Structure guided design of biparatopic nanobodies that potently neutralize SARS-CoV-2 and variants

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has demanded unprecedented attention to developing therapeutic reagents. The favorable physical properties of synthetic nanobodies (sybodies) make them attractive candidates for investigations of their ability to block viral entry. By X-ray crystallography we have determined the structural basis of the interaction of a panel of sybodies (Sb14, Sb16, Sb45 and Sb68) with the SARS-CoV-2 receptor binding domain (RBD): binary complexes of Sb16–RBD and Sb45–RBD; a ternary complex of Sb14–RBD–Sb68, and Sb45–RBD–Sb68, and Sb16 unliganded. Sb16 and Sb45 bind the RBD at its interface with ACE2 with similar footprints but position their complementarity determining regions (CDR)2 and CDR3 in diametrically opposite orientations. The ternary structure of the Sb45–RBD–Sb68 complex reveals that while Sb45 binds squarely at the ACE2 interface, Sb68 binds at the periphery of the ACE2 interface. The ternary structure reveals that a large surface area of the RBD may be captured by multiple sybodies. We also determined cryo-EM structures of Sb45 bound to the SARS-CoV-2 spike protein. Superposition of the X-ray structures of sybodies onto the trimeric spike protein cryo-EM map indicates that some sybodies may bind in both "up" and "down" configurations. Structural insights gained from these sybody structures were used to design novel highly potent biparatopic sybodies which bind the RBD tightly and neutralize SARS-CoV-2 and variants.