

2023 Biophysical Society
CryoEM Subgroup
Sat Feb 18

WHAT TO UPLOAD WITH YOUR SINGLE PARTICLE EM MANUSCRIPT

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Scripps Research

<https://lander-lab.com/validation.pdf>



Community recommendations on cryo-EM data archiving and validation

Outcomes of a wwPDB/EMDB workshop on cryo-EM data management, deposition and validation, held January 23 & 24 2020, EMBL-EBI, Hinxton UK

Kleywegt, Adams, Butcher, Lawson, Rohou, Rosenthal, Subramaniam, Topf, Abbott, Baldwin, Berrisford, Bricogne, Choudhary, Croll, Danev, Ganesan, Grant, Gutmanas, Henderson, Heymann, Huiskonen, Istrate, Kato, Lander, Lok, Ludtke, Mitsuoka, Pie, Pintilie, Richardson, Sachse, Salih, Scheres, Sorzano, Stagg, Wang, Warshamanage, Westbrook, Winn, Young, Burley, Hoch, Kurisu, Patwardhan, Velanka

PUBLICATION IN PREPARATION

**UPLOAD YOUR MAPS, MODELS,
AND VALIDATION REPORTS
WITH YOUR MANUSCRIPT!**

Semantics

- ▶ EM maps are NOT “electron density”
- ▶ “Dose” is a volumetric measurement, reported in \AA^3
 - ▶ Instead of “total dose,” use “electron exposure” or “fluence”
 - ▶ Instead of “dose rate,” use “exposure rate” or “flux”

Sample Preparation:

- ▶ **Concentration of sample applied to the grid**
- ▶ **Type of grid used (hole size, spacing, etc.)**
- ▶ **Device and settings used for glow discharge of EM grids**
- ▶ **Type of blotting paper used for EM grid preparation**
- ▶ **Device and settings used for blotting and vitrification**

Data collection & Processing

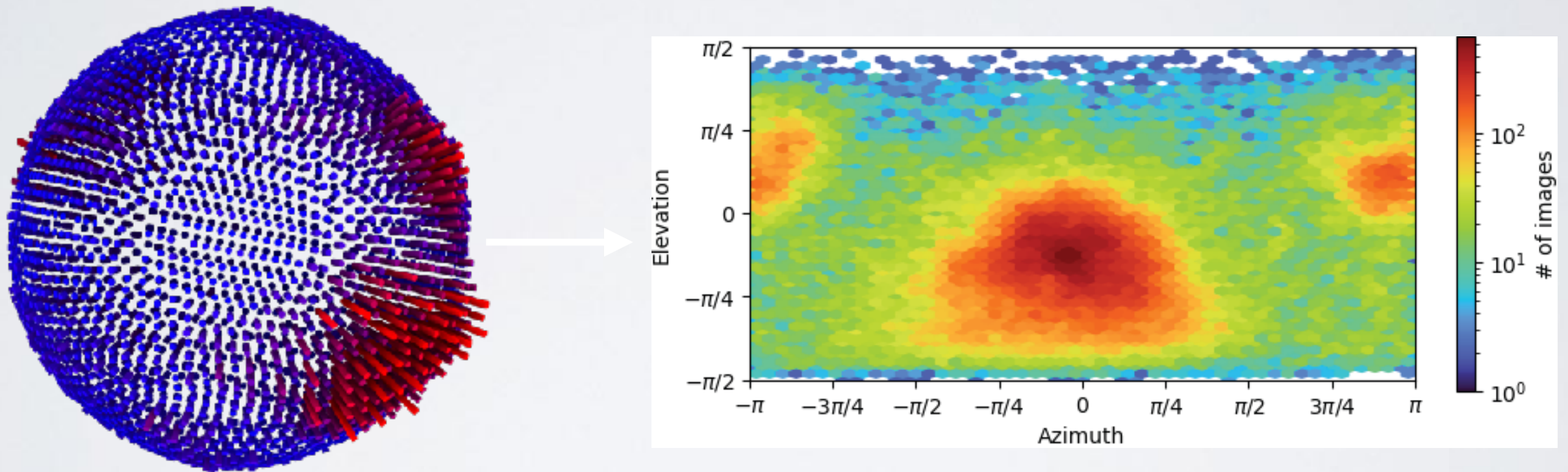
- ▶ Software was used for data collection, and how data were acquired (image shift or stage position). If image shift, specify if beam-tilt compensation used, and the maximum image shift.
- ▶ All non-default parameters used for classification and refinement (mask diameters, tau-fudge, e-step, # of classes, initial low pass filter, # of iterations, etc.)
- ▶ Detailed description of the atomic modeling methodology, including any relevant Phenix refinement parameters and any constraints (secondary structure, Ramachandran, etc.)

What to include in the supplement

- ▶ “representative” raw micrographs. They should be large enough so that particles are visible
- ▶ representative 2D averages showing well-defined structural details and a range of views
- ▶ Data processing workflow - include as much as you can (all classes, pixel size/scaling factor for each step of processing, 3D masks)

What to include in the supplement

- ▶ Euler Distribution Plot FOR EVERY MAP



What to include in the supplement

- ▶ **The FSC....**
 - ▶ **FSC IS NOT A MEASURE OF RESOLUTION!**

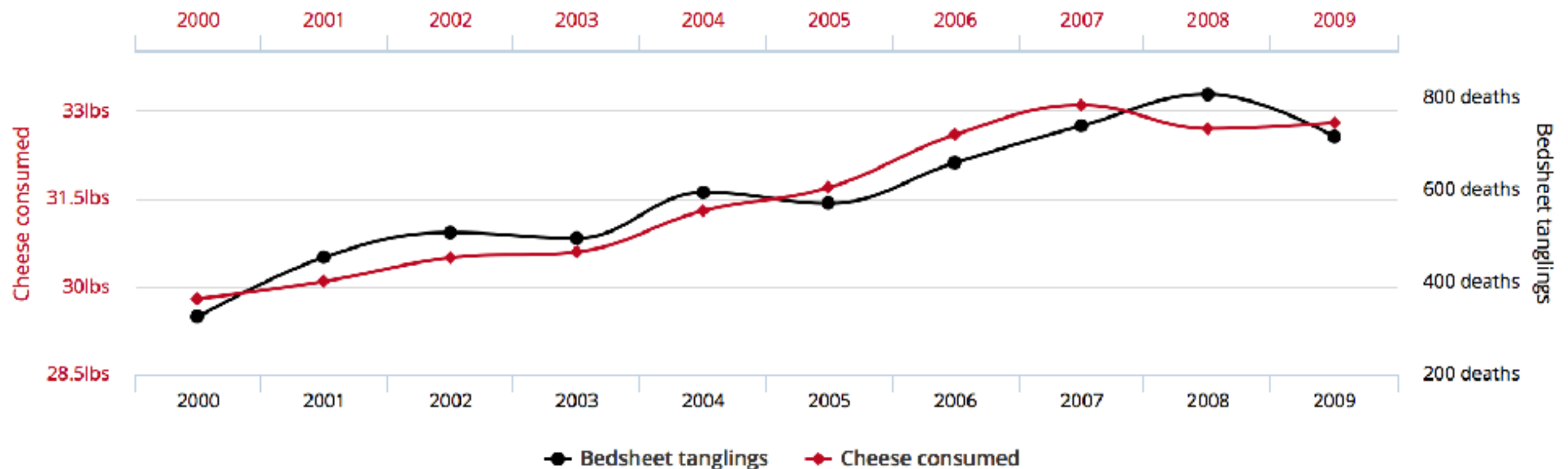
Be wary of correlation

Per capita cheese consumption
correlates with



Number of people who died by becoming tangled in their bedsheets

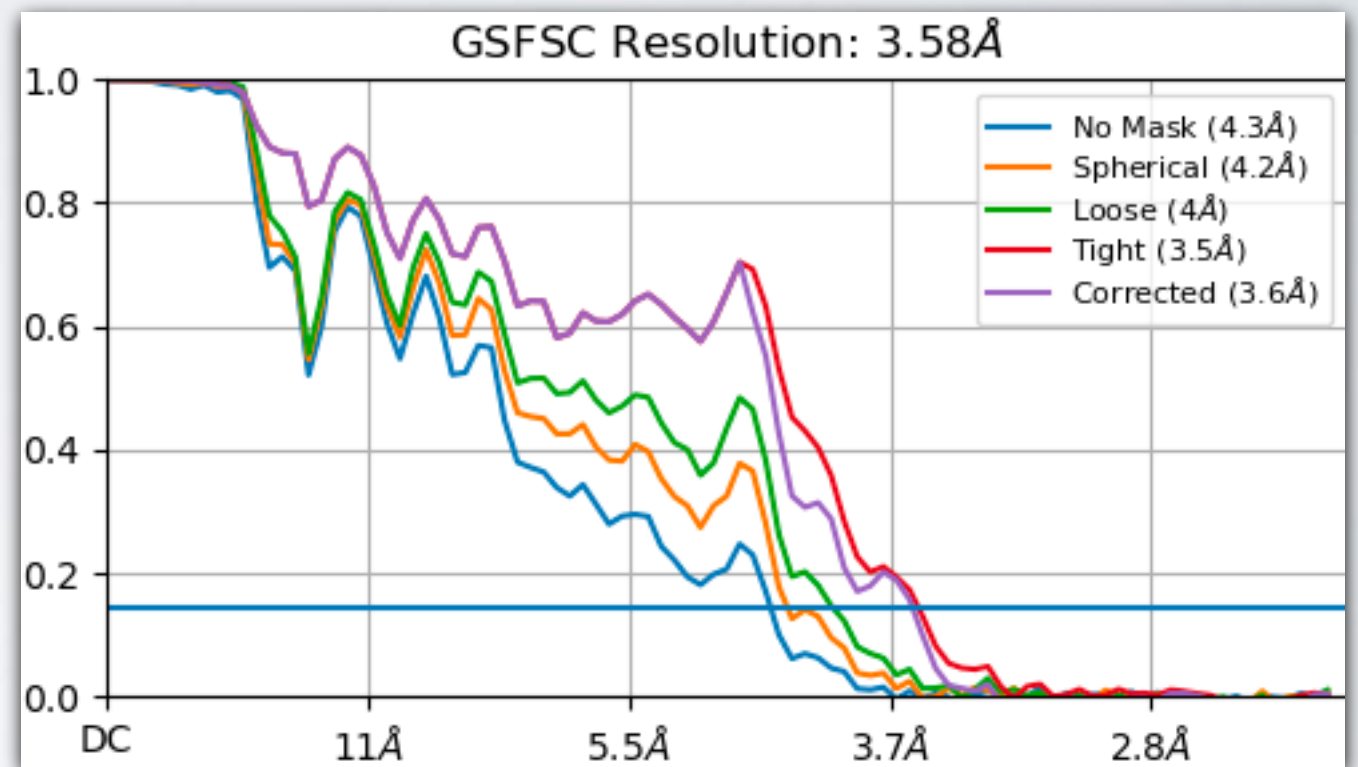
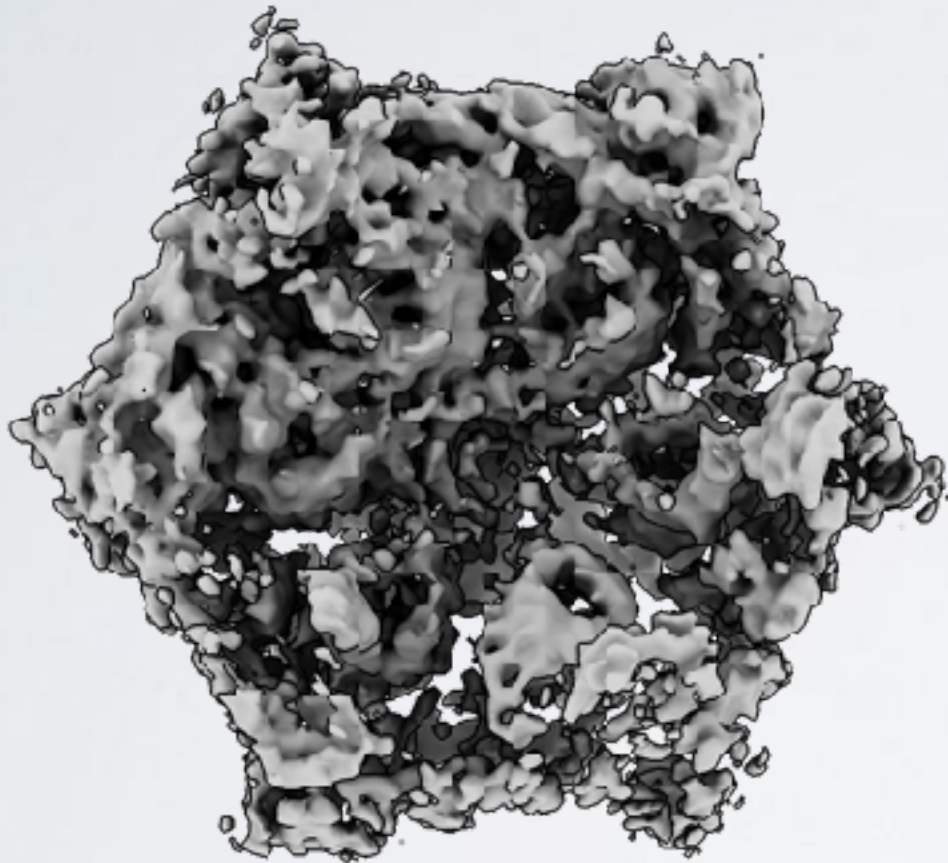
Correlation: 94.71% ($r=0.947091$)



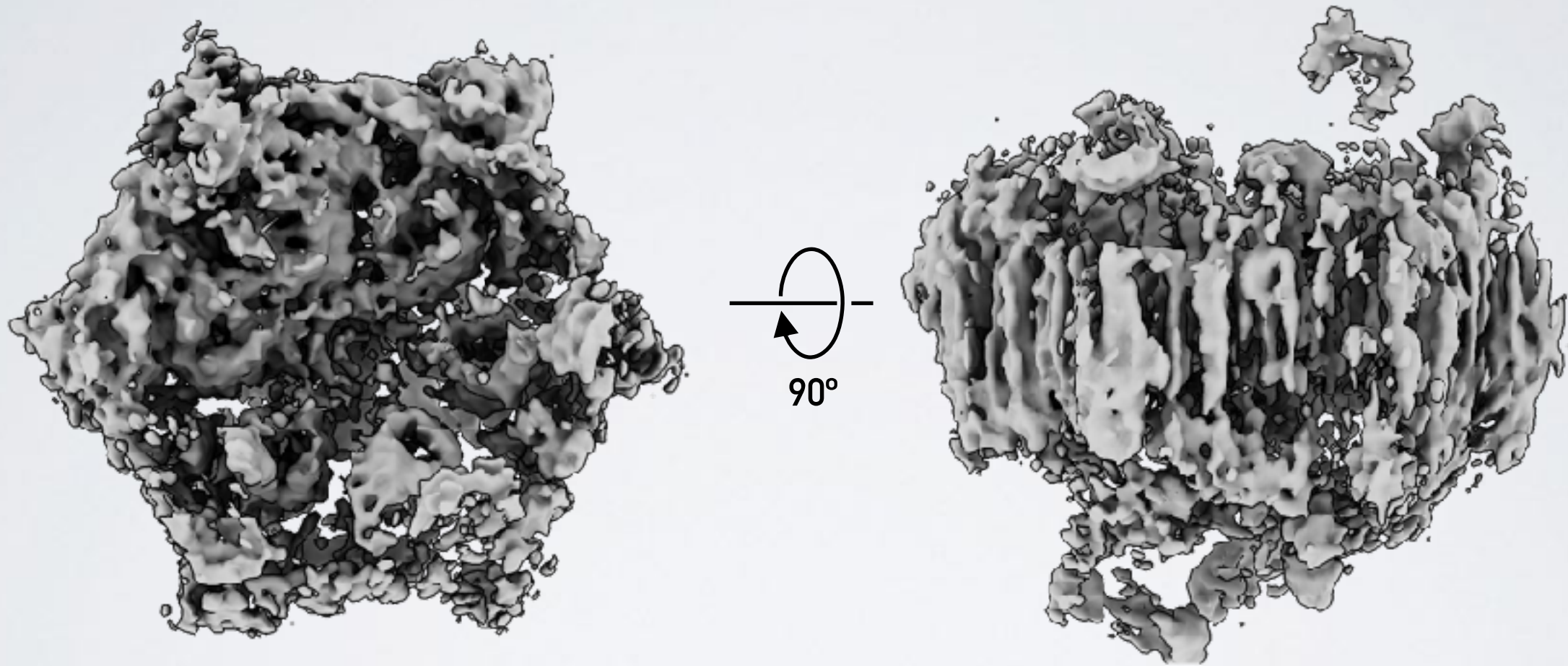
tylervigen.com

Data sources: U.S. Department of Agriculture and Centers for Disease Control & Prevention

Reported Resolution: 3.6 Å



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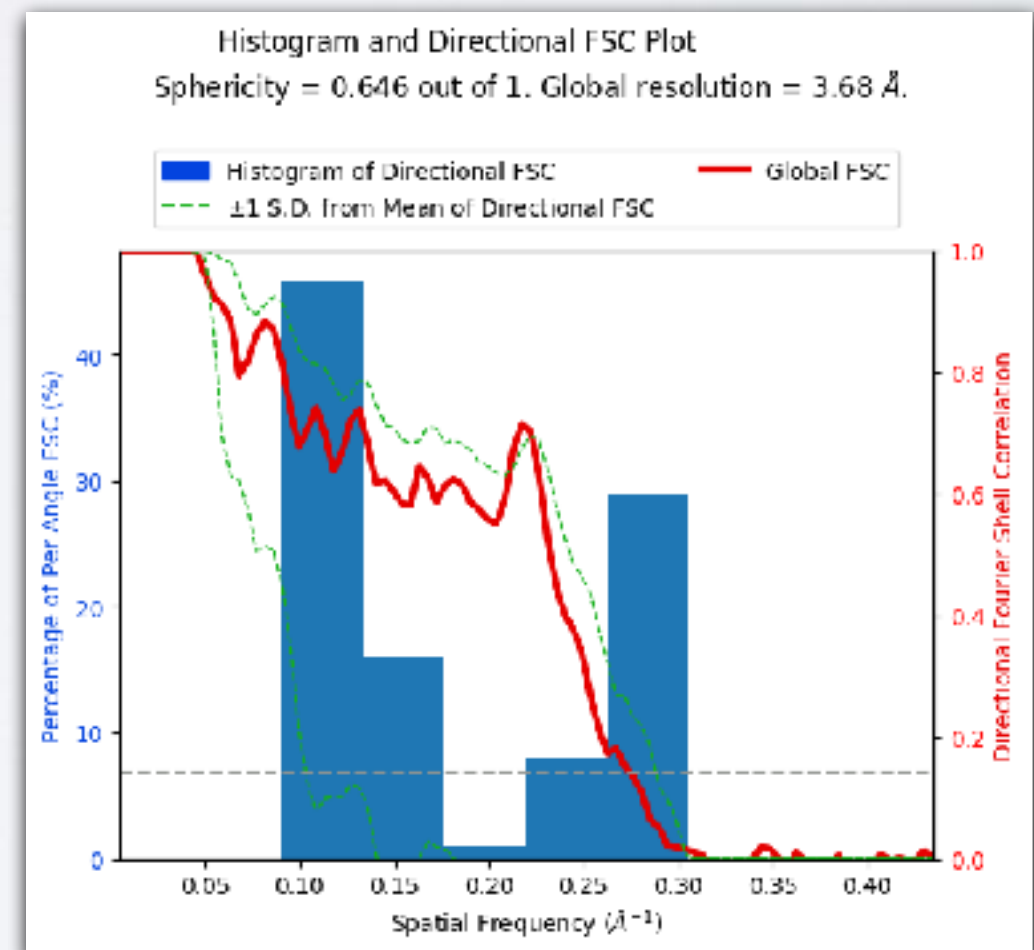
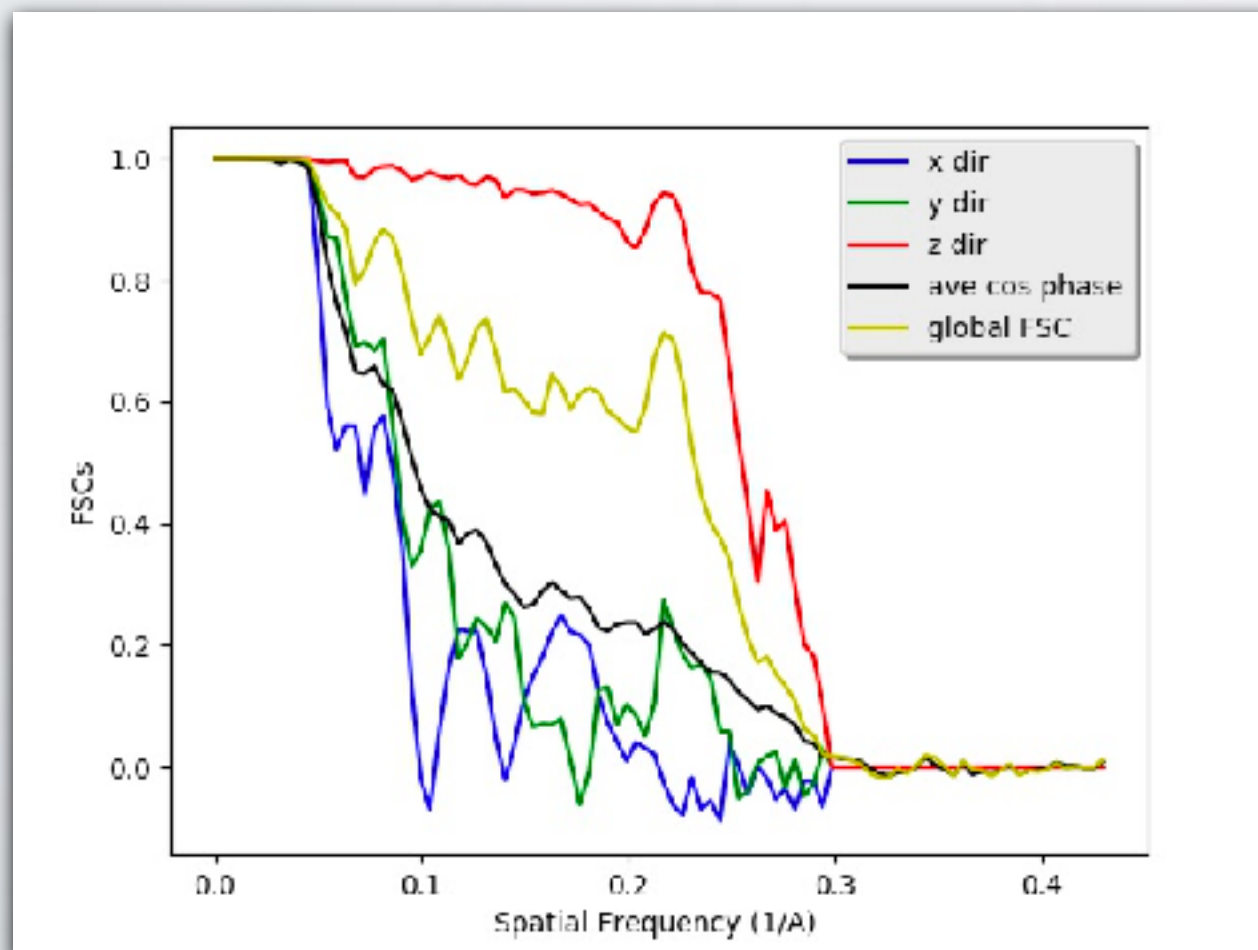
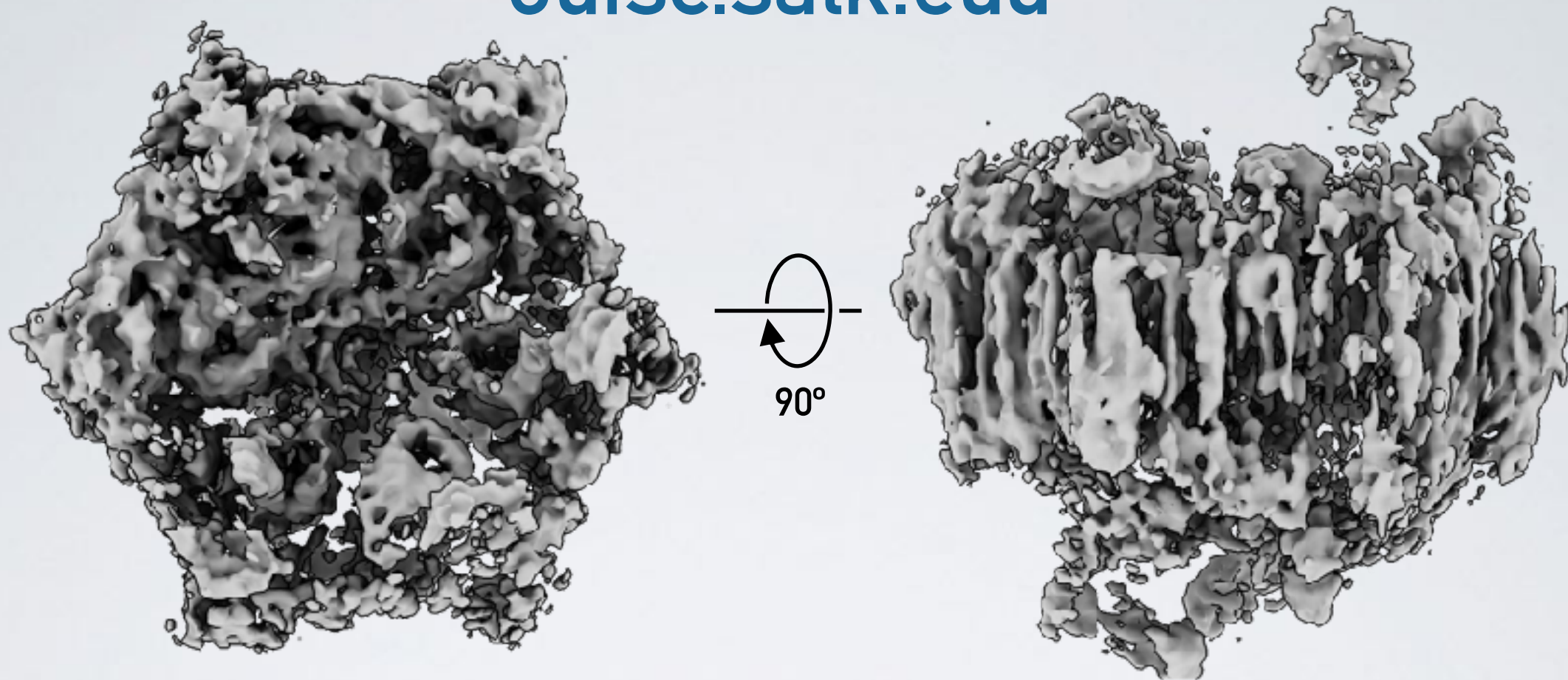
Remote 3DFSC Processing Server

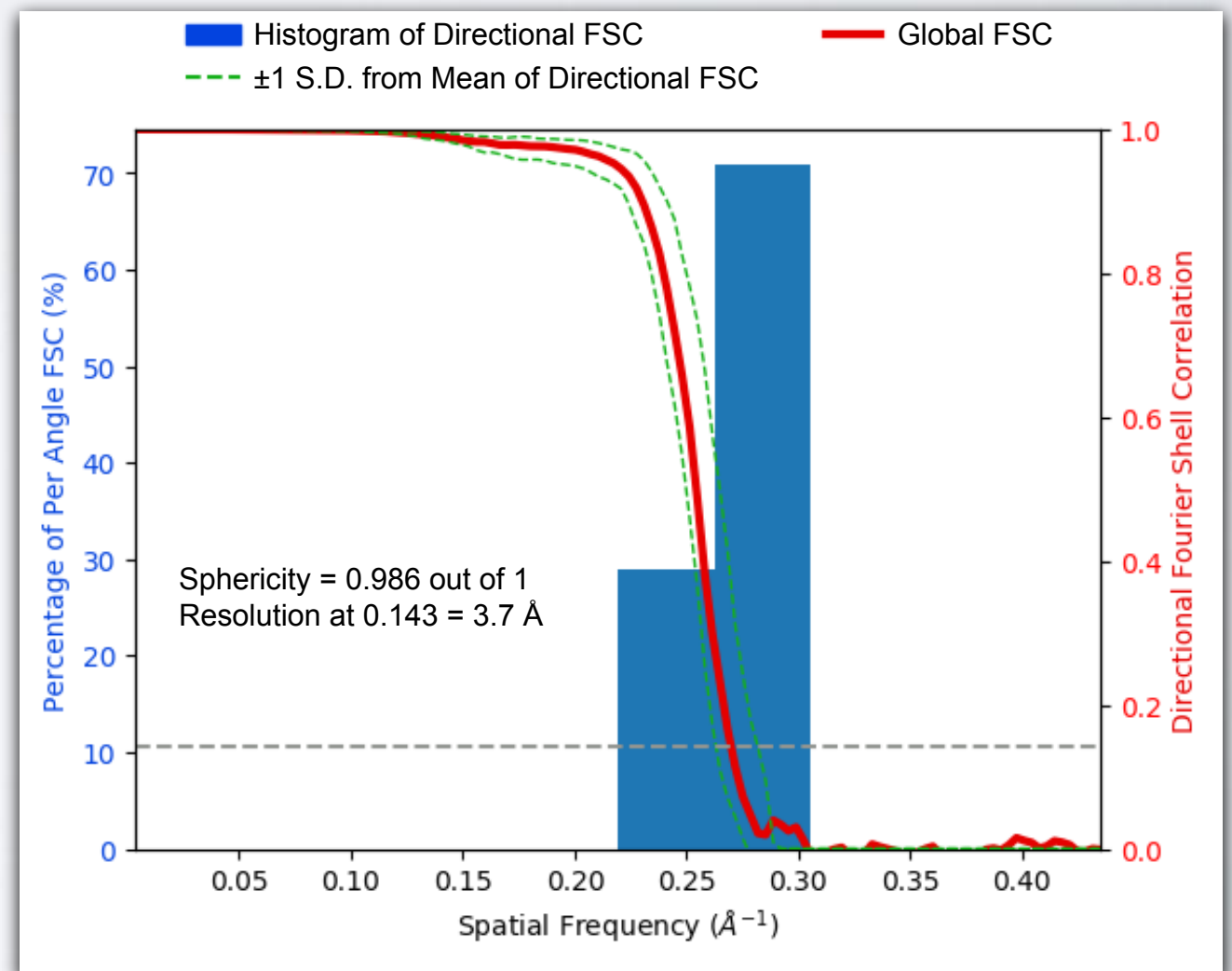
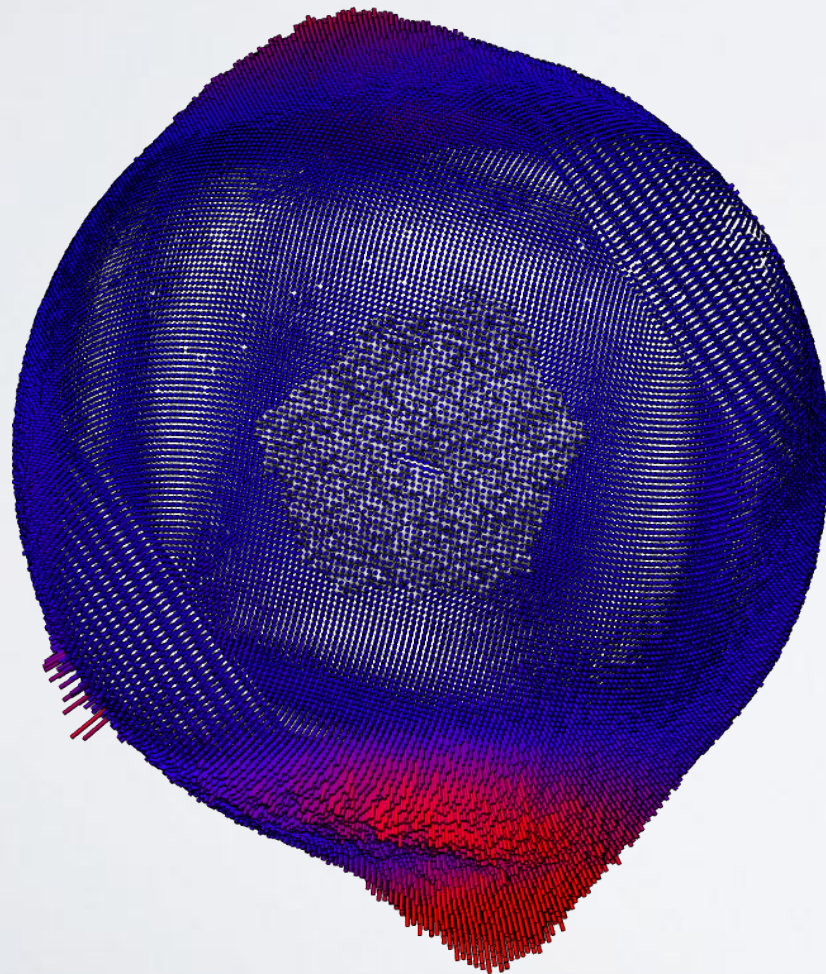
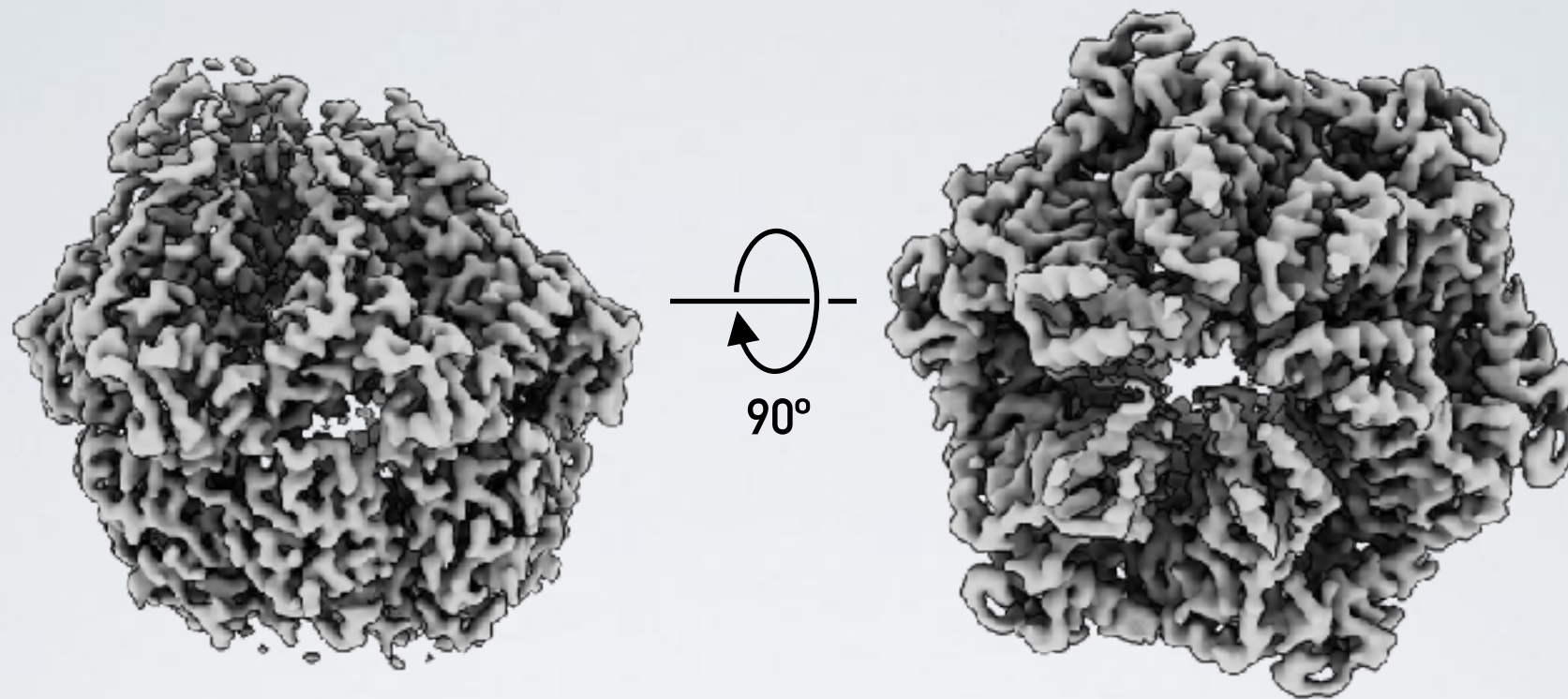
This is an application for remotely processing the 3D Fourier shell correlation of cryoEM maps.

Instructions:

- 1) Click "Register" on the navigation bar and follow the instructions to create an account.
- 2) Navigate to the processing form via the "Submit job" link.
- 3) Enter your email address and other required parameters in the form. You must upload a job name, two half maps (.mrc format), a full map (also .mrc format), and an appropriate pixel size. Click "Submit job".
- 4) You should receive an email to confirm your processing job. If you do not receive an email, please check your spam folders.
- 5) When your job is complete, you will receive another email with a link to view the results.







Reporting Resolution Anisotropy

Measuring the effects of particle orientation to improve the efficiency of electron cryomicroscopy

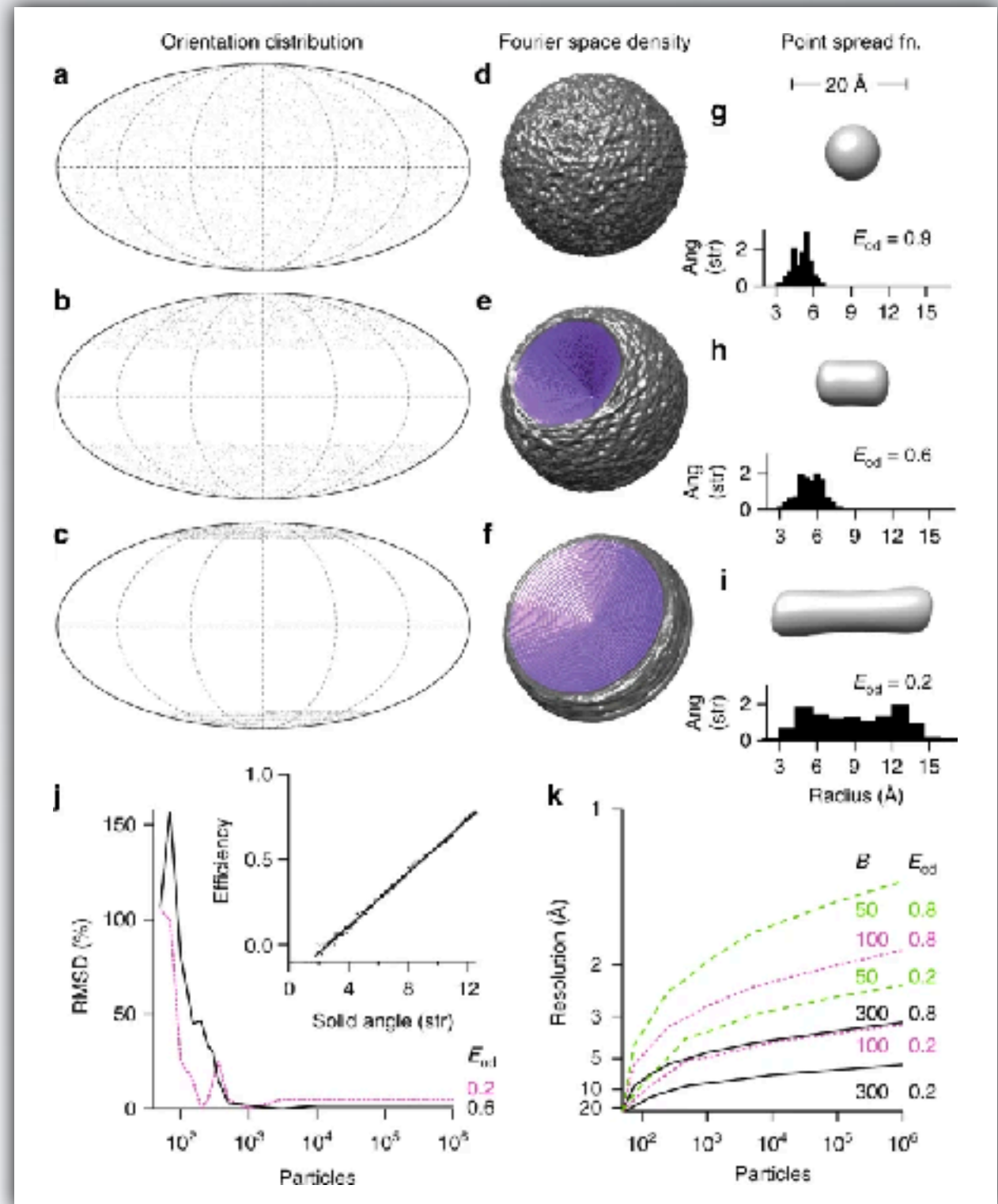
Katerina Naydenova & Christopher J. Russo

Nature Communications 8, Article number: 629 (2017) | [Cite this article](#)

Defines uniformity of resolution by characterizing the point spread function of the map (E_{od})

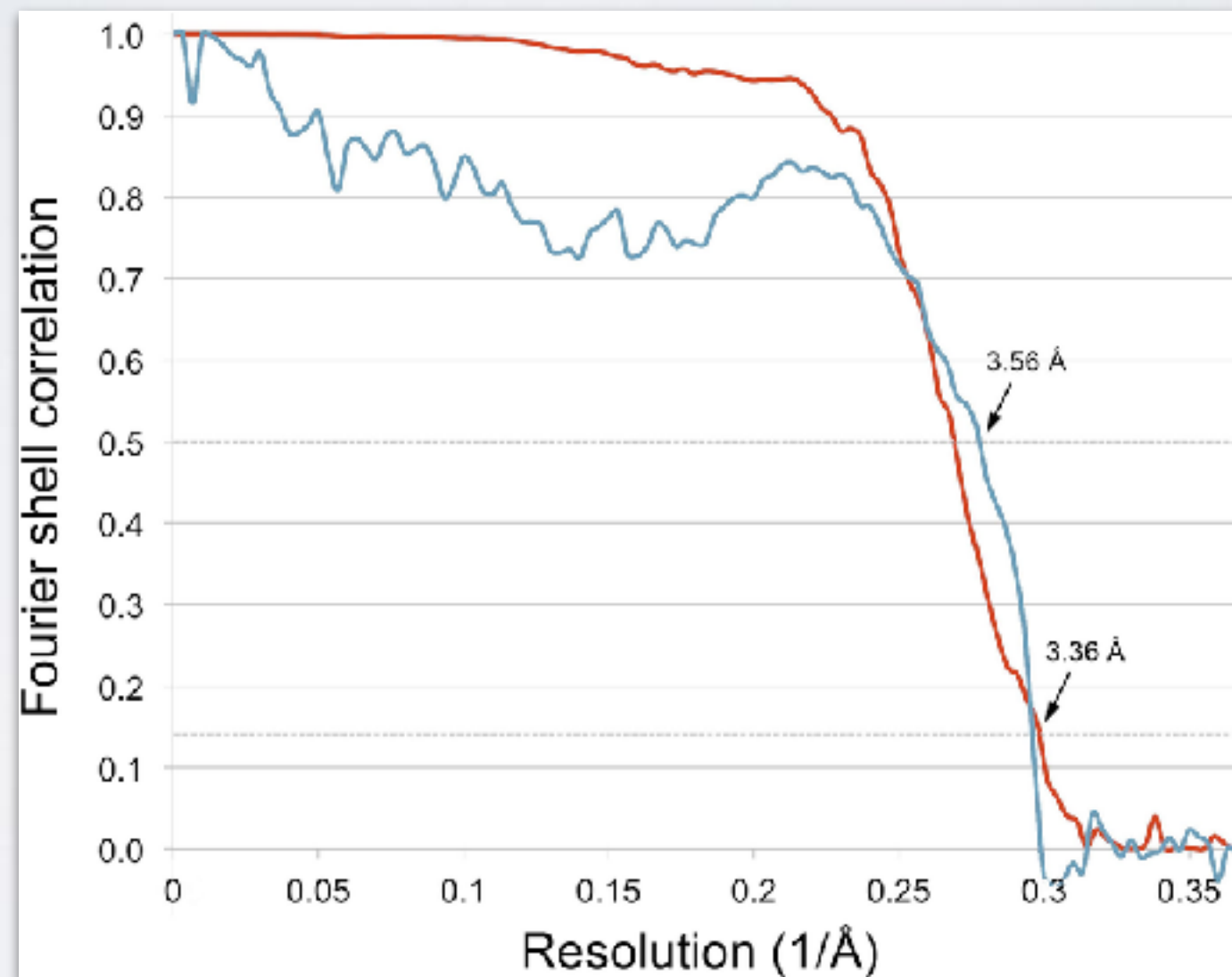
Download cryoEF:

<https://www.mrc-lmb.cam.ac.uk/crusso/cryoEF/>

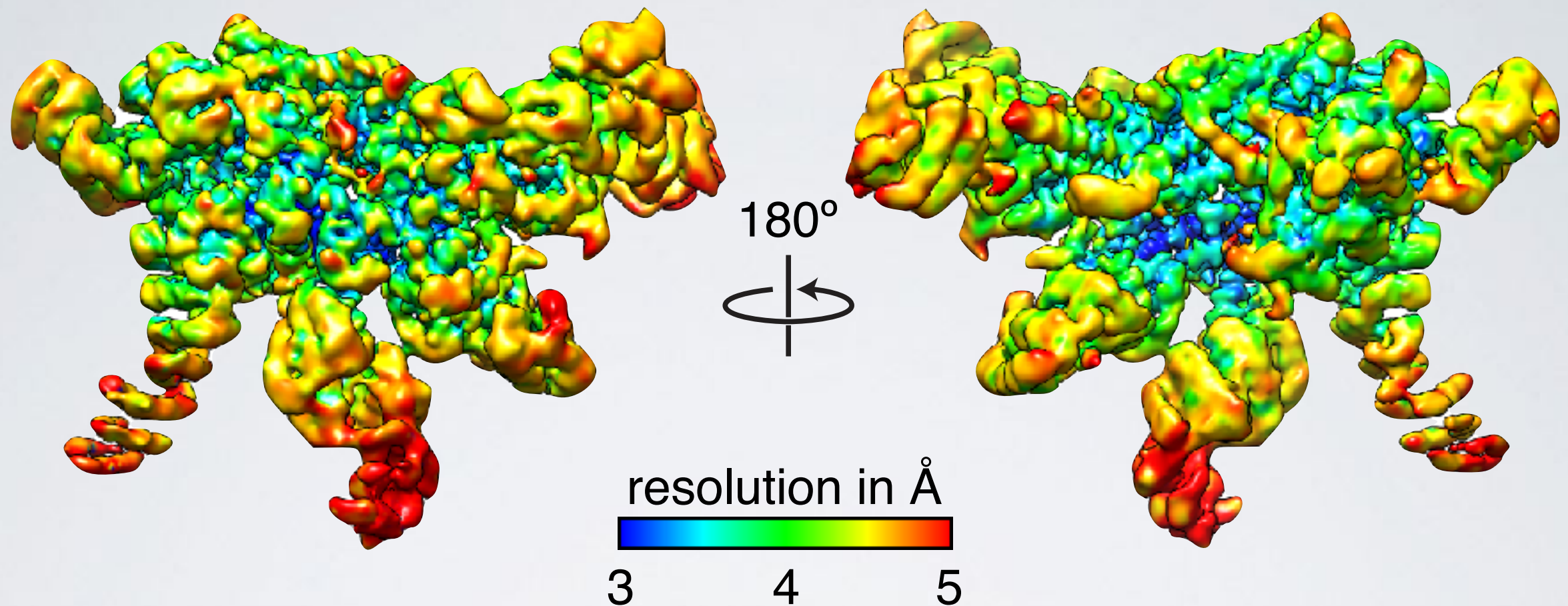


What to include in the supplement

- ▶ 3D FSC
- ▶ A Model-to-map FSC



What to include in the supplement



LOCAL RESOLUTION MAP FOR EACH MAP

Resmap - compares power of Fourier components

Bsoft - calculates windowed FSCs

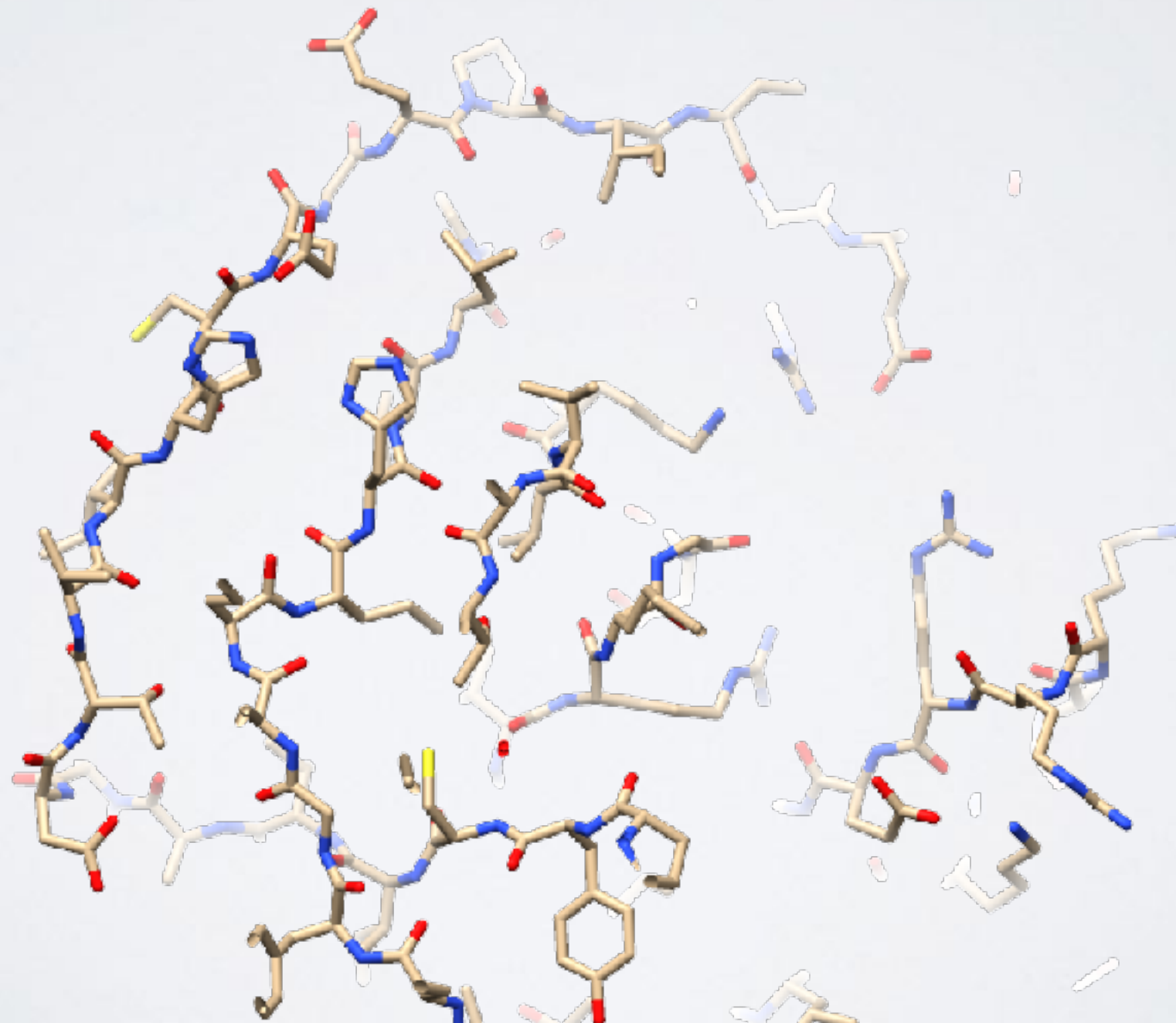
RELION - calculates windowed FSCs

Sparx - calculates local variance from 2D images

CryoSPARC - calculates windowed FSCs

What to include in the supplement

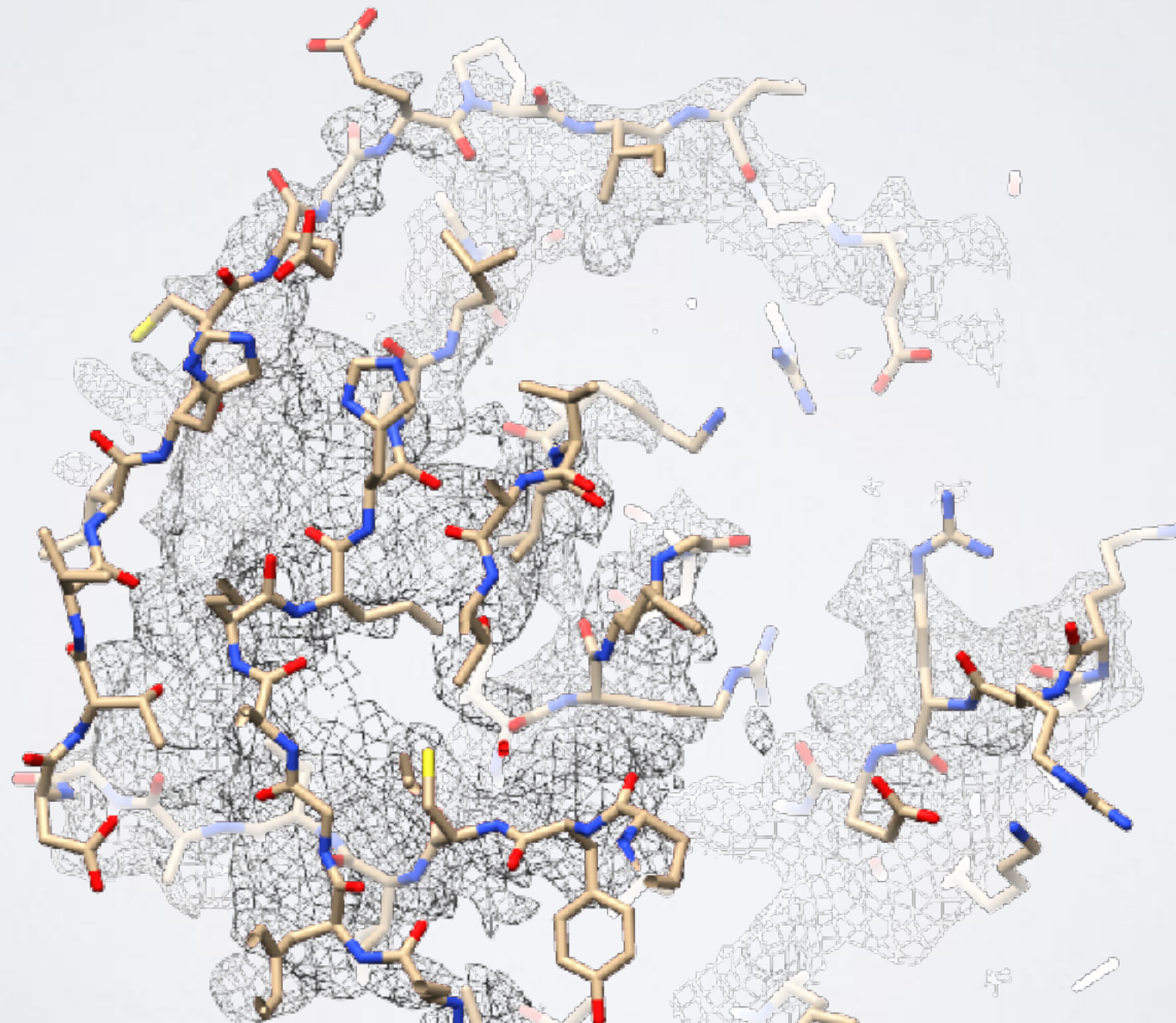
- ▶ If there are important interactions described in the text, include figures that include the EM density to support the modeled structure and interactions.



EMD-3295
2.3 Å

What to include in the supplement

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EMD-3295
2.3 Å

What to include in the supplement

- ▶ "Zoned" densities: Either the density for surrounding residues should be included in the figure, or a supplementary figure depicting this region with density should be included.

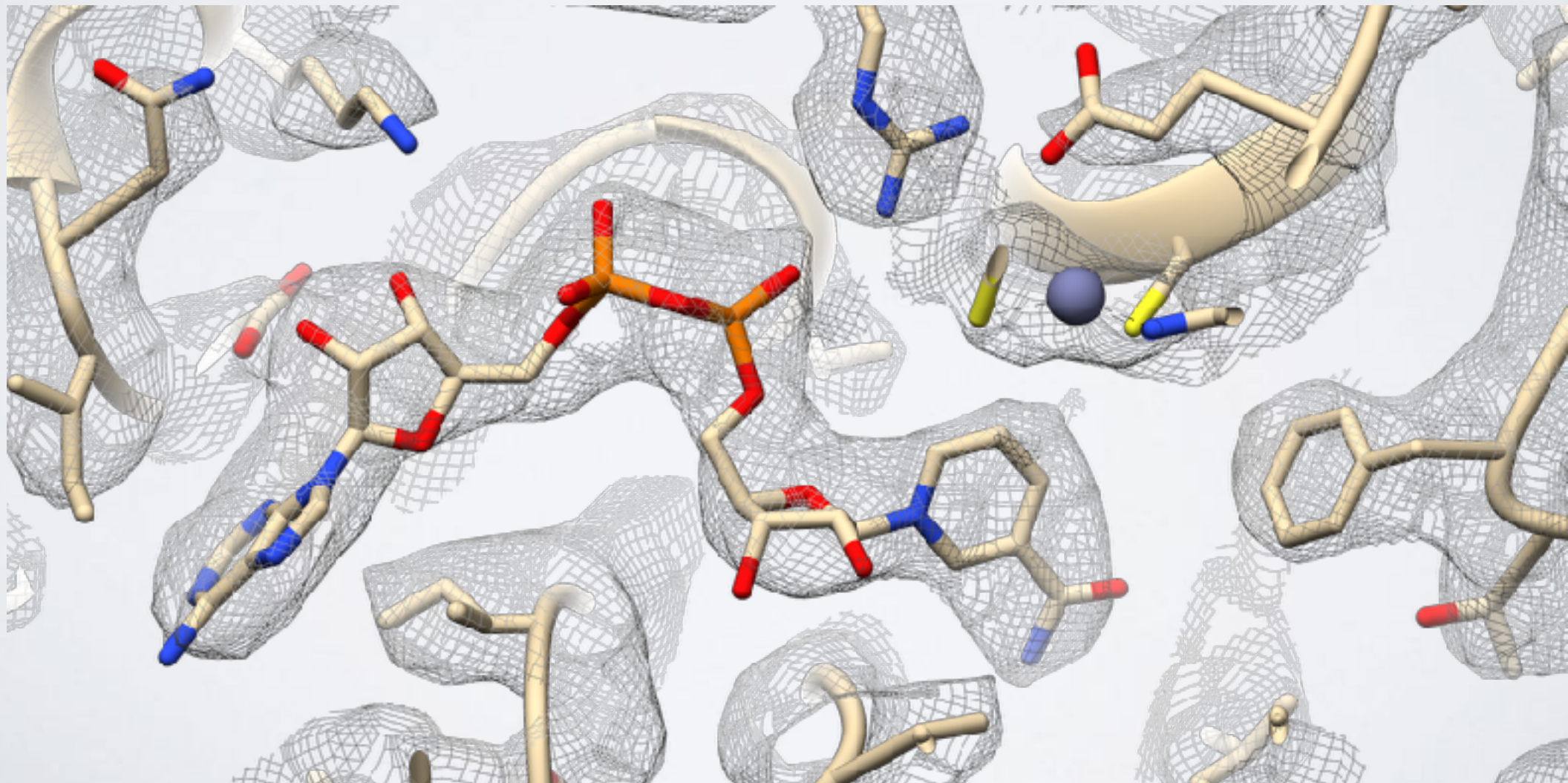


TABLE 1 - Data collection

- ▶ EMDB & PDB IDs
- ▶ Microscope & Detector
- ▶ Magnification & pixel size of images in Å
- ▶ Voltage (kV)
- ▶ Defocus Range (µm)
- ▶ Total electron exposure & Exposure rate (fluence & flux)
- ▶ # of frames collected per movie
- ▶ Energy filter slit width (eV, if applicable)
- ▶ Automation software (EPU, SerialEM, Leginon, Latitude...)
- ▶ # of micrographs used

TABLE 1 - Data processing

- ▶ Image Processing package(s) used for reconstruction
- ▶ Particle numbers: # extracted, # used for 3D, # in final map
- ▶ Estimated error of translations/rotations (RELION only)
- ▶ Resolutions: unmasked & masked FSCs at 0.143
- ▶ Local resolution range (a histogram SI fig is preferable)
- ▶ 3D FSC sphericity value
- ▶ Map sharpening B factor (\AA^2) / (B factor Range)

TABLE 1 – Modeling

- ▶ Atomic modeling refinement package
- ▶ Model composition (protein, ligands, DNA/RNA)
- ▶ CCvolume/CCmask
- ▶ B-factors of protein residues & ligands
- ▶ R.m.s. deviations from ideal values
- ▶ MolProbity score
- ▶ Clash score
- ▶ Poor rotamers (%)
- ▶ Ramachandrans (Favored, Outliers (%))
- ▶ CaBLAM outliers (%)
- ▶ EMRinger score
- ▶ Average Q-score

<https://lander-lab.com/validation.pdf>

