DRUG REPURPOSING TO FIGHT SARS-COV-2 SILENCING OF THE HOST IMMUNE RESPONSE

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IDENTIFYING A VIRUS-HOST PROTEIN INTERACTION WITH THE POTENTIAL TO REVERSE VIRUS-INDUCED DISEASE PHENOTYPES

INTRODUCTION

The development of a new drug is a costly and lengthy process. As the world faces the Covid-19 pandemic, there is an urgent need to identify drugs that stop this virus proliferation and propagation—and we need to do this quickly.

Drug repurposing is a discovery strategy that identifies drugs known to be safe for humans as potential treatments for diseases. Recent research has revealed a rising number of diverse coronaviruses circulating among various bat species and other animals, suggesting the likely inevitability that new highly pathogenic coronaviruses will invade human populations in the future. [4]

Previous BIOVIA blog posts [7, 8] have demonstrated how scientists are using structure-based and virtual screening approaches to select existing drugs based on their *in silico* propensity to target viral protease and possibly reduce virus proliferation.

However, to date, no known drug has really stood out. [5] Therefore, we need to consider other approaches.

In addition to hijacking the cellular machinery for its own replication, the SARS-CoV-2 virus also interferes with several cellular mechanisms leading to fatal patient symptoms[6] (e.g., cytokine storm, inflammatory syndrome, the inhibitory effect of early innate immunity delaying immune response and promoting virus replication, etc.).

In the context of complex cellular mechanisms, signaling pathways designed in systems biology can greatly help to predict the best way to reduce the pathogenicity or toxicity effects of a virus on the body. Once we design and scientifically validate these signaling pathways, we can use them to test new therapeutic hypotheses. In this way, systems biology creates new opportunities that allow the scientific community to accelerate the investigations of potential therapeutic solutions such as reusing existing drugs, already clinically tested and known to be safe, or defining new drug combinations to treat Covid-19.

However, the amount of data collected worldwide from various Covid-19 initiatives is growing rapidly and becoming increasingly difficult to federate, maintain and utilize for the prospective and systematic selection of candidate drugs. Currently no commercial tool allows scientists to leverage this data in searching for new therapeutic treatments with high probability of success in clinical trials. As a result, scientists lose time, as they repeat steps or slowly come up to speed with knowledge that others have already acquired or understood.

In response to these obstacles, BIOVIA Dassault Systèmes' Living Map application offers capabilities that help accelerate the discovery process of mono- or multi-target therapeutic solutions by leveraging our growing body of knowledge in an efficient, maintainable and accurate way.

CHALLENGES

It is challenging to identify drugs or drug combinations that can reverse the SARS-CoV-2 effects on host cell signaling systems because:

- Validated mathematical and biological models are scarce.
- There are not many references supporting scientific facts about a virus that has recently emerged.
- Assessing the effects of polypharmacological drugs or drug combinations addressing multiple targets in biological systems is extremely complex when using classical simulation tools.
- Manually gathering and aggregating knowledge and evidence from various databases requires time-consuming/ error-prone steps.

OBJECTIVES

By combining BIOVIA Pipeline Pilot technology with the Living Map collaborative pathway modeling and simulation capabilities, we aim to facilitate:

- Access to documented expert knowledge on viral mechanisms of diseases
- Validation of mathematical and biological models for in silico experimentation or precision medicine
- Identification of potential existing drugs or combinations of drugs from referenced drug databases
- The ability to propose multiple targets

BIOVIA LIVING MAP INTRODUCTION

BIOVIA Living Map is a service on the Dassault Systèmes **3DEXPERIENCE**® platform that provides automated knowledge access, modeling and simulation capabilities for a given scientific context such as systems biology.

The BIOVIA Living Map application currently leverages these capabilities to allow system biologists and mathematicians to design and simulate Boolean biological networks. It comes with a set of scientifically validated Boolean signaling pathways that scientists can use to modify existing experiments, create new experiments and test new hypotheses.

A key feature of BIOVIA Living Map is its ability to automate entity and relation mapping to renowned cross-references, thereby scientifically validating the designed model. We generically refer to the aggregation of a Living Map model such as a pathway and its cross-references as a knowledge map.

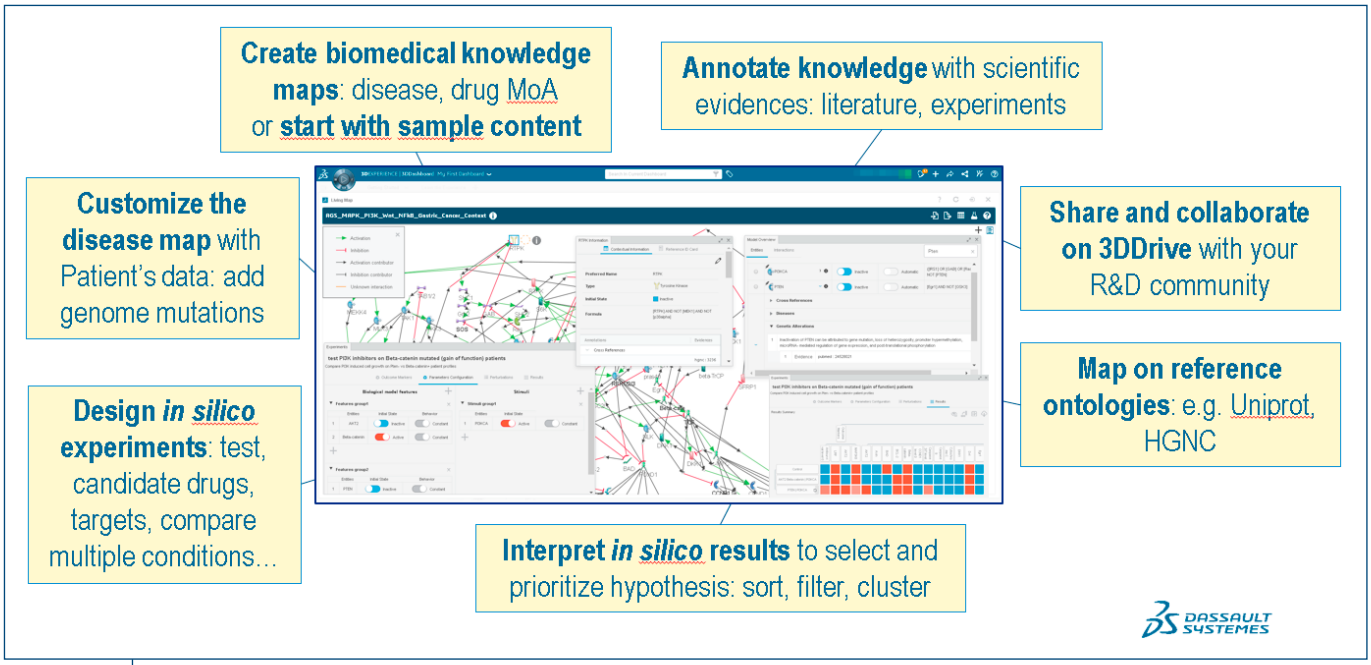


Figure 1: Key features of the BIOVIA Living Map application

Scientists can annotate a Living Map model with evidence supporting links to reference databases. They can annotate almost anything including entities, interactions and reasons for modelling. Annotations, especially annotations with evidence, improve the confidence level of the BIOVIA Living Map.

Users can visualize entities according to their function or nature such as gene, kinase, transmembrane receptor, complex, phenotype, etc. BIOVIA Living Map automatically transforms and interprets interactions (activations and inhibition relationships) between entities as Boolean equations during simulation. Therefore, a Living Map model in BIOVIA Living Map is a dynamic, qualitative network that enables the design and simulation of in silico experiments (see Figure 1). You can test research hypotheses by varying conditions such as the initial states of entities (present or absent), gene knockout, drug effects, etc. In addition to modeling and simulation, BIOVIA Living Map accelerates the collaborative collection and annotation of expert knowledge about diseases and its reuse across the R&D community.

SARS-2 MODELING OF SARS-COV-2 EFFECTS ON INNATE IMMUNITY

Identification and selection of relevant cell signaling pathways

As of today, the system biology of SARS-2 is scant, and scientists have only partially validated it. In order to model mechanisms and knowledge representing the activity of SARS-2 on human cells, we first carried out a search for articles in public databases (Signor, Pubmed, Uniprot, IntAct) that contain scientific experiments describing the biological mechanisms of coronaviruses at the human cell level.

We decided to focus on early phases of viral infection and designed our models as such. By targeting the early immune response (innate immunity), we intended to investigate new therapeutic intervention points in such a way that we could better control virus replication and propagation.

Based on these preliminary literature investigations, we selected one pathway from the Signor database [1] and three cell-signaling cascades described by Fung TS et al [2]:

- "Three branches of UPR signaling pathway activated and regulated by HCoV infection,"
- "Activation and modulation of MAPK signaling pathways by HCoV infection"
- "Type I interferon induction and signaling during HCoV infection and modulatory mechanisms"

Model creation and validation

We imported and merged the initial set of 3 signaling pathways in BIOVIA Living Map (see Figure 2). To create a Living Map model dedicated to COVID-19, we reviewed all entities and interactions that had major impacts in SARS-CoV-2. More specifically, using protein similarity calculations between the known sequence of SARS-CoV-2 and the other coronaviruses, we removed nonspecific SARS-CoV-2 viral proteins with a score below 85% similarity.

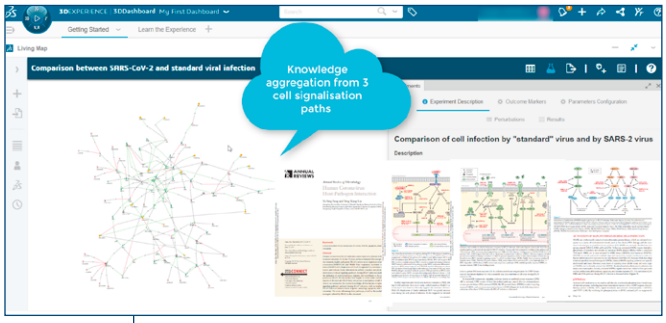
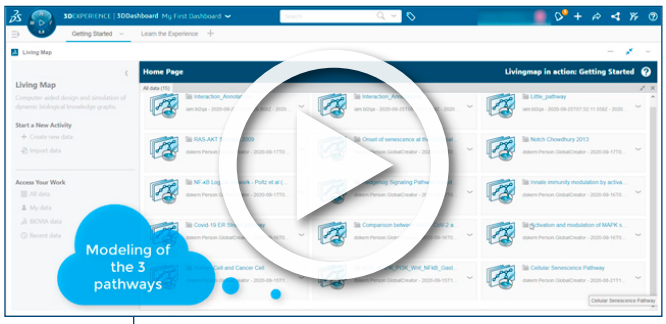


Figure 2: Knowledge aggregation of the 3 cell signalization pathways in the context of SARS-Cov2 infection

We then enriched the model by adding entities and interactions with references to scientific evidence (with data extracted from official databases: Uniprot, HGNC…) and citations with links to articles to confirm and explain the action mechanisms, the influence of an entity, etc. For example, it was necessary to crosscheck various articles in order to establish the initial / basal state of certain entities, in the context of this disease, so that the simulation results presented the expected behavior, as scientific papers did not include such information.



Video 1: Construction of a COVID-19 disease model and testing of innate immunity, inflammation and proliferation outcomes

After creating the model, we crosschecked the behavior described by Fung et al. [2] to ensure that our model respected the expected state of the phenotypes. We achieved this by defining experiments with different parameters and conditions (presence / absence of the virus) and launching simulations. It was sometimes necessary to change the model to reproduce what we had observed in vitro. We annotated all modifications and modeling reasons.

INVESTIGATION OF DRUG REPURPOSING BY SIMULATION

We have collected and integrated approved drugs known to modulate the activity of at least one protein target present in the model. With the integration and annotation of drug profiles, we enable the exploration of drug repurposing opportunities to counteract the inhibition of the innate immune response by SARS-CoV-2.

The International Union of Basic and Clinical Pharmacology / British Pharmacological Society (IUPHAR/BPS) database is a comprehensive, expert-curated, open-access database of information on drug targets and the substances acting on them (see Figure 3).

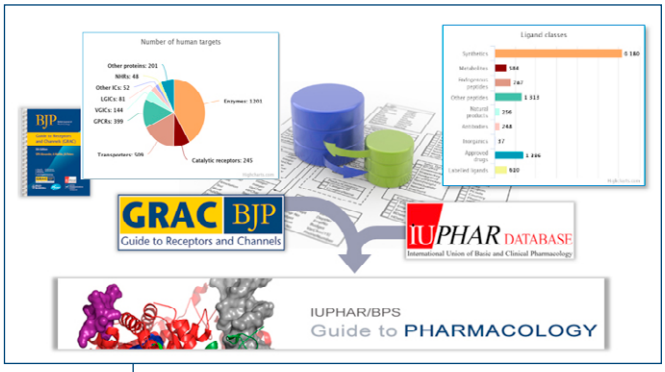


Figure 3: IUPHAR/BPS database used for drug repurposing

After developing a BIOVIA Pipeline Pilot Protocol to reassemble and filter IUPHAR [3] database tables (see Figure 4), we produced a custom pharmacological dataset consisting of (3,232) drug-effect-target triplets for all approved drugs (any modality) irrespective of their therapeutic indication. We used Uniprot cross-references as the shared identifier to map IUPHAR targets on protein entities in our model and found 33 pharmacological relations. We added 15 distinct drugs modulating 7 distinct targets to the model. We manually added additional drugs and their mechanism of action (e.g., chloroquine), extracted from recent published literature, to the model.

We simulated the effect of all approved drugs on our model using a simple Boolean simulation. We also screened all possible pairwise combinations of drugs (105). Finally, we investigated and compared the effects of the resulting therapeutic scenarios on the innate immunity and virus replication phenotypes in non-infected tissue vs. a non-coronavirus infection or a SARS-CoV-2 infection.

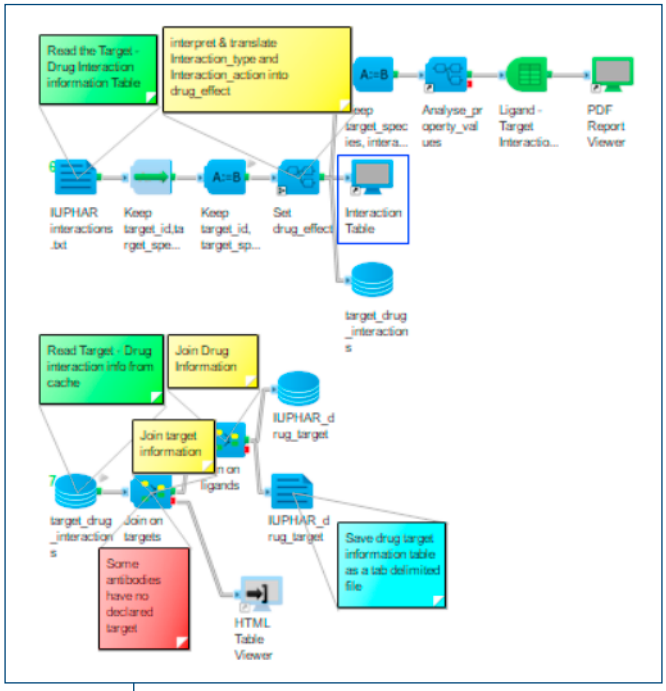


Figure 4: BIOVIA Pipeline Pilot protocol for drug repurposing strategy (extract).

RESULT

None of the 15 approved drugs or their pairwise combinations had a favorable effect on the targeted phenotypes (e.g., early innate immunity, pro-inflammatory cytokines, virus replication). However, we did find experimental drugs that demonstrated an inhibition effect on the coronavirus PLP protein [9]. The PLP (protease and papain-like protease) are essential coronavirus enzymes required for polypeptide processing during viral maturation. In addition, PLP cleaves host proteins to evade innate anti-viral immune responses [10]. In our model, inhibition of the interaction between PLP and ISG15 (Interferon-stimulated gene 15) causes the reactivation of the innate immunity leading to the silencing of the virus replication (see Figure 5).

Our approach highlights the potential role of PLP-ISG15 signaling as an escape mechanism to the early response to the SARS-CoV-2 infection. A recent publication [11] strengthening this hypothesis states that the overexpression of ISG15 in a cellular model of infections, as well as the statistical enrichment of gene expression networks associated with interferon, mediated immune response.

Figure 5: Comparative simulation of Non coronavirus (Non-CoV) viral infection and SARS-CoV-2 effects on host response: Contrary to Non-CoV, SARS-CoV-2 proteins silence the innate immunity leading to virus replication. Inhibition of the interaction between the viral PLP protein and the host ISG15 protein restores early immune response and blocks the virus replication.

CONCLUSION

In short, with BIOVIA Living Map, scientists can collaborate, understand and annotate the mechanisms of a disease on the **3D**EXPERIENCE® platform. Biologists can build and use models to investigate R&D hypotheses by simulation. In this work, we have constructed a dynamic model by assembling generic coronavirus cell signaling pathways published before the emergence of the COVID-19 pandemic. We have customized and validated our model with more specific and recent SARS-CoV-2 knowledge. We then launched simulations to investigate candidate drugs for repurposing and simulations to propose new candidate therapeutic intervention points.

Although the system did not predict an approved drug to reverse the virus-induced disease phenotypes, we identified a virus-host protein interaction with the potential of being effective. Our model suggests that inhibition of the PLP-ISG15 interaction would restore the innate immunity, likely leading to a reduction of virus replication at an early stage during the course of virus infection.

The recent publication of a SARS-CoV-2 PLP crystallographic structure [12] paves the way for the guided design of such inhibitors using molecular modeling and simulation as described by A. Goupil-Lamy et al. [8]

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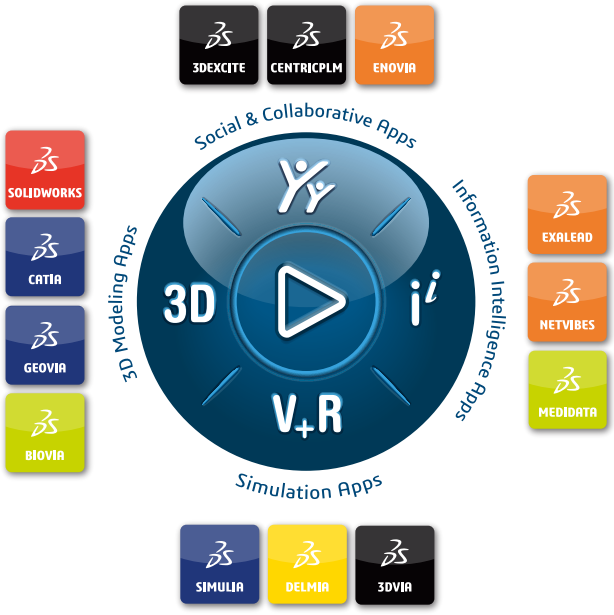

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