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Title: CD4-binding obstacles in conformational transitions and allosteric communications of HIV gp120
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Submit Date: Dec 21, 2019

Dear Dr. Li:

Thank you for submitting the above-named article to BBA - Biomembranes. Also thank you for responding positively to our previous review reports.

We have completed the review of your manuscript and a summary of the comments from two original reviewers is appended below. One reviewer is now happy with this version, while the second reviewer still has a few issues that could be improved or clarified. Please carefully revise the paper according to the reviewers' comments. Please note that your revised article will be re-evaluated.

We look forward to receiving your detailed response and your revised article.

The revised version of your submission is due by Mar 23, 2020.

Yours sincerely,

Hans J. Vogel, Ph.D.
Executive Editor
BBA - Biomembranes

Please see Reviewers' comments:

Reviewer #1: No further comments.

Reviewer #2: The authors addressed most of the comments raised by the reviewers, but the study still needs experimental validation to the theoretical computational work. The author should introduce any mutation that was reported to stabilize an intermediate Env state to their model, follow the effect on gp120 conformation during molecular simulations and compare the conformation to their 4 intermediates. Without experimental confirmation the work lacks strong scientific basis.

Response:
We thank the reviewer for careful reading and constructive comments on our manuscript.

This manuscript reported here is only a purely theoretical work. In our study, two comparative MD simulation systems of open-state gp120 under the CD4-stripped and CD4-exist conditions were performed to investigate CD4 binding effects on the molecular dynamics, thermodynamics, and kinetics of gp120. We think that controlling a single variable parameter (CD4 exists or not) in the same experimental environment (open-state gp120 without considering residue mutations) can be a reasonable design for our study.

Unfortunately, our laboratory has no conditions to carry out HIV wet experiments. However, some experimental reports, e.g. (Herschhorn et al., **2016**, *MBio*) listed by the reviewer, about mutation effect on gp120 conformation may support our study. Based on reviewer's previous comment, we *in silico* introduced all amino acids to position 193 and evaluated mutation effects on stability of open (PDB ID: 3J70), metastable conformations from MSM (Fig. 4 in main text), and closed (PDB ID: 5FYJ) gp120 by using a theoretical tool (Pandurangan et al., **2017**, *Nucleic Acids Res.*). Consistent with the experimental study (Herschhorn et al., **2016**, *MBio*), our assessment indicates that L193R stabilizes the intermediate conformations which has been found in the metastable conformation from MSM (Fig. 4E).

Mutation effects (**ΔΔG, positive value indicates that the mutation stabilizes the conformation**) of leucine 193 on the stability of open (PDB ID: 3J70), metastable conformations from MSM (Fig. 4 in main text), and closed (PDB ID: 5FYJ).

Mutation	open (PDB ID: 3J70), metastable conformations from MSM (Fig. 4 in main text), and closed (PDB ID: 5FYJ)							
	open	A (ligand_free)	A (CD4_bound)	B	C	D	E	closed
L193A	-0.3	-0.36	-0.36	-0.36	-0.39	-0.36	-0.39	-3.91
L193G	-0.44	-0.2	-0.57	-0.2	0.13	-0.2	0.13	-3.09
L193N	-0.18	-1.31	-1.31	-1.31	-0.81	-1.22	-0.81	-3.06
L193D	-0.79	-1.43	-1.43	-1.43	-1.34	-1.53	-1.34	-3.8
L193C	0.15	-0.19	-0.19	-0.19	0.08	-0.13	0.08	-0.85
L193Q	-0.72	-1.07	-1.07	-1.07	-1.04	-1.07	-1.04	-2.32
L193E	-0.69	-0.85	-0.86	-0.85	-0.83	-0.85	-0.83	-2.38
L193R	3.29	2.83	2.83	2.83	3.29	2.83	3.29	-2.11
L193H	-0.31	-0.77	-0.81	-0.81	-0.45	-0.75	-0.45	-1.52
L193I	-0.05	0.04	0.04	0.04	-0.05	0.04	-0.05	0.52
L193K	-0.71	-1.36	-1.36	-1.36	-1.22	-1.36	-1.22	-3.16
L193M	-0.32	-0.41	-0.41	-0.41	-0.33	-0.41	-0.33	-1.1

L193F	-0.33	-0.37	-0.37	-0.37	-0.33	-0.37	-0.33	-0.78
L193P	-2.19	-2.1	-2.1	-2.1	-2.19	-2.1	-2.19	-3.98
L193S	-1.45	-1.58	-1.58	-1.58	-1.58	-1.58	-1.58	-3.31
L193T	-1.46	-1.34	-1.34	-1.34	-1.42	-1.28	-1.42	-2.13
L193W	-0.09	-0.15	-0.16	-0.16	-0.09	-0.16	-0.09	-1.51
L193Y	-0.24	-0.47	-0.49	-0.47	-0.24	-0.47	-0.24	-1.36
L193V	-0.03	0.05	0.05	0.05	-0.03	0.05	-0.03	-1.45

The low, intermediate, and high FRET values are NOT related to energy but to distance. The authors should correct the discussion with the accurate definition. Open state is the intermediate FRET state and NOT high FRET. The closed conformation is metastable and therefore is found at HIGH energy prior to CD4 binding.

Response:
We fixed the misstatement about the open state in this revision.

Due to significant debate about the structure that represents the Env closed conformation, the authors should add a comment/definition that in their work they refer to the apparent closed conformation of gp120 as the closed state.

Response:
We defined the closed and open states more clearly in the Materials and Methods of this revision.

The numbering of Env residues are not consistent with the established standard. For example, Ala 395 is not part of the B20-B21 strands. The author should provide the accurate numbering to each reside discussed.

Response:
We corrected the residue number according to HIV-1 HXBc2 (uniprot ID: Q75760) in this revision.