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Off-Target Identification

High Accuracy Prediction of MAPK14 Off-Targets – a case study

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Introduction

During the discovery and development of new drugs, candidates with undesired and potentially harmful side effects can arise at all stages, which poses a significant scientific and economic risk. Most of such phenotypic side-effects can be attributed to the drug candidate binding to unintended proteins, so-called off-targets. Therefore, identifying potential off-targets early is of utmost importance in order to mitigate any downstream risks.

To demonstrate the validity and usability of our *DiscoveryEngine* as an off-target identifier, PharmAI and 2bind jointly applied a combination of knowledge-based *in silico* off-target screening and state-of-the-art biophysics to rapidly identify off-targets for a MAPK 14 inhibitor in a case study.

Out of 13 predicted off-targets, six were confirmed to interact with the inhibitor *in vitro*, which translates to an **exceptional hit rate of 46%.**

PharmAI's off-target identification is a unique service with an exceptionally high hit rate.

Off-Target Services

A typical off-target screening project can be completed within 7 weeks, excluding protein sourcing and compound procurement. The synergistic benefits of the latest AI technology and MicroScale Thermophoresis (MST) from 2bind, allows to launch *in silico* and *in vitro* pipelines instantaneously and in parallel with the knowledge of the target being the only requirement.

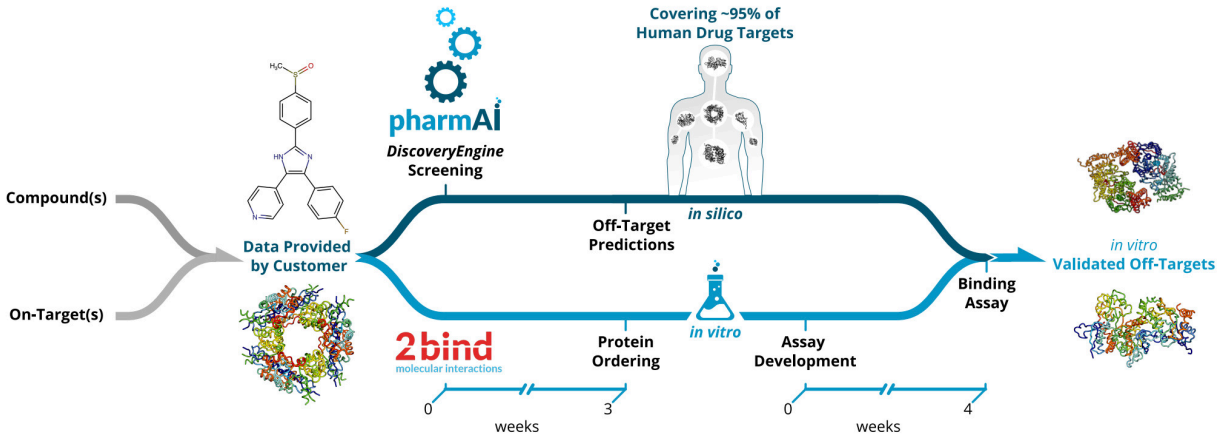

Figure 1: Workflow of the PharmAI-2bind Off-Target Identification Service. Upon the definition of the compound(s) and/or on-targets(s) of interest, *in silico* and *in vitro* pipelines are launched in parallel. The PharmAI *DiscoveryEngine* rapidly predicts potential off-targets, covering 95% of the known human drug targets. Predicted proteins are then purchased and assay development starts as soon as the *in silico* data is ready.

Case Study: MAPK14 Off-Target Screening

Starting with the MAPK14 inhibitor SB203580, we applied the PharmAI *DiscoveryEngine* and identified 167 proteins as potential off-targets for SB203580. Out of these proteins, 56 were already known to bind SB203580 *in vitro*. From the remaining 111 unknown targets, the top 13 that were commercially available were purchased in small quantities for biophysical testing.
SB203580 showed binding to six of these proteins in the 2bind Off-Target MST assay, translating to an exceptional hit rate of 46% (see Table 1).

	Validated Off-Target	Gene	UniProt AC	K_D	Sequence-Identity to MAPK14	Closest Binder (% Similarity)
	Serine/threonine-protein kinase/endoribonuclease IRE1 *endoplasmatic reticulum stress sensor*	ERN1	O75460	2.05±0.52 μM	8.30%	 CHEMBL3660376 (36%)
	Hematopoietic prostaglandin D synthase *production of prostanoids in the immune system*	HPGDS	O60760	30.87±4.54 μM	9.61%	 CHEMBL442461 (41%)
	Glutamate receptor 2 *excitatory synaptic transmission*	GRIA2	P42262	>500 μM	6.76%	 CHEMBL3799697 (31%)
	Nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1 *part of NAD biosynthesis*	NMNAT1	Q9HAN9	>500 μM	8.59%	n/a
	Fatty acid-binding protein 4 *lipid transport protein*	FABP4	P15090	>500 μM	6.65%	 CHEMBL248145 (42%)
	Menin *scaffold protein and regulator of gene transcription*	MEN1	O00255	>500 μM	8.71%	 CHEMBL3696205 (37%)

Table 2: Validated off-targets for the kinase inhibitor SB203580. For each protein, the determined K_D to SB203580 and the sequence identity to MAPK14 is given. Additionally, the chemically most similar compound to SB203580 with reported activity [14] for each protein is shown.

The results clearly show that our methodology allows to fine-tune target selectivity right from the very beginning of the drug discovery pipeline, reducing the downstream risks and costs. Knowing the target classes can also shed light on whether the compound will be a specific binder. Furthermore, the comprehensive knowledge of potential binding partners besides the primary target can guide and aid the molecular design in order to increase selectivity during the medicinal chemistry optimization.

Our case study clearly demonstrates the synergy between highly accurate knowledge-based *in silico* off-target prediction using the PharmAI *DiscoveryEngine* and fast and ultra-low sample consumption biophysical testing achieved with the 2bind Off-Target MST assay. Consequently, our combined efforts efficiently identify and validate off-targets at a large scale.

Associated Publication

Kaiser, F., Plach, M.G., Leberecht, C., Schubert, T. and Haupt, V.J. (2020). *In Silico Driven Prediction of MAPK14 Off-Targets Reveals Unrelated Proteins with High Accuracy.* bioRxiV, doi:10.1101/2020.07.24.219071 https://www.biorxiv.org/content/10.1101/2020.07.24.219071v1

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[3] Haupt, VJ, Daminelli, S, Schroeder, M (2013). *Drug Promiscuity in PDB: Protein Binding Site Similarity Is Key*. PLoS ONE, 8, 6:e65894.

[4] Kaiser, F., Plach, M.G., Leberecht, C., Schubert, T. and Haupt, V.J. (2020). In Silico Driven Prediction of MAPK14 Off-Targets Reveals Unrelated Proteins with High Accuracy, bioRxiV, doi: https://doi.org/10.1101/2020.07.24.219071

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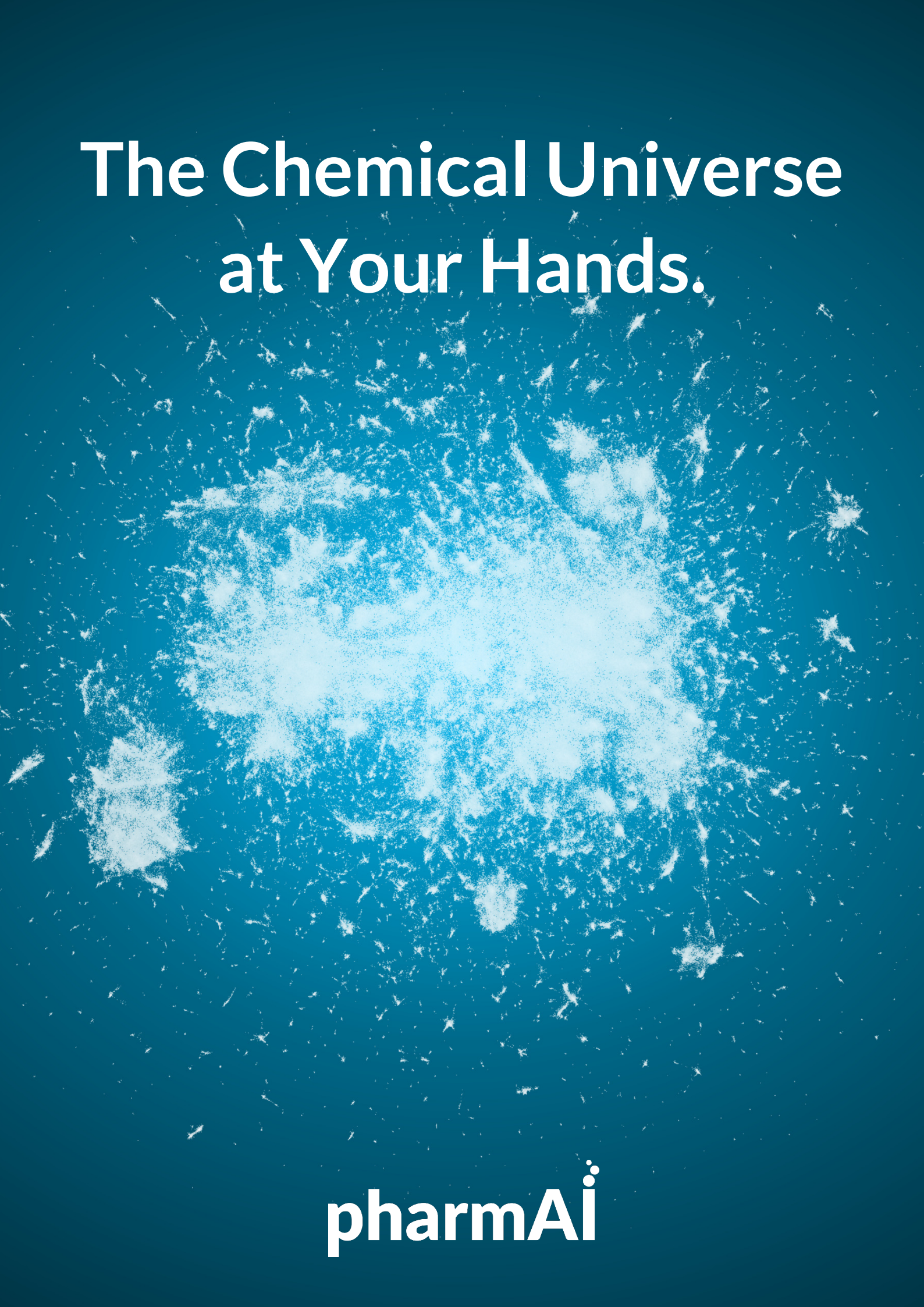

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