



Direct-to-Biology Platforms to Accelerate Drug Discovery

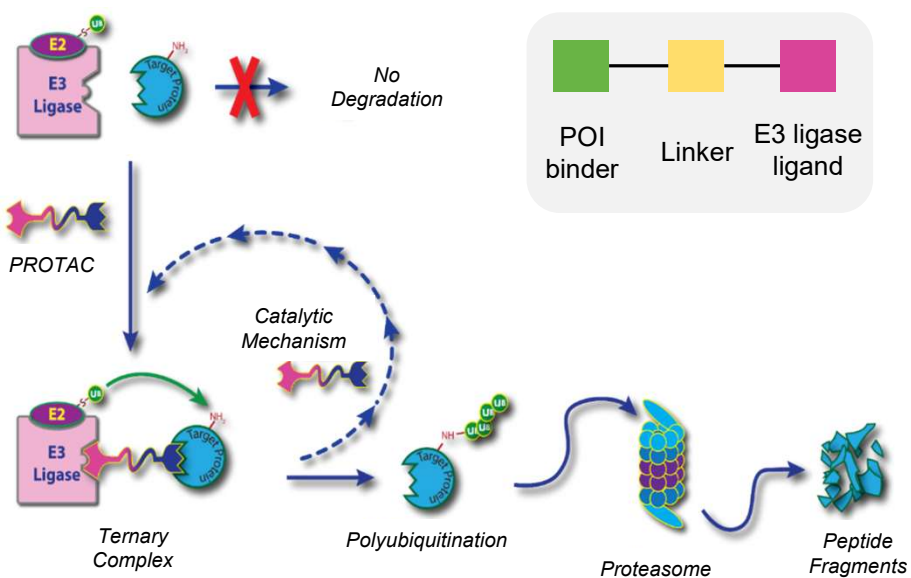
EFMC-YSN Lunch and Learn – 14th October 2025

Agenda



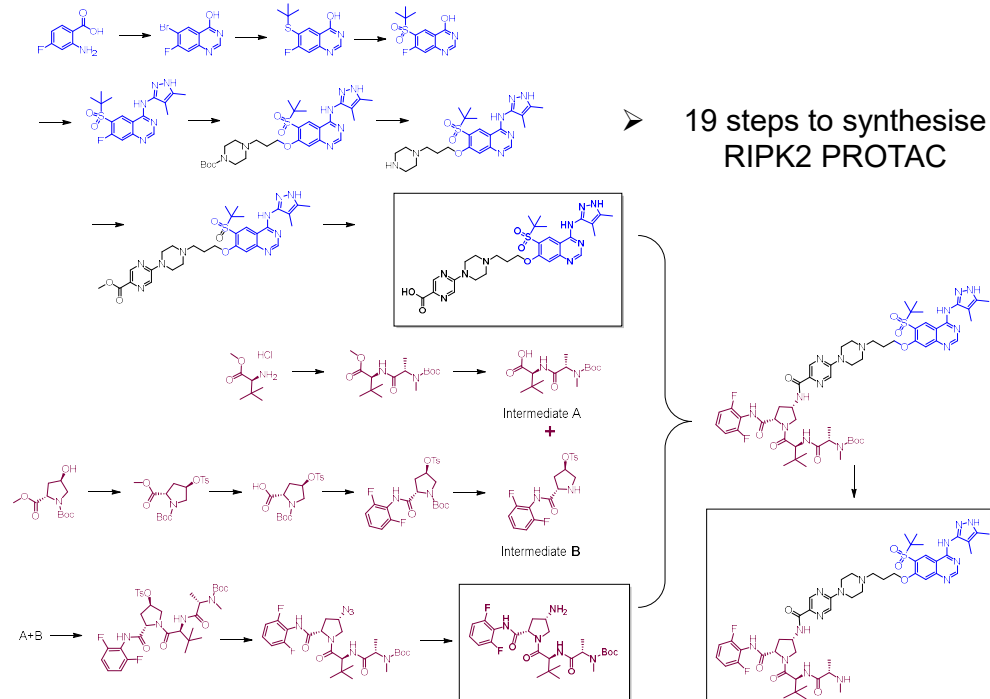
1. Introduction to PROTAC discovery
2. What is Direct-to-Biology (D2B)?
3. Use of D2B in PROTAC discovery
4. Experimental setup and validation process for new transformations
5. Literature examples
6. PhD project
7. Case studies from Andreas and Cassie
8. Q&A

PROTAC Discovery

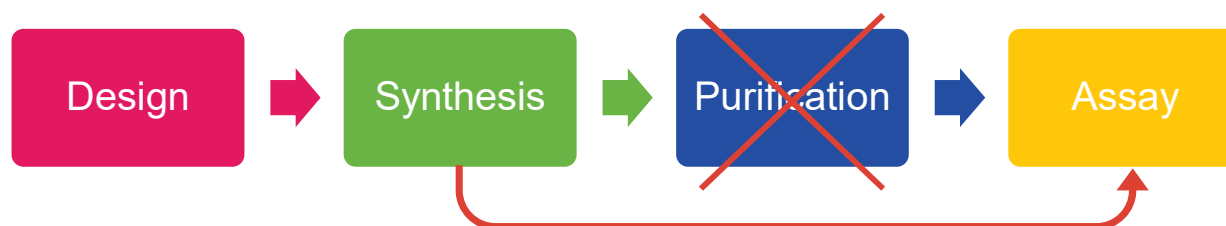


- Degradation potency is linked to ternary complex formation rather than binding affinity, thus structure-activity relationships (SAR) are challenging to predict, and significant trial-and-error is required to identify potent new degraders.

PROTAC = proteolysis targeting chimera
POI = protein-of-interest



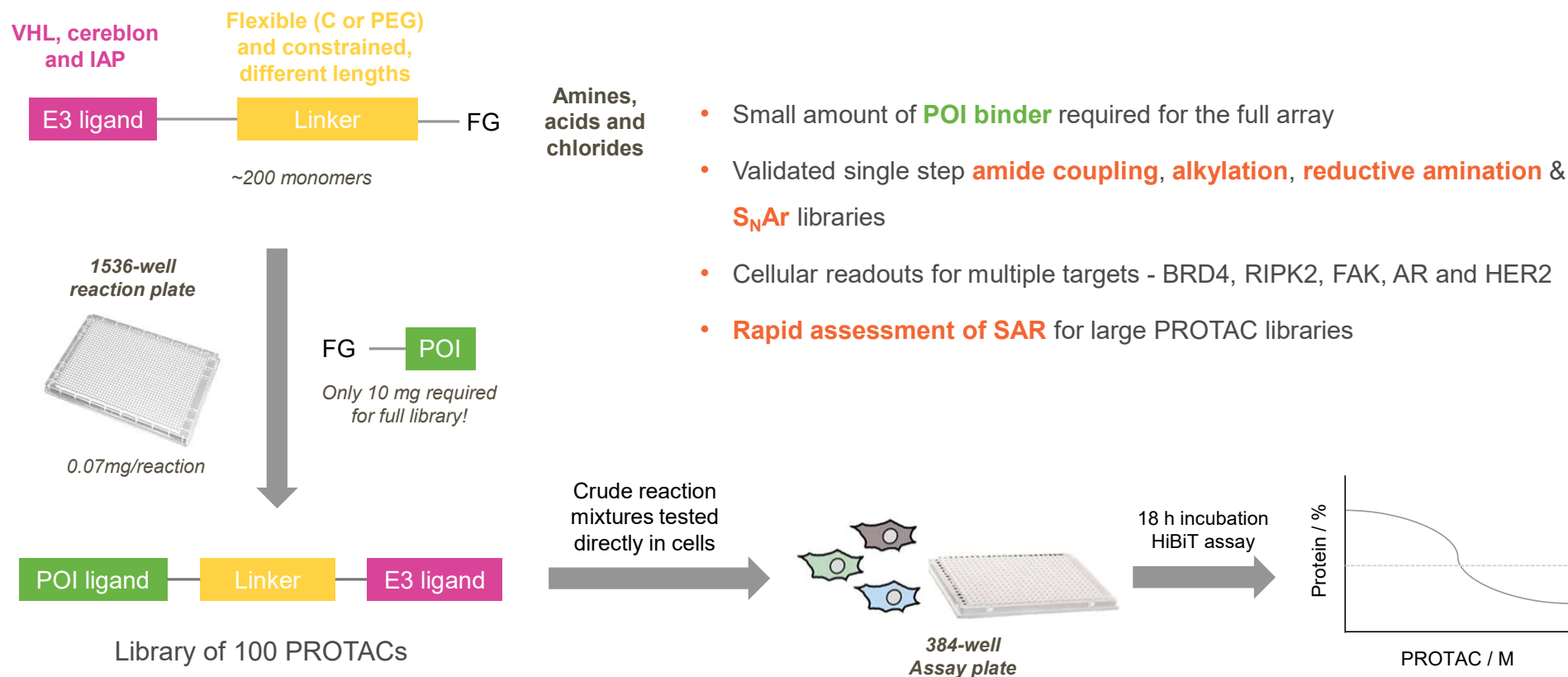
Introduction to Direct-to-Biology



Crucial Considerations

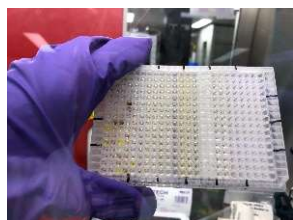
- How are your reagents affecting the assay readout?
- Is the data generated robust and comparable to purified samples?
- Is plate-based chemistry representative of batch scale?

PROTAC Direct to Biology (D2B)



100s of PROTACs with cellular degradation data within weeks

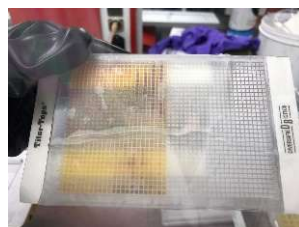
What does Direct-to-Biology look like in practice?



Prepare stock solutions and dispense onto 384 well plate



Mosquito liquid handling robot transfers reagents into correct wells on reaction plate



Reactions left for 24 h at room temperature in 1536 well plate



LCMS analysis gives % purity of each product



Products plated into columns 1 and 13 of a 384-well plate for serial dilutions

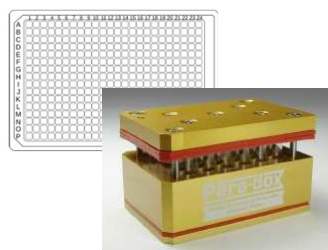
Alternatives for an academic setting might include:



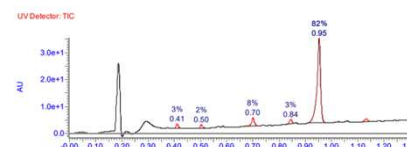
Dispensing from vials



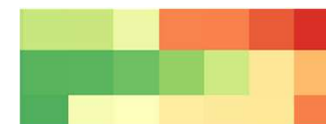
Multichannel pipettes or other liquid handling equipment



Different plate sizes or parallel chemistry setups



Option to assume full conversion after optimising reaction conditions

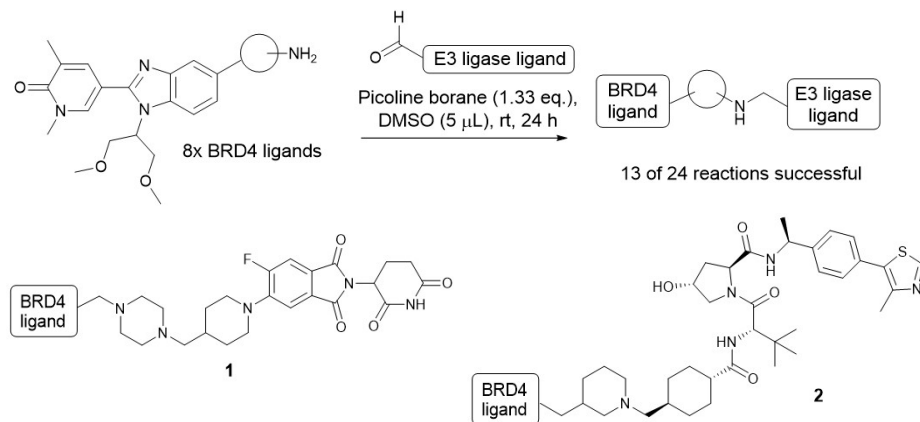


Option to run fewer points on concentration curve or single concentration assay

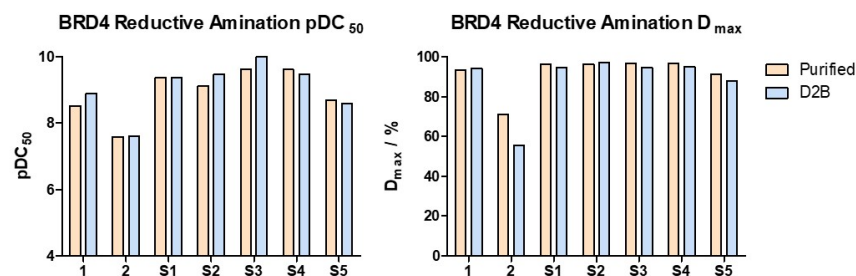
Validation of New Reaction Conditions

Reductive Amination

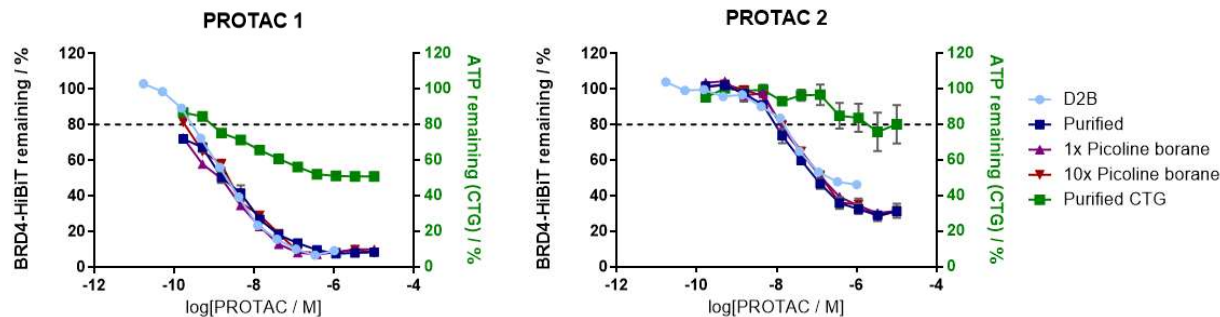
1. Condition optimisation



2. Correlation with purified samples



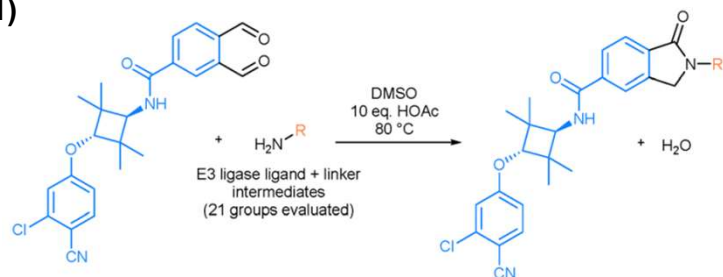
3. Additional controls - reagent spiking and cell viability assessment



Literature Examples

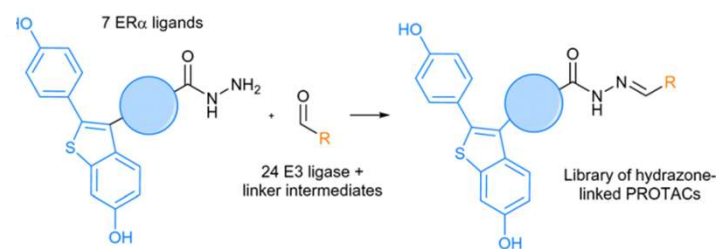
New reaction development: Release of water as the only by-product

1)



Guo et al., *Eur. J. Med. Chem.*, **2022**, 236, 114317.

2)



Roberts et al., *ACS Chem. Biol.*, **2020**, 15, 1487-1496.

Novel applications - assay design: Counter-screening to identify glues that are dependent on cereblon binding

3)

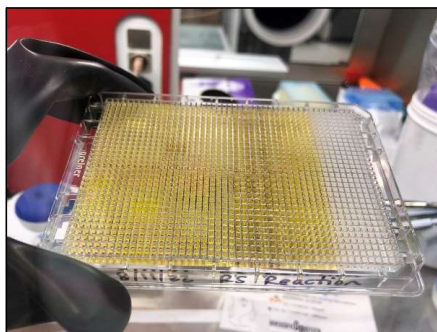
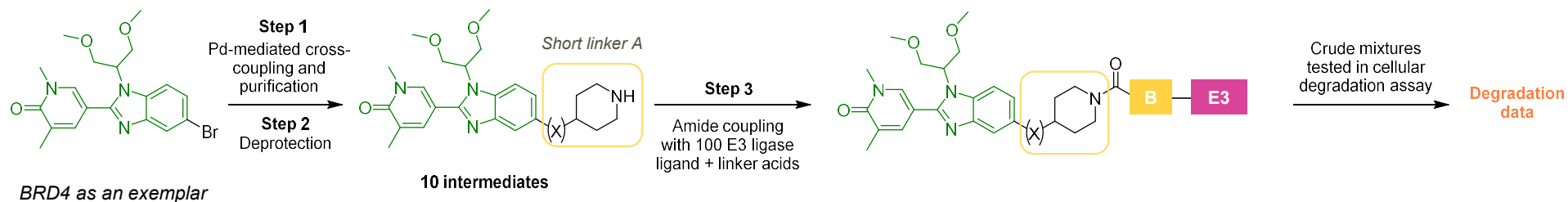
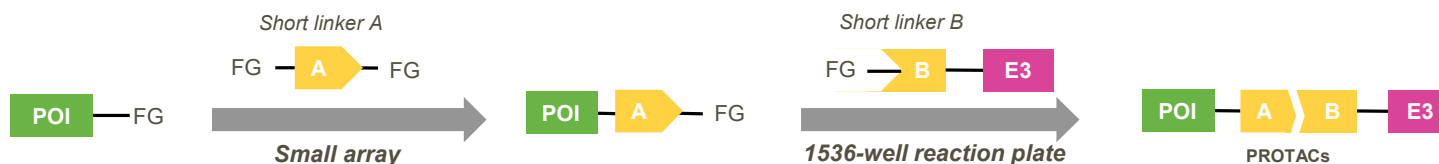


Li et al., *Eur. J. Med. Chem.*, **2023**, 258, 115567.

For a comprehensive overview, see tutorial review: *Innovative, Combinatorial and High-Throughput Approaches to Degradar Synthesis*, R. Stevens et al., *Chem. Soc. Rev.*, **2024**, 53, 4838-4861.

Multistep D2B to generate large PROTAC libraries

1. Pd-mediated sp^2 - sp^3 cross-coupling; 2. Deprotection; 3. Amide coupling



- Synthesised **900/1131** PROTACs in one experiment (success rate > 80%)
- Allows SAR to be quickly assessed – synthesis and testing completed by one scientist in **3 weeks**

3-Step D2B Workflow Developed to Assess SAR

Profiling 900 BRD4 PROTACs in a single experiment

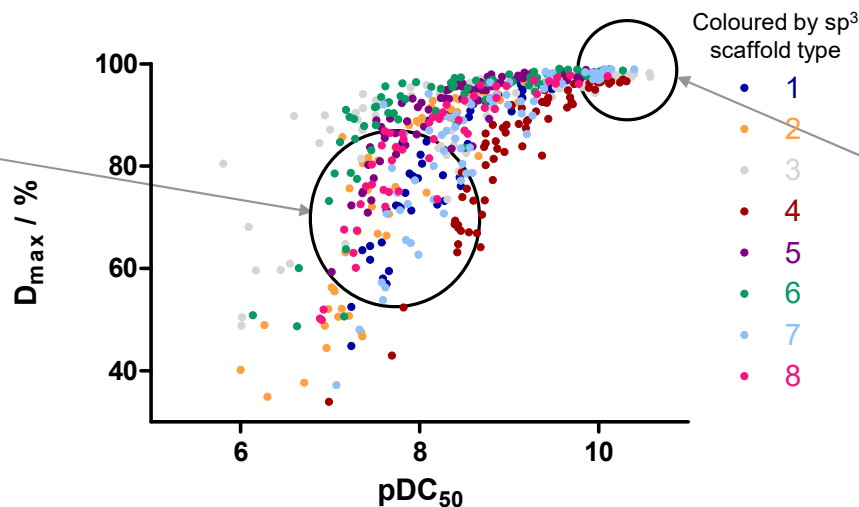
Examples with VHL:

PROTAC 5

Long flexible linker
pDC₅₀ 7.4 D_{max} 81 %

PROTAC 6

Short flexible linker
pDC₅₀ 7.3 D_{max} 90 %



Examples with CRBN:

PROTAC 7

15-atom flexible linker
pDC₅₀ 10.2 D_{max} 98 %

PROTAC 8

4-atom semi-constrained linker
pDC₅₀ 10.5 D_{max} 98 %

Example with VHL:

PROTAC 9

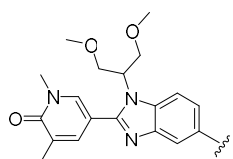
12-atom semi-constrained linker
pDC₅₀ 10.3 D_{max} 97 %

POI ligand

Linker

Linker

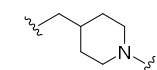
E3 ligand



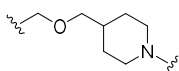
BRD4 binder

sp³ linkage e.g.

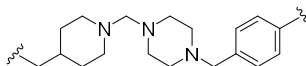
Scaffold 3



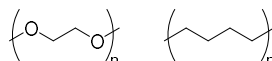
Scaffold 4



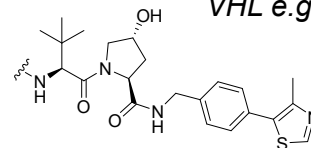
Semi-constrained e.g.



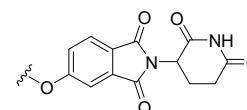
Flexible e.g.



VHL e.g.



Cereblon e.g.



Overall trends observed:

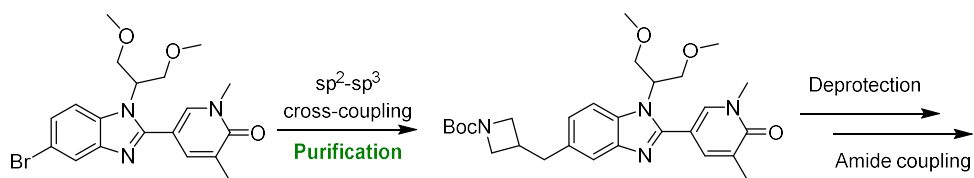
Cereblon typically more potent

12-15 atoms = optimum linker

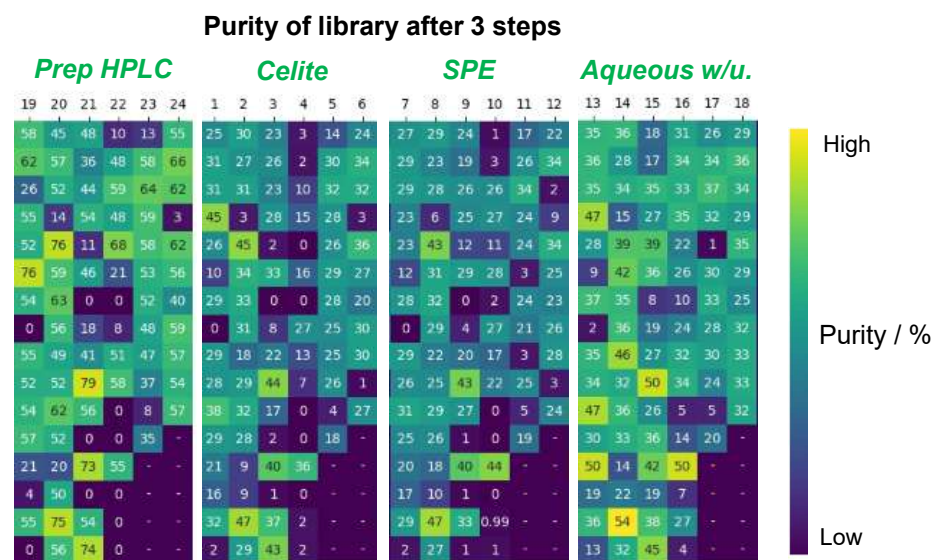
Choice of sp³ linkage is important

Three-step D2B with Pd-mediated cross-coupling

Strong correlation with purified samples after 3 steps with only one cartridge purification



Different purification methods trialled following step 1



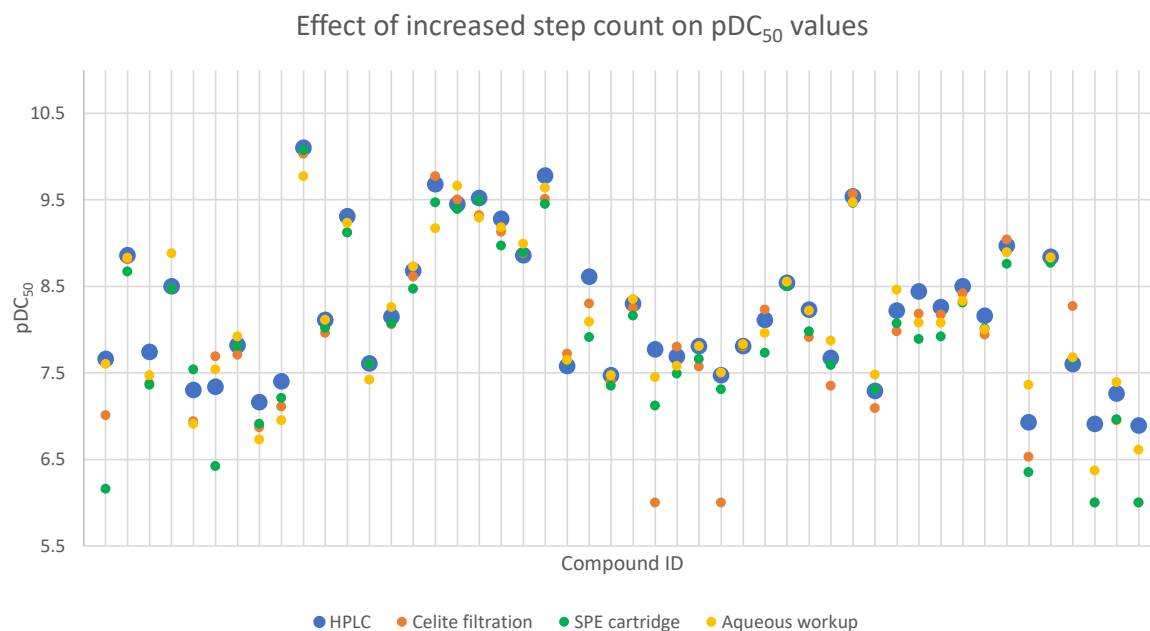
PROTAC purity typically between 20 and 40% after 3 steps

SPE = solid-phase extraction

Stevens et al., RSC Med. Chem., 2025, 16, 1141-1150.

How much purity do you really need?

pDC₅₀ values following multistep D2B chemistry - PROTAC purities of 20-40%



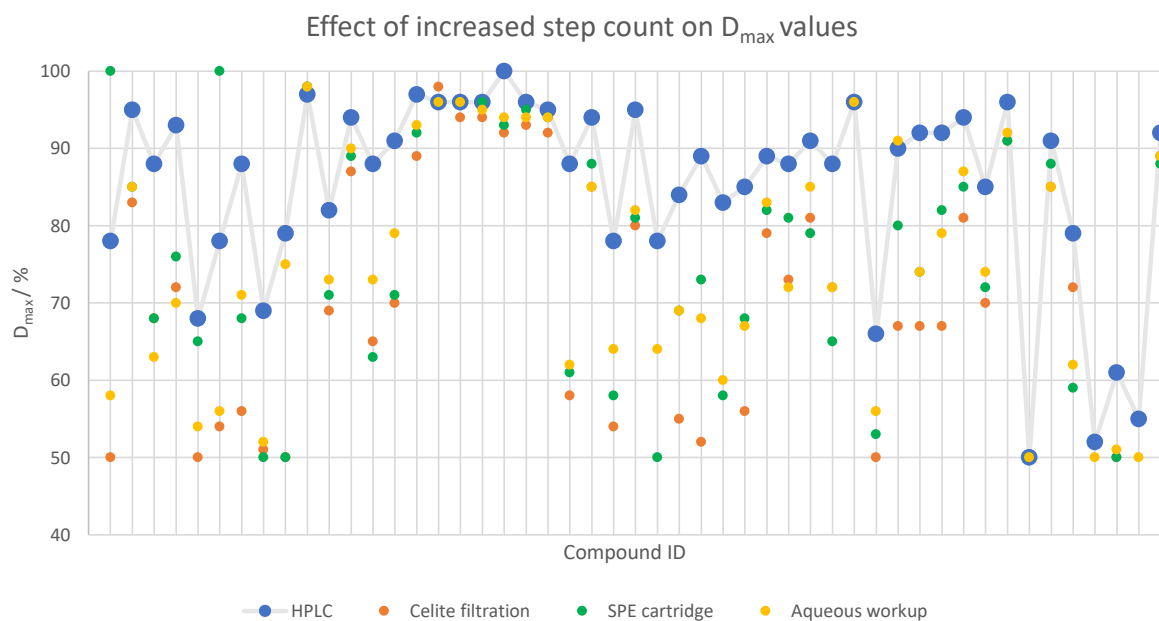
Of 49 samples, 94% of pDC₅₀ values lie within 1 log unit of the HPLC batch and 84% within half a log unit

Stevens et al., RSC Med. Chem., 2025, 16, 1141-1150.

For other experiments assessing purity requirements in PROTAC D2B see: Plesniak et al., ACS Med. Chem. Lett., 2023, 14, 1882-1890.

How much purity do you really need?

$D_{\max}$ values following multistep D2B chemistry - PROTAC purities of 20-40%



$D_{\max}$ is affected much more significantly than pDC50 when sample purity is low

Stevens et al., RSC Med. Chem., 2025, 16, 1141-1150.

For other experiments assessing purity requirements in PROTAC D2B see: Plesniak et al., ACS Med. Chem. Lett., 2023, 14, 1882-1890.

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EFMC
 Young
 Scientists
 Network



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Bicycle



Application of Multistep D2B to Discover a CNS-Active GSK3 Degradar

Andreas Holmqvist

PhD researcher, Centre for Targeted Protein Degradation, Dundee



Impact of D2B in R&D at AstraZeneca: An Industry Perspective

Cassie Pratley

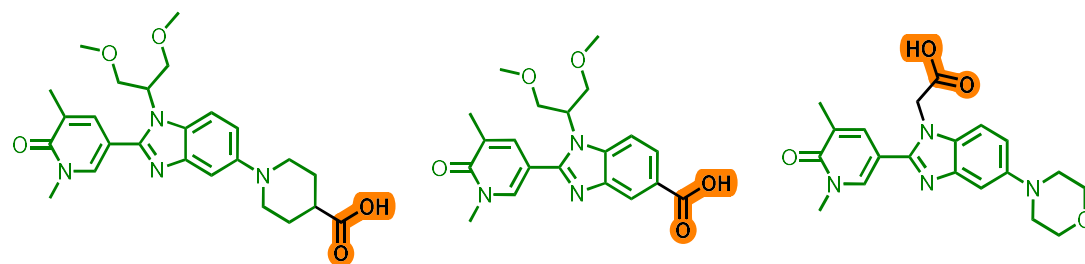
Senior Scientist, AstraZeneca



Thank you for listening!

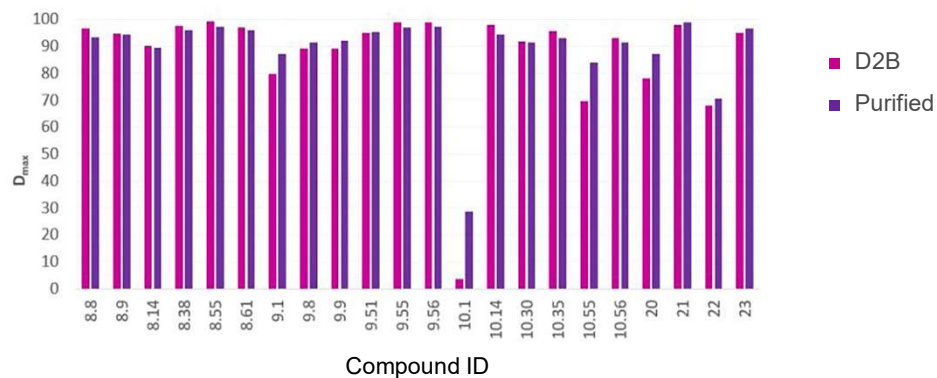
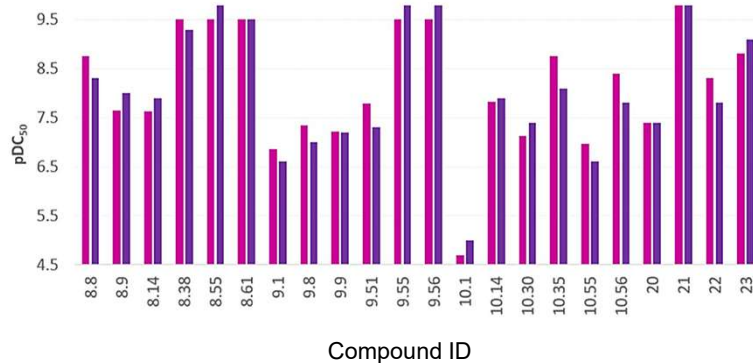
Any questions?

PROTAC Direct to Biology (D2B)



BRD4 ligands – 3 linking vectors

Excellent correlation across a range of potencies



How much purity do you really need?

- Work from AstraZeneca models partial conversion of starting material to product by titrating pure PROTAC with the respective starting materials
- Not a true model of partial conversion but still shows overall impact
- 50-100% purity gives pDC50 values within approximately half a log unit
- 10% purity can give an indication of degradation, but $D_{\max}$ values are inaccurate
- Important to note that every target may be different

