Accelerated Direct-to-Biology Facilitated by Plate-Based Reaction Optimisation and a Partial PROTAC® Library

Domainex
Enrich your Medicines Pipeline

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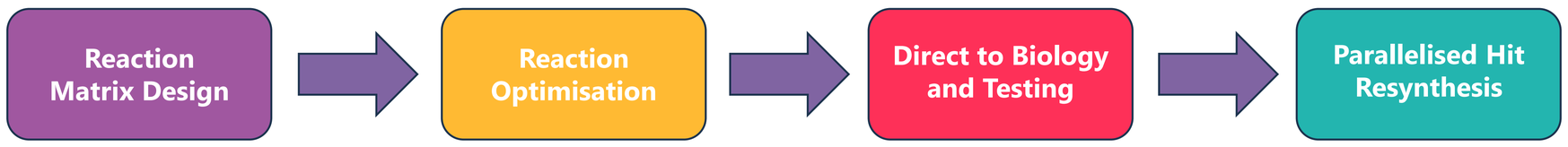
Introduction to Accelerated Synthesis Workflow

What is Direct-to-Biology? Direct-to-Biology, or D2B, is the nano scale synthesis of hundreds of fully elaborated compounds and subsequent testing of unpurified reaction mixtures in a biochemical or cell-based assay.

- Biological assay extensively tested to ensure reproducibility between D2B and purified samples
- Top-performing compounds resynthesised and purified in parallel to validate observed biological readout
- Facilitates the synthesis of **Pro**teolysis **ta**rgeting **c**himeras (**PROTACs®,** PROTAC® is a registered trademark of Arvinas Operations, Inc., and is used under license), which are typically more difficult to synthesise using traditional round-bottomed-flask chemistry

Accelerated Synthesis Workflow:

Our standardised workflow allows for highly successful SAR generation with a rapid turnaround time



Reaction Optimisation

At Domainex, reaction optimisations are conducted in plate format, with the advantage that:

- Up to 96 reactions can be conducted in parallel, with a total turnaround time of 3 days
- Less than 3 mg of starting material is used per well
- Standardised procedure ensures data is reliable
- Green procedure, using 100 µL solvent per reaction

Plates are designed using all available literature and in-house specialist knowledge, maximising the likelihood of finding the ideal set of reaction conditions

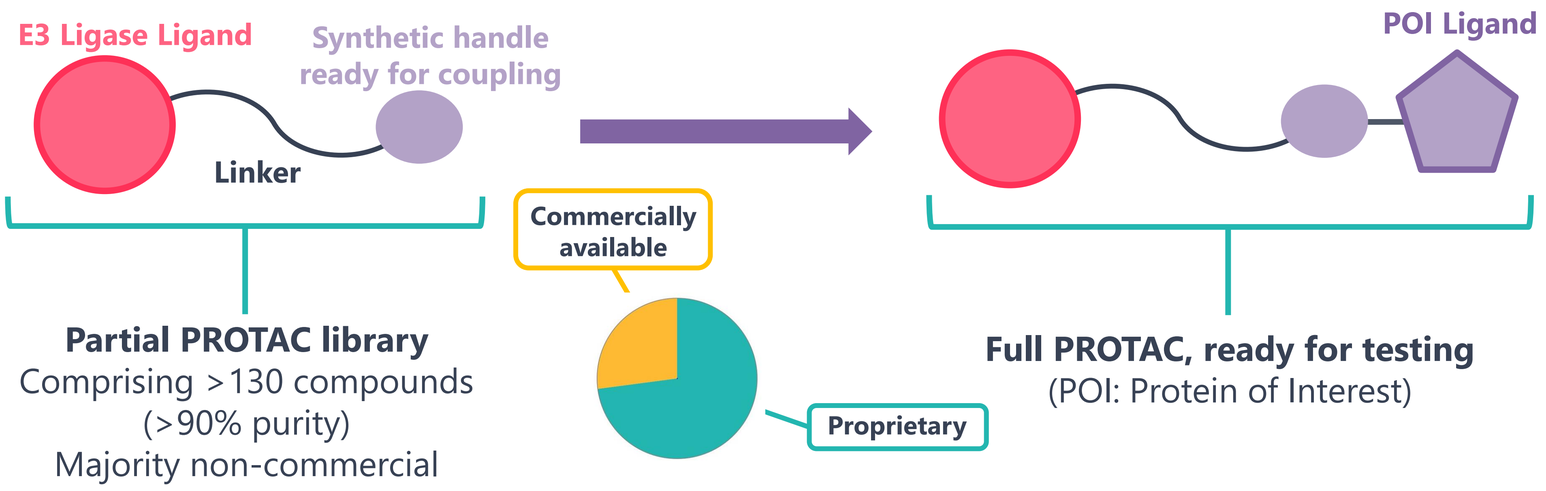
Direct-to-Biology

Using state-of-the-art equipment, up to 384 reactions can be setup in less than 1 h, dramatically increasing the productivity of the bench chemist.

- Highly efficient way-of-working: ~0.075 mg (250 nmol) of starting material used per reaction
- Standardised, automated reaction setup reduces the chance of random errors
- Optimised HPLC method allows full plate QC to be run in less than 16 h

Direct to Biology
Galaxy of opportunity

Partial PROTACs Platform



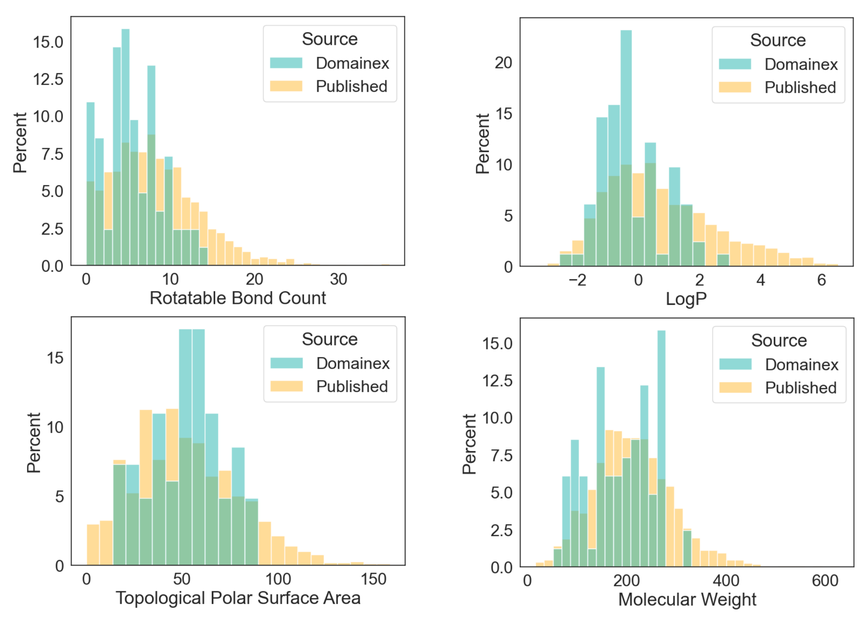
Partial PROTAC library
Comprising >130 compounds
(>90% purity)
Majority non-commercial

Full PROTAC, ready for testing
(POI: Protein of Interest)

The Domainex partial PROTAC toolbox was built for physicochemical diversity and reduced CRBN neo-substrate degradation in mind, and features a wide variety of:

- Linker lengths and types
- Flexible, semi-flexible and rigid linkers
- Different exit vectors (composition, position…)
- CRBN (IMiDs and non-IMiDs based) and VHL ligands

Property Comparison to Published Linkers



Case Study: Synthesis of Aurora A Degraders

Target: Aurora Kinase (overexpressed in human tumors)

- **Positive control:** JB170
  - Potent and highly specific PROTAC-mediated Aurora-A (Aurora Kinase) degrader (DC_{50}=28 nM) by linking Alisertib, to the CRBN-binding molecule Thalidomide
- **Chemistry:** Amide coupling of 40 representative partial PROTACs to Alisertib (POI ligand) in plate-based chemistry

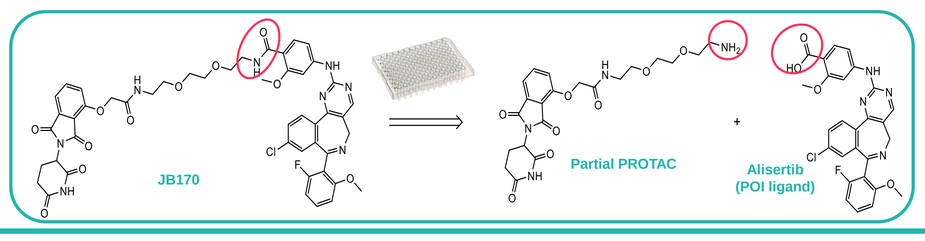
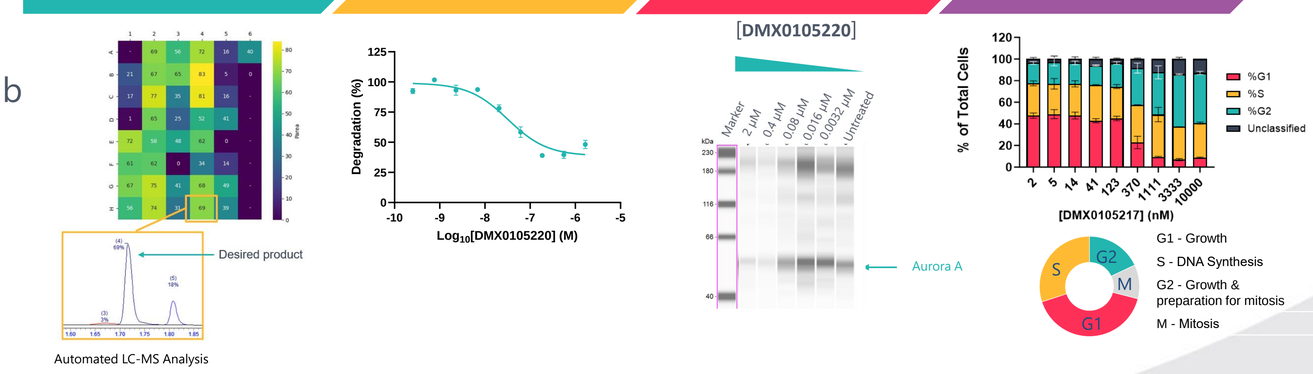


Plate based chemistry using proprietary partial PROTAC toolbox → HiBit Screen for degradation → Orthogonal JESS assay for Aurora A levels → Flow cytometry assay for cell cycle disruption



Conclusions

- State-of-the-art accelerated workflow for medicinal chemistry, using a synergistic combination of high throughput reaction optimisation, ready-to-couple partial PROTACs library and D2B workflow now validated on in-house project, with new degraders identified by D2B
- Now applied across medicinal chemistry portfolio for our clients

Domainex welcomes interest from any potential collaborators, industrial or academic. If you would like to learn more about our drug-discovery platform, please contact: **enquiries@domainex.co.uk**

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