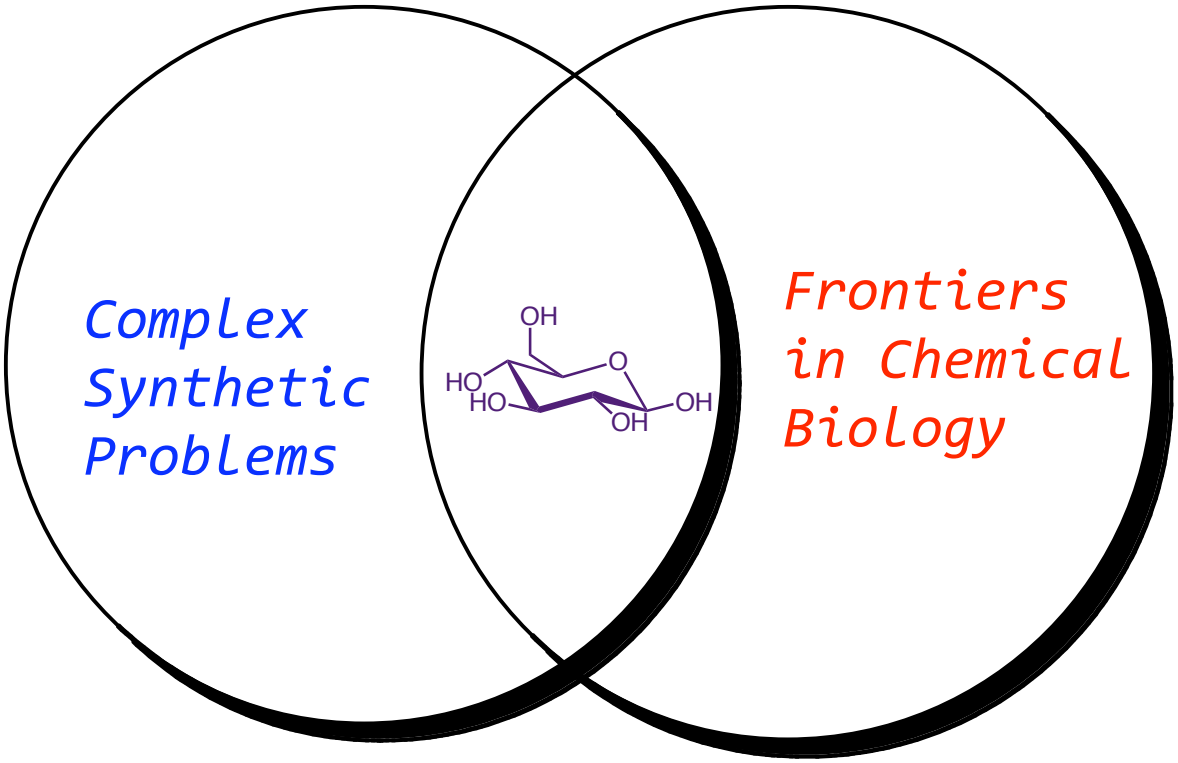
Challenges and Strategies in the Synthesis and Study of Glycans



Melissa M. Sprachman
October 15, 2011

Presentation Outline

- History and Nomenclature
- Current Significance of Glycochemistry/glycobiology
- Strategies for and Challenges in Glycan Synthesis
- Methods for Studying Glycans
- Glycan Perception: The Application of Bioorthogonal Chemistry

Historical Discoveries in Glycan Chemistry

1891 (Nobel prize in 1902)

H. Emil Fischer: structural proof of glucose and other monosaccharides

1916

J. MacLean: heparin isolation and use as an anticoagulant

1929

P.A. Levene: structure of 2-deoxyribose in DNA

1929 (Nobel prize in 1937)

W.N. Haworth: monosaccharide ring structures (pyranose, furanose)

1936 (Nobel prize in 1947)

C.F. Cori and G.T. Cori: role of glucose-1-phosphate in glycogen synthesis

1949 (Nobel prize in 1970)

L. Leloir: role of nucleotide sugars in glycan biosynthesis

1961-1965 (Nobel prize in 1974)

G.E. Palade: ER-Golgi pathway for glycoprotein biosynthesis and secretion

1970

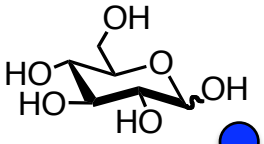
K.O. Lloyd, J. Porath, I. J. Goldstein: affinity purification of glycoproteins using lectins

1986

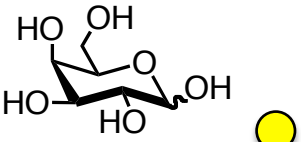
P.K. Qasba, J. Shaper, N. Shaper: cloning of the first animal glycosyltransferase

Essentials of Glycobiology; Varki, A.; Cummings, R. D.; Esko, J. D.; Freeze, H. H.; Stanley, P.; Bertozzi, C. R.; Hart, G. W.; Etzler, M. E., Eds., 2009.

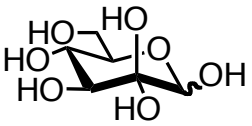
Residues in Mammalian Cells



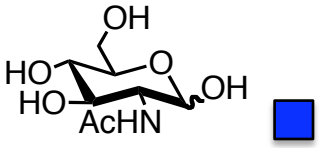
Glucose (Glc)



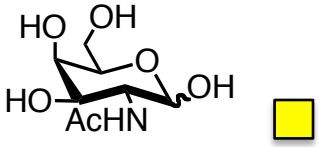
Galactose (Gal)



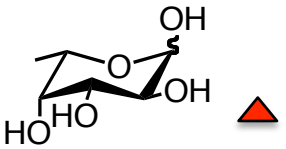
Mannose (Man)



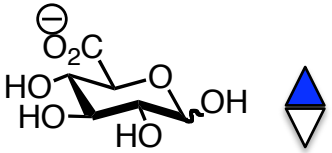
N-acetylglucosamine (GlcNAc)



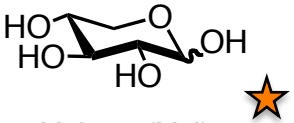
N-acetylgalactosamine (GalNAc)



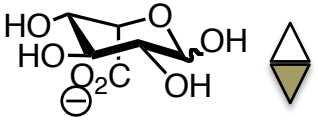
Fucose (Fuc)



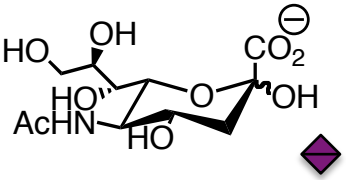
Glucuronic acid (GlcA)



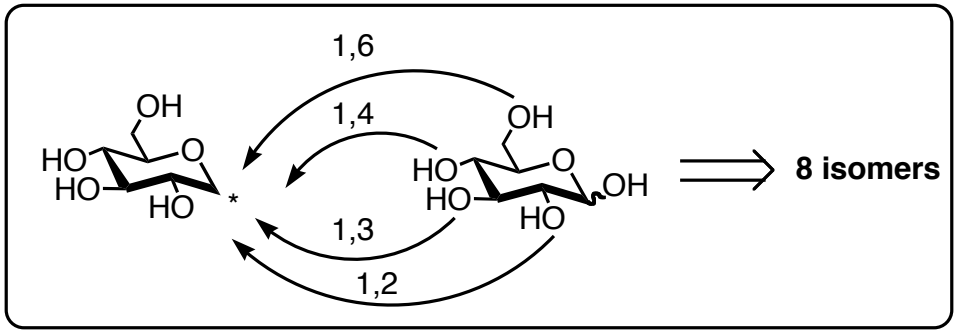
Xylose (Xyl)

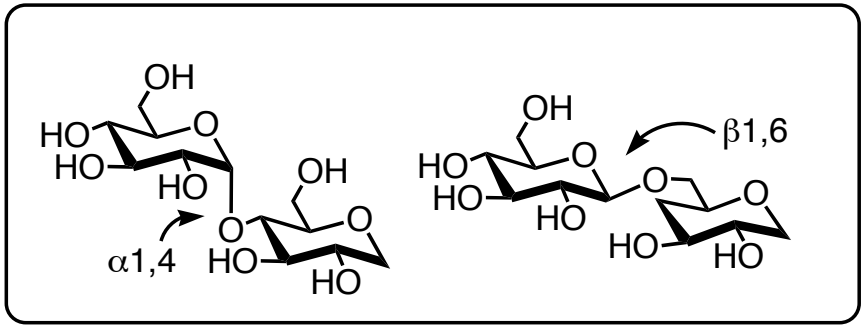


Iduronic acid (IdA)



Sialic acid (Sia)





"Chemical Glycobiology: why now?"*

"Carbohydrates have long been underappreciated by the scientific community, and many scientists approach the complex structures and elaborate nomenclature of carbohydrates with trepidation."

-Joshua Finkelstein (Nature, Senior Editor 2007)

" Progress in understanding and exploiting the molecular basis of carbohydrate recognition is hampered by the lack of a direct link between genome sequence and carbohydrate structure. Access to complex carbohydrate structures would facilitate their study, but facile and scalable production of such structures remains a major roadblock." (Richard Field, 2011)

*Seeberger, P. H. *Nature Chem. Biol.* **2009,** *5,* 368-372.
Finkelstein, J. *Nature* **2007,** *446,* 999.
Field, R. *Nature Chem. Biol.* **2011,** *7,* 658-659.

"Chemical Glycobiology: why now?"*

"But many researchers still express frustration when glycans are implicated at the nexus of their system of study. One fundamental problem is that glycans have complex, branched structures and are intrinsically heterogeneous. Thus, the vast majority of glycoproteins, which are estimated to comprise 50% of eukaryotic proteomes, have not been well-characterized at a molecular level.

In cases where the structural details of protein-associated glycans are defined, their functions are still mostly unknown. Our current view of glycobiology therefore remains largely descriptive and focused at the cellular, rather than the molecular, level."

Prescher, J. A.; Bertozzi, C.R. *Cell* **2006,** *126,* 851-854.

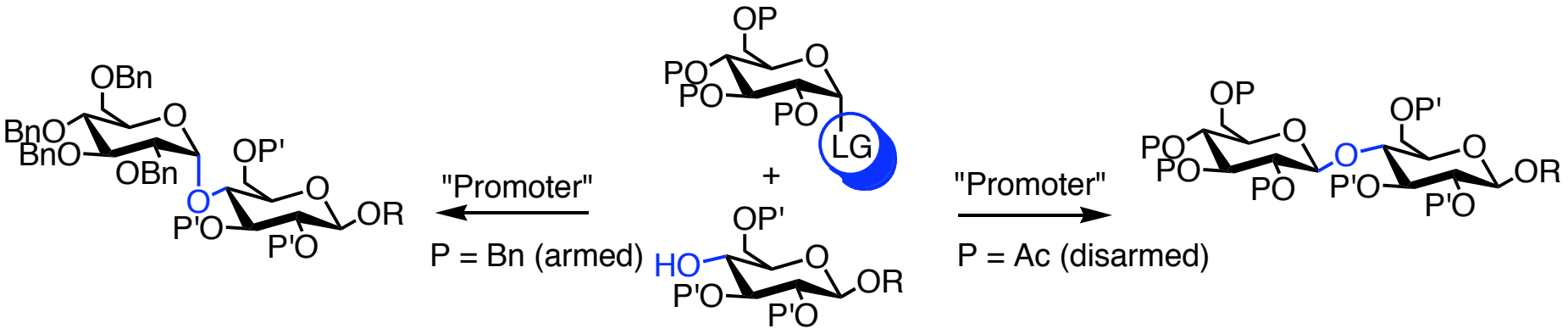
In Summary….

- Glycosylation events are post- or co-translational modifications
- Glycans have immense structural complexity and diversity
- Synthetic access to complex glycans is still a burden
- The functions of many glycans are yet to be determined

Part I. Current Methods and Opportunities in Oligosaccharide Synthesis

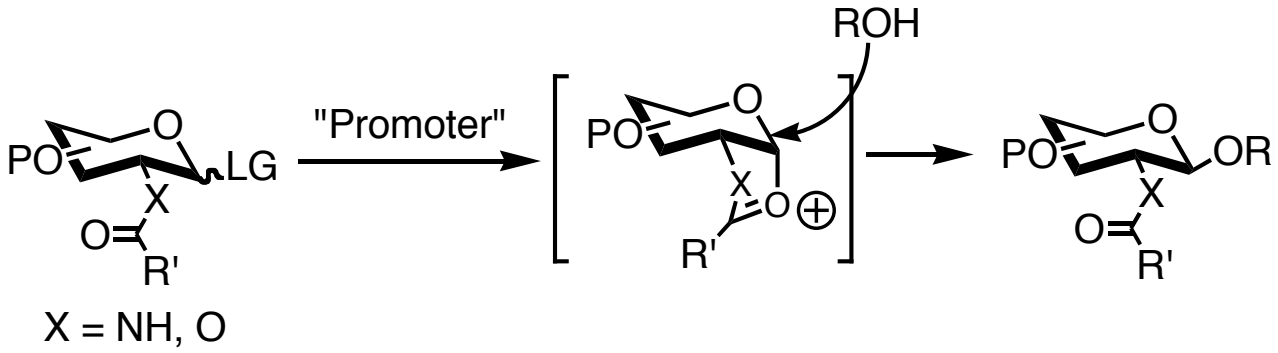
Oligosaccharide Synthesis (Classical Approaches)

Glycosyl Bond Formation:



Mootoo, D.R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B, *J. Am. Chem. Soc.* **1988,** *110,*583-5584.

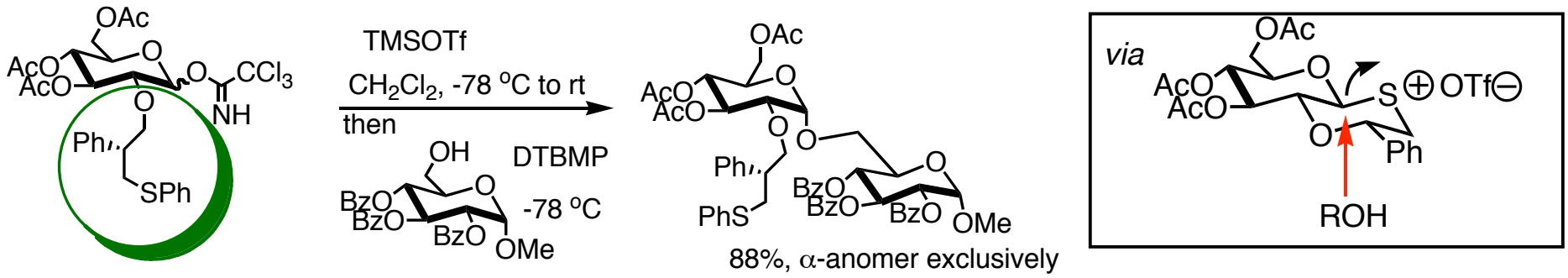
Neighboring Group Participation:



Kiessling, L. L.; Splain, R. A. *Annu. Rev. Biochem.* **2010,** *79,* 619-653.

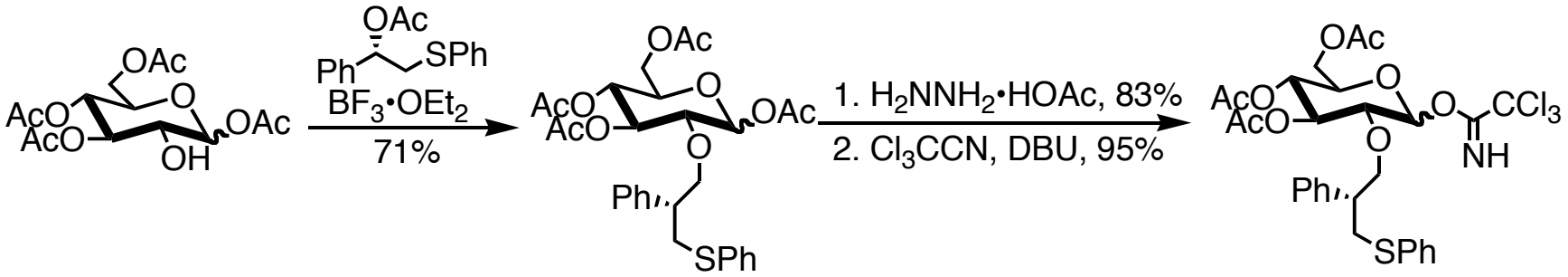
New Techniques in Oligosaccharide Synthesis

A New Take on NGP: the (*S*)-(phenylthiomethyl)benzyl moeity:



DTBMP = 2,6-di-*t*-butyl-4-methylpyridine

The "PG" is easily introduced:

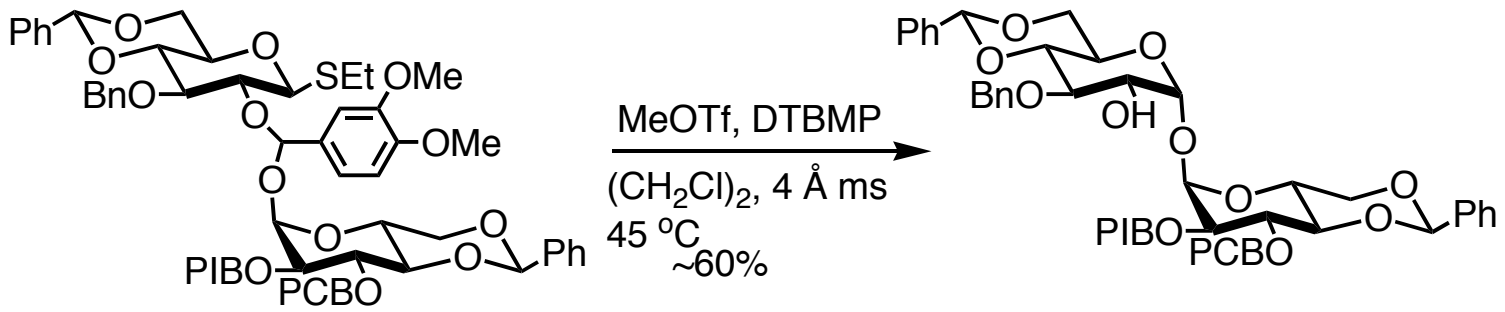


The group is readily removed using BF_3•OEt_2 and HOAc.

Kim, J. H.; Yang, H.; Park, J.; Boons, G. J. *J. Am. Chem. Soc.* **2005,** *127,* 12090-12097.

Additional "Tricks" for Controlling Selectivity

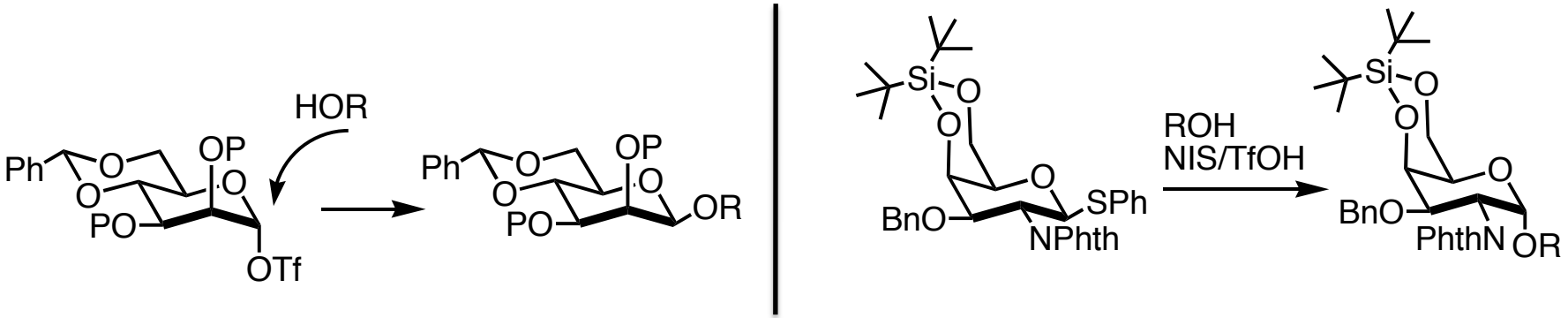
Intramolecular aglycon delivery (IAD) (tethering):



PIB = *p*-iodobenzyl
PCB = *p*-chlorobenzyl

Leigh, C. D.; Bertozzi, C.R. *J. Org. Chem.* **2008,** *73,* 1008-1017.

Steric and electronic effects:



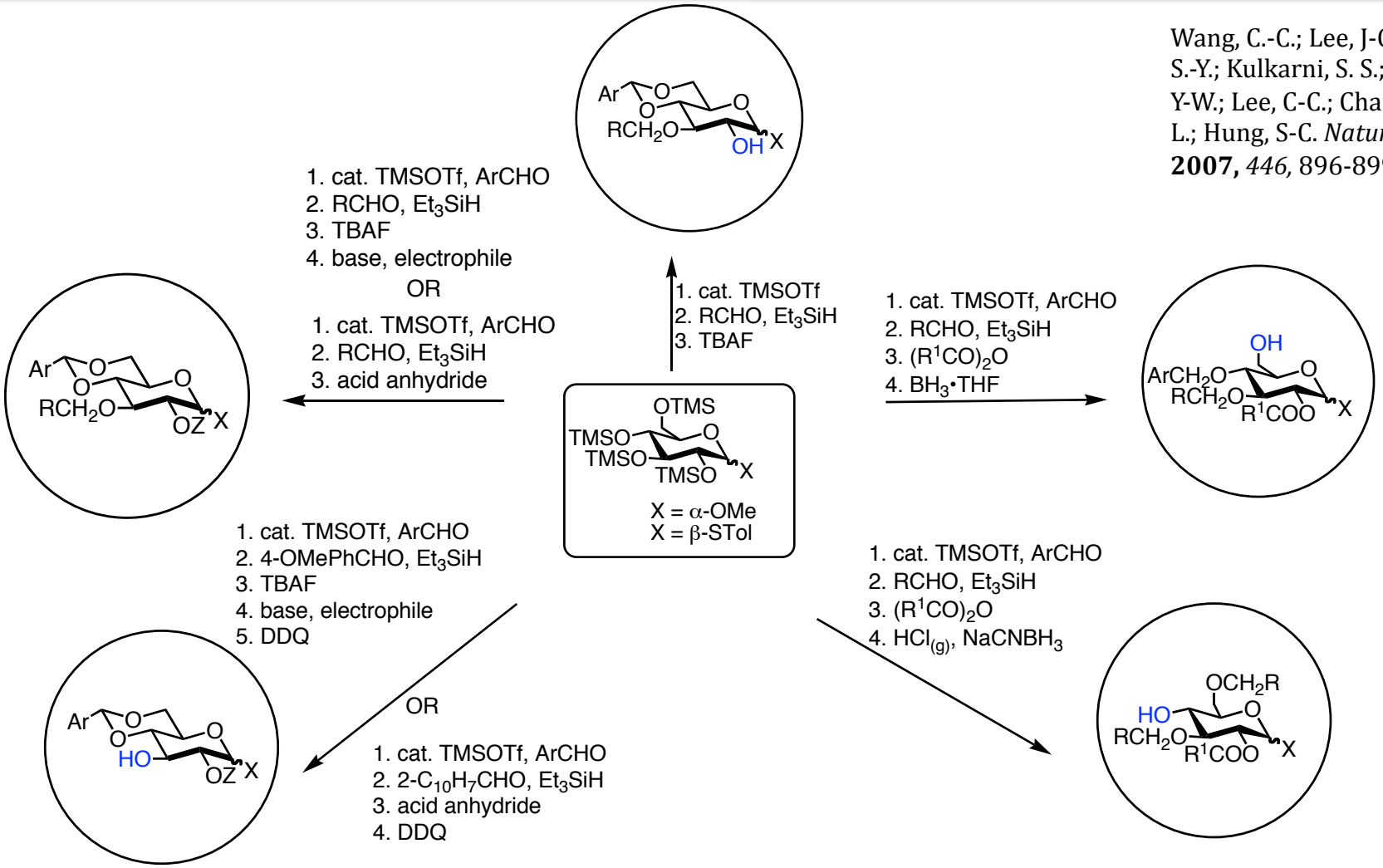
Boltje, T. J.; Buskas, T.; Boons, G.-J. *Nature Chem.* **2009,** *1,* 611-622.

Frontiers in Oligsaccharide Synthesis: Not an attempt to reinvent the wheel

- 1 Pot Reactions
- Streamlined/orthogonal protecting group strategies
- Polymer-supported oligosaccharide synthesis
- Chemoenzymatic synthesis

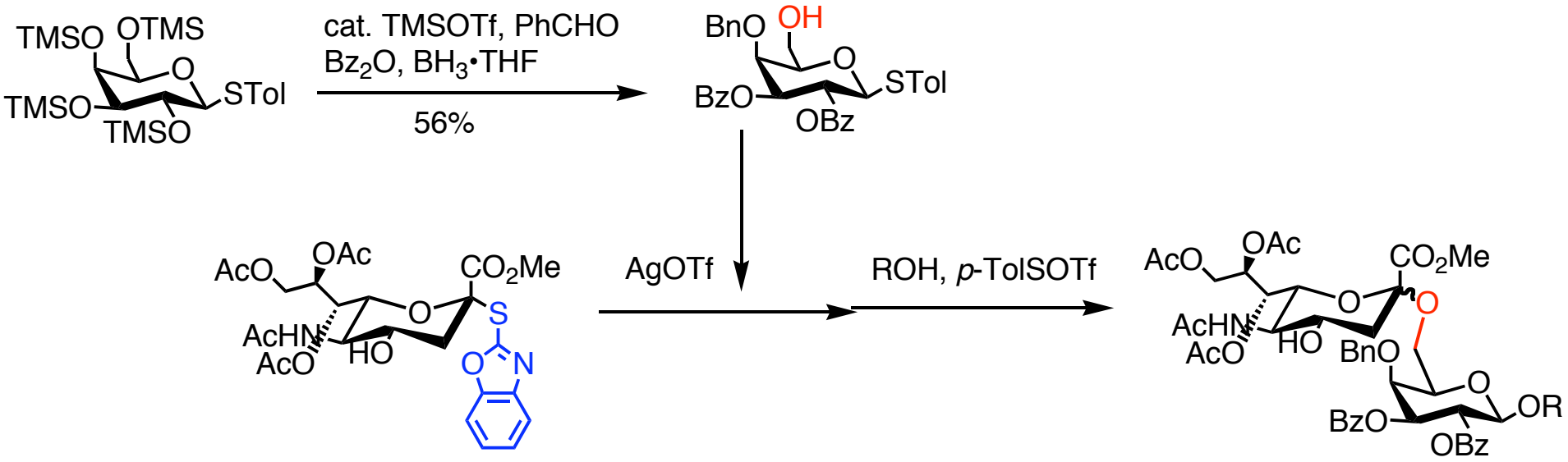
Reviews: Boltje, T. J.; Buskas, T.; Boons, G.-J. *Nature Chem.* **2009,** *1,* 611-622.
Chemoenzymatic synthesis of oligosaccharides: Kadokawa, J. *Chem. Rev.* **2011,** *111,* 4308-4345.

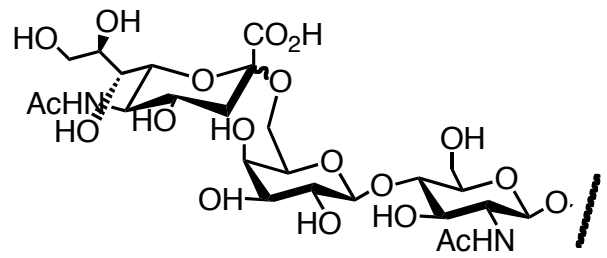
One Pot Protection and Glysosylation Strategies



Wang, C.-C.; Lee, J-C.; Luo, S.-Y.; Kulkarni, S. S.; Huang, Y-W.; Lee, C-C.; Chang, K-L.; Hung, S-C. *Nature,* **2007,** *446,* 896-899.

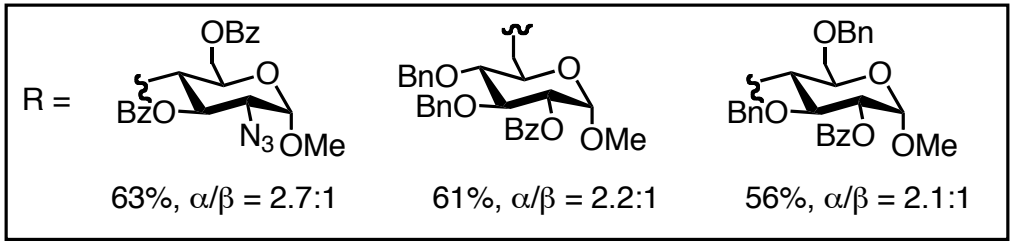
Application of One-Pot Methods to Oligosaccharide Synthesis





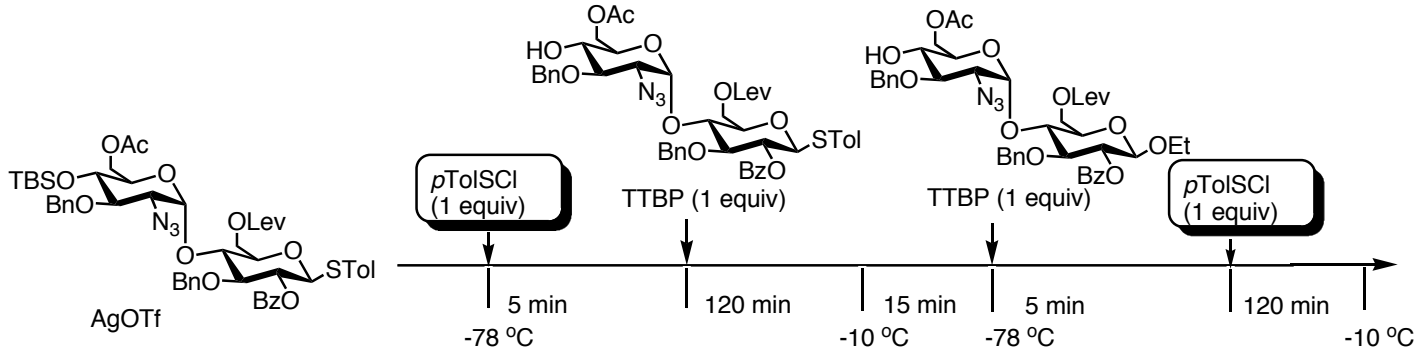
TrisaccharideSAα2,6Galβ1,4GlcNHAc

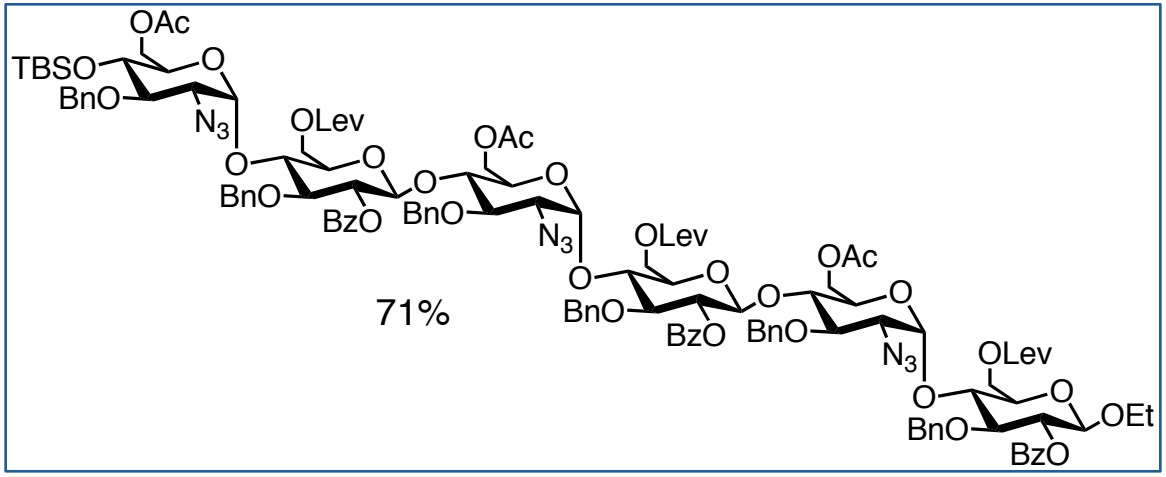
-identified as a host-cell receptor for haemagglutinin binding during viral infection of the H5N1 avian influenza virus



Wang, C.-C.; Lee, J-C.; Luo, S.-Y.; Kulkarni, S. S.; Huang, Y-W.; Lee, C-C.; Chang, K-L.; Hung, S-C. *Nature,* **2007,** *446,* 896-899.

Combinatorial Synthesis of Heparin Analogs





The authors applied this methodology to the synthesis and evaluation of libraries of heparin and heparin sulfate analogs.

Wang, Z.; Xu, Y.; Yang, B.; Tiruchinapally, G.; Sun, B.; Liu, R.; Dulaney, S.; Liu, J.; Huang, Z. *Chem. Eur. J.* **2010,** *16,* 8365-8375.

Solid-Supported Oligosaccharide Synthesis

- A fully automated synthetic system for oligosaccharides is a reachable goal, but it is yet to be attained.

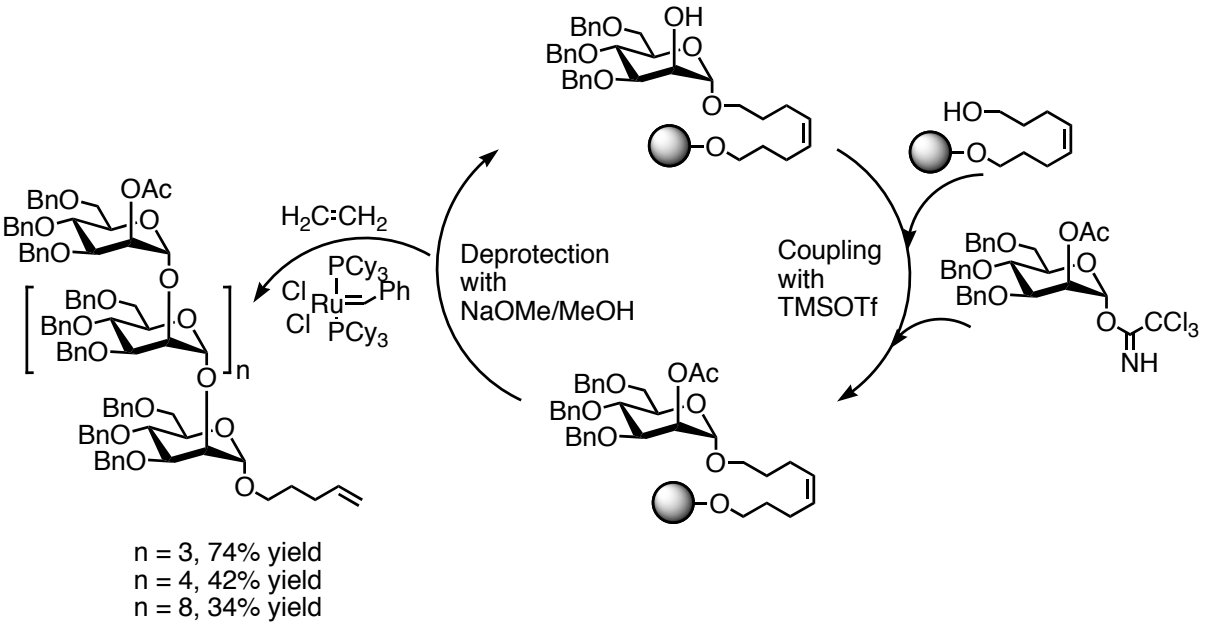
- Oligosaccharide synthesis has the problems of stereogenic centers and multiple reactive sites not found in peptide or nucleotide synthesis

- Using bioinformatic analysis, Seeberger and co-workers have shown that ~75% of the mammalian glycome could come from just 36 monosaccharide building blocks. A set of 65 monosaccharide building blocks would be required to produce 90% of mammalian structures.

Werz, D. B.; Ranzinger, R.; Herget, S.; Adibekian, A.; von der Lieth, C.-W.; Seeberger, P. H. *ACS Chem Biol.* **2007,** *2,* 685-691.

Automated Oligosaccharide Synthesis

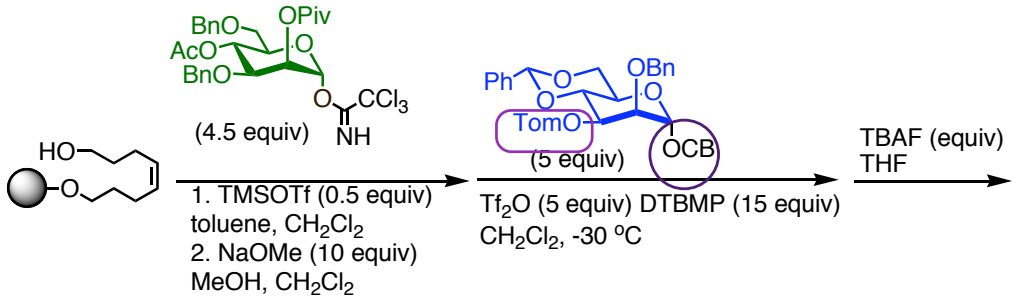
- The first automated synthesis was reported by Seeberger and coworkers in 2001 (Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science,* **2001,** *291,* 1523):

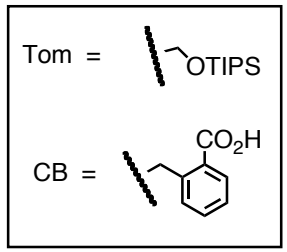


Seeberger, P. H. *Chem. Soc. Rev.* **2008,** *37,* 19-28.

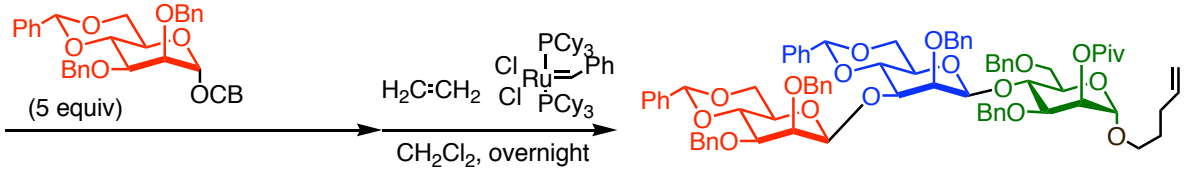
Challenging Bonds with Solid Supports

Installation of the β-mannosidic linkage:



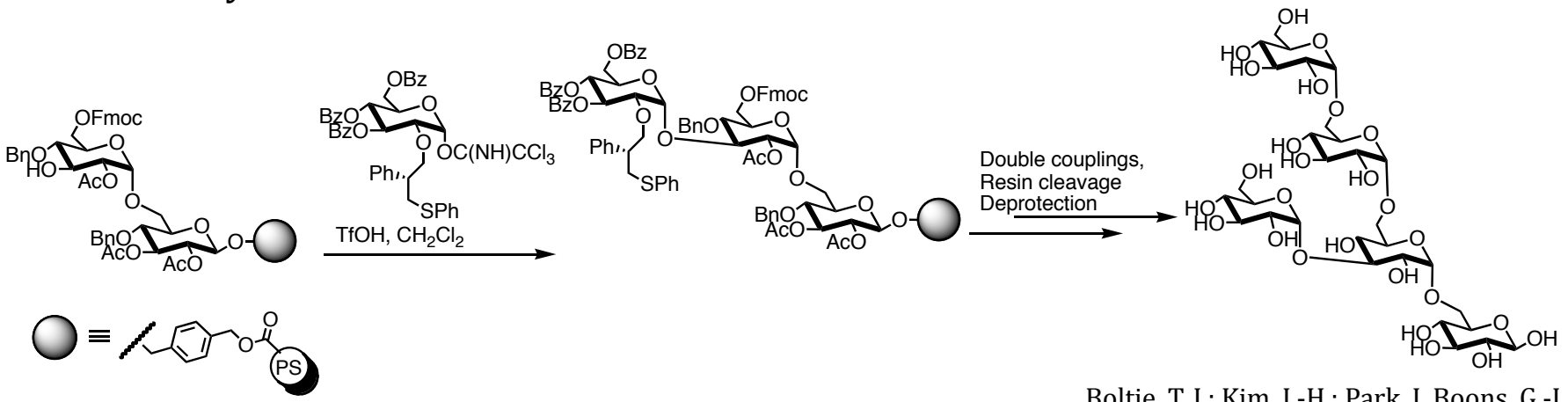


Codee, J. D. C.; Krock, L.; Castagner, B.; Seeberger, P. H. *Chem.-Eur. J.,* **2008,** *14,* 3987-3994.



50% from resin
mixture of anomers 8:1:3:1 in favor of desired

Installation of 1,2-*cis*-glycosidic linkages using a chiral auxiliary:

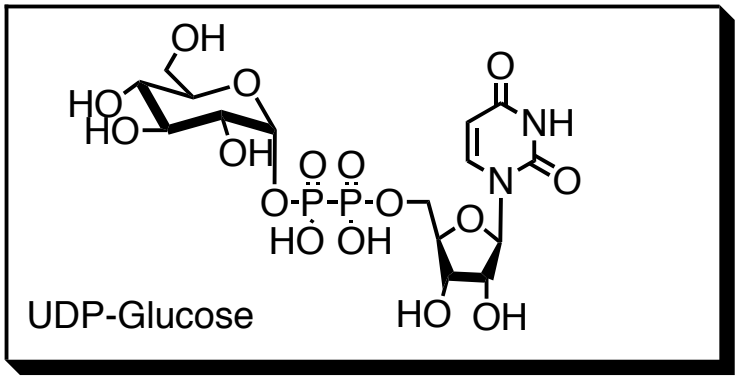


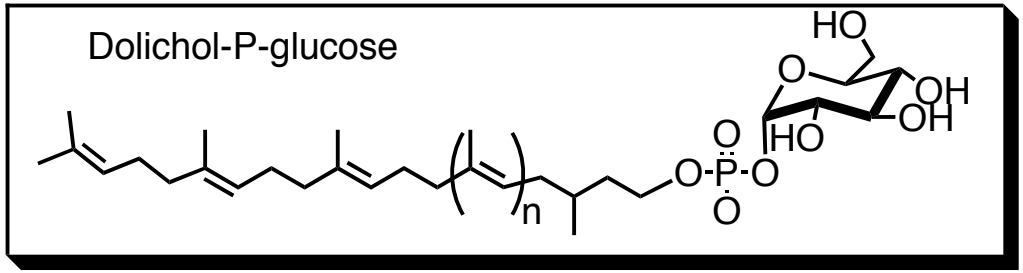
Boltje, T. J.; Kim, J.-H.; Park, J. Boons, G.-J. *Nature Chem.* **2010,** *2,* 552-557.

Glycan Processing Enzymes and Application to Glycan Synthesis

Enzymes Involved in Glycan Processing

Donors: nucleotide sugars and dolichol-phosphate-linked monosaccharides and oligosaccharides





Also Dolichol-P-mannose; Dolichol-P-P-(glucose3-mannose9-GlcNAc2; Undecaprenyl-P-P-*N*-acetylmuramic acid-pentapeptide-GlcNAc

Also UDP-galactose, UDP-xylose, UDP-*N*-acetylgalactosamine, UDP-glucuronic acid, GDP-mannose, GDP-fucose, CMP-sialic acid

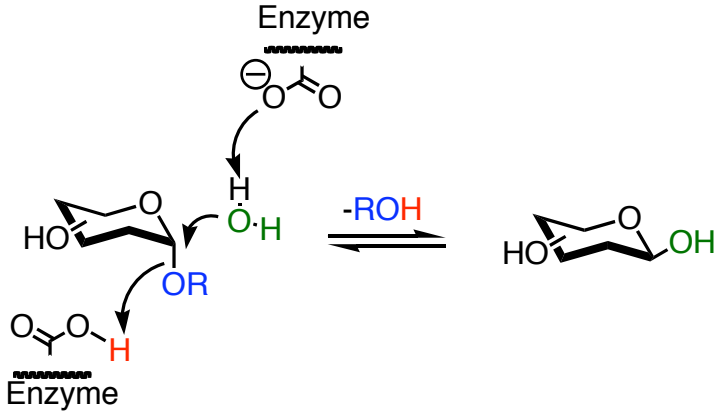
Acceptors: Oligosaccharides, monosaccharides, proteins, lipids, DNA

Enzymes Involved in Glycan Processing

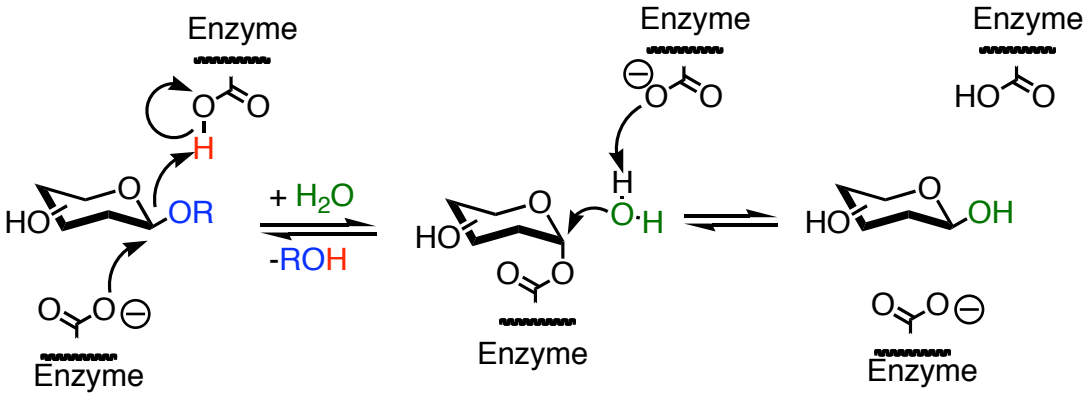
Breaking Glycosidic Bonds: The Action of Glycosidases:

-Inhibitors of glycosidases do exist; glycosidases are more promiscuous than glycosyltransferases

Mechanism of Inverting Glycosidases:



Mechanism of Retaining Glycosidases:

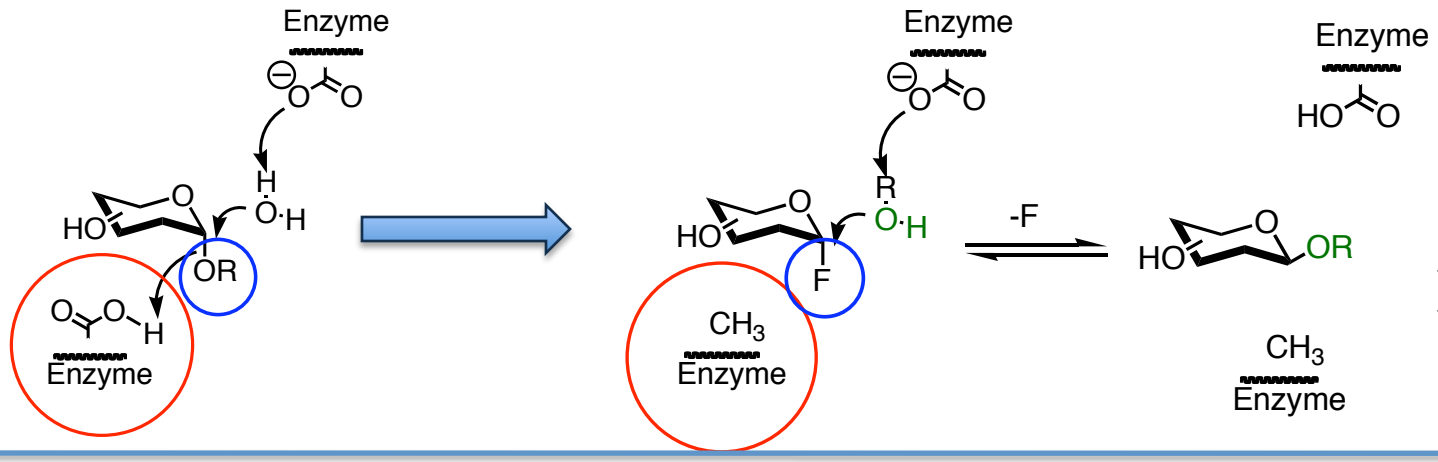


Drawbacks to use in chemoenzymatic synthesis:

-Low yielding (thermodynamically unfavored direction)
-Products are often substrates! (Product degradation)

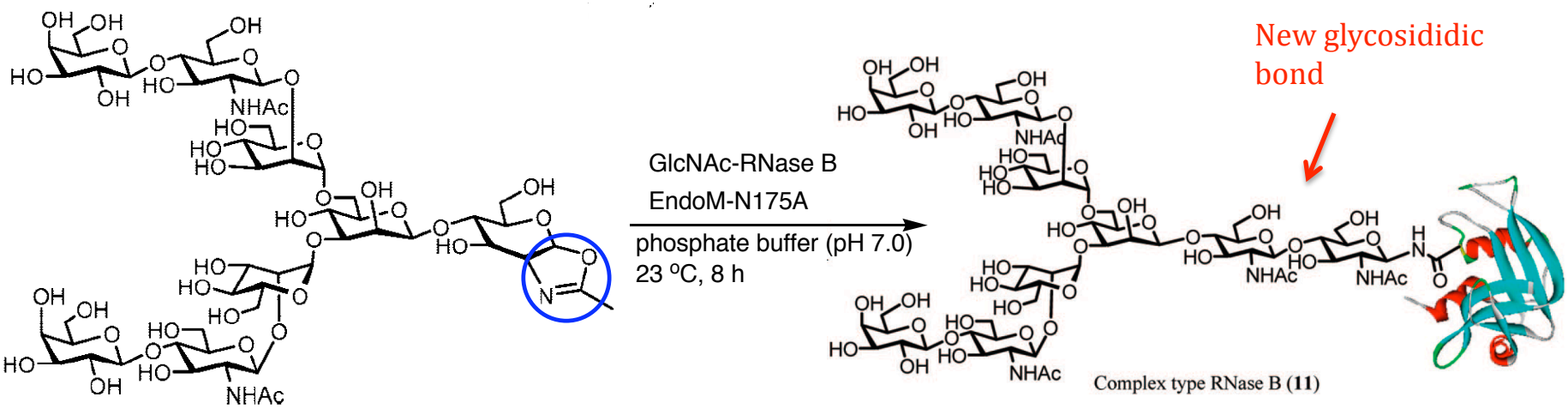
Kiessling, L. L.; Splain, R. A. *Annu. Rev. Biochem.* **2010,** *79,* 619-653.
Boltje, T. J.; Buskas, T.; Boons, G.-J. *Nature Chem.* **2009,** *1*, 611-622.

Glycosynthase Development



Hancock, S. M.; Vaughan, M. D., Withers, S. G. *Curr. Opin. Chem. Biol.* **2006,** *10,* 509-519.

Use of an oxazoline-containing substrate for glycosidic bond formation *via* a glycosynthase:



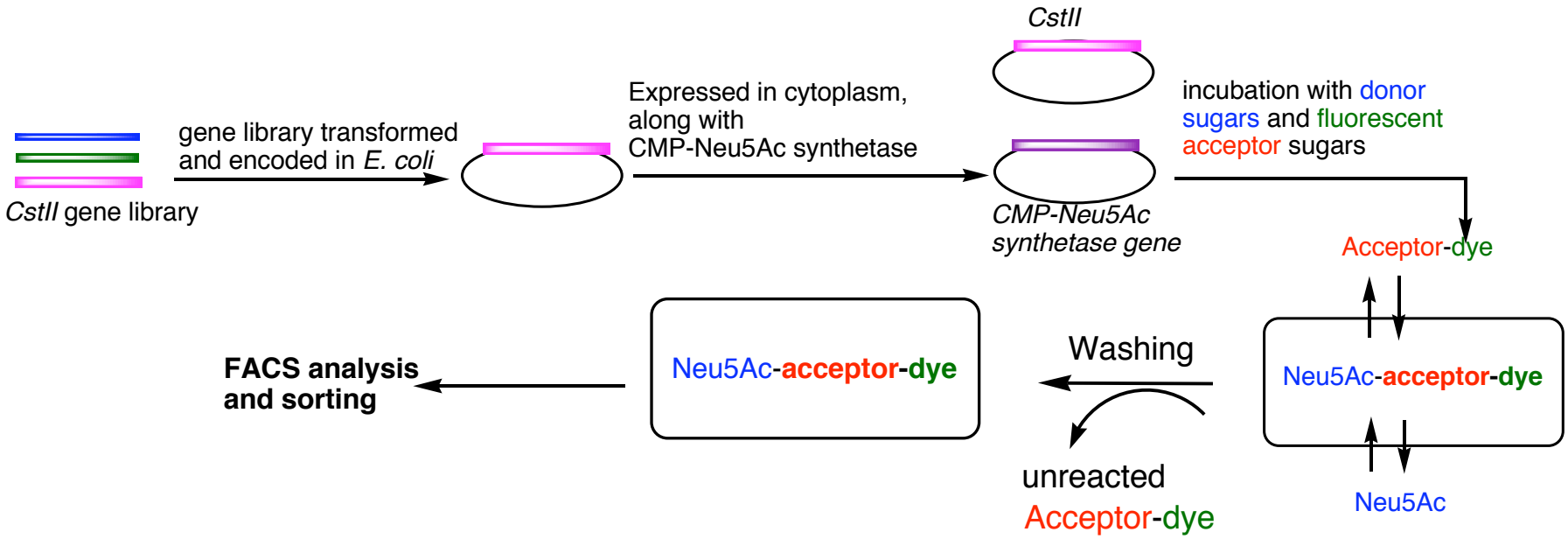
Problem: Need High-Throughput Screens for Novel Glycosynthase Enzyme

Huang et al. *J. Am. Chem. Soc.* **2009,** *131,* 2214-2223.

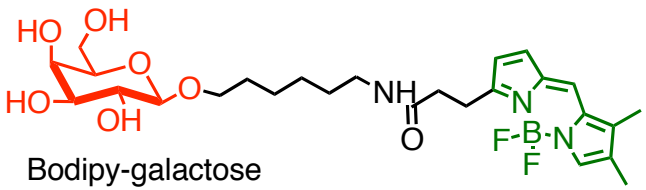
Kiessling, L. L.; Splain, R. A. *Annu. Rev. Biochem.* **2010,** *79,* 619-653.
Boltje, T. J.; Buskas, T.; Boons, G.-J. *Nature Chem.* **2009,** *1*, 611-622.

High Throughput Glycosynthase Screens

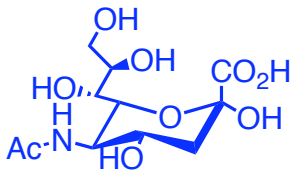
Fluorescence-Activating Cell Sorting



Example fluorescent acceptor sugar



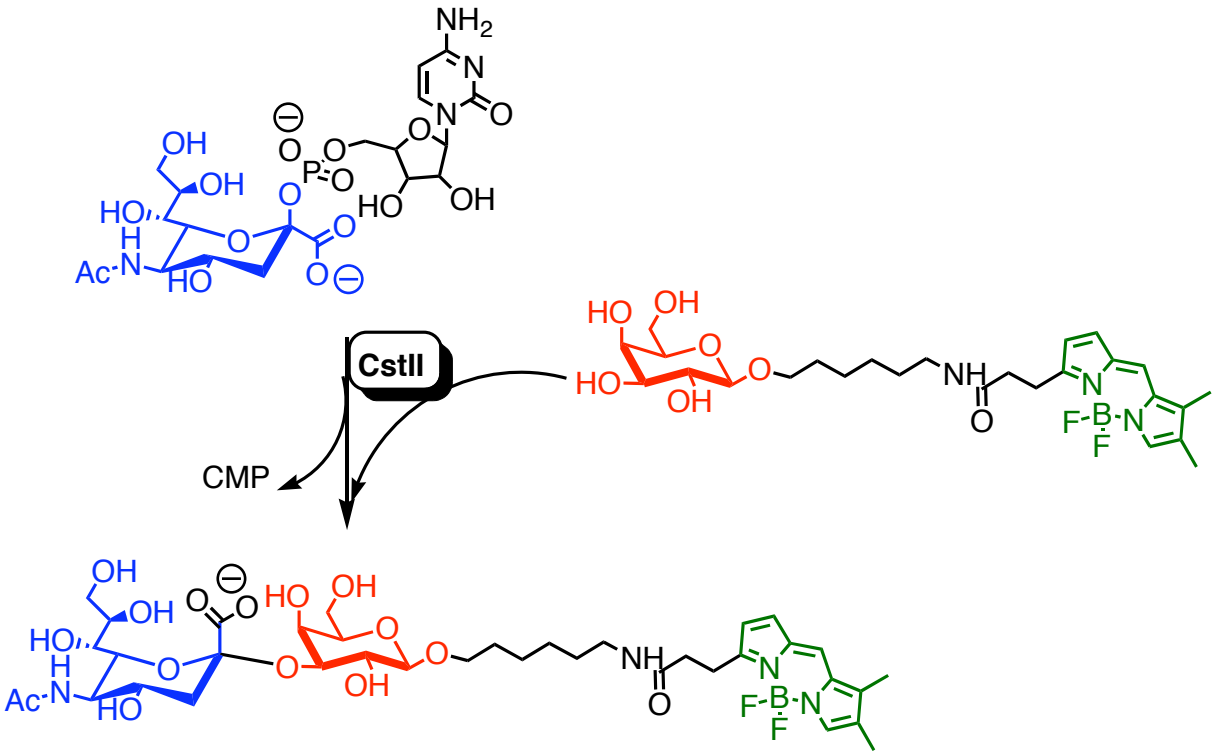
Bodipy-galactose



Neu5Ac
(sialic acid)

Withers et al. *Nature Methods* **2006,** *3,* 609-614.

The Sialyltransferase Reaction in Detail

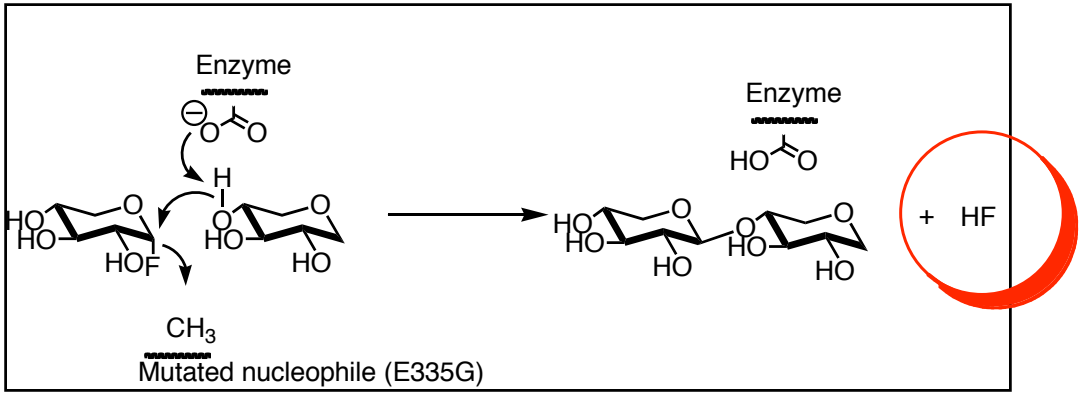


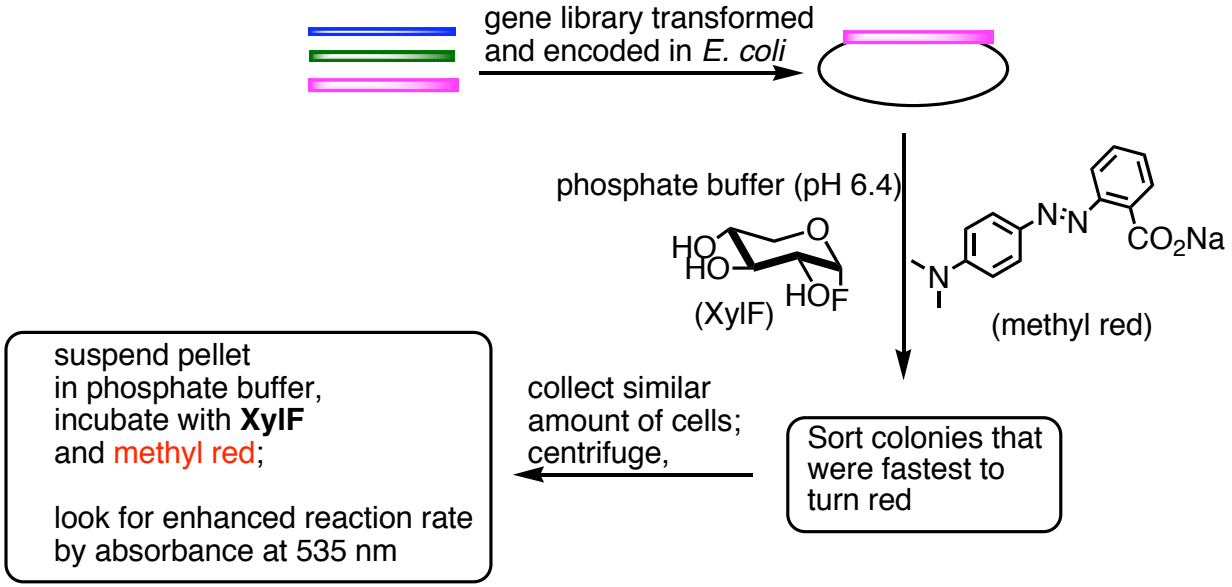
CstII = sialyltransferase from *Campyobacter jejuni*

Chiu, C.P.; Watts, A. G.; Lairson, L. L.; Gilbert, M.; Lim, D.; Wakarchuck, W. W.; Withers, S. G.; Strynadka, N. C. J. *Nat. Struct. Mol. Biol.* **2004,** *11,* 163-170.

High Throughput Glycosynthase Screens

pH Based Screening:

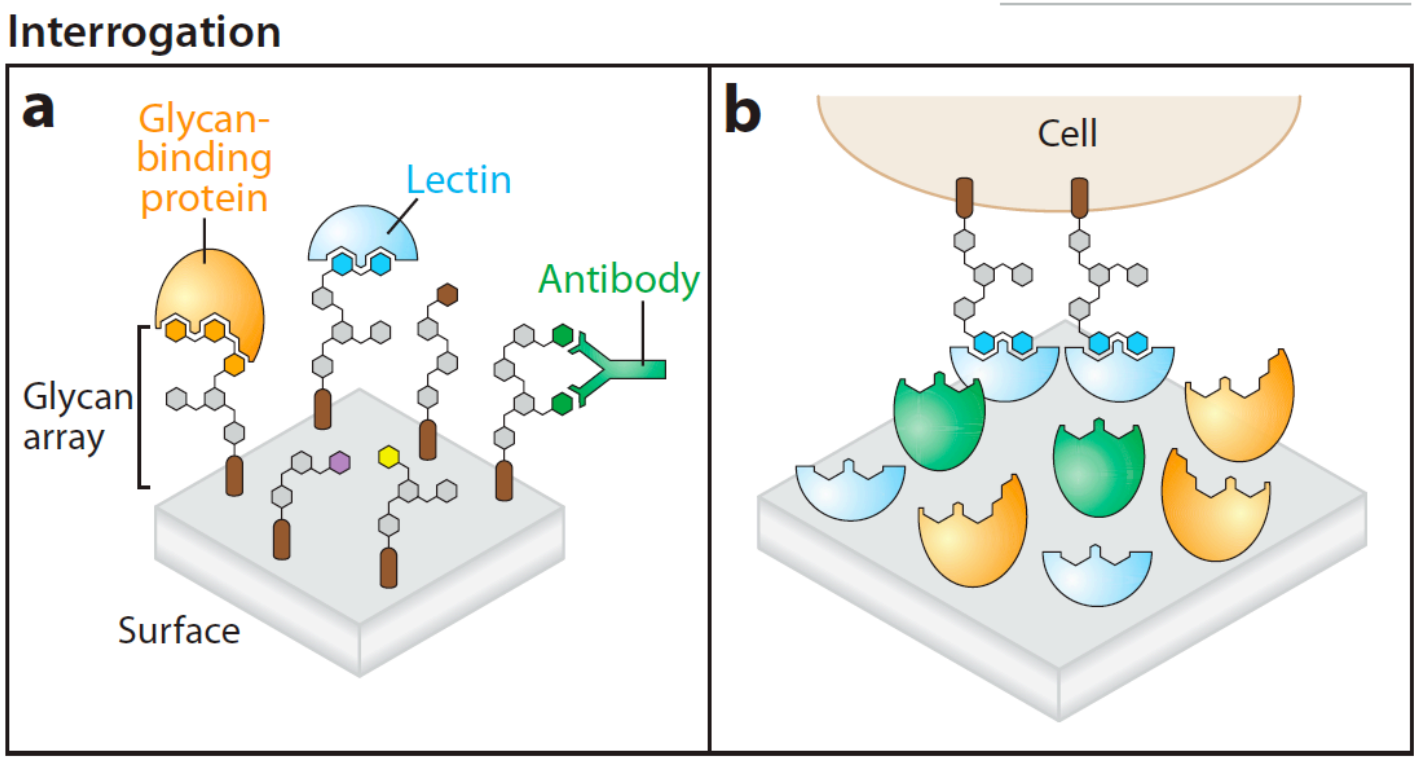




Ben-David, A.; Shoham, G.; Shoham, Y. *Chem. Biol.* **2008,** *15,* 546-551.

Part II.
Methods for Studying Glycans

Probing the Glycome



-Involves the study of interactions between natural glycans and binding partners.

-To be successful, you need 1) readily available natural glycans and/or novel glycans and 2) arrays bearing either glycoconjugates or lectins

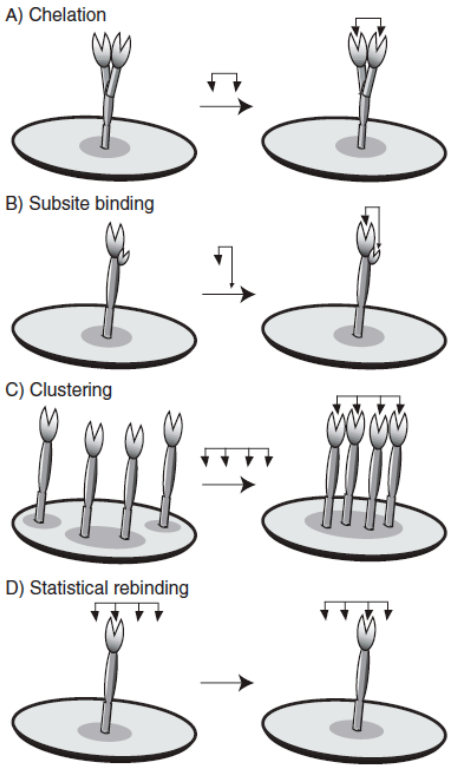
Kiessling, L. L.; Splain, R. A. *Annu. Rev. Biochem.* **2010,** *79,* 619-653.

Biological Roles of Glycans

- Protein trafficking
- Gene expression
- On surface of pathogens, serve as protective shield
- Target cell recognition/entering
- Protein-glycoconjugate interactions enable cells to communicate with their environments

Kiessling, L. L.; Splain, R. A. *Annu. Rev. Biochem.* **2010,** *79,* 619-653.

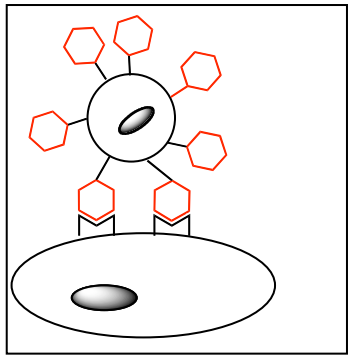
Glycan-Ligand Binding is Weak



Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Angew. Chem. Int. Ed.* **2006,** *45,* 2348-2368.

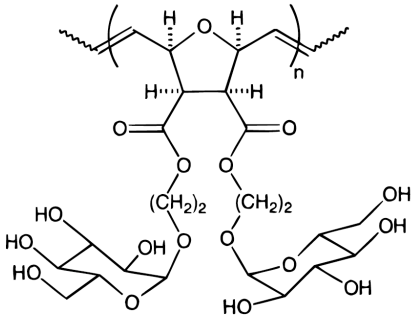
- Monovalent protein-glycan interactions have low binding dissociation constants (10^{-4} to 10^{-3} M)
- Often, binding is multivalent (increases apparent binding constant)
- Ensures only cells with correct receptor-ligand pairs form stable interactions.

Makes characterization of interactions difficult!!



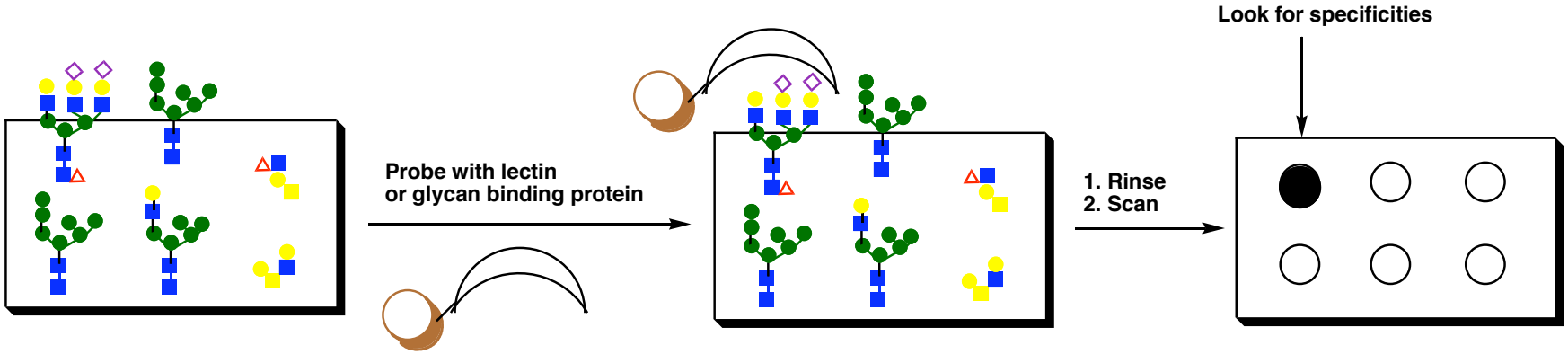
Multivalent ligands have been evaluated ->

See: Mortell, K. H.; Weatherman, R. V.; Kiessling, L. L. *J. Am. Chem. Soc.* **1996,** *118,* 2297, Gestwicki, J. E.; Cairo, C. W.; Strong, L. E.; Oetjen, K. A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002,** *124,* 14922, and the *Angew.* review cited.



Kiessling, L. L.; Splain, R. A. *Annu. Rev. Biochem.* **2010,** *79,* 619-653.

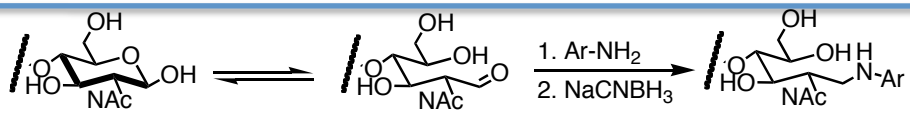
Glycan Arrays for Interrogation



Limitation: Supply of Oligosaccharides!

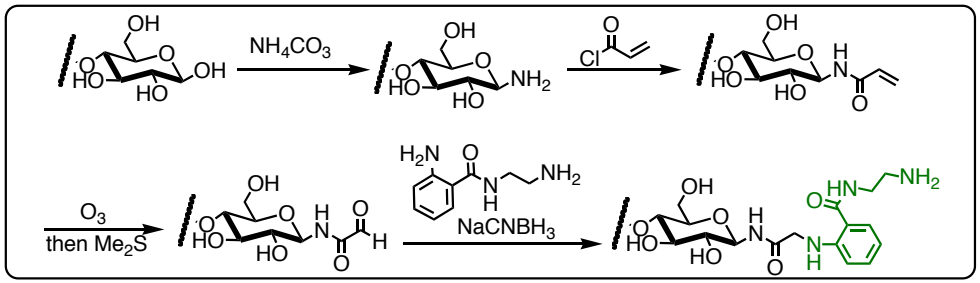
Krishnamoorthy, L.; Mahal, L. K. *ACS Chem. Biol.* **2009,** *4,* 715-732.

Methods for glycan immobilization:

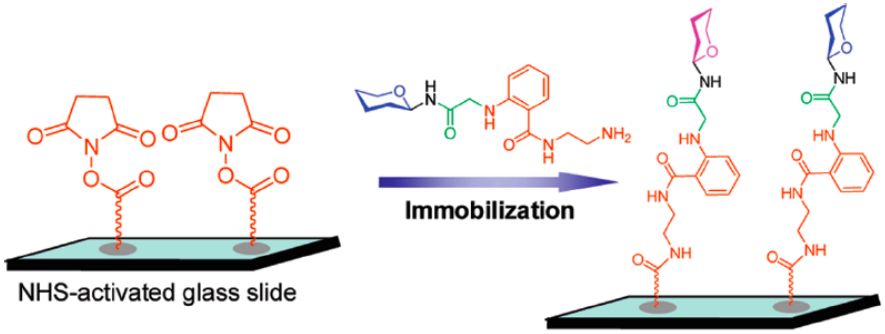


Oyelaran, O.; Gildersleeve, J. C. *Curr. Opin. Chem. Biol.* **2009,** *13,* 406-413.

Modification to achieve ring-closed, fluorescently labeled glycans:

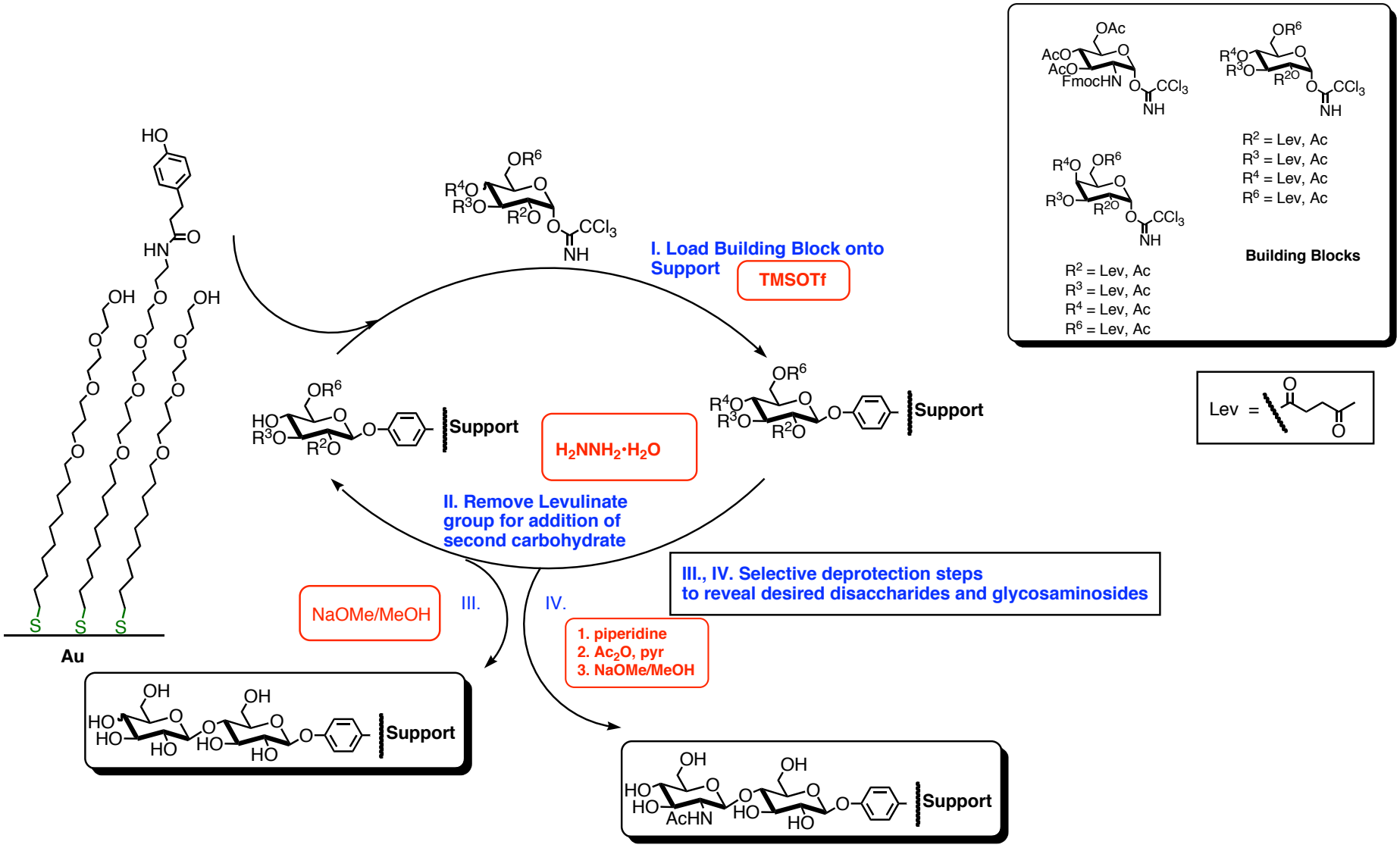


Song, Z.; Lasanajak, Y.; Xia, B.; Smith, D. F.; Cummings, R. D. *ACS Chem. Biol.* **2009,** *4,* 741-750.



Copied from: Park, S.; Sung, J.-W.; Shin, I. *ACS Chem. Biol.* **2009,** *4,* 699-701.

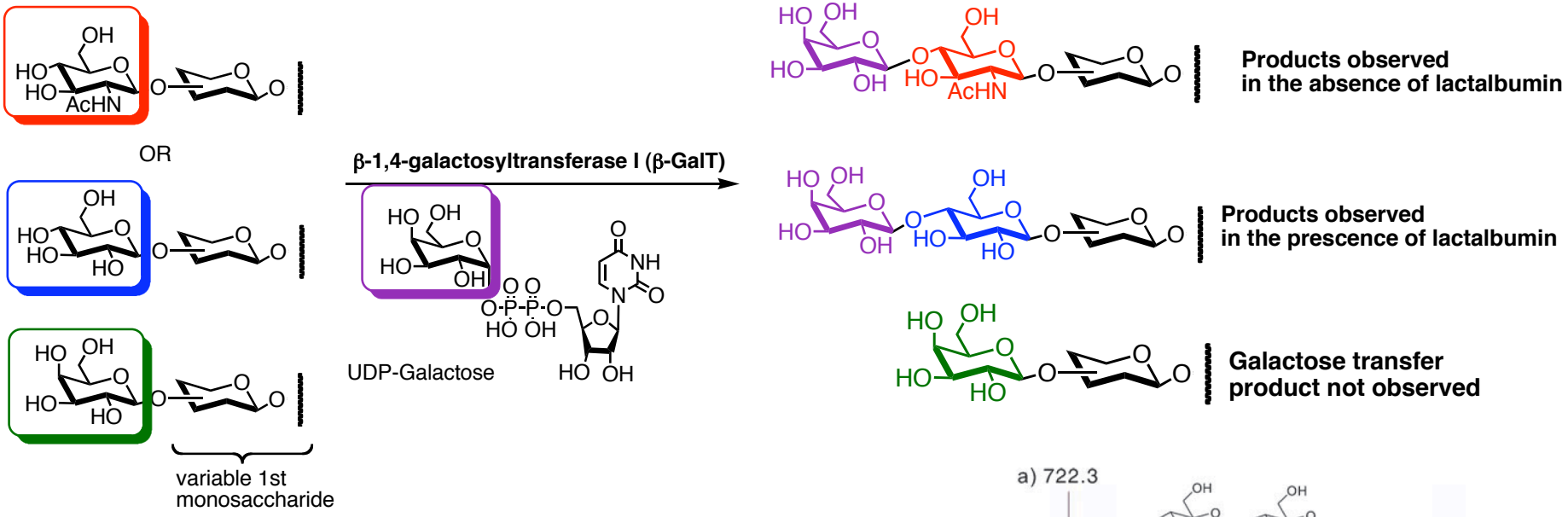
On-Chip Joint Synthesis and Assay

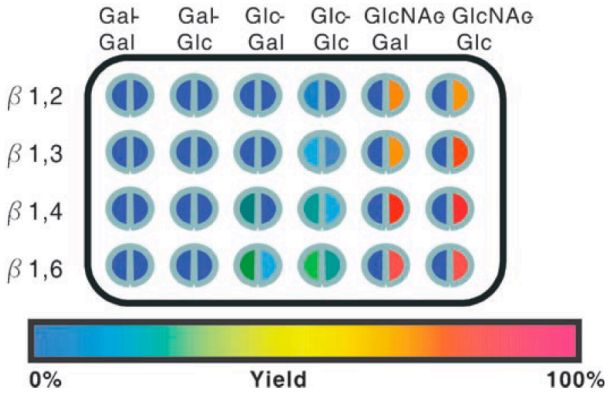


Ban, L.; Mrksich, M. *Angew. Chem. Int. Ed.* **2008,** *47,* 3396-3399.

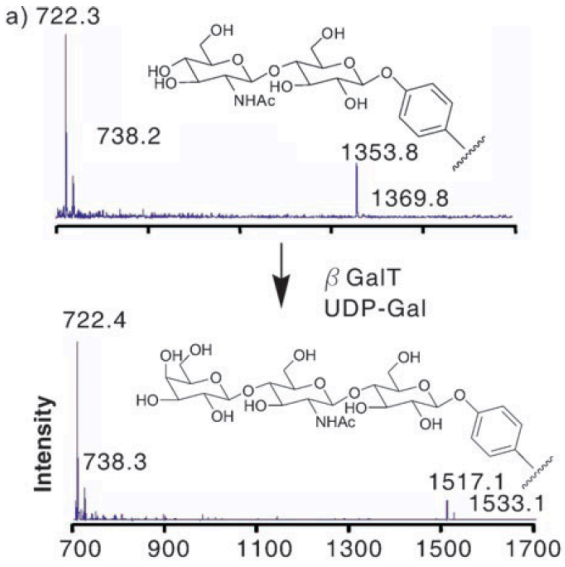
Use of the Array to Probe Enzyme Activity

Using the array to probe substrate specificity of bovine β-1,4-galactosyltransferase I:





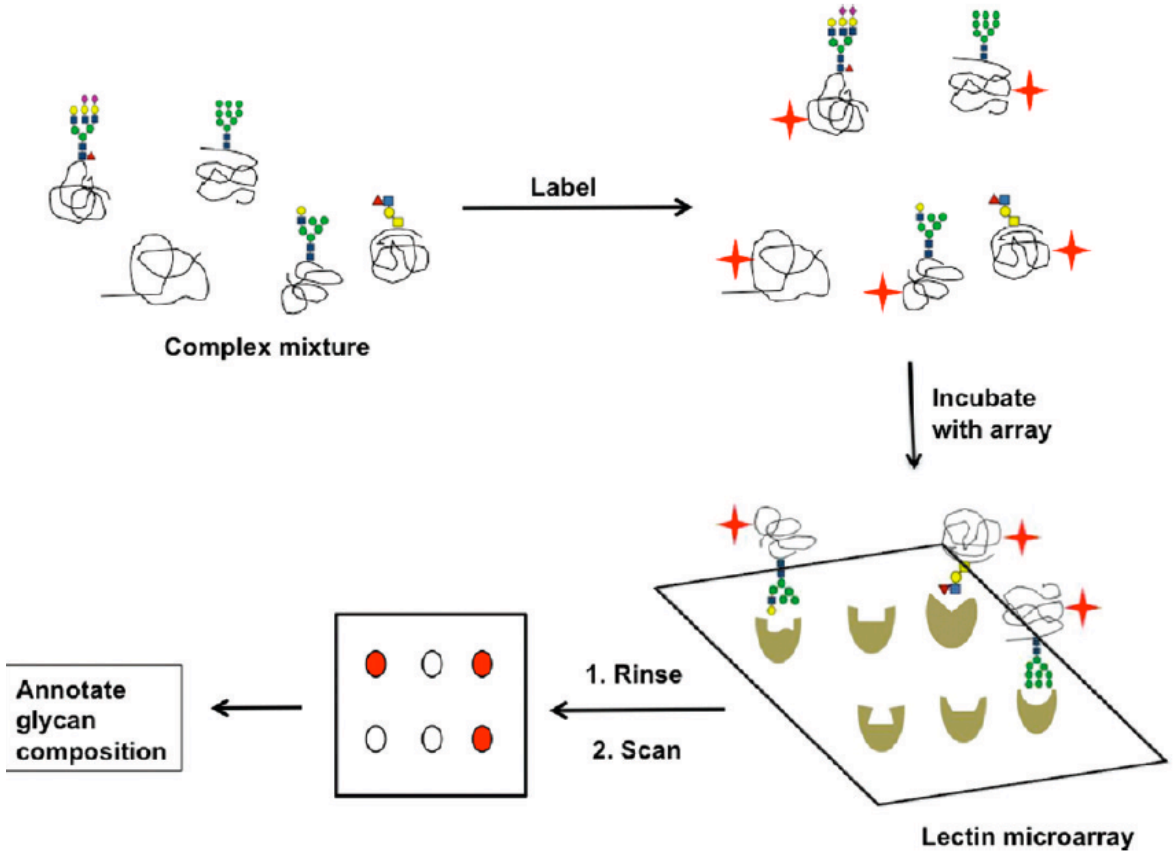
Analyzed By Self-assembled monolayers for matrix-assisted laser desorption time-of-flight spectrometry (SAMDI-TOF MS):



Ban, L.; Mrksich, M. *Angew. Chem. Int. Ed.* **2008,** *47,* 3396-3399.

Lectin Arrays

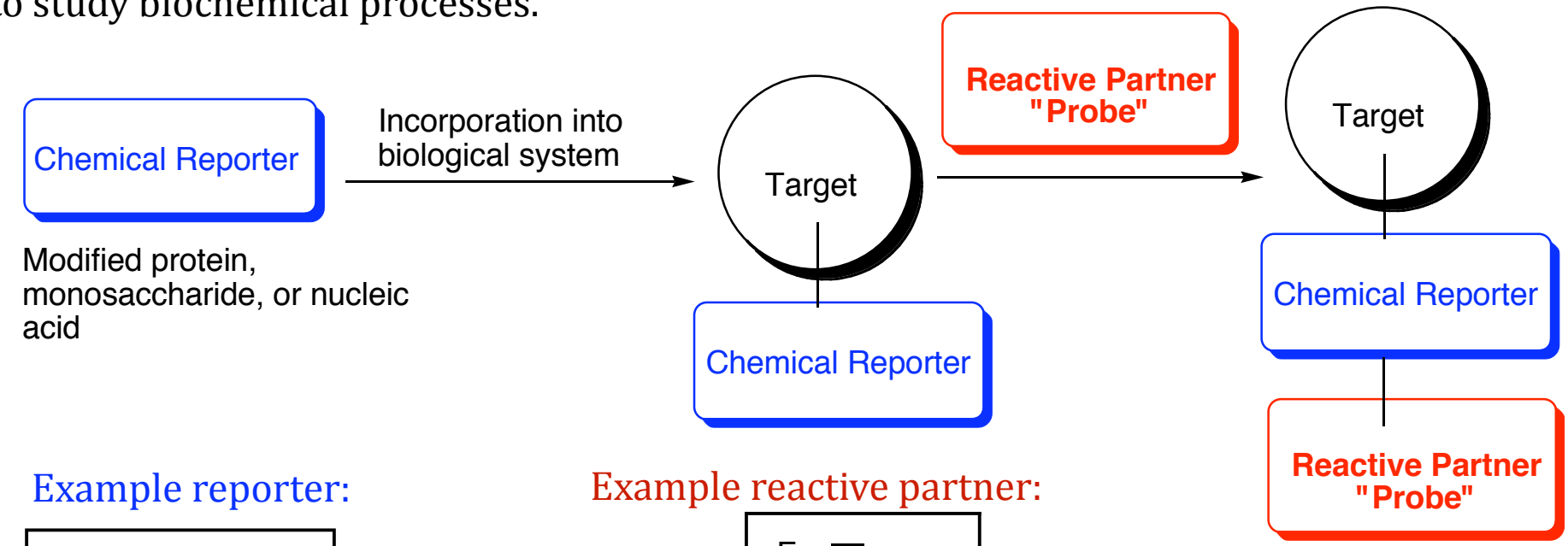
- **Advantages**
  - Array is multivalent
  - Lectins are characterized using glycan arrays; can get linkage specific information
  - Can observe many types of glycan conjugates simultaneously
- **Drawbacks**
  - Only applicable to motifs complimetary to lectins on array
  - Some plant lectins are glycosylated; problematic if probed with samples having native lectins



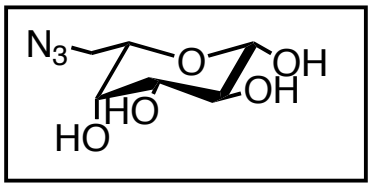
Copied from:Krishnamoorthy, L.; Mahal, L. K. *ACS Chem. Biol.* **2009,** *4,* 715-732.

Glycan Imaging through Bioorthogonal Chemistry

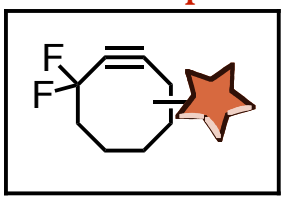
General Principle: Use of "man-made" chemical tools as biologically inert reporters to study biochemical processes.



Example reporter:



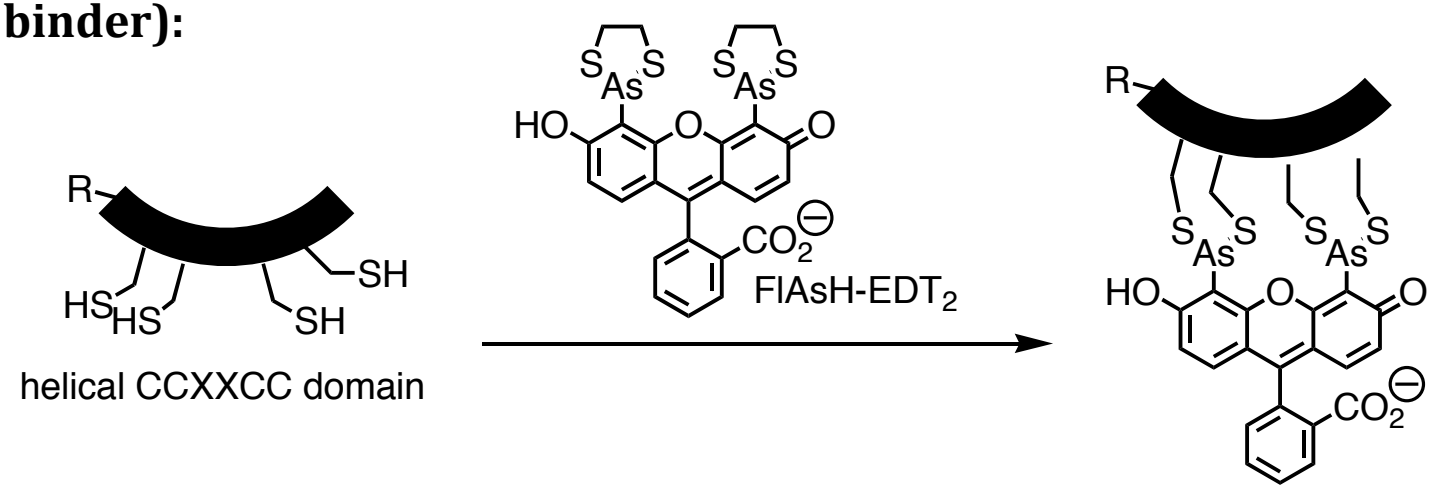
Example reactive partner:



Reviews: Sletten, E. M.; Bertozzi, C.R. *Angew. Chem. Int. Ed.* **2009,** *48,* 6974-6998
Boyce, M.; Bertozzi, C. R. *Nature Methods* **2011,** *8,* 638-642.
Agard, N. J.; Bertozzi, C. R. *Acc. Chem. Res.* **2009,** *42,*788-797.

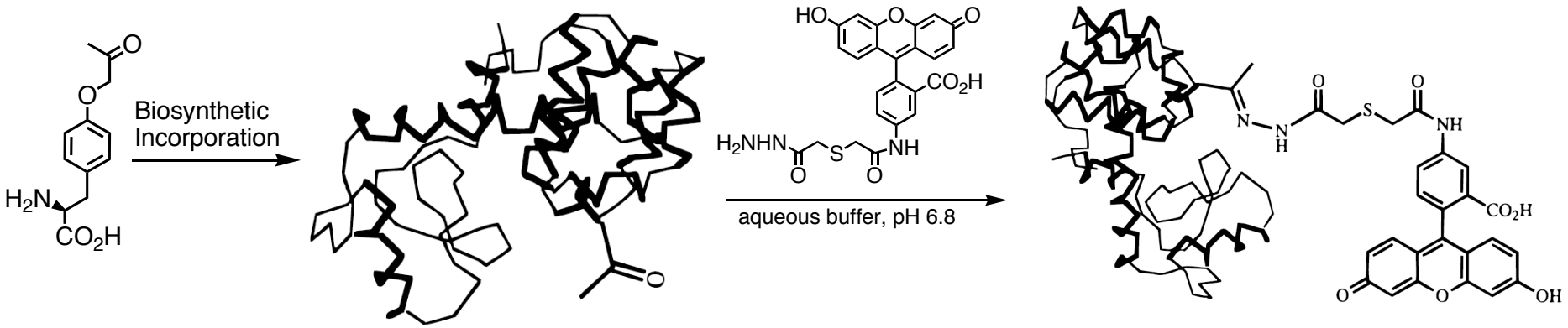
Early Work on Bioorthogonal Reactions

Early work: selective imaging of proteins using FlAsH (Fluorescein arsenical hairpin binder):



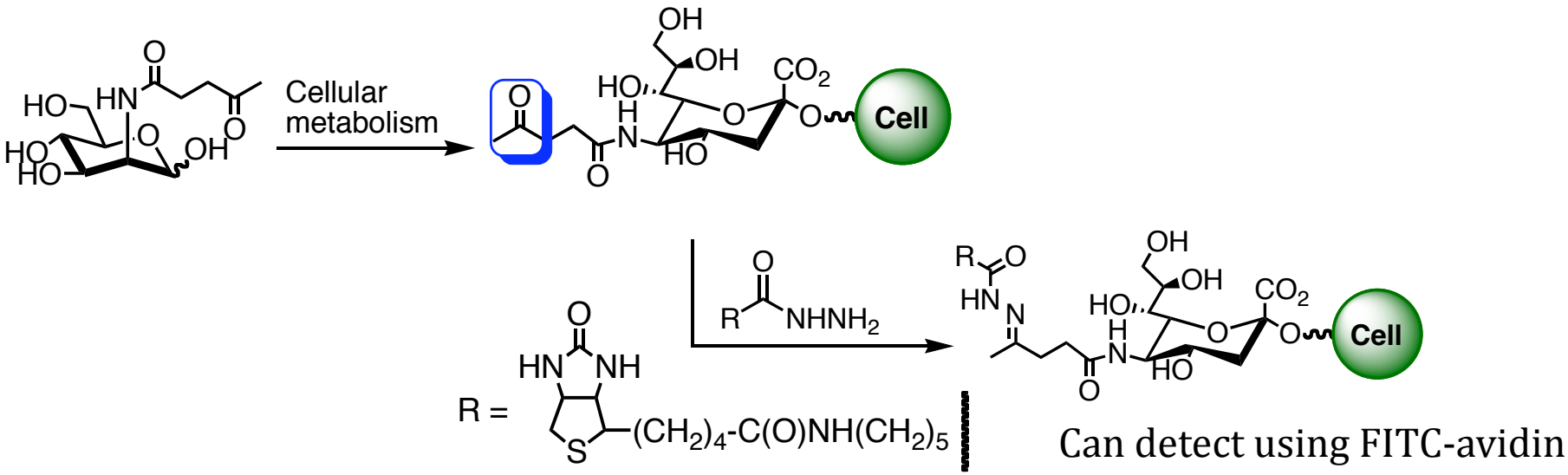
Griffin, B. A.; Adams, S. R.; Tsien, R. Y. *Science* **1998,** *281,* 269-272.

Site specific protein modification using a ketone tag:



Cornish, V. W.; Hahn, K. M.; Schultz, P. G. *J. Am. Chem. Soc.* **1996,** *118,* 8150-8151.

Extending Bioorthogonal Reactions to Glycans



Can detect using FITC-avidin

Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997,** *276,* 1125-1128.

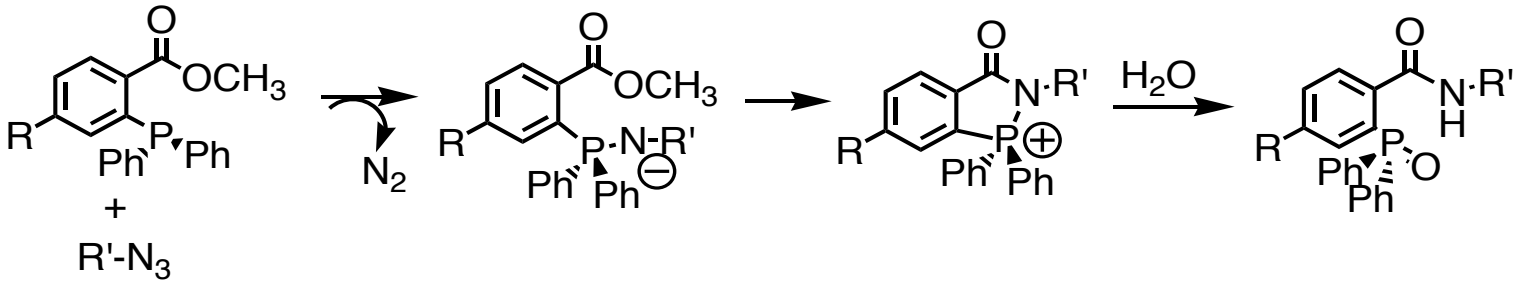
Drawback: Ketones and aldehydes *are* found in intracellular metabolites, including free sugars, pyruvate, and lipid catabolites.

Looked to completely non-endogenous functional groups for "universal bioorthogonality

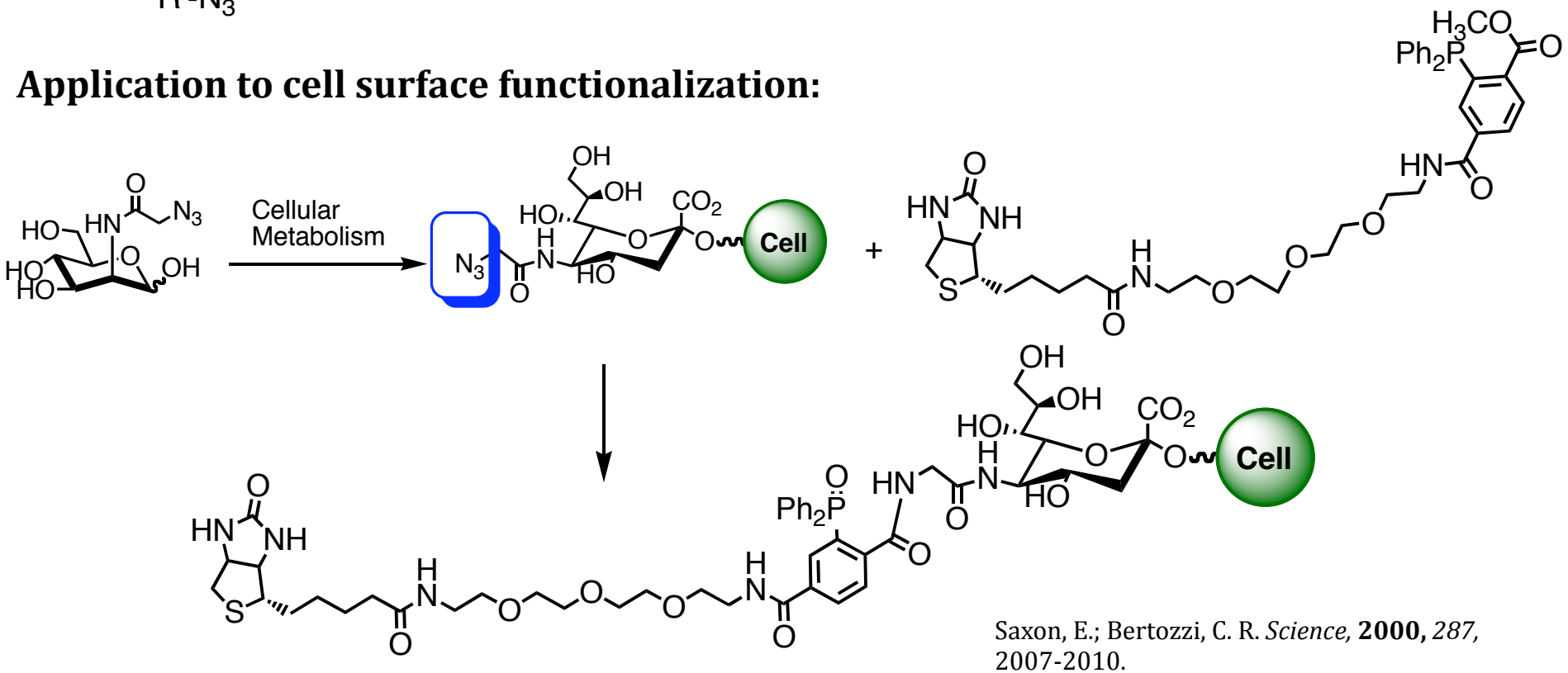
Boyce, M.; Bertozzi, C. R. *Nature Methods* **2011,** *8,* 638-642.

Development of the Staudinger Ligation

Modified Staudinger reaction for use in physiological systems:

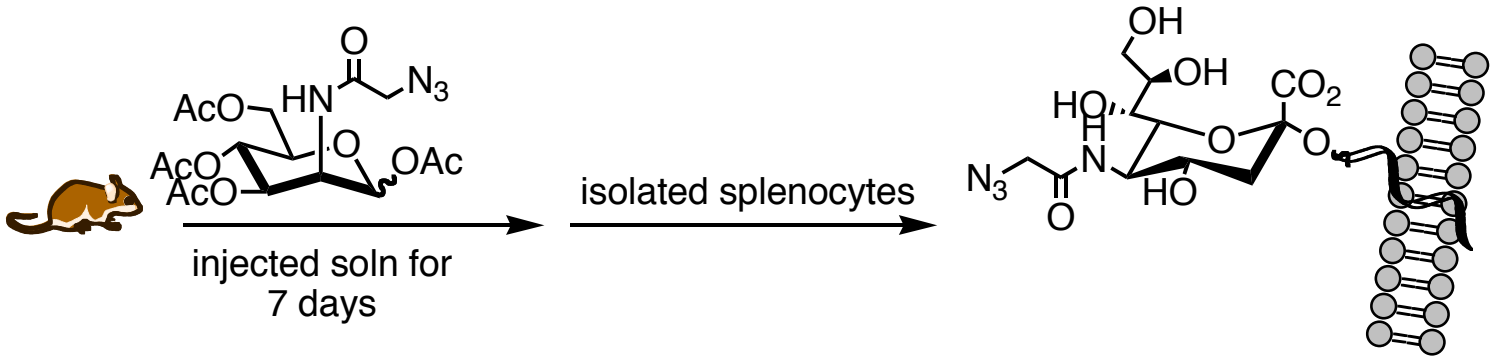


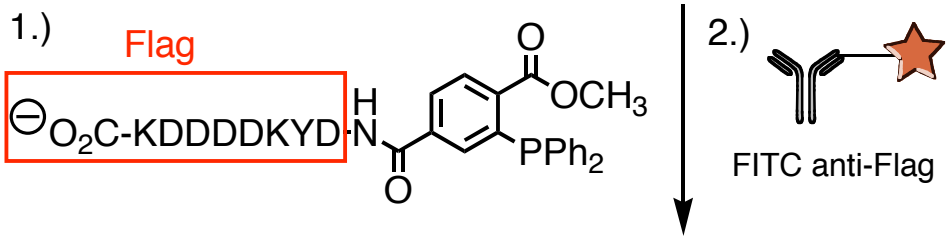
Application to cell surface functionalization:

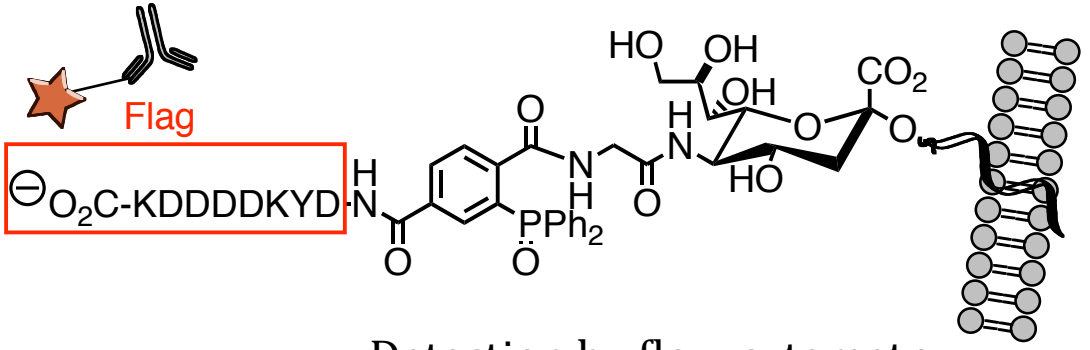


Saxon, E.; Bertozzi, C. R. *Science,* **2000,** *287,* 2007-2010.

Cellular Modification in Mice







Detection by flow cytometry

Prescher, J. A.; Dube, D. H.; Bertozzi, C. R. *Nature,* **2004,** *430,* 873-877.

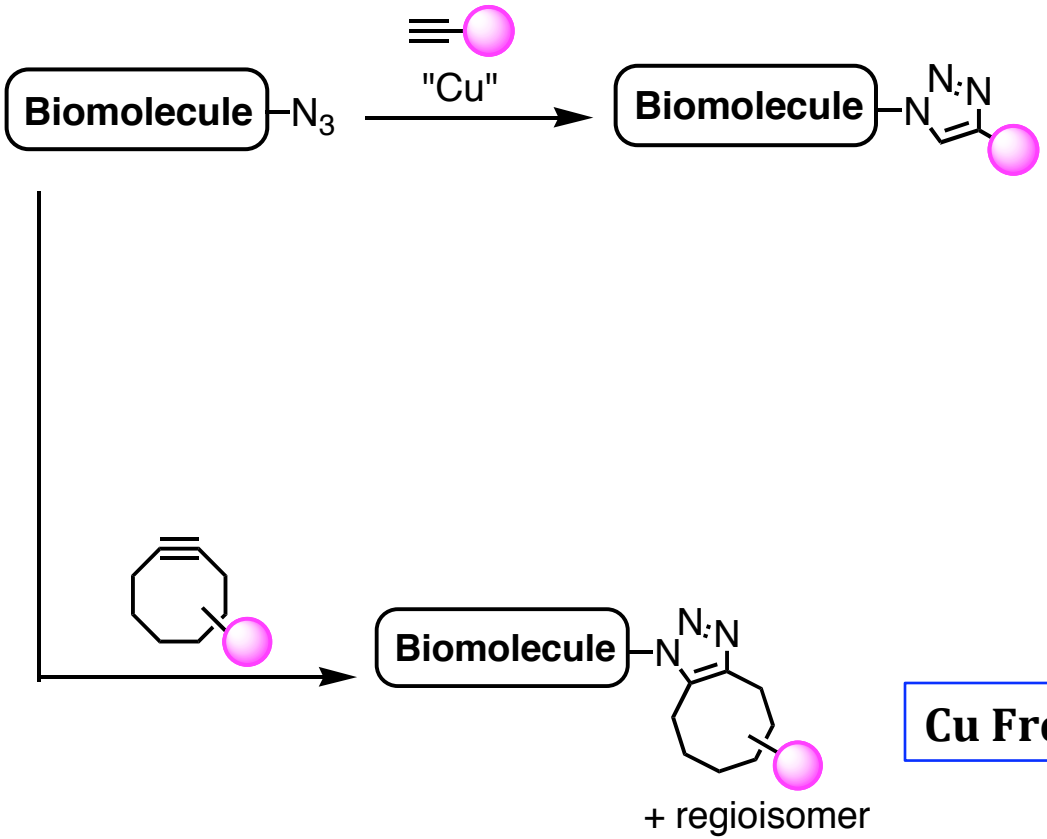
Drawback to the Staudinger ligation: Second order rate constant on the order of $10^{-3}M^{-1}s^{-1}$; necessitates high concentrations of phosphine (> 250 μM)

Sletten, E. M.; Bertozzi, C.R. *Angew. Chem. Int. Ed.* **2009,** *48,* 6974-6998

Bioorthogonal Click Chemistry

"Indeed, the azide has an alternative mode of reactivity, the 1,3-dipolar cycloaddition with alkynes…The Staudinger ligation may therefore be the first in a future arsenal of chemical reactions used to probe biology in living animals."

Prescher, J. A.; Dube, D. H.; Bertozzi, C. R. *Nature,* **2004,** *430,* 873-877.



Has been used in bioconjugation (attachment of dyes to cowpea mosaic virus; Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M.G. *J. Am. Chem. Soc.* **2003,** *125,* 3192

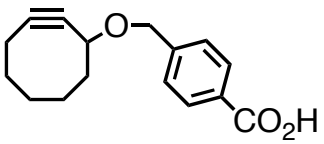
Drawback: Toxicity of copper salts in living organisms

Cu Free Click Chemistry

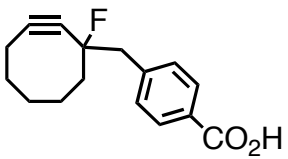
Sletten, E. M.; Bertozzi, C.R. *Angew. Chem. Int. Ed.* **2009,** *48,* 6974-6998

Cyclooctyne Click Reagents

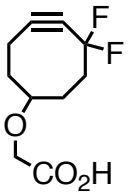
Second order rate constant for reaction with alkyl azide:



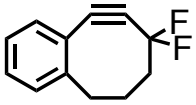
$k = 10^{-3} M^{-1}s^{-1}$



ca. 60-fold rate increase



$k = 10^{-1} M^{-1}s^{-1}$



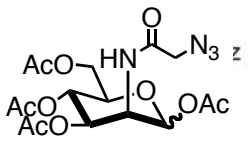
(stabilized by β-cyclodextrin)

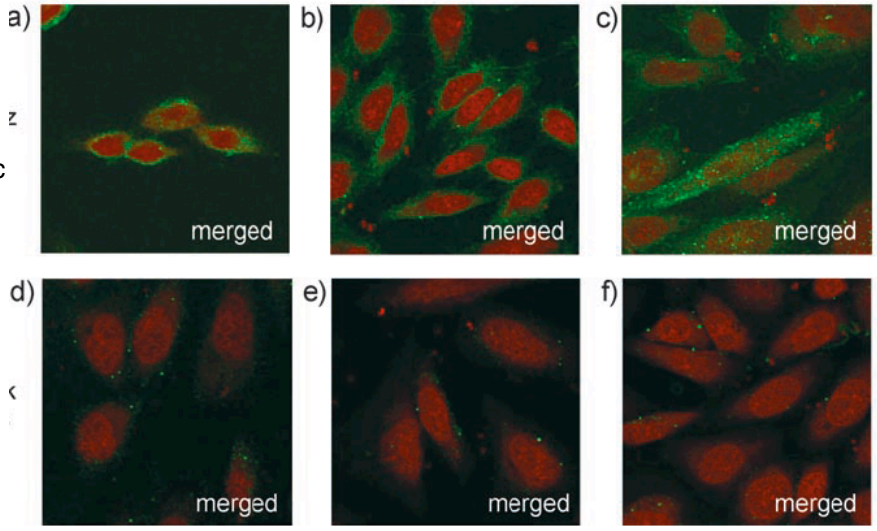
$k = 0.22\ M^{-1}s^{-1}$

Sletten, E. M.; Bertozzi, C.R. *Angew. Chem. Int. Ed.* **2009,** *48,* 6974-6998

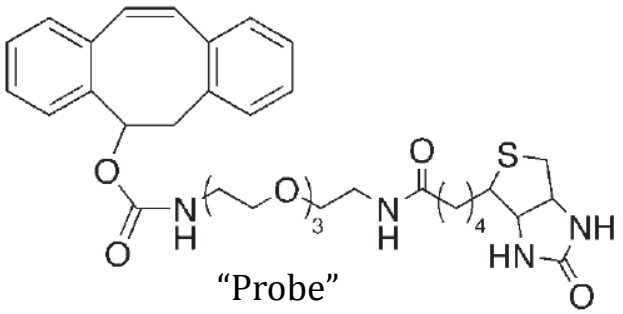
Sletten, E. M.; Nakamura, H.; Jewett, J. C.; Bertozzi, C.R. *J. Am. Chem. Soc.* **2010,** *132,* 11799.

Incubated with "probe": 1 h 4 oC 1 h, rt 1 h, rt
Incubated with avidin-Alexa-Fluor 488: 15 min, 4 oC 15 min, 4 oC 15 min, 4 oC then 1 h 37 oC





"Blank" cells



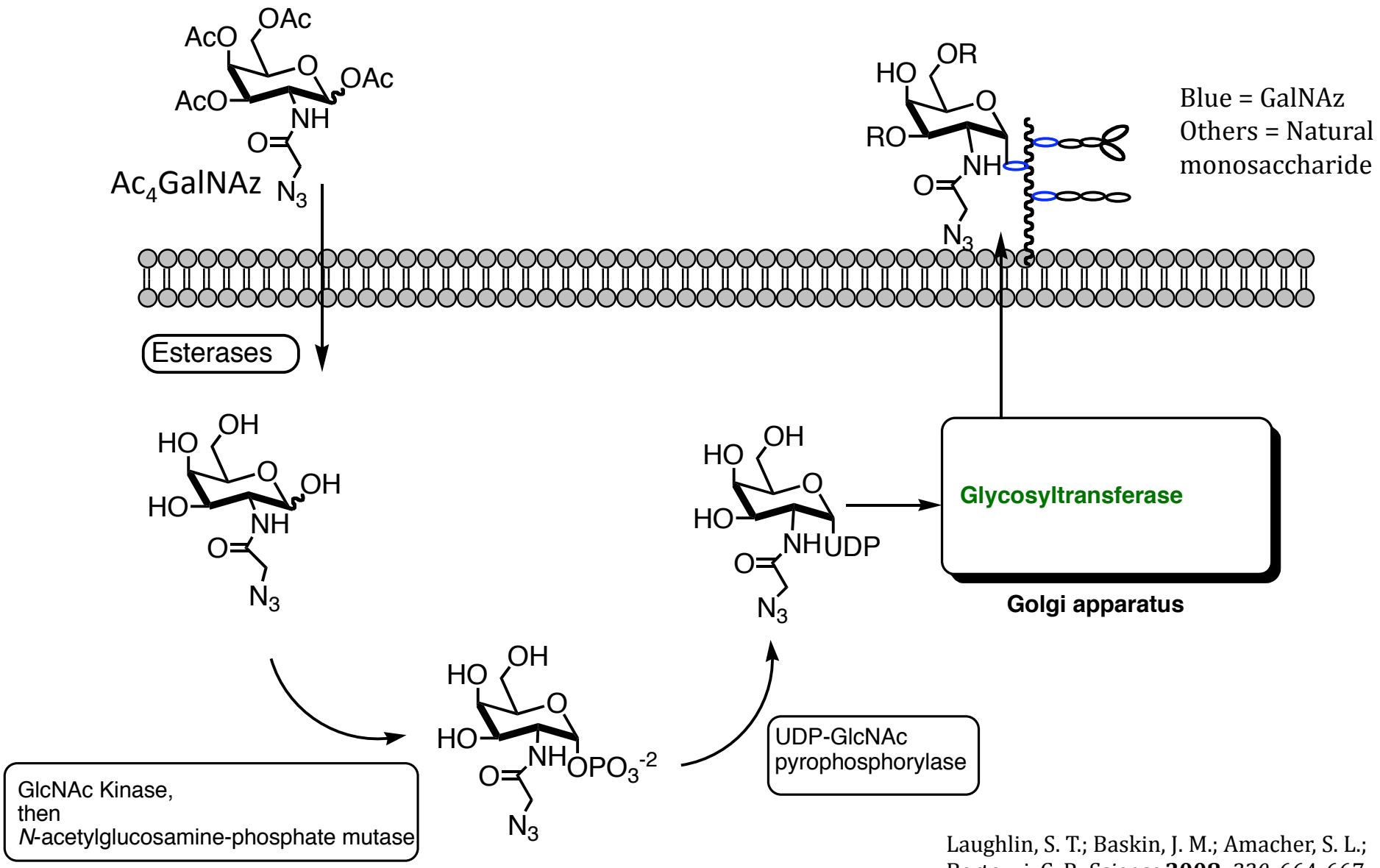
"Probe"

Note: Cells were stained with far-red fluorescent dye TO-PRO after washing and fixing

Ning, X.; Guo, J.; Wolfert, M. A.; Boons, G.-J. *Angew. Chem. Int. Ed.* **2008,** *120,* 2285-2287.

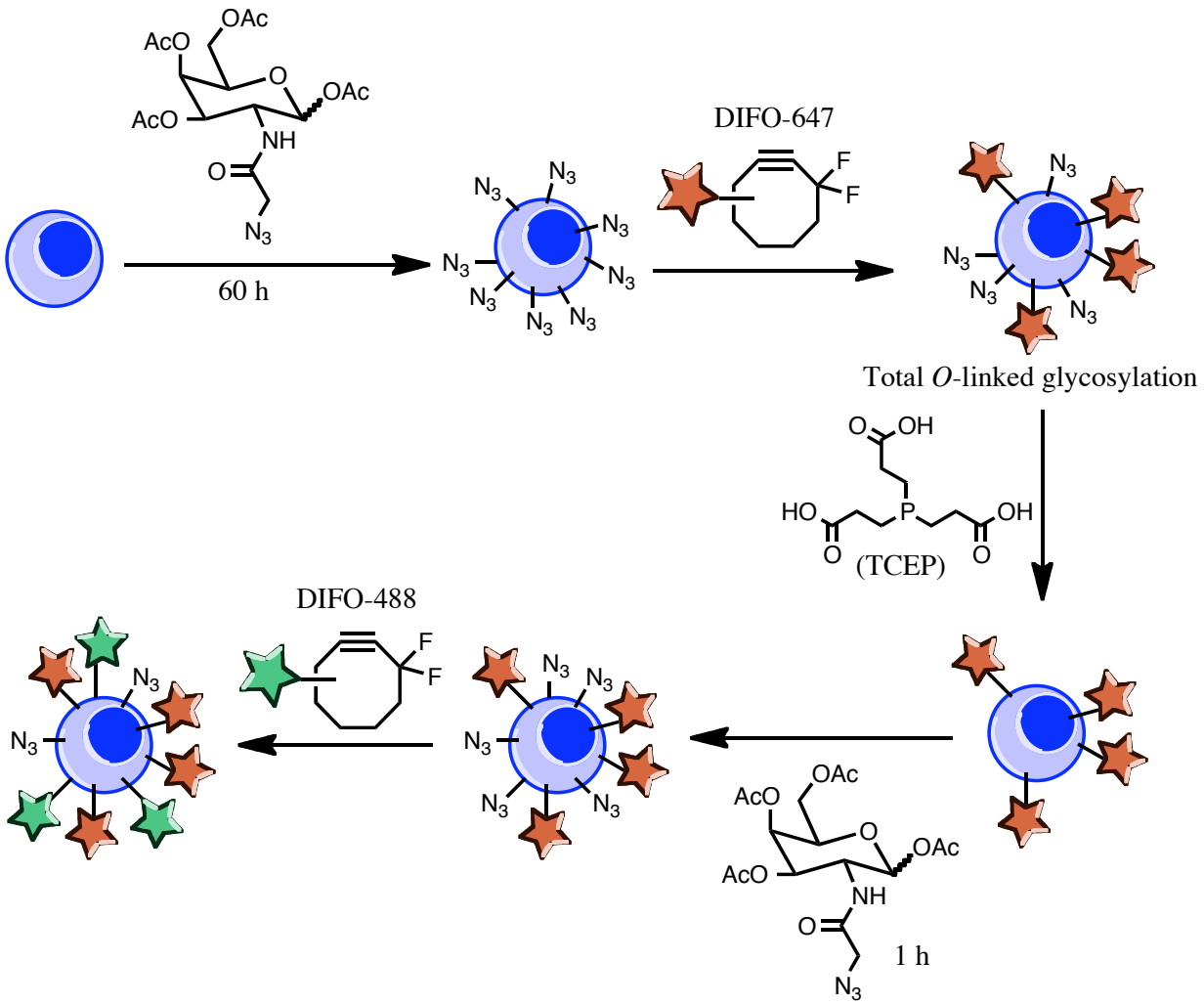
Imaging of Zebrafish Development

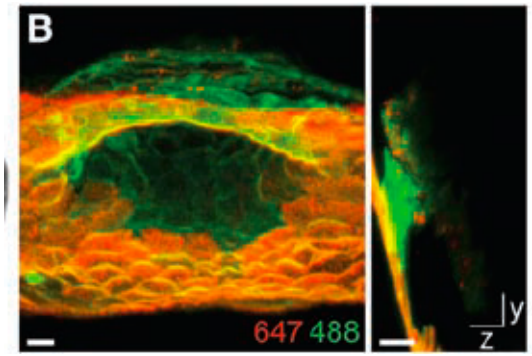
Exploiting the GalNAc Salvage Pathway:



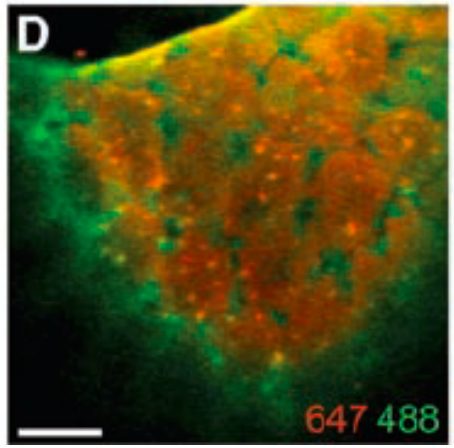
Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008,** *320,* 664-667.

Imaging Zebrafish *in vivo*





Zebrafish mouth region



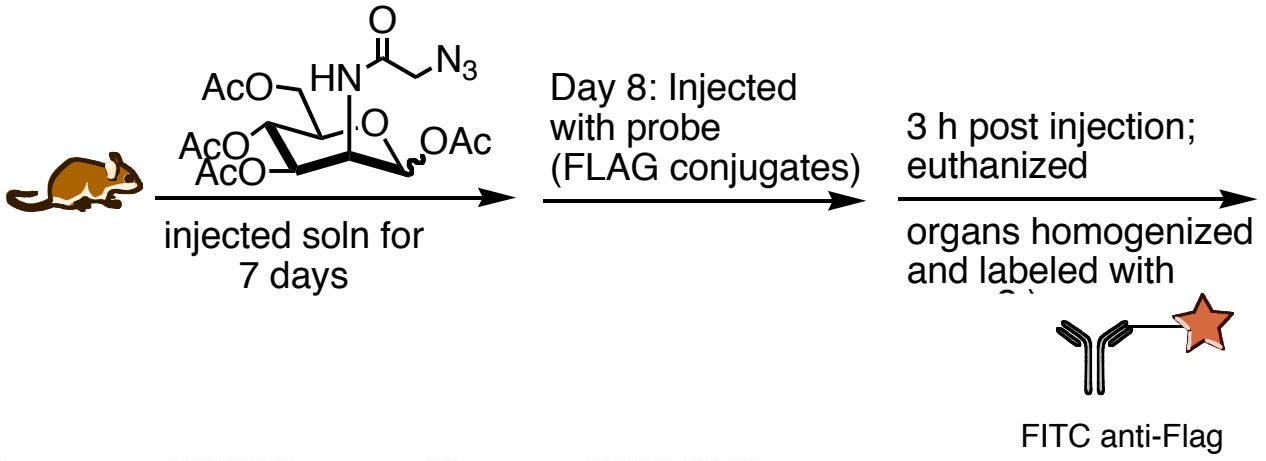
Zebrafish pectoral fin

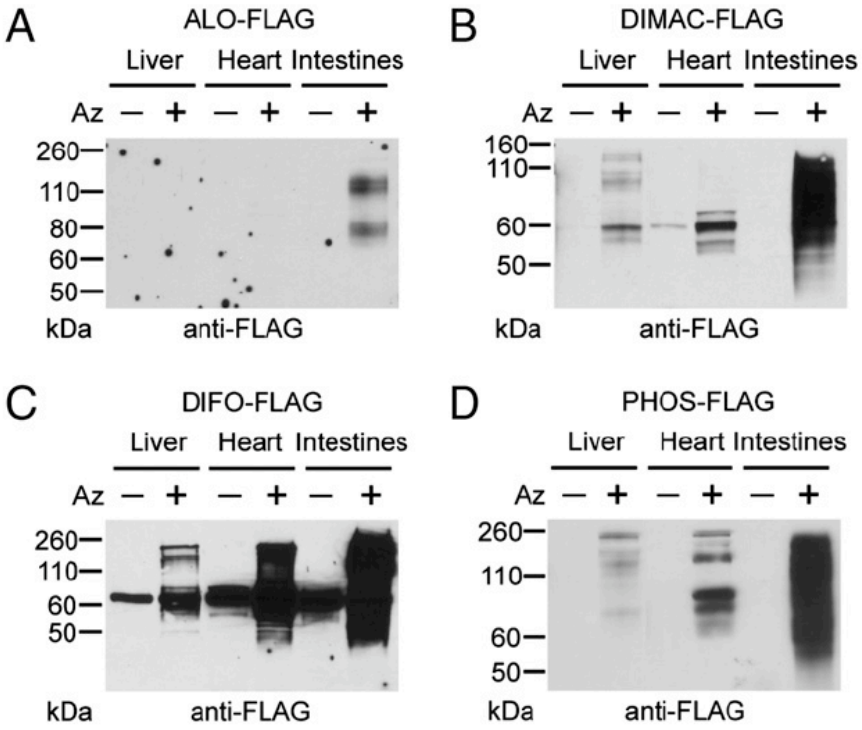
Laughlin, S. t.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008,** *320,* 664-667.

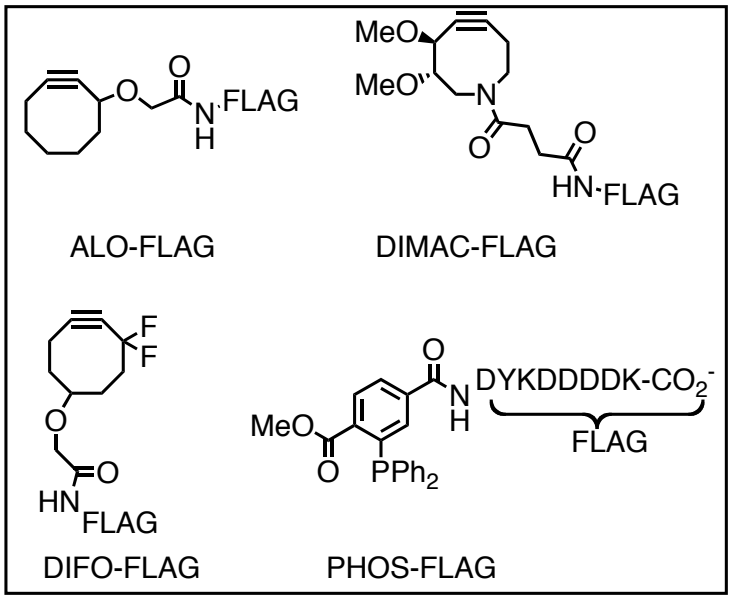
See also Agard, N. J.; Bertozzi, C. R. *Acc. Chem. Res.* **2009,** *42,* 788-797.

Extension to fucosylation: *ACS Chem Biol* **2011,** *6,* 547.

Studies in Mice (Part 2)







Chang, P. V.; Prescher, J. A.; Sletten, E. M.; Baskin, J. M.; Miller, I. A.; Agard, N. J.; Lo, A.; Bertozzi, C. R. *PNAS* **2010,** *107,* 1821-1826.

Bioorthogonal Imaging: Conclusions

•Bioorthogonal labels can be introduced metabolically into cells and whole organisms by taking advantage of glycan salvage pathways

•The Bertozzi group has shown that real-time imaging of glycan populations in developing zebrafish is possible

•Several probes (phosphines and octynes) have been evaluated for feasibility in reporting both *in vitro* and *in vivo*

•Future extensions of the work involve evaluating the metabolism of bioorthogonal probes

Summary and Outlook

- After over 100 years of studying saccharide chemistry, challenges for chemists remain:
  - 1) How can we efficiently synthesize diverse sets of oligosaccharides?
  - 2) Can we develop effective methods for detection of glycans, both *in vitro* and *in vivo*?

Key References

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