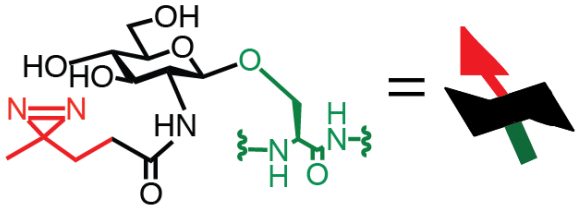
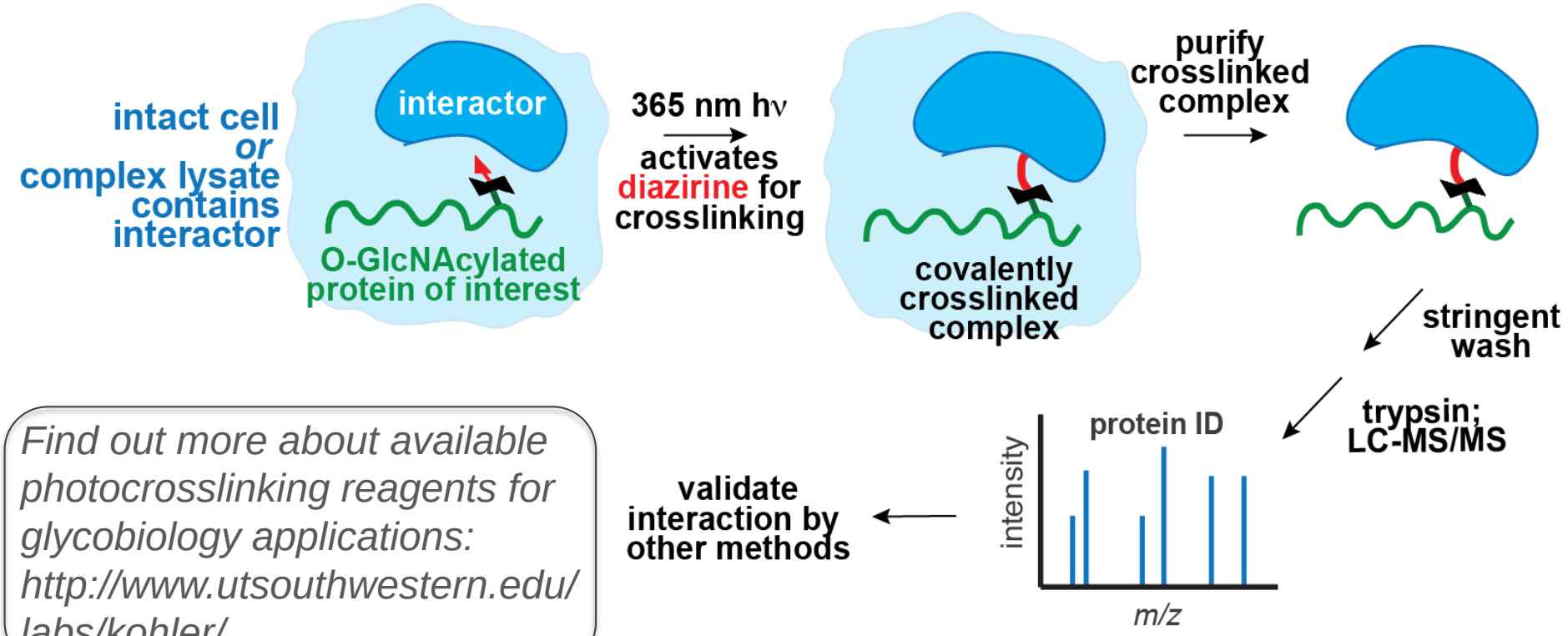
Photocrosslinking tools to covalently capture interaction partners of O-GlcNAcylated proteins

Jennifer Kohler lab
UT Southwestern



O-GlcNDAz is a photocrosslinking analog of O-GlcNAc that can be added chemoenzymatically to purified proteins or metabolically to proteins in living cells.

O-GlcNDAz crosslinking protocol

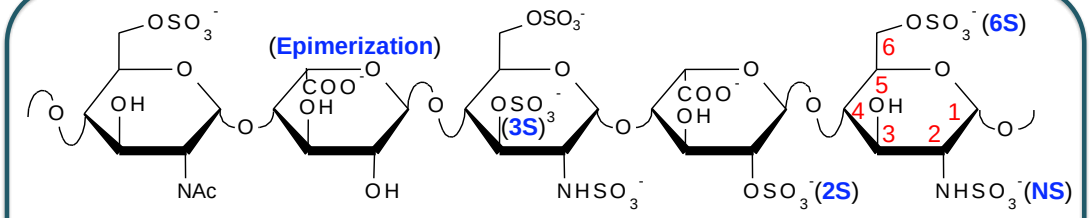


Find out more about available photocrosslinking reagents for glycobiology applications: http://www.utsouthwestern.edu/labs/kohler/

Cell library for heparan sulfate (HS) structure-function studies

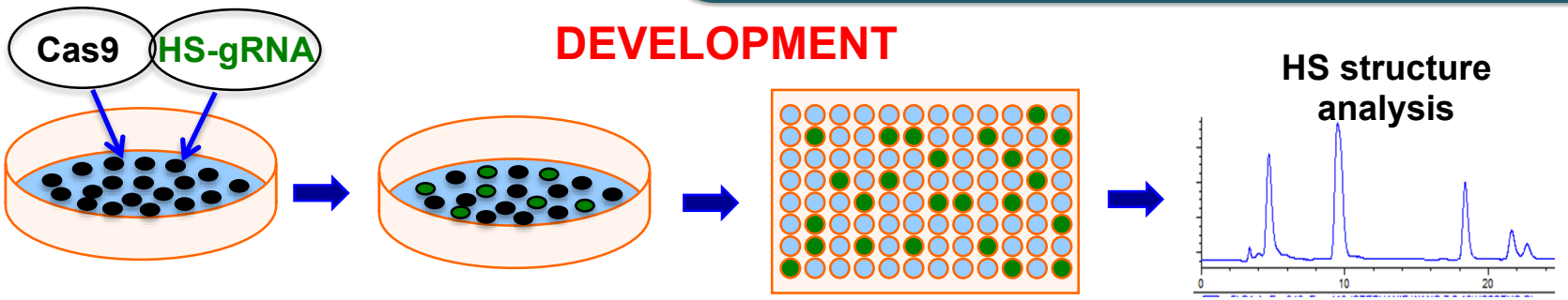
Lianchun Wang,
lwang@ccrc.uga.edu
Complex Carbohydrate Res. Ctr.
University of Georgia

Available HS-gRNAs and cells:
http://ccrc.uga.edu

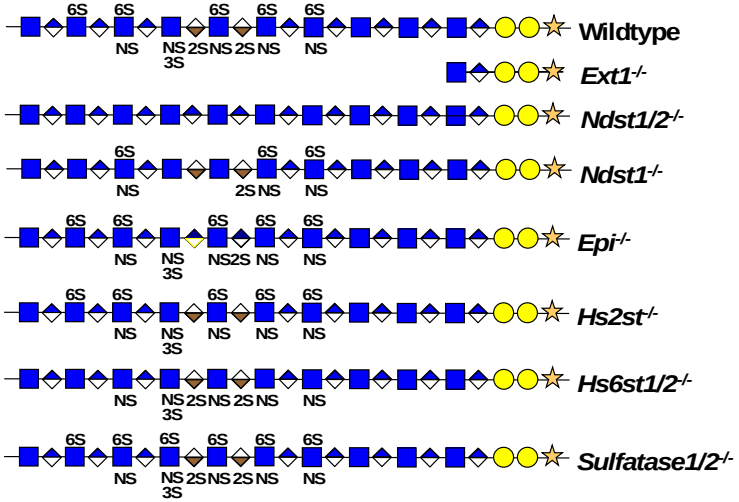


The **modifications** (epimerization and various sulfations) create **unique ligand-binding sites** in heparan sulfate

DEVELOPMENT



HS mutant cell library

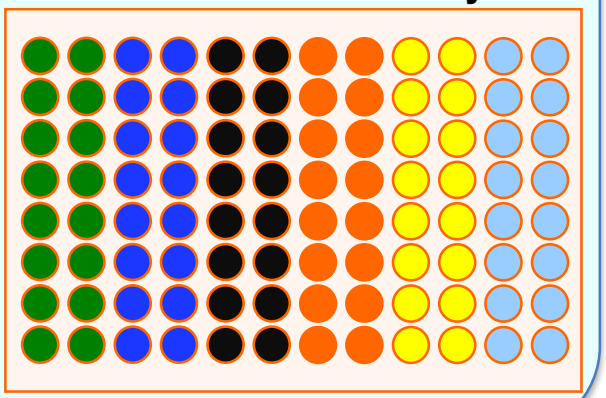


APPLICATION

Measure:

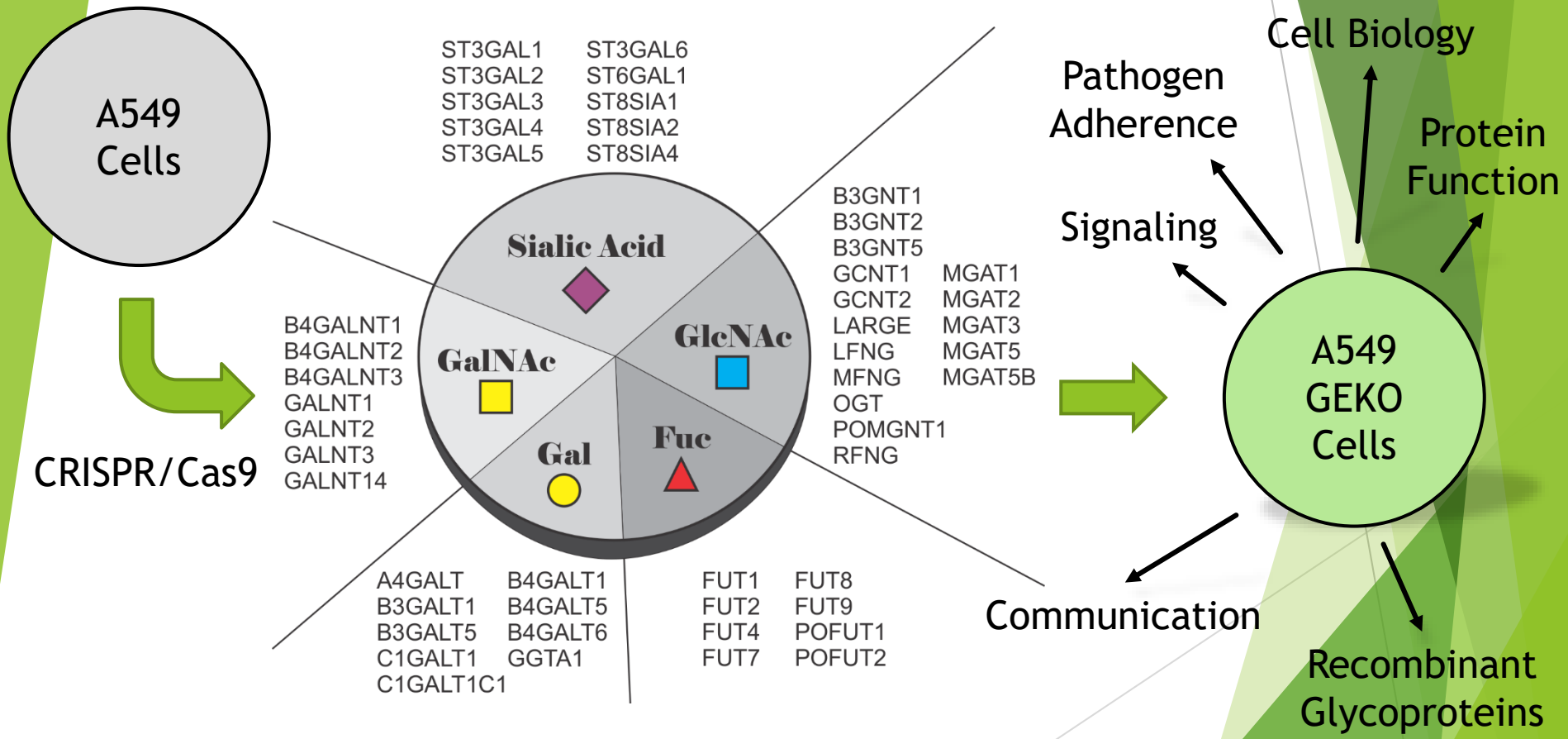
HS-ligand interactions on cell surface and cell responses

HS mutant cell array

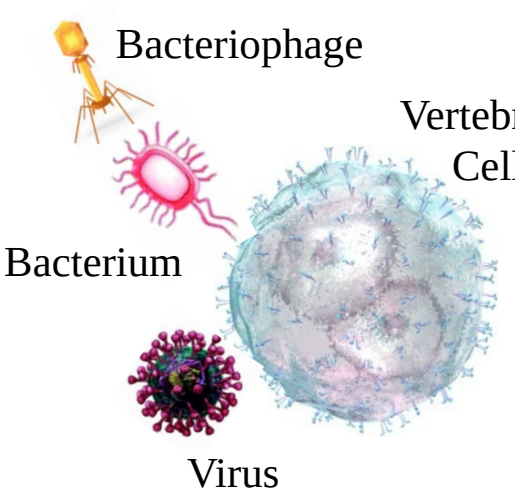


GEKO Technology

Glycome-Enhanced KnockOut cell lines lacking selected glycosyltransferases
https://case.edu/med/pathology/faculty/cobblab/GEKO.html



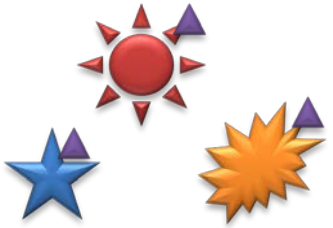
Contact: Brian A. Cobb, PhD, brian.cobb@case.edu



Bacterial Adhesins
Viral Hemagglutinins
Phage Endosialidase

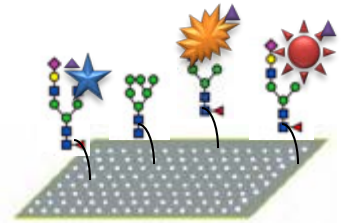
Cloning
Expression
Tagging

+ Mutated non-binding controls

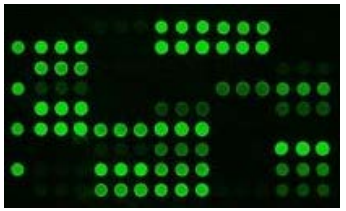


A Toolkit of Sialoglycan-Recognizing Probes (SGRPs)

Ajit Varki, UCSD, PI
Paul Sullam, UCSF, Co-PI
Colin Parrish, Cornell, Co-PI
Xi Chen, UC Davis, Co-PI



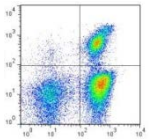
Characterize recognition properties by sialoglycan Array



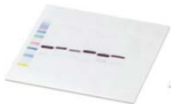
Selection of SGRPs that detect the sialome

IHC

ELISA



FACS



Western Blot

Validate by Western blotting, FACS, ELISA and IHC

Test Properties of Common Sialidases on Sialoglycan Array

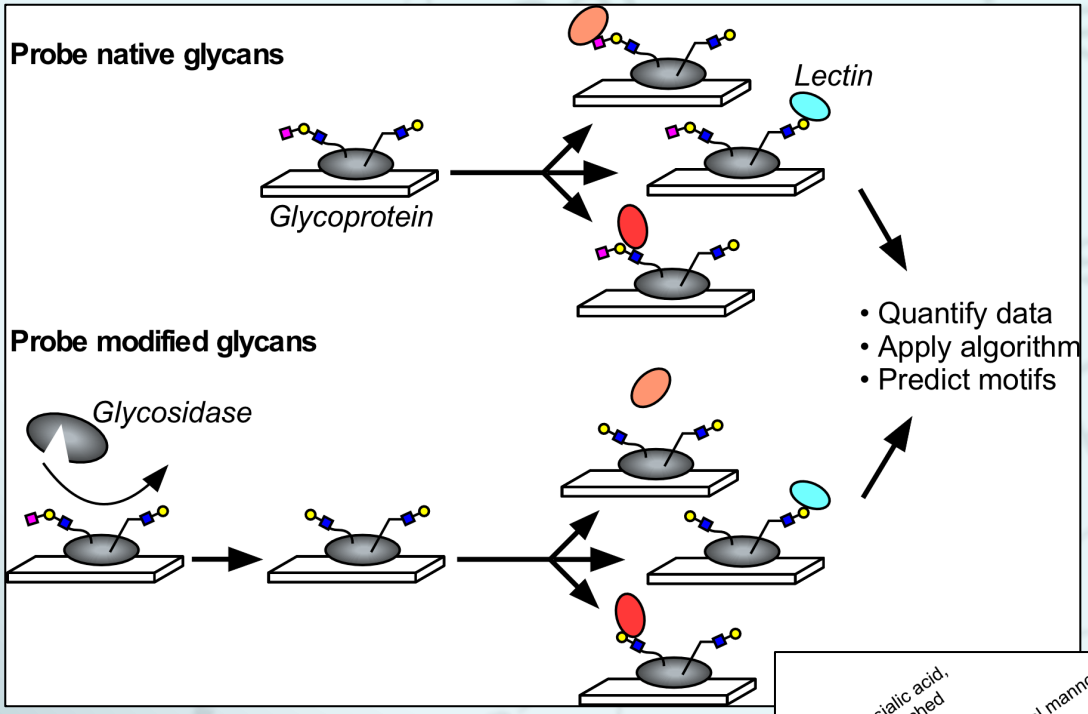
- **Explore novel sialoglycomes**
- **Detect sialic acid variations**
- **Track sialoglycome changes**
- **Predict cellular and molecular functions**
- **Predict genetic basis of sialoglycome changes**

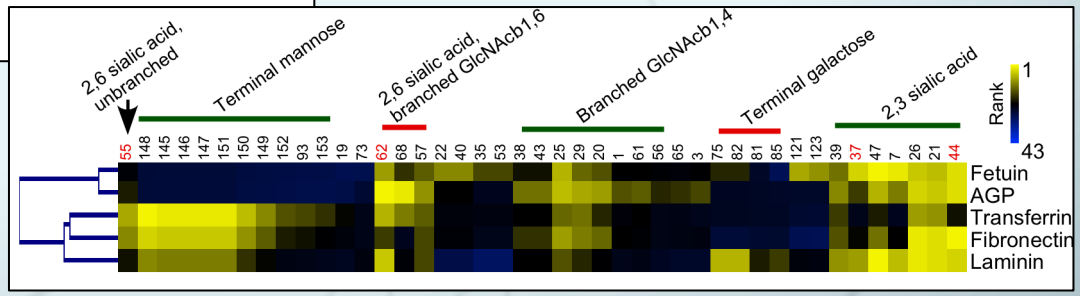
Compare with Traditional Sialic acid-binding Lectins

On-Chip GMAP

Glycan Modification and Probing

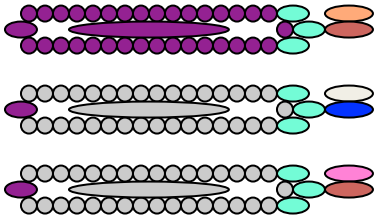
A method for analyzing protein glycosylation using tiny amounts of material





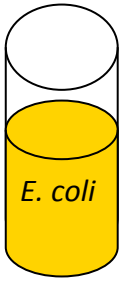
Contact: Brian Haab, PhD; Van Andel Research Institute; brian.haab@vai.org

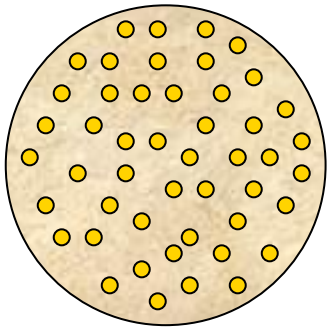
Glycan-specific Phage Displayed scfv Recombinant Antibody Selection and Characterization Approach



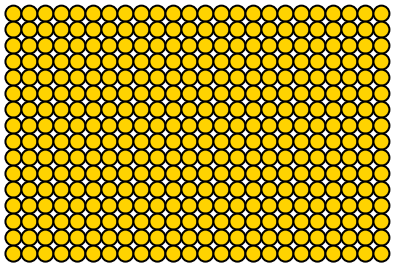
2.9 Billion Phage Displayed scfv

Phage scfv seletion on bitonylated glycan monosaccharide



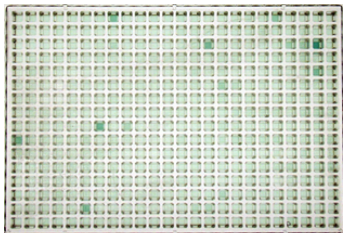


E. coli colonies

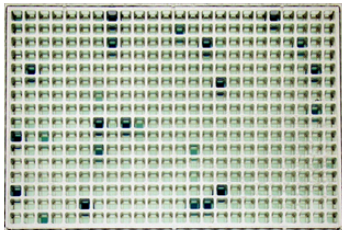


384 *E. coli* expressing scfv

ELISA

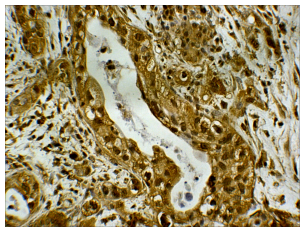


scfv on (–) control

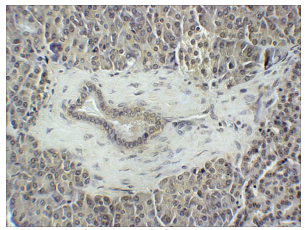


scfv on (+) control

Immunohistochemistry



scfv on (+) control



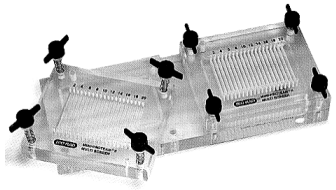
scfv on (-) control

+scfv

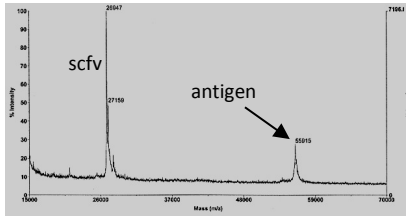
+scfv

+scfv

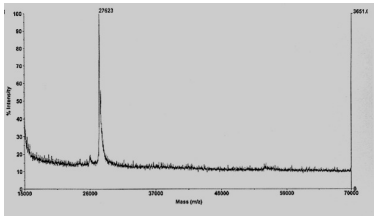
Western blot on cell lysates



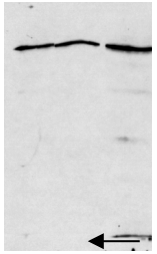
Bio-Rad Multi-Screen Apparatus



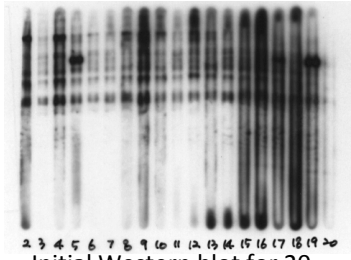
scfv Immuno-precipitation m ass spectrometry (+) control



scfv Immuno-precipitation m ass spectrometry (-) control



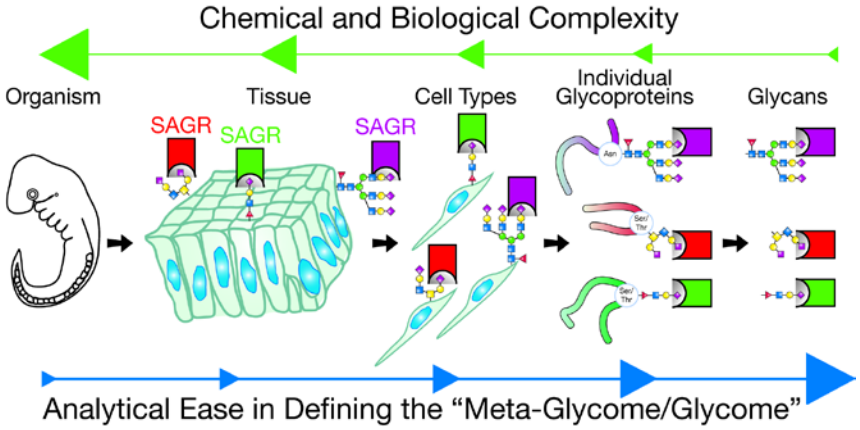
Optimized Western blo t For 3 scfv on cell lysates



Initial Western blot for 20 ELISA (+) scfv on cell lysates

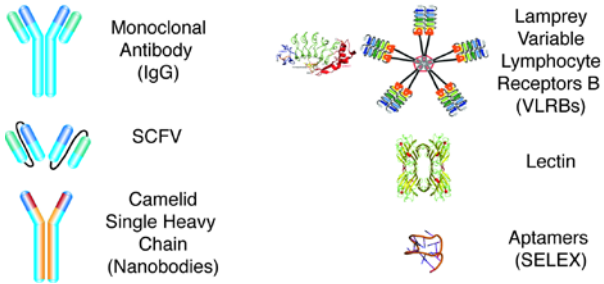
Lejla Pasic and Ray Mernaugh, Vanderbilt University

Smart Anti-Glycan Reagents to Generate the Human Glycome Atlas
(Richard D. Cummings Lab – Harvard Medical School, HMS Center for Glycoscience)



Smart Anti-Glycan Reagent (SAGR) – a recombinant reagent that specifically recognizes a glycan determinant; SAGRs are typically antibody-based

Examples of SAGRs

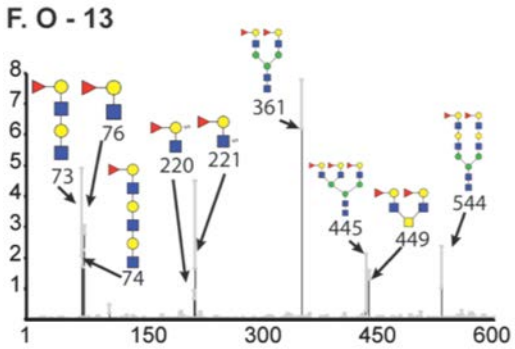


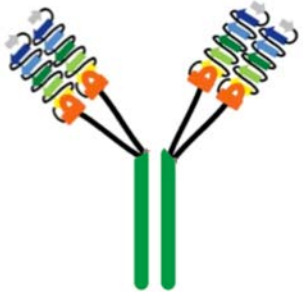
Immunization Strategy

Sea Lamprey Larvae  ~4in long

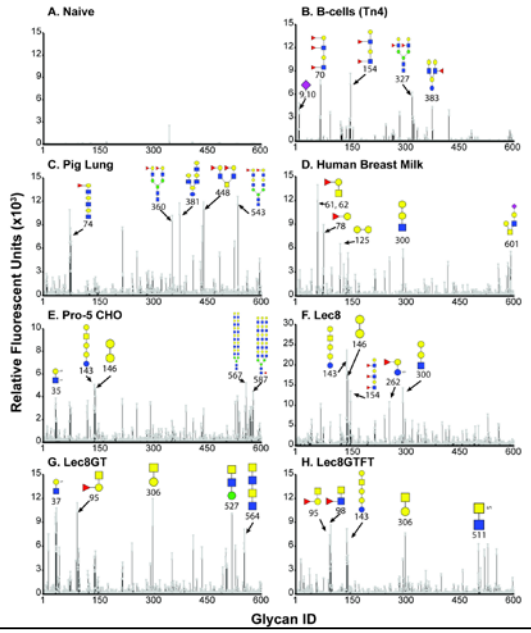
(step 1) Immunize a lamprey larvae; **(step 2)** screen anti-glycan antibodies in sera on glycan microarrays; **(step 3)** generate a yeast surface display (YSD) library; **(step 4)** enrich for yeast expressing desired variable lymphocyte receptors (VLRBs) anti-glycan antibodies; **(step 5)** sequence the genes encoding the VLRBs; **(step 6)** prepare recombinant Ig chimeras of the anti-glycan reagent (VLRB-FC)

Example of a Unique VLRB that was Recovered using MACS, FACS and Microarray Enrichment





Representative immune lamprey sera on the CFG glycan microarray



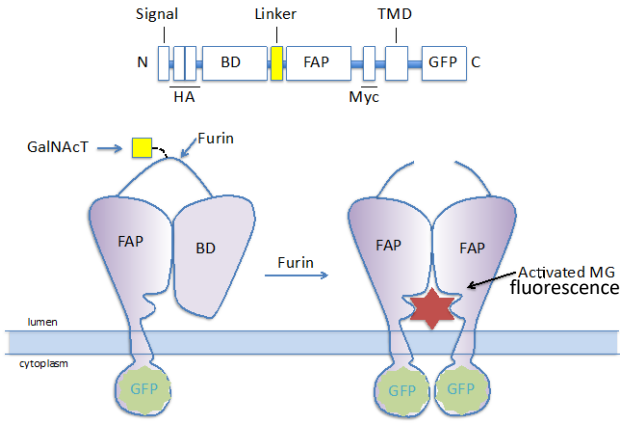
Exciting applications of the technology: (1) Make specific VLRBs to glycan antigens; (2) use VLRBs to map glycan expression; (3) use VLRBs to block glycan interactions important in biology; (4) replace murine mAbs with VLRBs

Supported by: NIH/NCI U01CA199882

In vivo Activity Detection of GalNAc Transferase Isozymes by Protein-Based Fluorescence Sensors

Adam D. Linstedt, Carnegie Mellon University, linstedt@cmu.edu

Sensor Design

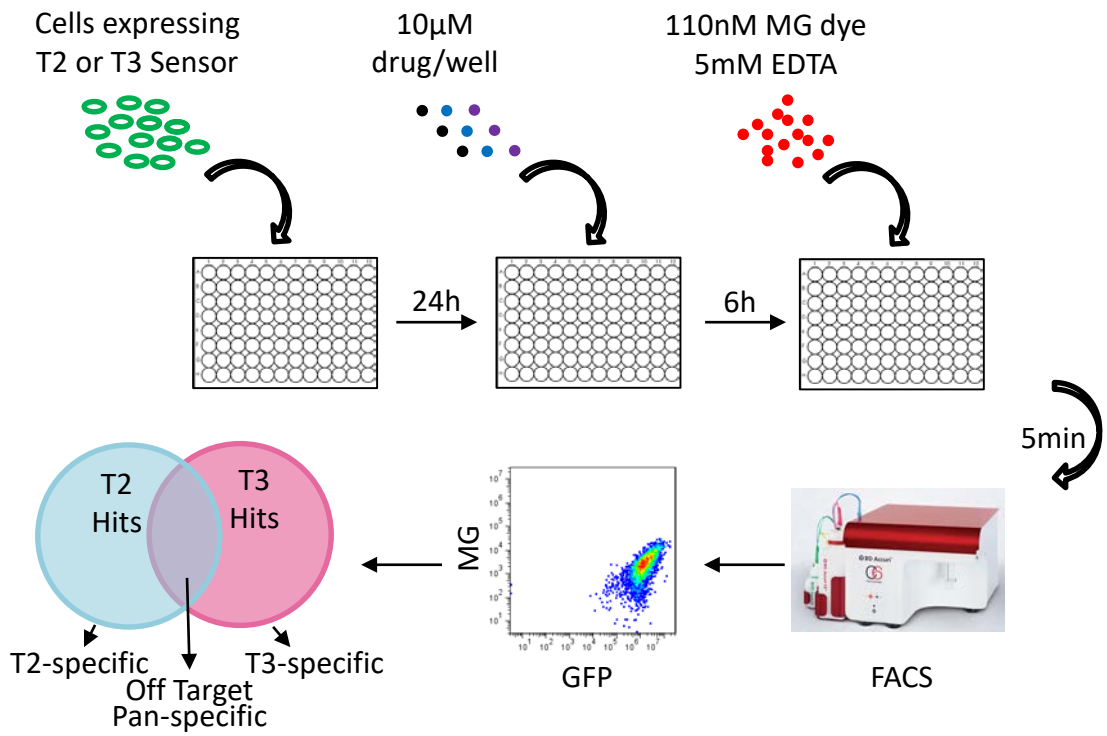


Example Linker: GalNAc-T3 acceptor site

-HFNTPIPRRHTRSAEDDG-

Furin cleavage site

Example use: Screen for drugs against O-glycosylation



Other Uses

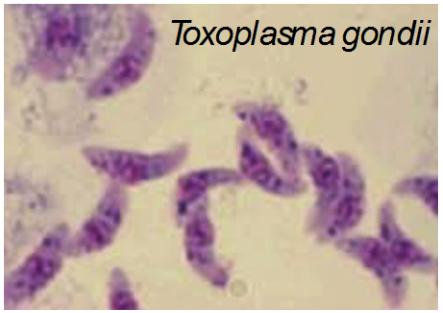
*Assay O-glycosylation isozyme activity *in situ*
*Define isozyme-specific consensus sequences
*Discover novel glycan masking sites
*Identify regulators of O-glycosylation

Ref: Song L, Bachert C, Linstedt AD (2016). Methods Mol. Biol. 1496:123-131. PMID: 27632006

Chris West,
Rick Tarleton &
Lance Wells,
Univ. of Georgia

Tools to Test Functional Interactions with Glycosylation

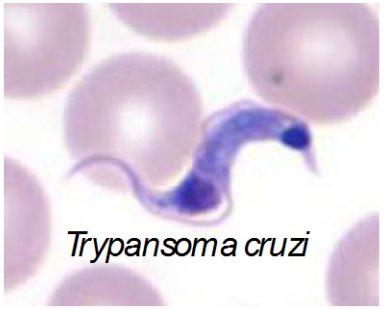
1* *Invasion* *Egress* *Motility* *Dormancy* *Virulence* *Persistence* *Di erentiation* *Proliferation* *Glycoconjugate*
Favorite Parasite Property or Function



2 Is Glycosylation Involved?

3 Predict Glycan Type

3′- See Parasite Glycan Tables



4 Select Relevant Glycogene(s)

4′- See Glycogene Table Resource

5 Retrieve Validated CRISPR guide-DNA

5′- See Resource of guide DNA sequences, guide DNA plasmids, select disrupted strains

6 Edit Glycogene

Cas9

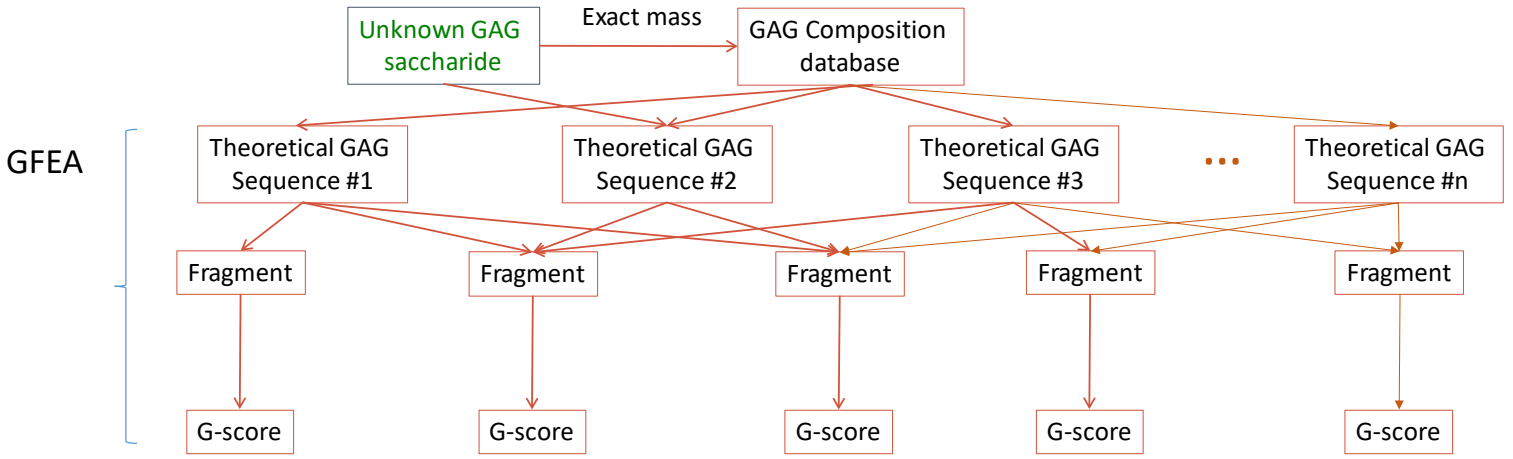
6′- Con rm Glycosylation E ect: PCR and/or Glycomics; See Resource of Glycome Pro les

* Follow the green arrows to ask if glycosylation is involved. The orange detours guide you to resources to assist you along the way.

Algorithms for interpretation of electron activated dissociation (ExD) tandem mass spectra of glycosaminoglycans (GAGs)

Contact
Joe Zaia (jzaia@bu.edu), Dept. of Biochemistry, Center for Biomedical Mass Spectrometry, Boston University

- GAGfinder: assigns elemental formulas/compositions to product ions in GAG ExD tandem mass spectra
- GAGfragDB: a database of GAG product ions
- GFEA: GAG Fragment Enrichment Analysis for scoring GAG tandem mass spectra



We maintain a set of heparan sulfate saccharide standards available for distribution:
http://www.bumc.bu.edu/pgsl/gag-synthetic-saccharides-available/