Identification of a high-affinity antagonistic nanobody against a challenging GPCR target

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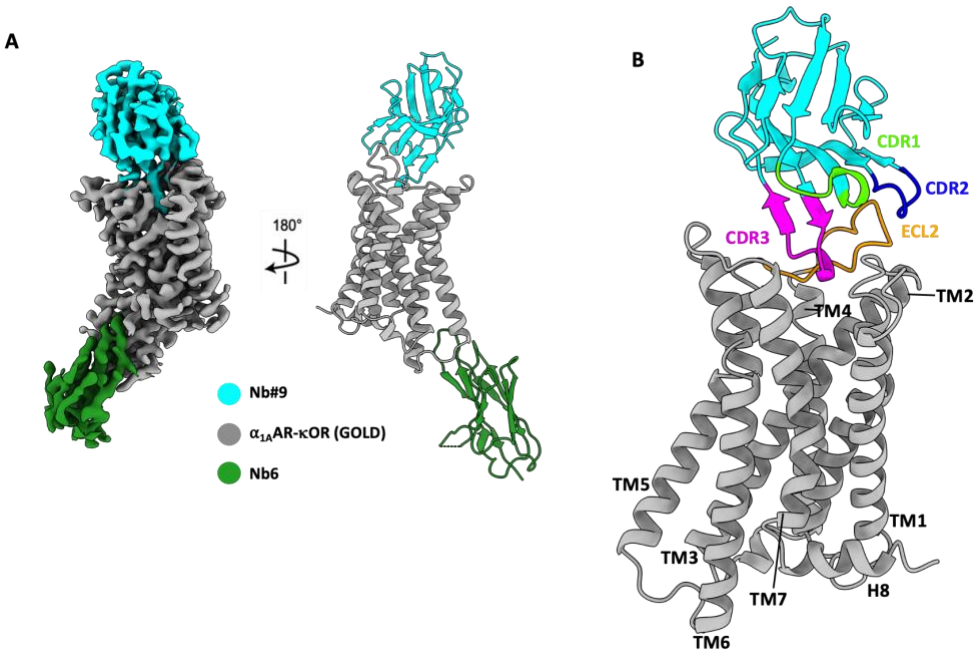
The α_1-adrenergic receptors (α_1-AR) are a family of Class A G protein-coupled receptors (GPCRs) that play an essential role in mediating the physiological function of noradrenaline through the sympathetic nervous system. The lack of subtype-selective tools for the three subtypes of the α_1-AR family (α_{1A}, α_{1B} and α_{1D}) has long been a bottleneck to studying and understanding their specific roles and distribution. Consequently, this lack of selective tools has hindered the validation of the therapeutic potential of the α_1-AR family. Further, the α_{1A}- and α_{1B}- subtypes are proposed to mediate opposing pathophysiological effects in cardiovascular and CNS indications. We have used combined structural biology and antibody discovery workflows to address this shortfall by identifying α_{1A}-adrenoceptor subtype-selective heavy-chain antibody fragments (nanobodies).

Here, we report approaches to isolate a panel of subtype-selective α_{1A}-adrenoceptor nanobodies that recognise therapeutically relevant epitopes on the extracellular solvent's accessible binding pocket. A representative nanobody (Nb#9) has been extensively pharmacologically characterised and demonstrates high-affinity and functional antagonism. Further, by solving the cryo-EM structure of Nb#9 bound to the α_{1A}-AR we have shown it achieves this functional effect by CDR3 engaging with the drug binding pocket and the other CDRs making extensive contacts across the extracellular loops of the receptor. These nanobodies represent the first subtype selective antibodies for an adrenergic receptor and will be valuable tools in studying the specific biology of α_{1A}-AR.



Architecture of the Nb#9-α_{1A}AR-κOR (GOLD)-Nb6 complex.