CASE STUDY

MatchMaker Proteome Screening identified more drug–target interactions than CETSA® MS, KiNativ™ or affinity purification

MatchMaker compared with experimental approaches for target deconvolution

Opportunity
The Ligand Express platform, powered by the in silico MatchMaker Proteome Screening technology was compared to three different Mass Spectrometry (MS) based deconvolution approaches.

Technology
Known interactors were cross referenced against both the MS-based and MatchMaker Proteome Screening results.

Solution
MatchMaker identifies a greater proportion of the known interactors, demonstrating that an in silico technology can provide useful insights into a drug's polypharmacology.

Experimental and in silico approaches for target deconvolution

Insight into a small molecule's polypharmacology permits the prioritization of candidates in drug development, enables drug repositioning, and informs on safety by identifying off-target binding which may result in deleterious side effects. Mass Spectrometry (MS) based deconvolution methods including cellular thermal shift assay combined with MS (CETSA® MS)[1], KiNativ™[2] and affinity purification[3] are experimental approaches commonly applied to elucidate a drug's polypharmacology, with KiNativ™ designed specifically for profiling kinase inhibitors. In collaboration with researchers from AstraZeneca, we compared our Deep Learning-based MatchMaker Proteome Screening technology to multiple MS-based target deconvolution methods in identifying proteins with IC50 measurements <10 µM. MatchMaker predicts binding between small molecules and proteins by using both protein and chemical structures. MatchMaker provides a ranked list, ranging from proteins most likely to bind the query small molecule to those least likely to bind, across the structurally characterized human proteome.

Polypharmacological profiles across technologies

Two compounds were selected for analysis, the first with accompanying data on results from CETSA® MS (250 proteins), KiNativ™ (120 proteins) and individual assays (IC50 on 101 proteins) and the second with data on results from CETSA® MS (543 proteins), affinity purification mass spectrometry (AP-MS) (436 proteins) and individual assays (IC50 on 94 proteins). Proteins which exhibited IC50 values <10µM in the individual assays were considered known interactors. Cyclica's Ligand Express platform which features the MatchMaker's proteome screening results was used to evaluate the polypharmacology of the compounds, leading to a ranked list of predicted binders for each compound comparable to similar lists provided by the experimental methods. We evaluated Ligand Express and the experimental approaches by how highly the known interactors ranked in the respective lists. One on-target protein was also known for each compound. The identity of the compounds and on-targets is not disclosed in this note.

MatchMaker identifies the greatest proportion of known interactors

Results for the first compound displayed limited overlap between the targets identified by the MS-based technologies and those highly ranked by MatchMaker (**Figure 1A**). Greatest agreement was observed between targets identified by MatchMaker and KiNativ™ rather than those between the MS-based approaches. KiNativ™ yielded the highest rank for the on-target (rank 1), followed by

MatchMaker (rank 36) whereas CETSA® MS did not identify the on-target. Of the 27 known interactors, MatchMaker identified the greatest proportion (62%) compared to both KiNativ™ (14%) and CETSA® MS (0%) within the top 100 ranks (**Figure 1B**). Extending to the top 400, MatchMaker captures additional targets, identifying 85% of known targets. In KiNativ™'s entire list (120 proteins) a total of 22% of those proteins with IC50 values <10μM were identified, whereas 3% was identified when considering the entire list for CETSA® MS (250 proteins).

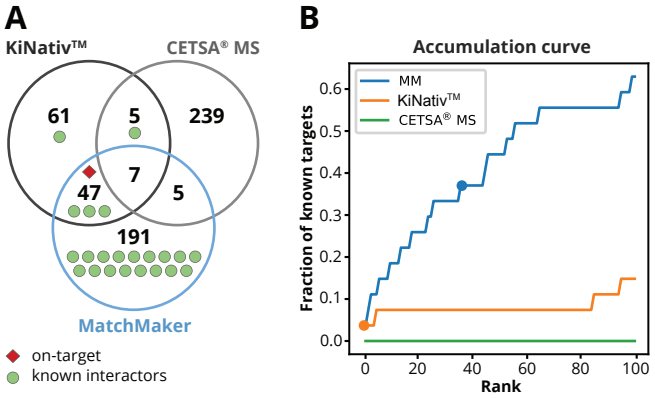


Figure 1. A) Overlap in the targets identified between KiNativ™, cellular thermal shift assay combined with MS (CETSA® MS) and MatchMaker for the first compound. A total of 61, 239 and 191 proteins were exclusively identified by KiNativ™, CETSA® MS and MatchMaker respectively. **B)** Fraction of known targets ranking higher than a given rank across target deconvolution approaches. Circles indicate rank of the on-target (KiNativ™ rank 1, MM rank 36, CETSA® MS not identified). Ranks extend to 100 as the lists of all three approaches extended to at least this value. MM (MatchMaker Proteome Screening), CETSA® MS (cellular thermal shift assay combined with MS).

For the second compound, limited target overlap was observed between the three target deconvolution approaches (**Figure 2A**), with CETSA® MS and affinity purification mass spectrometry (AP-MS) yielding the greatest degree of overlap. CETSA® MS yielded the highest rank for the on-target (rank 8), followed by AP-MS (rank 12) and then MatchMaker (rank 231). Of the 28 known interactors, CETSA® MS and AP-MS identified the greatest proportion (17.8%) within the top 100, while MatchMaker identified 7.1%. Extending to the top 400 ranks for all methods, MatchMaker identifies the greatest proportion at 53.5% while both CETSA® MS and AP-MS remained at 17.8% (**Figure 2B**). Of the 28 known interactors, 13 were non-kinases and 15 were kinases. Neither CETSA® MS nor AP-MS identified any of the non-kinase

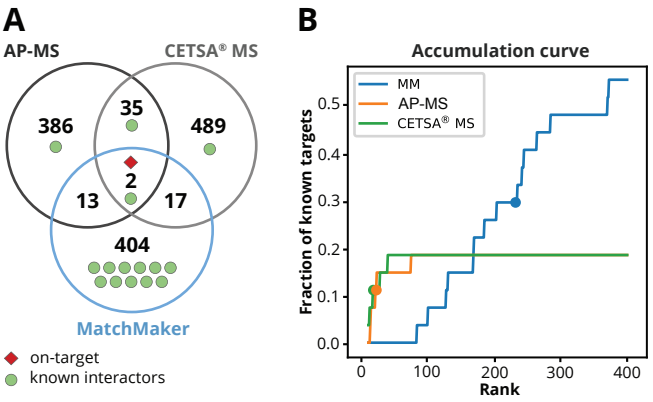
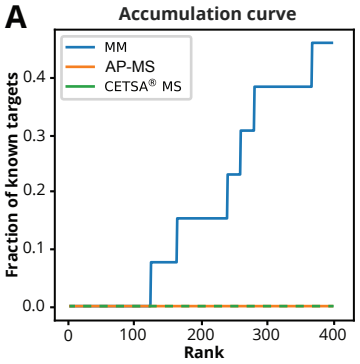
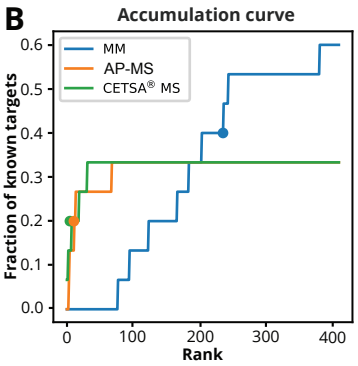


Figure 2. A) Overlap in the targets identified between cellular thermal shift assay combined with MS (CETSA® MS), affinity purification mass spectrometry (AP-MS) and MatchMaker Proteome Screening for the second compound. A total of 489, 386 and 404 proteins were exclusively identified by CETSA® MS, AP-MS and MatchMaker respectively. **B)** Fraction of known targets ranking higher than a given rank across target deconvolution approaches. Circle indicates rank of on-target (CETSA® MS rank 8; AP-MS rank 12; MM rank 231). MM (MatchMaker Proteome Screening), CETSA® MS (cellular thermal shift assay combined with MS).

binders, whereas MatchMaker identified 46% within the top 400 (**Figure 3A**). Of the 15 kinase targets, both CETSA® MS and AP-MS identified 33% within the top 100 whereas MatchMaker identified 13%. Extending to the top 400, the percentage for MatchMaker increases to 60% whereas CETSA® MS and AP-MS remain at 33% (**Figure 3B**).

Figure 3. A) Fraction of known non-kinase targets ranking higher than a given rank across target deconvolution approaches. Ranks extend to 400 as the lists of all three approaches extended to at least this value.





B) Fraction of known kinase targets ranking higher than a given rank across target deconvolution approaches. Circle indicates rank of on-target (CETSA® MS rank 8; AP-MS rank 12; MM rank 231). MM (MatchMaker Proteome Screening), CETSA® MS (cellular thermal shift assay combined with MS), AP-MS (affinity purification mass spectrometry).

In-silico target deconvolution approach provides useful insight into drug interactions

Protein drug interactions predicted by MatchMaker Proteome Screening and by MS-based deconvolution approaches (cellular thermal shift assay combined with mass spectrometry, affinity purification mass spectrometry and KiNativ™) for two compounds were compared to interactors individually assayed with IC50 values <10μM. For both compounds, MatchMaker identified a greater proportion of the known interactors, although for the second compound the MS based approaches identified known targets early within the ranks. For the second molecule, the first known binder is ranked at 76, which has the appearance of underperformance for MM. However, because 70 out of the 75 top ranked proteins (five proteins have IC50 values >10μM) have not been tested in the individual assays, it is possible that the 70 top proteins, of which 68 are kinases, are actual binders rather than false positives. An obvious follow-up to this study would involve additional testing of the second molecule against the top proteins in the MatchMaker ranked list. This study demonstrates that MatchMaker, a purely in-silico approach, is able to provide useful insights into drug interactions, which should help reduce the duration of the drug discovery process. Our findings, that some of the known targets identified solely by MatchMaker, have established links to side effects also support its value in safety profiling.

References

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