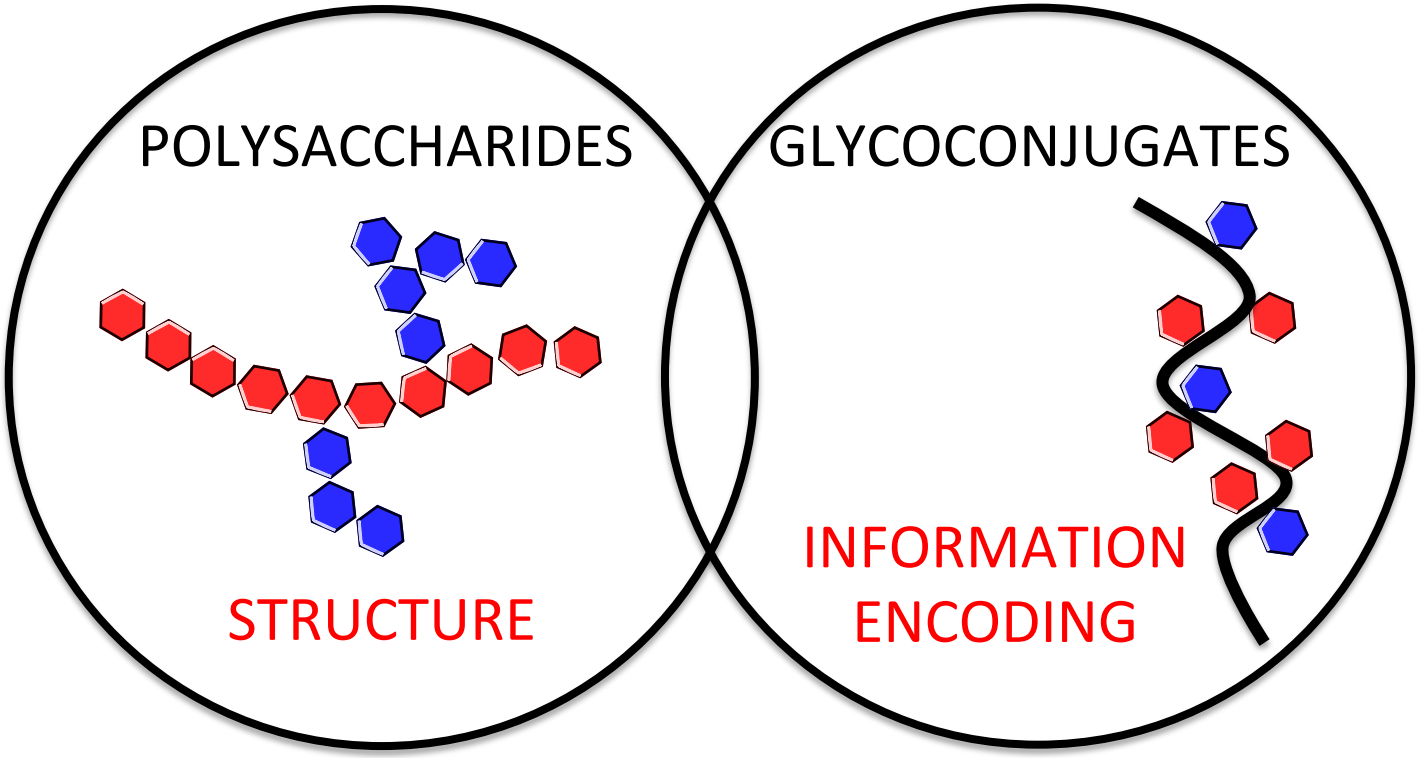
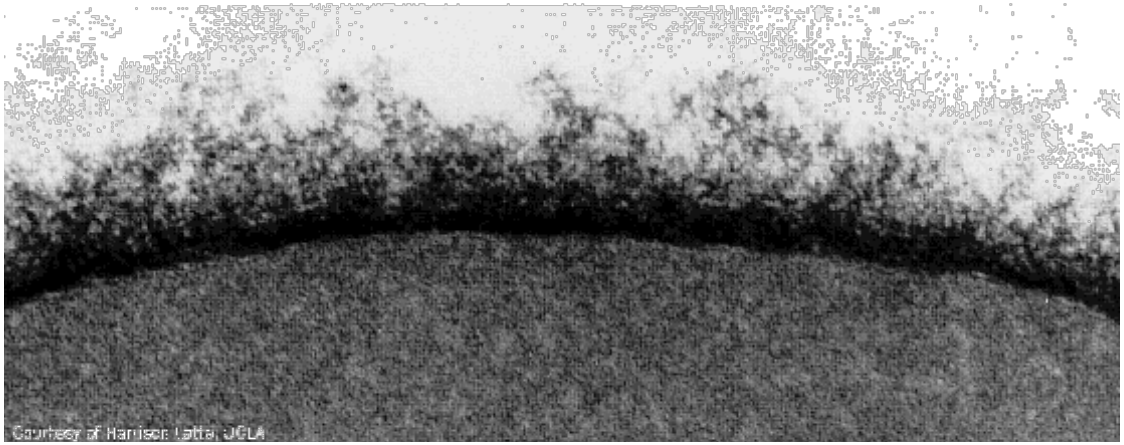
The nanoscale dimension of “glycobiology”

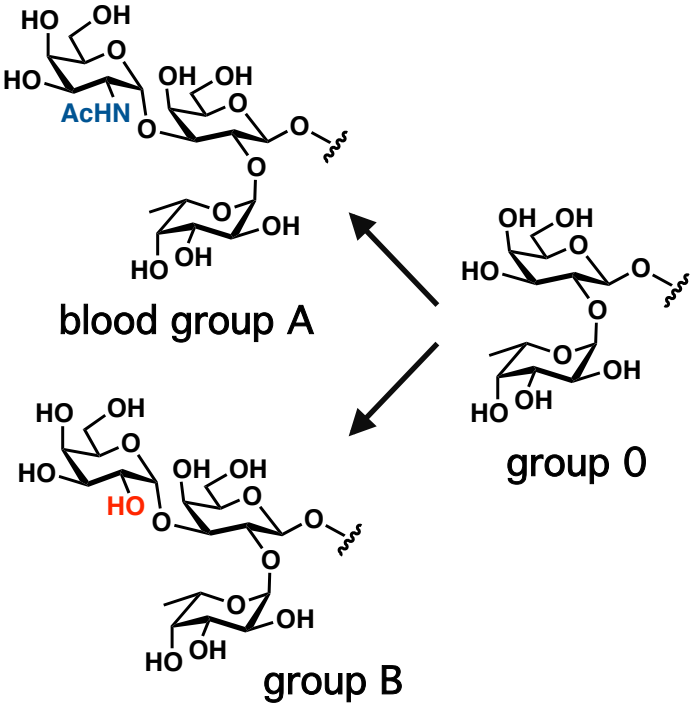


- The study of glycobiology necessitates material science approaches

Beyond the Genome: Encoding information with glycans

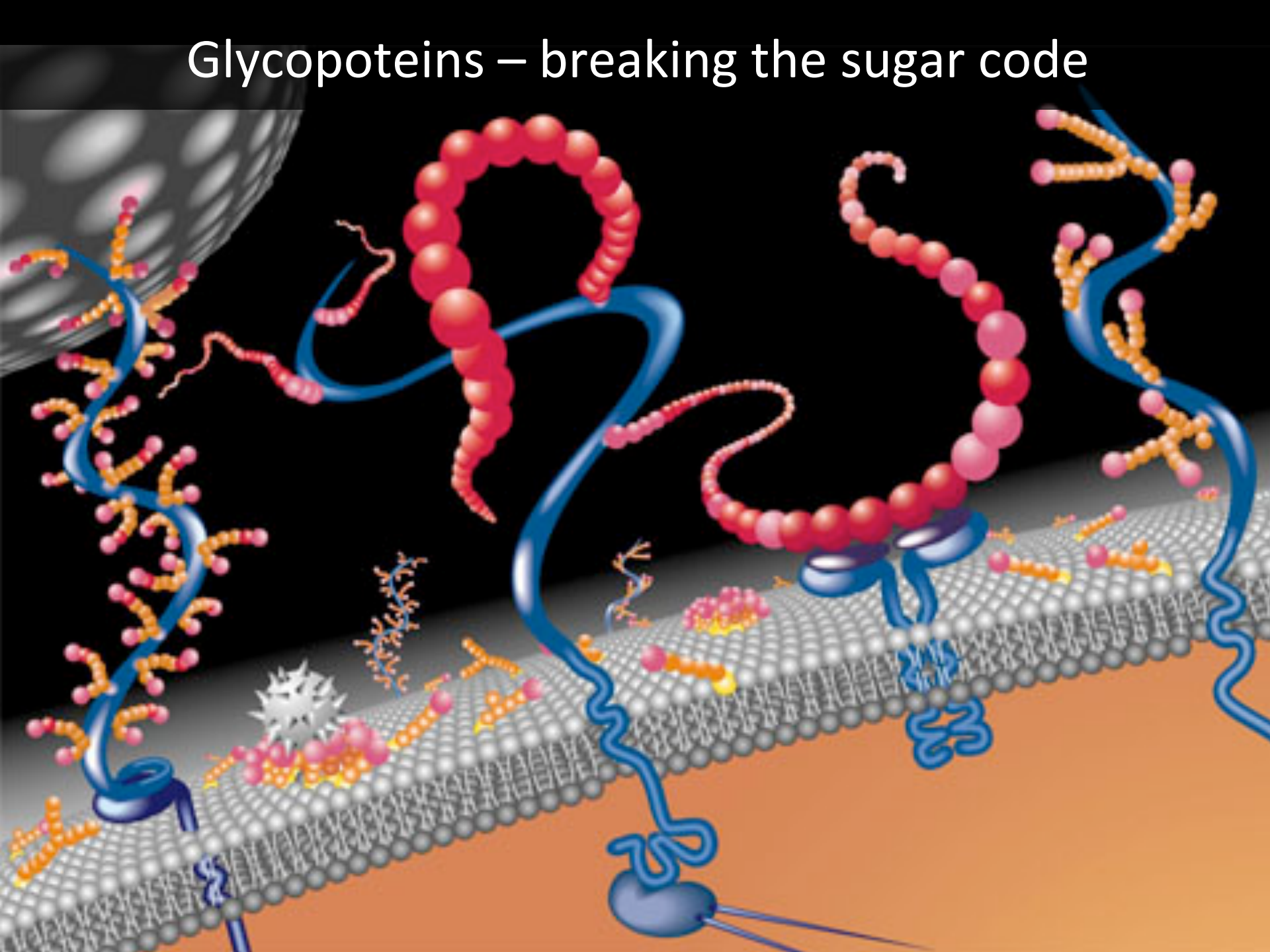


EM image of the Glycocalyx of an erythrocyte

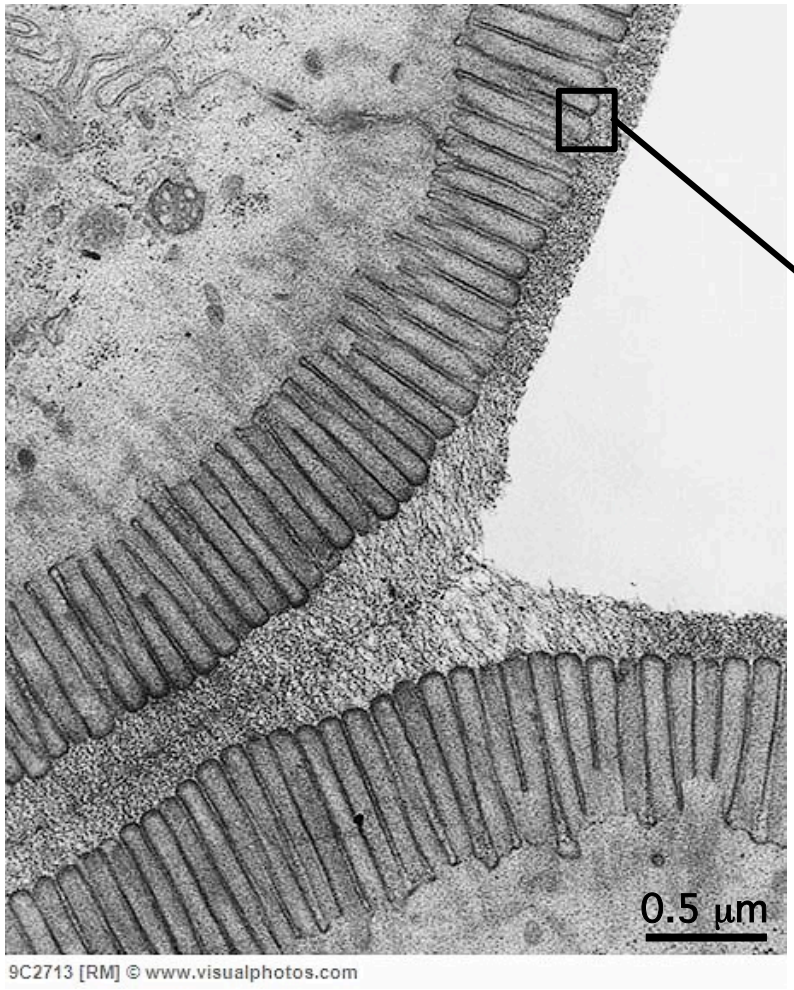


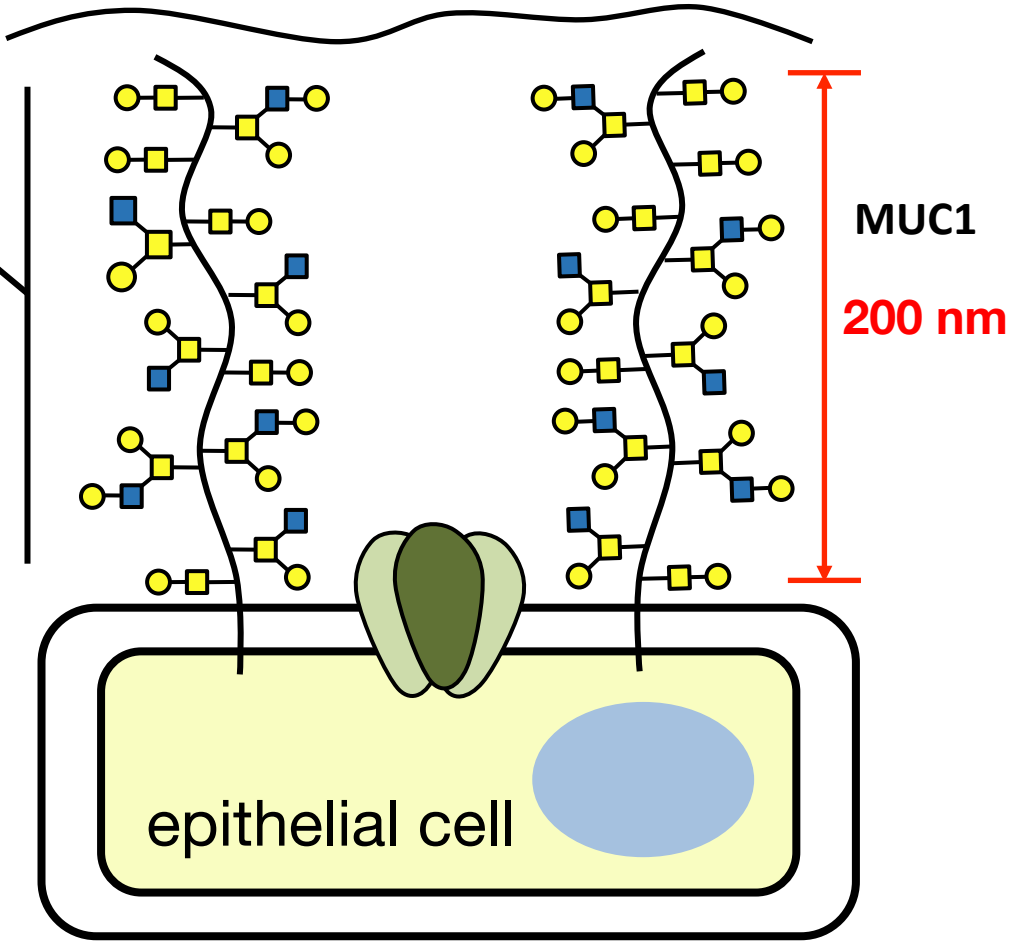
- Glycans are not encoded by genes
- Glycan structures reflect physiological state of cells
- Glycans can create diversity beyond that of the Genome and the Proteome
- Glycans are difficult to synthesize and characterize

Glycopoteins – breaking the sugar code



Mucin glycoproteins protect surfaces of epithelial cells

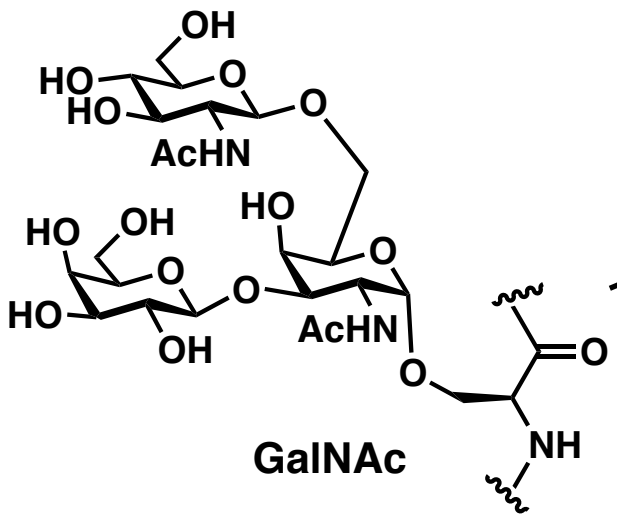




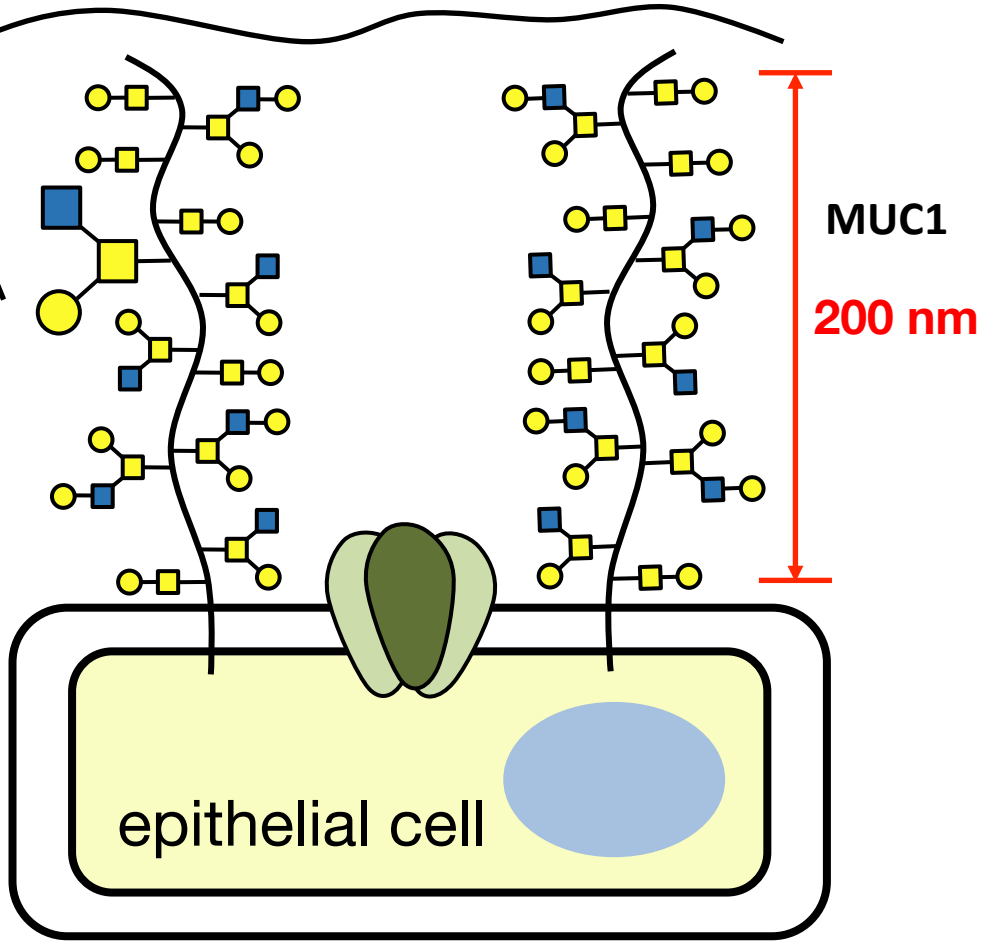
Intestinal epithelium, TEM
Don W. Fawcett

Essentials of Glycobiology
http://www.ncbi.nlm.nih.gov/books/NBK1908/

Mucins carry a diverse range of branched glycan structures

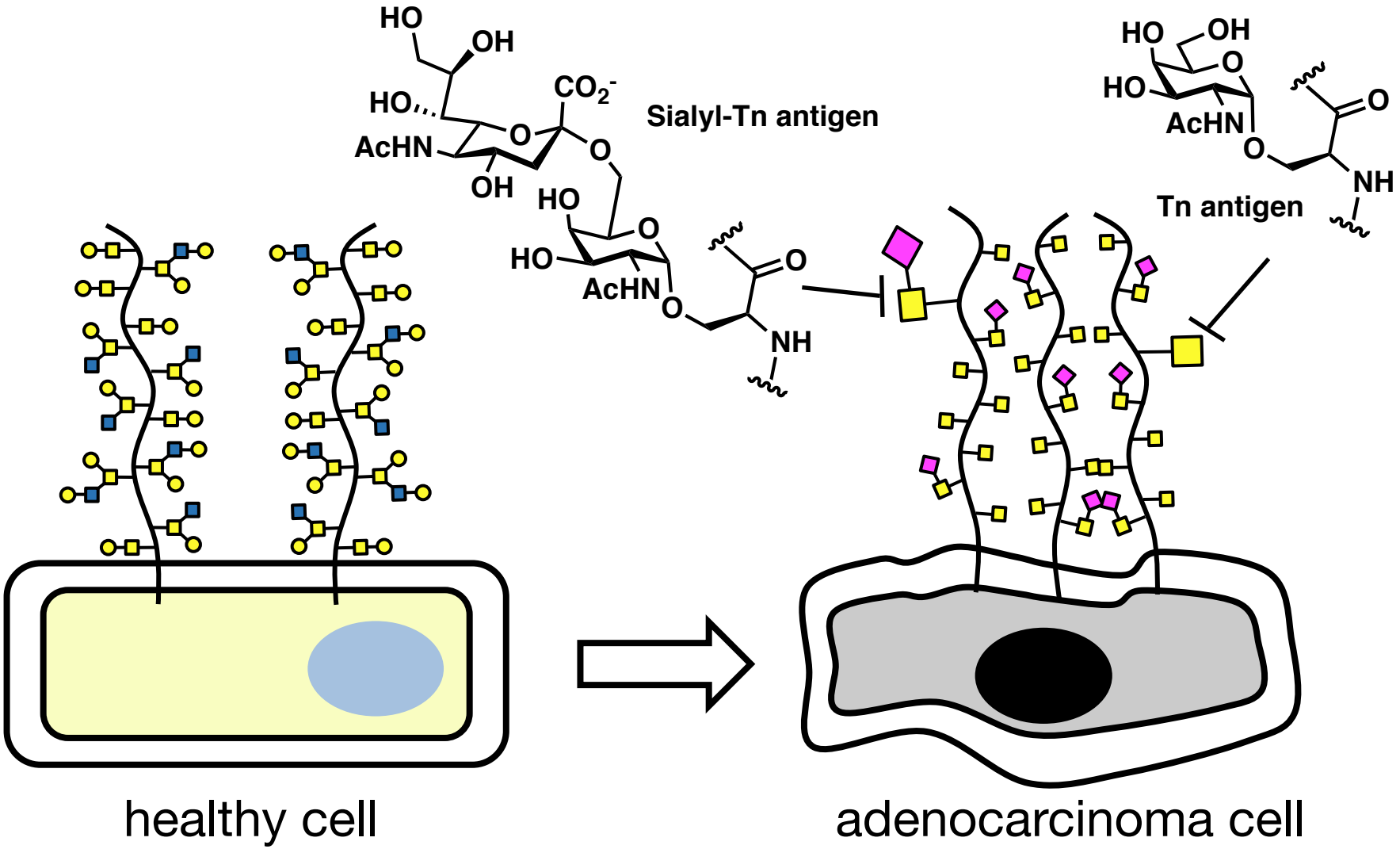


branched glycans attached through a core GalNAc sugar



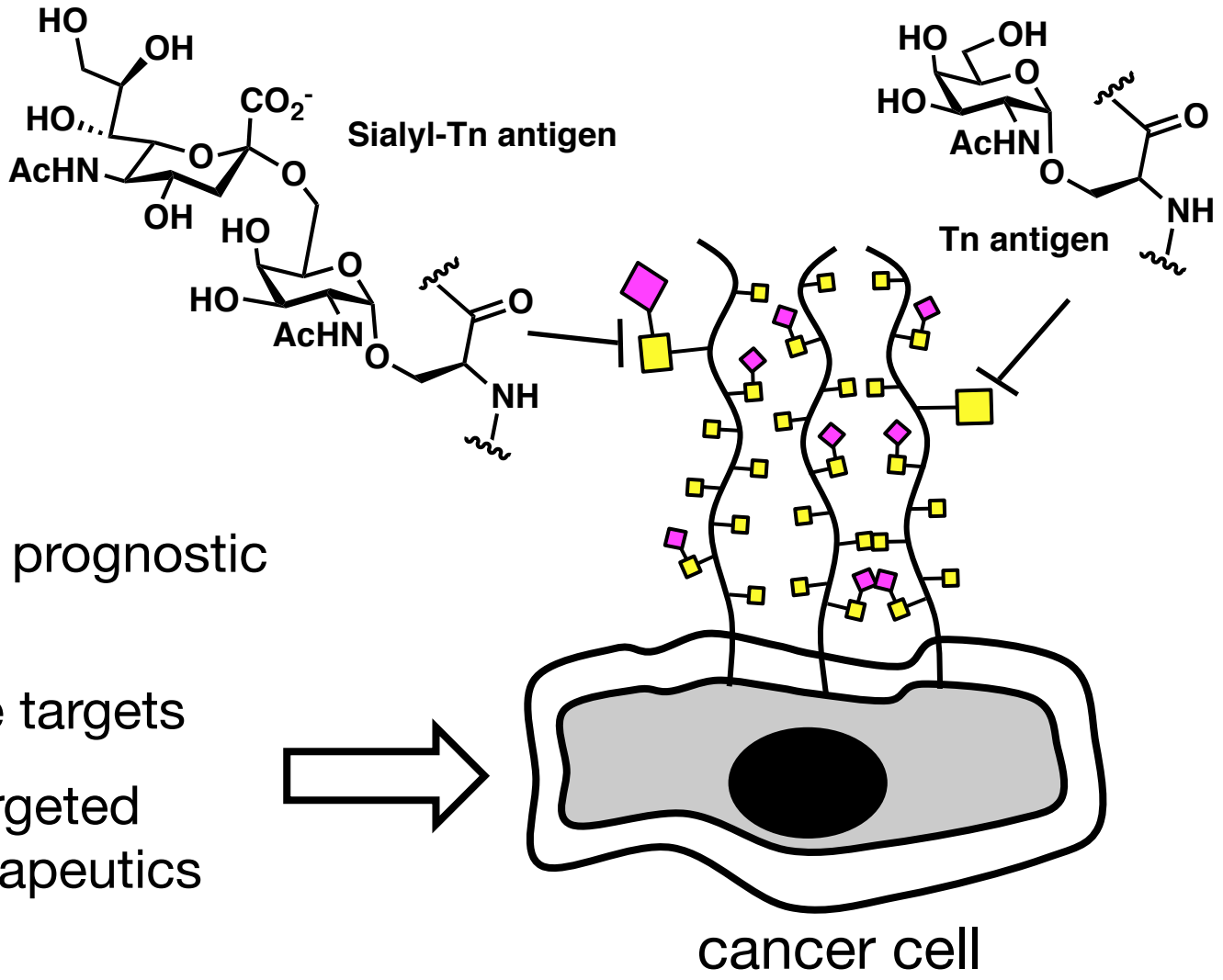
Singh and Hollingsworth *Trends Cell Biol. 16*, 467 (2006)

Mucin glycosylation changes in response to altered physiological state



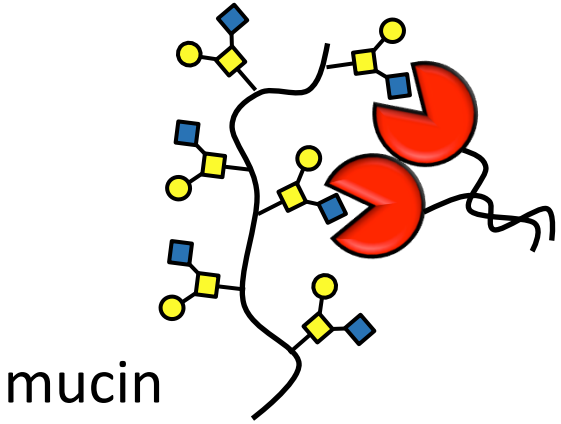
Singh and Hollingsworth *Trends Cell Biol. 16*, 467 (2006)

Cancer-specific glycans can be explored for therapeutic gains



- diagnostic and prognostic biomarkers
- cancer vaccine targets
- antigens for targeted delivery of therapeutics

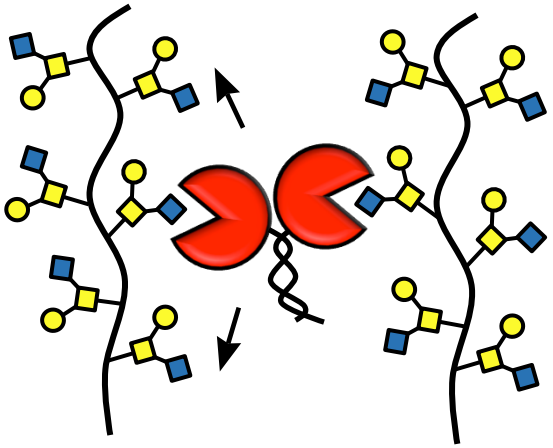
Specificity of glycan recognition is achieved through multivalency



face-to-face

low binding affinity for single glycans (K_d ~ 100 µM)

multivalency enhances binding avidity (K_d~ 10 nM)



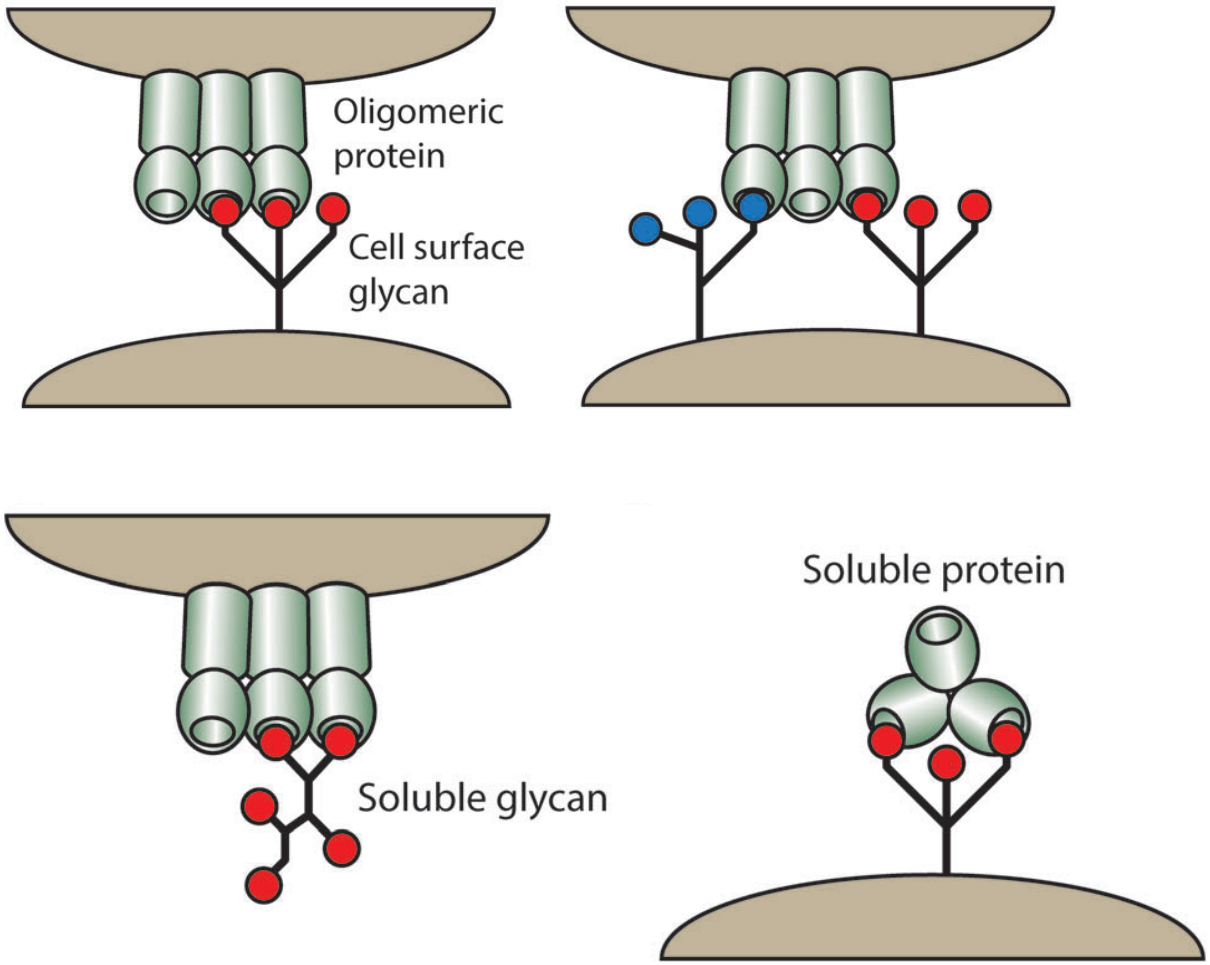
bind-and-slide

enhanced avidity is the result of prolonged persistence time of the complex

crosslinking is associated with activation of signaling cascades

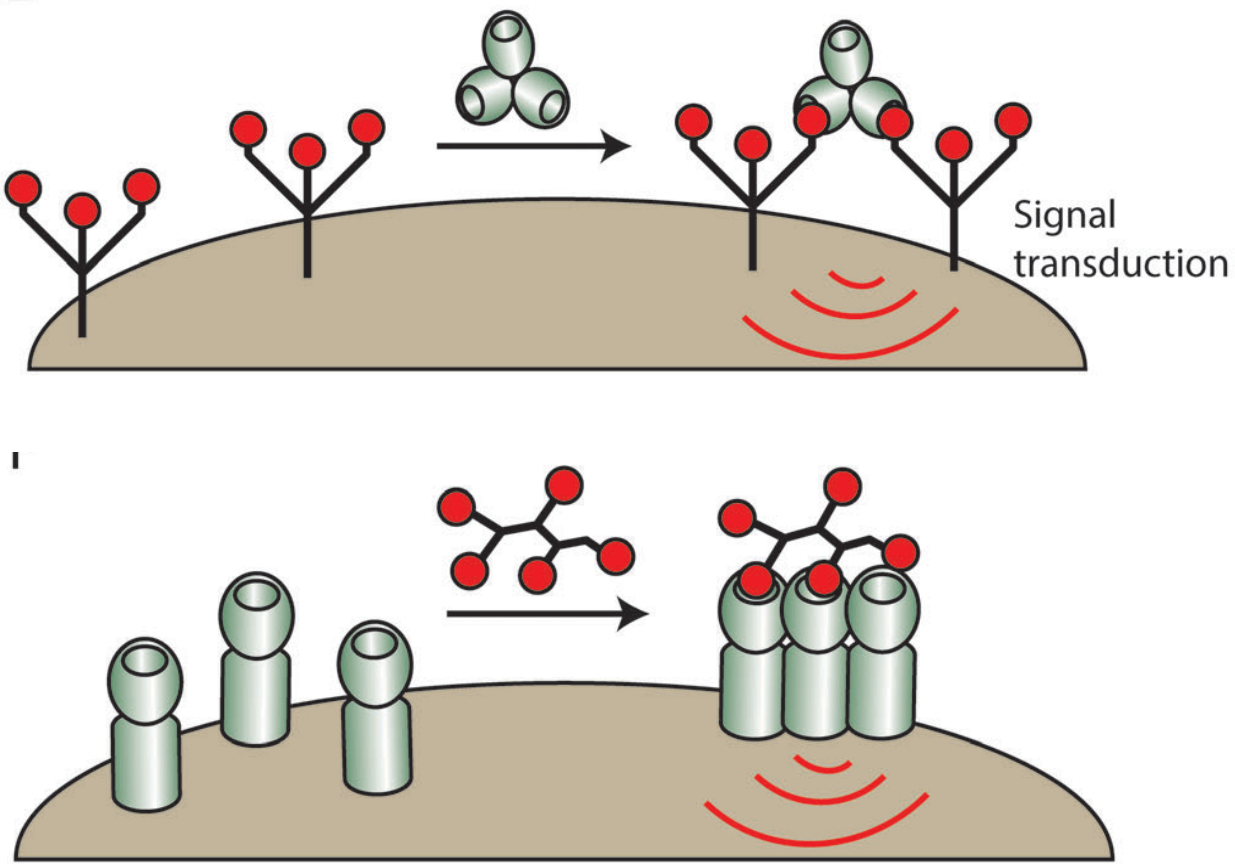
Brewer *J. Biol. Chem.* *282*, 28256 (2007)

Glycan multivalency in receptor binding



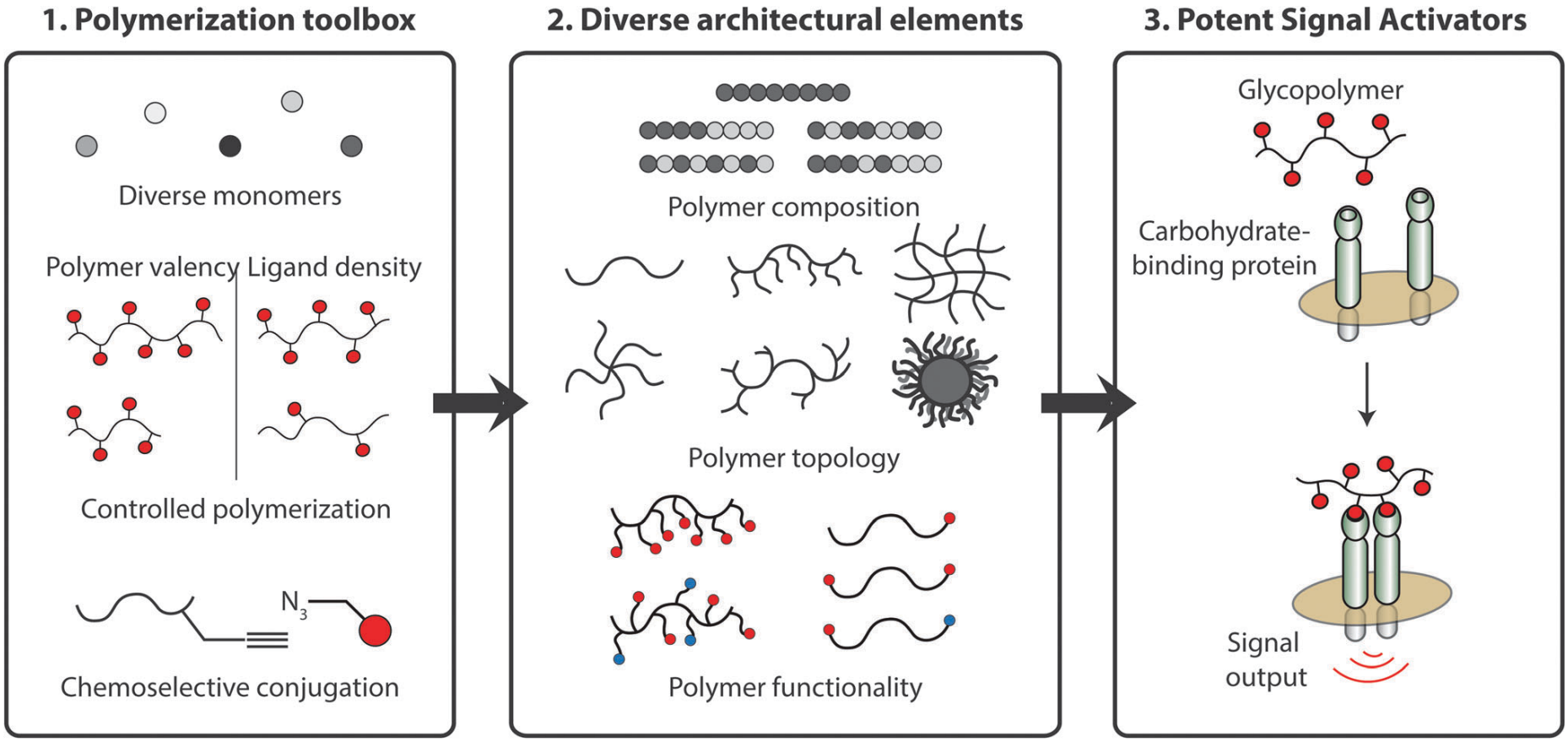
Kiessling and Grim *Chem. Soc. Rev.* *42*, 4476 (2013)

Receptor oligomerization and signal transduction



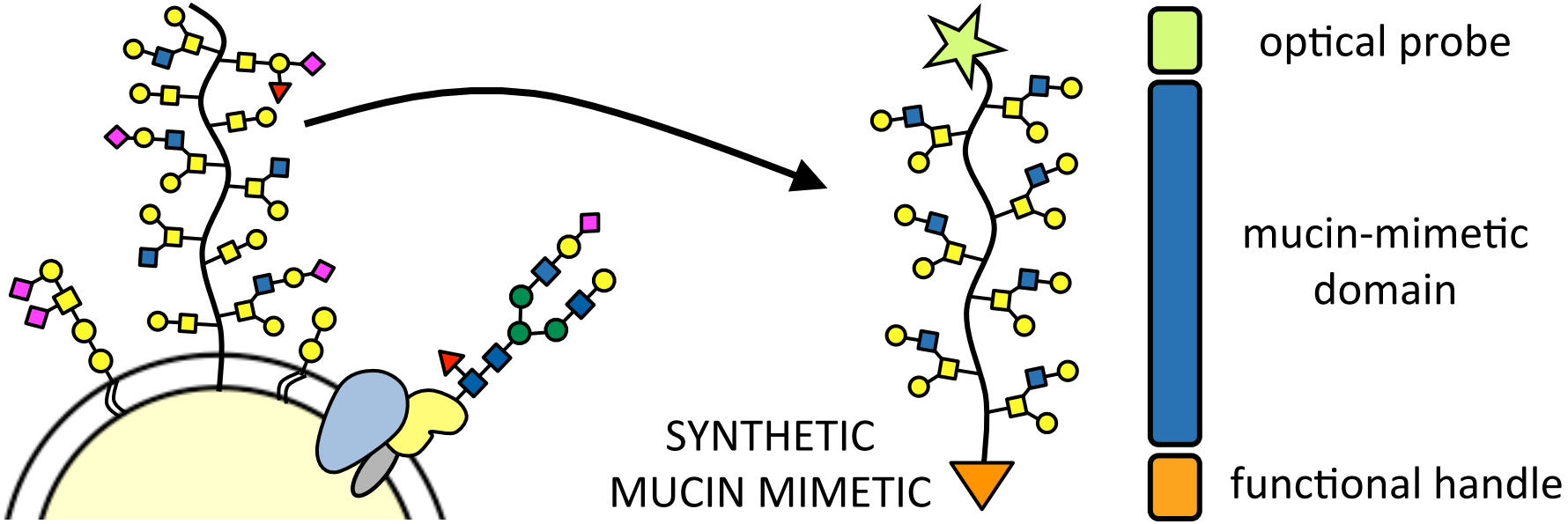
Kiessling and Grim *Chem. Soc. Rev.* *42*, 4476 (2013)

Glycopolymers – versatile mucin mimetics



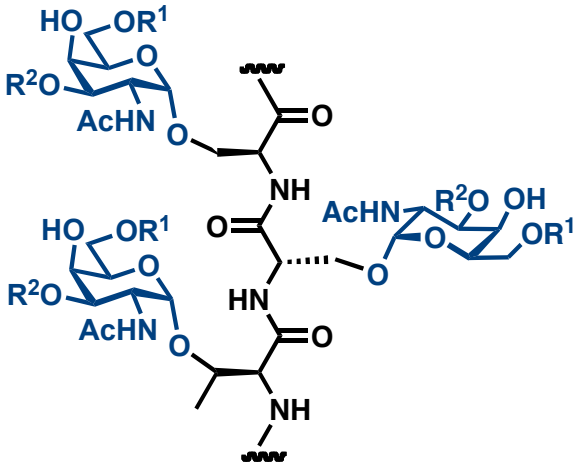
Kiessling and Grim *Chem. Soc. Rev.* *42*, 4476 (2013)

RAFT glycopolymers to probe mucin biology

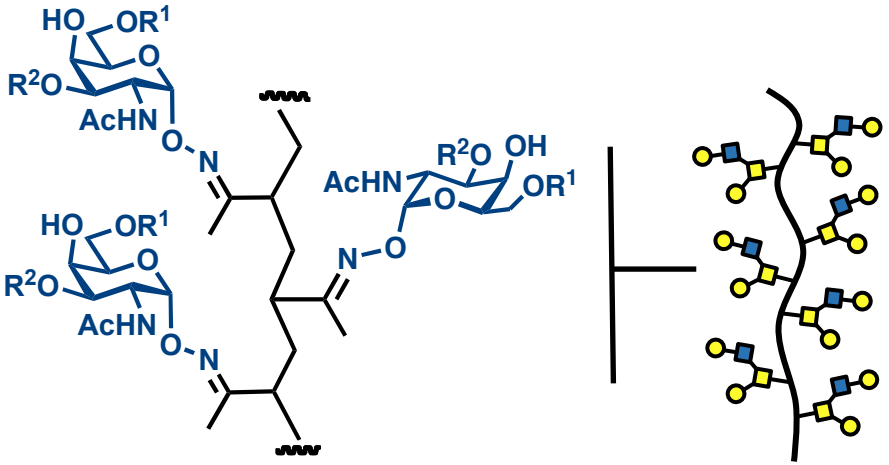


- synthetically tractable
- uniform glycan structures and programmable valency
- extended, mucin-like architectures
- well-defined lengths matching the dimensions of native mucins
- functionalized for surface immobilization and probe conjugation

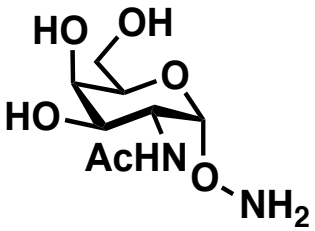
Design of mucin-mimetic glycodomain



native mucin

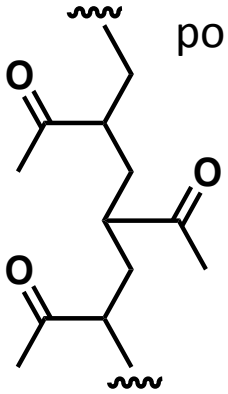


mucin-mimetic domain



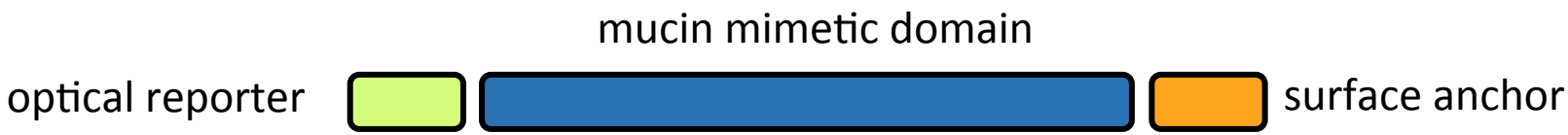
aminooxy-GalNAc

+

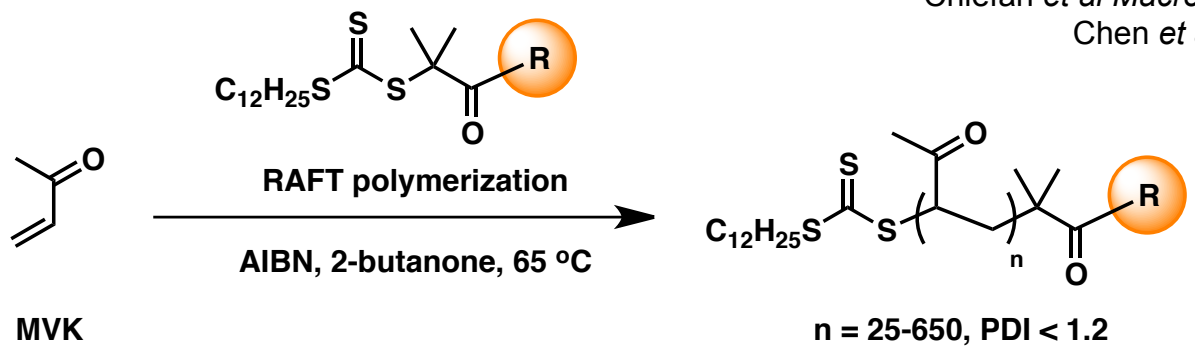


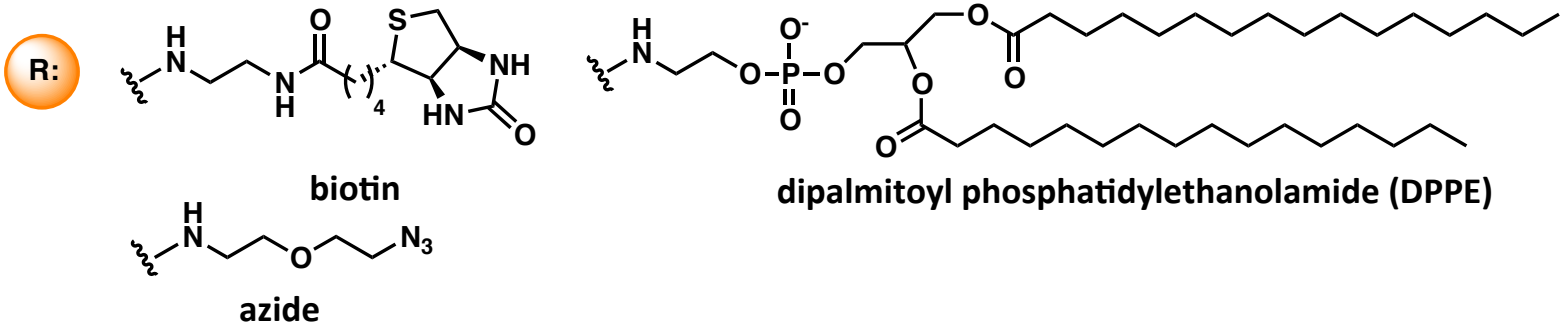
poly(methylvinyl ketone)
(pMVK) backbone

Synthesis of mucin-mimetic backbone via RAFT polymerization



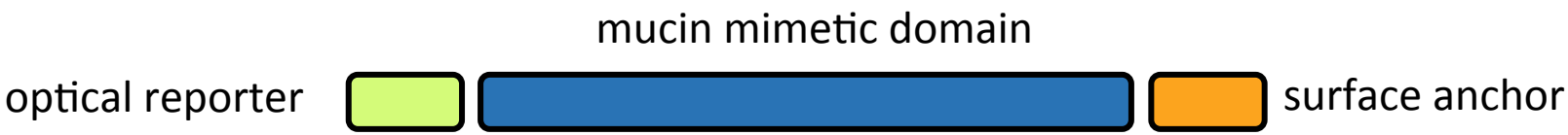
Chiefari *et al Macromolecules 31*, 5559 (1998)
Chen *et al JACS 129*, 10086 (2007)

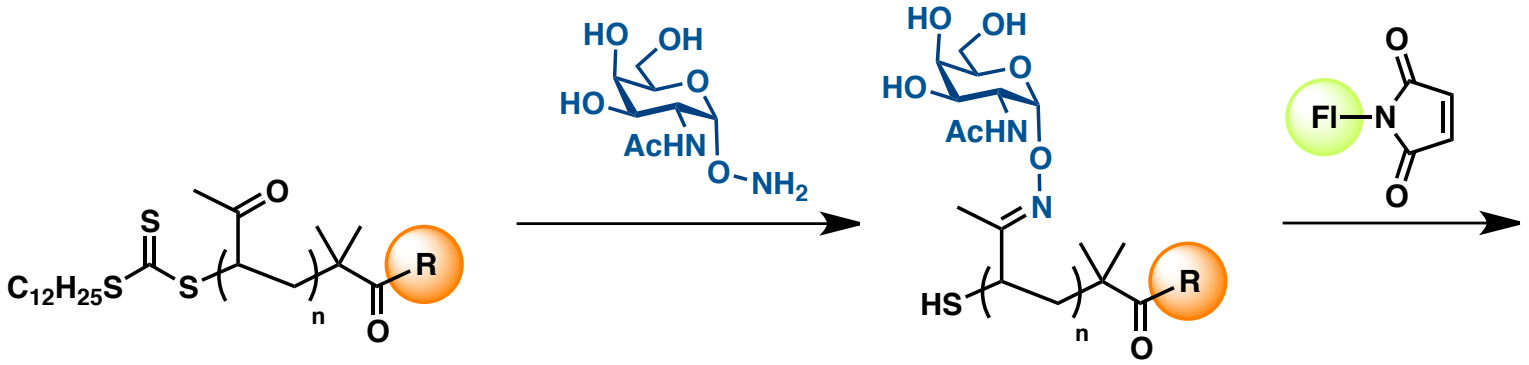


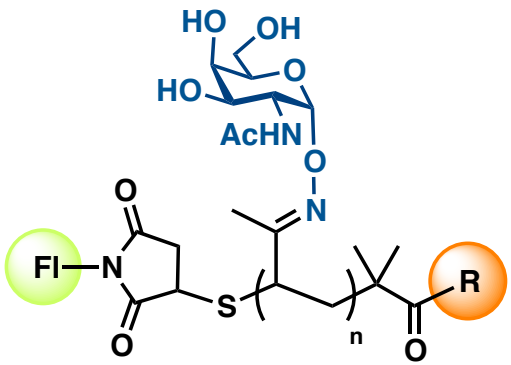


Angew. Chem. Int. Ed. 48, 4973 (2009)

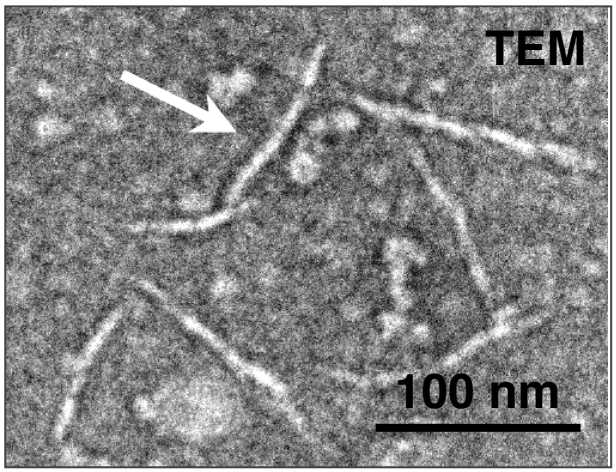
Synthesis of mucin mimetics







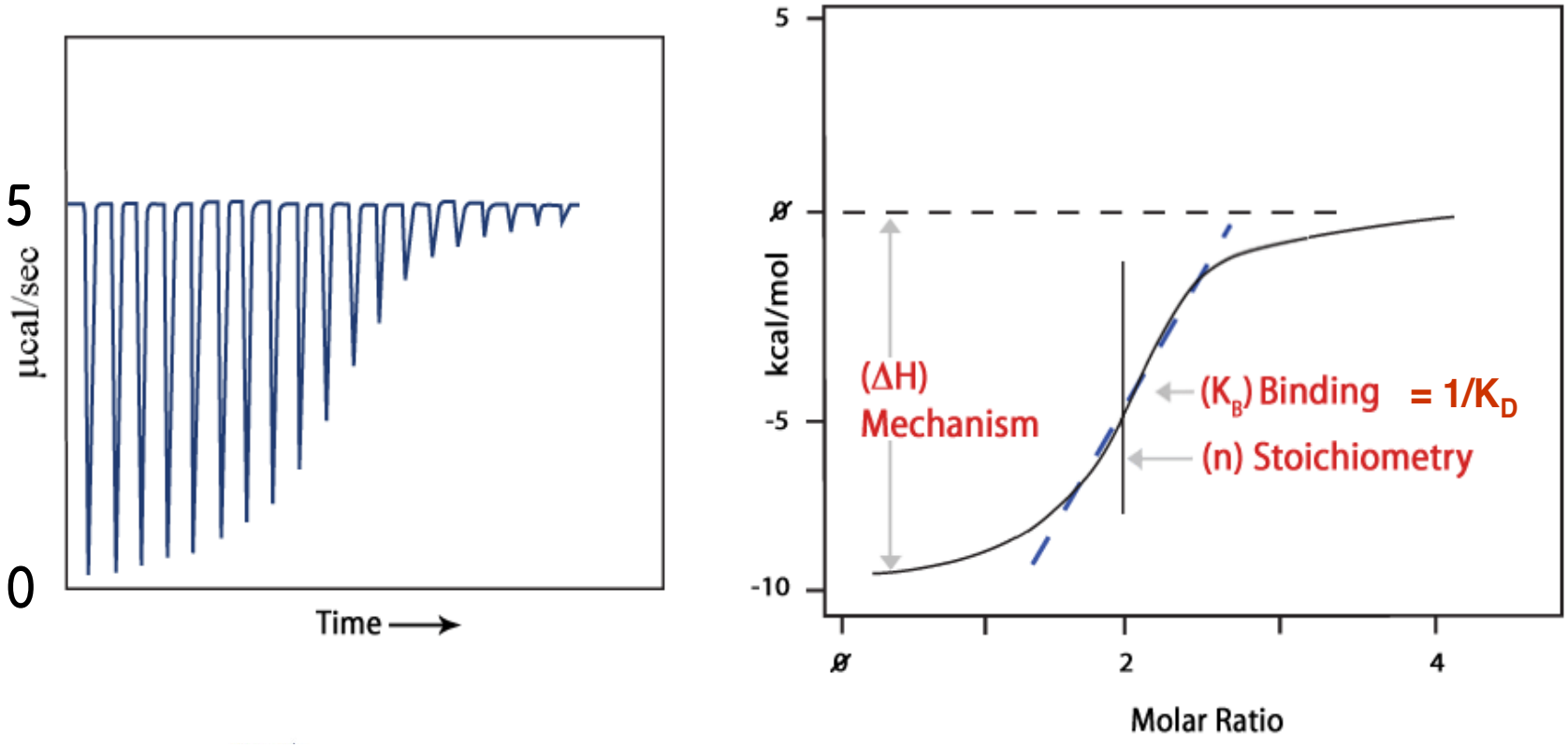
Fl = TAMRA or Alexa Fluor 488



Angew. Chem. Int. Ed. 48, 4973 (2009)

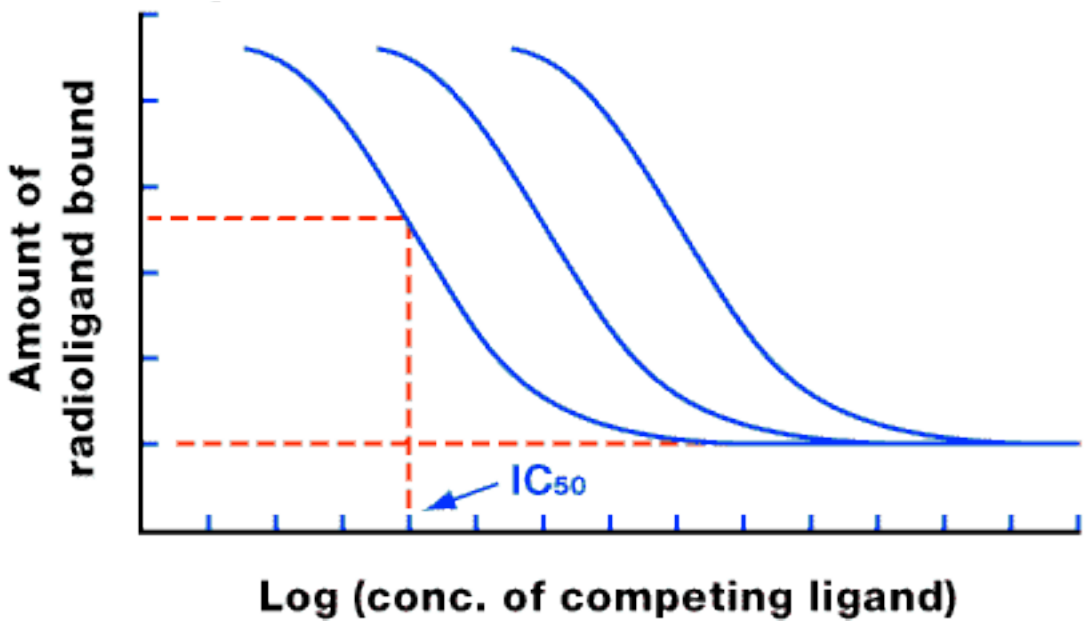
Methods to measure interactions of glycoconjugates with proteins

Isothermal titration calorimetry (ITC):



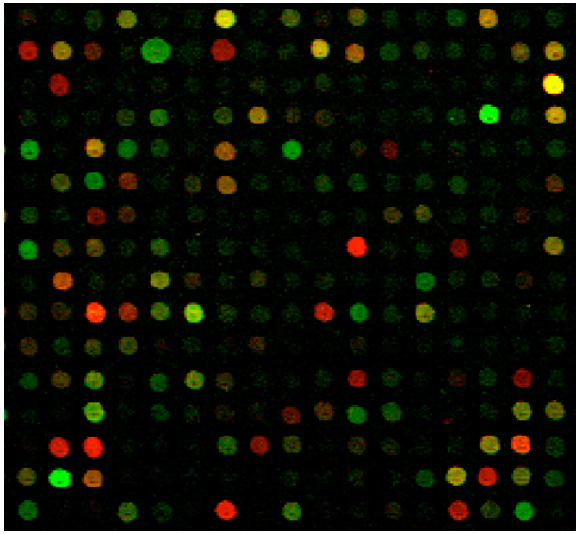
Methods to measure interactions of glycoconjugates with proteins

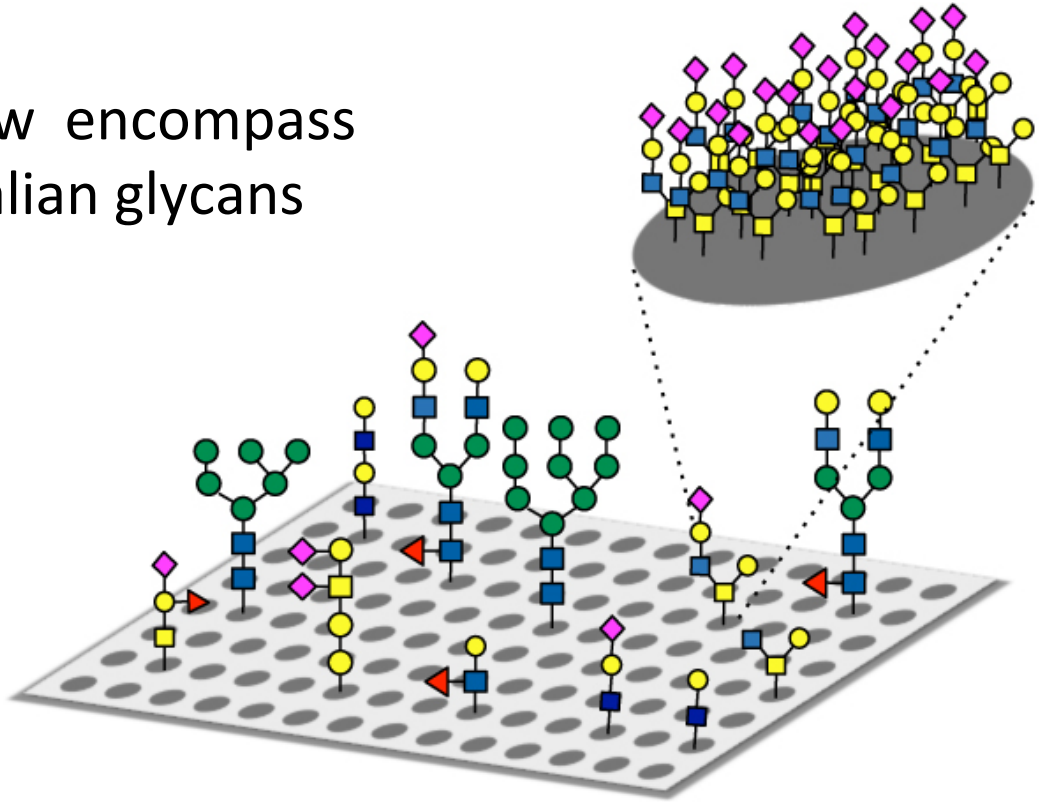
Competition assays with soluble glycoconjugates:



Glycan arrays enable high-throughput analysis of lectin specificities

current microarrays now encompass more than 600 mammalian glycans

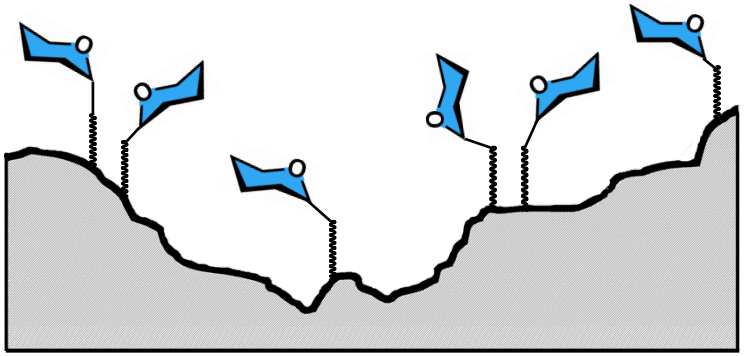




Rillahan and Paulson *Annu. Rev. Biochem. 80*, 797 (2011)

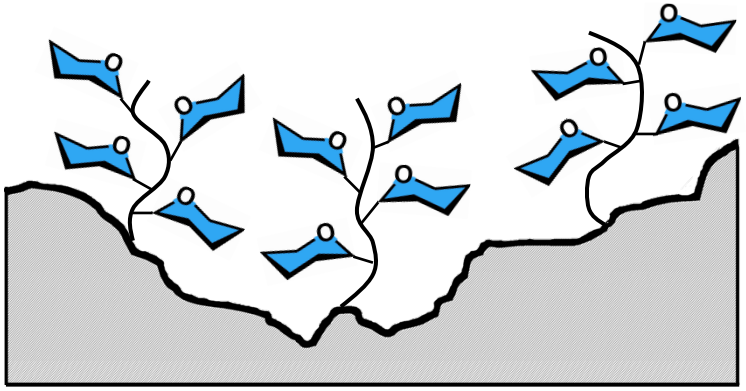
Mucin mimetics allow for control over glycan presentation in microarrays

CONVENTIONAL ARRAY



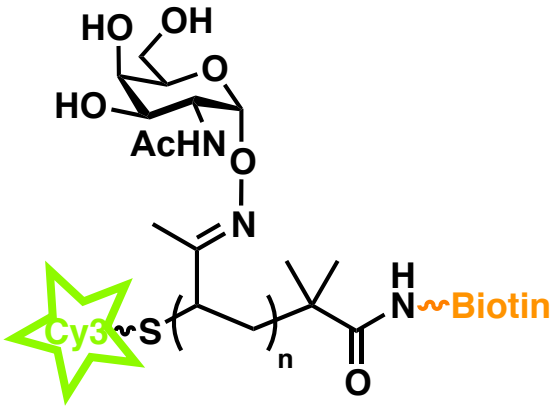
poorly controlled glycan presentation

MUCIN MIMETIC ARRAY

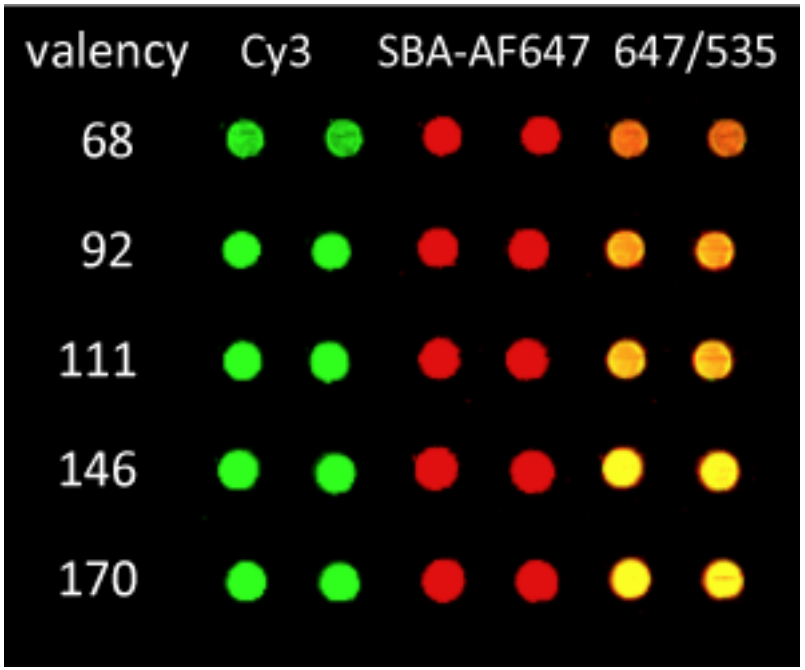


polymer structure dictates spatial glycan arrangements

Evaluation of the effects of GalNAc valency on lectin binding



mucin mimetic series:
n = 205
GalNAc valency: 70-170
maximum length ~ 25 nm

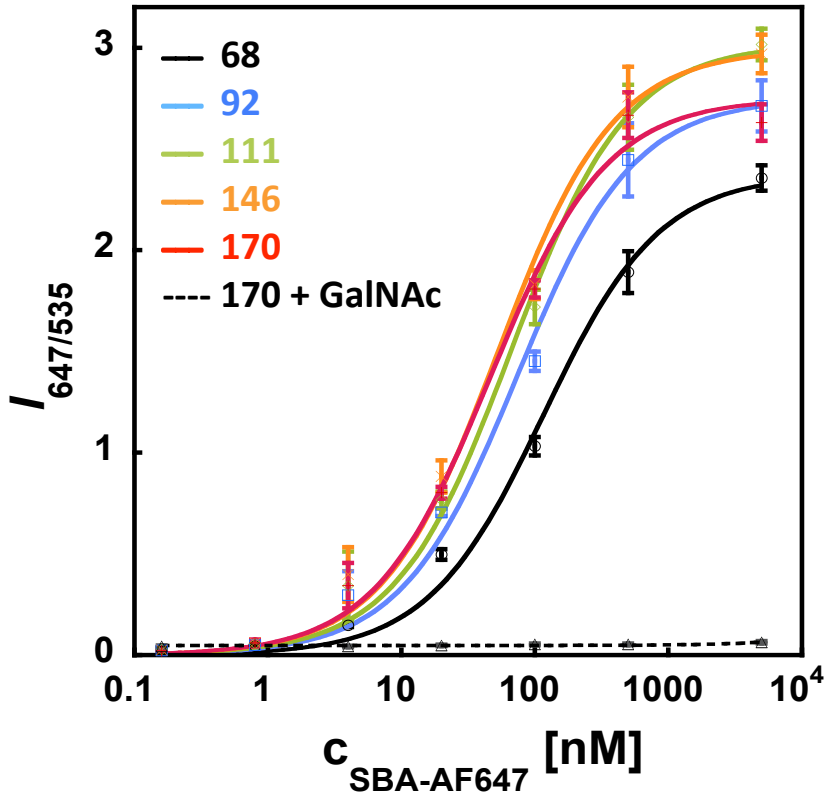


polymer spacing on array ~ 35 nm

The polymers were recognized by soybean agglutinin (SBA)

J. Am. Chem. Soc. 134, 15732 (2012)

Quantitative evaluation of binding avidities

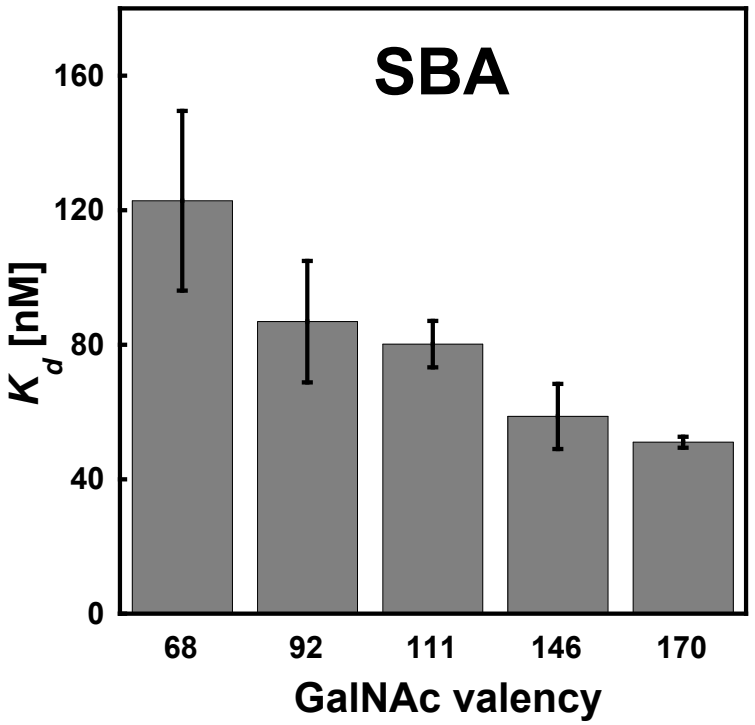


$$I_{647/535,obs} = \frac{I_{647/535,max}}{\frac{K_d}{[L]} + 1}$$

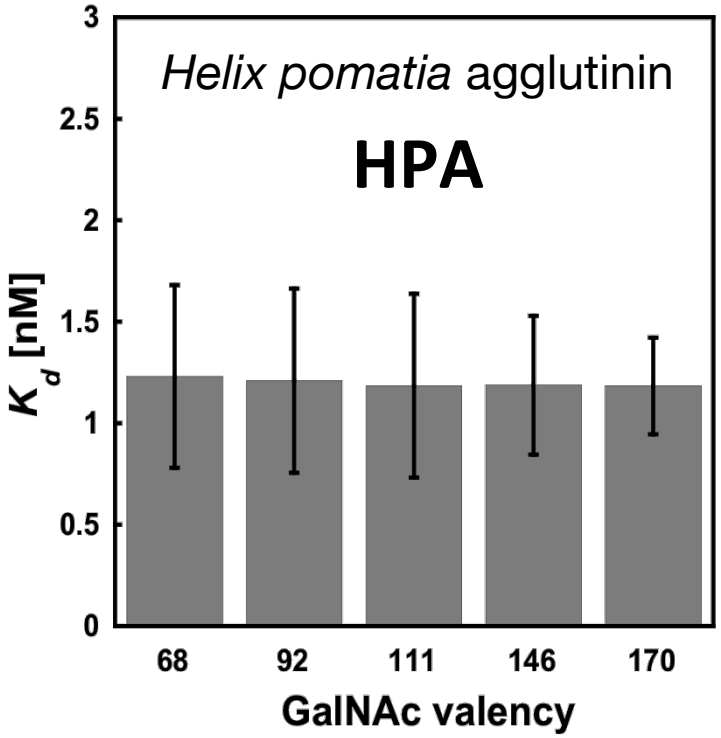
Gordus and MacBeath *J. Am. Chem. Soc. 128*, 13668 (2006)

Mucin mimetic array reveals differences in lectins' sensitivity to GalNAc valency

valency-dependent binding



valency-independent binding

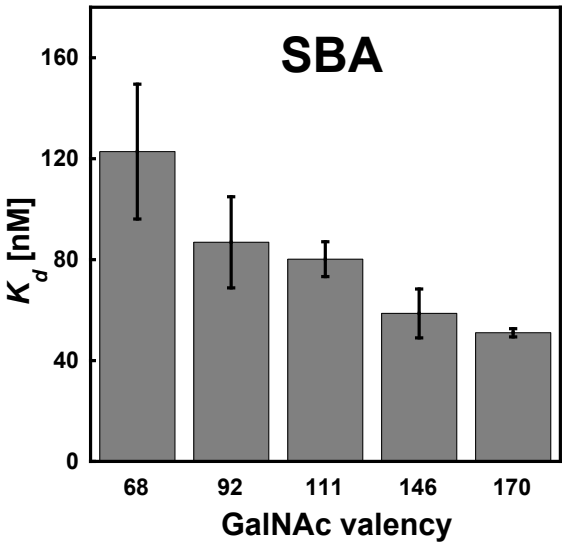


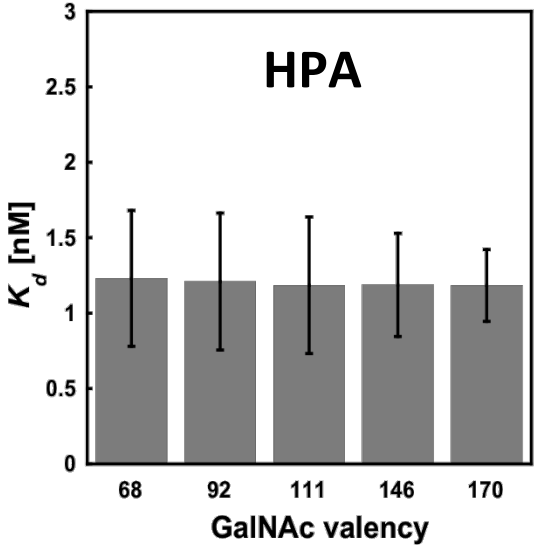
Godula and Bertozzi *J. Am. Chem. Soc. 134*, 15732 (2012)

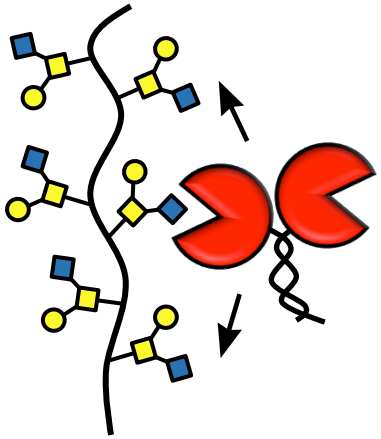
Brewer *J. Biol. Chem. 282*, 28256 (2007)

Oyelaran *et al J. Proteom. Res. 8*, 3529 (2009)

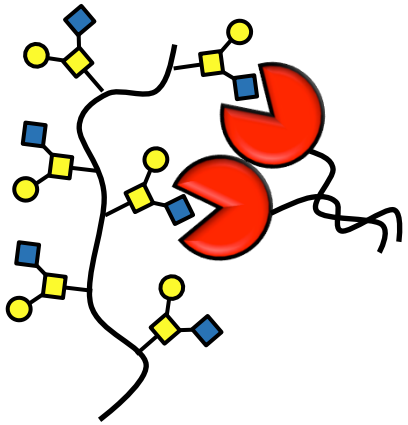
The two profiles represent the basic modes of lectin recognition







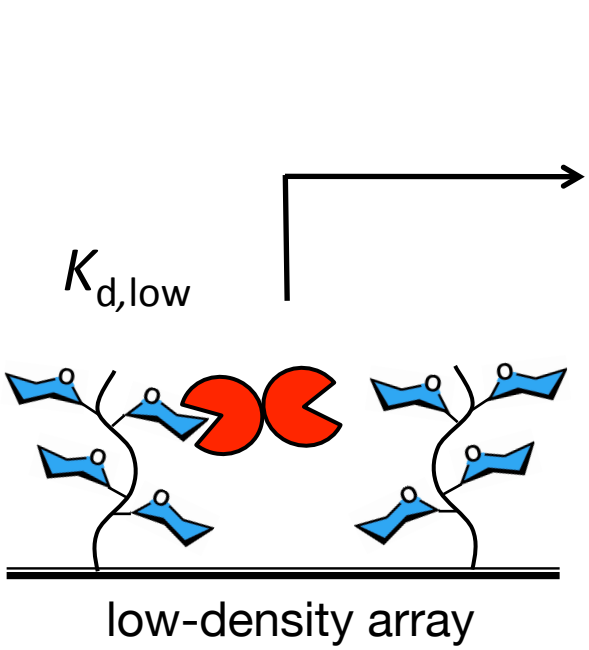
bind-and-slide



face-to-face

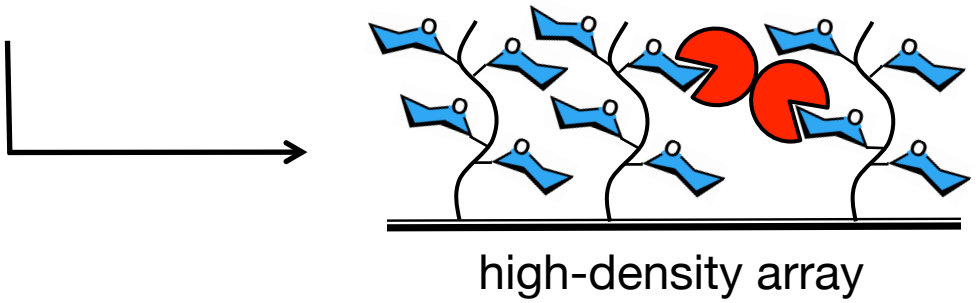
Brewer *J. Biol. Chem.* **2007**, *282*, 28256

Mucin mimetic array can reveal higher-order binding interactions



DISCRETE COMPLEX

$K_{d,high} \cong K_{d,low}$

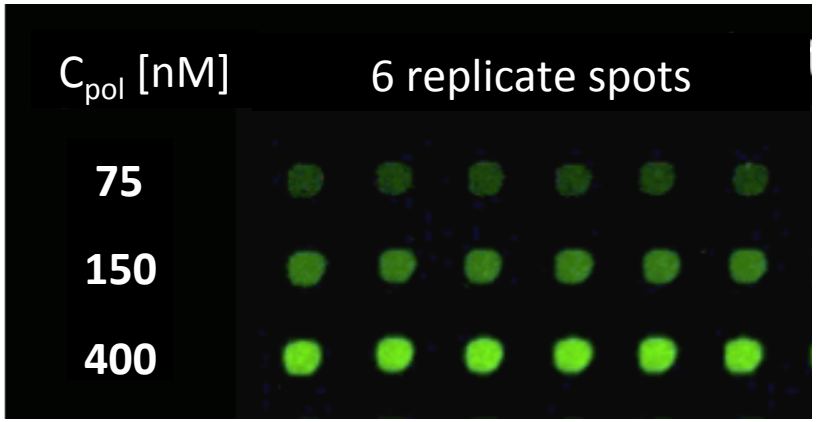


CROSSLINKED COMPLEX

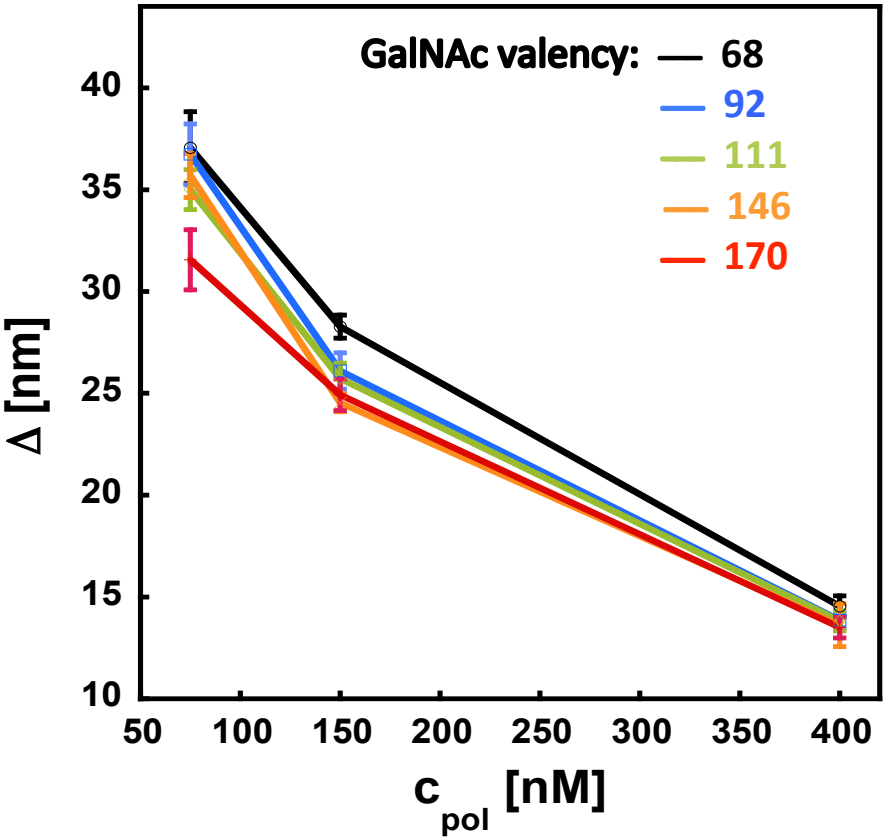
$K_{d,high} < K_{d,low}$

J. Am. Chem. Soc. 134, 15732 (2012)

Mucin mimetic array with variable glycopolymer surface density

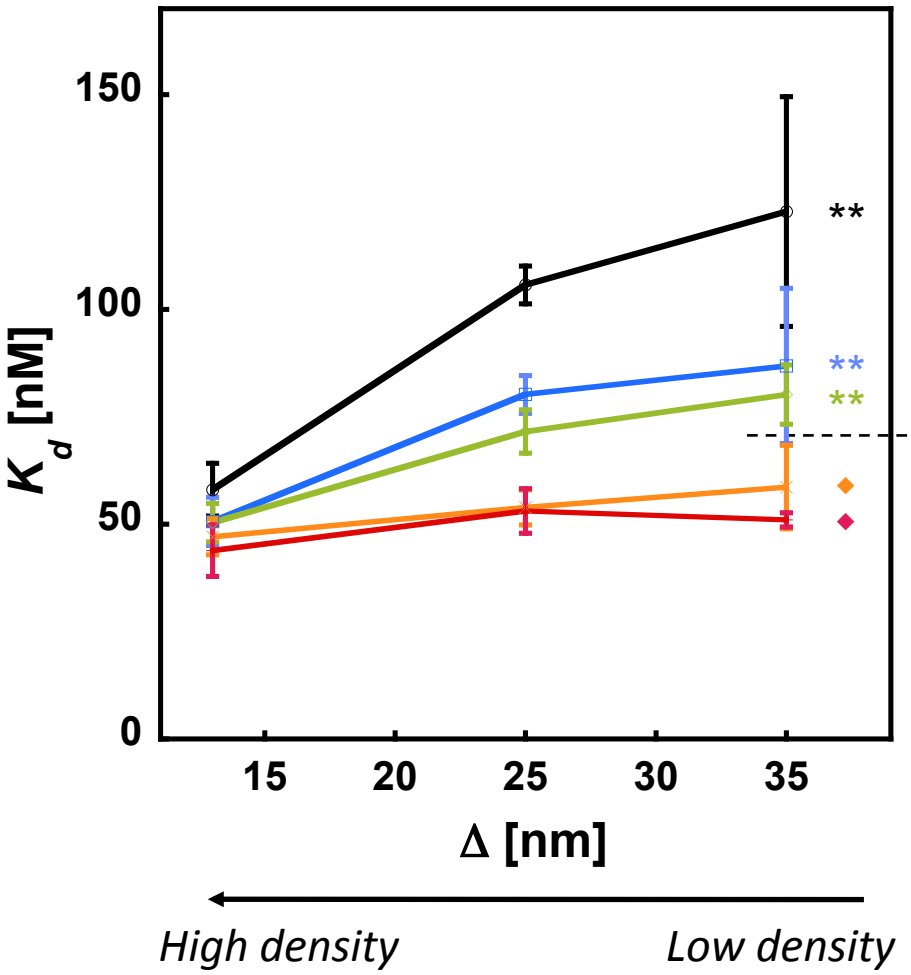


average polymer spacing



J. Am. Chem. Soc. 134, 15732 (2012)

SBA crosslinks mucin mimetics at valencies below 110



GalNAc valency

68

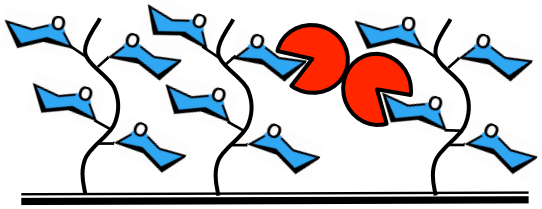
92

111

146

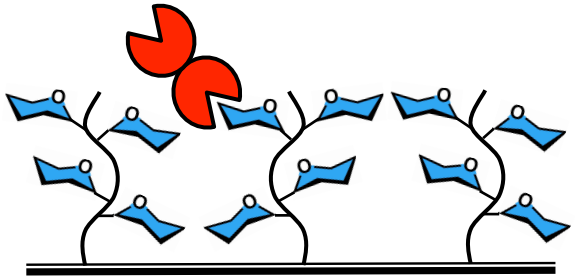
170

CROSSLINKING



$K_{d,high} < K_{d,low}$

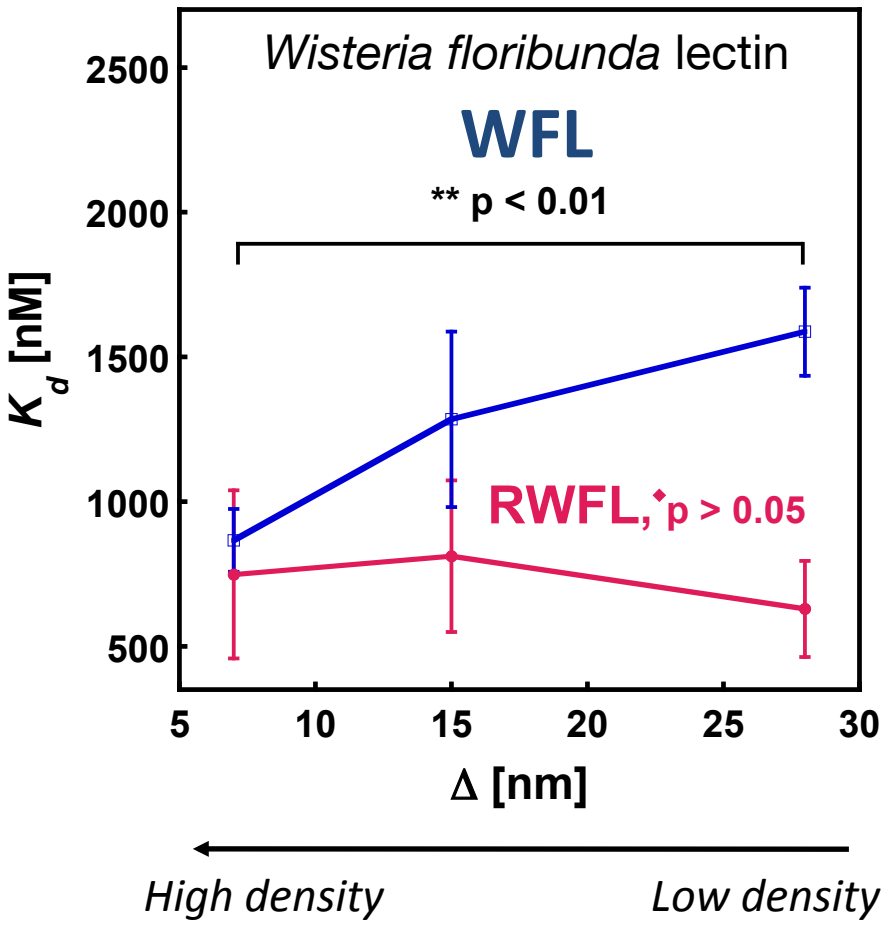
1:1 COMPLEX

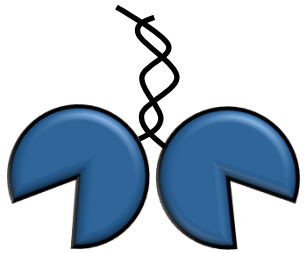


$K_{d,high} \cong K_{d,low}$

J. Am. Chem. Soc. 134, 15732 (2012)

No crosslinking is observed in monomeric lectins

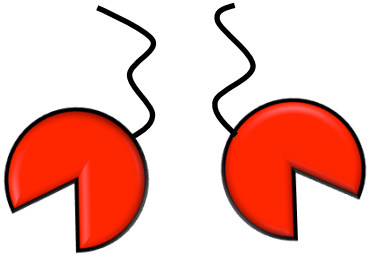




WFL

disulfide-bridged agglutinating form

1. DDT
2. 2-vinylpyridine

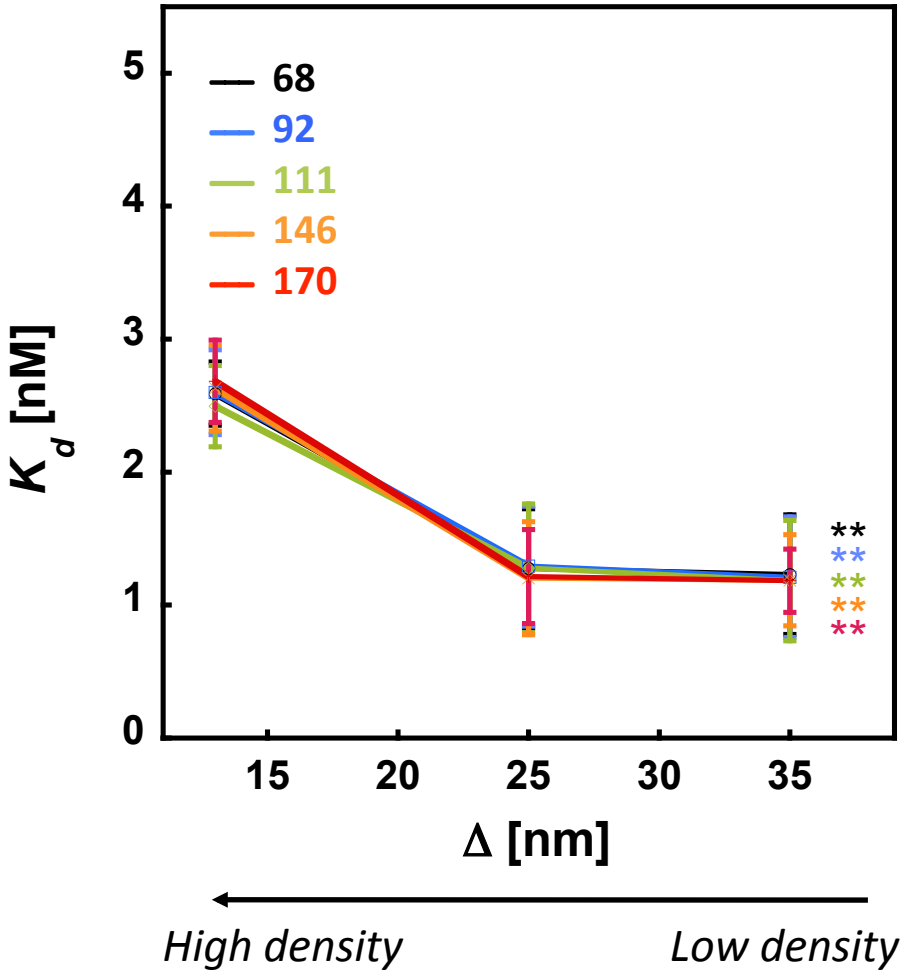


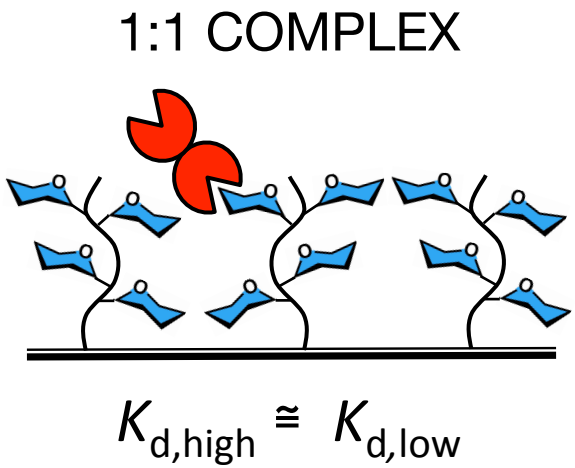
RWFL

reduced non-agglutinating form

J. Am. Chem. Soc. 134, 15732 (2012)

Helix pomatia agglutinin (HPA) does not crosslink the surface-bound mimetics

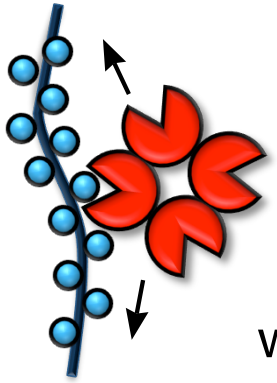




J. Am. Chem. Soc. 134, 15732 (2012)

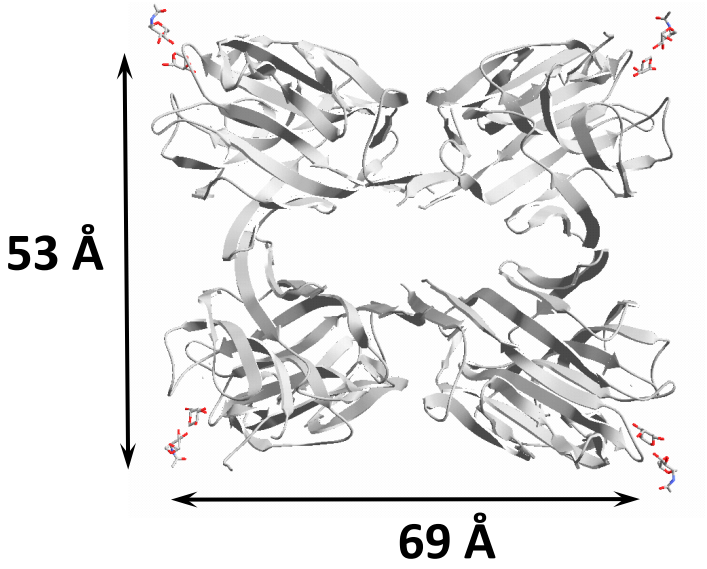
Lectin structure is likely to influence binding mode and ability to crosslink mucins

soybean agglutinin

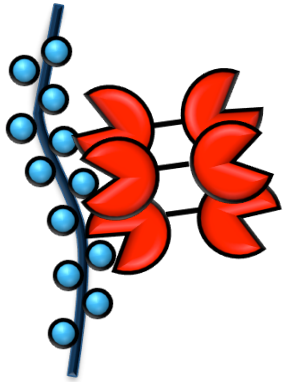


valency-dependent binding

propensity to crosslink with a valency threshold

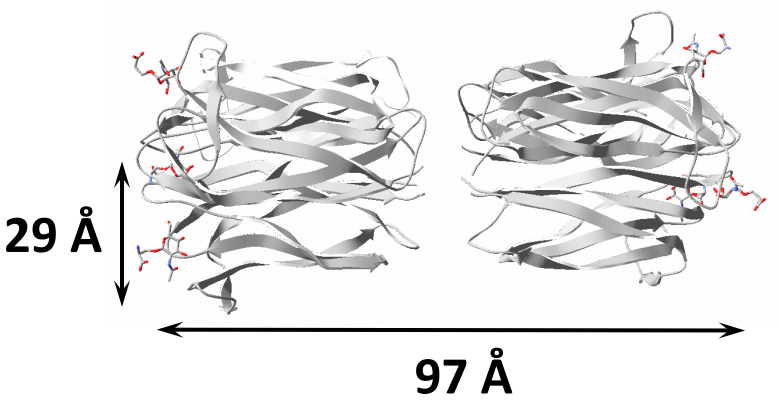


helix pomatia agglutinin



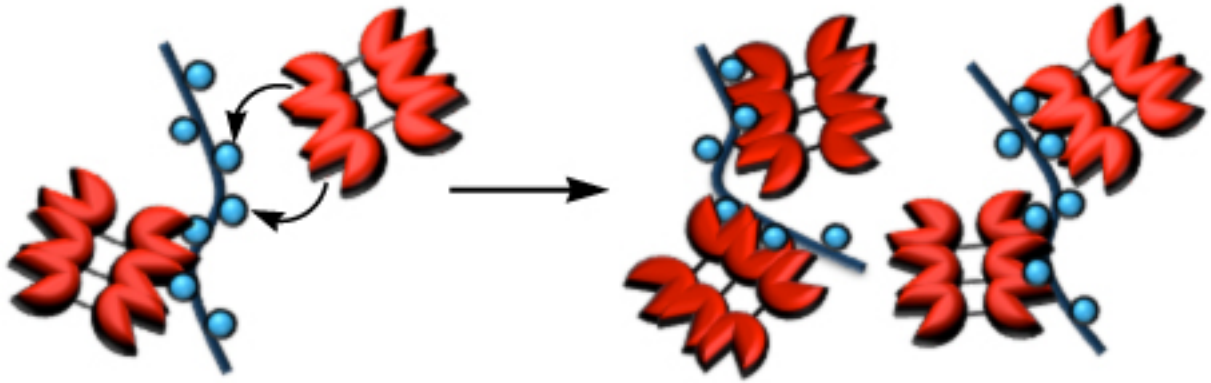
valency-independent binding

no crosslinking

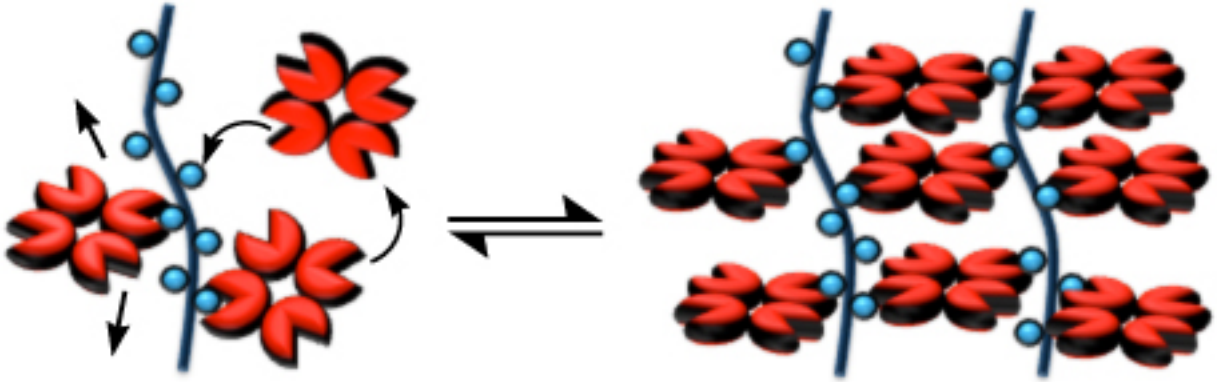


Gupta *et al Biochemistry 33*, 7495 (1994)
Sanchez *et al J. Biol. Chem. 281*, 20171 (2006)

Hypothesis: mode and kinetics of binding likely determine the extent of crosslinking

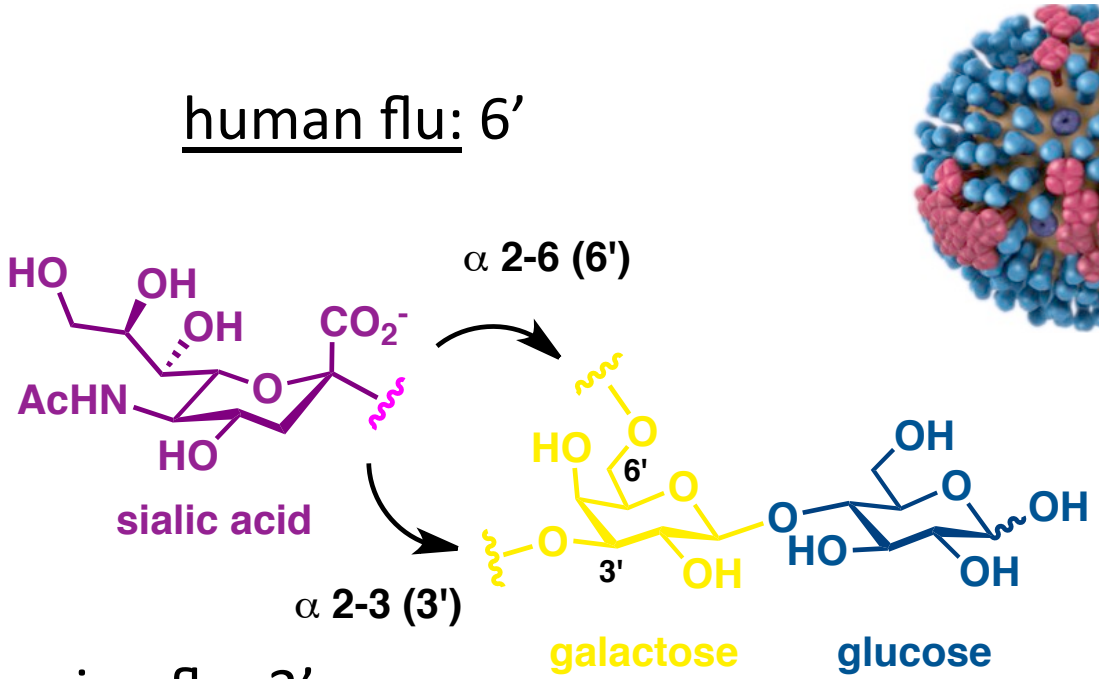


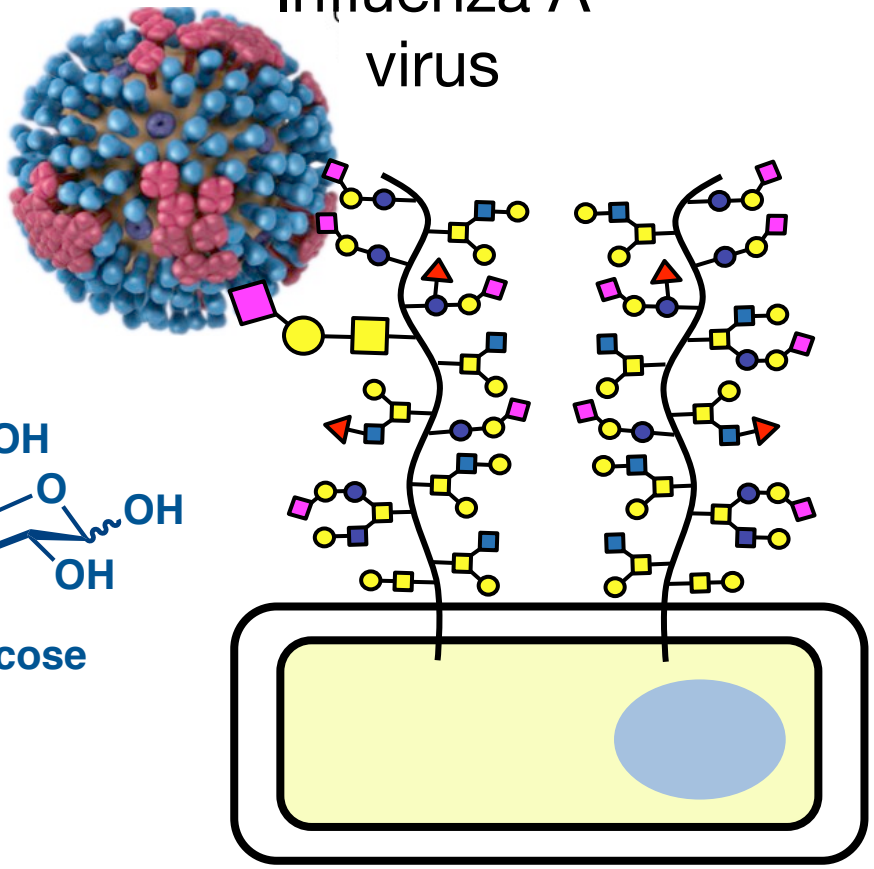
face-to-face binding
rapid and “irreversible”



bind-and-slide mode
reversible and dynamic

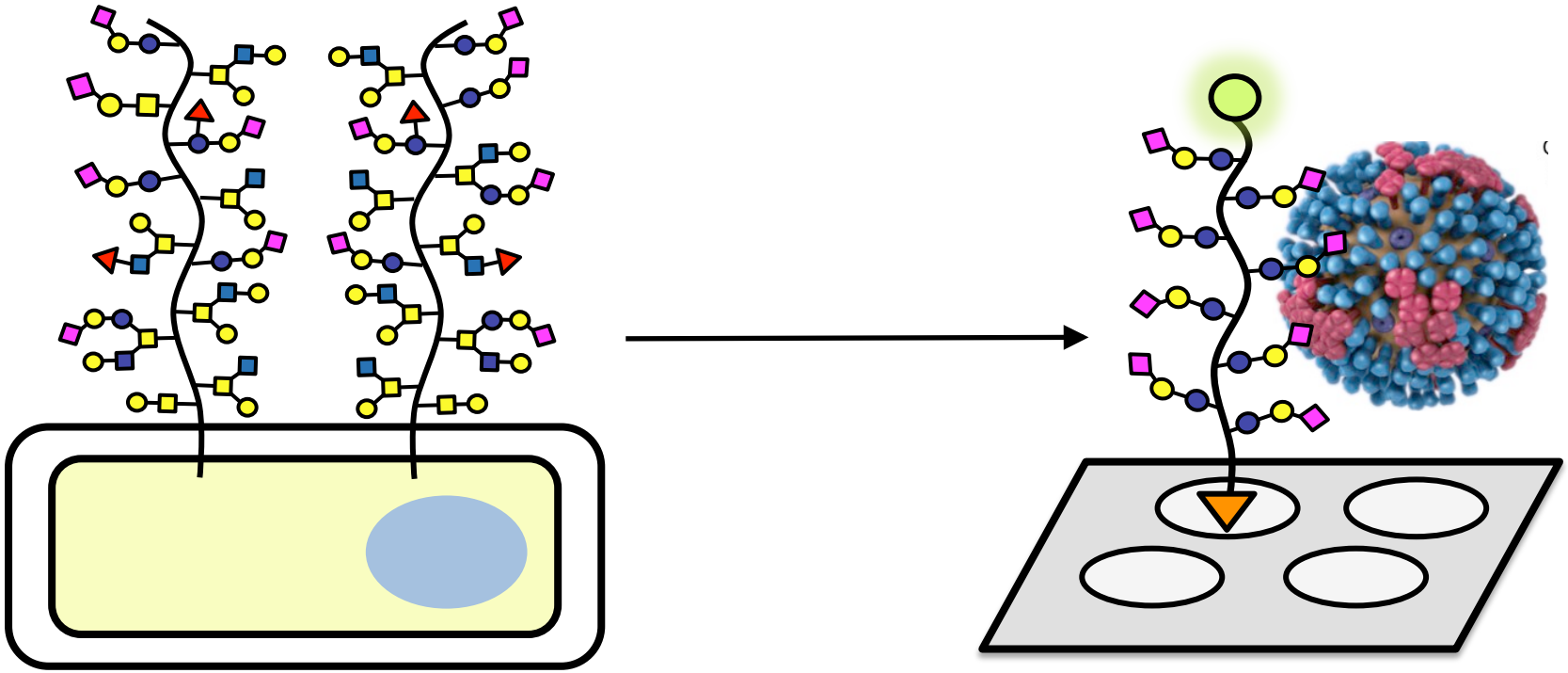
Pathogens utilize cell surface glycans to gain entry into host cells





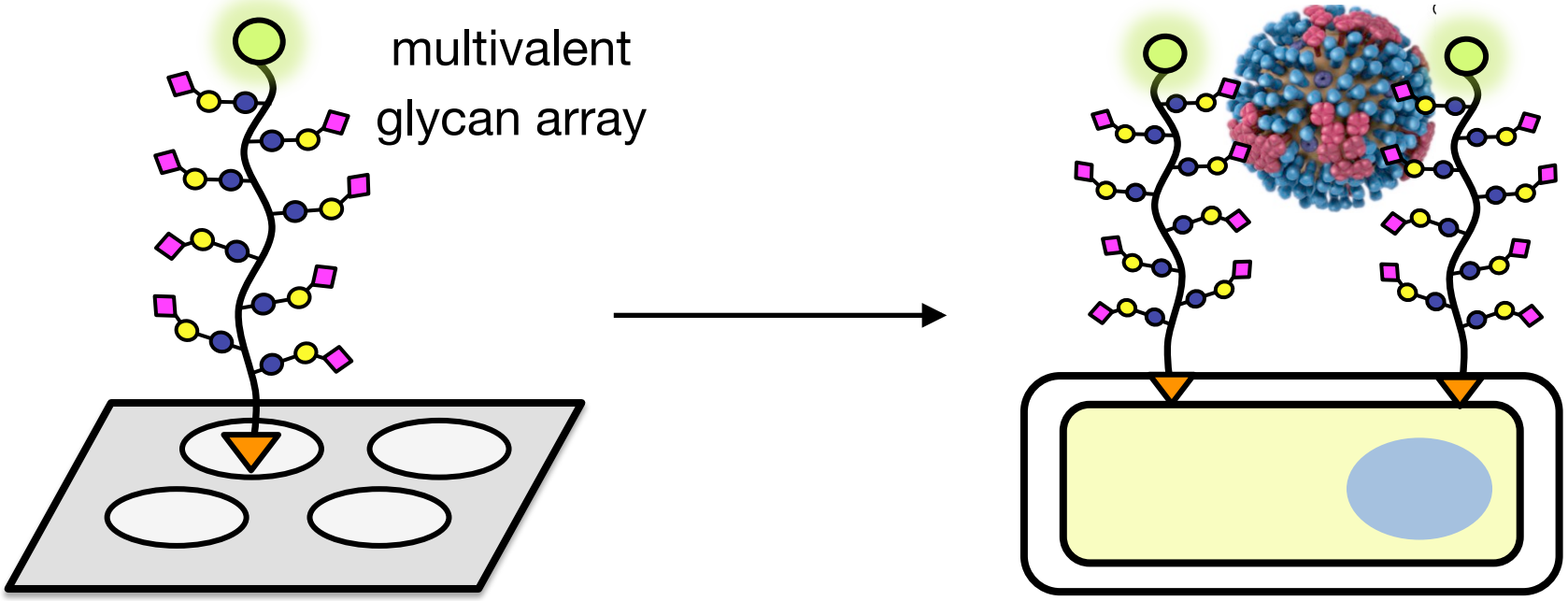
swine flu: 3’ and 6’
recognizes both linkages

Array platform to study Influenza A interactions with surface glycans



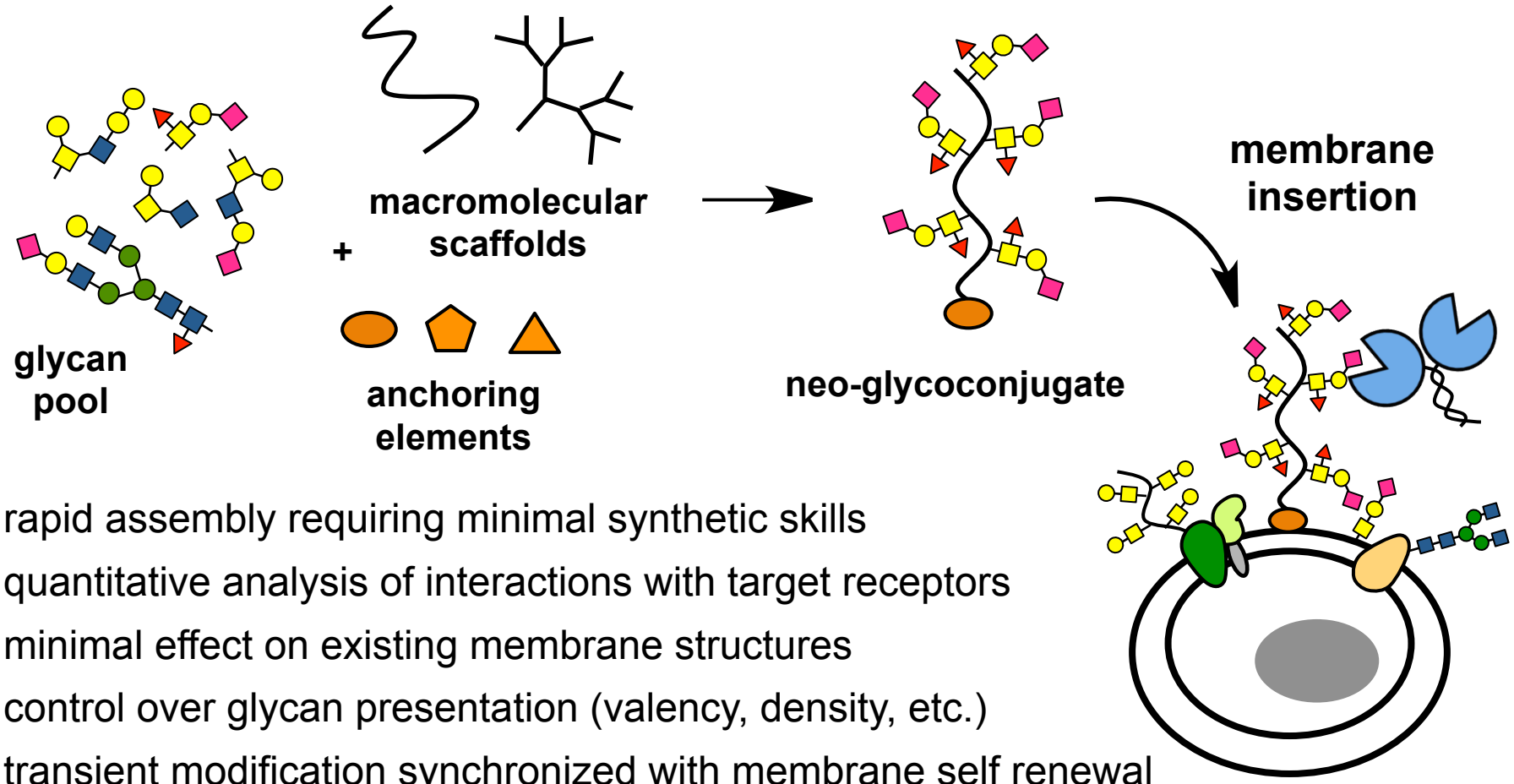
Huang, Cohen, Fisher, Schooley, Gagneux, Godula *ChemComm* (ASAP 2015)

Modeling infection: the "There and back again" approach



Chris Fisher

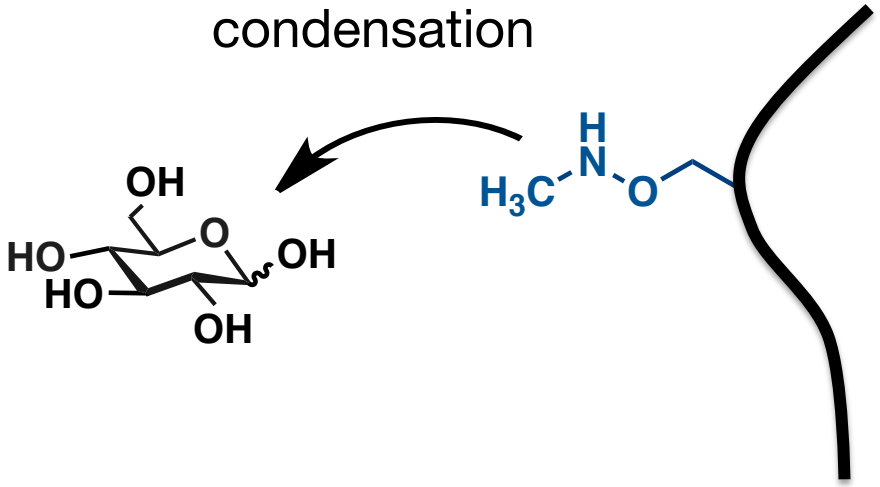
The “There and back again” strategy

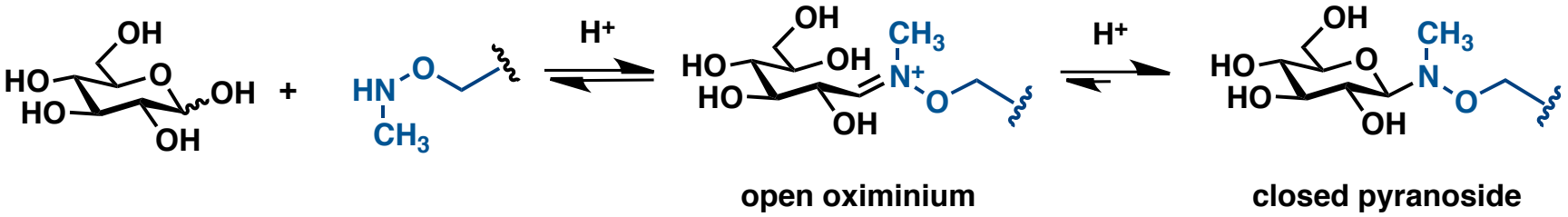


- rapid assembly requiring minimal synthetic skills
- quantitative analysis of interactions with target receptors
- minimal effect on existing membrane structures
- control over glycan presentation (valency, density, etc.)
- transient modification synchronized with membrane self renewal

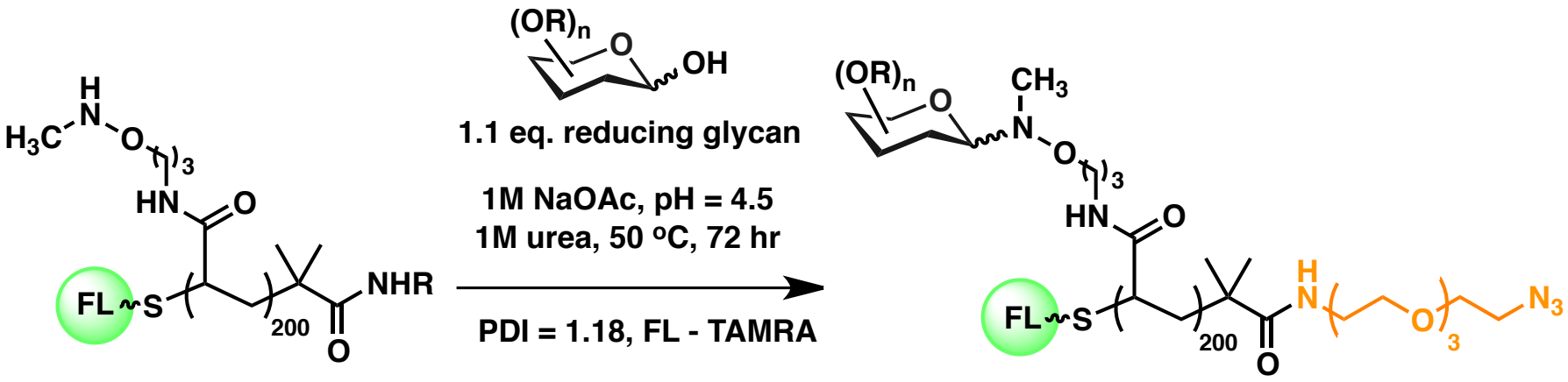
Godula and Bertozzi *JACS 132*, 9963 (2010)
Godula, Rabuka, Umbell, Bertozzi and Parthasarathy *JACS 131*, 10263 (2009)
Rabuka, Forstner, Groves, Bertozzi *JACS 130*, 5947 (2008)

Glycopolymer assembly via "reverse" ligation of reducing glycans





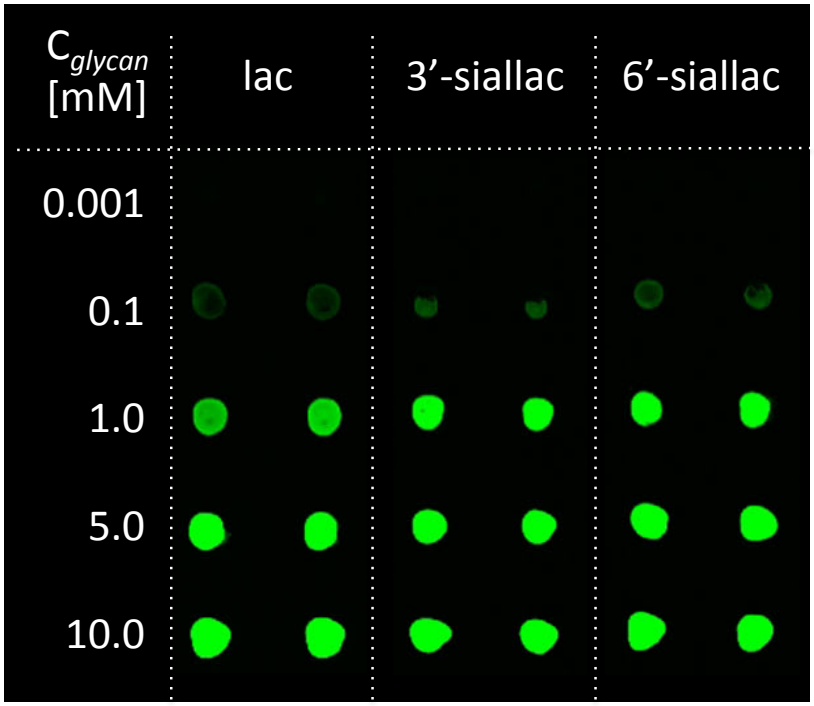
Assembly of mucin mimetics for sialoglycan display in microarrays

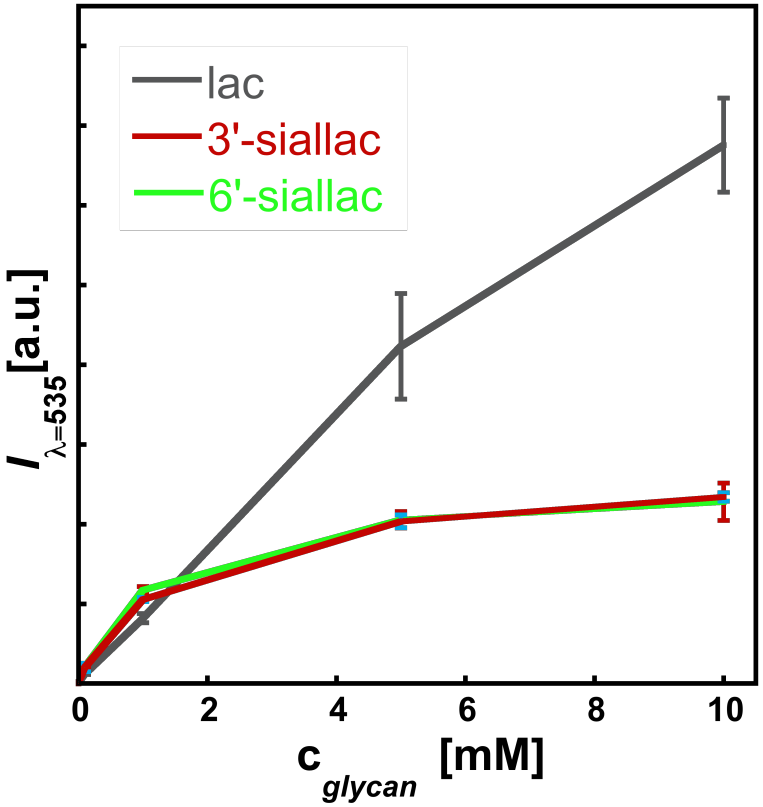


lactose	(α2-3) 3'-sialyllactose	(α2-6) 6'-sialyllactose
ligation efficiency: 70% glycan valency: 140	ligation efficiency: 45% glycan valency: 90	ligation efficiency: 45% glycan valency: 90

Huang, Cohen, Fisher, Schooley, Gagneux, Godula *ChemComm* (ASAP 2015)

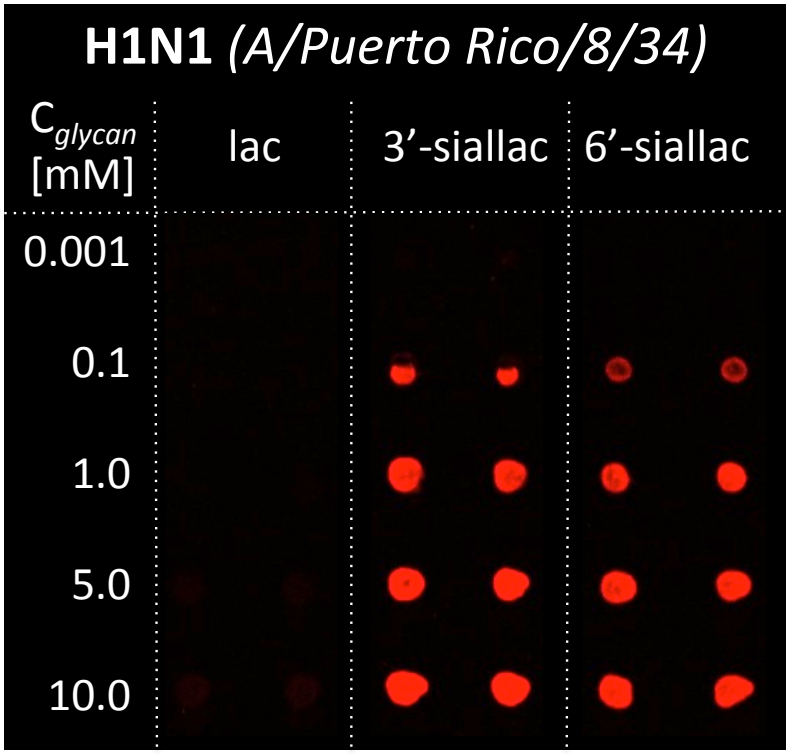
Construction and characterization of a density variant sialoglycan array

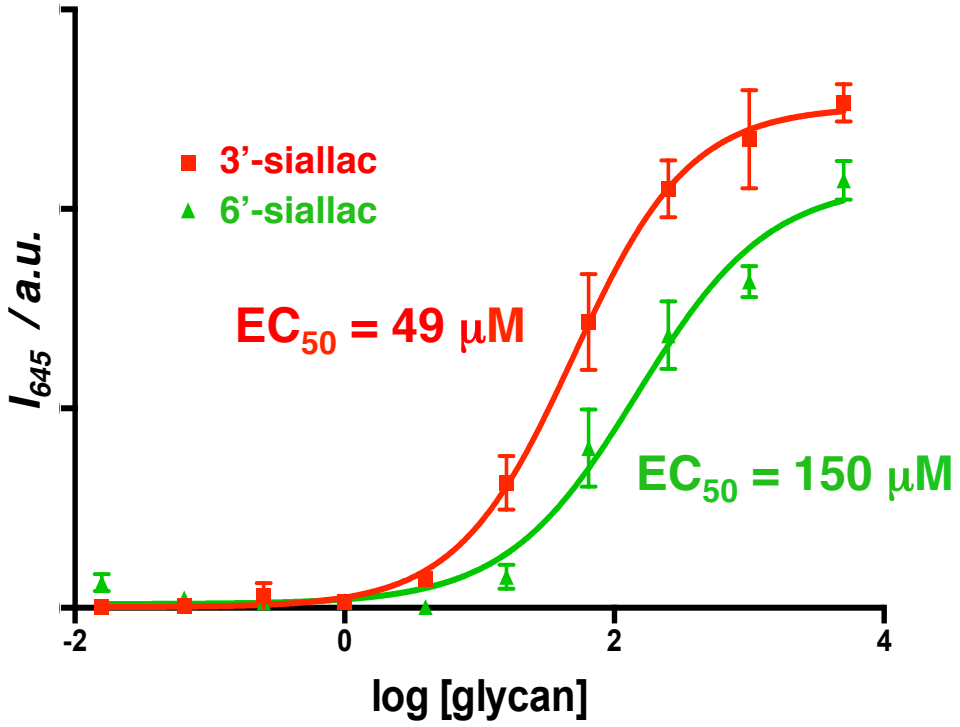




Huang, Cohen, Fisher, Schooley, Gagneux, Godula *ChemComm* (2015)

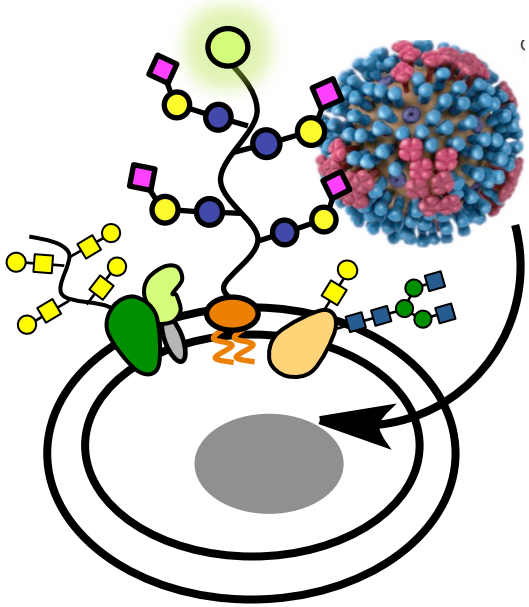
Binding of H1N1 Influenza virus to mucin-mimetic glycan array





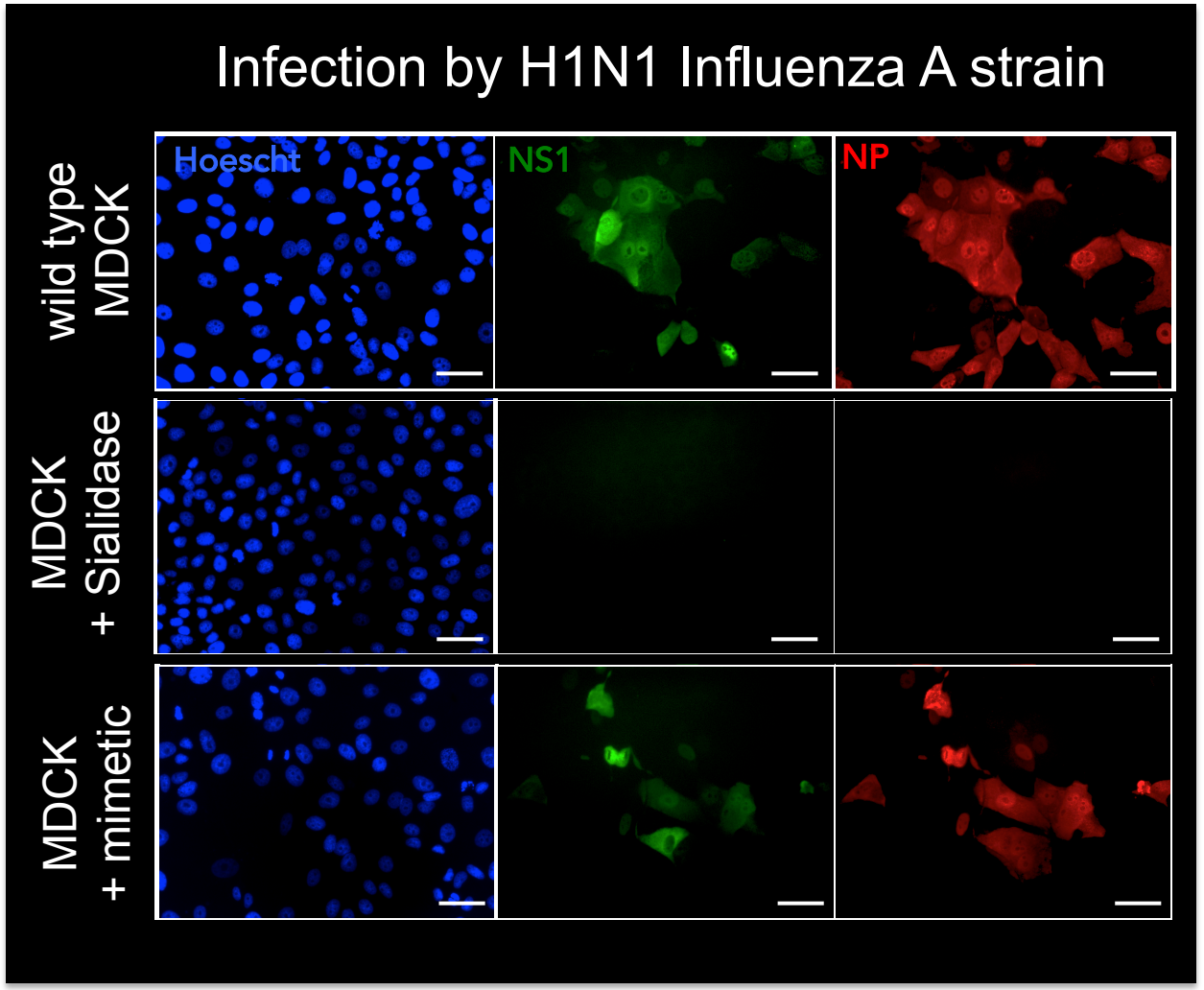
Huang, Cohen, Fisher, Schooley, Gagneux, Godula *ChemComm* (2015)

Priming the glycocalyx of MDCK cells for Influenza A infection

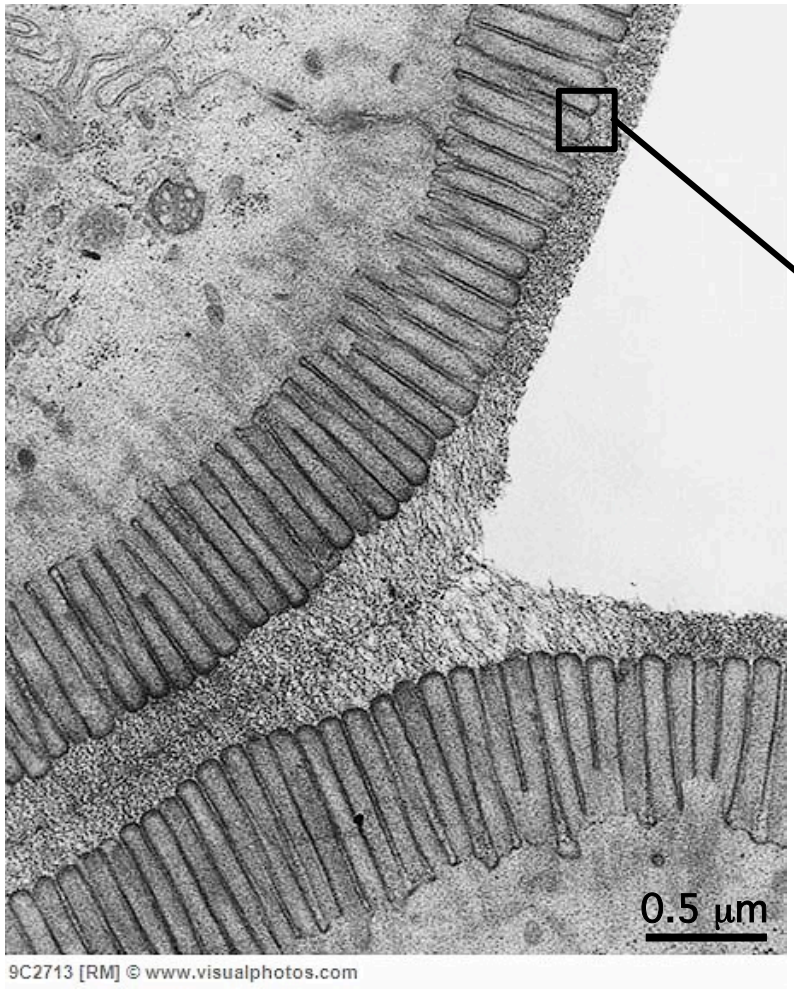


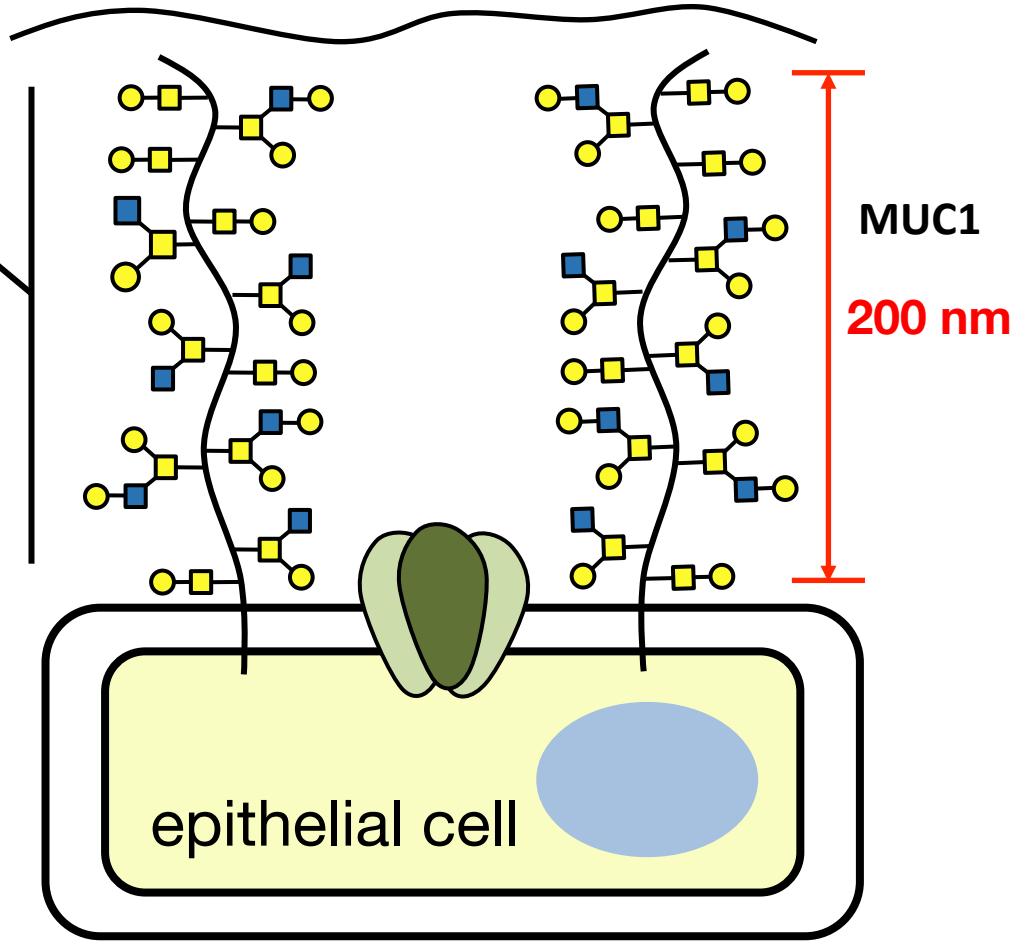
MDCK cell

6'-siallyllactose



Mucins create physical barriers





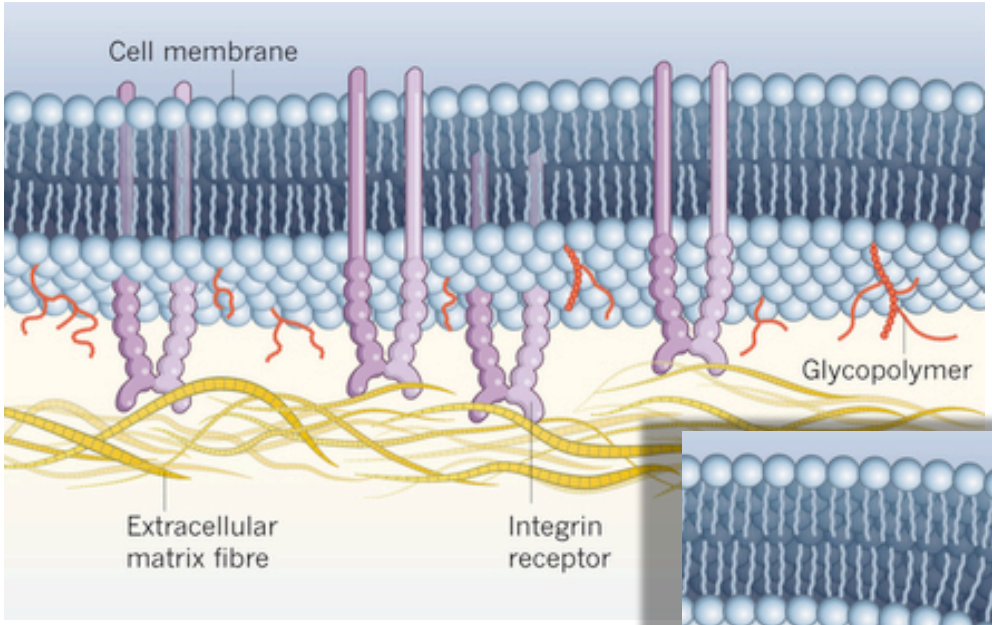
Intestinal epithelium, TEM
Don W. Fawcett

Essentials of Glycobiology
http://www.ncbi.nlm.nih.gov/books/NBK1908/

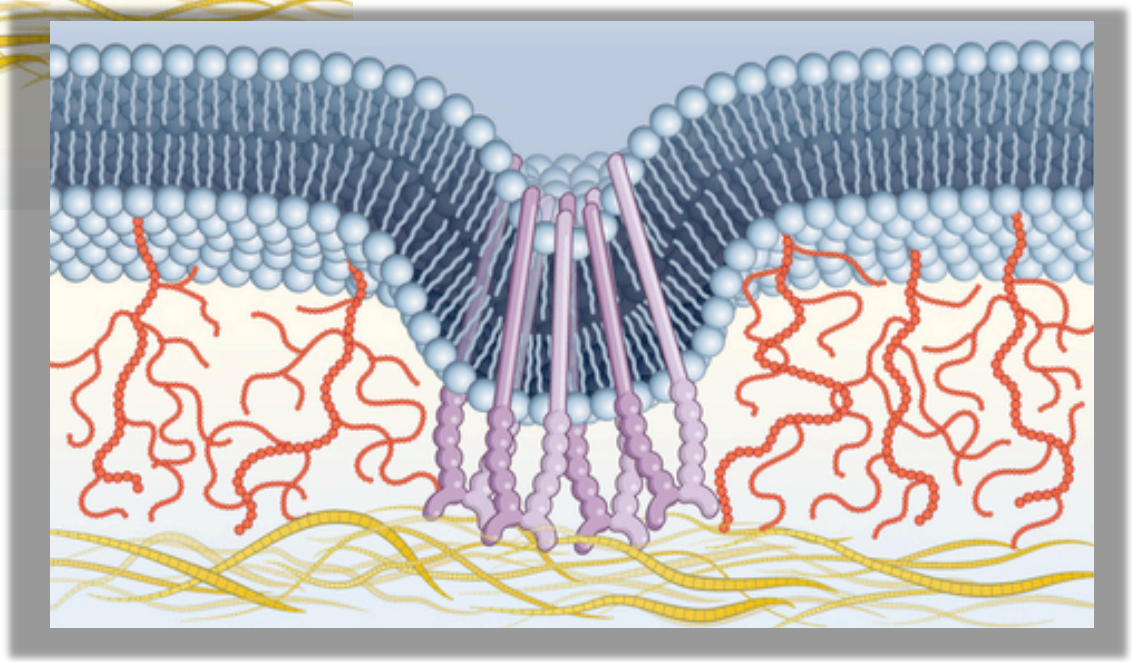
The mucin paradox

- bulky glycoproteins, such as MUC1, are overexpressed in aggressive cancers
- mucins may provide protection and enable survival in host tissues
- the rigidity of mucins may affect cancer cell adhesion and spreading

Mucin may promote formation of focal adhesions in soft tissues

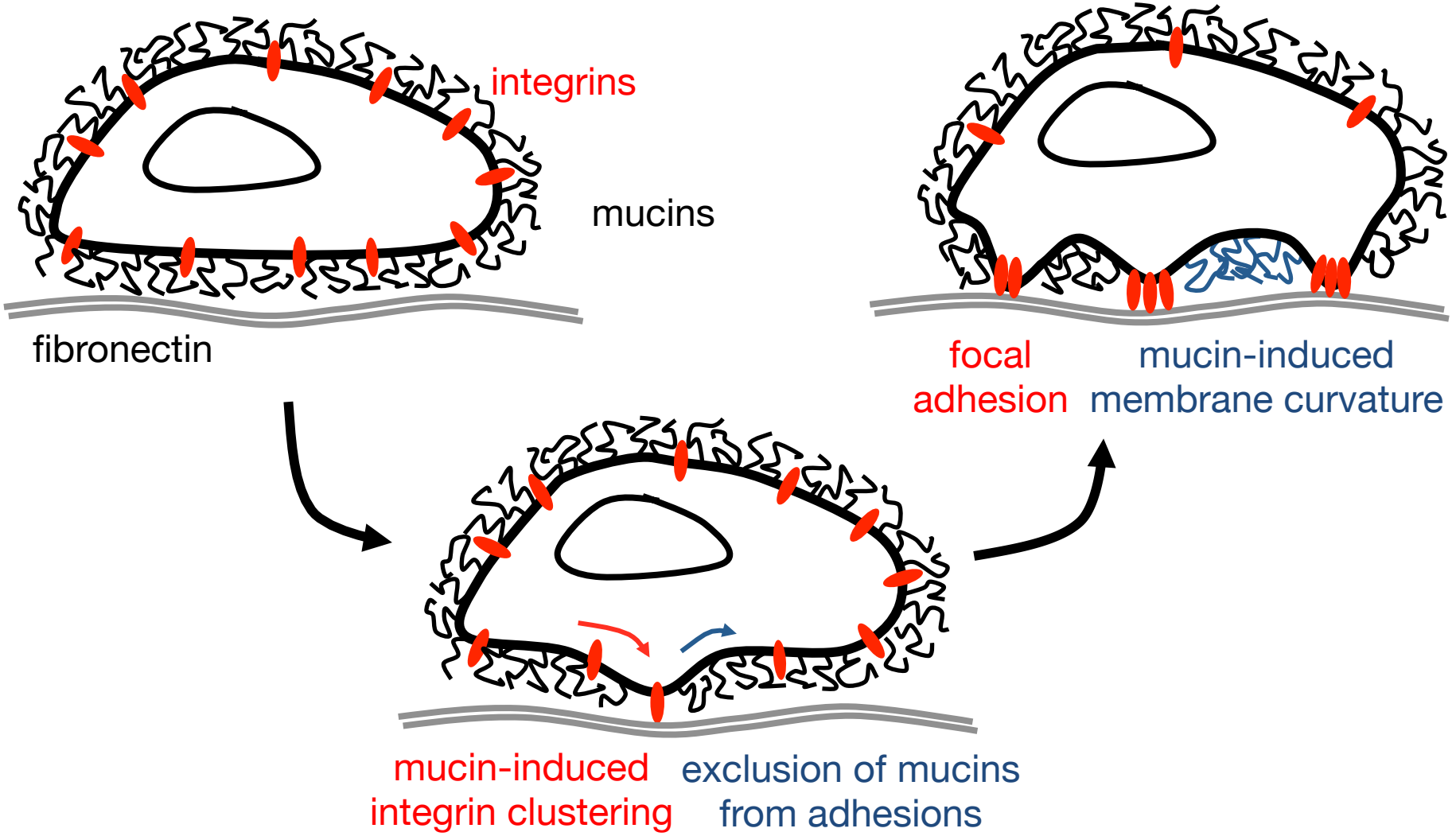


integrin clustering is required for survival and proliferation



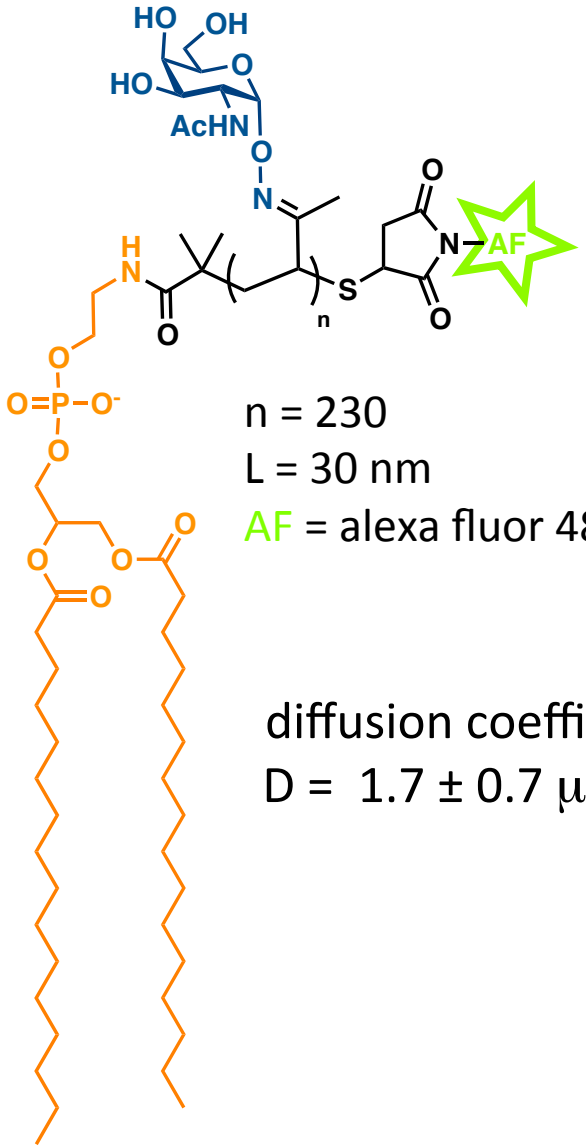
Paszek *et al Nature 511*, 319 (2014)

Model for mechanical priming of cancer cells for adhesion



Paszek *et al. PLoS Comp. Biol. 5,* e1000604 (2009)

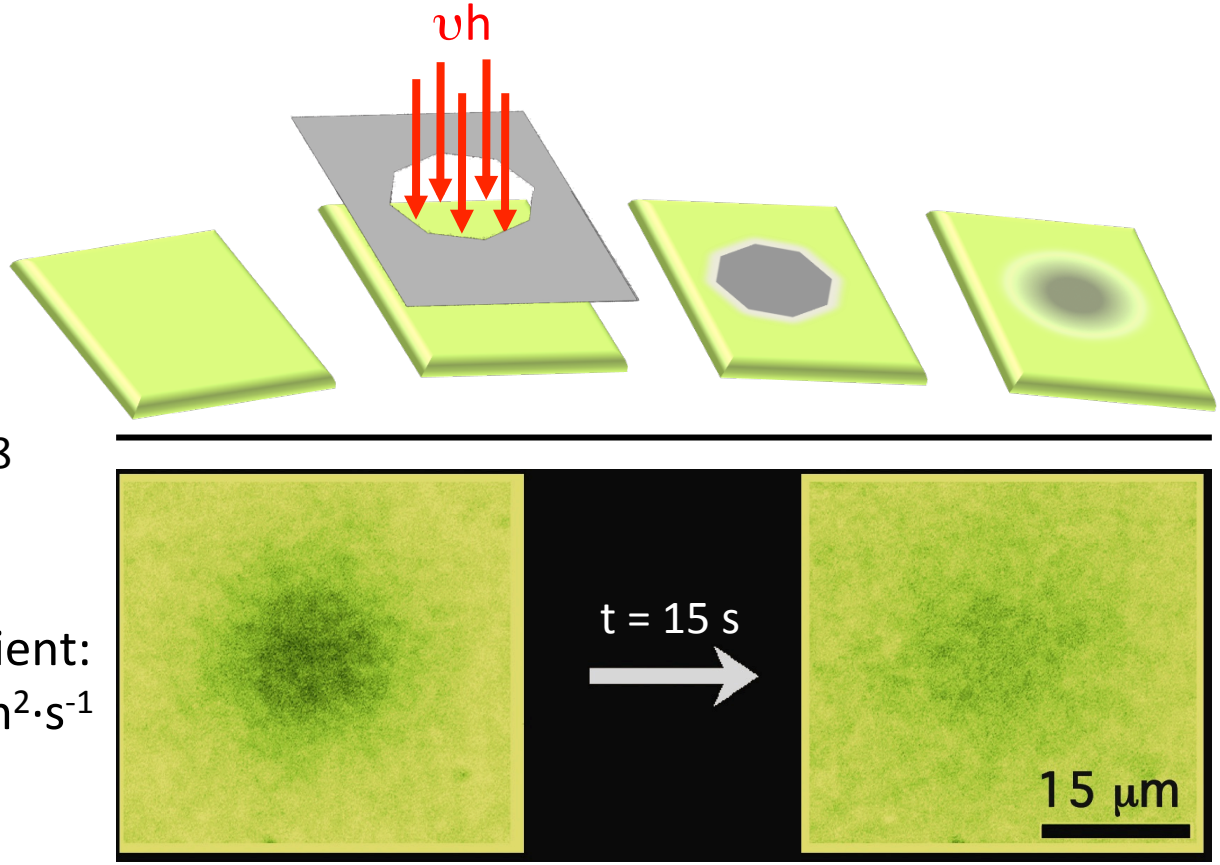
Lipid-terminated mucin mimetics are fluid in supported lipid bilayers



n = 230
L = 30 nm
AF = alexa fluor 488

diffusion coefficient:
D = 1.7 ± 0.7 $\mu m^2 \cdot s^{-1}$

Fluorescence recovery after photobleaching:

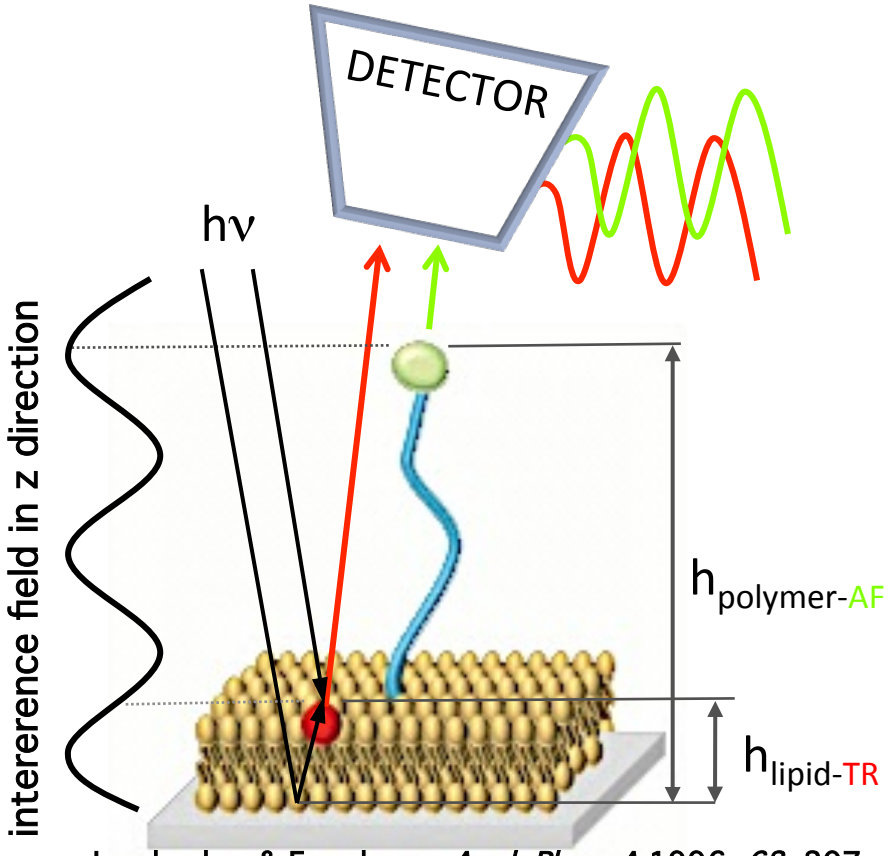


lipid: dioleyl phosphatidylcholine (D_{lipid}= 1.2 ± 0.4 $\mu m^2 \cdot s^{-1}$)

J. Am. Chem. Soc. 131, 10263 (2009)

Mucin mimetics accommodate upright orientation in supported lipid bilayers

Fluorescence Interference Contrast (FLIC) Microscopy



Lambacher & Fromherz, *Appl. Phys. A* 1996, *63*, 207
Boxer *et al. Langmuir* 2005, *21*, 4976

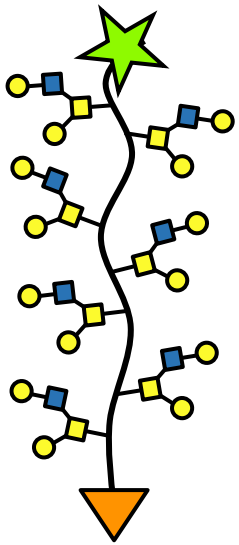
for ~30 nm mucin mimetic:

$h_{lipid\text{-}TR}$	5.5 ± 0.9 nm
$h_{mimetic\text{-}AF}$	16.7 ± 1.1 nm
Δh	11.2 ± 1.2 nm

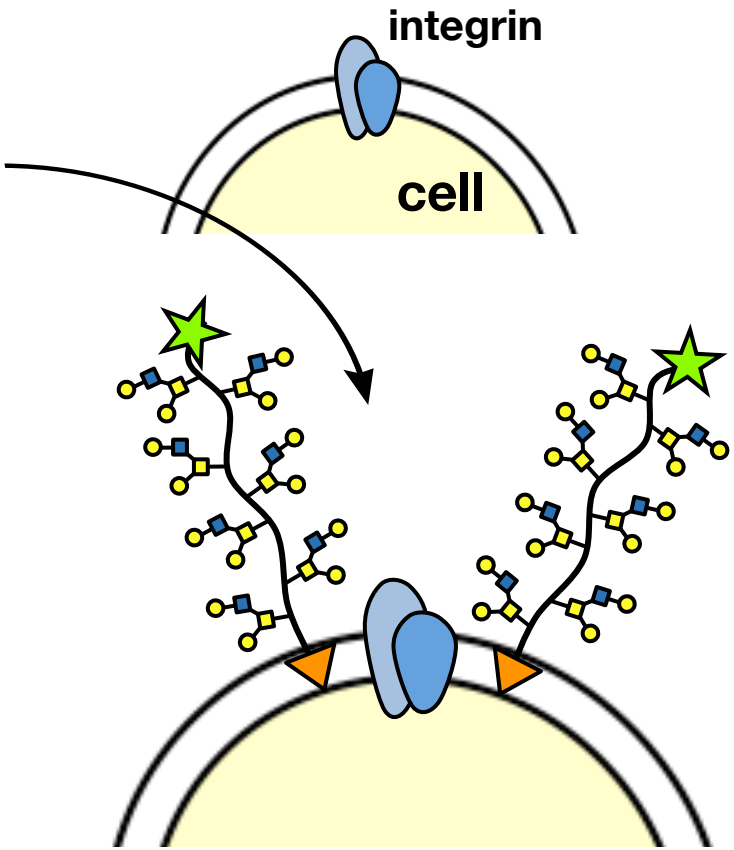
$\Delta h \sim {}^1/_2 L = 15$ nm

J. Am. Chem. Soc. 131, 10263 (2009)

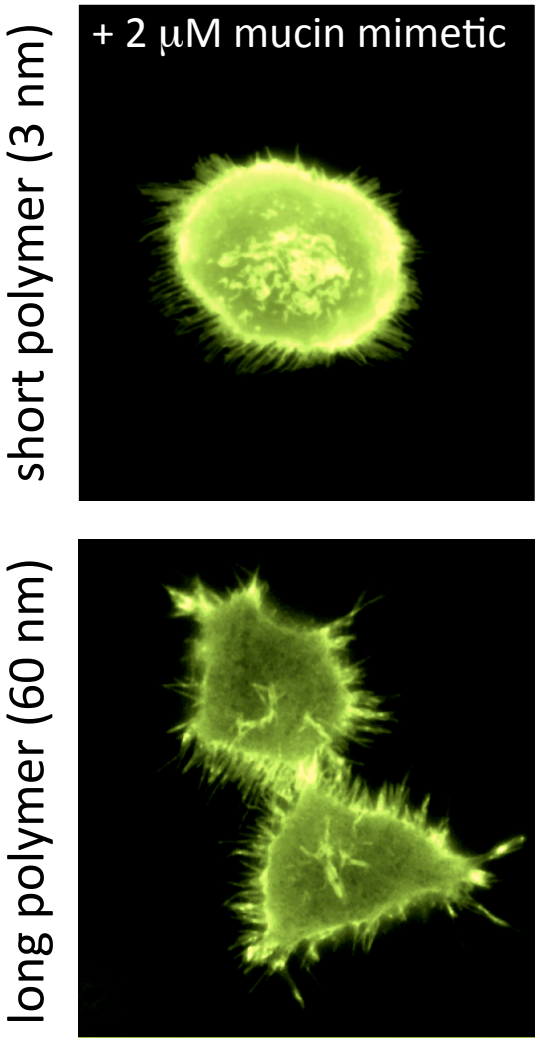
Construction of a synthetic Glycocalyx

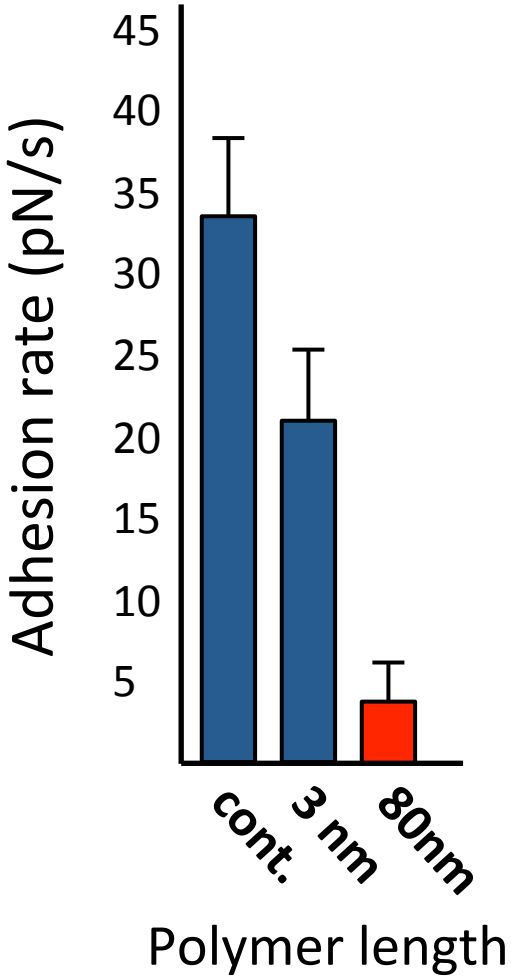


mucin mimetic length
~ 3, 20, and 80 nm



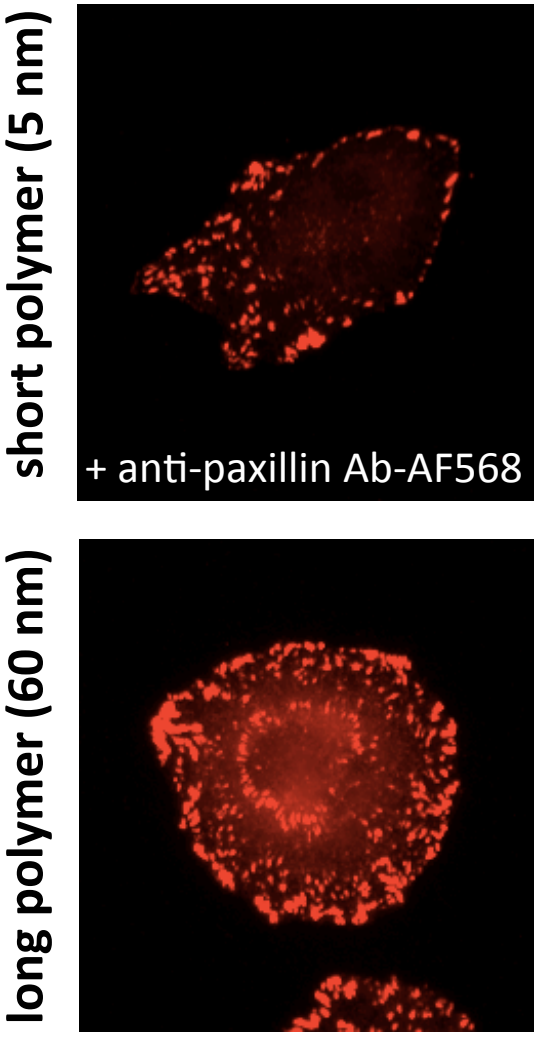
Mucin mimetics slow adhesion of MCF10A epithelial cells to fibronectin substrates

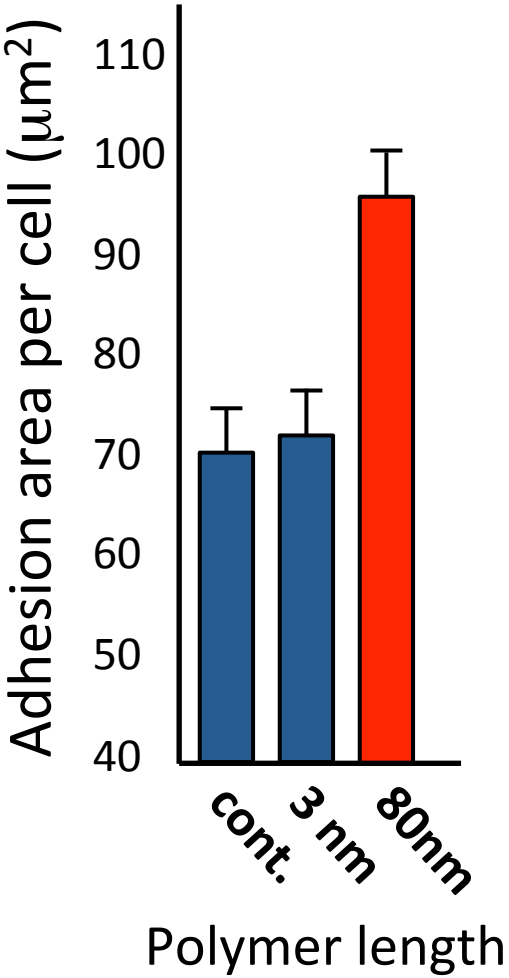




Paszek *et al Nature 511*, 319 (2014)

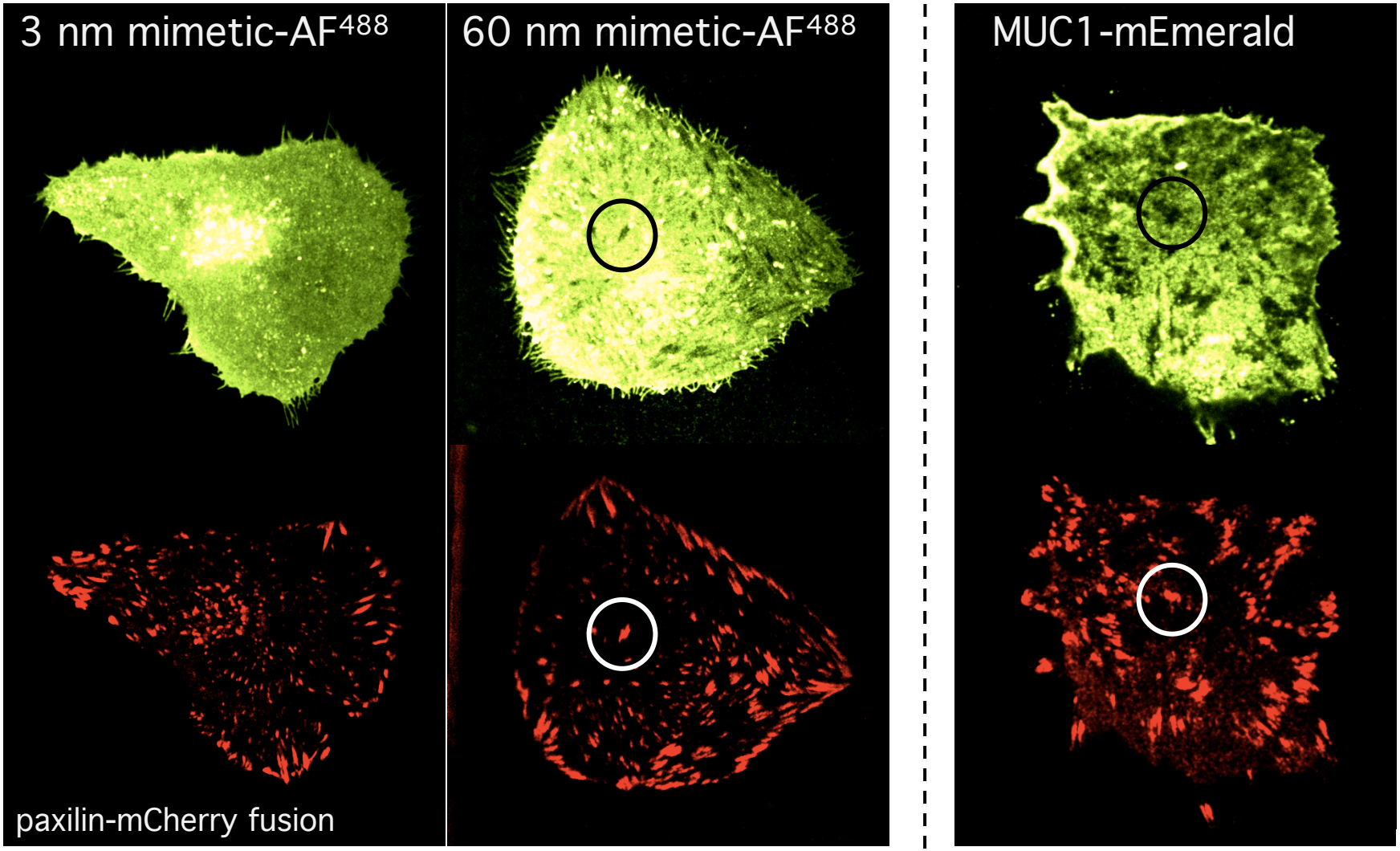
Long mucin mimetic drives the formation of focal adhesions





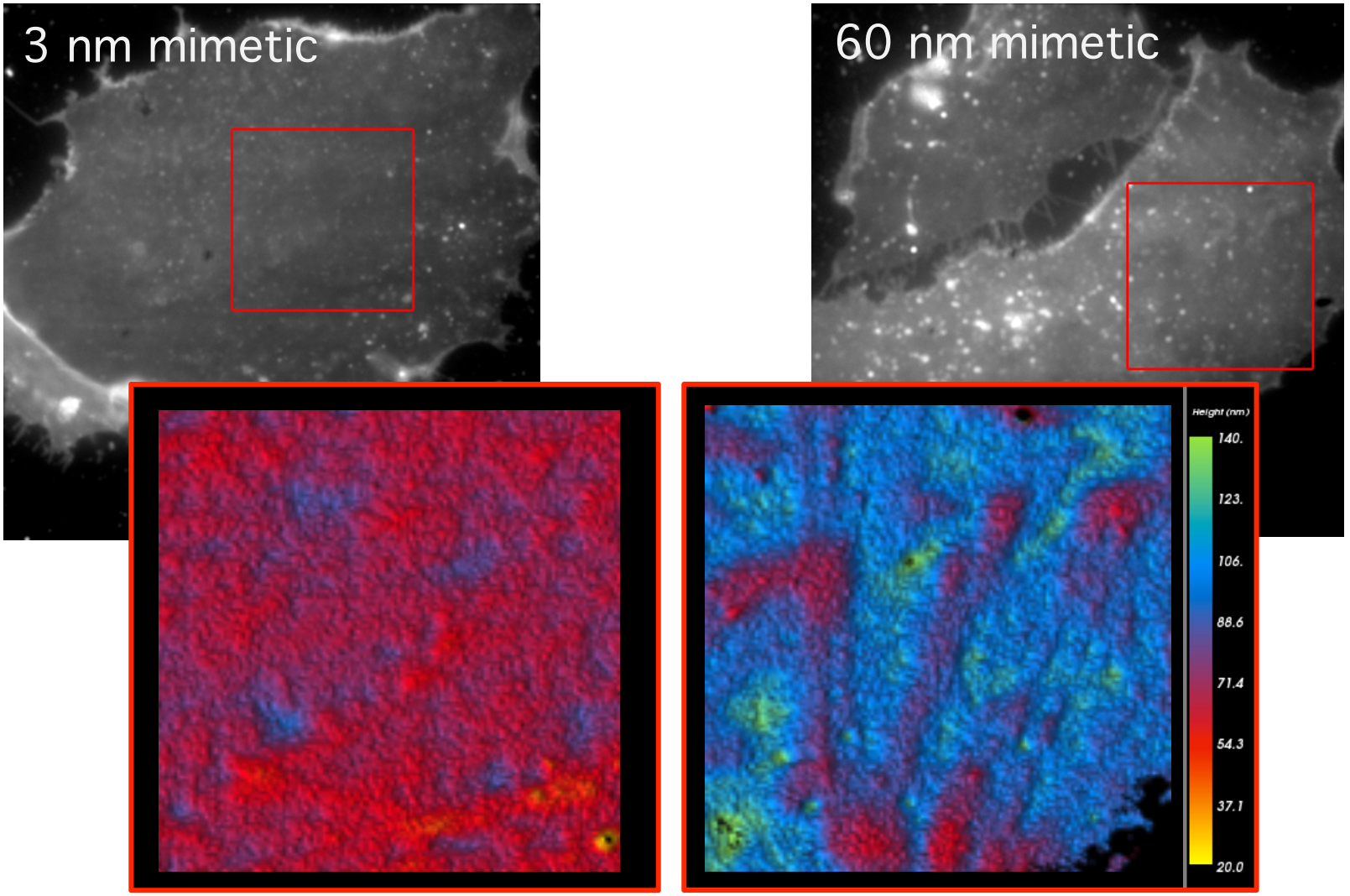
Paszek *et al Nature 511*, 319 (2014)

Long mucin mimetics are excluded from focal adhesions



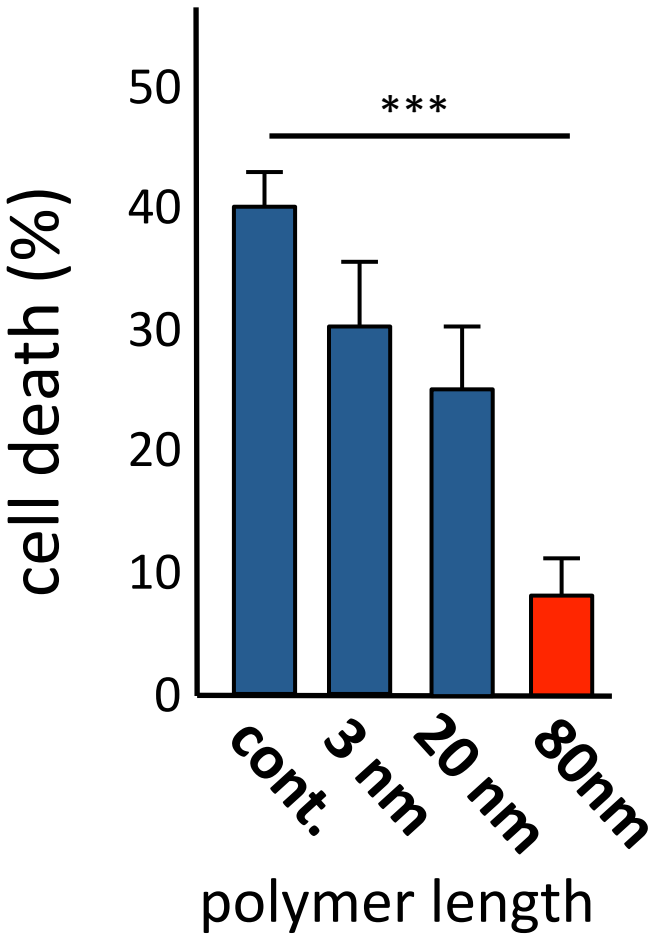
Paszek *et al Nature 511*, 319 (2014)

Interferometric imaging shows membrane topography altered by long mucin mimetics



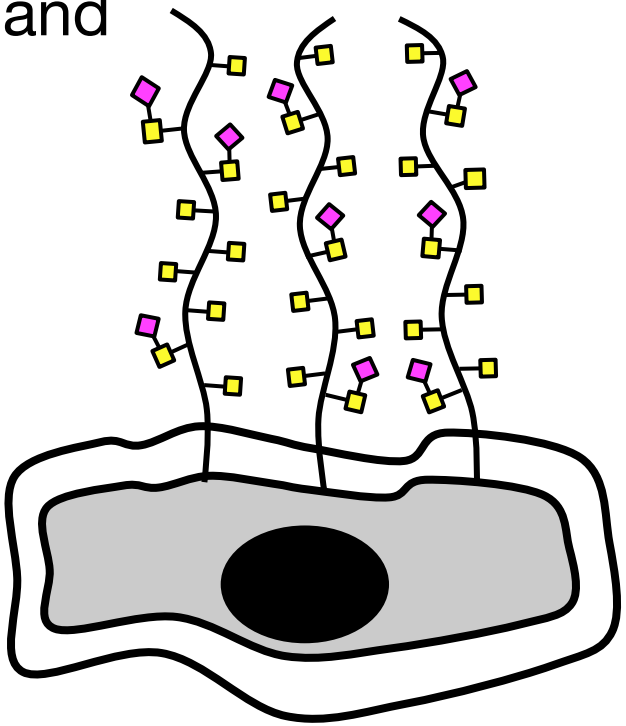
Paszek *et al Nature 511*, 319 (2014)

Long mucin mimetics enhance cell survival in a soft matrix



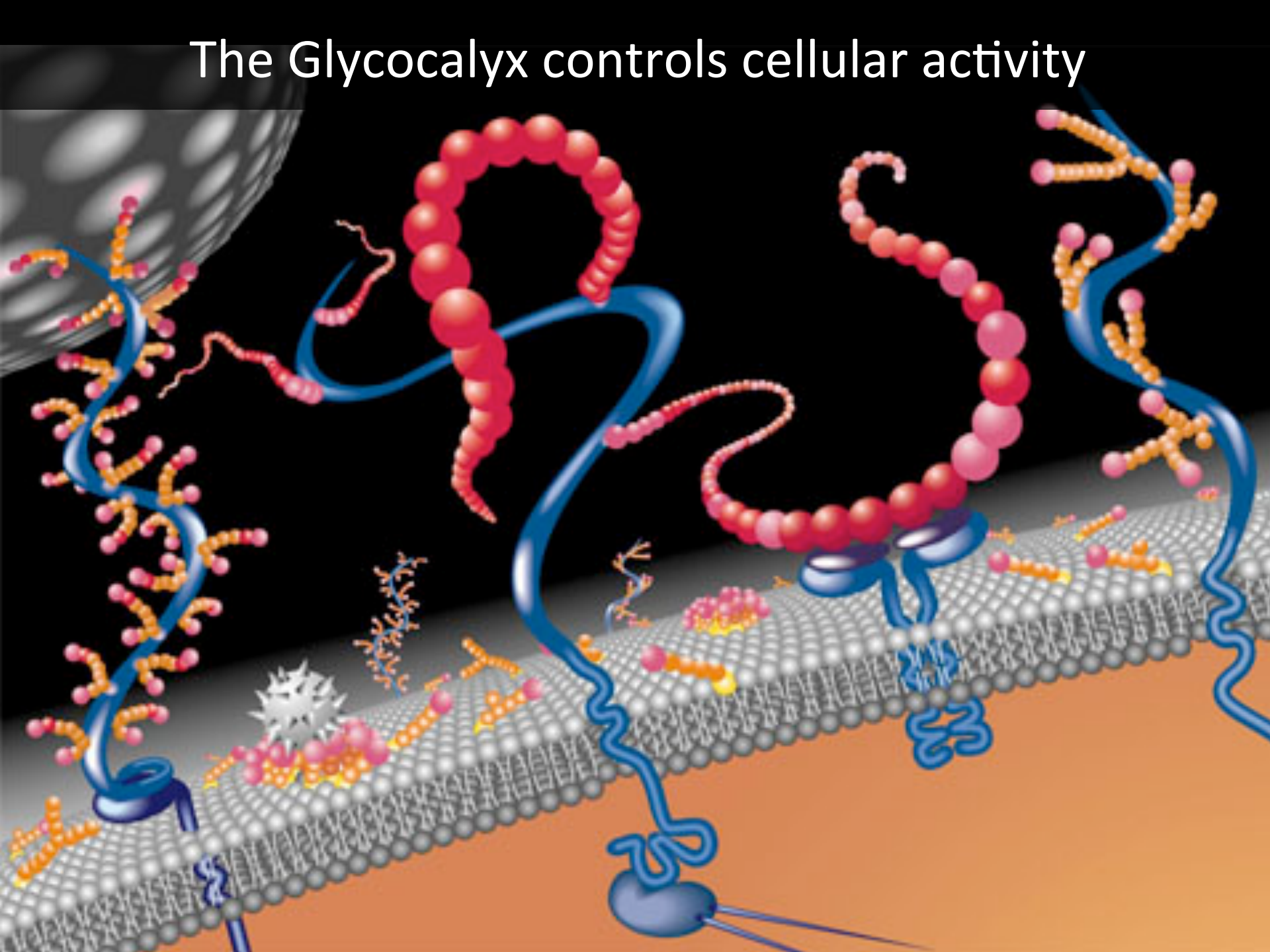
Conclusion:

The biomechanical properties of the Glycocalyx allow cancer cells to adhere and survive in soft tissues.

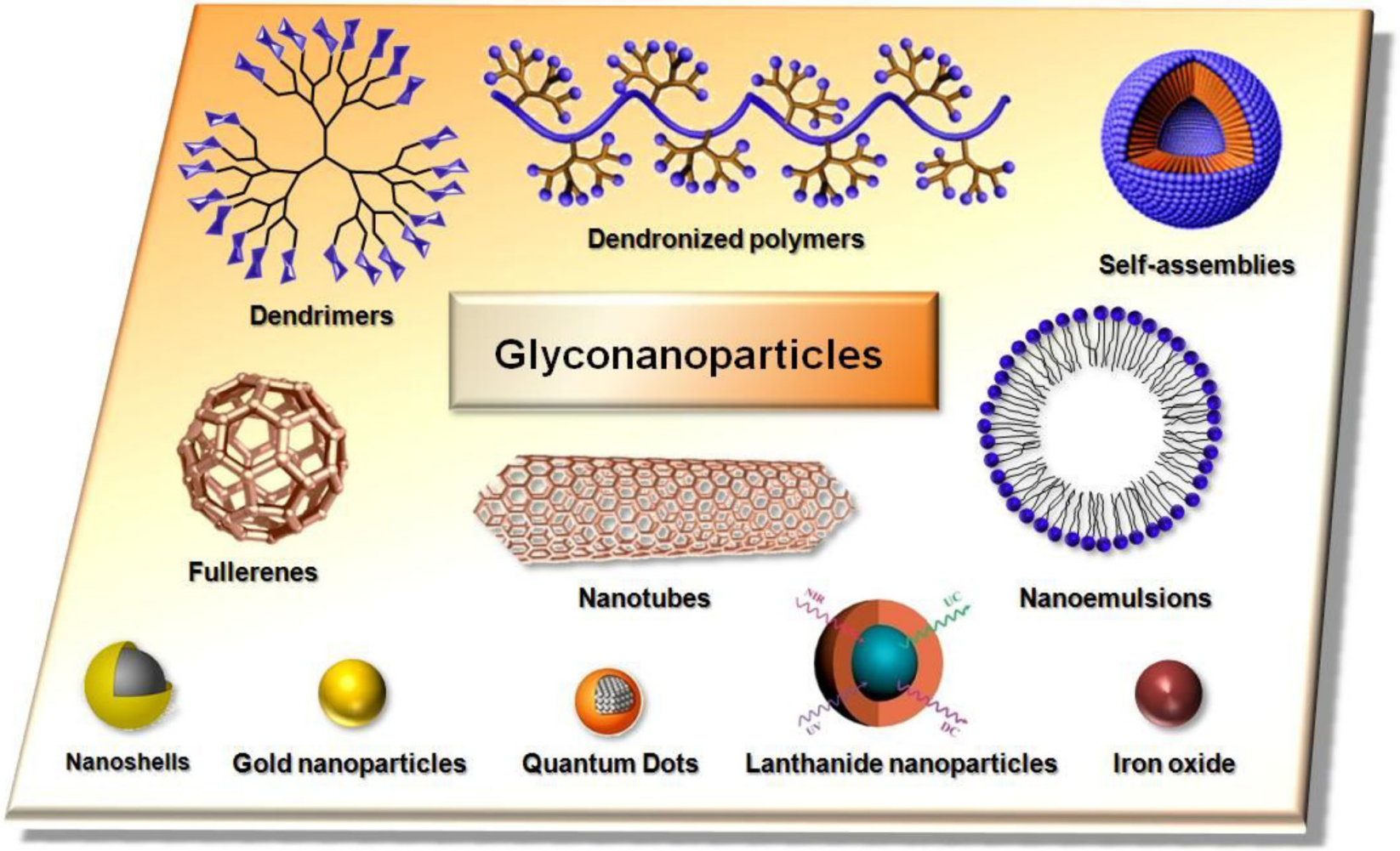


Paszek *et al Nature 511*, 319 (2014)

The Glycocalyx controls cellular activity

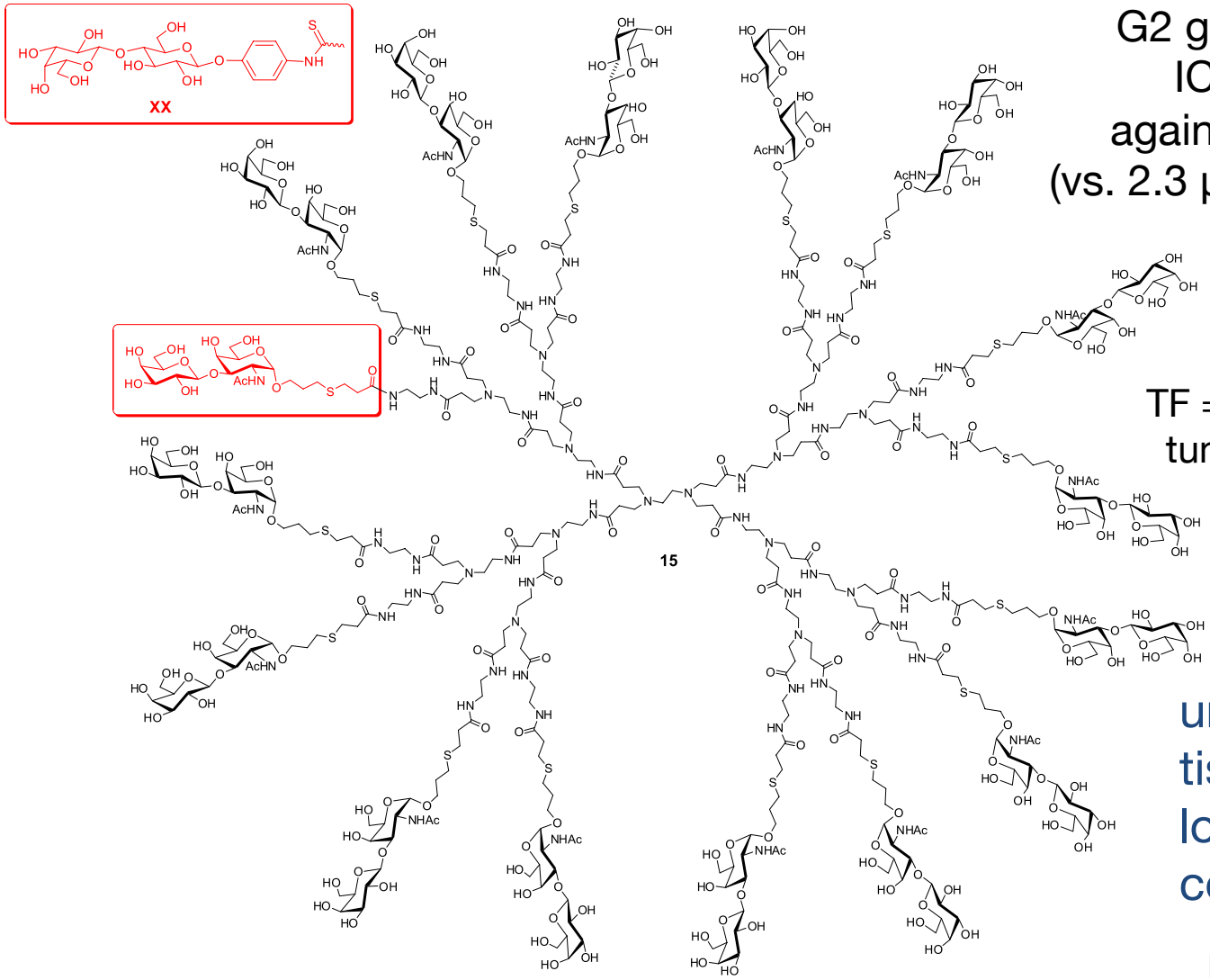


Glycomaterials to probe and influence glycan interactions



Roy et al *BJPS 49*, 85 (2013)

Glycodendrimers – soluble multivalent ligands to stimulate antibody response



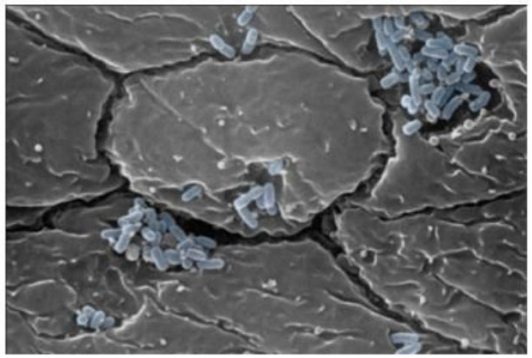
G2 glycodendrimer
IC_{50} = 1.2 nM
against TF antibody
(vs. 2.3 μM for TF antigen)

TF = *Thomsen-Friedenreich*
tumor associated antigen

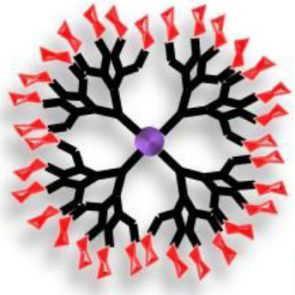
uniform architecture
tissue penetrability
low valency
compact size

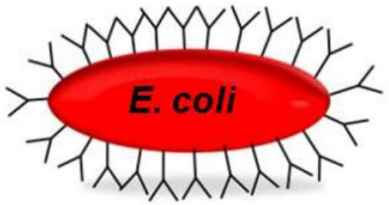
Roy et al *BJPS 49*, 85 (2013)

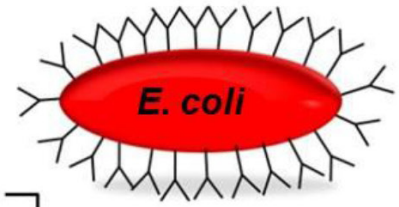
Glycodendrimers – soluble multivalent ligands to inhibit pathogen interactions

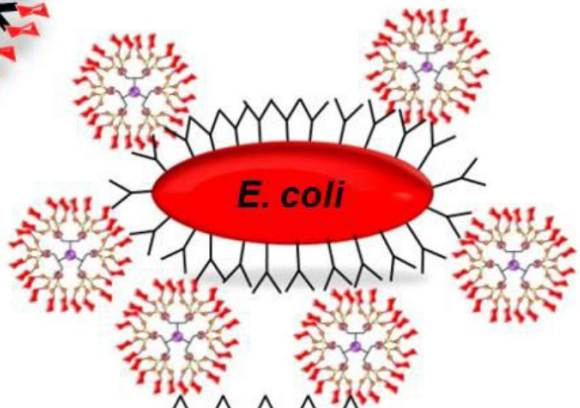


Glycodendrimers



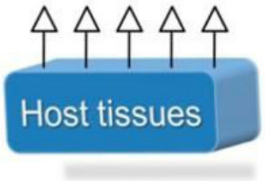


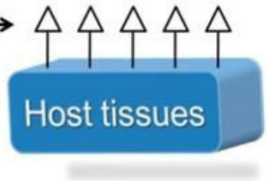


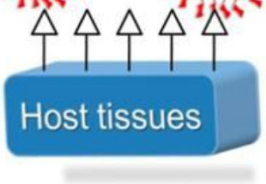


Sugars

Inhibition

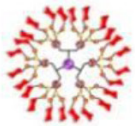






Adhesion

Glycodendrimer



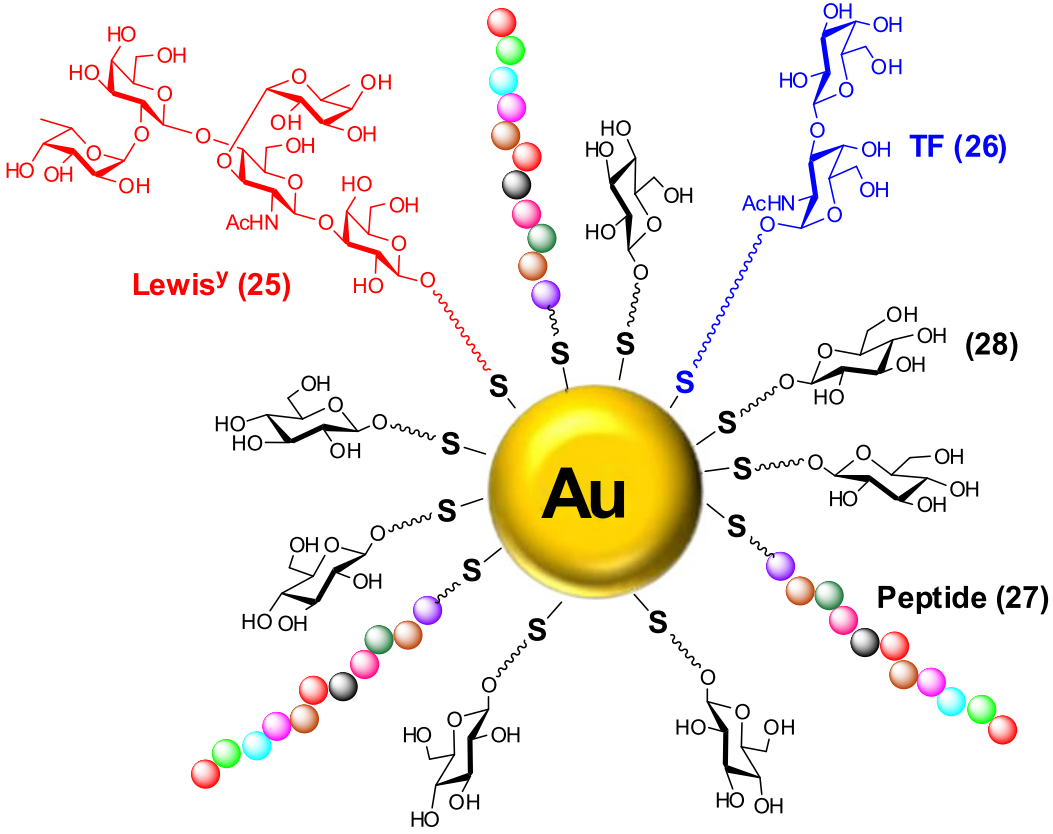
Infection

No Infection

G2 mannosylated glycodendrimer
500-fold more potent than mannose

Roy et al *BJPS 49*, 85 (2013)
Turnbull, Stoddart, Rev. Mol. Biol. *90*, 231 (2002)

Glyco-nanoparticles for biomedical use



multifunctional surfaces
optical properties
label-free detection (Au)

Roy et al *BJPS 49*, 85 (2013)