# Multistep HTC-D2B PROTAC Synthesis Enabled by Late-Stage $sp^2$-$sp^3$ Cross-Coupling Chemistry

Rebecca Stevens[1,2], Afjal Miah[1], Glenn Burley[2]

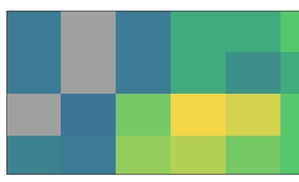
1) GSK, Medicines Research Centre, Stevenage, UK
2) Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK

**Develop late-stage C($sp^2$)-C($sp^3$) cross coupling conditions** → **Apply conditions to generate PROTAC intermediates** → **Synthesise diverse PROTAC library using D2B platform** → **Identify novel PROTAC hits**

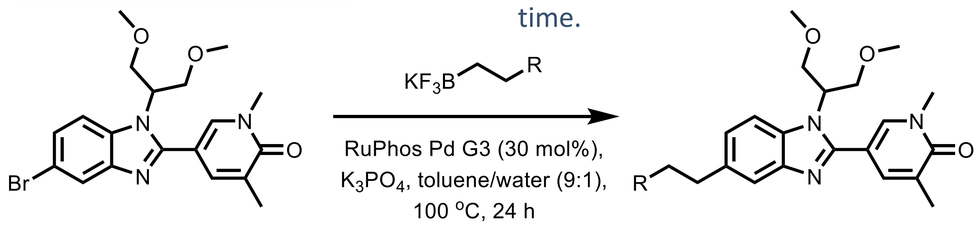
## AIMS

- Identify C($sp^2$)-($sp^3$) cross-coupling conditions suitable for use in a multistep High-Throughput-Chemistry Direct-to-Biology (HTC-D2B) workflow to expand the GSK PROTAC platform capabilities.
- Utilise new conditions to synthesise diverse libraries of PROTACs, exploring wider chemical space and physchem properties.

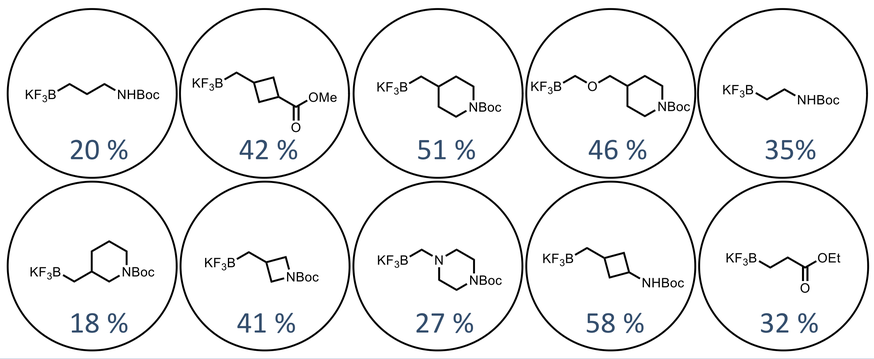
## LATE-STAGE FUNCTIONALISATION

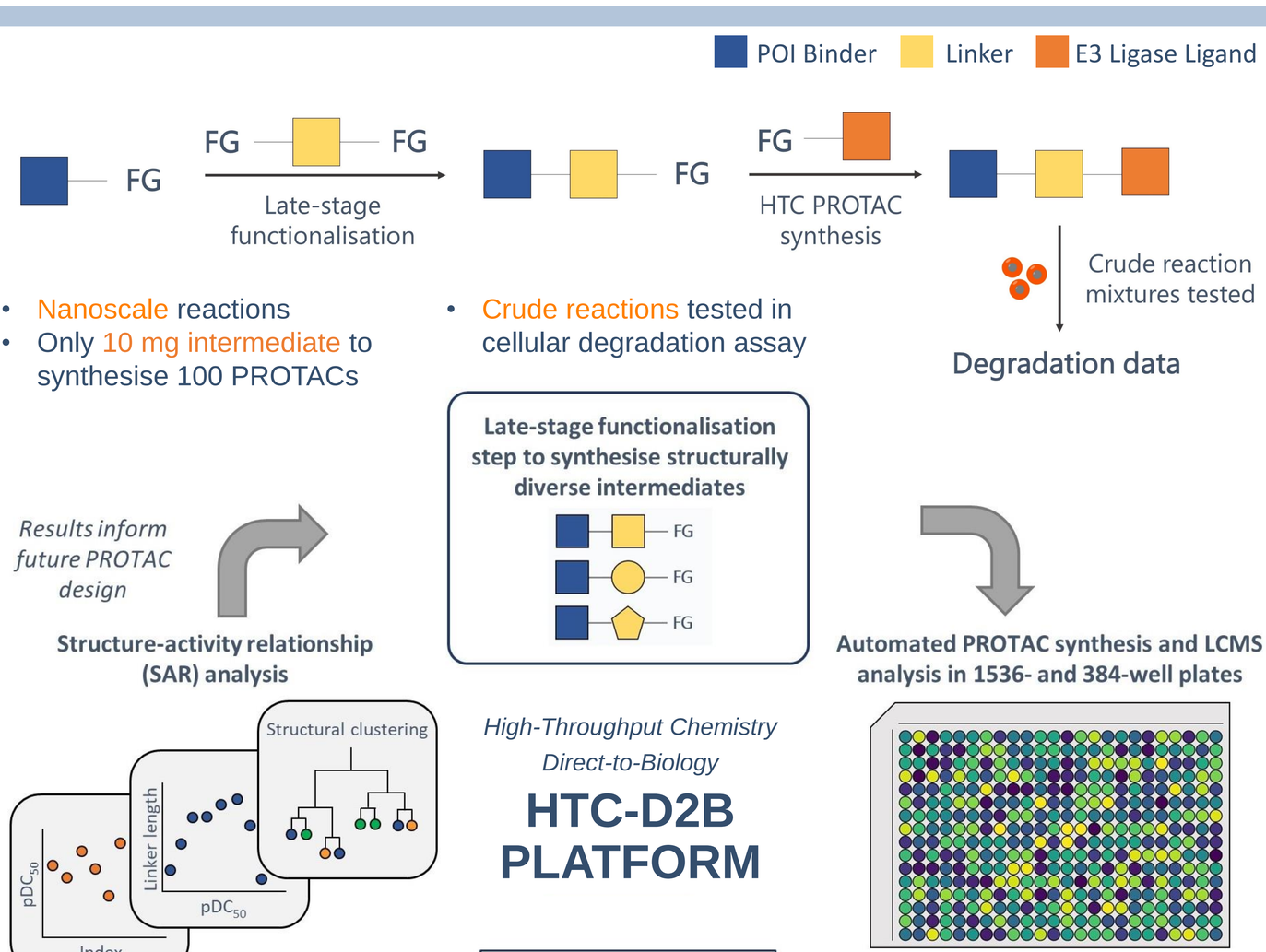



- High-throughput experimentation aided identification of suitable conditions.
- Screened catalysts and % loading, bases, solvents, temperature, reaction time.




- Substrate scope investigated in plate format - conditions suitable for 3 different fully-functionalised POI binders and a wide range of linkers.
- Reactions scaled up with BRD4 binder in moderate to good yields:



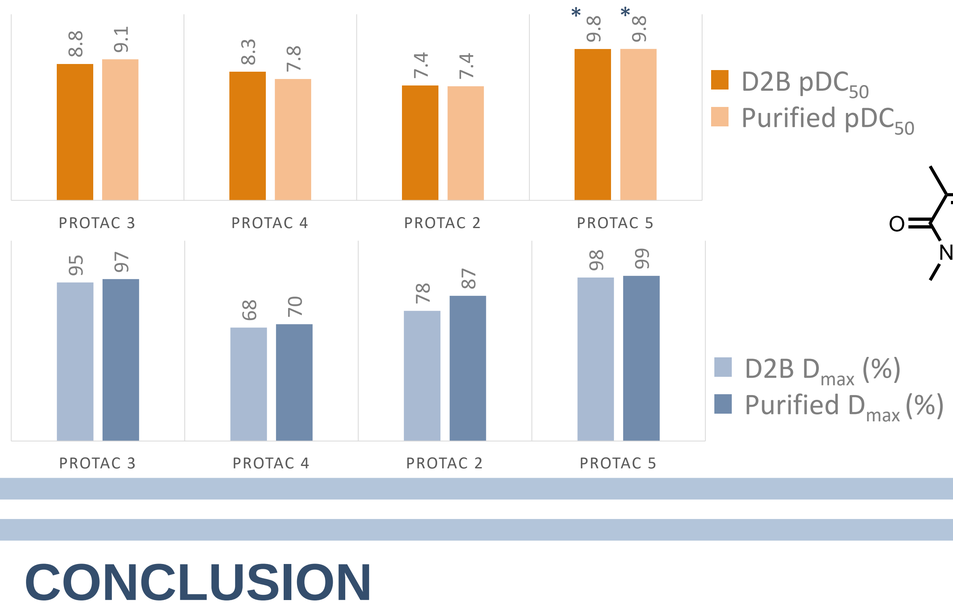



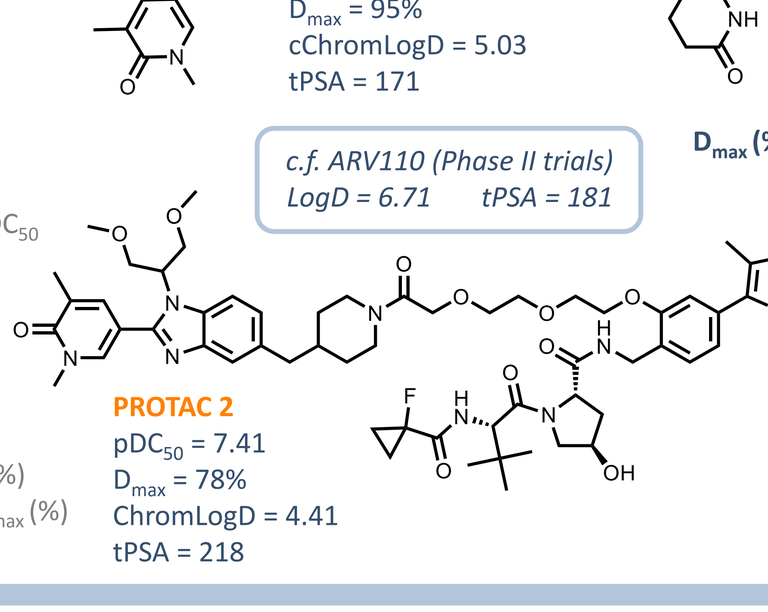

- Nanoscale reactions
- Only 10 mg intermediate to synthesise 100 PROTACs
- Crude reactions tested in cellular degradation assay

- 700 reactions carried out with chemistry success rate of ~70%, to synthesise 500 PROTACs in one month - assay to be run in the next month. Platform fully validated for a first set of 74 PROTACs, biological data below.

## IDENTIFICATION OF NOVEL BRD4 HITS

- First set of 74 PROTACs identified many highly potent compounds, several with desirable phychem properties for oral bioavailability.
- Identified novel hits for BRD4, employing both cereblon and VHL.
- 4 hits resynthesised and purified as controls – excellent correlation between D2B and purified samples.



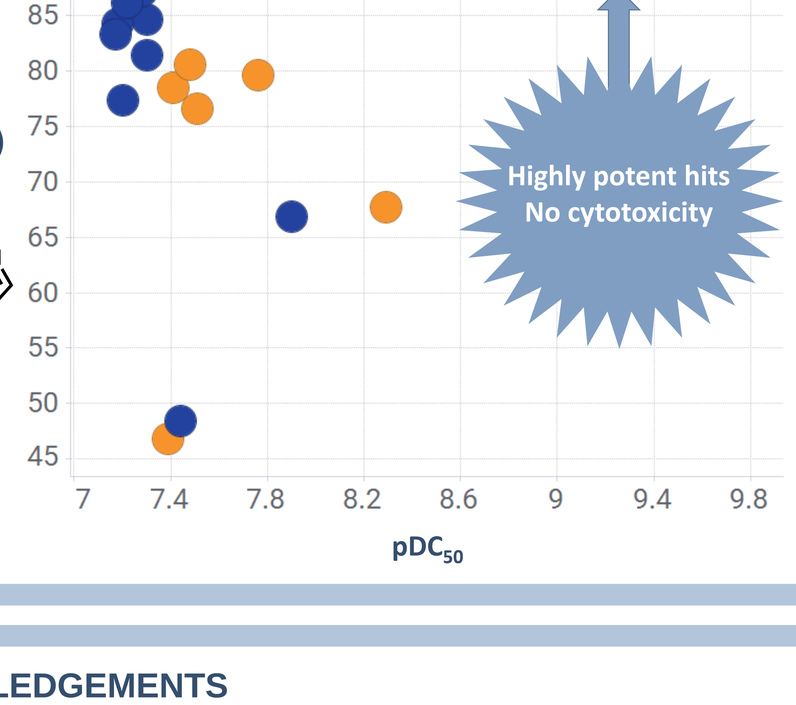
**PROTAC 1**
$pDC_{50}$ > 9.77*
$D_{max}$ = 95%
cChromLogD = 5.03
tPSA = 171

*c.f. ARV110 (Phase II trials)*
*LogD = 6.71 tPSA = 181*



**PROTAC 2**
$pDC_{50}$ = 7.41
$D_{max}$ = 78%
ChromLogD = 4.41
tPSA = 218

*$pDC_{50}$ of 9.77 = limit of assay (rounded to 1 dp in bar chart); colours represent two series of intermediates; n=1 for all assay data presented.




## CONCLUSION

- High-throughput experimentation enabled chemistry optimisation of a late-stage C($sp^2$)-C($sp^3$) coupling.
- 500 PROTACs have been synthesised in one month, with 74 novel BRD4 PROTACs already tested in HiBiT assay.
- D2B identified many highly potent hits, with purified compounds showing close correlation and no cytotoxicity.

## ACKNOWLEDGEMENTS

Thanks to Diana Klimaszewska, Justyna Macina and Peter Stacey from Protein Degradation for running the first set of biological assays, and for their training in the relevant biology for future assays.

*Presented at RSC BMCS 3rd Synthesis in Drug Discovery and Development – May 2023*