Development of high-binding potent nanobodies against SARS-CoV-2 spike protein

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Camelid single-domain antibodies, also known as nanobodies, can be readily isolated from naïve libraries for specific targets but often bind too weakly to their targets to be immediately useful. Laboratory-based genetic engineering methods to enhance their affinity, termed maturation, can deliver useful reagents for different areas of biology and potentially medicine. Using the receptor binding domain (RBD) of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein and a naïve library, we generated closely related nanobodies with micromolar to nanomolar binding affinities. By analyzing the structure–activity relationship using X-ray crystallography, cryoelectron microscopy, and biophysical methods, we observed that higher conformational entropy losses in the formation of the spike protein–nanobody complex are associated with tighter binding. To investigate this, we generated structural ensembles of the different complexes from electron microscopy maps and correlated the conformational fluctuations with binding affinity. This insight guided the engineering of a nanobody with improved affinity for the spike protein. This result offers a guiding principle for the rational maturation of nanobodies directed against the spike. High-binding potency nanobodies have been shown to be effective in animal models; thus, this technology could have application in future pandemics.

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