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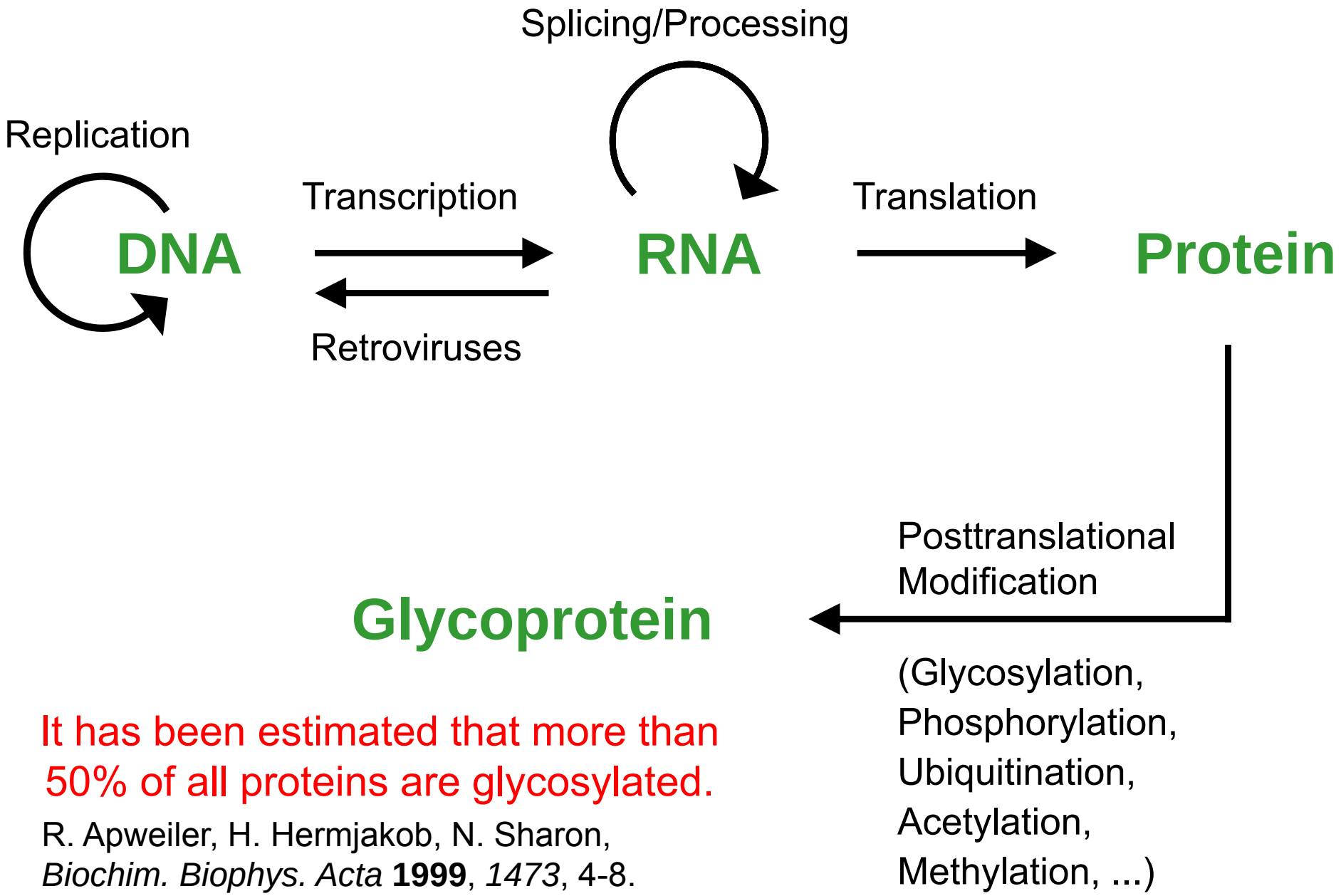
Chemical Biology of Cellular Carbohydrates

Valentin Wittmann
Universität Konstanz

XVIIth Ischia Advanced School of Organic Chemistry (IASOC Conference), Ischia, 28.09.2016

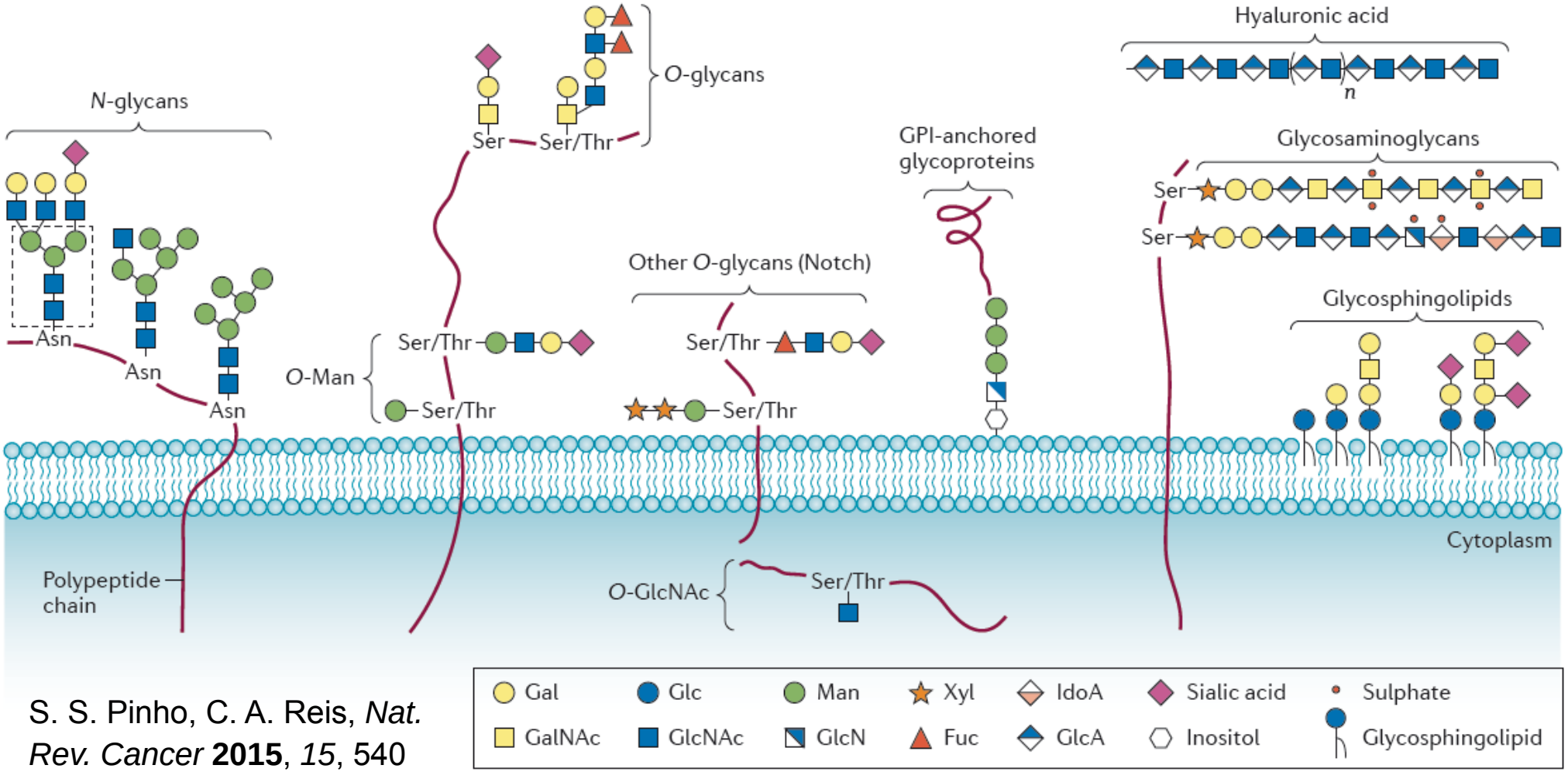
Central Dogma of Molecular Biology



It has been estimated that more than 50% of all proteins are glycosylated.

R. Apweiler, H. Hermjakob, N. Sharon, *Biochim. Biophys. Acta* **1999**, *1473*, 4-8.

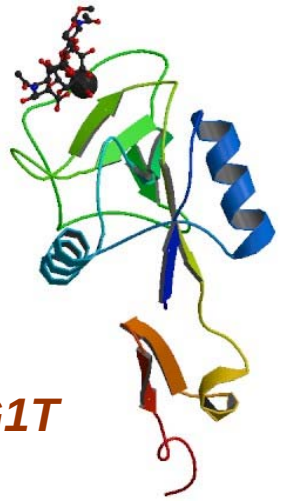
Common Classes of Glycoconjugates



S. S. Pinho, C. A. Reis, *Nat. Rev. Cancer* **2015**, *15*, 540

1. **Mechanistic investigation of carbohydrate-lectin interactions**
2. **Tracing carbohydrates in living cells**

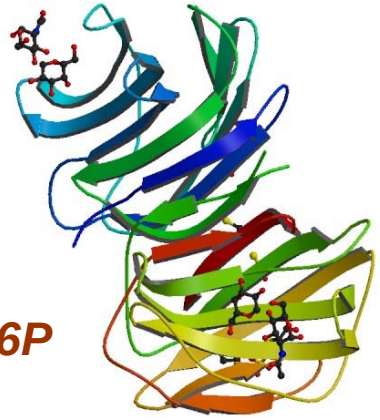
Carbohydrate-Lectin Interactions



1G1T

E-selectin • sLe^x

$K_D \approx 700\ \mu M$



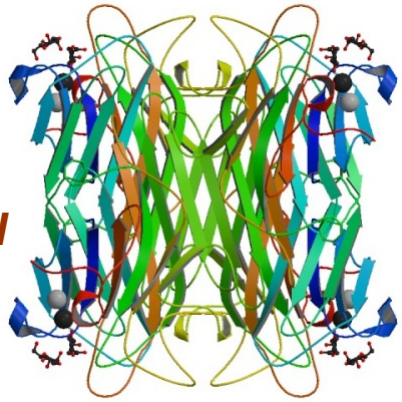
1W6P

galectin-1 • LacNAc

$K_D \approx 91\ \mu M$

Increased binding affinity through

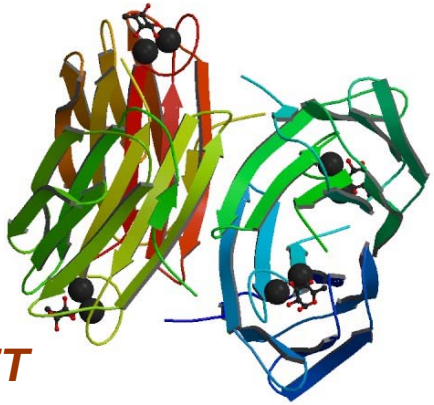
Multivalency



1I3H

Con A • Man(α1-2)Man

$K_D \approx 24\ \mu M$

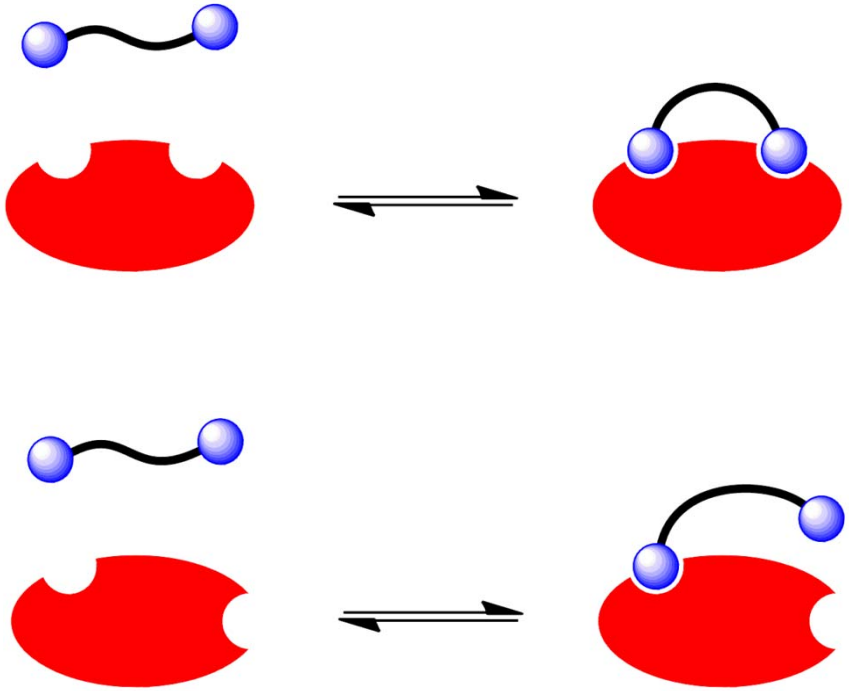


1GZT

Lec B (PA-IIL) • Fuc

$K_D \approx 2.9\ \mu M$

Multivalent Carbohydrate-Lectin Interactions



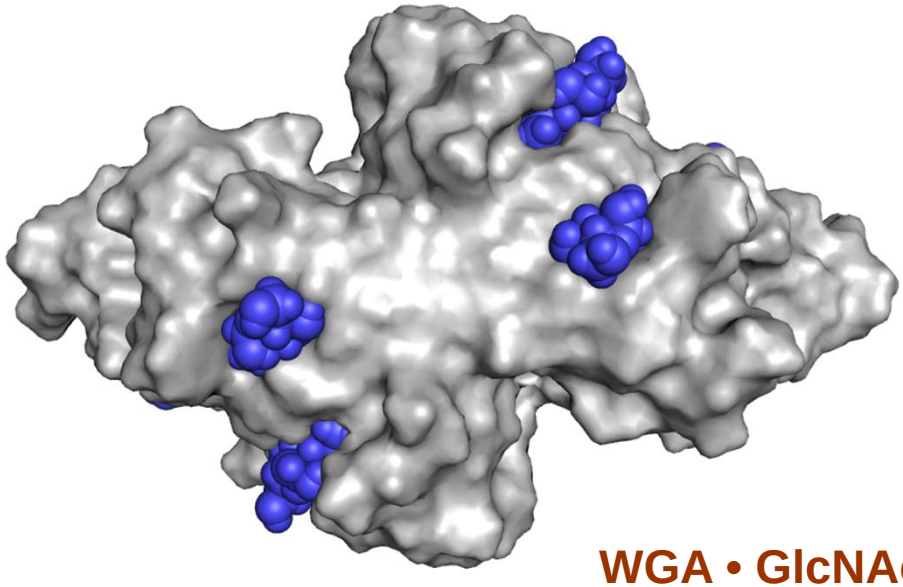
- Increased affinity even if spacer is too short
- Binding modes?

Strong interest in understanding the molecular mechanisms of multivalency

V. Wittmann, R. J. Pieters, *Chem. Soc. Rev.* **2013**, *42*, 4492

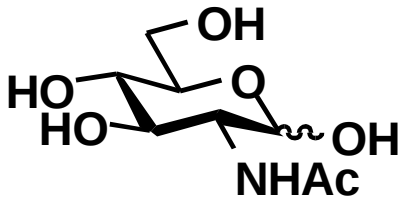
Model Lectin: Wheat Germ Agglutinin (WGA)

- Chitin-binding lectin from *Triticum vulgaris*
- Stable homo dimer: 2 x 171 amino acids
- 8 Binding sites per dimer (4 ‘primary’ and 4 ‘secondary’)

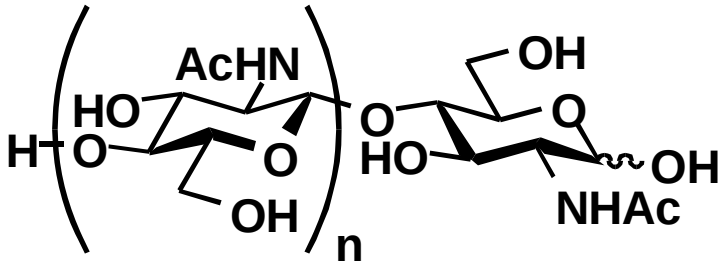


WGA • GlcNAc
(PDB ID: 2UVO)

- Carbohydrate ligand:



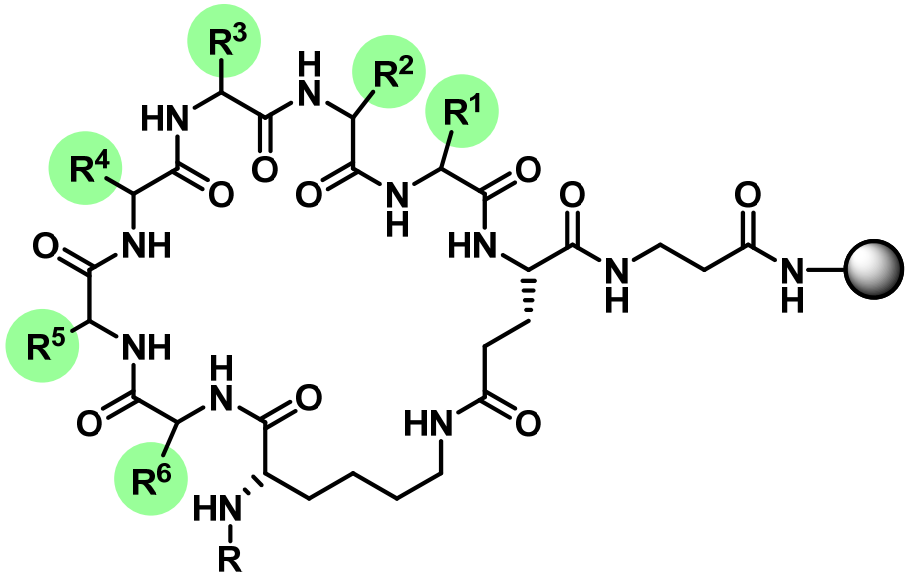
and



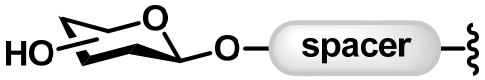
GlcNAc
K_d: 1–5 mM

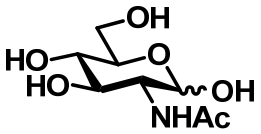
Chitobiose (n = 1)
K_d: 50–200 μM

Spatial Screening of Wheat Germ Agglutinin (WGA) Ligands



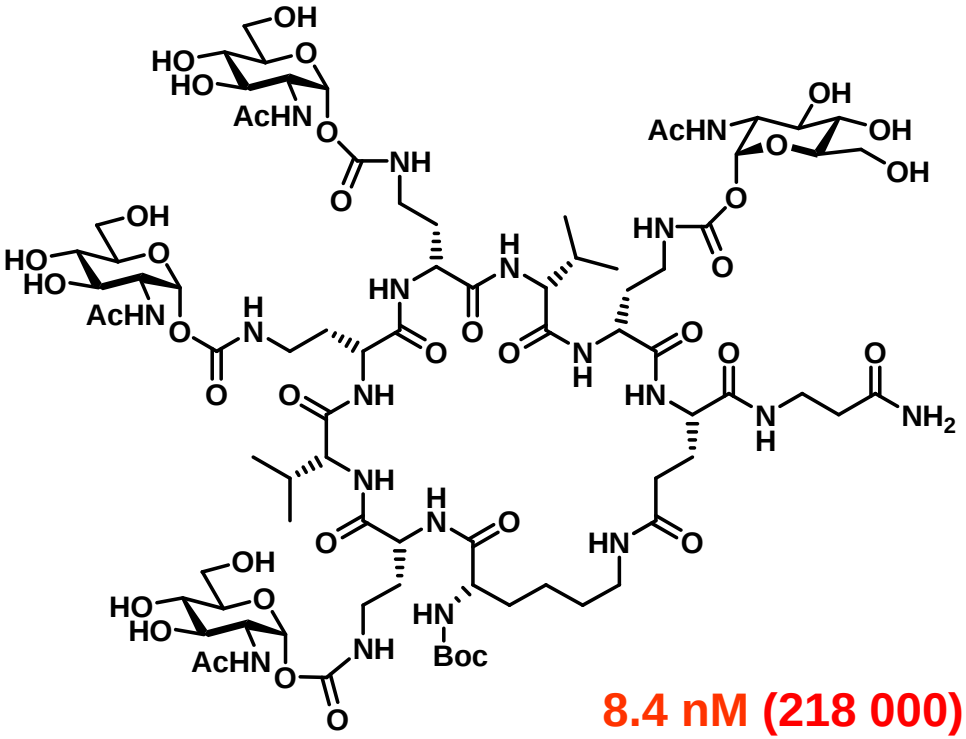
R^n = amino acid side chain

or 



1.83 mM (1)

Absolute (and relative) K_D values from ITC



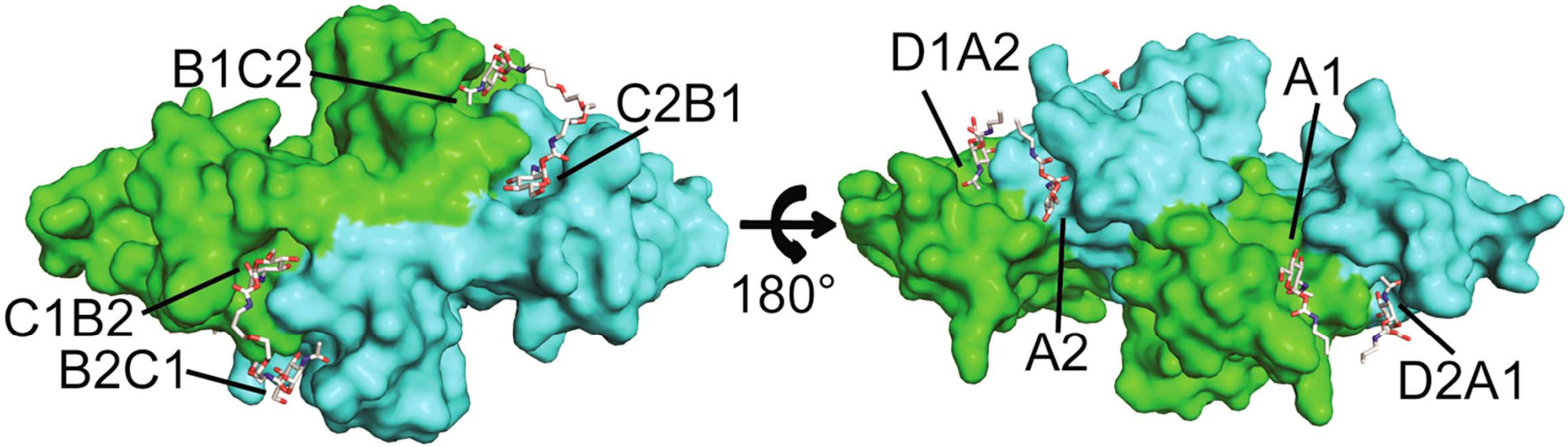
8.4 nM (218 000)

Angew. Chem., Int. Ed. **2000**, *39*, 4348

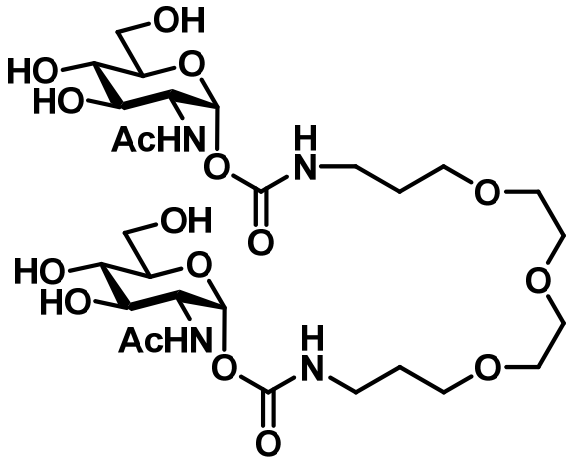
Angew. Chem., Int. Ed. **2004**, *43*, 900

J. Am. Chem. Soc. **2010**, *132*, 8704

1.7 Å Structure of WGA 3 in Complex with Divalent Ligand



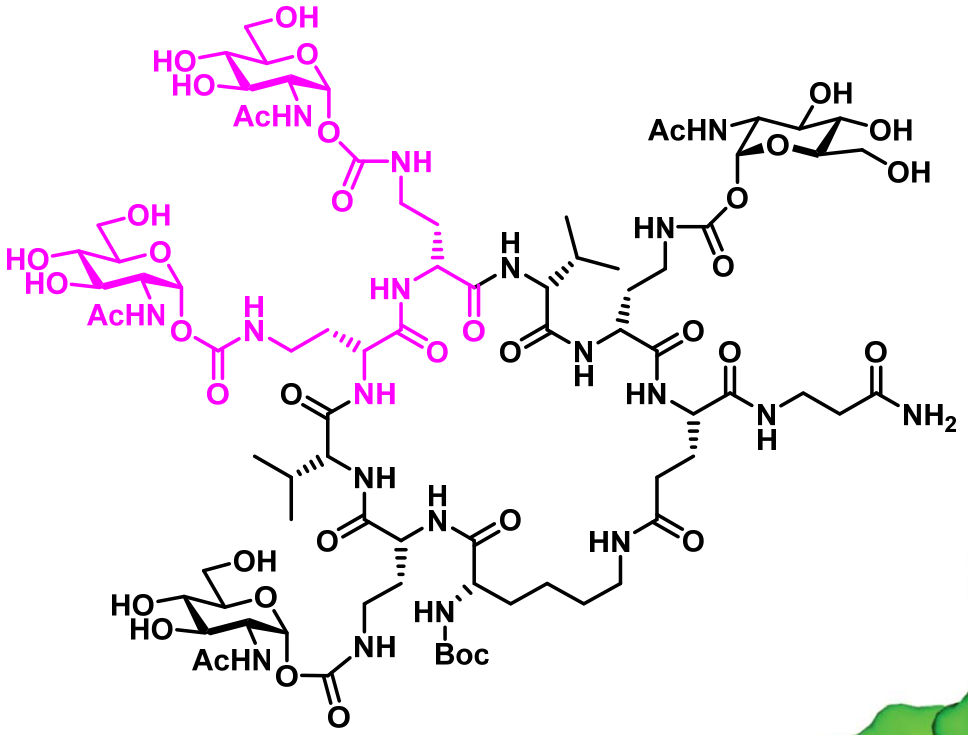
(PDB ID: 2X52)



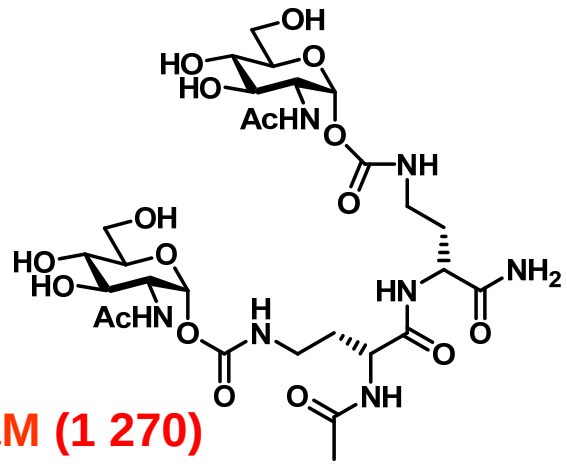
- 4 molecules of divalent ligand span pairs of binding sites.
- For the first time, all 8 binding sites are simultaneously occupied.
- Increased binding affinity of the divalent ligand allows identification of secondary binding sites.

J. Am. Chem. Soc. **2010**, *132*, 8704

2.75 Å Structure of Complex with Tetravalent Cyclic Peptide

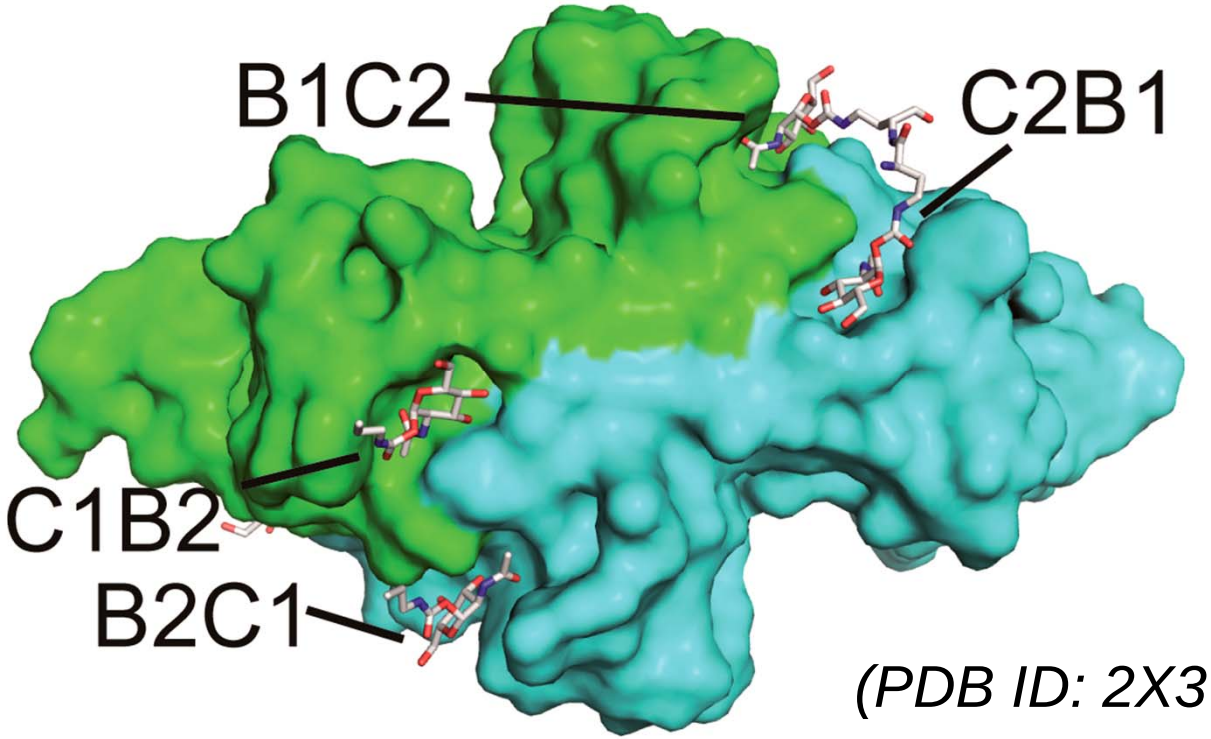


8.4 nM (218 000)



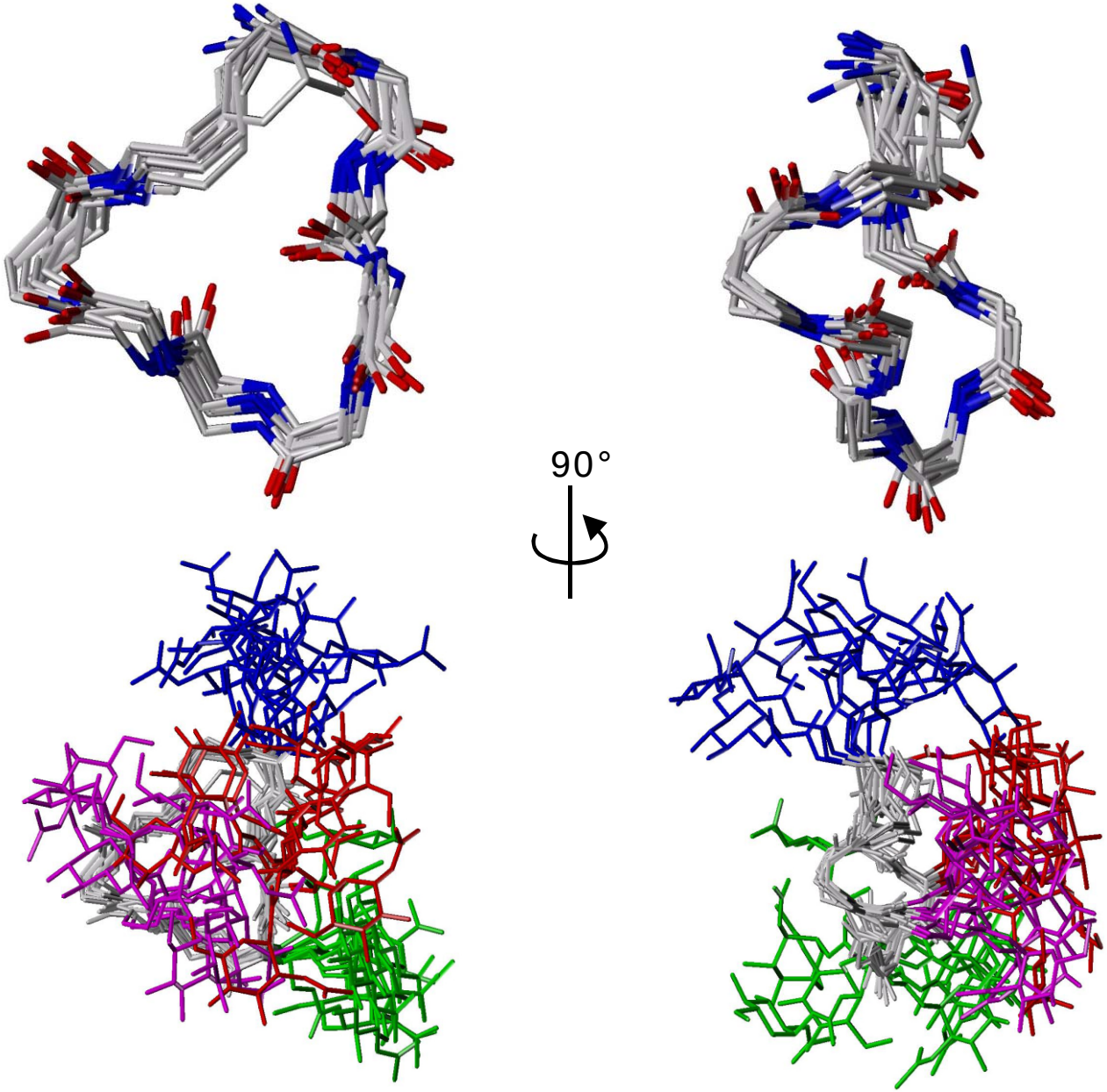
1.44 μM (1 270)

- two WGA dimers in asymmetric unit
- 11 out of 16 binding sites occupied
- remaining sites obstructed by crystal contacts



(PDB ID: 2X3T)

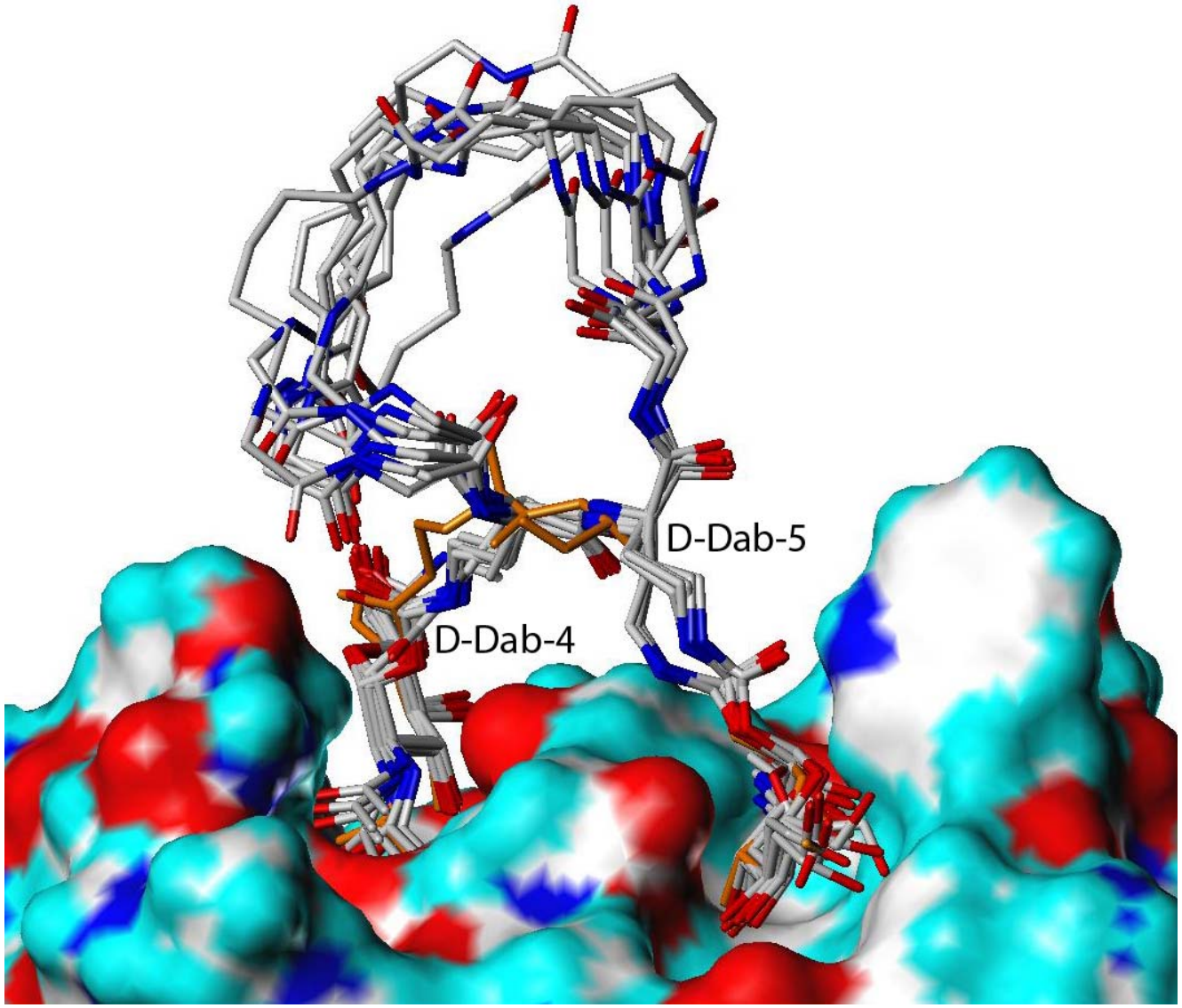
J. Am. Chem. Soc. **2010**, *132*, 8704



Ensemble of the dominant family of conformers of the tetravalent neoglycopeptide determined by NMR spectroscopy

Johannes Beck, Heiko Möller

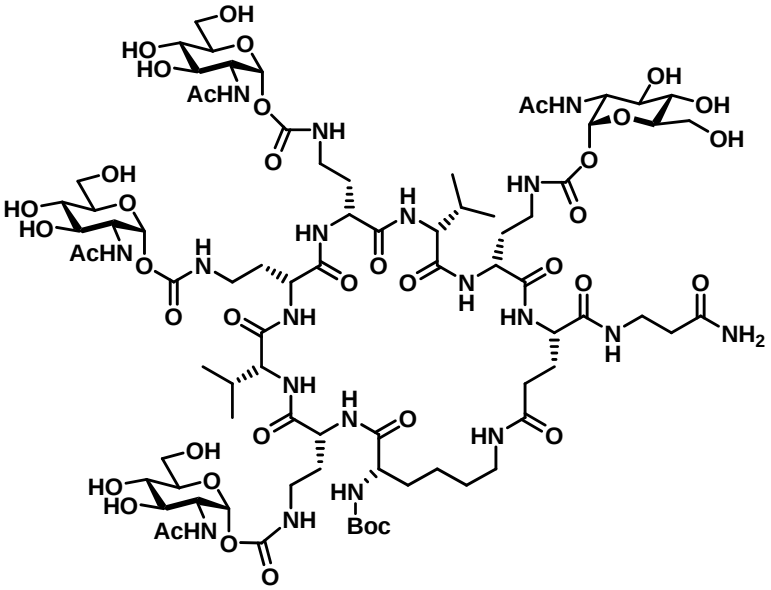
Superposition of NMR and X-ray Structure



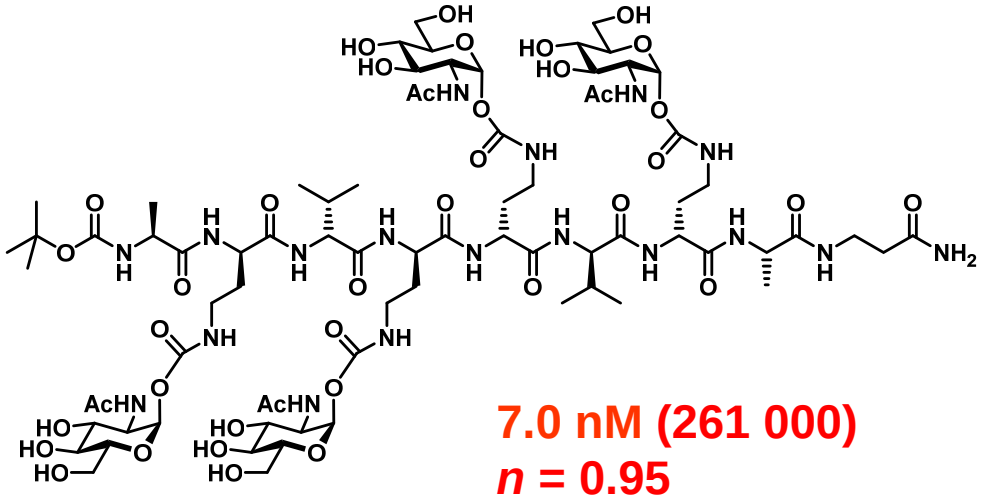
Backbone structure resolved in the crystal fits very well to the dominant conformational family determined by solution NMR.

J. Am. Chem. Soc. **2010**, *132*, 8704

Absolute (and Relative) K_D Values from ITC

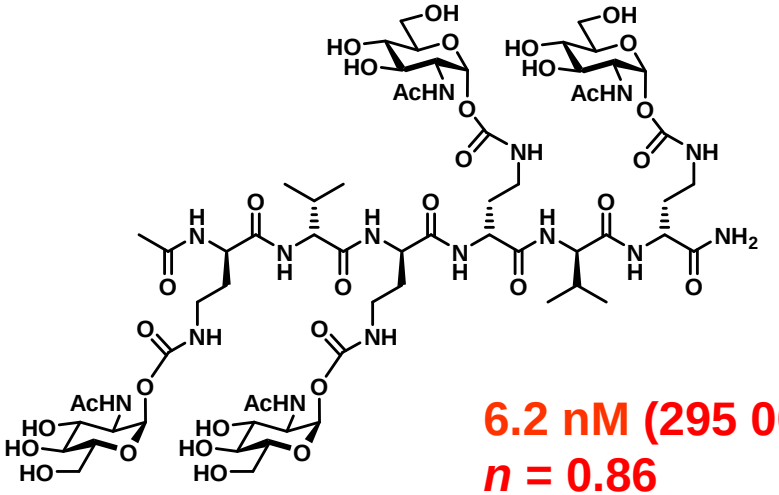


8.4 nM (218 000)
n = 0.84



7.0 nM (261 000)
n = 0.95

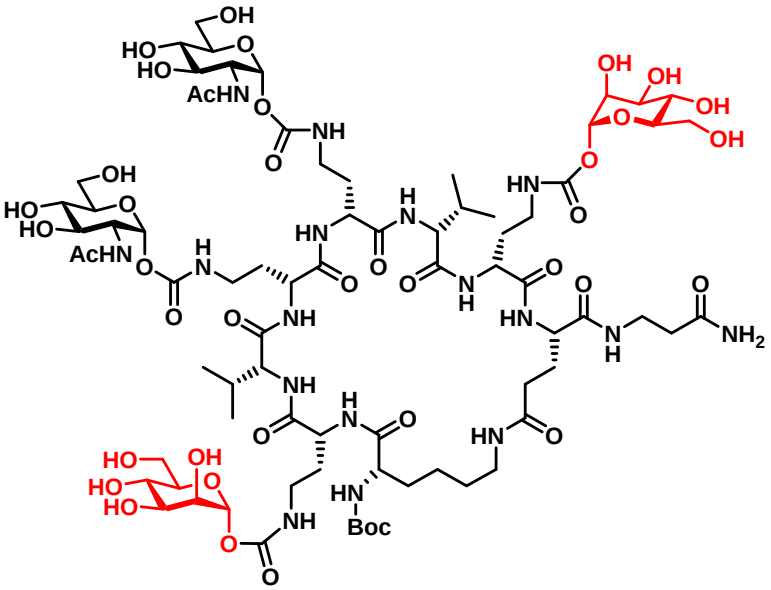
Cross-linking
(precipitate formation)



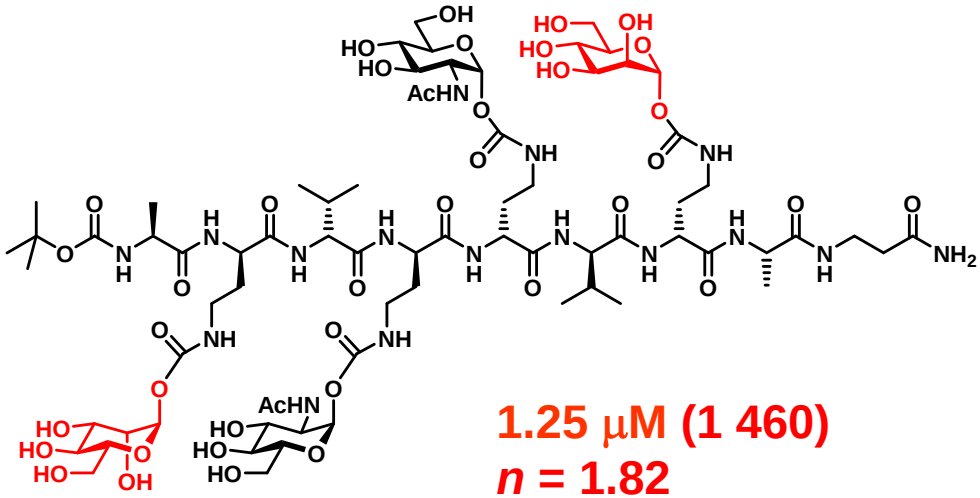
6.2 nM (295 000)
n = 0.86

P. Rohse, V. Wittmann, *Chem. Eur. J.* **2016**, *22*, 9724-9733

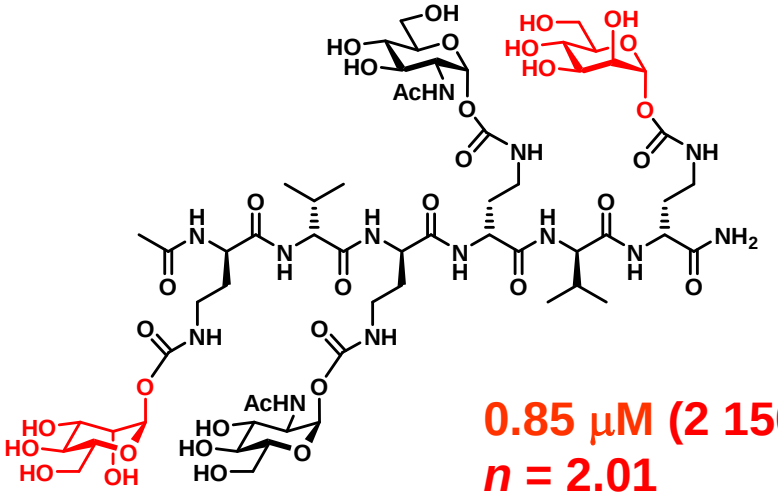
Absolute (and Relative) K_D Values from ITC



1.43 μM (1 280)
***n* = 1.81**



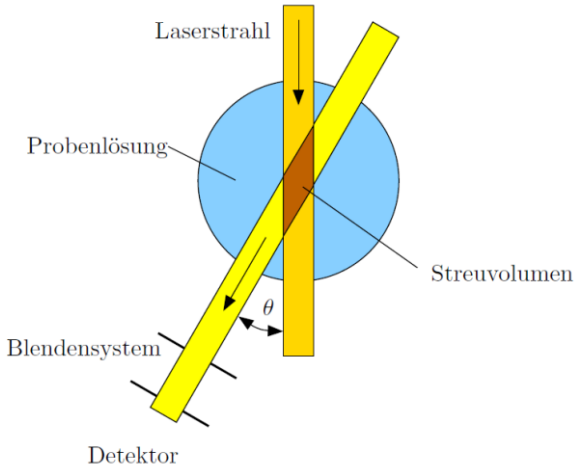
1.25 μM (1 460)
***n* = 1.82**



0.85 μM (2 150)
***n* = 2.01**

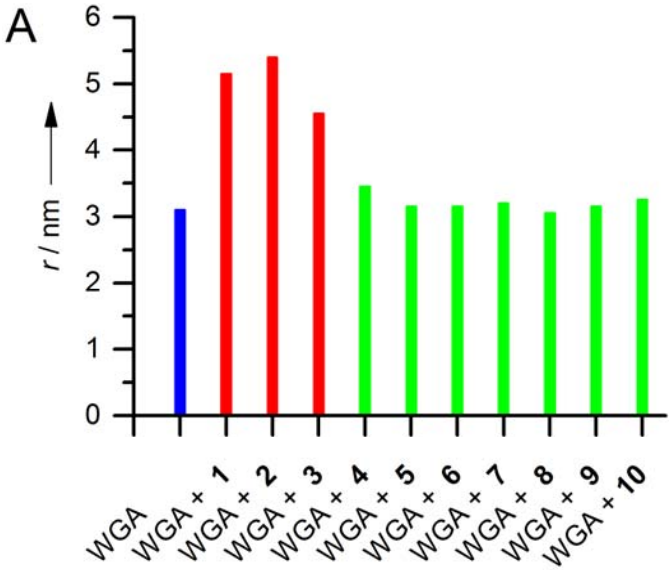
P. Rohse, V. Wittmann, *Chem. Eur. J.* **2016**, *22*, 9724-9733

DLS Measurements

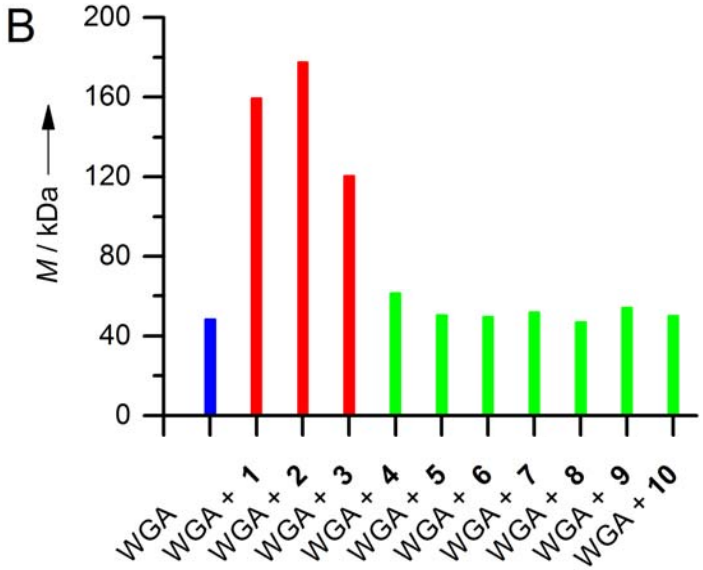


- Measurement of scattered light
- Influenced by speed of Brownian motion of particles in solution
  - ➢ Size of particles can be determined

Hydrodynamic radius



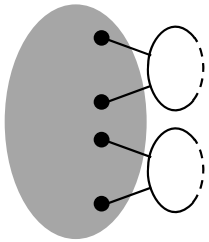
Molecular weight (calculated)



P. Rohse, V. Wittmann, *Chem. Eur. J.* **2016**, *22*, 9724-9733

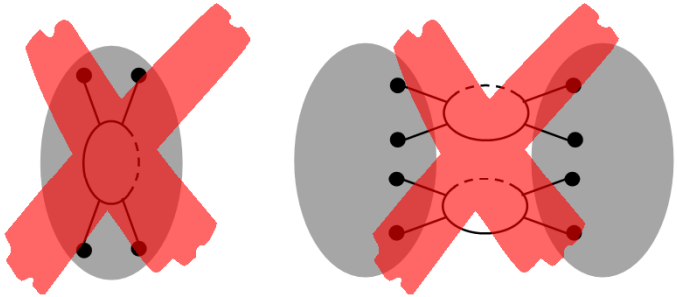
Plausible Binding Modes

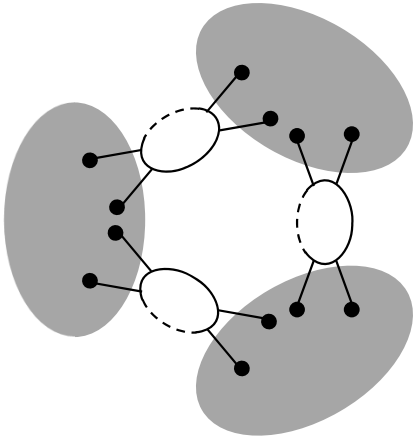
Divalent Ligands

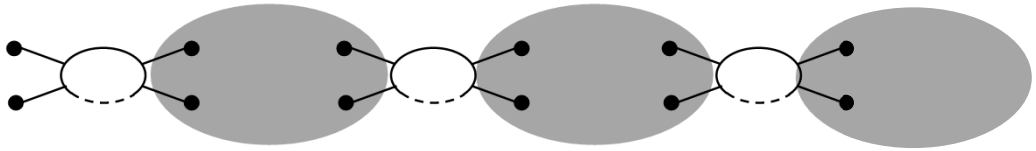


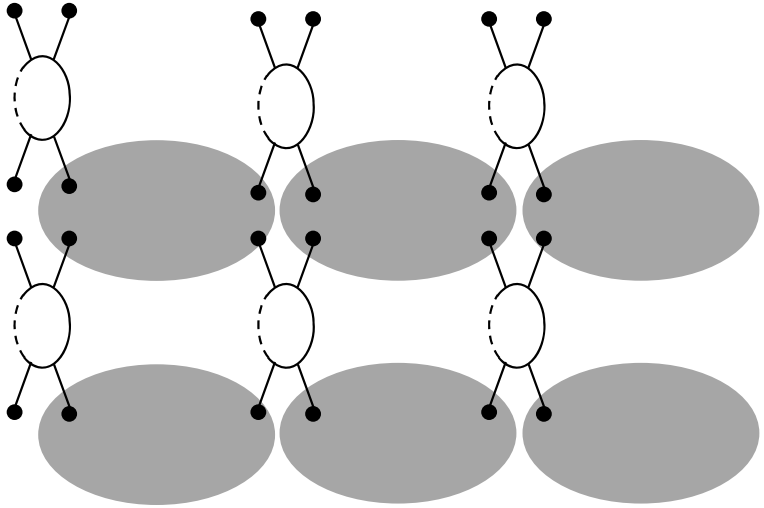
L : P
2 : 1

Tetravalent Ligands





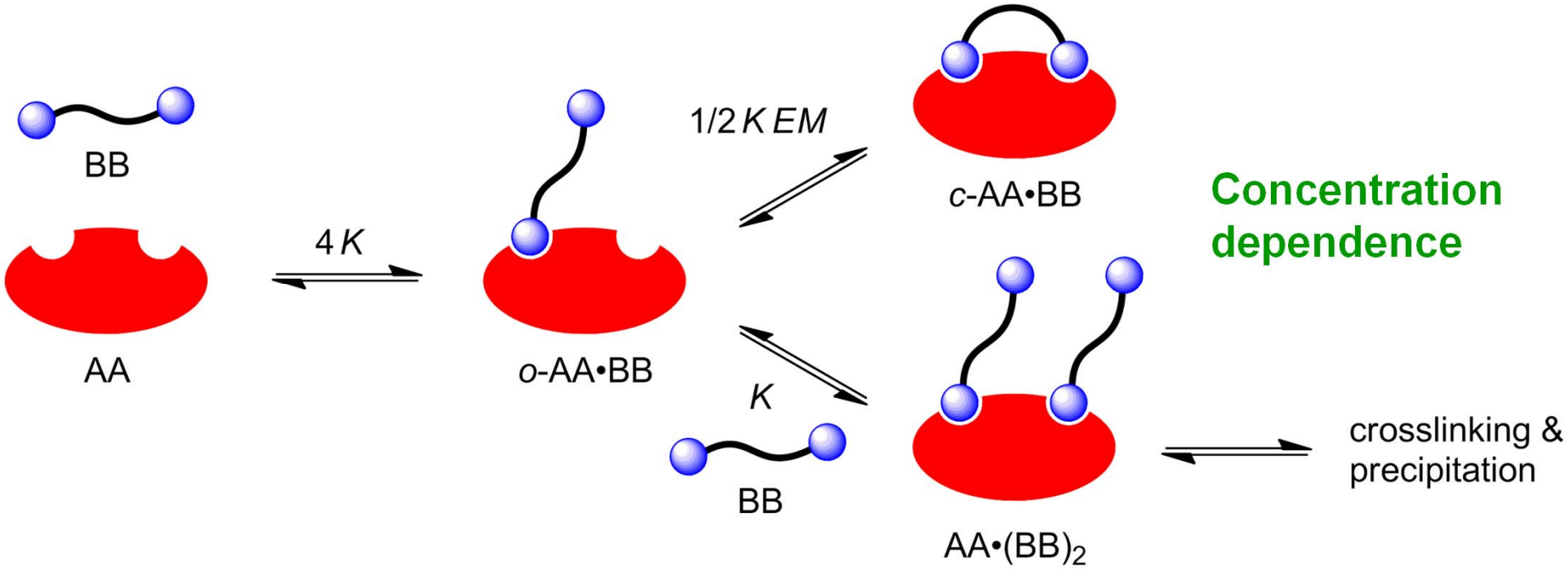




L : P
1 : 1

P. Rohse, V. Wittmann, *Chem. Eur. J.* **2016**, *22*, 9724-9733

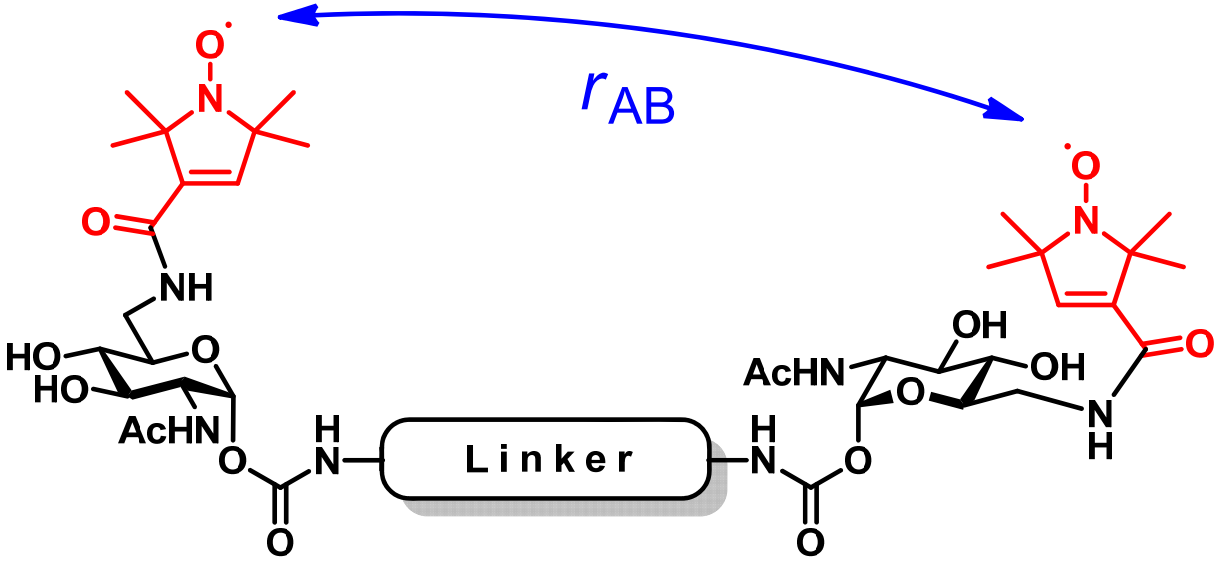
Multivalent Carbohydrate-Lectin Interactions

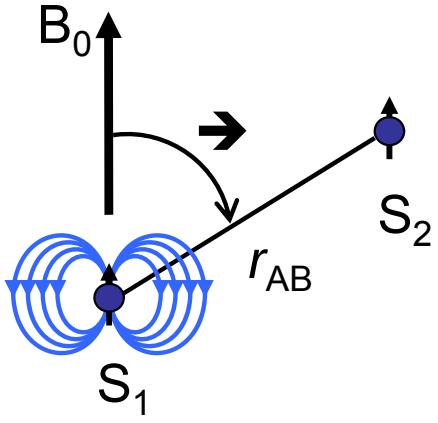


K: microscopic intermolecular association constant
EM: microscopic effective molarity

C. A. Hunter, H. L. Anderson, *Angew. Chem., Int. Ed.* **2009**, *48*, 7488
V. Wittmann, *Curr. Opin. Chem. Biol.* **2013**, *17*, 982

EPR-Spectroscopy: Distance Measurements in (Frozen Glassy) Solution



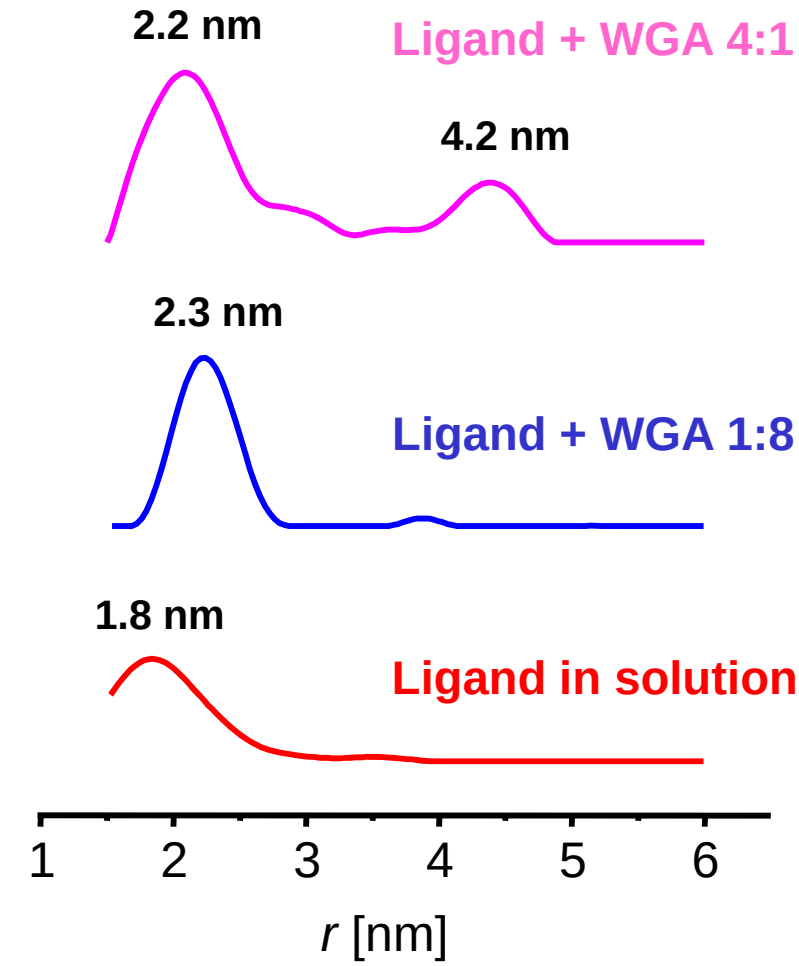


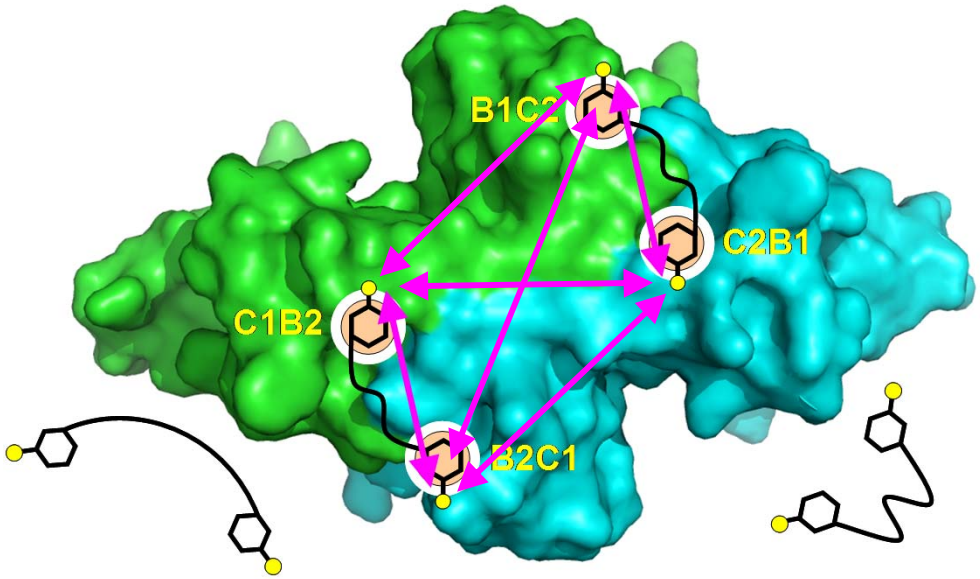
$$\omega_{dd} = \frac{\mu_0 g_A g_B \beta_e^2}{4\pi\hbar}\left(3\cos^2\theta - 1\right)\frac{1}{r_{AB}^3}$$

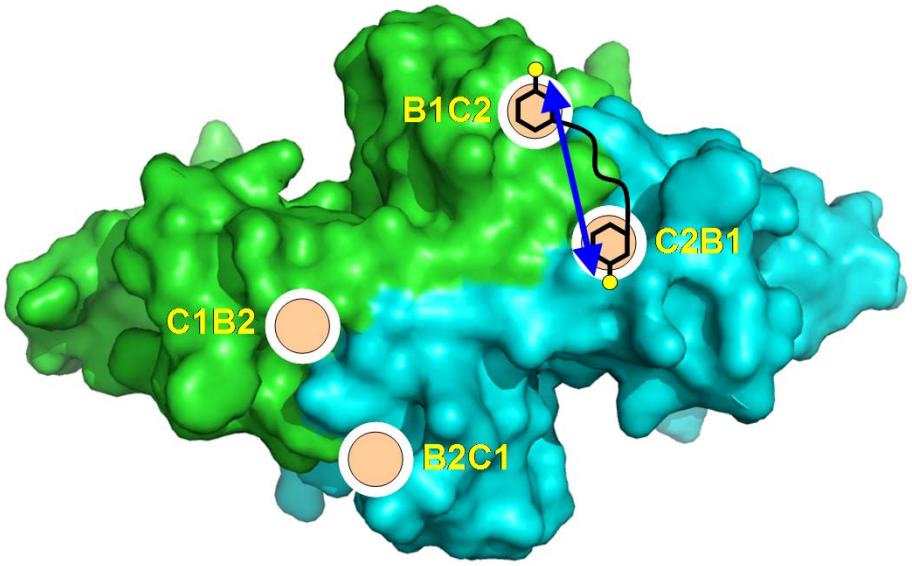
Dipolar interaction contains information about the distance r_{AB} between two coupled spins S_1 and S_2

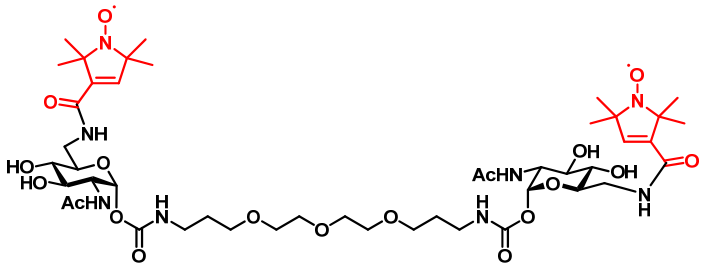
Angew. Chem., Int. Ed. **2011**, *50*, 8428

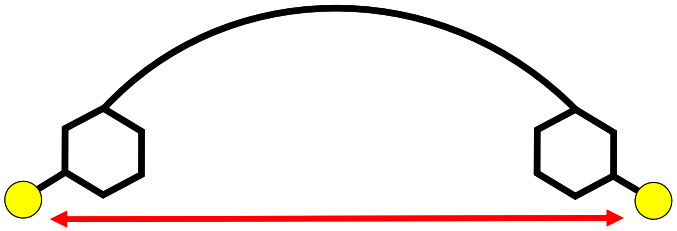
EPR-Derived Distance Distributions

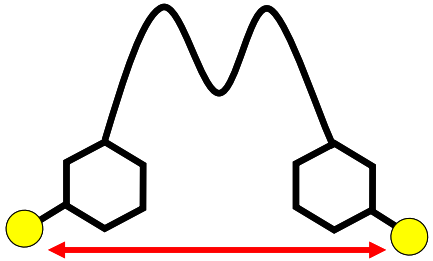






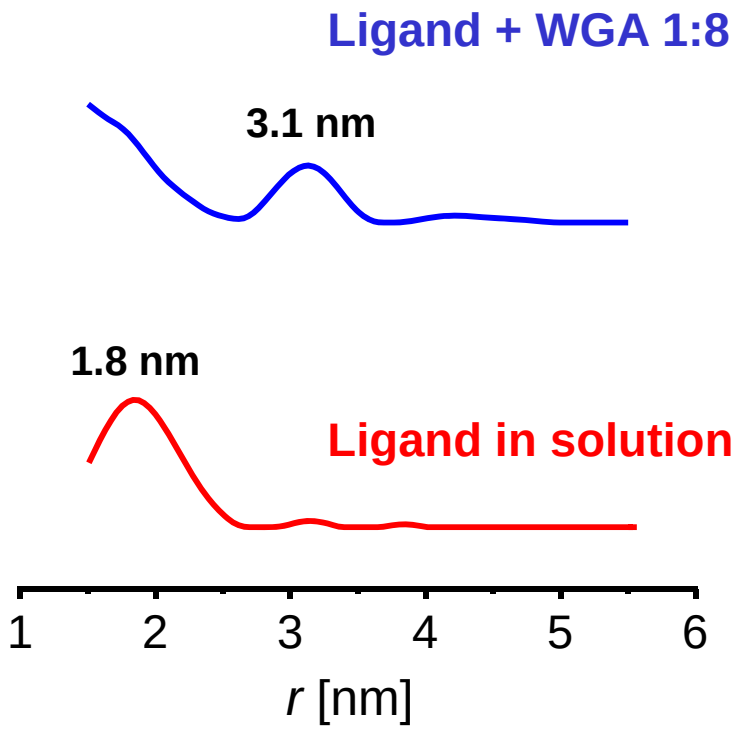


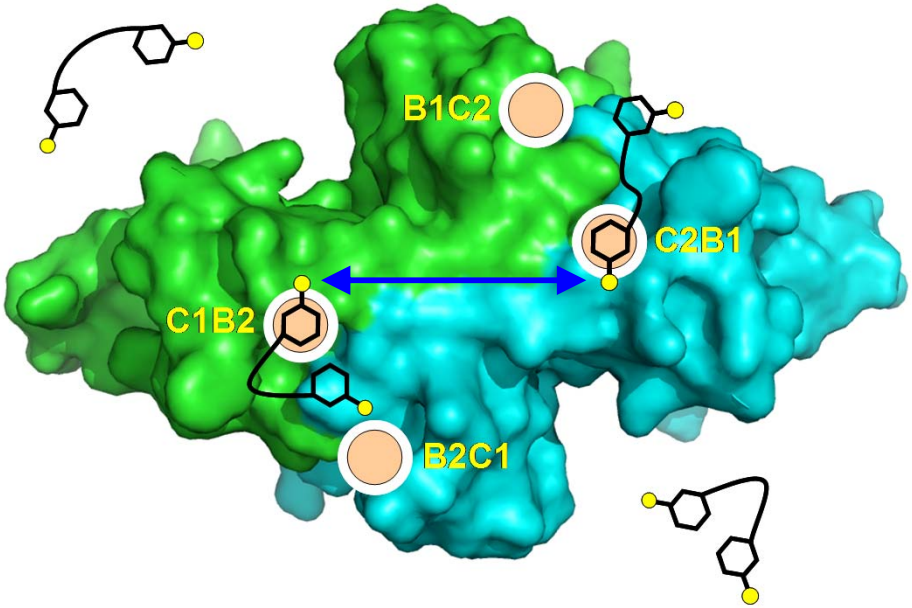


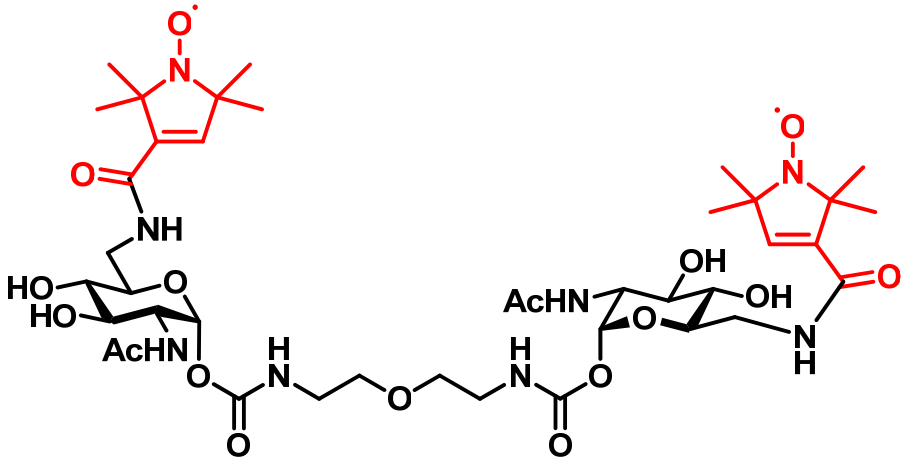


Angew. Chem., Int. Ed. **2011**, *50*, 8428

EPR-Derived Distance Distributions

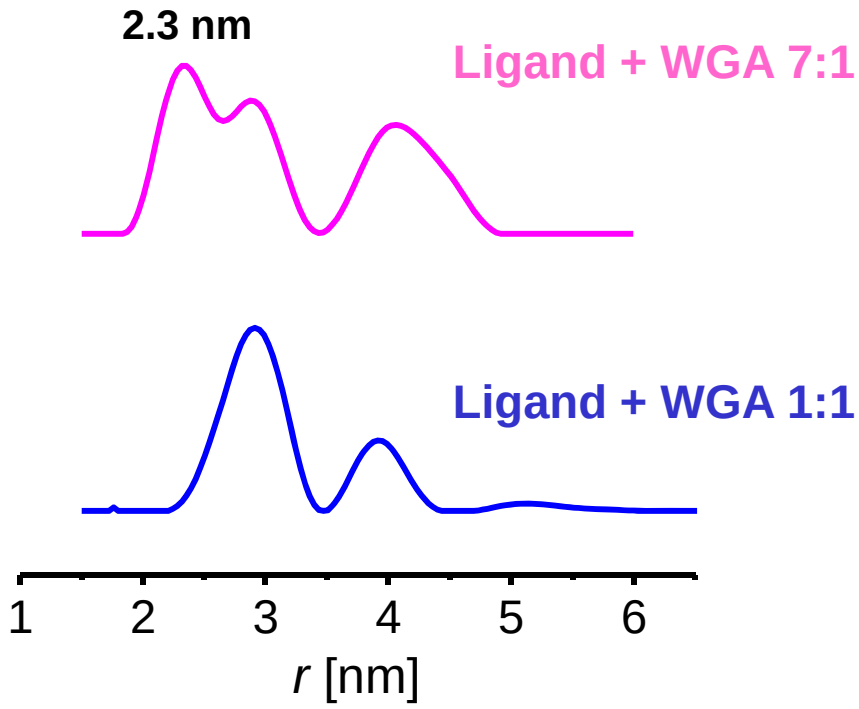


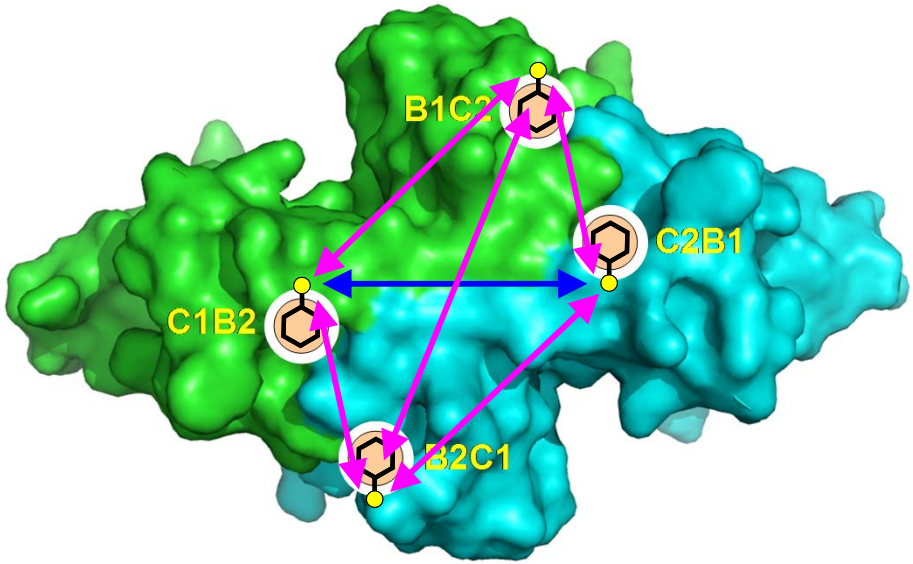


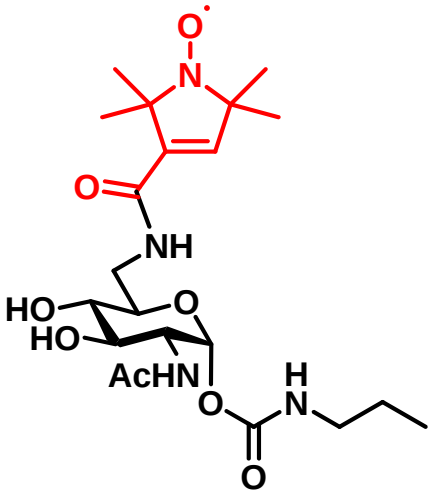


Angew. Chem., Int. Ed. **2011**, *50*, 8428

EPR-Derived Distance Distributions



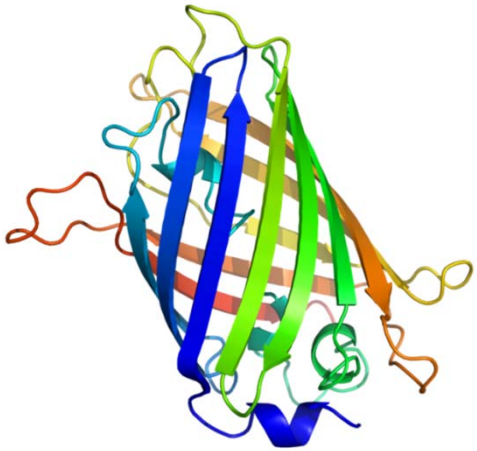


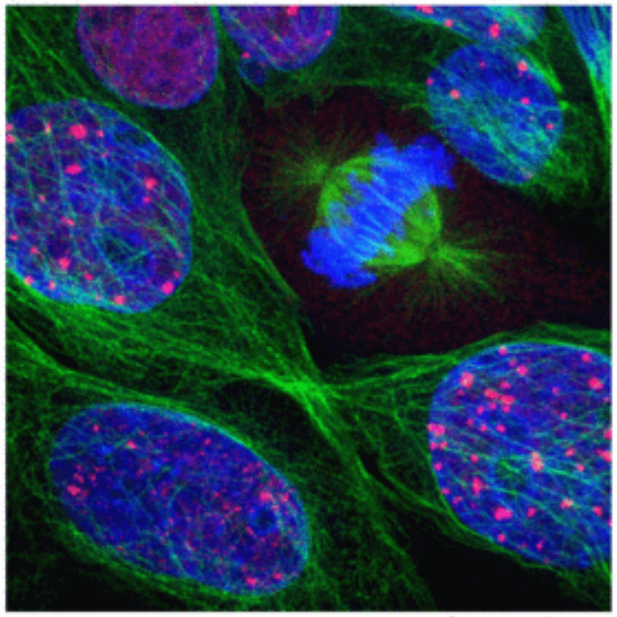


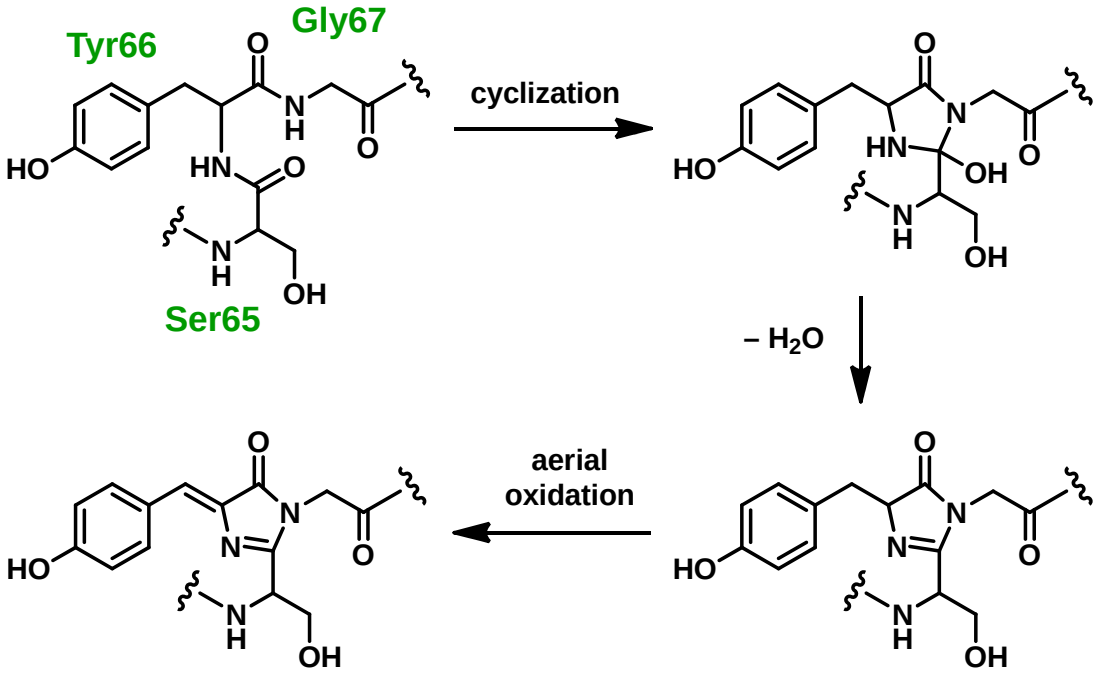
Angew. Chem., Int. Ed. **2011**, *50*, 8428

Protein Labeling: GFP Fusion Proteins

Green Fluorescent Protein (GFP)







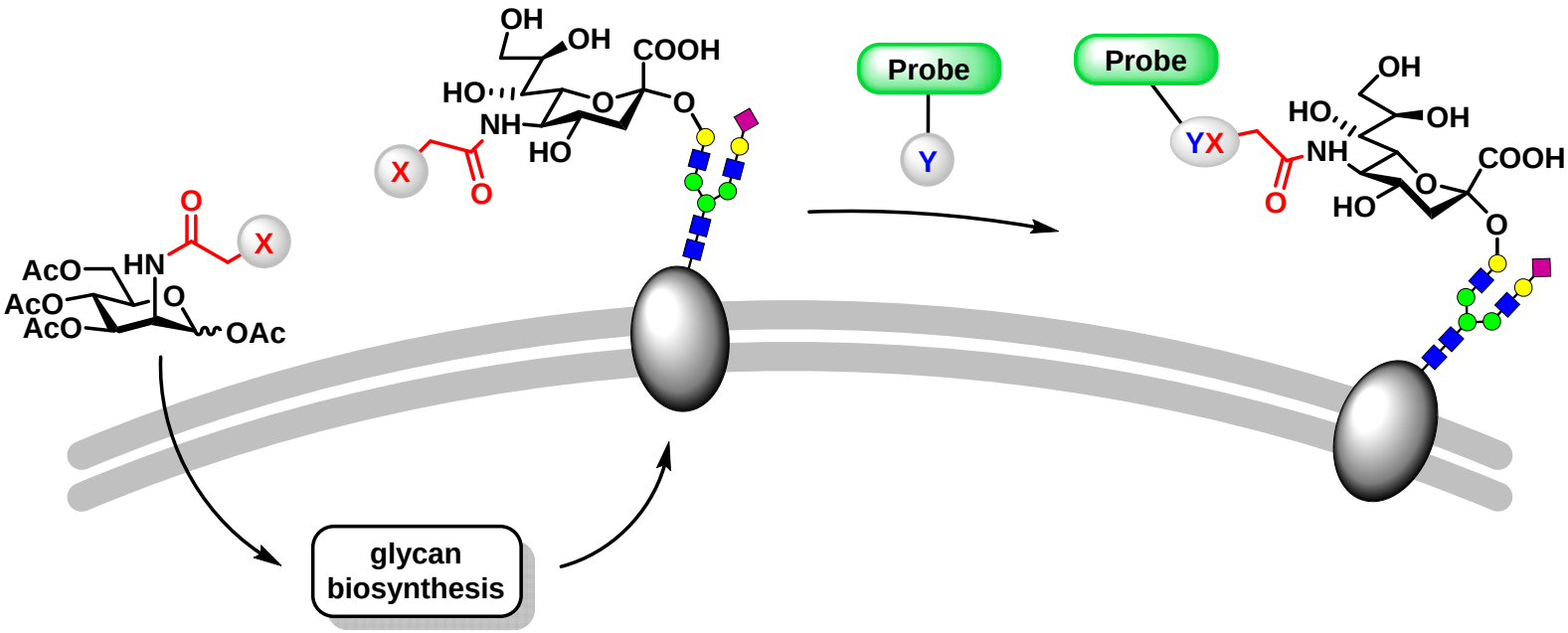
2008 Nobel Prize in Chemistry

Martin Chalfie

Osamu Shimomura

Roger Y. Tsien

Metabolic Glycoengineering (MGE)



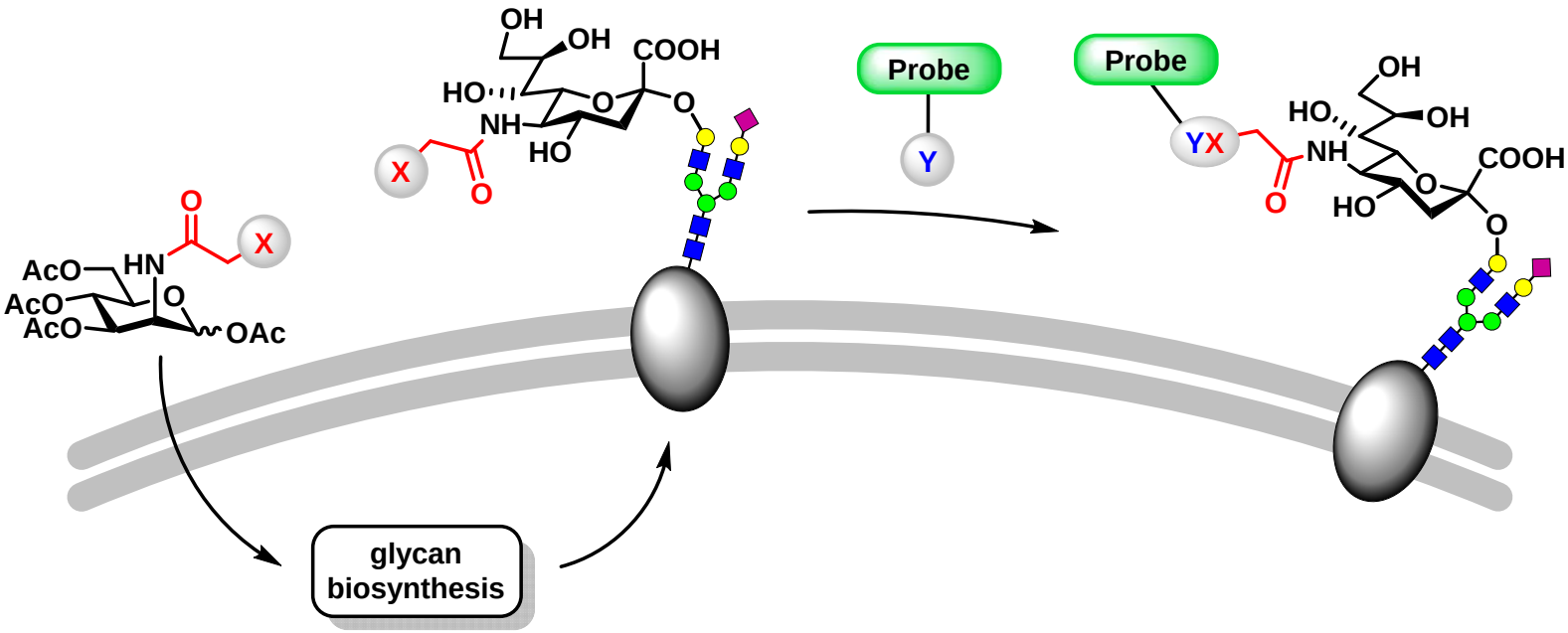
ManNAc derivatives → sialic acids bound to O- and N-glycans

GlcNAc derivatives → O-GlcNAc

GalNAc derivatives → mucin-type O-glycans

Interconversion can occur (catalyzed by epimerases).

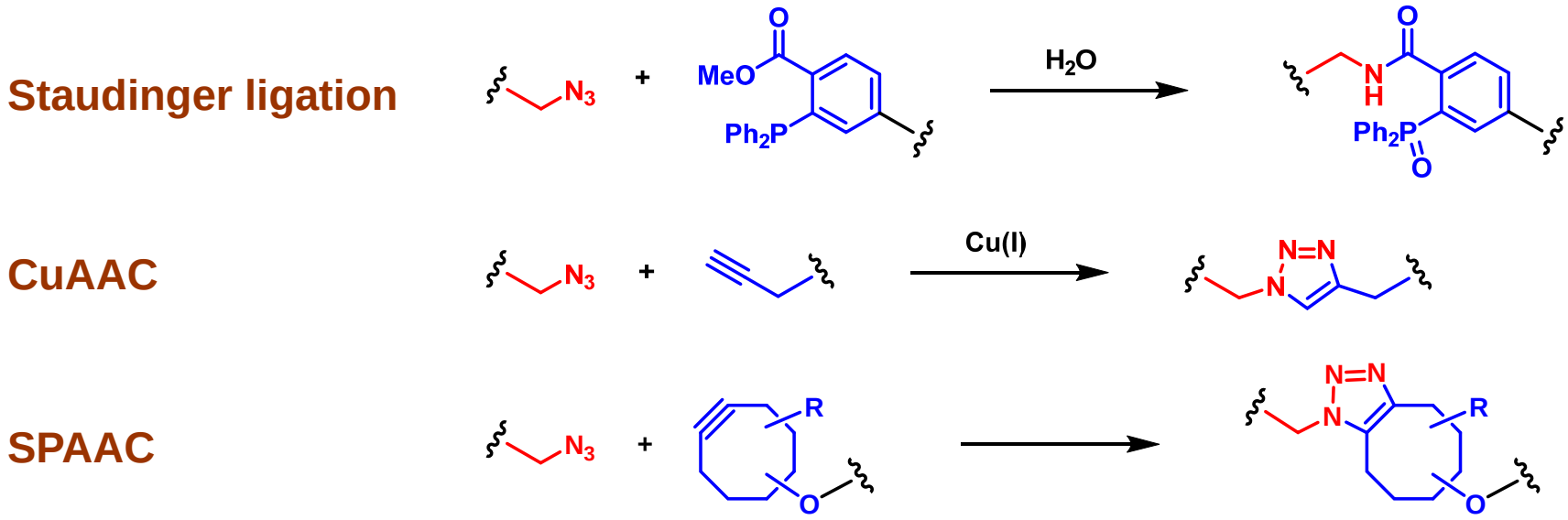
Metabolic Glycoengineering (MGE)



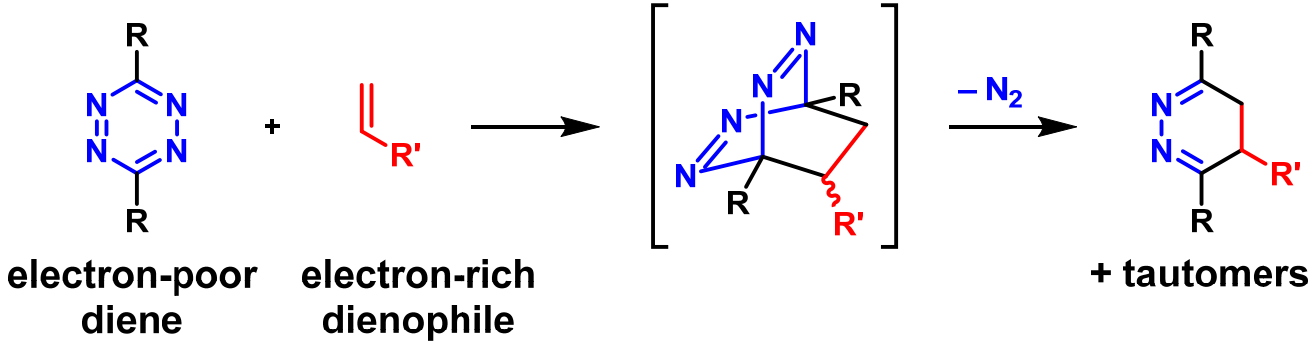
Challenges

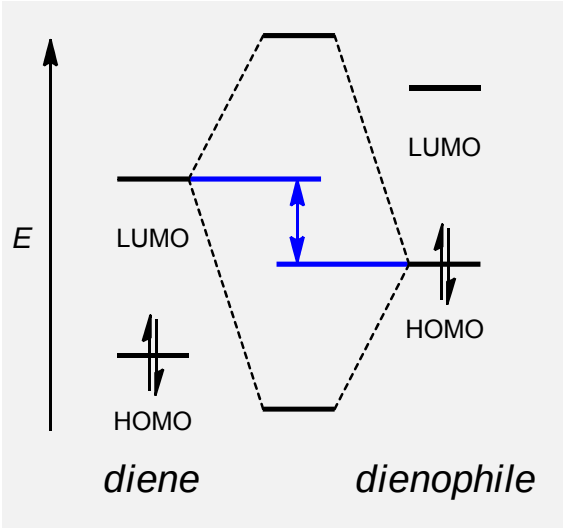
- Simultaneous detection of two (or more) different sugars
- Detection of glycans inside living cells
- Protein-specific detection of glycosylation

Bioorthogonal Ligation Reactions for MGE



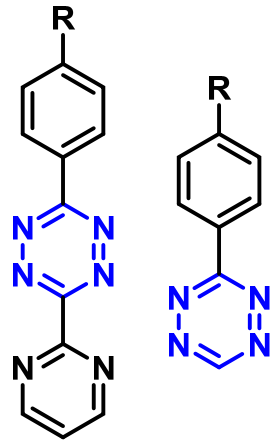
Inverse-Electron-Demand Diels-Alder (DAinv) Reaction

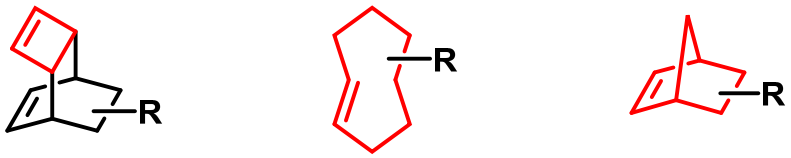




Carboni & Lindsey 1959, Sauer 1962

2008: First application to bioconjugation

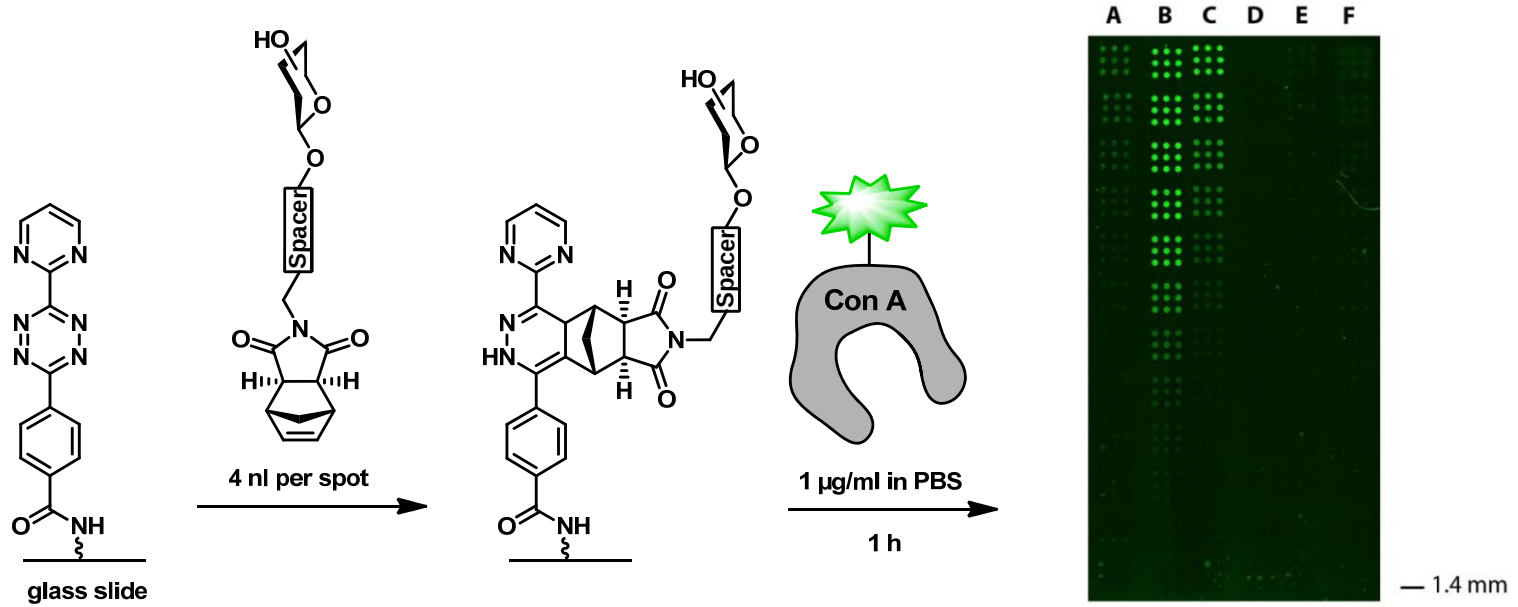




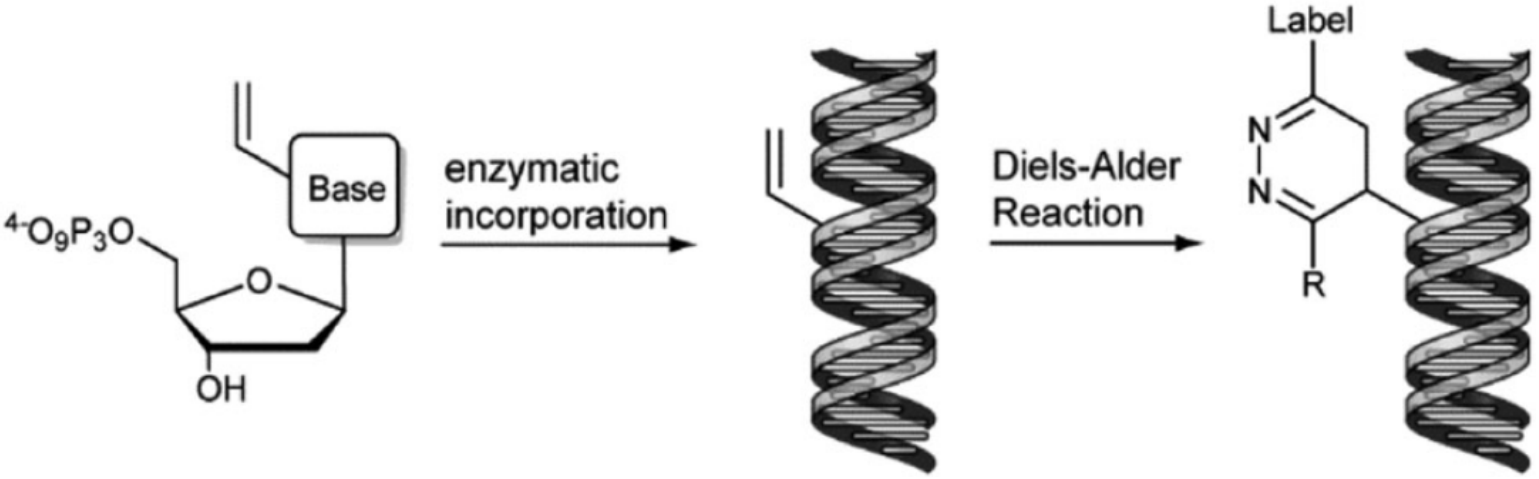
Braun/Wiessler: *Drug Des. Dev. Ther.* **2008**, *2*, 289
Fox: *J. Am. Chem. Soc.* **2008**, *130*, 13518
Weissleder/Hilderbrand: *Bioconjugate Chem.* **2008**, *19*, 2297

➢ **very fast** ➢ **irreversible** ➢ **metal-free** ➢ **bioorthogonal**

Application of the DAinv Reaction for Bioconjugation

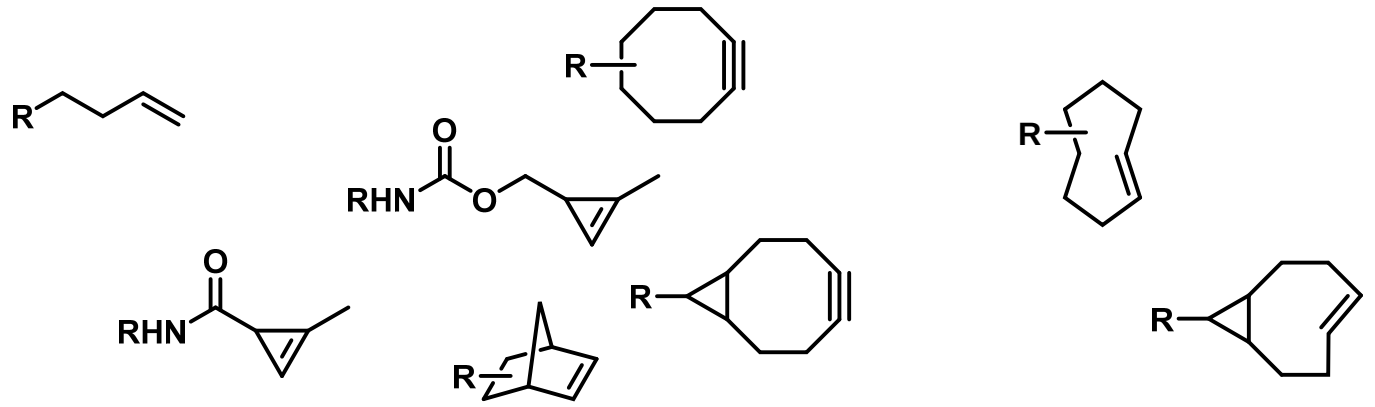


Chem. Eur. J. **2012**, *18*, 6548



Chem. Commun. **2014**, *50*, 10827

Dienophiles for the DAinv Reaction



inverse-electron-demand Diels-Alder reaction

Staudinger

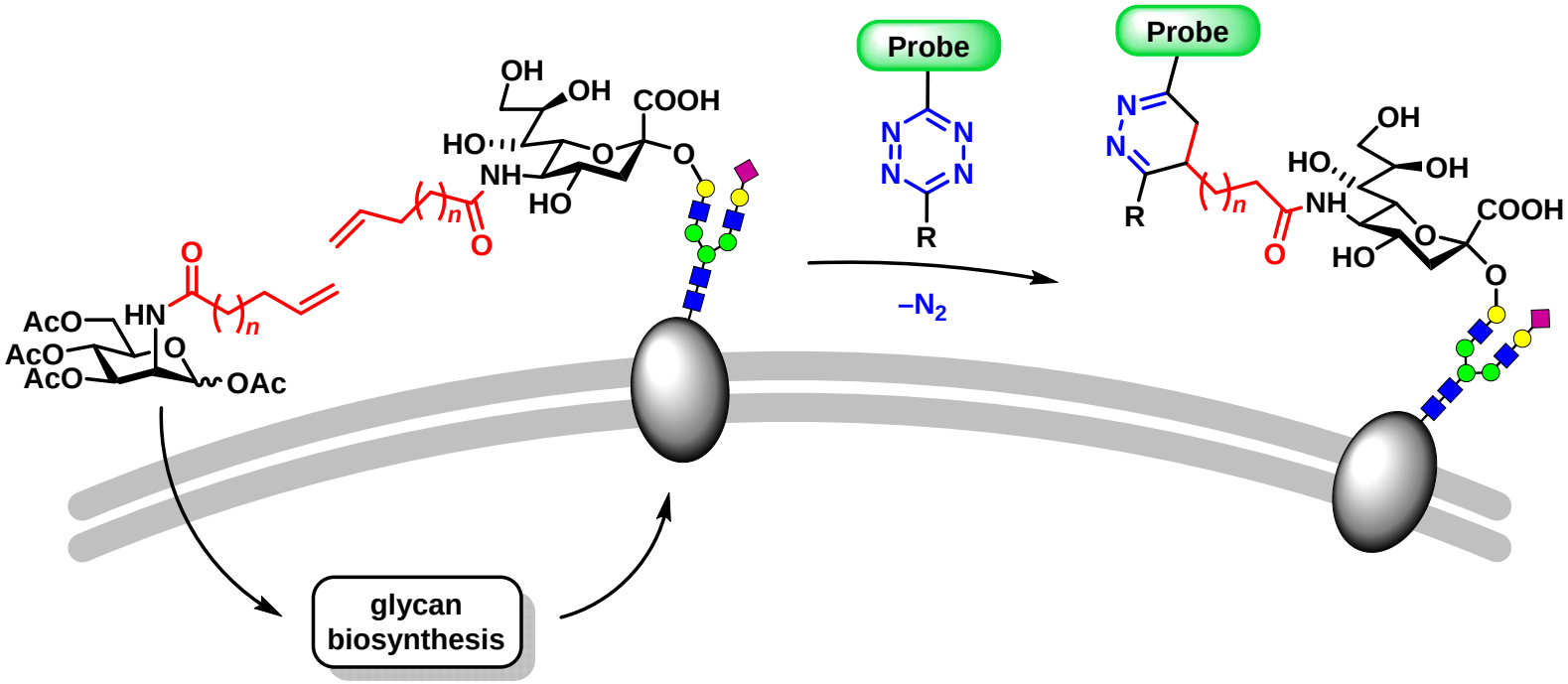
SPAAC

CuAAC

10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 1 10^{1} 10^{2} 10^{3} 10^{4} 10^{5}

Rate constant / $M^{-1}s^{-1}$ →

Metabolic Glycoengineering with the DAinv Reaction

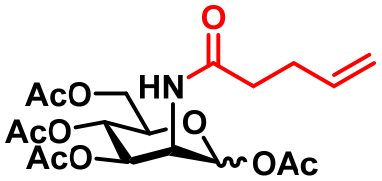


Angew. Chem., Int. Ed. **2013**, *52*, 4265

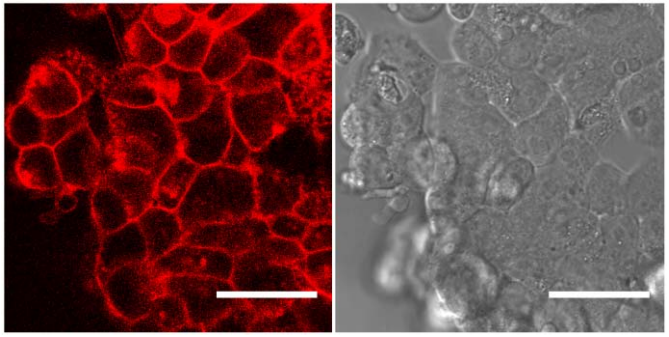
Metabolic Glycoengineering with the DAinv Reaction

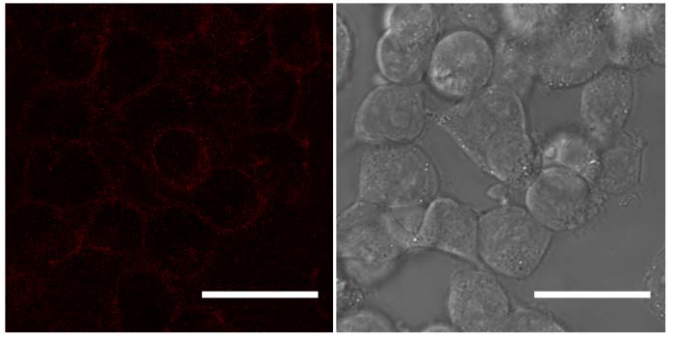
Fluorescence images of living HEK 293T cells

k = 0.021 $M^{-1}s^{-1}$

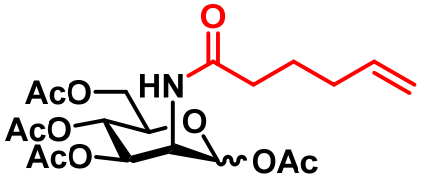


$Ac_4ManNPtl$

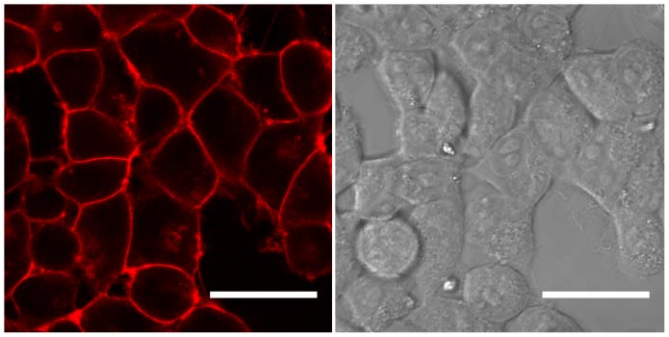


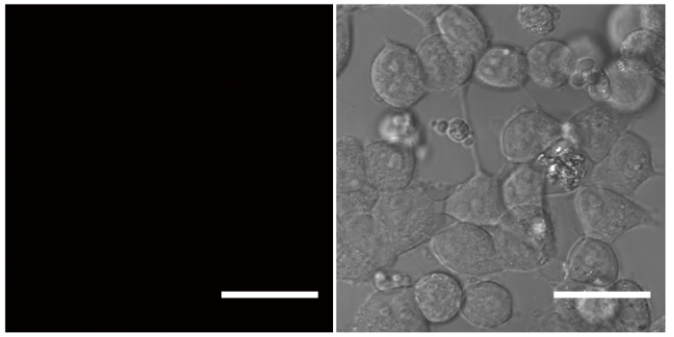


k = 0.041 $M^{-1}s^{-1}$



$Ac_4ManNHxl$

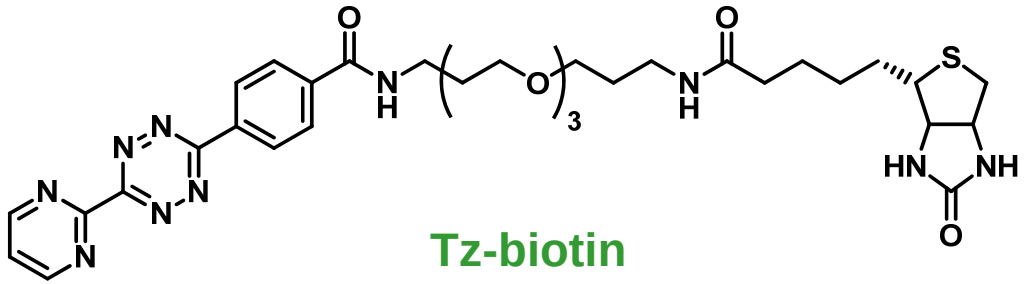




Scale bar: 30 μm

Cells grown with 100 μM sugar for 2 days, incubated with Tz-biotin, and then AlexaFluor®647-streptavidin

Control (no sugar)

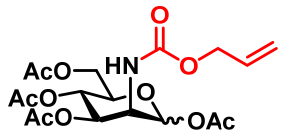


Tz-biotin

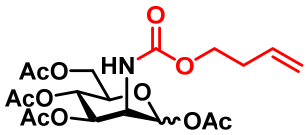
Angew. Chem., Int. Ed. **2013**, *52*, 4265

Metabolic Glycoengineering with Carbamates

Fluorescence images of living HEK 293T cells

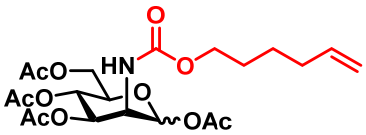


Ac_4ManNAloc



Ac_4ManNBeoc

Ac_4ManNPeoc



Ac_4ManNHeoc

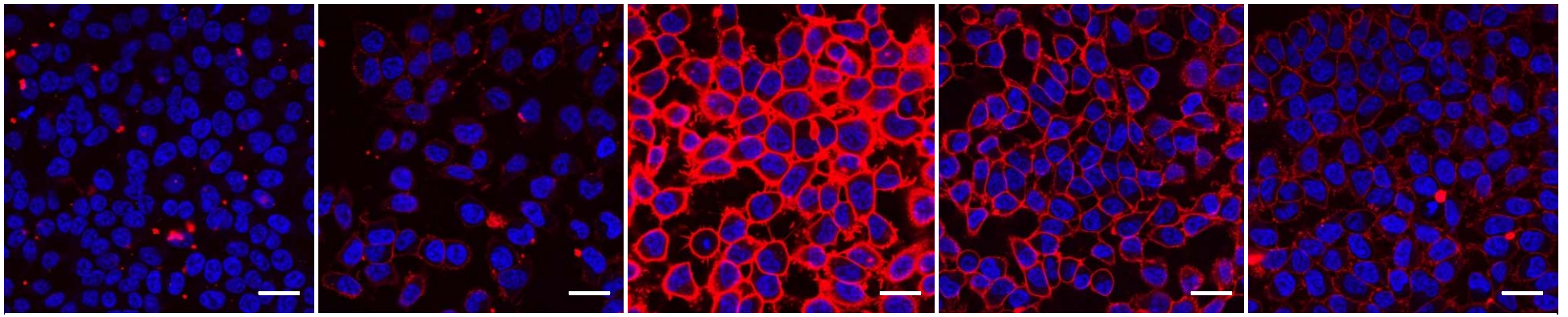
k_2:	0.0015 $M^{-1}s^{-1}$	0.014 $M^{-1}s^{-1}$	0.017 $M^{-1}s^{-1}$	0.074 $M^{-1}s^{-1}$

Control (no sugar)

DAinv reactivity

metabolic incorporation

50 % **13 %** **6 %** **n/d**



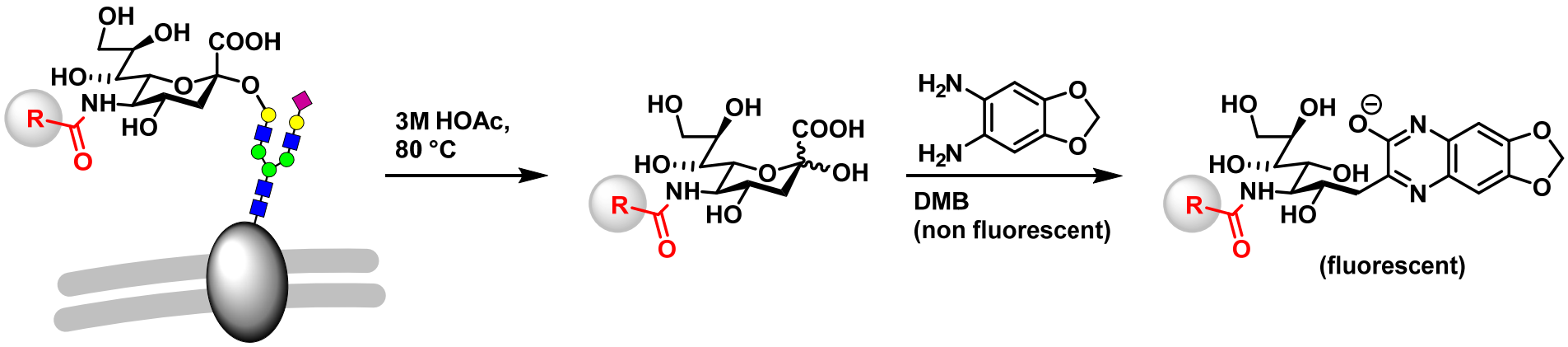
Scale bar: 30 µm

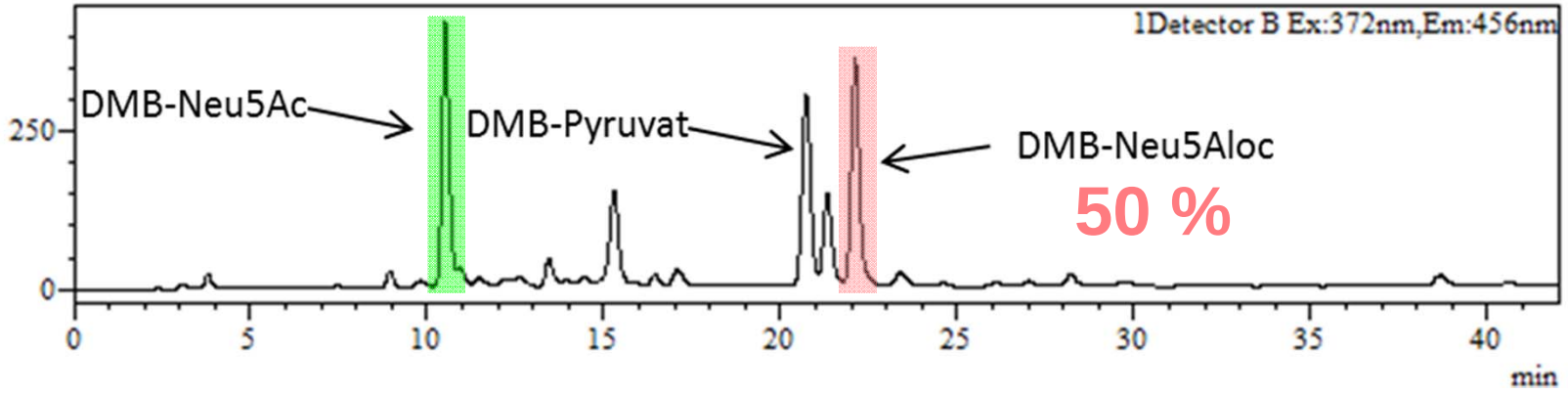
Cells were grown with 100 µM sugar for 2 days, then incubated with Tz-biotin (1 mM, 6 h, 37 °C) and Alexa-Fluor®647-streptavidin (20 min, 37 °C).

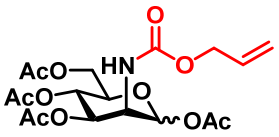
Chem. Eur. J. **2014**, *20*, 16502

... and Jeremias Dold

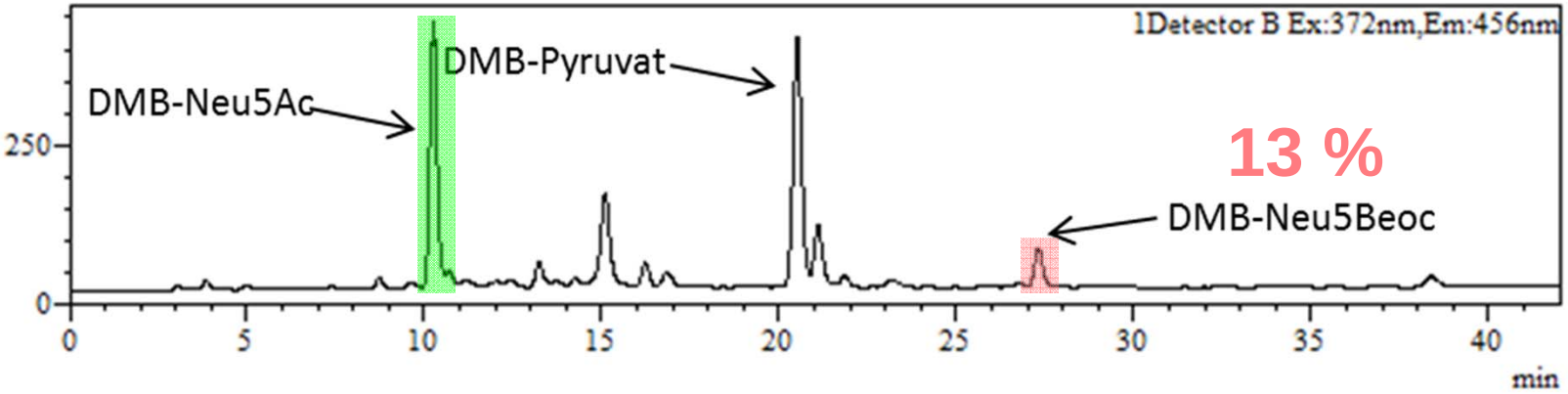
DMB Labeling of Released Sialic Acids

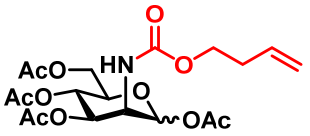






$Ac_4ManNAloc$



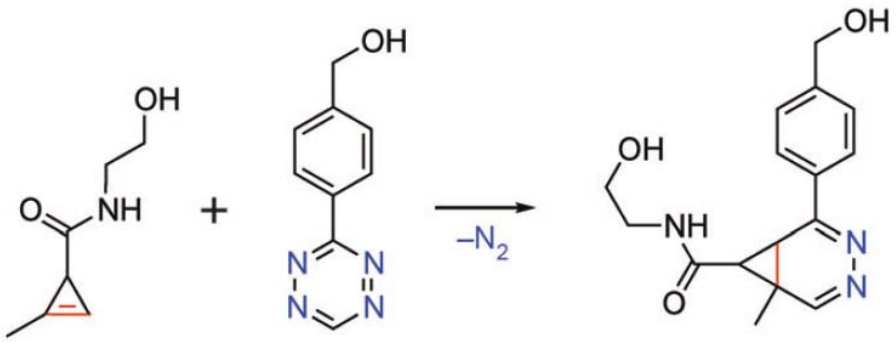


$Ac_4ManNBeoc$

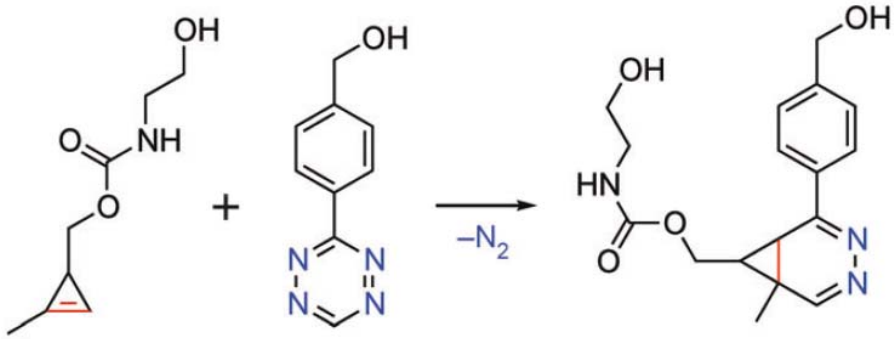
Jeremias Dold

Accelerating the DAinv Reaction

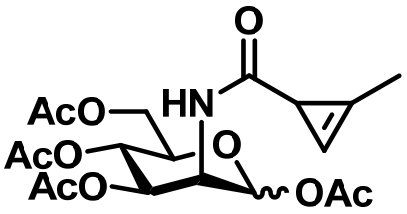
k = 0.137 $M^{-1}s^{-1}$



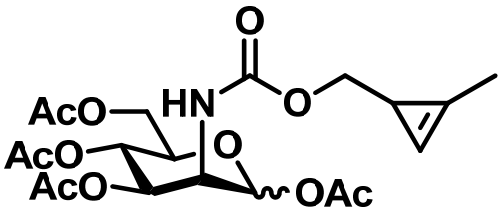
k = 13 $M^{-1}s^{-1}$



Devaraj group, *Angew. Chem., Int. Ed.* **2012**, *51*, 7476



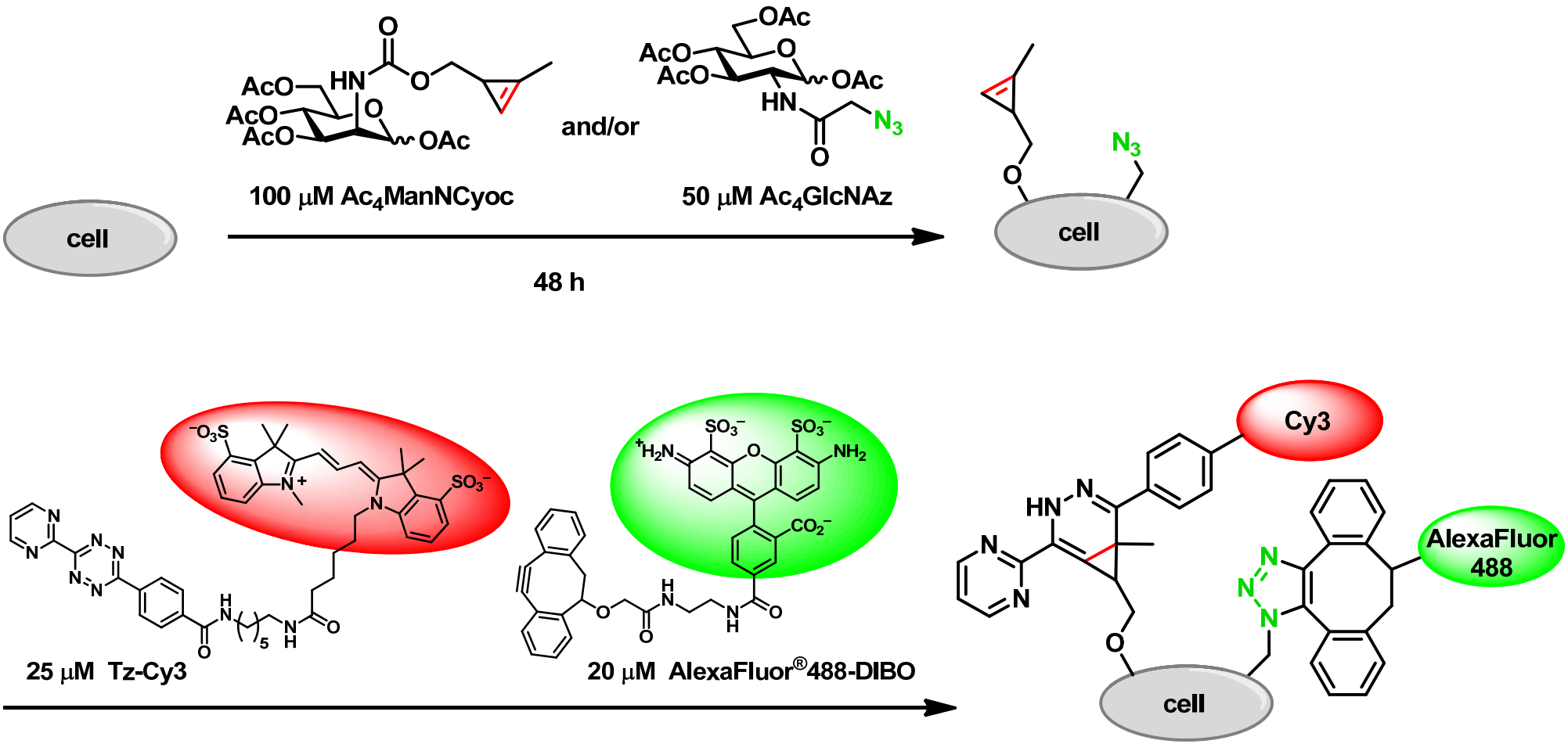
Devaraj group
ChemBioChem **2013**, *14*, 205



Wittmann group
Bioconjugate Chem. **2014**, *25*, 147

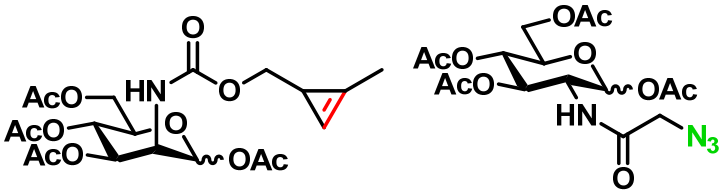
See also: J. A. Prescher,
Mol. BioSyst. **2014**, *10*, 1693

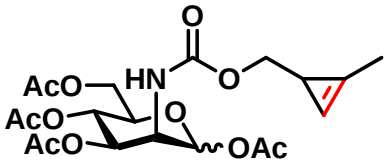
Dual-Labeling Strategy (HEK 293T Cells)

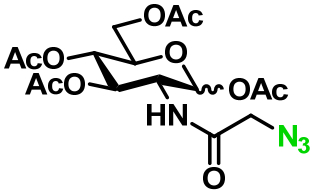


Bioconjugate Chem. **2014**, *25*, 147

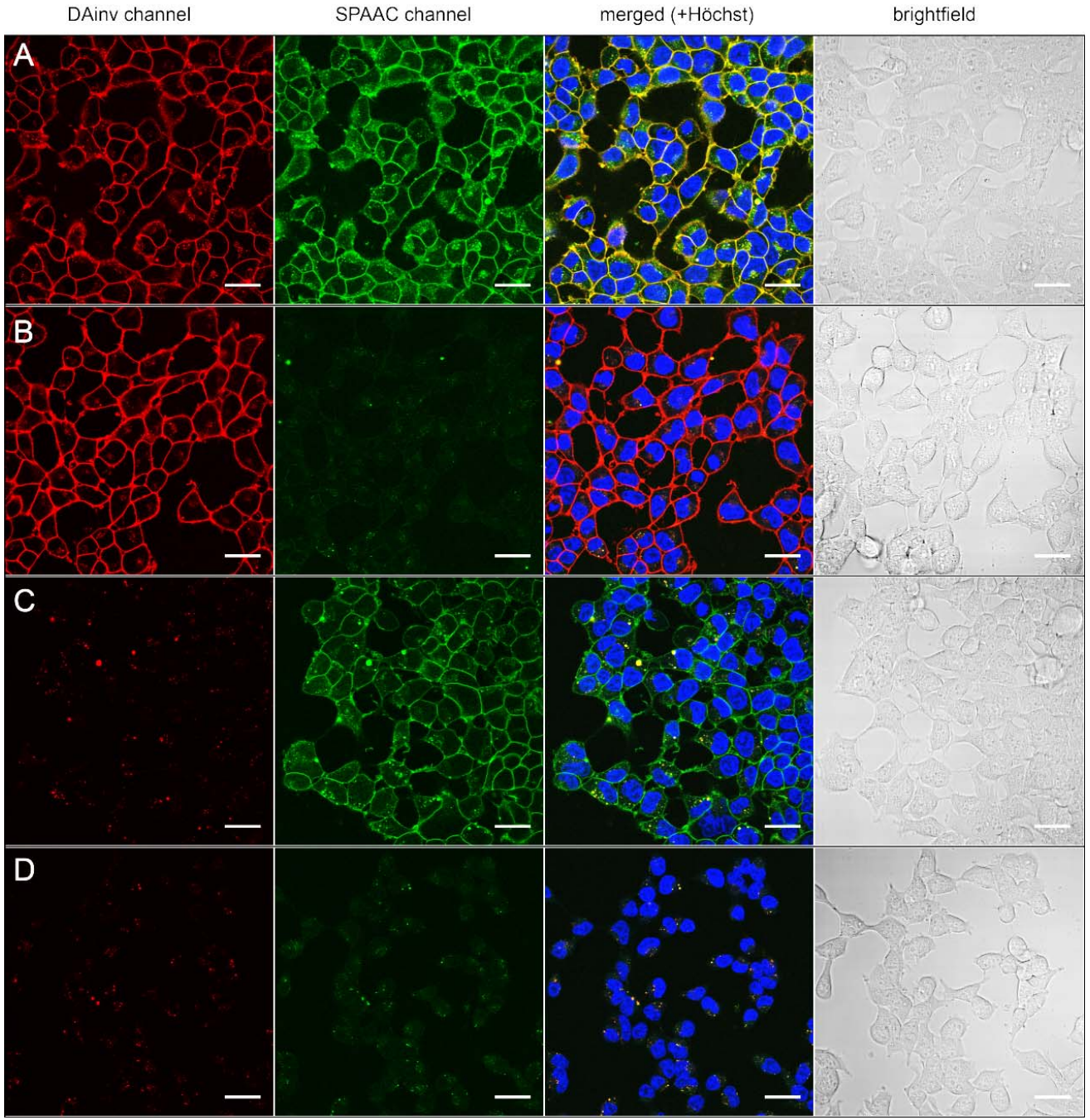
Dual-Labeling Strategy (HEK 293T Cells)





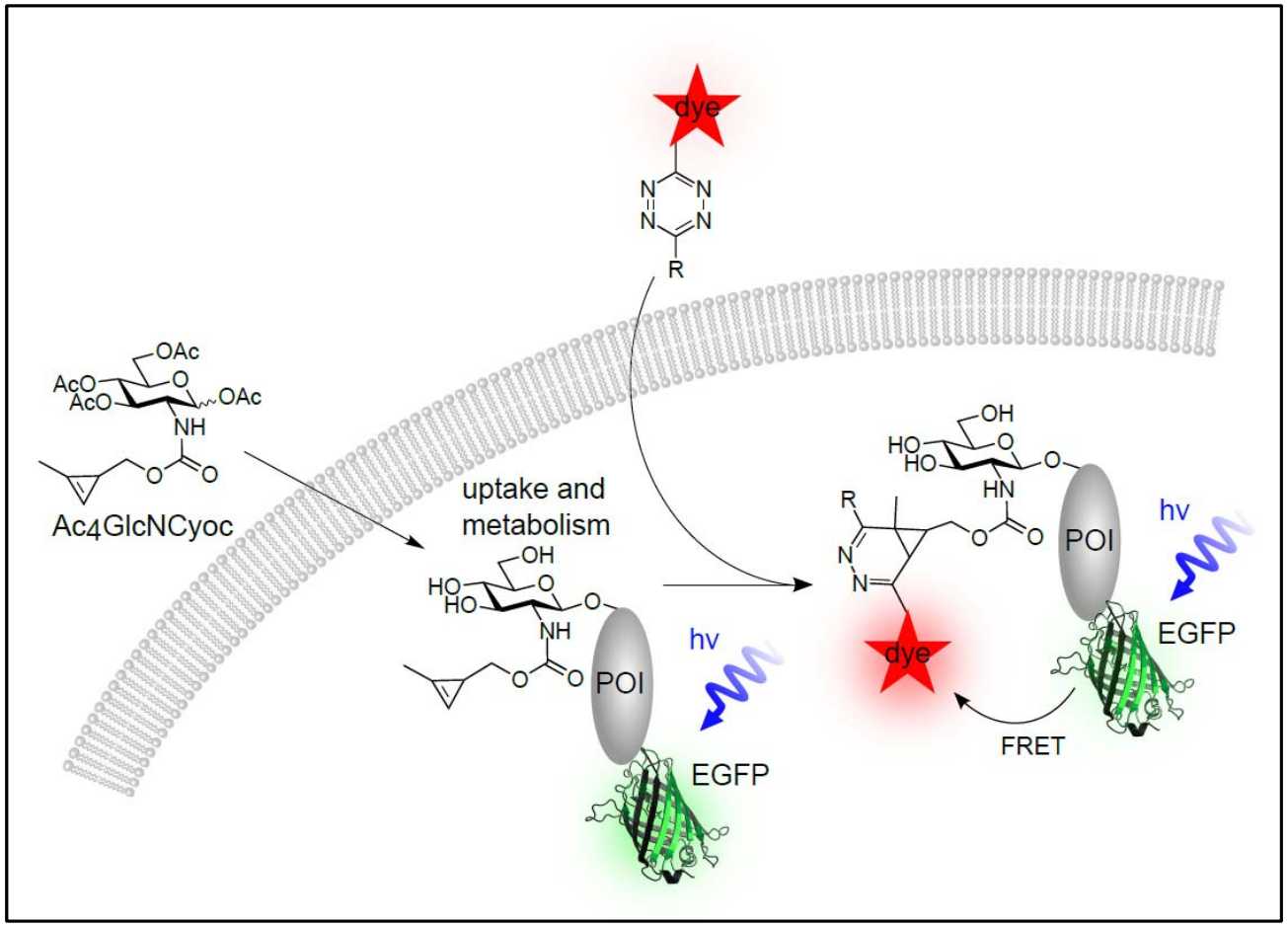


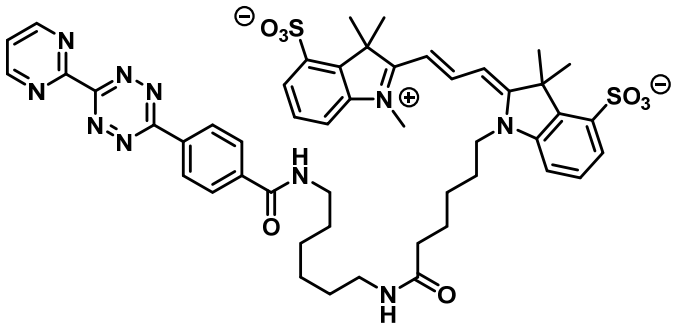
Control (no sugar)



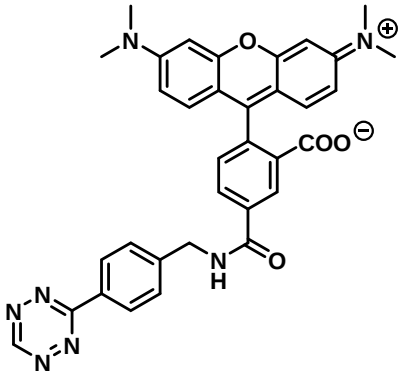
Bioconjugate Chem. **2014**, *25*, 147

Visualization of Protein-Specific Glycosylation in Living Cells





Cy3-Tz (reacts more specific)



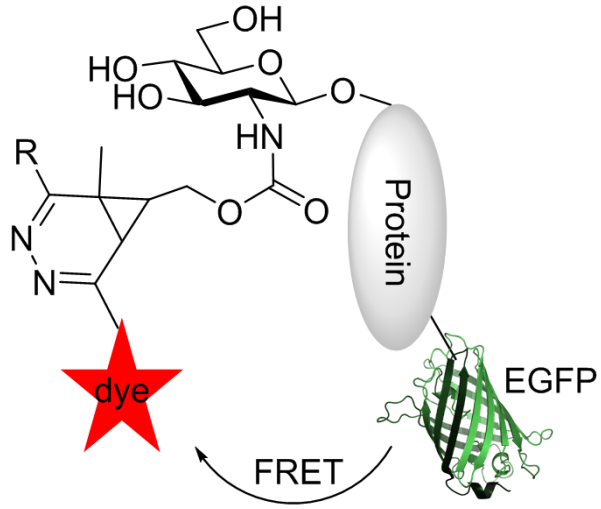
TAMRA-Tz (cell-permeable)

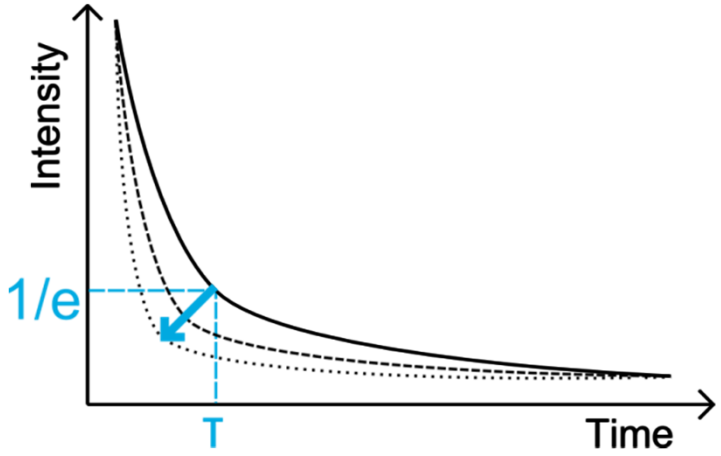
Detection of FRET with high contrast even in presence of a large excess of acceptor fluorophores by **fluorescence lifetime imaging microscopy (FLIM)**

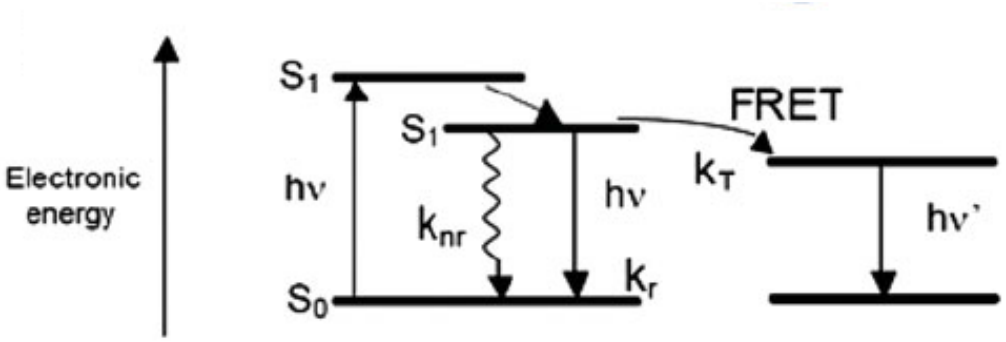
Proteins of interest (POI): O-GlcNAc transferase (OGT), forkhead transcription factor Foxo1, tumor suppressor p53, serine/threonine kinase Akt1

Fluorescence Lifetime Imaging Microscopy (FLIM)

The fluorescence lifetime is the average time that a molecule spends in the excited state.



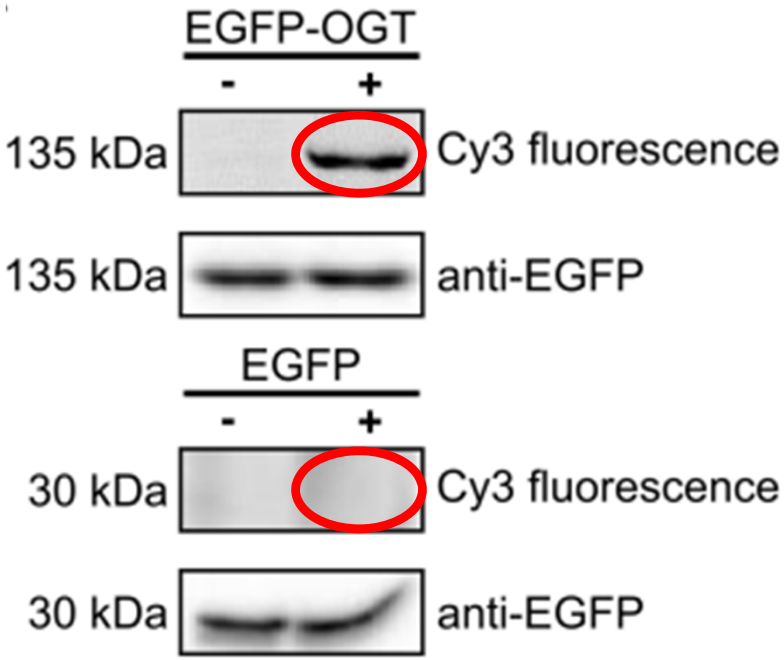




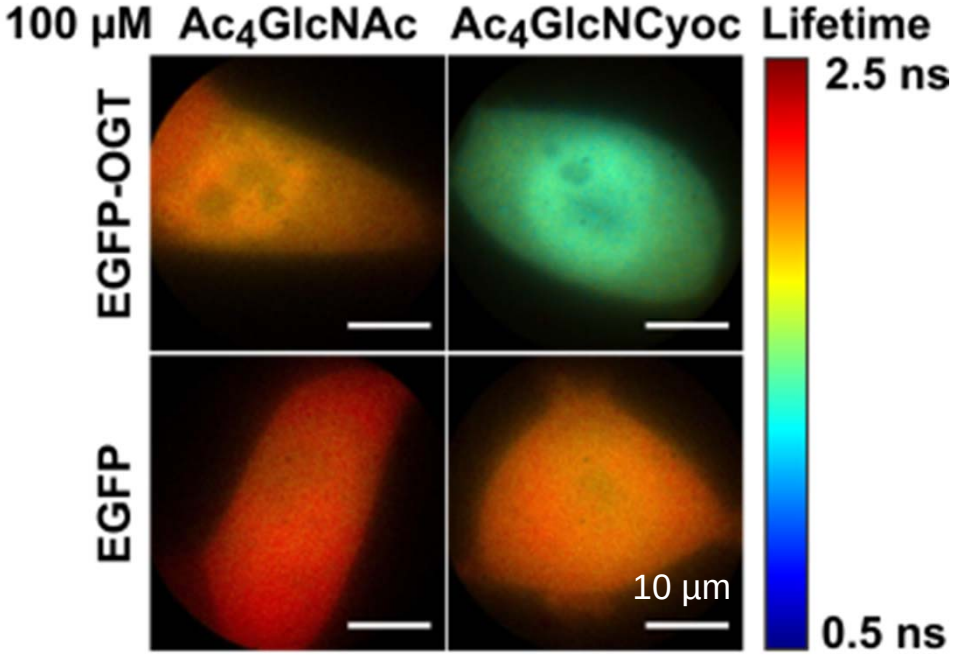
J. R. Lakowicz et al., *Biophys. J.* **1984**, *46*, 463-477

Visualization of O-GlcNAcylation of OGT inside Living Cells

HEK293T cells were transfected with OGT-EGFP or EGFP constructs and treated with Ac_4GlcNAc (–) or Ac_4GlcNCyoc (+) for 20 h.



Western blot analysis after immuno-precipitation using an anti-GFP antibody and labeling with Cy3-tetrazine.

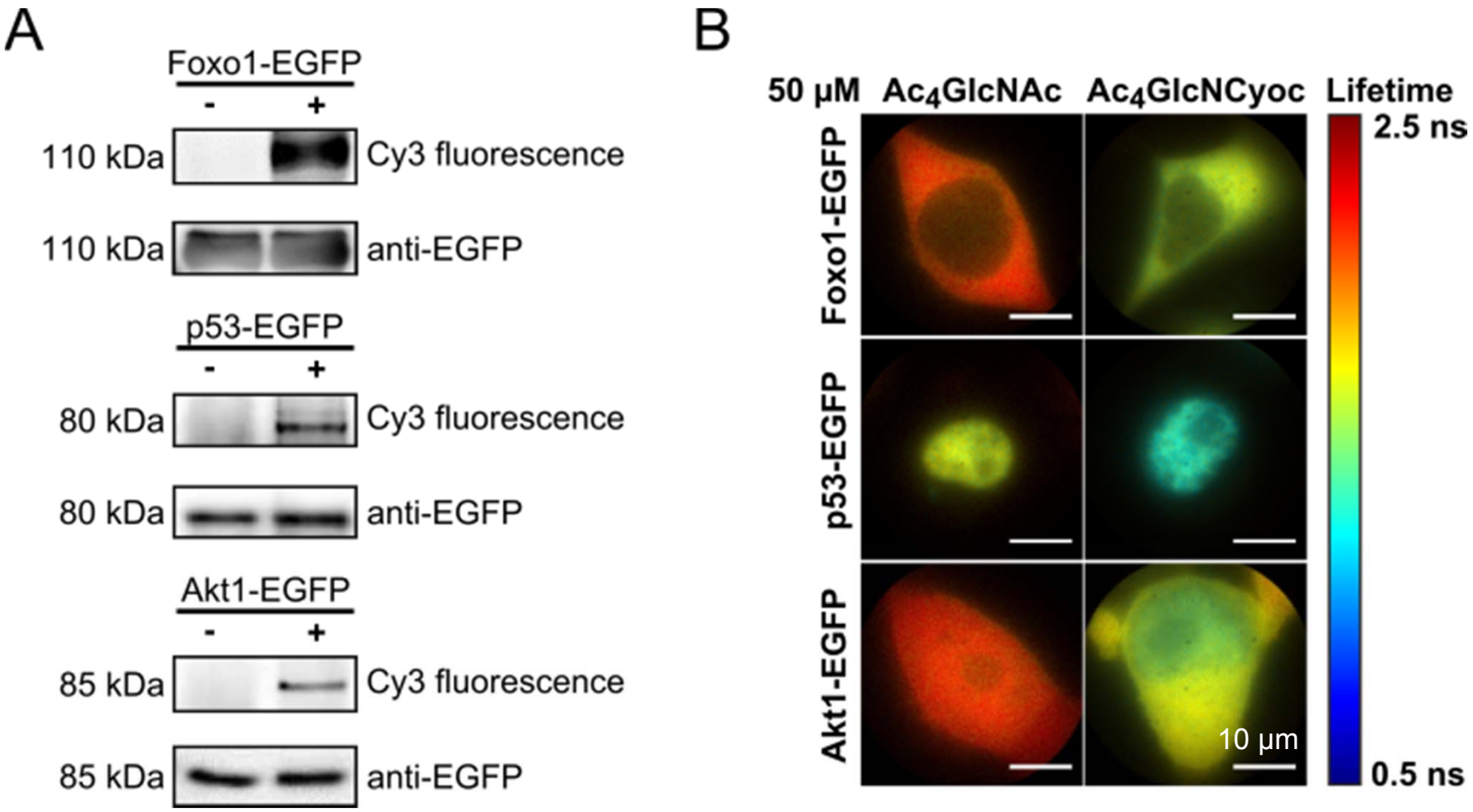


Representative fluorescence modulation lifetime images of individual cells after labeling with TAMRA-tetrazine.

F. Doll, A. Buntz, A.-K. Späte, V. F. Schart, A. Timper, W. Schrimpf, C. R. Hauck, A. Zumbusch, V. Wittmann, *Angew. Chem. Int. Ed.* **2016**, *55*, 2262-2266

Visualization of Protein-Specific Glycosylation in Living Cells

O-GlcNAcylation of Foxo1, p53, and Akt1 visualized by FLIM-FRET microscopy of individual cells



F. Doll, A. Buntz, A.-K. Späte, V. F. Schart, A. Timper, W. Schrimpf, C. R. Hauck, A. Zumbusch, V. Wittmann, *Angew. Chem. Int. Ed.* **2016**, *55*, 2262-2266

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The group

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Dr. Andrea Niederwieser
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Prof. Dr. W. Reutter (FU Berlin)
Prof. Dr. H. Scherer (FU Berlin)
Prof. Dr. G. Mayer (U Bonn)

SFB 1214

SFB 969

SPP 1623

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