

Abstract

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 of broadly neutralising antibodies (bNAbs), but HIV evades immune detection through sequence mutations, extensive 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.

Objectives

High-resolution structure of soluble HIV-1 Envelope (SOSIP) with bovine bNAbs:

- Structural elucidation of soluble Env (SOSIP) of HIV-1 in complex with bovine bNAbs containing a long CDHR3 region.

Method

- MEL-1872: affinity-purified using Protein G resin followed by SEC purification
- MEL-1872 Fab: generated by papain digestion of MEL-1872 antibody
- AD8 SOSIP: affinity-purified using 2G12-conjugated Sepharose 4B resin followed by SEC purification
- Negative staining of AD8 SOSIP
- Cryo-EM of AD8 SOSIP conjugated with Mel-1872 Fab

Introduction

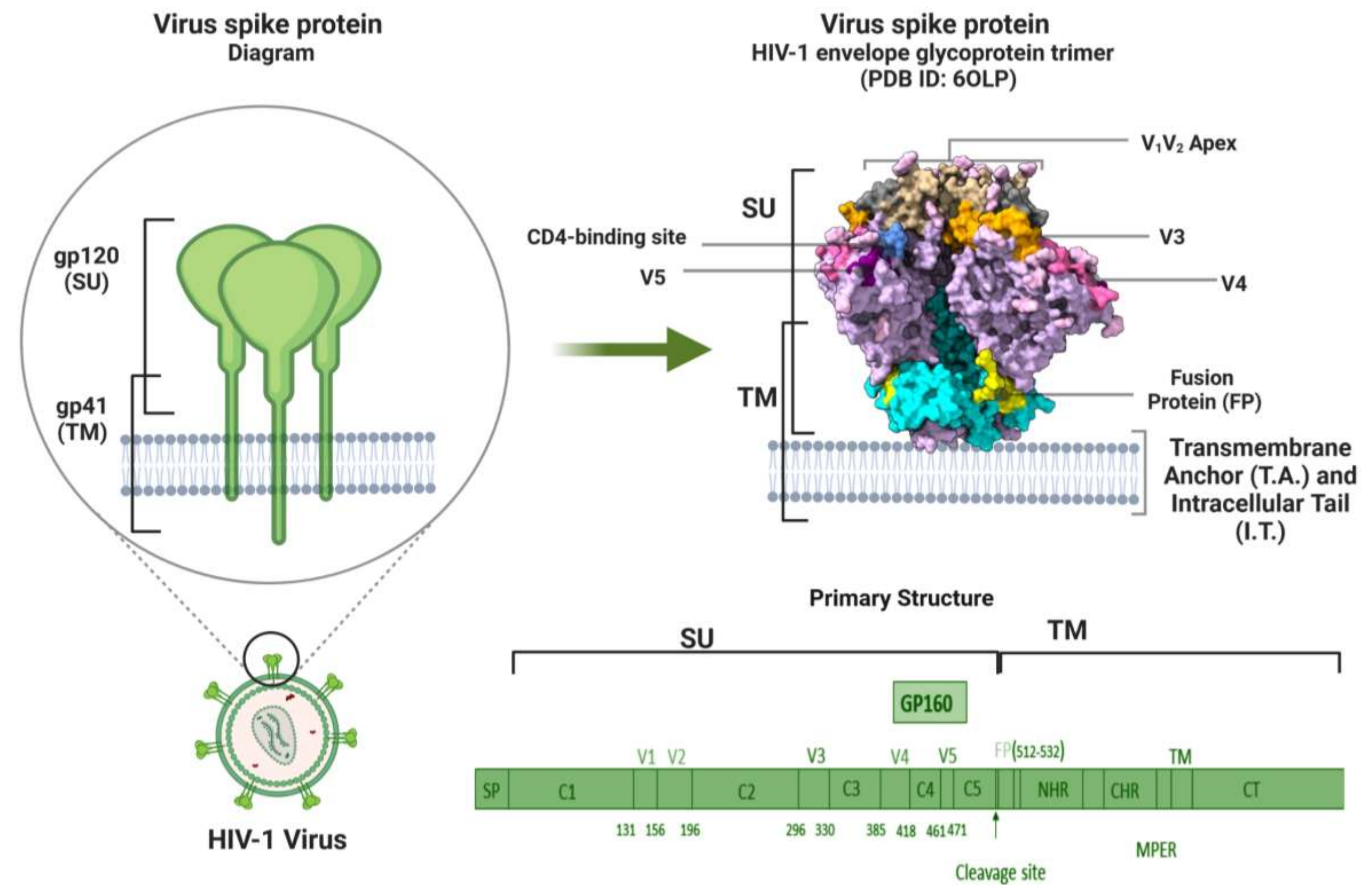
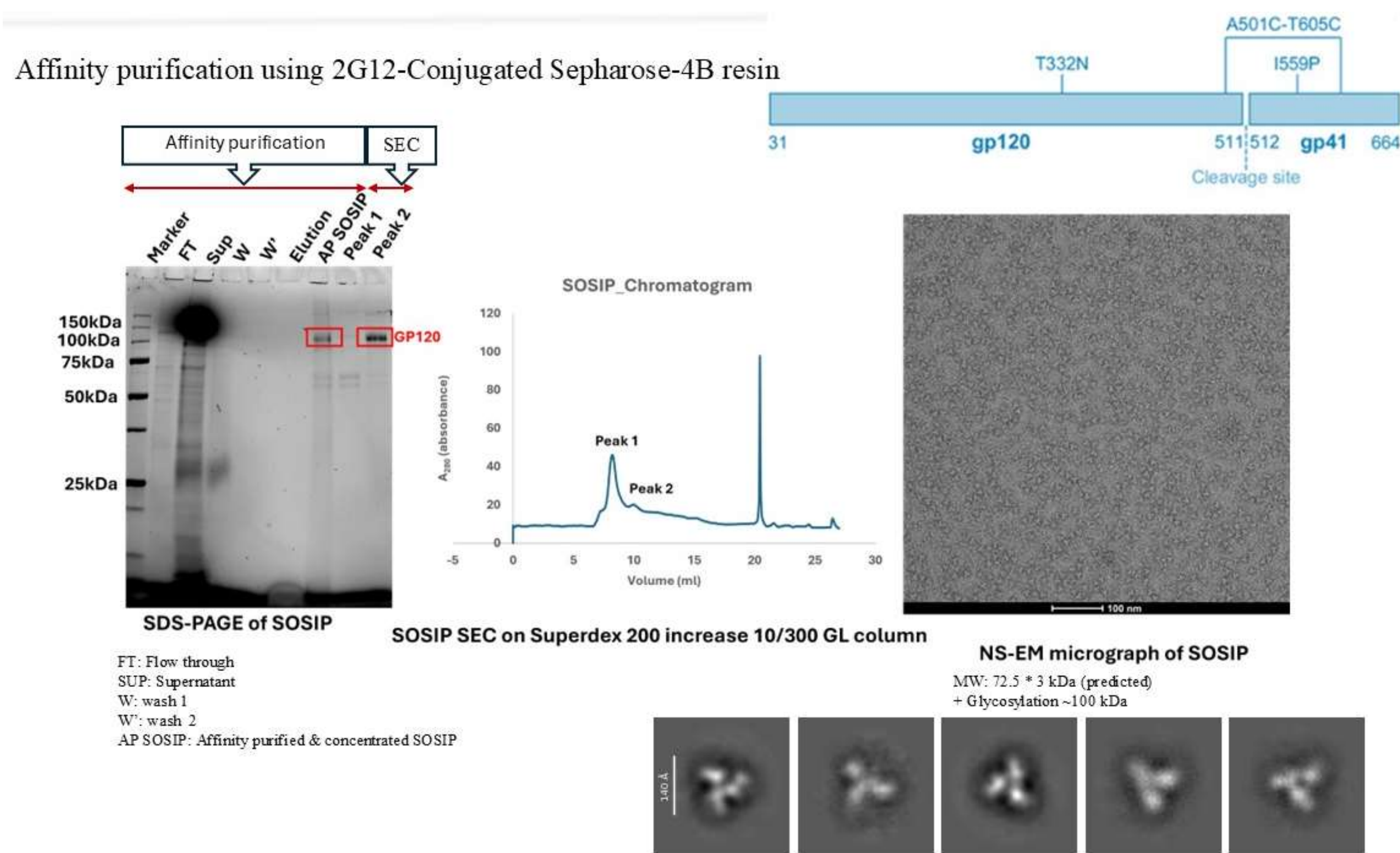


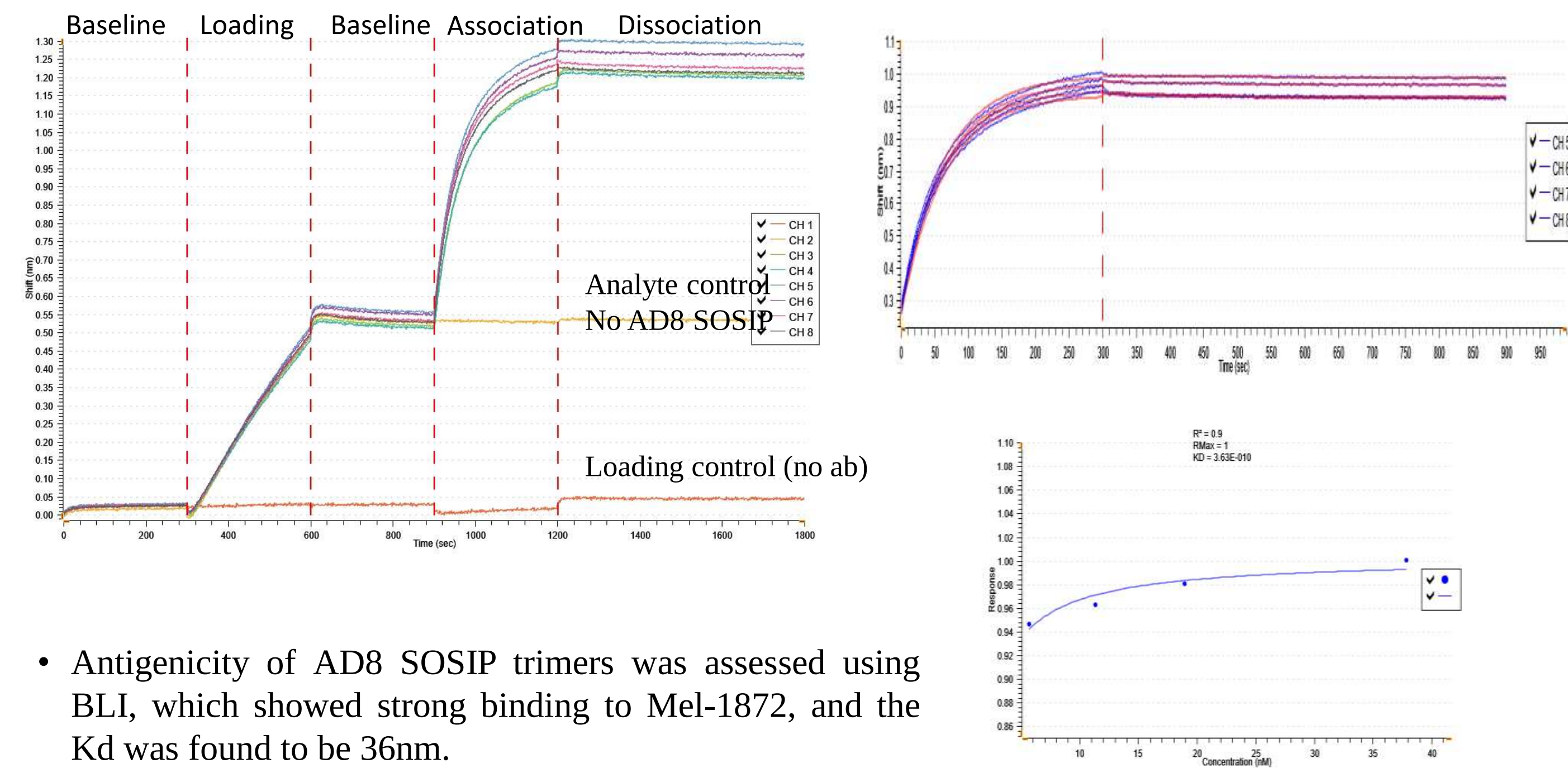
Figure 1. The HIV-1 Env is the only protein present on the surface of the HIV-1 virion. It has a surface unit (gp120) and a transmembrane unit (gp41). The schematic diagram shows the HIV-1 virion with its Env protein, depicting conserved and variable regions from N-terminal to C-terminal and bAb targeting sites on the trimeric structure of the HIV-1 Env heterodimer.

Results

AD8 SOSIP purification using 2G12-Conjugated Sepharose 4B resin and SEC



Bi-layer Interferometry (BLI) for binding analysis of MEL-1872 antibody with AD8 SOSIP



- Antigenicity of AD8 SOSIP trimers was assessed using BLI, which showed strong binding to Mel-1872, and the Kd was found to be 36nm.

Cryo-EM structure determination of AD8 SOSIP with Mel-1872 Fab

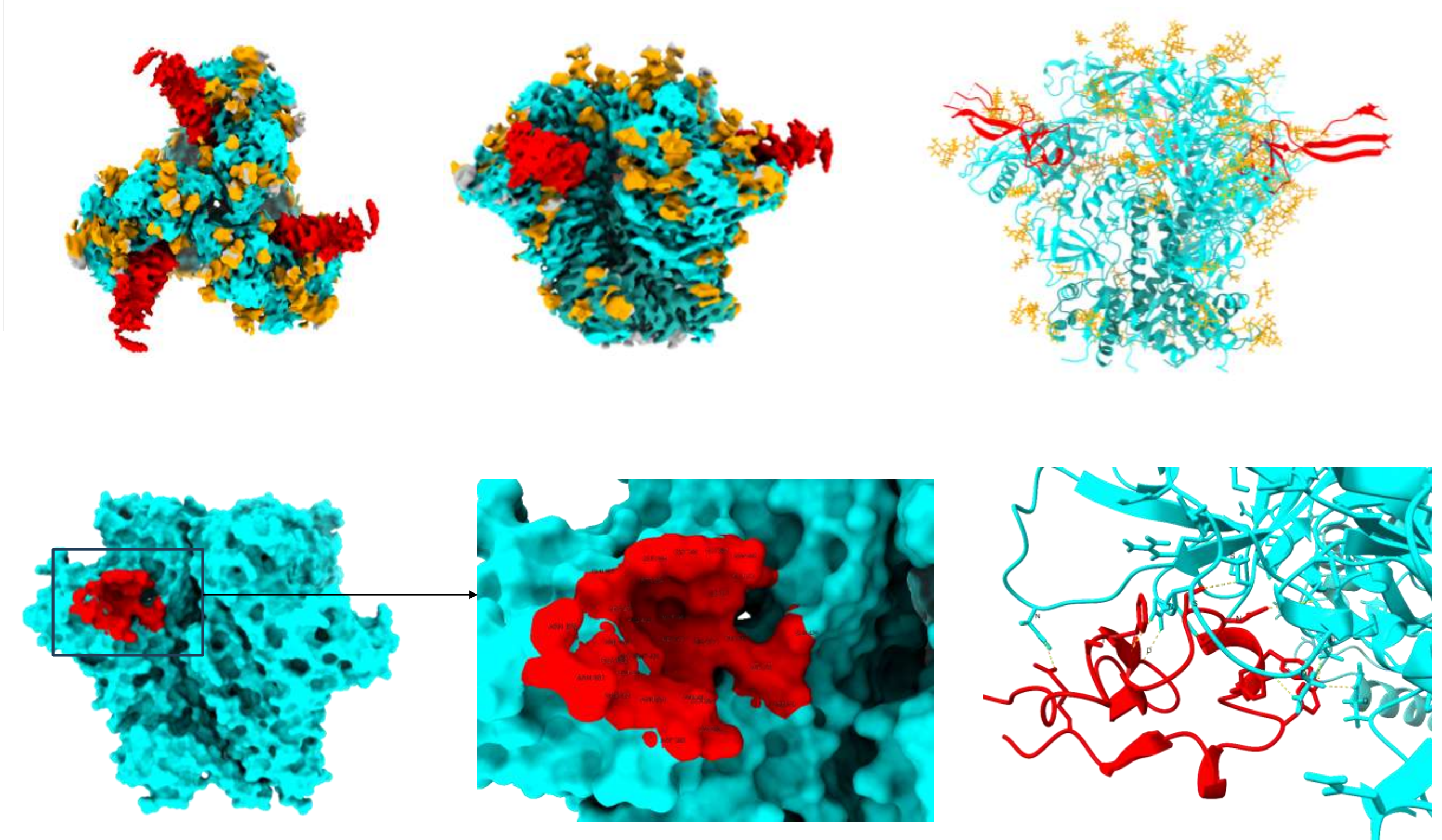
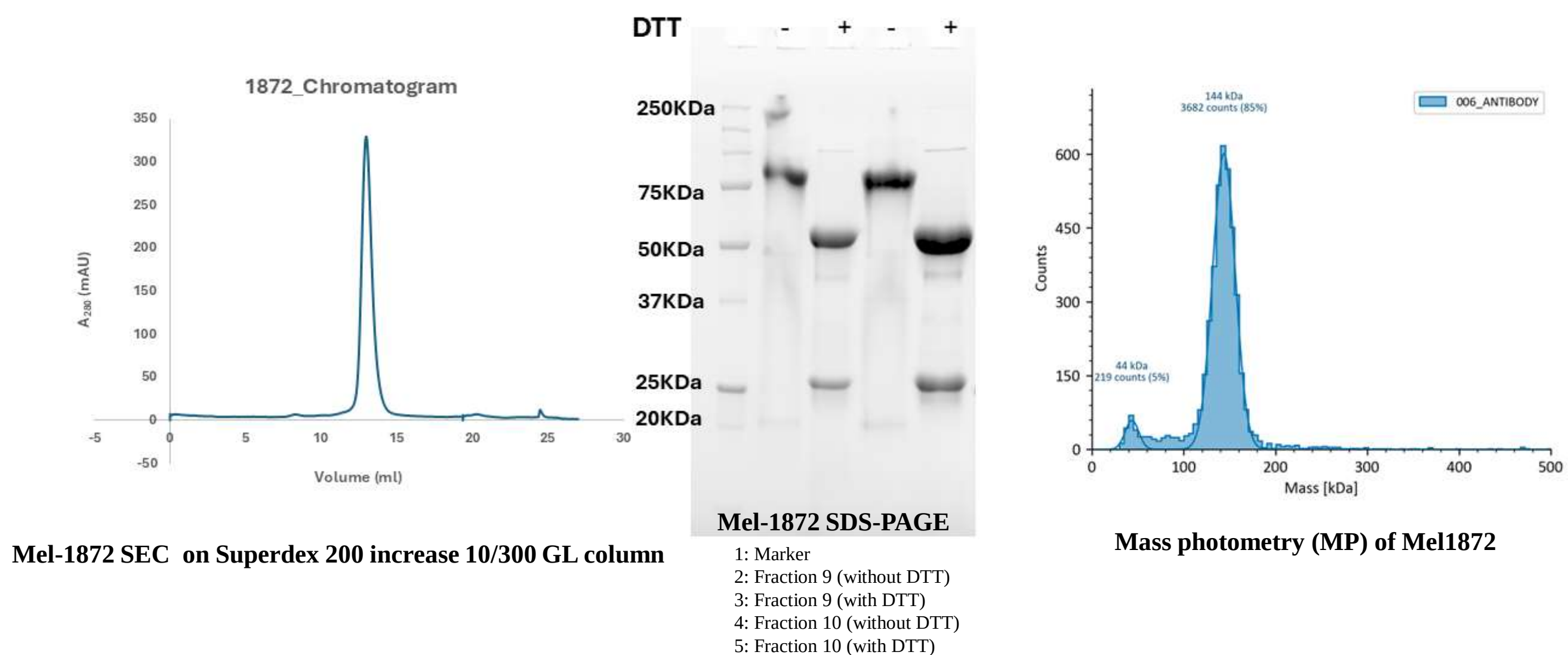


Figure 3. Top and side views of the cryo-EM map of the HIV Env trimer in complex with MEL-1872 Fab. The model was built according to the cryo-EM map. The MEL-1872 footprint on the HIV-1 Env trimer is shown in red, and the residues of the AD8 SOSIP trimer are marked in black. The H-binding residues of MEL-1872 with the AD8 SOSIP protomer were labelled, and the H-Bonds among them were shown in yellow.

- The NS-EM (negative-stained electron microscopy) and MP (mass photometry) of purified AD8 SOSIP showed that it existed mainly as trimers.

Mel-1872 (bovine bNAb) purification using protein G resin and SEC



- The SEC and MP of purified AD8 SOSIP showed the homogeneity of purified MEL-1872 Antibody

Conclusion

- MEL-1872 is a bovine bNAb targeting the CD4-binding site of the HIV-1 envelope protein.
- MEL-1872 CDRH3 contains a knob and stalk, which are alone stable and can be used as a prophylaxis

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