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 (at least formally):

- 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", "error 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; useful for noise 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)?**