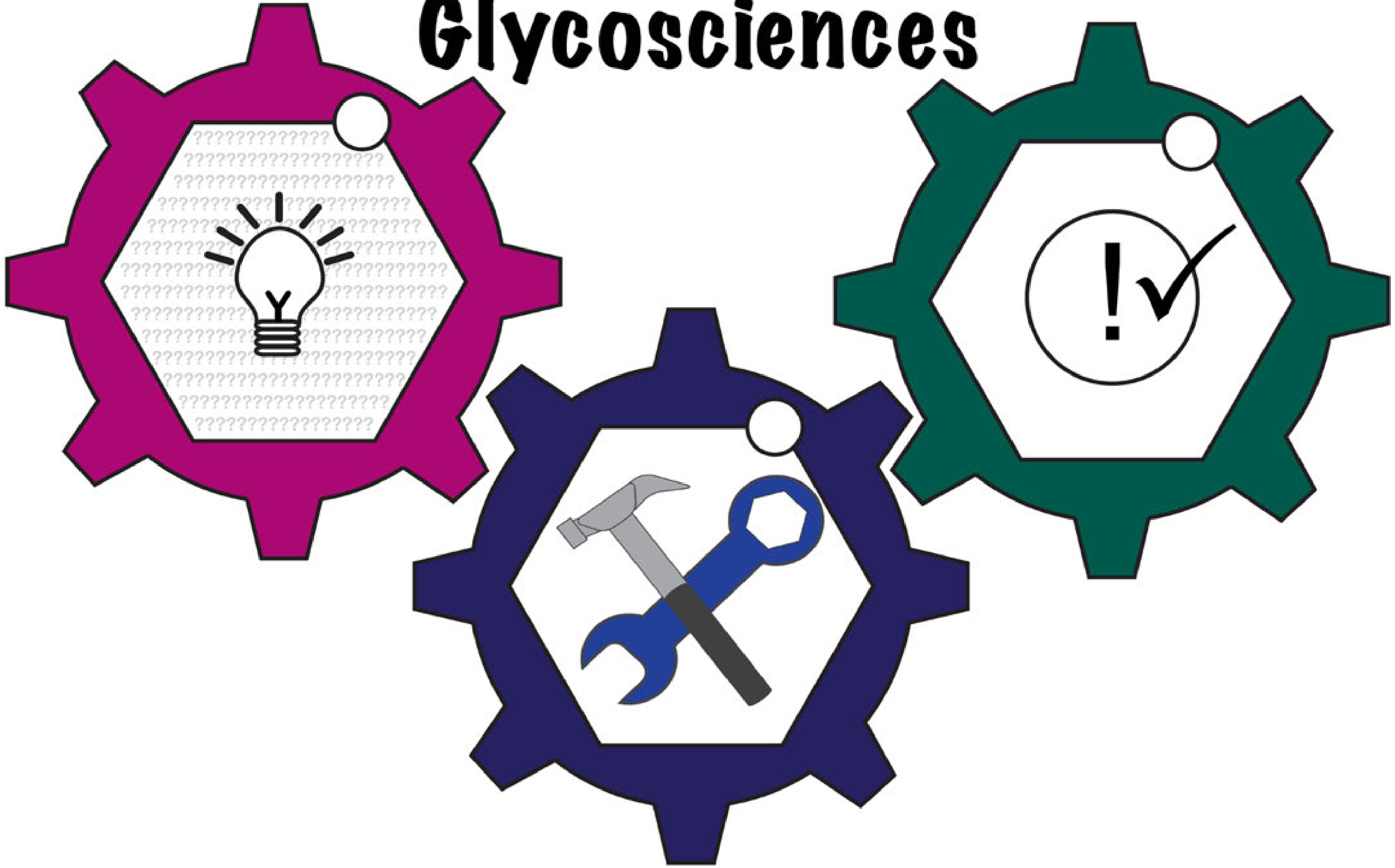
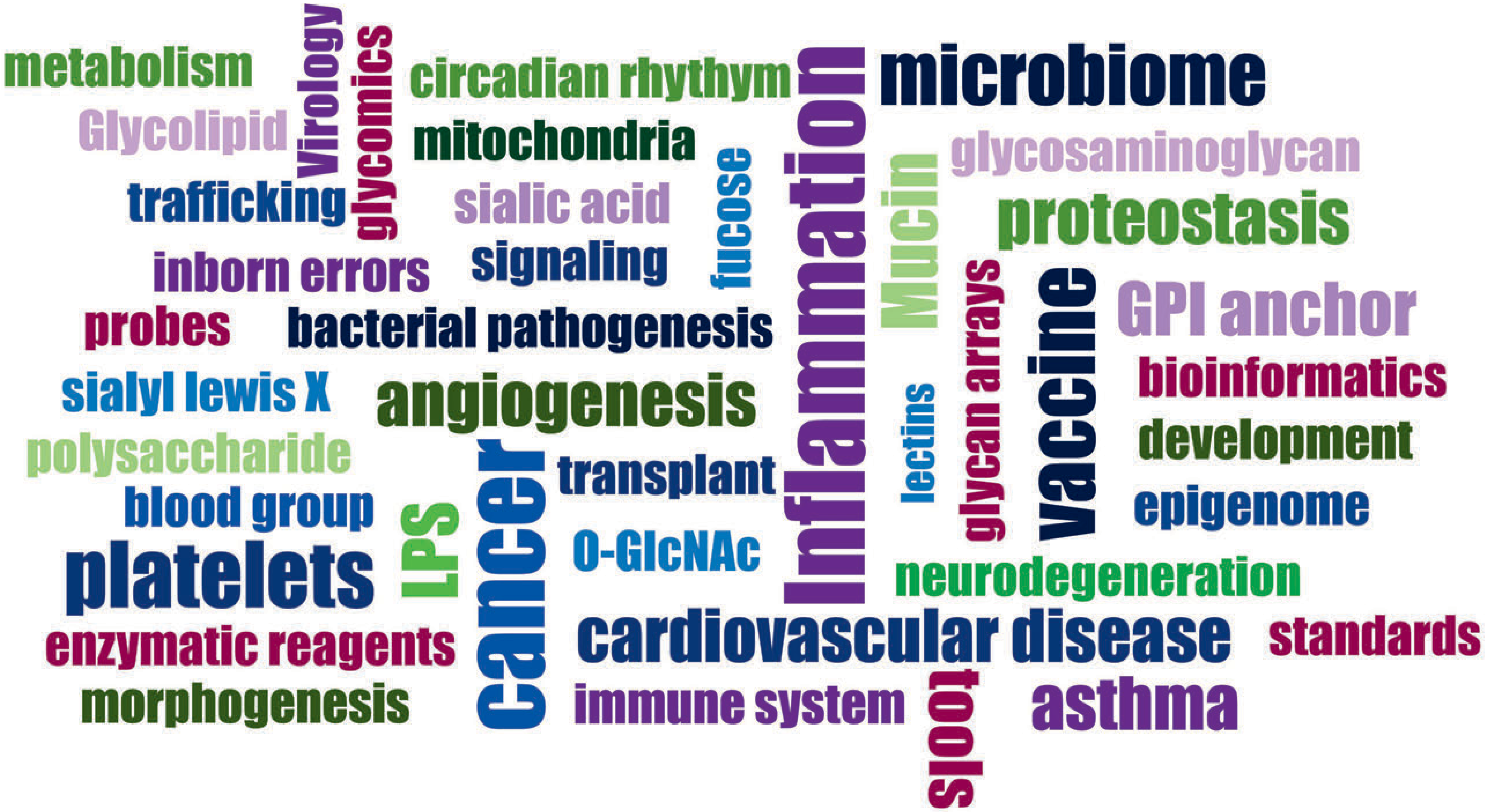
Emerging Technoloiges in the Glycosciences



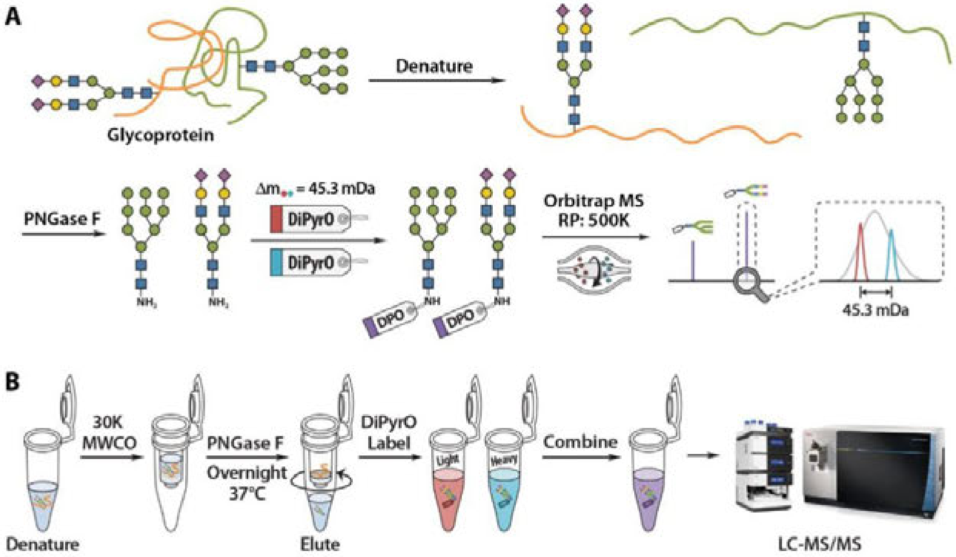
Glycans impact many fields



Mass-Spec approaches for characterizing and quantifying glycans

Multiplex Chemical Tags For High-Throughput Glycan and Glycopeptide Quantitation and Characterization

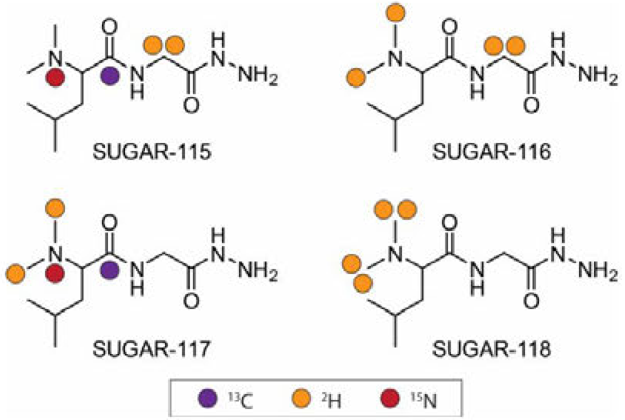
Workflow for the relative quantification of MS1-level DiPyrO-labeled N-glycans



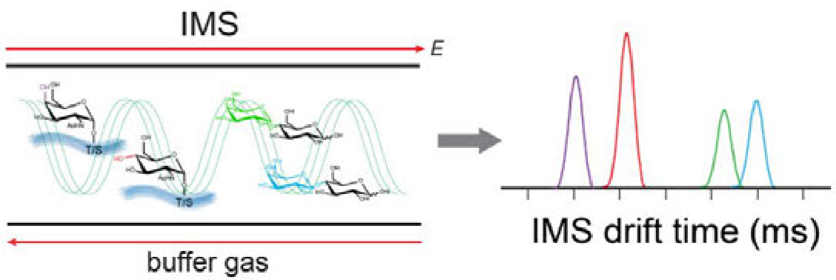
Patent pending

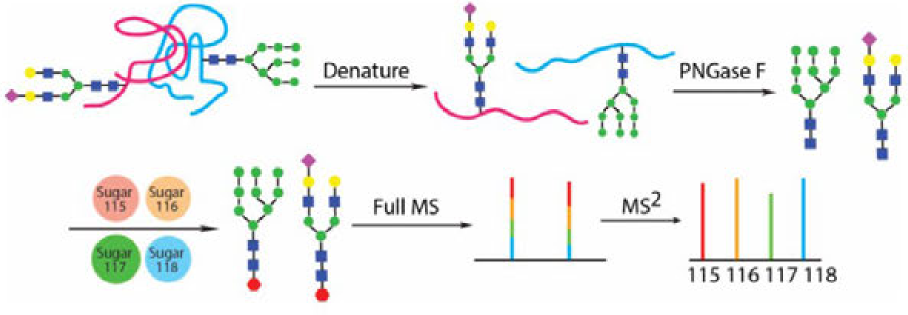
Contact: Lingjun Li
University of Wisconsin-Madison
Email: lingjun.li@wisc.edu
www.lilabs.org

Isobaric multiplex reagents for carbonyl containing compounds (SUGAR) tags for high-throughput MS2-level glycan quantitation



Ion mobility mass spectrometry for enhanced separation of glycan isomers





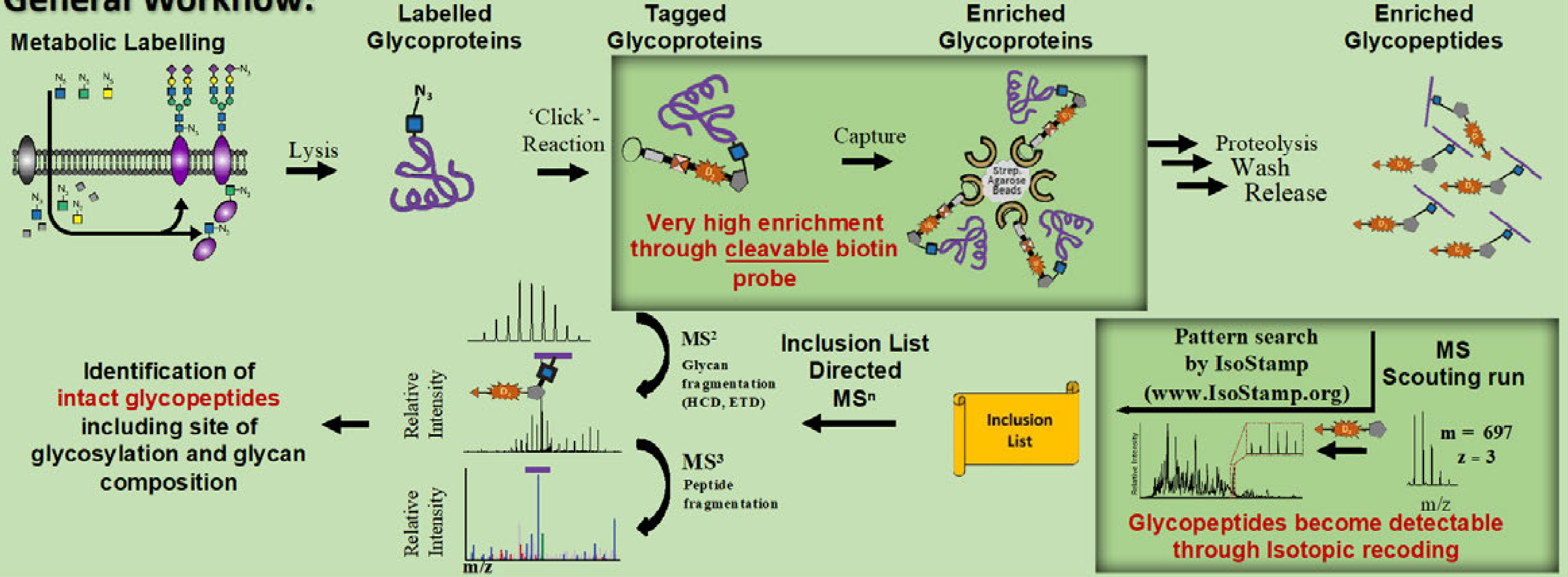
Mass-Spec approaches for characterizing and quantifying glycans

IsoTaG based Glycoproteomics

Making Glycoproteomics via Mass Spectrometry More Accessible to the Greater Scientific Community

Contact: Bertozzi Lab: Carolyn R. Bertozzi, Marc D. Driessen
Pitteri Lab: Sharon J. Pitteri, Abel Bermudez
isotag_glyco@stanford.edu & www.IsoStamp.org

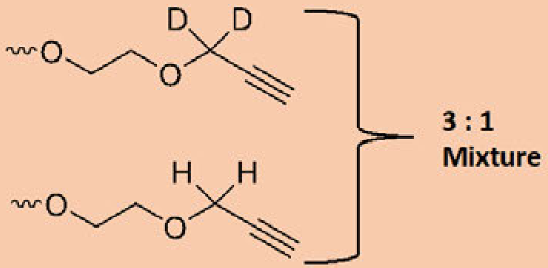
General Workflow:



Generation 2 "Flagship" probe:

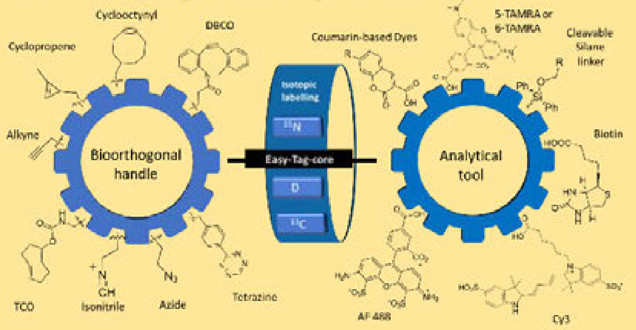
Alkyne based D_2-Probe

(Available for dissemination and testing by interested Labs)



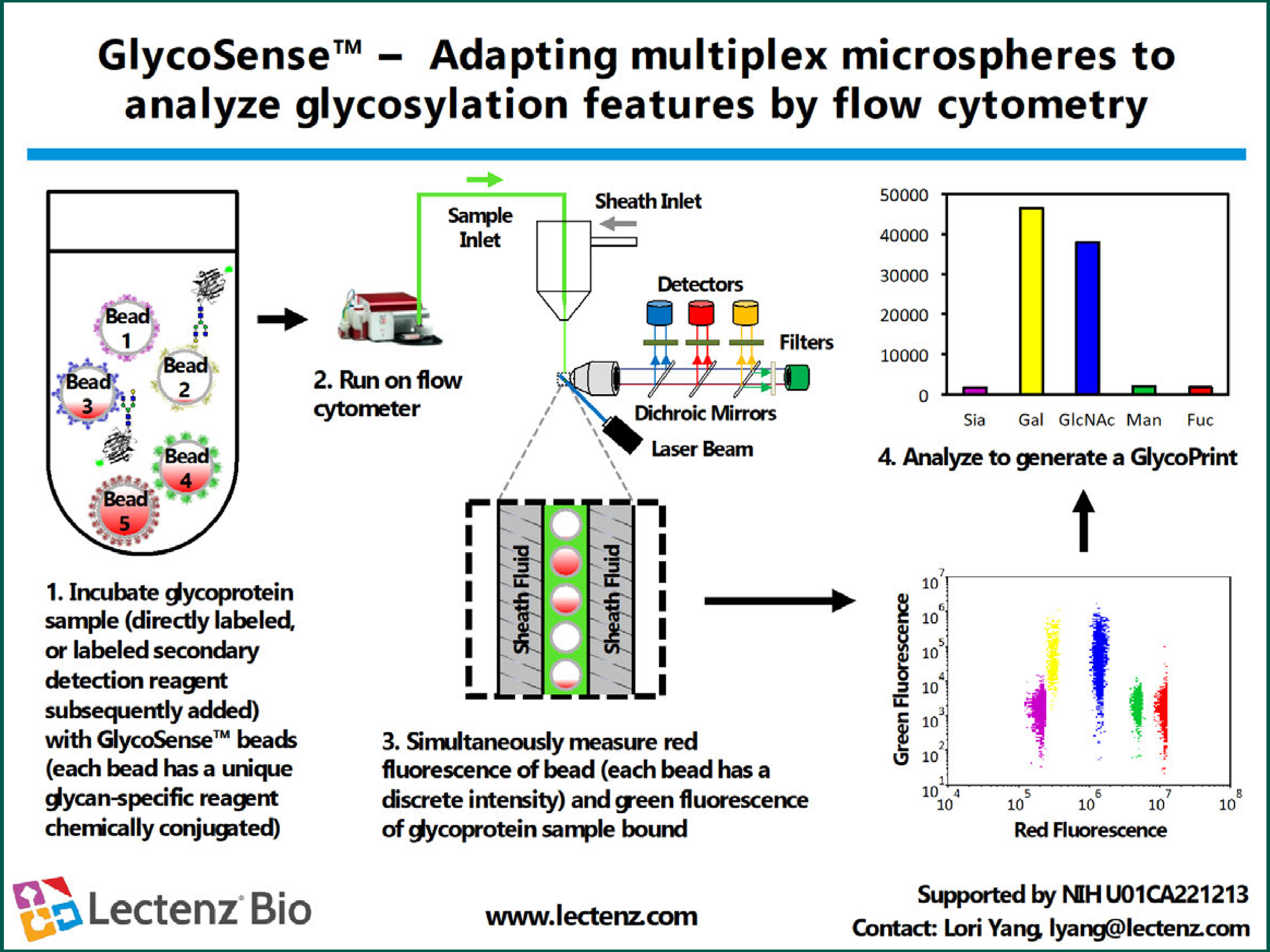
Next-Generation "Easy-TaG" probe:

- -fully modular
- -max functionality
- -var. applications
- -multiplexing
- -easy synthesis



Patent pending

Profiling glycans in complex mixtures



Profiling glycans in complex mixtures

Luminex Multiplex Glycan Array (LMGA): A high throughput and high content platform for glycan binding proteins

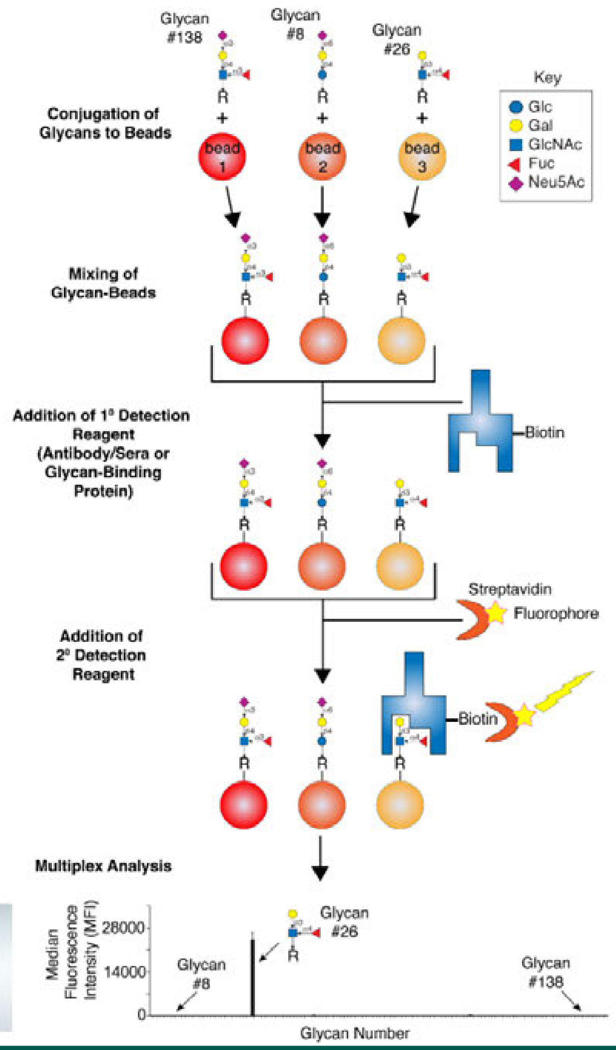
Jin-Xiong She, PhD & Sharad Purohit, PhD

Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta University, Augusta, GA

Jshe@augusta.edu

Capable of analyzing 500 glycans and 384 samples within one day:

- Validated using CLIA standard
- Highly reproducible
- Highly sensitive
- High throughput
- High content
- Greta for biomarker discovery



nature COMMUNICATIONS

1R21 CA199868
5UO1 CA221242

Profiling glycans in complex mixtures

Next Generation Glycan Microarray Enabled by Next Generation Sequencing

Maomao Yan, Yi Lasanajak, David F. Smith, Xuezheng Song

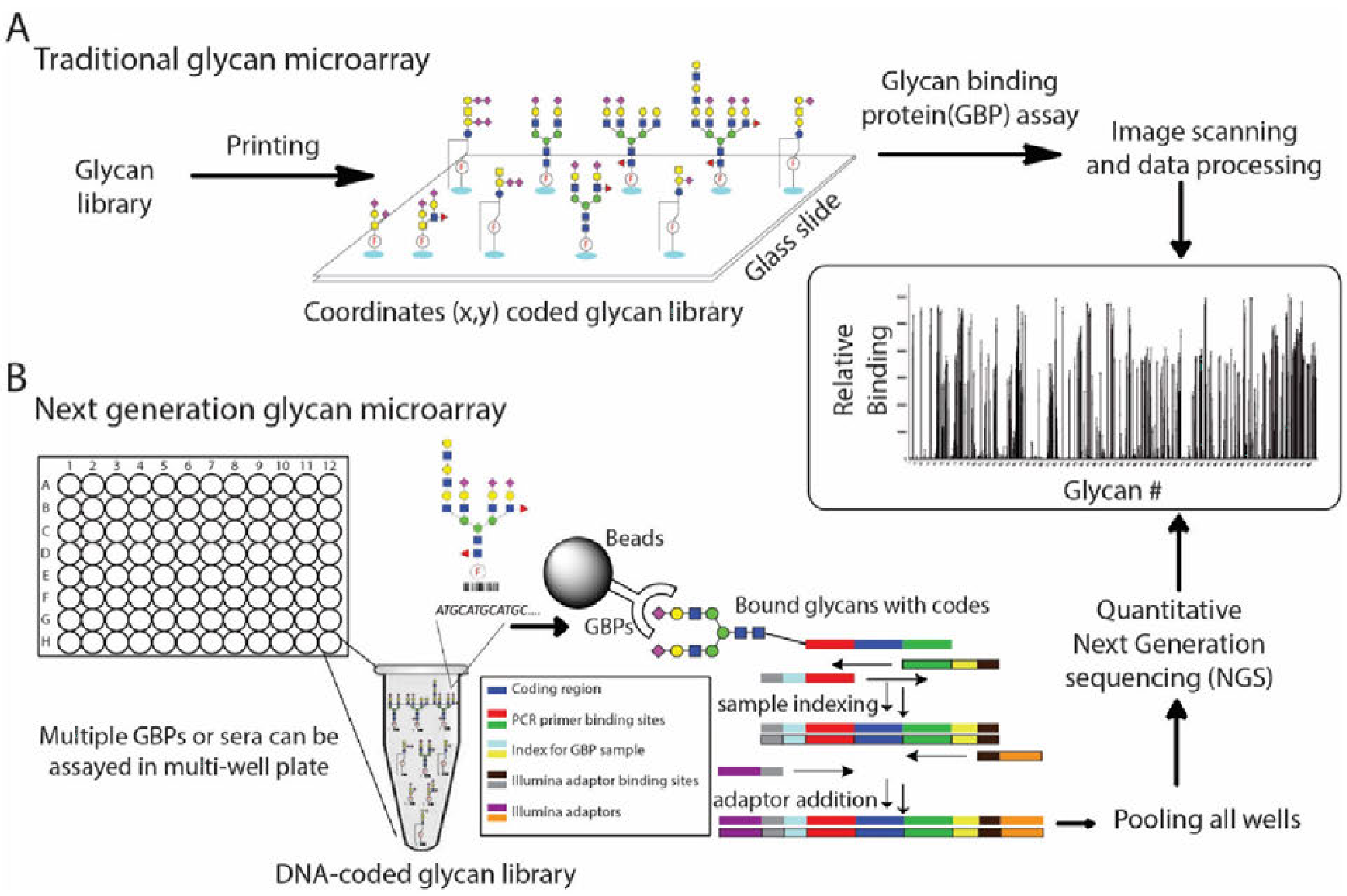
Emory Comprehensive Glycomics Core, Emory University School of Medicine, Atlanta, GA 30322, USA

Limitations of the Traditional Glycan Microarray include:

1. Requires specialized equipment
2. Glycan Capacity limited
3. Signal is relative fluorescence
4. Image processing required
5. Limited dynamic range
6. Not high throughput
7. Cannot analyze intact cells

Next Generation Glycan Microarray:

1. No specialized equipment
2. Unlimited glycan capacity
3. Signal is copy number from NGS
4. Eliminates image processing
5. Dynamic range expanded
6. High throughput format
7. Compatible with intact cells



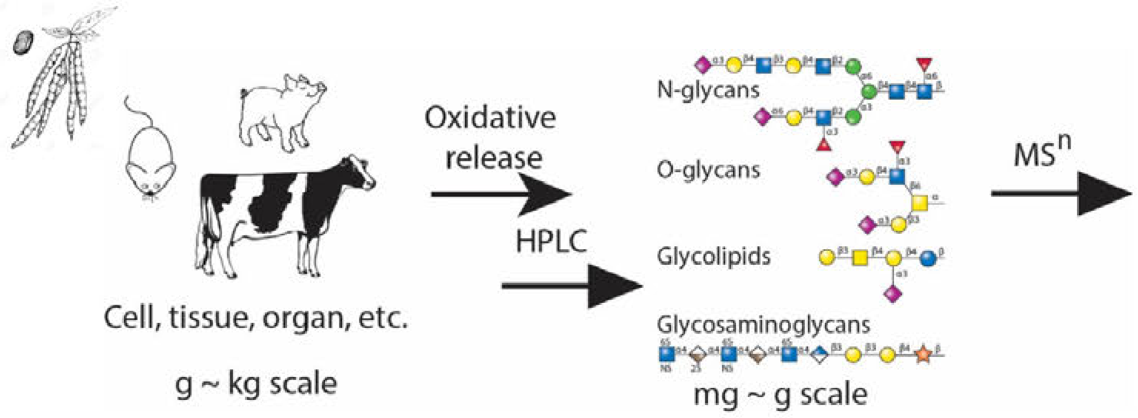
Supported by Common Fund Glycoscience 1R21GM122632

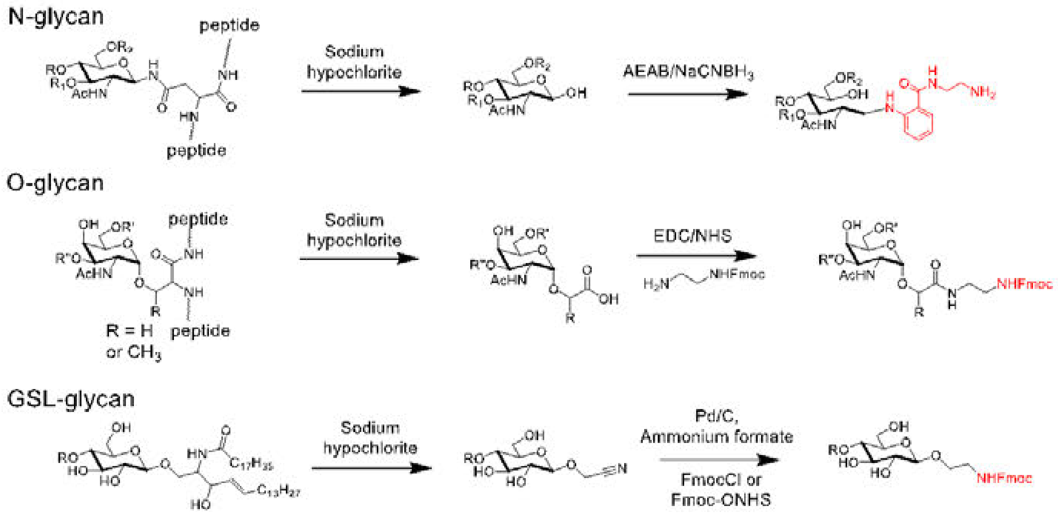
Novel Techniques for Releasing Glycans

Large Scale N-glycan Preparation from Soy Proteins by Oxidative Release of Natural Glycans (ORNG)

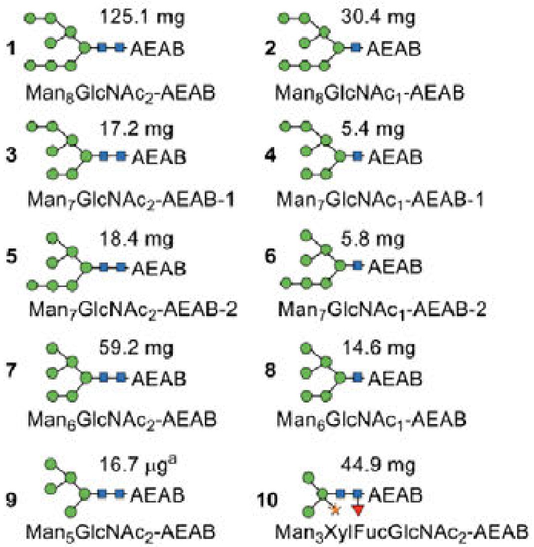
Yuyang Zhu, Yi Lasanajak, David F. Smith, and Xuezheng Song

Emory Comprehensive Glycomics Core, Emory University School of Medicine, Atlanta, GA 30322, USA





High Mannose N-Glycans >95% pure



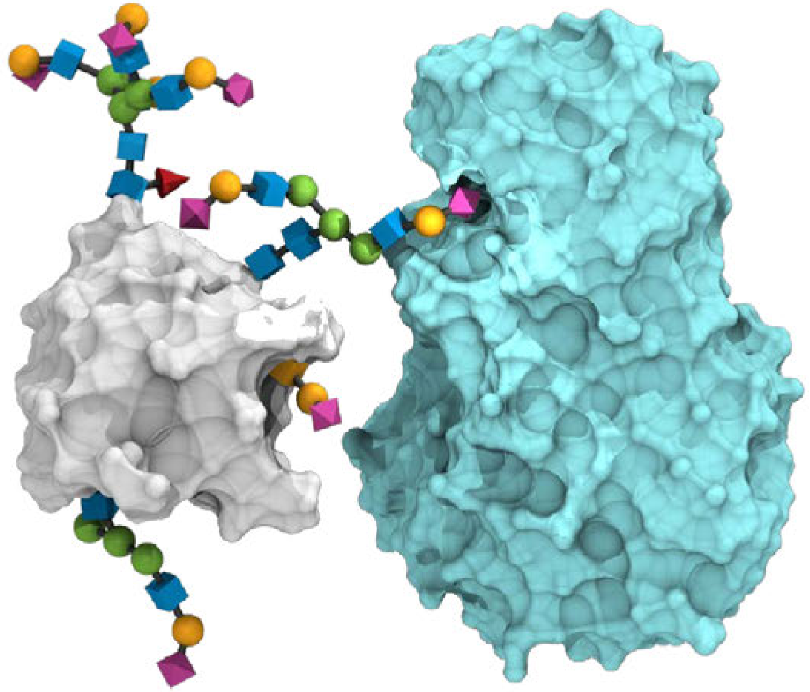
Available from NatGlycan, LLC
http://natglycan.com

Supported by Common Fund Glycoscience U01GM116254, and STTR grant R41GM122139.

Glycan binding proteins for detecting and enriching glycans

SiaFind Lectenz® – Reagents for the detection and enrichment of sialylated glycoproteins

- Novel reagents specific for sialic acid
  - Pan-Specific
  - α2,3-Specific
- Engineered from a neuraminidase
- Recombinantly produced
- Monomeric
- Offering
  - Native
  - Biotinylated
  - Affinity Column



Lectenz Bio

www.lectenz.com/shop

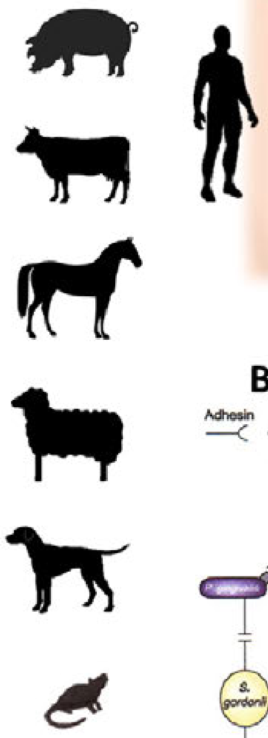
Supported by NIH R44GM113351, R44OD024964

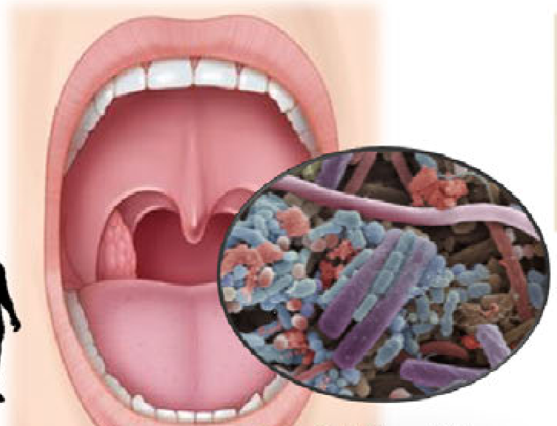
Contact: Lori Yang, lyang@lectenz.com

Glycan binding proteins for detecting and enriching glycans

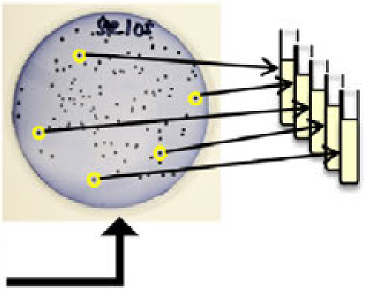
Harnessing the Oral Microbiome to Create Novel Glycan-Binding Molecules

PIs: **Stefan Ruhl**, University at Buffalo & **Paul Sullam**, UCSF

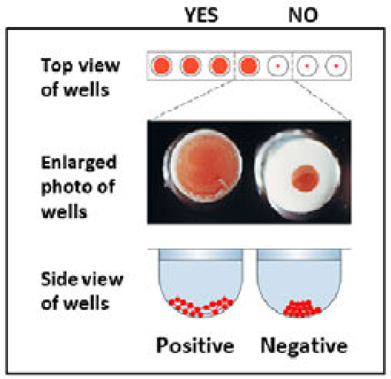




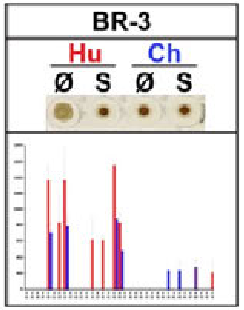
Oral Microbiome



Bacteria binding

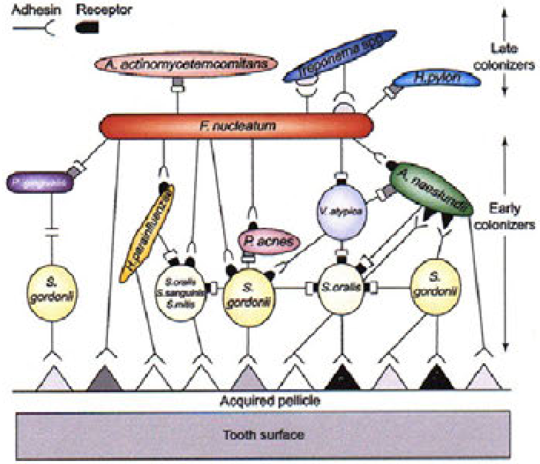


2004 Pearson Education, Inc., Benjamin Cummings



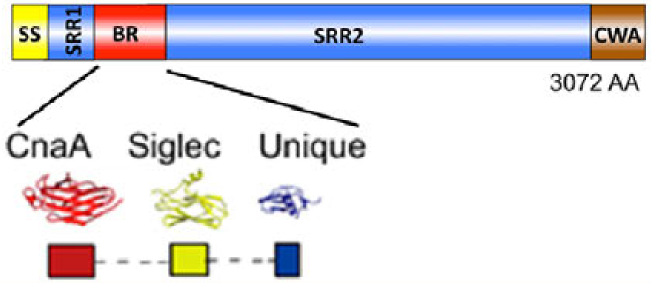
Determination of Binding on Sialoglycan Array

Bacterial Lectin Adhesins



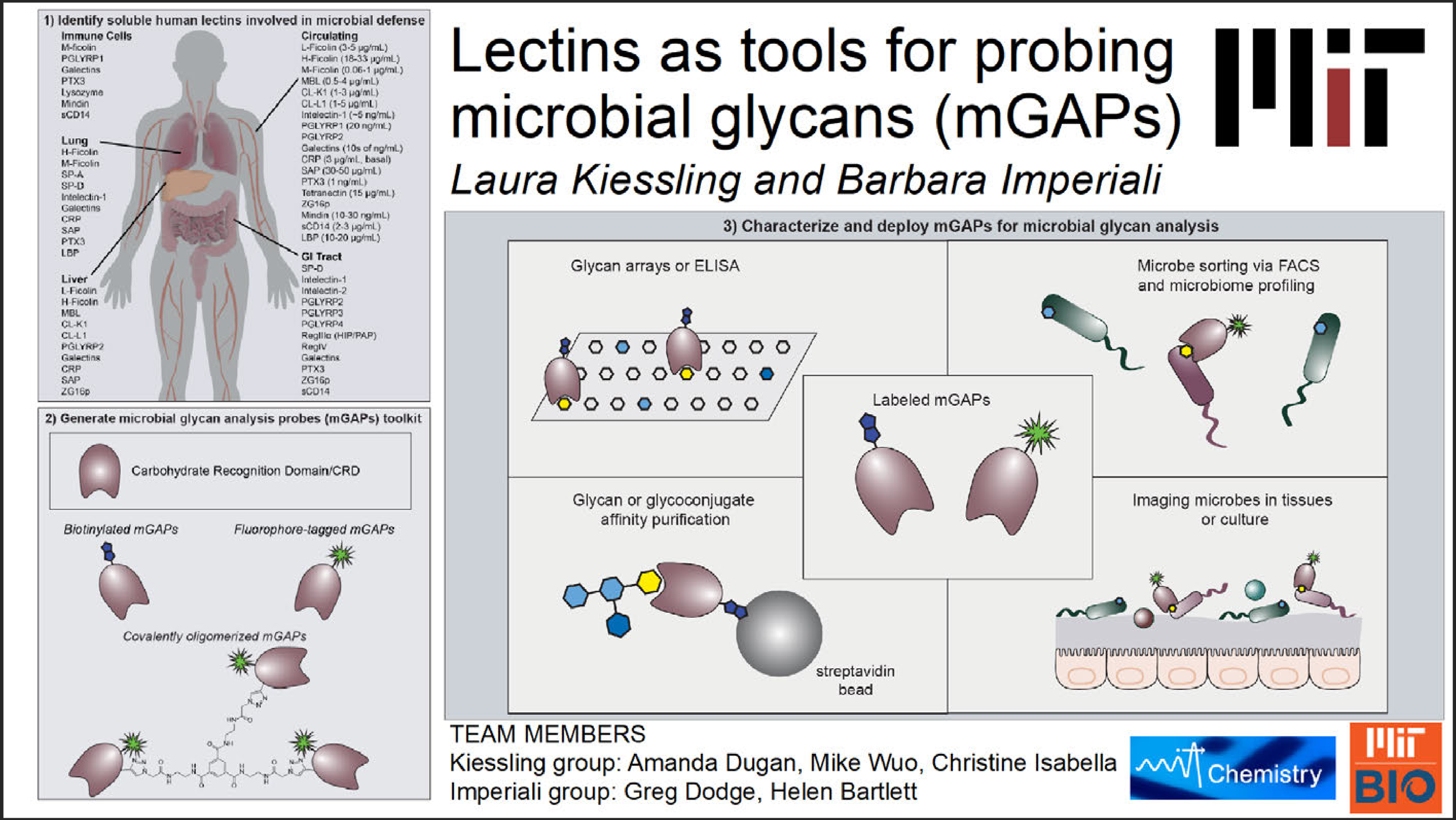
Kolenbrander *et al.*, 2002

Expression of Lectin / Siglec Domains



→ Use tagged recombinant lectin domains as tools to probe biological samples and tissues

Glycan binding proteins for detecting and enriching glycans



Detecting changes in the biosynthesis of glycans

Fluorescent biosensors for nucleotide sugars

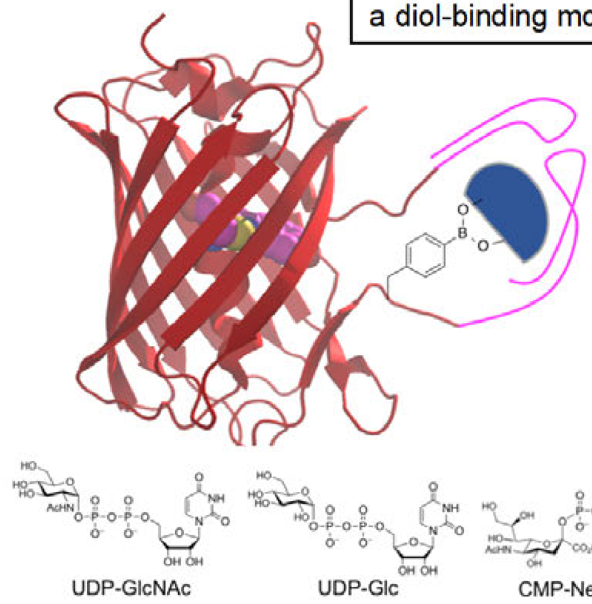
Boronic acid-containing peptide aptamer-based fluorescent proteins (BapaFPs) to detect nucleotide sugar dynamics in live cells

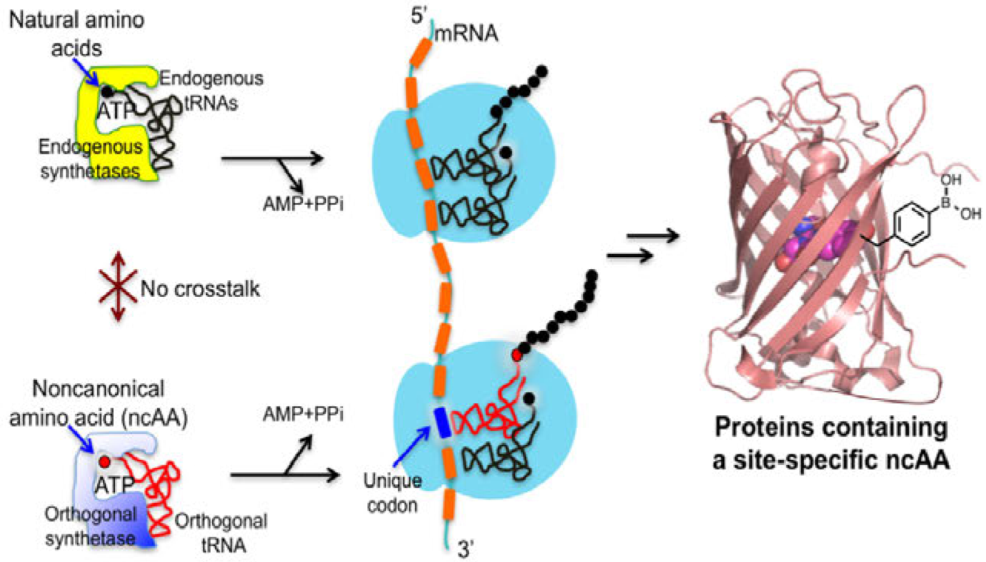
Contact: Prof. Huiwang Ai, University of Virginia
huiwang.ai@virginia.edu
https://med.virginia.edu/ai-lab/

The Ai Lab
Engineer Proteins to Image Bioactivity

General concept:

"Boronic acid as a diol-binding motif"

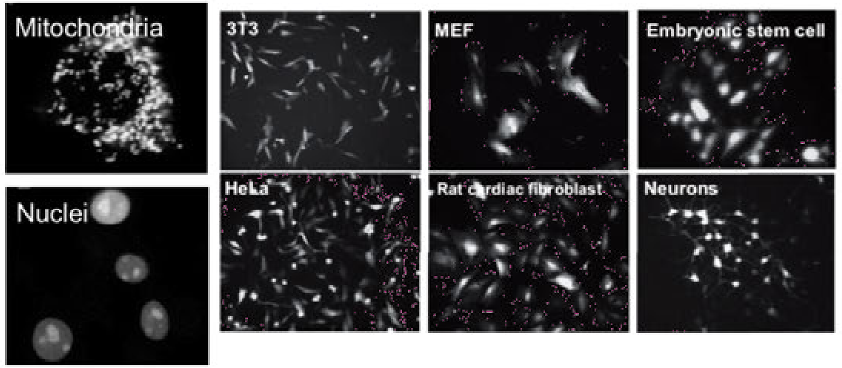




Genetic code expansion in living cells

A baculoviral system to deliver the BapaFP sensor into mammalian cells

Chatterjee, Ai, Schultz, et al., *PNAS*, 2013, 110: 11803-11808.

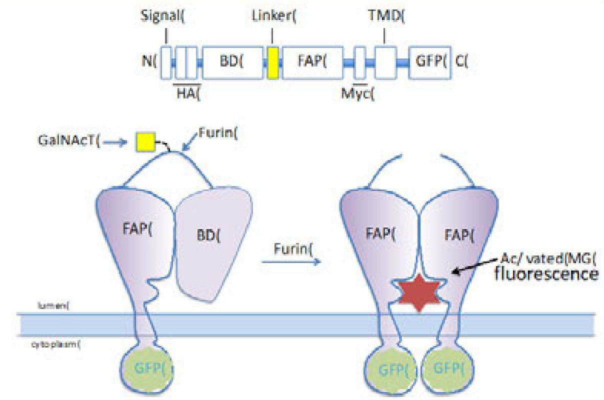


Detecting changes in the biosynthesis of glycans

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

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

Sensor Design

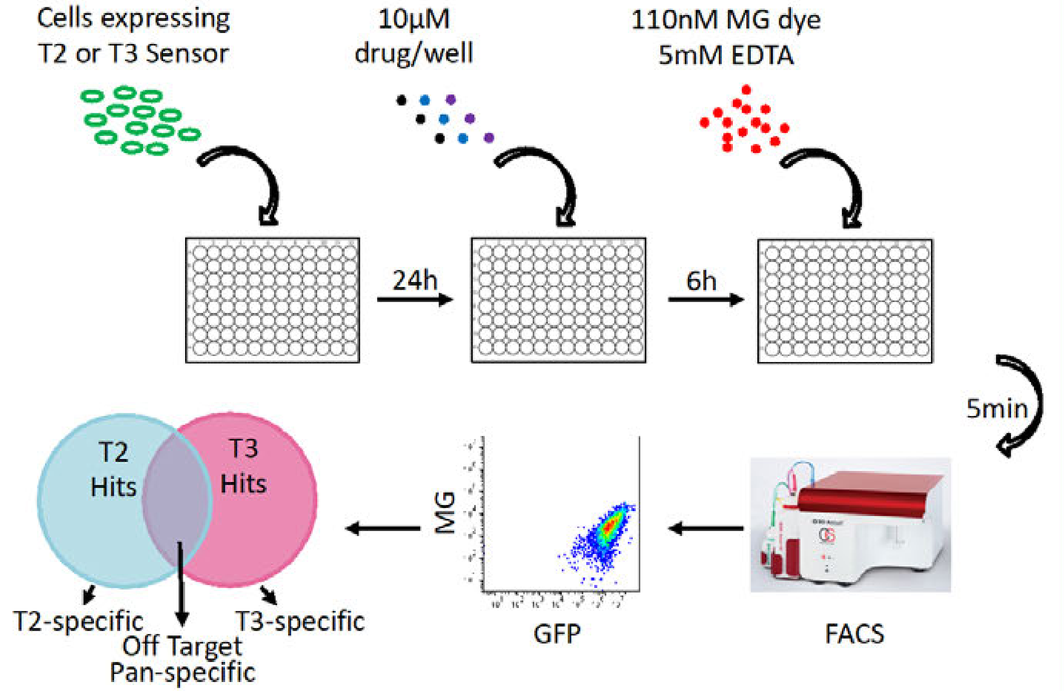


Example Linker: GalNAc-T3 acceptor site

-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

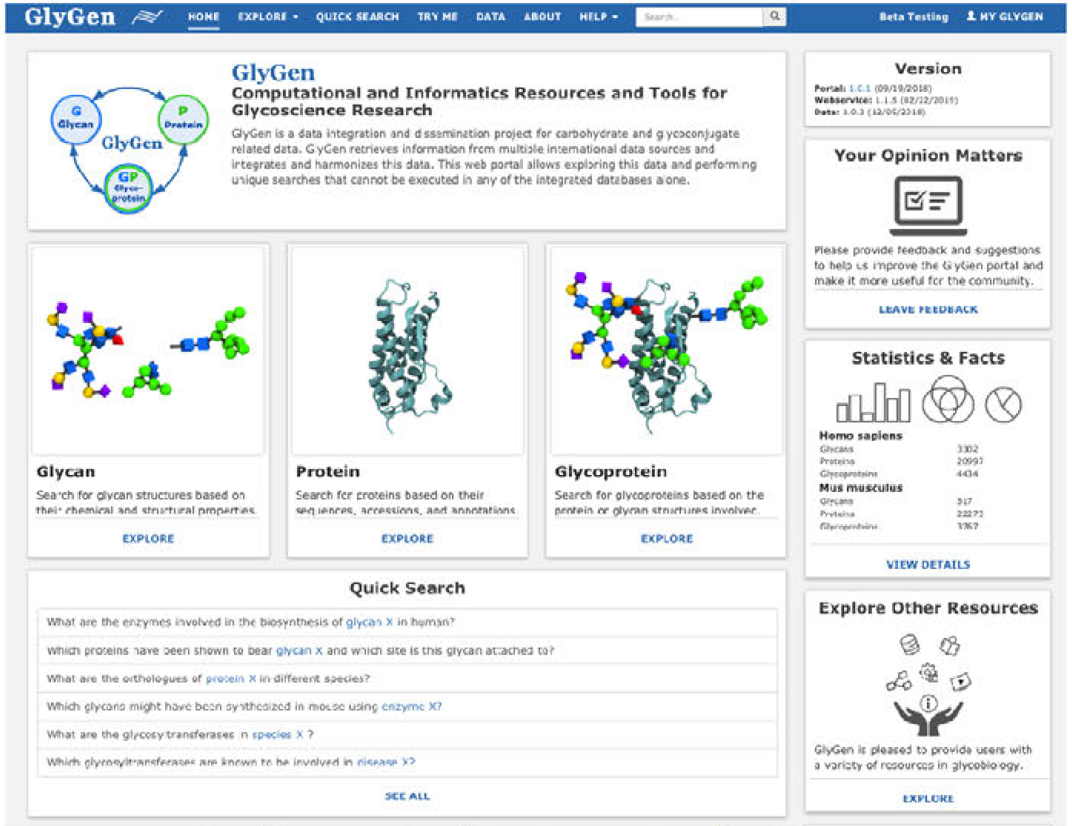
Bioinformatics Tools for Studying GLycans

GlyGen.org

Computational and informatics Resources for Glycoscience

GlyGen Portal

- User friendly interfaces
- Searches by glycan, protein, and glycoprotein
- Quick search to answer user specific questions.
- Online library of resources
- Helpful tutorials
- Use of CC BY 4.0 and GNU GPU v3.0 public licenses



http://glygen.org, http://data.glygen.org, http://api.glygen.org

GlyGen Data Collection

- Data collection from multiple international resources
- Data integration with intensive data quality control
- Metadata for the integrated dataset is made available in the readme using BioCompute Object schema
- Data can be accessed via data page, APIs and RDF tripestore (coming soon)

Raja Mazumder mazumder@gwu.edu *The George Washington University, Washington, DC.*
Will York will@ccrc.uga.edu *Complex Carbohydrate Research Center, University of Georgia, Athens, GA.*

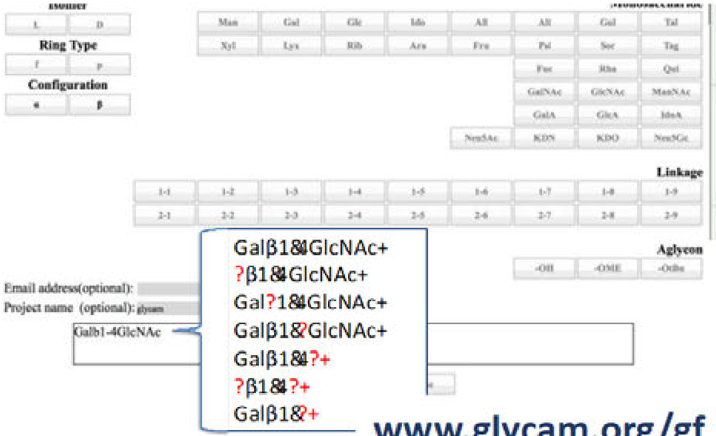
NIH Grant - U01 GM125267-01

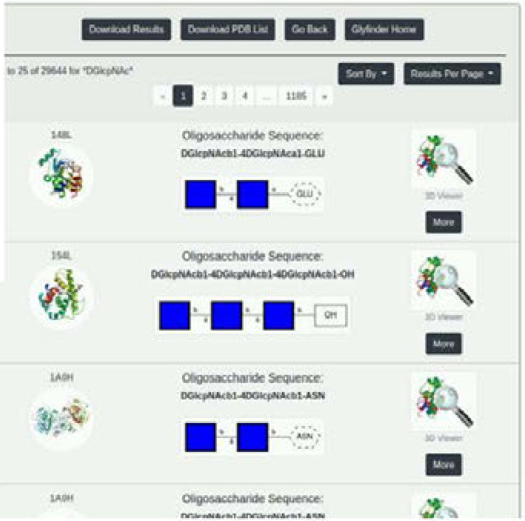
Bioinformatics Tools for Studying GLycans

Detecting and Curating Oligosaccharides in the PDB: GlyFinder and GlyProbity (Woods Group, UGA and ww-PDB)

1) Create a Search and Retrieval Tool "GlyFinder"

- As of July 2018, there were **141,616** protein structures in the PDB
- it has been impossible to accurately say how many protein structures contain carbohydrates!





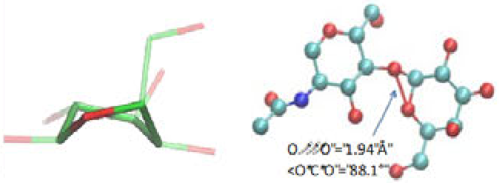
www.glycam.org/gf

N-Linked Glycans

- PDBs with N-linked glycans: 5,238 or 3.7% of all PDB entries
- N-linked glycans detected: 25,923 or 25.7% of the sugars detected
- On average, there are approximately 5 N-linked glycans per glycoprotein.

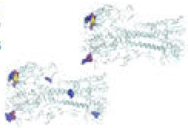
2) Create a Tool "GlyProbity" to Objectively Assess the Quality of Carbohydrate Structures.

- The format, in terms of interface design, and content, will reflect that of MolProbity



GlyProbity Summary output for PDB ID 1RVZ

Image of 1RVZ, highlighting the glycans



Click on any field in the table below for detailed output.

Topology		Geometry Outliers:	
Monosaccharides Detected	21	Glycosidic Phi angles	0
Residue Distribution		Glycosidic Psi angles	0
Monosaccharides	3	Glycosidic Omega angles	0
Oligosaccharides	6	Ring pucker	0
Carbohydrate Context		Unclear linkage definition	3
Covalently linked to protein	-	Bond lengths	0
Non-covalent complex	6	Bond angles	3
Monosaccharide average B-factor (± Stdev)	69 ± 22	Other exocyclic torsion angles	3
		Distorted amide group	3
Possible missing atoms	6	Uncertain anomeric configuration	6
Possible extra atoms	3	Non-bonded contact	0
Misnamed residues	0	Total Errors	27

Bioinformatics Tools for Studying GLycans

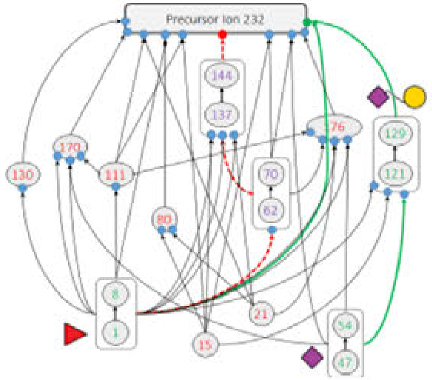
Glycoscience Software Products from U01CA221234 and R21HL131554

Assignment of glycomics and glycoproteomics LC/MS data

GlycReSoft

www.bumc.bu.edu/glycresoft

- LC-MS preprocessing
- Glycomics profiling
- Glycoproteomics (HCD only)



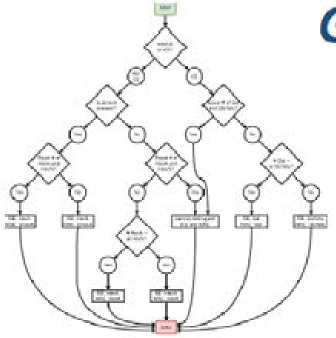
GlycoDeNovo

www.bumc.bu.edu/msr/software

Identification of glycans from ExD tandem mass spectra

- Topology reconstruction
- Candidate ranking by supporting peak counts
- IonClassifier candidate ranking

GAGFinder



Glycosaminoglycan ExD tandem MS assignment software.

- Returns a list of peaks with annotations for a GAG composition given precursor m/z and charge.

www.bumc.bu.edu/msr/software

Chemical Probes

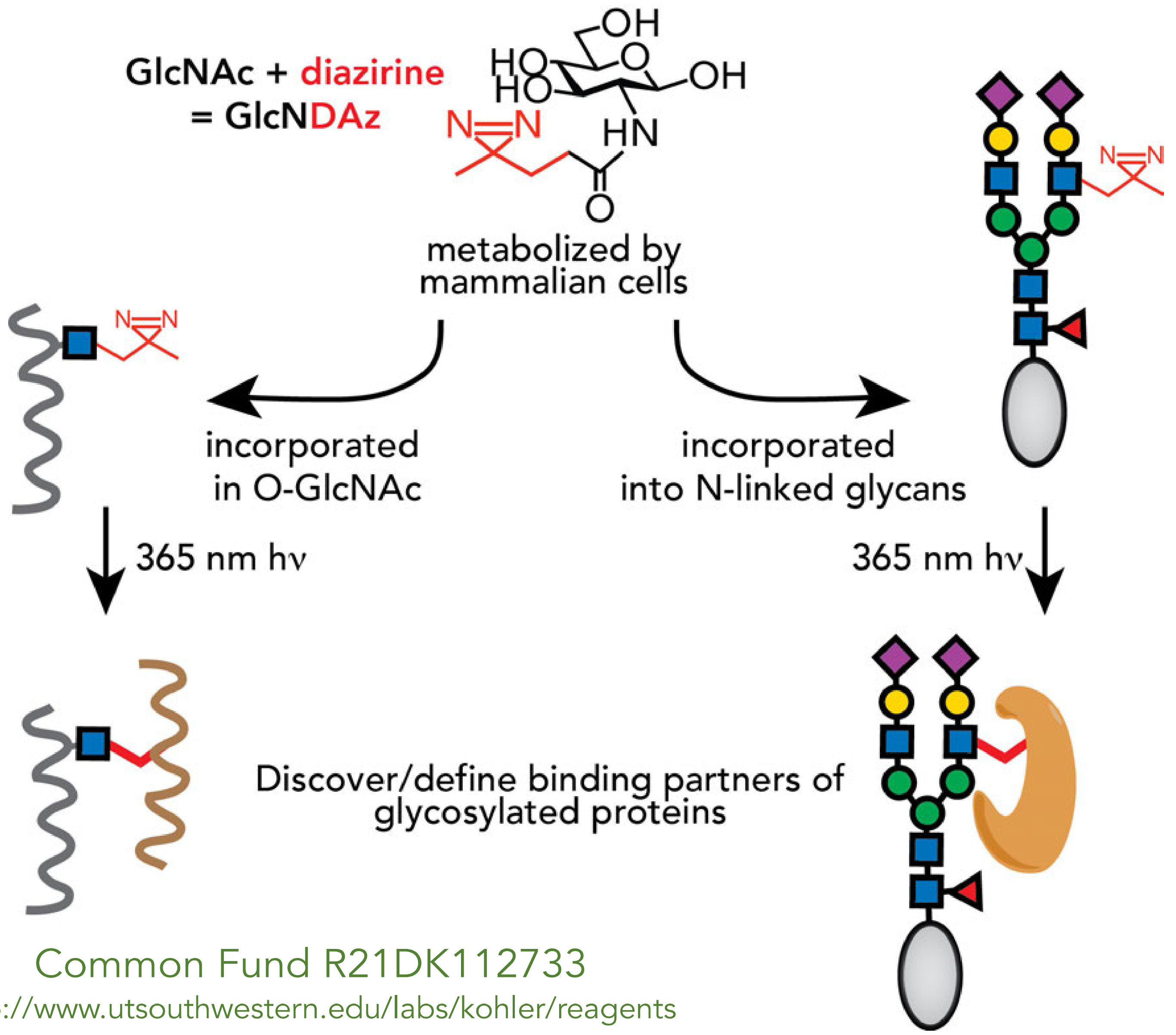
Metabolically-incorporated photoactivatable crosslinking sugar probes

<u>*GlcNDAz method*</u>:
PNAS (2012) 109:4834

<u>*GlcNDAz application*</u>:
PNAS (2018) 115:5956

Not depicted
<u>*Crosslinking sialic acid (SiaDAz)*</u>:
JACS (2008) 130:3278

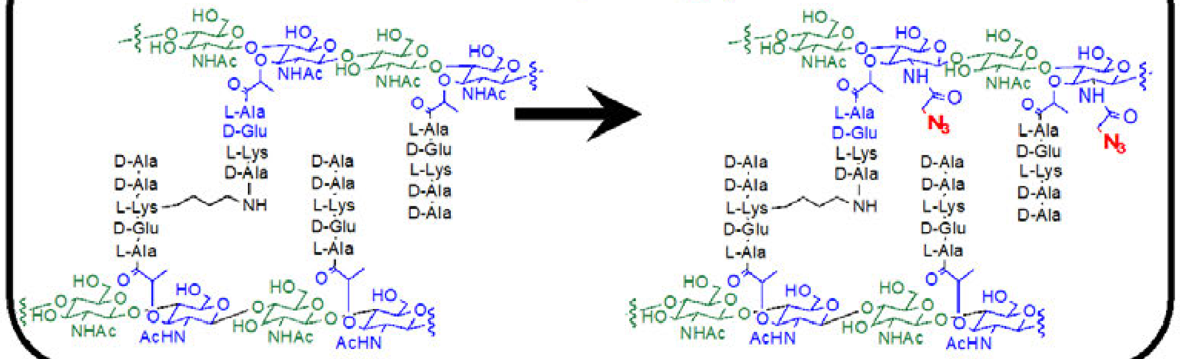
<u>*SiaDAz application*</u>:
eLife (2015) 4:e09545



Common Fund R21DK112733
http://www.utsouthwestern.edu/labs/kohler/reagents

Labeling the Carbohydrate Core of the Peptidoglycan

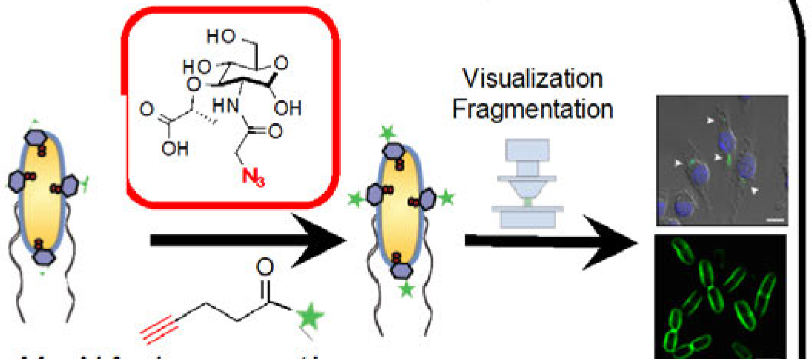
Bacterial Peptidoglycan



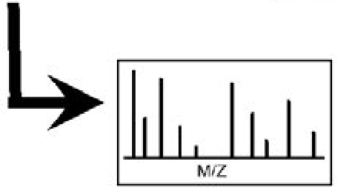
U01 Team:

Catherine L. Grimes
(U. of Delaware)
Nina R. Salama
(Fred Hutch. Cancer Center)
M. Sloan Siegrist
(UMass Amherst)

Metabolic Incorporation



MurNAc incorporation : *Nature Comm* (2017) 8: 15015



MurNAc derivatives: *JACS* (2018) *140: 9458*

Target Organisms

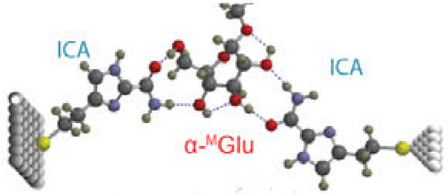
Non-pathogens	**Gram-positive:**	**Gram-negative:**
	Bacillus subtilis	*Escherchia coli*
	Lactobacillus acidophilus	*Pseudomonas putida*
	Streptomyces coelicolor	*Caulobacter crescentus*
		Related disease
Pathogens	**Gram-positive:**	
	Mycobacterium tuberculosis	Tuberculosis
	Staphylococcus aureus	Abscesses, sinusiti
	Streptococcus pneumoniae	Pneumonia
	Gram-negative:	
	Helicobacter pylori	Gastritis, gastric ulcers stomach cancer
	Salmonella enterica	Salmonellosis
	Yersinia enterocolitica	Yersiniosis
	Vibrio cholerae	Cholera
	Campylobacter jejuni	Gastroenteritis

1U01CA221230 Contact: Catherine L Grimes: cgrimes@udel.edu

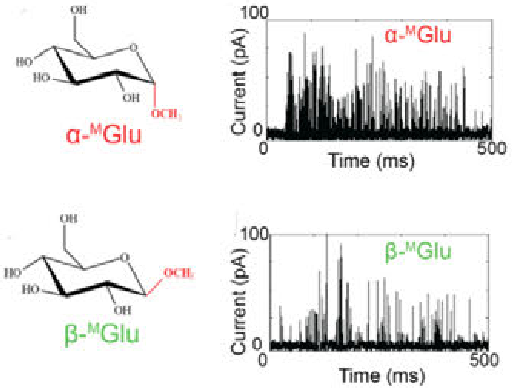
Studying Proteoglycans

Single Molecule Analysis of Glycosaminoglycans (GAG) using Recognition Tunneling (RT) Nanopores

RT as a tool for analyzing sugars



RT signals are produced when an analyte bridges two functionalized electrodes (ICA)

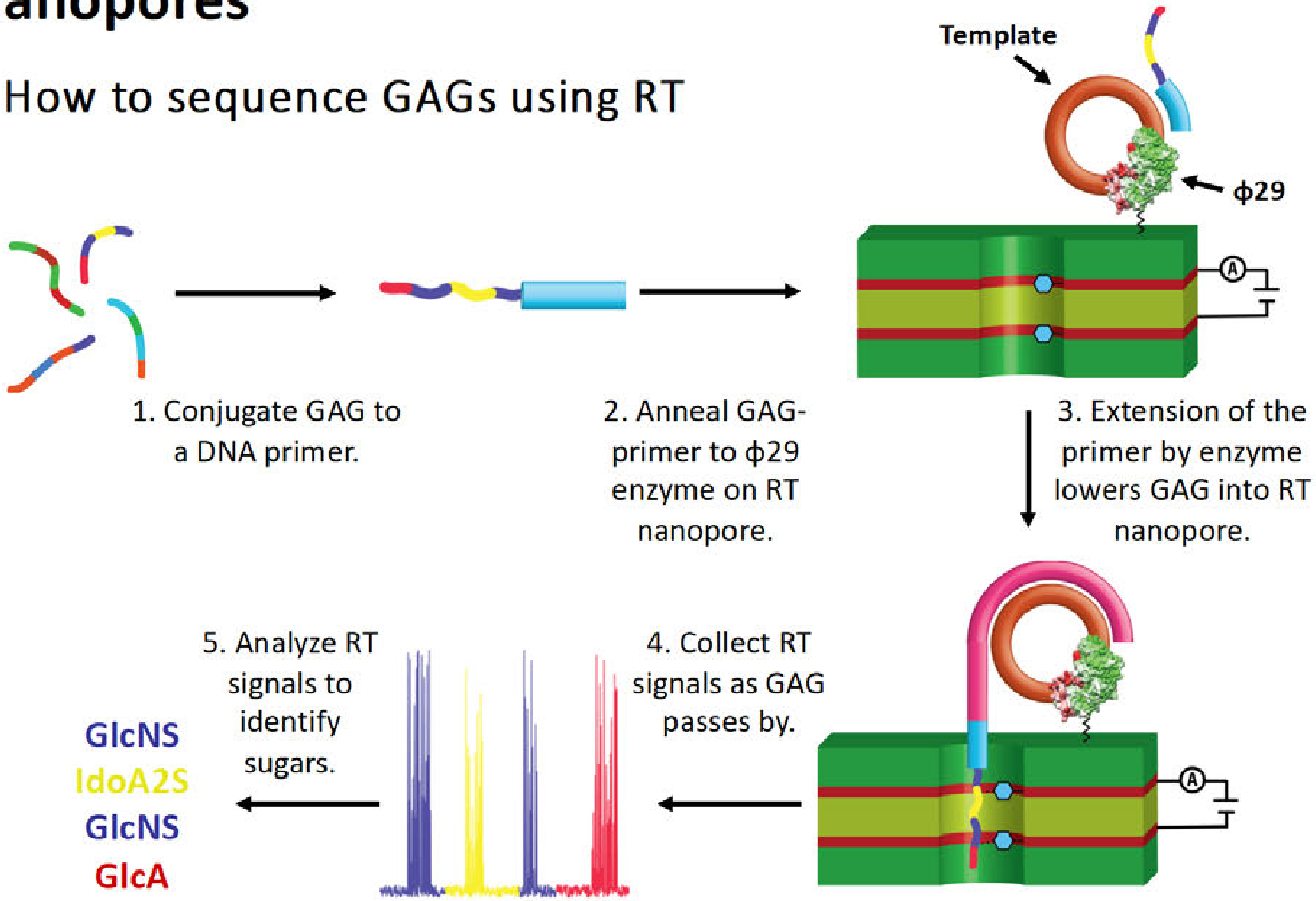


RT signals can be used to identify sugars

Im et al. *Nat. Commun.* (2016) 7:13868

Arizona State University

How to sequence GAGs using RT

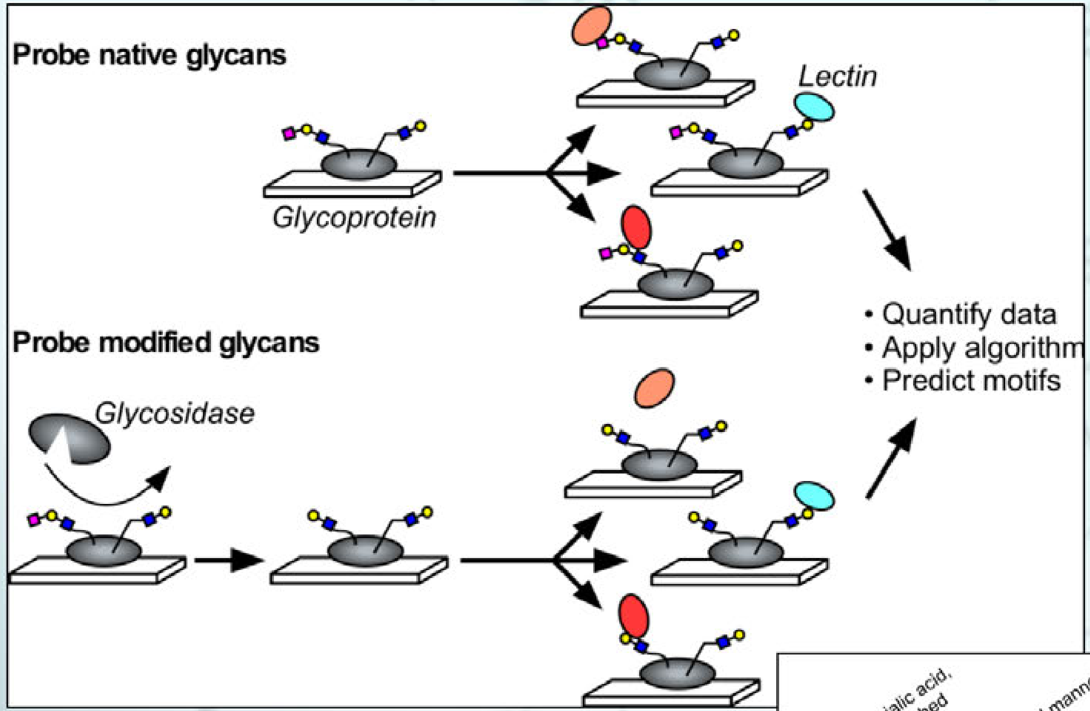


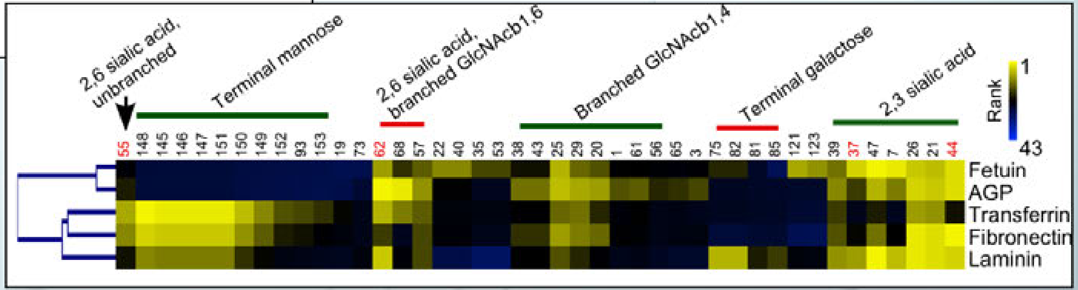
Support : NIH R21GM118339 & U01CA221235

Contact : Xu Wang (xuwang@asu.edu)
Stuart Lindsay (Stuart.Lindsay@asu.edu)

On-Chip GMAP | Glycan Modification and Probing

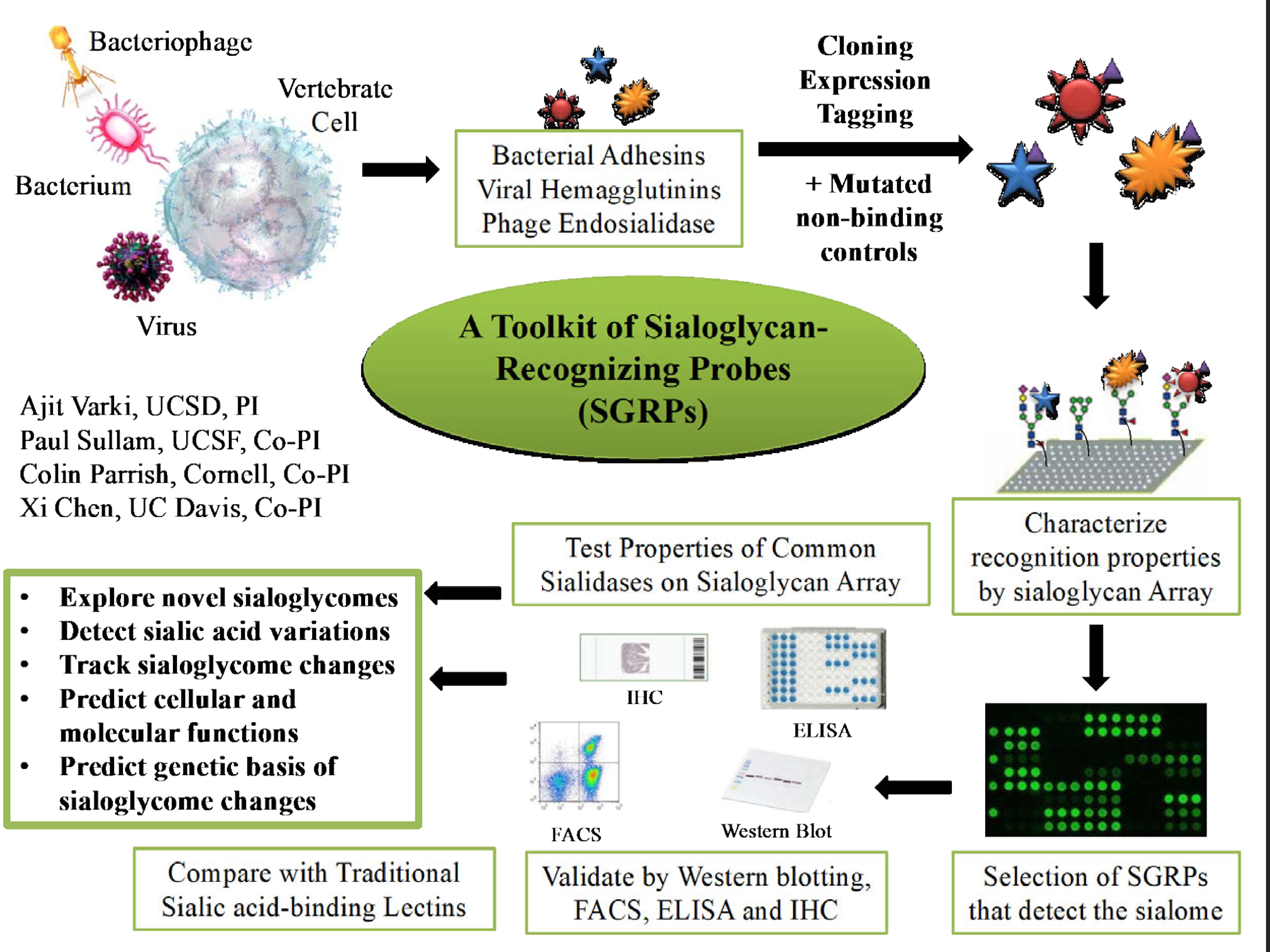
A method for analyzing protein glycosylation using tiny amounts of material





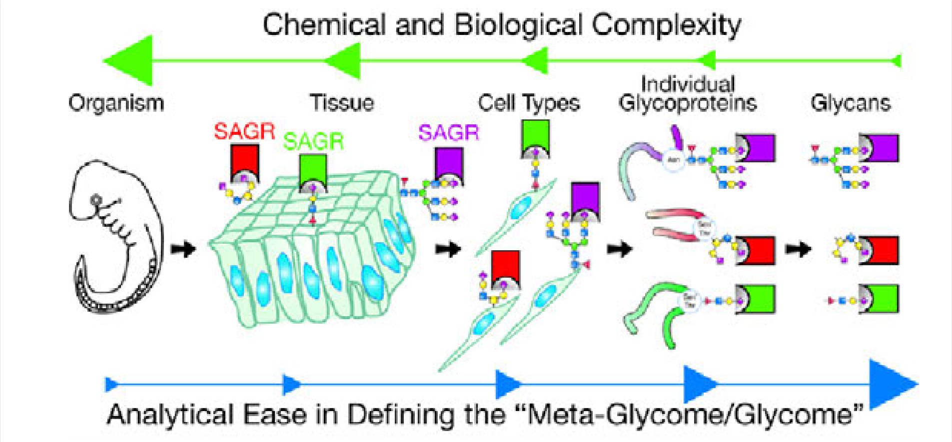
Contact: Brian Haab, PhD; Van Andel Research Institute; brian.haab@vai.org

Glycan binding proteins for detecting and enriching glycans



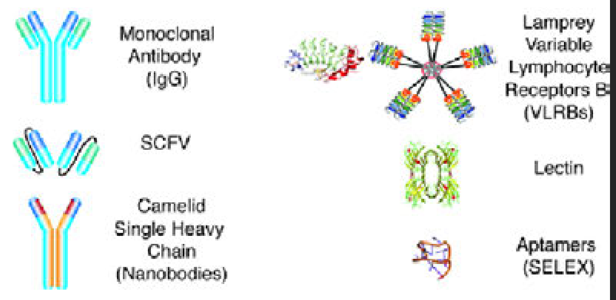
Glycan binding proteins for detecting and enriching glycans

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

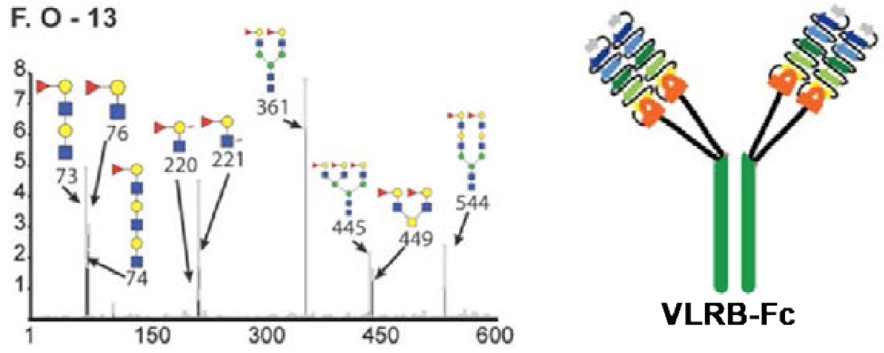


Immunization Strategy

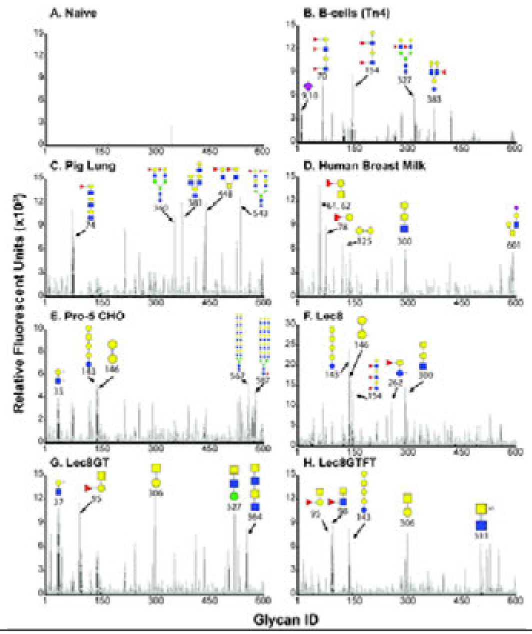
Sea Lamprey Larvae  ~4in long

(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

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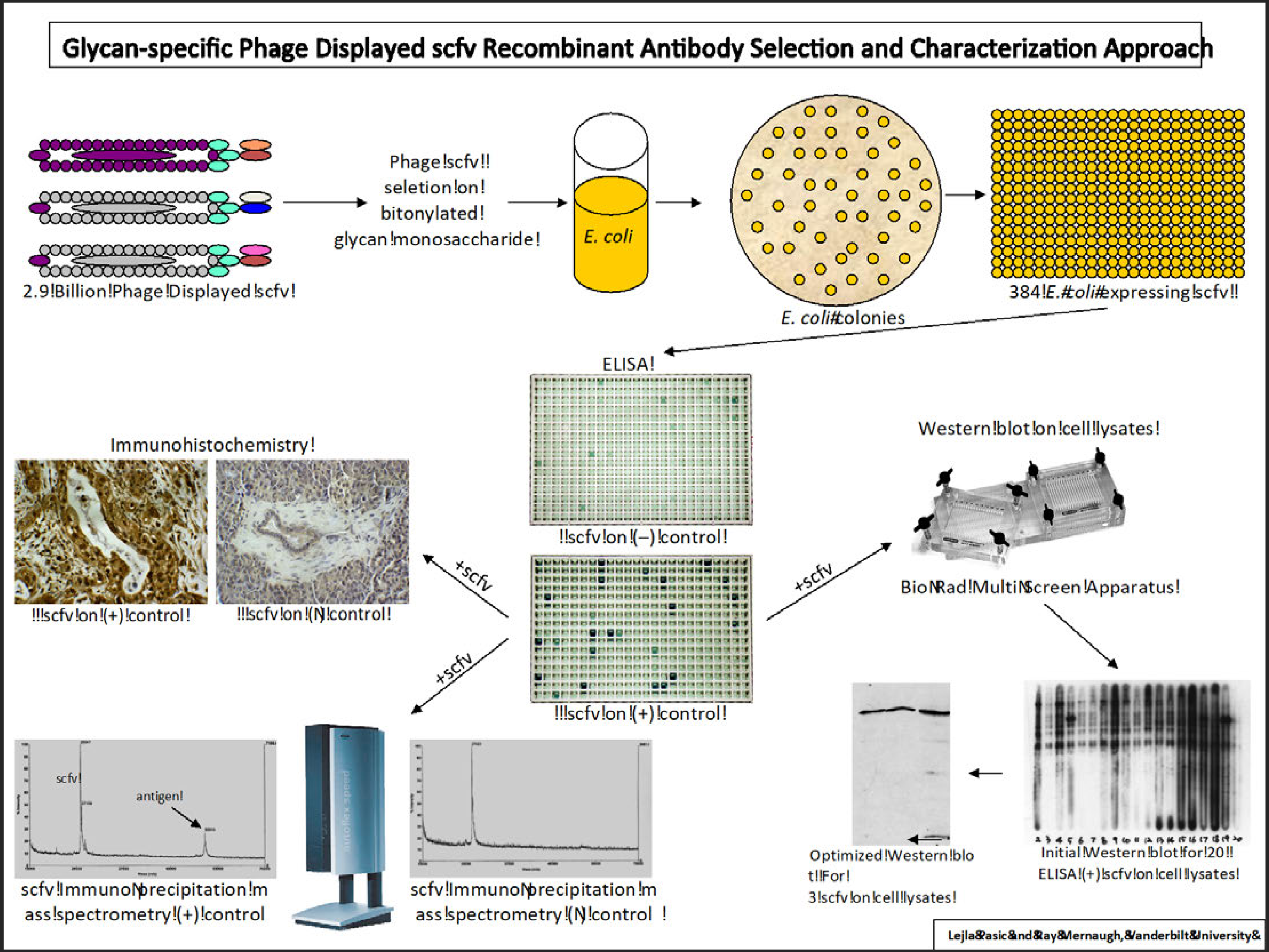
Glycan binding proteins for detecting and enriching glycans



Studying Proteoglycans

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

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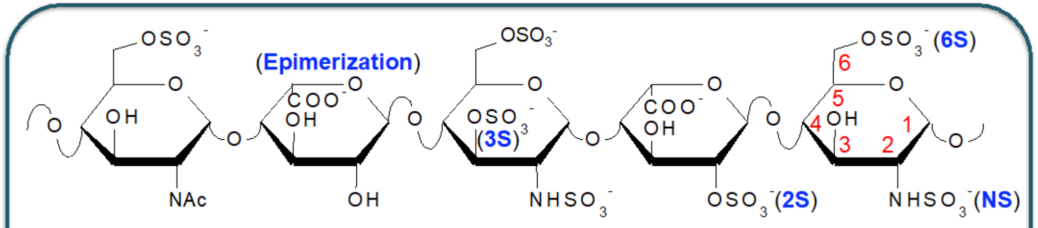
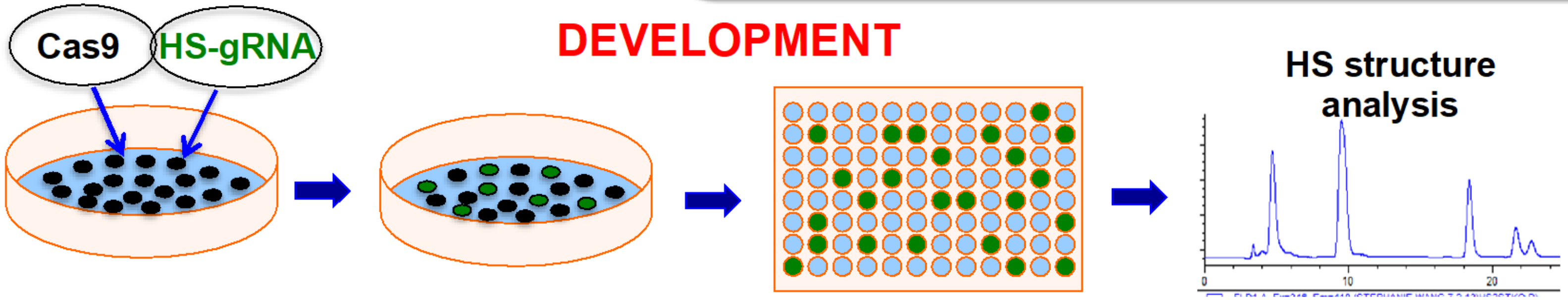
Available HS-gRNAs and cells:
http://ccrc.uga.edu



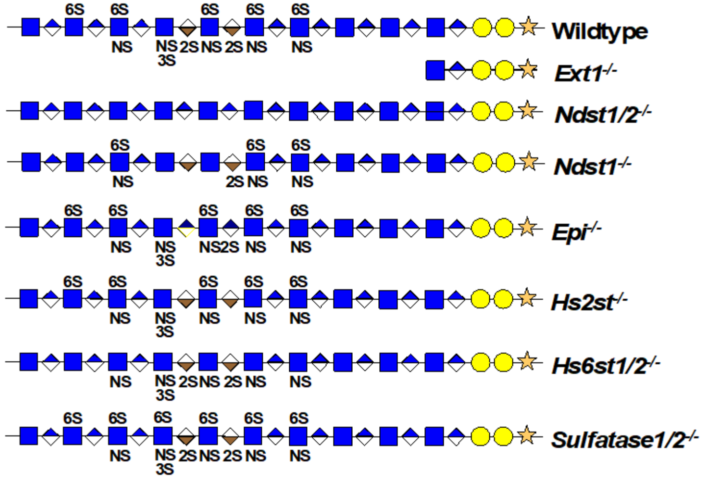
The **modifications** (epimerization and various sulfations) create **unique ligand-binding sites** in heparan sulfate

DEVELOPMENT



Wildtype cell → **Transfected cell pool** → **Cell cloning** → **Cell characterization**

HS mutant cell library

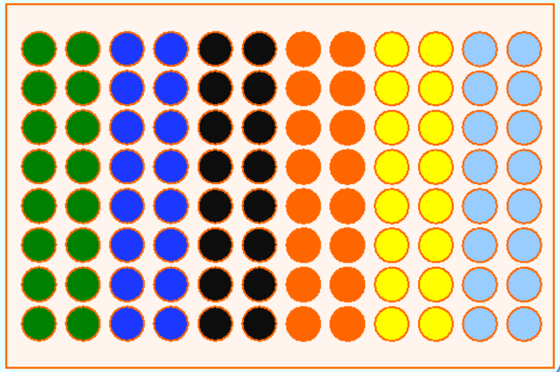


APPLICATION

Measure:

HS-ligand interactions on cell surface and cell responses

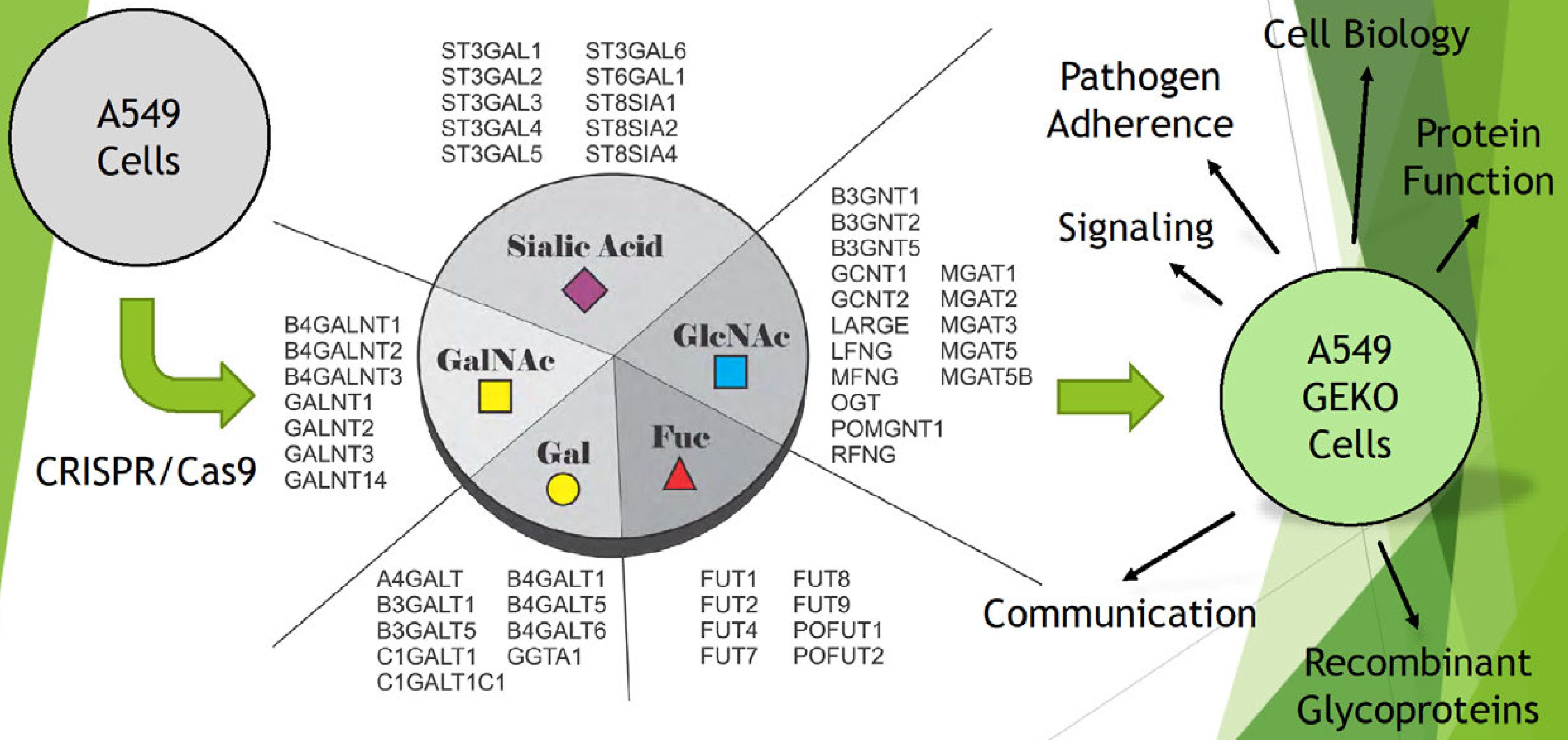
HS mutant cell array



Manipulating GLycans

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

Manipulating GLycans

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

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.

Want to Learn More:
https://commonfund.nih.gov/glycoscience

Glycoscience