Structural and Dynamic Characterization of mCD4-binding HIV-1 Envelope Glycoproteins

Rachel M. Kinzelman[1,2], Thaddeus M. Davenport[1,2], Julie M. Overbaugh[1,3], Kelly K. Lee[1,2]

[1] Pathobiology, University of Washington, Seattle, WA, USA.
[2] Medicinal Chemistry, University of Washington, Seattle, WA, USA.
[3] Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA, USA.

Introduction

SHIVs are valuable tools that combine SIV and HIV genes in order to replicate within non-human primates. An ideal SHIV would be capable of infecting non-human primates while retaining antigenicity associated with a wild-type HIV. Since HIV-1 envelope glycoprotein (Env) confers cell tropism, it is necessary to utilize an Env that can infect non-human primate CD4+ T cells. Mutations have been found that allow Env to use macaque CD4 receptor (mCD4) for infection. Two of these mutations, A204E[1] and S375W[2], enable Env isolates to utilize mCD4, but differ in their antigenicity. As Env is a dynamic protein that can shift between closed/unbound and open/CD4-bound states, this suggests that there may be differences in the mechanisms that allow the two mutations to bind mCD4. The mutations A204E, located adjacent to the bridging sheet, and S375W, in the CD4-binding site, were introduced into BG505 SOSIP.664 Env constructs and the trimers were purified using our epitope-independent method[3]. Using a combination of structural and biophysical approaches, we aim to correlate Env structure with its interaction with mCD4 and neutralizing antibodies (nAbs).

Hydrogen/Deuterium Exchange Mass Spectrometry suggests no difference in dynamics

Previous HDX-MS experiments in the Lee lab have shown that introducing the A204E mutation into BG505 allowed the SOSIP to sample between the prefusion (closed) and activated (open) conformations. Repeats of the experiment resulted in differing results. Instead, peptides in A204E that exhibited conformational sampling previously were observed to take up deuterium similarly to WT BG505 **(Fig 1)**.

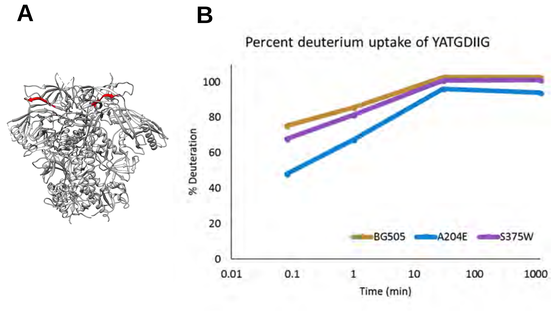


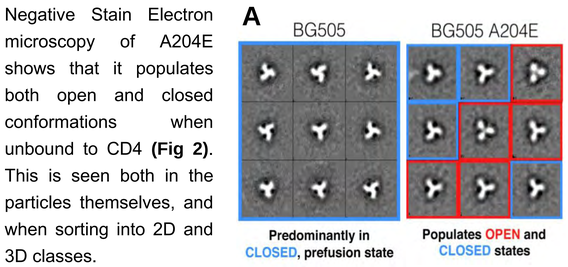
Fig 1. Similar exchange profiles suggest no difference in conformation between A204E, S375W, or BG505. **A.** Peptide YATGDIIG in Env (red), part of V3 loop that is exposed in the open state. PDB 4ZMJ **B.** Deuterium uptake in YATGDIIG in A204E, S375W, and BG505 WT.

Acknowledgments

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Electron Microscopy indicates that A204E adopts both open and closed conformations

Negative Stain Electron microscopy of A204E shows that it populates both open and closed conformations when unbound to CD4 **(Fig 2)**. This is seen both in the particles themselves, and when sorting into 2D and 3D classes.



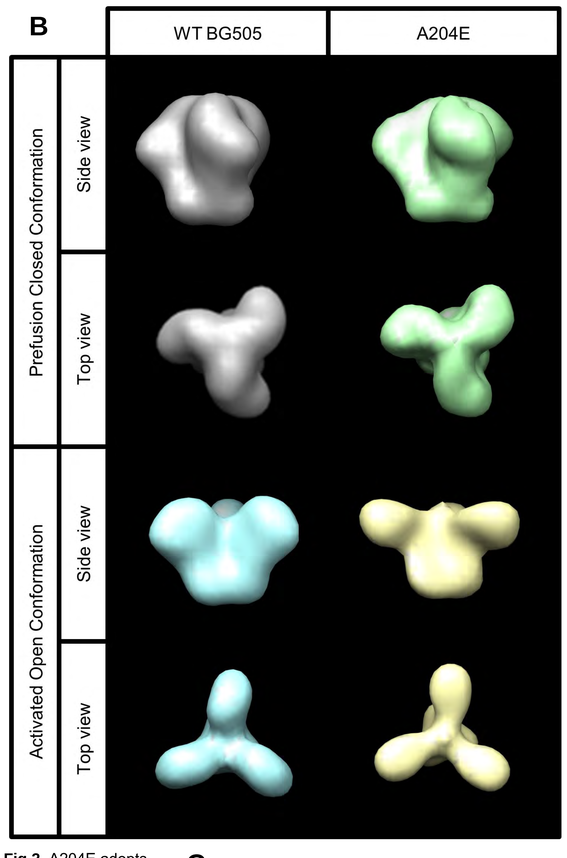
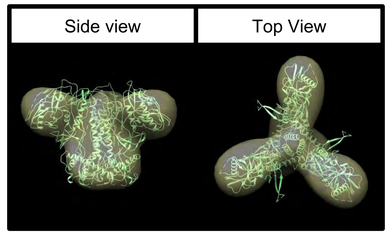


Fig 2. A204E adopts both open and closed structures. **A.** Negative stain particles of A204E. **B.** Model of open and closed conformations of A204E. PDB: 4ZMJ, 5THR. Closed: 19.5Å, 7153 particles. Open: 27Å, 2814 particles. **C.** Open model of A204E docked with Env open conformation crystal structure. PDB: 5THR.



A204E antigenicity is altered compared to S375W and BG505 Wild Type

Previous research in the Overbaugh lab found that antibodies specific to quaternary epitopes of Env had either increased or reduced neutralization potential in mCD4 adapted mutants[4]. To investigate further, Biolayer interferometry (BLI) using antibodies that target epitopes found in both the open and closed conformations of Env was performed on the two mutations and WT BG505 **(Table 1)**.

Antibody	Epitope	SOSIP conformation needed
17b	Coreceptor binding site	Open
PGT145	Trimer apex	Closed
VRC01	CD4 binding site	Either

Table 1: Antibody specificity for Env.

A204E has altered antigenicity compared to BG505 WT and S375W **(Fig 3)**.

- A204E was able to bind both 17b and PGT145.
- S375W cannot bind 17b.
- BG505 cannot bind 17b.

This indicates that A204E is able to sample the open state, whereas S375W and BG505 remain in the closed state.

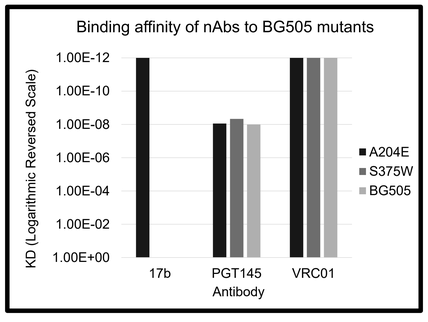


Fig 3. A204E binds 17b and PGT145, suggesting both open and closed conformations.

Structure of A204E-17b Fab Complex

Negative stain electron microscopy of A204E complexed with 17b fab confirmed that the mutant is able to bind 17b in the open conformation. Additional images will be taken to increase resolution and with varying amounts of 17b Fab.

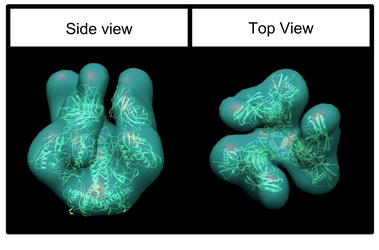
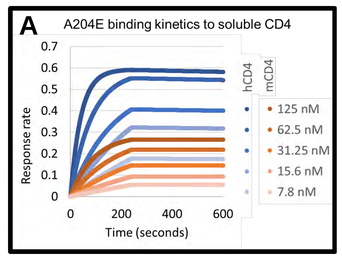


Fig 4. Negative stain EM model of A204E-17b Fab complex docked with Env-17b crystal structure. PDB: 5THR. Resolution: ~24Å, 3841 particles.

A204E and S375W bind more readily to human CD4 than to rhesus macaque CD4

BLI of soluble CD4 with the two mutants showed that, despite the mutations that allow for mCD4-binding, the Envs retain a higher initial binding affinity for human CD4 (hCD4) **(Fig 5)**. This was not unexpected, as these mutations should not change the affinity for hCD4 and would likely increase it by stabilizing the CD4-bound conformation once bound to CD4.



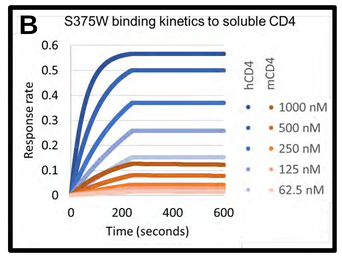


Fig 5. Binding kinetics of SOSIPs with soluble CD4. **A.** A204E had a K_{ON} of 1.9E5 with hCD4 and 8.7E4 with mCD4. **B.** S375W had a K_{ON} of 1.9E4 with hCD4 and 5.7E3 with mCD4.

Compared to A204E, S375W has a lower affinity for mCD4

A204E has a stronger affinity for mCD4 than S375W does. The association rate is higher and once bound, it has a lower dissociation rate **(Table 2)**. This is likely because A204E more easily samples the open, CD4-bound conformation, whereas S375W retains a more closed conformation until binding CD4.

Env Mutation	K_{ON} (1/Ms)	K_{OFF} (1/s)	K_D (M)
A204E	8.74E+04	<1.0E-07	<1.0E-12
S375W	5.70E+03	7.60E-05	1.33E-08

Table 2: Affinity constants of Env mutations for mCD4.

Conclusions

S375W is able to retain a native conformation when unbound to CD4, yet is able to bind to mCD4. The mutation allows for increased binding to hCD4 and mCD4, and most likely stabilizes the CD4-bound conformation once bound to CD4. This is in contrast to A204E which can sample the open conformation when unbound to CD4. A204E's higher affinity for hCD4 and mCD4 is likely because HIV-Envs that have a more open conformation have been found to more readily bind to CD4.

References

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