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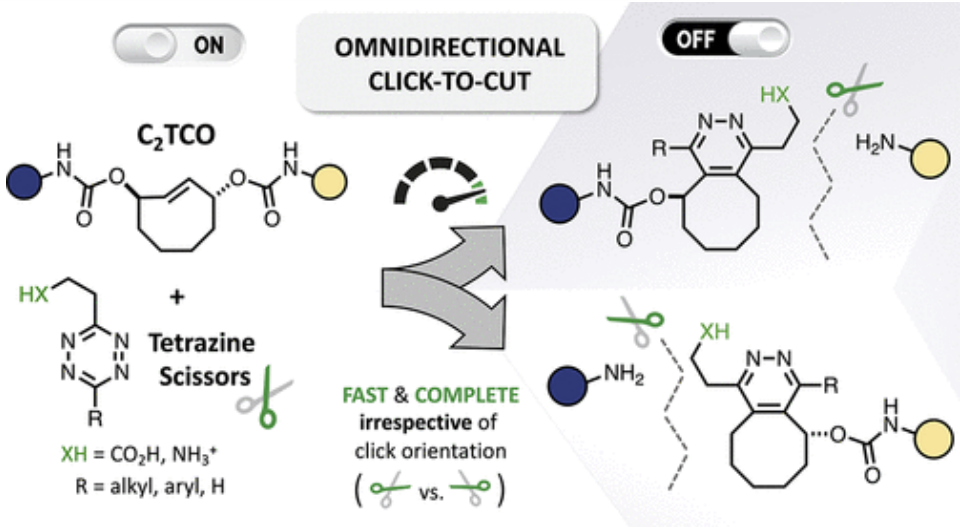
Article

Literature Report VI

A Cleavable C_2-Symmetric *trans*-Cyclooctene Enables Fast and Complete Bioorthogonal Disassembly of Molecular Probes

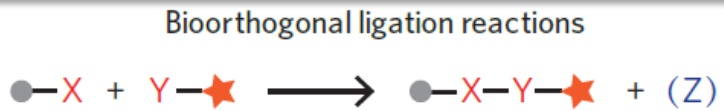
Martin Wilkovitsch,[$] Maximilian Haider,[$] Barbara Sohr, Barbara Herrmann, Jenna Klubnick, Ralph Weissleder, Jonathan C. T. Carlson,[*] and Hannes Mikula[*]

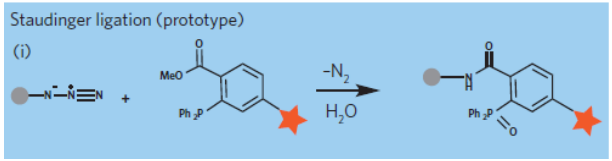


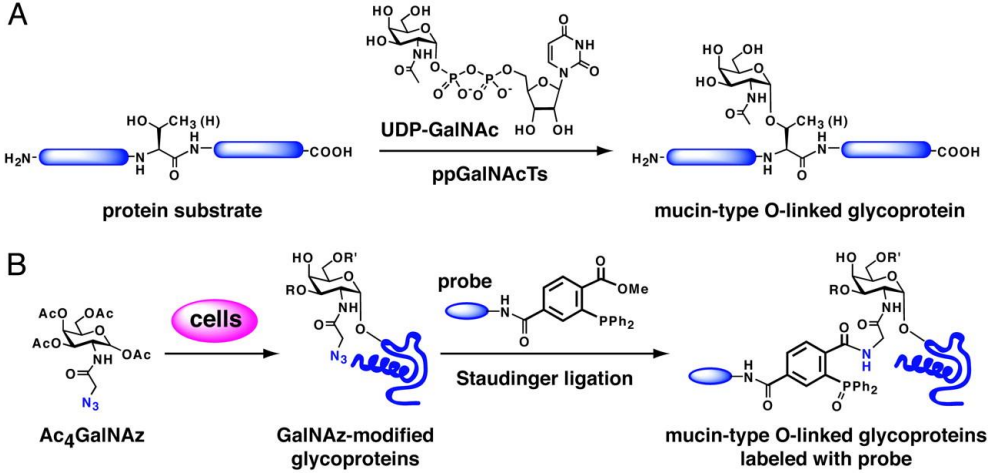
Reporter: Jin Li

Date: 2020-11-05

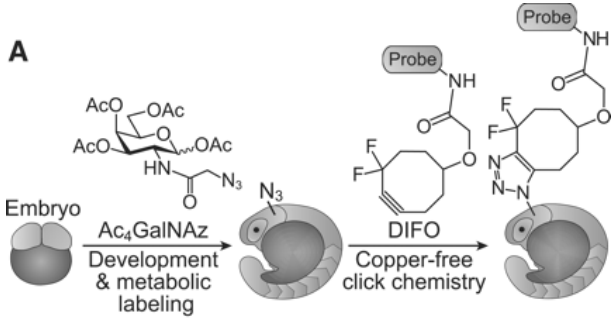
A brief history of bioorthogonal chemistry



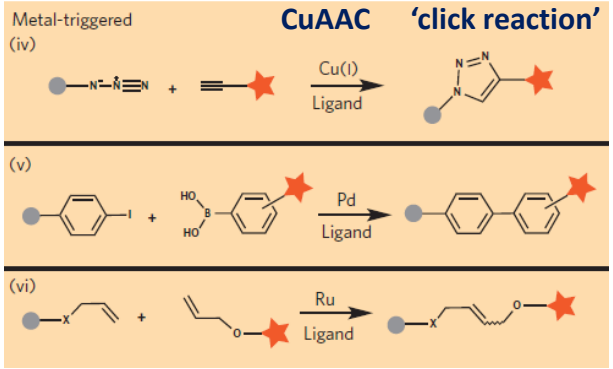




Applied the Staudinger ligation reaction on living cells and was the first to use the term 'bioorthogonal chemistry'

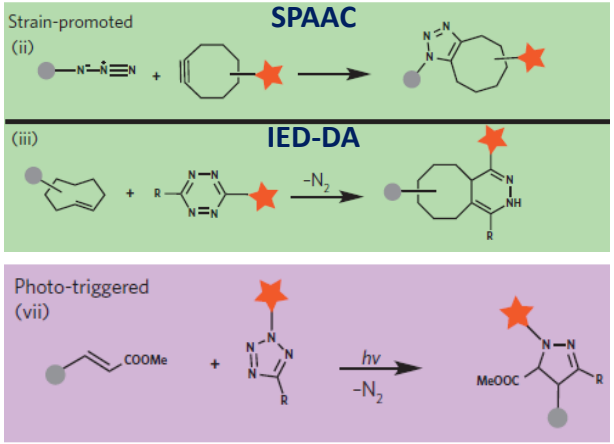


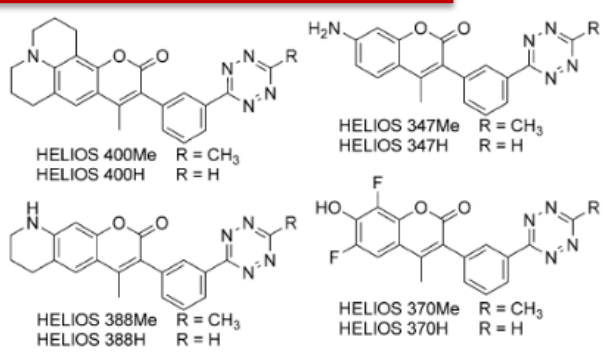
In Vivo Imaging of Membrane-Associated Glycans in Developing Zebrafish

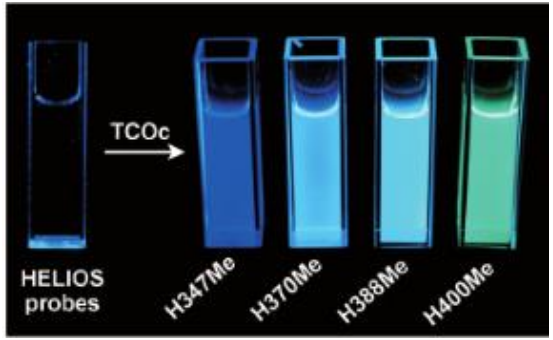


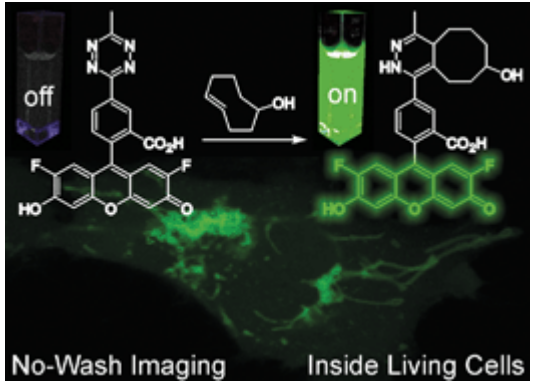
PNAS., 2003, 25, 14846; Science 2008, 320, 664; Nature Chemical Biology, 2016, 12, 129

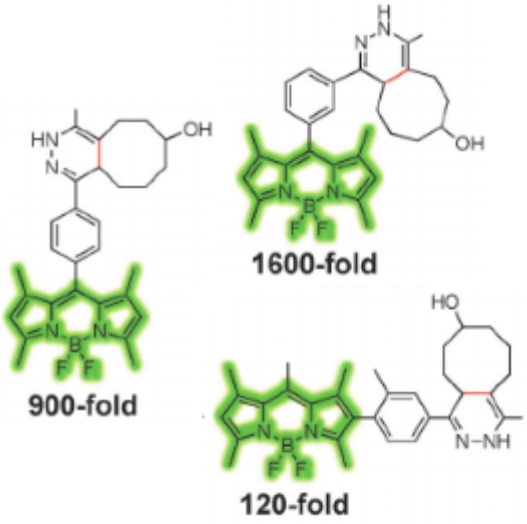
A brief history of bioorthogonal chemistry

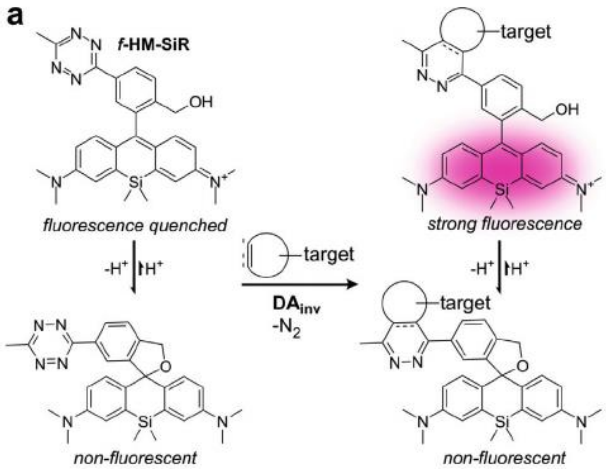






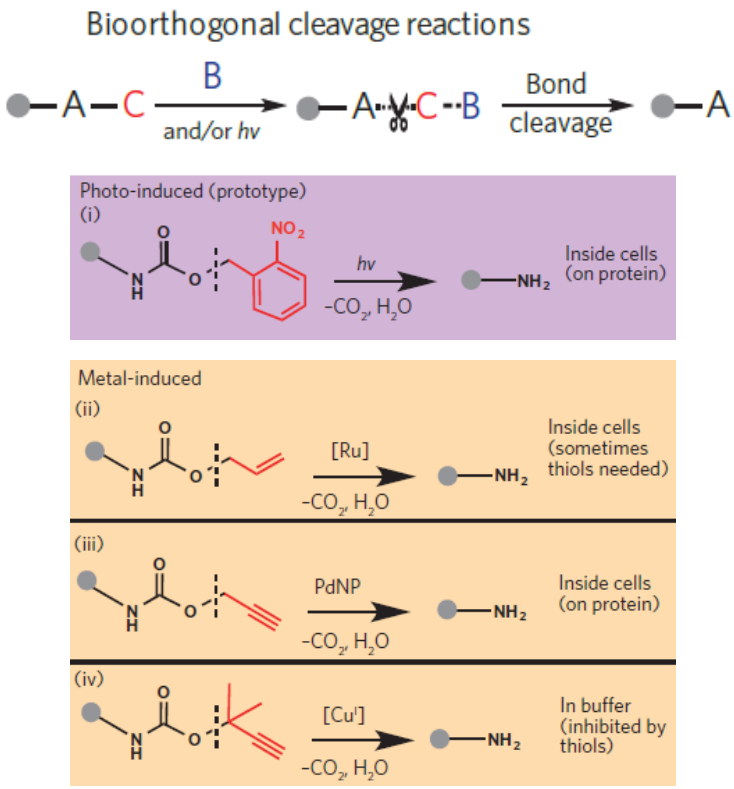


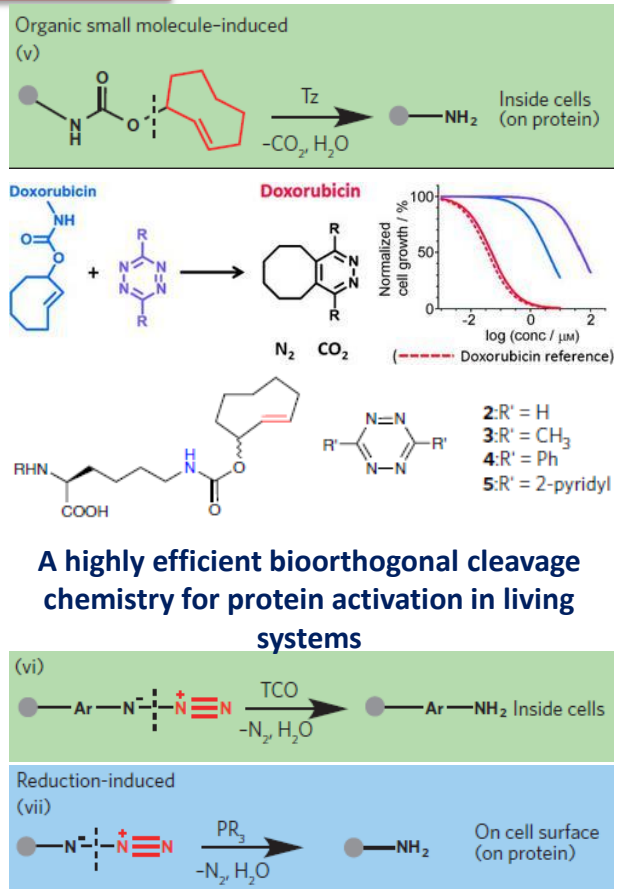




Nature Chemical Biology, **2016**, 12, 129; *Angew. Chem., Int. Ed.*, **2013**, 52, 6917; . *Angew. Chem., Int. Ed.*, **2014**, 53, 7531; *Chem. Sci*., **2017**, 8, 1506; *Angew. Chem., Int. Ed*., **2020**, 59, 804

Emerging bioorthogonal reactions beyond ligation

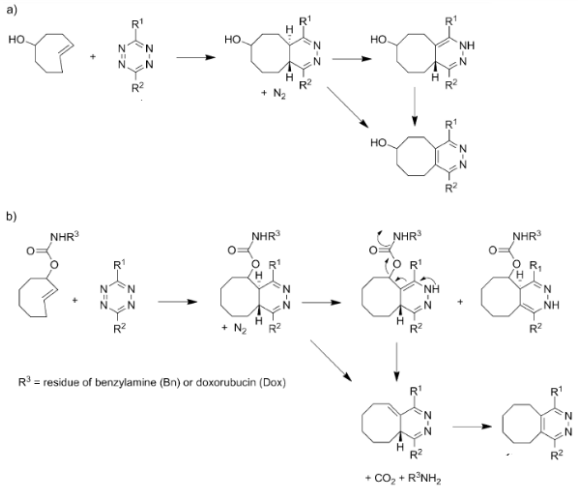


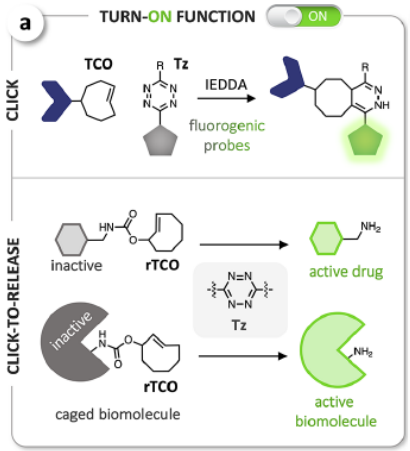


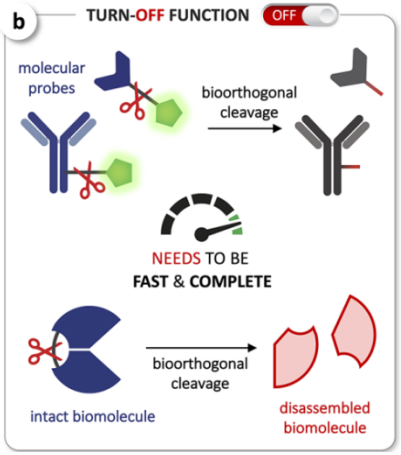
A highly efficient bioorthogonal cleavage chemistry for protein activation in living systems

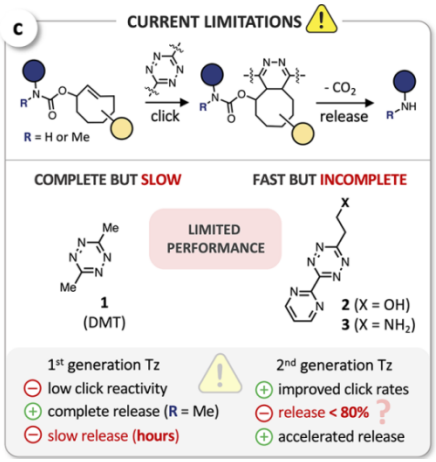
Nature Chemical Biology, **2016**, 12, 129; *Angew. Chem. Int. Ed.*, **2013**, 52, 14112; *Nat. Chem. Biol.* **2014**, 10, 1003

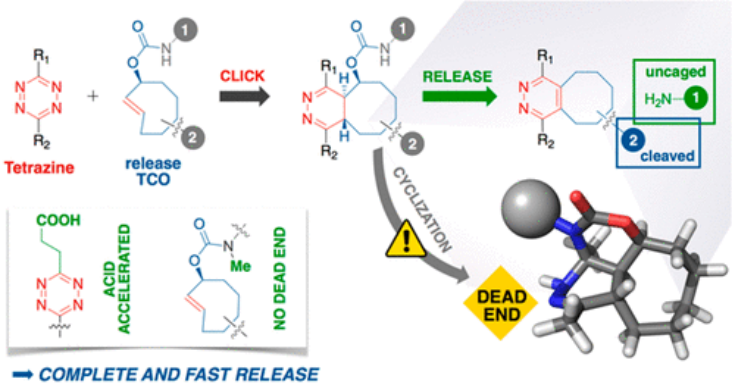
IEDDA and its potential release mechanisms





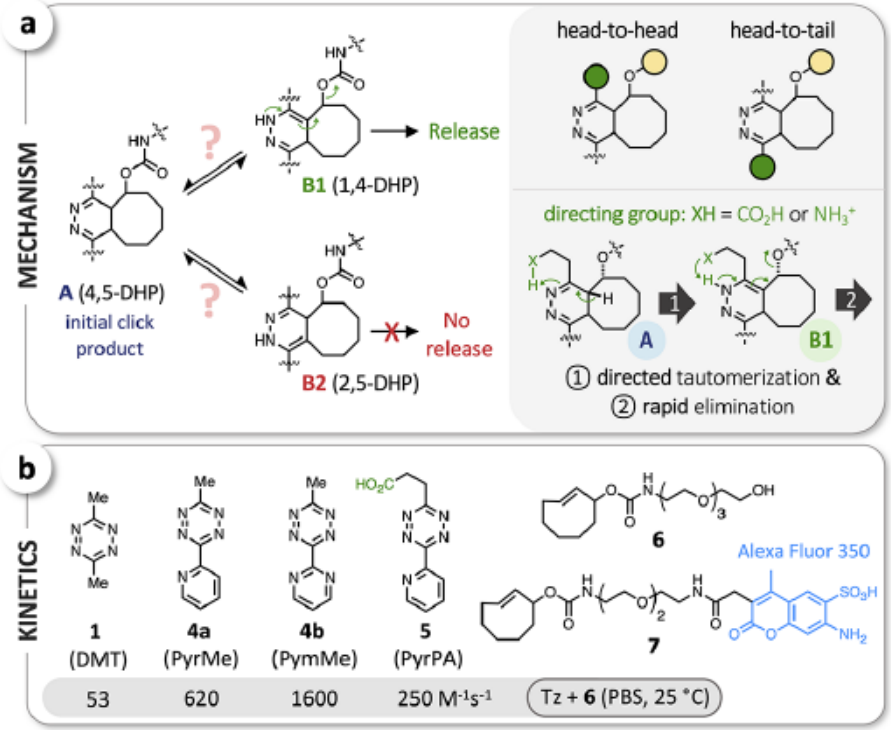


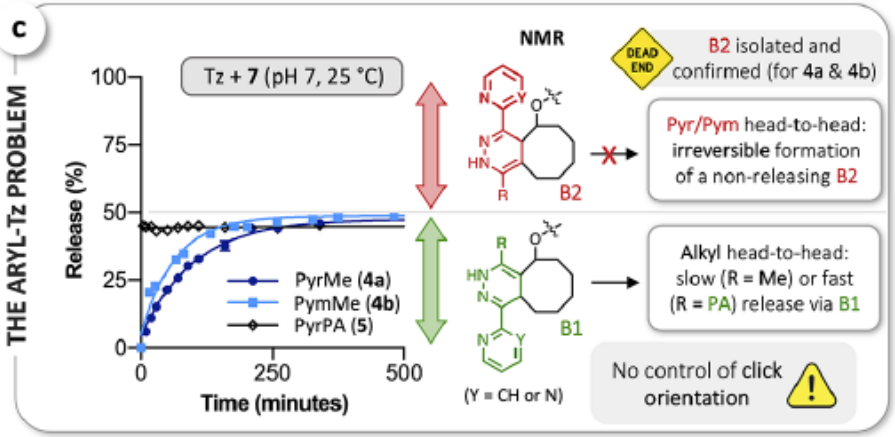




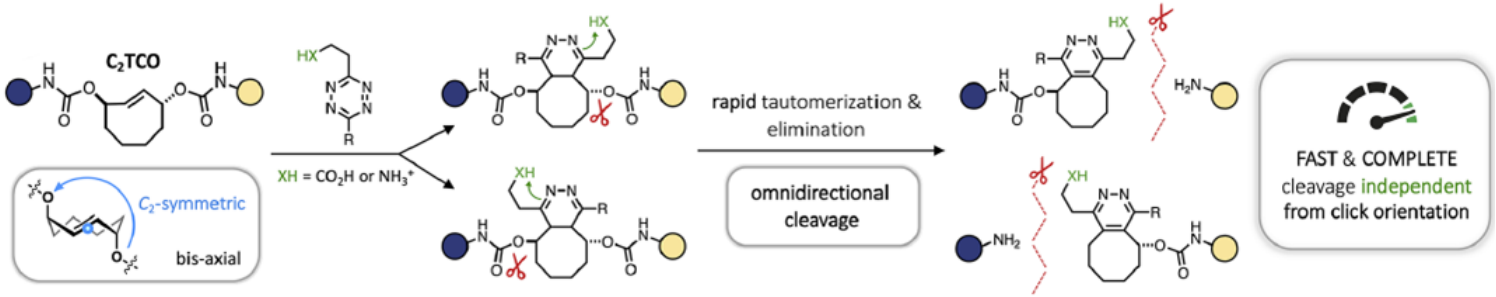
Angew. Chem., Int. Ed. 2013, 52, 14112；J. Am. Chem. Soc. 2018, 140, 3603

Bioorthogonal-triggered release

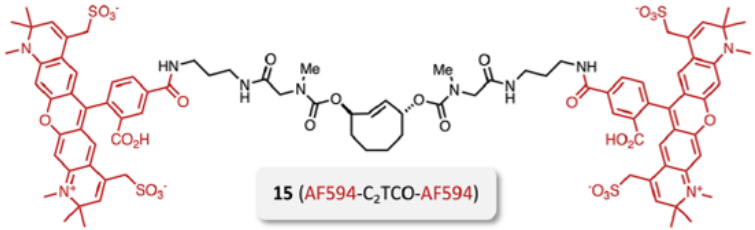




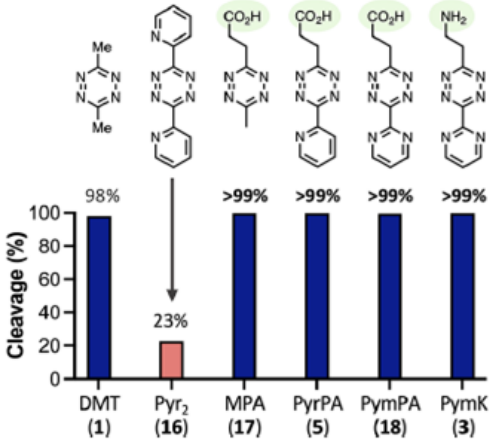
C_2-symmetric trans-cyclooctene



Tz-triggered cleavage of C_2TCO



b **Cleavage efficiency** (**endpoint**, pH 7.4)
15 (25 µM) + Tz (50-100 µM) → HPLC



c **Click kinetics** (PBS, 37 °C)

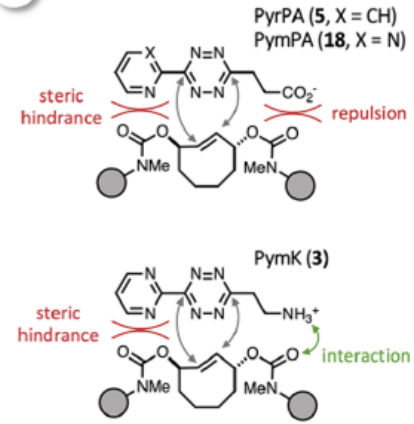
Tz + Sar-C_2TCO-Sar (**13**)
(stopped-flow measurements)

Tz	k ($M^{-1}s^{-1}$)
DMT (**1**)	17
MPA (**17**)	3.3
PyrPA (**5**)	**11**[a]
PymPA (**18**)	**24**
PymK (**3**)	**240**

10X

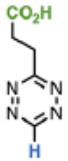
[a] 23-fold slower than **5** + rTCO (at 25 °C, Fig. 2b)

d



H-tetrazines

a H-TETRAZINES

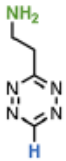


HPA + **15**: >99% cleavage (endpoint, PBS)

75 $M^{-1}s^{-1}$ †

HPA (**19**)

† 7-fold faster than PyrPA (**5**)



HK + **15**: >99% cleavage (endpoint, PBS)

390 $M^{-1}s^{-1}$ ‡

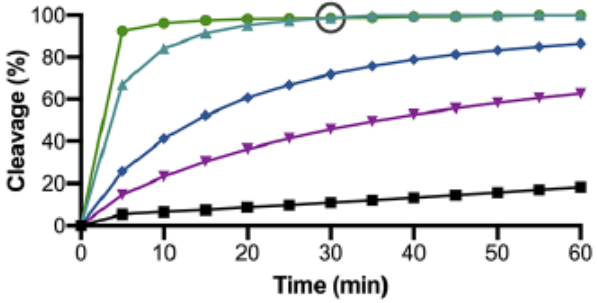
HK (**20**)

‡ 60% faster than PymK (**3**)

b FAST & COMPLETE CLEAVAGE

10 µM **15** + 20 µM **Tz** (pH 7.4)

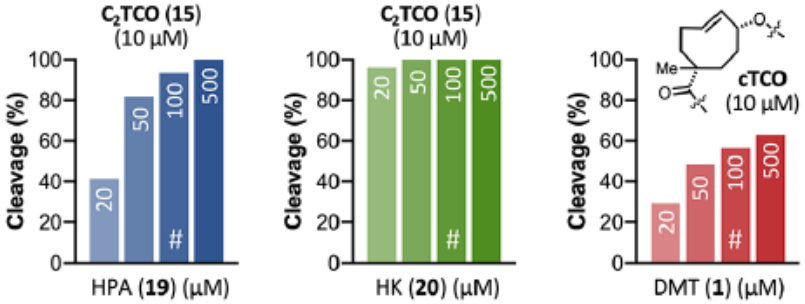
99% cleavage in **30 min**



NO intermediates (**A**, **B**) detected → INSTANTANEOUS RELEASE

c C_2TCO vs. known TCO linker (cTCO)

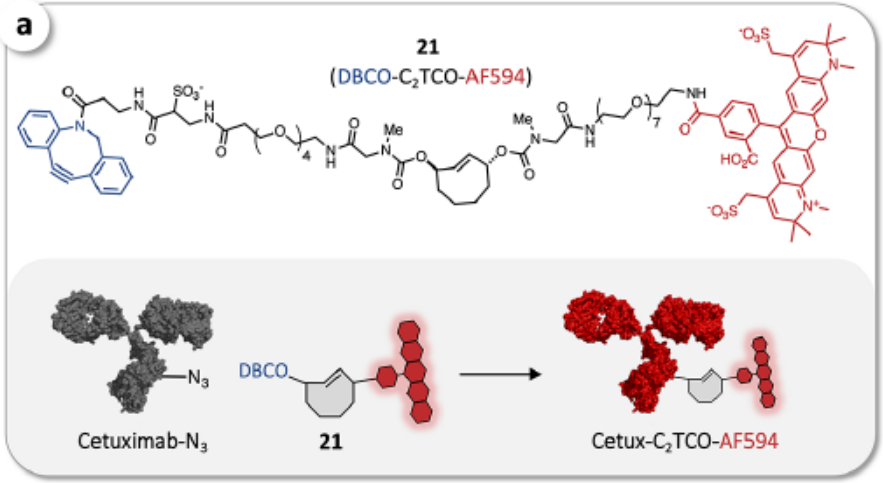
Cleavage after 10 minutes at increasing Tz concentration (PBS, 37 °C)

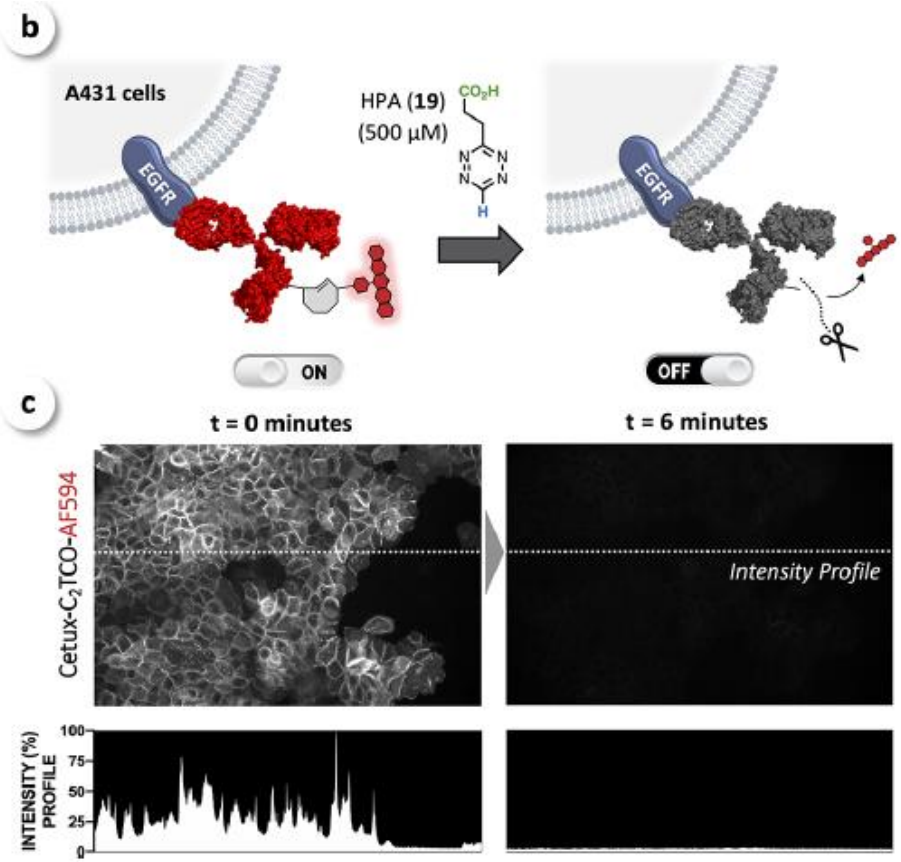


(#) Reactions at 100 µM Tz (PBS, 37 °C)

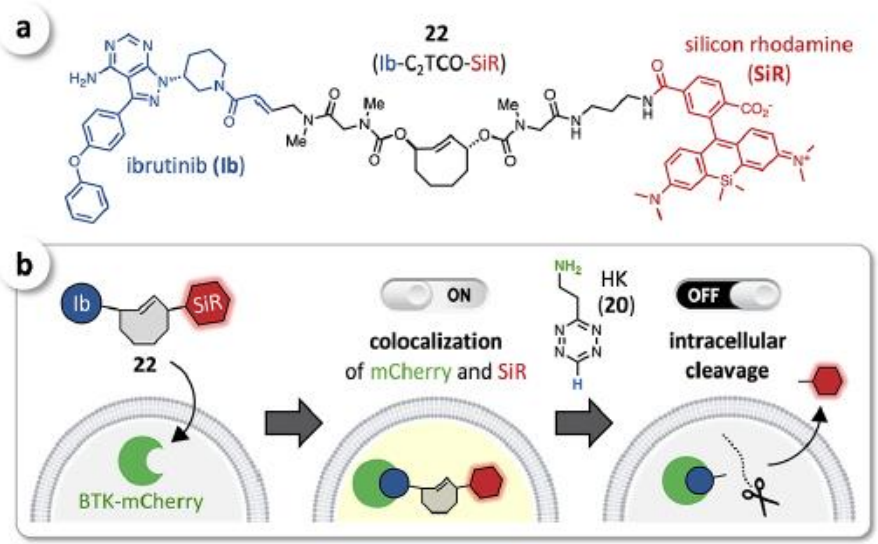
C_2TCO + HPA (**19**)	C_2TCO + HK (**20**)	cTCO + DMT (**1**)
99% click: ~11 min	99% click: ~2 min	99% click: ~14 min ⚠
99% cleavage: < 12 min	**99% cleavage: < 3 min**	**99% cleavage: > 1 h**

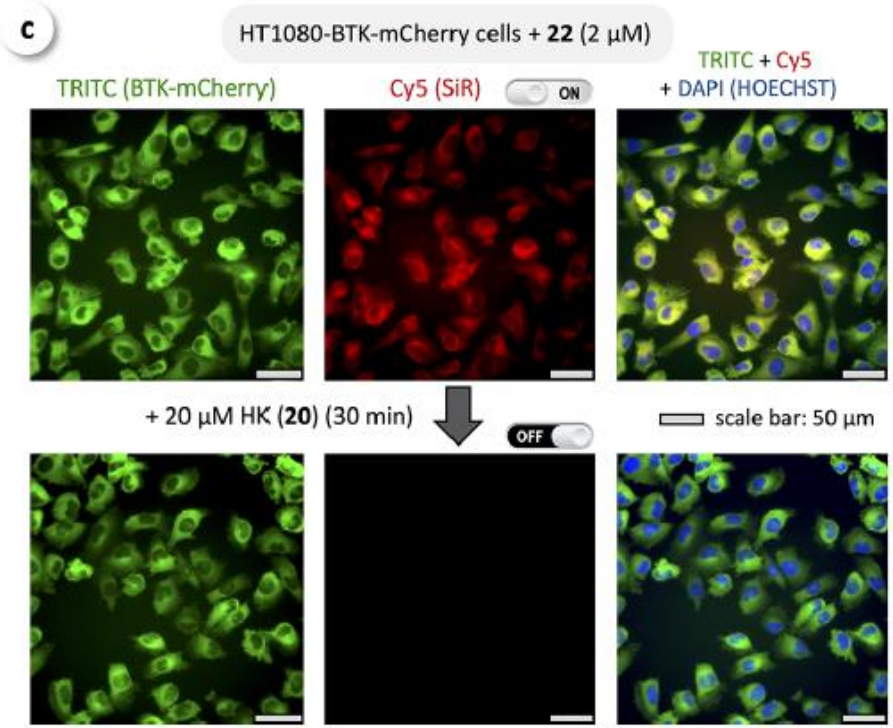
Extracellular Cleavage of Antibody-C_2TCO Conjugates





Intracellular Cleavage of Fluorescent C_2TCO-Probes





Thanks for your attention!