University of Zurich[UZH]

Harnessing photochemistry in radiotracer synthesis

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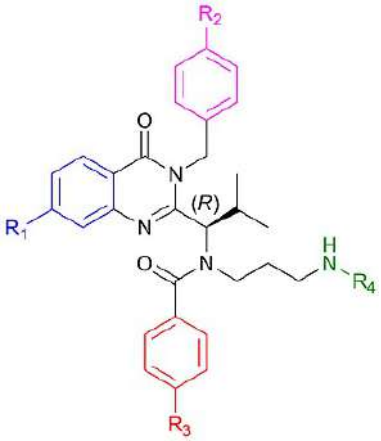
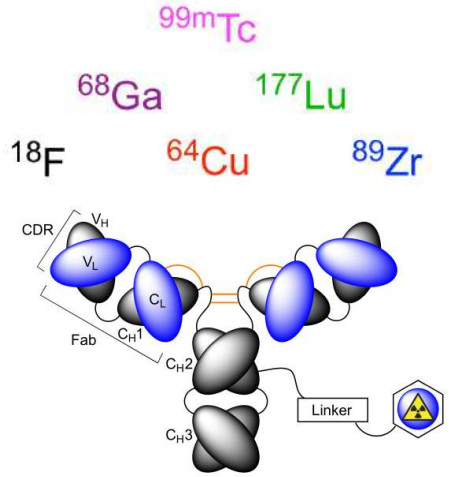
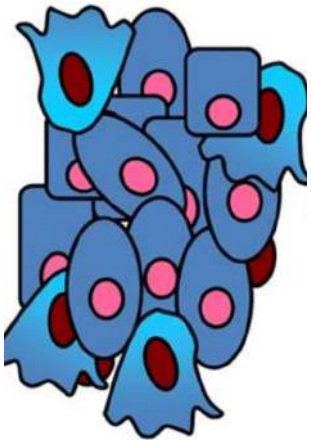
From chemistry to imaging science…



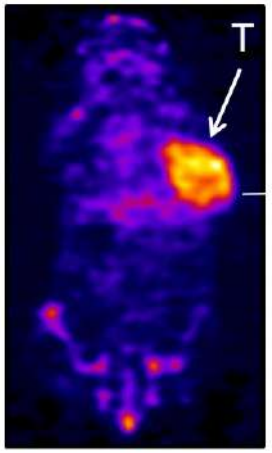
Synthetic medicinal chemistry



Radiochemistry



Cells



Animals

Humans

Current research areas

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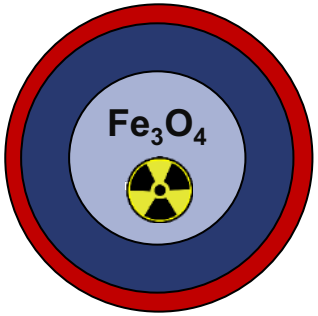
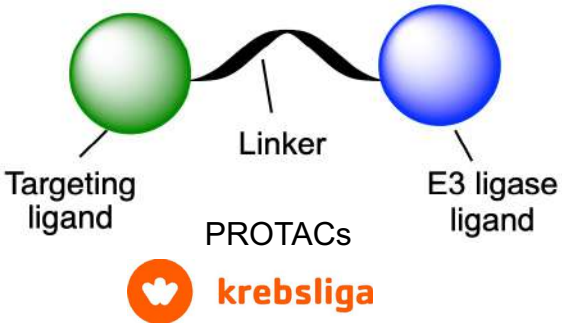


erc NanoSCAN

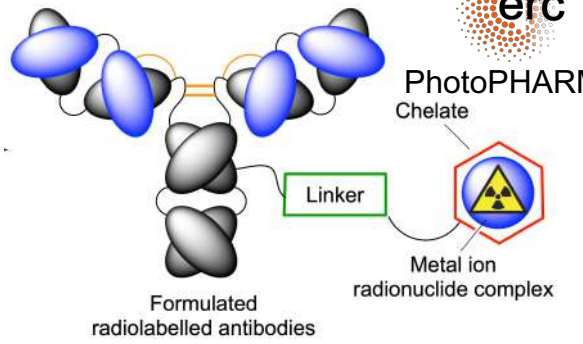
Nanoparticle radiochemistry for 'theranostics' (PET/MRI)



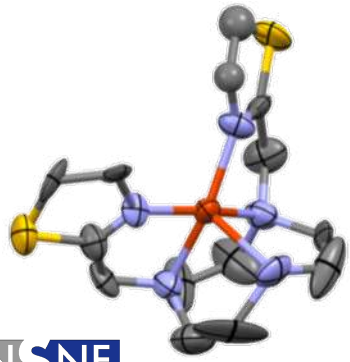
PROTACs

krebsliga

Companion diagnostics & novel therapy

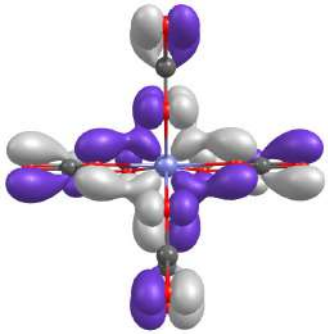


Bioconjugate chemistry
Photo(radio)chemistry

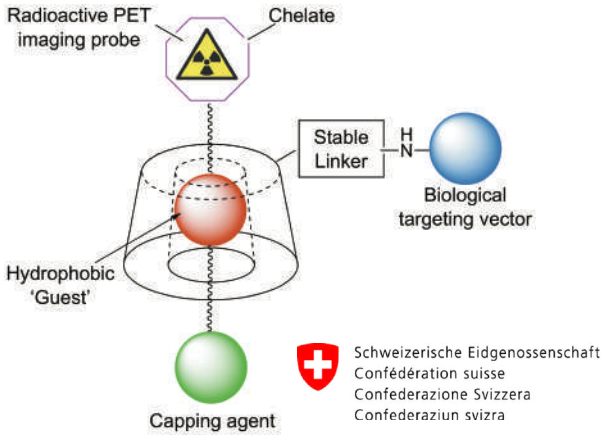


FNSNF

Chelate design & radiosynthesis (Cu, Ga, Zr, Lu, In, As ions)

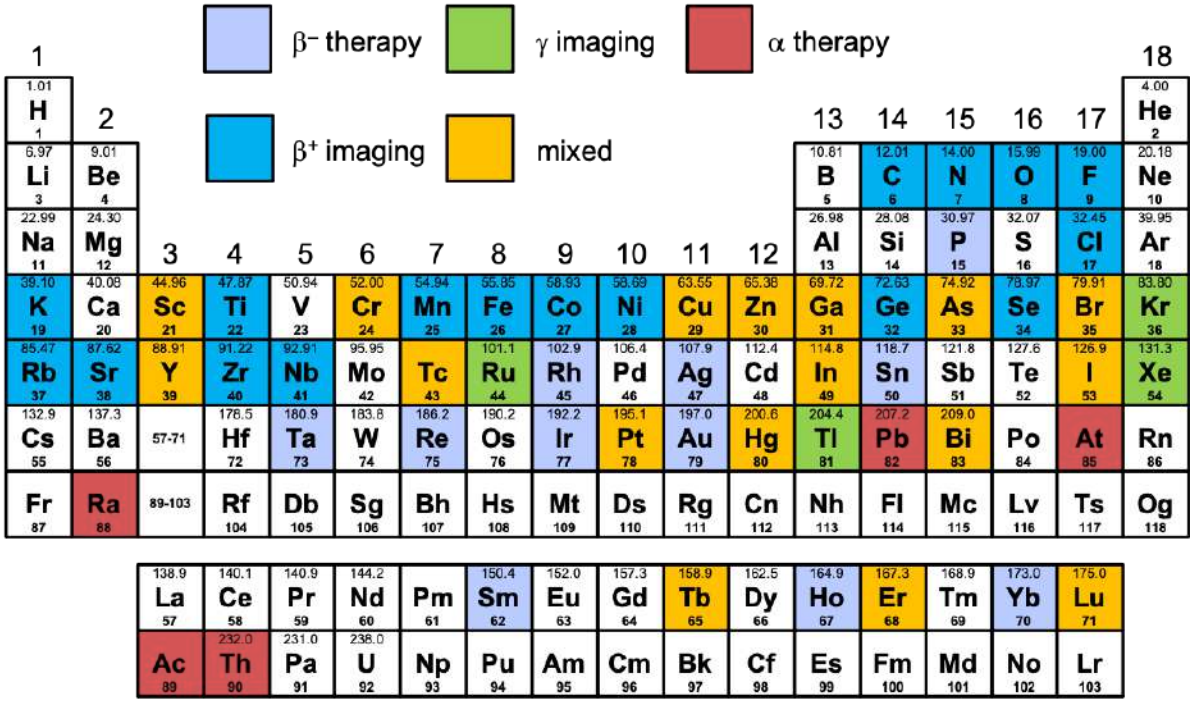


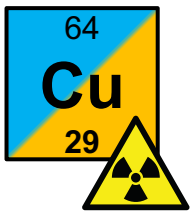
Density functional theory in radiotracer design

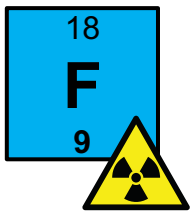


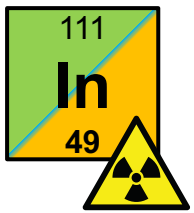
Supramolecular radiochemistry

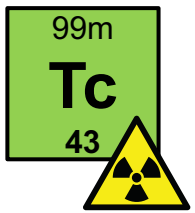
Radionuclides in Nuclear Medicine

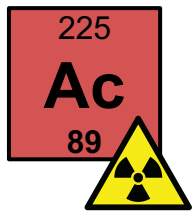












70, 71, 72, 74, 76, 77

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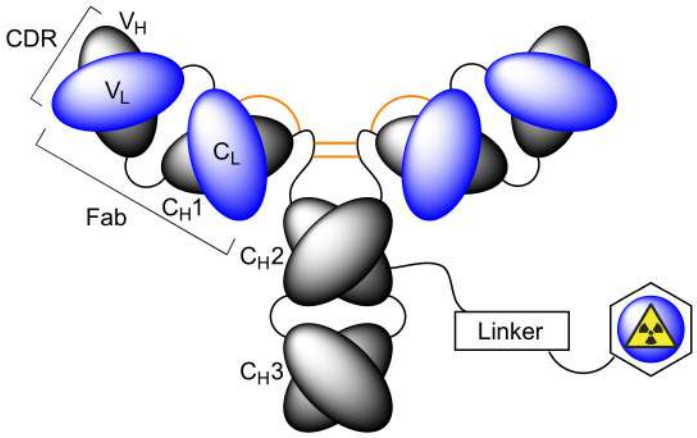
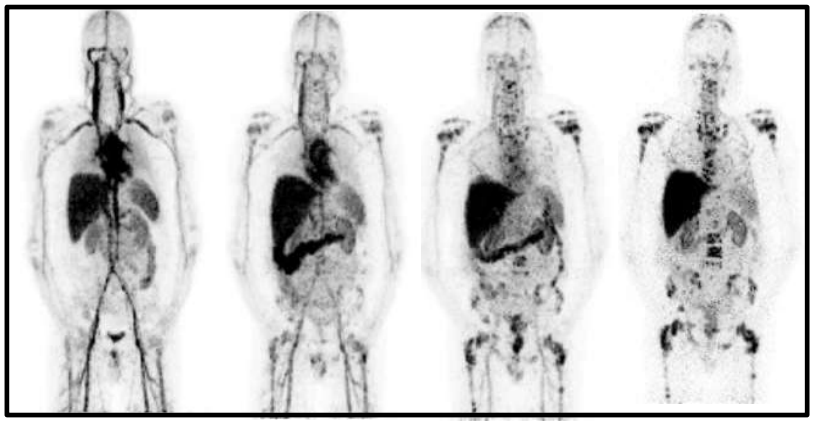
^{89}Zr radiotracers for immunoPET in cancer



[^{89}Zr]ZrDFO-J591 imaging of PSMA



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*#1 Highest Cited Article in Nucl. Med. Biol. since **2008***

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*Journal of Nuclear Medicine Editor's Choice Award, **2011***

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*Highlighted as the Cover Picture, Cancer Discovery, April **2012***

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*Highlighted as the Cover Picture, J. Nucl. Med., January **2013***

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Pandit-Tasker *et al.*, Clin. Cancer Res. **2015**, *21(23)*, 5277

Rylova *et al.*, J. Nucl. Med., **2016**, *57*, 96-102.

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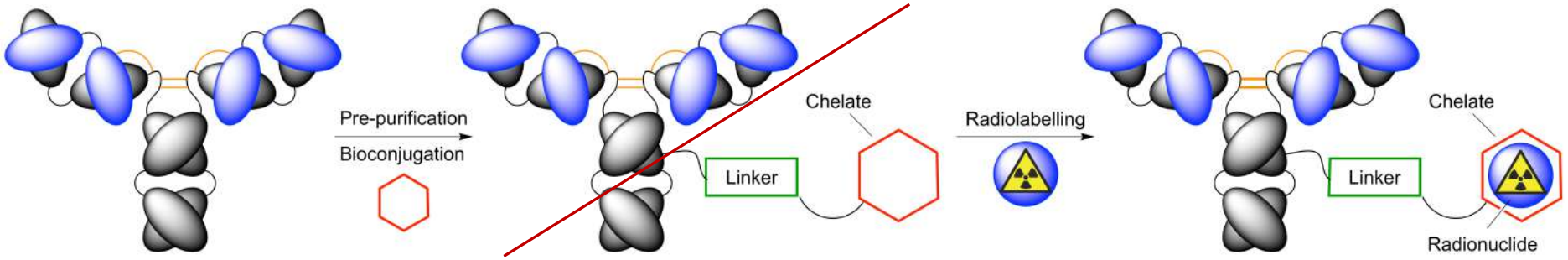
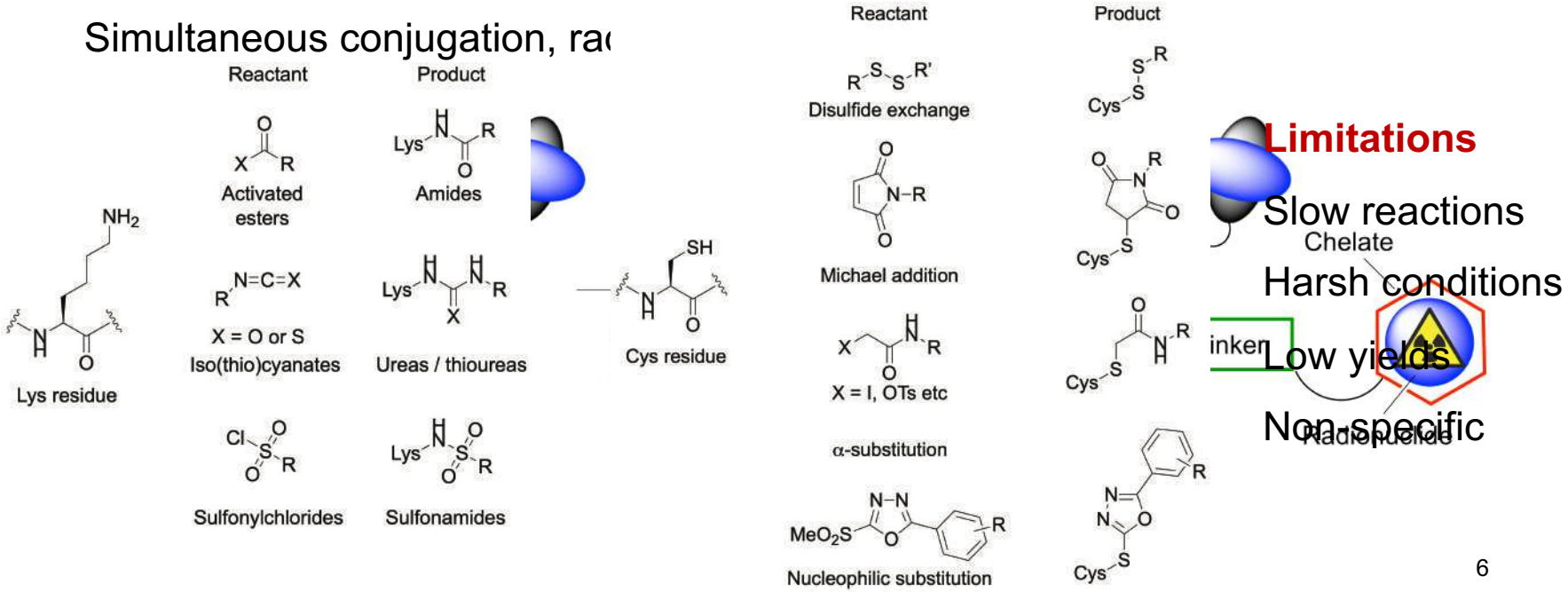
Technological advances in radiolabelled proteins

Traditional methods require pre-purification and two-step chemistry

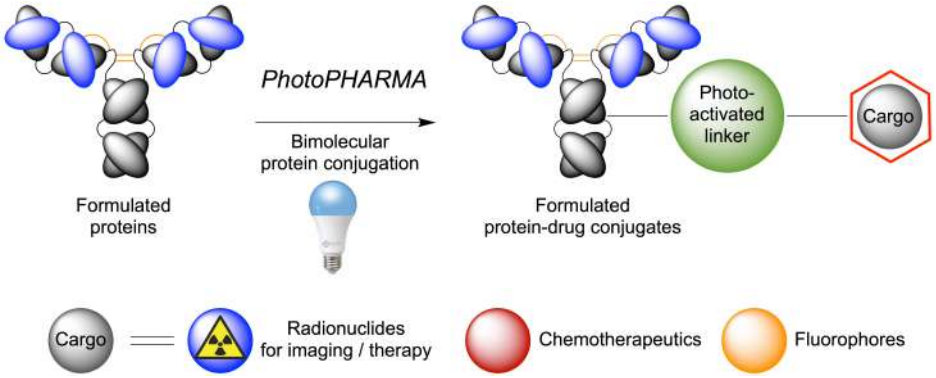


Simultaneous conjugation, ra



Light-induced protein conjugation for imaging and therapy

PhotoPHARMA



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Eichenberger *et al. Chem. Comm.*, **2019**, *55*, 2257-2260
Patra *et al. iScience*, **2019**, *13*, 416-431
Fay and Holland, *J. Nucl. Med.*, **2019**, *60*, 587-591
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Holland *et al.*, *Chem. Eur. J.*, **2020**, *26,* 33-48
Klingler *et al.*, *J. Nucl. Med.*, **2020**, *61*, 1072-1078
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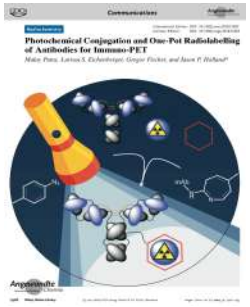
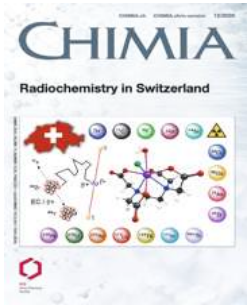
Guillou *et al.*, *Chem. Eur. J.*, **2020**, *26*, 7185-7189
Guillou *et al.*, *Nat. Protocol*, **2020**, *15*, 3579–3594
Fay and Holland, *Chem Eur. J.*, **2021**, *27*, 4893-4897
Guillou *et al.*, *Bioconjugate Chem.*, **2021**, *in press*
Klingler *et al.*, **2021**, *submitted*
Guillou *et al.*, **2021**, *submitted*
…



ACIE

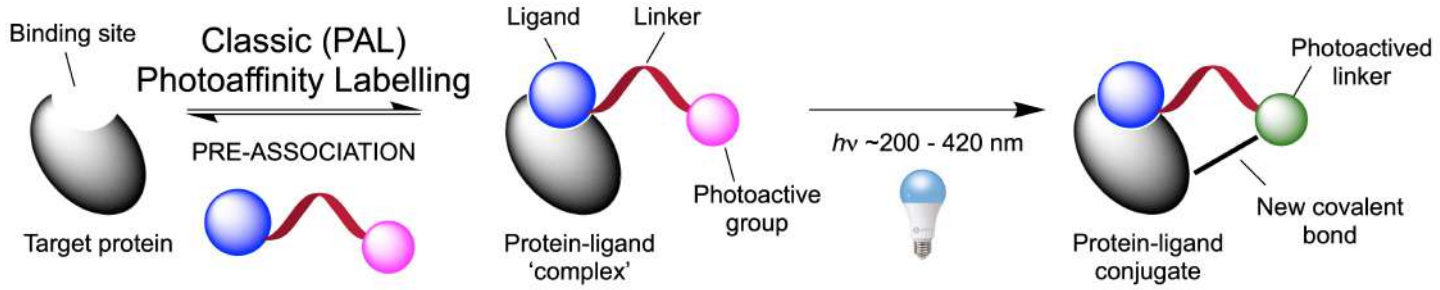
Chem Eur J

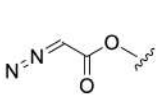


iScience

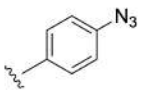
Chem Eur J

Photoactivated chemistry

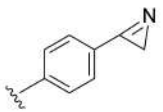




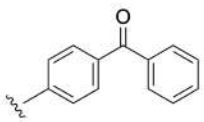
Diazo acetyls
Singh *et al.* 1962



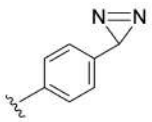
Aryl azides (ArN_3)
Fleet *et al.* 1969



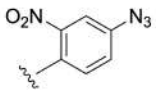
Aryl azirines
Padwa *et al.* 1971



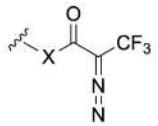
Benzophenones
Galardy *et al.* 1973



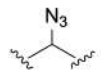
Aryl diazirines
Smith *et al.* 1973



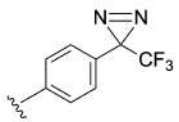
m-nitroaryl azides
Staros *et al.* 1974



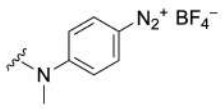
2-diazo-3-trifluoropropionyl
Chowdhry *et al.* 1976



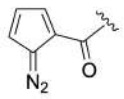
Aliphatic azides
Stoffel *et al.* 1978



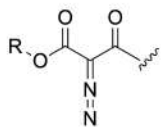
Trifluoromethyl
phenyldiazirines
Brunner *et al.* 1980



Benzyldiazonium salts
Goeldner *et al.* 1980

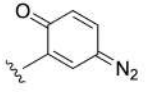


Diazocyclopentadienyls
Nielsen *et al.* 1983



Alkyldiazomalonyls
Nielsen *et al.* 1983

Tetrafluoroaryl azides
Young *et al.* 1989

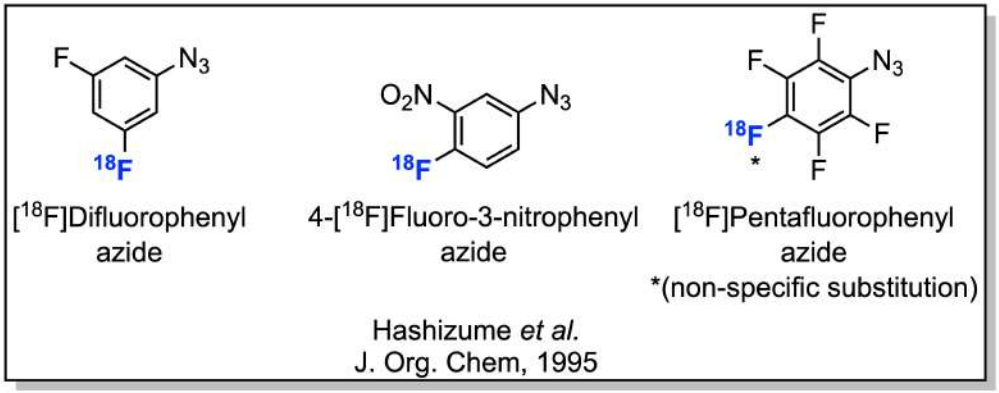


Diazocyclohexadienones
Goeldner *et al.* 1989

p-Azidopyridines
Kolpashchikov *et al.* 1999

Holland *et al.*, *Chem. Eur. J.*, **2020**, *26,* 33-48

Photochemistry & Radiochemistry: State of Play

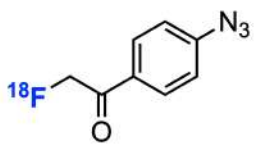


[^{18}F]Difluorophenyl azide

4-[^{18}F]Fluoro-3-nitrophenyl azide

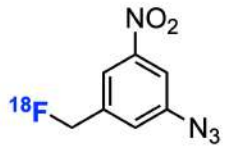
[^{18}F]Pentafluorophenyl azide
*(non-specific substitution)

Hashizume *et al.*
J. Org. Chem, 1995



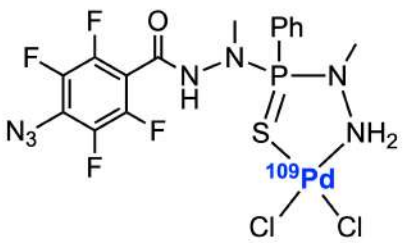
[^{18}F]APF

Wester *et al.*
Nucl. Med. Biol. 1996

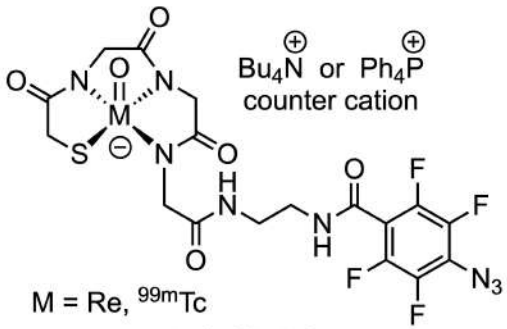


[^{18}F]ANBF

Lange *et al.*
J. Labelled Compd Radiopham. 2002

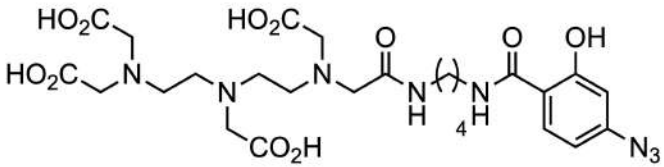


Pandurangi *et al.*
Photochem. Photobiol. 1997



$M(=O)MAG_3$
tetrafluorophenyl azides

Pandurangi *et al.*
J. Org. Chem. 1997
J. Org. Chem. 2002

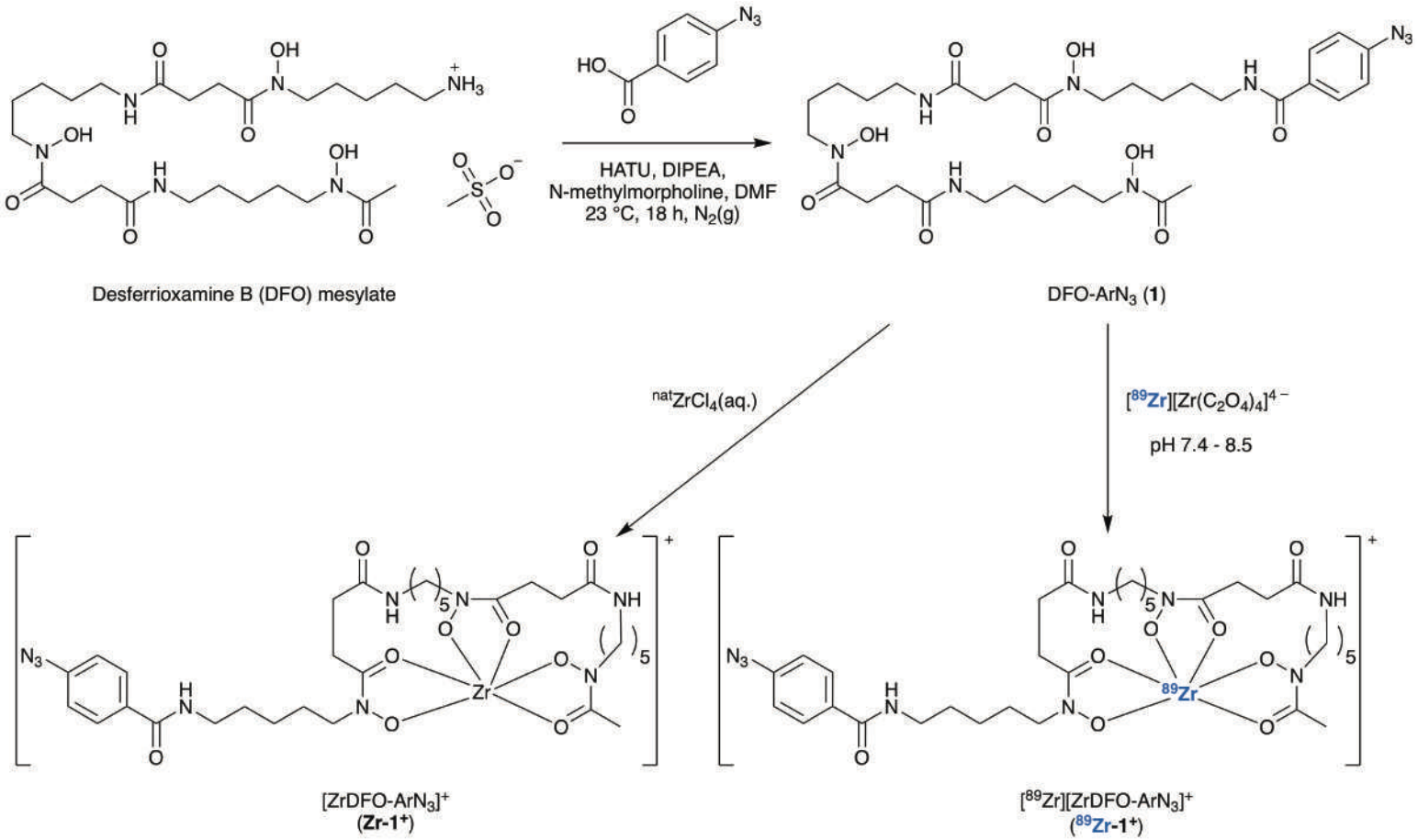


[^{111}In]labeled DTPA-ASBA

Nishikawa *et al.*
Bioconj. Chem. 2003

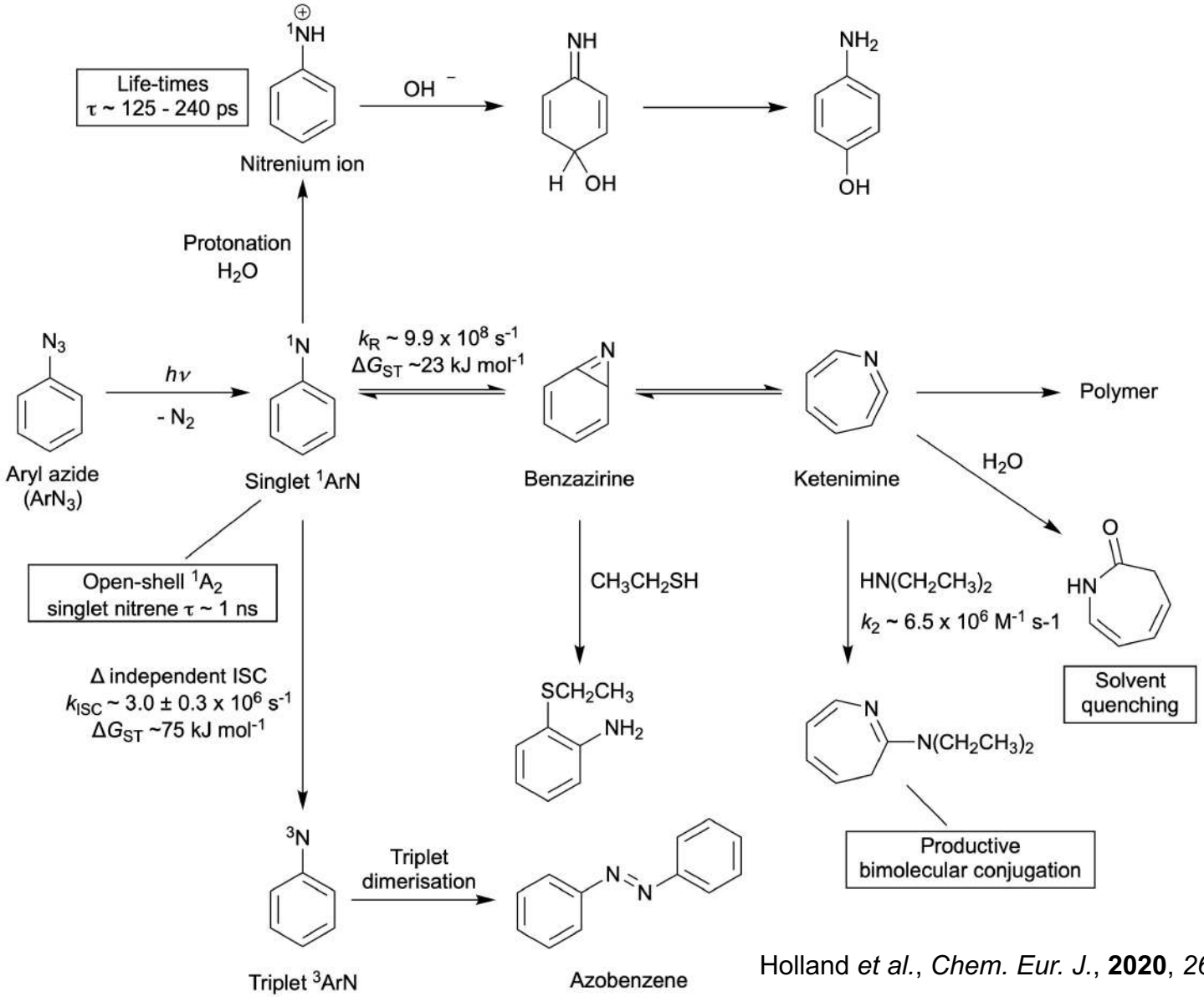
Photoactive chelates: DFO-ArN$_3$

- Desferrioxamine B (DFO) is the gold standard for $^{89}Zr^{4+}$ coordination



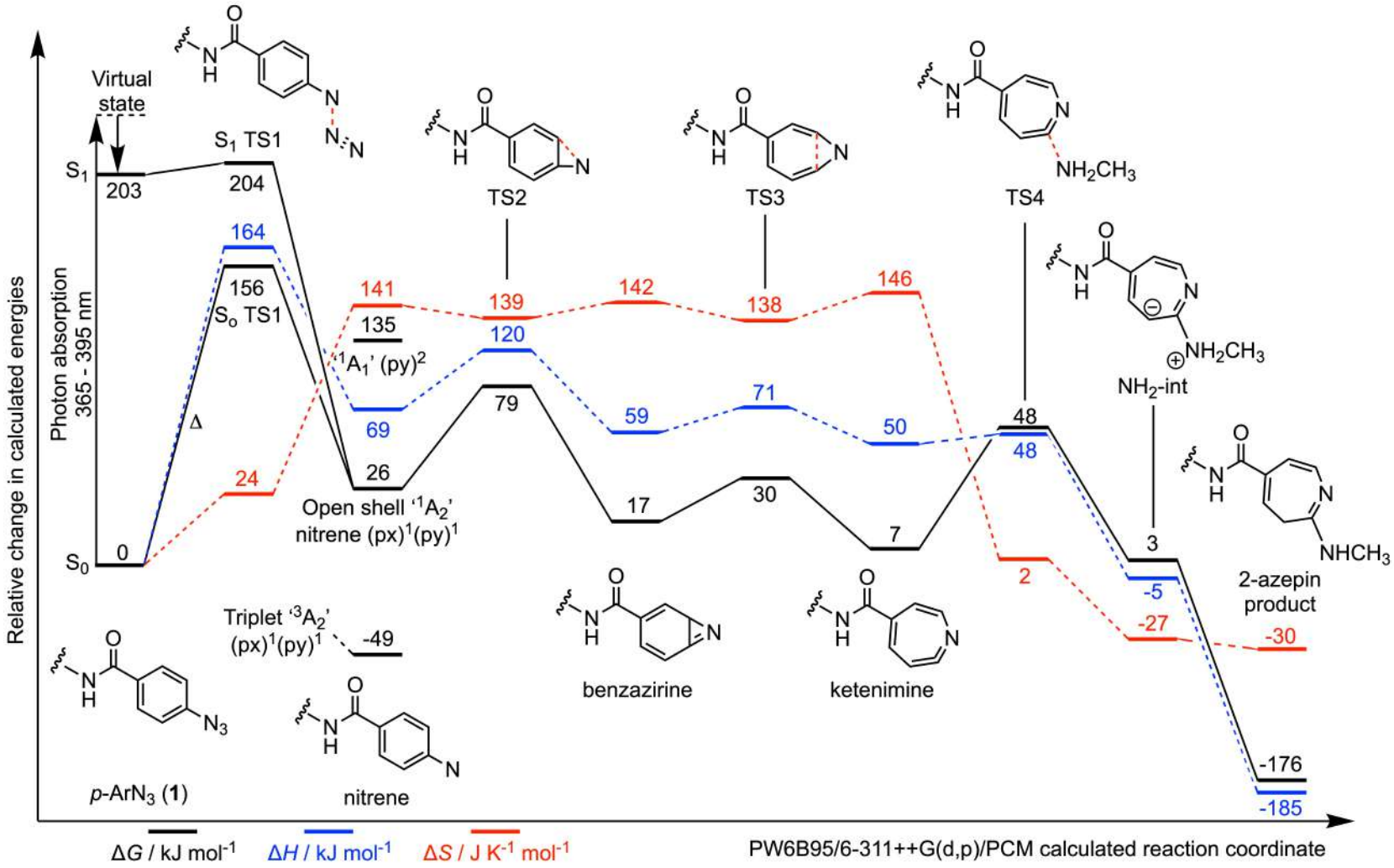
Patra *et al. iScience*, **2019**, *13*, 416-431

Aryl azide (ArN_3) photoinitiation mechanism



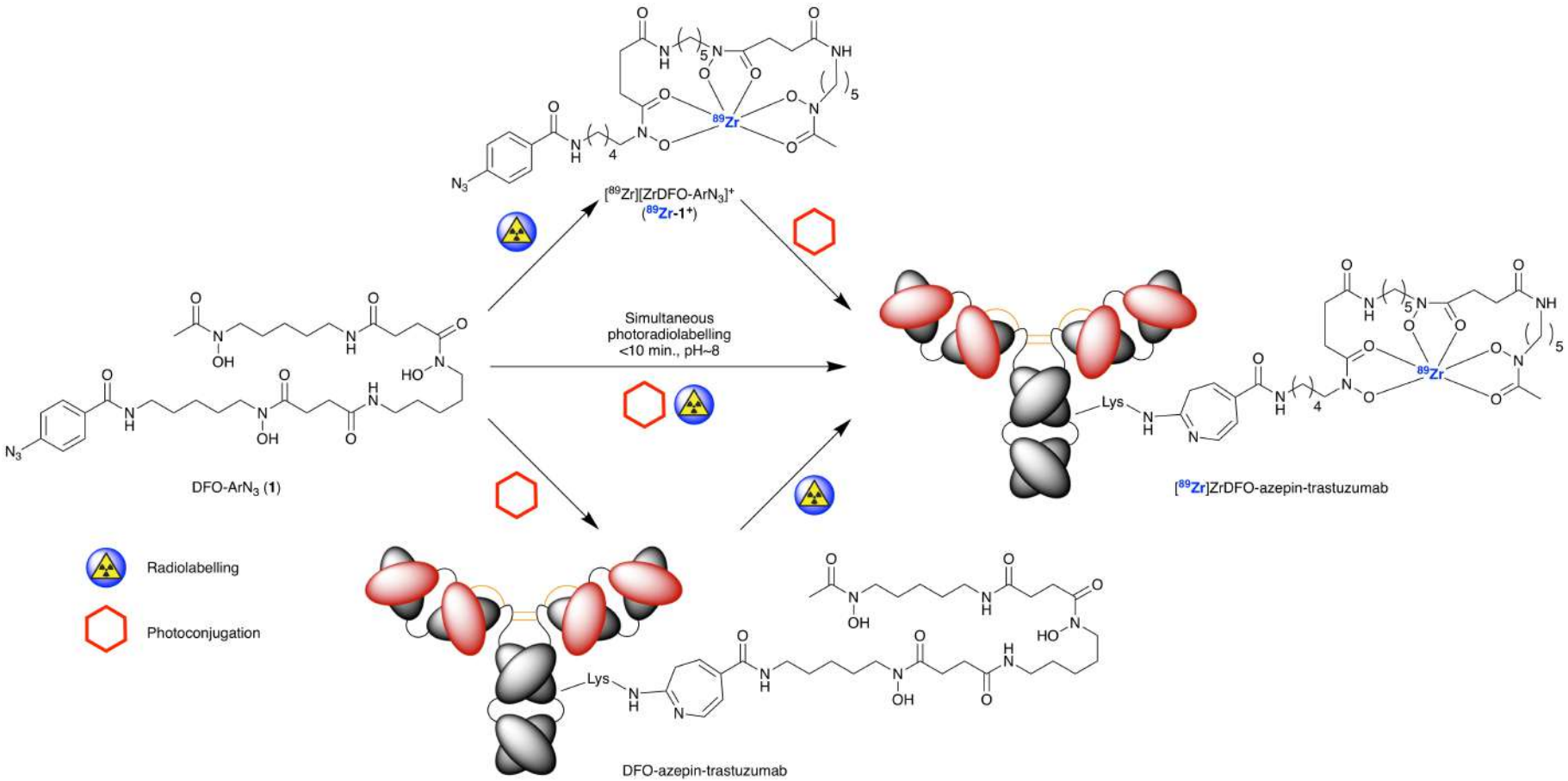
Holland *et al.*, *Chem. Eur. J.*, **2020**, *26,* 33-48

DFT calculated reaction coordinate



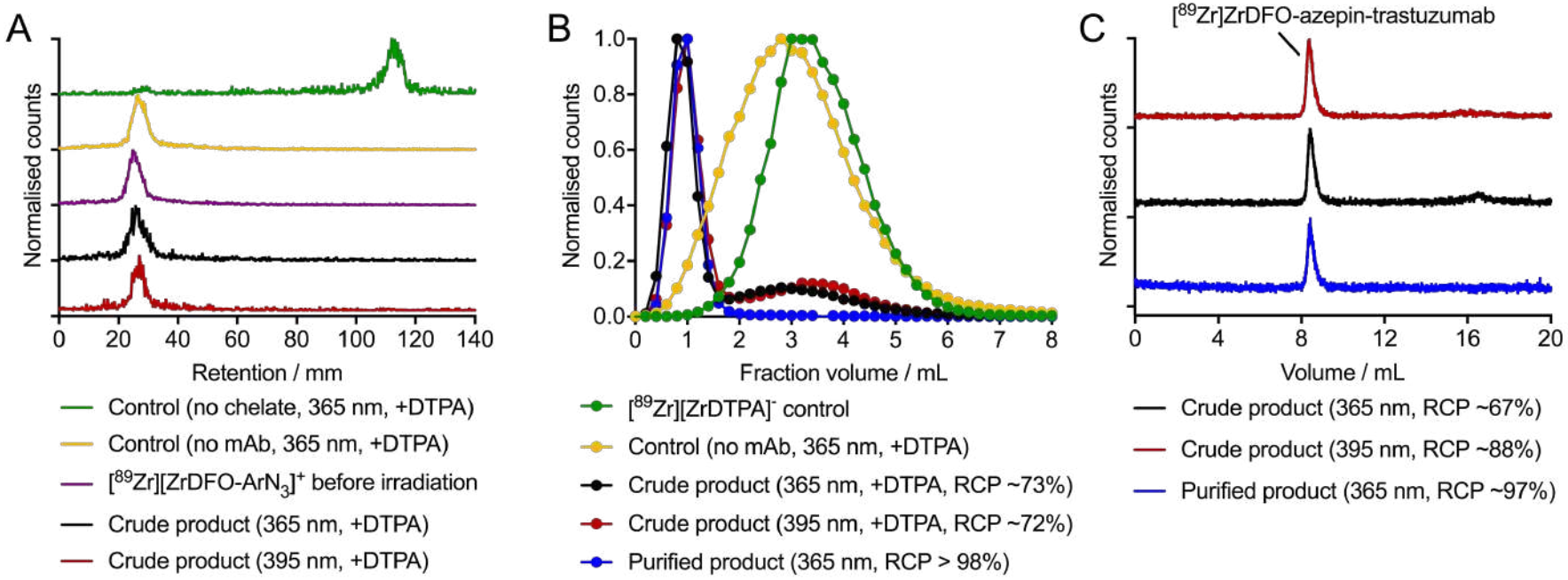
Patra *et al., iScience*, **2019**, *13*, 416-431

Simultaneous photoradiolabelling with ^{89}Zr



One-pot radiosynthesis of ^{89}ZrDFO-azepin-trastuzumab

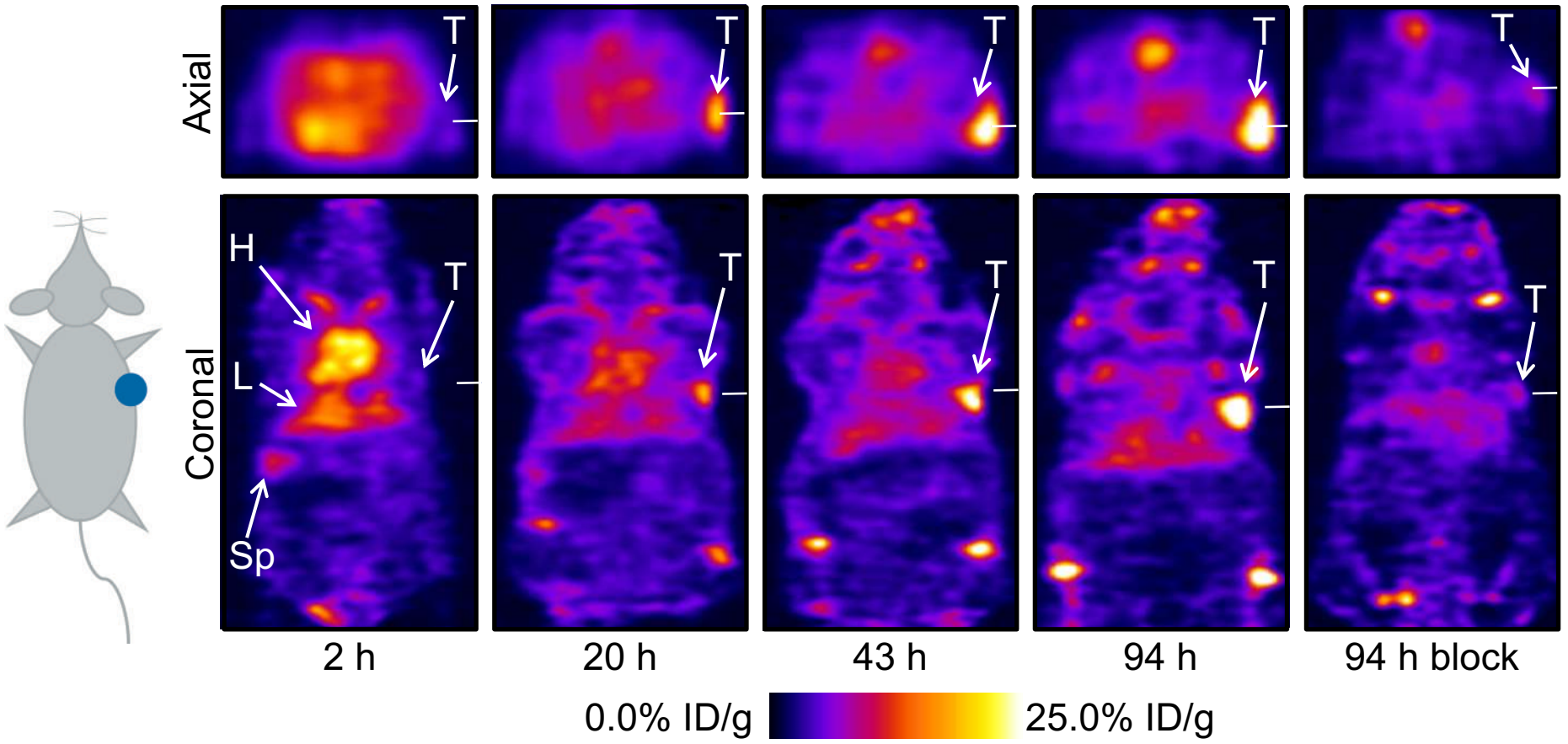
- One-pot, simultaneous photochemical conjugation and ^{89}Zr-radiolabelling
- ^{89}Zr radiolabeling of DFO works at pH 6 – 9
- Pre-purified or fully formulated mAbs
- Two-step process: photoradiochemical conversion efficiency (PCE) ~3%
- **One-pot simultaneous process: PCE ~75%**



Patra *et al. iScience*, **2019**, *13*, 416-431

^{89}ZrDFO-azepin-trastuzumab: PET imaging

- PET imaging in SK-OV-3 tumours (**HER2/*neu* positive**)

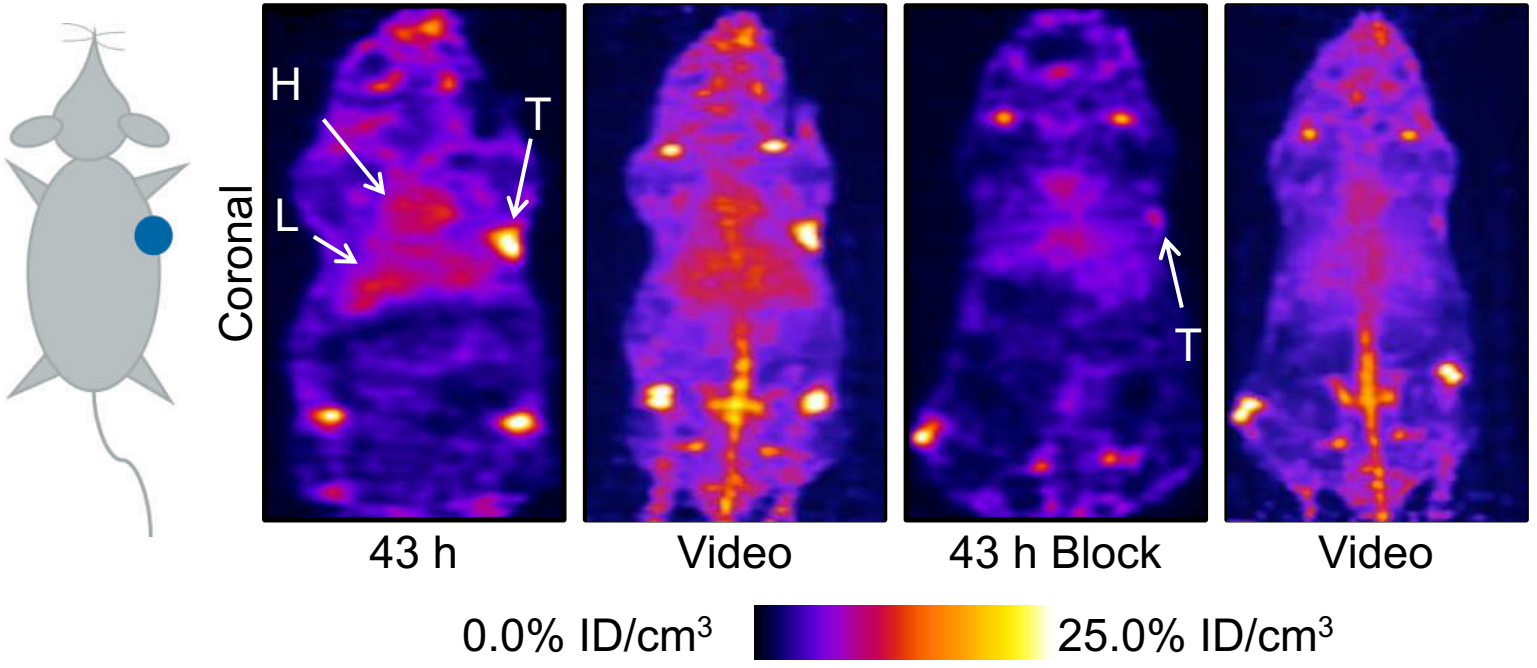


Patra *et al. iScience*, **2019**, *13*, 416-431

Maximum intensity projection images / videos

Maximum intensity projection (MIP) images and videos

- ^{89}ZrDFO-azepin-trastuzumab PET in mice bearing SK-OV-3 tumours
- Methods suitable for automation and potentially kit-based radiochemistry
- *'Instant'* radiolabelling using **formulated** antibodies in <10 min.

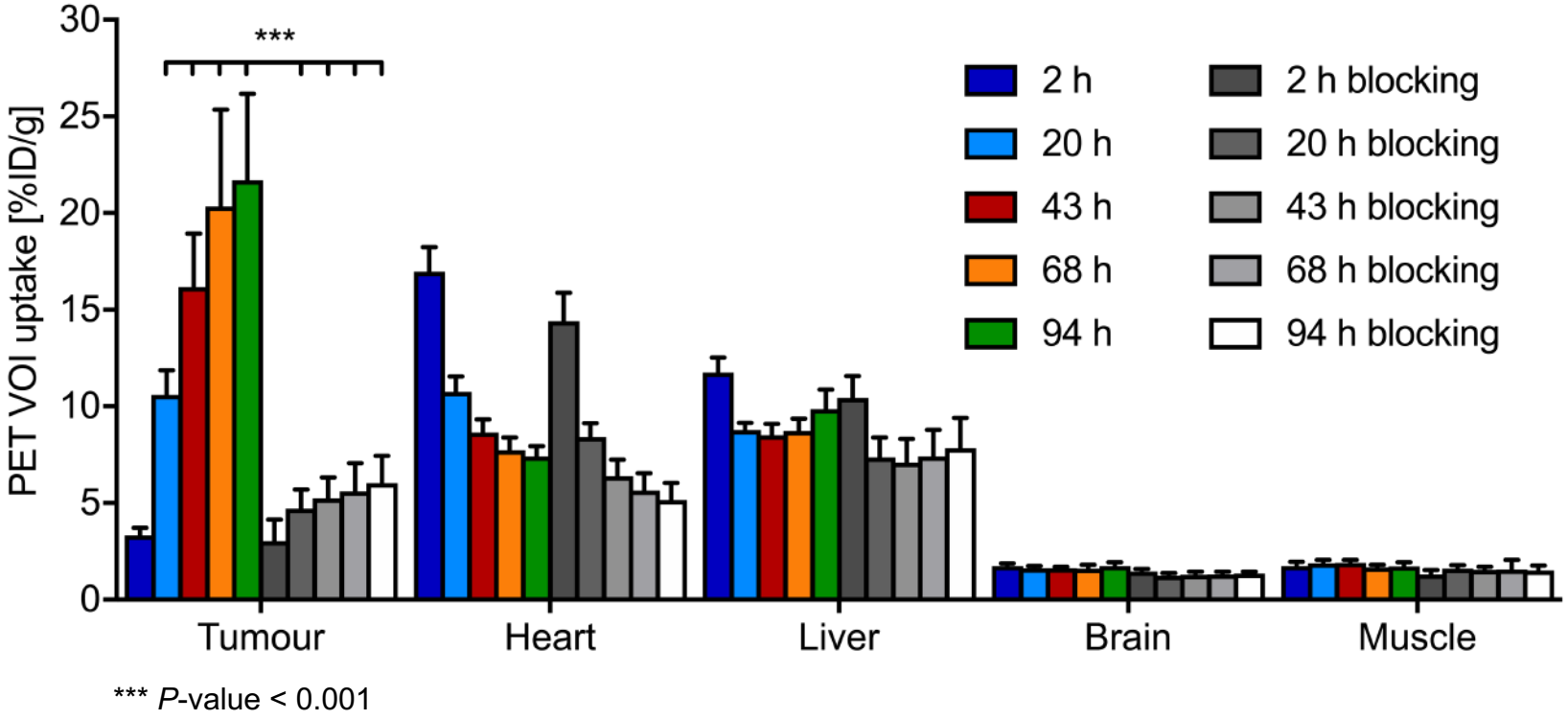


Patra *et al. iScience*, **2019**, *13*, 416-431

PET quantification

^{89}ZrDFO-azepin-trastuzumab

Volume of interest (VOI) analysis of PET imaging data

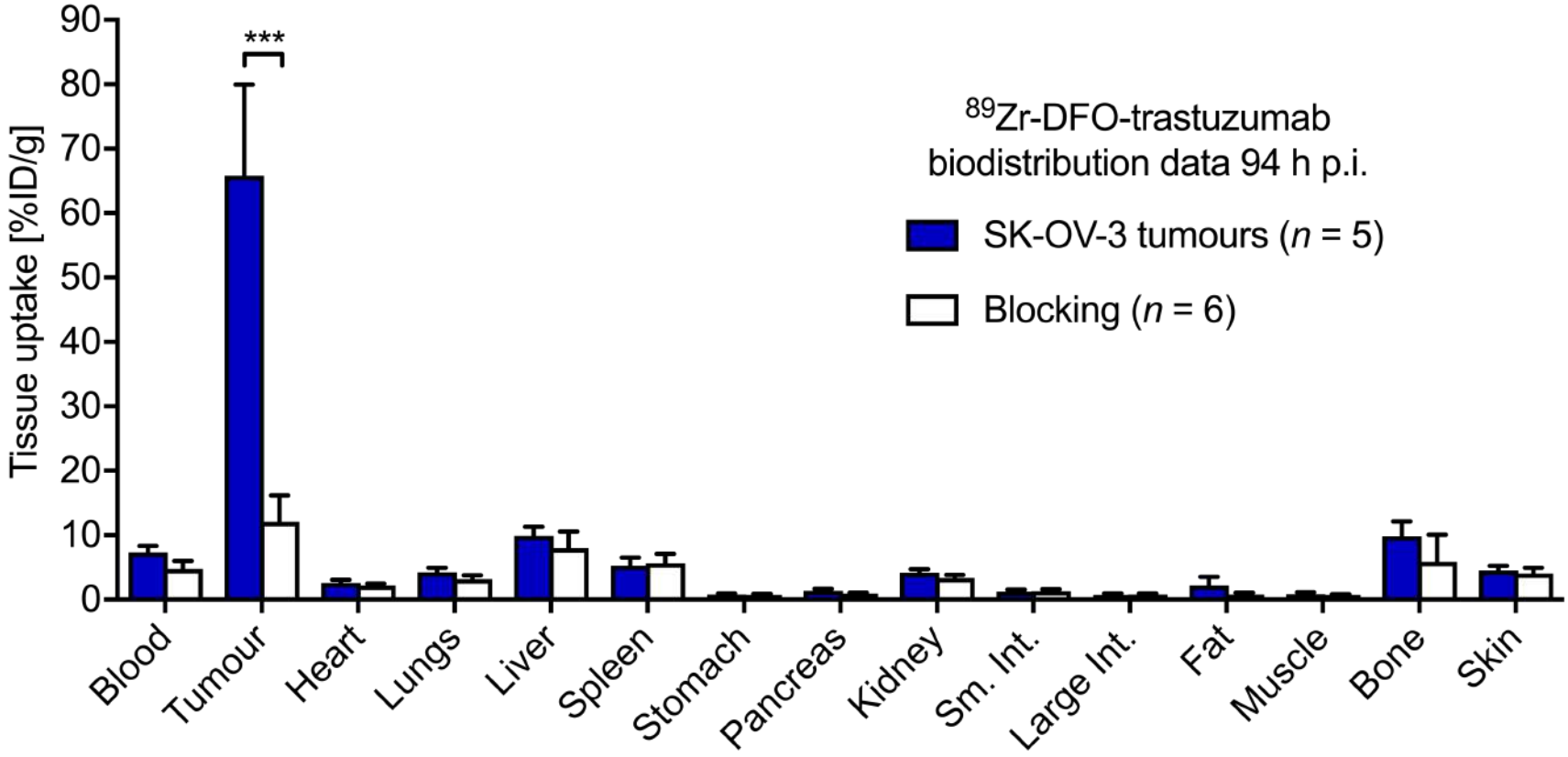


*** *P*-value < 0.001

Patra *et al.* iScience, **2019**, *13*, 416-431

Biodistribution data

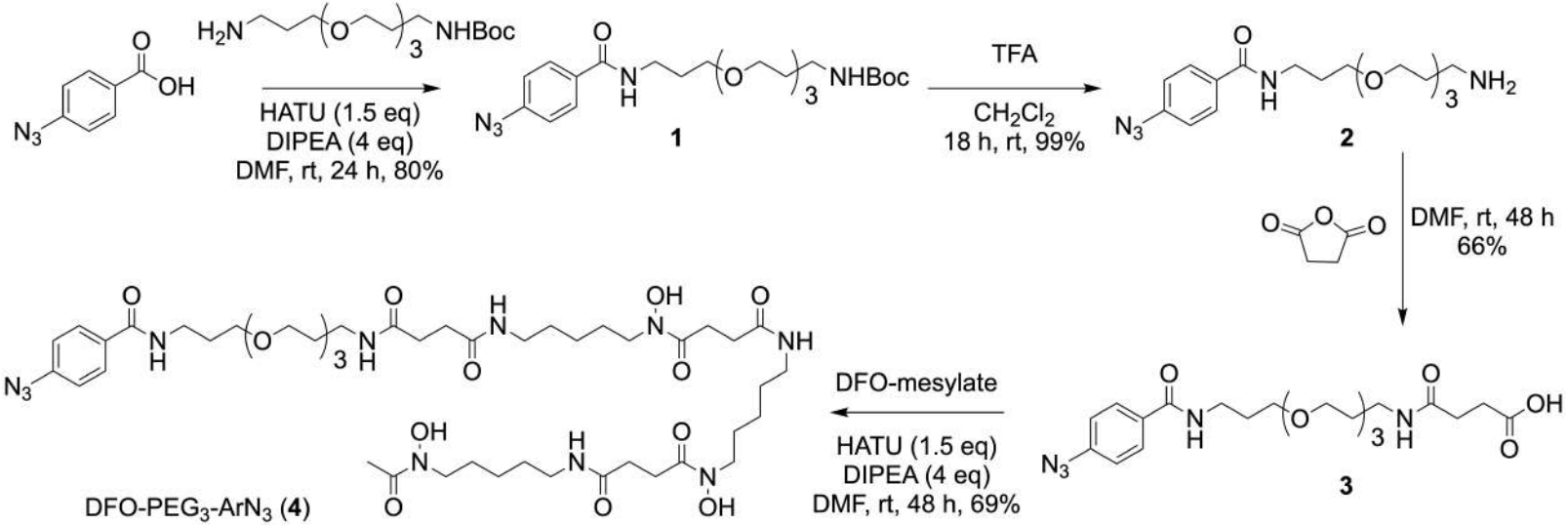
Ex vivo analysis of ^{89}ZrDFO-azepin-trastuzumab distribution

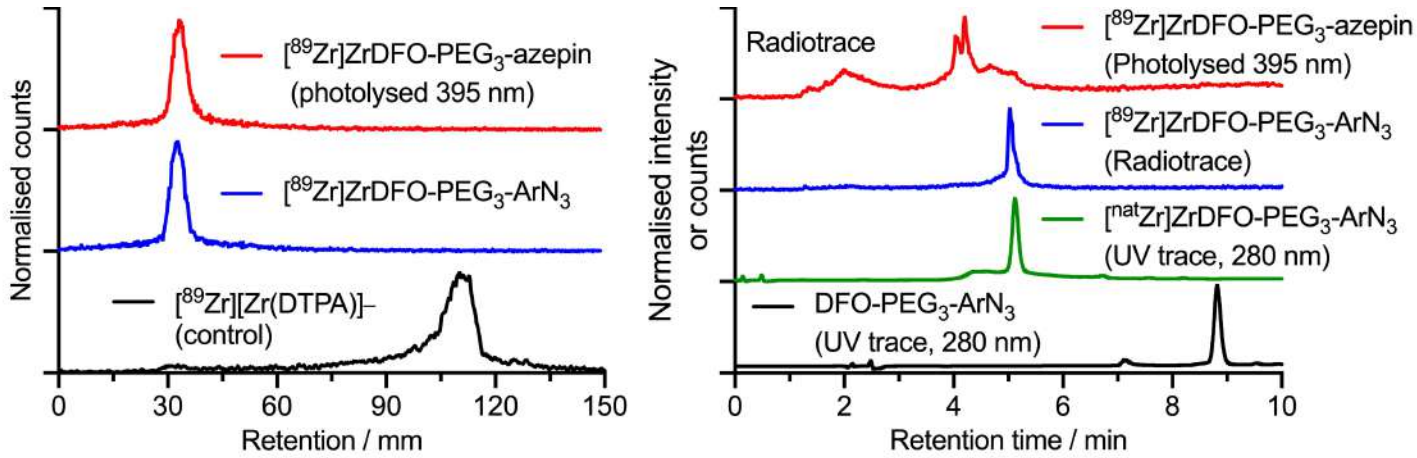


Patra *et al.* iScience, **2019**, *13*, 416-431

Water-soluble DFO-PEG$_3$-ArN$_3$

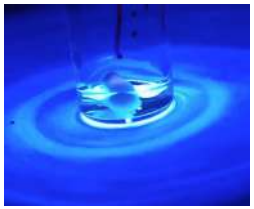
- Functionalisation of the free NH_2 of DFO (usually) compromises water solubility

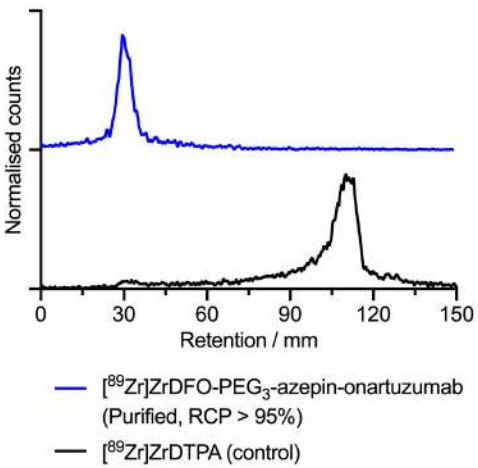


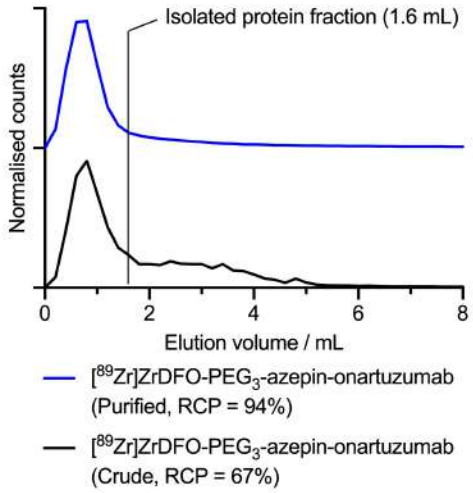


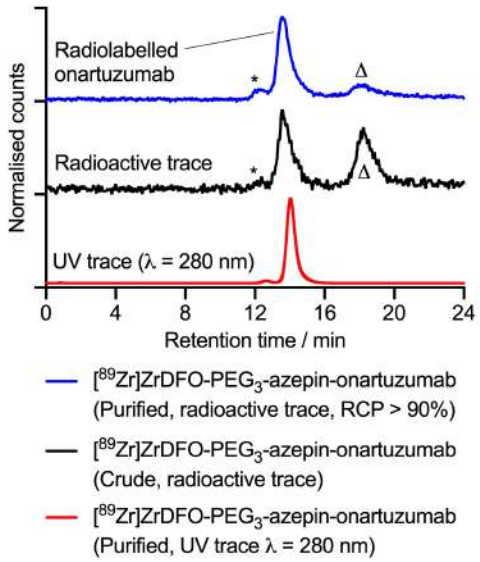
Guillou *et al.*, *Chem Eur J.* **2020**, *26*, 7185-7189

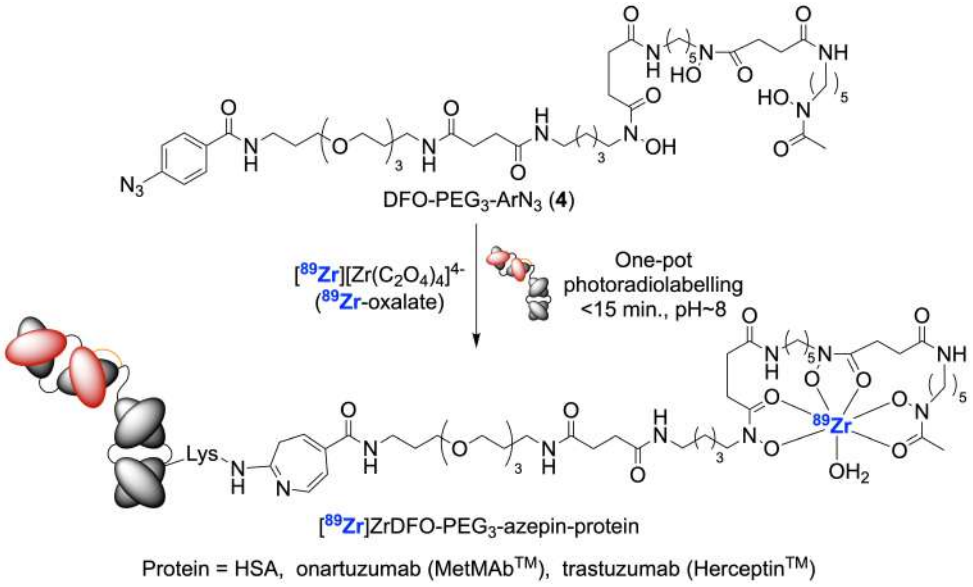
Photoradiochemistry with [^{89}Zr]ZrDFO-PEG$_3$-ArN$_3$











Decay-corrected RCYs (n = 3 / each)

72.9 ± 1.9% (HSA, 395 nm)

70.7 ± 2.3% (HSA, 365 nm)

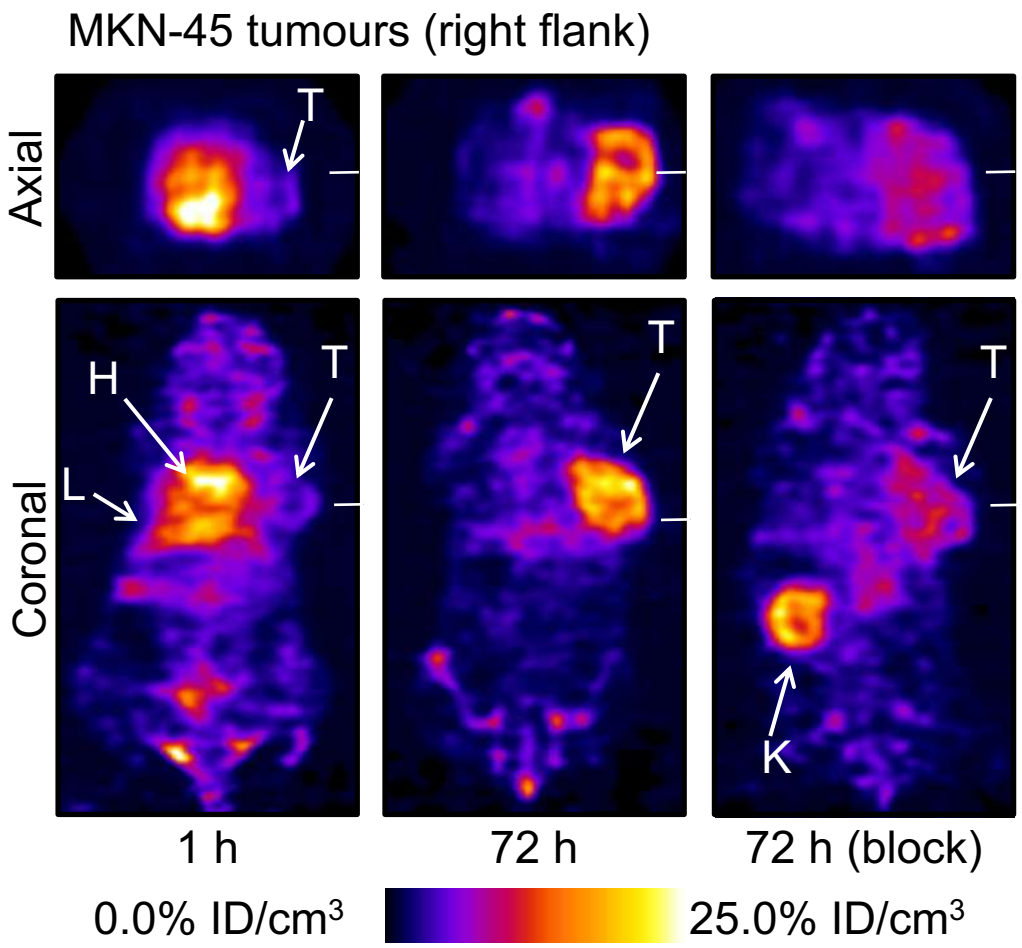
64.5 ± 6.7% (MetMAb™, 395 nm)

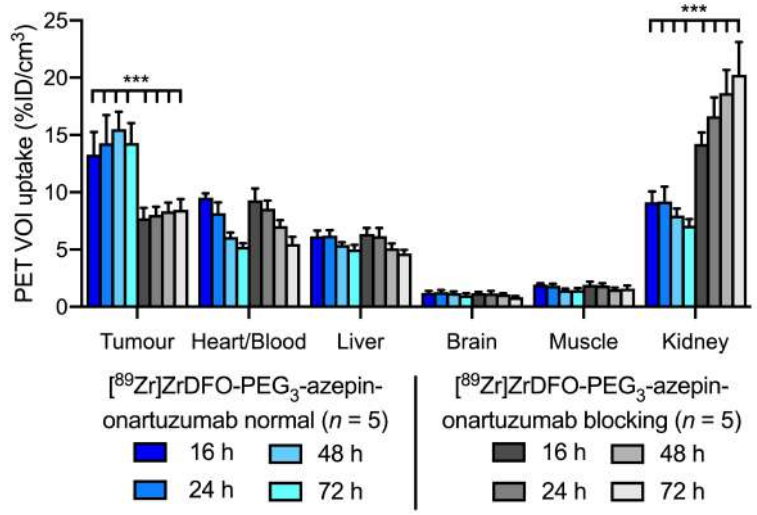
58.3 ± 3.4% (Herceptin™, 395 nm)

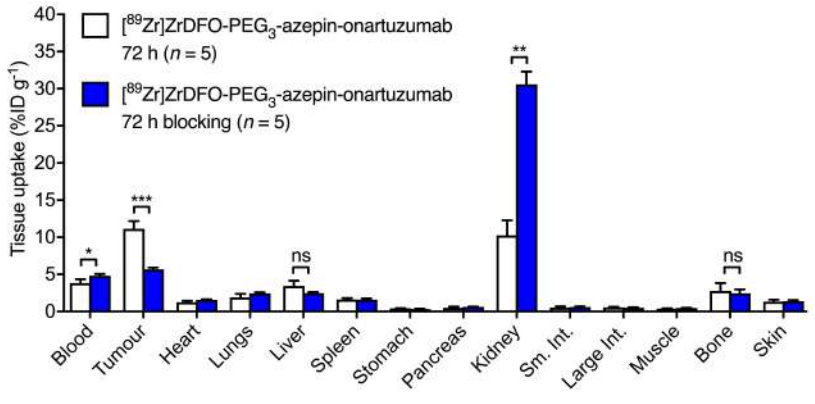
Guillou *et al.*, *Chem Eur J.* **2020**, *26*, 7185-7189

[^{89}Zr]ZrDFO-PEG$_3$-azepin-onartuzumab

- PET imaging and biodistribution analysis



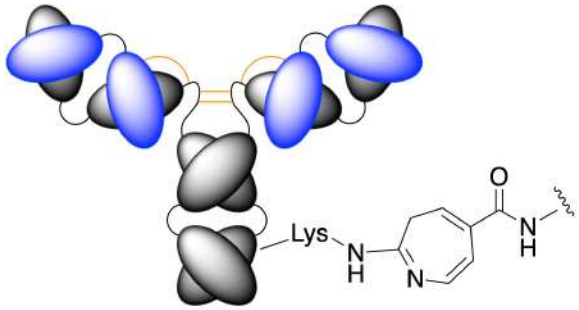


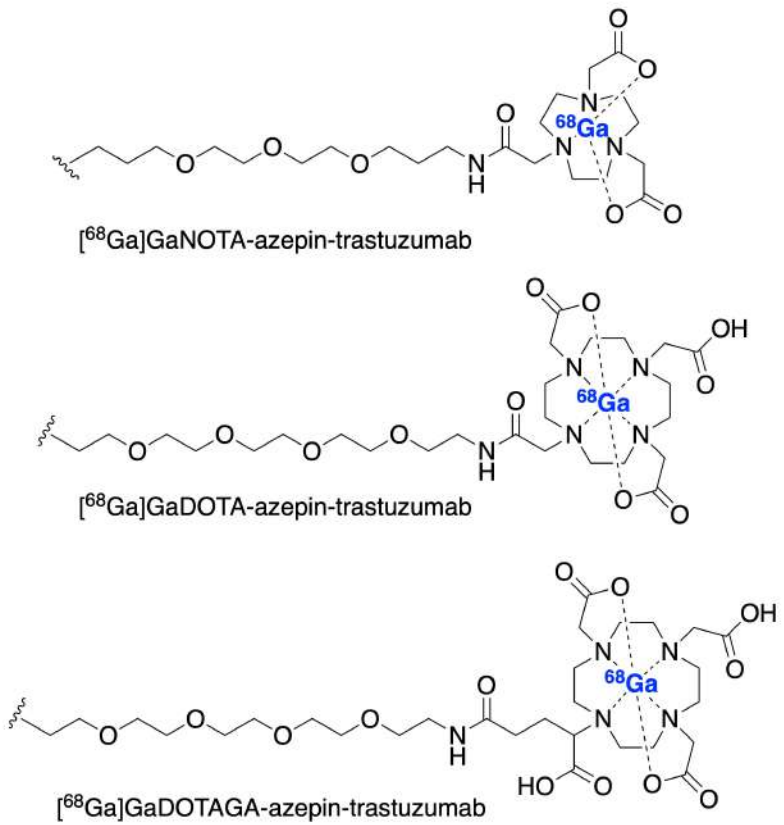


Guillou *et al.*, *Chem Eur J.* **2020**, *26*, 7185-7189

Photoactivatable chelates: *aza*-macrocycles

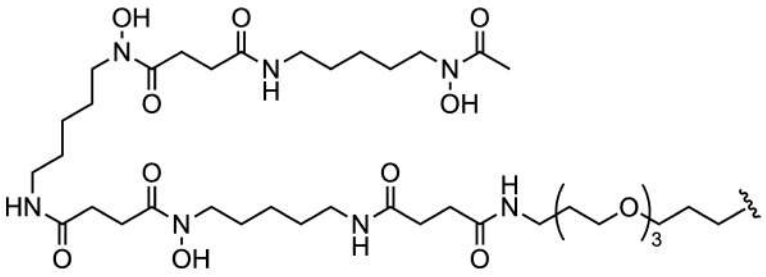
- Complexes with variable overall charge (M^{3+} complexes: +1, 0, –1 at pH7.4)
- Suitable for use with $^{64}Cu^{2+}$, $^{68}Ga^{3+}$, $^{90}Y^{3+}$, $^{111}In^{3+}$, $^{177}Lu^{3+}$, $^{225}Ac^{3+}$ etc





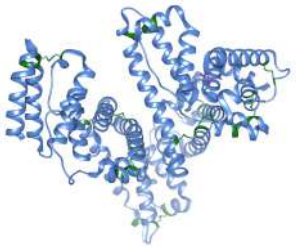
Eichenberger *et al. Chem. Commun.*, **2019**, *55*, 2257-2260
Gut and Holland, *Inorg. Chem.*, **2019**, *58(18),* 12302-12310
Fay *et al.*, *Bioconjugate Chem.*, **2019**, *30*, 1814-1820
Gribi and Holland, **2021**, *in preparation – ^{99m}Tc / Re*
Esteban Flores *et al,* **2021,** *in preparation – ^{64}Cu*

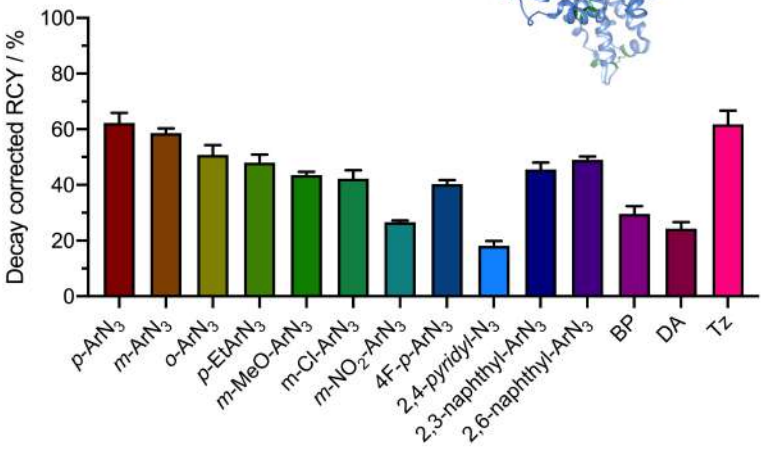
The chemical scope of light-induced protein ligation

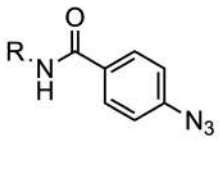


Photoactivatable
Desferrioxamine B (DFO)-PEG_3 derivatives

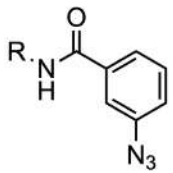
One-pot photoradiolabelling of human serum albumin (HSA)



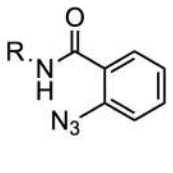




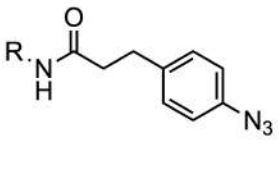
para-ArN_3 (**1**)



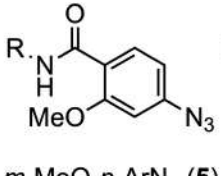
meta-ArN_3 (**2**)



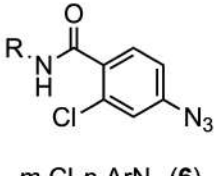
ortho-ArN_3 (**3**)



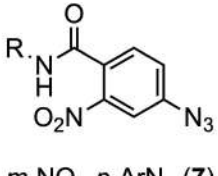
p-EtArN_3 (**4**)



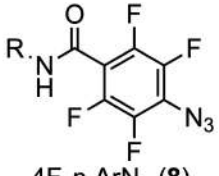
m-MeO-*p*-ArN_3 (**5**)



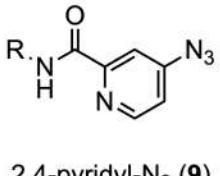
m-Cl-*p*-ArN_3 (**6**)



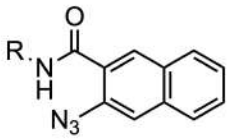
m-NO_2-*p*-ArN_3 (**7**)



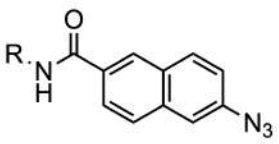
4F-*p*-ArN_3 (**8**)



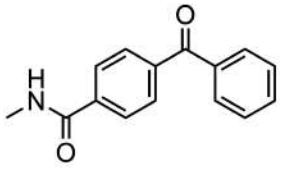
2,4-pyridyl-N_3 (**9**)



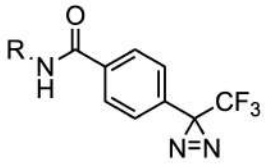
2,3-naphthyl-N_3 (**10**)



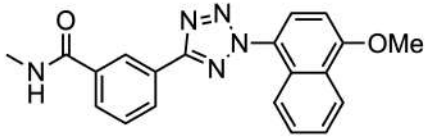
2,6-naphthyl-N_3 (**11**)



Benzophenone (BP, **12**)
(biradicals)



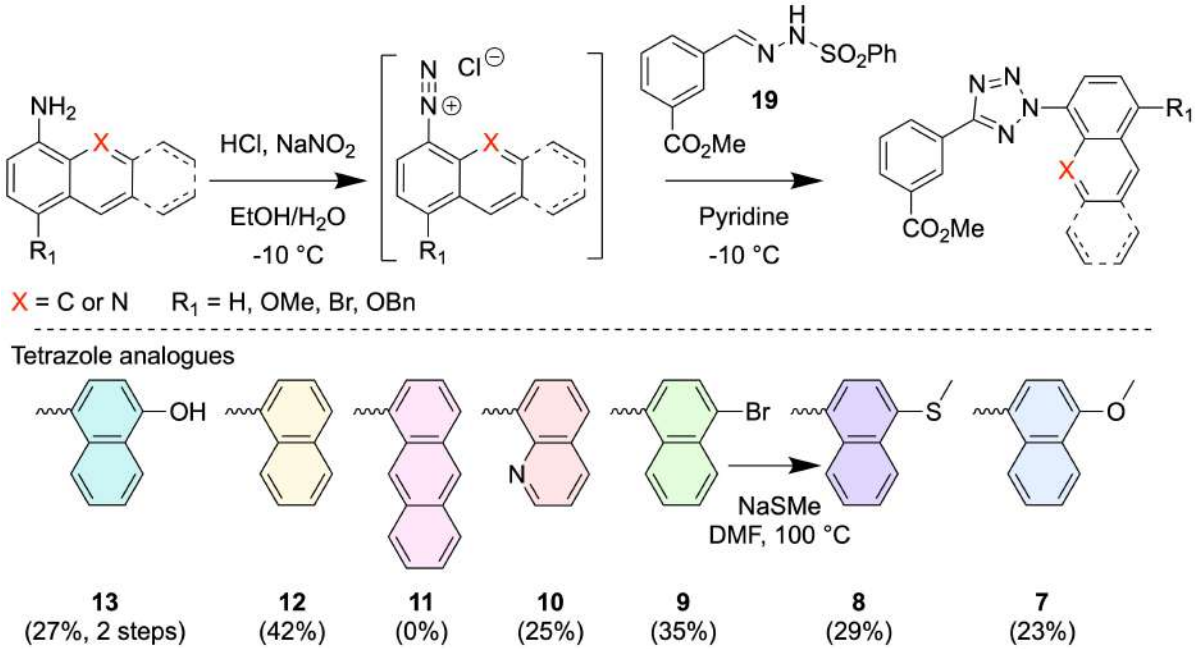
Diazirine (DA, **13**)
(carbenes)

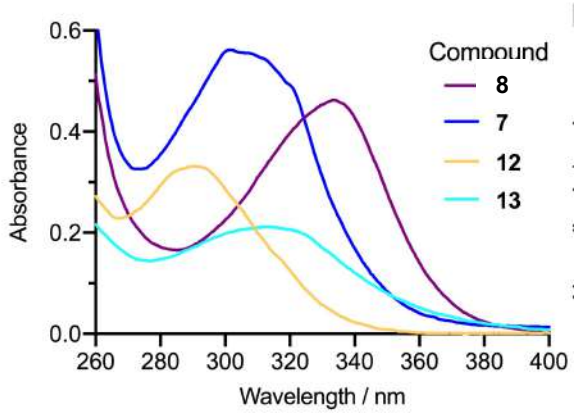


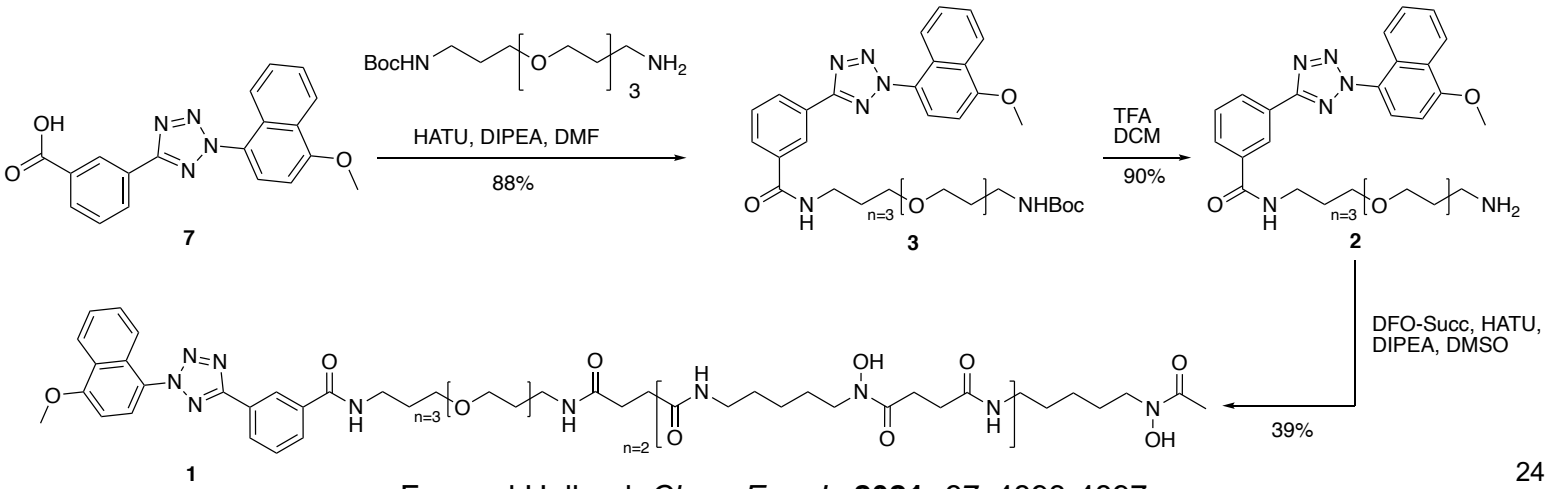
Tetrazoles (Tz, **14**)
(nitrile imines)

Guillou *et al.*, *Nat. Protocol*, **2020**, *15*, 3579–3594
Earley *et al.*, **2021**, *in preparation*

Auxochromic tuning DFO-tetrazole photoreactivity

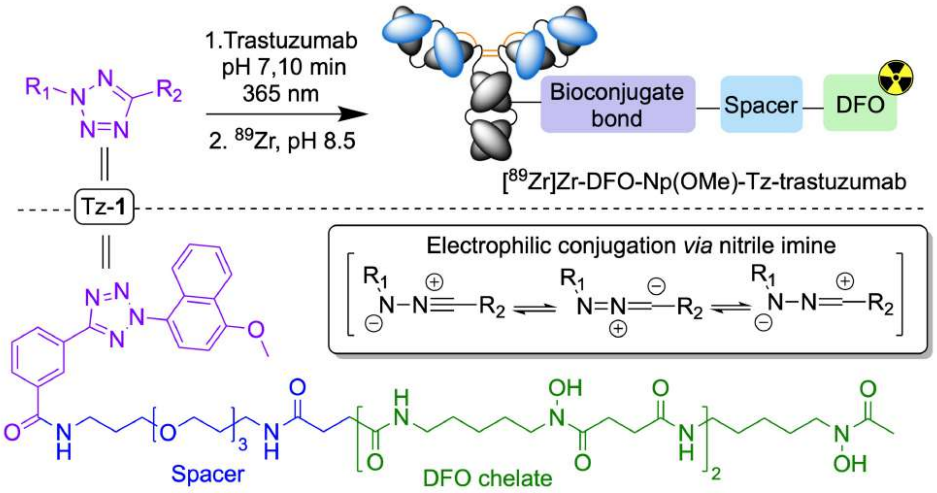


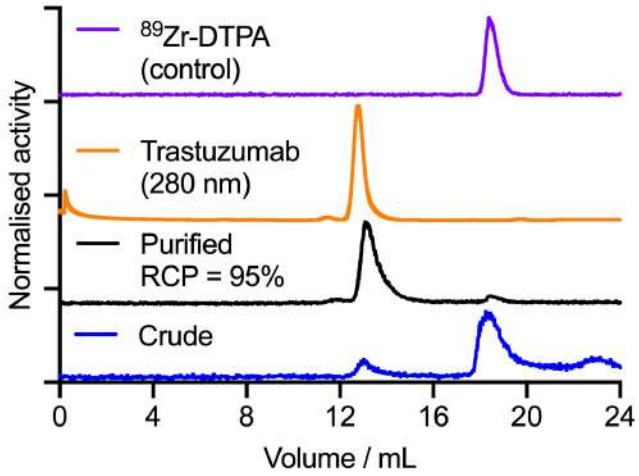


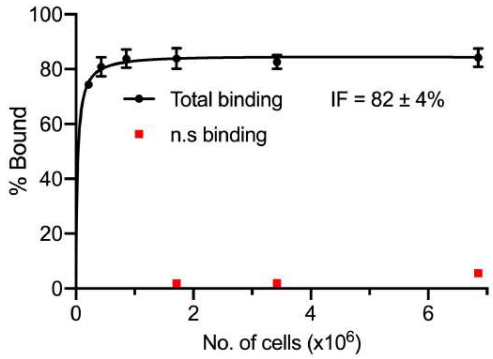


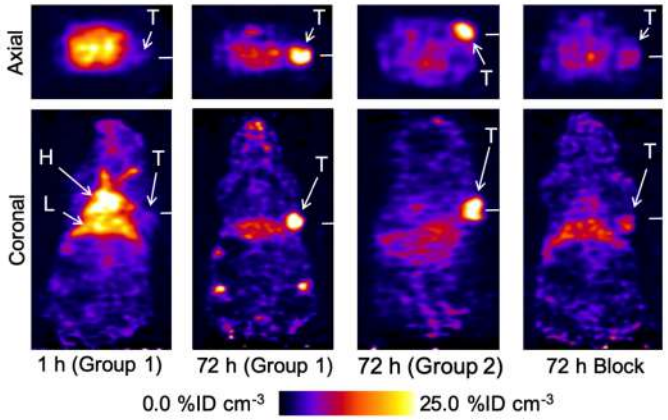
Fay and Holland, *Chem Eur. J.*, **2021**, *27*, 4893-4897

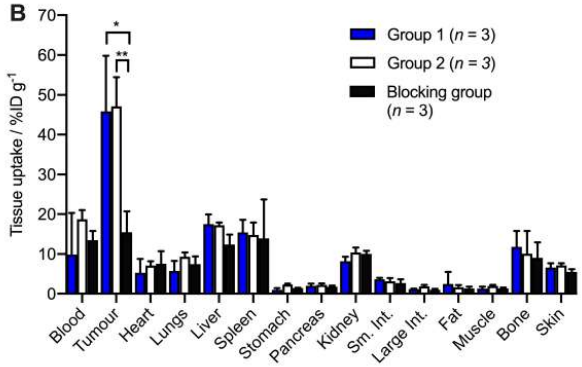
Tetrazole photoradiochemistry: ^{89}ZrDFO-Tz-trastuzumab





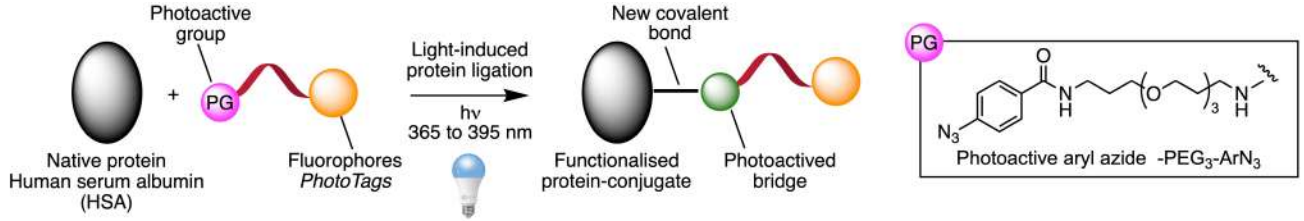


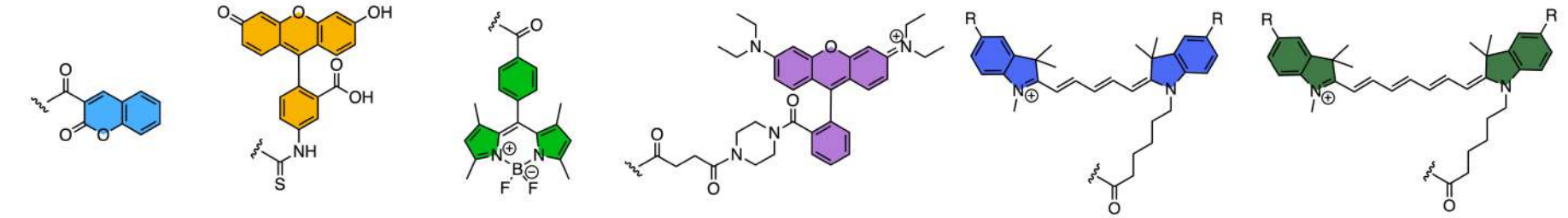




Fay and Holland, *Chem Eur. J.*, **2021**, *27*, 4893-4897

Photoactivatable fluorophores: *PhotoTags*

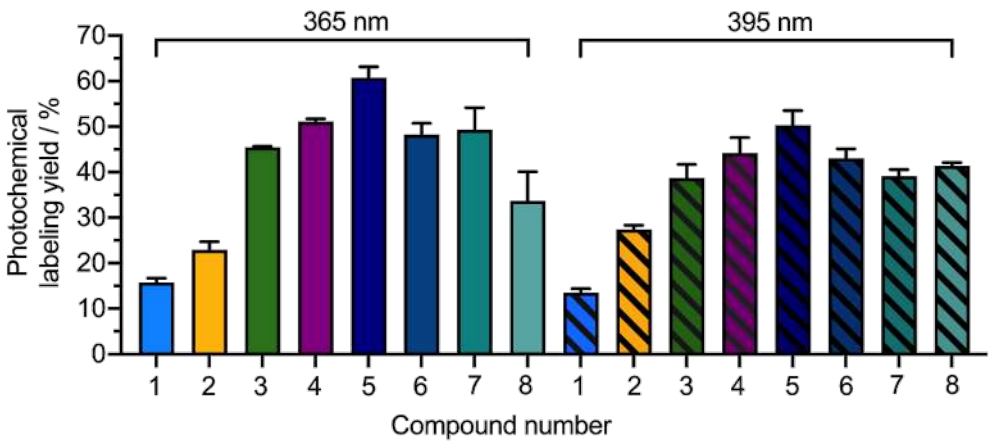


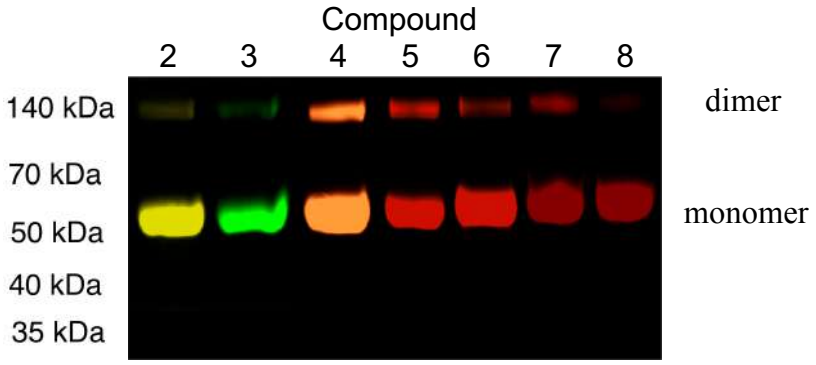


Coumarin-PEG$_3$-ArN$_3$ (**1**) Fluorescein-PEG$_3$-ArN$_3$ (**2**) BODIPY-PEG$_3$-ArN$_3$ (**3**) RhodamineB-PEG$_3$-ArN$_3$ (**4**)

R = H, Cy5-PEG$_3$-ArN$_3$ (**5**)
R = SO$_3$H, sulfo-Cy5-PEG$_3$-ArN$_3$ (**6**)

R = H, Cy7-PEG$_3$-ArN$_3$ (**7**)
R = SO$_3$H, sulfo-Cy7-PEG$_3$-ArN$_3$ (**8**)

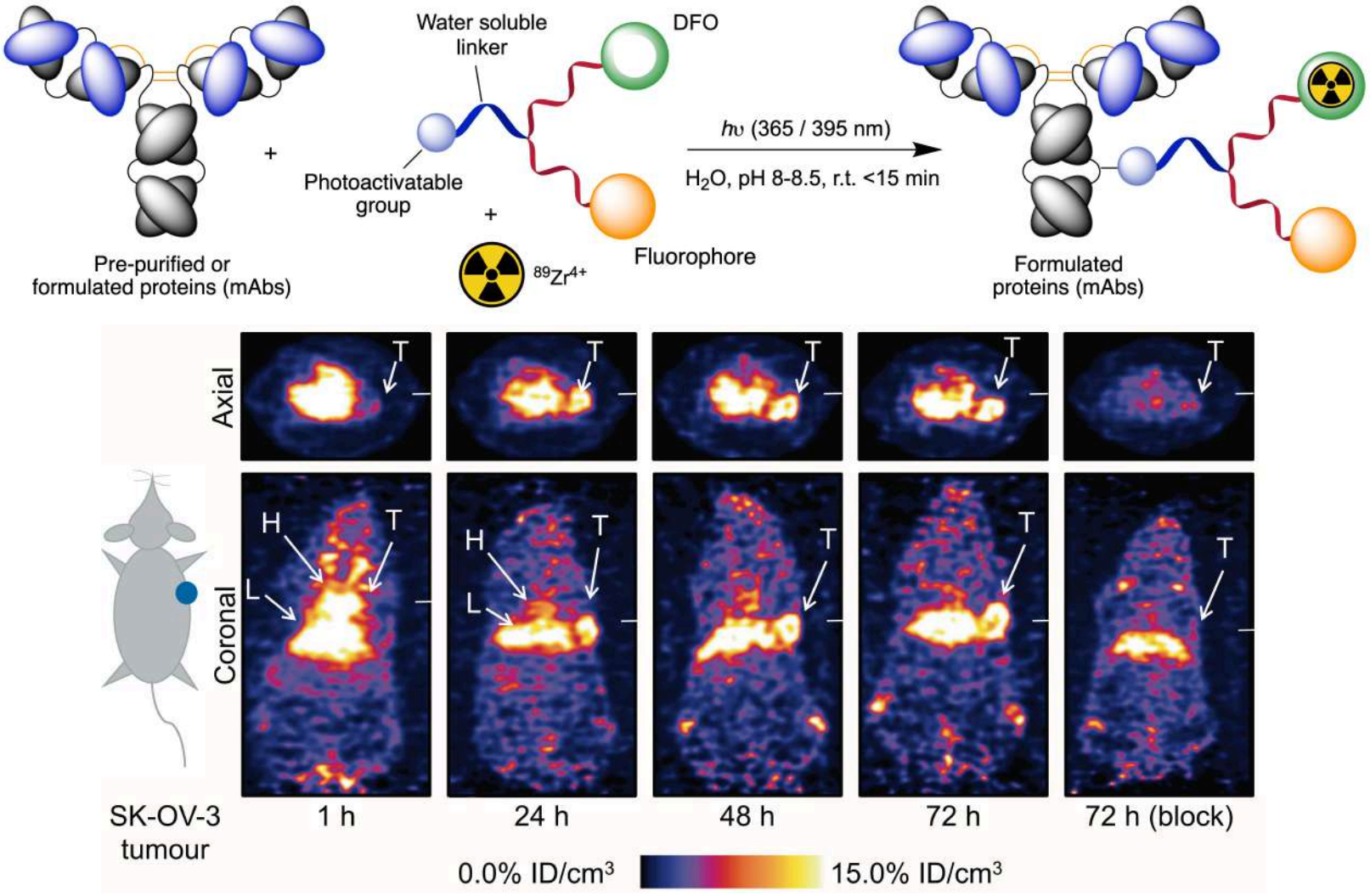




SDS-PAGE analysis visualised by fluorescence imaging

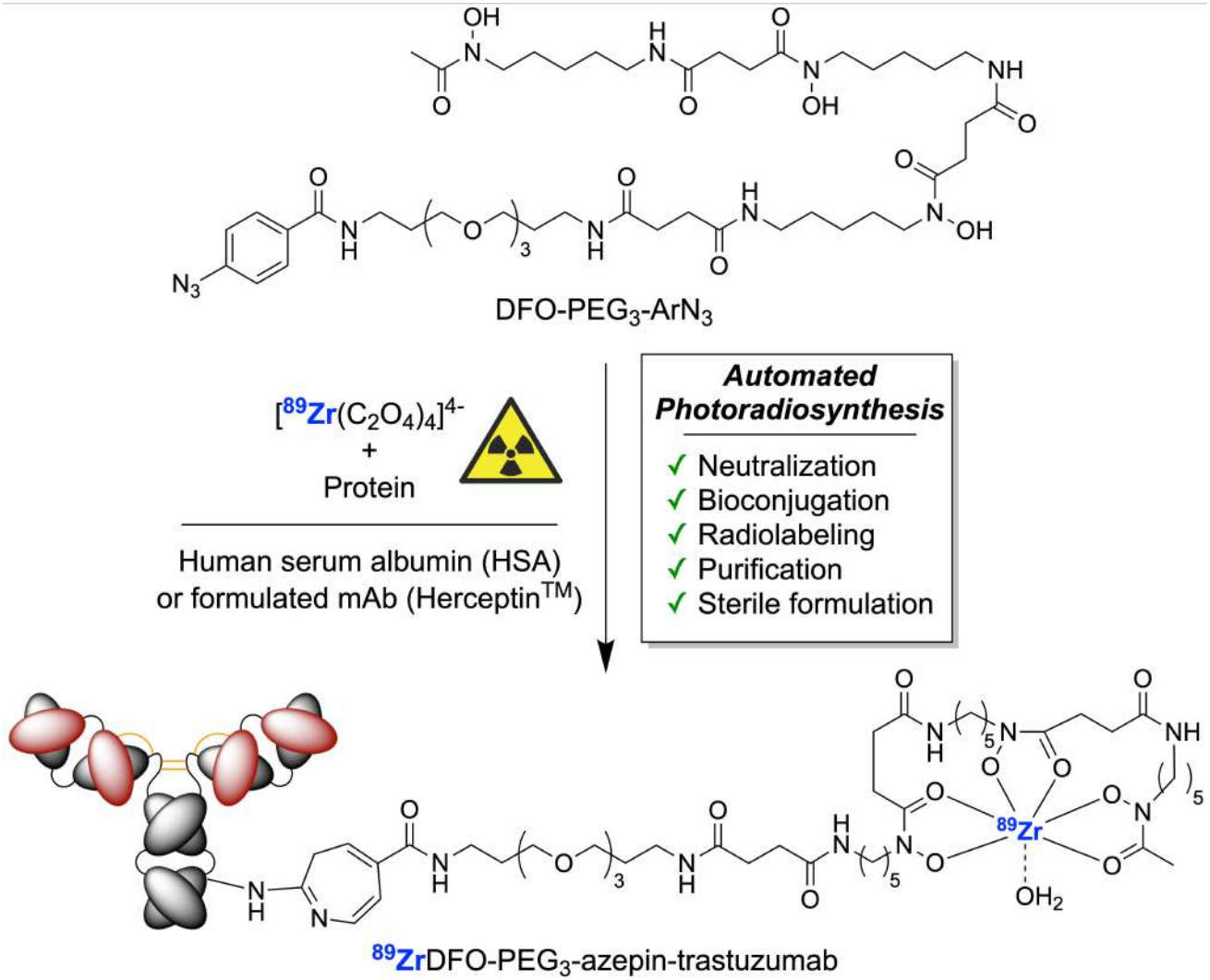
Fay *et al.*, *Org. Lett.*, **2020**, *22*(*9*), 3499-3503
Guillou *et al.*, **2021**, *submitted*

Dual-modality probe design: PET/optical



Guillou *et al.*, **2021**, *submitted*

Automated radiochemistry & translation



Klingler and Holland, **2021**, *submitted*

Radiosynthesiser design: ALISI 0.0

Open-source microcontrollers
(Arduino & breakout boards)

Liquid pathways (cassette)

- 3-way switching valves
- Digital servo motors
- Single-use sterile materials

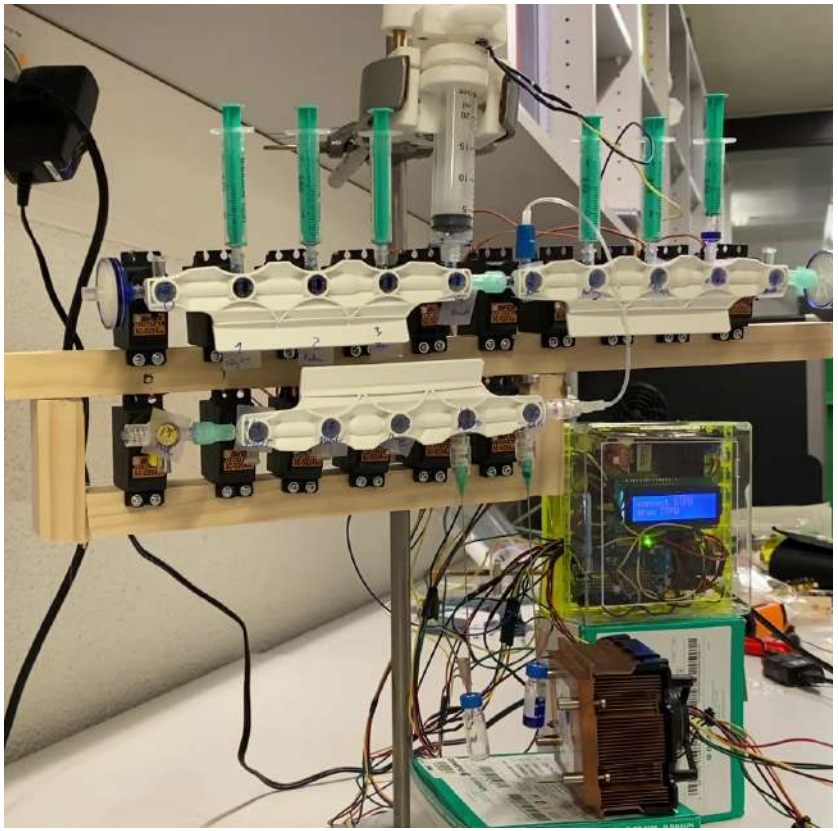
Liquid transfer

- Pneumatically-driven
- Syringe pump

Photoreactor

- ~3 W output LED, λ = 365 nm
- Cooled with an old CPU fan

Wood frame, fastened together with screws and hot glue

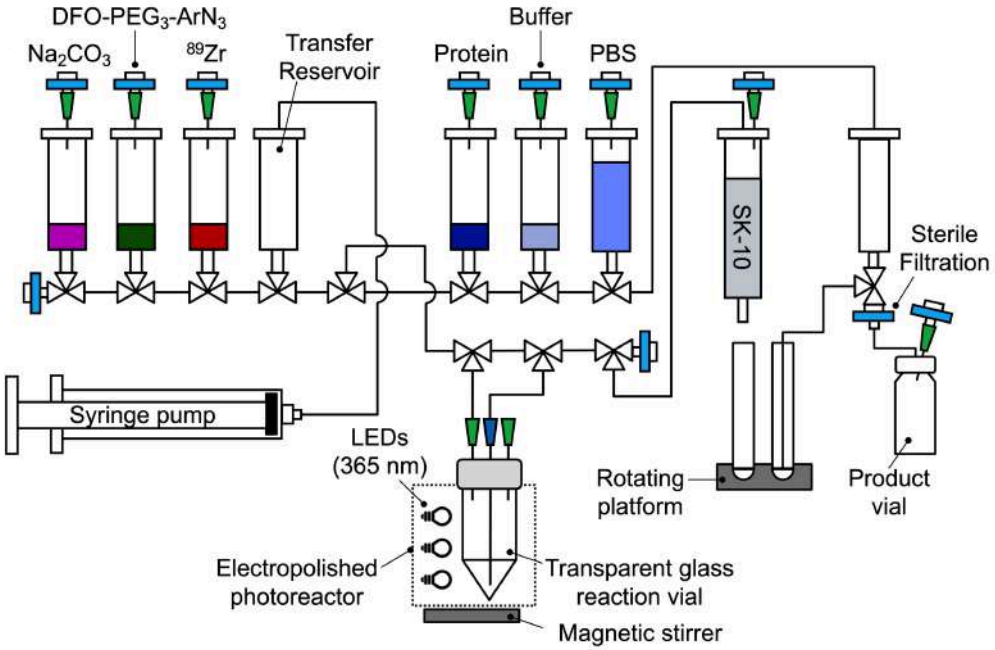


Radiosynthesiser design: ALISI 1.0

ADD MAN FACTORY

Custom design and construction with additive manufacturing (**3D-printing**)

- LASER-cut encasing
- Custom servo-motor plates & valve holders
- Syringe pump
- Sterile, single-use cassettes
- Electropolished stainless steel photoreactor
- Custom LED cooling
- LCD display and control button
- Sarcastic comments included!



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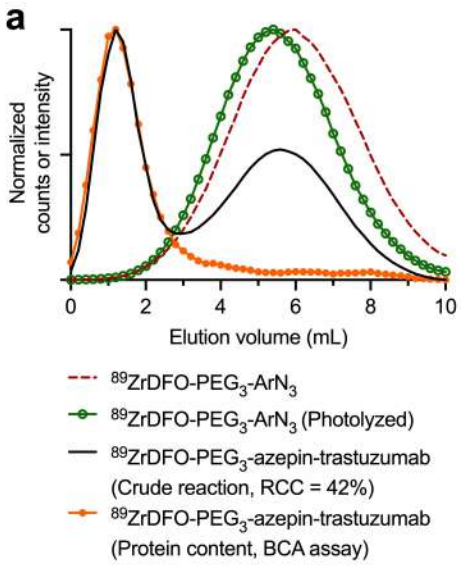
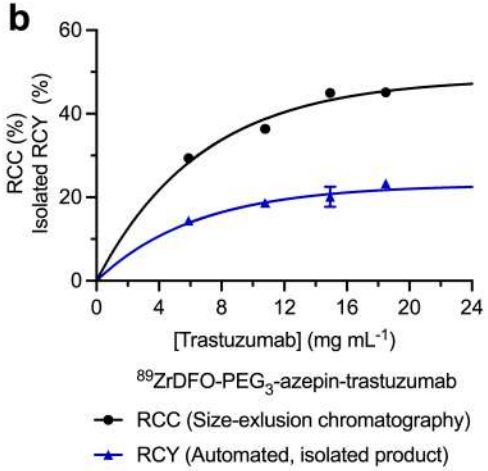
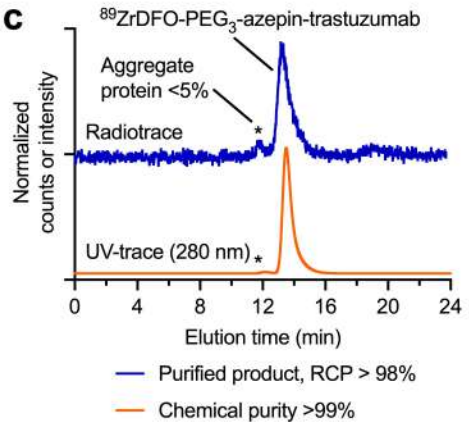
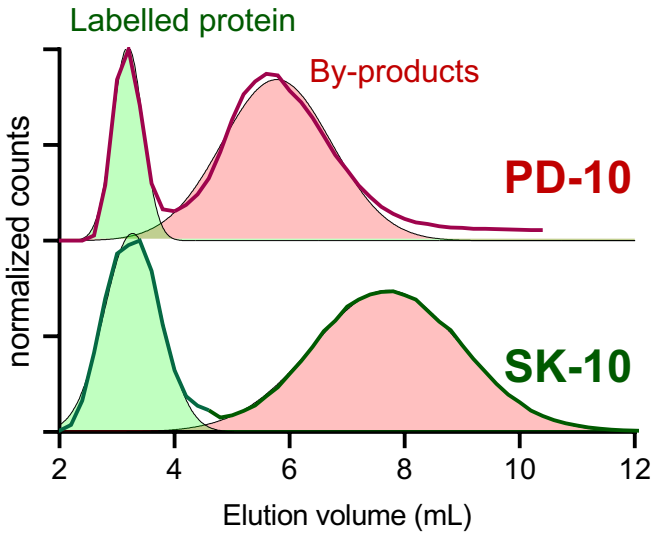
ALISI – automated photoradiosynthesis







Isolated, formulated, d.c.
RCY (mAb) = 20.1 ± 2.4% (*n* = 3)

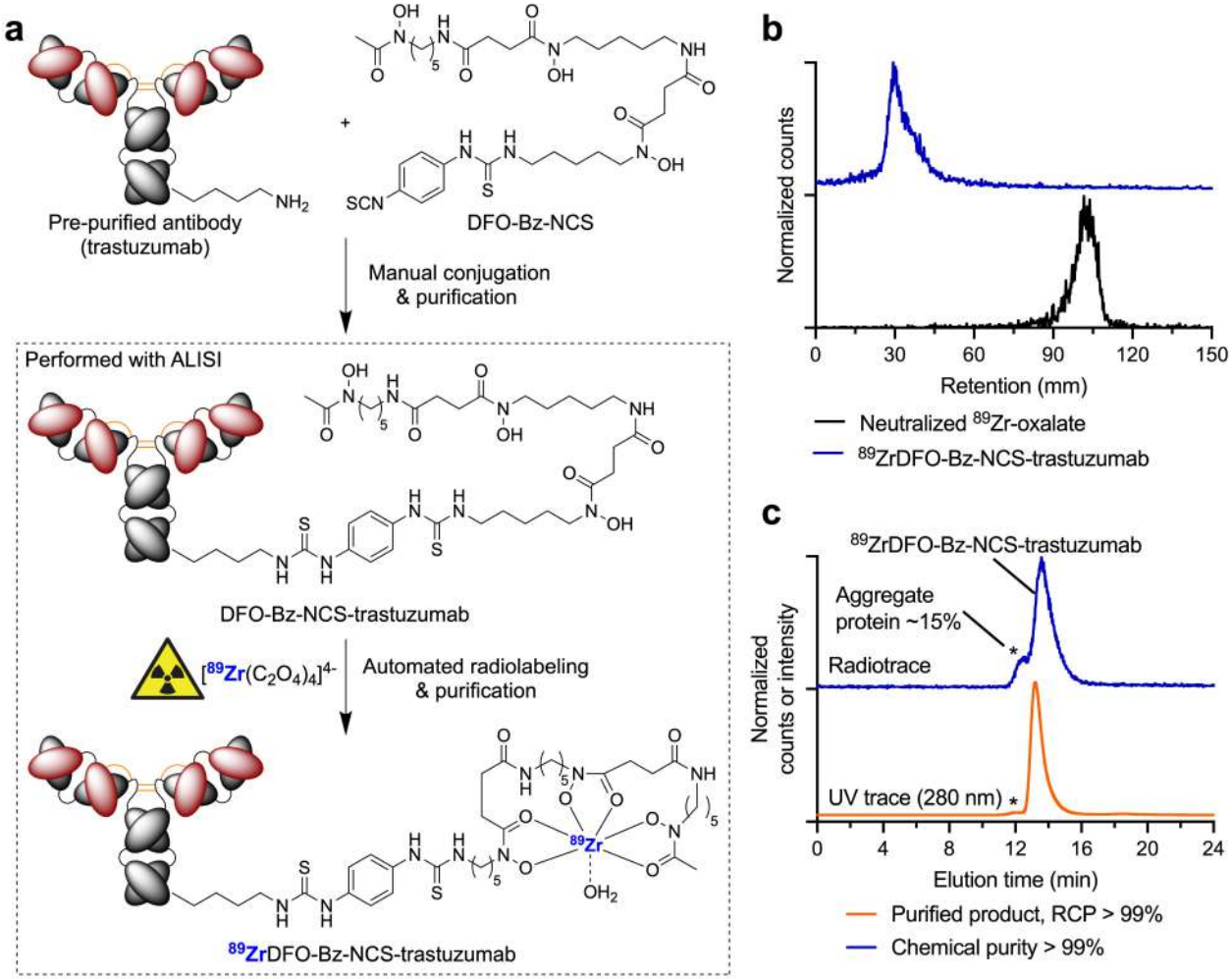


Optimising the mAb purification step:

- **PD-10** columns use Sephadex G-25
- **SK-10** columns use Sephadex G-100
- Improved purification of large proteins
- Strive for RCP >99% (vs. >90%)

Klingler and Holland, **2021**, *submitted*

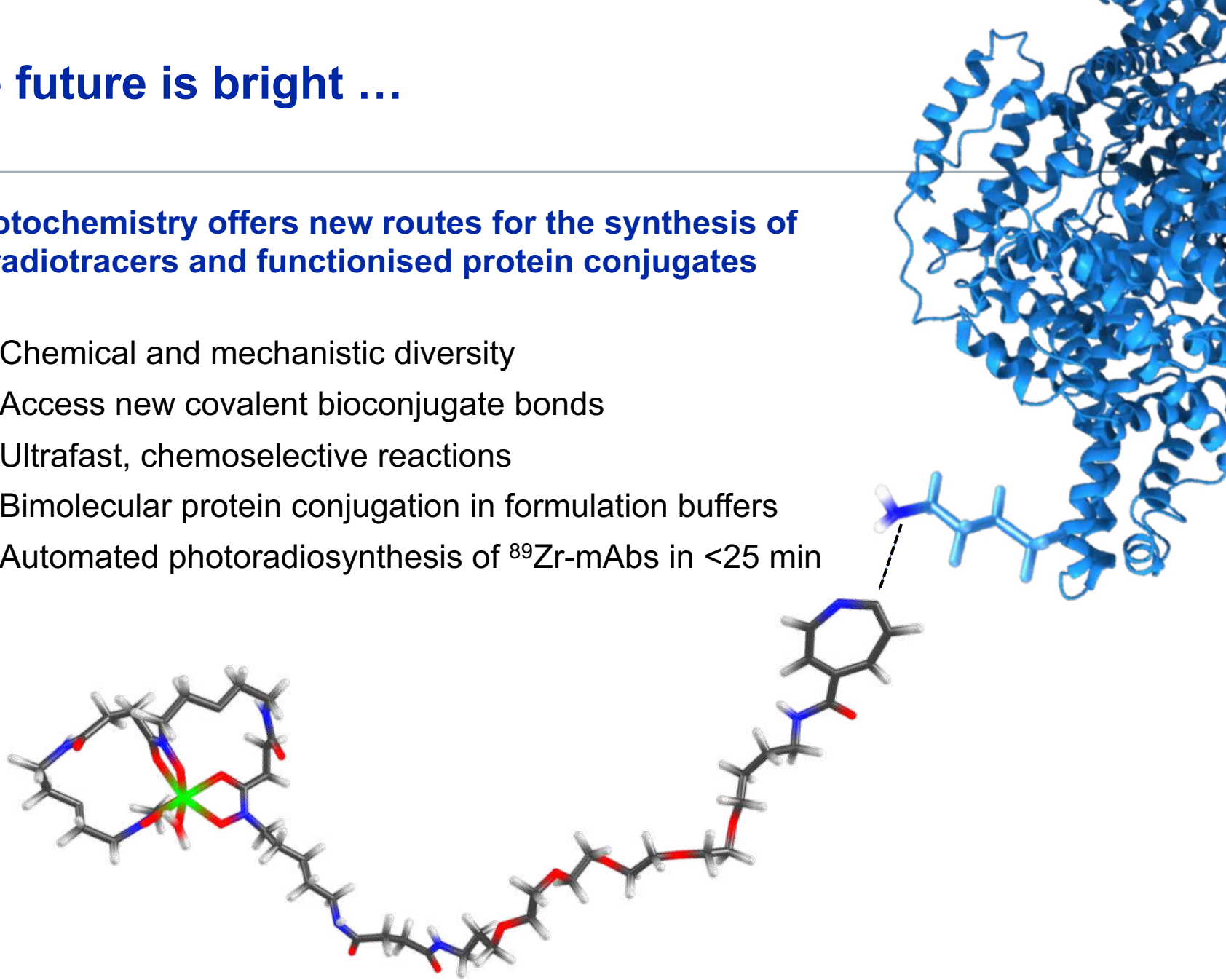
ALISI – automated conventional radiolabelling



The future is bright …

Photochemistry offers new routes for the synthesis of radiotracers and functionised protein conjugates

- Chemical and mechanistic diversity
- Access new covalent bioconjugate bonds
- Ultrafast, chemoselective reactions
- Bimolecular protein conjugation in formulation buffers
- Automated photoradiosynthesis of ^{89}Zr-mAbs in <25 min



Acknowledgements

University of Zurich[UZH]

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Thank you
Any questions, comments or suggestions?

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