

# Structure and Dynamics of the Native HIV-1 Env Trimer

James B. Munro,<sup>a</sup> Walther Mothes<sup>b</sup>

Department of Molecular Biology & Microbiology, Tufts University School of Medicine, Boston, Massachusetts, USA<sup>a</sup>; Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, Connecticut, USA<sup>b</sup>

**HIV-1/AIDS remains one of the worst pandemics in human history. Despite tremendous efforts, no effective vaccine has been found. Recent reports give new insights into the structure and dynamics of the HIV-1 Env trimer and renew hopes that a better understanding of Env will translate into new vaccine candidates and more-effective antiretroviral therapies.**

**H**IV type 1 (HIV-1) is the etiological agent of the AIDS pandemic. Over 70 million people have been infected by the virus, and ~39 million have died from AIDS-related illness. Antiretroviral therapy has reached ~11 million people and allows many HIV-1-infected people a long life span. However, the life-long chemotherapy is not without side effects, and the cancer burden among HIV-1-infected people remains elevated. Despite recent hopes that a cure might be possible (1–3), the more that we learn about the viral reservoir, the more that we realize how difficult it will be to eradicate HIV-1 in an infected patient (4–6). Given this situation, a low-cost preventative, such as a vaccine or an adeno-associated virus-delivered prophylactic, might be the preferred response to protect the human population (7). However, despite tremendous efforts, no effective vaccine has been found. This is due largely to specific features of the envelope glycoprotein (Env), which is uniquely exposed on the surface of the virion and as such is the primary target of antibodies.

HIV-1 Env is synthesized as a gp160 precursor and processed into a trimer of a heterodimer containing gp120 and gp41 subunits. HIV-1 Env promotes entry into target cells by recognizing cellular receptors and fusing viral and cellular membranes. The gp120 receptor-binding domain of Env first engages cellular CD4. This interaction leads to a conformational rearrangement in Env that results in presentation of the coreceptor-binding site. Interaction with the coreceptor triggers the gp41 membrane fusion domain to mediate virus entry.

Why is it so hard to generate a vaccine against HIV-1? In addition to recognizing cellular receptors, Env has evolved effective means of concealing functional centers from attack by antibodies. The Env trimer has three distinct features that make it an evasive machine that escapes neutralizing antibodies. First, Env is covered by a dense glycan layer that makes up half of its total molecular weight. This glycan shield restricts access of immunoglobulins to 97% of the Env surface (8). Second, the protein surface undergoes unusually rapid sequence variation. Approximately 50% of the Env surface has genetic variability greater than 10%. Taken together, these two factors result in only 2% of the Env surface being accessible to immunoglobulins with genetic variability of less than 10% (8). Third, the Env trimer has significant structural flexibility. Env can adopt a closed conformation, in which functional centers are masked while still responding to interactions with the receptor and coreceptor. The high glycosylation and the conformationally dynamic nature of Env has for many years impeded its structural characterization. Recent advances give new insights into the structure and dynamics of the HIV-1 Env trimer (8–11) and renew hopes that a better understanding of the HIV-1 Env trimer will

translate into new vaccine candidates and more-effective antiretroviral therapies.

We set out to advance the understanding of the conformational dynamics of the native Env trimer. Available structural data on the intact trimer at low resolution indicated large-scale rearrangements in which the V1V2 loop located at the tip of the trimer opens in response to CD4 and coreceptor mimics (12). Given the scale of this conformational change and the known time scale of HIV-1 entry, we therefore expected dynamics in the range of milliseconds to seconds. One method that provides access to conformational changes on this time scale is single-molecule fluorescence resonance energy transfer (smFRET). We therefore developed smFRET imaging methods to elucidate the conformational changes of HIV-1 Env on the surfaces of native HIV-1 virions (9). The application of smFRET to HIV-1 Env required the site-specific incorporation of fluorophores into the native trimer. To this end, we inserted two 6- to 12-amino-acid peptides into variable loops of the gp120 domain of Env, which allowed enzymatic labeling with donor and acceptor fluorophores. Peptides were placed into the V1 loop of gp120, known to open in response to CD4 (12), and into V4 or V5, which served as points of reference from which to observe V1 repositioning. Labeling sites that did not result in significant loss of infectivity or neutralization sensitivity compared to that of wild-type Env were identified. To ensure that only a single fluorescently labeled gp120 molecule was present on the surface of the virus, wild-type HIV-1 was cotransfected at a ratio of 40:1 over the dually tagged plasmid during generation of the virus. Virions were dually labeled enzymatically, purified, surface immobilized, and imaged via prism-based total internal-reflection fluorescence (TIRF) microscopy, which allows for the observation of conformational transitions in hundreds of individual molecules simultaneously over extended periods of time (in approximately minutes) (13).

Surprisingly, the unliganded HIV-1 Env on the surfaces of native virions was found to be dynamic after sampling of at least

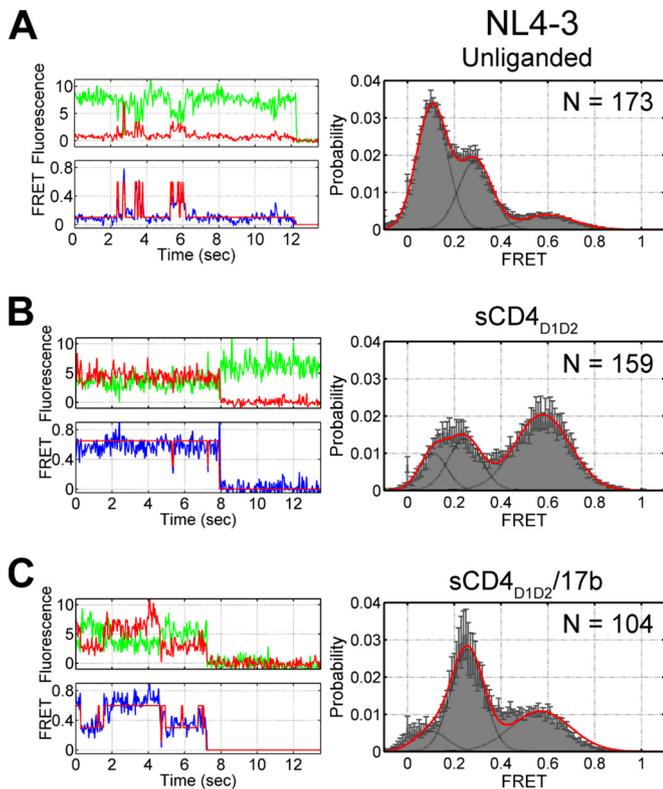
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Address correspondence to James B. Munro, James.Munro@tufts.edu, or Walther Mothes, walther.mothes@yale.edu.

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**FIG 1** HIV-1 Env is conformationally dynamic and has inherent access to conformational states that are stabilized by soluble CD4 and the coreceptor surrogate antibody 17b. (A) HIV-1 virions containing a single dually labeled Env molecule among native trimers were immobilized on the surfaces of passivated quartz microscope slides and imaged via TIRF microscopy. Representative fluorescence [Cy3B, donor, green; Cy5(4S)-cyclooctotetraene (COT) acceptor, red] (top left) and FRET (blue) trajectories (bottom left) obtained from a single HIV-1 NL4-3 Env protein on the surface of an intact virion are shown. Idealization of FRET trajectories (red) was achieved by fitting each trace to a three-state Markov model. (Top right) FRET trajectories from individual unliganded HIV-1NL4-3 Env proteins were compiled into a population FRET histogram and fit to the sum of three Gaussian distributions (red) with means of 0.1, 0.3, and 0.6 (black). (B, C) Fluorescence and FRET trajectories and FRET histograms in the presence of soluble CD4 (B) or soluble CD4 and coreceptor-mimicking antibody 17b (C). (Adapted from reference 9 with permission of the publisher.)

three distinct conformations, observed as low-, intermediate-, and high-FRET states (9) (Fig. 1). The low-FRET state was the most populated state and thus must reflect the closed ground state conformation of the prefusion Env, an interpretation that was confirmed studying ground state-stabilizing mutations. The two higher-FRET states were stabilized to various extents by soluble CD4 (sCD4) and the coreceptor-mimicking antibody 17b. When introduced together, sCD4 and 17b stabilized the intermediate-FRET state, which thus likely represents the coreceptor-stabilized state. Different responses to sCD4 for distinct HIV-1 isolates did not allow a precise assignment of the high-FRET state. Importantly, because all three FRET states were observed in the absence of ligands, conformations that are stabilized by CD4 and coreceptor are therefore intrinsically accessible to the unliganded HIV-1 Env.

We also compared the conformational samplings of Env for the laboratory-adapted neutralization-sensitive HIV-1 isolate NL4-3 with those of the neutralization-resistant clinical isolate

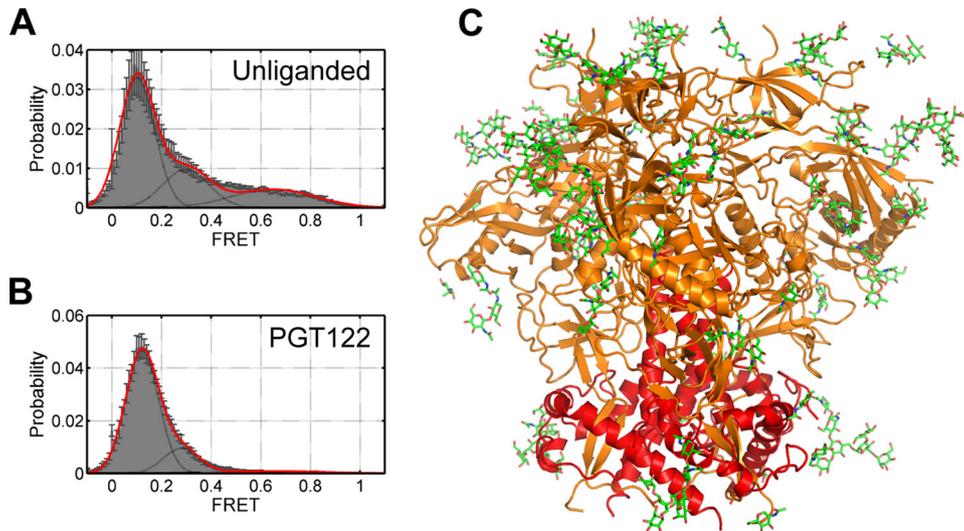
JR-FL. In contrast to NL4-3, which opened relatively frequently, the neutralization-resistant JR-FL Env rarely opened, and as a consequence, fewer molecules had access to the coreceptor-stabilized state. Given that JR-FL evolved under pressure from the host immune system to conceal functional centers and thus escape neutralization by antibodies, this provided the first dynamics-based rationale for differential neutralization sensitivity and resistance among HIV-1 isolates.

Hidden Markov modeling of the smFRET trajectories from both strains revealed the relative frequencies of the observed transitions and confirmed that HIV-1 Env is intrinsically capable of sampling three conformations. The CD4<sup>-</sup> and coreceptor-stabilized states are less energetically stable and thereby provide a mechanism by which vulnerable functional states are protected from antibodies. Consistently with this model, antibody 17b required extended incubation times to stabilize the activated state in the absence of sCD4. This analysis also indicated that the high-FRET state is a necessary intermediate during the activation of HIV-1 Env by CD4 and the coreceptor-mimicking antibody 17b. This analysis thus directly visualized the sequence of molecular events, underlying the two-step activation of HIV-1 Env by CD4 and coreceptor.

The establishment of an smFRET assay for the conformational state of native HIV-1 Env molecules on the surfaces of virions allowed us to visualize the conformational consequences of broadly neutralizing antibodies and small-molecule inhibitors. Surprisingly, multiple broadly neutralizing antibodies, despite engaging the Env trimer in very different ways, stabilized the ground state conformation (9) (Fig. 2A and B). The ground state stabilization of Env by these broadly neutralizing antibodies indicates that they recognize the closed conformation of HIV-1 Env. Our data are consistent with observations by Guttman and colleagues, who used hydrogen-deuterium exchange to arrive at a similar conclusion (14). Moreover, the especially strong ground state stabilization by antibodies such as PGT145 (9) and PGT122 (8) (Fig. 2A and B) also raises the possibility that in addition to binding the closed conformation, broadly neutralizing antibodies may inhibit transition out of the ground state, thereby preventing activation of Env. A potent small-molecule HIV-1 entry inhibitor, BMS-626529 (15), similarly stabilized the closed ground state. This suggests that ground state stabilization may be a general means of inhibiting HIV-1 entry.

A possible rationale for ground state stabilization as an antiviral strategy is provided by our observation that the unliganded HIV-1 Env has intrinsic access to the activated conformational states stabilized by CD4 and the coreceptor-mimicking antibody 17b. This may indicate that access to the open conformations is required for function because binding of CD4 and coreceptor may depend on the capture of preexisting conformations. Data from us, Guttman and colleagues, and others strongly suggest a conformational capture mechanism during binding of antibodies such as 17b (9, 14). Whether CD4 binding occurs in the same manner is a subject of current research.

The observed strong ground state stabilization of HIV-1 Env by PGT122 may in retrospect explain why this antibody was able to reduce conformational heterogeneity and allow the crystallization of a soluble trimer (8, 10) (Fig. 2C). Moreover, our smFRET data identify this structure as the ground state conformation of the Env trimer (8). Since broadly neutralizing antibodies specifically recognize this conformation, any immunogen intended to elicit



**FIG 2** Broadly neutralizing antibodies recognize the closed ground state conformation. (A, B) An experiment was performed as described for Fig. 1A for the unliganded dually labeled HIV JR-FL Env (A) and for JR-FL Env in complex with broadly neutralizing PGT122 (B), which reduces the occupancy of the high-FRET state and stabilizes the ground state. (Panels A and B are adapted from reference 8 with permission of the publisher.) (C) Structure of the closed ground state conformation of the soluble HIV-1 Env trimer with the gp120 subunit in orange, gp41 in red, and glycan shield in green. (Panel C is provided courtesy of Marie Pancera, Jonathan Stuckey, and Peter D. Kwong.)

these potent antibodies must present this conformation. Therefore, the vaccine research field is currently engineering Env mutants that lock the trimer in that ground state conformation. These locked trimer scaffolds will not transition to the receptor-stabilized states and will thus not present highly immunogenic open conformations to the immune system, which only distract the immune response and do not result in protective immunity.

These considerations explain why recent advances in the understanding of the structure and dynamics of the HIV-1 Env trimer gives hope that new effective vaccine scaffolds can be generated (8, 9). However, many questions remain to be addressed. The biggest hurdle that requires solving is how a vaccine based on one HIV-1 strain can elicit protection against heterologous challenge by a different HIV-1 strain. Also, while recent structures of the Env trimer represent critical advances (8, 10, 11, 16), they are all based on the use of engineered soluble trimers, and it remains an open question as to what extent these artificially stabilized trimers faithfully reflect all features of the wild-type HIV-1 Env trimer (17). Many additional aspects of how the HIV-1 Env fusion machine promotes viral entry remain to be understood. While we could demonstrate that gp120 resembles an allosteric immune evasion machine that has intrinsic access to all conformational states required for function, the underlying fusion machine, gp41, is expected to undergo irreversible conformational changes following triggering by the coreceptor that ultimately lead to membrane fusion. Last but not least, researchers studying other viral glycoproteins may ask themselves if their viral glycoproteins may similarly present a dynamic equilibrium of various conformations that may better explain observed phenotypes. It is our hope that smFRET technologies that allow the visualization of the conformational state of viral glycoproteins on the surfaces of native virions may play a pivotal role in these future studies.

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