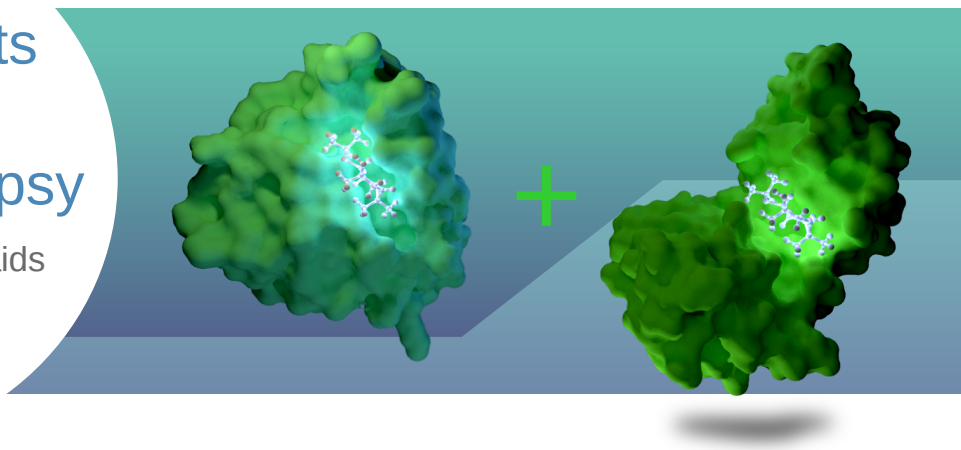# Cyclica's DIVE$_X$ Predicts Synergistic Drug Combinations for Epilepsy

Comparing proteome docking profiles aids in the identification of synergistic drug combination

PROBLEM
A collection of brain illness monotherapies were examined for potential synergistic drug combinations for the treatment of epilepsy.

TECHNOLOGY
Ligand Express™ tools:
PROBE$_X$ Proteome Docking,
DIVE$_X$ Systems Biology Discovery Platform

SOLUTION
Possible drug combinations were screened using PROBE$_X$ proteome docking software. DIVE$_X$ compared proteome docking profiles to identify drug pairs with an increased likelihood for drug synergy .

INTRODUCTION

Combinations of approved therapeutics can be an effective way to find novel and efficacious treatments for diseases, with multicomponent drugs being used to treat cancer, HIV, and diabetes. In ideal cases, drug combinations are synergistic or supra-additive; however, they can also be antagonistic, which can be harmful. Historically, synergistic drugs are well tolerated and safe due to improved target precision within disease specific pathways, which resultis in lower drug doses[1]. Unfortunately, predicting synergistic drug combinations is challenging, with most drug combinations discovered by costly empirical tests[2]. The effectiveness of multicomponent drugs has been attributed to the modulation of multiple targets within disease pathways[3]; however, having unique mechanisms of action is insufficient for identifying synergistic pairs, with other considerations (e.g. toxicity) needing exploration.

In this study, Cyclica's scientists wanted to explore drug synergy prediction. Cyclica examined potential multicomponent drugs for epilepsy, a chronic brain condition associated with convulsive seizures. Researcher's began by screening drugs of interest through PROBE$_X$, which produced unique proteome docking profiles for each drug consisting of a ranked list of putative ligand-protein interactions. Next, DIVE$_X$ analytical tools restricted protein results to include only those experimentally linked to epilepsy. Hypothesizing that the drug combinations with the highest chance of success at synergy were those that had distinctive protein targets, Cyclica researchers used functionality within DIVE$_X$ to measure overlap between each drug's modified proteome dataset. Investigation into shared proteins also revealed potential for cytotoxic effects. Using these approaches, Cyclica identified one drug pair with superior unique protein hits and predicted that it had the greatest likelihood of synergy.

METHODOLOGY

Six drugs were chosen for this initial study examining possible drug synergy. These drugs included: Gabapentin (Neurontin), an anticonvulsant; Levetiracetam (Keppra), an antiseizure drug; Phenytoin (Dilantin), reduces nonconvulsive seizures following brain injury; Ethosuximide (Emeside), attenuates acute seizures following brain injury; Dextromethorphan (DM), NMDA receptor antagonist; and Simvastatin (Zocor), inhibitor of HMGCoA reductase used for managing cholesterol. While approved for use in different disease indications, both Dextromethorphan and Simvastatin have been shown to have a mediating effect on seizures[4,5]. For each drug, proteome docking profiles were generated using PROBE$_X$. Using DIVE$_X$ cross-referencing functionality, these profiles were limited to epilepsy-associated gene as defined by DisGenNet (umls:C0014544). Pairwise combinations of proteome docking profile subsets were systematically compared for their similarity. A similarity scoring function was developed, where a high weighted score suggested a greater number of distinct epilepsy-related protein interactions for each compound. The weighted scoring function takes into account both the normalized docking score for each drug in the pairing, and an association scored derived from DisGenNet representing that proteins association to epilepsy. The weighted scores were visualized on a heatmap, and the most likely synergistic drug combination was identified. Additionally, the shared protein targets of paired drugs were investigated for biological consequences like adverse/toxic effects.

RESULTS

The weighted scoring function, based on Euclidean distance (**Equation 1**), was calculated for each possible drug combination using the methodology described. Results are visualized as a heatmap (**Figure 1**),

$$\text{Distance Score} = \sqrt{\sum_{i}^{\text{all hits}} (W_i A_i - W_i B_i)^2}$$

Equation 1. Euclidean distance measurement comparing binding profiles of Epilepsy-associated proteins i, between compounds A and B. A_i and B_i denote normalized docking scores for each compound. W_i represents the association score of protein i with Epilepsy, as defined by DisGeNet.

with the scores enumerated and coloured depending on its magnitude. Based on the data available, we inferred that the combination of Dextromethorphan and Simvastatin has the greatest potential to confer complementary polypharmacological actions on Epilepsy-associated proteins, demonstrating the largest magnitude of distinct protein hits with an overall score of 1.42. In contrast, Gabapentin and Levetiracetam shared many predicted targets, as noted by the lower distance score of 0.22. Interestingly, Simvastatin (the HMGCoA reductase inhibitor) and Dextromethorphan (NMDA antagonist), showed the highest number of unique protein hits, which aligns with their distinct biological functions and consequently, their diversity of known protein targets. A notable pairing amongst the anti-convulsant drugs was that of Phenytoin and Levetiracetam, a combination that has been shown to be supra-additive in the literature[6].

	Ethosuximide	Phenytoin	Dextromethorphan	Simvastatin	Gabapentin	Levetiracetam
Ethosuximide	0.00	0.60	0.91	1.16	0.46	0.41
Phenytoin	0.60	0.00	0.41	1.19	0.81	0.79
Dextromethorphan	0.91	0.41	0.00	1.42	1.13	1.15
Simvastatin	1.16	1.19	1.42	0.00	0.97	1.01
Gabapentin	0.46	0.81	1.13	0.97	0.00	0.22
Levetiracetam	0.41	0.79	1.15	1.01	0.22	0.00

Figure 1. A heatmap showing the weighted Euclidean distance between the different drug pairings (the more saturated the blue, the greater the difference between the two drugs binding profiles)

If supra-additive drug combinations arise as a feature of having different targets, then the combination of Dextromethorphan and Simvastatin represents the most promising pairwise combination. However, there are other considerations when determining drug pairings, a major one being the safety of the drug pairing. When using $DIVE_X$ to examine the protein targets common to Dextromethorphan and Simvastin, it became clear that these two compounds share predicted interactions with several drug-metabolizing proteins (**Figure 2**). This may place the drug pairing at risk for potential toxic effects, particularly with respect to hepatotoxicity. Thus, evaluating potential synergistic drug pairings should take into consideration unique proteome landscapes in order to account for harmful drug-drug contraindications.

SUMMARY

Cyclica researchers explored the capability of Ligand Express™ to explore synergistic drug combinations. Pre-selecting a list of six epilepsy-related drugs, Cyclica researcher's employed its proteome docking platform $PROBE_X$ to generate distinct profiles for each drug. $DIVE_X$ was used on these profiles to exclude proteins unrelated to epilepsy. These profile subsets were then examined for their uniqueness in pairwise combinations for all six drugs and weighted Euclidean distances scored the diversity of protein 'hits'. It was postulated that the

pairing with the most promise was Dextromethorphan and Simvastatin, as these had the greatest number of unique protein targets associated with epilepsy. However, Dextromethorphan and Simvastatin were also predicted to interact with certain metabolizing proteins, which elevates the chance of hepatotoxicity occuring with this pairing. Accounting for
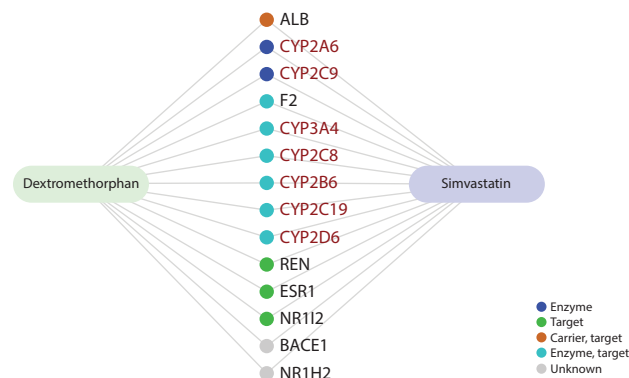

Figure 2. Visualization of predicted associations between Epilepsy-related genes and the pairing of Dextromethorphan & Simvastatin. Genes are represented as circles (coloured according to biological process, see in-figure legend). Highlighted in **red** are proteins linked to drug metabolism.

toxicity is yet another consideration for predicting effective drug combinations. Furthermore, Cyclica's $PROBE_X$ and $DIVE_X$ from the Ligand Express™ suite, will better inform predictions about synergistic drug combinations by providing a holistic view of all disease-associated proteins, a feat that is impossible on other platforms.

RESOURCES

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