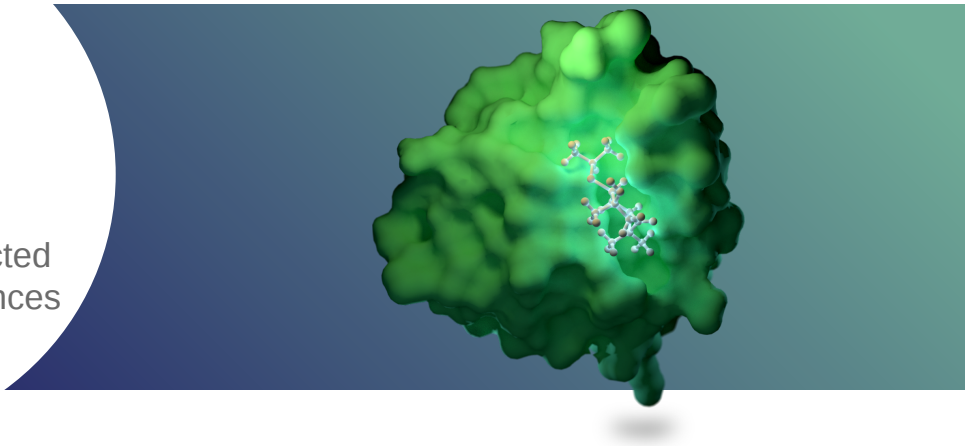
CASE STUDY

CuresWithinReach CYCLICA

SWITCH$_X$ Guides Drug Repurposing Study

Top drug repositioning candidates selected by anticipating the biological consequences of predicted ligand-protein interactions



PROBLEM

Cures Within Reach researchers required prioritization of compounds from a set of repurposing candidates for treatment of Idiopathic Pulmonary Fibrosis (IPF)

TECHNOLOGY

Ligand Express™ tools: PROBE$_X$ Proteome Docking, SWITCH$_X$ Ligand Effect Prediction, DIVE$_X$ Systems Biology Discovery Platform.

SOLUTION

Proteome-docking profiles generated for drug repurposing candidates using PROBE$_X$. Top IPF therapies identified by SWITCH$_X$ and interrogated using DIVE$_X$.

INTRODUCTION

Drug repurposing is quickly becoming a preferred methodology for developing therapies. Repurposing reduces the cost and time associated with producing drugs de novo and has become a major pipeline for many pharmaceutical companies[1]. Dedicated drug repurposing approaches aim to discover therapeutics for specific diseases. *Cures Within Reach* researchers identified several lead drug repurposing candidates with possible indications towards the treatment of Idiopathic Pulmonary Fibrosis (IPF)[2]. PROBE$_X$ produced proteome docking profiles for these lead compounds, and were then analyzed within the context of IPF using SWITCH$_X$ and DIVE$_X$. These analyses identified three lead small molecules, along with a short-list of potential protein targets for rational *in vitro* verification.

METHODOLOGY

Drug repurposing candidates were screened on Cyclica's PROBE$_X$ to generate their unique proteome docking profiles. An initial round of analysis discovered shared protein targets among the drug candidates that were potential targets for treating IPF pathogenesis. To supplement this initial analysis, several interrogation methods from Cyclica's platform were utilized. A curation procedure, informed by systems biology and input from a field expert of IPF biology, limited each drug candidate's proteome profile to a set consisting of only IPF-specific targets. Ligand-protein interactions from the curated data sets were analyzed by SWITCH$_X$ to determine the modulatory effect of predicted binding events (i.e. ligand binding activates or inhibits). When necessary, predicted ligand-protein poses were compared to known drug-protein structures to inform effector classification. Finally, DIVE$_X$ visualized protein networks assembled by their IPF-related biological roles, which provided a holistic overview of pertinent protein targets.

SWITCH$_X$ is a recently developed addition to Cyclica's platform providing chemogenomics insights into the likely biological consequences of a predicted ligand-protein interaction. SWITCH$_X$ is a database mining tool pulling biochemical data from trusted repositories (e.g. PubMed, PubChem, etc. containing > 1,000,000 unique records) to determine frequency of recorded modulatory activity for a particular protein (i.e. all known instances of activation, inhibition, or other). Combining the curated results from PROBE$_X$ with SWITCH$_X$ allowed for the classification of predicted ligand-protein interactions linked to IPF. In some cases, SWITCH$_X$ reports an activator/inhibitor distribution that is inconclusive for predicting effector type; in these cases, ligand co-occupation of a predicted binding site with either known inhibitors or activators implies the nature of the predicted ligand-protein interaction.

Using the network visualizer from DIVE$_X$, all protein hits identified from PROBE$_X$ were linked to functional biological annotations in accordance to Gene Ontology (GO) terms relevant to IPF. Specifically, 'child' terms were assembled for the following biological processes: autophagy, aging, mitochondrial homeostasis, apoptosis, oxidative stress, and fibrogenesis. Visualization facilitates identification of probable protein targets for observed biological effects, and represents other possible starting points for experimental validation efforts. Taken together, the collection of *in silico* analyses can produce a holistic profile for each repurposing target, streamlining lead prioritization initiatives and providing a rational starting point for further validation studies.

RESULTS

One of the lead repurposing candidates, Imipramine (*Tofranil*), will serve as a representative example for Ligand Express™ analysis and

results. Imipramine's PROBE$_X$ proteome docking profile was subjected to a curating procedure which identified 51 potential protein interactions immediately relevant to IPF. SWITCH$_X$ was then used to identify how these interactions would likely modulate the protein target. Five of these predictions are listed in **Table 1.**

Table 1. SWITCH$_X$ predictions for Imipramine. (*) predictions were manually verified by examining the putative binding site.

Target Protein	% Positive Regulators (n)	% Negative Regulators (n)	Predicted Effect	Therapeutically relevant to IPF?
TGFβR1	3.0 (11)	83.5 (405)	Inhibition*	Yes
TAB1	0.0 (0)	100.0 (81)	Inhibition	Yes
ARK - 2	3.3 (15)	96.7 (443)	Inhibition	No
JMJD2A	0.0 (0)	98.0 (48)	Inhibition	No
PI3K γ	0.0 (0)	99.8 (517)	Inhibition	Yes

The modulation of IPF-linked proteins may modify disease progression as predicted by the right most column of the table. In order to designate whether the anticipated modulatory action of an Imipramine-protein interaction would be beneficial as an IPF treatment, the protein's role in IPF biology was examined and used to assign its significance in the disease state (e.g. predicted inhibition of an overexpressed and disease-promoting protein would be therapeutically relevant). Imipramine's interaction with TGFβR1 displayed an activator/inhibitor distribution that precludes its immediate classification; hence, Imipramine's predicted binding site was superimposed with known inhibitors/activators (**Figure 1**). Upon inspection, it became clear that Imipramine binds in an analogous fashion to a known inhibitory molecule and was therefore classified as a likely inhibitor of TGFβR1 activity. Of the 51 ligand-proteins interactions linked to IPF, 15 were predicted to remedy the disease state, 5 interactions could have a counterproductive effect, and the remainder had undetermined consequences.

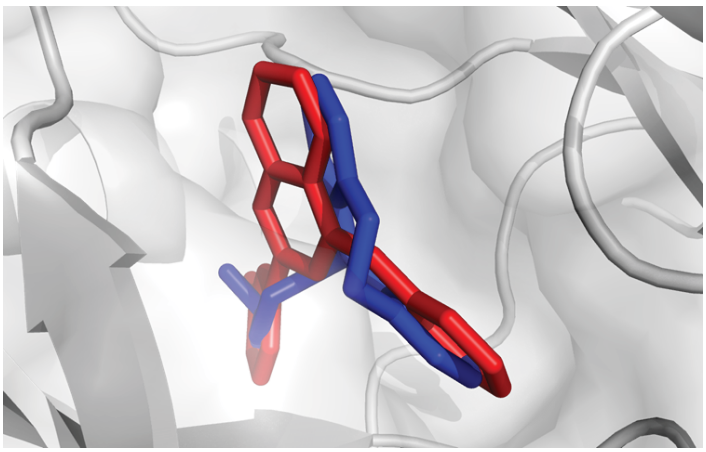


Figure 1. Visualization of Imipramine(blue) docked to TGFβR1 and the co-crystallized inhibitory quinazoline (red). This interaction was predicted to be inhibitory in nature.

An additional level of interrogation was provided by DIVE$_X$'s functional annotation network visualization, which connects all drug-protein interactions that may be relevant to IPF, providing an overview of disease relevant biological processes and targets (**Figure 2**). This graphic highlights several protein interactions predicted by PROBE$_X$ that have immediate therapeutic relevance to IPF.

The aforementioned analytical methods were employed for all drug candidates. By comparing the results of the analytical methods described above, three compounds had properties that distinguished them as having best potential as an IPF monotherapy.

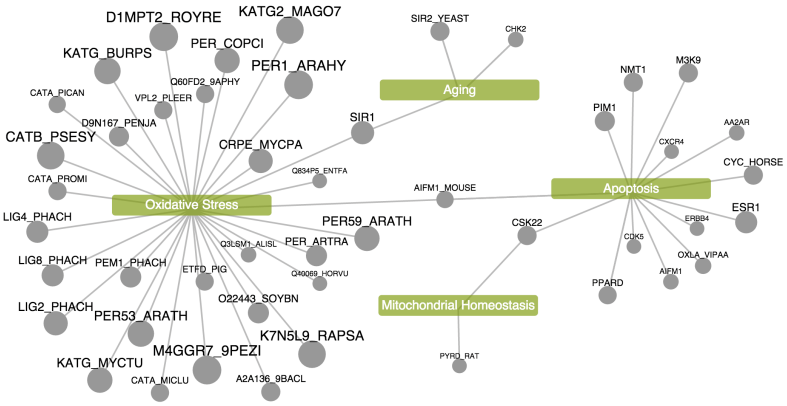


Figure 2. Visualization of interconnectivity between top protein hits for Imipramine mapped to genes and their products (i.e. proteins) associated with IPF processes. Radii of circles and font size represent the likelihood of the predicted interaction.

SUMMARY

A collection of drug repurposing candidates for IPF was screened using PROBE$_X$, and further analyzed using SWITCH$_X$ and DIVE$_X$ tools. SWITCH$_X$, a data mining tool that predicts consequences of drug binding, helped characterize predicted IPF protein interactions. Certain results from SWITCH$_X$ suggested obvious effector classification, leading to direct inference of a specific modulation type; in cases of ambiguity, inspection of the binding site, co-visualized with both known inhibitors/activators provided a measure of differentiation. Importantly, many of the predicted drug-protein interactions remain ambiguous in terms of modulatory effect and would require additional investigation using appropriate biochemical techniques. A visualization of proteins associated wtih IPF, along with relevant biological processes, provided an additional degree of data interrogation. Three repurposing candidates were highlighted as having desired modulatory effects on IPF-associated proteins, and these drugs also had pronounced connectivity with relevant cellular functions within the IPF proteomic network . Furthermore, this analysis allows *Cures Within Reach* to strategically proceed with these three compounds, using newly identified proteins highlighted in this study as a starting point for any necessary biochemical analysis.

RESOURCES

1. Curtis R. Chong & David J. Sullivan, Jr. New uses for old drugs. *Nature* **448**, 645–646 (2007).

2. Sgalla, G., Biffi, A. & Richeldi, L. Idiopathic pulmonary fibrosis: Diagnosis, epidemiology and natural history. *Respirology* **21**, 427–437 (2016).

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