

I have produced and published several negative-stain EM 3D models for antibodies in complex with the HIV envelope protein (the spike found on its outer surface)(1-4) and have also done EM work with the influenza hemagglutinin in complex with an antibody (*manuscript submitted*). I also anticipate analyzing new complexes once we have antibody sequences from COVID-19 donors.

My laboratory is currently working on multiple complexes suitable for cryo-EM studies. The molecular weights of our complexes range from 360 kDa to ~600 kDa. We have analyzed several complexes by negative stain EM and have attained good 2D class averages (Figs. 1 and 2). To attain higher resolution structures, we have begun preparing cryo-EM samples for one of the complexes (Fig. 1B). We have not been able to get good ice or consistency for this. We have tried increasing blotting time on the Vitrobot to up to 12 seconds and still have thick ice. We have also tried altering the temperature at which we freeze our samples (the original ones were done at 4 degrees and we have switched over to room temperature), but we have yet to analyze those samples.

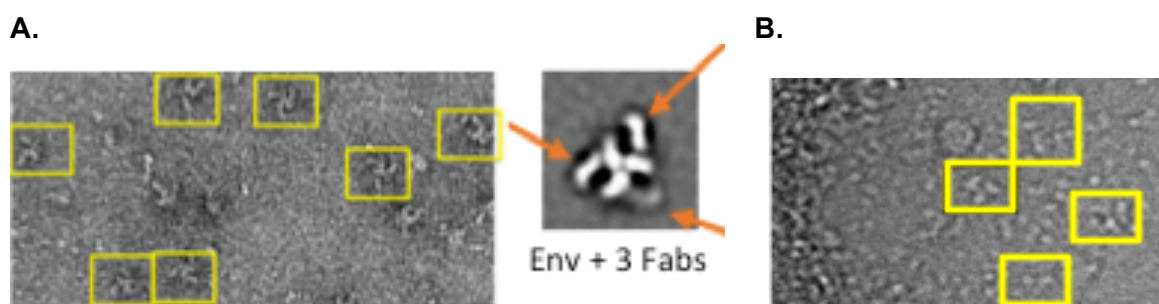


Figure 1. Complexes of Fab with an HIV Envelope (Env) protein. (a) Negative stain EM of the Fab-Env complex. Particles representing Envs fully saturated with Fabs are boxed in raw images (left) and representative 2D class average determined by particle picking using EMAN2 is shown (right). **(b) cryo-EM of the Fab-Env complex.** Particles representing Env with Fab are boxed in raw images (Note, color was inverted to improve visibility). Grid carbon is on the left.

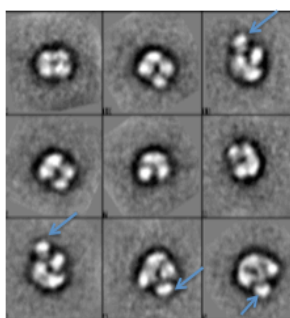


Figure 2. Negative stain EM 2D class averages of HIV Env in complex with a different Fab. Arrows point to representative bound Fab in several of the class averages.

References

1. M. Bonsignori *et al.*, Staged induction of HIV-1 glycan-dependent broadly neutralizing antibodies. *Sci Transl Med* **9**, (2017).
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3. W. B. Williams *et al.*, Initiation of HIV neutralizing B cell lineages with sequential envelope immunizations. *Nat Commun* **8**, 1732 (2017).
4. D. Easterhoff *et al.*, Boosting of HIV envelope CD4 binding site antibodies with long variable heavy third complementarity determining region in the randomized double blind RV305 HIV-1 vaccine trial. *PLoS Pathog* **13**, e1006182 (2017).