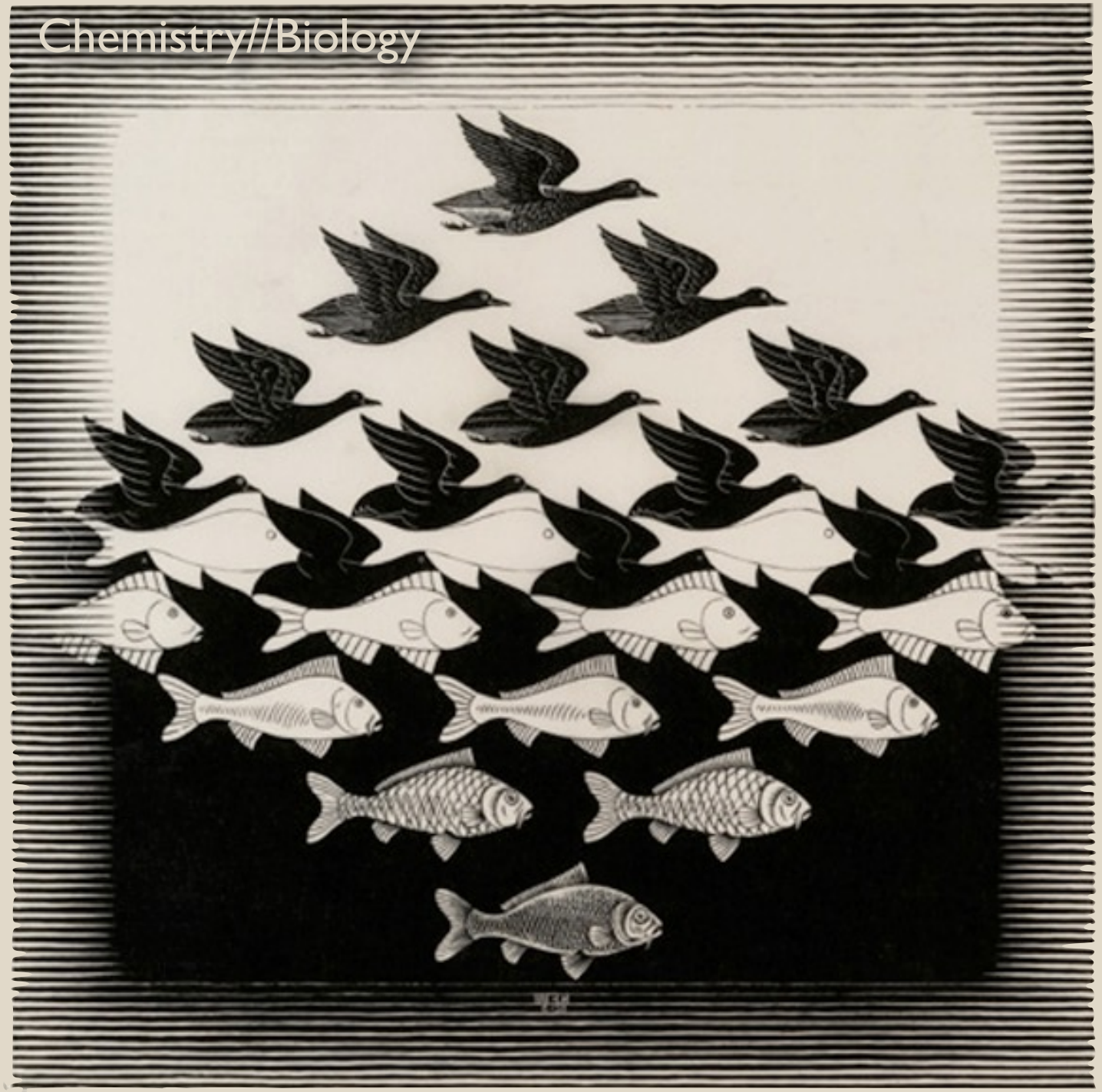
Chemical Biology in Drug Discovery

Chemistry//Biology

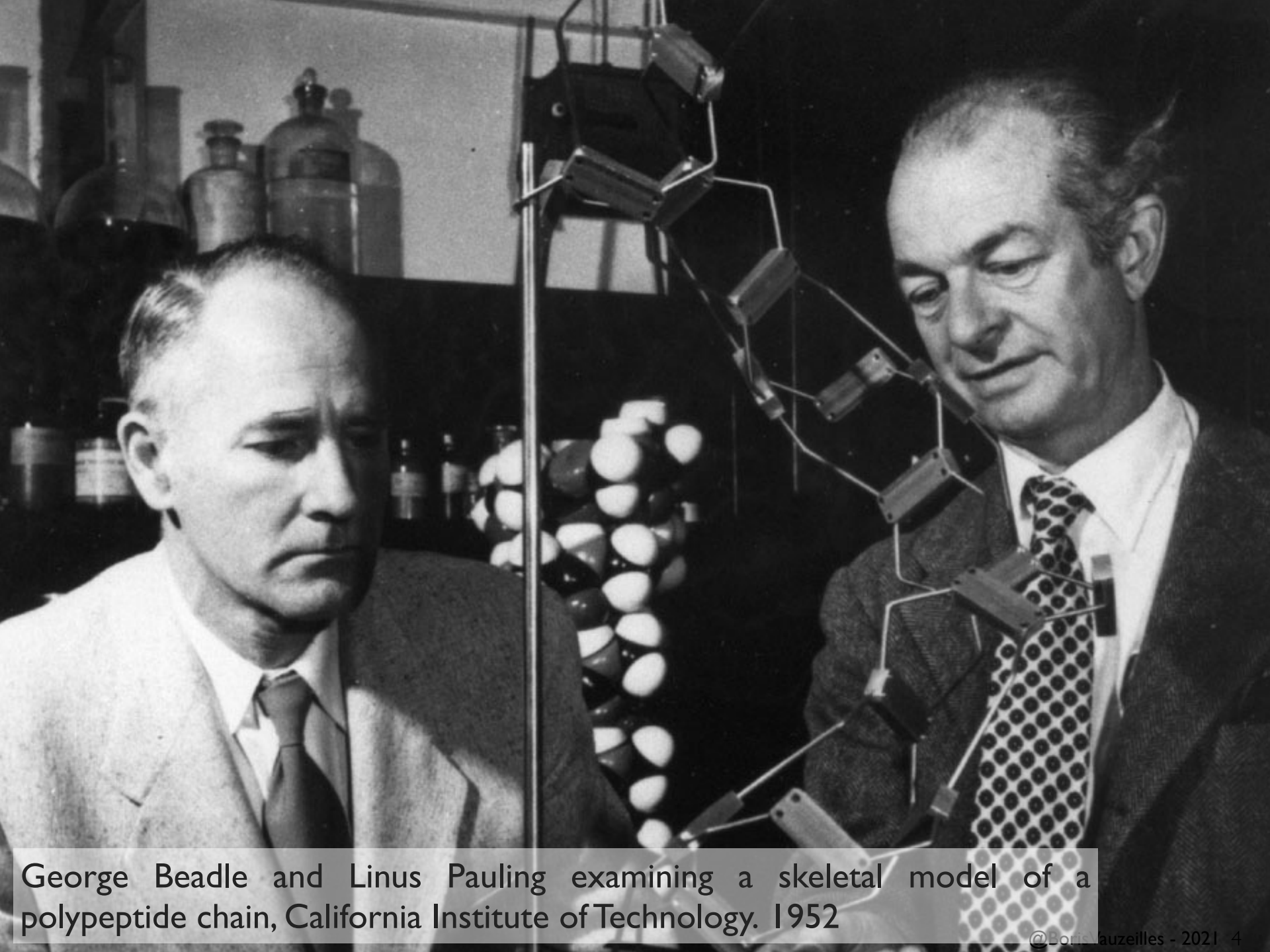


M. C. Escher
Sky and Water I
1938

CHEMICAL BIOLOGY

Collaboration between biology and chemistry at Caltech has already resulted in some impressive discoveries. Now a new grant promises to put the program on a long-term basis.

Engineering and Science - Feb. 1954



George Beadle and Linus Pauling examining a skeletal model of a polypeptide chain, California Institute of Technology. 1952

Dr. Linus Pauling
Division of Chemistry
California Institute of Technology
Pasadena 4, California

OCT 30 1945 REC'D

STANFORD UNIVERSITY, CALIFORNIA

October 26, 1945

Dear Pauling:

I am very happy to say the answer is "Yes". I am sure everything will work out well and I want to thank you for the role you have played in making it possible for us to become a part of the best chemical-biology group in the world.

Naturally I am anxious to see how we come out in the gamble with the Rockefeller Foundation and am therefore in favor of our getting under way with our application for a grant for chemical biology or whatever it is to be called. What is your notion as to the main outlines of the proposal? I have not thought much about the problem of what should be included in biology aside from immuno-genetics and chemical genetics but I think we should make the proposal broad enough to include photosynthesis, microbiology, virusology, isotope tracer work and conventional plant and animal chemistry. I agree with you that the main danger is that we will not be imaginative enough in drawing up the plan. After all we want to anticipate developments for 10 or 15 years or at least make room for them in our scheme of things.

Cordially yours.
G. W. Beadle

Biological Chemistry - Chemical Biology

Biological chemistry
Biochemistry

Study of chemical processes within and relating to living organisms

Chemical Biology - Biologie chimique - Chémobiologie

«Agreeing on a precise definition of chemical biology has been a persistent challenge for the field. We asked a diverse group of scientists to "define chemical biology" and present a selection of responses»

Nature Chemical Biology, **2015**, *11*, 378–379

"How would you define chemical biology?"

"Chemical biology is an area of research in which chemical and biological concepts and tools interact synergistically in the pursuit of new discoveries or technologies"

Carolyn R Bertozzi

Stanford University, Palo Alto, California, USA

Nature Chemical Biology, **2015**, *11*, 378–379

"How would you define chemical biology?"

"Chemical biology involves viewing the world around us, the living organisms and their environment, through the lens of a chemist, and taking advantage of the unique ability of chemists to not only study but also create new forms of matter at the molecular level for societal benefit."

Christopher J Chang

University of California, Berkeley and HHMI, Berkeley, California, USA

Nature Chemical Biology, **2015**, *11*, 378–379

"How would you define chemical biology?"

"It is chemistry brought to life: the science of applying chemistry to understand and perturb biological processes."

Stefan Kubicek

CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

Nature Chemical Biology, **2015**, *11*, 378–379

"How would you define chemical biology?"

"Let me know when you figure it out!"

Jennifer A Prescher
University of California, Irvine, California, USA

Nature Chemical Biology, **2015**, *11*, 378–379

"How would you define chemical biology?"

"Chemical biology is that field of science that is conducted by chemical biologists"

Brian K Shoichet

University of California, San Francisco, San Francisco, California, USA

Nature Chemical Biology, **2015**, *11*, 378–379

"How would you define chemical biology?"

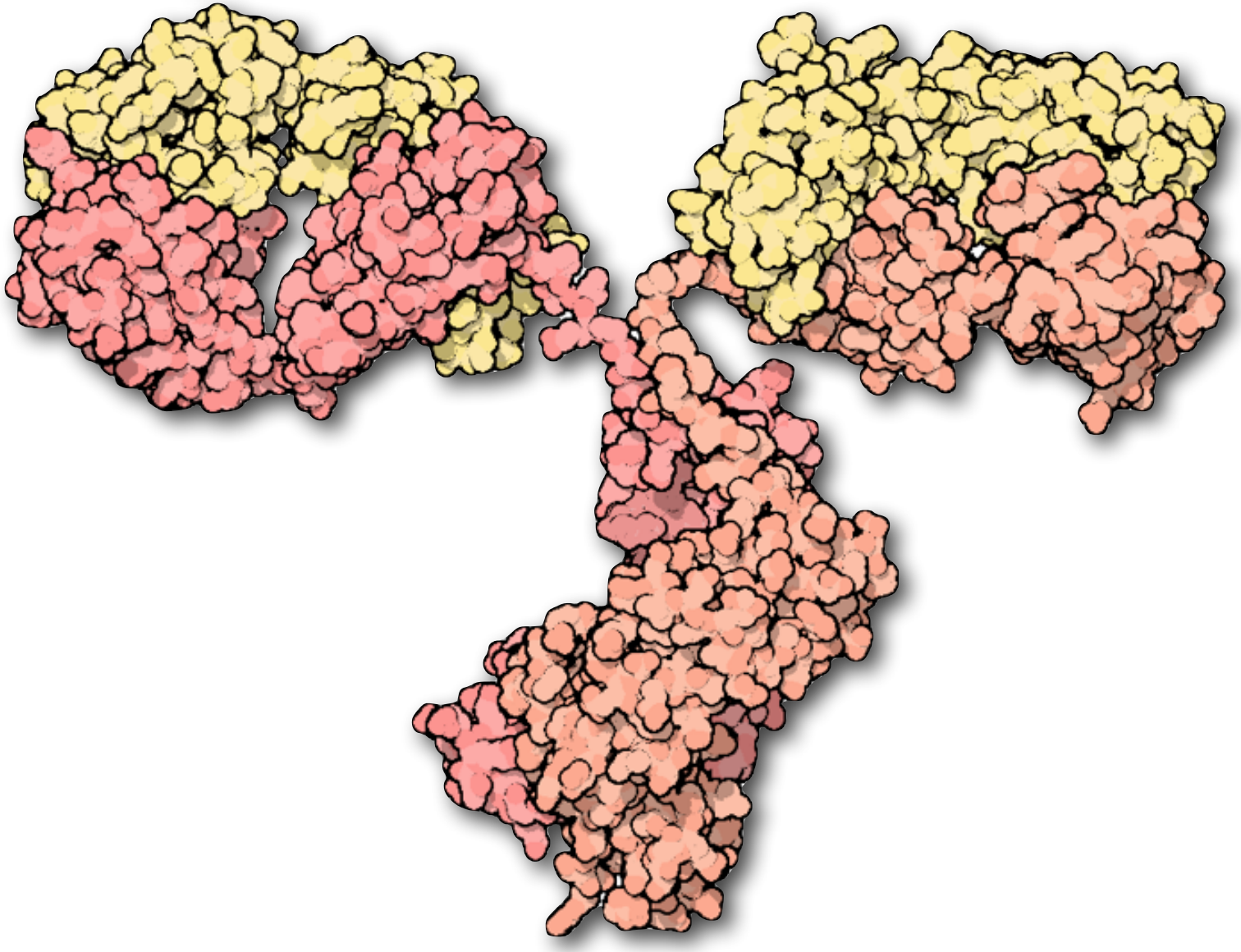
"Decoding the mysteries of human biology in the language of chemistry is an important scientific frontier"

Chaitan Khosla

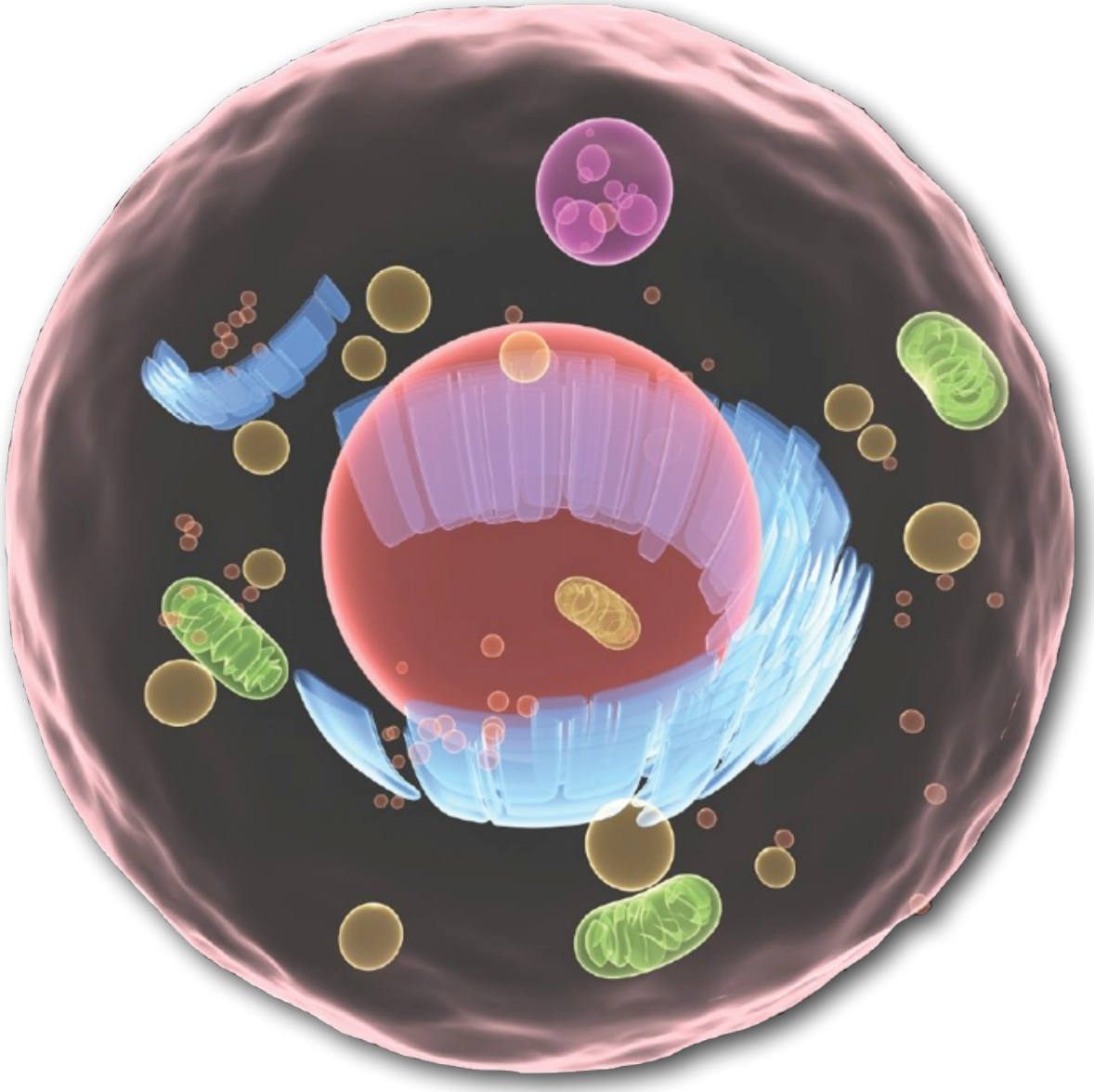
Stanford University, California, USA

Chemistry in a flask

Chemistry on biomolecules



Chemistry in a cell



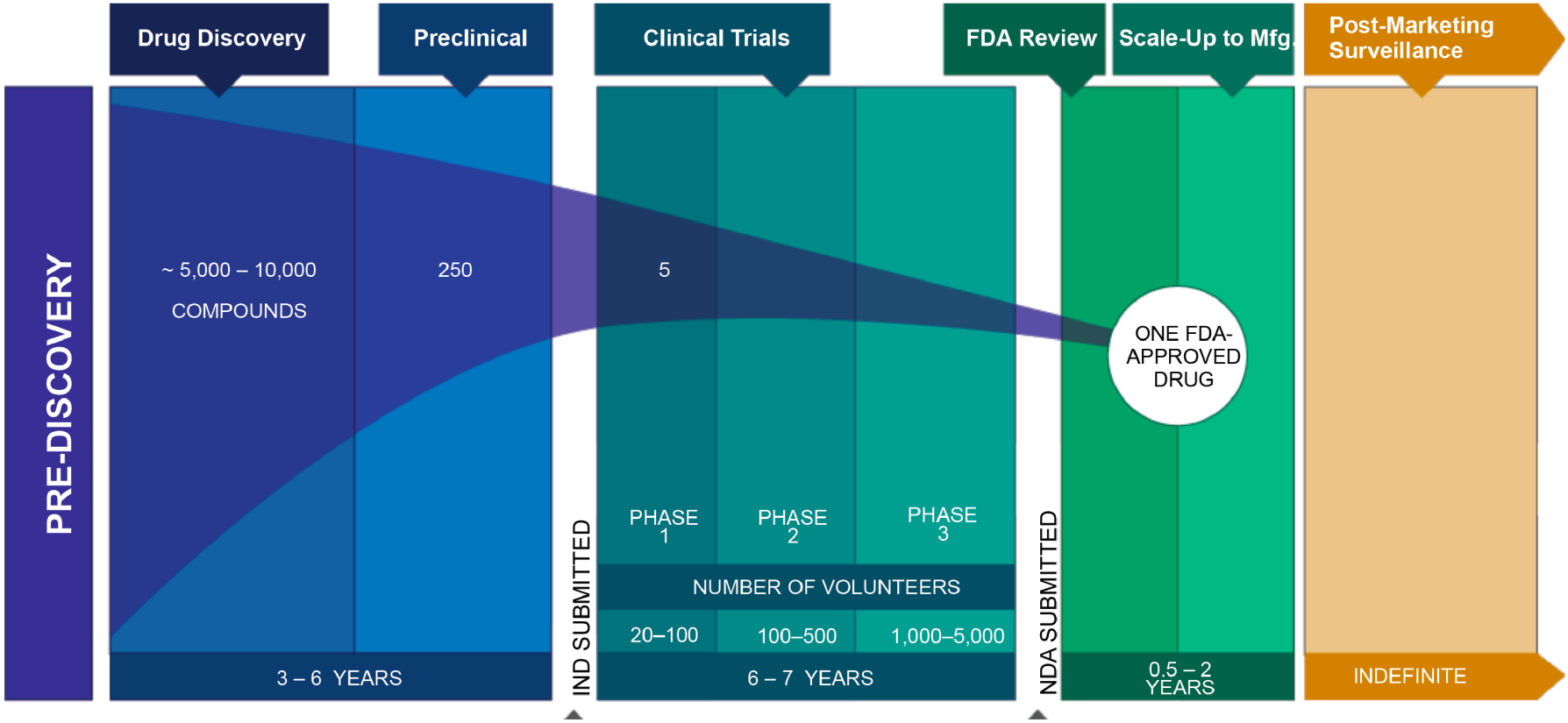
www.howitworksdaily.com

Chemistry in living organisms

Développement d'un médicament

- Il faut 12 ans de R&D et 250 à 800 millions d'euros pour mettre un médicament sur le marché.

- Environ 6000 molécules testées

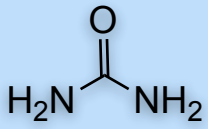


@BorisVauzeilles - 2002

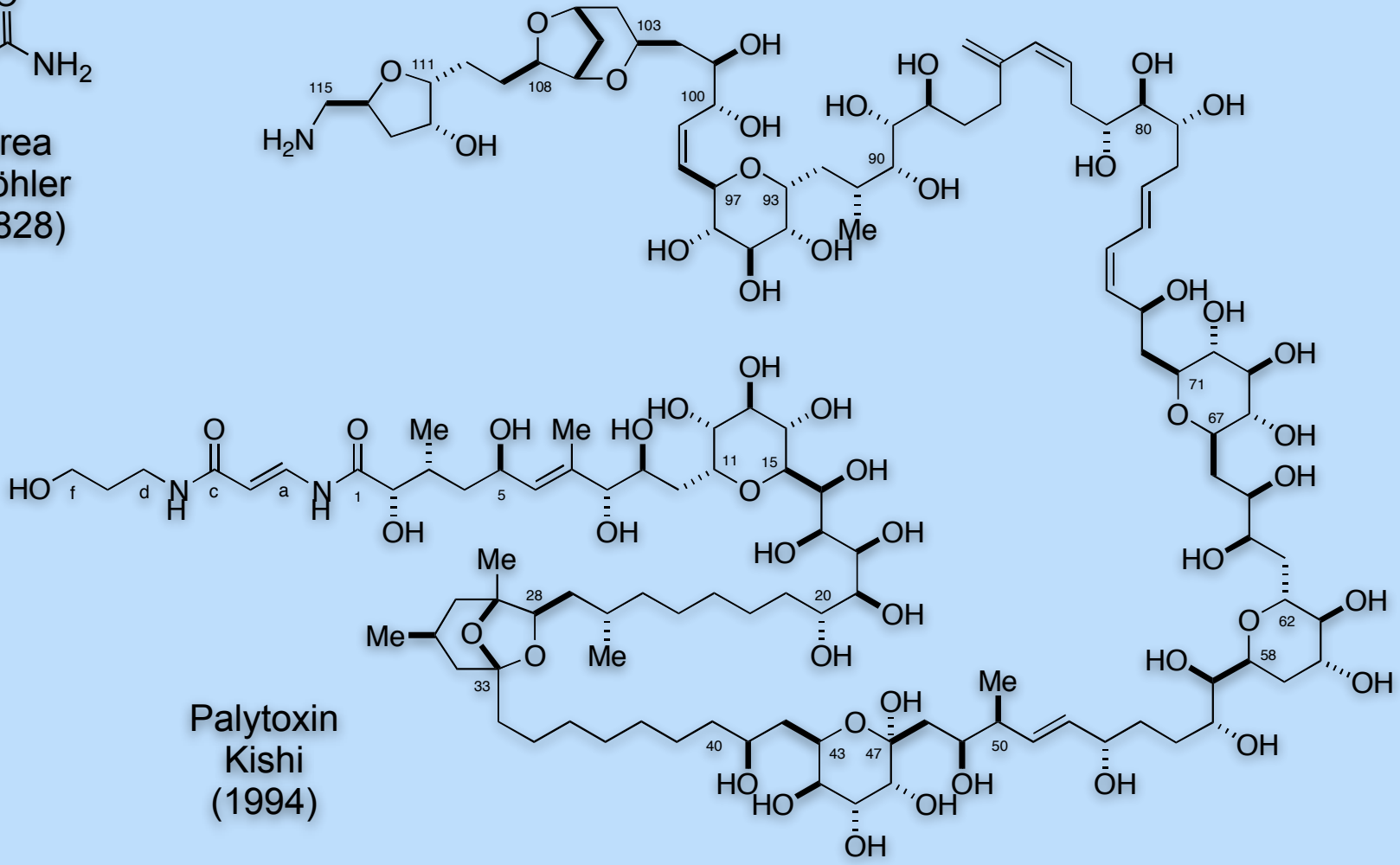
Bio

conjugation

Total Synthesis of Natural Products



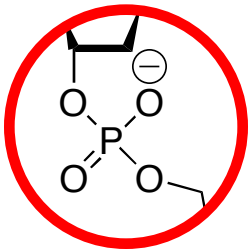
Urea
Wöhler
(1828)



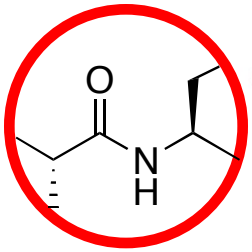
Palytoxin
Kishi
(1994)

Biological Macromolecules

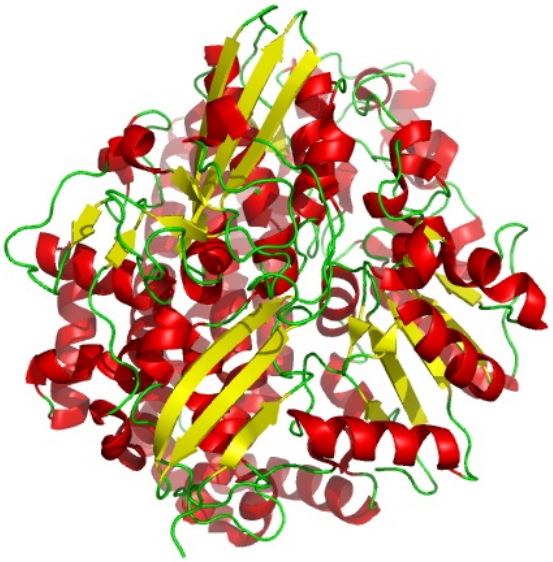
Acide nucléique



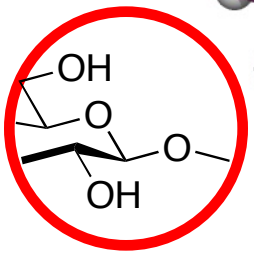
Phosphodiester



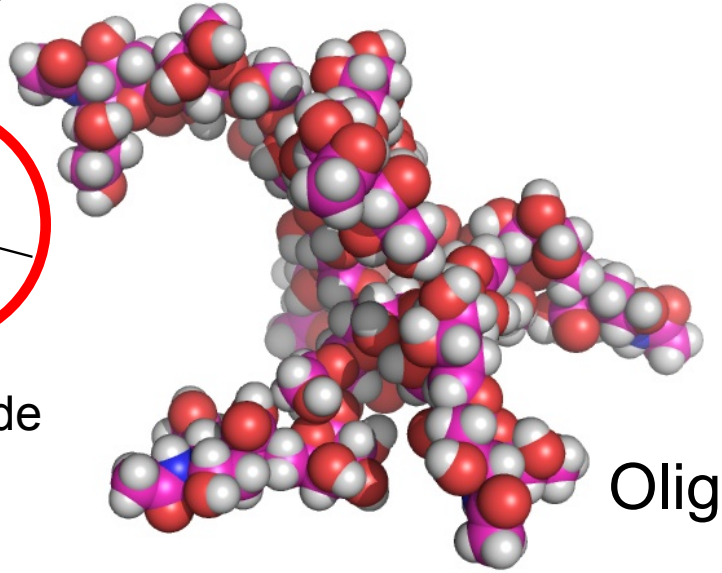
Amide



Protéine

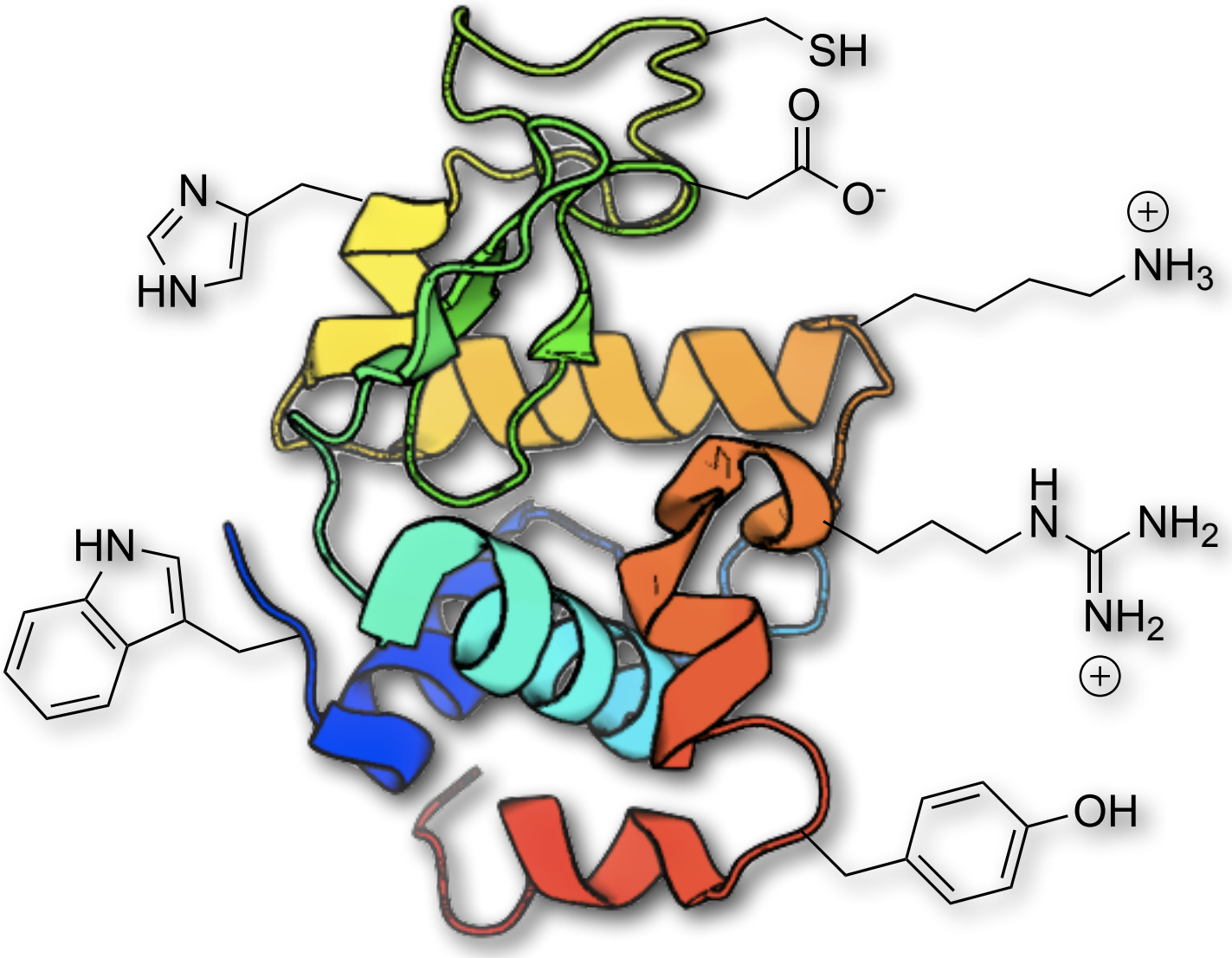


Glycoside



Oligosaccharide

Bioconjugation



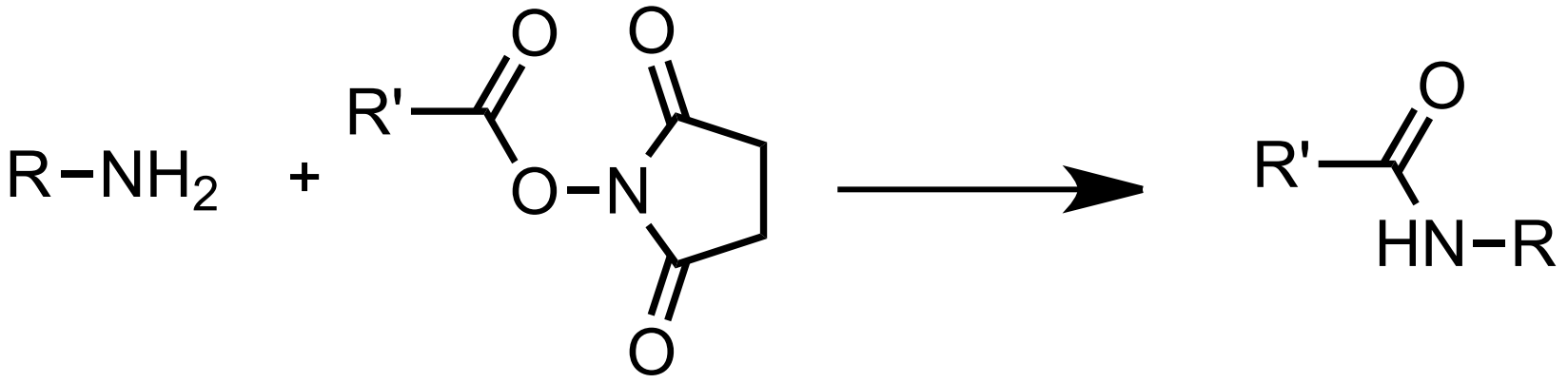
Relevant chemical transformations ?

Chemical Transformations Leading to Protein Covalent Modifications

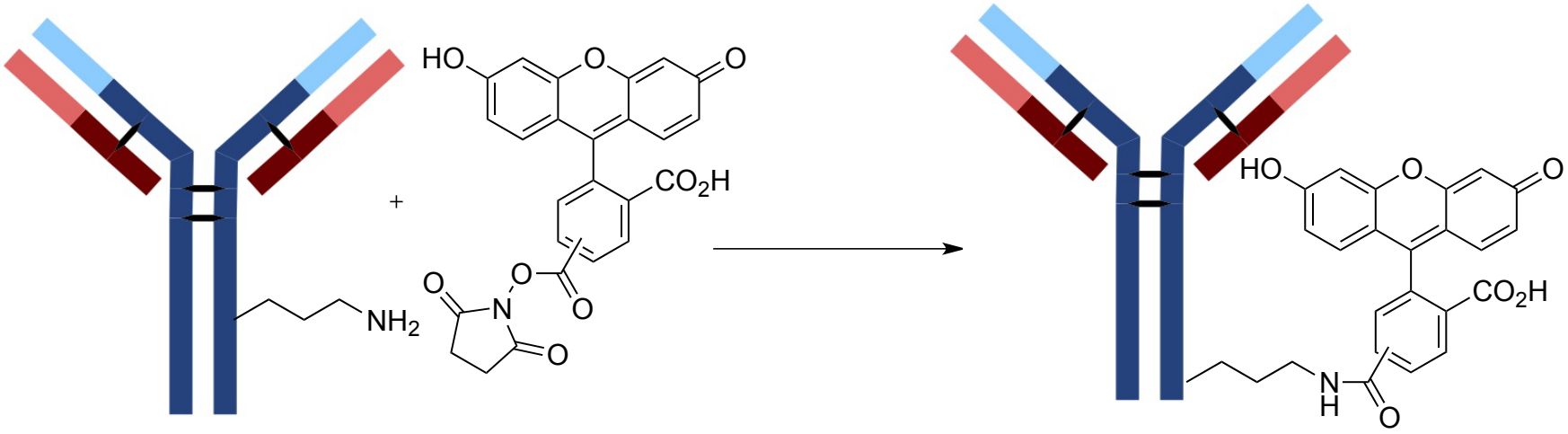
- Water Is the Sole Solvent
- A Neutral pH Is Required
- Ambient Temperature (Up to 40°C)
- Kinetics, which Adapted to the Observed Phenomenon (on the Hour Scale)
- Low Reactant Concentrations
- Nontoxic Reagents

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

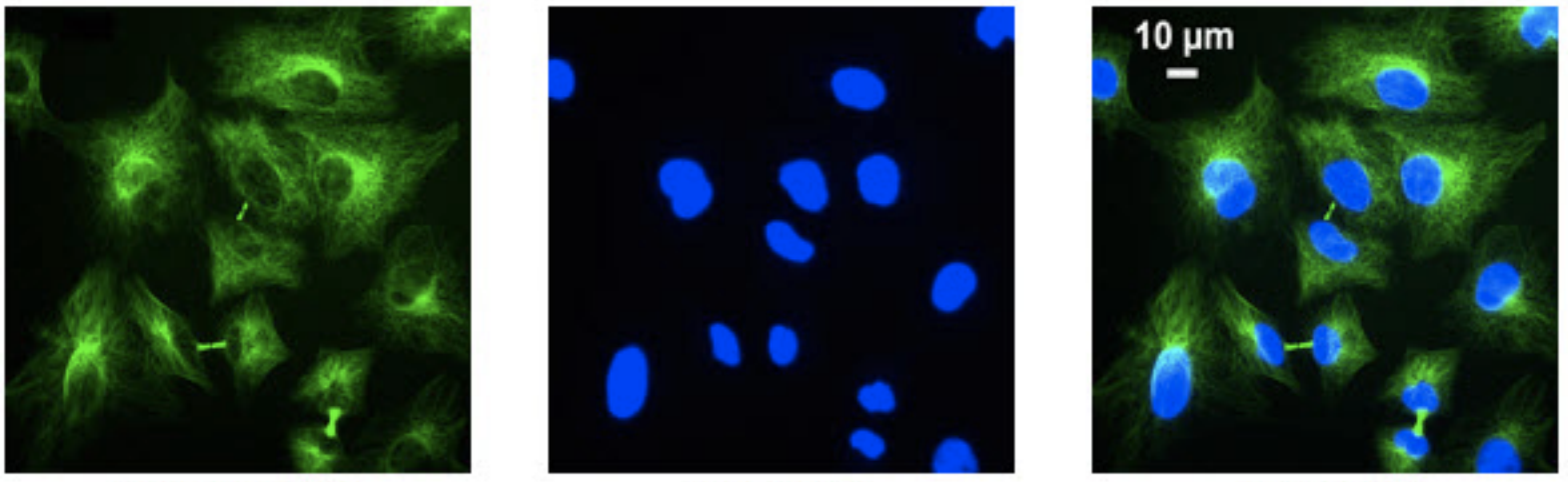
Bioconjugation - Aminolysis



Antibody labeling

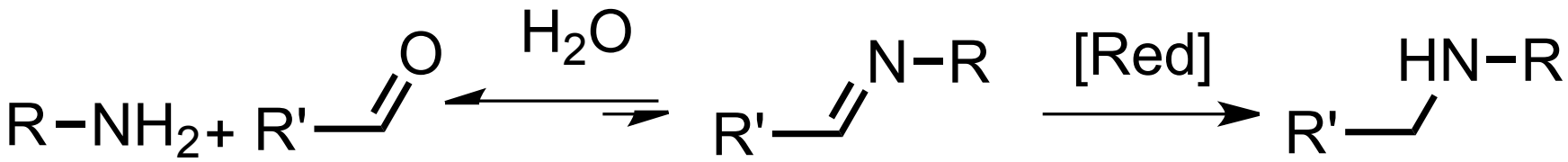


Tubulin imaging

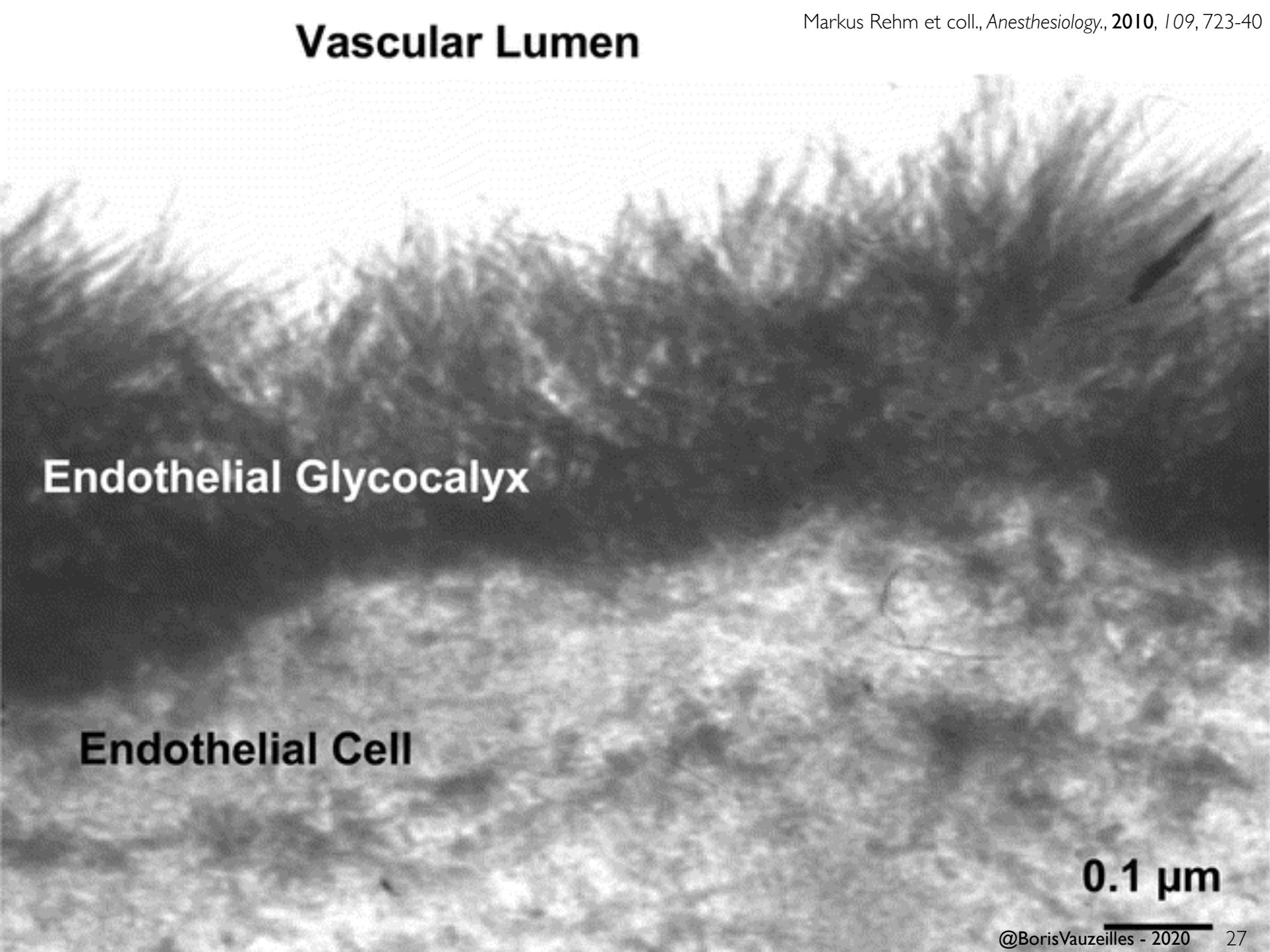


Detection of α-tubulin in A549 cells demonstrates use of fluorescein-labeled secondary antibody
Cells were grown in 96-well microplates for 18-20 hrs, fixed with 4% paraformaldehyde (Part No. 28906) and permeabilized with 0.1% Surfact-Amps X-100 (Part No. 28314). Cells were then probed with a mouse anti-α-tubulin 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

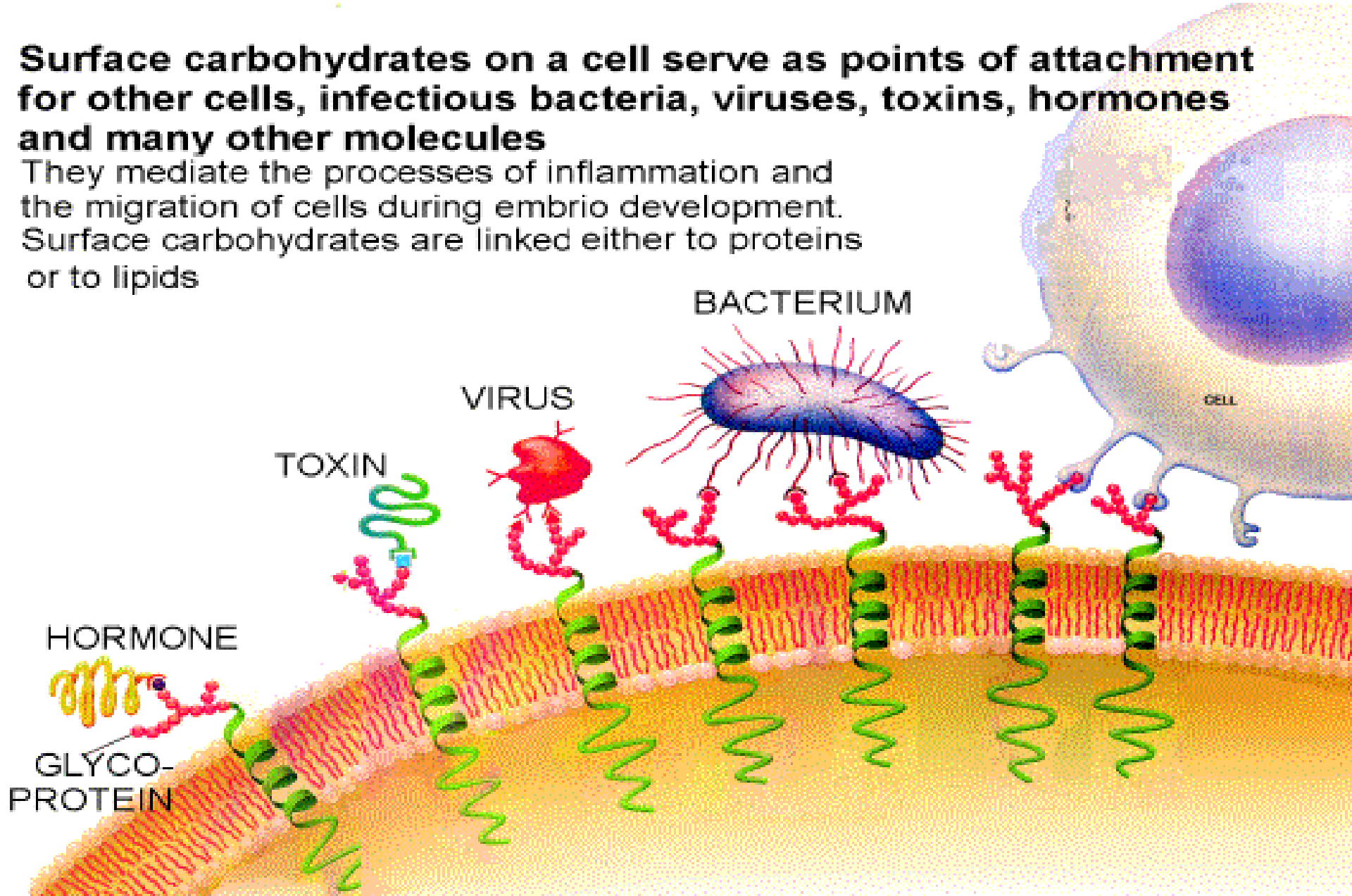


Markus Rehm et coll., *Anesthesiology.*, **2010**, *109*, 723-40

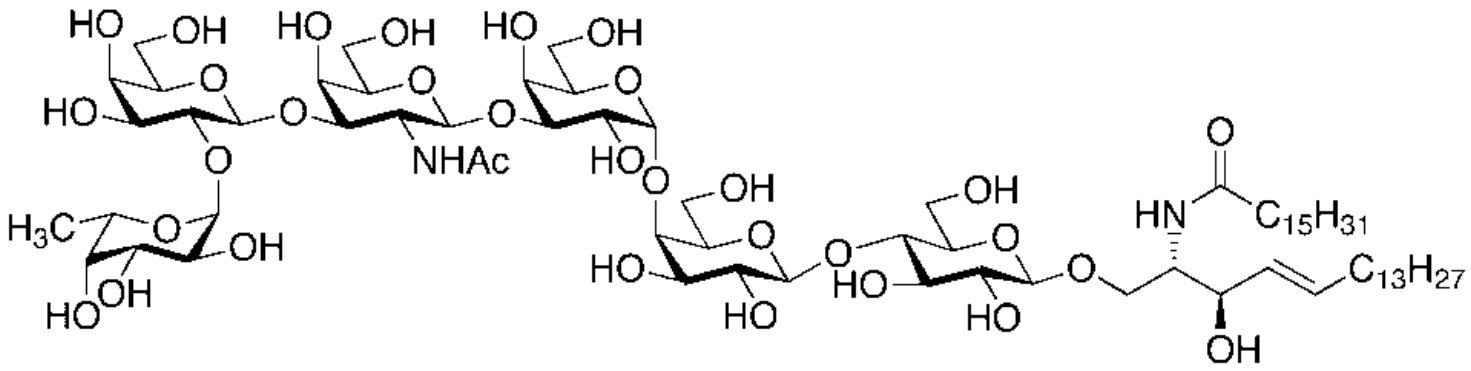


Surface carbohydrates on a cell serve as points of attachment for other cells, infectious bacteria, viruses, toxins, hormones and many other molecules

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



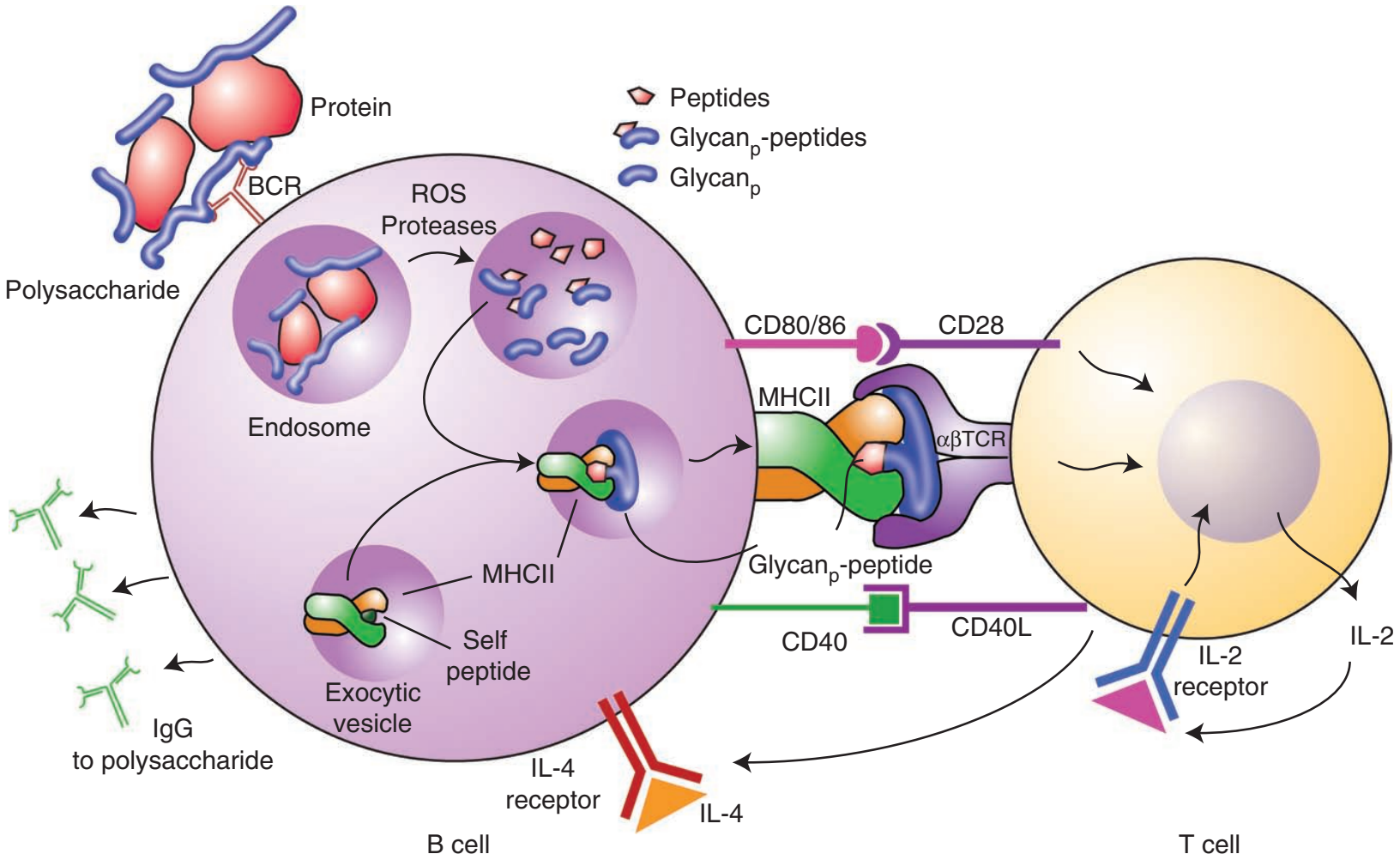
Tumor associated antigens



1: MBr1 antigen / Globo-H

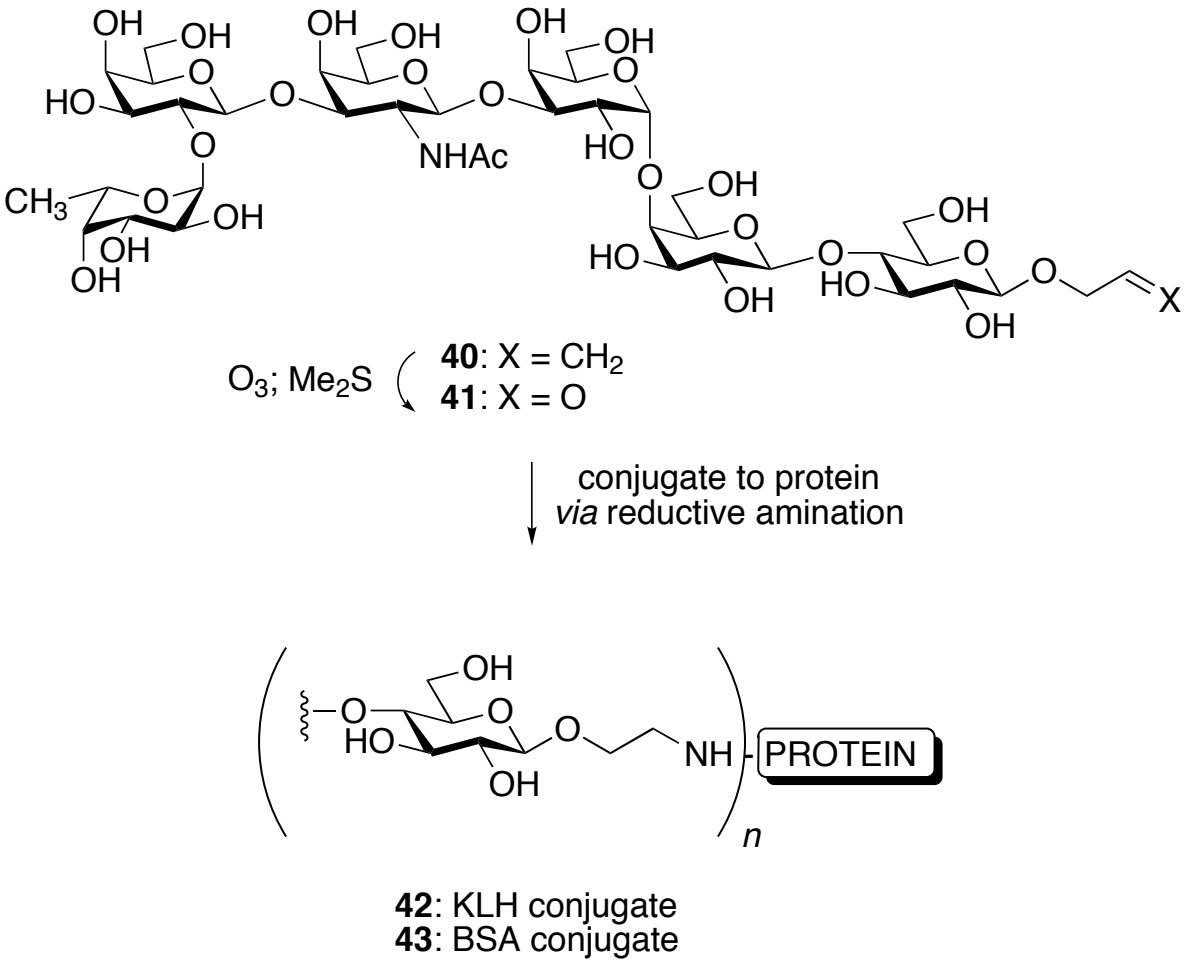
Samuel J. Danishefsky et coll., *Angew. Chem. Int. Ed.*, **2000**, *39*, 836-63

Glycoconjugate vaccines



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

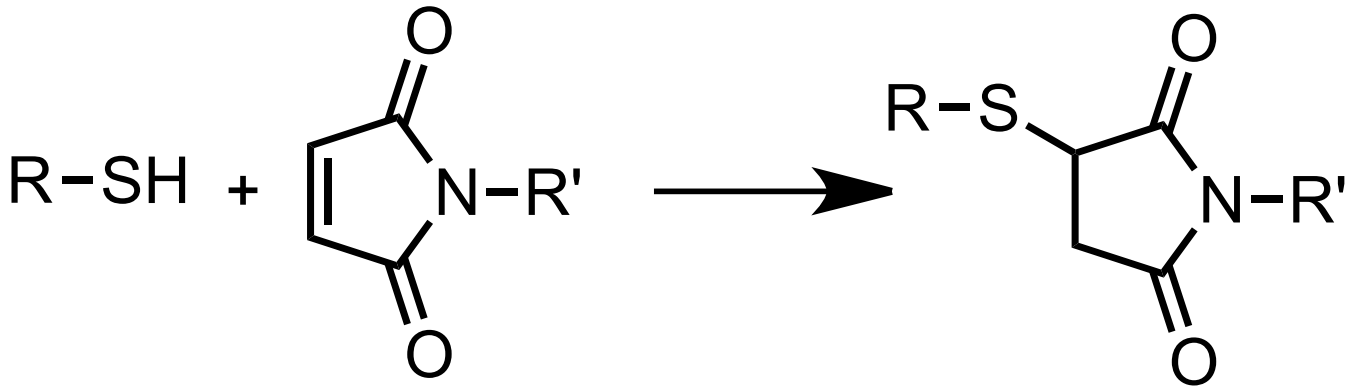
Anticancer vaccines ?



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



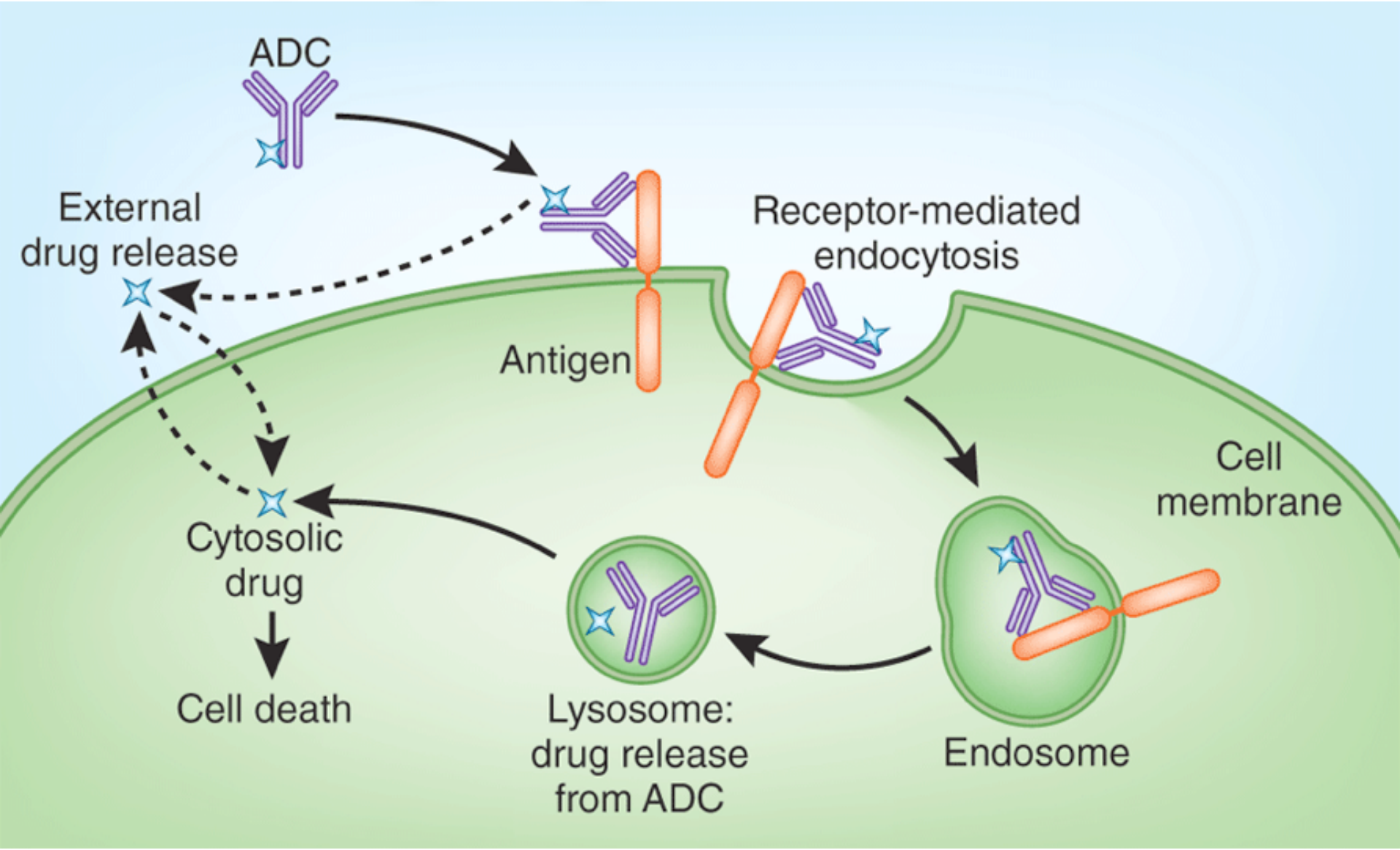
Paul Ehrlich

César Milstein and George Köhler

Indian Ocean sea hare

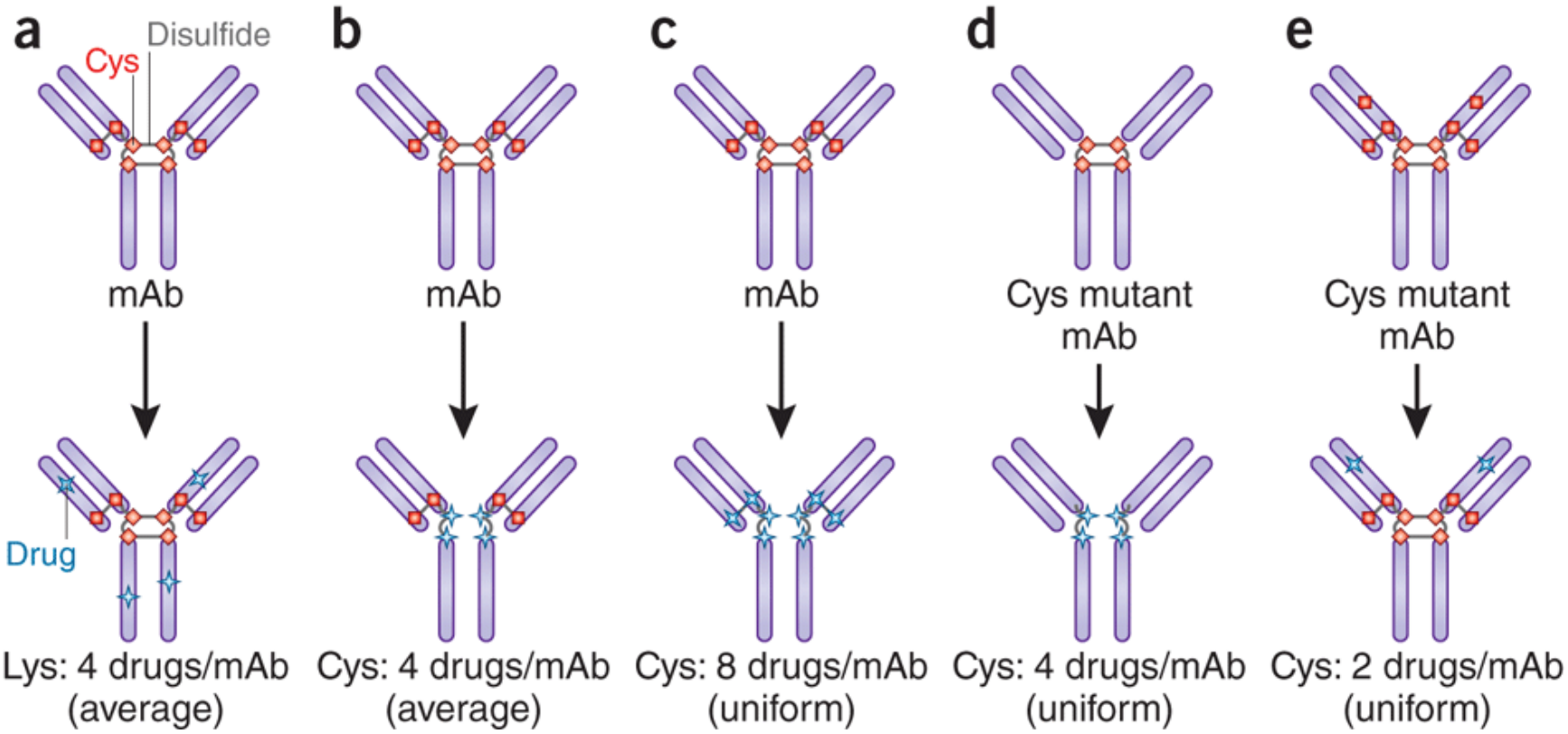
George Pettit

Antibody-Drug conjugates



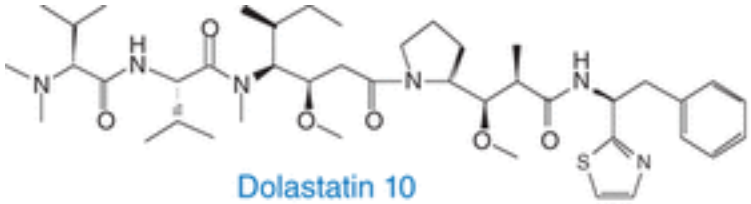
Peter D Senter & Eric L Sievers, *Nature Biotechnol.*, **2012**, *30*, 631-637

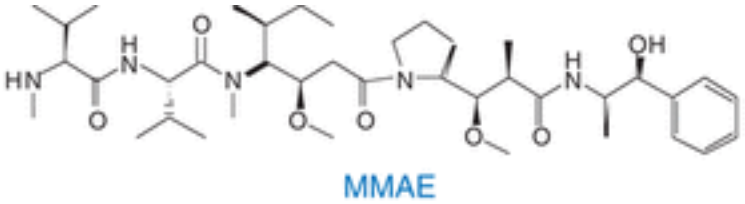
Conjugation technology

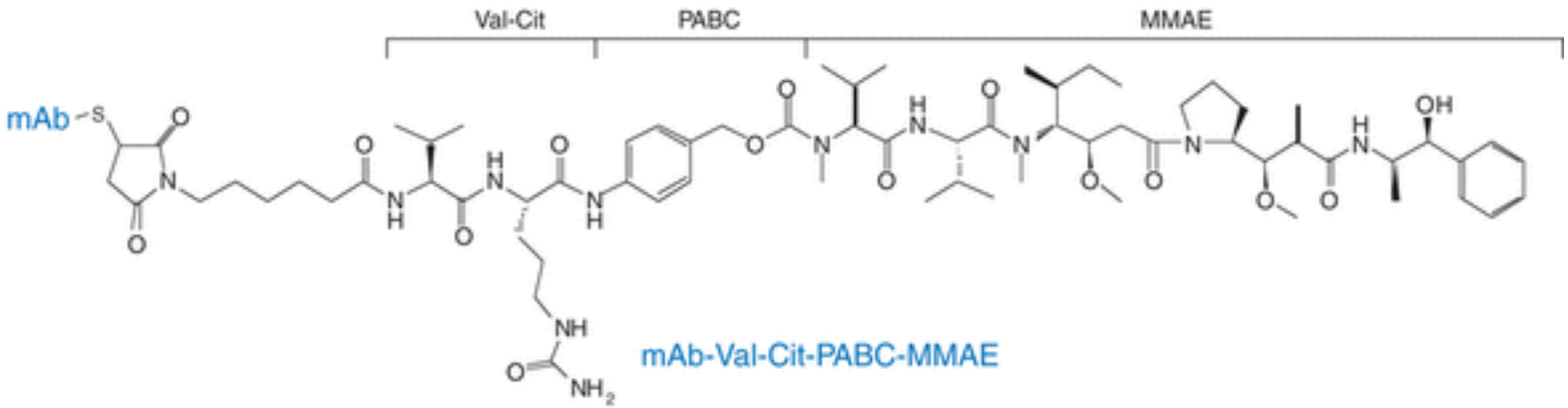


Peter D Senter & Eric L Sievers, *Nature Biotechnol.*, **2012**, *30*, 631-637

Choosing the linker

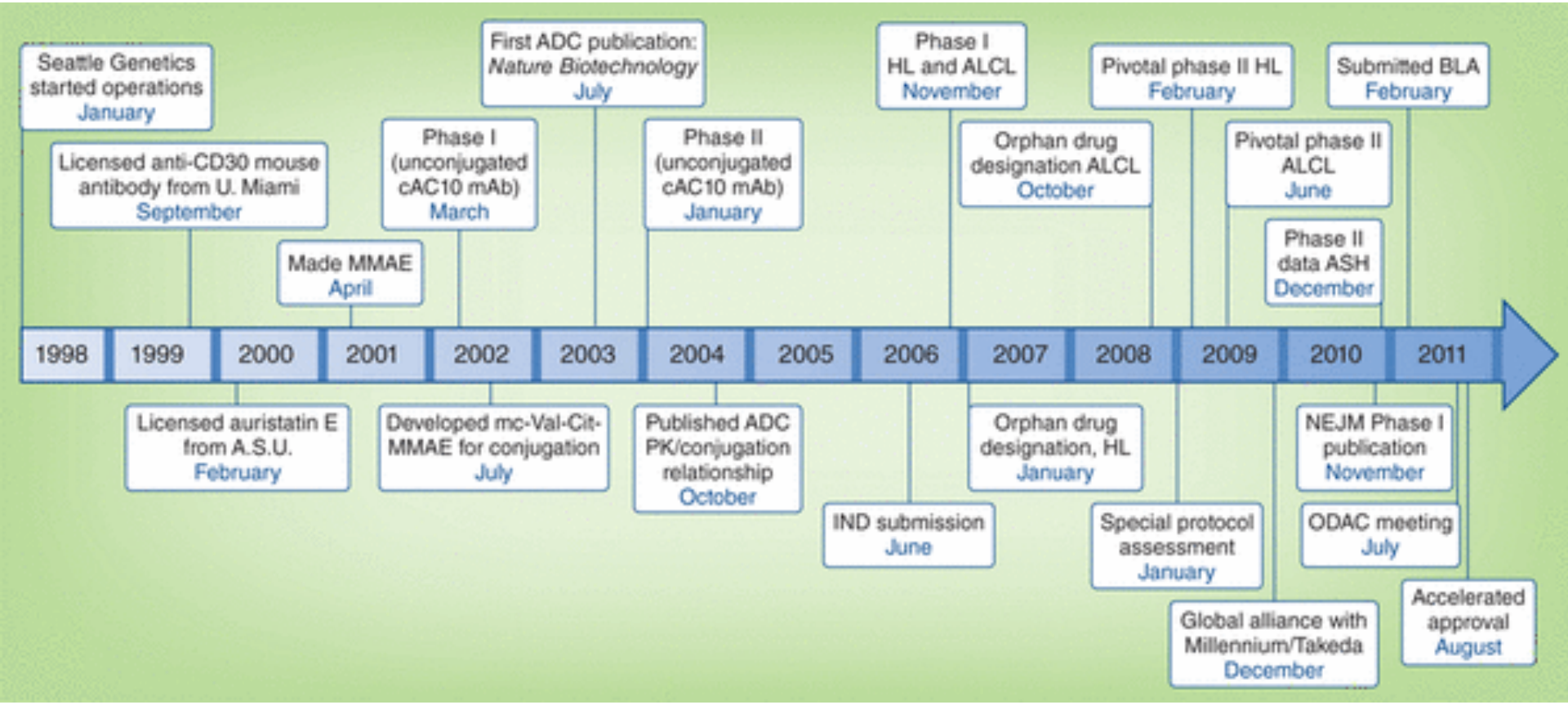






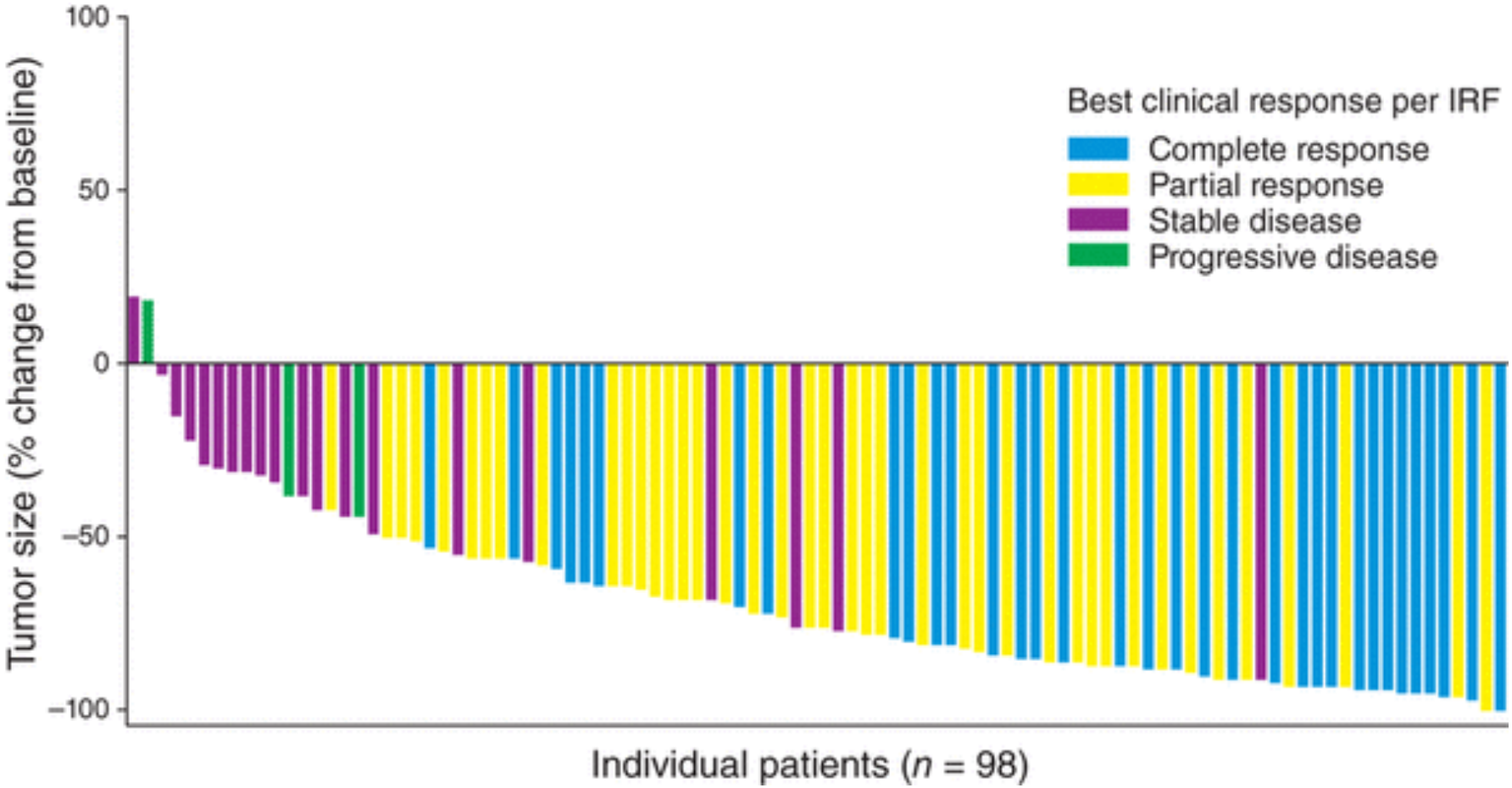
Peter D Senter & Eric L Sievers, *Nature Biotechnol.*, **2012**, *30*, 631-637

Approval

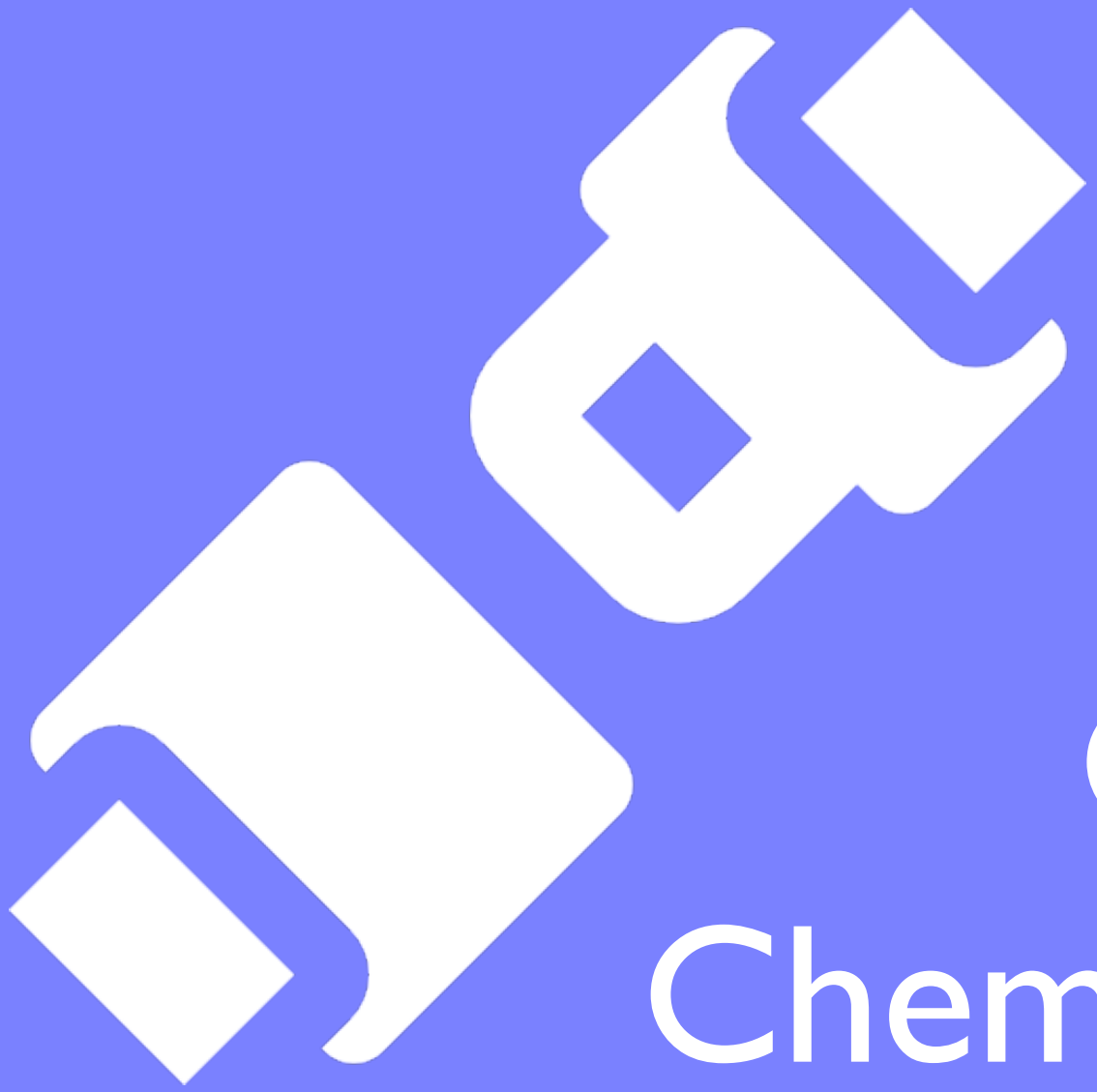


Peter D Senter & Eric L Sievers, *Nature Biotechnol.*, **2012**, *30*, 631-637

Phase II clinical trials... Brentuximab vedotin



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

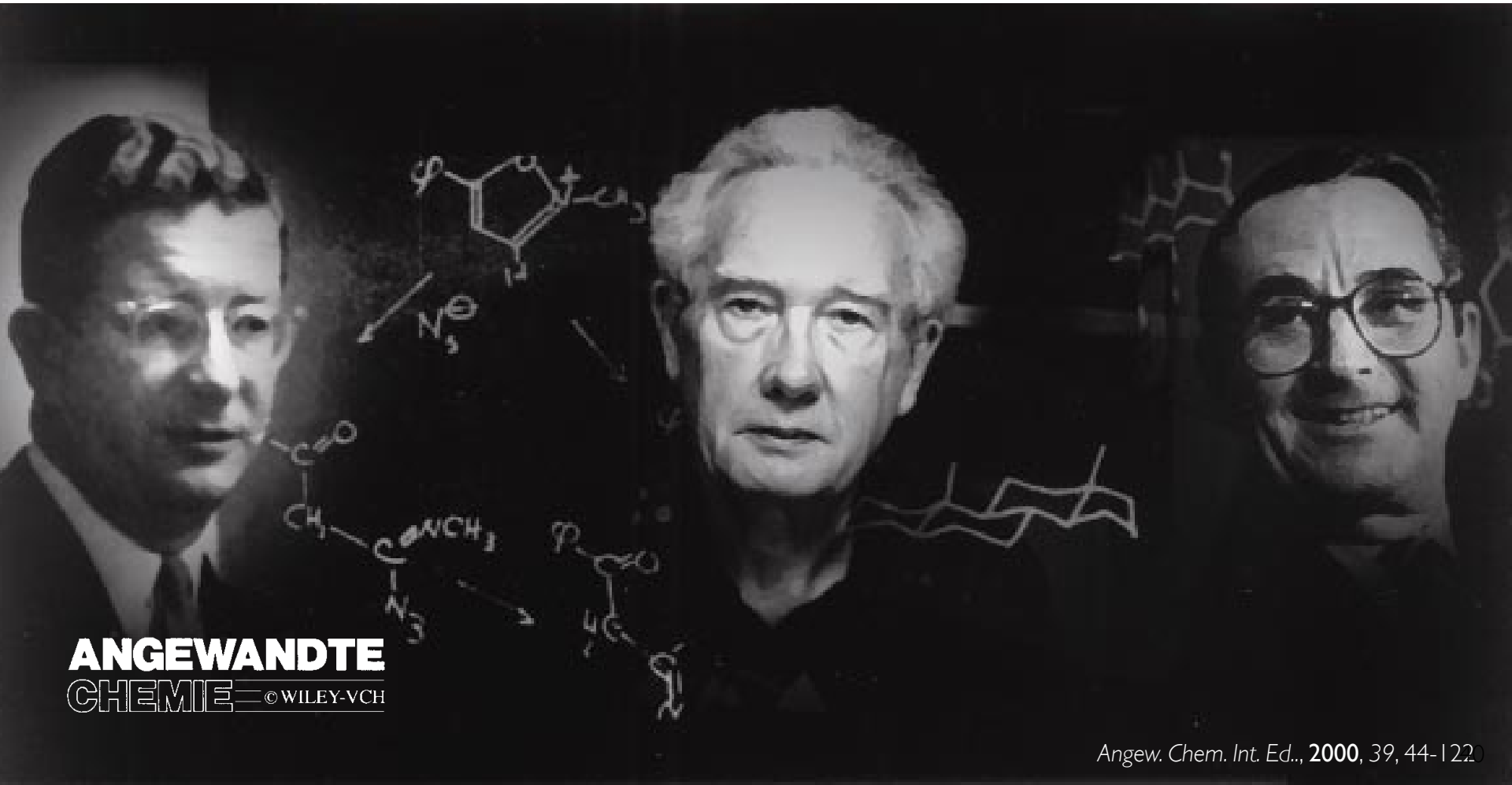


Click Chemistry

The Art and Science of Total Synthesis at the Dawn of the Twenty-First Century**

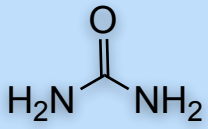
K. C. Nicolaou,* Dionisios Vourloumis, Nicolas Winssinger, and Phil S. Baran

Dedicated to Professor E. J. Corey for his outstanding contributions to organic synthesis

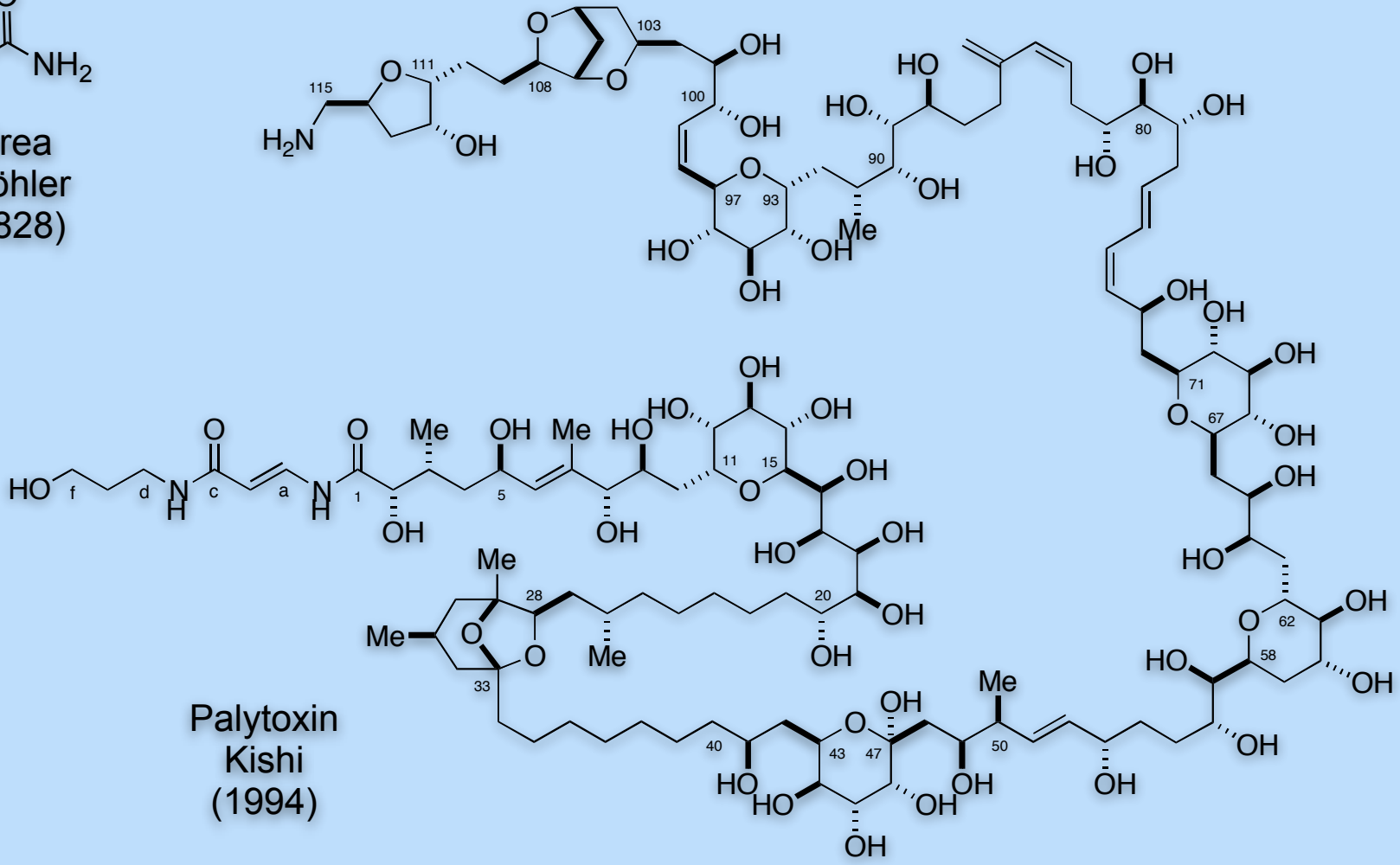


Angew. Chem. Int. Ed., **2000**, *39*, 44-122

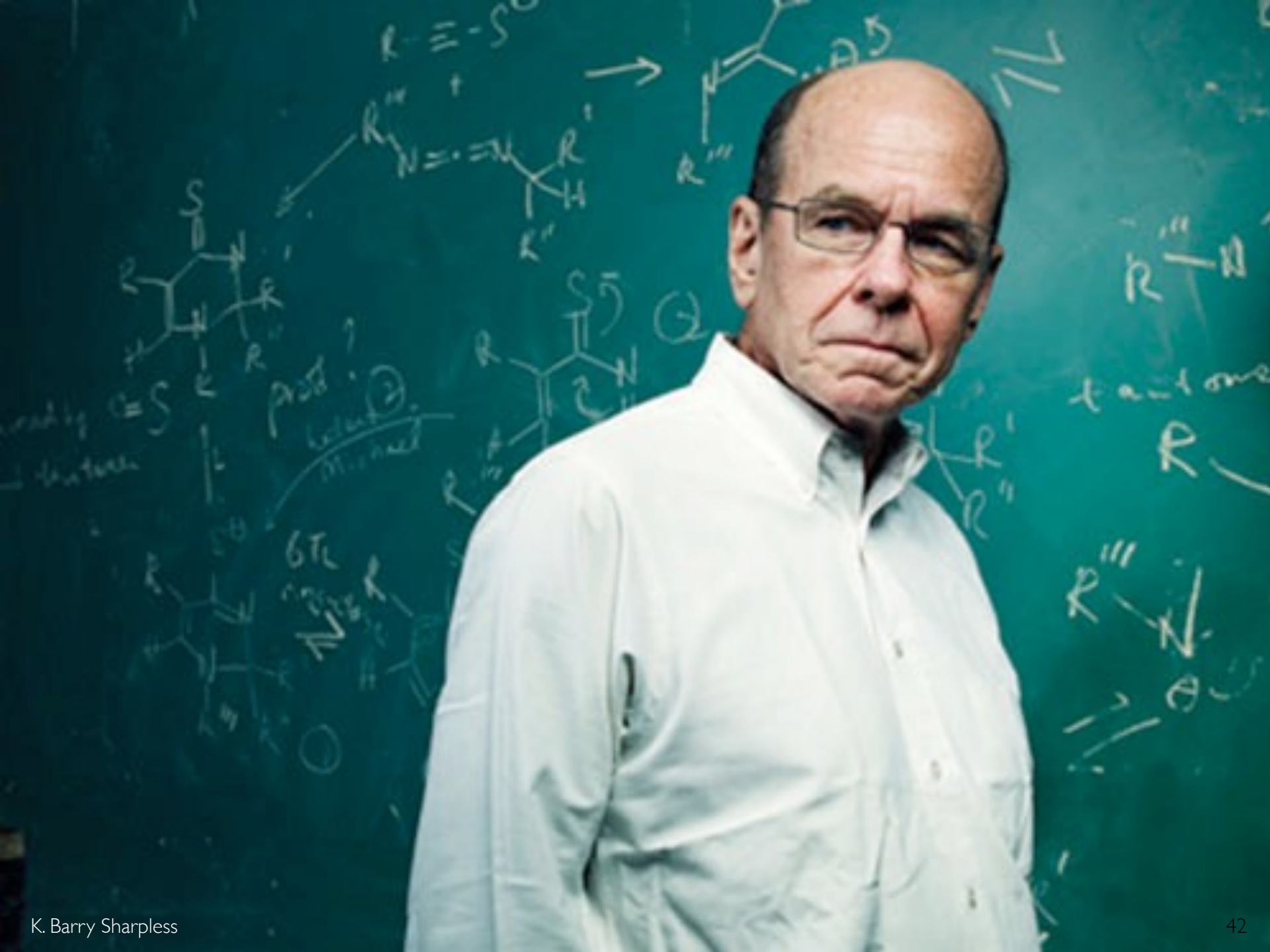
Total Synthesis of Natural Products



Urea
Wöhler
(1828)



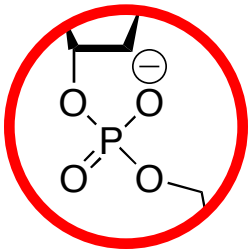
Palytoxin
Kishi
(1994)



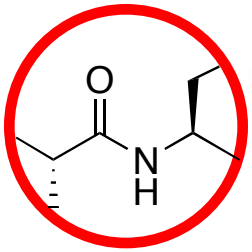
K. Barry Sharpless

Biomacromolecule assembly

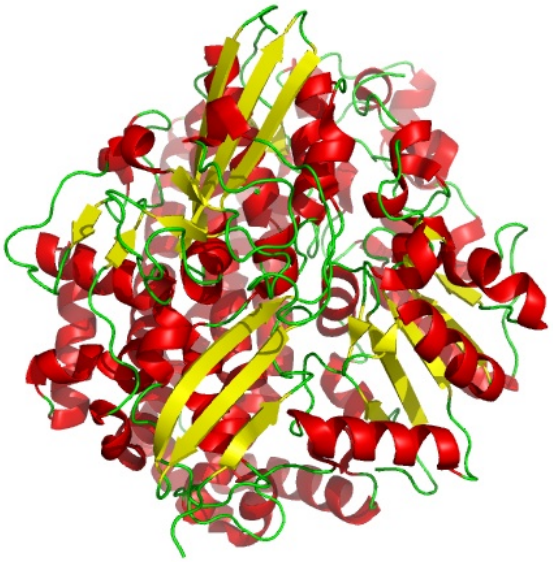
Acide nucléique



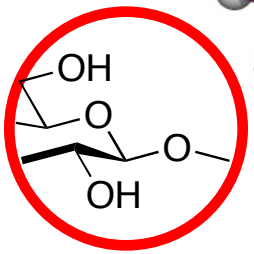
Phosphodiester



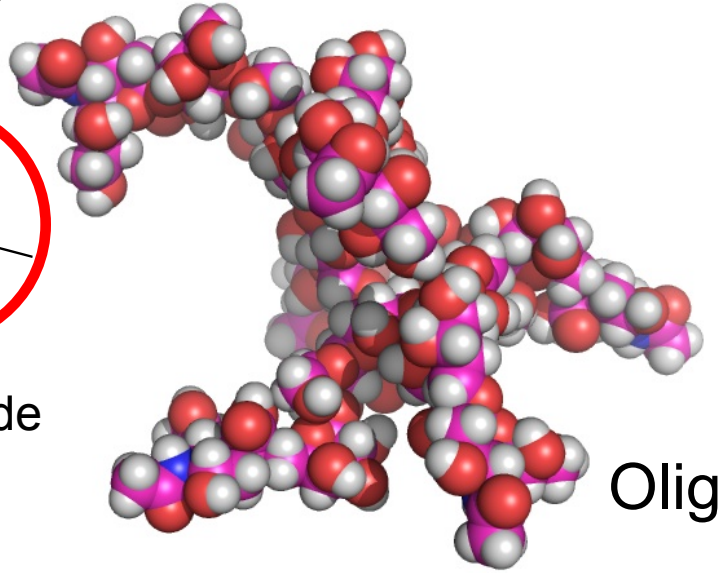
Amide



Protéine



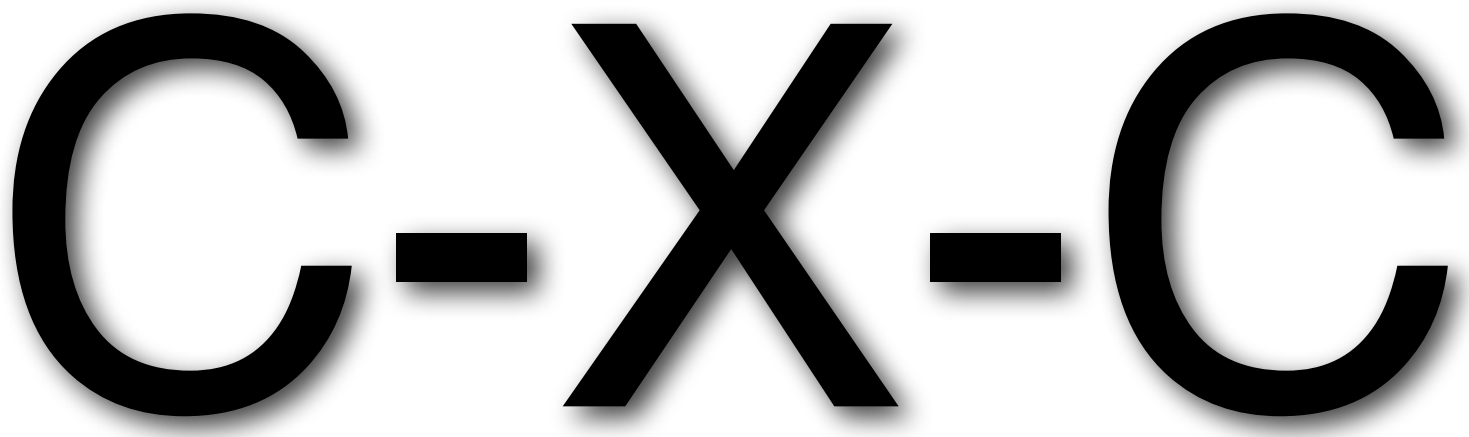
Glycoside



Oligosaccharide

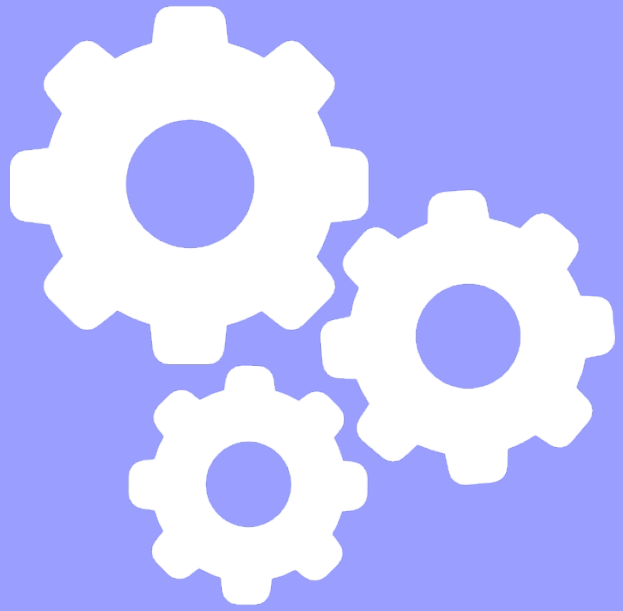
Click Chemistry

Diverse Chemical Function from a Few Good Reactions



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

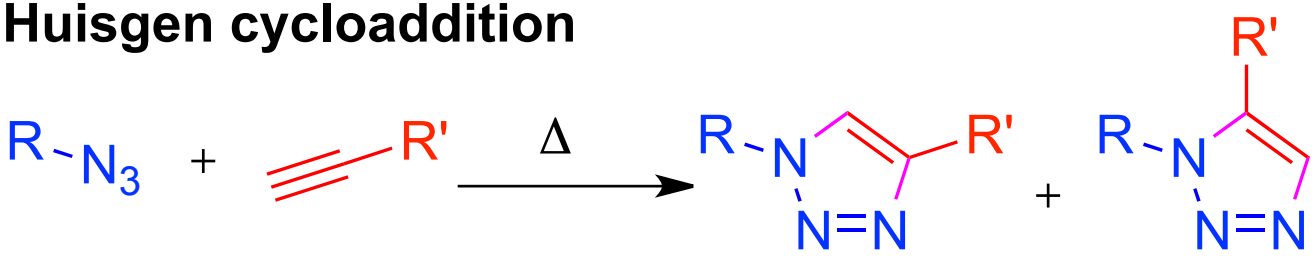
Structure



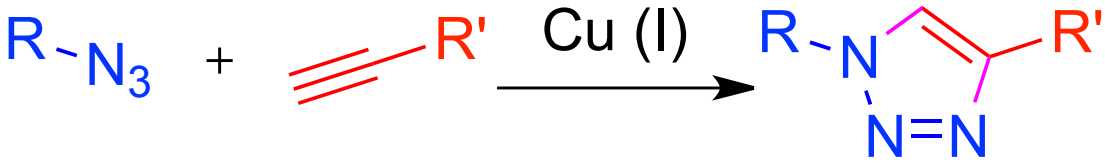
Function

Click chemistry - 2+3 Cycloadditions

Huisgen cycloaddition

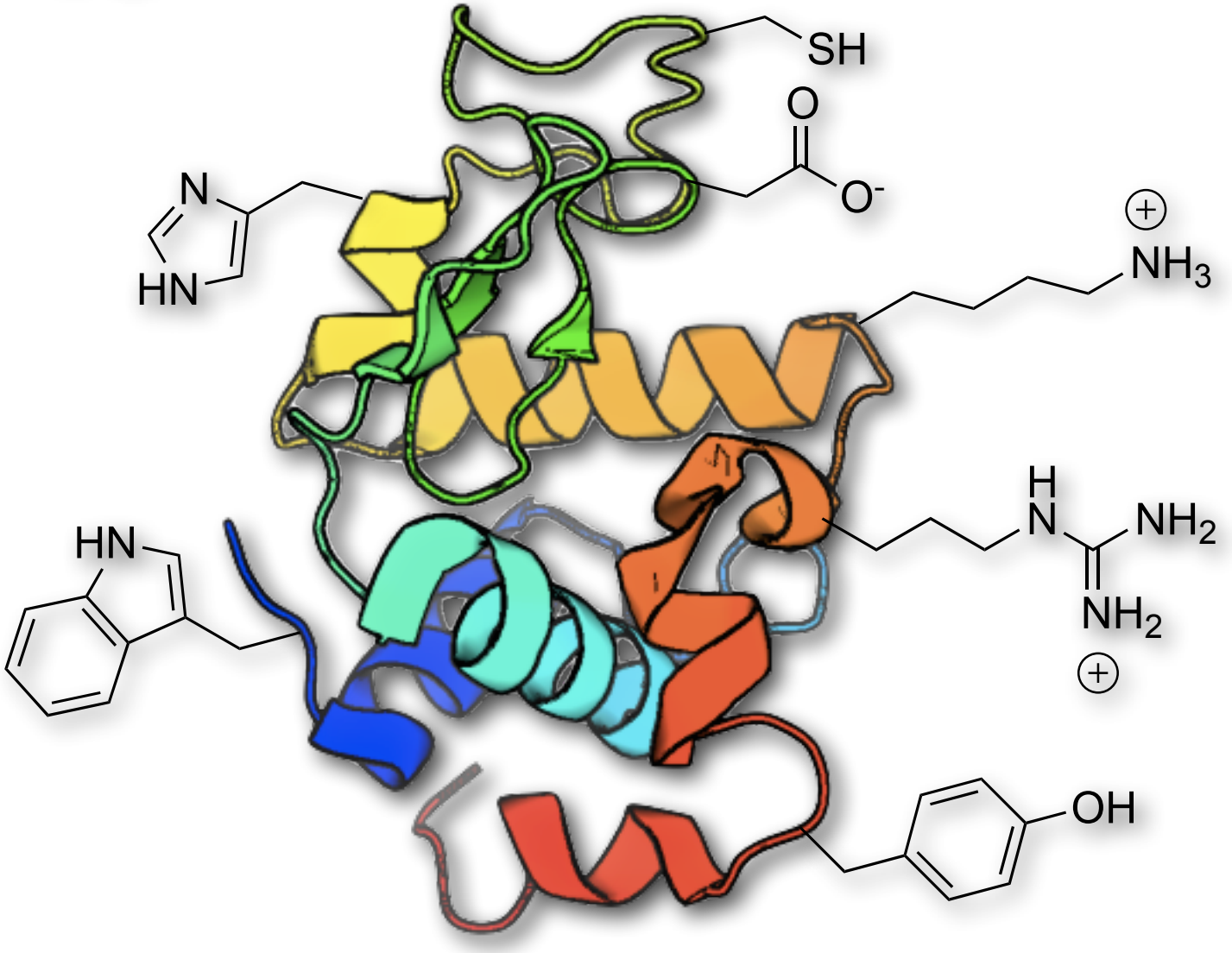


CuAAC

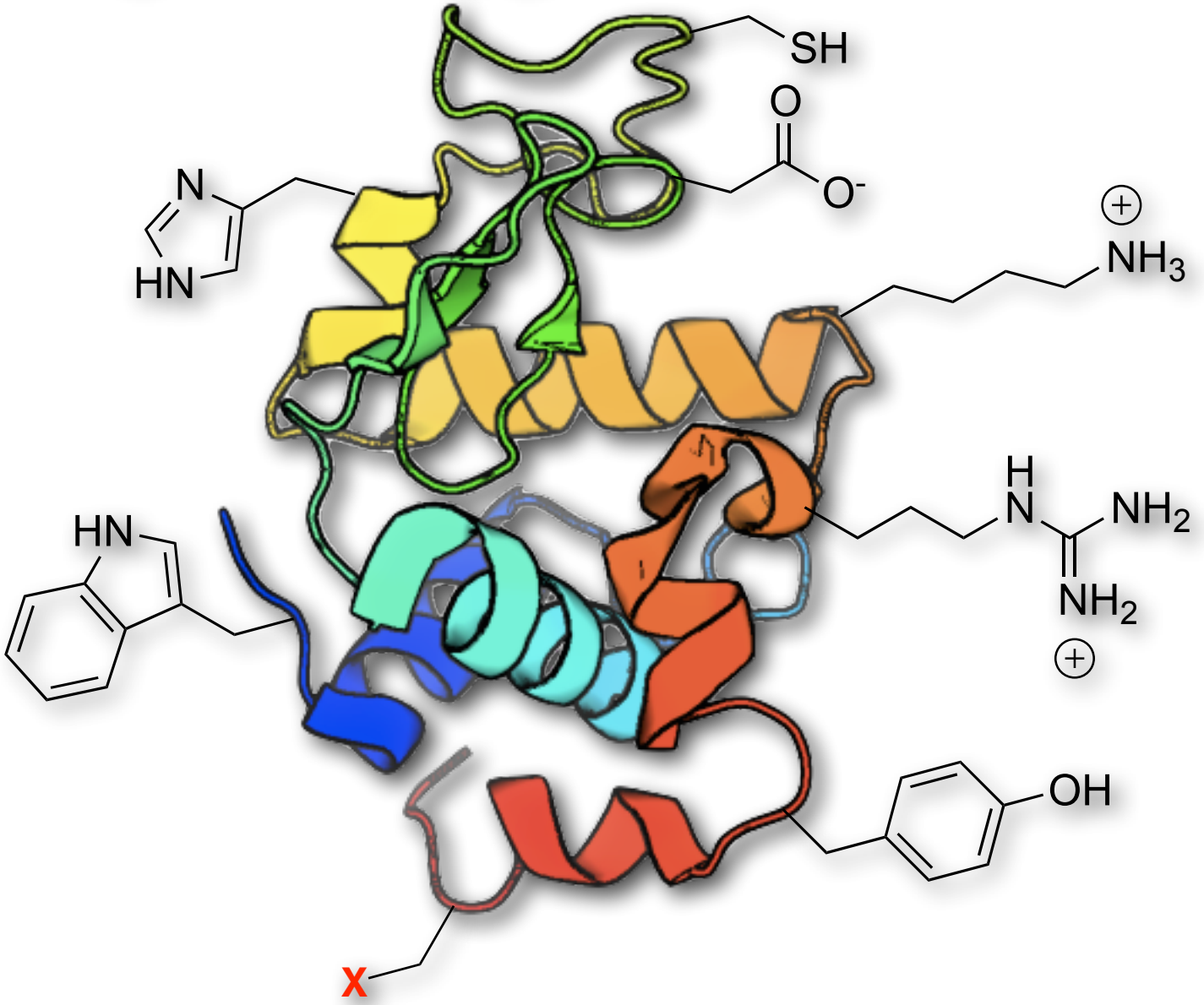


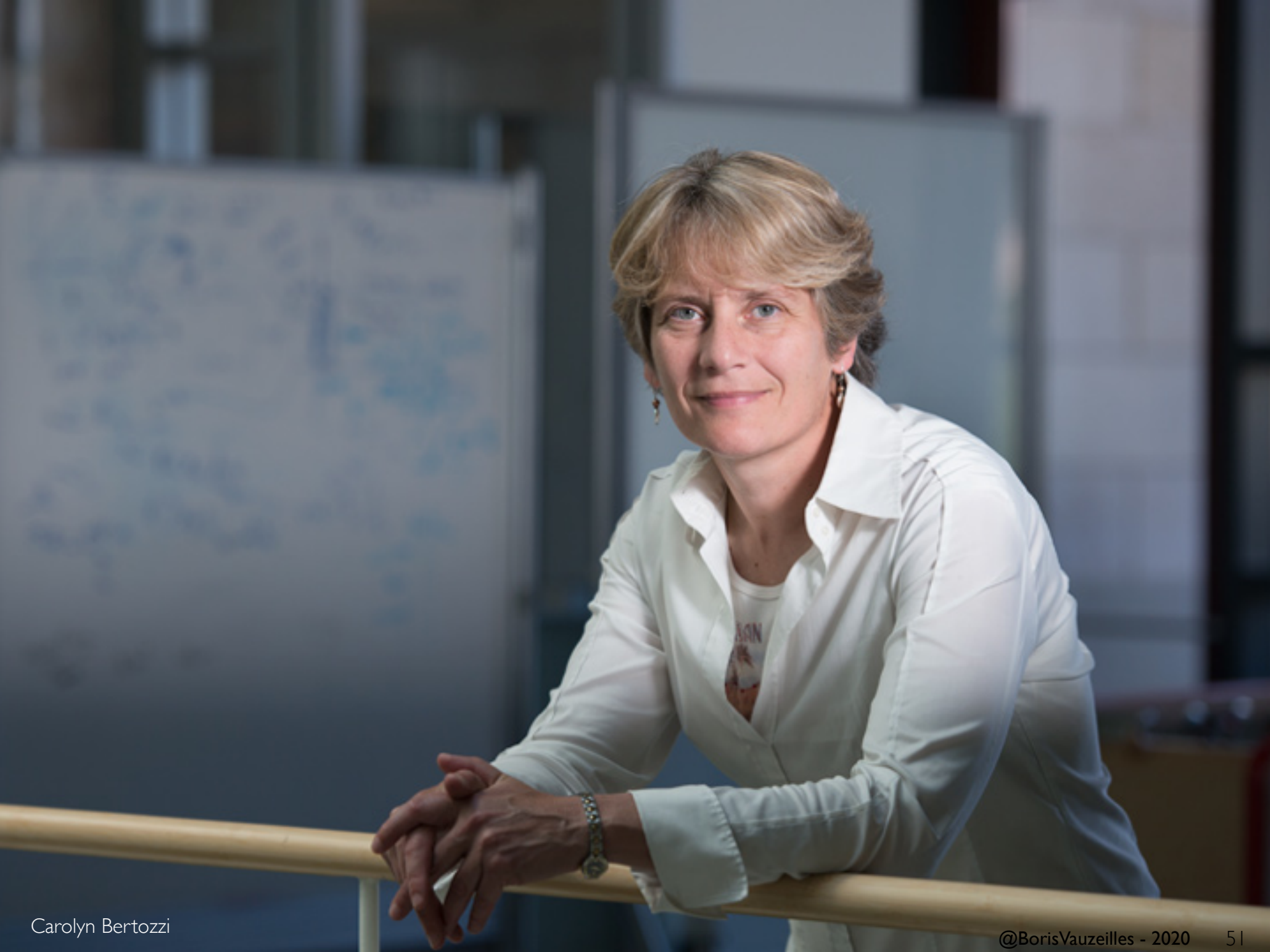
Bioorthogonal Chemistry

Bioconjugation



Bioorthogonal bioconjugation

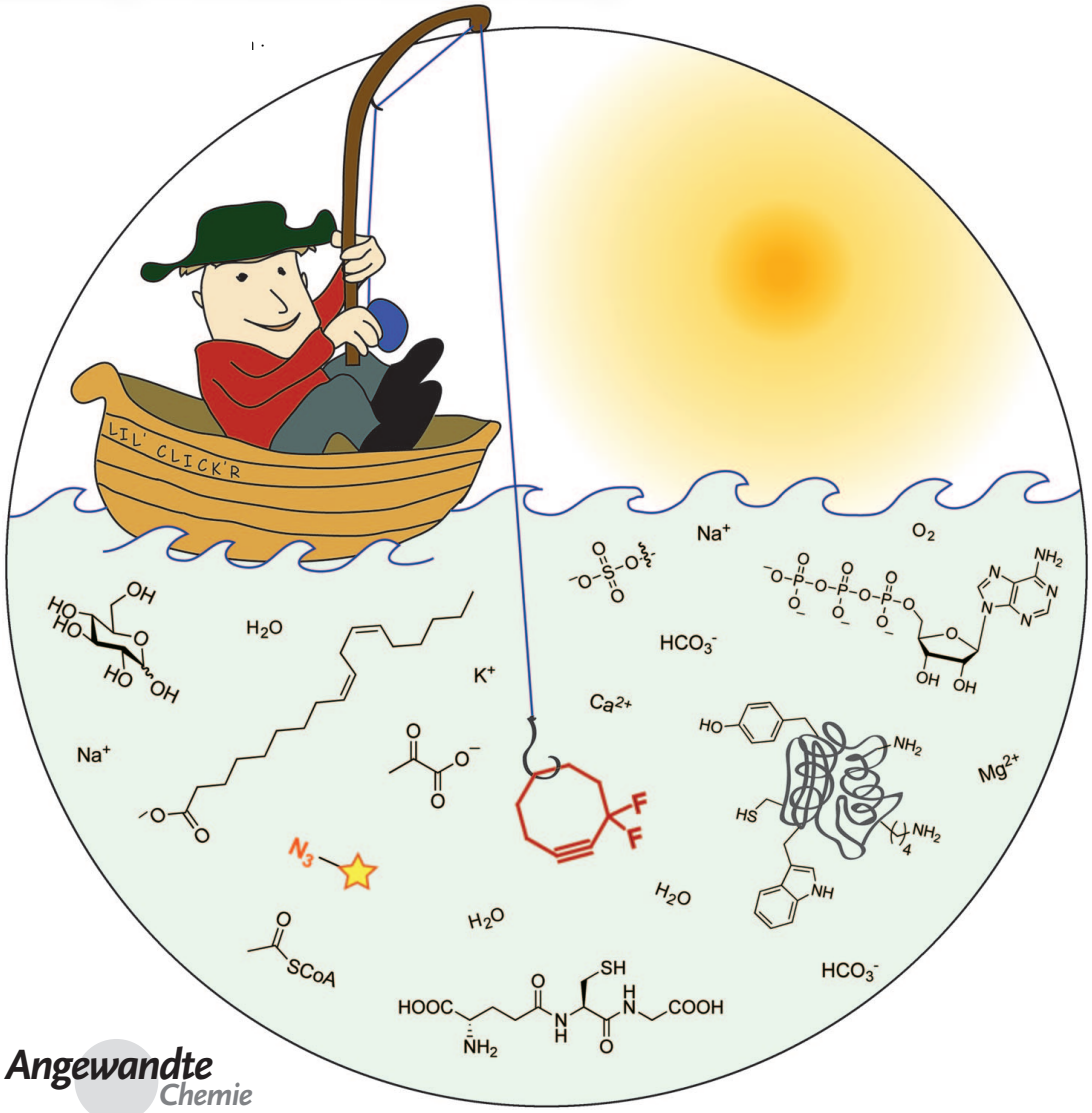




Carolyn Bertozzi

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

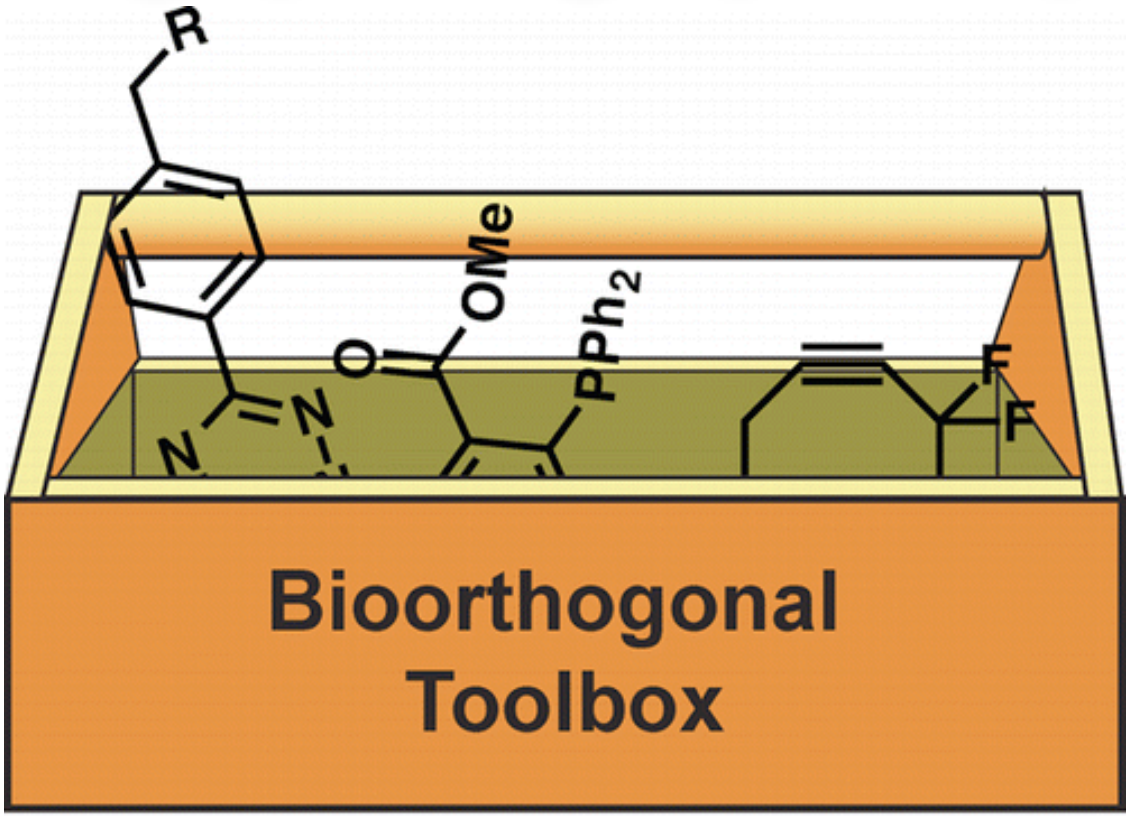
Relevant chemical transformations ?

Chemical Transformations Leading to Protein Covalent Modifications - in live cell or organism

- 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

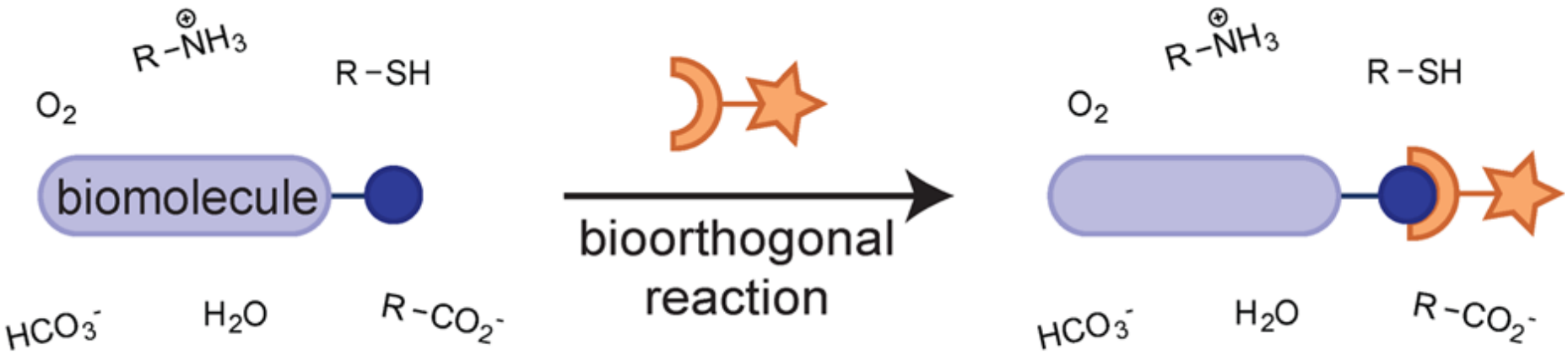
Mathieu Pucheault et coll., *Chem. Biol.*, **2010**, *39*, 213-27

Finding the right (bioorthogonal) chemistry



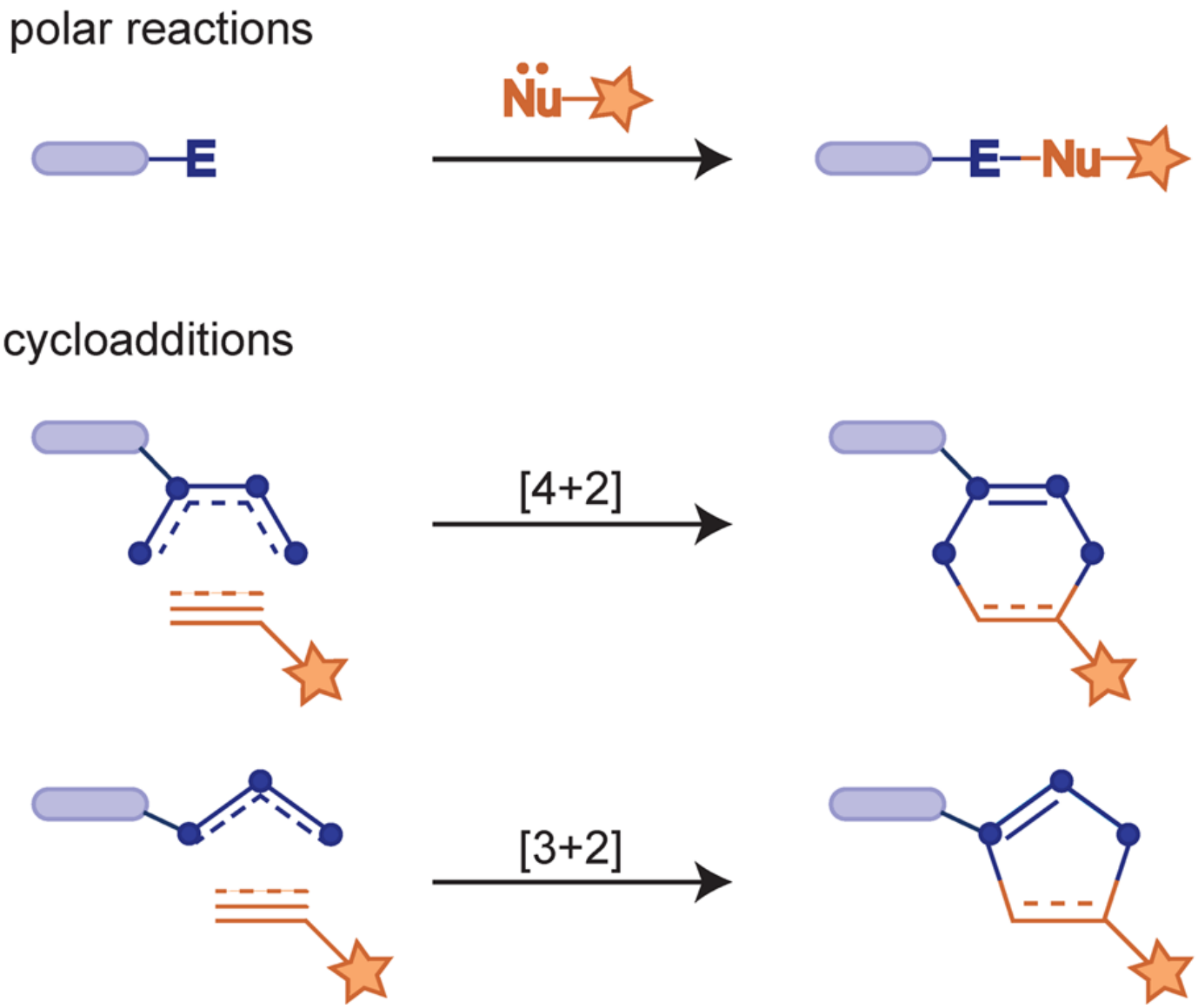
Jennifer A. Prescher *et coll.*, *ACS. Chem. Biol.*, **2014**, *9*, 592-605

Biocompatibility - Bioorthogonality



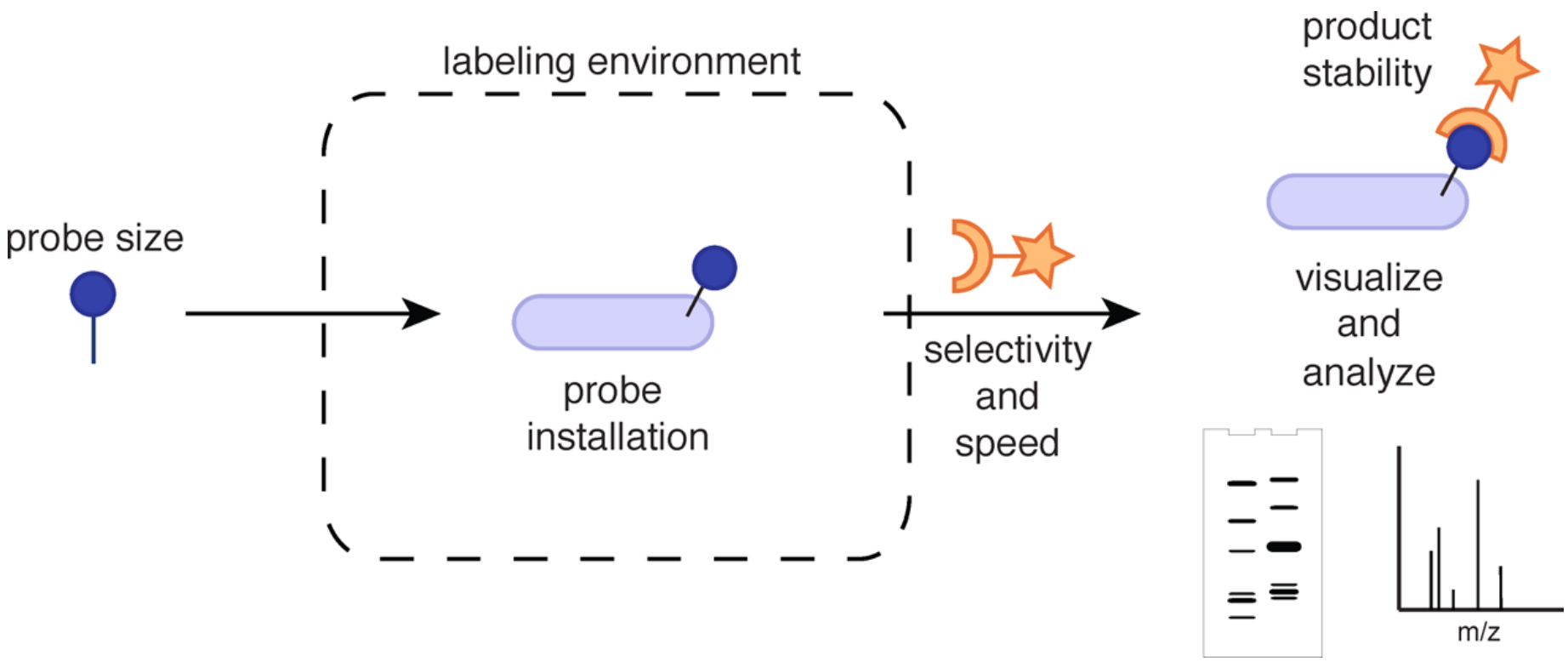
Jennifer A. Prescher *et coll.*, *ACS. Chem. Biol.*, **2014**, *9*, 592-605

Finding the right bioorthogonal reaction



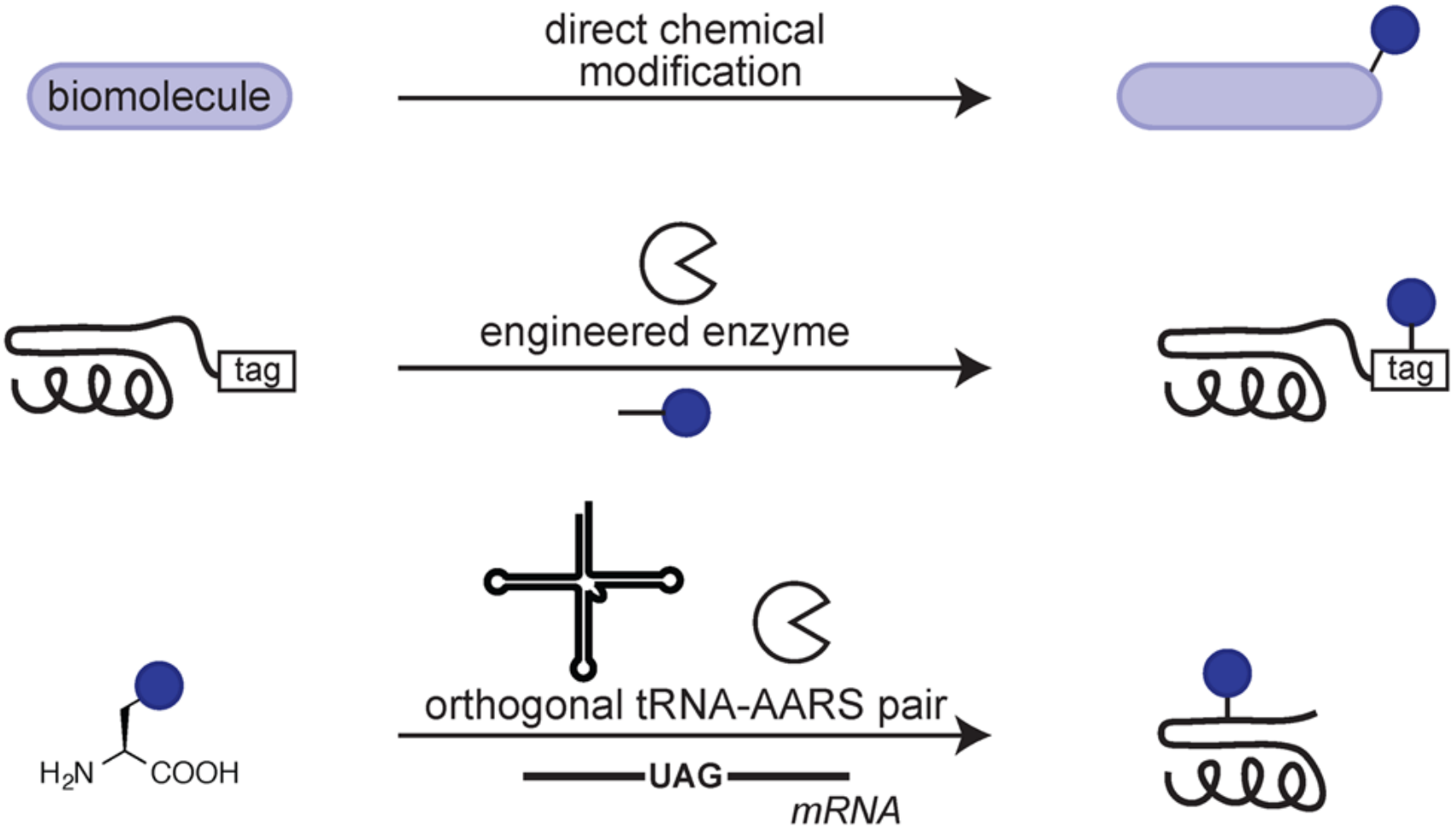
Jennifer A. Prescher *et coll.*, *ACS. Chem. Biol.*, **2014**, *9*, 592-605

Labeling biomolecules using bioorthogonal chemistry



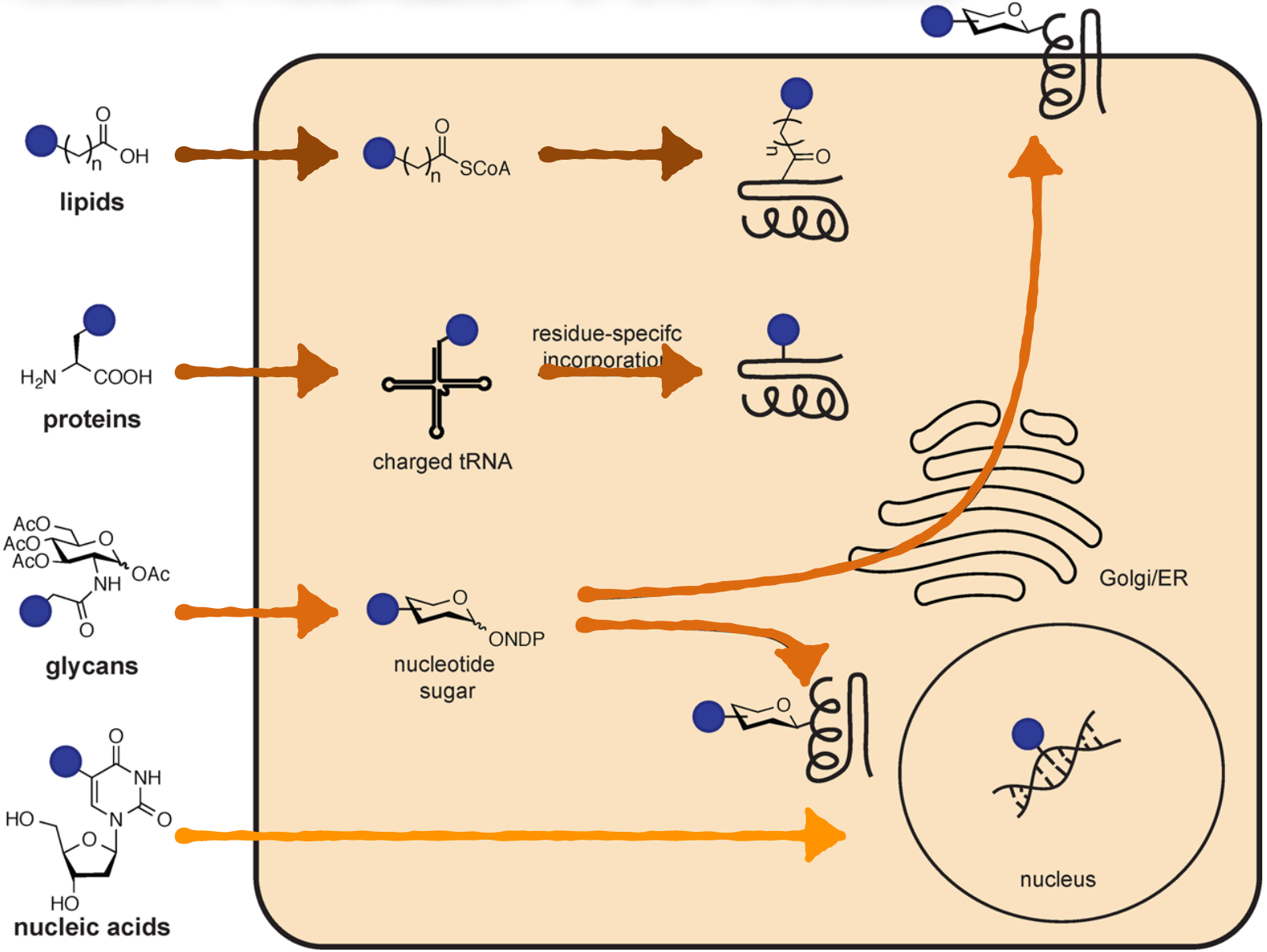
Jennifer A. Prescher *et coll.*, *ACS. Chem. Biol.*, **2014**, *9*, 592-605

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

Bioorthogonal chemistry - Staudinger ligation

Staudinger reduction

Hermann Staudinger

Azaylide (Iminophosphorane) formation

Triphenylphosphin-phenylimin.

$$(C_6H_5)_3P=N\cdot C_6H_5$$

Phenylazid reagiert mit Triphenylphosphin in unverdünntem Zustand sehr lebhaft unter starker Erwärmung. Die Reaktion wird deshalb am besten in ätherischer Lösung vorgenommen. Dabei kann auch beim Arbeiten unter starker Kühlung das primäre Additionsprodukt nicht isoliert werden, sondern es bildet sich sofort unter Stickstoffentwicklung das obige Phosphiniminderivat.

H.ermann Staudinger, Jules Meyer. *Helv. Chim. Acta*, **1919**, *2*, 635-646

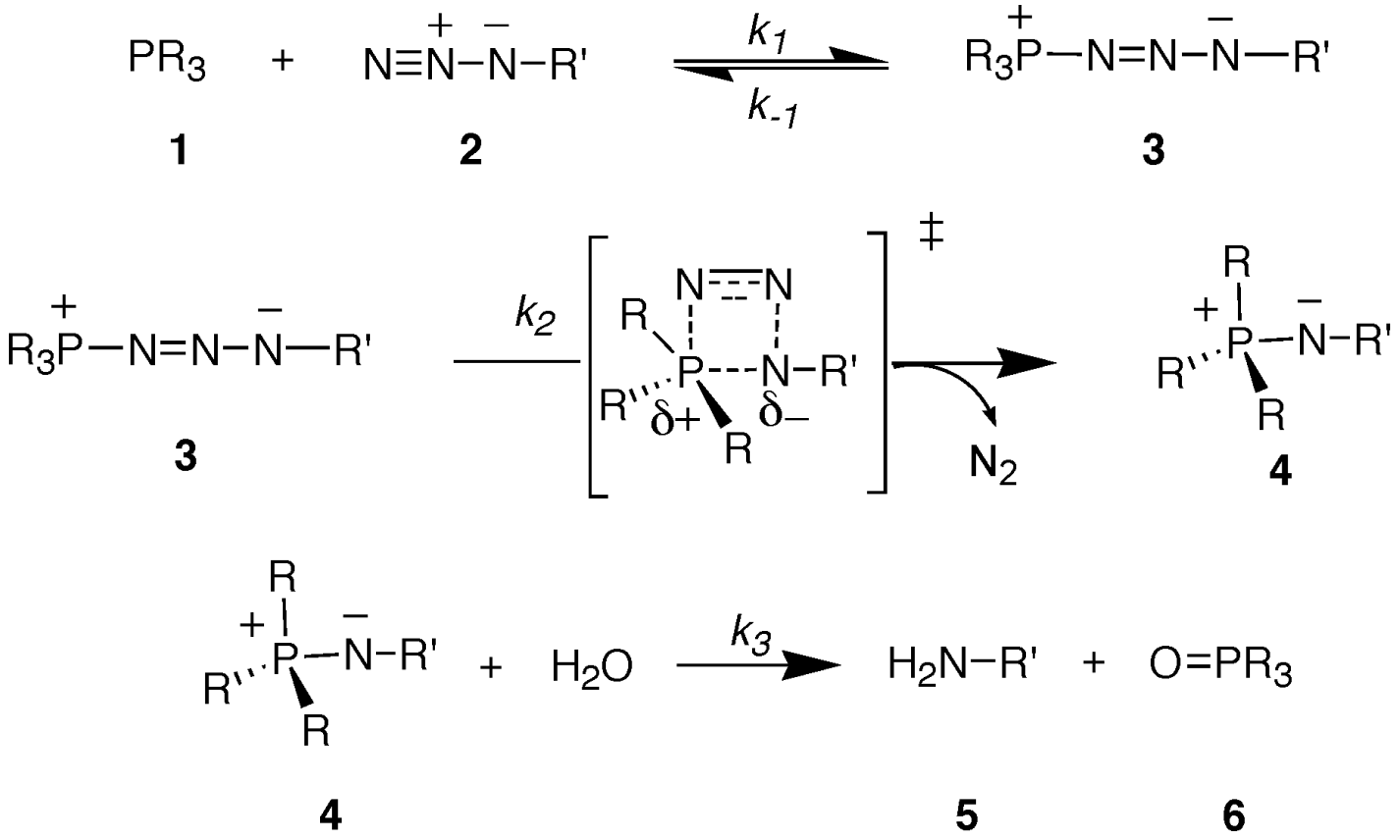
Hydrolysis

Reaktionen des Phosphiniminderivates.

Das Phosphiniminderivat hat schwach basische Eigenschaften. In verdünnter Salzsäure und Schwefelsäure ist es etwas löslich, beim Kochen wird das Produkt in Phosphinoxyd und Anilin resp. Anilinsalze hydrolysiert.

H.ermann Staudinger, Jules Meyer. *Helv. Chim. Acta*, **1919**, *2*, 635-646

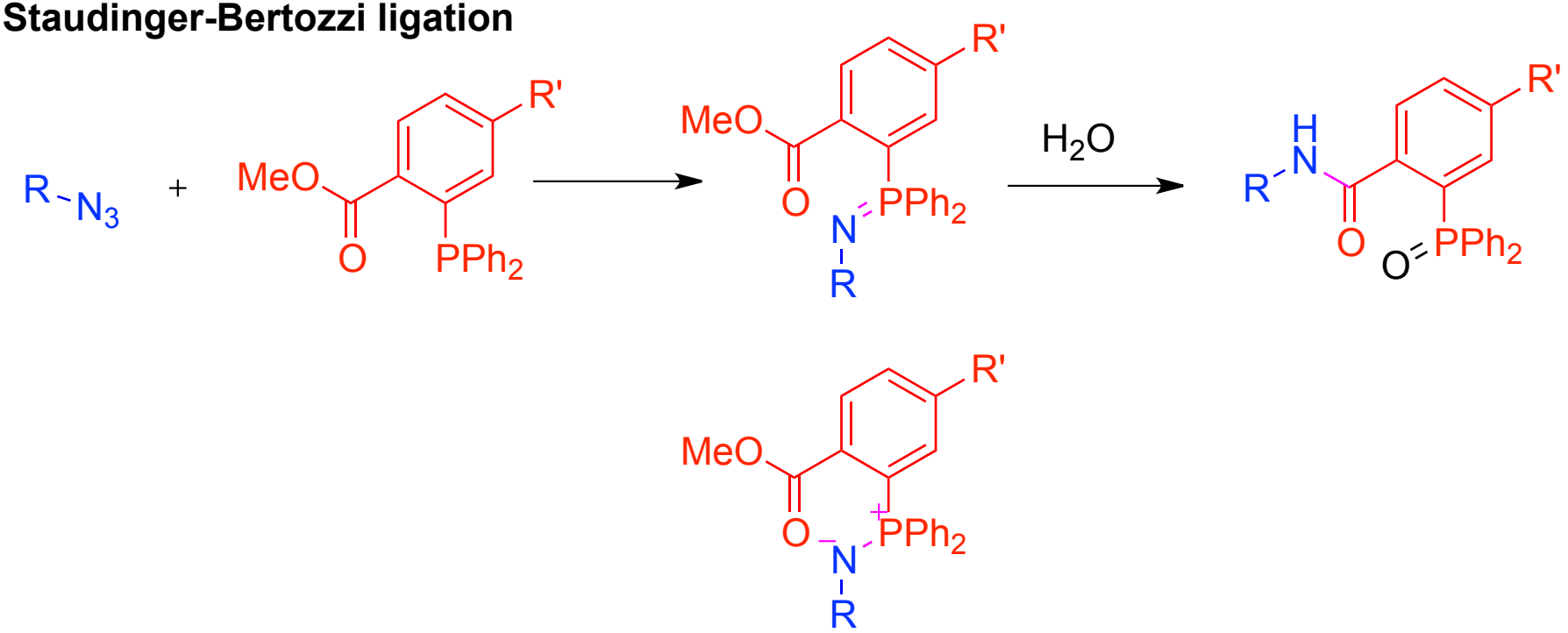
Mechanism



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

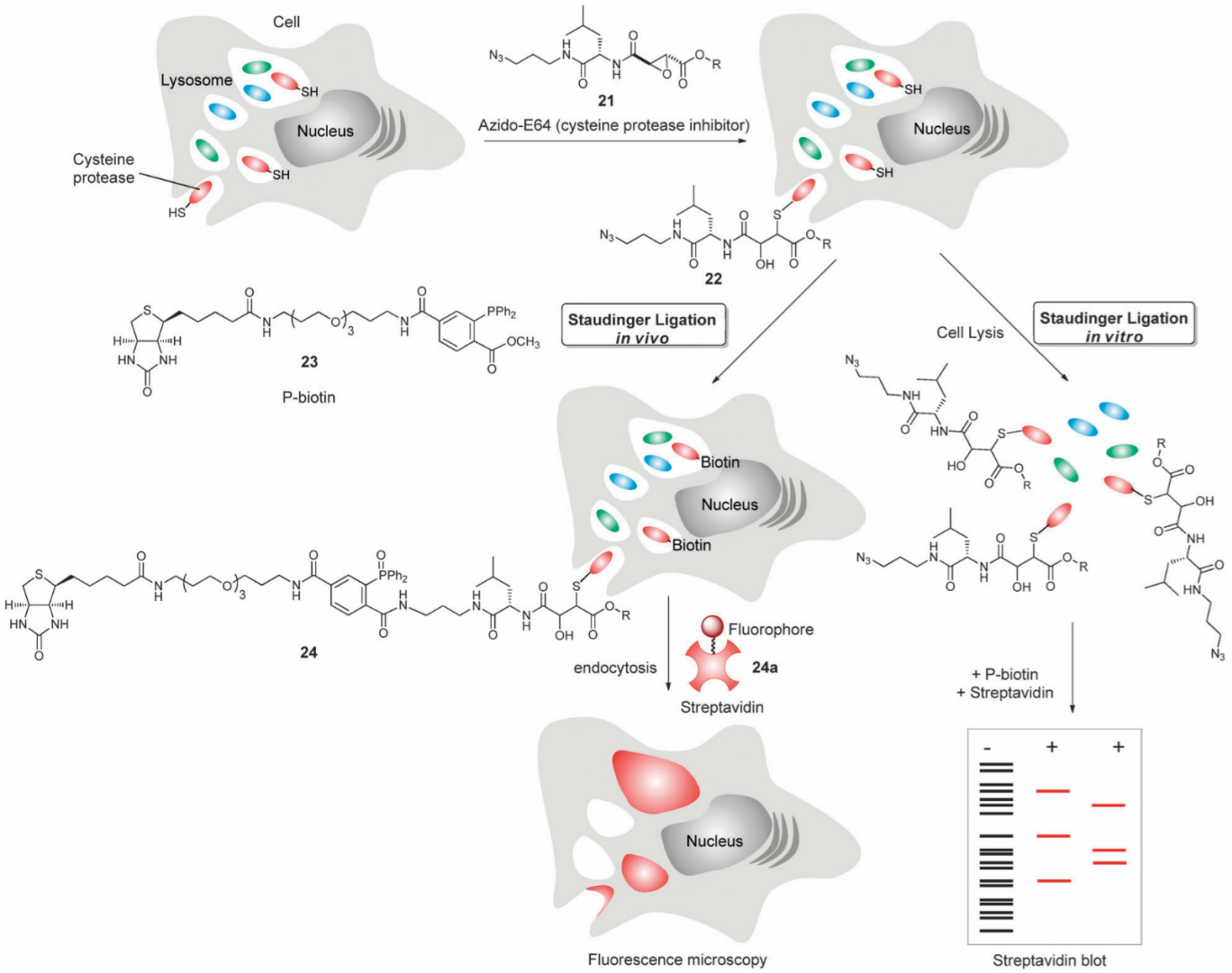
Bioorthogonal chemistry - Staudinger ligation

Staudinger-Bertozzi ligation



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

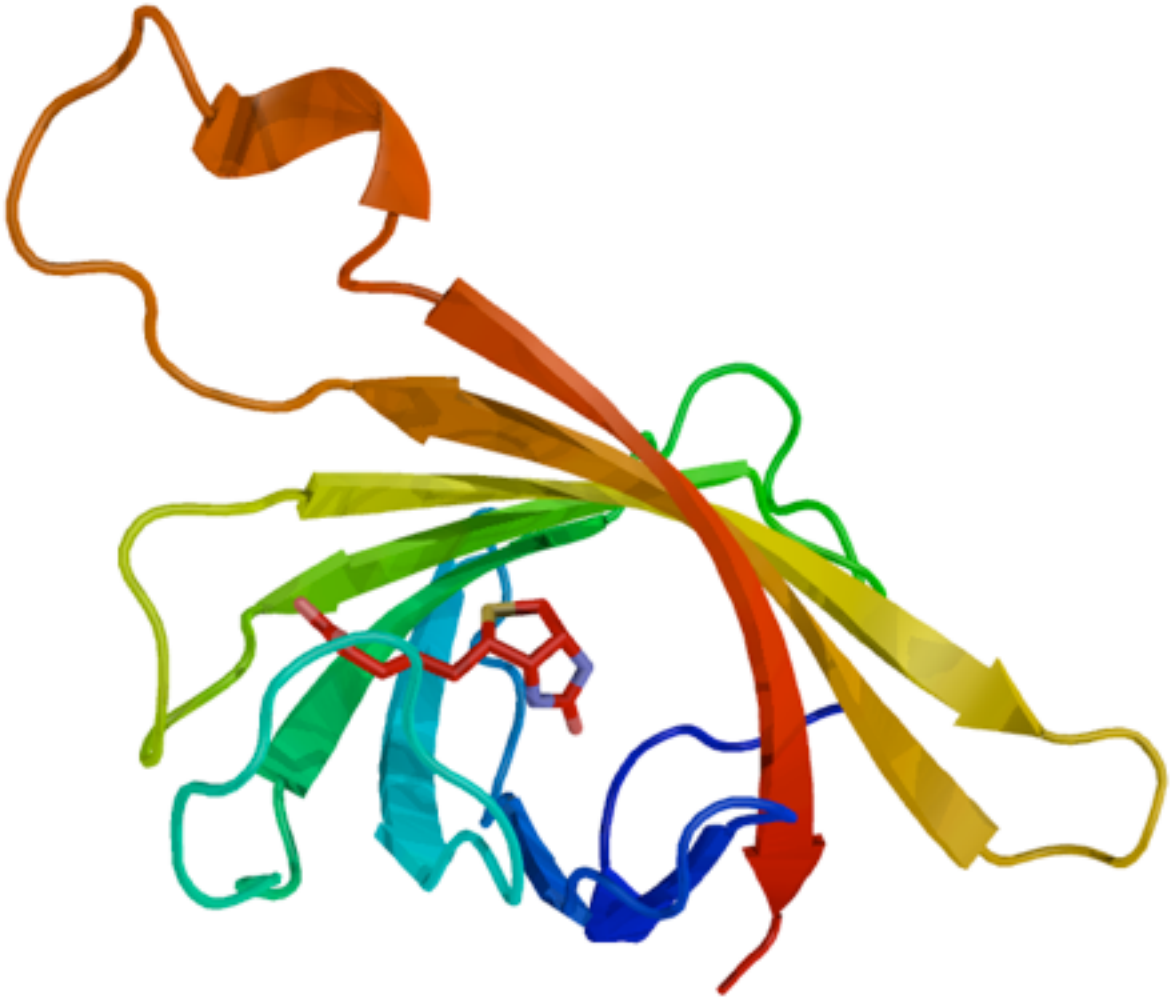
Applications of Staudinger Ligation



Scheme 5 Biotin labelling of proteins through the addition of P-biotin **23** in cells (left) and *in vitro* in a streptavidin blot after cell lysis (right).[67,68]

H. L. Ploegh *et coll.*, *ACS Chem. Biol.*, **2006**, *1*, 713–723 and *J. Am. Chem. Soc.*, **2007**, *129*, 2744–2745.
Review: S. Bräse *et coll.*, *Chem. Soc. Rev.*, **2011**, *40*, 4840–4871

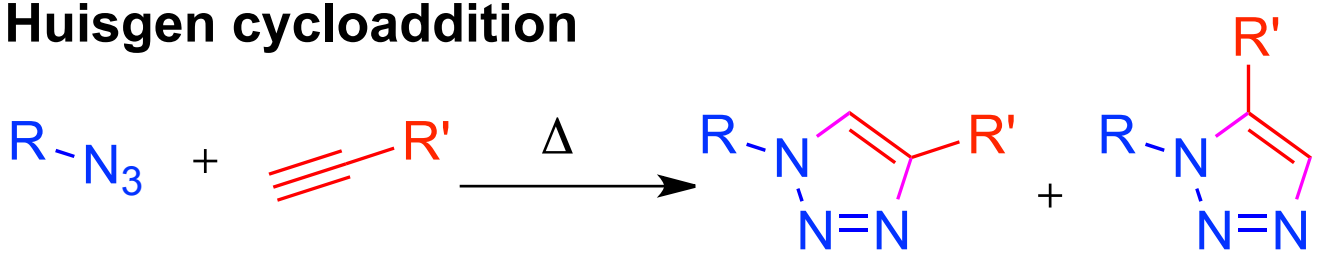
Biotin - Avidin interaction



$K_D \approx 10^{-15}$ M

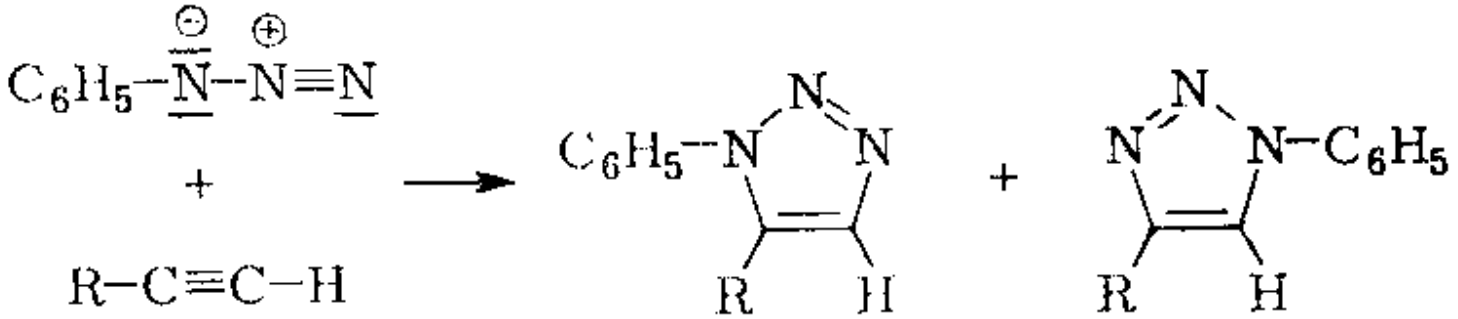
Bioorthogonal chemistry - 2+3 Cycloadditions

Huisgen cycloaddition



Rolf Huisgen

Huisgen Cycloaddition

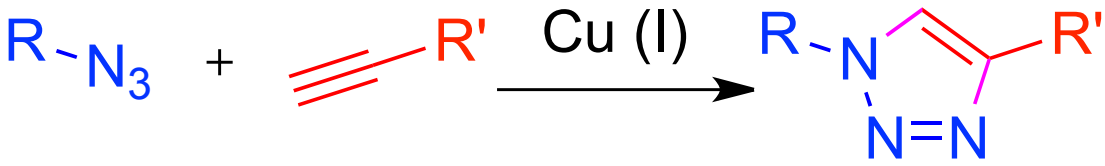


$R = C_6H_5$	52%	43%
$R = CH(OC_2H_5)_2$	23%	52%

Rolf Huisgen, *Angew. Chem. Int. Ed.*, **1963**, *2*, *565-632*

Bioorthogonal chemistry - 2+3 Cycloadditions

CuAAC



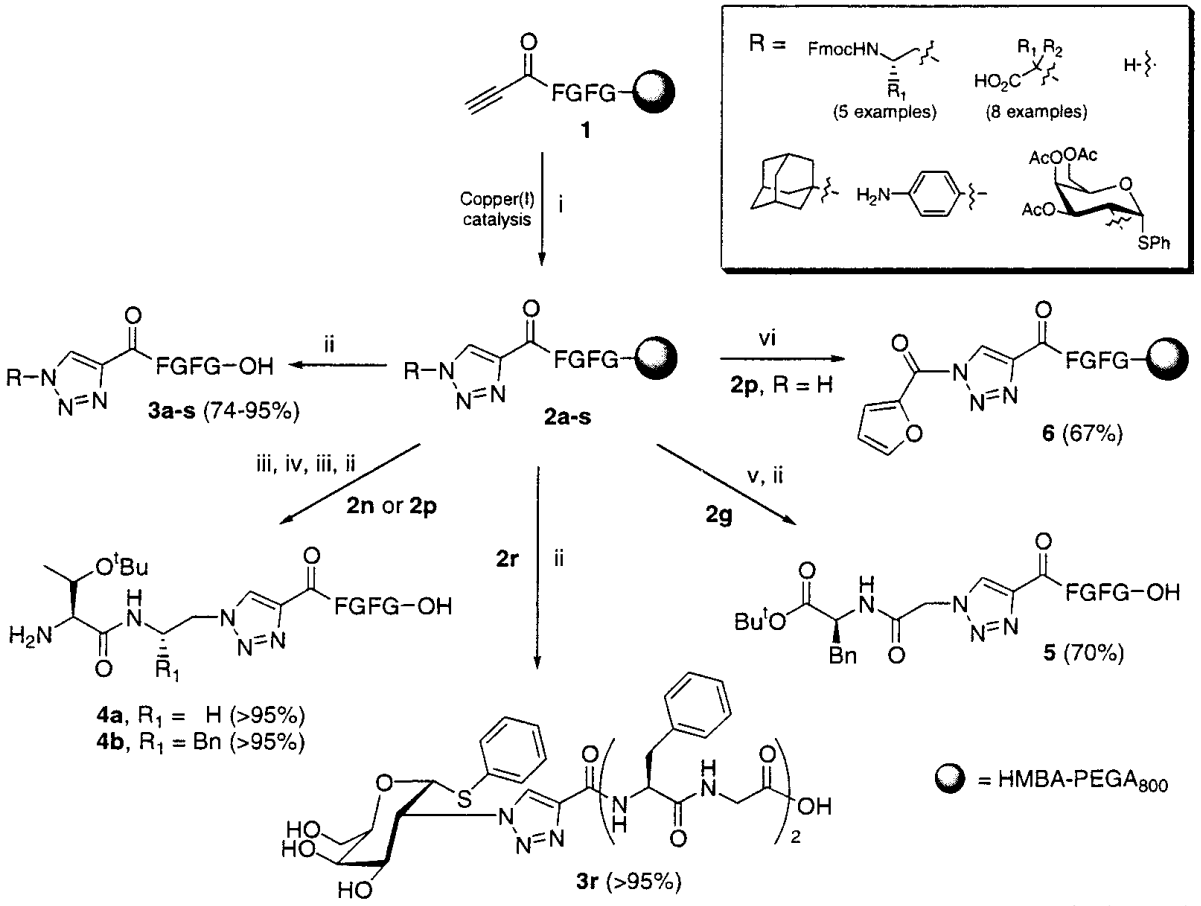
CuI

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

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

Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides

Christian W. Tornøe, Caspar Christensen, and Morten Meldal*

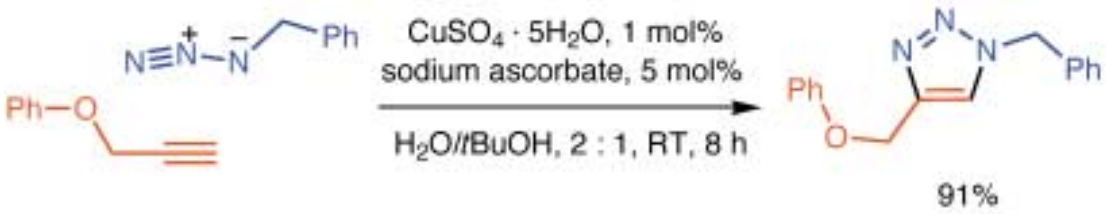


Received December 14, 2001
Published on Web 04/02/2002

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

A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes**

Vsevolod V. Rostovtsev, Luke G. Green,
Valery V. Fokin,* and K. Barry Sharpless*



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

Received: April 29, 2002

Cell penetrating peptide

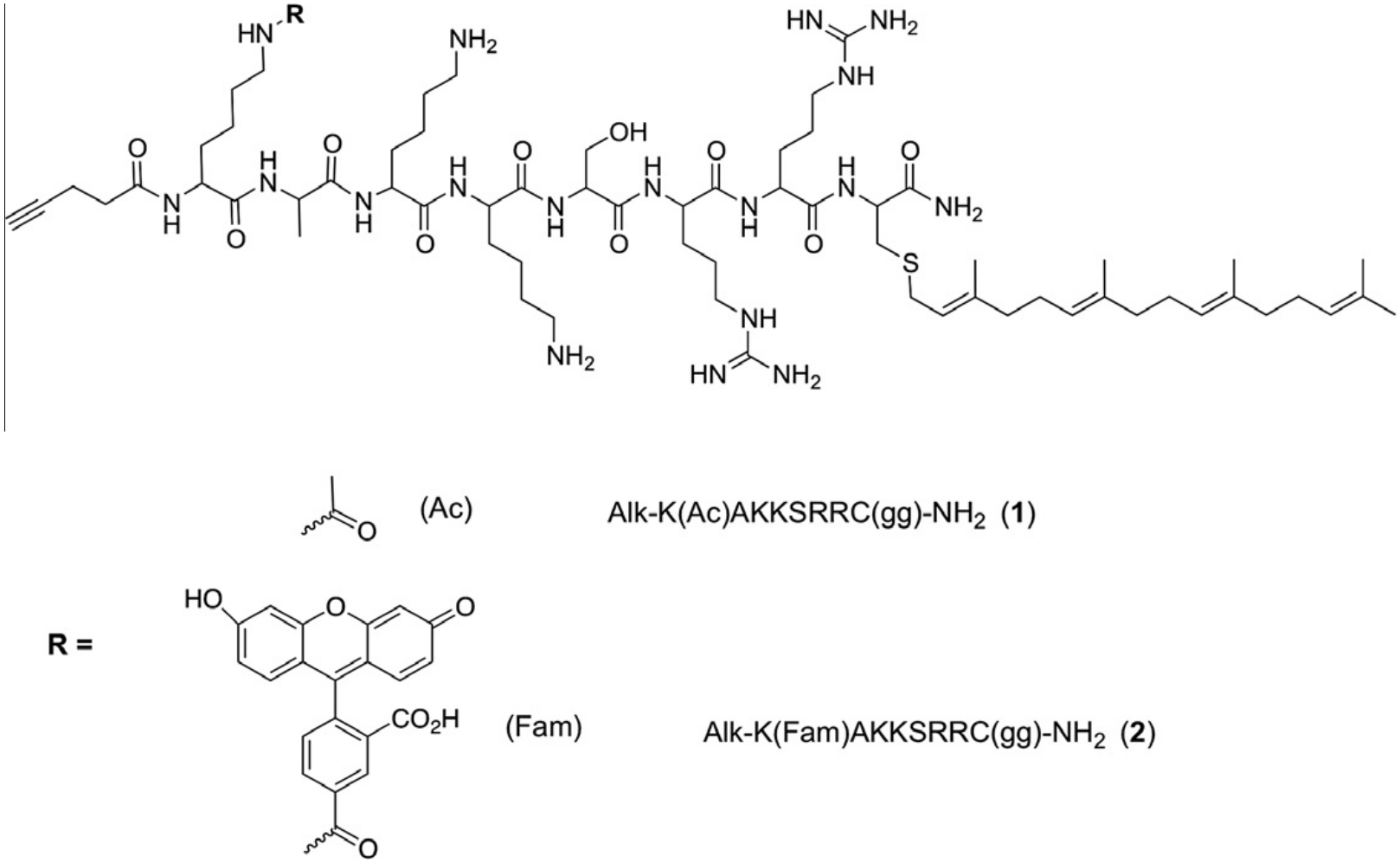


Figure 1. The sequences of the peptides used in this study.

M. D. Distefano *et coll., Bioorg. Med. Chem. Lett.,* **2011**, *21*, 4998-5001

Imaging

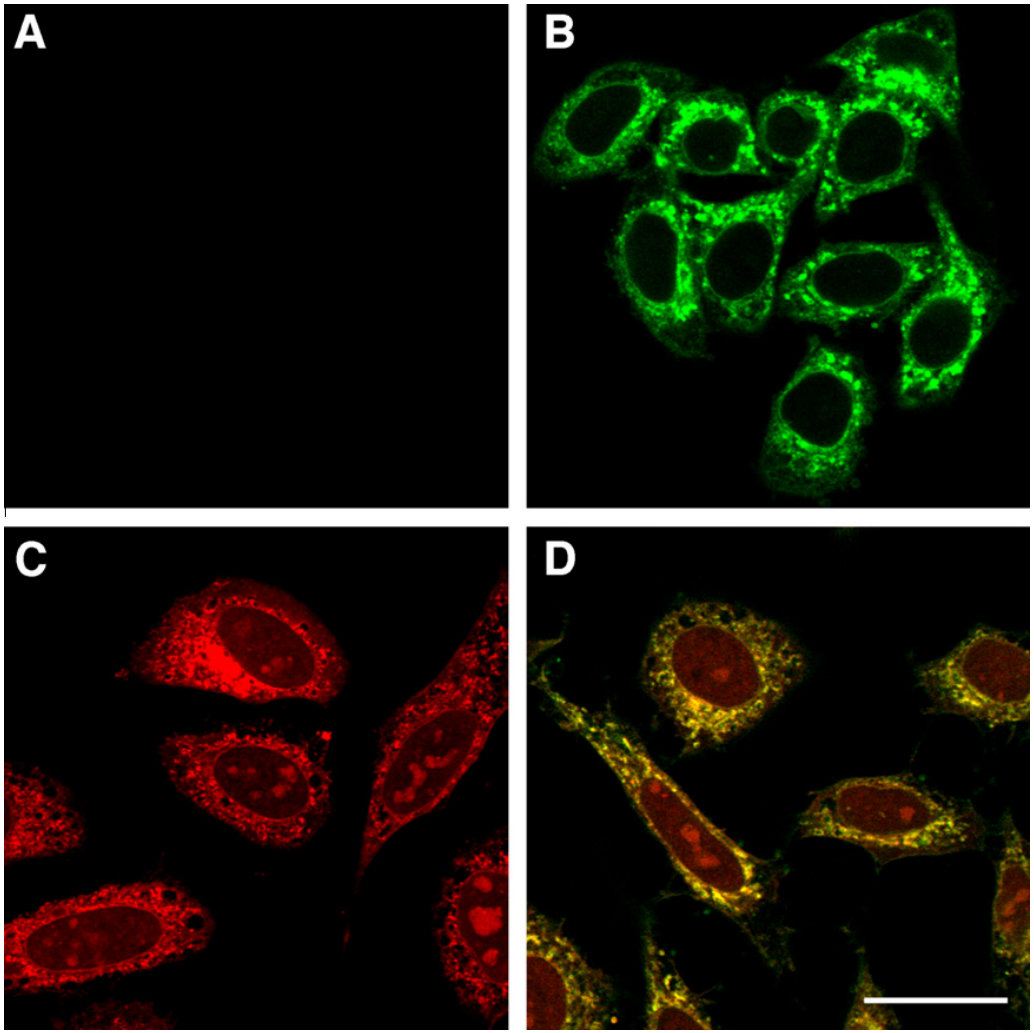
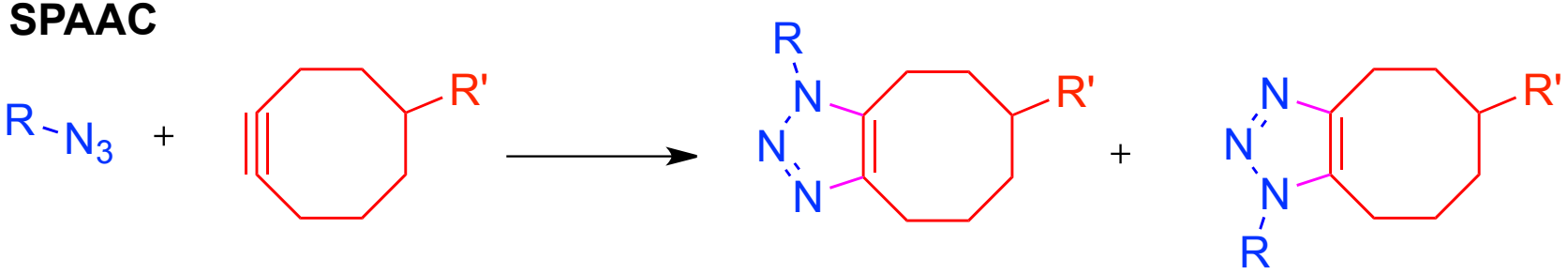


Figure 2. HeLa cells incubated with peptides 1 or 2 at 1 μM for 2 h. Panel (A) Click reaction performed on cells that were not treated with an alkyne peptide, showing no background labeling by TAMRA-N_3. Panel (B) Peptide 2 visualized with green 5-Fam fluorescence. Panel (C) Peptide 1 monitored by click reaction with TAMRA-N_3 on fixed cells. Panel (D) Peptide 2 monitored by click reaction with TAMRA-N_3 on fixed cells and green 5-Fam fluorescence; the yellow color indicates co-localization of 5-Fam and TAMRA fluorophores, now present on the same peptide. The size bar represents a distance of 25 μm.

Bioorthogonal chemistry - 2+3 Cycloadditions

SPAAC



Georg Wittig

Strain Promoted Alkyne Azide Cycloaddition

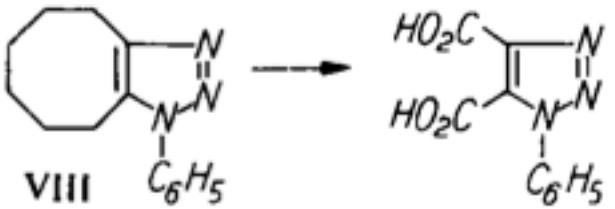
GEORG WITTIG und ADOLF KREBS

Zur Existenz niedergliedriger Cycloalkine, I[1)]

Aus dem Institut für Organische Chemie der Universität Heidelberg

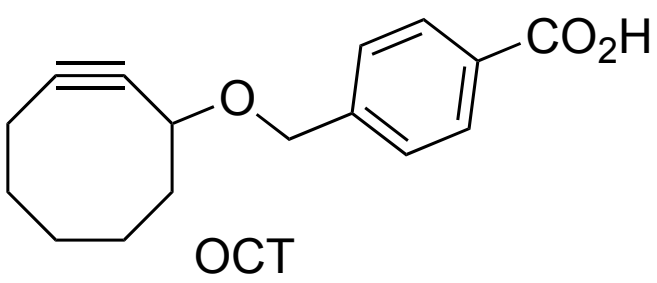
(Eingegangen am 24. Mai 1961)

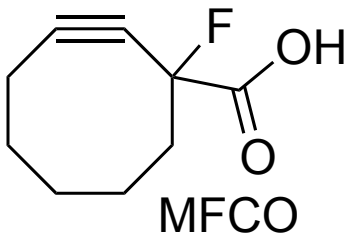
Als besonders additionsfreudig erwies sich *Phenylazid*, das nach A. T. BLOMQUIST und LIANG HUANG LIU[5)] mit Cyclooctin explosionsartig zu einer nicht näher untersuchten viskosen Flüssigkeit reagierte. Nach eigenen Untersuchungen erhielt man dabei in 73-proz. Ausbeute das bei 84—85° schmelzende Triazolderivat VIII, das identisch mit einem nach K. ALDER und G. STEIN[20)] bereiteten Präparat war und mit Kaliumpermanganat zur bekannten *1-Phenyl-1.2.3-triazol-dicarbonsäure-(4.5)* [21)] oxydiert werden konnte:

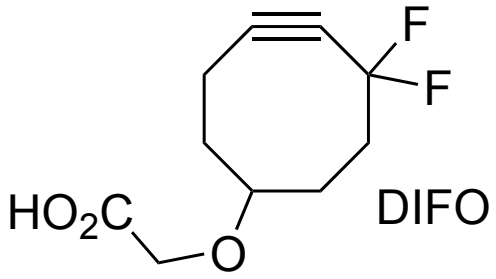


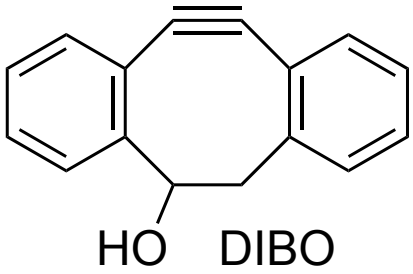
VIII

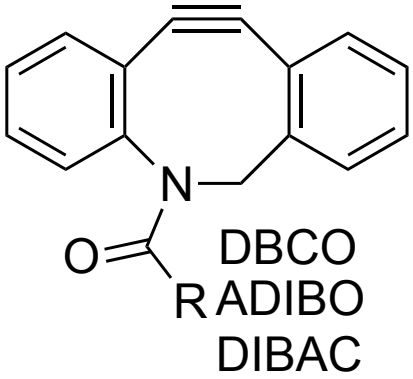
Cyclooctynes

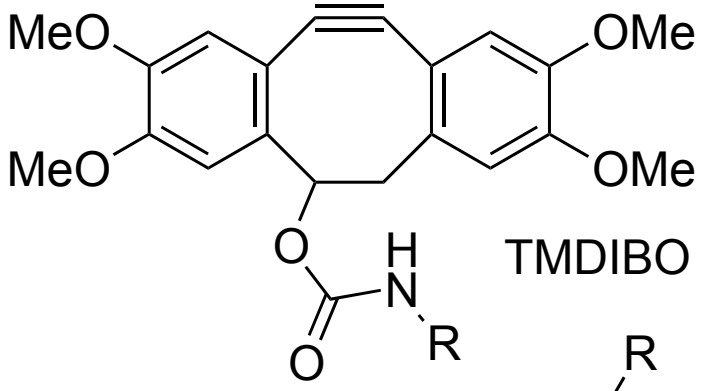


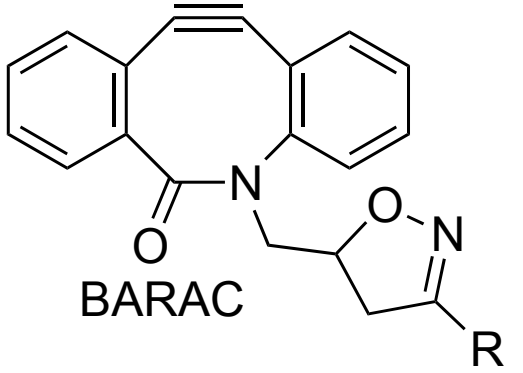


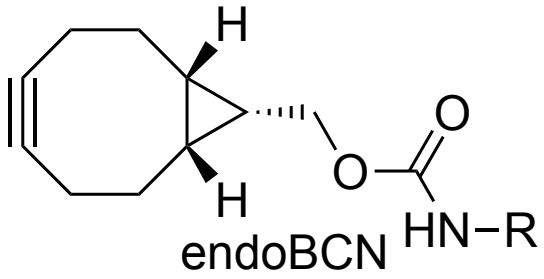


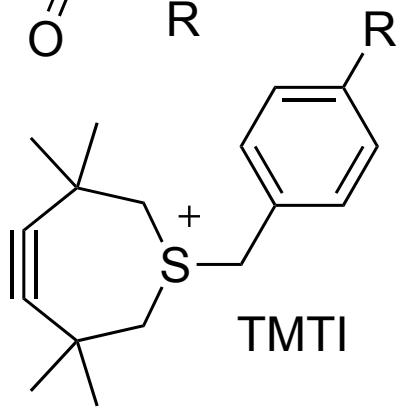








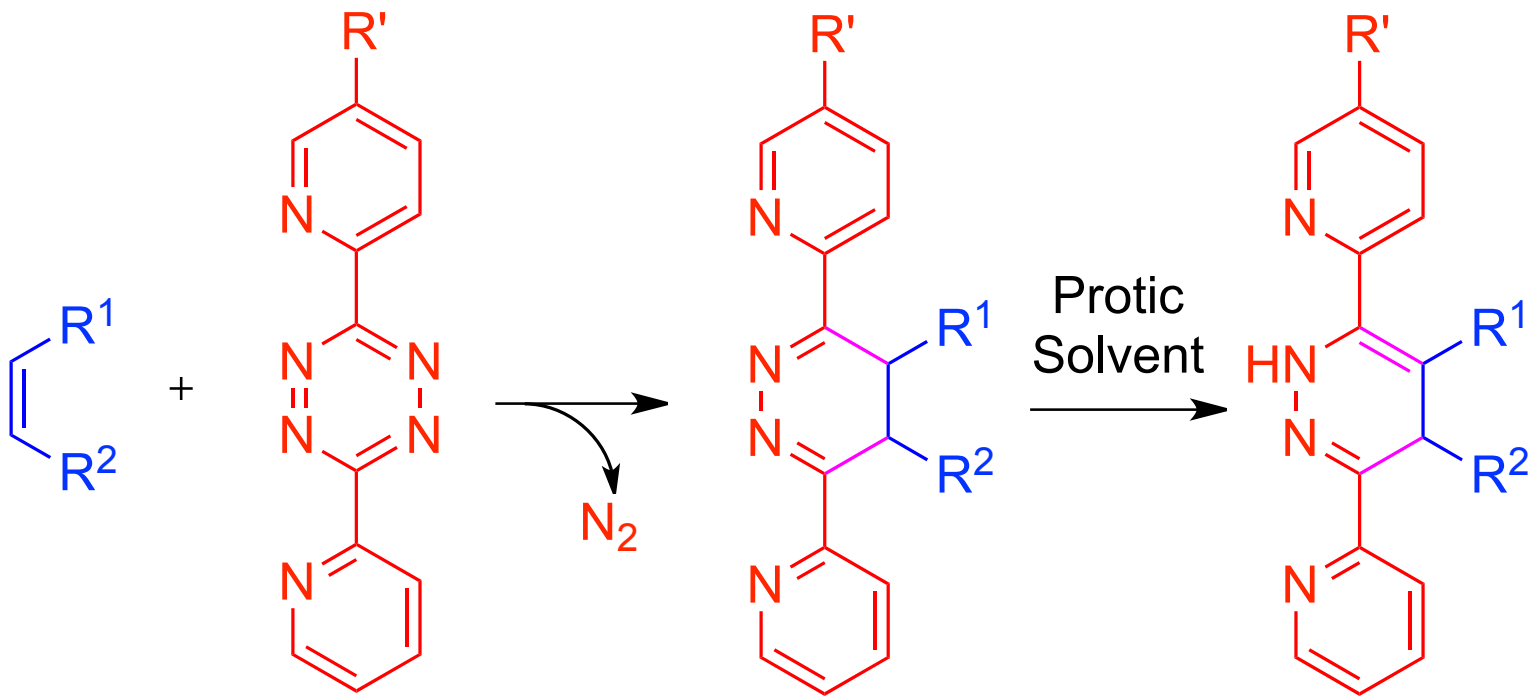




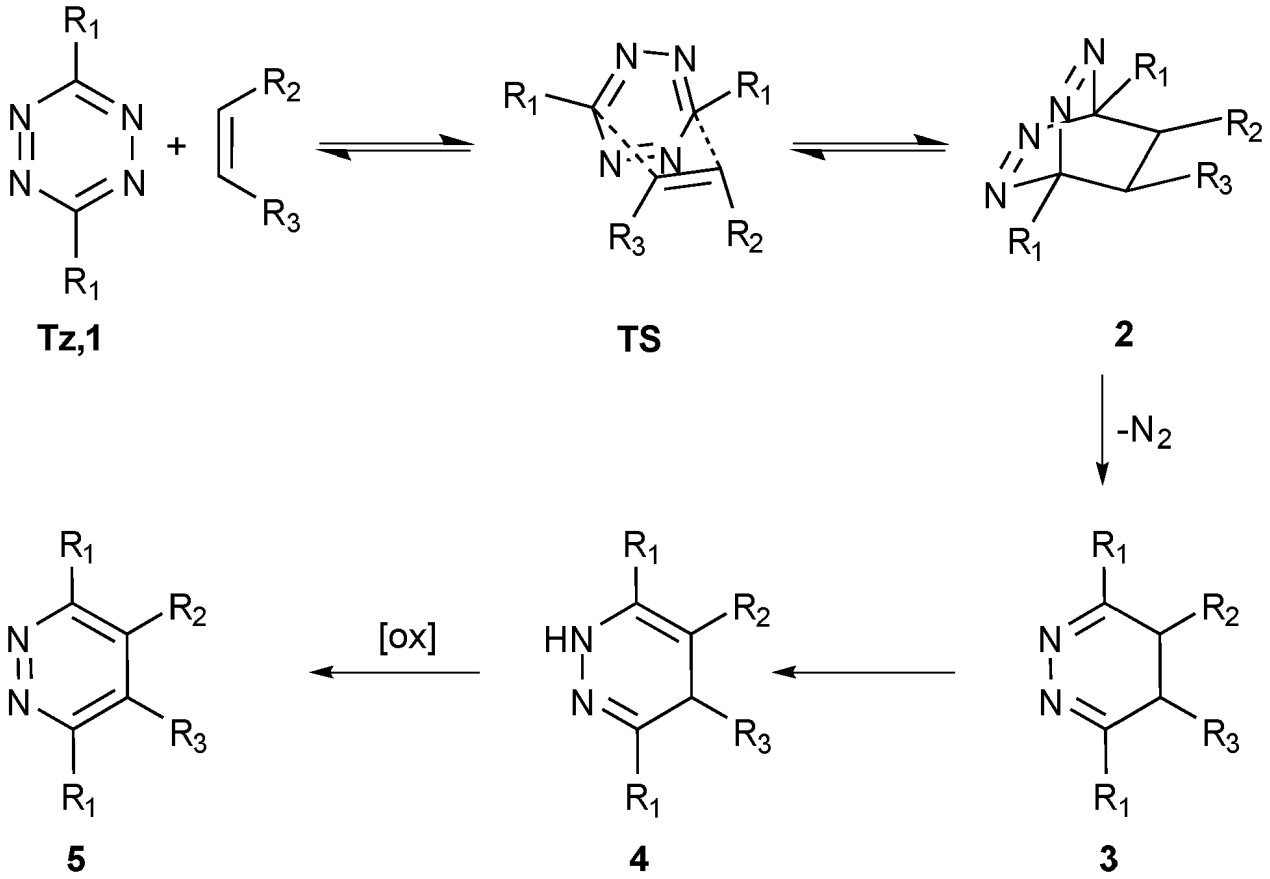
B. Vauzeilles *et coll.*, *Actualité Chimique*, **2015**, *393-394*, 24-30

Bioorthogonal Chemistry -

Inverse electron demand Diels Alder



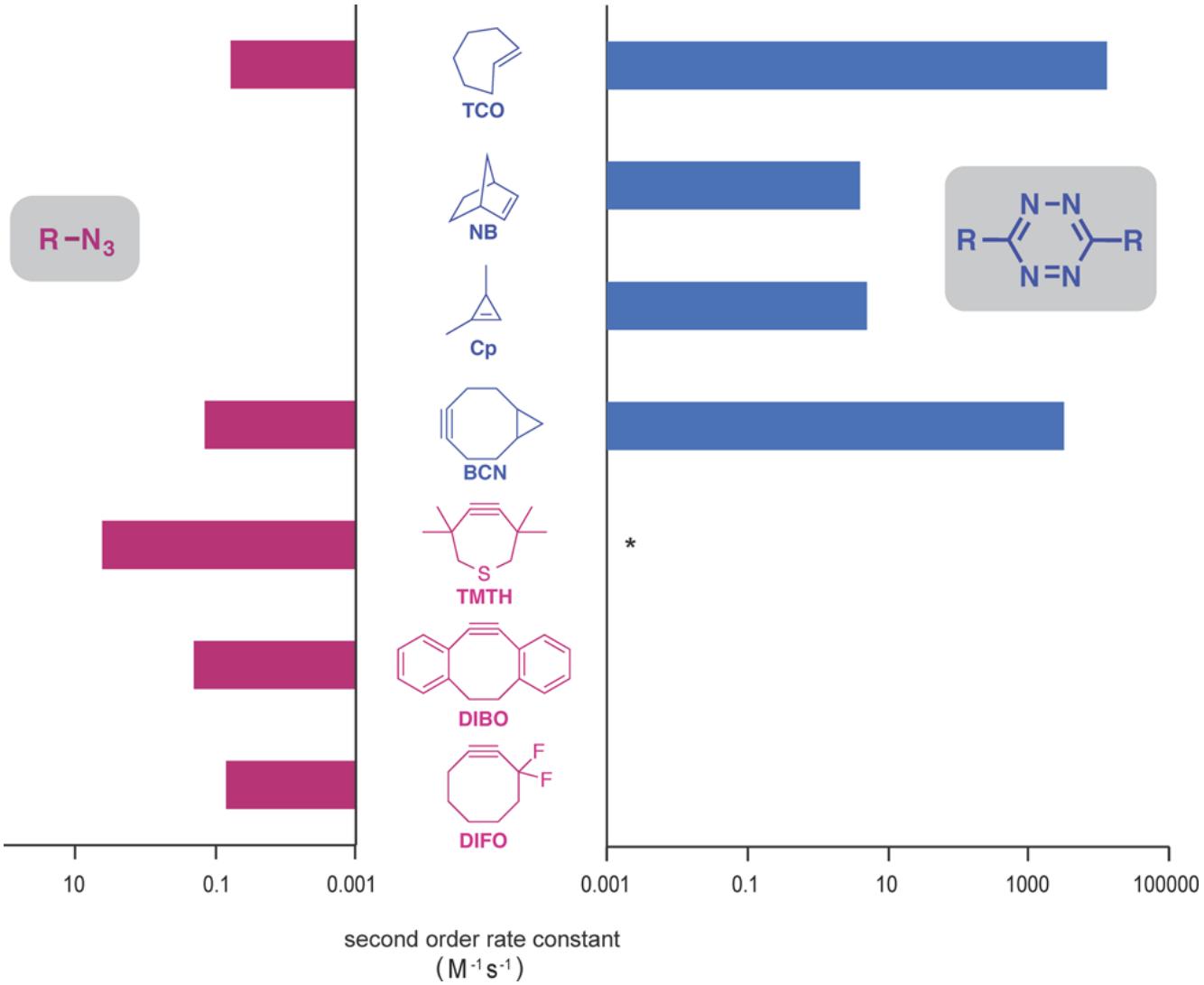
Tetrazine-ene iEDDA



Scheme 1 iEDDA reaction scheme.

Astrid-Caroline Knall and Christian Slugovc, *Chem. Soc. Rev.*, **2013**, *42*, 5131-42

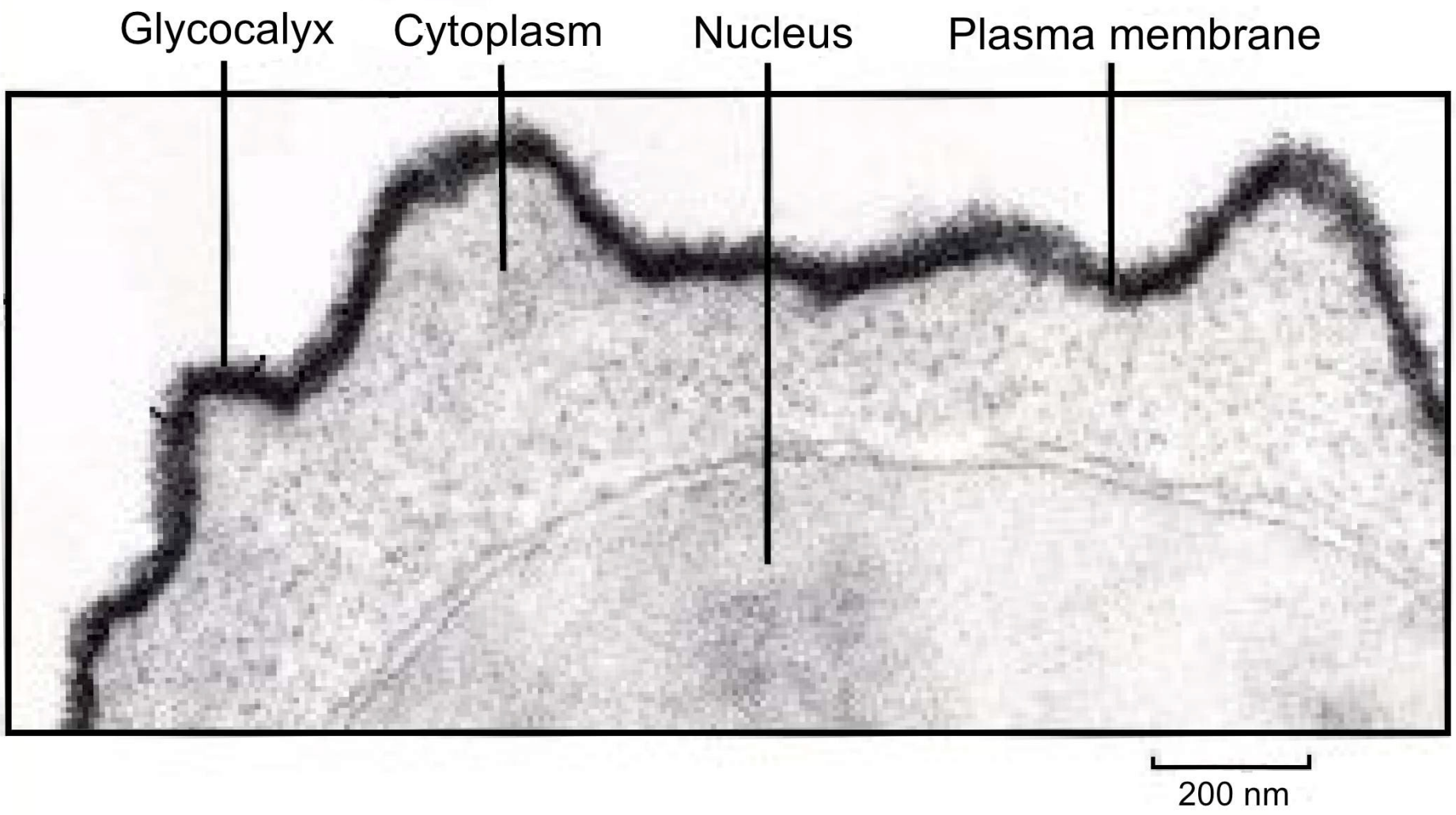
Relative reactivities

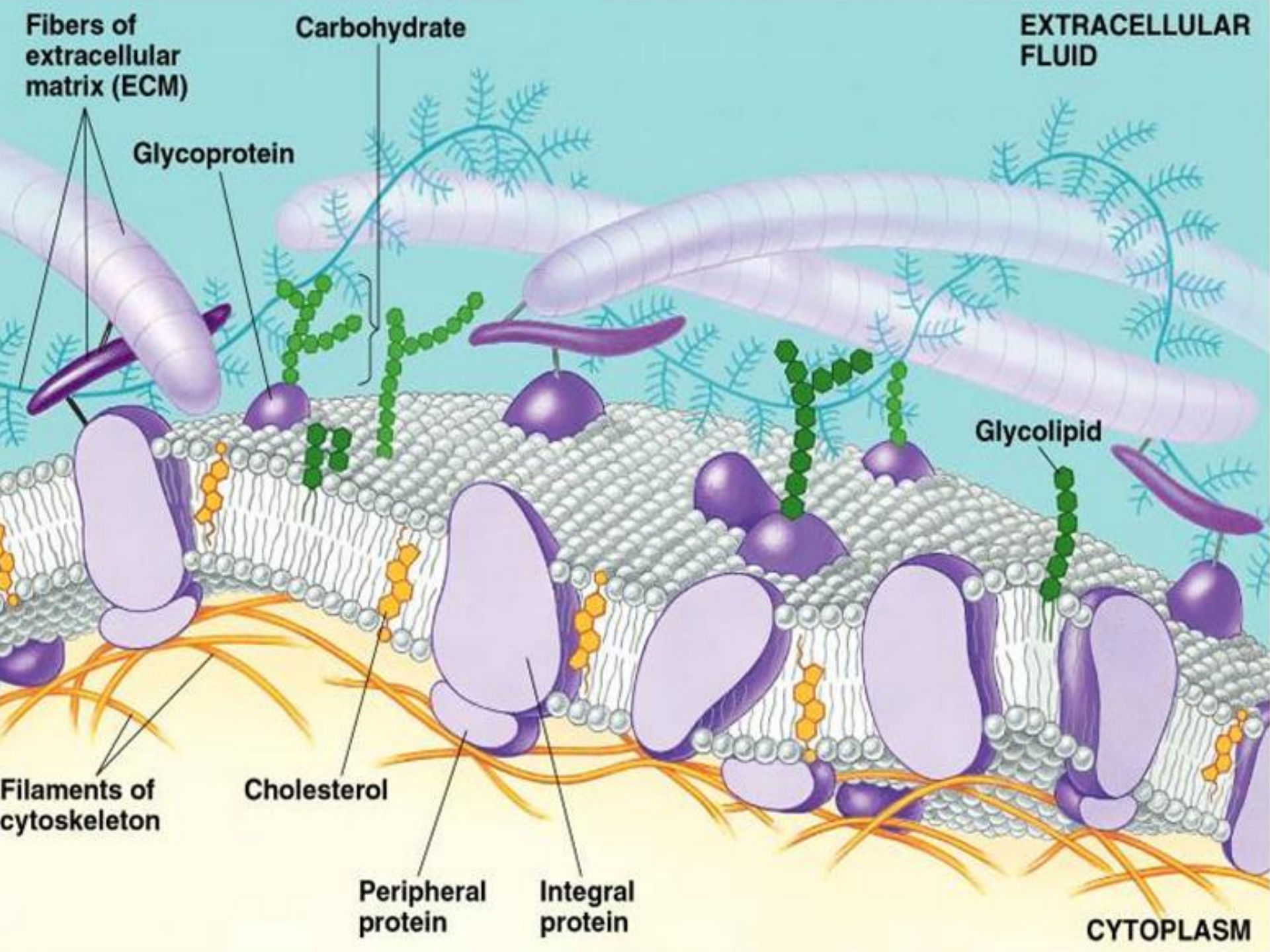


Jennifer A. Prescher *et coll.*, *ACS. Chem. Biol.*, **2014**, *9*, 592-605

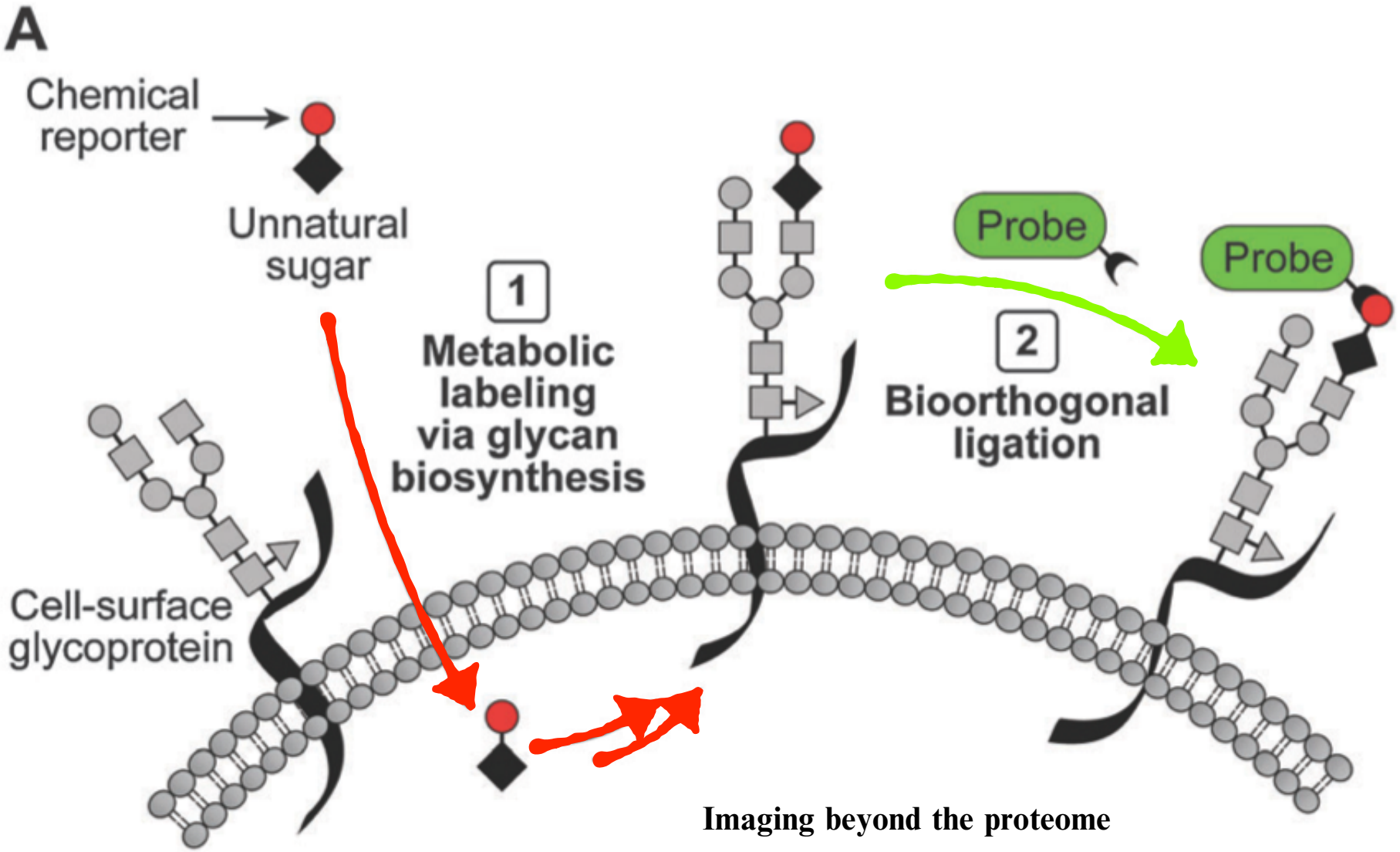
Metabolic glycan labeling

Cell surface




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

Metabolic Glycan Labeling

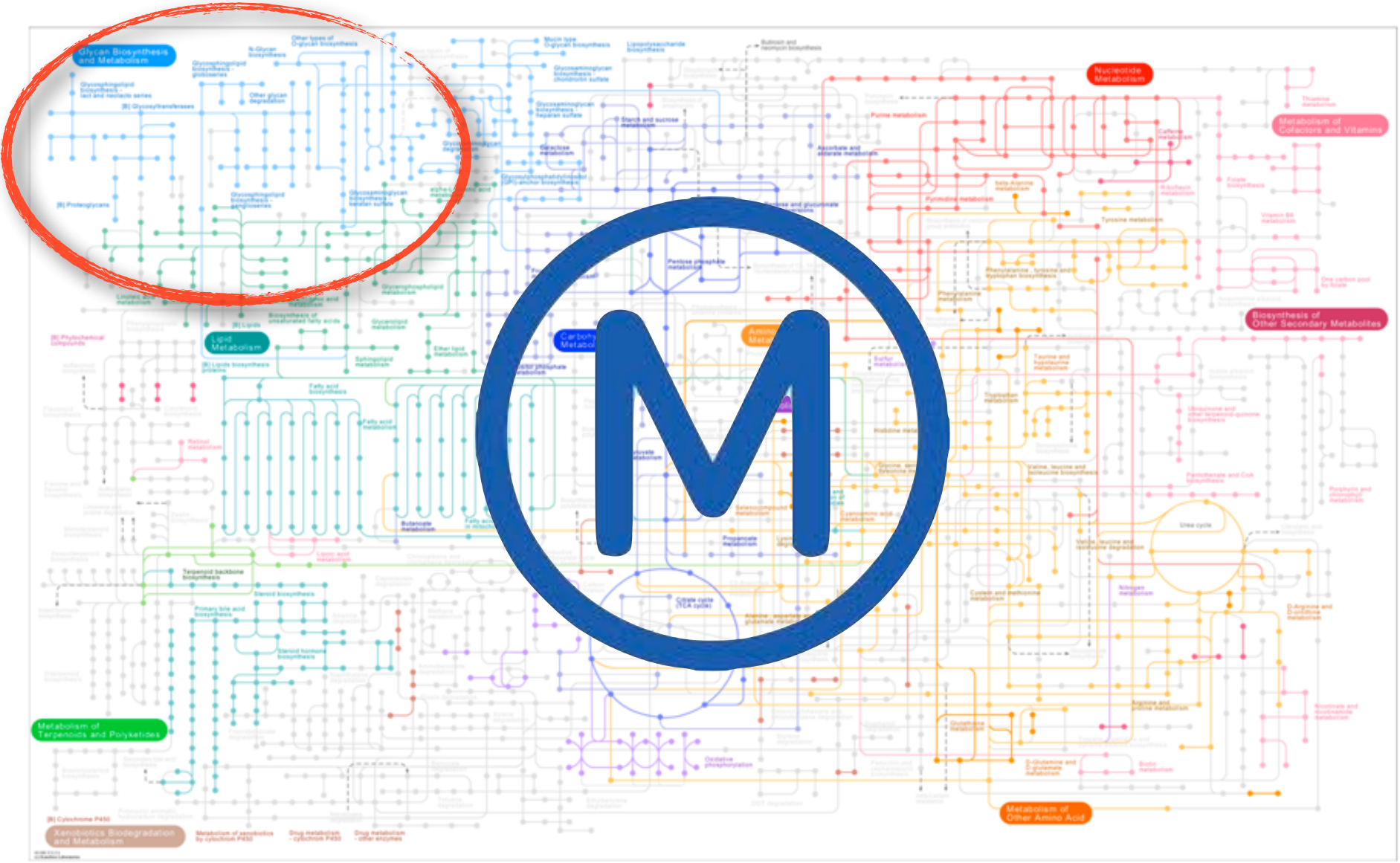


Imaging beyond the proteome

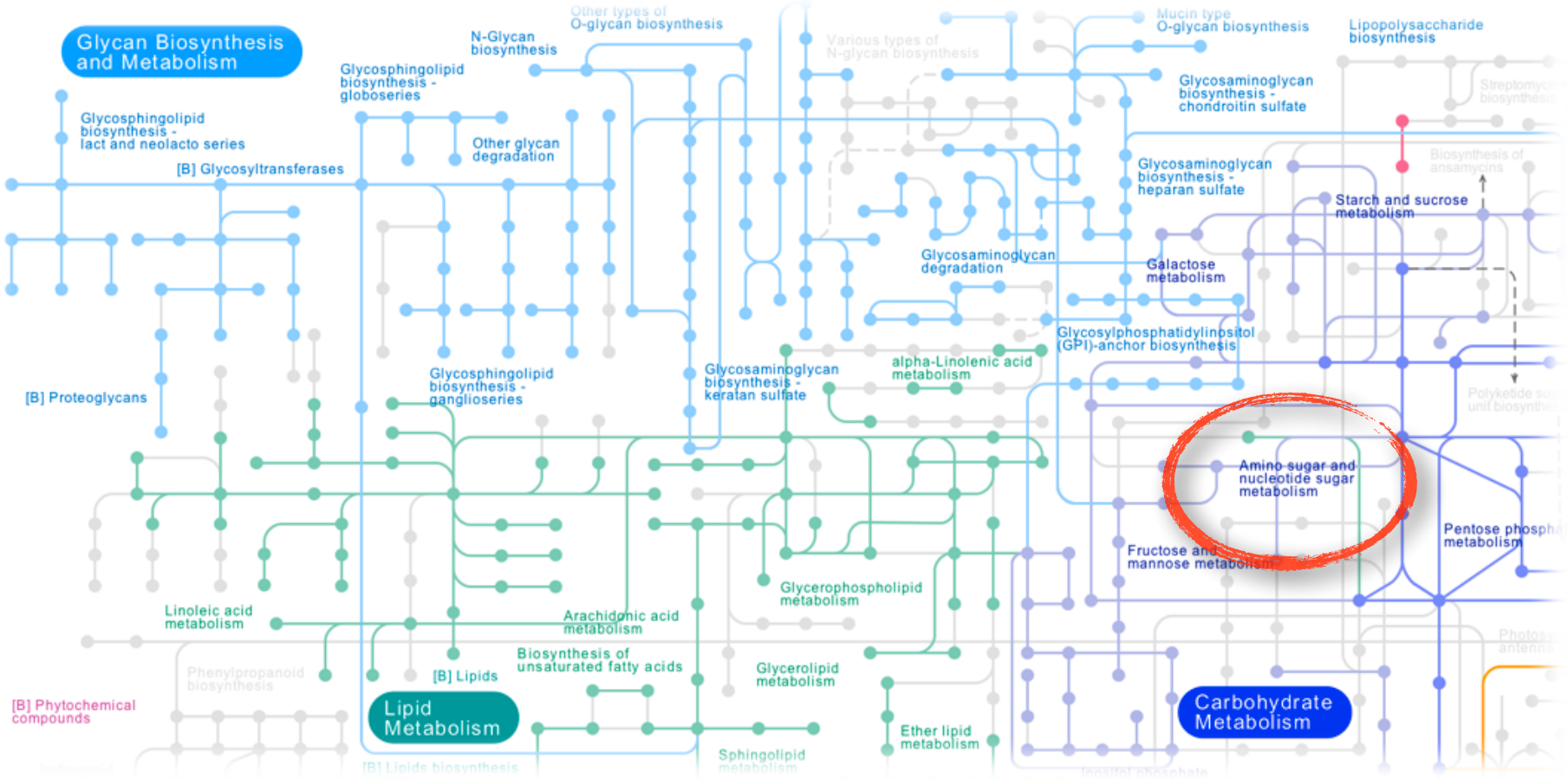
Pamela V. Chang[a] and Carolyn R. Bertozzi*[abc]

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

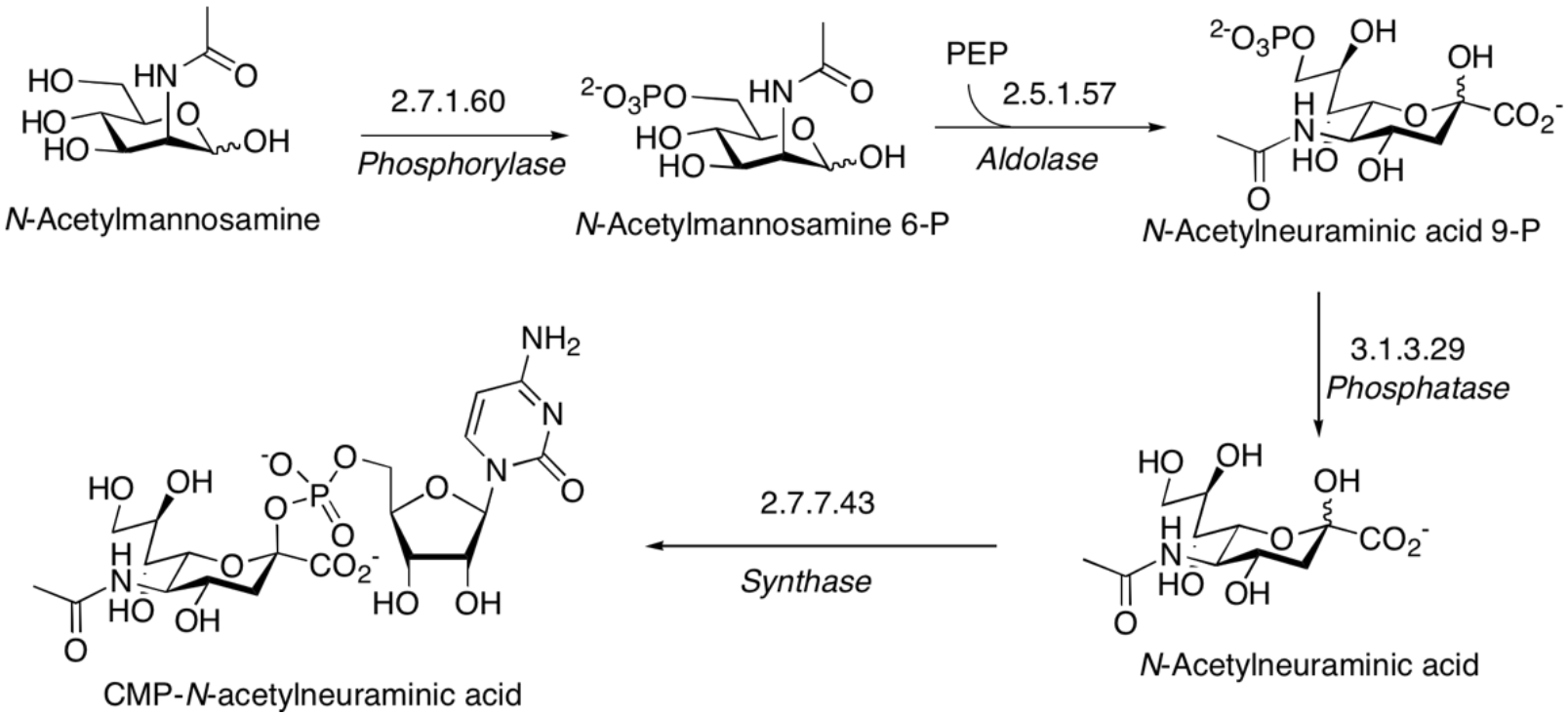
Metabolic Oligosaccharide Engineering



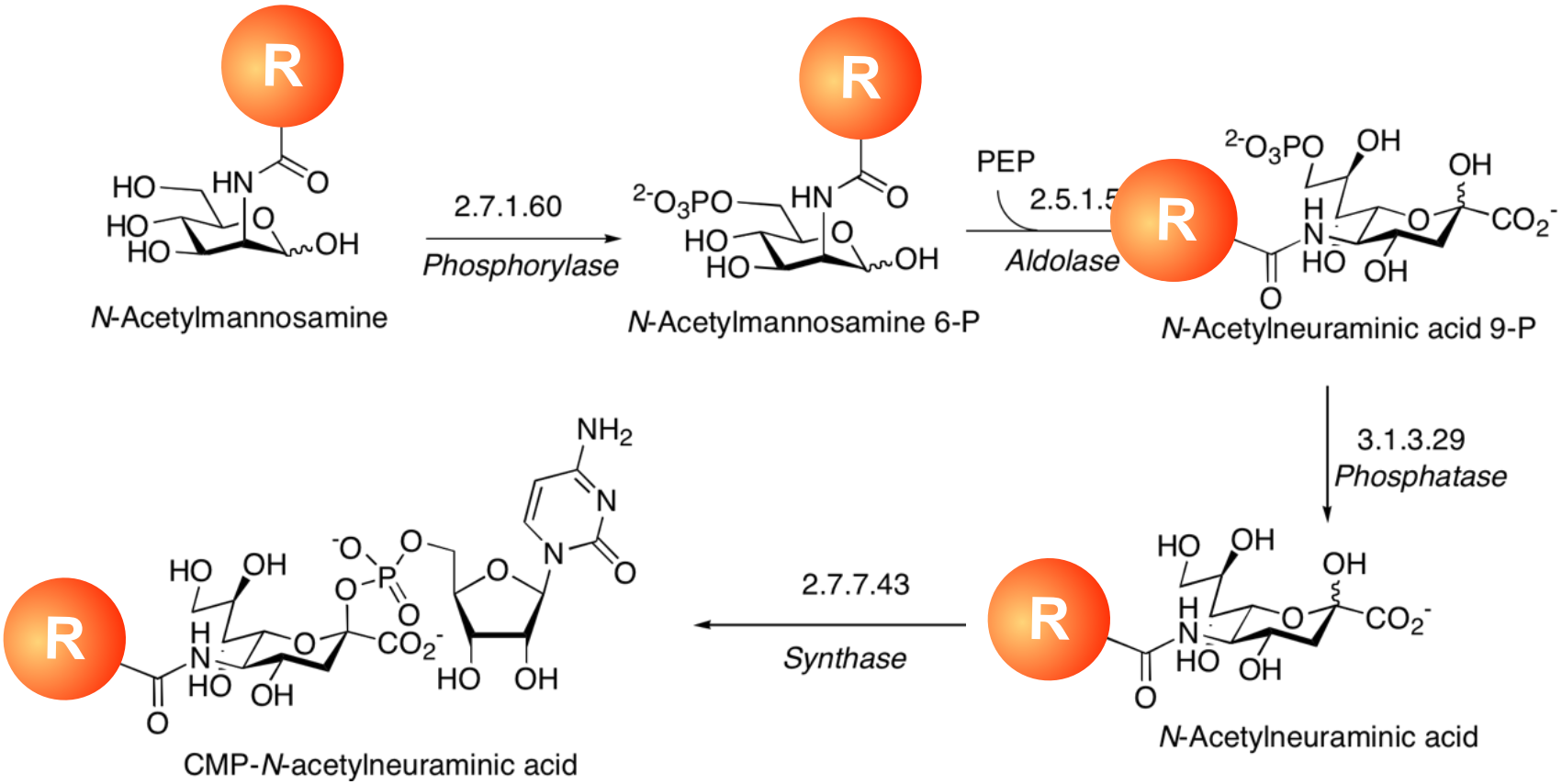
Metabolic Oligosaccharide Engineering



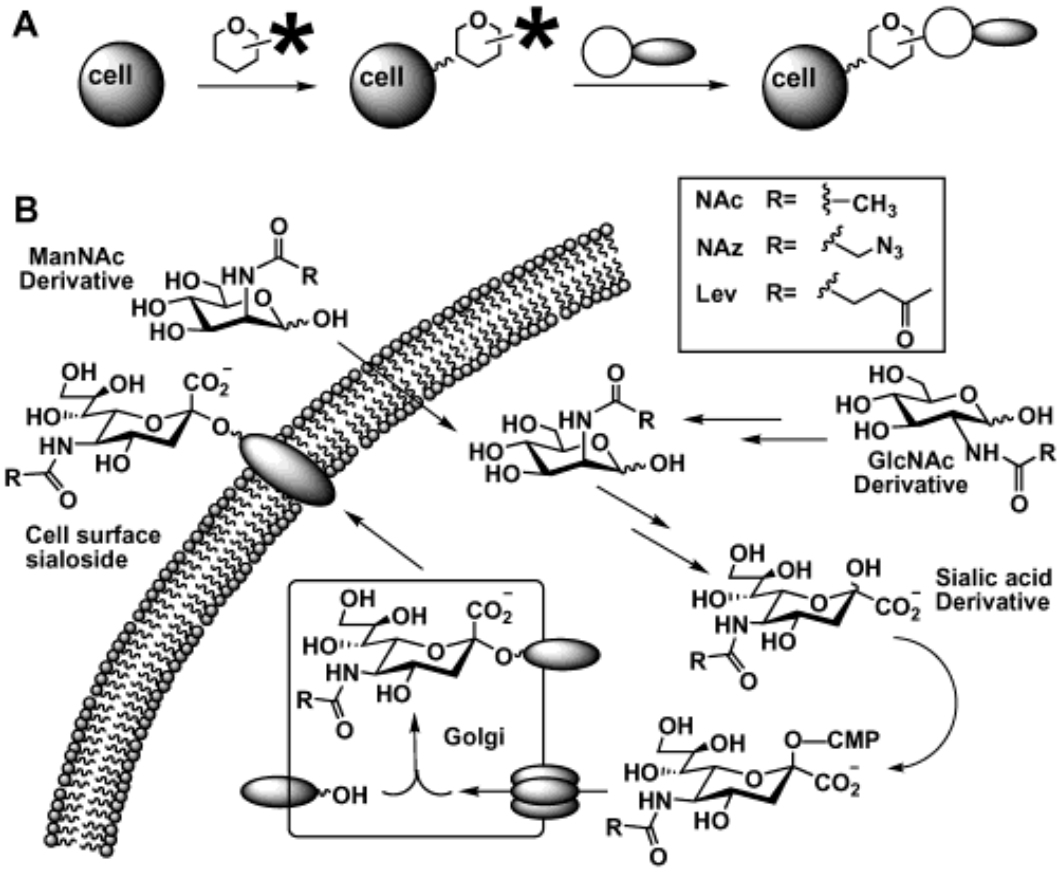
N-Acetyl neuraminic acid



N-Acetyl neuraminic acid



Azides

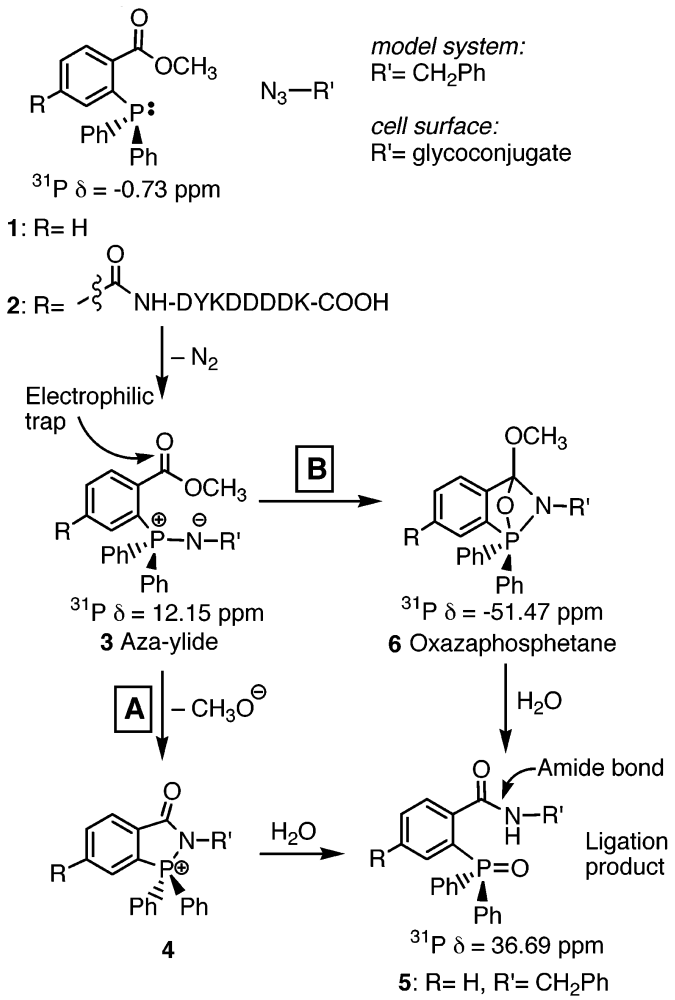


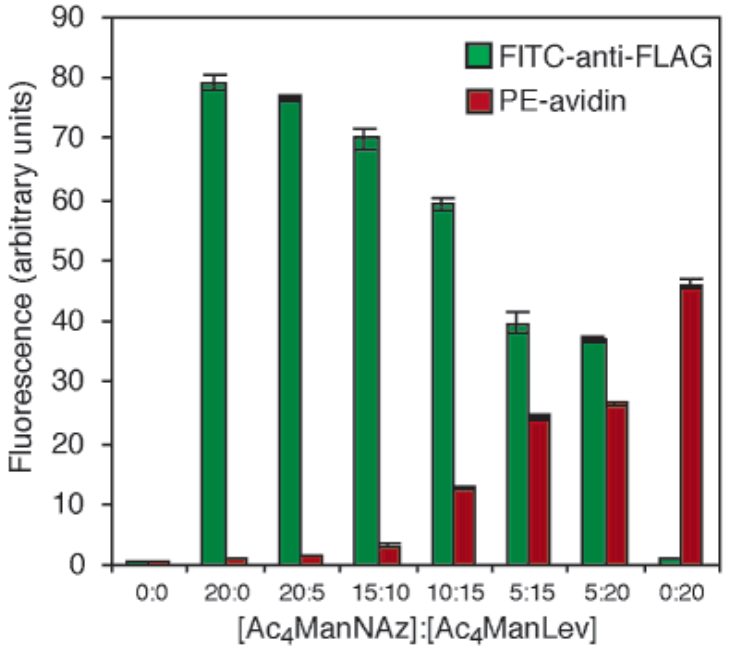
J|A|C|S
ARTICLES
Published on Web 11/23/2002

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*,†,‡

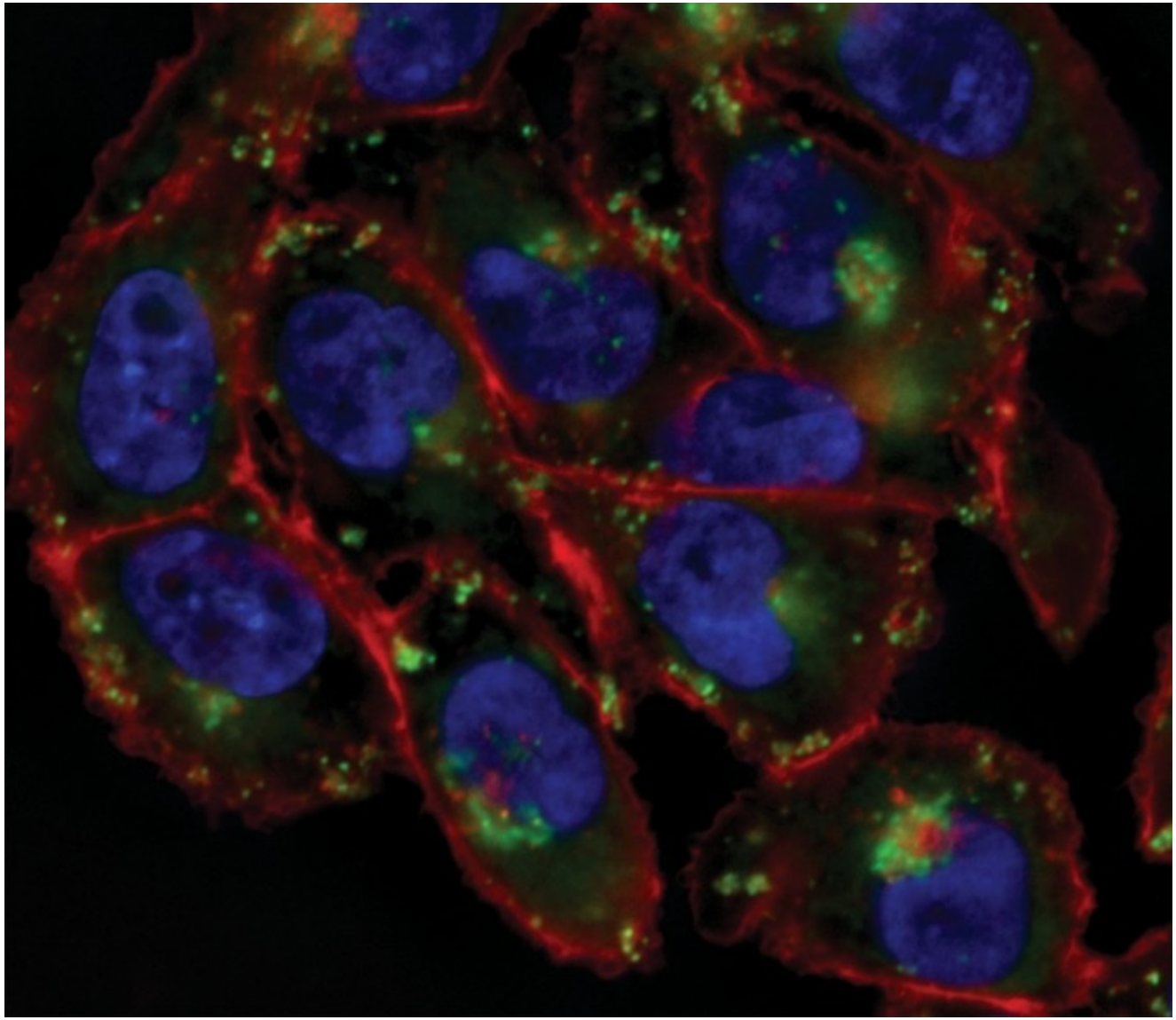
Staudinger ligation





FLAG peptide, NH_2-DYKDDDDK-COOH
PE, phycoerythrin

Copper-free click chemistry

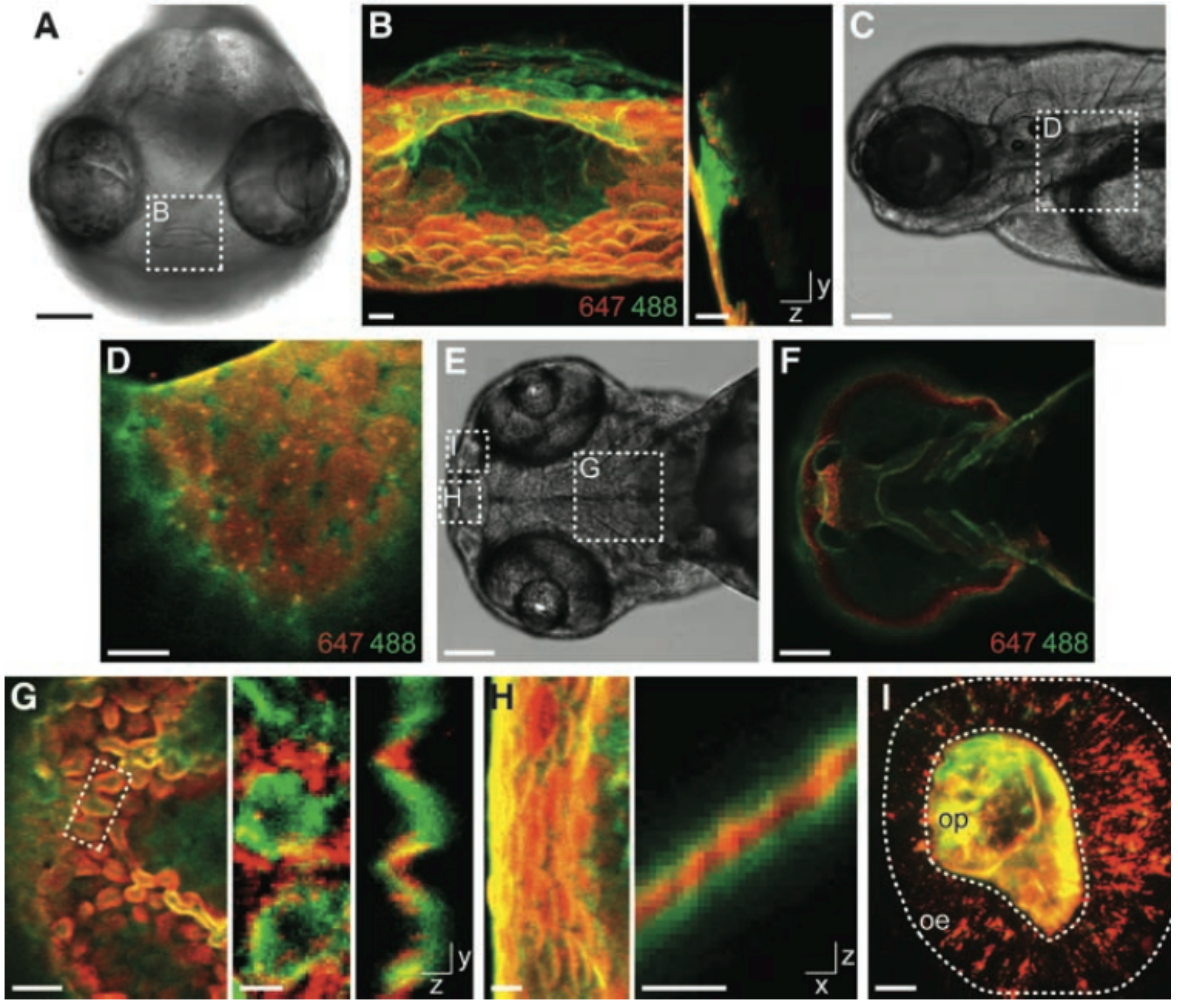


CHO
Nucleus
Golgi
Glycans

Zebrafish embryos

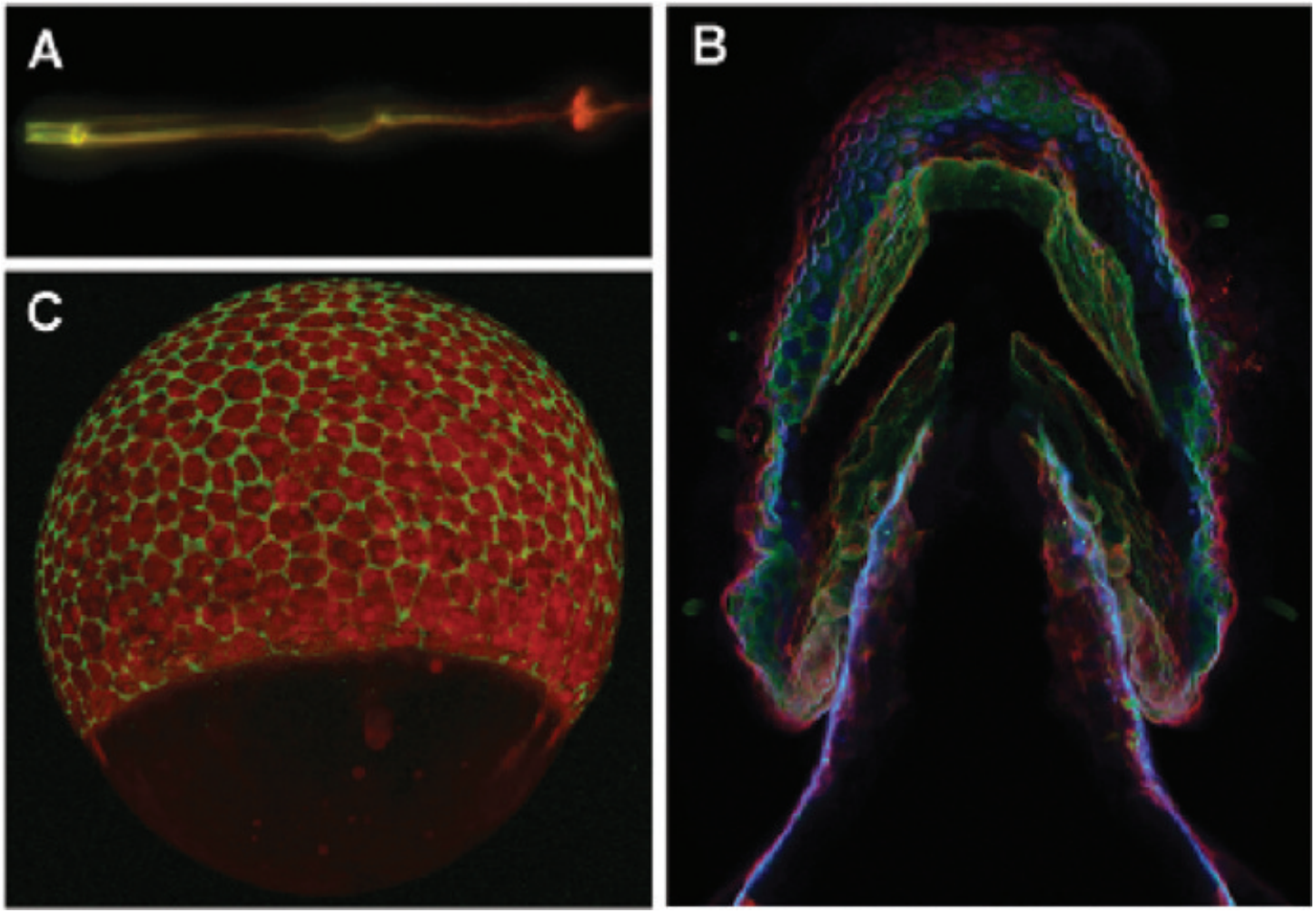
In Vivo Imaging of Membrane-Associated Glycans in Developing Zebrafish

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



Science, 2008

C. elegans / Zebrafish

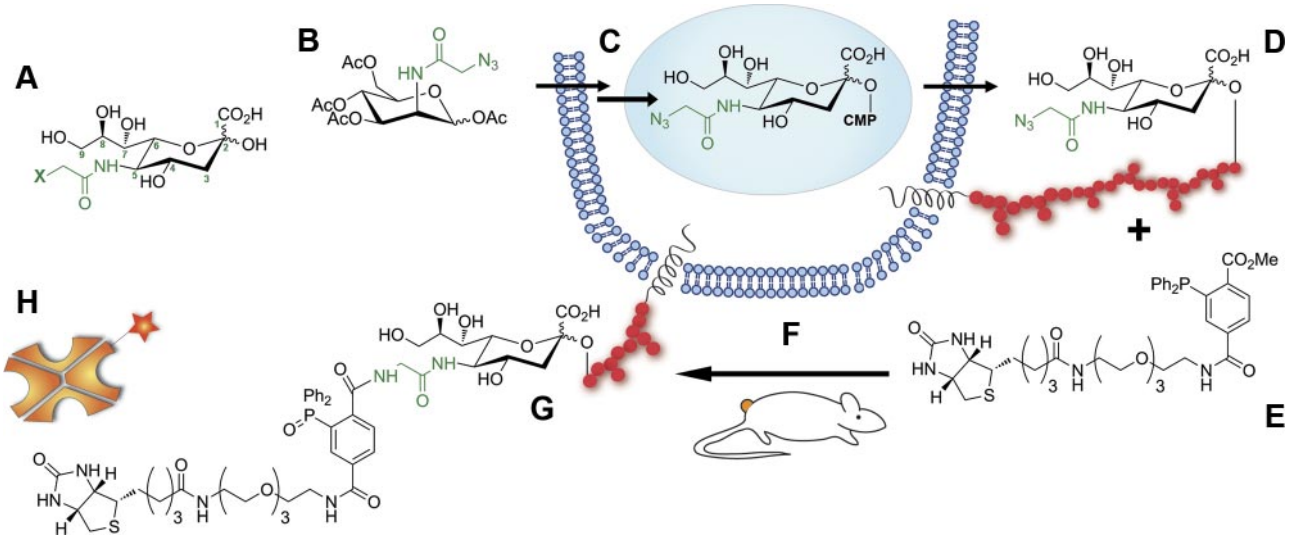


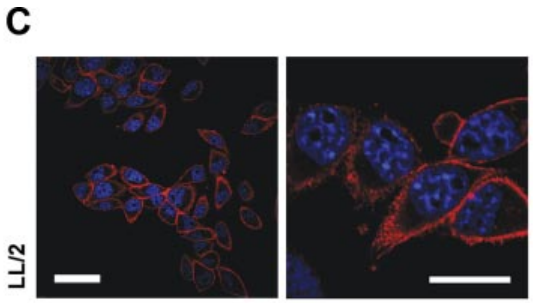
Sialylated tumor glycan imaging

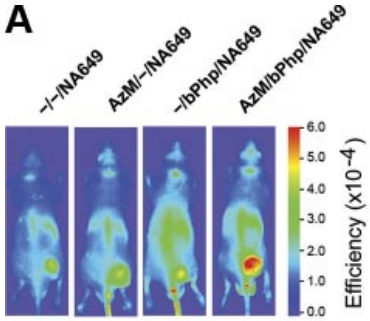
Imaging sialylated tumor cell glycans *in vivo*

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.





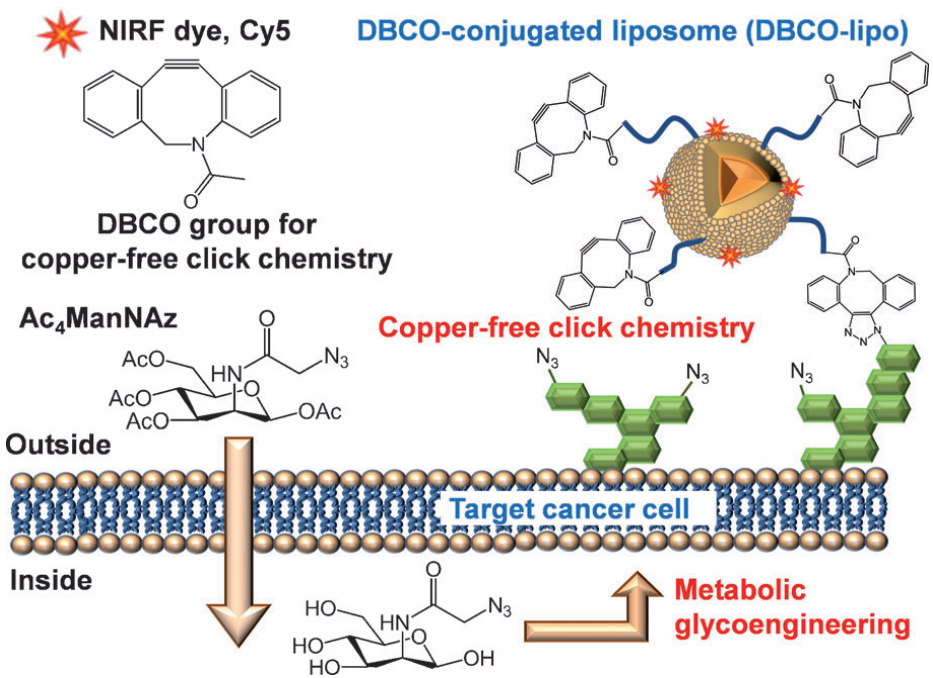


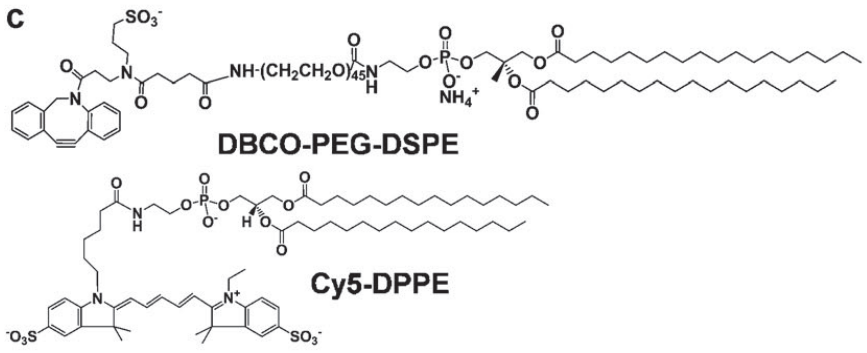
Tumor-targeting

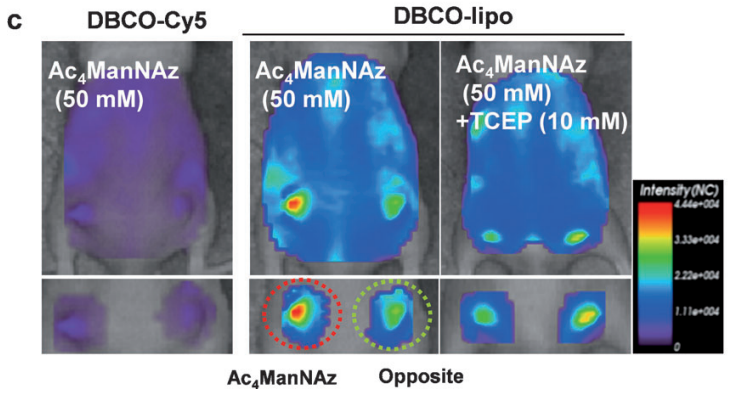
Bioorthogonal Copper-Free Click Chemistry In Vivo for Tumor-Targeted Delivery of Nanoparticles**

*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





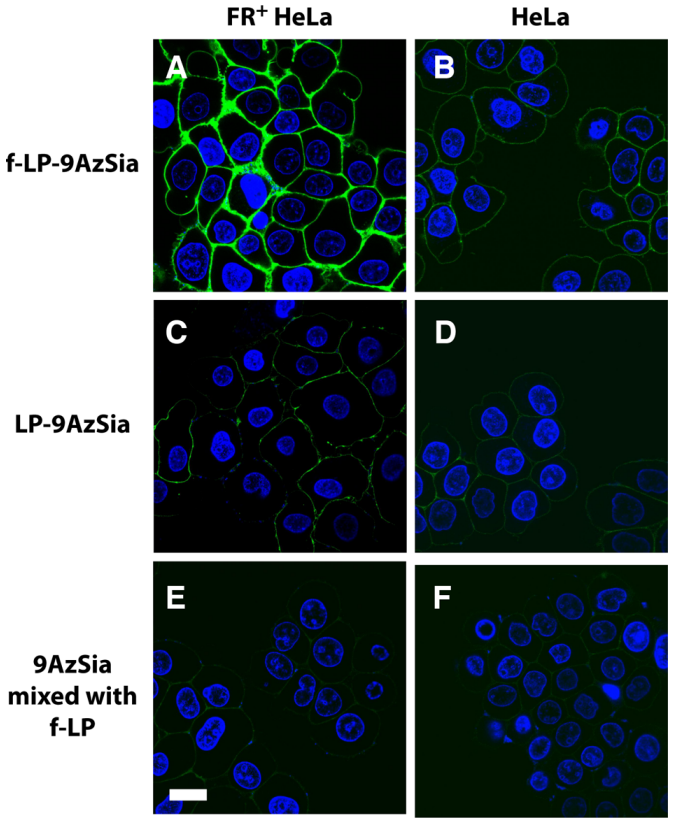


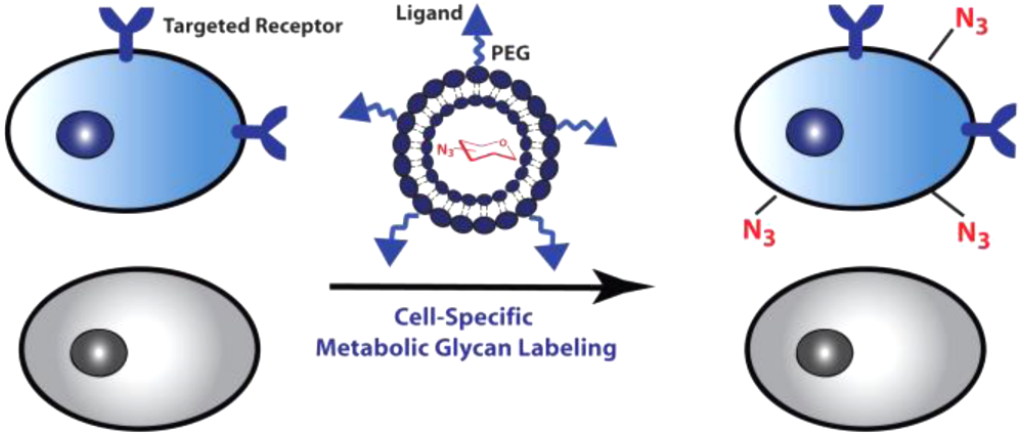
Cell-selective glycan labeling

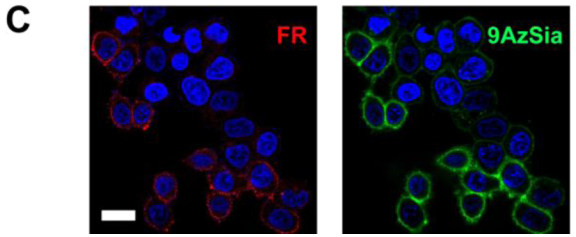
Cell-Selective Metabolic Glycan Labeling Based on Ligand-Targeted Liposomes

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

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







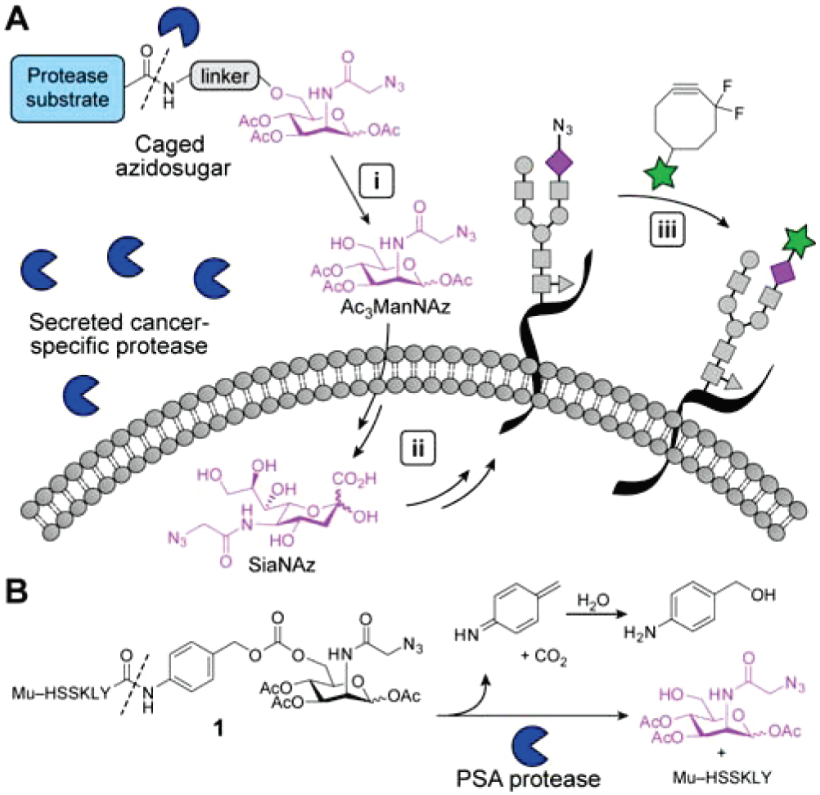
MCF-7 and FR+ HeLa

Caged metabolic precursors

A Strategy for the Selective Imaging of Glycans Using Caged Metabolic Precursors

Pamela V. Chang, Danielle H. Dube,† Ellen M. Sletten, and Carolyn R. Bertozzi*

J. AM. CHEM. SOC. 2010, *132*, 9516–9518



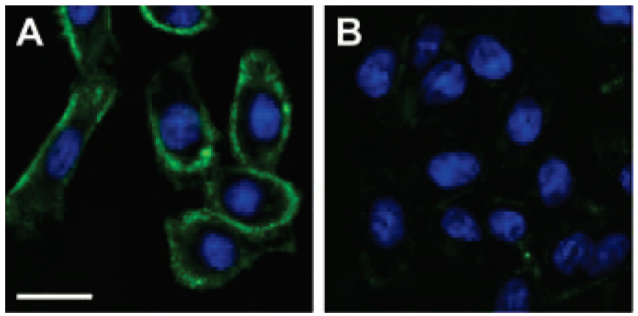
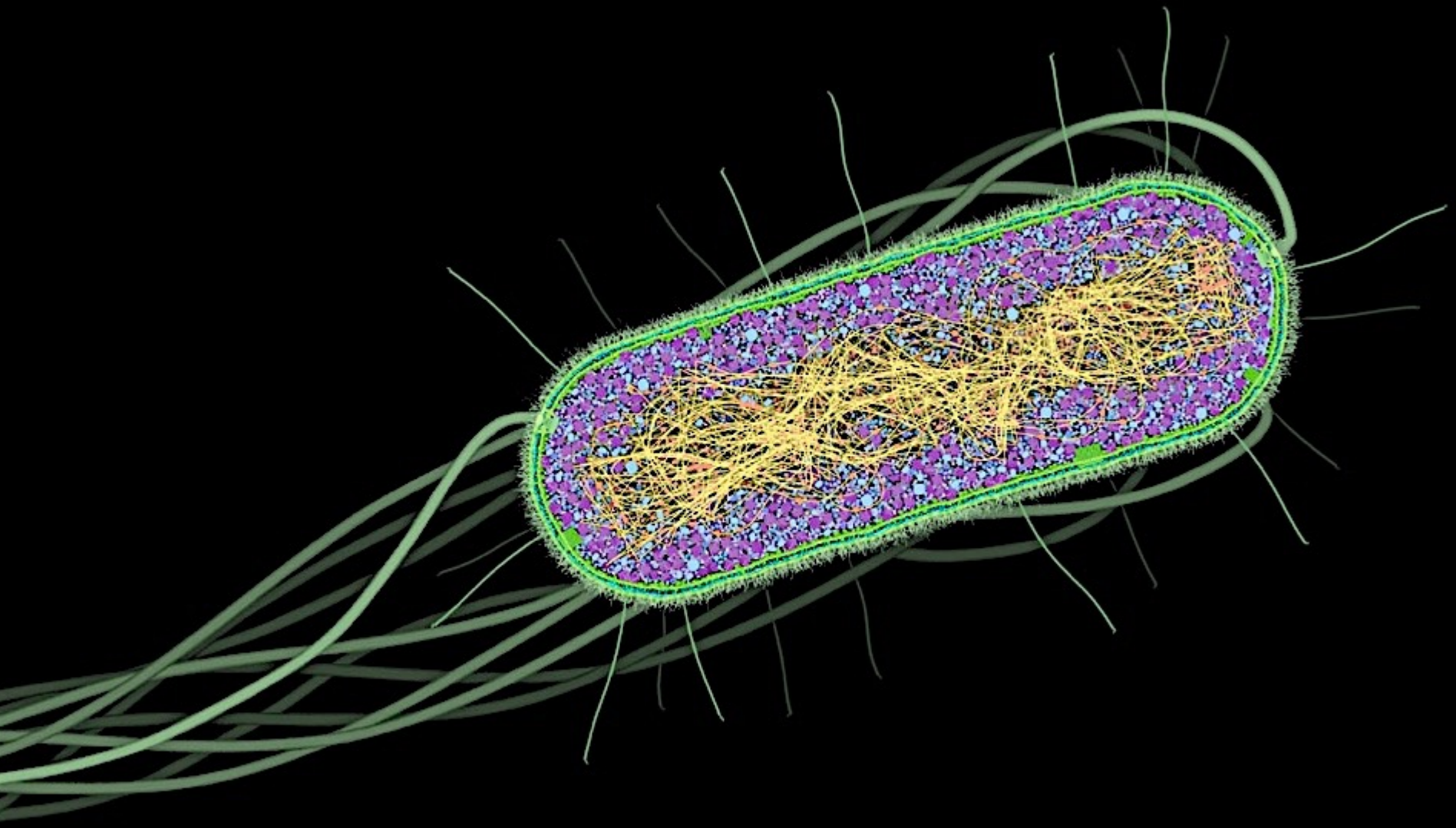


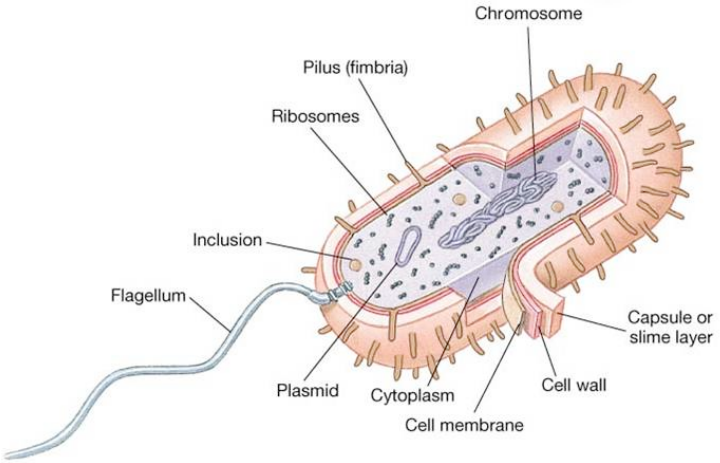
Figure 4. Selective imaging of cells using **1** in the presence of PSA. Fluorescence microscopy analysis of CHO cells treated with **1** (100 μM) and (A) PSA (50 μg/mL) or (B) HK PSA (50 μg/mL), followed by DIFO−biotin (100 μM) and a quantum dot 605−streptavidin conjugate. Green = Texas Red channel; Blue = DAPI channel. Scale bar = 20 μm.

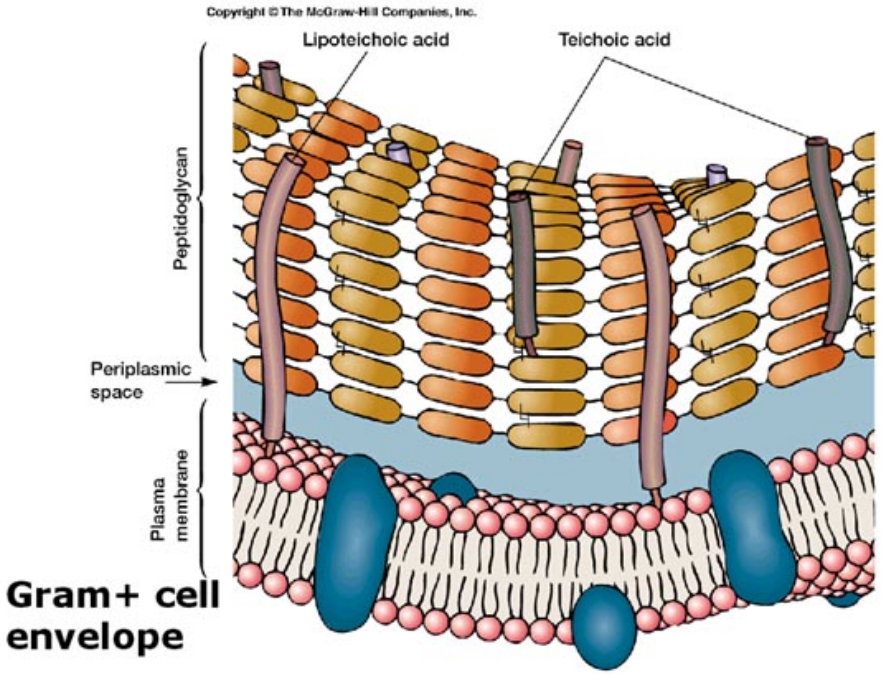
Metabolic Lipopolysaccharide Labeling

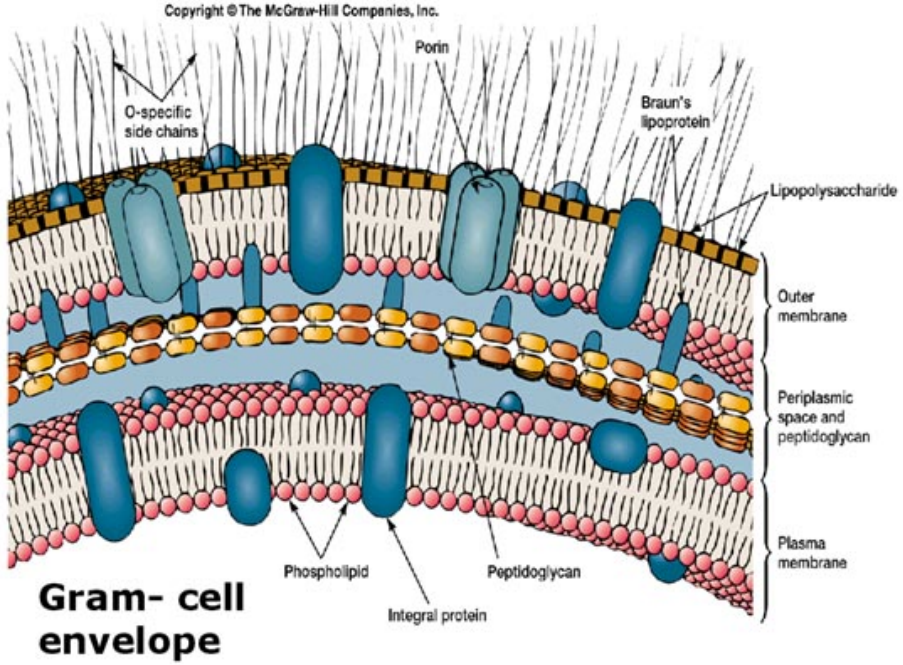


detecting live bacteria

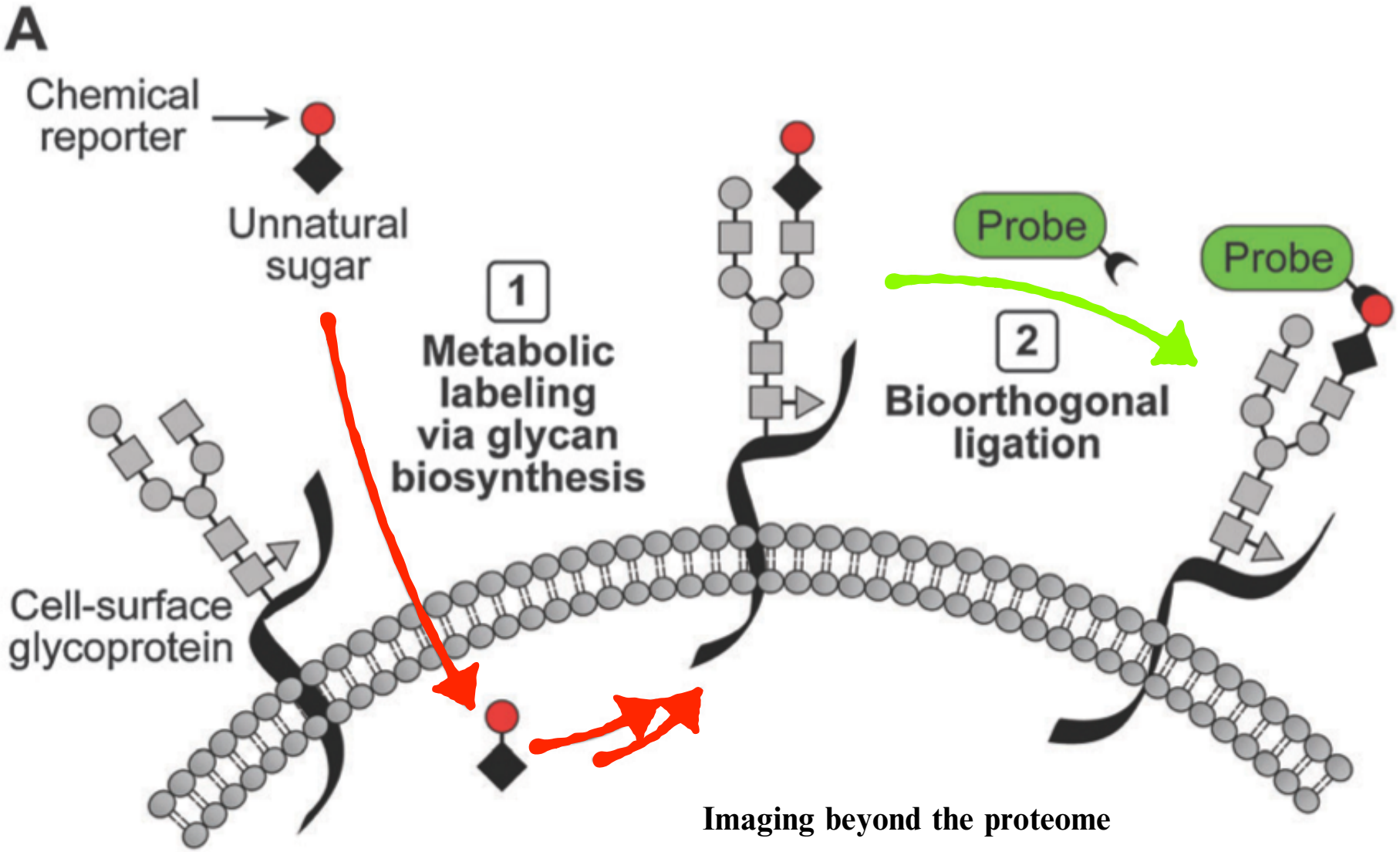
Structure of bacterial cell envelope







Metabolic Glycan Labeling

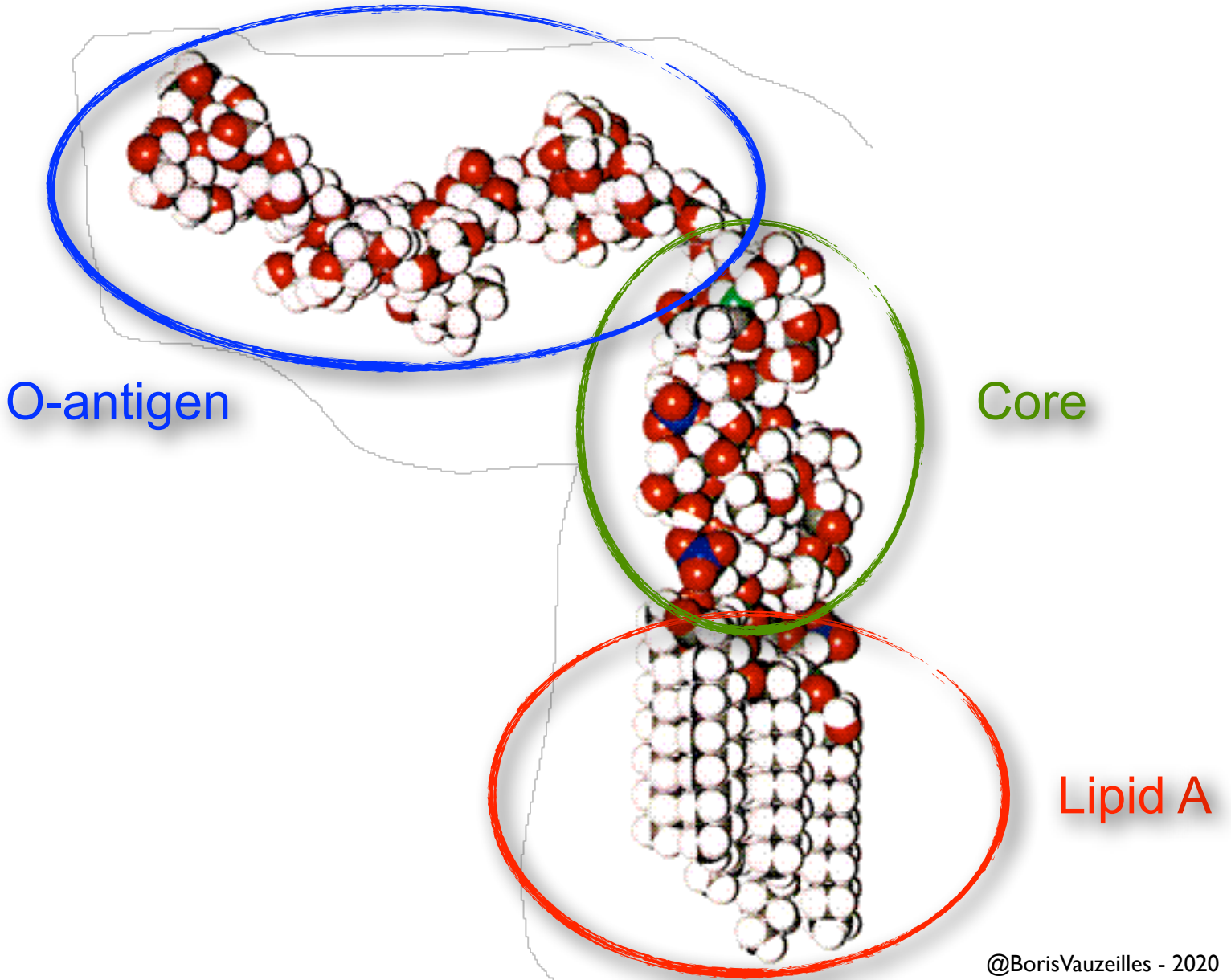


Imaging beyond the proteome

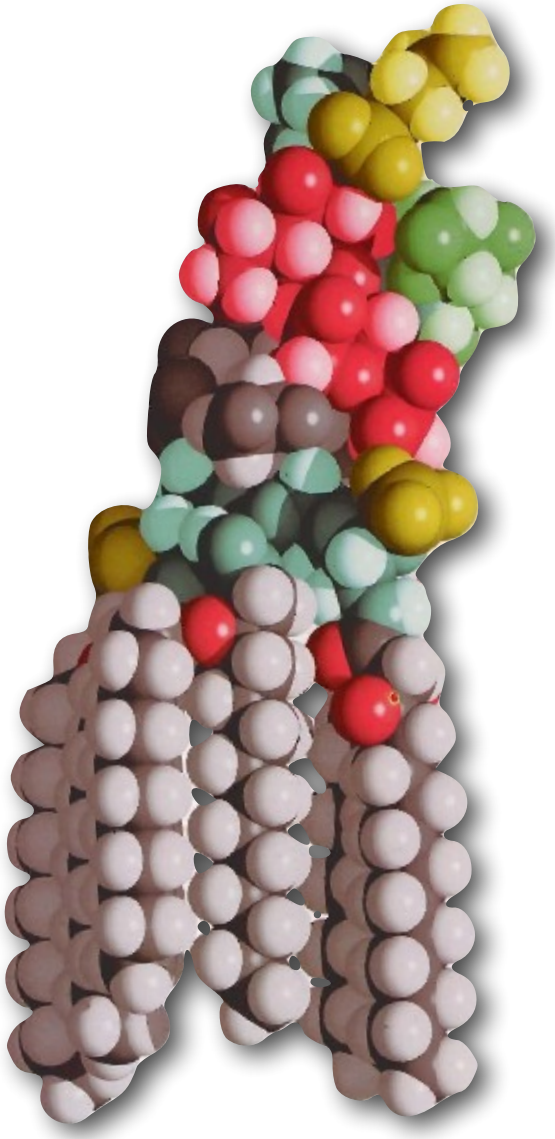
Pamela V. Chang[a] and Carolyn R. Bertozzi*[abc]

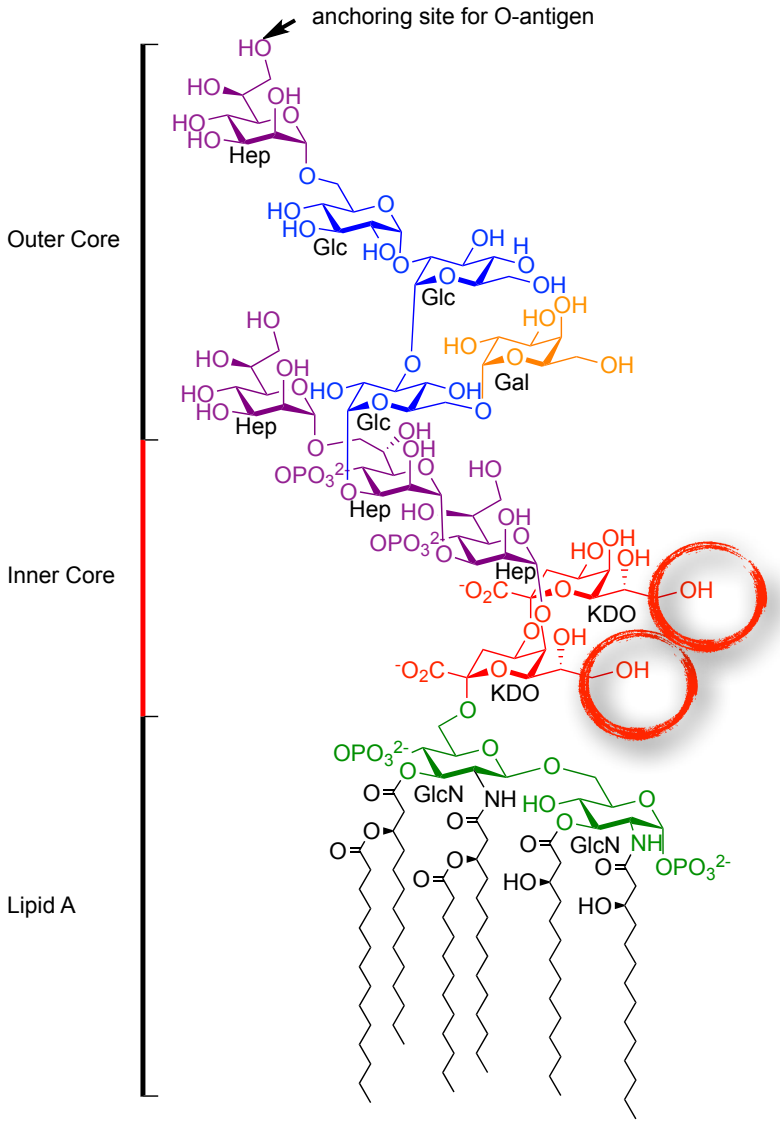
Chem. Commun., 2012, **48**, 8864–8879

Lipopolysaccharide



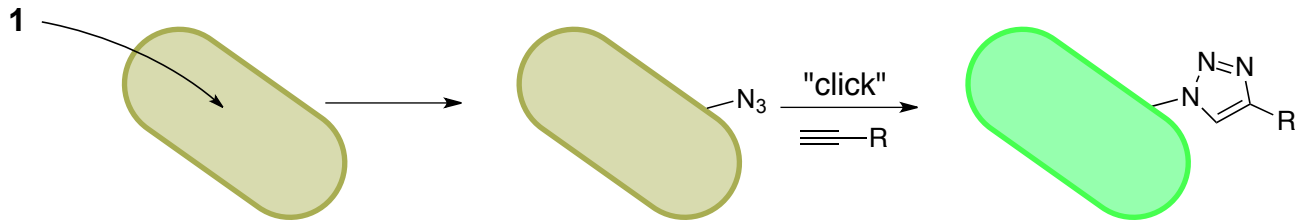
Structure of E. coli LPS

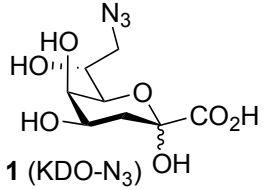




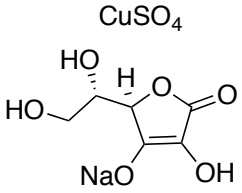
clicking bugs

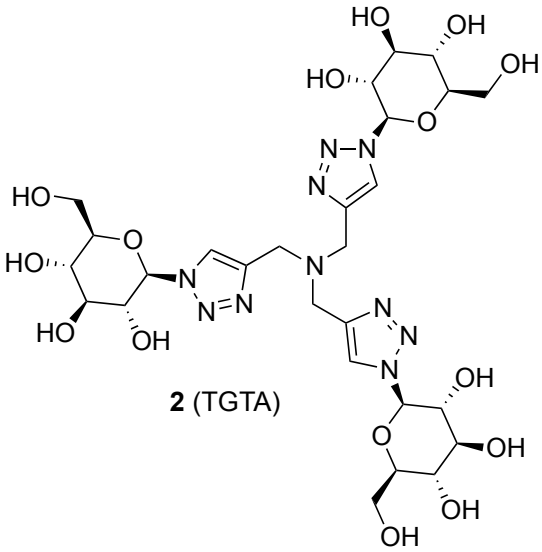
Click-chemistry



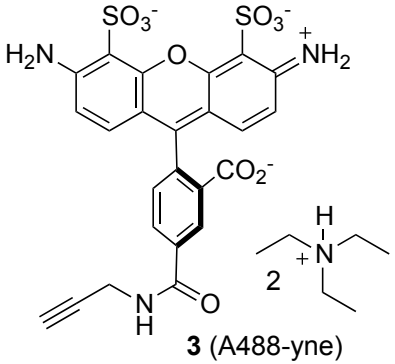


1 (KDO-N_3)



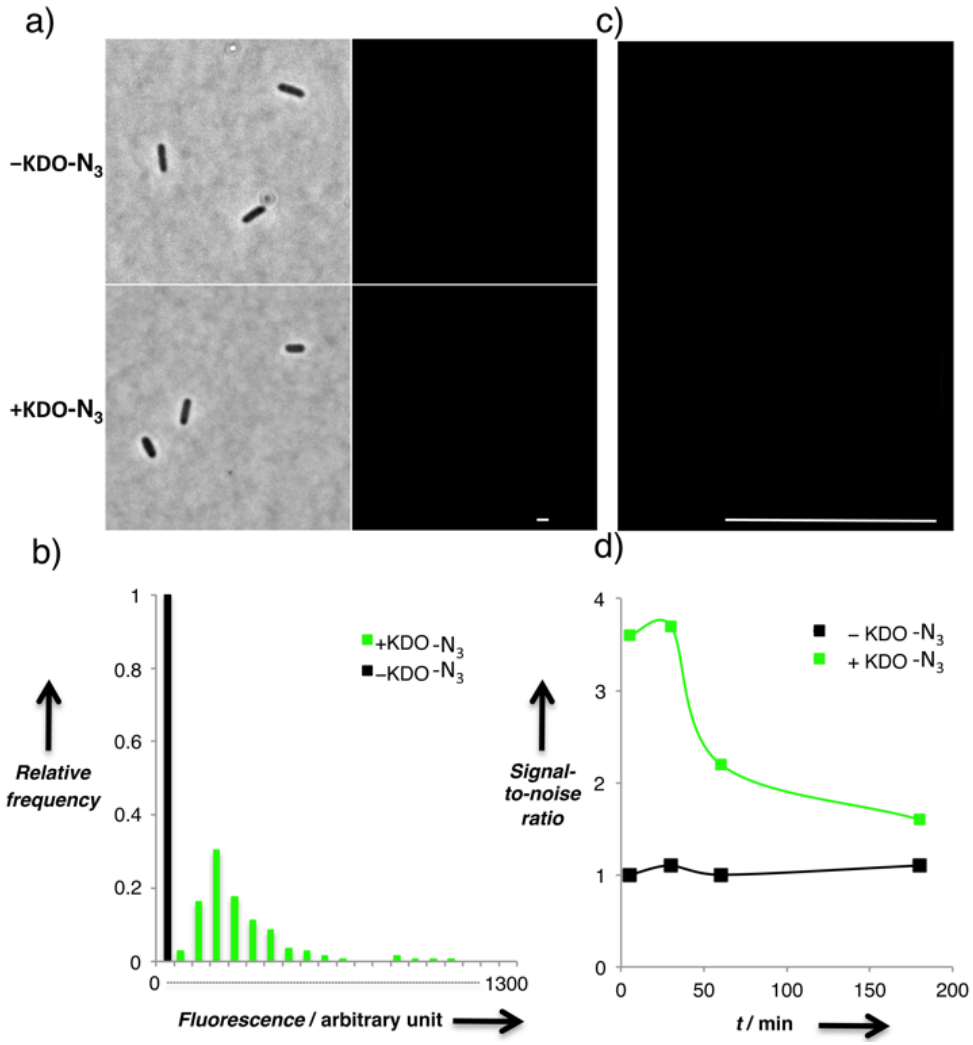


2 (TGTA)



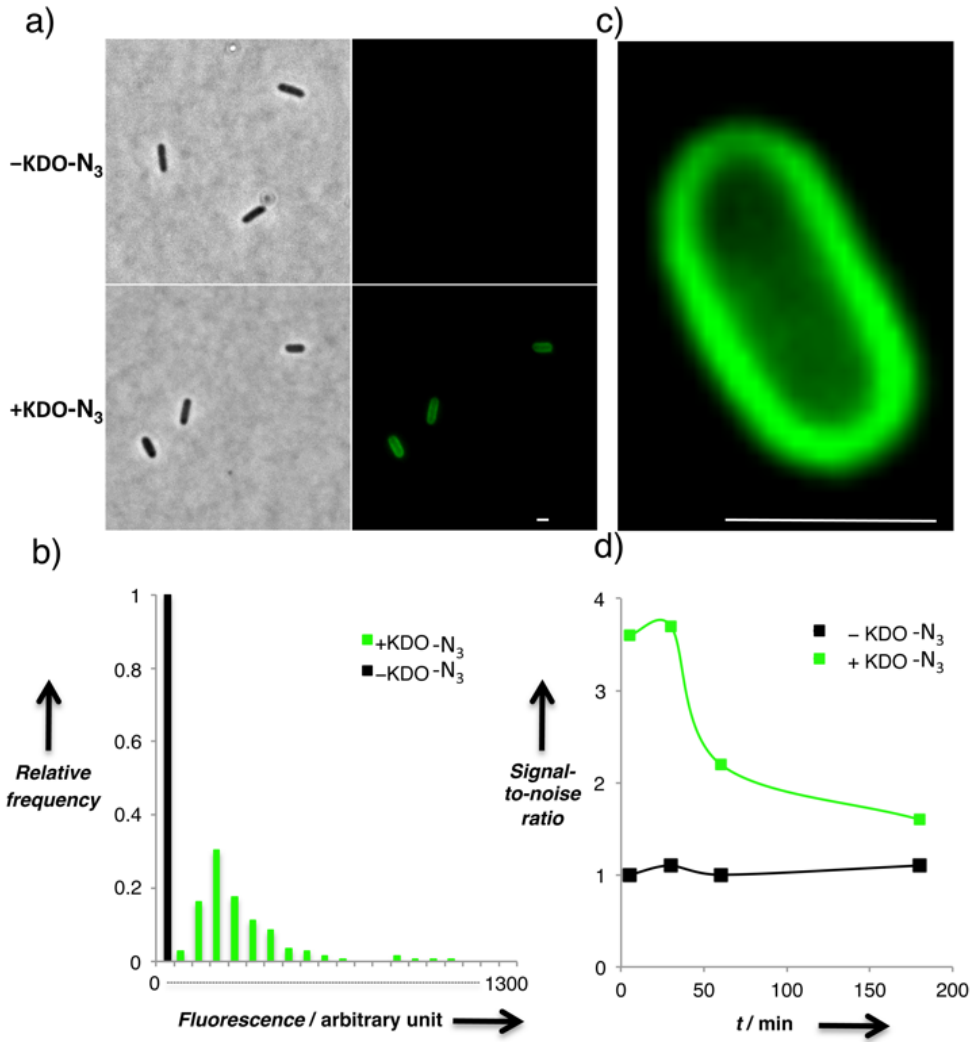
3 (A488-yne)

E. coli labeling



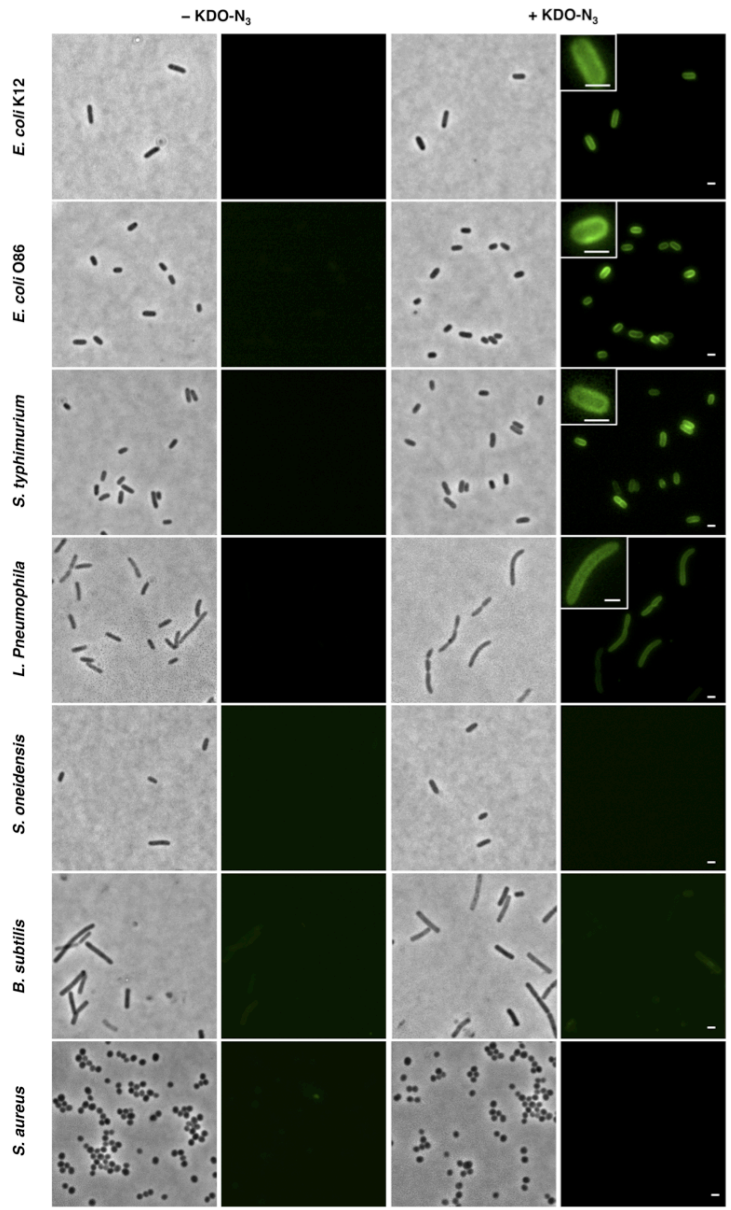
A. Dumont, A. Malleron, M. Awwad, S. Dukan*, B. Vauzeilles*, *Angew. Chem. Int. Ed.*, **2012**, *51*, 3143-3146

E. coli labeling



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

Other species - Kdo-N_3



Escherichia coli K12

Gram -

Escherichia coli O86

Gram -

Salmonella typhimurium

Gram -

Legionella pneumophila

Gram -

Shewanella oneidensis

Gram - / KDO -

Bacillus subtilis

Gram +

Staphylococcus aureus

Gram +

Legionella pneumophila

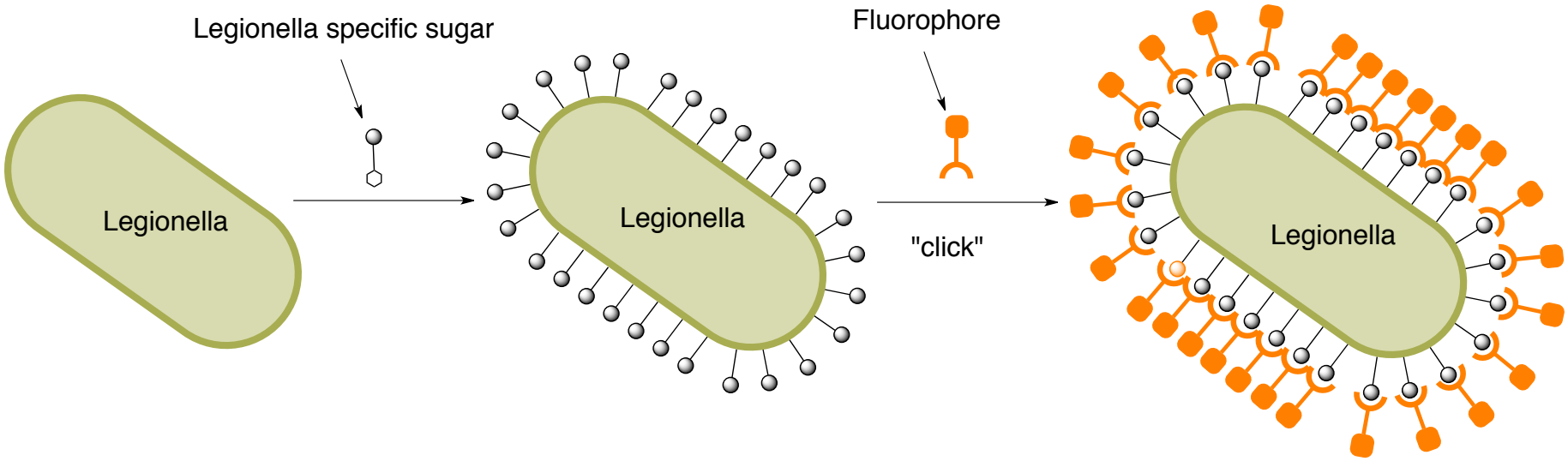
Bellevue-Stratford Philadephia

1976

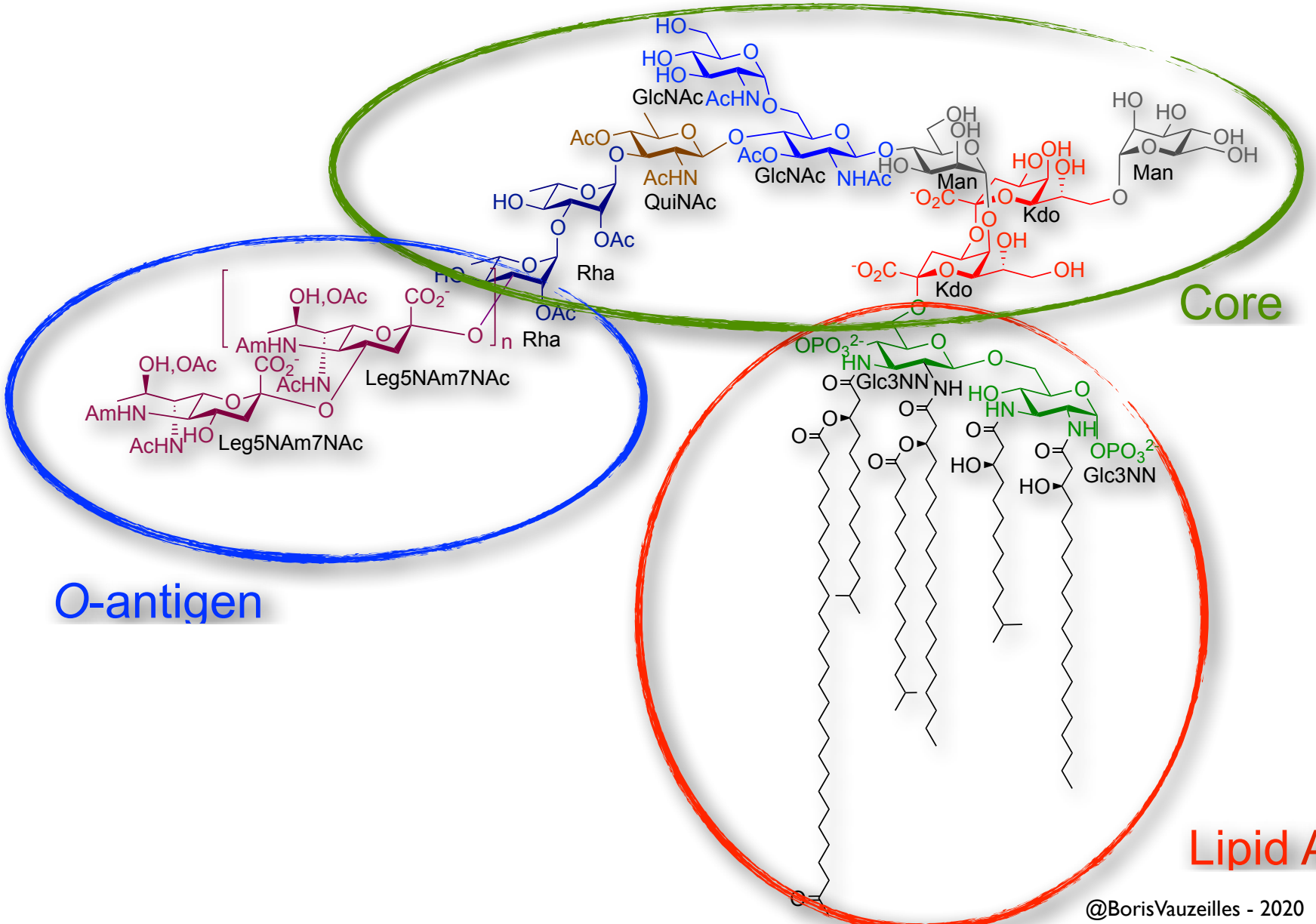
Philadelphia 1976 - Convention of the American Legion

221 people infected - 34 died (15%)

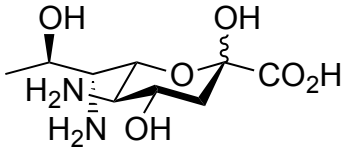
Detection strategy



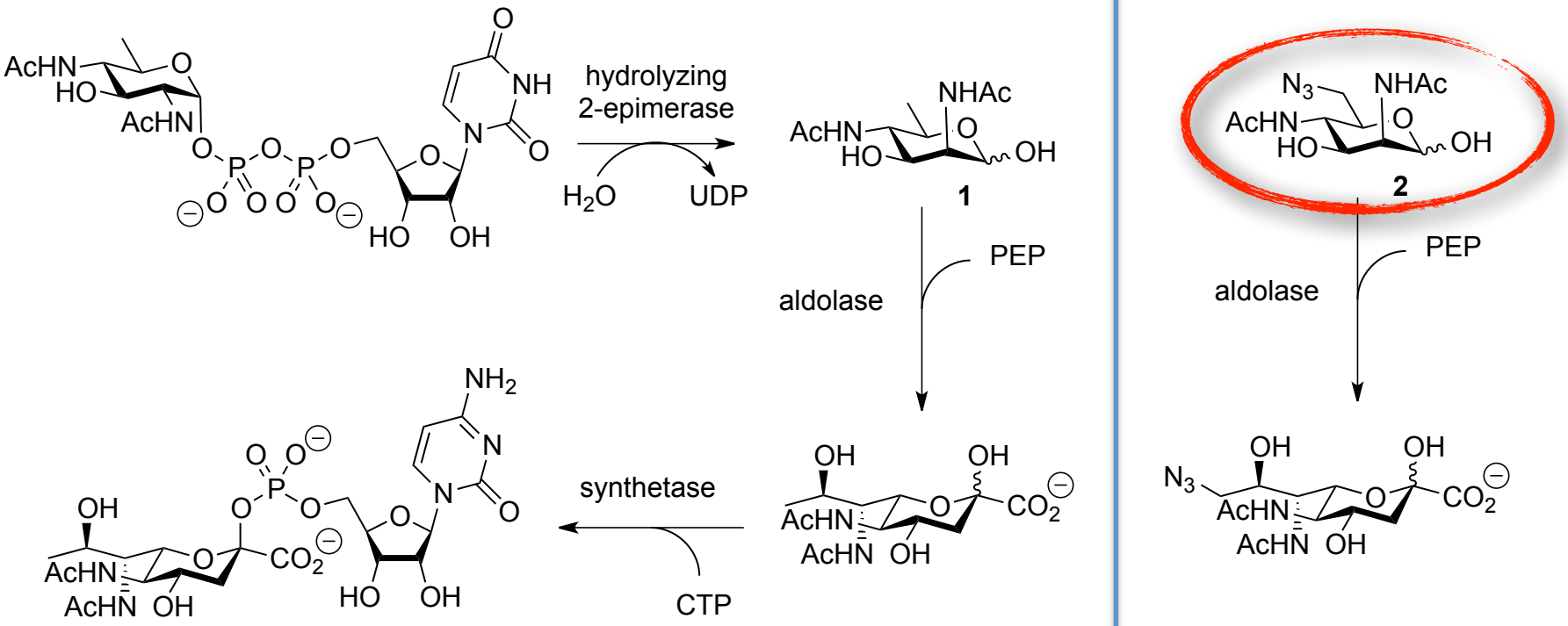
LPS of *Legionella pneumophila* serogroup 1



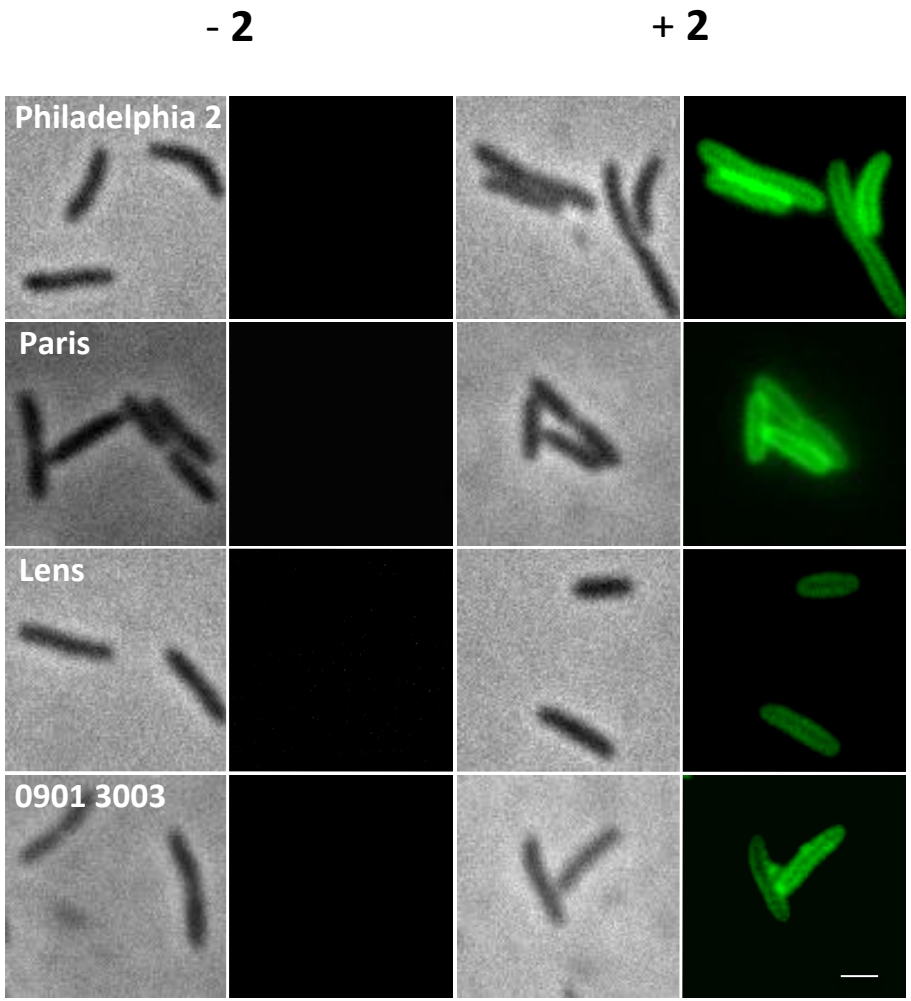
Legionella pneumophila - Sg1



Legionaminic acid (Leg)



L. pneumophila serogroup 1

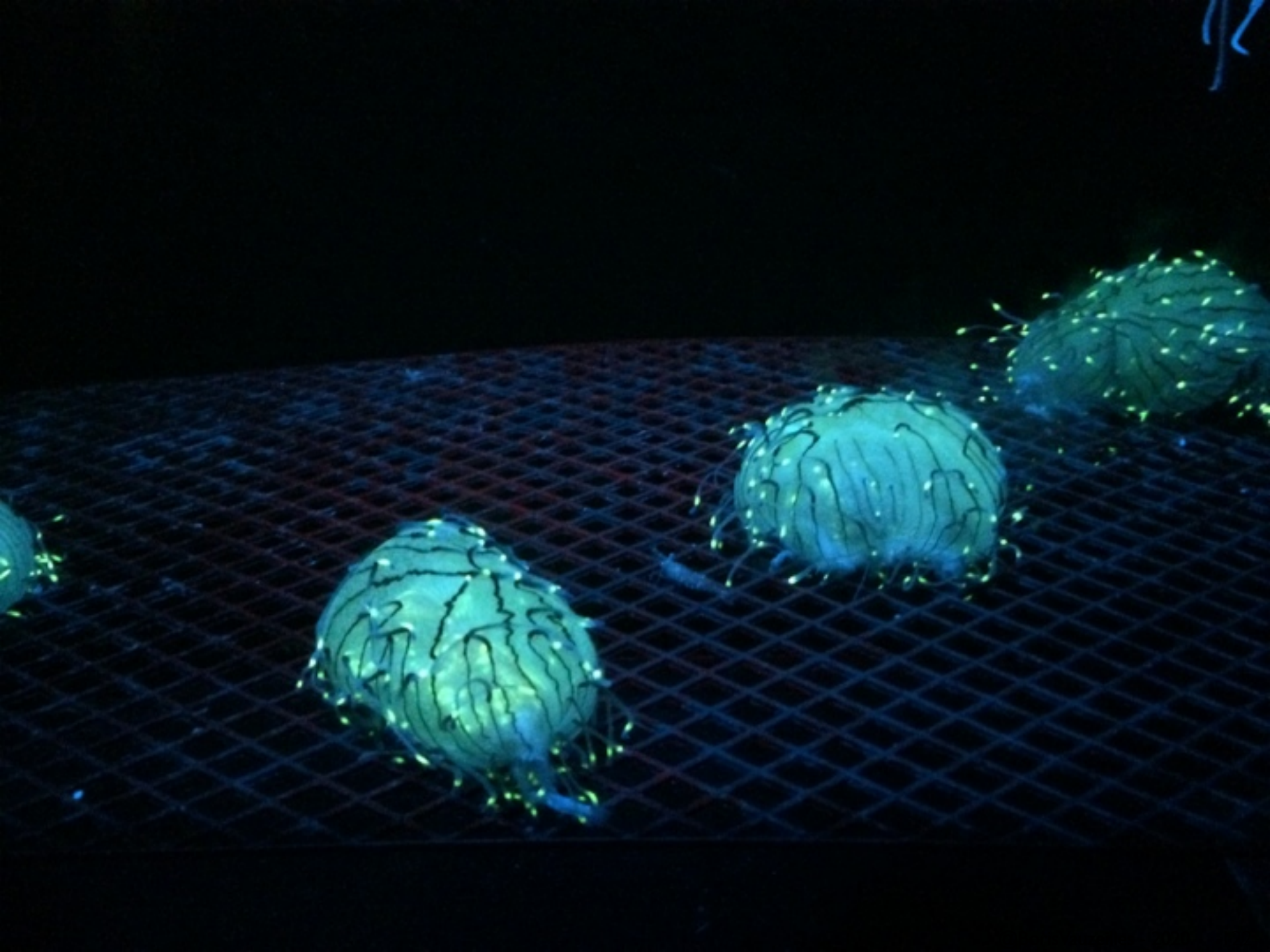


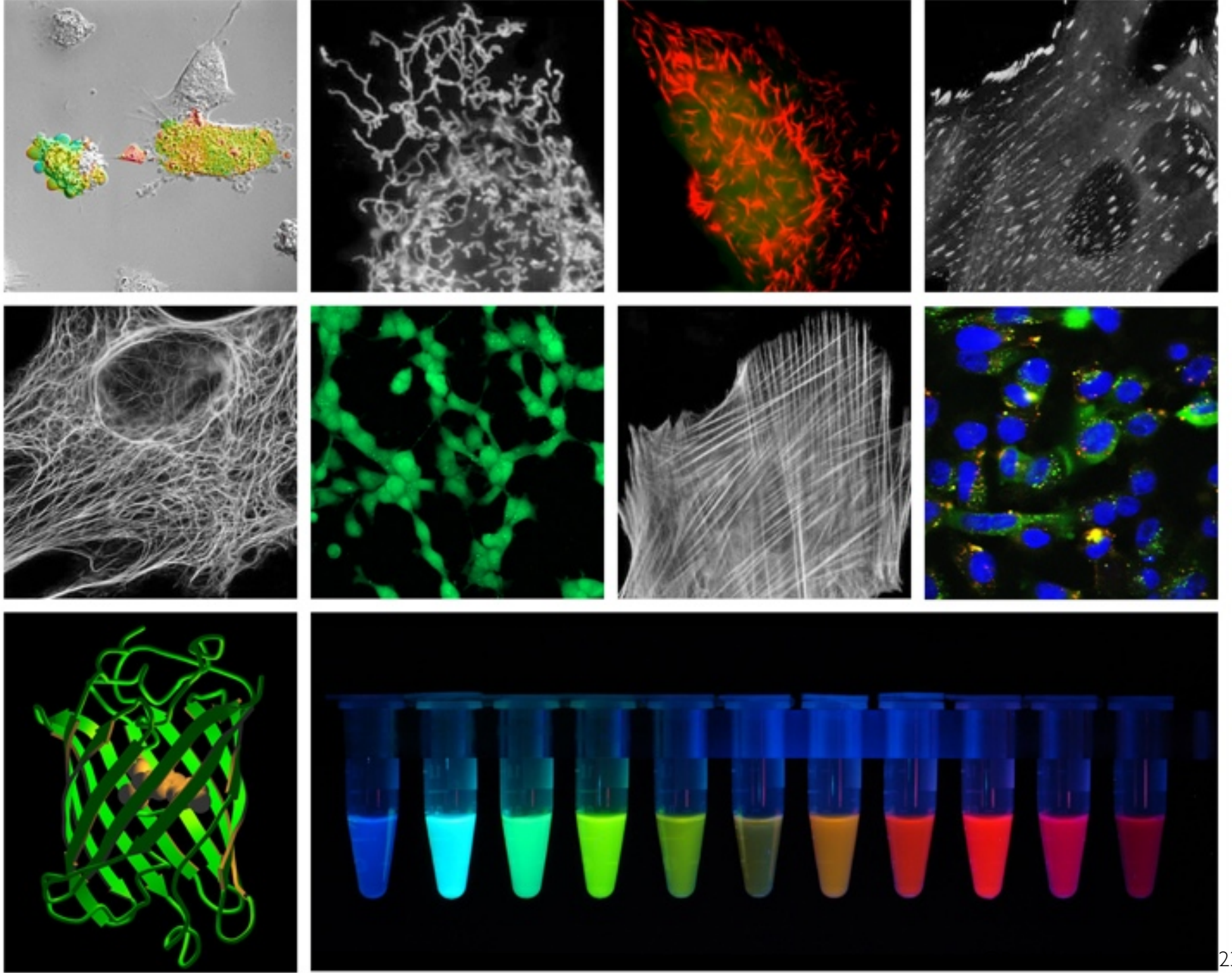
J. Mas Pons, A. Dumont, G. Sautejeau, E. Fugier, A. Baron, S. Dukan*, B. Vauzeilles*, Angew. Chem. Int. Ed., **2014**, *53*, 1275-1278

Labeling proteins *in vivo*

Fluorescent proteins

Roger Y. Tsien





SNAP-Tag - CLIP-Tag

The Nobel Prize in Chemistry 2015

Photo: Cancer Research UK

Tomas Lindahl

Prize share: 1/3

Photo: K. Wolf/AP Images for HHMI

Paul Modrich

Prize share: 1/3

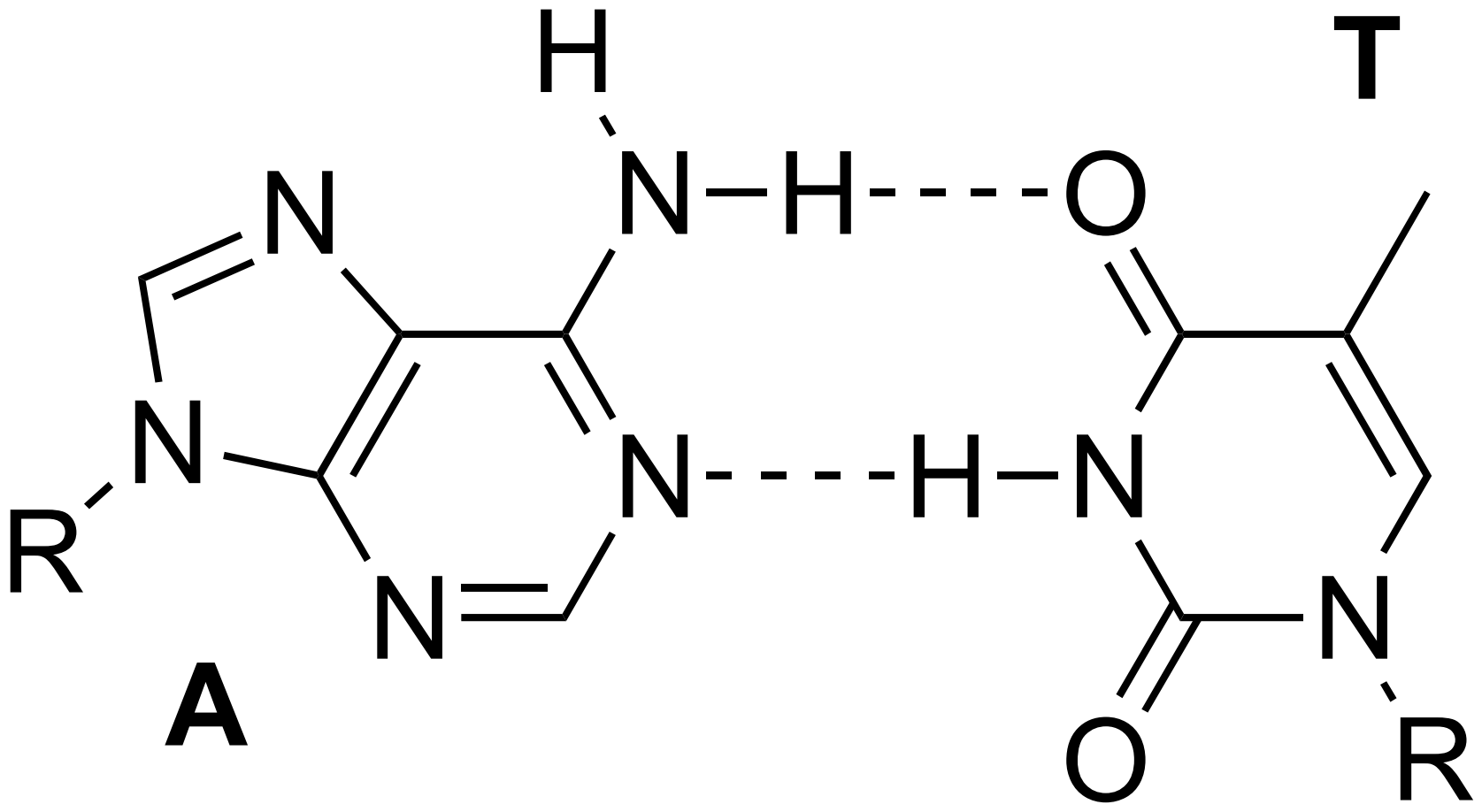
Photo: M. Englund, UNC-School of Medicine

Aziz Sancar

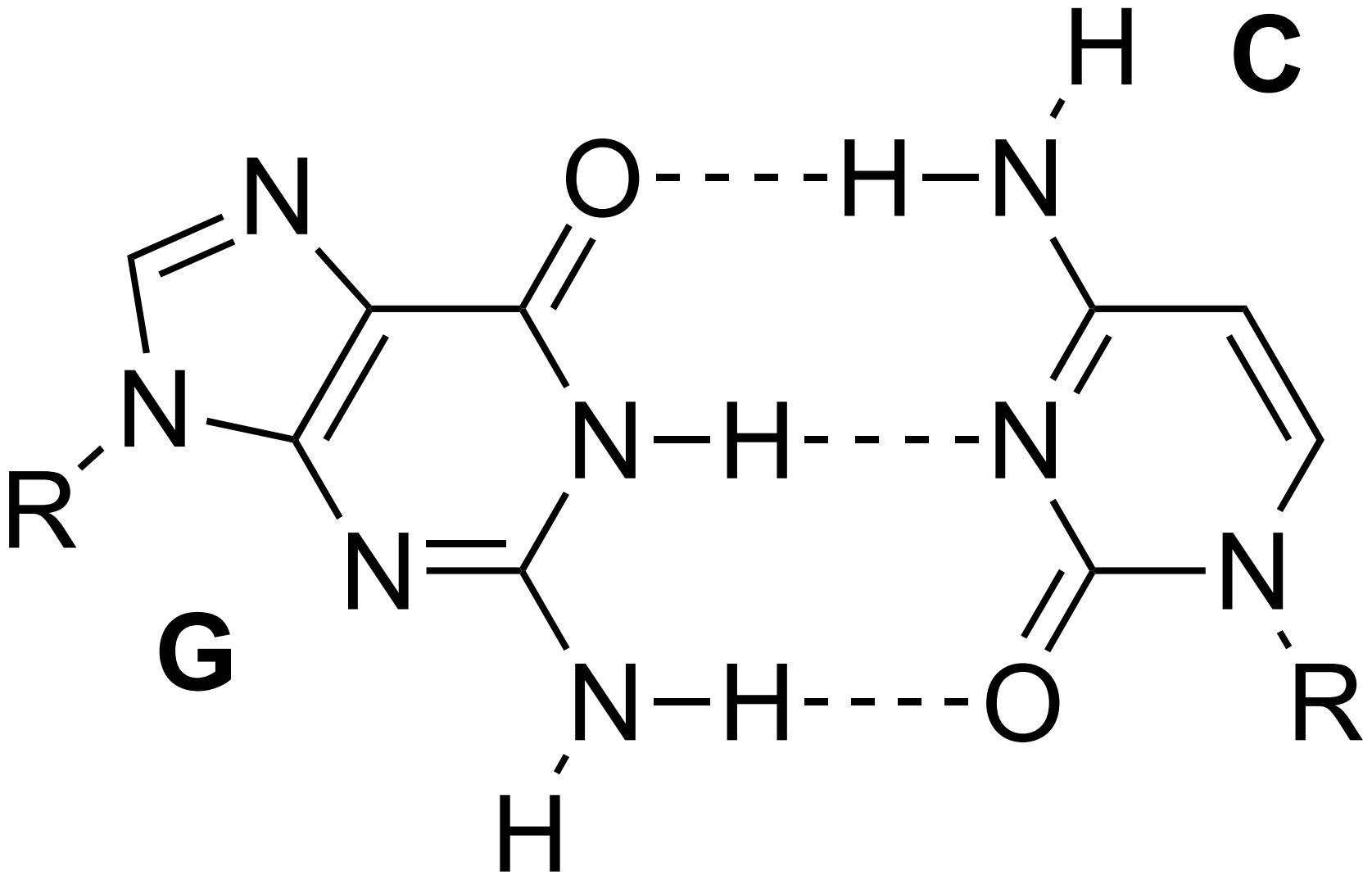
Prize share: 1/3

The Nobel Prize in Chemistry 2015 was awarded jointly to Tomas Lindahl, Paul Modrich and Aziz Sancar *"for mechanistic studies of DNA repair"*.

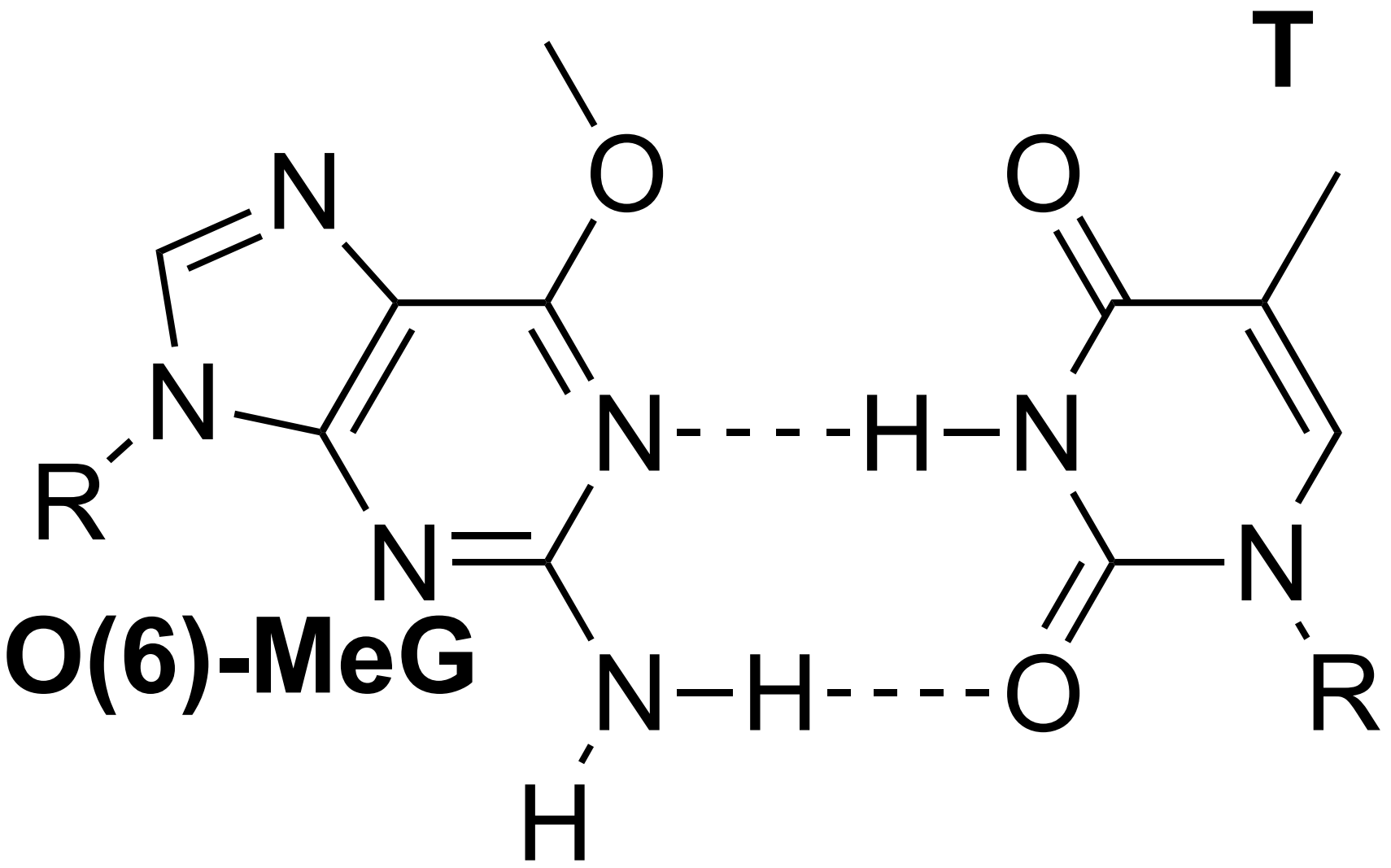
A•T base pair



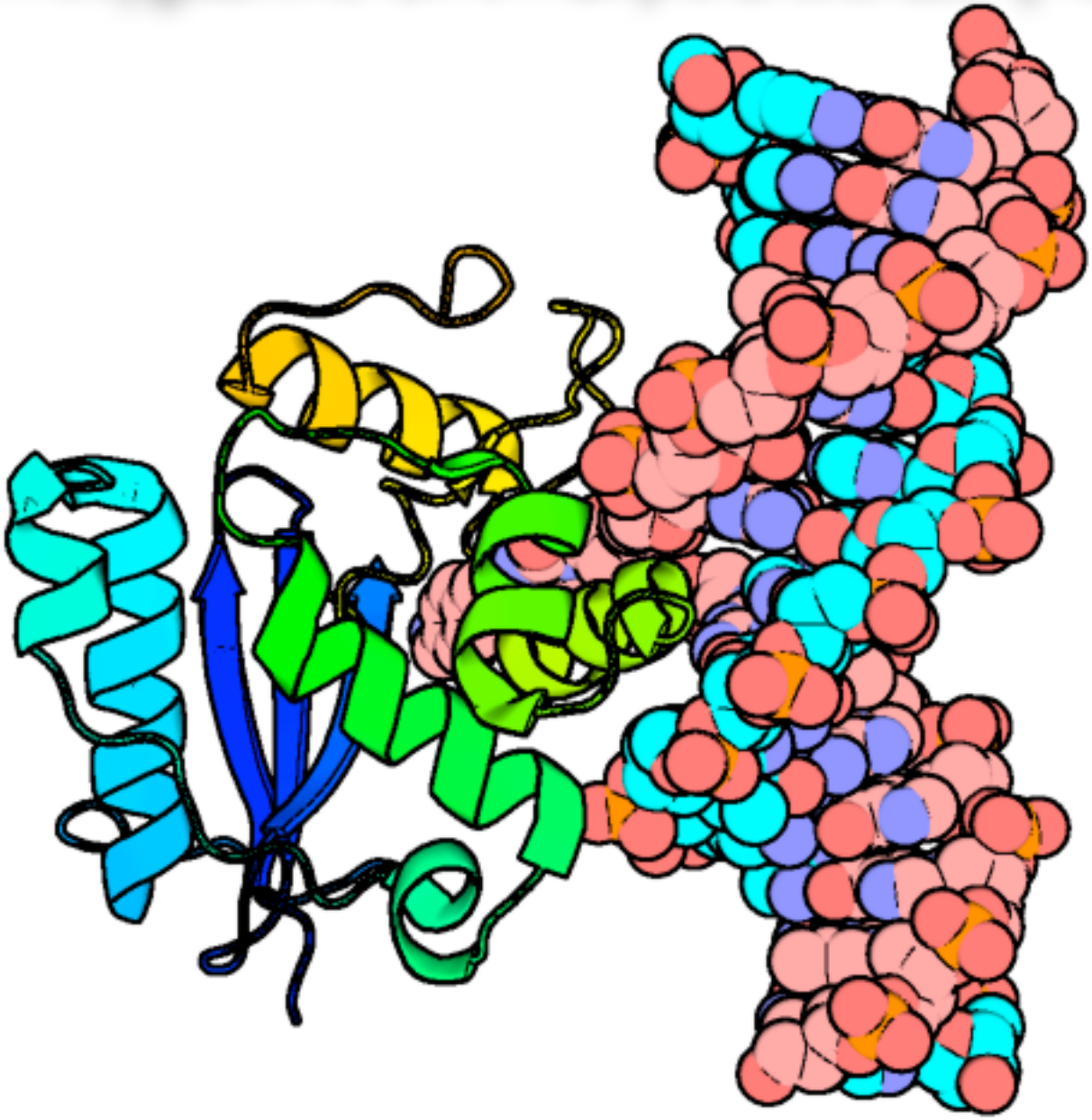
G•C base pair



O(6)-MeG•T

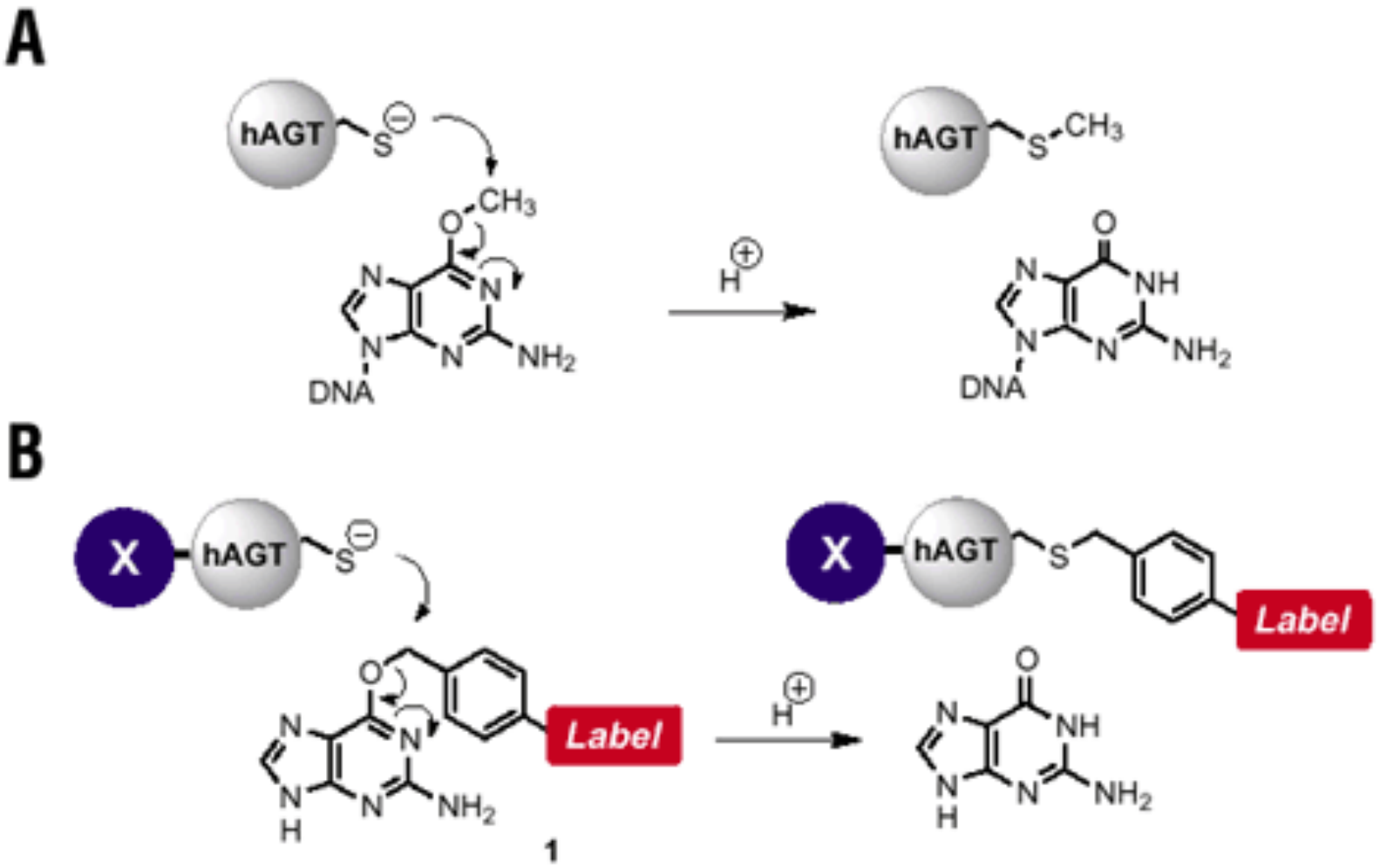


O6-Alklyguanine-DNA alkyltransferase (AGT)



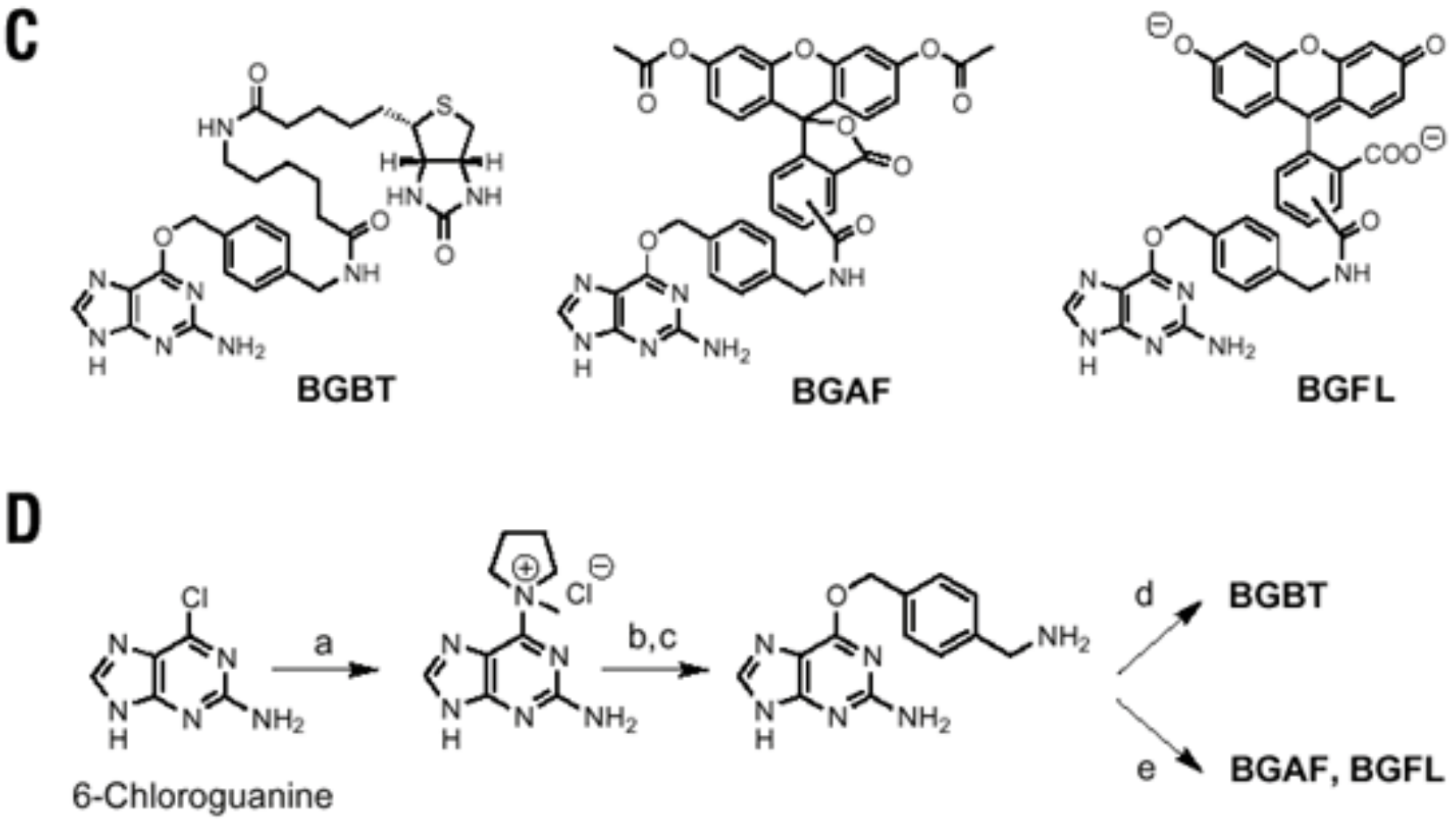
Kai Johnsson

SNAP-Tag



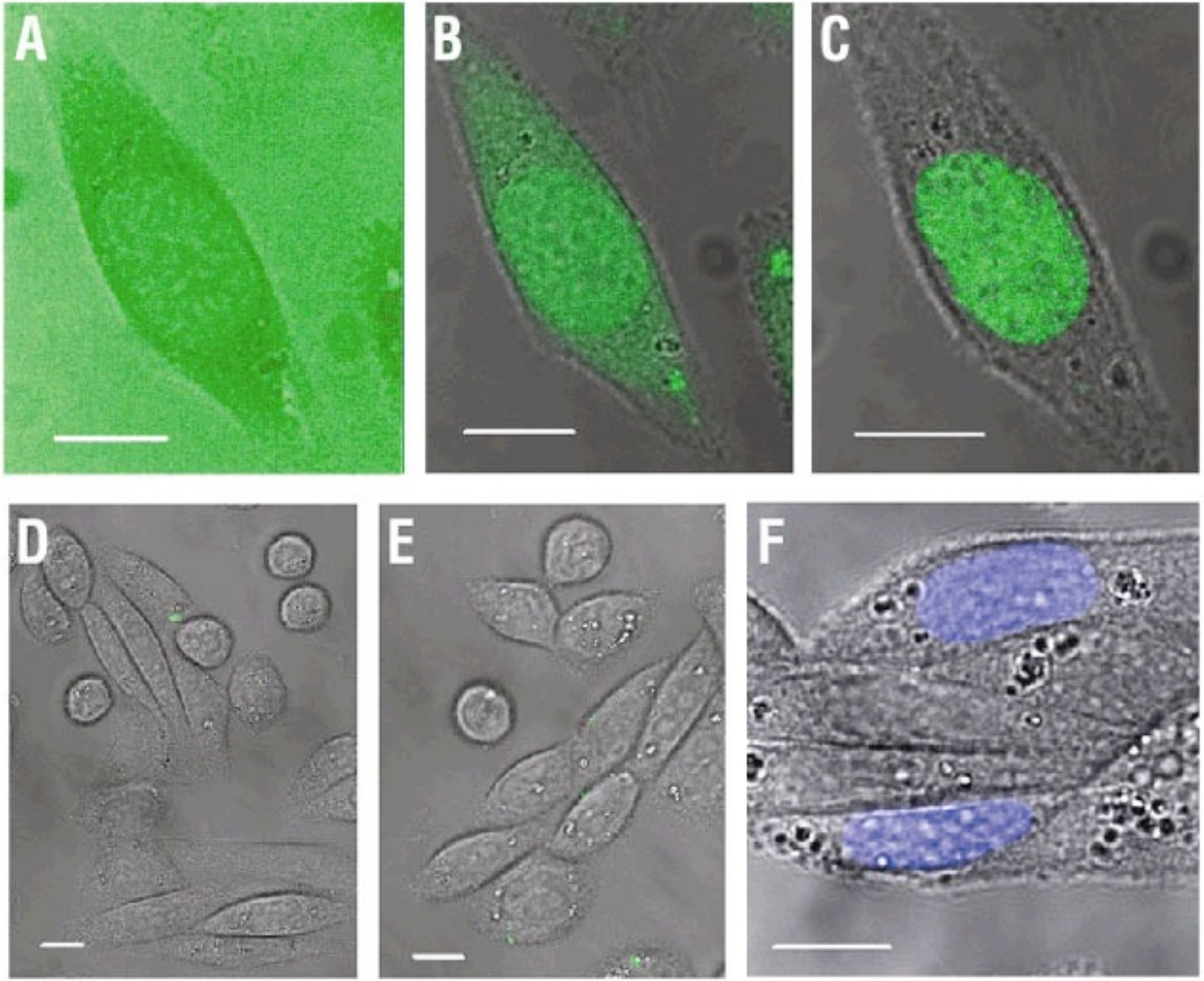
K. Johnsson *et coll., Nat. Biotechnol.*, **2003**, *21*, 86-89

SNAP-Tag

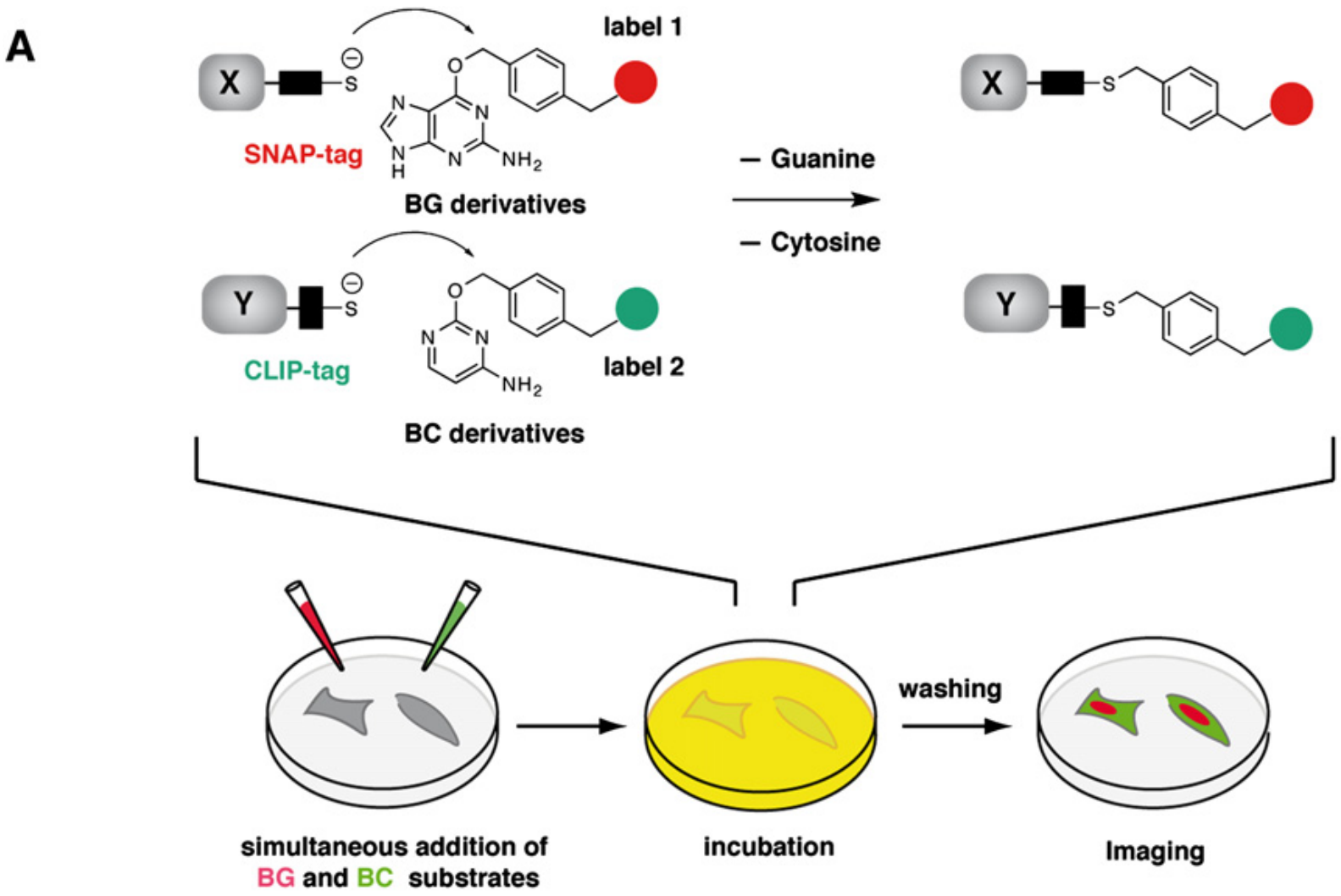


(a) 1-methylpyrrolidin, N,N-dimethylformamide (DMF), 66%; (b) 2,2,2-trifluoro-N-(4-hydroxymethyl-benzyl)-acetamide, potassium tert-butoxide, DMF, 88%; (c) K_2CO_3, methanol, 85%; (d) N-(+)-biotinyl-6-aminocaproic acid N-succinimidyl ester, triethylamine, DMF, 69%; (e) 5(6)-carboxyfluorescein diacetate N-succinimidyl ester (mixture of isomers), triethylamine, DMF, 8% (BGFL), 2% (BGAF)

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

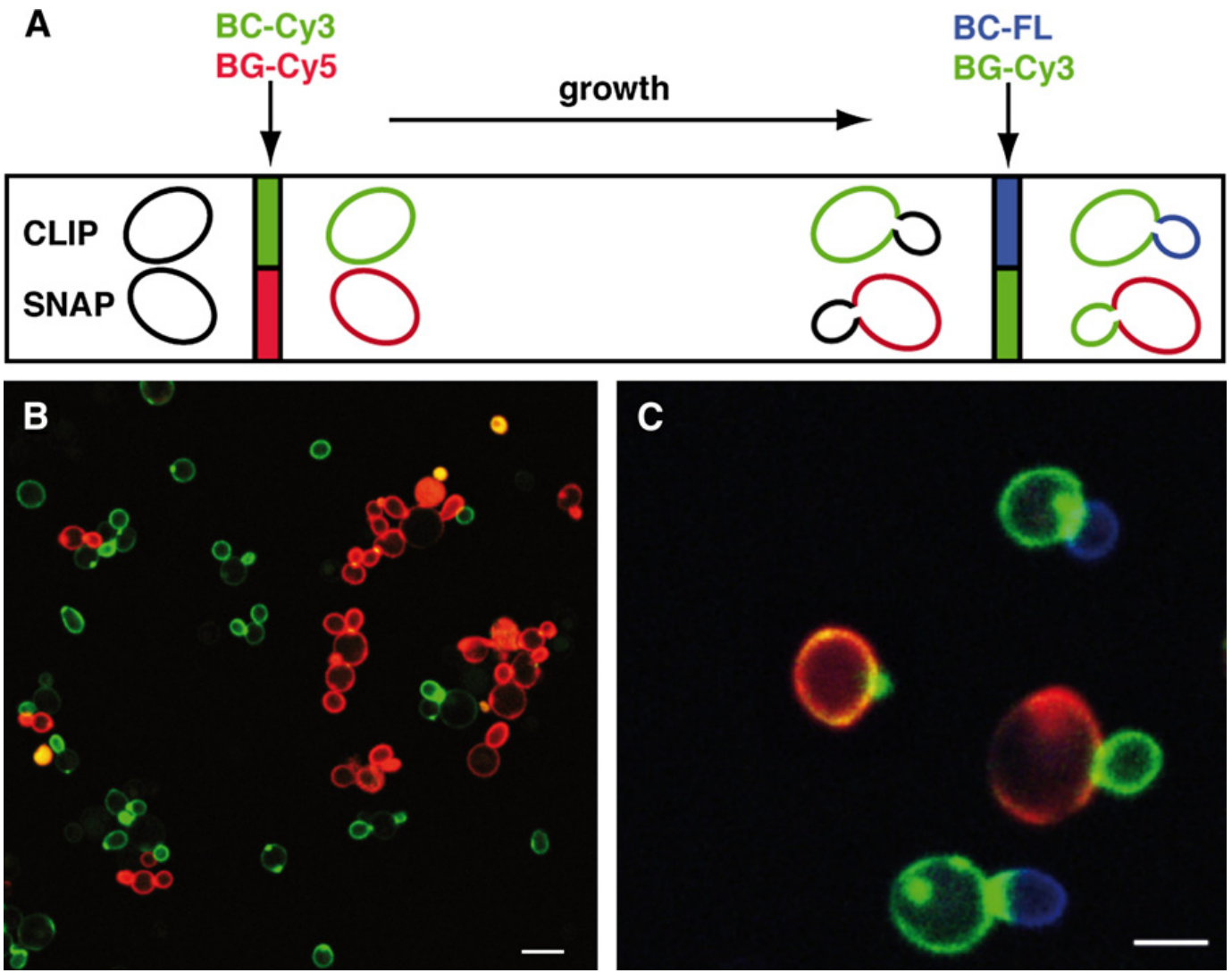

A
B
C
D
E
F

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

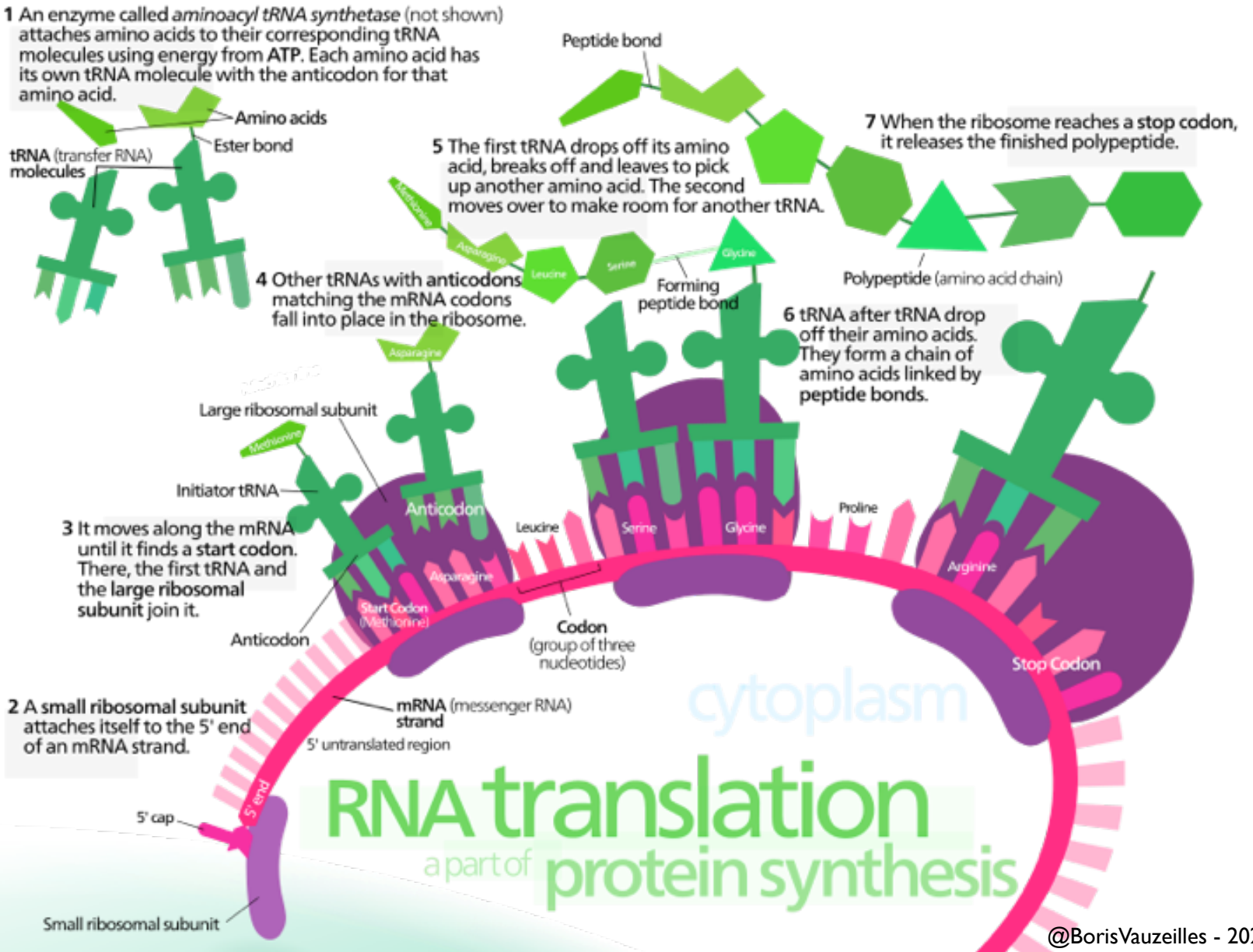
nonpolar | polar | basic | acidic | (stop codon)

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

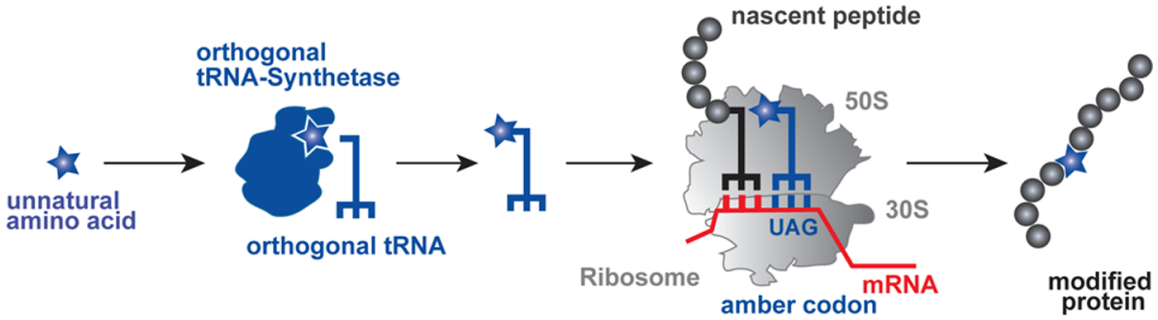


Figure 16. Expanding the genetic code. An unnatural amino acid (blue star), added to the cell growth medium, is specifically recognized by an orthogonal aminoacyl tRNA synthetase and attached to an orthogonal amber suppressor tRNA, which is decoded by the ribosome in response to an amber codon (UAG) 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...

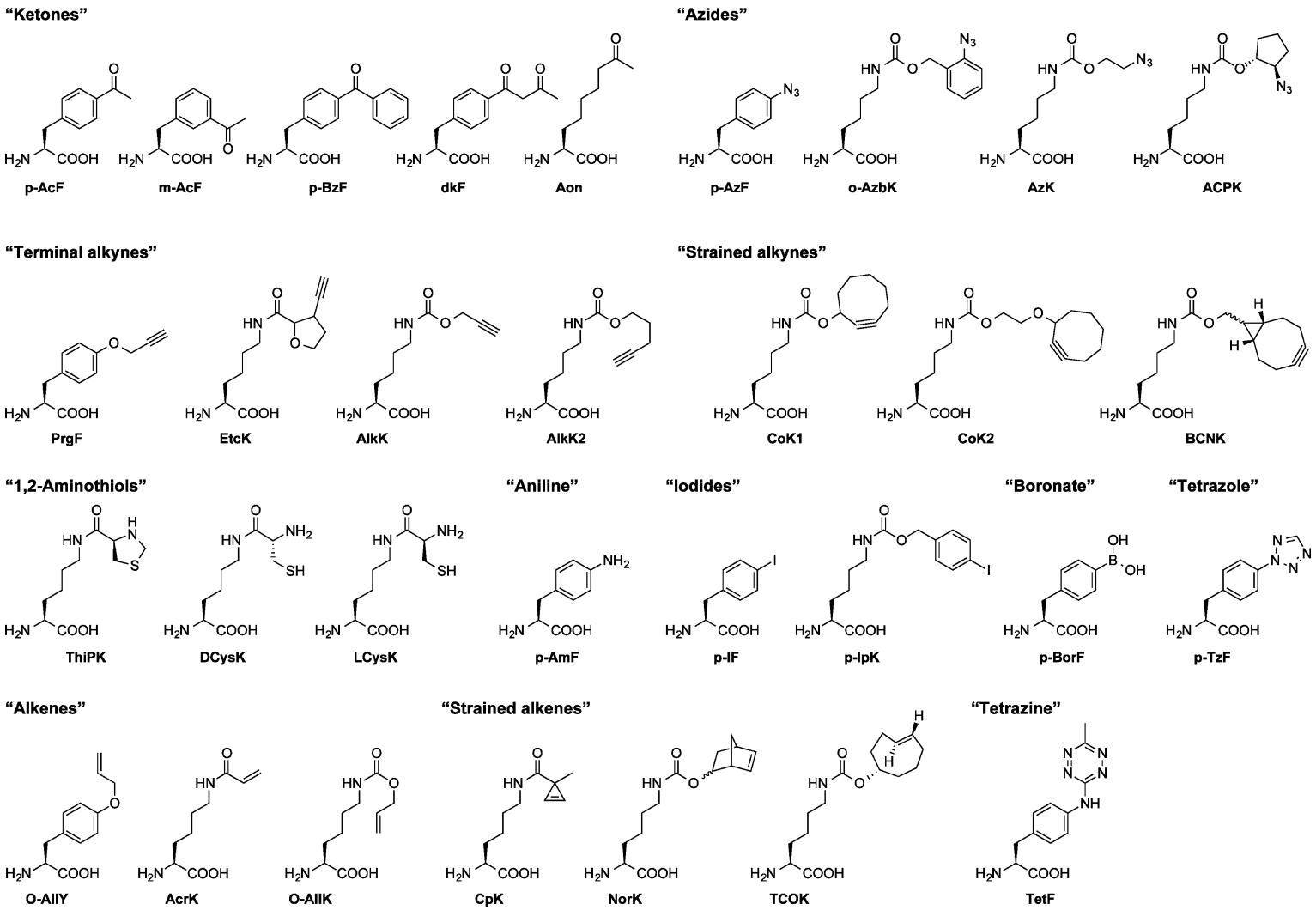
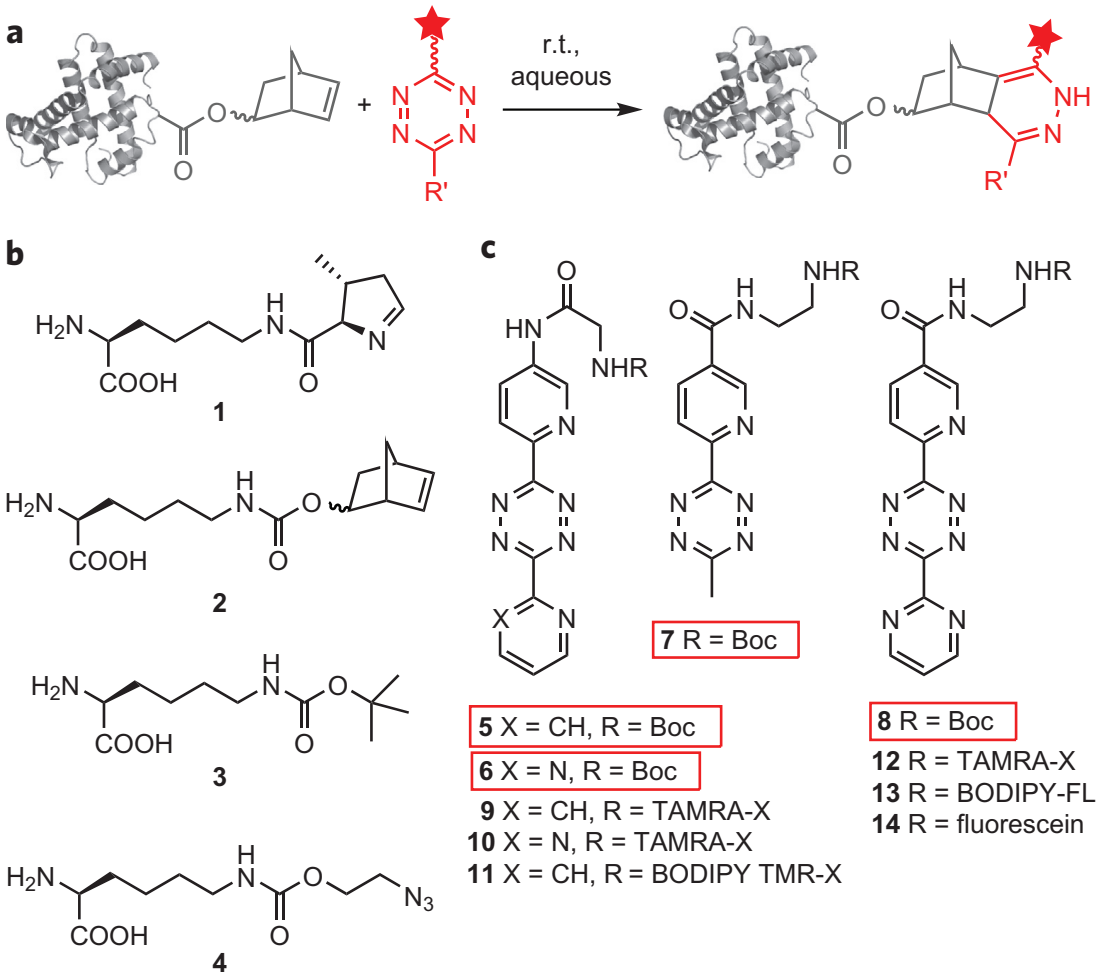


Figure 34. Structural formulas of unnatural amino acid useful for chemoselective labeling that have been incorporated site-specifically into proteins via genetic code expansion.

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...

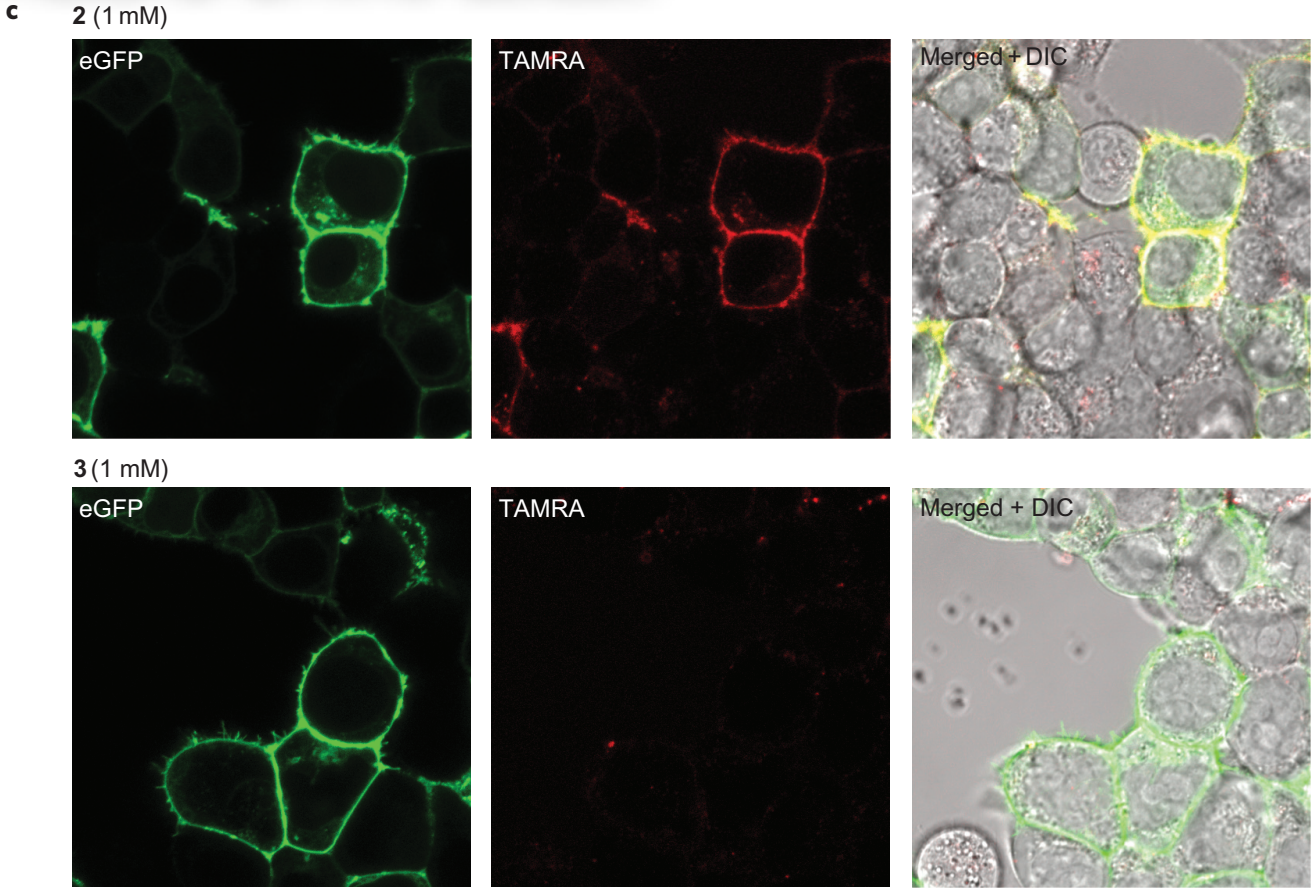
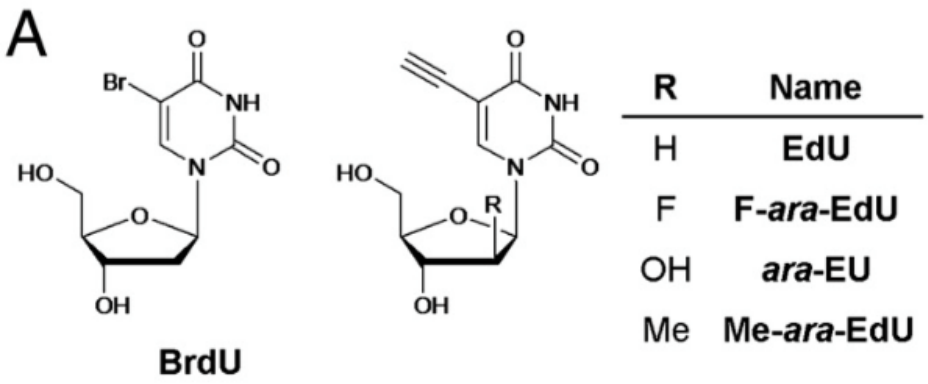


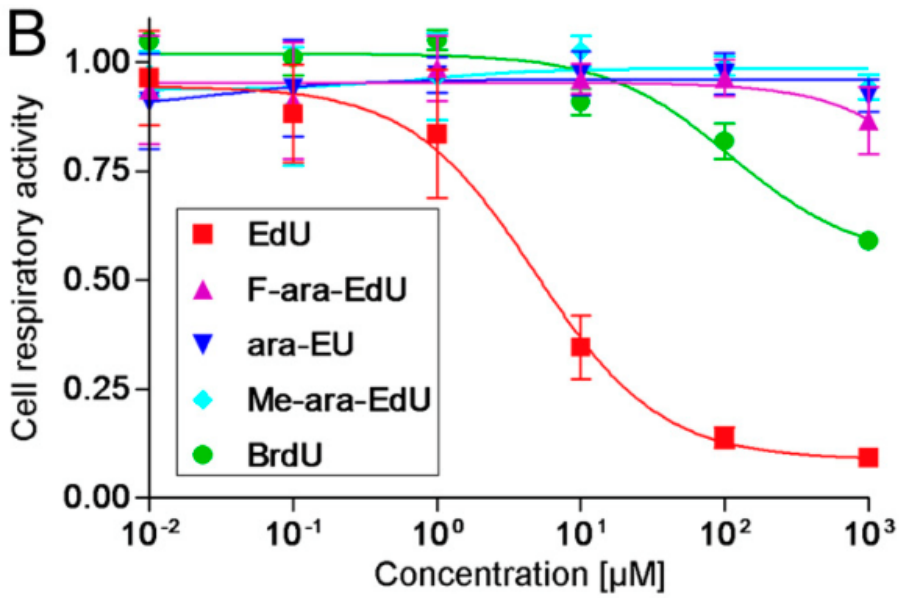
Figure 4 | Site-specific incorporation of 2 into proteins in mammalian cells and the specific labelling of EGFR-GFP on the cell surface with 9. a, Cells that contain the PylRS/$tRNA_{CUA}$ pair and the mCherry(TAG)eGFP-HA reporter produced GFP only in the presence of **2**. **b**, Western blots confirm 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**.

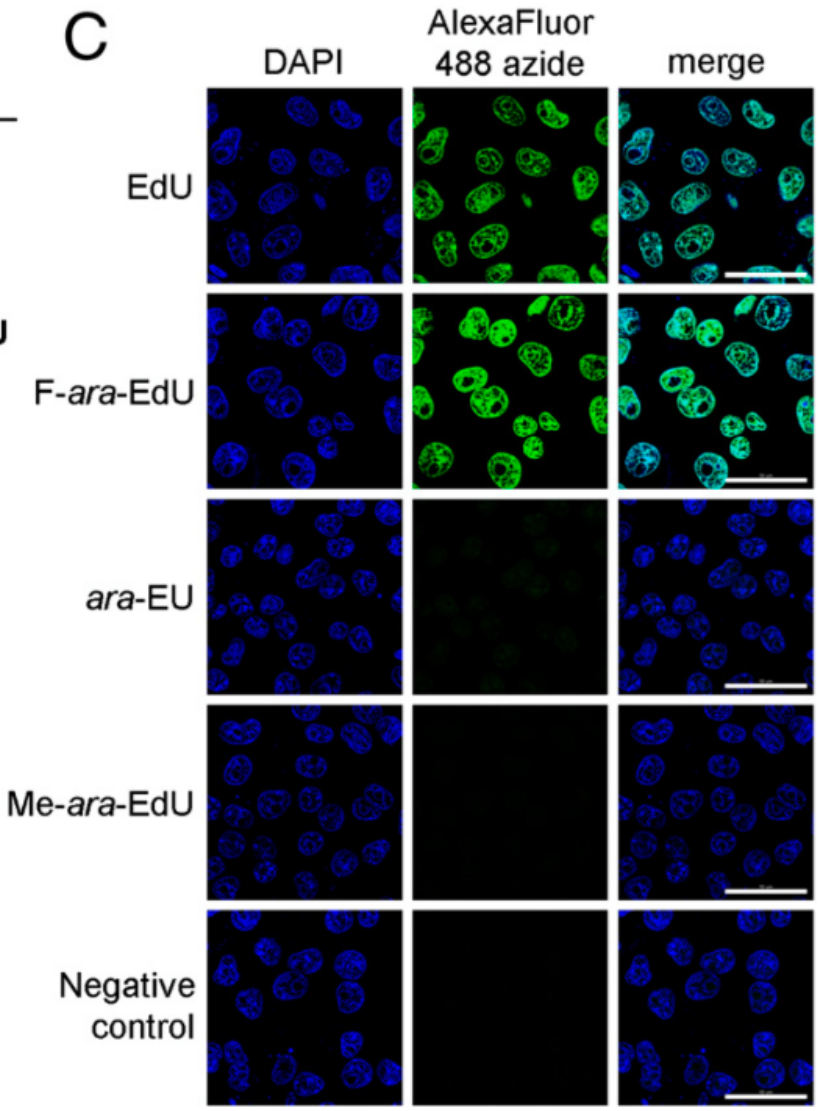
Jason Chin *et coll., Nature Chem.*, **2012**, *4*, 298-304

Labeling DNA

Non natural arabinosyl nucleosides



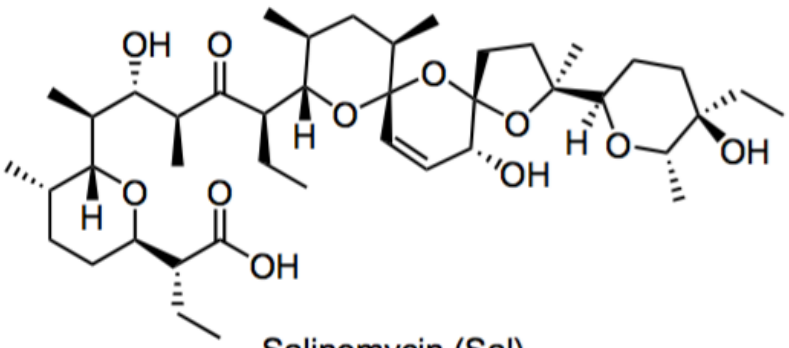




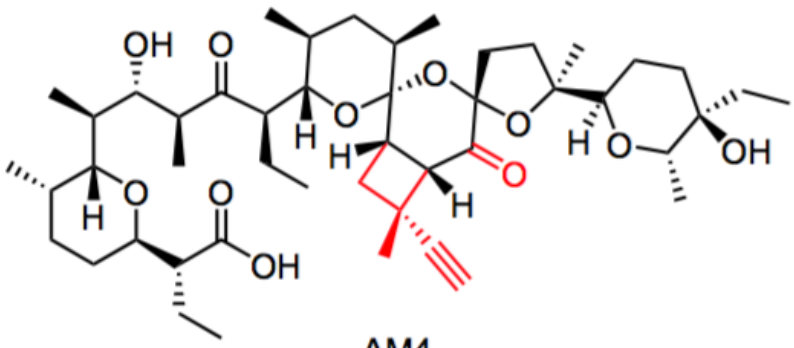
Nathan W. Luedtke *et coll., Proc. Natl. Acad. Sci. USA*, **2011**, *108*, 20404-20409

Visulazing Small molecules

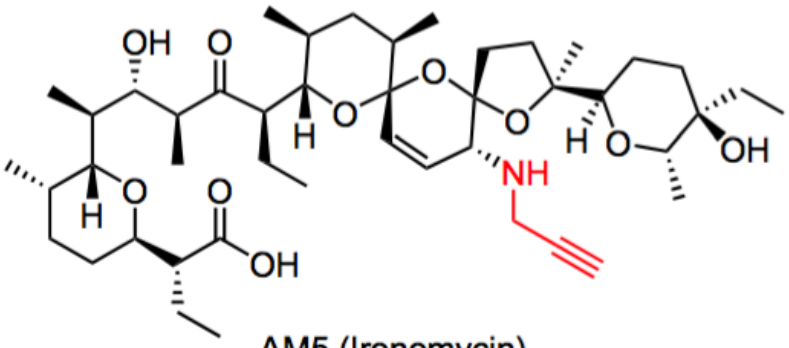
Salinomycin probes



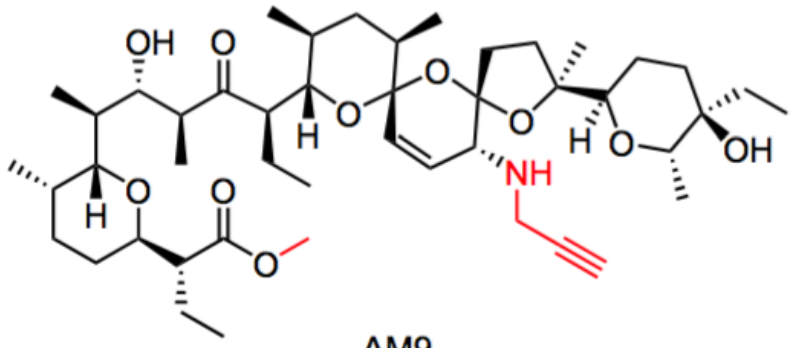
Salinomycin (Sal)



AM4



AM5 (Ironomycin)



AM9

Raphaël Rodriguez *et coll., Nature Chem.*, **2017**, *9*, 1025-1033
Review: Raphaël Rodriguez *et coll., Nature Rev. Chem.*, **2018**, *2*, 202-215

Salinomycin probes

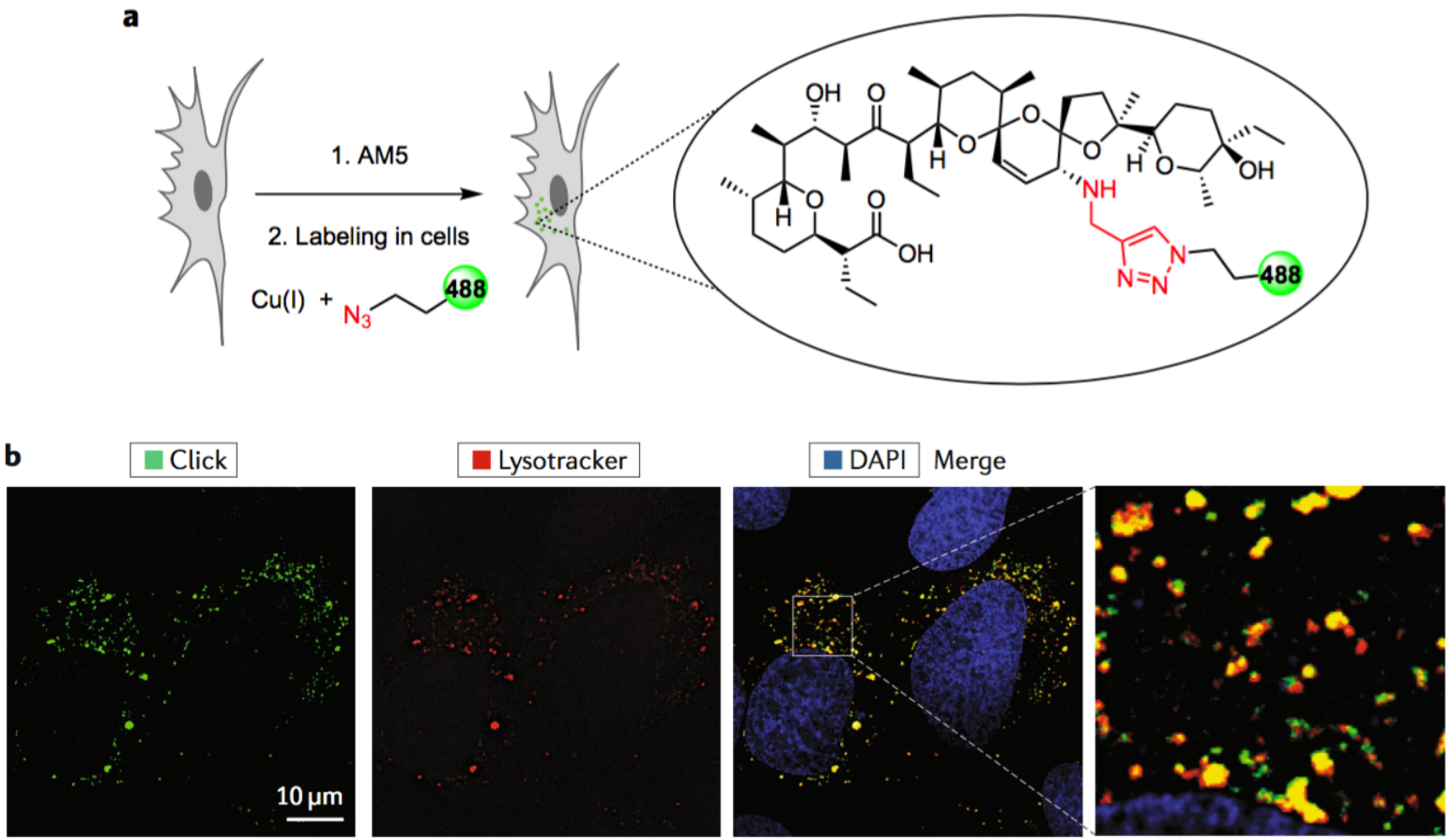
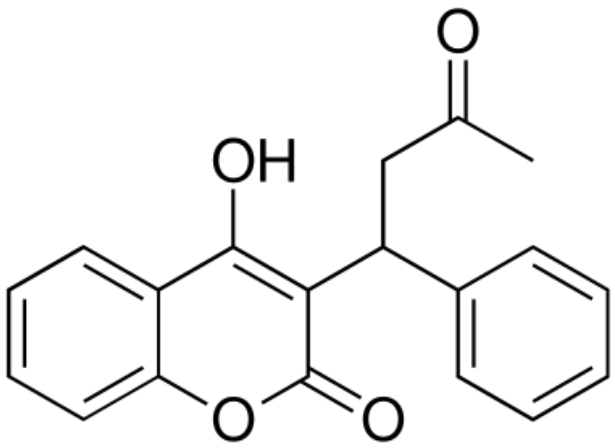


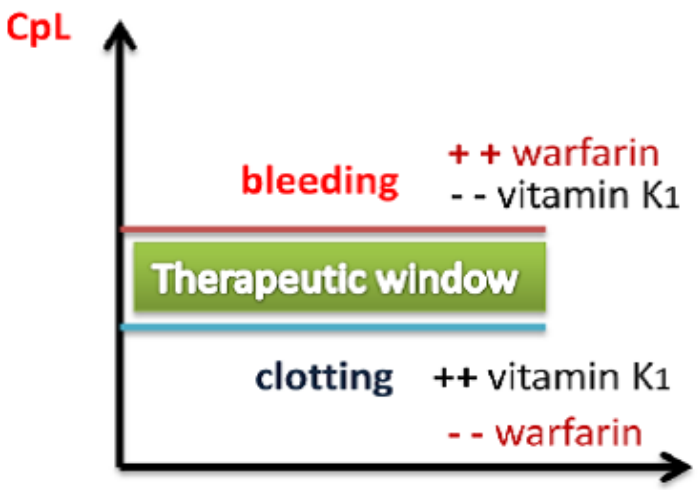
Fig. 7 | **Visualizing a clickable small molecule that targets lysosomal iron. a** | Molecular structure of salinomycin and the clickable surrogate ironomycin. **b** | Fluorescence microscopy image of the colocalization of labelled ironomycin (green) with the lysosomal marker Lysotracker (red) in HMLER human mammary cells. 4´,6-Diamidino-2-phenylindole (DAPI; blue) stains nuclear DNA in the merged image. Magnified image is 6×. Part **b** adapted from REF.[119], Springer Nature Limited.

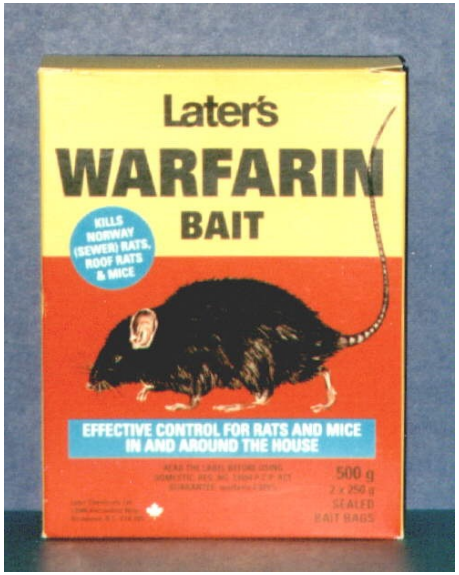
Raphaël Rodriguez *et coll., Nature Chem.*, **2017**, *9*, 1025-1033
Review: Raphaël Rodriguez *et coll., Nature Rev. Chem.*, **2018**, *2*, 202-215

Inactivating Drugs

Warfarin - Anticoagulant







https://en.wikipedia.org/wiki/Warfarin

Click & Clear

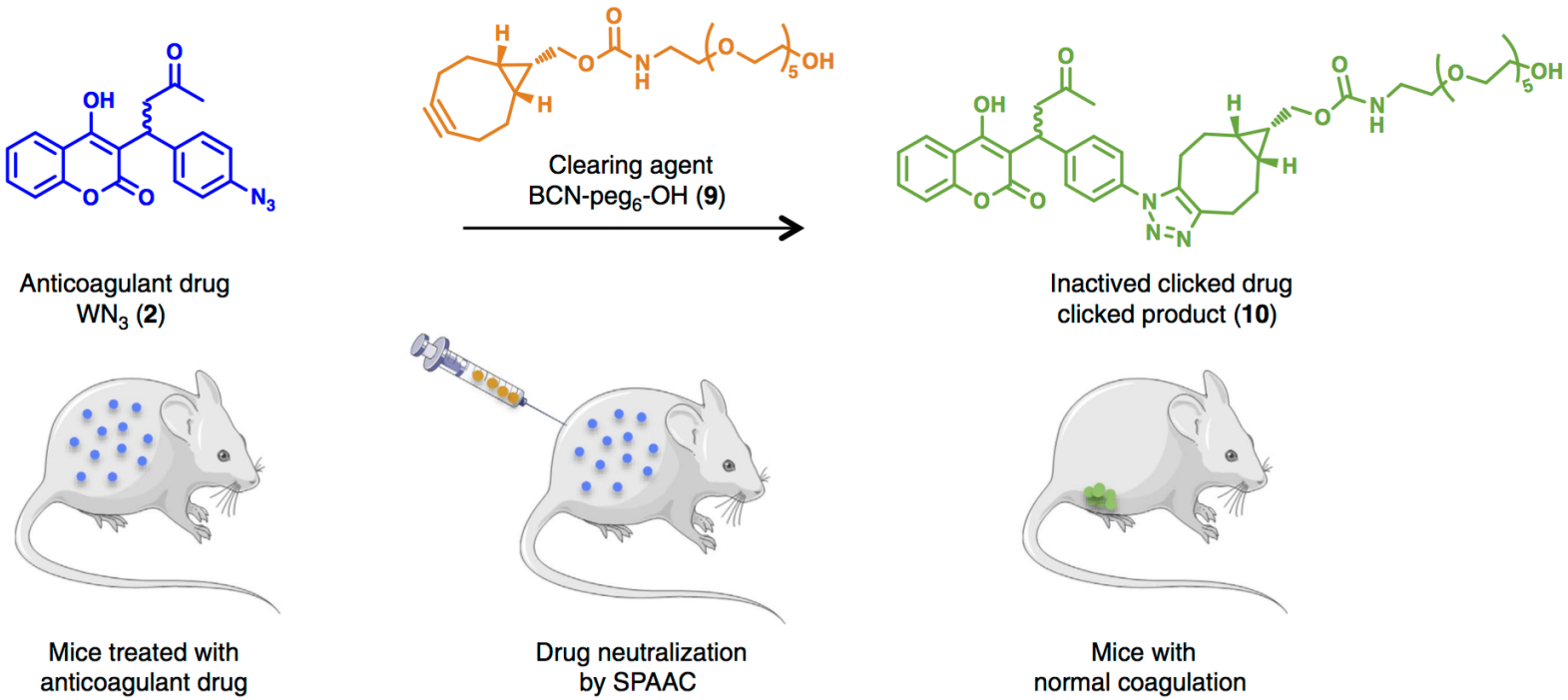


Figure 1 | 'Click and Clear' strategy. Biological inactivation and fast clearance of a circulating drug, Warfarin-N_3 **2**, by *in vivo* Strain Promoted Alkyne Azide Cycloaddition (SPAAC) reaction. A mouse submitted to anticoagulant therapy (WN_3, **2**) is treated with a clearing agent (BCN-peg_6-OH, **9**) prone to react with the anticoagulant drug. *In vivo* bio-orthogonal reaction between circulating WN_3 **2** and BCN-peg_6-OH **9** leads to the formation of an inactivated compound **10** which is readily cleared from the bloodstream, restoring normal coagulation activity. The pictures of mice and syringe have been downloaded from Servier Medical Art Database which provides these illustrations through the Creative Commons license (https://creativecommons.org/licenses/by/3.0/).

Alain Wagner *et coll.*, *Nature Commun.*, **2017**, *8:*15242

Target Identification

Target vs Phenotypic-based Drug Discovery

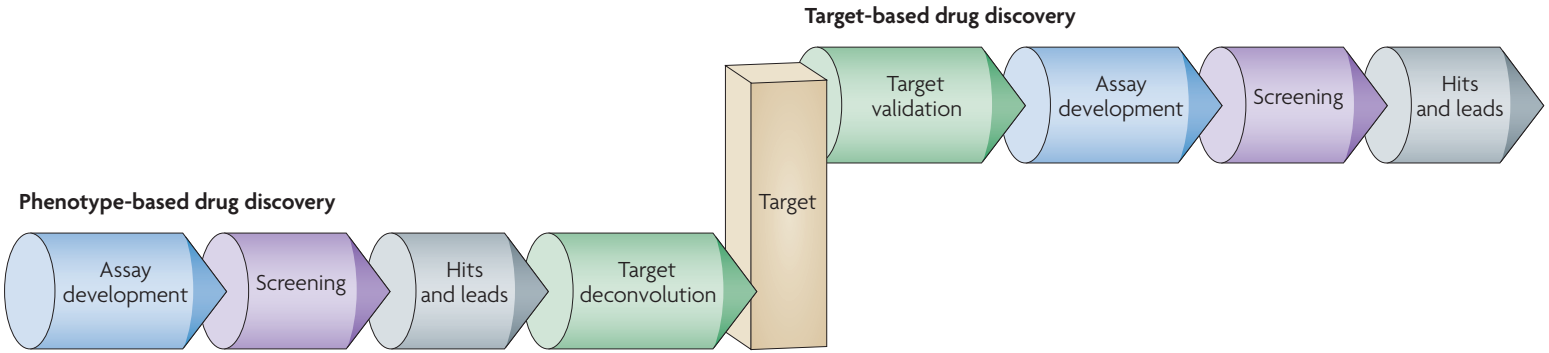
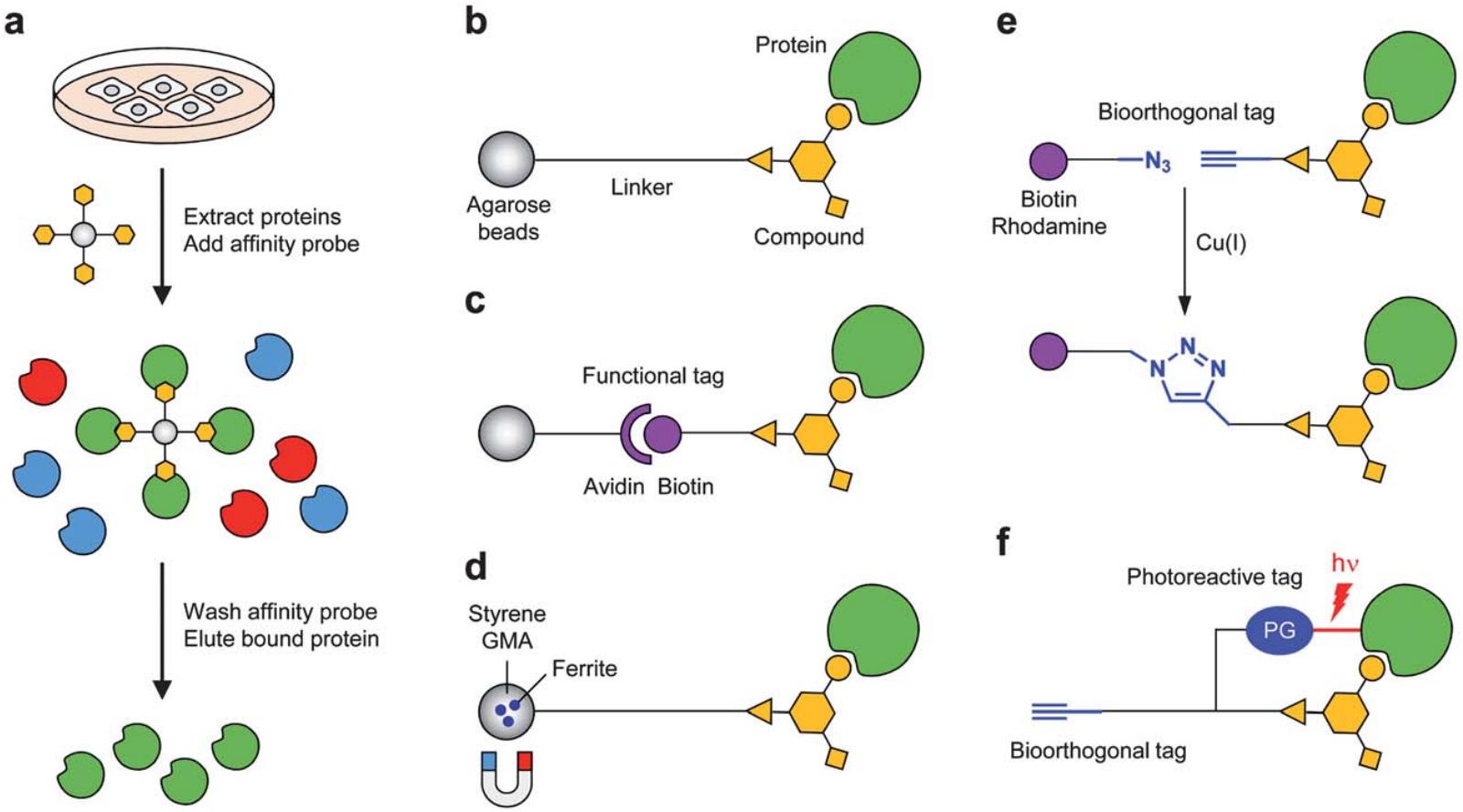


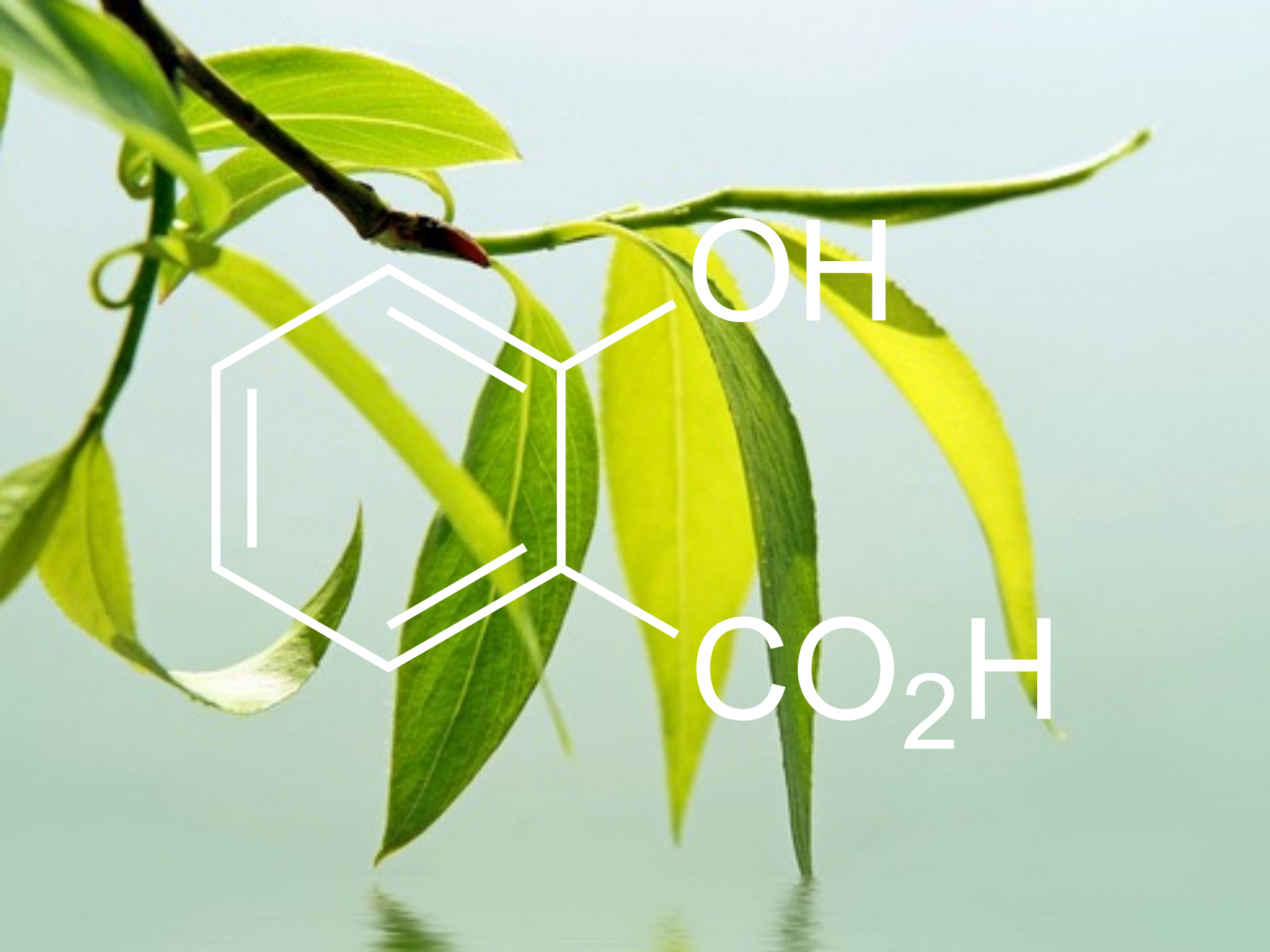
Figure 1 | **Phenotype-based versus target-based drug discovery.** The diagram illustrates the early phase of drug discovery, in which the aim is to identify target and lead molecules. In the phenotype-based approach, lead molecules are obtained first, followed by target deconvolution to identify the 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.

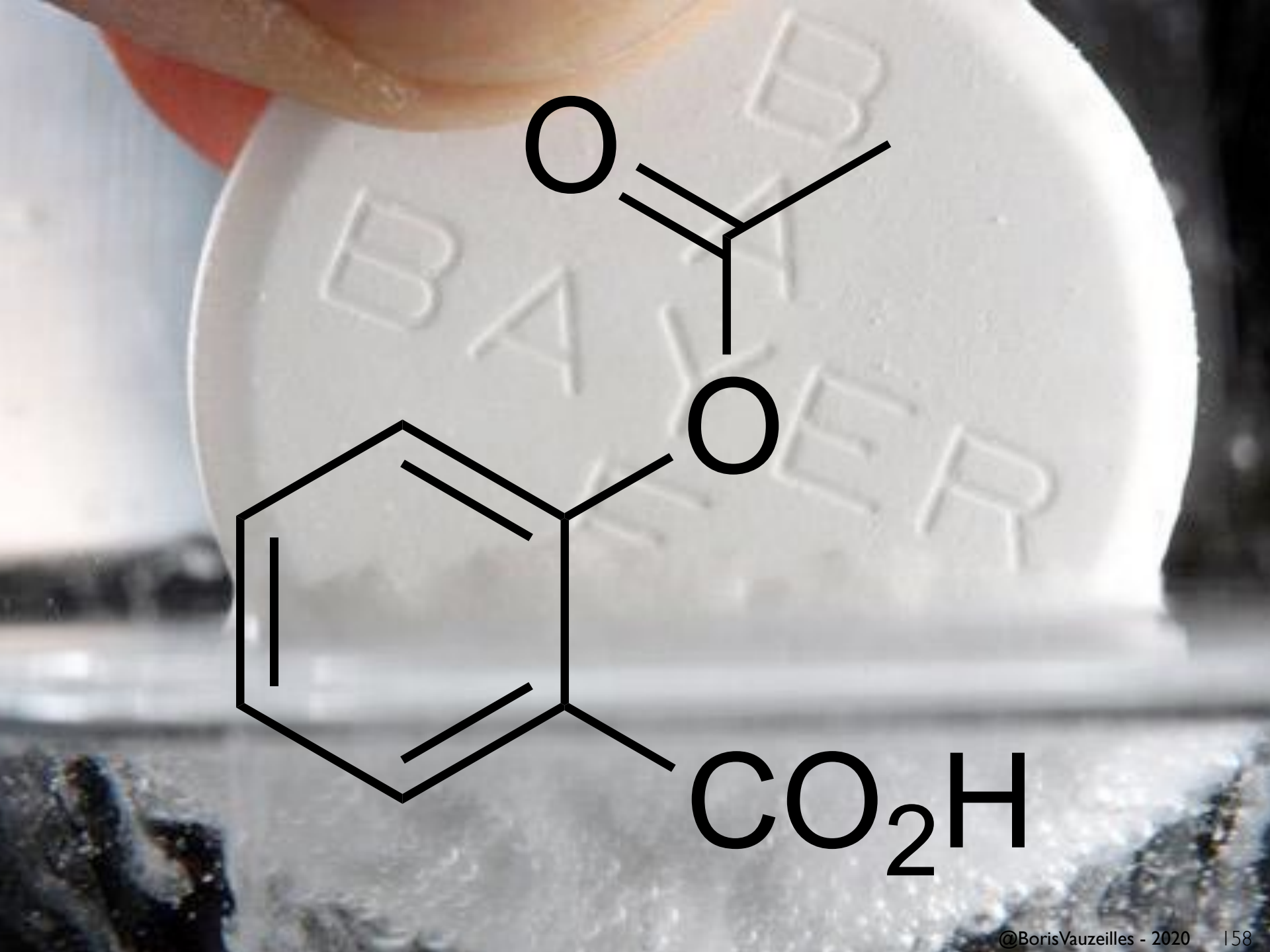
G.i Gaviraghi *et coll.*, *Nat. Rev. Drug. Discov.*, **2007**, *6*, 891-903

Affinity purification

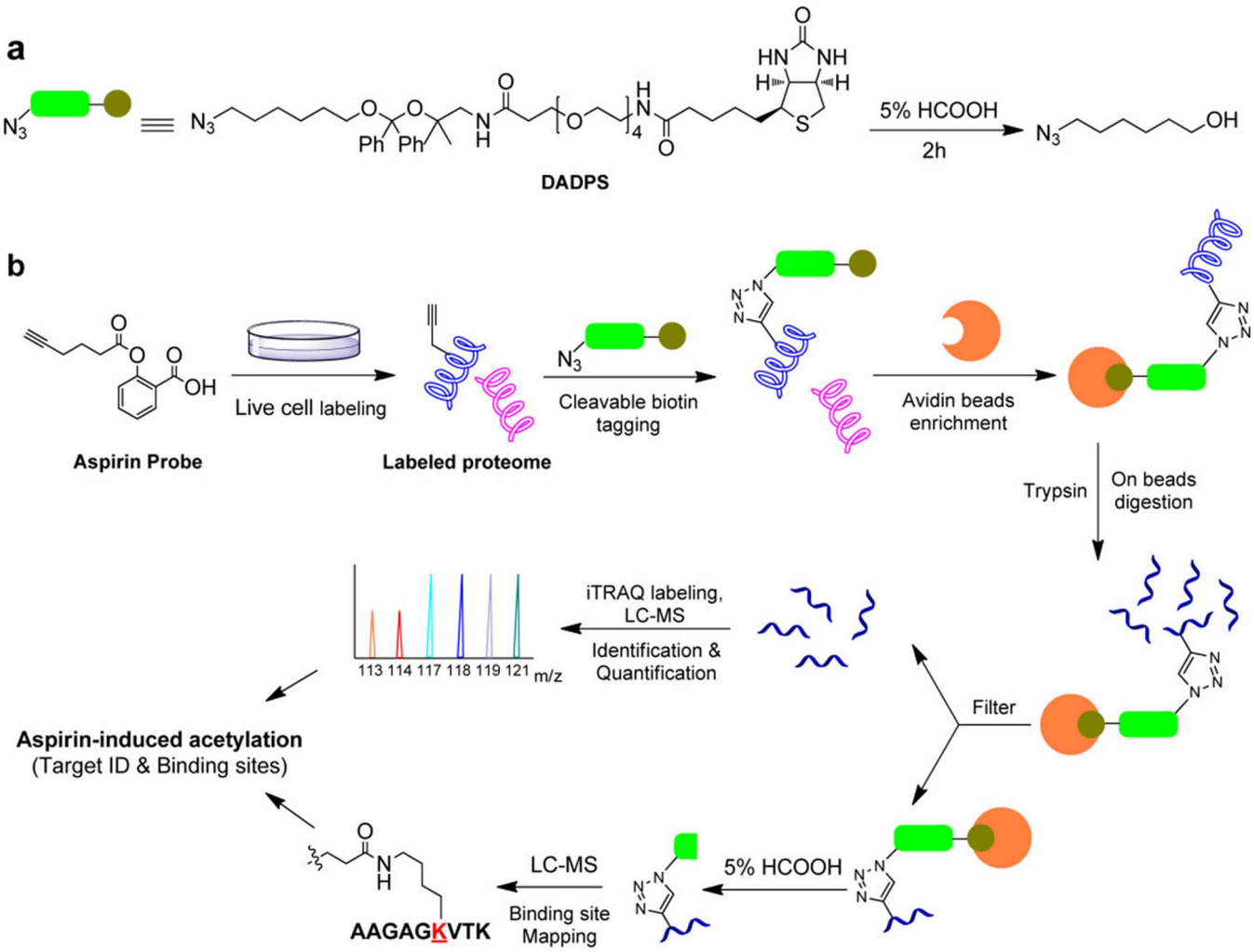


H. Osada *et coll.*, *MedChemComm*, **2014**, 5, 277-287


OH
CO2H

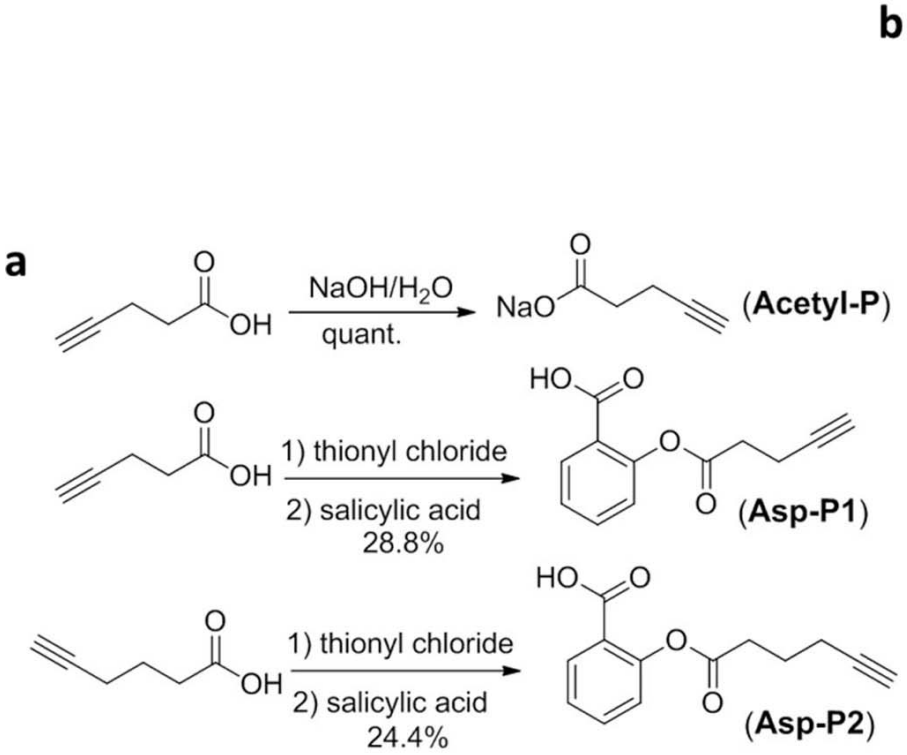

O
O
CO_2H

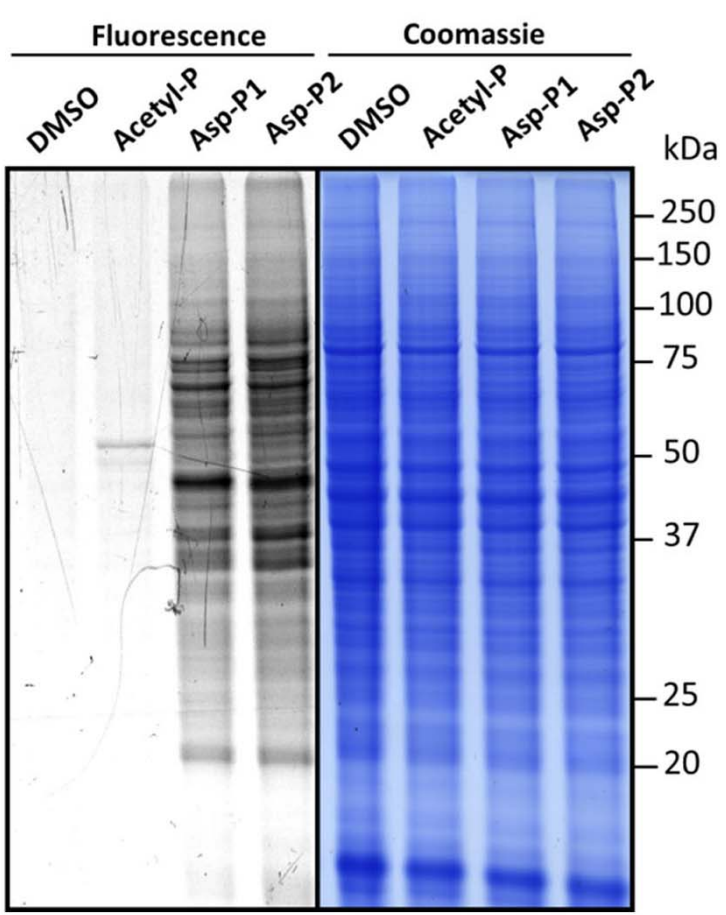
Target identification strategy



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

Targets





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

Aspirin Targets

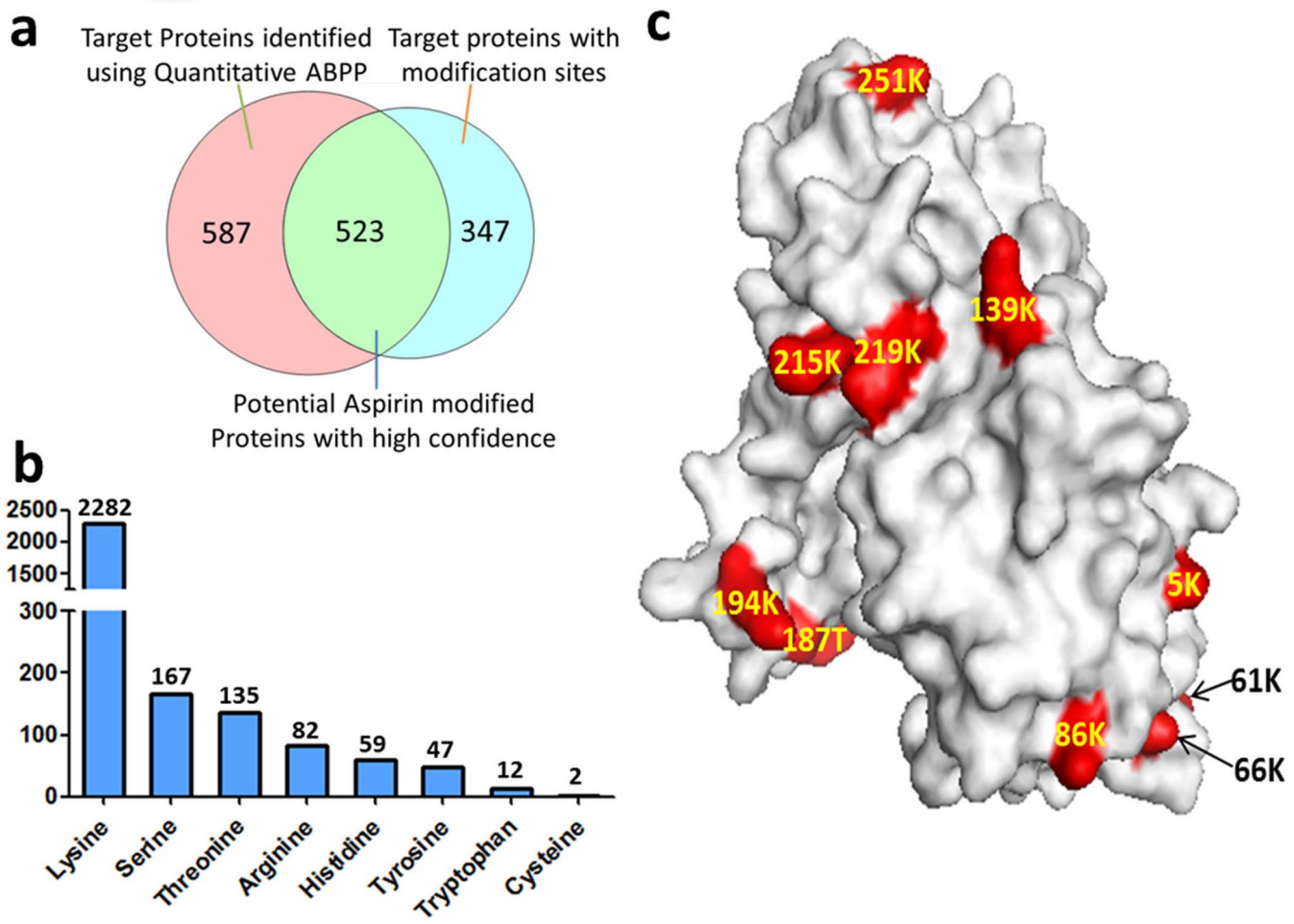
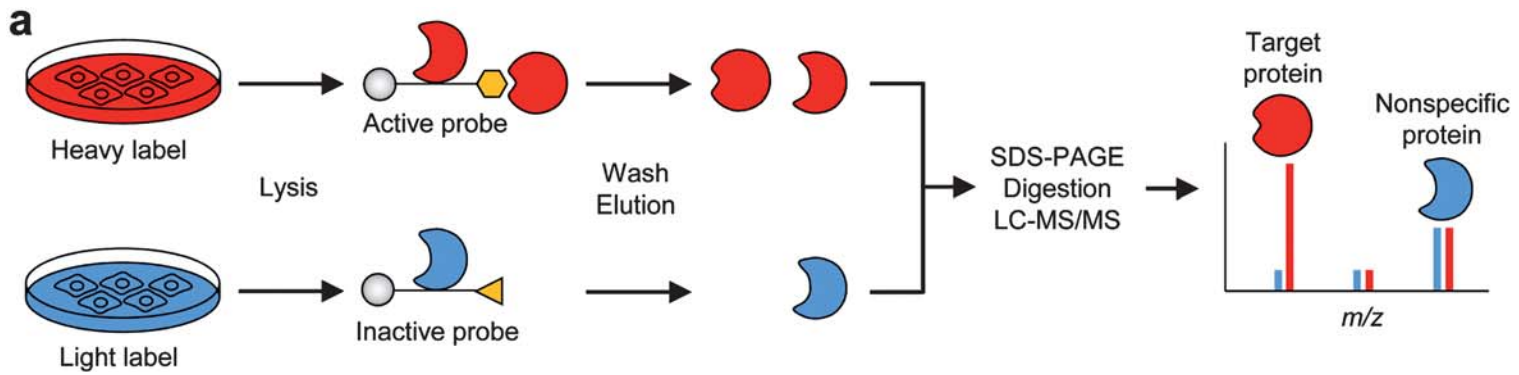


Figure 3 | Summary of aspirin modified proteins and amino acid residues. (a) Numbers of proteins identified using quantitative ABPP, proteins with modification sites identified and proteins confirmed with high confidence; (b) Numbers of the aspirin-modified amino acid residues. Numbers on top of the 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

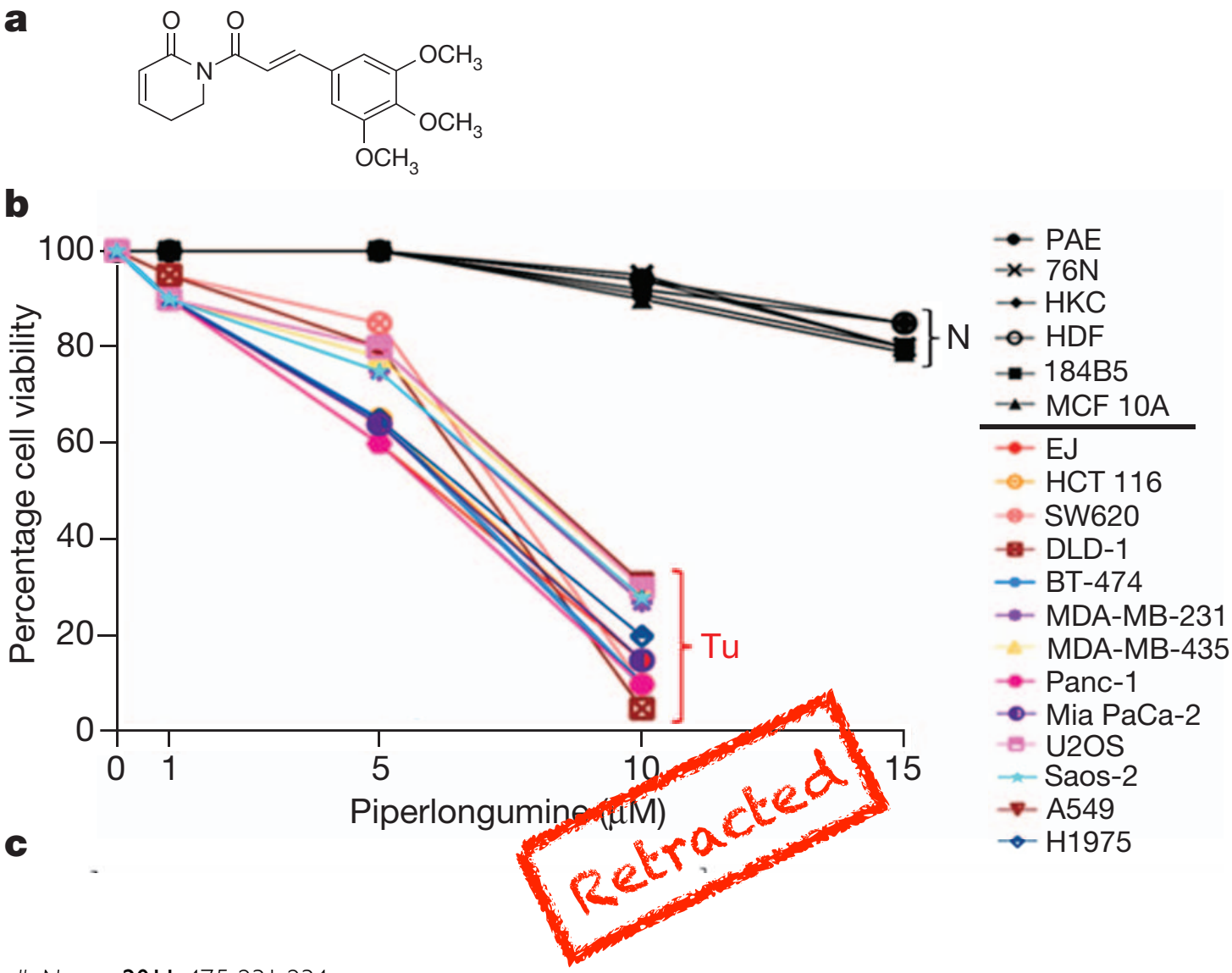
SILAC

Stable isotope labeling by amino acids in cell culture



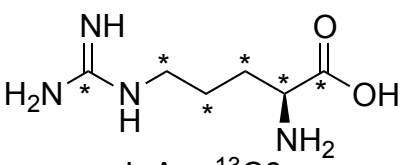
H. Osada *et coll.*, *MedChemComm*, **2014**, *5*, 277-287

Piperlongumine selectively kills cancer cells

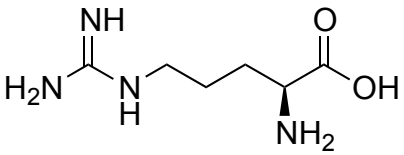


S. L. Schreiber *et coll.*, *Nature*, **2011**, 475, 231-234

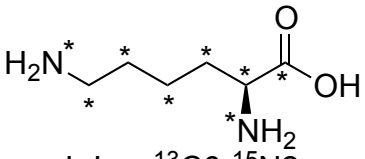
Target identification using SILAC



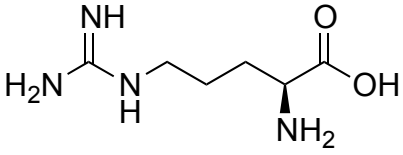
L-Arg-^{13}C6



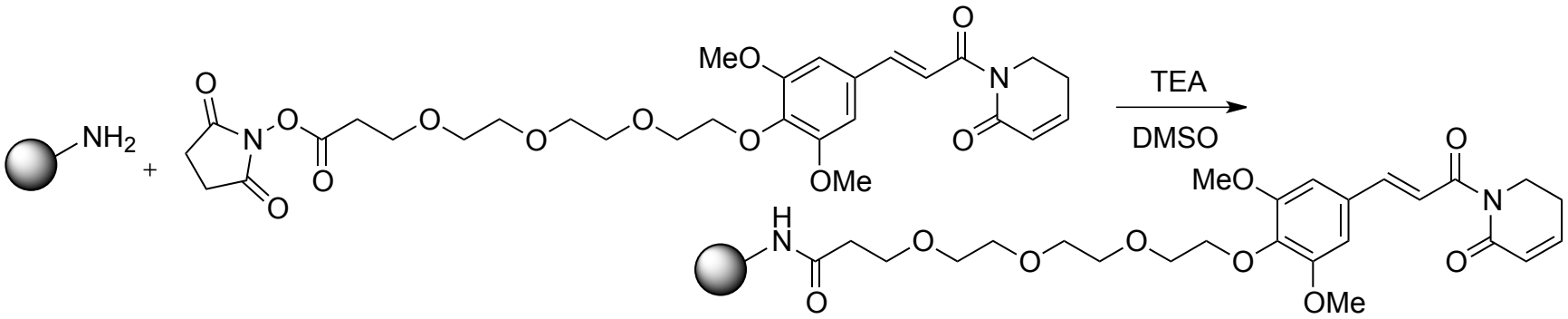
L-Arg

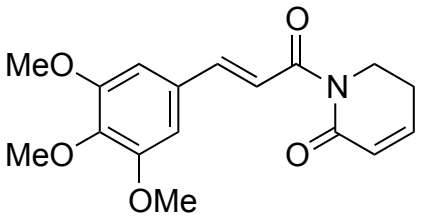


L-Lys-^{13}C6-^{15}N2



L-Lys



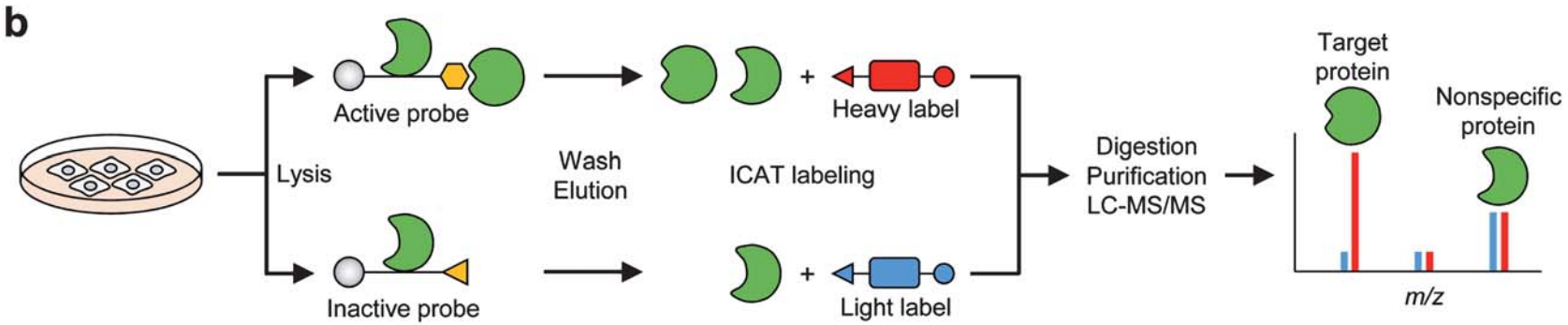


70, 14 & 7 fold excess

S. L. Schreiber *et coll.*, *Nature*, **2011**, 475, 231-234

ICAT

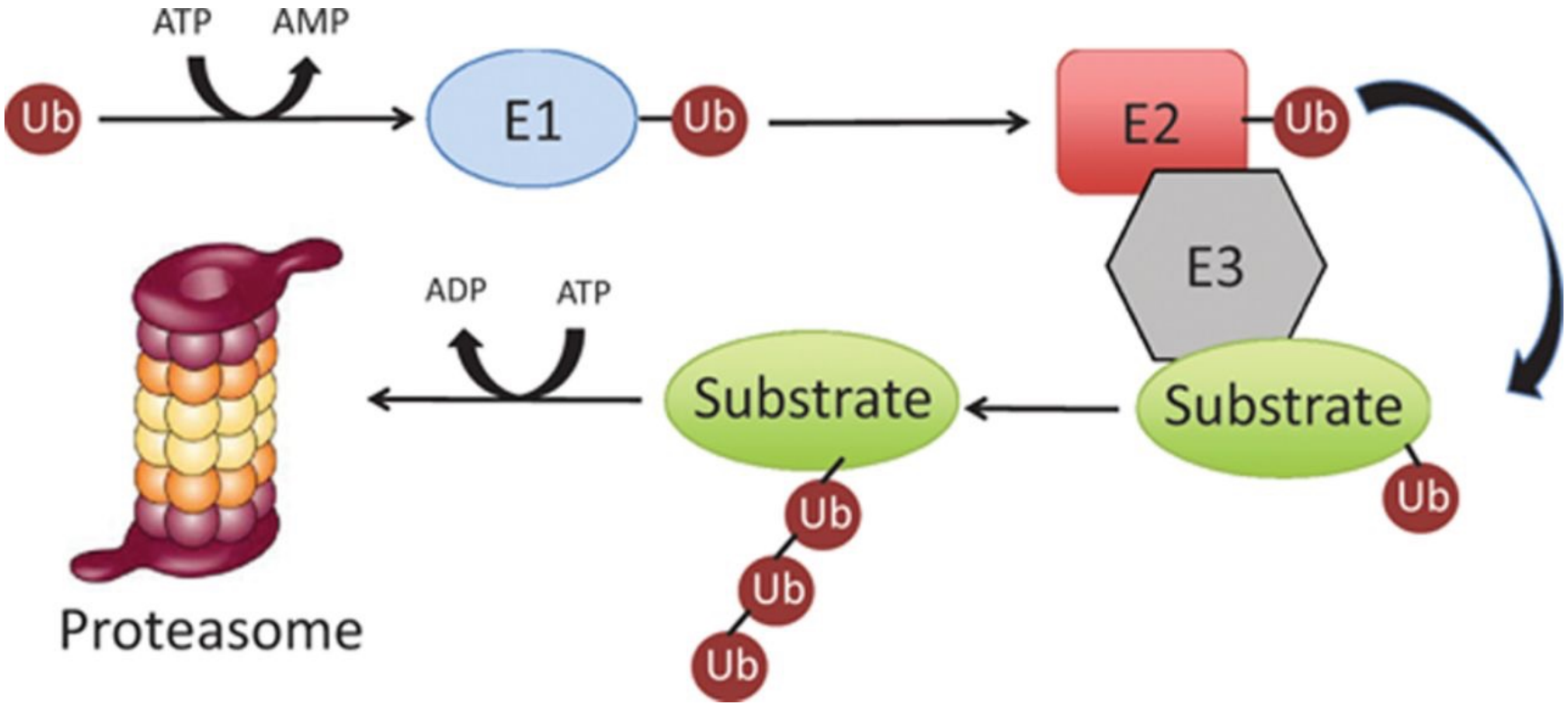
Isotope-coded affinity tag



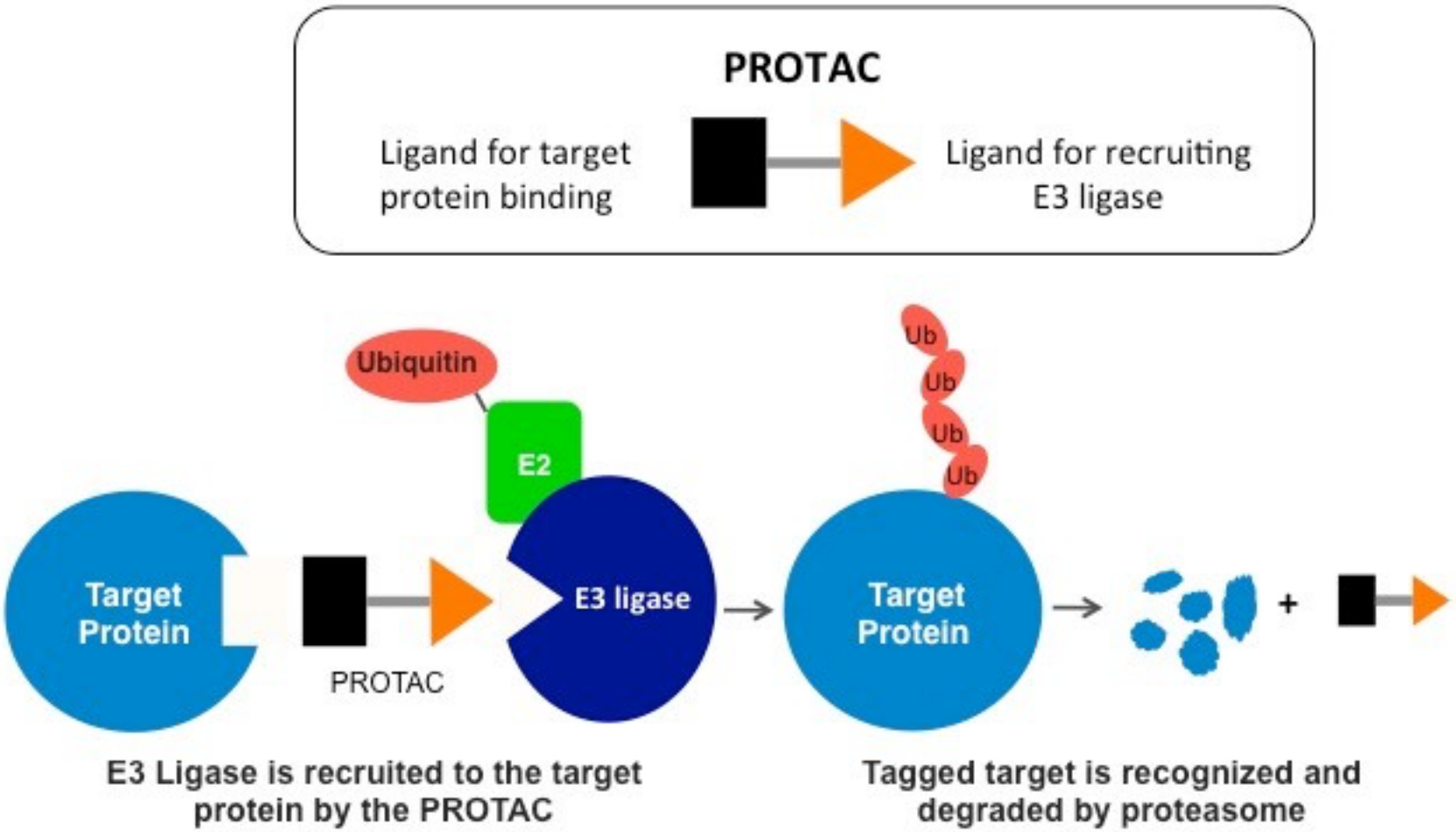
H. Osada *et coll.*, *MedChemComm*, **2014**, *5*, 277-287

Target Elimination

Ubiquitin-Proteasome system



PROteolysis Targeting Chimera



Craig Crews *et coll.*, *Proc. Natl. Acad. Sci. USA.*, **2001**, *98*, 8554-8559