BCH210H Assignment 2

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Introduction:

[General Info] Coronaviruses (CoV) is a big family of RNA viruses causing respiratory tract infections that can be mild or lethal in human and birds. SARS outbreak in 2002-2004 and MERS since 2012 both belong to coronavirus family. (Wikipedia) Currently, a novel coronavirus pandemic Covid-19 firstly occurred in Wuhan in December 2019 has spread to the world and caused more than forty-five million confirmed cases and one million deaths. The coronavirus was then officially named as severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) by the WHO (Zheng, 2020).

[Epidemiology] SARS-CoV-2 mainly influences people's cardiovascular and immune system. The lungs are the primary damage site. Common symptoms include "cough, fever, dyspnea, musculoskeletal symptoms (myalgia, joint pain, fatigue), gastrointestinal symptoms, and anosmia". (Carfi, 2020). Although most patients' symptoms are not serious, about 15% has severe pneumonia and about 5% eventually develop to acute respiratory distress syndrome (ARDS), septic shock or multiple organ failure. (Cao, 2020). Jorden (2020) found that older people, males, and people with hypertension, diabetes, cardiovascular disease, or chronic lung disease are more likely to have severe symptoms or death. SARS-Cov-2 is spread mainly through human-to-human transmission with high infectiousness. Therefore, preventing it from outbreaking is difficult. Also, patients are very likely to have persistent symptoms after acute illness (Carfi, 2020). ***[Immunology]*** Research found that inhibiting the excessive inflammatory response can be a potential therapy for COVID-19. Invasive mechanical ventilation and receiving oxygen are proved to be effective in reducing mortally possibilities.

[Virology and Physiology] How SARS-Cov-2 influences cardiovascular and immune systems is associated with two membrane proteins, including Angiotensin-converting enzyme 2 (ACE2) and spike glycoprotein (S protein). ***[Role of S protein]*** Spike glycoprotein is a class one viral fusion protein located in SARS-COV-2's outer membrane (Walls, 2017). Each S-protein is composed of S1 and S2 subunits. During the viral infection, the S-protein is cleaved into S1 and S2. S1 binds to ACE-2 enzyme, and S2 cleaved further for membrane fusion. Both of the two processes promote SARS-Cov-2 infection. (Dalan, 2020) ***[Role of ACE2]*** ACE2 is a single pass type 1 membrane-bound aminopeptidase that is located on the surface of many cell types and tissues, such as lungs, heart, blood vessels, kidney, liver and gastrointestinal tract. ACE2 is identified as a functional receptor for SARS-Cov-2. It binds to S-protein of SARS-Cov-2 to allow virus transmission. Also, it plays an important role in the renin-angiotensin-aldosterone system (RAAS) pathway by converting ANG II to other molecules to counteract ANG II's effects. Insel (2020) believe that an abnormally high ANG II activity determines the severity of the symptoms induced by SARS-Cov-2.

Membrane-Protein Structures:

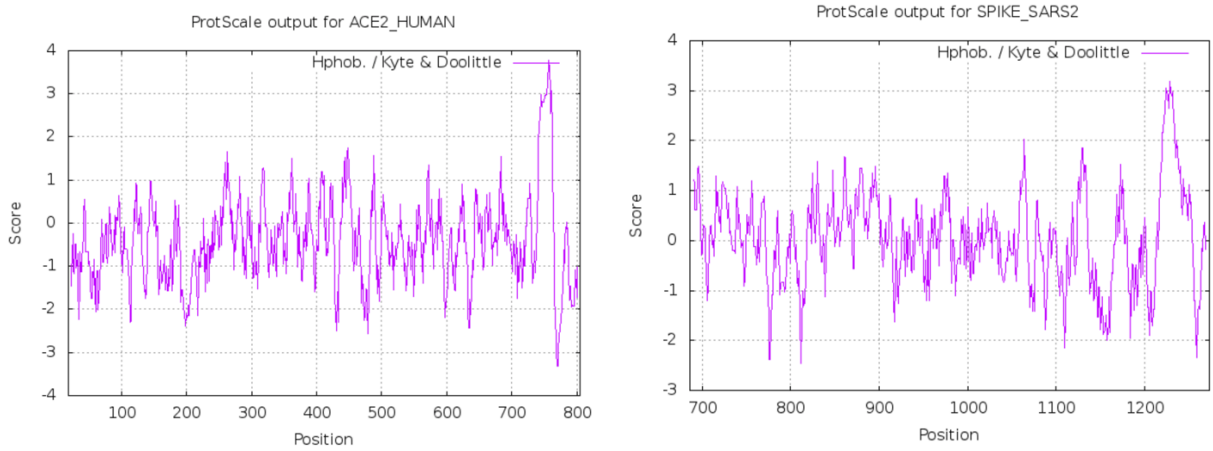

Figure (left): ACE2 Hydropathy Plots (18-805 residues) with transmembrane portion (741-761 residues)
Figure (right): Spike Glycoprotein Hydropathy Plots S2 chain (686-1273 residues) with transmembrane portion (1214-1234 residues)

[Common]ACE2 and S protein are both type 1 integral membrane protein (Belouzard, 2012), which means that the amino terminal is outside the membrane and is single pass. It completely spans the membrane and is hard for purification. (Slides 4.3) According to the hydropathy plots, the hydropathy index are positive for more than 20 amino acids in position 741-761 in the left figure, and 1214-1234 in the right figure, which proves that the portions are transmembrane.
[S protein structure] S glycoprotein consists of a short intracellular tail, a transmembrane anchor and a receptor-binding ectodomain. The ectodomain contains amino-terminal S1 subunit (14-685 residues) that provides adhesion to the target cell and carboxy-terminal S2 subunit (686-1273 residues) that ensures membrane-fusion. The S1 and S2 subunits remain non-covalently associated in the metastable pre-fusion S trimer. (Walls, 2016) In S1 subunit, there is an N-terminal domain (14-305 residues) and a receptor-binding domain (319-541 residues). The receptor-binding domain is positively charged and binds to the cell receptor ACE2. In S2 subunit, there is fusion peptide (788-806 residues) and cytoplasm domain (1237-1273 residues). Fusion peptide is composed mainly of hydrophobic residues, such as glycine or alanine, which can disrupt and connect lipid bilayers of the host cell membrane to mediate membrane fusion. (Huang, 2020). The cleavage site of S1 and S2 still remains unknown.
[ACE structure] ACE2's structure has a deep channel (19-615 residues) on the top of the molecule with the catalytic site. (Lan et al. 2020) The channel is surrounded by negatively charged ridges, which allows binding of positively charged receptor binding domain of S-glycoprotein. These ridges contain residues D136, E150, N154, D157, D292, D295, D299. Also, three hydrophobic regions including Phe, Trp, and Tyr residues are found close to negatively charged ridges to facilitate binding. (Probakaran, 2004)
[Post translational modifications] Both proteins undergo glycosylation and methylation. Glycosylation is the process to link sugar chains to Asn or Ser/Thr. This form of post-translational modification mostly takes place in the ER and Golgi. Glycosylation of membrane proteins and lipids can lead to increased diversity of complexity. (Slides 7.2). However, Sun et al. (2020) found that glycosylation cannot directly increase binding affinity. Methylation is a process to link methyl group to the proteins. "57E, 68K, and 329E sites in ACE2, which surround its binding site with S protein's receptor binding domain is completely methylated." (Sun et al.,

2020) Methylation increases the hydrophobicity of these sites to facilitate binding. Also, ACE2 undergoes hydroxyprolines. The extra hydroxyl group can increase hydrophilicity of proline.

Protein-Protein Structures:

[Protein-protein interaction] The binding of SARS-Cov-2 and ACE2 can be proved by crystallization of SARS-CoV-2 RBD and ACE2 complex. Shang et al. (2020) show that a N-O bridge is formed between Arg439 in the S1 receptor binding domain and Glu329 at the outer surface of the ACE2. Shang et al. (2020), in another paper, investigated binding and protease activation of SARS-Cov-2 spike using biochemical and pseudoviral entry assays. He found that spike protein should be proteolytically cleaved at the S1/S2 boundary at PPC cleavage site during viral packaging. The cell surface protease TMPRSS2 and lysosomal proteases cathepsins are involved in the cleavage. In this case, S1 dissociates and S2 undergoes a dramatic structural change to fuse membranes. After the membrane is fused, the dissociated S1's receptor binding domain recognized ACE2 as its receptor and promote for receptor-binding and immune evasion.

[Hypothesis] My hypothesis is that the mutation of key residues in SARS-Cov-2 spike glycoprotein's receptor binding domain could block the interaction between S protein and ACE2. As discussed in the previous sections, receptor binding domain (RBD) recognizes and binds to ACE2. Therefore, mutation of key residues might influence RBD's functions, thus blocks the interaction. The experiment I designed is to prepare two sets of S protein and ACE2 protein. One set is control group with normal S protein and ACE2 protein. Another set is experimental group with normal ACE2 protein and S protein that has key residues, such as Arg439 (form N-O bridge), mutated. Both of the two groups are examined in an environment that is suitable for S protein and ACE2 protein interaction. Whether interaction is taking place can be examined by mass spectrometry as the mass of peptides will be larger in the bound proteins. If the binding interaction is decreased or inhibited, it means that the key residue tested(mutated) plays an important role in functions of the receptor binding domain and can possibly contribute to treatment options. If the binding interaction is not influenced, it means that the key residue tested(mutated) cannot block the interaction.

[Conclusion] In understanding the underlying biochemical mechanism in COVID-19, I found that the application of biochemistry can help us analyze the mechanism in a molecular level, which give us a better understanding of the problem and the ability to predict experiment results. For example, with the hydropathy plots, we can predict the transmembrane portion of the two proteins. Also, we can design experiment more purposefully if we can predict experiment results.

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