Bioorthogonal chemistry for chemical biology applications

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1. Concepts of bioorthogonal chemistry

Chemical biology



Background

Need for holistic view of living systems (within cells too)

Tracking in native environment

Natural reporters (e.g. Trp)

GFPs (size, monomodal, only directly encoded proteins)

Antibody conjugates (more general, multimodal, size)

Background

► Tagging biomolecules (traditional ways – SH, NH₂ etc)

Chemical reactions blending

Simplicity of GFP

High specificity of antibodies

Versatility of small molecule probes

Bioorthogonal reactions

Background

Non-native functions or non-native combinations of natural functions

- Stable under physiological conditions
- Biocompatible and biotolerable starting materials and products
- Stable products
- Small functions
- Fast, selective, high yielding reactions with complementary function

Bioorthogonal reactions

CLICK REACBIOCOMPATIBLE REACTIONSselective high yields modular inoffensive by	TIONS
BIOCOMPATIBLE REACTIONS selective high yields modular inoffensive by	
	products
physiologically stable non toxic materials small functionsready availabit benign solvent stereospecifici non-sensitive fast	lity ty materials

BIOORTHOGONAL REACTIONS

Bioorthogonal schemes

- Two-step labeling strategy
- Incorporation of a bioorthogonal function
- Modulation with a signaling unit

Bioorthogonal modules

Target biomolecule : protein, lipid, nucleic acid etc.

Chemical reporter : non-native, non-perturbing chemical handles that can be modified in living systems through highly selective reactions with exogeneously delivered probes

Signaling unit : fluorescent, radioactive, NMR active nuclides, PET etc.

Implementation of chemical reporters

Metabolism (bioorthogonalized metabolites)

- Genetic encoding (non-canonical amino acids)
- Enzymatically (e.g. PCR)

Chemical ligation (unique natural motifs)

- C. R. Becer, R. Hoogenboom, U. S. Schubert (2009) Angew. Chem. Int. Ed. 48, 4900
- K. E. Beatty (2011) Mol. BioSyst. 7, 2360

2. Bioorthogonal reactions



Bioorthogonal reactions

Polar reactions

Nuclepohiles and elecrophiles are involved

Schiff bases of hydrazines and oximes

Cycloadditions

- Dipolar cycloadditions
- Diels-Alder reactions

Miscallenious reactions

- C. R. Becer, R. Hoogenboom, U. S. Schubert (2009) Angew. Chem. Int. Ed. 48, 4900
- K. E. Beatty (2011) Mol. BioSyst. 7, 2360

2.1. Polar reactions

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Polar reactions

- Oxime ligations
- Staudinger ligation (azides + phosphanes)
- Cyanobenzothiazole condensation
- ► Native chemical ligation
- Unnatural motifs (His₆, Cys₄)

- C. R. Becer, R. Hoogenboom, U. S. Schubert (2009) Angew. Chem. Int. Ed. 48, 4900
- K. E. Beatty (2011) *Mol. BioSyst.* 7, 2360

Aldehyde / ketone condensations



Aldehydes / ketones + amines

Aldehydes / ketones are relatively rare

Amines are present everywhere

Equilibrium process - oxo form is favored at physiological conditions)

Alpha-effect nucleophiles - hydrazine, aminooxy compounds, Schiff base is favored

• P. Crisalli, E. T. Kool (2013) J. Org. Chem. 78, 1184

Oxime ligations

- ► Rate 10⁻³ M⁻¹s⁻¹
- Products can hydrolyze slowly in cells
- Ketones and aldehydes are present in sugars (not truly bioorthogonal)
- More stable product with aminooxy-tryptamine

• P. Crisalli, E. T. Kool (2013) J. Org. Chem. 78, 1184

Oxime ligations – aminooxy tryptamine¹⁷



• P. Agarwal et al. (2012) PNAS. 110, 46

Azides as chemical reporters

- Abiotic, absent from living systems
- Stable in water, resistant to oxidation (unstable at elevated temperatures)
- Biocompatible (eg. AZT, but inorganic azides are toxic)
- Soft electrophile do not react with "hard" nucleophiles (thiols can reduce it at higher temperatures)
- Dipole, reacts readily with dipolarophiles
- Aryl azides may photodegrade
- Large energy content



Staudinger reaction

Azide acts as an electrophile (Soft)



Staudinger ligation

The phosphane contains an intramolecular electrophilic trap



• Saxon et al. (2000) *Science*,287, 2007.

Staudinger ligation

Fully biocompatible

Slow kinetics ($k_2 = 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$)

EDGs to phosphine accelerates a bit

► High concentration, high background fluorescence

Spontaneous oxidation of phosphane

Phosphanes are metabolized by cythochromes

[•] Saxon et al. (2000) Science,287, 2007.

Cyanobenzothiazole ligation



Fully biocompatible

Good kinetics ($k_2 = 9.2 \text{ M}^{-1} \text{ s}^{-1}$)

Side reactivity with thiols

• Rao et al. (2009) Angew. Chem. Int. Ed., 51, 9658.

Native Chemical Ligation (NCL)



• Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. (1994). Science, 266, 776

Native chemical ligation

► N- terminal cysteine, selenocysteine

- Thioester
- High yields
- Thiols catalyze the first step
- Mainly for post-synthetic /translational modifications or fragment condensation.
- Thioester synthesis is challenging

Thiol oxidation

• Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. (1994). Science, 266, 776

Genetically encoded tags (Cys₄)
CCXXCC (X= Pro, Gly) – singularity is ensured
Biarsenical probes FIAsH and ReAsH tagging
Isolated Cys residues do not perturb
Fluorogenic
Limited to proteins

His6 tags (Ni-NTA probes)

• Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. (1994). Science, 266, 776



• B. A Griffin, et al. (1998). *Science, 281*, 269



• B. A Griffin, et al. (1998). *Science, 281*, 269



- Mainly for affinity chromatography
- B. A Griffin, et al. (1998). Science, 281, 269

2.2. Cycloadditions



- Azide acts as a dipole
- Alkynes are also rare, inert and stable functions
- Reaction is thermodynamically favored, but requires activation
- Heat, EWG groups or catalyst



• Huisgen, R. (1963) Angew. Chem. Int. Ed. 2, 565.

Cu(I) catalyzed – "click"- reaction



- V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless (2002) *Angew. Chem. Int. Ed.* 41, 2596.
- C. W. Tornøe, C. Christensen, M. Meldal (2002) J.Org. Chem. 67, 3057

Generally usable

Regiospecific

► Fast

► Wide variety of solvents, temperatures

Ascorbate generates Cu(I), and complexes

Copper cytotoxicity

- V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless (2002) Angew. Chem. Int. Ed. 41, 2596.
- C. W. Tornøe, C. Christensen, M. Meldal (2002) J.Org. Chem. 67, 3057





Coordinating ligands



Effects of ligands - reaction speed


Effects of ligands - cytotoxicity



• W. T. Worell et al. (2013) *Science, 340*, 457.

Strain promoted azide-alkyne cycloaddition



- C. P. Ramil, Q. Lin (2013). *Chem. Commun. 49*, 11007
- C. R. Bertozzi, et al., (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 1821

Strain promoted azide-alkyne cycloaddition

- Decrease energy of LUMO
- Decrease gap between HOMO and LUMO
- Increase ground energy by distortion
- Approach TS structure by distortion



• D. H. Ess et al. (2008) Org. Lett. 10, 1633

Reactivity of cyclooctynes

- Electron withdrawing substituents
 Ring fusion
 Incorporation of sp² atoms
- Stability vs. reactivity
 A^{1,3} strain
 Long synthetic routes

Cyclooctyne reactivity



• C. P. Ramil, Q. Lin (2013). Chem. Commun. 49, 11007

Cyclooctyne reactivity





- ~ 25 kJ/mol ⊿G[‡]
- $k_2 = 0.05 \text{ M}^{-1}\text{s}^{-1}$

• K. Chenoweth, D. Chenoweth, W. A. Goddard III. (2009) Org. Biomol. Chem. 7, 5255.

Cyclooctyne reactivity







9-TS1 ∆E[‡]=12.7 (∆G[‡]=25.3)



10-TS1 ΔE[‡]=11.0 (ΔG[‡]=23.3)



4,5

5,6





9-TS2 ∆E[‡]=12.0 (∆G[‡]=24.9)

10-TS2 ΔE[‡]=11.3 (ΔG[‡]=23.5)



ΔE[‡]=10.0 (ΔG[‡]=22.1)

• K. Chenoweth, D. Chenoweth, W. A. Goddard III. (2009) Org. Biomol. Chem. 7, 5255.

Cyclooctyne stability



- 5 % decomposition after 44 h. at 25 °C, water / acetonitrile
- No decomposition after 2 h at 37 °C, 30% decomposition after 9 h
- ▶ 9 % reaction with glutathione at 25 °C after 14 h

• B. R. Varga, M. Kállay, K. Hegyi, Sz. Béni, P. Kele (2012) Chem. Eur. J. 18, 822.

Comparision of cyclooctynes



• B. R. Varga, M. Kállay, K. Hegyi, Sz. Béni, P. Kele (2012) Chem. Eur. J. 18, 822.

Photoinduced SPAAC



Fluorogenic SPAAC



- A. Poloukhtine et al. (2009) J. Am. Chem. Soc. 131,15769.
- J. C. Jewett, C. R. Bertozzi (2011) Org. Lett. 13, 5937.

Reaction with nitrones



- One order of magnitude faster (nitrone: 10⁻² 10 M⁻¹ s⁻¹) Nitril oxide: k₂ = 10-30 M⁻¹ s⁻¹)
- Especially in water

• N. Xinghai (2010) Angew. Chem. Int. Ed. 49, 3065.

Inverse electron demand Diels-Alder reaction



Tetrazines with strained alkenes / alkynes



Very fast (k₂ = 1 - 10³ M⁻¹s⁻¹)
 Stability vs. selectivity
 Difficult synthesis

A.C. Knall, C. Slugovc (2013) Chem. Soc. Rev. 42, 5131.

Diels-Alder reactions



A.C. Knall, C. Slugovc (2013) Chem. Soc. Rev. 42, 5131.

Diels-Alder reactions



A.C. Knall, C. Slugovc (2013) Chem. Soc. Rev. 42, 5131.

Mechanism



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Diels-Alder reactions



- Lower LUMO energy of diene (tetrazine)
- Steric demand of substituents
- Stability vs. reactivity

Steric demand vs. LUMO energy

Tetrazine	LUMO (eV)	k ₂ (vinyl-ether) / M ⁻¹ s ⁻¹
	0.28	1.20 × 10 ⁻³
$\xrightarrow{Et_2N} \qquad $	0.46	8.0 × 10 ⁻³
$\xrightarrow{Et_2N}_{O} \xrightarrow{N}_{N} \xrightarrow{N}_{N} \xrightarrow{N}_{N} CH_3$	0.83	1.45 × 10 ⁻³
$\xrightarrow{Et_2N}_{O}\xrightarrow{N}_{N}\xrightarrow{N}_{N}\xrightarrow{N}_{N}H$	0.71	2.40 × 10 ⁻²

G. B. Cserép, O. Demeter, E. Bätzner, M. Kállay, H-A. Wagenknecht, P. Kele In preparation

Reactivity vs stability



G. B. Cserép, O. Demeter, E. Bätzner, M. Kállay, H-A. Wagenknecht, P. Kele In preparation

Cross-reactivity



• T. Plass et al (2012) Angew. Chem. Int. Ed., 51, 4166

Cross-reactivity



Cross-reactivity



Photoinduced tetrazole-alkene cycloaddition





Fast

Spatial and temporal resolution

► Fluorogenic, but UV excitation



Fast

Spatial and temporal resolution

EWGs on Ar increases HOMO energy, and reaction rate

Fluorogenic, but UV excitation (305 nm works the best)

• K. V. reyna et al (2011) Acc. Chem. Res., 44, 828



• Yu et al (2011) Bioorg. Med,. Chem. Lett., 21, 5033.

Tetrazole	$\lambda_{\max} \stackrel{b}{=} (nm)$	ε ₃₀₂ (M ⁻¹ cm ⁻¹)	ε ₃₆₅ (M ⁻¹ cm ⁻¹)	ε ₃₉₅ (M⁻¹ cm⁻¹
<i>p</i> -amino ^c	310	20500	3500	N.D.
1	338	7103	11908	1935
2	324	16415	1813	1042
3	332	25838	907	682
4	346	28892	35177	6167
5	360	9035	30819	8184

• Yu et al (2011) Bioorg. Med,. Chem. Lett., 21, 5033.

2.3. Miscallaneous ligations



Quadricyclane ligation



- Quadricyclane is abiotic stable (fully saturated)
- Highly strained (80 kcal/mol)
- Reacts with electron poor π-systems (but not with simple alkenes or cyclooctynes) k₂ = 0.25 M⁻¹ s⁻¹
- Diethyldithiocarbamate prevents photoreversion
- E. Sletten et al (2011) J. Am. Chem. Soc., 133, 17570.

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Tyrosine specific tagging



mega-Stokes, near infra-red dye



G. B. Cserép, A. Herner, O. S. Wolfbeis, P. Kele (2013) Bioorg. Med. Chem. Lett. 23, 5776

Tyrosine specific tagging



G. B. Cserép, A. Herner, O. S. Wolfbeis, P. Kele (2013) Bioorg. Med. Chem. Lett. 23, 5776

Cysteine targeting



G. B. Cserép, Zs. Baranyai, D. Komáromy, K. Horváti, Sz. Bősze, P. Kele Under review

Others...

Ru-catalyzed olefin metathesis

- Tetrazines with isonitriles
- Suzuki and Sonogashira couplings

• E. Sletten et al (2011) *J. Am. Chem. Soc.*, *133*, 17570.

Summary

Reaction type	Reactant 1	Reactant 2	Approximate rate constant (M ⁻¹ s ⁻¹)	Comments	References
Aldehyde/ketone condensation	O R H(CH₃)	$ \begin{array}{c} H_2N-O-R \\ O \\ H_2N-N \\ H \\ R \end{array} $	0.001 (H ₂ O)	adducts prone to hydrolysis; aniline catalyst can be used	Jencks 1959
		H ₃ C-N-O N HO O Pictet- Spengler	0.26 (100 mM sodium phosphate)	reaction provides more stable C-C linkages	Agarwal 2013
Staudinger ligation	R-N ₃	O O O O Me PPh ₂	0.003 (PBS)	phosphines susceptible to oxidation	Saxon 2000
Cyanobenzothiazole condensation		S C≡N	9.19 (PBS)	side reactivity with free thiols	Rao 2009

• E. Sletten et al (2011) J. Am. Chem. Soc., 133, 17570.

Summary

CuAAC	R−N ₃	──R, Cu(I)	k _{obs} 10-100 (10-100 μΜ Cu)	copper catalyst required	Tornoe 2002
Strain-promoted azide-alkyne cycloadditions (SPAAC)		OCT, DIFO, BCN	0.0012-0.14 (ACN)	no metal catalyst; some octynes susceptible to thiol attack	Agard 2004
		BARAC, DIBO, DIBAC	0.17-0.96 (ACN)		Jewett 2010
Alternative 1,3-dipolar cycloadditions		R	0.013-3.9 (ACN/H ₂ O)	some nitrones susceptible to hydrolysis	McKay 2010
	R—≡ [⊕] N-O	H	30 (H ₂ O)	nitrile oxide generated <i>in situ</i> (photolysis)	Gutsmiedl 2009
		OMe	0.15-58 (1:1 ACN:PBS)	nitrile imine generated in situ (photolysis)	Yu 2012
	$\stackrel{\oplus}{\underset{R}{\overset{\ominus}}} \overset{\ominus}{\underset{N}{\overset{\otimes}}}$	R	13.5 (ACN/H ₂ O)	diazo generated from azide precursor	McGrath 2012
	R−N ₃	O R R	70,000-106,000 (H ₂ O)	oxanorbornadiene susceptible to reactivity with basic amino acids	van Berkel 2007
Summary

Inverse Electron-Demand Diels-Alder (IED-DA)	R	$R \xrightarrow{N=N} R'$ N-N	210-2,800,000 (PBS, 37°C)	TCO can isomerize over time	Blackman 2008
	R		0.12-9.46 (95:5 H ₂ O:MeOH)	norbornene and functionalized cyclopropenes are shelf stable	Devaraj 2008
	H Me		0.03-13 (12-15% DMSO in PBS)		Yang 2012 Patterson 2012
Hetero-Diels-Alder	RS	O N	0.0015 (5:1 H ₂ O:MeOH)	quinone methide generated <i>in situ</i>	Li 2013
Miscellaneous ligations	R	XR, Ru(II)	0.03-0.3 (PBS/tBuOH)	ruthenium catalyst required	Lin 2013
	R	Ph Ni Ph S ^S S Ph	0.25 (PBS/EtOH)	requires nickel-stabilization of pi-electrons	Sletten 2011
	⊕ ⊝ R−N≡C	R- N-N R'	0.12-0.57 (THF/H ₂ O)	products can hydrolyze in water	Stockmann 2011
	Ar-X	R-B(OH) ₂ , [Pd]	N/A	palladium catalyst required; boronic acids are moderately cytotoxic	Chalker 2009
	Ar-X	──R, [Pd]		palladium catalyst required	Kodama 2007