Reply to “The Broadly Neutralizing, Anti-HIV Antibody 4E10: an Open and Shut Case?”

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We thank Drs. Strong and Finton for their interest in our work involving the broadly neutralizing anti-HIV-1 antibody 4E10 (1). In that study, we determined the crystal structure of the unbound form of the Fab region of 4E10, as well as that of non-neutralizing mutants with the peptide epitope bound (1). In particular, the conformation of the critical CDR-H3 loop in the crystal structure of the unbound Fab differed greatly from that previously observed for the Fv version of the same antibody (2). Collectively, our data support the existence of a preformed crevice for binding of the helical membrane-proximal external region (MPER) epitope.

In the accompanying letter (3), Strong and Finton concur with the main tenet of our article, i.e., that 4E10 must “recognize an antigenic structure more complex than just the linear α -helical epitope.” However, in the same letter these authors disagree with our interpretation of the structural data corresponding to the new unbound form of the Fab fragment and with our criticism of the structure of the unbound form of the Fv version (2). Strong and Finton argue that the low pH of the crystallization solution and the use of the shorter Fv construct are not compelling arguments to explain the conformational discrepancies with respect to the Fab. They argue that the Fv construct of 4E10 “retains the structure, binding, and neutralization properties of the Fab” (4). In addition, they explain that the thermodynamic signature of the binding (low entropic component) would not be consistent with an order-disorder transition during binding of the epitope peptide in solution. Moreover, based on a qualitative crystal contact analysis, they argue that our structure of the Fab in the unbound state does not reflect the “fully-free state of 4E10, but, unfortunately, recapitulates the ligand-bound state, resolving the potential contradiction.” The closed conformation observed in the Fv construct may generate a lipid-binding site representing an alternative conformation to the epitope-binding structure, implying that a conformational transition must occur between both forms for effective engagement of the gp41 antigen (2, 4).

We find the arguments expressed in the letter by Strong and Finton reasonable, but only to some extent. For example, we acknowledge that the discrepancy between the crystal structures of the unbound form of the Fab and Fv constructs does not by itself constitute sufficient evidence to completely rule out the existence of large conformational changes in the CDR-H3 loop (2). In addition, neither the crystal structure of the unbound Fv nor the “shut-open” mechanism precludes the existence of a distinct unbound form of the antibody competent in binding to the helical epitope. In the framework of the model described in Strong and Finton’s letter, the structure described in our paper would correspond to the “open-unbound” antibody.

Nevertheless, a fair critical assessment of the unbound struc-

tures should take into account the entire data set available in our study (1) and the structures deposited in the PDB structural database. A quantitative analysis of the crystal contacts with PISA (5) shows that, compared to that in the structure of Fab with the epitope bound (6), only a small fraction of the paratope interface ($250 \text{ \AA}^2$, i.e., 35% of the paratope surface) of the Fab in the unbound form is involved in intermolecular contacts with other Fab chains in the crystal lattice (Table 1). The small value of the contact interface suggests that crystal-packing forces in the unbound form of the Fab cannot effectively mimic the binding of the peptide epitope. In addition, the highly dynamic nature of the residues of the paratope of Fab in the absence of peptide (Fig. 5 in reference 1) is not consistent with the establishment of specific intermolecular interactions with other molecules of Fab in the crystal, and therefore crystal lattice forces in this region are unlikely to govern the conformation of the complementarity-determining region (CDR) loops.

In contrast, and as determined by the same type of analysis, the intermolecular contact interface of residues of the paratope in the structure of unbound Fv is surprisingly large ($534 \text{ \AA}^2$, 75% of the paratope surface), more than double that of the unbound Fab and approaching the value determined for the complex between antibody and peptide (Table 1). Moreover, the interface surface involving the critical CDR-H3 loop (responsible for the conformational change reported in the Fv construct [5]) even exceeds the value determined for 4E10 Fab in the bound form. Taken together, these observations suggest that the conformation of the paratope may be influenced more by nonnative interactions in the unbound form of the crystallized Fv construct than that in the crystallized Fab.

Finally, the existence of a “shut” conformation may not be compatible with previous mutational studies (1, 7). As we described in our study, the thermodynamic and kinetic evidence gathered from three different versions of CDR-H3 (wild type, delta-loop, and the $W_{H100}D$ $W_{H100B}D$ double mutant, termed WDWD) is essentially indistinguishable among the three constructs examined. These data may be difficult to reconcile with the shut conformation because some of the residues modified, such as

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TABLE 1 Contact interface area of 4E10 antibodies

Structure	Contact interface				Reference
	Paratope ^a (Å ²)	%	CDR-H3 (Å ²)	%	
2FX7 ^b	714	100	221	100	6
5CIP ^c	250	35	148	67	1
4LLV ^c	534	75	348	158	2

^a Paratope is defined as CDR-H1, -H2, -H3, and CDR-L2 (1).

^b Contact interface between the reference Fab antibody and the 4E10 peptide epitope (6).

^c Intermolecular contact interface with other molecules of antibody in the crystal lattice.

Trp_{H100b} (deleted in the delta-loop construct or mutated to Asp in WDWD), interact with other regions of the paratope in the shut conformation of unbound Fv. As a consequence, if this conformation were relevant for binding the epitope in solution, we would expect to observe some differences in the thermodynamic (7) and/or kinetic (transition state) parameters (1), but that was not the case.

In summary, we believe that the published structure of the Fab in the unbound form (1) adequately represents the antigen-binding competent form of the antibody.

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