

Conformational Changes in HIV-1 Env Trimer Induced by a Single CD4 as Revealed by Cryo-EM

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The HIV-1 Envelope (Env), a heterotrimeric glycoprotein on the virus surface, mediates attachment and entry into host cells, and is also the sole target for neutralizing antibodies. HIV-1 Env has a number of defenses that protect its most vulnerable regions from antibody recognition, which include glycan shielding, variable loops, and conformational masking. These defenses pose a challenge to both vaccine and therapeutics development [1]. The functional requirement of entry, however, necessitates conservation of receptor binding sites, as well as their exposure at specific points on the entry pathway (Figure 1). An understanding of the interactions of HIV-1 with cellular receptors, and of its mechanism of entry, is therefore, intricately tied with efforts to develop an effective vaccine or therapeutic.

The binding site on Env for its primary receptor CD4 is a known target of naturally elicited broadly neutralizing antibodies on HIV-1 Env, and has been extensively targeted by vaccine and drug design efforts. Until recently, the structural information available for CD4 binding was limited to structures of CD4-bound monomeric gp120 obtained by x-ray crystallography [2]. Structural determination of the initial contact on the HIV-1 Env trimer was a challenge because CD4 binding triggered conformational changes and opening of the trimer. Here we describe the initial contact of HIV-1 Env with its primary receptor CD4, captured using a 2-disulfide stabilized soluble, Env construct (DS-SOSIP) [3], and visualized at 6.8 Å using cryo-electron microscopy [4]. The cryo-EM map revealed a single CD4 molecule wedged between two gp120 protomers of a closed HIV- Env trimer. This structure expands the previously defined footprint of the functional CD4 binding site and defines initial CD4-induced structural changes of the Env trimer. Docking onto the pre-fusion, closed trimer revealed that CD4-binding site directed antibodies can reach into an interprotomer groove and make direct contact with the newly discovered site of quaternary CD4 interaction, thus highlighting the mimicry of this initial receptor contact by the immune system. Additional investigation of the single CD4-bound conformation show that binding of a single CD4 is sufficient to transition HIV-1 Env trimer to a more open conformation [5], characterized by separation of gp120 protomers, and loss of CD4-quaternary contacts.

Our results provide insight into the initial CD4 contact and CD4-induced conformational change, with implications for therapeutics and vaccine design targeting the CD4-binding site. They also reveal the utility of cryo-EM in studying Env conformation and entry intermediates, and in providing information on heterogeneous, asymmetric populations that may not be amenable to crystallization. In future studies,

we plan to continue to combine x-ray crystallography (for high resolution information on subunit structure) and cryo-EM (for visualizing heterogeneous and asymmetric populations) to characterize entry intermediates further along the entry pathway.

References:

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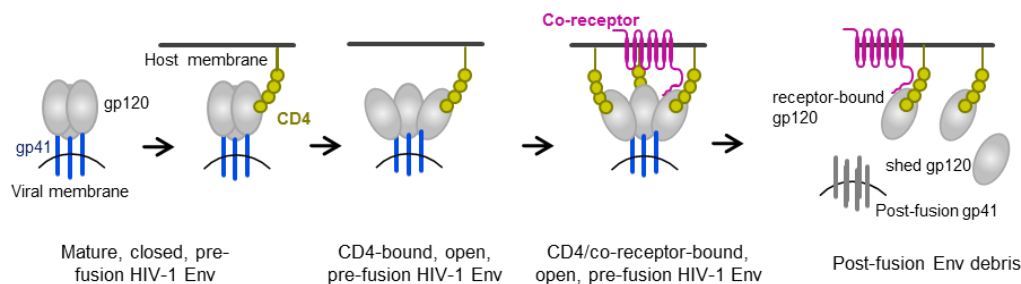


Figure 1. Conformational dynamics of HIV-1 entry. Mature HIV-1 Env prior to receptor activation adopts a closed conformation. On engaging CD4 receptor, the trimer transits to an open conformation, interacts with co-receptor and undergoes additional conformational changes to effect viral and host cell membrane fusion.

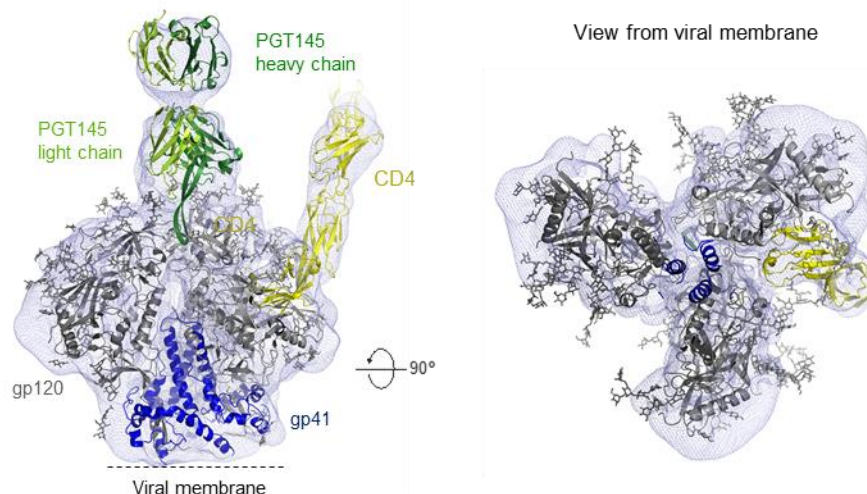


Figure 2. Cryo-EM structure of the initial interaction between HIV-1 Env and CD4 receptor. Left. Side view of a ternary complex comprising DS-SOSIP HIV-1 Env trimer, 4-domain sCD4 and Fab from the broadly neutralizing antibody PGT145, with a 6.8 Å resolution EM-reconstructed density shown in light blue mesh. Right. View from the viral membrane of the complex rotated 90°. PGT145 is a pre-fusion, closed conformation specific, broadly neutralizing anti-Env antibody that was used in the structural determination to further reinforce the pre-fusion, closed Env conformation in the complex.