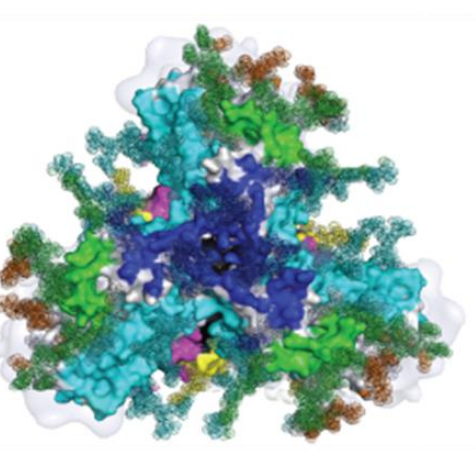


# Determination of the First High-Resolution Structure of HIV-1 Envelope in Complex with Macaque CD4

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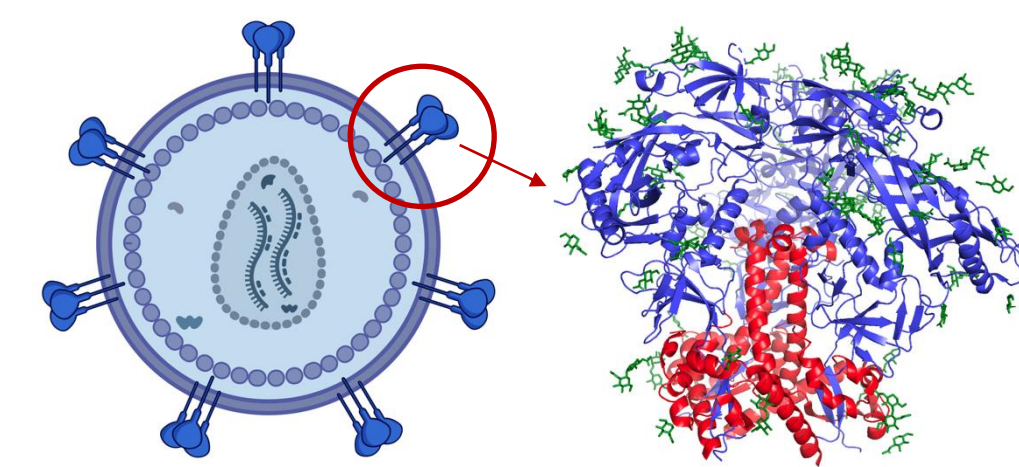
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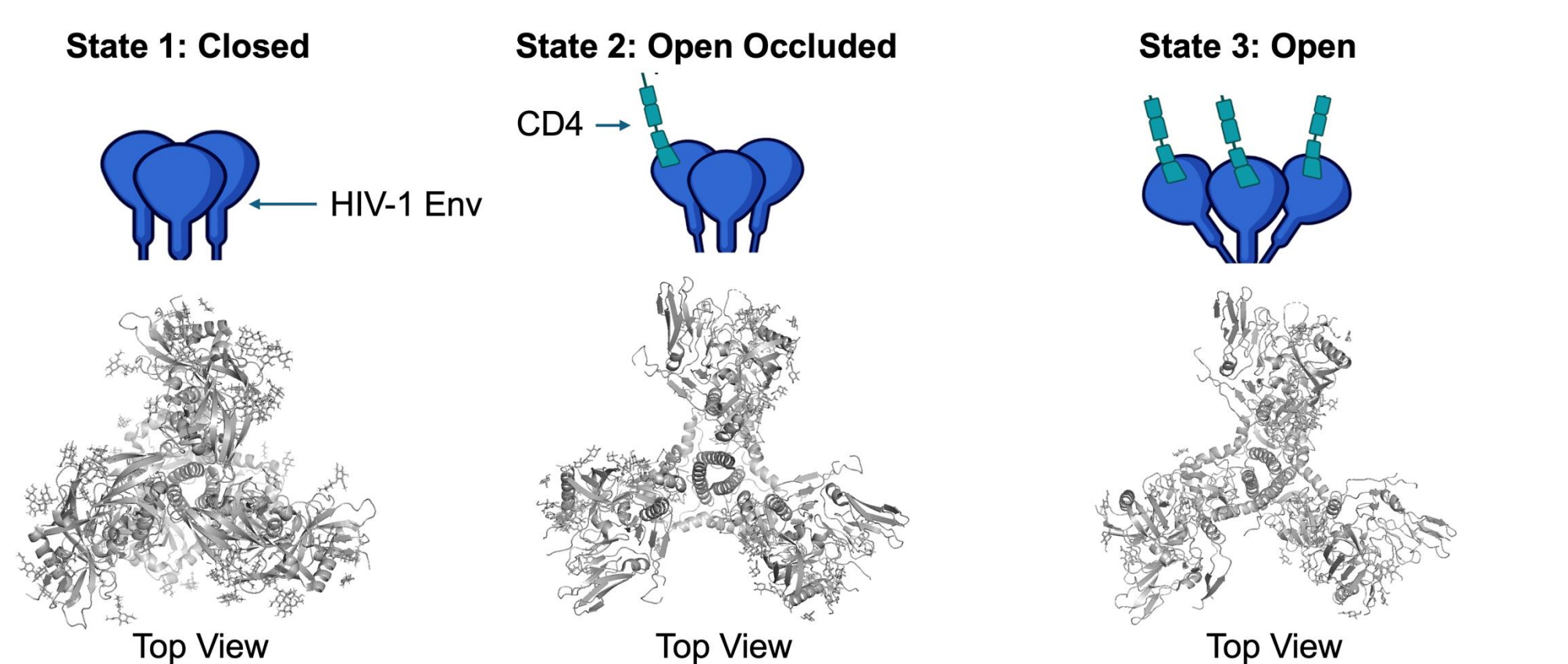
## Introduction

### Human Immunodeficiency Virus 1 (HIV-1) is a global health burden

- 1.3 million new cases and 630,000 deaths in 2024
- No vaccines or cures
- Envelope (Env) glycoprotein trimer is the only viral antigen expressed on the virus

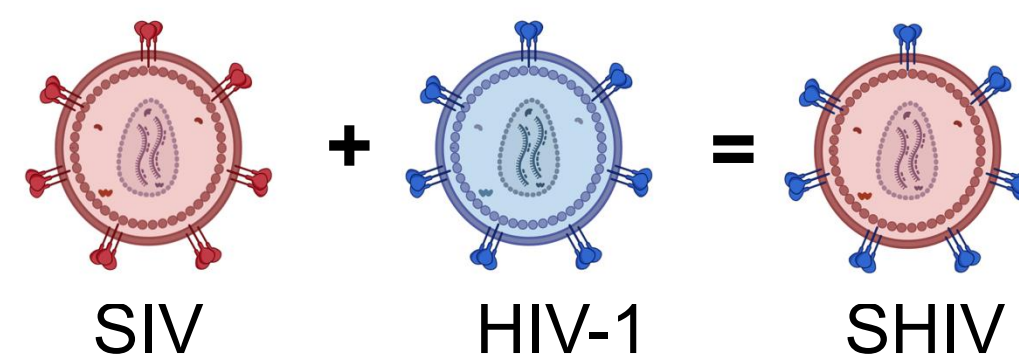


### CD4-induced conformational changes in Env



### SHIVs: surrogate challenge viruses encode HIV-1 Env to model HIV-1 infection in rhesus macaques

- Macaque models are critical for preclinical evaluation of therapeutics and vaccines
- HIV-1 does not cause persistent infection in macaques
- SHIVs – chimeric viruses encoding HIV-1 Env and SIV accessory genes



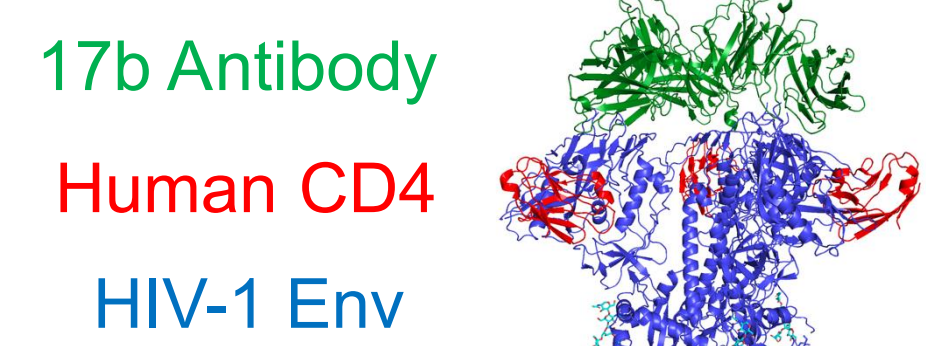
**Major Issue:** SHIVs require alteration of HIV-1 Env to efficiently utilize suboptimum macaque CD4 receptor for viral entry and SHIV replication.

**Solution:** Rationally design Envs with minimal changes by comprehensively analyzing structures of HIV-1 Env in complex with macaque CD4

### HIV/AIDS field is lacking a structure of HIV-1 Env with macaque CD4

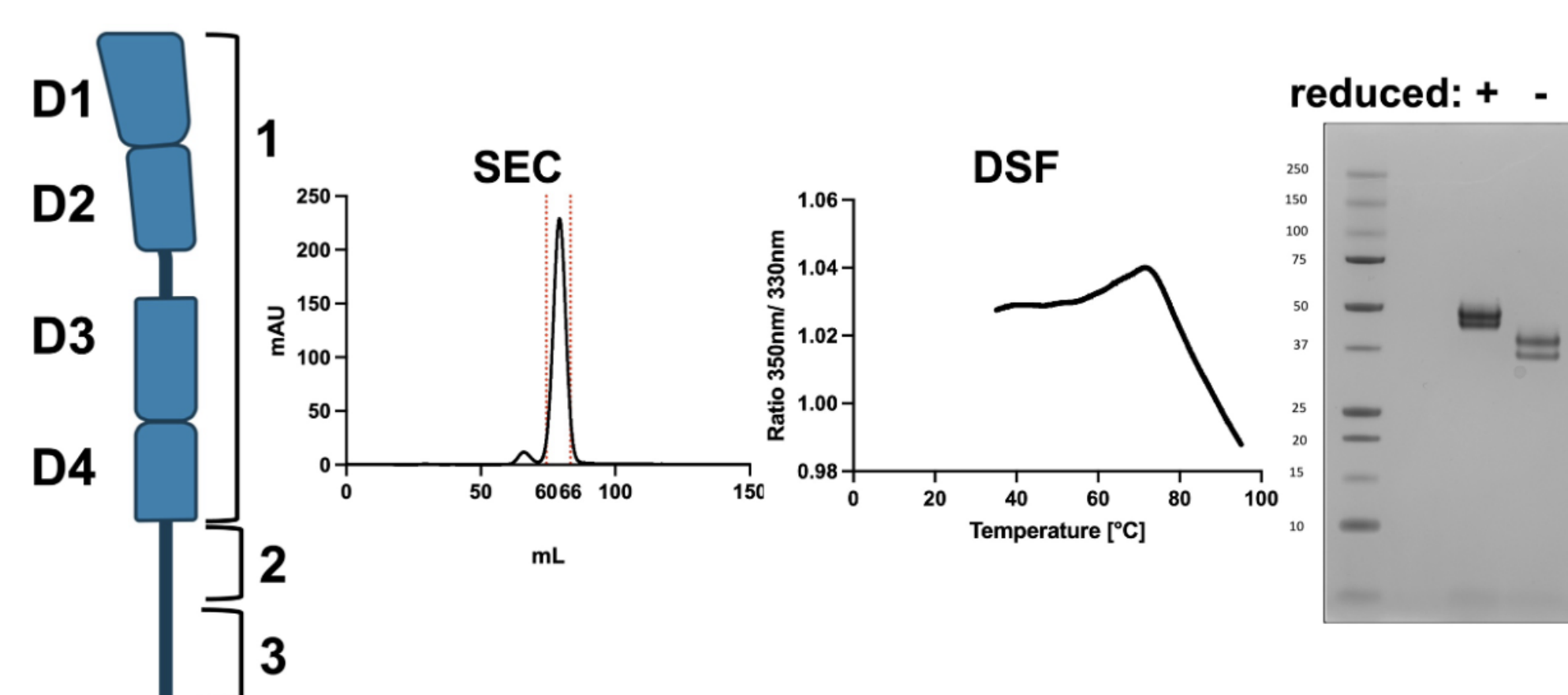
Currently, all Env changes **must** be evaluated using macaque challenge studies, which are *extremely* labor-intensive and expensive.

Macaque CD4 protein would enable structural evaluation of Env-macaque CD4 binding to identify residues critical for CD4 binding and SHIV replication.



HIV-1 Env + human CD4    HIV-1 Env + macaque CD4

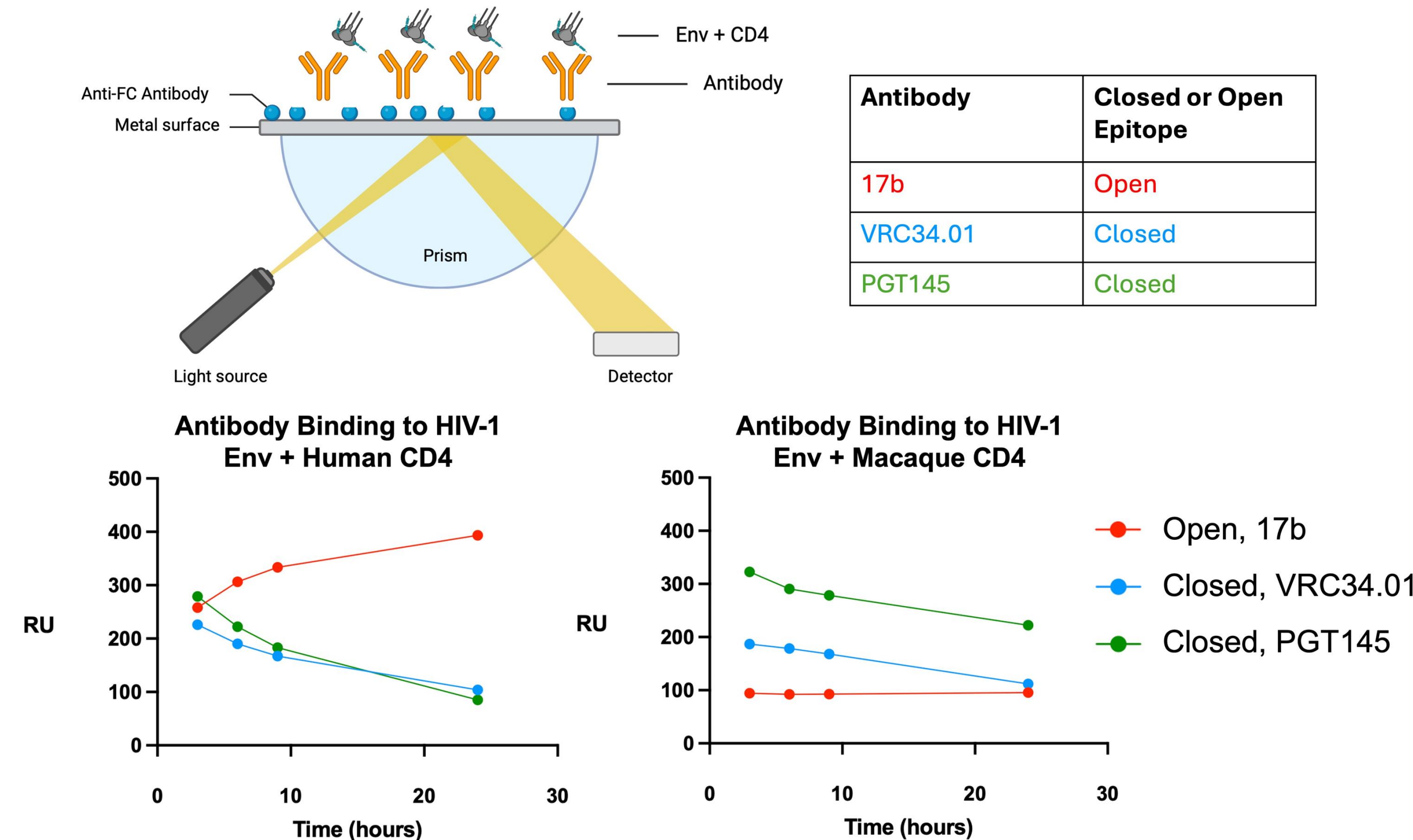
## Purification of soluble 4 domain macaque CD4



**Figure 1. Purification of soluble macaque CD4.** Schematic of CD4 showing: 1) D1-to-D4 extracellular, 2) transmembrane, and 3) cytoplasmic domains. Size exclusion chromatogram (SEC), differential scanning fluorimetry (DSF), and SDS-Page of purified soluble macaque CD4.

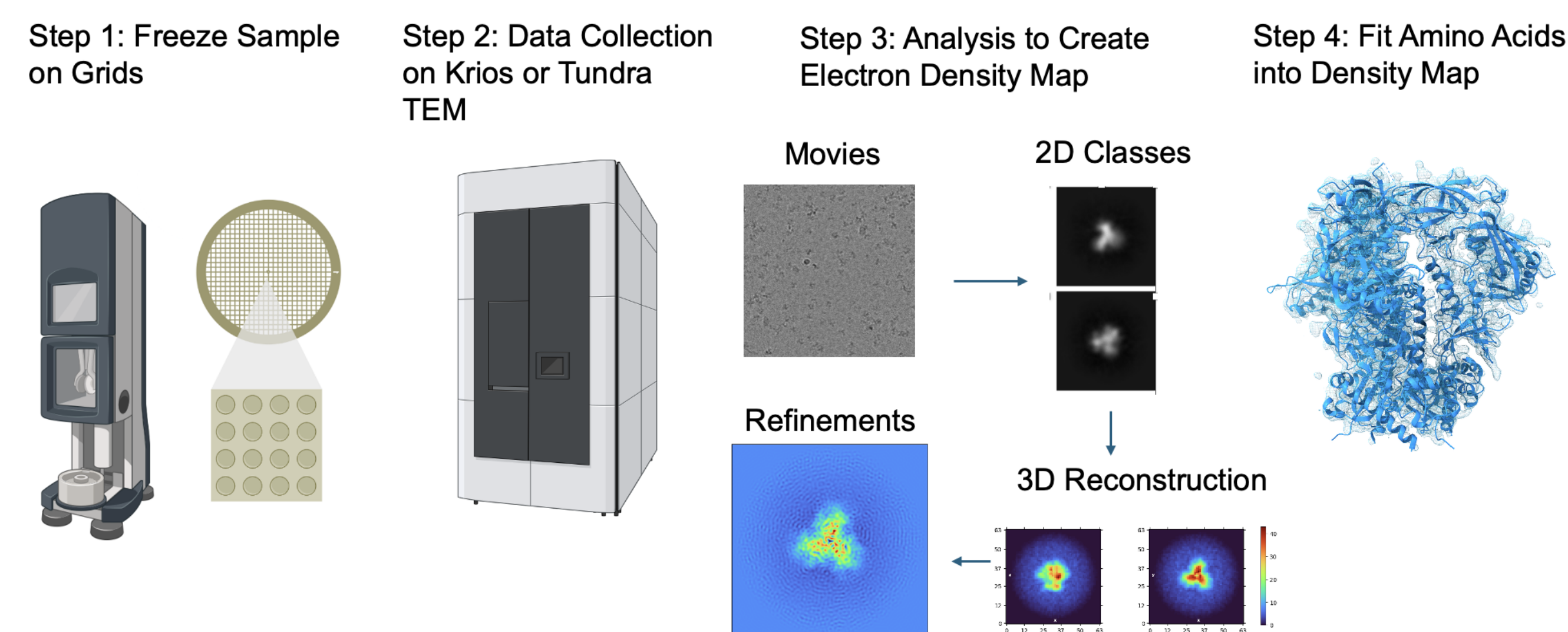
## Env-CD4 binding assay

Goal: To determine whether macaque CD4 induces similar conformational changes in Env as human CD4



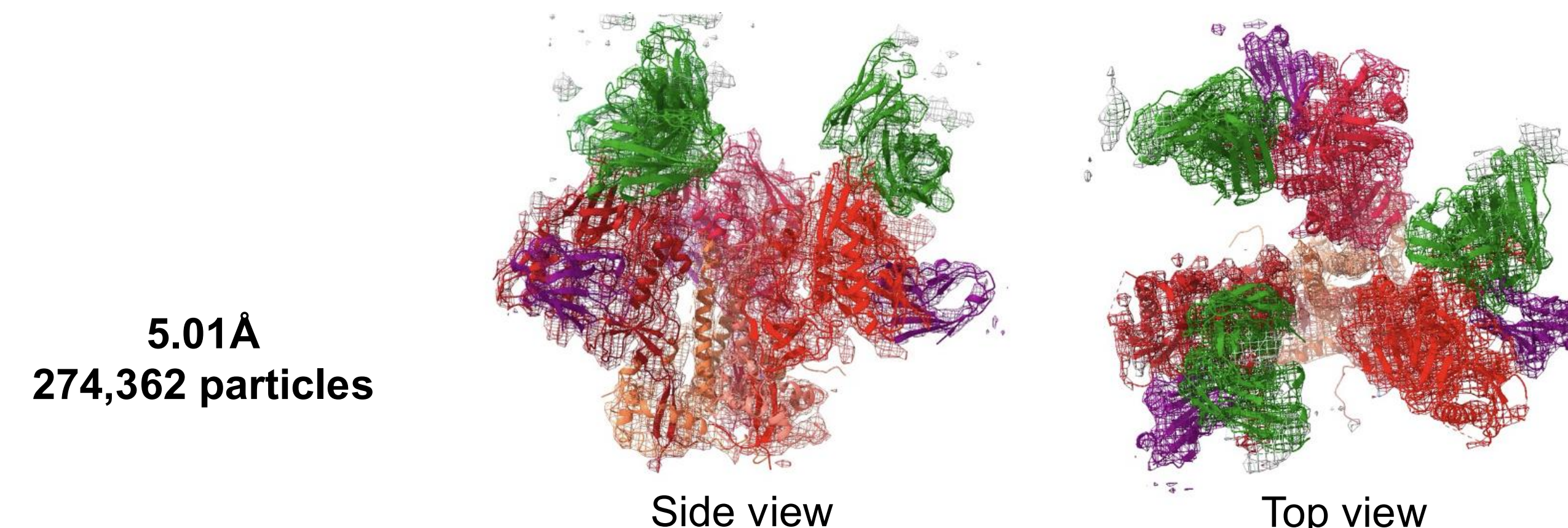
**Figure 2. Surface plasmon resonance (SPR) assay monitoring Env openness.** Env was incubated at 25°C with either soluble human CD4 or macaque CD4. At different time-points after incubation, binding to 17b, VRC34.01, and PGT145 was measured.

## Structural determination utilizing cryo-EM



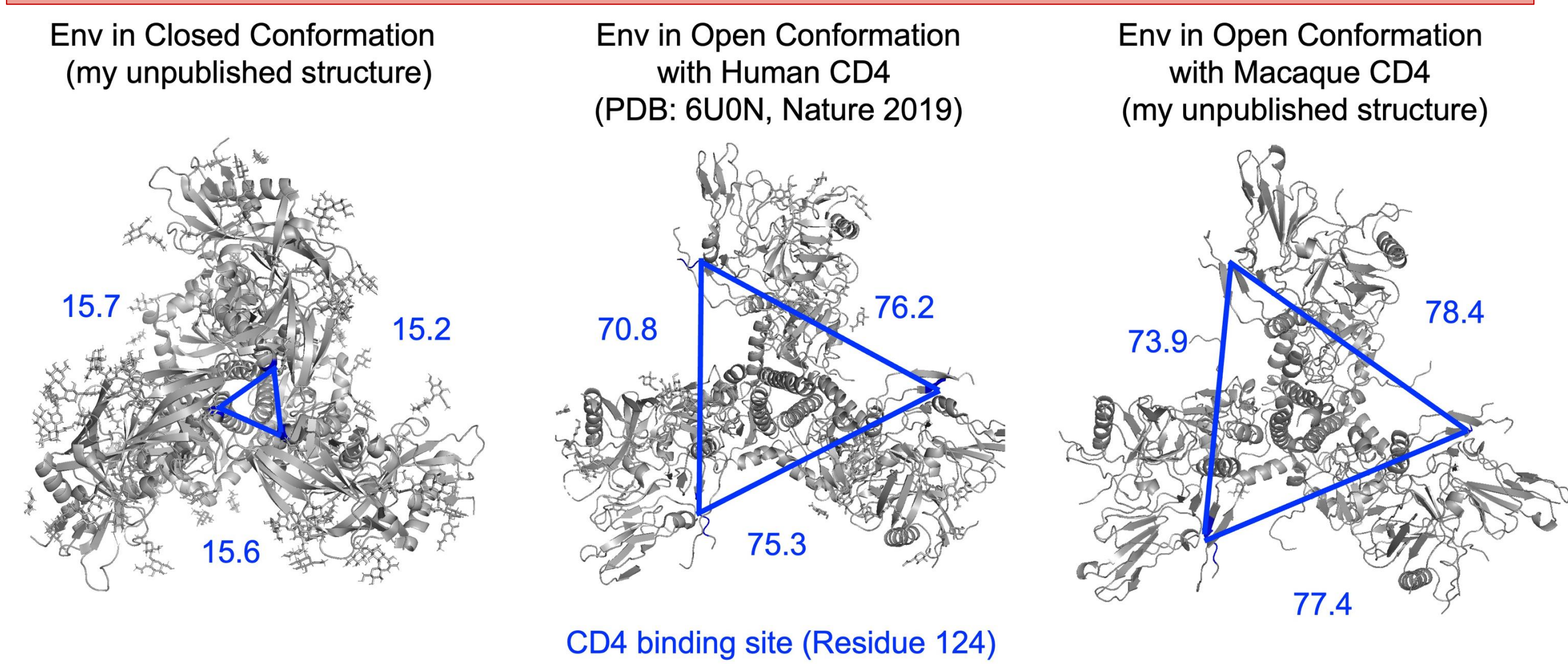
**Figure 3. Cryo-electron microscopy workflow used for structural determination.** Samples are vitrified on carbon grids, imaged during data collection, and processed utilizing cryoSPARC for 3D reconstruction, followed by model building with fitting amino acids in the density map.

## Solved structure of HIV-1 Env in complex with macaque CD4 and 17b



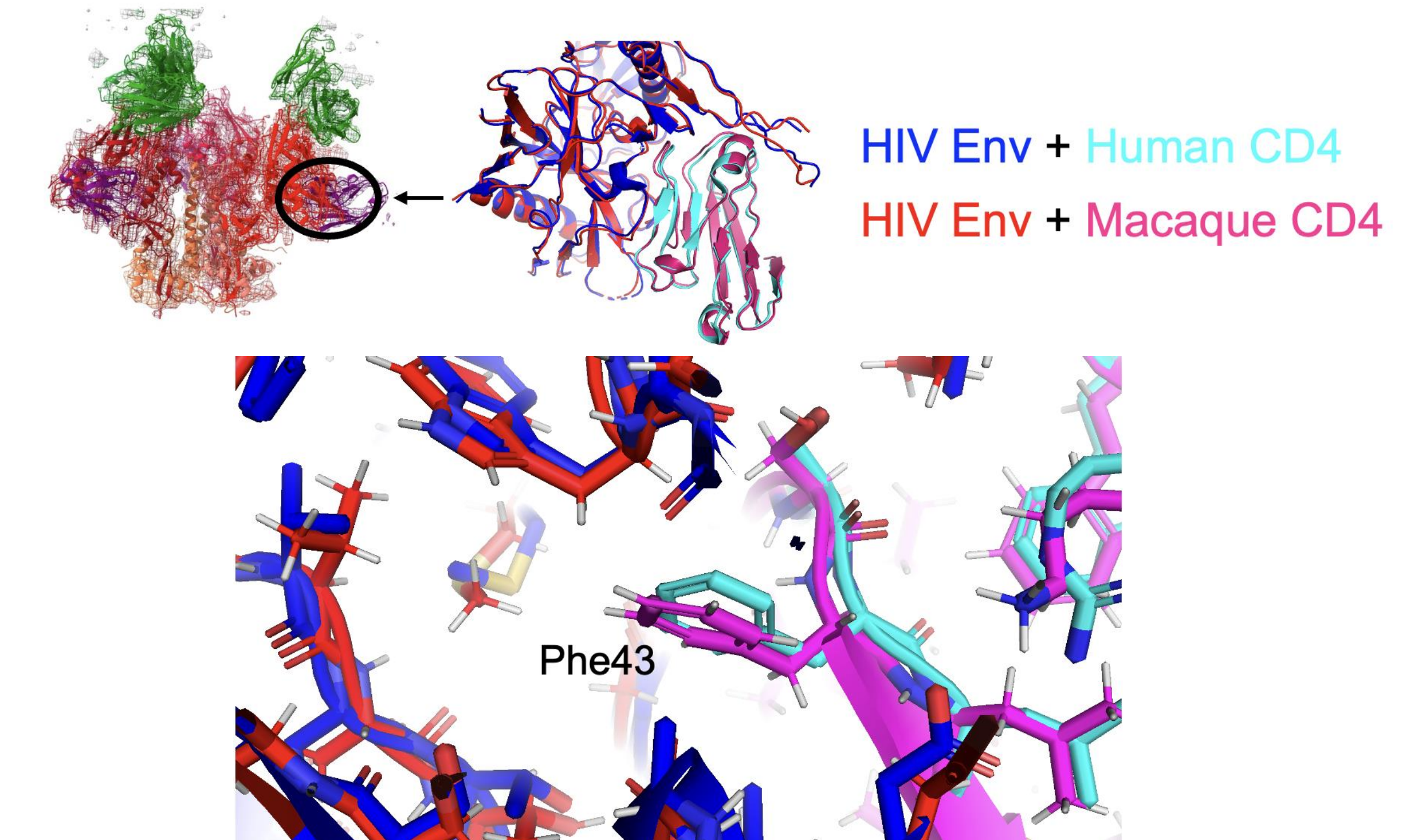
**Figure 4. Structure of BG505 SOSIP Env trimer bound to macaque CD4 and 17b Fab.** Env protomers and domains are shown in distinct colors. Env was incubated with macaque CD4 for 24 hours at 25°C, followed by a 30-minute incubation with 17b Fab. Samples were vitrified on R1.2/1.3 carbon grids and data collected on a ThermoFisher Scientific Titan Krios microscope. Data was processed in cryoSPARC, and structural visualization and model building were performed using ChimeraX, PyMOL, and Coot.

## HIV-1 Env bound to macaque CD4 reaches fully open state



**Figure 5. Extent of Env openness measured as the distances between residue 124 in the CD4-binding site.** Distances (Å) between residue 124 in each Env protomer were measured in PyMOL to quantify the extent of Env opening.

## HIV-1 Env-CD4 binding site does not show large changes between human vs. macaque CD4



**Figure 6. Overlay of HIV Env bound to human CD4 and macaque CD4.** Overlay of these complexes highlights structural similarities between the two CD4-bound states. HIV Env + human CD4 structure corresponds to PDB ID 9D90. Phe-43 binding cavity of overlay is shown to demonstrate similarity in two structures.

## Conclusions

- Obtained first-ever structure of HIV-1 Env in complex with macaque CD4
- HIV-1 Env bound to macaque CD4 reaches a fully open state.
- Differences in conformational-specific antibody binding to Env-CD4 indicate differences in CD4 binding kinetics for human versus macaque CD4
- High resolution Cryo-EM structure of Env-Macaque CD4 will be critical for rationally designing SHIVs with engineered changes in HIV-1 Env that can use suboptimal macaque CD4 for viral entry**
- Limitations:
  - Utilized stabilized, soluble version of HIV-1 Env trimer
  - HIV-1 subtype-specific differences may be observed for Env-CD4 interaction

## Acknowledgements

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