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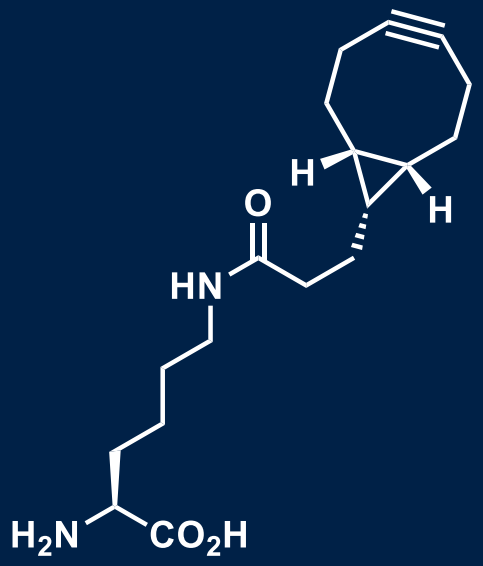
Alkyne labels and reporter groups in bioorthogonal chemistry

A review of the recent literature



Thursday December 12, 2013

Outline

- What is bioorthogonality?
- Historic background and development
- Why bioorthogonal chemistry?
  - Advantages
  - Requirements
- Reporter groups and how to install them
- Commonly encountered labels
- Alkyne reactions
  - CuAAC
  - SPAAC
  - SPANC
  - SPATL
  - Sonogashira cross-coupling
- Mutual orthogonality of bioorthogonal reactions

What does ‘bioorthogonality’ mean?

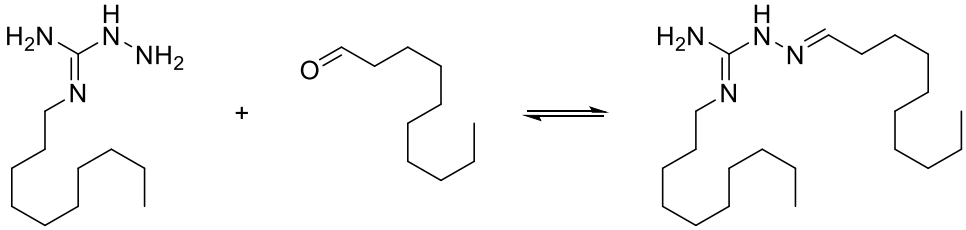
- Coined by Prof. Carolyn Bertozzi (UC Berkeley) in 2003
- Formalised by Prof. Herman Overkleeft (Leiden) in 2005

«Bioorthogonal is defined as: not associated with – and chemically inert to – the conditions in the particular physiological system.»

- This means
  - Non-native reactants
  - Selective reactions under physiological conditions

Prescher, J. A. and Bertozzi, C. R. *Nature Chem. Bio.* **2005**, *1*, 13
van Swieten, P. F. *et al. Org. Biomol. Chem.* **2005**, *3*, 20

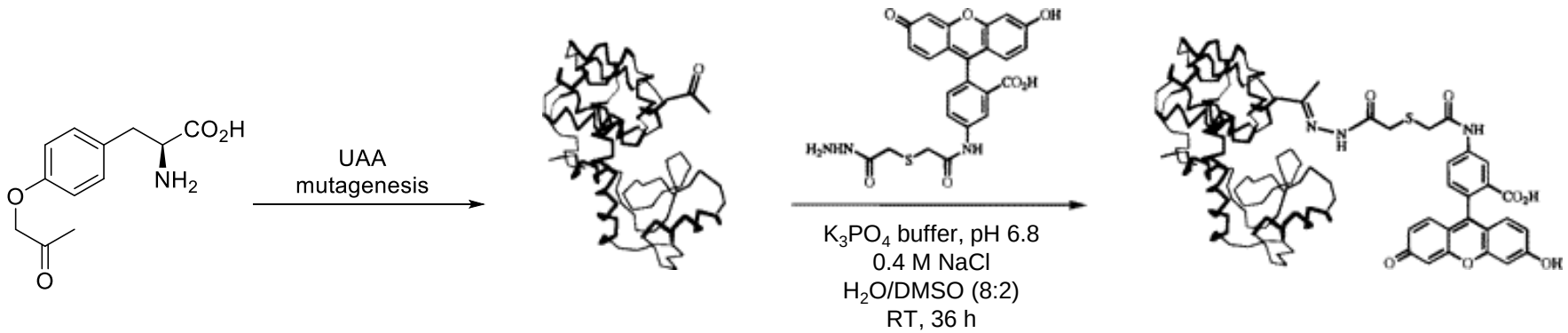
The development of bioorthogonal strategies



- *In vivo* self-assembly of cytotoxic hydrazones
  - Erythrocyte lysis after 80 min at 28 µM under physiological conditions
  - Human HeLa cells and *E. coli* J96

Rideout, D. *Science* **1986**, *223*, 561

The development of bioorthogonal strategies



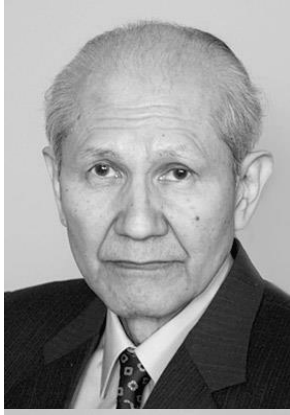
- Previously: Functionalisation of cysteine thiols
- Development of unnatural amino acid (UAA) mutagenesis (1989)
- Two-step strategies
  - *In vitro* incorporation of unnatural reporter groups
  - Posttranslational labelling *in vitro* with fluorescin hydrazide

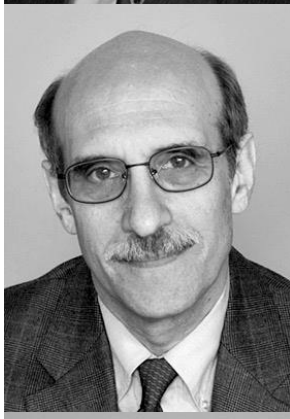
Noren, C. J. *et al. Science* **1989**, *244*, 4901
Cornish, V. W. *et al*. *J. Am. Chem. Soc*. **1996**, *118*, 8150

Green fluorescent protein (GFP)

- Isolated from jellyfish *Aequorea Victoria*
- Revolutionised cell biology from 1994 onwards
  - Visual tags – protein localisation
  - Biosensors
    - Report on i.e. gene expression or pH
  - Mutants of various colours

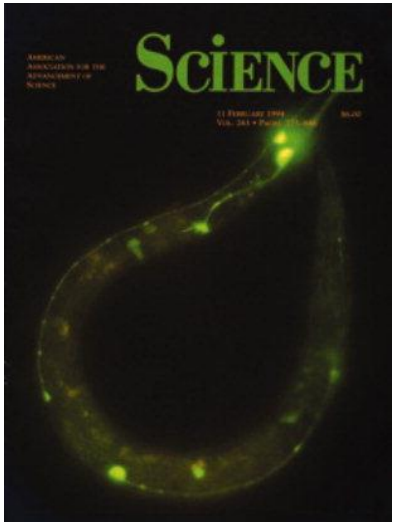
- The Nobel Prize in Chemistry, 2008
  - Prof. Em. Osamu Shimomura (Boston University)
  - Prof. Martin Chalfie (Columbia University)
  - Prof. Roger Y. Tsien (UC San Diego)



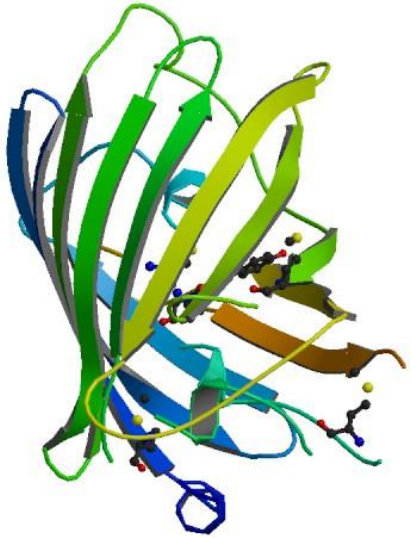


Chalfie, M. *et al*. *Science* **1994**, *263*, 802
Remington, S. J. *Protein Science* **2011**, *20*, 1509
«The Nobel Prize in Chemistry 2008». *Nobelprize.org*. Nobel Media AB 2013. Web: 6 Dec 2013. <http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2008/>

Green fluorescent protein (GFP)



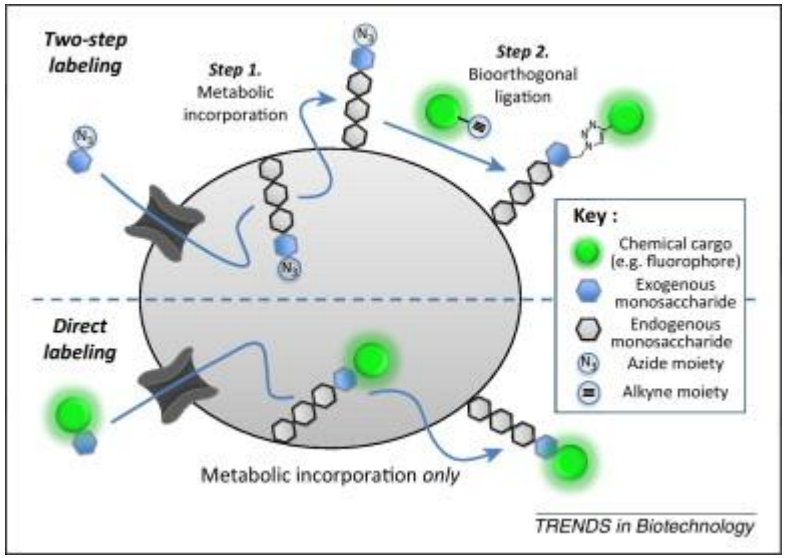
- Limitations
  - Only applicable to proteins
  - Large size, structural perturbation
  - Visualisation by optical methods only
- Bioorthogonal chemistry seeks to overcome the limitations
  - GFP is NOT a bioorthogonal reporter
  - Presently remains the method of choice



Prescher, J. A. and Bertozzi, C. R. *Nature Chem. Bio.* **2005**, *1*, 13
Chalfie, M. *et al*. *Science* **1994**, *263*, 802
PDB ID: 1EMA; Ormö, M. *et al. Science* **1996**, *273*, 1392
Remington, S. J. *Protein Science* **2011**, *20*, 1509

The advantages of bioorthogonal chemistry

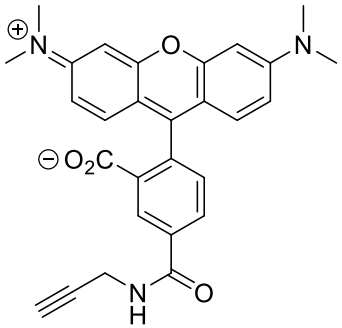
- Two-step strategy
  - Biomolecule functionalised by incorporation of *reporter group*
  - Selective tagging with exogenously delivered *label*
- Applicable to all biomolecules (in theory)
- Small size
  - Non-perturbing
  - Better access to intracellular and extravascular compartments
- Bioorthogonality = selectivity
  - Highly selective reactions
  - Low background – sensitive
  - Labelling *in vivo* – in whole organisms!



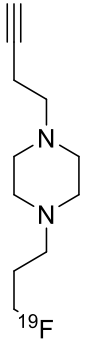
Prescher, J. A. and Bertozzi, C. R. *Nature Chem. Bio.* **2005**, *1*, 13
Gautam, S. *et al. Trends in Biotechnology* **2013**, *31*, 258

The advantages of bioorthogonal chemistry

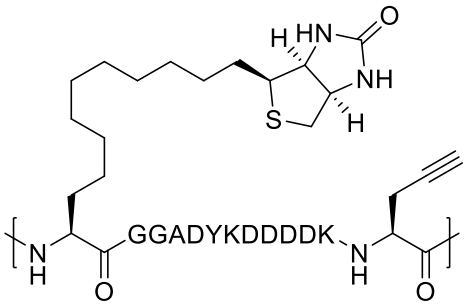
- Versatile and divergent
  - Two-step labelling enables various functionalisation of same reporter group



Fluorescent label



Radiolabel



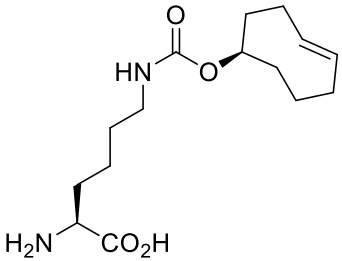
Biotin

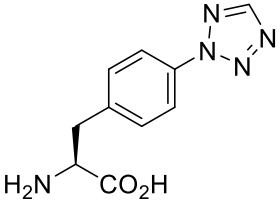
Prescher, J. A. and Bertozzi, C. R. *Nature Chem. Bio.* **2005**, *1*, 13; Krueger, T. G. and Imperiali, B. *ChemBioChem* **2013**, *14*, 788; Pretze, M. *et al. ChemMedChem* **2013**, *8*, 935

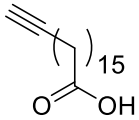
The requirements for bioorthogonal chemistry

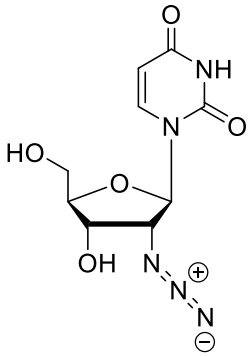
- Toxicity
  - Catalysts
  - Ligands
  - Byproducts
- Solubility
- Kinetics
  - Concentration
  - Bioavailability
  - Physiological conditions (pH, temperature)
- Selectivity
  - Chemoselectivity
    - Orthogonal to endogenous functionality
    - Mutual orthogonality
  - Regioselectivity

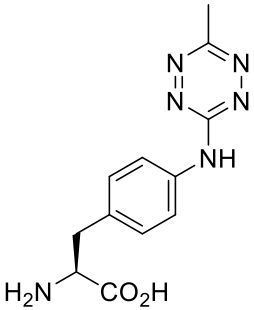
Frequently used reporter groups

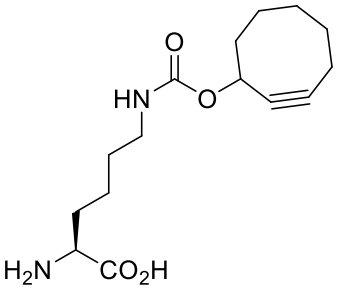


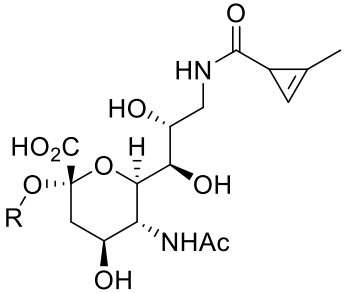






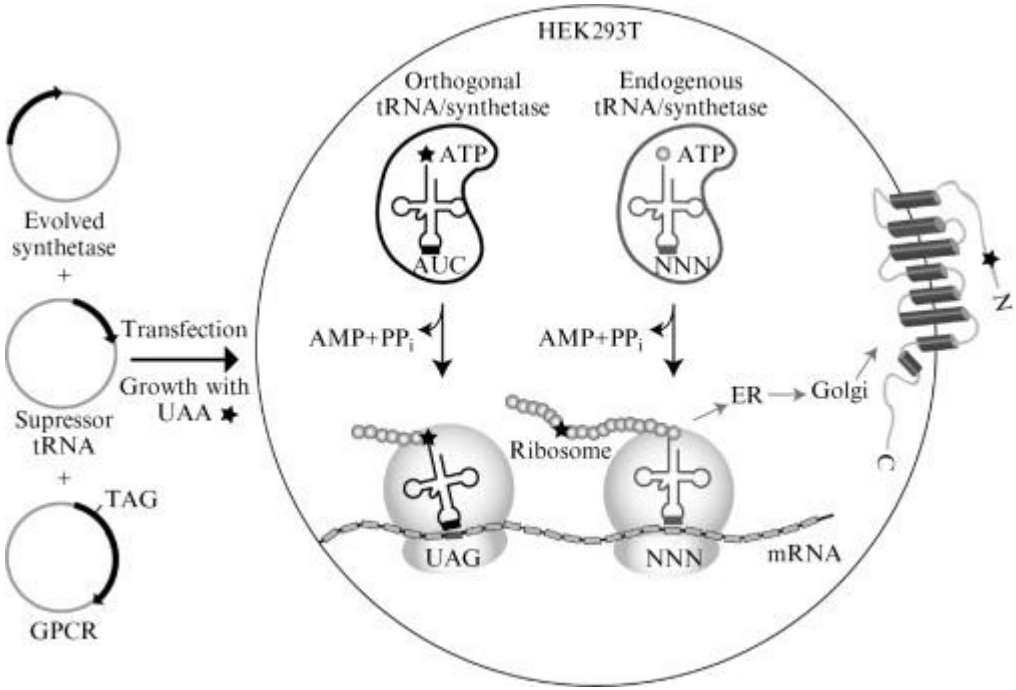






Seitchik, J. L. *et al. J. Am. Chem. Soc*. **2012**, *134*, 2898; Yount, J. S. *et al. Bioorg. Med. Chem.* **2012**, *20*, 650; Fauster, K. *et al. ACS Chem. Biol.* **2012**, *7*, 581; Lang, K. *et al. J. Am. Chem. Soc.* **2012**, *134*, 10317; Plass, T. *et al. Angew. Chem. Int. Ed.* **2012**, *51*, 4166; Wang, J. *et al. J. Am. Chem. Soc.* **2010**, *132*, 14812; Patterson, D. M. *et al. J. Am. Chem. Soc.* **2012**, *134*, 18638

How to install a reporter group



- Proteins
  - Unnatural amino acids (UAA)
  - Residue-specific manner
    - Incubation of auxotrophic strains with UAA
    - Multiple labelling
  - Site-specific manner
    - Expanding the genetic code
      - Engineering mutially selective tRNA and aminoacyl-tRNA synthethase
      - Transfection of encoding genes into cells
    - Using the cell's translational machinery to incorporate bioorthogonal reporter groups

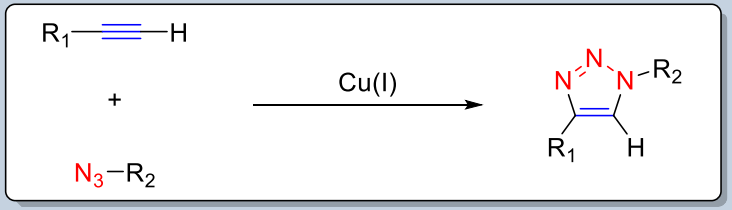
Huber, T. *et al. Methods in Enzymology*, **2013**, *520*, 281

How to install a reporter group

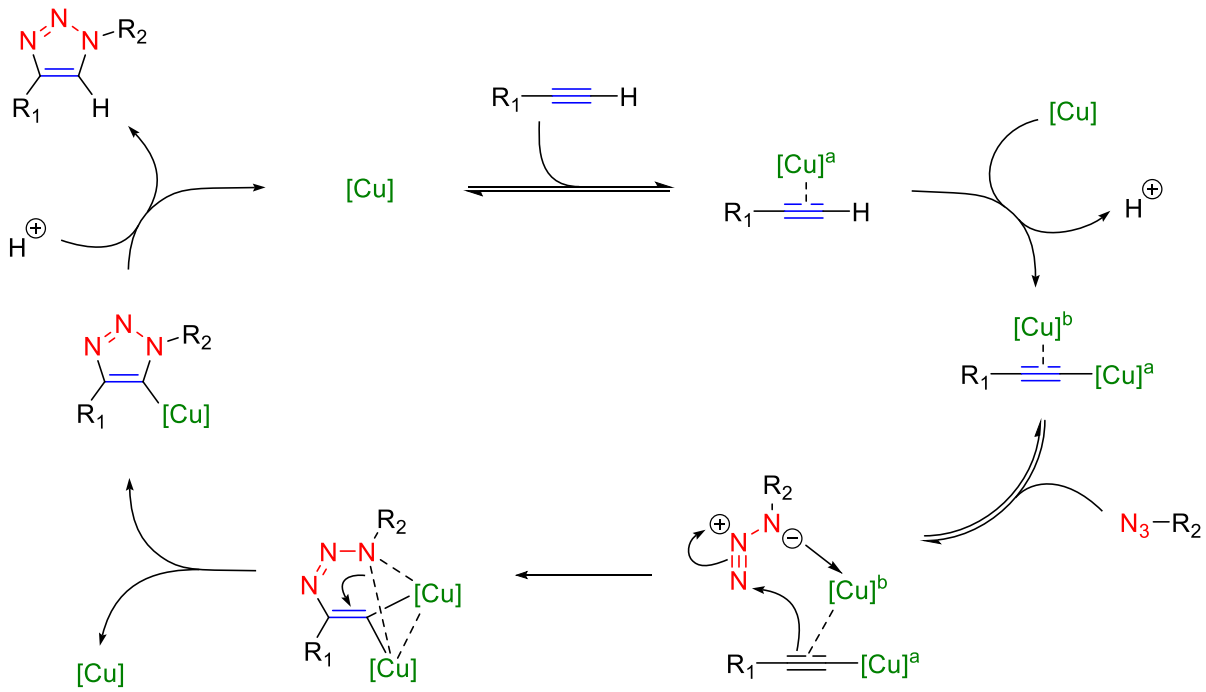
- Lipids, glycans and glycoconjugates
  - Metabolic incorporation (resembles residue-specific incorporation for proteins)
  - Feeding labelled metabolites through endogenous biosynthetic pathways
  - Exploit promiscuous enzymes, avoid the stringent ones

- Latent labelling
  - Reporter groups incompatible with cellular machinery
  - Extra level of modification – first install small, non-reporting chemical handle
  - Enzymatic modification to install the actual reporter group

The Cu Catalysed Alkyne-Azide Cycloaddition (CuAAC)

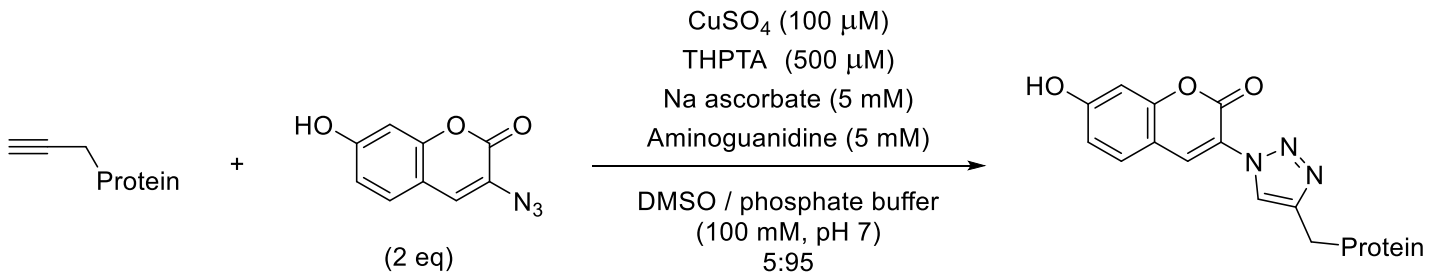


The mechanism of CuAAC

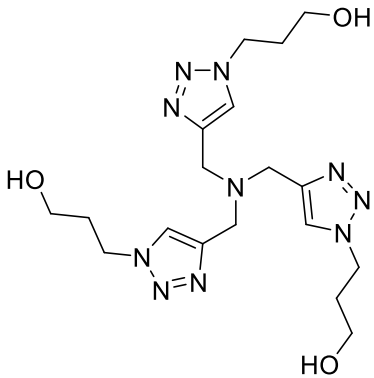


Worrell, B. T. *et al. Science* **2013**, *340*, 457

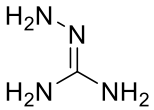
Optimised conditions for CuAAC



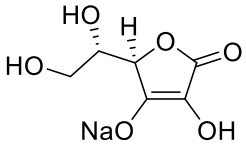
- Sodium ascorbate generates Cu(I) in situ
- 50 μM < [Cu] < 100 μM
  - Exception: Substrate contains Cu binding motifs (≤ 0.5 mM)
  - Other metals, e.g. Zn(II), compete with Cu binding
- ≥ 5 eq. tris(triazolylmethyl)amine ligand
- Aminoguanidine intercepts ascorbate oxidation byproducts
- DMSO solubilises hydrophobic domains
- Buffers: 6.5 ≤ pH ≤ 8.0
- Free thiols (glutathione) strong inhibitors



THPTA



Aminoguanidine

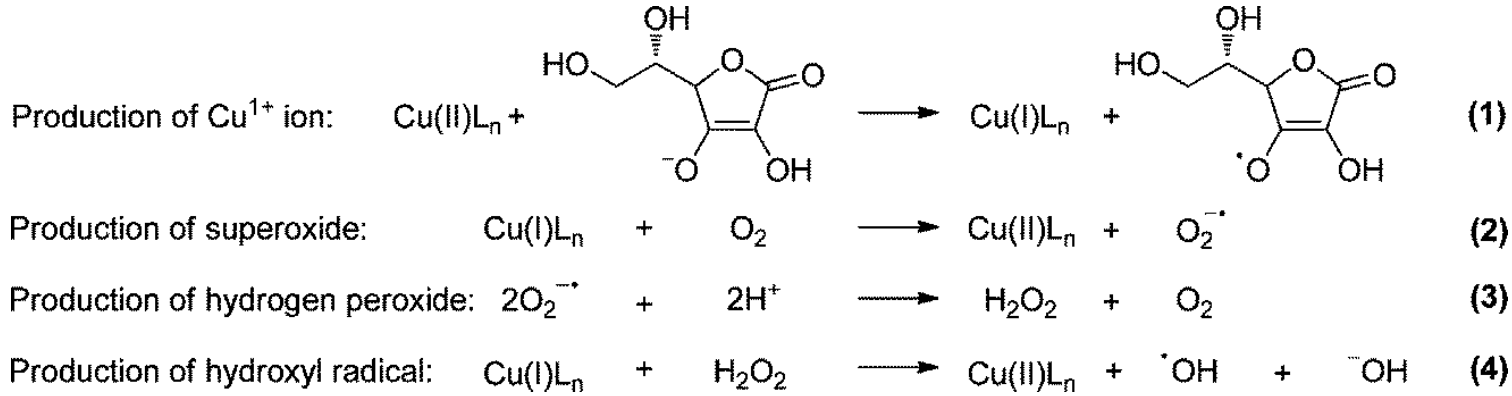


Sodium ascorbate

Hong, V. *et al. Angew. Chem. Int. Ed.* **2009**, *48*, 9879

The cytotoxicity of copper

- Thiolophilicity of Cu(I)
- Cu(II) and sodium ascorbate generate reactive oxygen species (ROS)

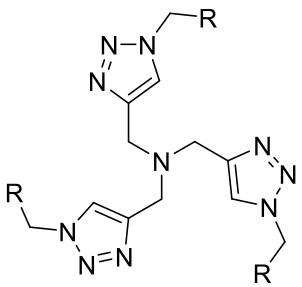


- Dependant on reduction potential of Cu-ligand complex
  - Cu complexes (porphyrins) can even protect against oxidative stress
  - Ligand tuning of toxicity and reactivity

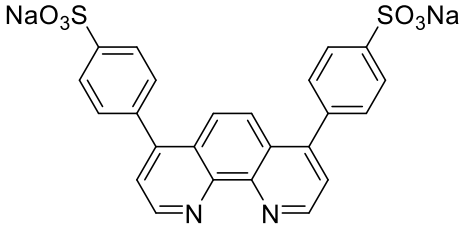
Kennedy, D. C. *et al. J. Am. Chem. Soc.* **2011**, *133*, 17993

Dealing with cytotoxicity

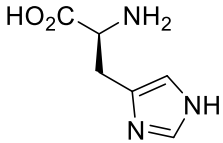
		IC_{50} values for MTT toxicity assays in human cells			
Ligand	Cu(II/I) reduction potential (mV) vs NHE	Huh7.5 cells	MDA-MB-468 cells	Hek293T cells	Hela cells
SO_4^{2-}	0	73.9 μM	124 μM	107 μM	50.5 μM
L-histidine	–120	>1000 μM	>1000 μM	>1000 μM	>1000 μM
EDTA	N/A	118 μM	192 μM	111 μM	116 μM
TBTA	260	16.3 μM	29.6 μM	13.5 μM	12.2 μM
BPS	140	0.74 μM	8.65 μM	33.0 μM	45.6 μM
THPTA	300	93.3 μM	22.4 μM	117 μM	183 μM



TBTA R = Ph
THPTA R = CH_2CH_2OH



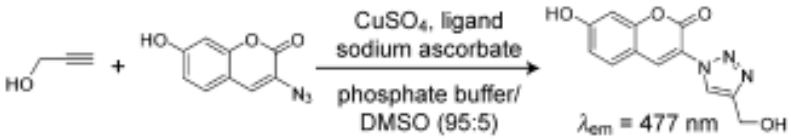
BPS

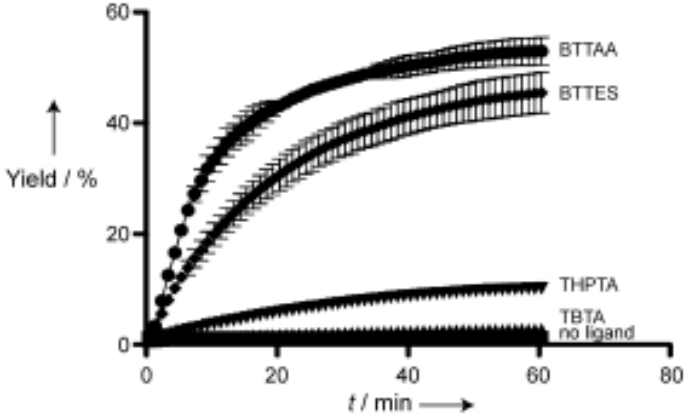


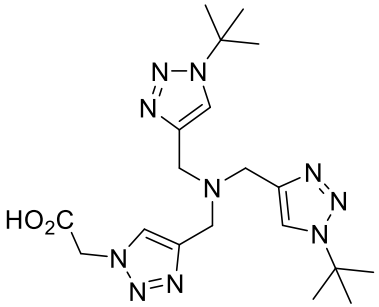
(L)-Histidine

Hong, V. *et al. Angew. Chem. Int. Ed.* **2009**, *48*, 9879
Kennedy, D. C. *et al. J. Am. Chem. Soc.* **2011**, *133*, 17993

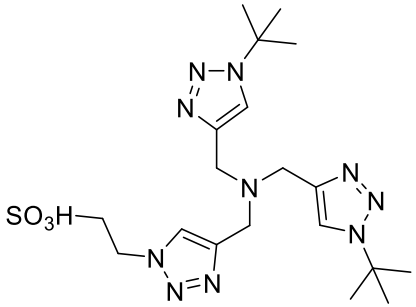
Ligand rate acceleration







BTTAA

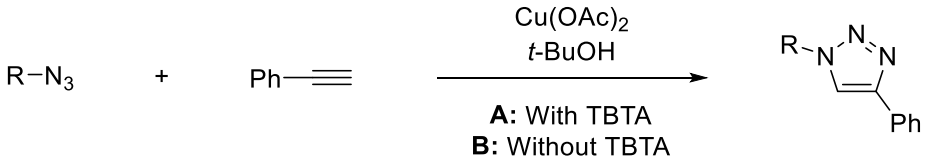


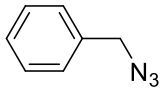
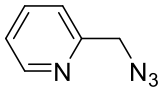
BTTES

Besanceney-Webler, C. *et al. Angew. Chem. Int. Ed.* **2011**, *50*, 8051

Chelation assisted CuAAC

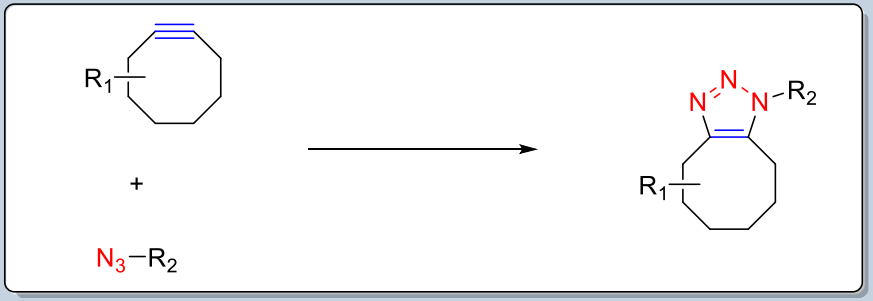
- Privileged substrates
  - 2-picolyl azides
  - 2-azidomethyl quinolines



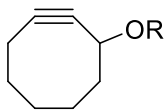
	Substrates	
Conditions		
A: With TBTA	35 min (100%)	< 5 min (100%)
B: Without TBTA	90 min (100%)	60 min (100%)

Brotherton, W. S. *et al. Org. Lett.* **2009**, *11*, 4954; Michaels, H. A. and Zhu, L. *Chem. Asian J.* **2011**, *6*, 2825; Kuang, G.-C. *et al. J. Am. Chem. Soc.* **2011**, *133*, 13984; Uttamapinant, C. *et al. Angew. Chem. Int. Ed.* **2012**, *51*, 5852

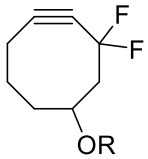
The Strain Promoted Alkyne-Azide Cycloaddition (SPAAC)



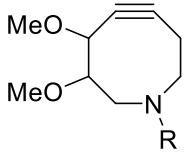
Improving the kinetics of SPAAC



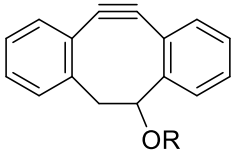
OCT
(2004)
k_2 = 0.0012 $M^{-1}s^{-1}$



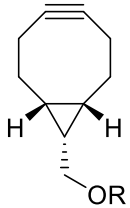
DIFO
(2008)
k_2 = 0.076 $M^{-1}s^{-1}$



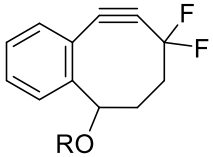
DIMAC
(2008)
k_2 = 0.0030 $M^{-1}s^{-1}$



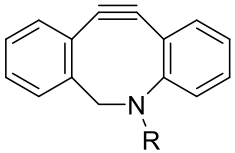
DIBO
(2008)
k_2 = 0.17 $M^{-1}s^{-1}$



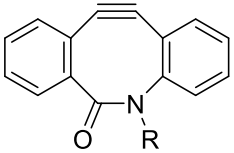
BCN
(2010)
k_2 = 0.14 $M^{-1}s^{-1}$



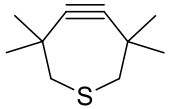
DIFBO
(2010)
k_2 = 0.22 $M^{-1}s^{-1}$



DIBAC
(2010)
k_2 = 0.31 $M^{-1}s^{-1}$



BARAC
(2010)
k_2 = 0.96 $M^{-1}s^{-1}$

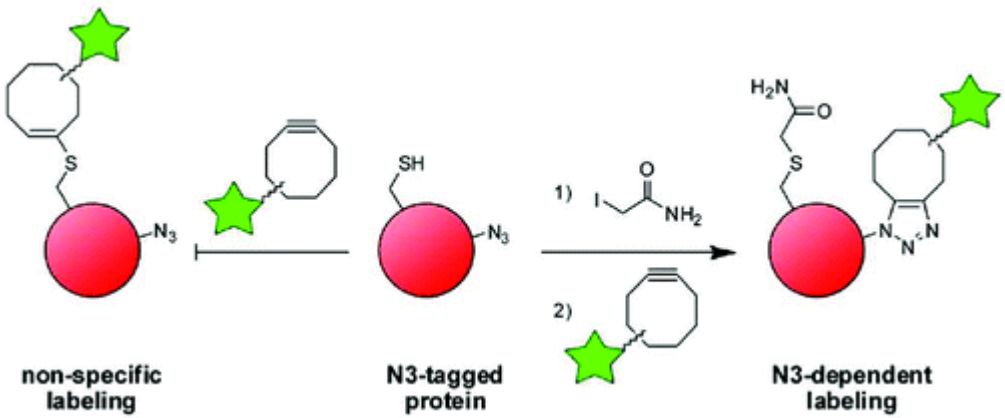


TMTH
(2012)
k_2 = 4.0 $M^{-1}s^{-1}$

Agard, N. J. *et al. J. Am. Chem. Soc.* **2004**, *126*, 15046; Codelli, J. A. *et al. J. Am. Chem. Soc.* **2008**, *130*, 11486; Ning, X. *et al. Angew. Chem. Int. Ed.* **2008**, *47*, 2253; Debets, M. F. *et al*, *Chem. Commun.* **2010**, *46*, 97; Dommerholt, J. *et al. Angew. Chem. Int. Ed.* **2010**, *49*, 9422; Sletten, E. M. *et al. Org. Lett.* **2008**, *10*, 3097; Jewett, J. C. *et al. J. Am. Chem. Soc.* **2010**, *132*, 3688; Sletten, E. M. *et al. J. Am. Chem. Soc.* **2010**, *132*, 11799; de Almeida, G. *et al. Angew. Chem. Int. Ed.* **2012**, *51*, 2443

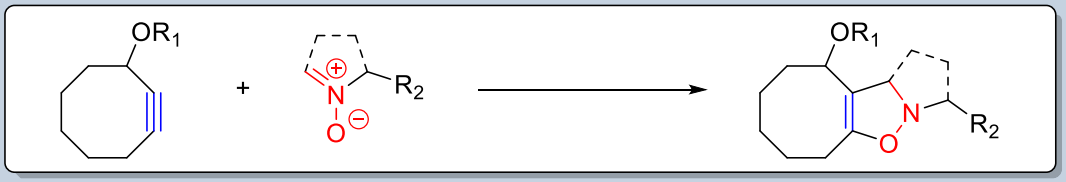
Warning: Cyclooctynes are not fully bioorthogonal!

- Free cysteine sidechains may undergo thiol-yne additions
- Background labelling reduces sensitivity
- Preincubation with iodoacetamide



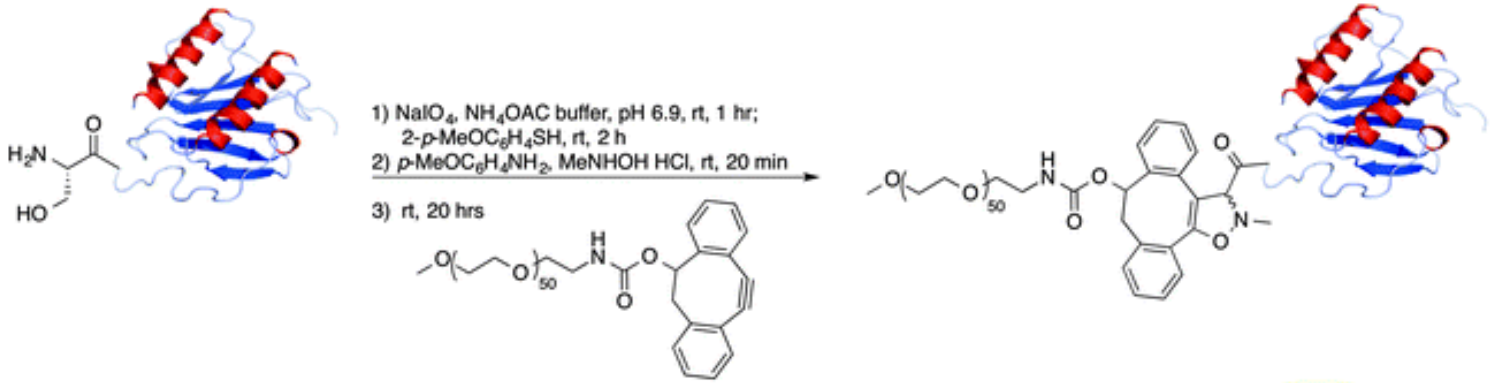
van Geel, R. *et al. Bioconjugate Chem.* **2012**, *23*, 392

Strain-Promoted Alkyne-Nitrone Cycloaddition (SPANC)



The SPANC reaction

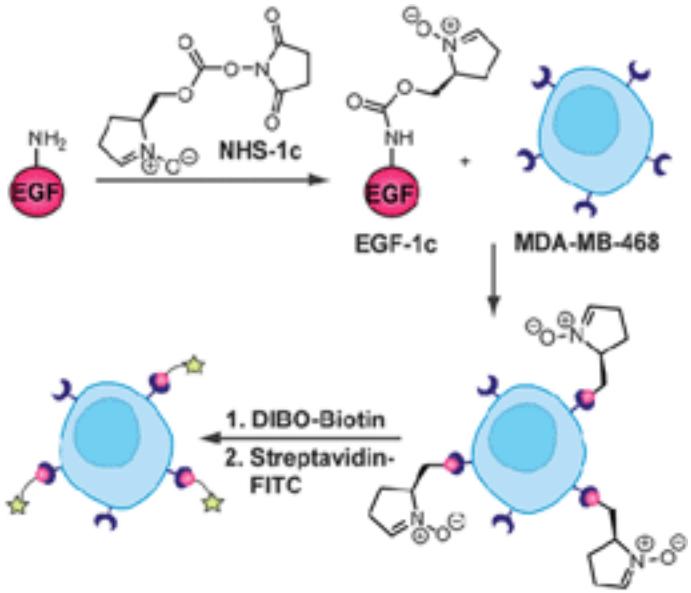
- Reported in 2010 by van Delft and co-workers
- 30 to 60 times faster than SPAAC
- *In situ* generated nitrone
- PEGylation of IL-8

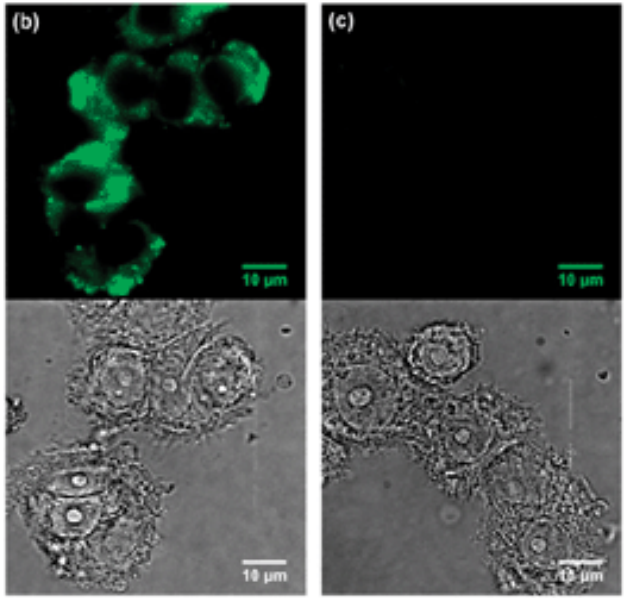


Ning, X. *et al. Angew. Chem. Int. Ed.* **2010**, *49*, 3065; Ramil, C. P. and Lin, Q. *Chem. Commun.* **2013**, *49*, 11007

SPANC with cyclic nitrones

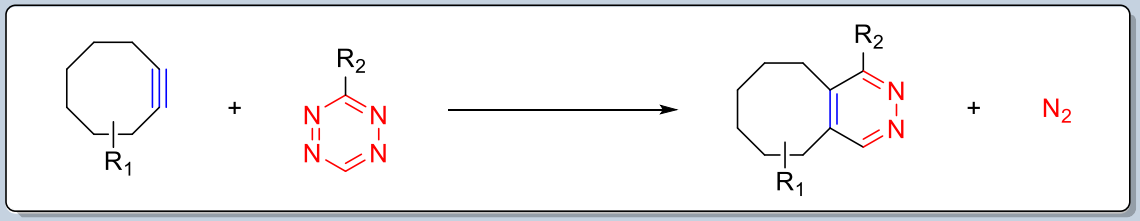
- Faster than non-cyclic
- Activity-based probing



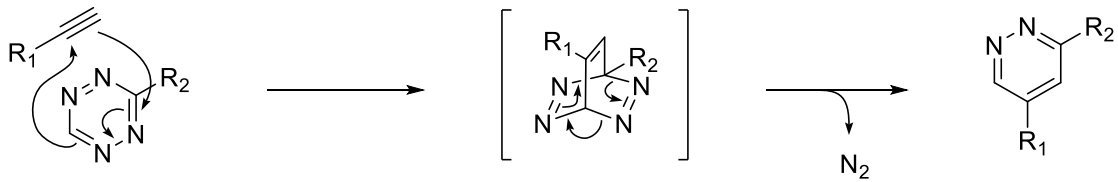


McKay, C. S. *et al. Chem. Commun.* **2010**, *46*, 931; McKay, C. S. *et al. Chem. Commun.* **2011**, *47*, 10040

Strain-Promoted Alkyne-Tetrazine Ligation (SPATL)



SPATL = Reverse electron demand DA

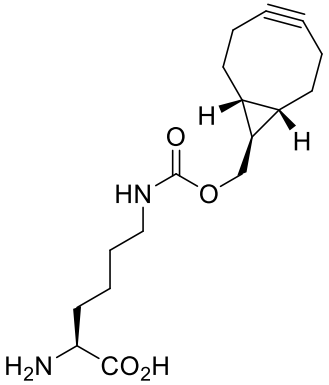


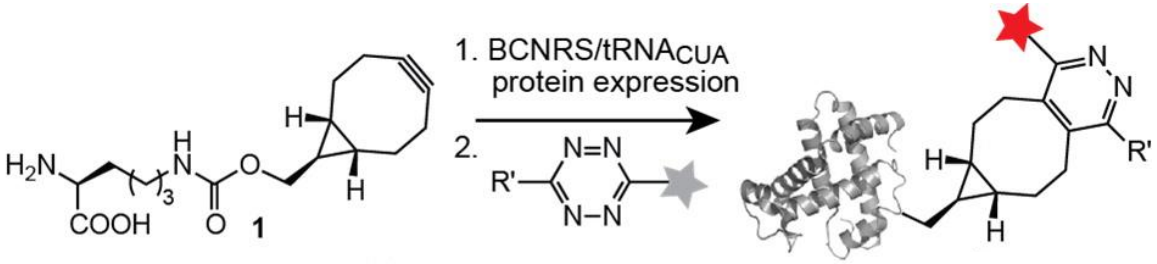
- Developed for strained *alkenes* in 2008
  - Extremely fast – $k_2 \approx 2000\ M^{-1}\ s^{-1}$
  - *trans*-Cyclooctene
  - Norbornene
  - Cyclopropene

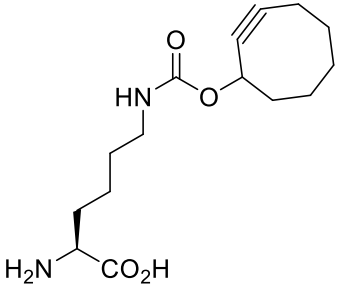
Blackman, M. L. *et al. J. Am. Chem. Soc.* **2008**, *130*, 13518
Devaraj, N. K. *et al. Bioconjugate Chem.* **2008**, *19*, 2297

Unnatural amino acids as reporter groups

- Few examples with alkynes
- Genetically encoded unnatural amino acid reporter groups
- BCN-amino acid: Single regioisomer!
  - FRET experiments
- «Turn-on» fluorescence effect

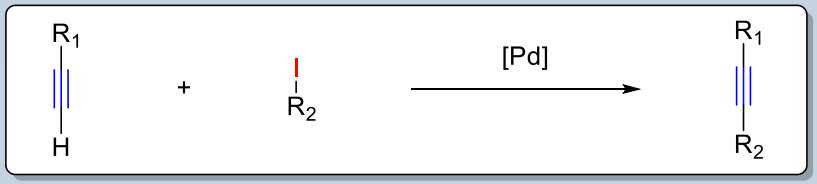






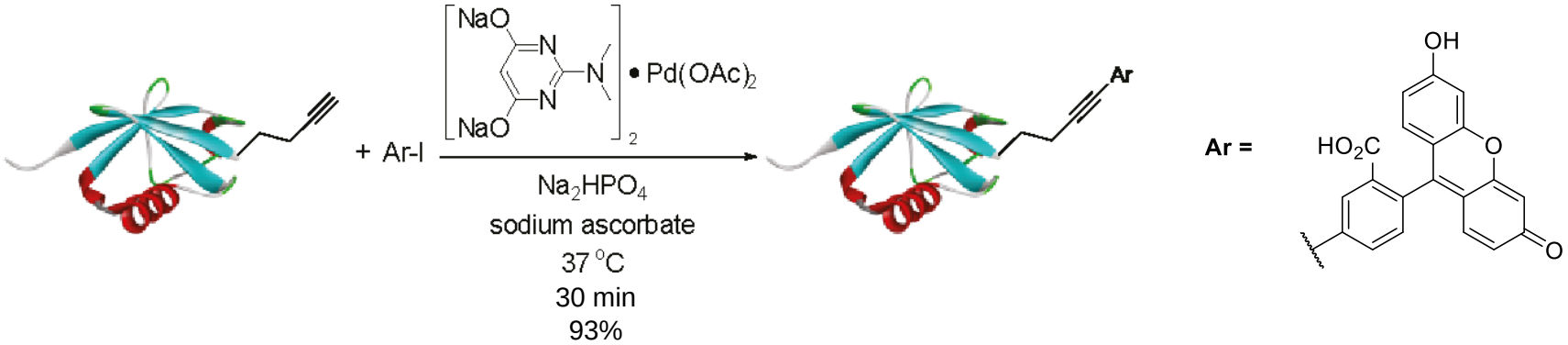
Lang, K. *et al. J. Am. Chem. Soc.* **2012**, *134*, 10317
Borrmann, A. *et al. ChemBioChem* **2012**, *13*, 2094
Plass, T. *et al. Angew. Chem. Int. Ed.* **2012**, *51*, 4166

The Sonogashira Cross-Coupling Reaction



Is bioorthogonal Sonogashira possible?

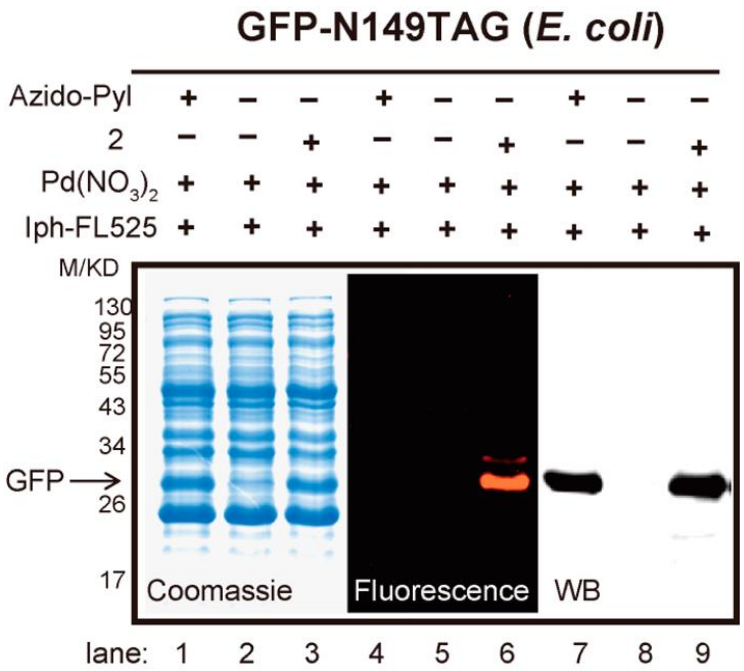
- Cytotoxicity of copper
- Water soluble Pd complex (50 eq)
- 50 eq of fluorescin iodide
  - µM concentrations
- *In vitro* and *in vivo* (*E. coli* cell surface proteins)

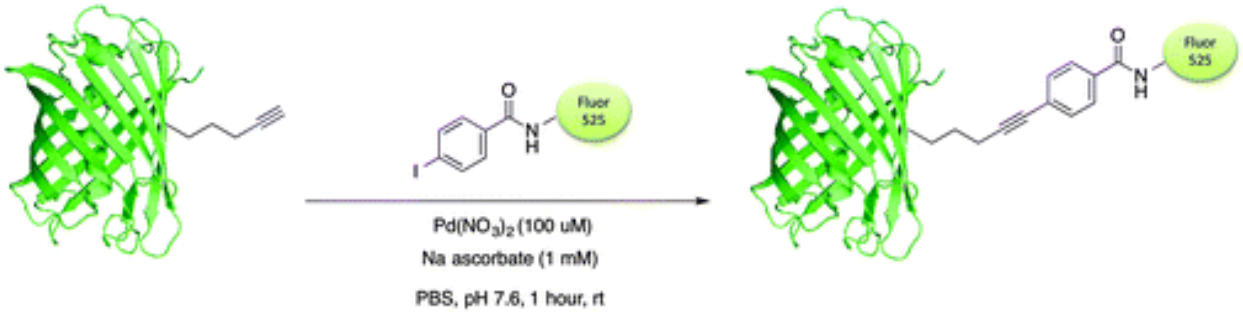


Li, N. *et al. J. Am. Chem. Soc.* **2011**, *133*, 15316

Ligand free Sonogashira

- Bioavailable – inside Gram-negative bacteria
- No apparent Pd cytotoxicity
- Lower concentrations of Pd (10 eq)



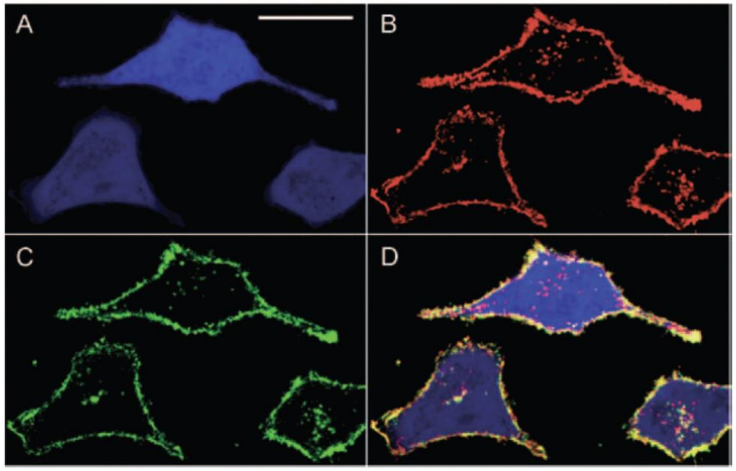


Li. J. *et al. J. Am. Chem. Soc.* **2013**, *135*, 7330; Ramil, C. P. and Lin, Q. *Chem. Commun.* **2013**, *49*, 11007

Mutual Orthogonality of Bioorthogonal Transformations

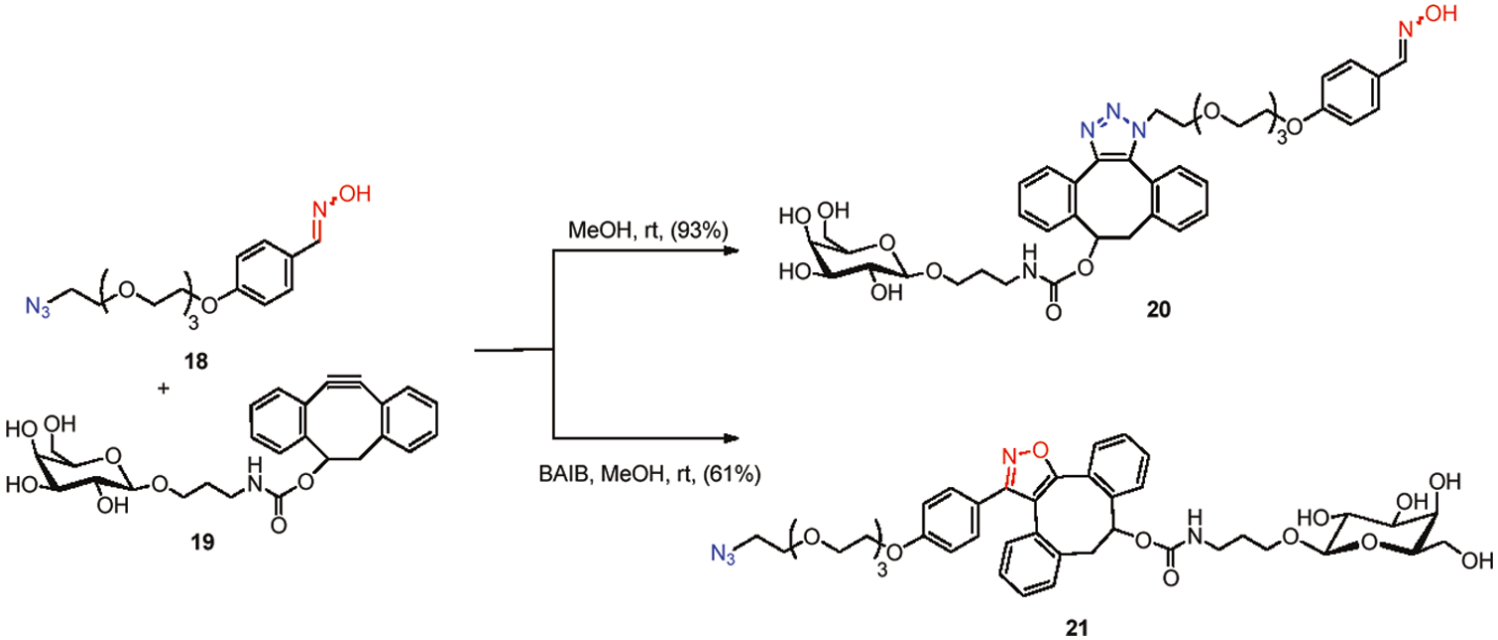
What is the point?

- Two- or threefold labelling
  - Different probes
  - Selectively
- Spatially or temporally separated labels
  - Transmembrane proteins
  - Protein kinetics and dynamics
- More data from a single experiment



Carroll, L. *et al. Org. Biomol. Chem.* **2013**, *11*, 5772

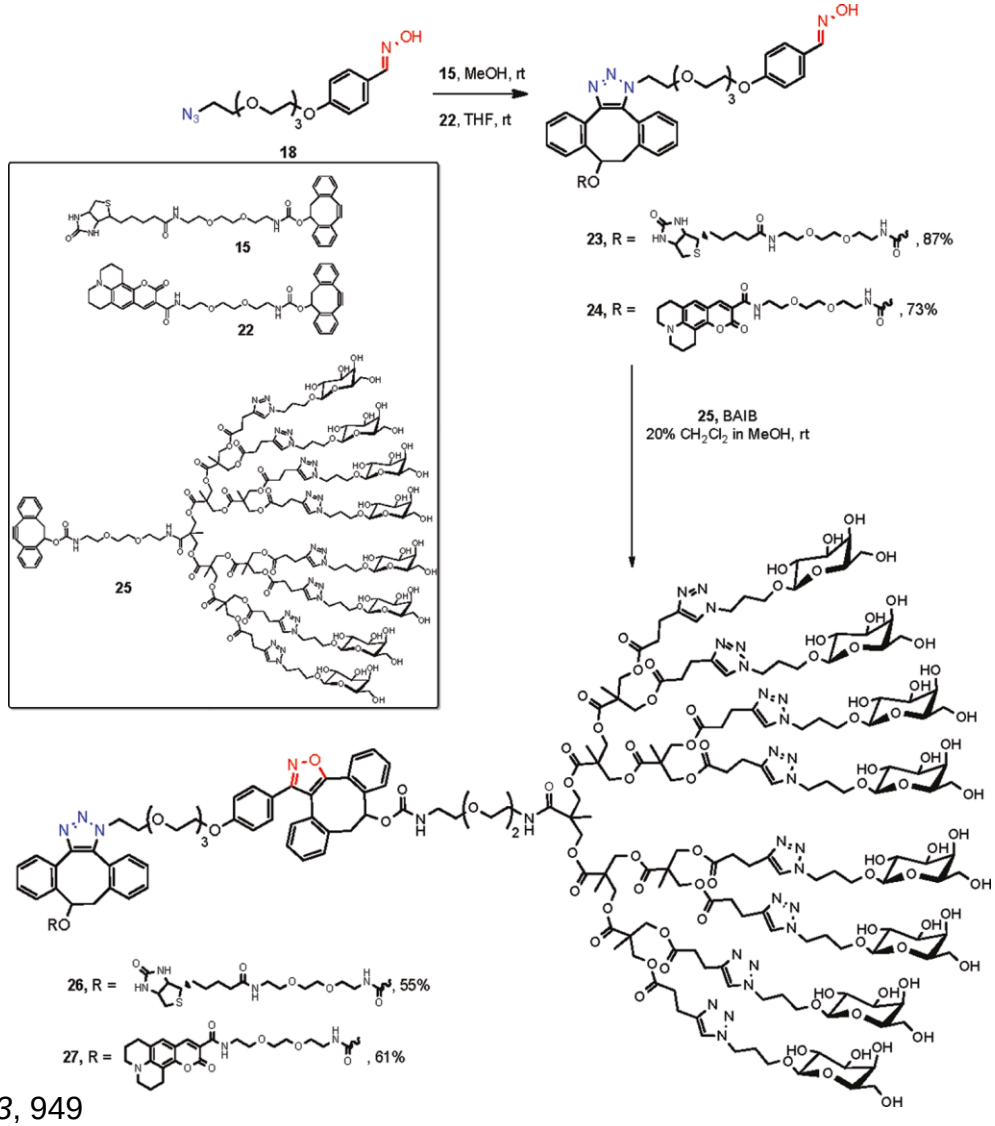
SPAAC vs SPANOC



Sanders, B. C. *et al. J. Am. Chem. Soc.* **2011**, *133*, 949

SPAAC vs SPANOC

- Latent labelling



Sanders, B. C. *et al. J. Am. Chem. Soc.* **2011**, *133*, 949

Summary