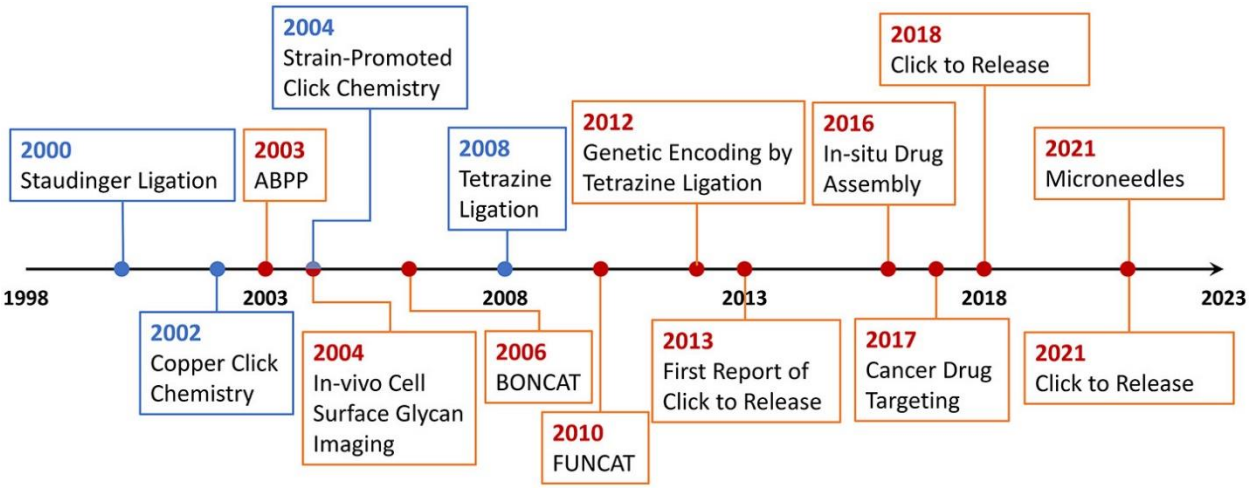
Bioorthogonal Chemistry 101

Introduction

Bioorthogonal chemistry is a set of reactions that can take place in biological environments without affecting biomolecules or interfering with biochemical processes. For this purpose, the reaction must meet the following requirements: fast, efficient, and specific.

- **pH**: The reaction must occur at the temperatures and pH of physiological environments.
- **Efficient**: The reaction must provide products selectively and in high yields and must not be affected by water or endogenous nucleophiles, electrophiles, reductants, or oxidants found in complex biological environments.
- **Fast**: the reaction must be fast, even at low concentrations, and must form stable reaction products.
- **Specific**: The reaction should involve functional groups not naturally present in biological systems

History



Rate constants comparisons

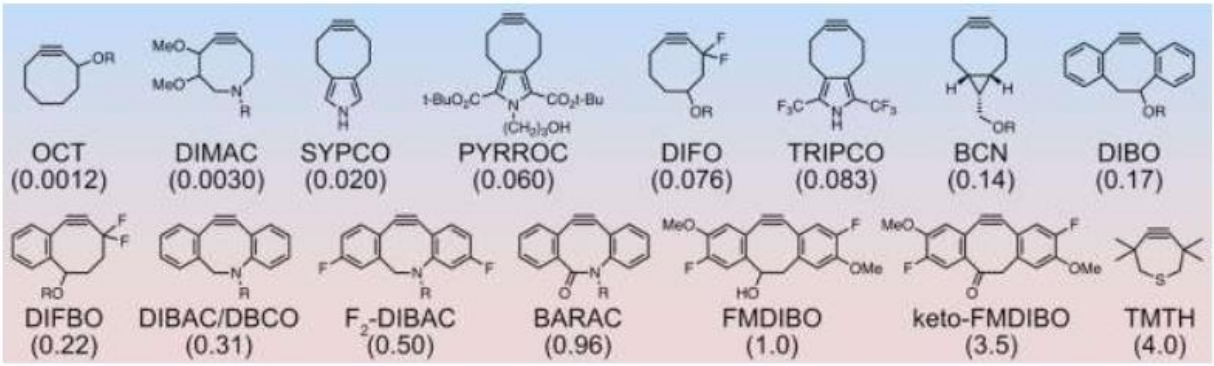


Figure 1. Chemical structures of various cyclooctyne derivatives in order of reactivity toward azides, rate constants (k_2, $M^{-1}\cdot s^{-1}$) for each of the derivatives are given in parentheses.

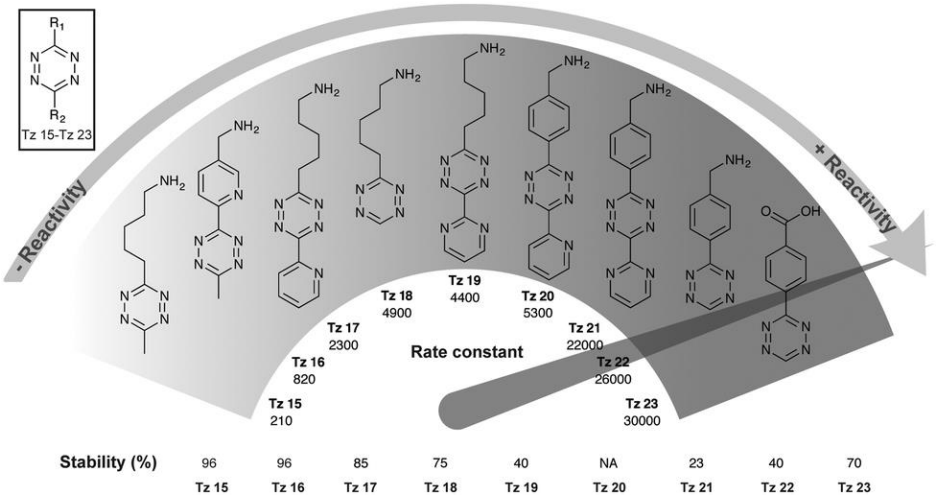
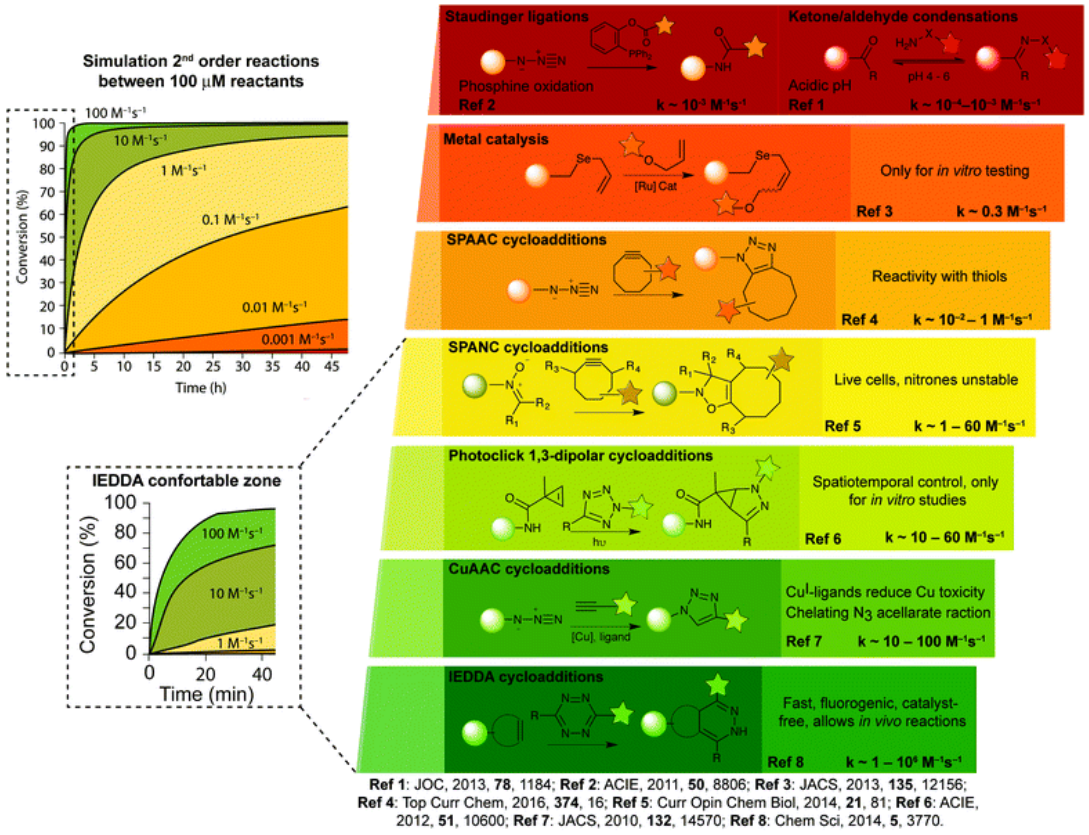


Fig. 2 Second order rate constants of selected tetrazines with TCO in PBS at 37 °C and corresponding stability assessed in PBS at 37 °C for 10 h. NA, not assessed



Ref 1: JOC, 2013, **78**, 1184; **Ref 2**: ACIE, 2011, **50**, 8806; **Ref 3**: JACS, 2013, **135**, 12156; **Ref 4**: Top Curr Chem, 2016, **374**, 16; **Ref 5**: Curr Opin Chem Biol, 2014, **21**, 81; **Ref 6**: ACIE, 2012, **51**, 10600; **Ref 7**: JACS, 2010, **132**, 14570; **Ref 8**: Chem Sci, 2014, **5**, 3770.

Fig. 3 Examples of bioorthogonal reactions useful for bioconjugation and general comments about their utility and challenges.

Strengths and weaknesses of bioorthogonal reactions

Table 1. Summary of Strengths and Weaknesses of Bioorthogonal Reactions

biorthogonal reactions	advantages	disadvantages
Staudinger ligation	Azides and phosphines are biocompatible, stable amide linkages	Slow reactions, phosphines prone to oxidation
CuAAC (azide + alkyne)	Fast reactions, *k* ~10-100($M^{-1}S^{-1}$) with 20uM Cu(I). Good regioselectivity	Despite some ligands such as THPTA to stabilize copper catalysts, copper toxicity remains a concern
SPAAC (azide + DBCO)	No use of copper catalysts *k*~ 1-60 ($M^{-1}S^{-1}$)	1.Reactions slower than CuAAC, bulky cyclooctynes difficult to incorporate into biomolecules 2.Preferred solvent: ethanol (10-40%) or DMSO(up to 60%)/ PBS buffer 3. pH < 5.5 rxn slow down because low stability DBCO? 4. thiol, sodium azide reacts with DBCO
IEDDA (TCO + Tz)	Very fast reactions, *k* ~ 1-10^6 ($M^{-1}S^{-1}$)	TCO has lower stability in aqueous environments

- CuAAC: Copper-Catalyzed Azide–Alkyne Cycloaddition;
- SPAAC: strain-promoted azide–alkyne cycloaddition;
- IEDDA: inverse electron demand Diels–Alder reaction