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Dear *eLife* Editorial Board,

We are writing to submit our manuscript “*Mapping mutational effects along the evolutionary landscape of HIV envelope*” for consideration as a Research Article.

In this paper, we address a question of fundamental importance to the evolution of HIV (and of proteins more generally): How rapidly do the effects of mutations shift during evolution?

To systematically answer this question, we use deep mutational scanning to quantify how all amino-acid mutations to HIV’s Env protein affect the growth of two transmitted-founder strains of virus. We then compare these measurements between the two Envs to identify sites where the effects of mutations have changed. Interestingly, the patterns are somewhat different than have been found in earlier studies of protein evolution – we find much more evidence of long-range epistasis, for instance. We suggest that this is due to the remarkable conformational complexity of Env relative to most other proteins that have been studied in a similar fashion.

In addition, we compare our data to Env’s evolution among natural HIV sequences. We identify sites that are evolving both faster and slower in nature than required by simple selection for viral growth in cell culture. We show that these sites appear to be under selection from immunity, thereby explaining why their natural evolutionary patterns cannot be fully explained by simple functional constraints.

Our work will be of strong interest to three groups of scientists: HIV virologists, evolutionary biologists, and protein biochemists. This broad range makes our study appropriate for *eLife*. Below we highlight points that will interest each of these groups:

1. Our study is the first large-scale characterization of mutational effects to transmitted-founder strains of HIV. In particular, we characterize the functional effects of all amino-acid mutations to BG505, which is the most widely used strain in both structural biology and vaccine development. This makes our work highly relevant to those fields.
2. Our study is one of the most systematic examinations of epistatic shifts during long-term protein evolution, and certainly examines the most complex protein studied in this respect. As we show, this complexity leads to some new findings

with respect to the prevalence of long-range epistasis. This makes our work highly relevant to ongoing investigations in evolutionary biology of the role of epistasis.

3. We show that our work can be used to better identify sites under strong pressure from immunity in HIV's natural evolution. This map of sites of immune selection is highly relevant to viral evolution.

Finally, we make all of the experimental and computational work highly transparent and open, consistent with the ethos of *eLife*. We have provided supplementary files that contain a well-documented Jupyter notebook that reproduces *all* the computational analyses. We have also submitted the HIV mutant libraries generated in this study to the NIH AIDS Reagent Program repository, where they will be freely available to academic researchers. Therefore, both our computational and experimental work will be easy for others to build on as they wish.

We suggest that the following individuals would be appropriate Reviewing Editors:

- **Pamela Bjorkman** is an expert in the study of HIV Env.
- **Richard Neher** is an expert on HIV evolution.

Our paper touches on HIV virology, protein evolution, and viral evolution. We therefore request reviewers with expertise in each of these areas.

- Relevant experts in HIV virology:
 - **Peter Kim** (<http://peterkimlab.stanford.edu/>): kimpeter@stanford.edu
 - **Alexandra Trkola** (<http://www.virology.uzh.ch/de/research/gtrkolad.html>): trkola.alexandra@virology.uzh.ch
 - **Andrew Ward** (<https://ward.scripps.edu/>): andrew@scripps.edu
 - **James Munro** (<http://sackler.tufts.edu/Faculty-and-Research/Faculty-Research-Pages/James-Munro>): james.munro@tufts.edu
 - **Susan Zolla-Pazner** (<http://icahn.mssm.edu/profiles/susan-zolla-pazner>): susan.zolla-pazner@mssm.edu
- Relevant experts in protein evolution:
 - **Georgii Bazykin** (<http://faculty.skoltech.ru/people/georgiibazykin>): g.bazykin@skoltech.ru
 - **David McCandlish** (<https://www.cshl.edu/research/faculty-staff/david-mccandlish/>): mccandlish@cshl.edu
 - **David Pollock** (<http://www.evolutionarygenomics.com/>): David.Pollock@UCDenver.edu
 - **Joe Thornton** (<http://voices.uchicago.edu/thorntonlab/>): joet1@uchicago.edu
- Relevant experts in viral evolution:
 - **Claus Wilke** (<http://wilkelab.org/>): wilke@austin.utexas.edu
 - **Ben Murrell** (<http://profiles.ucsd.edu/benjamin.murrell>): bmurrell@ucsd.edu

- **Alexandra Walczak** (<https://www.phys.ens.fr/~awalczak/>):
awalczak@lpt.ens.fr
- **Simon Frost** (<https://www.vet.cam.ac.uk/directory/sdf22@cam.ac.uk>):
sdf22@cam.ac.uk

Thanks for your time and consideration.

Sincerely,



Jesse D. Bloom, Ph.D.