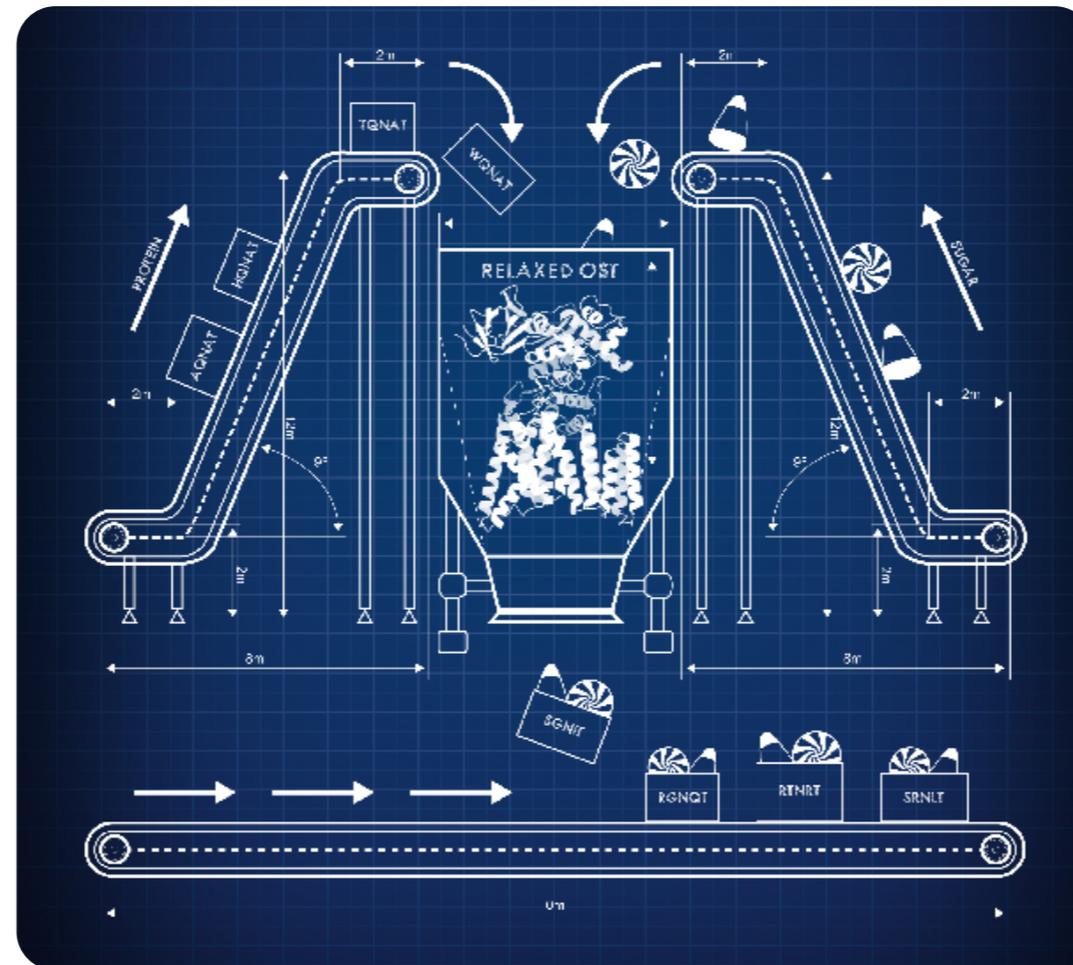


Synthetic glycobiology: designing and engineering glycomolecules inside and outside of cells



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Acknowledgements

Bacterial glycosylation



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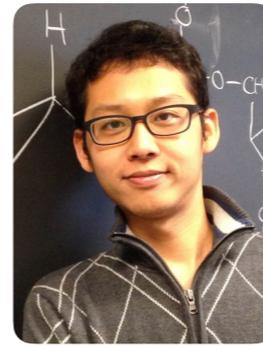


Cameron
Glasscock



Prof. Markus Aebi
(ETH-Zurich)

Cell-free glycosylation



Thapakorn
Jaroentomeechai



Jessica
Stark (NWU)



Weston
Kightlinger (NWU)



Prof. Mike Jewett
(NWU)

Biomembrane microfluidics



Alicia
Aquino



Zach
Manzer



Ferra
Pinnock



Prof. Susan Daniel
(Cornell)

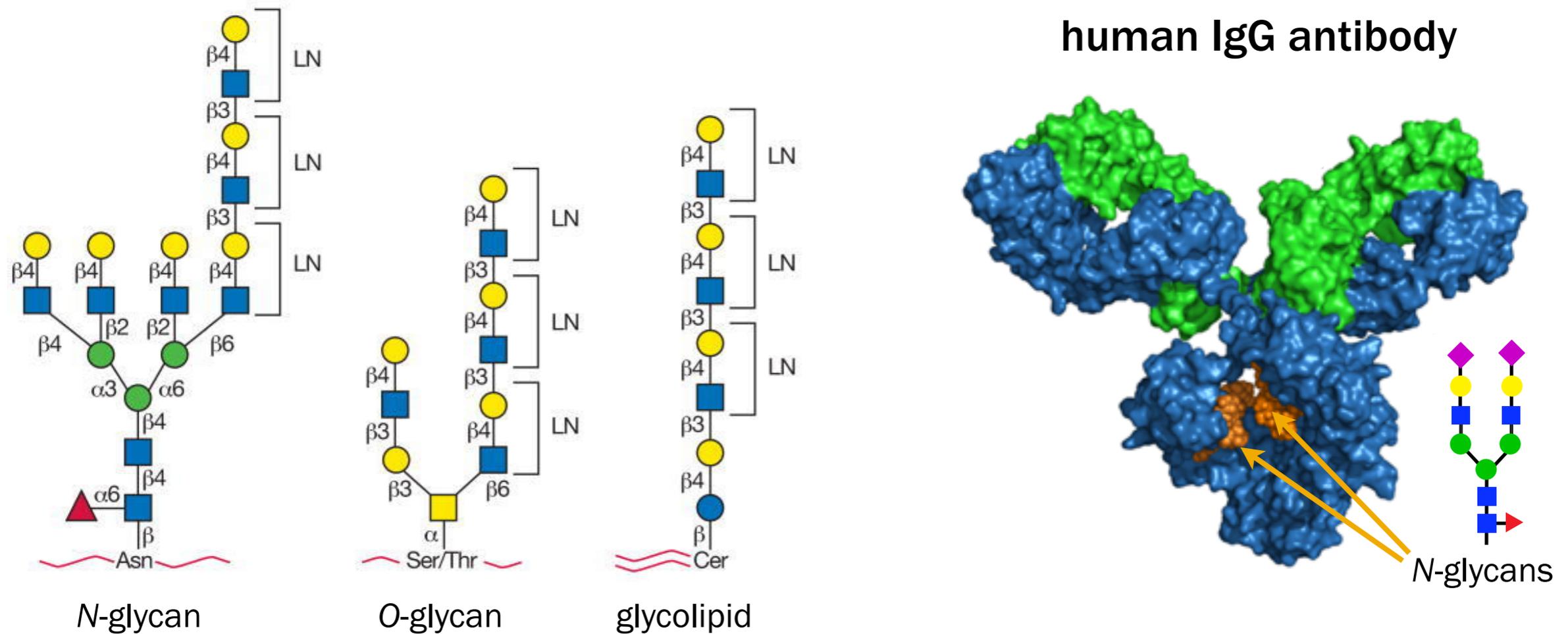
NSF funding



CMMI-1728049*
CBET-1402843
CBET-1605242
MCB-1413563

*Spatio-temporal biomanufacturing of post-translationally modified proteins using biomembrane microfluidics

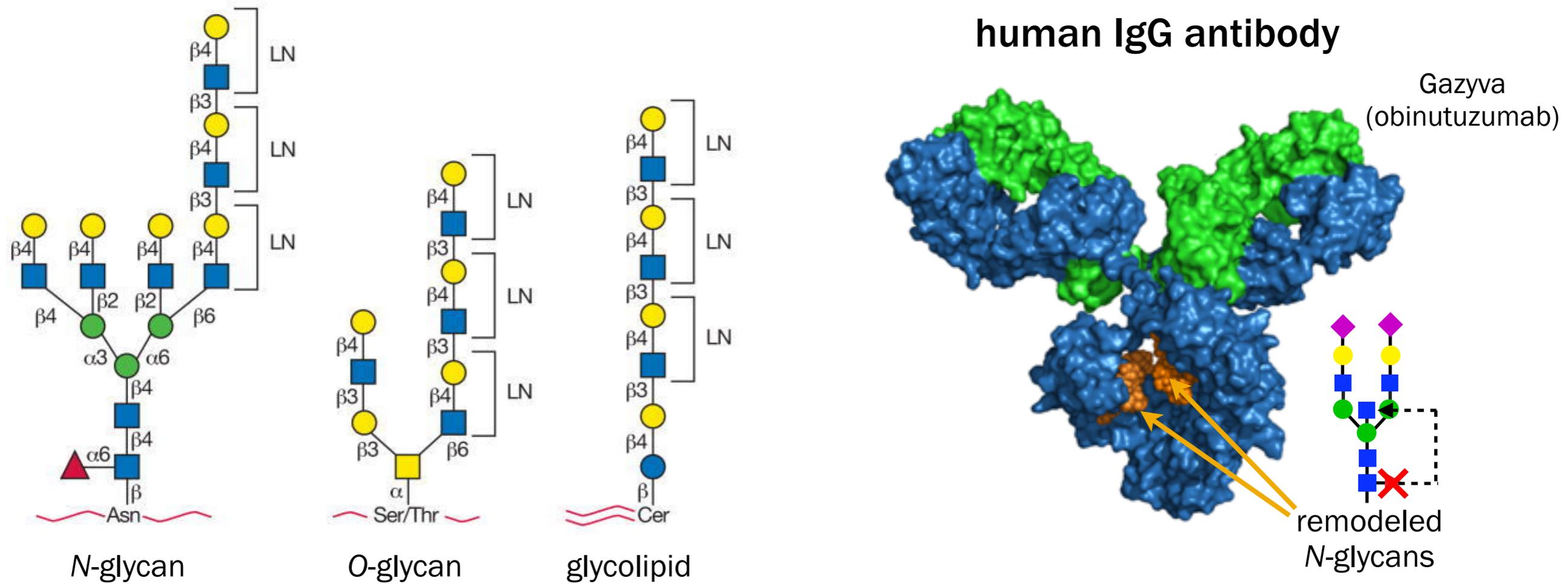
Glycosylation is an important protein modification



Stanley, Cummings et al. (2009) *Essentials of Glycobiology*

Sheridan (2007) *Nat Biotechnol*
 Jefferis (2009) *Nat Rev Drug Discov*
 Keys and Aebi (2017) *Curr Opin Syst Biol*

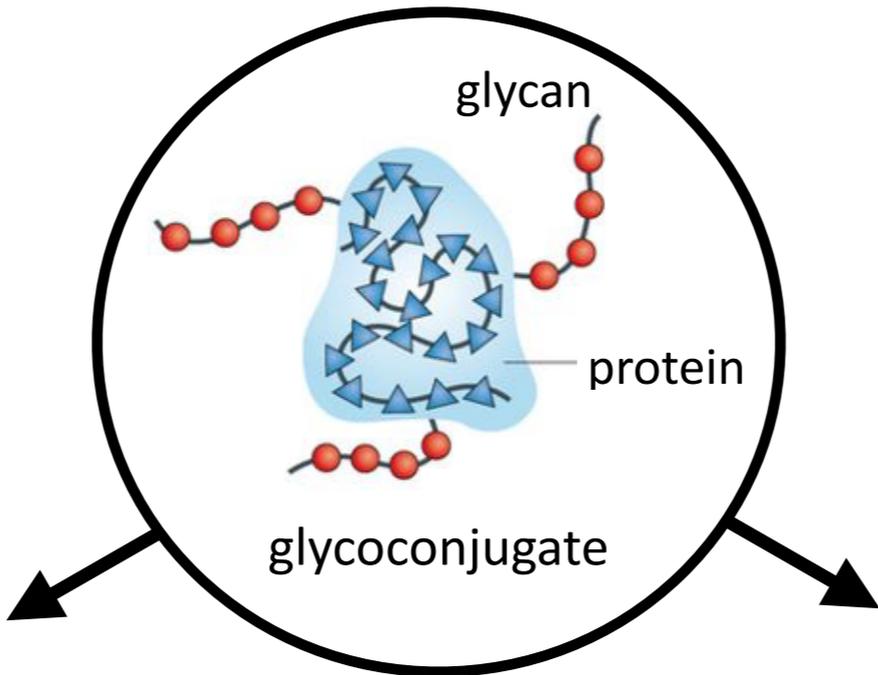
Glycosylation is an important protein modification



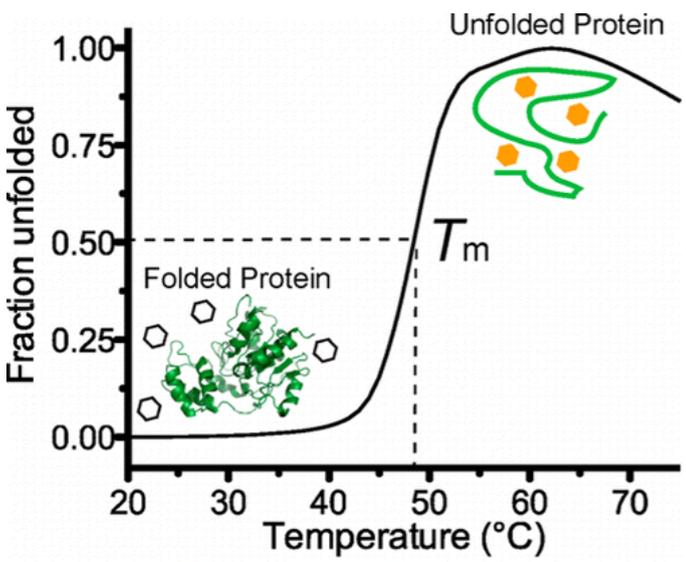
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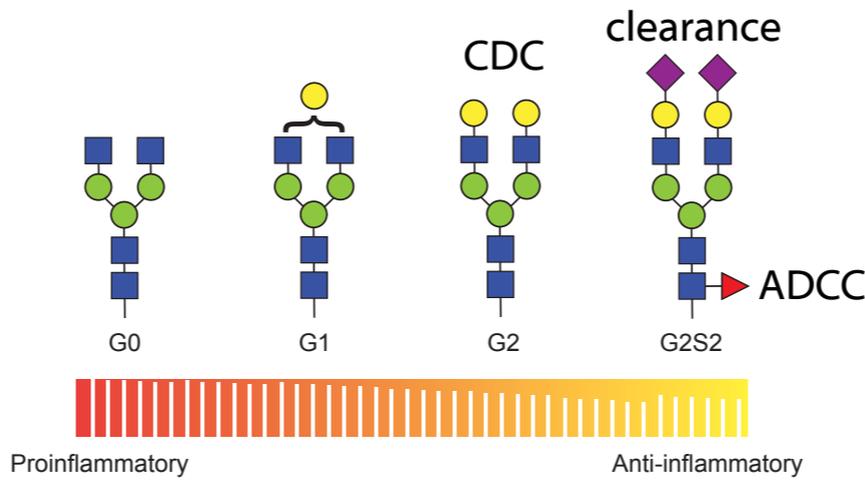
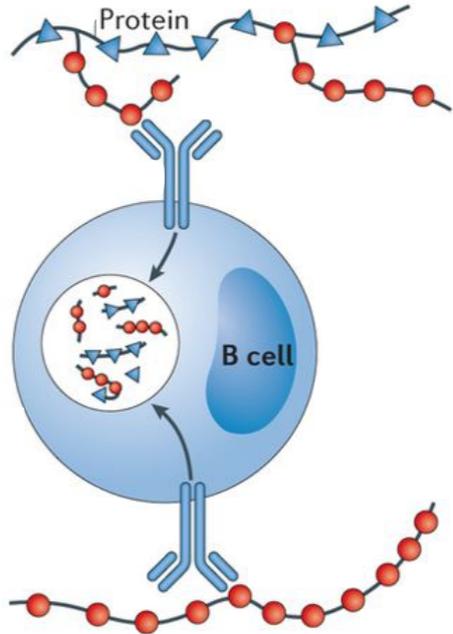
Controlling specific glycan structures is a key challenge



understanding and engineering protein function

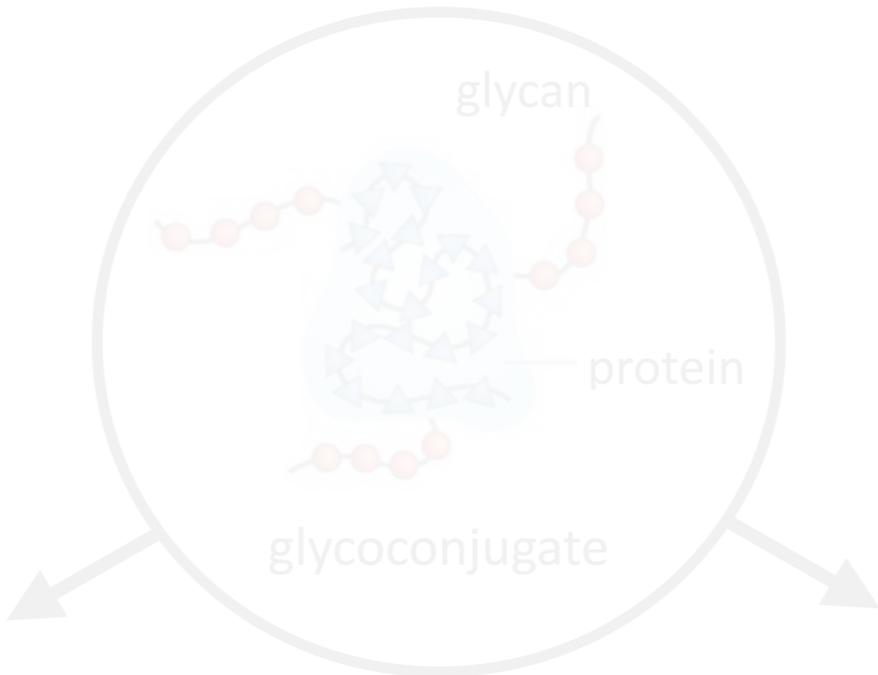


tailoring immunological responses



engineering next-generation medical reagents

Controlling specific glycan structures is a key challenge

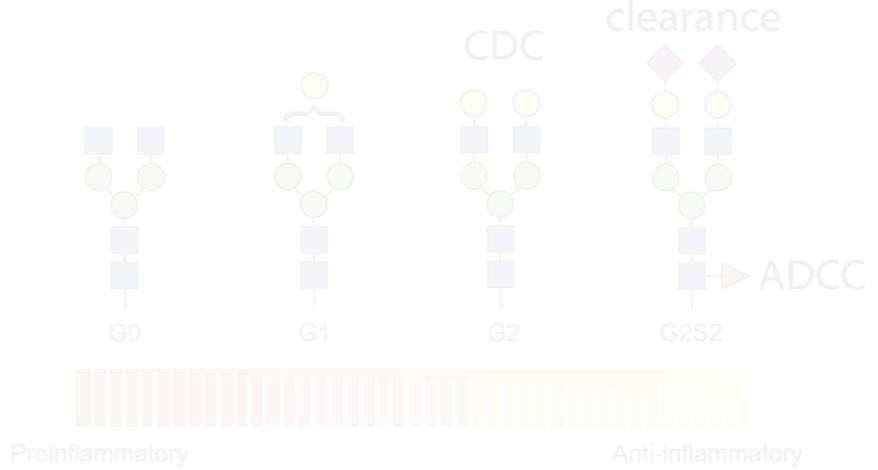
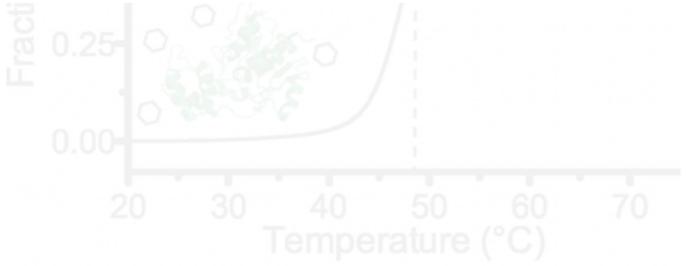


understanding and engineering protein function

tailoring immunological responses



Only a small part of glycoprotein structural space has been explored and access to novel glycoproteins is limited by available production routes

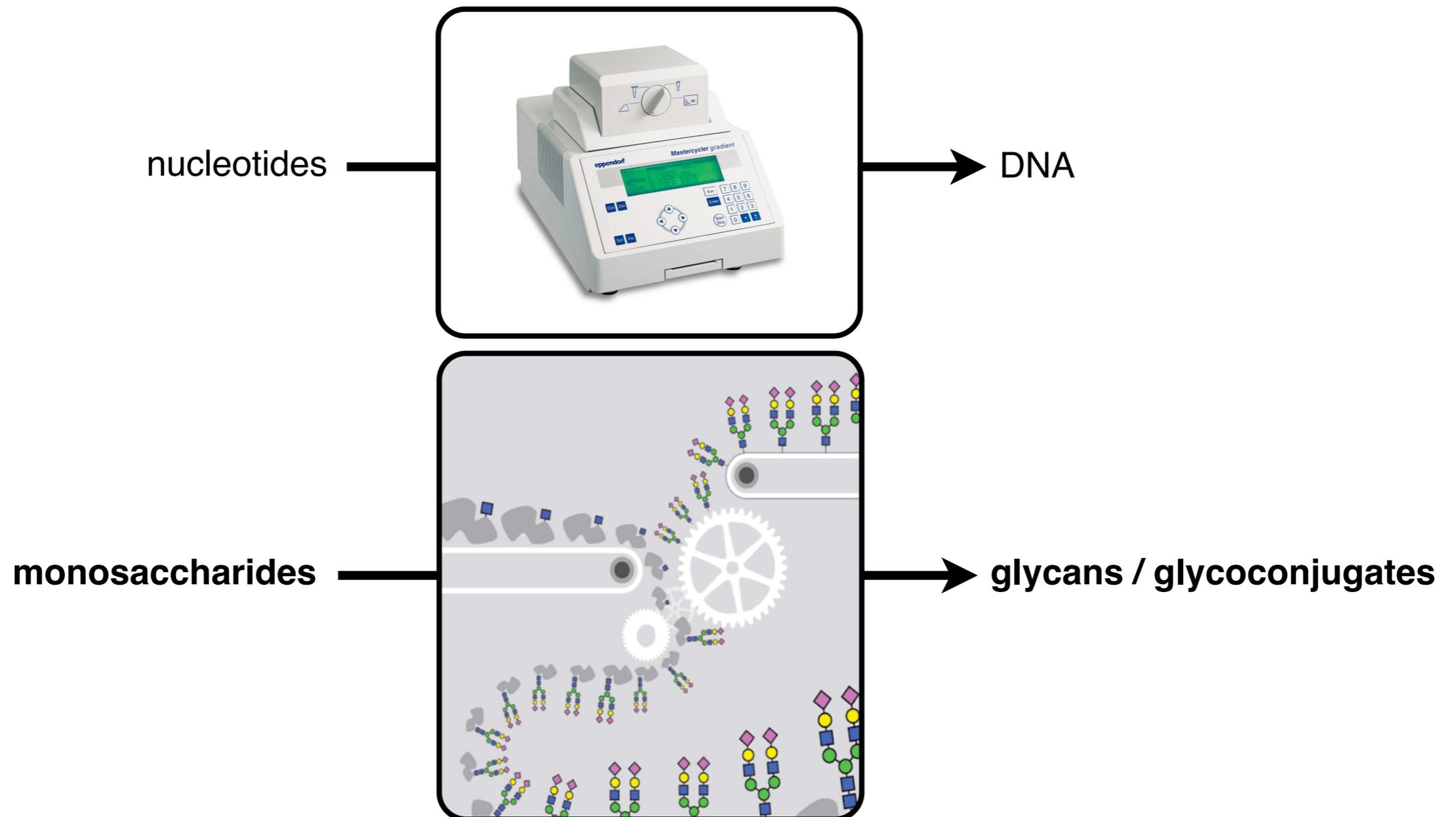


engineering next-generation medical reagents

Accelerating glycoscience: the need for new tools

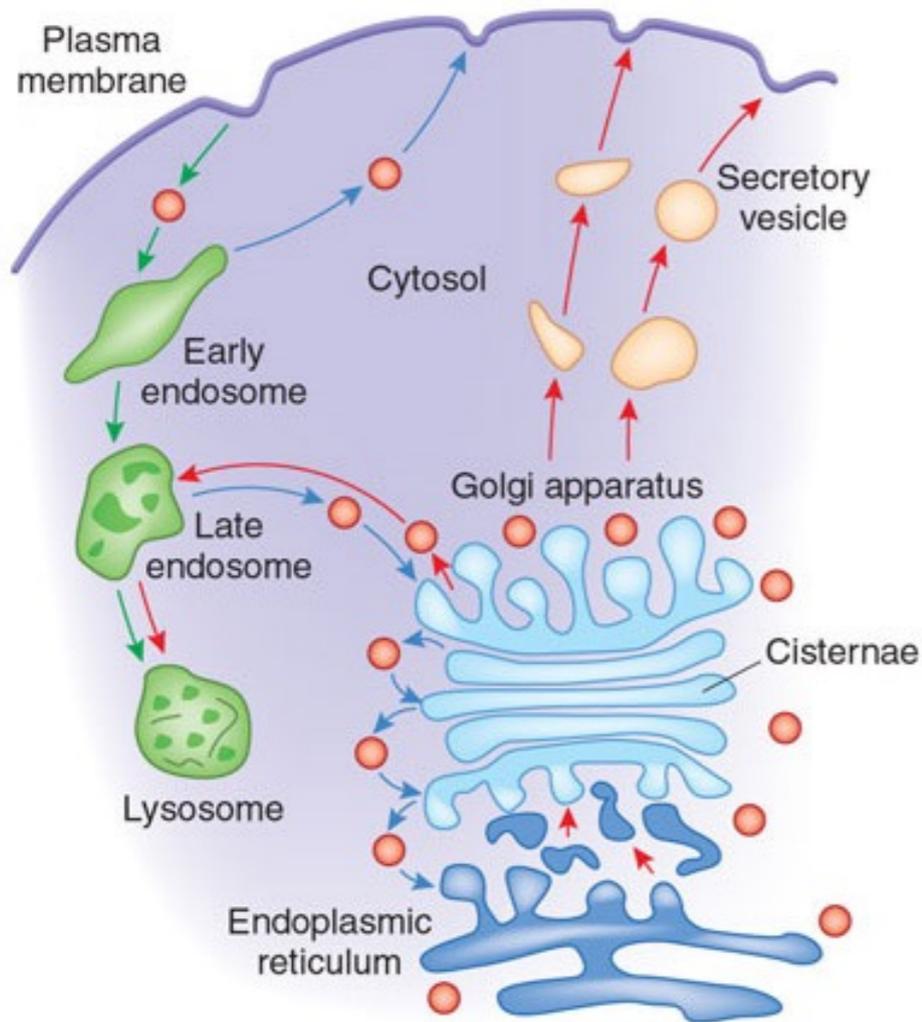
“The development of transformative methods for the **facile synthesis of carbohydrates and glycoconjugates** should be a high priority for the NIH, NSF, DOE, and other relevant stakeholders.”

-Transforming Glycoscience: A Roadmap for the Future, NRC Report 2012

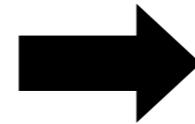
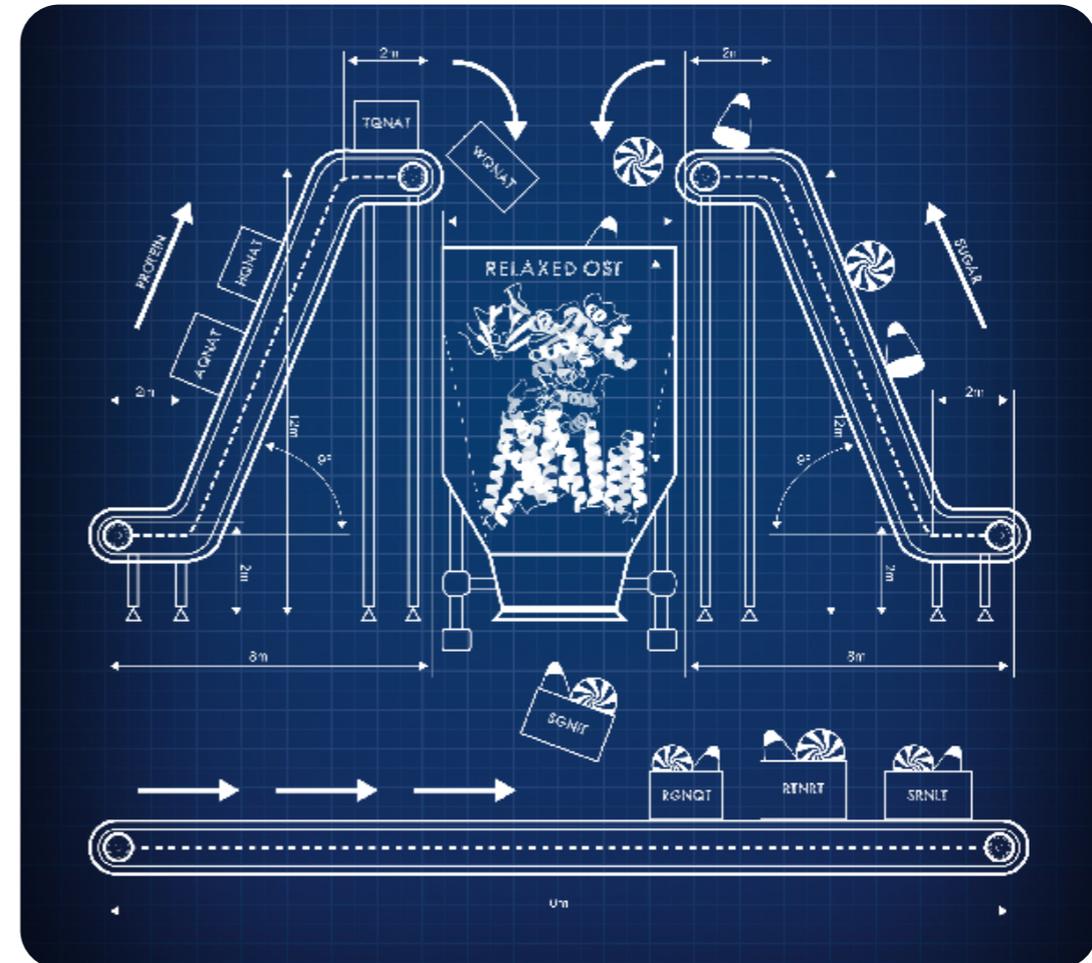


Can we re-create this complex cellular process?

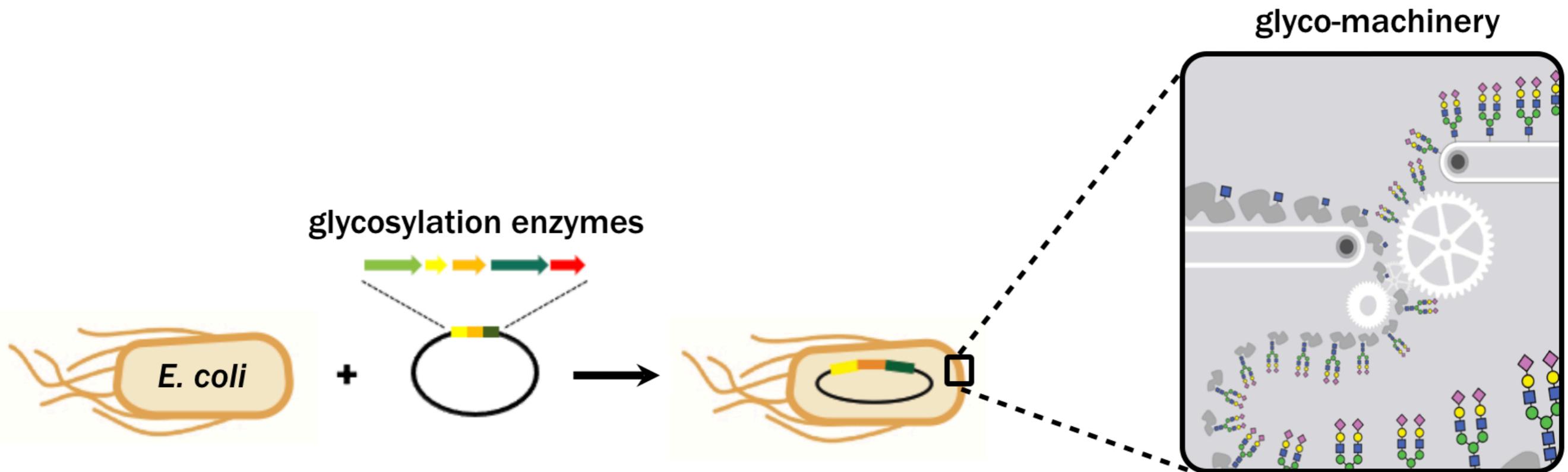
Natural glycan processing in mammalian cells



Synthetic assembly line system for controllable protein glycosylation



Reprogramming bacteria as glycoprotein factories

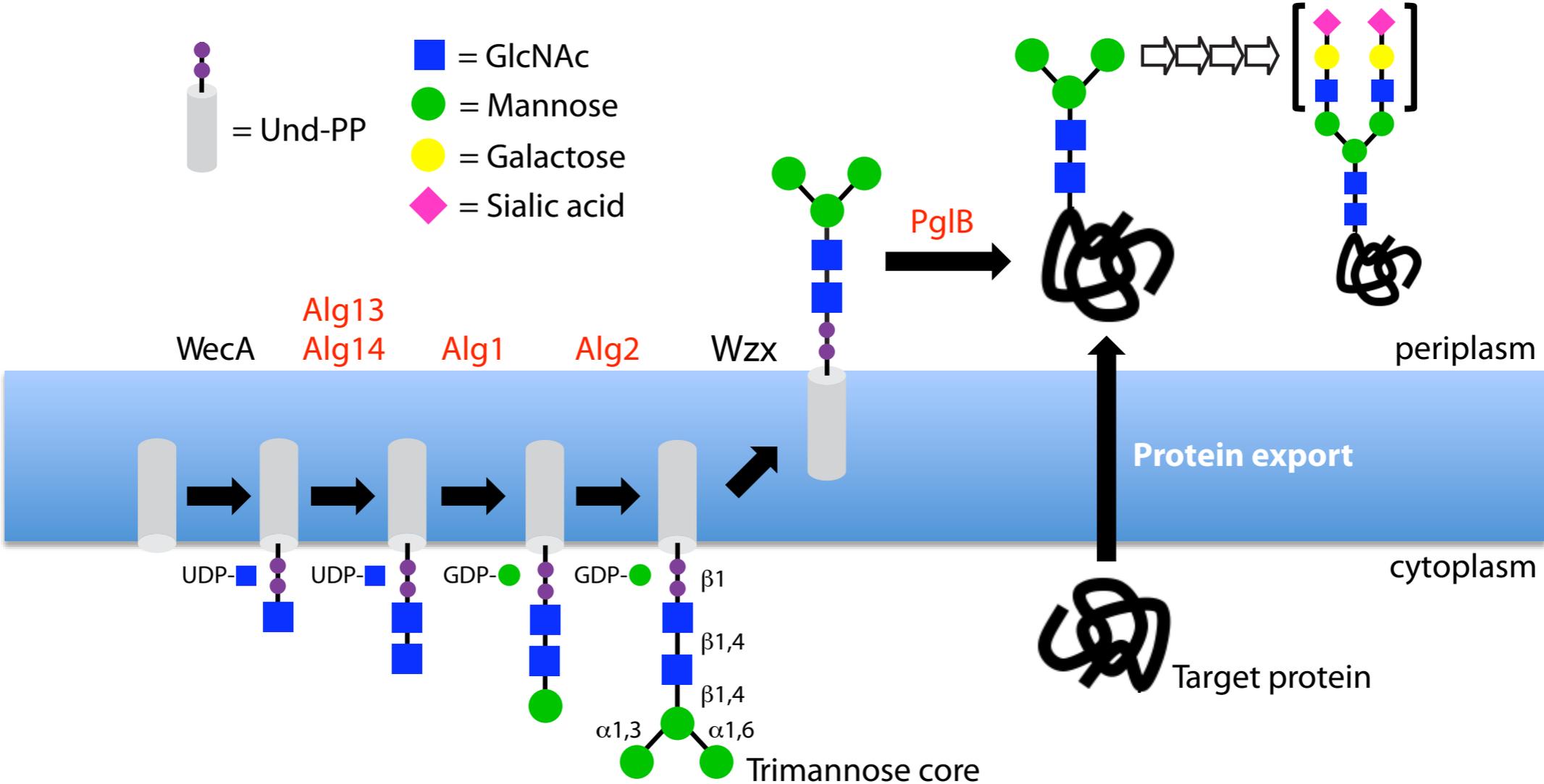


Rational design of **orthogonal glycosylation pathways** that are uncoupled from evolutionary constraints, and selectively abstracted from cellular regulation

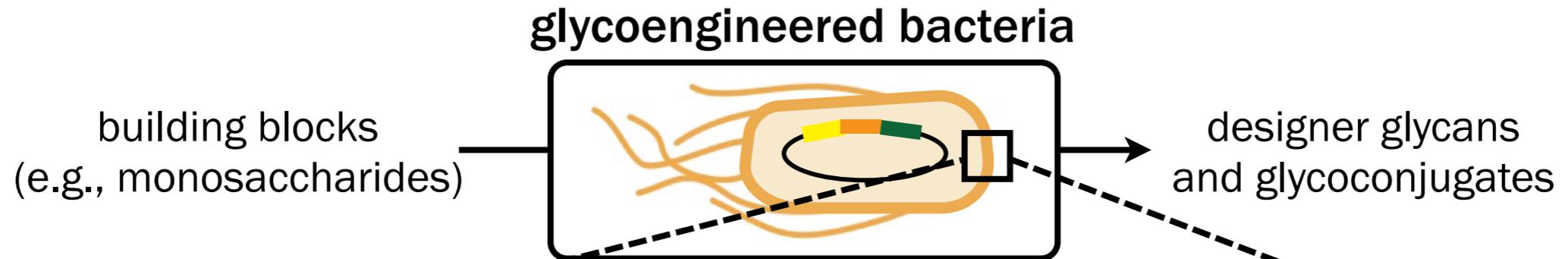
- emerging approach to making biology more amenable to engineering
- glycoforms made from recombinant DNA technology
- modular “plug and play” glycosylation systems
- versatile biosynthetic tool for glycoscience and glycomedicine
- model system to better understand glycosylation

Rational design of orthogonal glycosylation pathways

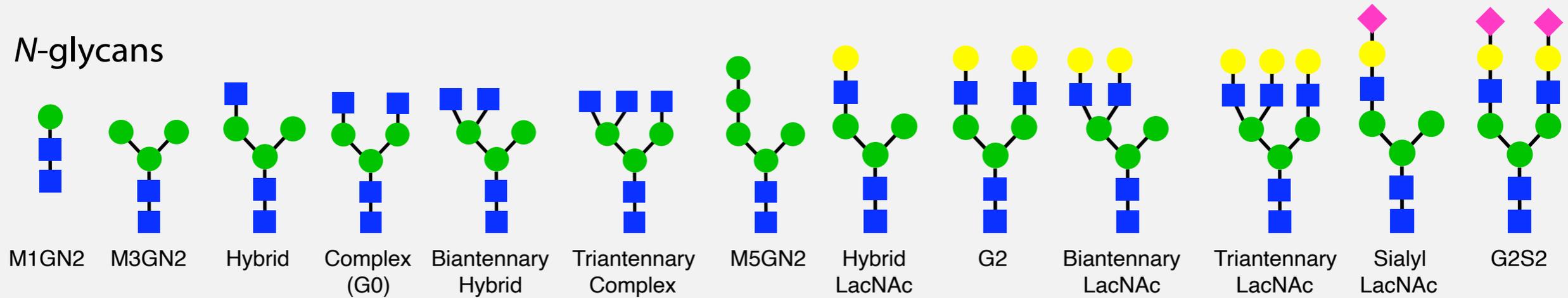
“Glycans-by-Design”



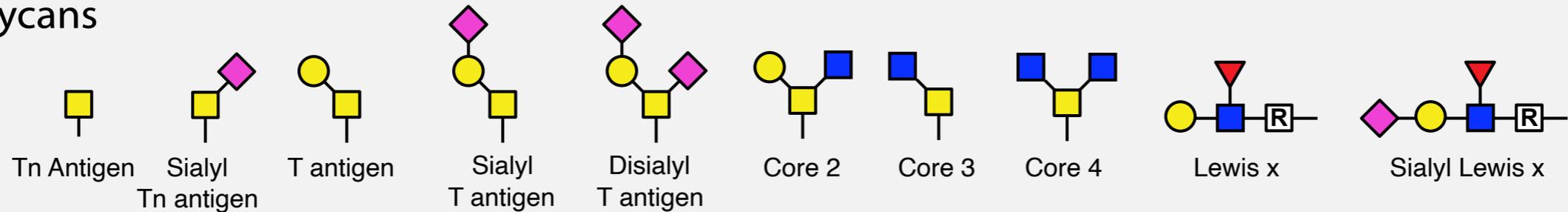
Biosynthetic routes to glycoprotein structural space



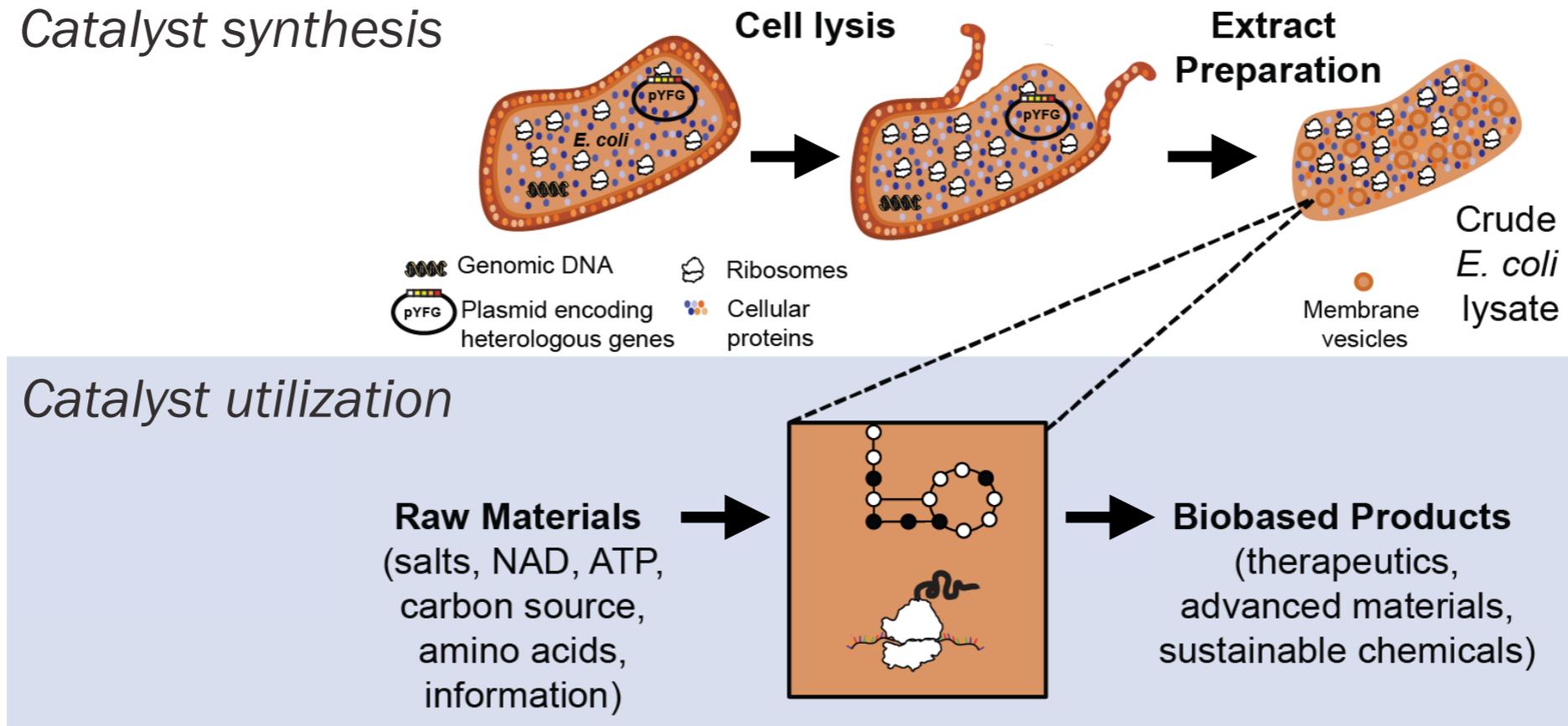
N-glycans



O-glycans



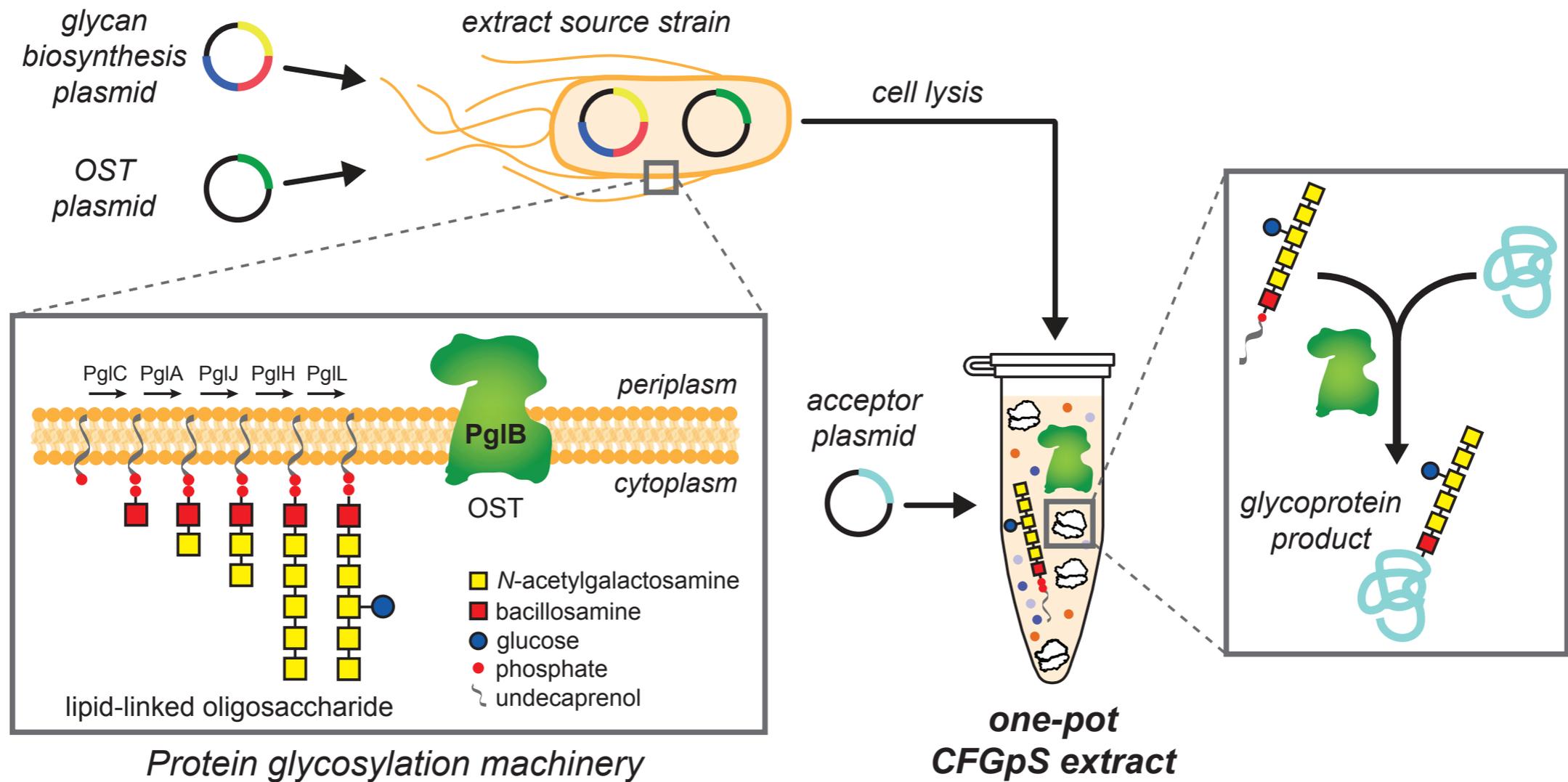
Cell-free biology is the activation of complex biological processes without using intact living cells



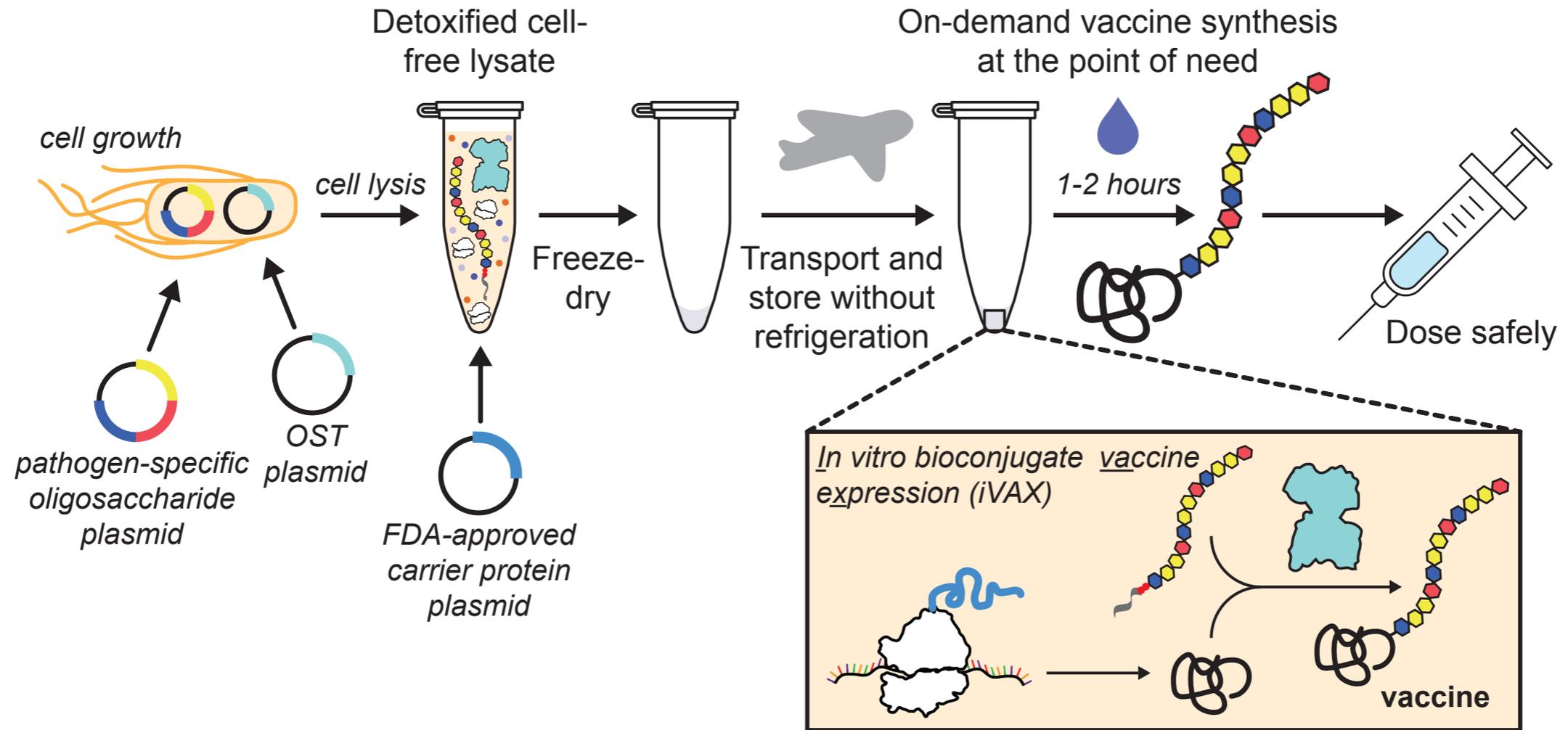
- Separate catalyst synthesis (growth) from catalyst utilization (production)
- Lack structural boundaries (i.e., direct access to the reaction)
- Direct resources towards the exclusive production of one product

Glycoengineering without borders: cell-free glycosylation

Cell-free lysates enriched with functional glycosylation machinery

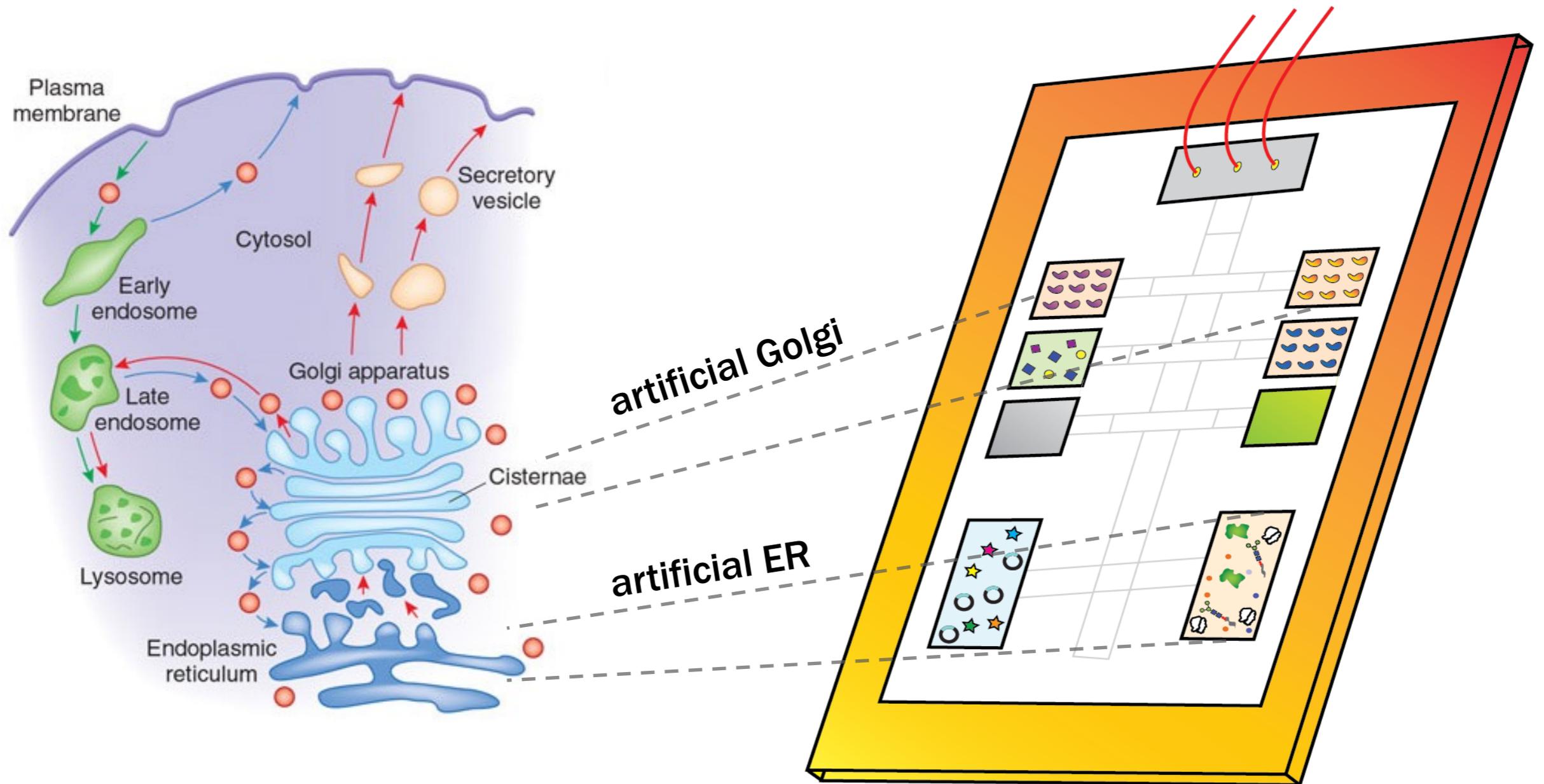


On-demand, portable, low-cost vaccine biomanufacturing

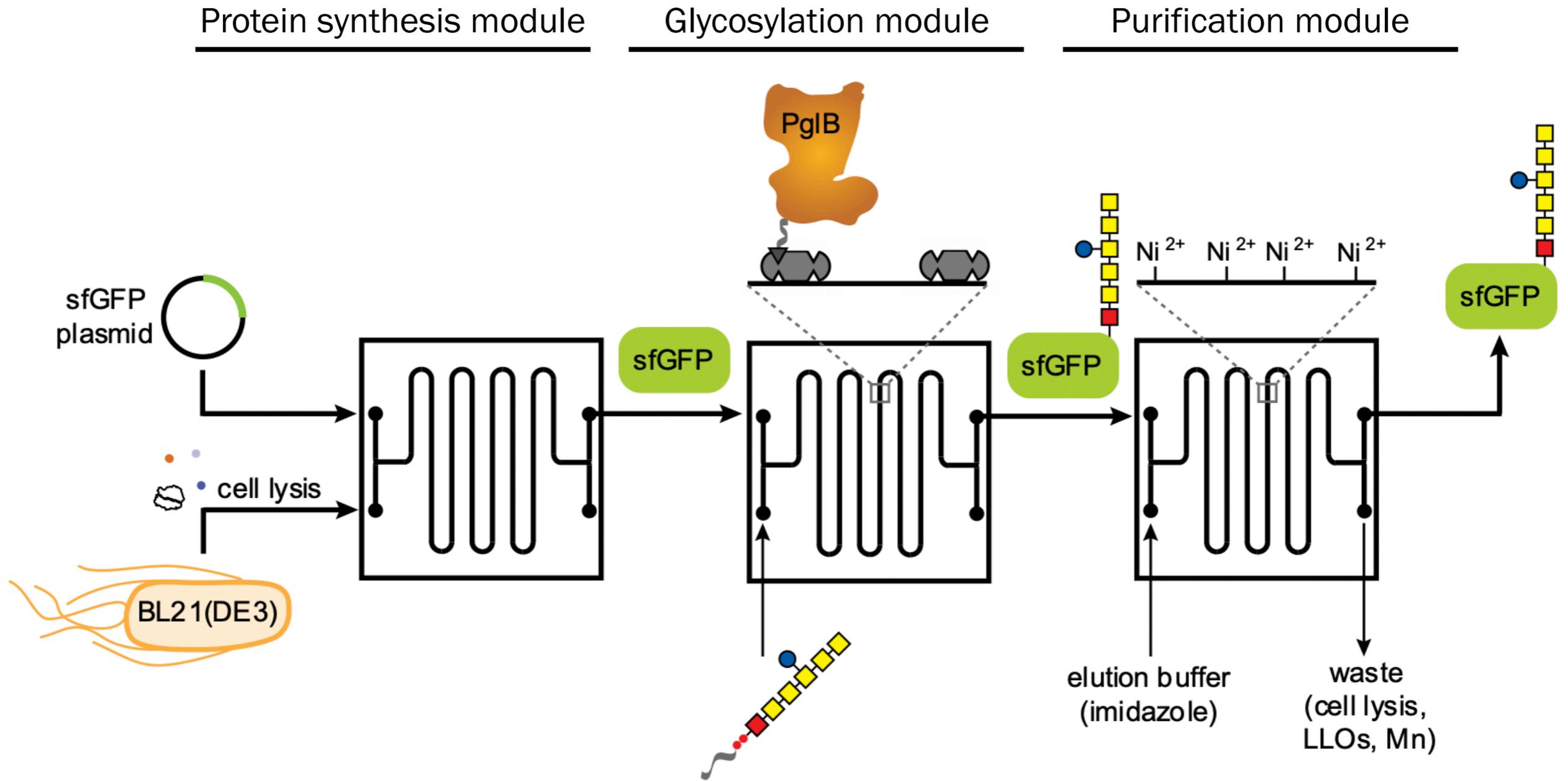


Nature-inspired glycosylation-on-a-chip

Hypothesis: spatial and temporal organization of protein glycosylation pathways can be recapitulated using biomembrane microfluidic device

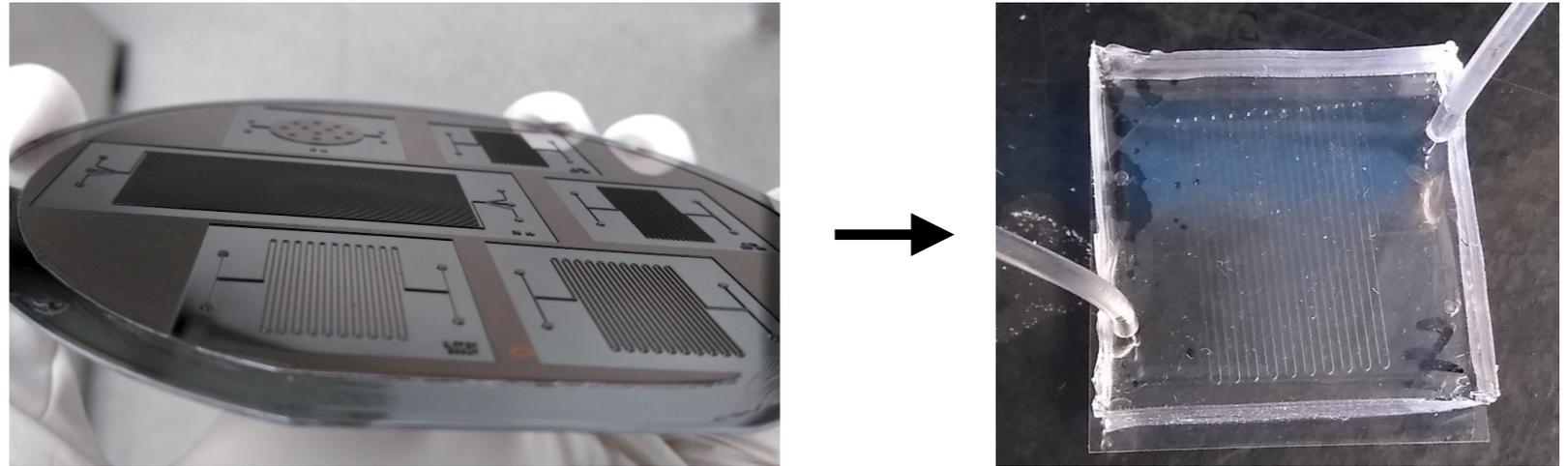


First-generation glycosylation-on-a-chip technology

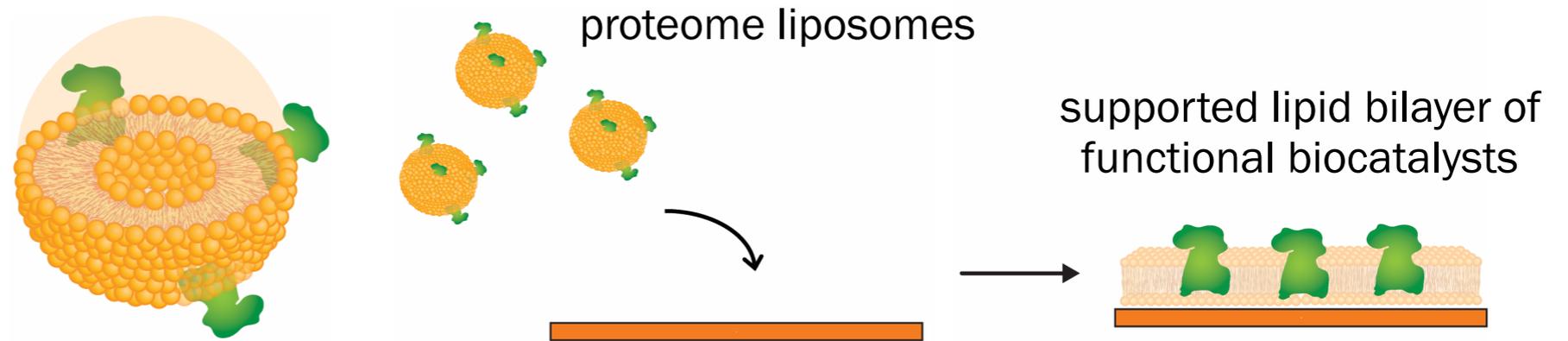


Design-build-test cycles for glycosylation-on-a-chip

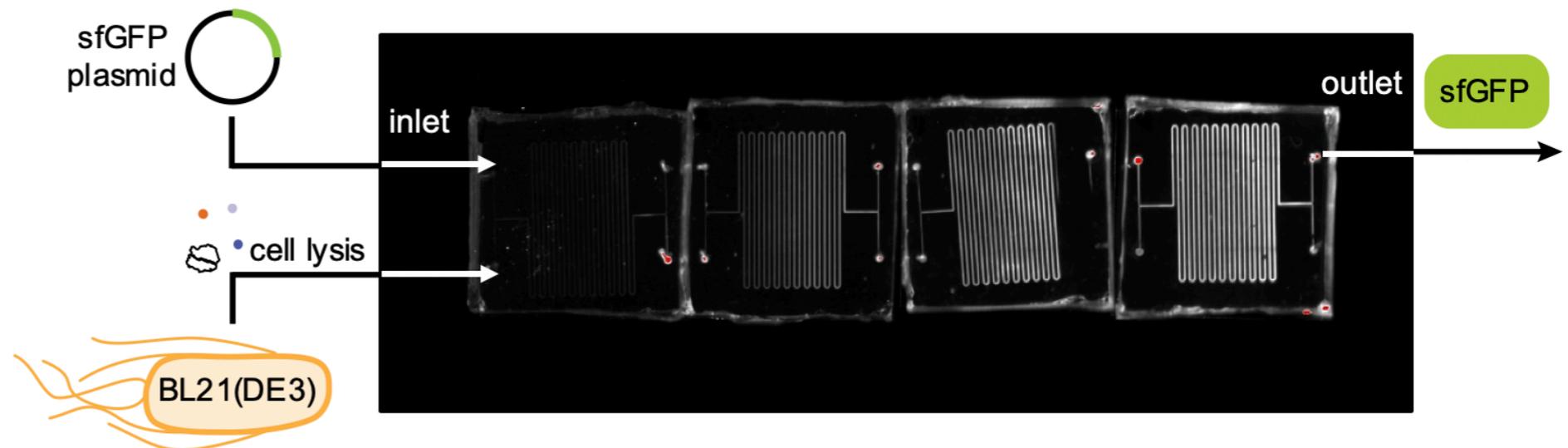
Device fabrication



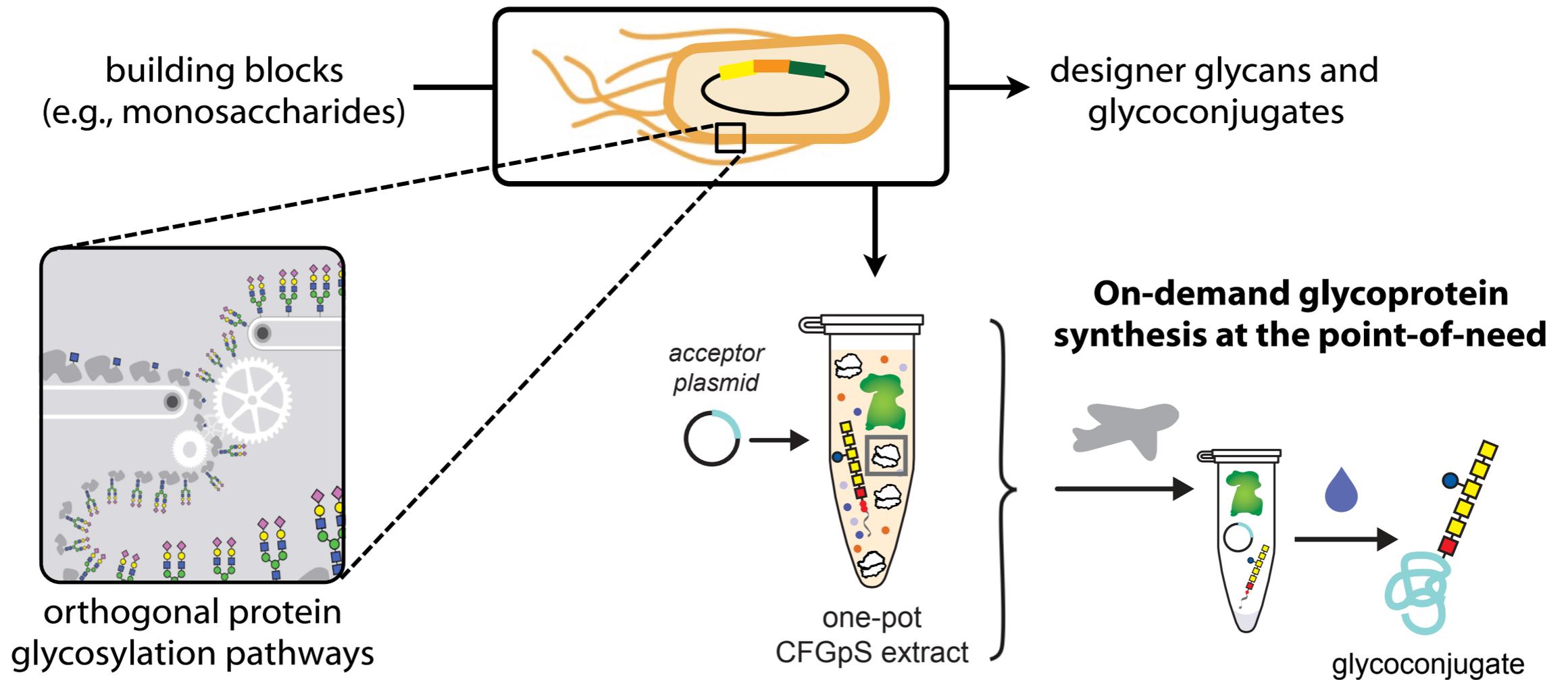
Molecular integration



Prototype evaluation



Summary



Bacteria as living factories for **orthogonal protein glycosylation systems**

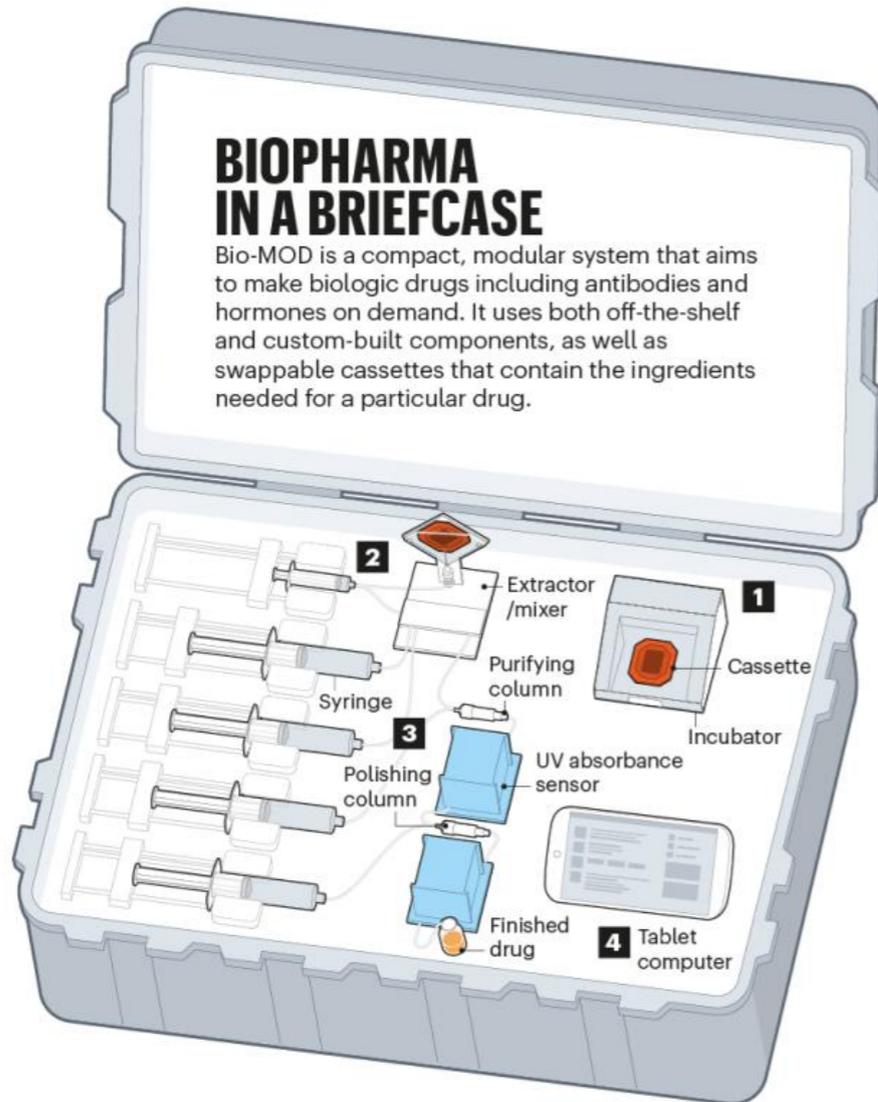
Cell-free biosynthesis of glycoproteins via lysates enriched with glycosylation machinery

Integration of cell-free glycosylation with nanotechnology yields **glycosylation-on-a-chip**

Synthetic systems for **understanding rules of life**

Future directions...

Medicines-on-Demand



Protocell Engineering

