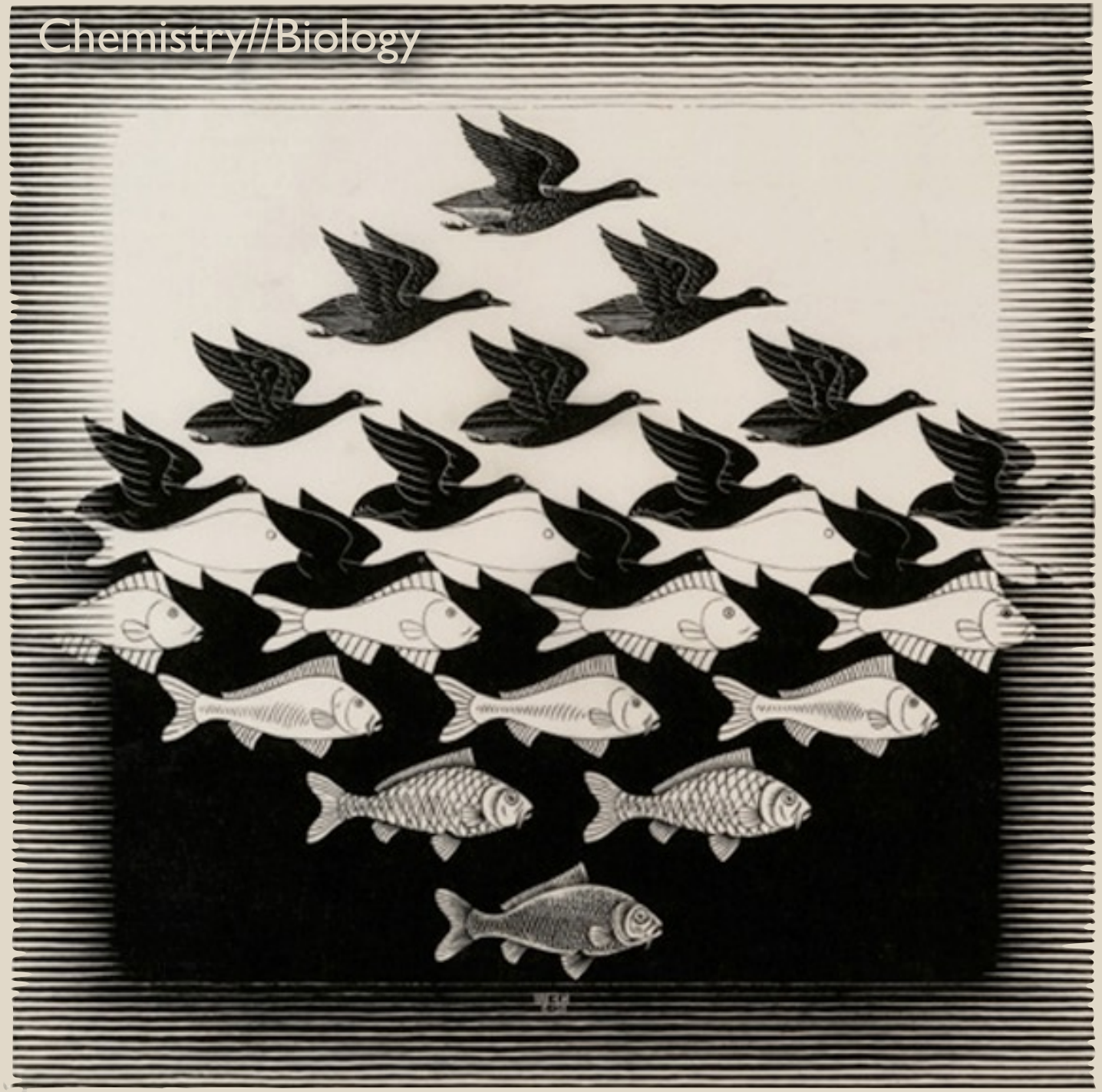
Activation et étiquetage des protéines et biomolécules

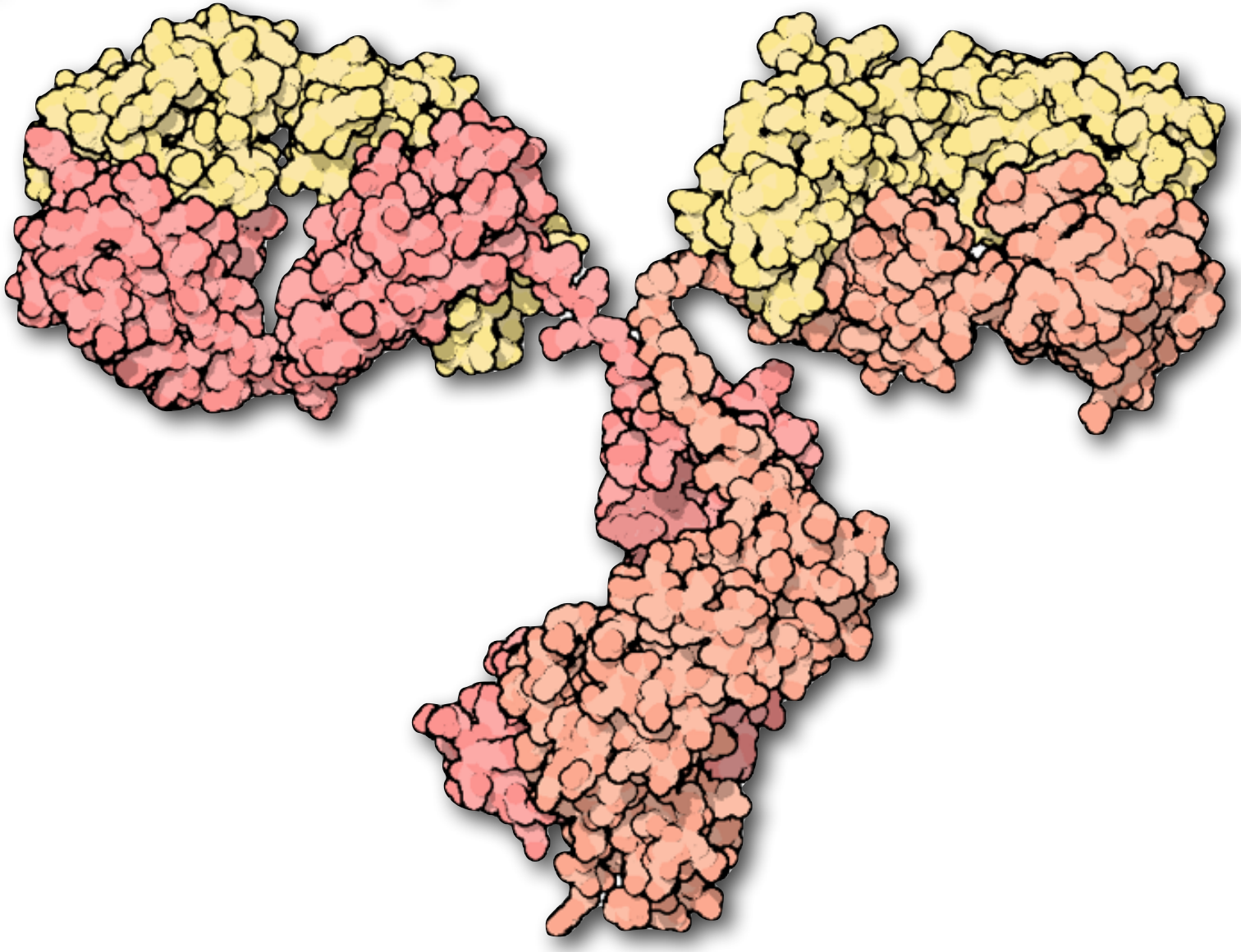
Chemistry//Biology



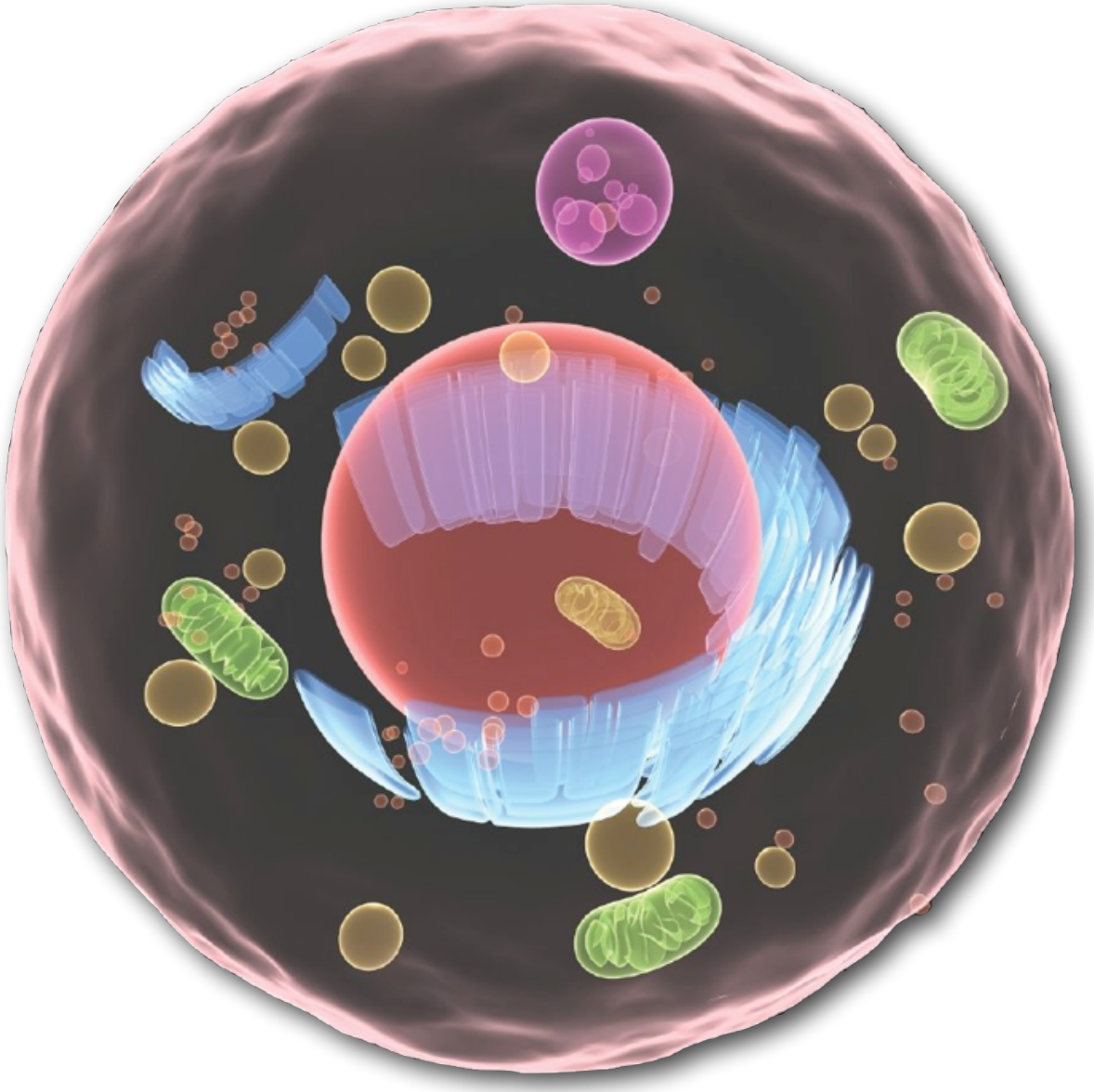
M. C. Escher
Sky and Water I
1938

Synthetic chemistry in a flask

Synthetic chemistry on biomolecules



Synthetic chemistry in a cell

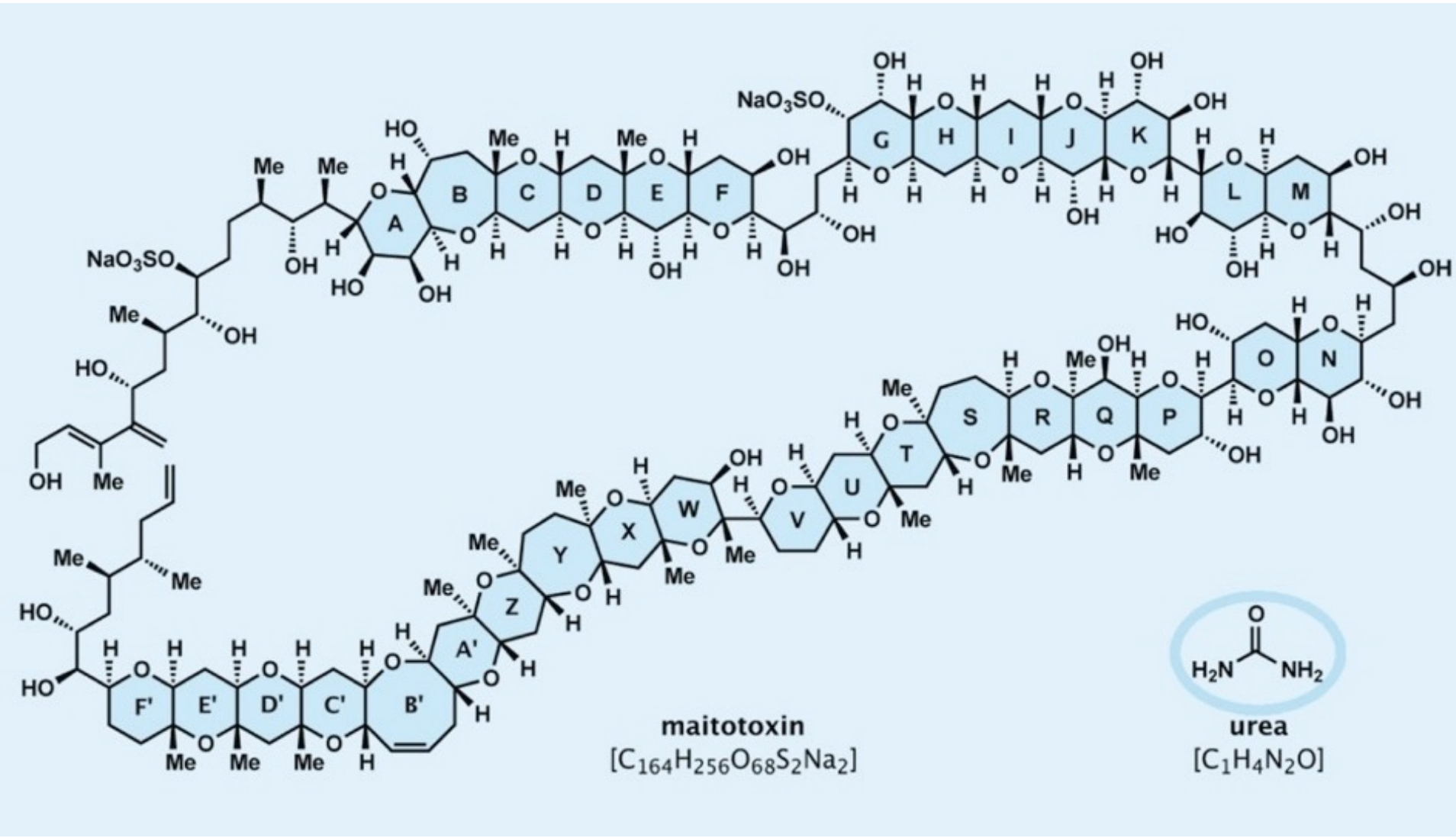


Synthetic chemistry in living organisms

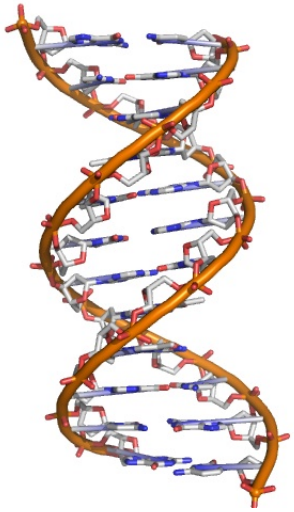
Photo by Ricky Kharawala on Unsplash

Bio conjugation

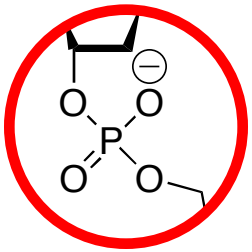
Natural products synthesis



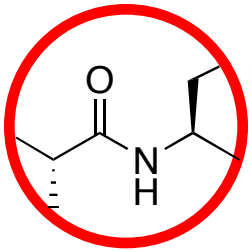
Macromolécules biologiques



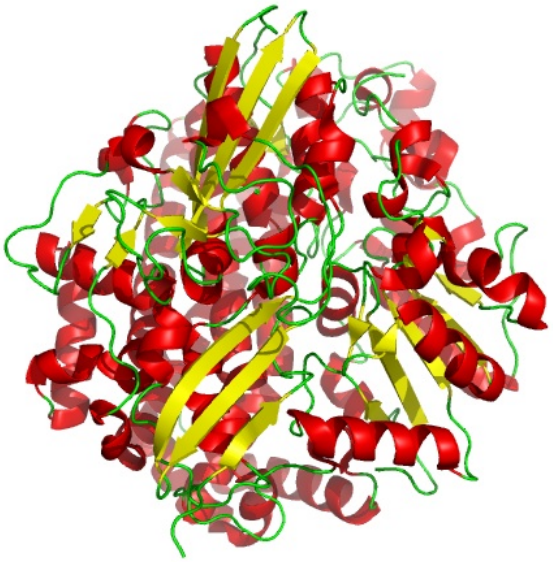
Acide nucléique



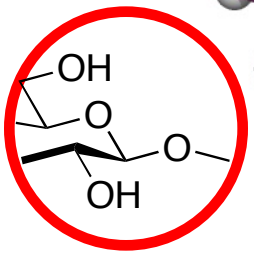
Phosphodiester



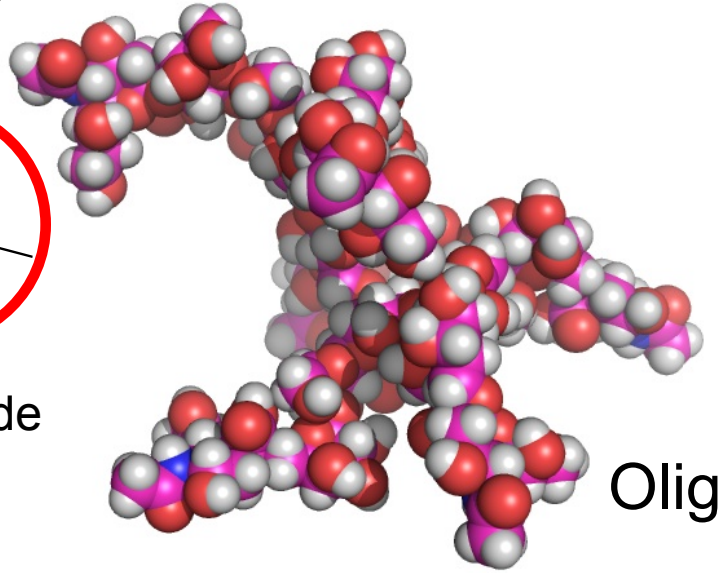
Amide



Protéine

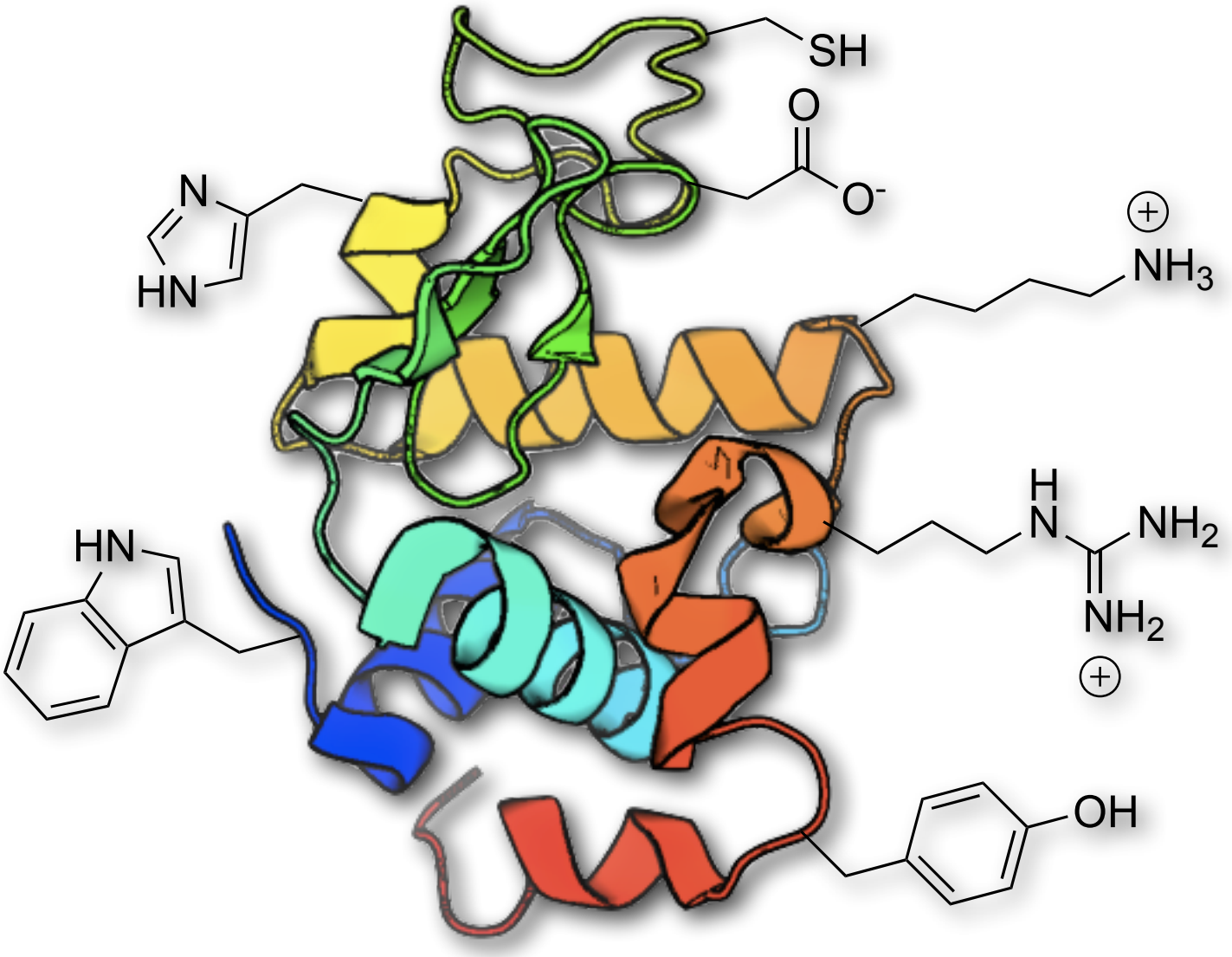


Glycoside



Oligosaccharide

Bioconjugation



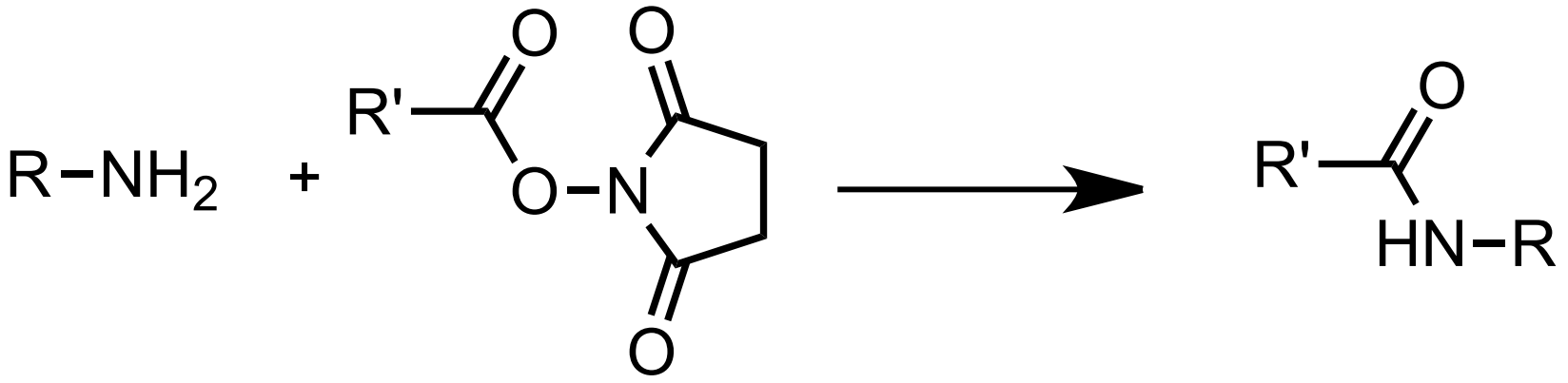
Relevant chemical transformations ?

Chemical Transformations Leading to Protein Covalent Modifications

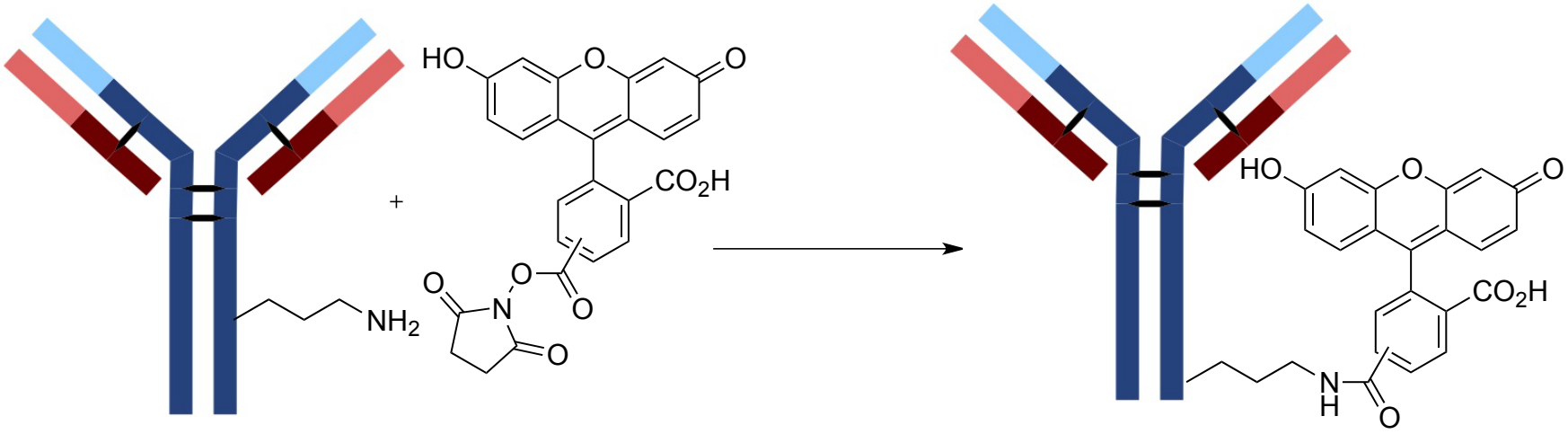
- Water Is the Sole Solvent
- A Neutral pH Is Required
- Ambient Temperature (Up to 40°C)
- Kinetics, which Adapted to the Observed Phenomenon (on the Hour Scale)
- Low Reactant Concentrations
- Nontoxic Reagents

Mathieu Pucheault et coll., *Chem. Biol.*, **2010**, *39*, 213-27

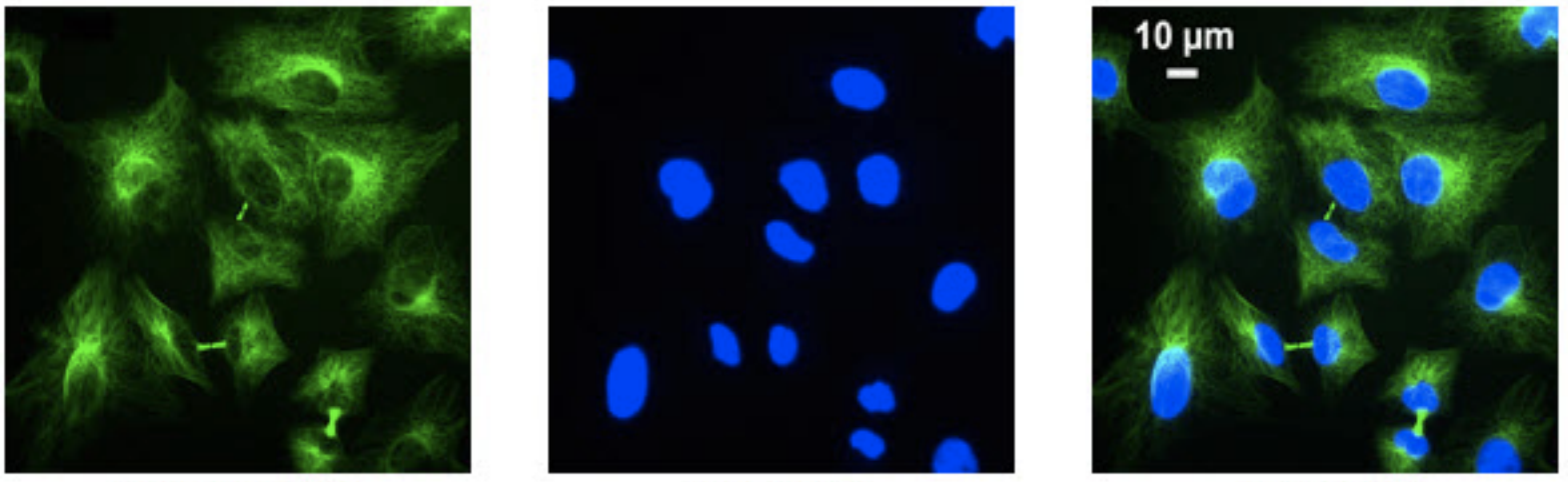
Bioconjugation - Aminolysis



Antibody labeling

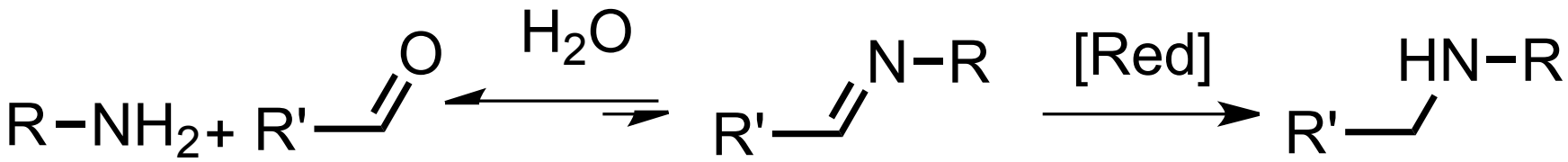


Tubulin imaging

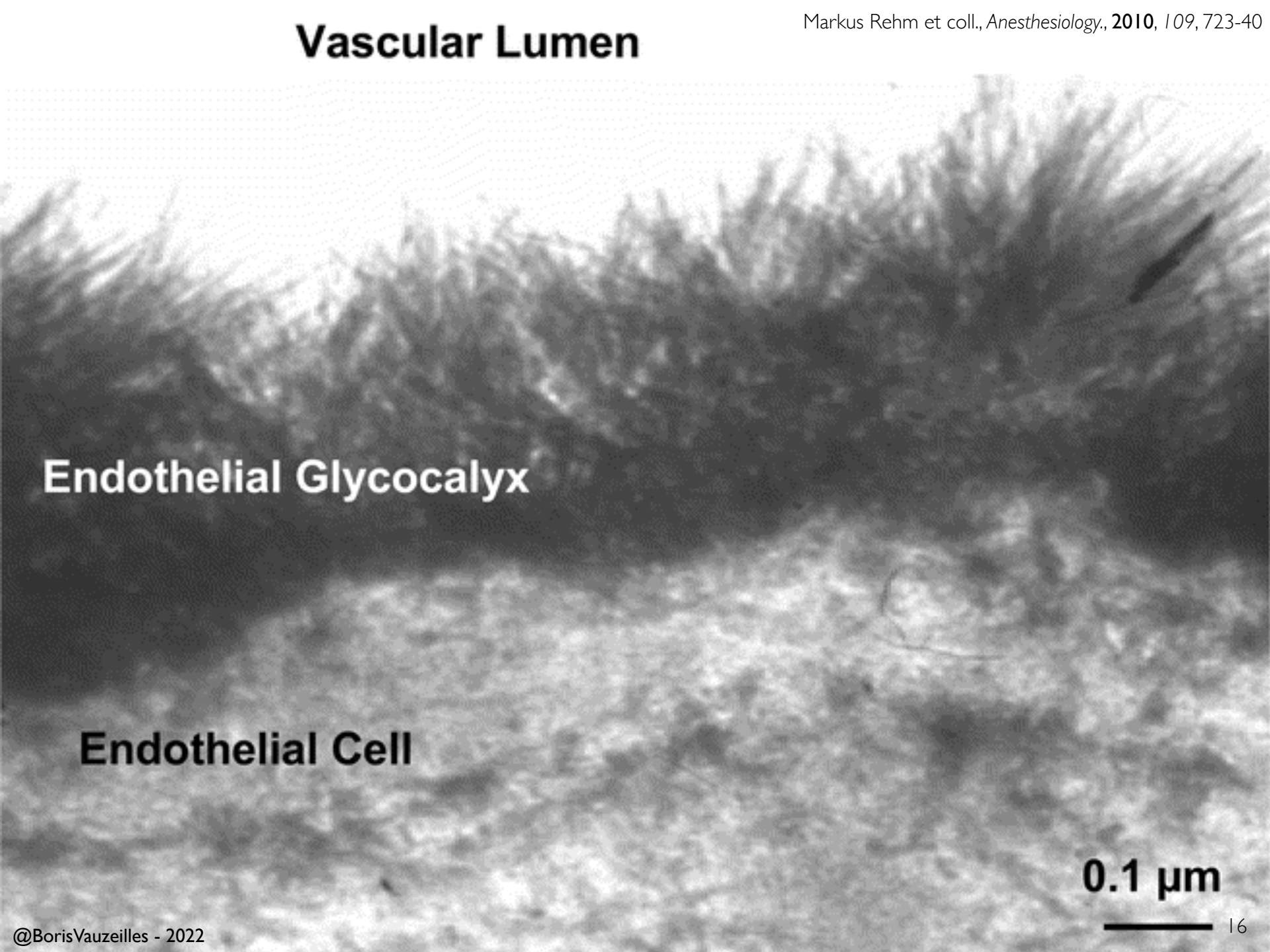


Detection of α-tubulin in A549 cells demonstrates use of fluorescein-labeled secondary antibody
Cells were grown in 96-well microplates for 18-20 hrs, fixed with 4% paraformaldehyde (Part No. 28906) and permeabilized with 0.1% Surfact-Amps X-100 (Part No. 28314). Cells were then probed with a mouse anti-α-tubulin primary antibody (0.4µg/mL) and Fluorescein-goat anti-mouse secondary antibody (2µg/mL). Nuclei were labeled with Hoechst Dye. Images were acquired by fluorescence microscopy. **A.** Fluorescence image shows a delicate network of α-tubulin (pseudo-colored green) located exclusively in the cytoplasm. **B.** Nuclear counterstain with Hoechst Dye (pseudo-colored blue) **C.** Merged image.

Bioconjugation - reductive alkylation

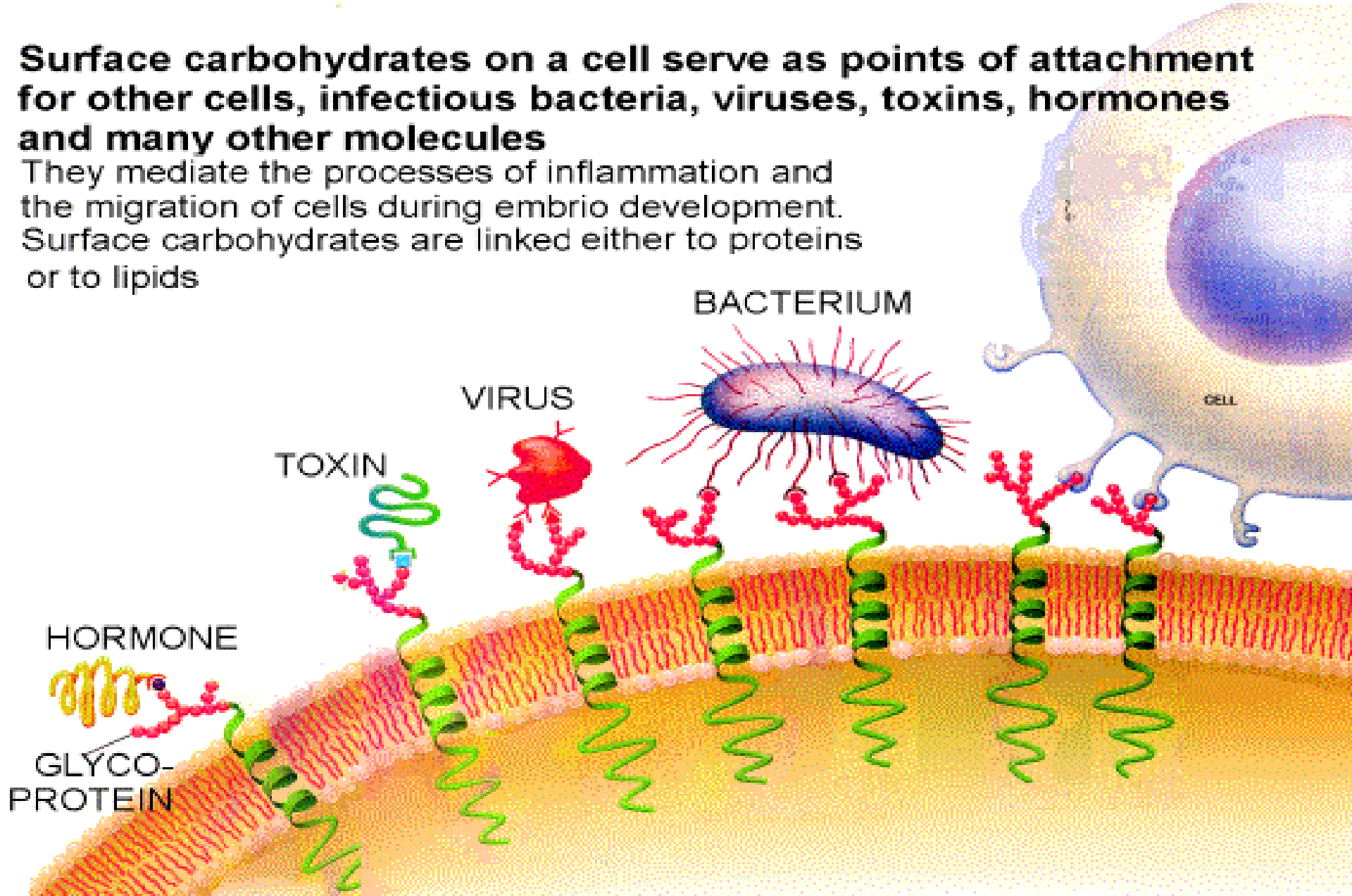


Markus Rehm et coll., *Anesthesiology.*, **2010**, *109*, 723-40

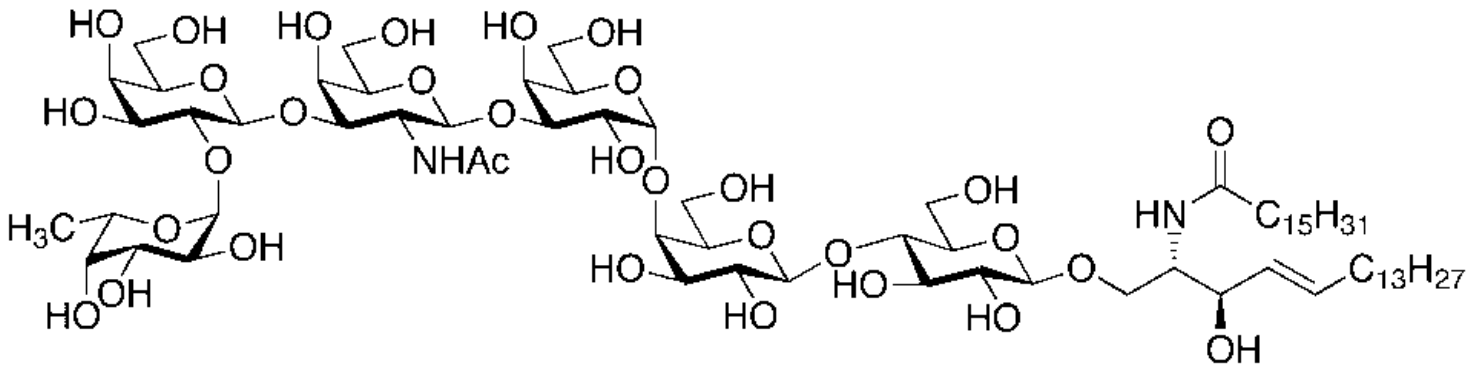


Surface carbohydrates on a cell serve as points of attachment for other cells, infectious bacteria, viruses, toxins, hormones and many other molecules

They mediate the processes of inflammation and the migration of cells during embrio development.
Surface carbohydrates are linked either to proteins or to lipids



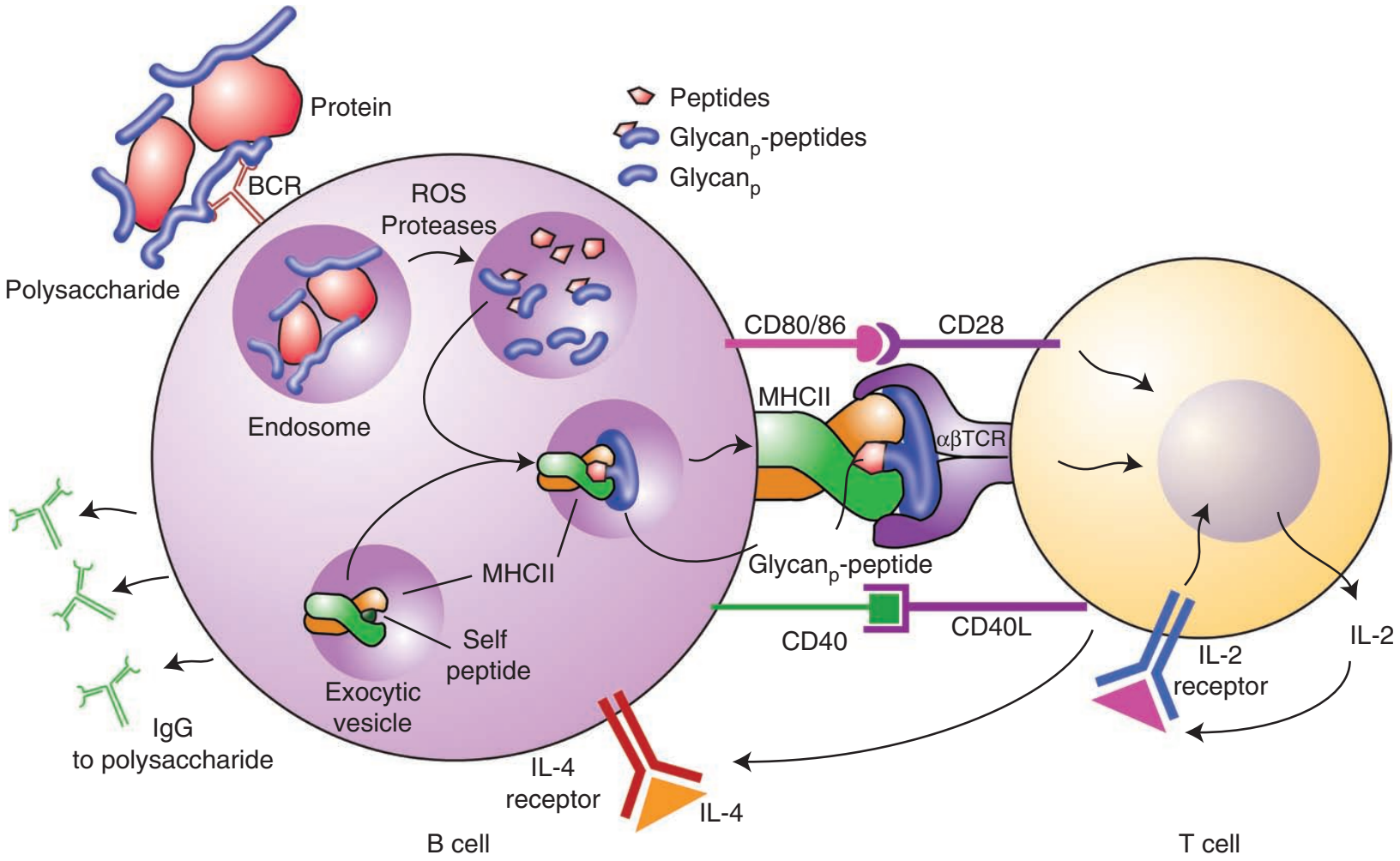
Tumor associated antigens



1: MBr1 antigen / Globo-H

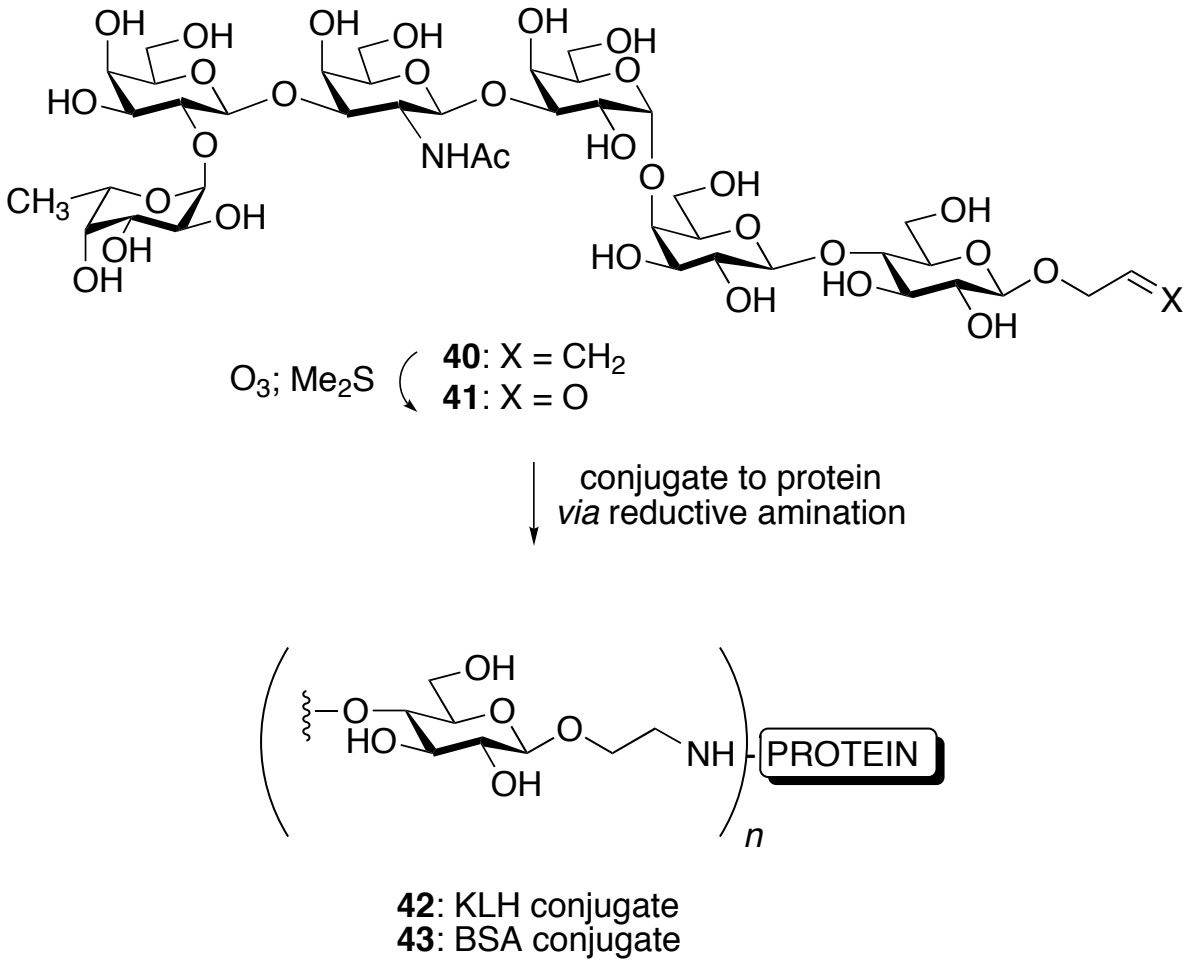
Samuel J. Danishefsky et coll., *Angew. Chem. Int. Ed.*, **2000**, *39*, 836-63

Glycoconjugate vaccines



Dennis L. Kasper et coll., *Nature Med.*, **2011**, *17*, 1602-1610

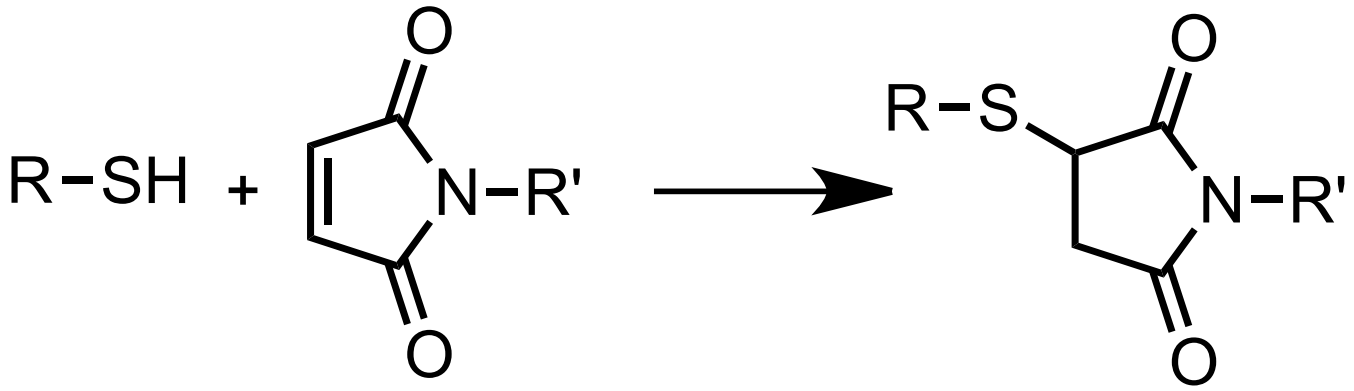
Anticancer vaccines ?



Scheme 8. Synthesis of the Globo-H – KLH vaccine construct.

Samuel J. Danishefsky et coll., *Angew. Chem. Int. Ed.*, **2000**, *39*, 836-63

Bioconjugation - Michael addition



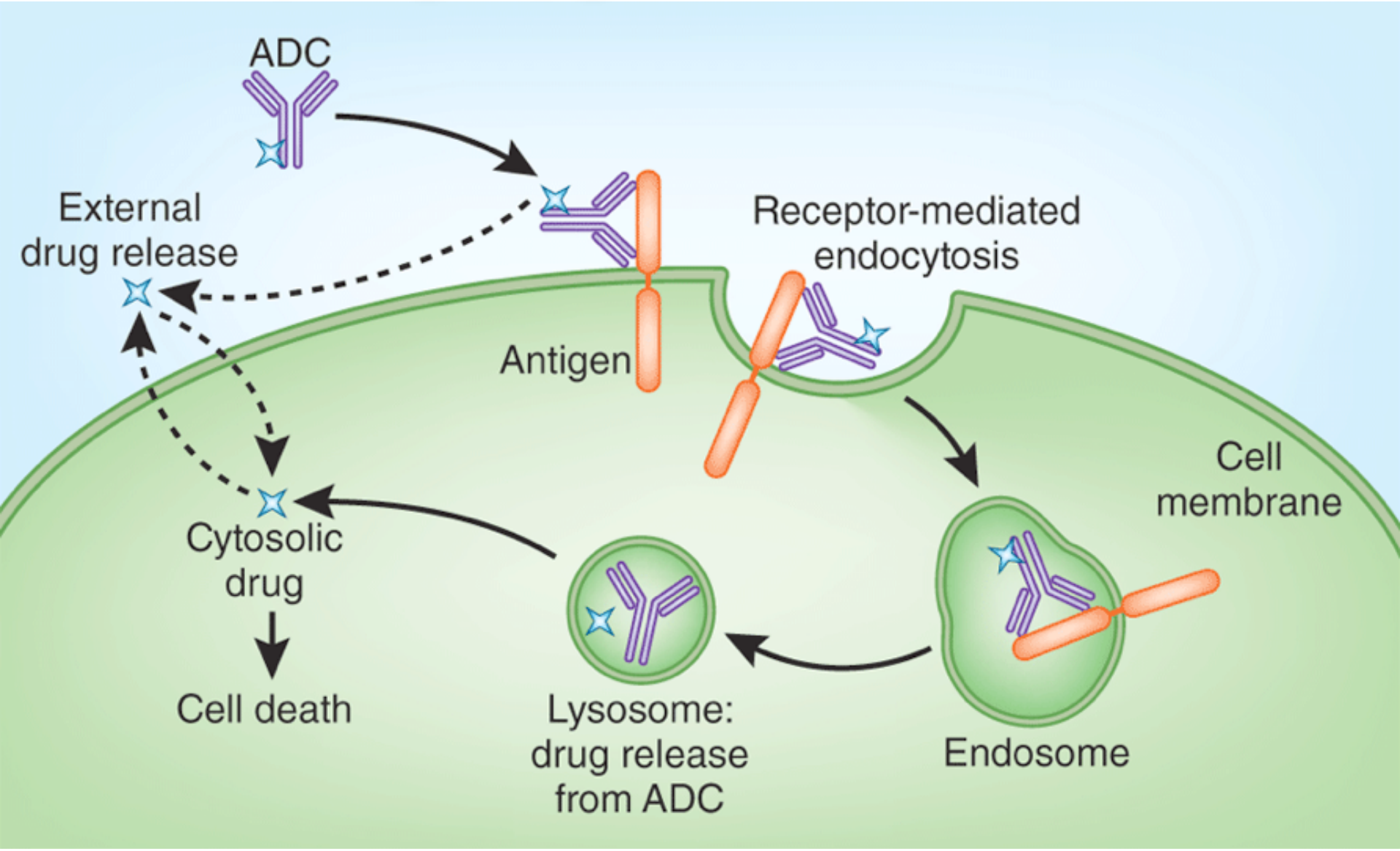
Paul Ehrlich

César Milstein and George Köhler

Dolabela auricularia

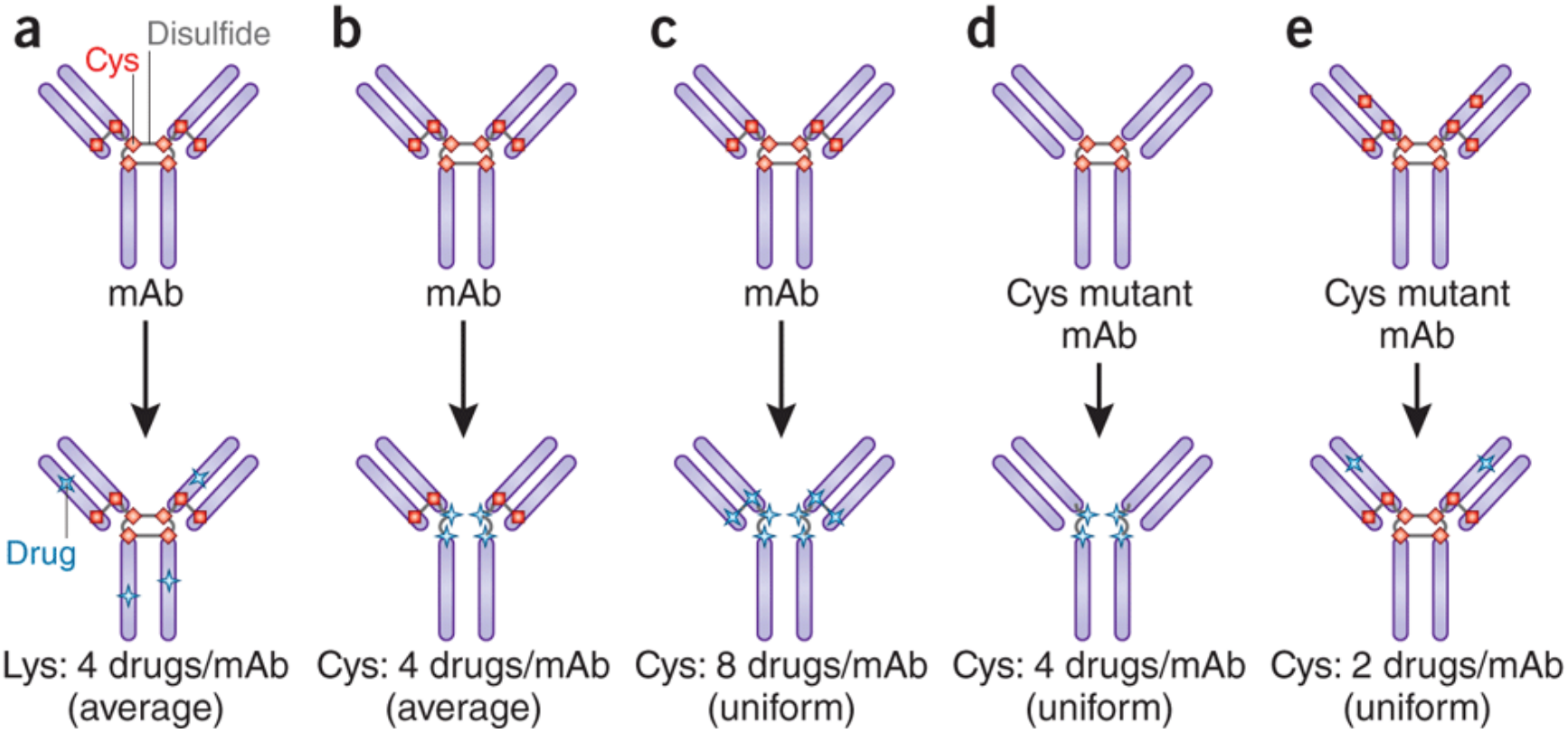
George Pettit

Antibody-Drug conjugates



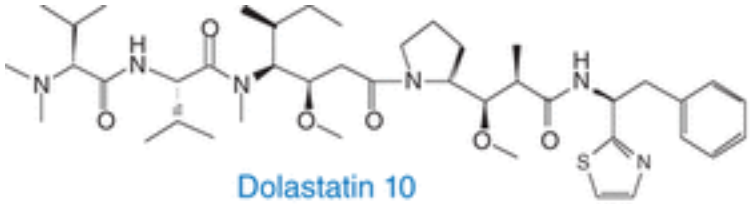
Peter D Senter & Eric L Sievers, *Nature Biotechnol.*, **2012**, *30*, 631-637

Conjugationtechnology

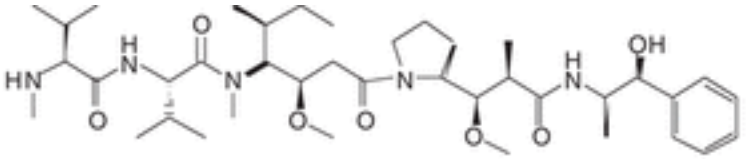


Peter D Senter & Eric L Sievers, *Nature Biotechnol.*, **2012**, *30*, 631-637

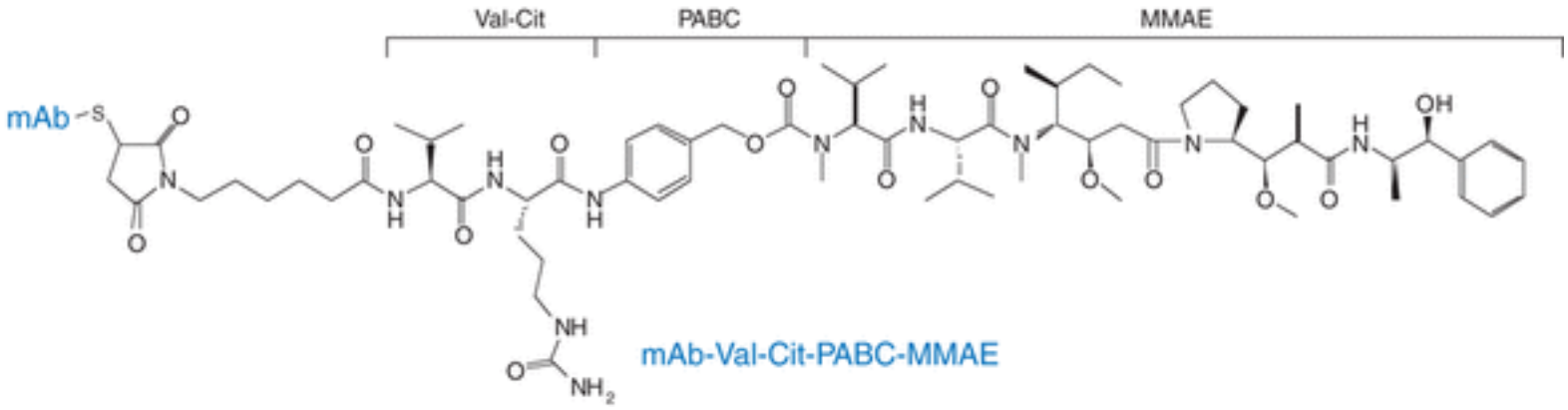
Choosing the linker



Dolastatin 10

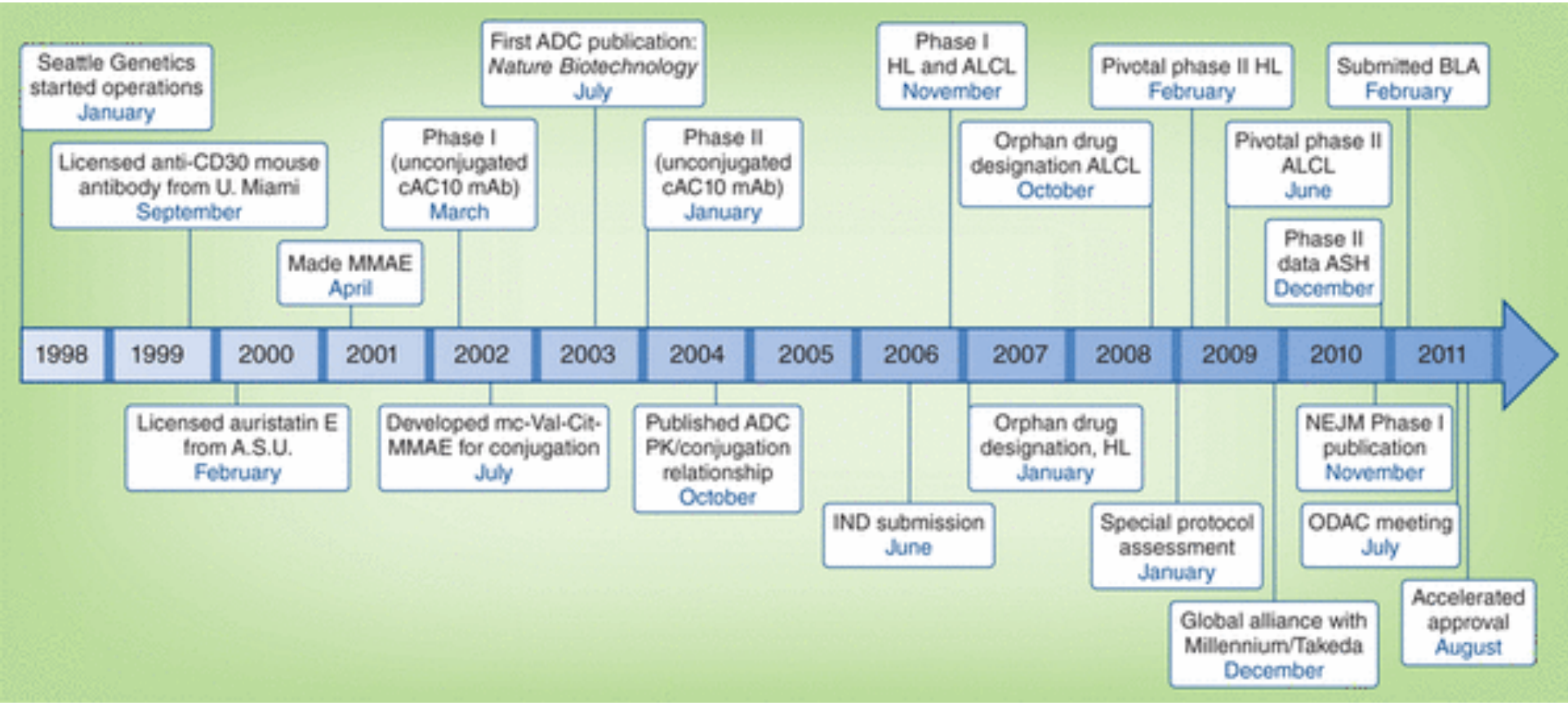


MMAE



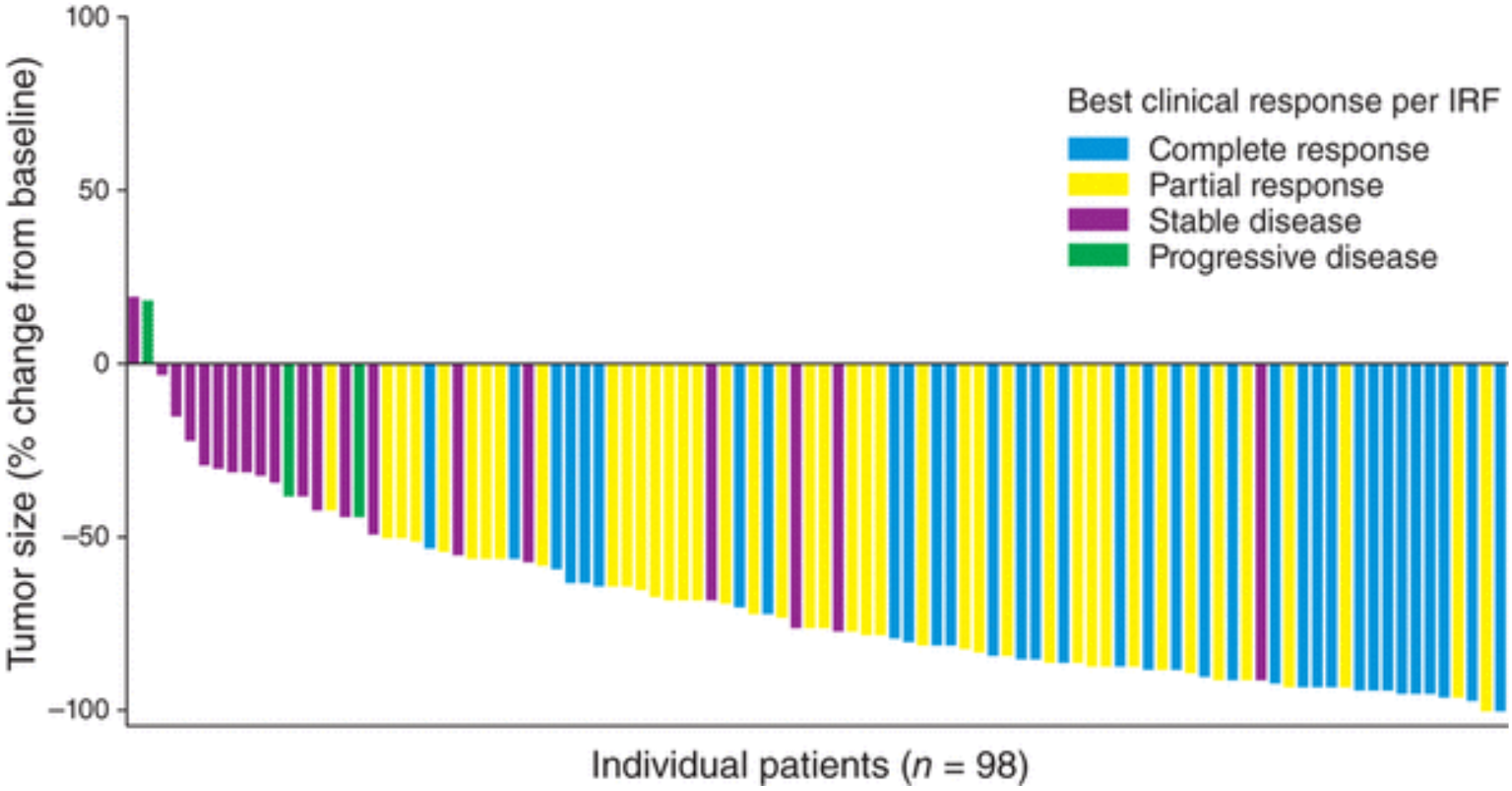
Peter D Senter & Eric L Sievers, *Nature Biotechnol.*, **2012**, *30*, 631-637

Approval



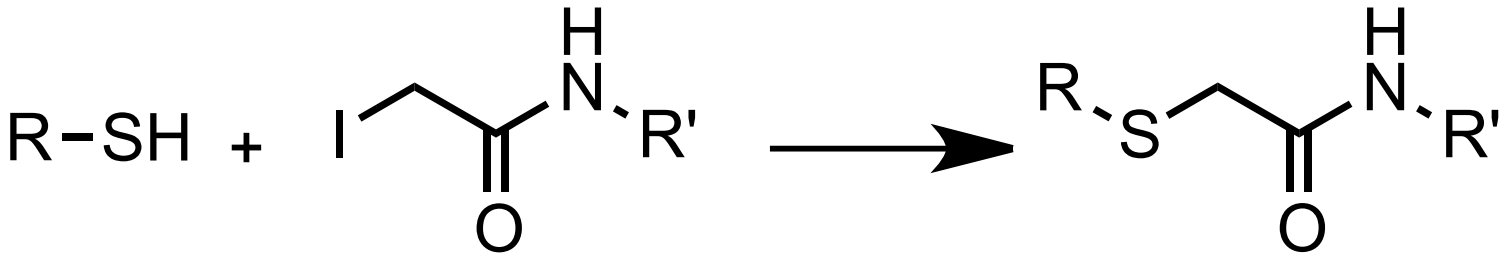
Peter D Senter & Eric L Sievers, *Nature Biotechnol.*, **2012**, *30*, 631-637

Phase II clinical trials...



Peter D Senter & Eric L Sievers, *Nature Biotechnol.*, **2012**, *30*, 631-637

Bioconjugation - Alkylation



Probing cysteine oxydation

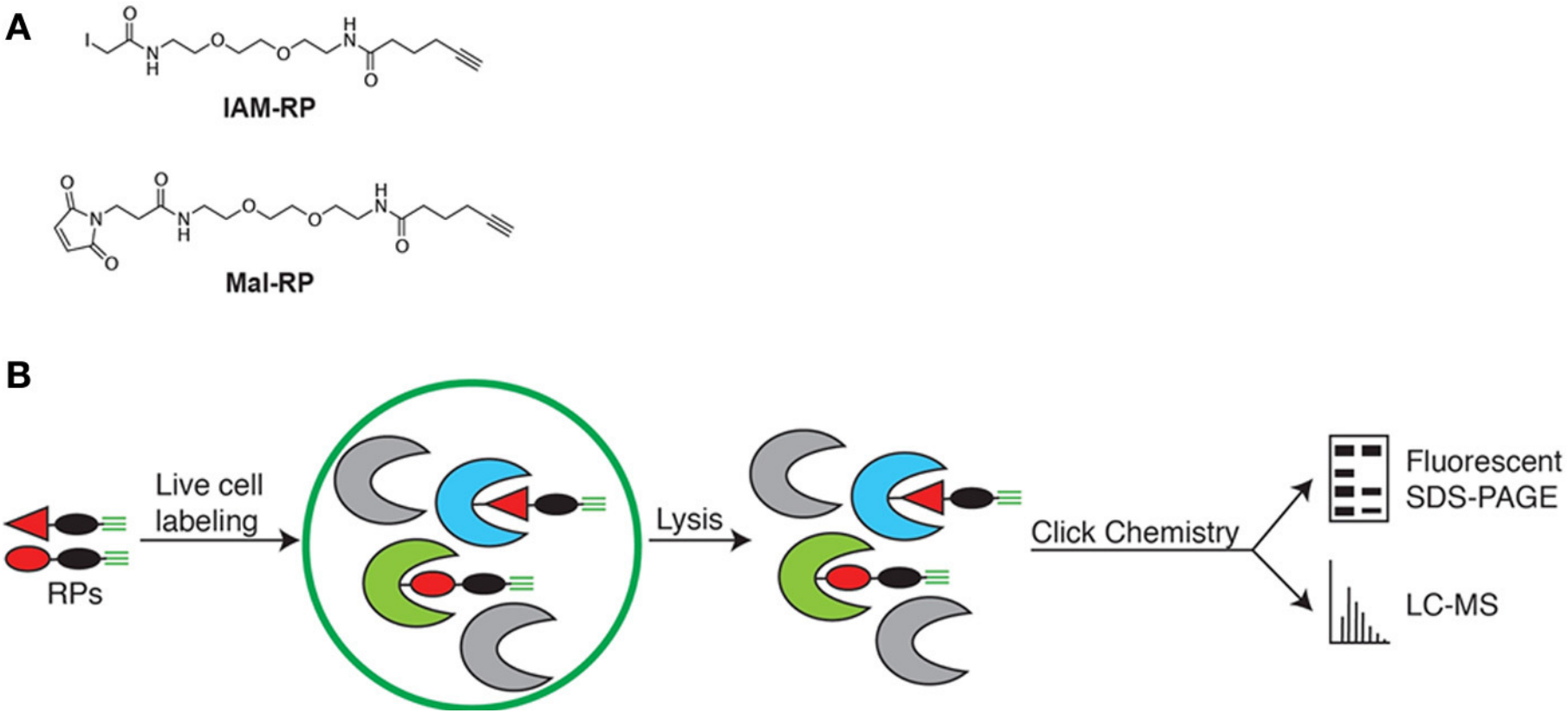
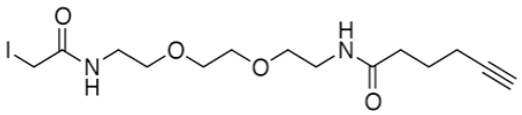


FIGURE 1 | (A) Chemical probes for *in vivo* labeling of reduced cysteine thiols. IAM-RP and Mal-RP. **(B)** Schematic of *in vivo* chemical proteomics approach for analysis of redox-sensitive *Synechococcus* 7002 proteins.

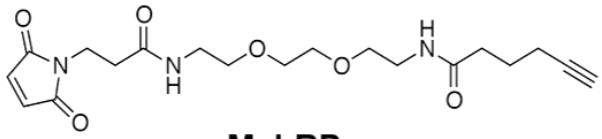
A.T. Wright *et coll.*, *Front. Microbiol.*, **2014**, *5*, 325

Thiol reactive probes

a

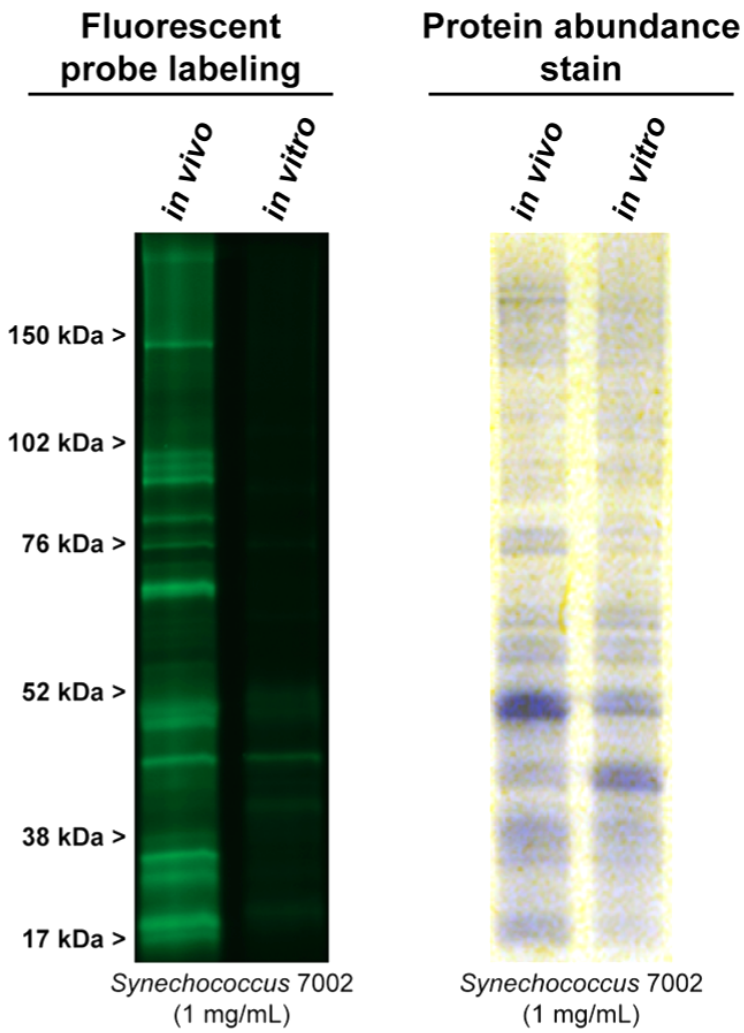


IAM-RP



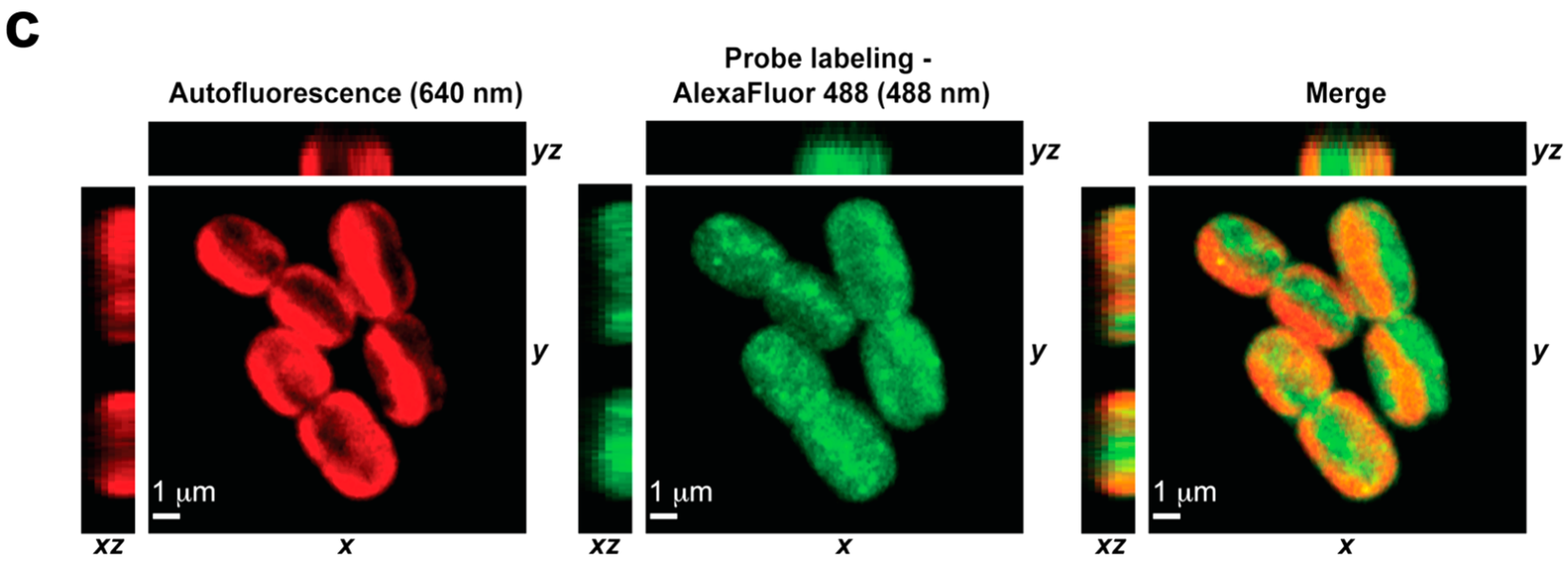
Mal-RP

b

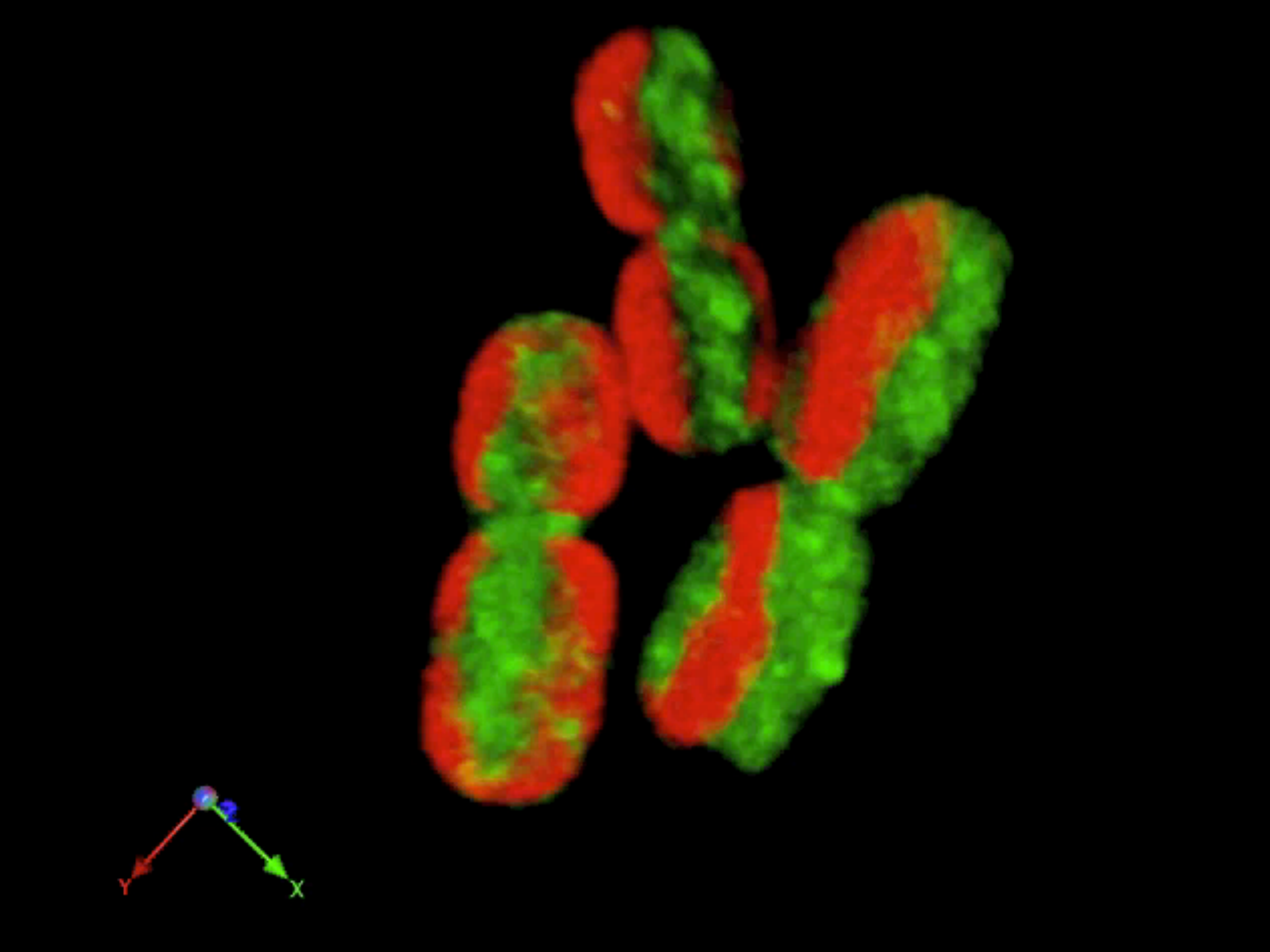


A. T. Wright *et coll.*, *Front. Microbiol.*, **2014**, *5*, 325

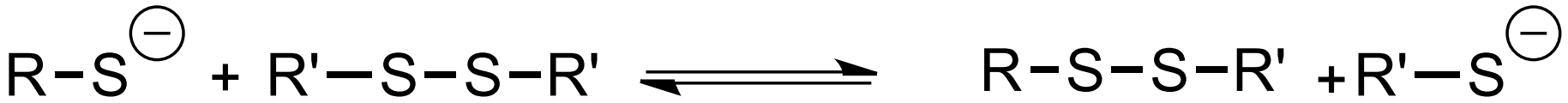
Labeling thiols *in vivo*



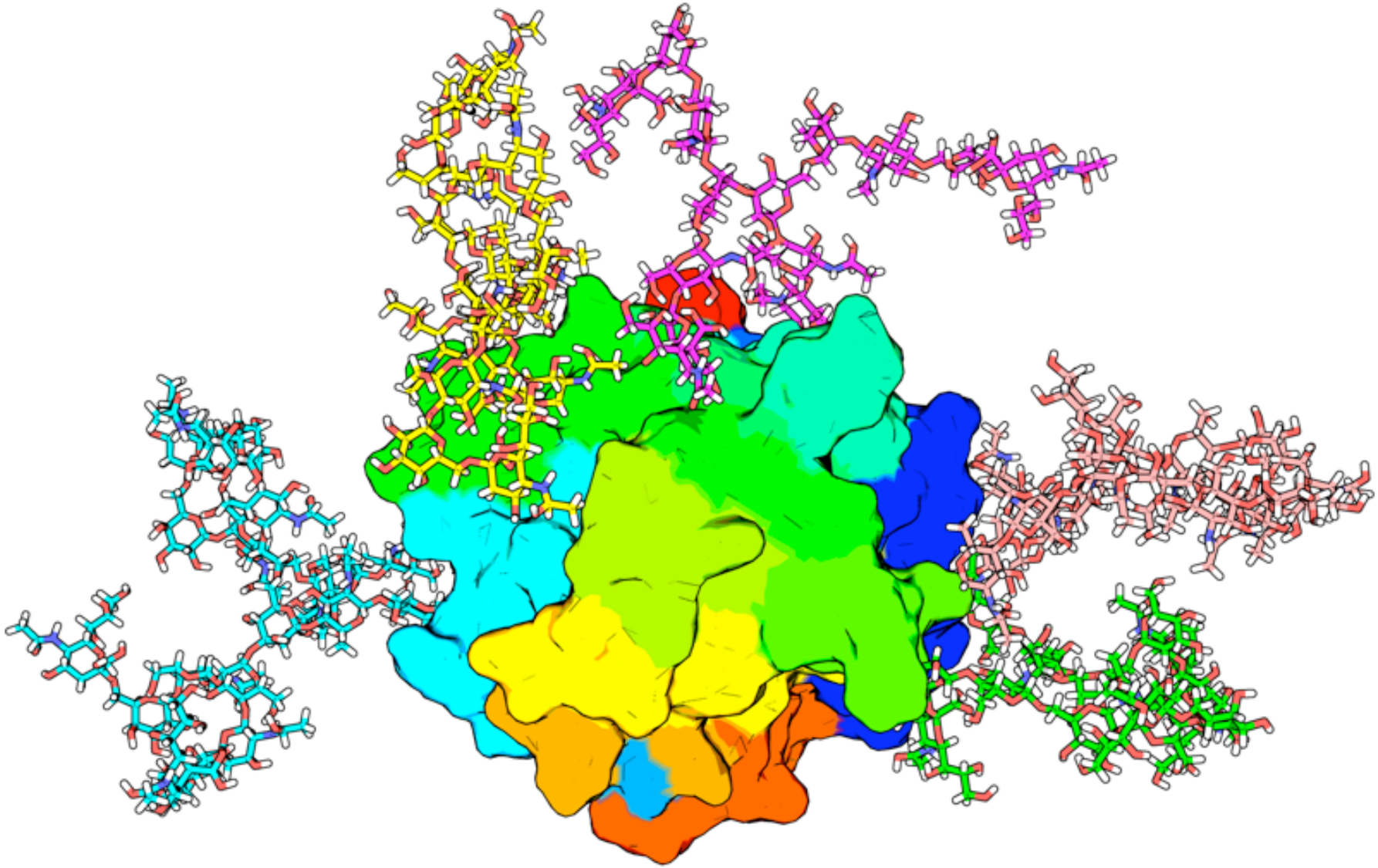
A. T. Wright *et coll.*, *Front. Microbiol.*, **2014**, *5*, 325


Y
X

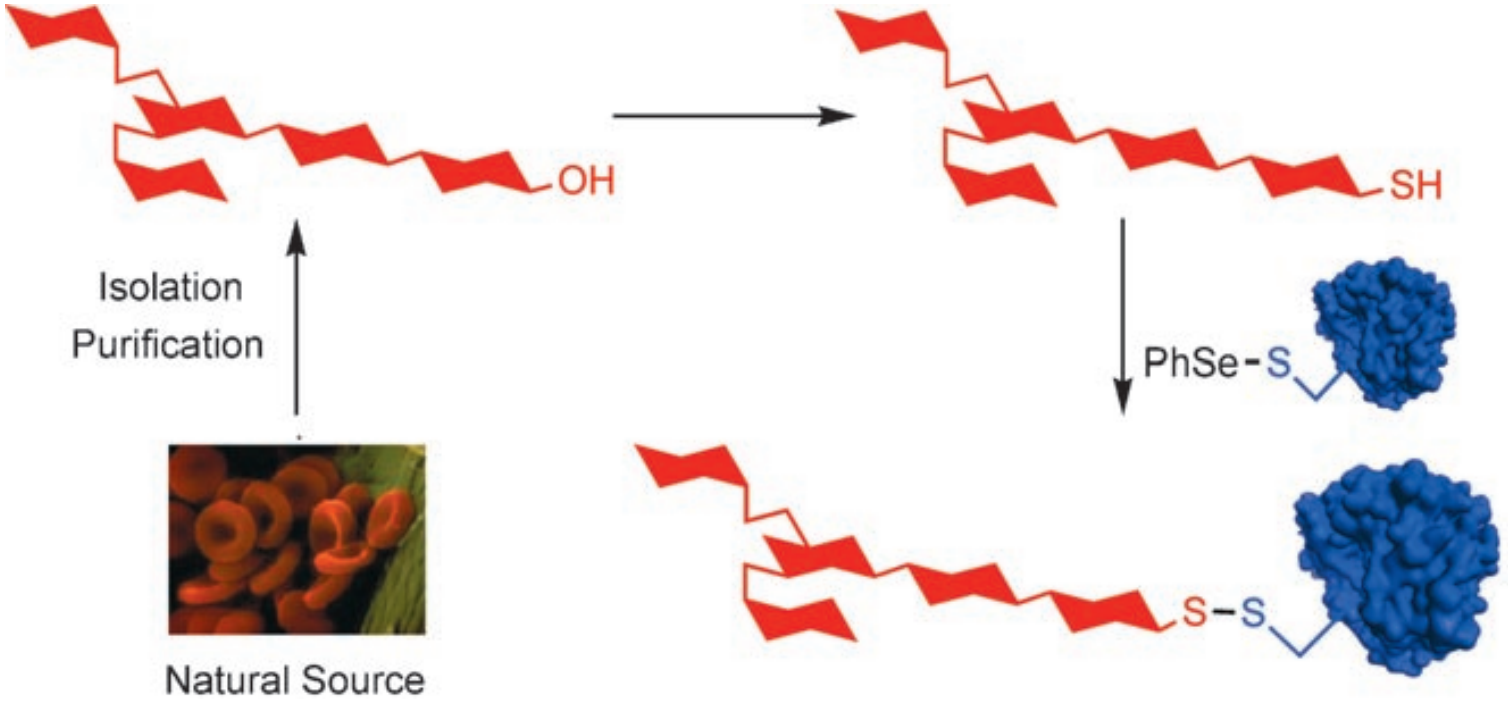
Bioconjugation - Thiol exchange



Glycoproteins



Neoglycoproteins

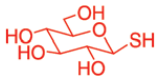
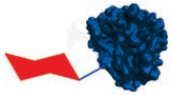
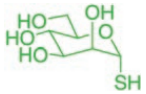
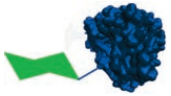
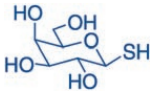
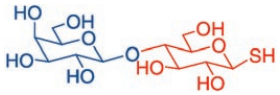
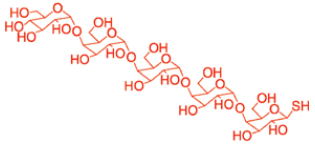
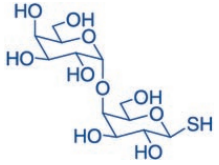
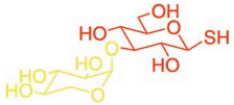
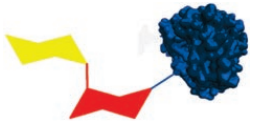


Scheme 2. One-pot protein glycosylation with reducing sugars isolated from natural sources.

G. J. L. Bernardes, D. P. Gamblin, B. G. Davis, *Angew. Chem. Int. Ed.*., **2006**, *45*, 4007-4011

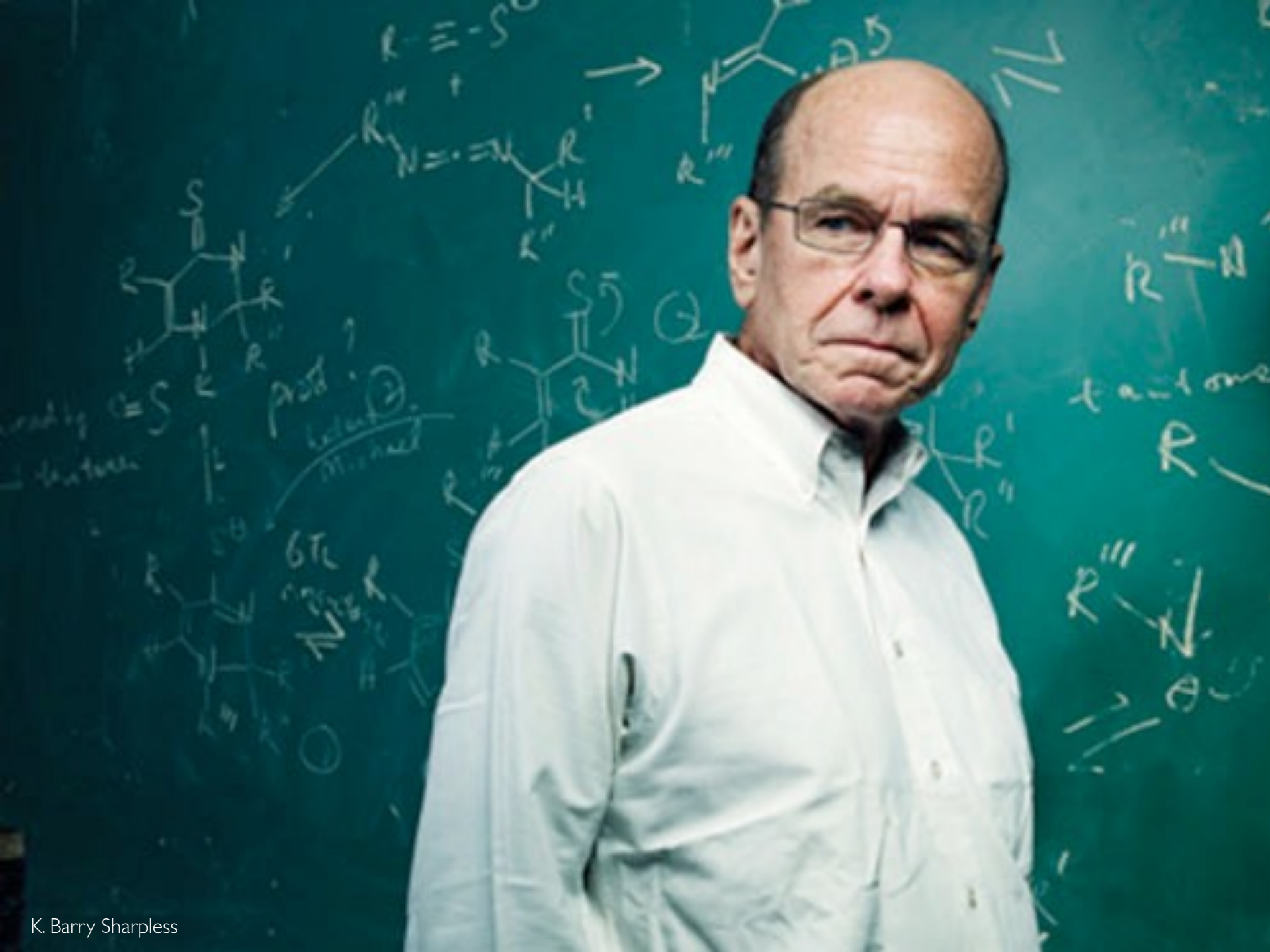
Proof of concept

Table 4: Two-step strategy for direct protein conjugation from deprotected reducing sugars through LR and Glyco–SeS.[8]

Sugar	Thiol product[a]	Protein product[b]	Conversion [%][c]	ESI-MS Found (calcd)
Glc			**32a** >95	27066 (27064)
Man			**32b** >95	27064 (27064)
Gal			**32c** >95	27064 (27064)
Lactose (Galβ1,4Glc)			**32d** >95	27234 (27225)
Maltopentaose $(Glc\alpha1,4)_4Glc$			**32e** >95	27722 (27711)
Galabiose (Gaα1,4Gal)			**32f** >95	27224 (27225)
(Xylα1,3Glc)			**32g** >95	27194 (27194)

[a] Typically, LR (1.2 equiv) was added to a solution of the deprotected sugar in anhydrous dioxane and left to stir at 110 °C for 48 h; see the Supporting Information for more details. [b] Typically, crude thiol (20–50 equiv), in water, was added to preactivated SBLS156C-SePh in CHES (70 mM), MES (5 mM), $CaCl_2$ (2 mM); pH 9.5. After 30 min at RT, the reaction was analyzed by LC–MS; [c] Conversion determined by ESI-MS.

Click Chemistry



K. Barry Sharpless

Click Chemistry

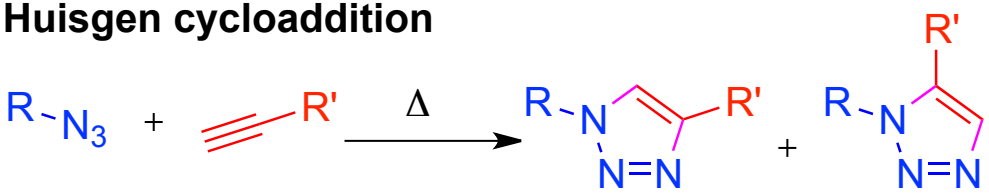
Diverse Chemical Function from a Few Good Reactions

C-X-C

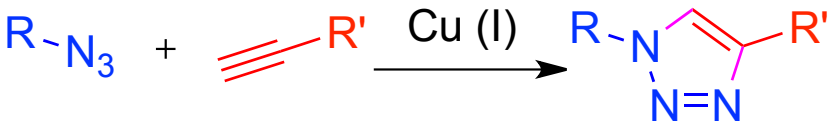
H. C. Kolb, M. G. Finn, and K. B. Sharpless, *Angew. Chem. Int. Ed.*., **2001**, *40*, 2004-2021

Click chemistry - 2+3 Cycloadditions

Huisgen cycloaddition



CuAAC

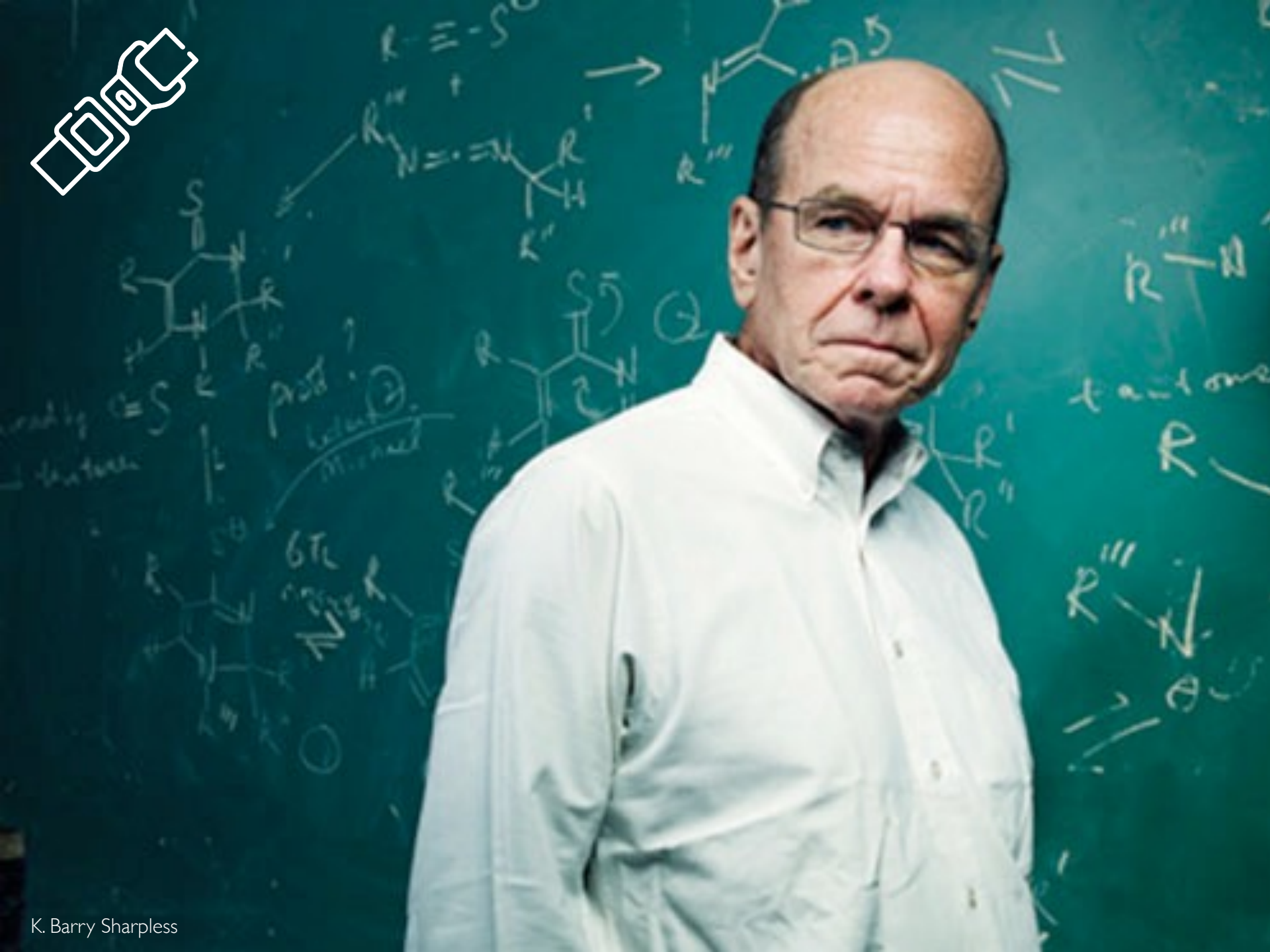


Meldal *et coll., J. Org.. Chem..*, **2002**, *67*, 3057-64

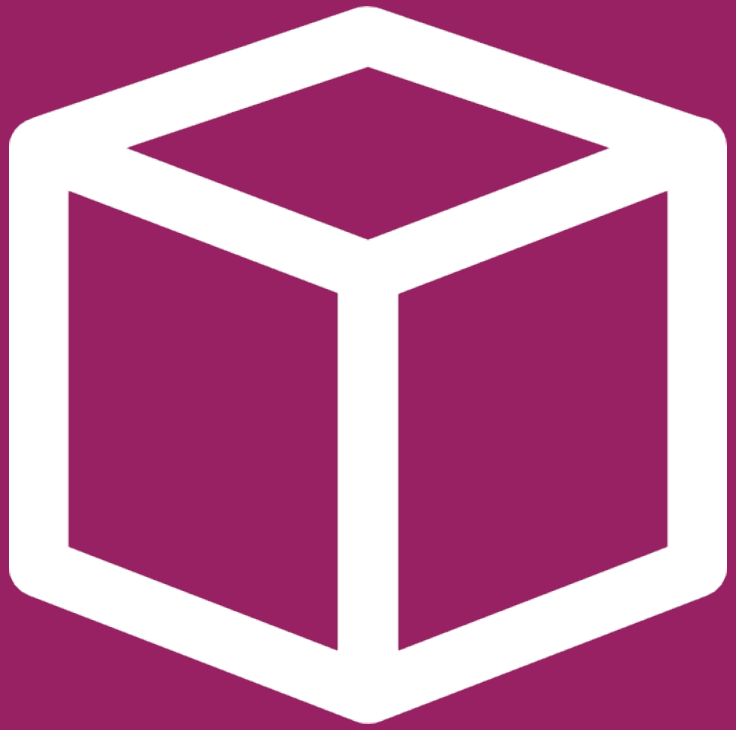
Sharpless *et coll., Angew. Chem. Int. Ed.*, **2002**, *41*, 2596-99

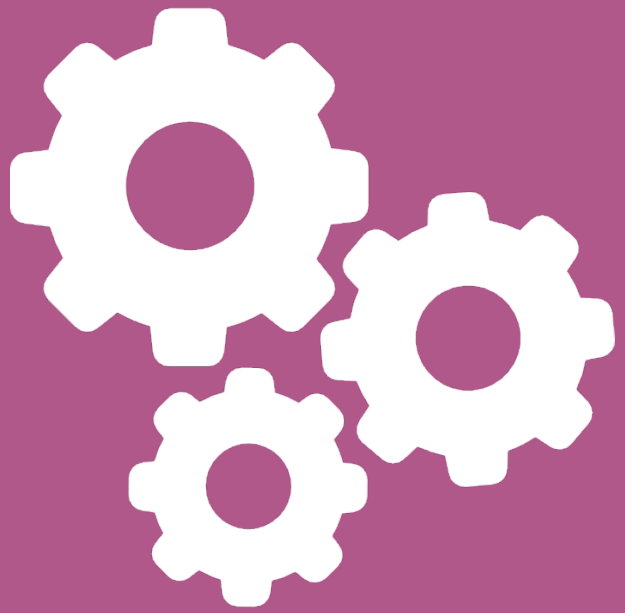
Morten P. Meldal

Icon made by Freepik from www.flaticon.com



K. Barry Sharpless





Structure

Function

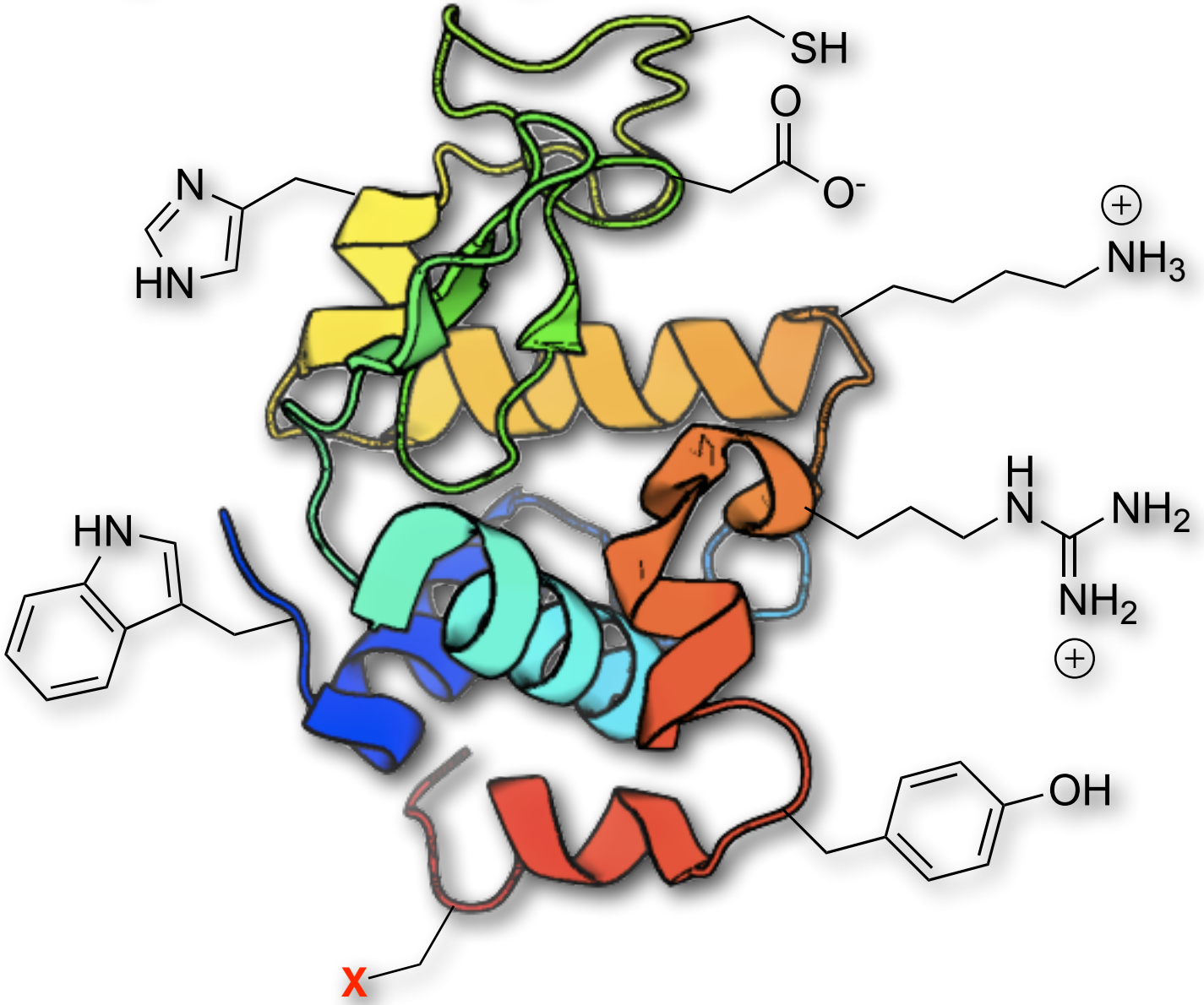
Icon made by Cole Bemis from www.flaticon.com

Icon made by Freepik from www.flaticon.com

Bioorthogonal Chemistry

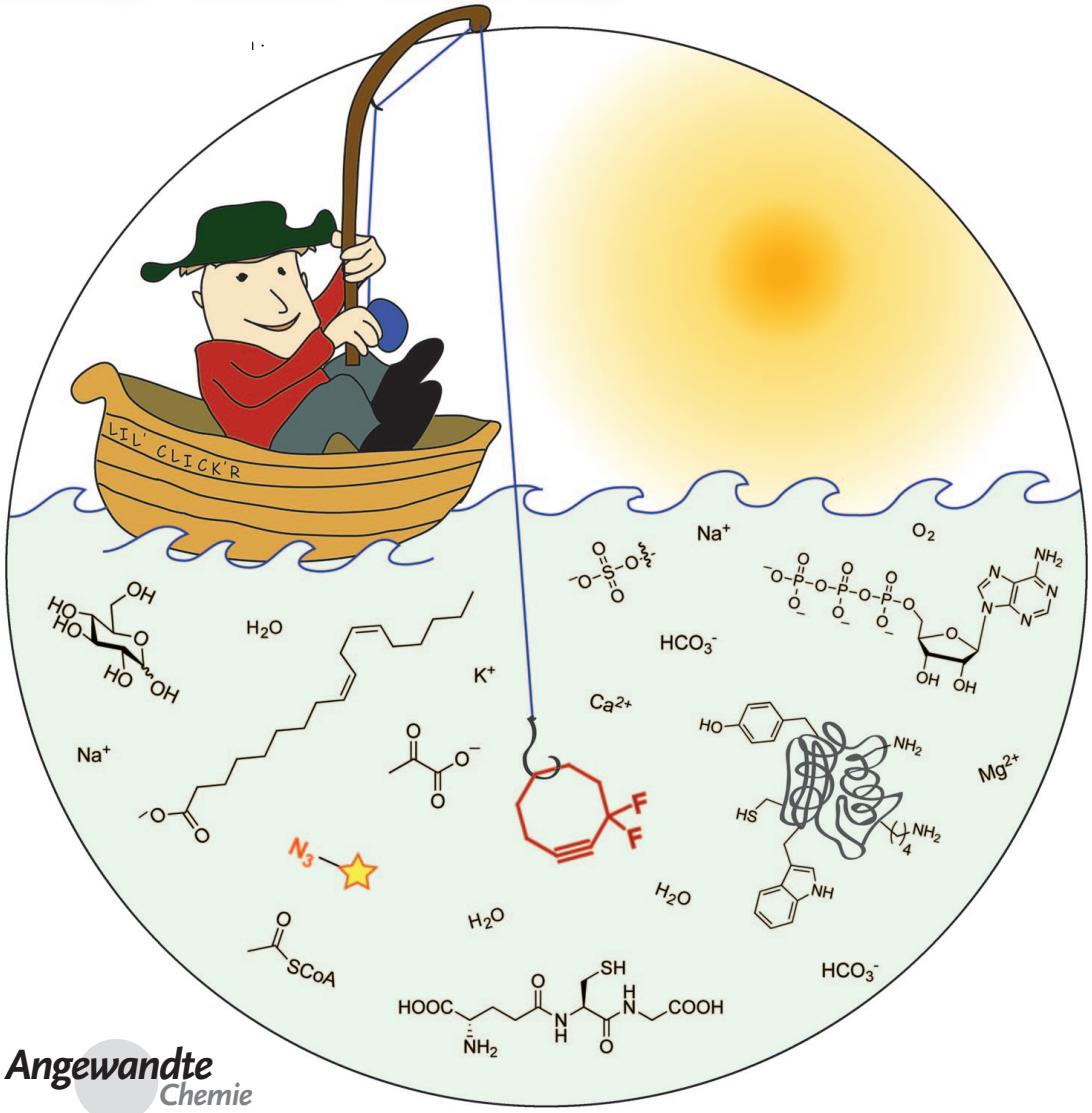
Carolyn Bertozzi

Bioorthogonal bioconjugation



Bioorthogonal chemistry

Fishing for Selectivity in a Sea of Functionnality



Angewandte Chemie

Carolyn R. Bertozzi et coll., *Angew.. Chem. Int. Ed.*, **2009**, *48*, 6974-98

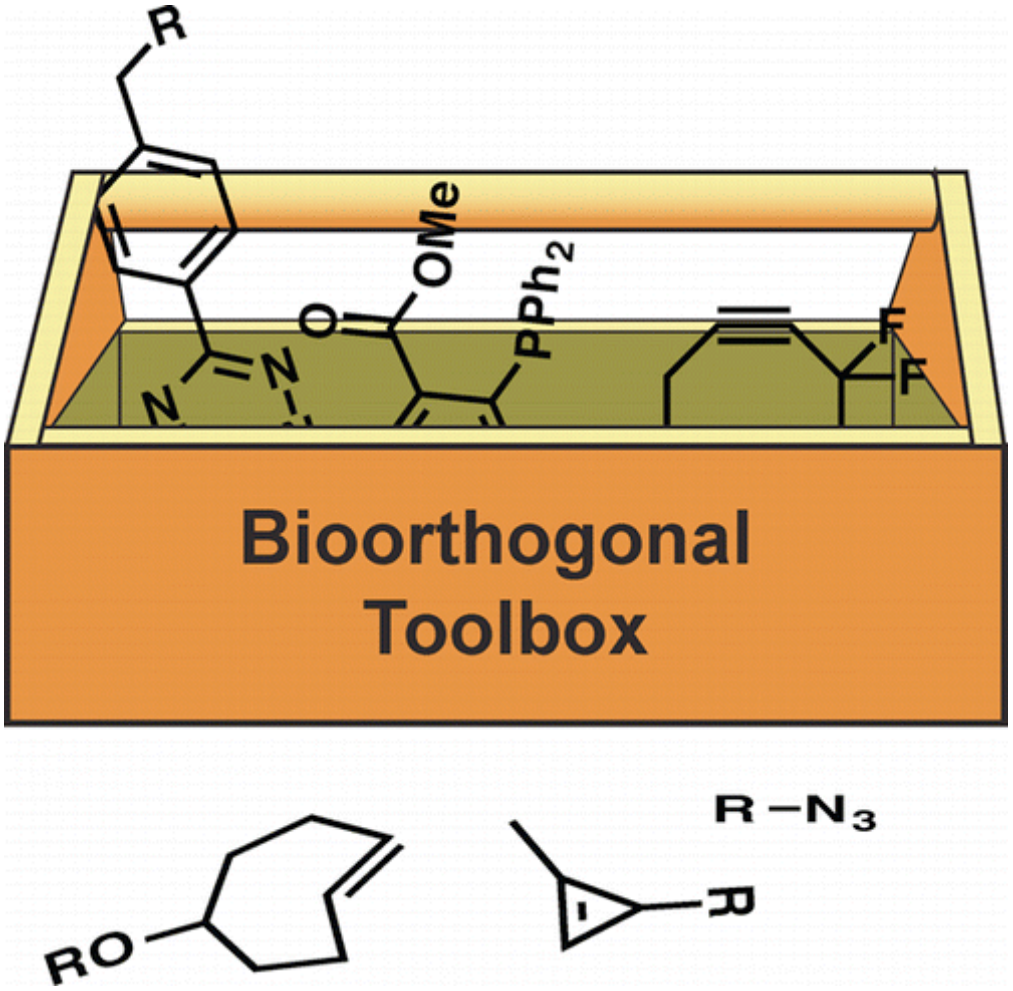
Relevant chemical transformations ?

Chemical Transformations Leading to Protein Covalent Modifications

- Water Is the Sole Solvent
- A Neutral pH Is Required
- Ambient Temperature (Up to 40°C)
- Kinetics, which Adapted to the Observed Phenomenon (on the Hour Scale)
- Low Reactant Concentrations
- Nontoxic Reagents

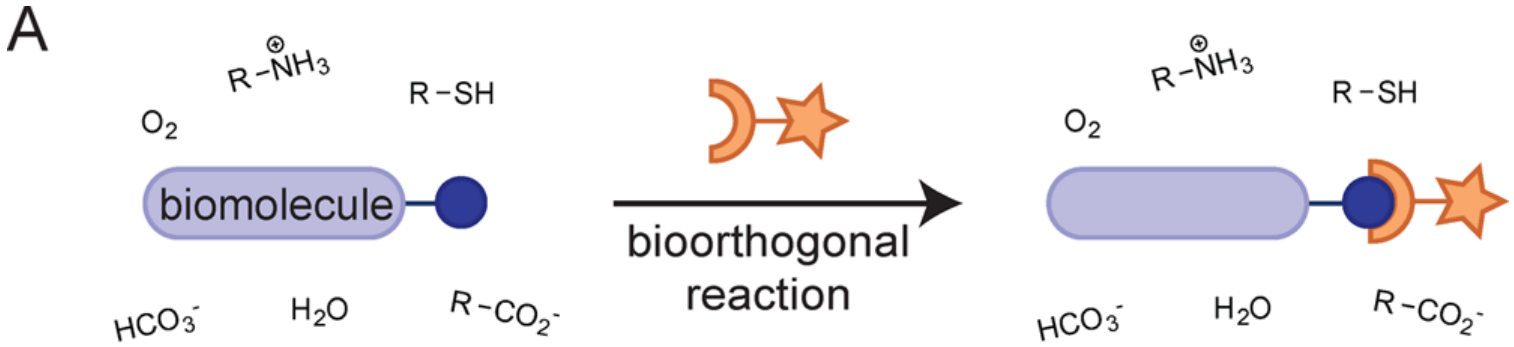
Mathieu Pucheault et coll., *Chem. Biol.*, **2010**, *39*, 213-27

Finding the right (bioorthogonal) chemistry



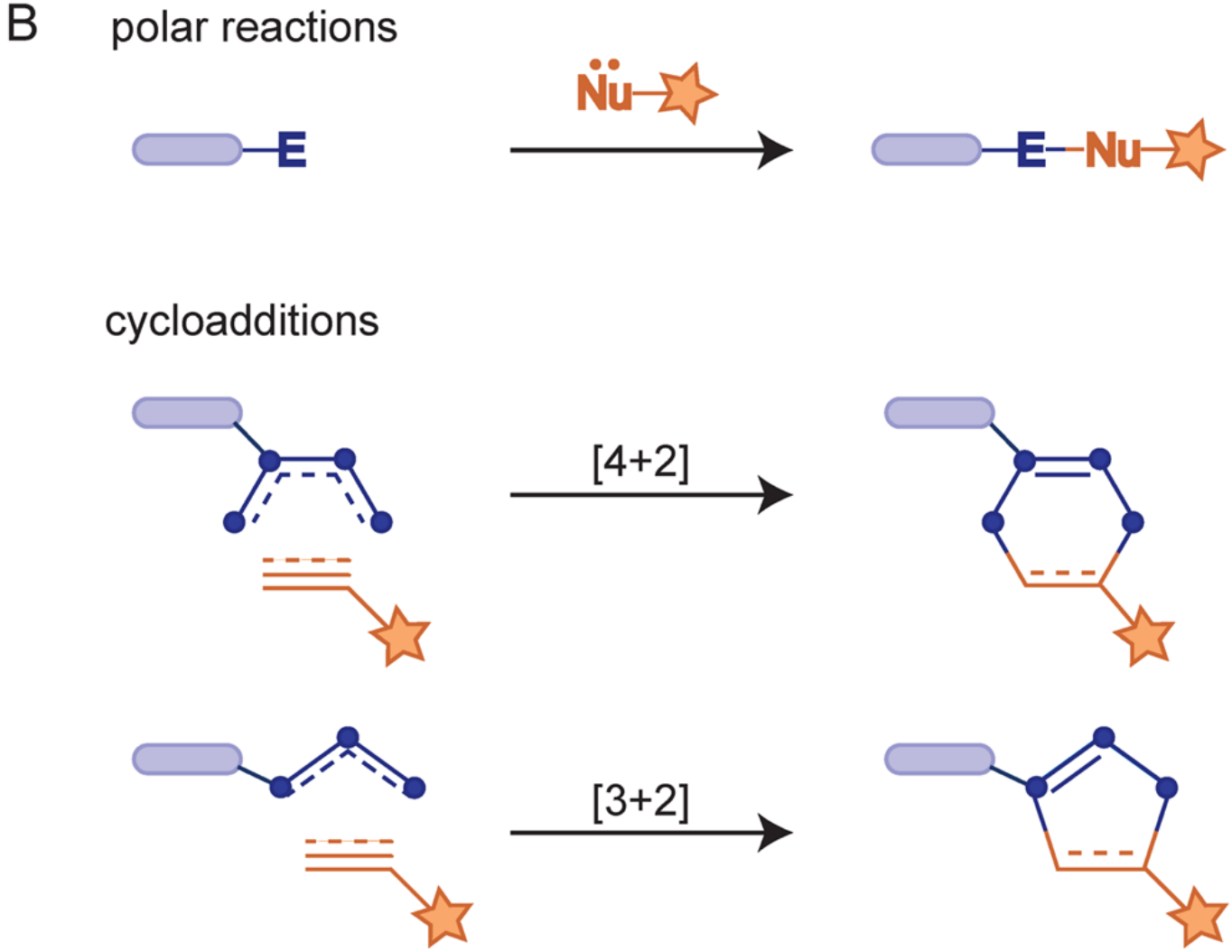
Jennifer A. Prescher *et coll.*, *ACS. Chem. Biol.*, **2014**, *9*, 592-605

Finding the right bioorthogonal reaction



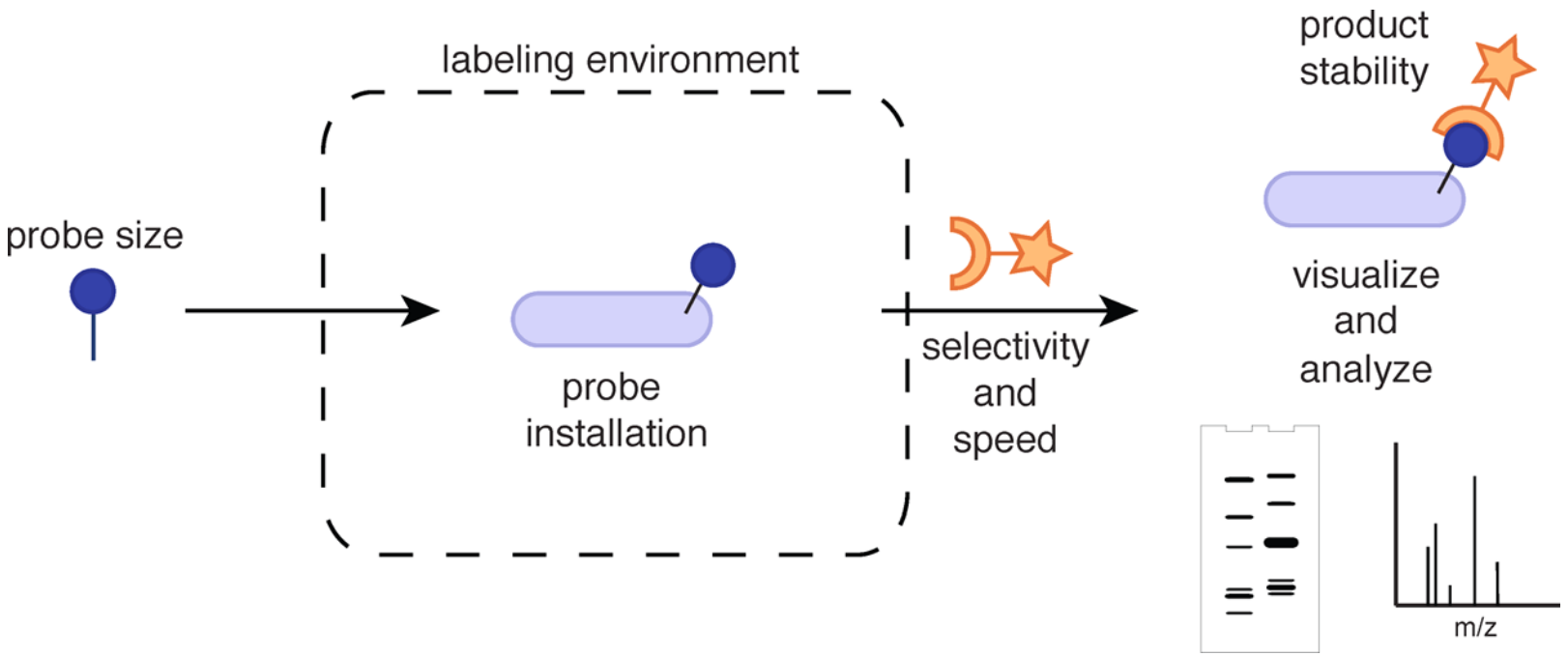
Jennifer A. Prescher *et coll.*, *ACS. Chem. Biol.*, **2014**, *9*, 592-605

Finding the right bioorthogonal reaction



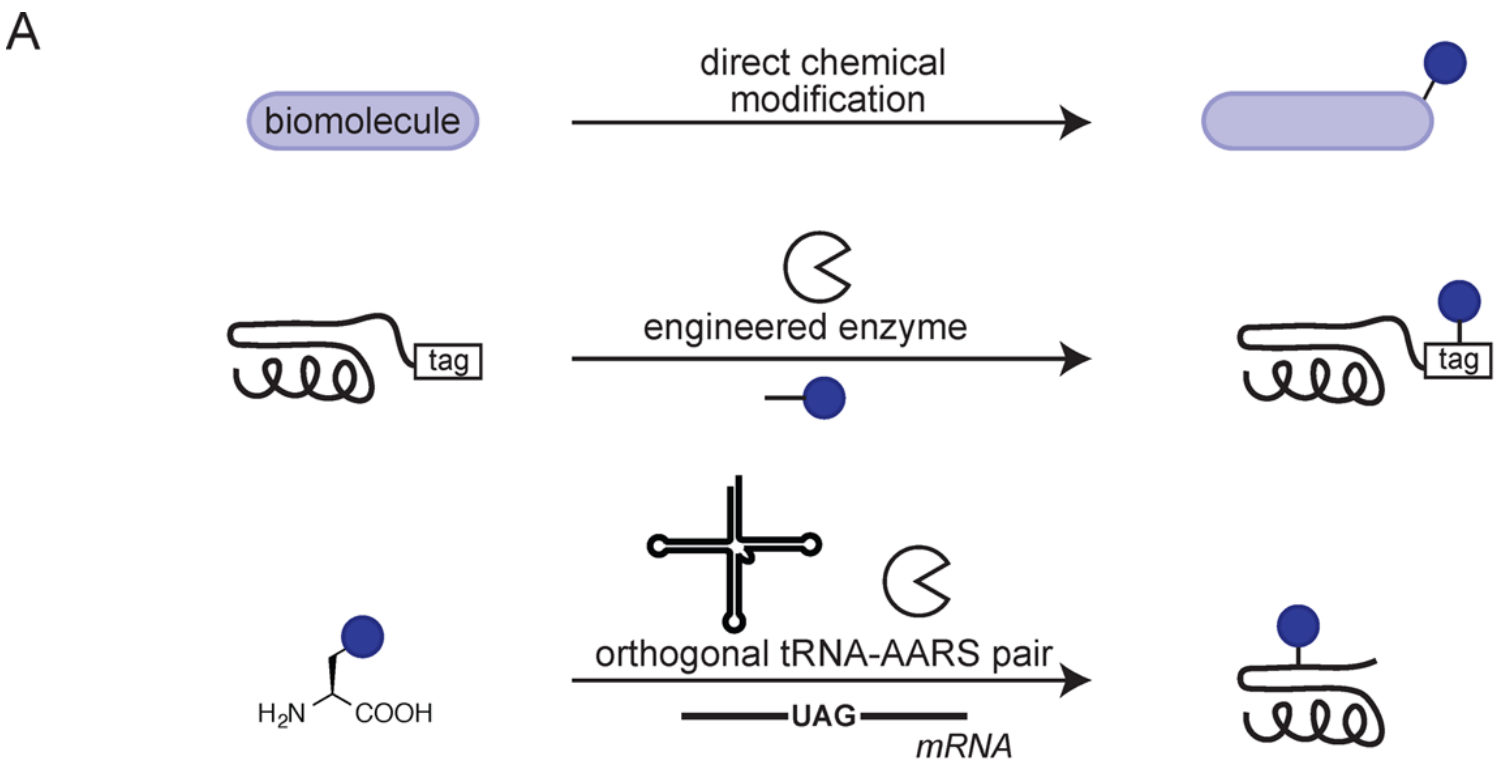
Jennifer A. Prescher *et coll.*, *ACS. Chem. Biol.*, **2014**, *9*, 592-605

Labeling biomolecules using bioorthogonal chemistry



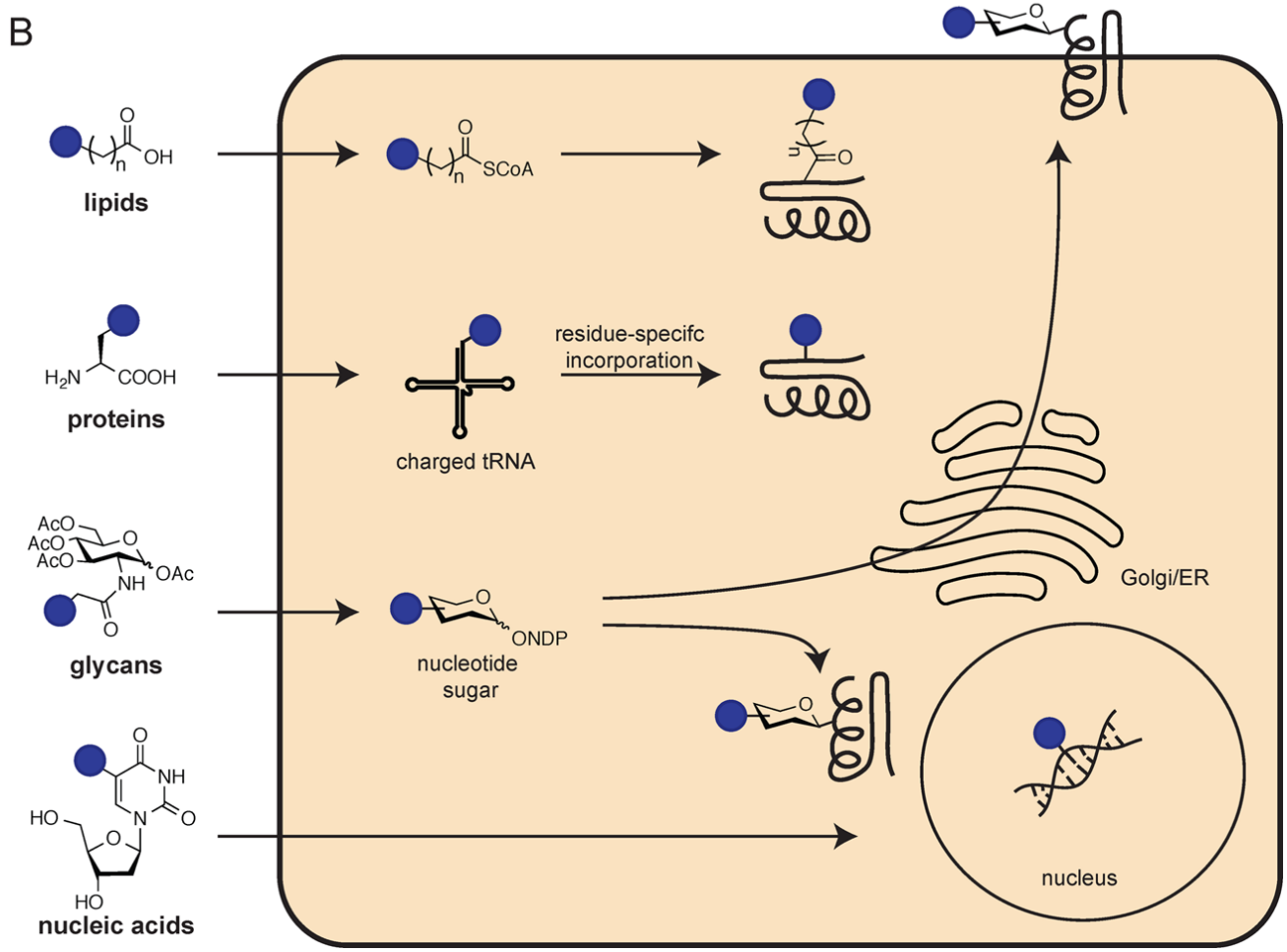
Jennifer A. Prescher *et coll.*, *ACS. Chem. Biol.*, **2014**, *9*, 592-605

Modification of protein targets



Jennifer A. Prescher *et coll.*, *ACS. Chem. Biol.*, **2014**, *9*, 592-605

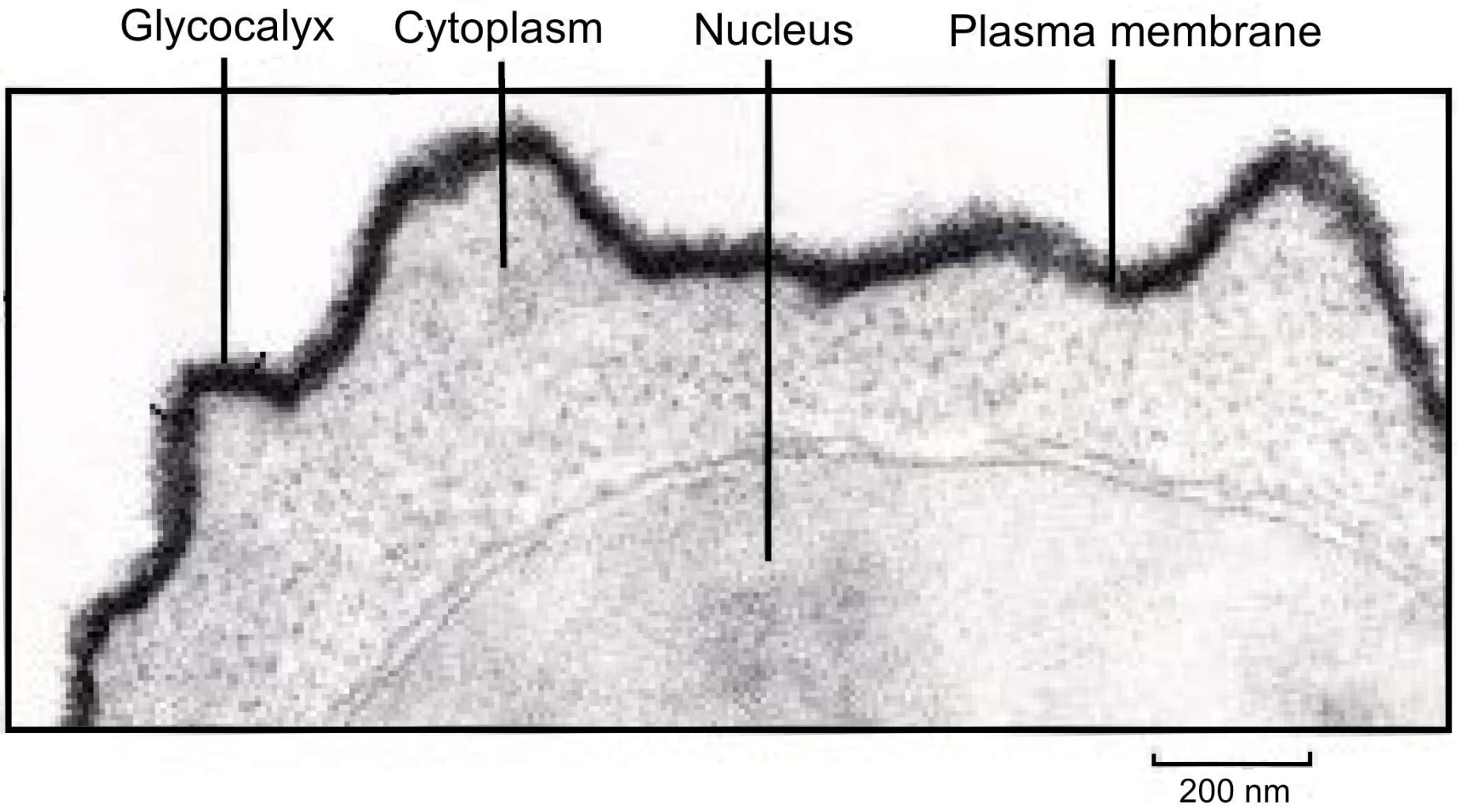
Metabolic modification of biomolecules

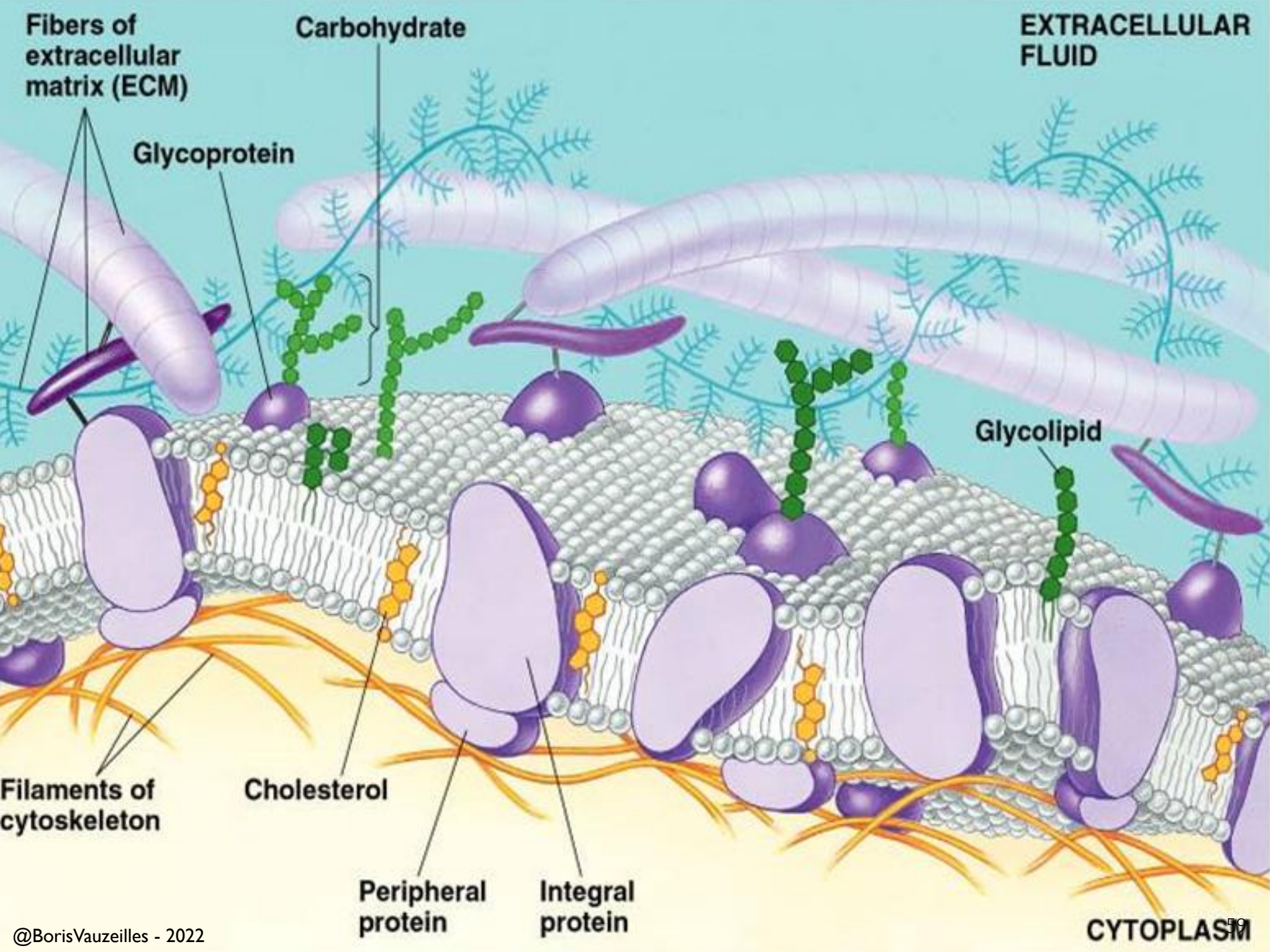


Jennifer A. Prescher *et coll.*, *ACS. Chem. Biol.*, **2014**, *9*, 592-605

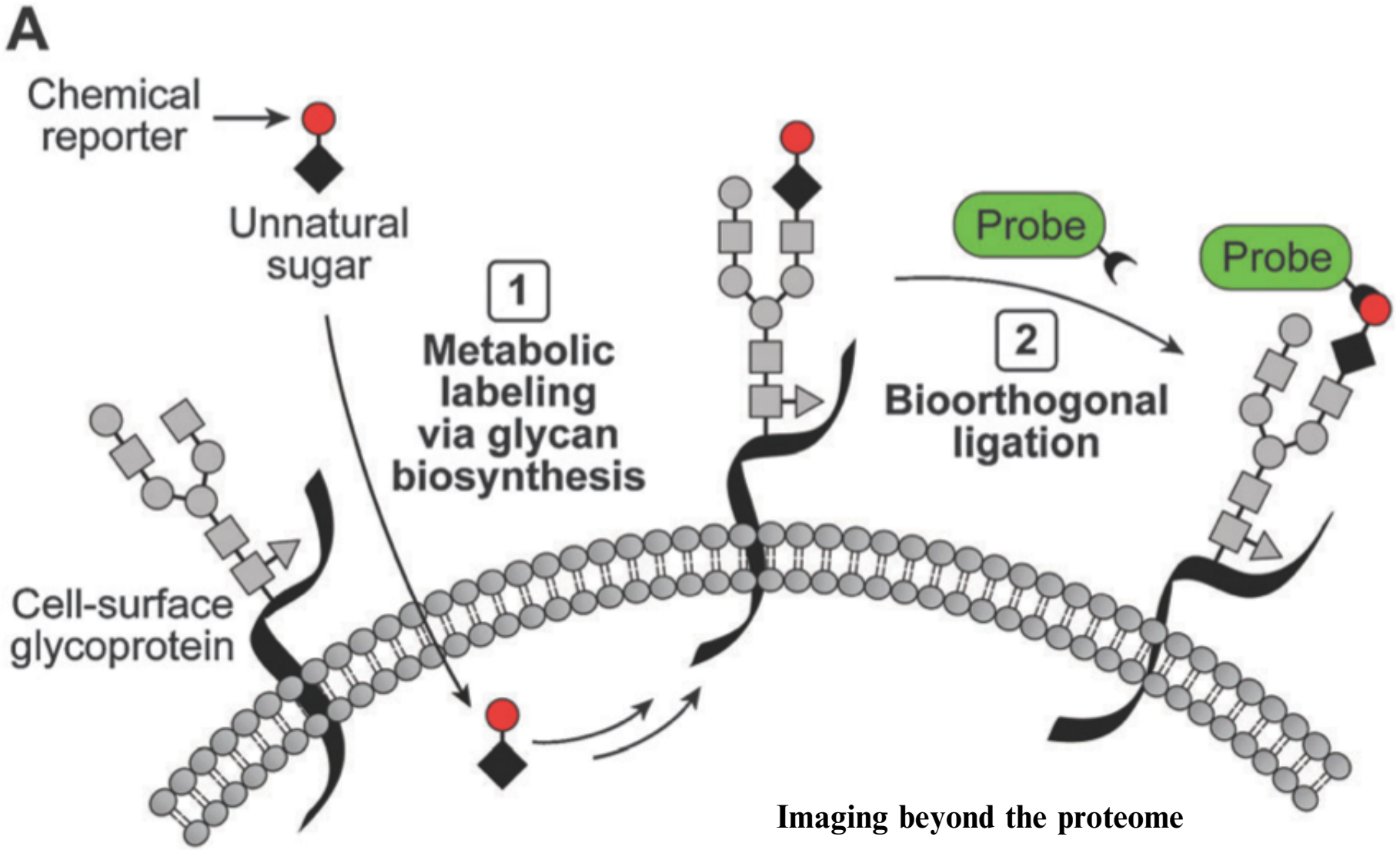
Metabolic glycan labeling

Cell surface




Fibers of extracellular matrix (ECM)
Carbohydrate
EXTRACELLULAR FLUID
Glycoprotein
Glycolipid
Filaments of cytoskeleton
Cholesterol
Peripheral protein
Integral protein
CYTOPLASM

Metabolic Glycan Labeling

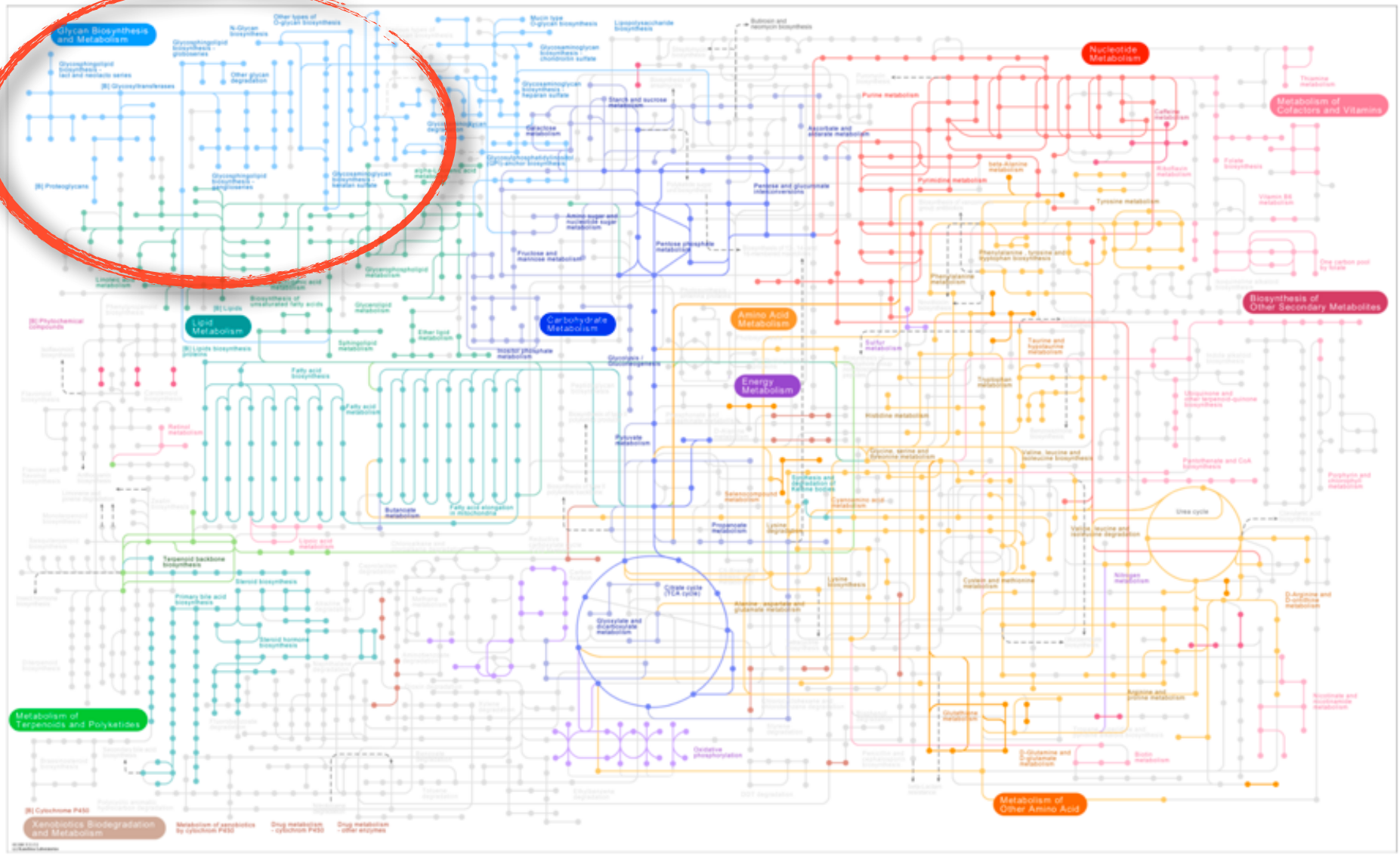


Imaging beyond the proteome

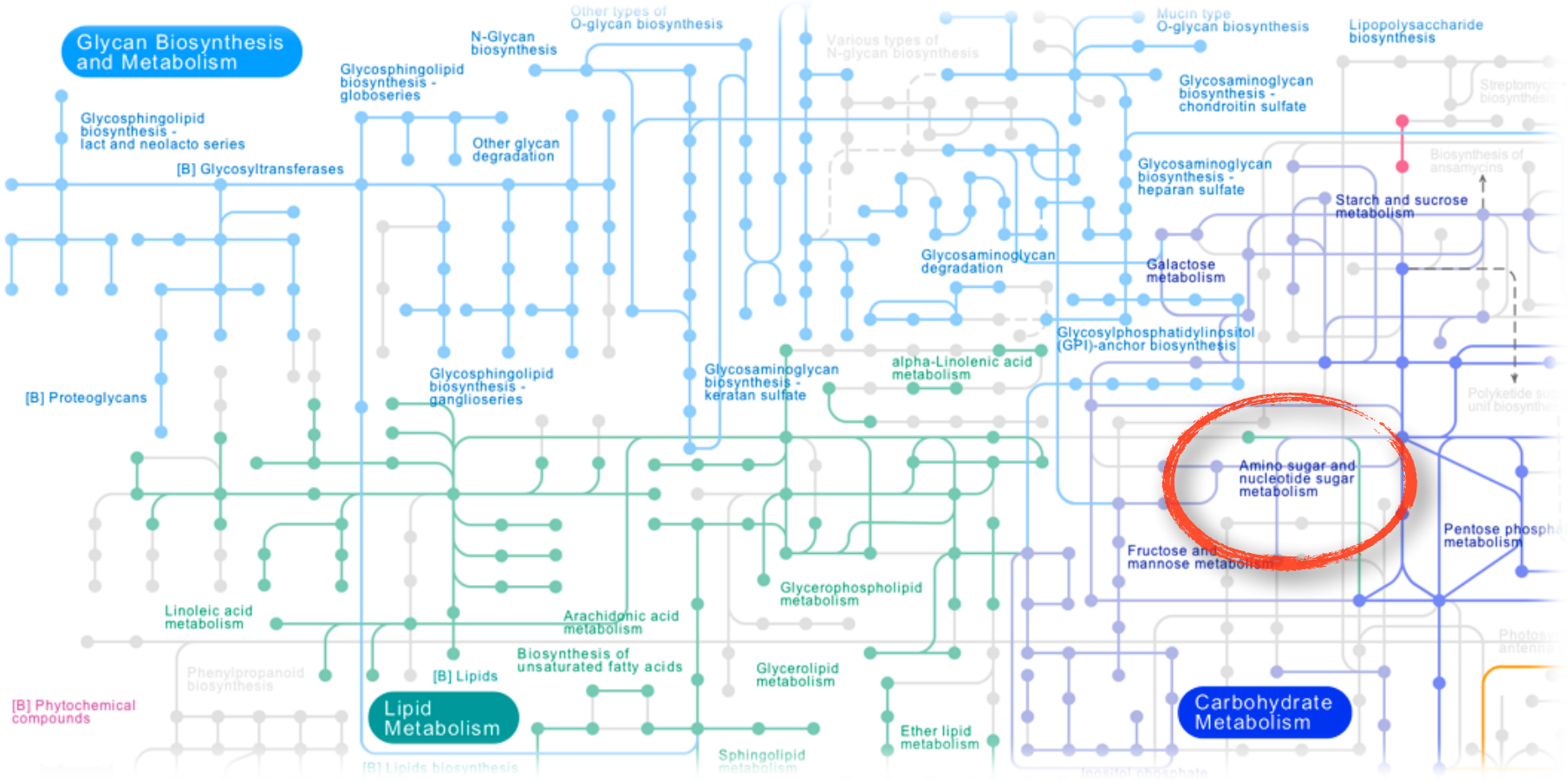
Pamela V. Chang[a] **and Carolyn R. Bertozzi***[abc]

Chem. Commun., 2012, **48**, 8864–8879

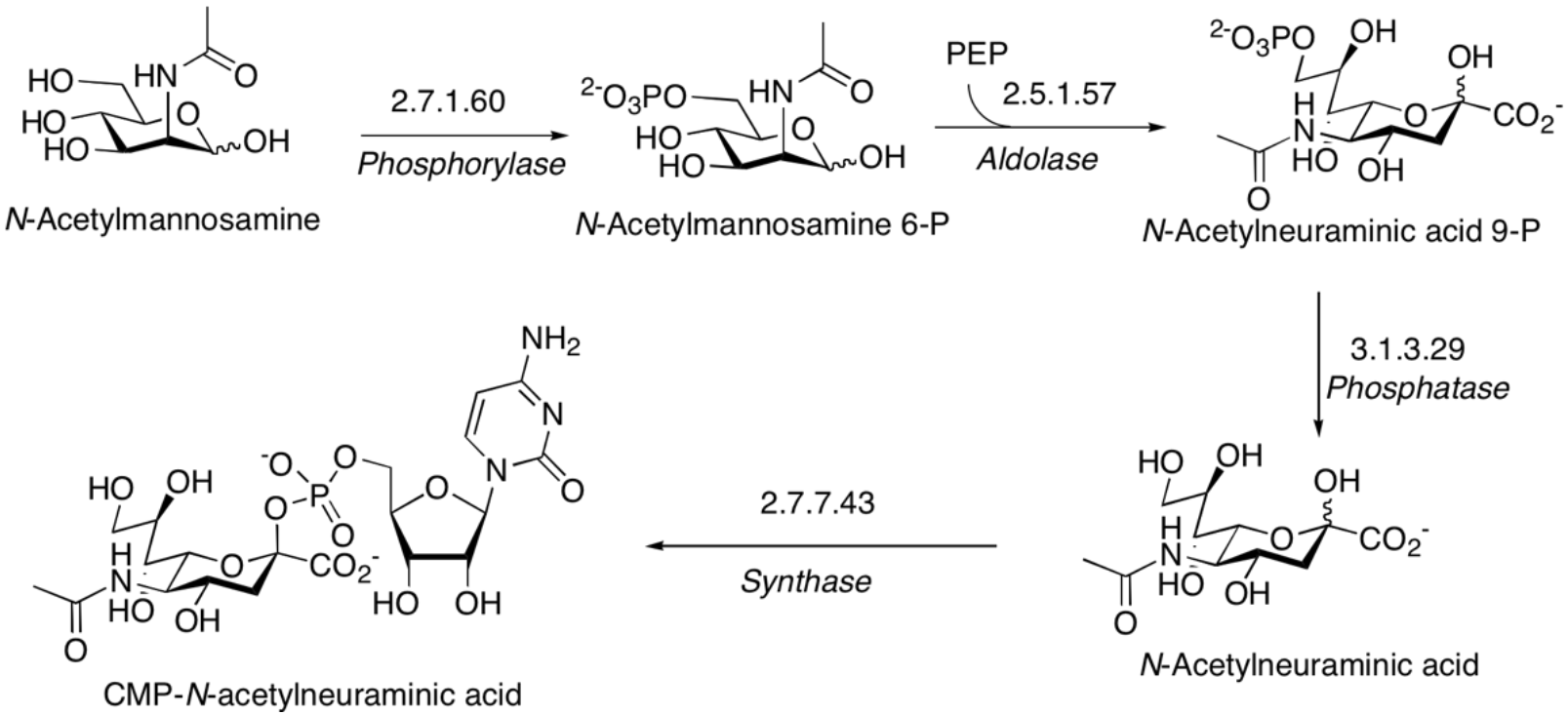
Metabolic Oligosaccharide Engineering



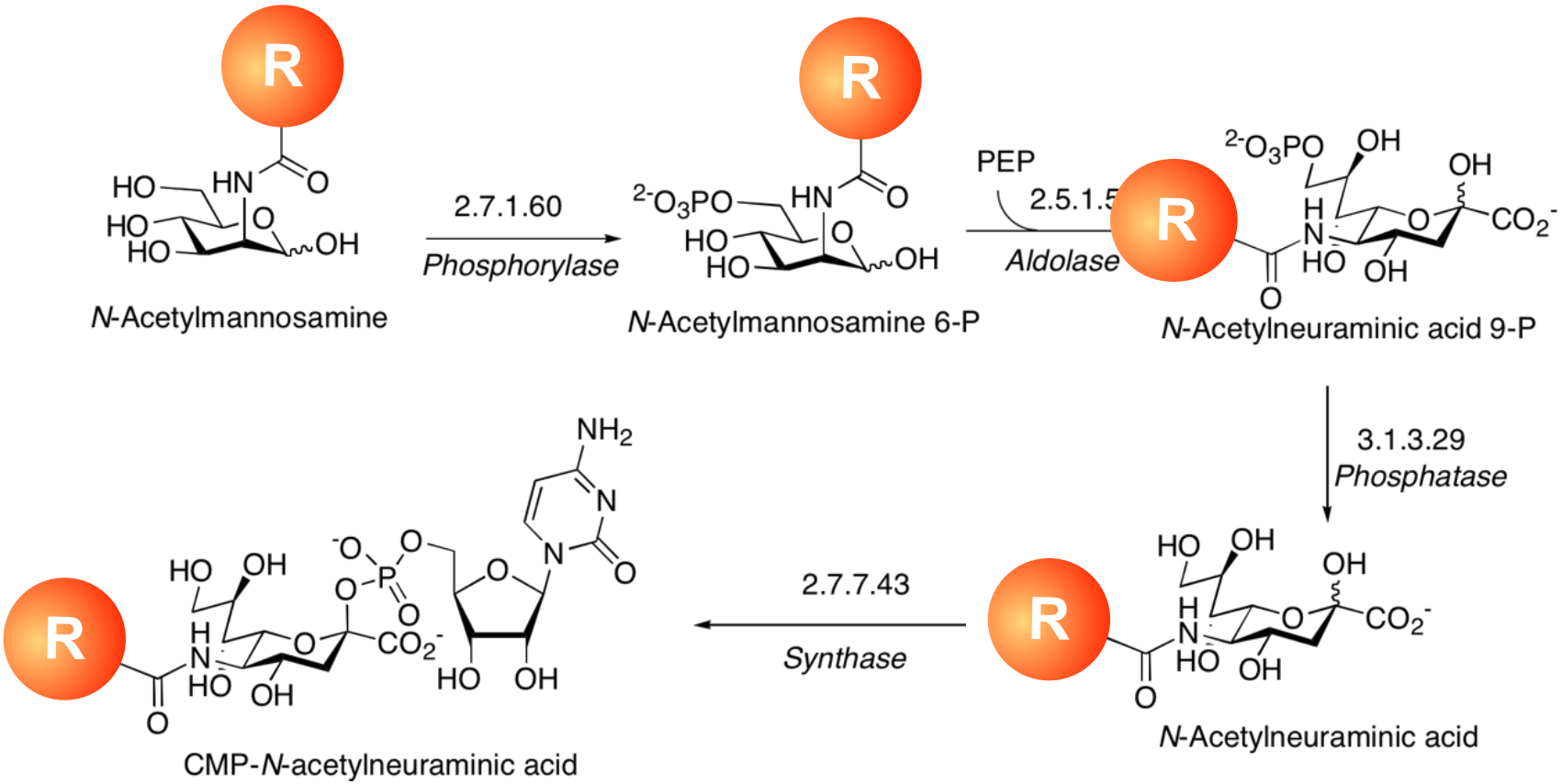
Metabolic Oligosaccharide Engineering



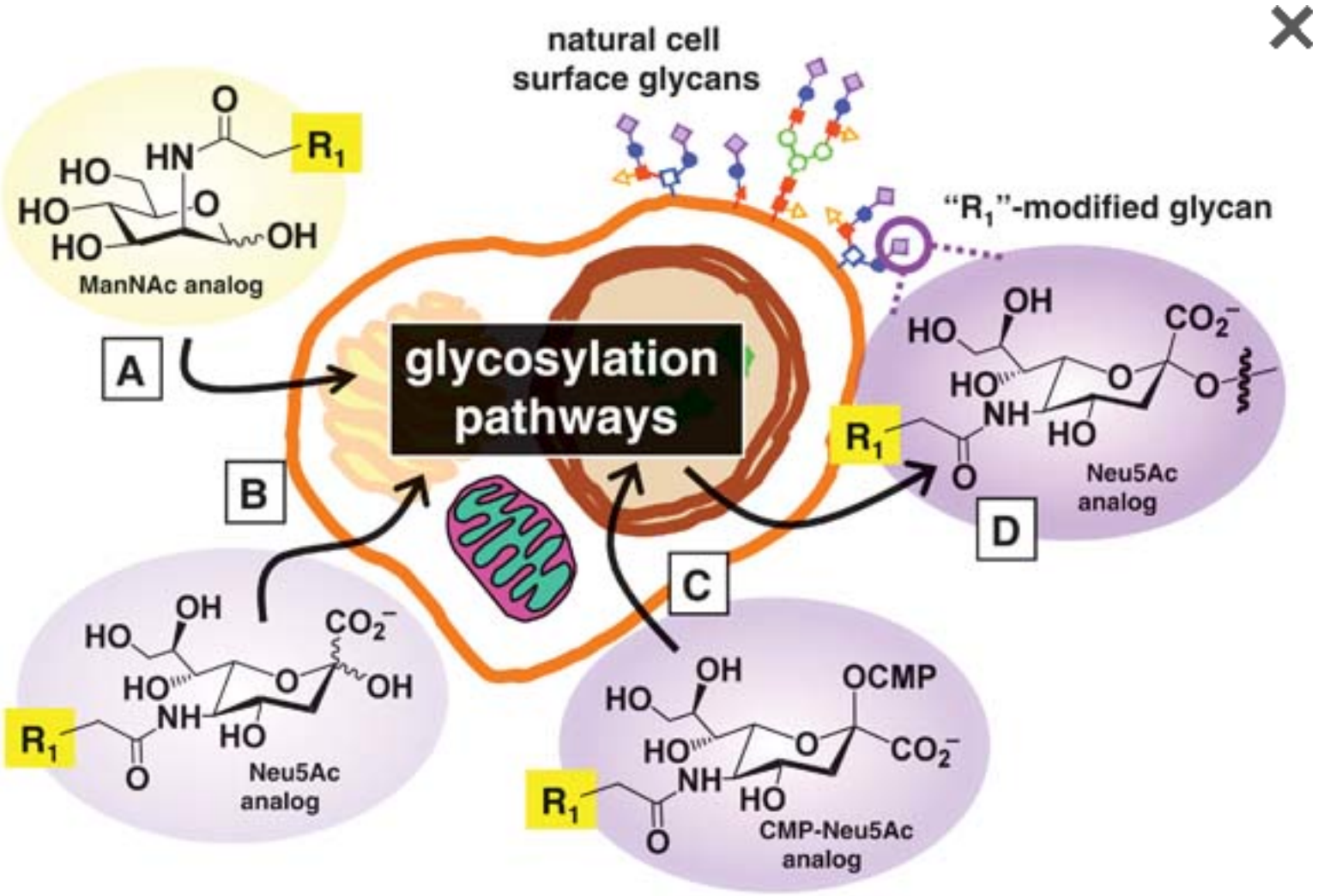
N-Acetyl neuraminic acid



N-Acetyl neuraminic acid

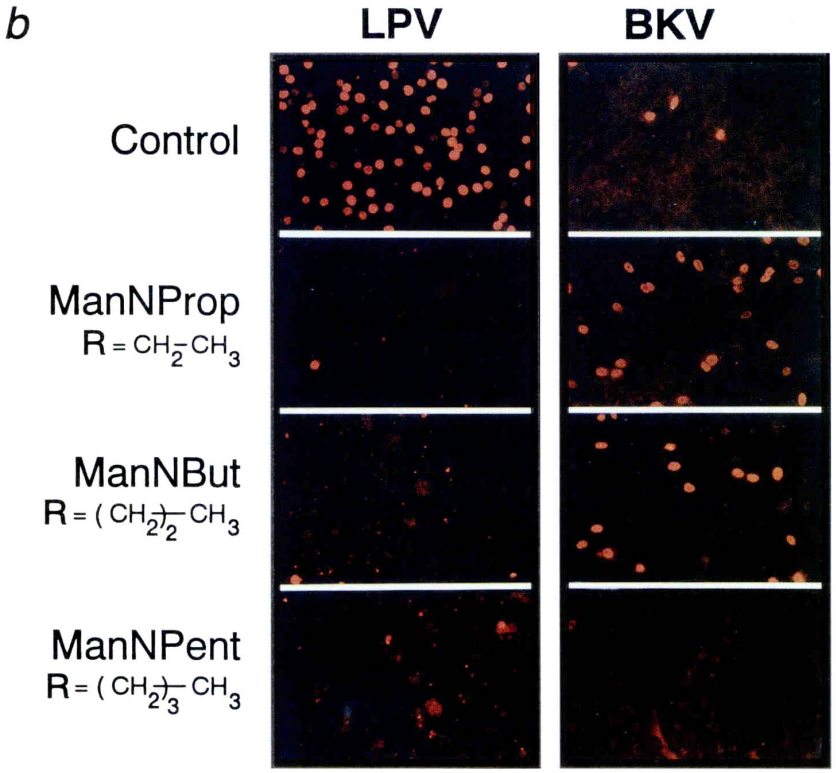


Cell-surface glycan engineering



Cell surface engineering

FIG. 4. **Reduction or enhancement of host cell susceptibility to LPV or BKV infection by pretreatment with sialic acid precursor analogues.** *a*, the chair conformation of the applied *N*-substituted D-mannosamines is shown with *R* indicating the modified *N*-acyl group. *b*, host cell lines BJA-B and Vero were cultured for 48 h in the presence of the sialic acid precursor analogues ManNProp, ManNBut, or ManNPent or as control an equivalent volume of PBS and subsequently infected with LPV or BKV, respectively. Virus-infected cells were identified by immunofluorescence staining for LPV and BKV capsid proteins 48 h after infection (this time allows the completion of only one viral replication cycle). Similar numbers of cells are present in microphotographs of each *panel* as determined by nuclear counterstaining (not shown).



THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 270, No. 3, Issue of January 20, pp. 1308–1314, 1995
Printed in U.S.A.

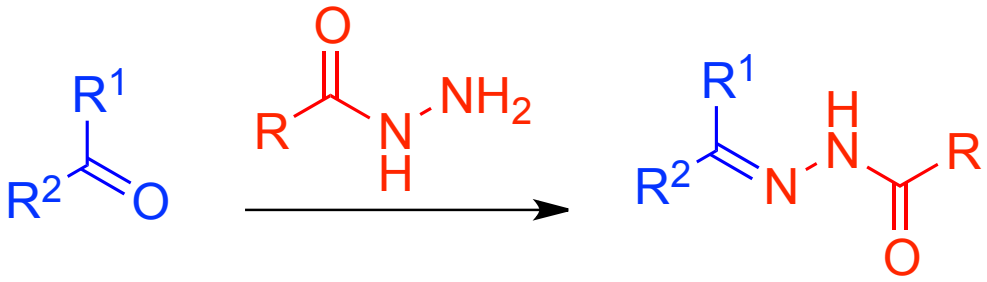
Biosynthetic Modulation of Sialic Acid-dependent Virus-Receptor Interactions of Two Primate Polyoma Viruses*

(Received for publication, September 14, 1994, and in revised form, November 2, 1994)

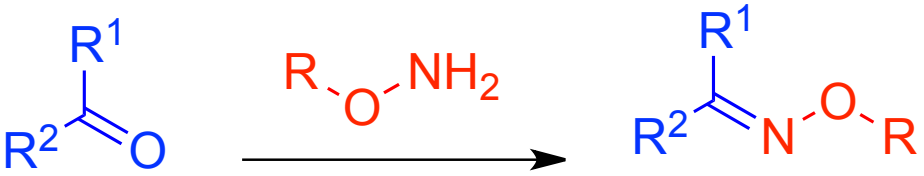
Oliver T. Keppler‡, Peer Stehling§, Markus Herrmann‡, Holger Kayser§, Detlef Grunow§, Werner Reutter§, and Michael Pawlita‡¶

Bioorthogonal chemistry - polar ligations

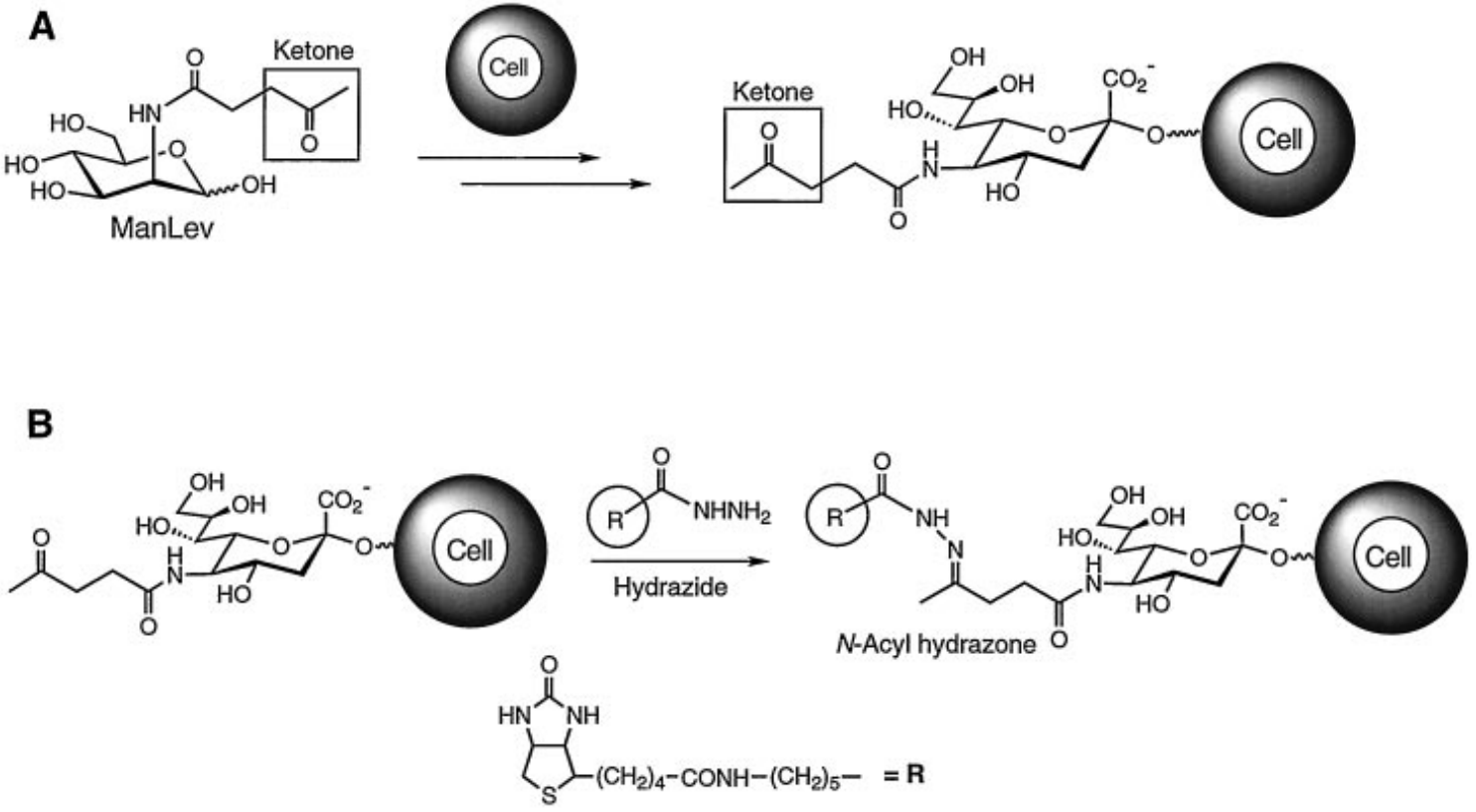
Hydrazone ligation



Oxime ligation

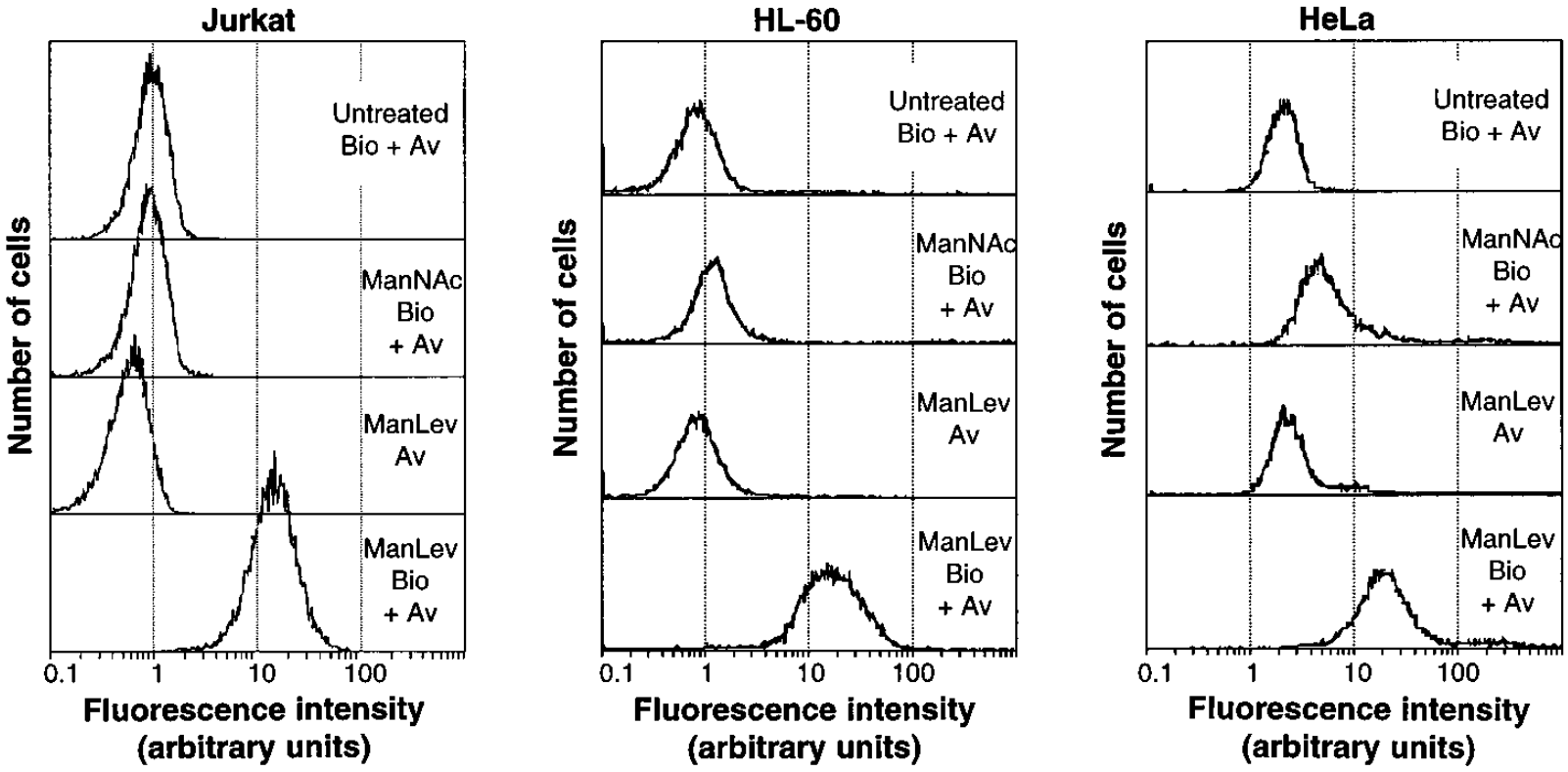


Ketone incorporation on cell surface



Engineering Chemical Reactivity on Cell Surfaces Through Oligosaccharide Biosynthesis
Lara K. Mahal, *et al.*
Science **276**, 1125 (1997);
DOI: 10.1126/science.276.5315.1125

Ketone incorporation on cell surface

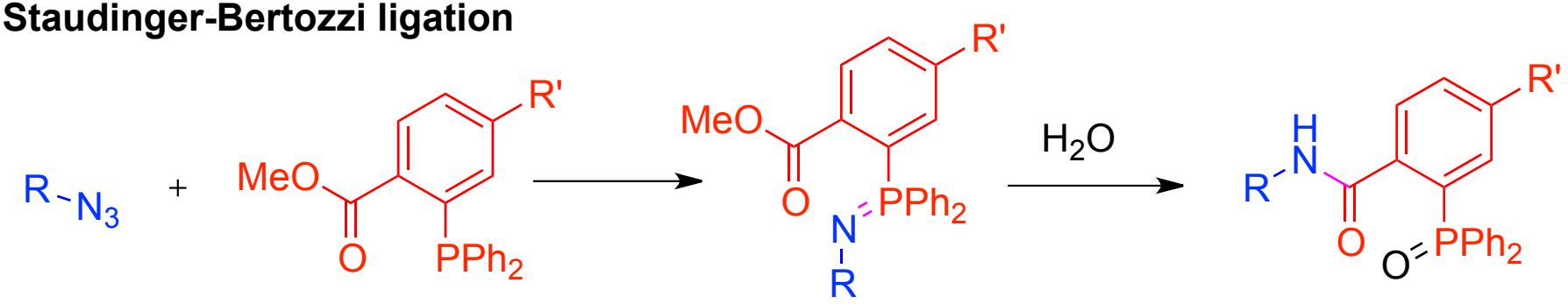


FITC-Avidin

Engineering Chemical Reactivity on Cell Surfaces Through Oligosaccharide Biosynthesis
Lara K. Mahal, *et al.*
Science **276**, 1125 (1997);
DOI: 10.1126/science.276.5315.1125

Bioorthogonal chemistry - Staudinger ligation

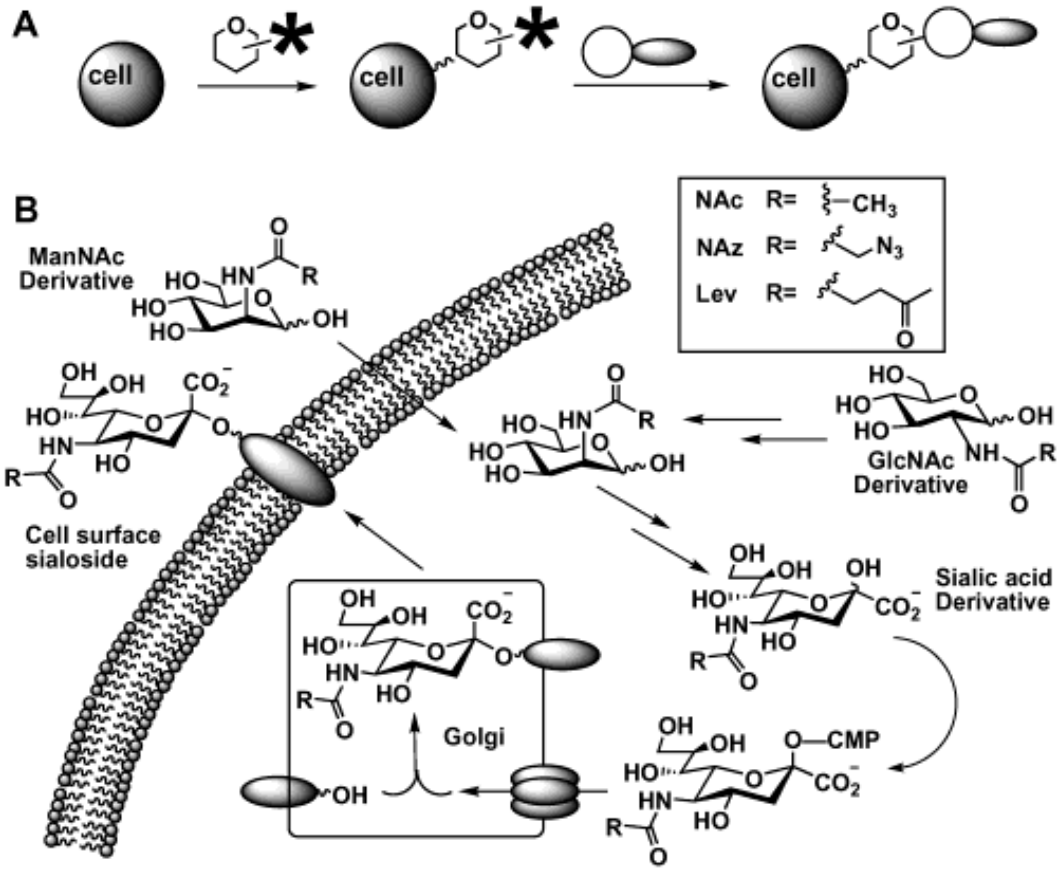
Staudinger-Bertozzi ligation



C. R. Bertozzi *et coll.*, *J. Am. Chem. Soc.*, **2005**, *127*, 2686-2695

Hermann Staudinger

Azides

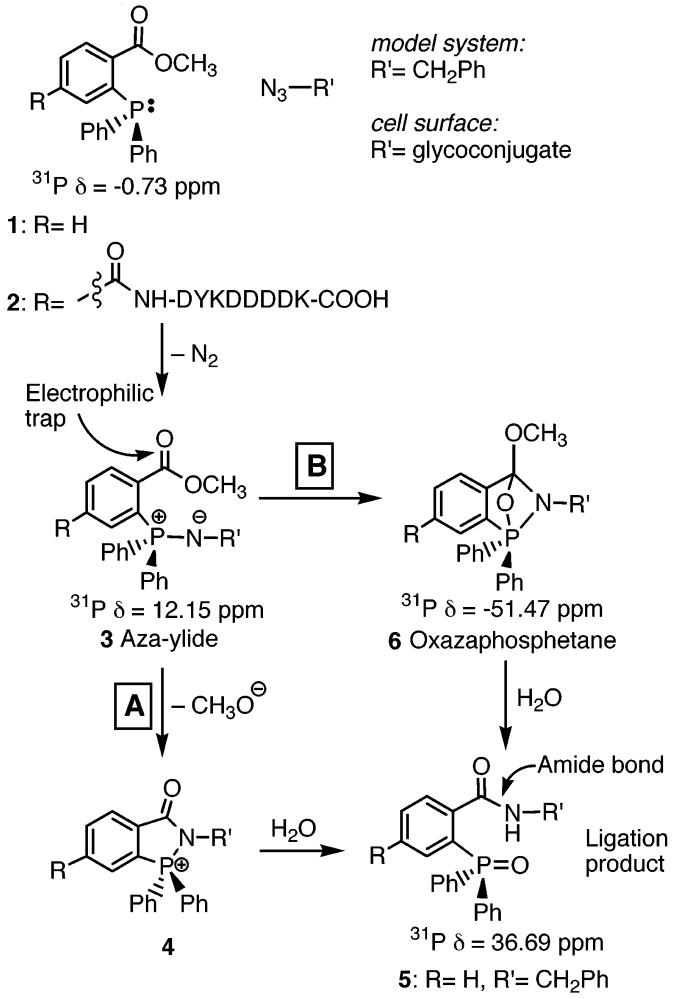


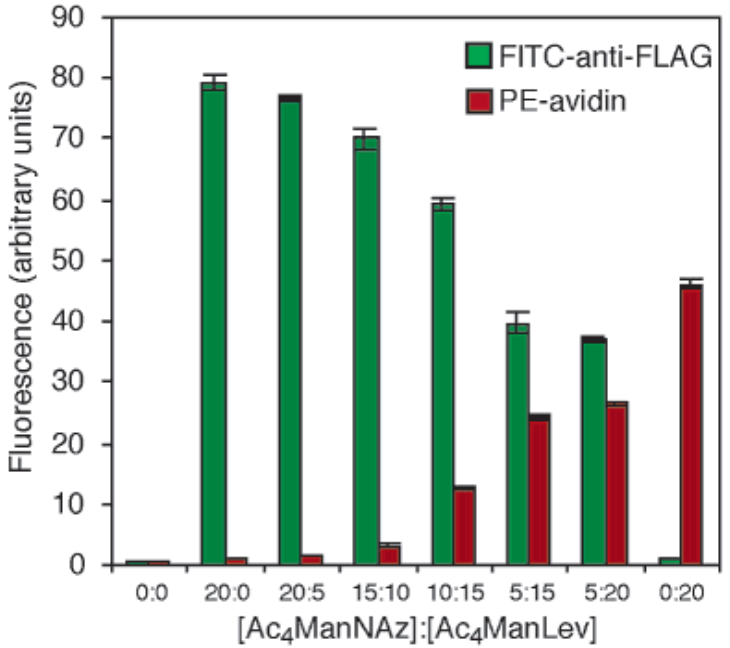
J|A|C|S
ARTICLES
Published on Web 11/23/2002

Investigating Cellular Metabolism of Synthetic Azidosugars with the Staudinger Ligation

Eliana Saxon,† Sarah J. Luchansky,† Howard C. Hang,† Chong Yu, Sandy C. Lee, and Carolyn R. Bertozzi*,†,‡

Staudinger ligation

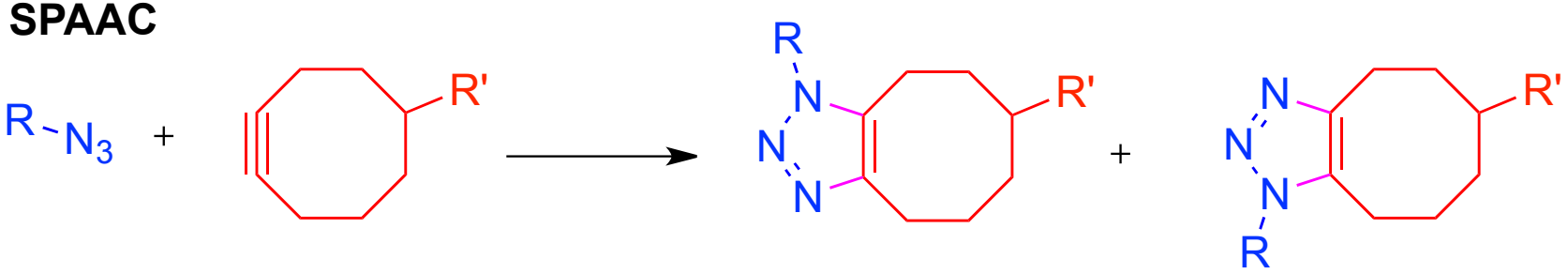




FLAG peptide, NH_2-DYKDDDDK-COOH
PE, phycoerythrin

Bioorthogonal chemistry - 2+3 Cycloadditions

SPAAC



Copper-free click chemistry

J|A|C|S COMMUNICATIONS

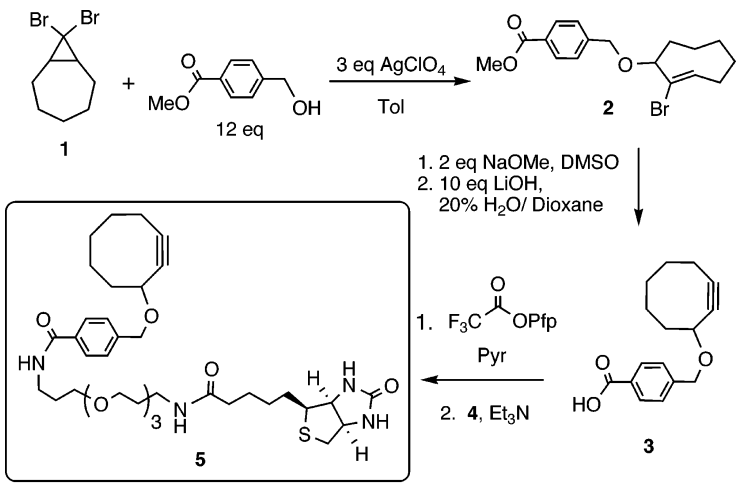
Published on Web 11/02/2004

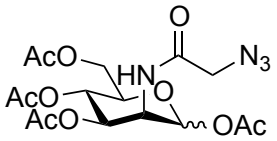
A Strain-Promoted [3 + 2] Azide - Alkyne Cycloaddition for Covalent Modification of Biomolecules in Living Systems

Nicholas J. Agard, Jennifer A. Prescher, and Carolyn R. Bertozzi*

Departments of Chemistry and Molecular and Cell Biology and Howard Hughes Medical Institute, University of California, and Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720

Received August 19, 2004; E-mail: crb@cchem.berkeley.edu





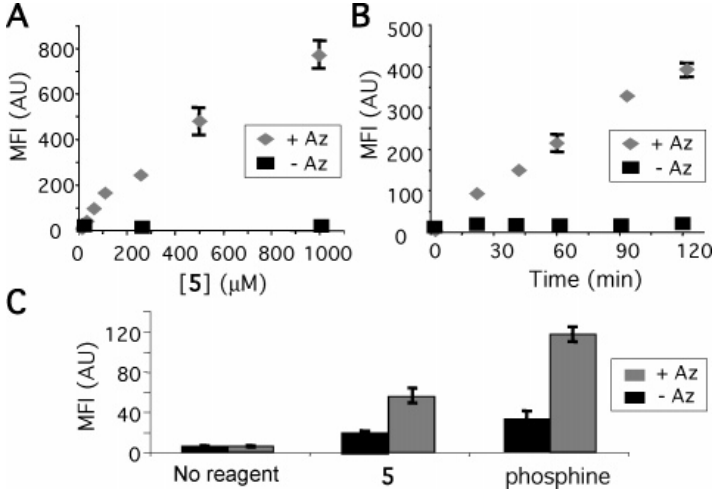
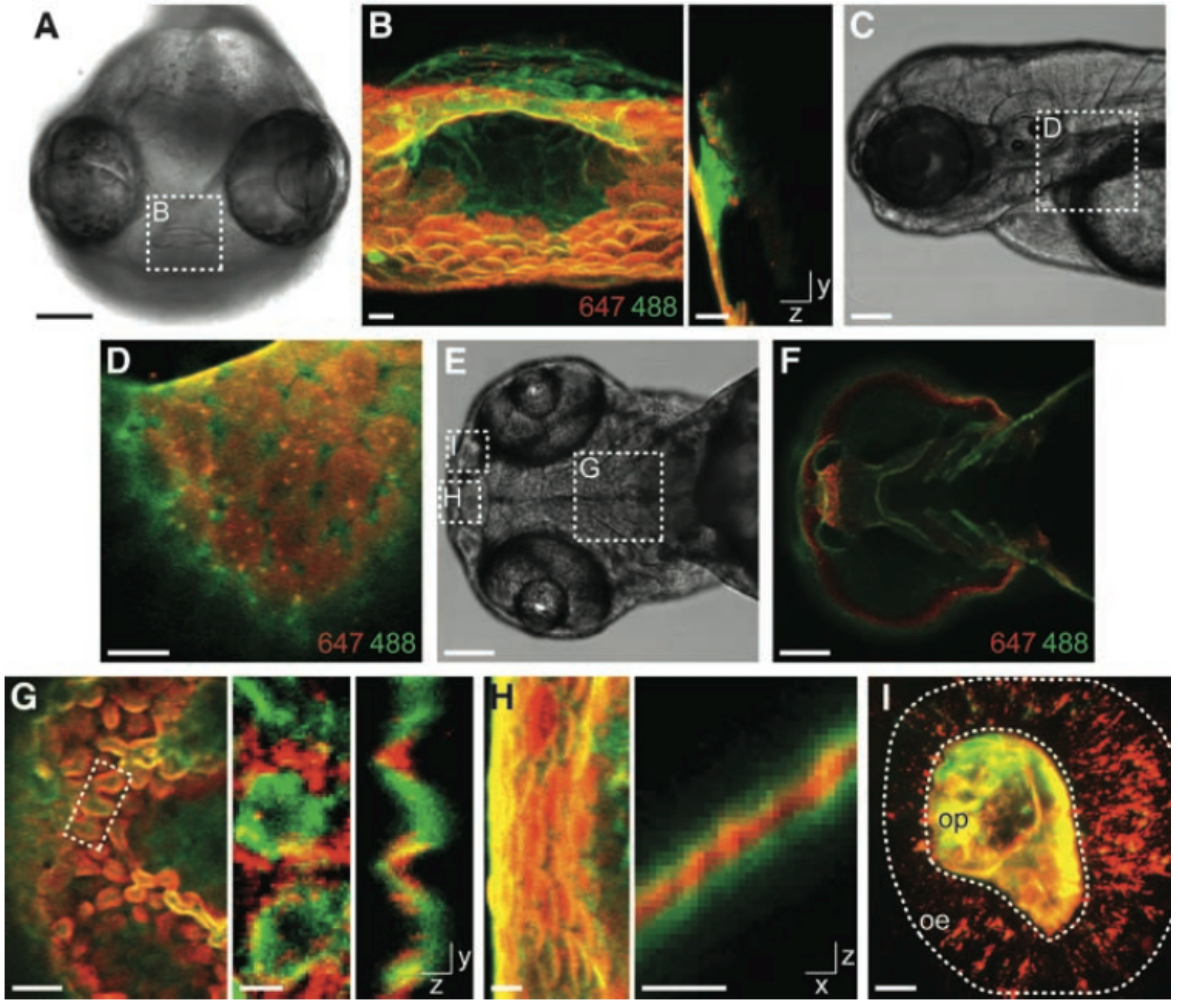


Figure 3. Cell-surface labeling with compound **5**. Jurkat cells were incubated in the presence (+ Az) or absence (- Az) of 25 μM $Ac_4ManNAz$ for 3 d. (A) The cells were reacted with various concentrations of **5** for 1 h at room temperature and treated with FITC - avidin; mean fluorescence intensity (MFI) was determined by flow cytometry. (B) Cells were incubated with 250 μM **5** at room temperature and analyzed as in A. (C) Cells were incubated with 100 μM probe for 1 h at room temperature and analyzed as in A. Error bars represent the standard deviation from three replicates. AU) arbitrary fluorescence units.

Zebrafish embryos

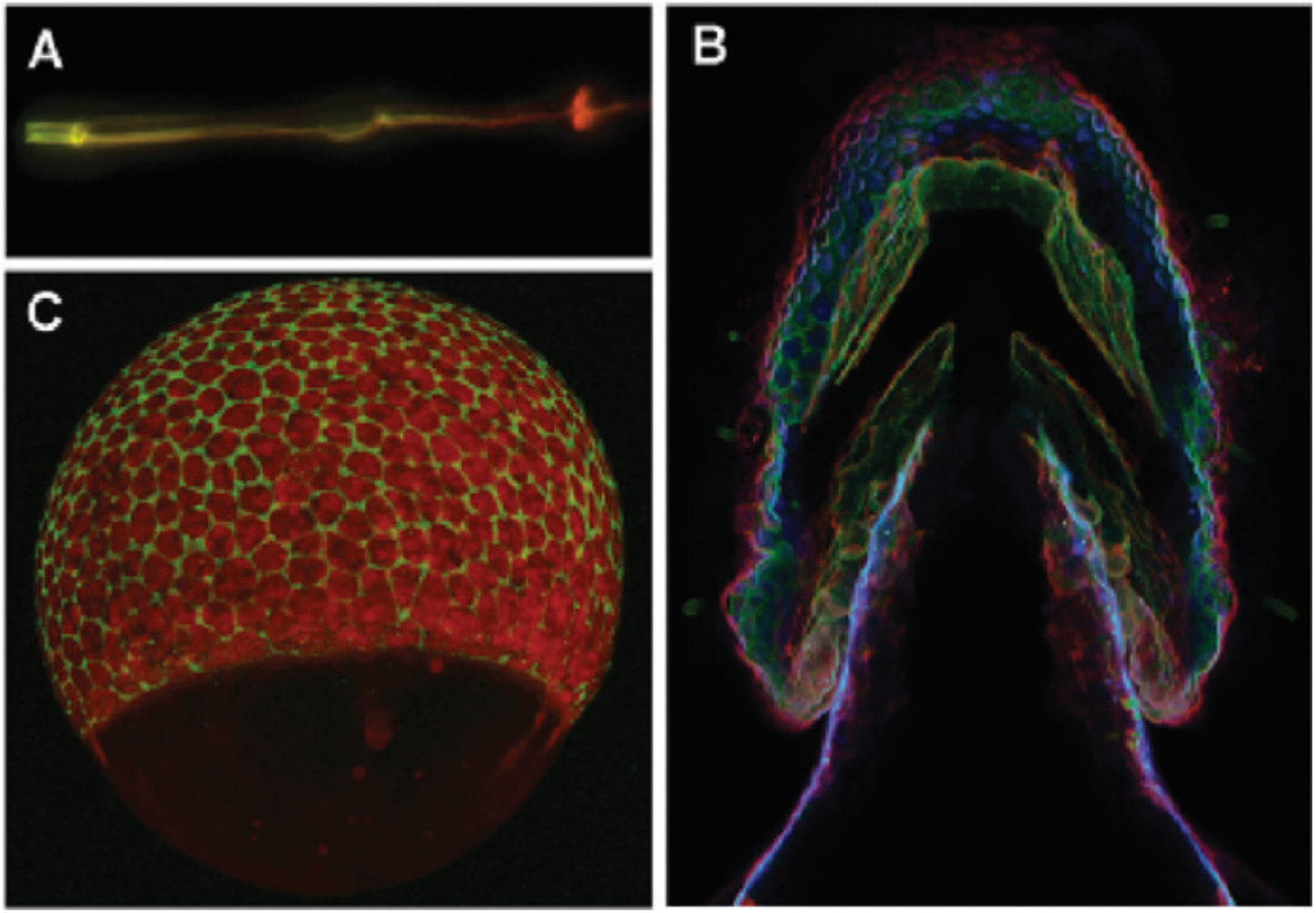
In Vivo Imaging of Membrane-Associated Glycans in Developing Zebrafish

Scott T. Laughlin,[1]* Jeremy M. Baskin,[1]* Sharon L. Amacher,[2] Carolyn R. Bertozzi[1,2,3,4]†



Science, 2008

C. elegans / Zebrafish

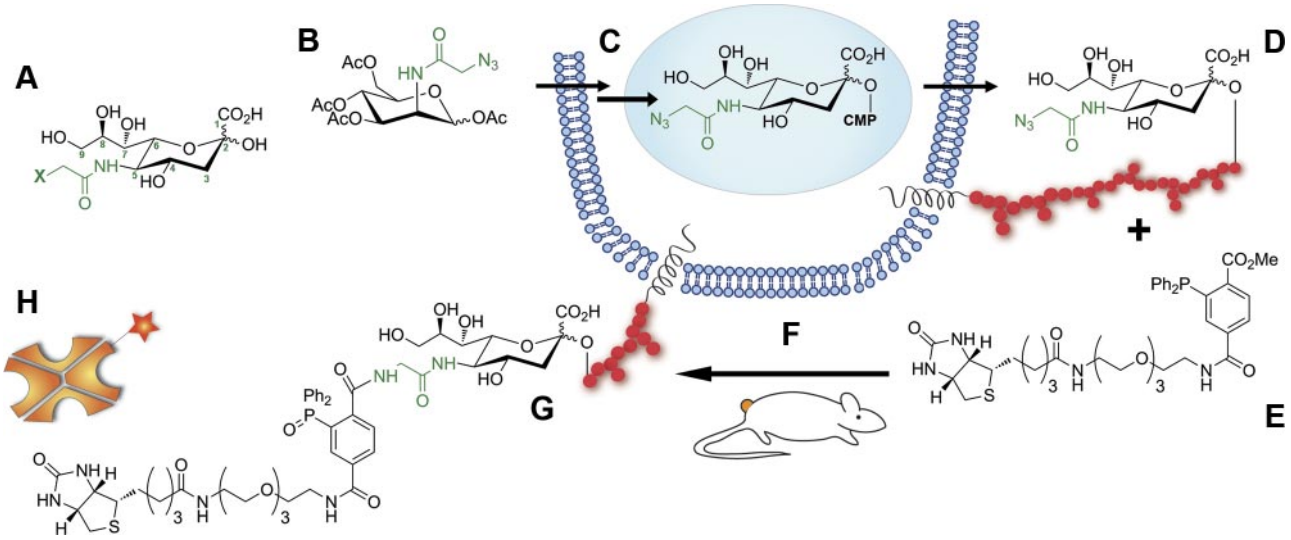


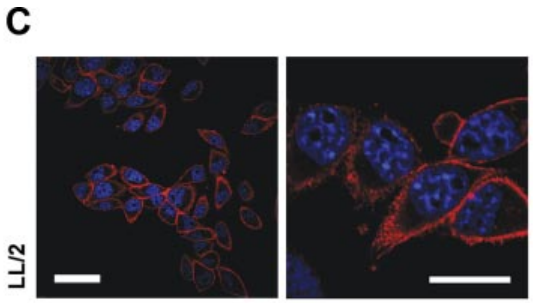
Sialylated tumor glycan imaging

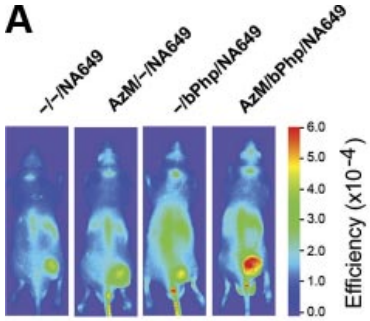
Imaging sialylated tumor cell glycans *in vivo*

André A. Neves,* Henning Stöckmann,*,‡ Rebecca R. Harmston,* Helen J. Pryor,* Israt S. Alam,* Heather Ireland-Zecchini,* David Y. Lewis,* Scott K. Lyons,* Finian J. Leeper,‡ and Kevin M. Brindle*,†,1

FASEB J **2011**, *25*, 2528-2537.





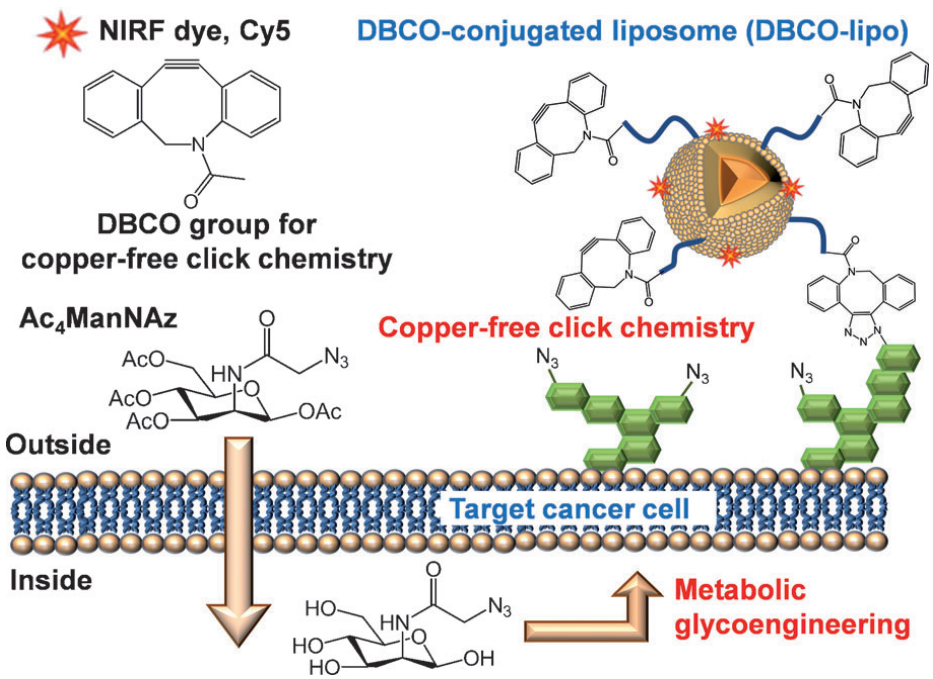


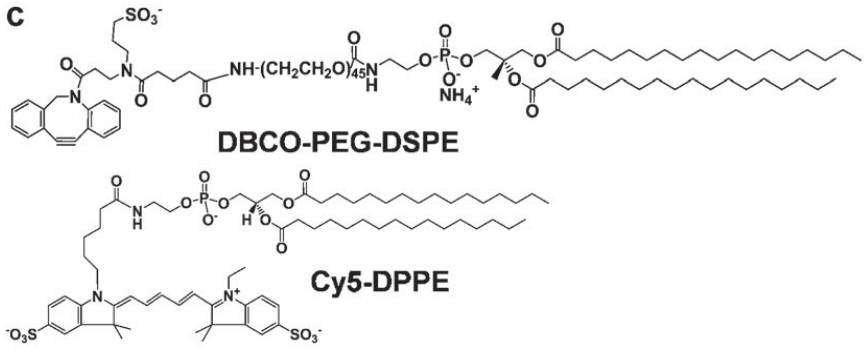
Tumor-targeting

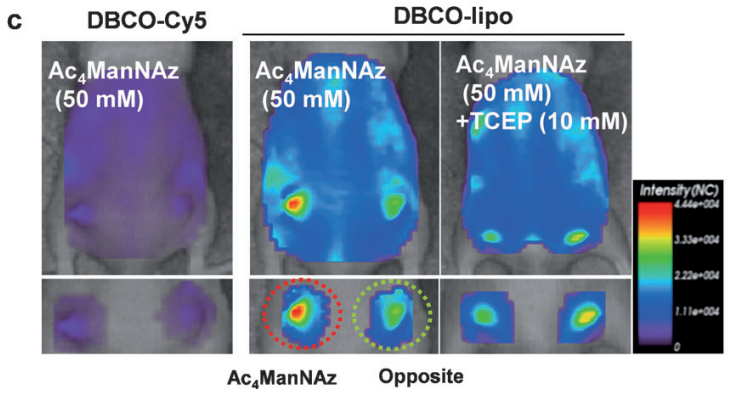
Bioorthogonal Copper-Free Click Chemistry In Vivo for Tumor-Targeted Delivery of Nanoparticles**

*Heebeom Koo, Sangmin Lee, Jin Hee Na, Sun Hwa Kim, Sei Kwang Hahn, Kuiwon Choi, Ick Chan Kwon, Seo Young Jeong, and Kwangmeyung Kim**

Angew. Chem. Int. Ed. **2012**, *51*, 1 – 6





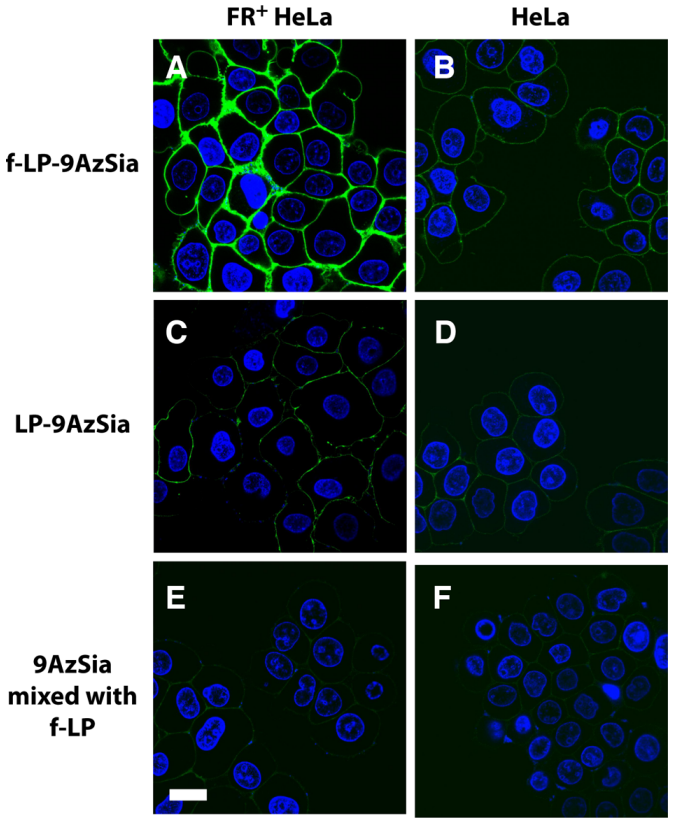


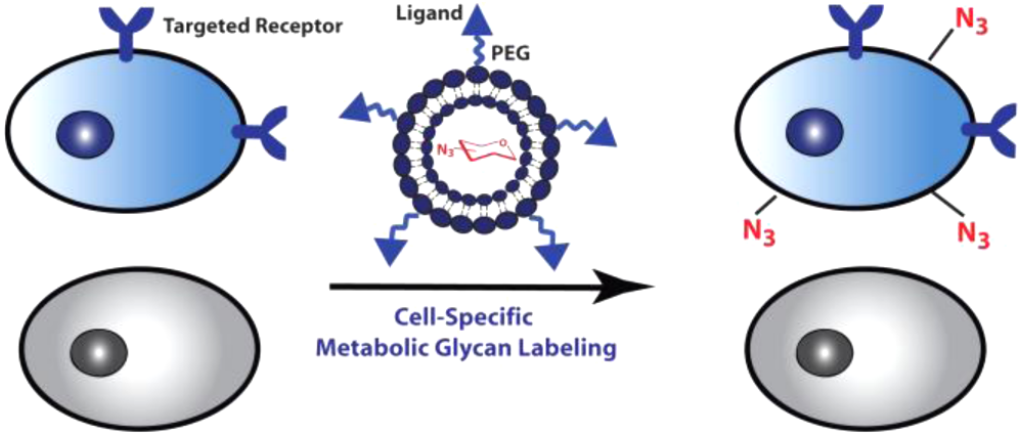
Cell-selective glycan labeling

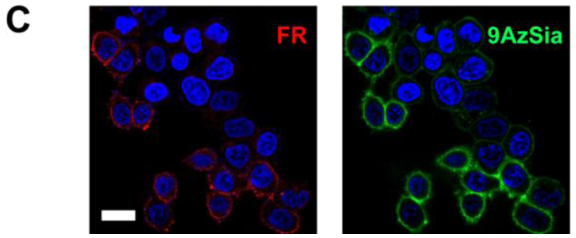
Cell-Selective Metabolic Glycan Labeling Based on Ligand-Targeted Liposomes

Ran Xie,[†,‡] Senlian Hong,[¶,‡] Lianshun Feng,[†] Jie Rong,[†] and Xing Chen*[,†,§,∥]

J. Am. Chem. Soc. 2012, 134, 9914–9917

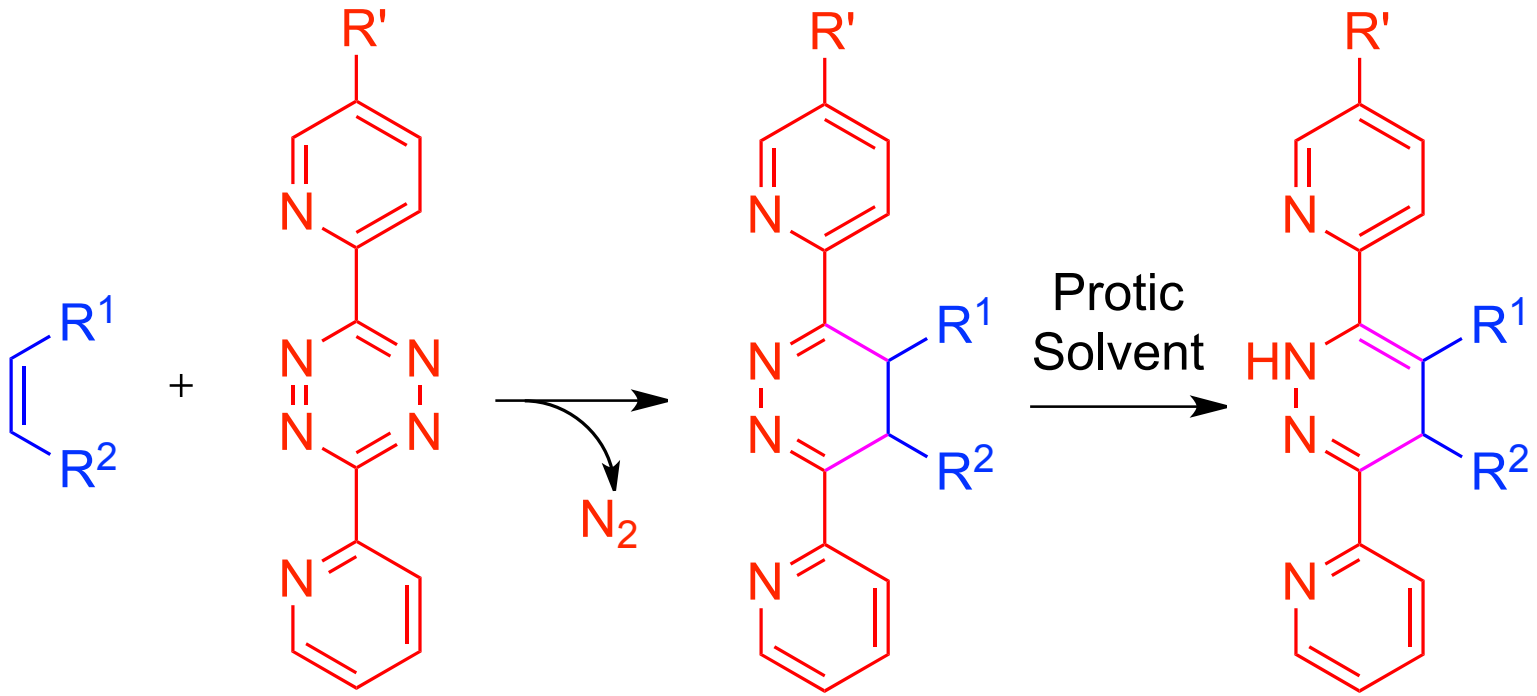




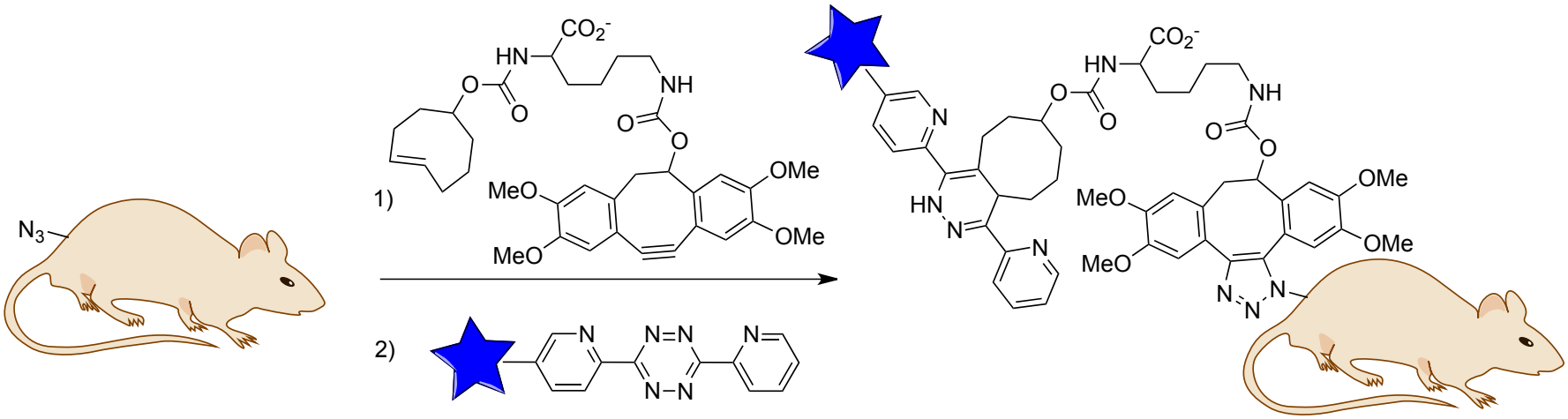


MCF-7 and FR+ HeLa

Bioorthogonal Chemistry -
Inverse electron demand Diels Alder



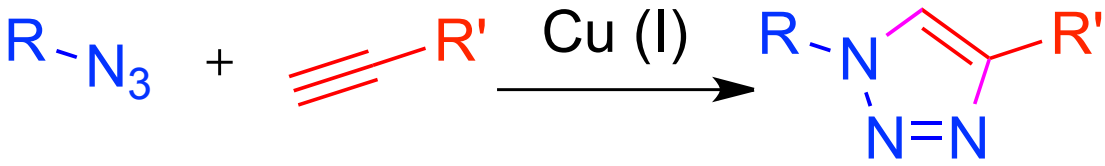
Sequential double click



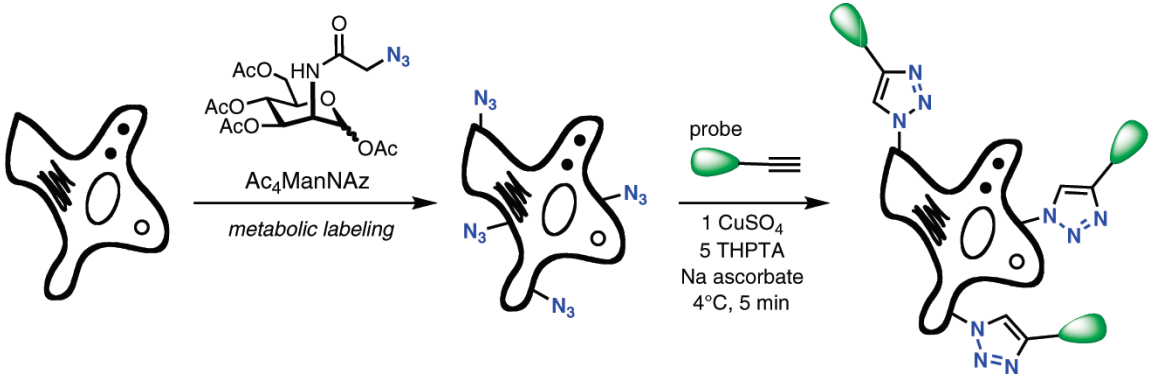
F. J. Leeper., K. M. Brindle *et coll.*, *Chem. Eur. J.*, **2013**, *24*, 934-941

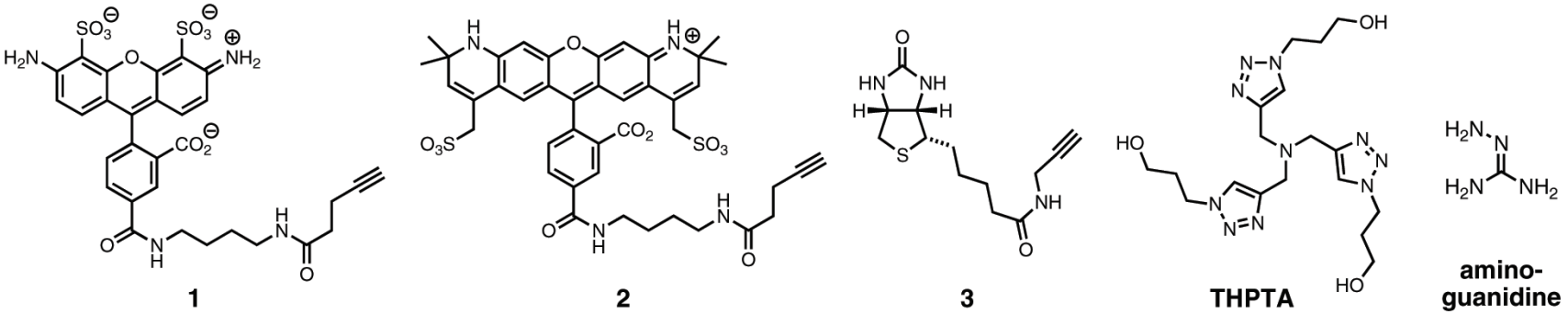
Bioorthogonal chemistry - 2+3 Cycloadditions

CuAAC



Copper-catalyzed click chemistry





Bioconjugate Chem. **2010,** 21, 1912–1916

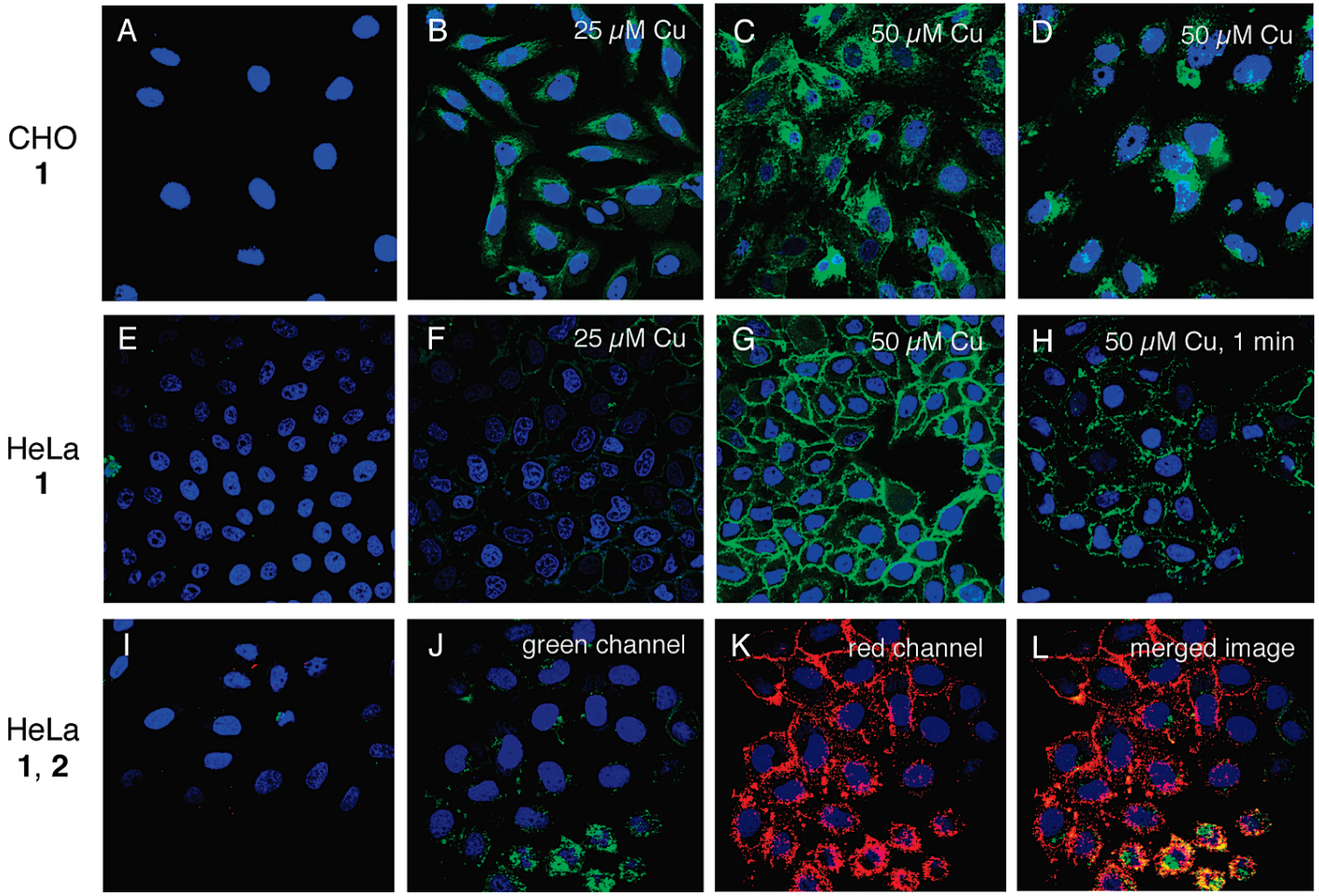
Labeling Live Cells by Copper-Catalyzed Alkyne - Azide Click Chemistry

Vu Hong,[§,†] Nicole F. Steinmetz,[§,‡] Marianne Manchester,[|] and M. G. Finn*[,†]

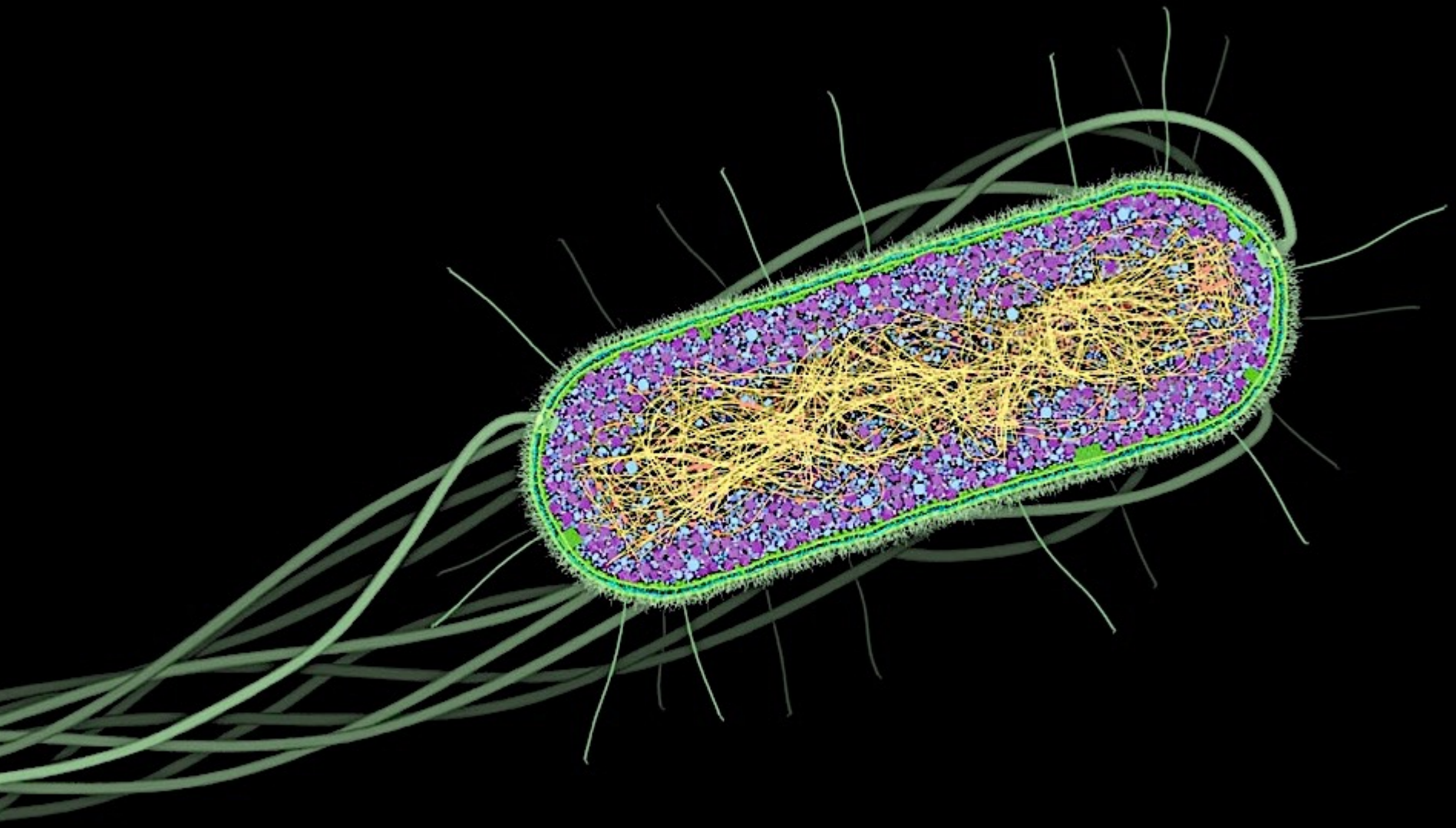
Department of Chemistry and The Skaggs Institute for Chemical Biology, Department of Cell Biology and Center for Integrative Molecular Biosciences, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, 9500 Gilman Drive, La Jolla, California.

Received June 17, 2010; Revised Manuscript Received August 24, 2010

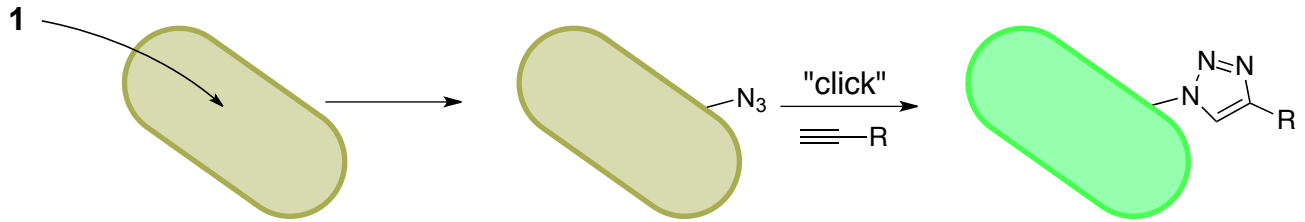
Copper-catalyzed click chemistry

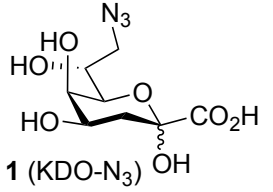


Metabolic Lipopolysaccharide Labeling



Click-chemistry





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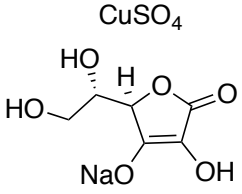
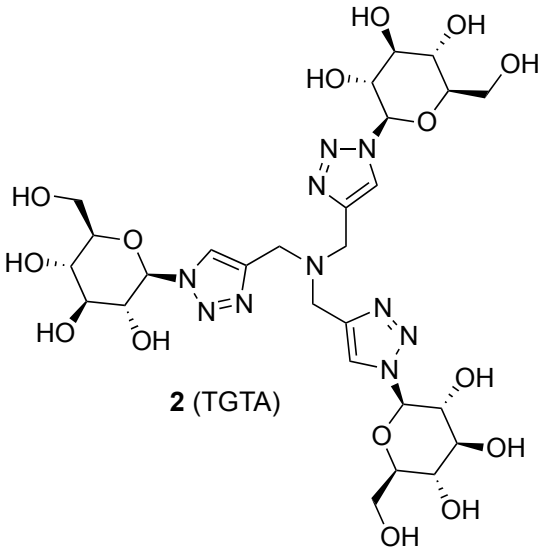
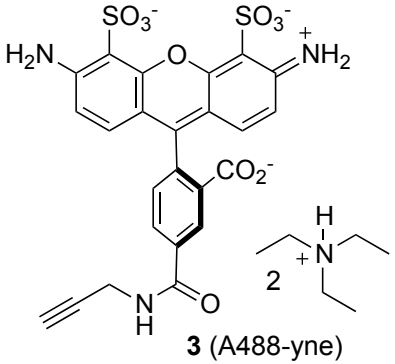


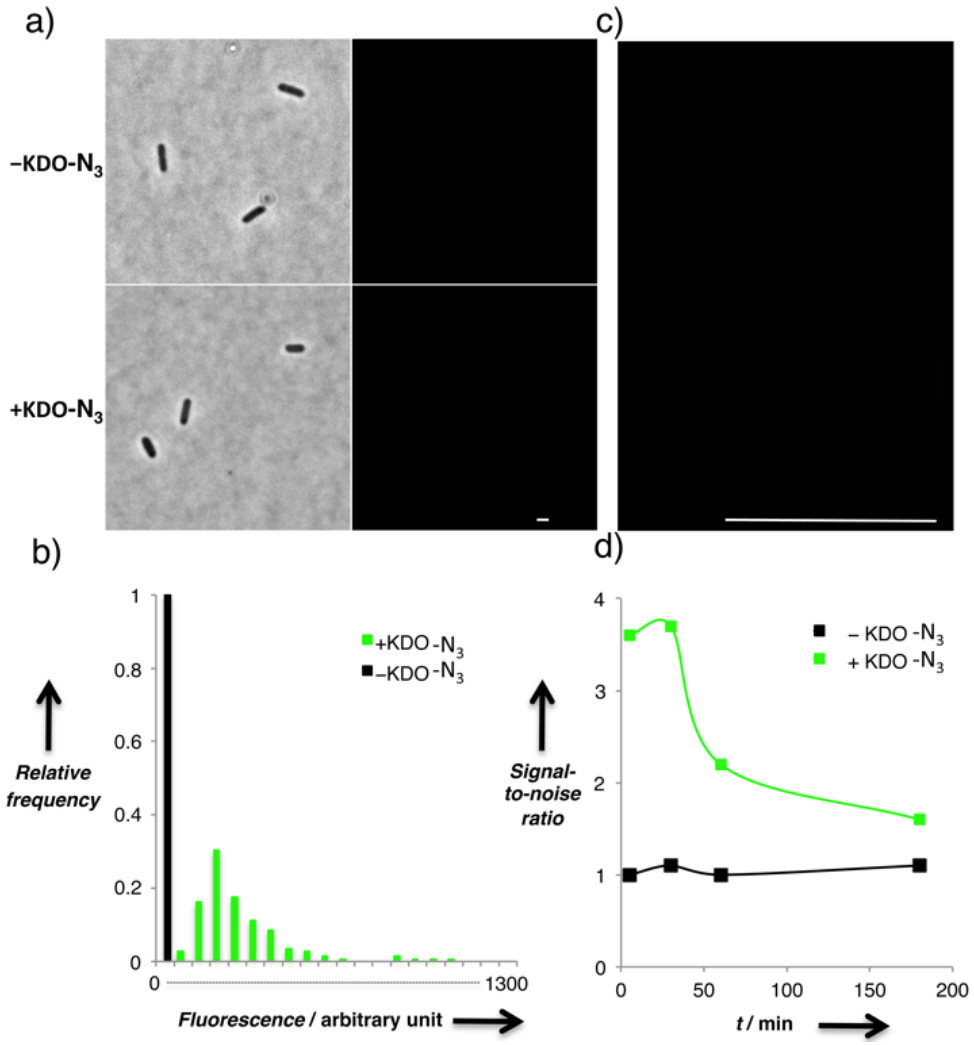


2 (TGTA)



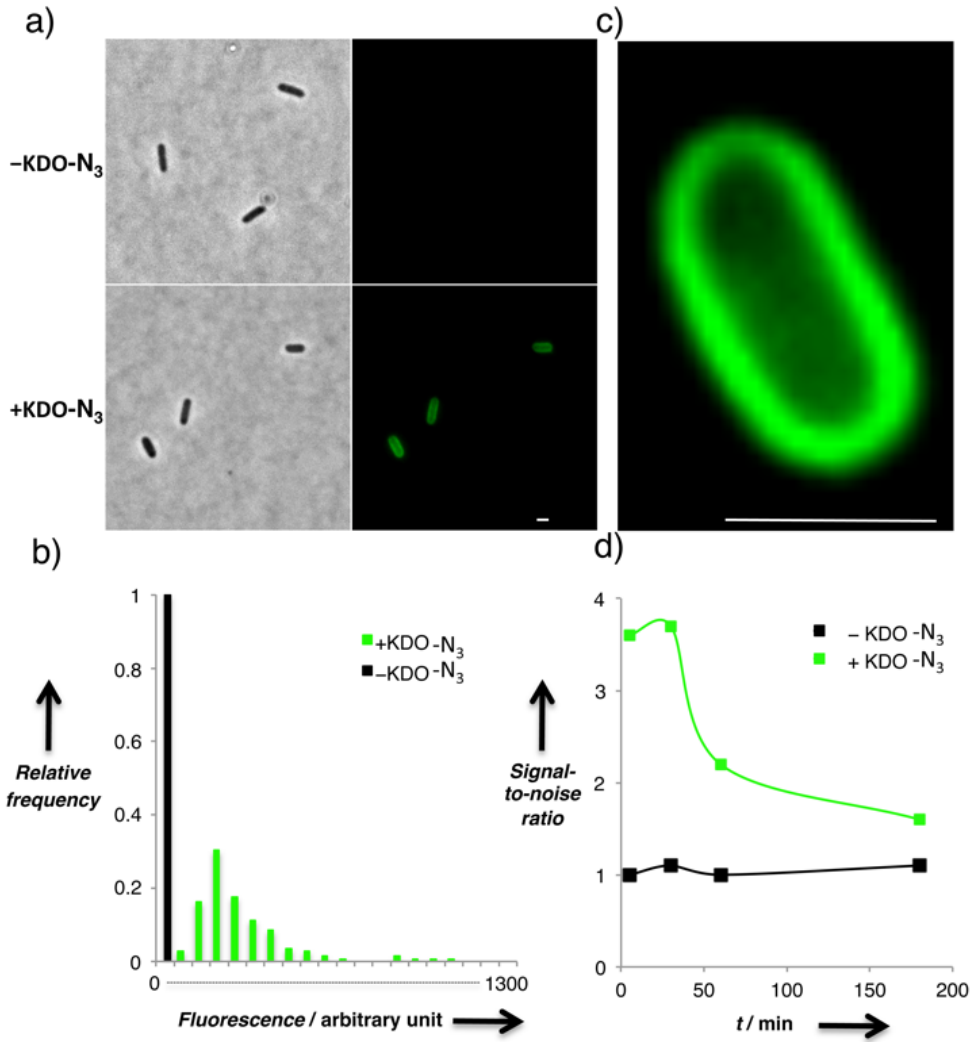
3 (A488-yne)

E. coli labeling



A. Dumont, A. Malleron, M. Awwad, S. Dukan*, B. Vauzeilles*, *Angew. Chem. Int. Ed.*, **2012**, *51*, 3143-3146

E. coli labeling

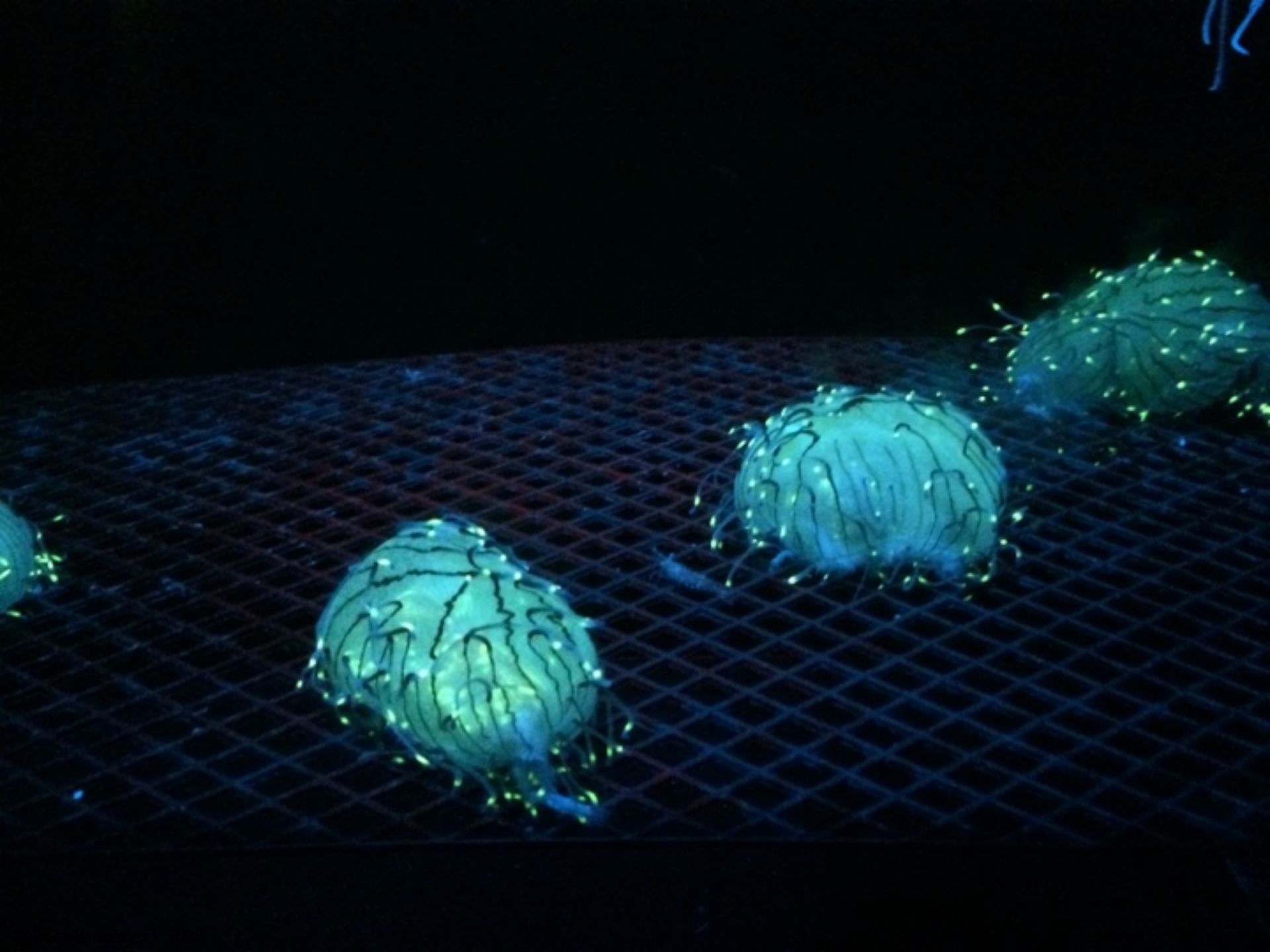


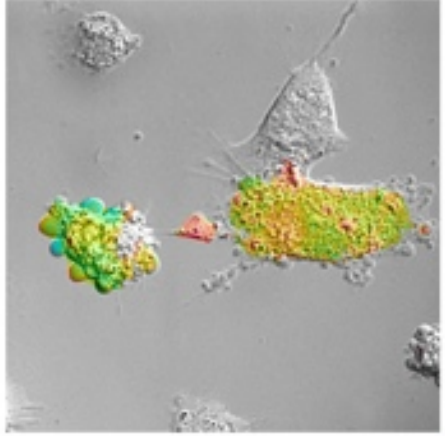
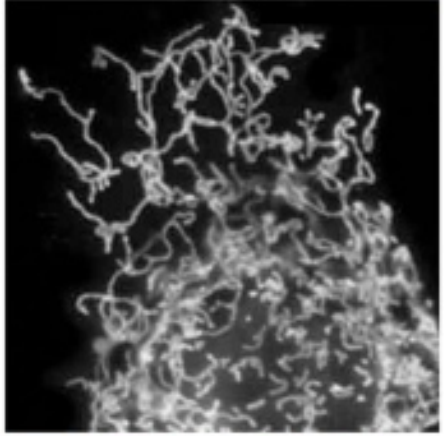
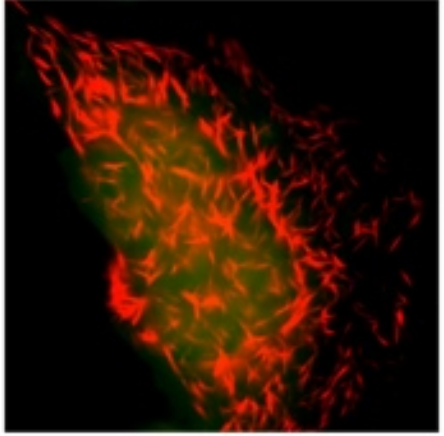
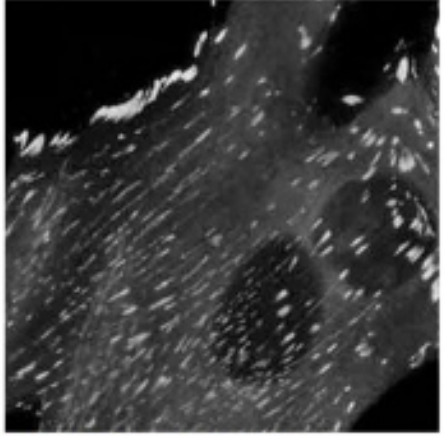
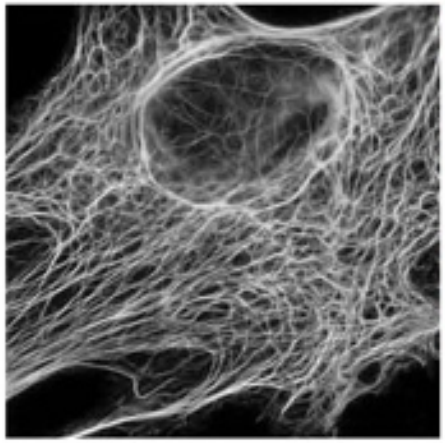
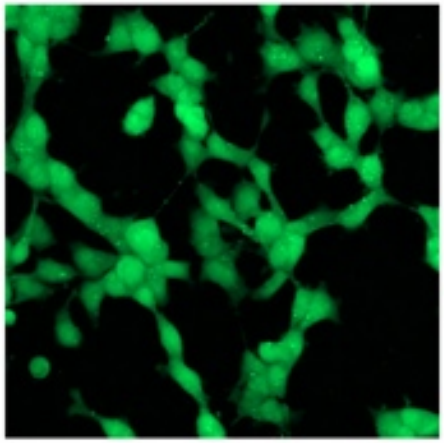
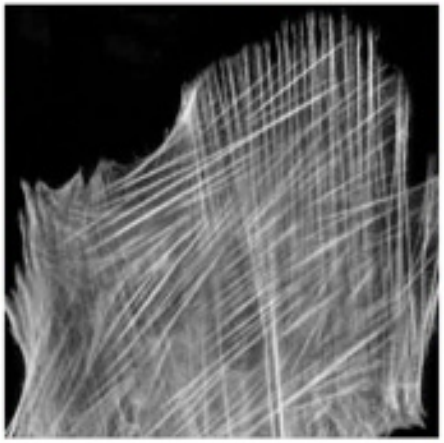
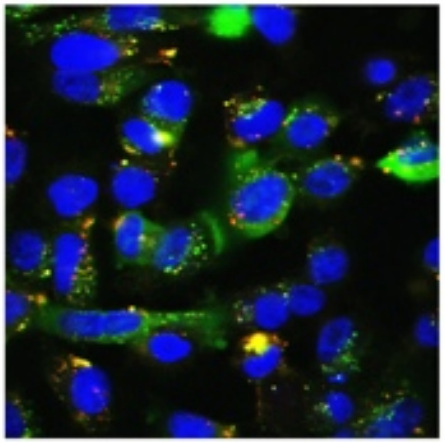
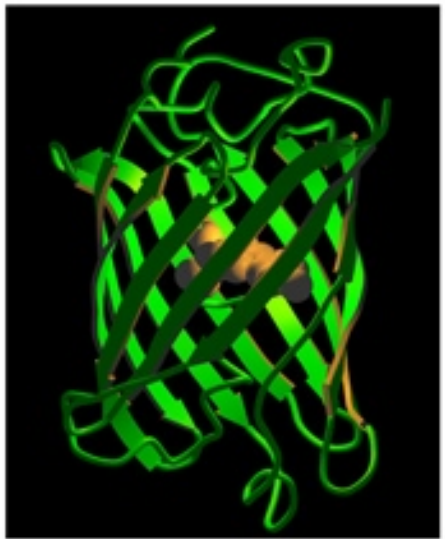
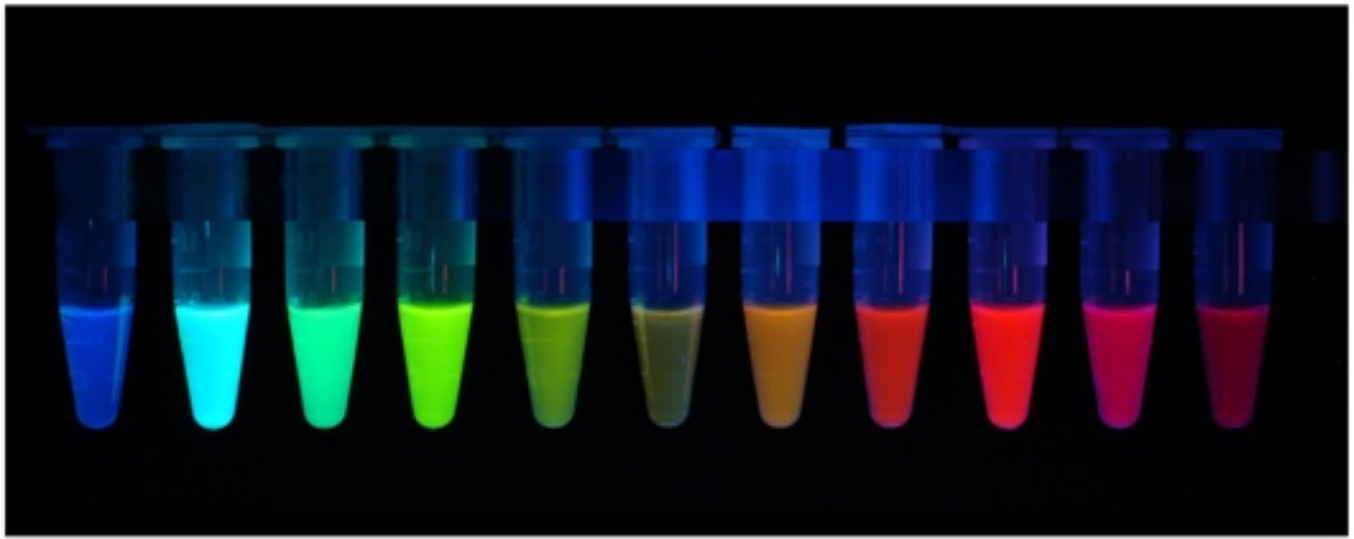
A. Dumont, A. Malleron, M. Awwad, S. Dukan*, B. Vauzeilles*, *Angew. Chem. Int. Ed.*, **2012**, *51*, 3143-3146

Labeling proteins *in vivo*

Fluorescent proteins

Roger Y. Tsien

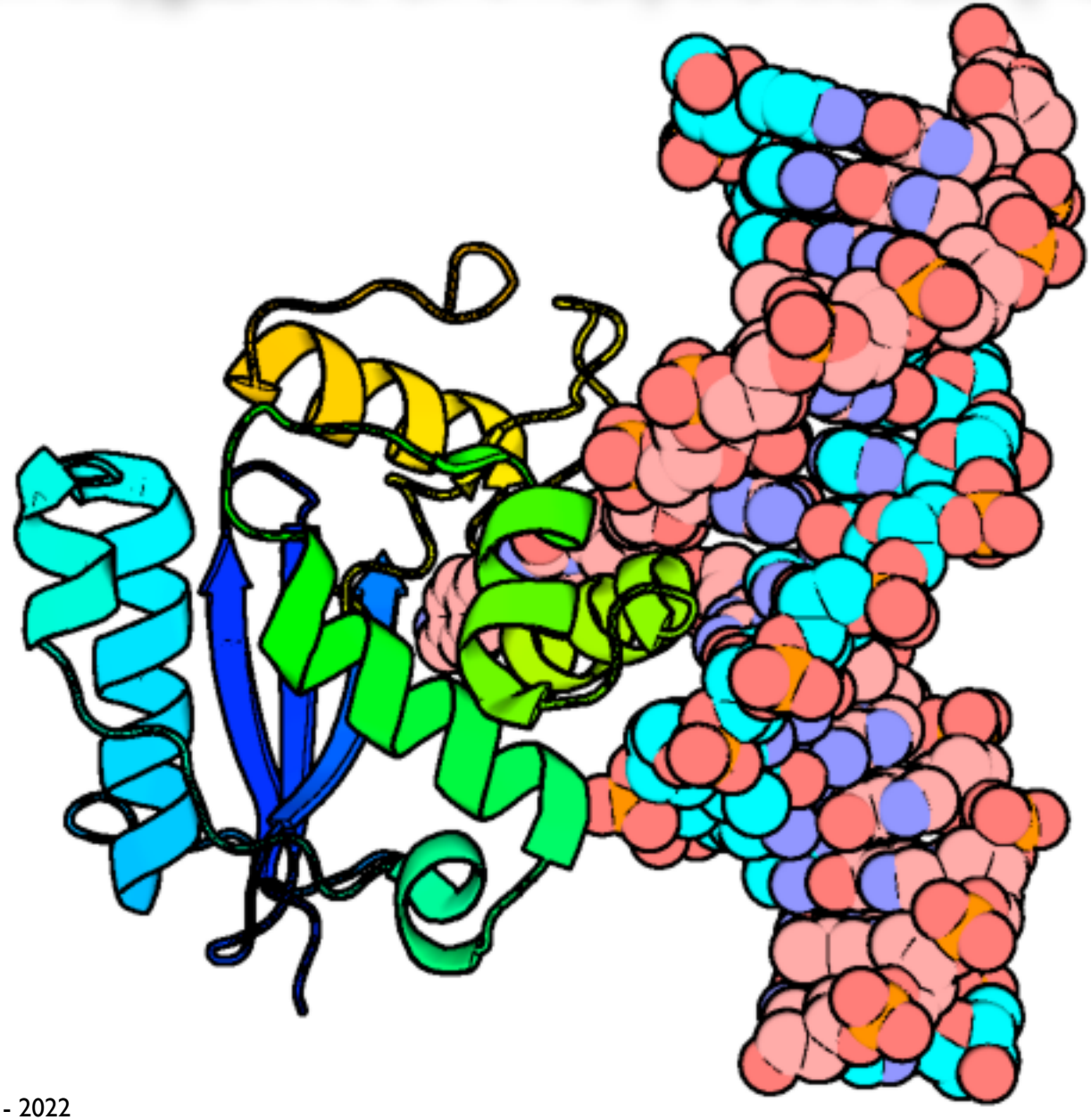


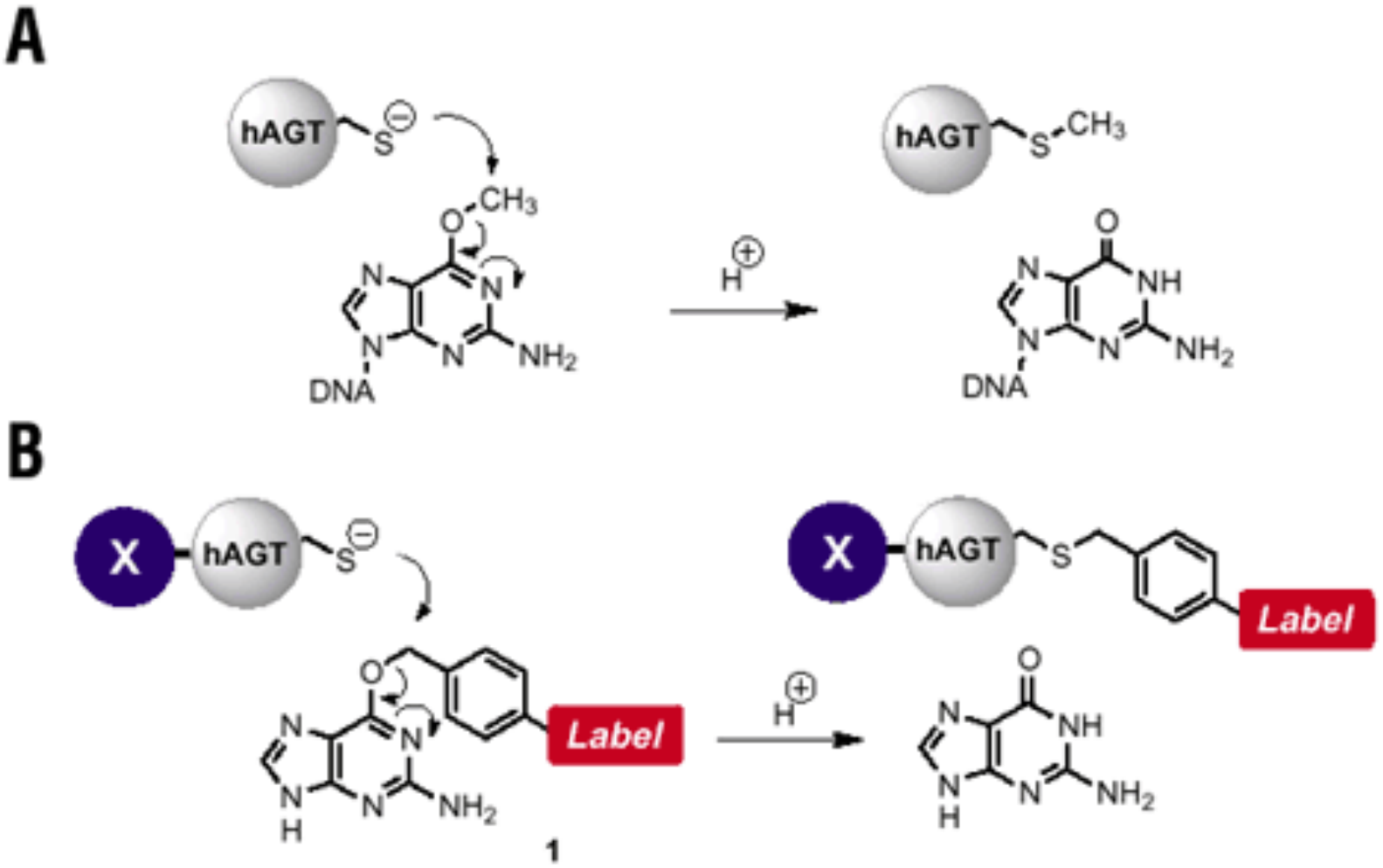
SNAP-Tag - CLIP-Tag

Kai Johnsson

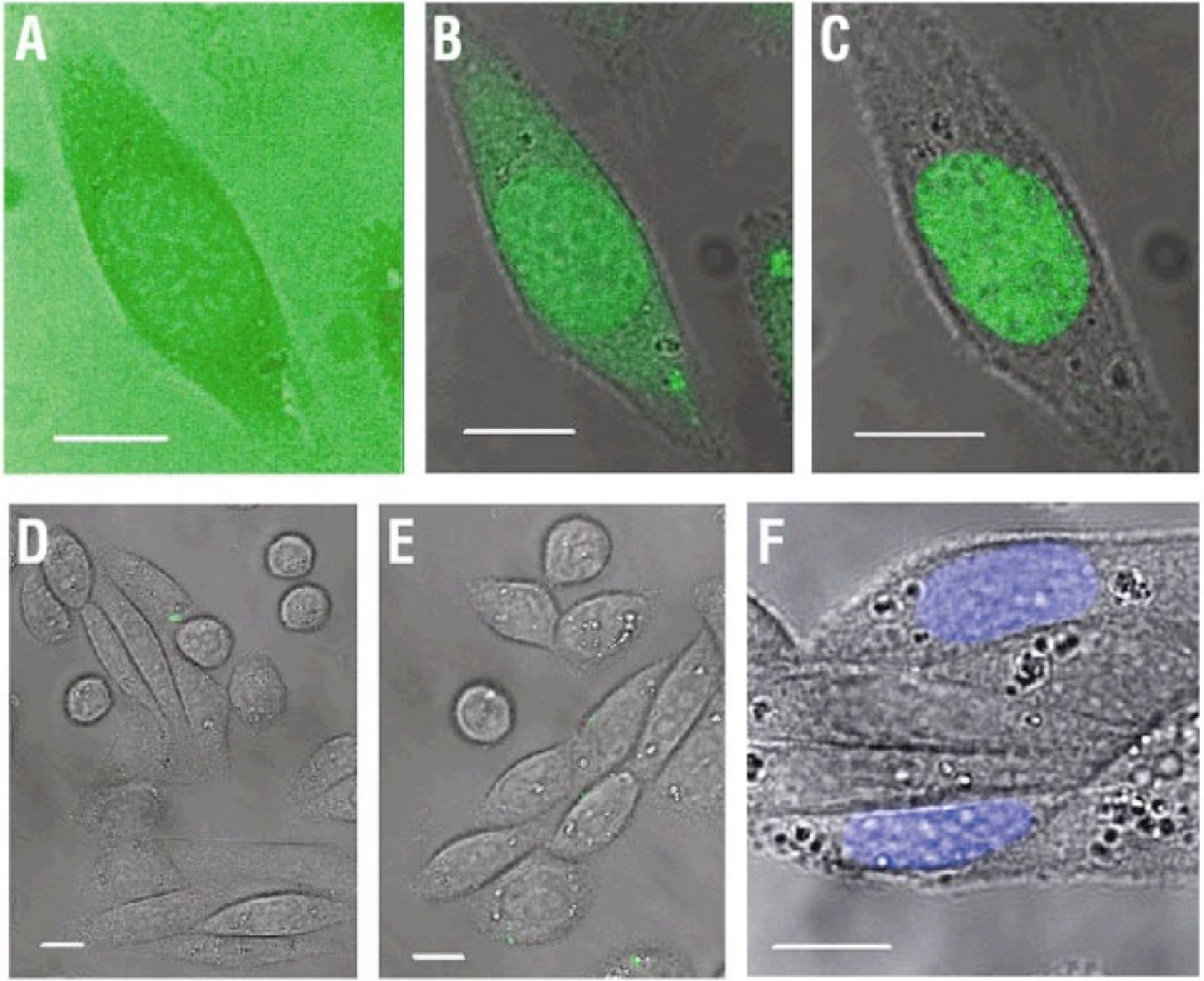
O6-Alklyguanine-DNA alkyltransferase (AGT)



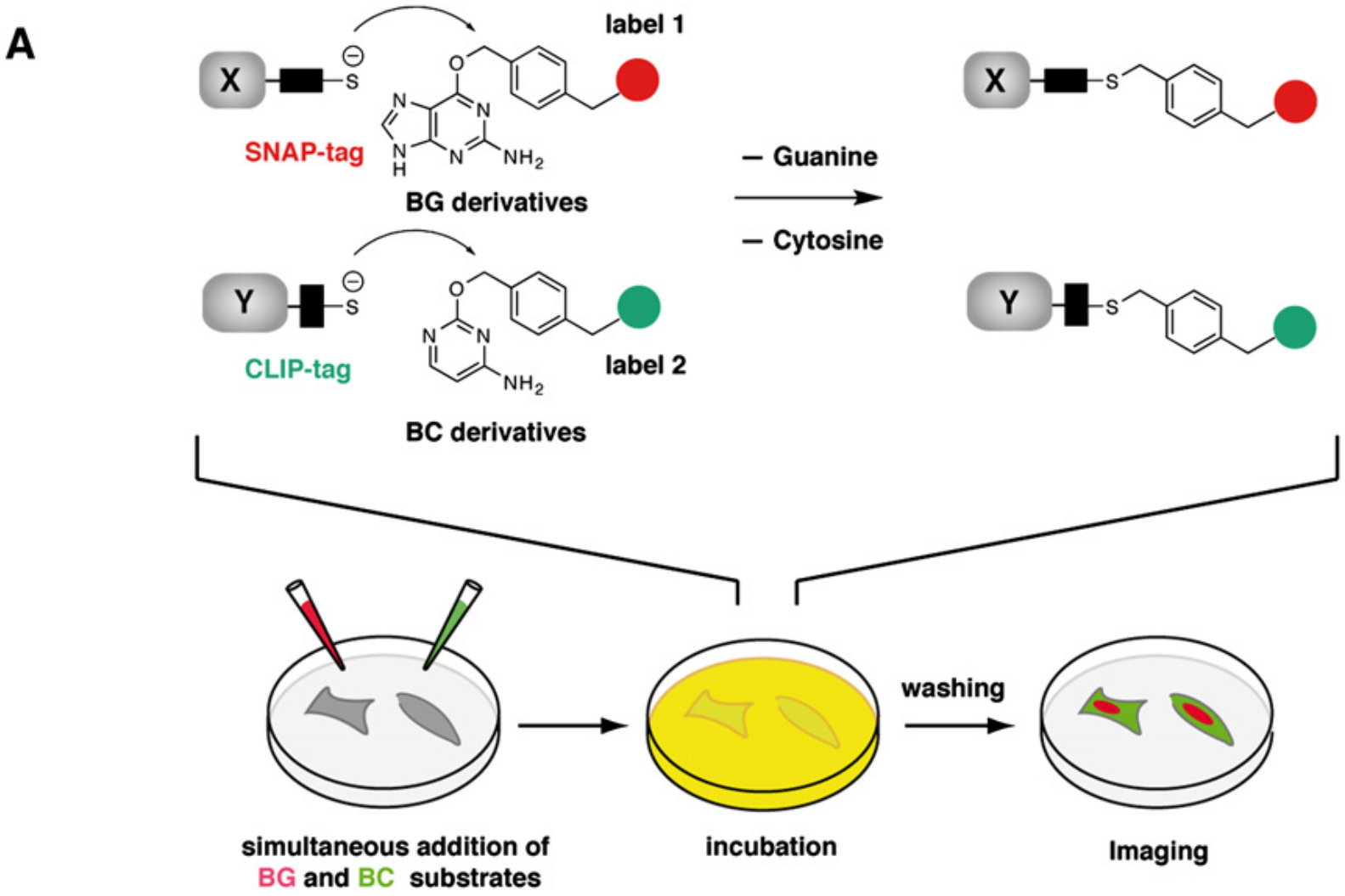
SNAP-Tag



K. Johnsson *et coll., Nat. Biotechnol.*, **2003**, *21*, 86-89

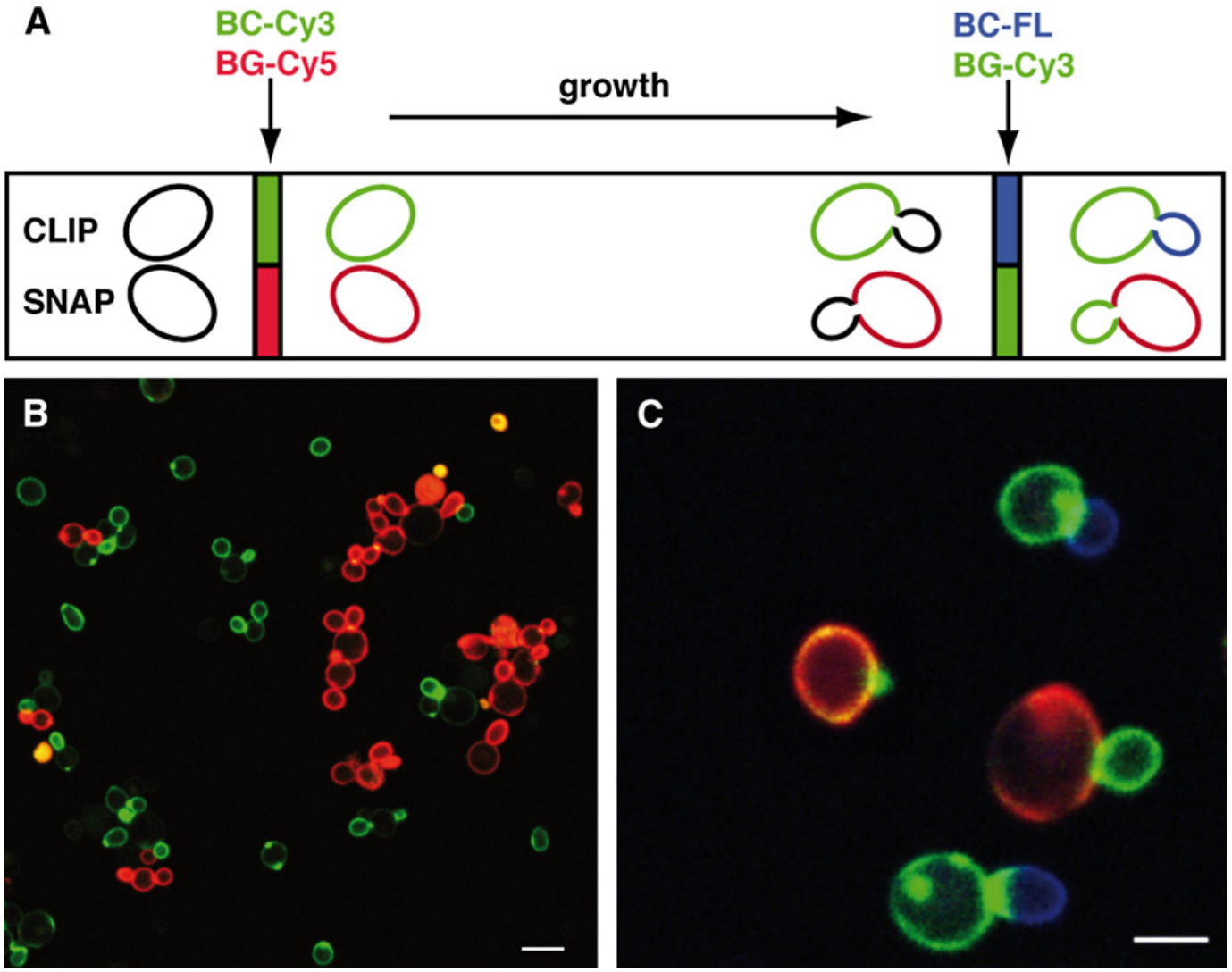

A
B
C
D
E
F

CLIP-Tag



A. Gautier, K. Johnsson *et coll., Chem. Biol.*, **2008**, *15*, 128-136

Yeast labeling



A. Gautier, K. Johnsson *et coll., Chem. Biol.*, **2008**, *15*, 128-136

Expanding the genetic code

Peter G. Schultz

Genetic code... RNA

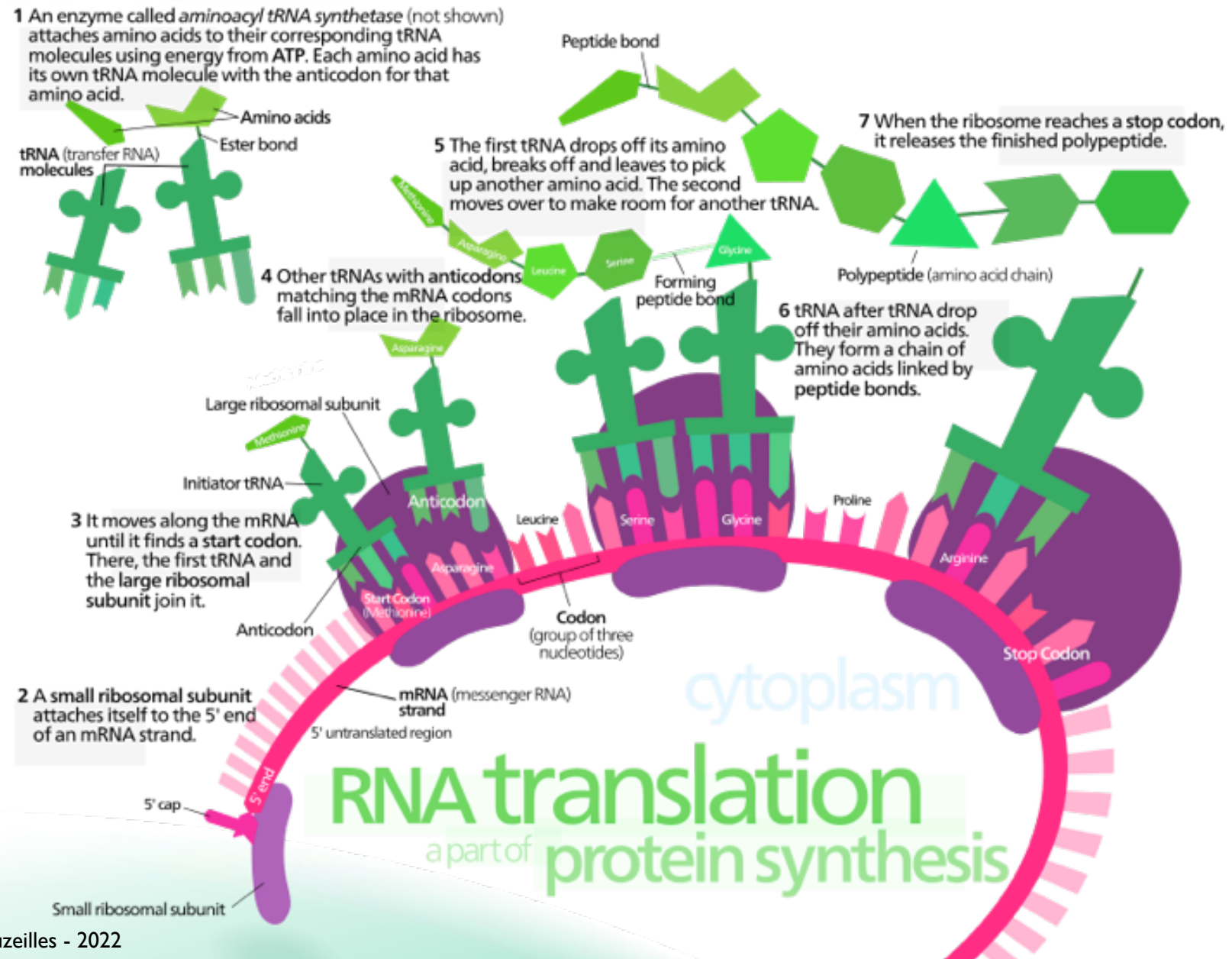
nonpolar | polar | basic | acidic | (stop codon)

Standard genetic code

1st base	2nd base								3rd base
	U		C		A		G		
U	UUU	(Phe/F) Phenylalanine	UCU	(Ser/S) Serine	UAU	(Tyr/Y) Tyrosine	UGU	(Cys/C) Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	(Leu/L) Leucine	UCA		UAA	Stop (*Ochre*)	UGA	Stop (*Opal*)	A
	UUG		UCG		UAG	Stop (*Amber*)	UGG	(Trp/W) Tryptophan	G
C	CUU		CCU	(Pro/P) Proline	CAU	(His/H) Histidine	CGU	(Arg/R) Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	(Ile/I) Isoleucine	ACU	(Thr/T) Threonine	AAU	(Asn/N) Asparagine	AGU	(Ser/S) Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	AUG[A]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GUU	(Val/V) Valine	GCU	(Ala/A) Alanine	GAU	(Asp/D) Aspartic acid	GGU	(Gly/G) Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GUG		GCG		GAG		GGG		G

https://en.wikipedia.org/wiki/Translation_(biology)

RNA Translation



Expanding the genetic code

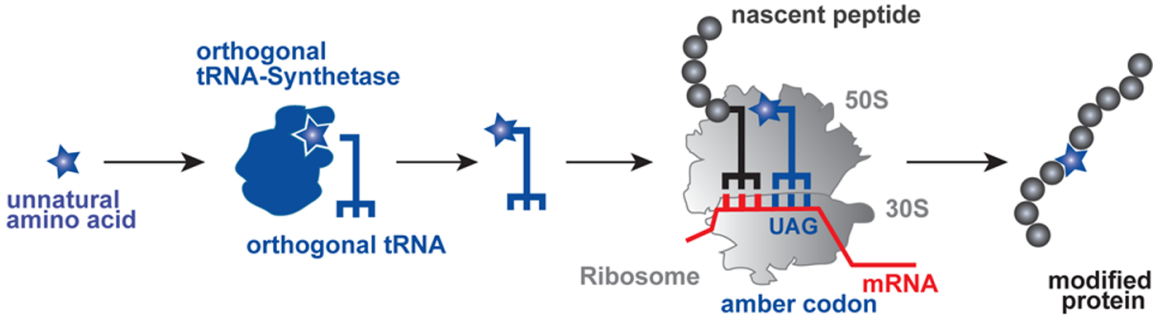


Figure 16. Expanding the genetic code. An unnatural amino acid (blue star), added to the cell growth medium, is specifically recognized by an orthogonal aminoacyl tRNA synthetase and attached to an orthogonal amber suppressor tRNA, which is decoded by the ribosome in response to an amber codon (UAG) introduced into the gene of interest, allowing the synthesis of a protein with a site-specifically introduced unnatural amino acid.

Peter-G. Schulz *et coll., Methods.*, **2005**, *36*, 227-238
Jason Chin *et coll., Chem. Rev.*, **2014**, 114, 4764-4806

Non natural amino acids...

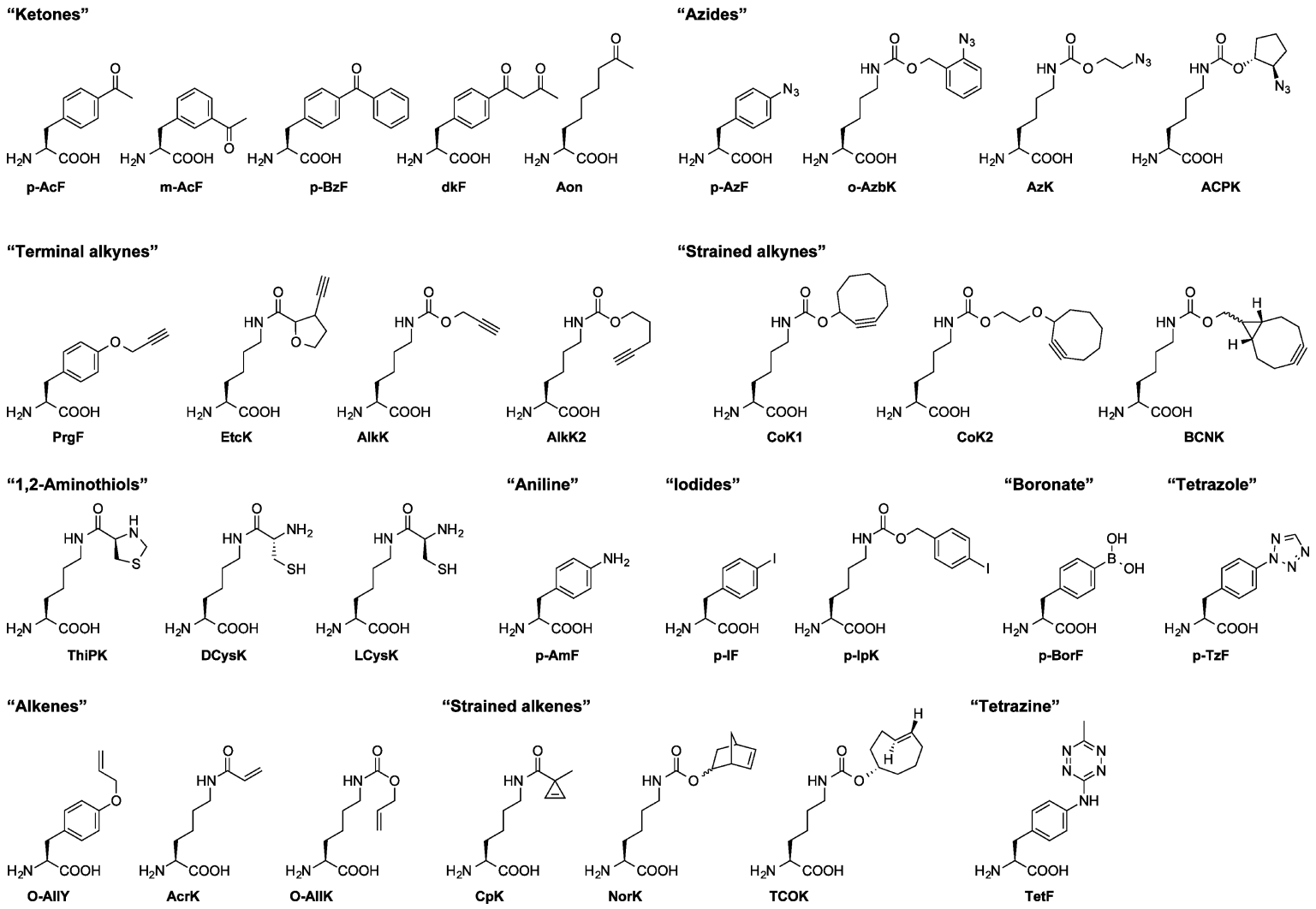
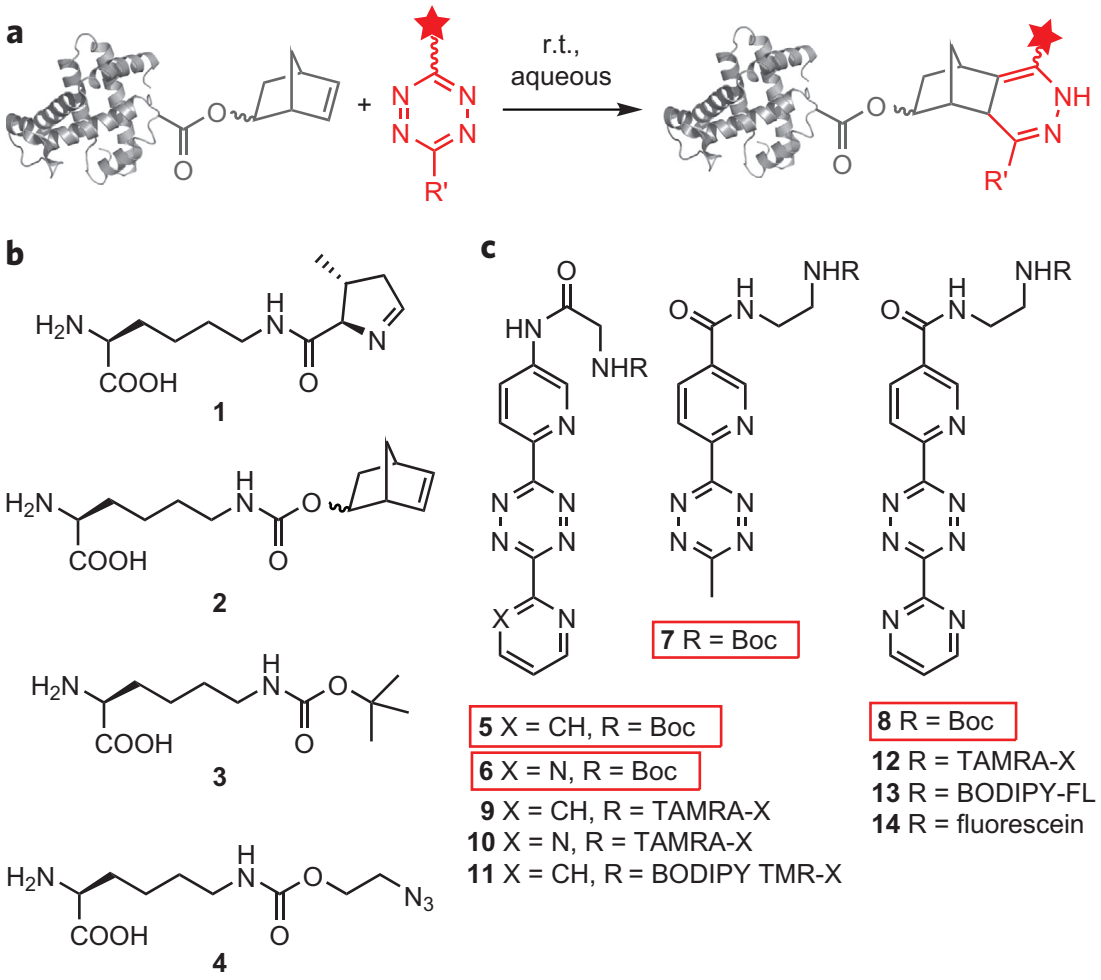


Figure 34. Structural formulas of unnatural amino acid useful for chemoselective labeling that have been incorporated site-specifically into proteins via genetic code expansion.

Jason Chin *et coll., Chem. Rev.*, **2014**, 114, 4764-4806

Jason Chin

Non-natural amino acids...



Jason Chin *et coll.*, *Nature Chem.*, **2012**, *4*, 298-304

Non natural amino acids...

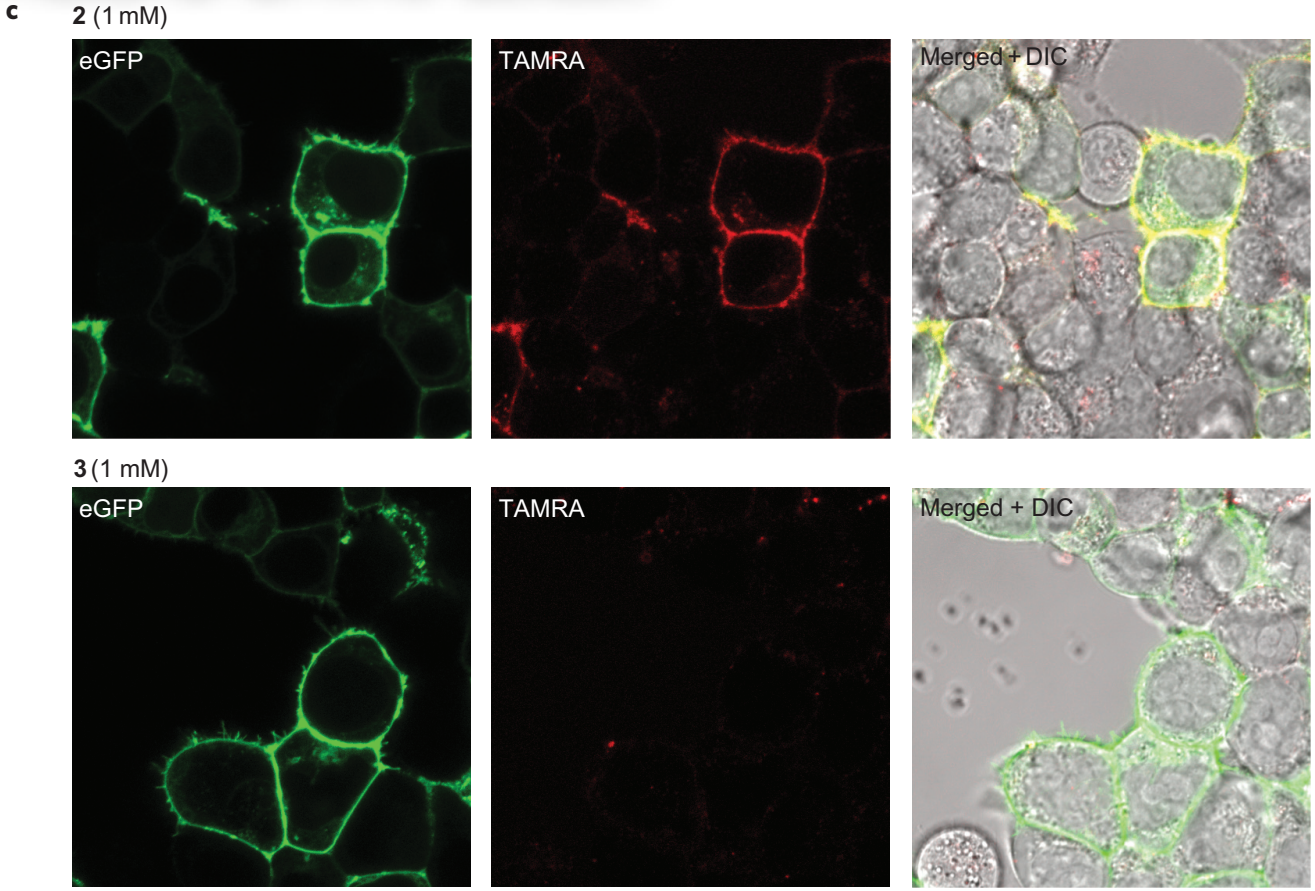
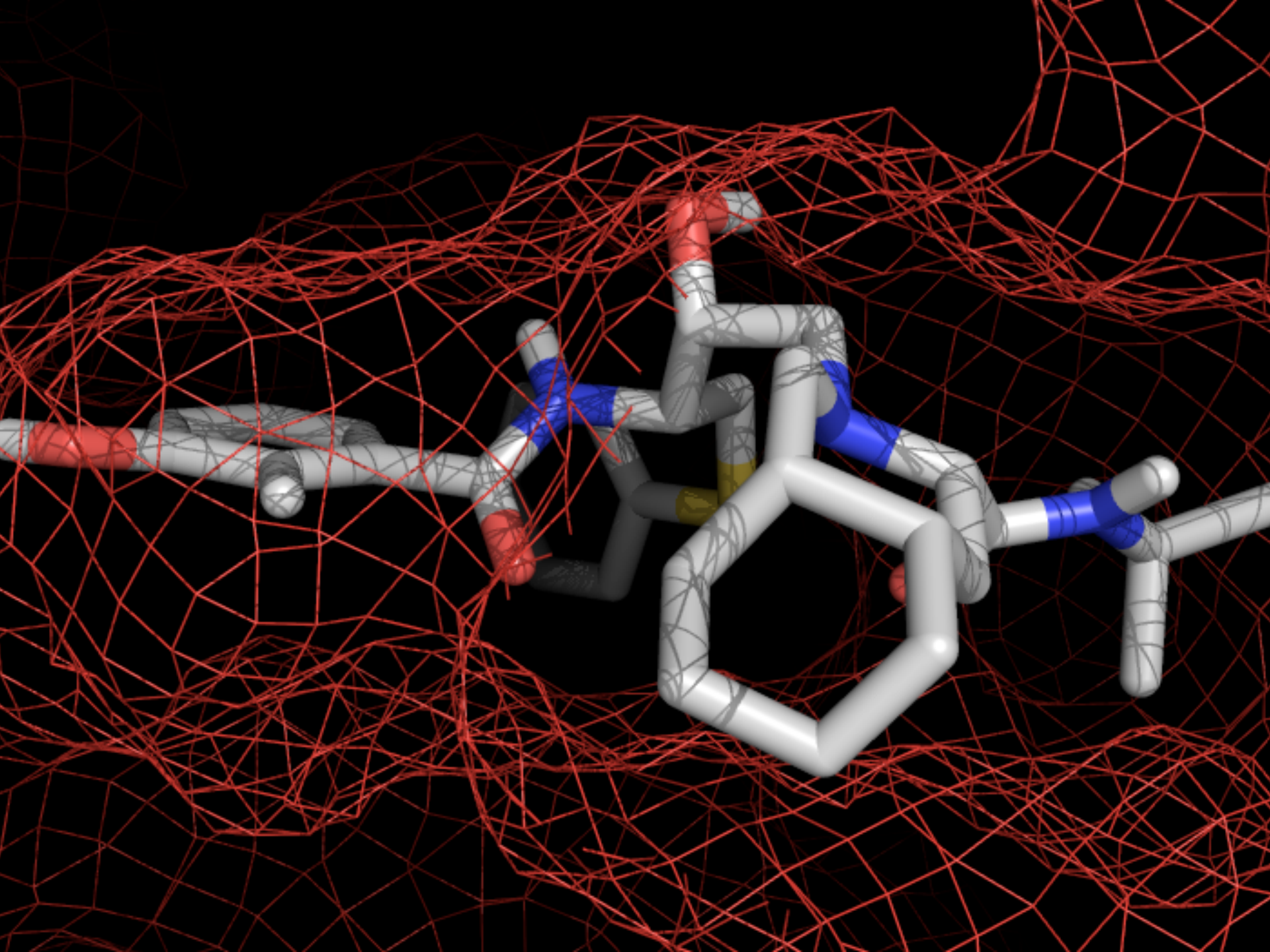


Figure 4 | Site-specific incorporation of 2 into proteins in mammalian cells and the specific labelling of EGFR-GFP on the cell surface with 9. a, Cells that contain the PylRS/$tRNA_{CUA}$ pair and the mCherry(TAG)eGFP-HA reporter produced GFP only in the presence of **2**. **b**, Western blots confirm that the expression of full length mCherry(TAG)eGFP-HA is dependent on the presence of **2**. **c**, Specific and rapid labelling of a cell surface protein in live mammalian cells. EGFR-GFP that bears **2** or **3** at position 128 is visible as green fluorescence at the membrane of transfected cells (left panels). Treatment of cells with **9** (200 nM) leads to selective labelling of EGFR that contains **2** (middle panels). Right panels show merged green and red fluorescence images, DIC = differential interference contrast. Cells were imaged four hours after the addition of **9**.

Jason Chin *et coll.*, *Nature Chem.*, **2012**, *4*, 298-304

Target Identification



Target vs Phenotypic-based Drug Discovery

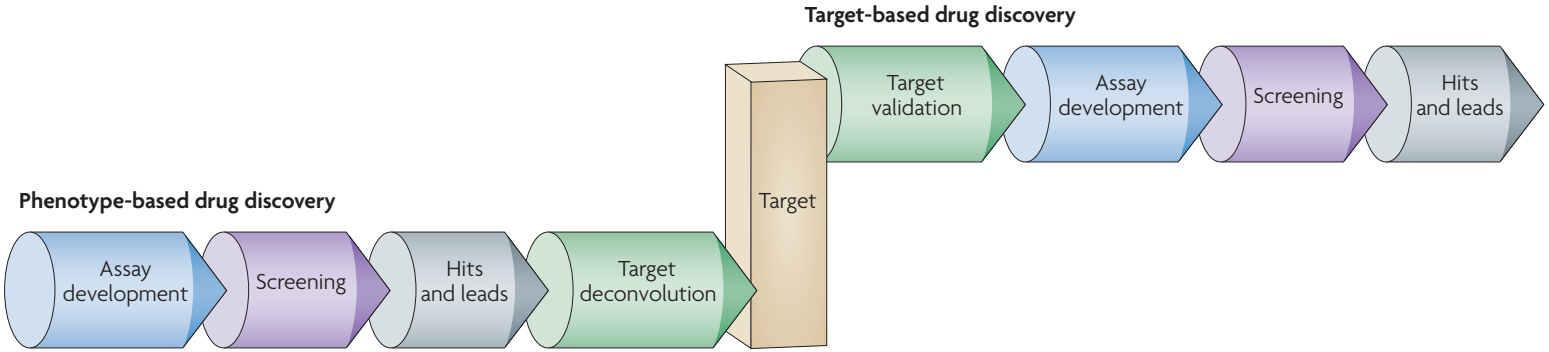
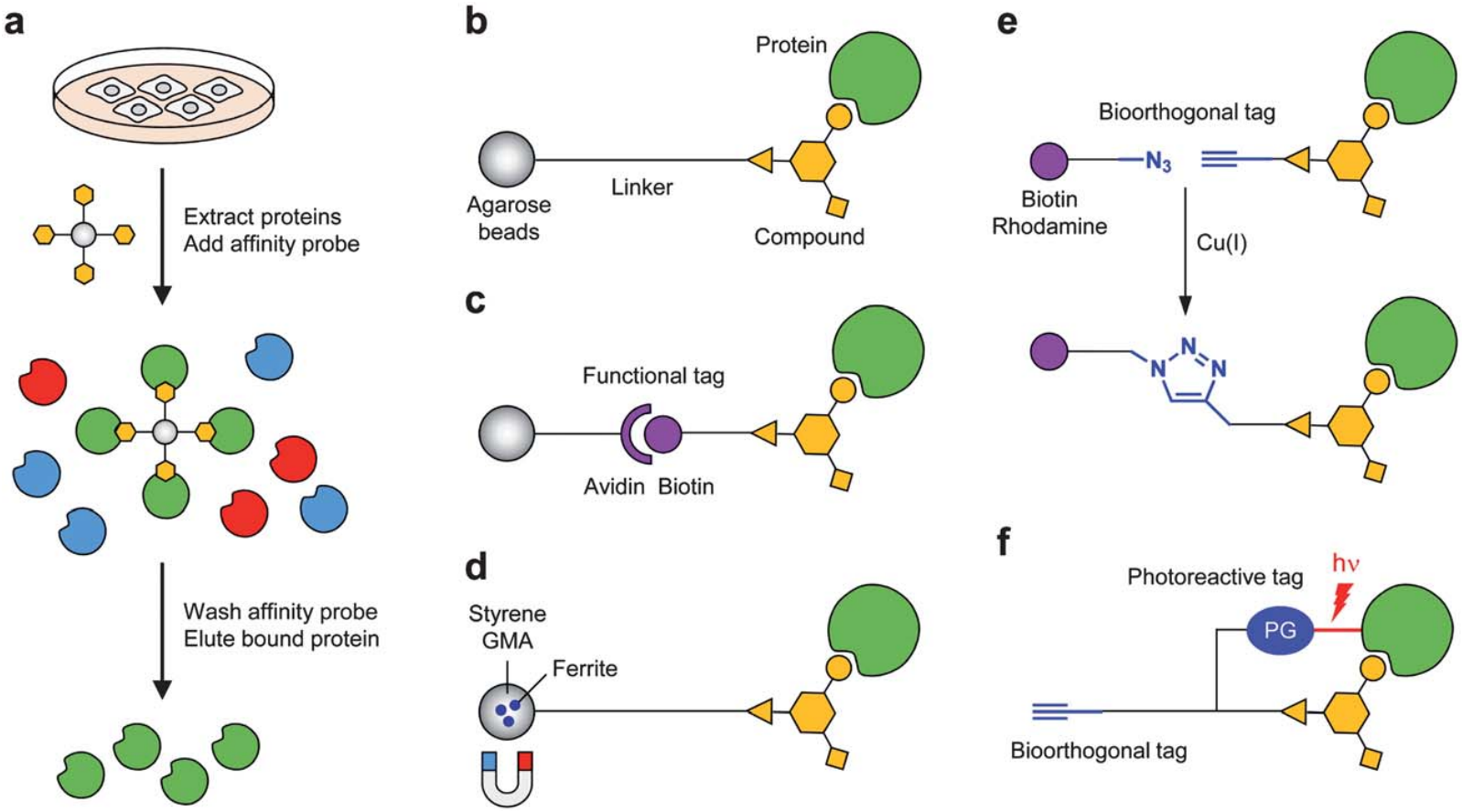


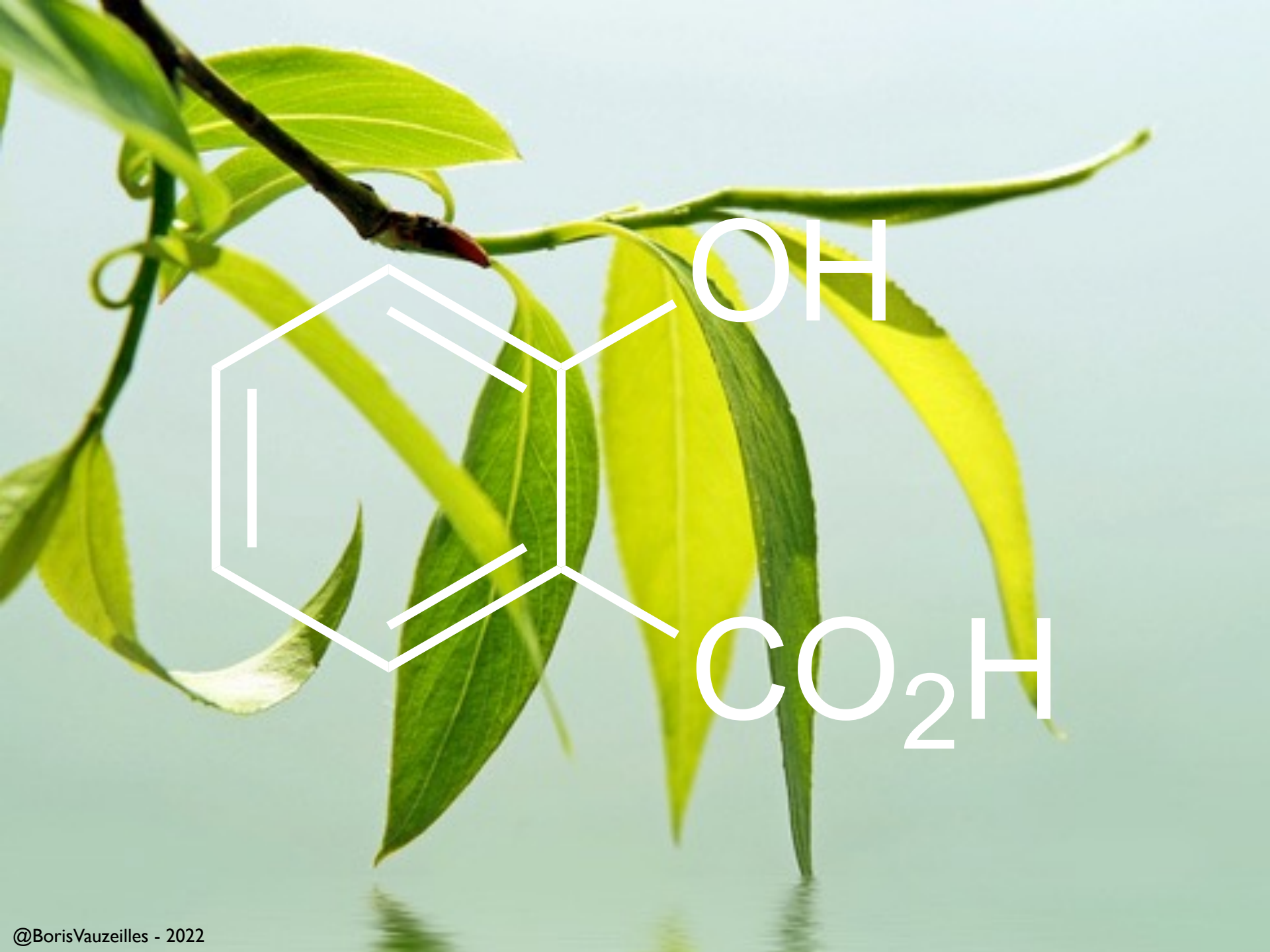
Figure 1 | **Phenotype-based versus target-based drug discovery.** The diagram illustrates the early phase of drug discovery, in which the aim is to identify target and lead molecules. In the phenotype-based approach, lead molecules are obtained first, followed by target deconvolution to identify the molecular targets that underlie the observed phenotypic effects. In the target-based approach, molecular targets are identified and validated before lead discovery starts; assays and screens are then used to find a lead.

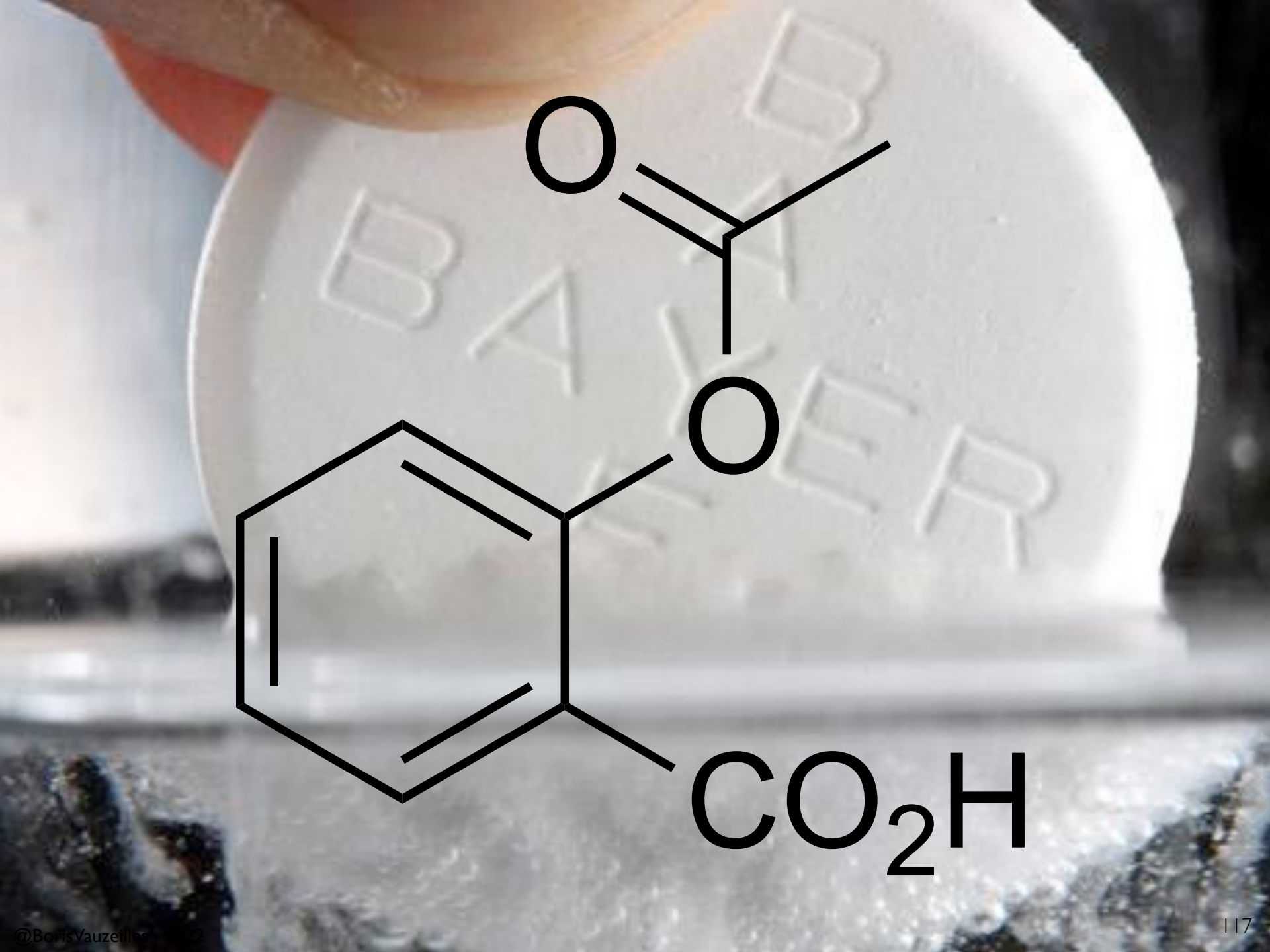
G.i Gaviraghi *et coll.*, *Nat. Rev. Drug. Discov.*, **2007**, *6*, 891-903

Affinity purification

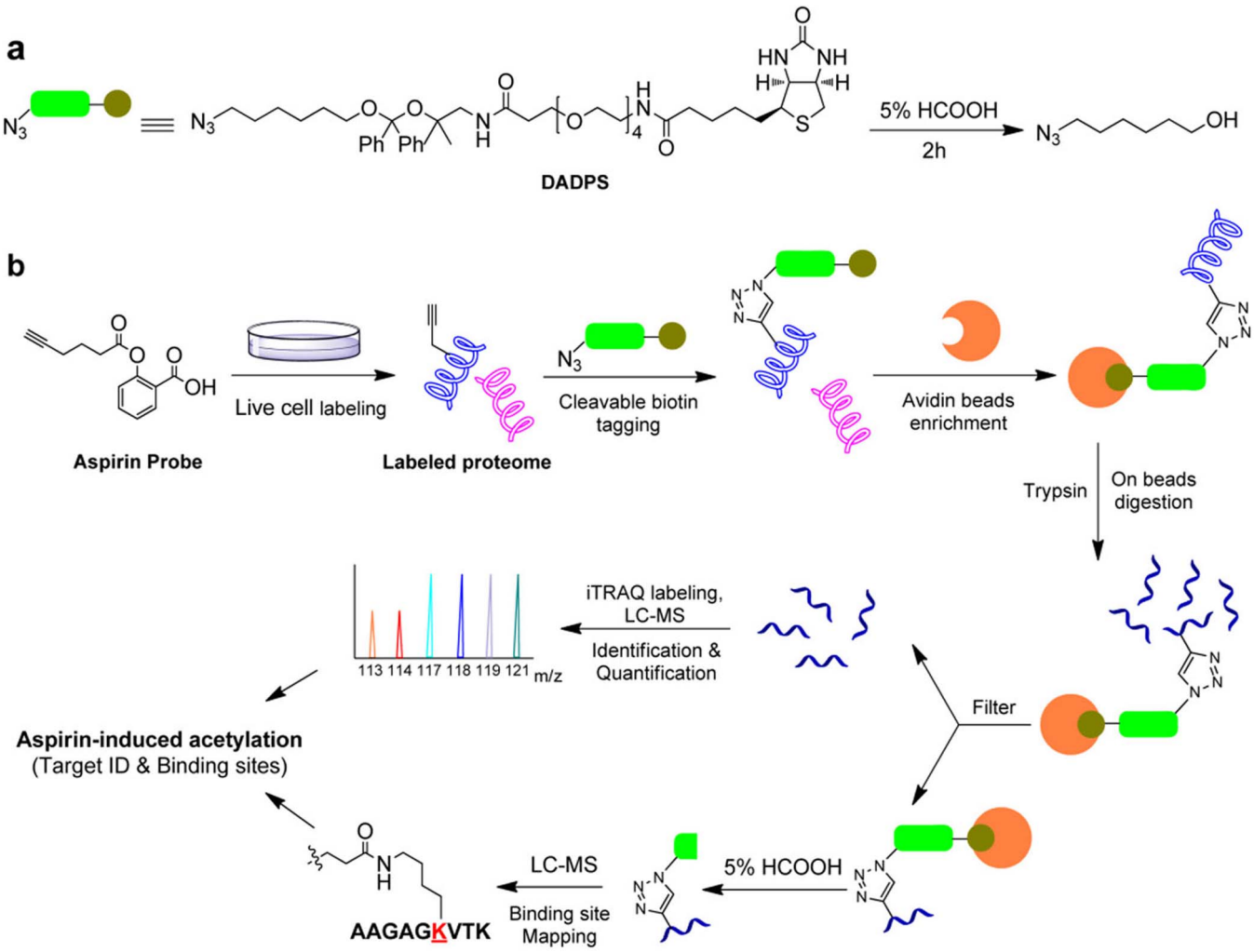


H. Osada *et coll.*, *MedChemComm*, **2014**, *5*, 277-287



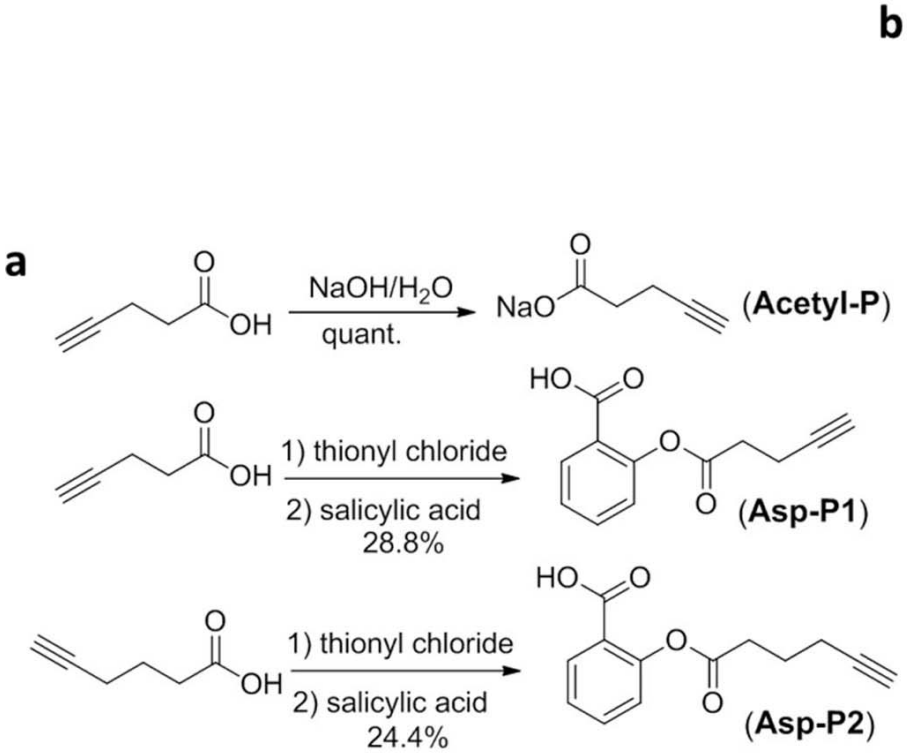

O
O
CO_2H

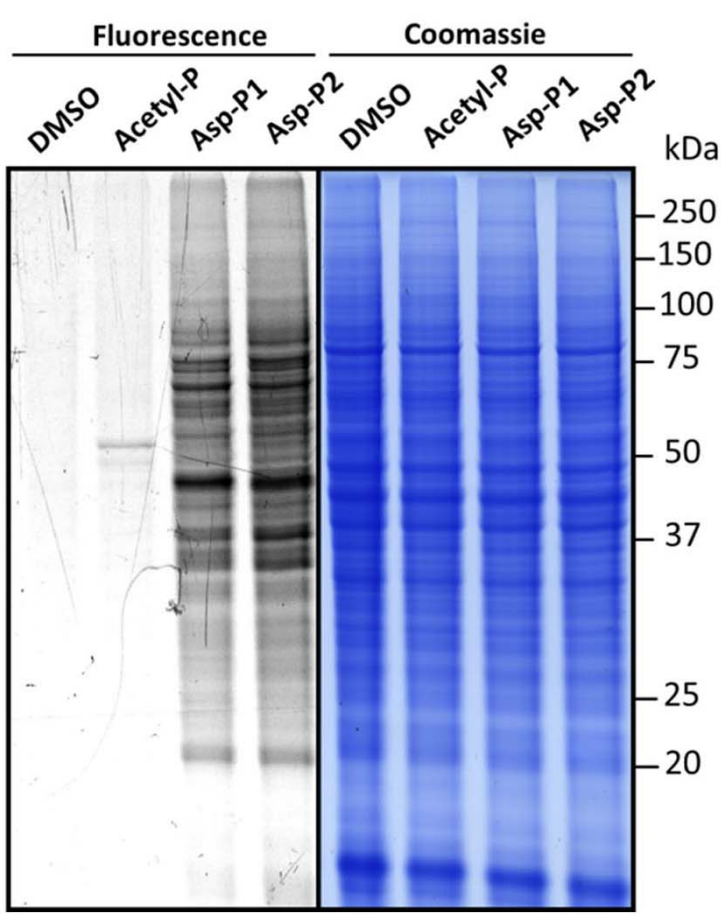
Target identification strategy



Q. Lin *et coll.*, *Scientif. Rep.*, **2015**, 5, 7896

Targets





Q. Lin *et coll.*, *Scientif. Rep.*, **2015**, *5*, 7896

Aspirin Targets

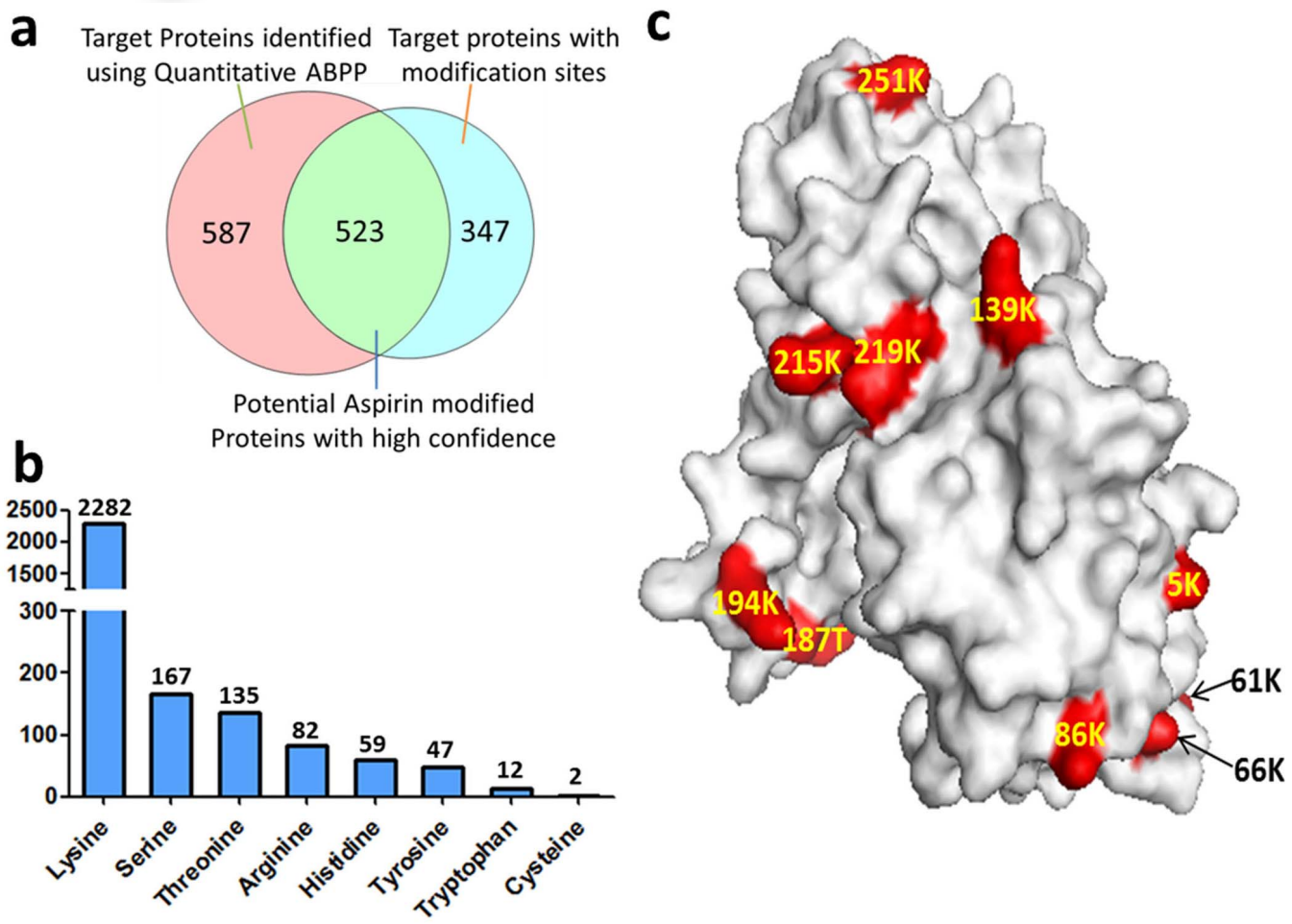


Figure 3 | Summary of aspirin modified proteins and amino acid residues. (a) Numbers of proteins identified using quantitative ABPP, proteins with modification sites identified and proteins confirmed with high confidence; (b) Numbers of the aspirin-modified amino acid residues. Numbers on top of the columns are the numbers of peptides modified by **Asp-P2**; (c) Locations of aspirin-modified residues in the protein GAPDH.

Q. Lin *et coll.*, *Scientif. Rep.*, **2015**, 5, 7896

Chimie Combinatoire Dynamique

Jorge Luis Borges (1899 – 1986)

tout ce que je vais vous dire est deja ecrit dans la bibliotheque...

Livre **upkmdr xh** Page **297**

rien de ce que
je vous
raconte n est
ecrit dans la
bibliotheque...

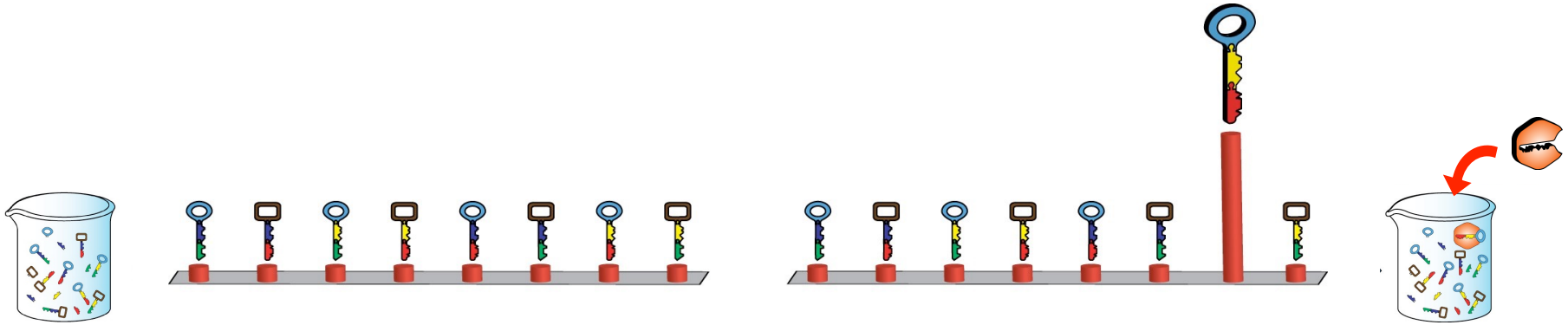
Livre **arxdkofq** Page **93**

$$K_a = \frac{[A \bullet B]}{[A][B]}$$

$$A + B \rightleftharpoons A \bullet B$$

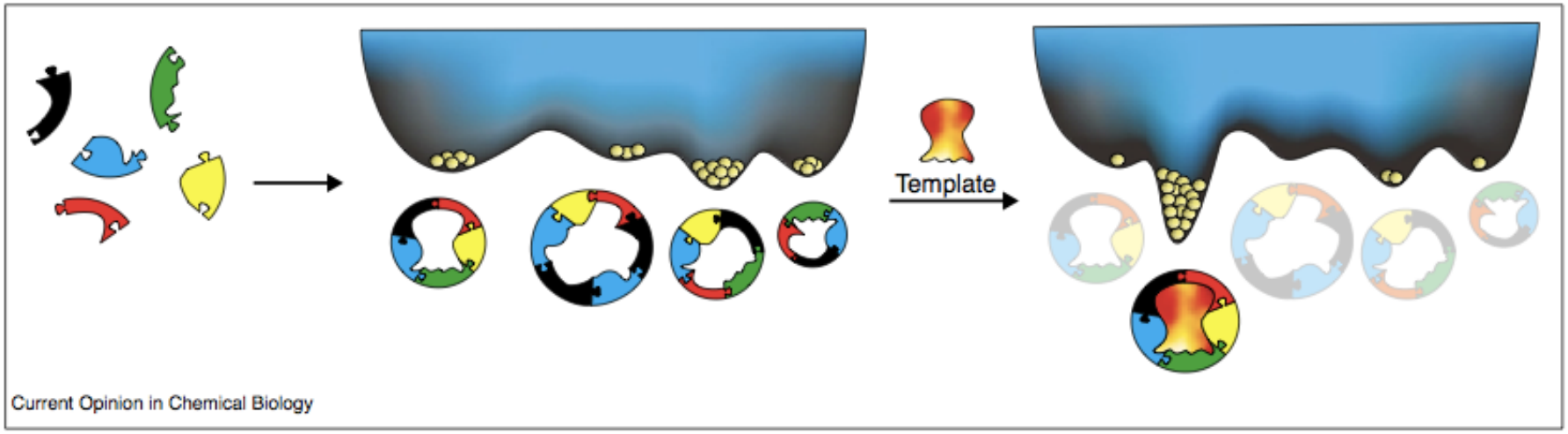
Chimie Combinatoire Dynamique

I. Huc, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106



Reviews: I. Huc, R. Nguyen, *Comb. Chem. High Throuhput Screening*, **2001**, *4*, 109
S. Otto, R. L.E. Furlan, J. K.M. Sanders, *Curr. Opin. Chem. Biol.*, **2002**, *6*, 321
O. Ramström, T. Bunyapaiboonsri, S. Lohmann, J.M. Lehn, *Biovhim. Biophys. Acta*, **2002**, *1572*, 178

Chimie Combinatoire Dynamique



A small dynamic combinatorial library and its free energy landscape showing the effect of adding a template that strongly and selectively binds to one of the equilibrating species.

Bibliothèques Combinatoires Virtuelles

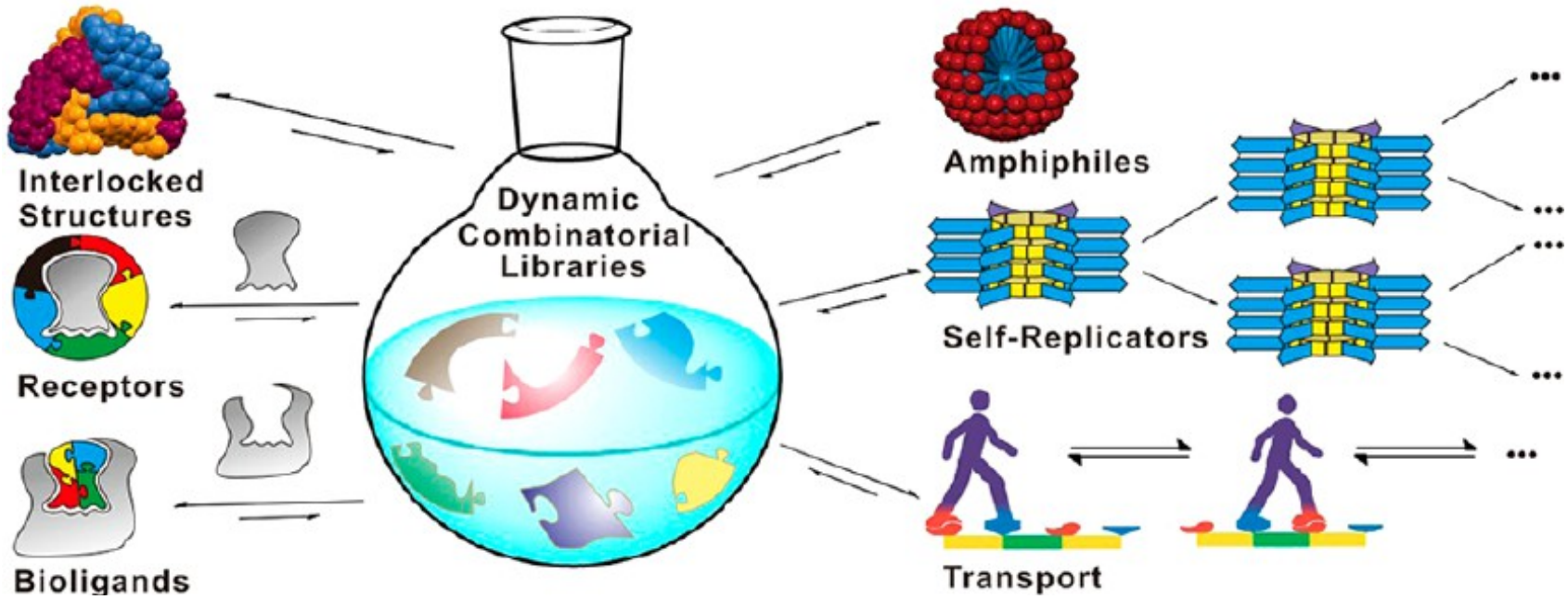
Dynamic combinatorial chemistry is a conceptually different approach that rests on supramolecular chemistry

It relies on a **reversible connection process** for the spontaneous and continuous generation of all possible combinations of a set of basic components, thus **making virtually available all structural and interactional features** that these combinations may present.

I. Huc, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1997**, 94, 2106

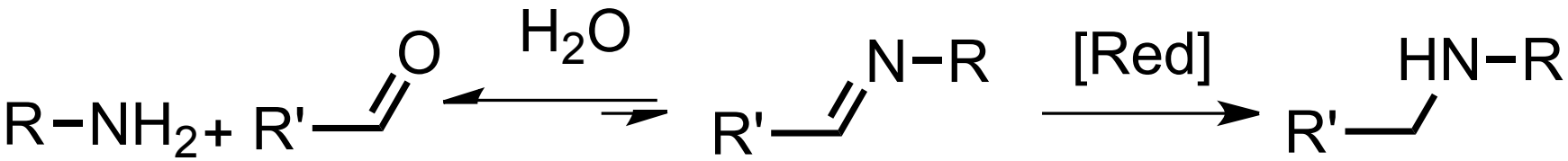
Cibler des protéines

Bibliothèques combinatoires dynamiques

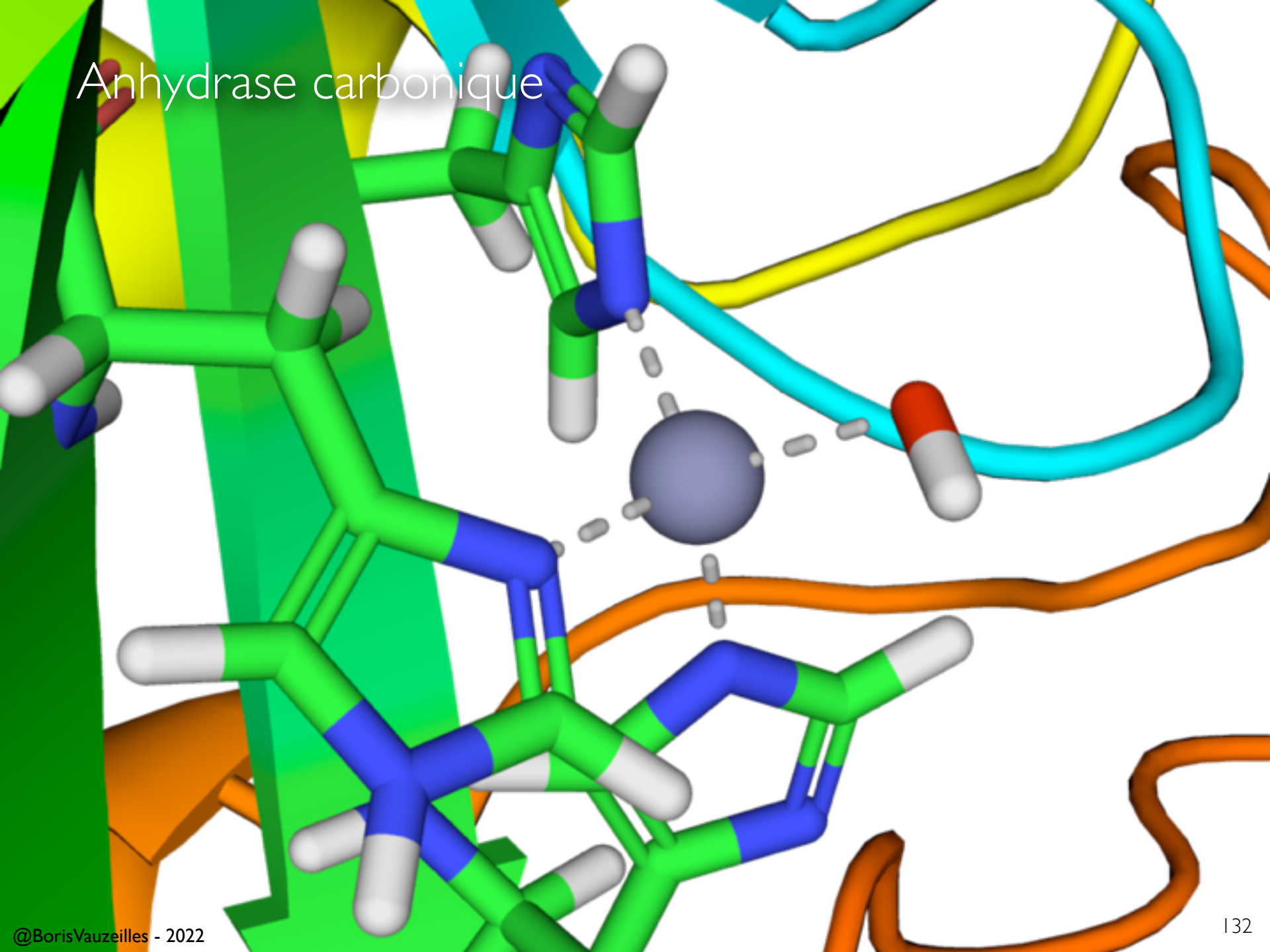


Sijbren Otto *et coll.*, *J. Am.. Chem. Soc.*, **2013**, 135, 9222-39

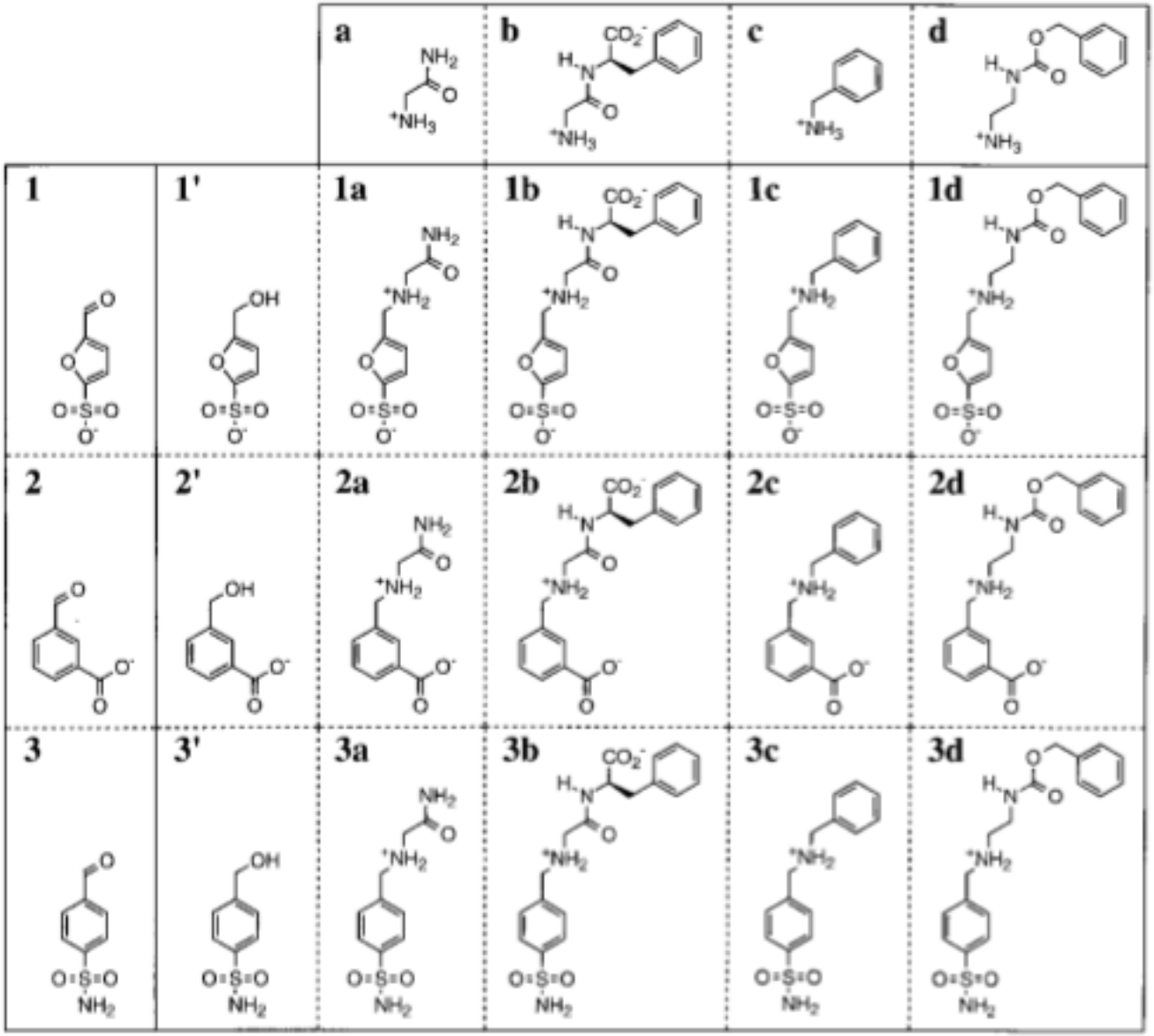
Réactions réversibles - Formation d'imines



Anhydrase carbonique



Inhibiteurs de l'anhydrase carbonique II



I. Huc, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1997**, 94, 2106

Profil HPLC

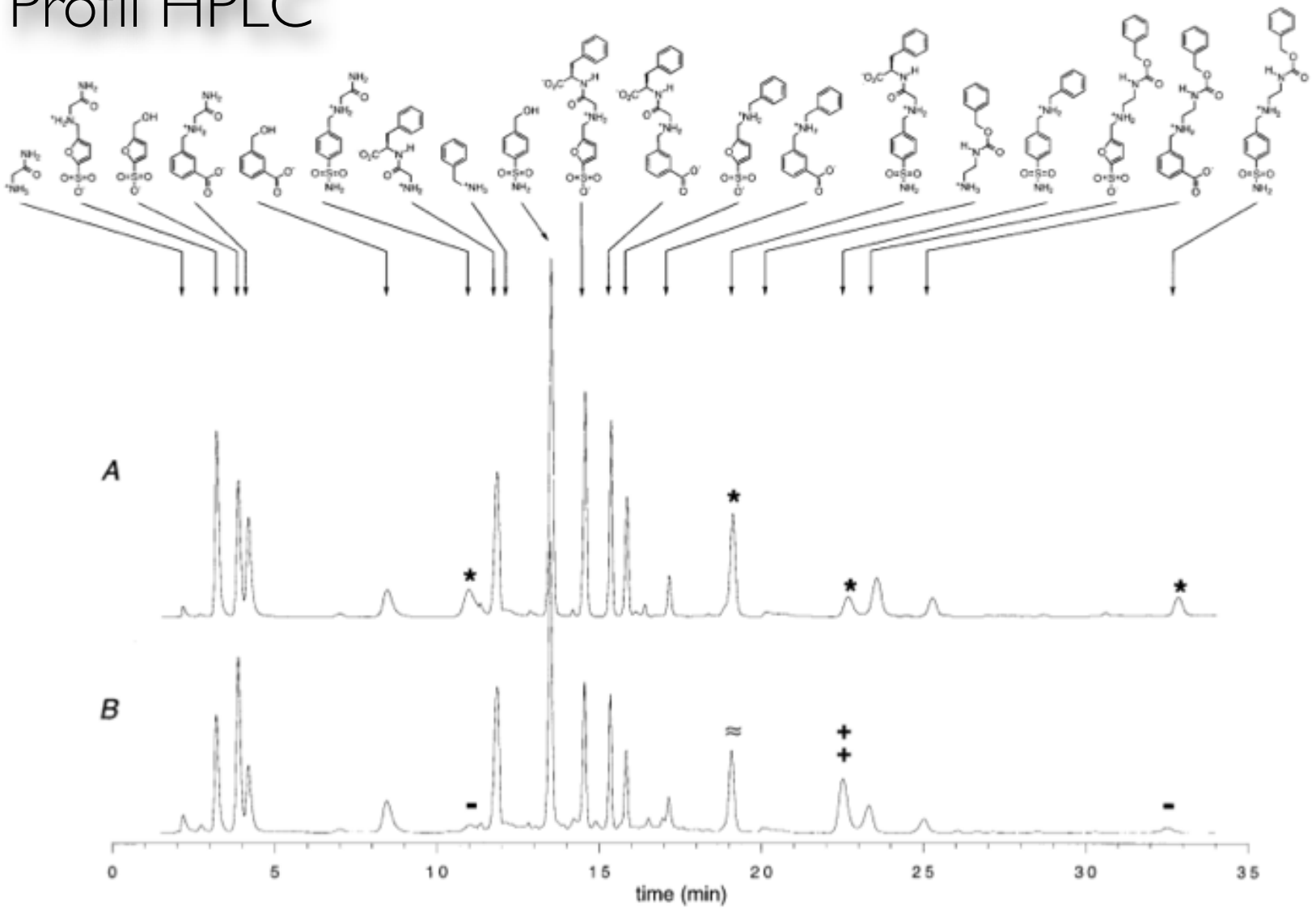


FIG. 3. HPLC traces of the final reaction mixtures of **a** + **b** + **c** + **d** (each at 4 mM) with **1** + **2** + **3** (each at 0.4 mM) and $NaBH_3CN$ (1.2 mM) in aqueous phosphate (20 mM, pH 6). (*A*) Trace corresponds to the reaction without additive. (*B*) Trace corresponds to the reaction in the presence of 1 equivalent (0.4 mM) of CA.

Product amplification

Table 1. Relative proportions between the products **3c** and **3a, 3b,** or **3d,** obtained with and without enzyme in reactions involving the aldehyde **3** and two competing amines **c** and **a, b,** or **d**

Starting materials	Normalized proportion of the products	Normalized value
3, c, a	$(\mathbf{3c/3a})_{rel}$	15
3, c, b	$(\mathbf{3c/3b})_{rel}$	4.5
3, c, d	$(\mathbf{3c/3d})_{rel}$	21
3, c, d, I	$(\mathbf{3c/3d})_{rel}$	2

Value of x means that the ratio between the two products is x times higher with CA than without CA. **I** is the inhibitor hexyl 4-sulfamoyl-benzoate.

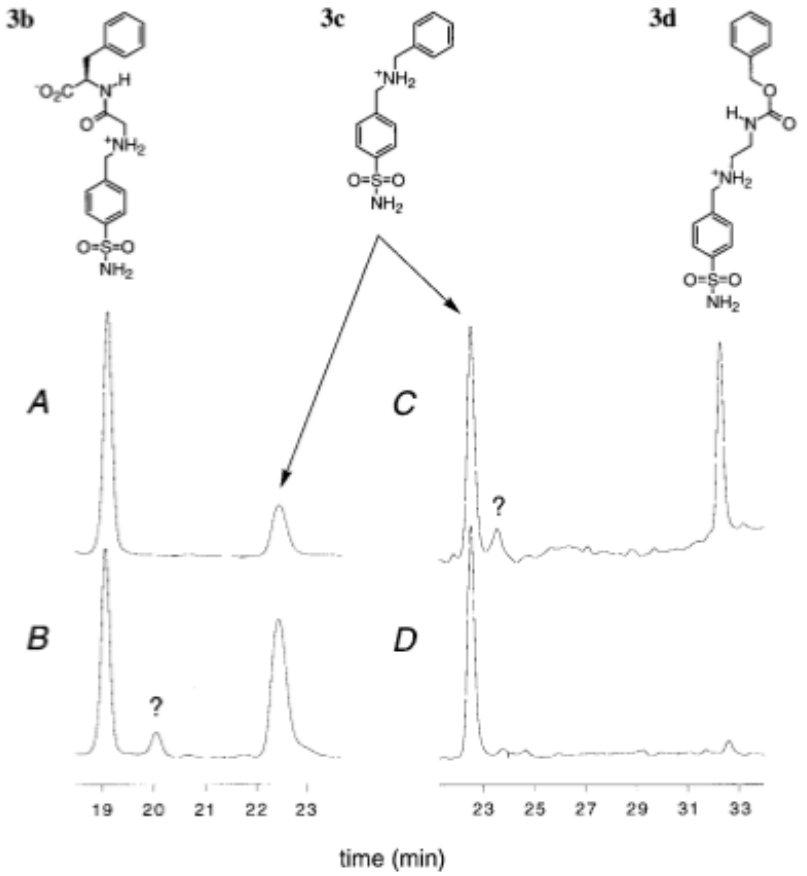
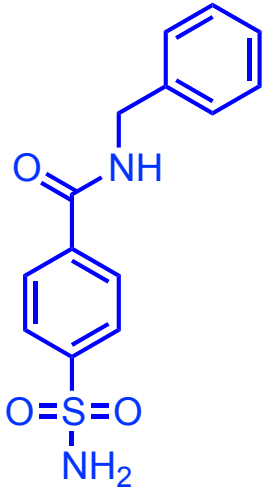
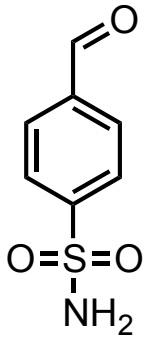


FIG. 4. HPLC traces of the final reaction mixtures of **3** (0.4 mM) with **b** + **c** (each at 2 mM) (*A* and *B*) or **c** + **d** (each at 2 mM) (*C* and *D*) and $NaBH_3CN$ (1.2 mM) in aqueous phosphate (20 mM, pH 6). (*A* and *C*) Traces correspond to the reaction without additive. (*B* and *D*) Traces correspond to the reaction in the presence of 1 equivalent (0.4 mM) of CA. Question marks indicate unidentified impurities.

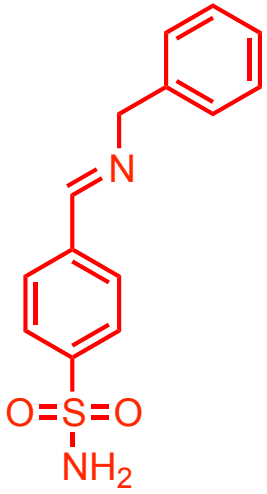
Bilan



Inhibiteur connu
1.1 nM

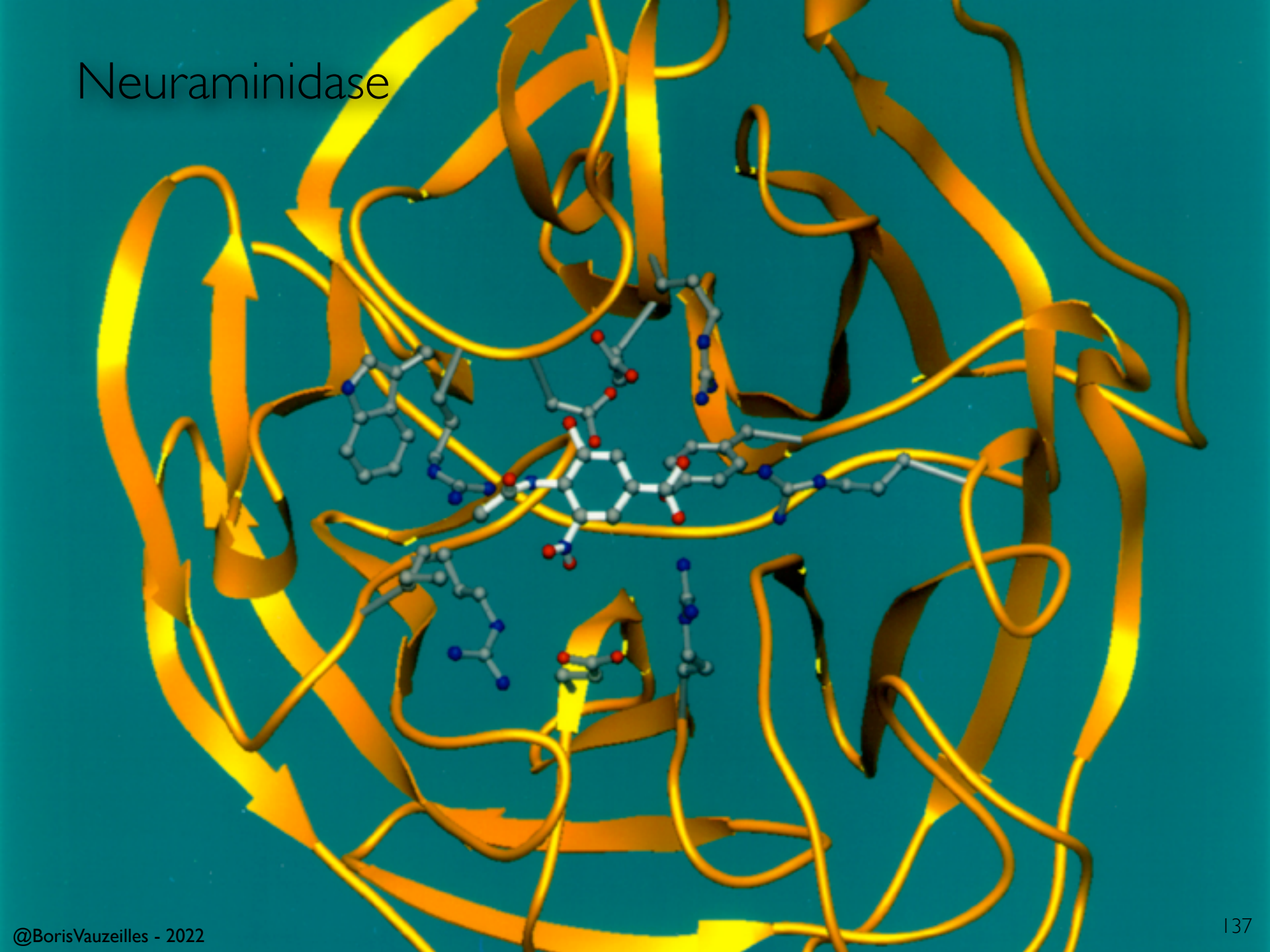


Plateforme
submicromolaire

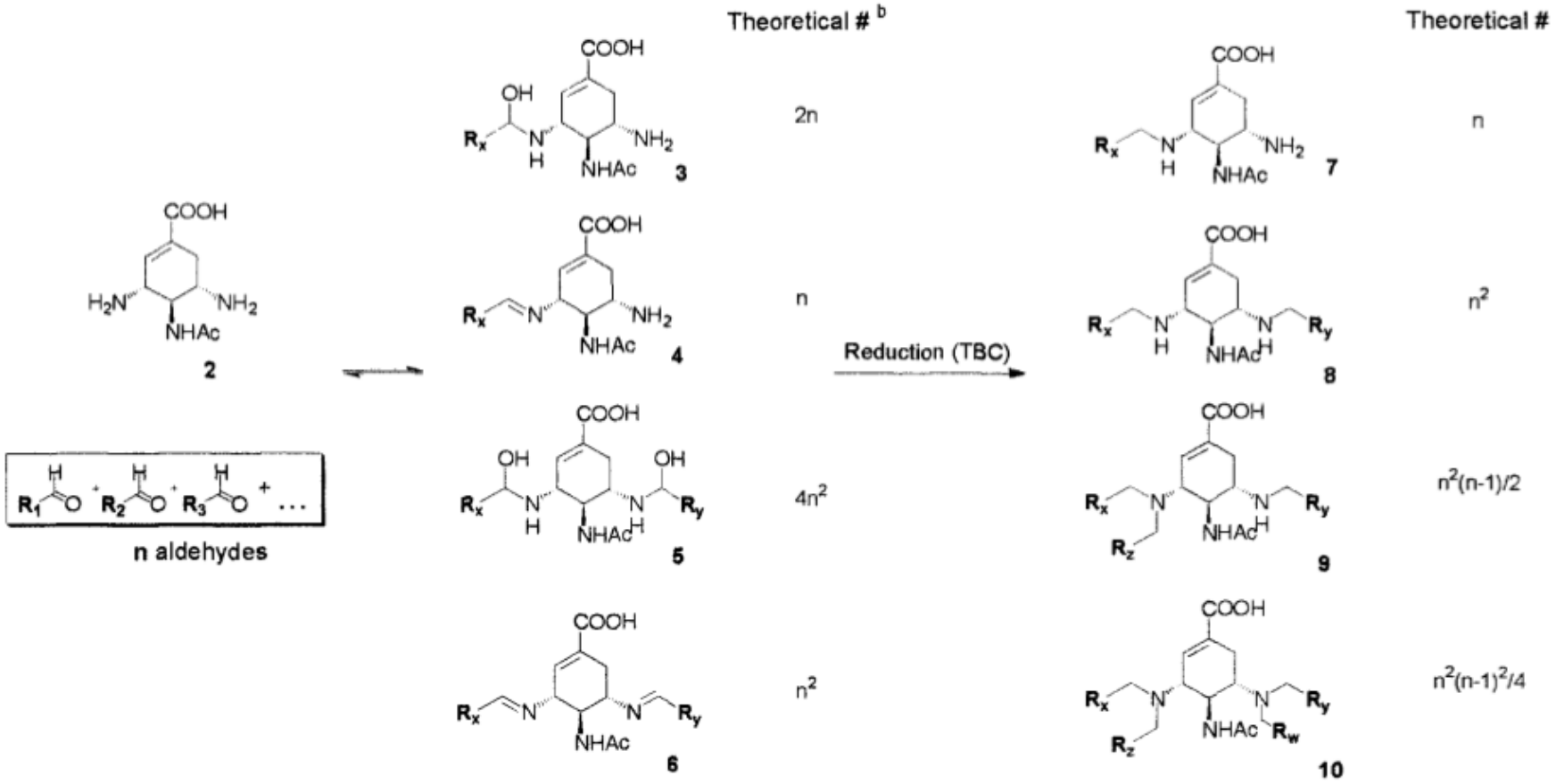


Hit

Neuraminidase



Neuraminidase



Scheme 1. Structures of the scaffold and library composition. [a], Hemiaminal species are restricted to those detected by NMR. [b], Numbers of compounds **3** and **5** account for diastereomers.

A.V. Eliseev *et al.*,*Proc. Natl. Acad. Sci. USA* **2002**, 99, 3382; J. *Med. Chem.*, **2003**, 46, 356

Bibliothèque combinatoire dynamique

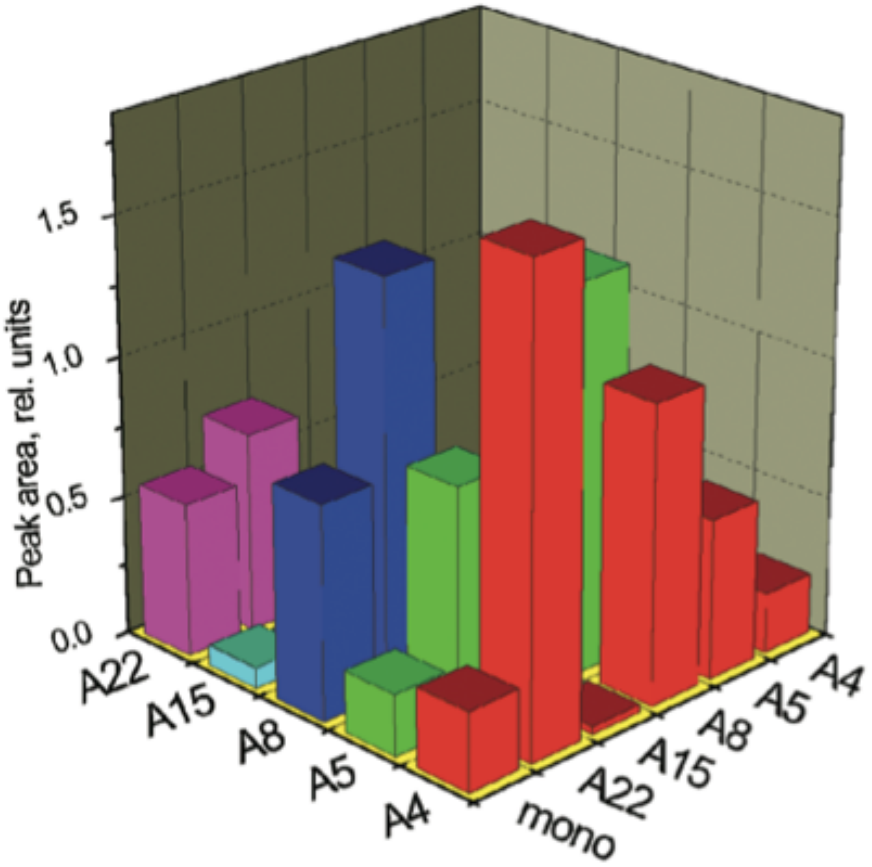


Fig. 1. Mono- and disubstituted components (structures **7** and **8**, respectively) detected in a static library of potential neuraminidase inhibitors (see *Materials and Methods* for synthesis protocol) after 24 h of incubation. Heights of the bars correspond to peak areas of the compound masses in the extracted ion chromatograms.

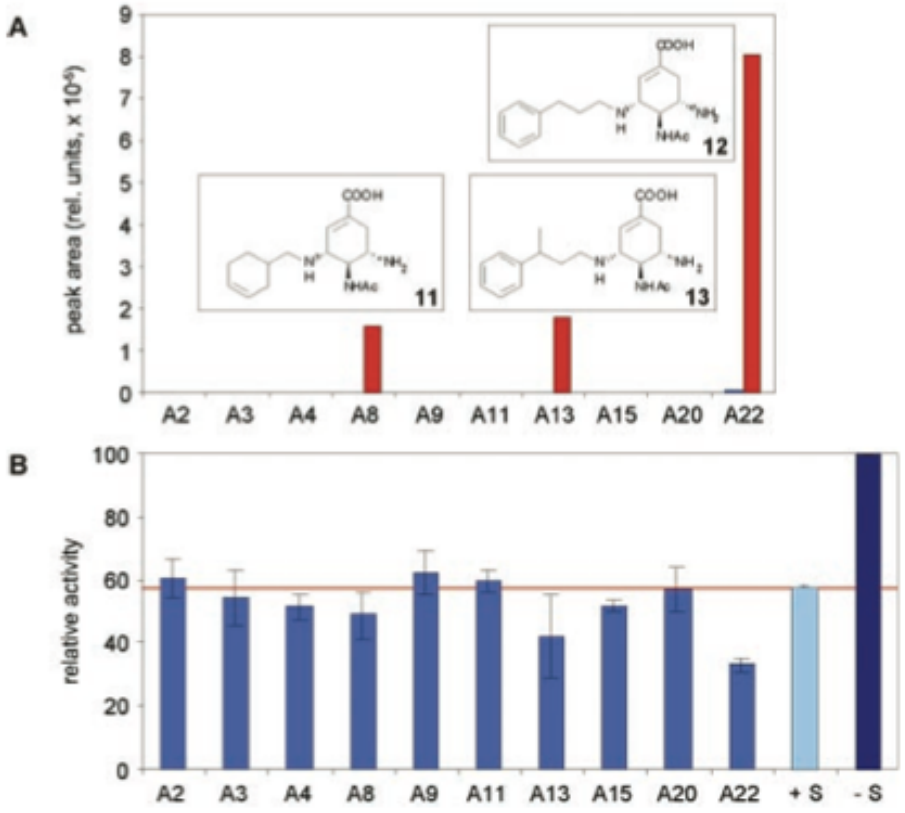
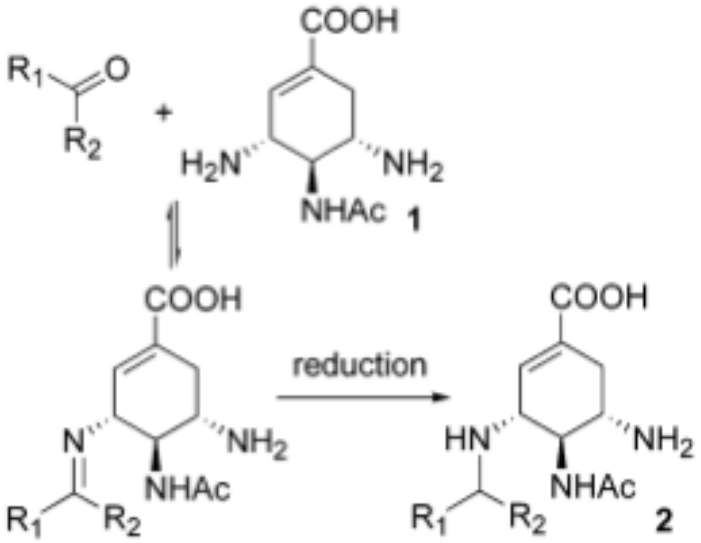
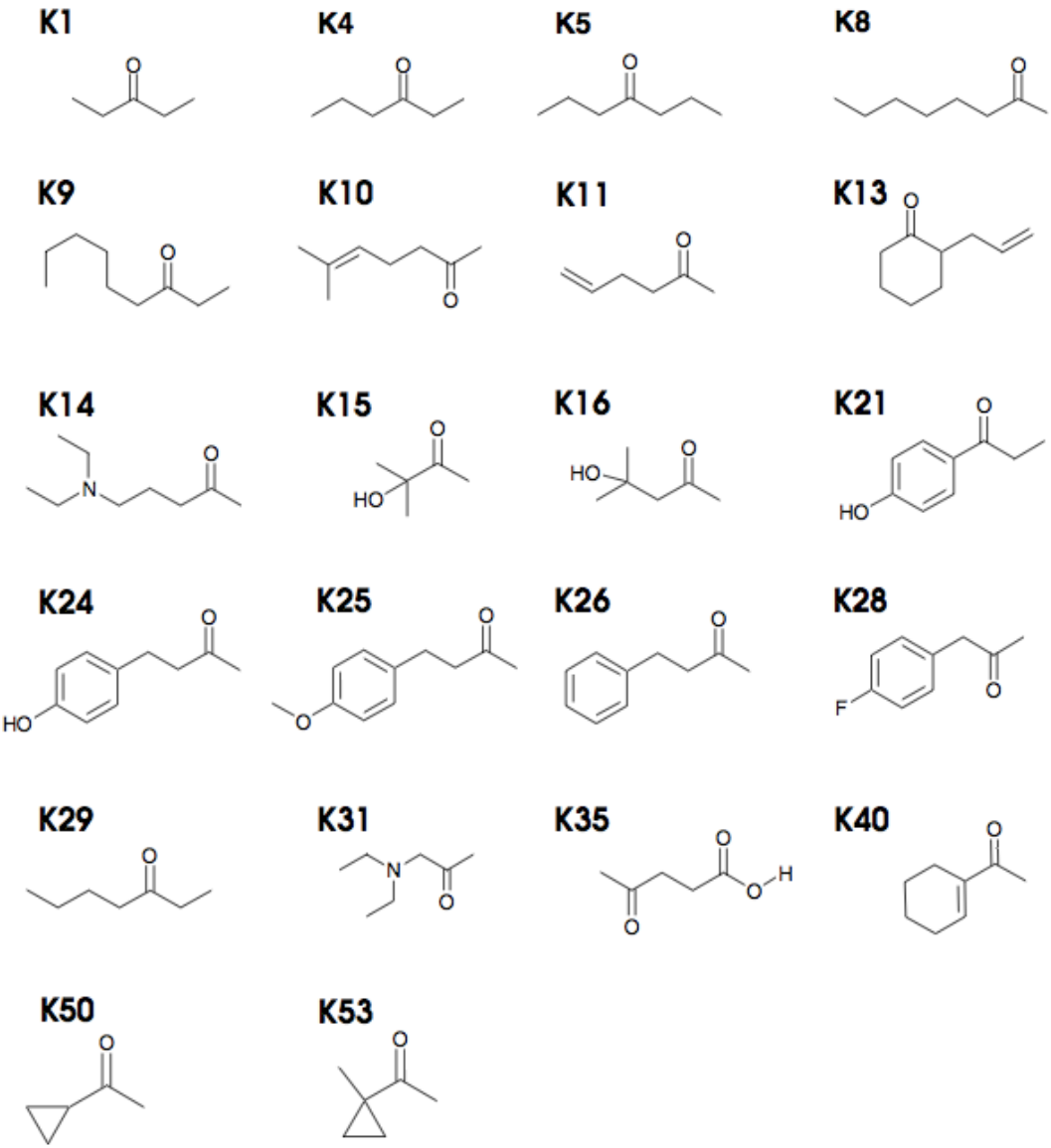


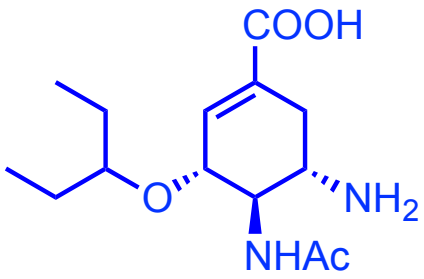
Fig. 2. (*A*) Library components of general structure **7** formed in the presence (red bars) and in the absence (blue bars, where applicable) of 10 μM neuraminidase on incubation of the mixture of **2** (10 μM) with A2-A4, A8, A9, A11, A13, A15, A20, and A22 (200 μM each) and 100 μM tetrabutylammonium cyanoborohydride (TBC) for 12 h. See *Materials and Methods* for analytical protocol. (*B*) Inhibition of neuraminidase by mixtures of **2** (10 μM) with individual aldehydes, as marked on the axis (2 mM each). Control bars correspond to the enzyme activity without inhibitors (−S) and with the scaffold **2** alone (+S).

Cétimines

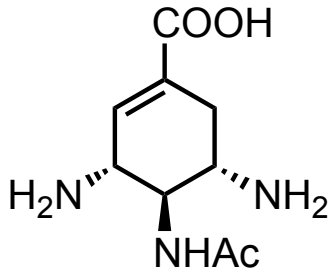

COOH
R_1
O
R_2
+
H_2N
NH_2
NHAc 1
COOH
N
NH_2
NHAc
R_1
R_2
reduction
COOH
HN
NH_2
NHAc
R_1
R_2
2


K1
K4
K5
K8
K9
K10
K11
K13
K14
K15
K16
K21
K24
K25
K26
K28
K29
K31
K35
K40
K50
K53

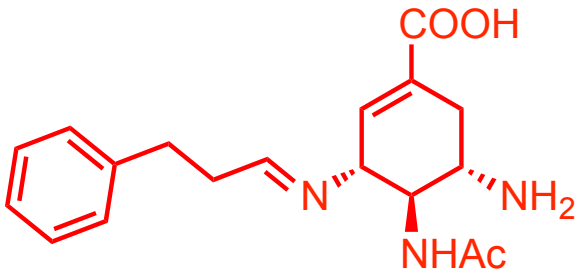
Bilan



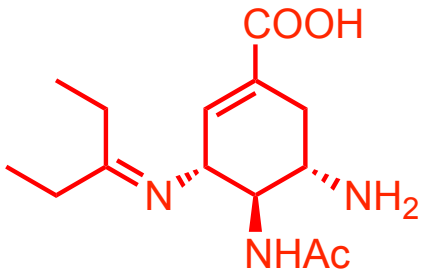
Inhibiteur connu
1.3 nM



Plateforme
31 μM



Hit 1
1.6 μM



Hit 2
85 nM

A.V. Eliseev *et al.*,*Proc. Natl. Acad. Sci. USA* **2002**, 99, 3382; J. *Med. Chem.*, **2003**, 46, 356

Synthèse accélérée par la cible

Chimie click *in situ*

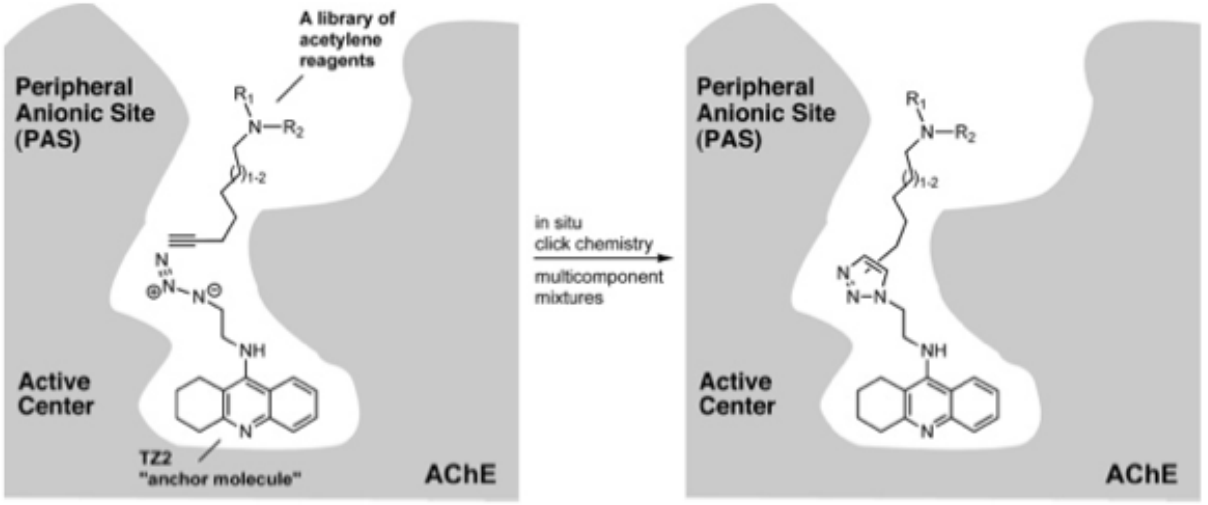


Figure 2. In situ click chemistry screening for AChE inhibitors, containing novel peripheral site ligands.

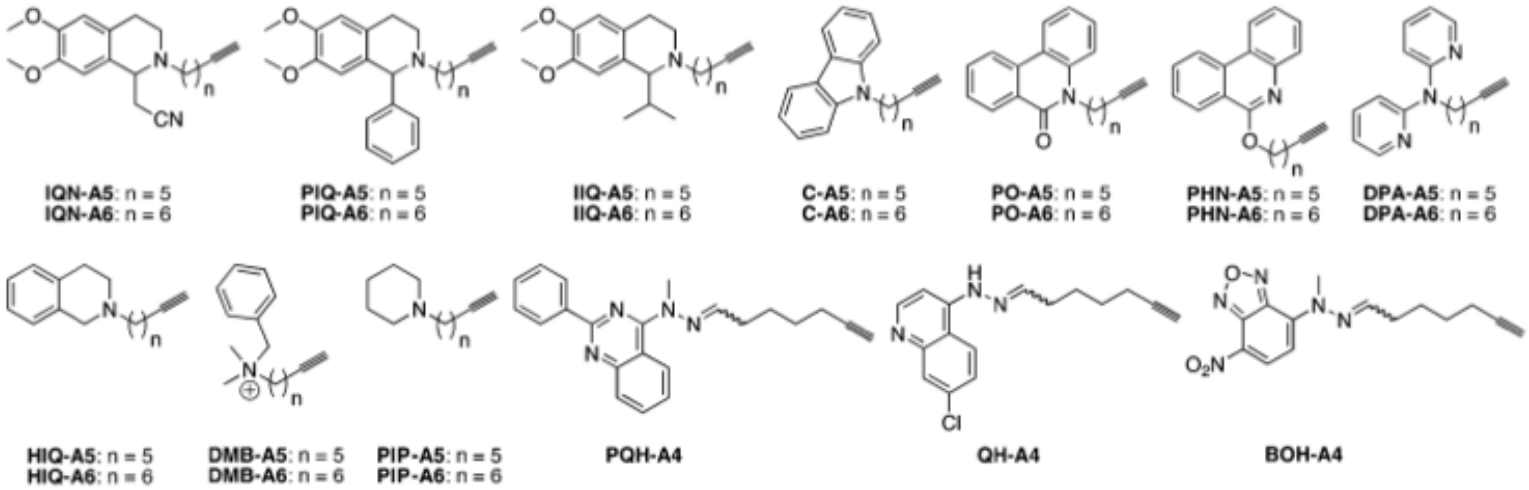
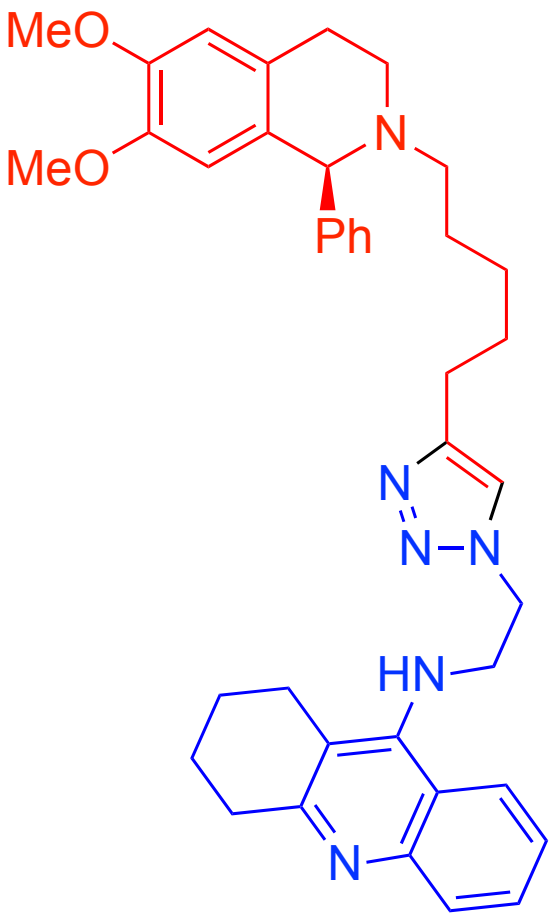


Figure 3. A library of 23 acetylene reagents for in situ click chemistry screening. All chiral compounds are racemic.

Inhibiteur femtomolaire



Ki = 33 fM (AChE anguille)

The End