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Computation-aided novel epitope prediction by targeting spike protein's functional dynamics in Omicron

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1 The ever-growing crisis imposed by Omicron

The global corona virus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has lasted for more than 3 years and resulted in about 657 million infections and 6.6 million deaths as of date 05 January, 2023 (https://covid19.who.int/). The latest variant of concern (VoC), Omicron, is leading a new wave of infections globally[1]. Although small molecule inhibitors are emerging to show antiviral activities for SARS-CoV-2[2-3], only limited drugs have been approved (*e.g.*, remdesivir and baricitinib). Vaccination remains the preferred protection method, however, extra vaccine dose is often required to effectively neutralize Omicron[4]; especially for the continuous evolution SARS-CoV-2 variants by constant mutations, escape from neutralizing antibodies is still a major concern that challenges the effectiveness of existing vaccines[5]. This global public health crisis urgently demands developing effective antibodies against the Omicron.

2 Epitope loss in Omicron

Mutations in the S protein of Omicron caused more epitope loss[7]. The trimeric S protein on the surface of SARS-CoV-2 is the prime antibody target because of its accessibility and its important role in mediating virus fusion into host cell[8]. The protomer of S protein consists mainly of the S1 and S2 subunits (Fig. 1A). Most epitopes of existing antibodies are found in the S1 subunit, with majority in the receptor binding domain (RBD) and some in the N-terminal domain (NTD)[9]. SARS-CoV-2 and its VoCs are able to escape existing antibodies through continuously mutating residues in the epitope regions[7]. The epitope loss is particularly the case for the Omicron VoC[10]. The severe epitope loss in Omicron calls for new and more precise epitope prediction, especially the epitopes that are conserved and are resilient to mutations.

3 pH-regulated functional dynamics of the S protein

Various S protein Cryo-EM structures have been resolved to demonstrate the intrinsic dynamic nature of the S protein. Of particular interest is the dynamics underlying the RBD positioning process. In the trimeric S protein, the positioning of the RBD with respect to the NTD (from neighboring protomer) determines the accessibility of the RBD to host cell angiotensin conversion enzyme 2 (ACE2) (Fig. 1A). The repositioning of the RBD is crucial for both ACE2 binding and immune evasions[6,11]. However, molecular basis governing the positioning of the RBD is still unclear[6]. pH has long been recognized as an important factor to regulate protein conformational dynamics[12]. A recent study clearly shows how pH regulates the RBD positioning of the S protein and how such a regulation is vital to viral infectivity[6]. Specifically, in the endosomal entry pathway of SARS-CoV-2 into host cell, pH decreases from 6 (early endosomes) to 5 (late endosomes) and to as low as 4 (in lysosomal)[6]. During this entry process, the S protein exhibits a pH-dependent ACE2 binding and conformational changes. Theoretically, the trimeric

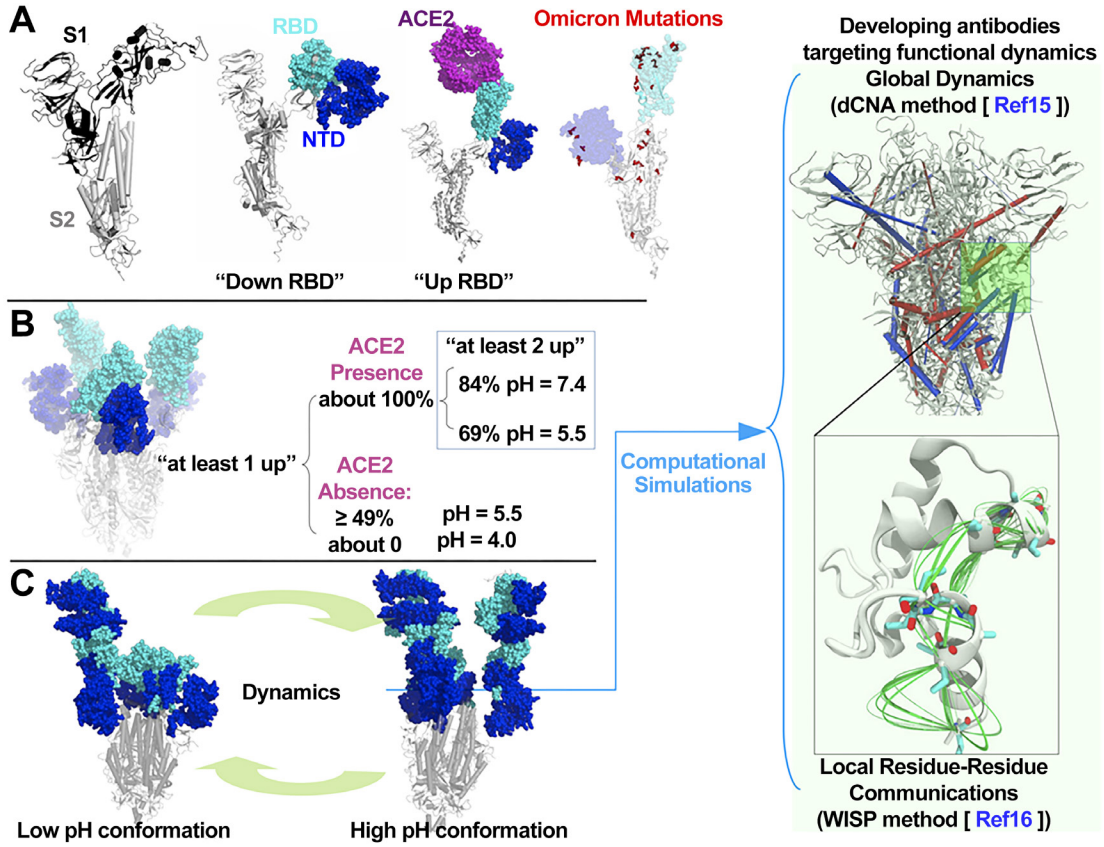

Fig. 1 Sipke protein structure and dynamics

(A) The protomer of S protein showing S1 and S2 subunits. S1 contains the RBD and NTD domains. The RBD has "down" and "up" configurations, and the 'up' configuration is required for binding host ACE2; (B) Experimentally observed pH-regulated S protein conformational plasticity during the endosomal entry of SARS-CoV-2 into host cell[6]; (C) The functional dynamics underlying the pH-regulated S protein plasticity is integral to SARS-CoV-2 infectivity. Computational simulations are able to decipher such dynamics and identify conserved regions that may have antigenicity; RBD, receptor binding domain; ACE2, angiotensin conversion enzyme 2; NTD, N-terminal domain.

S protein can have up to three ACE2 bound to its RBD domains. It is observed that pH affects the number of ACE2 bound to S protein: at neutral pH = 7.4 the percentage of "at least two ACE2 bound" conformation is around 88% versus that of 69% at pH = 5.5[13]. This implies that pH mediates the S protein's ability to take the "up RBD" configuration. This mediation effect is more obvious at acidic pH: lowering pH from 5.5 to 4.0 dramatically affects the repositioning of the RBD, resulting in nearly 100% "down RBD" configuration which precludes any ACE2 binding (Fig. 1B). Structurally, this pH-regulated RBD repositioning is attributed to a switch located remotely from the RBD[6]. This switch changes its folding status with changing pH and mediates the RBD repositioning. The distant nature between the switch and the RBD highlights the importance of functional dynamics in the S protein, as the altered folding status of the switch must transmit to the RBD through such a dynamics network. This pH-regulated functional dynamics is a signature of SARS-CoV-2's S protein and is expected to be highly conserved across all SARS-CoV-2 VoCs including Omicron, as if not, the Omicron's invasion ability would be greatly compromised. Indeed, it has been widely accepted that proteins' dynamic fluctuations are evolutionarily optimized for function[14]. Therefore, targeting the pH-regulated functional dynamics in the S protein for new epitope prediction is likely an effective strategy of designing antibodies against Omicron.

4 Leveraging computational simulations to decipher the functional dynamics

We propose here that extensive molecular dynamics (MD) simulations coupled with post-processing dynamics network analysis aid us to decipher the functional dynamics in the S protein (Fig. 1C). MD simulations have found their great success in revealing protein dynamics and relating them to protein

functions[15]. In the case of the S protein, on the basis of sufficient sampling by extensive MD simulations, we could identify the global dynamics network using methods such as difference contact network analysis (dCNA)[15]. Specifically, we compared the dynamics (measured by residue-residue contact network) of two S protein ensembles simulated at different pH values to tease out the dynamics network that is sensitive to pH changes[15]. Besides, local subtle changes in the dynamics can change protein functions without large conformation changes[16]. It has been reported that a new P1 variant of SARS-CoV-2 enhances antibody resistance without causing global changes of the spike protein[17], signifying the importance of such local changes. These local dynamics can be derived from MD trajectories using the method such as the weighted implementation of suboptimal paths (WISP) tool[16], which identifies the key residues constituting the signaling pathways between user-selected source and sink residues (Fig. 1C). Therefore, the combination of extensive MD simulations and dynamics network analysis can reveal the global functional dynamics and the local signaling pathways. These components are expected to be highly conserved in the S protein as they are directly related to the virus's infectivity[6]. Thus we could target these components to find new epitopes in the S2 subunit, which are outside the traditional RBD and NTD domains, as it has been suggested that the S2 subunit has the potential to become antigenic[18].

5 Conclusion

The greatly increased transmissibility and vaccine escapes of the Omicron call for new epitope prediction. Finding highly conserved epitopes on the S protein is fundamental to combat SARS-CoV-2 infection but the mission is non-trivial[7]. Although several computation models have been proposed to predict potential epitopes on the S protein[19-20], these predictions are generally based on limited S protein structures and the predicted epitopes are likely facing the same challenge of being mutated in new S variants. In this perspective, we propose to target the functional dynamics, especially those related to the virus infectivity, to find a highly conserved region, with the rationale that variants that disturb these functional dynamics network would abolish infectivity. Benefiting from our current and growing knowledge about various S protein Cryo-EM structures obtained along the pH-regulated SARS-CoV-2 entry process into host cell[6], the functional dynamics connecting these structures are within the reach of our hand through computational simulations plus state-of-the-art dynamic network analysis tools. Epitopes comprising the residues involved in the functional dynamics are conserved and might be used to develop long-term effective antibodies against Omicron and possible newer SARS-CoV-2 variants.

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Conflicts of interests

Yang B F is the Editor-in-Chief of Frigid Zone Medicine. Zhang Y is an Editorial Board Member. The article was subject to the journal's standard procedures, with peer review handled independently of these Members and their research groups.

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