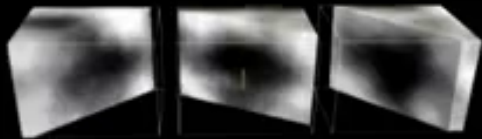




# Image Optimization Strategy

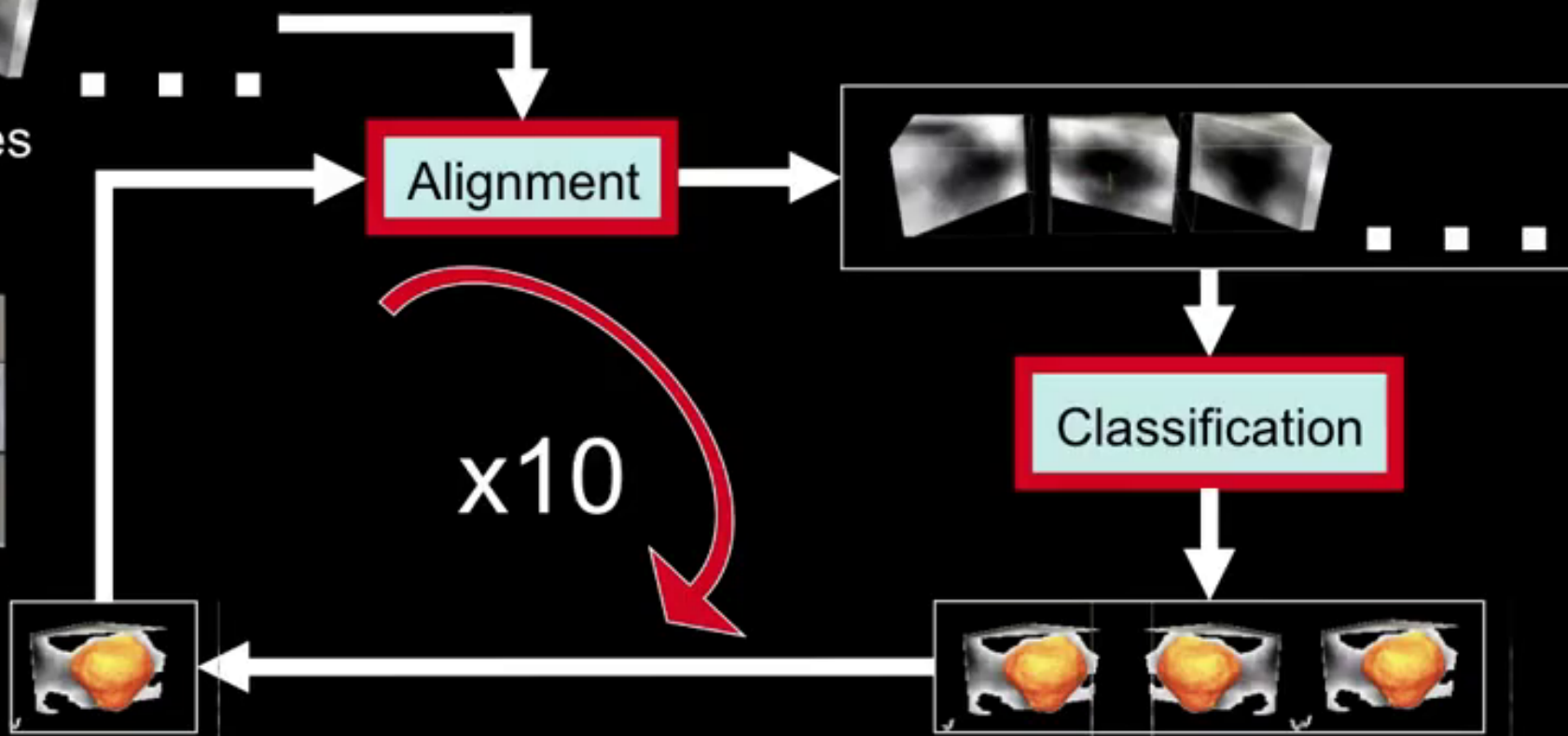


4000 sub-volumes



# Image Optimization Strategy

4000 sub-volumes



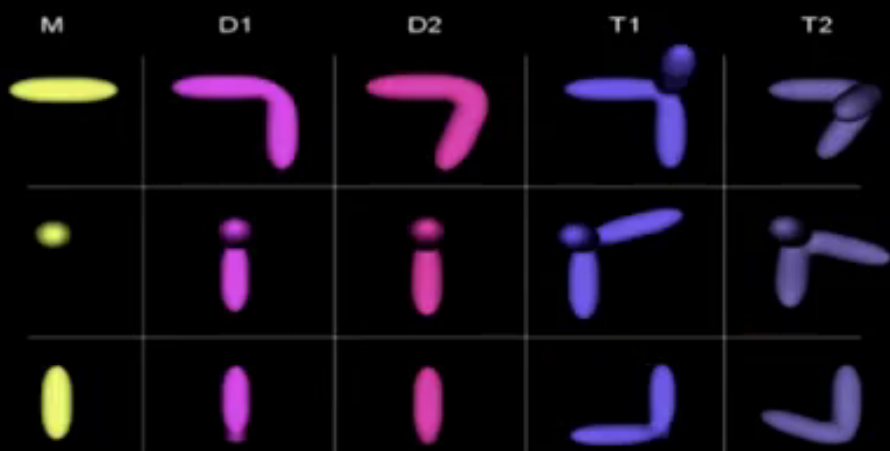
# 3D Image Alignment



$$\min_{\theta, \phi, \psi, \vec{t}} d(\text{img}_1, \text{img}_2)$$

The diagram illustrates the 6 degrees of freedom (DOF) for 3D image alignment. It shows two 3D volumes, each composed of blue, red, and gray planes. The first volume is in its reference orientation. The second volume is transformed by a combination of rotation and translation. The rotation is defined by three angles:  $\theta$  (roll, around the vertical axis),  $\phi$  (pitch, around the horizontal axis), and  $\psi$  (yaw, around the diagonal axis). The translation is represented by the vector  $\vec{t}$ .

- 6 DOF problem: Speed-up in Fourier domain

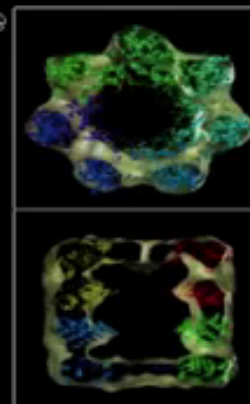
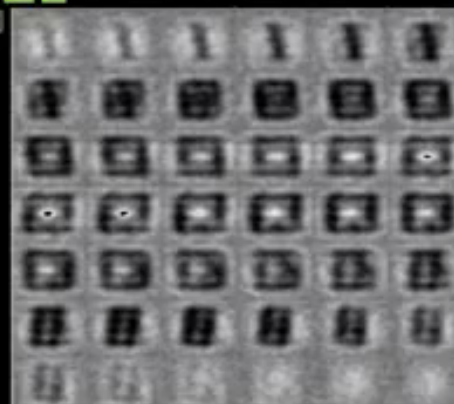
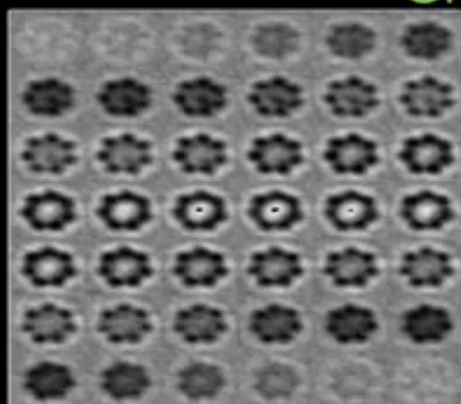
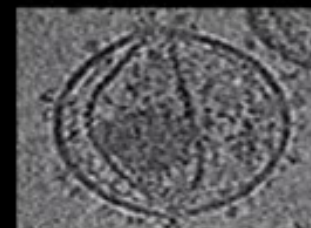


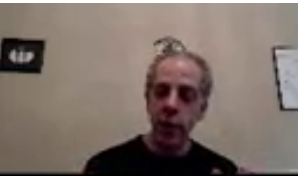
Phantoms



HIV

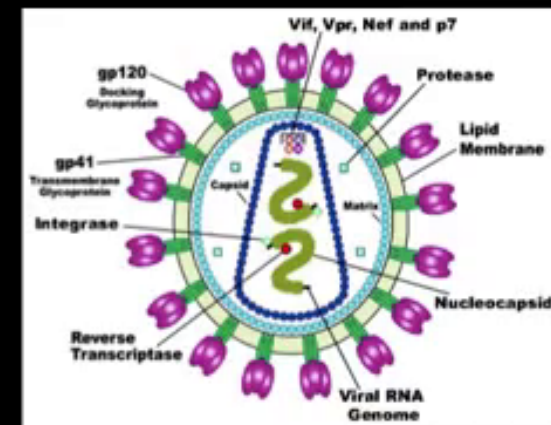
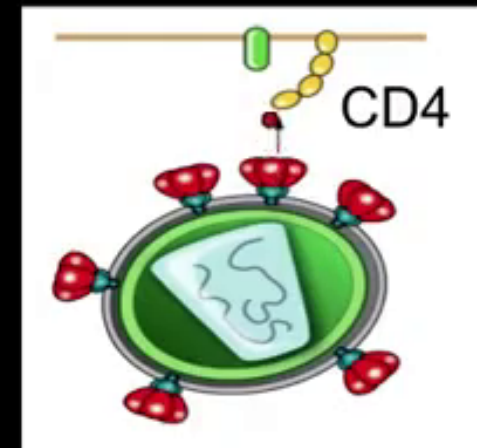
GroEL

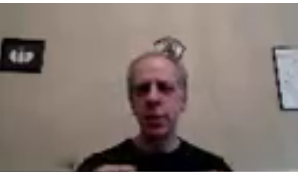




# HIV envelope glycoproteins

- Mediate virus binding to the cell surface receptor CD4 on target cells to initiate infection
- Functional unit is a trimer of **gp120** (surface glycoprotein) and **gp41** (transmembrane unit).
- Structure of components available.
- Structure of the trimer remains elusive.





## LETTERS

**Molecular architecture of native HIV-1 gp120 trimers**Jun Liu<sup>1\*</sup>, Alberto Bartesaghi<sup>1\*</sup>, Mario J. Borgnia<sup>1\*</sup>, Guillermo Sapiro<sup>2</sup> & Sriram Subramaniam<sup>1</sup><sup>1</sup>Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland 20892, USA. <sup>2</sup>Department of Electrical and Computer Engineering, University of Minnesota, Minneapolis, Minnesota 55455, USA.

\*These authors contributed equally to this work.

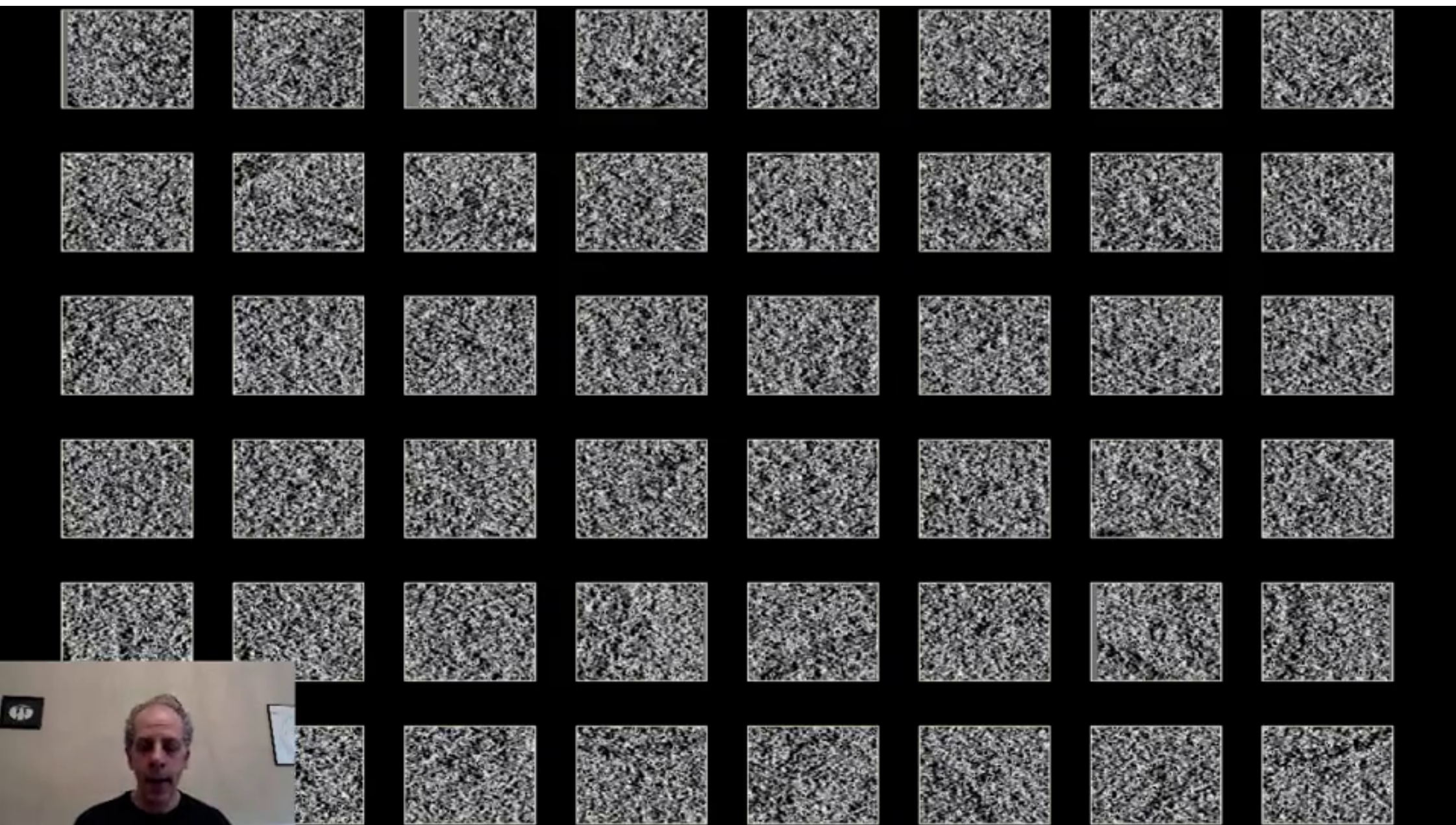
- Use cryo-electron tomography combined with 3D image averaging and classification
- Report 3D “snapshots” of trimeric spike:
  - Unliganded state
  - Complex with broadly neutralizing b12
  - Ternary complex with CD4 and 17b



# Imaging the spike at different states

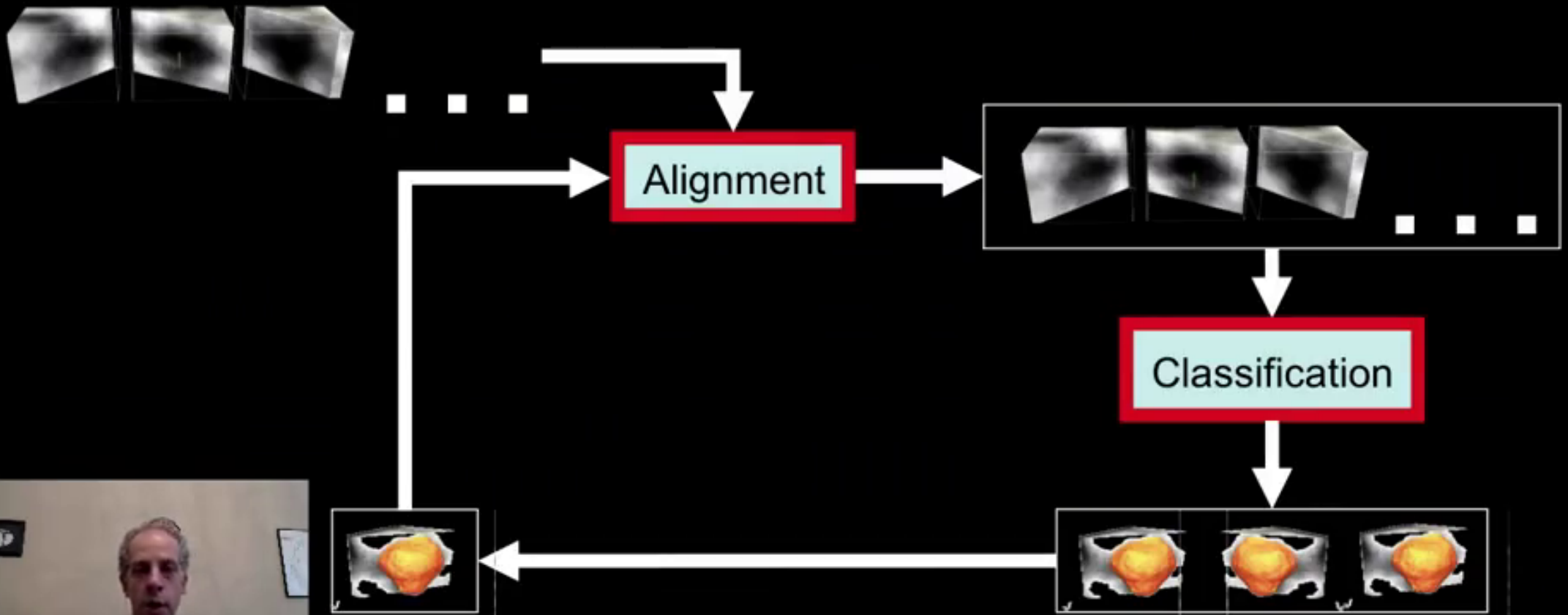
- 80 tilt series, 400 virus, 4K spikes
  1. Unliganded state
  2. Complex with b12
  3. Ternary complex with CD4 and 17b



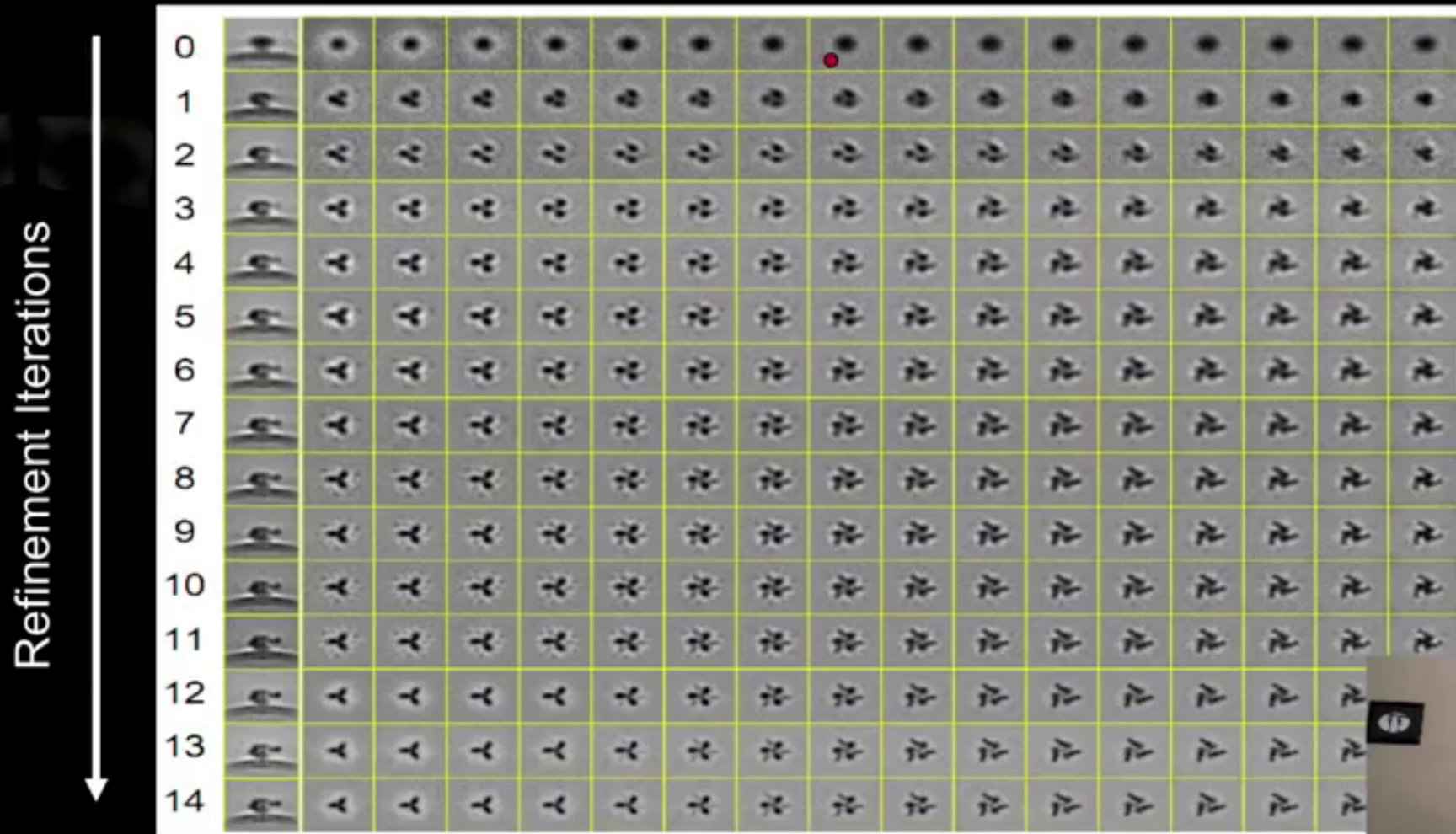


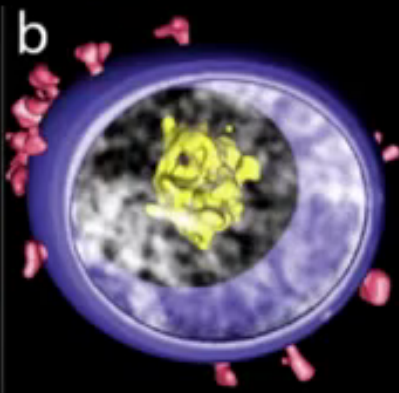
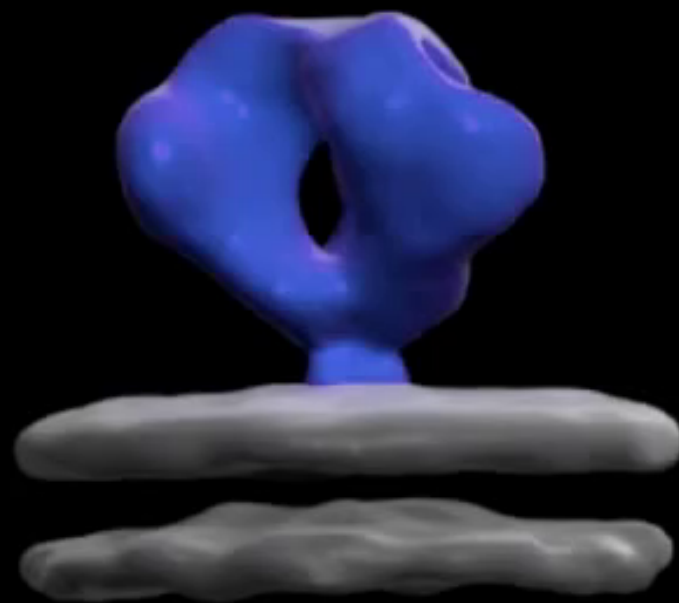


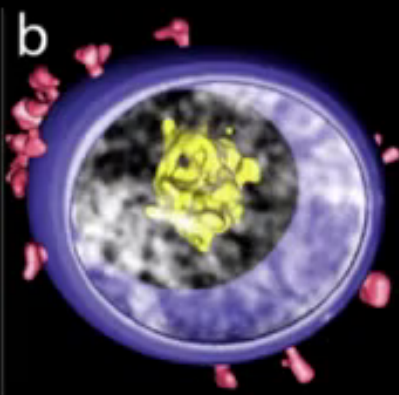
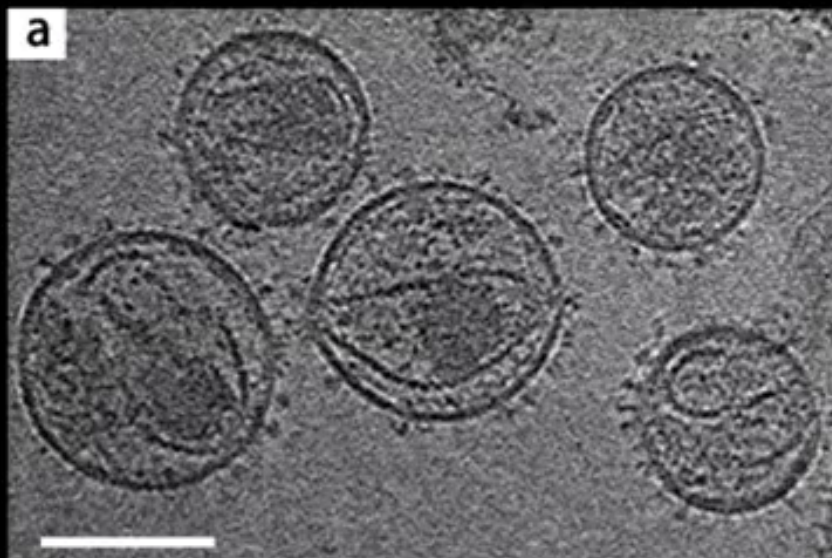
# Image Refinement Loop



# Image Refinement Loop



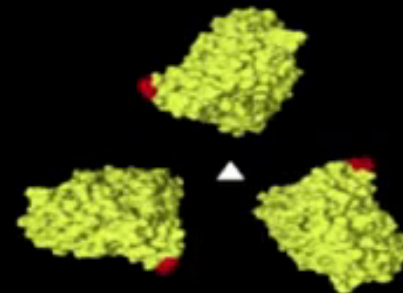
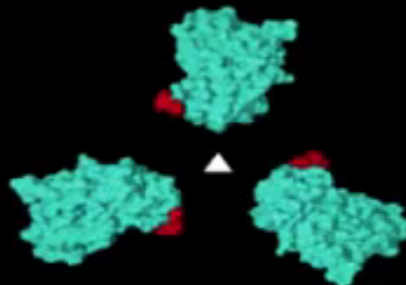
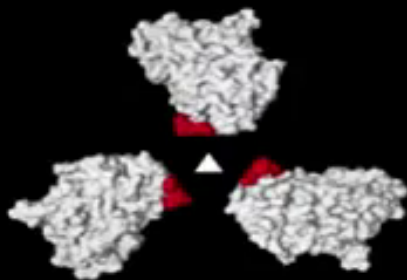
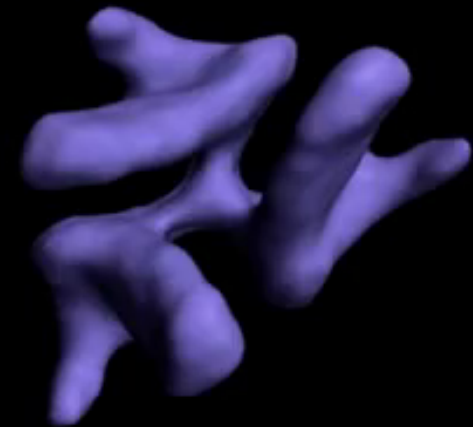
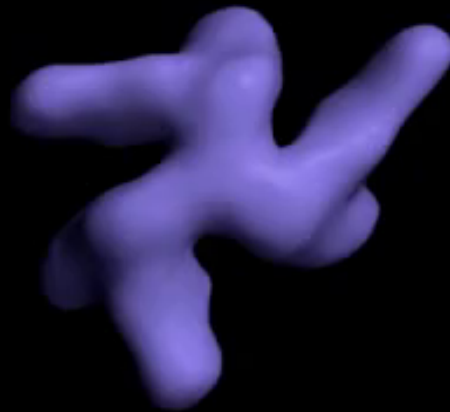








# Piecing it all together



unliganded

b12-bound

CD4-bound

# Conformational changes of the trimeric spike that occur upon CD4 binding

