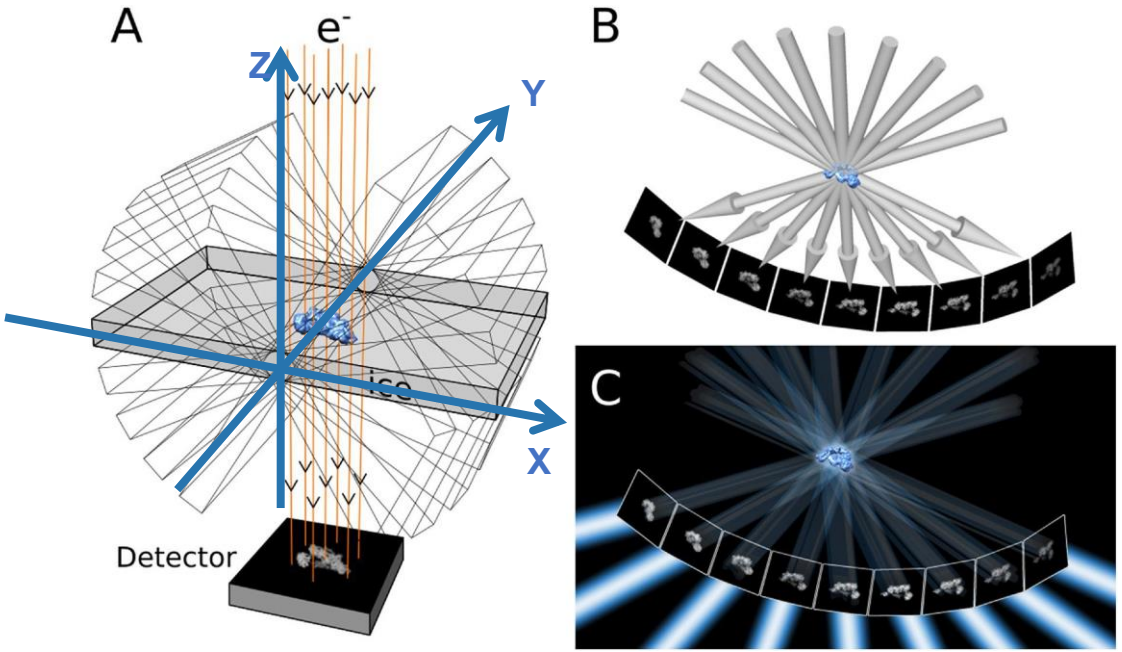
UCLA

Isotropic reconstruction for electron tomography with deep learning

Yun-Tao Liu
Postdoc

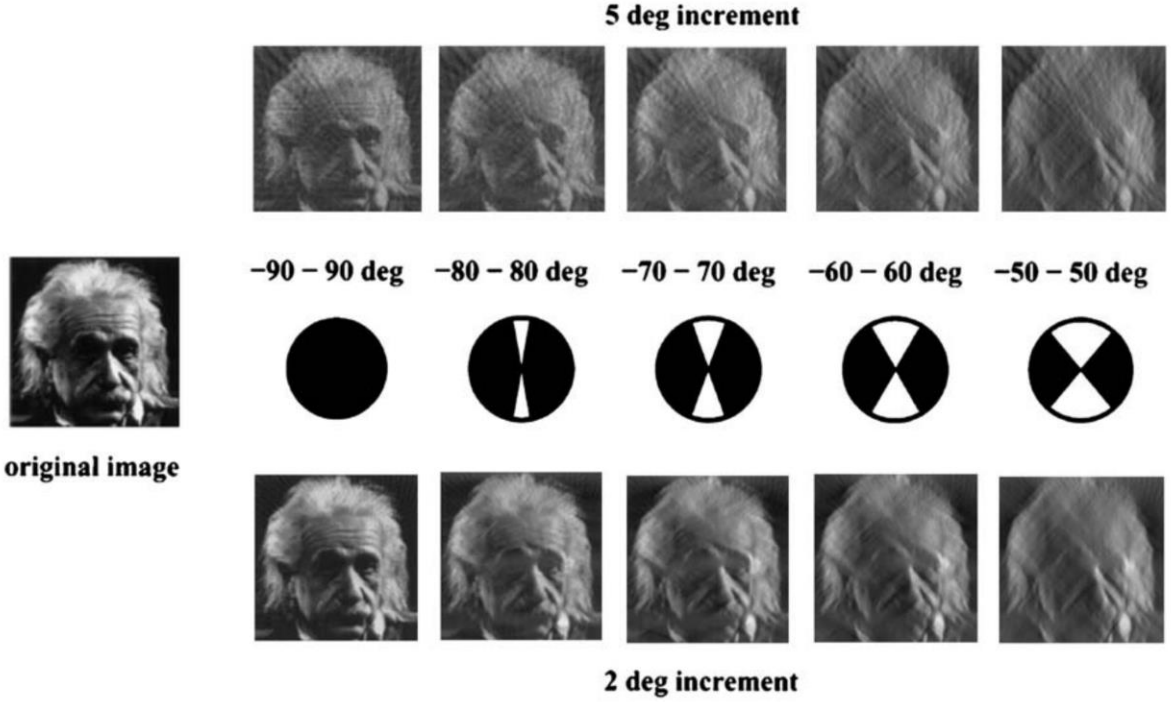
Hong Zhou lab at California Nanosystems Institute, UCLA

cryo electron tomography and 3D reconstruction

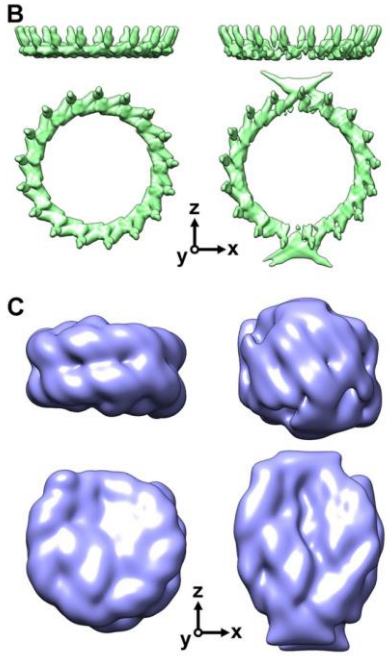


1. **Low signal to noise ratio**: limited electron dose distributed on the images.
2. **Missing wedge artifact**: limited tilt angle range

The influences of missing wedge on cryo-tomograms

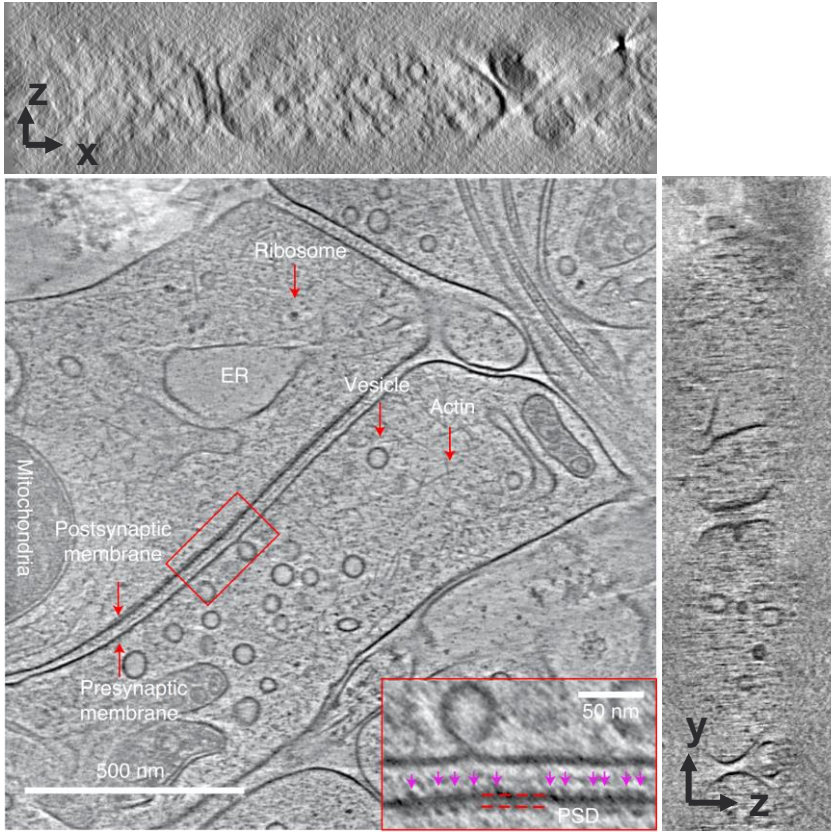


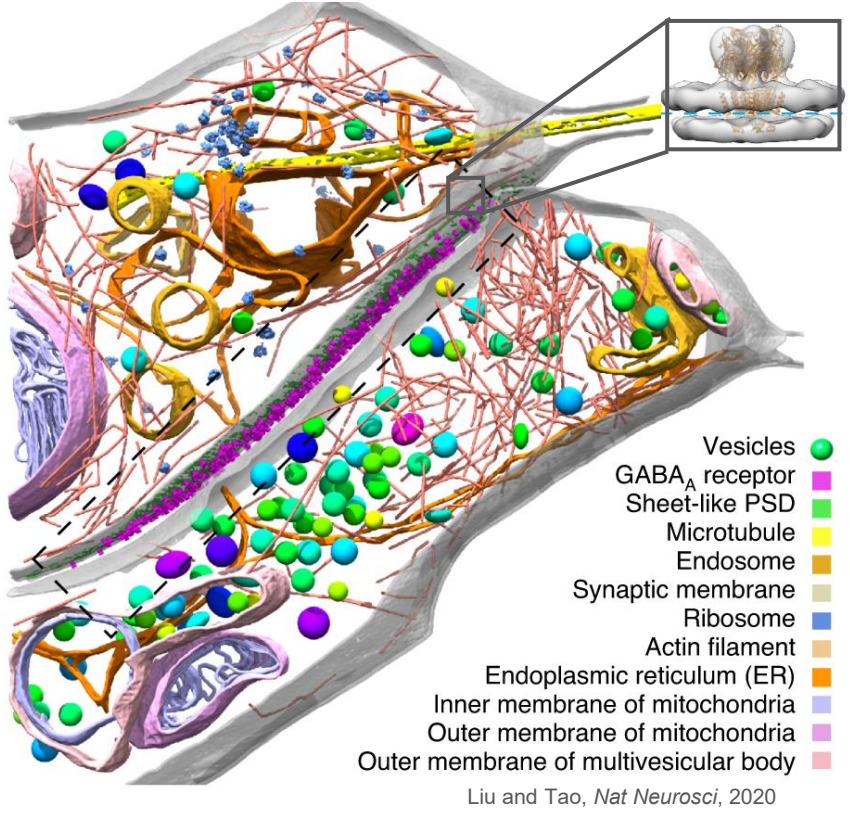
Nudelman F, de With G, Sommerdijk NAJM ,Cryo-electron tomography: 3- dimensional imaging of soft mater, 2011, Soft Mater (7)



Ng and Gan, MBoC 2019

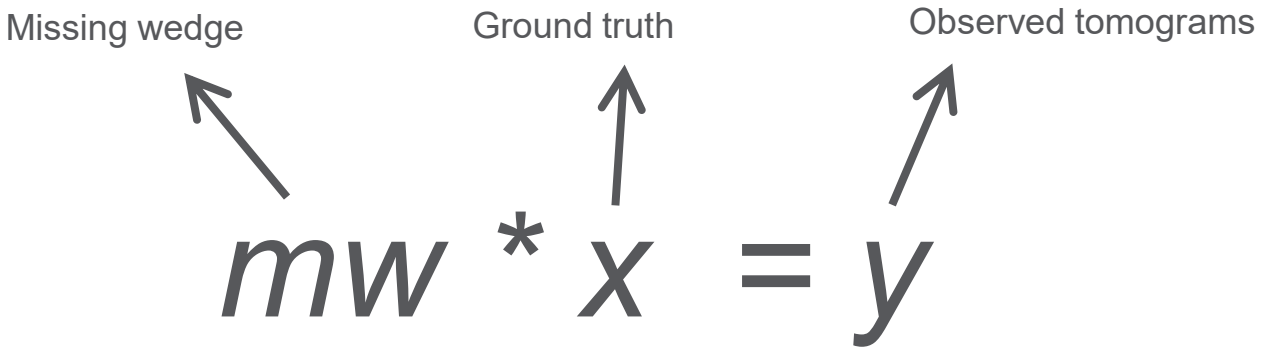
Missing wedge in cellular tomography





Liu and Tao, *Nat Neurosci*, 2020

Formulation of missing wedge problem

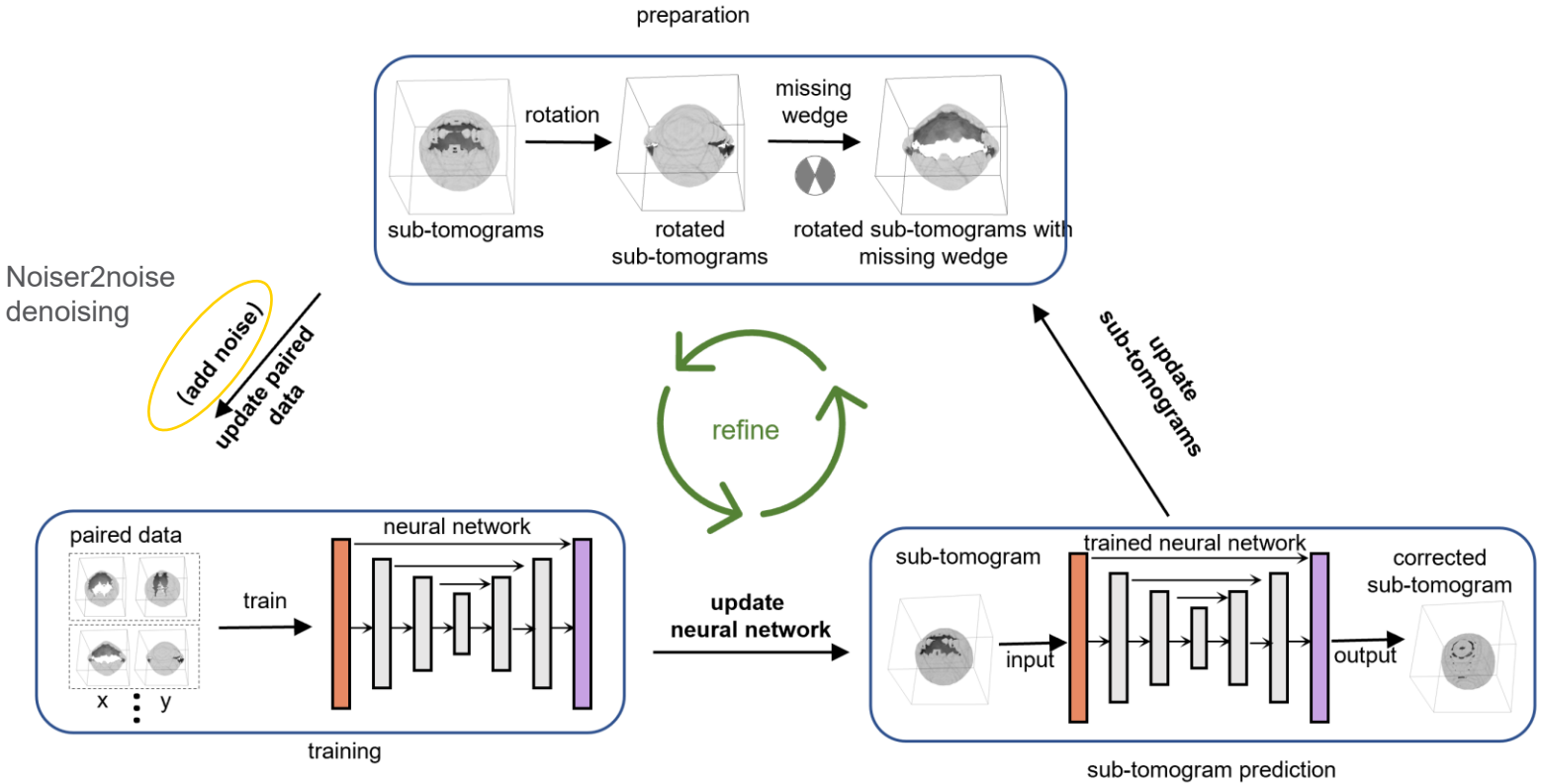


1. No ground truth
2. Ensure the AI-generated information is trustworthy

IsoNet's self-supervised strategy

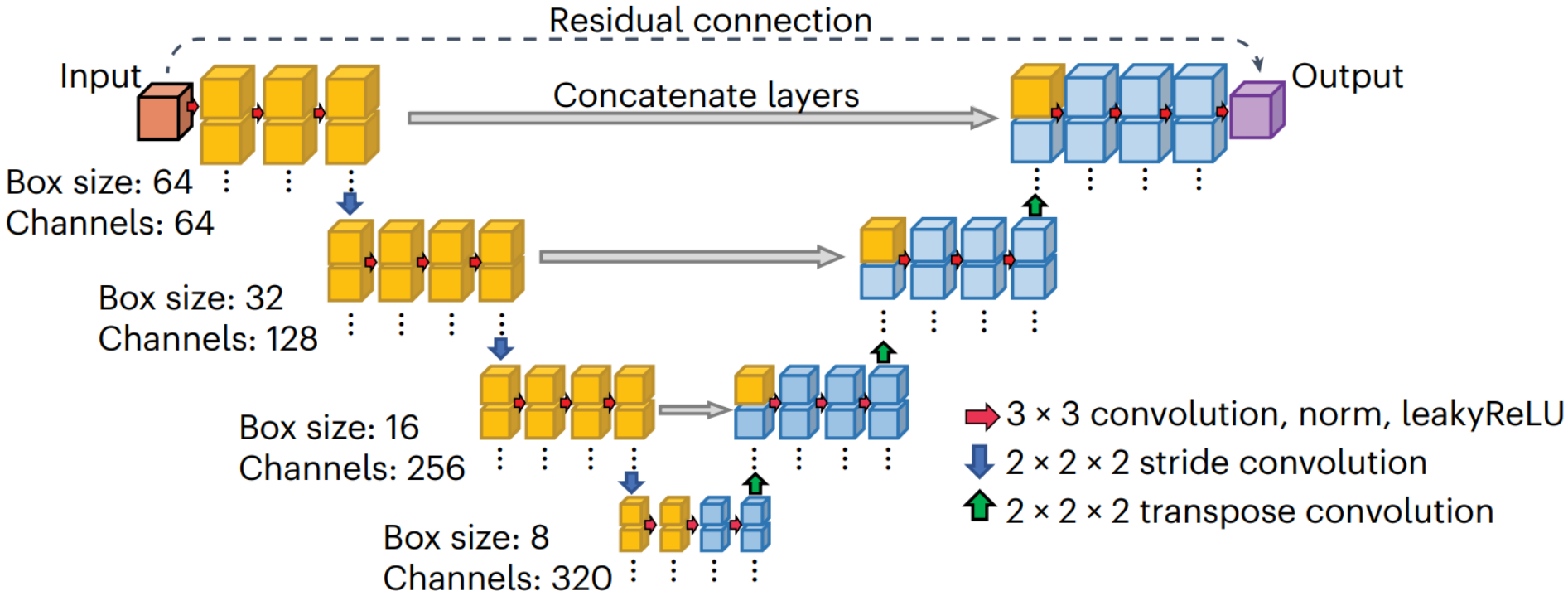
1. Assume the observed data is "ground truth"
2. (in silico) Remove some information from "ground truth"
3. Pretend we do not know that information
4. Train a neural network to predict the missing information from the remaining data
5. Improving "ground truth" with neural network and goto step1

IsoNet: Isotropic reconstruction of ET with deep learning

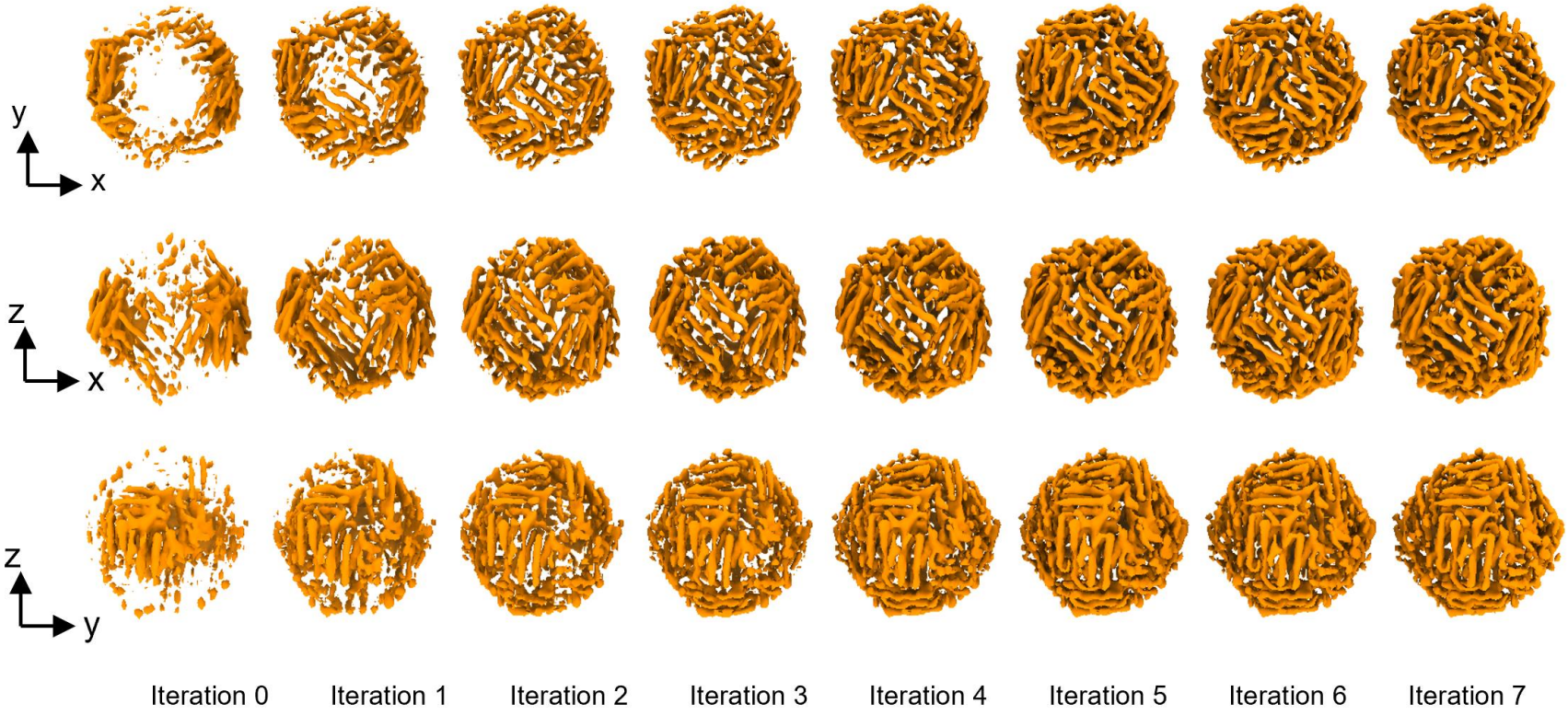


Liu et,al. Nat. Comm. 2022

UNet architecture

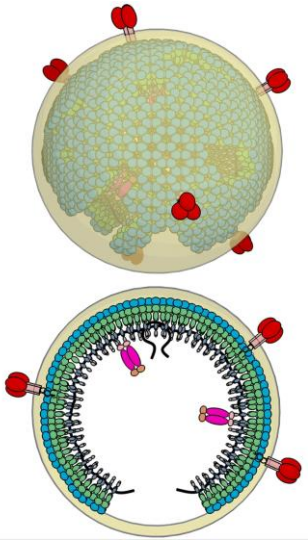


IsoNet tested on simulated data of apoferritin

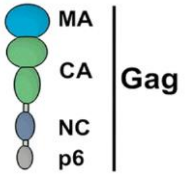


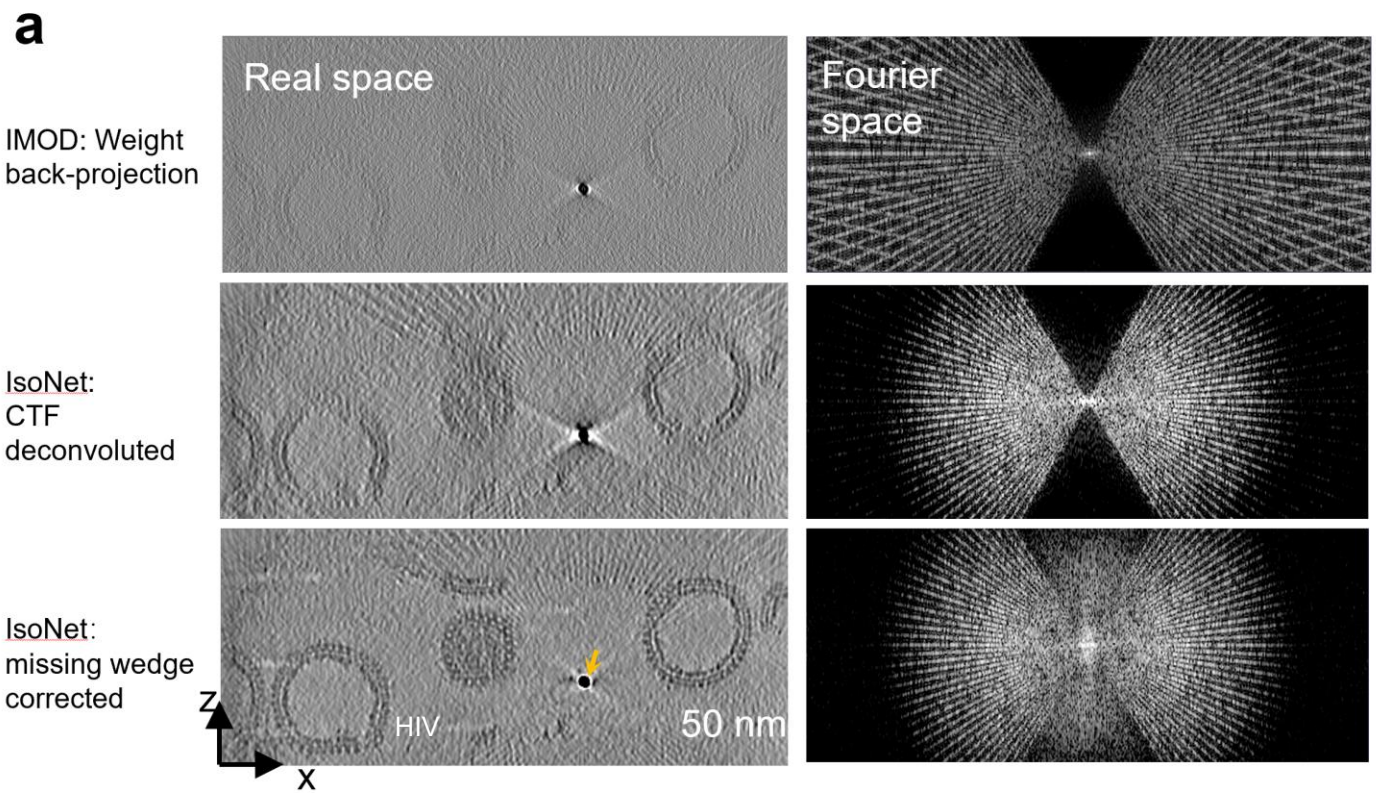
IsoNet fills the information in missing wedge region

Immature HIV

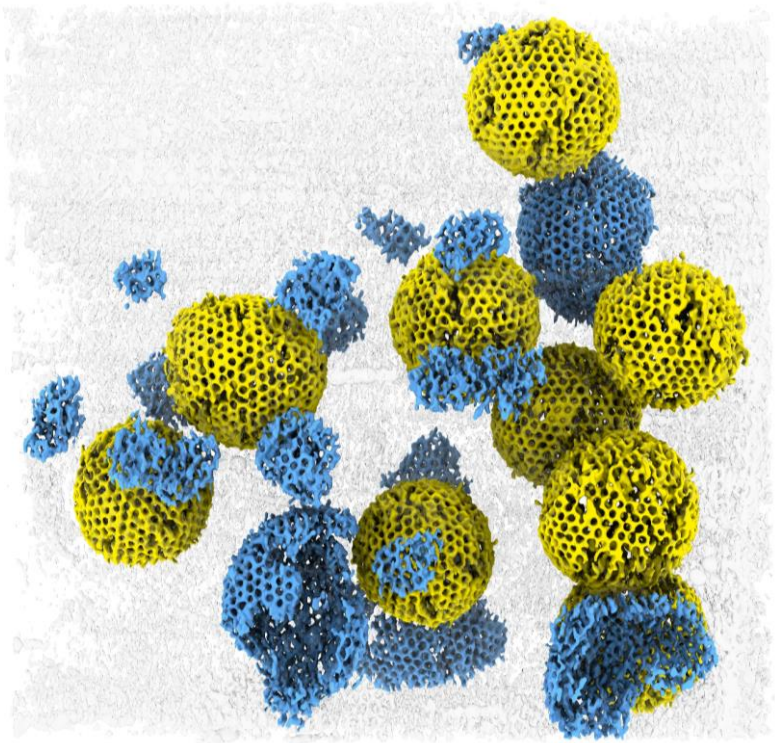


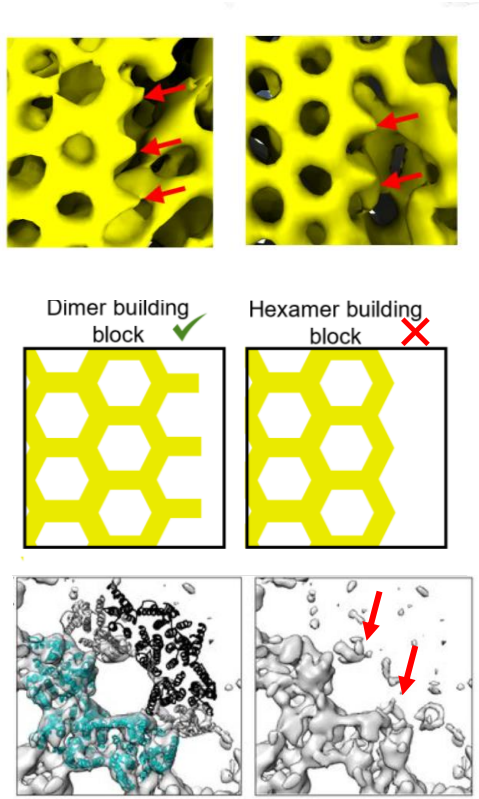
Chojnacki et, al. *Retrovirology* 2018





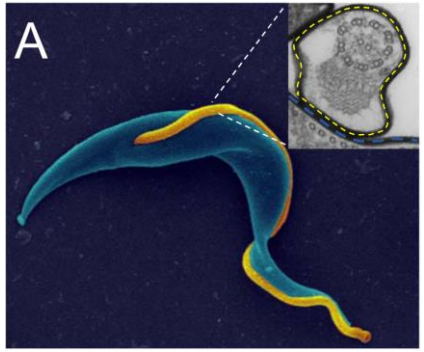
IsoNet reveals lattice defects of immature HIV



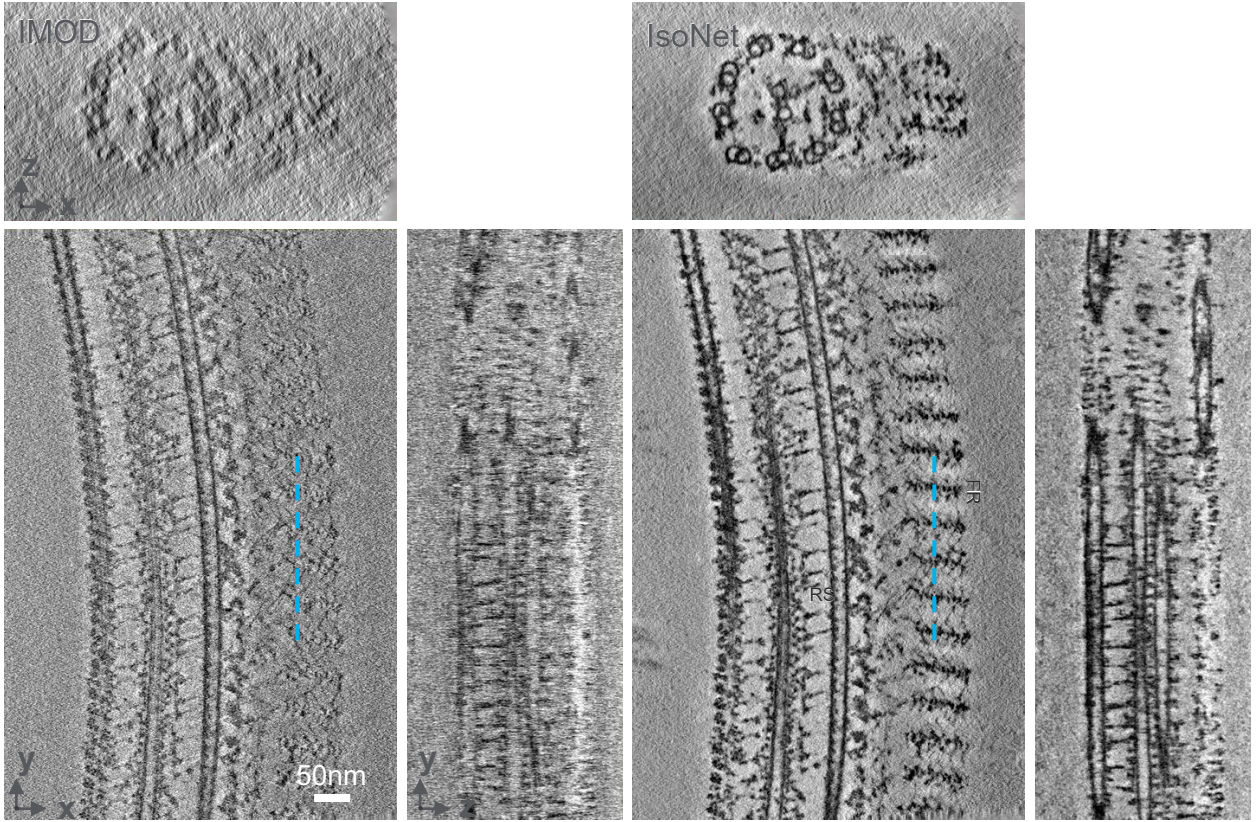


Tan et, al. PNAS 2021

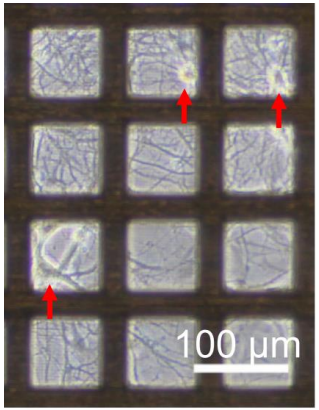
IsoNet reveals architecture of a eukaryotic flagella



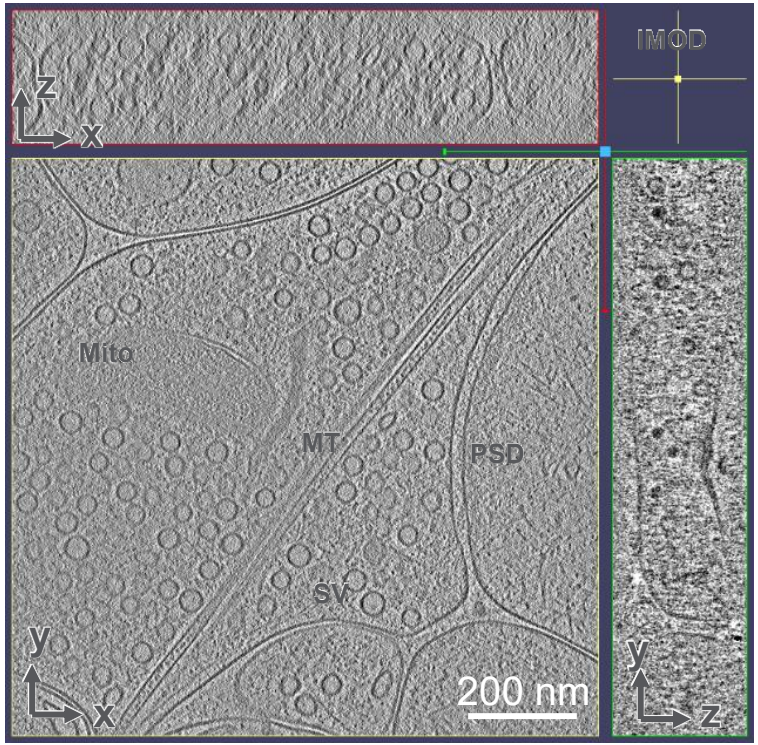
Imhof & Zhang et, al. 2019

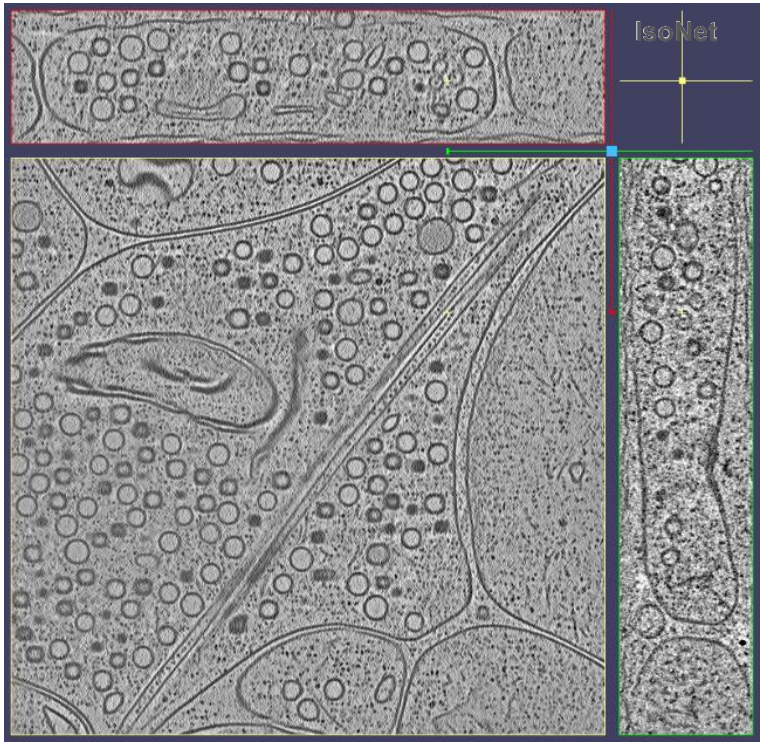


IsoNet recovered missing information in a tomogram of neurons



Tao & Liu et, al. 2018





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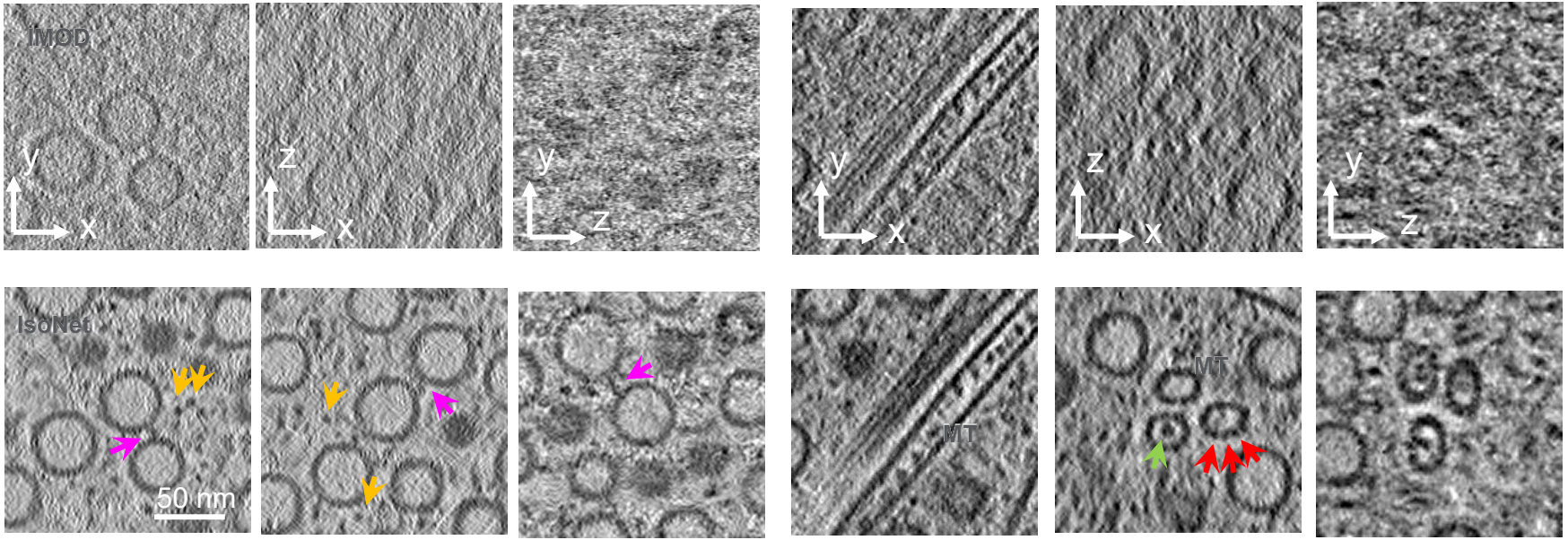
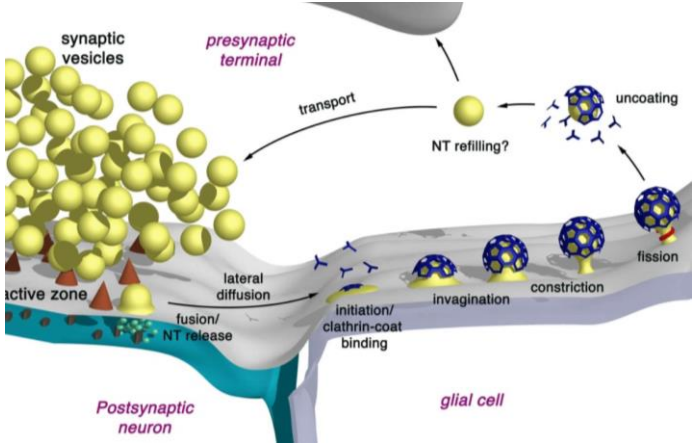
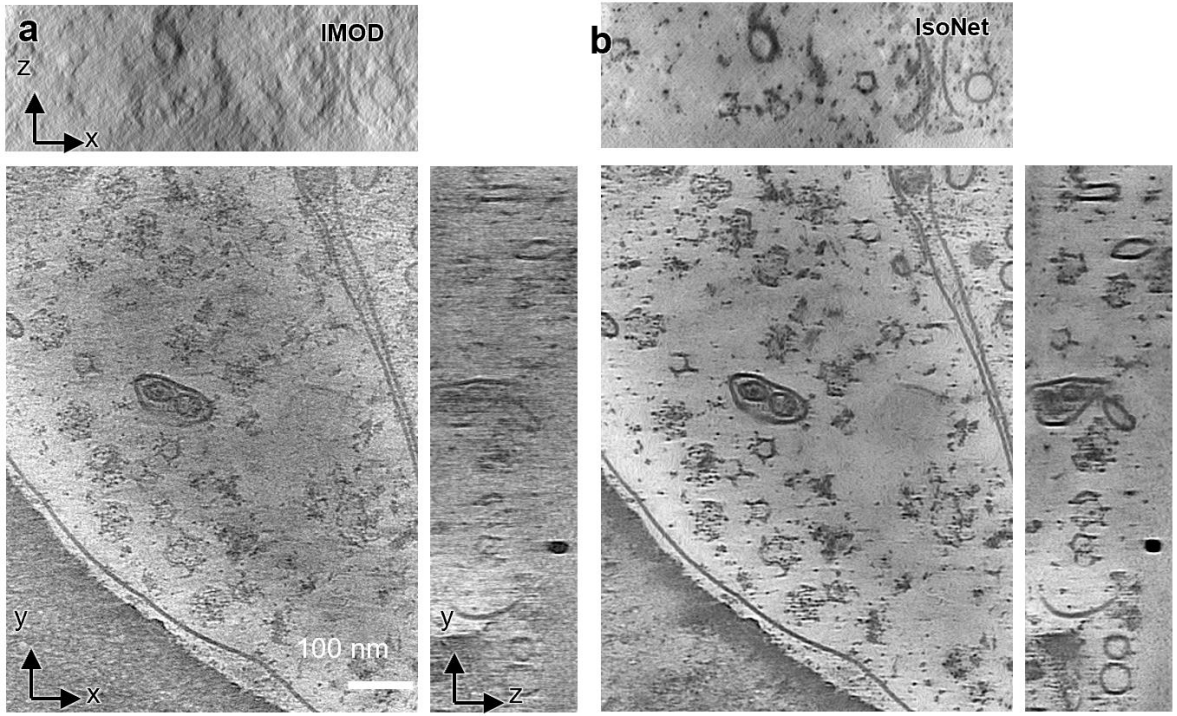
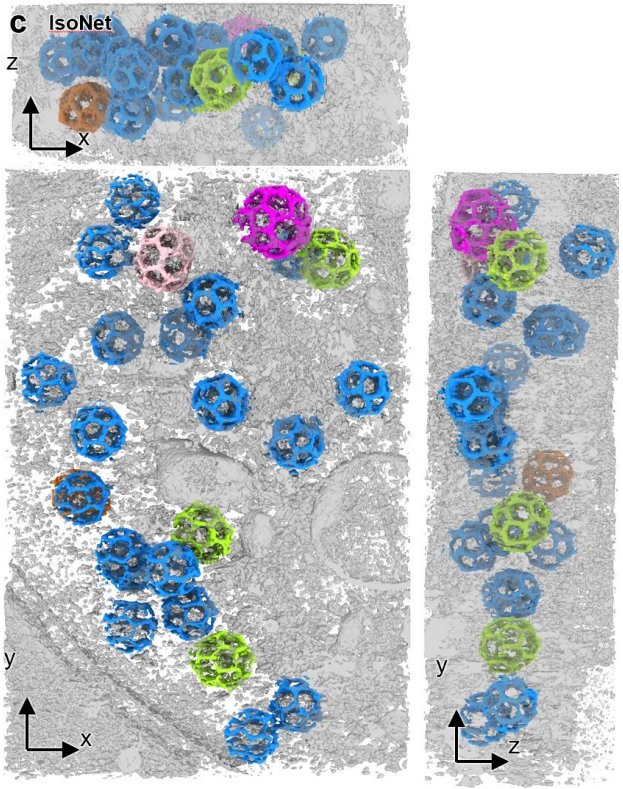


Visualization of clathrin cages in a neuronal synapse



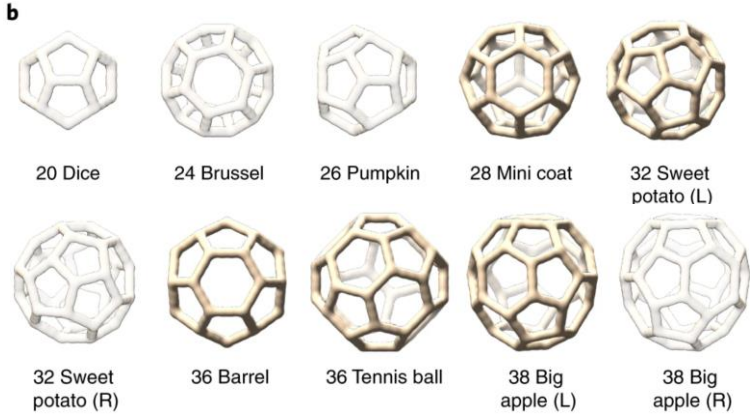


In situ structure of clathrin cages without averaging



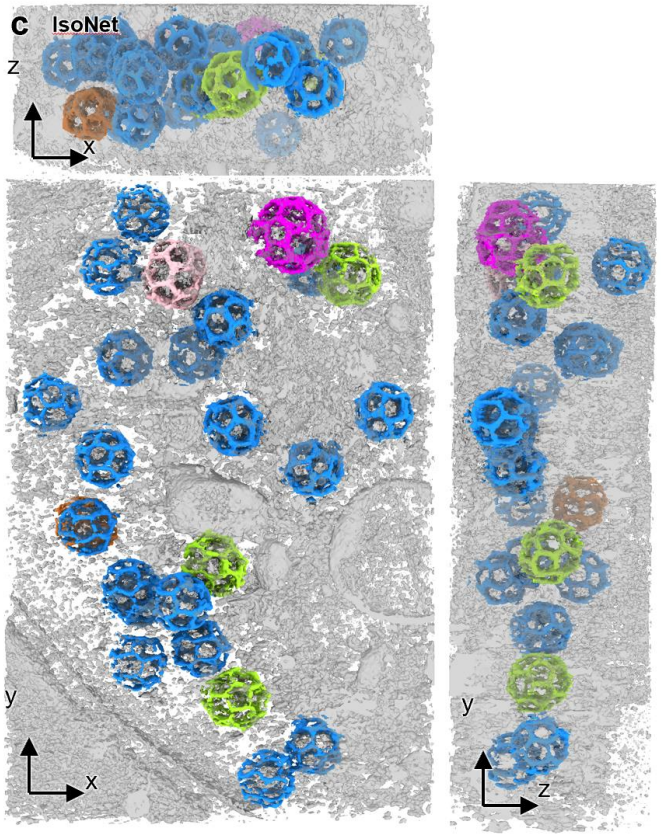
In situ structure of clathrin cages without averaging

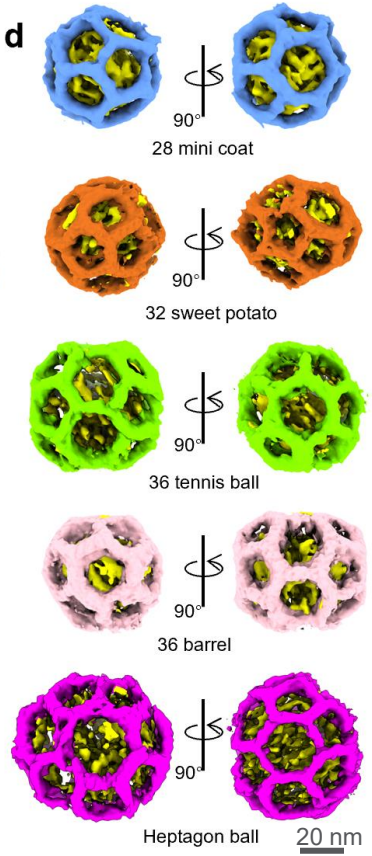
Possible clathrin structures



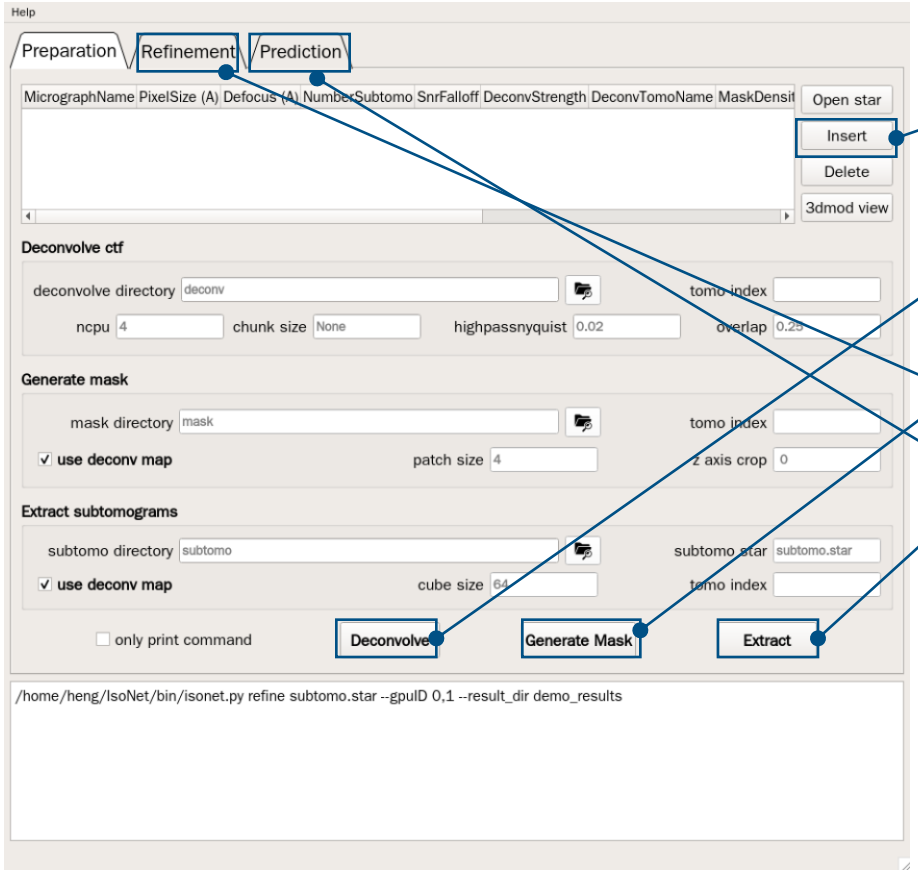
In vitro assembled

Morris et, al. NSMB 2019





GUI for IsoNet



Add tomogram

CTF deconvolution to improve contrast

Automatic mask generation
to avoid empty, carbon, platinum areas

Prepare subtomogram data for training

Actual iterative training

Apply neural network to tomograms

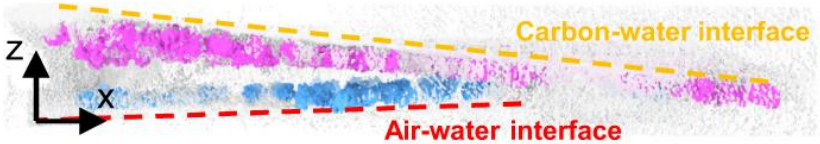
https://isonetcryoet.com/docs.html

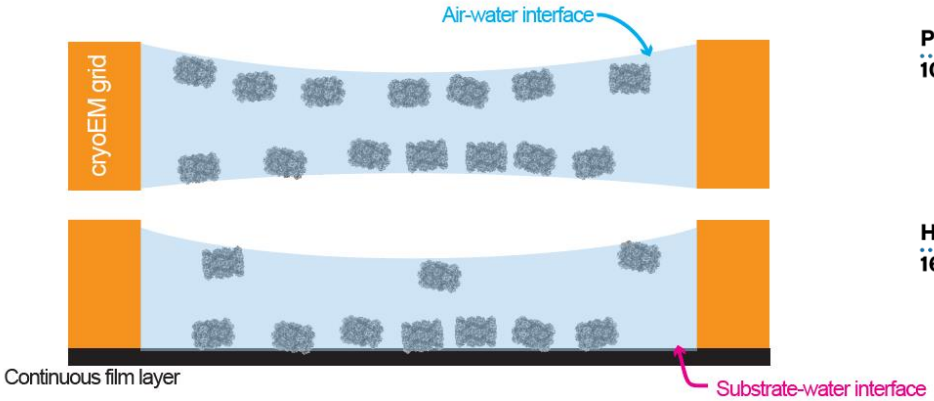
Single Particle IsoNet (spIsoNet)

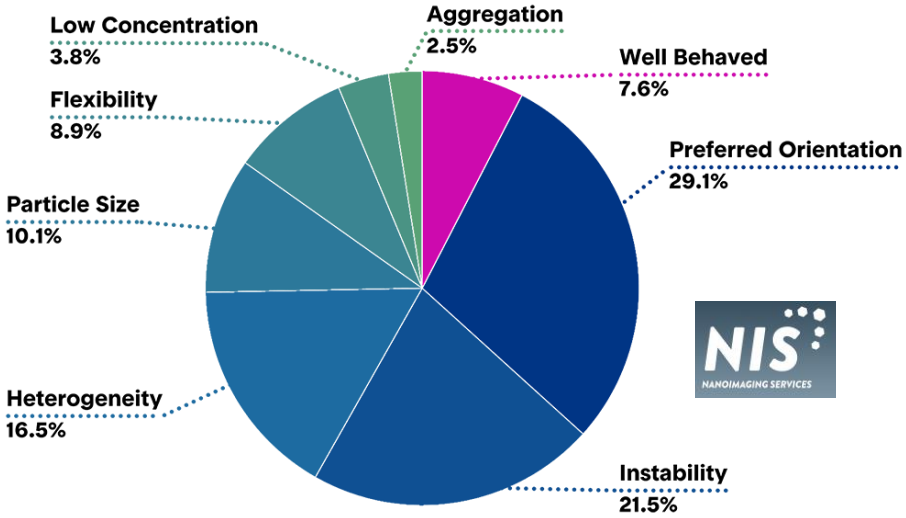
Using the information recovery ability to assist cryoEM single particle analysis and subtomogram averaging.

Liu, Fan et, al. bioxriv 2024, Nat. Methods 2025

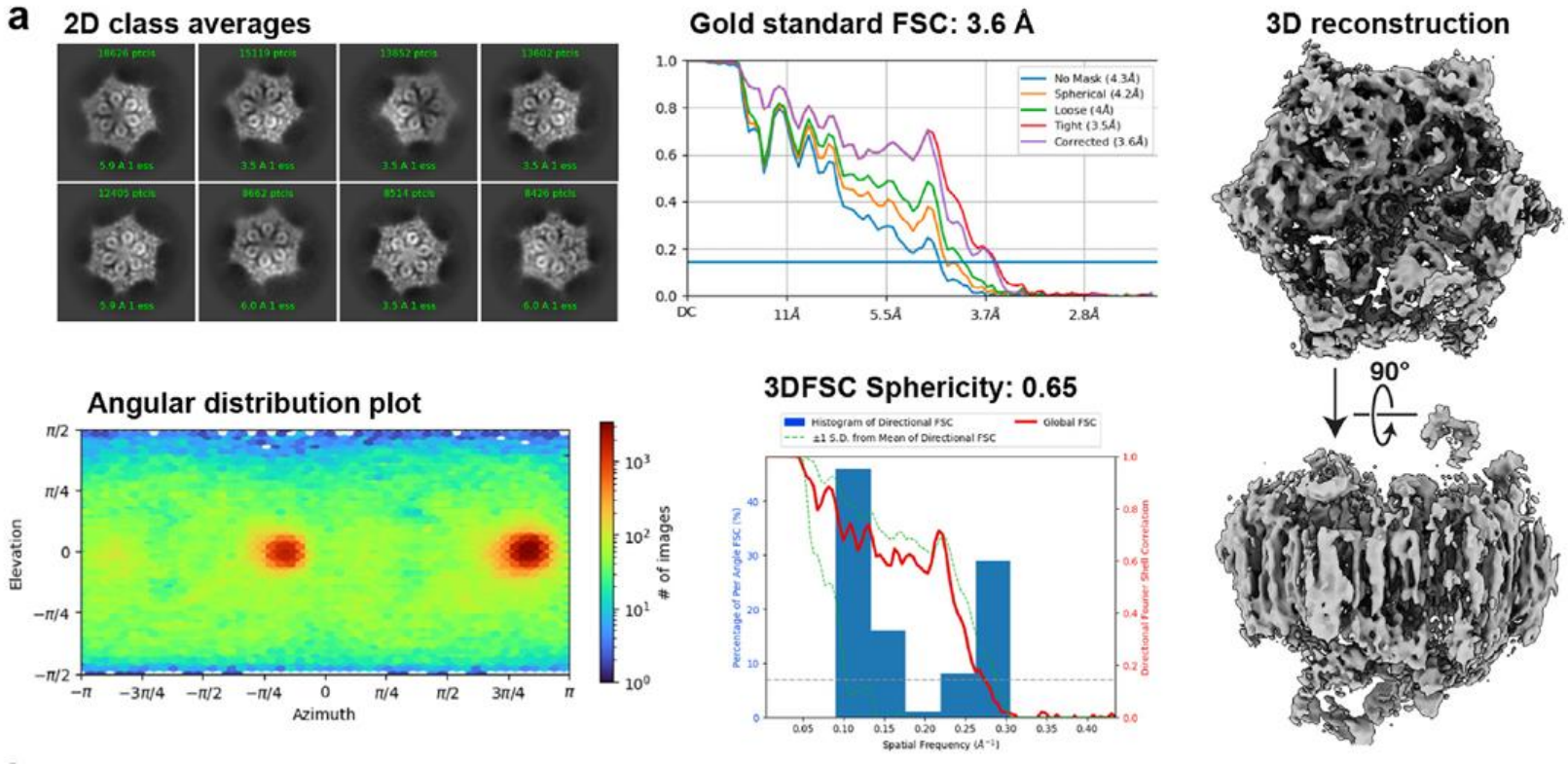
Preferred orientation is a common problem in cryoEM







Preferred orientation induces artifacts



Lander, current opinion in structural biology, 2024

single particle IsoNet

Special designs in spIsoNet

- 3D FSC to represent preferred orientation
- **End-to-end** training without refine iterations
- **Simultaneous** Noise2noise denoising and missing information recovery
- spIsoNet **regularization** for particle alignment

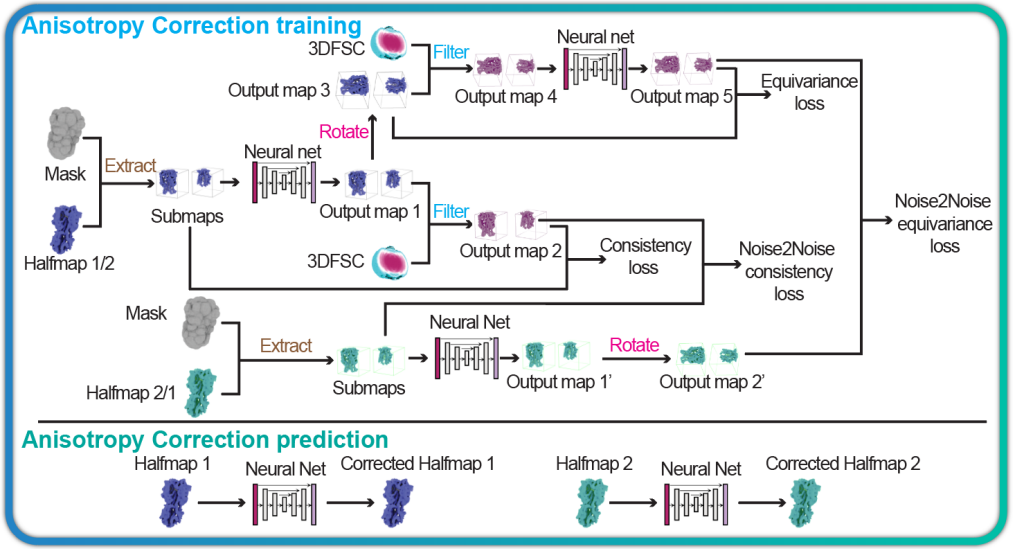
Shared property with cryoET IsoNet

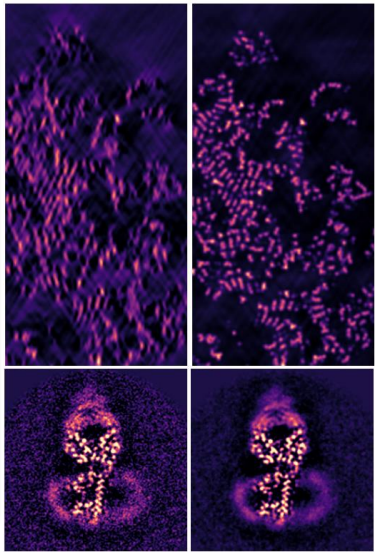
- Learn from your experimental data, no external information or assumption

https://github.com/IsoNet-cryoET/spIsoNet

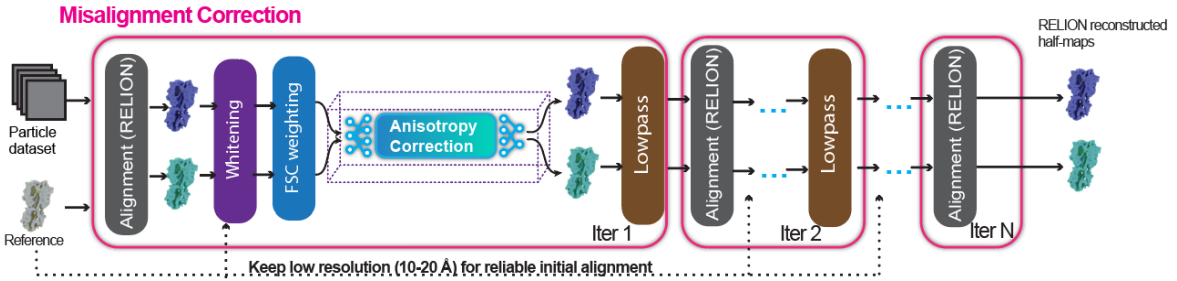
*sp*IsoNet Implementation: Two Modules

anisotropy correction

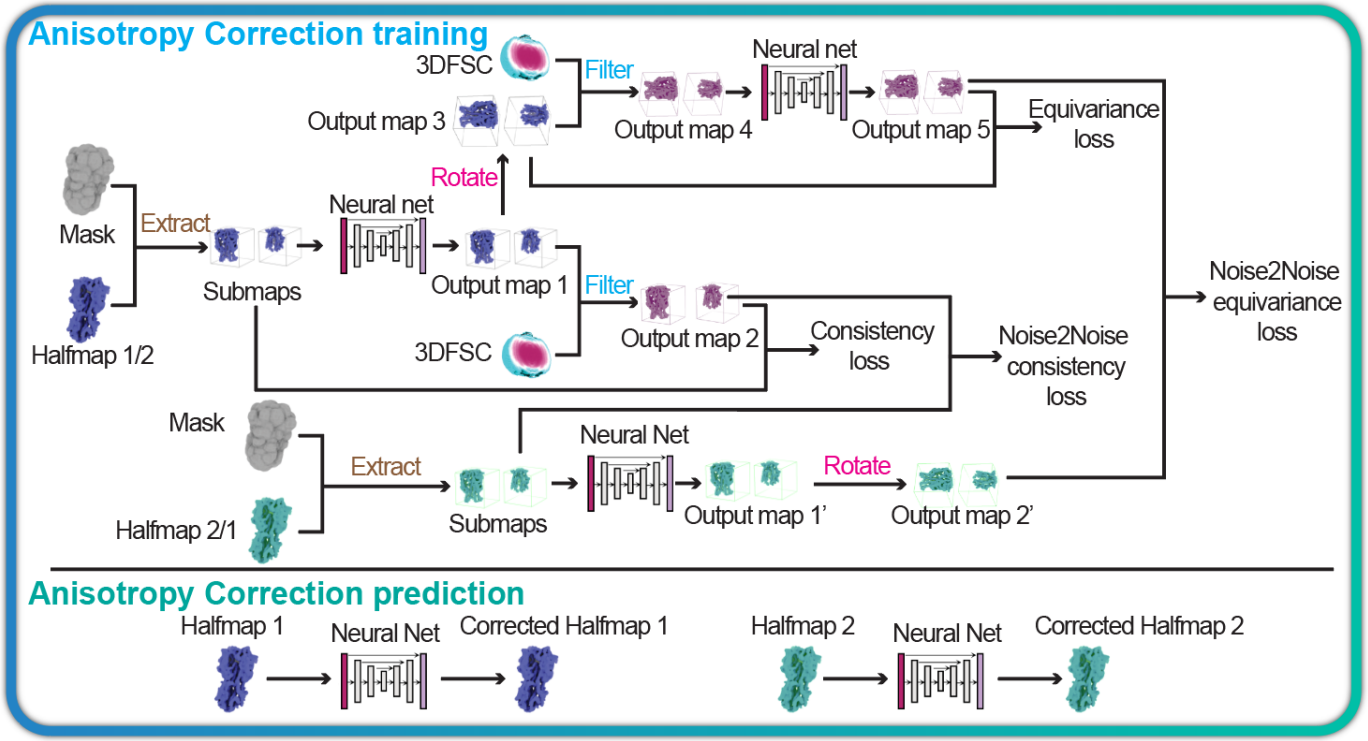


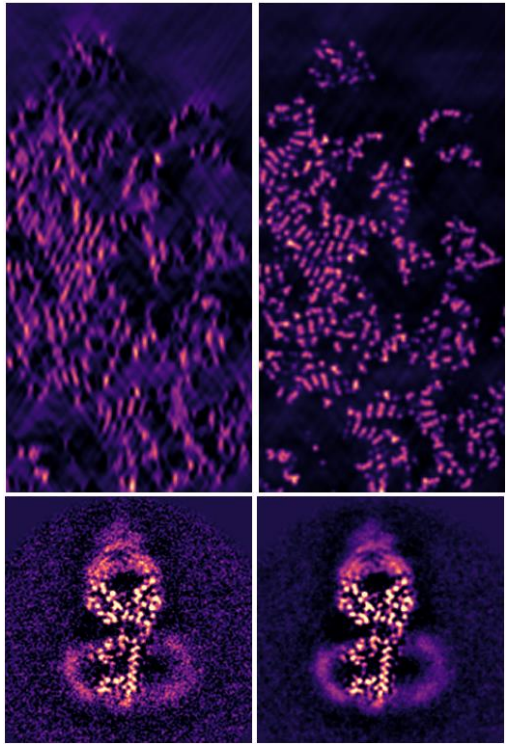


misalignment correction



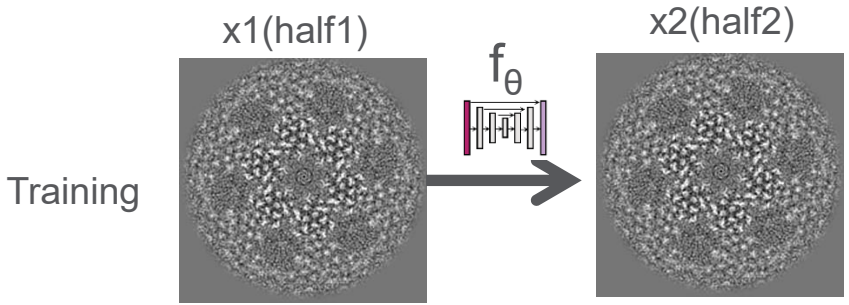
Anisotropy Correction module



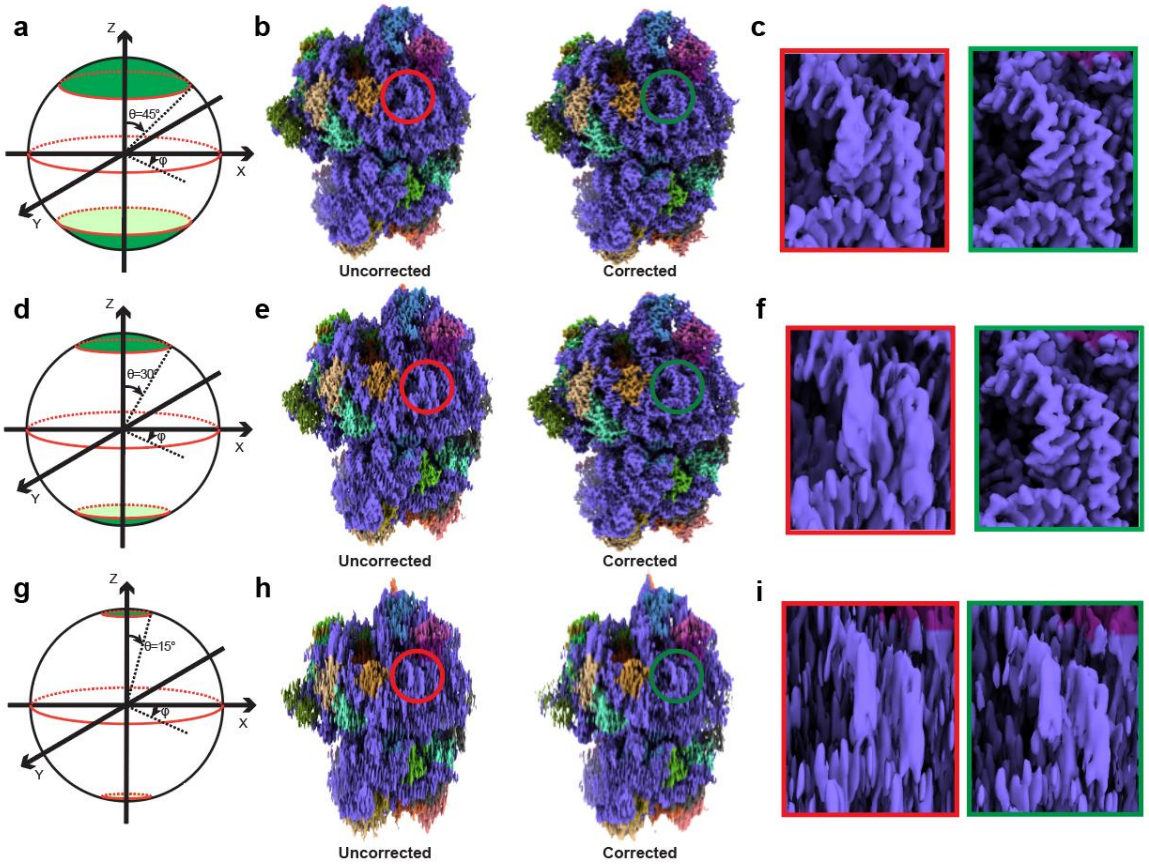


Design of the losses

- **Consistency loss:**
  Guiding recovery in under-sampled Fourier regions;
- **Equivariance loss:**
  Learn to recover in silico removed information in rotated maps;
- **Noise2Noise loss:**
  Uses pairs of noisy inputs to denoise without ground truth;

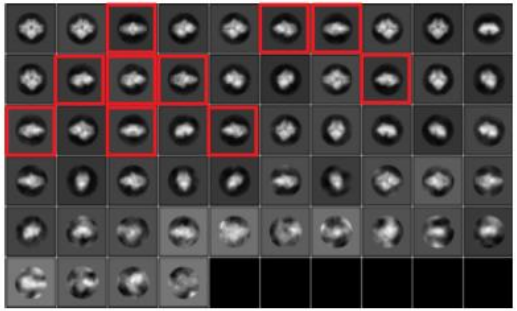


Performance of the anisotropy correction module of *sp*IsoNet

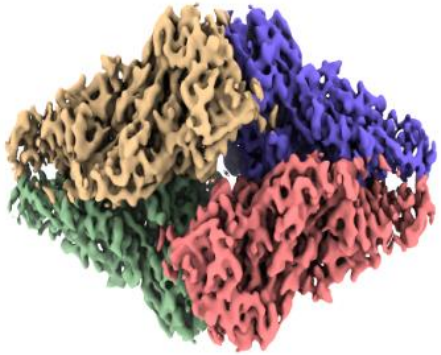


Anisotropy correction for β-Galactosidase

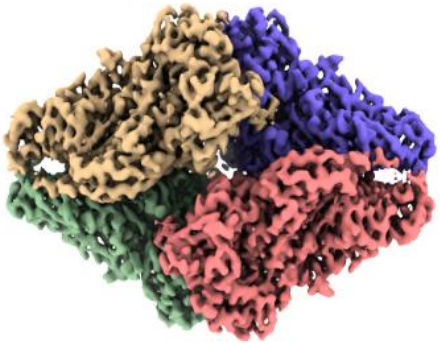
a



Only side view, 1513 particles

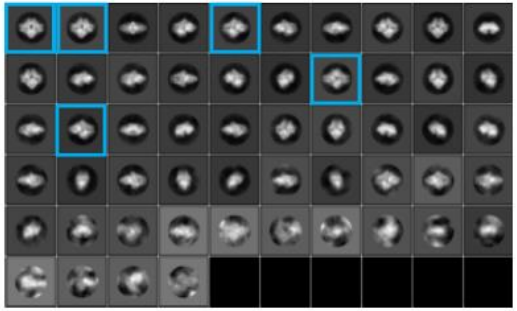


Uncorrected

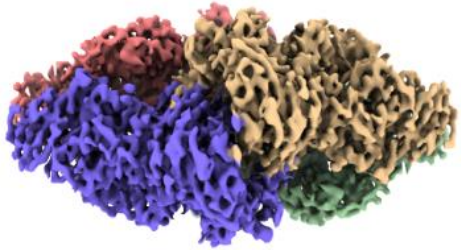


Corrected

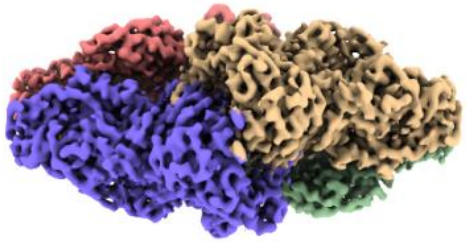
b



Only top view, 950 particles

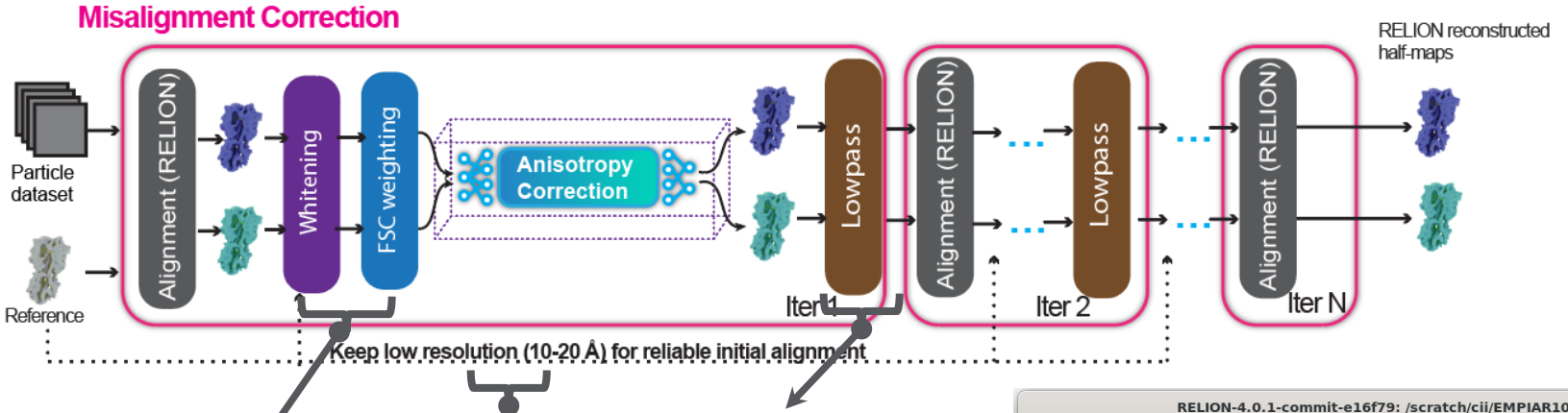


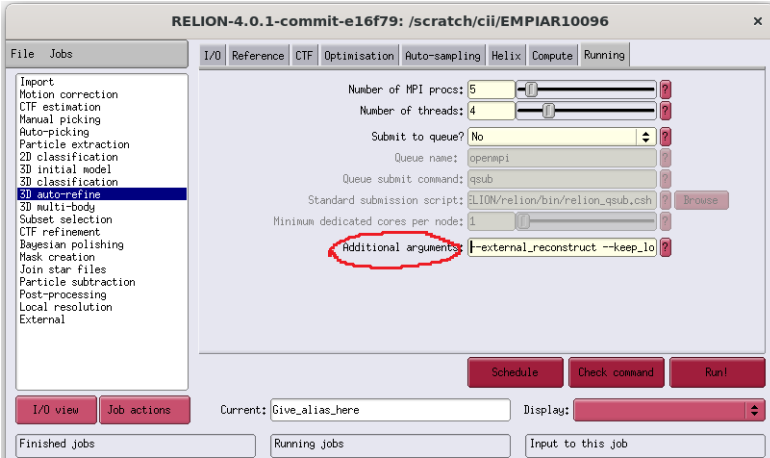
Uncorrected



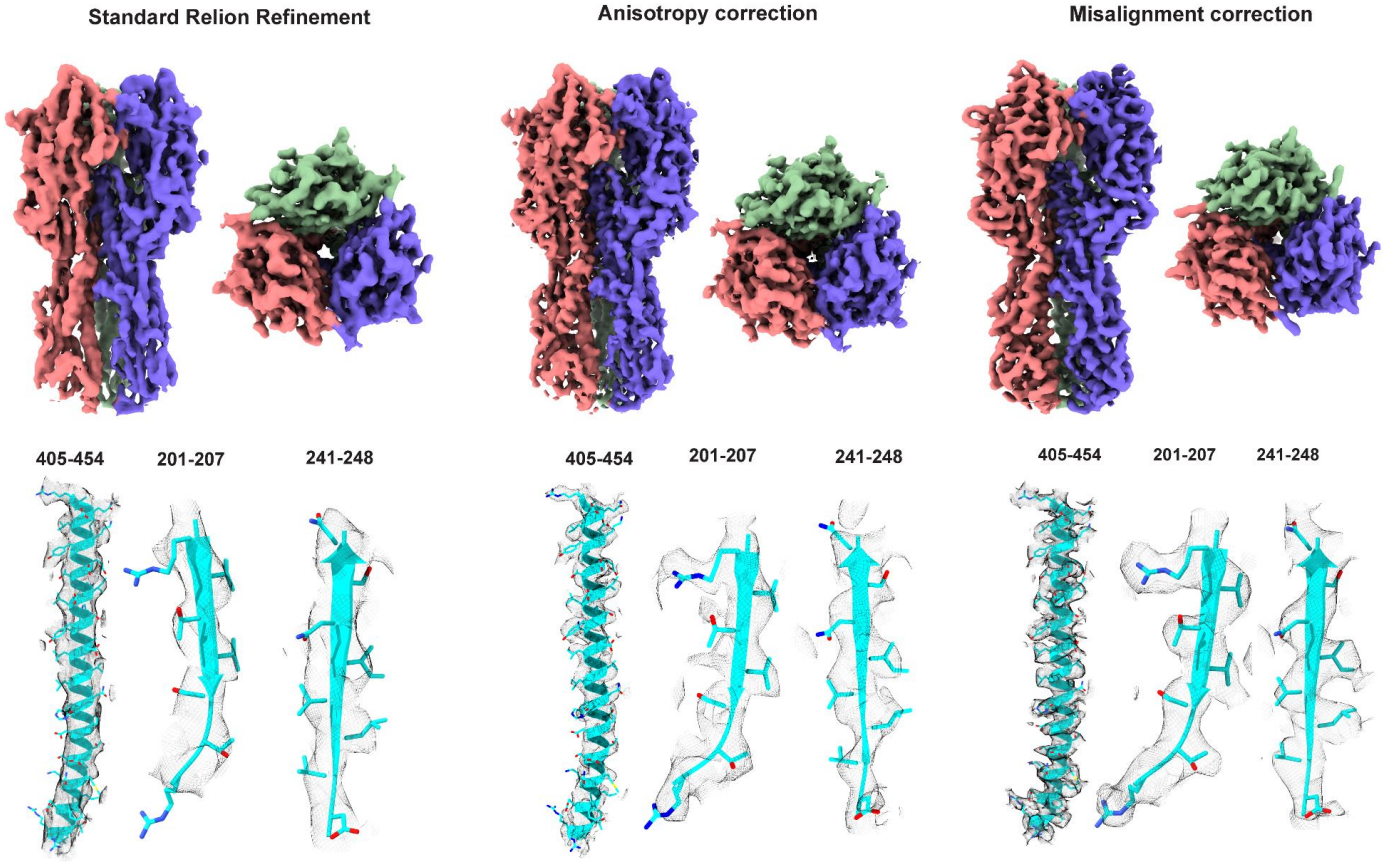
Corrected

Misalignment correction module

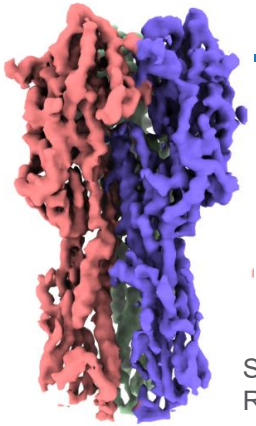




Tilted dataset of hemagglutinin

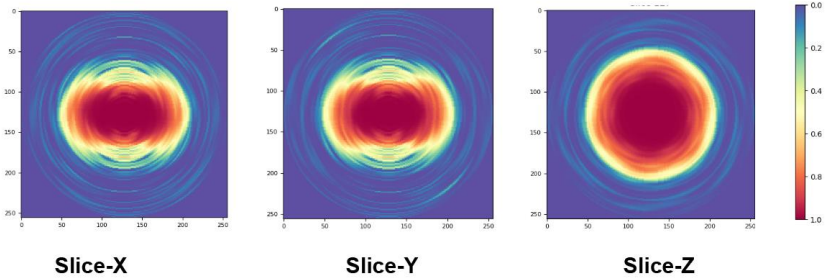


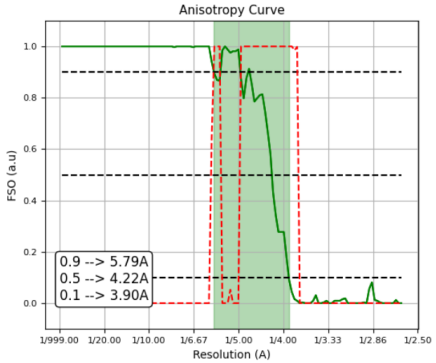
Performance of misalignment correction



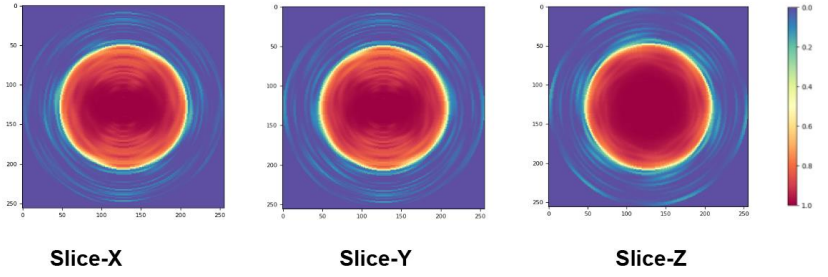
Standard RELION

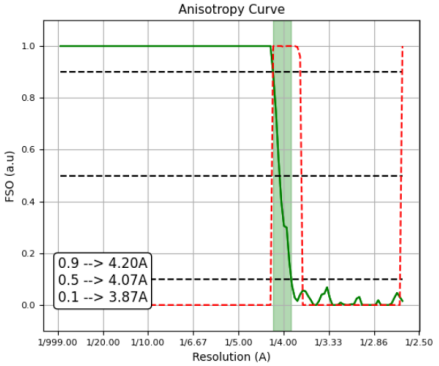
Standard Relion Refinement

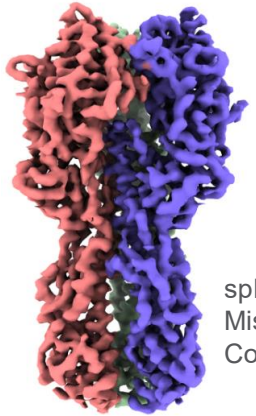




Misalignment Correction



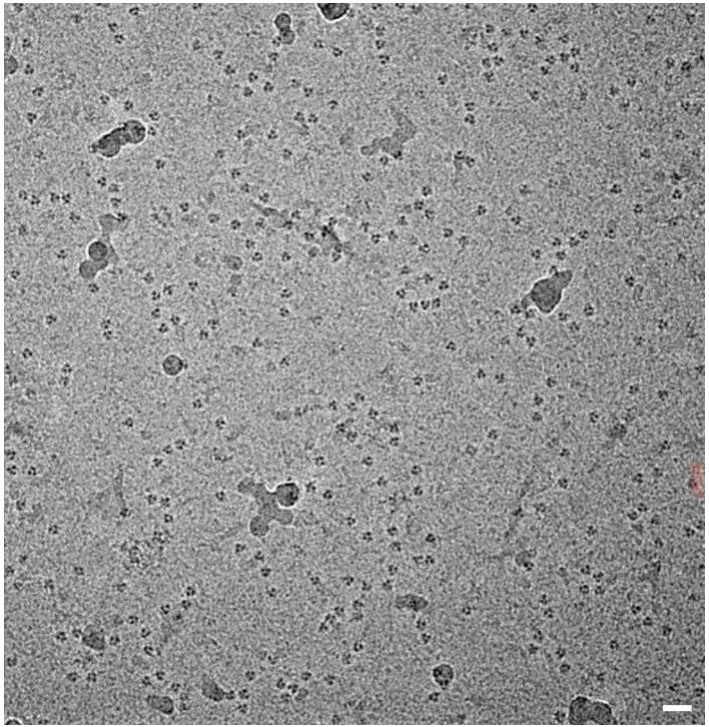


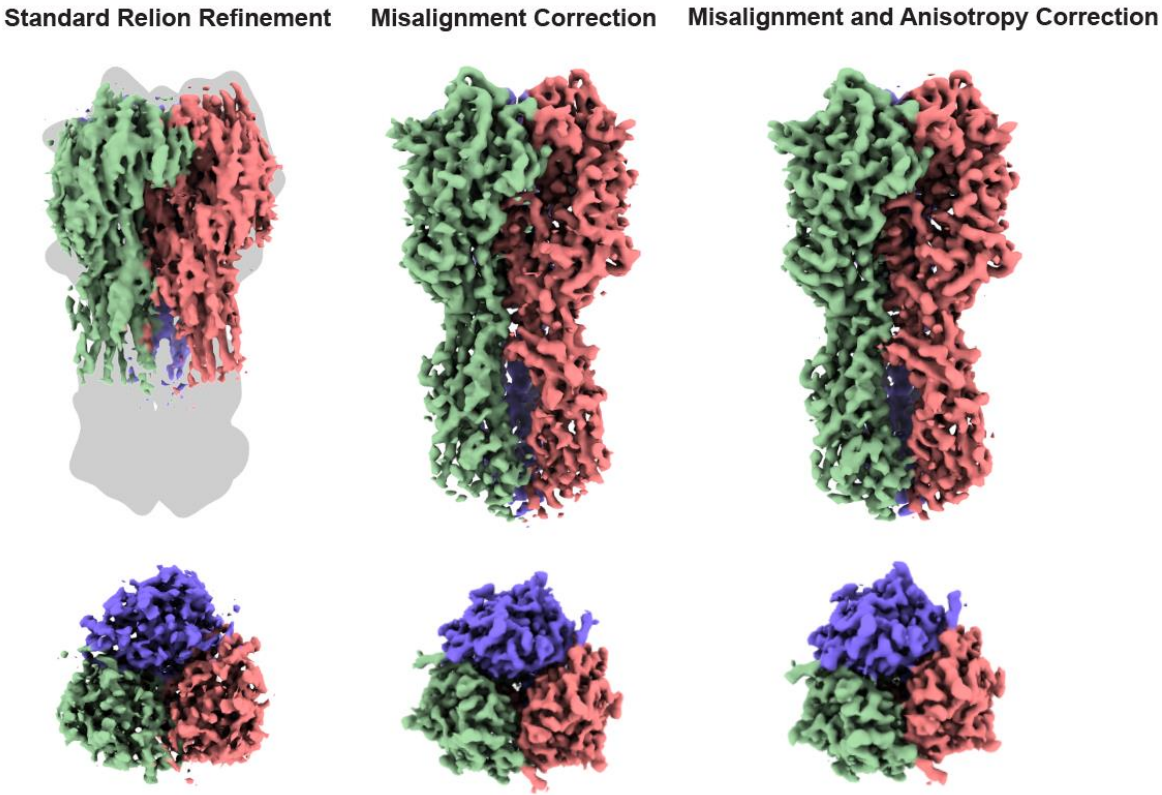


spIsoNet Misalignment Correction

!!! The misalignment correction map is directly reconstructed by RELION

Extreme case almost no side view

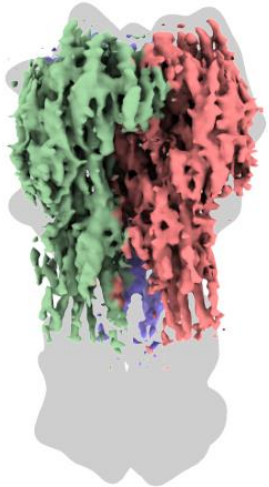




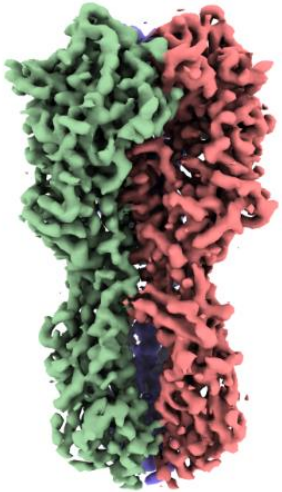
Tan et al., Carragher & Lyumkis, 2017, Nature Methods

Near-atomic structure of hemagglutinin from non-tilt dataset

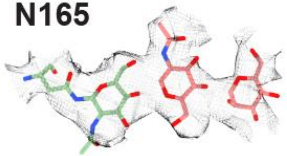
Standard Relion Refinement



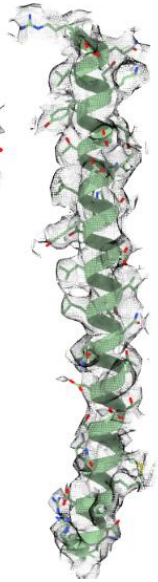
Misalignment and Anisotropy Correction



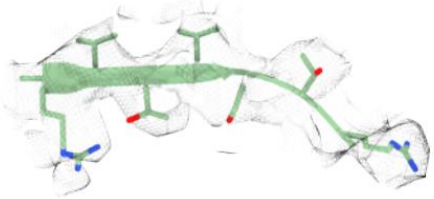
N165



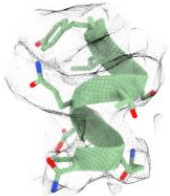
405-454



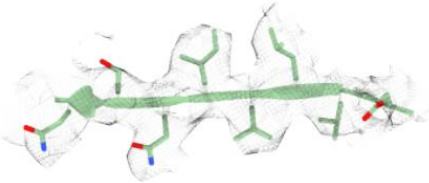
201-207



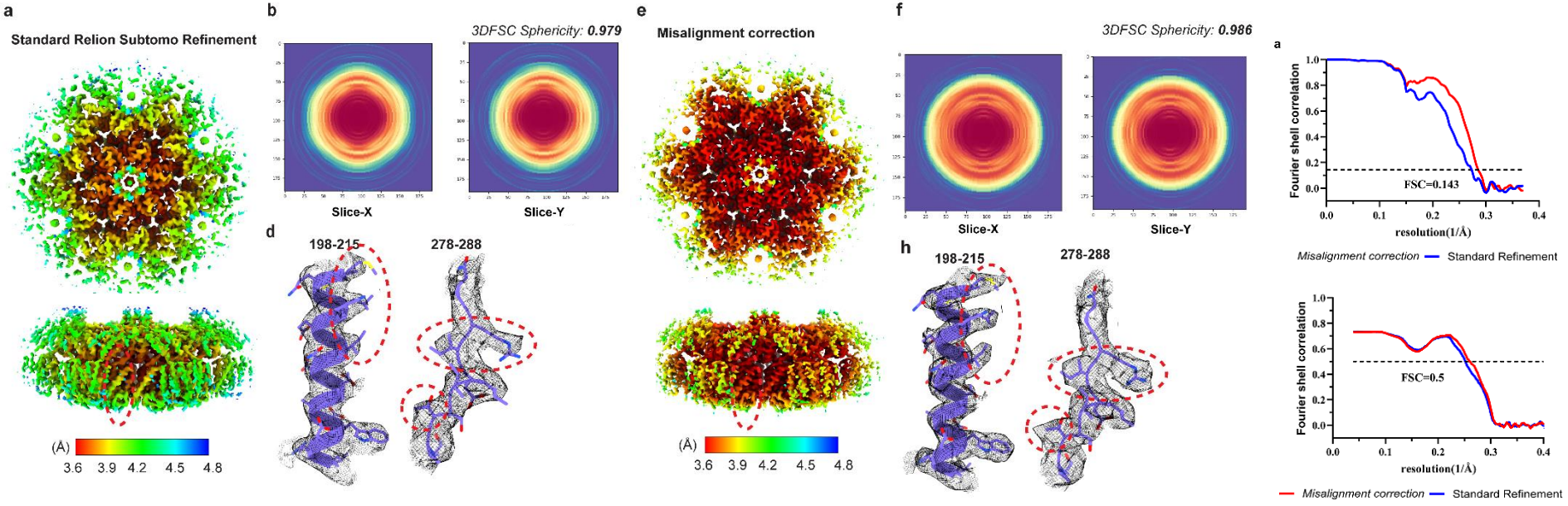
188-195



241-248



splsoNet improves subtomogram averaging



Isotropic and denoised map improve subtomogram alignment

Liu et,al, 2025, Nature Methods

Summary

- Self-supervised deep learning from only observed data, leveraging the fact that the true signal distribution invariant to rotation and translation
- IsoNet to recover missing-wedge for molecular sociology interpretation of cellular cryoET.
- spIsoNet: the preferred orientation can now be compensated computationally instead of physical and chemical treatments.

Thank You

https://github.com/IsoNet-cryoET

California NanoSystems Institute, UCLA

- Prof. Hong Zhou
- Dr. Hongcheng Fan
- Dr. Hui Wang

University of Science and Technology of China

- Heng Zhang
- Dr. Changlu Tao
- Prof. Guoqiang Bi

Recommended spIsoNet strategies to address "preferred" orientation problem

fast map anisotropy correction

① particle dataset → Standard refinement (e.g. relion / cryosparc) → SpIsoNet anisotropy correction

misalignment and anisotropy correction

② particle dataset → SpIsoNet misalignment correction ..... SpIsoNet anisotropy correction

severe misalignment and anisotropy correction

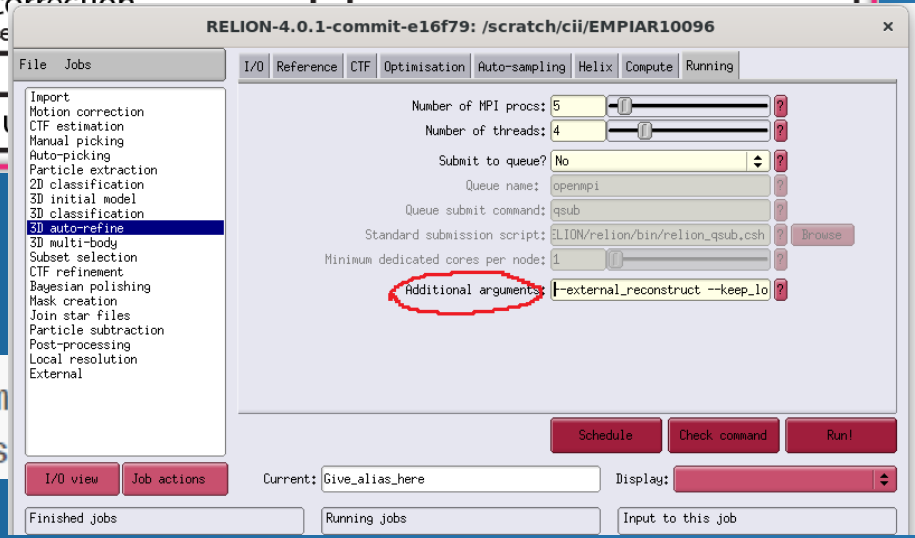
③ particle dataset → spIsoNet misalignment correction
Keep low resolution (10-20 Å) from reference

relatively unifor

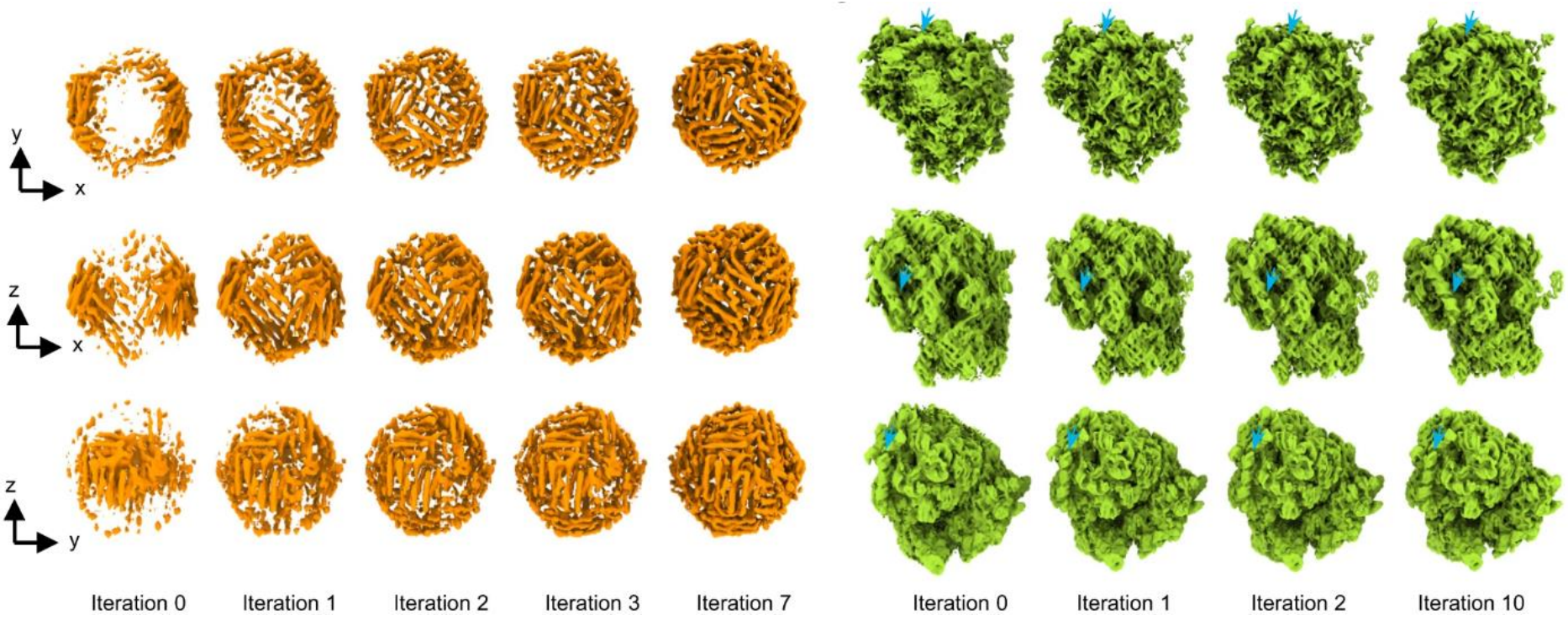
misalignment correction

anisotropy correction

```
spisonet.py reconstruct emd_8731_half_m
aniso_file FSC3D.mrc --mask emd_8731_ms
```



Validation using simulated data of symmetric (apoferritin) and asymmetric (ribosome) structures



IsoNet package available at **GitHub** and **SBGrid** (Liu & Zhang *et al*., bioRxiv, 2021; Nat Comm, 2022)