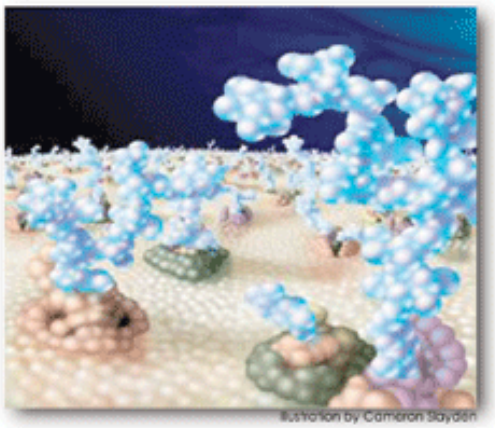
The Career of Carolyn Bertozzi

Group Meeting: November 12, 2008

David A Nagib



Key Reviews

Prescher, J. A.; Bertozzi, C. R. Chemistry in Living Systems. Nature Chem. Biol. 2005, 1, 13-21.

Dube, D. H.; Bertozzi, C. R. Metabolic Oligosaccharide Engineering as a Tool for Glycobiology. Curr. Opin. Chem. Biol. 2003, 7, 616

Bertozzi, C. R.; Kiessling, L. L. Chemical Glycobiology. Science 2001, 291, 2357-2364.

Seminal Publications

In vivo Imaging of Membrane-Asociated Glycans in Developing Zebrafish. Science 2008, 320, 664-667.

Chemical Remodelling of Cell Surfaces in Living Animals. Nature 2004, 430, 873-877.

A Small Molecule Modulator of Poly-a2,8-Sialic Acid Expression on Cultured Neurons and Tumor Cells. Science 2001, 294, 380-382.

Cell Surface Engineering by a Modified Staudinger Reaction. Science 2000, 287, 2007-2010.

Engineering Chemical Reactivity on Cell Surfaces Through Oligosaccharide Biosynthesis. Science 1997, 276, 1125-1128.

The Career of Carolyn Ruth Bertozzi

Biographical Notes

■ Education (b. 1966 - Boston, MA)

A.B.: Harvard University (1988); J. Grabowski – photoacoustic calorimetry

Bell Labs (1988); C. Chidsey – electron transfer materials
- Coadsorption of Ferrocene-Terminated and Unsubstituted Alkanethiols on Gold: Electroactive Self-Assembled Monolayers. *J. Am. Chem. Soc.* 1990, **112**, 4301-4306

Ph.D.: University of California, Berkeley (1993); M. D. Bednarski – oligosaccharide interactions
- Carbon-Linked Galactosphingolipid Analogs Bind Specifically to HIV-1 gp120. *J. Am. Chem. Soc.* 1992, ***114***, 1063
- Antibody Targeting to Bacterial Cells Using Receptor-Specific Ligands. *J. Am. Chem. Soc.* 1992, **114**, 2242
- A Receptor-Mediated Immune Response Using Synthetic Glycoconjugates. *J. Am. Chem. Soc.* 1992, **114**, 5543

Postdoc: University of California, San Francisco (1996): S. D. Rosen – leukocyte trafficking
- The Selectins and Their Ligands. *Curr. Opin. Cell Biol.* 1994, **6**, 663

■ Current Professional Appointments

T.Z. and Irmgard Chu Distinguished *Professor of Chemistry* at UC Berkeley
Professor of Molecular and Cell Biology at UC Berkeley
Investigator of the Howard Hughes Medical Institute
Director of the Molecular Foundry, a nanoscience institute at the Lawrence Berkeley National Laboratory

■ Notable Awards

Ernst Schering Prize (2007); Havinga Medal, Univ. Leiden (2005); Iota Sigma Pi Agnes Fay Morgan Research Award (2004);
Member: National Academy of Sciences (2005) & American Academy of Arts and Sciences (2003); Fellow: AAAS (2002);
Irving Sigal Young Investigator Award of the Protein Society (2002), ACS Award in Pure Chemistry (2001);
Presidential Early Career Award in Science and Engineering (PECASE) (2000); Joel H. Hildebrand Chair (1998-2000);
Arthur C. Cope Scholar Award (ACS) (1999); MacArthur Foundation Award (1999) Horace S. Isbell Award in Carbohydrate Chemistry (ACS) (1997)

■ Editorial boards: *Curr Opin in Chem Biol* (Editor-in-Chief), *ACS Chem Biol, Perspectives in Med Chem*

■ Publications:

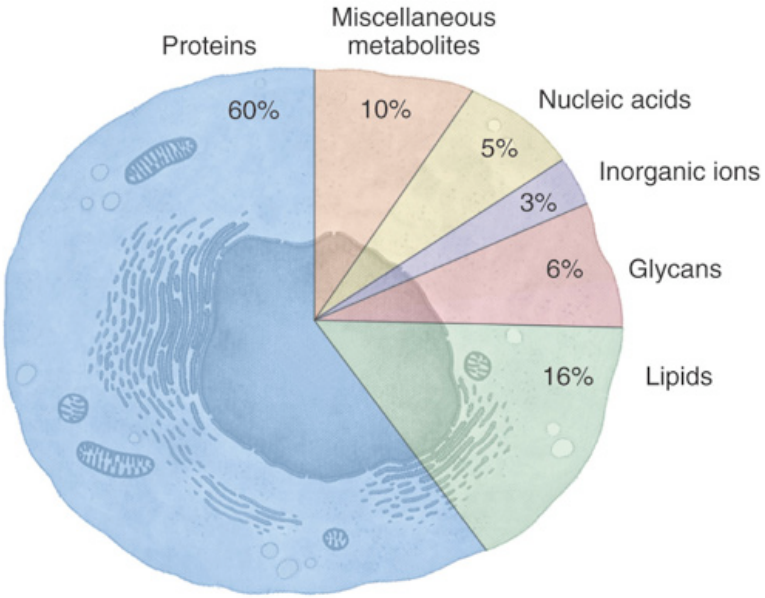
2 books, 14 reviews (including ones in Science, Nature, Cell, Chem. Soc. Rev., & Acc. Chem. Res.), and
>200 publications (20 publications in 2008 alone, including in *Science*, *JACS* (4), *Angew* (2), *Biochem* (2), & *PNAS*)

The Bertozzi Group

Research Areas: Background

- Living systems: composed of networks of interacting biopolymers, ions, and metabolites

Composition of typical mammalian cell



Genomics & Proteomics:
~20,000 genes ➔ ~100,000 modified proteins

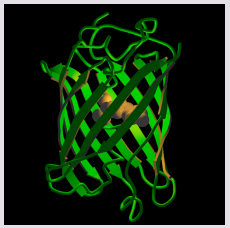
More than half the proteins are modified with glycans, lipids, or other metabolites

Complex array of cellular processes

… cannot be observed by examination of isolating purified biomolecules

Goal: Track molecules within their native environs

Most popular ('08 Nobel-winning) tagging strategy:
Green fluorescent protein (GFP)



Applications (many):
Protein expression & localization

Limitations:
Large structural perturbations may influence expression, localization, or function

Not amenable to glycans, lipids, nucleic acids, or 1000s of small organic metabolites

New approach: Since glycosylations are the most complex and ubiquitous of the types of post-translational modifications, an oligosaccaride-based probe could better elucidate their role in cell recognition & inter-cellular communication

The Bertozzi Group

Research Areas: Challenges & Strategy

- Key Questions

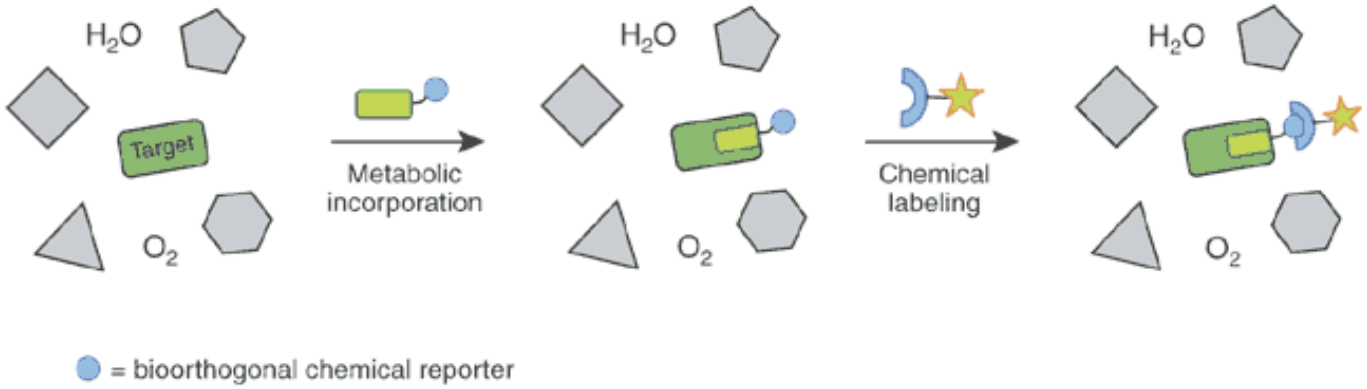
Which sugar moieties - glycans or oligosaccharides - are attached to which proteins?

How do glycosylation patterns differ from human and bacterial or cancerous cells?

Does the pattern change over the course of a person or disease's lifetime?

Is there a sugar code, comparable to the genetic code?

- Approach: Employ chemical tools to uncover the role of cell surface oligosaccarides by designing (1) a new, synthetic probe, which allows for the (2) detection and isolation of (3) proteins, glycans, and lipids



The bioorthogonal chemical reporter strategy

Non-native, non-perturbing chemical handles that can be (1) introduced via cellular metabolism, and (2) modified in living systems through highly selective reactions with exogenously delivered probes

Bioorthogonal chemical reporters

Design of a chemical reporter and bioorthogonal reaction

- Requirements for bioorthogonality

1) *Reactive*: should involve a rapid reaction, unaided by auxiliary reagents, with innocuous (or no) byproducts

   • similar to antibody-antigen kinetics

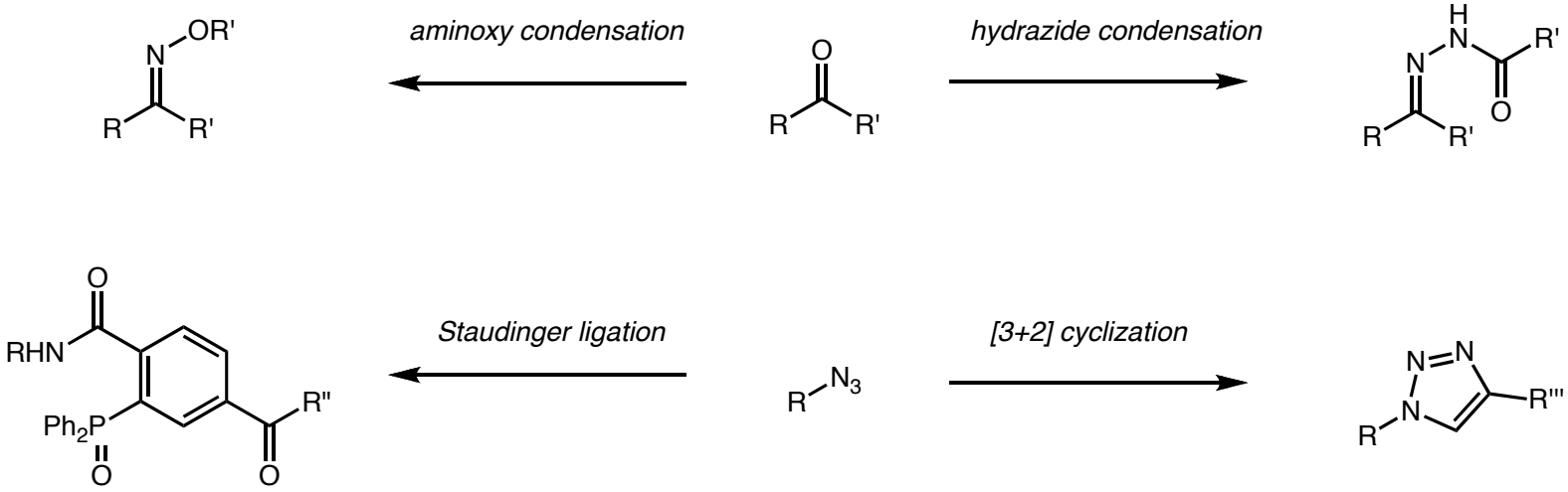
2) *Selective:* must avoid the abundance of nucleophiles, reducing agents, and other functionality present in cells

   • amines, isothiocyanates, thiols, and maleimides ➔ too promiscuous (may label irrelevant targets)

3) *Robust:* must possess adequate metabolic stability and bioavailability

   • physiological environment, typically 37°C, pH 6-8

- Potential reactions

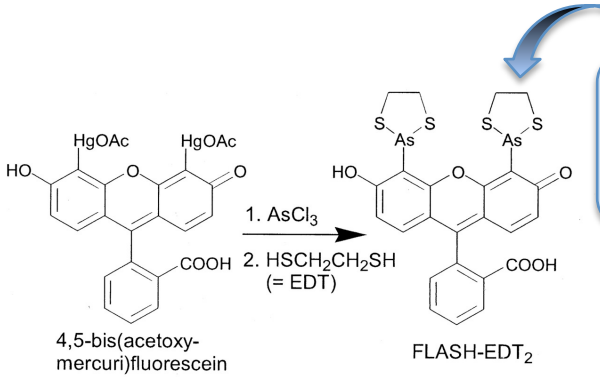


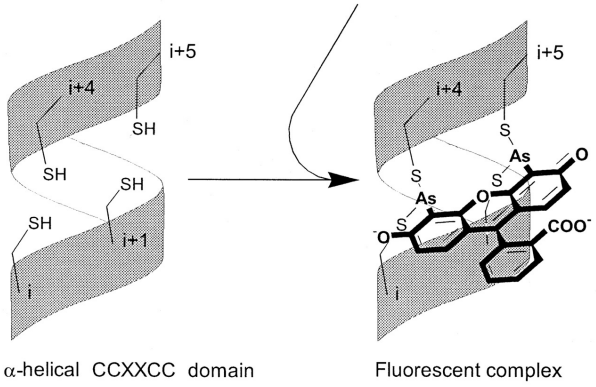
State of the Art

Bioorthogonal chemical reporters: *pre-Bertozzi*

- Peptide sequences: tetracysteine motif

A hexapeptide chemical reporter (CCXXCC) can be:
(1) genetically incorporated into proteins, and subsequently
(2) covalently labeled in living cells with membrane-permeant biarsenical dyes





Target specificity:
EDT protecting group prevents promiscuous labeling of isolated cysteine residues

Minimal structural perturbation:
Cys-Cys-Pro-Gly-Cys-Cys
(relative to GFP; 26.9 kDa)

*Successfully employed to image a variety of proteins, previously known to be perturbed by GFP labeling.

↓

Other peptide/reporter pairs:
Histidine-rich peptides & Ni probes
Acidic peptides & luminescent lanthanides

↓

However, processes still limited to the tagging of proteins & not-amenable to glycans/lipids

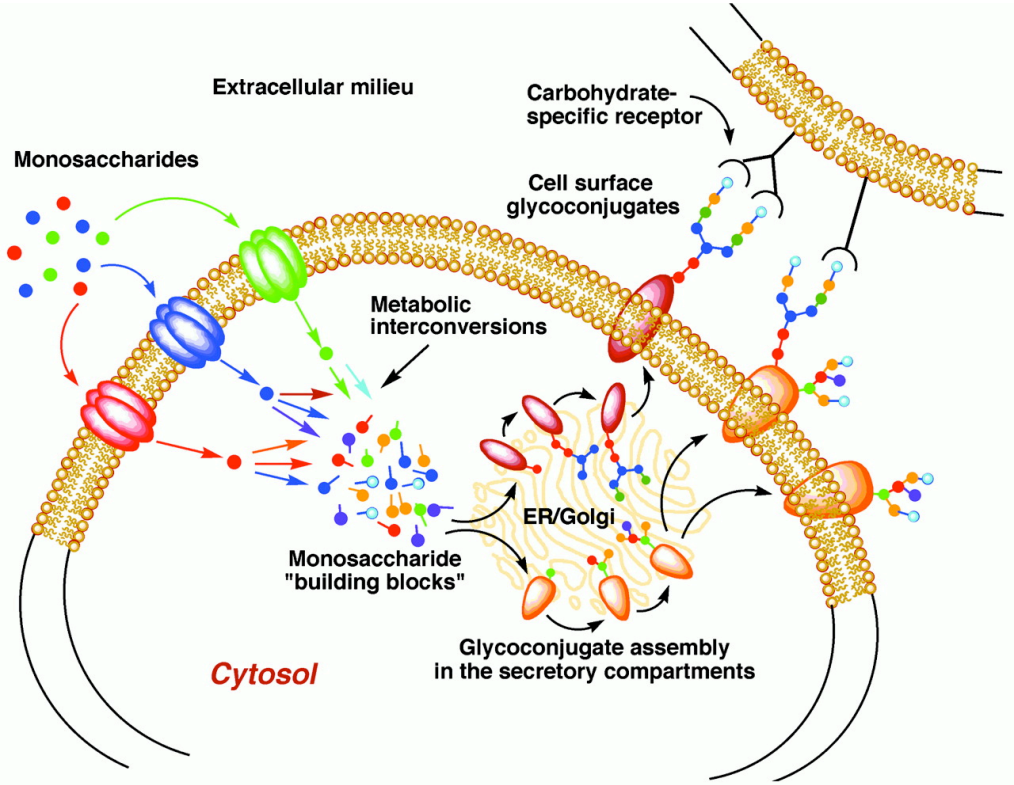
Griffin, B.A. Griffin, B.A., Adams, S.R. & Tsien, R.Y. *Science* **1998,** *281*, 269-272.

The Bertozzi Approach

Probing sugars

- *Metabolic oligosaccharide engineering*

•Unnatural monosaccharides are taken up by cells, transformed by the cell's biosynthetic machinery, and ultimately incorporated into glycoconjugates. Some are secreted or remain in the cell; the majority become cell surface glycoproteins

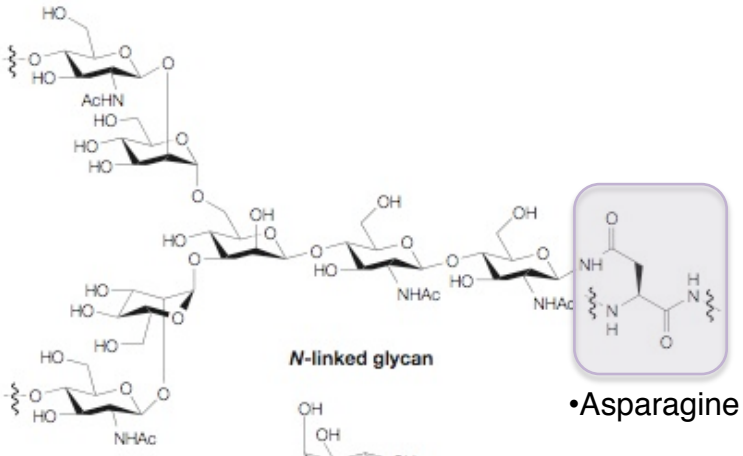


Review: Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357-2364.

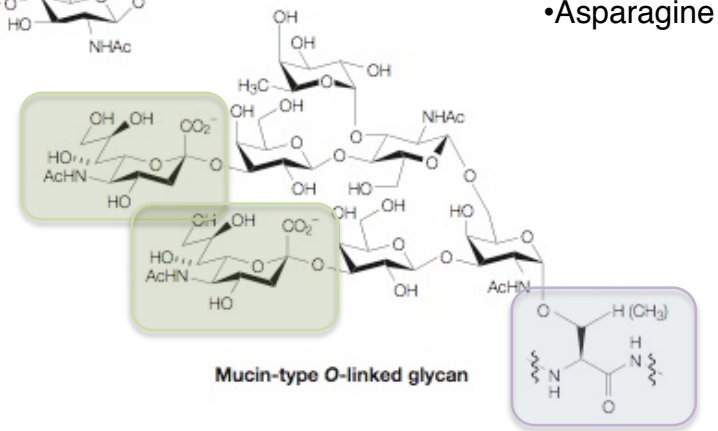
Glycobiology 101

The diverse array of glycan structures

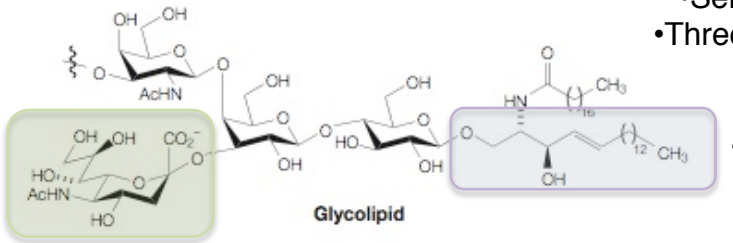
- *Major classes of glycan structures*



•Asparagine



•Serine
•Threonine

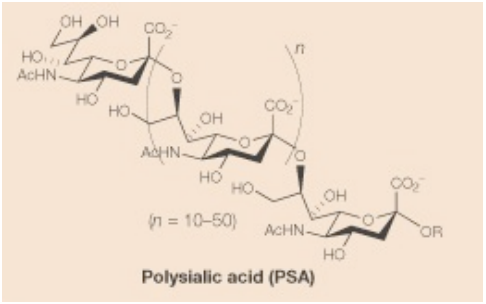


•Lipids

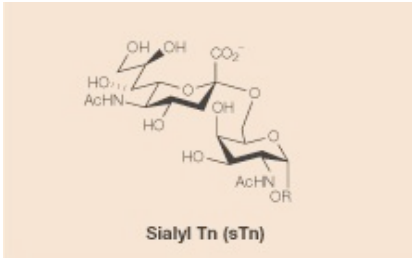
•Sialic acid – typical monosaccharide cap of olicosaccharides

- *Cancer associated glycans*

•Altered glycosylation patterns are a hallmark of the tumor phenotype



•Polysialic acid (PSA) expression is normally restricted to embryonic development; overexpressed in tumors



•High levels of the capping monosaccharide sialic acid suggests high metastatic ability of many types of cancer (acidic functionality may promote entry into bloodstream)

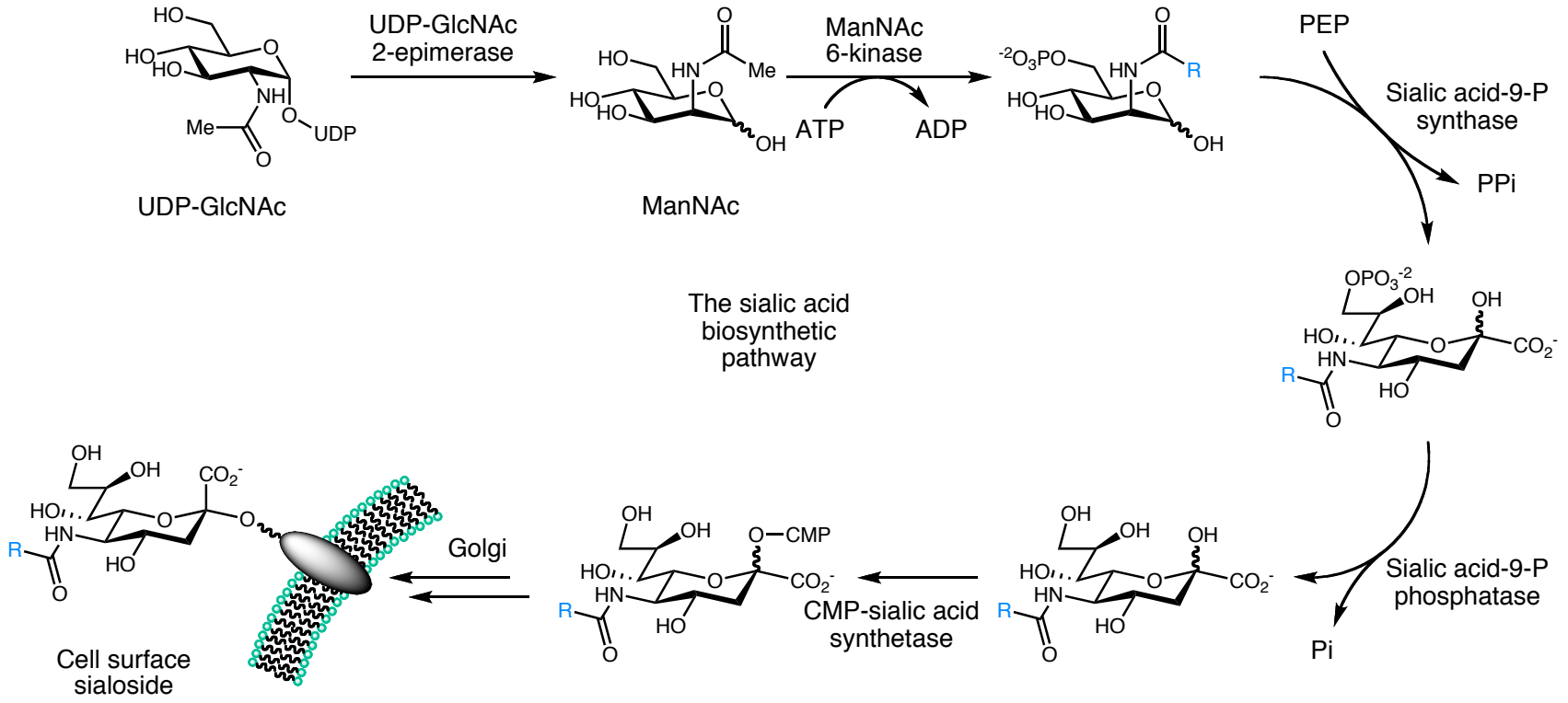
Dube, D. H.; Bertozzi, C. R. *Nature Rev. Drug Disc.* **2005**, *4*, 477-88.

The Bertozzi Approach

Probing sialosides

- *Metabolic oligosaccharide engineering*

•Unnatural monosaccharides are taken up by cells, transformed by the cell's biosynthetic machinery, and ultimately incorporated into glycoconjugates. Some are secreted or remain in the cell; the majority become cell surface glycoproteins



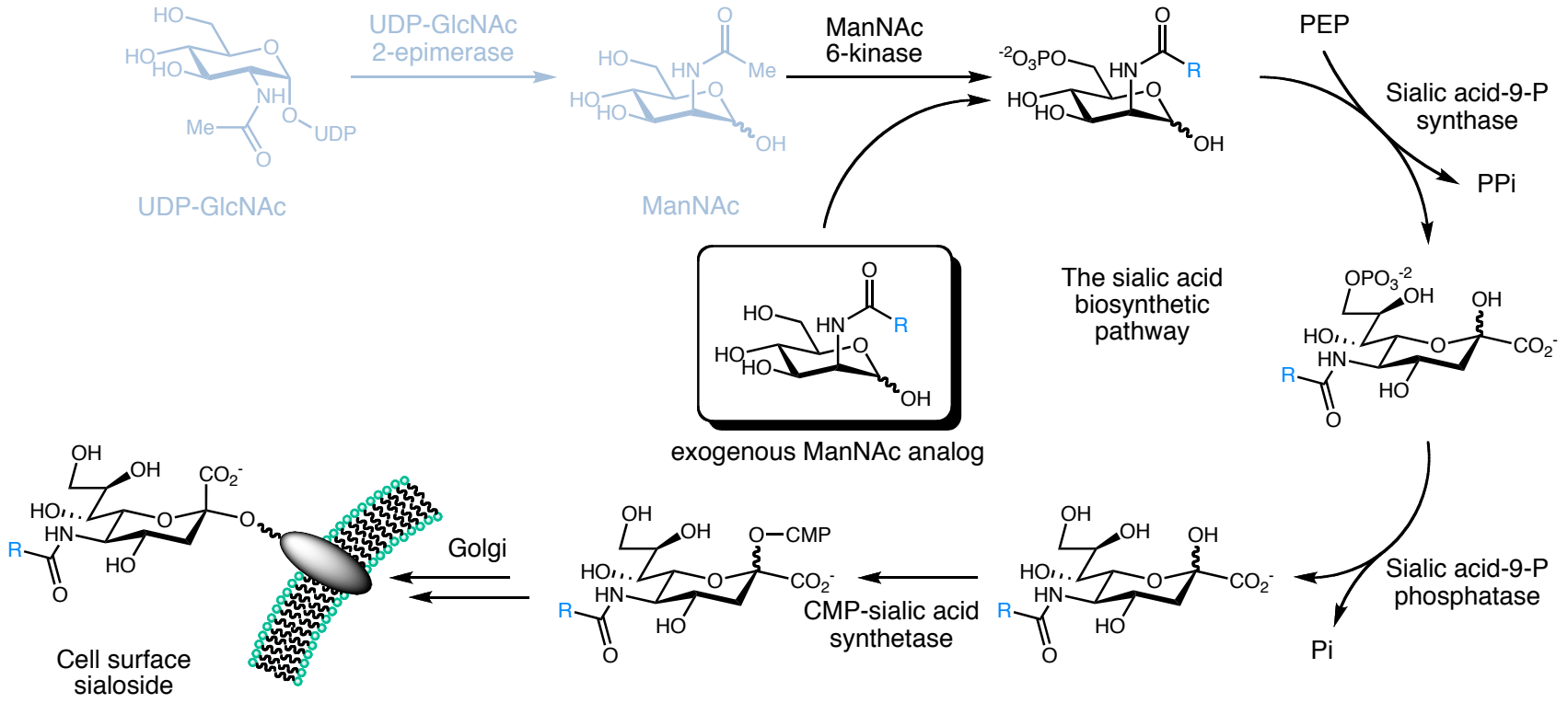
Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125-1128.

The Bertozzi Approach

Probing sialosides

- *Metabolic oligosaccharide engineering*

•Unnatural N-acyl manosamines are taken up by cells, transformed by the cell's biosynthetic machinery, and ultimately incorporated into sialosides. Some are secreted or remain in the cell; the majority become cell surface glycoproteins



- The sialic acid biosynthetic pathway is permissive of unnatural ManNAc analogs (at the NAc position)
- ManNAc 6-kinase is the bottleneck enzyme, which allows for competitive introduction of exogenous analogs

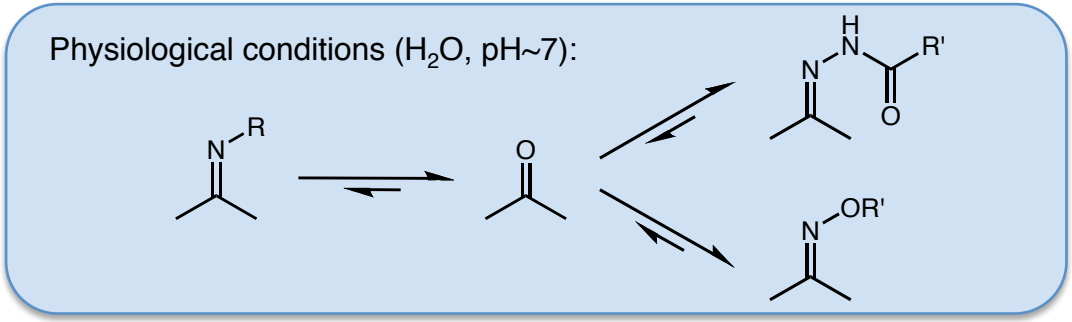
Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125-1128.

Bertozzi's chemical reporters

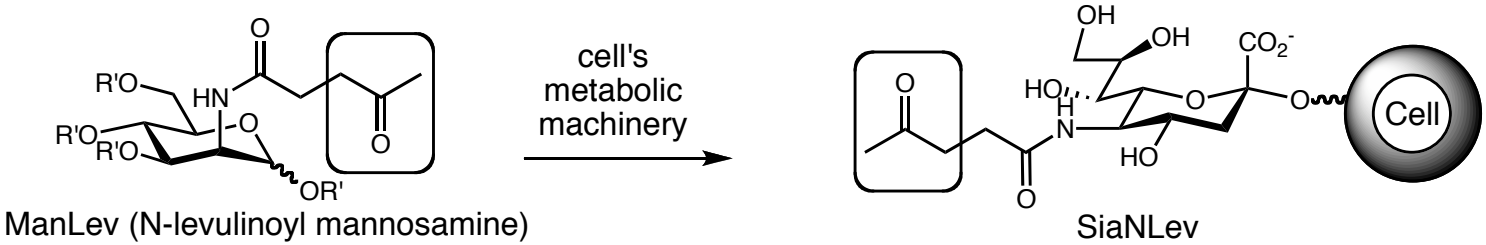
The carbonyl group

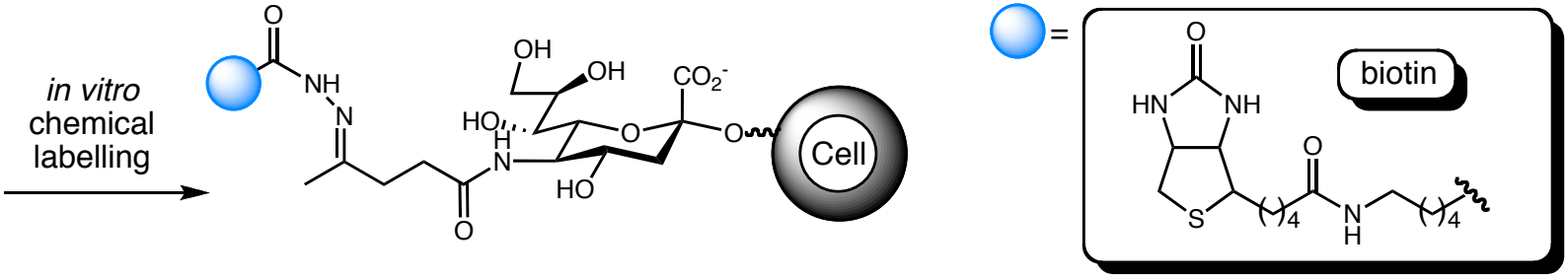
- Ketones and aldehydes

(1) Very small functional group will not perturb system
(2) Stabilized Schiffs bases (oximes & hydrazones) are favored in water and quite stable under physiological conditions



- Biosynthetic incorporation of ketone groups into cell-surface-associated sialic acid & subsequent chemical labelling



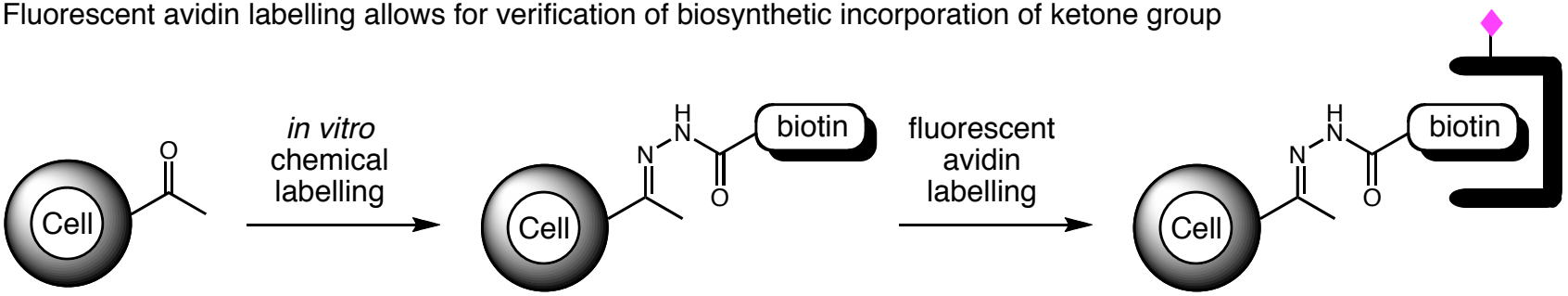


Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125-1128.

Initial Success

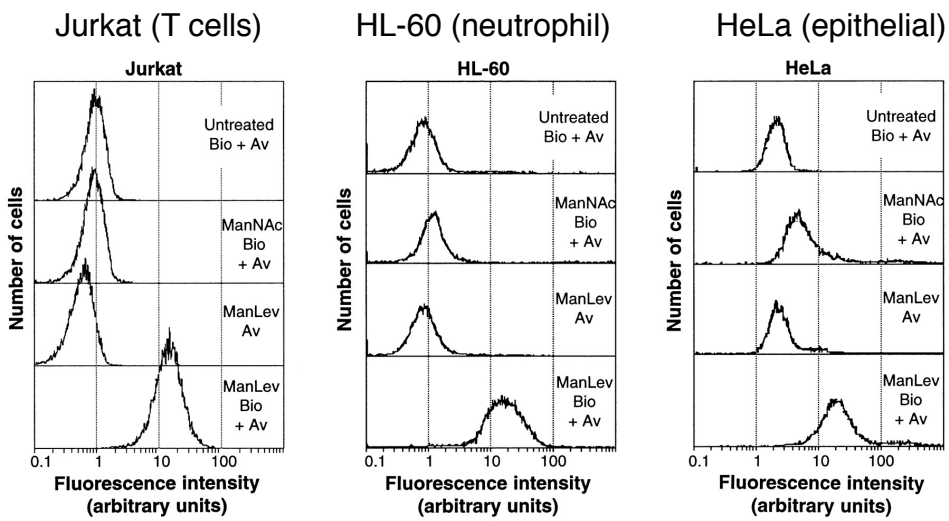
The carbonyl group as chemical reporter

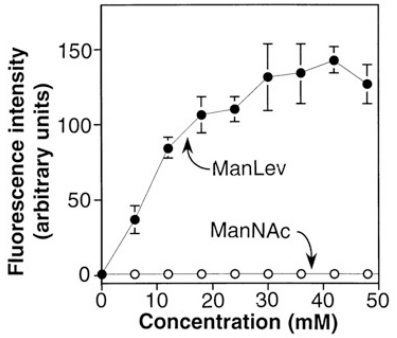
- Fluorescent avidin labelling allows for verification of biosynthetic incorporation of ketone group



Avidin is a tetrameric protein which binds to biotin with high affinity and selectivity ($K_D \approx 10^{-15}$ M)

- Quantitative analysis (via flow cytometry) confirmed ketone expression in three human cell lines





- Ketone incorporation is dose-dependant and saturatable

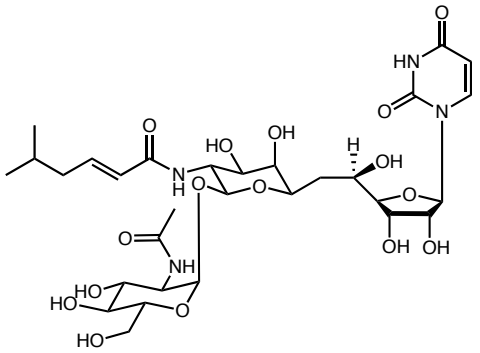
~1.8 x 10^6 probes/cell

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

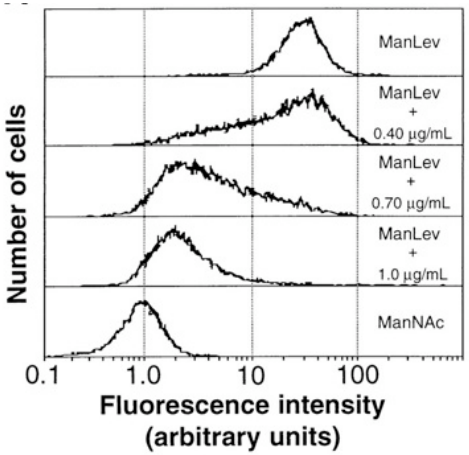
Controls & Applications

The carbonyl group as chemical reporter

- Demonstrated the ketone groups are displayed on the cell surface as sialoglycoconjugates



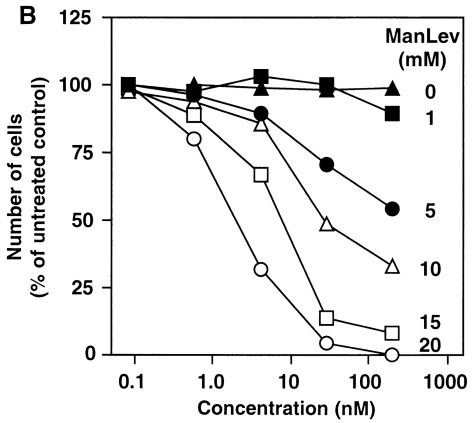
Tunicamycin: inhibits N-linked protein glycosylation (common in Jurkat T cells)

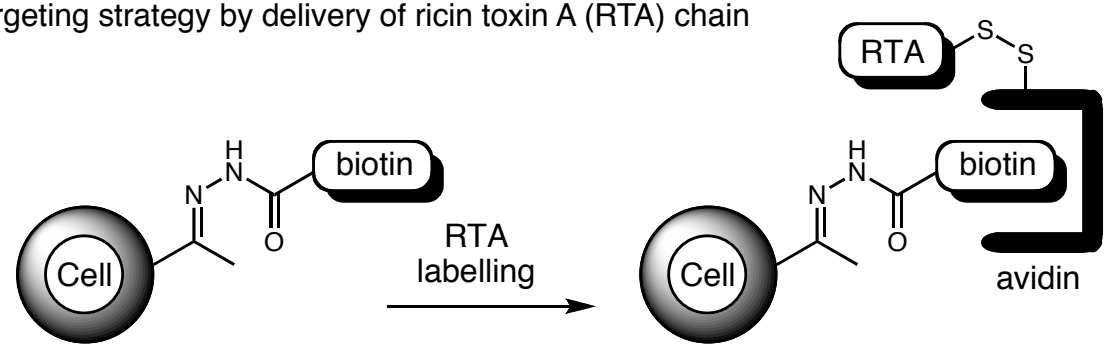


- Tunicamycin inhibits ketone incorporation on Jurgat cells in a dose-dependant fashion

- HL-60 and HeLa cells are unperturbed by tunicamycin, but instead inhibited by α-benzyl N-acetyl-galactosamine (an inhibitor of O-linked glycosylation), consistent with high mucin expression on myeloid and epithelial cells

- Demonstrated the oncological immunotargeting strategy by delivery of ricin toxin A (RTA) chain





- Reducing environment of cell breaks disulfide linkage and releases toxins into cell.
- Death of cells within 3 days represent successful drug-targeting.

> 700,000 ketones/cell: lethal doses (LD_{50}) 1-10nM

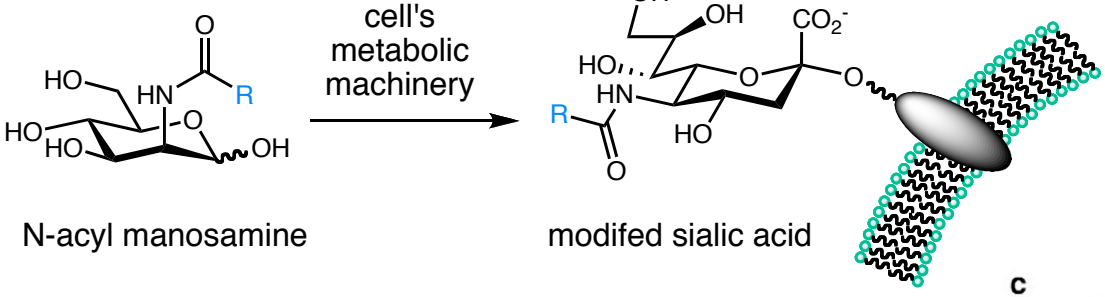
< 50,000 ketones/cell: no toxicity

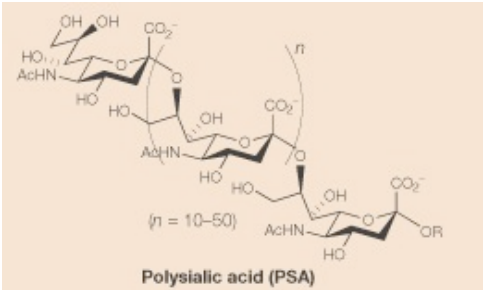
Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125-1128.

Anti-tumor applications

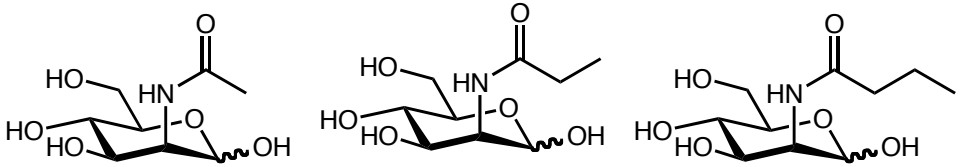
The chemical reporter as a small molecule inhibitor

- Polysialic acid (PSA) expression is a post-translational modification of neural proteins, commonly overexpressed in tumors





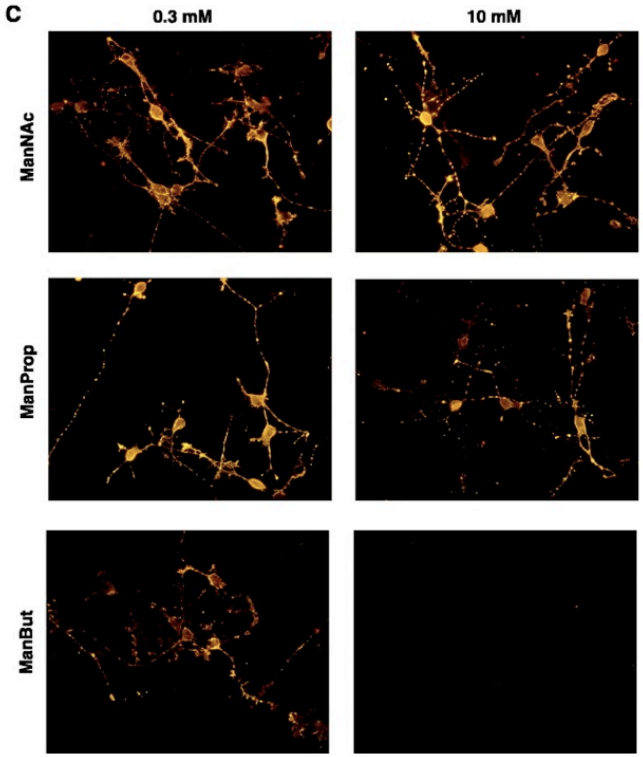
- N-acetylmannosamine (ManNAc) is naturally converted to sialic acid



- ManNAc & ManNProp do not hinder PSA expression in tumors

ManNBut abrogates staining by PSA antibody in a dose-dependent manner

(i.e. ManNBut serves as a small-molecule, metabolic inhibitor of PSA expression)

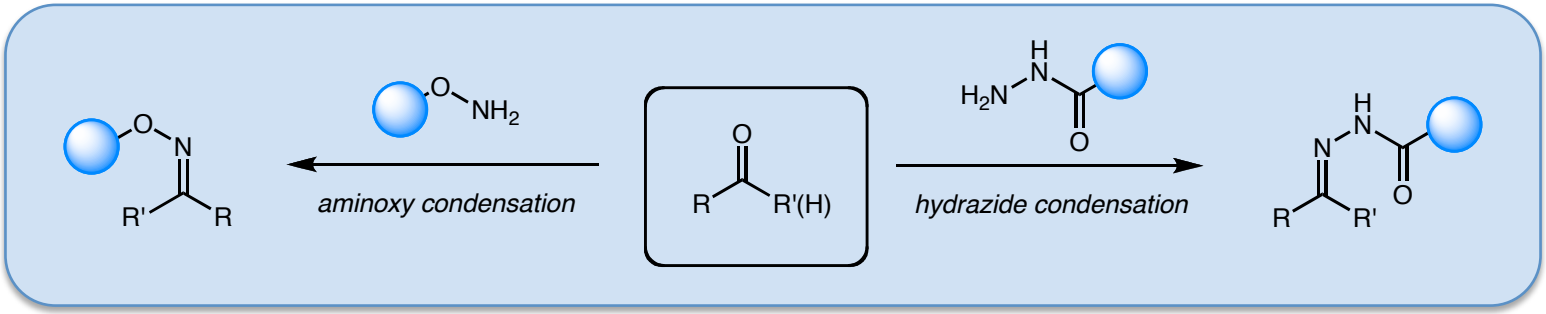


Mahal, L. K.; Charter, N. W.; Angata, K.; Fukuda, M.; Koshland, D. E., Jr.; Bertozzi, C. R. *Science* **2001**, *294,* 380-382.

Scope & Limits

1st generation chemical reporters

- Ketones and aldehydes



* *Proof of principle*: small, organic molecule as a non-perturbing chemical reporter of glycans & lipids

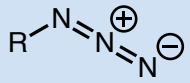
- *Bioorthogonal vs biorestricted*

  - Optimal pH = 5-6, not achievable in most tissues *in vivo* (restricted to specific cultured cells; *in vitro*)
  - Endogenous keto-metabolites interfere with oxime/hydrazone chemistry (i.e. sugars, pyruvates, oxaloacetate, and various cofactors, such as pyridoxal phosphate)
  - Restricted to environs devoid of carbonyl electrophiles; cell surface & extracellular environment

The Azide

2nd generation chemical reporters

- Bioorthogonal functional group: *the azide*



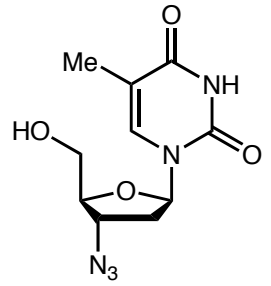
azide

- absent from nearly all naturally occuring species (except for a unialgal metabolite)
- mildly electrophilic; does not react with hard nucleophiles (i.e. amines)
- does not react appreciably with H_2O
- resistant to oxidation
- only 3 atoms

- Common misconceptions of azide stability & toxicity

Myth: azides are unstable to heat	*Truth*: azides are prone to decomposition at elevated temperatures (>100°C), but are quite stable at physiological temperatures
Myth: azides are unstable to light	*Truth*: azides are well-known photocrosslinkers, but do not photodecompose in the presence of ambient light
Myth: azides are toxic	*Truth*: the azide anion (i.e. NaN_3) is a widely used cytotoxin, but organic azides have no intrinsic toxicity

- Current applications in medicine
  - reverse transcriptase inhibitors, such as AZT, used to treat HIV
  - azido group increases lipophilicity, which promotes more facile permeation of cell membranes as well as the blood-brain barrier

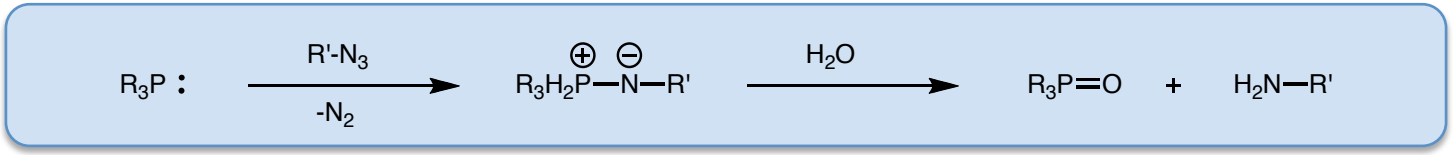


azidothymidine (AZT)

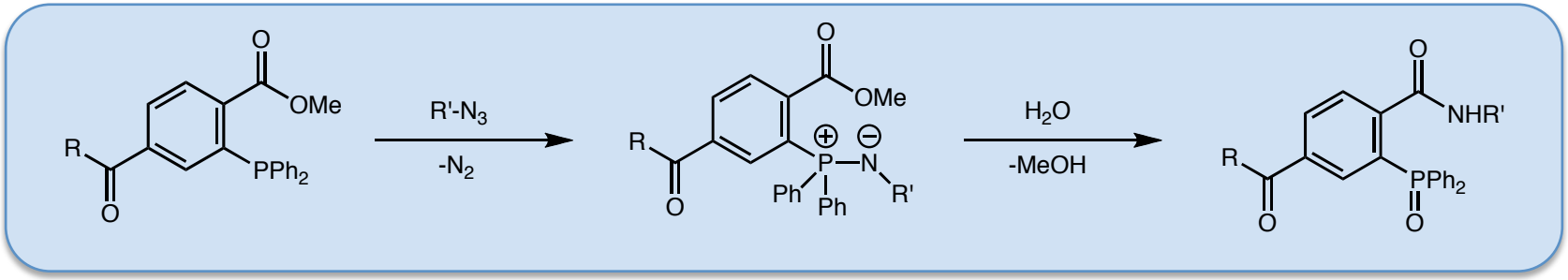
Staudinger ligation

2nd generation chemical reporters

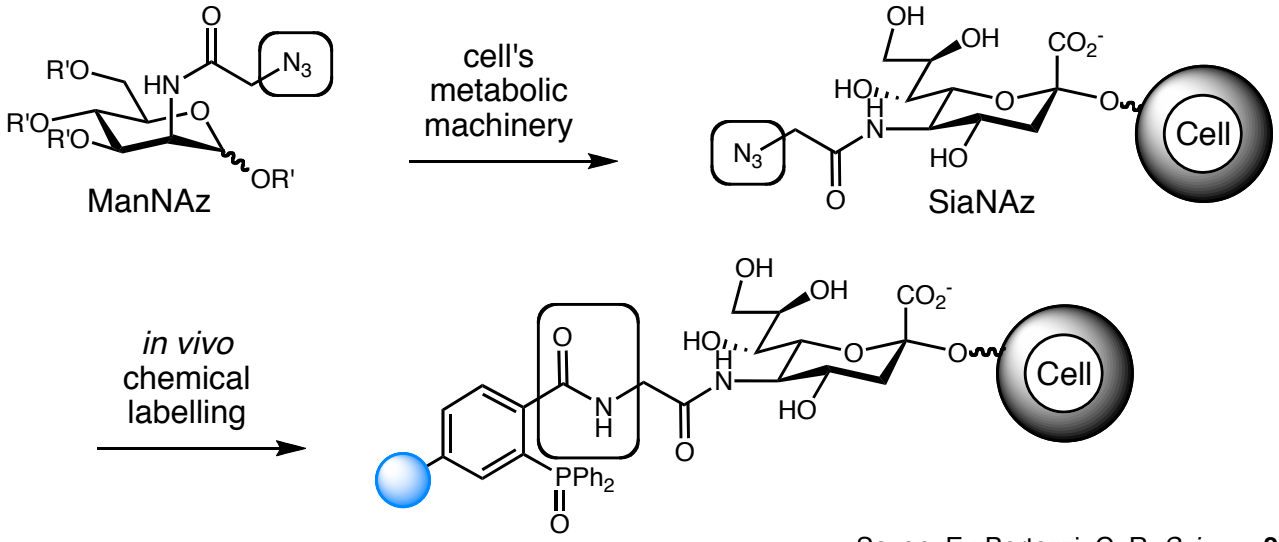
- Classical Staudinger reaction



- Modified Staudinger reaction: *introduction of an intramolecular trap*



- Staudinger ligation as bioorthogonal chemical reaction under physiological conditions (H_2O, pH ~7)

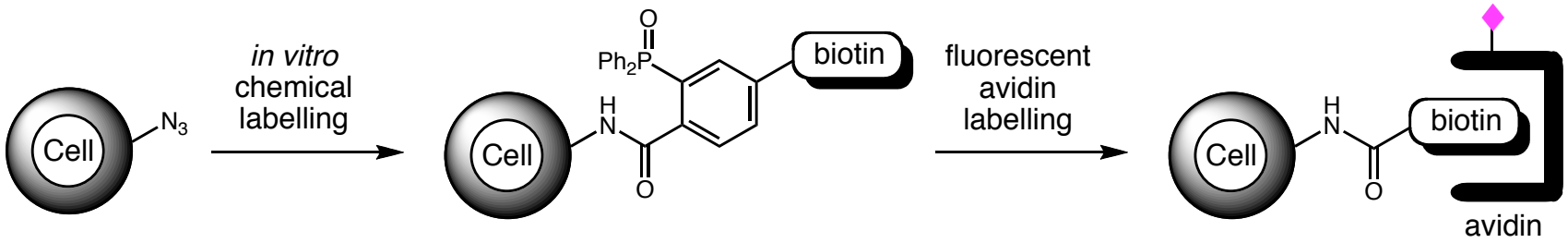


Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007-2010.

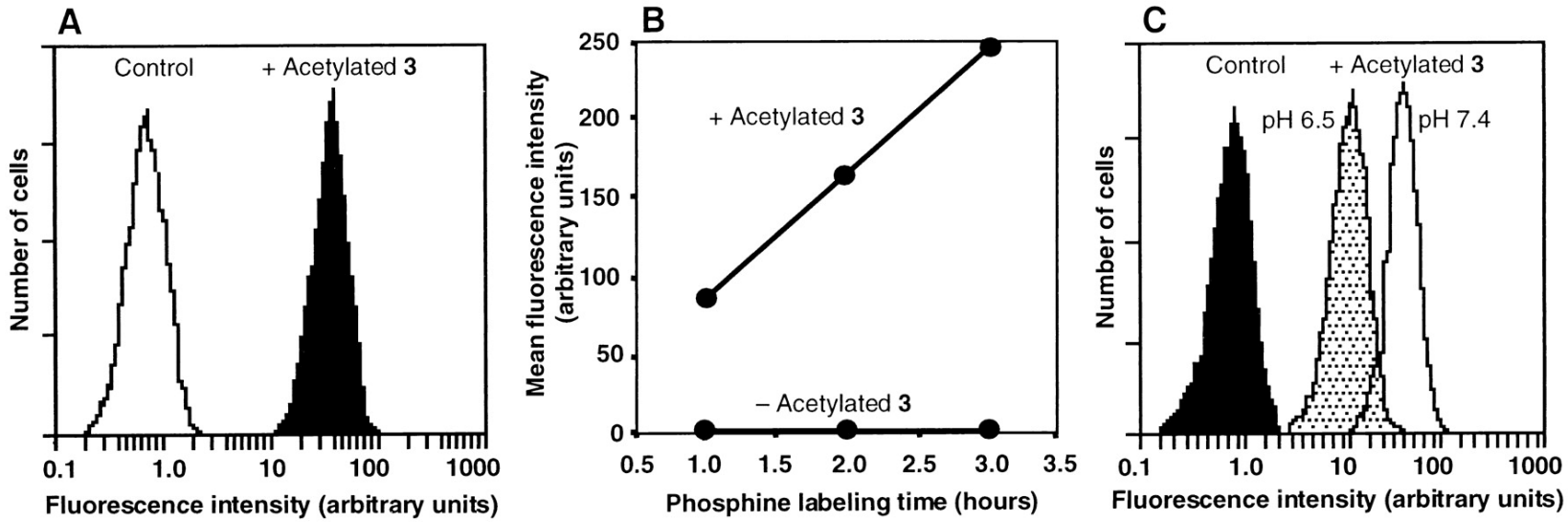
Cell surface engineering via Staudinger ligation

The azide as chemical reporters

- Fluorescent avidin labelling allows for verification of biosynthetic incorporation of azide group



- Quantitative analysis (via flow cytometry) confirmed azide expression in three human cell lines



- Two-fold fluorescence vs ketones
- No change in growth rate 3 days after modified Staudinger reaction

- Dose & time -dependant

~850,000 probes/cell (in only 1 hr)
~4.5x10^6 probes/cell (in 3 hr)

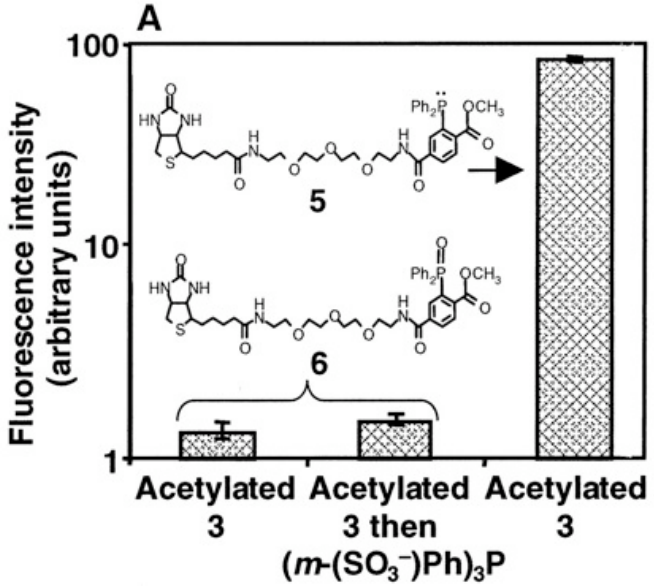
- Works in less acidic media than carbonyl reporters
- Acidic conditions are deleterious

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

Cell surface engineering via Staudinger ligation

Verification of cell surface selectivity & bioorthogonality

- *Cell surface selectivity:* nonspecific acylation must be ruled out

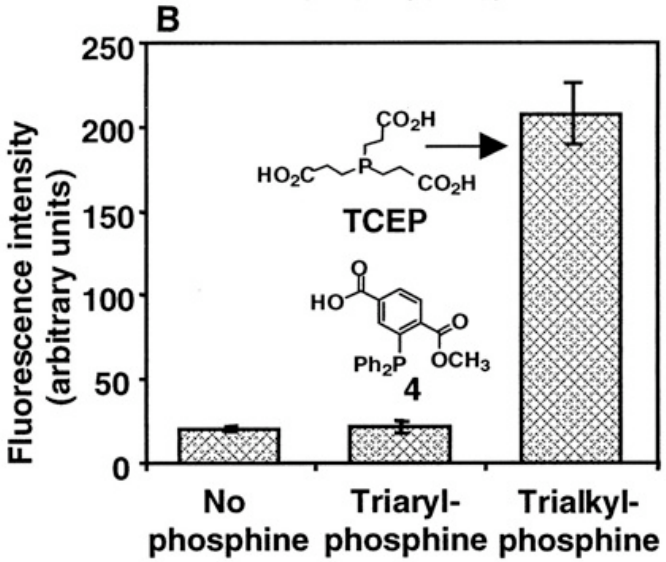


Classical Staudinger reaction does NOT associate with synthetic phosphine oxide probe (A)

… even when azides are intentionally reduced (B)

... versus normal IM ligation (C)

- *Chemical orthogonality:* both participants must not engage functional groups endogenous to cells



Absence of free sulfhydryl groups (A)

Triaryl phosphine does NOT reduce disulfides (B)

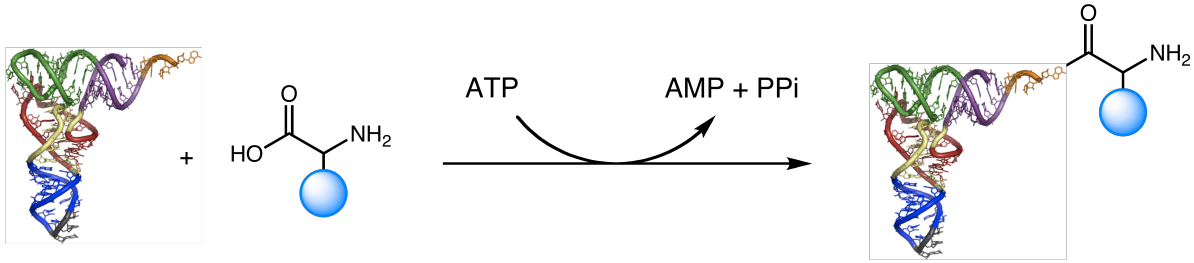
Trialkyl phosphine DOES generate appreciable sulfides (C)

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

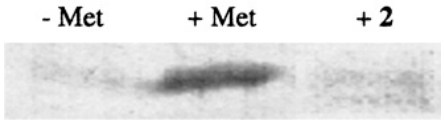
Protein modification

Additional applications of the Staudinger ligation

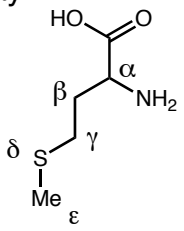
- Incorporation of azides into recombinant proteins requires initial uptake by methionyl-tRNA



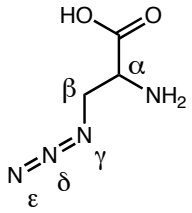
- Detection via ^{32}P-pyrophosphate exchange assay



AZA



methionine
Met



azidoalanine
AZA

Kiick, K. L.; Saxon, E.; Tirrell, D. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99,* 19-24.

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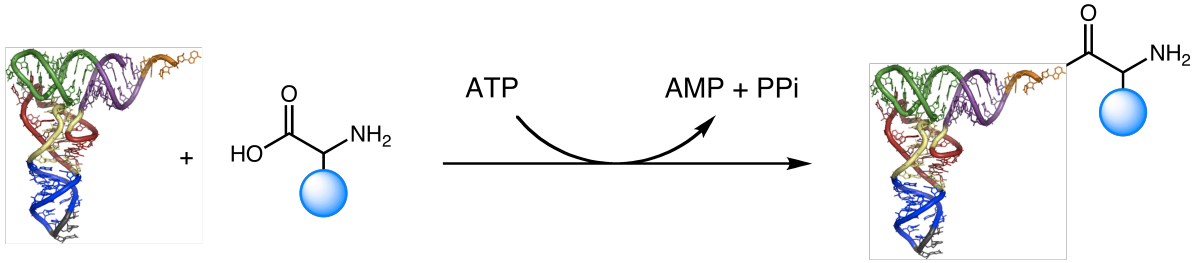

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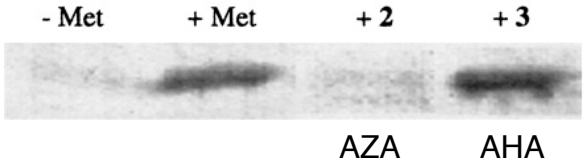
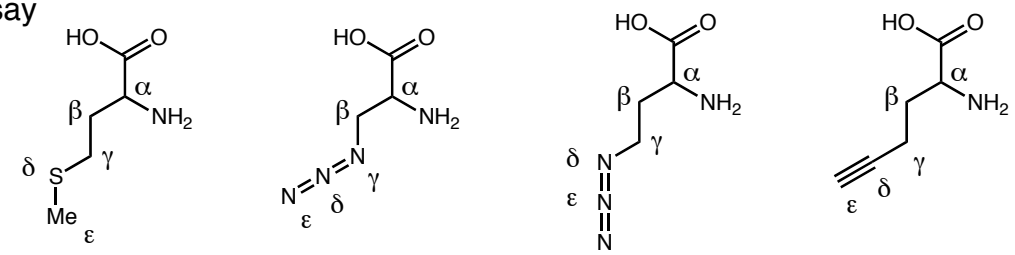
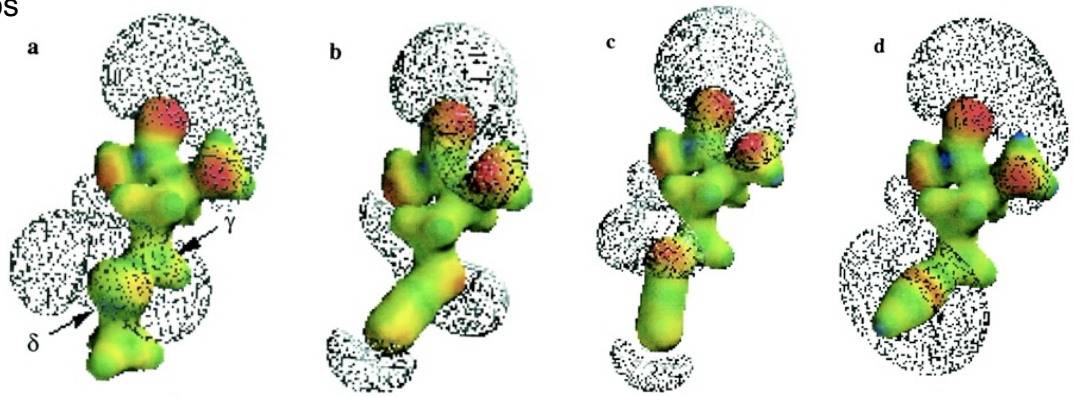




methionine **Met** | azidoalanine **AZA** | azidohomoalanine **AHA** | 2-amino-5-hexynoic acid

- Electron density & isopotential surface maps



- Isopotential surface most highly extended at
  - δ position: Met & AHA
  - γ position: AZA

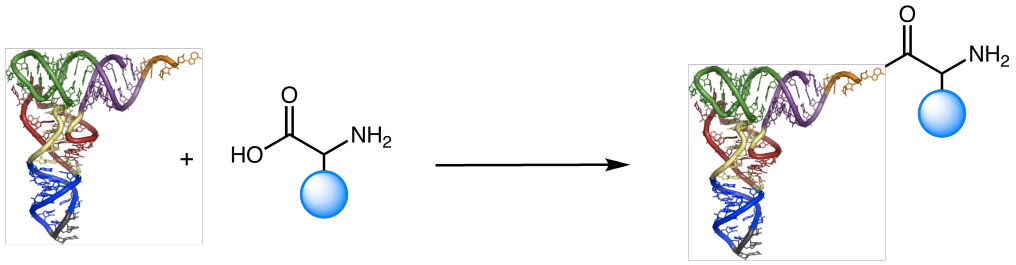
- e- density at δ position may form H-bonds with Tyr-260 and Leu-13 in the active site of the MetRS enzyme, which functionalizes the methionyl-tRNA

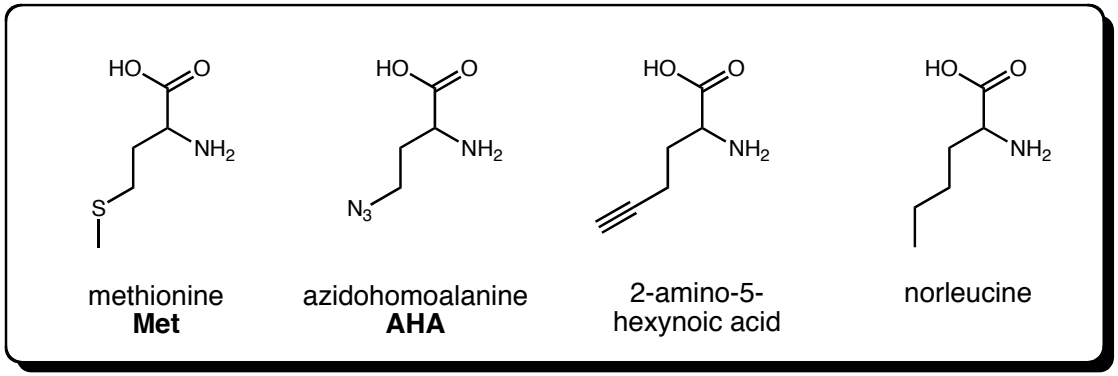
Kiick, K. L.; Saxon, E.; Tirrell, D. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99,* 19-24.

Azide incorporation

Chemoselective modification of recombinant proteins

- The rate of activation of the modified amino acids by MetRS was determined *in vitro*





methionine **Met**

azidohomoalanine **AHA**

2-amino-5-hexynoic acid

norleucine

- Quantification of the rate of activation of MetRS

a.a. analog	k_{cat}/k_m (s^{-1} μM^{-1})	k_{cat}/k_m (rel to Met)
Met	5.47×10^{-1}	1
AHA	1.42×10^{-3}	1/390
hexynoic	1.16×10^{-3}	1/500
norleucine	5.22×10^{-4}	1/1050

• Both the *homo*-azide and alkyne chains were incorporated into MetRS at similar rates

Kiick, K. L.; Saxon, E.; Tirrell, D. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99,* 19-24.

Azide incorporation

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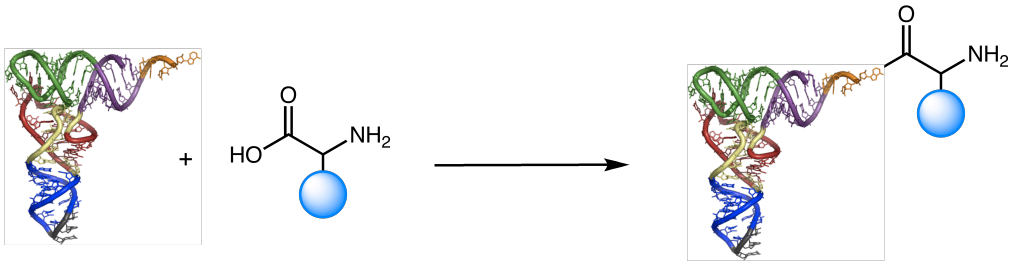
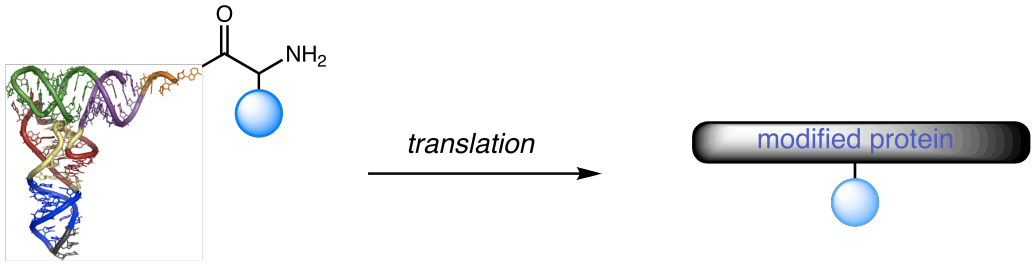


- Translational activity was then assessed using a methionine auxotroph of *E. coli*



- Quantification of the rate of activation of MetRS and corresponding synthesized protein, mDHFR

a.a. analog	k_{cat}/k_m (s^{-1} μM^{-1})	k_{cat}/k_m (rel to Met)	relative protein yield
Met	5.47×10^{-1}	1	100 %
AHA	1.42×10^{-3}	1/390	100 %
hexynoic	1.16×10^{-3}	1/500	100 %
norleucine	5.22×10^{-4}	1/1050	57 %

- Both the *homo*-azide and alkyne chains were incorporated into MetRS at similar rates
- Both supported complete protein synthesis
- Complementary mass spec & peptide sequencing analyses confirmed azide (AHA) incorporation, with ~96% replacement of methionine

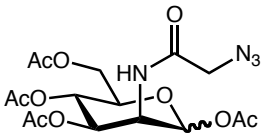
Kiick, K. L.; Saxon, E.; Tirrell, D. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99,* 19-24.

In living organisms

Metabolic oligosaccharide engineering & Staudinger ligations in mice

- Metabolic oligosaccharide engineering performed *in vivo* in a living animal for the first time

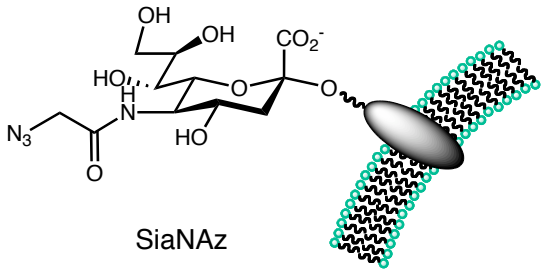
+



$Ac_4ManNAz$

(1) Inject $Ac_4ManNAz$ solution for 7 days

(2) Isolate splenocytes



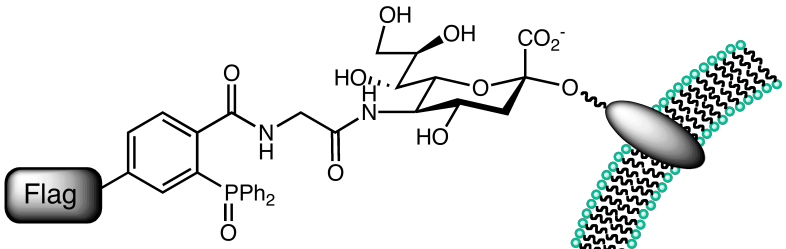
SiaNAz

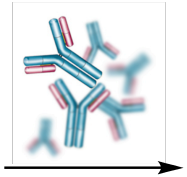
- Peracetylated ManNAz ($Ac_4ManNAz$) will passively diffuse into cells more readily than the free ManNAz
- Carboxyesterases, which exist at high levels in rodent serum, convert $Ac_4ManNAz$ to ManNAz relatively quickly

- Staudinger ligations performed *ex vivo* on isolated mouse splenocytes

SiaNAz

ex vivo chemical labelling





Fluorescent antibody detection

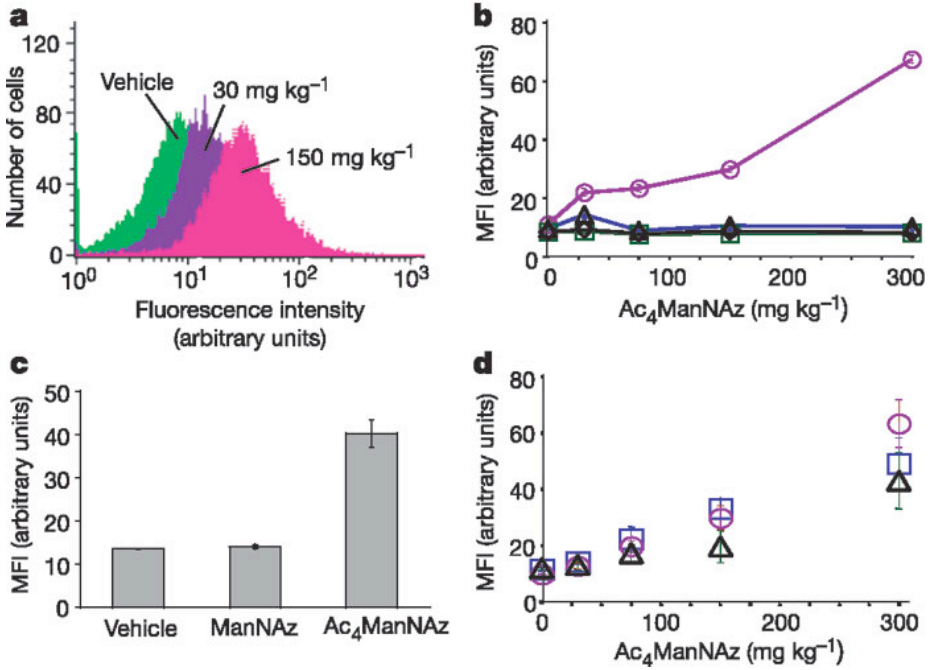
- Mice are euthanized on the 8th day and their splenocytes (cells rich in sialosides) are isolated
- Staudinger ligation is performed *ex vivo* with a phosphine bearing a Flag peptide, whose antibody is fluorescent

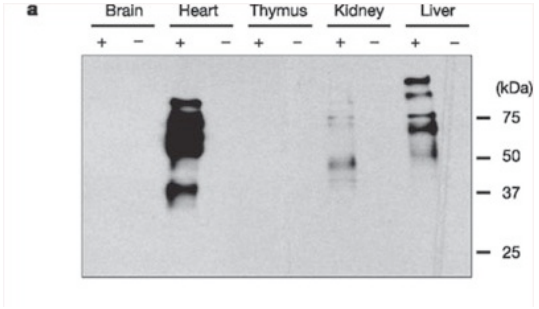
Prescher, J. A.; Dube, D. H.; Bertozzi, C. R. *Nature* **2004**, *430*, 873-877.

In living organisms

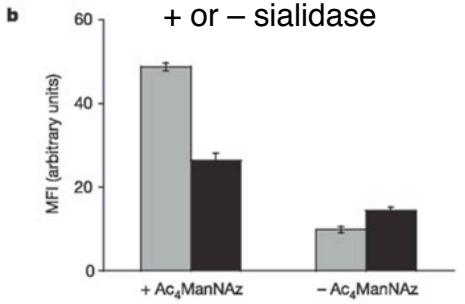
Metabolic oligosaccharide engineering & Staudinger ligations in mice

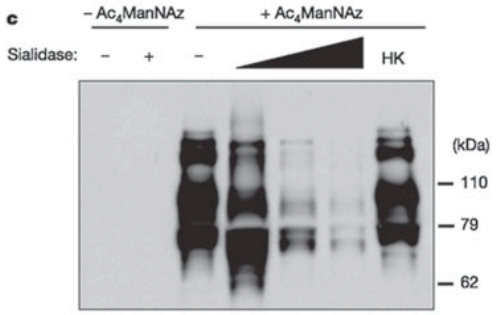
- Ac_4ManNAz is metabolized *in vivo*





+ or – sialidase





- Flow cytometry analysis similar to *in vitro* studies

  • No adverse physiological effects on mice over 7 days, as determined by monitoring feeding habits, weights, and overall activity

  • 3% of natural sialic acid in the heart were replaced with SiaNAz

  • Liver is known to secrete numerous sialylated glycoproteins

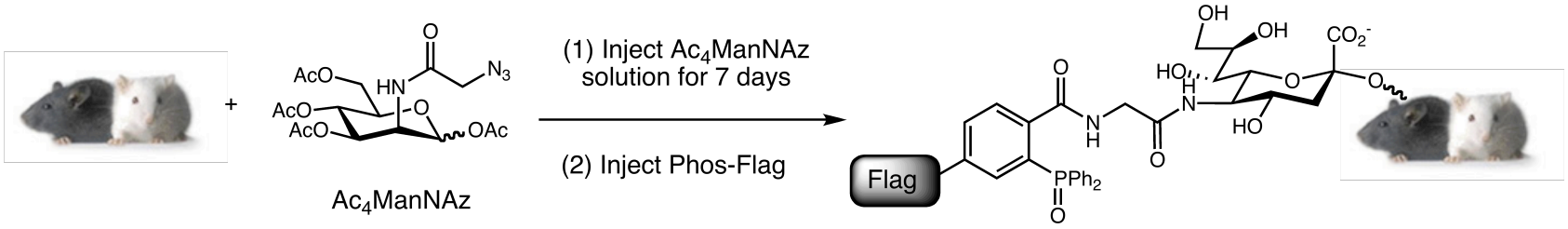
  • However, heart & kidney labelling may be a product of significantly lower levels of UDP-GlcNaC-2-epimerase (ManNAc)

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

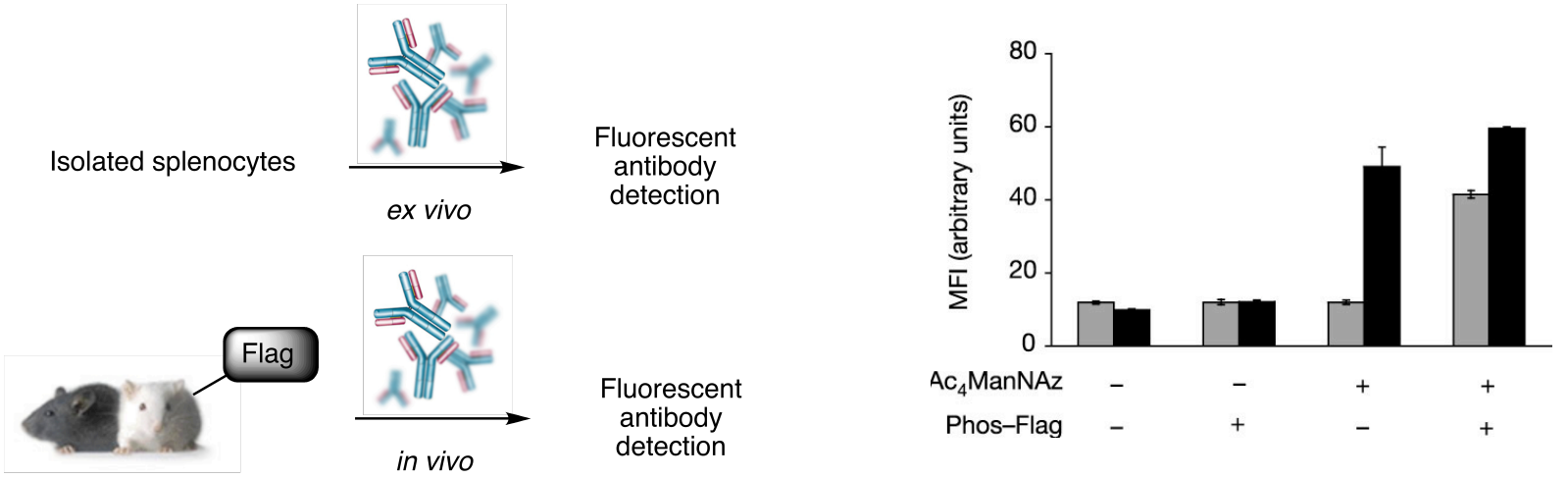
In living organisms: in vivo

Metabolic oligosaccharide engineering & Staudinger ligations in mice

- Metabolic oligosaccharide engineering AND Staudinger ligations performed *in vivo* in a living animal for the first time



- Staudinger ligations then performed both *ex vivo* on isolated mouse splenocytes AND *in vivo* in living mice



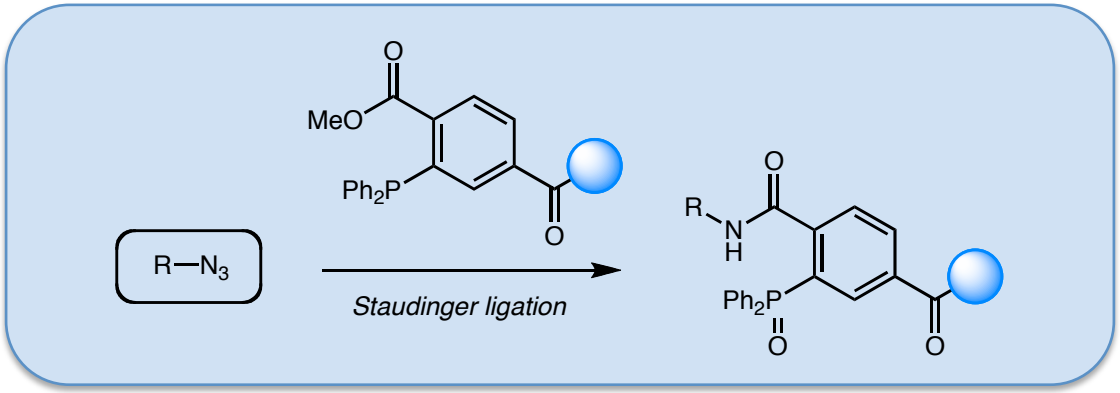
- Gray bars represent *in vivo* Staudinger ligation
- Black bars represent the same, with additional *ex vivo* Staudinger ligation

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

Scope & Limits

2nd generation chemical reporters

- Azides & phosphines



• *Bioorthogonal*: azides & triaryl phosphines are abiotic functional groups that undergo reaction at pH~7 with no toxic effects

- *Applications*

  • modify glycans on *living* cells: *Science* **2000** *287,* 2007 and animals: *Nature* **2004**, *430*, 873

  • enrich glycoprotein subtypes from various proteomes: *Proc. Natl. Acad. Sci. USA* **2003,***100,* 14846

  • impart new functionality to recombinant proteins: *Biochemistry* **2004,** *43,* 12358

  • "traceless Staudinger" is also now available to exclude phosphine oxide from product: *Org. Lett.* **2000***, 2,* 2141

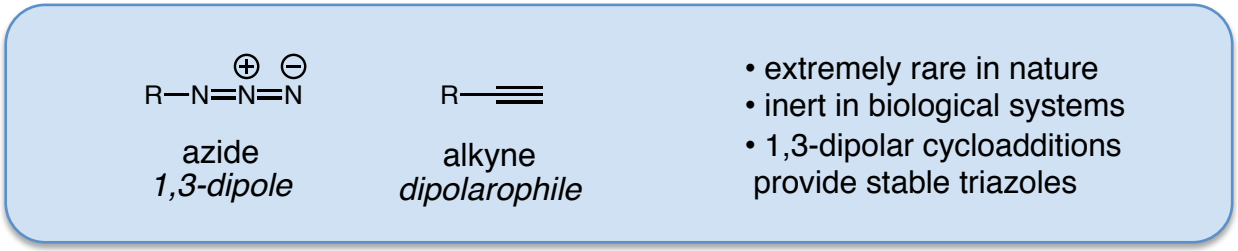
- *Remaining limitations*

  • oxidation of phosphine by air or metabolic enzymes is the only side reaction that diminishes the scope of the probe

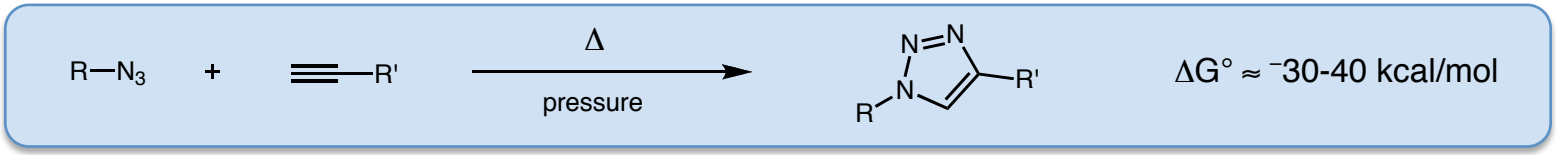
Click chemistry

3rd generation chemical reporters

- Bioorthogonal functional groups: *the azide & the alkyne*



- Huisgen [3+2] cycloaddition



- Click chemistry

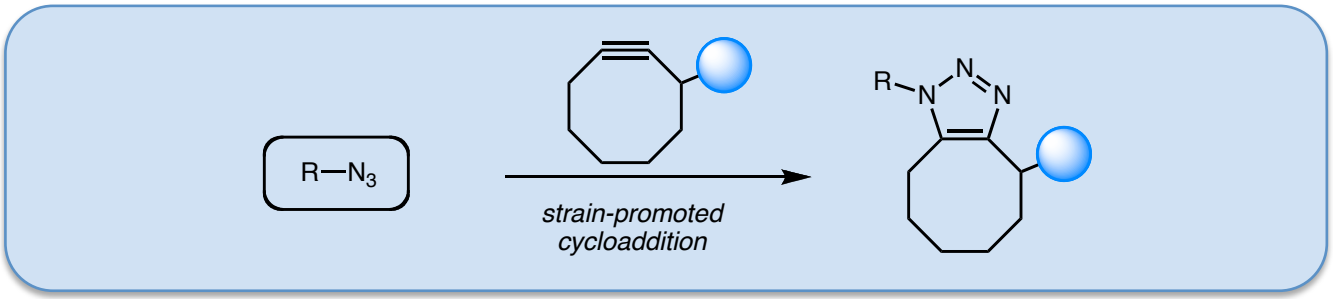
  - Huisgen [3+2] cycloaddition is thermodynamically favorable; however, requires elevated temperatures or pressures
  - Alkynes can be activated by appending α-esters; but prone to Michael additions (NOT bioorthogonal)
  - Sharpless' Cu(I)-catalyzed cycloaddition: *accelerated ~10^6-fold (25 x faster than Staudinger ligation in cell lysates)*
  - Reaction proceeds regioselectively in physiological conditions with no background labelling
  - *Cellular toxicity* of Cu(I) catalyst is only major limitation

Huisgen, R. *Angew. Chem. Int. Edn. Engl.* **1963**, *2*, 565–598.
Rostovtsev, V.V., Green, L.G., Fokin, V.V. & Sharpless, K.B. *Angew. Chem. Int. Edn. Engl.* **2002**, *41,* 2596–2599.

Strain-promoted cycloaddition

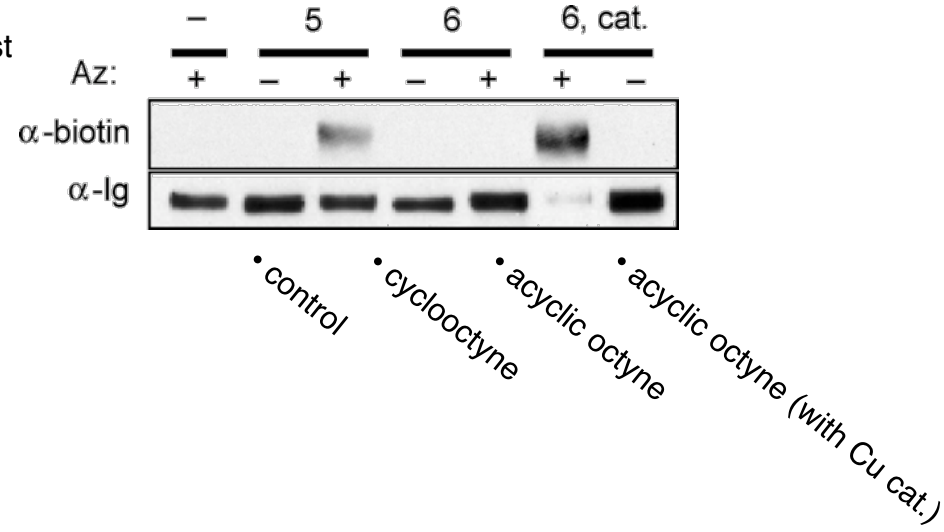
3rd generation chemical reporters

- Catalyst-free [3+2] cycloaddition



- Reaction features

  - Eight-membered ring creates ~18 kcal/mol of strain; released in transition state, upon reaction with azide
  - Reaction proceeds at room temperature, without catalyst



- Applications

  - Labeled molecules both *in vitro* and on cell surfaces without observable toxic effects

- Limitations

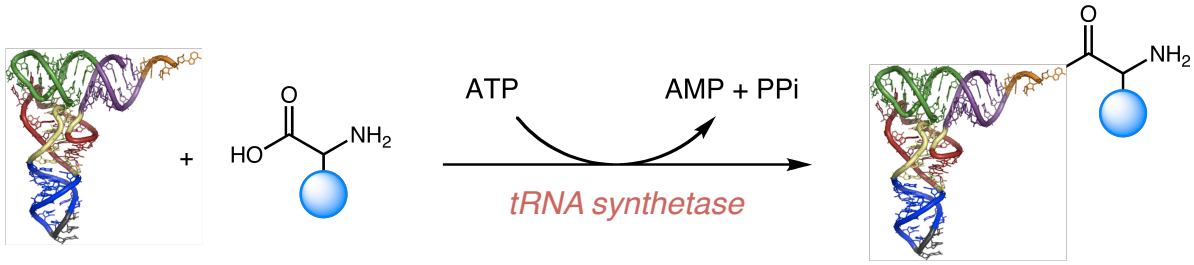
  - Slow (i.e. 2nd order rate constant: ≈ 0.0012 M^{-1} s^{-1}) compared to Staudinger ligation: ≈ 0.0025 M^{-1} s^{-1}

Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126,* 15046-15047.

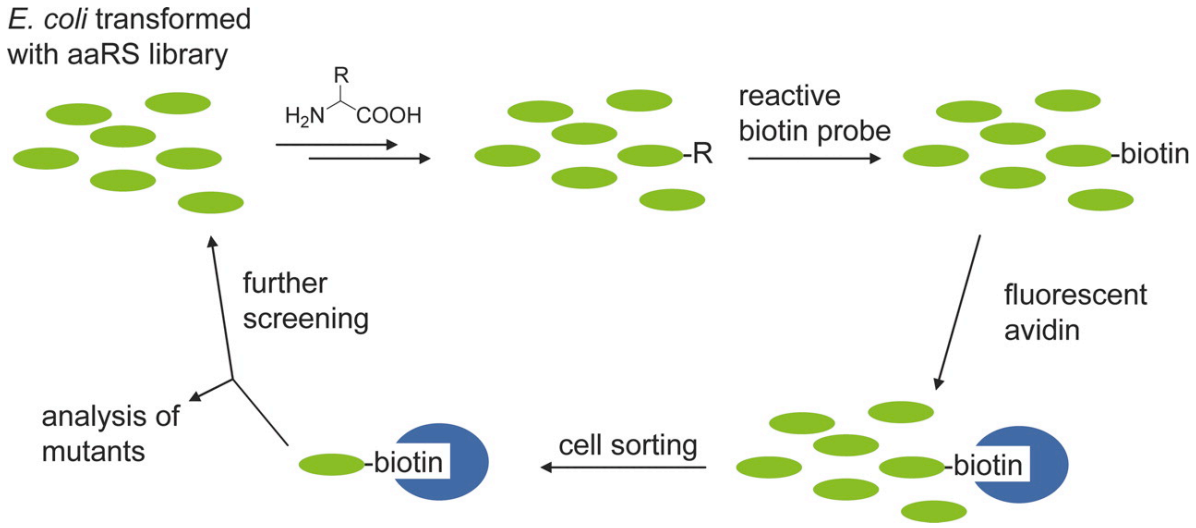
Protein modification

Applications of the strain-promoted cycloaddition

- Incorporation of azides into recombinant proteins requires initial uptake by methionyl-tRNA



- A new screening procedure for the identification of *tRNA synthetase* activity was developed based on cell surface probes

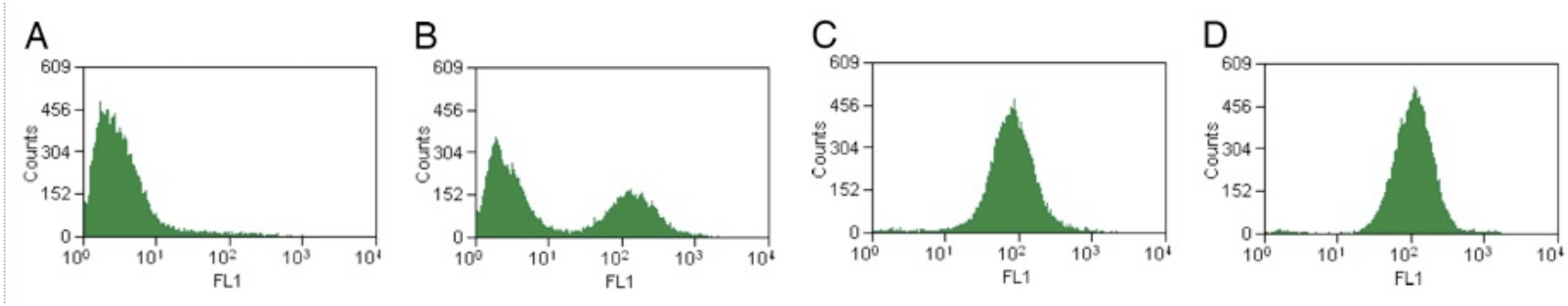


Link, A. J.; Vink, M. K.; Agard, N. J.; Prescher, J. A.; Bertozzi, C. R.; Tirrell, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103,* 10180-10185.

Protein modification made easy

Identification of 3 mutant MetRS strains

- Fluorescence histograms of cells expressing azide-amino acids



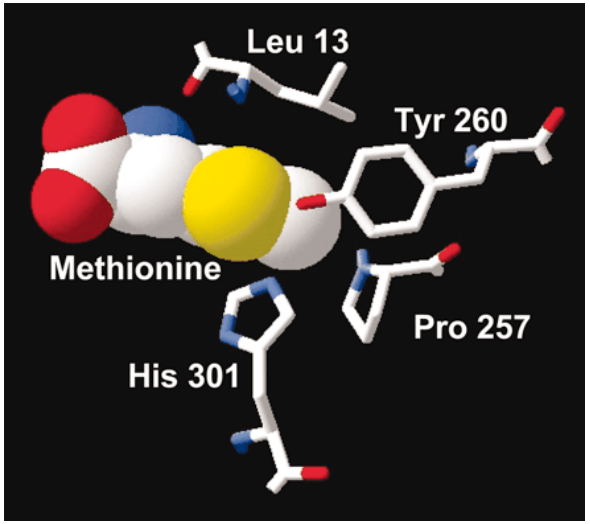
wild-type MetRS

wild-type MetRS
+ top 1% of cells from A

mutant MetRS #1

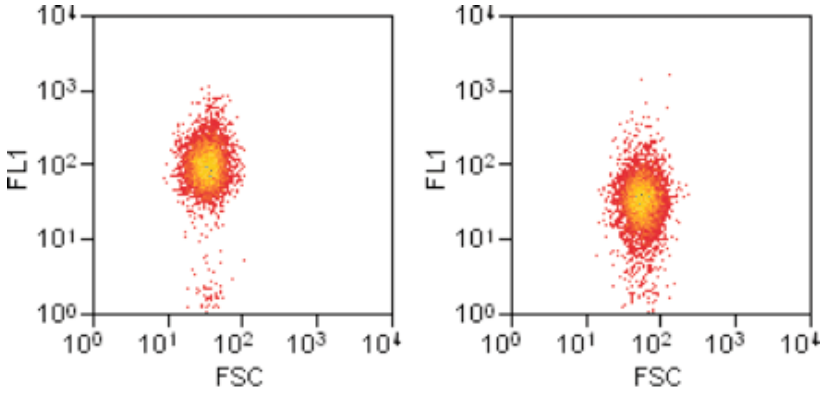
mutant MetRS #2

- Mutations found in MetRS clones that enable incorporation of azide-amino acids were all located in the binding site



Click chemistry

Cu-catalyzed vs strain-promoted
(3-fold difference)

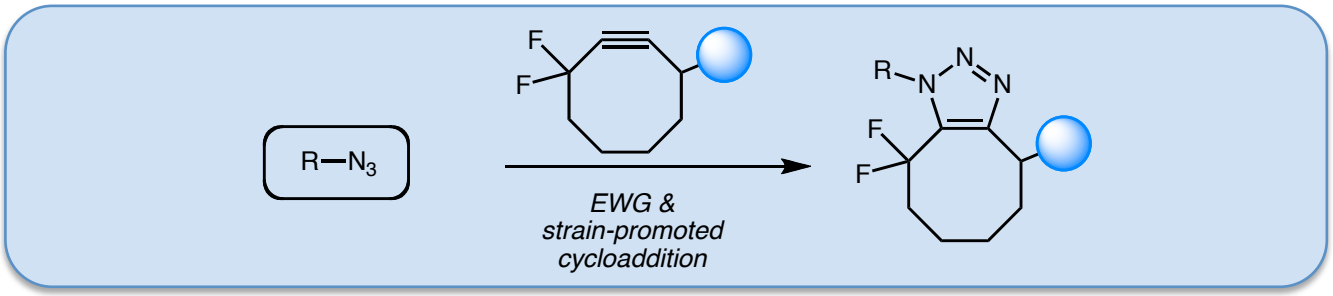


Link, A. J.; Vink, M. K.; Agard, N. J.; Prescher, J. A.; Bertozzi, C. R.; Tirrell, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103,* 10180-10185.

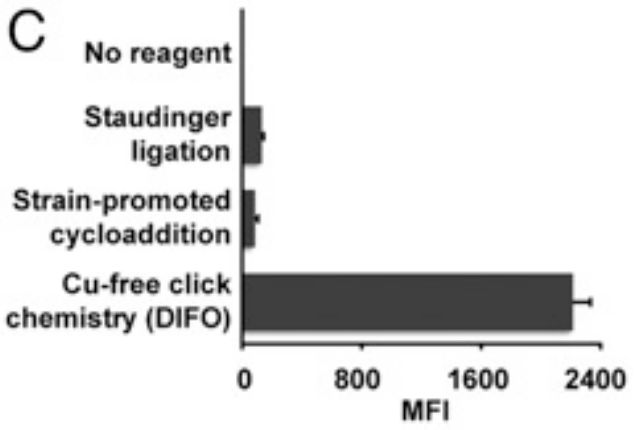
In vivo click chemistry

2nd generation Cu-free click chemistry reagents

- Electron-withdrawing groups, in addition to ring strain, significantly enhance the rate of [3+2] cycloaddition



- Reaction features
  - Bioorthogonal, *more selective* than previous reactions
  - Fast*** Staudinger ligation: ≈ 0.0025 M^{-1} s^{-1}
    Cu-catalyzed click: ≈ 0.0625 M^{-1} s^{-1} (*ex vivo*)
    1st generation click: ≈ 0.0012 M^{-1} s^{-1}
    2nd generation click: ≈ 0.076 M^{-1} s^{-1}



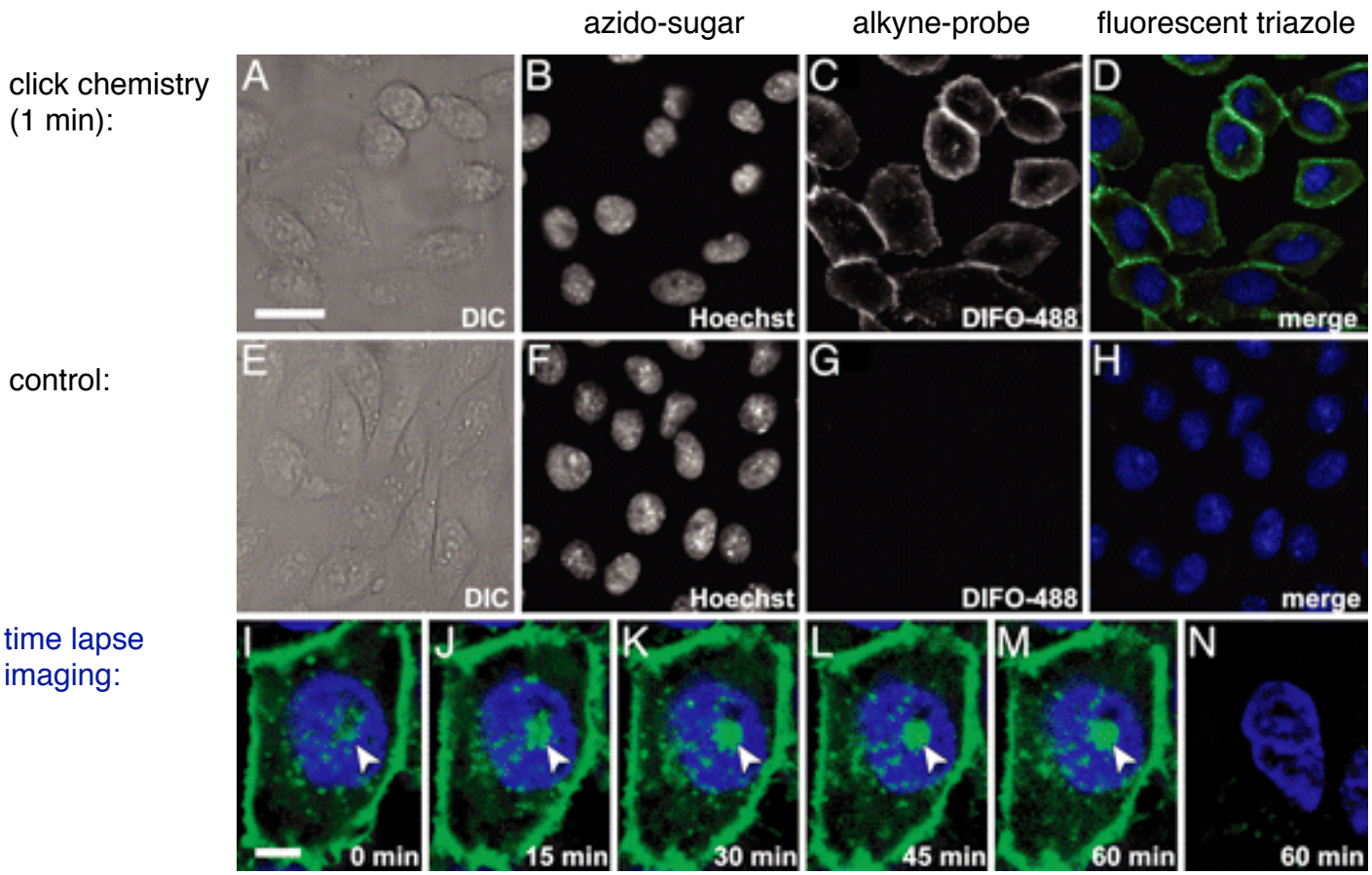
- Applications
  - Dynamic processes in living cells are now accessible upon removal of cytotoxic copper catalyst

Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104,* 16793-16797.

In vivo imaging

Chinese hamster ovary (CHO) cells

- *In vivo* imaging of glycoproteins in living Chinese hamster ovary (CHO) cells via rate-enhanced, Cu-free click chemistry



- Time lapse imaging monitors the trafficking of the labeled cell-surface glycans.
- Strong signal colocalized with transferrin uptake to endosomes and a Golgi marker, saturated at 30 min ($t_{1/2} \approx 15$ min)

Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104,* 16793-16797.

Dynamic in Vivo *imaging*

Chinese hamster ovary (CHO) cells

- Dynamic *in vivo* imaging of glycan trafficking using different color fluorescent labels

Day 1:

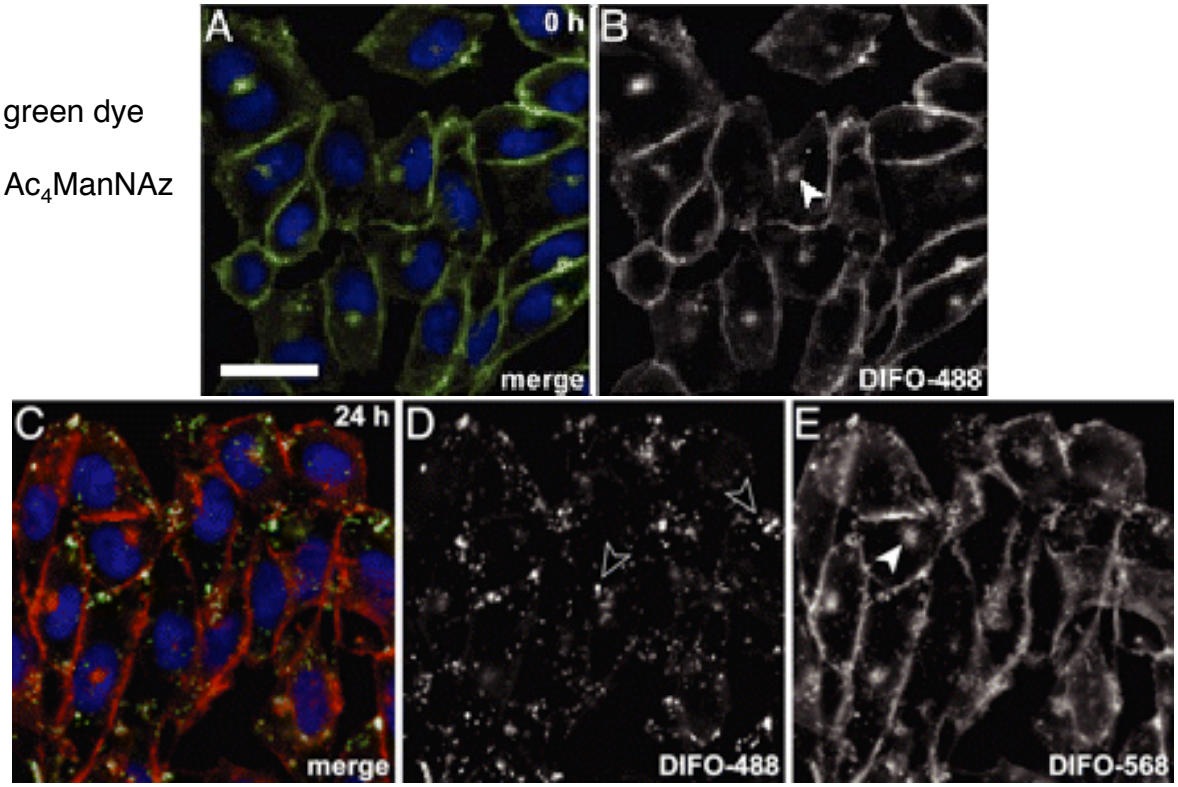
click chemisry w/ green dye

… resubjected to Ac_4ManNAz sugar media …

Day 2:

click chemisry w/ red dye

Green ➔ golgi!



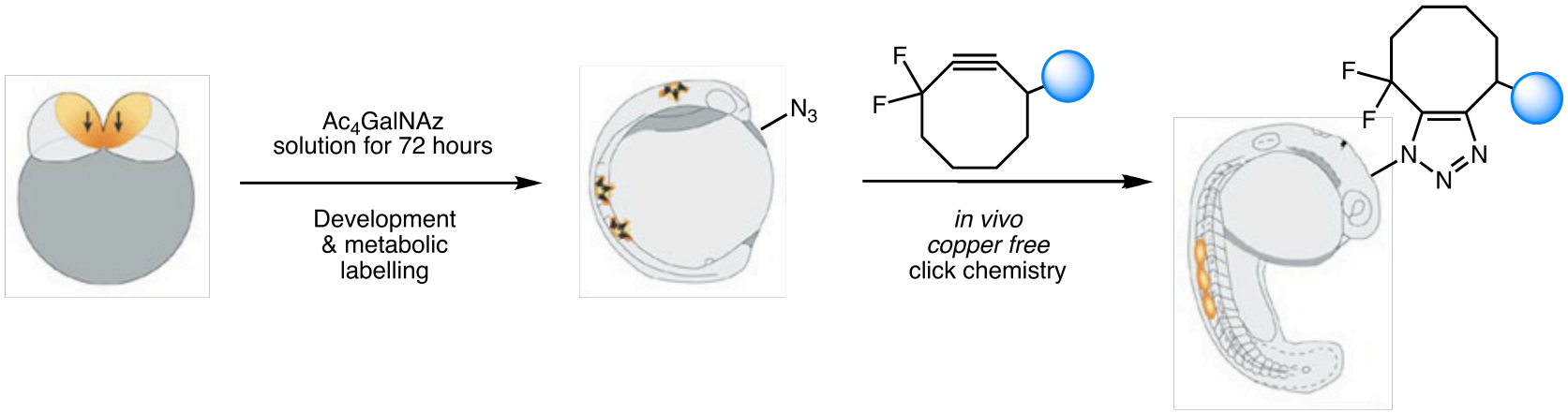
*** Provides a new platform for *evaluating the kinetics of glycan internalization and subcellular partitioning* to the endosomal, Golgi, and lysosomal compartments on the minute, hour, and day time scales ***

Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104,* 16793-16797.

Imaging zebrafish glycans

From cell cultures to living organisms

- Dynamic *in vivo* imaging of zebrafish was chosen as a model organism because of its amenability to optical imaging



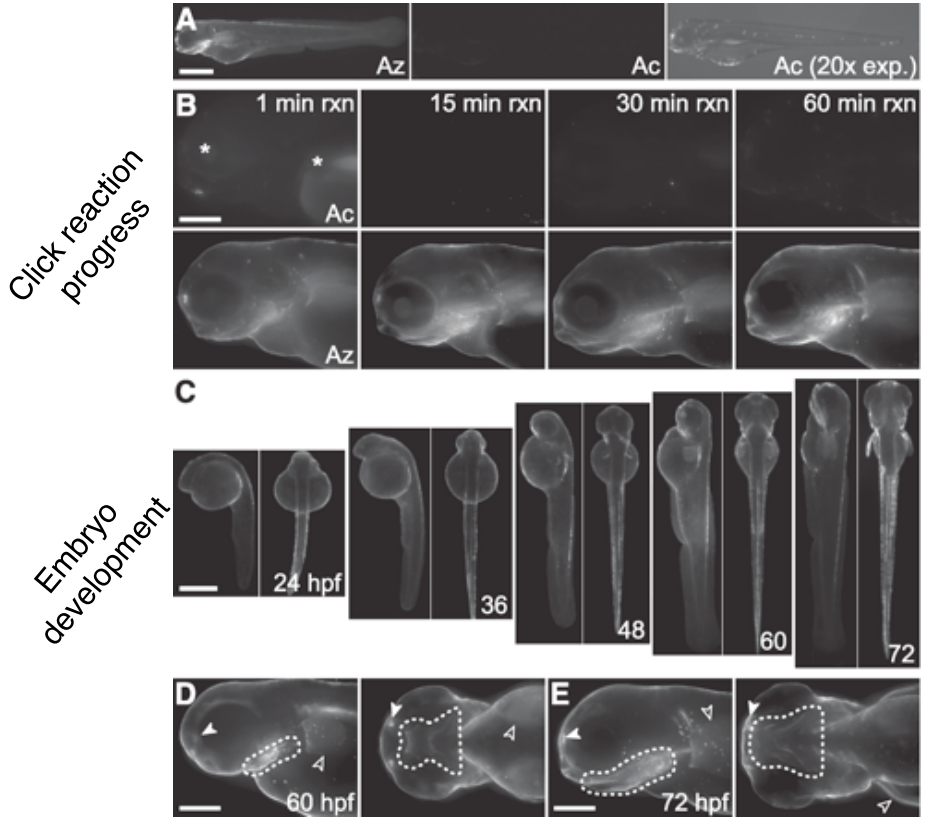
- Robust dose-dependant metabolic labelling was observed, similar to that of mammalian cells (with no background).
- No observed toxicity resulting from treatment with Ac_4GalNAz or DIFO reagents.
- Several glycoproteins (β-hexosamine, β-integrin, lysosome-associated membrane protein, nicastrin scavenger receptor B, and Thyl) were isolated and identified, corresponding to known or predicted sites of mucin-type O-linked glycosylation.

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

Imaging zebrafish glycans

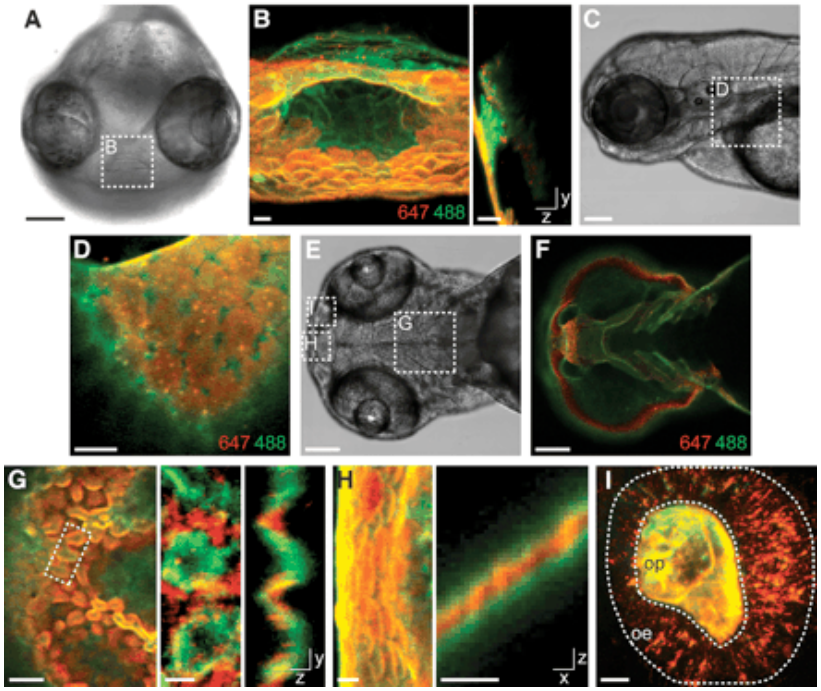
From cell cultures to living organisms

- *in vivo* imaging revealed differences in the cell-surface expression, intracellular trafficking, and tissue distribution of glycans



Global patterns of glycosylation:
Concentration in olfactory organs (solid arrow), pectoral fins (hollow arrow), and pharyngeal epidermis in jaw (dotted line)

Identification of temporally distinct glycan populations during zebrafish development using two-color labeling



(A) frontal view; (B) mouth; (C) lateral view; (D) pectoral fin; (E-F) ventral view; (G) jaw; (H) mouth; (I) olfactory

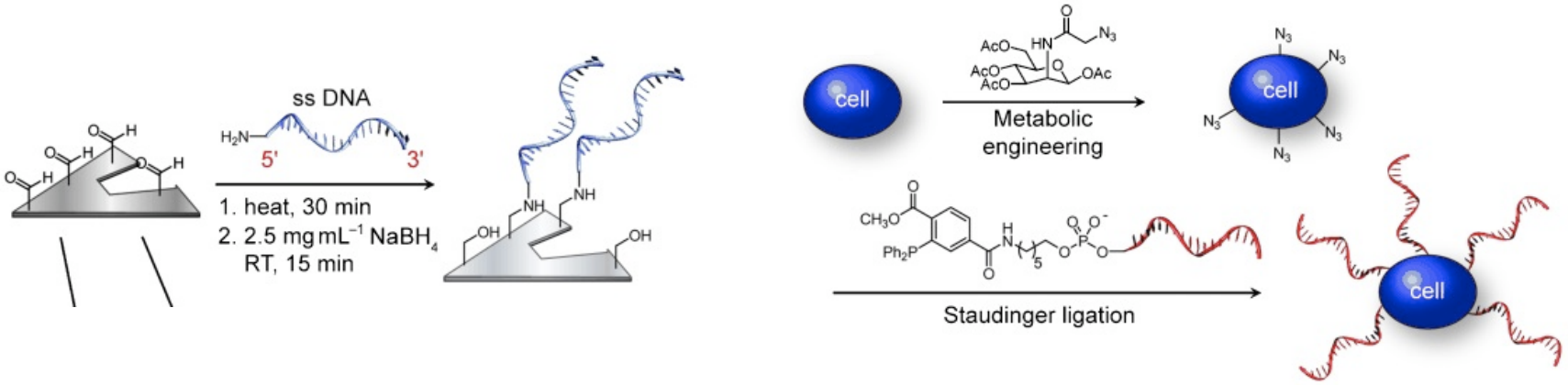
(Red) old glycans; (Green) new glycans (1 hour later)

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

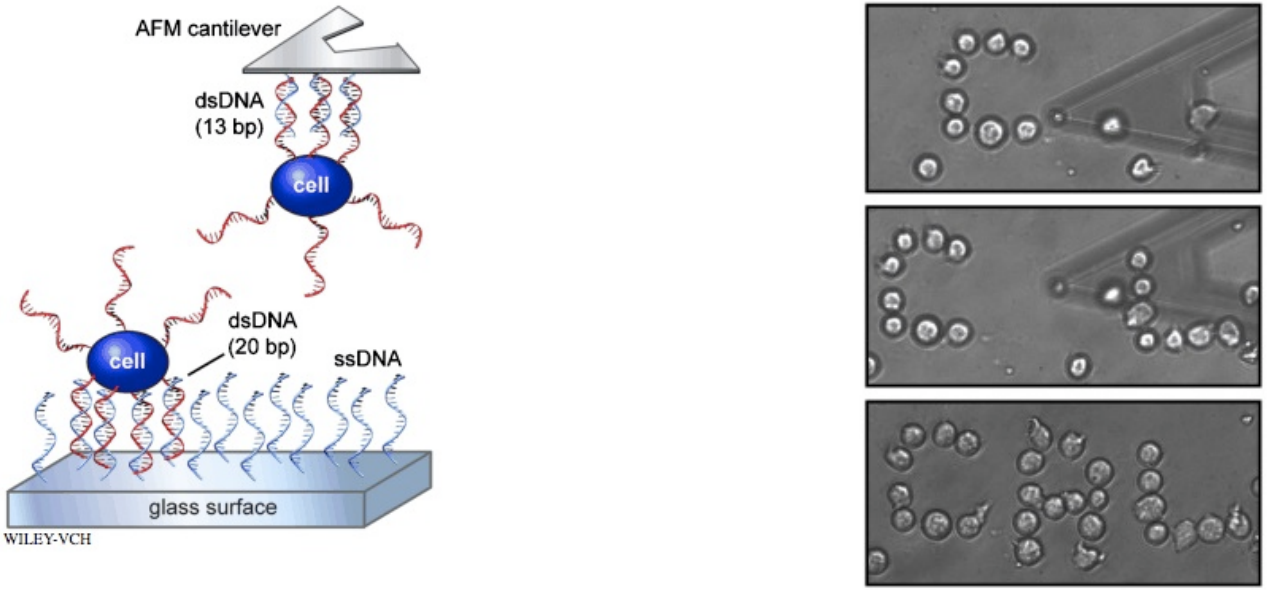
DNA cell adhesion

Multi-disciplinary applications of chemical reporters

- DNA-coated AFM cantilevers and DNA-bearing cells are prepared independantly



- Cell adhesion and the patterning of live cells is promoted by complimentary base pairing

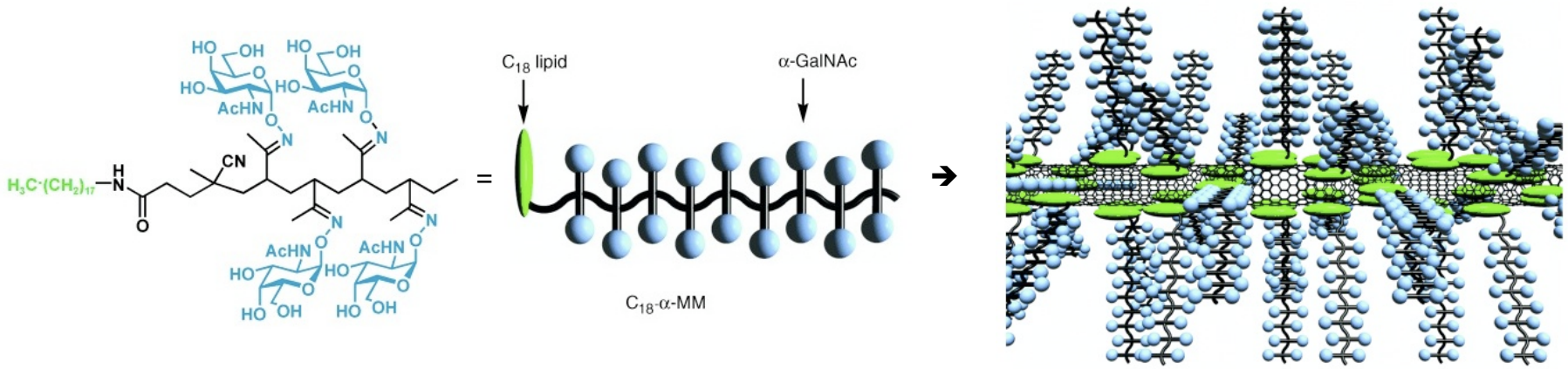


Hsiao, S. C.; Crow, A. K.; Lam, W. A.; Bertozzi, C. R.; Fletcher, D. A.; Francis, M. B. *Angew. Chem. Int. Ed. Engl.* **2008**, *120*, 8601-8605.

Medicinal nanotubes

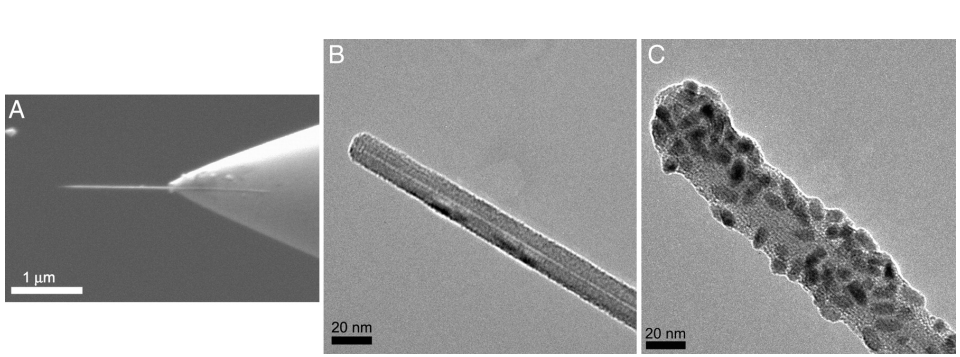
Multi-disciplinary applications of chemical reporters

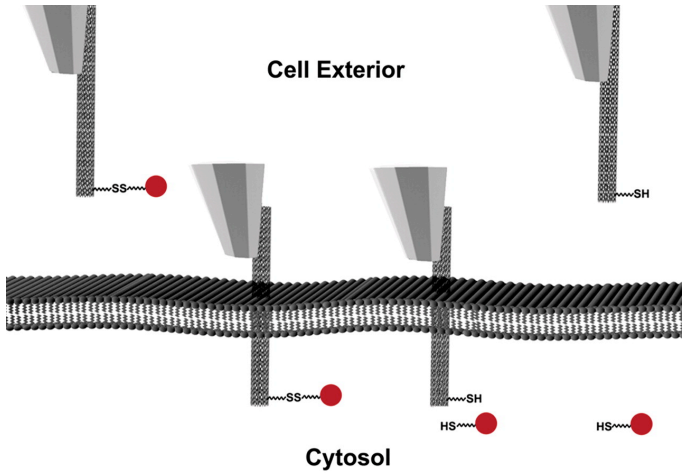
- Biomimetic engineering of carbon nanotubes (CNTs) as mucin mimics, conveys aqueous solubility properties to CNTs



Chen, X.; Lee, G. S.; Zettl, A.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2004,** *43*, 6112-6116.

- A nanotube attached to an atomic force microscope (AFM) tip serves as a “nanoneedle”



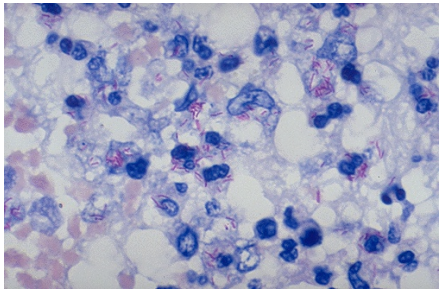


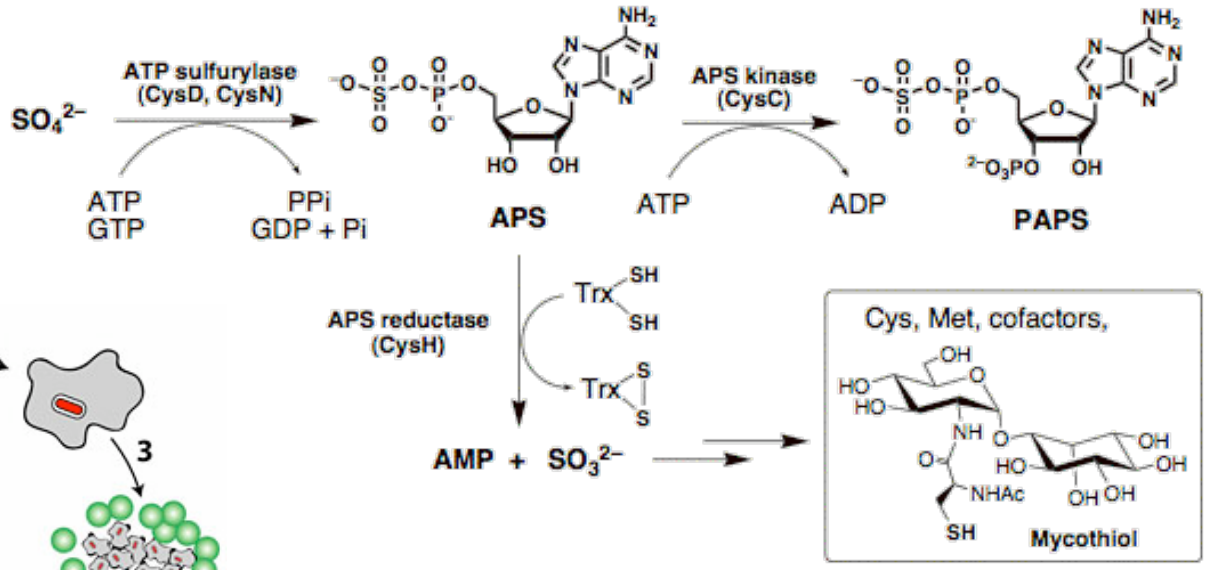
Chen, X.; Kis, A.; Zettl, Z.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104,* 8218-8222.

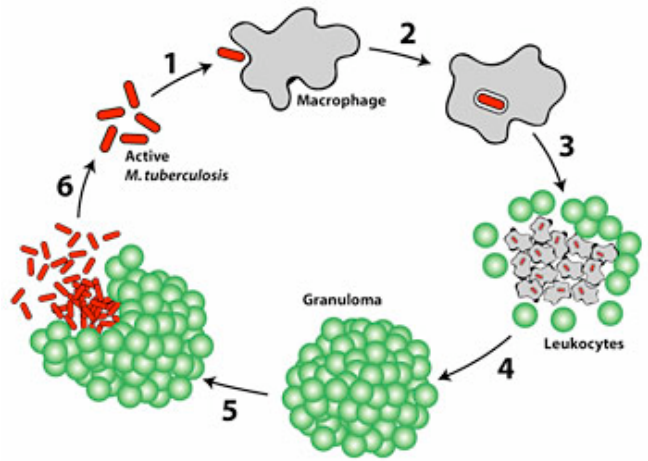
M. tuberculosis

Additional research areas in the Bertozzi group

- Sulfate metabolism & assimilation in *M. tuberculosis* mycobacteria is vital for survival in heavily oxidative environments







Goal: to develop small molecule inhibitors of ATP sulfurylase as potential drug leads against *M. tuberculosis*

Review: Schelle, M. W.; Bertozzi, C. R. *ChemBioChem* **2006**, *7,* 1516-24.

Summary

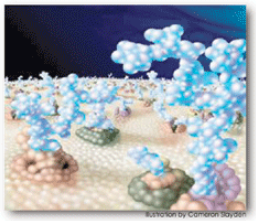
The Bertozzi Group

■ Research theme:

To employ chemical tools to uncover the role of cell surface oligosaccarides involved in cell recognition & intercellular communication

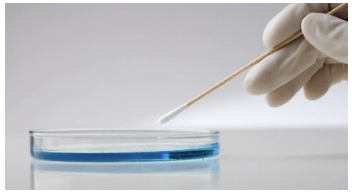
1996

1997
Introduced concept of metabolic oligosaccharide engineering



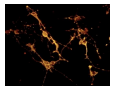
Science **1997**, *276*, 1125-1128

2000
1st Staudinger ligation
in vivo azide add'n; *in vitro* ligation



Science **2000**, *287,* 2007-2010

2001
tumor inhibition by small molecule



Science **2001**, *294,* 380

2002
azides on recombinant proteins

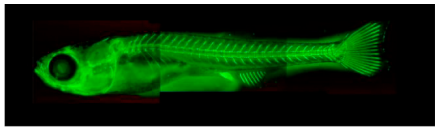
2004
Staudinger ligation in living animals
in vivo azide; *in vivo* ligation

Nature **2004**, *430,* 873-877

2006
azide tolerant tRNA mutants via Cu-free click

2007
in vivo click chemistry

2008
click chemistry in living animals: *in vivo* imaging of zebrafish



Science **2008**, *320,* 664-667