Tailored Fragment Screening Strategies for Diverse Fragments

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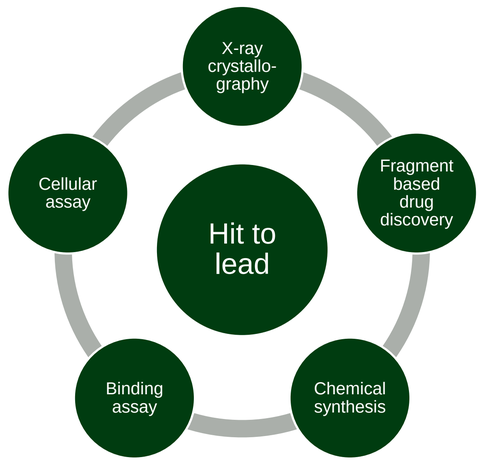
Abstract

Fragment-based drug discovery (FBDD) identifies low MW compounds that bind weakly to druggable protein targets. The key hit-finding techniques are surface plasmon resonance (SPR) and nuclear magnetic resonance (NMR). In contrast, covalent binders (with electrophilic warheads) are most found using MS combining intact mass by RPLC-MS (overall distribution of bound ligands), and peptide mapping (site distribution/specificity).

Efficient screening processes rapidly generate hits, that are confirmed by more detailed measurements and orthogonal techniques. Examples of each are presented

Non covalent Fragment screening

Protein production → Feasibility → Screening → Hits → Hit confirmation



Fragment Library

External or internal library

Example: NovaFrag library

- 1200 cpds
- Fully qualified library
- Good coverage of chemical space
- Significant 3D/sp3
- Synthetically tractable
- Rapid follow-up by SAR by catalog
- IP free

Screening Capacity

SPR Cytiva Biacore 8K:

- 1 week screen: singletons at 1 conc
- 1 week screen: hits at 3 concs
- 1 week dose-response: K_D

NMR Bruker Ultra High-field 600 MHz –1 GHz:

- Cocktails of 5 cpds:
- Ligand observed
- NMR experiments: STD, WaterLOGSY, T2/T1rho
- Fully automated: SampleJet, TECAN robot
- 2 weeks for set-up
- Min 4 weeks for screening

Target Carboxylases

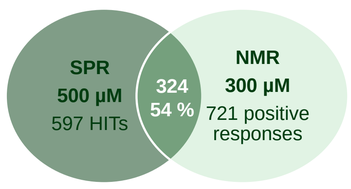
- Parallel LO-NMR (cocktails) & SPR screen
- Client library of 2000 fragments
- 700+ NMR detected fragment
- Hit rate ~35 % (unusual, high)
- 20 common HITs between SPR and NMR

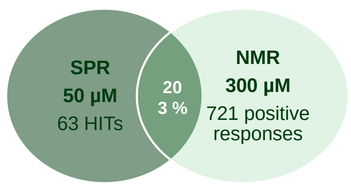
Target Acetyltransferase

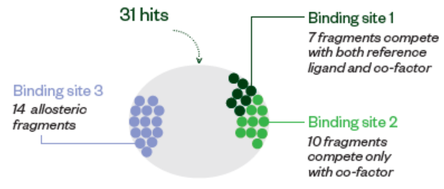
- Parallel LO-NMR & SPR screen
- 1250 NovaFrag library
- 31 common detected fragment HITs
- Two successive competition assays by NMR
- Kinetic profiling of selected hits by SPR
- 3 categories defined

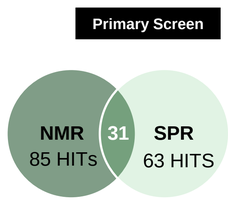
Target Phosphatase

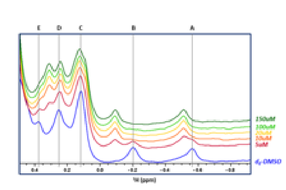
- 1D ^{1}H-NMR, Protein Observed
- Mixture of 12 Fragments
- Hits validated with ^{13}C-CYS-labeled protein











Covalent Fragment screening

LC-MS: Intact mass

Feasibility	Pre-Screening	Hit validation/counter-screen	Binding site mapping	Titration of confirmed hits
1 week	1 -3 weeks	1 week	2 weeks	1 week

Incubation optimization

- Target residue accessibility
- Warhead reactivity (chloractamides, acrylamides etc.)
- Protein stability

PL1 Binding (%)

	15min			1h			5h			24h		
	ACR	Iac	ClAc	ACR	Iac	ClAc	ACR	Iac	ClAc	ACR	Iac	ClAc
5µM - 10 eq	0.0%	39.0%	0.0%	0.0%	78.6%	0.0%	0.0%	98.2%	2.6%	0.0%	100.0%	16.2%
5µM - 50 eq	0.0%	84.8%	0.0%	0.0%	97.3%	0.0%	1.5%	N/A	20.2%	43.2%	100.0%	66.1%
5µM - 100 eq	0.0%	93.6%	0.0%	0.0%	100.0%	1.4%	24.3%	N/A	47.7%	78.2%	100.0%	89.0%

PLn Binding (%)

	15min			1h			5h			24h		
	ACR	Iac	ClAc	ACR	Iac	ClAc	ACR	Iac	ClAc	ACR	Iac	ClAc
5µM - 10 eq	0.0%	39.0%	0.0%	0.0%	84.0%	0.0%	0.0%	99.2%	2.6%	0.0%	100.0%	16.2%
5µM - 50 eq	0.0%	89.4%	0.0%	0.0%	99.0%	0.0%	1.5%	N/A	20.2%	43.2%	100.0%	66.1%
5µM - 100 eq	0.0%	97.0%	0.0%	0.0%	100.0%	1.4%	24.3%	N/A	47.7%	78.2%	100.0%	89.0%

Average number of ligand covalently bound

	15min			1h			5h			24h		
	ACR	Iac	ClAc	ACR	Iac	ClAc	ACR	Iac	ClAc	ACR	Iac	ClAc
5µM - 10 eq	0.0	0.3	0.0	0.0	0.3	0.0	0.0	0.4	0.0	0.1	0.3	0.2
5µM - 50 eq	0.0	0.9	0.0	0.0	1.4	0.0	0.1	1.5	0.1	0.4	1.3	0.6
5µM - 100 eq	0.0	1.6	0.0	0.0	2.2	0.1	0.2	2.3	0.4	0.6	2.0	0.8

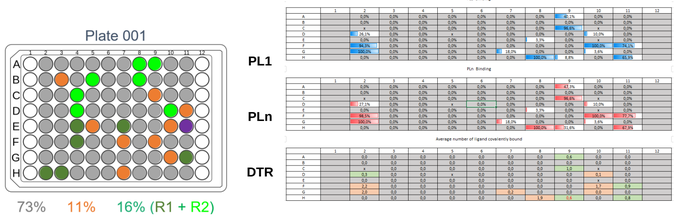
$$PL1\ binding\ (\%) = \frac{I\ PL1}{\sum I\ (P+PLn)}$$

PL1: *Protein + 1 ligand*
PLn: *Protein + n ligands*
DTR: *Drug targeted Ratio*

$$PLn\ binding\ (\%) = \frac{\sum I\ (PLn)}{\sum I\ (P+PLn)} \qquad DTR = \frac{\sum I\ (PLn \times n)}{\sum I\ (PLn)}$$

Throughput ~250 cpds/day

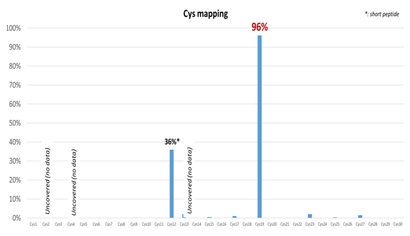
- Client or commercial library
- Automated data processing: UniDec + KNIME
- Ranking of compounds
- Counterscreen on mutated or subdomain of the protein



- Grey : (PL1 = PLn = DTR = 0) and Orange : (PLn ≤ 15 %, DTR < 0.3) or (PLn = 100 %, DTR > 2) ➔ **discarded**
- **R1** (Rank 1) : (PL1 ~ PLn > 50 % and 0.7 ≤ DTR ≤ 1.5) ➔ **Rank 1 cpds**
- **R2** (Rank 2) : (PL1 ~ PLn > 25 % and 0.3 ≤ DTR ≤ 0.6) ➔ **Rank 2 cpds**

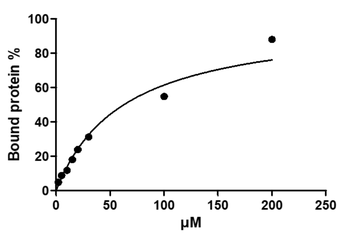
Binding site identification

- Bottom-up approach
- Quantification
- Determination of site specificity



k_{inact}/Ki

- MedChem follow-up



Summary

The examples illustrate efficient fragment screening workflows based on several biophysical techniques.
Non-covalent fragment hits were found by screening a chemically diverse library. SPR and NMR each generate some unique hits but may also be used as orthogonal means of hit confirmation prior to SAR enablement by X-ray crystallography and hit -to- lead programs.
Covalent fragment screening by MS has been applied to multiple targets with a wide variety of warheads leading to the identification and characterization of specific and potent covalent binders.

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