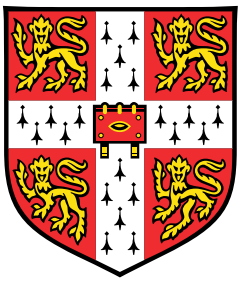
Accelerating the Design-Make-Test cycle of Drug Discovery with Machine Learning



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Declaration

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university. This dissertation is my own work and contains nothing which is the outcome of work done in collaboration with others, except as specified in the text and Acknowledgements. This dissertation contains fewer than 65,000 words including appendices, bibliography, footnotes, tables and equations and has fewer than 150 figures.

William McCorkindale
January 2023

Acknowledgements

TODO - finish acknowledgements And I would like to acknowledge ...

- Alpha
- Collaborators
- DeepMind?
- Cambridge friends
- Family

Abstract

Drug discovery follows a design-make-test cycle of proposing drug compounds, synthesising them, and measuring their bioactivity, which informs the next cycle of compound designs. The challenges associated with each step leads to the long timeline of preclinical pharmaceutical development. This thesis focuses on how we can use machine learning tools to accelerate the design-make-test cycle for faster drug discovery.

We begin with the design of new compounds, looking at the initial stage of fragment-based hit finding where only the 3D coordinates of fragment-protein complexes are available. The standard approach is to "grow" or "merge" nearby fragments based on their binding modes, but fragments typically have low affinity so the road to potency is often long and fraught with false starts. Instead, we can reframe fragment-based hit discovery as a denoising problem – identifying significant pharmacophore distributions from an "ensemble" of fragments amid noise due to weak binders – and employ an unsupervised machine learning method to tackle this problem. We construct a model that screens potential molecules by evaluating whether they recapitulate those fragment-derived pharmacophore distributions. We show that this approach outperforms docking on distinguishing active compounds from inactive ones on historical data. Further, we prospectively find novel hits for SARS-CoV-2 Mpro and the Mac1 domain of SARS-CoV-2 non-structural protein 3 by screening a library of 1B molecules.

After identifying hit compounds, we enter the the hit-to-lead stage where we wish to optimise their molecular structures to improve bioactivity. Framing bioactivity modelling as classification of active/inactive would not allow us to rank compounds based on predicted bioactivity improvement, while the low number of active compounds and the measurement noise make a regression approach challenging. We overcome this challenge with a learning-to-rank framework via a classifier that predicts whether a compound is more or less active than another using the difference in molecular descriptors between the molecules as input. This allows us to make use of inactive data, and threshold the bioactivity differences above measurement noise. Validation on retrospective data for Mpro shows that we can outperform docking on ranking ligands, and we prospectively screen a library of 8.8M molecules and arrive at a potent compound with a novel scaffold.

After designing a drug candidate one needs to find a synthesis route to actually make the molecule in the real world. An exciting approach is to use deep learning models trained on patent reaction databases, but they suffer from being opaque black-boxes. It is neither clear if the models are making correct predictions because they inferred the salient chemistry, nor is it clear which training data they are relying on to reach a prediction. To address this issue, we developed a workflow for quantitatively interpreting a state-of-the-art deep learning model for reaction prediction. By analysing chemically selective reactions, we show examples of correct reasoning by the model, explain counterintuitive predictions, and identify Clever Hans predictions where the correct answer is reached for the wrong reason due to dataset bias.

Testing a drug candidate typically involves obtaining a pure sample of the molecule, and then measuring its bioactivity in solution via an assay. While necessary for maximum accuracy, compound purification can be time-consuming and costly. We investigated whether we needed compound purification at all for training machine learning bioactivity models by assaying crude reaction mixtures instead of pure samples. This approach allowed us to obtain bioactivity data in higher throughput and train useful models for identification of false negative assay measurements, as well as prospective screens.

The research presented in this thesis highlights the promise of applying machine learning in accelerating the design-make-test cycle of drug discovery. This thesis concludes by outlining promising research directions for applying machine learning within drug discovery.

Table of contents

Preface

Chapter 1 (TODO - add links) talks about ...

In Chapter 2 we discuss ... This work resulted in the publication of the following article:

> William McCorkindale, Ivan Ahel, Haim Barr, Galen J. Correy, James S. Fraser, Nir London, Marion Schuller, Khriesto Shurrush, Alpha A. Lee. Fragment-Based Hit Discovery via Unsupervised Learning of Fragment-Protein Complexes.

Specify who did what

Chapter 3:

> Aaron Morris, William McCorkindale, The COVID Moonshot Consortium, Nir Drayman, John D. Chodera, Savaş Tay, Nir London and Alpha A. Lee. Discovery of SARS-CoV-2 main protease inhibitors using a synthesis-directed de novo design model, *Chem. Commun.*, 2021,57, 5909-5912

Chapter 4

> Dávid Péter Kovács, William McCorkindale and Alpha A. Lee. Quantitative interpretation explains machine learning models for chemical reaction prediction and uncovers bias. *Nature Communications* volume 12, Article number: 1695 (2021)

D.P.K. and W.M. implemented the algorithms, D.P.K. trained the models, designed the experiments and analysed the model attributions for the various reaction classes. W.M. implemented Tanimoto splitting and applied reaction templates for counting statistics and artificial dataset generation. A.A.L. supervised and directed the project. All authors discussed the results and approved the manuscript. D.P.K. and W.M. contributed equally to this study.

Chapter 5:

The final chapter ...

Chapter 1

Introduction

The discovery of new pharmaceuticals traditionally follows the design-make-test paradigm, where molecules are repeatedly proposed, synthesized, and assayed. Drug candidates are designed based on some hypothesis relating chemical structure to drug activity, which gets updated in light of new activity results. This cycle repeats as the molecular search space narrows down until a candidate molecule satisfies the necessary activity/selectivity/toxicity criteria.

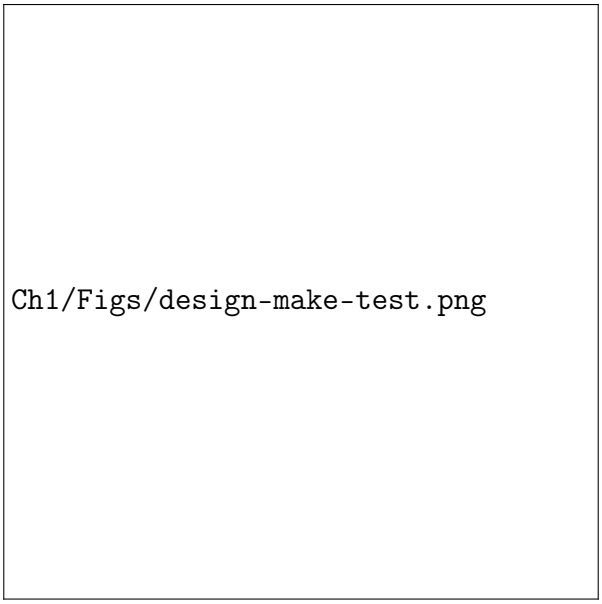

Fig. 1.1 An overview of the design-make-test cycle in drug discovery.

While computational methods have long been used in various stages of the cycle, there has been a recent surge in applying artificial intelligence to drug discovery following its success in various other fields, most notably computer vision and natural language processing. Since

molecular assaying is largely an automated process the application focus has been on 'design' and 'make' [**?**], for example in modelling quantitative structure-activity relationships (QSAR), designing generative models for proposing drug candidates, and planning retrosynthesis routes (Fig **??**).

As the field of data-driven drug discovery matures beyond merely adapting the latest state-of-the-art machine learning (ML) methods, the present challenge is to tailor ML models specifically for the unique problems and situations faced in pharmaceutical chemistry. This report summarizes my efforts over the past year to play a part in this challenge with intuitions based on physical science. These consist of three separate tasks, one on 'Make' and two on 'Design':

- **Interpreting learnt chemical principles from Molecular Transformer:** a state-of-the-art reaction prediction model (Molecular Transformer) was investigated with input and data attribution methods to discern whether the model had learnt chemically reasonable patterns of reactivity, or had simply succumbed to hidden bias in the datasets.

- **Exploiting molecular shape for property prediction:** a descriptor of atomic positions known as SOAP, which has seen widespread use in condensed matter physics due to its symmetry-invariance properties, was utilized in a Gaussian Processes model and shown to be competitive with other state-of-the-art models on predicting bioactivity. It was also demonstrated that ensembling models with diverse representations led to further predictive power.

- **Designing Sars-CoV-2 MPro inhibitors:** An initiative known as COVID Moonshot [**?**] was established to search for inhibitors of the Sars-CoV-2 main protease (MPro), crowd-sourcing drug candidate designs from the scientific community. In the early stages of the project, I utilised a genetic algorithm with SOAP descriptors for combining disparate fragment hits; in the most recent stage, I implemented a graph siamese network to learn how to rank the activity of assayed molecules, which was then used to suggest new candidates via computational screening of a constructed library.

The lessons learnt from these projects are used to inform possible avenues of future research, which are discussed in the final chapter of this report.

Chapter 2

Fragment-Based Hit Discovery via Unsupervised Learning of Fragment-Protein Complexes

In this work, we describe an end-to-end hit detection approach that bridges the the paradigms of both fragment-based drug design and virtual screening. Our method, named FRESCO, utilises unsupervised learning to learn pharmacophore distributions directly from experimental 3D fragment-protein structures. The trained model evaluates whether or not a particular compound possesses pharmacophores matching the distribution exhibited by the bound fragments, replicating the intuition of a medicinal chemist deducing spatial correlations between pharmacophores from different fragments. Our approach is computationally validated with a retrospective study on SARS-CoV-2 main protease (Mpro) ligands using data from COVID Moonshot [**?**], showing high enrichment. Then, we conduct an experimental search for novel hits for Mpro and the Mac1 domain of SARS-CoV-2 non-structural protein 3 (nsp3) by scoring a library of 1.4 billion purchasable compounds from EnamineREAL, resulting in 1 (novel?) hit for MPro and 2 (novel?) hits for Mac-1. (Scaffold exploration of the detected hits hopefully lead to novel potent ligands!) Our results are the first experimentally validated demonstration of hit detection via a fully computational workflow starting directly from an experimental fragment screen.

2.1 Introduction

TODO - shorten introduction, put some of it in discussion?

Developing a new drug from original idea to the launch of a finished product is a complex process which can take 12–15 years and cost in excess of $1 billion [**?**]. A key step in the

early stages of the drug discovery process following the identification of a biological target is hit detection. Broadly speaking, a 'hit' is a compound that interacts with the identified target sufficiently strong enough to act as a starting point for optimisation of the compound structure towards a candidate drug.

Approaches towards hit detection generally involve the screening of libraries of compounds. For example, in high throughput screening (HTS) often hundreds of thousands of chemical compounds are synthesised and tested, requiring substantial resources as well as complex logistics. While experimental techniques such as DNA-Encoded libraries are being developed to increase the efficiency of large-scale compound screening [**?**], there has been a growing push towards conducting hit detection computationally instead to decrease the cost and accelerate this step of the drug discovery process [**?**].

In this approach, known as virtual screening, a computational scoring function is used to estimate the potency of a compound. After computing the scores for all of the compounds in a library, only those ranked highly by the scoring function are chosen for synthesis and experimental validation. Currently the predominant scoring function used to conduct a virtual screen is molecular docking. In molecular docking, the 3D conformation of a ligand and the target are explicitly modelled and a physics-based simulation of the binding process is conducted, with the calculated energy of the bound ligand as the score. Although this approach has yielded success [**?** **?**], correctly performing molecular docking is non-trivial and the deficiencies of molecular docking for bioactivity prediction are well-documented [**?** **?**].

An alternative to these methodologies is fragment-based drug discovery (FBDD). In this approach, a library of very low molecular weight compounds ('fragments' typically less than 18 nonhydrogen atoms [**?**]) are screened at high concentrations alongside the generation of bound fragment-protein structures via X-ray crystallography or cryo-EM. By obtaining these experimental structures and examining the binding interactions between individual fragments with protein residues, fragments can be used as building blocks for larger molecules by linking or merging together disparate fragments in order to increase potency. Conceptually, FBDD is based on a coarse-graining of fragments to specific moieties or groups that are associated with interactions to the target, with the goal of maintaining and optimising these interactions in larger molecules.

Although there exist some computational approaches for supporting FBDD, for example hot spot analysis and pocket druggability prediction [**?**], at present the main procedure of selecting which fragments to merge and how to do so remains largely intuition-based and human-driven. (and fraught to error? citation needed [**?**])

In this work, we describe an end-to-end hit detection approach that bridges the the paradigms of both fragment-based drug design and virtual screening. Our method, named FRESCO,

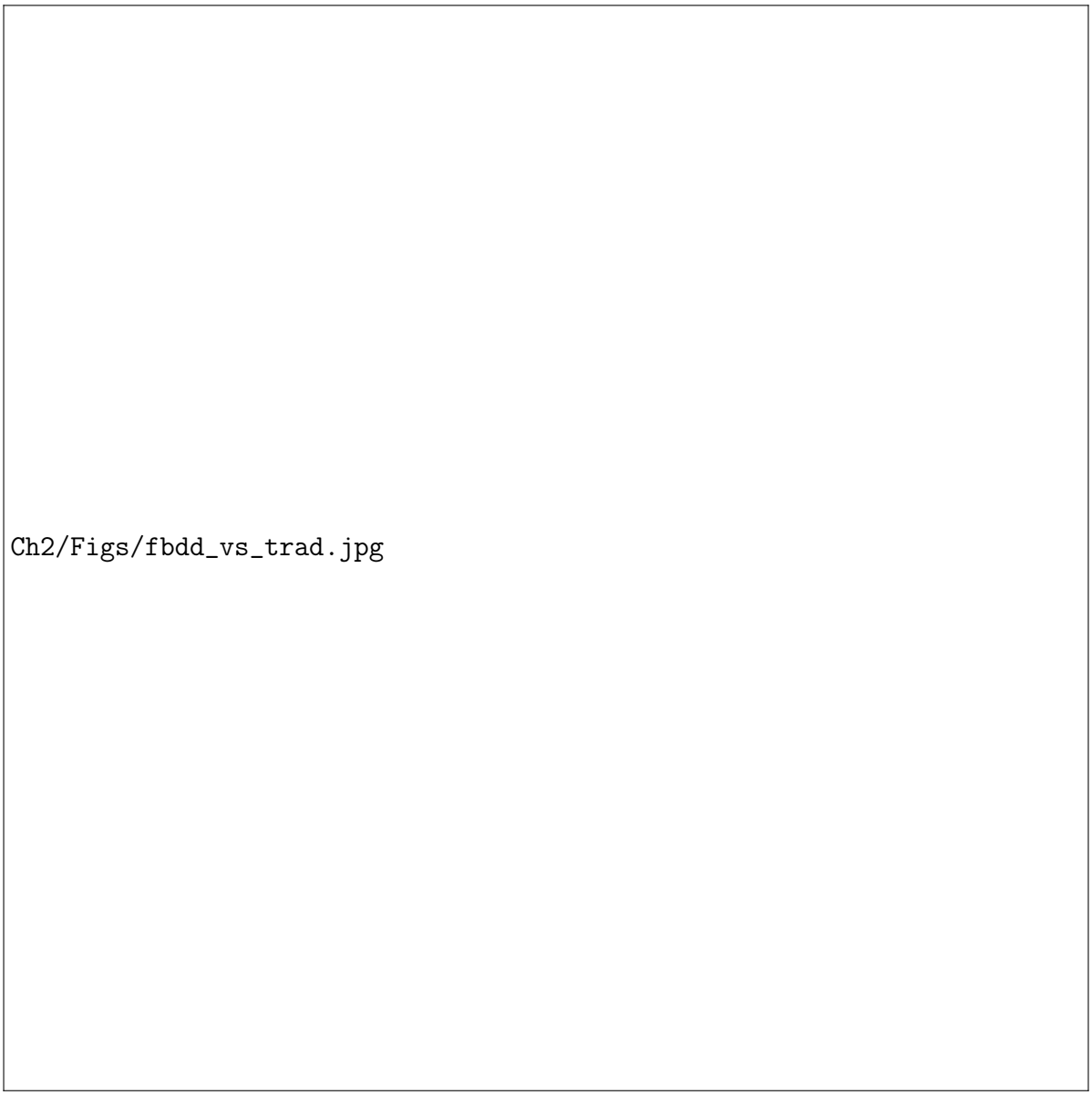

Fig. 2.1 An illustration comparing fragment-based drug discovery to traditional approaches.

utilises unsupervised machine learning to learn pharmacophore distributions directly from experimental 3D fragment-protein structures. The trained model acts as a scoring function that can be used to perform virtual screening, evaluating whether or not a particular compound possesses pharmacophores matching the distribution exhibited by the bound fragments.

This methodology aims to replicate the intuition of a medicinal chemist preforming fragment-based drug discovery, abstracting fragments to pharmacophores and deducing spatial correlations between pharmacophores from different fragments. As a matter of fact, we go beyond the typical strategem of growing one individual fragment independently of the others, or merging two particular fragments - by training our model on all of the fragment-protein structures we leverage information from all existing fragments, ensuring no pharmacophore correlations are overlooked in the hit detection process.

We first computationally validate our approach with a retrospective study on bioactivity prediction for SARS-CoV-2 main protease (Mpro) ligands using data from COVID Moonshot [**?**], showing high enrichment. Then, we conduct an experimental search for novel hits for Mpro and the Mac1 domain of SARS-CoV-2 non-structural protein 3 (nsp3) by performing a virtual screen with FRESCO on a library of 1.4 billion purchasable compounds from EnamineREAL. This resulted in 1 (novel?) hit for MPro and 2 (novel?) hits for Mac-1, with crystallographic poses for the Mac-1 hits. Follow-up compounds for performing scaffold exploration of the detected hits were synthesised and assayed demonstrating credible structure-activity relationships, confirming that the detected hits were genuine.

Our method is unique not only in its philosophy, but also its use of unsupervised learning in the form of kernel density estimation. Although there has been a rapid growth in machine learning methods applied to drug discovery in recent years particularly in QSAR/molecular property prediction, the vast majority of them are supervised learning techniques requiring not merely the existence of experimental assay data, but its existence in sufficient quantity and quality to train a useful model. The nature of the problem of hit detection in early-stage drug discovery is one where such data in nonexistent, which underlies the necessity of having methods for directly performing hit detection from fragment screen data.

The only comparable methods that the authors are aware of is recent work in using deep generative models for proposing merges between two fragments (DeLinker [**?**], SyntaLinker [**?**], and Develop [**?**]). These approaches differ for ours in several ways: Firstly, these models all require human intervention from an expert in choosing which fragments to merge, or what pharmacophoric constraints need to obeyed, whereas our model is fully end-to-end. Secondly, these methods utilise neural network-based generative models which are very sensitive to training hyperparameter choice in general (citation about mode collapse [**?**]), and for molecular generation in particular known to propose invalid and/or unsynthesizable molecules [**?**]. In

contrast, our method relies on kernel density estimation which is simple, robust and free from hyperparametear tuning, and we we explicitly only screen purchasable, easily-synthesised molecules. Lastly, the proposed models have only been studied computationally and lack validation in the real world.

Our results are the first experimentally validated demonstration of hit detection via a fully computational workflow starting directly from an experimental fragment screen. This work opens the door for bridging fragment-based drug discovery with virtual screening.

2.2 Results

2.2.1 Computational Retrospective Study

To validate that our hypothesised methodology has merit, a computational study was conducted on the SARS-CoV-2 main protease (Mpro). The COVID Moonshot campaign [**?**] was established as an open science effort towards developing a patent-free antiviral drug for the SARS-CoV-2, specifically targeting the inhibition of Mpro as that would prevent the virus from further replication. Throughout the campaign, activity data consisting of the structures of molecules that were synthesised and assayed was continually released. As a proof-of-concept, the model predictions for the assayed molecules are compared against the measured activity and analysed.

Firstly, the FRESCO model was fit on publicly reported crystallographic structures of non-covalent fragments bound to the SARS-CoV-2 Mpro protein [**?**]. Next, conformer coordinates for Moonshot compounds reported before March 22nd 2021 were obtained from parallel work within the Moonshot consortium performing docking studies on Mpro (TODO - citation/explanation?). Pharmacophore features were generated from these conformers and the molecules were scored using the model. The enrichment factor for picking compounds above an IC50 of 10is computed as we are most interested in the ability of this model to sample potent compounds. We also compute the enrichment from ranking the compounds with the Chemgauss4 scoring function.

The results are shown in Fig. **??** illustrating high enrichment, validating the hypothesis that it is possible to correlate bioactivity with unsupervised learning of fragment pharmacophore distributions. The date 21st July 2021 marks when additional experimental data was released and the direction of the COVID Moonshot campaign shifted from hit detection to optimisation of existing lead compounds [**?**]. The optimisation process relies on the improvement of energetic interactions between the ligand and residues in the active site, likely growing beyond the volume covered by the initial fragment hits and so cannot in principle be captured by

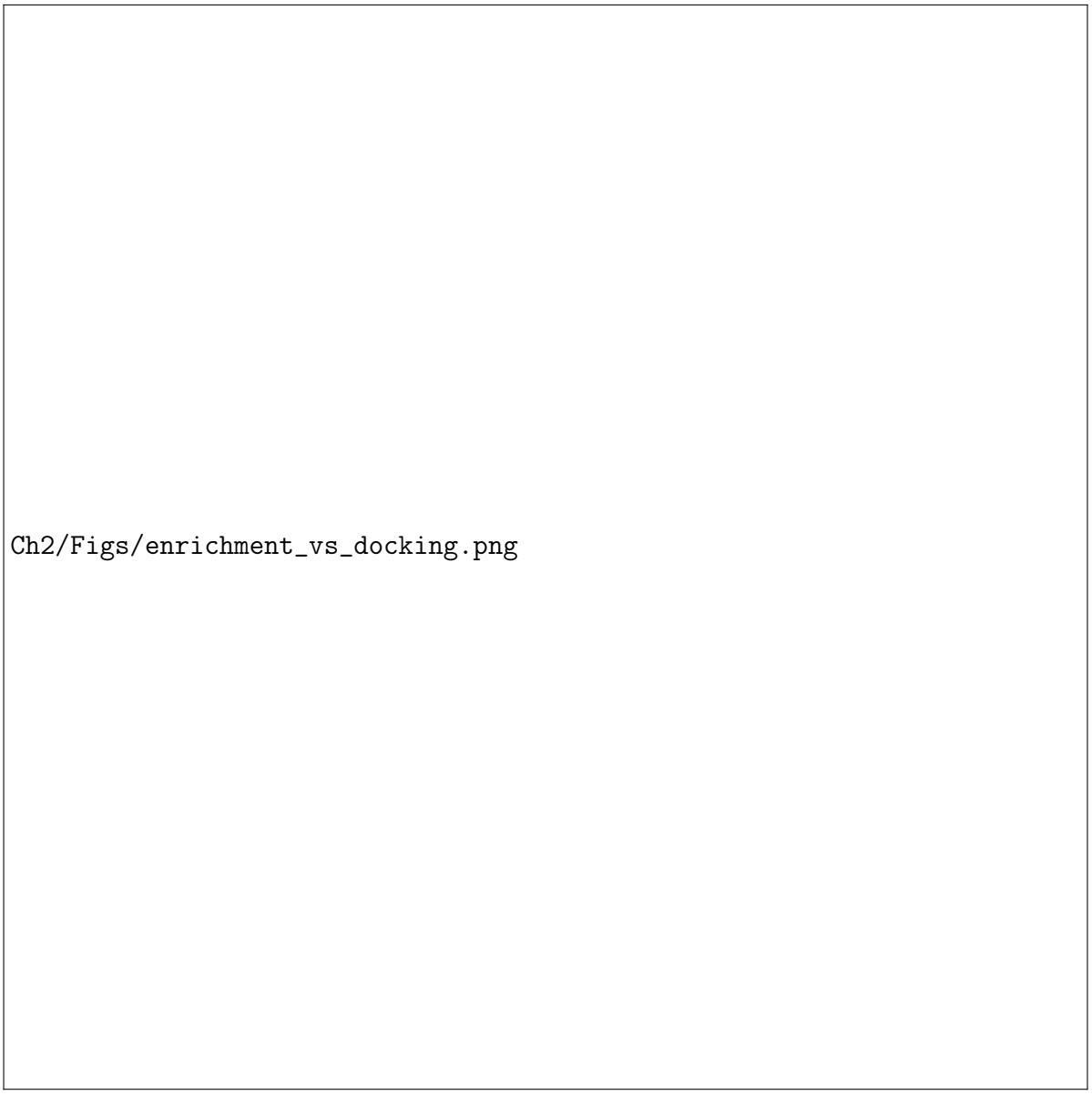

Fig. 2.2 FRESCO is able to detect potent compounds purely based on unsupervised learning. High enrichment relative to docking is achieved when performing a retrospective study on activity data COVID Moonshot.

FRESCO. This is consistent with the observed decrease in enrichment relative to docking when using data from latter stages of Moonshot where more of the submitted compounds are designed for reaching nanomolar affinity. This shows the value in using FRESCO for hit detection in the early stages of a drug discovery campaign.

TODO - Which enrichment curves to show? Put some in the SI? Should I include ZINC graph?

2.2.2 Experimental Prospective Study

After confirming the merit of this approach via a retrospective computational study, a prospective experimental search for novel hits was performed to demonstrate the capability of this methodology. We study two targets: the main protease (Mpro) of SARS-CoV-2 and the Mac1 domain of SARS-CoV-2 non-structural protein 3 (nsp3).

For both targets we followed the same workflow to discover novel hits. We first fit FRESCO models on experimental fragment-protein crystal complexes, and used the models to screen the VirtualFlow [**?**] library of commercially available compounds. The top predicted compounds were filtered by their physical properties and were clustered by structural similarity. The centroids of the 50 most populous clusters were selected as hit candidates and ordered for synthesis and testing. Details on the methodology can be found in Sec. **??**.

Mpro

For Mpro, 38 compounds were successfully synthesised and assayed. One of the compounds, WIL-UNI-d4749f31-37, was recorded with an IC50 of 25.8measured via fluorescence assay while the remaining compounds were found to be inactive. To confirm that the compound activity was not a false positive (eg measured potency due to assay interference) and that genuine ligand-protein interactions existed, a follow-up series of 8 compounds (ALP-UNI-ed5cdfd2) consisting of structural perturbations to the molecular scaffold was also synthesised and assayed. All 8 compounds exhibited inhibition at high concentrations and one compound (ALP-UNI-ed5cdfd2-1) had a lower IC50 of 19.4, demonstrating a genuine structure–activity relationship SAR for this hit compound.

Mpro order 1st batch = WIL-UNI-d4749f31, order 2nd batch = WIL-UNI-2a57d06c (no actives).

Mac-1

For Mac-1, 52 compounds were successfully synthesised and assayed. Two of the compounds show non-negligible activity at high concentration - at 250, compound Z5551425673 (as a

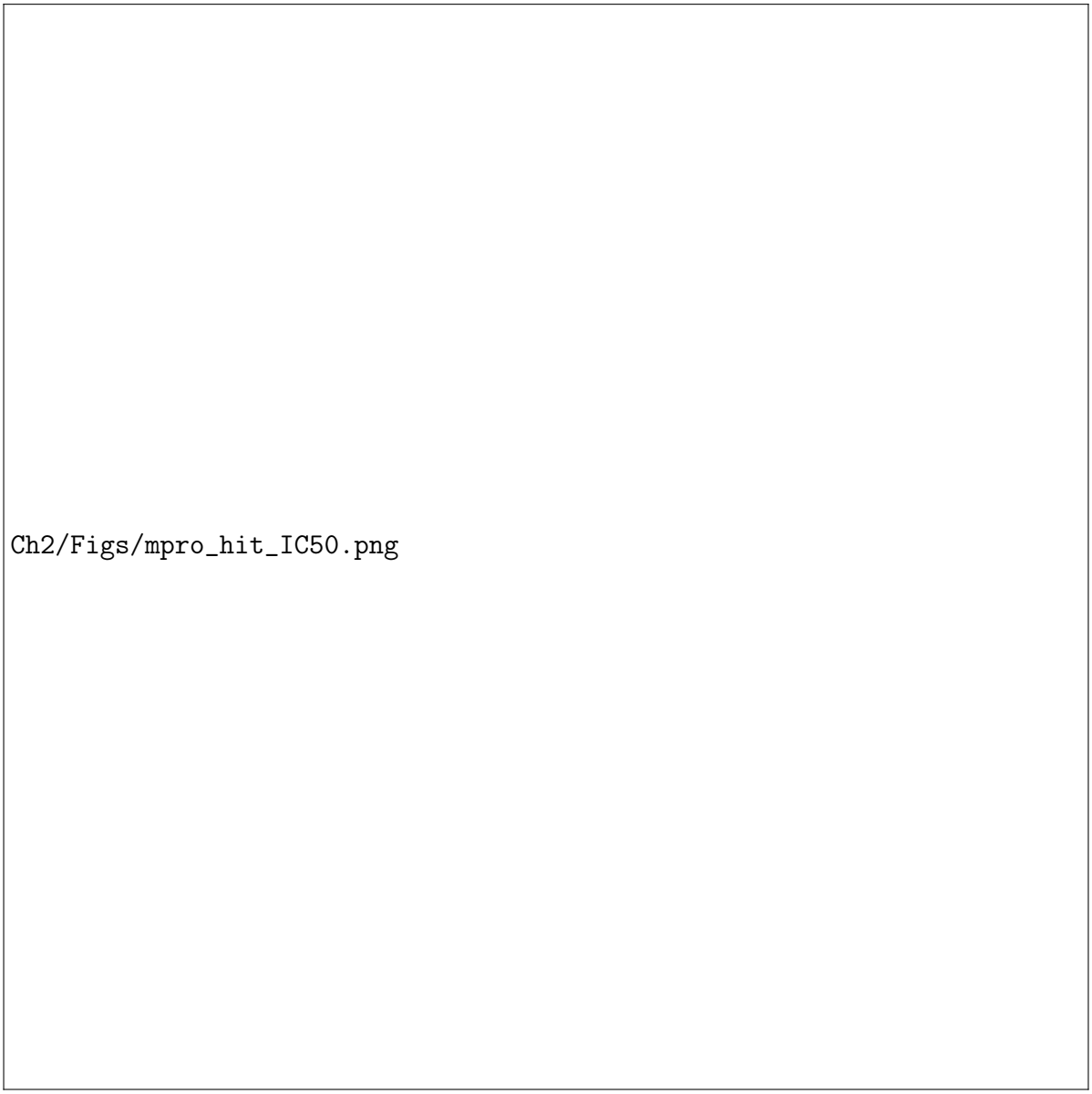

Fig. 2.3 Dose-Response curve for WIL-UNI-d4749f31-37 and follow-up compounds demonstrating SAR relationship.

racemic mixture) has an inhibition of 30.1% , while compound Z1102995175 has 24.8%. In addition, crystallographic structures of Z5551425673 (modelled as the S-stereoisomer) bound to the active site was also found alongside that of 3 other compounds (Z2890189003, Z2890182452, Z1423250928), confirming that Z5551425673 is a true hit.

As with Mpro, a follow-up series of compounds were designed to perturb the chemical structure of Z5551425673 in order to confirm the existence of SAR for this compound against Mac-1. 26 compounds were ordered and ... TODO.

TODO - table and structures in SI

2.3 Discussion

TODO - improvements that could be made? Future extensions? Transfer learning between targets? Docked fragments? Ensemble dock scores with FRESCO scores?

2.4 Conclusion

2.5 Methods

2.5.1 Datasets

The crystal structures were downloaded from `Fragalysis`. For Mpro, non-covalent fragments from the XChem fragment screen [**?**] were used while for Mac-1 both XChem and UCSF fragment data were used.

The Moonshot activity data for the retrospective study was accessed in Mar 22nd 2021. The IC50 values in that dataset, as well as in the prospective study on Mpro were measured from a fluorescence based enzyme activity assay.

For Virtual Screening, we utilize a published dataset of more than 1.4 billion commercially available molecules from EnamineREAL & ZINC15 in a ready-to-dock format [**?**].

2.5.2 Model Construction

The model used in this work takes as input the 3D pharmacophore distribution of a candidate molecule, and evaluates the log-probability that the distribution matches that of the fragment screen on the target site.

The 3D pharmacophore distribution of a molecule is obtained by extracting pharmacophores from the molecular SMILES and their corresponding conformer coordinates, and then evaluat-

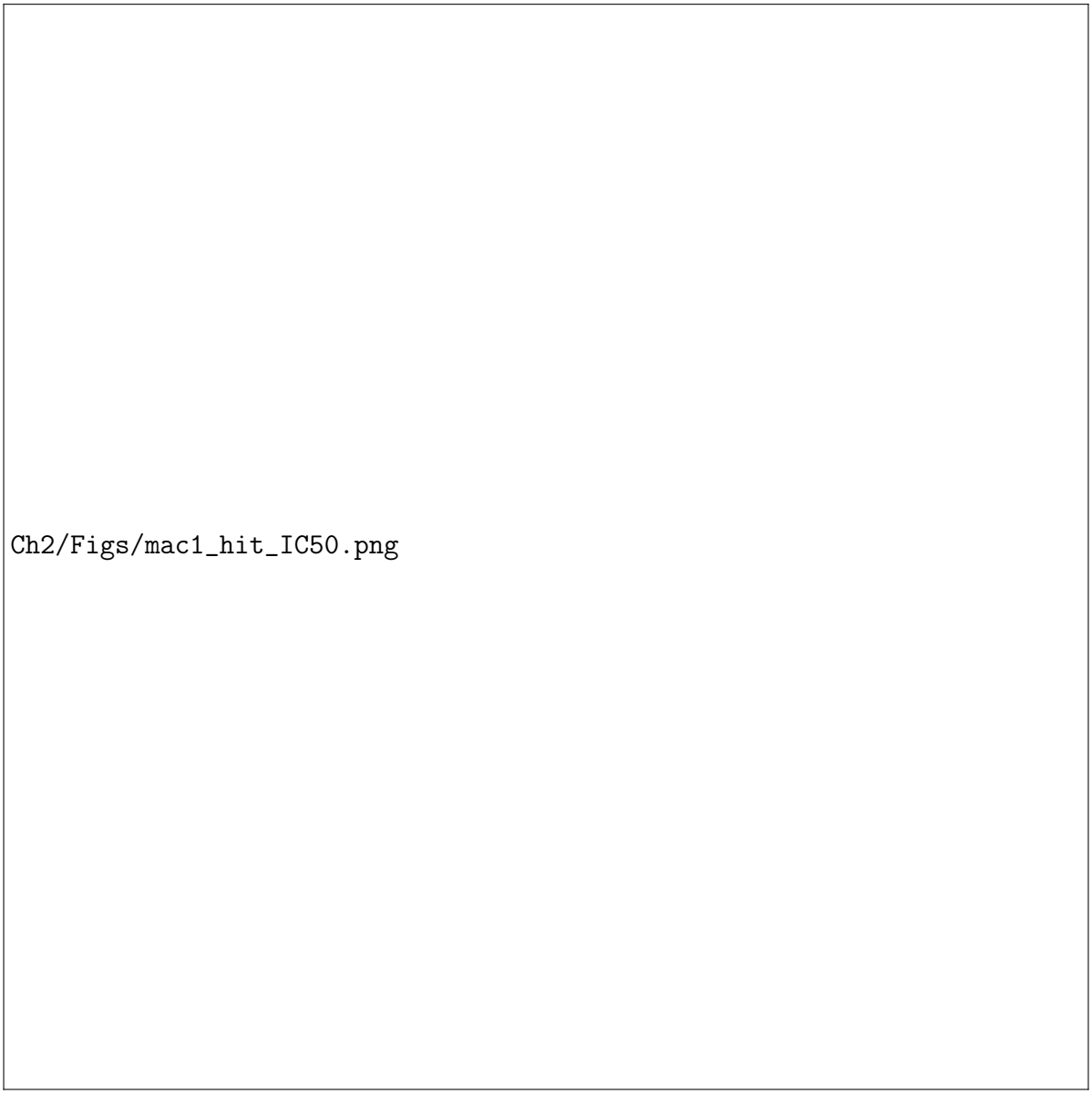

Fig. 2.4 Dose-Response curve for Z5551425673 and follow-up compounds demonstrating SAR relationship. Also showing crystal structure TODO.

ing the pairwise distance matrix between all possible pharmacophore pairs (eg Donor-Donor & Aromatic-Acceptor). SMARTS pattern matching following default pharmacophore definitions in RDKit were used to extract pharmacophores from the fragment SMILES. The pharmacophores considered are hydrogen bond donors, hydrogen bond acceptors, and aromatic rings. The coordinates of each pharmacophore are defined as the average over the atoms in the pharmacophore (eg the position of an aromatic pharmacophore from a benzene ring would be the mean of the coordinates of the 6 carbon atoms in the ring).

For some fragments, multiple crystallographic poses are recorded. To account for this, we weigh the contribution of each fragment structure to the overall fragment pharmacophore distribution by $\frac{1}{n}$ where n is the number of conformations recorded for each conformer. In addition, we exclude the counting of correlations between pharmacophores from the same fragment - only correlations between different fragments are measured. This is to avoid spurious intra-fragment correlations that have nothing to do with binding to the binding site - strong correlations in pharmacophore distribution between multiple independent fragments are indicative of useful binding interactions and these are what we hope to capture with this methodology.

The bandwidth for KDE fitting was chosen for each system using the Improved Sheather-Jones algorithm [**?**] (implemented in KDEpy). KDEs of the systems are then constructed using the chosen bandwidths with `scikit-learn` for technical ease of use in evaluating probabilities. The `scikit-learn` implementation relies on a relatively slow tree-based algorithm that searches over the training datapoints - to increase the efficiency of virtual screening, computationally fast approximations of the KDEs are made using the `scipy interp1d` function. Comparisons of the KDE bandwidth can be found in supplementary information.

Virtual screening of molecular libraries is done by evaluating the probability of the pharmacophore distribution of each molecule using the KDEs. For each pharmacophore combination, the mean log-probability of the distribution is calculated. The overall score for the molecule is returned as the mean log-probability over all of the pharmacophore combinations.

TODO - description of runtime? Emphasise no GPUs needed?

2.5.3 Compound Selection

After conducting a virtual screen, the top-500k predictions were selected and filtered to remove undesirable properties. A series of successive filtering steps were performed: first, only molecules with physical properties in well-understood "lead-like" chemical space [**?**] were kept. Secondly, the sum of the number of hydrogen bond donors and hydrogen bond acceptors were constrained to an upper limit of 8 as we noticed that the model tended to pick "messy" molecules. Then, we remove molecules that match known filters for pan-assay interference

compounds (PAINS) [**?**] as well as filters for covalent substructures (eg furan, thiophene, nitro groups). Duplicate tautomers for each molecule are also removed. Finally, for ease of synthetic accessibility, we only consider molecules with less than two chiral centers.

The top-50k molecules remaining from the filtering were then clustered via Butina Clustering [**?**] with a Tanimoto distance threshold of 0.2. This resulted in 24748 and 22358 clusters for Mpro and Mac-1, respectively. For both targets the centroids of the 50 most populous clusters (or the closest purchasable analogue if it wasn't available) were chosen as the candidate compounds. These compounds were ordered for synthesis from Enamine which resulted in 38 and 52 successfully made molecules for Mpro and Mac-1, respectively.

TODO - Confirm made/assayed molecule numbers.

2.6 Author Contributions

WM and AAL designed the study. WM and AAL devised the predictive model and WM implemented it. WM and AAL wrote the original draft, all authors commented on it.

WM acknowledges the support of the Gates Cambridge Trust. AAL acknowledges the Winton Programme for the Physics of Sustainability.

All code used for this work can be found in the GitHub repo https://github.com/wjm41/frag-pcore-screen. Supplementary figures and tables can be found in an accompanying file.

Chapter 3

Discovery of SARS-CoV-2 main protease inhibitors using a synthesis-directed de novo design model

Electronic Supplementary Information (ESI) contains experimental and assay details. Our training set, de novo design method and generated molecules are available on https://github.com/wjm41/mpro-rank-gen.

The SARS-CoV-2 main viral protease (M^{pro}) is an attractive target for antivirals given its distinctiveness from host proteases, essentiality in the viral life cycle and conservation across coronaviridae. We launched the COVID Moonshot initiative to rapidly develop patent-free antivirals with open science and open data. Here we report the use of machine learning for *de novo* design, coupled with synthesis route prediction, in our campaign. We discover novel chemical scaffolds active in biochemical and live virus assays, synthesized with model generated routes.

Coronaviruses are a family of pathogens that is frequently associated with serious and highly infectious human diseases, from the common cold to the SARS-CoV pandemic (2003, 774 deaths, 11% fatality rate), MERS-CoV pandemic (2012, 858 deaths, 34% fatality rate) and most recently the COVID-19 pandemic (ongoing pandemic, 1.7 million deaths up to Dec 2020).

The main protease (M^{pro}) is one of the best characterized drug targets for direct-acting antivirals [**?** **?**]. M^{pro} is essential for viral replication and its binding site is distinct from known human proteases, thus inhibitors are unlikely to be toxic [**?** **?**]. Moreover, the high degree of conservation across different coronaviruses renders M^{pro} targeting a fruitful avenue towards pan-cornavirus antivirals [**?**]. To date, most reported M^{pro} inhibitors are

peptidomimetics, covalent, or both [**?**]. Peptidomimetics are challenging to develop into oral therapeutics, and covalent inhibitors incur additional idiosyncratic toxicity risks. We launched the COVID Moonshot consortium in March 2020, aiming to find oral antivirals against COVID-19 in an open-science, patent-free manner [**?**].

Here we report the prospective use of a simple model to rapidly expand hits. Starting from 42 compounds with IC_{50} within assay dynamic range ($< 100\mu$M) and 515 inactives, our model designed 5 new compounds predicted to have higher activity, together with predicted synthetic routes. All designs were were chemically synthesized and experimentally tested, and 3 have measurable activity against $\mathrm{M}^{\mathrm{pro}}$. The top compound has comparable $\mathrm{M}^{\mathrm{pro}}$ inhibition to the best in the training set, but with a different scaffold, and is active against the OC43 coronavirus in a live virus assay.

Algorithmic *de novo* design aims to automatically generate compounds that are chemically diverse, synthetically accessible and biologically active [**?**]. Classic approaches apply heuristics to fragment and modify known active compounds, with the region of chemical space explored and synthetic accessibility constrained by those rules [**? ? ?**]. Recent machine learning approaches explore chemical space in more abstract molecular representation space [**? ?**], but this often comes at the expense of synthetic accessibility [**?**]. Our approach builds on rule-based fragmentation and molecule generation, but employs a method that combines regression and classification amid noisy data, and use of machine learning to predict synthesis routes. Our model comprises two parts: compound prioritisation and chemical space exploration.

Our compound prioritisation model aims to predict whether a designed compound is likely to be an improvement in activity over the incumbent. However, as is typical in the hit-expansion stage, bioactivity modelling is hindered by insufficient data where the majority of compounds are inactive, and noisy data as measurement variability increases for lower affinity compounds. Thresholding the data and framing the problem as classification of active/inactive would not allow us to rank compounds based on predicted improvement over the incumbent, yet the amount of measured bioactivity data and the measurement noise makes a regression approach challenging.

To overcome both challenges, we develop a learning-to-rank framework [**? ?**]. Rather than training a regression model to predict the IC_{50} of a compound, we instead train a classifier to predict whether a compound is more or less active than another compound, with the input to the model being the *difference* in molecular descriptors between the molecules (see Figure **??** for a schematic). This model accounts for both compounds with IC_{50} measurements and compounds that are simply inactive – active compounds are ranked by their IC_{50}, all inactives with no measurable IC_{50} are considered less active than active compounds, and inactive-inactive pairs are ignored. Further, we account for noise by only considering IC_{50}

Ch3/Figs/ranking-model.pdf

Fig. 3.1 Relative ranking of ligands can be predicted by our learning-to-rank machine learning model. (A) A schematic of the model setup. A classifier takes the difference in pharmacophore fingerprint between two molecules and predicts where one molecule is more or less active than the other. (B) The Receiver Operating Characteristic curve of classifying whether a molecule is more/less active than the other. AUC 95% CI reported in main text.

differences amongst actives above 5 μM. We use the FastAI Tabular model [**?**], with input features generated from concatenated Morgan, Atom Pair, and Topological Torsion fingerprints implemented in RDkit [**?**], and dataset was randomly split into training (80%) and testing (20%); details about model implementation can be found in ESI and source code.

Figure **??** shows that our binary ranking model achieves an AUC of 0.88 (95% CI: [0.83,0.96]) in ranking ligands within the test set, and AUC for 0.94 (95% CI: [0.91,0.98]) where we compare a ligand in the training set against another ligand in the test set; the latter is more relevant as our goal is finding ligands more active than the best incumbent. The 95% confidence interval is computed using bootstrapping. We also compare our model against OpenEye's FRED hybrid docking mode as implemented in the "Classic OEDocking" floe, a physics-based docking algorithm, on the Orion online platform, which achieves AUC of 0.72; 95% CI: [0.722,0.723] (see ESI for implementation details). Note that docking does not require ligand bioactivity as training data, thus is not a directly comparison to machine learning. In the Supplementary Material, we discuss that our model ranks ligands better than a model that directly learns IC_{50} (AUC = 0.86; 95% CI: [0.71,0.95]).

Beyond train-test split, model performance can be evaluated from a time-split. Five months have elapsed from the time we deployed our model to select compounds to writing up the manuscript. During that time, the COVID Moonshot Consortium (a team of expert medicinal chemists) has independently designed, synthesised and tested 356 compounds [**?**], out of which 15% were better than the top 2 compounds (having IC_{50} comparable within error) in our dataset. Table **??** shows that our model has an enrichment factor of $\sim$2, i.e. if we rescore the 356 compounds synthesized by the medicinal chemistry team using our model, and pick the top 1%-10% percentile, the proportion of molecules that would be better than the top 2 compounds would be $\sim$2x higher than human selection.

Having demonstrated the accuracy of our ranking model, we now turn to chemical space exploration. We first consider a set of chemically reasonable perturbations (e.g. amide to retroamide, amide to urea), which is applied to the whole set of active molecules. We then fragment along synthetically accessible bonds (e.g. amides and aromatic C-C and C-N), and reconnect the synthons to generate an exhaustive library. The resulting library of 8.8 million

Percentile	1%	2.5%	10%
Enrichment Factor	1.7	2.3	1.7

Table 3.1 Enrichment factor for the time-split dataset, where we consider model performance on data arriving after the model has been deployed to generate compounds for synthesis and testing.

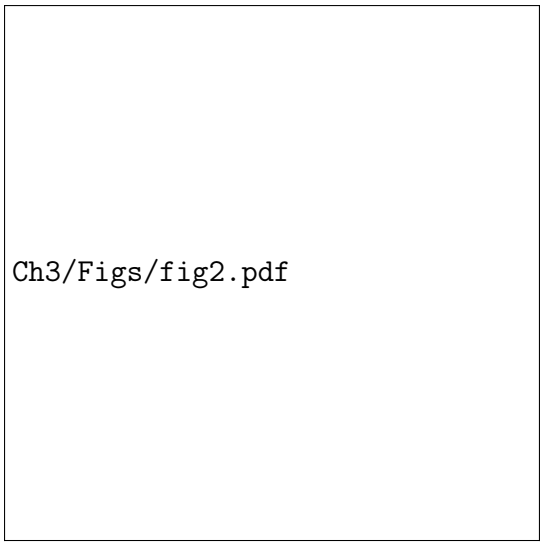

Fig. 3.2 Our synthesis-driven design model prioritises molecular scaffold that are not in the top hits. (A) The 5 compounds selected by our methodology for synthesis and testing. (B) The top 3 compounds from the training set, with potency and cytotoxicity measurements.

Fig. 3.3 Model generated synthetic schemes that are experimentally validated. Schemes (A)-(E) show the synthesis schemes generated by our model (grey) and experimental schemes for Compounds **1**-**5**. The ESI contains experimental procedures provided by our contract research organisation.

generated molecules is scored using our ranking model by the probability of having a higher potency compared to the most potent molecule in the dataset.

Although virtual "reactions" were used to generate new molecules, the synthons are not necessarily off-the-shelf nor the reactions optimal. As such, we use a retrosynthesis predictor to triage based on synthetic accessibility. We fed top hits into Manifold, our platform for synthesis route prediction (https://postera.ai/manifold). Manifold searches for synthetic routes starting from purchasable molecules. The underlying technology is based on Molecular Transformer, a machine learning model for reaction prediction using sequence-to-sequence translation [**?** **?**]. The top 5 molecules with predicted routes <4 steps were synthesised and tested (Figure **??**A). For comparison, the most potent molecules from the training set are shown in Figure **??**B; $\mathbf{1}-\mathbf{5}$ have Tanimoto similarity <0.48 (1024-bit ECFP6) to every molecule in the training set.

Figure **??** shows that for Compounds **1**, **2**, **4** and **5** our retrosynthesis algorithm generates successful routes, thus provides a reasonable estimate of synthetic complexity. The syntheses were carried out at the Wuxi AppTec and compounds were assayed as received. Minor variations in building blocks were employed depending on what was readily available. We note that our algorithm failed to estimate the synthetic complexity of Compound **3**. The final amide formation step was unexpectedly challenging, and no desired product was seen

Fig. 3.4 Three compounds generated using our synthesis-directed model exhibit Mpro activity. Our most active compound has measurable antiviral activity against the OC43 coronavirus and no measurable cytotoxic effect ($CC_{50}(A549) > 100\mu M$). 95% CI: IC50 (Mpro) – Compound 1 [3.42,4.86] μM, Compound 2 [15.1,16.5] μM, Compound 3 [48.8,69.4] μM; EC50 (OC43) – Compound 1 [10.1, 18.4] μM. See ESI for assay details.

despite significant efforts in condition screening. Compound **3** was furnished via an alternative strategy, employing an Ullmann coupling to arylate the amide, which was not predicted by our approach.

Compounds **1**-**5** were tested for Mpro activity using a fluorescence assay. Figure **??** shows that Compounds **1**-**3** have IC_{50} within assay dynamic range ($< 100\mu M$), and Compound **1** has $IC_{50} = 4.1\mu M$. Compound **1** is further assayed in live virus assays, with the less pathogenic OC43 coronavirus, showing $EC_{50} = 13\mu M$ and is not cytotoxic ($CC_{50} > 100\mu M$ against A549 cell line; CC_{50} is the concentration required to cause 50% cell death). We employ OC43 as a rapid surrogate assay for SARS-CoV-2 as the former can be done in a BSL-2 rather than BSL-3 lab. Interestingly, the top non-cytotoxic hit of the training set (TRY-UNI-2eddb1ff-7) does not show OC43 activity, showcasing the utility of using generative models to suggest new scaffolds with complementary physicochemical properties.

In summary, we demonstrated the utility of a *de novo* design model, guided by estimation of synthetic complexity, for generating ideas in hit expansion. At the time of writing, the quinolone series is undergoing optimisation by the COVID Moonshot initiative (https://postera.ai/covid). Data for Compound **1**-**5** is registered as the `ALP-POS-ddb41b15` series on the Moonshot platform.