*N-GLY*canyzer: PAT Toolkit for Near Real-Time Monitoring of Monoclonal Antibody (mAb) N-Glycosylation

Mr. Aron Gyorgypal & Prof. Shishir P.S. Chundawat*

[1]Department of Chemical and Biochemical Engineering

Rutgers, The State University of New Jersey

Piscataway, New Jersey 08854

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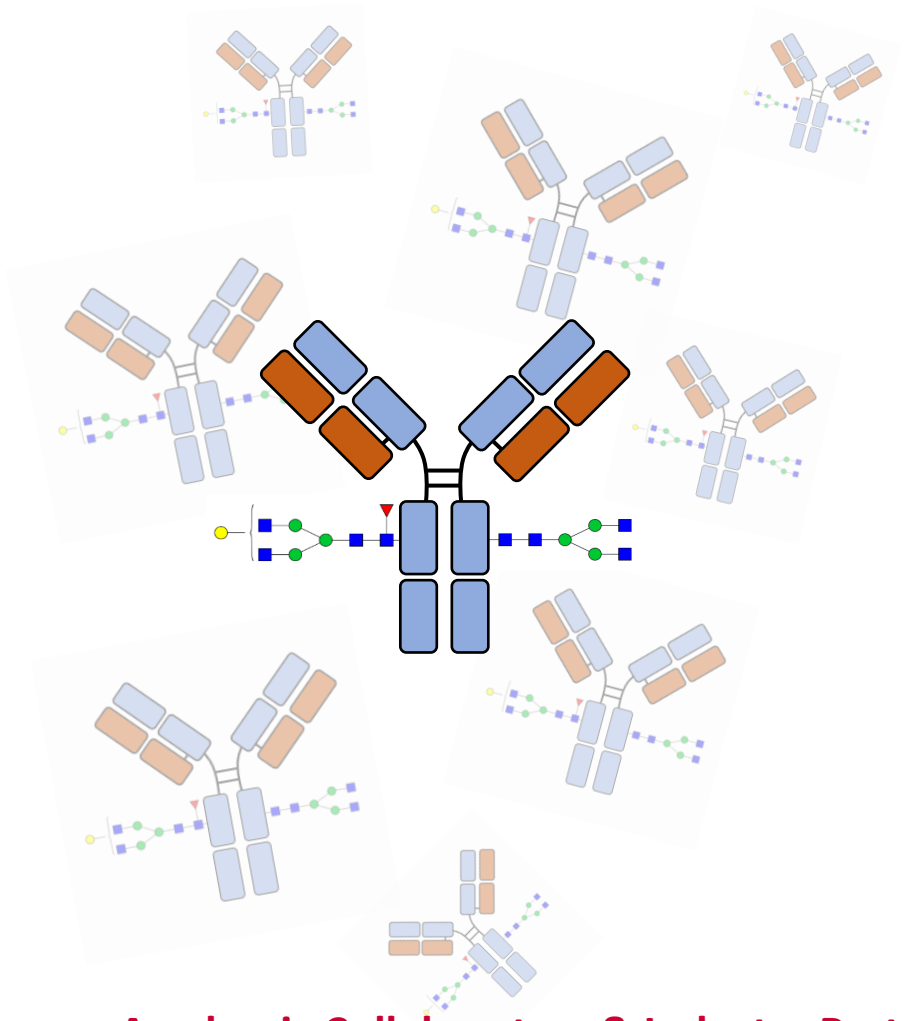
Acknowledgments



Chundawat Lab Members & Rutgers Alumni

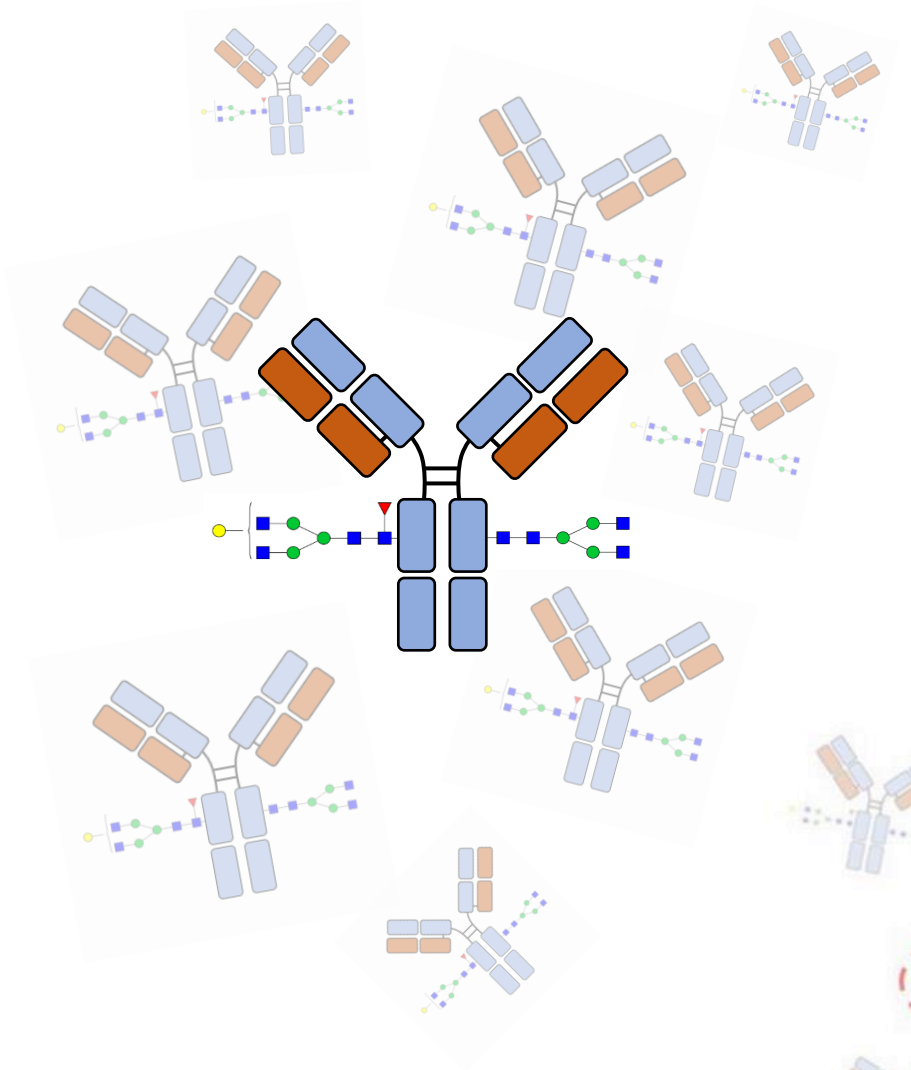
Academic Collaborators & Industry Partners

- Oscar Potter, Wayne Heacock, and Agilent Team
- Rutgers/Delaware FDA Project Team
- FIALabs, Genscript Biotech, Eppendorf, Kaiser

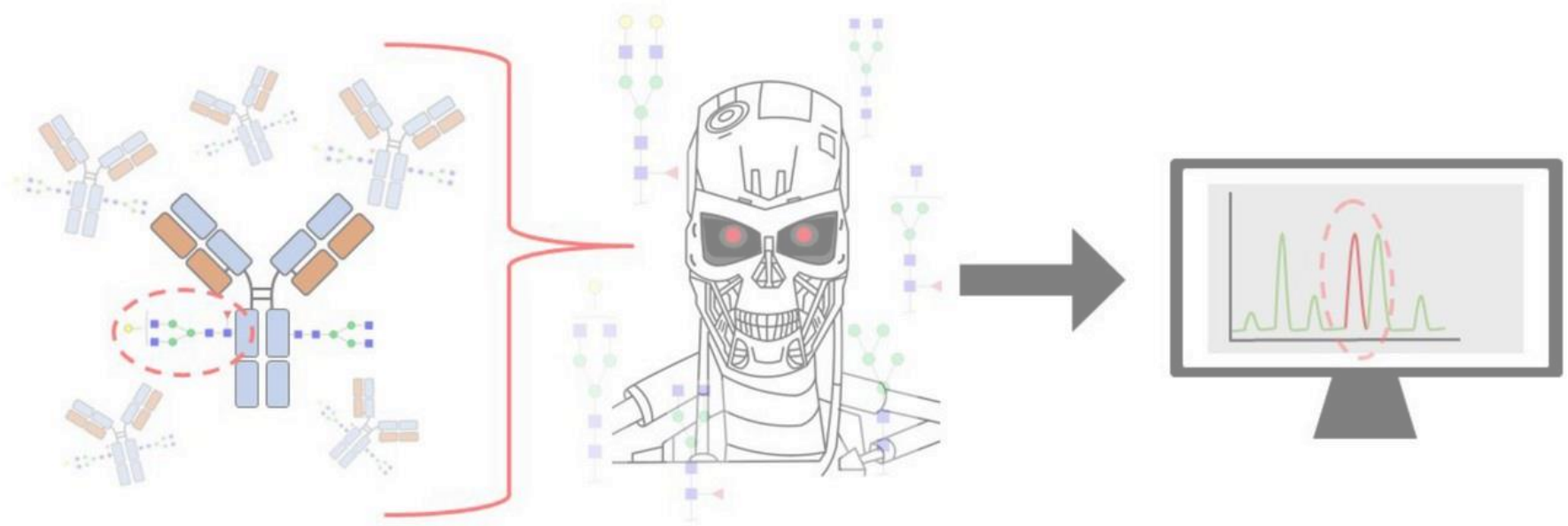
Project Funding & Support

CBER Award 1R01FD006588

Outline



- Overview to Chundawat Research Lab at Rutgers
- Project Background
  - mAbs, Glycosylation, & Continuous Manufacturing
- Experimental Methods
  - N-GLYcanyzer at-line PAT toolkit integration
- Results
  - System optimization with Agilent
- Conclusions & Future Work



Overview of Chundawat Research Lab at Rutgers University

Multi-Disciplinary Expertise in Glycosciences and Glycoengineering at Rutgers University

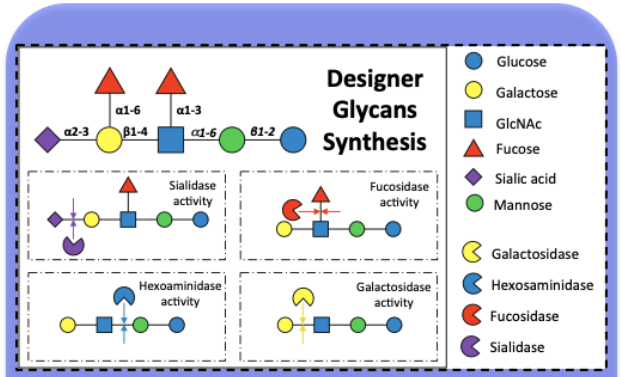
- **Carbohydrate-active Enzymes (CAZymes) & Protein Engineering**
  - *Protein Structure Modeling, Engineering, Expression, & Purification*
  - *Protein-Ligand Binding Molecular Dynamic/Docking Simulations*
- **Designer Glycans & Glycoconjugates Synthesis**
  - *Chemo-enzymatic Pathways for Glycans Synthesis*
  - *Designer Biopharmaceutical Biologics (Glycoproteins)*
- **Single-Molecule (SM) Imaging Based Bio-Engineering Toolkit Development**
  - *Multiplexed Protein-Glycan Force Spectroscopy*
  - *Cell/Protein-Glycan Interactome & Glycomics*
- **Bioprocess Engineering & Continuous Biomanufacturing**
  - *Biomanufacturing, Protein Bioseparations, & Bioprocess Modeling*
  - *Biomass Biorefining for Biofuels & Biochemicals Production*

Proteins

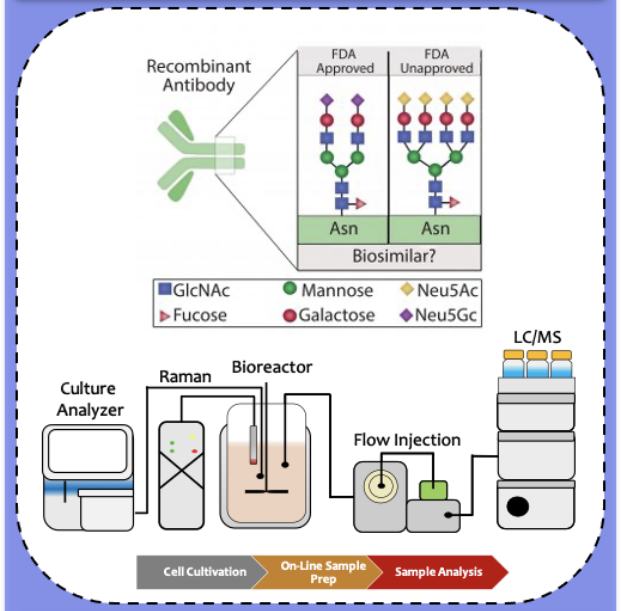
Glycans

NanoTools

Bioprocess

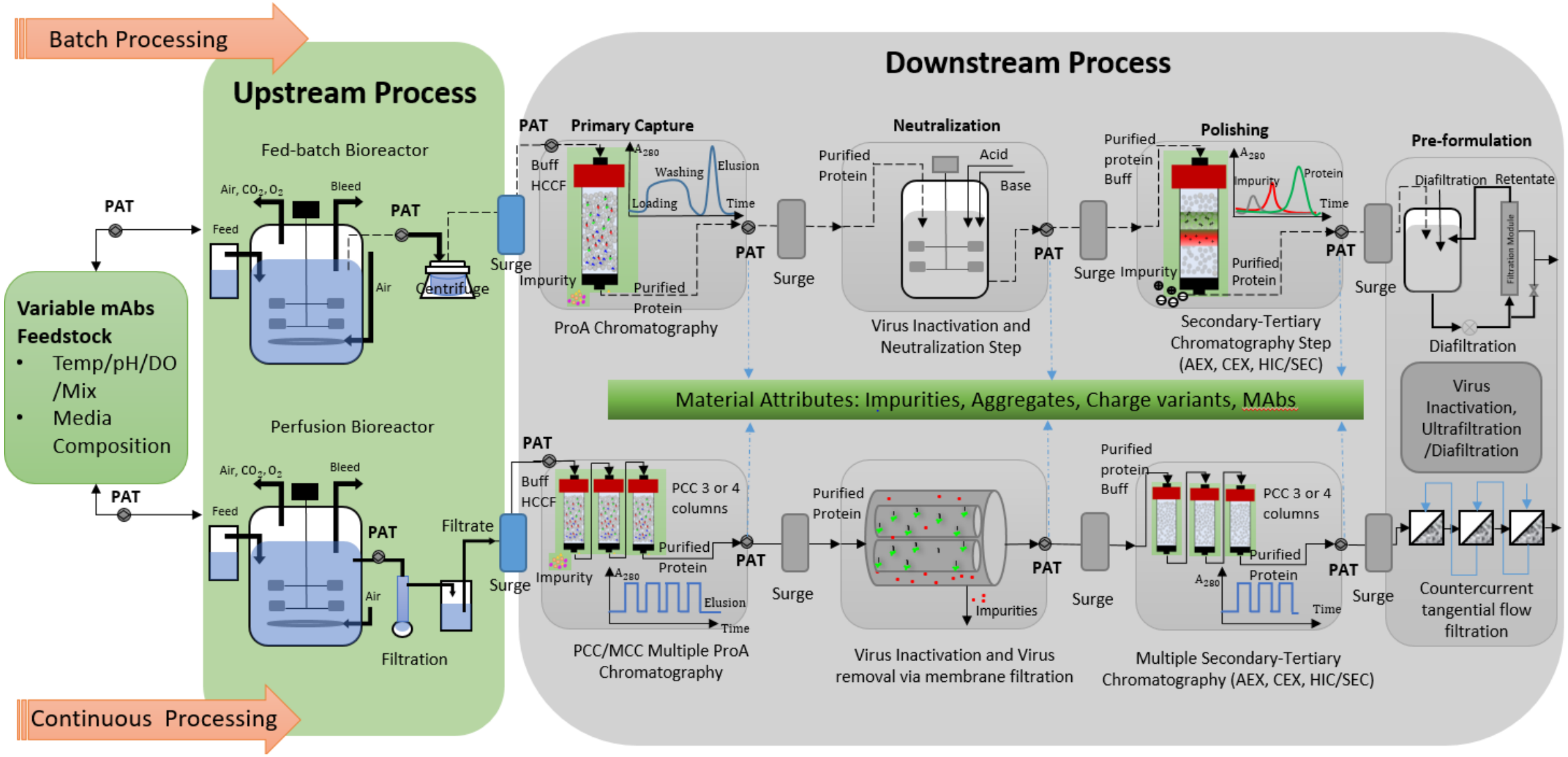


Chemoenzymatic synthesis of designer oligosaccharides



Real-time characterization of antibody drug glycosylation

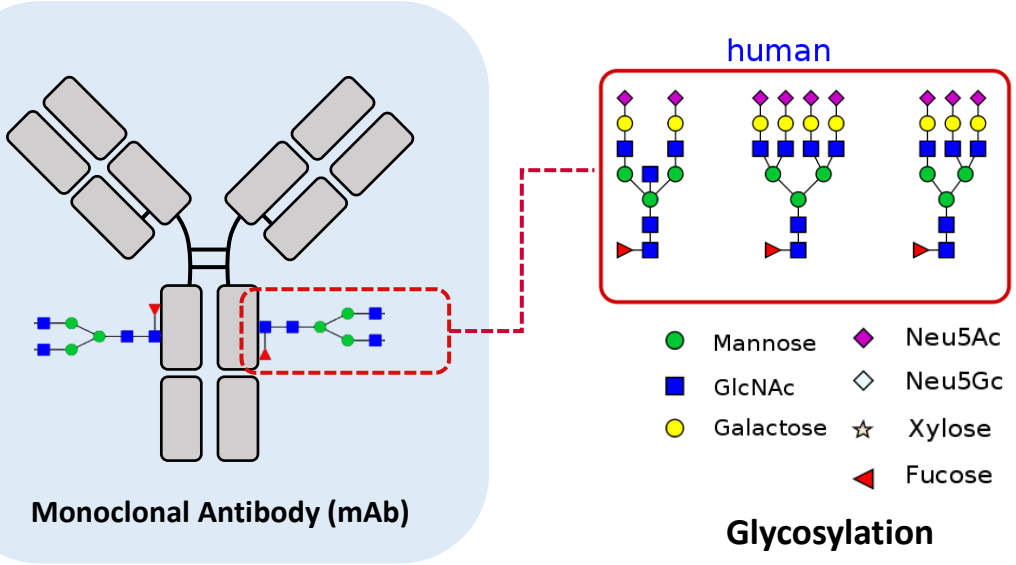
Background: Continuous Biomanufacturing of Biological Drugs

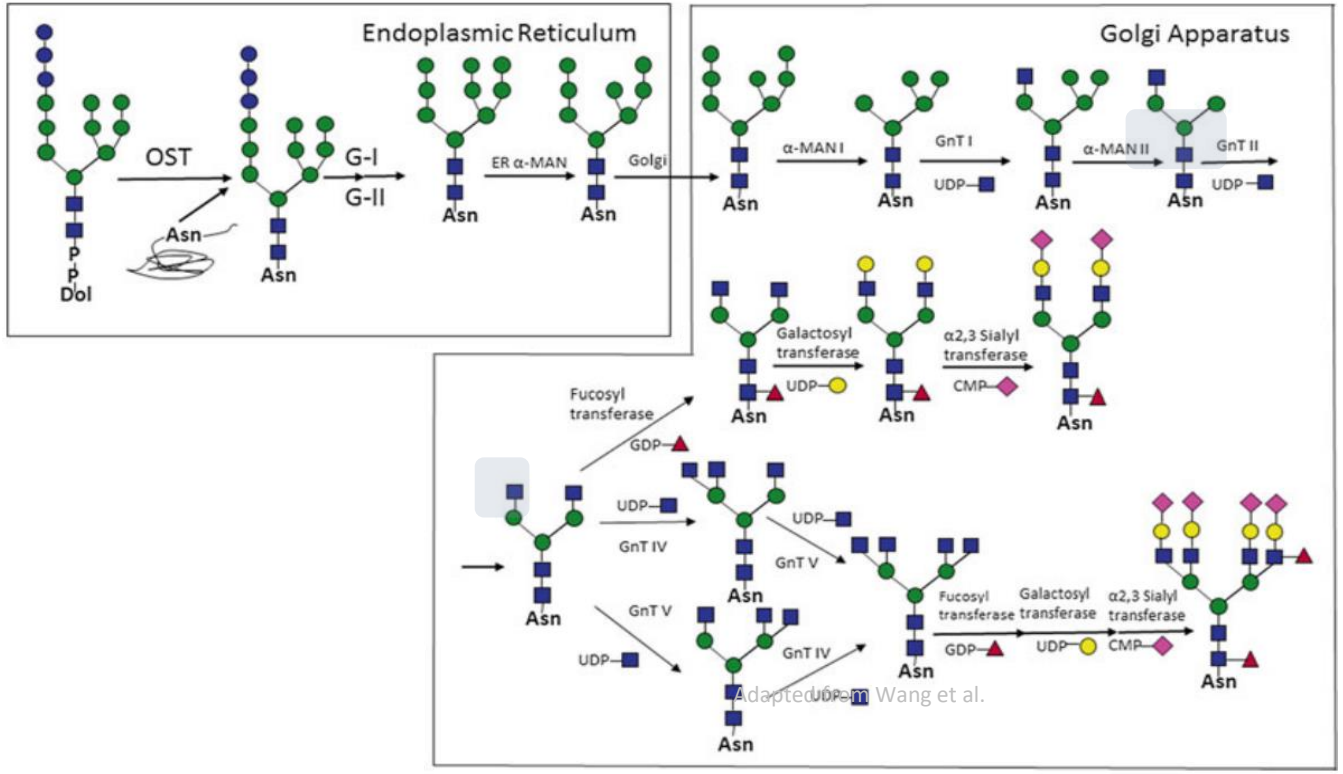


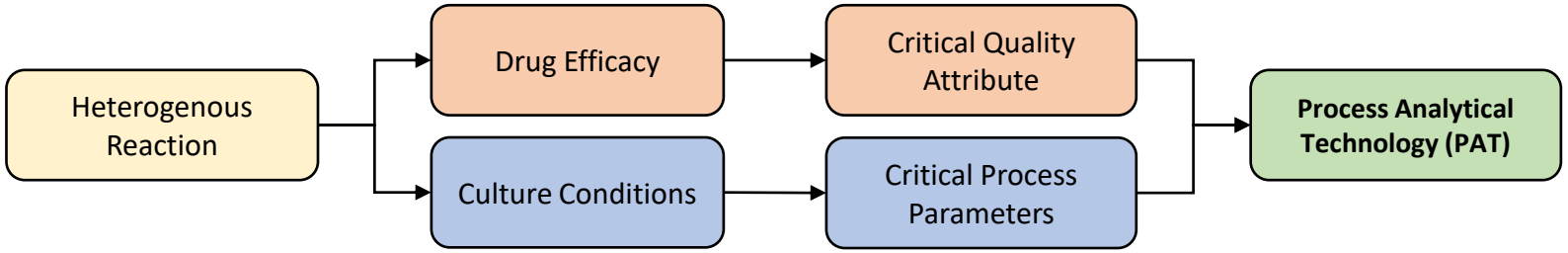
How to monitor drug quality (e.g., glycoproteins) during advanced biomanufacturing using PAT?

Background: Biologics or mAb N-Glycosylation

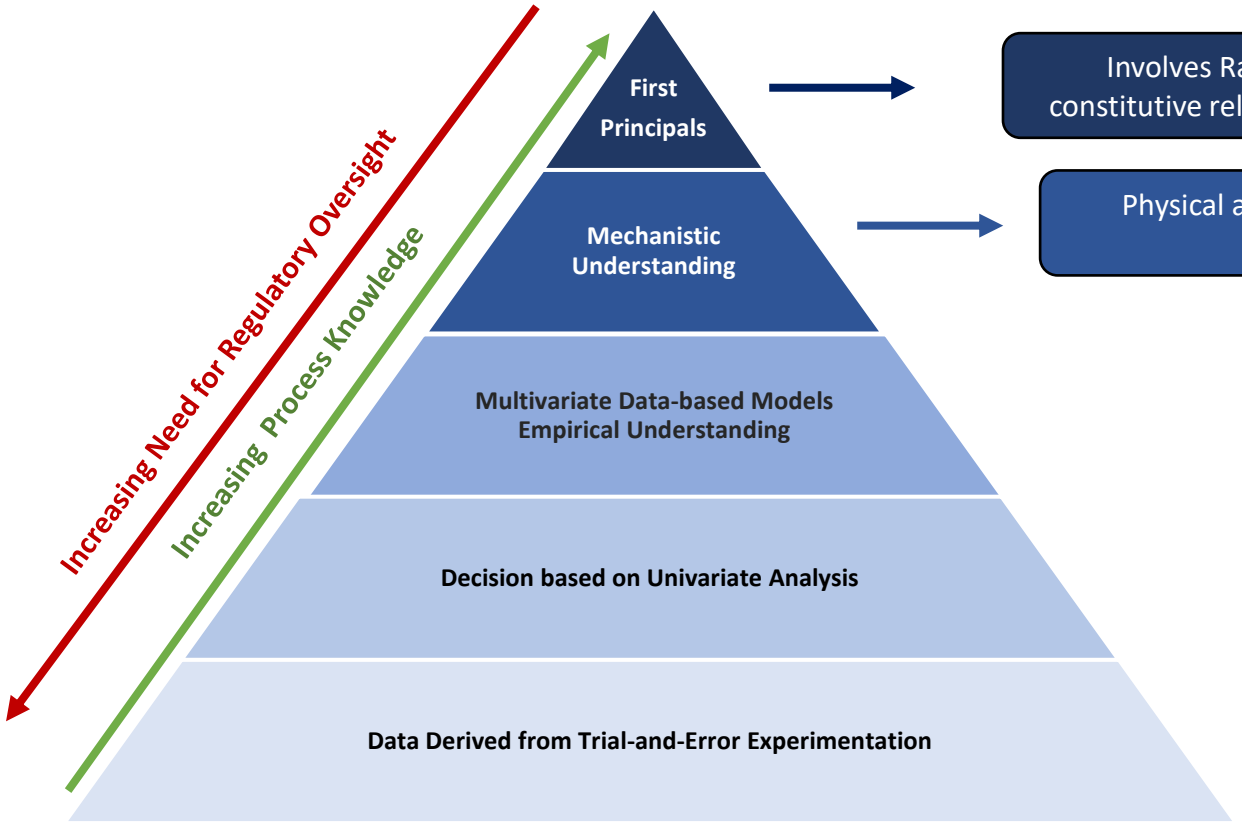
Wang, Q., Stuczynski, M., Gao, Y., & Betenbaugh, M. J. (2015). Strategies for Engineering Protein N-Glycosylation Pathways in Mammalian Cells. In A. Castilho (Ed.), *Glyco-Engineering: Methods and Protocols* (Vol. 1321, pp. 287–305). Springer New York. https://doi.org/10.1007/978-1-4939-2760-9_20







Background: PAT Regulatory Framework for Drug Manufacturing

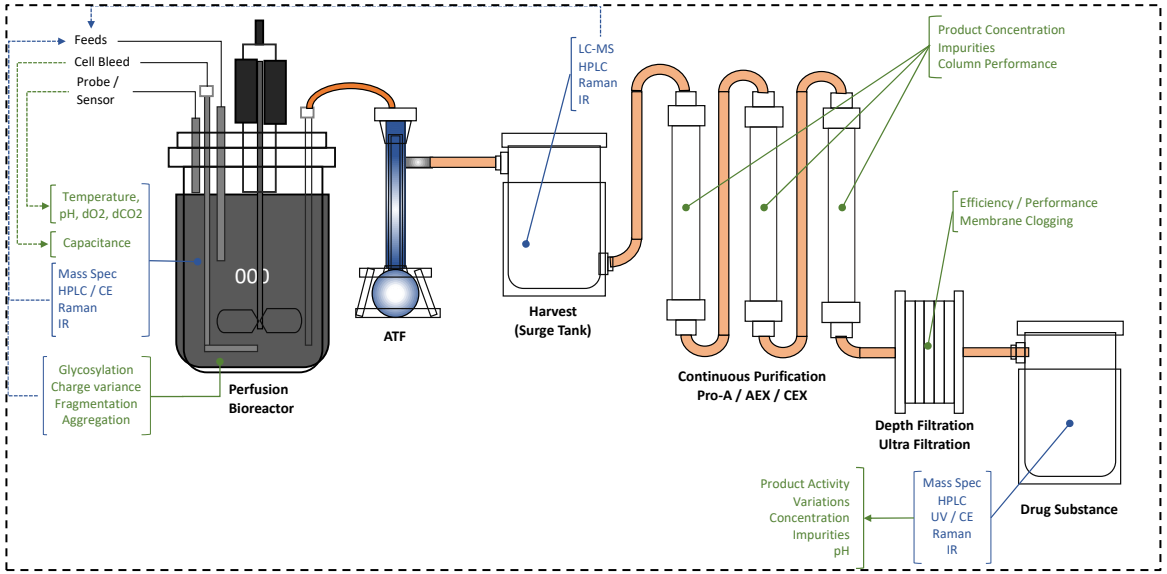


Involves Rather complete mechanistic understanding (RXN stoich; kinetics, constitutive relationship between heat / mass/ momentum conservation equations

Physical and biochemical principles that constitute model equations based off experimental data

Process Analytical Technology (PAT)

Mechanism to design, analyze and control a bioprocess

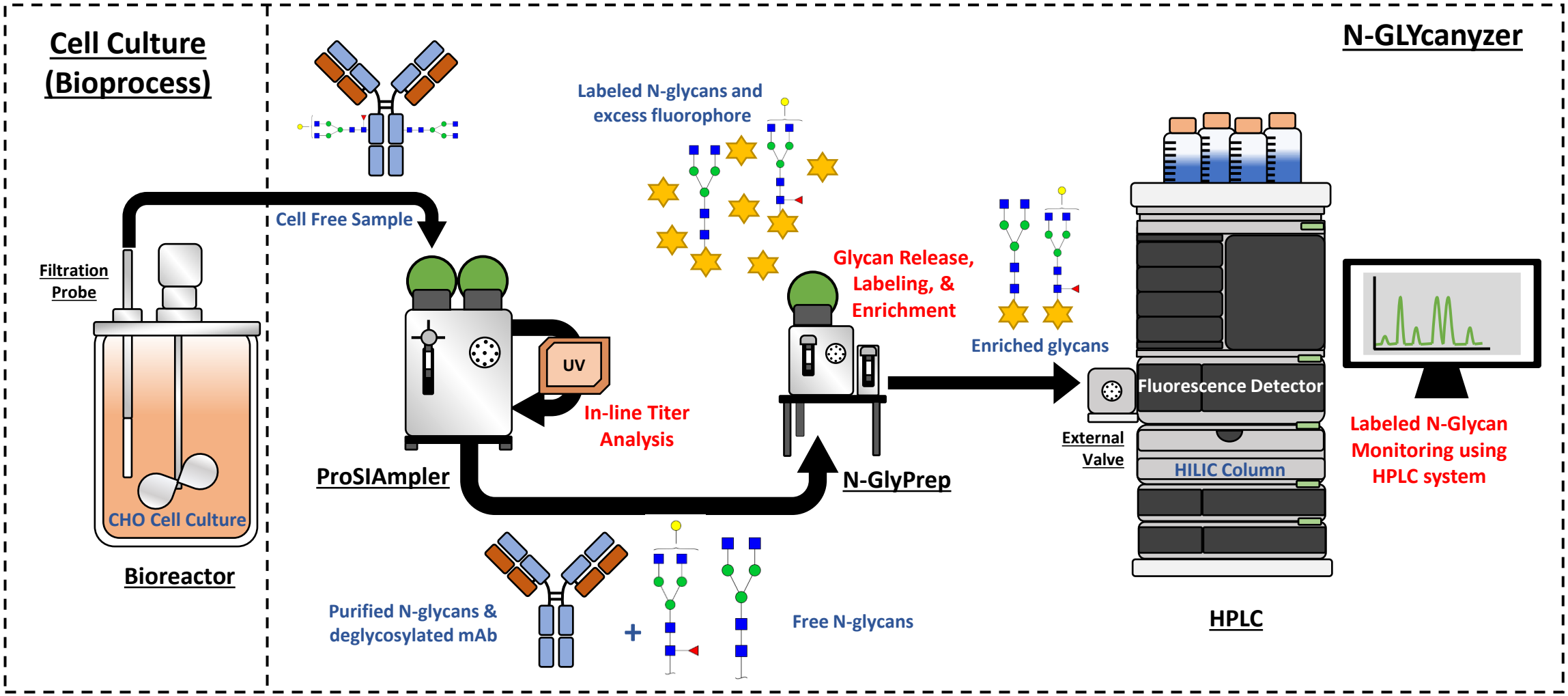


Rutgers Continuous mAb Production Line (w/Integrated PAT)

Hong, M. S., et al. "Process analytical technology and digital biomanufacturing of monoclonal antibodies." *Biopharm. Process* 23 (2020): 122-125.

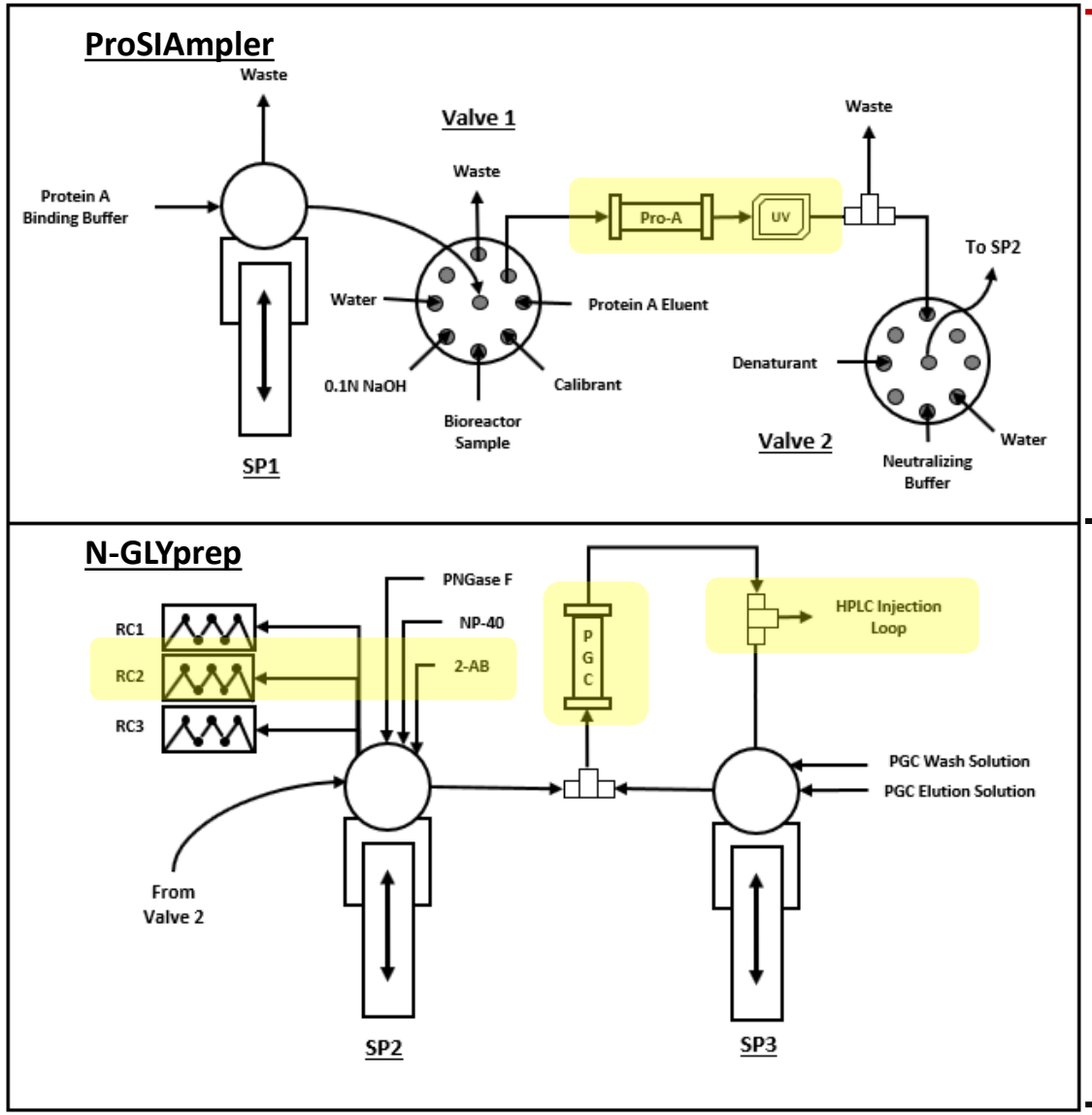
V. Chopda *et al.*, "Recent advances in integrated process analytical techniques, modeling, and control strategies to enable continuous biomanufacturing of monoclonal antibodies," *J. Chem. Technol. Biotechnol.*, no. April, p. jctb.6765, May 2021.

N-GLYcanyzer PAT workflow enables real-time glycosylation PAT



Gyorgypal, A., & Chundawat, S. P. S. (2021). An Integrated Process Analytical Platform for Automated Monitoring of Monoclonal Antibody N-linked Glycosylation. BioRxiv, 2021.11.14.468439. https://doi.org/10.1101/2021.11.14.468439

GLYcanyzer PAT Process Flow Diagram



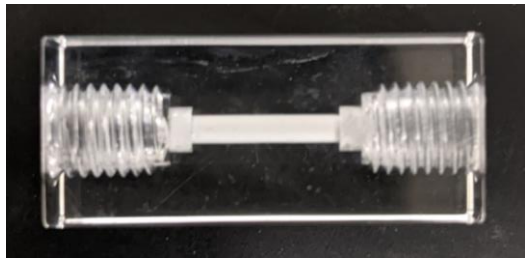
ProSIAmpler
Cell-free sampling from reactor
mAb purification (Protein A Affinity Chromatography)
In-Line UV for mAb titer analysis

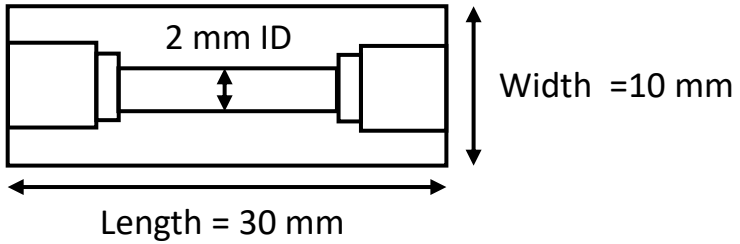
N-GLYprep
mAb denaturation
mAb deglycosylation (removal of glycan from mAb)
Glycan fluorescent labeling
Glycan Enrichment (removal of excess fluorophore)

Entire PAT toolkit is under software control running a fully-customizable python code and is integrated with Agilent LC

Protein A mAb capture is highly reproducible & customizable

A.





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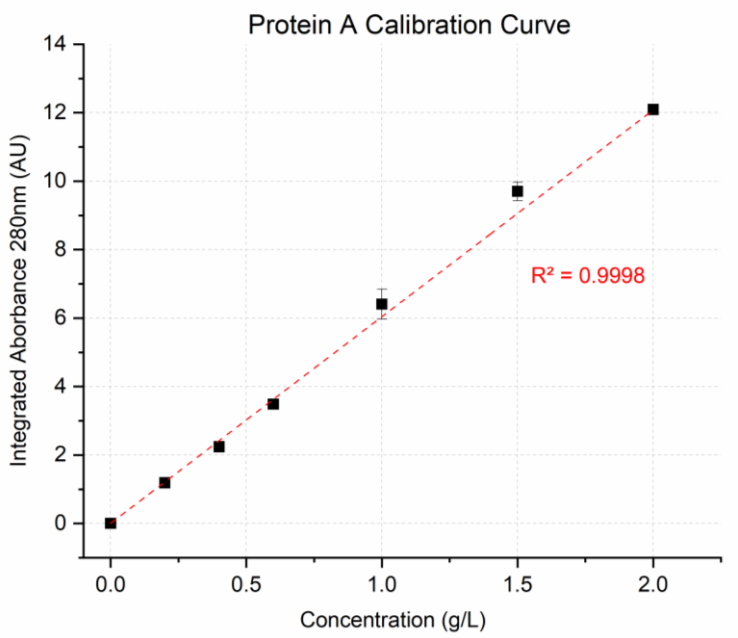

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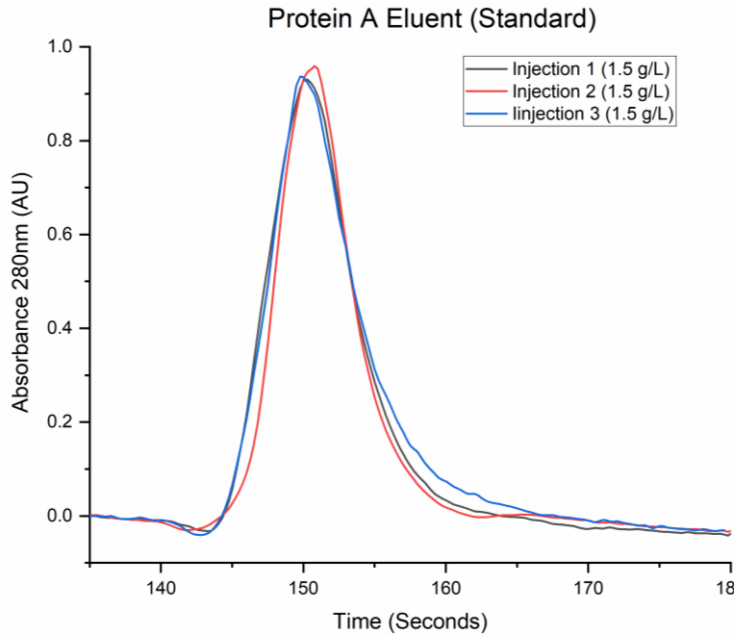
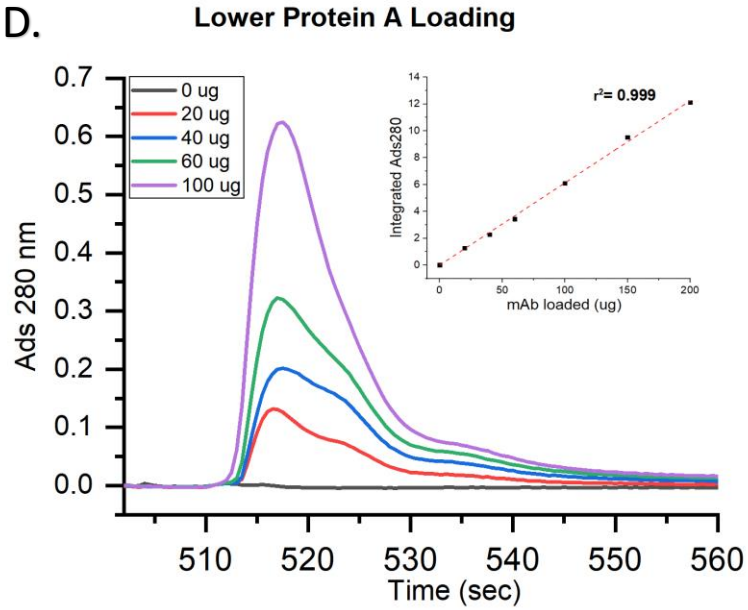
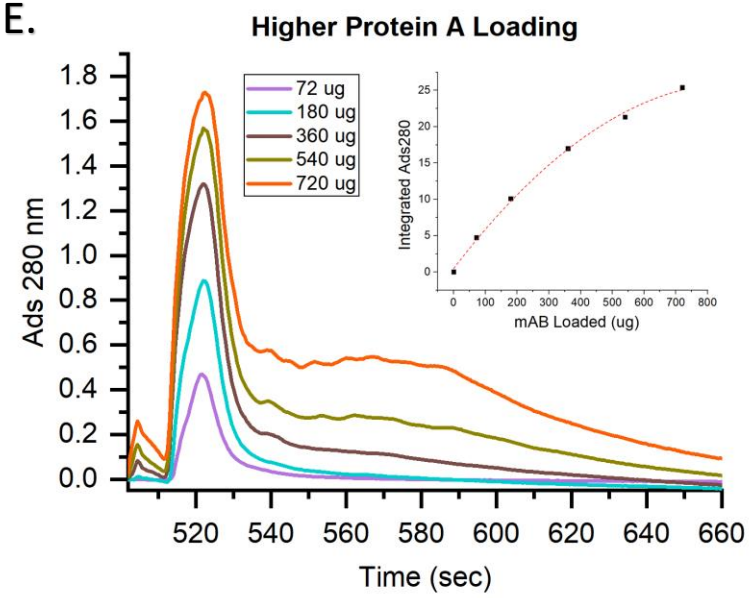


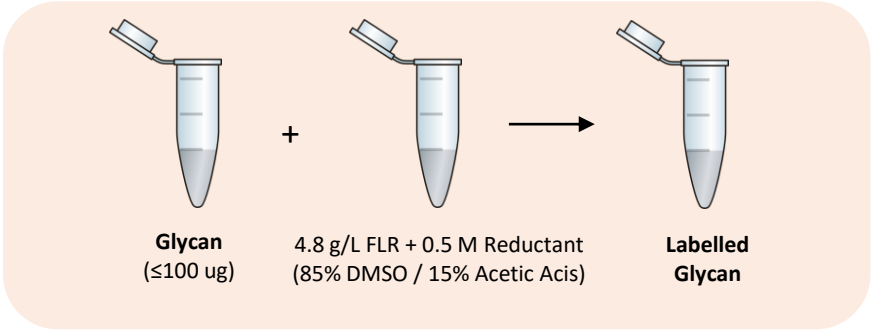
D.

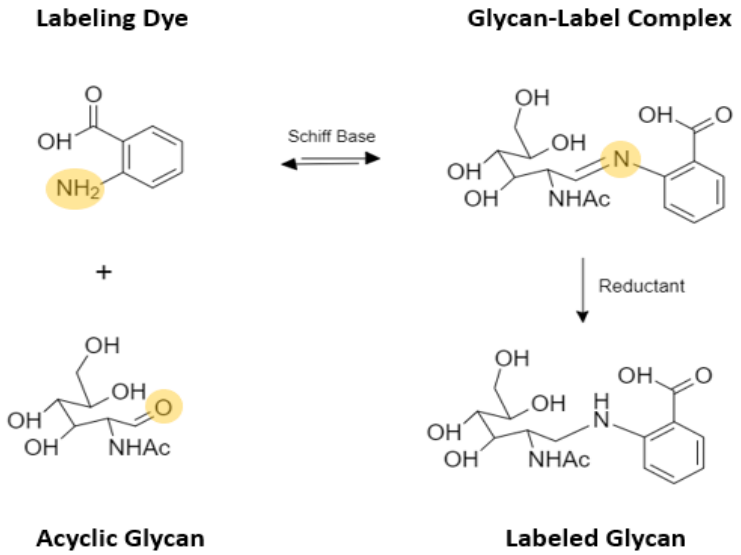


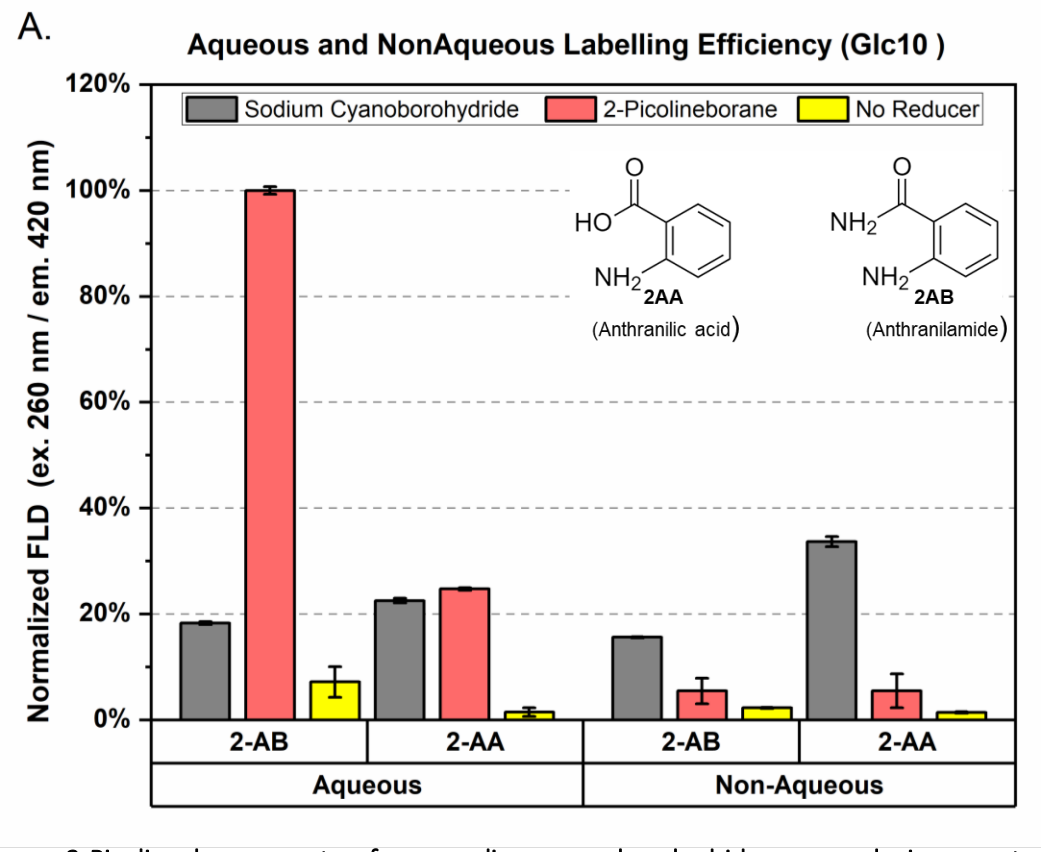
E.



Glycan labeling is optimized under aqueous conditions to enable long-term PAT deployment







2-Picoline borane outperforms sodium cyanoborohydride as a reducing agent under aqueous conditions for 2-AB fluorophore labeling of dextran. Here, (4A) shows the labeling efficiency of the 10-mer of glucose (i.e., maltodecaose) in the dextran ladder.

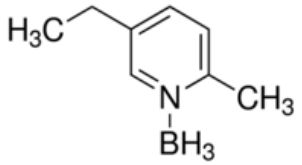
Entire PAT toolkit is has been optimized for labeling glycans with standard 2-AB fluorophore but has been customized with Agilent's IPC dye too!

Labeled glycan can be rapidly enriched using PGC to remove unlabeled dye

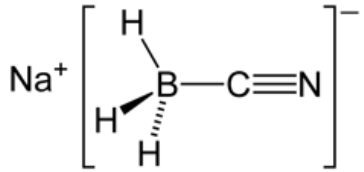
- Retention is thought to occur through a combination of factors interactions between the mobile phase and analytes promoting or discouraging retention.
- Hydrophobic interactions promote retention by pushing analytes out of solution toward the PGC surface
- **Unpublished work shows Agilent HILIC guard column works as well as PGC for labeled glycan cleanup as well**

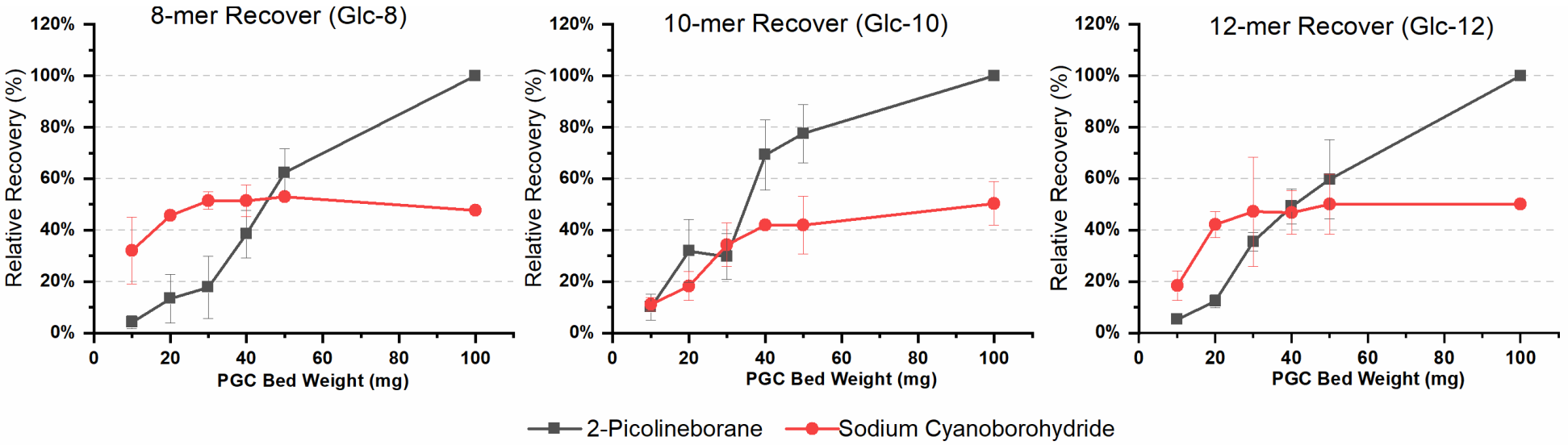
Reductants:

2-Picoline Borane

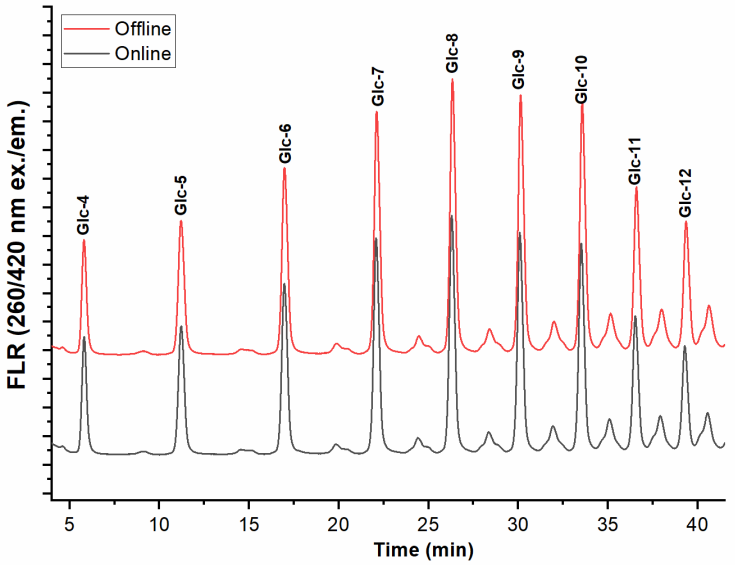


Sodium Cyanoborohydride

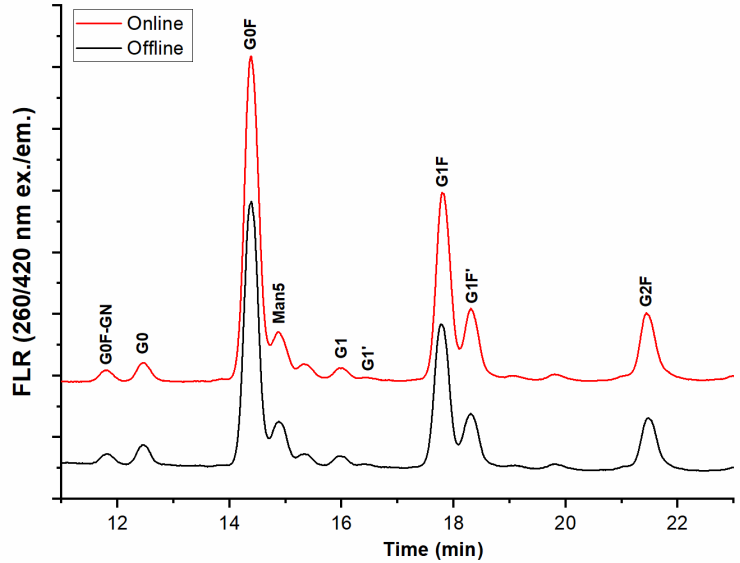




Integrated N-GLYcanyzer PAT with Agilent HPLC: Summary of on-line vs. offline reproducibility



Glucose Oligomer	Online (n=2)						Offline (n=2)					
	Relative Abundance			Retention Time (mins)			Abundance			Retention Time (mins)		
4	6.1%	±	0.5%	5.80	±	0.04	6.2%	±	0.1%	5.86	±	0.04
5	8.8%	±	0.5%	11.21	±	0.01	8.6%	±	0.1%	11.30	±	0.04
6	11.6%	±	0.2%	16.96	±	0.03	12.5%	±	0.2%	17.04	±	0.03
7	14.0%	±	0.3%	22.09	±	0.03	13.5%	±	0.2%	22.16	±	0.01
8	15.5%	±	0.1%	26.31	±	0.02	15.9%	±	0.3%	26.37	±	0.03
9	14.7%	±	0.3%	30.10	±	0.02	15.5%	±	0.3%	30.16	±	0.03
10	13.6%	±	0.3%	33.53	±	0.04	13.2%	±	0.3%	33.57	±	0.06
11	8.7%	±	0.3%	36.55	±	0.05	8.1%	±	0.2%	36.58	±	0.02
12	6.9%	±	0.5%	39.31	±	0.05	6.5%	±	0.2%	39.35	±	0.04



Glycan	Online (n=3)						Offline (n=3)					
	Relative Abundance			Retention Time (mins)			Abundance			Retention Time (mins)		
G0F-GN	1.4%	±	0.1%	11.76	±	0.07	1.5%	±	0.1%	11.85	±	0.10
G0	2.2%	±	0.1%	12.41	±	0.06	2.7%	±	0.2%	12.50	±	0.11
G0F	43.5%	±	0.2%	14.34	±	0.07	43.5%	±	0.1%	14.42	±	0.10
Man5	6.9%	±	0.2%	14.82	±	0.07	7.2%	±	0.2%	14.91	±	0.08
G1	1.5%	±	0.3%	15.94	±	0.06	1.4%	±	0.2%	16.00	±	0.08
G1F	25.1%	±	0.4%	17.75	±	0.09	25.3%	±	0.2%	17.80	±	0.08
G1F'	9.9%	±	0.1%	18.27	±	0.09	9.6%	±	0.2%	18.33	±	0.09
G2F	9.6%	±	0.2%	21.39	±	0.08	10.2%	±	0.4%	21.44	±	0.11

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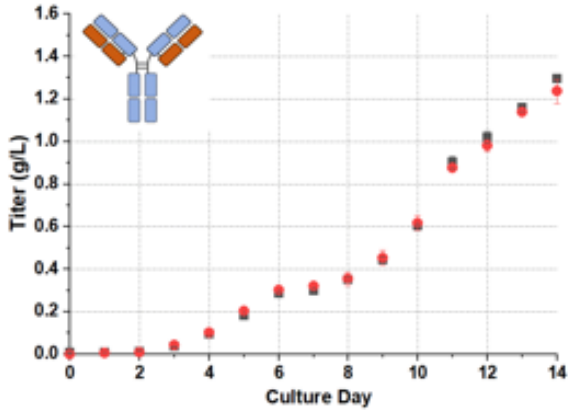
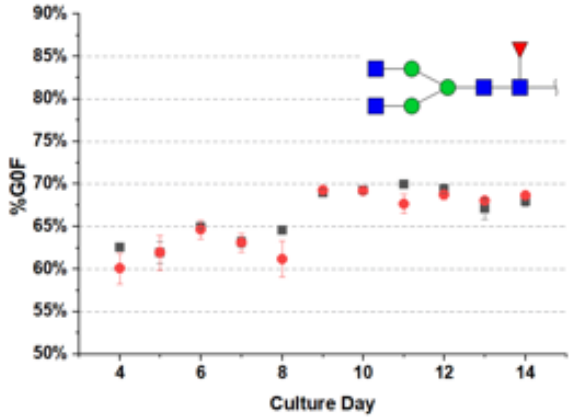
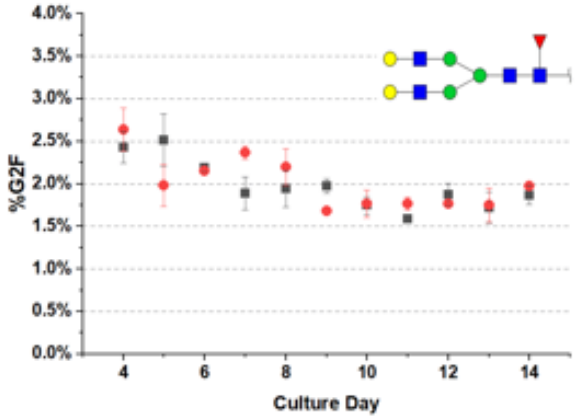
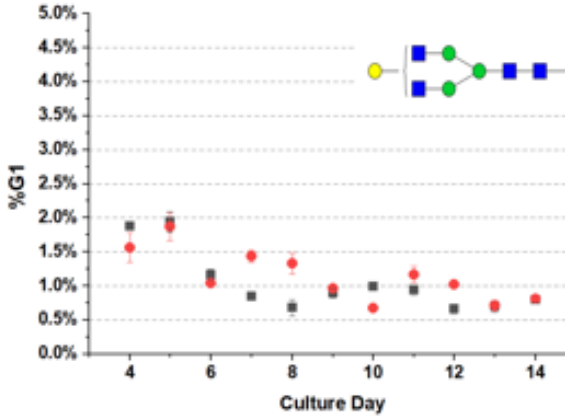
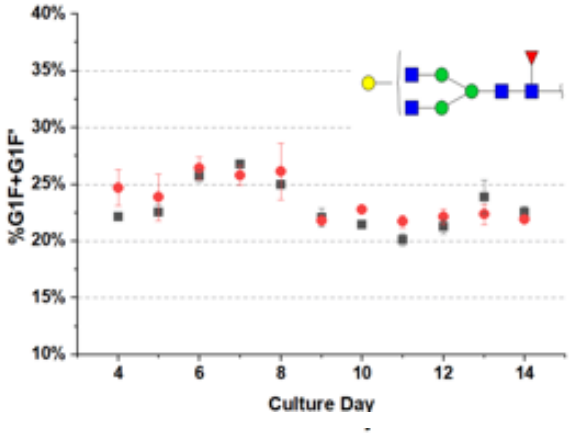
N-GLYcanyzer allows continuous monitoring of mAb glycosylation using 14-day cell culture

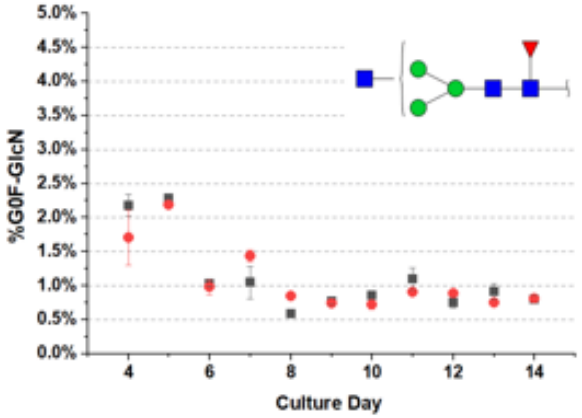


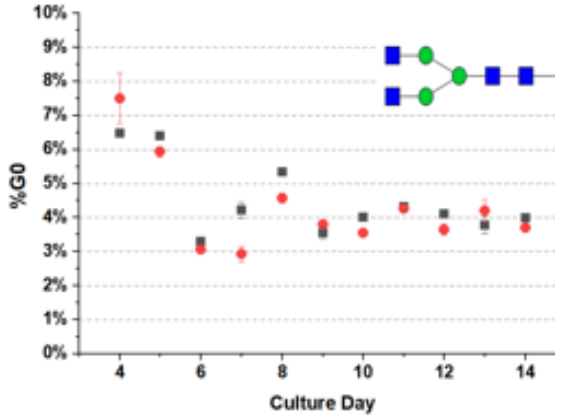


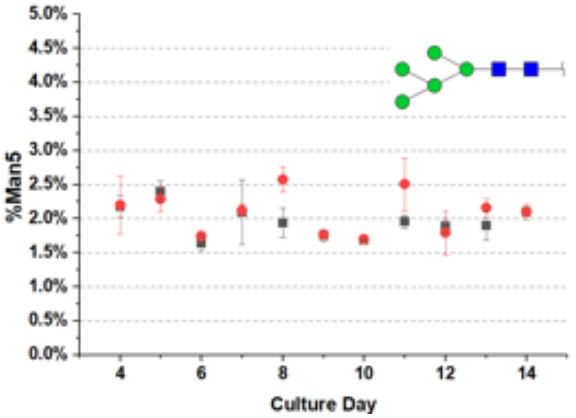












2AB Labeling Method (Pro):
Cost per sample is very cheap
Well-studied in open literature

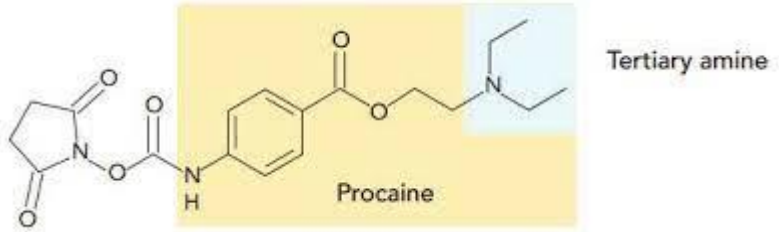
2AB Labeling Method (Con):
Sample to analysis time ~ 3 hours

N-GLYcanyzer can use Agilent's Instant PC glycan labeling methodology

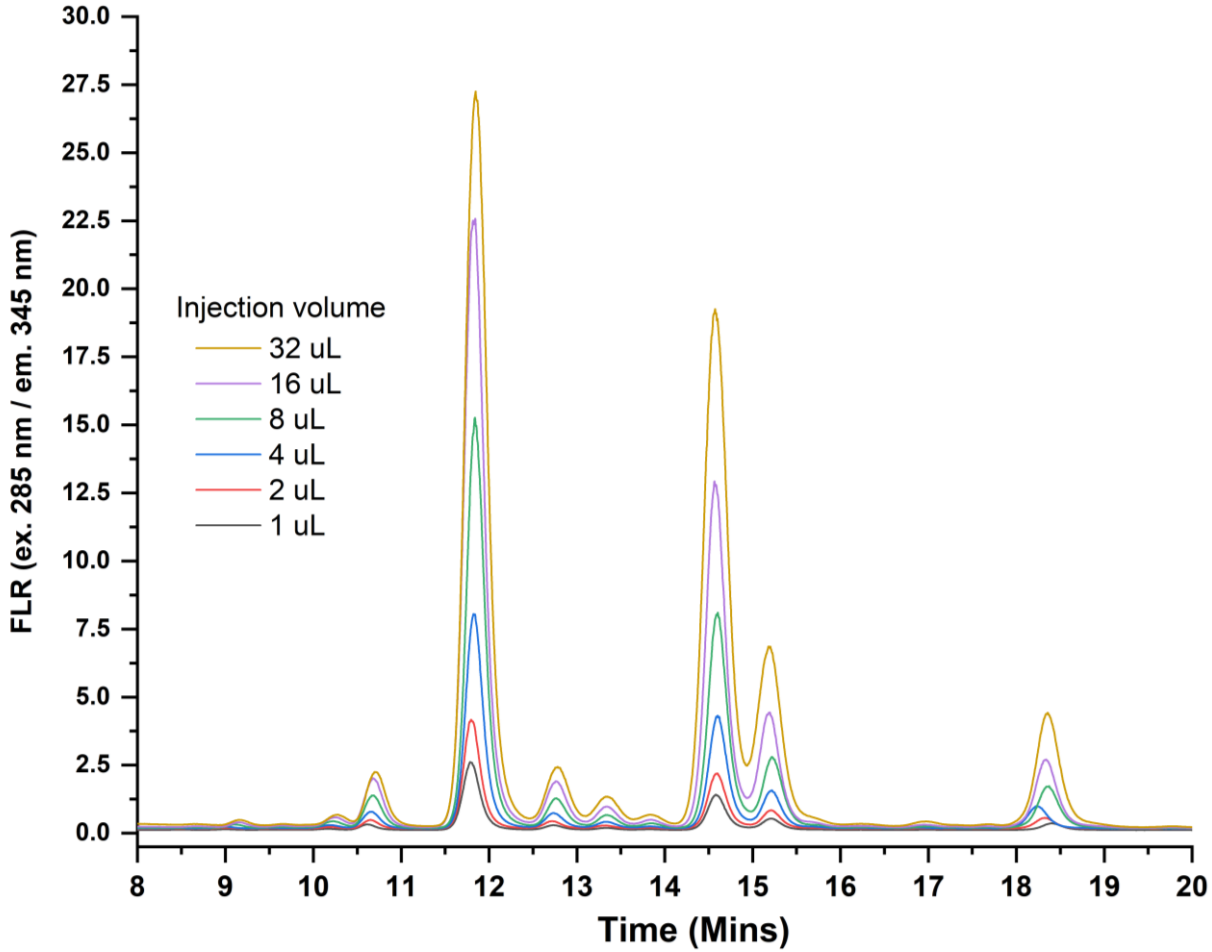
InstantPC Labeling Method (Pros):

Increase in FLR intensity vs 2AB, MS-compatible

Sample to analysis time less than 45 minutes



InstantPC Label



		1 uL	2 uL	4 uL	8 uL	16 uL	32 uL
INT FLR Intensity	G0F-GN	0.55	0.92	1.23	2.42	4.77	5.88
	G0	2.87	4.57	8.37	15.90	27.11	31.88
	G0F	35.68	57.11	117.05	229.81	375.87	478.44
	Man5	2.28	3.63	7.29	14.26	23.30	29.82
	G1	0.93	1.38	2.79	5.33	8.34	11.98
	G1F	20.54	32.92	66.82	129.88	212.67	367.04
	G1F'	7.49	11.62	22.86	46.05	76.41	132.91
	G2F	4.19	6.52	13.26	26.54	42.48	75.54
Relative Abundance	G0F-GN	0.7%	0.8%	0.5%	0.5%	0.6%	0.5%
	G0	3.9%	3.9%	3.5%	3.4%	3.5%	2.8%
	G0F	47.9%	48.1%	48.8%	48.9%	48.8%	42.2%
	Man5	3.1%	3.1%	3.0%	3.0%	3.0%	2.6%
	G1	1.2%	1.2%	1.2%	1.1%	1.1%	1.1%
	G1F	27.6%	27.7%	27.9%	27.6%	27.6%	32.4%
	G1F'	10.0%	9.8%	9.5%	9.8%	9.9%	11.7%
	G2F	5.6%	5.5%	5.5%	5.6%	5.5%	6.7%

Unpublished data: In collaboration with Agilent (Oscar Potter, Aron Gyorgypal, Shishir Chundawat)

Future Work & Impact

Preprint Publication:

Gyorgypal, A., & Chundawat, S. P. S. (2021). An Integrated Process Analytical Platform for Automated Monitoring of Monoclonal Antibody N-linked Glycosylation. *BioRxiv*, 2021.11.14.468439. https://doi.org/10.1101/2021.11.14.468439

New Results

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An Integrated Process Analytical Platform for Automated Monitoring of Monoclonal Antibody N-linked Glycosylation

Aron Gyorgypal, Shishir P.S. Chundawat

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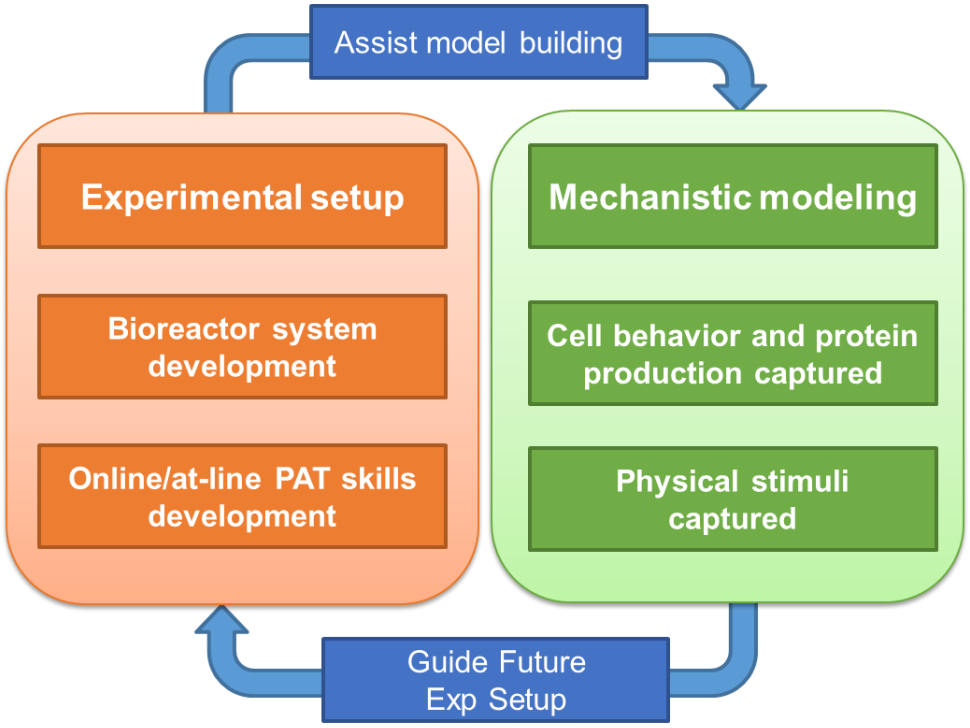
This article is a preprint and has not been certified by peer review [what does this mean?].



Future Work:

- Modulate mAb glycosylation and collect real time data to understand temporal change in mAb glycosylation
- Use N-GLYcanyzer PAT tool for real-time process control

Broader Impact:

- Enabling advanced process control through rapid N-Glycan Analysis
- Increase understanding of process design space and influence of glycosylation with critical process parameters

Contact details to support ongoing research program at Rutgers!

- **Address:**

Office:

Engineering Wing C, Room C150A

Labs:

Basement Labs C009, C003, C004
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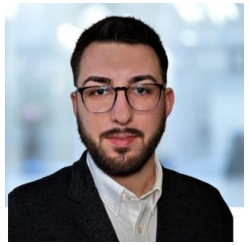


Mr. Aron Gyorgypal

- **Email:**

shishir.chundawat@rutgers.edu

- **Twitter:**

@sps_chundawat

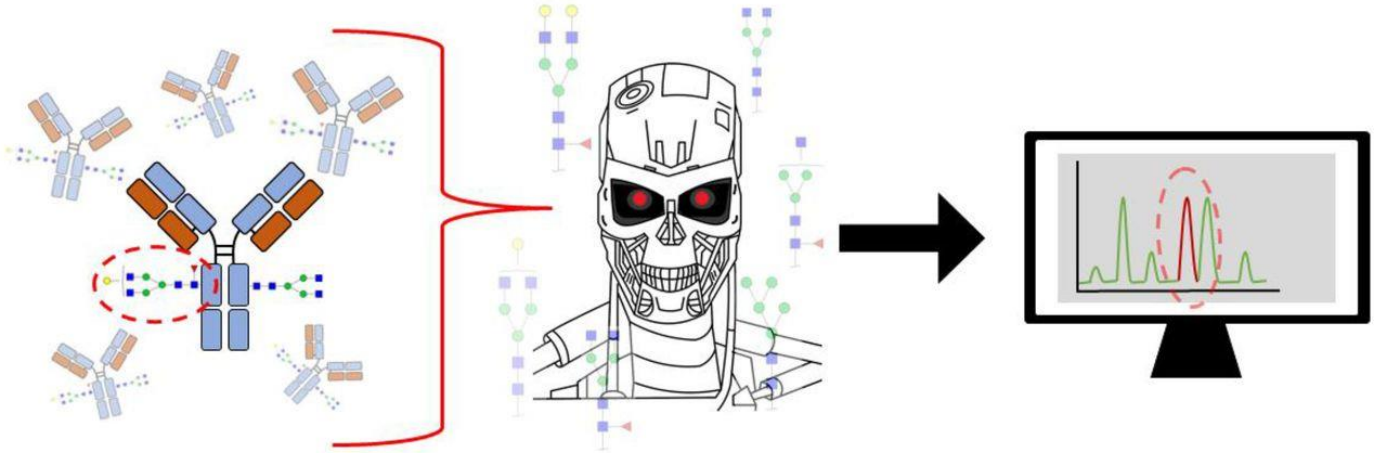
- **Office Phone:**

848-445-3678

- **Research Website Link:**

http://chundawat.rutgers.edu

N-GLYcanyzer Project Team at Rutgers University



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