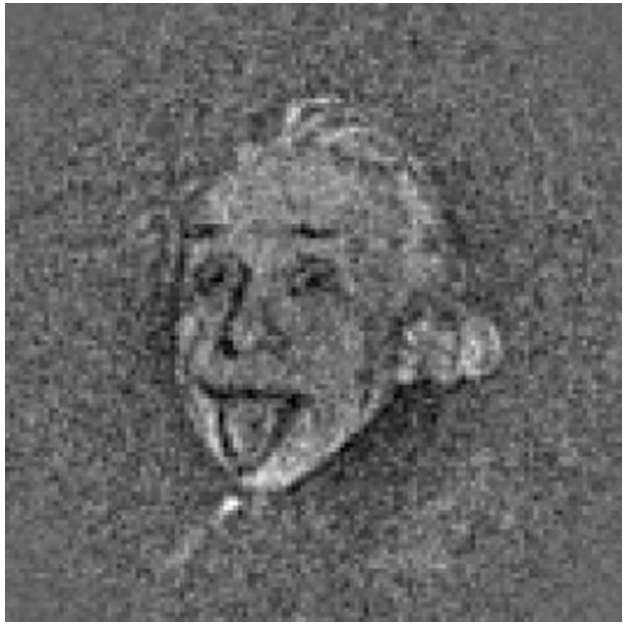
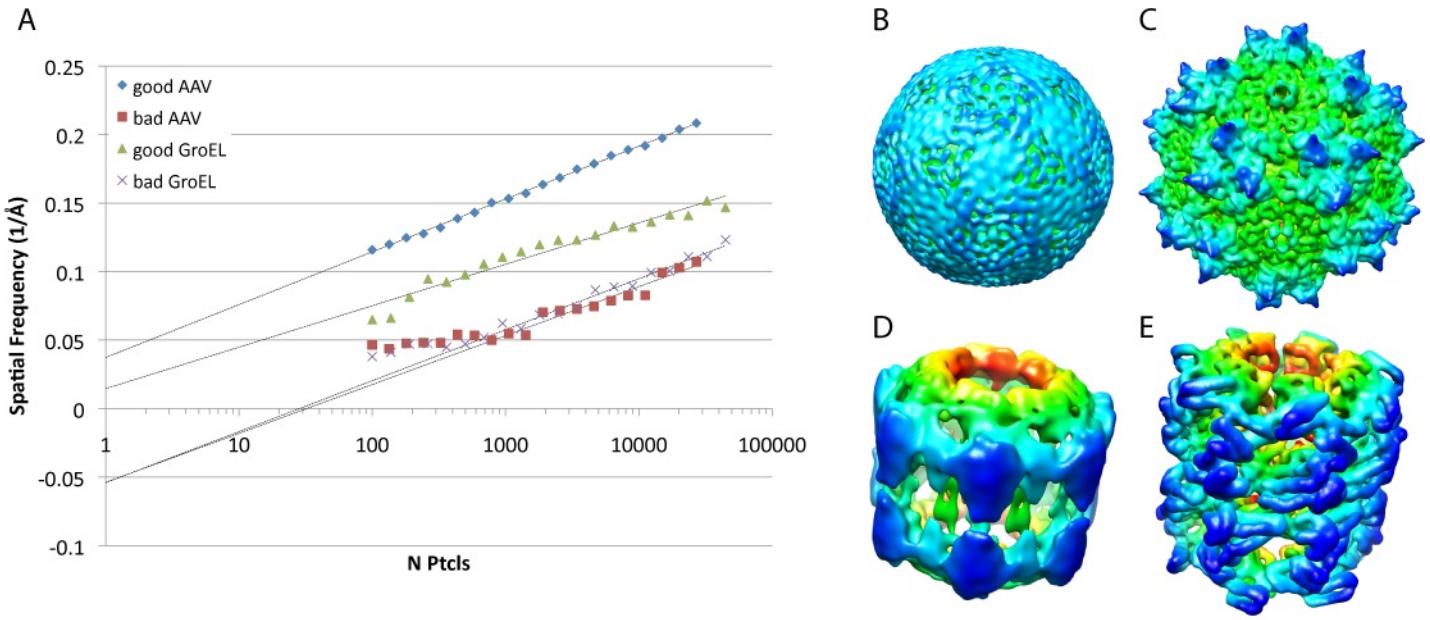
Single particle caveats

- Initial model bias
  - Since the images are so noisy, it is possible to regenerate an image of a reference from aligning pure noise



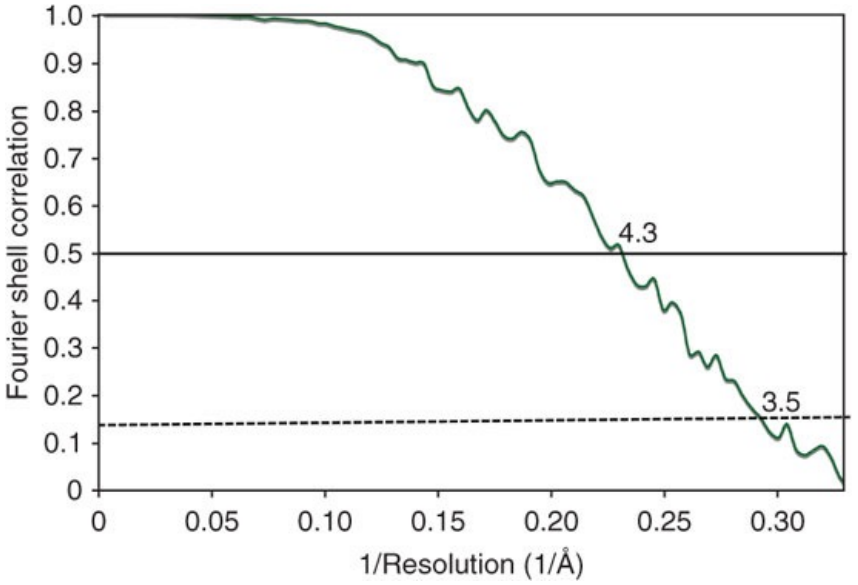
It is possible to get a “high resolution” structure from a completely incorrect reconstruction

Figure 3

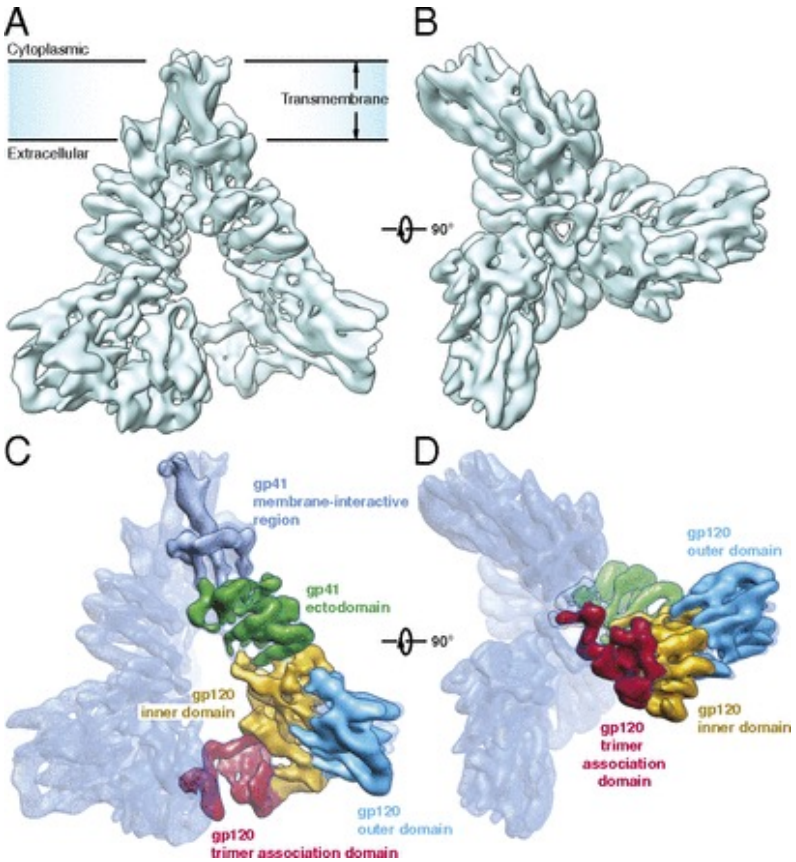


Artificially inflated resolution

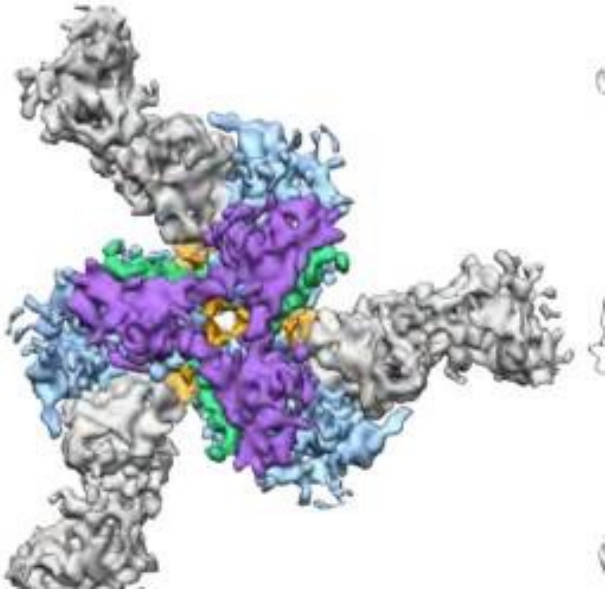
- Reconstruction may be “correct”, i.e. show the expected biological features, but the reported resolution may be more than is justified
  - Arises from noise bias
    - Through iterations of refinement noise can start to correlate with itself
    - Shifts FSC curve to the right



Infamously “wrong” structure of HIV trimer



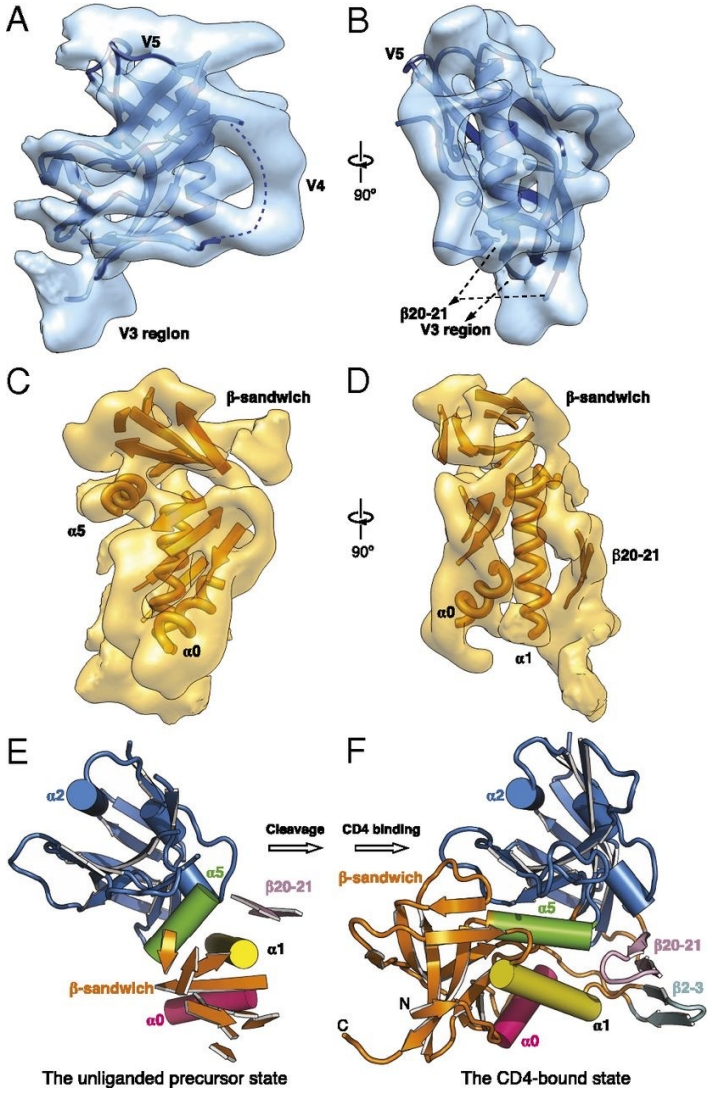
Vs



Mao et al. PNAS 2013

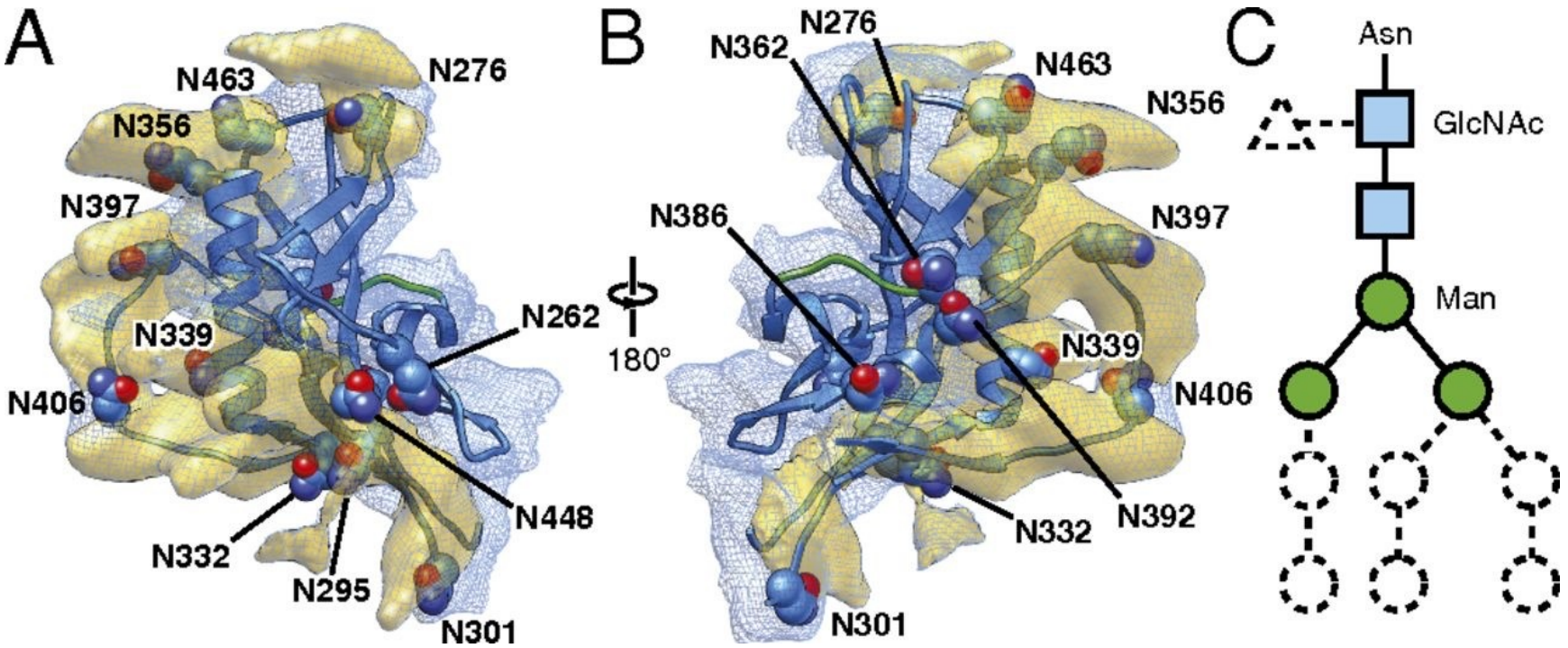
Lyumkis et al. Nature 2013

Mao *et al.* sins



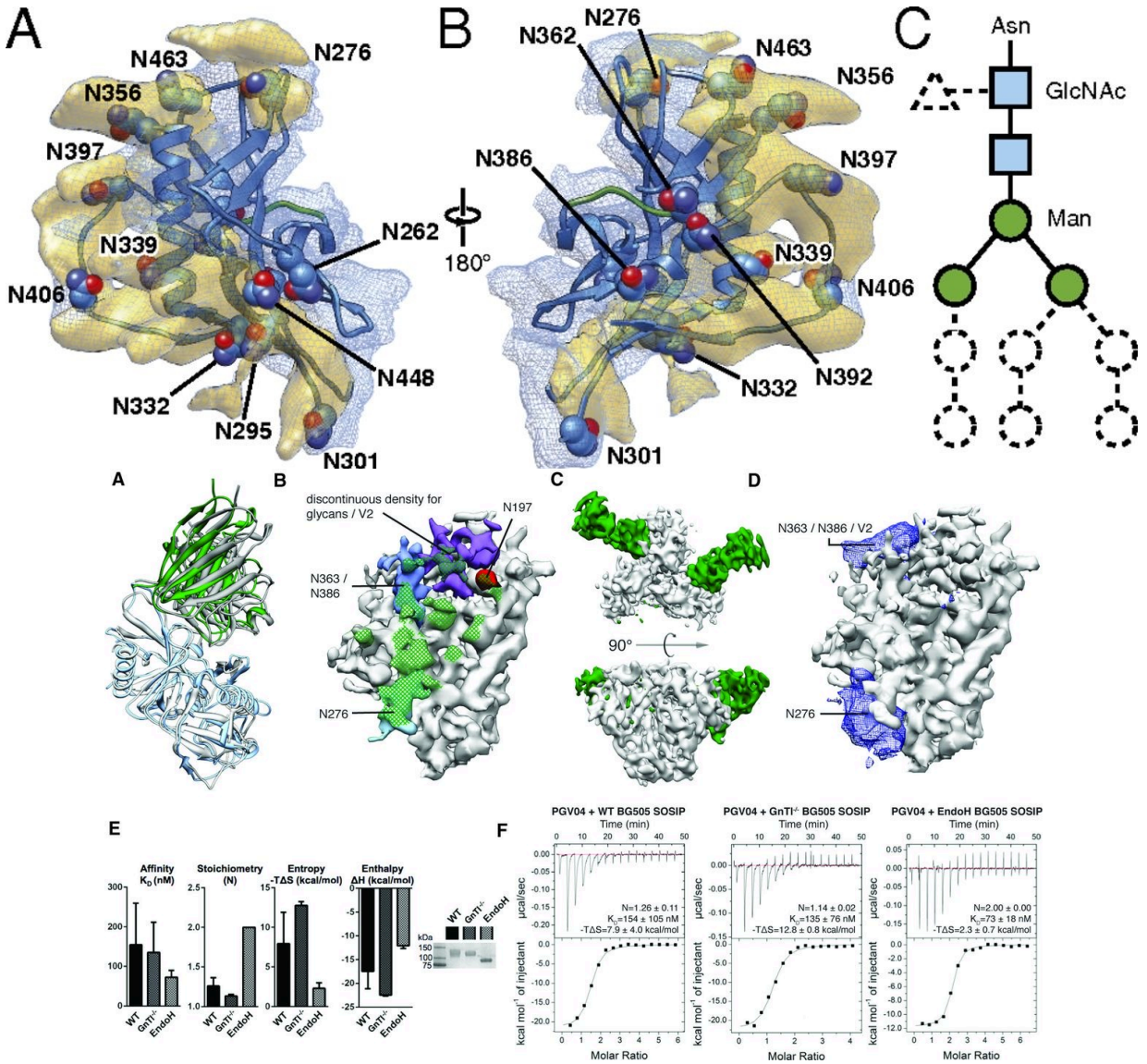
- Flexible fitting with a huge conformational change
  - No validation – Booooo!
  - Extraordinary claims require extraordinary evidence

Mao *et al.* sins

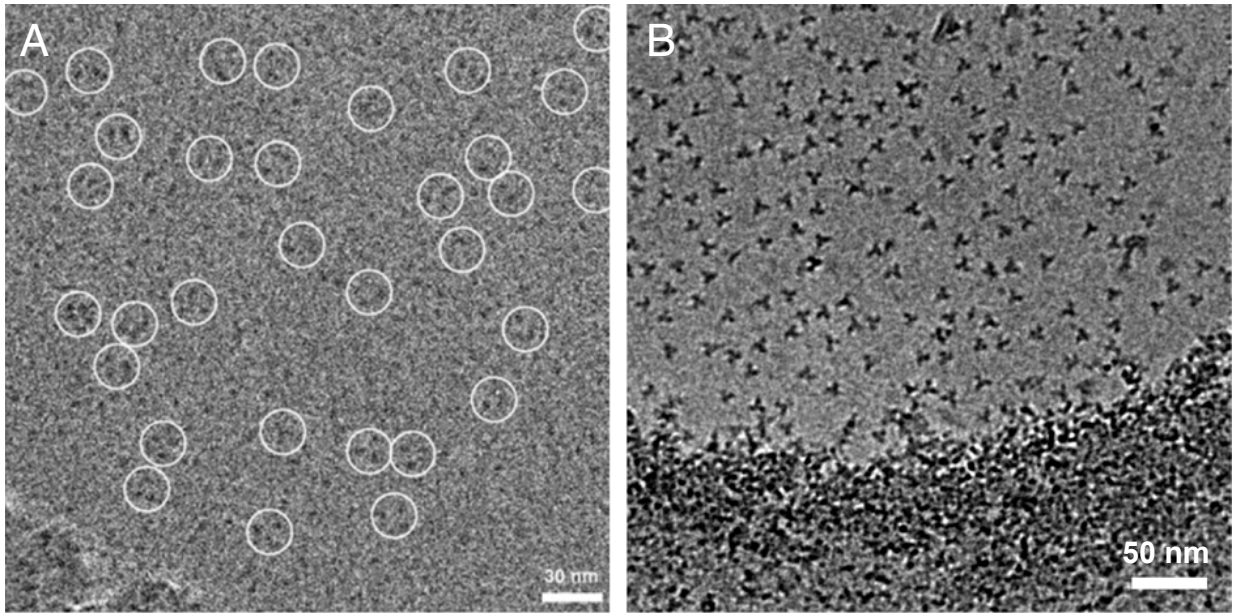


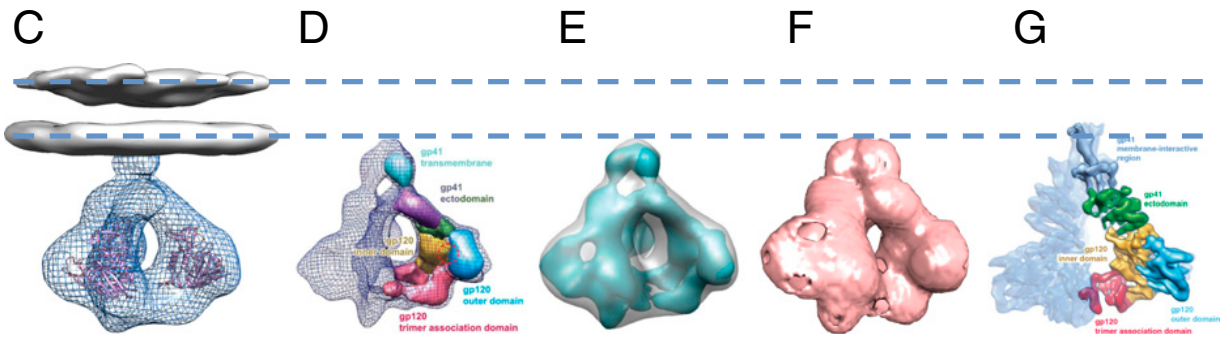
- Occam's Razor was not employed
  - Why should we think that those densities don't belong to the protein?

Compare to Lyumkis *et al.*



Other sins –particles are nearly invisible





Three response papers claim Mao *et al.* is artifactual

- *Finding trimeric HIV-1 envelope glycoproteins in random noise* – Van Heel, PNAS, 2013
- *Structure of trimeric HIV-1 envelope glycoproteins* – Subramanaim, PNAS, 2013
- *Avoiding the pitfalls of single particle cryo-electron microscopy: Einstein from noise* – Henderson, PNAS, 2013