tagworks PHARMACEUTICALS

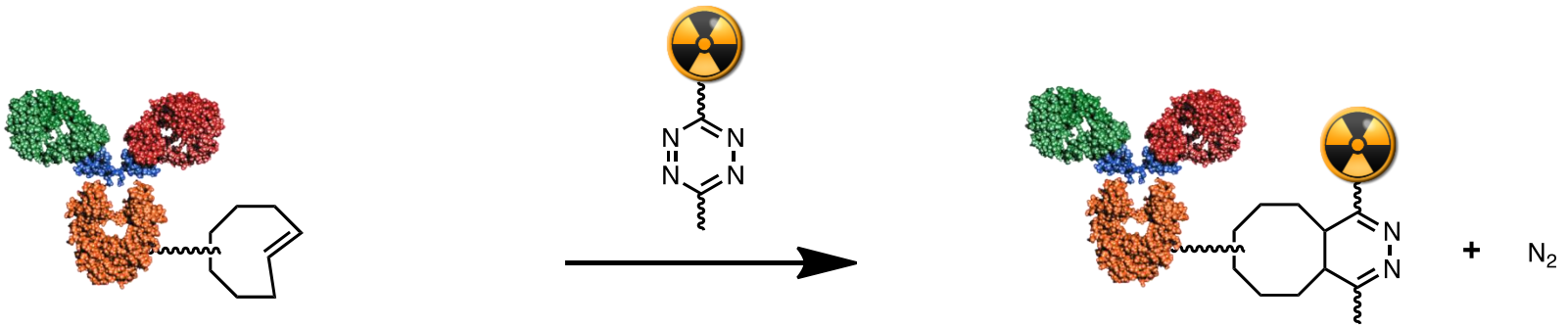
In vivo click chemistry

Marc Robillard

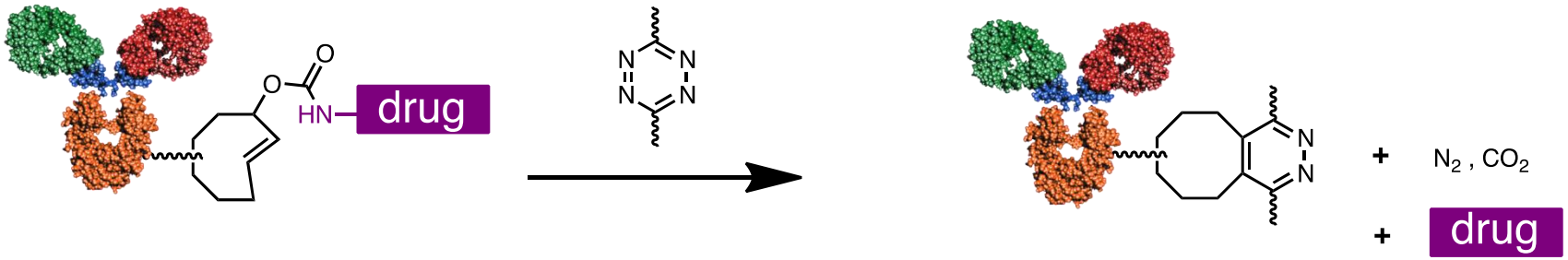
THERACAT, Barcelona, 31-5-18

On-target actuation of tagged antibodies

Click tags for Pretargeted Radioimmunoimaging & Radioimmunotherapy (RIT)

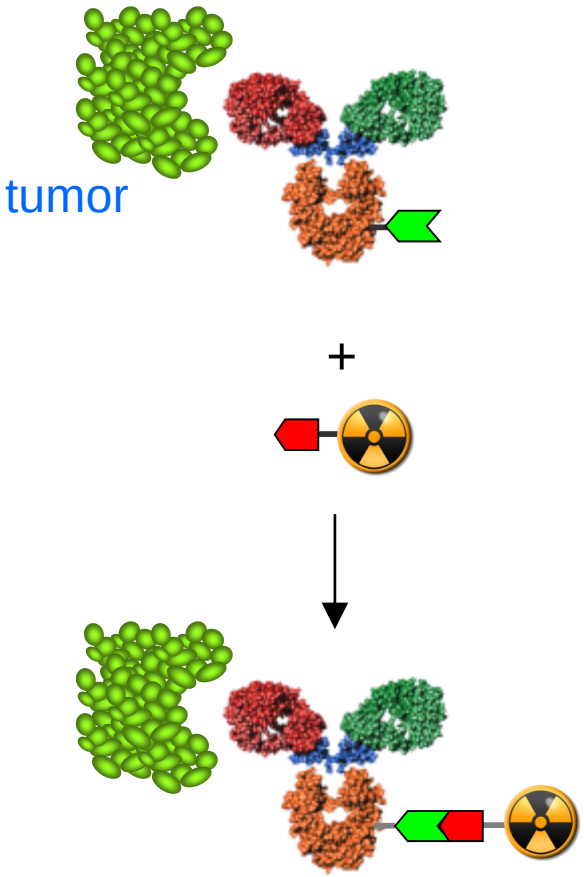


Click-cleavable linkers for non-internalizing Antibody-Drug Conjugates (ADC)



Pretargeting..

..improves radioimmunotherapy and -imaging of tumors via a 2-step tumor targeting scheme



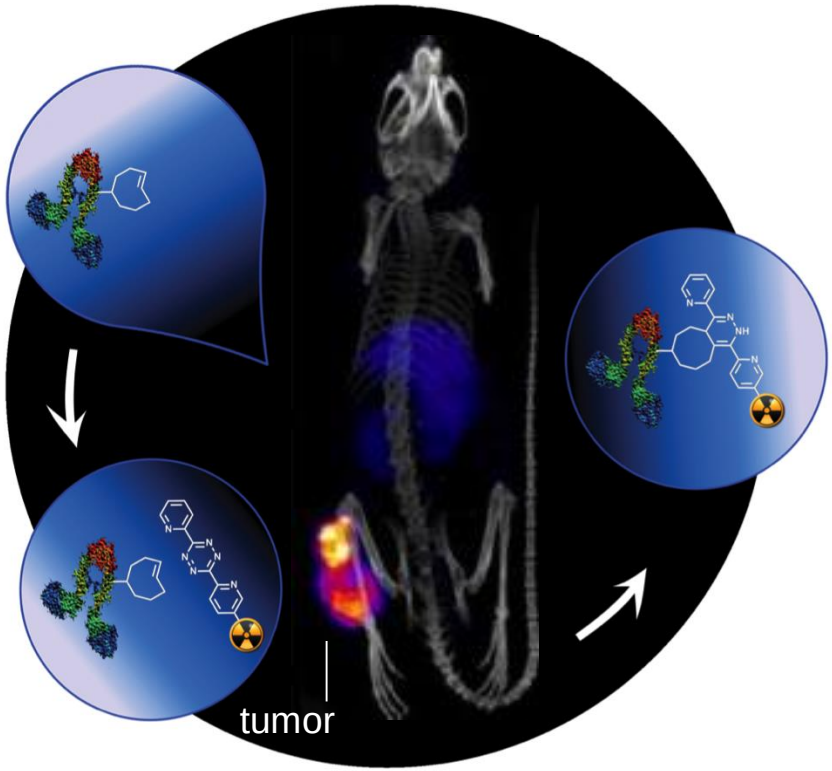
Step 1:
Slow tumor binding with antibody

Step 2:
Fast binding with small probe with

High tumor-background ratio &
increased efficacy

Circumventing biological recognition may facilitate repeat procedures

Click pretargeting



Selective binding of radioactive probe to chemical tag of antibody via bioorthogonal reaction

Very fast coupling system (up to k_2 ~ 3 x 10^5 $M^{-1}s^{-1}$) approaching streptavidin-biotin. High in vivo stability of tag ($t_{1/2}$ 10 days) and reaction product (>> 1 week)

Boosts target-to-blood ratios: improved imaging for e.g. companion diagnostics (^{18}F-tetrazines), and increased tumor dose in radioimmunotherapy

Low likelihood of immunogenicity compared to biological pretargeting components: repetition

Universal & straightforward tag conjugation with minimal perturbation: antibodies, fragments, peptides, particles, ..

Rossin *et al.*, *Angew. Chem. Int. Ed.* **2010**, *49*, 3375–3378

Fox *c.s. J. Am. Chem. Soc.* **2008**, *130*, 13518

On-target actuation of tagged antibodies

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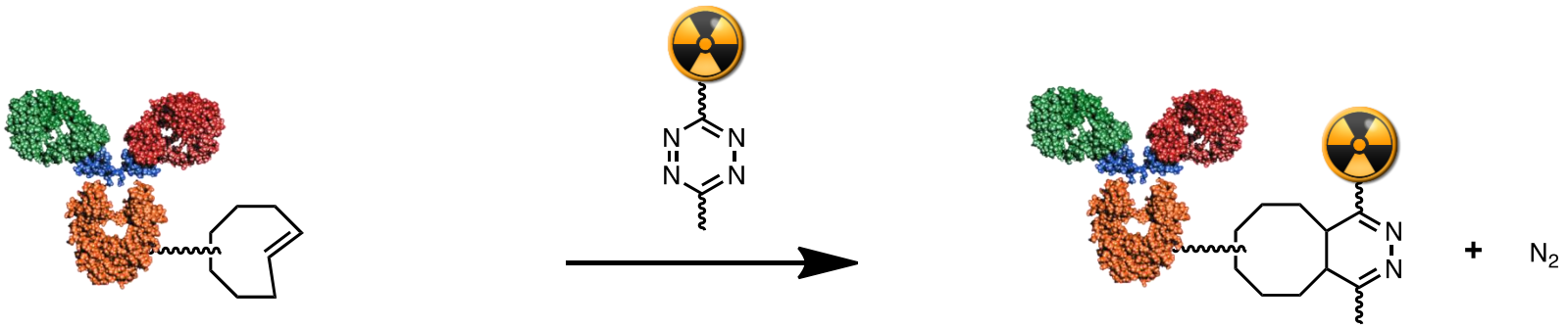
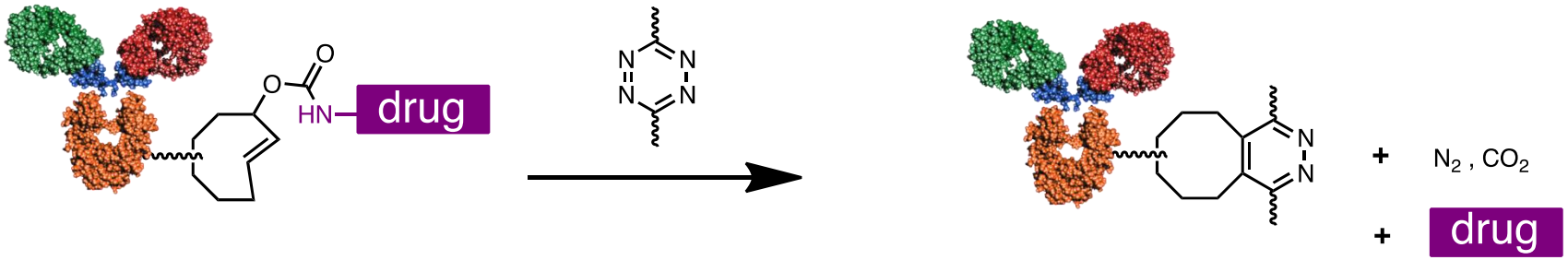
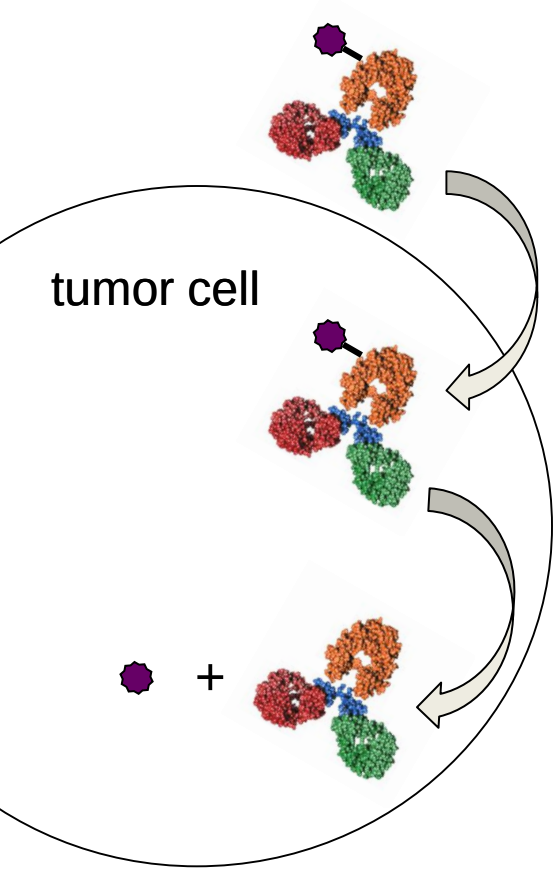


Click-cleavable linkers for non-internalizing Antibody-Drug Conjugates (ADC)



Antibody-Drug Conjugates (ADC)

Current systems are based on intracellular toxin release by enzymes or thiols

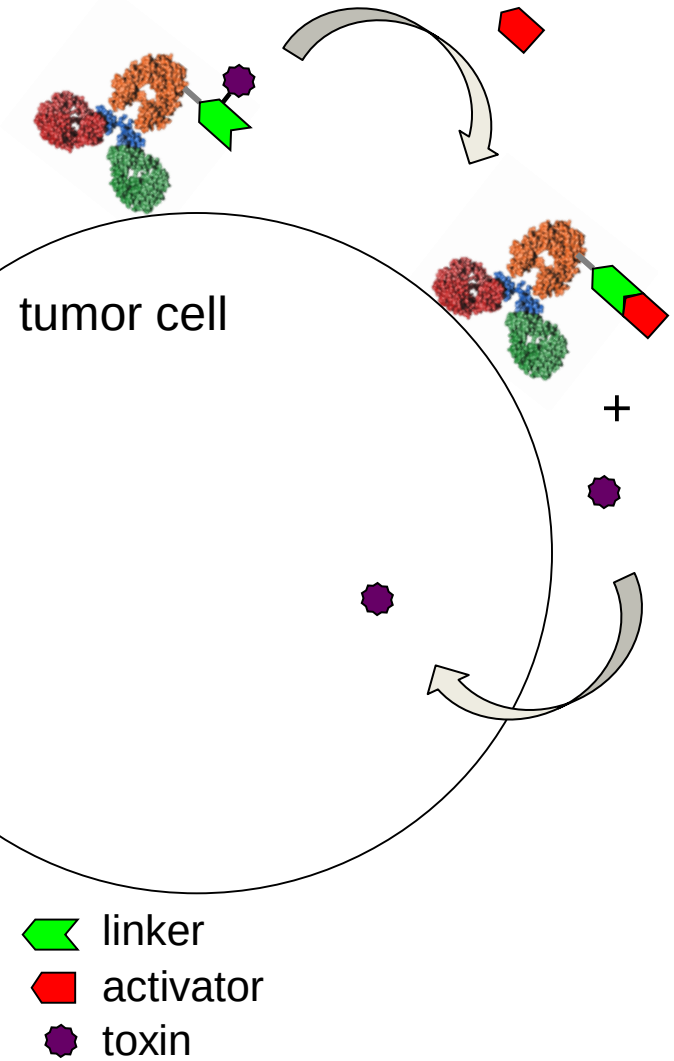


Issues

- Limited to efficiently internalizing receptors
- Shortage of suitable ADC targets in solid tumors
- Less effective in heterogeneous or poorly penetrated tumors, i.e. solid tumors

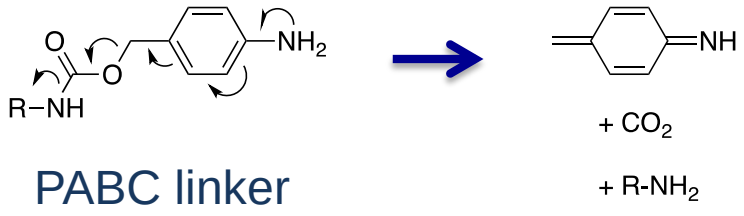
toxin

Click-to-Release Antibody Drug Conjugates

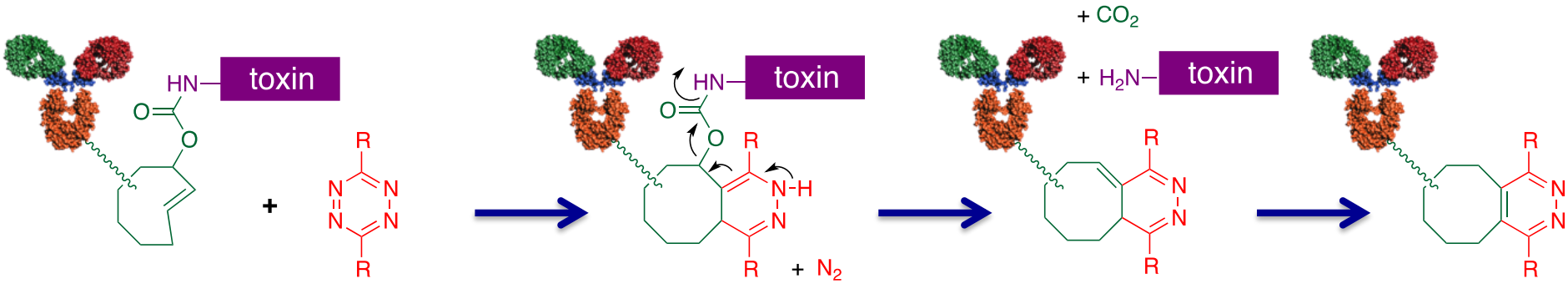


- Stable ADC linker, cleaved by a chemical probe in vivo
- 2 steps: after ADC has cleared from blood, probe is administered, triggering toxin release @ tumor
- Modification of in vivo validated pretargeting tech
- Expands the range of ADC targets: non-internalizing receptors, extracellular matrix constituents, stroma, etc
- Advantageous in heterogeneous or poorly penetrated tumors
- Universal & temporally controlled release independent from tumor biology
- Well suited for mAb fragments, or full mAbs in combination with a clearing agent

Moving from Click to Unclick



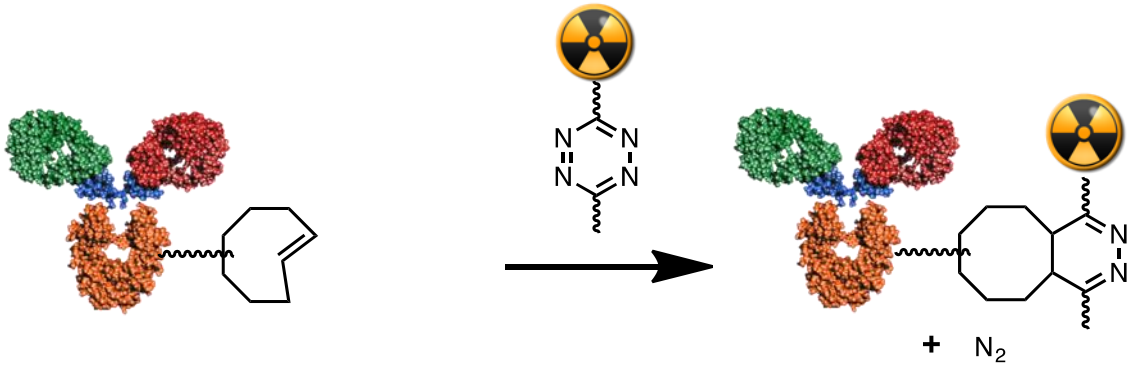
PABC linker

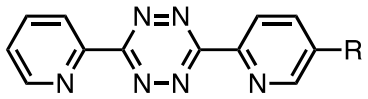


Versteegen *et al., Angew. Chem. Int. Ed.* **2013**, *52,* 14112–14116

Click-to-Release - lower reactivities

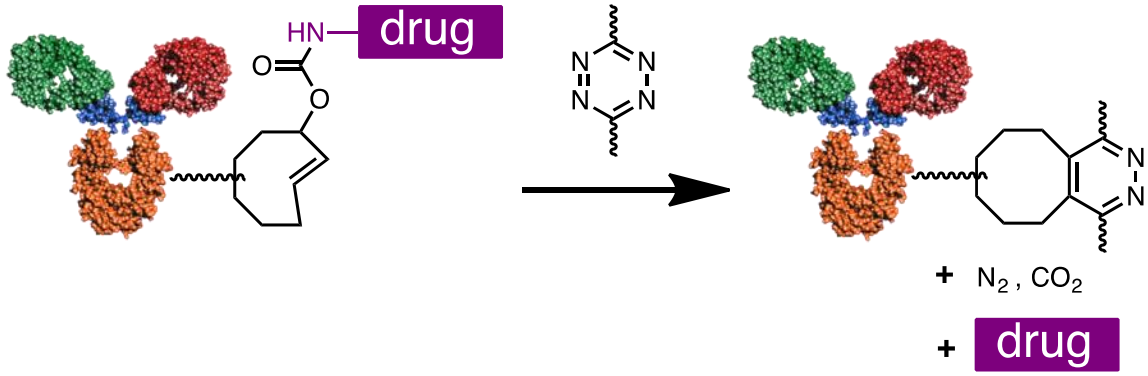
Click tags for Pretargeted Radioimmunoimaging & Radioimmunotherapy (RIT)

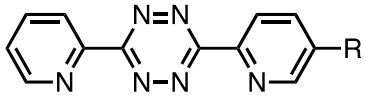




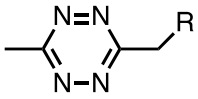
- $k_2 > 1.3 \times 10^5\ M^{-1}s^{-1}$

Click-cleavable linkers for non-internalizing Antibody-Drug Conjugates (ADC)





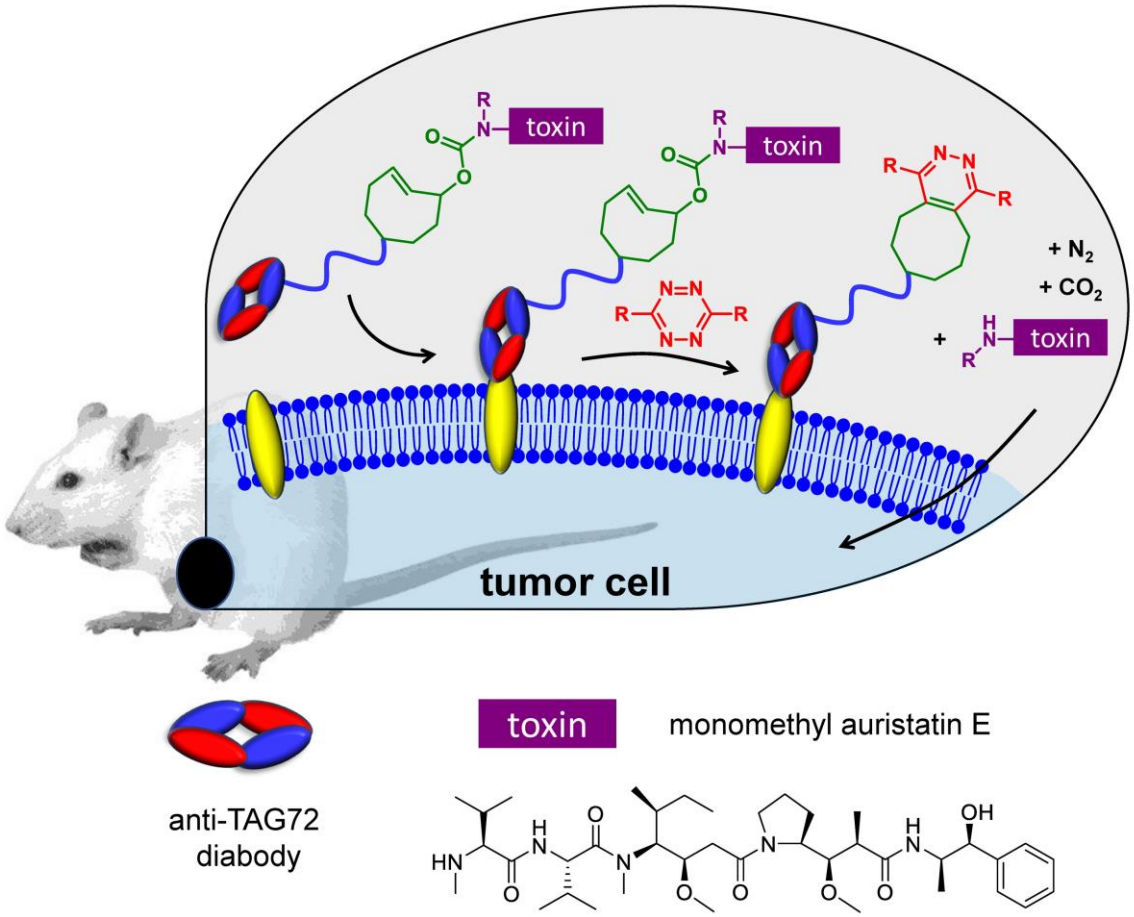
- $k_2 \sim 4 \times 10^3\ M^{-1}s^{-1}$
- release: 10 %



- $k_2 \sim 55\ M^{-1}s^{-1}$
- release: 85 %

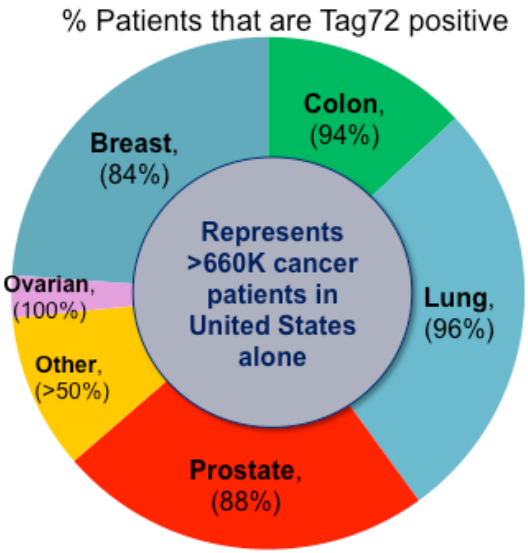
Click-to-Release of MMAE from TAG72-targeted diabody

Objectives: faster clearing ADC, improved activator, more potent drug



TAG72:

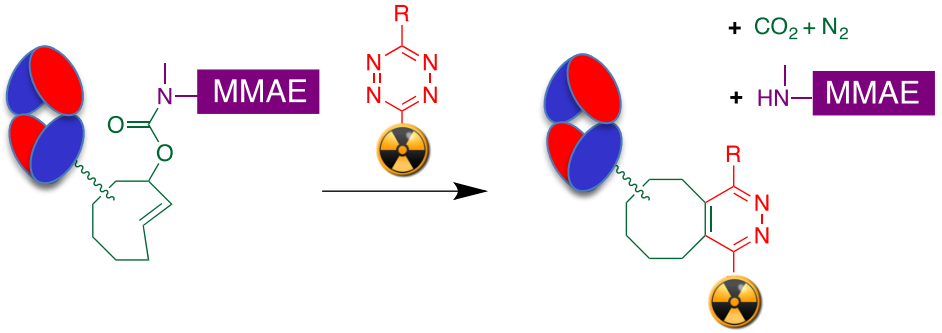
extracellular cell membrane target, non-internalizing, low shedding

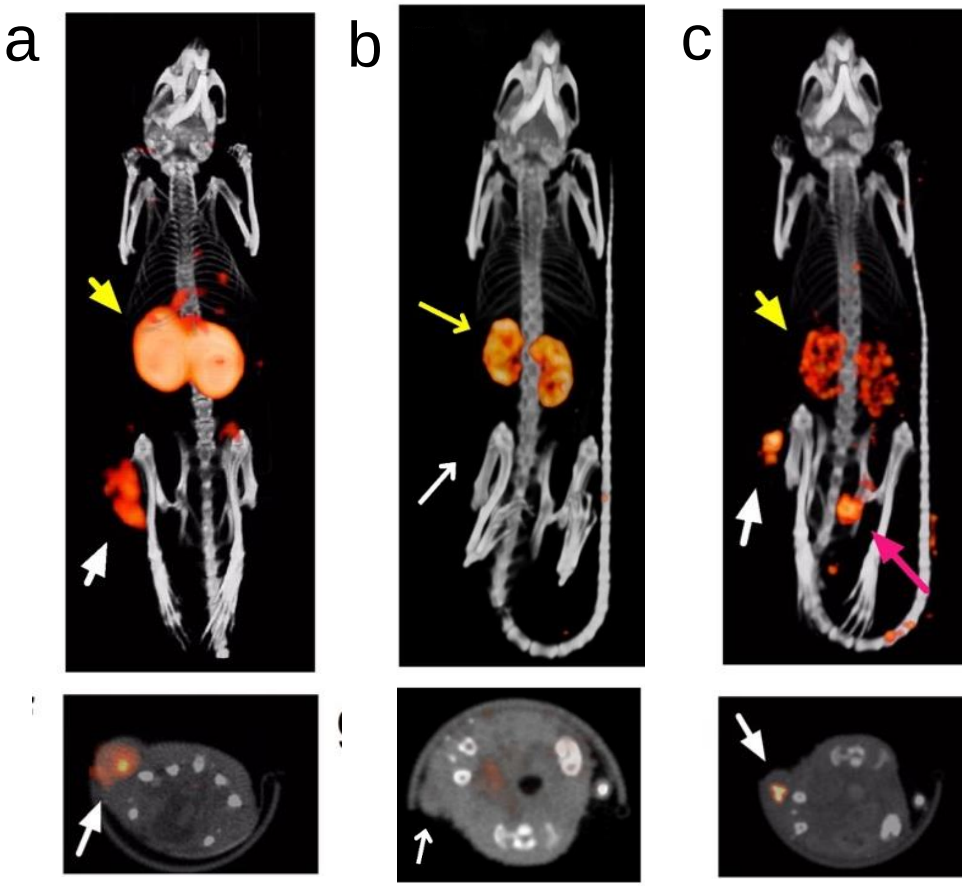


Rossin *et al.*, *Nat. Commun.* **2018**

Click-to-Image-Release

LS174T-mice inj. 1) tc-ADC; 2) 1 eq ^{111}In-Tz @ 48h; 3) imaging/biodistribution @ 51h



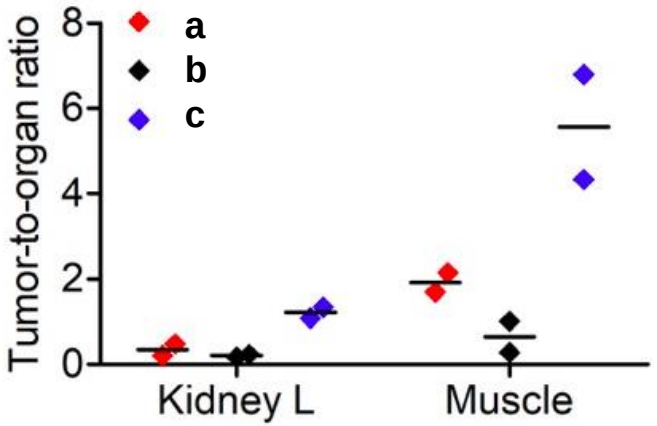


0.33 ± 0.07 %

0.11±0.06 %

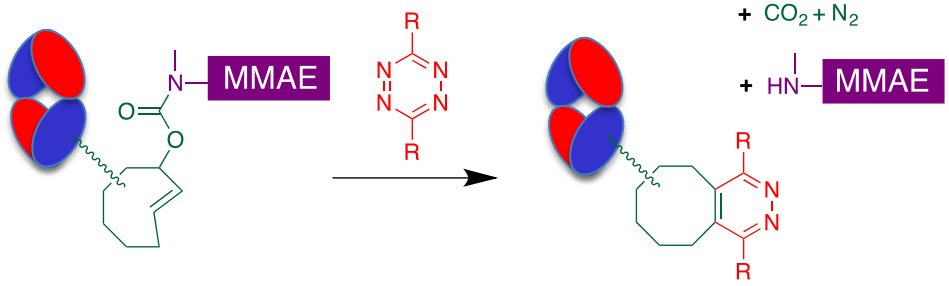
6 % ID/g

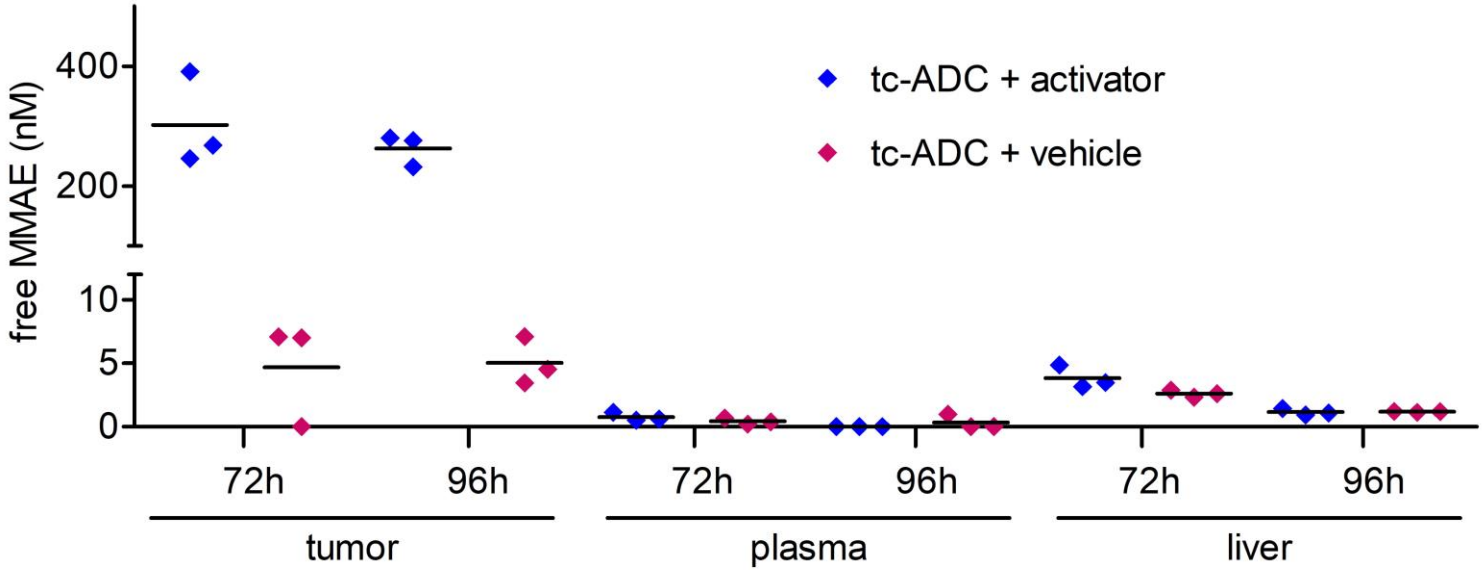
a) tc-ADC + ^{111}In-activator
b) ^{111}In-activator
c) tc-ADC + ^{111}In-probe



Free MMAE concentrations in vivo

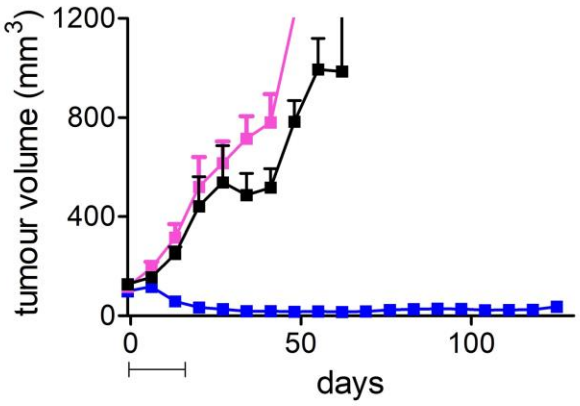
LS174T-mice inj. 1) tc-ADC (0.033 µmol/kg) , 2) activator (0.335 mmol/kg; 48h), biodistribution @ 72 and 96 h, MMAE extraction

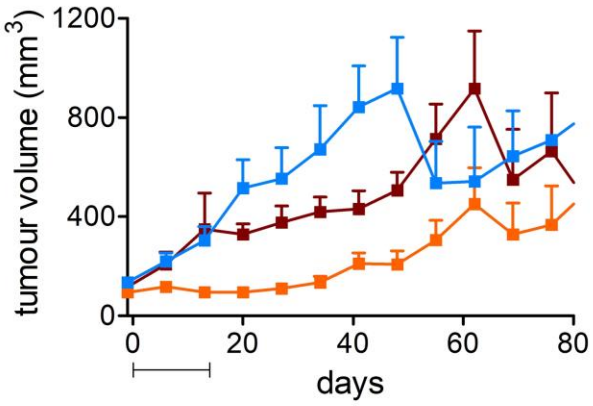


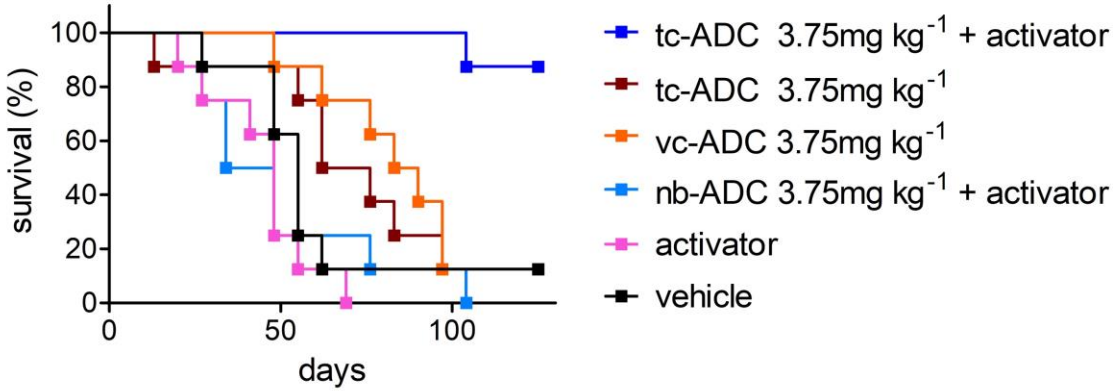


Therapeutic efficacy in OVCAR-3 tumor bearing mice

Mice inj. within 2 weeks with 4 cycles of 1) tc-ADC, 2) activator (0.335 mmol/kg; 48h)

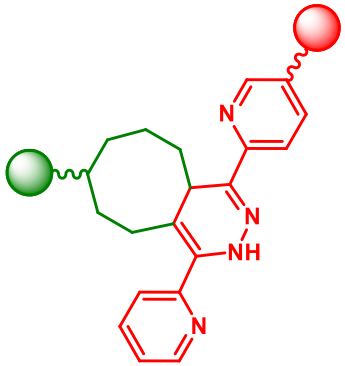






ADC	diabody	R
tc-ADC	anti-TAG72	-TCO-MMAE (**1**)
vc-ADC	anti-TAG72	-val-cit-MMAE (**2**)
nb-ADC	anti-PSMA	-TCO-MMAE (**1**)

Plans within Theracat



Click

- Radiolabel and image catalysts in vivo. Depending on the nature of the catalyst the radiolabeling will occur pre- or post-catalyst administration.

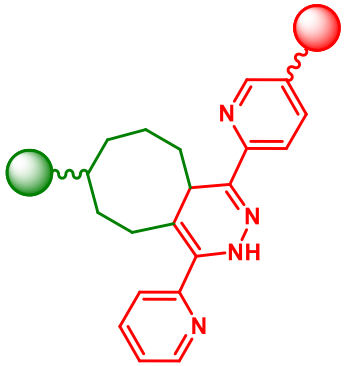
Unclick

- Activate target-localized catalysts, enabling temporal control over catalyst activity
- Selectively destabilize catalyst-containing nanoparticles at the target site, liberating the catalysts and enabling efficient catalyst-substrate interaction.

Neither click or unclick

- Image catalysts activity in vivo by using radioimaging agents designed to localize at the target following catalyst-mediated uncaging

Plans within Theracat



Deliverables ESR12, WP4

- **R8.1**: In vivo imaging of nanoparticle- and protein-based catalysts (D3.1);
- **R8.2**: In vivo imaging of radiolabeled substrate being activated by catalyst (D3.2);
- **R8.3**: In vivo release and/or activation of a target-bound catalyst by click-release chemistry (D3.3)

Planned secondment(s) (host, start month, duration):

- EDI, M12, 2 months
- BAS, M18, 3 months