Some basic concepts of cryo-EM & 3D reconstruction

Correct terms are important (be precise and rigorous in science :-)

By cryo-EM, we obtain:

- a "3D reconstruction" (initial or refined)
- a "cryo-EM map" or "density map"
- a "structure"

technically:

- back-projection
- angular reconstitution
- random conical tilt
- tilt series / tomogram

NOT:

- an "envelope" (would be SAXS or neg. stain. EM)
- a "volume", units would be Å^3 (e.g. volume of a pocket, volume x density = mol. mass)
- a "surface", units would be Å^2 (e.g. interaction surface between 2 proteins)
- a "model", would be a molecular model *fitted to* the map (crystallography/cryo-EM)
  or a model *compatible with* SAXS data or NMR restraints;
  other "models": "homology model", "hypothetical model", "working model"

Some basic concepts of cryo electron microscopy

Correct terms are important:

A classification is based on a statistical analysis:

- multivariate statistical analysis (MSA) provides information on variance (variability) which serves to merge similar images into class averages (classes); is *independent* of a reference
- classes *are NOT*: the sum of images that correlate best with a reference (through a multi-reference alignment)

Some basic concepts of cryo electron microscopy

Basic aspects:

- "resolution" corresponds to "spatial frequency" in image processing (1/ Å)
- Nyquist frequency is = 2 x pixel size, e.g. 1 Å / pixel → Nyquist = 2 Å
- interpolations during 2D image alignment and 3D reconstruction limit the possible resolution to about 2/3 of the Nyquist frequency, i.e. here ~ 3 Å *(exception: super-reso)*

pixels in 3D: "voxel"

Consider:

- any correlation calculation (e.g. alignment) is biased by the reference used
- resolution estimation, criteria used:
  - 0.5, arbitrary, historically from the virus field, tends to underestimate resolution
  - 0.143 (Henderson) and ½ bit (van Heel)
  - 3 σ, not used anymore (over-estimation)
  - features in the map: can we see dsRNA helices (~10-12 Å resolution), α-helices (~8 Å), ß-sheets (~5 Å) or side chains (4-2.5 Å depending on size)?

Basic Steps in Single particle image processing and 3D reconstruction

I. Pre-processing

- Digitization of micrographs (negatives); not needed if CCD/CMOS images
- particle selection, « boxing »
- correction of the contrast transfer function
- band-pass filtering and normalisation of particle images

II. Structure determination

- particle centering / alignments
- MSA (multivariate statistical analysis) + classification
- angle assignment
  - angular reconstitution
  - projection matching
- 3D reconstruction
- structure refinement
- resolution assessment
- map interpretation; fitting of known structures, atomic model building…