# High-resolution structure of soluble HIV-1 envelope (SOSIP) with bovine bNAbs using cryo-EM

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**Background:**
HIV, which causes AIDS, has led to 40 million deaths worldwide and currently affects 39 million people. Although antiretroviral therapy helps people live longer, HIV cannot be cured, and treatment is challenged by drug resistance and new variants. So far, RV144 is the only HIV vaccine to show partial success, but its effectiveness is moderate.
The HIV envelope (Env) protein is the primary target for broadly neutralising antibodies (bNAbs), but HIV evades immune detection via sequence mutations, heavy glycosylation, and conformational changes. Additionally, survivors of chronic HIV infection generate bNAbs targeting state-1 (closed conformation) of the Env protein. We have developed bovine bNAbs targeting these conformations through sequential immunisations, which are being explored to inform vaccine design.
**Methods:**
AD8 SOSIP was expressed in Expi293F cells and purified using affinity and size-exclusion chromatography. Bovine bNAbs (MEL-1872 and MEL-9351) and their Fab fragments underwent similar purification. Binding kinetics were assessed by biolayer interferometry (BLI). For structural analysis, AD8 SOSIP was incubated with Fab fragments, and the resulting complexes were purified and vitrified for cryo-electron microscopy (cryo-EM). Single-particle analysis enabled reconstruction of the complexes and characterisation of antibody–epitope interactions.
**Results:**
Cryo-EM data were collected for AD8 SOSIP conjugated with Fab fragments of MEL-1872 bovine bNAbs. The reconstructed cryo-EM map of AD8 SOSIP–MEL-1872 Fab complexes achieved a global resolution of 2.8 Å at an FSC cutoff of 0.143. The resolved structure revealed atomic details of MEL-1872 epitopic-paratopic interactions. AD8 SOSIP interacted with MEL-1872 CDRH3-harbouring residues via its CD4 binding loop, D loop, and V5 loop residues through hydrogen bonding and salt bridges. Additionally, binding kinetics between

MEL-1872 and AD8 SOSIP were determined using BLI. The dissociation constant (KD) was found to be 36 nM.

**Conclusion:**

MEL-1872 is a bovine bNAb targeting the CD4-binding site of the HIV-1 envelope protein, with potential applicability for informing phylaxis and the development of next-generation HIV vaccines.