Glycoscience: The art of making sugars of different kinds

Sriram Neelamegham

Chemical & Biological Engineering and Clinical Translational Research Center

State University of New York, Buffalo, NY, USA

University at Buffalo
The State University of New York

BUFFALO

Demystifying the Glycosciences

- *Glycobiology*: Study of the function of sugars attached to proteins and membranes, including protein- and lipid- linked sugars.
- *Glycoconjugates*: Formed when mono-, oligo- or poly-saccharides attach to proteins and lipids. This occurs in ER and Golgi (mostly).
- *Glycans*: Carbohydrate entity attached to proteins and lipids. Found outside cells, in cytoplasm (complex) and nucleus decorating transcription factors (simple).
- *Lectins*: Glycan binding proteins

Function of glycans

- Structural component of cell wall and extracellular matrix proteins *[Cancer and stem cell biomarkers]*
- Intra- and extra-cellular trafficking of glycoconjugates *[Protein therapeutics half-life]*
- Cell adhesion: during cell-cell and cell-matrix interaction. *[Inflammation, human-virus and human-microbiome interactions]*
- Cell signaling: Intracellular and extracellular *[transcription factor regulation. O-GlcNAc formation on Ser/Thr competes with phosphorylation]*

Why is glycosylation so complicated?

A. It is not part of the central dogma – not part of regular course work.

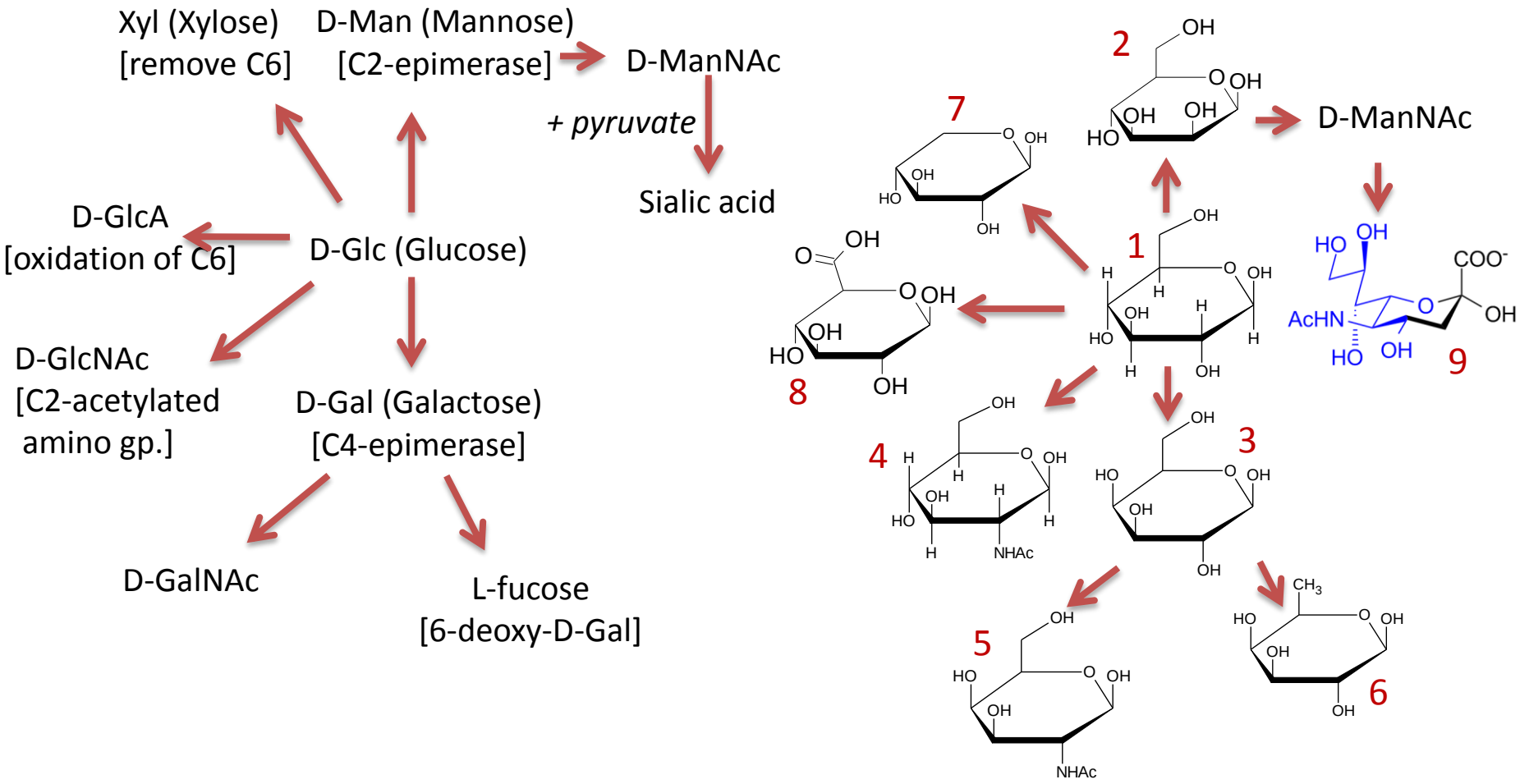
B. Because the biochemists made it look more complicated than it really is:
   - Database lists 700 different monosaccharides. In humans there are only 9!
   - They said that there are 10^{12} possible carbohydrates. In reality it is closer to 10^{2}-10^{3} classical structures.
   - The names are so complicated. Why doesn't everyone just use IUPAC nomenclature

C. Because it is complicated

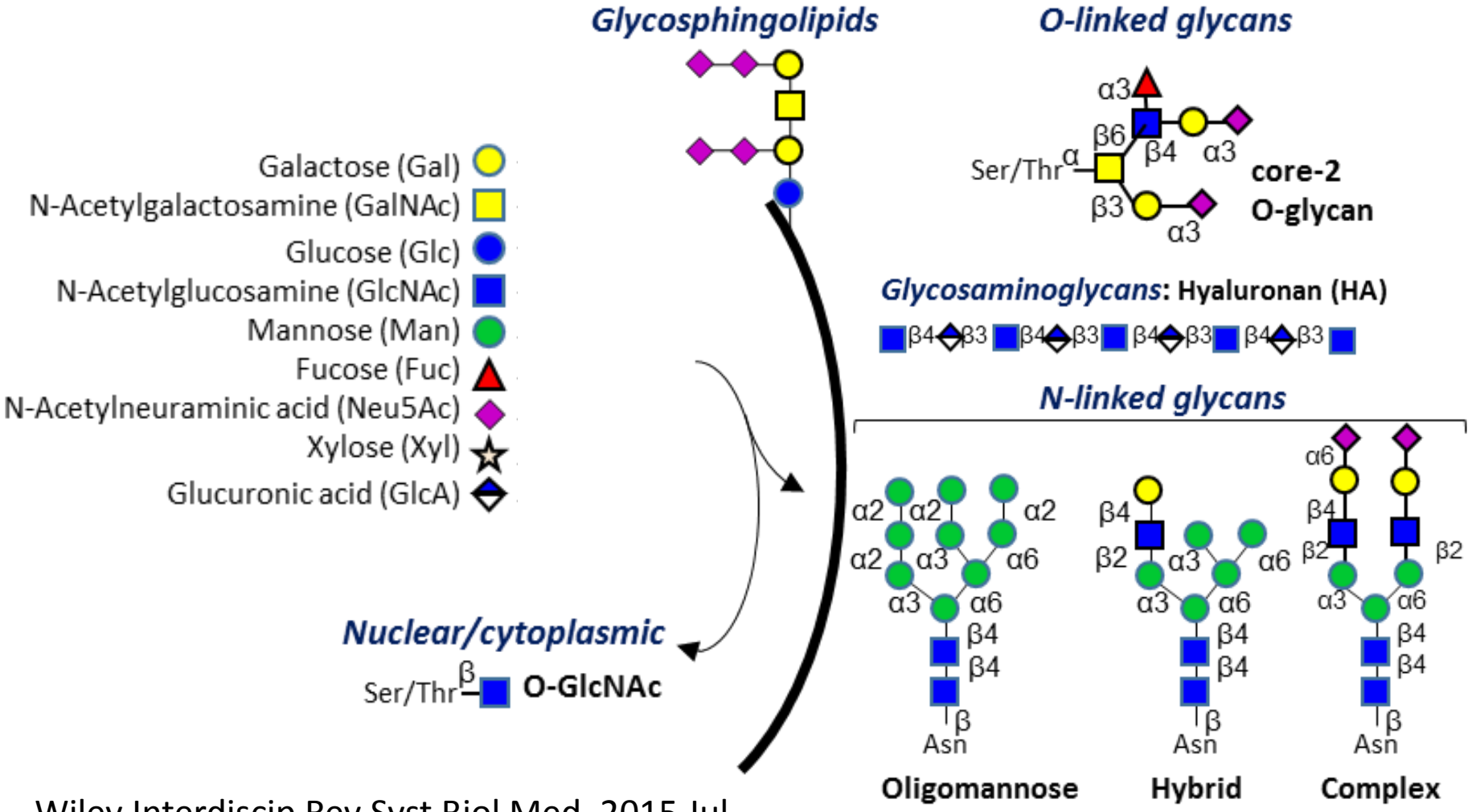
Common monosaccharides

- *Most glycans are hexose sugars*: 4 chiral carbons resulting in 16 different molecules (epimers and enantiomers).
- But many are of relative low abundance. Humans are made of 9 major monosaccharides.

All sugars are similar in structure

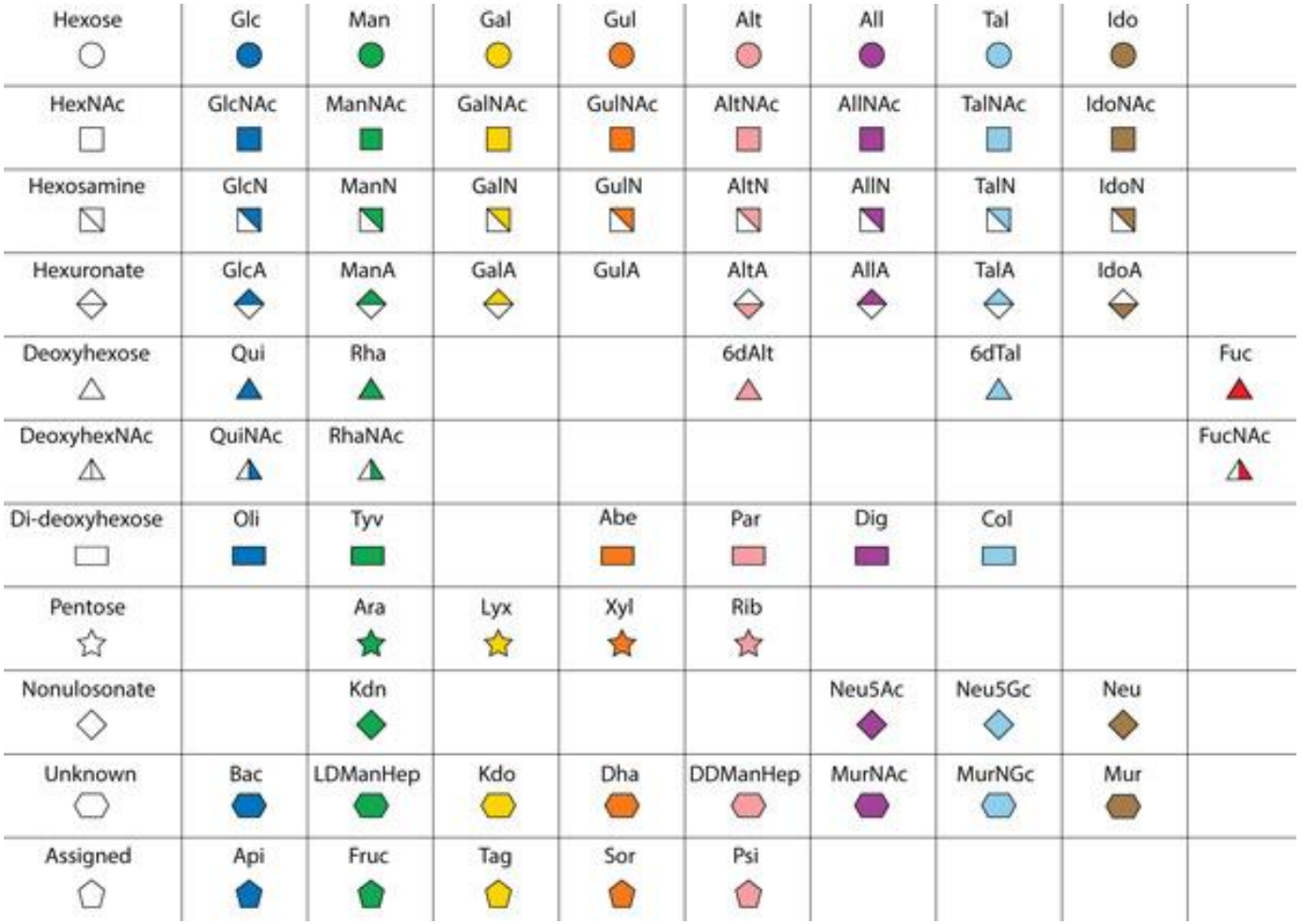


Different families of glycans



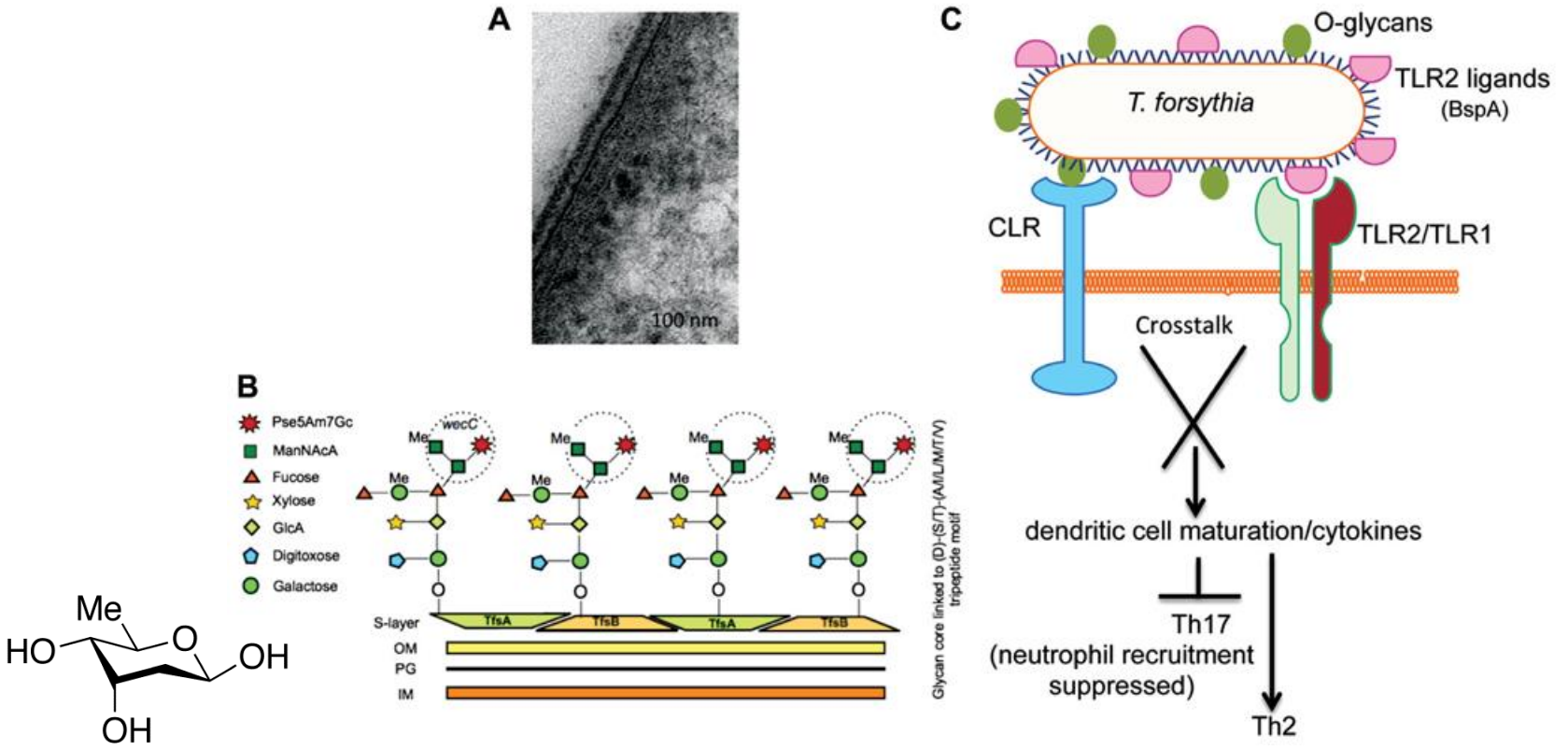
Wiley Interdiscip Rev Syst Biol Med. 2015 Jul-Aug;7(4):163-81

Of course there are more sugars in plants and the microbiome…



http://www.ncbi.nlm.nih.gov/books/NBK310273/

… and more on bacteria that regulate immune function



A. TEM of *T. forsythia* showing glycans. **B.** Schematic *O*-glycan core to protein. Terminal trisaccharide is circled. **C.** Immune signaling by C-type lectin-like receptor (CLR) and TLR2 activated by *O*-glycans and TLR2 ligands (e.g., BspA) orchestrates.

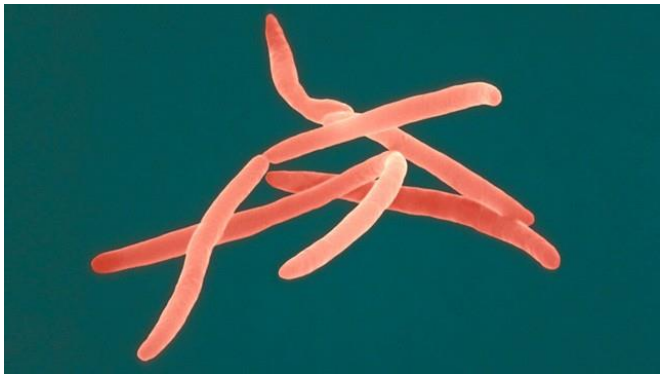
Front. Microbiol., 17 October 2013

Relevance to human diseases

- Human glycans bind bacterial lectin-like adhesins
- Bacterial carbohydrates bind mammalian lectins
- Bacterial carbohydrates mimic human glycans, and condition the microbiome

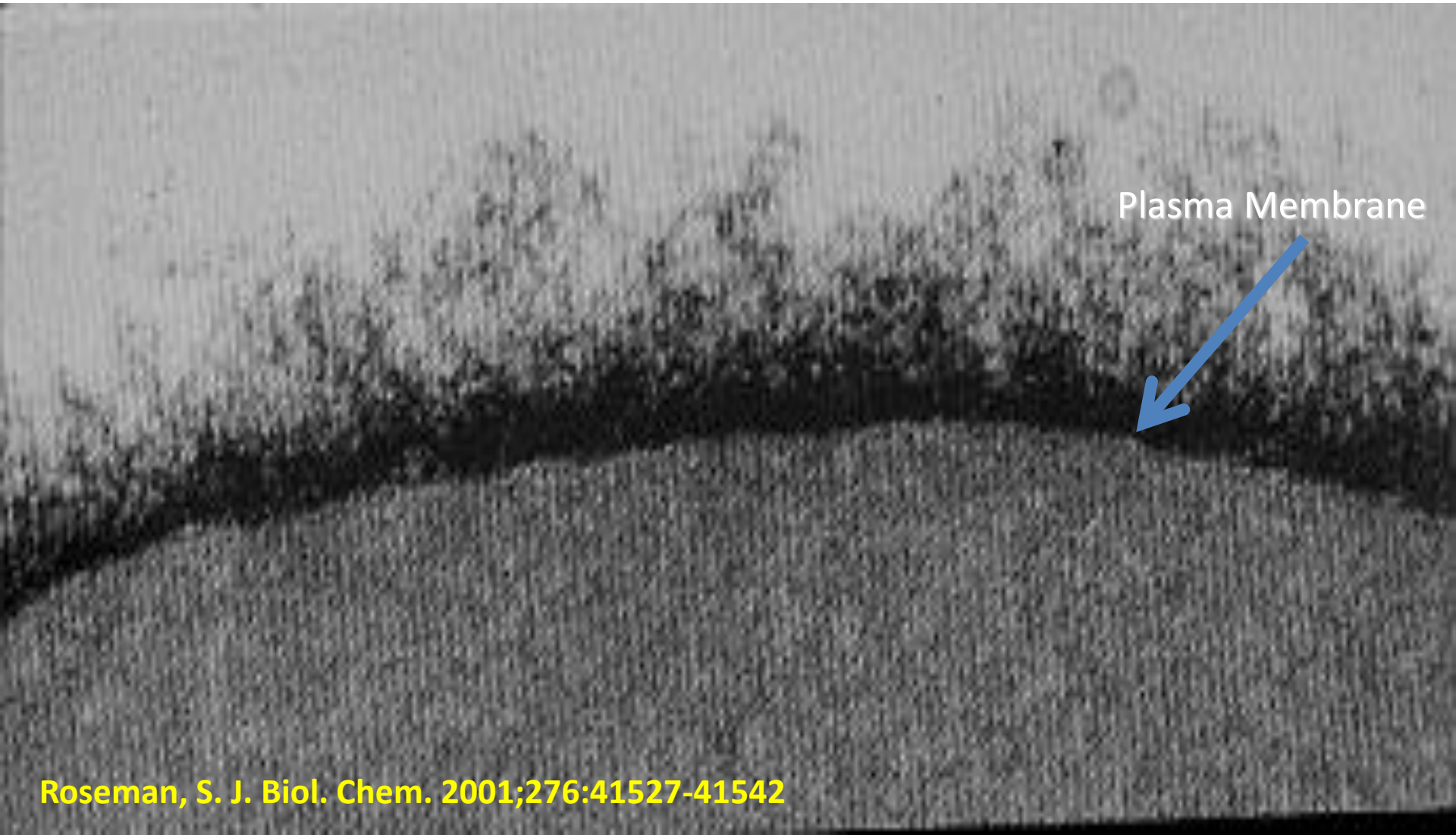
Fusobacterium nucleatum

- Plays a role in periodontal disease.
- Associated with colon cancer

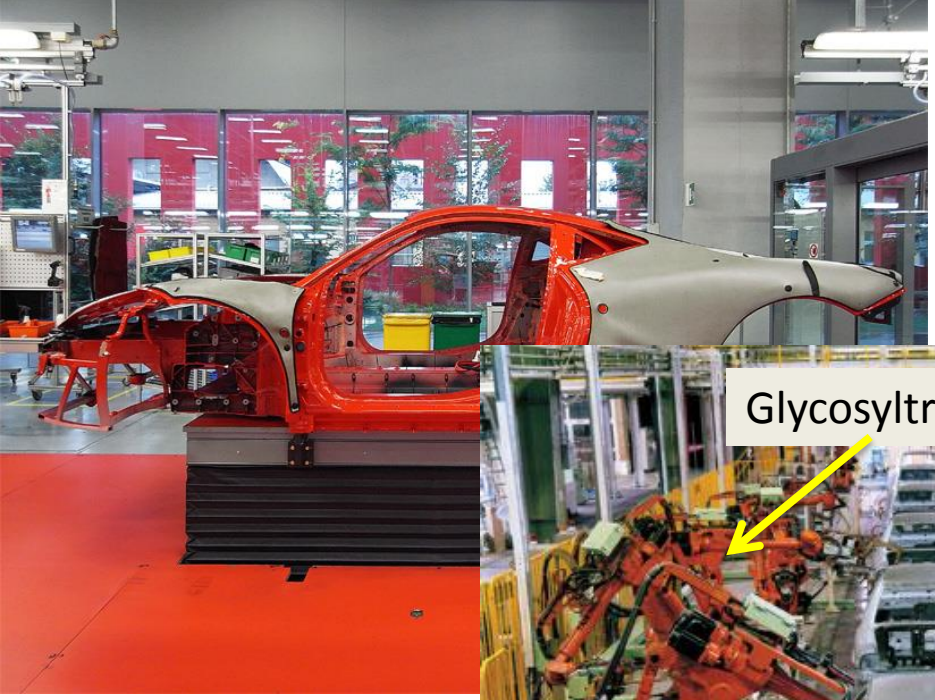


wikipedia

Glycocalyx – Physically big



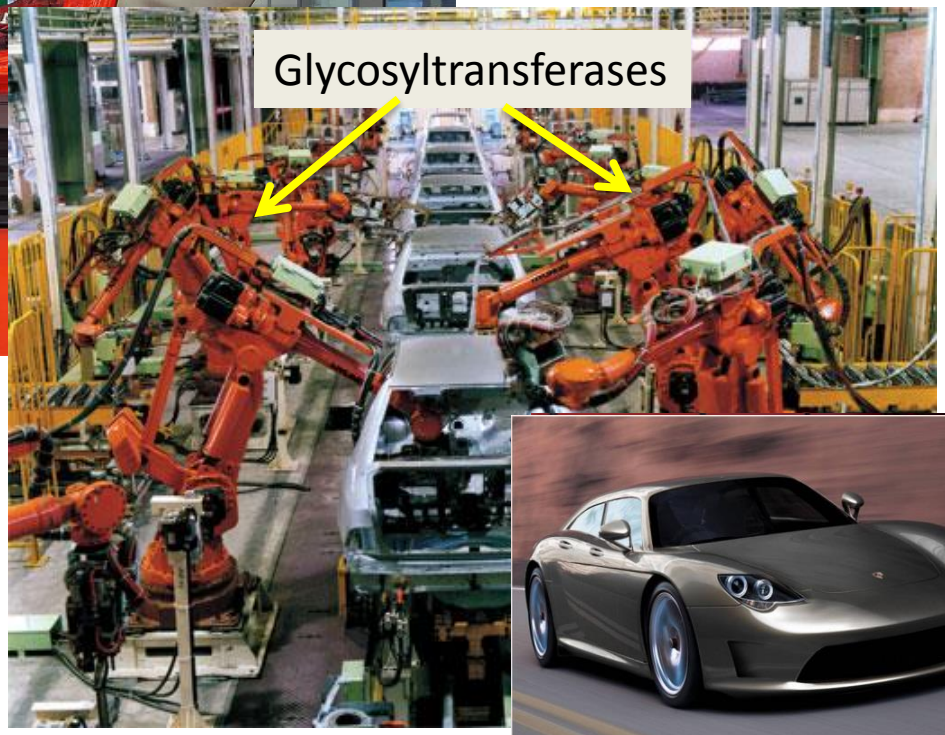
Roseman, S. J. Biol. Chem. 2001;276:41527-41542



Translated protein

Post-translational modification

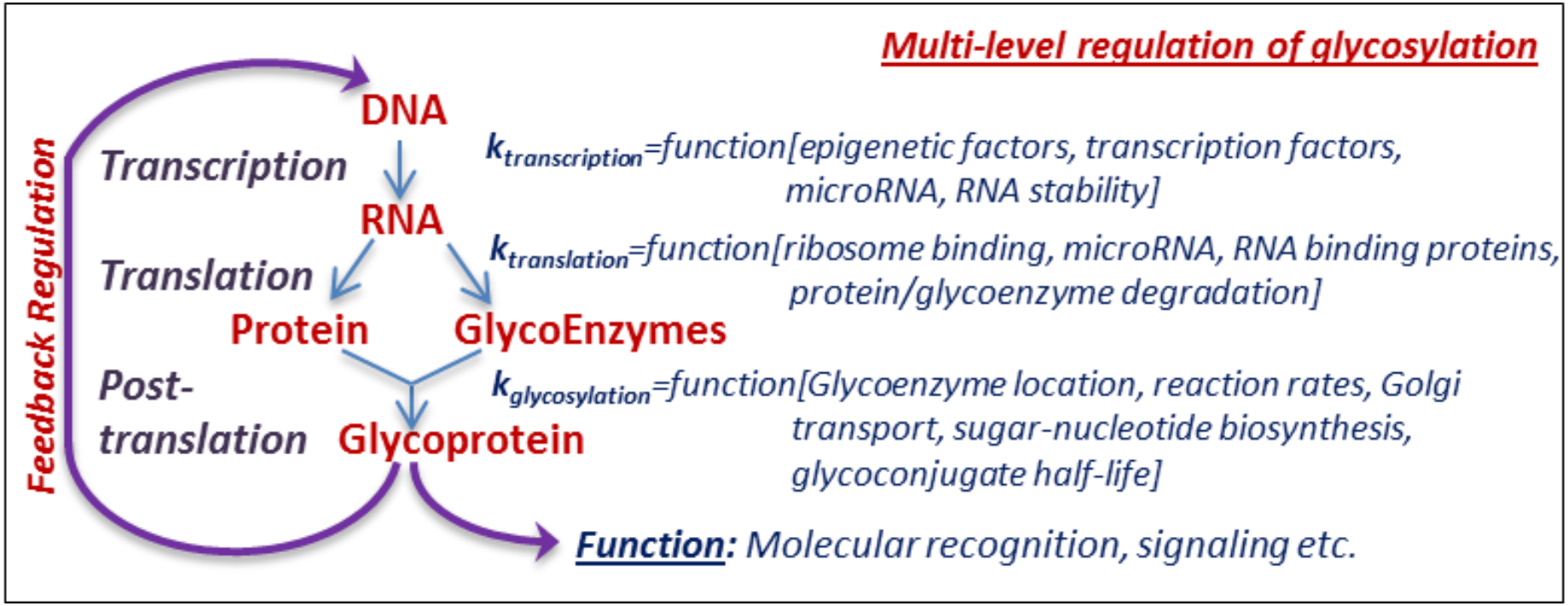
Functional protein



Ribosome

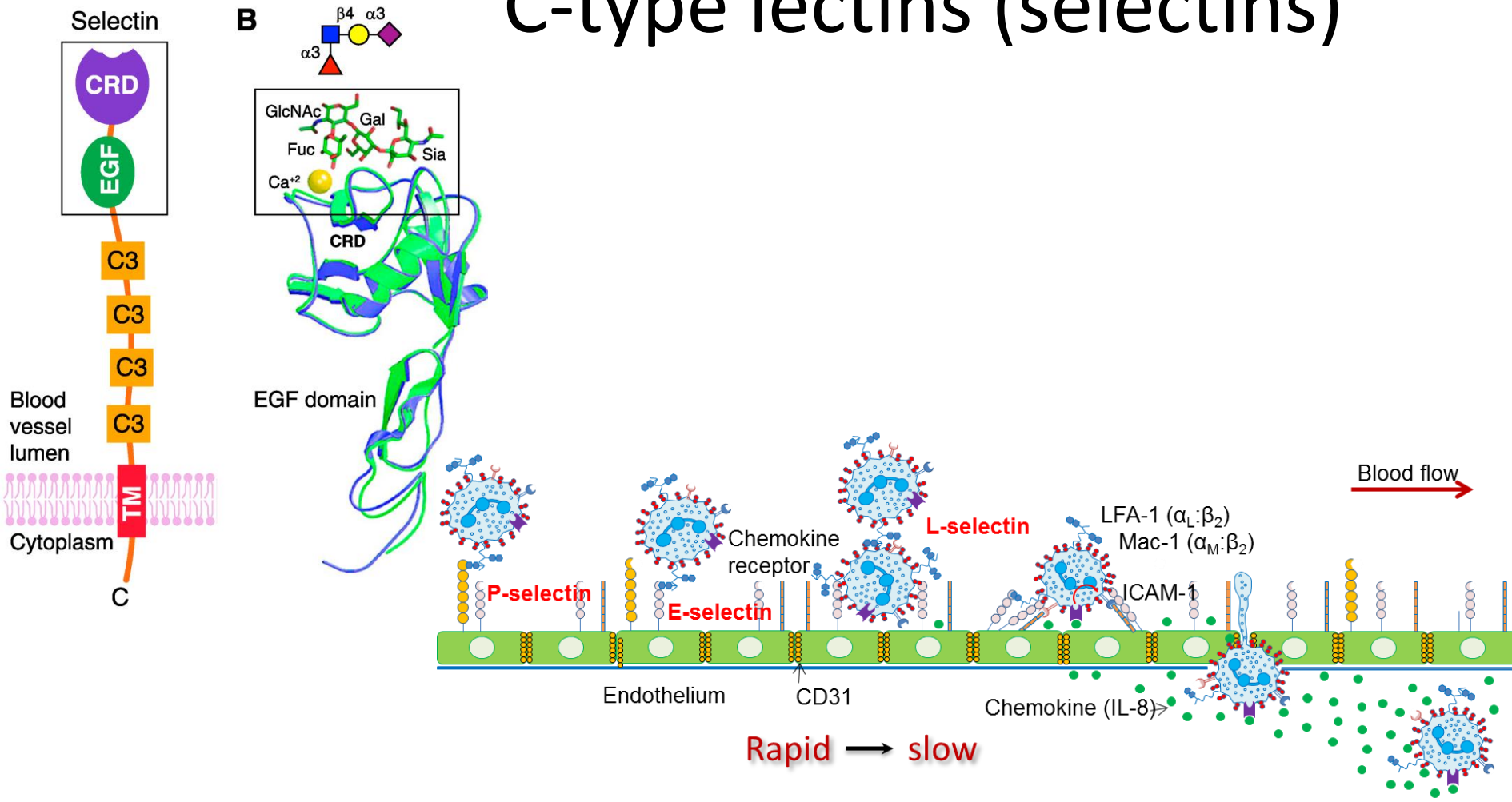
ER/Golgi

Cell-surface



Neelamegham and Mahal, Current Opinion of Structural Biology, 2016

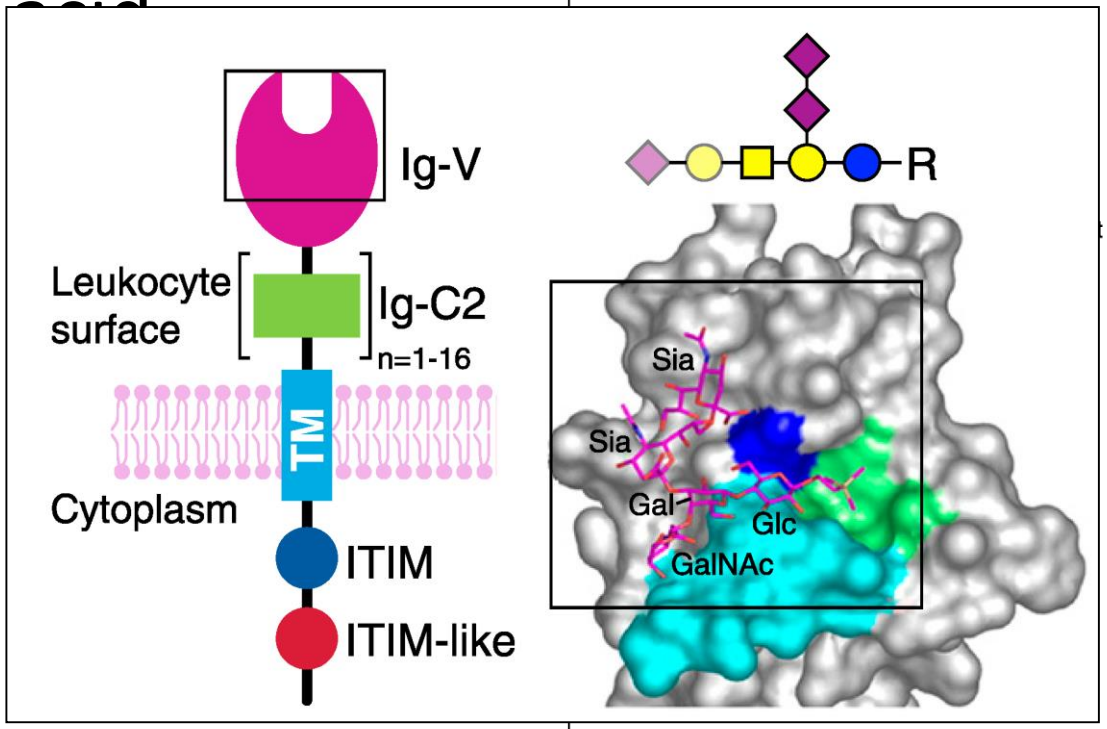
Lectins: Glycan binding proteins
C-type lectins (selectins)



Cell. 2000 Oct 27;103(3):467-79.

Siglecs

- Contains shallow Arg that binds sialic

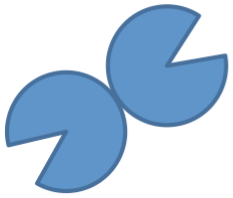


Schnaar, J. Leuk. Biol, June, 2016

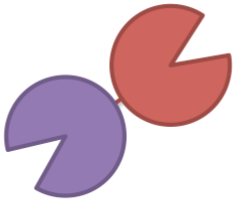
TABLE 1. Human siglecs.

Siglec	Alias	Cell type expression	Ig domains	ITIM / DAP12	glycan binding preferences[a]
1	sialo-adhesin CD169	macrophages, monocytes	17		α3 β4
2	CD22	B cells	7	ITIM	6S α6 β4
3	CD33	myeloid progenitors, macrophages, monocytes, microglia, granulocytes	2	ITIM	α6 β4
4	MAG	myelinating cells	5		α3 β4
5		neutrophils, monocytes, B cells	4	ITIM	α8 α6
			3	ITIM	α6
			3	ITIM	α8 α3 β4
			3	ITIM	6S α3 β4
			3	ITIM	6S α3 β4
			5	ITIM	α6 β4
			5	ITIM	α8 α3
			3	DAP12	α8 α3 α6
			2	DAP12	α6
16		macrophages, microglia	5	DAP12	α8 α3

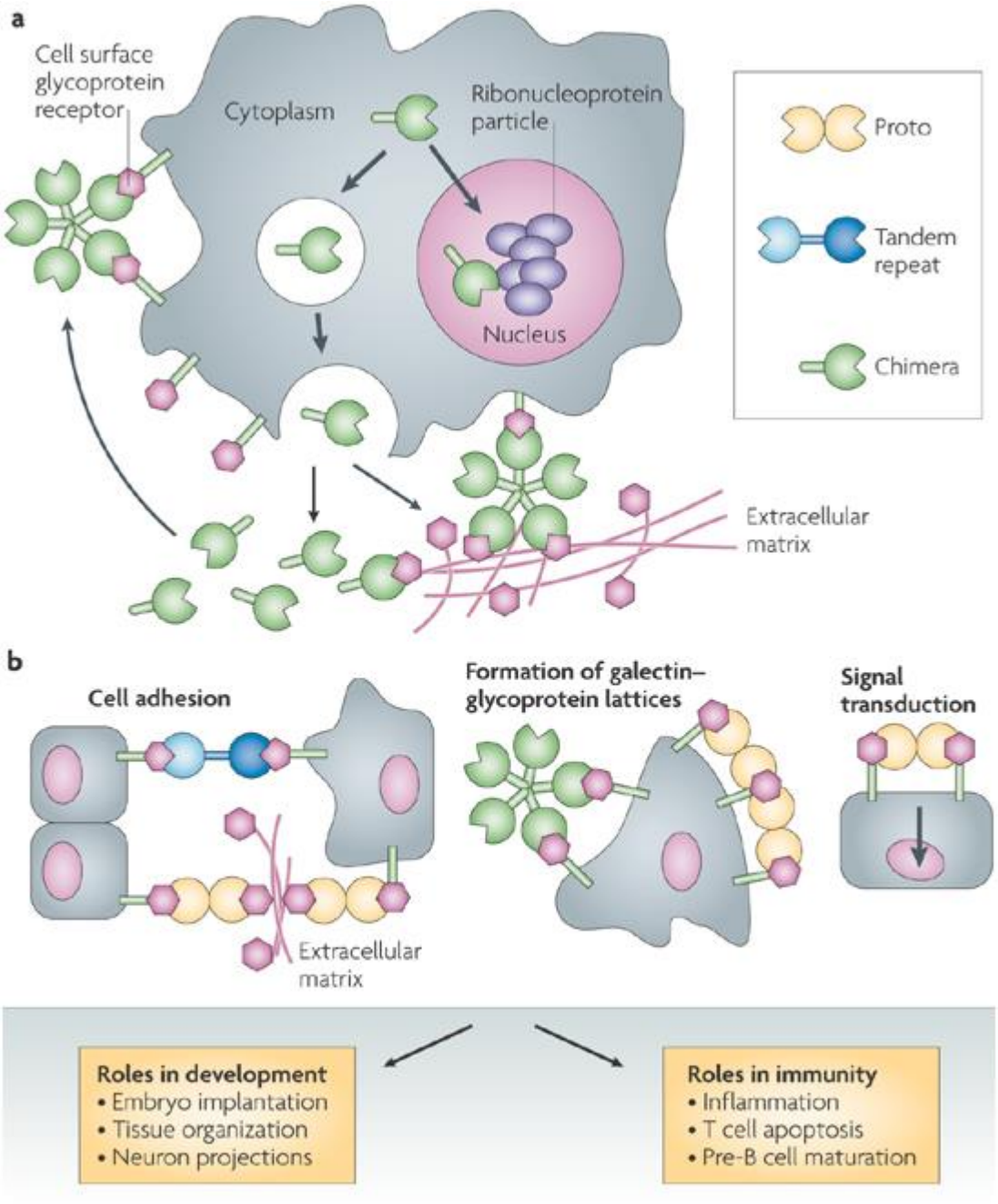
Galectins



Dimeric
Galectin-1, -2, -5, -7, -10, -11, -13, -14 & -15

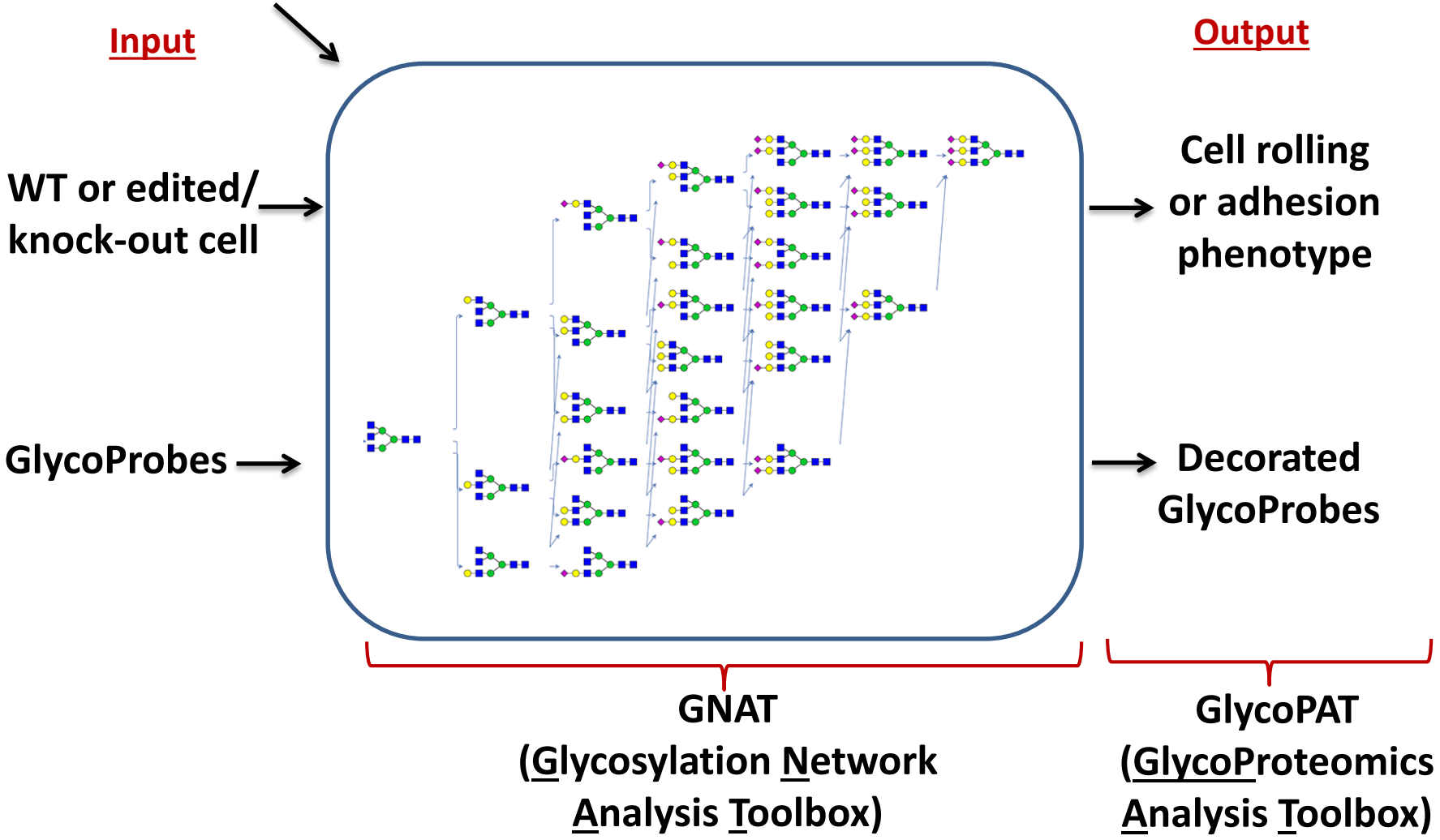


Tandem
Galectin-4, -6, -8, -9 & -12



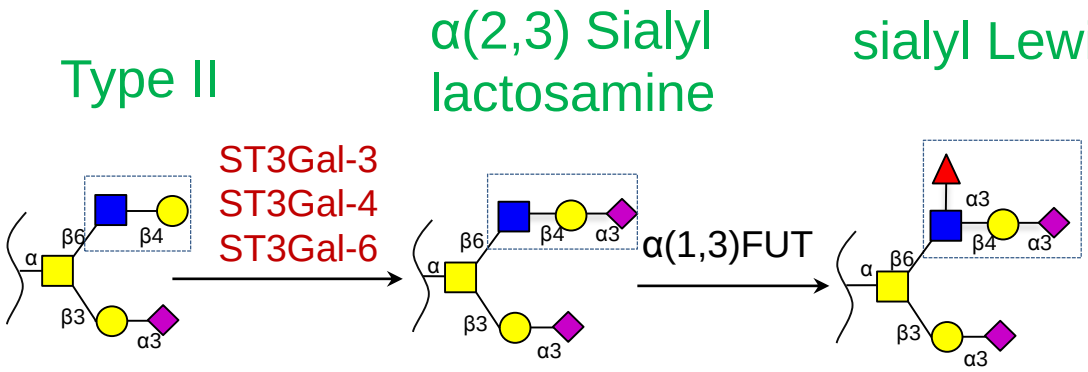
Nature Reviews Microbiology 7, 424-438 (June 2009)

Systems Glycobiology



http://sourceforge.net/projects/gnatmatlab/

Which α(2,3)sialyltransferase, ST3Gal-3, -4 or -6, contributes to human selectin-ligand biosynthesis?



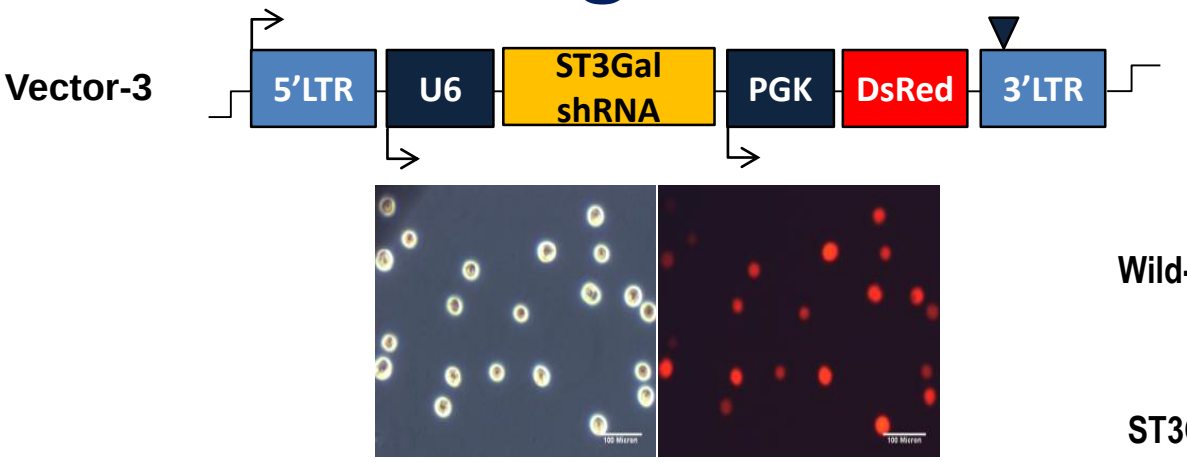
Sugar	*Mono.*
◆	Sialic acid
●	Galactose
●	Mannose
■	GlcNAc
■	GalNAc
▲	Fucose

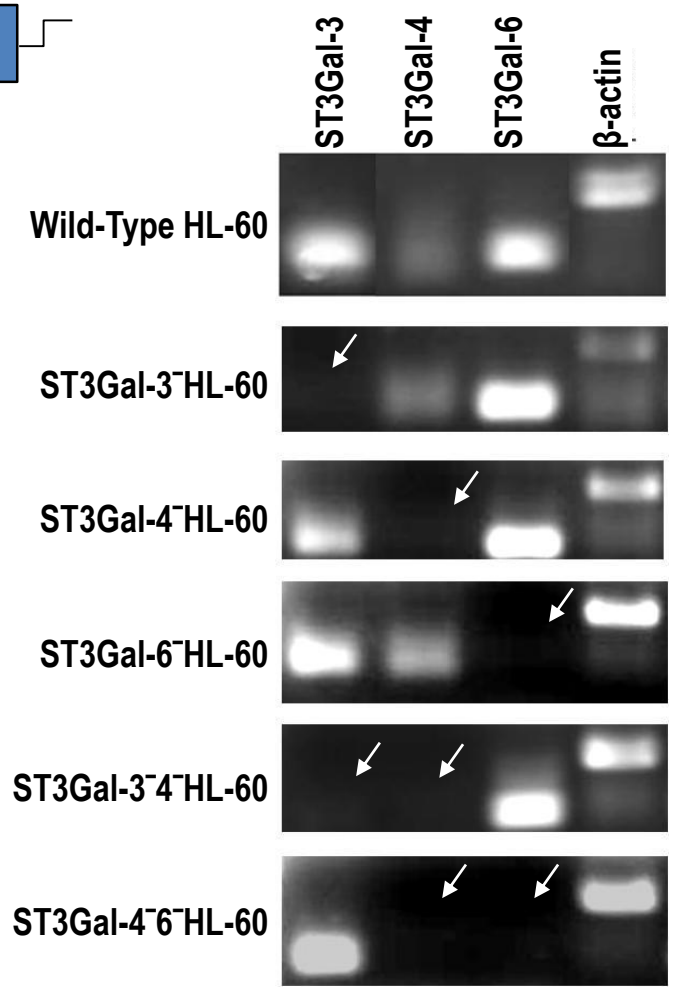
Alexander Buffone

Nandini Mondal

Mondal *et al. Blood*. 125:687-96, 2015.

Making ST3Gal $^{-}$ HL60 cells

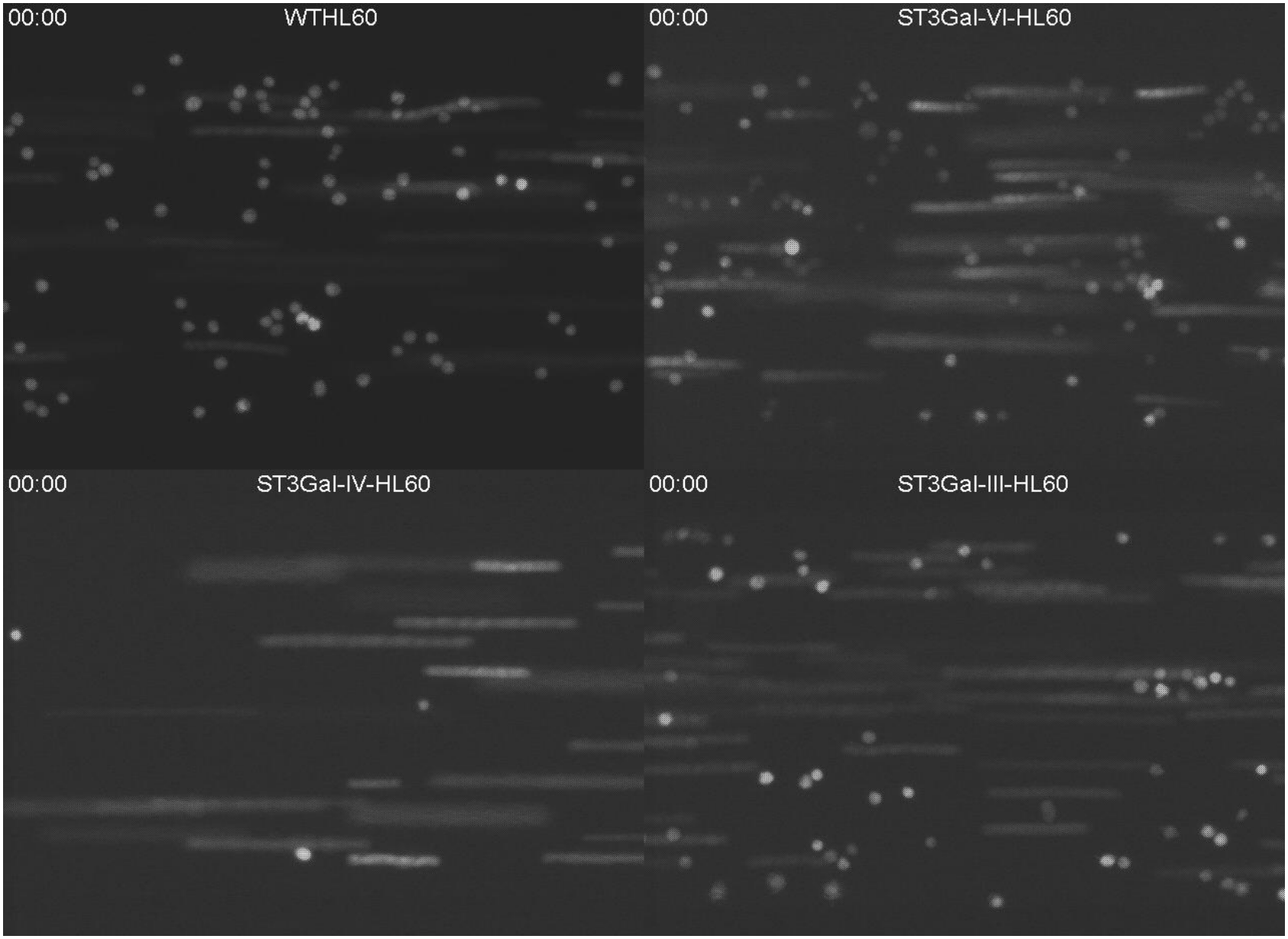




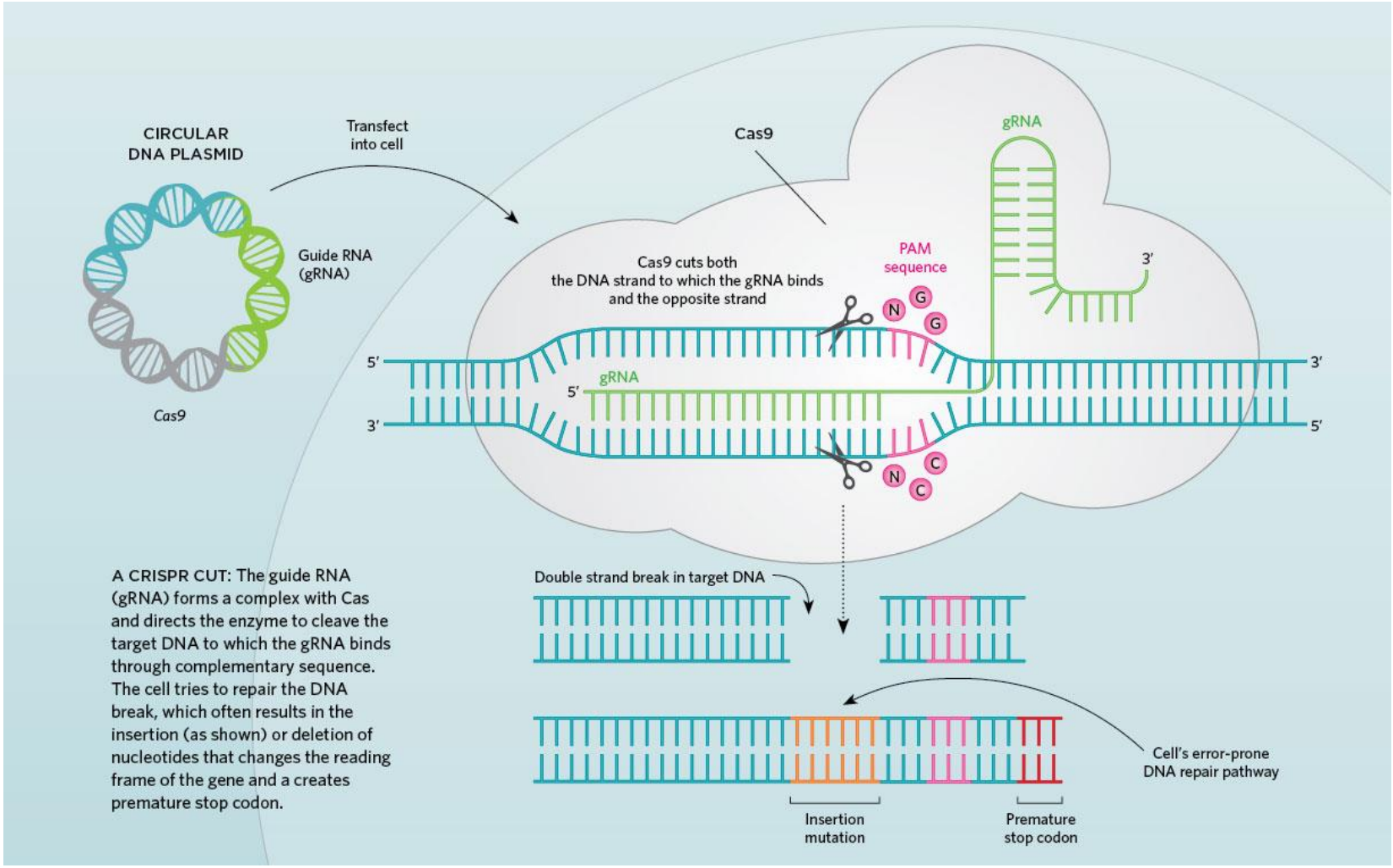
- Single knock down cells
  - ST3Gal-3 $^{-}$
  - ST3Gal-4 $^{-}$
  - ST3Gal-6 $^{-}$
- Dual knock down cells
  - ST3Gal-3 $^{-}$ 4 $^{-}$
  - ST3Gal-4 $^{-}$ 6 $^{-}$

Buffone *et al. J Biol Chem*. 288(3):1620-33, 2013.

HL60 cell rolling on E-Selectin

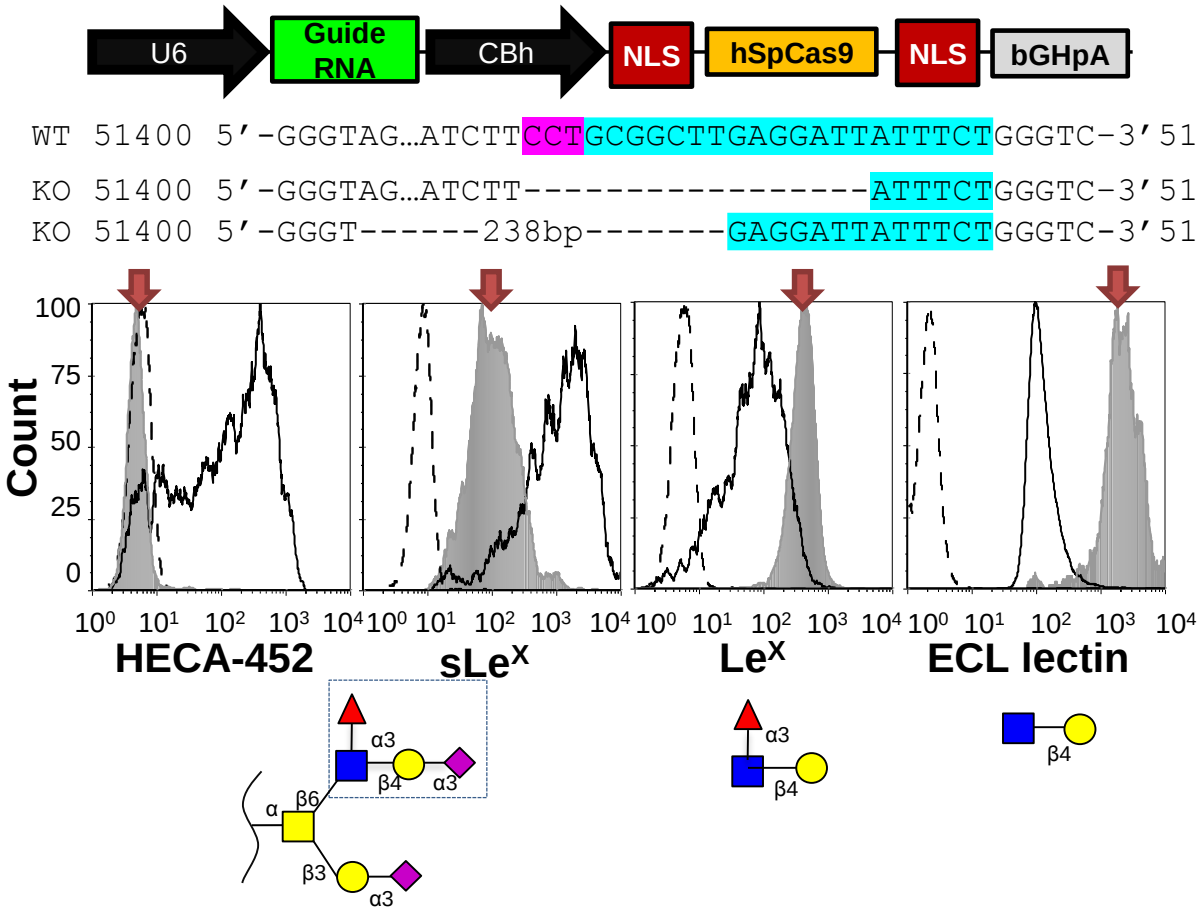


Genome Editing with CRISPR-Cas9

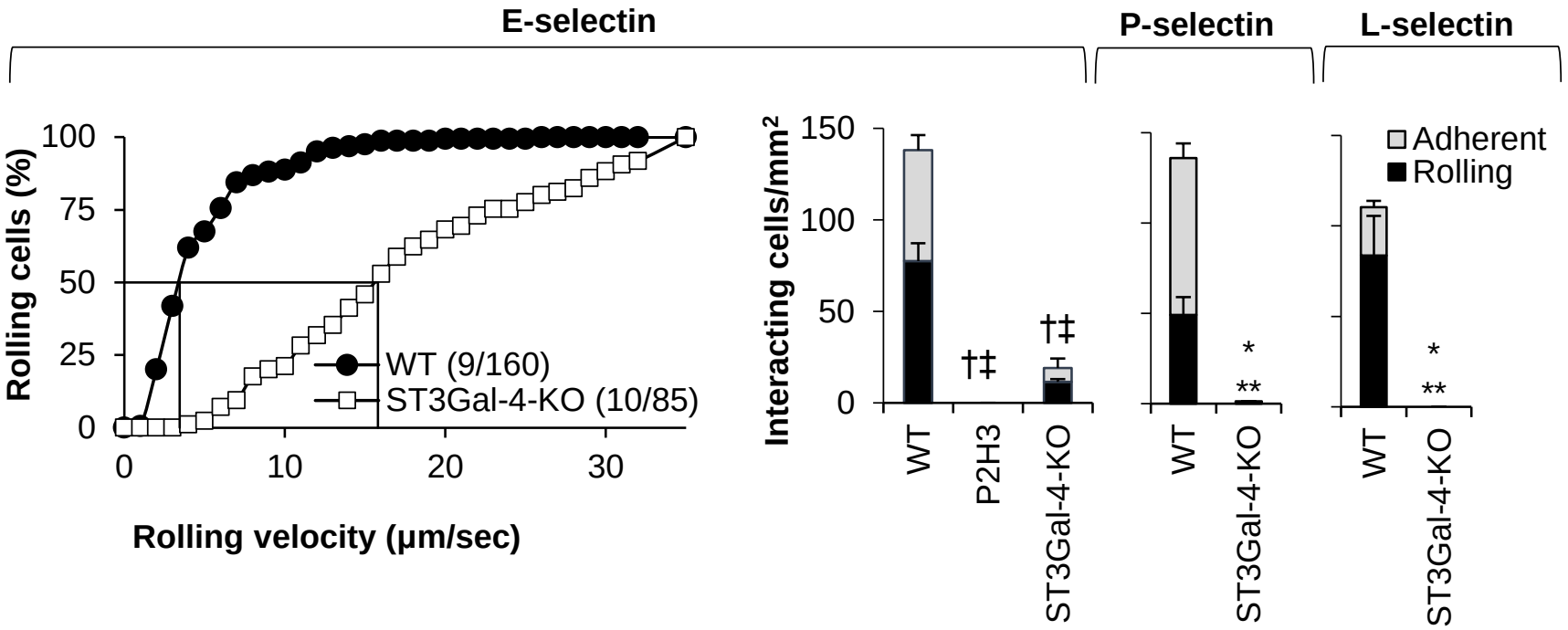


The Scientist, 2014

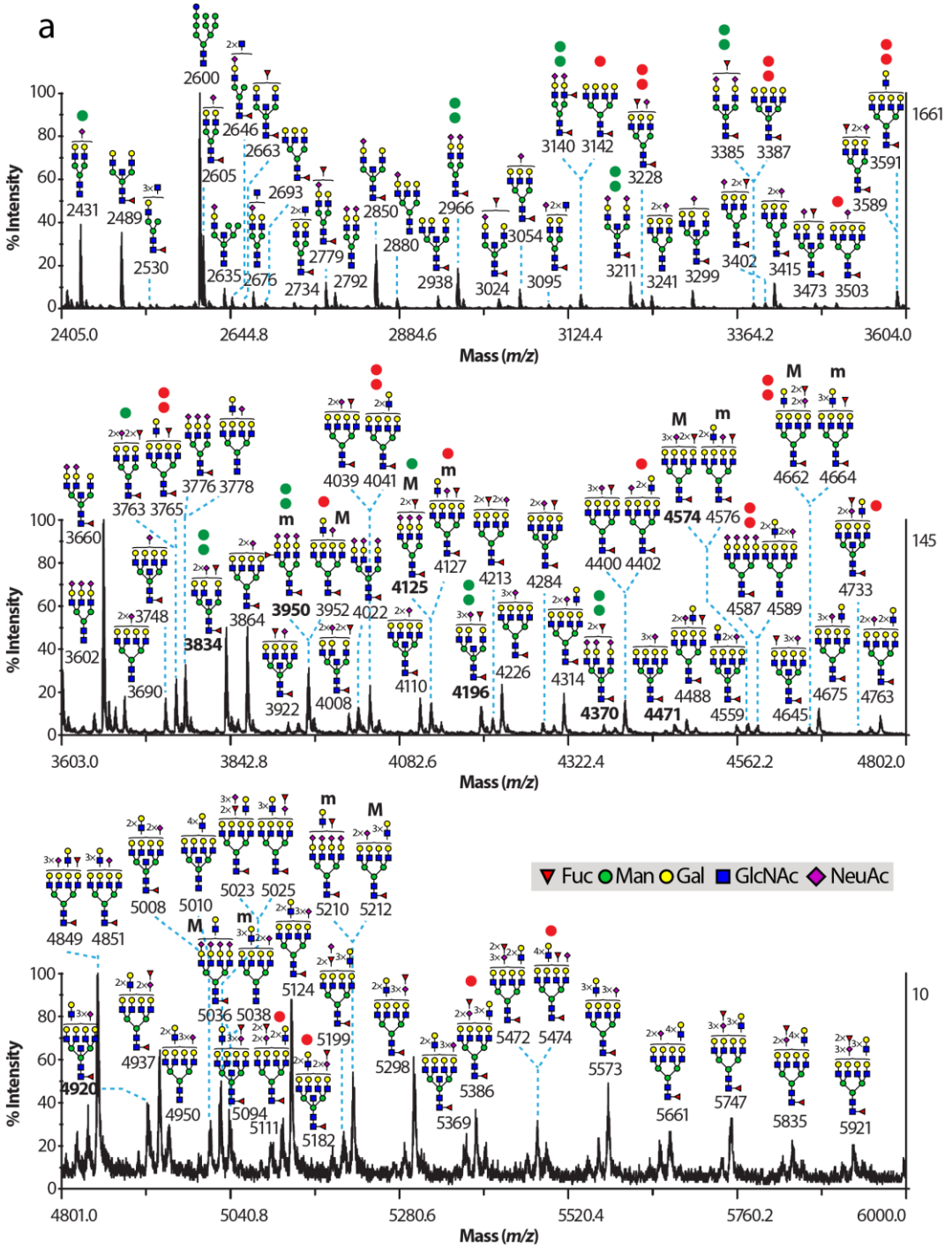
HL-60 ST3Gal-4 knock out generated using CRISPR/Cas9



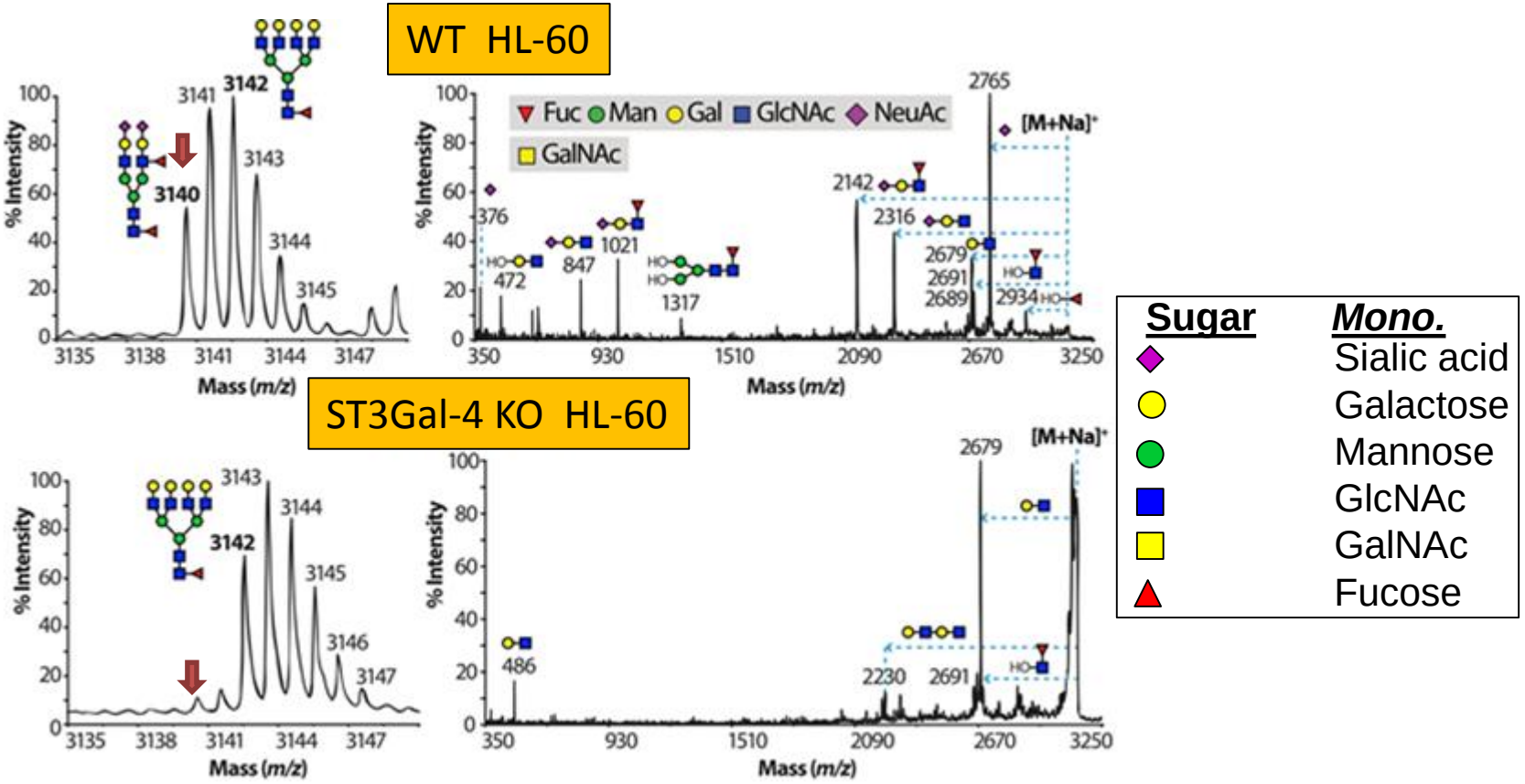
Abrogation of leukocyte rolling on all selectins



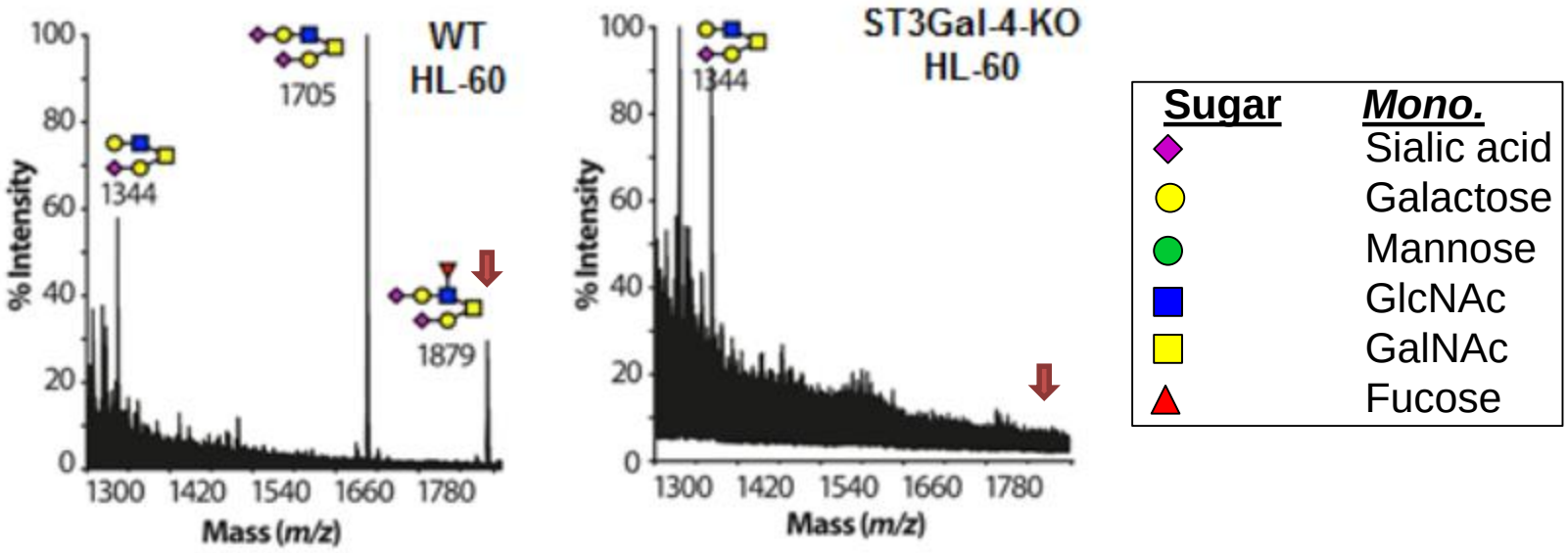
Profiling glycans



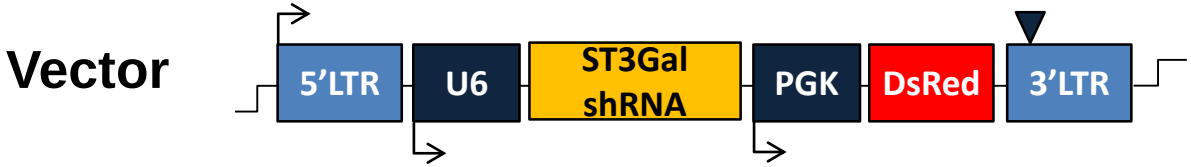
N-glycans: ST3Gal-4 controls sLe^X biosynthesis

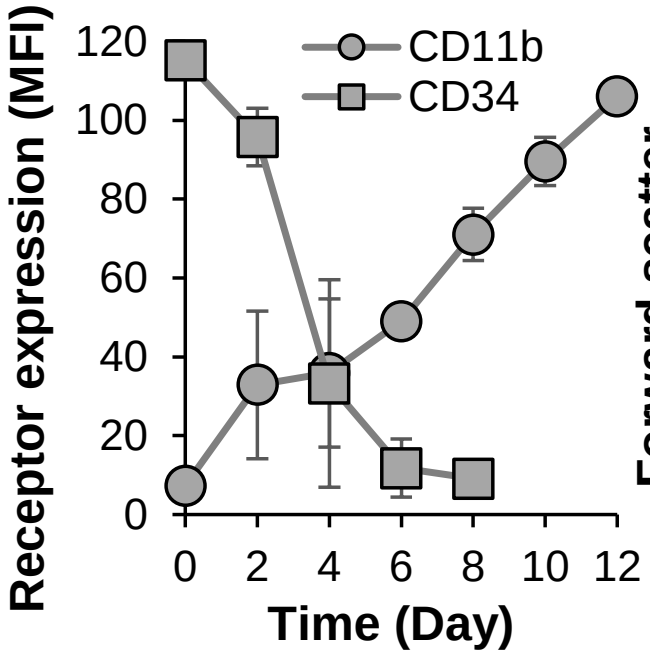


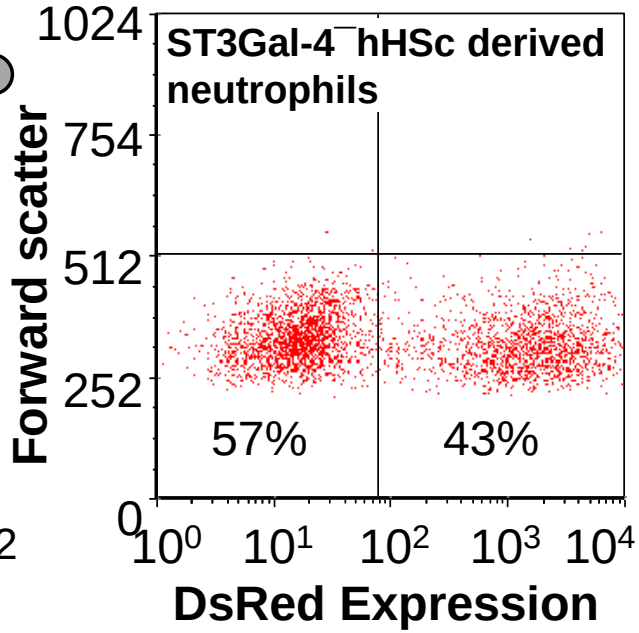
O-glycans: ST3Gal-4 deletion abrogates sLe^X biosynthesis



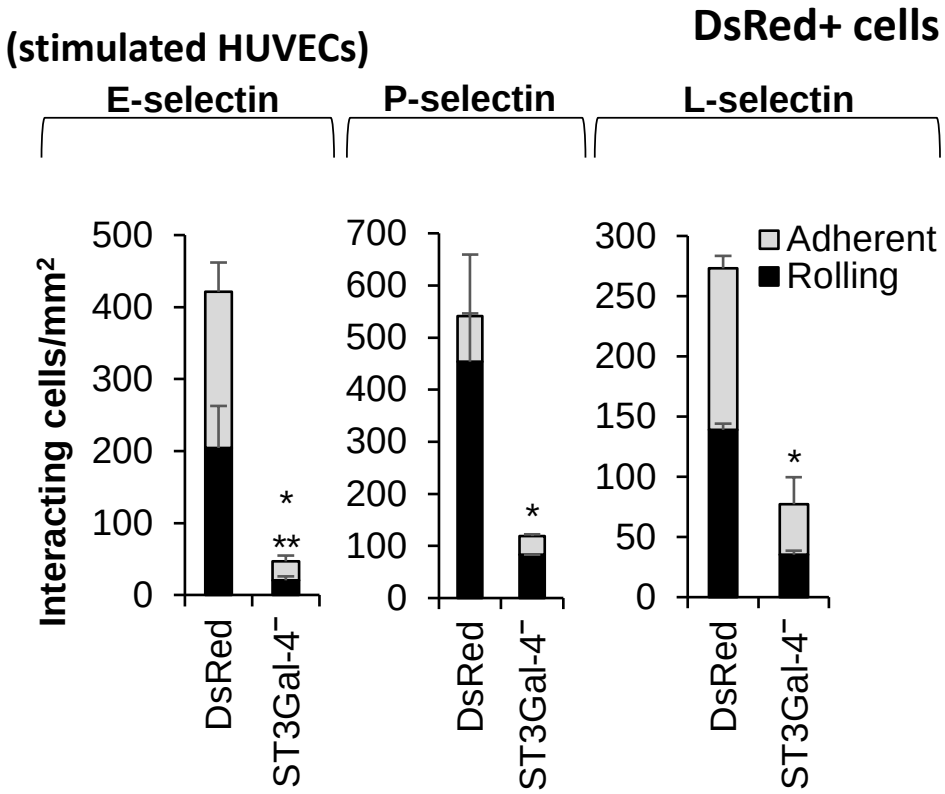
Human neutrophils derived from hematopoietic stem cells (HSCs)





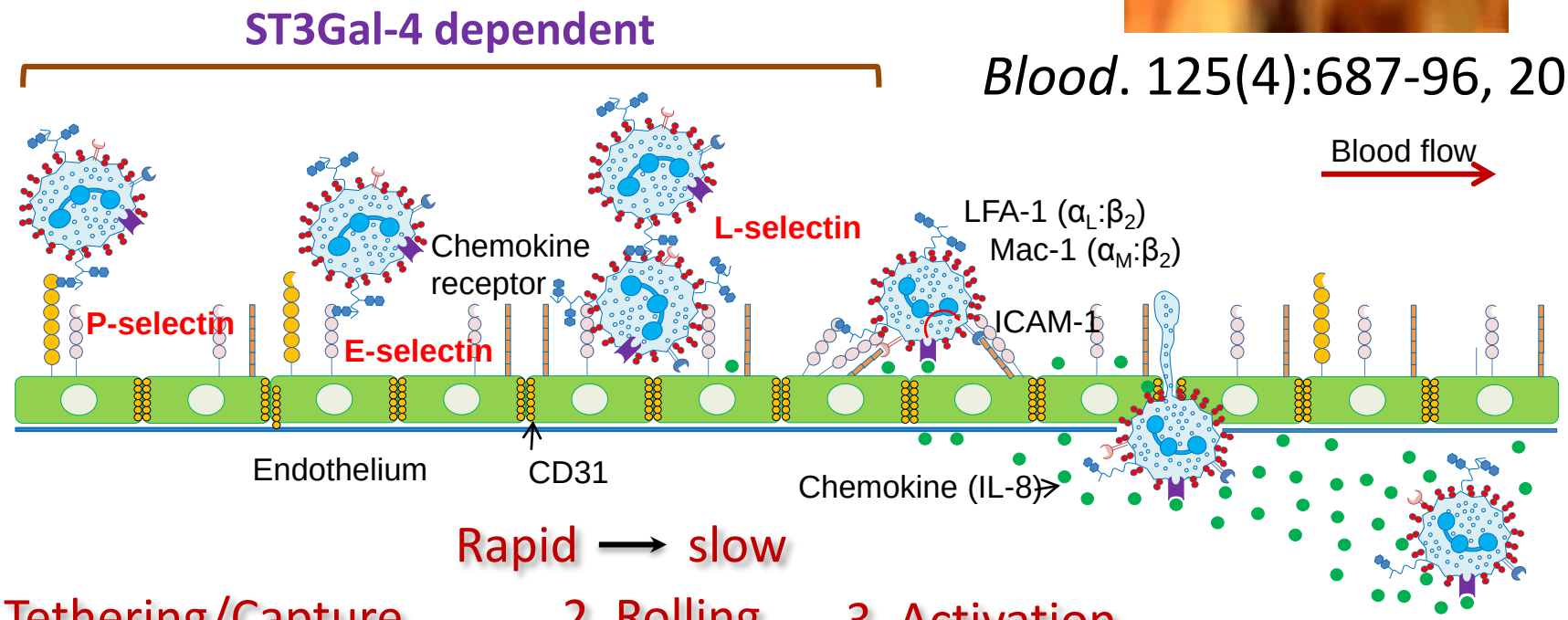


Human neutrophil rolling on selectins



The multistep cell adhesion cascade

Blood. 125(4):687-96, 2015



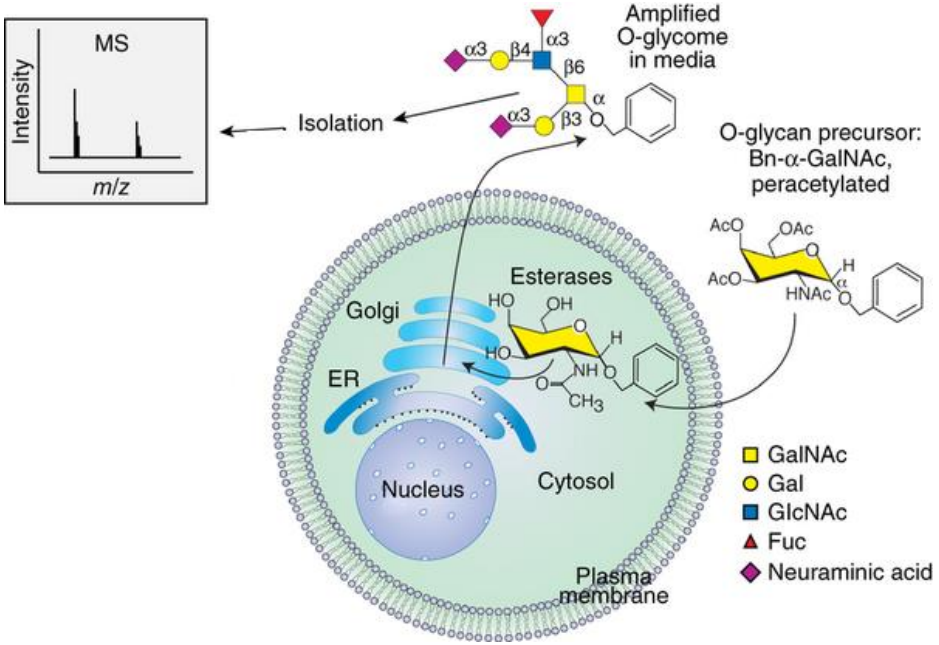
Rapid → slow

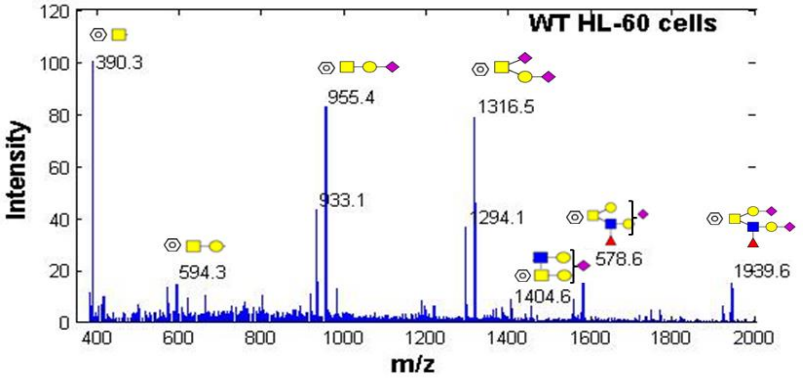
1. Tethering/Capture
2. Rolling
3. Activation
4. Firm adhesion & diapedesis

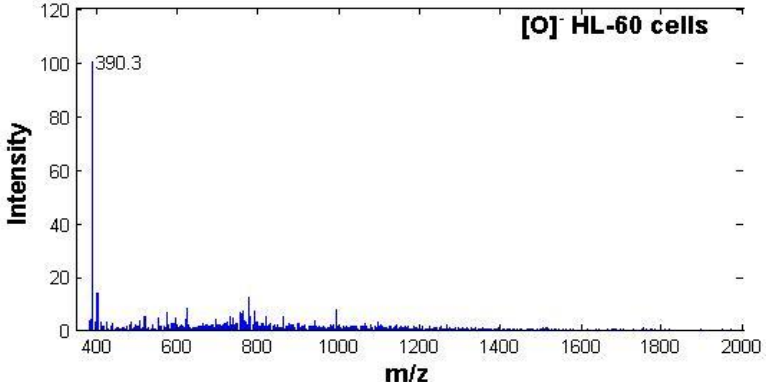
Glycan dependent

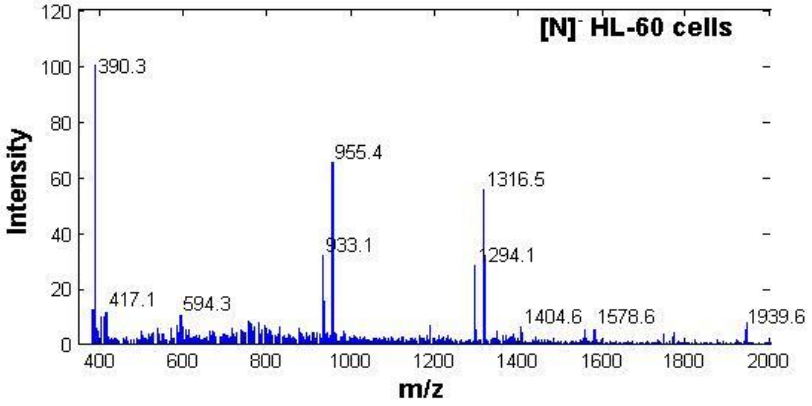
Glycan independent

Rapid glycan profiling and chemical synthesis



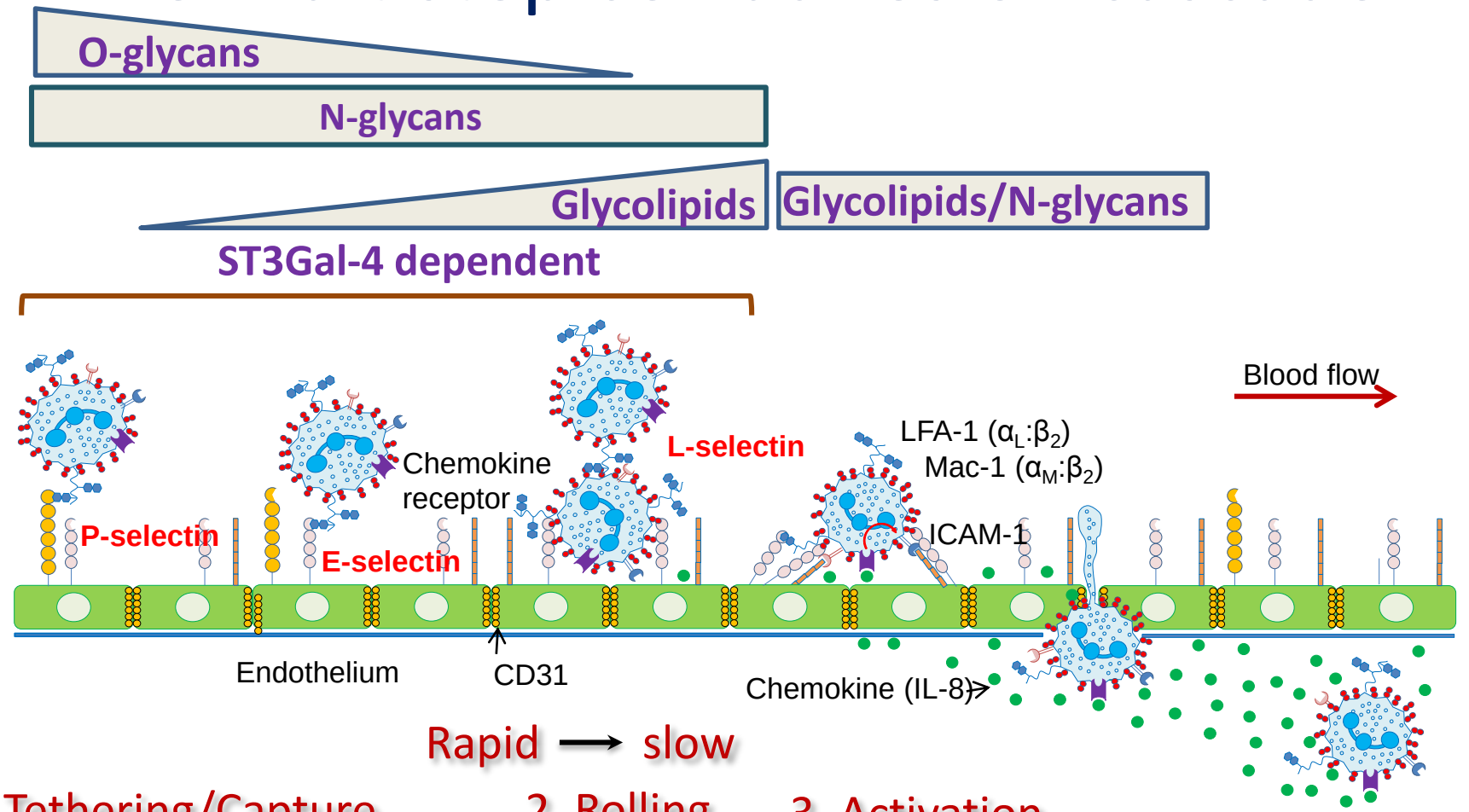




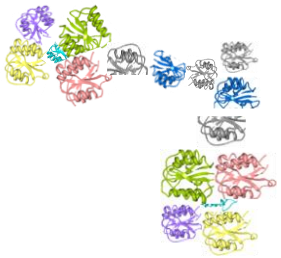


Nature Methods 13, 81–86 (2016)

The multistep cell adhesion cascade

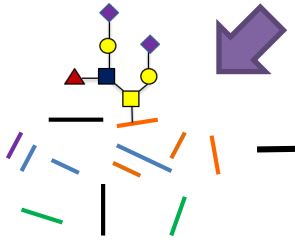


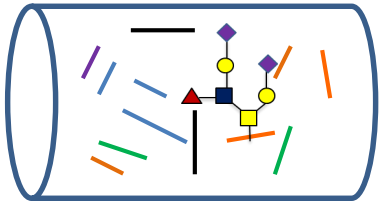
GlycoPAT: High-throughput glycoproteomics analysis



Protease Digestion

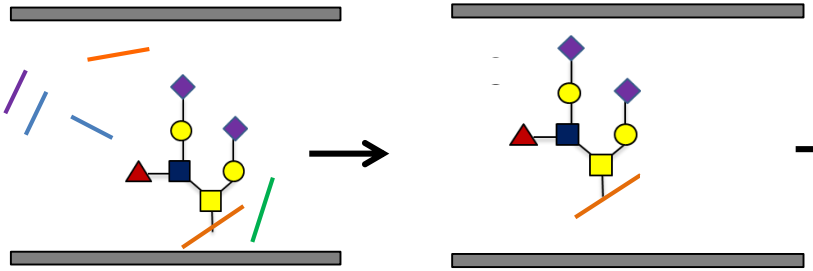
(Trypsin, Glu-C etc.)





nanoLC

Tandem mass spectrometry in Orbitrap

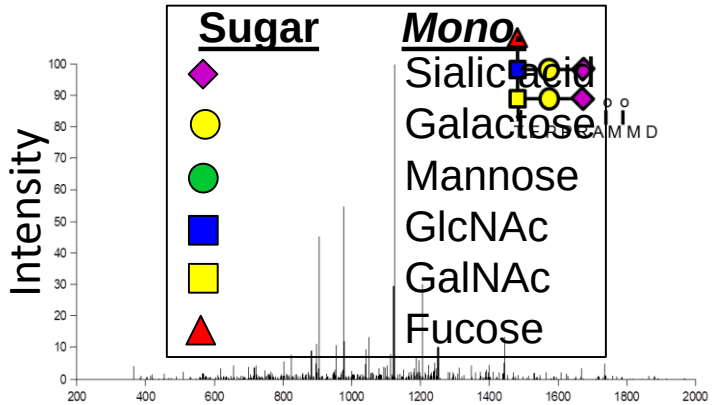


MS^1

Separation based on precursor m/z

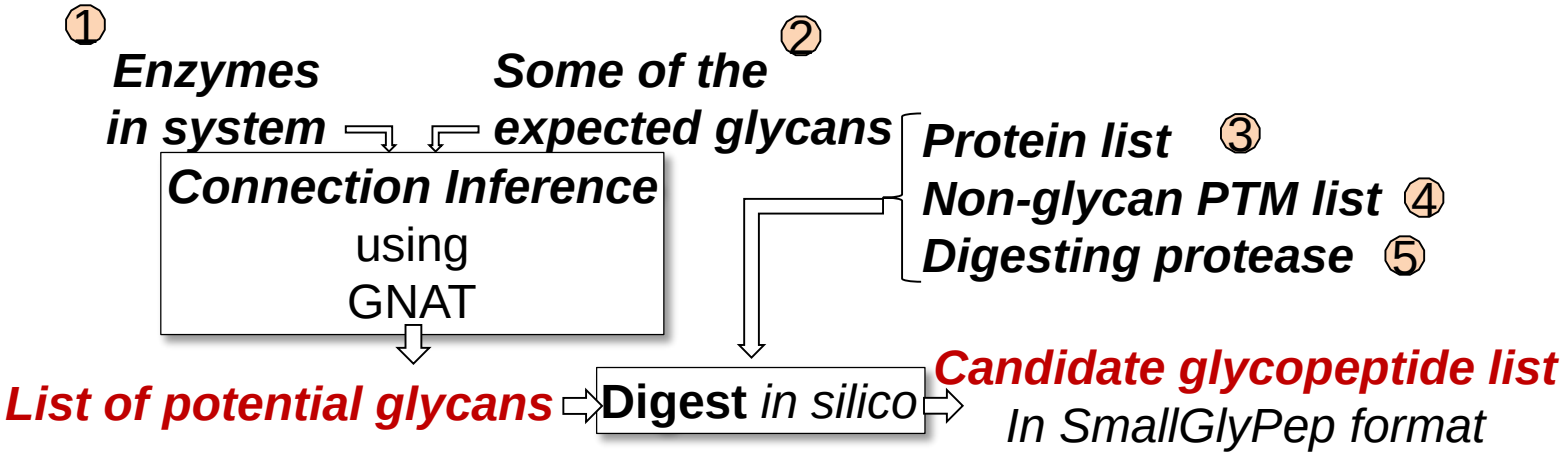
MS^2

fragmentation

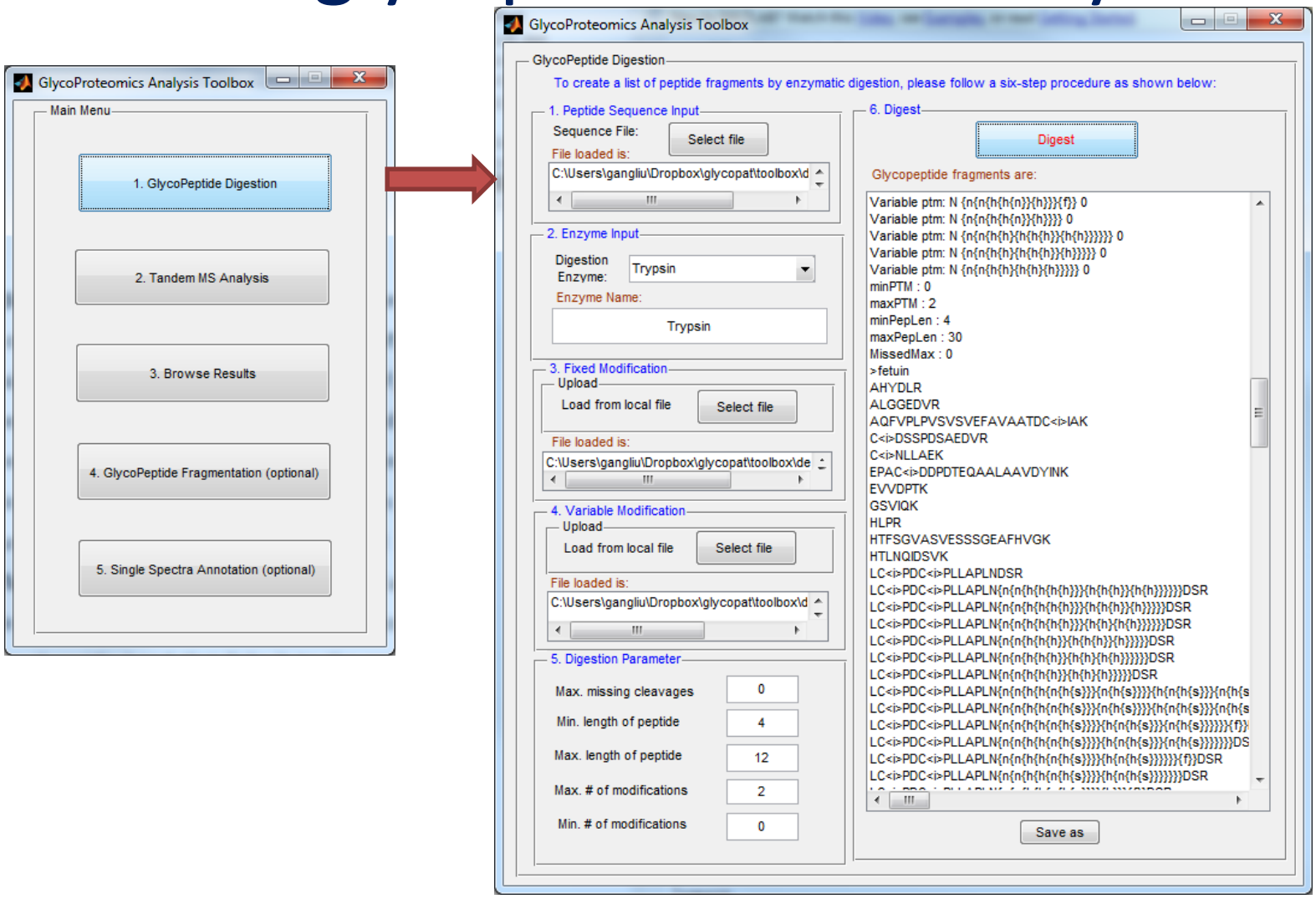


With Jun Qu

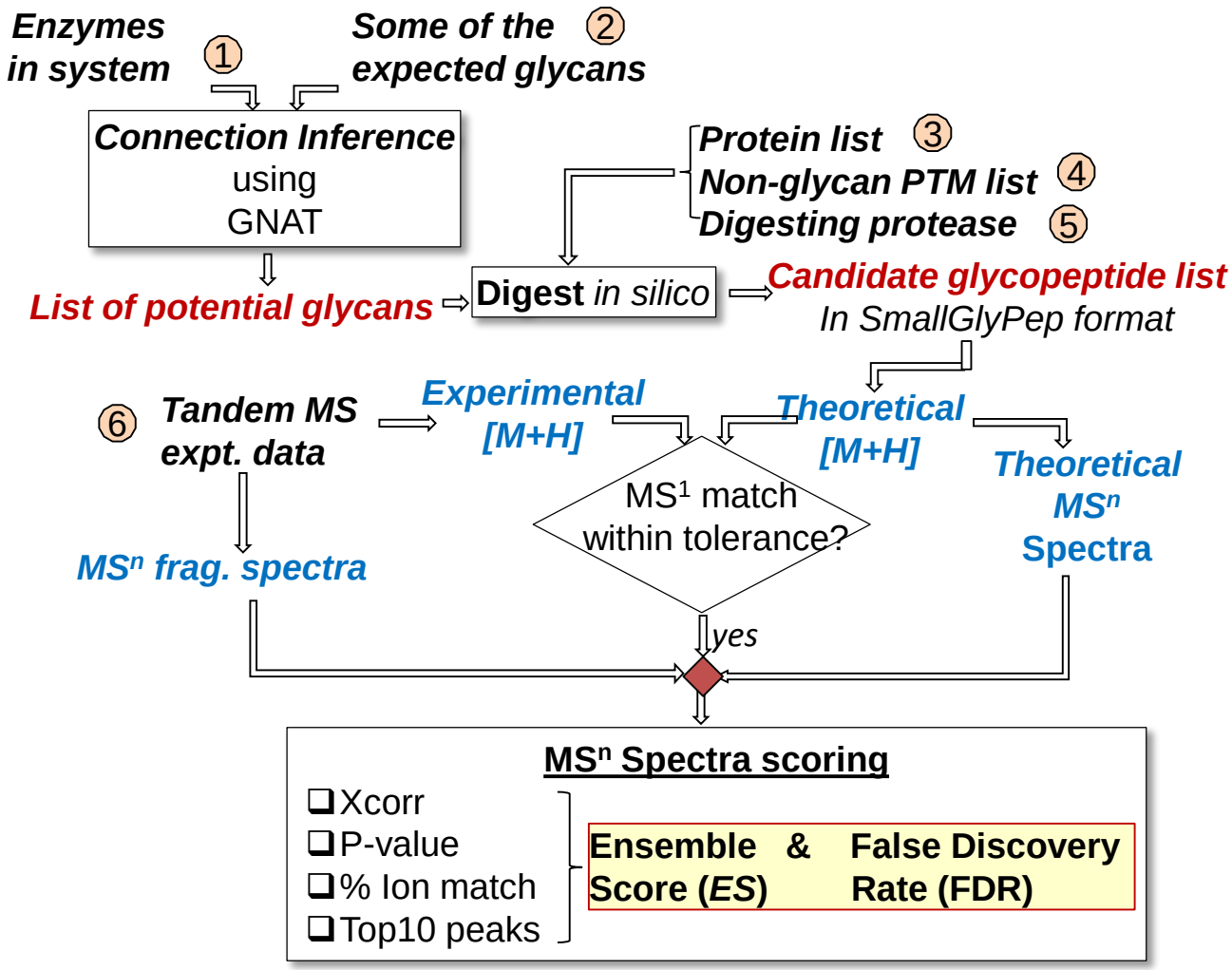
Overall algorithm



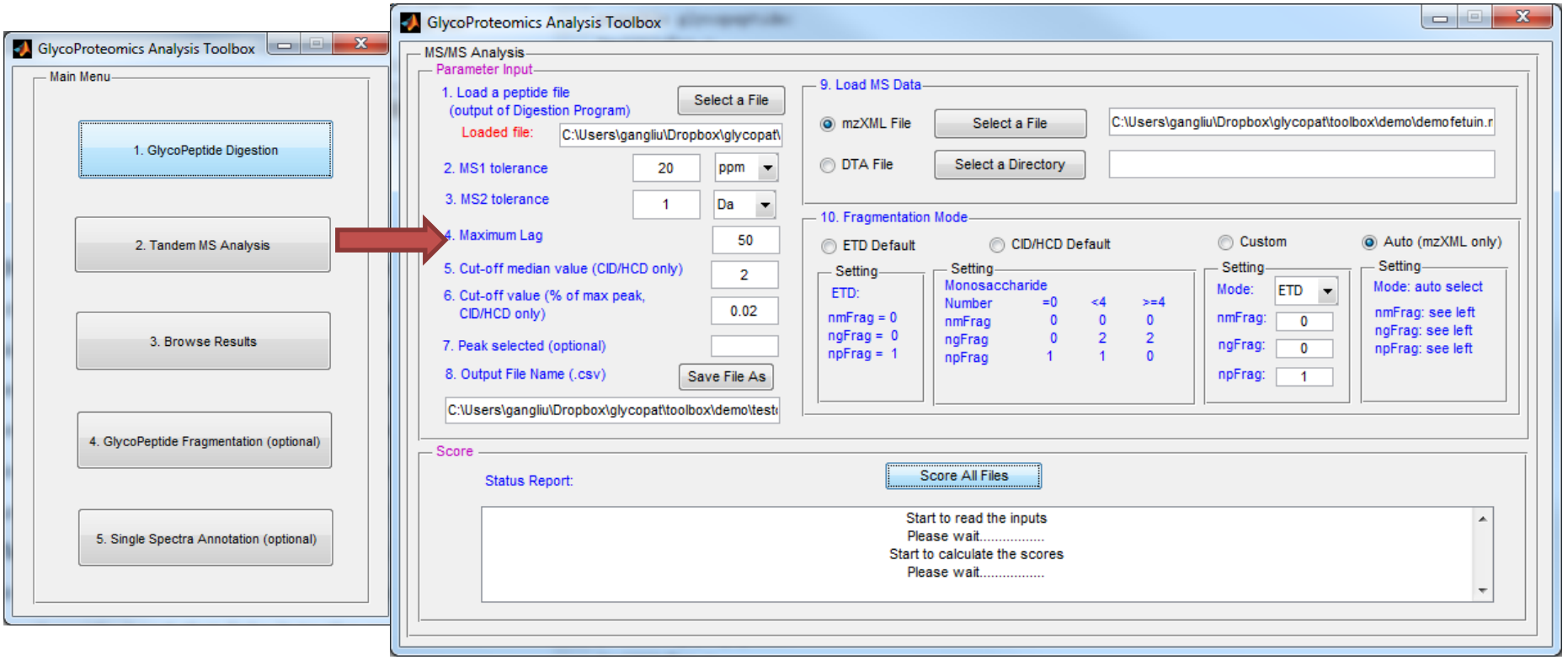
GlycoPAT: High-throughput glycoproteomics analysis



Overall algorithm



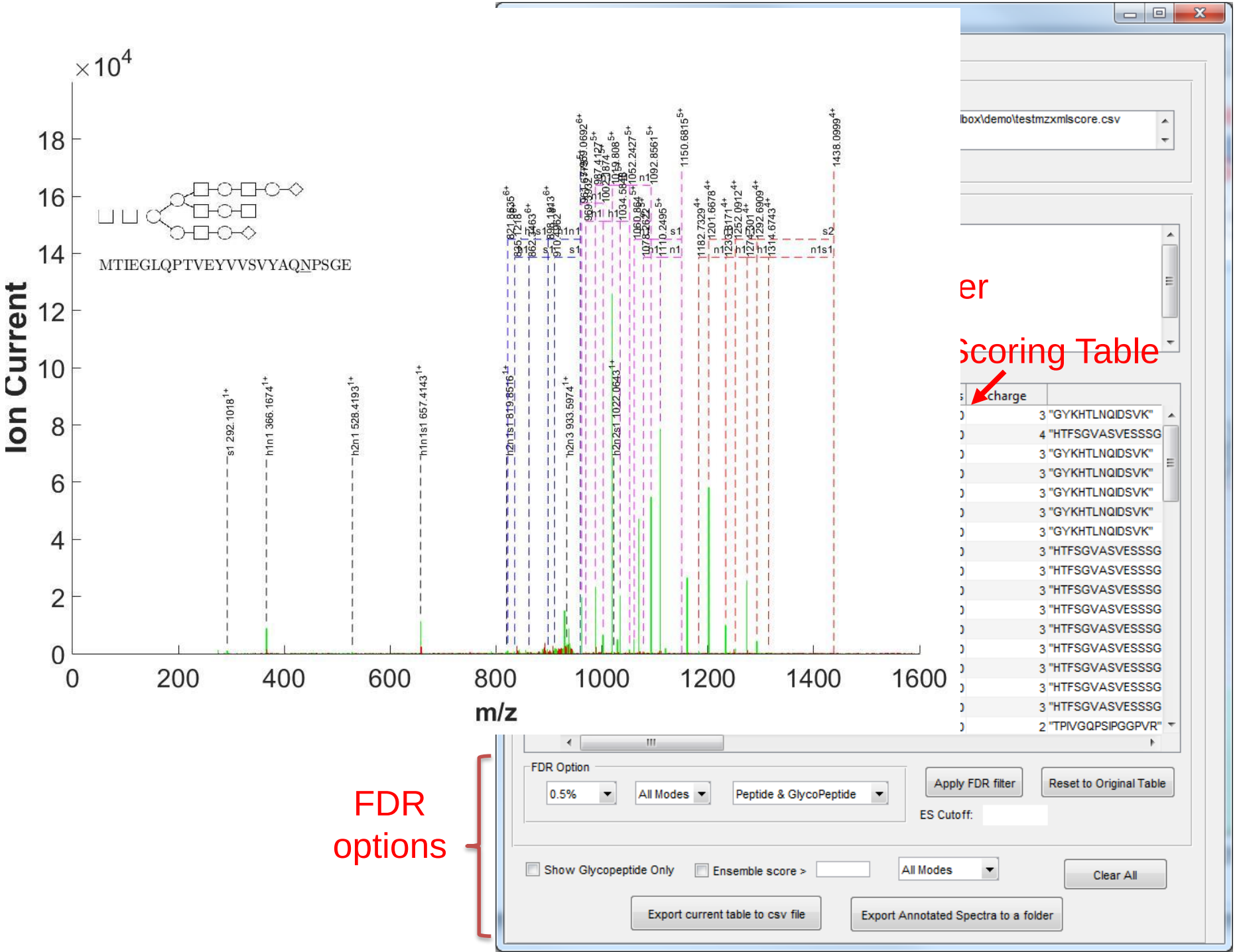
GlycoPAT: High-throughput glycoproteomics analysis



Scoring parameters

- Xcorr
- P-value
- Top10
- % ion-match

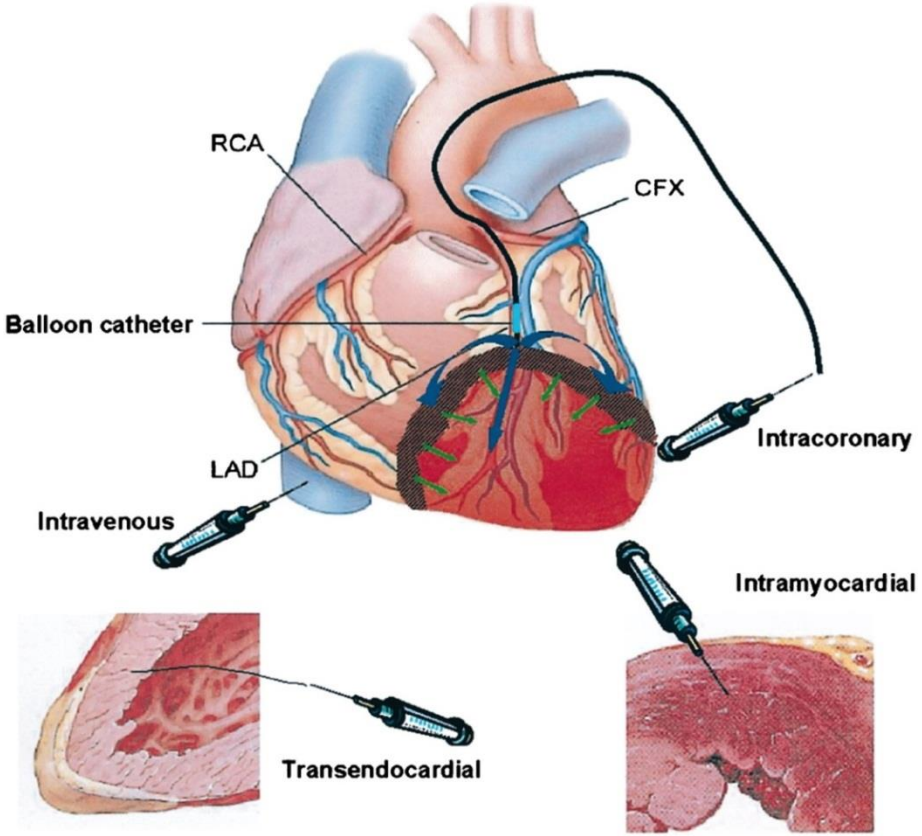
→ **Ensemble score**


×10^4
Ion Current
m/z
MTIEGLQPTVEYVVSVYAQNPSGE
s1 292.1018 1+
h1n1 366.1674 1+
h2n1 528.4193 1+
h1n1s1 657.4143 1+
h2n1s1 819.8516 1+
h2n3 933.5974 1+
h2n2s1 1022.0643 1+
1150.6815 5+
1438.0999 4+
Scoring Table
charge
"GYKHTLNQIDSVK"
"HTFSGVASVESSSG
FDR Option
0.5%
All Modes
Peptide & GlycoPeptide
Apply FDR filter
Reset to Original Table
ES Cutoff:
Show Glycopeptide Only
Ensemble score >
All Modes
Clear All
Export current table to csv file
Export Annotated Spectra to a folder
FDR options

Characterization of:

- Simple standard proteins
- Mixtures of standards
- Plasma cryoprecipitate from 5cc of blood

Cell Adhesion GlycoEngineering



- MSCs (Mesenchymal stem cells) and CDCs (Cardiosphere derived stem cells) are promising cellular therapuetics.
- However, promising results from animal studies are not translating to clinical benefits
- Local infusion to damaged tissue is not beneficial
- **Systemic infusion proximal to the therapeutic site**
  - **minimally invasive**
  - **allows repeated treatment**

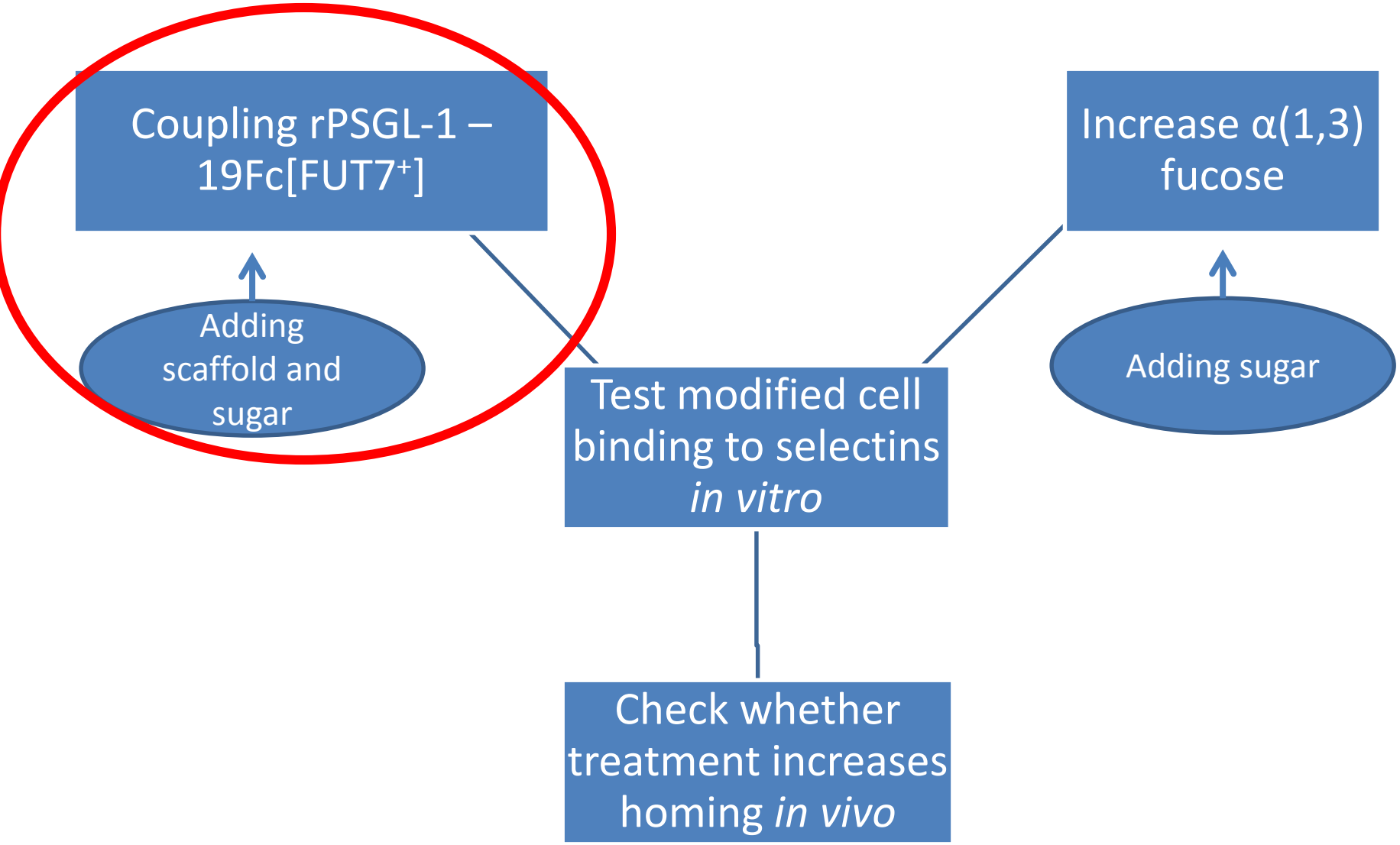
Strauer B E , and Kornowski R Circulation. 2003;107:929-934

Goals

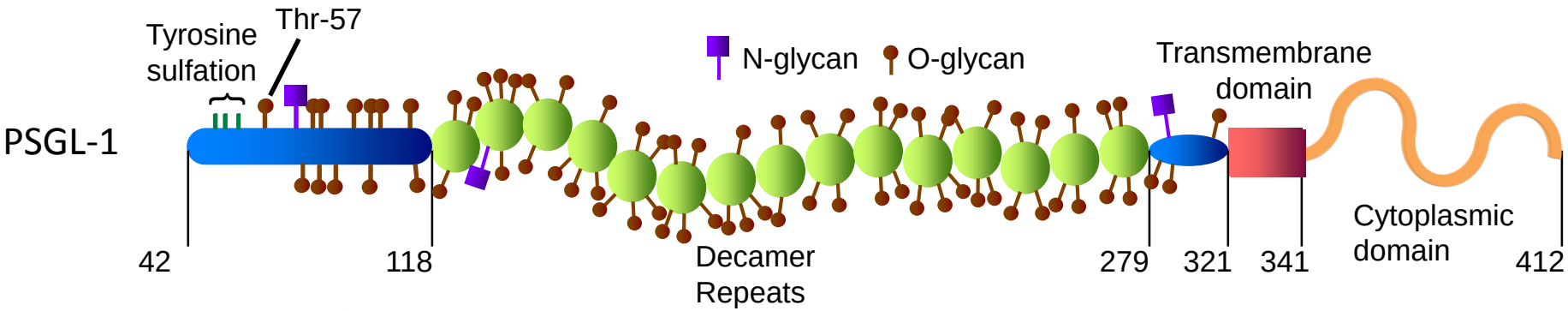
- Make stem cells home to sites of inflammation, much like white blood cells
- Create methods that can be used in a clinical setting with minimal effort
- Move studies to clinically relevant large animals

Chi Lo

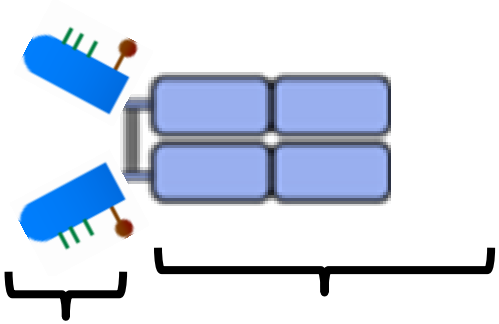
Experimental plan



Recombinant PSGL-1

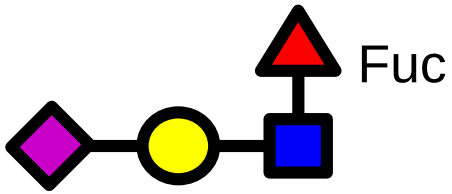


19Fc [FUT7^{+}]



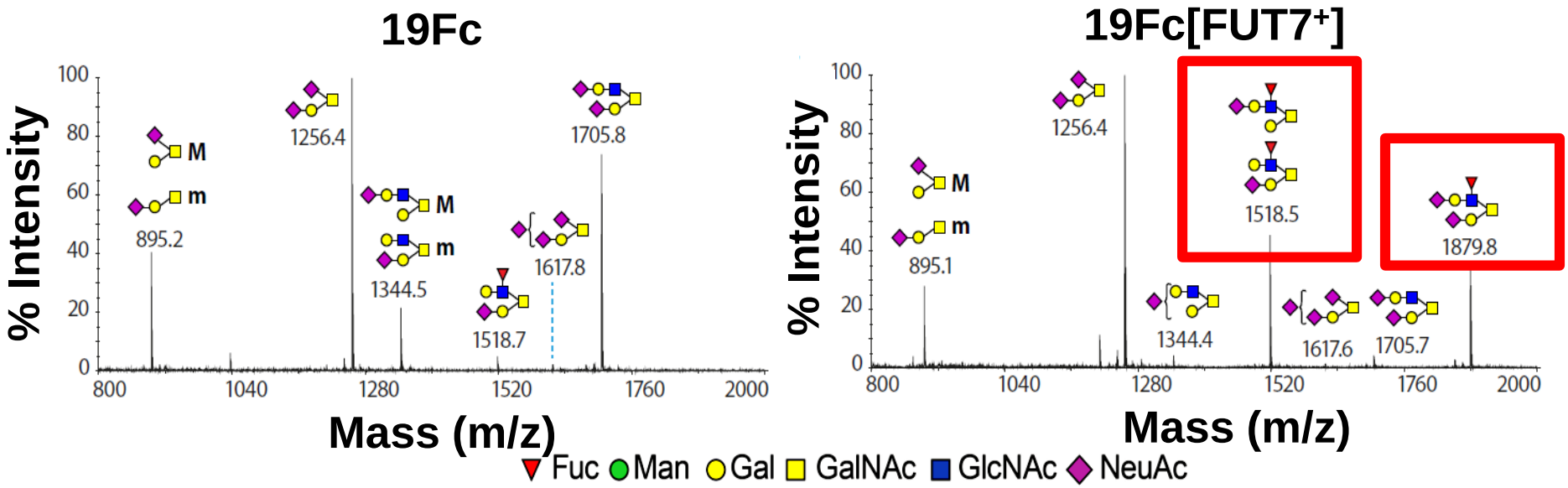
19 aa PSGL-1

Human IgG_1



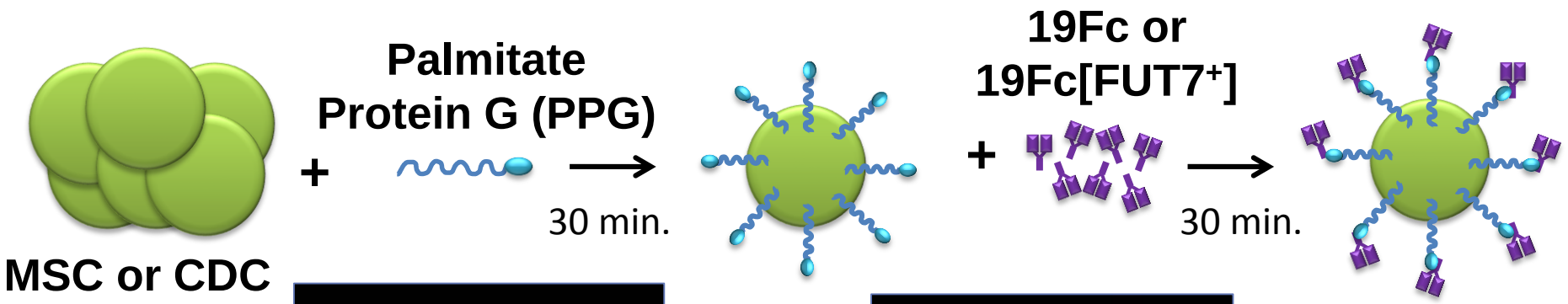
Sialyl Lewis-x

Characterization of 19Fc

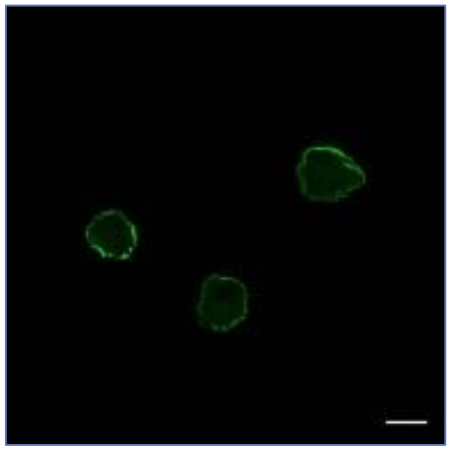


Lo, C.Y., et al. Biomaterials, 2013. **34**(33): p. 8213-22.

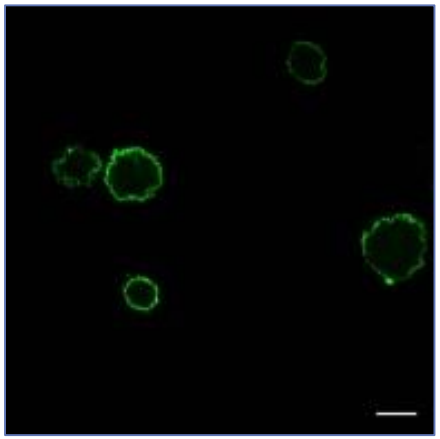
Coupling recombinant PSGL-1

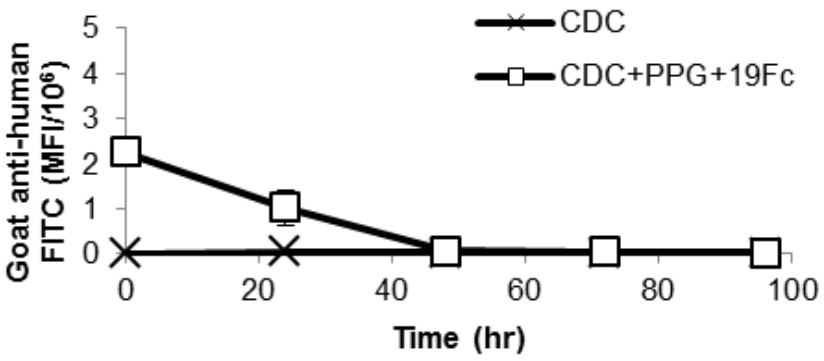


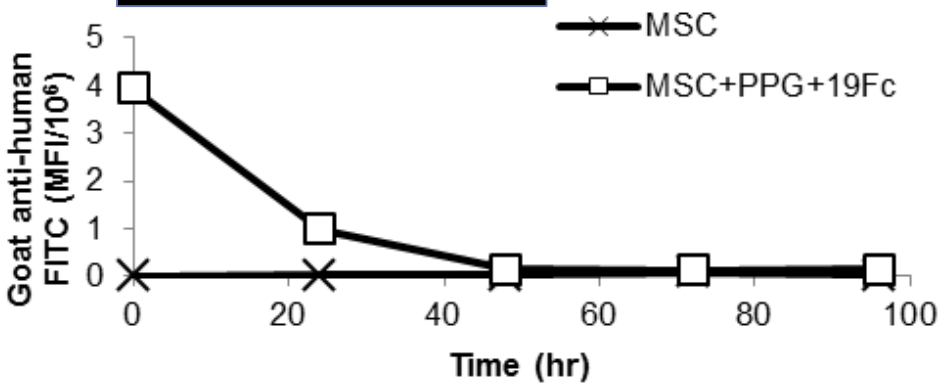
CDC



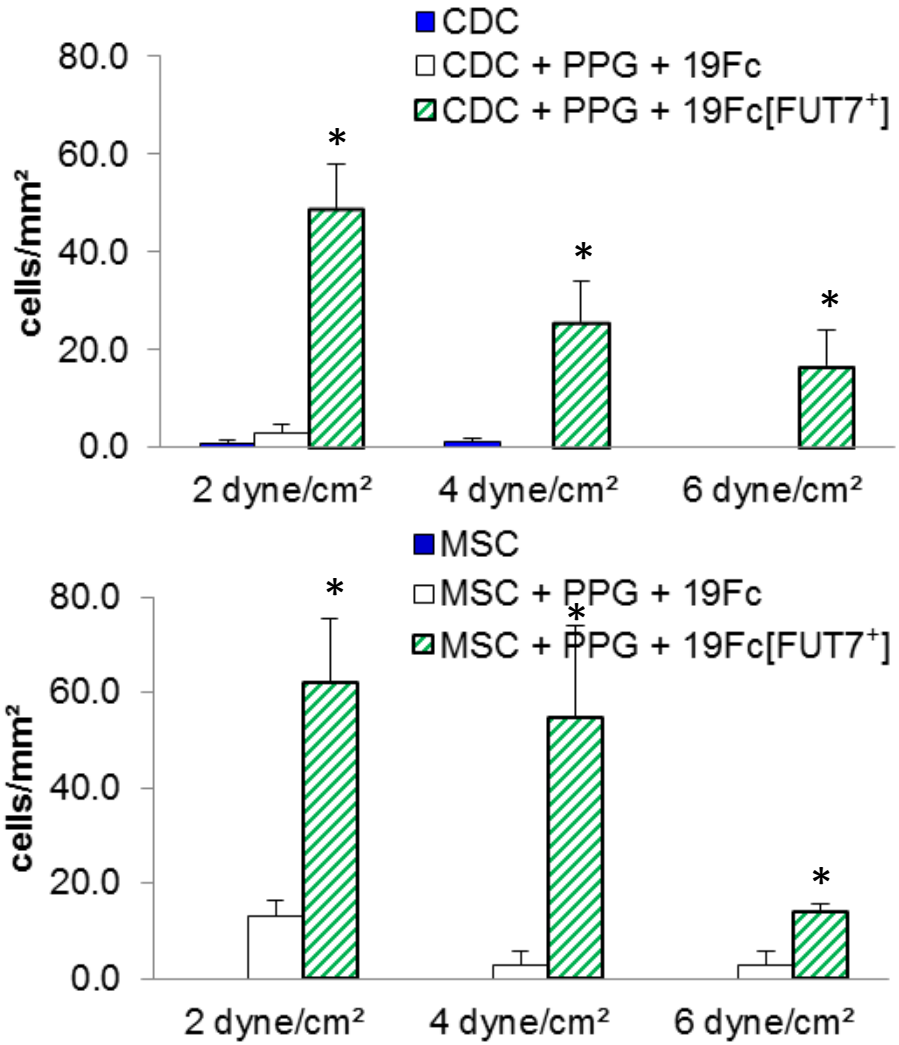
MSC



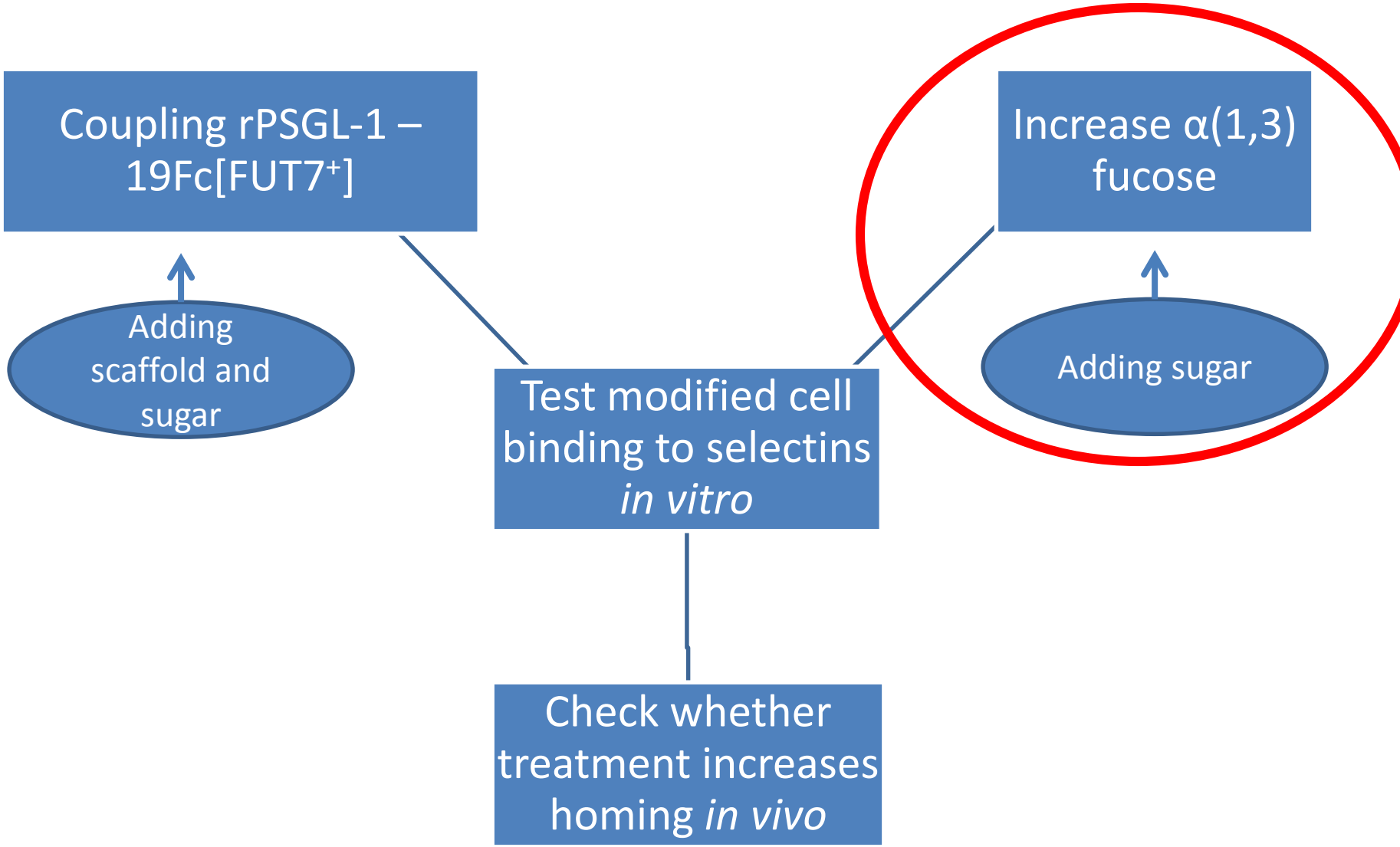




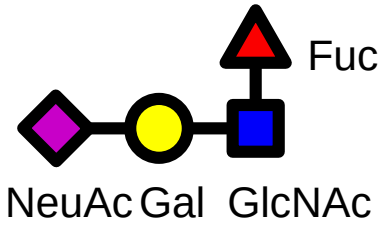
19Fc coupling with PPG increases interactions with P-selectin



Experimental plan

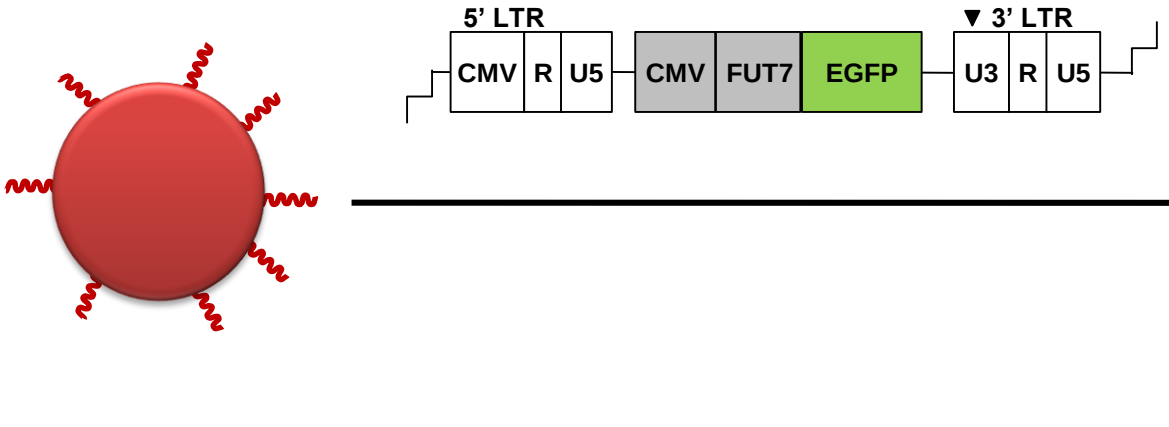


Over-expression of FUT7



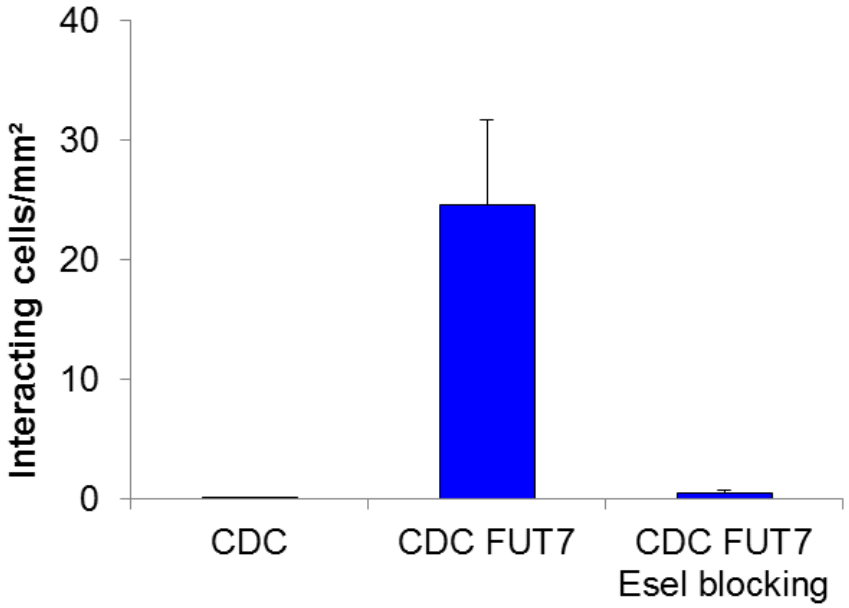
Sialyl Lewis-x

Lentiviral constructs



Fucose

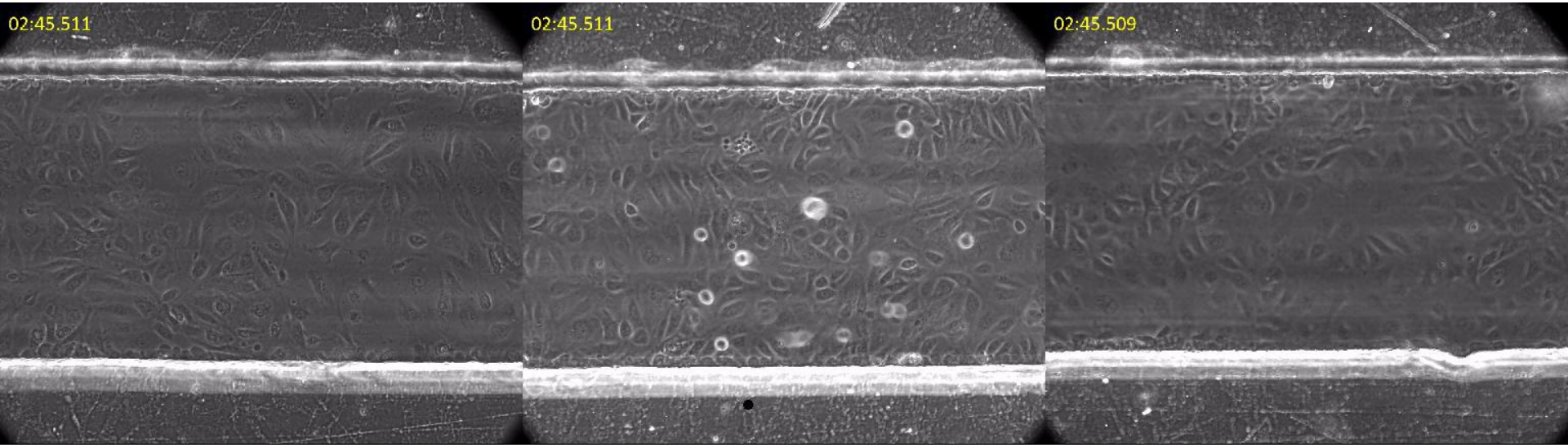
FUT7 increases CDC interactions with stimulated HUVECs



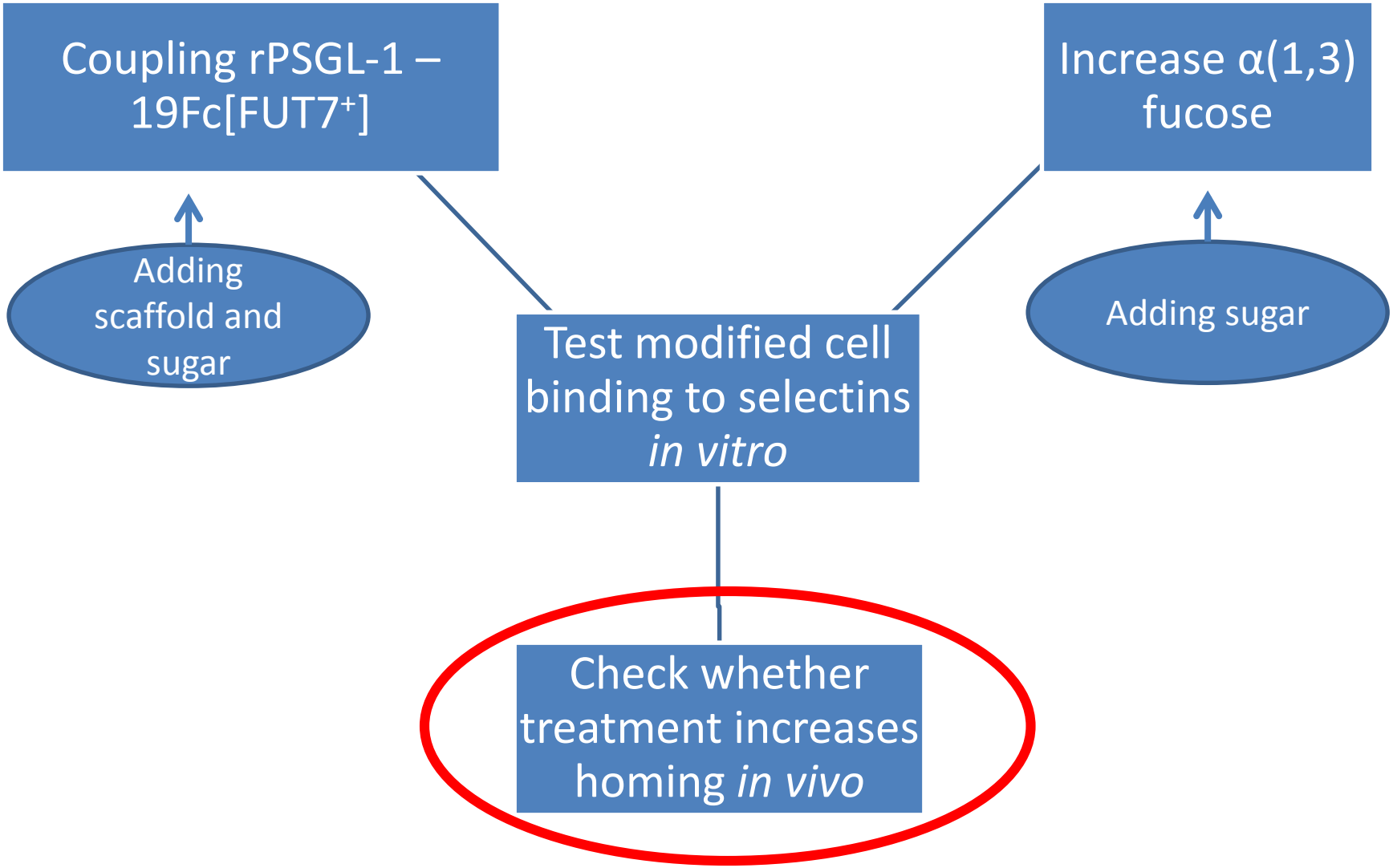
CDC

CDC FUT7

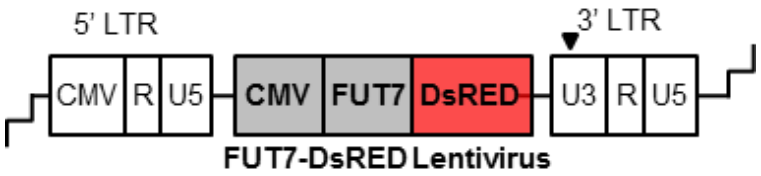
CDC FUT7 +
Esel blocking

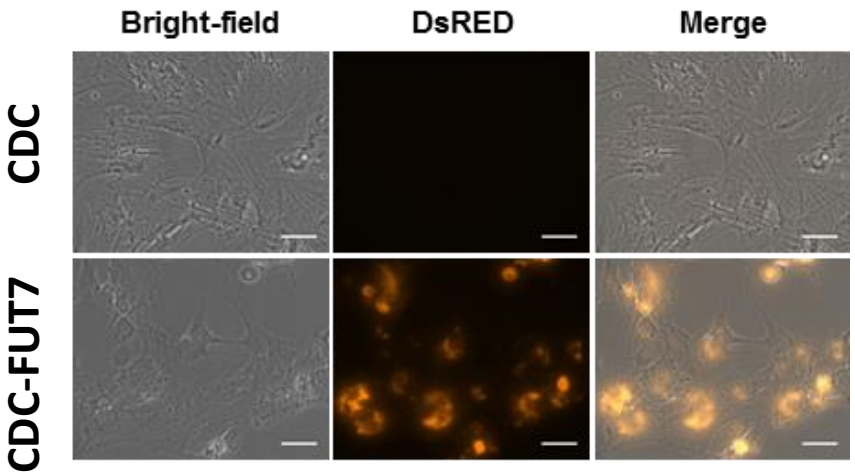


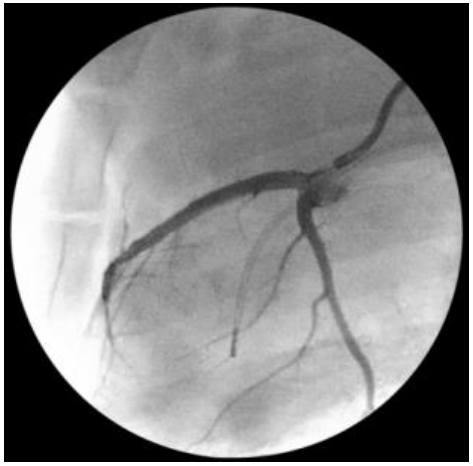
Large animal studies: Swine



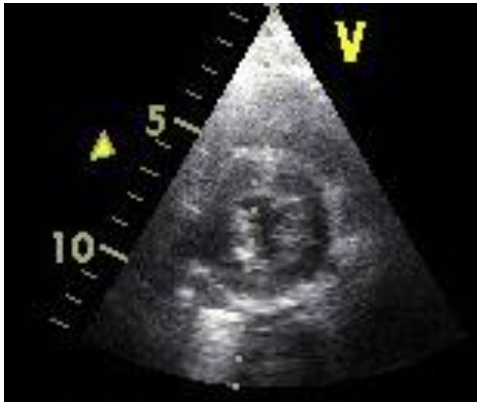
Pig experiments: 30 min. brief ischemia-reperfusion of LAD





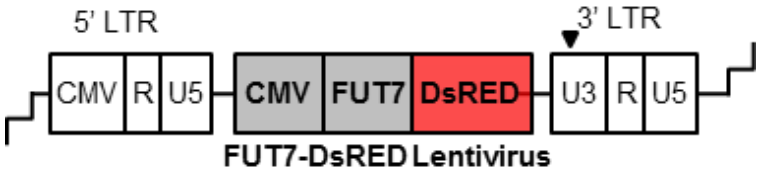


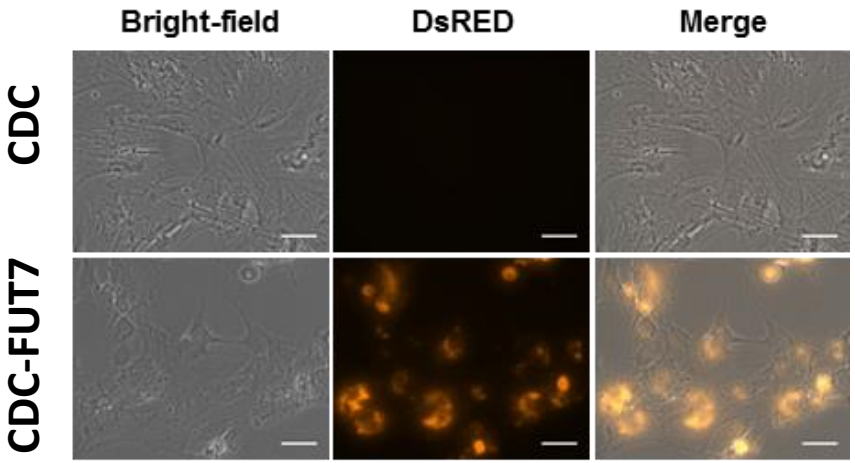
Catheter placement for cell injection

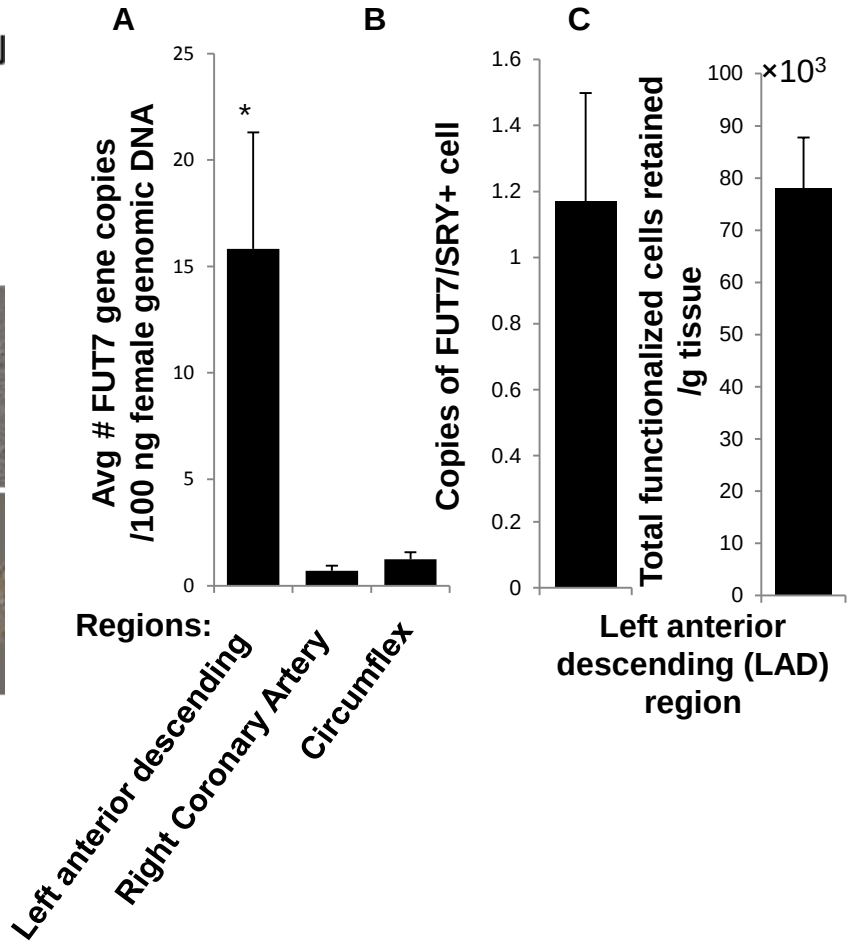


echocardiogram

Pig experiments: 30 min. brief ischemia-reperfusion of LAD







Lo, C.Y. *et al*. Biomaterials (2016), 74: 19-30

Conclusion

- Glycosylation is a poorly communicated field--its easier than it looks
- There are tremendous opportunities for exploration:
  - New basic science
  - New applications
  - New drugs

The final frontier--- boldly go where no (wo)man has gone before

Acknowledgements

Current lab members:

Anju Kelkar, Ph.D. Arezoo Momeni

Kai Cheng

Hannah W

Xinheng Y

Collaborat

Aristotelis ollege-London

S. G. Sam

John Cant

Joseph La lo, NY

Funding s

NIH Heart, Lung and Blood Institute: Systems Biology program

American Heart Association

NY State Stem Cell Program

National Heart, Lung, and Blood Institute

American Heart Association | American Stroke Association. life is why

NYSTEM NEW YORK STATE STEM CELL SCIENCE