Computational chemistry-based improvement of bioconjugation reactions

2022/12/22

Literature seminar

M2 Habazaki

Contents

Features of Bioconjugations

Bioconjugations

- **The reaction of two molecules and a crosslinking agent that covalently links the components together.**
- At least one of the molecules is **of biological origin or is a fragment or derivative of a biomolecule**.
- Its use is **directed toward biological or life science applications**.
  - Assay and Quantification
  - Detection, Tracking, and Imaging
  - Purification, Capture, and Scavenging
  - Therapeutics and *In Vivo* Diagnostics
  - Vaccines and Immune Modulation

Bioorthogonal ligations

- **Unnatural functional group**
- High site specificity
- Within the complex milieu of a cell or a cell lysate

Chemoselective modifications

- **Natural functional group (mainly amino acid)**
- Immediate accessibility without the need for more specialist techniques

Hermanson *Bioconjugate Techniques* Elsevier Science, **2013**

Spicer, Davis *Nat. Commun.* **2014**, *5*, 4740

Requirements of Bioconjugations

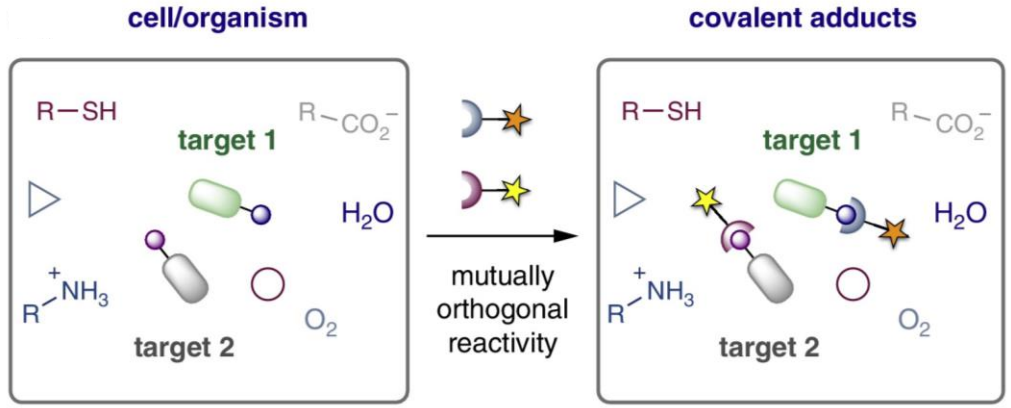
Fundamental requirements for bioconjugation reactions

- Mutual reactivity in a physiological environment (<37 °C, pH 6–8, aqueous solvent)
- **Rapid reaction**
- Near total conversion to generate homogenous constructs
- **High stability of reagents and adducts**
- High chemoselectivity
- Innocuous (or no) byproducts
- Not to disrupt biomoleculars' architecture and/or function

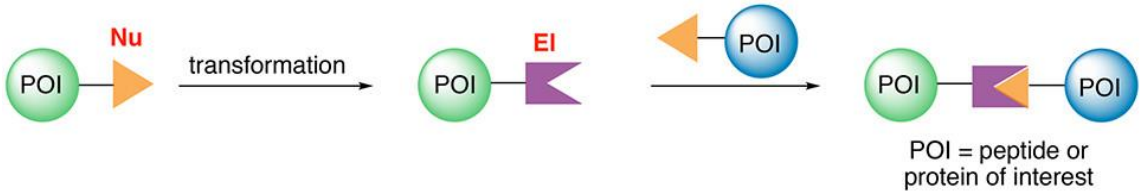
Requirements for more effective bioconjugation tools

- **Mutual orthogonality (a)**
- **Modularity (b)**
- Metabolic introduction of a unique functional group

(a) Mutual orthogonality



(b) Modularity



Prescher, Bertozzi *Nat. Chem. Biol.* **2005**, *1*, 13–21
Bird, Zhou *et al. Bioconjugate Chem.* **2021**, *32*, 2457–2479
Shih, Kamber, Prescher *Curr. Opin. Chem. Biol.* **2014**, *21*, 103–111
Baumann, Hackenberger *et al. J. Am. Chem. Soc.* **2020**, *142*, 9544−9552
Spicer, Davis *Nat. Commun.* **2014**, *5*, 4740

Importance of Mechanistic Elucidation and Improvement of Bioconjugations

Challenging points

- **The prerequisites critically restrict the scope of chemical reactions** that can be performed in a biological context.

Key point in development

An in-depth understanding of mechanisms and structural effects is essential for accessing new and improved versions of bioconjugation chemistry.

- Mechanistic principles
- Structure–reactivity trends

One Powerful Strategy (Today's Topic)

Computational Mechanistic Analysis using DFT Calculation to Improve Bioconjugation Reactions

Deb, Franzini *et al. Chem. Rev.* **2021**, *121*, 6850−6914

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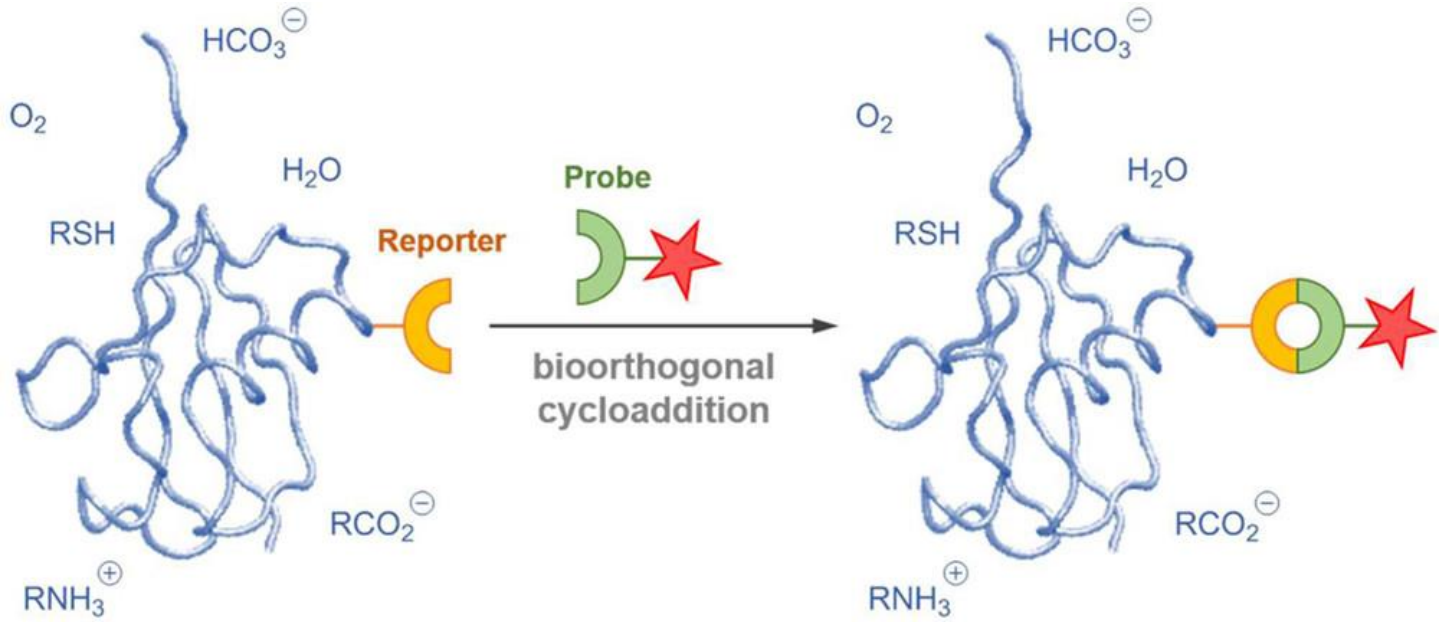
Features of Bioorthogonal Cycloadditions

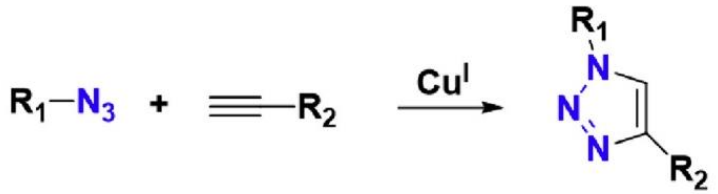
Bioorthogonal Cycloadditions

- **The most studied bioorthogonal reactions**
- **Fast reactions**, **Good regioselectivity**, **Well-established chemistry**
- **The contributions of computations** to this field began in the past decade and have matured.

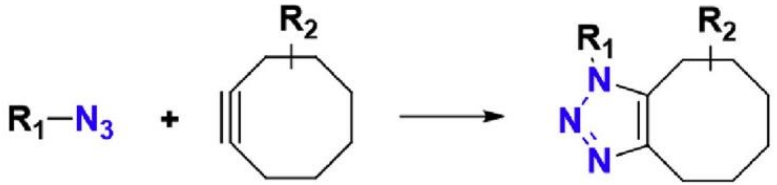


Classical click and bioorthogonal reactions

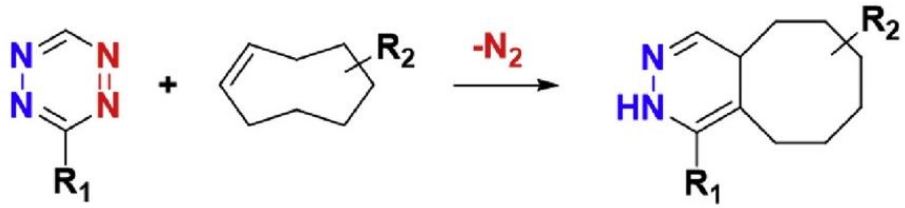
Cu-catalysed azide-alkyne cycloaddition (CuAAC)
(2002) metal-mediated



Strain-promoted azide-alkyne cycloadditions (SPAAC)
(2004) metal-free



Inverse electron-demand Diels-Alder cycloadditions (IEDDA, Tetrazine ligation)
(2008) metal-free



Liu, Liang, Houk *Acc. Chem. Res.* **2017**, *50*, 2297–2308

L'Islea, Unciti-Broceta *et al. Curr. Opin. Chem. Biol.* **2021**, *61*, 32−42

Computational Chemistry in Cycloaddition Reactions

Distortion/Interaction (D/I) model

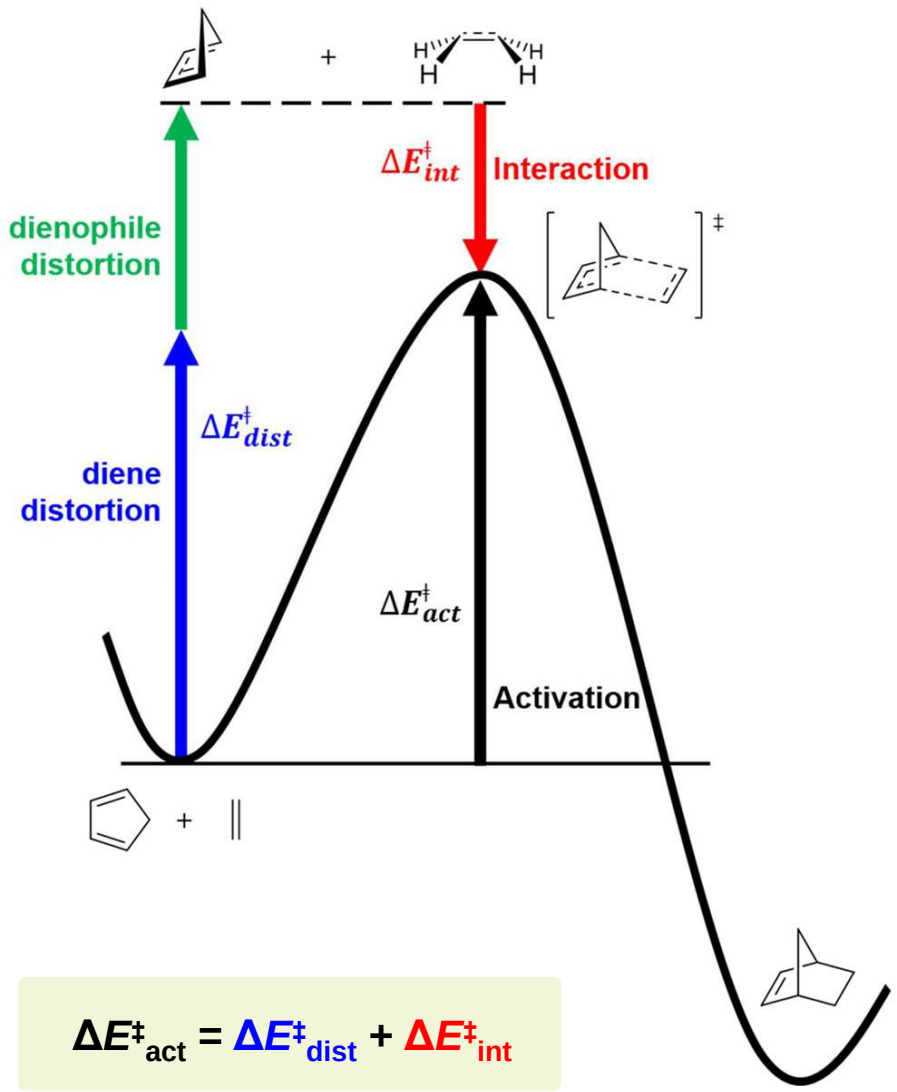
- A relatively new tool for chemists **to understand reactivities and selectivities**
  (By Prof. Morokuma → Prof. Houk & Prof. Bickelhaupt)

- ΔE_{act} is decomposed into two contributions:
  ① **The distortion energy** associated with the structural distortion (ΔE_{dist})
  ② **The interaction** between these increasingly distorted reactants (ΔE_{int})

※ **Δ*E*** (Electronic energy) & **Δ*G*** (Gibbs free energy)

- ***E***: The energy of the electrons in atoms, ions, or molecules, which are bound by the electric field of the nucleus
- $G = H - TS = (U + pV) - TS = (E + E_{trans} + E_{rot} + E_{vib}) + pV - TS$
- Arrhenius equation: $k = A \exp(-\Delta G^{‡} / k_B T)$

E → for analysis of reactivity trend, ***G*** → for prediction of reaction rate

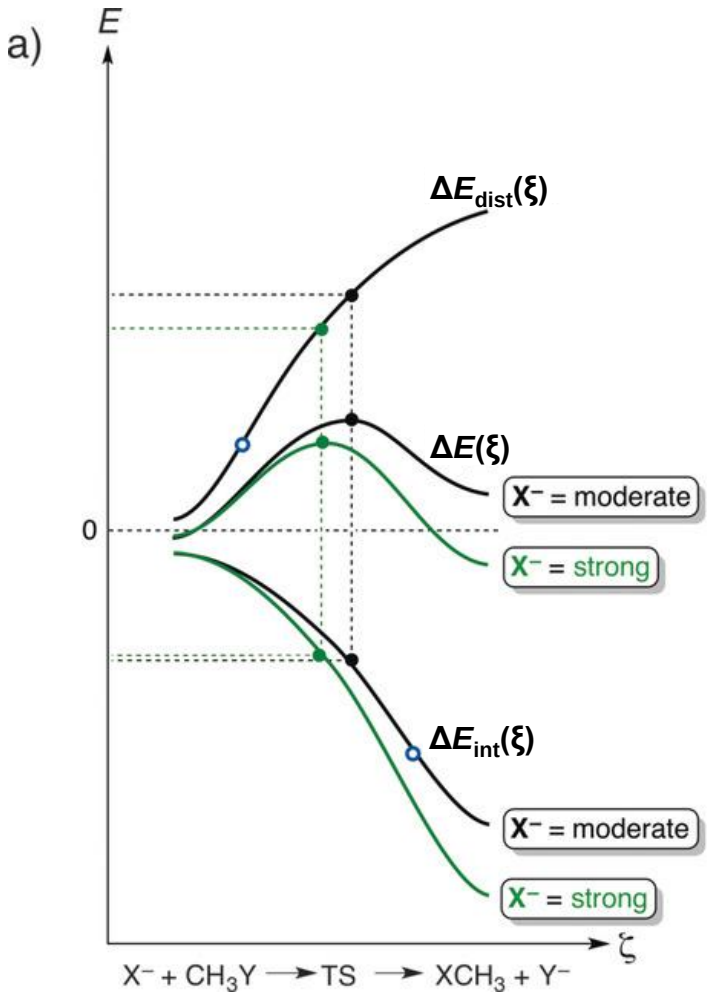


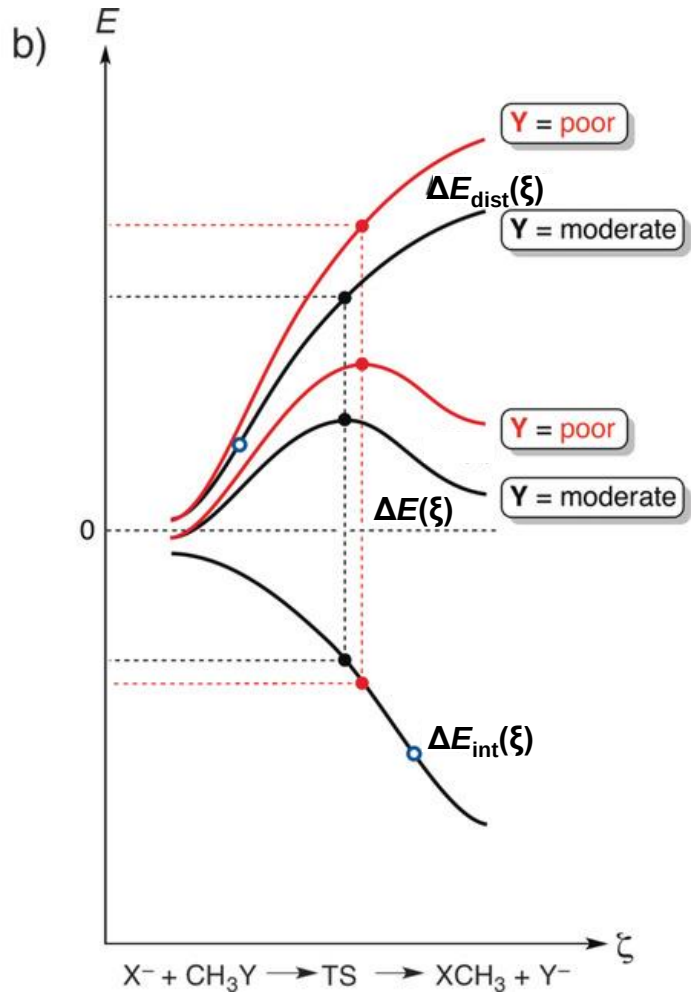
$\Delta E^{‡}_{act} = \Delta E^{‡}_{dist} + \Delta E^{‡}_{int}$

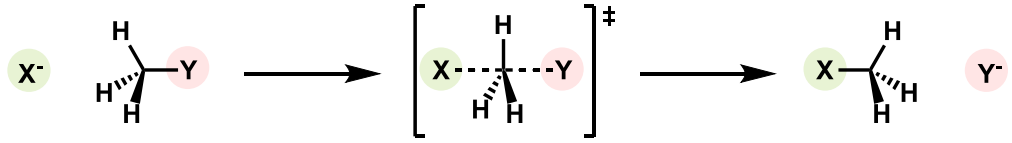
Bickelhaupt, Houk *et al. Angew. Chem., Int. Ed.* **2017**, *56*, 10070–10086
Liu, Liang, Houk *Acc. Chem. Res.* **2017**, *50*, 2297–2308

Distortion/Interaction (D/I) Model

Example in S_N2 reactions







X^- = Nucleophile

Y = Leaving group

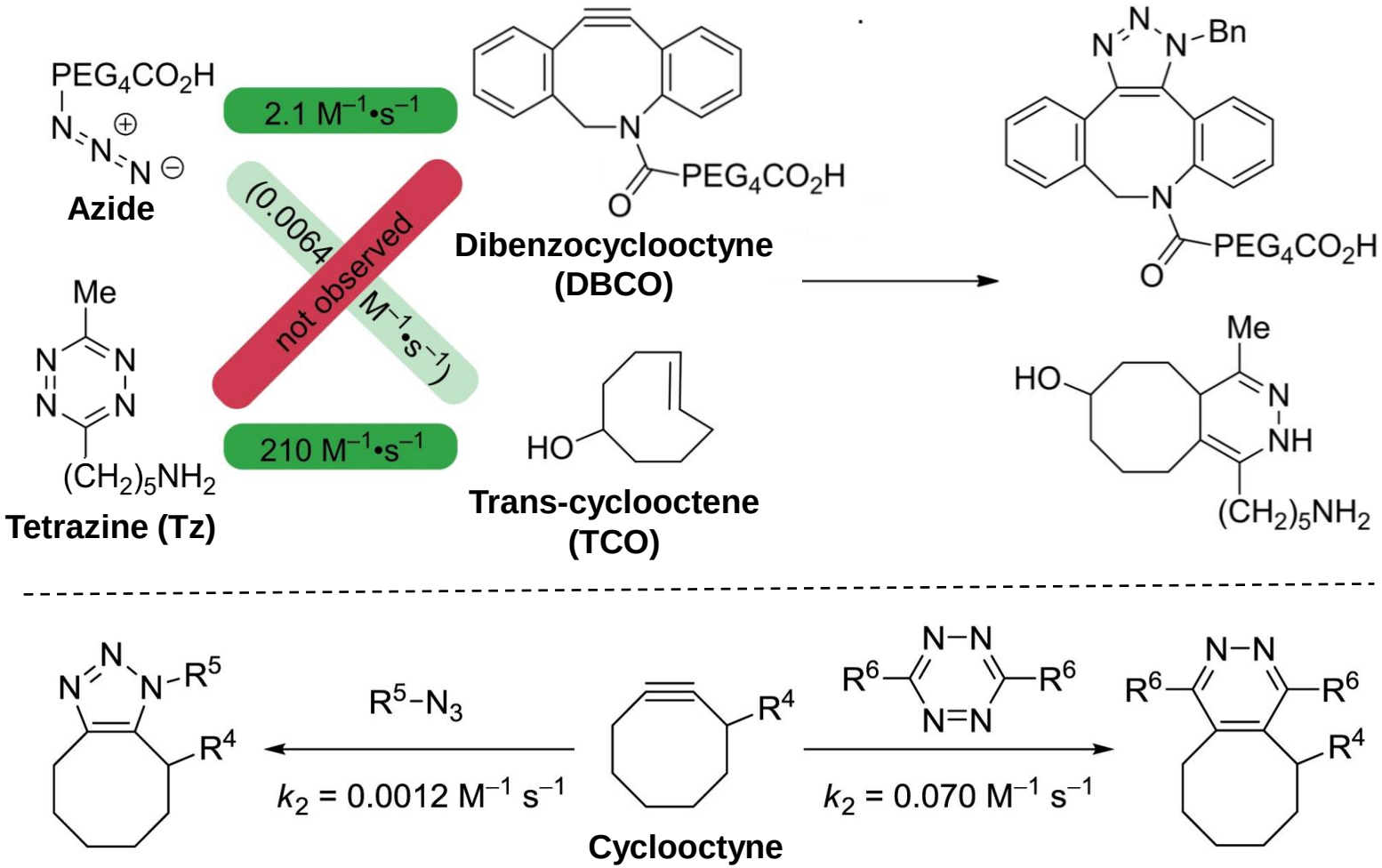
a. A better nucleophile
→ A stronger Lewis basicity
→ Lowering the S_N2 barrier by **enhancing the stabilizing interaction**

b. A poorer leaving group
→ A higher energy penalty to lengthen a stronger C−Y bond
→ Raising the S_N2 barrier because of **a more destabilizing distortion curve**

Bickelhaupt, Houk *et al. Angew. Chem., Int. Ed.* **2017**, *56*, 10070–10086

Elucidation of Mutual Orthogonality in Bioorthogonal Cycloadditions

The mutual orthogonality of azide–DBCO and tetrazine–TCO cycloadditions



Question

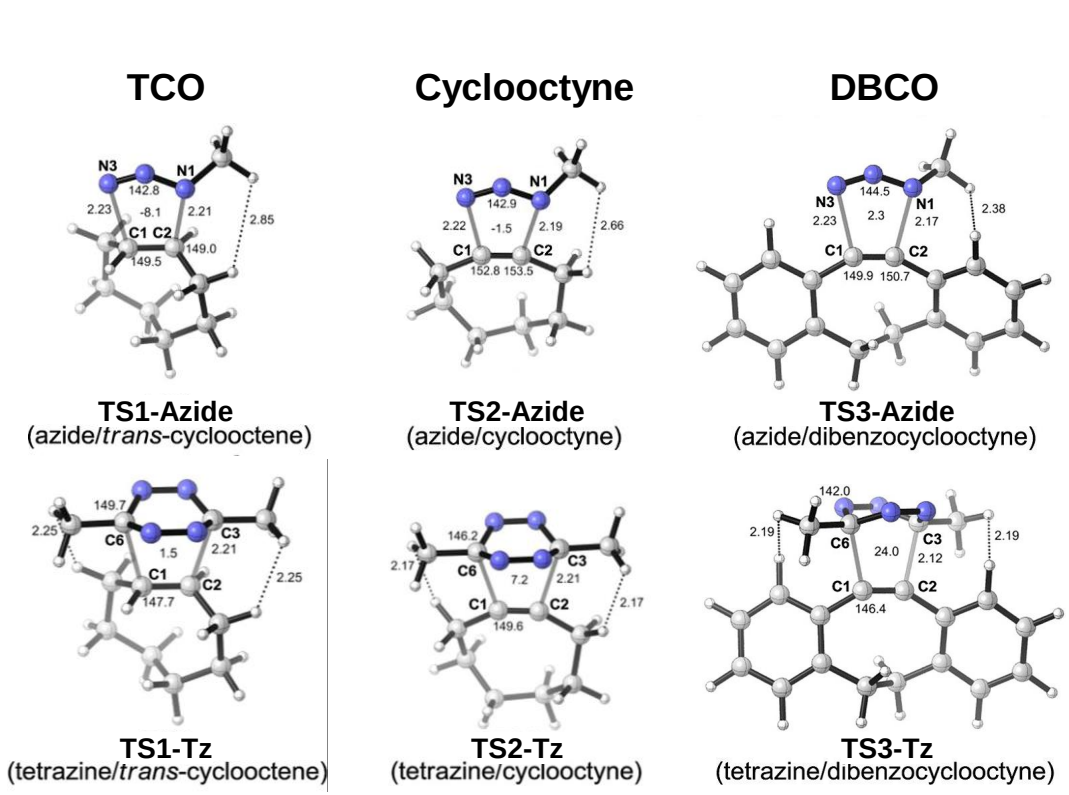
Why do their selectivities differ dramatically?

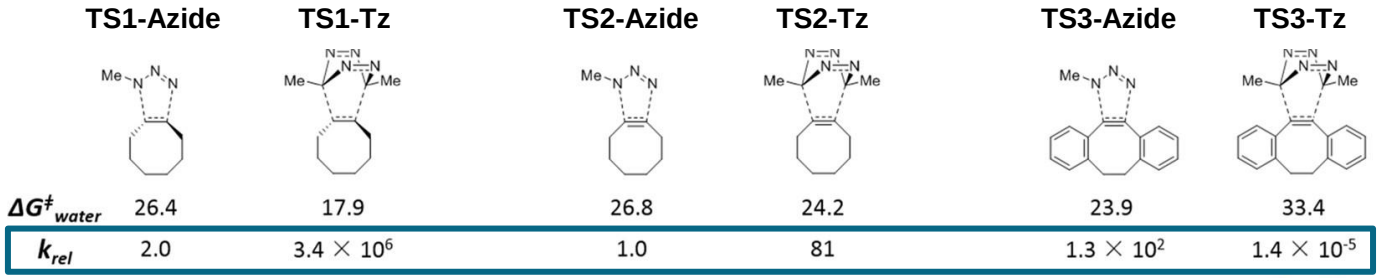
DFT-calculation approach

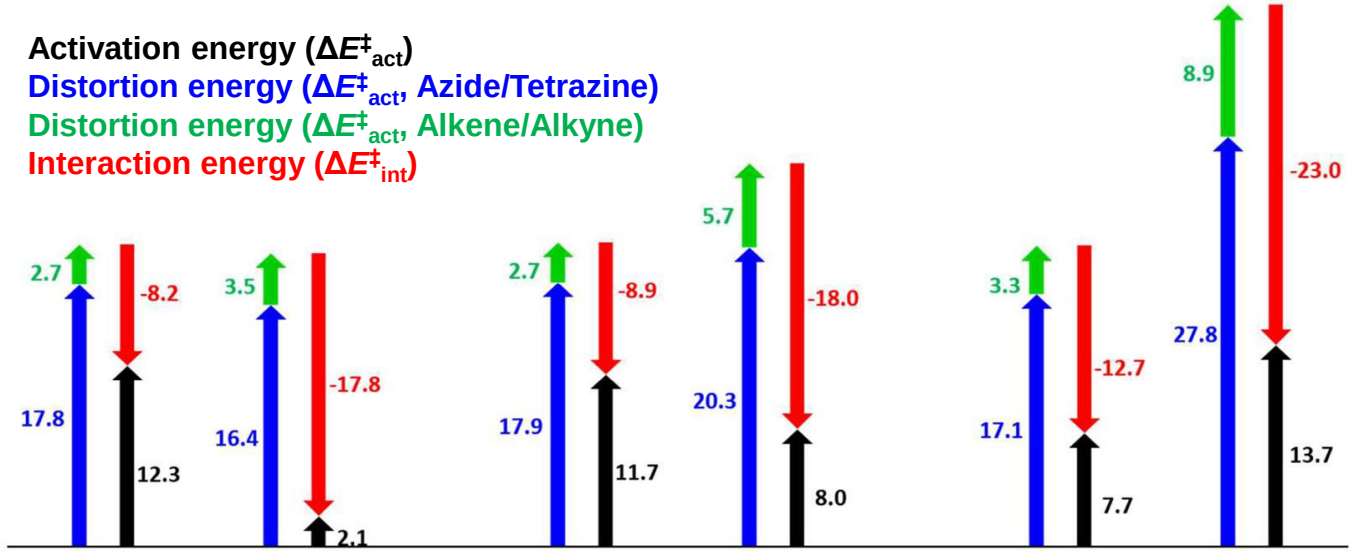
- **Point out the factors** that control the reactivity patterns
- **Develop a set of design principles** for new orthogonal cycloadditions
- **Predict** that two new bioorthogonal reagents

Karver, Weissleder, Hilderbrand *Angew. Chem., Int. Ed.* **2012**, *51*, 920
Liang, Houk *et al. J. Am. Chem. Soc.* **2012**, *134*, 17904–17907
Hu, Schomaker *ChemBioChem* **2021**, *22*, 3254–3262

Elucidation of Mutual Orthogonality in Bioorthogonal Cycloadditions







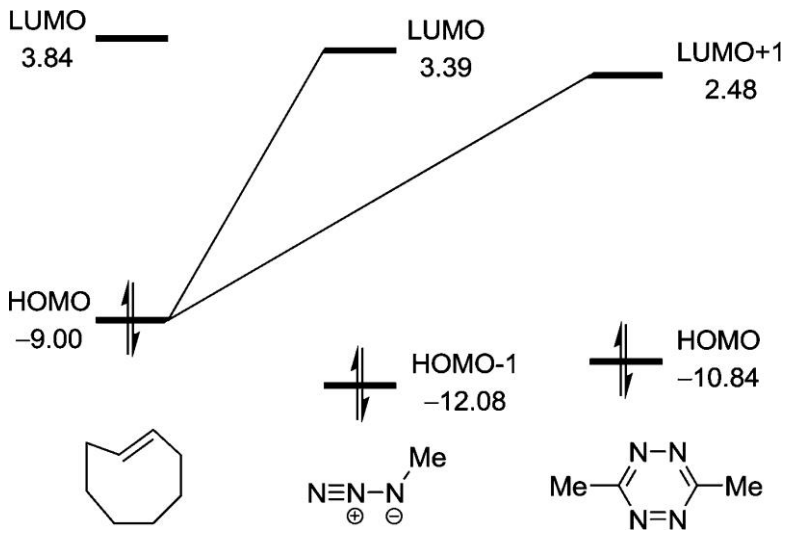
- Trans-cyclooctene (TCO):
  The interaction energy of TS1-Tz is much larger than that of TS1-Azide.
- Dibenzocyclooctyne (DBCO):
  The extremely sluggish kinetics of the DBCO–tetrazine cycloaddition is mainly **due to very high distortion energy**.

Liang, Houk *et al. J. Am. Chem. Soc.* **2012**, *134*, 17904–17907

Liu, Liang, Houk *Acc. Chem. Res.* **2017**, *50*, 2297–2308

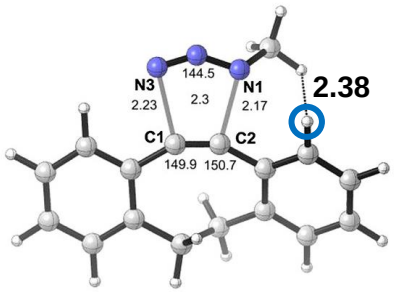
Elucidation of Mutual Orthogonality in Bioorthogonal Cycloadditions

Trans-cyclooctene (TCO): ΔE_{int}

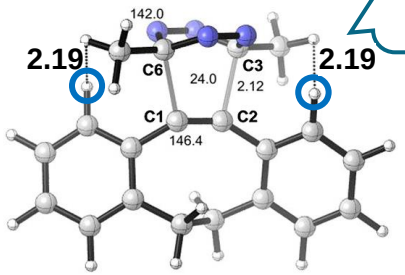


- Frontier molecular orbital (FMO) analysis
- **The smaller orbital energy gap between TCO and tetrazine** makes the favorable orbital interaction in TS1-Tz stronger than that in TS1-Azide.

Dibenzocyclooctyne (DBCO): ΔE_{dist}

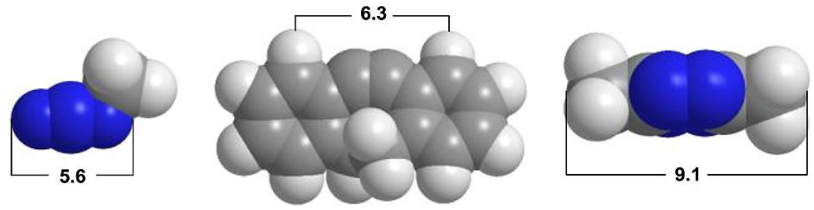


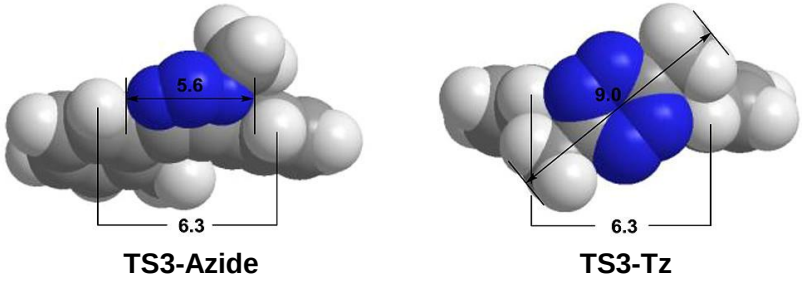
TS3-Azide
(azide/dibenzocyclooctyne)



TS3-Tz
(tetrazine/dibenzocyclooctyne)

Close to the sum of van der Waals radii





- The structural analysis of transition states
- Because of **the great steric hindrance** caused by the two aryl hydrogen atoms (marked ○), the steric effect overwhelms the electronic effect, leading to the exclusive azide selectivity.

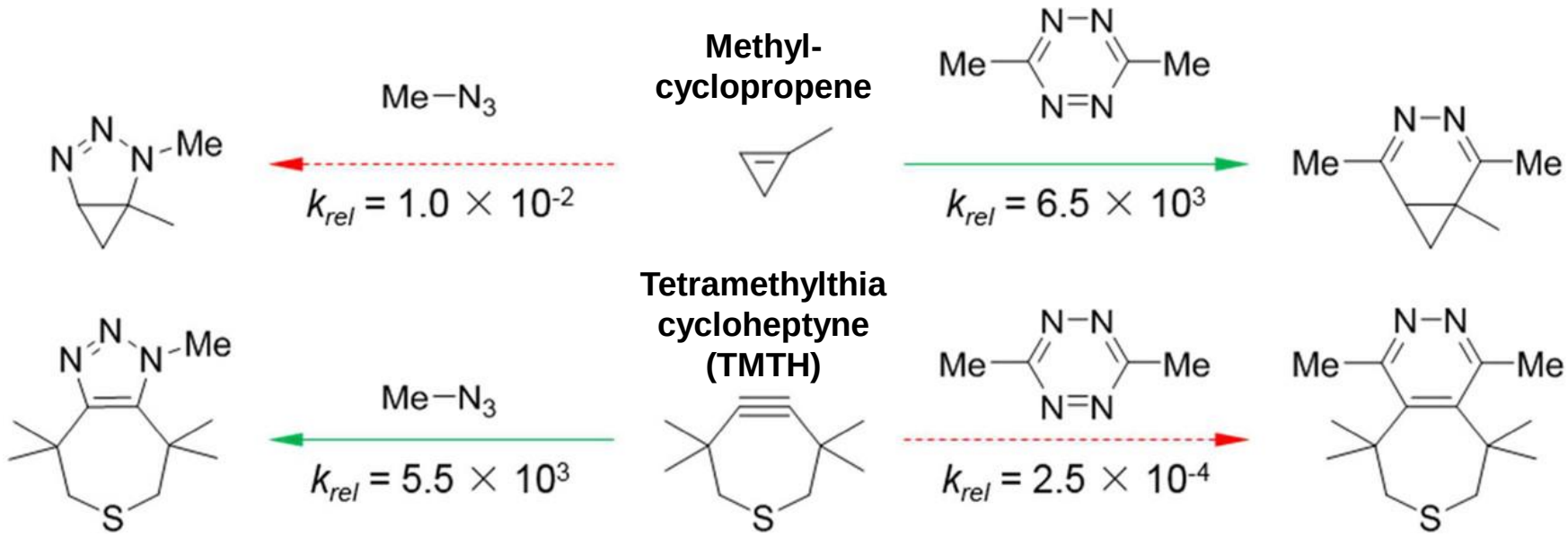
Liang, Houk *et al. J. Am. Chem. Soc.* **2012**, *134*, 17904–17907

Calculation-based Design in Bioorthogonal Cycloadditions

Principles for the design of orthogonal reaction pairs

- **Intrinsically more reactive substances can be made less reactive by increasing the distortion controlled by steric effects**

Prediction of Mutual Orthogonality of Two New Bioorthogonal Reagents



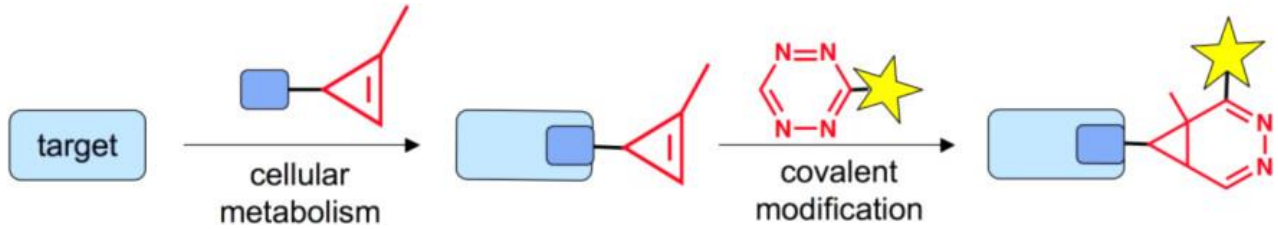
Liang, Houk *et al. J. Am. Chem. Soc.* **2012**, *134*, 17904–17907

Liu, Liang, Houk *Acc. Chem. Res.* **2017**, *50*, 2297–2308

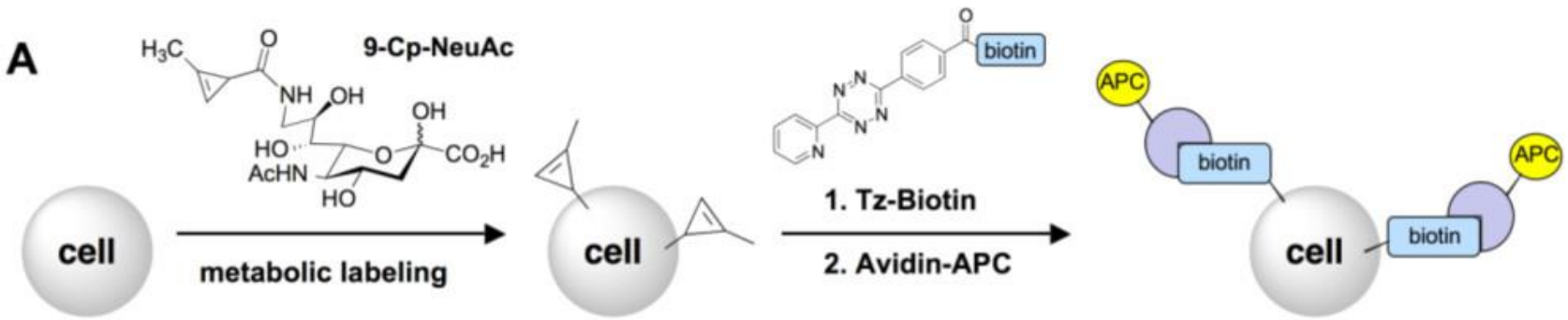
Application of Methylcyclopropene-Tetrazine Conjugation in Living Systems

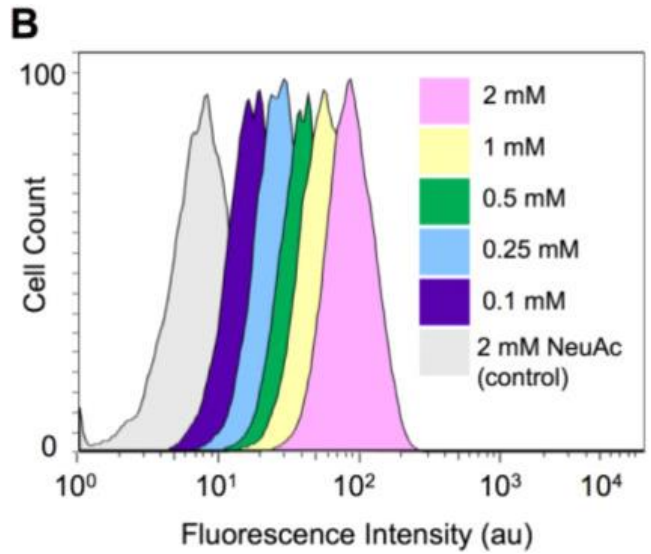
Application as a new bioorthogonal reaction

- *In-vitro* selective protein modification
- **Metabolic incorporation** and labeling of cyclopropenes onto live cell surfaces



- Elucidation of the substituent effects on tetrazine–cyclopropene ligation
- Identification of new mutual orthogonal reaction pairs
- Demonstration of *in-cell* protein labeling



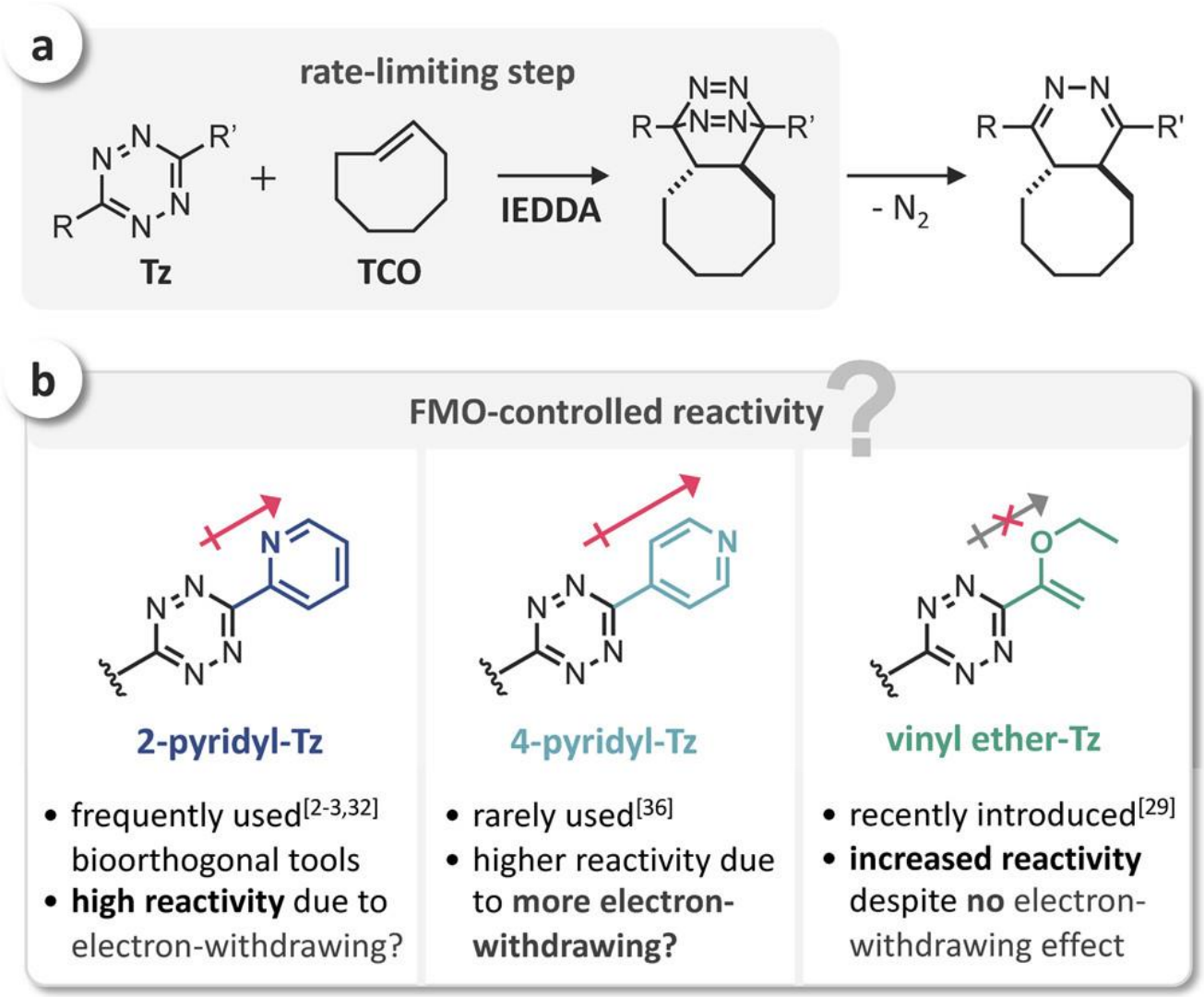


Patterson, Prescher *et al. J. Am. Chem. Soc.* **2012**, *134*, 18638–18643

Liu, Liang, Houk *Acc. Chem. Res.* **2017**, *50*, 2297–2308

Uncovering the Key Mechanism of the Reactivity/Stability Trade-Off

The influence of aryl substituents of tetrazine on the click reactivity



Question

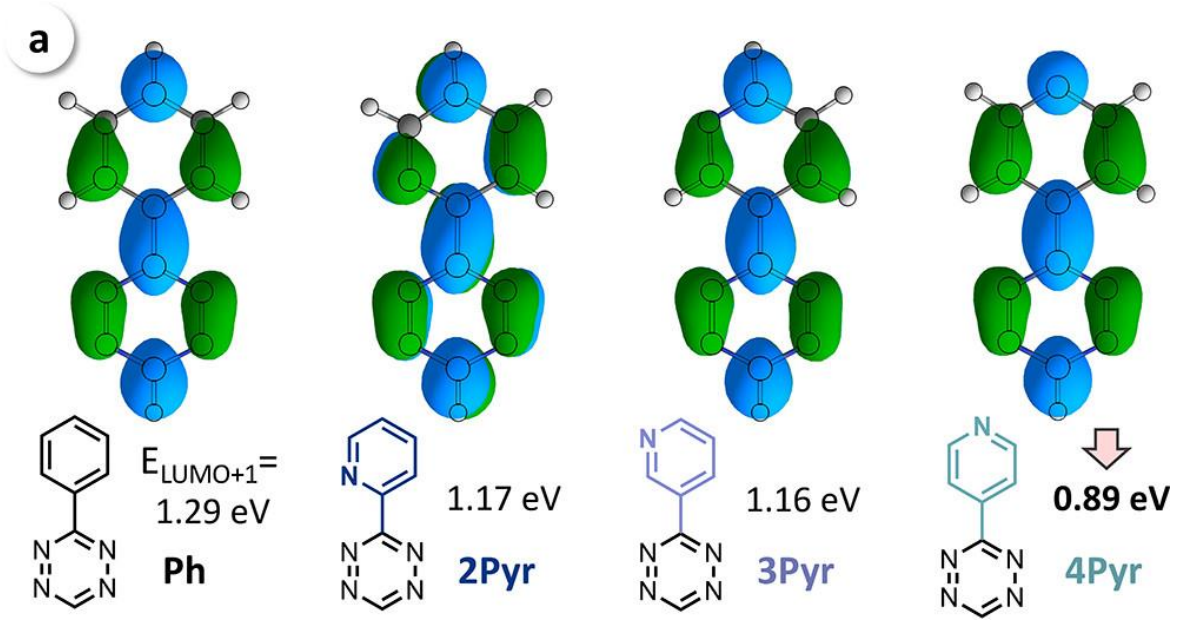
- Are there any **yet overlooked mechanistic aspects** other than FMO interactions that have a crucial effect on the reactivity of tetrazine?

- **Revealing the key mechanism of the substituent effects** on Tz-TCO cycloaddition using **Distortion/Interaction model**.

Svatunek, Houk, Mikula *et al. J. Am. Chem. Soc.* **2022**, *144*, 8171–8177

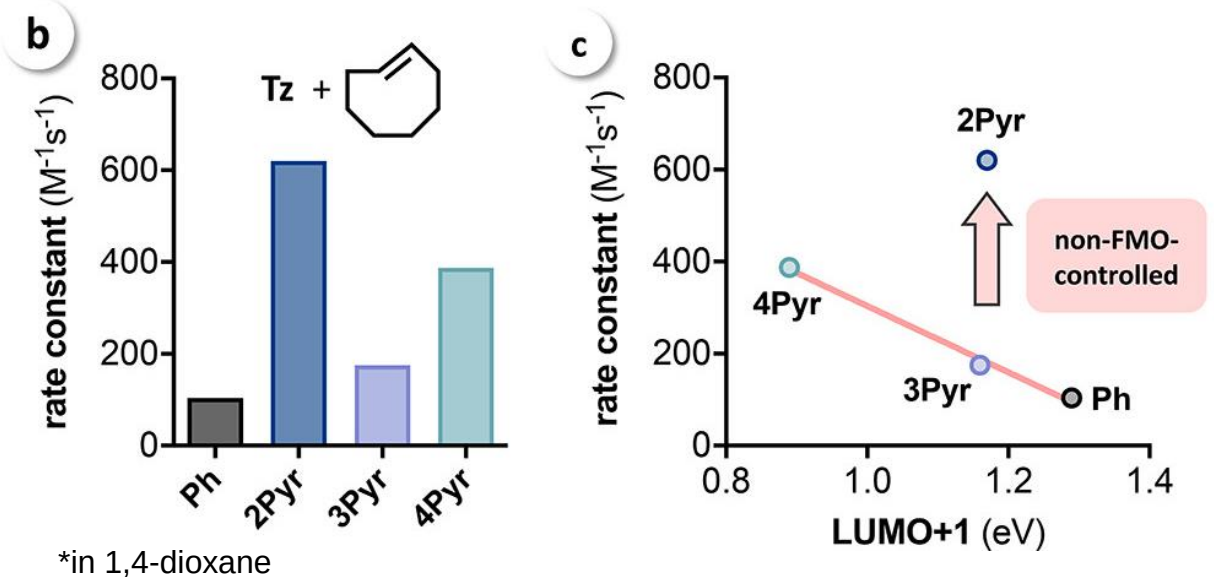
Relationship of the Orbital Energies and the Reaction Rates

FMO analysis using DFT calculation



- As expected, the order of the orbital energies was **Ph** > **2Pyr, 3Pyr** > **4Pyr**

Experimental measurement of reaction rate



*in 1,4-dioxane

- The reactivity trend for **Ph**, **3Pyr**, and **4Pyr** seems to be governed by FMO interactions.

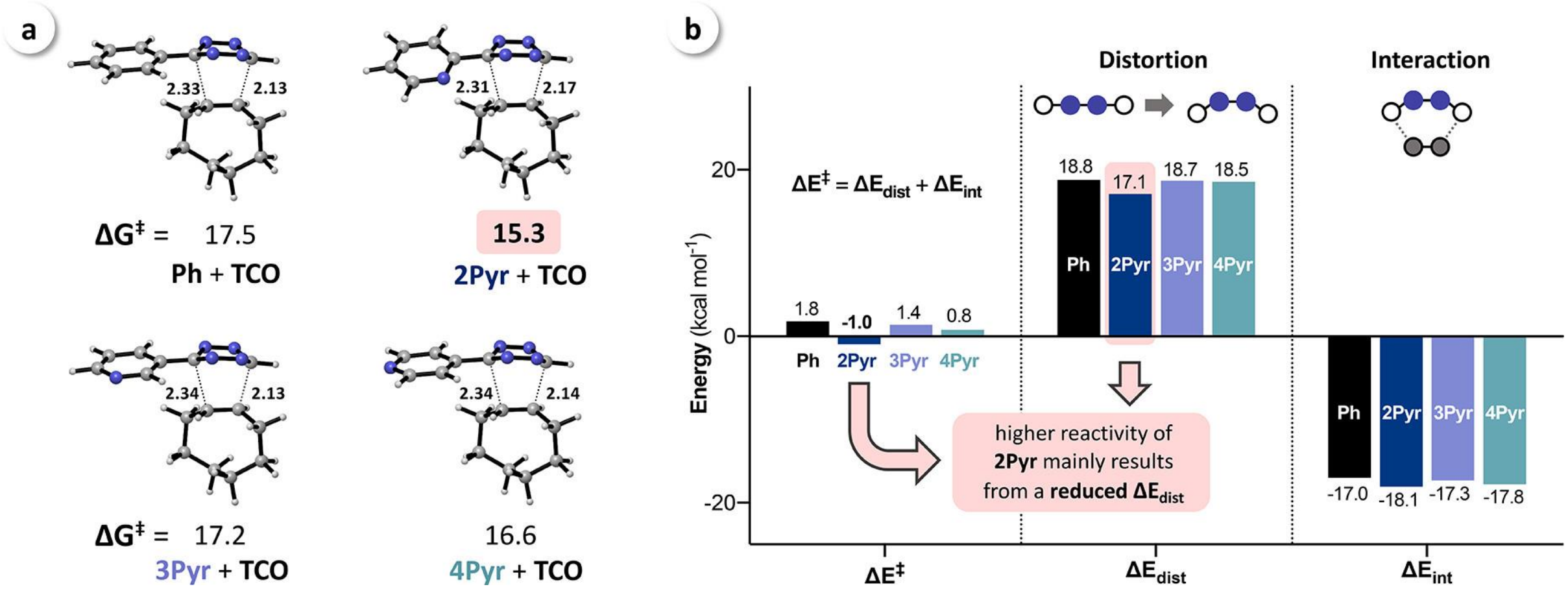
⇕

- **2Pyr** is **significantly more reactive** (>3-fold) than expected based on the respective orbital energy.

→ The high IEDDA reactivity of **2Pyr cannot be attributed to the electron-withdrawing effect.**

Svatunek, Houk, Mikula *et al. J. Am. Chem. Soc.* **2022**, *144*, 8171–8177

Distortion/Interaction Analysis of Tetrazine-TCO IEDDA Reactions

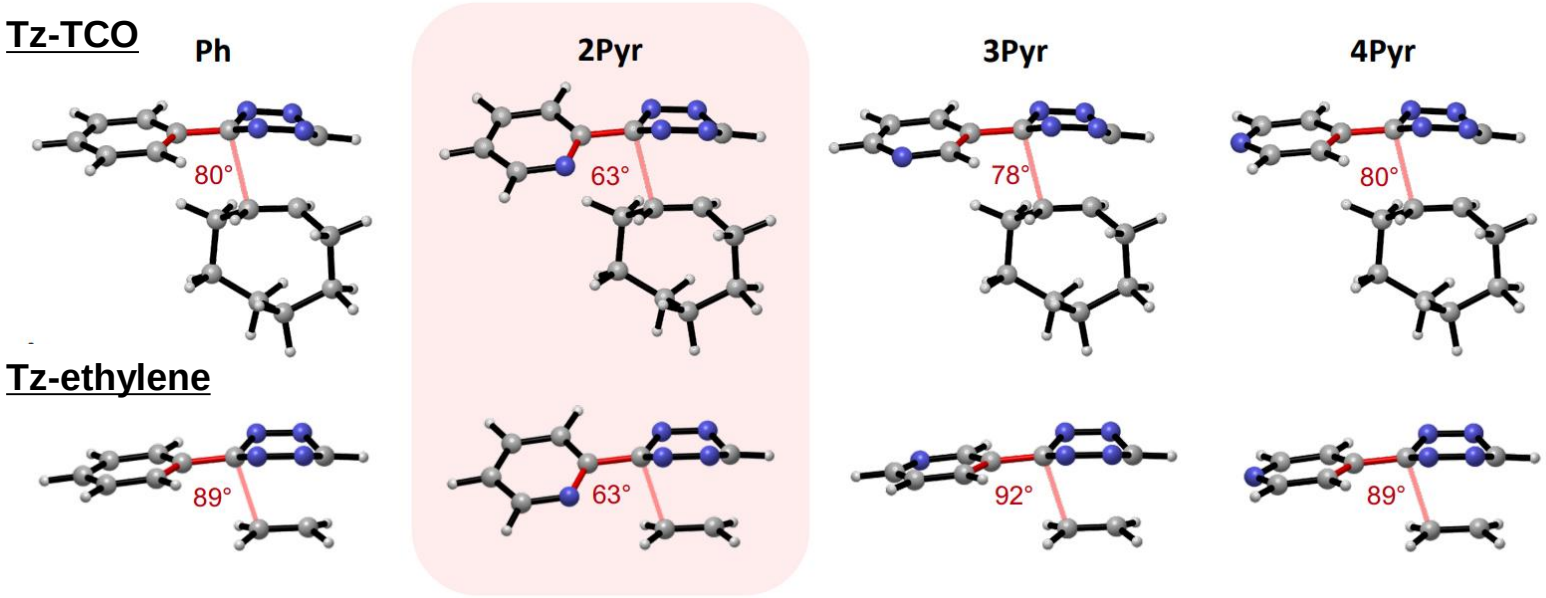


- ΔE_{dist} for **2Pyr** is about 1.5 kcal/mol lower than that for **Ph**, **3Pyr**, and **4Pyr**.

  → The increased reactivity of **2Pyr** with TCO is **mainly caused by a reduced distortion energy**.

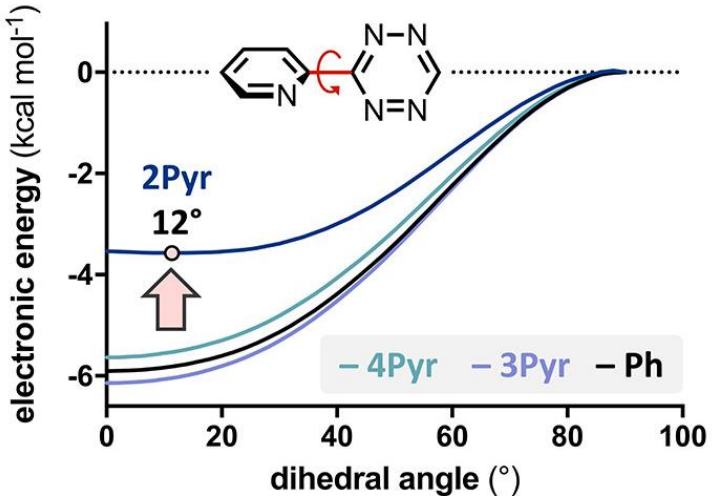
Svatunek, Houk, Mikula *et al. J. Am. Chem. Soc.* **2022**, *144*, 8171–8177

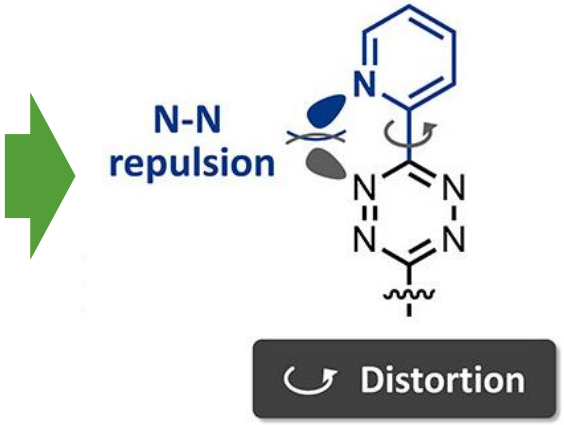
Uncovering the Key Role of Distortion in Tetrazine-TCO IEDDA Reactions



- In **Ph**, **3Pyr**, and **4Pyr**, the aryl moiety is **tilted only in the reaction with TCO** due to the steric demand of the allylic CH_2.
- For **2Pyr**, **a much stronger tilt** was observed with TCO, which **did not change with ethylene**.

→ **An intrinsic property** of 2-pyridyl-Tz rather than forced by steric interactions.



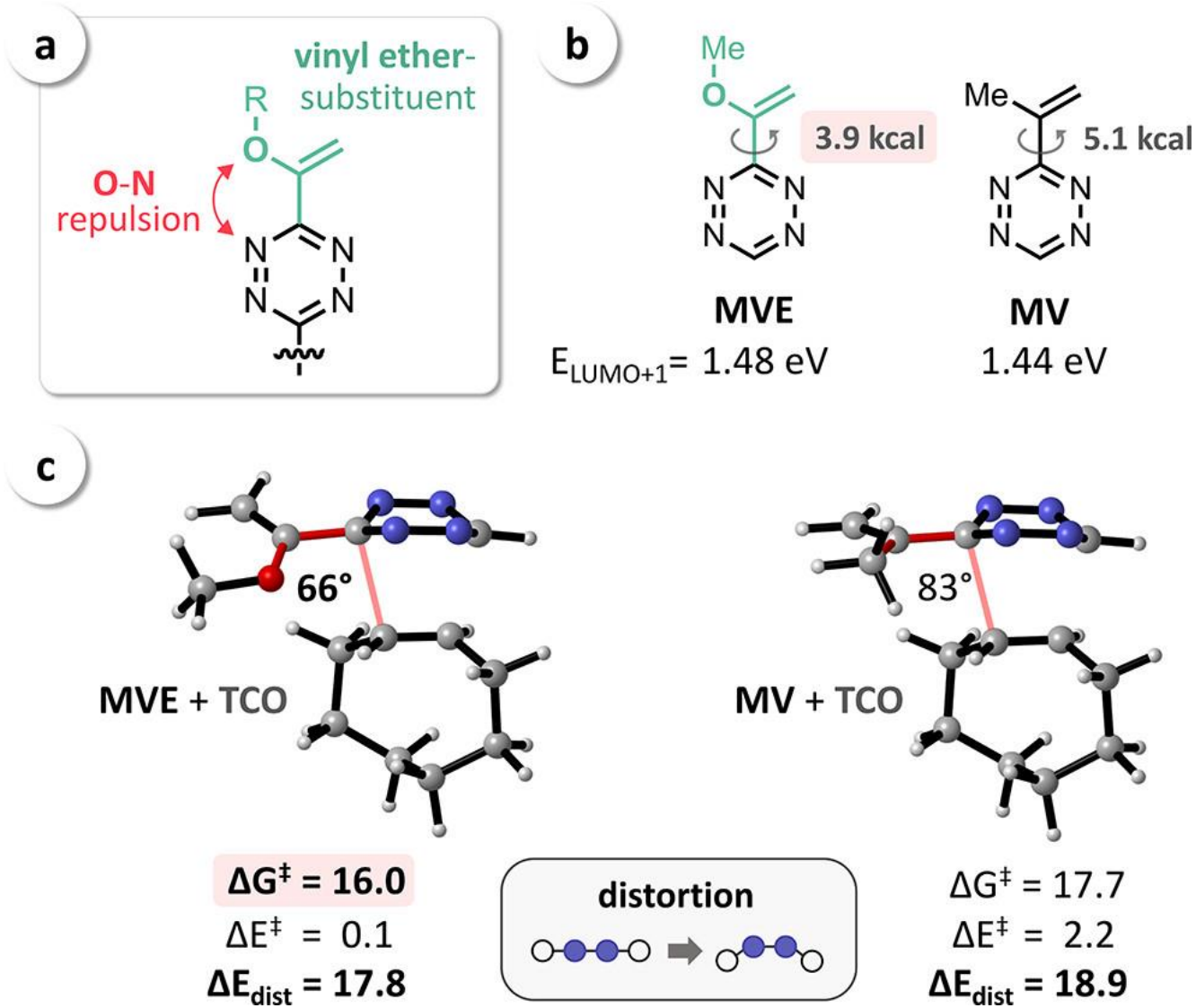


- A **nitrogen–nitrogen interaction** destabilizes the 2-pyridyl-Tz.

→ The key factor of the reactions of 2-pyridyl-Tz with TCOs is **the reduced Tz distortion energies caused by *N-N* repulsive intramolecular interactions**.

Svatunek, Houk, Mikula *et al. J. Am. Chem. Soc.* **2022**, *144*, 8171–8177

Repulsive *O–N* Interaction Increases the Reactivity of Vinyl Ether-Tz

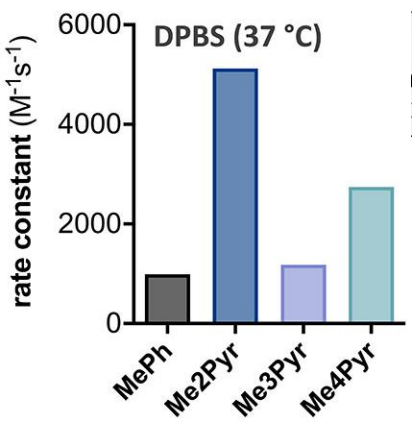


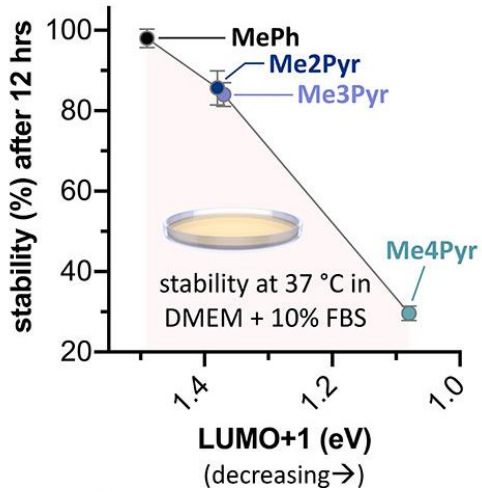
- Reduced rotational barrier
- Non-electron-withdrawing character
- Significantly stronger tilt of the vinyl–Tz bond
- Low distortion energies (ΔE_{dist})
- Low free energy of activation ($\Delta G^{\ddagger}$)

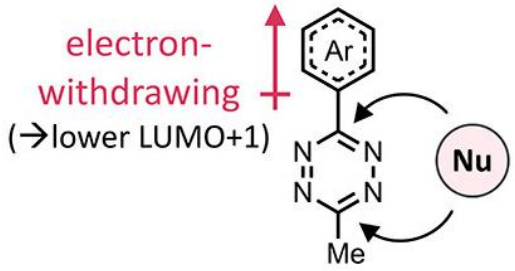
***O–N* repulsion plays a crucial role** regarding the potentially increased reactivity of **MVE**

Svatunek, Houk, Mikula *et al. J. Am. Chem. Soc.* **2022**, *144*, 8171–8177

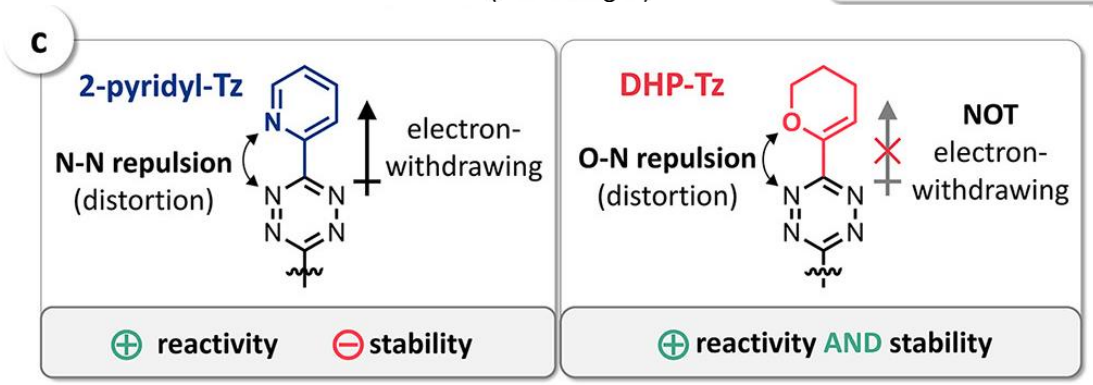
Uncovering the Key Role of Distortion in The Reactivity/Stability Trade-Off

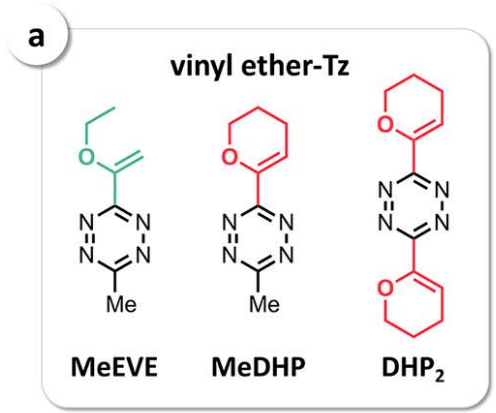


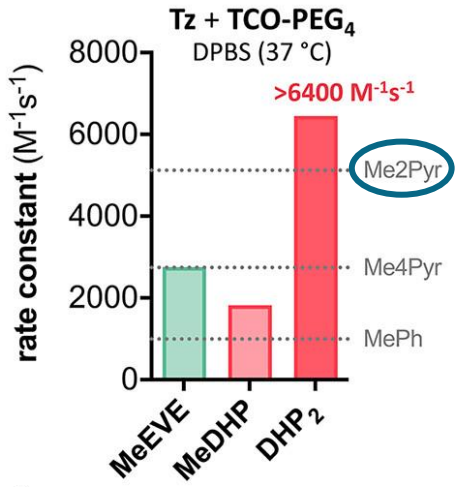


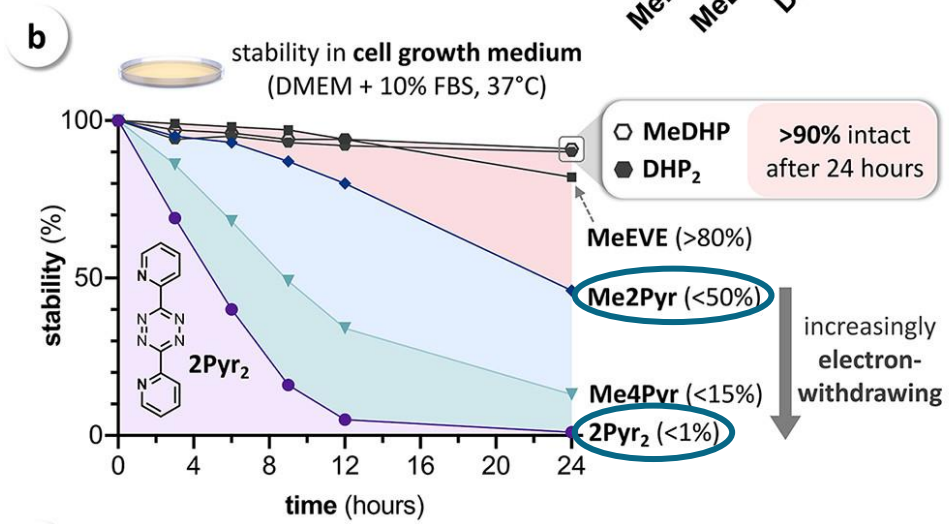


electron-withdrawing substituents accelerate **Tz degradation**







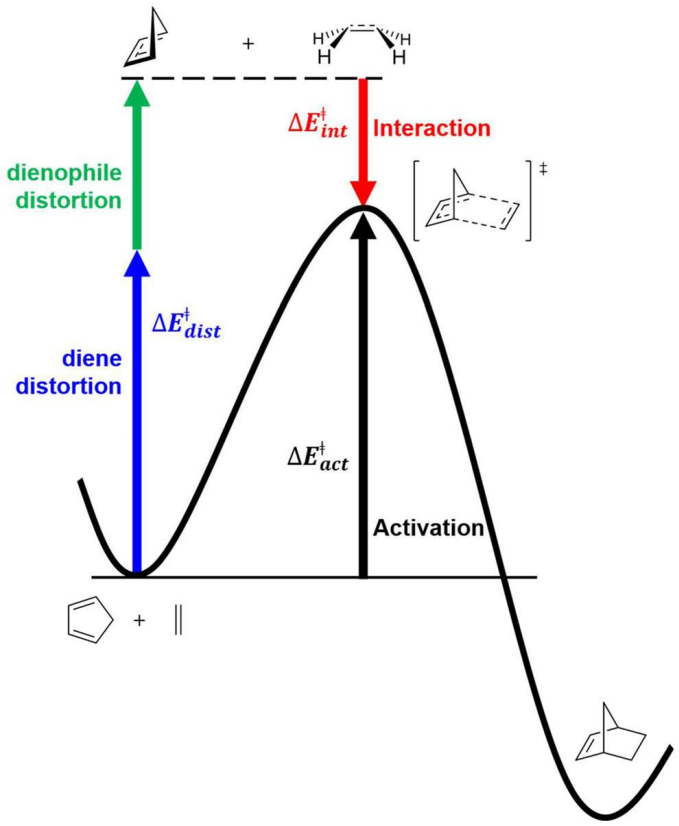


- **Non-electron-withdrawing DHP substituents** can be used to **significantly increase the IEDDA reactivity** of Tz while **maintaining a high compound stability**.

Svatunek, Houk, Mikula *et al. J. Am. Chem. Soc.* **2022**, *144*, 8171–8177

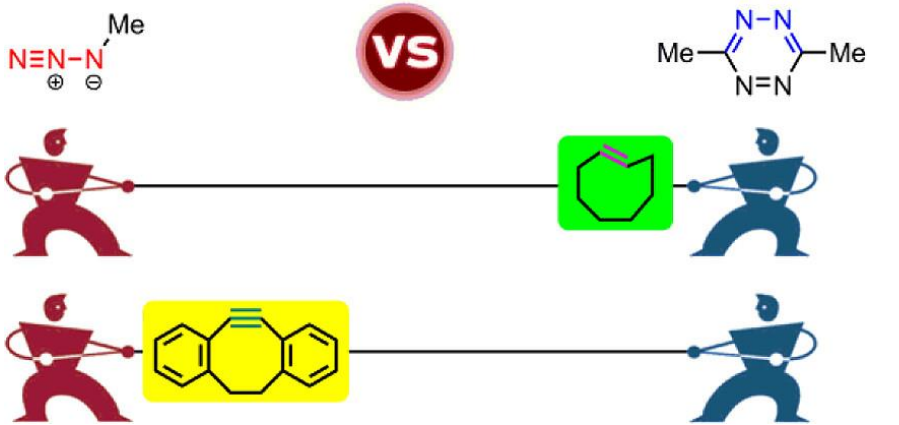
Short Summary

Transition states analysis

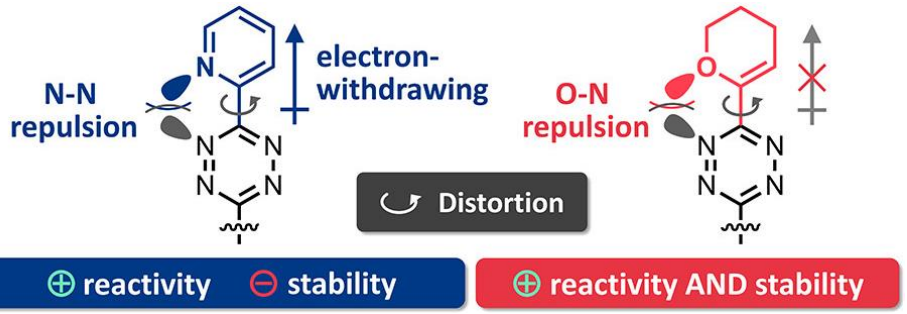


Distortion/Interaction (D/I) model

Generalized principles development

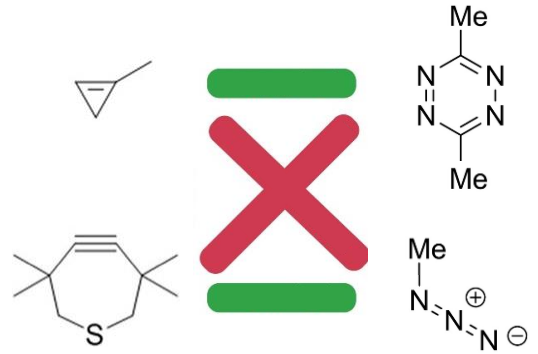


Mutual orthogonality



Reactivity / stability trade-off

New design



New Mutual orthogonal pair

New structure with high reactivity & stability

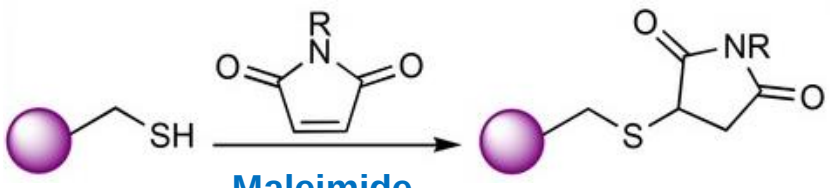
Contents

Chemoselective Cysteine Bioconjugation

Cys selective bioconjugation

- ✔ **A low natural abundance** on accessible protein surfaces
- ✔ **The unique nucleophilic properties** of the sulfhydryl group

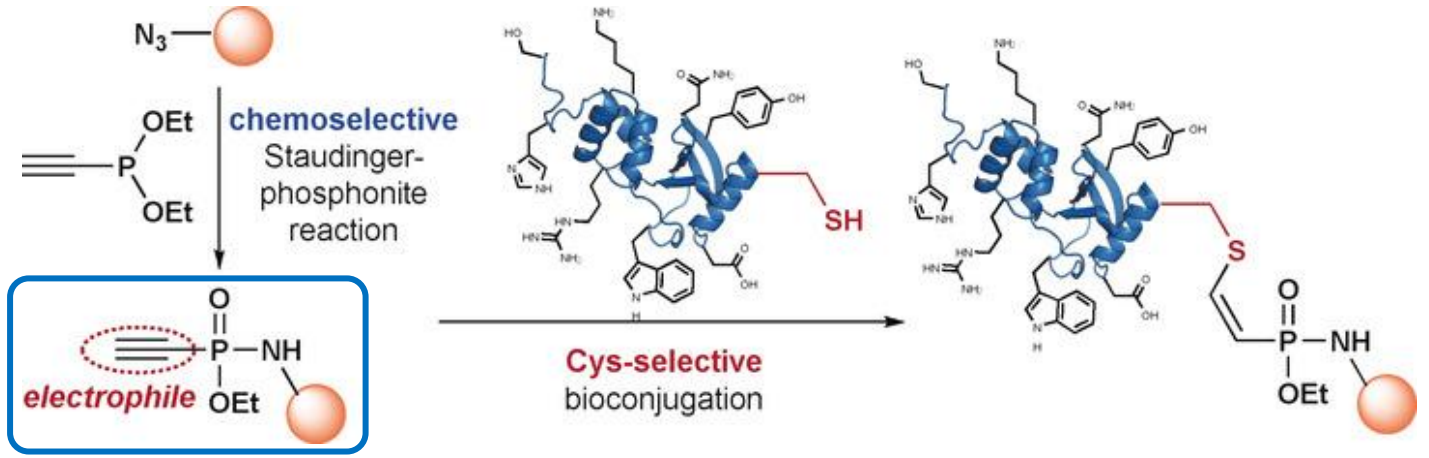
The most widely used method

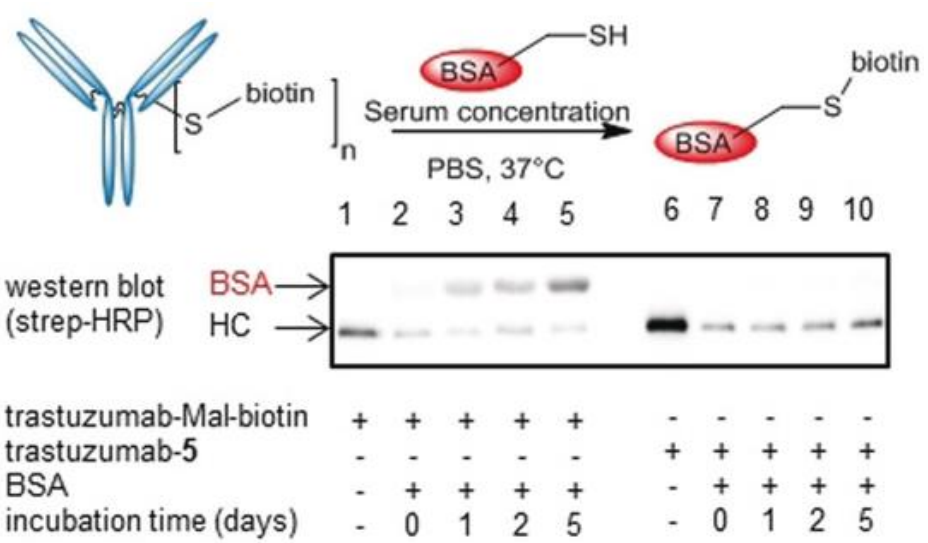


- ✔ **Rapid kinetics**
- ✘ **Stability** (Retro-Michael addition in the presence of external thiols)

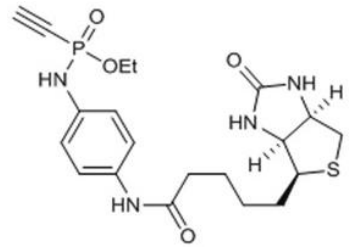
Gunnoo, Madder *et al. ChemBioChem* **2016**,*17*, 529–553

Ethynyl phosphorus(V) electrophiles





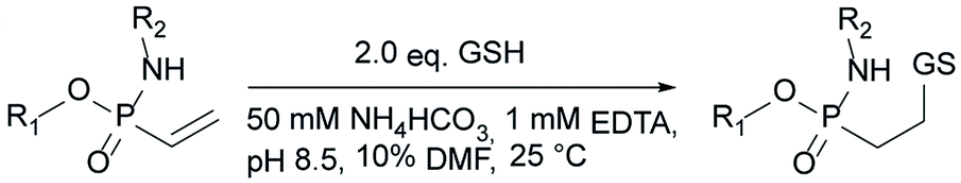
5 =



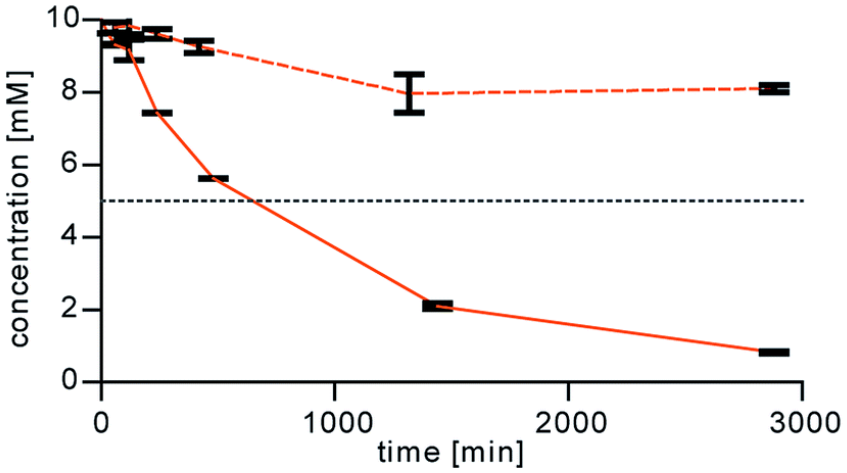
- ✔ **Cys selectivity**
- ✔ **Modularity**
- ✔ **Stability**

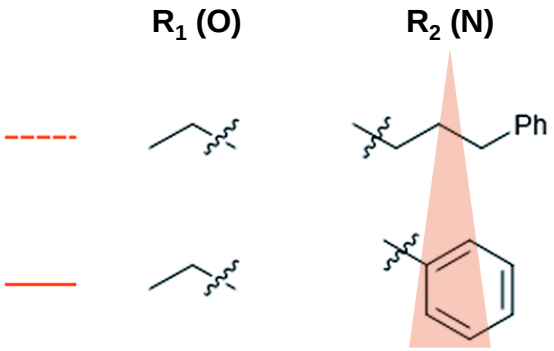
Kasper, Hackenberger *et al. Angew. Chem. Int. Ed.* **2019**, *58*, 11625–11630

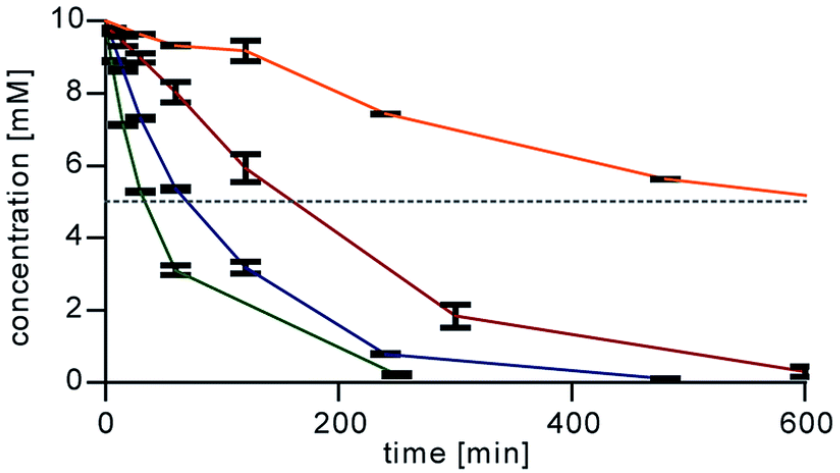
Effects of Substituents around Phosphorus on Thiol Addition Reactivity

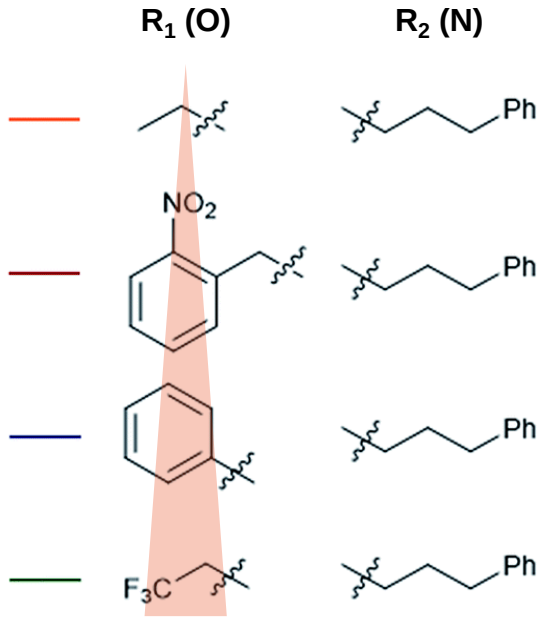


R_1 = ethyl, 2-nitrobenzyl, phenyl, trifluoroethyl
R_2 = phenyl, 3-phenylpropyl





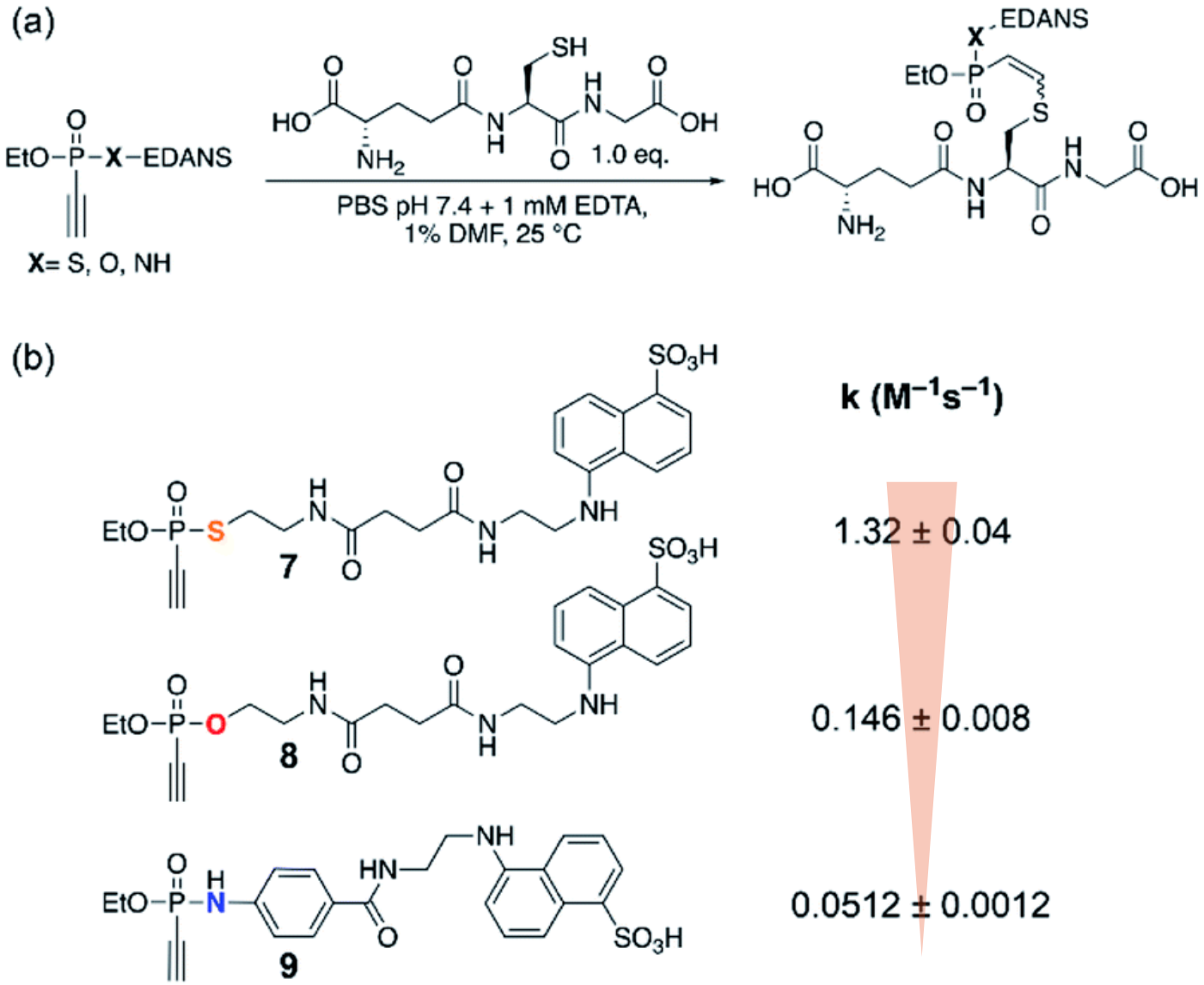




- **Electron-withdrawing substituents** at the *N*- and the *O*-residue **increased the speed** of the thiol addition.

Kasper, Hackenberger *et al. Chem. Sci.* **2019**,*10*, 6322–6329

The Mechanism behind Enhanced Reactivity of Ethynyl P(V) Electrophiles



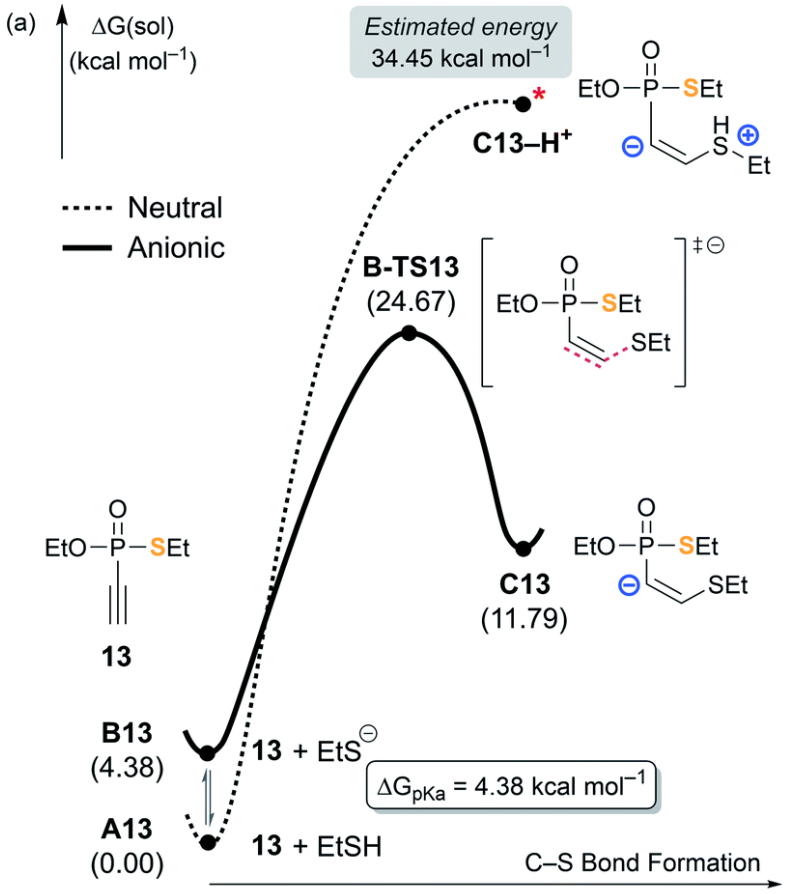
- **The heteroatoms** bound to the central phosphorus atom have **a notable influence on the overall thiol addition reactivity**.
- **Not consistent with simple electronegativity considerations** (S: 2.5, O: 3.5, N: 3.0).

Pursue a mechanistic rationale using DFT calculation

Park, Sun, Baik, Hackenberger *et al. Chem. Sci.* **2021**, *12*, 8141–8148

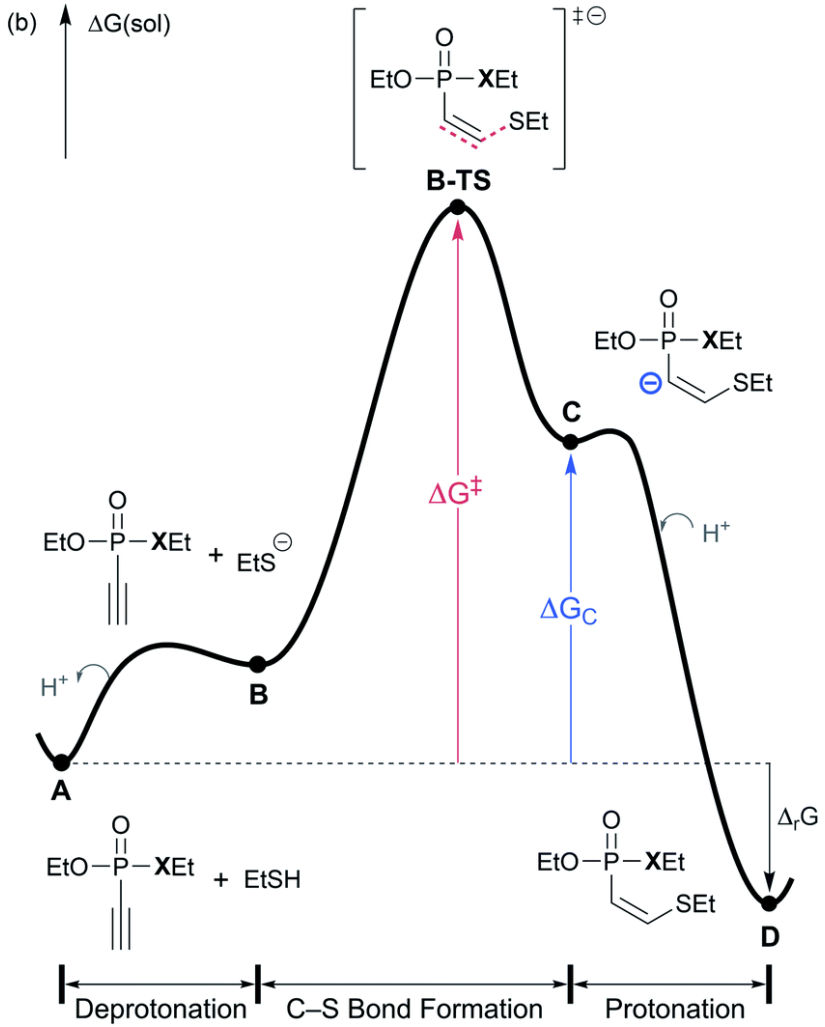
The Mechanism behind Enhanced Reactivity of Ethynyl P(V) Electrophiles

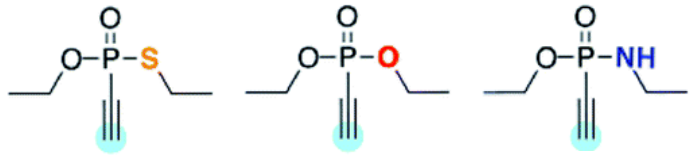
Possible timings for deprotonation



- The thiol cannot form an addition product
- **The thiolate addition is feasible**

Computationally examined mechanistic pathway



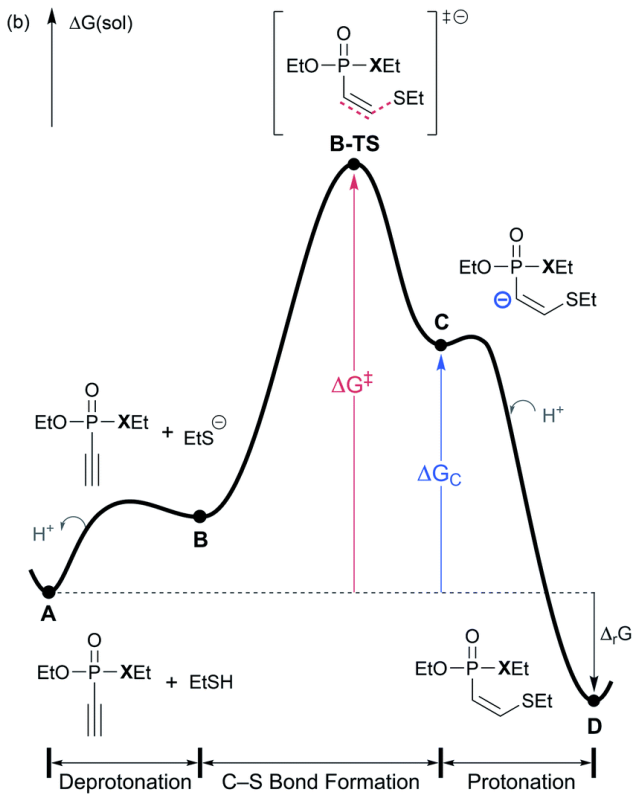


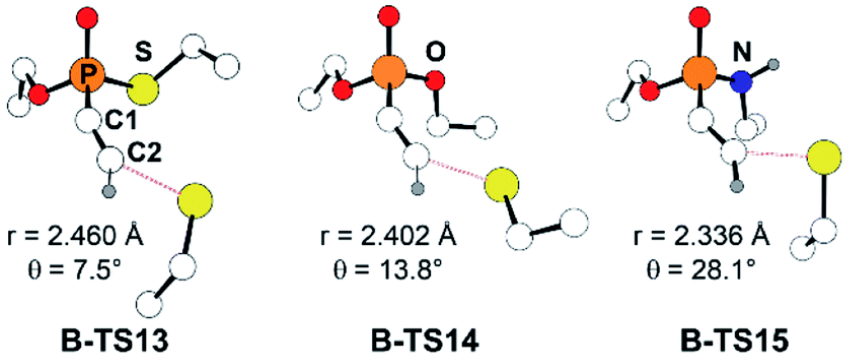
Substrate		$\Delta G^{\ddagger}$
X = S	13	24.67
X = O	14	26.89
X = NH	15	30.20

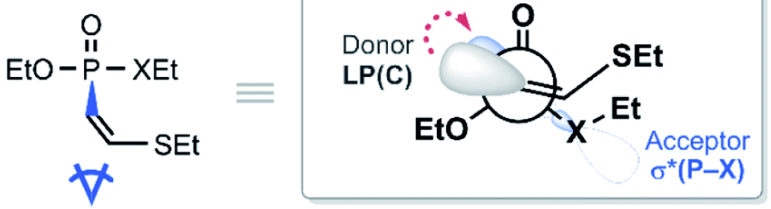
- **Calculated barriers correlate well** with the trends observed in the experimental rates.

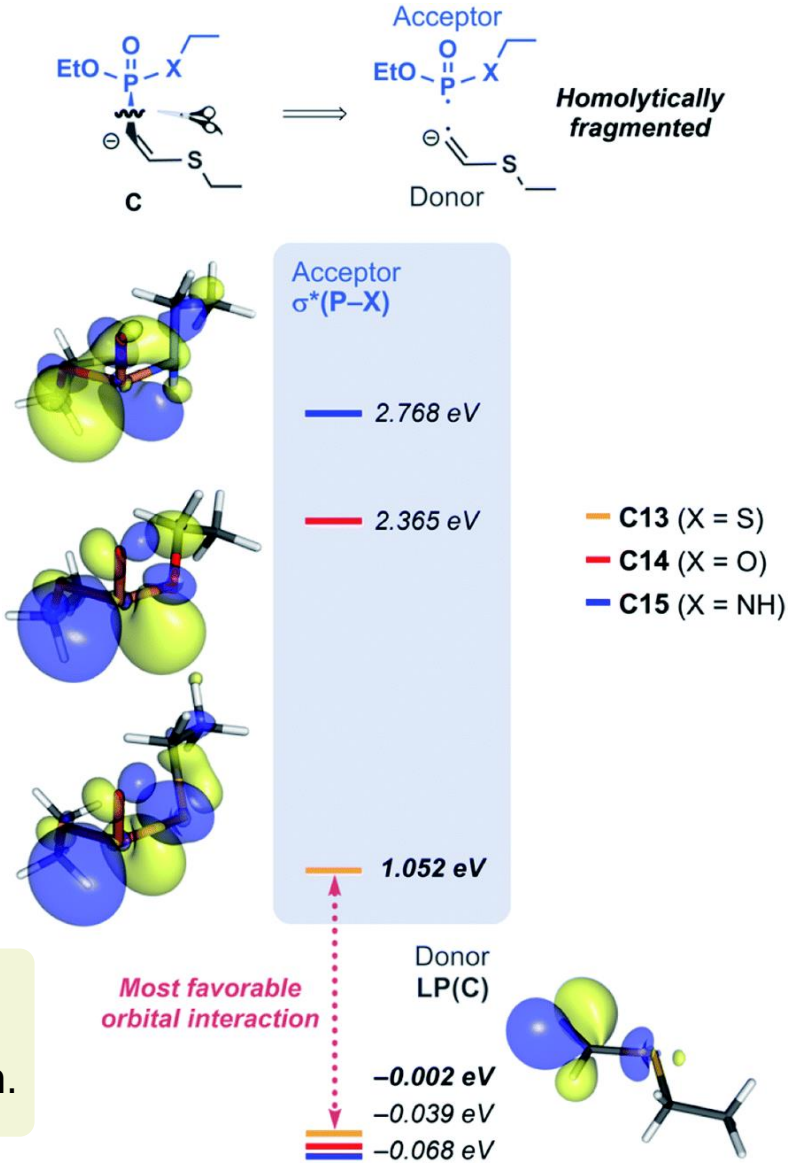
Park, Sun, Baik, Hackenberger *et al. Chem. Sci.* **2021**, *12*, 8141–8148

The Mechanism behind Enhanced Reactivity of Ethynyl P(V) Electrophiles









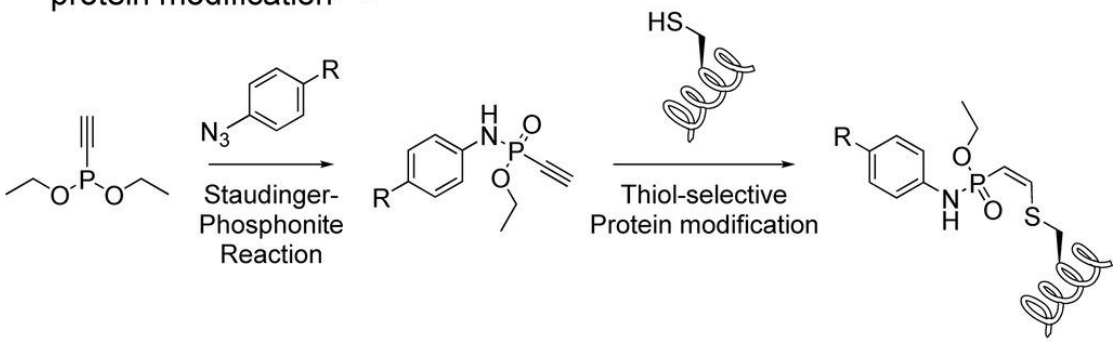
- The X–P–C1–C2 dihedral angles are within 50° → **Stereoelectronic interaction**
- **The P–X antibonding orbital** accepts the electron density through **hyperconjugation**.

Park, Sun, Baik, Hackenberger *et al. Chem. Sci.* **2021**, *12*, 8141–8148

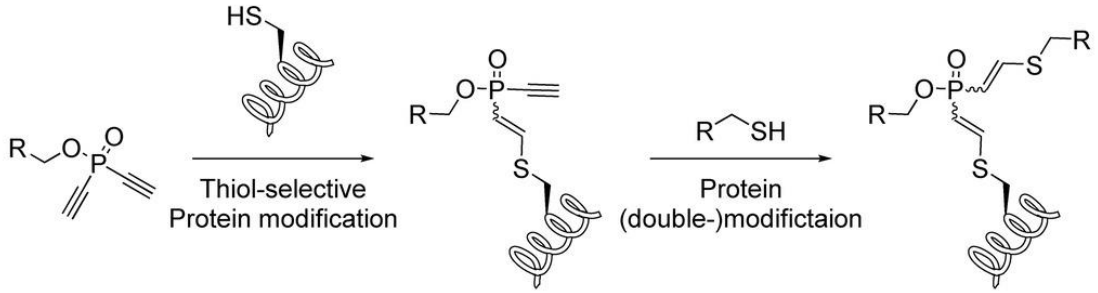
DFT-Guided Discovery of Ethynyl-Triazolyl-Phosphinates as Modular Electrophiles

Previous modular ethynyl P(V) electrophiles

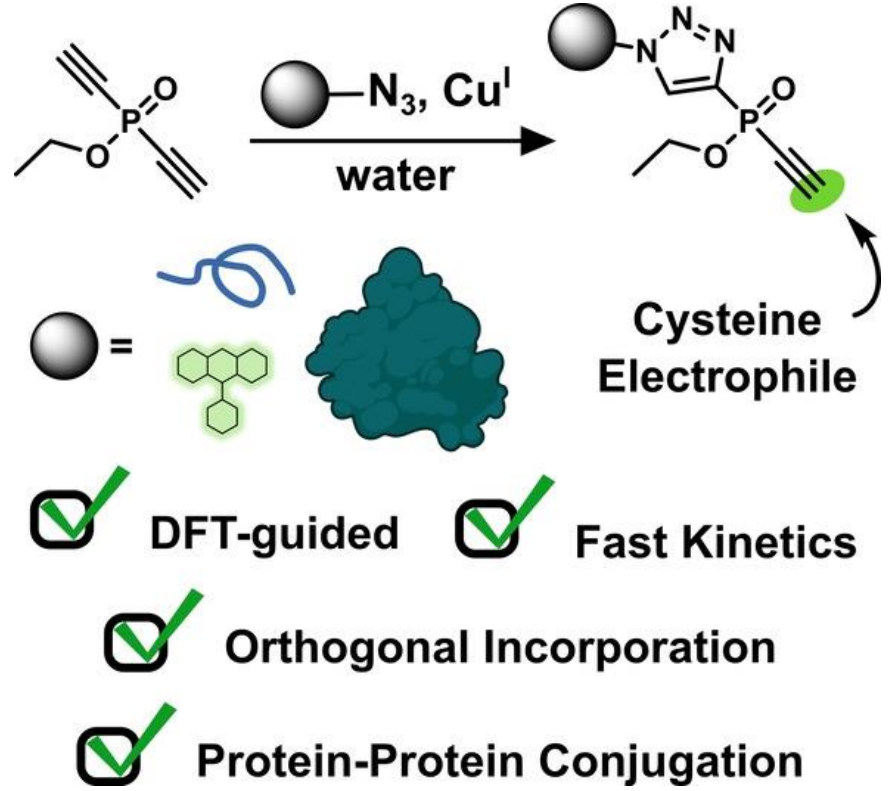
A. Staudinger induced electrophilic phosphonamidates for Cys-selective protein modification[35]



B. Diethynyl-phosphinates for Cys-selective antibody rebridging and protein (double-)modification[34]



Discovery of new modular ethynyl P(V) electrophiles

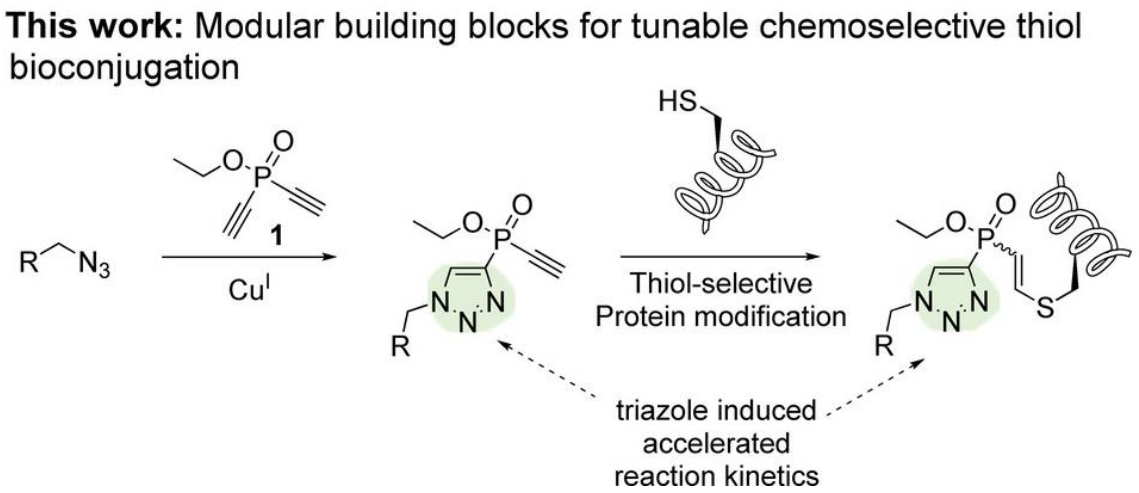


- **Ethynyl-Triazolyl-Phosphinates (ETPs)** as readily accessible, fast, and highly selective thiol-electrophiles, **guided by DFT-based computer models**.

Stieger, Baik, Hackenberger *et al. Angew. Chem. Int. Ed.* **2022**, *61*, e202205348

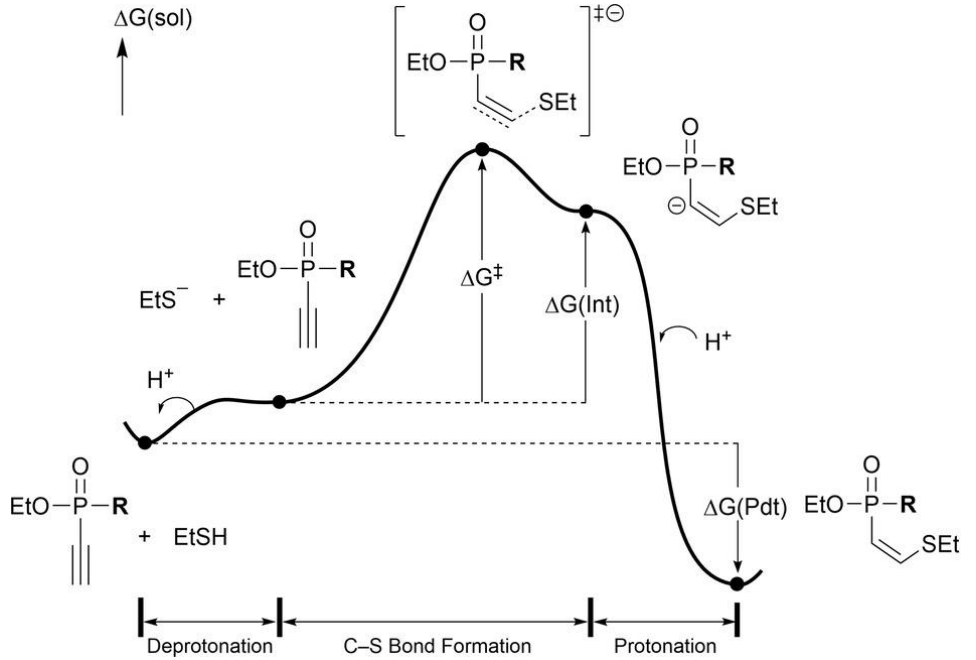
DFT-Guided Discovery of Ethynyl-Triazolyl-Phosphinates as Modular Electrophiles

Design to increase the reaction speed of ethynyl P(V) electrophiles



- **The bond P–XEt** located in an antiperiplanar position to the lone-pair played a decisive role in **accepting the electron density**.

→ Use **heterocyclic substituents** to achieve both **an electron-withdrawing inductive effect** and **π-conjugation with the lone-pair electrons**.



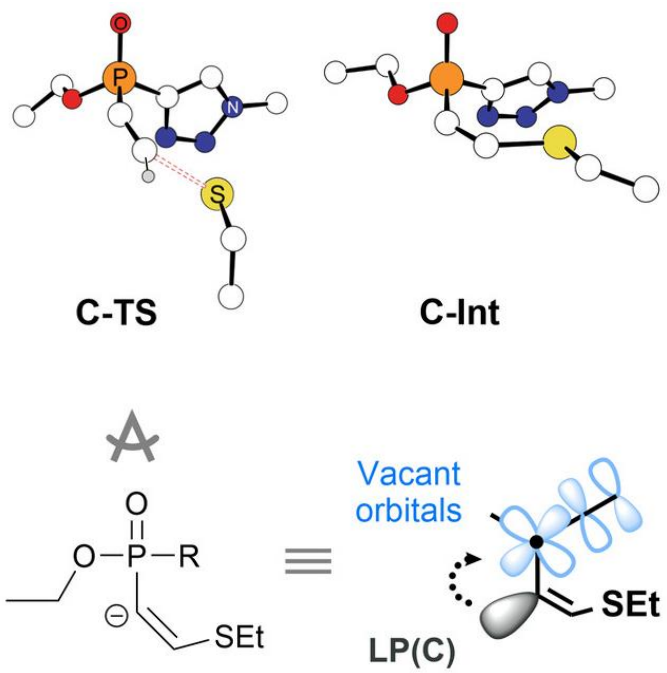
Easily accessed via Cu^{I}-catalyzed azide-alkyne cycloaddition

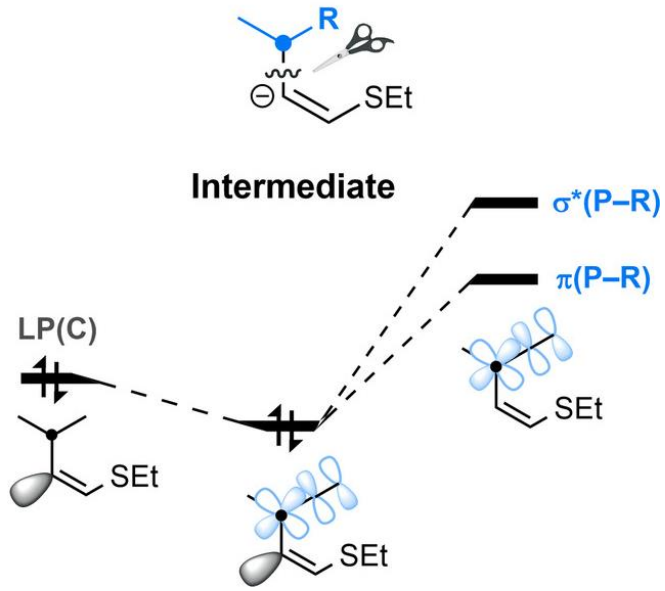
-R	A	B	C	D	E	F
ΔG‡	18.11	18.05	19.95	19.00	19.53	23.34
ΔG(Pdt)	–21.18	–20.94	–18.56	–20.31	–19.68	–18.77

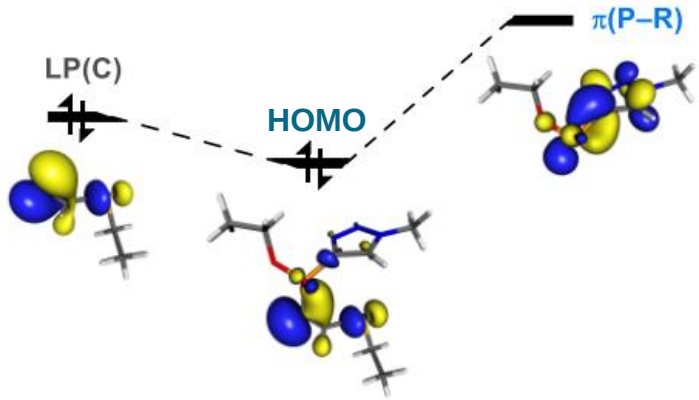
Stieger, Baik, Hackenberger *et al. Angew. Chem. Int. Ed.* **2022**, *61*, e202205348

DFT-Guided Discovery of Ethynyl-Triazolyl-Phosphinates as Modular Electrophiles

The substituents influence of the π-system to enhance the reactivity





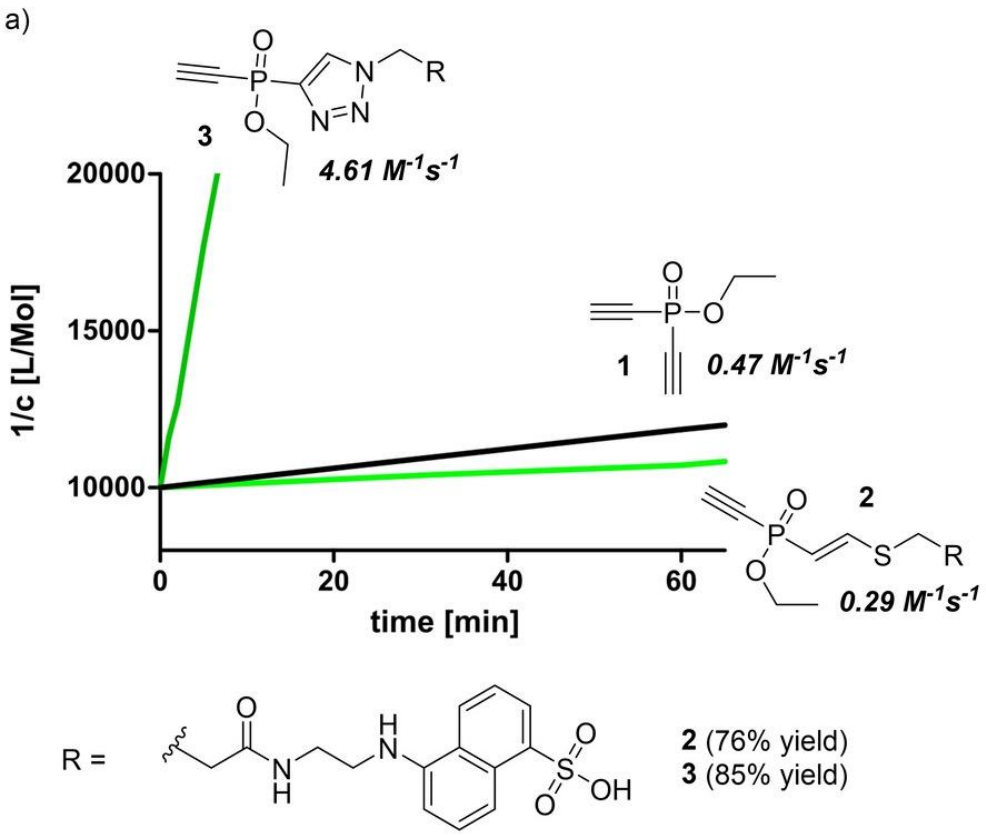


- **Delocalization of electrons into vacant orbitals related to the P−R bonds** is feasible.

Stieger, Baik, Hackenberger *et al. Angew. Chem. Int. Ed.* **2022**, *61*, e202205348

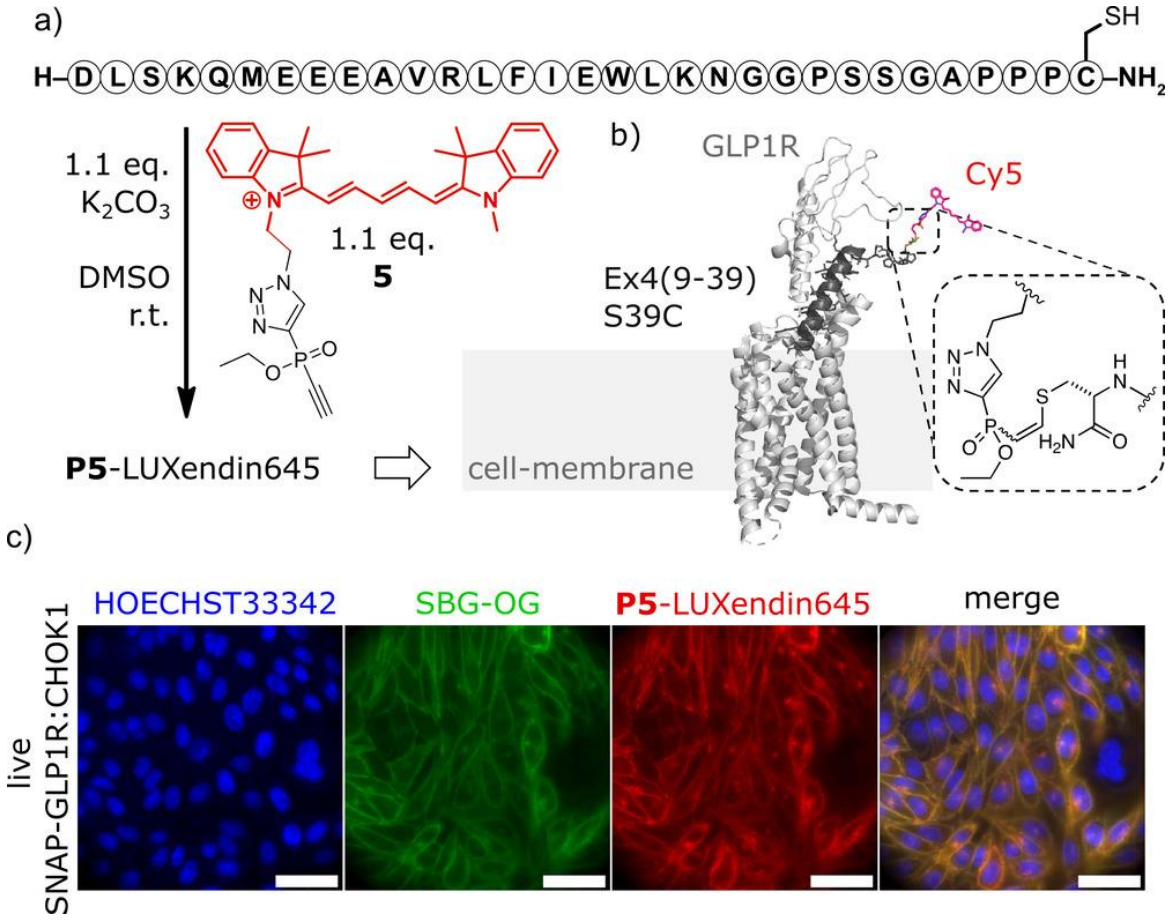
Application of Ethynyl-Triazolyl-Phosphinates as Modular Electrophiles

The experimental thiol addition kinetics



- **ETP electrophile (3) showed accelerated reaction kinetics**, which is in agreement with the DFT-calculated reaction barriers

Cysteine-selective peptide modification



- **P5**-LUXendin645 specifically labelled CHO-K1 cells stably expressing SNAP-GLP1R.

Stieger, Baik, Hackenberger *et al. Angew. Chem. Int. Ed.* **2022**, *61*, e202205348

Contents

Summary & Perspective

Challenges of Bioconjugations

- **The critically restricted scope of chemical reactions** that can be performed in a biological context.

Computational Mechanistic Analysis

In-depth insights and understanding about structure–reactivity trends from **transition state analysis**

→**Constitute a solid foundation** for extremely fast, stable reactions with high utility

Combination with Chemical Ingenuity

Improve and expand the bioconjugations, and open new capabilities in the life sciences