



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



## Lucidenic acid A inhibits the binding of hACE2 receptor with spike protein to prevent SARS-CoV-2 invasion

Juan Xu<sup>a,b,1</sup>, WenTao Yang<sup>a,b,1</sup>, YiFeng Pan<sup>c</sup>, HaiShun Xu<sup>a</sup>, Liang He<sup>d</sup>, BingSong Zheng<sup>a</sup>, YingQiu Xie<sup>f</sup>, XueQian Wu<sup>a,b,e,\*</sup>

<sup>a</sup> State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Lin'an, 311300, PR China

<sup>b</sup> Zhejiang Provincial Key Laboratory of Characteristic Traditional Chinese Medicine Resources Protection and Innovative Utilization, Zhejiang A&F University, Lin'an, 311300, PR China

<sup>c</sup> Hangzhou Xihu District Center for Disease Control and Prevention, Hangzhou, 310030, PR China

<sup>d</sup> Zhejiang Provincial Academy of Forestry, Hangzhou, 310000, Zhejiang, China

<sup>e</sup> National Innovation Alliance of Lingzhi and Sanyeqing Industry, Lin'an, 311300, China

<sup>f</sup> Department of Biology, School of Sciences and Humanities, Nazarbayev University, Nur-Sultan, Kazakhstan

### ARTICLE INFO

Handling Editor: Dr. Jose Luis Domingo

*Chemical compounds studied in this article:*  
Lucidenic acid A (PubChem CID: 14109375)

*Keywords:*

Anti-SARS-CoV-2

*Ganoderma lucidum* triterpenoids

FRET test

Natural products

Omicron variant

### ABSTRACT

High infection caused by mutations of SARS-CoV-2 calls for new prevention strategy. *Ganoderma lucidum* known as a superior immunoenhancer exhibits various antiviral effects, whether it can resist SARS-CoV-2 remains unclear. Herein, virtual screening combined with in vitro hACE2 inhibition assays were used to investigate its anti SARS-CoV-2 effect. Potential 54 active components, 80 core targets and 20 crucial pathways were identified by the component-target-pathway network. The binding characters of these components to hACE2 and its complexes with spike protein including omicron variant was analyzed by molecular docking. Lucidenic acid A was selected as the top molecule with high affinity to all receptors by forming hydrogen bonds. Molecular dynamics simulation showed it had good binding stability with the receptor proteins. Finally, in vitro FRET test demonstrated it inhibited the hACE2 activity with IC<sub>50</sub> 2 μmol/mL. Therefore, lucidenic acid A can prevent the virus invasion by blocking hACE2 binding with SARS-CoV-2.

### 1. Introduction

The recent emergence of SARS-CoV-2 Omicron variant has been a big concern because of its high infectivity. It contains 30 amino acid mutations on spike protein (Yin et al., 2022), which weakens the neutralization of existing vaccine antibodies and monoclonal antibodies (Planas et al., 2022), thus it is urgent to develop therapeutic strategies that can resist the persistent emergence of SARS-CoV-2 variants (Garcia-Beltran et al., 2021; Nile et al., 2021). SARS-CoV-2 consists of four proteins: spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N). Among them, S protein exists as a trimer on the surface of the virus and is the key protein for binding to human angiotensin-converting enzyme 2 (hACE2) during viral infection. hACE2 is regarded as the key host receptor for SARS-CoV-2 to enter target cells and trigger the viral life cycle (Hikmet et al., 2020). The variation of SARS-CoV-2 enhances the binding ability of S protein to hACE2 (Lin

et al., 2022). Since most of the current strategies resisting SARS-CoV-2 are to neutralize the virus with monoclonal antibodies targeting the S protein, and to block SARS-CoV-2's replication and proliferation (Taylor et al., 2021). There is no effective antiviral drug or vaccine dealing with the constantly emerging variants of SARS-CoV-2 for a long time. If the binding of SARS-CoV-2 with hACE2 is blocked, it can effectively prevent SARS-CoV-2 from infecting human cells and achieve the purpose of preventing and controlling COVID-19. Given that SARS-CoV-2 itself is highly susceptible to mutation, inhibiting the binding activity of hACE2 may be an effective way to improve population immunity to SARS-CoV-2 infection. It has been shown that small molecule active ingredients can inhibit the entry of respiratory syncytial virus into the body and prevent viral infection (Ren et al., 2020). For instance, Curcumin in the Chinese herb turmeric could prevent neocrown pneumonia infection by inhibiting pathogen entry (Maruyama et al., 2021).

Traditional Chinese medicine (TCM) with a long and prominent

\* Corresponding author. Hangzhou City, Zhejiang Province, PR China.

E-mail address: [wxq@zafu.edu.cn](mailto:wxq@zafu.edu.cn) (X. Wu).

<sup>1</sup> Equal contribution.

application, is a rich source of many small molecule active ingredients. The natural bioactive compounds isolated from TCM provide a huge, unexplored chemical structural diversity and play a major part in the fight against infectious diseases (Rivero-Segura and Gomez-Verjan, 2021). It has been reported that extracts or related bioactive compounds from more than 30 medicinal plants displayed antiviral action against coronavirus infection by inhibiting viral attachment and penetration, viral RNA and protein synthesis, and viral key proteins (Remali and Aizat, 2021). Fungal bioactive compounds such as polysaccharides, terpenes and cordycepin etc. also provide therapeutic potential in the prevention of coronavirus as protease inhibitors and immunomodulators (Suwannarach et al., 2020). *Ganoderma lucidum* (*G. lucidum*), a well-known traditional Chinese medicine, belongs to the Polyporaceae family and is a homology of medicine and food, which is rich in natural bioactive components (Ahmad, 2018). As a functional food, *G. lucidum* can be used to improve the low immune function in various diseases. The fruiting body, mycelia and spores of *G. lucidum* contain nearly 400 different bioactive components. The main components of *G. lucidum* with outstanding pharmacological activity are polysaccharides, triterpenes, nucleosides and sterols (Xu et al., 2021). It has been used to treat a variety of diseases in China such as liver disease, glomerulonephritis, hypertension, and tumors etc. (Dhar and Bhattacharjee, 2021). It also exhibits admirable antiviral effects against viruses like HIV-1, enteroviruses (Lin et al., 2020). Recently, a L-fucose-containing polysaccharide of *G. lucidum* was shown outstanding antiviral efficacy in the cell-based anti-SARS-CoV-2 assay, the virus content in hamsters infected with SARS-CoV-2 was only half that of the control group (water) (Jan et al., 2021), yet its antiviral mechanism remains unclear for undefined structure of the polysaccharide. It is also unclear whether other small molecule active ingredients such as triterpenes and sterols can resist to SARS-CoV-2. Briefly, as a superior immunoenhancer (Wu et al., 2020), *G. lucidum* has great potential to resist SARS-CoV-2. It is of significant importance to explore its inhibitory effects on SARS-CoV-2 especially in terms of preventing the virus invasion by block the binding of SARS-CoV-2 with the receptor hACE2, and it will shed new lights on the molecular mechanism of *G. lucidum* prevention on virus infection.

## 2. Materials and methods

### 2.1. Filtrating the active ingredients of *G. lucidum*

The components of *G. lucidum* were found through the systematic pharmacological platform of traditional Chinese medicine (TCMSP) (Ru et al., 2014). According to oral availability (OB)  $\geq 30\%$  and drug-likeness (DL)  $\geq 0.18$ , the active components of *G. lucidum* were preliminarily filtrated to obtain the effective active components. The target was predicted by SwissTarget Prediction database (Gfeller et al., 2014), and the target gene name corresponding to the target protein was obtained and regulated by Uniprot database (UniProt Consortium, 2019).

### 2.2. CCOVID-19 targets and common targets prediction

"COVID-19" was used as the keyword to explore the potential targets of SARS-CoV-2 in GeneCards database (Stelzer et al., 2011), and the targets highly correlated with COVID-19 were screened out based on the scores. Only when the scores were greater than 5 could the targets be settled as COVID-19 targets. In order to explore the interaction between *G. lucidum* drug-related targets and COVID-19 targets, the Jvenn platform was used to intersect the two kinds of targets and draw the Venn diagram.

### 2.3. Protein-protein interaction (PPI) map

The antiviral target genes were introduced into the STRING database to construct the PPI network model (Szklarczyk et al., 2019). The PPI

information table of the potential antiviral target proteins was obtained, then the PPI network was further visualized and topologically analyzed by Cytoscape 3.8.2. Analysis topology parameters were set up using Network-Analyzer of Cytoscape 3.8.2. The size and color of nodes in the network were positively correlated with the values. Its function was described by analyzing the biological processes it participated in.

### 2.4. KEGG and GO enrichment

Metascape database summarizes many authoritative data resources, such as GO, KEGG, UniProt and DrugBank. The anti-COVID-19 targets of *G. lucidum* were input into the Metascape platform with  $P < 0.01$ . The GO enrichment analysis and KEGG pathway annotation analysis were carried out, and the chart was drawn on the online platform.

### 2.5. Construction of compound-target-pathway network

The compound-target-pathway network was constructed by Cytoscape 3.8.2, and the network was analyzed by using the built-in tool network analyzers with parameters including connectivity (Degree), media (Betweenness) and compactness (Closeness). The components of *G. lucidum*, targets and pathways of COVID-19 were input into Cytoscape 3.8.2 to construct the anti-COVID-19 "component-target-pathway" network of *G. lucidum*, and the core targets and main active components were analyzed according to the parameters.

### 2.6. Molecular docking

The structures of the key active components of *G. lucidum* were obtained from TCMSP database and PubChem database, and the energy of the structures were minimized by Chem3D. The three-dimensional crystal structure of hACE2 (PDB code: 1r42) and spike-ACE2 complex (PDB code: 6m0j), spike(Omicron variant)-ACE2 complex (PDB code: 7t9l) were downloaded from PDB database, and saved as receptor files separately by pymol software. Water molecules and small molecular ligands of the receptors were further deleted (Moors, 2020). Molecular docking was carried out by Autodock Vina. The potential SARS-CoV-2 inhibitors were screened out according to their interaction with the receptors.

### 2.7. Molecular dynamics simulation

100 ns molecular dynamics (MD) simulation was performed using GROMACS 2022 on spike + hACE2 complexes (including Omicron variation) with or without lucidenic acid A, and solely on hACE2 with lucidenic acid A. Firstly, a dodecahedral simulation box was constructed, and the spike + hACE2 complex or hACE2 was placed in the center of the box. Water molecules (TIP3P) were added to the remaining volume of the box, and then chlorine/sodium atoms were added to neutralize the system. The energy of each system was minimized by the fastest descent algorithm. To equilibrate the systems, a two-step equilibration of NVT and NPT was performed for 100 ps using the leapfrog algorithm to keep the pressure of each system at 1 bar and the temperature controlled at 300 K. The final output simulation files were used to calculate RMSD (root mean square deviation), RMSF (root mean square fluctuation), Rg (radius of gyration), free energies, and decomposition of energy terms for visualization and analysis. Moreover, the H-bond and non-bonded interaction patterns were also taken as the evaluation parameters.

### 2.8. hACE2 inhibition activity assay

The inhibitory activity of the compound on hACE2 was detected by fluorescence resonance energy transfer (FRET) (Algar et al., 2019). The protease detection method based on FRET was as follows: the screened components with potential inhibitory effect were prepared in different concentrations (0.02–2  $\mu\text{mol/mL}$ ) by dissolving with DMSO, 5  $\mu\text{L}$  of

each concentration was added to 93  $\mu\text{L}$  (92  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  hACE2 Enzyme) Assay Reagent. The mixed solution was added to the black 96-well plate, and 2  $\mu\text{L}$  fluorescence substrate was added to each well. The mixed solution was incubated at 37  $^{\circ}\text{C}$  for 45 min, and the fluorescence was determined by a multi-function enzyme labeling instrument. The excitation wavelength was 325 nm and the emission wavelength was 393 nm.

### 3. Results and discussions

#### 3.1. Screening and target prediction of active components of *G. lucidum*

A total of 242 components in *G. lucidum* were obtained in TCMSP. Further, 61 compounds were selected meeting the parameter which was set as  $\text{OB} \geq 30\%$  and  $\text{DL} \geq 0.18$ . After target prediction by Swisstarget prediction database, the components with no antiviral targets were removed. 54 components with effective targets and 574 target proteins were screened out, including 44 kinds of triterpenes and 10 kinds of sterols (Table S1). From the preliminary screening, there was a variety of potential anti-SARS-CoV-2 active components in *G. lucidum*.

#### 3.2. Acquisition of related targets of COVID-19

1229 COVID-19 viral targets were acquired from Genecards database. According to experience, targets with scores greater than 5 were set as COVID-19 targets, and the repetition value was deleted. Finally, 791 COVID-19-related targets were obtained. Then, by taking an intersection of the *G. lucidum* antiviral targets and COVID-19 targets, 80 COVID-19 targets of *G. lucidum* were obtained as shown in (Fig. S1). According to these antiviral targets of *G. lucidum*, the effective components of *G. lucidum* were further analyzed.

#### 3.3. The PPI map of the targets of the intersection of *G. lucidum* and COVID-19

The potential antiviral target genes of *G. lucidum* were imported into the STRING database to build a network model. Biological species was selected as "Homo sapiens", and the minimum threshold was selected as "highest confidence". Further visual analysis of the network was conducted through Cytoscape 3.8.2, the results are shown in (Fig. 1). Taking the values as the standard, the target genes with greater scores were screened: STAT3, MAPK3, MAPK1, TP53, TNF, MAPK8, MAPK14, LCK, IL6. These targets are related to inflammation, viral infection, tumor,

and other pathways. STAT3 acts as a transcriptional activator (Hegde et al., 2022), it has been shown that inhibition of the activation of STAT3 cytokine signaling pathway is beneficial to the treatment of pulmonary inflammation caused by virus (Pandey et al., 2022). Tumor necrosis factor (TNF) family refers to a group of cytokines that can cause cell death (apoptosis) (Wu et al., 2021). The combination of anti-inflammatory drugs and antiviral drugs targeting TNF significantly improved clinical diseases (Pandey et al., 2022). MAPK 1, 3, 8 and 14 are members of the mitogen-activated protein kinases family which participate in cell proliferation, differentiation, transcriptional regulation and so on. The invasion mechanism of SARS-CoV-2 leads to the failure of cells to inhibit MAPK14 (p38) signal through hACE2 activity, giving rise to increased inflammation, vasoconstriction and thrombosis (Pandey et al., 2022). TP53 produces stress response to different cells and regulates the expression of genes, so as to induce apoptosis, senescence cell cycle arrest, metabolic changes or DNA repair (Sivakumar et al., 2022). SARS-CoV-2 infection may regulate immune inflammatory response and induce lymphocyte apoptosis by promoting the activation of TP53 (Xiong et al., 2020). hACE2 can affect the expression of TP53 in pulmonary endothelial cells, and the deletion of TP53 binding site leads to the enhancement of hACE2 promoter activity (Zhang et al., 2021). LCK gene is a member of the protein tyrosine kinase family, and the encoded protein is a key signal molecule for T cell selection and maturation in development (Weiße et al., 2021). IL6 encodes a cytokine that plays a role in inflammation and B cell maturation, which can cause fever in patients with autoimmune diseases or infections, mainly acute and chronic inflammation (Dolsen and Harvey, 2021). Therefore, it is speculated that the active components of *G. lucidum* can treat COVID-19 by acting with inflammation, viral infection, tumor and other targets.

#### 3.4. Pathway-target-component network construction

1779 GO entries were selected, including 1628 entries for biological processes (BP), 56 Cellular component (CC) entries for cell composition, 95 entries for molecular function (MF). According to the degree of enrichment, the top 20 of each project are selected. From those GO term, it was suggested that *G. lucidum* mainly participates in response to toxic substance, response to injury, lymphocyte activation, protein serine/threonine kinase activity, and binding to protein kinase. They may play roles in preventing virus invasion, inflammation, cell metabolism, and immune response (Fig. 2A). A total of 309 KEGG enrichment pathways were obtained, which were mainly involved in Kaposi sarcoma associated herpesvirus infection, hepatitis B, hepatitis C, Epstein-Barr virus

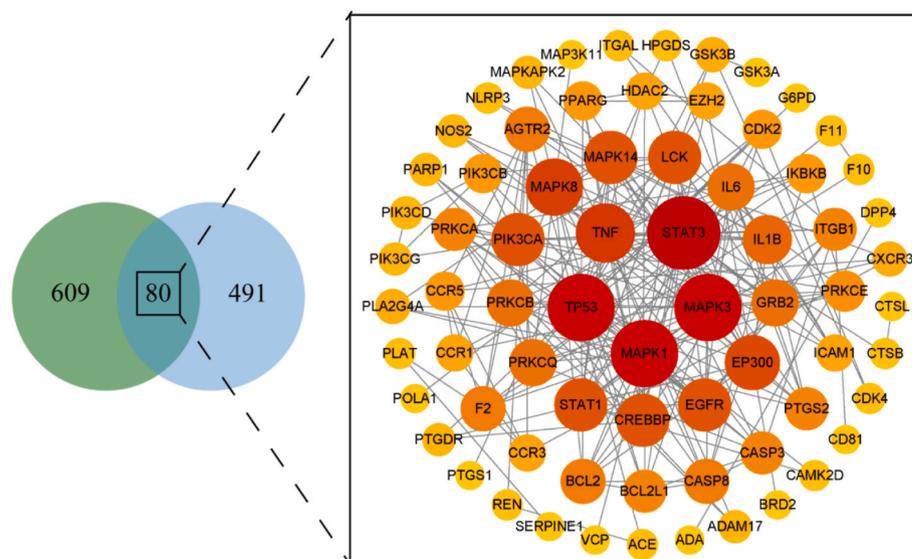


Fig. 1. PPI network of *G. lucidum* and COVID-19 intersection targets.

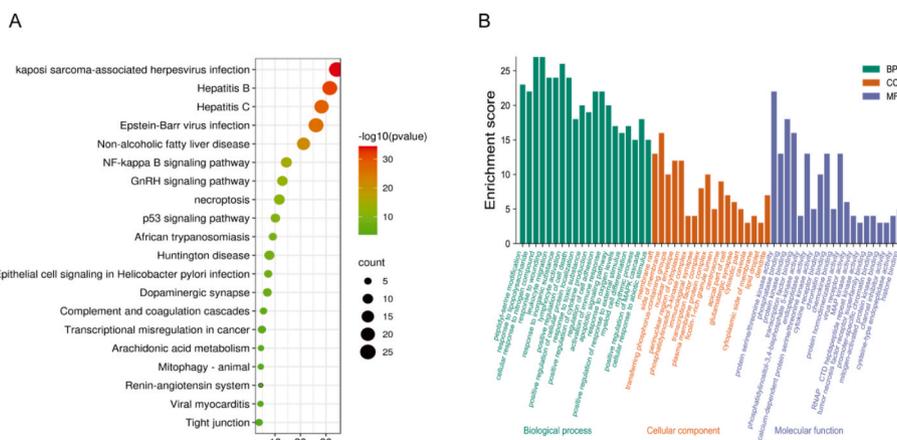


Fig. 2. Go enrichment and KEGG enrichment analysis of potential antiviral targets of *G. lucidum*.

infection, NF-κB signal pathway and so on. Those with higher enrichment scores were all related to antiviral and anti-inflammatory pathways. It was reported that triterpenoids from the alcoholic extract of *G. lucidum* had anti viral activities against a variety of disease causing viruses such as HSV1, HSV2 and influenza etc. (Zhu et al., 2015). Ganoderic acid T-Q and TR have been reported as neuramidase inhibitors of H5N1 and H1N1 (Bharadwaj et al., 2019). It can be inferred that *G. lucidum* plays an antiviral role by regulating multiple targets and pathways (Fig. 2B).

Network analysis showed that each potential antiviral compounds of *G. lucidum* could act on multiple targets. 54 selected compounds interacted with 80 targets, and the 80 targets converged to 20 pathways. The

network consisted of 153 nodes and 934 edges, and the average number of neighbors, the characteristic path length, the network centralization, the network density and the network heterogeneity were 12.078, 2.528, 0.313, 0.079, and 0.774 respectively (Fig. 3). These 54 potential anti-SARS-CoV-2 components of *G. lucidum* acted on 80 targets related to COVID-19, and these 80 targets corresponded to 20 major signal pathways. Among them, the ones with higher correlation degree were Kaposi sarcoma associated herpesvirus infection, and infection of Epstein-Barr virus, hepatitis B, and hepatitis C, which were closely related to anti-virus.

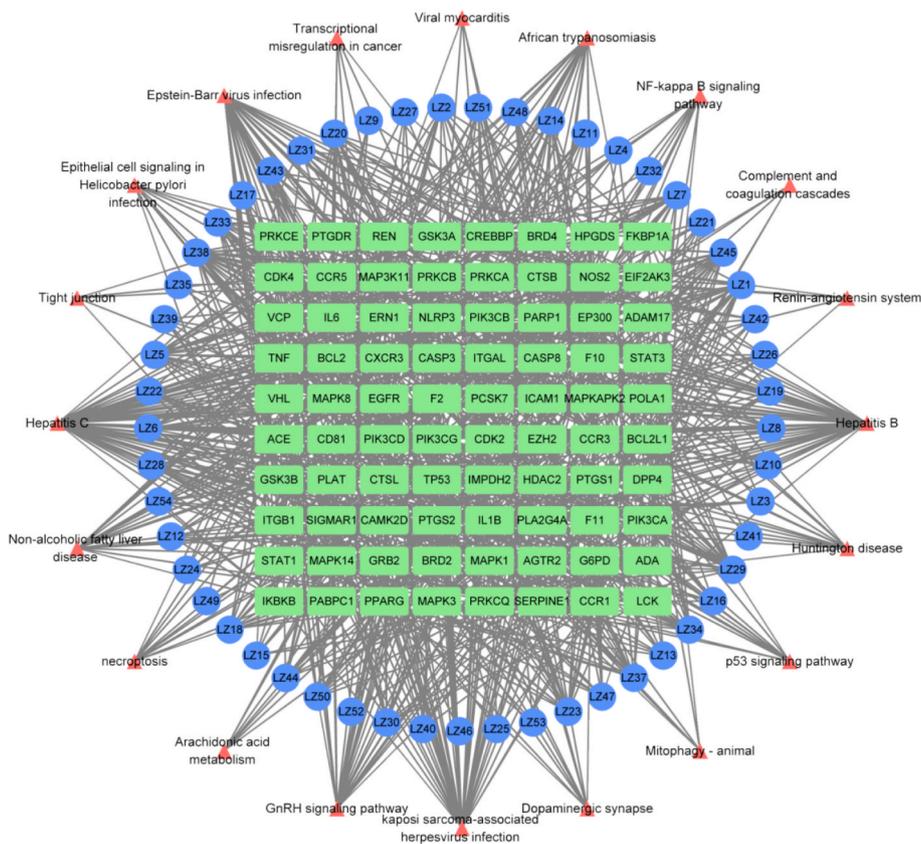


Fig. 3. Network of *G. lucidum* with pathway-target-compound analysis. The blue round represents *G. lucidum* active compounds, green square represents predicted targets, red triangle represents relevant pathways. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

### 3.5. Docking of the screened components of *G. lucidum*

In order to carry out molecular docking, 54 potential antiviral components of *G. lucidum* were docked with hACE2, spike-hACE2 complex and spike (Omicron variant)-hACE2 complex respectively. The results showed that most of the components had good binding energy, and the binding sites were very similar, mainly concentrated in the hydrophobic pockets of protein receptors (Fig. 4A), suggesting they had good binding activity with all the receptors. The active components in the top five were screened out separately. The docking binding energy of lucidinic acid A was the best in the three receptors among the top five substances (Table S2), indicating that lucidinic acid A had strong binding affinity to the target proteins.

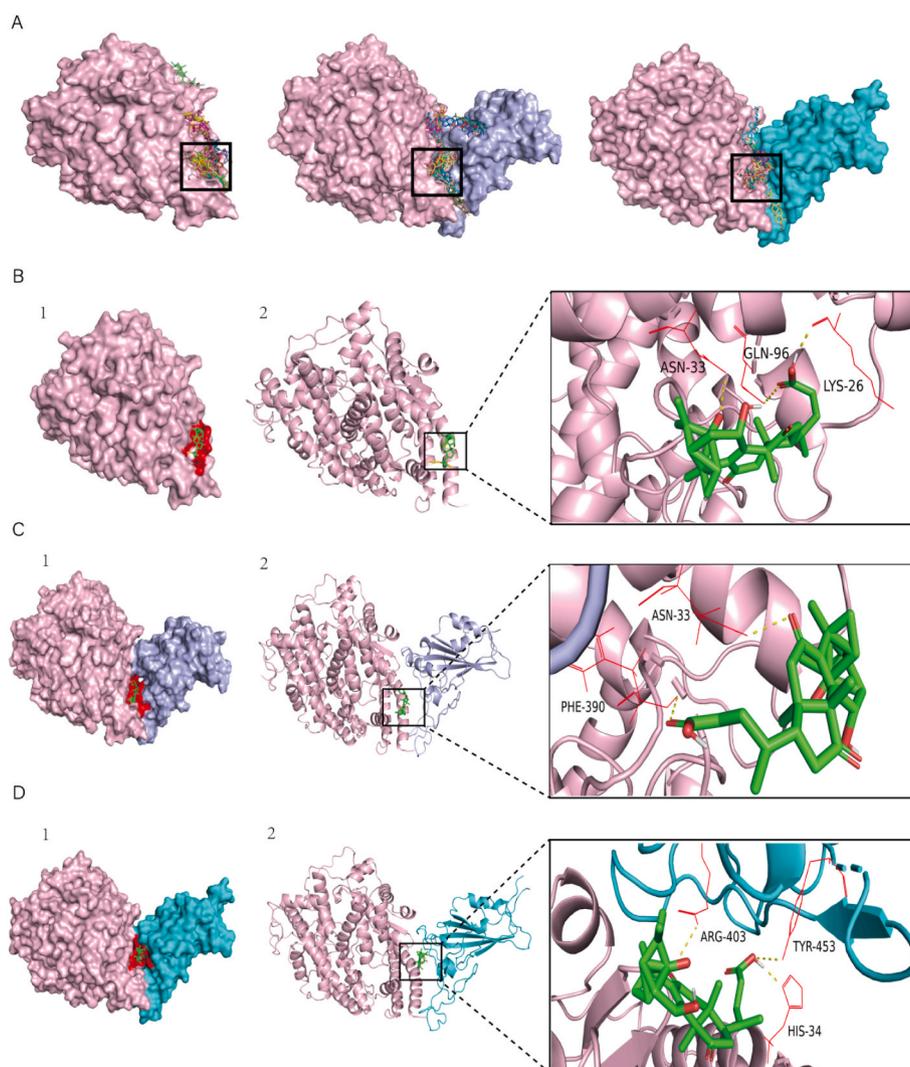
It was shown that lucidinic acid A interacted with the amino acid residues Gln96, Asn33 and Lys26 of hACE2 protein to form hydrogen bond interaction (Figs. 4B-2), suggesting it had strong binding ability to hACE2. According to Ortega et al., Asn33, Asn 34 and Lys26 of hACE2 were necessary for its binding to Leu 455 and Asp 405, the key residues of SARS-CoV-2 spike protein (Ortega et al., 2020), so the formation of hydrogen bond between lucidinic acid A and hACE2 led to competition for the binding sites of spike protein as to block the virus invasion. It is suggested that lucidinic acid A might be a potential inhibitor of SARS-CoV-2.

Importantly, the binding activities of lucidinic acid A with spike-

hACE2 complexes (including Omicron variant) were explored to better understand its mechanism of action on SARS-CoV-2. In terms of the binding mode between lucidinic acid A and spike-hACE2 complex, lucidinic acid A was embedded to the hydrophobic pocket of the spike-hACE2 complex with the binding site between hACE2 and the spike protein (Figs. 4C-1), and it also formed hydrogen bonds with the Asn33 residue of hACE2 (Figs. 4C-2). As for the docking with spike (Omicron variant)-hACE2 complex, it binded to the similar hydrophobic pocket (Figs. 4D-1). Besides the hydrogen bond interaction with hACE2 at His 34 residue, it interacted with the amino acid residues Tyr453 and Arg 403 of spike (Omicron variant) protein (Figs. 4D-2), which may explain why lucidinic acid A had higher affinity to the spike (Omicron variant)-hACE2 complex. Although lucidinic acid A docked with the two kinds of complexes similar to its interaction with hACE2, the binding energies were higher than that of hACE2 alone. The possible reason is that allosteric hACE2 caused by the exist of spike protein make it more affiliative to lucidinic acid A. To sum up, lucidinic acid A could effectively suppress the combination of hACE2 with SARS-CoV-2 and its variants so as to prevent the virus invasion.

### 3.6. Molecular dynamic simulation and inhibition assay

In order to observe whether lucidinic acid A can inhibit the binding activity of spike proteins (including Omicron variation) with hACE2, the

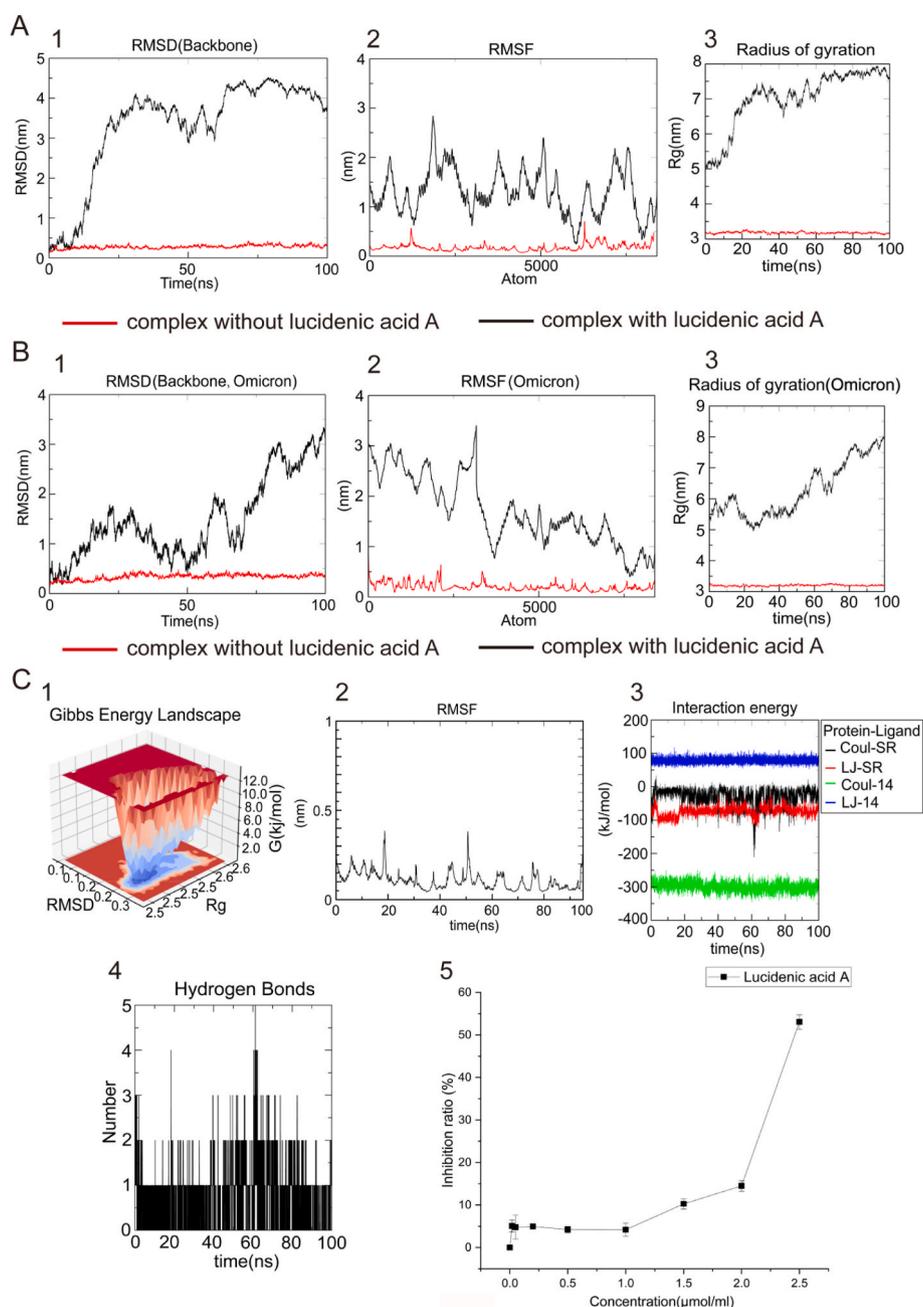


**Fig. 4.** Molecular docking of components of *G. lucidum* (A); Molecular docking of lucidinic acid A with the hACE2 (1r24) (B), spike-hACE2 complex (6m0j) (C) and spike (Omicron variant)-hACE2 complex (7t9l) (D).

best conformation in the molecular docking results was selected and 100 ns MD simulation was carried out. Parameters including RMSD (root mean square deviation), RMSF (root mean square fluctuation), and Rg (radius of gyration) were used to evaluate the stability of the spike-hACE2 complexes, and the high deviation of RMSD and drastic fluctuation of RMSF and Rg indicate weak stability (Yu et al., 2021). As shown in the results, the binding of hACE2 with the spike protein (including Omicron variation) was quite stable for the RMSD, RMSF and Rg had no significant differences during the whole simulation process without lucidenic acid A (the red lines in Fig. 5A and B). However, spike + hACE2 complexes (including Omicron variation) became unstable after the adding of lucidenic acid A. It was observed that the RMSD of spike + hACE2 with lucidenic acid A deviated obviously between 0.2 nm and 3.7 nm (the black line in Figs. 5A-1), and the RMSD deviation of spike +

hACE2 (Omicron variation) was remarkable as well with lucidenic acid A (the black line in Figs. 5B-1). The fluctuations of RMSF of the two complexes were also obvious with the variation greater than 2 nm in the presence of lucidenic acid A (the black lines in Figs. 5A-2 and 5B-2). Moreover, there were violent fluctuations in the Rg of the two complexes with lucidenic acid A (the black lines in Figs. 5A-3 and 5B-3). These results showed that lucidenic acid A might inhibit the combination of the spike proteins (including Omicron variation) with hACE2, which was in accordance with the results of molecular docking.

To further verify whether lucidenic acid A attenuates the binding of the spike proteins to ACE2 by inhibiting ACE2 activity, molecular dynamics simulation of hACE2 alone with lucidenic acid A, and in vitro inhibition activity of lucidenic acid A on hACE2 were carried out respectively. It was shown in the molecular dynamics simulation results



**Fig. 5.** Molecular dynamic simulation of the spike + hACE2 complex with (in black line) and without (in red line) lucidenic acid A (A), the spike (omicron variation)+hACE2 complex with (in black line) and without (in red line) lucidenic acid A (B), and hACE2 with lucidenic acid A (C1–C4); the inhibition rate curve of lucidenic acid A on hACE2 (C5). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

that the RMSD of the hACE2 backbone was stable between 0.18 nm and 0.22 nm (Figs. 5C-1), and no significant differences were found between the initial and final RMSDs throughout the simulation (0.1 nm and 0.2 nm), indicating that lucidenic acid A and hACE2 were bound well to each other. In addition, slight fluctuations of residues were observed from the RMSF plots in the range of 0.053 nm–0.38 nm (Figs. 5C-2). As shown in Figs. 5C-1, the Rg values of hACE2 with lucidenic acid A remained almost constant (2.50–2.60 nm). Apart from the above parameters, the H-bond and non-bonded interaction patterns, and the interaction energies of hACE2 with lucidenic acid A were evaluated. Gibbs free energy was mainly concentrated in 2–4 kJ/mol, remaining relatively stable. The number of hydrogen bonds was mainly concentrated in 1–2 during the simulation (Figs. 5C-4), which was close to the molecular docking results. The average short-range Coulombic (Coul-SR) energy, the short-range Lennard-Jones (LJ-SR) energy and the total interaction energy were  $-29.94 \pm 3.9$ ,  $-74.38 \pm 3.8$ , and  $-104.32 \pm 7.7$  kJ/mol respectively (Figs. 5C-3), and all the interaction energies maintained stability during the simulation, suggesting that the combination of hACE2 with lucidenic acid A had favorable binding and folding stability, and high compactness as well. Consequently, it was inferred that lucidenic acid A might restrain the binding of the spike proteins to hACE2 via suppressing the binding activity of hACE2. In order to verify this hypothesis, we used the cutting test based on fluorescence resonance energy transfer (FRET) to determine the IC50 of lucidenic acid A on hACE2. At low concentration, the inhibition rate of lucidenic acid A on hACE2 was about 5%, and when the concentration reached 2.5  $\mu\text{mol}/\text{mL}$ , the inhibition rate reached 55%. The calculated IC50 was about 2  $\mu\text{mol}/\text{mL}$  (Fig. 5C-). In conclusion, the anti-COVID-19 activity of lucidenic acid A might be related to its inhibition on hACE2 binding with

SARS-CoV-2, resulted in the blockage of the virus invasion.

### 3.7. Mechanism of lucidenic acid a blocking SARS-CoV-2 cell entry

The SARS-CoV-2 infection cycle begins with viral recognition and binding to the surface receptors, which subsequently induces the viral endocytosis into host cell. It undergoes conformational changes and proteolytic changes of the S protein, then fuses with the cell membrane, transcription and replication of viral RNA by using endogenous organelles to translate its replicase (Bharathi et al., 2022). Genomic RNA (gRNA), as the translation template of polymeric protein pp1a and pp1ab, is cleaved to form non structural proteins (NSPs) and promote the formation of double membrane vesicles in cell membrane. Sub-genomic RNA is produced during genomic transcription/replication, which translates structural proteins; and finally, the viral vesicles is assembled and released (Russo et al., 2020) (Fig. 6).

It has been shown that Omicron mutant strains have a greater ability to bind hACE2 compared to wild-type coronavirus (Shah and Woo, 2022). Receptor binding is a key step in SARS-CoV-2 invasion, it is a potential antiviral solution to prevent viral entry into host cells by inhibiting its combination to the receptor (Omotuyi et al., 2022). It was reported that lucidenic acid A can inhibit PMA-induced MMP-9 activity and has anti-invasive effects on hepatocellular carcinoma cells (Weng et al., 2007). In this study, lucidenic acid A interacted with the amino acid residues Gln96, Asn33 and Lys26 of hACE2 protein to form hydrogen bond interaction. Asn33 and Lys26 are the key amino acid residue of hACE2 binding to spike protein. Meanwhile in vitro inhibitory effect of lucidenic acid A on hACE2 binding activity indicated lucidenic acid A had a good inhibitory effect on hACE2 with IC50 2  $\mu\text{mol}/\text{mL}$ .

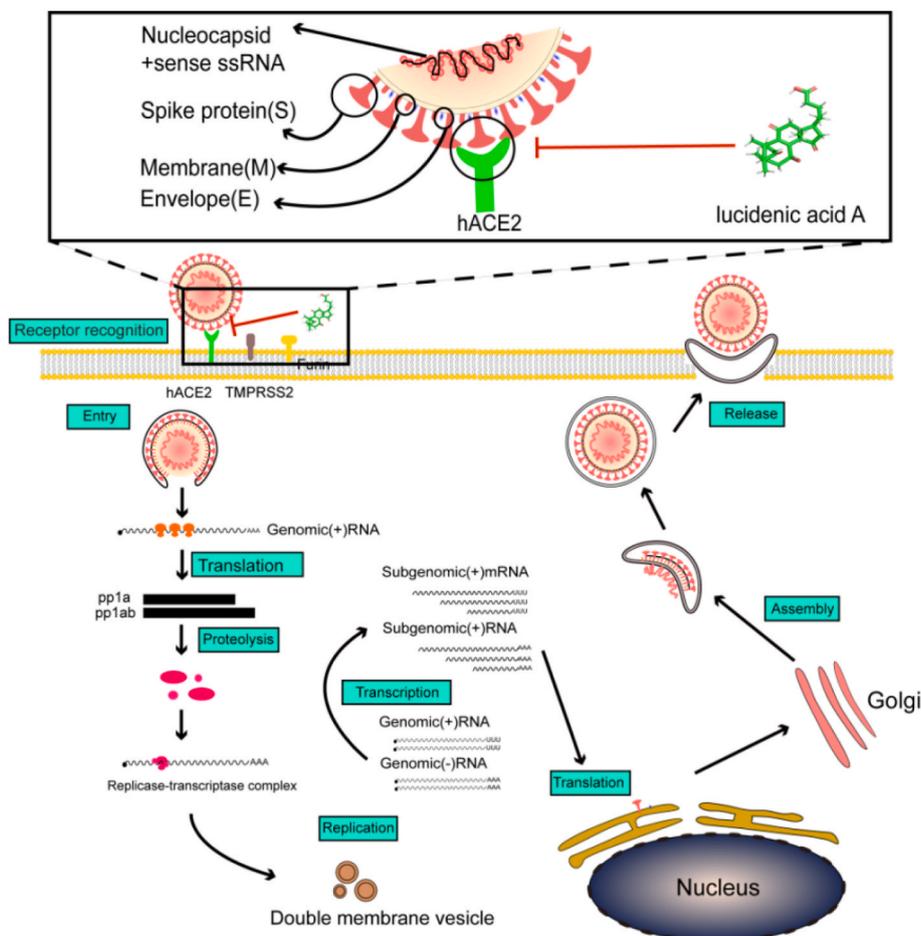


Fig. 6. A proposed schematic mechanism of lucidenic acid A inhibiting COVID-19 by blocking SARS-CoV-2 cell entry.

Consequently, lucidenic acid A probably inhibited COVID-19 by preventing SARS-CoV-2 from binding to hACE2. It has great potential in the prevention and treatment of COVID-19.

#### 4. Conclusion

In this study, we screened the active small molecules in *G. lucidum* that could effectively inhibit COVID-19 through network pharmacology. It was found that the potential antiviral small molecules of *G. lucidum* were mostly triterpenes and sterols, which mainly acted on anti-inflammatory and antiviral signaling pathways such as Kaposi sarcoma associated herpesvirus, hepatitis B, hepatitis C and Epstein-Barr virus etc. Therefore, *G. lucidum* may play a role in the prevention and treatment of COVID-19 through multi-targets and multi-pathways. Based on molecular docking, we screened out lucidenic acid A which exhibited a high binding capacity to hACE2. Molecular dynamics simulation further showed favorable binding stability, folding stability, and high compactness of the lucidenic acid A and hACE2 complex. In-vitro inhibitory effect of lucidenic acid A on hACE2 binding activity indicated lucidenic acid A had a good inhibitory effect on hACE2 with IC50 2  $\mu\text{mol/mL}$ . Moreover molecular dynamics simulations of spike + hACE2 (including Omicron variation) with and without lucidenic acid A manifested that the complexes of spike + hACE2 (including Omicron variation) were very stable, but became unstable and fluctuated greatly with the adding of lucidenic acid A. Consequently, lucidenic acid A inhibits the binding activity of hACE2 receptor with spike protein to prevent SARS-CoV-2 invasion.

#### CRedit authorship contribution statement

**Juan Xu:** Conceptualization, Methodology, Software, Validation, Writing – original draft, Writing – review & editing, Funding acquisition. **WenTao Yang:** Conceptualization, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. **YiFeng Pan:** Writing – review & editing. **HaiShun Xu:** Formal analysis, Writing. **Liang He:** Methodology, Validation, Data curation. **BingSong Zheng:** Writing – review & editing, BiZeng Mao, Writing – review & editing. **YingQiu Xie:** Writing – review & editing. **XueQian Wu:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

The data that has been used is confidential.

#### Acknowledgements

The authors acknowledge that the present study is supported by Natural Science Foundation of Zhejiang Province (grant number 19C010004), Key Research and Development Project of Zhejiang Province (grant number 2017C02012-4), and Scientific research and development fund of Zhejiang Agriculture and Forestry University (grant number 2016FR010).

#### Abbreviations

G	lucidum Ganoderma lucidum
hACE2	Human angiotensin converting enzyme 2
FRET	fluorescence resonance energy transfer
GLS G	lucidum Sterols

TNF	Tumor necrosis factor
IL-6	Interleukin-6
NF- $\kappa$ B	NF-kappaB inhibitor alpha
MAPK	Mitogen-activated protein kinase
p38	Phosphatidylinositol 3-kinase
STAT3	Signal Transducer And Activator Of Transcription 3
TP53	Tumor Protein P53
LCK	LCK Proto-Oncogene

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fct.2022.113438>.

#### References

- Ahmad, M.F., 2018. Ganoderma lucidum: persuasive biologically active constituents and their health endorsement. *Biomed. Pharmacother.* 107, 507–519.
- Algar, W.R., Hildebrandt, N., Vogel, S.S., Medintz, I.L., 2019. FRET as a biomolecular research tool - understanding its potential while avoiding pitfalls. *Nat. Methods* 16 (9), 815–829.
- Bharadwaj, S., Lee, K.E., Dwivedi, V.D., Yadava, U., Panwar, A., Lucas, S.J., Pandey, A., Kang, S.G., 2019. Discovery of *G. lucidum* triterpenoids as potential inhibitors against Dengue virus NS2B-NS3 protease. *Sci. Rep.* 9 (1), 19059.
- Bharathi, M., Sivamaruthi, B.S., Kesika, P., Thangaleela, S., Chaiyasut, C., 2022. Silico screening of bioactive compounds of representative seaweeds to inhibit SARS-CoV-2 ACE2-bound omicron B.1.1.529 spike protein trimer. *Mar. Drugs* 20 (2), 148.
- Dhar, S., Bhattacharjee, P., 2021. Promising role of curcumin against viral diseases emphasizing COVID-19 management: a review on the mechanistic insights with reference to host-pathogen interaction and immunomodulation. *J. Funct. Foods* 82, 104503.
- Dolsen, M.R., Harvey, A.G., 2021. IL-6, sTNF-R2, and CRP in the context of sleep, circadian preference, and health in adolescents with eveningness chronotype: cross-sectional and longitudinal treatment effects. *Psychoneuroendocrinology* 129, 105241.
- Garcia-Beltran, W.F., Lam, E.C., St Denis, K., Nitido, A.D., Garcia, Z.H., Hauser, B.M., Feldman, J., Pavlovic, M.N., Gregory, D.J., Poznansky, M.C., Sigal, A., Schmidt, A.G., Iafate, A.J., Naranbhai, V., Balazs, A.B., 2021. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell* 184 (9), 2372–2383 e9.
- Gfeller, D., Grosdidier, A., Wirth, M., Daina, A., Michielin, O., Zoete, V., 2014. SwissTargetPrediction: a web server for target prediction of bioactive small molecules. *Nucleic Acids Res.* 42 (Web Server issue), W32–W38.
- Hegde, M., Daimary, U.D., Kumar, A., Chinnathambi, A., Alharbi, S.A., Shakibaei, M., Kunnumakkara, A.B., 2022. STAT3/HIF1A and EMT specific transcription factors regulated genes: novel predictors of breast cancer metastasis. *Gene* 818, 146245.
- Hikmet, F., Mear, L., Edvinsson, Å., Micke, P., Uhlén, M., Lindskog, C., 2020. The protein expression profile of ACE2 in human tissues. *Mol. Syst. Biol.* 16 (7), e9610.
- Jan, J.T., Cheng, T.R., Juang, Y.P., Ma, H.H., Wu, Y.T., Yang, W.B., Cheng, C.W., Chen, X., Chou, T.H., Shie, J.J., Cheng, W.C., Chein, R.J., Mao, S.S., Liang, P.H., Ma, C., Hung, S.C., Wong, C.H., 2021. Identification of existing pharmaceuticals and herbal medicines as inhibitors of SARS-CoV-2 infection. *Proc. Natl. Acad. Sci. U. S. A.* 118 (5), e2021579118.
- Lin, Y.L., Shih, C., Cheng, P.Y., Chin, C.L., Liou, A.T., Lee, P.Y., Chiang, B.L., 2020. A polysaccharide purified from *Ganoderma lucidum* acts as a potent mucosal adjuvant that promotes protective immunity against the lethal challenge with enterovirus A71. *Front. Immunol.* 11, 561758.
- Lin, S., Chen, Z., Zhang, X., Wen, A., Yuan, X., Yu, C., Yang, J., He, B., Cao, Y., Lu, G., 2022. Characterization of SARS-CoV-2 Omicron spike RBD reveals significantly decreased stability, severe evasion of neutralizing-antibody recognition but unaffected engagement by decoy ACE2 modified for enhanced RBD binding. *Signal Transduct. Targeted Ther.* 7 (1), 56.
- Maruyama, T., Kobayashi, S., Nakatsukasa, H., Moritoki, Y., Taguchi, D., Sunagawa, Y., Morimoto, T., Asao, A., Jin, W., Owada, Y., Ishii, N., Iwabuchi, Y., Yoshimura, A., Chen, W., Shibata, H., 2021. The curcumin analog GO-Y030 controls the generation and stability of regulatory T cells. *Front. Immunol.* 12, 687669.
- Mooers, B.H.M., 2020. Shortcuts for faster image creation in PyMOL. *Protein Sci.* 29 (1), 268–276.
- Nile, S.H., Nile, A., Jalde, S., Kai, G., 2021. Recent advances in potential drug therapies combating COVID-19 and related coronaviruses-A perspective. *Food Chem. Toxicol.* 154, 112333.
- Omotuyi, O., Olubiyi, O., Nash, O., Afolabi, E., Oyinloye, B., Fatumo, S., Femi-Oyewo, M., Bogoro, S., 2022. SARS-CoV-2 Omicron spike glycoprotein receptor binding domain exhibits super-binder ability with ACE2 but not convalescent monoclonal antibody. *Comput. Biol. Med.* 142, 105226.
- Ortega, J.T., Serrano, M.L., Pujol, F.H., Rangel, H.R., 2020. Role of changes in SARS-CoV-2 spike protein in the interaction with the human ACE2 receptor: an in silico analysis. *EXCLI J* 19, 410–417.
- Pandey, P., Al Rumaih, Z., Kels, M., Ng, E., Kc, R., Chaudhri, G., Karupiah, G., 2022. Targeting ectromelia virus and TNF/NF- $\kappa$ B or STAT3 signaling for effective treatment of viral pneumonia. *Proc. Natl. Acad. Sci. U. S. A.* 119 (8), e2112725119.

- Planas, D., Saunders, N., Maes, P., Guivel-Benhassine, F., Planchais, C., Buchrieser, J., Bolland, W.H., Porrot, F., Staropoli, I., Lemoine, F., Péré, H., Veyer, D., Puech, J., Rodary, J., Baele, G., Dellicour, S., Raymenants, J., Gorissen, S., Geenen, C., Vanmechelen, B., Schwartz, O., 2022. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. *Nature* 602 (7898), 671–675.
- Remali, J., Aizat, W.M., 2021. A review on plant bioactive compounds and their modes of action against coronavirus infection. *Front. Pharmacol.* 11, 589044.
- Ren, J.L., Zhang, A.H., Wang, X.J., 2020. Traditional Chinese medicine for COVID-19 treatment. *Pharmacol. Res.* 155, 104743.
- Rivero-Segura, N.A., Gomez-Verjan, J.C., 2021. In silico screening of natural products isolated from Mexican herbal medicines against COVID-19. *Biomolecules* 11 (2), 216.
- Ru, J., Li, P., Wang, J., Zhou, W., Li, B., Huang, C., Li, P., Guo, Z., Tao, W., Yang, Y., Xu, X., Li, Y., Wang, Y., Yang, L., 2014. TCMSp: a database of systems pharmacology for drug discovery from herbal medicines. *J. Cheminf.* 16 (6), 13.
- Russo, M., Moccia, S., Spagnuolo, C., Tedesco, I., Russo, G.L., 2020. Roles of flavonoids against coronavirus infection. *Chem. Biol. Interact.* 328, 109211.
- Shah, M., Woo, H.G., 2022. Omicron: a heavily mutated SARS-CoV-2 variant exhibits stronger binding to ACE2 and potently escapes approved COVID-19 therapeutic antibodies. *Front. Immunol.* 12, 830527.
- Sivakumar, S., Qi, S., Cheng, N., Sathe, A.A., Kanchwala, M., Kumar, A., Evers, B.M., Xing, C., Yu, H., 2022. TP53 promotes lineage commitment of human embryonic stem cells through ciliogenesis and sonic hedgehog signaling. *Cell Rep.* 38 (7), 110395.
- Stelzer, G., Dallah, I., Stein, T.I., Satanower, Y., Rosen, N., Nativ, N., Oz-Levi, D., Olender, T., Belinky, F., Bahir, I., Krug, H., Perco, P., Mayer, B., Kolker, E., Safran, M., Lancet, D., 2011. In-silico human genomics with GeneCards. *Hum. Genom.* 5 (6), 709–717.
- Suwannarach, N., Kumla, J., Sujarit, K., Pattananandecha, T., Saenjum, C., Lumyong, S., 2020. Natural bioactive compounds from fungi as potential candidates for protease inhibitors and immunomodulators to apply for coronaviruses. *Molecules* 25 (8), 1800.
- Szklarczyk, D., Gable, A.L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N.T., Morris, J.H., Bork, P., Jensen, L.J., Mering, C.V., 2019. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* 47 (D1), D607–D613.
- Taylor, P.C., Adams, A.C., Hufford, M.M., de la Torre, I., Winthrop, K., Gottlieb, R.L., 2021. Neutralizing monoclonal antibodies for treatment of COVID-19. *Nat. Rev. Immunol.* 21 (6), 382–393.
- UniProt, Consortium, 2019. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res.* 47 (D1), D506–D515.
- Weiß, J., Rosemann, J., Müller, L., Kappler, M., Eckert, A.W., Glaß, M., Misiak, D., Hüttelmaier, S., Ballhausen, W.G., Hatzfeld, M., Haemmerle, M., Gutschner, T., 2021. Identification of lymphocyte cell-specific protein-tyrosine kinase (LCK) as a driver for invasion and migration of oral cancer by tumor heterogeneity exploitation. *Mol. Cancer* 20 (1), 88.
- Weng, C.J., Chau, C.F., Chen, K.D., Chen, D.H., Yen, G.C., 2007. The anti-invasive effect of lucidenic acids isolated from a new *Ganoderma lucidum* strain. *Mol. Nutr. Food Res.* 51 (12), 1472–1477.
- Wu, X., Cao, J., Li, M., Yao, P., Li, H., Xu, W., Yuan, C., Liu, J., Wang, S., Li, P., Wang, Y., 2020. An integrated microbiome and metabolomic analysis identifies immunoenhancing features of *Ganoderma lucidum* spores oil in mice. *Pharmacol. Res.* 158, 104937.
- Wu, B., Zhao, T.V., Jin, K., Hu, Z., Abdel, M.P., Warrington, K.J., Goronzy, J.J., Weyand, C.M., 2021. Mitochondrial aspartate regulates TNF biogenesis and autoimmune tissue inflammation. *Nat. Immunol.* 22 (12), 1551–1562.
- Xiong, Y., Liu, Y., Cao, L., Wang, D., Guo, M., Jiang, A., Guo, D., Hu, W., Yang, J., Tang, Z., Wu, H., Lin, Y., Zhang, M., Zhang, Q., Shi, M., Liu, Y., Zhou, Y., Lan, K., Chen, Y., 2020. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. *Emerg. Microb. Infect.* 9 (1), 761–770.
- Xu, J., Xiao, C., Xu, H., Yang, S., Chen, Z., Wang, H., Zheng, B., Mao, B., Wu, X., 2021. Anti-inflammatory effects of *Ganoderma lucidum* sterols via attenuation of the p38 MAPK and NF- $\kappa$ B pathways in LPS-induced RAW 264.7 macrophages. *Food Chem. Toxicol.* 150, 112073.
- Yin, W., Xu, Y., Xu, P., Cao, X., Wu, C., Gu, C., He, X., Wang, X., Huang, S., Yuan, Q., Wu, K., Hu, W., Huang, Z., Liu, J., Wang, Z., Jia, F., Xia, K., Liu, P., Wang, X., Song, B., Xu, H.E., 2022. Structures of the Omicron spike trimer with ACE2 and an anti-Omicron antibody. *Science* 375 (6584), 1048–1053.
- Yu, Z., Kan, R., Ji, H., Wu, S., Zhao, W., Shuiian, D., Liu, J., Li, J., 2021. Identification of tuna protein-derived peptides as potent SARS-CoV-2 inhibitors via molecular docking and molecular dynamic simulation. *Food Chem.* 342, 128366.
- Zhang, Y., Niu, G., Flisikowska, T., Schnieke, A., Flisikowski, K., 2021. A tissue- and gender-specific regulation of the SARS-CoV-2 receptor ACE2 by P53 in pigs. *Biochem. Biophys. Res. Commun.* 553, 25–29.
- Zhu, Q., Bang, T.H., Ohnuki, K., Sawai, T., Sawai, K., Shimizu, K., 2015. Inhibition of neuraminidase by *Ganoderma* triterpenoids and implications for neuraminidase inhibitor design. *Sci. Rep.* 5, 13194.