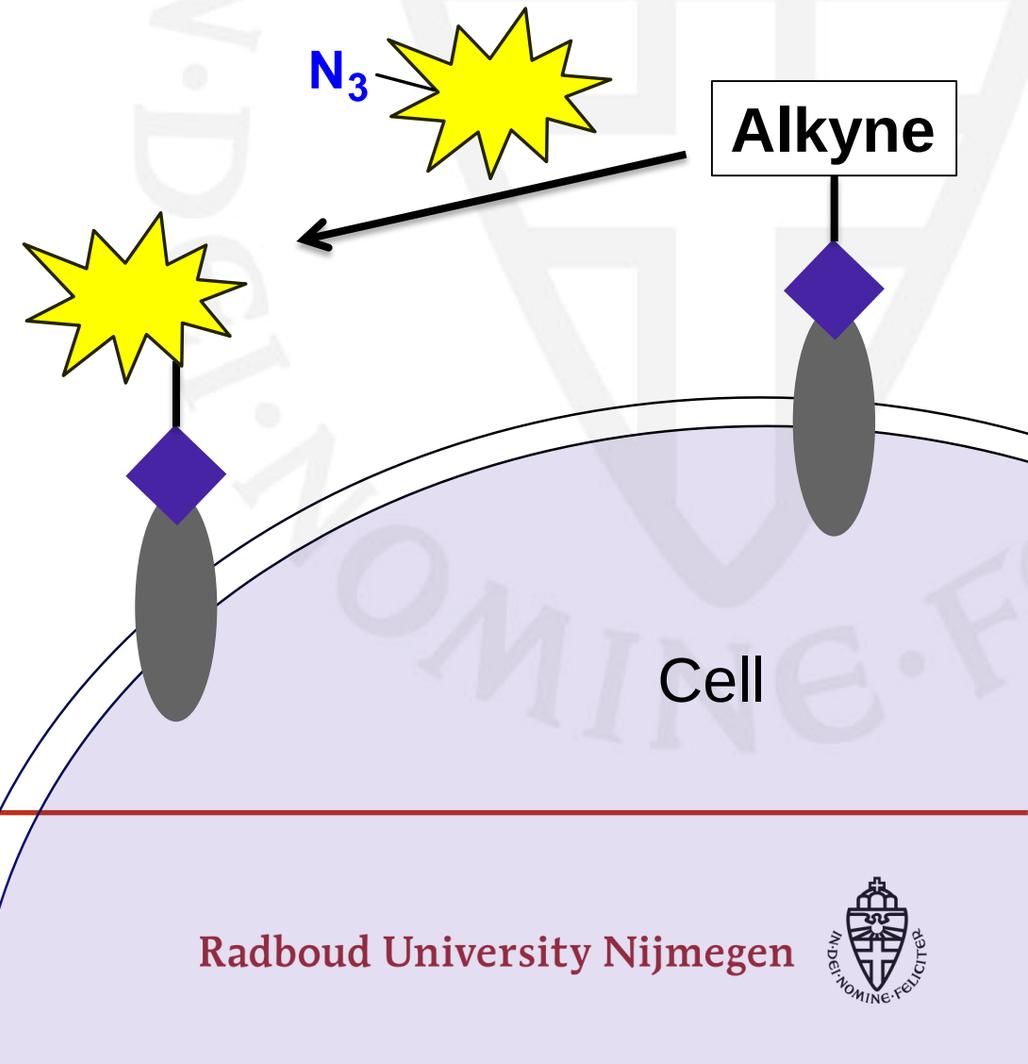
1) J.A. Prescher & Carolyn R Bertozzi, *Nat. Chem. Biol.* **2005**
2) Pamela V. Chang, Carolyn R. Bertozzi, Peng Wu *et al*. Angew. Chem. **2009**
3) Wong C.H. *et al*, PNAS, **2007**

= ManNac = Sialic acid = Protein

Making the chemical reporter visible

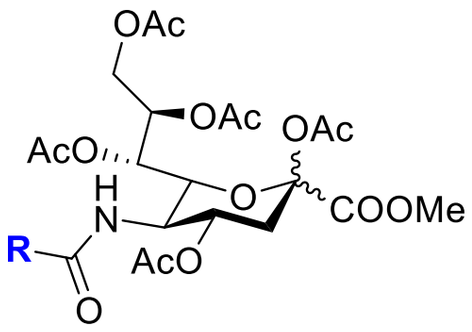


- The two reactants are unique in nature
- They only react with each other
- The rest of the cell remains the same

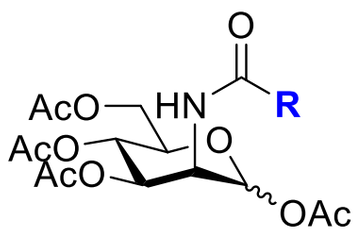


1) Wong C.H. *et al*, PNAS, **2007**
2) Kennedy *et al* **2011** *J. Am. Chem. Soc.*

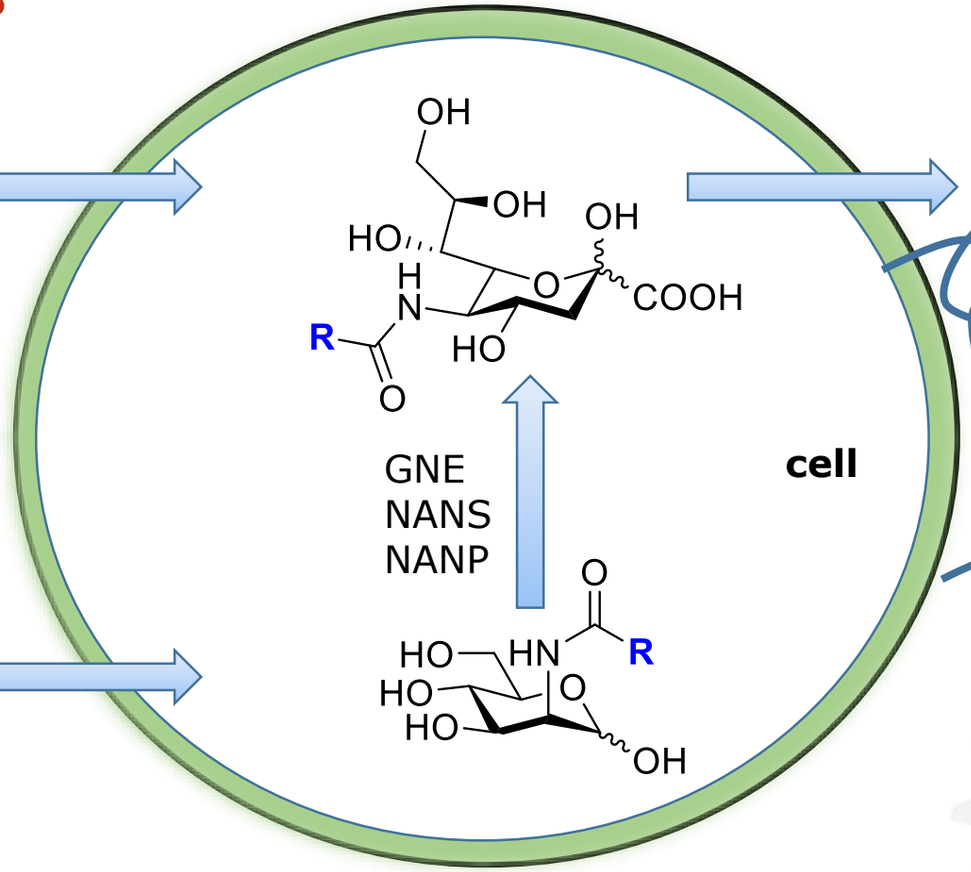
Labeling Sialic Acids

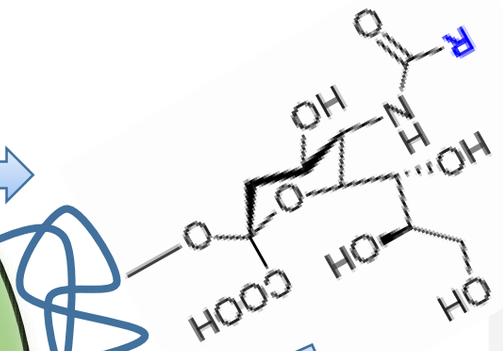


$Ac_5SiaNPoc$, **R** = $OCH_2C{\equiv}CH$
$Ac_5SiaNAz$, **R** = CH_2N_3

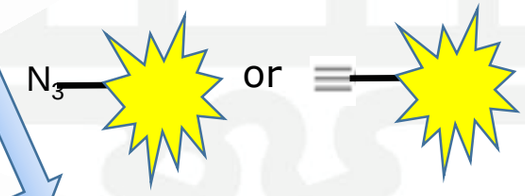


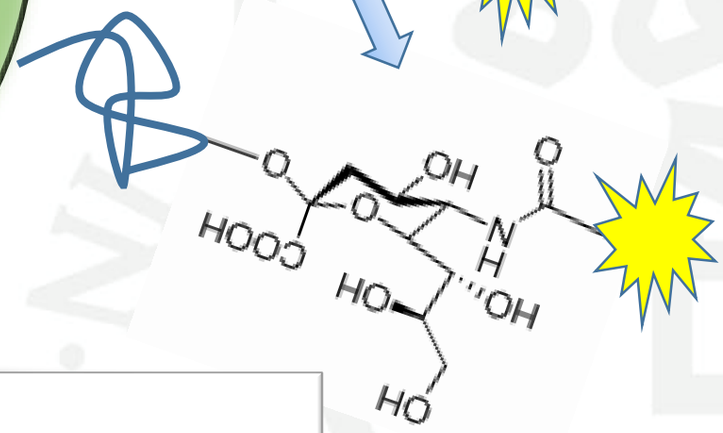
$Ac_4ManNPoc$, **R** = $OCH_2C{\equiv}CH$
$Ac_4ManNAz$, **R** = CH_2N_3

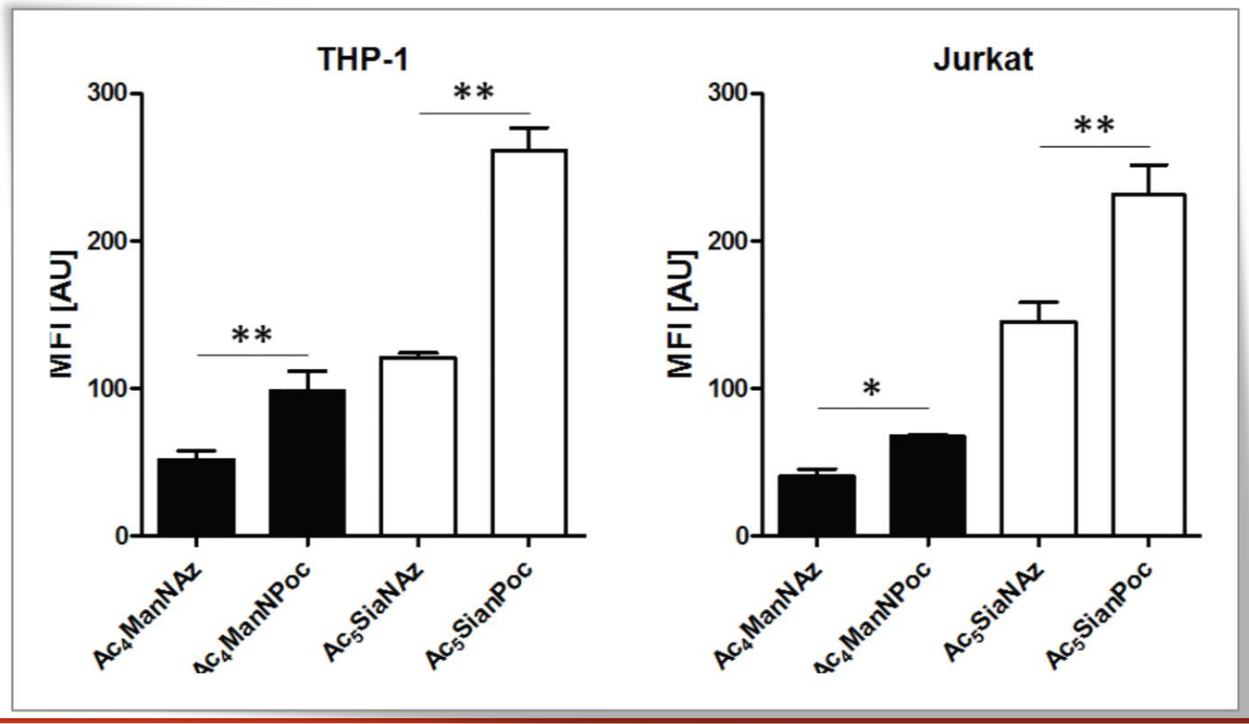


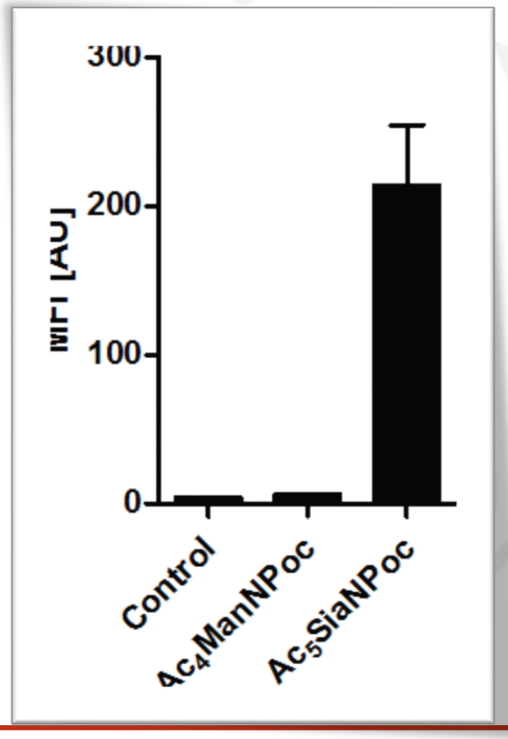


Cu(I)
Histidine









T.-L. Hsu, S. R. Hanson, K. Kishikawa, S.-K. Wang, M. Sawa, C.-H. Wong, *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 2614-2619
P. V. Chang, X. Chen, C. Smyrniotis, A. Xenakis, T. S. Hu, C. R. Bertozzi, P. Wu, *Angew. Chem., Int. Ed.* **2009**, *48*, 4030-4033.
Luchansky, S.J., Goon, S. & Bertozzi, C.R., *Chembiochem,* **2004**, *5*, 371-4.
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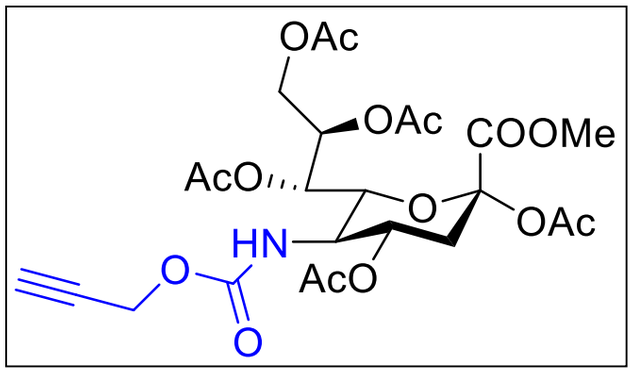

Alkyne

Cell membrane

Cell

GNE/NANS/NANP

CMAS/ST

Alkyne

Feedback Inhibition

= ManNac

= Sialic acid

= Protein

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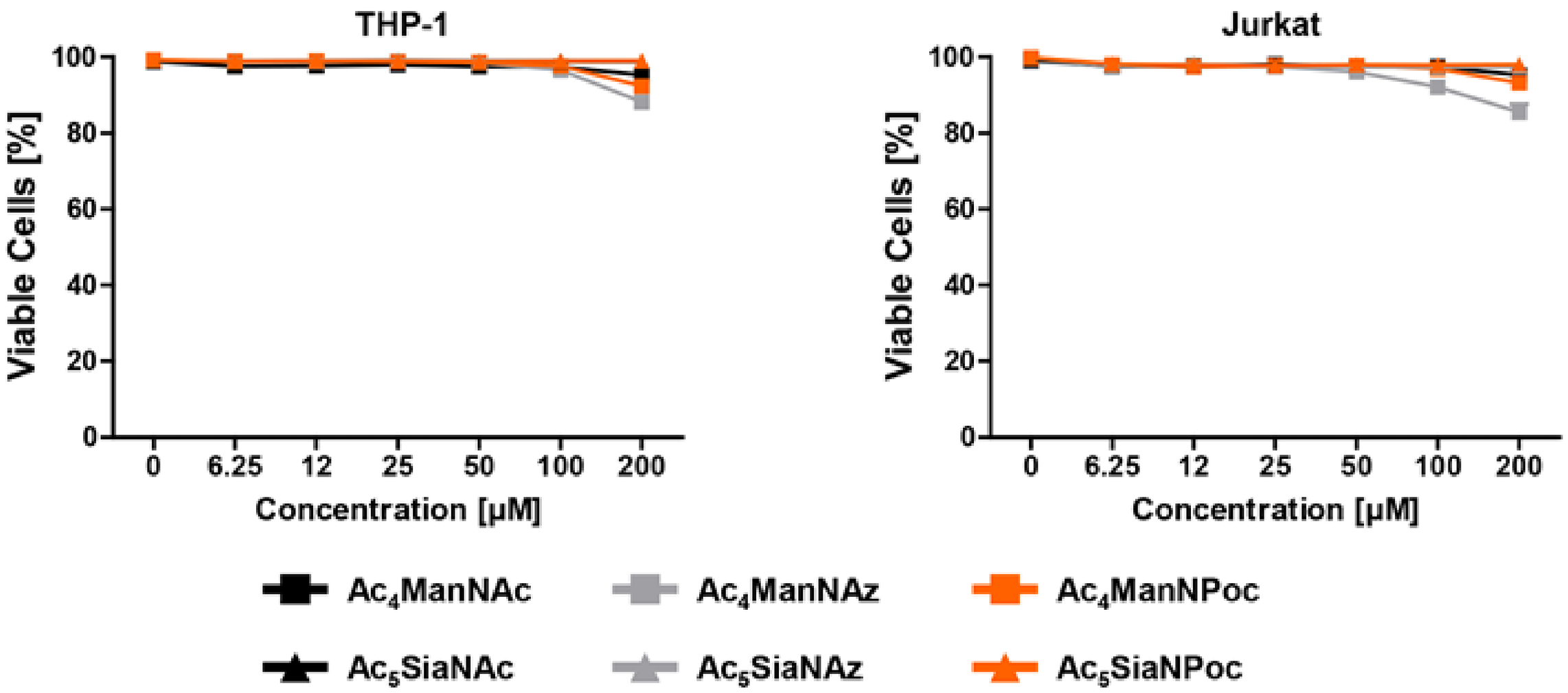
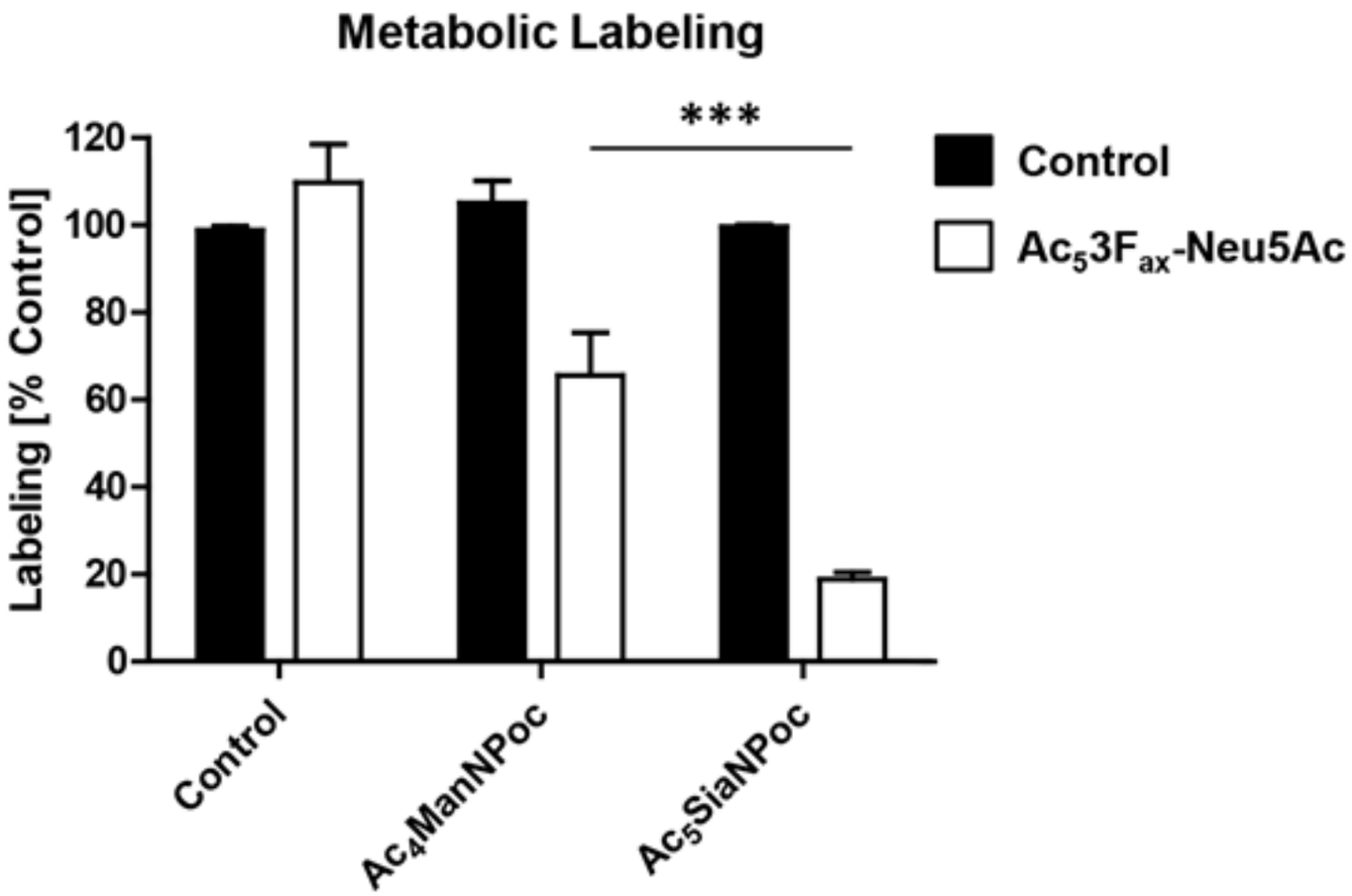
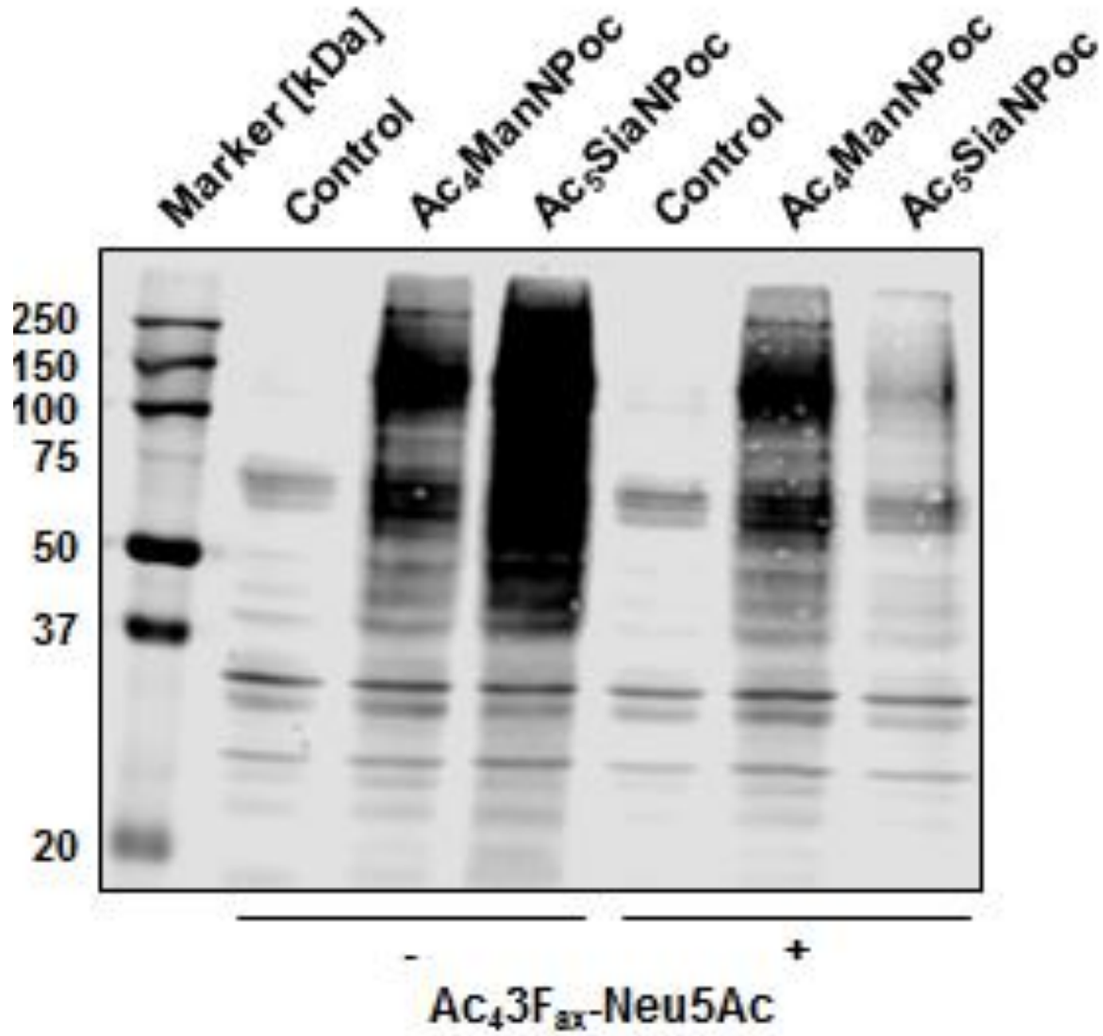
Sialic acid analogues are less toxic

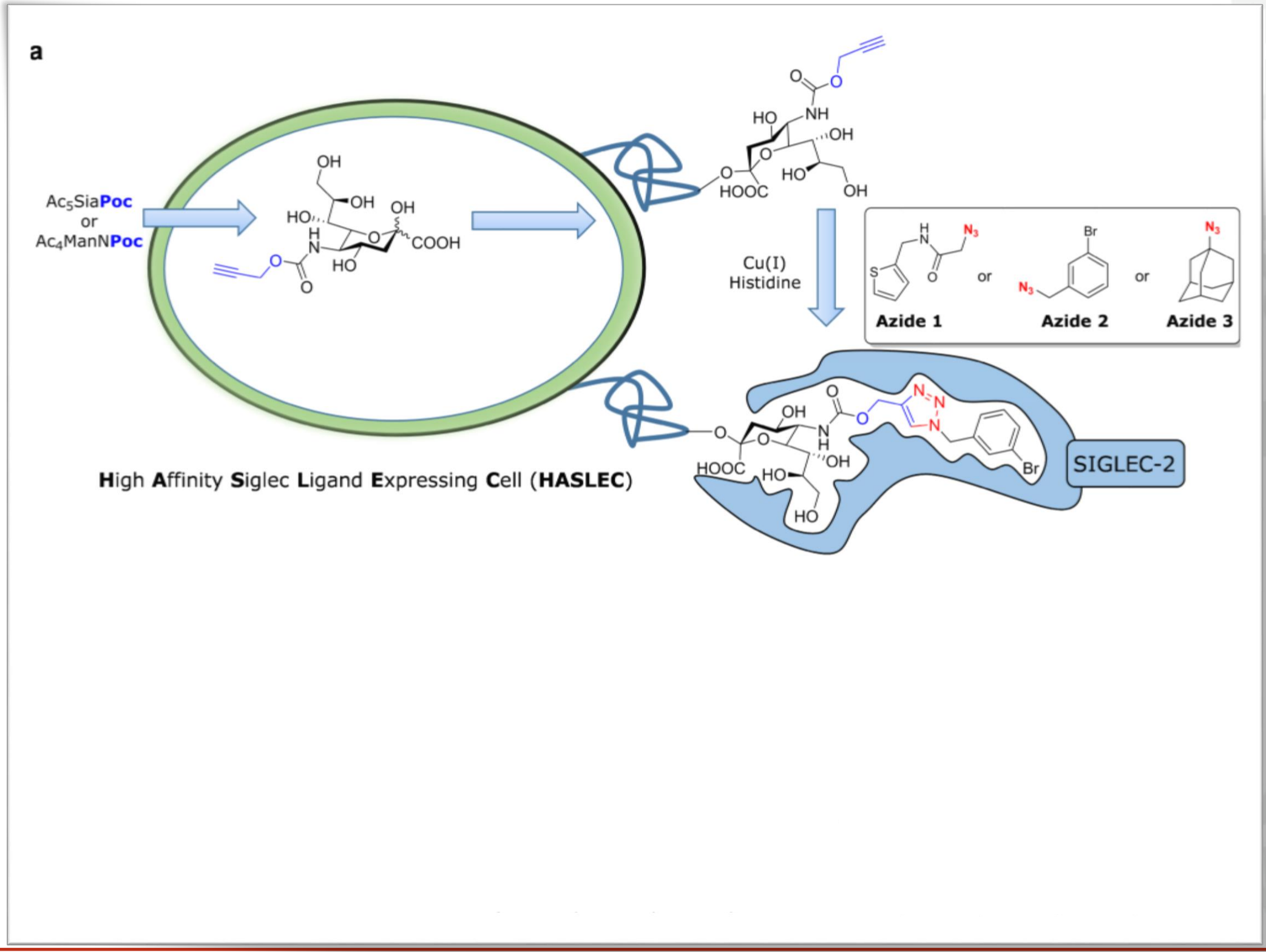


Sialic analogues are more specific





High Affinity Siglec Ligand Expressing Cells (HASLECs)

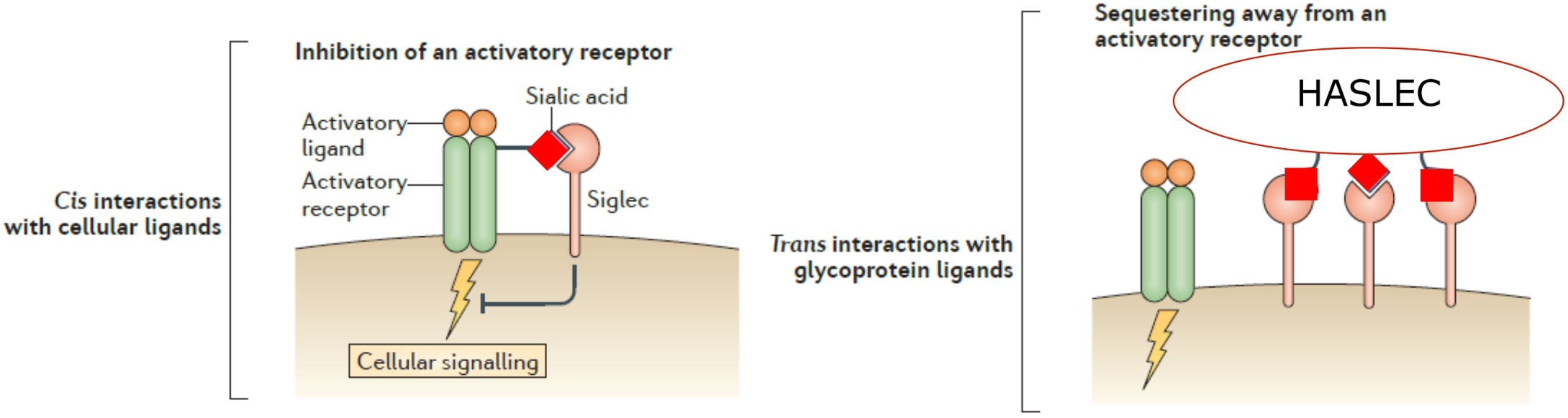


Heise T., Büll C., Adema G.J., Boltje T.J. *et.al. ACS. Chem. Biol. ASAP*

Future work, more design and screening

- **More screening**
- More ligands
- Different positions on the sialic acid
- **Functional experiments**
- HASLEC/SIGLEC containing cell binding
- Functional consequences of HASLEC binding: Immune activation/suppression

= Sialic acid = High Affinity SIGLEC Ligand



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Hudak J.E., Canham S.M., Bertozzi C.R., *Nat. Chem. Biol.,* **2014**, *1*, 69-75

Acknowledgements

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RIMLS

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- Christian Büll
- Danielle Beurskens

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