New therapeutic modalities

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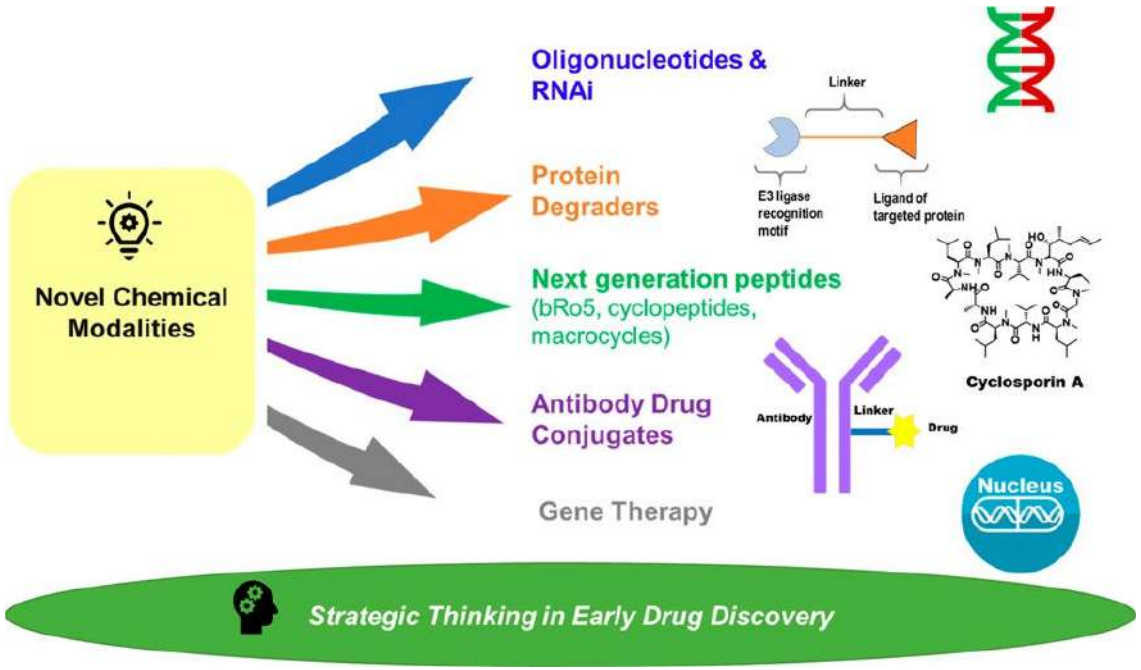
School of Pharmacy and Food Sciences, 7th March 2025

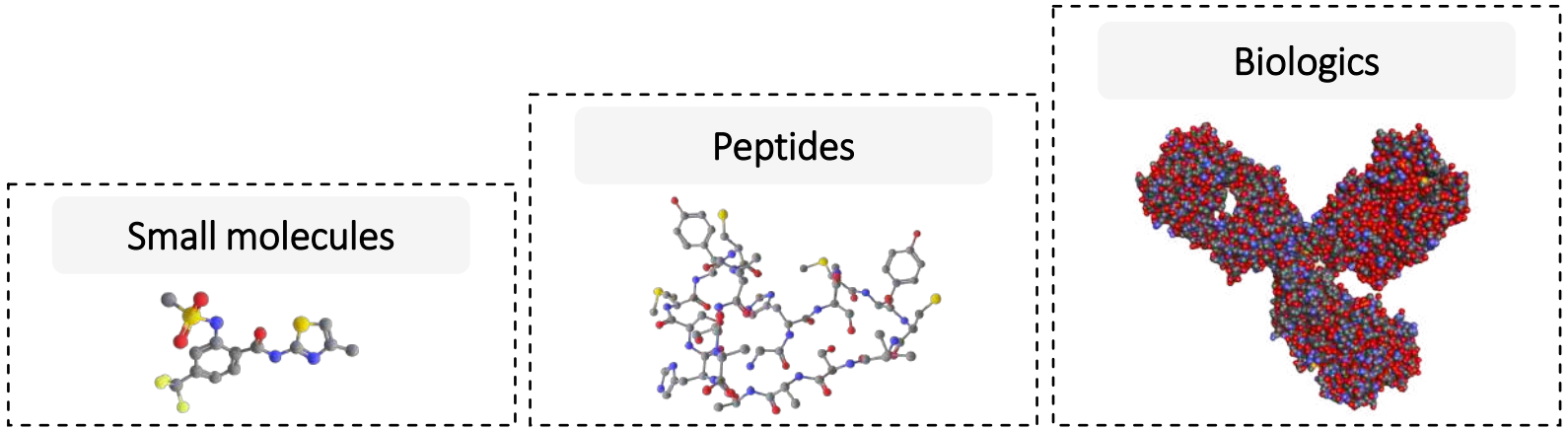
New therapeutic modalities



M.-J. Blanco *et al. ACS Med. Chem. Lett.* **2020**, *11*, 228

- **Therapeutic peptides**
- **Antibody-drug conjugates (ADCs)**
- **Targeted protein degradation (TPD)**

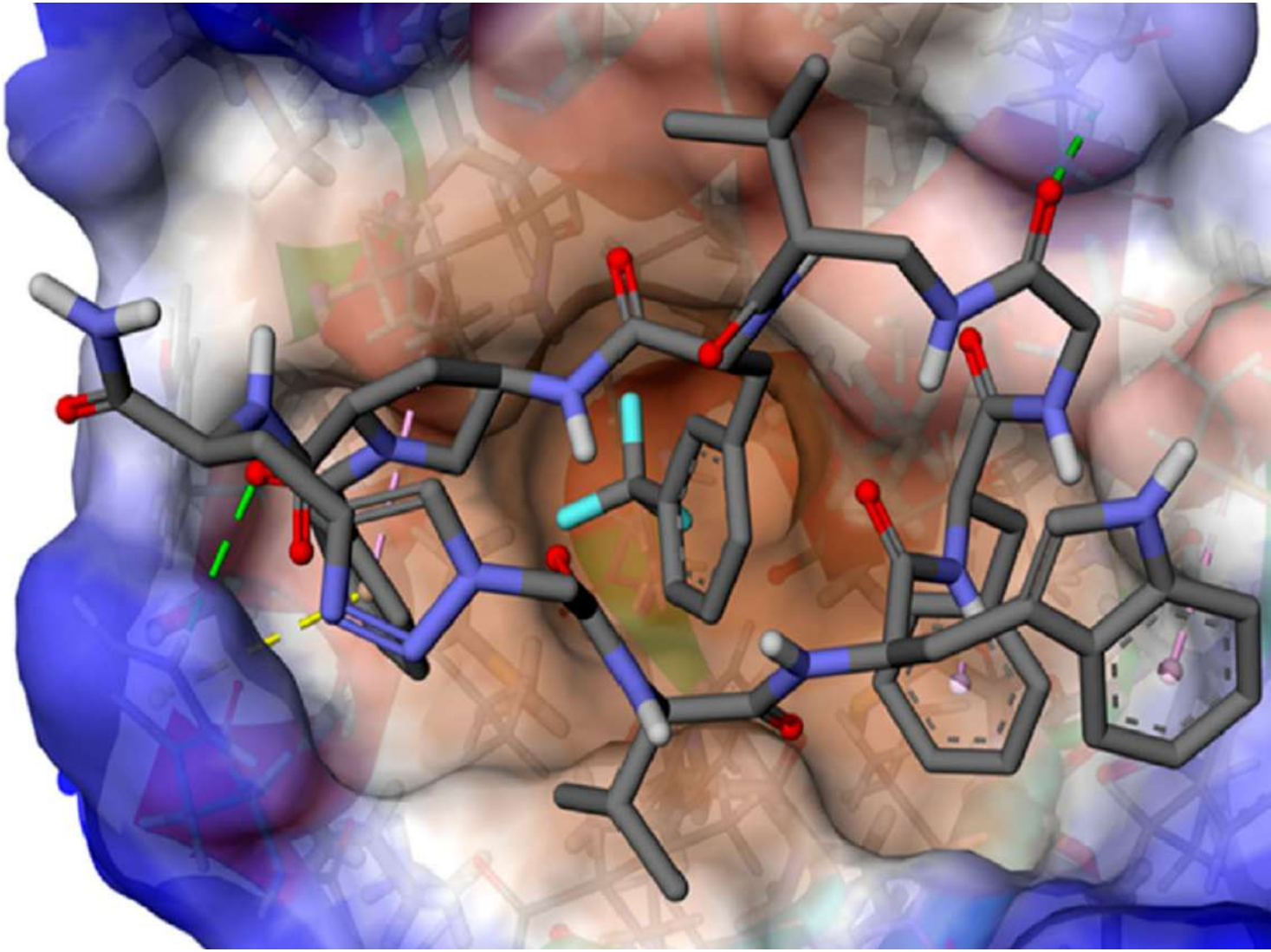
Classification of therapeutics



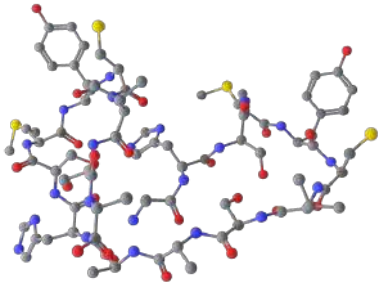
Size (Da)	**< 1000**	**1000-5000**	**> 5000**
Targets	Small, well-defined pockets	Large, shallow surfaces	Extracellular proteins
Binding affinity	Moderate	High	High
Target specificity	Moderate	High	High
Oral bioavailibility	Often	Rarely	No
Membrane permeability	Often	Rarely	No
Plasma half-life	Hours	Minutes to hours	Weeks
Production cost	Low	Moderate	High
Off-target toxicity	Yes	No	No
Immunogenicity	No	No	Yes

Henninot *et al. J. Med. Chem.* **2018**, *61*, 1382; La Manna et al. *Int. J. Mol. Sci.* **2018**, *19*, 2714

Photoswitches for peptide stapling



Peptides



Major limitations:

- Poor enzymatic stability
- Poor membrane permeability

MACROCYCLISATION

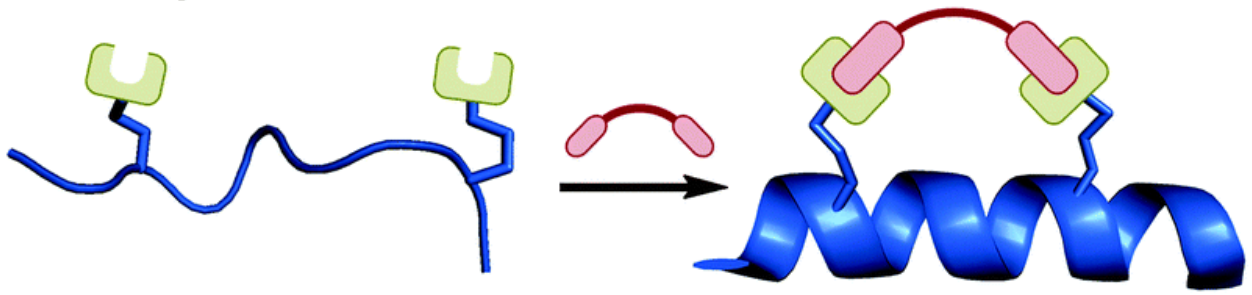
Peptide stapling

Reinforcing the alpha helical conformation:

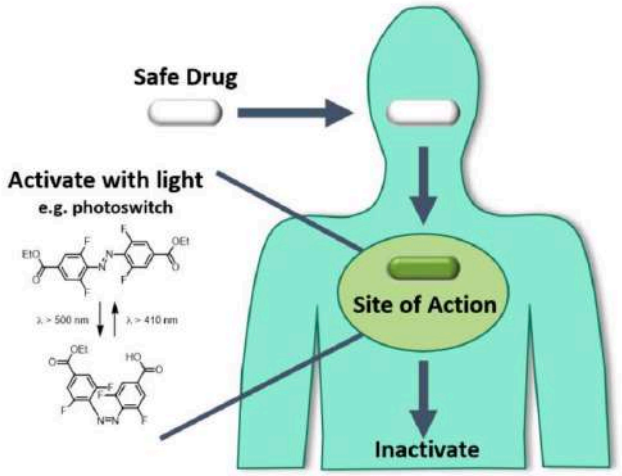
↑ stability

↑ potency

↑ permeability



Lau *et al. Chem. Soc. Rev.* **2015**, *44*, 91



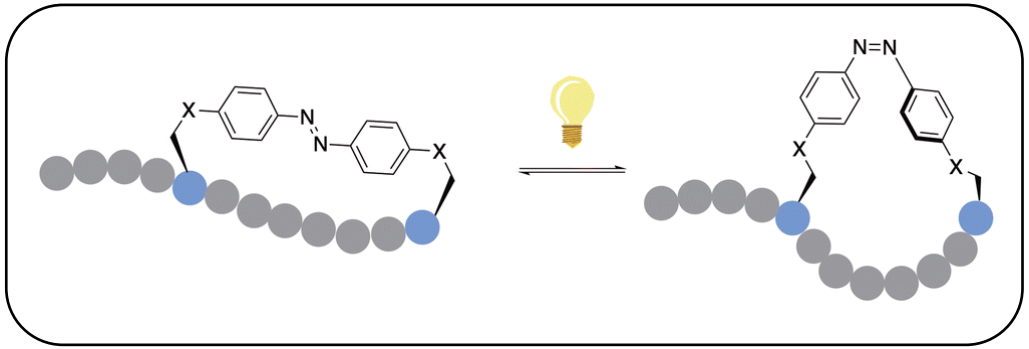
Photopharmacology

Staple peptides represent an ideal ‘substrate’ for **photopharmacology**, given the importance of structural changes for their PK profile and biological activity.

The incorporation a platform able to undergo *cis-trans* isomerization upon irradiation with light enables the formation of **photoswitchable peptides**.

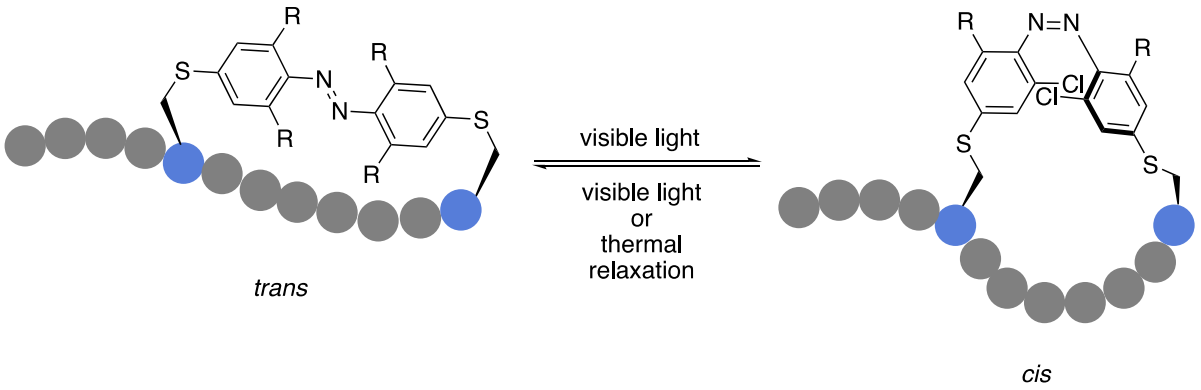
Azobenzene photoswitches

- ✓ Easy synthesis
- ✓ Highly photostable
- ✓ Readily undergo *cis-trans* isomerisation

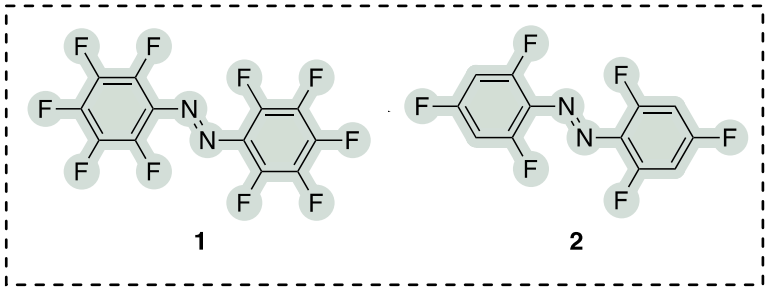


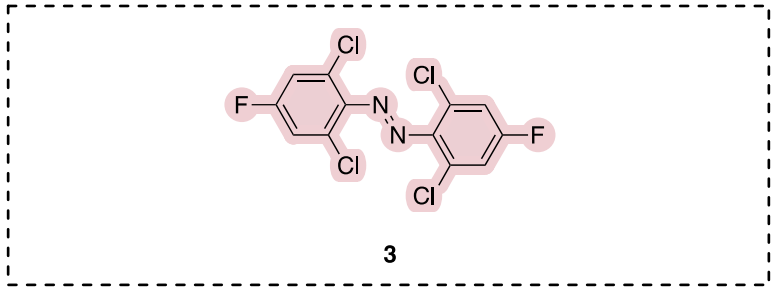
Chem. Rev. **2018**, *118*, 10710; *ChemPhotoChem* **2019**, *3*, 431; *Angew. Chem. Int. Ed.* **2009**, *48*, 1484; *ChemBioChem* **2019**, *20*, 1417; *ACS Cent. Sci.* **2022**, *8*, 57; *J. Am. Chem. Soc.* **2013**, *135*, 9777

Objectives



- Visible-light azobenzene platform
- Cysteine selective peptide stapling
- Stapling *via* S_NAr





✓ Green-light isomerisation

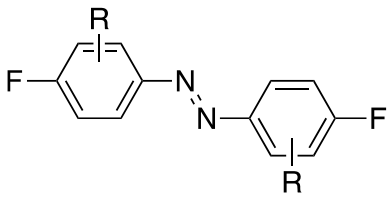
✓ One-step synthesis

✓ Red-light isomerisation

✓ One-step synthesis

Kapun *et. Al. RSC Chem. Biol.*, **2024**,*5*, 49

Reactivity assessment

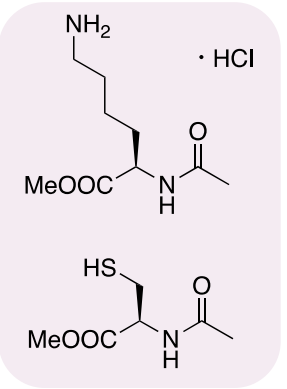


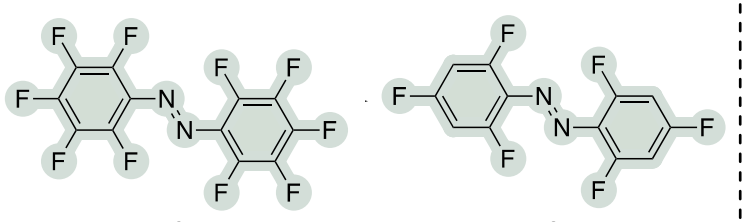
+ amino acid (2 eq)

Tris (2-4 eq)

DMF
rt
2 h

Products analysis by LCMS, ^{1}H and ^{19}F NMR

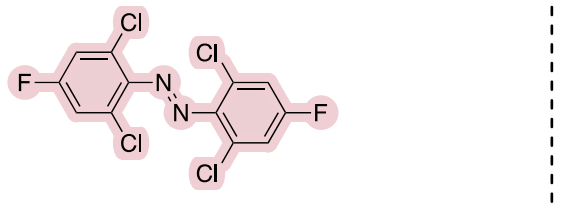




1 2

✗ Poly-substitution at *ortho* position

✗ Lysine reactivity



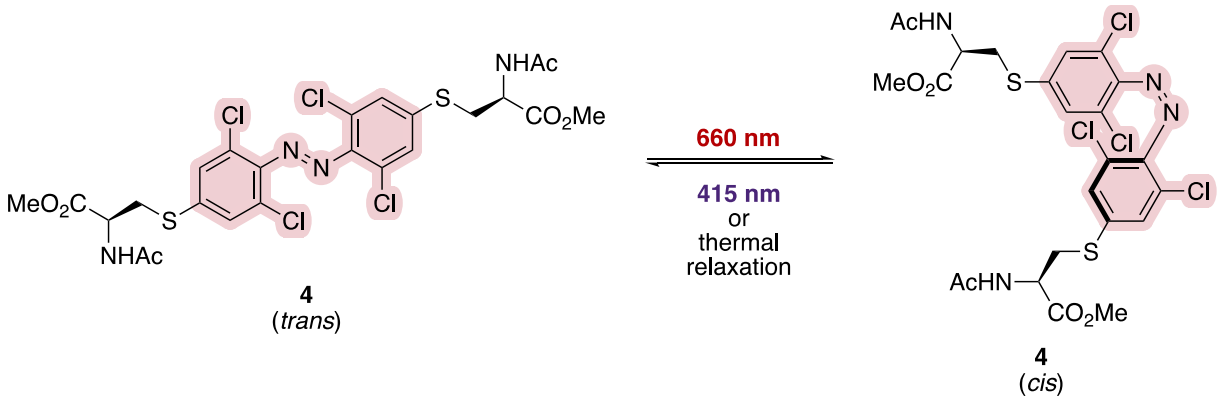
3

✓ Highly selective for *para* position

✓ Cysteine selective

Spokoyny *et al. J. Am. Chem. Soc.* **2013**, *135*, 5946; Kapun *et al. RSC Chem. Biol.* **2024**, *5*, 49

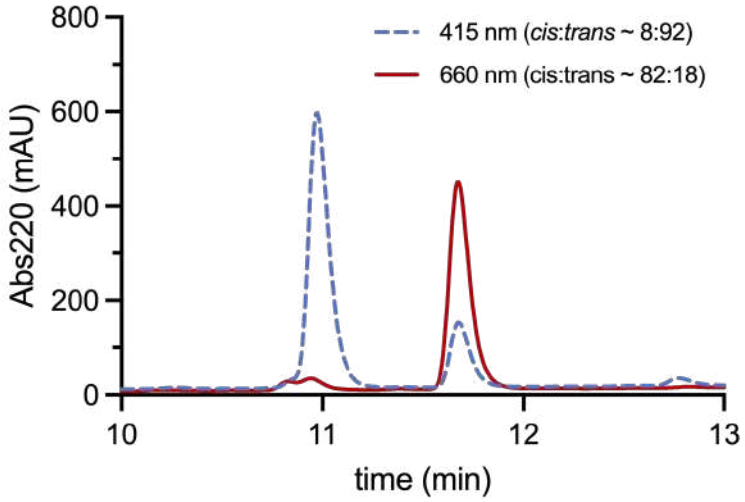
Photochemical Characterisation



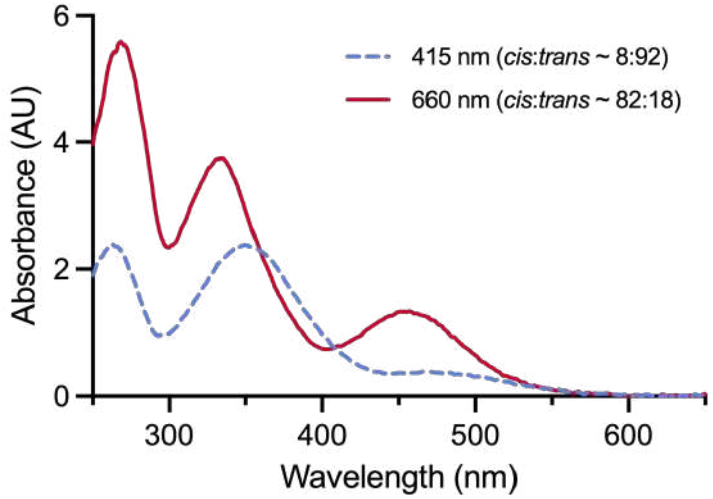
4 (*trans*)

4 (*cis*)

HPLC analysis



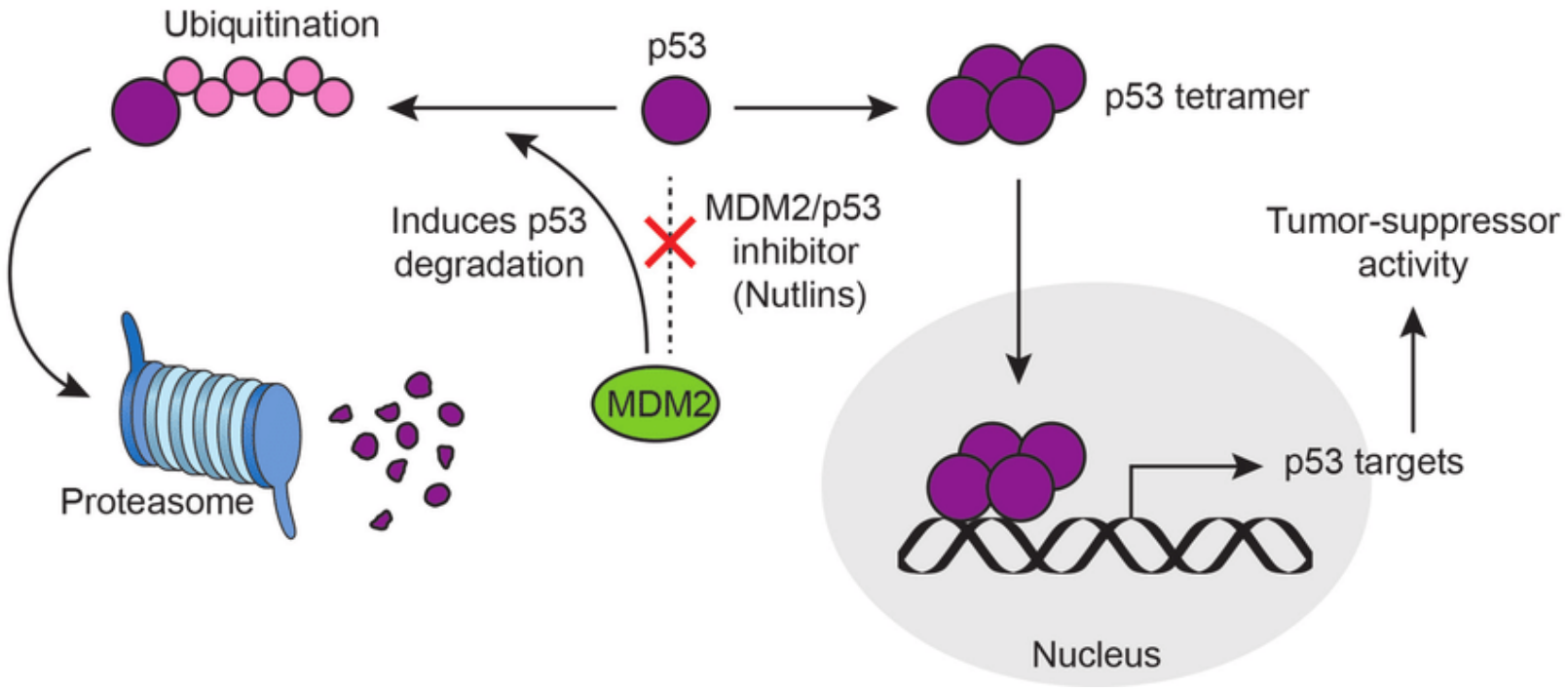
UV–vis analysis



500 µM in H_2O/MeCN 50:50. The spectra were recorded upon 30 min and 90 min irradiation with 415 nm and 660 nm LED lights, respectively.

Kapun *et. Al. RSC Chem. Biol.*, **2024**,*5*, 49

Proof-of-concept: MDM2–p53 inhibition

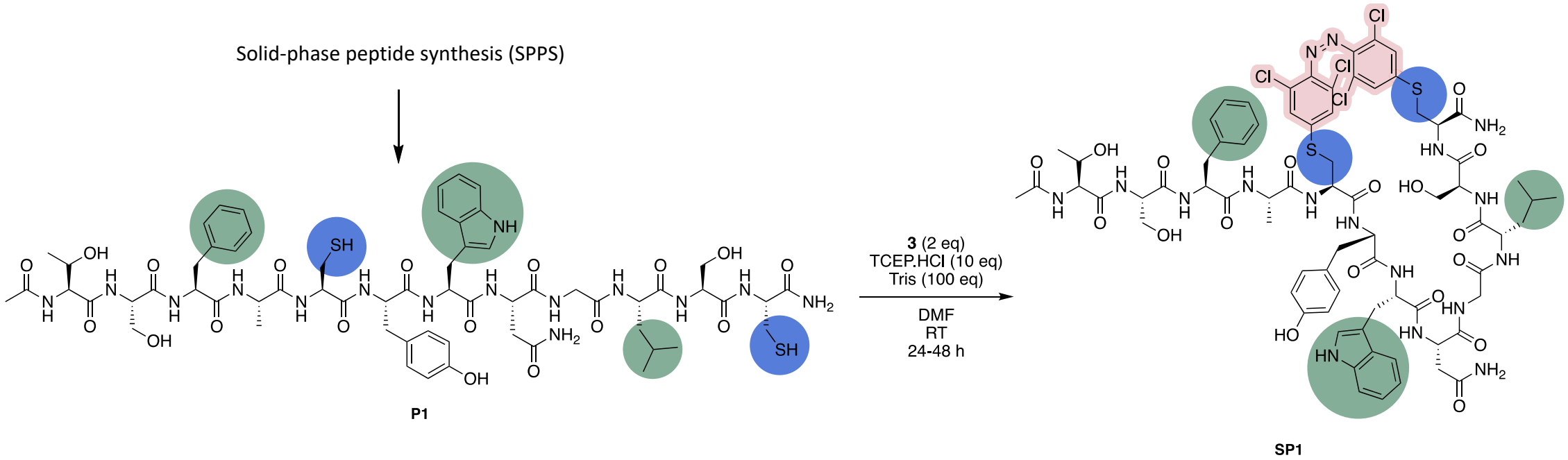


Inhibition of the p53/MDM2 interaction has been shown to efficiently rescue p53 from degradation, thus recovering its tumour suppressor activity

Huang *et al. Cell Res.* **2016**, *26*, 484 ; Kapun *et al. RSC Chem. Biol.* **2024**, *5*, 49

Proof-of-concept: staple peptide

The photoswitchable *ortho*-chloro AB was applied to a derivative of **PMI** (Ac-TSFAEYWNNLSP-NH_2), a potent inhibitor p53–MDM2 interaction



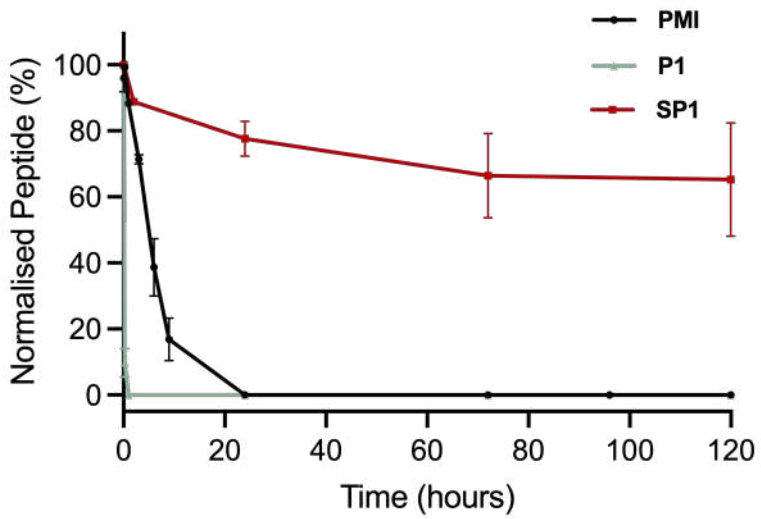
Substitutions at positions 5 and 12 to facilitate (*i*, *i*+7) peptide stapling, allowing for the incorporation of the staple without disrupting the interaction with MDM2, driven by the 'hot spot' residues (Phe3, Trp7 and Leu10)

Kapun *et. al. RSC Chem. Biol.*, **2024**,*5*, 49

Stability studies

Peptide	Sequence
PMI	Ac-TSFAEYWNNLSP-NH_2
P1 (Cys derivative)	Ac-TSFACYWNGLSC-NH_2
SP1	Ac-TSFAXYWNGLSX-NH_2

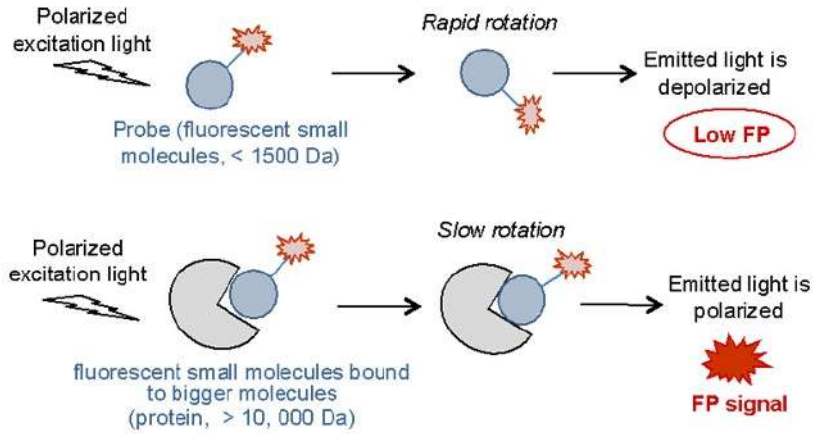
Stability in human serum



Incubated at 37 °C and monitored throughout 5 days by HPLC

Kapun *et. al. RSC Chem. Biol.*, **2024**,*5*, 49

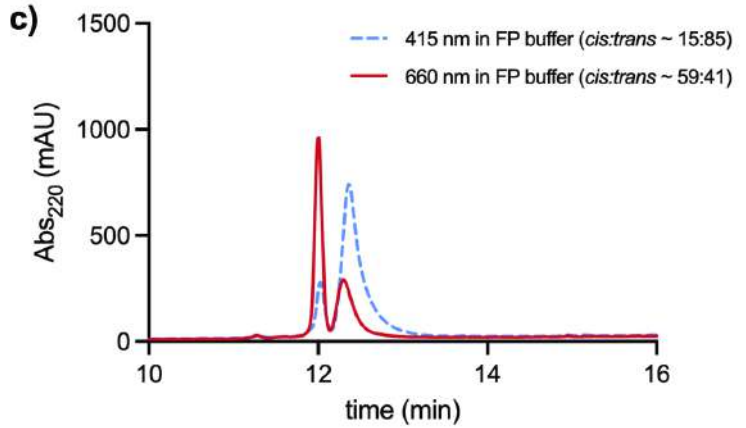
Competitive fluorescence polarisation (FP) assay
Determination of binding affinity of peptides for MDM2 direct FP, followed by competitive FP assay



Arkin, M.R. *et al. Assay Guidance Manual Eli Lilly & Company and the National Center for Advancing Translational Sciences* **2004**

Peptide	Sequence
PMI	Ac-TSFAEYWNNLSP-NH_2
FP tracer (K_d = 7.6 nM)	TAMRA-RFMDYWEGL-NH_2
P1	Ac-TSFACYWNGLSC-NH_2
SP1	Ac-TSFAXYWNGLSX-NH_2

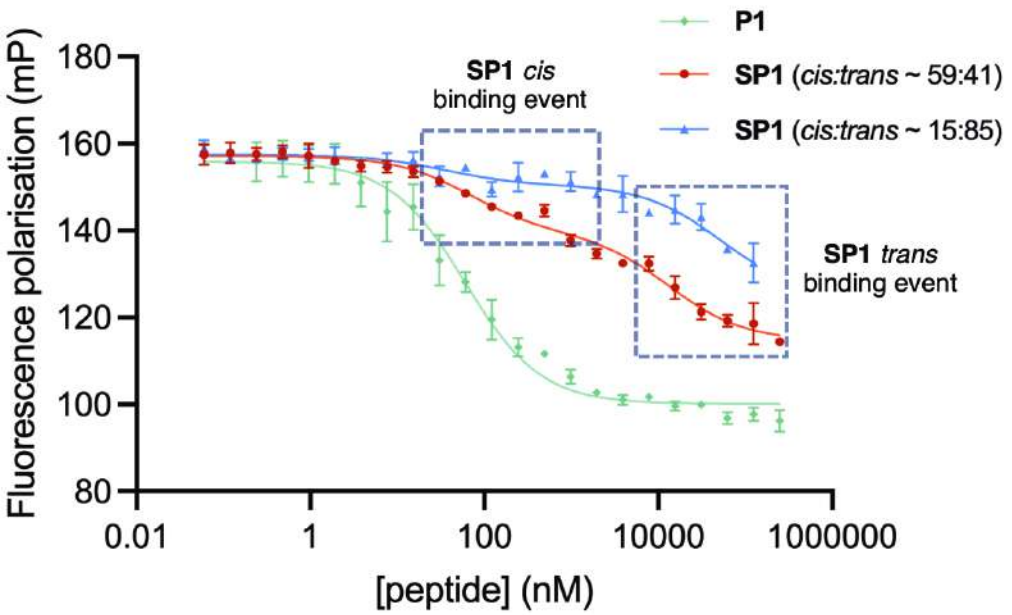
HPLC analysis in FP buffer



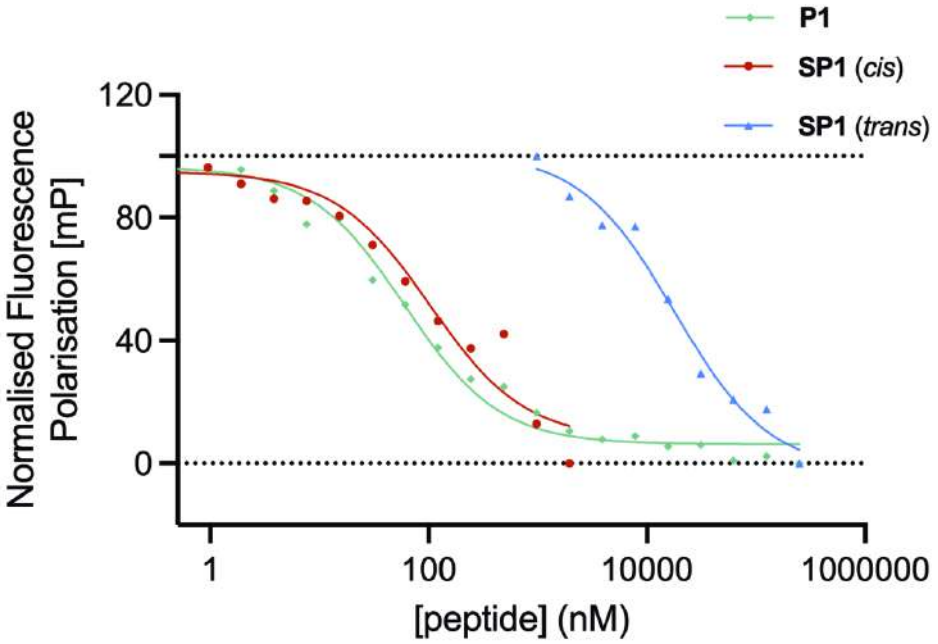
FP buffer (PBS, 0.05% (v/v) Tween-20, 3% DMSO)

Kapun *et. al. RSC Chem. Biol.*, **2024**,*5*, 49

Competitive FP assay



Normalised FP curves



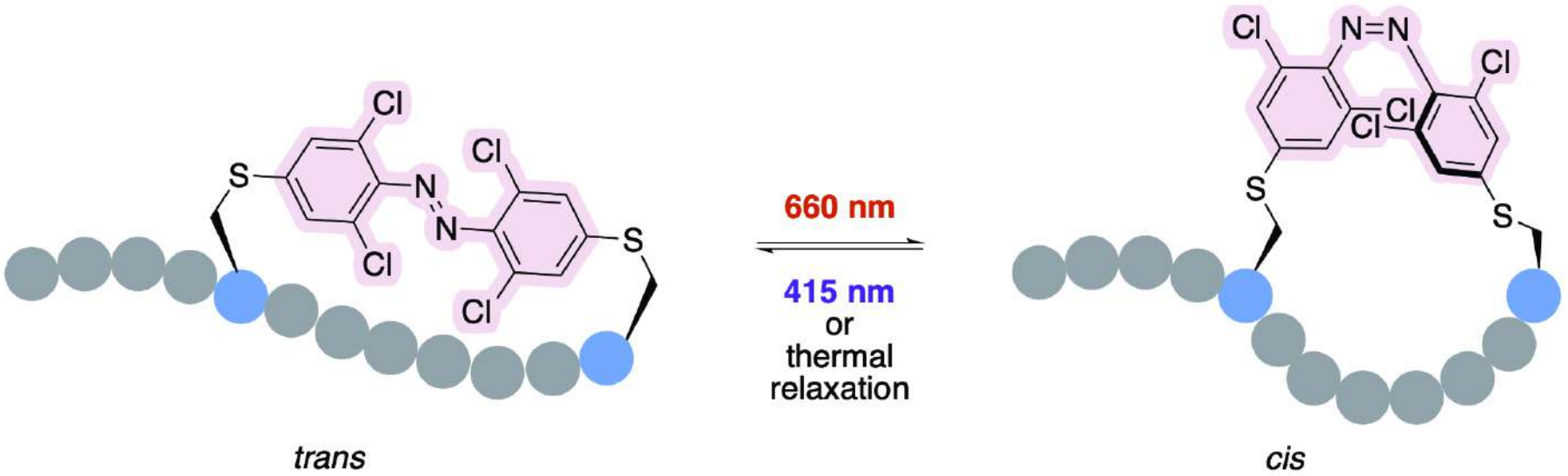
Peptide	PMI	P1	SP1 (*trans*)	SP1 (*cis*)
K_i (nM)	0.78 ± 0.36	7.93 ± 3.83	> 13000	54 ± 4.9

>240-fold higher affinity by *cis* isomer

Kapun *et. al. RSC Chem. Biol.*, **2024**,*5*, 49

Conclusions

ortho-chloro azobenzene photoswitch for peptide stapling



- ✔ Use of red light
- ✔ Cysteine-selective peptide stapling via S_NAr
- ✔ Light induced changes in peptide conformation
- ✔ >240-fold difference in binding affinity for MDM2 upon *trans-cis* isomerisation

Kapun *et. al. RSC Chem. Biol.*, **2024**,*5*, 49

Tetra DVP

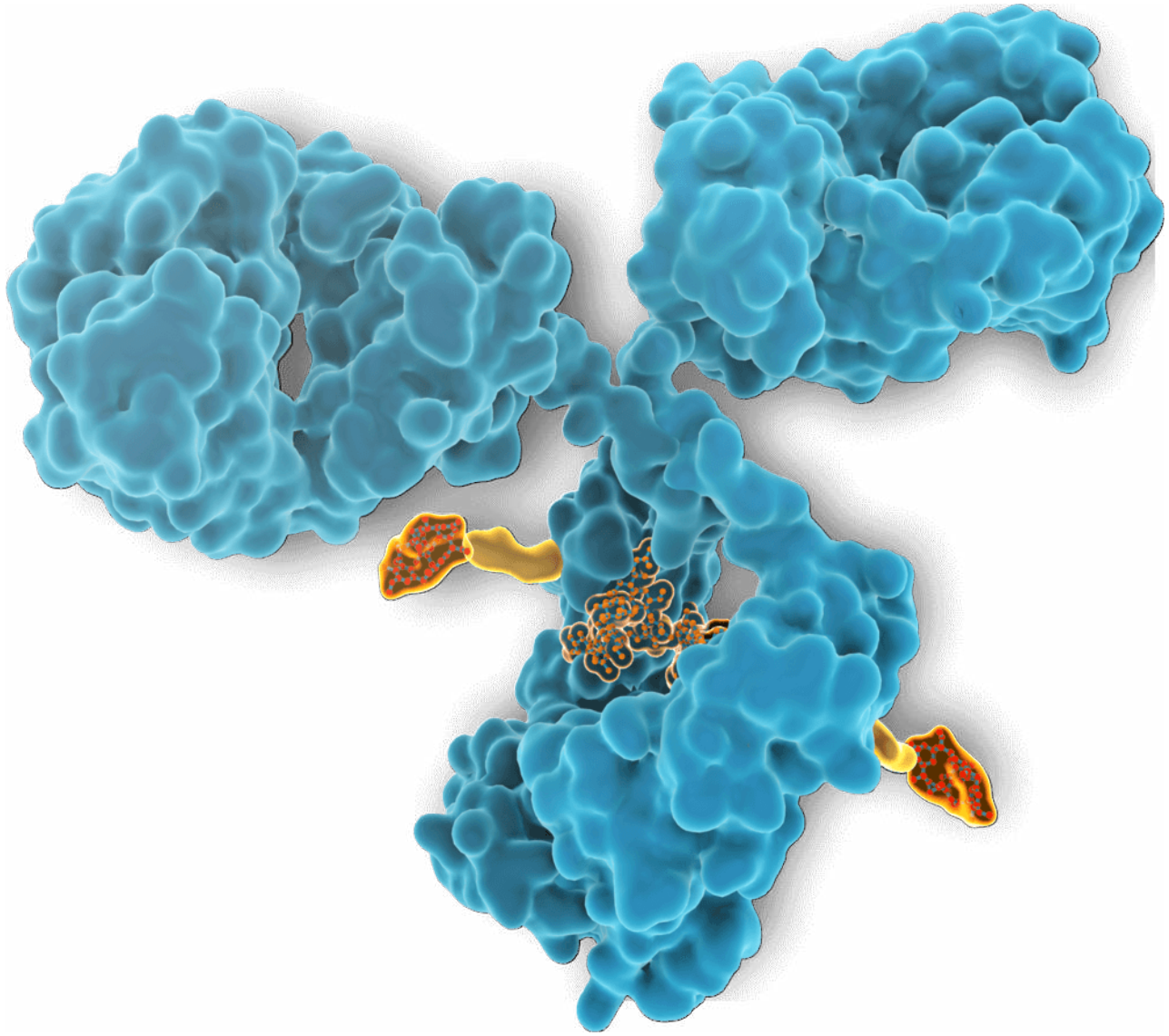
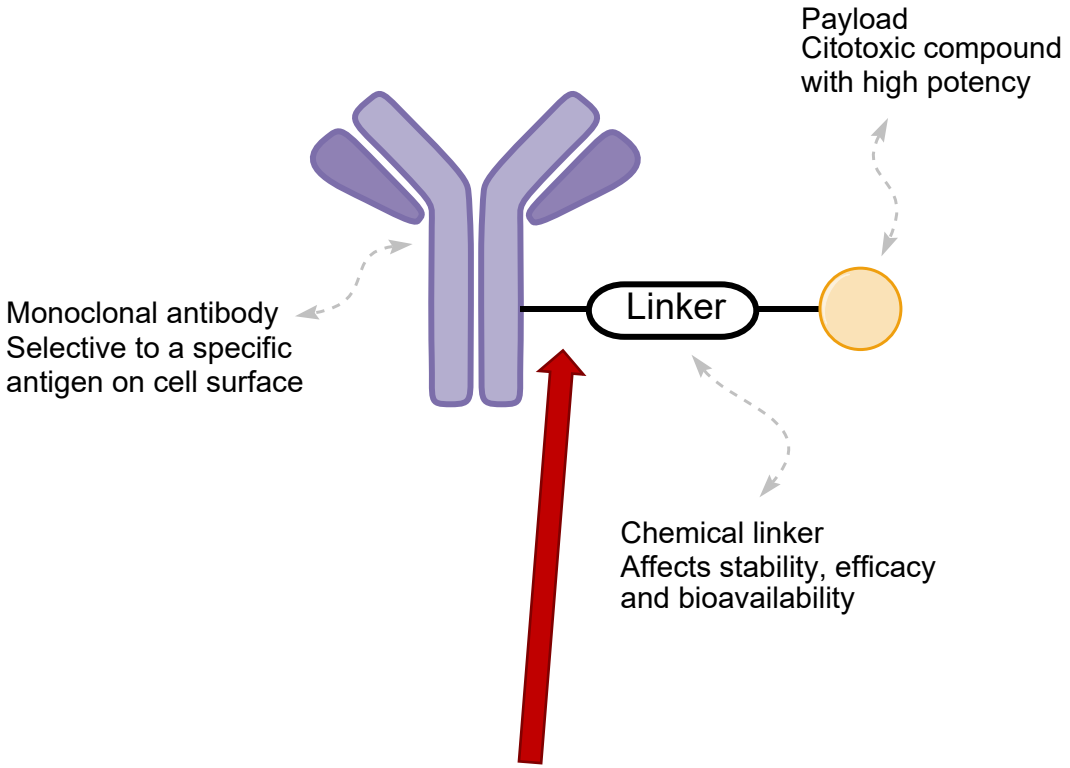


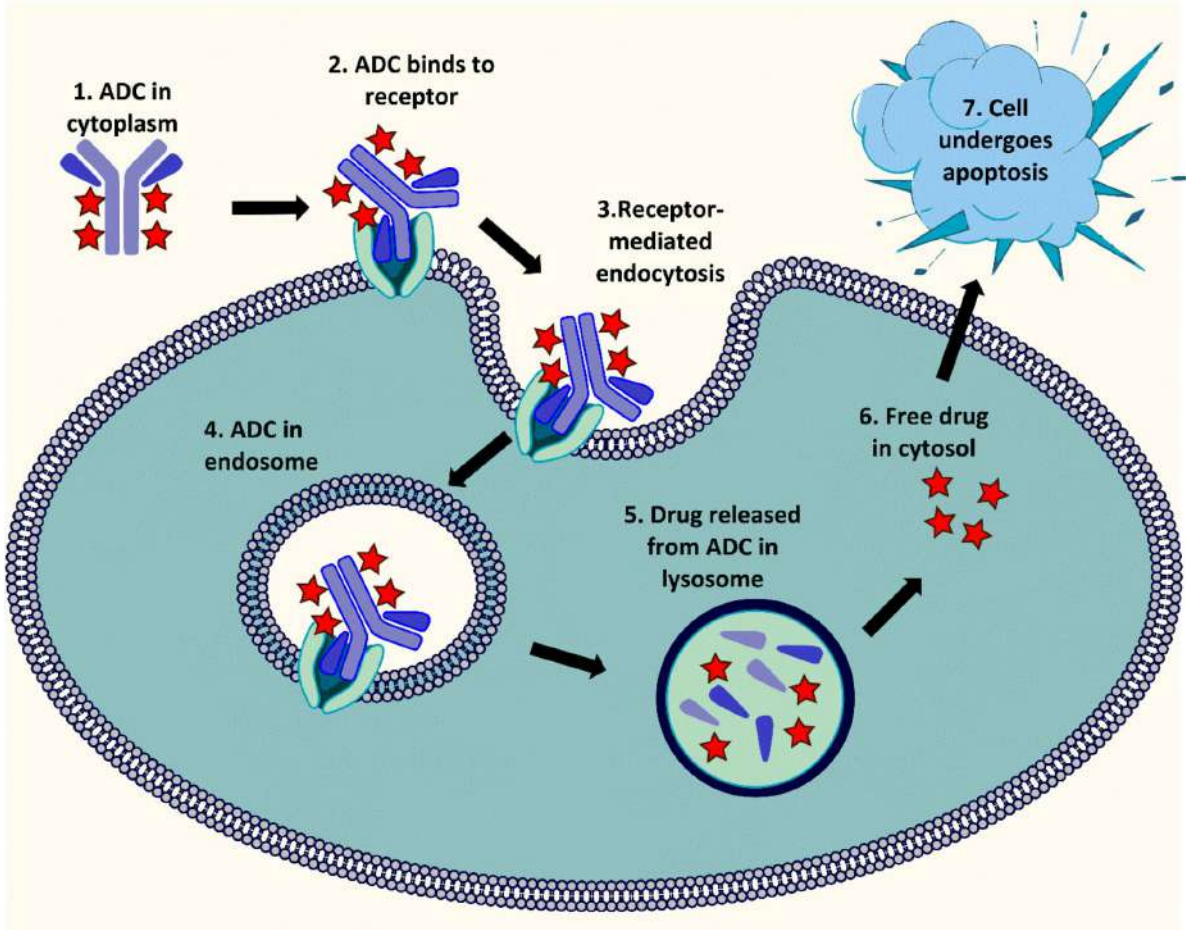
Figure from *Araris Biotech AG* website

What is an ADC?



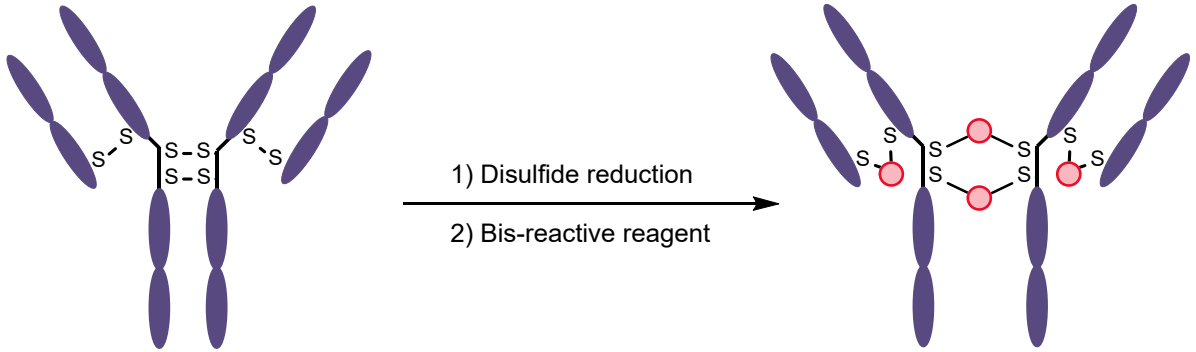
Antibody-linker attachment

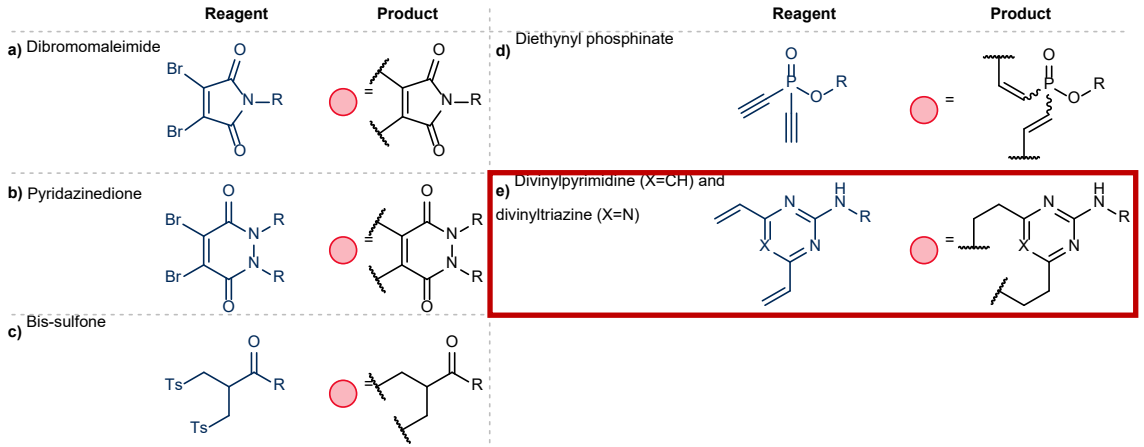
How ADCs work?



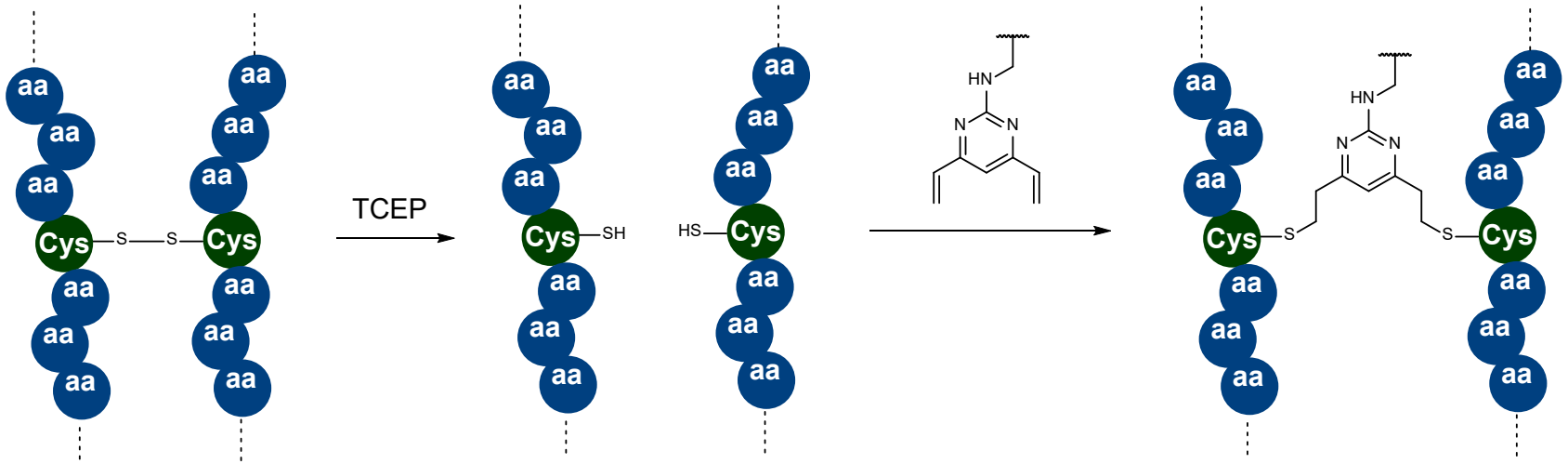
Chen, H. *et al*. *Molecules* **2017**, *22*, 1281

Disulfide re-bridging (interchain cross-linking)





Divinylpyrimine (DVP)



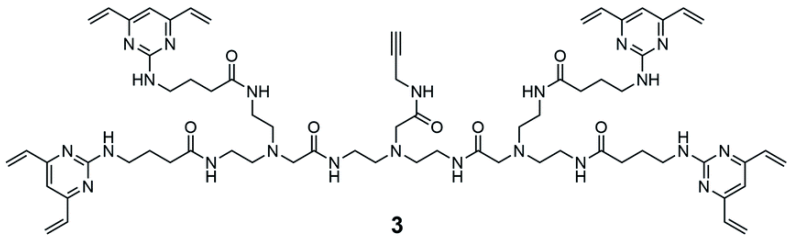
DVP conjugation Properties:

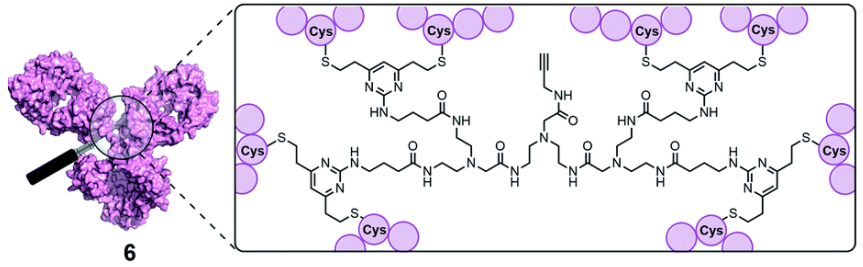
- Fast
- Irreversible
- Stable
- Chemoselective
- **Consistent DAR**
- Facile synthesis

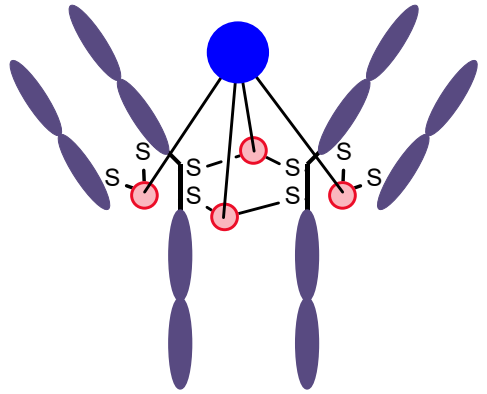
S. Walsh *et al. Chem. Sci.* **2019**,*10*, 694

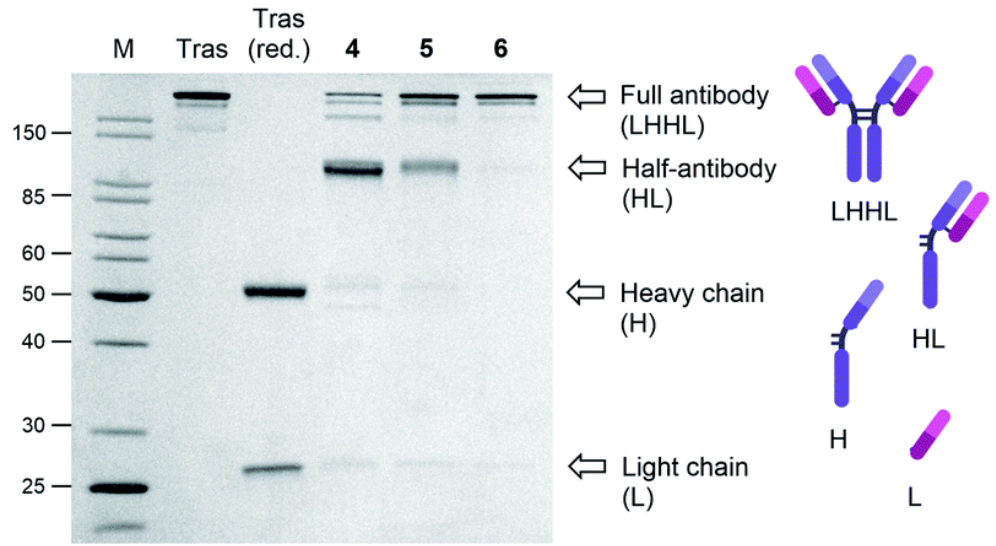
DAR = drug-antibody ratio → Described as an average of cytotoxic units per antibody

DAR consistency is a major limitation of ADCs → **Tetra-divinylpyrimidine (tetra-DVP)**









Tras = Trastuzumab

4 from DVP

5 from bis-DVP

6 from tetra-DVP

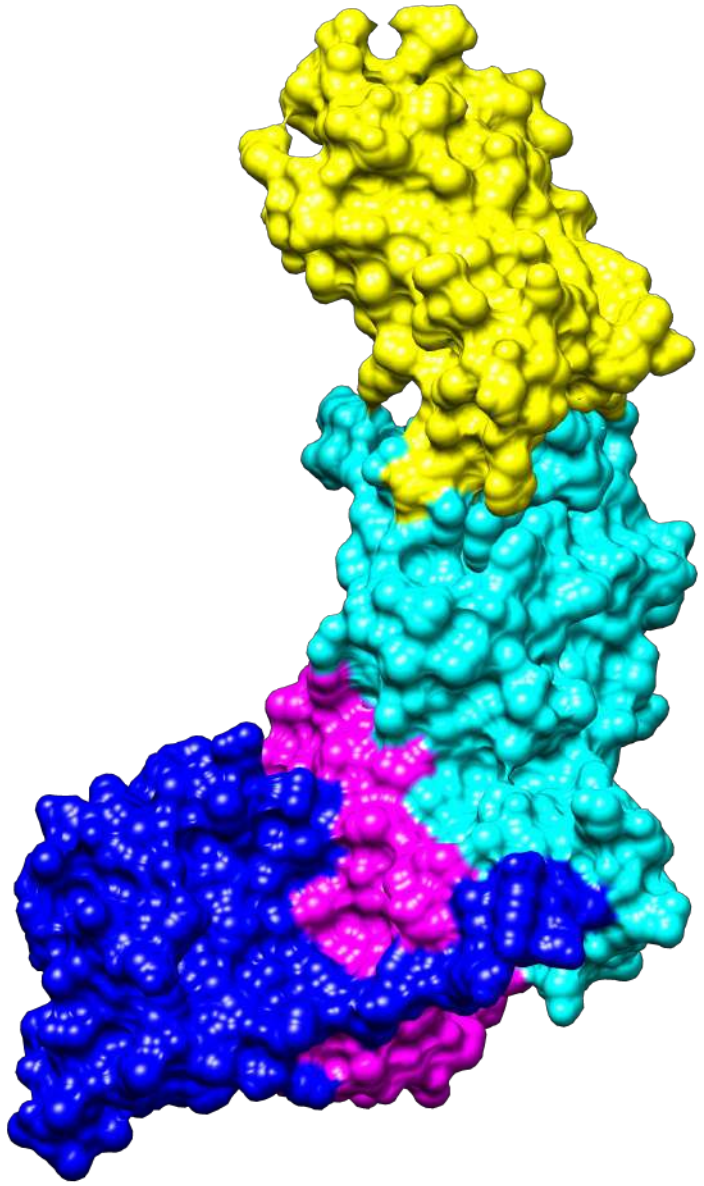
Functionalisable group

↓

Modular cargo loading (consistent DAR)

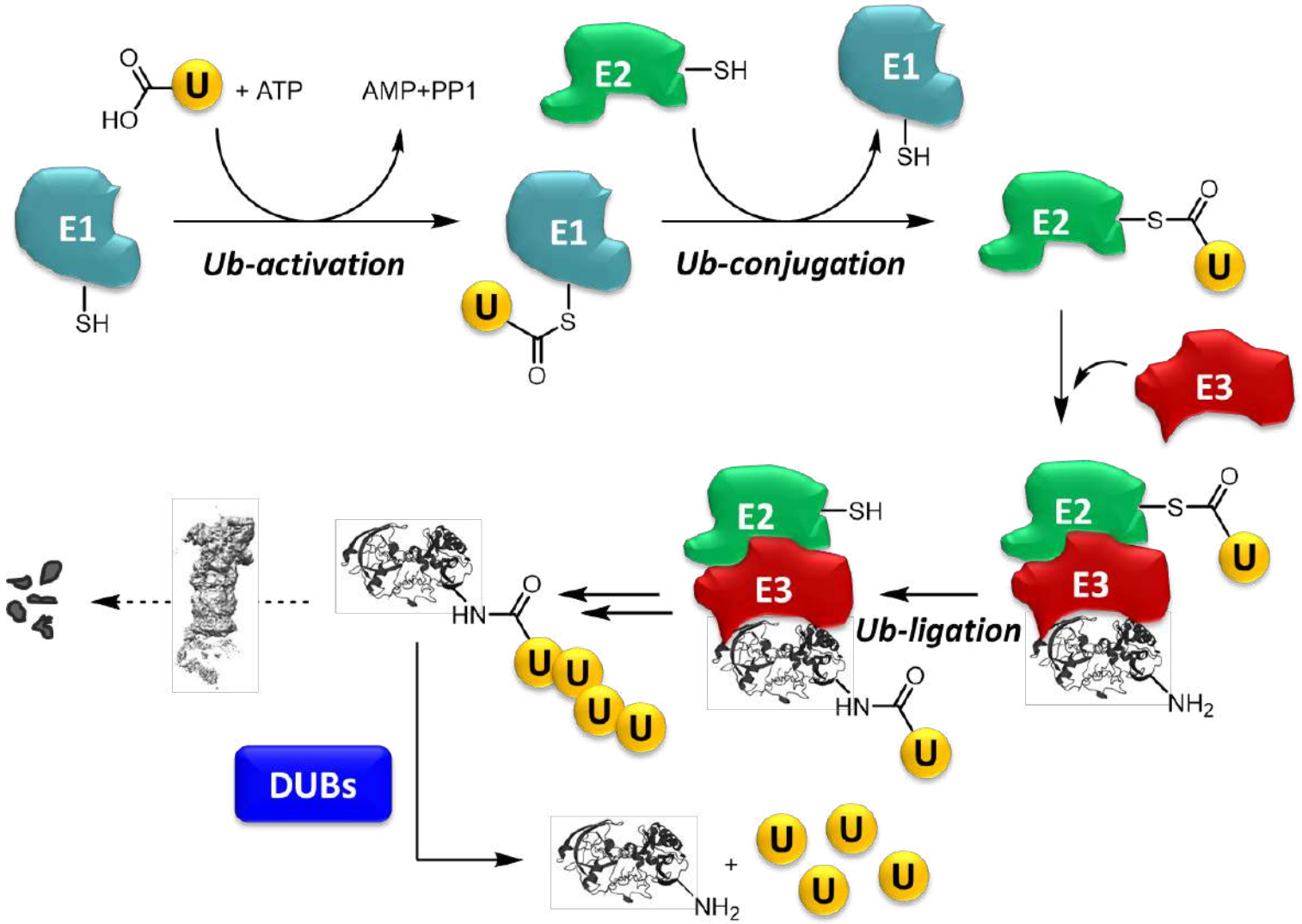
F.M. Dannheim *et al. Chem. Sci.* **2022**,*13*, 8781

Ubiquitin-specific protease 2 targeted degraders (USP2-TD)



PDB: 5T35

Ubiquitin proteasome system



Targeting **deubiquitinating enzymes (DUBs)** offers the opportunity to modulate the UPS with increased specificity, reduced toxicity profiles.

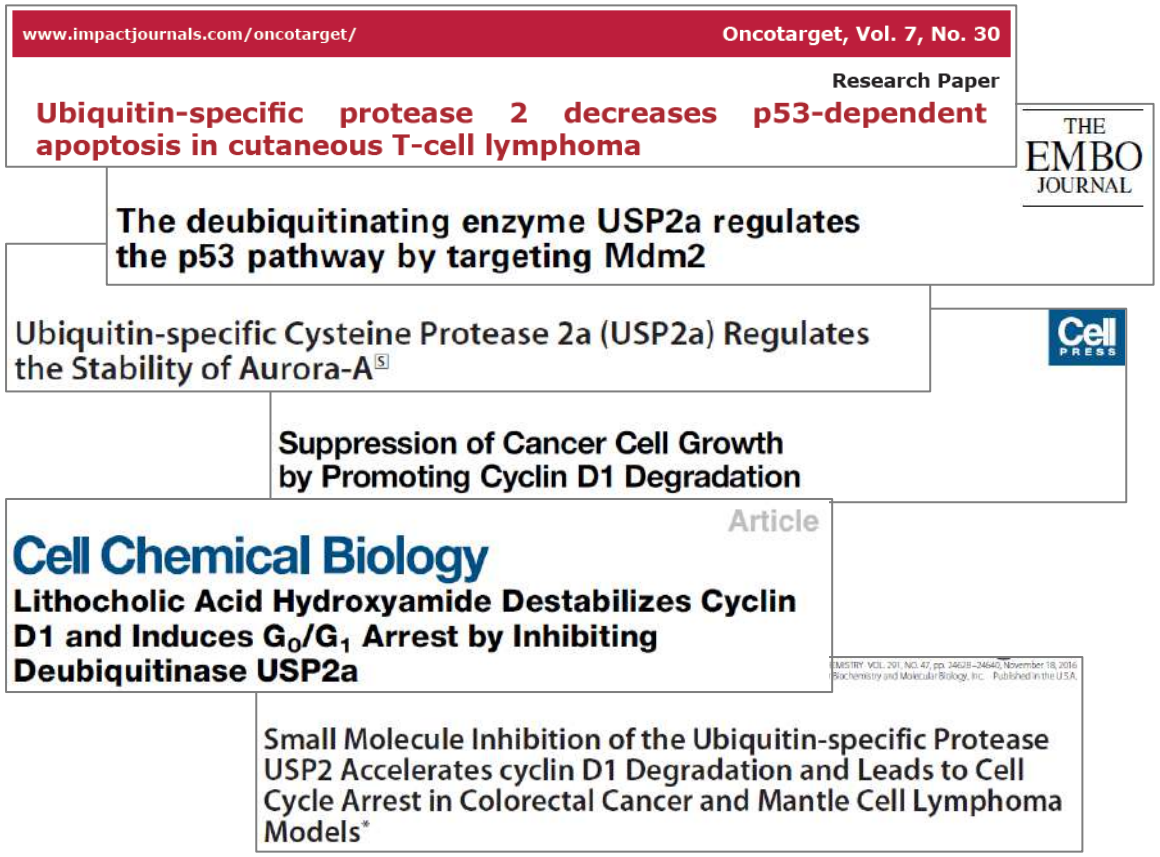
Target validation of DUBs currently represents a priority in the field since we are lacking appropriate **chemical probes** for most of them.

Ubiquitin-specific protease 2 (USP2)

Ubiquitin-specific protease 2 (USP2)

Why USP2?

USP2 is an oncoprotein responsible for the stabilisation of different cell-cycle modulators and signalling pathway factors that lead to **cancer cell survival, proliferation and metastasis**.



Oncogenic substrates of USP2

- MDM2
- Aurora A
- Cyclin A1/D1
- MDM4
- FAS
- c-Myc
- Twist
- AIF
- TRAF2
- EGFR

↑ USP2 or **↑ substrate**

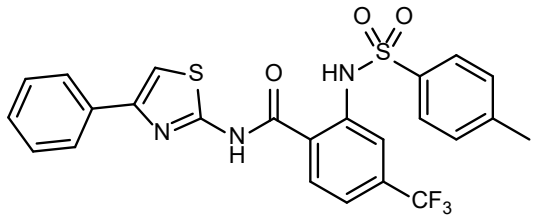
USP2-associated cancers:

- Triple-negative breast
- Prostate
- Ovarian
- Cutaneous T-cell lymphoma
- Colon
- Breast
- Mantle cell lymphoma
- Lung
- Glioblastoma

Targeting USP2

ML364, a “chemical probe”?

USP2 chemical inhibition has been barely explored while it has been extensively studied by biologists during the last decade, using ML364 as chemical probe.



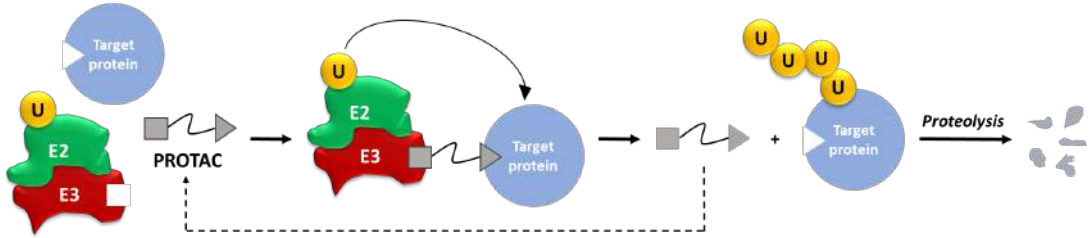
ML364

	Chemical probe standards	ML364 profile
✘ Potency (functional assay)	< 100 nM	1.1 µM
✘ Potency (cellular assay)	< 1 µM in cellular assay	Low µM
✘ Mechanism of action	Elucidated	Unknown
✘ Selectivity	> 30-fold within the family	USP8 IC_{50} = 0.95 µM
✘ Negative control	Inactive analogue	Structurally “related”

Poor quality chemical probe

M. Davis *et al. J. Biol. Chem.* **2016**, *291*, 24628.

Proximity-induced approach

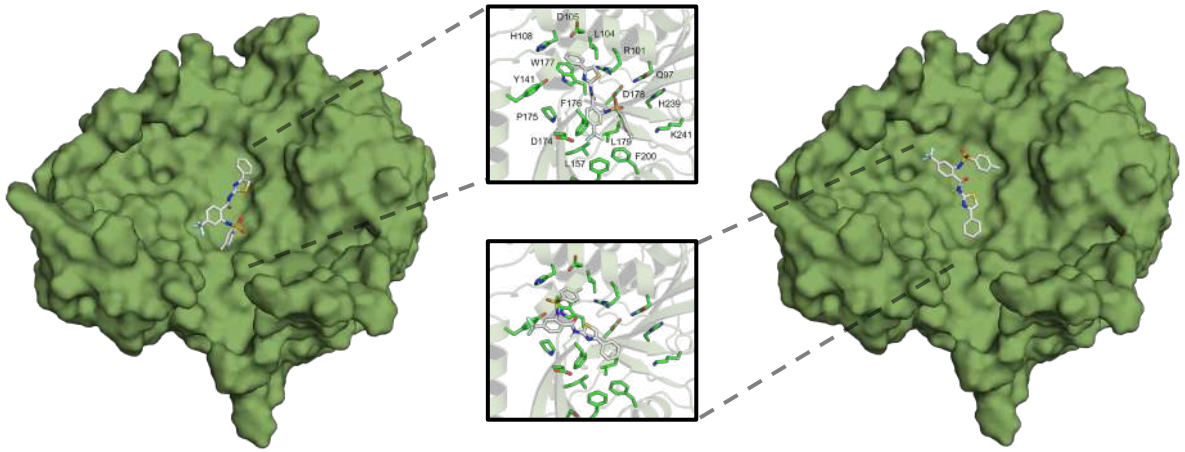


ML364-based USP2 degraders

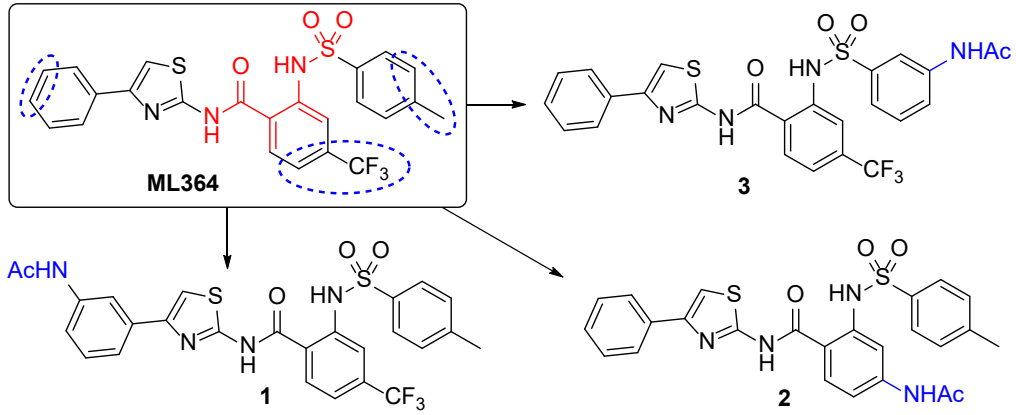
↑ **potency:** catalytic function and depletion of the target
↑ **selectivity:** ternary complex formation

The discovery of an appropriate chemical probe that can be used for a confident interrogation and further understanding of USP2 biology and disease.

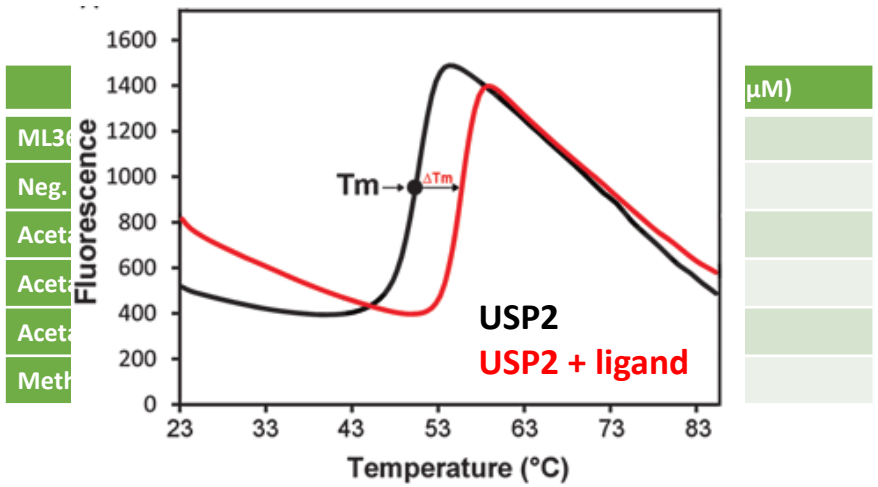
Exit vector investigation



Docking calculations predicted two preferential binding modes at the PPI surface with Ub.

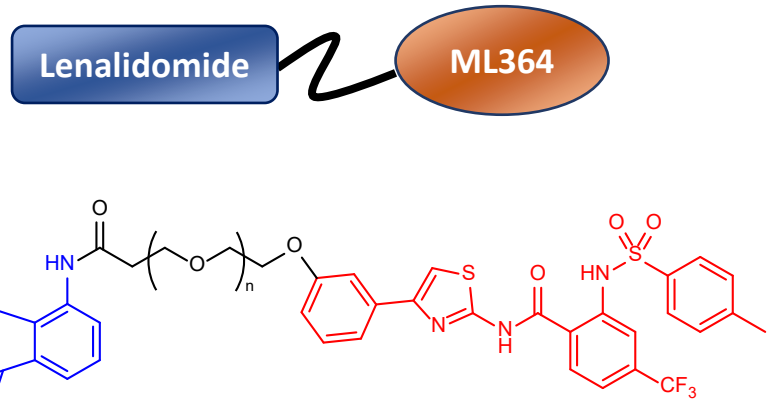


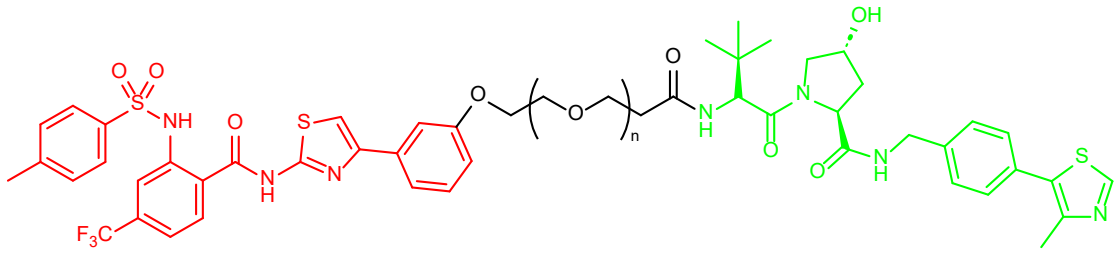
Differential Scanning Fluorimetry (DSF)



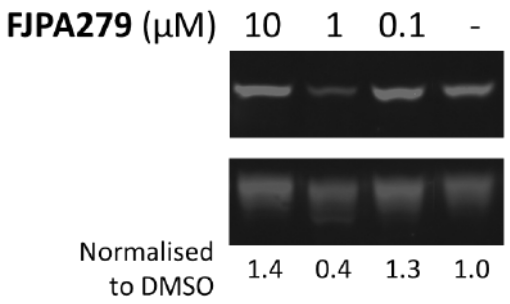
USP2 degradation

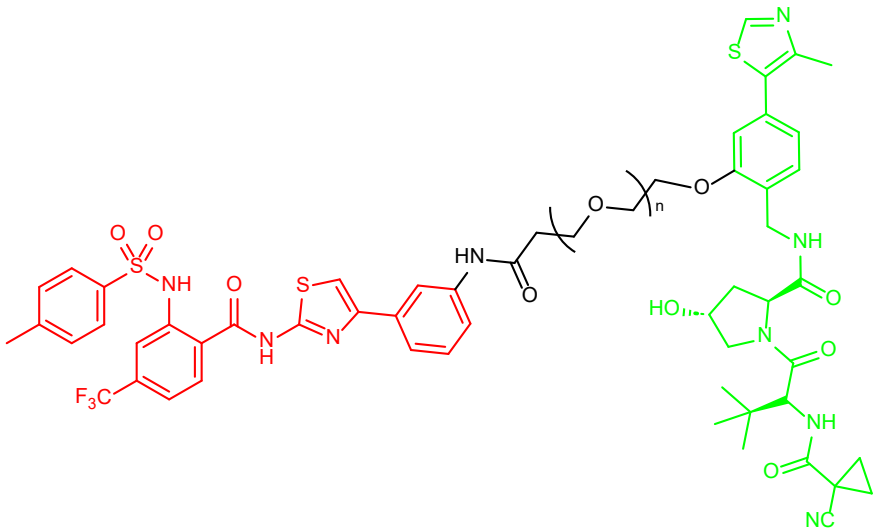
Heterobifunctional degrader candidates





Degradation assays





FJPA279, a VH032-based compound, was identified as a partial degrader of USP2 at 1 μM, after 24 h treatment in MCF-7 cells.

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