Na"Nobody" Likes Covid-19!: The Role of Nanobodies in Fighting COVID-19

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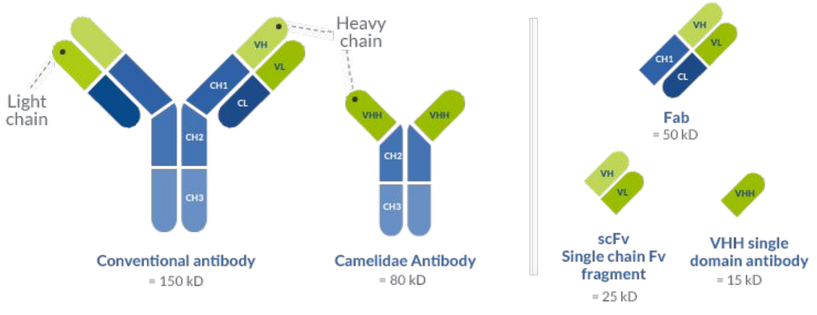
Abstract

The SARS-CoV-2 pandemic has placed increased stress and importance on the development of new and effective treatments for COVID-19. According to Huo et. al, nanobodies are a new and promising treatment that may be the future of preventing COVID-19 and other pathogens. Nanobodies are a monomeric protein consisting solely of heavy chain amino acids with only one chain in its Receptor Binding Domain(RBD), in contrast to the antibody that consists of two heavy and two light chains in its RBD. The structural difference affects antibodies' and nanobodies' function in reference to the binding to the SARS-CoV-2 virus' antigen (the spike), and their structural difference allows for a comprehensive treatment against COVID-19 by providing unique opportunities in their application. Our research and 3-D modeling using jMol suggests 3 main binding locations of the C5 nanobody onto the spike: the primary binding site (I) consists of a core arginine to glucose magnetic bond surrounded by a ring of hydrophobic side chains; the secondary binding site (II) is a cluster of hydrophilic side chains; finally, the third binding site (III) is a small hydrophobic pair. Binding sites I and III are concentrated on the face oriented away from the center of the spike while site II is oriented towards the center. Site II does not entirely cover the back face of the connection, impling an instability. Further inspection reveals a largely hydrophilic back face that, while not interfacing with the spike, would stabilize against other binded nanobodies. The additional support indicates that nanobodies bind best in a trimer, a point corroborated by empirics in Güttler et al and Huo et al. In addition to nanobodies binding ability and simple structure, they can also be produced synthetically granting immediate care. The Mahtomedi MSOE Center for BioMolecular Modeling MAPS Team used 3-D modeling and printing technology to compare the structural and functional properties of nanobodies and antibodies in fighting SARS-CoV-2 virus.

What are Nanobodies?

Antibodies are Y-shaped proteins utilized by the body's immune system to fight pathogens. To do this, the antibody identifies a unique molecule of the pathogen, called the antigen, and marks it. The antibody then attaches to the antigen to fight it off. Nanobodies, a type of antibody, have been discovered, and may be beneficial in fighting off viruses, such as COVID-19, more efficiently. In the most simplistic terms, a nanobody is a single-domain antibody, derived from the alpaca heavy chain HCAB antibodies. It is an antibody fragment consisting of one monomeric variable antibody domain, and these monomers react together to form the 3-D networks in a nanobody. These nanobodies can be created by splitting Immunoglobulin G, which is a dimeric antibody commonly found in humans. Similar to traditional antibodies, nanobodies bind selectively to a specific antigen to help fight it off. However, nanobodies only contain the VHH (single variable) domain, while a regular antibody has a VH (heavy chain) domain and a VL (light chain) domain. This structural variation causes many differences between conventional antibodies and nanobodies. Regular antibodies have flat surfaces, whereas nanobodies are able to bind to clefts, making them able to bind to a variety of surfaces. Nanobodies are smaller and more consistent than traditional antibodies are, making them beneficial for usage in treatments, such as fighting COVID-19.

Figure 1 ("Single Domain Antibodies")



Nanobody-Spike Binding

The three main binding sites described in the abstract are listed in order of importance. Binding site I is also divided into two parts. The first part is a magnetic bond consisting of an arginine side chain (positively charged) on the nanobody and a glucose side chain (negatively charged) on the spike. This connection forms a salt bridge as observed in Huo et al., thus creating an actual physical connection. The surrounding hydrophobic atoms serve as part 2 in the binding site. Site II is to the left of Site I (see Fig. 2A) and is the main hydrophilic binding site. Additionally, we find minor two minor chains that appear to be oriented towards the binding site but are notably farther away than the rest. Finally, site III, the smallest of the three, is a hydrophobic binding site, above and to the right of site I. It consists of a phenylalanine chain on both the nanobody and spike. Although small, it appears to provide crucial support to the outside of the connection and slots the nanobody into the odd shaped tail of the nanobody (see Fig. 2). These binding sites, however, don't provide total coverage of the receptor-binding-domain of the spike (RBD). The backside has no apparent binding site. We argue, instead, that because of the shape of the nanobodies and spike, the largely hydrophilic interior face of the nanobody loosely binds to other nanobodies forming a group of three (a trimer). This theory is supported by Güttler et al. The most potent nanobody triad increase in potency by "30-000-fold," from it's monomeric counterpart. For our specific nanobody, the binding affinity (K_D) increased ~90-fold (Huo et. al.).

Nanobody Models

Fig. 2A

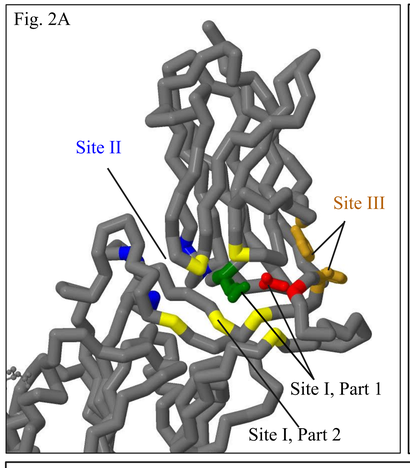


Fig. 2B

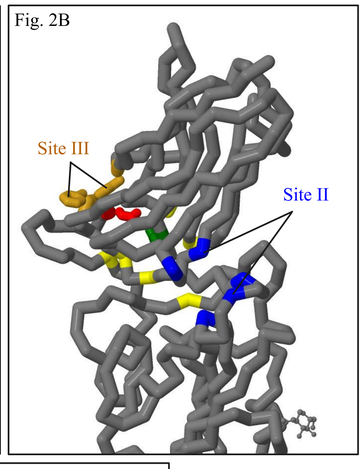


Fig. 3

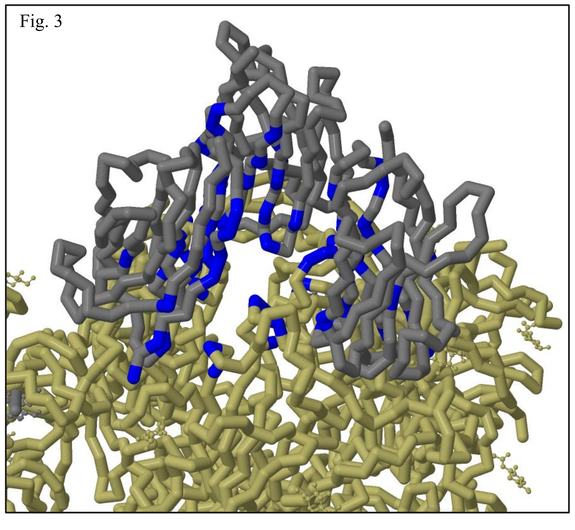


Figure 2A Site I, Part 1: Arginine (+) is green; Glucose (-) is red. Site I, Part II: Hydrophobic chains shown in light yellow. Site III: Shown in dark yellow.
Figure 2B Site II: Chains are shown in blue.
Figure 3 Three Nanobodies in a trimeric formation (grey) bound to a COVID-19 spike (beige) in the all "down" formation. Blue signifies a hydrophilic sidechain; note the concentration of hydrophilic sidechains near the center of the trimer.
(PDB 7OAN)

How Nanobodies Disrupt COVID

SARS-CoV-2, like all other viruses, seeks to enter and then commandeer human cells in order to replicate. COVID-19 specifically targets the human angiotensin-converting enzyme 2 (hACE2) as its path into our cells. The role of the nanobody, as previously described in "What are Nanobodies," is to disrupt COVID-19's antigen, the spike. The spike consists of three independent RBDs that alternate between "up" (active) and "down" (inactive) positions (Huo et al.). Additionally, in order for the RBD to interact with hACE2, at least one of the three RBDs must be in the up position (Huo et al.). In order to totally neutralize a spike, nanobodies must bind to all three of its RBDs. While monomers (nanobodies not grouped into a trimer) are able to bind to both up and down positions, the trimeric formation of the C5 (see Fig. 3) is limited to binding to a spike protein when all three RBDs are in the down position due to the odd geometry of the spike and nanobody trimer. However, the trimeric compound maintains a much stronger binding affinity compared to the monomeric nanobody, despite any possible issues with "up" or "down" RBDs. Then, once the trimer is bound to the spike, the nanobody serves as a flag to the immune system to entirely destroy the now neutralized COVID-19 virus.

Conclusion

Nanobodies, single domain antibodies, are derived from alpacas. Their structure differs from the conventional antibody because of their single VHH domain, as opposed to the traditional VH domain and VL domain. These nanobodies have characteristics that could be beneficial in fighting diseases such as COVID-19, due to nanobodies' smaller size and ability to be more consistent throughout the body. The shape of the nanobody and its spike allows it to form bonds between the hydrophilic interior face of the nanobody and other nanobodies to create a trimer. The three receptors on the nanobody alternate between up and down positions. In order to gain maximum binding strength, the receptors must all be in the down position to catch the spike. This process stops the spike protein from binding to the human cell, stopping the infection of COVID-19. In addition to battling COVID-19, the nanobody can provide treatment and resources in other applications. The nanobody has been used in the field of in vivo imaging, and its ability to be easily manufactured allow it to be used as treatments for cancer, malignant tumors, and human bacterial infections.

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