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AKM Azad *

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Network Medicine-Based Approach Reveals Cyclosporine and Selinexor Drug Combination as an Effective Therapy against SARS-COV-2

AKM Azad

Department of Mathematics and Statistics, Faculty of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh 13318, Saudi Arabia; kazad@imamu.edu.sa

Abstract: The proliferation of SARS-COV-2 through enhanced viral replication and its subsequent triggering of cytokine storm, are some of the hallmark phenotypes in severe COVID-19 patient cases. Cyclosporine, an immunosuppressant drug and Selinexor, an inhibitor of nuclear transporter protein, both have been successfully demonstrated to be effective against SARS-COV-2 infection by targeting those functions individually. However, the highly multifactorial pathology of SARS-COV-2 infection hinders any mono-therapeutic strategy to become an optimal option. In this study, we assess the potential efficacy of the Cyclosporine-Selinexor combination on an integrated interactome by adopting a network-medicine-based repositioning technique, where disease proximity, functional proximity and their topological separation are evaluated, followed by a robust statistical significance test. Results have shown that both drug target modules are highly proximal to the SARS-COV-2 disease modules in terms of network topology and functional associations, in a statistically significant manner, individually. Functional enrichment of both drug modules and SARS-COV-2 infected modules has shown that two drugs target the functions related to viral replication and cytokine storm during infection. Moreover, a high degree of network separation been those two drug target modules has been observed, revealing "complementary exposure" patterns, rendering this drug combination as an effective one against SARS-COV-2 infection. We hope that our results will encourage researchers to further investigate the potency of Cyclosporine and Selinexor combination in vivo or in vitro, and ultimately lead that up to clinical trials to treat SARS-COV-2 patients.

Keywords: cyclosporine; selinexor; drug combination; network medicine; SARS-COV-2; functional enrichment; drug target

1. Introduction

The emergence of Coronavirus Disease 2019 (COVID-19) caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in late 2019, has triggered a global pandemic, costing more than 7.0 million lives to date and significant loss in the global economy [1]. Significant comorbid conditions are reported to be associated with the SARS-COV-2 infection, including asymptomatic infection, cardiac arrest severe respiratory illness and death [2]. The ability to quickly spread among the mass population, significant morbidity, and extremely high mortality rate has rendered the need to find therapeutic drugs with high efficacies urgent. The initial response to this SARS-COV-2-induced pandemic primarily involved symptomatic management and supportive care as there was and still hasn't been any drug developed exclusively for SARS-COV-2 disease. Despite the successful innovations and administrations of several vaccines to control the spread, threats to the pandemic havoc remained constant due to vaccine hesitancy, efficacy, treatments for high-risk groups, potential emergence of novel strains of the SARS-COV-2 virus, etc.

Network medicine is a promising approach in the field of bio-medicine, which has the potential to analytically decipher novel drug targets and therapeutic combinations for complex diseases, especially COVID-19, which has comorbidity with other disease conditions [3]. The key aspect of the approach is to utilize the network-based analysis of the inter-dependencies among the biological entities, such as genes, proteins, and drug molecules within a disease context. This has great potential to decipher the complex and multivariate notions of those entities. Moreover, it can reveal novel therapeutic

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targets and drug combinations from existing drugs, originally designed for other but relevant disease indications - a notion denoted as *drug repositioning*.

It is known that if a list of biomarkers is identified to be involved in a particular disease context, their interacting partners are more likely to share their contribution to a similar phenotype - altogether forming a localized network of genes/proteins/molecules that can be identified as "disease module" [4]. For a particular drug to be addressed as an effective therapeutic molecule, it has to be topologically proximal to the disease module, which can be quantified as the average shortest distances between the drug-target proteins/molecules (also known as "drug module") with those of the disease module [5].

Cyclosporine is an immunosuppressant drug that plays a vital role in modulating the responses of the immune system during SARS-COV-2 infection. It has been reported that it effectively inhibits calcineurin (a eukaryotic Ca(2+)- and calmodulin-dependent serinethreonine protein phosphatase), which proved to be effective against SARS-CoV, MERS-CoV (Middle East respiratory syndrome-related coronavirus) and HIV (human immunodeficiency syndrome) viruses [6]. Cyclosporine also can reduce inflammatory cytokine production during the SARS-COV-2 infection process. Moreover, it can interfere with viral replication by inhibiting the cellular functions that are critical for SARS-COV-2 entry. On the other hand, Selinexor works as an inhibitor of the Exportin 1 (XPO1) protein, which is responsible for the transport of molecules including ORF3b, ORF9b and nucleocapsid, out of the nucleus [7]. It is reported that Selinexor prevents the proliferation of SARS-COV-2 via activating the release of anti-inflammatory cytokines [8]. In a *in vivo* study, Selinexor drug has successfully controlled the cytokine storm in COVID-19 patients via downregulating the pro-inflammatory cytokines IL-1β, IL-6, IL-10, IFN-γ, TNF-α, and GMCSF. Moreover, it has been reported that Selinexor may disrupt the SARS-COV-2 replication in severe COVID-19 cases by inhibiting its target protein, XPO1 [9].

Therefore, we hypothesized that the combination of Cyclosporine and Selinexor will offer a unique therapeutic strategy as it may integrate Cyclosporine's potential immunomodulatory and antiviral effects with Selinexor's ability to disrupt viral protein export and viral replication processes. In this research, we adopted the network-medicine approach to assess the viability of this unique combination. Here, their individual drug-target networks' topological and functional proximity with SARS-COV-2 induced disease module are assessed followed by their statistical significance tests. A network separation metric is evaluated to justify their ability to target different segments of the SARS-COV-2-induced disease module for enhanced efficacy. Moreover, cross-talk analysis of enriched functions of drug modules and their disease modules was conducted to decipher the complex mechanism underpinning their potential to be highly efficacious dual combinations against SARS-COV-2.

2. Materials and Methods

In this study, we systematically assess the combination of Cyclosporine and Selinexor drugs against SARS-COV-2 by adopting a network-medicine principle. The overall schematic diagram of this study is shown in Figure 1.

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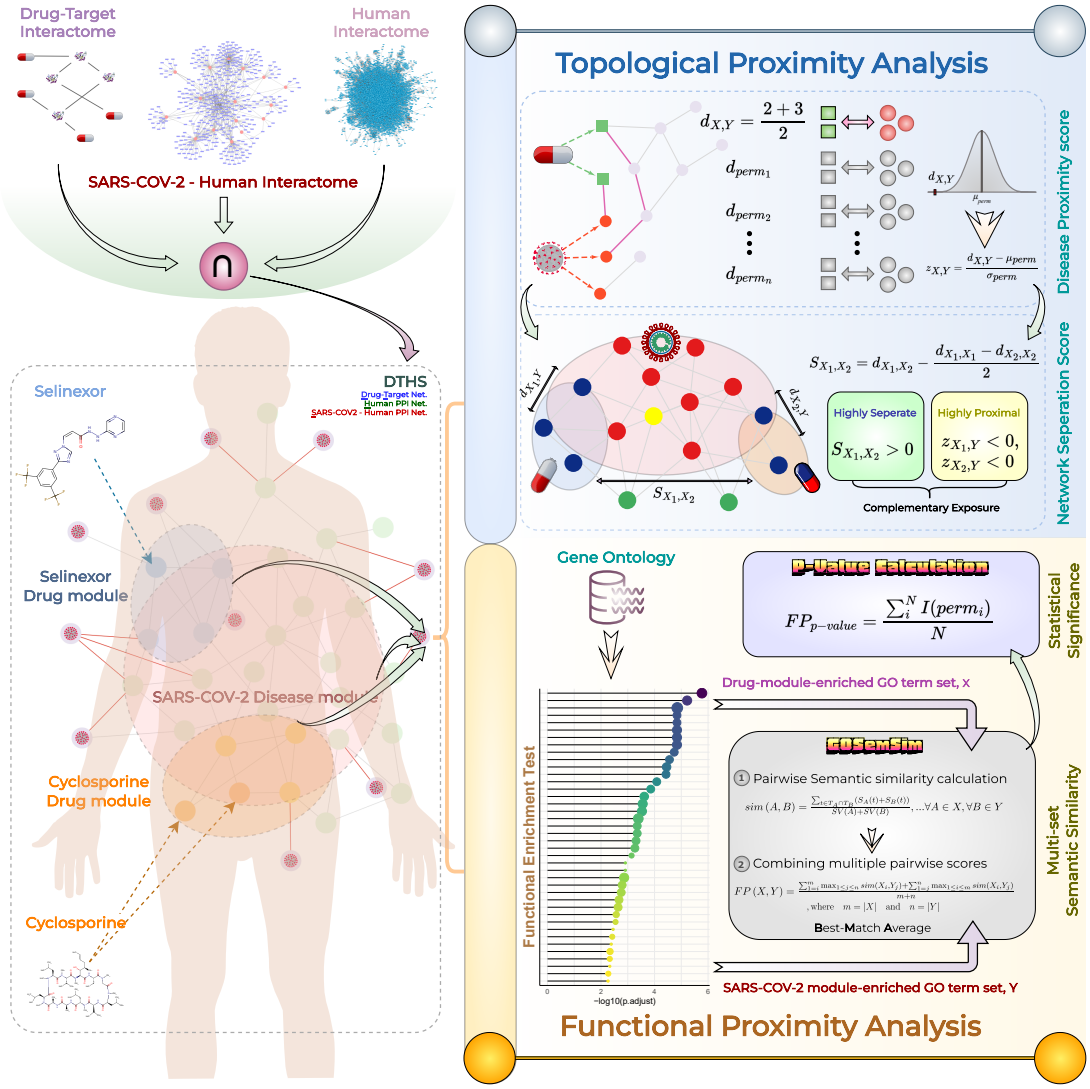

Figure 1. The overall schematic diagram for the Network-medicine-based analysis of Cyclosporine and Selinexor drug combination against SARS-COV-2 infection. First, the protein-protein interaction networks for two Drugs and their corresponding targets (with their immediate neighbourhoods), among Human and SARS-COV-2 proteins, human proteins, are collected and merged to form an integrated network, namely DTHS (Drug-Target, Human PPI and SARS-COV-2 - Human PPI). Next, topological and functional proximity measures are determined with statistical significance tests. Combined efficacy is also assessed through the quantification of their "complementary exposure" towards the SARS-COV-2 infection module via determining the separation strength of their respective drug modules in the context of SARS-COV-2 disease module

2.1. Data Collections

To conduct the network-based analysis for testing our hypothesis, i.e., the effectivity of the Cyclosporine-Selinexor combination in treating SARS-COV-2 infection, we collected drug and its target protein information from Drugbank [10]. To create host-pathogen interaction network, a comprehensive map of human (host) and SARS-COV-2 (pathogen) protein-protein interactions (PPI) were collected from Gordon *et al.* [11], whereas the human PPI data was collected from the Interologous Interaction Database (I2D) [12]. Next, for the functional enrichment of drug and disease protein modules, Gene Ontology (GO) based Biological Functions (BP) terms were collected from the Enrichr library [13]. Moreover, the gene symbol and protein identifiers were mapped by collecting the data from the UniProt database [14].

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2.2. Network Construction

To decipher the network-based perspective of a drug combination upon a disease module in the host interactome, the comprehensive network construction is considered the backbone of the whole analysis. Here, we fused three types of PPI networks for Cyclosporine and Selinexor drug-pairs, where Cyclosporine and its target, and Selinexor and its target networks from DrugBank [10], human PPI network from I2D [12], and high confidence Human-SARS-COV-2 (i.e., host-pathogen) interaction network from Gordon *et al.* [11]. This fused network is named as Drug-Target, Human PPI and SARS-COV-2 - Human PPI, in short DTHS. This fusion was done via shared PPI, where the nodes were the HUGO gene symbols, and the edges were protein-protein interactions from respective sources, after mapping UniProt protein IDs.

2.3. Functional Enrichment of Drug Targets

To find the list of GO term-based biological functions, enriched with the drug module (drug proteins and their immediate PPI partner) and the disease module of genes/proteins, we conducted the over-representation test by employing hypergeometric test using *phyper* R function from *stats* R package. We removed the GO terms that have sizes less than 15 and sizes more than 100 to remove too specific or too generic terms, respectively. The background genes/proteins used for this study were a union of all the genes/proteins involved in the GO Biological functions and the genes/proteins in the DTHS network. The nominal p-values from the hypergeometric test are later corrected using the FDR correction technique and a threshold of < 0.05 is used to define the significantly enriched GO term-based biological functions.

Measuring Topological and Functional Proximity Scores for Both Cyclosporine-SARS-COV-2 and Selinexor-SARS-COV-2

2.3.1. Topological Proximity Calculation

It is understood that like other diseases, SARS-COV-2-affected host (Human) genes would form a modular pattern, where those affected genes will remain in close vicinity with each other via PPI connections [15]. Topological proximity scores the average shortest path length between the drug and disease modules of genes/proteins in an interactome. Let, X, and Y be two vectors of genes/proteins, indicating a set of target genes/proteins (in humans), and a set of disease genes/proteins that are perturbed by the SARS-COV-2 disease, respectively. If $x \in X$, and $y \in Y$ are those target genes/proteins and disease genes/proteins, respectively, then the topological proximity (TP) of a drug module (i.e., X) with the corresponding disease module (i.e., Y) is measured with the following equation:

$$d(X,Y) = \frac{1}{\|Y\|} \sum_{y \in Y} \min_{x \in X} d(x,y) \tag{1}$$

where the $d(x,y)$ is defined as the shortest distance between a target gene/protein x and a disease gene/protein y perturbed by SARS-COV-2. Next, this proximity score, $d(X,Y)$, is subjected to be tested for its statistical significance, which we outline in 2.5.

2.3.2. Functional Proximity Calculation

The functional proximity of a drug module with the SARS-COV-2 disease module assesses the impact of the drug upon the perturbed segment of the host interactome at the downstream level. This evaluation enhances our understanding, of where the topological proximity measurement falls short of providing. Here, the Gene Ontology-based biological functions were used to conduct the functional proximity measurement. First, the functions enriched by drug module and disease modules of genes/proteins were found [see 2.3]. Then semantic similarity scores were computed by employing these two sets of functions (i.e., one set for the drug module-enriched functions and the other set is for

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the SARS-COV-2 enriched functions) by using the *mgosim* function via *GOSemSim* R package [16]. It follows the approach defined by Wang *et al.*, which is summarized below.

Let, $DAG_A = (A, T_A, E_A)$ and $DAG_B = (B, T_B, E_B)$ are two Directed Acyclic Graphs for two GO Terms A and B, respectively, where $\forall A \in X$ and $\forall B \in Y$, and X and Y are two sets of enriched functions for drug-module and the disease module, respectively [Figure 1]. Here, T_A and E_A are set of GO terms and set their relationships, respectively, within the DAG_{A_i}, which consists of the GO term A and all of its ancestors. For the GO term B, the T_B and E_B can also be defined in a likewise manner. Next, a semantic value for a GO term A is defined as the sum of all the S-values, which is as follows:

$$SV(A) = \sum_{t \in T_A} S_A(t) \tag{2}$$

where, $S_A(t)$ is the S-value associated with the term A, which is defined as below:

$$\begin{cases} S_A(A) = 1 \\ S_A(t) = \max\{w_e * S_A(t') \mid t' \in \text{childrenof}(t)\} \text{ if } t \neq A \end{cases} \tag{3}$$

Next, the semantic similarity score is determined by employing the S-values and the individual semantic values SVs for each GO term, given $DAG_A = (A, T_A, E_A)$ and $DAG_B = (B, T_B, E_B)$, for GO term A and B, respectively.

$$sim(A, B) = \frac{\sum_{t \in T_A \cap T_B} S_A(t) + S_B(t)}{SV(A) + SV(B)} \tag{4}$$

Note, to find the functions enriched by the target proteins of a drug through the enrichment analysis [see 2.3], the list of target proteins was augmented with their immediate PPI partners within the comprehensive interactome, DTHS. This step offers a viable enrichment analysis as Selinexor had only one target protein, namely, XPO1. The semantic similarity measurement uses the topology and information content of the Gene Ontology graph to compare the GO functional terms of the drug module (Cyclosporine and Selinexor targets and their immediate PPI partners) and the SARS-COV-2 disease module. Pairwise semantic similarity scores for each pair of terms from drug modules and disease modules were aggregated using a best-match average (*bma*) strategy to find a combined semantic similarity by using the following equation, which is later defined as the functional proximity (*FP*) score between a drug module and SARS-COV-2 disease module. [17].

$$FP(X, Y) = \frac{1}{(m+n)} \times \left(\sum_{1=i}^{m} \max_{1 \leq j \leq n} sim(X_i, Y_j) + \sum_{1=j}^{n} \max_{1 \leq i \leq m} sim(X_i, Y_j)\right) \tag{5}$$

, where $m = |X|$ and $n = |Y|$

2.4. Measuring Network Separation Score for Cyclosporine and Selinexor Drugs

The network separation scores, namely S_{X_1,X_2} of Cyclosporine and Selinexor drugs state the extent their respective drug modules are topologically separated within the comprehensive interactome, DTHS. The potential importance of S_{X_1,X_2} are of two folds: the larger the magnitude of S_{X_1,X_2}, the more effective the Cyclosporine and Selinexor combination is and the lesser their synergistic toxicity is [18]. The quantification of S_{X_1,X_2} is determined by comparing mean shortest distances within their respective drug modules, i.e., d_{X_1,X_1} and d_{X_2,X_2} (intra-module), with the mean shortest distance with the genes/proteins of Cyclosporine drug module with those of Selinexor drug module i.e., d_{X_1,X_2} (inter-module). Note, here a drug module is composed of the target genes/proteins of a drug and its immediate PPI interactive partners in the host interactome. The equation for calculating S_{X_1,X_2} is given below:

$$S_{X_1,X_2} = d_{X_1,X_2} - \frac{d_{X_1,X_1} - d_{X_2,X_2}}{2} \tag{6}$$

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where d_{X_1,X_2} is defined as the average shortest distance between the genes/proteins in the Cyclosporine drug module with the nearest genes/proteins in Selinexor drug modules, and the genes/proteins in the Selinexor drug module with the nearest genes/proteins in Cyclosporine drug modules. The equation to calculate d_{X_1,X_2} is given below:

$$d_{X_1,X_2} = \frac{1}{\|X_1\| + \|X_2\|} \times \left(\sum_{x' \in X_1} \min_{x'' \in X_2} d(x', x'') + \sum_{x'' \in X_2} \min_{x' \in X_1} d(x', x'') \right) \tag{7}$$

2.5. Statistical Significance Analysis

Statistical significance analysis is important to outline the robustness of findings through any analytical approaches. Here, we have conducted several statistical techniques to find the significance of all the methodologies used. First, to find the significance of the functional enrichment using the over-representation analysis (ORA), a hypergeometric test was conducted, which yielded a statistical significance p-value of the enrichment itself, which was further corrected using the FDR-correction technique to get FDR-corrected p-values.

Next, the statistical significance of the topological proximity and functional proximity scores of Cyclosporine and Selinexor drug modules with SARS-COV-2 disease modules were tested against a reference distribution of such values, that are found through a permutation test with a certain number of iterations (N). At each iteration of the permutation test, a background network is constructed, where each genes/proteins of a drug module (i.e., Cyclosporine or Selinexor) DTHS network were permuted (*without repetition*), but maintaining the same size and degree distribution of that drug module. Next, for the topological proximity score, the observed score was converted to a z-score, with the aggregated mean (μ_{perm}) and standard deviation (σ_{perm}) of similar scores from all iterations of the permutation test (see below), and corresponding the left-tail p-value (i.e., $Pr(Z < z_{X,Y})$) was determined from the Z-distribution. Therefore, for a drug to be highly significant in terms of its topological proximity with the SARS-COV-2 disease module, the observed $z_{X,Y}$ has to be negative and its magnitude be very high.

$$z_{X,Y} = \frac{d(X,Y) - \mu_{perm}}{\sigma_{perm}}$$

To conduct the statistical significance test for the functional proximity score, we set a null hypothesis that the functional proximity score within a permutation (FP_{perm_i}) is greater or equal to that of the observed functional proximity score of a particular drug (FP_{obs}). This is outlined below:

$$H_0 : FP_{perm_i} \geq FP_{obs}$$

$$H_1 : FP_{perm_i} < FP_{obs}$$

The empirical p-value for rejecting the null hypothesis can be found below:

$$FP_{p-value} = \frac{\sum_i^N I(perm_i)}{N}$$
$$I(perm_i) = \begin{cases} 1 & \text{if } FP_{perm_i} \geq FP_{obs} \\ 0 & otherwise \end{cases} \tag{8}$$

3. Results

3.1. Monotherapeutic Targets of Cyclosporine and Selinexor Drugs for Treating SARS-COV-2 Patients

Cyclosporine is an immunosuppressive agent that works as an effective calcineurin inhibitor against SARS-CoV, MERS-CoV (Middle East respiratory syndrome-related coronavirus) and HIV (human immunodeficiency syndrome) viruses [6]. For SARS-CoV-2 infection, this drug has been tested

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in the Vero E6 cells model, along with other potential therapeutics, e.g. Remdesivir and diltiazem [19]. It has a list of four primary targets in Humans, namely, PPP3R2, PPIA, CAMLG, and PPIF. In our network-based study, it is shown that Cyclosporine may target and inhibit the cyclophilin activities of its target PPIA (peptidyl-prolyl isomerase activity) and its downstream kinase activities [Figure 2a]. Moreover, Selinexor has the potential role to suppress the pro-inflammatory cytokines during the SARS-COV-2 infection via releasing anti-inflammatory cytokines [8]. It has only one drug target, namely, XPO1, inhibition of which may contribute to blocking the nuclear export, RNA transport and inflammatory signalling, necessary for viral replication [7,20]. In this work, we report that inhibition of XPO1 through Selinexor can suppress the nuclear export of viral proteins, namely SARS-COV-2 ORF3A, SARS-COV-2 ORF9B, SARS-COV-2 Spike, and SARS-COV-2 ORF6 [7].

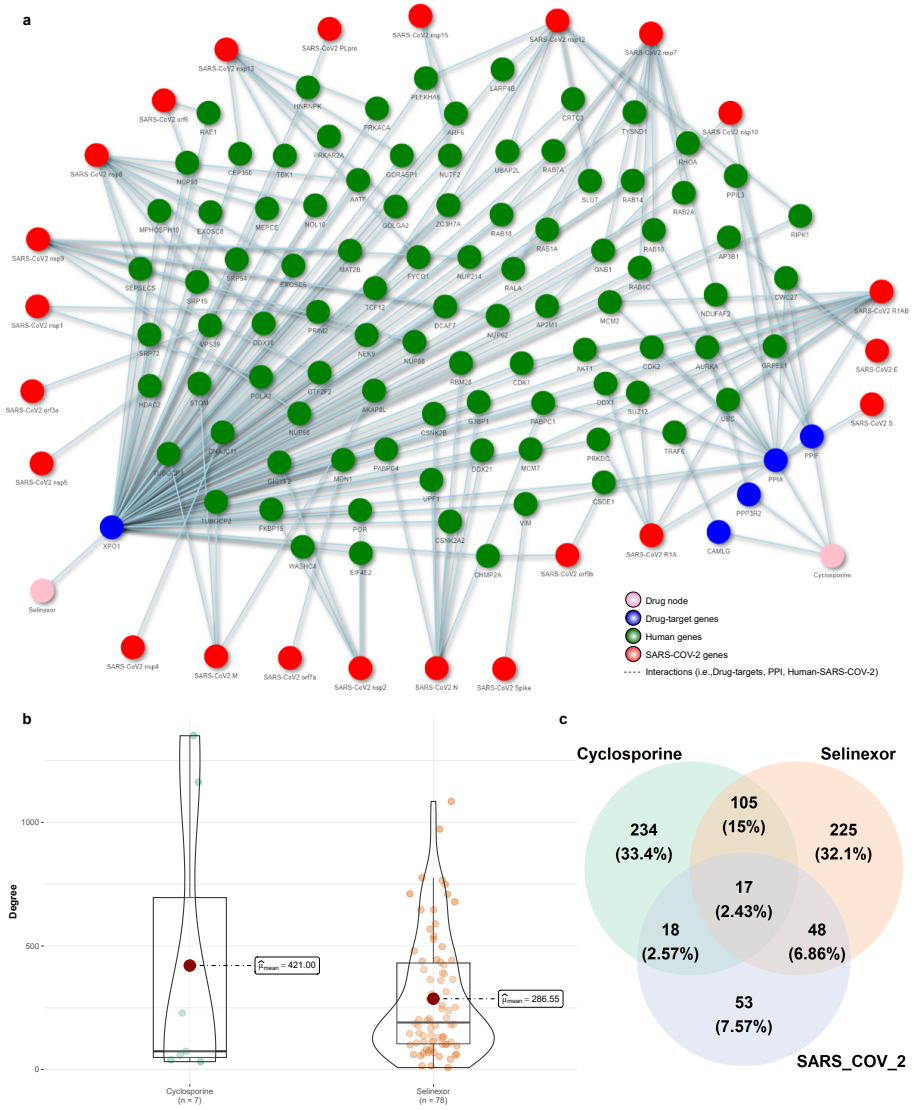

Figure 2. DTHS (Drug-Target, Human PPI and SARS-COV-2 - Human PPI) Network view of the network-based mechanism of actions of Cyclosporine and Selinexor drugs on SARS-COV-2 and human interactome, **(a)**. Degree distribution of both drugs in the DTHS, **(b)**, where for each drug, unique disease genes targeted by drug targets' PPI neighbourhood are considered [Table 1]. The number of biological functions from Gene ontology enriched by two drug modules (drug target and their immediate PPI partner) and the SARS-COV-2 disease module, and their overlaps are shown, **(c)**.

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3.2. DTHS Network Construction for Evaluating Complementary Exposure of Cyclosporine and Selinexor Combination against SARS-COV-2

To study the combination effects of Cyclosporine and Selinexor drugs on SARS-COV-2-affected human host interactome, we combined multiple networks, namely, Drug-target networks, Human PPI networks, and SARS-COV-2 and Human PPI networks. Table 1 shows the basic characteristics of complementary exposure of these two drugs on SARS-COV-2 disease modules [Figure 2a]. When we observed the degree distributions of only the unique disease genes (i.e., SARS-COV-2 affected ones) targeted by drug targets' PPI neighbourhood (shortest path length, $SPL = 1$), both drug modules revealed a high degree of connectivities within the SARS-COV-2 infected disease modules, where the mean degree scores were 421 $(n = 7)$ and 286 $(n = 78)$ [Figure 2b].

Table 1. DTHS (Drug-Target, Human PPI and SARS-COV-2 - Human PPI) Network characteristics of Cyclosporine and Selinexor combination therapy for SARS-COV-2: Coverage of COVID-19 genes the Drug targets and their PPI (Protein-Protein Interaction) neighbourhood (shortest path length, $SPL = 1$)

	Cyclosporine	**Selinexor**
Drug Targets (Human)	PPP3R2, PPIA, CAMLG, PPIF	XPO1
No. of PPI neighbours of Drug targets within the DTHS network	**356**	**1758**
No. of Disease genes as the PPI neighbours of Drug targets	**16**	**87**
Unique Disease genes targeted by drug targets' PPI neighbourhood	Total = **7** (UBC, CWC27, PPIL3, TRAF6, PPIA, NDUFAF2, GRPEL1)	Total = **78** (ARF6, G3BP1, CDK1, CSNK2A2, DCAF7, MCM7, PRKACA, PRKDC, GNB1, RAB1A, FKBP15, SUZ12, RAB14, AP3B1, PABPC4, RIPK1, HNRNPK, NUP62, STOM, GORASP1, SLU7, GTF2F2, RHOA, DDX1, VPS39, EIF4E2, RAB18, CSNK2B, DDX10, RAB7A, SRP19, RBM28, NOL10, UBAP2L, MPHOSPH10, AP2M1, RAB2A, NUP214, RALA, HDAC2, GOLGA2, WASHC4, TUBGCP3, POR, NUP98, PRKAR2A, NUTF2, RAE1, PRIM2, DDX21, UPF1, POLA2, CEP350, EXOSC5, GIGYF2, NUP58, PLEKHA5, CHMP2A, EXOSC8, SRP72, MEPCE, SRP54, TBK1, TUBGCP2, CRTC3, LARP4B, TCF12, NUP88, NEK9, SEPSECS, AATF, FYCO1, AKAP8L, DNAJC11, MDN1, TYSND1, MAT2B, ZC3H7A)
Pathogen genes targeted by drug targets or its PPI neighbourhood	Total = **10** (SARS-COV2 R1A, SARS-COV2 R1AB, SARS-COV2 N, SARS-COV2 ORF9B, SARS-COV2 NSP7, SARS-COV2 SPIKE, SARS-COV2 E, SARS-COV2 NSP12, SARS-COV2 S, SARS-COV2 NSP10)	Total = **22** (SARS-COV2 N, SARS-COV2 ORF9B, SARS-COV2 NSP15, SARS-COV2 R1AB, SARS-COV2 NSP9, SARS-COV2 NSP13, SARS-COV2 R1A, SARS-COV2 NSP7, SARS-COV2 NSP2, SARS-COV2 E, SARS-COV2 NSP12, SARS-COV2 PLPRO, SARS-COV2 M, SARS-COV2 ORF3A, SARS-COV2 NSP8, SARS-COV2 SPIKE, SARS-COV2 NSP10, SARS-COV2 NSP5, SARS-COV2 ORF6, SARS-COV2 NSP1, SARS-COV2 NSP4, SARS-COV2 ORF7A)

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3.3. Both Cyclosporine and Selinexor Are Significantly Proximal to SARS-COV-2 Disease Modules

The efficacy of a drug primarily depends on the way it affects the disease module that consists of the genes/proteins directly/indirectly affected by that particular disease, e.g., SARS-COV-2. This notion is directly associated with the fact that the drug targets remain within or immediate neighbourhood of the diseased-perturbed gene modules in the host PPI interactome. Here, for the dual combination of Cyclosporine and Selinexor, we observed their combined efficacy upon the SARS-COV-2 disease module through the network-medicine approach. Using the DTHS combined networks of the Cyclosporine shows the topological proximity value as a z-score of -4.67 along with the significance p-value of 1.49×10^{-6}. Moreover, Selinexor has also been found to be highly proximal to the disease module (i.e., SARS-COV-2 perturbed human genes) with topological proximity value as a z-score of -6.66 along with the significance p-value of 1.33×10^{-11}.

This result suggests that the targets of both of these drugs possess intimate connections with the gene modules perturbed by SARS-COV-2 infections within the human host by revealing a significant overlap between its target sub-network and the virus-altered molecular interactome. Moreover, the negative z-scores for both Cyclosporine and Selinexor underscore that such topological proximities with disease-altered interactome are not just a random closeness but also statistically robust, compared with several background simulations ($N = 500$) [Section 2.5]. Here, at each iteration, the background DTHS were permuted in such a way that the similar topological landscapes remained (i.e., similar degree distributions) intact, but the genes/proteins were randomly altered. Again, the negative z-score value indicates that within the distribution of permuted topological proximity scores, the observed scores are placed on the extremely left-sided, for which the probability, $Pr(Z < z)$ is extremely low (i.e., p-values of 1.49×10^{-6} and 1.33×10^{-11} for Cyclosporine and Selinexor, respectively. Therefore, it can be suggested that the combination of Cyclosporine and Selinexor could orchestrate a highly efficient therapeutic strategy against SARS-COV-2 viral onslaught with their profound topological proximities with the disease module.

3.4. Functional Pathways/Terms Enriched by Cyclosporine and Selinexor Drug Modules and SARS-COV-2 Disease Module

To observe the functional footprints of each drug, we conducted the GO term (biological processes only) enrichment analysis for the target genes of each drug and their immediate neighbourhood in the Human PPI interactome (shortest path length, $SPL = 1$) and the human genes perturbed by the SARS-COV-2 infection. The background gene set for this enrichment analysis was defined as the union of all the genes involved in all the GO terms used and the drug target genes along with their immediate neighbourhood. The list of GO Biological functions that had size < 15 or > 100 were pre-filtered before the enrichment analysis to remove too specific or too generic functional terms. Fisher's exact test was conducted as the over-representation analysis (ORA) of the input genesets, where the p-values of that statistical test were corrected using the FDR (False discovery rate) approach. Applying the FDR-adjusted p-value < 0.05 as a threshold, 374 [Figure 2c], 395 [Figure 2c] and 136 [Figure 2c] Biological processes were found significantly enriched for Cyclosporine drug module (target proteins and immediate PPI neighbourhoods), Selinexor drug module (target proteins and immediate PPI neighbourhoods), and SARS-COV-2 perturbed gene sets, respectively. Among these processes, 35 out of 374 functions were found common between Cyclosporine drug-induced and SARS-COV-2 perturbed disease modules and 18 of them were exclusively enriched by the Cyclosporine drug module only. Likewise, 65 out of 395 Biological processes were shared between the Selinexor drug module and the disease module and 48 of them were exclusively enriched by the Selinexor drug module only. Note, that only 17 biological processes that were enriched by SARS-COV-2 perturbed genes/proteins, were also enriched with the Cyclosporine and Selinexor drug modules (drug-target protein and their immediate PPI partners in Human interactome). Figure 3a, Figure 3b, and Figure 4 show the top 40 functions (ascending order of $adj.p$-values) that are significantly enriched with the Cyclosporine drug-module, Selinexor drug-module and the SARS-COV-2 infected disease module, respectively.

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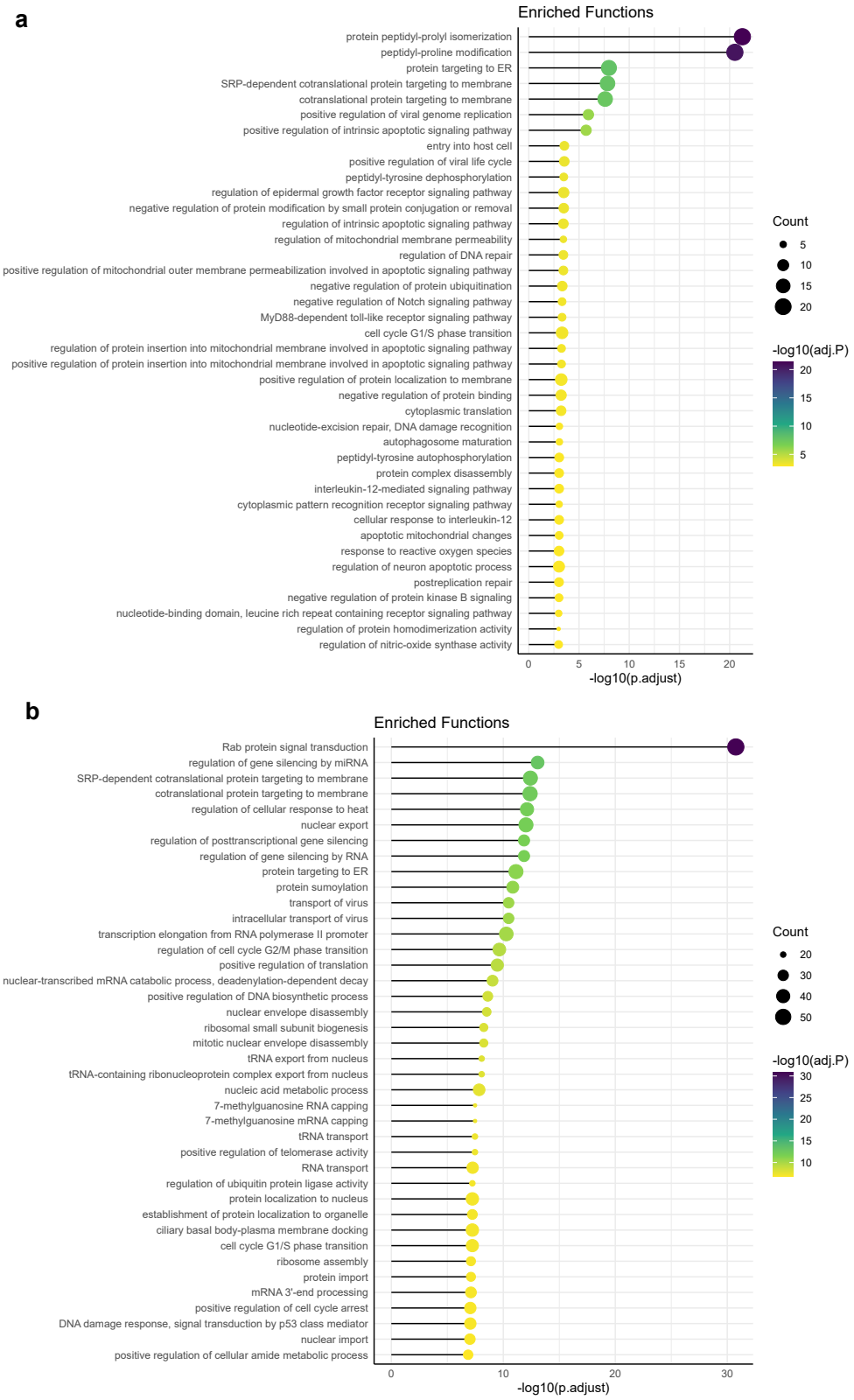

Figure 3. Functional enrichment results of **(a)** Cyclosporine and **(b)** Selinexor drug targets and the immediate neighbourhoods with GO biological processes. Here top 40 enriched terms for both drugs are shown, where the too-specific (size < 15) and too-general (size > 100) GO terms are excluded from the analysis apriori.

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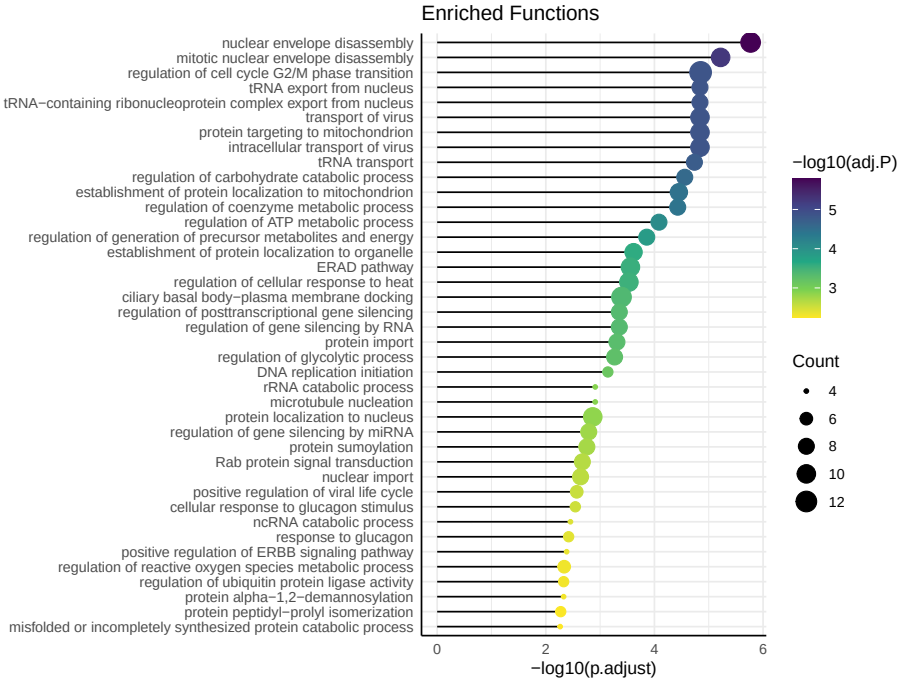

Figure 4. Functional enrichment results of SARS-COV-2 perturbed genes in human interactome. Here top 40 enriched terms for both drugs are shown, where the too-specific (size < 15) and too-general (size > 100) GO terms are excluded from the analysis apriori.

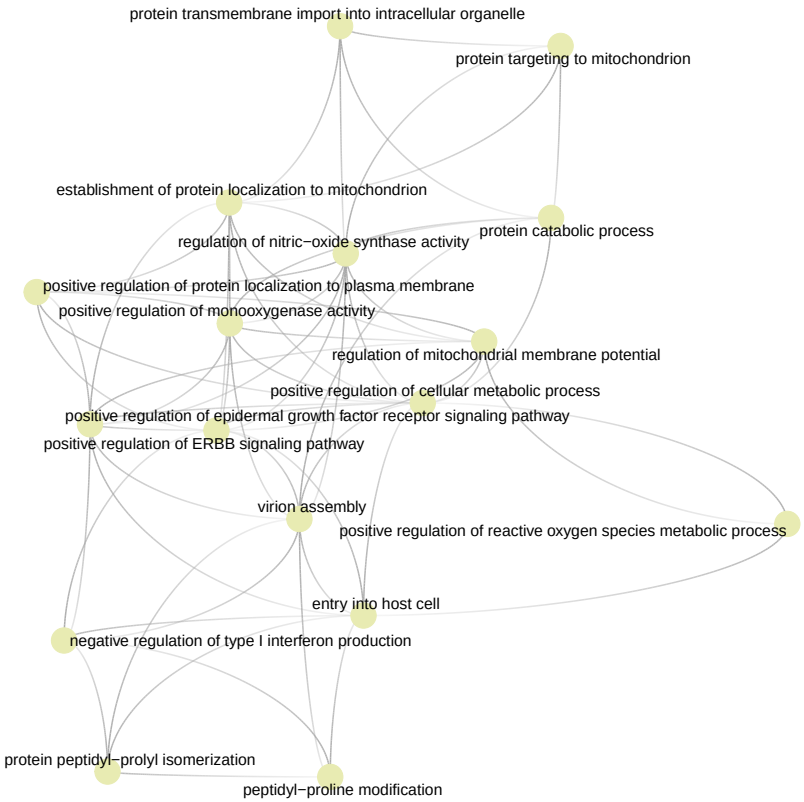

Figure 5. Crosstalk visualization of SARS-COV-2 induced biological functions that are **exclusively** enriched by the **Cyclosporine drug module**. Here nodes are significantly enriched functions and interactions among them represent their cross-talk via shared genes/proteins in the drug module.

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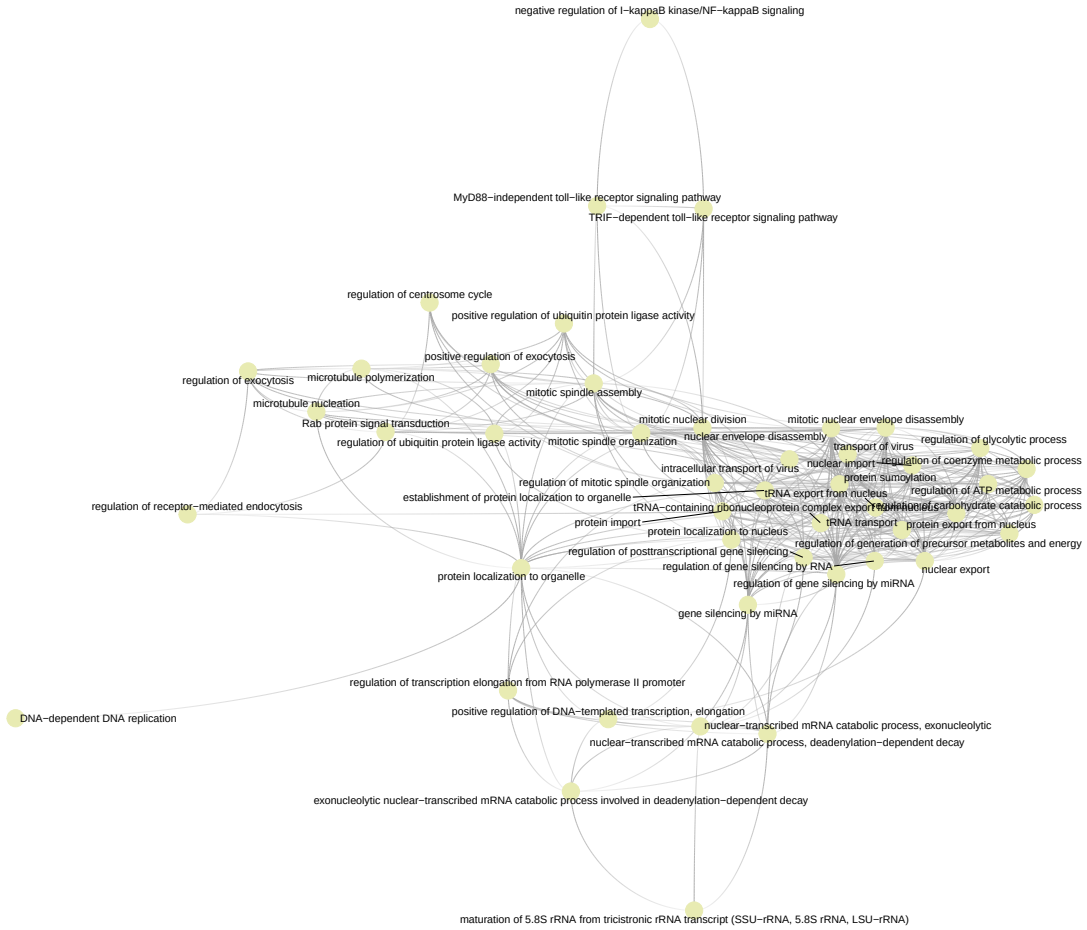

Figure 6. Crosstalk visualization of SARS-COV-2 induced biological functions that are **exclusively** enriched by the **Selinexor drug module**. Here nodes are significantly enriched functions and interactions among them represent their cross-talk via shared genes/proteins in the drug module.

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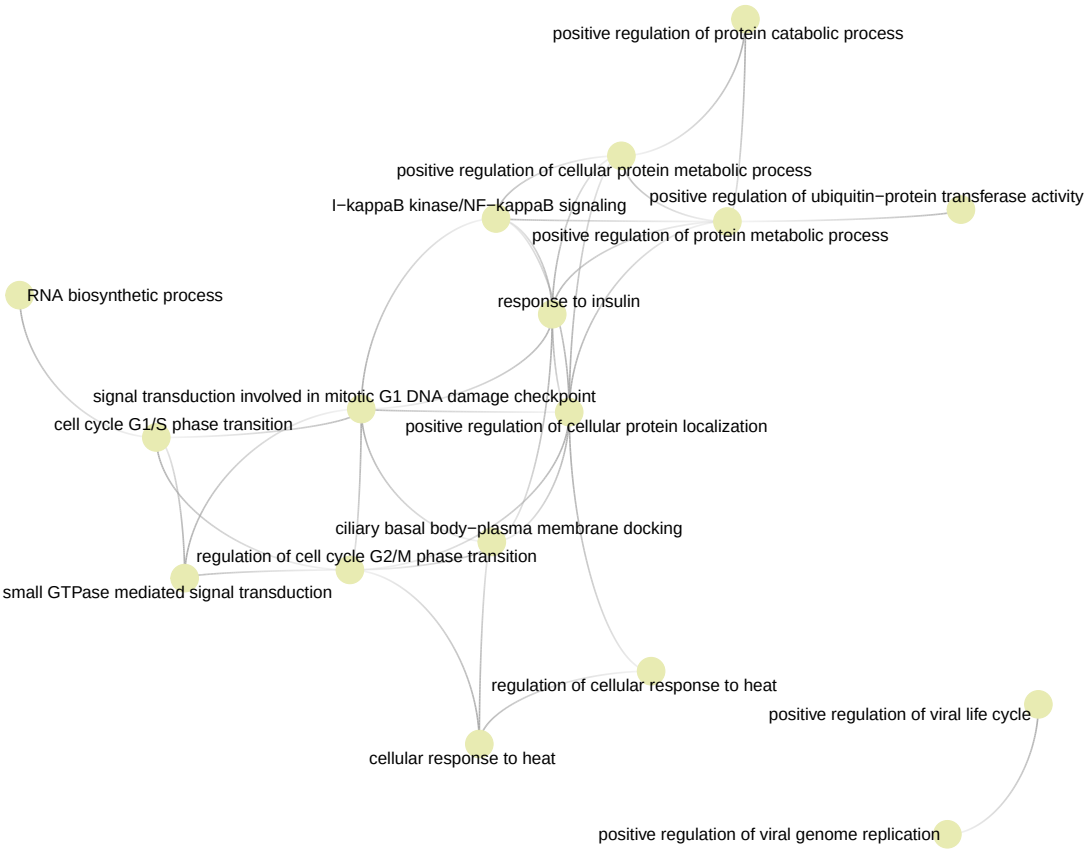

Figure 7. Crosstalk visualization of SARS-COV-2 induced biological functions that are **commonly** enriched by the **Cyclosporine and Selinexor drug modules**. Here nodes are significantly enriched functions and interactions among them represent their cross-talk via shared genes/proteins in the drug module.

3.5. Semantic Similarities Shown a Profound Functional Overlap between Drug-Target Enriched and SARS-COV-2 Enriched GO Term Sets

To further observe each drug's downstream effects on the SARS-COV-2 perturbed host interactome, we conducted the functional proximity analysis that measures the semantic similarity between the set of biological functions (i.e. GO Terms) enriched by targets of a drug and their immediate neighbourhoods and those enriched by the disease gene modules [Figure 2c]. Note, that the functional proximity scores are based on the semantic similarity measure, the larger the similarity scores, the larger the functional proximity. Hence a minimum value of 0 indicates the functional effect of a drug is completely different from the disease-induced pathways, whereas a maximum value of 1 demonstrates the complete overlap of biological functions induced by both the drug target and the disease.

In this study, we have observed that both Cyclosporine and Selinexor have shown high functional proximity scores individually with the SARS-COV-2 perturbed human interactome, with the values as 0.59 (p-value: 0.042) and 0.67 (p-value: 0.026). The statistical analysis revealed that the functional proximities of Cyclosporine and Selinexor with SARS-COV-2 perturbed human interactome were significantly higher (< 0.05) than those of the background DTHS networks, where each background DTHS network was the same perturbed DTHS network, which was used for the statistical analysis of topological proximity measurement [see the subsection 3.3].

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3.6. Cyclosporine and Selinexor Drug Modules Reveal Their Complementary Exposure Pattern in Targeting SARS-COV-2 Disease Module

A drug pair's efficacy also depends on its pharmacologically distinct effects on the disease module by targetting topologically separate parts of the interactome [18]. This notion is known as *complementary exposure* of two acting drugs, the quantification (network separation score) of which may putatively suggest how effective the combination would be on the disease module [Figure 1]. To determine this network separation score, the mean shortest distances of each drug module with disease modules were compared with the mean shortest distance between the two drug modules [18] [See the Methods section 2.4]. Therefore, higher the separation score indicates that the two drug modules are topologically highly separated but individually highly proximal to the disease module. Here, it is observed that both Cyclosporine and Selinexor drug modules were shown highly *complementary* with a separation score, $S_{X_1,X_2} = 0.972$ (> 0), while individually maintaining very high topological proximity scores, i.e., $z_{X_1,Y} = -4.67$ (p-value = 1.49×10^{-6}) and $z_{X_2,Y} = -6.66$ (p-value = 1.33×10^{-11}). This suggests that both these drugs can only be effective on the SARS-COV-2 disease module in a synergistic manner [Figure 1].

4. Discussions

In this study, we defined the SARS-COV-2 perturbed disease gene/protein module as the list of host genes that had direct PPI interaction from Gordon *et al.* [11]. The functional enrichment test of this disease module revealed a total of 136 significant biological functions ($adj.p$-value < 0.05) [Figure 2c]. Out of these SARS-COV-2-affected host functions, Cyclosporine and Selinexor drug modules (drug target proteins and their immediate PPI partners) induce 35 (2.57%) and 65 (6.86%) biological functions, respectively, whereas 17 (2.43%) functions were shared between these two sets. Literature evidence suggested that the processes, namely, positive regulation of protein metabolic process (GO:0051247), viral genome replication (GO:0045070) [21], regulation of cellular response to heat (GO:1900034) [22], cell cycle G1/S phase transition (GO:0044843) [23], I-kappaB kinase/NF-kappaB signalling (GO:0007249) [24], positive regulation of cellular protein localization (GO:1903829) [25], and positive regulation of viral life cycle (GO:1903902) [26] active in SARS-COV-2 conditions, which are also targeted by the Cyclosporine and Selinxor drug combination to prove its potency against the infection. Heat shock protein expressions are driven by various cellular stress conditions, primarily triggered by different comorbid conditions (e.g., cardiovascular diseases, chronic lung disease, and renal disease) during SARS-COV-2 infection [22]. The transition from the G1 to the S phase is affected by the SARS-COV-2-induced cell cycle arrest via the depletion of the cyclin D regulator [23].

However, among these 6 SARS-COV-2 perturbed biological functions, 3 functions were exclusively induced by the Cyclosporine drug module, which are Peptidyl-proline modification (GO:0018208), Virion assembly (GO:0019068), and protein peptidyl-prolyl isomerization (GO:0000413). Peptidyl-prolyl cis/trans isomerase Pin1 is responsible for SARS-COV-2 proliferation - a potential target for novel therapeutics [27,28]. For Selinexor, 29 SARS-COV-2-perturbed functions are exclusively induced by this drug module, including, I-kappaB kinase/NF-kappaB signalling (GO:0007249) [24], Rab protein signal transduction (GO:0032482) [29,30], nuclear import (GO:0051170) [31], regulation of ATP metabolic process (GO:1903578) [32], regulation of post-transcriptional gene silencing (GO:0060147) [33], and tRNA transport (GO:0051031) [34].

5. Conclusions

In this article, we have adopted the network-medicine-based analytical approach to examine the potency of the Cyclosporine and Selinexor drug combination in combating the SARS-COV-2 infection. Several key metrics are used to test our hypothesis, including topological proximity, functional proximity and network separation scores. Our results suggested that these two drugs show a "complementary exposure" pattern with the SARS-COV-2 perturbed human interactome (i.e., disease module) while maintaining statistically significant topological and functional proximity scores, individually. This

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renders the suggested drug combination a highly effective treatment strategy with potentially low toxicity for SARS-COV-2 infected patients. Thus it is worth investigating this combination further towards clinical trials to observe its true potential.

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