Journal of proteome research

pubs.acs.org/jpr

Reviews

Techniques and Strategies for Potential Protein Target Discovery and Active Pharmaceutical Molecule Screening in a Pandemic

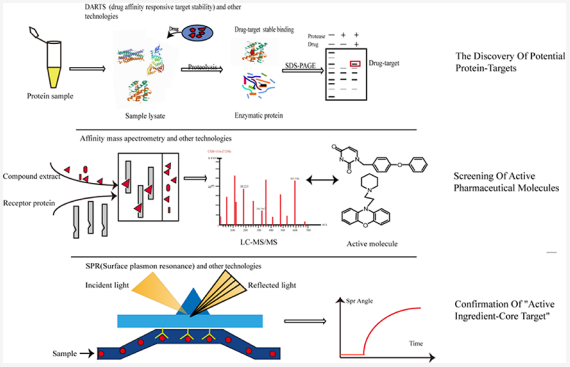
Hongxin Yu,[#] Chunyan Li,[#] Xing Wang,[#] Jingyi Duan, Na Yang, Lijuan Xie, Yu Yuan, Shanze Li, Chenghao Bi, Bin Yang,* and Yubo Li*

Cite This: *J. Proteome Res.* 2020, 19, 4242−4258

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Viruses remain a major challenge in the fierce fight against diseases. There have been many pandemics caused by various viruses throughout the world over the years. Recently, the global outbreak of COVID-19 has had a catastrophic impact on human health and the world economy. Antiviral drug treatment has become another essential means to overcome pandemics in addition to vaccine development. How to quickly find effective drugs that can control the development of a pandemic is a hot issue that still needs to be resolved in medical research today. To accelerate the development of drugs, it is necessary to target the key target proteins in the development of the pandemic, screen active molecules, and develop reliable methods for the identification and characterization of target proteins based on the active ingredients of drugs. This article discusses key target proteins and their biological mechanisms in the progression of COVID-19 and other major epidemics. We propose a model based on these foundations, which includes identifying potential core targets, screening potential active molecules of core targets, and verifying active molecules. This article summarizes the related innovative technologies and methods. We hope to provide a reference for the screening of drugs related to pandemics and the development of new drugs.



KEYWORDS: *pandemic, protein targets, active pharmaceutical molecules*

1. INTRODUCTION

The recent outbreak of novel coronavirus pneumonia (COVID-19) is undoubtedly an urgent global public health problem. The mortality and morbidity of COVID-19 are still rising. The World Health Organization assessed the new coronavirus pneumonia as a global pandemic on March 11, 2020.[1,2] A pandemic is a disease that spreads around the world with a large number of infections. For example, influenza A H1N1 in 2009, severe acute respiratory syndrome (SARS) in 2003, and AIDS (HIV/AIDS) first discovered in 1981.[3] These diseases had a serious impact on economic development and human health.

Vaccination is a safe and effective means of prevention and treatment in the face of a pandemic. However, there are also difficulties in developing vaccines, such as long development time, virus mutation, related technical support, and the need for large-scale production.[4,5] Because of these problems, it has brought considerable challenges to the research and development of vaccines. As biological macromolecules, proteins play a vital role in the development of the pandemic. On the one hand, the specific binding of active ingredients can prevent and alleviate disease. On the other hand, it is an essential receptor for viruses, participating in virus replication and causing disease.[6] Proteins play a vital role in finding potential therapeutic targets, developing vaccines and drugs, and preventing and treating pandemic diseases. Therefore, exploring the role of protein molecules in pandemic diseases is of great value for the prevention and treatment of pandemics.

In summary, this article reviews the pathogenesis of pandemic influenza and the process of proteins in the body participating in the disease. This article has a deep understanding of the disease from the molecular level. It proposes a model of "the discovery of potential protein targets, the screening of active pharmaceutical molecules, and the confirmation of "active ingredient-core target": first, using relevant technology to capture key potential targets for specific diseases; second, using affinity mass spectrometry and other technology to quickly and efficiently identify the active

Special Issue: Proteomics in Pandemic Disease

Received: May 31, 2020
Published: September 22, 2020

molecules of the core target;[7] and finally, in vitro and in vivo experiments were used to verify the "active molecule−target protein" interaction. We hope that the research process of the techniques and strategies introduced in this review can provide a reference for the treatment of pandemic diseases (Figure 1).

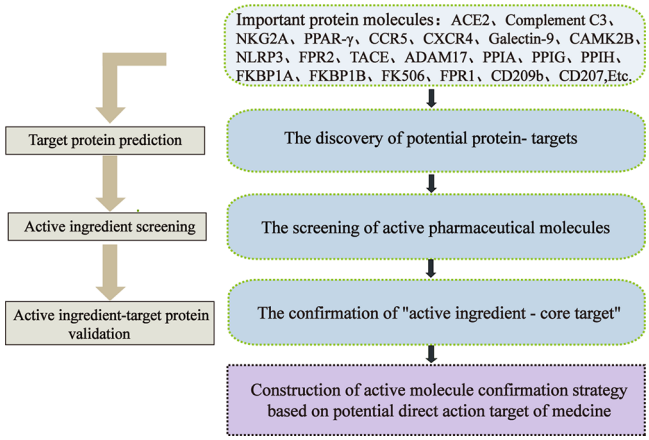


Figure 1. Construction of a molecule confirmation strategy based on the potential direct action target of medicine.

2. POTENTIAL PROTEIN TARGETS IN A PANDEMIC

As everyone knows, the novel coronavirus pneumonia COVID-19 has caused huge casualties and serious economic losses, which has posed a threat to the world. Therefore, it is urgently necessary to understand the pathogenesis of the pandemic, including COVID-19, and develop effective medicines to treat the disease. This part will summarize the pathogenesis of previous pandemics, such as COVID-19, AIDS, H1N1, SARS, plague, etc., and the role of proteins in the process of disease. We hope to have some inspiration on the research of therapeutic medicines.

2.1. Potential Protein Targets in COVID-19

The novel coronavirus pneumonia (COVID-19), which attacked in the world in 2019, is now reported to be caused by a new type of coronavirus (SARS-CoV-2),[8] which is a highly infectious and highly pathogenic acute respiratory disease. Since its occurrence, the disease has seriously endangered human health and has been listed as a global pandemic by the World Health Organization. The pathogen, the new coronavirus SARS-CoV-2, the severe acute respiratory syndrome virus SARS-CoV, and the Middle East respiratory syndrome MERS-CoV virus, belongs to the β-coronavirus family. Because of the difference in the spike protein (s), SARS-CoV-2 has a higher transmission efficiency, and through the spike protein (s) attached to the receptor ACE2 into human cells, causing serious damage to the heart and lungs and other organs.[9,10] For the exploration of the pathogenesis of COVID-19, a mouse model is the most suitable. Zhao's team[11] developed the model by replicating the defective adenovirus (AD5-HACE2) and delivering human ACE2 to the mouse, which developed pneumonia. In the study, researchers treated mice with recovered patient plasma and remxavir the day before infection and found that the virus was cleared faster and the disease infection was reduced. The establishment of a mouse model has great significance to study the pathogenesis of and vaccine against COVID-19. ACE2, an angiotensin-converting enzyme 2, is widely expressed in the heart, intestine, lung, and other tissues. It is an important regulatory protein in the renin-angiotensin system (RAS) to maintain physiological function, which lyses AngII as Ang (1−7), and Ang (1−7) can specifically bind to the Mas protein and play an anti-inflammatory role.[12] Sun et al.[13] speculated that the combination of SARS-CoV-2 and ACE2 depleted ACE2 and inhibited the pathway ACE2/Ang (1−7)/Mas, which led to the imbalance of the RAS system, increasing inflammatory factors, which aggravated the deterioration of pneumonia. Zhao et al.[14] found that ACE2 was highly expressed in lung cells using high-throughput single-cell sequencing analysis technology, and its wide expression in AT2 may explain the serious alveolar injury after infection. ACE2 is a key receptor for viral transmission and an important target for drug development. In view of this, the development of ACE2 receptor inhibitors and the blocking of virus invasion vectors will be a potential therapeutic direction. Related studies[15,16]

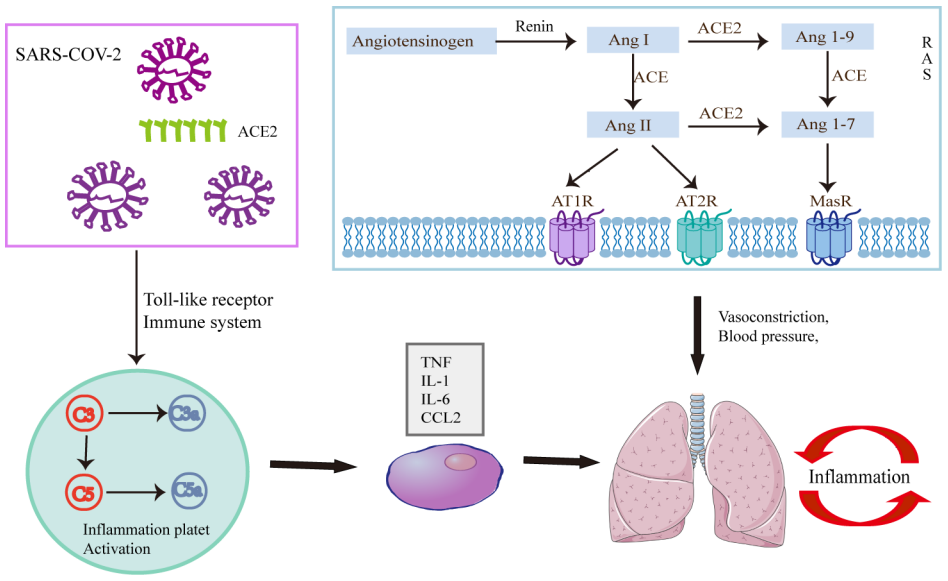


Figure 2. Pathogenesis and complement activation of novel coronavirus pneumonia.

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

found that the use of angiotensin-converting enzyme inhibitors (ACEIs) and TMPRSS inhibitors of s protein bound with the ACE2 receptor can reduce the probability of lung injury. In addition, ACE2 was found to be highly expressed in patients' cardiomyocytes. Lu et al.[17] used TargetScan for bioinformatics search to predict the mirnas that regulate ACE2, and combined with RT-PCR and Western blot for verification. The final results showed that Mir-200C could regulate the expression of ACE2 in myocardial cells, and the researchers reported the first miRNA candidate that could target ACE2 in myocardial cells. These results are of reference value for an in-depth study of the pathogenesis of COVID-2019 and the development of therapeutic medicines. In addition, complement C3 is a kind of β2-globulin synthesized from the liver, which is the key substance of the immune pathway. It was found that[18] when SARS-CoV-2 combined with ACE2, the level of angiotensin II increased, further activating complement, which was related to acute respiratory failure (ARDS) and systemic coagulopathy. Mastaglio et al.[19] interfered with AMY-101, a complement C3 inhibitor, which can reduce the secretion of inflammatory factors during ARDS, indicating that complement C3 is an important protein in the process of COVID-19 disease. Also, NSP15 endoribonuclease plays an equally important role in virus invasion as ACE2. According to studies, NSP15 can replicate the genetic material of the virus. Chikhale et al. made use of molecular docking and kinetic studies to screen and identify them and found that the effective components of Asparoside-C, Asparoside-F, and Asparoside-D had good binding ability to the binding domain of this protein. The screening of antiviral drugs has a certain reference significance.[20,21] The pathogenesis and complement activation pathways of novel coronavirus pneumonia are shown in Figure 2.

Cytokine storm is another important factor affecting COVID-19 disease, which is characterized by the excessive release of inflammatory factors and chemokines by cells such as dendritic cells, and an imbalance of immune response, thus aggravating pneumonia. Huang et al.[22] found that the levels of IL2, IL7, MIP1A, TNF, and other inflammatory factors in ICU patients in Wuhan were higher than those in non-ICU patients, and the elderly and patients with complications were more likely to be infected. The immune response mediated by cytokine storm is correlated with the development of the disease, so inhibiting the release of inflammatory cytokines and enhancing the immune response are effective treatments. NKG2A receptor is one of the NK cell inhibitory receptors. Zheng et al.[23] studied the overexpression of NKG2A in patients with SARS-CoV-2 infection, which reduced the number and function of NK and CD8 + T cells, and destroyed the immune response. Furthermore, Van et al.[24] used monalizumab, an NKG2A inhibitor, and the results showed that it prevented tumor growth in mice and brought the disease under control. On the basis of the above experiments, it is of great significance to study the inhibitors of the NKG2A receptor to restore the immune function of NK and CD8+ T cells. Heat shock protein 60(HSP60), a heat stress protein, could promote the secretion of inflammatory cells, and is related to cardiovascular diseases such as hypertension and inflammatory response. In COVID-19, patients with hypertension have increased levels of heat shock protein 60-(HSP60), which causes acute heart damage and heart failure and finally could aggravate their condition. Therefore, the pharmacological inhibition of heat shock protein 60(HSP60) could reduce inflammation.[25] It is worth noting. Studies have found that sterol regulatory element binding protein-2 (SREBP-2) plays an important regulatory role in lipid metabolism. In COVID-19 patients, the SREBP-2 protein level is increased under the influence of the cytokine storm, while the total cholesterol level is low. Lee's team studied the expression of SREBP-induced cholesterol organism sestrin-1 and PCSK9, which inhibited the inflammatory response induced by SREBP-2, and added SREBP-2 and NF- B inhibitors to inhibit the cytokine storm in an infectious mouse model. These results suggest that SREBP-2 may be a key therapeutic target for preventing the cytokine storm and lung injury.[26] Besides, the peroxisome proliferator-activated receptor (PPAR)-γ plays a key regulatory role in anti-inflammatory responses, and recent studies have shown that it is a potential target for regulating cytokine storm.[27]

2.2. Potential Protein Targets in AIDS

AIDS, acquired immune deficiency syndrome, is caused by the human immunodeficiency virus (HIV), which could be transmitted through sex, blood, and mother-to-child routes, leading to the development of infections and tumors. According to statistics,[28] in 2016, the number of people infected with HIV in the world has reached 36.7 million, and the number of people dying from AIDS has reached 1 million. It could be seen that HIV is an infectious disease that seriously affects human health and the social economy. As we all know, pathogen HIV is a lentivirus, which is divided into subtypes HIV-1 and HIV-2. Although the two have similarities in gene arrangement and transmission mode, relatively speaking, HIV-1 is highly infectious and pathogenic.[29] Diseases caused by HIV-1 are mainly due to the combination of gp120 protein on the surface and the CD4 molecule on the surface of the host cell, which is expressed as a mature virus molecule in the cells and then leads to infection. This process is mainly related to an autoimmune response and ERK signaling pathway.[30,31] Moreover, Nef is a protein in range of 27−34 kDa, one of the early HIV-1 expressions of protein. The study found that Nef could adjust the host cell protein (such as virus receptor CD4 immune receptors, host a restrictive factor, etc.) in the functional status and the status of the virus replication by many ways. It is vital for the pathogenesis of the virus, which would be an important target of antiretroviral drug discovery.[32] Betzi et al.[33] found that compounds dlc27 and Nef: SH3 could be antagonistic to each other. First, 335 compounds were pre selected from 1420 compounds by using high throughput docking and gfscore evaluation scores. These compounds were further clustered by rtlbr based pharmacodynamic filter, and 33 candidates were retained. Ten of them were selected for experimental evaluation through chemical and geometric properties. Finally, NMR HSQC experiments confirmed that dlc27 interacted with Nef at the expected rtlbr. This study is conducive to finding antagonistic substances against Nef, which can resist virus replication. Therefore, by blocking the entry of the pathogen HIV into the cell or any link of its redevelopment, the way of pathogen infection is interrupted, which will bring the AIDS condition under control. Therefore, the confirmation of key proteins becomes the core of the research. CCR5 (c-c chemokine receptor type 5) is a membrane protein on the cell surface, which is an important auxiliary receptor for HIV-1 to invade the human body. More and more studies have been done on antagonists targeting CCR5. According to the research, the drug maraviroc (MVC),

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

which is the only FDA approved inhibitor drug, has a better clinical effect than other CCR5 antagonists, such as tak779, SCH-351125 (SCH-C), and cenicriviroc (CVC or TBR-652).[34−37] CXCR4, a member of the G protein-coupled transmembrane fragment receptor (GPCR) family, is also a coreceptor for the invasion of the HIV-1 virus with CCR5, playing an important role in the pathogenesis and providing a new method for the treatment of AIDS.[38] In the study of CXCR4 antagonist, in addition to the recognized Plerixafor (AMD-3100), which has a good inhibitory effect on HIV-1, dual antagonism has become a new method. For example, Gama et al.[39] isolated cinnamic acid, hexanoic acid, and laurate from *Euphorbia tirucalli* and proved that they could antagonize CCR5 and CXCR4 and have a certain antiviral effect. Galactose lectin-9 (galectin-9), a member of the lectin superfamily, plays an important role in physiological and pathological processes. Recently, it has been found that galectin-9 has a high concentration in HIV-infected patients and antiretroviral therapy patients, and activates ERK and CREB signaling pathways downstream of TCR in an lck-dependent way, releasing inflammatory factors to participate in the HIV-1 transcription process.[40,41] Currently, there is no good drug for the treatment of diseases in this field. In the future, an inhibitor or inhibition of galectin-9 pathway can be developed to prevent the infection of the HIV-1 virus.

2.3. Potential Protein Targets in H1NI

The outbreak of influenza A (H1N1) in Mexico in 2009, which infected 290 000 people, was the first influenza pandemic of the 21st century. In the past, the influenza virus occurred from 1918 to 1920 (Spain), 1957 to 1959 (H2N2, Asia), and 1968 to 1970 (H3N2, Hong Kong). It was a highly infectious acute respiratory infection, which seriously affected global public health.[42] Fever, vomiting, and muscle soreness were common symptoms after infection. And pneumonia and organ failure were usually associated with worse infected patients. The H1N1 virus binds to glycoproteins or glycolipids on the surface of host cells via hemagglutinin (HA), then carries out viral replication and infection of the organism. Related studies have shown that in the pathogenesis of H1N1, a large number of inflammatory factors are released and the immune function declines, which involves the MAPK pathway, toll-like receptor signaling pathway, apoptosis, and other pathways.[43−45] For the treatment of influenza viruses, neuraminidase inhibitors (NAIs) are currently effective antiviral drugs, which prevent virus replication by reducing the surface protease activity of the virus, such as oseltamivir. At the same time, the virus replication cannot leave the host factors, according to König et al.[46] using genome-wide RNAi screen for integrated system methods, 10 proteins related to the process of virus replication such as FGFR, GSK3B, CAMK2B, and PRSS35. Calcium/calmodulin-dependent protein kinase type 2 (CAMK2B) is a serine/threonine kinase, and the inhibitor KN-93 has a certain inhibition of influenza virus.[47] It provides a new strategy to understand the mechanics of H1N1 disease and find new therapeutic targets.

NLRP3 inflammasome, a protein complex with a molecular weight of about 700 kDa, plays an important role in the immune system and is closely related to metabolic disorders, multiple sclerosis, inflammatory bowel disease, and other disease processes.[48−50] Jia et al. found that during the H1N1 infection, inflammatory factors such as IL-1β and IL-18 were secreted due to the activation of NLRP3 inflammasome, exacerbating lung injury. Meanwhile, the mTOR-NF-κB-NLRP3 pathway was inhibited by the combination of drugs to reduce the level of inflammatory factors and slow down the infection.[51] The formyl peptide receptor 2 (FPR2), a member of the GPCR protein family, is associated with the host defense and inflammatory response.[52] Virus modifiable host factors are used to suppress host defense systems and enhance their transmission. Ampomah demonstrated that infection with the H1N1 PR8 influenza virus leads to increased expression of FPR2. This study found that FPR2 is regulated by the IFN-STAT3 pathway, inhibiting STAT3 phosphorylation in mouse bone marrow macrophages, inhibiting FPR2 induction, and resulting in a reduction in viral load. Finally, deletion of FPR2 in mouse BMDM resulted in a reduced viral load, indicating that FPR2 was beneficial to virus replication.[53] Also, Rahman[54] revealed that the monoclonal antibody FN-1D6-AI and antagonist WRW4 can significantly inhibit the viral replication (IAV A/PR/8/34(H1N1) process on A549 epithelial cells, indicating that FPR2 is an important target protein for the treatment of influenza virus infection.

2.4. Potential Protein Targets in SARS

Severe acute respiratory syndrome (SARS), which occurred in 2002−2004, is a viral respiratory disease caused by coronavirus SARS-CoV. This disease caused more than 8000 infections, more than 800 deaths, and severe economic losses. Similar to the pathogenesis of novel coronavirus in 2019, the virus binds to the receptor ACE2 and causes disease, resulting in damage to lungs and other organs and a decline in immune function.[55] Pathogen SARS-CoV and SARS-CoV2 are both β-coronavirus, and the difference of structural protein S is not as fast as that of SARS-CoV-2, but the pathogenicity is stronger.[56] According to recent studies, innate immunity and cytokine responses are involved in the occurrence and progression of SARS,[57,58] so the study of key proteins in the pathogenesis is conducive to finding new breakthroughs in the treatment of diseases. The expression of ACE2, angiotensin converting enzyme 2, is down-regulated during SARS-CoV virus infection, which is the key link of virus replication. Vincent et al. interfered with VeroE6 cells before and after infection with chloroquine, which has been proven to have antiviral drug activity, and found that chloroquine could destroy the terminal glycosylation state of ACE2 and had a good effect on the prevention and treatment of the disease.[59,60] TNF-α-converting enzyme (TACE) and ADAM17 are members of the ADAM protein family. They promote the binding of the virus to the ACE2 receptor during the process of viral infection and are particularly important in the process of disease infection. Haga et al. found that the expression of ACE2 in the extract of pseudovirus infected VeroE6 cells decreased, while the expression of 80 kDa protein in the supernatant increased, but the antibody to the cytoplasmic domain of ACE2 did not detect the peptide of 80 kDa, and then C peptidase activity was detected in the supernatant of cultured cells treated with SARS-S. TACE is a key cytokine in SARS-S-induced ACE2 abscission. In order to verify this conclusion, it was found that TAPI-0 effectively reduced the C-peptidase activity in the culture supernatant by using TACE inhibitors. Further Western blotting analysis showed that the inhibitor could inhibit SARS-S-induced ACE2 shedding.[61] ADAM17 protein has been proven to be a candidate medium for stimulating ACE2 shedding in another study. In this experiment, Western blotting analysis and fluorescent peptide Mca-APK (Dnp) determination were done

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

in the extract collected from HEK-ACE2 cells, and it was confirmed that ACE2 was exfoliated by extracellular domain, and then this view was confirmed by protein analysis of siRNA transfected cells.[62] At the same time, as shown by Western blotting, the natural inhibitor NTIMP-3 inhibited ACE2 shedding in a dose-dependent manner. In summary, the hydrolytic shedding of cell surface proteins is an important mechanism for regulating their expression and function. Therefore, the study of TACE and ADAM17 inhibitors may be helpful to inhibit ACE2 shedding and resist virus binding to receptors, which is an important protein.

In addition, Pfefferle et al.[63] screened host proteins with high Nsp1 binding index to the nonstructural protein Nsp1 of SARS-CoV by whole genome analysis (HTY2H), including PPIA, PPIG, PPIH, FKBP1A, FKBP1B, and FK506, and intervened with inhibitor CspA in the experiment. The results showed that these proteins could inhibit the occurrence of important CnA/NFAT immune regulatory pathways to resist virus invasion, indicating that these proteins provide a new target for antiviral infection. However, the mechanism of action needs to be clarified through further research.

2.5. Potential Protein Targets in Plague

Plague is a natural focus disease caused by *Yersinia pestis*. The main modes of transmission are (1) rats and fleas, (2) skin contact, (3) respiratory tract transmission, high fever, cough, dyspnea, and toxemia after infection. The pandemic of the virus appeared in the 6th, 14th and 19th centuries. It is highly contagious and has a high case fatality rate. It ranks first among 39 legal infectious diseases and is destructive to human health.[64] In the course of disease, T cell signal transduction is an important pathway of plague. For example, Olson et al.[65] found that pathogens activate the MyD88 pathway to release cytokines such as IL-10 and IFN- β, which promotes lung growth during pneumonic plague.

Dhariwal et al.[66] found that the activation of MyD88 by TLR7 leads to the increase of the interferon level and aggravates the infection of *Yersinia pestis* pathogen. However, the host cell receptor plays a certain role. On the one hand, it can promote virus replication, and at the same time, the use of receptor antagonists can prevent transmission. Formyl peptide receptor (FPR1), a member of G protein coupled receptor (GPCR), has been found in which the pathogen *Yersinia pestis* binds to FPR1 in immune cells through the T3SS secretion system, which promotes viral factor replication and infection in new hosts. At the same time, the use of FPR1 inhibitors such as mAb can enhance the immune response and alleviate infection, indicating that FPR1 plays an important role in viral pathogenesis.[67] Recently, Yang et al.[68] found that *Yersinia pestis* invaded CHO-SIGNR1, but did not infect other CHO transducers, indicating that SIGNR1 (CD209b) is the core lipopolysaccharide receptor of *Yersinia pestis*. In order to verify the specificity of the interaction between *Yersinia pestis* and SIGNR1, the team further proved that SIGNR1 antibody, mannan, His-Mermaid, and CD66 can inhibit core LPS-SIGNR1 interaction. It is suggested that type c lectin receptor SIGNR1 (CD209b) plays a malignant role in virus transmission and is a new receptor for *Yersinia pestis* binding to host cells. Previously, the team found that CD207, a type II transmembrane C lectin with mannose binding specificity, is also a receptor protein that binds pathogens to host cells.[69] This study of receptor proteins will help to find new therapeutic targets.

Through the review of disease mechanism and related proteins, it is not difficult to find that immunity plays an important role in the process of disease. The immune system of the body is destroyed, the resistance is reduced, and the release of inflammatory factors will make the disease develop further, which provides an opportunity for the invasion of viruses and bacteria. Therefore, improving immunity is particularly important for disease prevention. At the same time, in the process of disease, related proteins are important carriers of the virus, which are closely related to the spread and deterioration of the disease. Therefore, finding potential proteins and understanding the mechanism of the disease will help to find ways to treat the disease.

3. THE DISCOVERY OF POTENTIAL PROTEIN TARGETS

Biomacromolecular proteins are an important part of organisms and play an important role in life activities. On the one hand, they could be used as vitamins or carriers of drug molecules to maintain normal physiological functions of the body. On the other hand, they could be used as viral vectors in a pandemic to disrupt the physiological balance and cause disease. Protein has gradually become a target that has pharmacodynamic functions in the body and can be acted on by drugs. It can effectively improve disease symptoms by regulating the physiological activity of the target, and the determination of the target is the core of elucidating the mechanism of action of Chinese medicine and the key to constructing the correlation between chemical composition and pharmacodynamic action, which is the basis for the development of modern new drugs. In addition, emerging drug target finding technology can determine the relationship between drugs and targets and then explore their new uses, providing new ways for disease treatment. Therefore, using certain techniques to discover key proteins and to find potential targets is of great significance to quickly find a breakthrough in the treatment of diseases.

3.1. Screening Potential Target Proteins Based on the Large Database

The continuous development of databases and bioinformatics technology has provided strong support for the development of network pharmacology in the era of big data. Network pharmacology, as a high-throughput technology for predicting drug targets, could quickly and inexpensively predict drug action targets, which lays a foundation for the application of medical network pharmacology. This big data network predicting target breaks through the traditional "single target" model. Analyzing the biological basis of drugs from a network perspective provides a research strategy for the screening of active ingredients. It could link the "full component-target" and "specific disease-target" networks in a high-throughput manner to reveal the potential targets and mechanism of action of active ingredients in regulated diseases. This method conforms to the overall view of TCM theory and opens up a new situation for the analysis of "multicomponent-multitarget-multipath" in the field of Chinese medicine. Wang et al.[70] used network pharmacology to screen 169 potential targets of the traditional Chinese medicine Ma Xing Shi Gan decoction for COVID-19, such as heat shock protein 90, RAC-alpha serine/threonine-protein kinase, transcription factor AP-1, etc., and enriched to the important inflammatory pathways about the extent of COVID-19 infection by KEGG. Cheng et al.[71] also

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

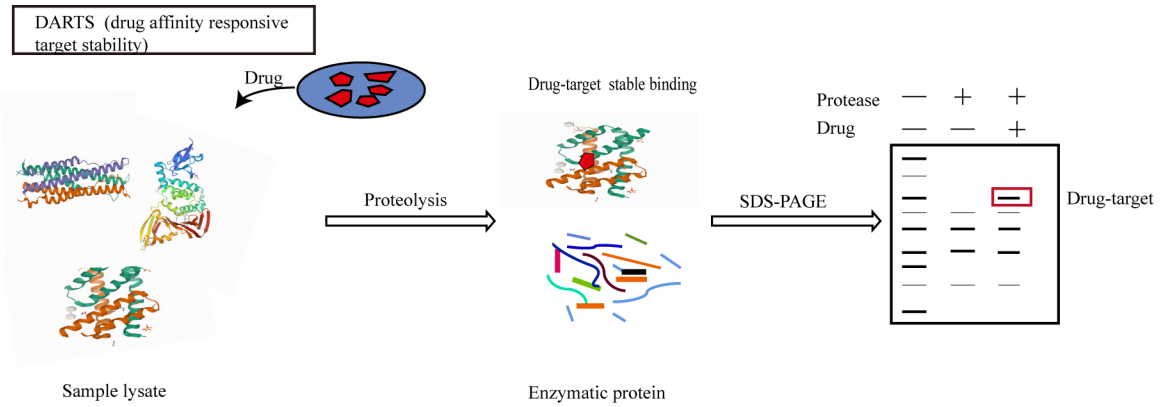


Figure 3. Technical route of drug affinity related target stability technology.

used this method to screen the targets for the antihepatic steatosis mechanism of calycosin, such as ALDH2, NPC1, P450, HMGB1, etc., and related targets were further confirmed by in vivo and in vitro experiments. In addition, Song et al.[72] found that celastrol exerted a therapeutic effect on rheumatoid arthritis by acting on multiple proteins such as MMP-9, COX-2, and c-Myc.

Therefore, network pharmacology is a powerful auxiliary method in the field of medical research. However, its platform still needs to be further supplemented and improved in the era of rapid data updating. In addition, how to effectively identify the interaction between multiple drugs and target proteins has become a problem in development.[73,74]

3.2. Proteomics-Based Label-free Small Molecule Probes to Screen Potential Target Proteins

The label-free probe technology is established based on proteomics. Proteins are in a dynamic equilibrium of different folding states in the organism, when a certain folding state specifically binds to small molecule compounds in traditional Chinese medicine, and the stability of protein fragments will increase immediately, mainly as an increase in quantity, enhanced thermal stability, and enhanced oxidation stability. The main manifestations are increased quantity, enhanced thermal stability, and enhanced antioxidant stability. At present, its methods are mainly divided into drug affinity related target stability technology (DARTS), thermal proteome profiling (TPP), and the stability of proteins from rates of oxidation (SPROX). Their basic principle is mainly to use protein separation, qualitative and quantitative analysis to track and identify specific binding proteins, so as to realize the screening of target proteins.[75−77]

DARTS technology is mainly used to analyze the binding fragments of proteins and small molecules to achieve the qualitative identification of proteins. In this method, the cells combined with the drug are lysed, and the total protein is extracted. Then, the small molecule drug is incubated and combined with the lysis solution, and an appropriate concentration of protease is added, and the mixed protein after the affinity reaction is subjected to enzymatic hydrolysis. Finally, Western blot, SDS-PAGE, and other methods could be used to identify the binding protein. (Technical route of drug affinity related target stability technology as Figure 3.) The TPP method is based on the increased thermal stability of the protein affinity small molecule, the protein fragment moves to the high-temperature end, and the target protein is identified through the protein abundance and temperature curve. In this method, drugs are added to the lysed protein, and then the protein is denatured by heating in a gradient. Finally, mass spectrometry is used to analyze the protein abundance, and then the melting curve is drawn to identify the bound protein fragments. The premise of applying the SPROX method is that after the protein is combined with the small molecule, there is methionine in the protein residue. The oxidation rate of methionine could be regulated by controlling the concentration of protein anticoagulant. According to the curve relation between oxidized product/nonoxidized product and the concentration of the anticoagulant, observe whether the intersection point of the curve moves toward the direction of the increase of the concentration of the anticoagulant, so as to judge whether the protein is a specific target protein. However, if there is no methionine in the protein residues bound to small molecules, it is impossible to detect. Therefore, the SPROX needs to combine the DARTS and the TPP to prove each other. Qin et al.[78] used DARTS technology to find that GNF-7 inhibitors target RIPK1 and RIPK3 kinases in combination, providing an important perspective for the treatment of acute kidney injury. Kirsch et al.[79] found the target protein nucleolar protein 14 (NOP14) of the anticancer drug vioprolide A (VioA) through the TPP method, which helps to better explore the mechanism of action. Li et al.[80] reported several experiments and finally screened out the target targets related to traditional Chinese medicine by applying label-free probe technology.

In summary, label-free probe technology could be used to screen the targets of complex active ingredients. First, full composition analysis of the compounds in the drug should be performed. Second, the target group of each chemical component is taken out, combined with specific disease analysis to clarify the target of drug action. The label-free probe technology could screen target proteins in a relatively large range and is beneficial to reveal unknown targets.

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

However, this technique tends to introduce errors into proteomic analysis, and its screening of target proteins in complex traditional Chinese medicine extracts has certain disadvantages compared with single-component target identification, and it is also difficult to clarify the interaction between the target and various chemical components.[81]

3.3. Screening of Potential Target Proteins Based on Probe-Labeled Small Molecule Technology

Probe labeling technology is that a small molecule substance is introduced into a group to be modified and used as a probe. After specifically identifying and binding to a potential target protein, the functional information on the target protein is obtained by mass spectrometry and other methods.

Affinity chromatography is the most commonly used method for target identification of active natural products. In this method, biotin or agar gel is added as an affinity tag on a small molecule and incubated with the protein extract. The target is separated from the unbound protein by elution, and the protein name is identified by SDS-PAGE analysis mass spectrometry.[82] Chen et al.[83] found that the target protein of ginseng (ginsenoside) with a fatigue-relieving effect in skeletal muscle tissue was creatine kinase (CK-MM) through affinity chromatography analysis technology, which was verified by molecular docking. Therefore, the mechanism of fighting against asthma action of ginseng is better explained. Zhang et al.[84] used this method to show that the doxofylline and β2-adrenergic receptors have a high junction and index, which may be an anti-asthma mechanism. This method has the advantages of high specificity and easy operation. However, the introduction of affinity tags with greater steric hindrance can easily lead to reduced or even lost activity of the compound. Therefore, we introduced affinity-based protein profiling (ABPP).

ABPP is currently a relatively mature probe labeling technology. It uses chemical methods to introduce groups such as fluorescence, which are used as active molecular probes, and then covalently binds to the active site of the target protein to enrich and separate the target protein that interacts with small molecules.[85] For example, Ma et al.[86] used ABPP and bioimaging techniques to reveal cell targets containing five photoreactive anticancer inhibitors such as ATP1A1, MDR1, and PARP1. In this technology, the probe synthesis is simple and could identify physiological targets containing active electrophilic framework substances. However, the identification of nonspecific proteins is the main problem of this technology development. The click reaction was proposed by Sharpless in 2001. This method creates a new chemical combination method based on C-X-C syntheses, such as copper-catalyzed azido-alkynyl cycloaddition reaction. It has the advantages of efficient and feasible operation and mild reaction conditions. For example, Zhang et al.[87] achieved immunofluorescence colocalization with the natural inhibitor AKT through the click reaction between the probe and the alkynyl CY5, further illustrating the anti-inflammatory mechanism of Swertiamarin, the component of honeysuckle. Jourdan et al.[88] found that antimalarial drugs invading pathogens are a random alkylation process, and using click chemistry and other technologies found that 25 proteins changed. However, the disadvantage of using this method is that the introduction of biorthogonal groups such as alkynyl groups on small molecules through organic synthesis will lead to a decrease in the activity of the compound.

3.4. Screening Potential Target Proteins Based on Information Biochip Technology

Information biochip technology is divided into gene chip and protein chip technology. It is mainly through the planar microprocessing technology, which is constructed on the solid chip and another microsurgery biochemical analysis system. It can accurately and quickly detect protein, nucleic acid, and other biological components with high flux and automation. The basic principle is that after the drug enters the human body, the pharmacokinetics molecules will interact with the cells to cause changes in the external (morphology) and internal (metabolism) of the cells, among which the internal changes are concentrated on the changes of gene expression or protein. Therefore, the mechanism of action of the drugs can be inferred by analyzing the different factors or proteins, and the drug target can be confirmed.[89,90] For example, Wei et al.[91] explored the target of Baixiangdan capsule in regulating premenstrual anxiety disorder based on gene transcription level and analyzed the differential gene expression profiles of the hippocampus in each experimental group by gene chip technology, and the real-time fluorescence quantitative detection results were consistent with the data shown in GeneChip, which confirmed the effective target gene of Baixiangdan capsule. Huang et al.[92] used protein chip technology to identify 69 candidate target proteins in discussing the effect and mechanism of Celastrol on human colorectal cancer.

In a comprehensive analysis, the application of information biochip technology has achieved high-throughput, high-efficiency, and automated screening of drug targets. However, as the intermediate medium of carrying genes or proteins, the chip has shortcomings such as poor reproducibility of experimental results and the limited scope of target analysis.

3.5. Screening Potential Target Proteins Based on New Chemical Materials

Magnetic nanoparticles (MNPs) are a new type of material with high development and application value in recent years, which are widely used in modern scientific fields such as biomedicine and nuclear magnetic resonance imaging. MNPs generally consist of magnetic cores composed of metal oxides such as iron, cobalt, and nickel and nanoparticles which are made up of a high molecular polymer/silicon/hydroxyapatite shell outside the magnetic core. It has magnetic directivity, under the action of an external magnetic field, and it could realize directional movement and facilitate positioning and separation from the medium. Its active polymer groups on the polymer shell can be combined with various biological molecules, such as protein and enzyme, etc. Currently, MNPs have mainly been applied in biomedicine, including magnetic drug targeting, nucleic acid/protein/virus detection.[93] Wang et al.[94] developed a technique for in vivo target recognition using nanoparticles, which provided a feasible way for drug target prediction. First, by using the hydroxyl structure of compound echinacoside and by the epoxy marker on the MNPs surface, we successfully prepared organ selective and recyclable MNPs. Then, through intravenous injection of MNPs in rat body it was distributed to a specific organ, and then it was swallowed later. The target protein can be captured in situ by drugs combined with MNPs. When MNPs is used to target the target, it should first identify the active ingredients, then classify all the components, and use the special structure

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

Table 1. Identification Technology for Finding Target Protein

number	method	principle	advantage	disadvantage
1	network pharmacology	computer model, constructing "disease, target, drug" network interaction map	It can predict the target of drug action quickly and at low cost, realize multicomponent, multitarget	virtual prediction
2	drug affinity related target stability technology (DARTS)	small molecules and protein binding stability enhance resistance to enzymatic hydrolysis	no need to modify small molecules	will be interfered by indirect targets
3	thermal proteome profiling (TPP)	The thermal stability of the protein affinity with small molecules is enhanced.	good stability, the advantages of a large number of identified proteins, and no need to use antibodies for detection	time-consuming, expensive, and limited detection of membrane proteins
4	stability of proteins from rates of oxidation (SPROX)	analyze the thermodynamic properties of protein folding or unfolding reactions using the oxidation rate of methionine residues in proteins mediated by H_2O_2	analyze multiple target proteins that interact with drugs, and quantify the affinity of ligand binding proteins	some proteins in the sample are easy to form precipitates and aggregates after heat denaturation
5	affinity chromatography	drugs or small molecules selectively purify the tightly bound target proteins, and then fully eluted with buffer to remove nonspecific binding proteins	efficient, fast, and easy	The introduction of affinity tags with greater steric hindrance can easily lead to reduced or even loss of compound activity.
6	affinity-based protein profiling (ABPP)	introduce active molecular probes to enrich and separate target proteins that interact with small molecules after covalently binding to the active site of the target protein	no need for tedious probe synthesis	probe labeled nonspecific protein
7	click reaction	C-X-C new chemical combination method based on synthesis	efficient and simple operation, mild reaction conditions	The introduction of bioorthogonal groups such as alkynyl groups will result in reduced compound activity.
8	information biochip technology	Pharmacodynamic molecules will interact with cells to cause changes in the external (morphology) and internal (metabolism) of the cell. The internal changes are concentrated in the changes in gene expression or protein. Through the analysis of differential genes or proteins.	high throughput, high efficiency, automation	The results have poor reproducibility and the scope of analysis target is limited.
9	MNPs	Nanoscale particles have magnetic guidance (targeting), under the action of an external magnetic field, they can achieve directional movement, which is convenient for positioning and separation from the medium.	new materials with high application value and wide application range	The precursor is expensive and toxic, and there are many factors that affect the particle size and performance of the nanomagnetic powder.

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

of various components combined with MNPs to capture the target protein.

Although MNPs is a star material in the field of nonmaterial science, it still has unavoidable problems in high-tech competition. For example, precursors are expensive and toxic, and their products cannot form stable dispersion systems. At the same time, many factors affect the size and performance of nanomagnetic powder. How to synthesize and control many factors is an important technical measure to obtain high-performance nanopowder. The identification technology for finding target protein as Table 1.

4. SCREENING OF ACTIVE PHARMACEUTICAL MOLECULES

Drug small molecules are closely related to human health. They could play a role in the treatment of diseases by carrying biological macromolecules and proteins in vivo. Therefore, searching for active components from drugs by targeting potential targets is a key task for treating diseases. Thus, this part continues to summarize the techniques of searching for potential active ingredients so as to provide the technical means for treating diseases.

4.1. Screening Potential Active Molecules Based on Chemical and Biological Techniques

Affinity mass spectrometry (AFMS) is a kind of bioanalytical technique that combines affinity ultrafiltration with high-performance liquid chromatography−mass spectrometry (LC/MS). On the basis of ultrafiltration, the chemical components of the affinity target protein are separated and screened. It is used in conjunction with LC/MS to quickly screen and identify active small molecules that have affinity with biological targets. When separating and screening active small molecules by affinity ultrafiltration, the small molecule compounds at the active sites of affinity target proteins are specifically bound by incubating the mixture extract of traditional Chinese medicine with target proteins, while the nonaffinity compounds are free, and then the proteins are denatured by acidic organic solvents, thereby eluting the specific ligand small molecules.[95] The eluent was injected into the LC/MS system for analysis of LC/MS under the positive and negative ion mode, the elution solution of traditional Chinese medicine was analyzed separately, and the total ion flow diagram of each material was obtained. MassLynx software could be used to check the chemical composition information on all kinds of Chinese herbal medicines according to the retention time, the number of fine quality, and the two level fragments of each chemical component, and confirm the chemical composition by standard proofreading.[96] Andrographis Herba (AH) has anti-inflammatory effects, and its main anti-inflammatory active ingredients are diterpenoids and flavonoids. Cyclooxygenase-2 inhibitor (COX-2) is expressed in large quantities in cells stimulated by inflammation. Jiao et al.[97] used bioaffinity ultrafiltration combined with UPLC-Q-TOF-MS method to screen the components of AH that bind to COX-2. The ultrafiltration method is used to filter out the components that are not combined with COX-2 in the incubation solution of AH ethanol extract and COX-2, and the ultrafiltrate containing enzyme conjugate is separated. In addition, the UPLC-Q-TOF-MS method was established to analyze the ultrafiltrate. In the final screening, five active compounds that specifically bind to COX-2 were found as COX-2 inhibitors. Affinity mass spectrometry based on the identified core targets to screen active molecules in Chinese medicine, the target protein, and Chinese medicine extract are less, and the sample solution does not need to be immobilized. However, in the process of screening ultrafiltration technology, the experimental results may be affected by factors such as ultrafiltration membrane material, target protein extracts dosage, and dissociation fluid selection, and there is false positive or false negative interference. Therefore, when screening the effective substance base of Chinese medicine with affinity mass spectrometry, we must take account of all interference factors to obtain ideal experimental results.[98]

4.2. Screening Potential Active Molecules Based on Molecular Biology Techniques

4.2.1. Molecular Biochromatography. Molecular biochromatography (MBC) is based on the principle of molecular specificity recognition. Using the different binding degree and affinity of different Chinese medicine components with various biological macromolecules, and the different retention time of each chemical component on the chromatographic column, the active substances of Chinese medicine could be screened out. In the experiment, the biological macromolecules were fixed on a certain carrier as the stationary phase of the chromatogram, the corresponding Chinese medicine extract was added to the mobile phase, and finally the chemical components of the traditional Chinese medicine that specifically bound to the target biological macromolecules were eluted and separated. The technique has the characteristics of good reproducibility and fast analysis speed, which provides a new way of thinking for the basic research of the pharmacodynamic substance of traditional Chinese medicine.[99] For example, Lv et al.[100] used this technology to discover several meaningful phloem epidermal growth factor receptor antagonists in the traditional Chinese medicine Marsdenia tenacissima. α1-Acid glycoprotein is a plasma protein, and most drugs are quantitatively bound to plasma protein. Wang et al.[101] used molecular biological chromatography with α1-acid glycoprotein as a stationary phase to screen the biologically active components of Salvia miltiorrhiza. The main active ingredients were separated by the isocratic elution method. Suitable chromatographic conditions for identification and analysis of tanshinone were chosen, and finally tanshinone IIA was determined as one of the main biologically active components. MBC could play an important role in the screening of active ingredients and the study of the role relationship. But it is not complete in simulating the internal environment. Moreover, problems such as difficulty in preparing stationary phase and short life of chromatographic column need to be solved.

4.2.2. Molecular Docking Technique. The molecular docking technique could predict the binding pattern between chemical components and direct target, and could quickly find the chemical components directly acting on the core target.[102] The technology is the use of computer software simulation, such as Dock and AutoDock, the ligand molecular biological small molecules (traditional Chinese medicine active substance) and the receptor macromolecules (proteins, enzymes, etc.) interaction. According to the calculated parameters, the binding pattern and affinity of the functional groups and the active sites of the target receptor of small molecules were predicted, and the interaction mechanism between the effective components of traditional Chinese medicine and the target was elucidated from the molecular level. At present, the commonly used molecular docking modes for virtual screening of active

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

small molecule substances of traditional Chinese medicine are flexible docking and semiflexible docking. The flexible docking method requires a sufficiently accurate molecular conformation; it is computationally intensive but inefficient. During the calculation of the semiflexible docking, the receptor conformation does not change, while the ligand conformation may change to some extent.[103] Therefore, it is a kind of virtual docking method often used in molecular docking.[103] Dioscin has strong anti-infection, antiallergic, antiviral, and antishock pharmacological effects. Yin et al.[104] used reverse docking technology to find potential targets of Dioscin in humans, rats, and mice. They used the automatic molecular docking software MDdock to molecularly dock the three-dimensional structure and three-dimensional crystal structure of dioscin. After that, potential targets were screened from the Potential Drug Target Database (PDTD), and the MetaCore platform was used to analyze biological activity, signal pathways, and regulatory networks. Finally, the most possible targets of diosgenin were predicted to be DNA topological recombinase I, DNA polymerase, and so on. In addition, Yu and other teams screened anti-COVID-19 coronavirus drugs through molecular docking, which is a good method for discovering antiviral drugs and analyzing the binding sites of drugs and targets.[105−107]

Molecular docking technology clarifies the mechanism of interaction between drugs and target proteins, and provides a basis for the mechanism of treatment of diseases. But this technology also has certain limitations. Its result is virtual data, which still needs to be verified in combination with in vivo experiments.[108]

5. CONFIRMATION OF "ACTIVE INGREDIENT-CORE TARGET"

The mode of action and structure−activity relationship between protein and drug play a key role in guiding drug design and synthesis. It is the material basis for rational drug use in life sciences, chemistry, and clinical medicine. Therefore, it is of great significance to study the mode of action between bioactive drug molecules and biomacromolecules. This section will summarize the technology of different active ingredients-protein binding methods, discuss their related principles, their respective advantages and disadvantages, and provide scholars with ideas for choosing appropriate technologies for experiments and better searching for drugs to treat diseases.

5.1. The Basis of Pharmacodynamic Substance Was Confirmed by Mass Spectrometry

5.1.1. Matrix-Assisted Laser Analytical Ionization Time-of-Flight Mass Spectrometry. Matrix-assisted laser analysis ionization time-of-flight mass spectrometry (MALDI-TOF-MS) could use a laser to illuminate the cocrystal film formed by the sample and the matrix. The matrix absorbs energy from the laser and transmits it to biomolecules. The ionization process is the process of ionizing biomolecules by transferring protons to or obtaining protons from biomolecules. The ion mass-to-charge ratio is proportional to its flight time. It is a soft ionization technology for the detection of mixtures and biological macromolecules. MALDI-TOF-MS has a high tolerance to salts and detergents in the analyte solution. Compared with traditional techniques, MALDI-TOF-MS has high accuracy, wide detection range, and good compatibility.[109] Kallsten et al.[110] compared four antibody-drug conjugates (ADCs) technologies through experiments. Trastuzumab-VcMMAE conjugates with different degrees of cysteine coupling were prepared. Among them, the spectral quality of antibody-drug conjugates was analyzed based on MALDI-TOF-MS. MALDI has a faster data processing speed and is more suitable for early screening of potential ADC structures. Because of the limited resolution of TOF for large ions, the measured peak width is relatively wide. At the same time, the deviation of the sample application program will affect the data results. It should be noted that it is necessary to search in a known spectrum library, so the mass spectrum database needs to be further improved. When this technology is used to confirm potential active molecules, the complex of "active small molecule-target protein" could be subjected to mass spectrometry to confirm the active molecule based on the molecular weight assigned. This technique also has disadvantages, such as the need for pretreatment, poor reproducibility, low signal noise ratio (SNR), and sensitivity, and the sample is susceptible to matrix interference.[111,112]

5.1.2. Native Electrospray Ionization Mass Spectrometry. Native electrospray ionization mass spectrometry (native ESI-MS) is increasingly used for quantitative analysis of biomolecular interactions. This analytical method could be used to analyze and detect biological macromolecules under close to natural physiological conditions, so as to maintain their complete structure and biological functions. In recent years, it has been widely used in the study of protein noncovalent complexes. This method could directly compare the signal strength of analytes of similar sizes (such as proteins and their counterparts with small molecules). It is applied to detect complexes with multiple binding balances and ligands of different molecular weights. The entire analysis process is accurate and efficient, so it is an effective method for studying "small molecule-protein" complexes.[113,114] Ma et al.[115] used native ESI-MS, showed that brominated flame retardant and thyroxin could form a stable complex in ammonium acetate buffer solution at 37 °C and physiological pH, and analyzed the binding ratio of them. Gavriilidou et al.[116] used ESI-MS to analyze the combination of compounds produced during fragment-based drug discovery and two proteins with different functions. In the native ESI-MS method, if the signal response of the analyte is not uniform, the peak ratio may be distorted, so this method is limited in the determination of a large oligomeric molecular complex.[117]

5.2. The Basis of Pharmacodynamic Substances Was Confirmed Based on Spectral Analysis

5.2.1. UV−Visible Absorption Spectrum. The UV−visible absorption spectrum is one of the most convenient techniques for the study of small molecule−protein interaction. Because of a part of the amino acid residues that make up the protein has UV absorption properties, the conformation of amino acid residues is determined by the microenvironment of protein molecules. The change of microenvironment will lead to the change of protein conformation and the change of UV−visible absorption spectrum of chromogenic groups.[118] Small molecules and proteins have their own ultraviolet absorption peaks at different wavelengths. Therefore, according to the difference of absorption spectra before and after binding of proteins and small molecules, it could be judged whether the two have interacted, and judge the dynamic change process of structure and conformation of protein molecules under the action of ligands. The UV−visible spectrum is generally complementary to other technologies. Human serum albumin (HSA) and bovine serum albumin (BSA) are the most

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

abundant proteins in plasma. Shaghaghi et al.[119] used the UV−visible absorption spectrum and Fourier transform infrared spectrum to analyze the conformational changes of the antidiabetes drug sitagliptin (SIT) and HSA/BSA protein and clarify the substance basis of efficacy. The results showed that the ultraviolet absorption spectrum of serum protein increased with the growth of the concentration of SIT. Infrared spectroscopy analysis found that the secondary structure of serum protein had slightly changed. This showed that SIT could change the secondary structure of serum albumins. Song et al.[120] showed the binding of copper regulatory protein CopC to curcumin by UV−visible absorption spectrum and fluorescence spectrum, and analyzed the negative spectral characteristics of curcumin-phosphatidylcholine complexes. And the results showed that protein β-sheet was reduced, and curcumin changes the conformation of CopC. This was related to curcumin's anticancer effect. The UV−visible absorption spectrum has the advantages of simple operation and fast speed, but there are still some limitations, such as it could not be used for substances with a weak UV signal.

5.2.2. Time-Resolved Fluorescence Spectroscopy. Because the red shift or blue shift of the maximum emission wavelength of the time-resolved fluorescence spectroscopy could reflect the polarity change of the microenvironment around amino acid residues, it is often used to research the interaction between proteins and drug molecules. Using time-resolved fluorescence spectroscopy to study the interaction between the proteins and drug molecules, the complex will cause static quenching. In order to show the binding strength of the complex, we could calculate the binding sites at different temperatures. In the experiment, the difference of wavelength between the excitation and emission monochromators was fixed, and the synchronous fluorescence spectrum scan was performed to obtain the fluorescence characteristic spectrum reflecting the microenvironmental changes of tyrosine and tryptophan residues. In the small molecule−protein complex system, with the increase of the small molecule content, the fluorescence intensity of the spectrum changes, and the maximum absorption wavelength of the complex shifts, resulting in the change of protein conformation.[121] Tang et al.[122] researched the binding effect of anthocyanin cyanidin-3-*O*-glucoside (C3G) with three proteins, among which the binding constant was analyzed by fluorescence spectrum, indicating the effect of free C3G in plasma.

5.2.3. NMR Spectroscopy. Nuclear magnetic resonance (NMR) spectroscopy is an application of the nuclear magnetic resonance effect of hydrogen-1 in a molecule to the nuclear magnetic resonance spectroscopy, which can be used to determine the molecular structure. This technique could be used to determine the molecular structure when the sample contains hydrogen, especially the isotope hydrogen-1. Moreover, it can provide structural information changes at the atomic level. During the operation, we should prepare a small molecule solution and small molecule protein recheck solution and finally put them in the nuclear magnetic tube for hydrogen spectrum analysis after we set reasonable nuclear resonance frequency, pulse sequence, scanning times, and other parameters. In the experiment, more attention should be paid to the choice of dissolving agent and the interaction time of small molecule−protein interaction.[123] By investigating the changes of peak area and chemical shift before and after the proton peak binding, the classification of various atoms could be determined. Thus, the chemical groups involved in the coordination reaction when the small molecule substances bind to the protein could be identified, and their structures could be inferred.[124] α1-Acid glycoprotein (AGP) is one of the important proteins in an inflammatory response. Becker et al.[125] used the NMR technology to explore many details of the binding interaction between propranolol and α1-acid glycoprotein, providing important insights for drug interactions. Nonspecific interactions that did not distinguish between the enantiomers of propranolol increase the contribution of the saturation transferred difference (STD) effect. Nuclear magnetic resonance spectroscopy has the advantages of fast detection speed and good sample integrity. With the improvement of technology, the application of NMR in various fields has been developed, but it also has some shortcomings, such as low sensitivity, signal overlap, and so on.

5.2.4. X-ray Diffraction Technique. X-ray diffraction (XRD) could provide accurate structural information about the interaction between proteins and small molecules. X-ray diffraction is to get the distribution of electron density in the crystal by using the diffraction effect of electrons on X-ray, and then analyze and obtain the position information on atoms, namely, the crystal structure. The association of X-rays with crystallography allows the study of various crystal structures, especially protein crystal structures. It is currently the most commonly used method to analyze the three-dimensional structure of a protein, which could significantly improve the signal-to-noise ratio of crystal diffraction data, thus improving the quality and resolution of crystal diffraction data.[126] Most of the antiandrogen drugs used in the treatment of prostate cancer target androgen receptor (AR). FKBP51 is a positive regulator of AR, and inhibiting the expression of FKBP51 can reduce the signal pathway of AR. P-nitrophenol (PNP) exerts its anti-androgen activity by binding to the estrogen receptor or androgen receptor, respectively. Wu et al.[127] hypothetically screened the small molecule inhibitors targeting FKBP51. The structure of the complex between small molecules and proteins was analyzed by X-ray diffraction, and the binding sites of PNP and FKBP51 proteins were found. The results showed that PNP molecules occupy the pocket of PPI catalysis in the FK1 region, making the structure stronger. Finally, the hot amino acid residues involved in PNP binding were found, which explained the binding mode of PNP and FKBP51 protein. 3C-like protease (3CLpro) and papain-like protease (PLpro), which are regarded as ideal targets for antiviral drugs, can process nonstructural proteins that are essential for virus replication. Su et al.[128] analyzed the anti-SARS-CoV-2 activity of Shuanghuanglian preparation in vitro. Baicalein and its derivatives are potent SARS-CoV-2 3CLpro inhibitors and one of the flavonoids with the highest content in *Scutellaria baicalensis*. The crystal structure of SARS-CoV-2 3CLpro combined with baicalein was determined by the X-ray diffraction technique. The analysis showed that baicalein highly binds to the center of the active site of the substrate by interacting with two catalytic residues, preventing the peptide substrate from approaching the active site. In recent decades, the X-ray diffraction technique is still an important technical means to determine the protein structure. However, it should be noted that, as shown in current research, the phase analysis of X-ray diffraction requires the cultivation of larger protein crystals. However, many proteins are not easy to crystallize, or single crystals cannot reach the crystallization conditions required for analysis, so they are not suitable for proteins that are hard to crystallize. In addition, this method has a longer

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

workflow. In any case, we believe that, with the emergence of a large number of high-resolution X-ray tools, the analysis of protein structure will rise to a new level, and X-ray diffraction technique will show more promising application prospects.

5.2.5. Microscale Thermophoresis Technique. Microscale thermophoresis is an optical method that could characterize the properties of biomolecules based on physical principles. In recent years, it has been applied to the fluorescence chromogenic quantitative technique of bioanalysis. It could monitor the changes of the mass, charge, hydration layer, and conformation of biomolecules by measuring the directional motion of particles in the microscopic temperature gradient. The microscale thermophoresis technique uses the fluorescence labeling method, and at least one of the above properties will change when the titrated nonfluorescent molecule binds to the labeled molecule. By sensitively and rapidly monitoring the thermal migration of fluorescently labeled molecules in solution or even in a complex matrix, the binding of target proteins to specific compounds could be quantified.[129] Guo et al.[130] confirmed the combination of Azacoccone E and 3-phosphoglycerate dehydrogenase (PHGDH) through microscale thermophoresis and cellular thermal shift assay (CETSA), which provided evidence for the treatment of cancer with this drug. Peng et al.[131] used this technique to find that lovastatin targeted binding protein MD-2 relieves neuropathic pain through the TLR4 pathway. Therefore, this technique could measure the interaction forces between biomolecules in the natural state environment, requires low experimental cost and sample size, could achieve high-throughput and high-efficiency determination, and could provide research combined with energy free energy, enthalpy, and entropy, and more parameters, such as information. However, the high price of the instrument and the inability to directly determine the specific binding sites are the main constraints for future development.

5.2.6. Surface Plasmon Resonance Technology. Surface plasmon resonance (SPR) is a biosensor analysis technology based on the physical and optical phenomena of surface plasmon resonance. It could maintain the natural activity of biomolecules without the use of fluorescent labeling or isotopic labeling. SPR instrument could detect the whole process of binding and dissociation of biomolecules such as small molecular-protein in real-time, obtain the binding kinetic parameters of their interaction, namely, the binding rate constant K_a, the binding equilibrium constant K_a, and the dissociation rate constant K_d. Finally, the small molecule-protein affinity is evaluated. It has the advantages of simple operation, less sample consumption, and rapid sensitivity.[132] Gu et al.[133] used SPR technology to study the binding protein of berberine BBR in the treatment of myeloma, which was UHRF1. Shao et al.[134] used SPR technology to verify that Ginsenoside Rb3 (G-Rb3) bound to the retinoid X receptor α (RXRα), which was a potential target for the treatment of heart failure. In addition, our research group screened liquiritin apioside (LA), a monomeric component in liquorice, by using SPR technology, which could strongly bind to the mineralocorticoid receptor (MR), and combined with other experiments to prove that the combination of the two could lead to myocardial fibrosis through the TGF-β1/p38 MAPK pathway, which was helpful to understand the mechanism of adverse reactions caused by drugs.[135] Although SPR technology is a recognized technology to confirm the combination of drug components and targets, it still has some disadvantages, such as low detection signal, long detection time, and difficult fixation of small molecules.

5.3. Confirm the Material Basis of Efficacy Based on Imaging Technology

5.3.1. Atomic Force Microscopy Imaging. Atomic force microscopy (AFM) has become an indispensable tool in the field of biomolecular research. It is a kind of image scanning probe microscope, which shows the surface properties of the sample by detecting the interaction forces between atoms with high spatiotemporal resolution. Therefore, it could evaluate the bonding effect of molecular size according to the different forces formed between the molecules. Sun et al.[136] analyzed the two-dimensional and three-dimensional morphology images of the complex of human serum albumin (HSA) and γ-lactone essences based on AFM. The study showed that the molecular height of the complex changed significantly, and its average height was about half of the free HSA. Receptor tyrosine kinases (RTKs) is a new target for tumor therapy. Epidermal growth factor receptor EGFR is one of the members of the RTKs family. EGFR could activate pathways involved in cell proliferation and cell survival. Zhang et al.[137] found that, with the change of resveratrol concentration, the expression of EGFR decreased, which further explained the anticancer activity. AFM imaging not only could avoid the modification of samples, but also observe the height changes of protein molecules before and after the combination of small molecules through different modes (such as tapping mode) and display the molecular morphology of proteins under physiological conditions. However, the disadvantage of AFM is its slow analysis speed.

5.3.2. Cryo-Electron Microscope. After the biological macromolecules are frozen rapidly, the sample is imaged by cryo-electron microscope (cryo-EM) at low temperature, and then the three-dimensional structure of the sample is obtained by image processing and reconstruction calculation. The cryo-EM technology, divided into single particle freezing electron microscopy, electron fault three-dimensional reconstruction, and electron crystallography mainly, has become an important method to research structural biology presently. There are three steps to study the three-dimensional structure of a protein by cryo-EM technology. First, the sample is frozen and fixed in liquid nitrogen and low temperature so that the water molecule transforms the form of a glass state. Then, sample images are recorded by microscope with a detector and lens system. Second, after processing the image signal, the three-dimensional structure is obtained by using the three-dimensional reconstruction technology.[138] Compared with other methods, the advantages of cryo-EM technology are not having to prepare crystals and keeping the activation of samples, but it has higher requirements for sample preparation and could not analyze the sample's components.

5.4. Confirm the Basis of Pharmacodynamic Substances Based on Cell Experiments

MTT colorimetry is usually used to detect the activity of cultured cells.[139] The value of optical density measured by enzyme marker was positively correlated with the number of living cells, but not correlated with the number of dead cells. MTT could be used to detect the activity of bioactive factors and confirm the composition of antidisease drugs. MTT could verify the balancing effect of active ingredients on diseases objectively. In cell experiments, the efficacy of active ingredients could be confirmed by comparing the cell vitality

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

and survival rate of the control group, the model group, and the drug administration group, respectively. However, in order to confirm whether the specific affinity target proteins of the active ingredient is effective or not, additional target protein inhibitor/agonist groups should be added, and the results of multiple groups should be analyzed statistically, so as to make clear that the protection of the active ingredient on normal cell growth is realized by the specific affinity target proteins.[140,141] However, according to the comprehensive analysis, the cell experiment is in vitro verification, which is different from the vital human signs, so it is necessary to conduct in vivo verification.

5.5. Confirm the Basis of Pharmacodynamic Substances Based on Animal Experiments

The main pharmacodynamic effects of drugs require at least in vivo and in vitro experiments and even two or more experimental verifications. Animals are used for in vivo experiments to verify the material basis of the efficacy. The animal is induced to establish a disease model by administration, and then the animal's serum or tissue is collected. These are used as pharmacodynamic data sources for the follow-up study of active molecules.

Investigating the effect of active molecules on the expression of target protein and downstream effector protein could confirm the mechanism of the two. The relative expression of genes was analyzed by RT-PCR method, total organ mRNA was extracted from the organs of animals, and then reverse transcription was carried out and the cDNA stock solution was diluted for real-time quantitative PCR reaction. Finally, some methods are used to analyze the relative gene expression, such as the 2-ΔΔCt method.[142] Western blot experiments could be used to detect changes in target proteins and downstream effector proteins.[143] After animal organ tissues were collected, tissue protein was extracted, and protein content was detected. After the target protein and effector protein were added to the buffer, SDS-polyacrylamide gel electrophoresis and protein electrotransfer were performed. After the membrane was transferred, the primary antibody was incubated, and the membrane was washed the next day and then transferred to the secondary antibody for the room temperature reaction, and finally chemiluminescence was added. The substrate reaction solution was developed and photographed in the ECL camera system.[144]

Rats or mice are generally used as simulated objects for animal experiments. Qingfei Xiaoyan Wan (QF) is a traditional Chinese medicine prescription for the treatment of respiratory diseases. Hou et al.[145] used *Pseudomonas aeruginosa* to induce acute pneumonia in mice given orally administered QF, and used quantitative RT-PCR to study the expression of key genes in the PI3K/AKT pathway and the Ras/MAPK pathway, which involve inflammation and immune response in the human body. The activation of transcription factor NF-κB could trigger the production of inflammatory diseases in the lungs. Arctigenin (ATG) derivatives, which are NF-κB inhibitors and the main active molecules of QF, are the main active ingredients that exert anti-inflammatory effects. Studies have found that ATG inhibits the expression of all genes, and the inhibitory effect of ATG is the strongest compared with the inhibition of other compounds. ATG is the most important component that inhibits the PI3K/AKT pathway and affects the expression of downstream genes NF-κB and MAP kinase. This study used RT-PCR technology to determine that ATG was the primary anti-inflammatory agent in QF, and successfully determined the anti-inflammatory mechanism of QF. Animal experiments have an important guiding role in inferring the clinical situation, and tests will be conducted on mice at the initial stage to verify the efficacy. However, because of the similarities and differences of species, the drug reactions of animals and humans are not exactly the same. At present, the target screening of chemical components is often based on the prototype components of the drug, and what really makes a difference is the form it takes in the body. Therefore, it is necessary to combine the metabolism research of the compound to conduct target screening.

6. CONCLUSION

Pandemics caused by viruses occur around the world. The mutation frequency, pathogenesis, and pathogenic signal pathways of influenza viruses need to be explored in depth. On the basis of the current level of scientific development, antiviral drug therapy will still be the main means of treating future viral infections. The development of drugs is a long process. A clear interpretation of the characteristics of the virus and the mechanism that causes the disease still requires greater investment. To find effective therapeutic drugs that can control the development of the disease, it is necessary to further understand and clarify the pathogenesis, and to dig deeper into the potential core targets. Because protein is considered to be a target that has medicinal functions in the body and could be acted upon by drugs, researchers are using advanced techniques to analyze protein structure and find drug-active molecules that bind to the protein. All in all, protein targets are crucial in fighting against pandemics and provide a theoretical basis for unknown pandemics in the future.

The impact of pandemics on mankind is far-reaching, and effective prevention and treatment methods are still an arduous task. Various technologies for screening out potential target proteins and potential active molecules are becoming mature, but the shortcomings that restrict the development of many technologies need to be further improved. This requires the combination of multiple technologies and complements with other technologies. The techniques and strategies mentioned in this article help to discover potential target proteins or active molecules, and we hope will provide certain references for disease treatment.

AUTHOR INFORMATION

Corresponding Authors

Bin Yang − *Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China*; Phone: +86-22-59596221; Email: yang3023008@163.com

Yubo Li − *Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China*; orcid.org/0000-0003-0455-0969; Email: yaowufenxi001@sina.com

Authors

Hongxin Yu − *Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China*

Chunyan Li − *Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China*

Xing Wang − *Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China*

Jingyi Duan − *Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China*

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

Na Yang − *Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China*
Lijuan Xie − *Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China*
Yu Yuan − *Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China*
Shanze Li − *Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China*
Chenghao Bi − *Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jproteome.0c00372

Author Contributions

#H.Y., C.L., and X.W. have contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China [No. 81873194] and Tianjin Talent Development Special Support Project for High Level Innovation and Entrepreneurship.

■ REFERENCES

(1) World Health Organization. (2020 Mar 11). https://www.who.int/zh/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020.
(2) Anderson, R. M.; Heesterbeek, H.; Klinkenberg, D.; et al. How will country-based mitigation measures influence the course of the COVID-19 epidemic? *Lancet* **2020**, *395*, 931−934.
(3) Grennan, D. What Is a Pandemic? *JAMA* **2019**, *321*, 910.
(4) Yuen, K. S.; Ye, Z. W.; Fung, S. Y.; Chan, C. P.; Jin, D. Y. SARS-CoV-2 and COVID-19: The most important research questions. *Cell Biosci.* **2020**, *10*, 40.
(5) Yang, L.; Tian, D.; Liu, W. Strategies for Vaccine Development of COVID-19. *Sheng Wu Gong Cheng Xue Bao.* **2020**, *36*, 593−604.
(6) Bojkova, D.; Klann, K.; Koch, B.; et al. Proteomics of SARS-CoV-2-infected host cells reveals therapy targets. *Nature* **2020**, *583*, 469.
(7) Lin, H.; Zhang, C.; Lin, Y.; et al. A strategy for screening trypsin inhibitors from traditional Chinese medicine based on a monolithic capillary immobilized enzyme reactor coupled with offline liquid chromatography and mass spectrometry. *J. Sep. Sci.* **2019**, *42*, 1980−1989.
(8) South, A. M.; Diz, D. I.; Chappell, M. C. COVID-19, ACE2, and the cardiovascular consequences. *Am. J. Physiol Heart Circ Physiol.* **2020**, *318*, H1084−H1090.
(9) Liu, Z.; Xiao, X.; Wei, X.; et al. Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2. *J. Med. Virol.* **2020**, *92*, 595−601.
(10) Rothan, H. A.; Byrareddy, S. N. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J. Autoimmun.* **2020**, *109*, 102433.
(11) Sun, J.; Zhuang, Z.; Zheng, J.; et al. Generation of a Broadly Useful Model for COVID-19 Pathogenesis, Vaccination, and Treatment. *Cell* **2020**, *182*, 734−743.
(12) Meng, Y.; Yu, C. H.; Li, W.; et al. Angiotensin-converting enzyme2/angiotensin-(1−7)/Mas axis protects against lung fibrosis by inhibiting the MAPK/NF-κB pathway. *Am. J. Respir. Cell Mol. Biol.* **2014**, *50*, 723−736.
(13) Sun, M. L.; Yang, J. M.; Sun, Y. P.; et al. Inhibitors of RAS Might Be a Good Choice for the Therapy of COVID-19 Pneumonia. *Zhonghua Jie He He Hu Xi Za Zhi.* **2020**, *43*, 219−222.
(14) Zhao, Y.; Zhao, Z.; Wang, Y.; Zhou, Y.; Ma, Y.; Zuo, W. Single-Cell RNA Expression Profiling of ACE2, the Receptor of SARS-CoV-2. *Am. J. Respir. Crit. Care Med.* **2020**, *202*, 756−759.
(15) Zhang, X.; Yu, J.; Pan, L. Y.; et al. ACEI/ARB use and risk of infection or severity or mortality of COVID-19: A systematic review and meta-analysis. *Pharmacol. Res.* **2020**, *158*, 104927.
(16) Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Kruger, N.; Herrler, T.; Erichsen, S.; Schiergens, T. S.; Herrler, G.; Wu, N.-H.; Nitsche, A.; Muller, M. A.; Drosten, C.; Pohlmann, S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **2020**, *181*, 271−280.e8.
(17) Lu, D.; Chatterjee, S.; Xiao, K.; Riedel, I.; Wang, Y.; Foo, R.; Bar, C.; Thum, T. MicroRNAs targeting the SARS-CoV-2 entry receptor ACE2 in cardiomyocytes. *J. Mol. Cell. Cardiol.* **2020**, *148*, 46−49.
(18) Magro, C.; Mulvey, J. J.; Berlin, D.; et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. *Transl Res.* **2020**, *220*, 1−13.
(19) Mastaglio, S.; Ruggeri, A.; Risitano, A. M.; et al. The first case of COVID-19 treated with the complement C3 inhibitor AMY-101. *Clin. Immunol.* **2020**, *215*, 108450.
(20) Khan, R. J.; Jha, R. K.; Singh, E.; et al. Identification of promising antiviral drug candidates against non-structural protein 15 (NSP15) from SARS-CoV-2: an in silico assisted drug-repurposing study. *J. Biomol. Struct. Dyn.* **2020**, 1.
(21) Chikhale, R. V.; Sinha, S. K.; Patil, R. B.; et al. In-silico investigation of phytochemicals from Asparagus racemosus as plausible antiviral agent in COVID-19. *J. Biomol. Struct. Dyn.* **2020**, 1.
(22) Huang, C.; Wang, Y.; Li, X.; et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **2020**, *395*, 497−506.
(23) Zheng, M.; Gao, Y.; Wang, G.; et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell. Mol. Immunol.* **2020**, *17*, 533−535.
(24) van Hall, T.; Andre, P.; Horowitz, A.; Ruan, D. F.; Borst, L.; Zerbib, R.; Narni-Mancinelli, E.; van der Burg, S. H.; Vivier, E. Monalizumab: inhibiting the novel immune checkpoint NKG2A. *J. Immunother. Cancer.* **2019**, *7*, 263.
(25) Jakovac, H. COVID-19 and hypertension - is the HSP60 culprit for the severe course and worse outcome? *Am. J. Physiol. Heart Circ. Physiol.* **2020**, *319*, H793.
(26) Lee, W.; Ahn, J. H.; Park, H. H.; Kim, H. N.; Kim, H.; Yoo, Y.; Shin, H.; Hong, K. S.; Jang, J. G.; Park, C. G.; Choi, E. Y.; Bae, J.-S.; Seo, Y.-K. COVID-19-activated SREBP2 disturbs cholesterol biosynthesis and leads to cytokine storm. *Signal Transduct. Target Ther.* **2020**, *5*, 186.
(27) Ciavarella, C.; Motta, I.; Valente, S.; Pasquinelli, G. Pharmacological (or Synthetic) and Nutritional Agonists of PPAR-γ as Candidates for Cytokine Storm Modulation in COVID-19 Disease. *Molecules* **2020**, *25*, 25−2076.
(28) Guan, Y. The United Nations has released its latest AIDS report. *Chin. J. Nat.* **2017**, *39*, 30.
(29) Sharp, P. M.; Hahn, B. H. Origins of HIV and the AIDS pandemic. *Cold Spring Harbor Perspect. Med.* **2011**, *1*, a006841.
(30) Espíndola, M. S.; Soares, L. S.; Galvão-Lima, L. J.; et al. HIV infection: focus on the innate immune cells. *Immunol. Res.* **2016**, *64*, 1118−1132.
(31) Furler, R. L.; Uittenbogaart, C. H. Signaling through the P38 and ERK pathways: a common link between HIV replication and the immune response. *Immunol. Res.* **2010**, *48*, 99−109.
(32) Shi, J.; Zhang, X. F.; Zheng, Y. H. The latest function of HIV-1 Nef protein and its enhancement of molecular mechanism of viral infectivity. *Acta Virol.* **2008**, *34*, 113−120.
(33) Betzi, S.; Restouin, A.; Opi, S.; et al. Protein protein interaction inhibition (2P2I) combining high throughput and virtual screening: Application to the HIV-1 Nef protein. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 19256−19261.

(34) Baba, M.; Nishimura, O.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Iizawa, Y.; Shiraishi, M.; Aramaki, Y.; Okonogi, K.; Ogawa, Y.; Meguro, K.; Fujino, M. A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 5698−5703.
(35) Strizki, J. M.; Xu, S.; Wagner, N. E.; Wojcik, L.; Liu, J.; Hou, Y. SCH-C (SCH 351125), an orally bioavailable, small molecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection in vitro and in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 12718−12723.
(36) Seto, M.; Aikawa, K.; Miyamoto, N.; Aramaki, Y.; Kanzaki, N.; Takashima, K.; Kuze, Y.; Iizawa, Y.; Baba, M.; Shiraishi, M. Highly potent and orally active CCR5 antagonists as anti-HIV-1 agents: synthesis and biological activities of 1-benzazocine derivatives containing a sulfoxide moiety. *J. Med. Chem.* **2006**, *49*, 2037−2048.
(37) D'Antoni, M. L.; Mitchell, B. I.; McCurdy, S.; et al. Cenicriviroc inhibits trans-endothelial passage of monocytes and is associated with impaired E-selectin expression. *J. Leukocyte Biol.* **2018**, *104*, 1241−1252.
(38) Kou, J.; Kuang, Y. Q. Mutations in chemokine receptors and AIDS. *Prog. Mol. Biol. Transl Sci.* **2019**, *161*, 113−124.
(39) Abreu, C. M.; Price, S. L.; Shirk, E. N.; Cunha, R. D.; Pianowski, L. F.; Clements, J. E.; Tanuri, A.; Gama, L. Dual role of novel ingenol derivatives from Euphorbia tirucalli in HIV replication: Inhibition of de novo infection and activation of viral LTR. *PLoS One* **2014**, *9*, e97257.
(40) Tandon, R.; Chew, G. M.; Byron, M. M.; et al. Galectin-9 is rapidly released during acute HIV-1 infection and remains sustained at high levels despite viral suppression even in elite controllers. *AIDS Res. Hum. Retroviruses* **2014**, *30*, 654−664.
(41) Colomb, F.; Giron, L. B.; Premeaux, T. A.; Mitchell, B. I.; Niki, T.; Papasavvas, E.; Montaner, L. J.; Ndhlovu, L. C.; Abdel-Mohsen, M. Galectin-9 Mediates HIV Transcription by Inducing TCR-Dependent ERK Signaling. *Front. Immunol.* **2019**, *10*, 267.
(42) Osztovits, J.; Balázs, C.; Fehér, J. H1N1-influenza-pandémia, 2009 [H1N1 influenza-pandemic, 2009]. *Orv. Hetil.* **2009**, *150* (50), 2265−2273.
(43) Wang, Q. W.; Su, Y.; Sheng, J. T.; et al. Anti-influenza A virus activity of rhein through regulating oxidative stress, TLR4, Akt, MAPK, and NF-κB signal pathways. *PLoS One* **2018**, *13*, e0191793.
(44) Ding, Z.; Sun, G.; Zhu, Z. Hesperidin attenuates influenza A virus (H1N1) induced lung injury in rats through its anti-inflammatory effect. *Antiviral Ther.* **2018**, *23*, 611−615.
(45) Liu, B.; Meng, D.; Wei, T.; Zhang, S.; Hu, Y.; Wang, M. Apoptosis and pro-inflammatory cytokine response of mast cells induced by influenza A viruses. *PLoS One* **2014**, *9*, e100109.
(46) König, R.; Stertz, S.; Zhou, Y.; et al. Human host factors required for influenza virus replication. *Nature* **2010**, *463*, 813−817.
(47) Sumi, M.; Kiuchi, K.; Ishikawa, T.; et al. The newly synthesized selective Ca2+/calmodulin dependent protein kinase II inhibitor KN-93 reduces dopamine contents in PC12h cells. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 968−975.
(48) Kim, Y. G.; Kim, S. M.; Kim, K. P.; Lee, S. H.; Moon, J. Y. The Role of Inflammasome-Dependent and Inflammasome-Independent NLRP3 in the Kidney. *Cells* **2019**, *8*, 1389.
(49) Zhen, Y.; Zhang, H. NLRP3 Inflammasome and Inflammatory Bowel Disease. *Front. Immunol.* **2019**, *10*, 276.
(50) Luo, B.; Huang, F.; Liu, Y.; et al. NLRP3 Inflammasome as a Molecular Marker in Diabetic Cardiomyopathy. *Front. Physiol.* **2017**, *8*, 519.
(51) Jia, X.; Liu, B.; Bao, L.; et al. Delayed oseltamivir plus sirolimus treatment attenuates H1N1 virus-induced severe lung injury correlated with repressed NLRP3 inflammasome activation and inflammatory cell infiltration. *PLoS Pathog.* **2018**, *14*, e1007428.
(52) Ye, R. D.; Boulay, F.; Wang, J. M.; et al. International Union of Basic and Clinical Pharmacology. LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. *Pharmacol. Rev.* **2009**, *61* (2), 119−161.
(53) Ampomah, P. B.; Moraes, L. A.; Lukman, H. M.; Lim, L. H. K. Formyl peptide receptor 2 is regulated by RNA mimics and viruses through an IFN-β-STAT3-dependent pathway. *FASEB J.* **2018**, *32*, 1468−1478.
(54) Rahman, F.; Chebbo, M.; Courtin, N.; Fotso, F. A.; Alessi, M. C.; Riteau, B. The Annexin A1 Receptor FPR2 Regulates the Endosomal Export of Influenza Virus. *Int. J. Mol. Sci.* **2018**, *19*, 1400.
(55) Báez-Santos, Y. M.; St. John, S. E.; Mesecar, A. D. The SARS-coronavirus papain-like protease: structure, function and inhibition by designed antiviral compounds. *Antiviral Res.* **2015**, *115*, 21−38.
(56) Rabaan, A. A.; Al-Ahmed, S. H.; Haque, S.; et al. SARS-CoV-2, SARS-CoV, and MERS-COV: A comparative overview. *Infez Med.* **2020**, *28*, 174−184.
(57) Totura, A. L.; Baric, R. S. SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon. *Curr. Opin. Virol.* **2012**, *2*, 264−275.
(58) Channappanavar, R.; Perlman, S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin. Immunopathol.* **2017**, *39*, 529−539.
(59) Savarino, A.; Boelaert, J. R.; Cassone, A.; Majori, G.; Cauda, R. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect. Dis.* **2003**, *3*, 722−727.
(60) Vincent, M. J.; Bergeron, E.; Benjannet, S.; et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virol. J.* **2005**, *2*, 69.
(61) Haga, S.; Yamamoto, N.; Nakai-Murakami, C.; Osawa, Y.; Tokunaga, K.; Sata, T.; Yamamoto, N.; Sa-sazuki, T.; Ishizaka, Y. Modulation of TNF-α-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-α production and facilitates viral entry. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 7809−7814.
(62) Lambert, D. W.; Yarski, M.; Warner, F. J.; et al. Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). *J. Biol. Chem.* **2005**, *280*, 30113−30119.
(63) Pfefferle, S.; Schöpf, J.; Kögl, M.; et al. The SARS-coronavirus-host interactome: identification of cyclophilins as target for pan-coronavirus inhibitors. *PLoS Pathog.* **2011**, *7*, e1002331.
(64) Gracio, A. J. d. S.; Gracio, M. A. A. Plague: A Millenary Infectious Disease Reemerging in the XXI Century. *BioMed Res. Int.* **2017**, *2017*, 1−8.
(65) Olson, R. M.; Dhariwala, M. O.; Mitchell, W. J.; Anderson, D. M. Yersinia pestis Exploits Early Activation of MyD88 for Growth in the Lungs during Pneumonic Plague. *Infect. Immun.* **2019**, *87*, e00757−18.
(66) Dhariwala, M. O.; Olson, R. M.; Anderson, D. M. Induction of Type I Interferon through a Noncanonical Toll-Like Receptor 7 Pathway during Yersinia pestis Infection. *Infect. Immun.* **2017**, *85*, e00570−17.
(67) Osei-Owusu, P.; Charlton, T. M.; Kim, H. K.; Missiakas, D.; Schneewind, O. FPR1 is the plague receptor on host immune cells. *Nature* **2019**, *574*, 57−62.
(68) Yang, K.; He, Y.; Park, C. G.; et al. Yersinia pestis Interacts With SIGNR1 (CD209b) for Promoting Host Dissemination and Infection. *Front. Immunol.* **2019**, *10*, 96.
(69) Yang, K.; Park, C. G.; Cheong, C.; et al. Host Langerin (CD207) is a receptor for Yersinia pestis phagocytosis and promotes dissemination. *Immunol. Cell Biol.* **2015**, *93*, 815−824.
(70) Wang, Y. X.; Ma, J. R.; Wang, S. Q.; et al. Utilizing integrating network pharmacological approaches to investigate the potential mechanism of Ma Xing Shi Gan Decoction in treating COVID-19. *Eur. Rev. Med. Pharmacol Sci.* **2020**, *24*, 3360−3384.
(71) Cheng, X.; Liu, N.; Liu, H.; Huang, N.; Sun, X.; Zhang, G. Bioinformatic and biochemical findings disclosed anti-hepatic steatosis mechanism of calycosin. *Bioorg. Chem.* **2020**, *100*, 103914.
(72) Song, X.; Zhang, Y.; Dai, E.; Du, H.; Wang, L. Mechanism of action of celastrol against rheumatoid arthritis: A network pharmacology analysis. *Int. Immunopharmacol.* **2019**, *74*, 105725.

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

(73) Zhang, R.; Zhu, X.; Bai, H.; Ning, K. Network pharmacology databases for traditional Chinese medicine: review and assessment. *Front. Pharmacol.* **2019**, *10*, 123.
(74) Zheng, J.; Wu, M.; Wang, H.; et al. Network pharmacology to unveil the biological basis of health-strengthening herbal medicine in cancer treatment. *Cancers* **2018**, *10*, 461.
(75) Ge, Y. W.; Wang, S. M.; Zhang, L. Y. Label-free small molecule probe and target discovery of traditional Chinese medicine. *Zhongguo Zhong Yao Za Zhi.* **2019**, *44*, 4152−4157.
(76) Jin, L.; Wang, D.; Gooden, D. M.; et al. Targeted Mass Spectrometry-Based Approach for Protein-Ligand Binding Analyses in Complex Biological Mixtures Using a Phenacyl Bromide Modification Strategy. *Anal. Chem.* **2016**, *88*, 10987−10993.
(77) Kaur, U.; Meng, H.; Lui, F.; et al. Proteome-Wide Structural Biology: An Emerging Field for the Structural Analysis of Proteins on the Proteomic Scale. *J. Proteome Res.* **2018**, *17*, 3614−3627.
(78) Qin, X.; Hu, L.; Shi, S. N.; et al. The Bcr-Abl inhibitor GNF-7 inhibits necroptosis and ameliorates acute kidney injury by targeting RIPK1 and RIPK3 kinases. *Biochem. Pharmacol.* **2020**, *177*, 113947.
(79) Kirsch, V. C.; Orgler, C.; Braig, S.; et al. The Cytotoxic Natural Product Vioprolide A Targets Nucleolar Protein 14, Which Is Essential for Ribosome Biogenesis. *Angew. Chem., Int. Ed.* **2020**, *59*, 1595−1600.
(80) Li, Y. B.; Lv, C.; Zhang, W. D. Methods and applications for identification and identification of non-labeled natural product targets. *Acta Pharmacol. Sinica* **2019**, *54*, 82−88.
(81) Chang, J.; Kim, Y.; Kwon, H. J. Advances in identification and validation of protein targets of natural products without chemical modification. *Nat. Prod. Rep.* **2016**, *33*, 719−730.
(82) Lomenick, B.; Olsen, R. W.; Huang, J. Identification of direct protein targets of small molecules. *ACS Chem. Biol.* **2011**, *6*, 34−46.
(83) Chen, F.; Zhu, K.; Chen, L.; et al. Protein target identification of ginsenosides in skeletal muscle tissues: discovery of natural small-molecule activators of muscle-type creatine kinase. *J. Ginseng Res.* **2020**, *44*, 461−474.
(84) Zhang, Y.; Zeng, K.; Wang, J.; Gao, H.; Nan, Y.; Zheng, X. Identifying the antiasthmatic target of doxofylline using immobilized β2 -adrenoceptor based high-performance affinity chromatography and site-directed molecular docking. *J. Mol. Recognit.* **2016**, *29*, 492−498.
(85) Wang, C.; Chen, N. Proteomic analysis based on activity. *Acta Chem. Sinica* **2015**, *73*, 657−668.
(86) Ma, N.; Zhang, Z. M.; Lee, J. S.; et al. Affinity-Based Protein Profiling Reveals Cellular Targets of Photoreactive Anticancer Inhibitors. *ACS Chem. Biol.* **2019**, *14*, 2546−2552.
(87) Zhang, M.; Ma, X.; Xu, H.; Wu, W.; He, X.; Wang, X.; Jiang, M.; Hou, Y.; Bai, G. A natural AKT inhibitor swertiamarin targets AKT-PH domain, inhibits downstream signaling, and alleviates inflammation. *FEBS J.* **2020**, *287*, 1816−1829.
(88) Jourdan, J.; Walz, A.; Matile, H.; et al. Stochastic Protein Alkylation by Antimalarial Peroxides. *ACS Infect. Dis.* **2019**, *5*, 2067−2075.
(89) Han, K.; Wang, M.; Zhang, L.; Wang, C. Application of molecular methods in the identification of ingredients in Chinese herbal medicines. *Molecules* **2018**, *23*, 2728.
(90) Chen, S. Q.; Dong, G. Q.; Sheng, C. Q.; Zhang, W. N. Advances in the identification of targets for active small molecule compounds. *J. Pharmaceut. Practice* **2016**, *34*, 97−102.
(91) Wei, S.; Sun, P.; Guo, Y.; Chen, J.; Wang, J.; Song, C.; Li, Z.; Xue, L.; Qiao, M. Gene expression in the hippocampus in a rat model of premenstrual dysphoric disorder after treatment with baixiangdan capsules. *Front. Psychol.* **2018**, *9*, 2065.
(92) Huang, X. P.; Chen, J. K.; W, X.; et al. Systematic identification of celastrol-binding proteins reveals that shoc2 is inhibited by celastrol. *Biosci. Rep.* **2018**, *38*, BSR20181233.
(93) El-Boubbou, K. Magnetic iron oxide nanoparticles as drug carriers: preparation, conjugation and delivery. *Nanomedicine (London, U. K.)* **2018**, *13*, 929−952.
(94) Wang, Y. H.; Song, X. M.; Jiang, Y.; Tu, P. F.; Zeng, K. W. Method for active ingredients' in vivo target identification of traditional Chinese medicine using magnetic nanoparticles. *Zhongguo Zhong Yao Za Zhi.* **2019**, *44*, 2657−2661.
(95) Chen, G.; Huang, B. X.; Guo, M. Current advances in screening for bioactive components from medicinal plants by affinity ultrafiltration mass spectrometry. *Phytochem. Anal.* **2018**, *29*, 375−386.
(96) Yuan, L.; Zhang, Z. Z.; Hou, Z. G.; Yang, B.; Li, A.; Guo, X.; Wang, Y.; Li, Y. B. Rapid classification and identification of complex chemical compositions in traditional Chinese medicine based on UPLC-Q-TOF/MS coupled with data processing techniques using the KuDieZi injection as an example. *Anal. Methods* **2015**, *7*, 5210−5217.
(97) Jiao, J.; Yang, Y.; Wu, Z.; et al. Screening cyclooxygenase-2 inhibitors from Andrographis paniculata to treat inflammation based on bio-affinity ultrafiltration coupled with UPLC-Q-TOF-MS. *Fitoterapia* **2019**, *137*, 104259.
(98) Zhou, H.; Wang, Y. M.; Zheng, Z.; Liu, S.; Liu, Z. Q. Advances in the application of affinity ultrafiltration mass spectrometry in the screening of active ingredients in traditional Chinese medicine. *J. Chin. Mass Spectrom. Soc.* **2018**, *39*, 641−652.
(99) Liu, Y. L.; An, Q.; Chen, S. E.; Zhao, L. S.; Liu, L. L. Advances in the application of biological macromolecules in the study of traditional Chinese medicine by molecular biological chromatography. *J. Pharm. Res.* **2019**, *38*, 652−656.
(100) Lv, Y.; Shi, X.; Fu, J.; Jia, Q.; Lin, Y.; Wang, H.; Yang, X.; Han, S. Screening potential antagonists of epidermal growth factor receptor from Marsdenia tenacissima via cell membrane chromatography model assisted by HPLC-ESI-IT-TOF-MS. *Biomed. Chromatogr.* **2019**, *33*, e4569.
(101) Wang, H.; Zou, H.; Kong, L.; Ni, J. Analysis of bioactive components in traditional Chinese medicines by molecular bio-chromatography with alpha1-acid glycoprotein stationary phase. *J. Basic Clin. Physiol. Pharmacol.* **2000**, *11*, 155−172.
(102) Pinzi, L.; Rastelli, G. Molecular Docking: Shifting Paradigms in Drug Discovery. *Int. J. Mol. Sci.* **2019**, *20*, 4331.
(103) Saikia, S.; Bordoloi, M. Molecular docking: challenges, advances and its use in drug discovery perspective. *Curr. Drug Targets* **2019**, *20*, 501−521.
(104) Yin, L.; Zheng, L.; Xu, L.; Dong, D.; Han, X.; Qi, Y.; Zhao, Y.; Xu, Y.; Peng, J. In-silico prediction of drug targets, biological activities, signal pathways and regulating networks of dioscin based on bioinformatics. *BMC Complementary Altern. Med.* **2015**, *15*, 41.
(105) Yu, R.; Chen, L.; Lan, R.; Shen, R.; Li, P. Computational screening of antagonists against the SARS-CoV-2 (COVID-19) coronavirus by molecular docking. *Int. J. Antimicrob. Agents* **2020**, *56*, 106012.
(106) Yu, J. W.; Wang, L.; Bao, L. D. Exploring the Active Compounds of Traditional Mongolian Medicine in Intervention of Novel Coronavirus (COVID-19) Based on Molecular Docking Method. *J. Funct. Foods* **2020**, *71*, 104016.
(107) Das, S.; Sarmah, S.; Lyndem, S.; Singha Roy, A. An investigation into the identification of potential inhibitors of SARS-CoV-2 main protease using molecular docking study. *J. Biomol. Struct. Dyn.* **2020**, 1.
(108) Wang, X.; Song, K.; Li, L.; Chen, L. Structure-based drug design strategies and challenges. *Curr. Top. Med. Chem.* **2018**, *18*, 998−1006.
(109) Machálková, M.; Schejbal, J.; Glatz, Z.; et al. A label-free MALDI TOF MS-based method for studying the kinetics and inhibitor screening of the Alzheimer's disease drug target β-secretase. *Anal. Bioanal. Chem.* **2018**, *410*, 7441−7448.
(110) Källsten, M.; Hartmann, R.; Artemenko, K.; et al. Qualitative analysis of antibody-drug conjugates (ADCs): an experimental comparison of analytical techniques of cysteine-linked ADCs. *Analyst* **2018**, *143*, 5487−5496.
(111) Longuespée, R.; Casadonte, R.; Kriegsmann, M.; et al. MALDI mass spectrometry imaging: A cutting-edge tool for fundamental and clinical histopathology. *Proteomics: Clin. Appl.* **2016**, *10*, 701−719.

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258

(112) Lu, M.; Cai, Z. Advances of MALDI-TOF MS in the analysis of traditional Chinese medicines. *Top. Curr. Chem.* **2012**, *331*, 143−164.
(113) Wongkongkathep, P.; Han, J. Y.; Choi, T. S.; Yin, S.; Kim, H. I.; Loo, J. A. Native top-down mass spectrometry and ion mobility MS for characterizing the cobalt and manganese metal binding of α-synuclein protein. *J. Am. Soc. Mass Spectrom.* **2018**, *29*, 1870−1880.
(114) Alu'datt, M. H.; Rababah, T.; Kubow, S.; Alli, I. Molecular changes of phenolic-protein interactions in isolated proteins from flaxseed and soybean using native-PAGE, SDS-PAGE, RP-HPLC, and ESI-MS analysis. *J. Food Biochem.* **2019**, *43*, e12849.
(115) Ma, J. Y.; Li, Y. W.; Mei, N.; Tian, H. D.; Wang, M. Study on the interaction between brominated flame retardants and thyroxine delivery protein by non-denaturated electrospray mass spectrometry. *J. Instrum. Anal.* **2018**, *37*, 525−531.
(116) Gavriilidou, A. F. M.; Holding, F. P.; Coyle, J. E.; Zenobi, R. Application of native ESI-MS to characterize interactions between compounds derived from fragment-based discovery campaigns and two pharmaceutically relevant proteins. *SLAS Discovery* **2018**, *23*, 951−959.
(117) Root, K.; Wittwer, Y.; Barylyuk, K.; Anders, U.; Zenobi, R. Insight into signal response of protein ions in native ESI-MS from the analysis of model mixtures of covalently linked protein oligomers. *J. Am. Soc. Mass Spectrom.* **2017**, *28*, 1863−1875.
(118) Zhang, L.; Liu, Q. Q.; Wu, C. L.; Wang, P.; Xu, X. L.; Han, M. Y. Advances in research methods for the interaction between polyphenols and proteins. *Sci. Technol. Food Ind.* **2018**, *39*, 340−345.
(119) Shaghaghi, M.; Dehghan, G.; Rashtbari, S.; Sheibani, N.; Aghamohammadi, A. Multispectral and computational probing of the interactions between sitagliptin and serum albumin. *Spectrochim. Acta, Part A* **2019**, *223*, 117286.
(120) Song, Z.; Yuan, W.; Zhu, R.; Wang, S.; Zhang, C.; Yang, B. Study on the interaction between curcumin and CopC by spectroscopic and docking methods. *Int. J. Biol. Macromol.* **2017**, *96*, 192−199.
(121) Xin, J. W.; Ma, H. Y.; Luo, J. J. Study on the interaction between cyproheptadine hydrochloride and bovine serum albumin by fluorescence spectrometry. *Chem. World* **2020**, *61*, 99−104.
(122) Tang, L.; Li, S.; Bi, H.; et al. Interaction of cyanidin-3-O-glucoside with three proteins. *Food Chem.* **2016**, *196*, 550−559.
(123) Taenzler, P. J.; Sadeghian, K.; Ochsenfeld, C. A dynamic equilibrium of three hydrogen-bond conformers explains the NMR spectrum of the active site of photoactive yellow protein. *J. Chem. Theory Comput.* **2016**, *12*, 5170−5178.
(124) Kim, J.; Liu, M.; Hilty, C. Modeling of polarization transfer kinetics in protein hydration using hyperpolarized water. *J. Phys. Chem. B* **2017**, *121*, 6492−6498.
(125) Becker, B. A.; Larive, C. K. Probing the binding of propranolol enantiomers to alpha1-acid glycoprotein with ligand-detected NMR experiments. *J. Phys. Chem. B* **2008**, *112*, 13581−13587.
(126) Lieberman, R. L.; Peek, M. E.; Watkins, J. D. Determination of soluble and membrane protein structures by X-ray crystallography. *Methods Mol. Biol.* **2013**, *955*, 475−493.
(127) Wu, D. Small molecules targeting FKBP51 inhibit the AR signaling pathway of prostate cancer. Lanzhou University, 2017.
(128) Su, H. X.; Yao, S.; Zhao, W. F.; et al. Anti-SARS-CoV-2 activities in vitro of Shuanghuanglian preparations and bioactive ingredients. *Acta Pharmacol. Sin.* **2020**, *41*, 1167−1177.
(129) Zheng, F. J.; Liu, Y. N.; Ma, X. H.; Li, X. L.; Ma, J.; Liu, N. Advances in biomedical research on microthermophore technology. *Chemistry of Life* **2016**, *36*, 443−448.
(130) Guo, J.; Gu, X.; Zheng, M.; Zhang, Y.; Chen, L.; Li, H. Azacoccone E inhibits cancer cell growth by targeting 3-phosphoglycerate dehydrogenase. *Bioorg. Chem.* **2019**, *87*, 16−22.
(131) Peng, Y.; Zhang, X.; Zhang, T.; et al. Lovastatin inhibits Toll-like receptor 4 signaling in microglia by targeting its co-receptor myeloid differentiation protein 2 and attenuates neuropathic pain. *Brain, Behav., Immun.* **2019**, *82*, 432−444.
(132) Senapati, S.; Biswas, S.; Manna, S.; Ros, R.; Lindsay, S.; Zhang, P. A y-shaped three-arm structure for probing bivalent interactions between protein receptor-ligand using AFM and SPR. *Langmuir* **2018**, *34*, 6930−6940.
(133) Gu, C.; Yin, Z.; Nie, H.; Liu, Y.; Yang, J.; Huang, G.; Shen, J.; Chen, L.; Fei, J. Identification of berberine as a novel drug for the treatment of multiple myeloma via targeting UHRF1. *BMC Biol.* **2020**, *18*, 33.
(134) Shao, M.; Guo, D.; Lu, W.; et al. Identification of the active compounds and drug targets of Chinese medicine in heart failure based on the PPARs-RXRα pathway. *J. Ethnopharmacol.* **2020**, *257*, 112859.
(135) Fan, S.; Gu, K.; Wu, Y.; et al. Liquiritinapioside - A mineralocorticoid-like substance from liquorice. *Food Chem.* **2019**, *289*, 419−425.
(136) Sun, Q.; Gan, N.; Zhang, S.; et al. Insights into protein recognition for γ-lactone essences and the effect of side chains on interaction via microscopic, spectroscopic, and simulative technologies. *Food Chem.* **2019**, *278*, 127−135.
(137) Zhang, L.; Yang, F.; Cai, J. Y.; Yang, P. H.; Liang, Z. H. In-situ detection of resveratrol inhibition effect on epidermal growth factor receptor of living MCF-7 cells by Atomic Force Microscopy. *Biosens. Bioelectron.* **2014**, *56*, 271−277.
(138) Henderson, L. D.; Beeby, M. High-throughput electron cryotomography of protein complexes and their assembly. *Methods Mol. Biol.* **2018**, *1764*, 29−44.
(139) Grela, E.; Kozłowska, J.; Grabowiecka, A. Current methodology of MTT assay in bacteria- A review. *Acta Histochem.* **2018**, *120*, 303−311.
(140) Qi, J.; Yu, J.; Tan, Y.; Chen, R.; Xu, W.; Chen, Y.; Lu, J.; Liu, Q.; Wu, J.; Gu, W.; Zhang, M. Mechanisms of Chinese medicine xinmailong's protection against heart failure in pressure-overloaded mice and cultured cardiomyocytes. *Sci. Rep.* **2017**, *7*, 42843.
(141) Tian, Y.; Du, Y.-Y.; Shang, H.; Wang, M.; Sun, Z.-H.; Wang, B.-Q.; Deng, D.; Wang, S.; Xu, X.-D.; Sun, G.-B.; Sun, X.-B. Calenduloside E analogues protecting H9c2 cardiomyocytes against H_2O_2-induced apoptosis: design, synthesis and biological evaluation. *Front. Pharmacol.* **2017**, *8*, 862.
(142) Chen, Y.S.; Li, S.P.; Xiao, H.; Xie, Z.Y.; Zhang, W. M.; Metastasis-associated gene 1 expression in human medulloblastoma and its association with invasion and metastasis in medulloblastoma Daoy cell lines. *GMR, Genet. Mol. Res.* **2016**, *15* (2). DOI: 10.4238/gmr.15027894
(143) Sajjad, S.; Do, M. T.; Shin, H. S.; et al. Rapid and efficient western blot assay by rotational cyclic draining and replenishing procedure. *Electrophoresis* **2018**, *39*, 2974−2978.
(144) Dai, G.; Ding, K.; Cao, Q.; et al. Emodin suppresses growth and invasion of colorectal cancer cells by inhibiting VEGFR2. *Eur. J. Pharmacol.* **2019**, *859*, 172525.
(145) Hou, Y.; Nie, Y.; Cheng, B.; et al. Qingfei Xiaoyan Wan, A traditional Chinese medicine formula, ameliorates Pseudomonas aeruginosa-induced acute lung inflammation by regulation of PI3K/AKT and Ras/MAPK pathways. *Acta Pharm. Sin. B* **2016**, *6*, 212−221.

https://dx.doi.org/10.1021/acs.jproteome.0c00372
J. Proteome Res. 2020, 19, 4242−4258