Bioorthogonal Chemistry: Enabling nCAA Protein Labelling

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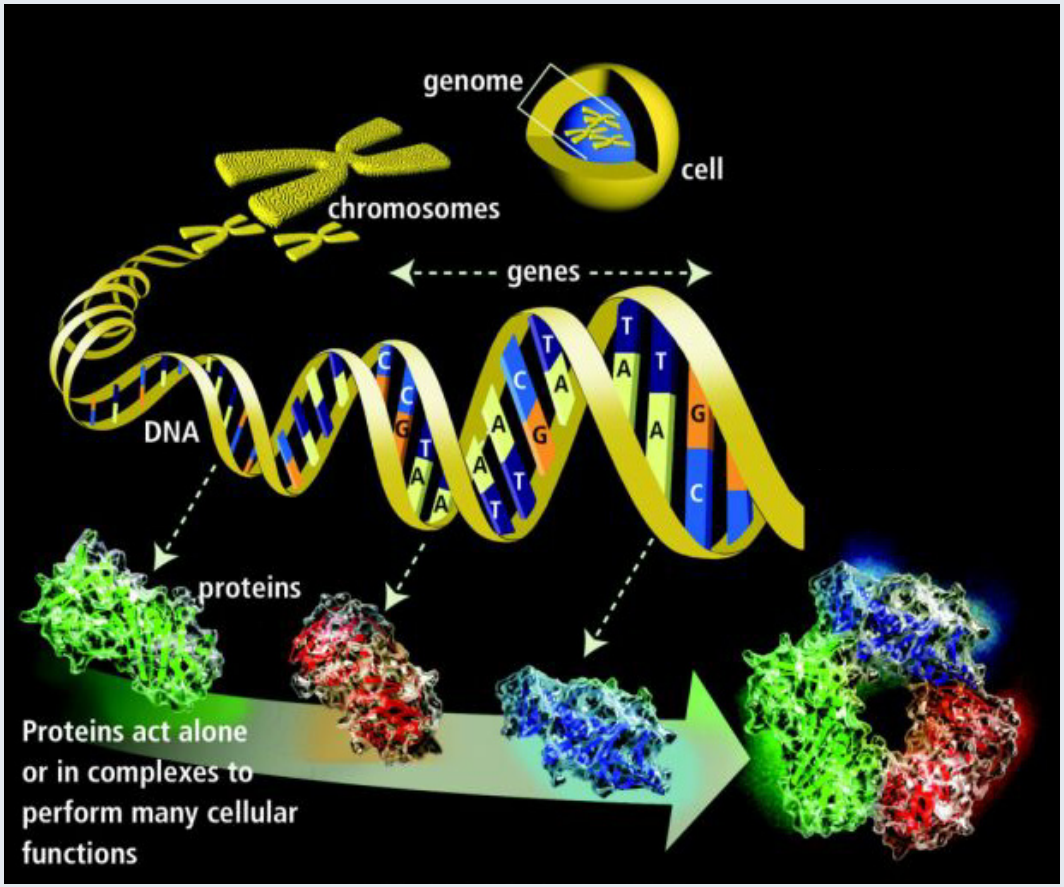
A. Manos-Turvey,

Wipf Group: Frontiers of Chemistry

Jan. 17th, 2015

From the Genome to Proteins

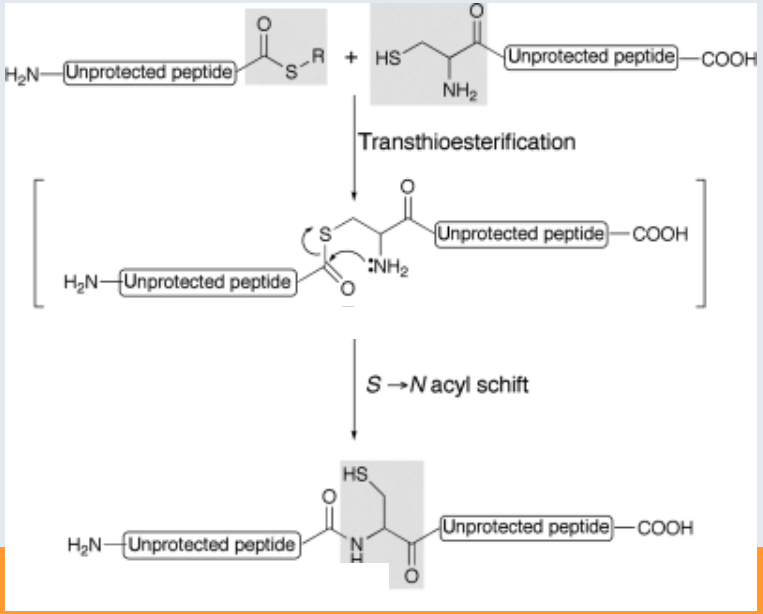
- **Pre-genomic era:** ended with the completion of the human genome project in 2003
- **Genomic era:** resulted in the rapid collection of immense databases of genomic sequences
- **Post-genomic era:** decipher the structural and functional information about biomolecules encoded by the genomic data
  - 3-4 D structure
  - Dynamics
  - Function

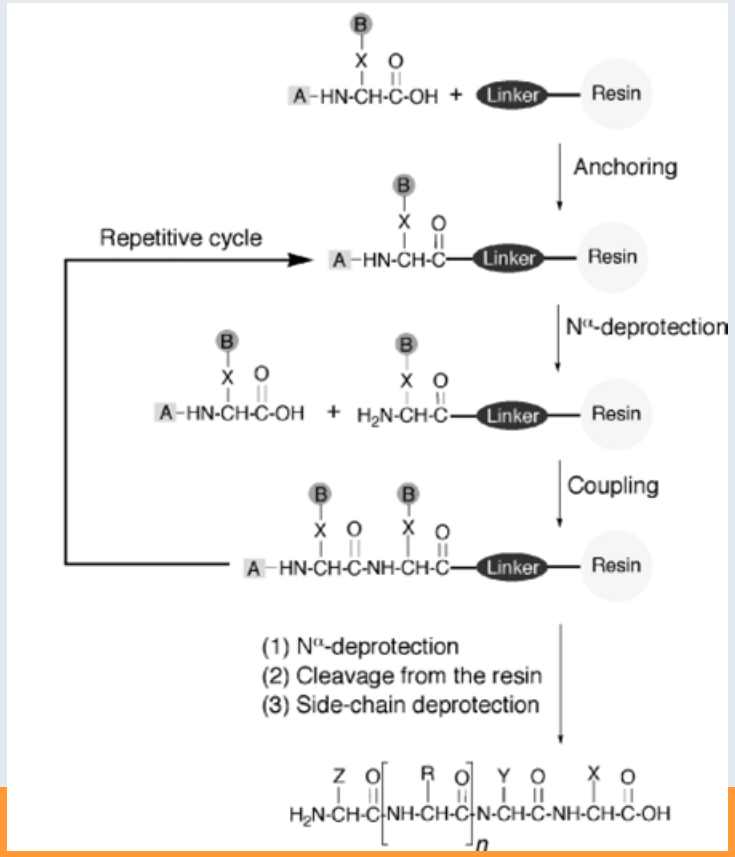


International Human Genome Sequencing Consortium, *Nature*, **2004**, *431*, 931-945
Image: http://www.howscienceismade.com/2013/06/writing-gene-nomenclature.html

Understanding Biology through Chemistry

- The ability to incorporate non-canonical amino acids (nCAAs) into proteins, allowing chemical probes to be synthesised, is aiding this search
- Methods of nCAA incorporation in proteins are well documented
  - Solid phase synthesis
  - Native chemical ligation
  - *In vitro* translation

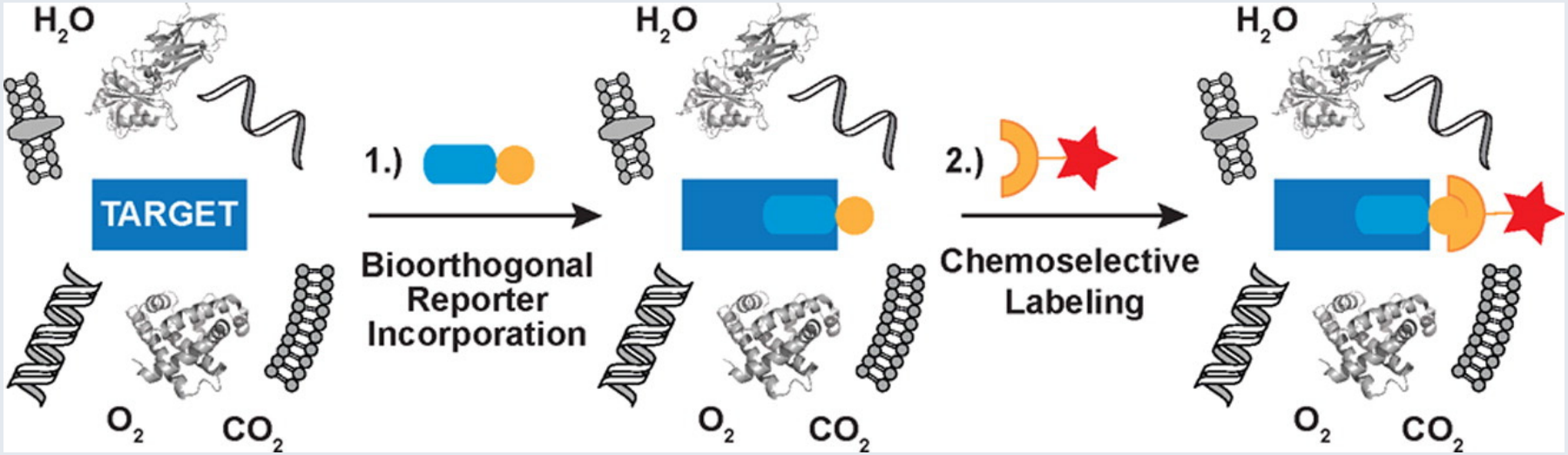




T. Kimmerlin, D. Seebach, *J. Peptide Res.*, **2005**, *65*, 229-260

Understanding Biology through Chemistry

- Proteins within the cells of interest require “bioorthogonal chemistry” to allow for labelling in the cellular environment.



K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Bioorthogonal Chemistry

- To be labelled a bioorthogonal reaction, a reaction must meet several criteria – many only meet some of these prescriptions
  - Incorporated functionality + probe react selectively with one another
  - Reactions yield stable covalent linkages with no by-products
  - Reactants must be kinetically, thermodynamically and metabolically stable, and non-toxic, prior to reaction
- Many chemoselective reactions have been found, but few are truly bioorthogonal
  - Many remain restricted to cell surface labelling or *in vitro* systems
  - Kinetics of the reaction are very important

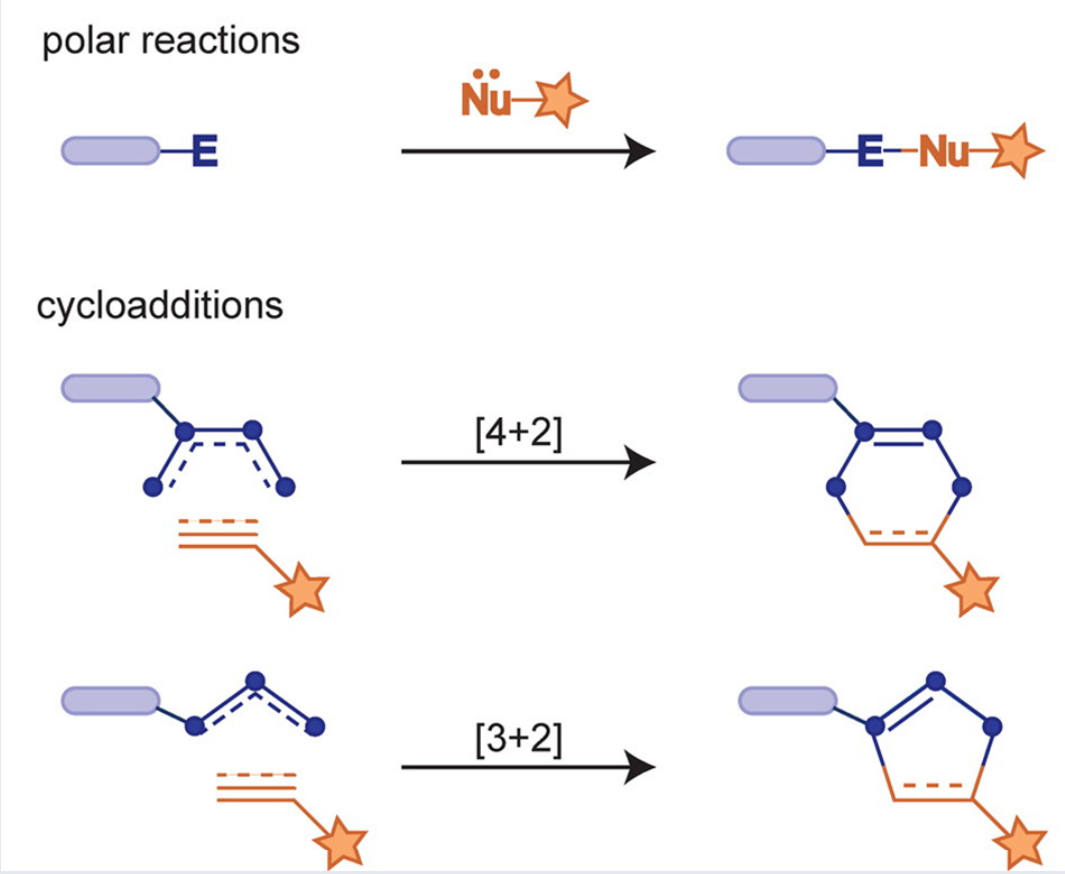
E.M. Sletten, C.R. Bertozzi, *Angew. Chem. Int. Ed.*, **2009**, *48*, 6974-6998
R.K. Lim, Q. Lin, *Chem. Commun.*, **2010**, *46*, 1589-1600

Bioorthogonal Chemistry Kinetics

- Biological processes are very rapid, bioorthogonal reactions need to be able to compete with these
- Bioorthogonal reactions often follow second-order kinetics and the rate of reaction is dependant upon:
  - [biomolecule]
  - [labelling reagent]
  - Second-order rate constant
- Difficulties:
  - Target biomolecules are usually in low abundance in their native environment
  - Higher conc. of labelling reagents can increase insolubility/chances of off-target effects
  - Need reactions which have higher intrinsic rate constants
- Faster chemoselective reactions are needed to increase utility of labelling

Bioorthogonal Chemical Reactions

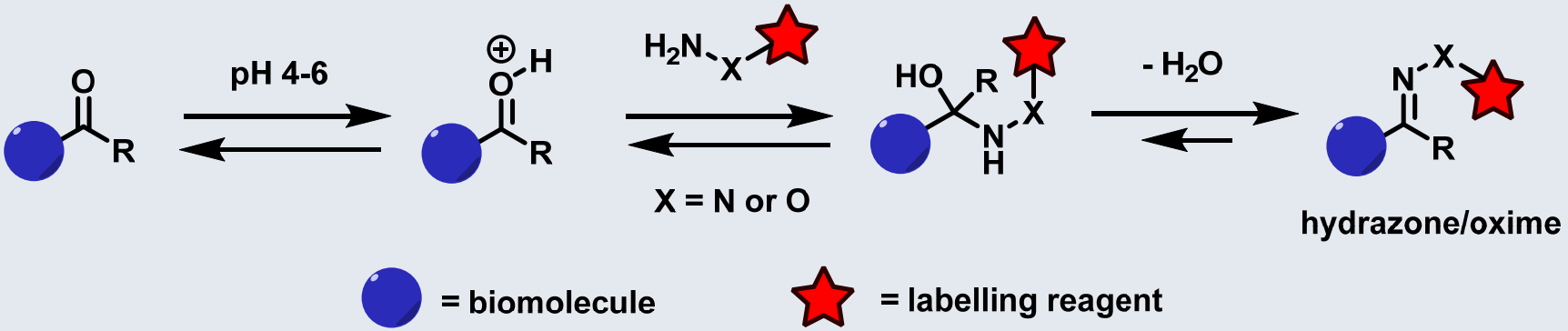
- The predominant transformations in the “bioorthogonal toolkit” are:



D.M. Patterson, L.A. Nazarova, J.A. Prescher, *ACS. Chem. Biol.*, **2014**, *9*, 592-605

Ketone/Aldehyde-Hydrazine/Alkoxylamine Reactions

- Aldehyde and ketone reactions were amongst the first bioorthogonal reactions to be identified
  - Ketones are preferable due to lower general activity under physiological conditions
- Under acidic conditions the carbonyl group is protonated and reacts with amines to form reversible Schiff base

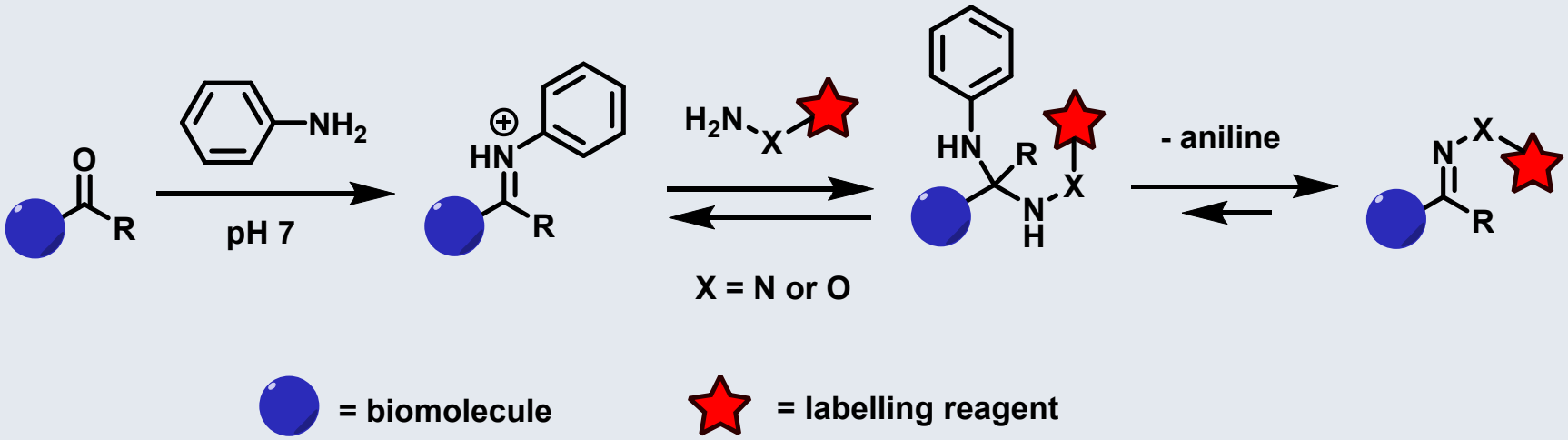


- Difficulties:
  - Commonly requires acidic pH
  - Slow kinetics (10^{-4}-10^{-3} $M^{-1}s^{-1}$)
  - Requires high [reagent] (mM)
  - Competition from naturally occurring aldehyde/ketone metabolites

K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Ketone/Aldehyde-Hydrazine/Alkoxylamine Reactions

Aniline activation:

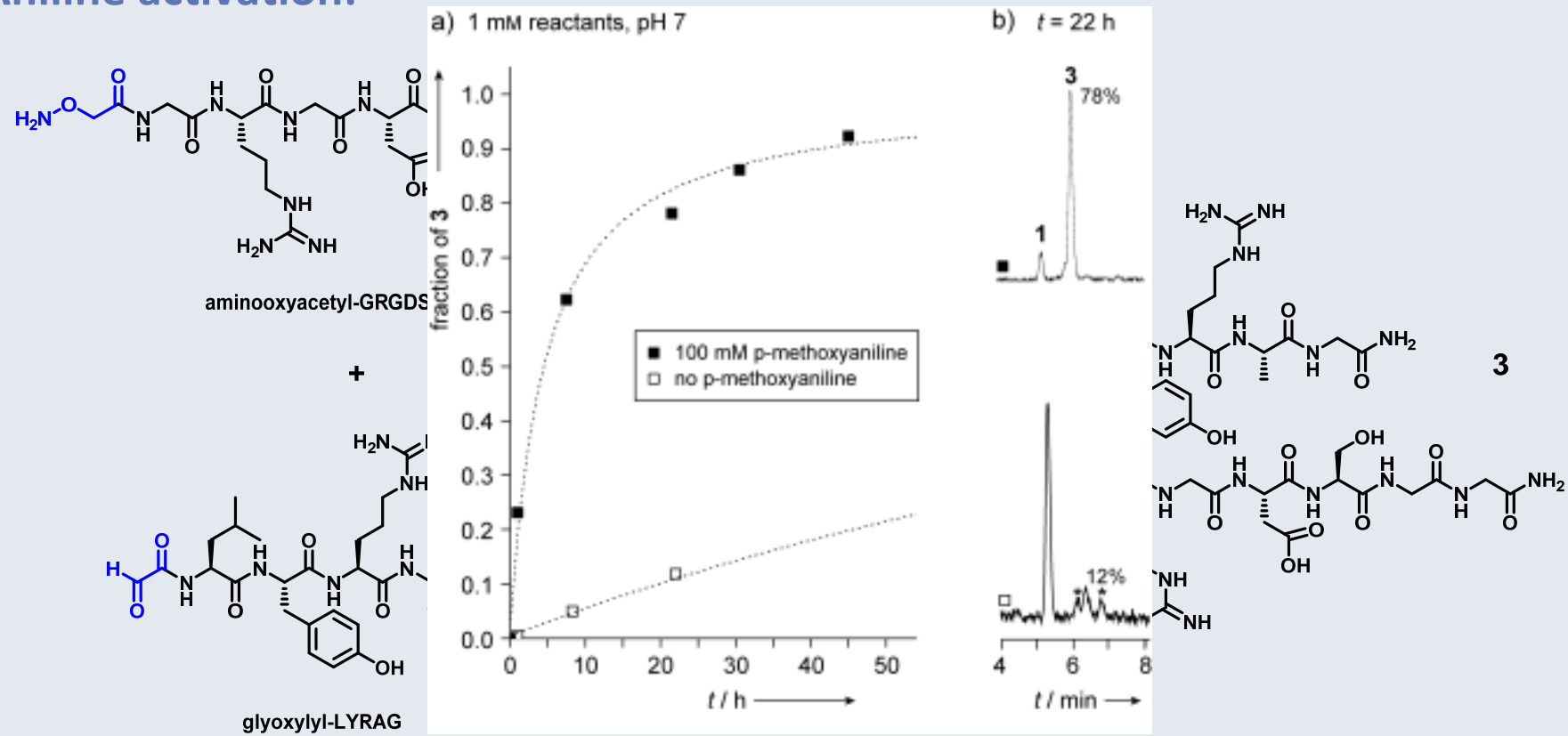


- Can be run at pH 7
- Accelerates the reaction > 40 fold (> 400 at pH 4.5)
- Formation of the aniline schiff base becomes the rate determining step
- Live cell surface labelling has proven successful

A. Dirksen, T.M. Hackeng, P.E. Dawson, *Angew. Chem. Int. Ed.*, **2006**, *45*, 7581-7584
J. Rayo, N. Amara, P. Krief, M.M. Meijler, *J. Am. Chem. Soc.*, **2011**, *133*, 7469-7475

Ketone/Aldehyde-Hydrazine/Alkoxylamine Reactions

Aniline activation:

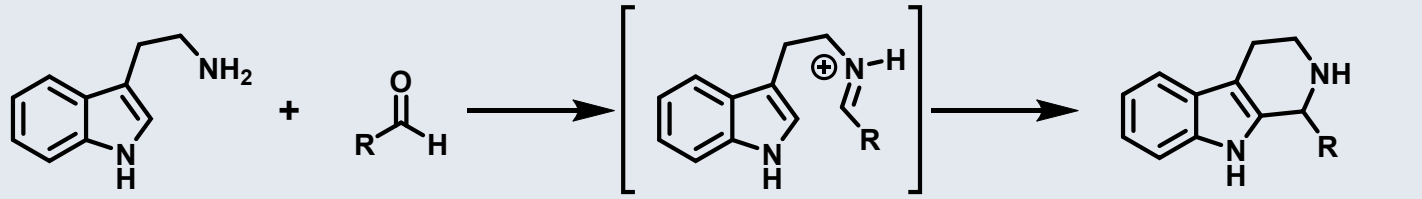


- Hydrazones and oximes can be hydrolytically cleaved over extended periods of time

A. Dirksen, T.M. Hackeng, P.E. Dawson, *Angew. Chem. Int. Ed.*, **2006**, *45*, 7581-7584

Ketone/Aldehyde-Hydrazine/Alkoxylamine Reactions

Pictet-Spengler Reaction



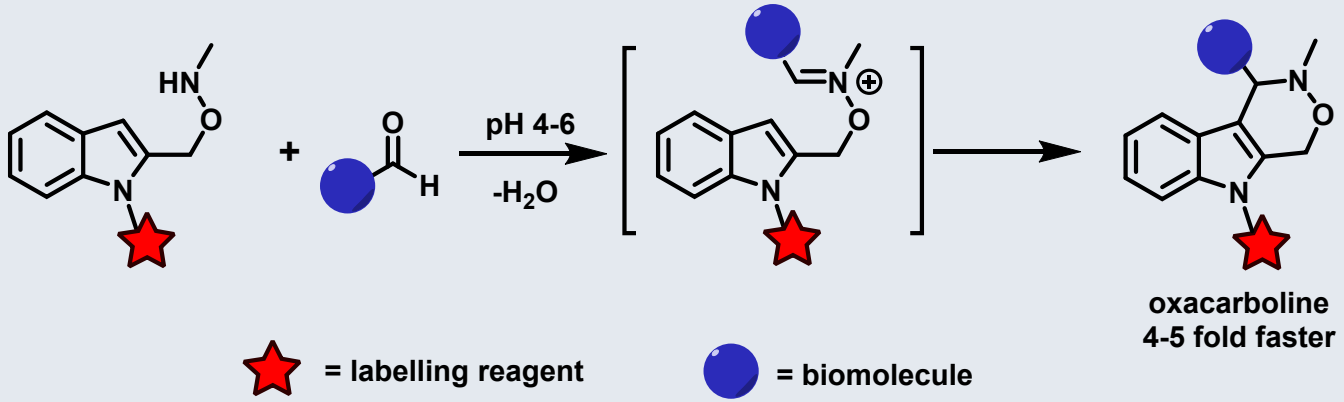
Tryptamine

tetrahydrocarboline

k_2 ~10^{-4} $M^{-1}s^{-1}$ at pH 4-5

Pictet-Spengler Ligation

- Accelerates the reaction in aqueous media



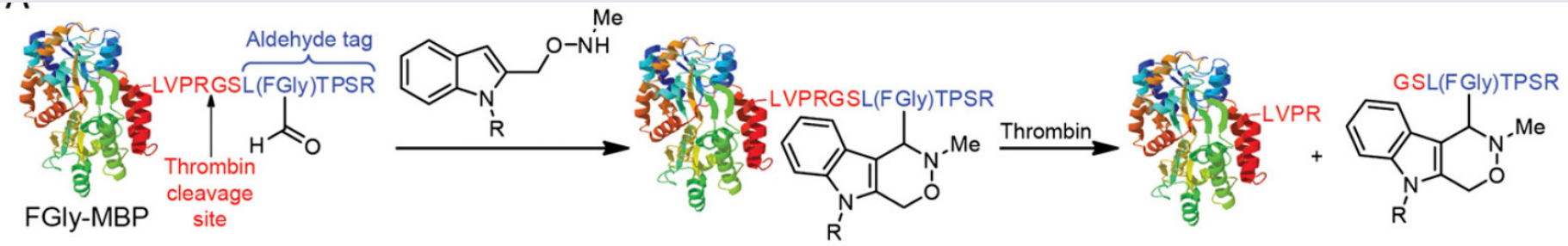
oxacarboline
4-5 fold faster

= labelling reagent

= biomolecule

P. Agarwal, J. van der Weijden, E.M. Sletten, D. Rabuka, C.R. Bertozzi, *PNAS*, **2013**, *110*, 46-51

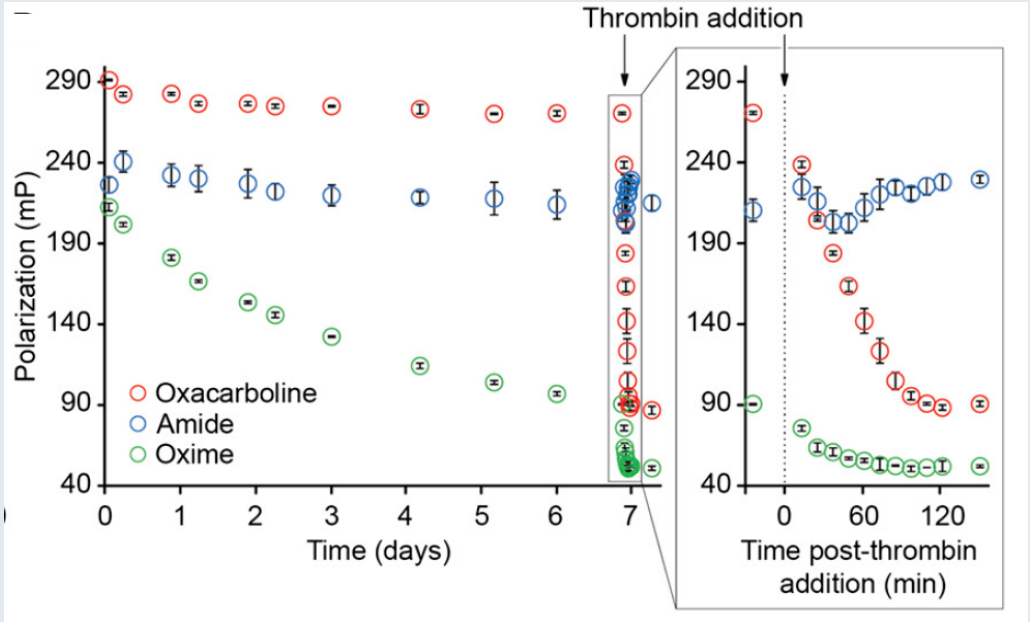
Ketone/Aldehyde-Hydrazine/Alkoxylamine Reactions



Pictet-Spengler Ligation

- Successfully used to label proteins and antibodies
- Shows heightened stability to its oxime counterparts

- Still requires acidic conditions
- Mainly *in vitro* utility

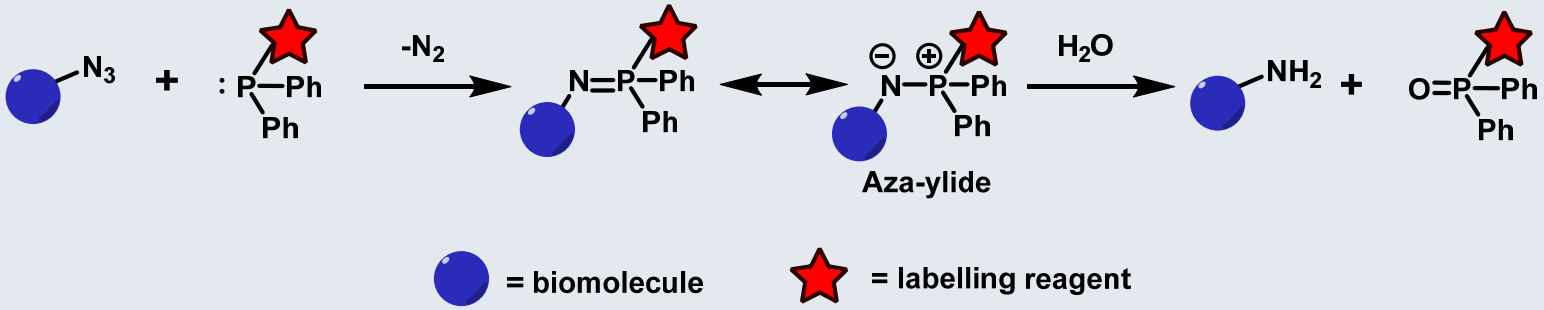


P. Agarwal, J. van der Weijden, E.M. Sletten, D. Rabuka, C.R. Bertozzi, *PNAS*, **2013**, *110*, 46-51

Azide-Phosphine (Staudinger) Reactions

- Azide groups are absent from biological systems
  - Small in size
  - Stable under physiological conditions
  - React with bioorthogonal triaryl phosphines

Staudinger Reduction



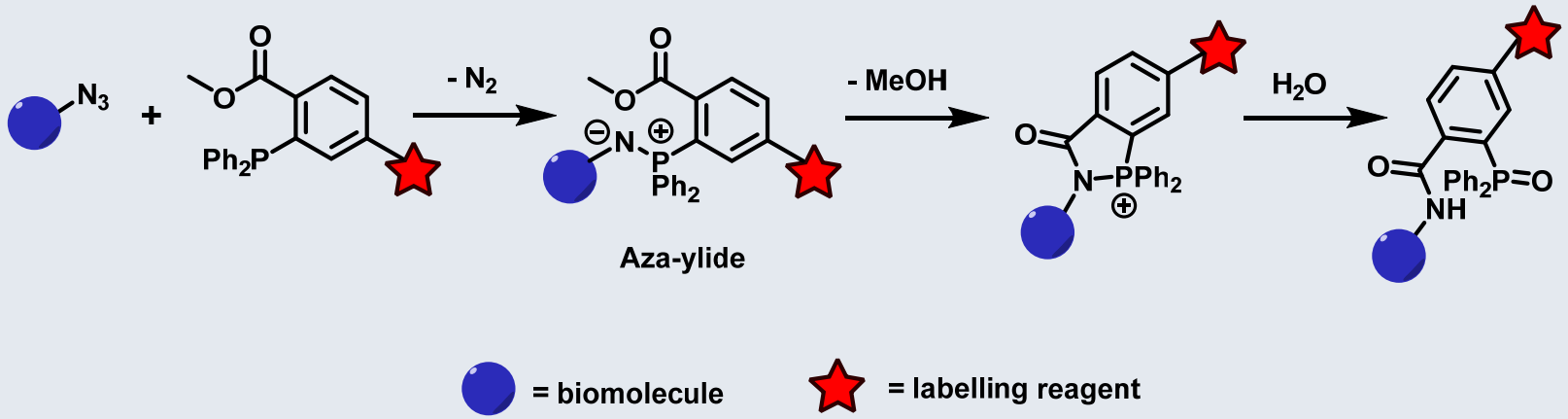
- Need to trap the reaction at the aza-ylide intermediate

K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Azide-Phosphine (Staudinger) Reactions

Staudinger Ligation

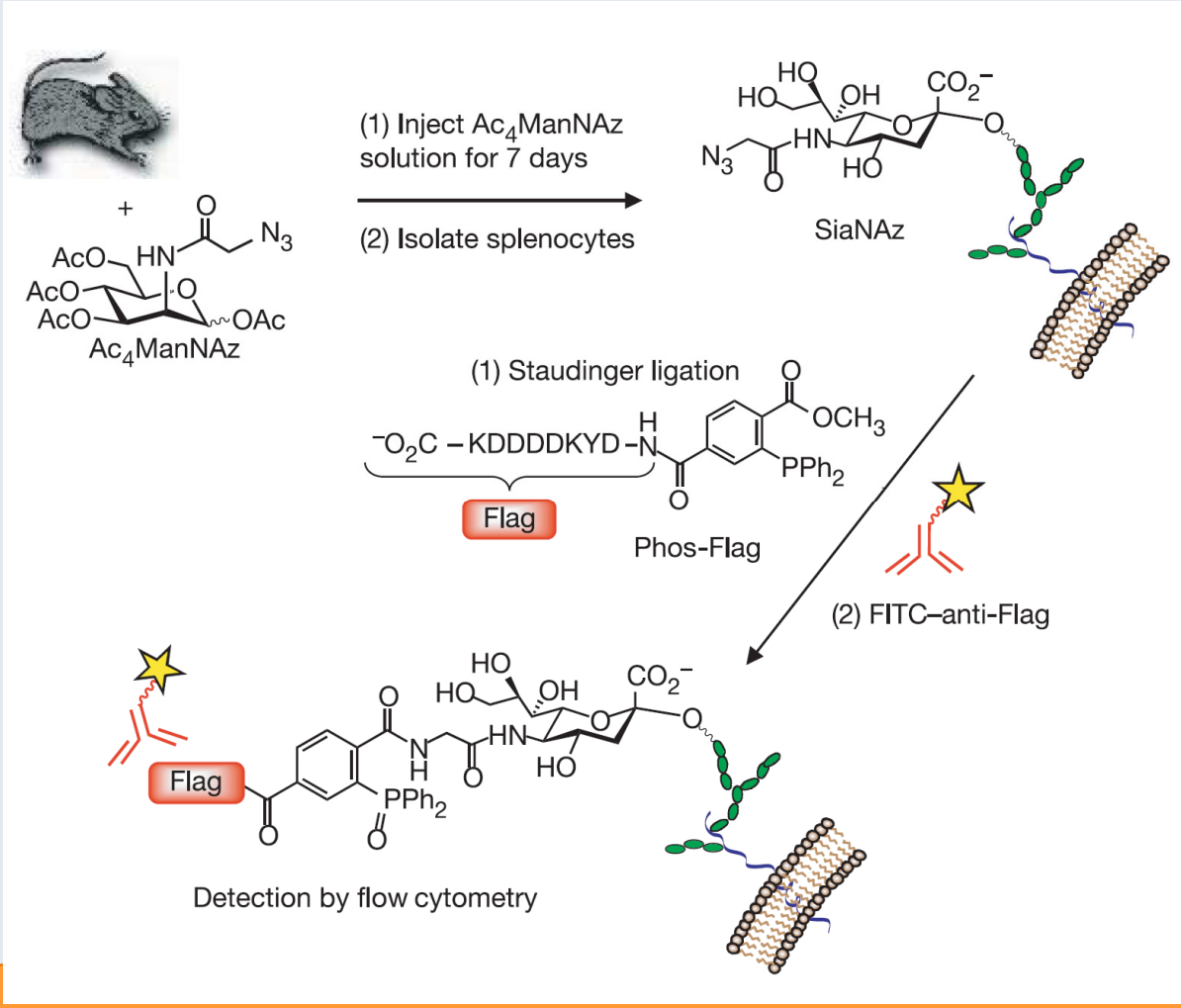
- Intermediate aza-ylide undergoes intramolecular formation to give an amide
  - A electrophilic trap was incorporated into the phosphine
  - Stable under physiological conditions
  - Reacts with bioorthogonal triaryl phosphines ($k_2 = 10^{-3}\ M^{-1}s^{-1}$)



E. Saxon, C.R. Bertozzi, *Science*, **2000**, *287*, 2007-2010
P. Shieh, C.R. Bertozzi, *Org. Biomol. Chem.*, **2014**, *12*, 9307-9320

Azide-Phosphine (Staudinger) Reactions

Staudinger Ligation

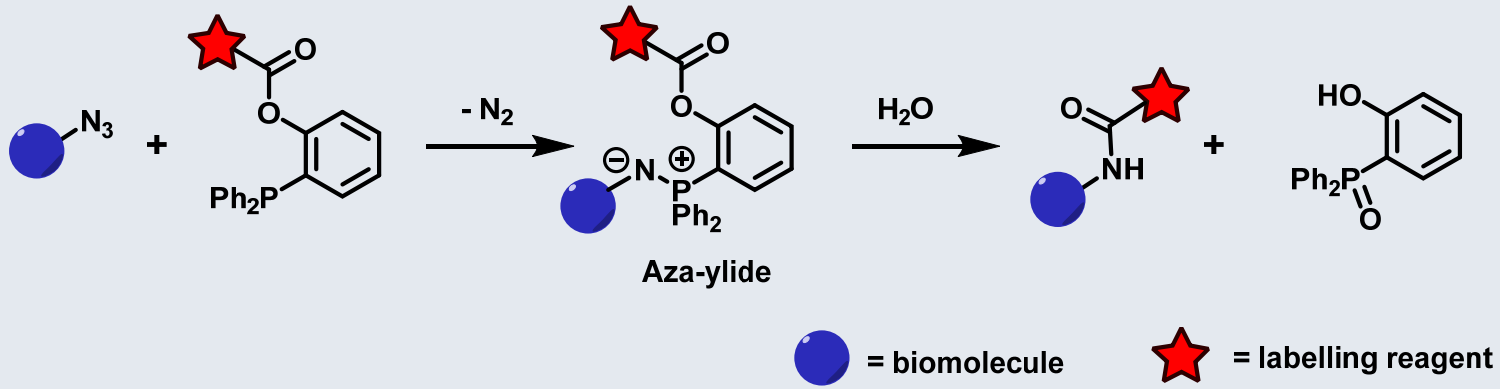


J.A. Prescher, D.H. Dube, C.R. Bertozzi, *Nature*, **2004**, *430*, 873-877

Azide-Phosphine (Staudinger) Reactions

Traceless Staudinger Ligation

- Final amide-linked product does not contain a phosphine oxide group
- Useful in the synthesis of peptides
  - Does not require a cysteine residue (unlike NCL)



General Difficulties

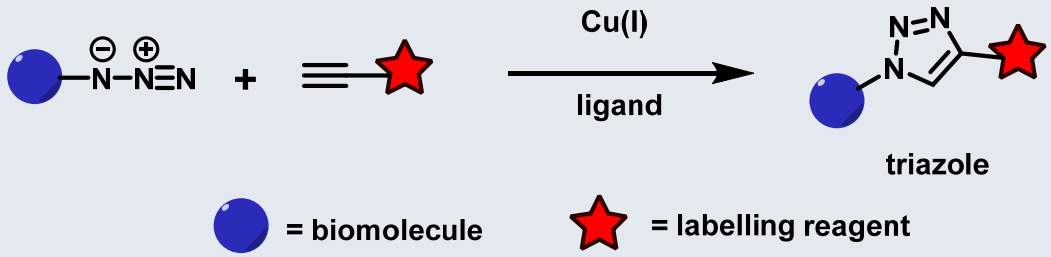
- Slow kinetics (10^{-3} $M^{-1}s^{-1}$)
  - Requires high [labelling reagent]
- Increasing nucleophilicity of phosphines leads to oxidation

E. Saxon, J.L. Armstrong, C.R. Bertozzi, *Org. Lett.*, **2000**, *2*, 2141-2143
K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Azide-Alkyne Reactions ([3+2] Cycloadditions)

- Azide groups are absent from biological systems
  - Small in size
  - Stable under physiological conditions
  - **React with physiologically stable alkynes**

Copper Catalysed Azide-Alkyne 1,3-Dipolar Cycloaddition (CuACC)



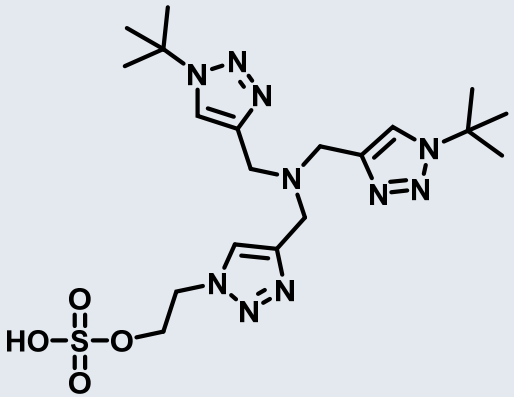
- Requires sufficient Cu(I) to maintain rate of reaction
  - *E. coli* stop growing after 16 h of 100 µM CuBr exposure
  - Mammalian cells tolerate < 500 µM of Cu(I) for ~ 1 h

H.C. Kolb, K.B. Sharpless, *Drug Discovery Today*, **2003**, *8*, 1128-1137
K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

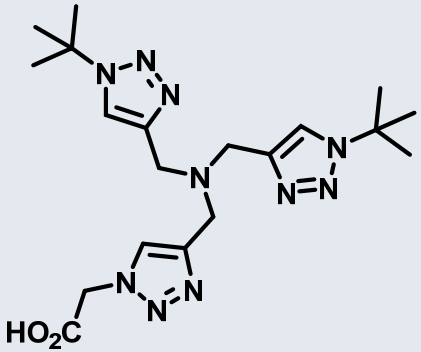
Azide-Alkyne Reactions ([3+2] Cycloadditions)

Copper Catalysed Azide-Alkyne 1,3-Dipolar Cycloaddition (CuACC)

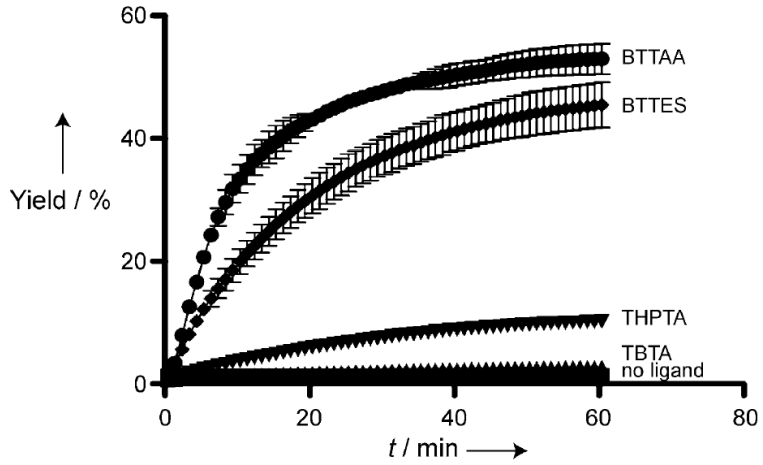
- **Water soluble ligands**
  - Reduces cytotoxicity by acting as reductants
  - Has led to faster reaction times, improved kinetics (k_2 = 10-200 $M^{-1}s^{-1}$)
  - Effective for cell surface labelling



BTTES



BTTAA



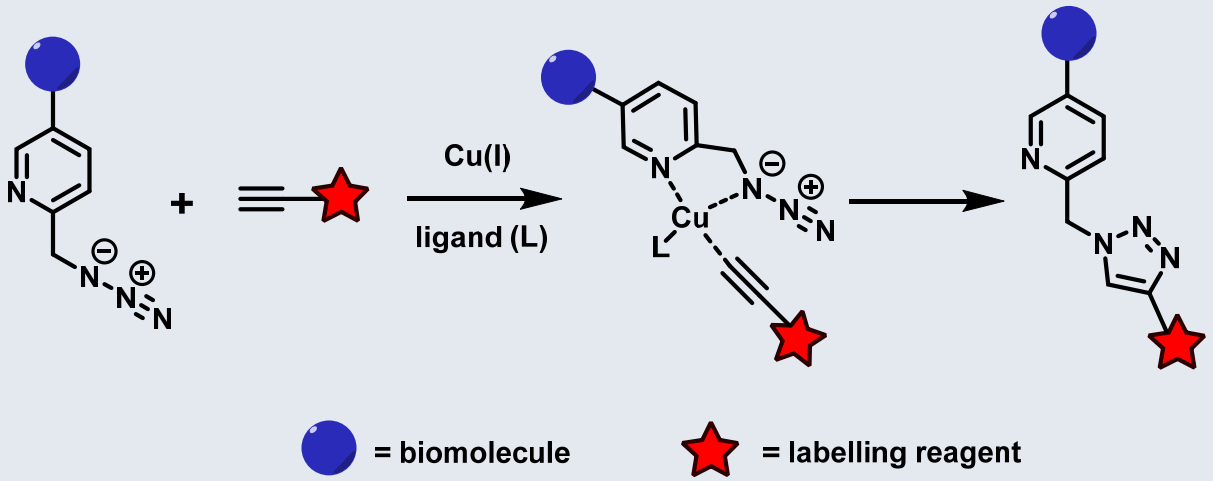
- Reaction conditions: propargyl alcohol (50 µM), 3-azido-7-hydroxycoumarin (100 µM), $CuSO_4$ (50 µM) ([ligand]/[CuSO4]=6:1), potassium phosphate buffer (0.1 M, pH 7.0)/DMSO=95:5, sodium ascorbate (2.5 mM), rt

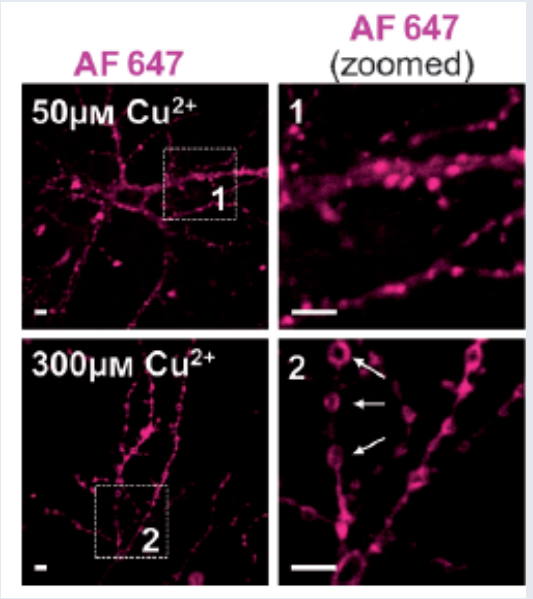
C. Besanceney-Webler, H. Jiang, T. Zheng, L. Feng, D. Soriano del Amo *et al.*, *Angew. Chem. Int. Ed.*, **2011**, *50*, 8051-8056
S.I. Presolski, V. Hong, S.-H. Cho, M.G. Finn, *J. Am. Chem. Soc.*, **2010**, *132*, 14570-14576

Azide-Alkyne Reactions ([3+2] Cycloadditions)

Copper Catalysed Azide-Alkyne 1,3-Dipolar Cycloaddition (CuACC)

- **Copper-chelating organic azides**
  - Raises the effective [Cu(I)] at the reaction site through proximal pyridine N
    - Reaction rate at 10 µM exceeds that of non-chelating variants at 100 µM
  - Can be used in concert with water soluble ligands ([Cu(I)] = 10 µM)
  - Effective for labelling proteins in live cells



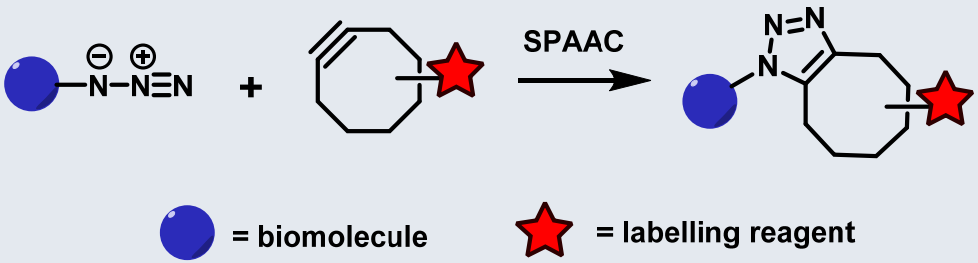


C. Uttampinant, A. Tangpeerachaikul, S. Grecian, S. Clarke, U. Singh, P. Slade, *et al.*, *Angew. Chem. Int. Ed.*, **2012**, *51*, 5852-5856
K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

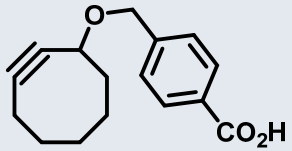
Azide-Alkyne Reactions ([3+2] Cycloadditions)

Strain-Promoted Alkyne-Azide Cycloaddition (SPAAC)

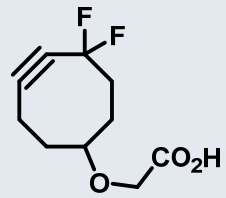
- Copper Free
  - Uses cyclooctynes as reagent
  - Shows no observable cytotoxicity
  - Some are now commercially available



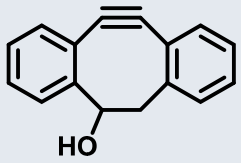
  - Original cyclooctyne bioorthogonal reactions are slower than Staudinger ligations
  - Improvements in derivatives has only led to rates of ~0.1 to 1 $M^{-1}s^{-1}$



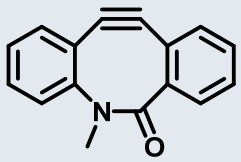
Oct
$k_2 = 2.4 \times 10^{-3}\ M^{-1}s^{-1}$



DIFO
$k_2 = 7.6 \times 10^{-2}\ M^{-1}s^{-1}$



DIBO
$k_2 = 5.7 \times 10^{-2}\ M^{-1}s^{-1}$



BARAC
$k_2 = 9 \times 10^{-1}\ M^{-1}s^{-1}$

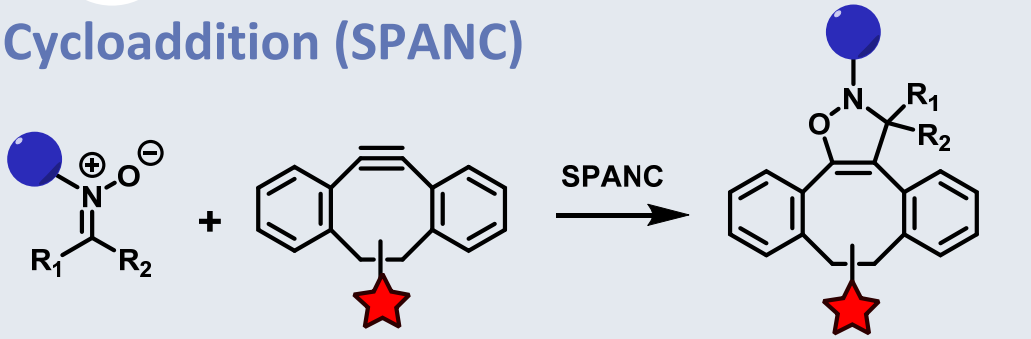
(k_2 = all for test reaction with benzyl azide)

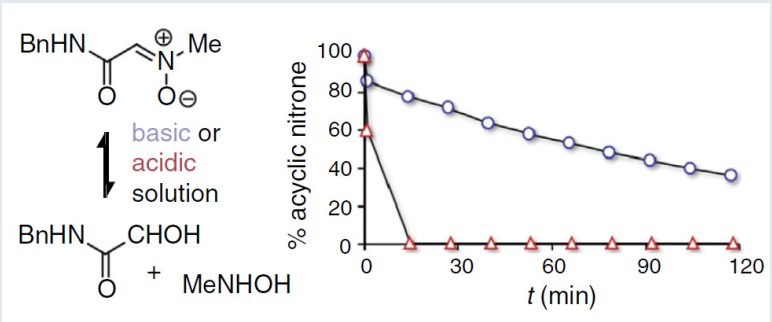
C.G. Gordon, J.L. Mackey, J.C. Jewett, E.M. Sletten, K.N. Houk, C.R. Bertozzi, *J. Am. Chem. Soc.*, **2012**, *134*, 9199-9208
J. Codelli, J.M. Baskin, N.J. Agard, C.R. Bertozzi, *J. Am. Chem. Soc.*, **2008**, *130*, 11486-11493
X. Ning, J. Guo, M.A. Wolfert, G.-J. Boons, *Angew. Chem. Int. Ed.*, **2008**, *47*, 2253-2255

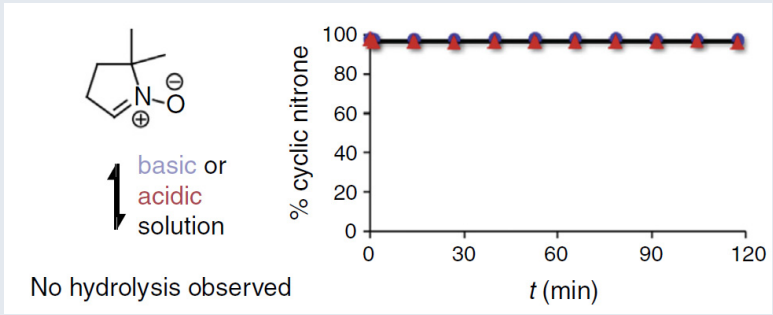
Nitrone-Cyclooctyne Reactions ([3+2] Cycloadditions)

Strain-Promoted Alkyne-Nitrone Cycloaddition (SPANC)

- Uses more reactive 1,3-dipole nitrones in place of azides
  - Rate constants up to 60 $M^{-1}s^{-1}$ in model reactions
  - 60 x faster than SPAAC
- Lower [reagent]
- Cyclic nitrones are more stable than acyclic counterparts
  - Susceptibility of nitrones to hydrolysis can be overcome



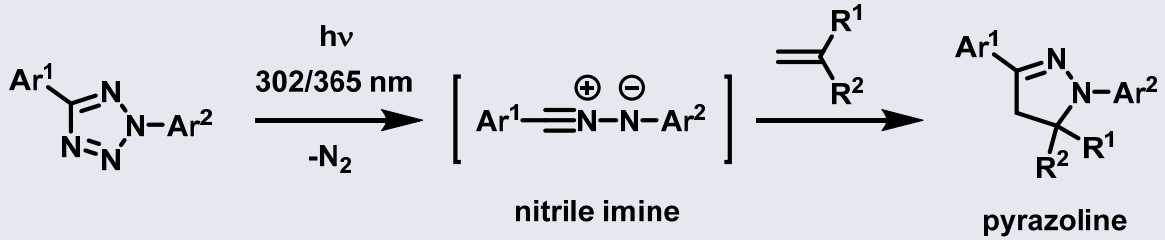




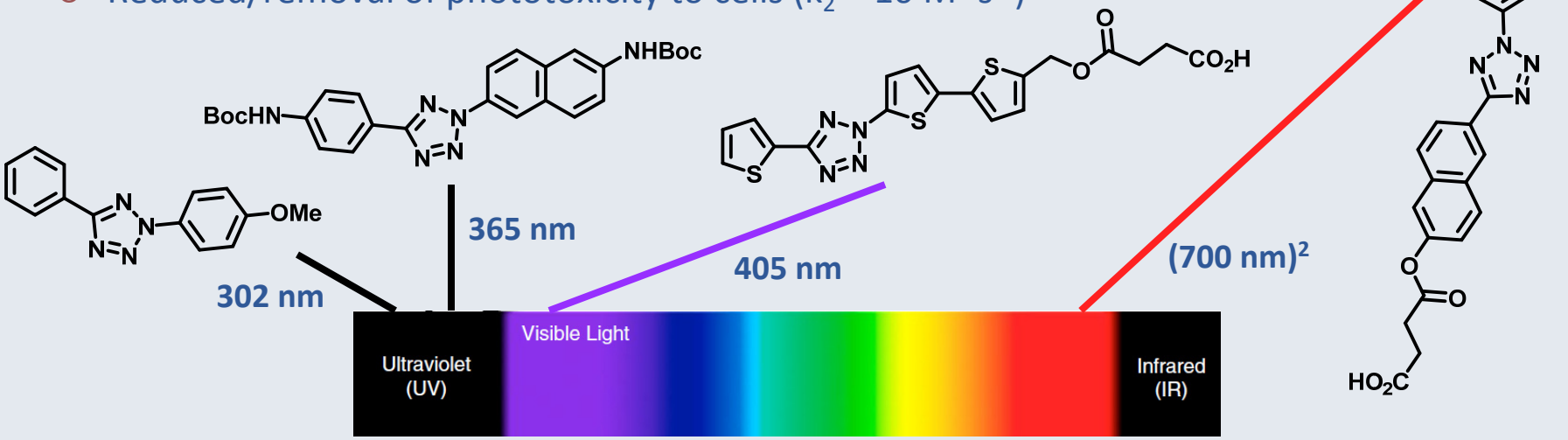
C.S. McKay, J. Moran, J.P. Pezacki, *Chem. Commun.*, **2010**, *46*, 931-933
C.S. McKay, M. Chigrinova, J.A. Blake, J.P. Pezacki, *Org. Biomol. Chem.*, **2012**, *10*, 3066-3070
D.A. MacKenzie, A.R. Sherratt, M. Chigrinova, L.L.W. Cheung, J.P. Pezacki, *Curr. Opin. Chem. Biol.*, **2014**, *21*, 81-88

Alkene-Tetrazole Reactions (Photoclick Cycloaddition)

- Tetrazoles activated with light will generate an imine *in situ* (1967)
  - An aqueous variant was first reported in 2008



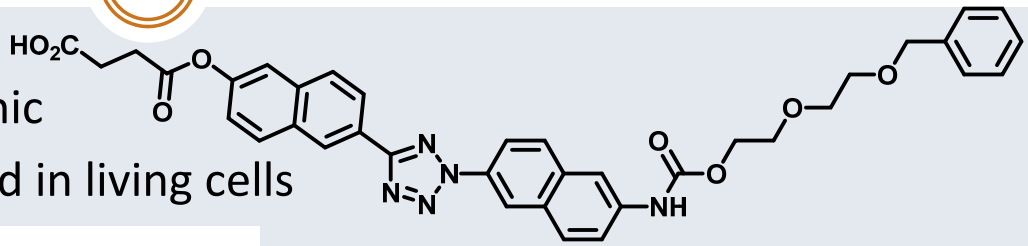
- Originally UV activated (4 min), now near IR excited (2 h)
  - Reduced/removal of phototoxicity to cells (k_2 ~ 10 $M^{-1}s^{-1}$)

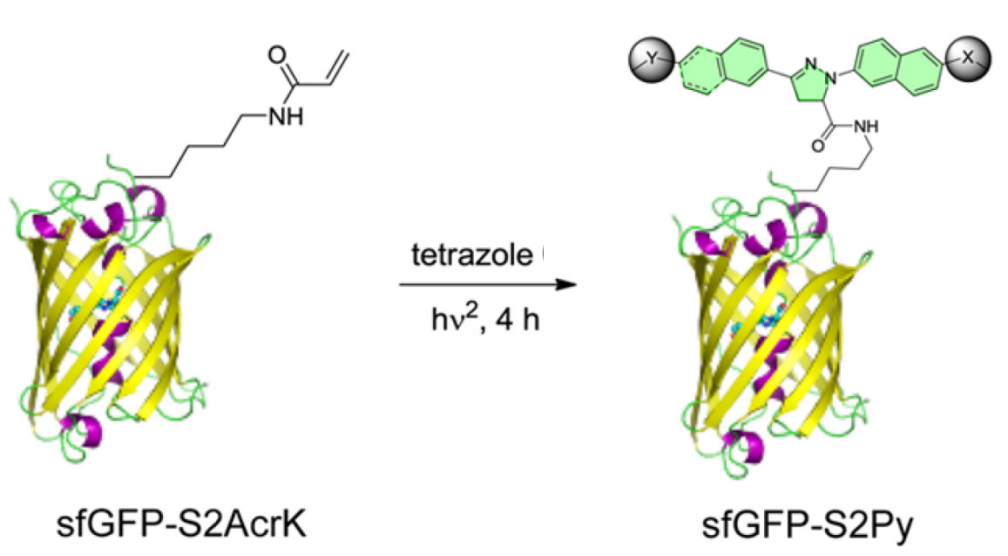


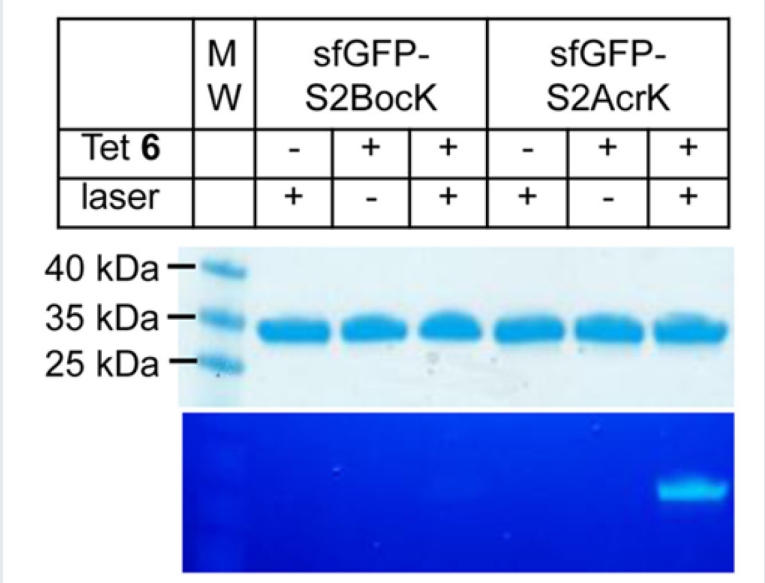
W. Song, Y. Wang, J. Qu, M.M. Madden, Q. Lin, *Angew. Chem. Int. Ed.*, **2008**, *47*, 2832-2835
C.P. Ramil, Q. Lin, *Curr. Opin. Chem. Biol.*, **2014**, *21*, 89-95

Alkene-Tetrazole Reactions (Photoclick Cycloaddition)

- Pyrazoline products are fluorogenic
- Has been used to label *in vitro* and in living cells







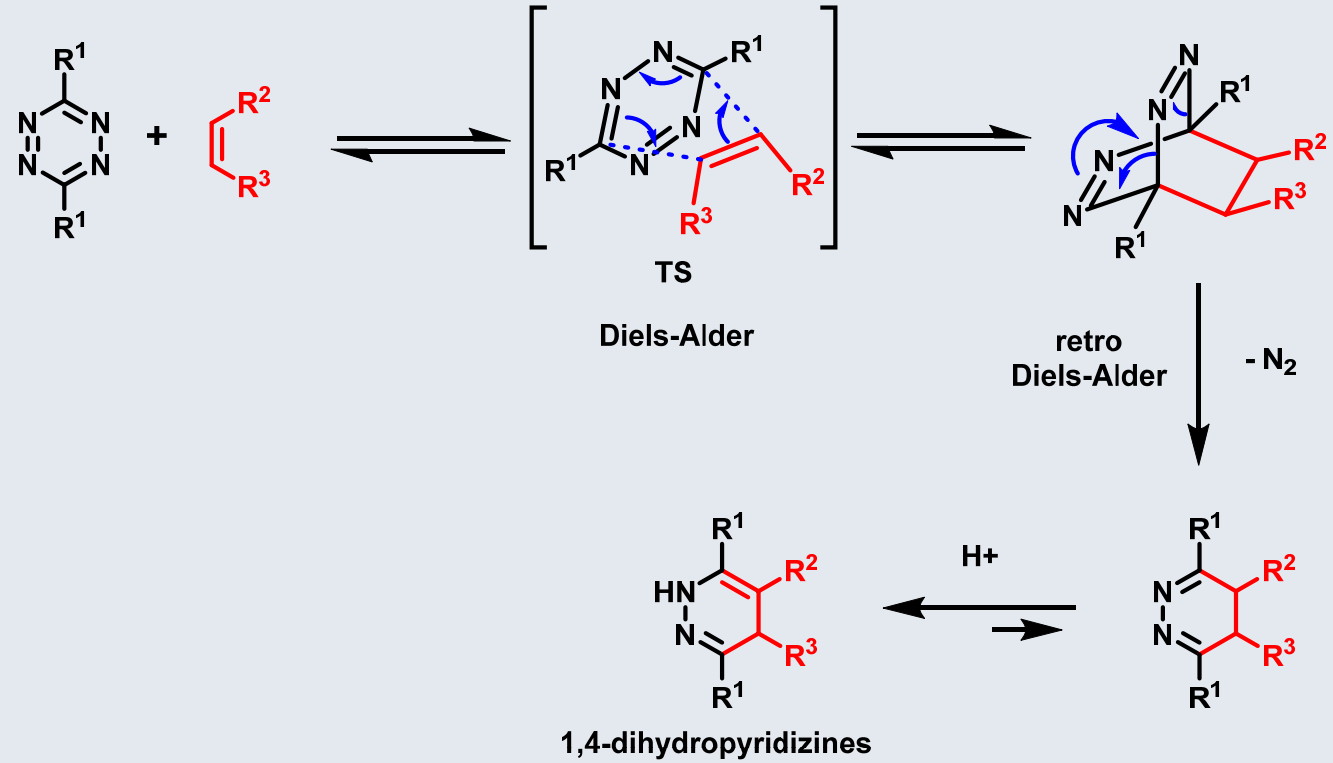
Difficulties

- Can be quenched by chloride ions/acidic conditions
- Internal *cis*-alkenes are endogenous in biological systems, could compete

Z. Yu, T.Y. Ohulchanskyy, P. An, P.N. Prasad, Q. Lin, *J. Am. Chem. Soc.*, **2013**, *135*, 16766-16769
C.P. Ramil, Q. Lin, *Curr. Opin. Chem. Biol.*, **2014**, *21*, 89-95
K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Strained Alkene/Alkyne-Tetrazine Reactions (Inverse-Electron-Demand Diels-Alder Cycloadditions)

- 1,2,4,5-Tetrazines are reacted with electron-rich dienophiles (alkenes) = iEDDA

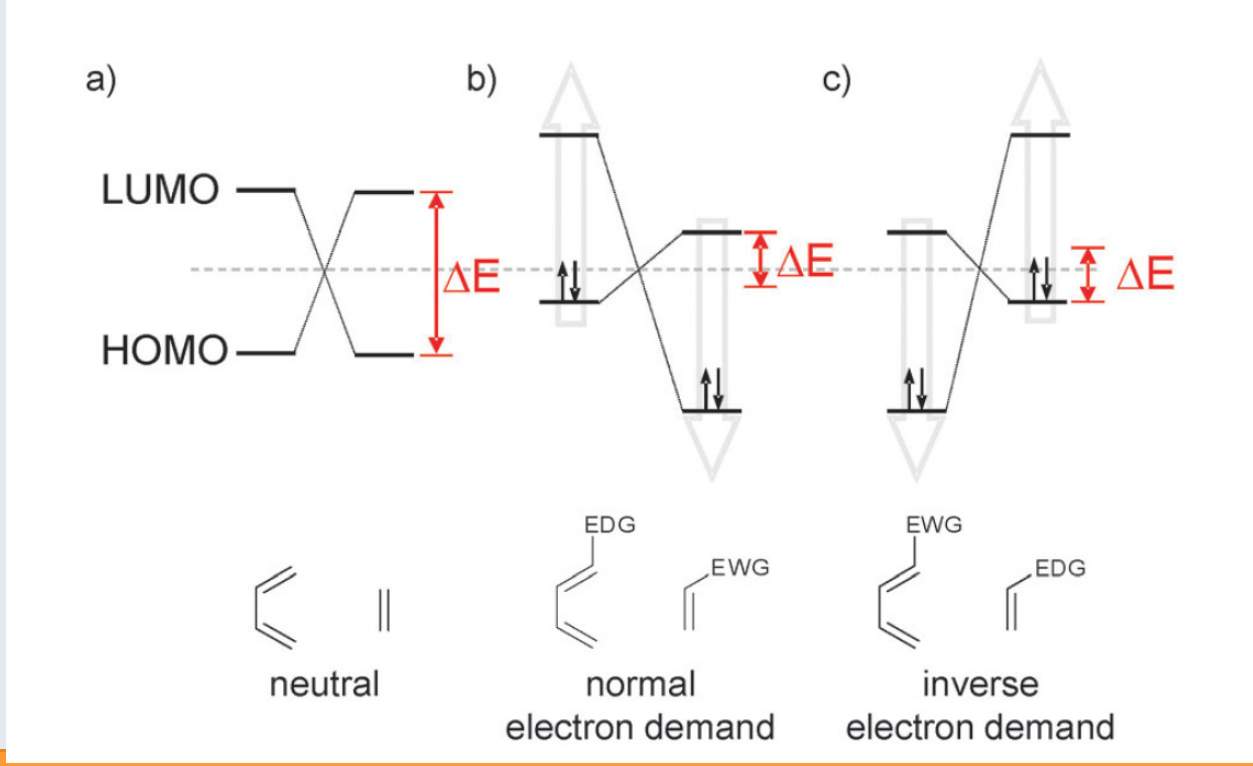


  - Highly strained alkenes (such as *trans*-cyclooctene) react very rapidly in organic solvent
  - Previously described tetrazines were not stable in H_2O

A.-C. Knall, C. Slugovc, *Chem. Soc. Rev.*, **2013**, *42*, 5131-5142
C.P. Ramil, Q. Lin, *Curr. Opin. Chem. Biol.*, **2014**, *21*, 89-95

Strained Alkene/Alkyne-Tetrazine Reactions (Inverse-Electron-Demand Diels-Alder Cycloadditions)

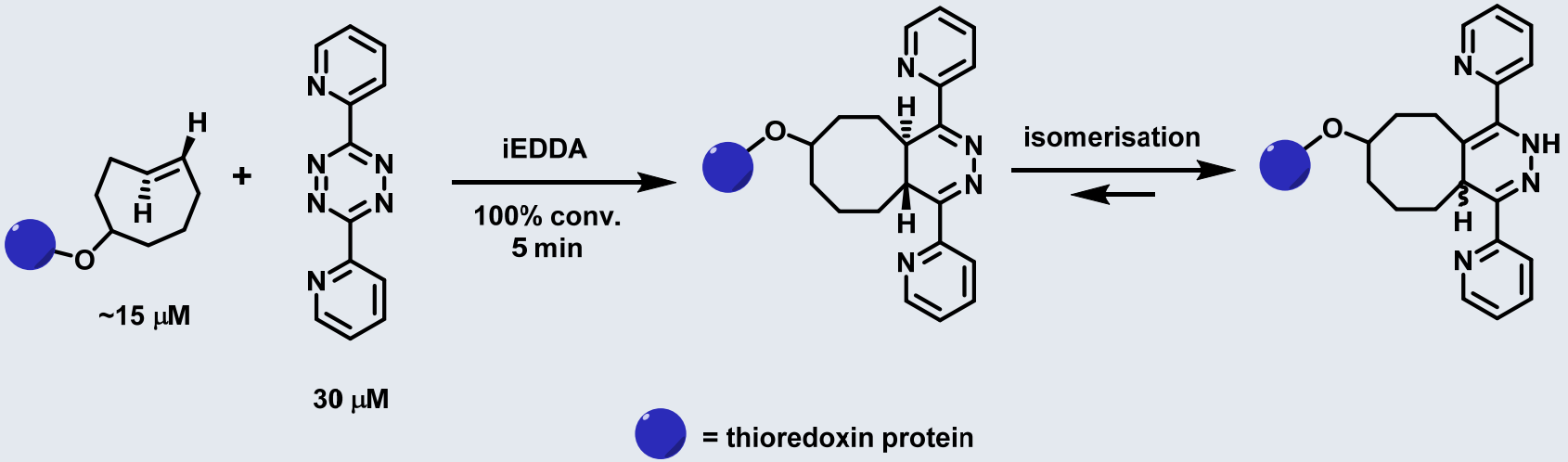
- Governed by the $HOMO_{dienophile}$ – $LUMO_{diene}$ gap
  - EWG groups at the 3- and 6-positions of the tetrazine lower the LUMO = Faster iEDDA
  - Dienophiles with EDG substituents raise the HOMO = Faster iEDDA
  - High ring strain in the dienophile helps reduce the activation energy



A.-C. Knall, C. Slugovc, *Chem. Soc. Rev.*, **2013**, *42*, 5131-5142
C.P. Ramil, Q. Lin, *Curr. Opin. Chem. Biol.*, **2014**, *21*, 89-95

Strained Alkene/Alkyne-Tetrazine Reactions (Inverse-Electron-Demand Diels-Alder Cycloadditions)

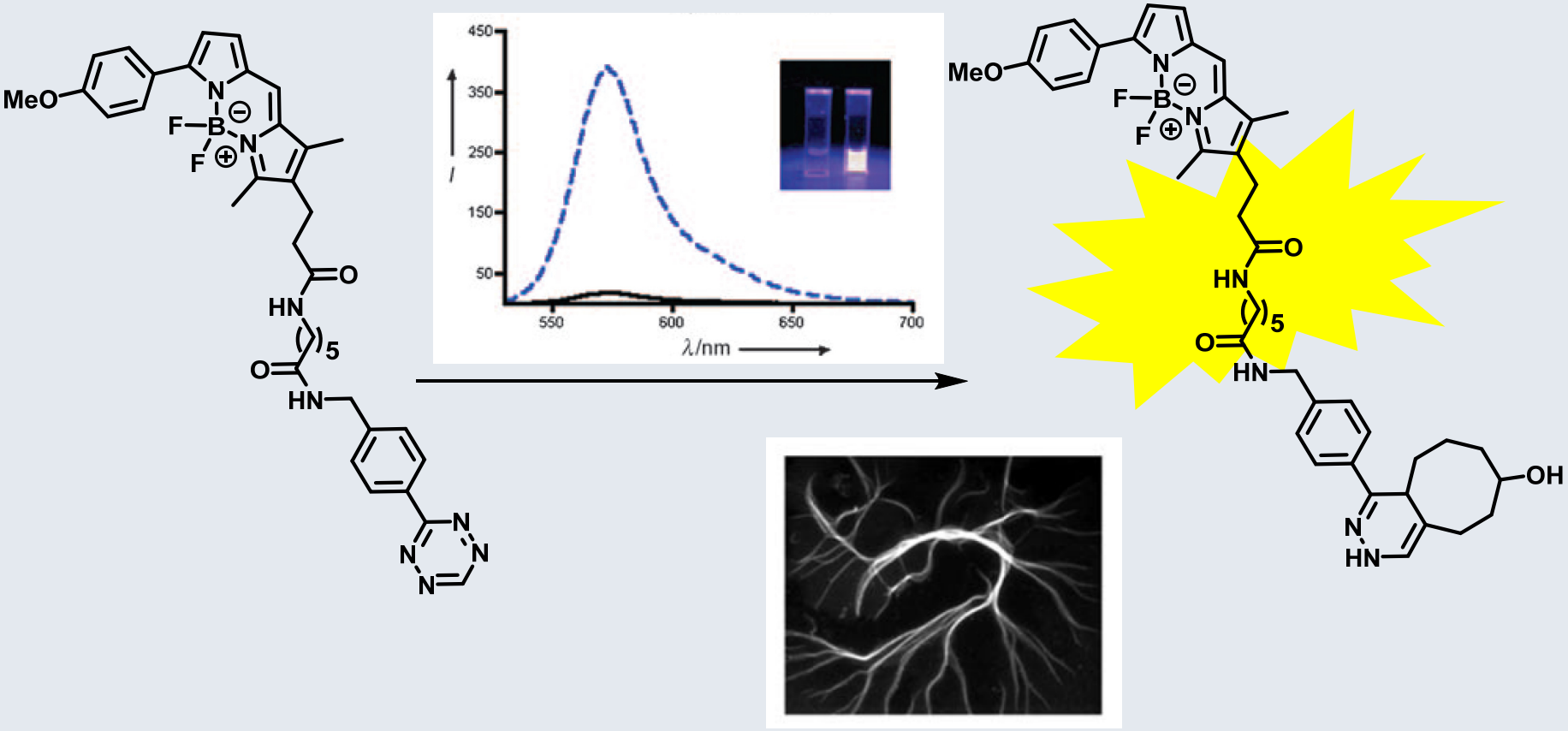
- iEDDA was first shown to be bioorthogonal in 2008
  - 3,6-Diaryl-*s*-tetrazines were found to be H_2O stable
  - Can be run in cell media and cell lysate with > 80% yield (k_2 = 2000 $M^{-1}s^{-1}$)
  - Successfully used to label proteins *in vitro* and *in vivo*



M.L. Blackman, M. Royzen, J.M. Fox, *J. Am. Chem. Soc.*, **2008**, *130*, 13518-13519

Strained Alkene/Alkyne-Tetrazine Reactions (Inverse-Electron-Demand Diels-Alder Cycloadditions)

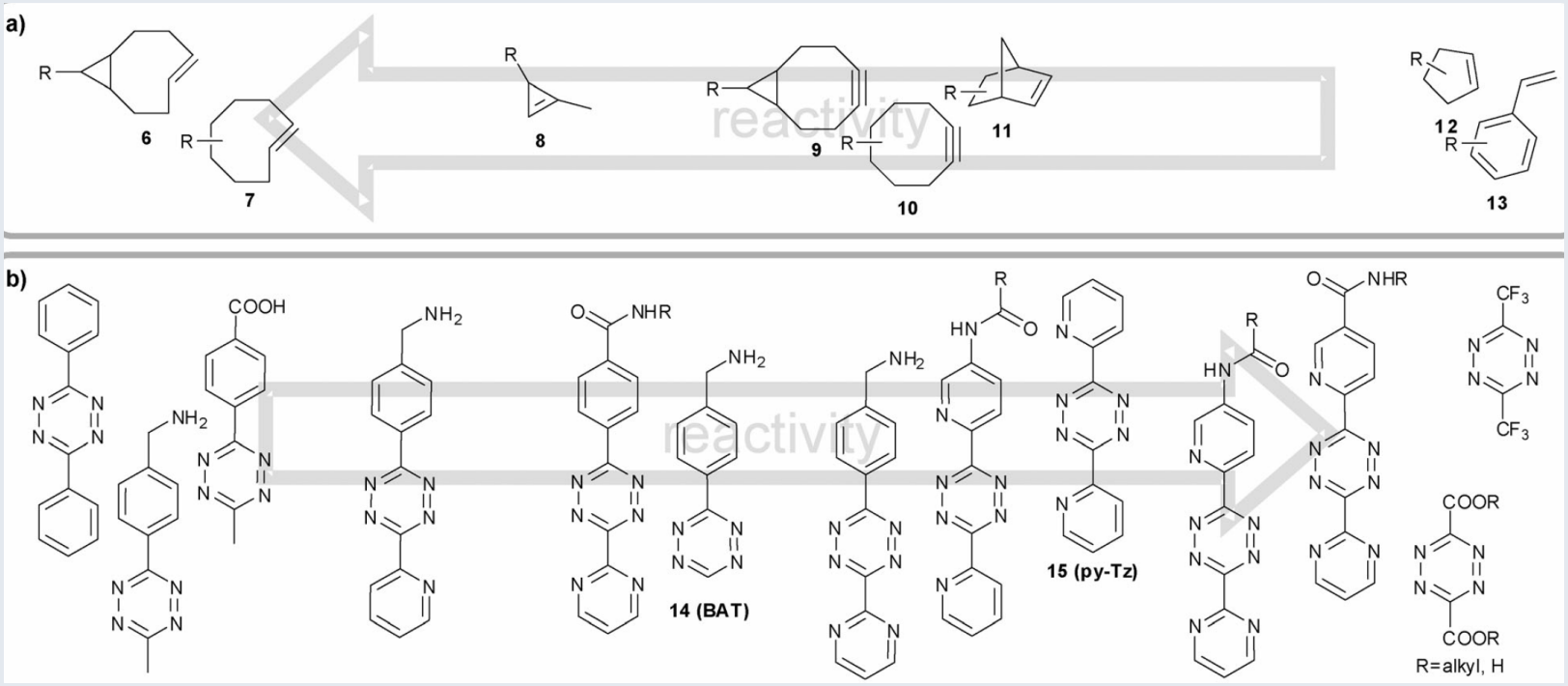
- Tetrazines can be conjugated to fluorophores to create fluorogenic products
  - The tetrazine quenches fluorescence until activation by iEDDA



N.K. Devaraj, S. Hiderbrand, R. Upadhyay, R. Mazitschek, R. Weissleder, *Angew. Chem. Int. Ed.*, **2010**, *49*, 2869-2872
C.P. Ramil, Q. Lin, *Curr. Opin. Chem. Biol.*, **2014**, *21*, 89-95

Strained Alkene/Alkyne-Tetrazine Reactions (Inverse-Electron-Demand Diels-Alder Cycloadditions)

- A range of strained alkenes/alkynes (a) and tetrazines (b) have now been investigated



Difficulties

- Tetrazine synthetic availability
  - Potentially explosive nature of synthetic precursors to tetrazines

A.-C. Knall, C. Slugovc, *Chem. Soc. Rev.*, **2013**, *42*, 5131-5142
C.P. Ramil, Q. Lin, *Curr. Opin. Chem. Biol.*, **2014**, *21*, 89-95

Incorporation of nCAAs for Bioorthogonal Reactions

- Proteins typically consist of 20 naturally occurring amino acids
- Syntheses of nCAAs bearing bioorthogonal groups are now well documented
- Highjacking protein translation can lead to successful nCAA incorporation
  - High fidelity of protein translation
  - Yields can be increased by encoding repressed translational promoters

Residue-specific

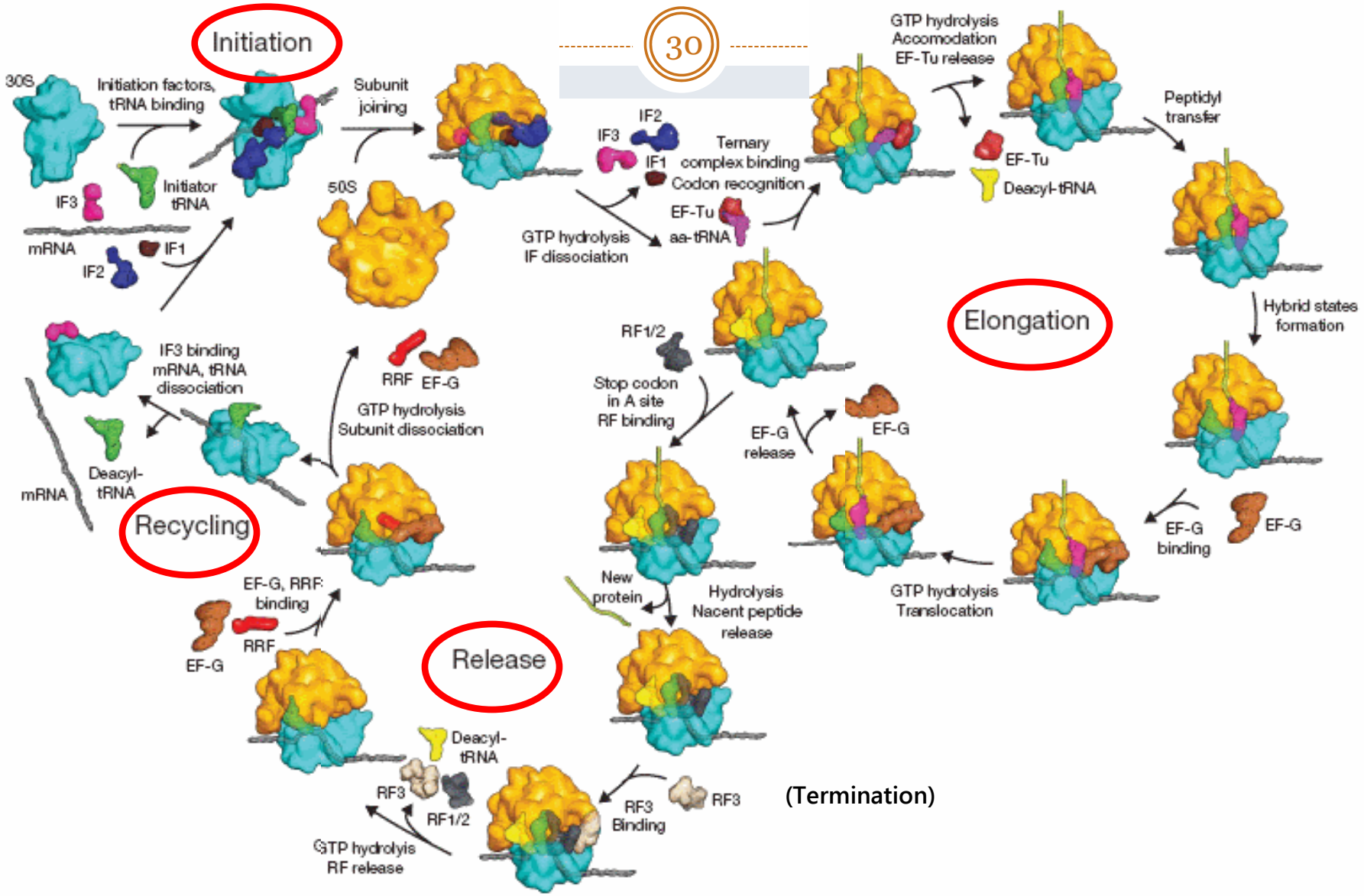
- 1950s – replace one of the 20 amino acids with a similar synthetic variant

Site-specific

- 1980s – a nCAA can be incorporated at a specific site within a protein, in the presence of the natural amino acids

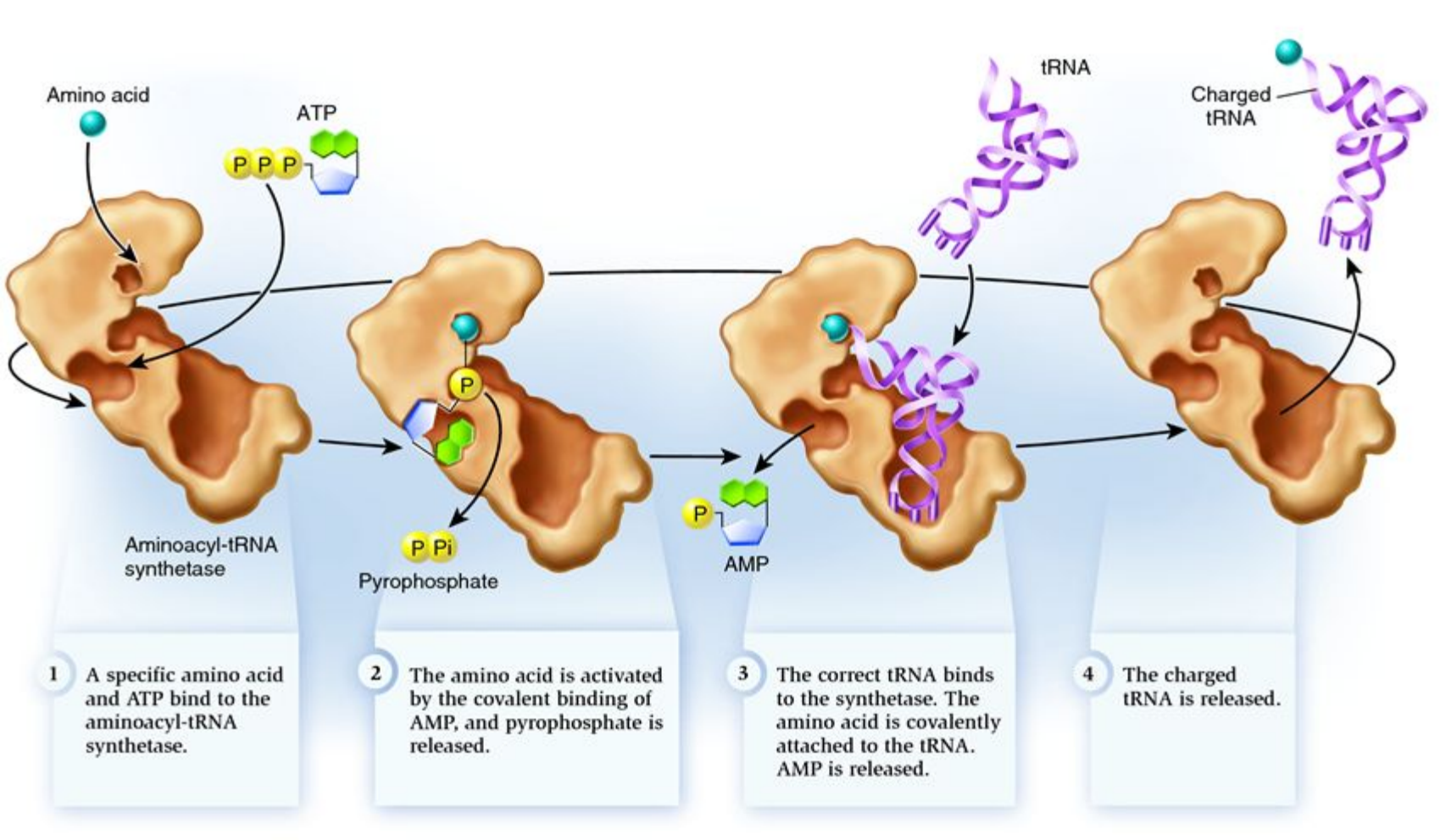
A.-C. Knall, C. Slugovc, *Chem. Soc. Rev.*, **2013**, *42*, 5131-5142
K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Protein Translation 101



T. M. Schmeing, V. Ramakrishnan, *Nature*, **2009**, *461*, 1234-1242

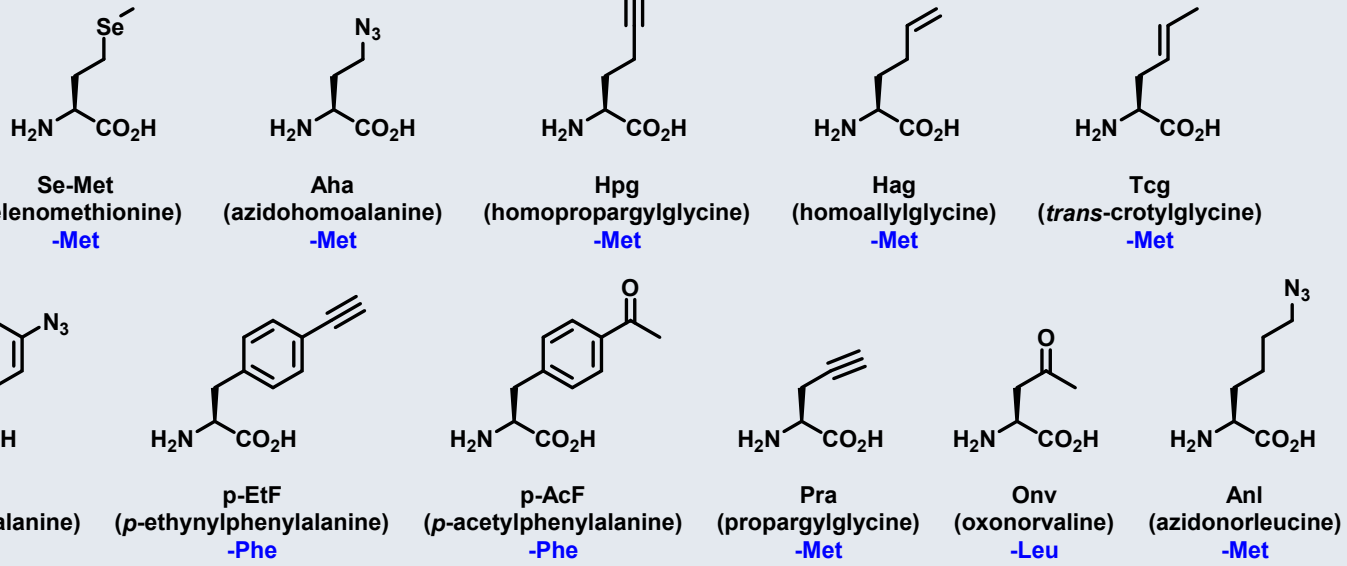
Amino Acid Transfer with tRNA Synthetases



M. Ibba, D. Söll, *Annu. Rev. Biochem.*, **2000**, *69*, 617-650
http://biomoocnews.blogspot.com/ Sept 10th 2014 Blog

Residue-Specific Incorporation of nCAAs

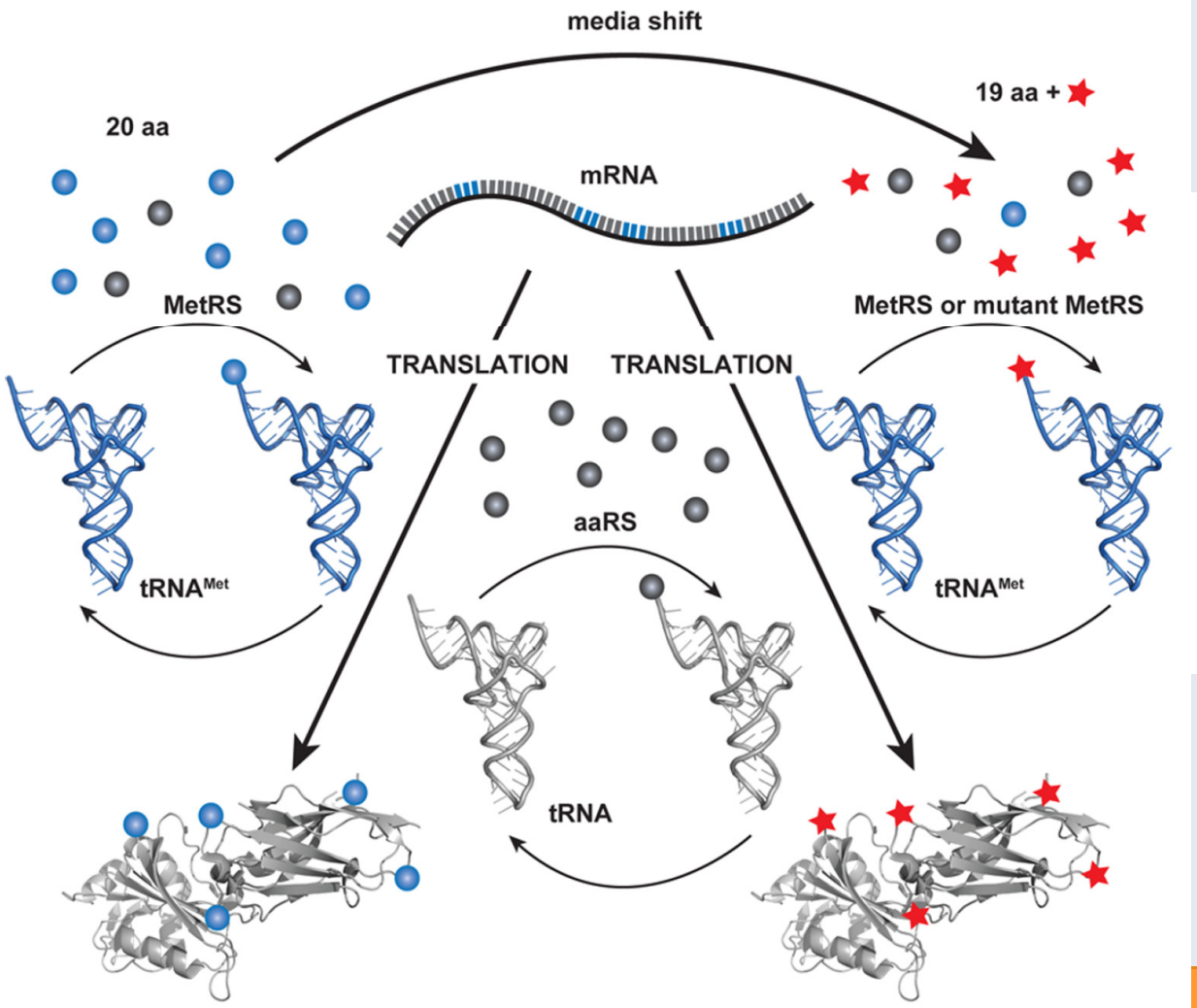
- Produces globally modified proteins
- Simplest variants require no genetic alterations of the target organism
  - Targets entire proteome of the cell/organism



- Three key requirements:
  - Natural amino acid must be encoded at the genetic level
  - The unnatural amino acid must be a substrate for the natural variants tRNA synthetase
  - The desired protein must be expressed when the unnatural amino acid is in the cell

K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Residue-Specific Incorporation of nCAAs



- = Met, or natural amino acids to be replaced
- = one of other 19 amino acids
- = unnatural amino acid
- = codon for Met or natural amino acid to be replaced
- = codons for other 19 natural amino acids

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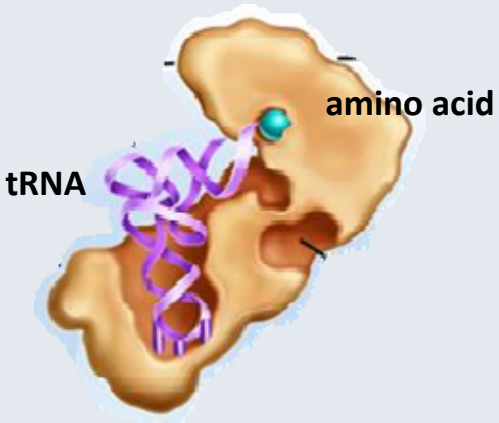
Residue-Specific Incorporation of nCAAs

- Greater alterations in nCAAs can be accomodated by mutating existing tRNA synthetase binding sites



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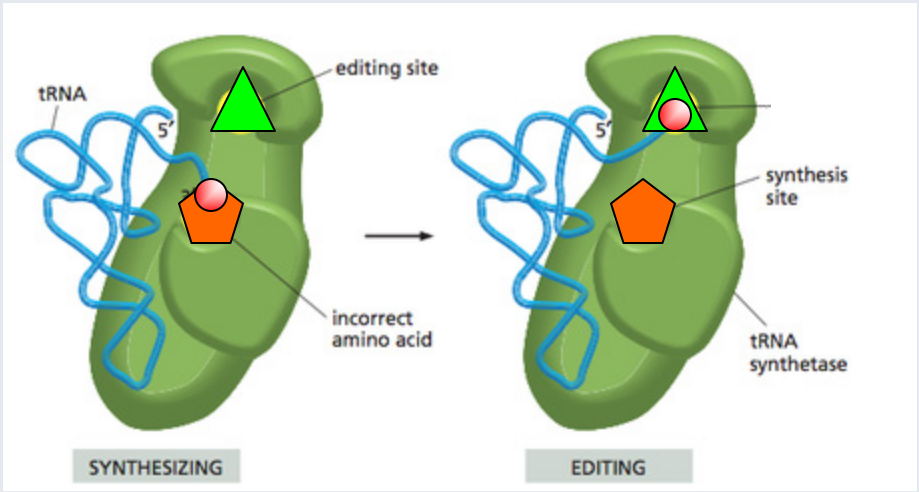
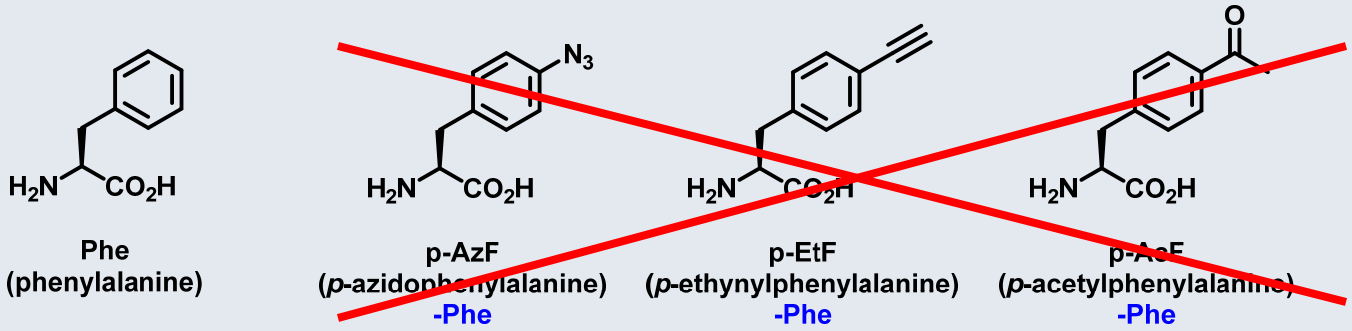


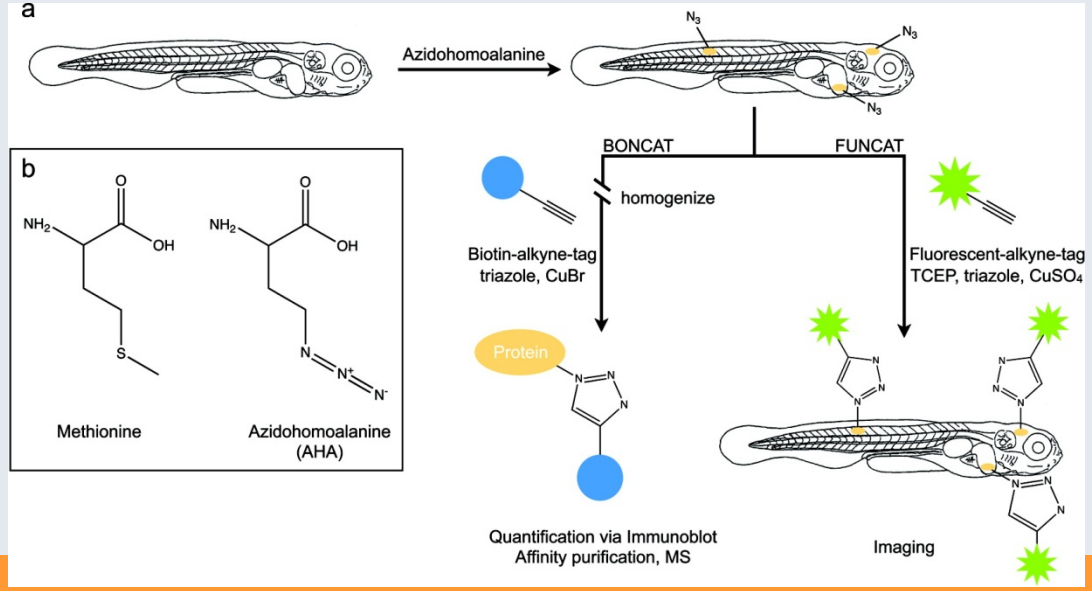


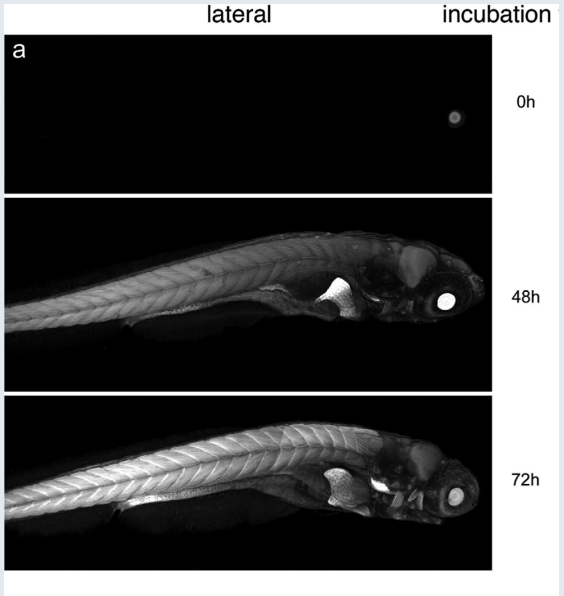
K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806
K. Kirshenbaum, I.S. Carrico, D.A. Tirrell, *ChemBioChem*, **2002**, *3*, 235-237
http://quizlet.com/30405369/bio-lecture-5-chapter-6-rna-to-protein-flash-cards/

Residue-Specific Incorporation of nCAAs

Uses

- BONCAT & FUNCAT (**B**io**o**rthogonal/**F**l**u**orescent **n**on**c**anonical **a**mino acid **t**agging)
  - Determine kinetics of protein synthesis and separate newly synthesised proteins from pre-existing proteome
  - Analysis of localised synthesis of proteins critical to cell function (such as axons)
  - Labelling of newly synthesised proteins in multicellular organisms
    - Now progressed to cell selective protein labelling



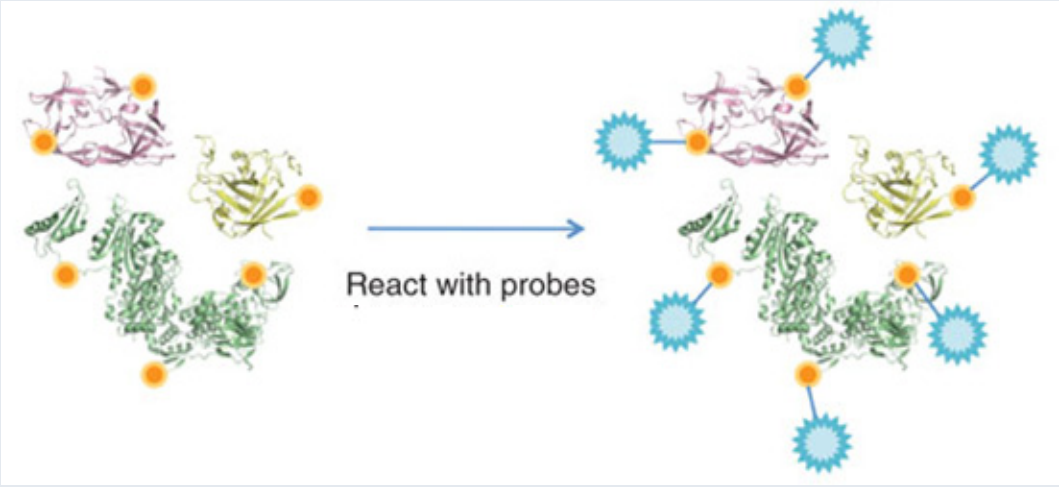


K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806
F.I. Hinz, D.C. Dieterich, D.A. Tirrell, E.M. Schuman, *ACS. Chem. Neurosci.*, **2012**, *3*, 40-49
F. Truong, T.H. Yoo, T.J. Lampo, D.A. Tirrell, *J. Am. Chem. Soc.*, **2012**, *134*, 8551-8556

Residue-Specific Incorporation of nCAAs

Difficulties

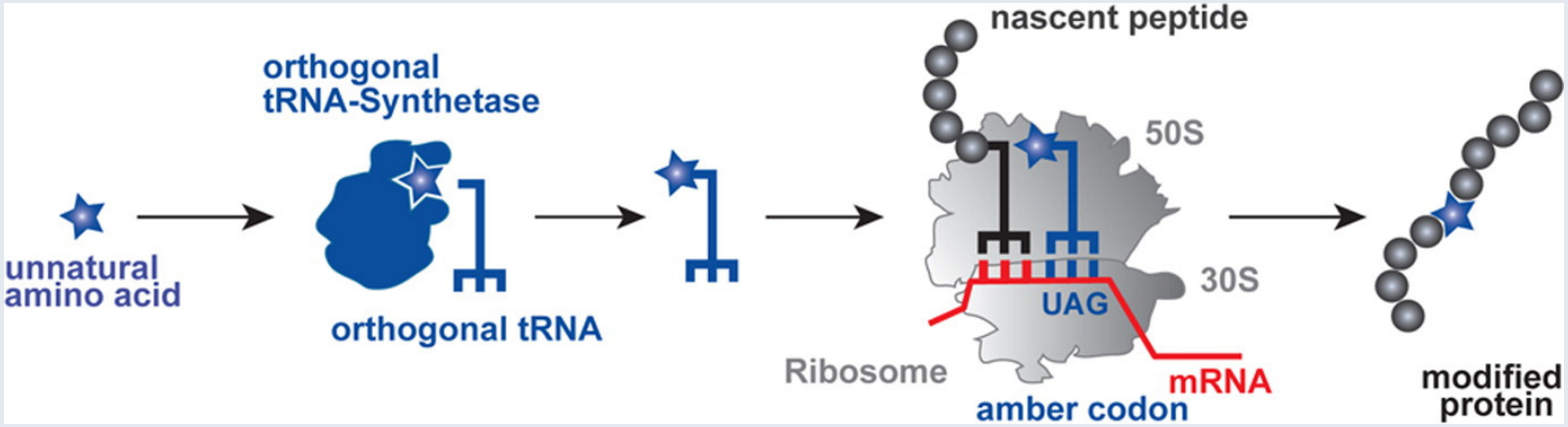
- Labels all proteins in an organism or cell unless specifically genetically altered
- Some nCAA incorporation can be toxic to the cells
  - Conc. and exposure time dependant
- Some nCAAs are poorly incorporated
  - Addressed by use of mutated tRNA synthetases that can be selectively expressed
- Limited by requirements of the cell, i.e. 20 natural amino acids



K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806
J.T. Ngo, J.A. Champion, A. Mahdavi, I.C. Tanrikulu, K.E. Beatty, R.E. Connor, T.H. Yoo *et al.*, *Nat. Chem. Bio.*, **2009**, 5, 715-717

Site-Specific Incorporation of nCAAs

- Uses genetic code expansion
- Allows a single amino acid of a peptide to be altered in the presence of the all 20 naturally occurring amino acids

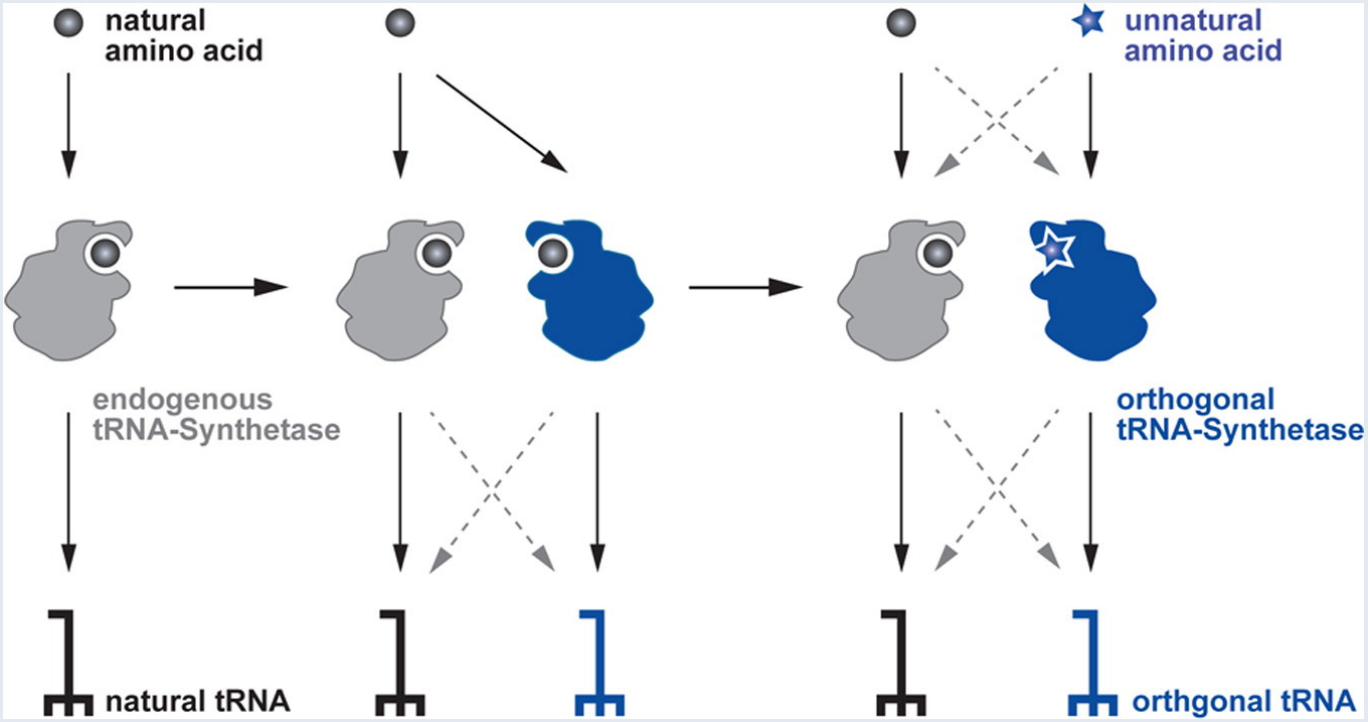


- Three key requirements:
  - An orthogonal amino-acyl-tRNA synthetase/tRNA pair
  - A blank codon to encode for a nCAA
    - Commonly the amber stop codon UAG (TAG) as 93% of *E.coli* genes end with TGA or TAA
  - Methods to ensure the transfer of the nCAA to the orthogonal tRNA occurs selectively

T.S. Young, P.G. Schultz, *J. Biol. Chem.*, **2010**, *285*, 11039-11044
K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Site-Specific Incorporation of nCAAs

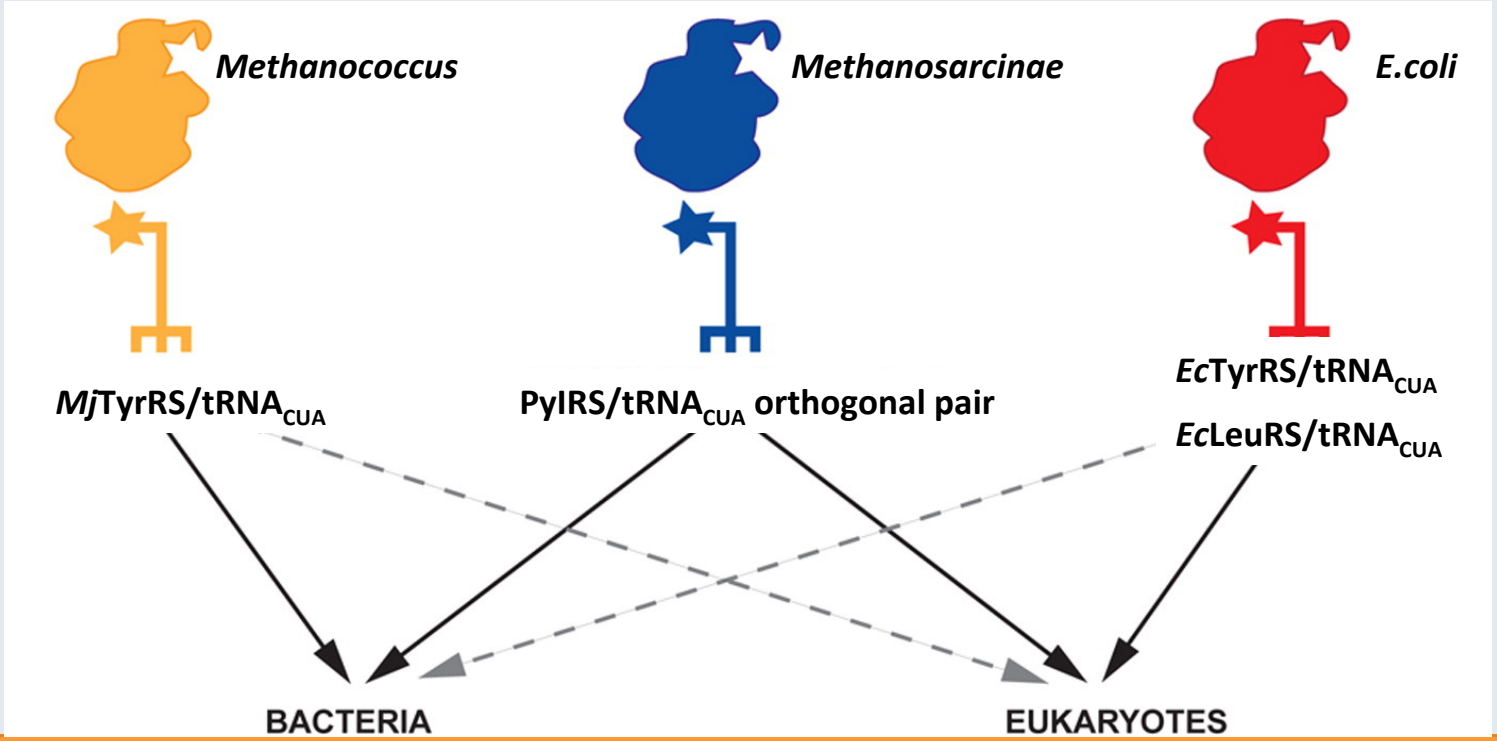
- Orthogonal tRNA synthetase: must not label any endogenous tRNA in a cell
- Orthogonal tRNA: must not be recognised by any endogenous tRNA synthetases
- **Exploit the tRNA-synthetase/tRNA differences between organisms**



K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Site-Specific Incorporation of nCAAs

- Four tRNAs (with desirable synthetase partner) that recognise amber stop codons have been identified and used in site-specific manipulations of proteins
  - tRNA may require mutation to recognise nCAAs
  - tRNA does not need to be altered to recognise a manufactured blank codon

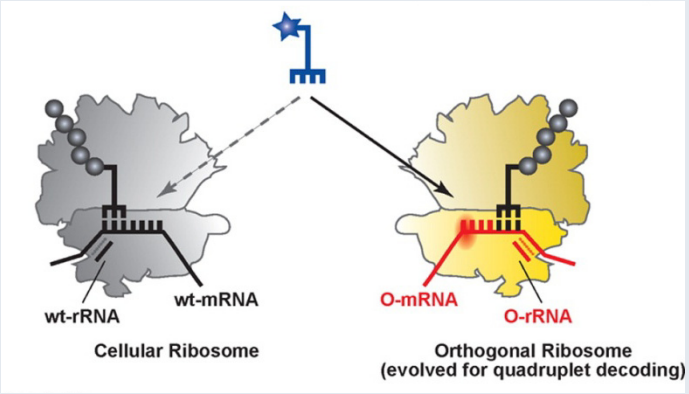


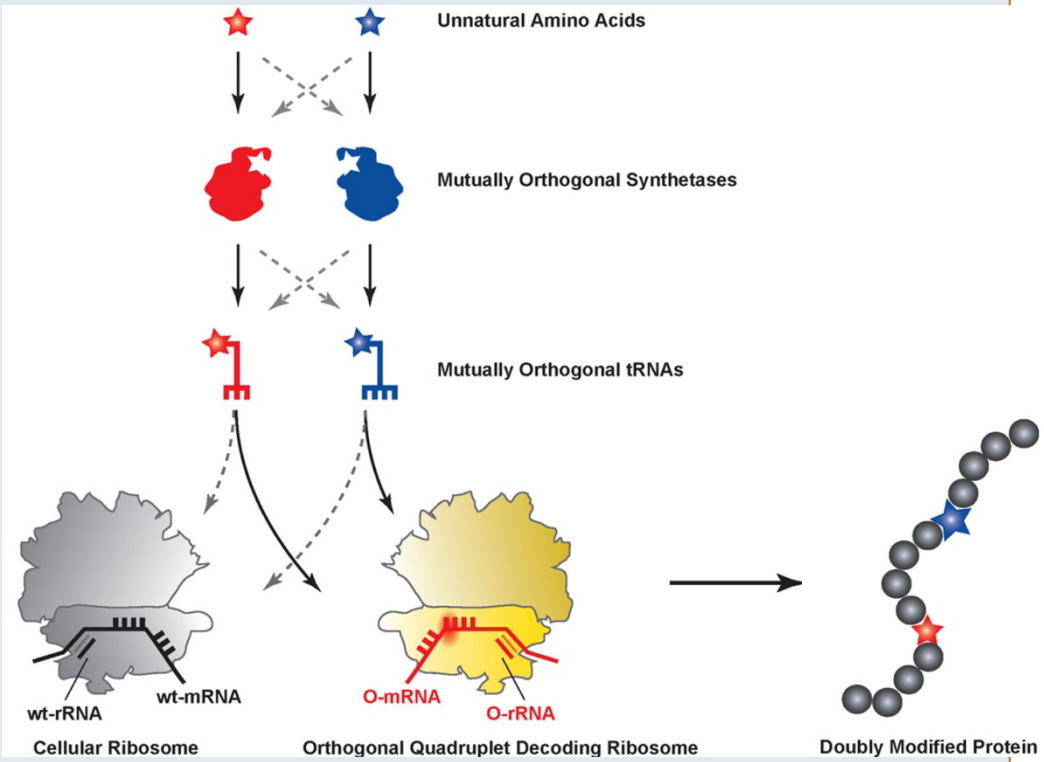
K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Site-Specific Incorporation of nCAAs

Orthogonal translation pathway

- A new genetic code, evolved to include quadruplet codons
  - New ribosome synthesis
    - Will accommodate quadruplet codon tRNA
  - New mRNA
  - New tRNA-synthetase/tRNA pairs
    - Could non-α-amino acids be used?



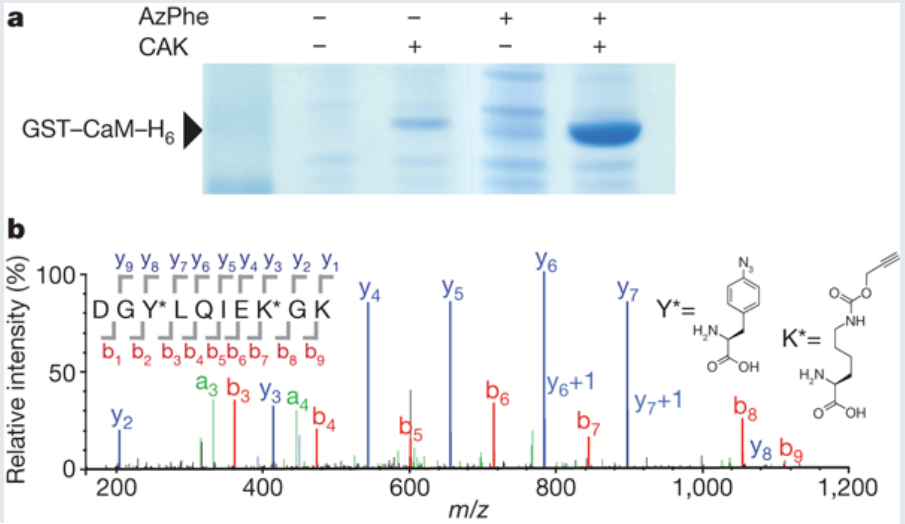


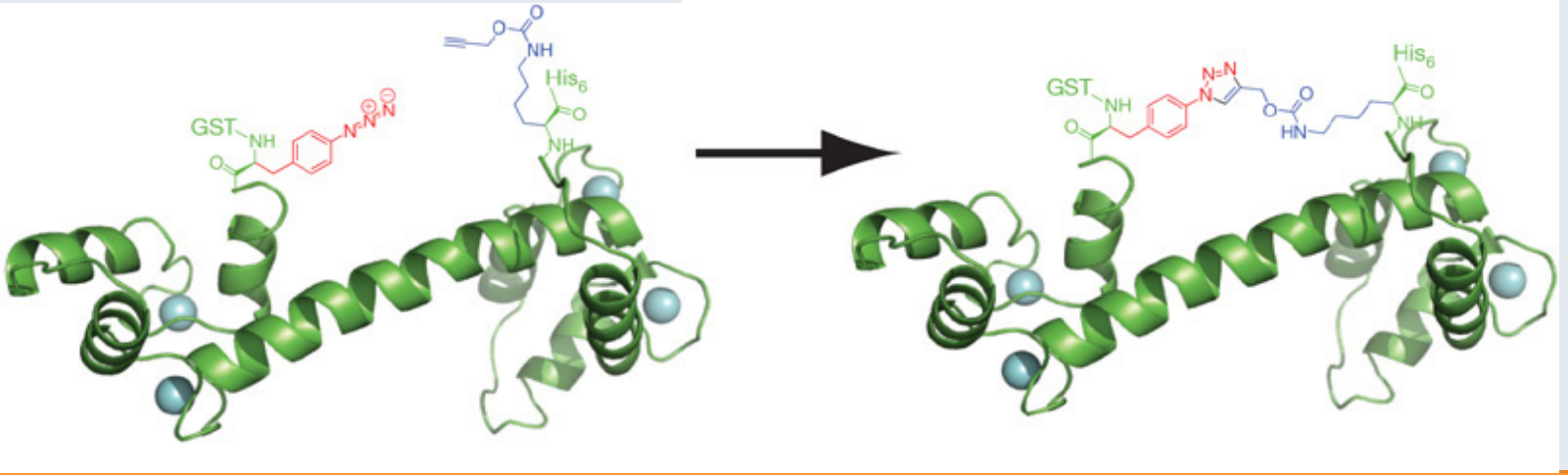
K. Wang, W.H. Schmied, J.W. Chin, *Angew. Chem. Int. Ed.*, **2012**, *51*, 2288-2297
K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Site-Specific Incorporation of nCAAs

Orthogonal translation pathway

- Used successfully to create a modified GST-calmodulin protein
  - Only synthesised if both nCAAs are incorporated
  - Has the potential to create new proteins and alter functions and structures



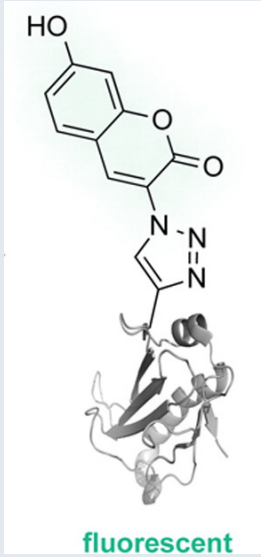


H. Neumann, K. Wang, L. Davis, M. Garcia-Alai, J.W. Chin, *Nature*, **2010**, *464*, 441-444
K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Site-Specific Incorporation of nCAAs

Uses

- More nCAAs have been successfully incorporated than in residue-specific experiments
- Help to investigate specific proteins within organisms and cells
- Being used to form antibody conjugates (such as ADC antibody-drug conjugates)
- Enables minimal disruption to normal protein folding
  - Can probe specific sites in the protein amenable to nCAA incorporation
- Can (and has) been adapted to DNA and RNA labelling (aptamers)
  - Could prove useful in early and specific cancer detection assays



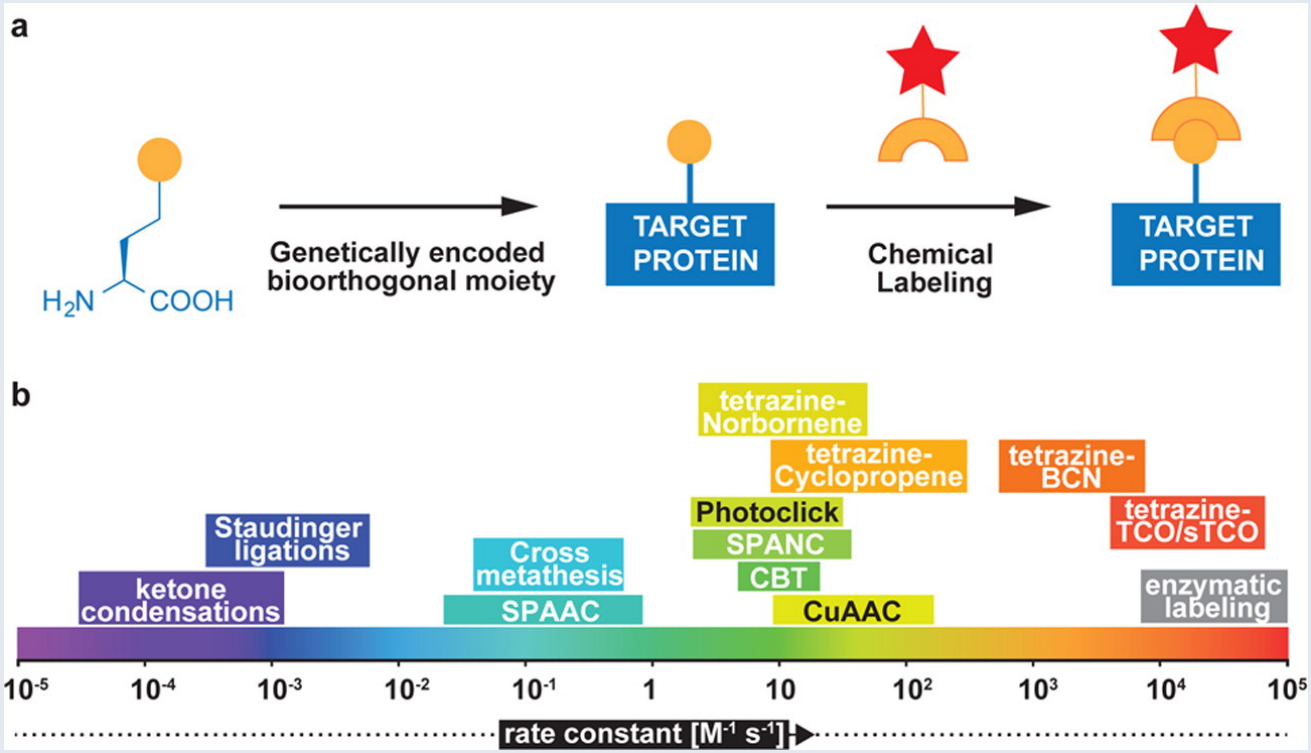
Difficulties

- The number of orthogonal tRNA-synthetase/tRNA pairs is small
  - Limit of two nCAAs that can be incorporated in any cell
  - The process of mutation and selection can be painstaking

K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806
S.B. Sun, P.G. Schultz, C.H. Kim, *ChemBioChem*, **2014**, *15*, 1721-1729

Conclusions and Scope

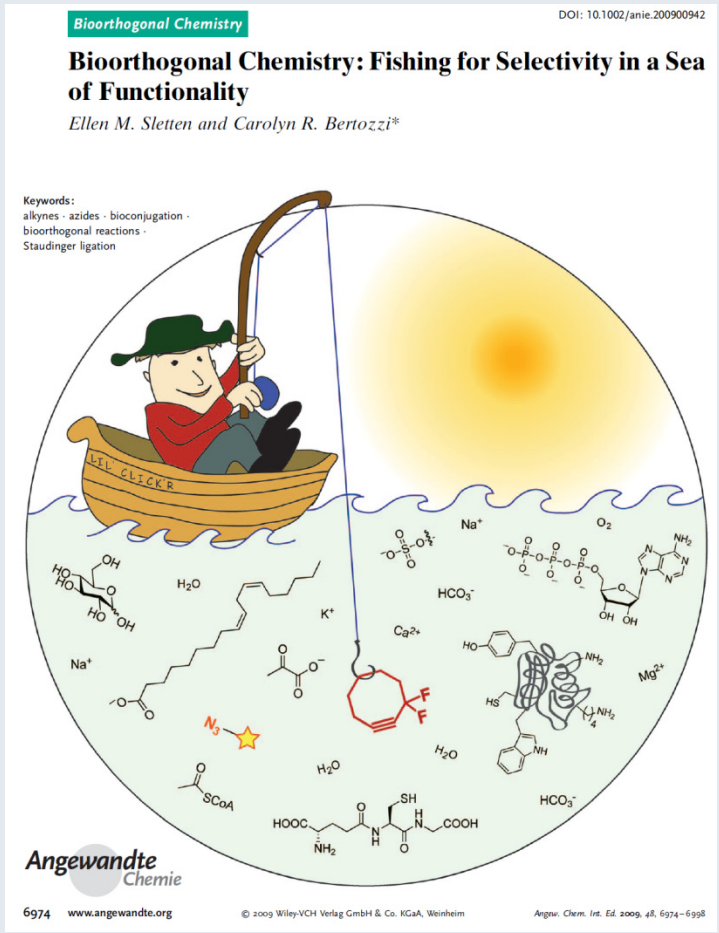
- Bioorthogonal chemistry has been developed
  - Chemoselective
  - Non-toxic/no byproducts



K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Conclusions and Scope

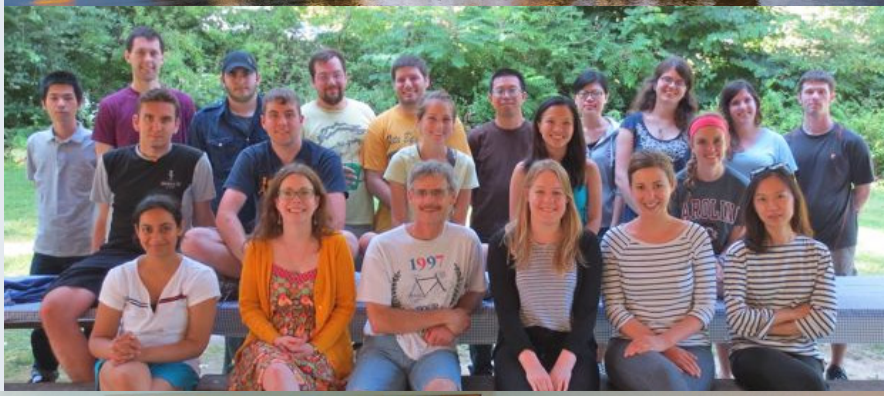
- Bioorthogonal chemistry has been developed
  - Reactions with faster kinetics are still needed
  - Need to investigate quantitative reaction specificity
  - Need to further optimise and analyse site-specific labelling in mammalian cells
- Some intracellular imaging difficulties need to be addressed
  - Selective imaging/washing out labelling probe
  - More easily adapted fluorescent probes are also needed to couple with this work
- As new reactions/reagents are discovered, the scope and utility of this field will increase

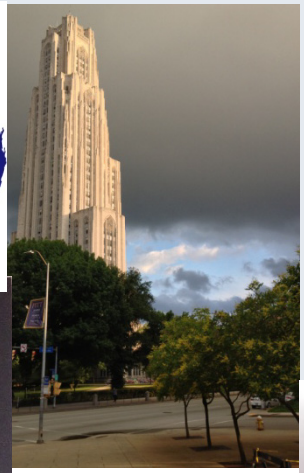


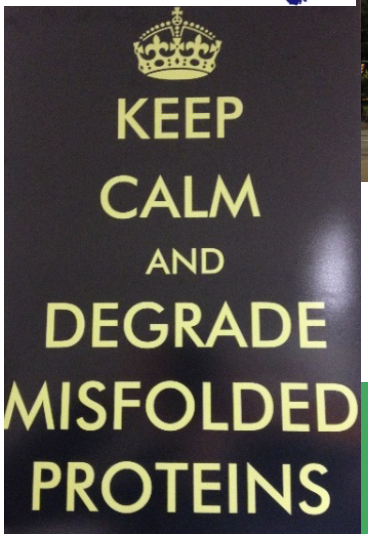
K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806
P. Shieh, C.R. Bertozzi, *Org. Biomol. Chem.*, **2014**, *12*, 9307-9320
H.-W. Shih, D.N. Kamber, J.A. Prescher, *Curr. Opin. Chem. Biol.*, **2014**, *21*, 103-111

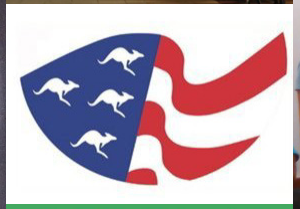
Acknowledgements

- Dr Peter Wipf
- Dr Jeff Brodsky
- Wipf and Brodsky Group Members (past and present)









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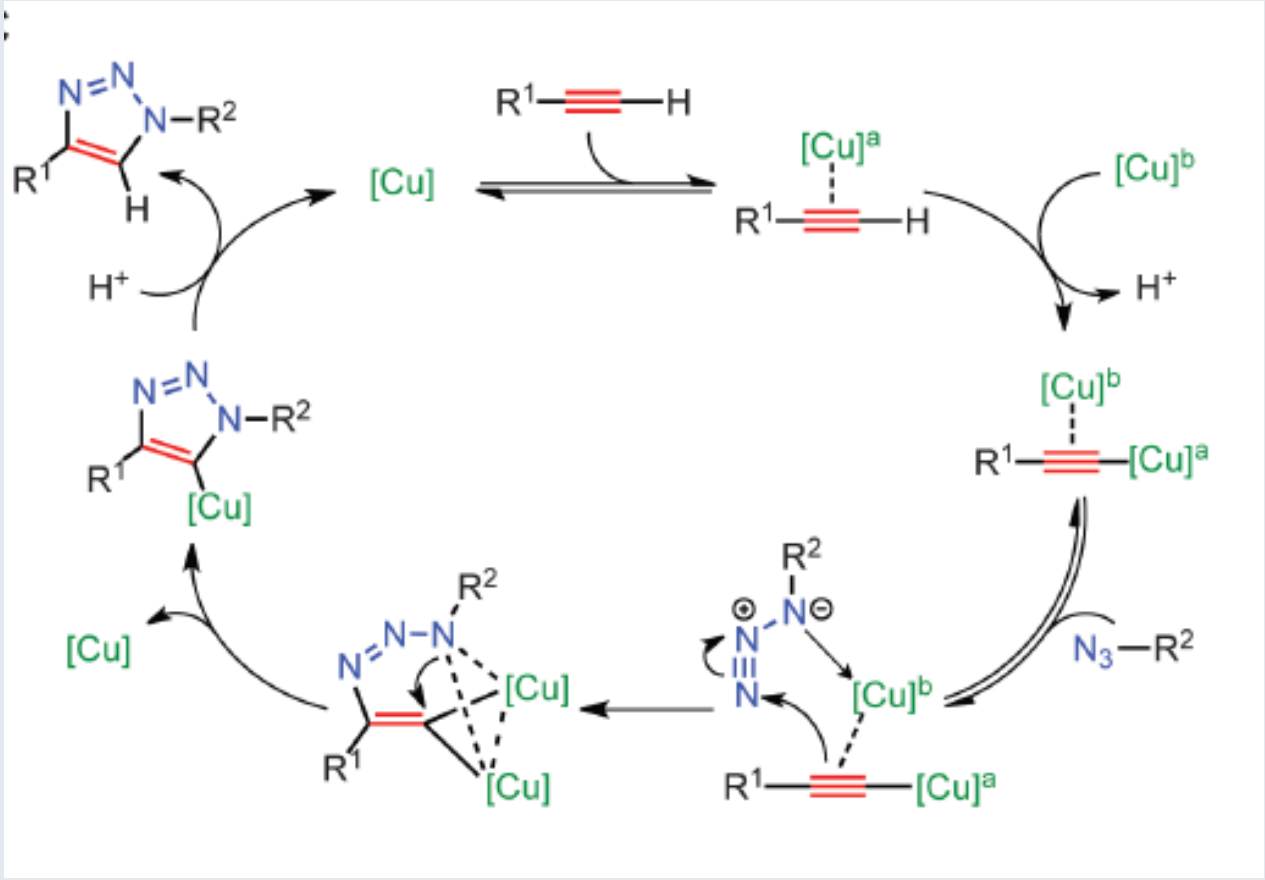
HOWARD HUGHES
MEDICAL INSTITUTE

Azide-Alkyne Reactions ([3+2] Cycloadditions)

Copper Catalysed Azide-Alkyne 1,3-Dipolar Cycloaddition (CuACC)



B.T. Worrell, J.A. Malik, V.V. Fokin, *Science*, **2013**, 457-460

Copper Catalysed Azide-Alkyne 1,3-Dipolar Cycloaddition (CuACC)

- **Water soluble ligands mechanism**

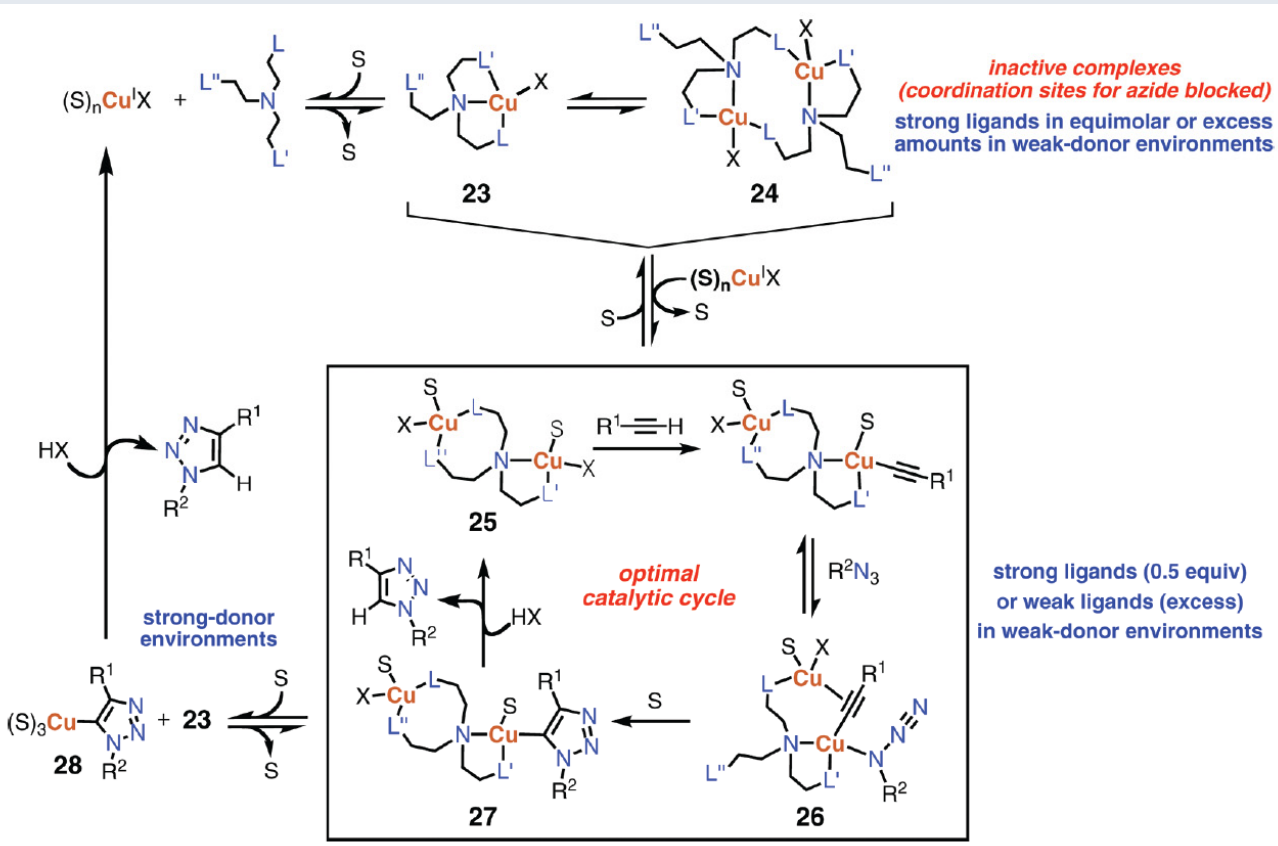
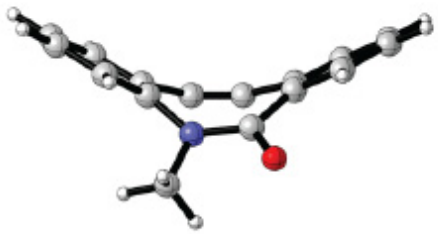


Figure 7. Proposed mono- and binuclear speciation in the CuAAC process. X = anionic donor such as σ-acetylide, halide, hydroxide, or triazolide; S = neutral intermolecular donor such as DMSO, other solvent, π-alkyne, or organic azide. Productive complexes are not formed when only one heterocyclic donor arm (L) exists.

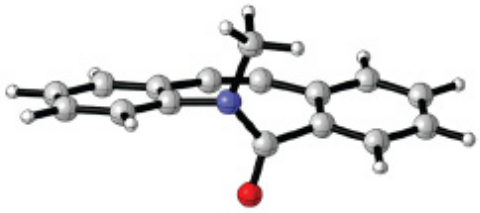
S.I. Presolski, V. Hong, S.-H. Cho, M.G. Finn, *J. Am. Chem. Soc.*, **2010**, *132,* 14570-14576

Azide-Alkyne Reactions ([3+2] Cycloadditions)

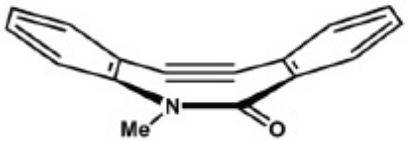
- **Calculated and X-ray strain of BARAC strained cyclooctyne**

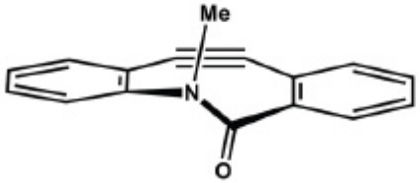


cis-BARAC

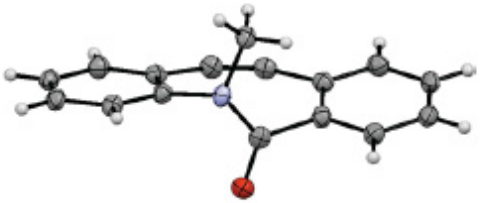


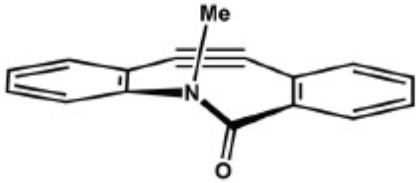
trans-BARAC





B3LYP/6-31G(d)



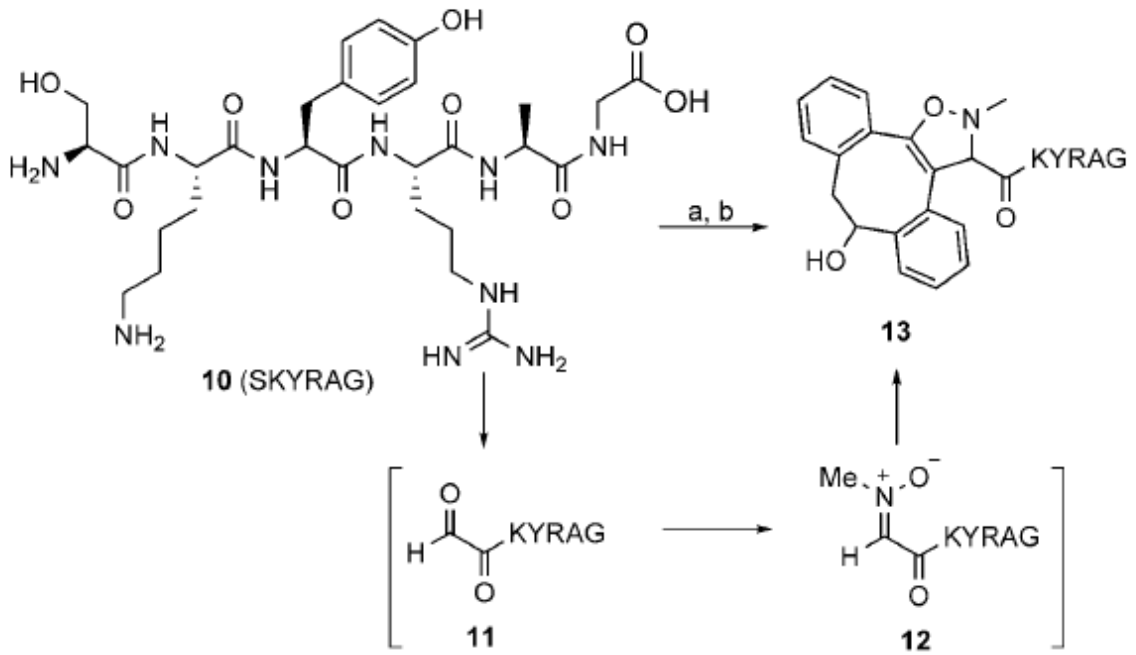


X-ray crystal structure

C.G. Gordon, J.L. Mackey, J.C. Jewett, E.M. Sletten, K.N. Houk, C.R. Bertozzi, *J. Am. Chem. Soc.*, **2012**, *134*, 9199-9208

Nitrone-Cyclooctyne Reactions ([3+2] Cycloadditions)

- **Synthesis of nitrone bound to biomolecules**

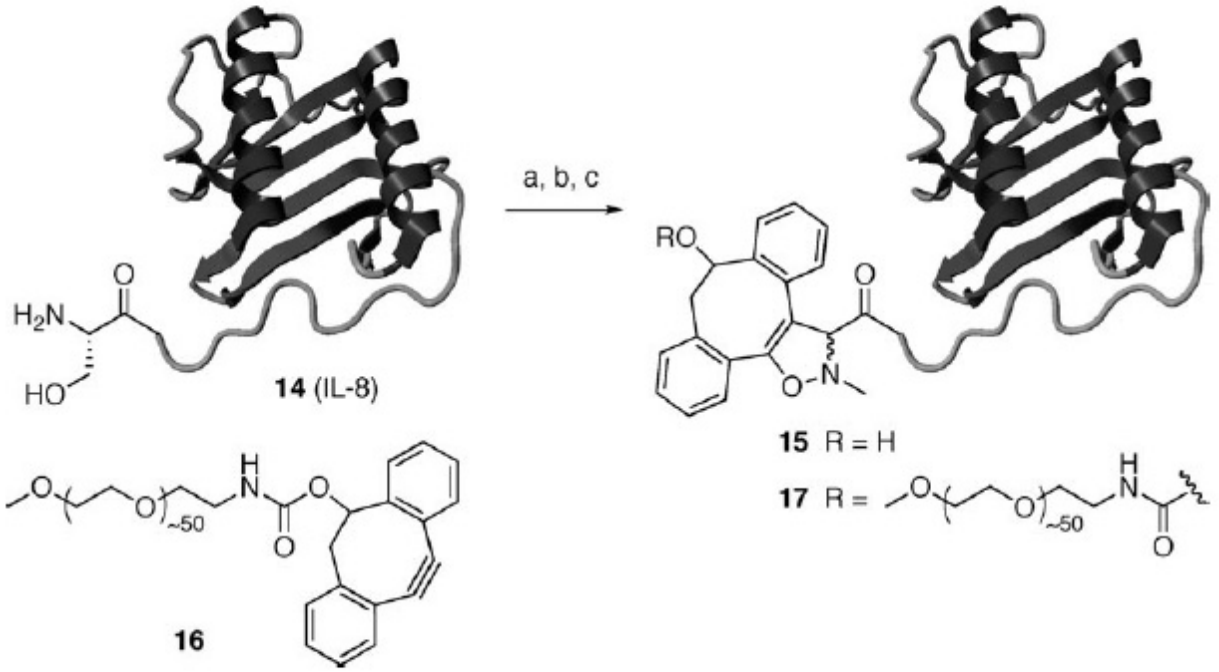


Scheme 1. One-pot N-terminal conjugation of a hexapeptide by SPANC: a) 1. $NaIO_4$, NH_4OAc buffer, pH 6.8, room temperature, 1 h; 2. *p*-$MeOC_6H_4SH$, room temperature, 1 h; then *p*-$MeOC_6H_4NH_2$, MeHNOH·HCl, room temperature, 20 min; b) **2**, room temperature, 1 h.

X. Ning, R.P. Temming, J. Dommerhalt, J. Guo, D.B. Ania *et al.*, *Angew. Chem. Int. Ed.*, **2010**, *49*, 3065-3068

Nitrone-Cyclooctyne Reactions ([3+2] Cycloadditions)

- **Synthesis of nitrone bound to biomolecules**



Scheme 2. One-pot N-terminal functionalization of IL-8 by SPANC: a) 1. $NaIO_4$, NH_4OAc buffer, pH 6.9, room temperature, 1 h; 2. *p*-$MeOC_6H_4SH$, room temperature, 2 h; b) *p*-$MeOC_6H_4NH_2$, MeNHOH·HCl, room temperature, 20 min; c) cyclooctynol **2** or PEG–cyclooctyne **16**, room temperature, 20 h.

X. Ning, R.P. Temming, J. Dommerhalt, J. Guo, D.B. Ania *et al.*, *Angew. Chem. Int. Ed.*, **2010**, *49*, 3065-3068

Nitrone-Cyclooctyne Reactions ([3+2] Cycloadditions)

- **Synthesis of nitrone bound to labelling tags**

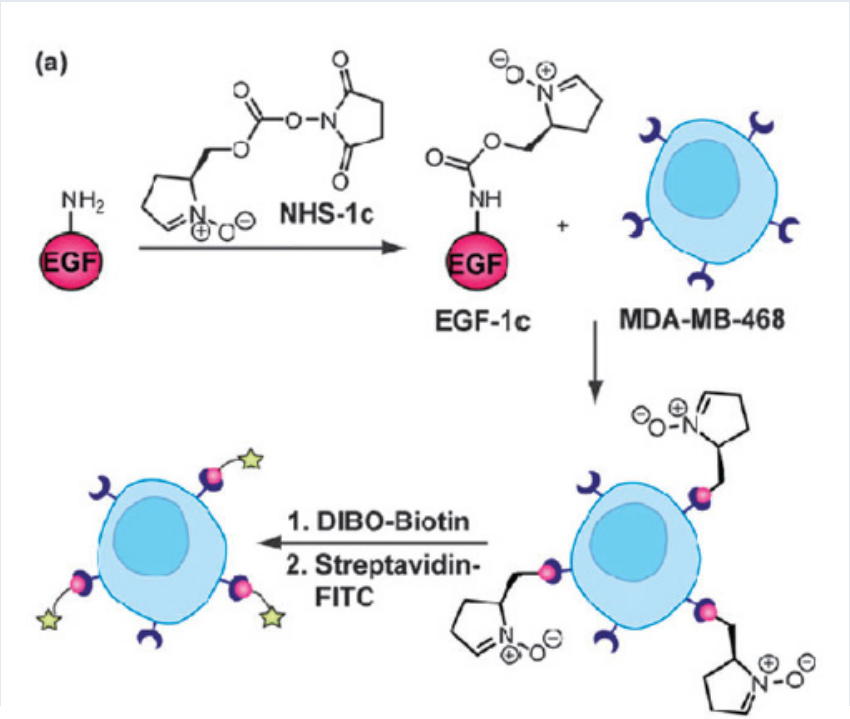
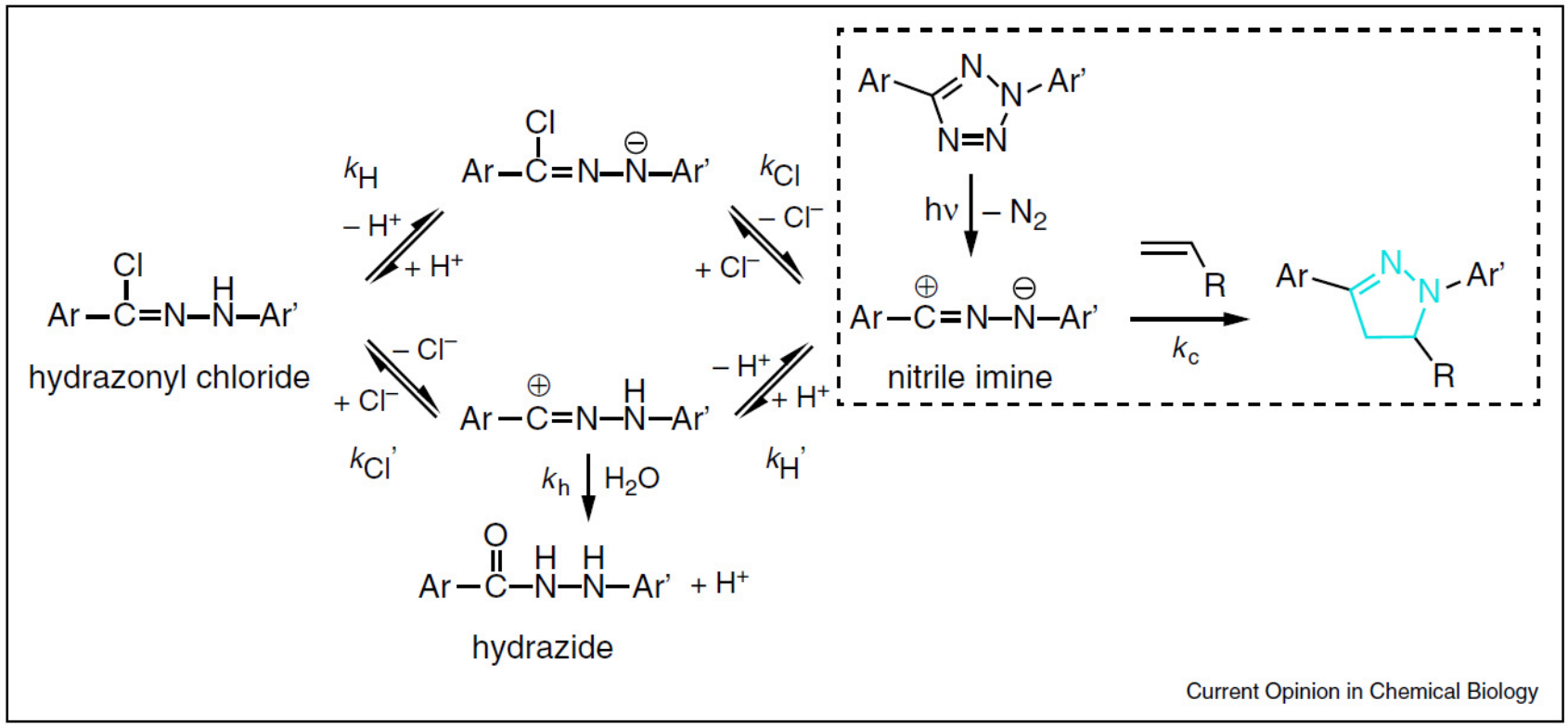


Fig. 1 *In situ* labeling of EGF–EGFR interactions *via* SPANC in MDA-MB-468 cells. (a) Cyclic nitrone modified EGF-**1c** bound to EGFR was labeled by SPANC with **2b**-biotin for 30 min prior to streptavidin-FITC fluorescent labeling. (b) Fluorescence (top) and

C.S. McKay, J.A. Blake, J. Cheng, D.C. Danielson, J.P. Pezacki, *Chem. Commun.*, **2011**, *47*, 10040-10042

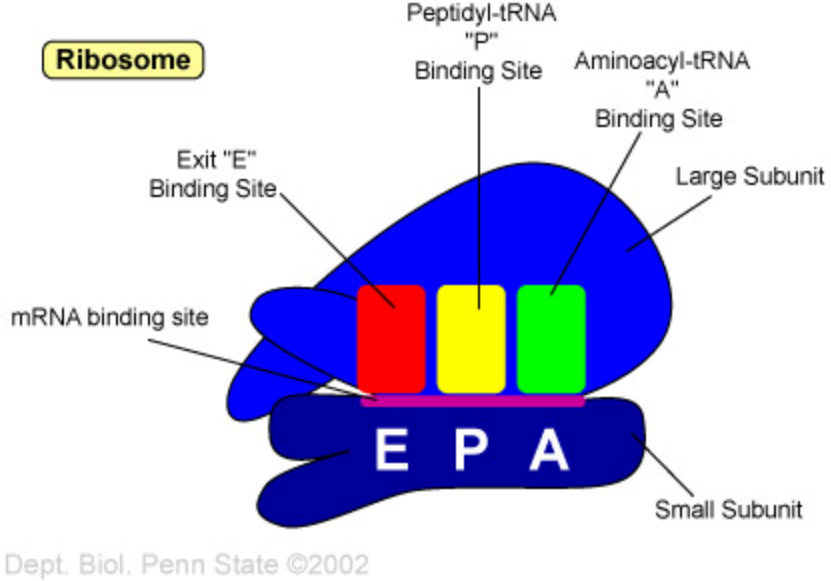
Alkene-Tetrazole Reactions (Photoclick Cycloaddition)

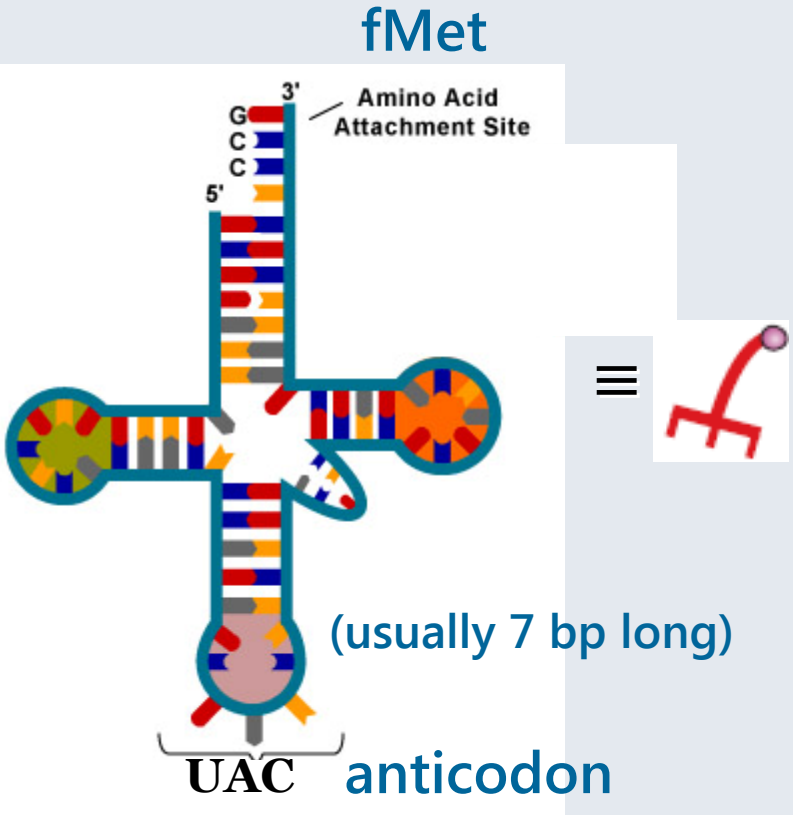
- **Mechanism of nitrile imine interconversion**



C.P. Ramil, Q. Lin, *Curr. Opin. Chem. Biol.*, **2014**, *21*, 89-95

Protein Translation 101 (Prokaryotes)





16S rRNA (30S subunit)

UCCUCC
AGGAGG

(usually 7 bp long)

5' mRNA

Shine-Delgarno sequence

(start) codon

T. M. Schmeing, V. Ramakrishnan, *Nature*, **2009**, *461*, 1234-1242
https://wikispaces.psu.edu/display/Biol230WCE/Protein+Translation