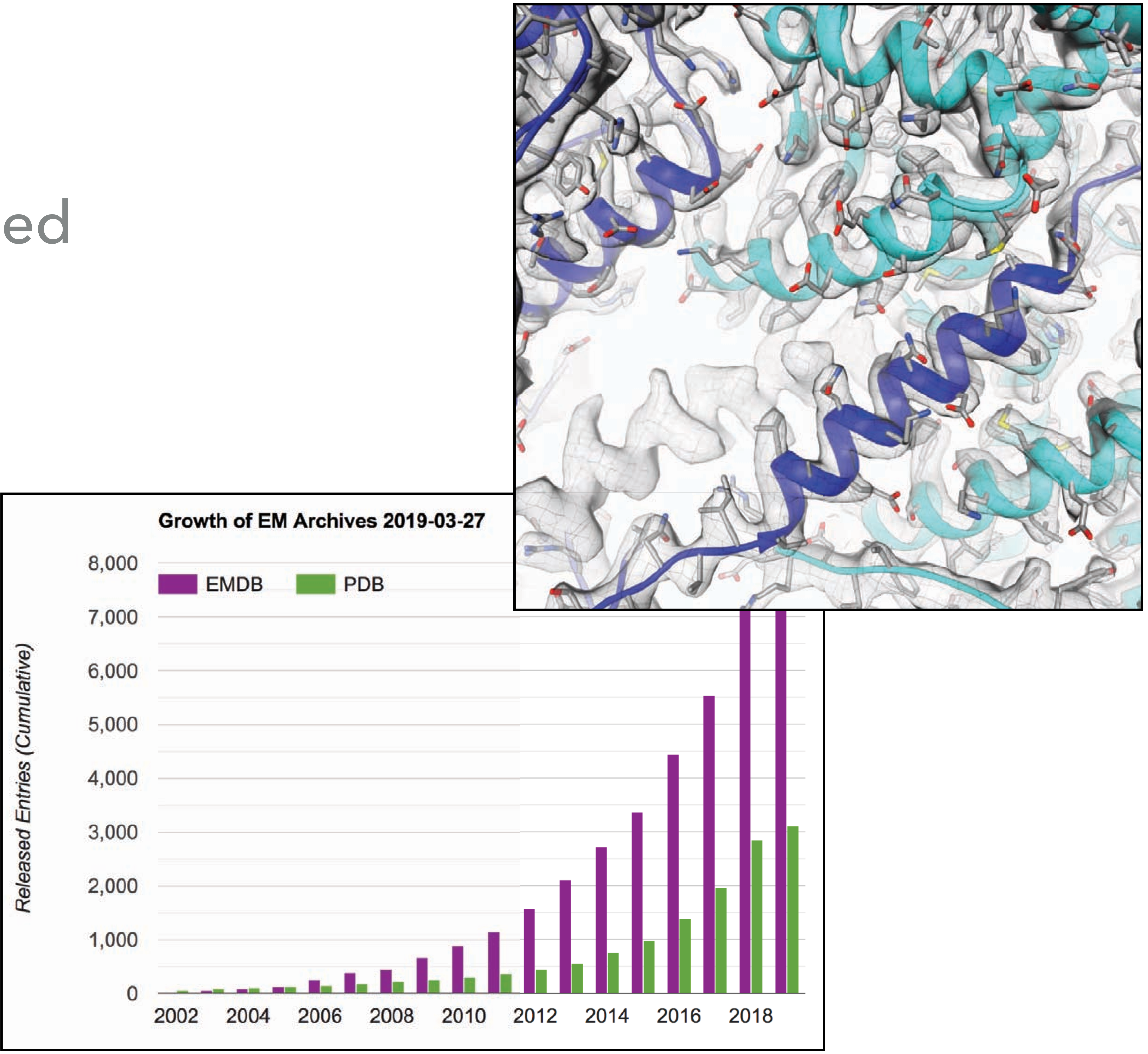
ELECTRON CRYO-MICROSCOPY

- ➡ ~15-20% growth per year in deposited structures over the last 10 years
- ➡ Smaller complexes (~50-100kDa)
- ➡ Larger complexes up to interacting cells
- ➡ Atomic resolution (>2Å resolution)



Growth of EM Archives 2019-03-27

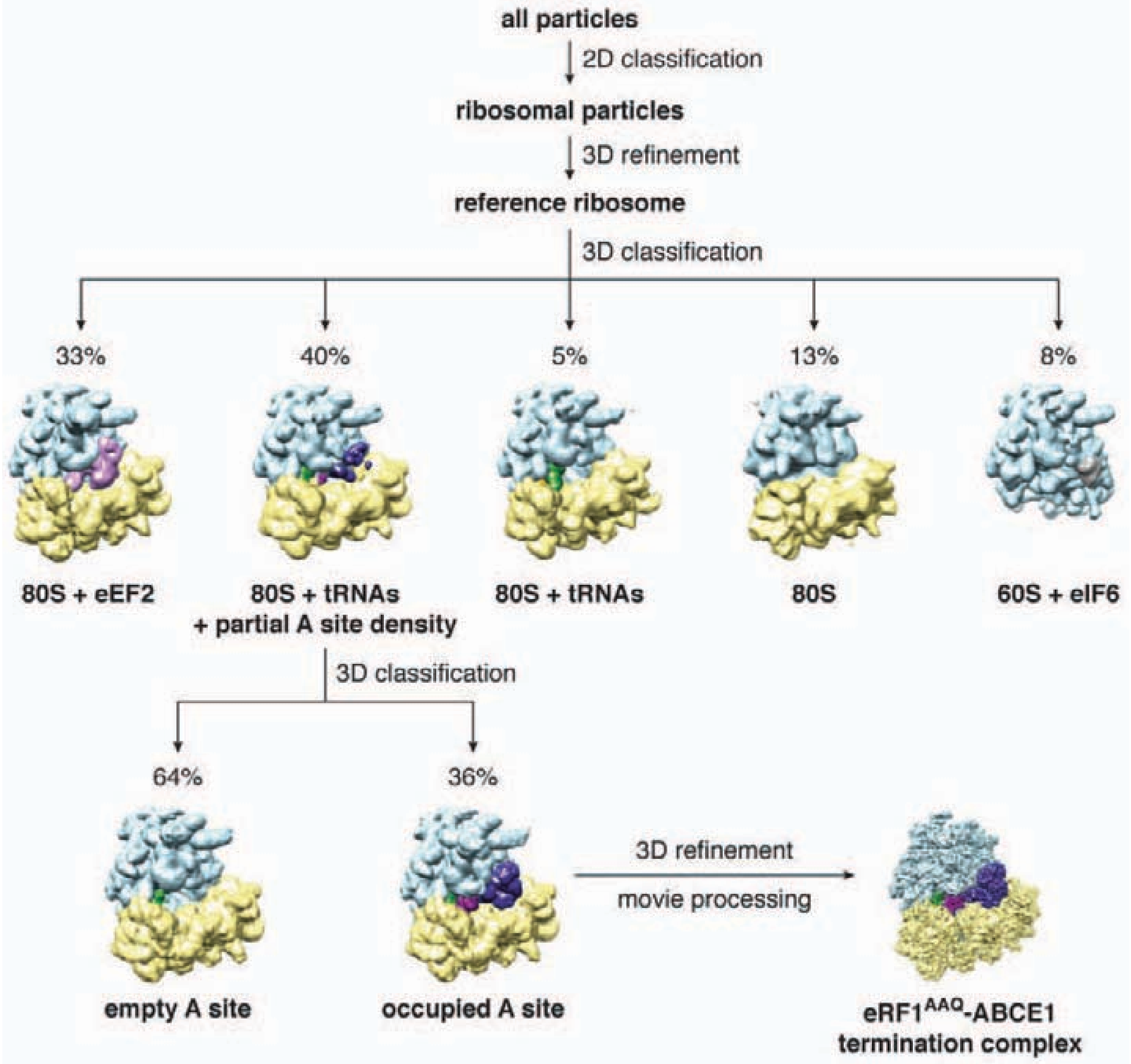
PUSHING THE BOUNDARIES OF CRYO-EM

Improved Electron cryomicroscopes

- Automated data acquisition
- Multiple sample holders

New Direct Detectors

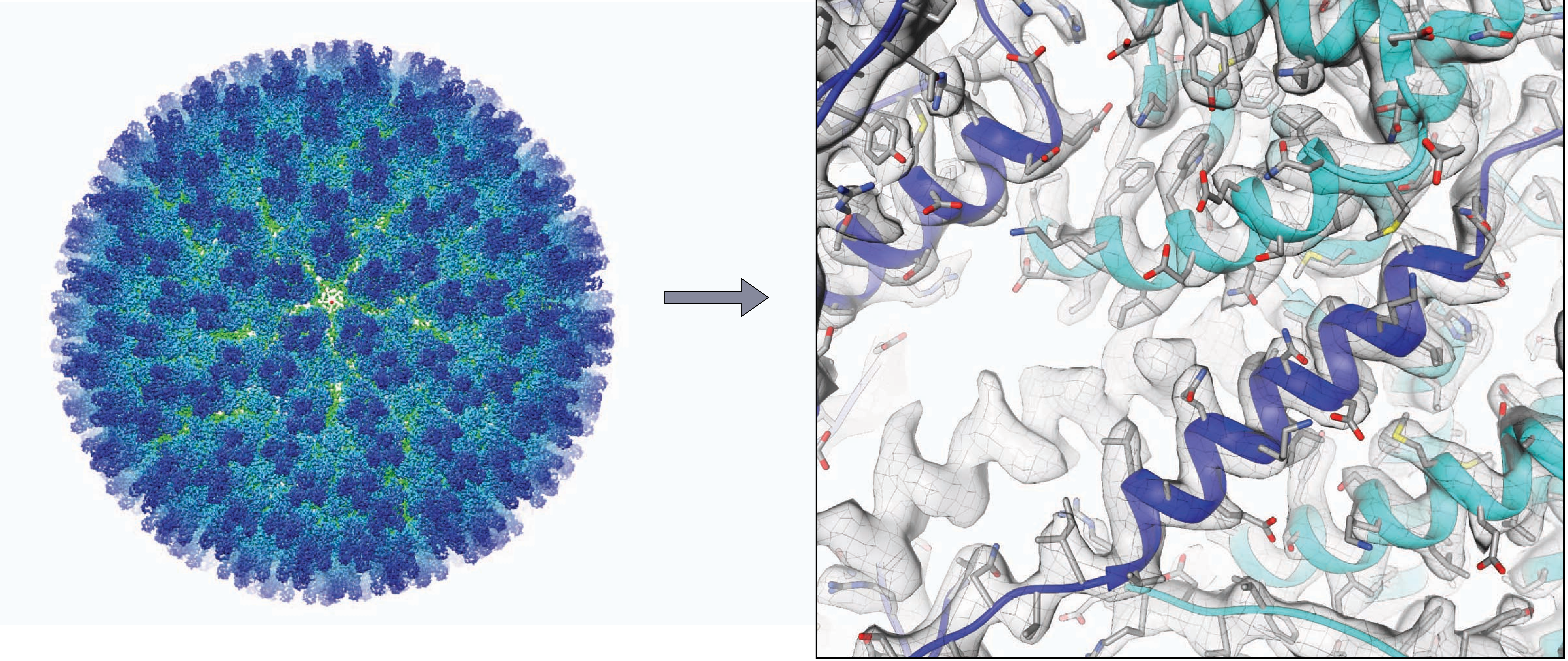
- Improved resolution and signal to noise ratio
- Movie mode
- CMOS sensors
  - Gatan K2 Summit
  - FEI Falcon
  - Direct Electron DE

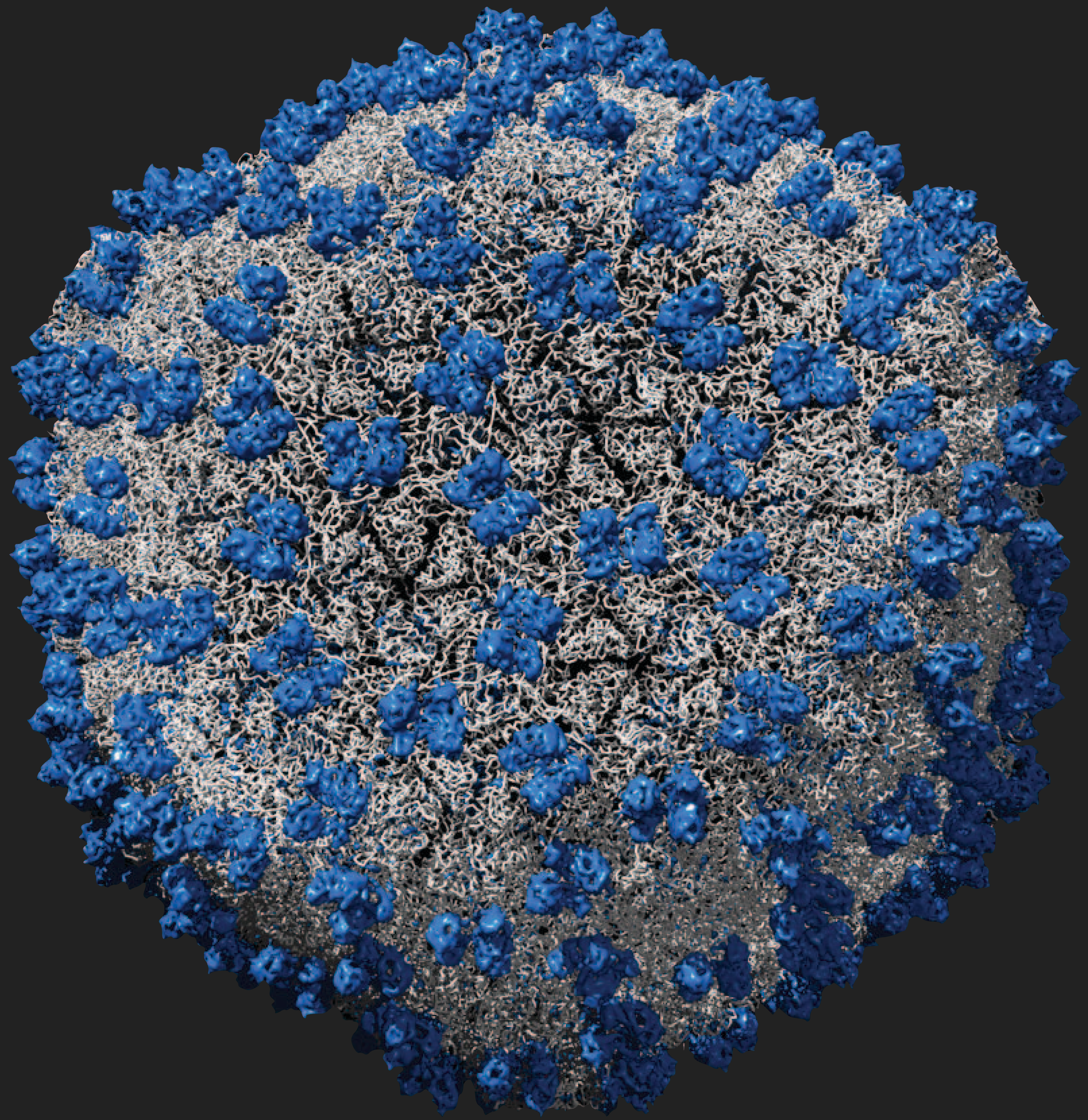


New Image Processing Schema

- Maximum Likelihood methods
- 3D Focus Classification methods
  - RELION
  - EMAN2

FROM MAP TO MODEL





UTMB CRYO-EM WORKSHOP

RESOLUTION, RESOLVABILITY AND CRYO-EM

Matthew Baker, Ph.D
Baylor College of Medicine

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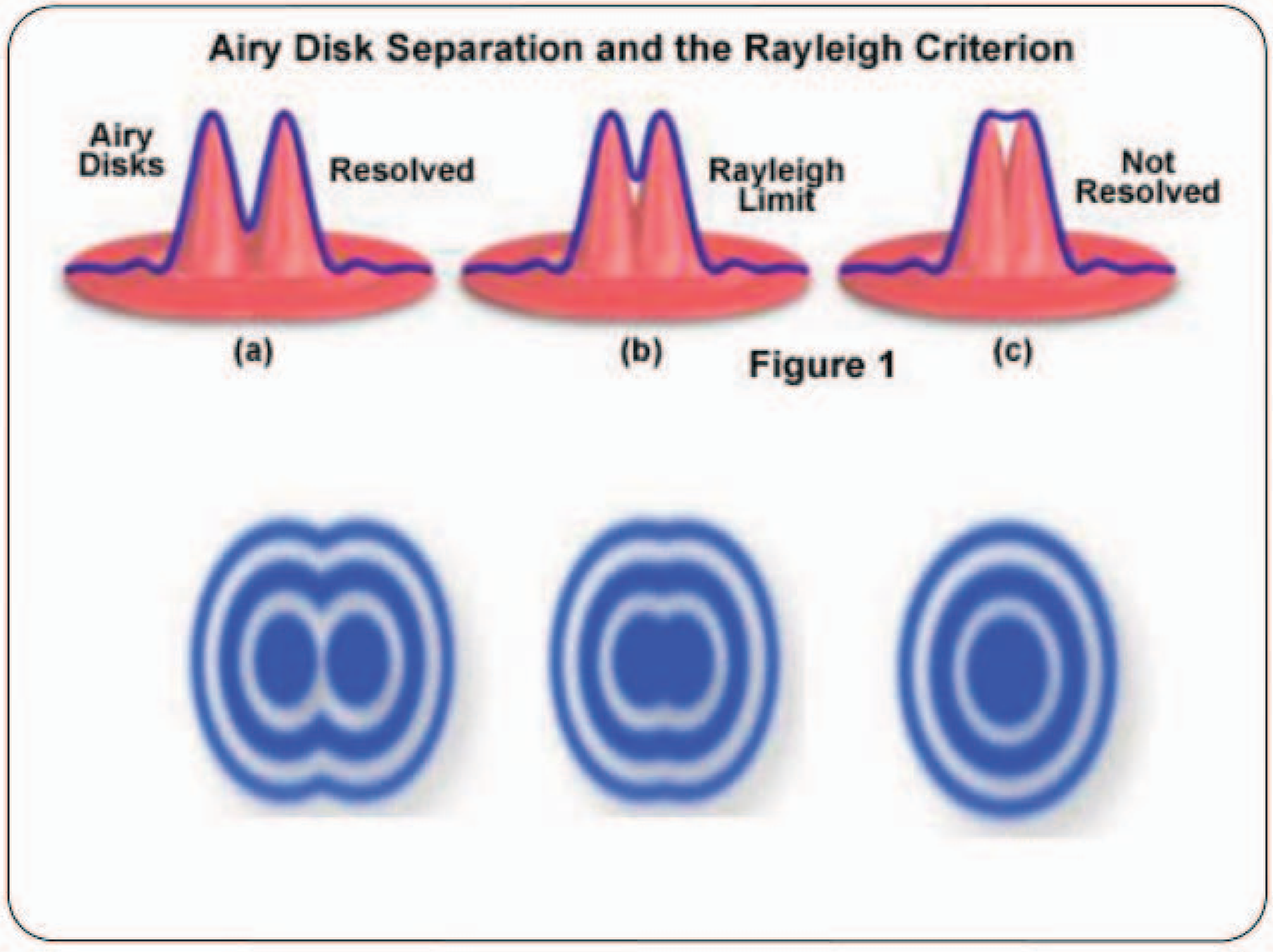
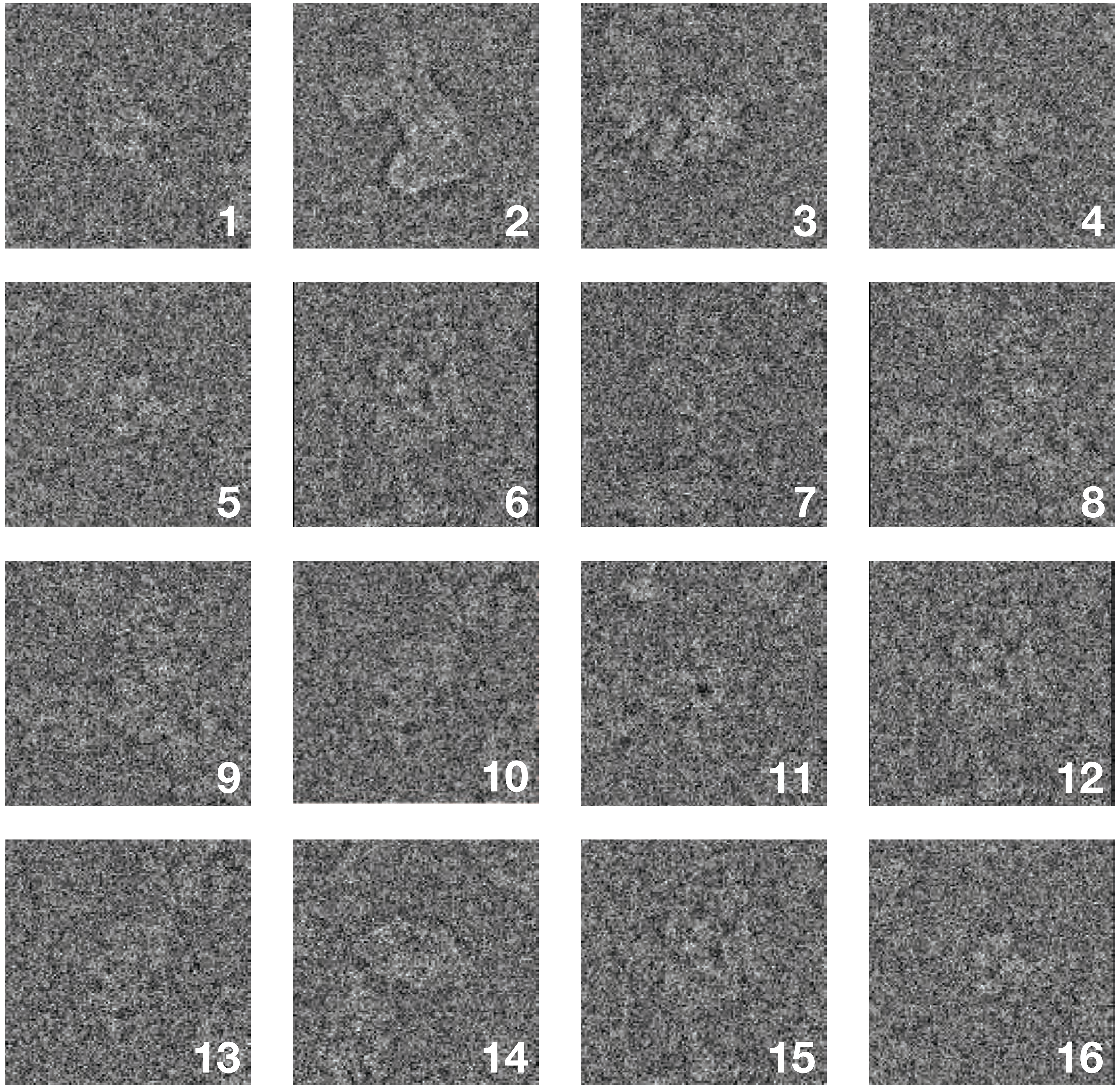
RESOLUTION

- Resolution is the ability to distinguish two points as separate structures rather a single feature.
- In practice, resolution is a measure of the level of detail

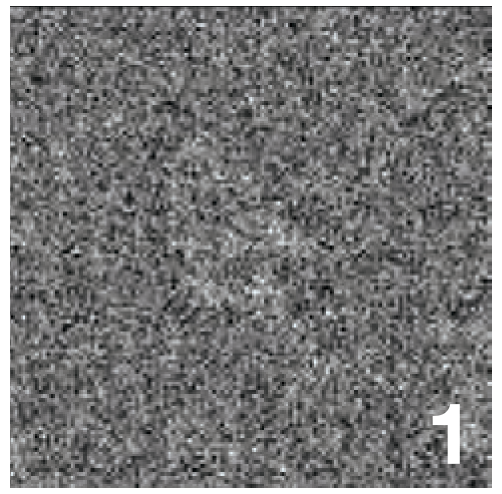


DEFINING RESOLUTION IN CRYO-EM

- Resolution is characterized by features in the density maps
- Fourier Shell Correlation (FSC) measures strength of features relative to noise
- Gold Standard Refinement
    - Data is split into 2 halves, reconstructed using two different starting models and compared
- A “threshold” is set to measure resolution



GOLD STANDARD REFINEMENT AND FSC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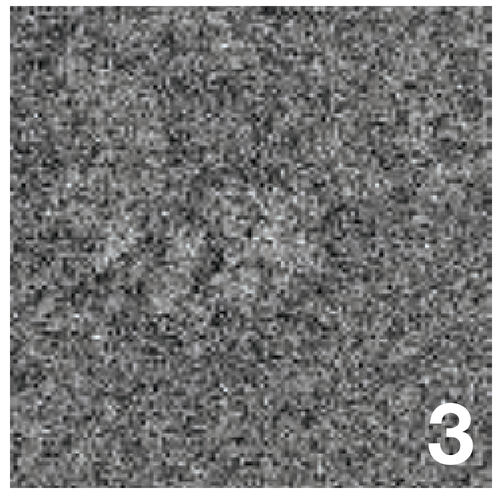
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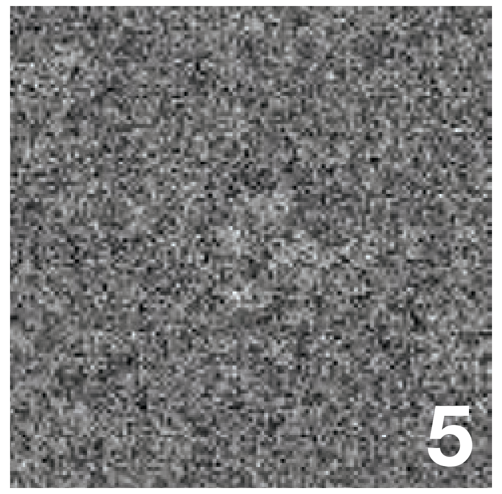
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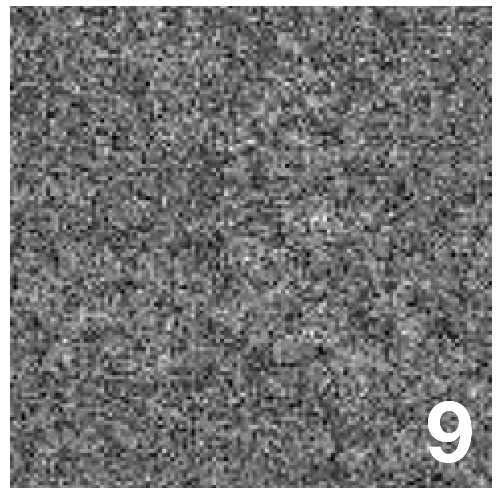
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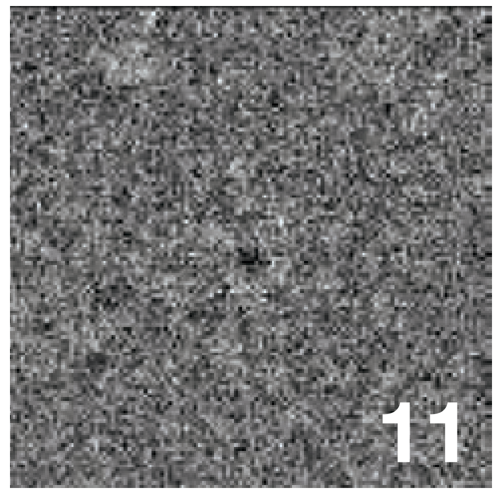
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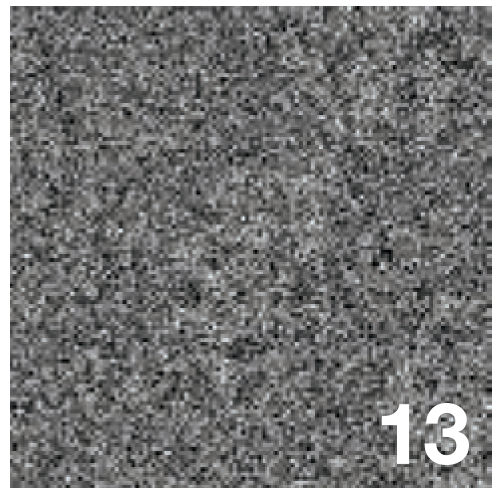
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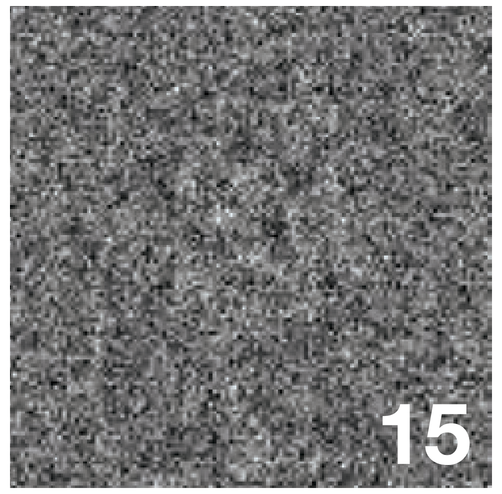
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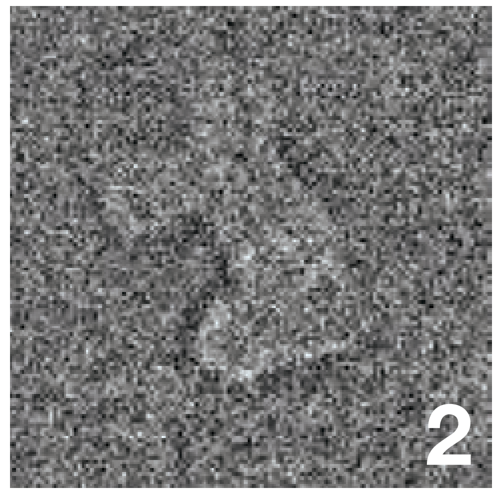
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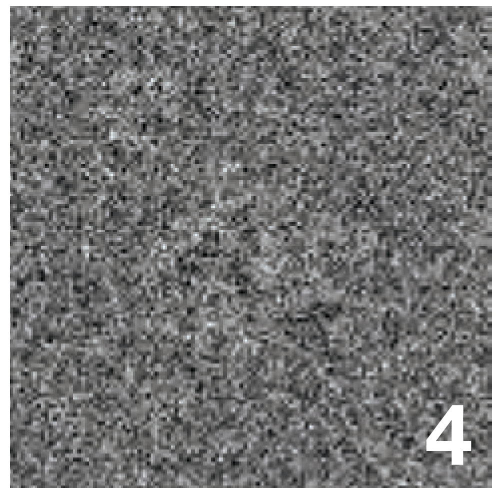
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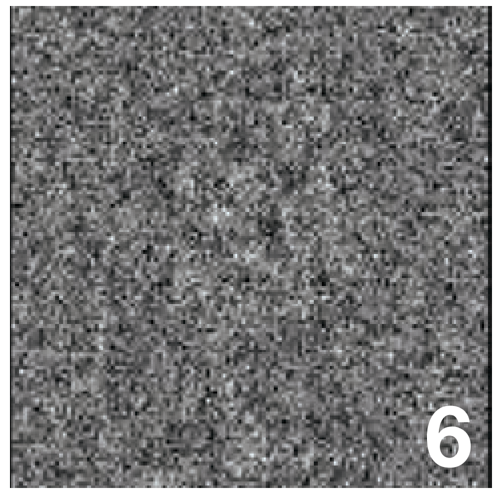
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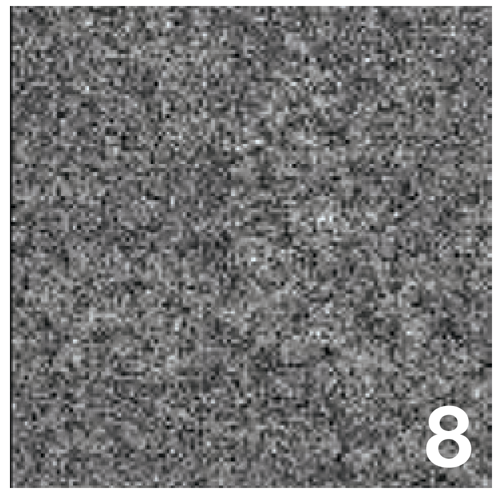
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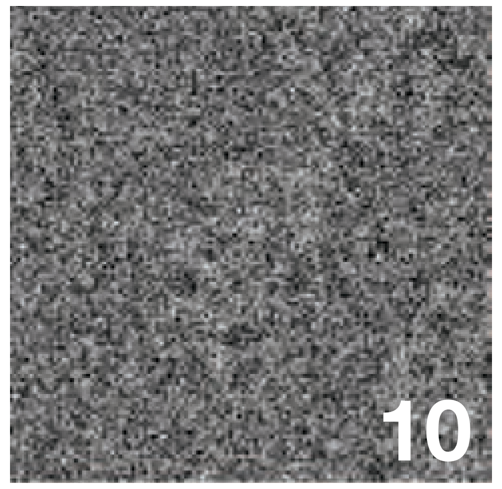
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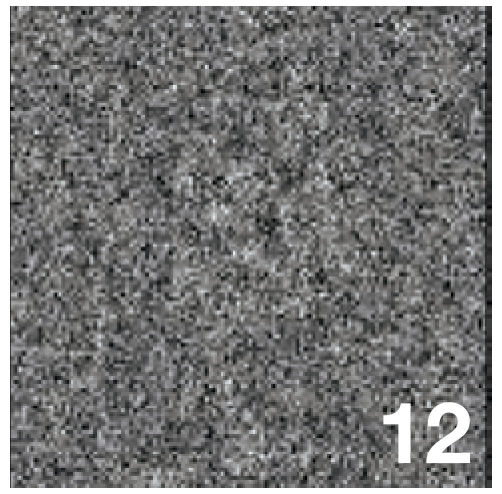
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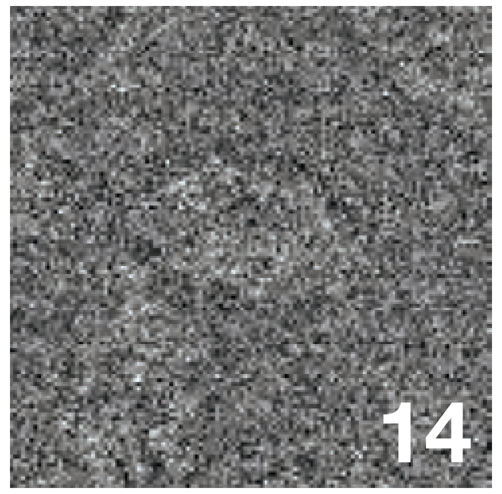
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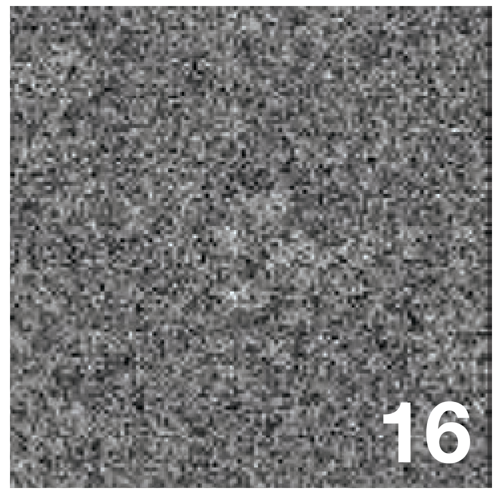
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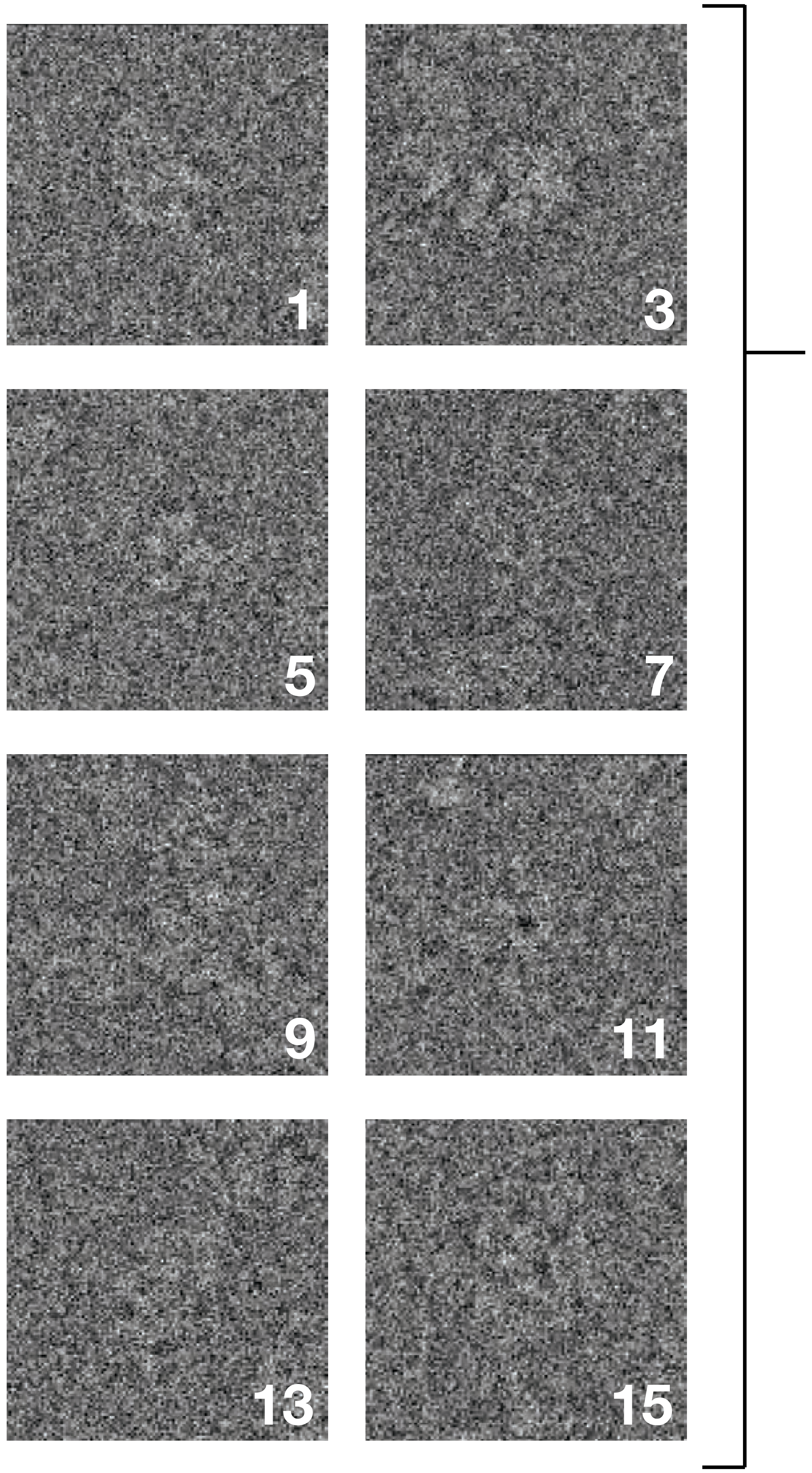
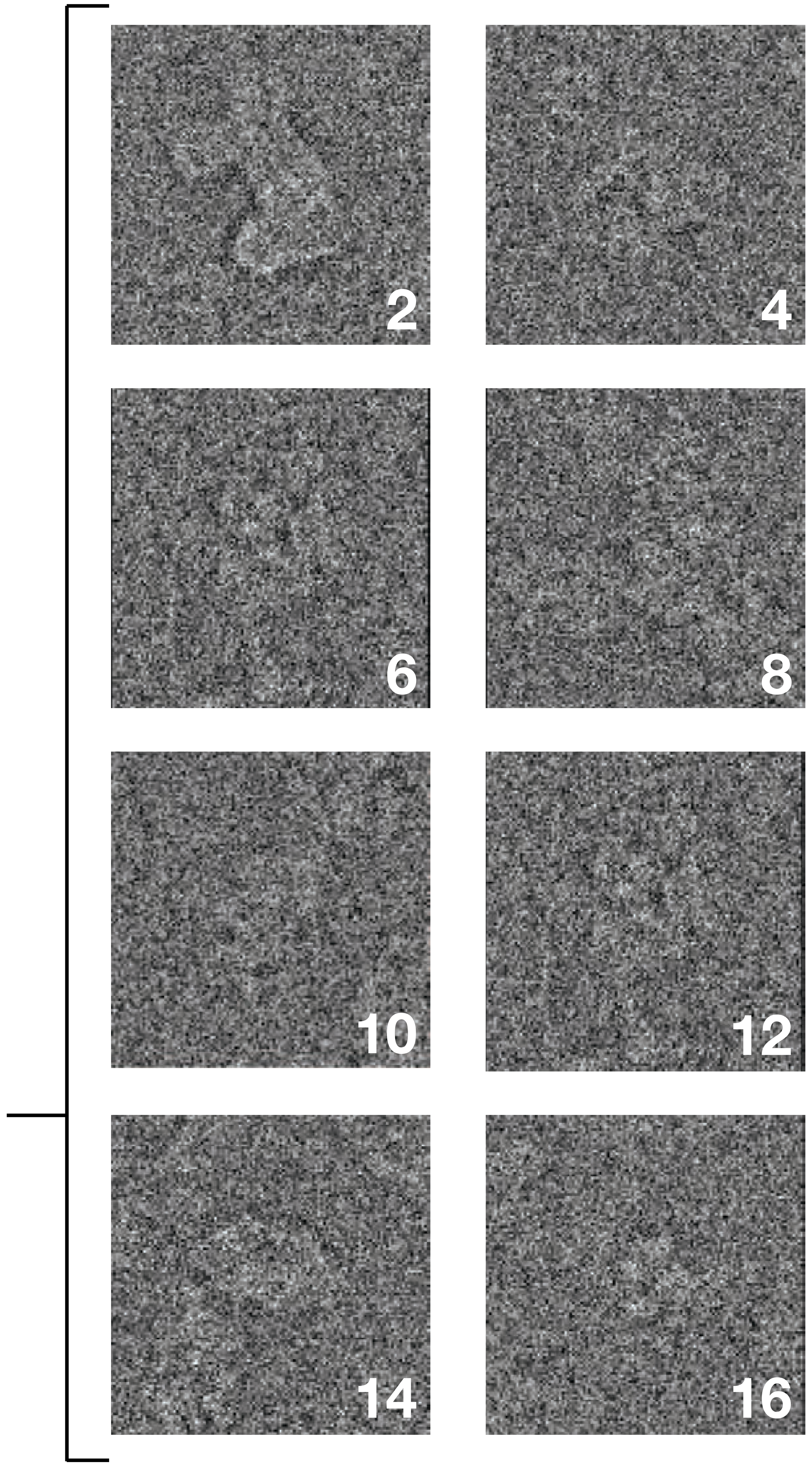
GOLD STANDARD REFINEMENT AND FSC

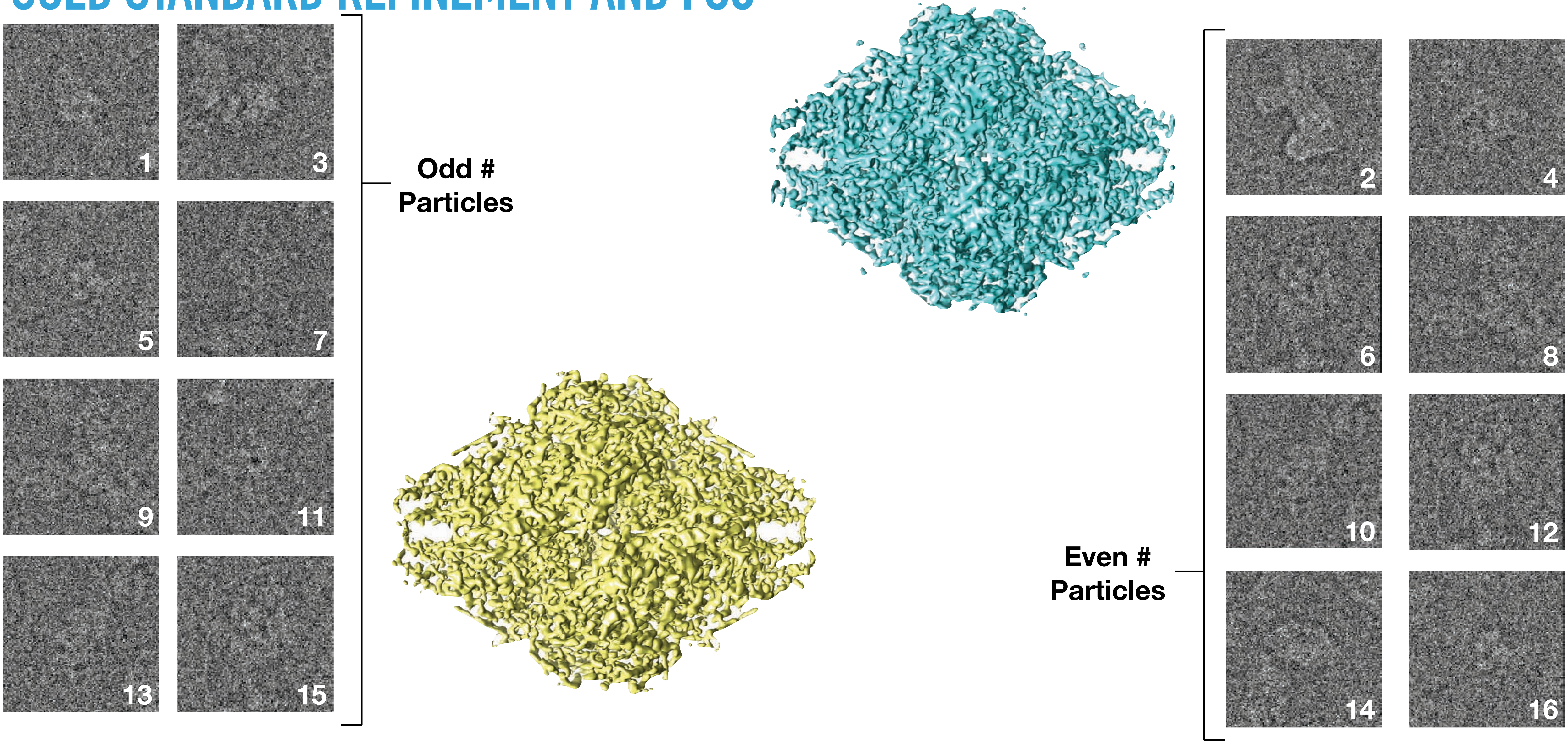


**Odd #
Particles**



**Even #
Particles**

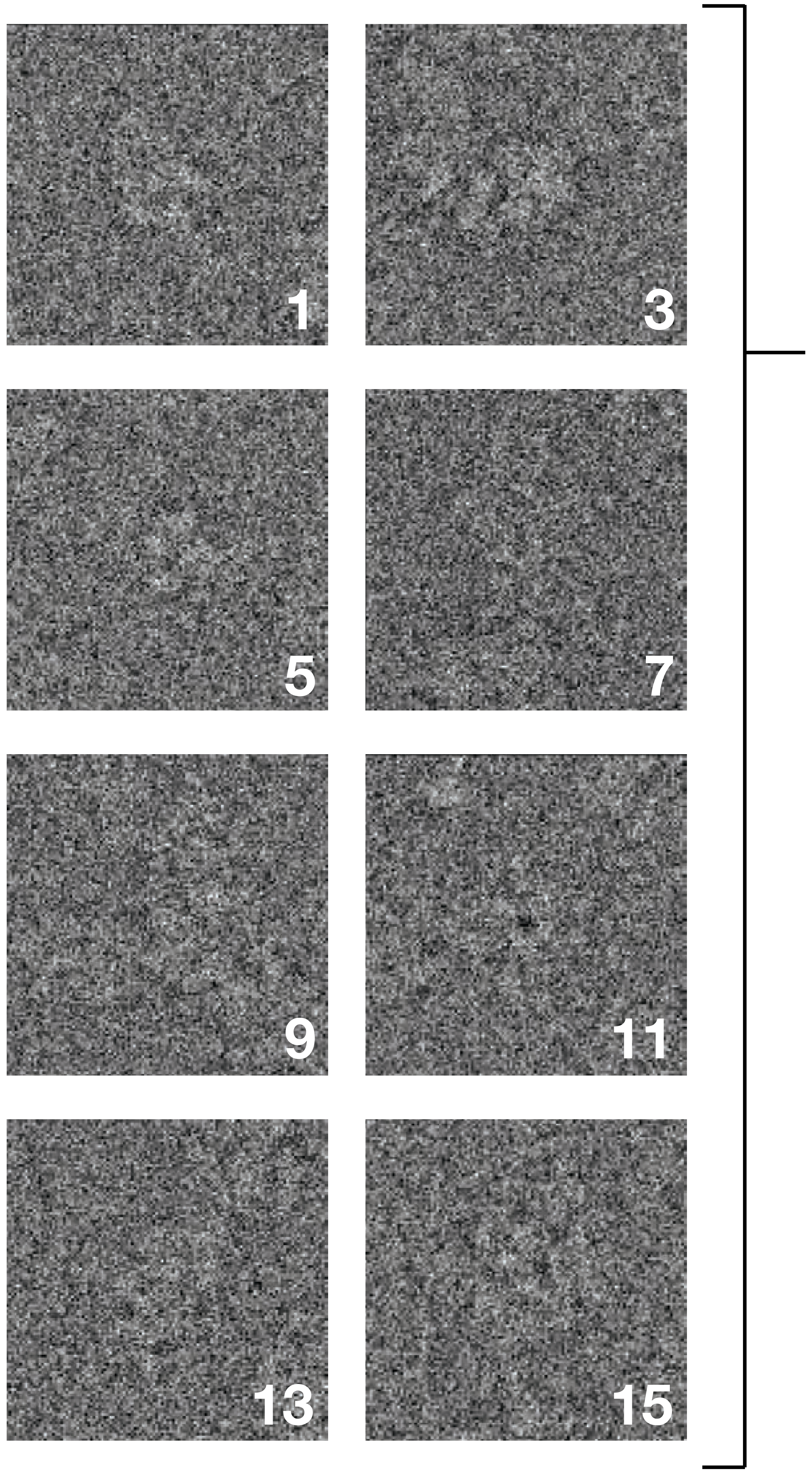
GOLD STANDARD REFINEMENT AND FSC



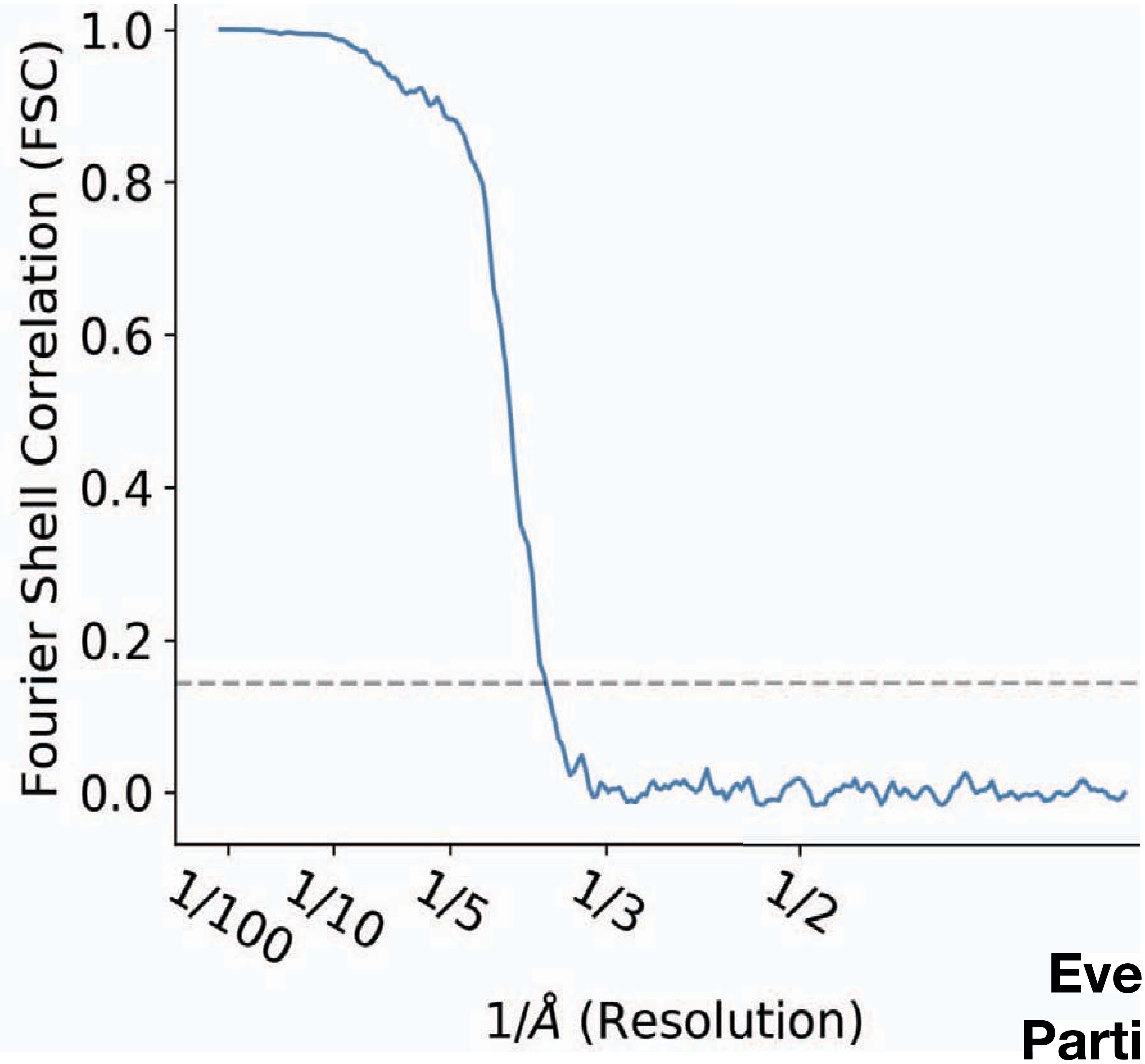
Odd # Particles

Even # Particles

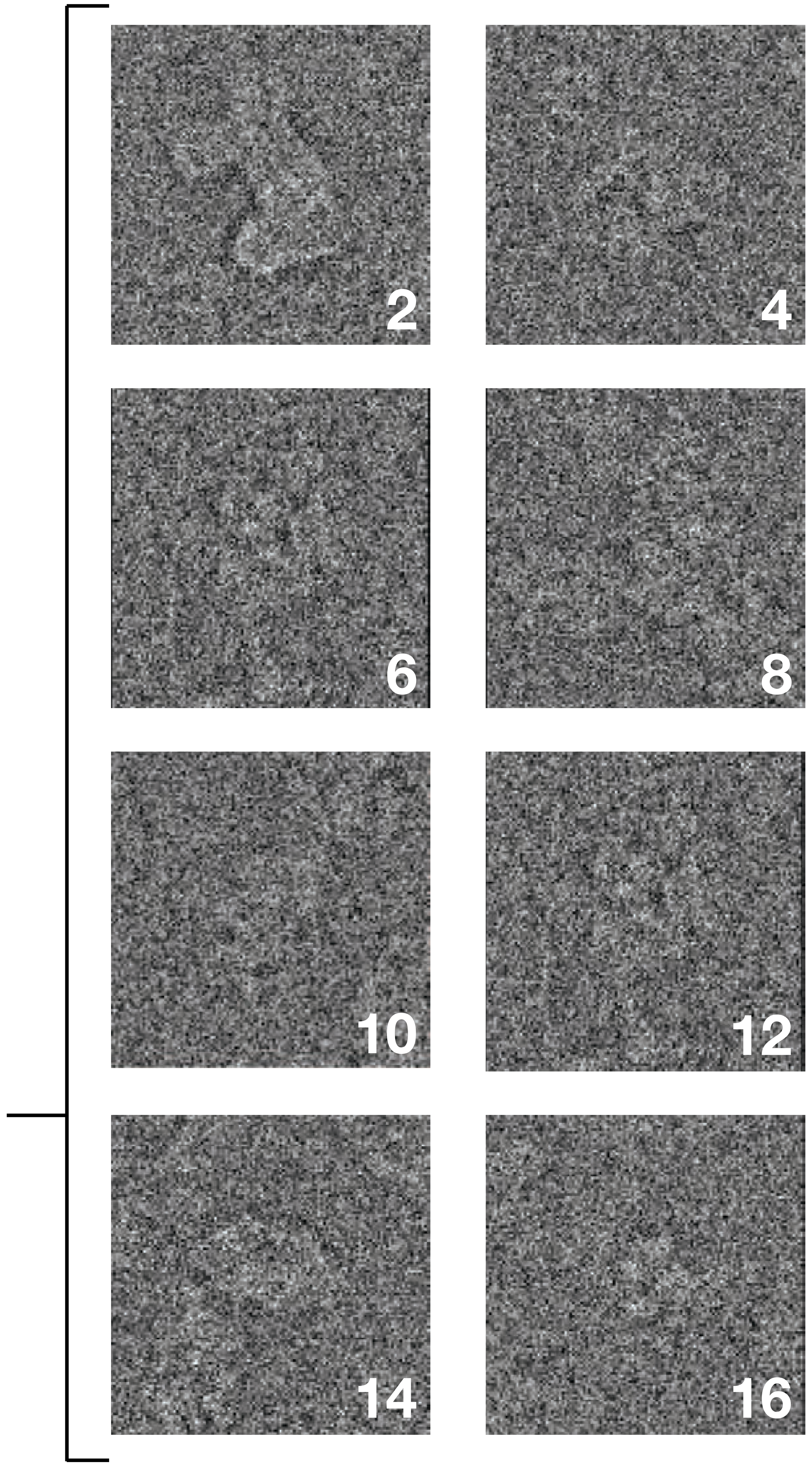
GOLD STANDARD REFINEMENT AND FSC



Odd # Particles



Even # Particles



CRYO-EM RESOLUTION AND GROEL

RESOLVABILITY MILESTONES

Subunit boundaries

Beta Sheets

Helix pitch

Large side chains

Rotamers

15Å

10Å

5Å

2Å

Alpha helices

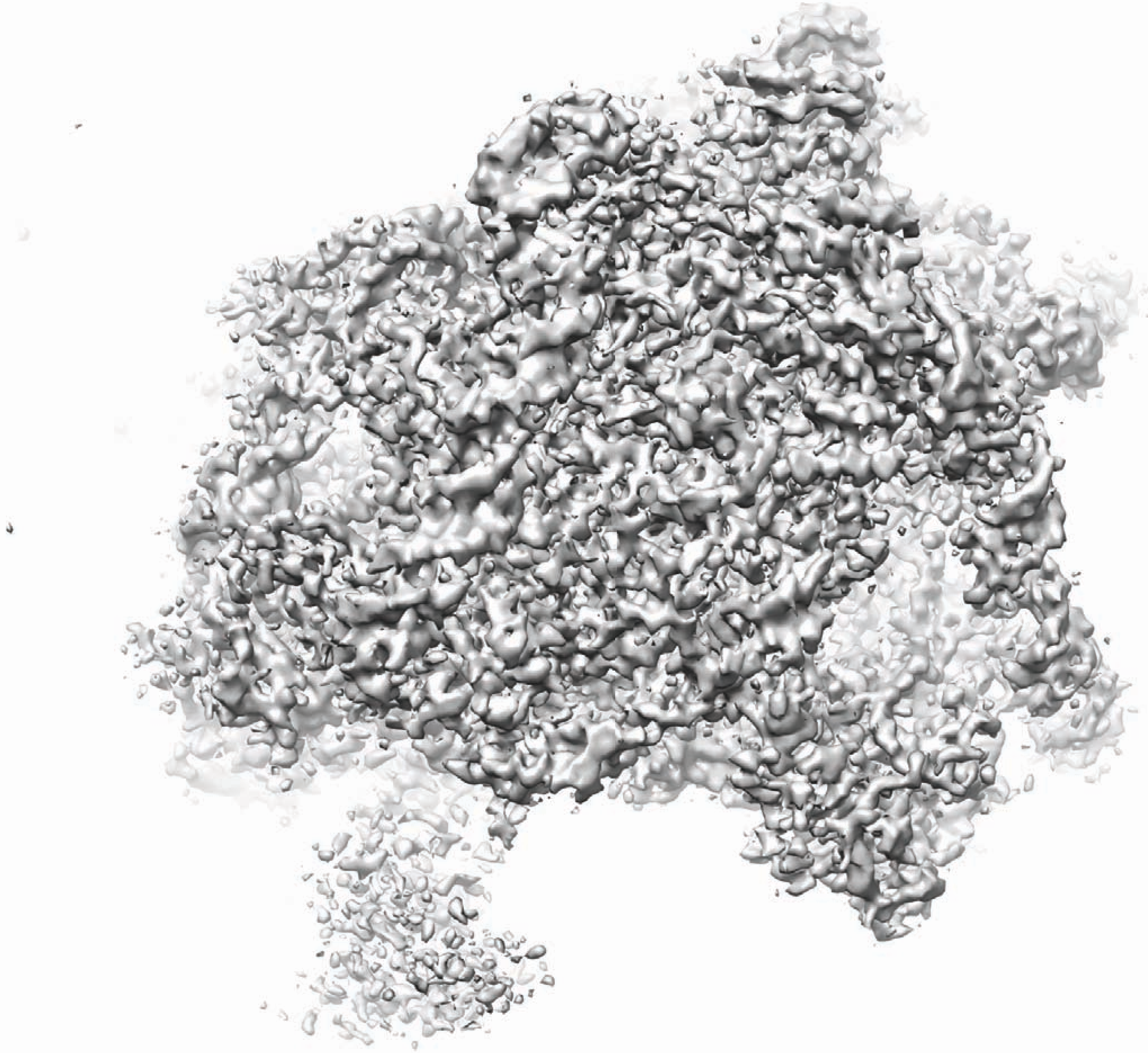
Subunit connectivity

Beta strands

Most side chains

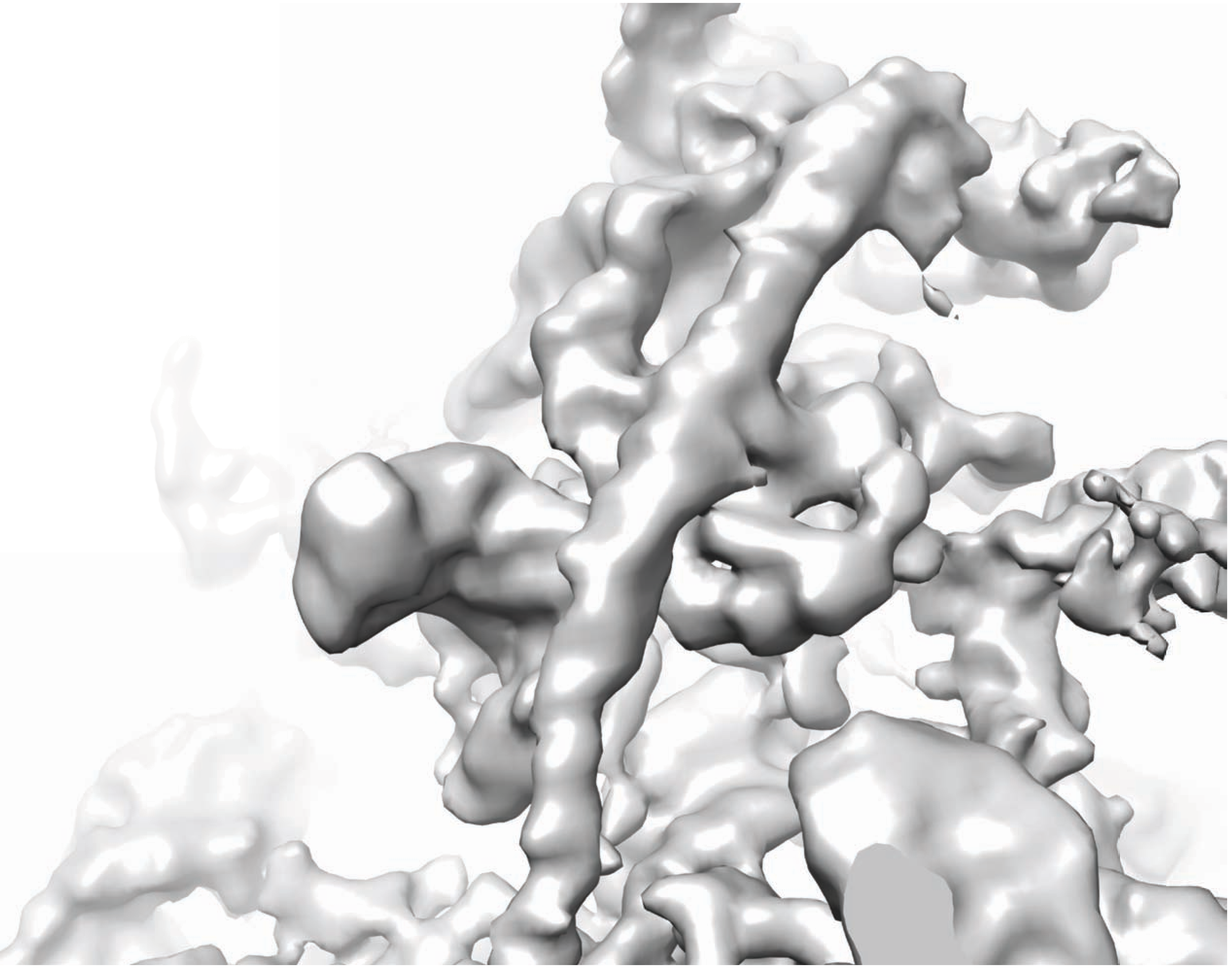
RESOLUTION VS. RESOLVABILITY

EMDB 3888: 4.2Å resolution (2017)

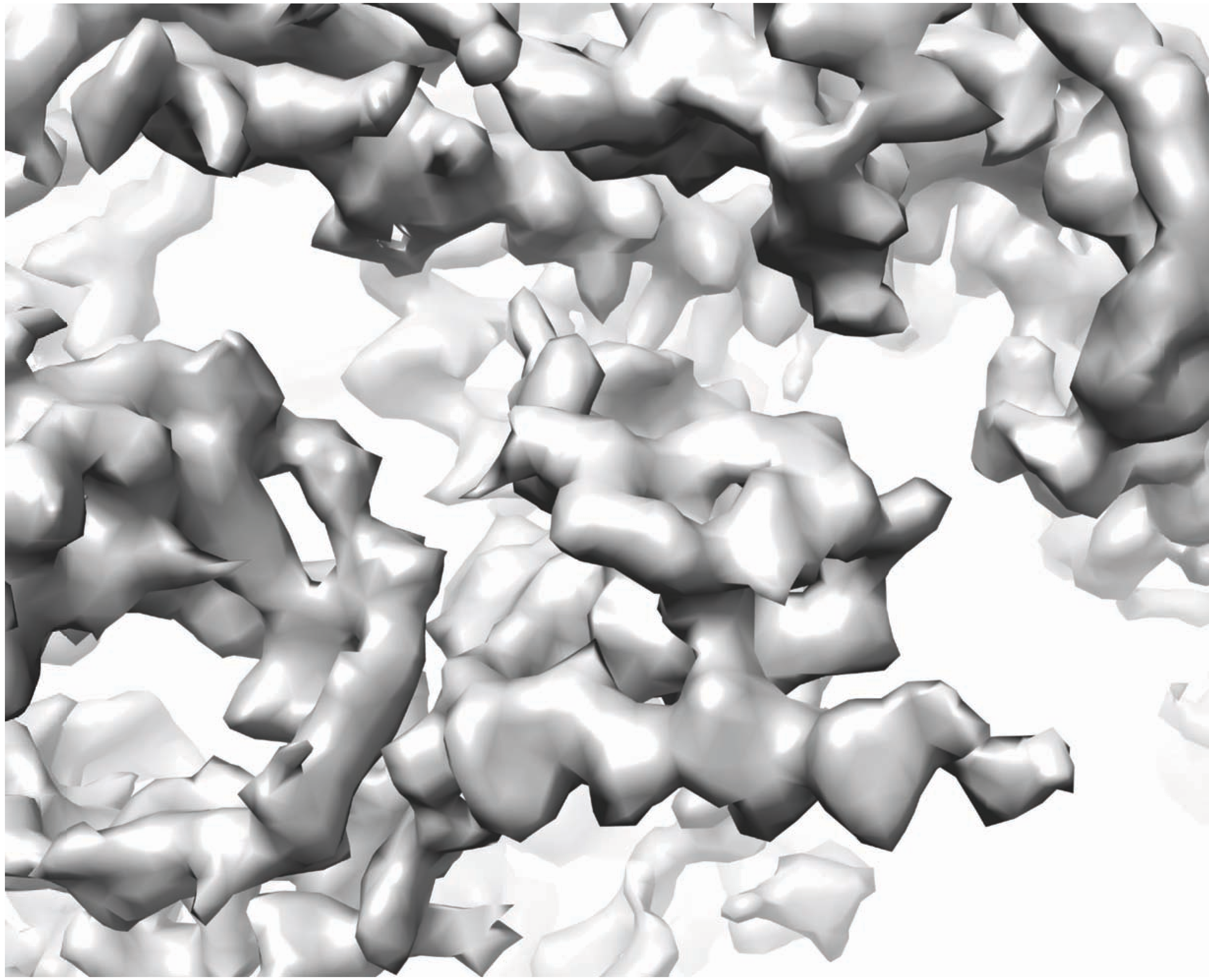


EMDB 3147: 4.2Å resolution (2015)

RESOLUTION VS. RESOLVABILITY



EMDB 3888: 4.2Å resolution (2017)



EMDB 3147: 4.2Å resolution (2015)

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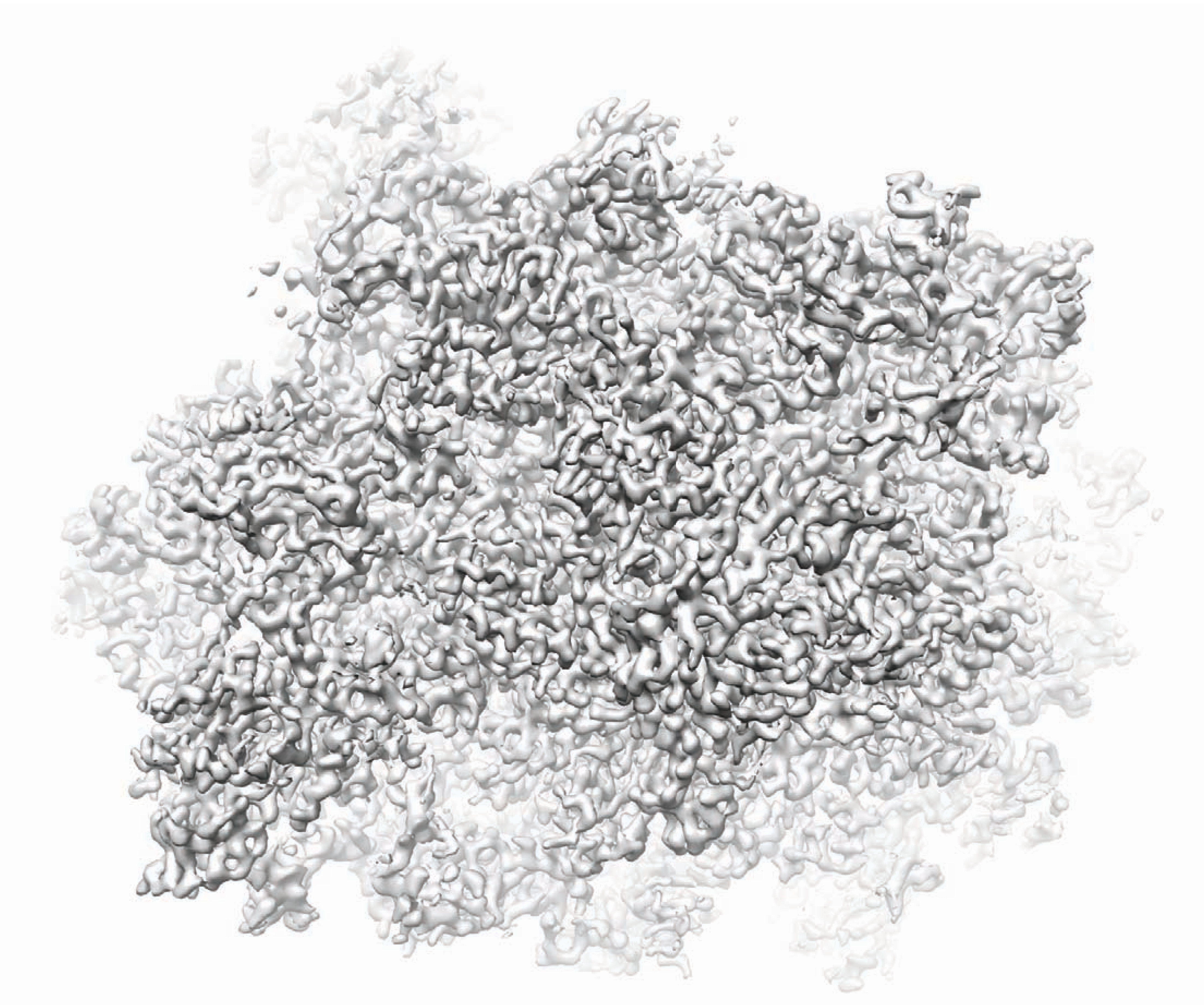
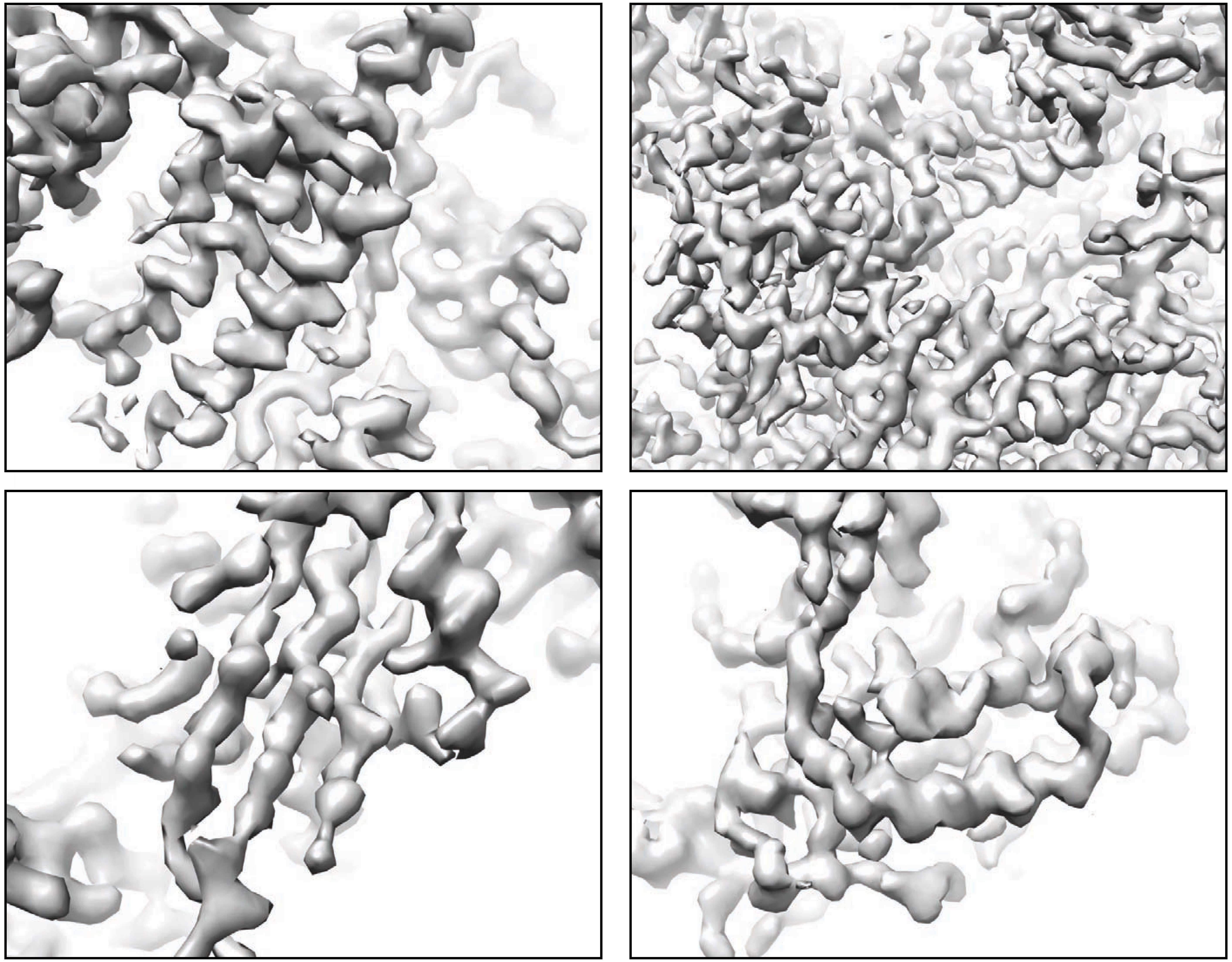
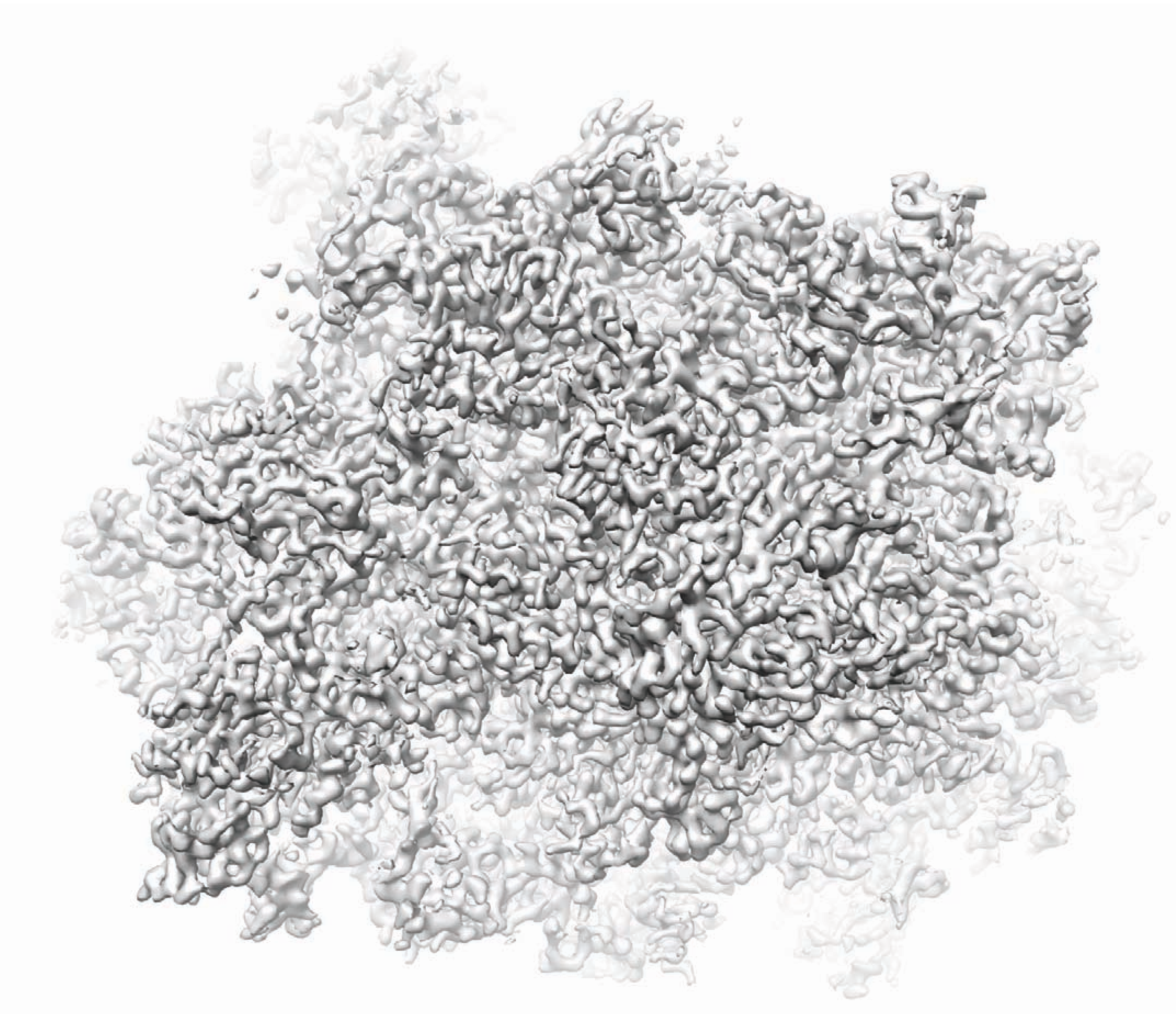


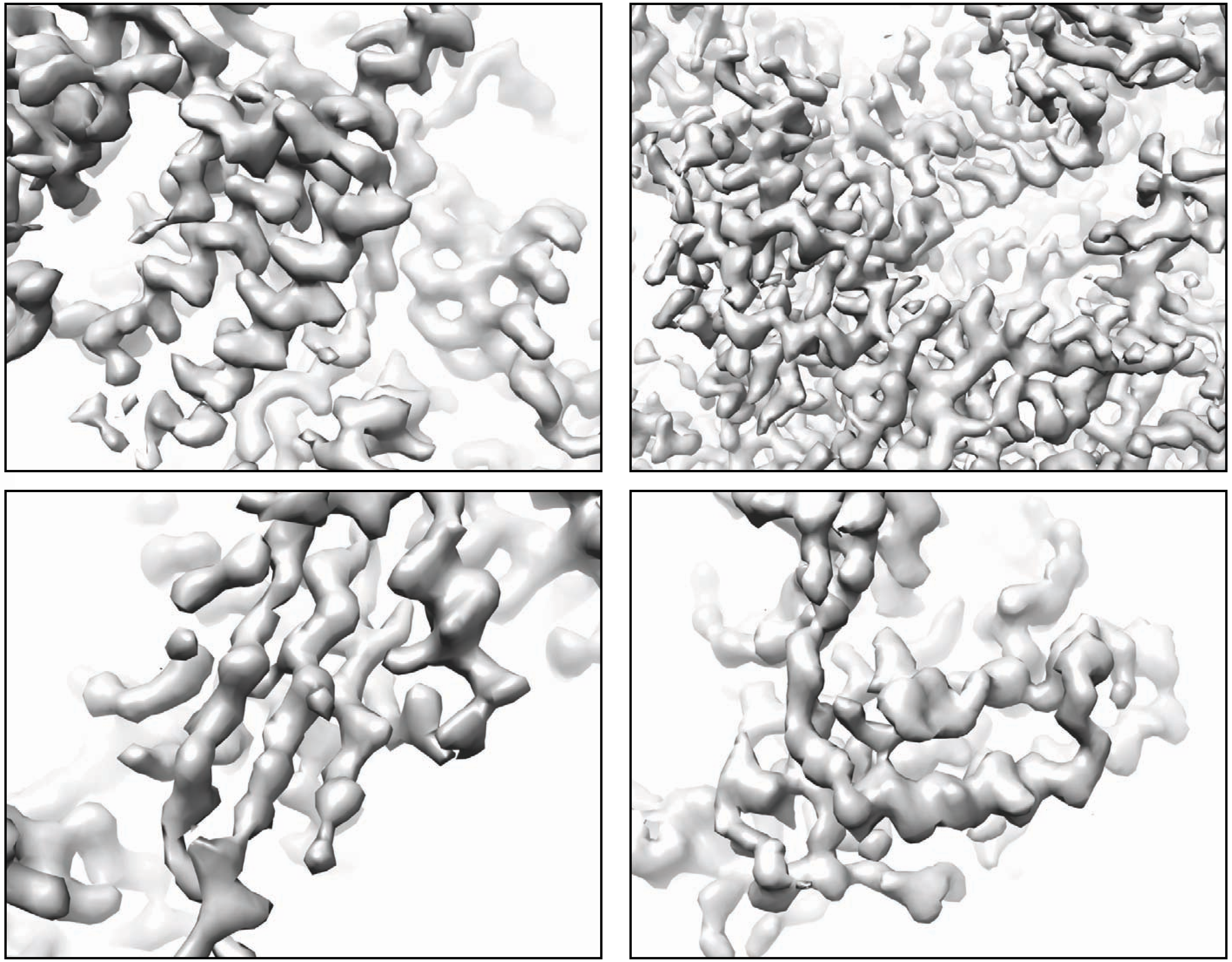
EMDB 0373



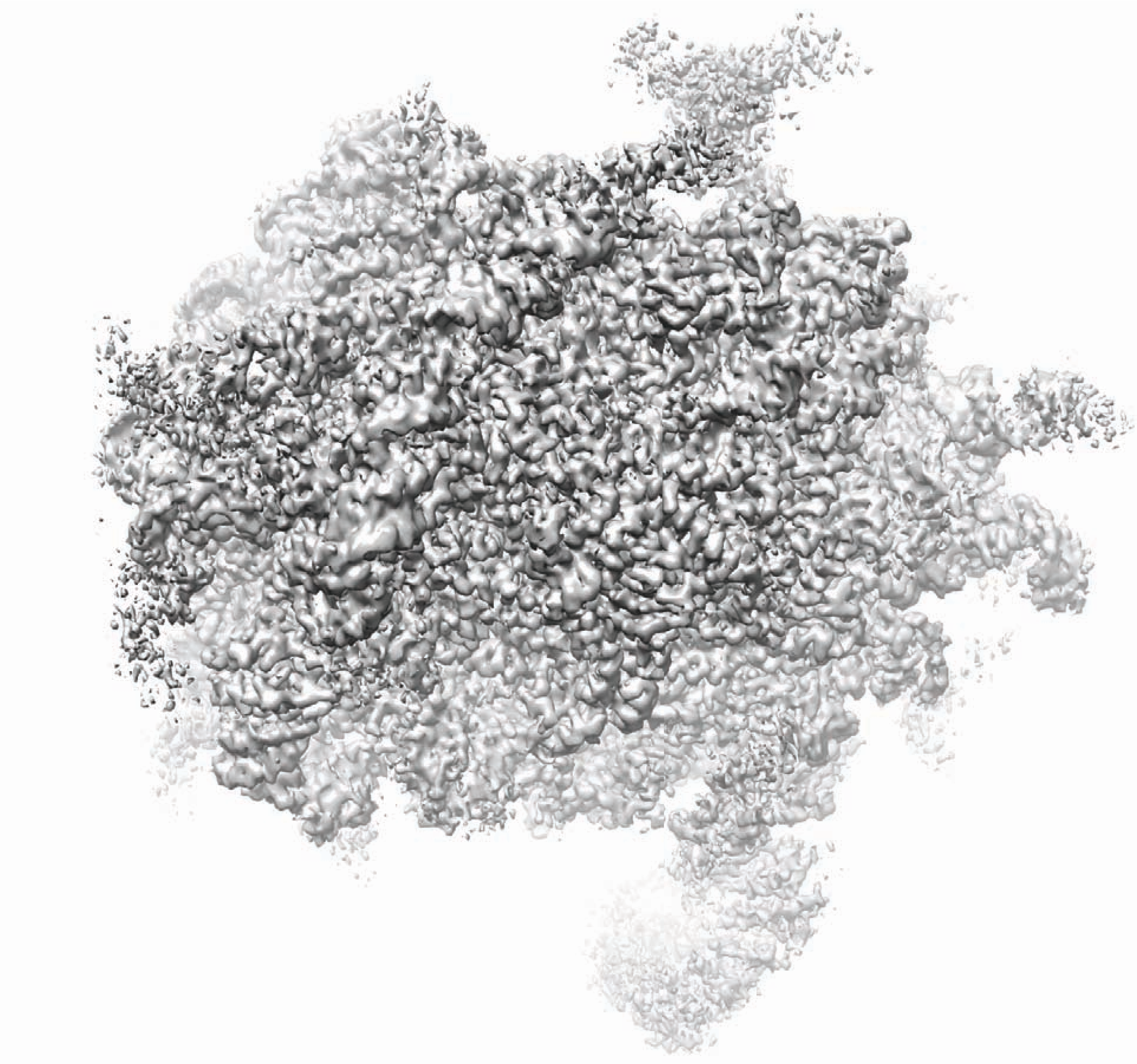
EXAMPLE 1



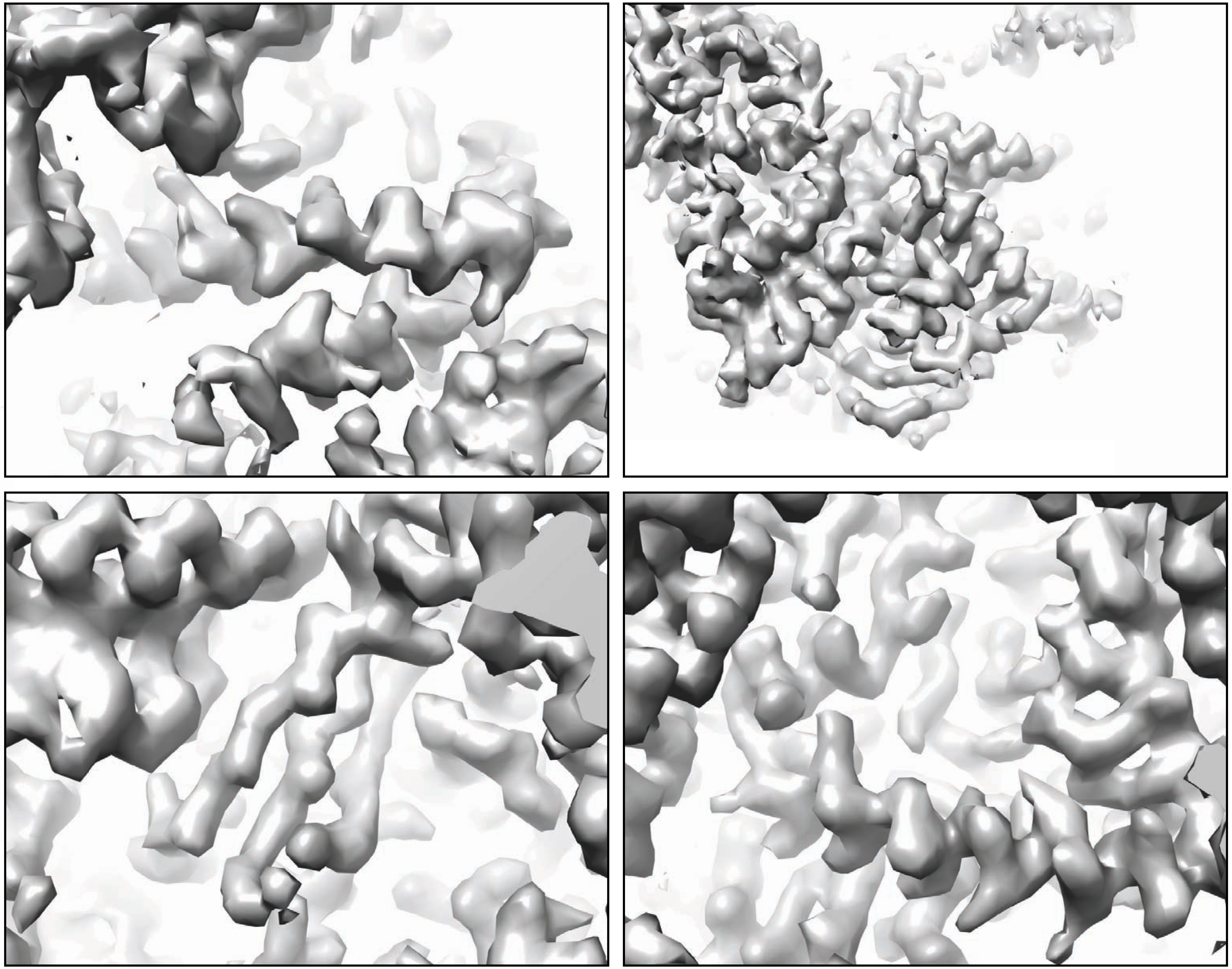
EMDB 0373 3.8Å resolution



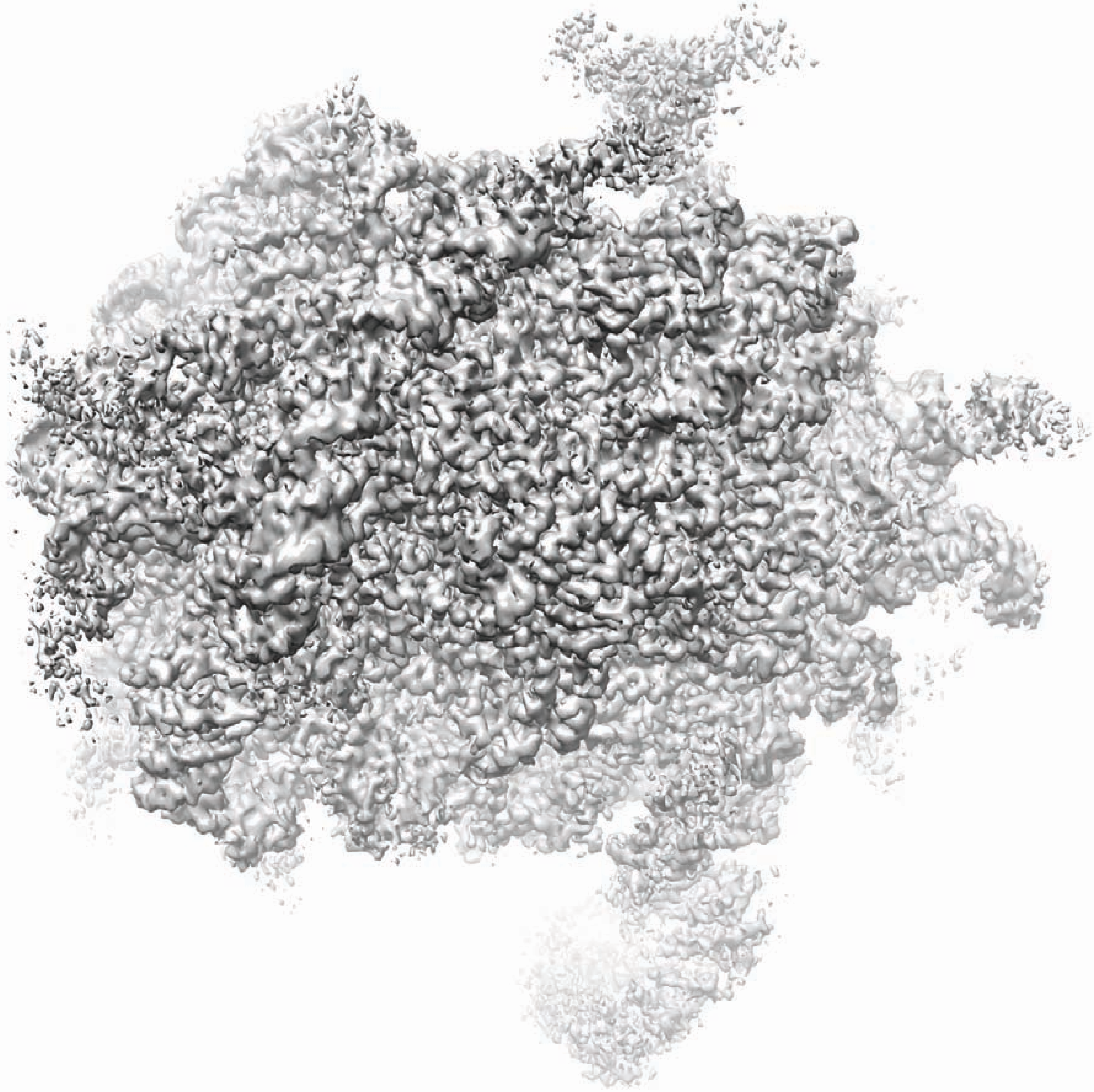
EXAMPLE 2



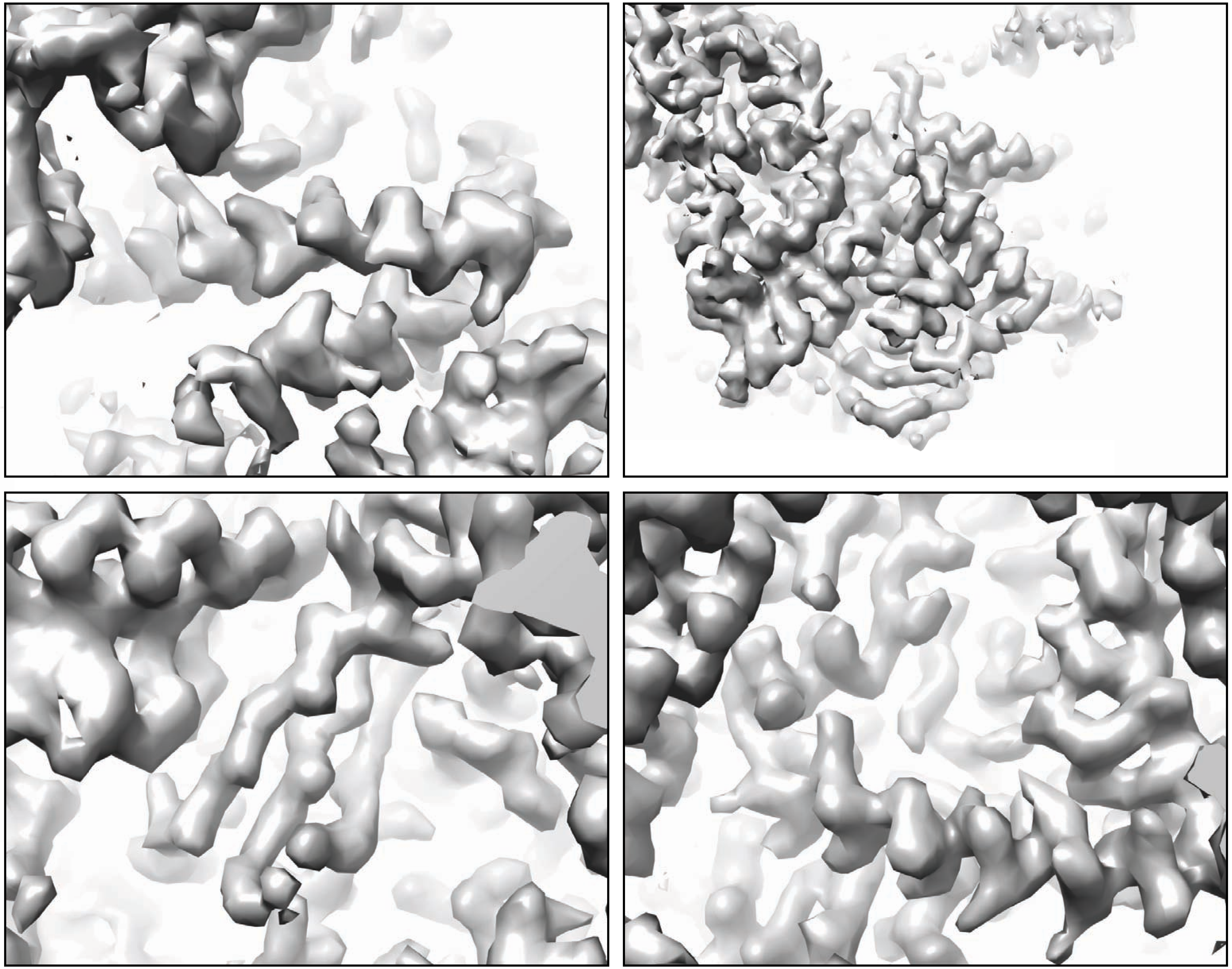
EMDB 2832



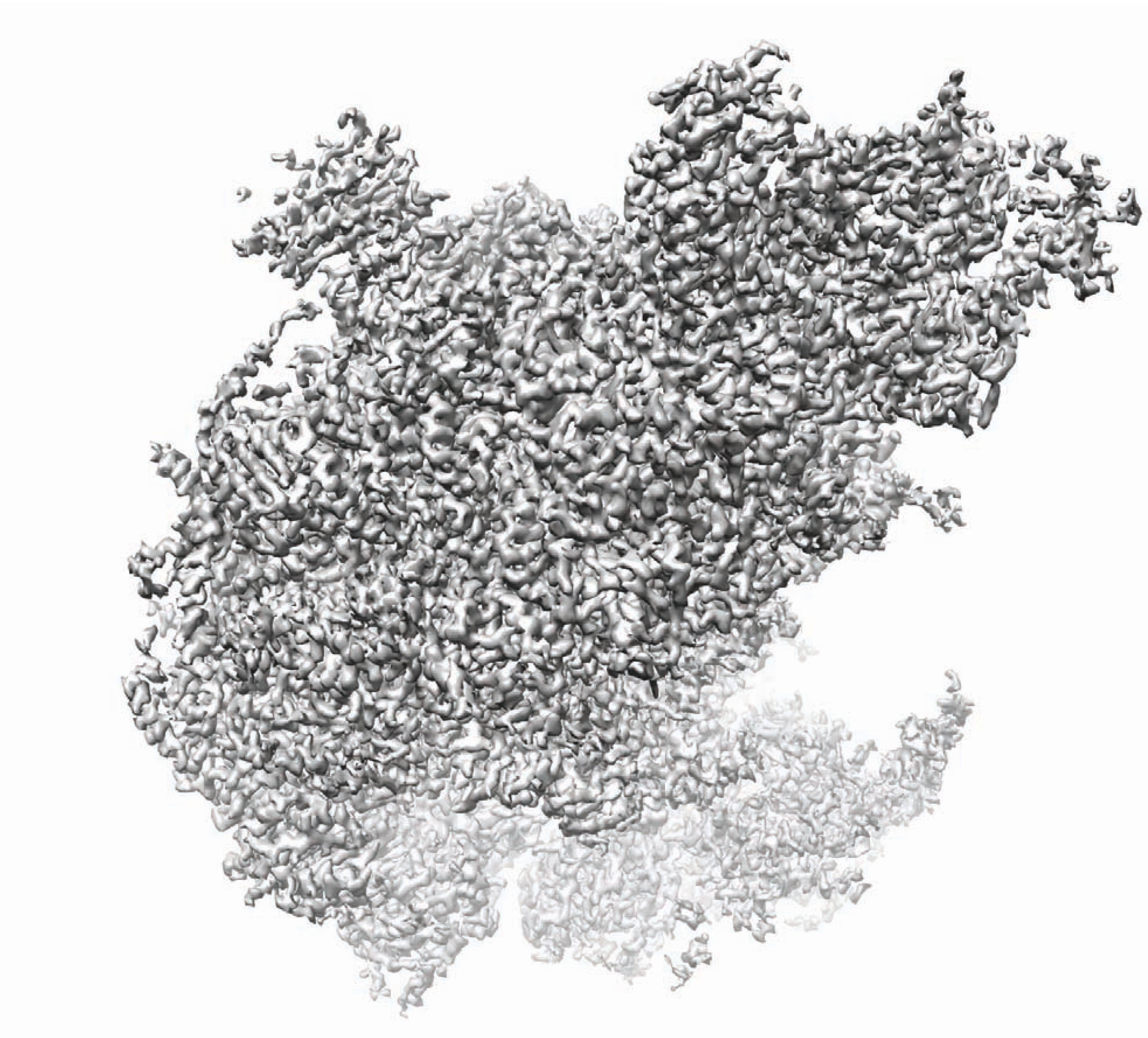
EXAMPLE 2



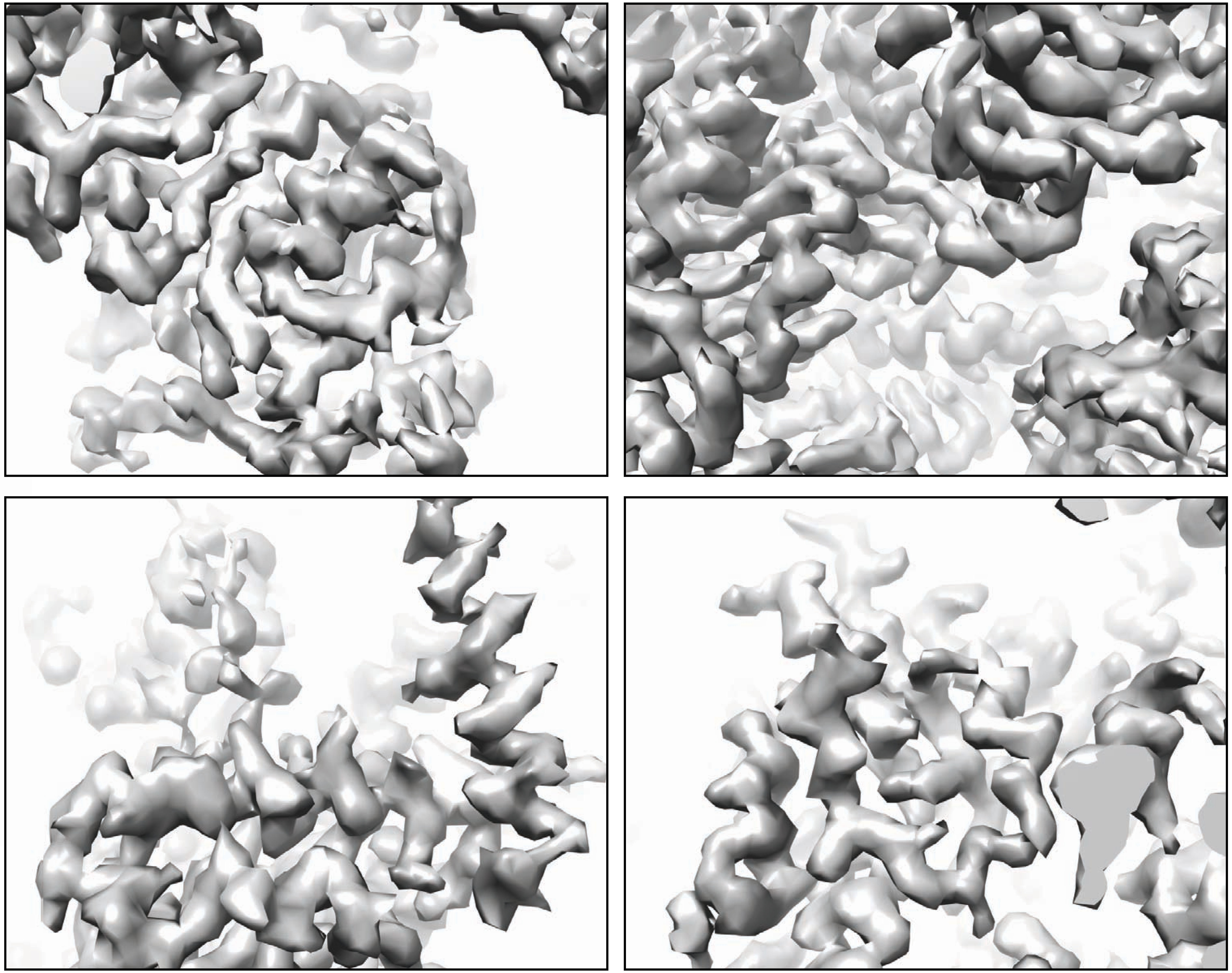
EMDB 2832 3.6Å resolution



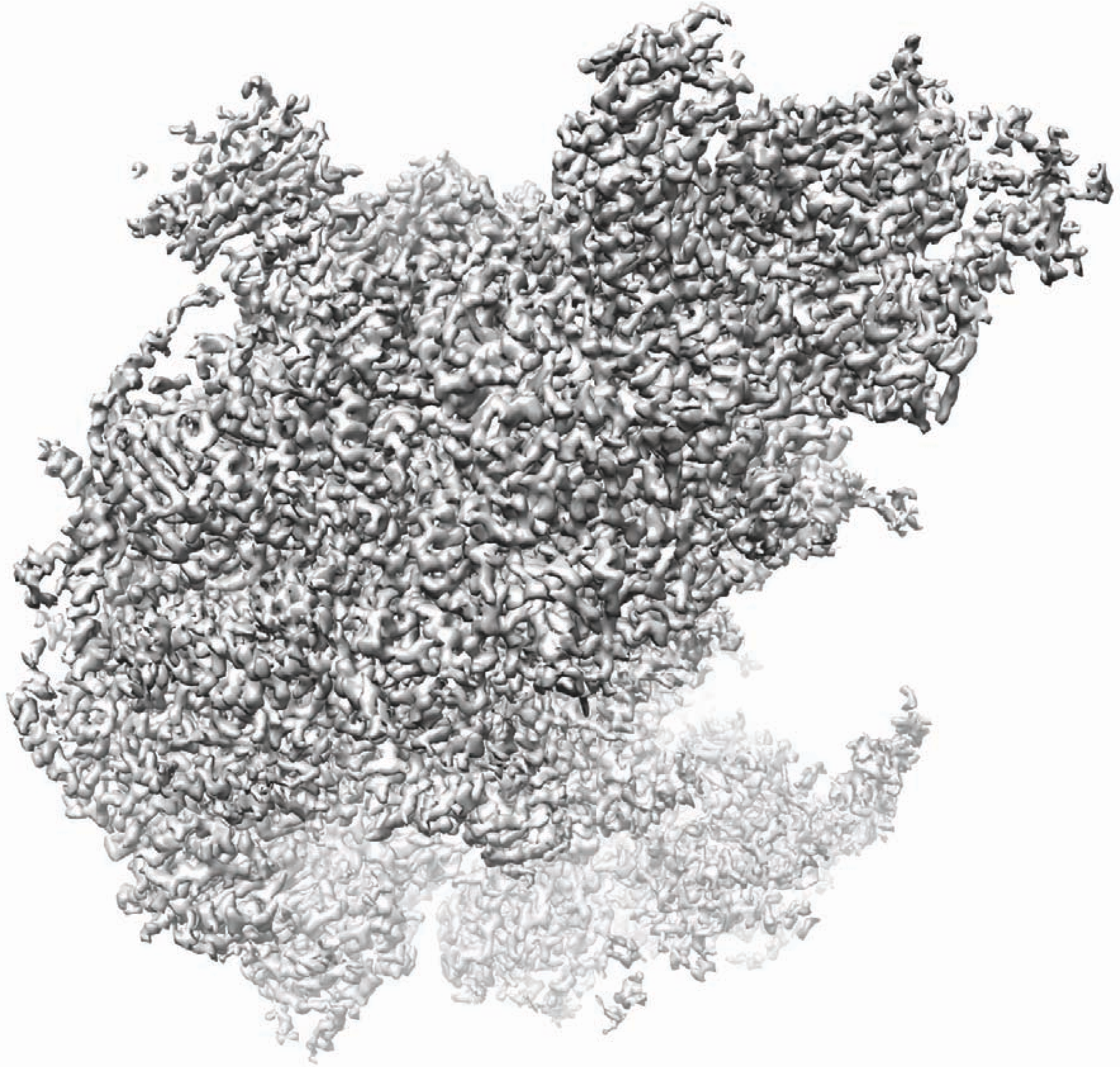
EXAMPLE 3



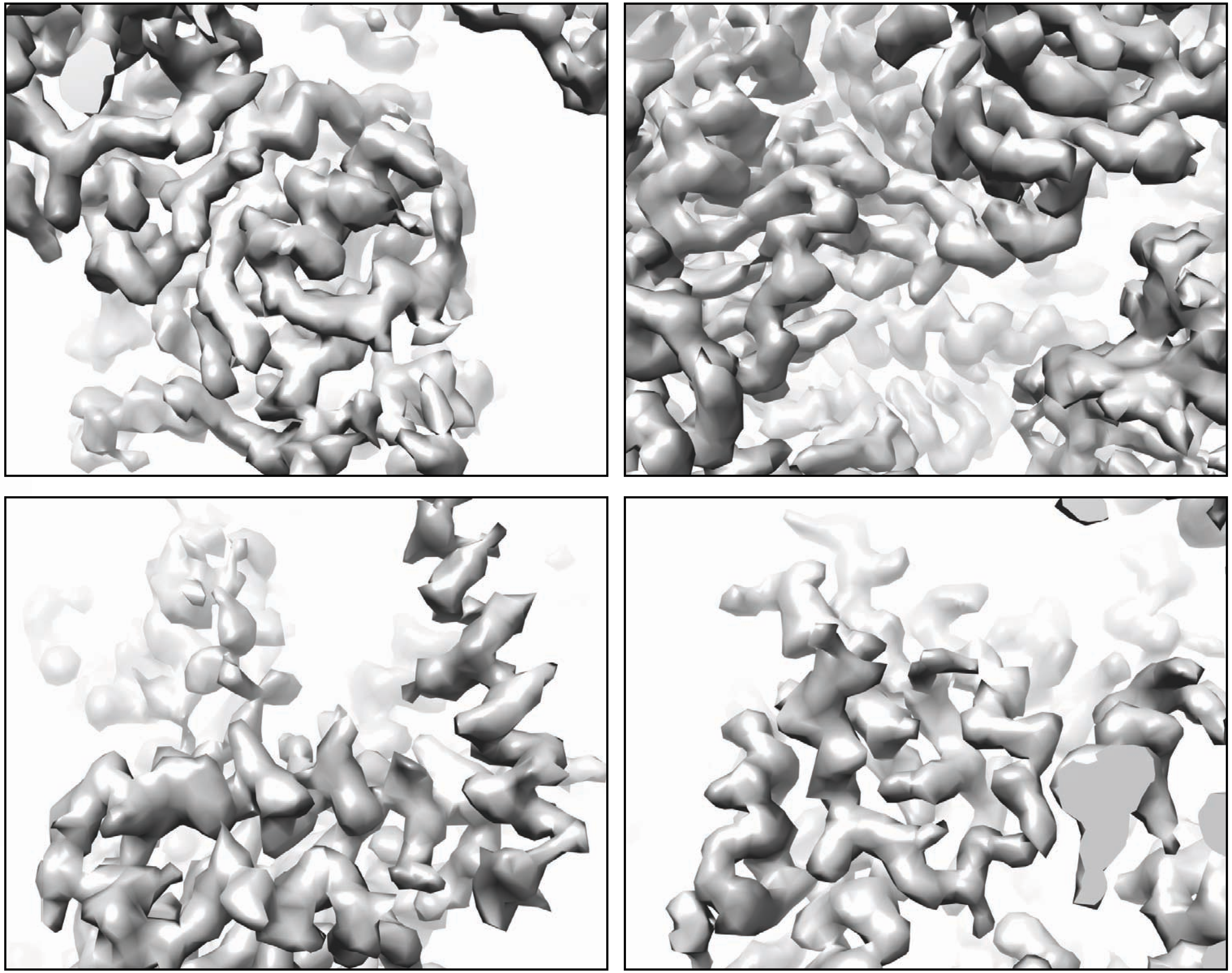
EMDB 6615



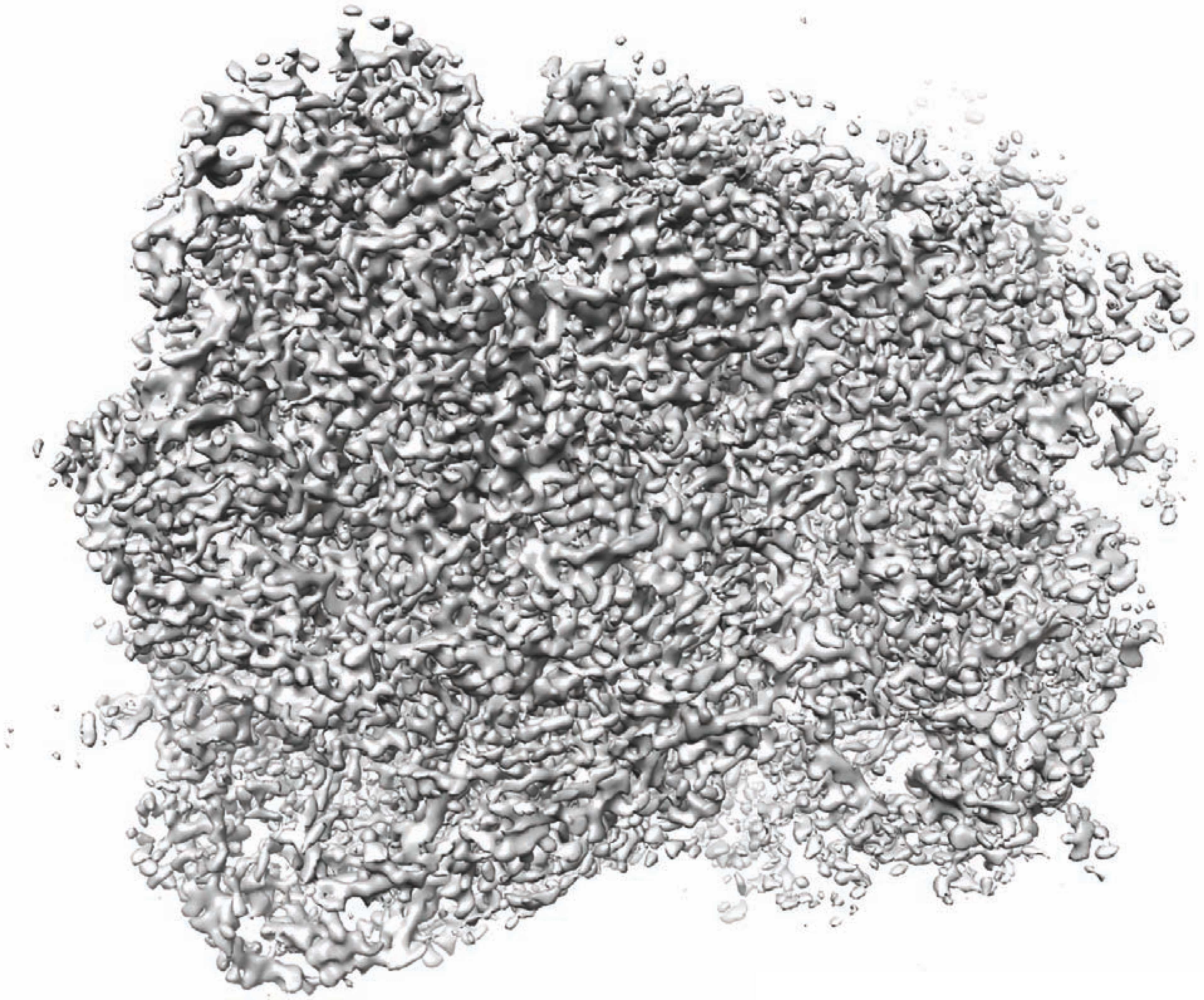
EXAMPLE 3



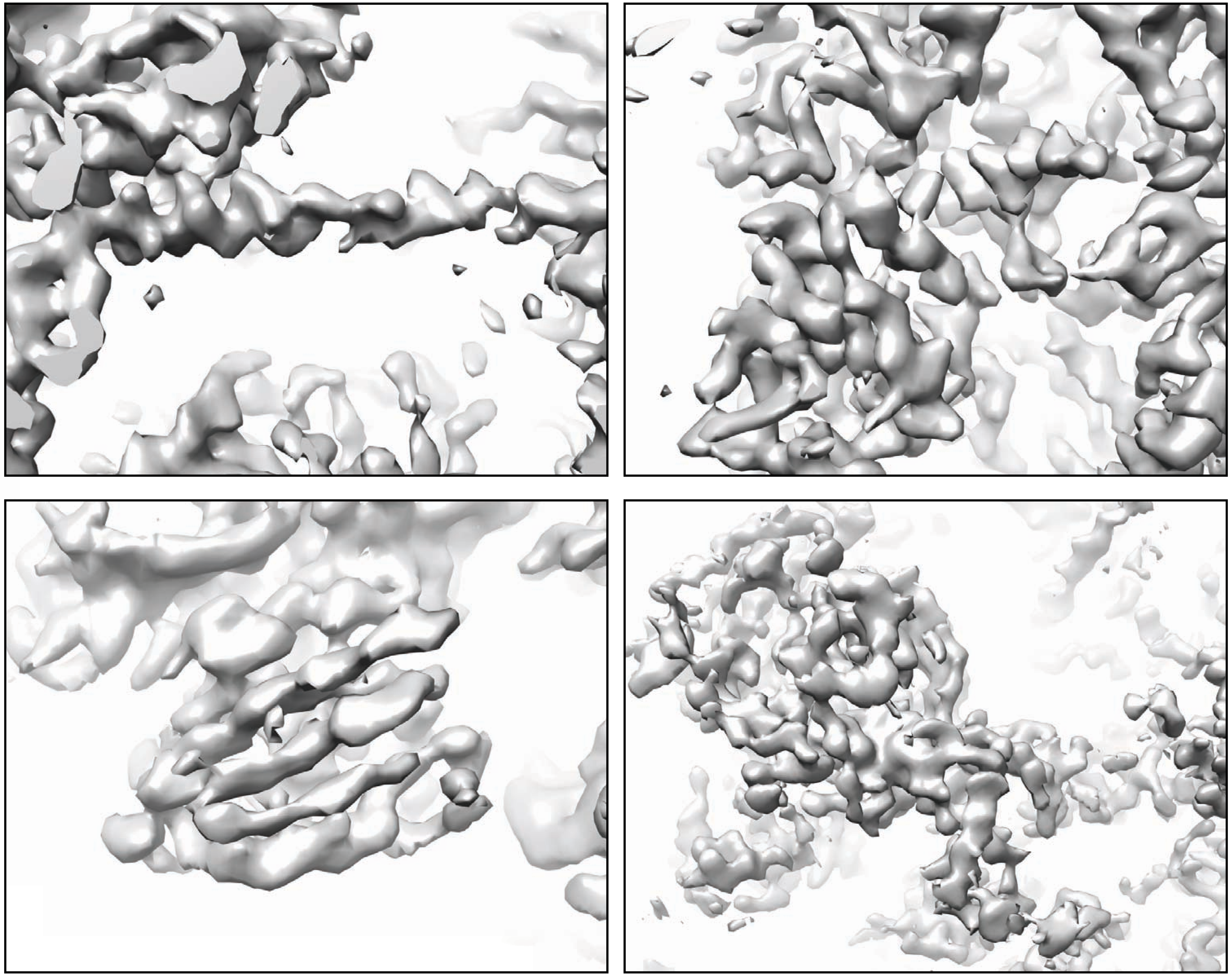
EMDB 6615 3.08Å resolution



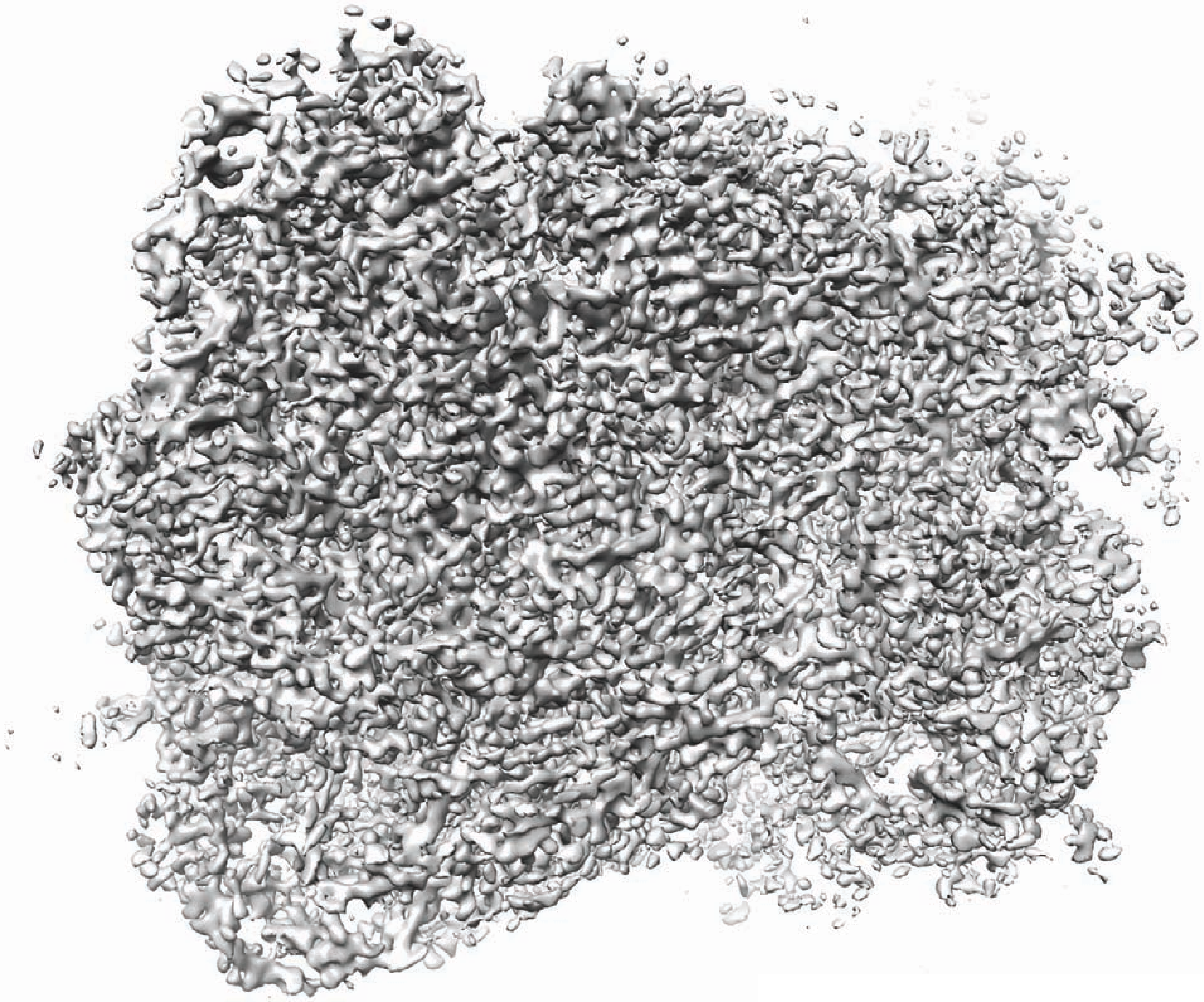
EXAMPLE 4



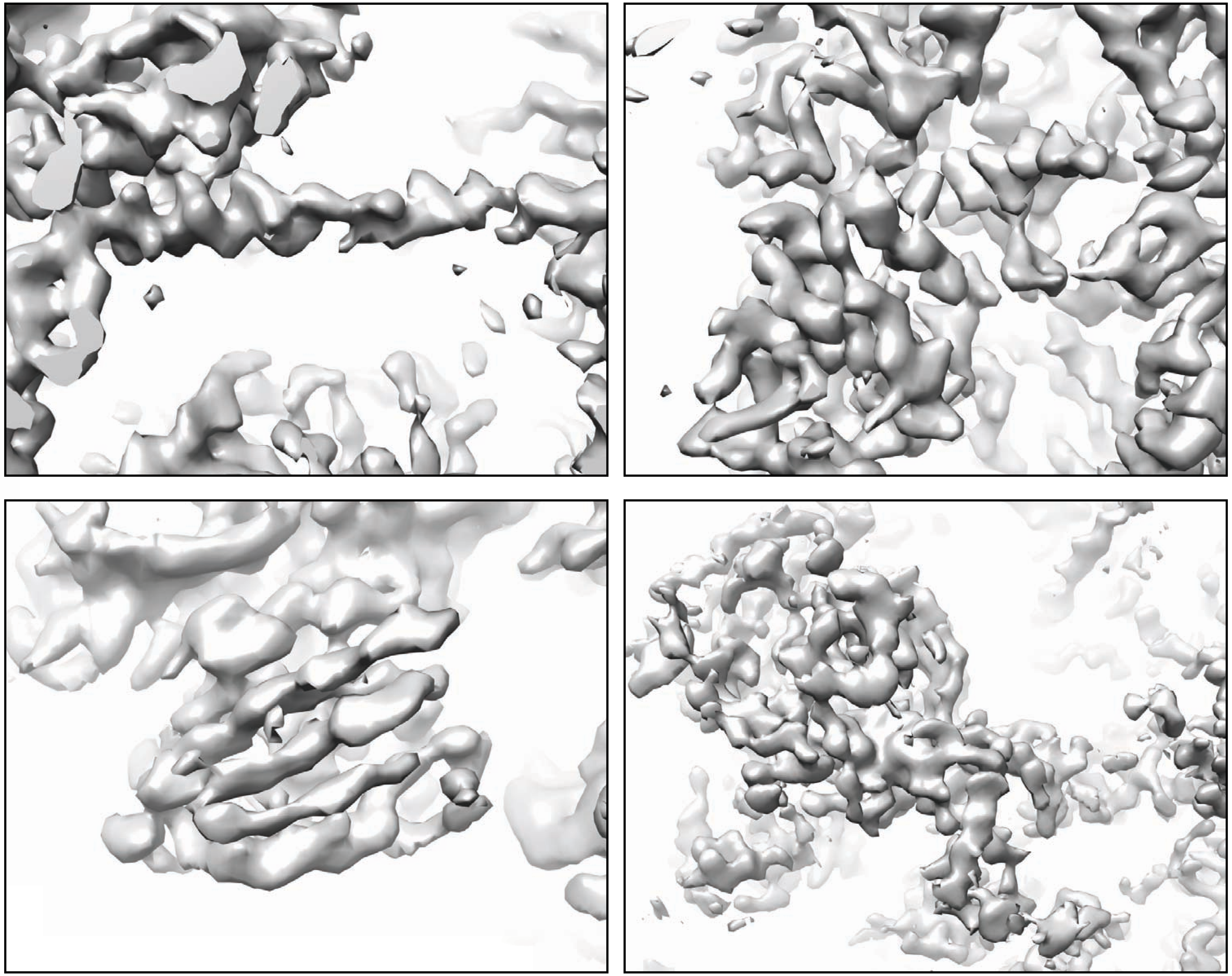
EMDB 0047



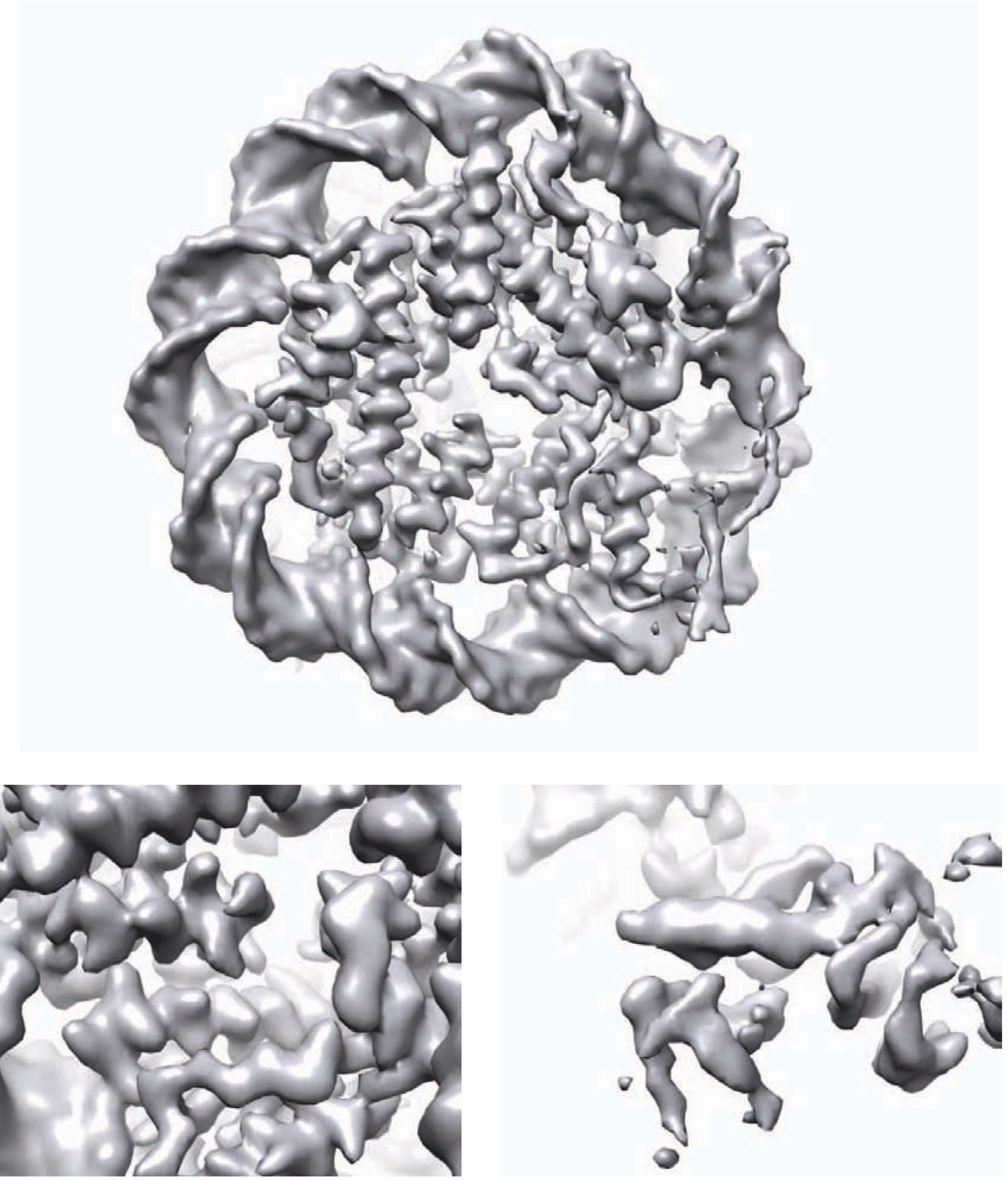
EXAMPLE 4



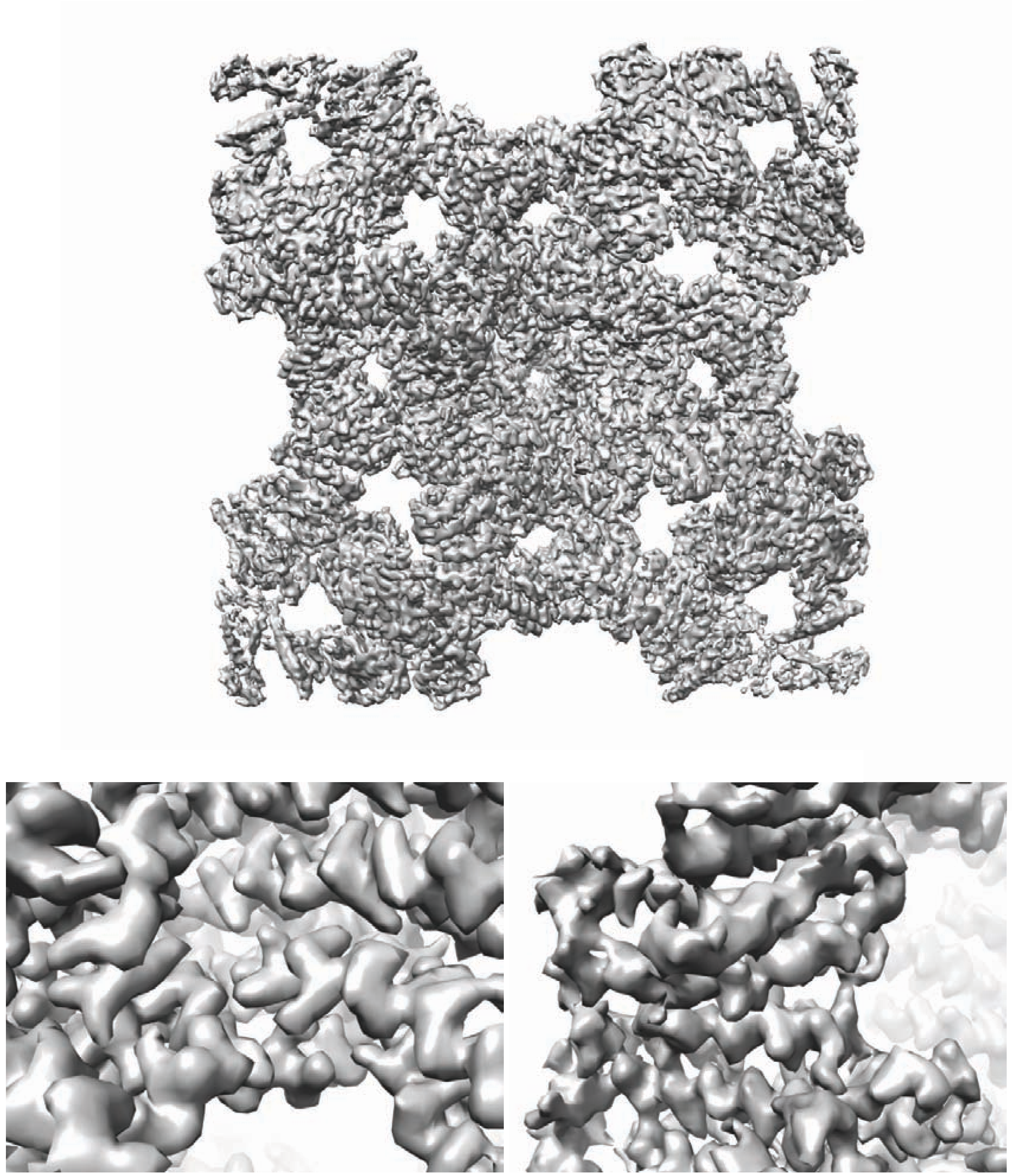
EMDB 0047 4.4Å resolution



ONE MAP, MULTIPLE RESOLUTIONS



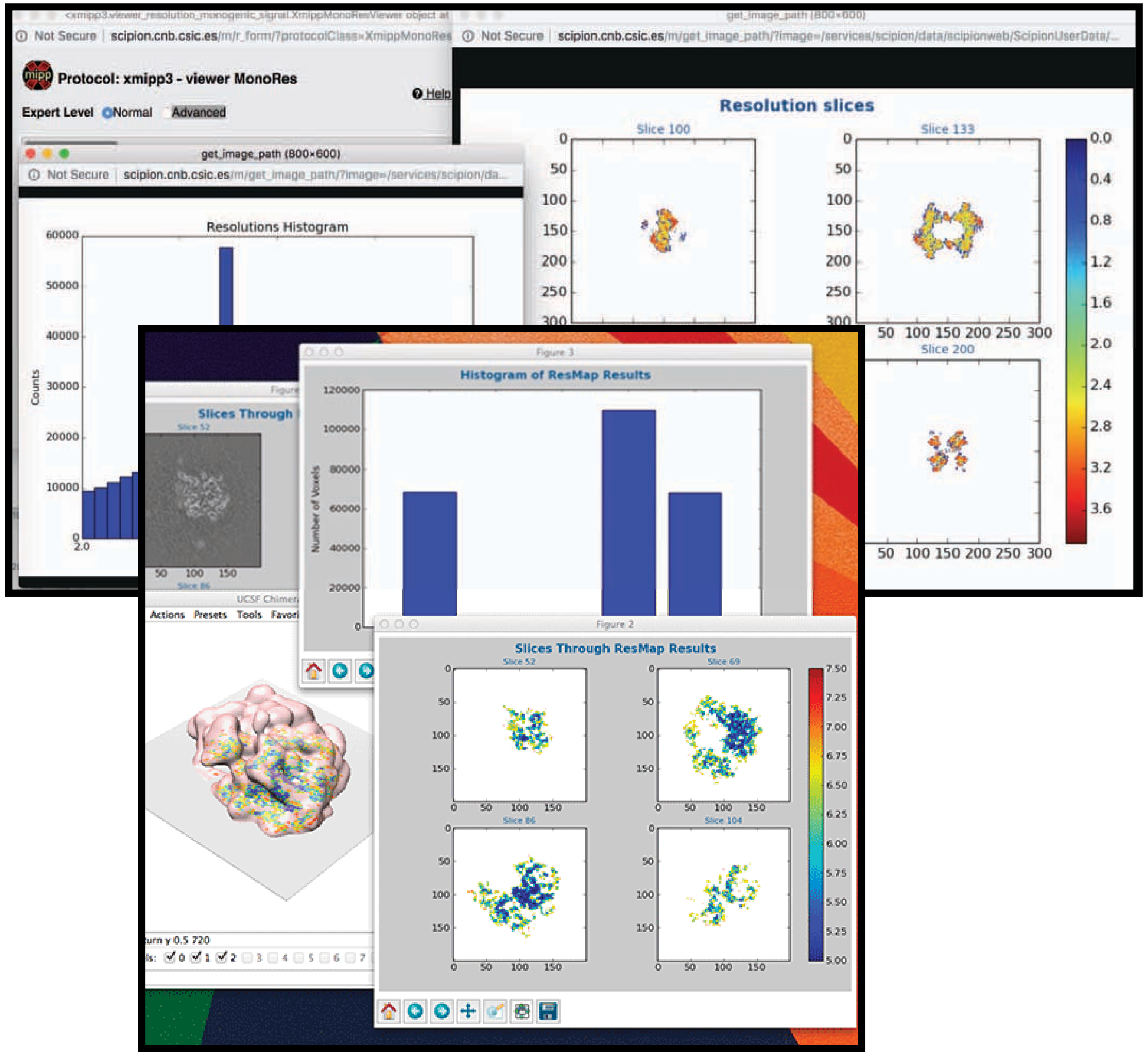
EMDB 0652: 3.5Å resolution



EMDB 8342: 3.6Å resolution

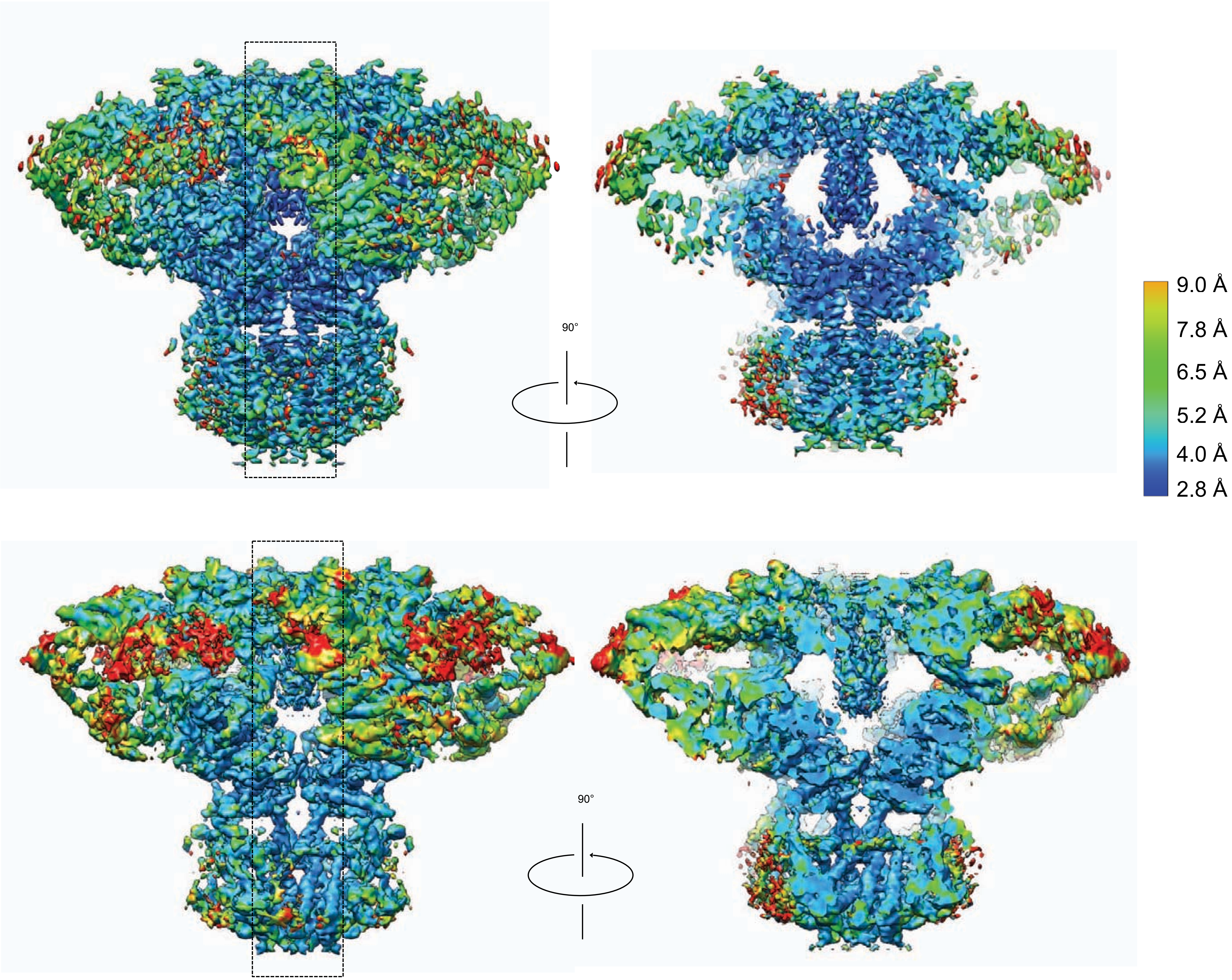
EMDB 6810: 5Å resolution

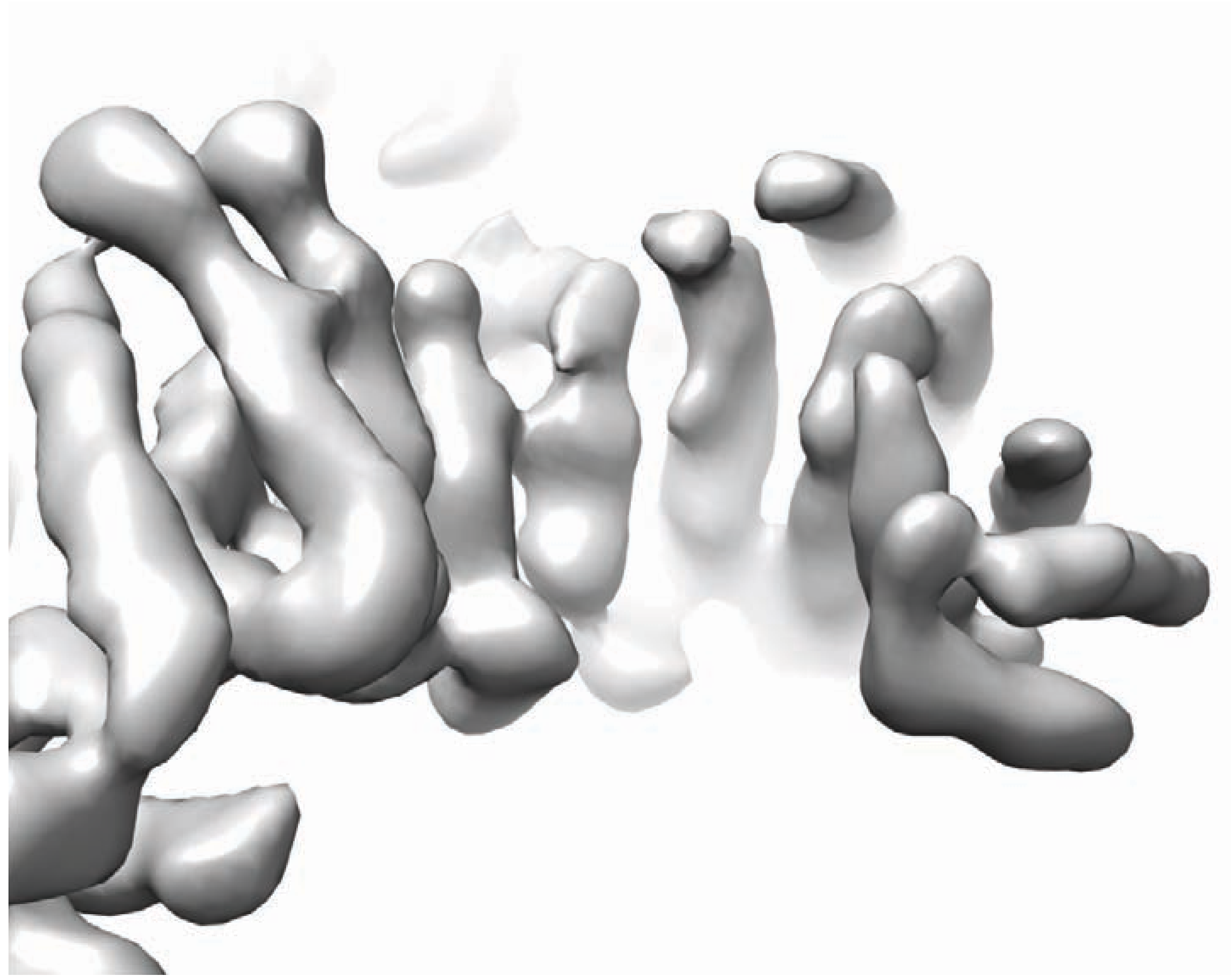
ESTIMATING LOCAL RESOLUTION

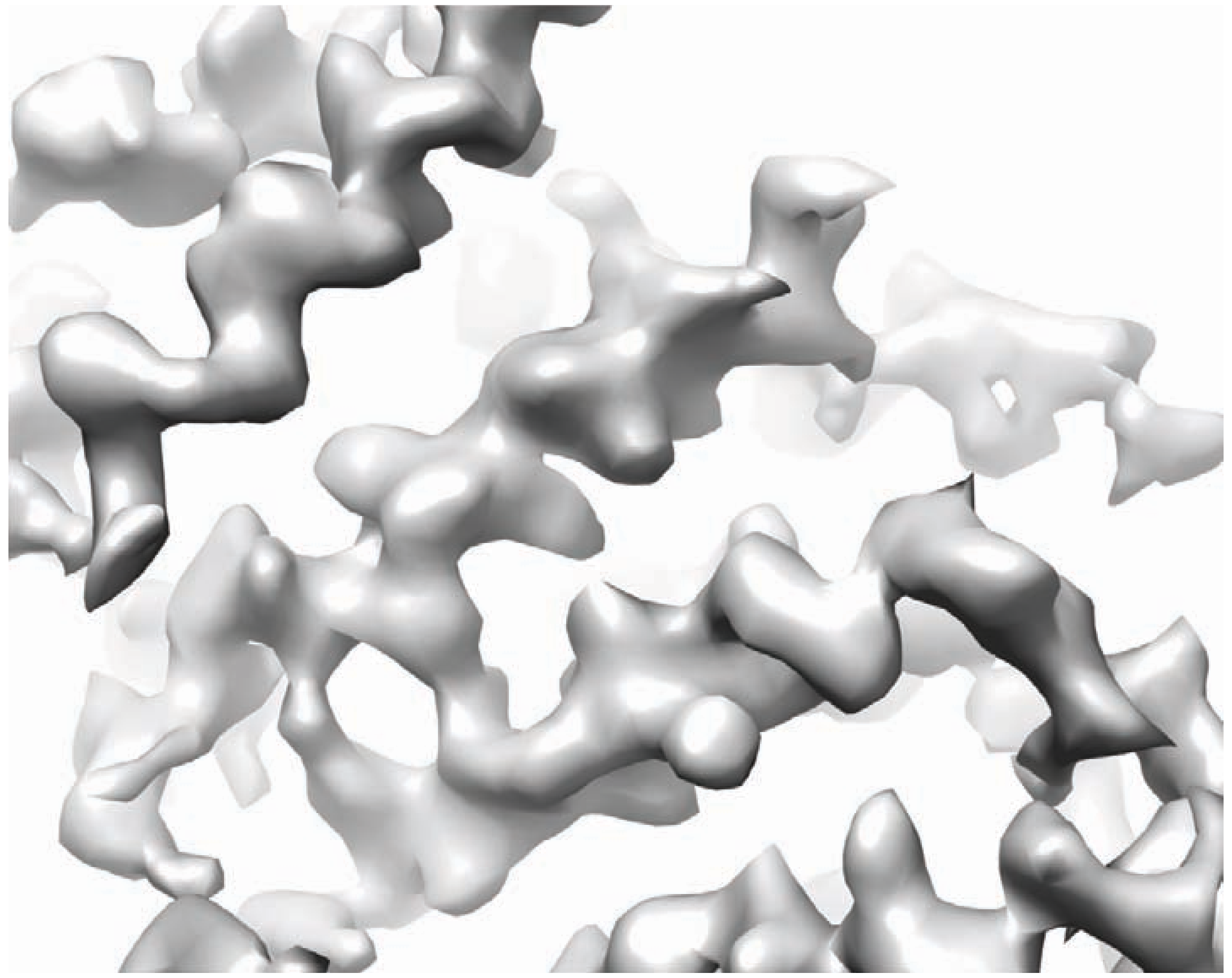


- Gold-standard methods cannot address local resolution differences in maps
  - Sample heterogeneity
  - Image processing errors
- Local resolution measures (such as ResMap, e2fsc.py, Biocres, MonoRes,variance maps) provide a per voxel statistical assessment of resolution
  - Resolution assessments can be mapped back to the density map in Chimera
- Local resolution aids in map analysis and model building

ESTIMATING LOCAL RESOLUTION

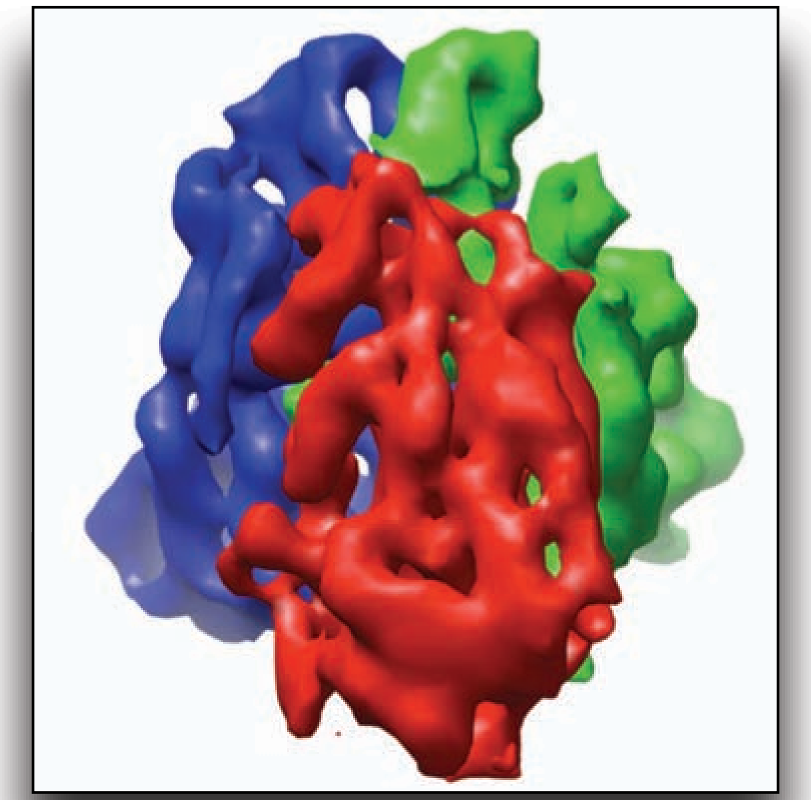




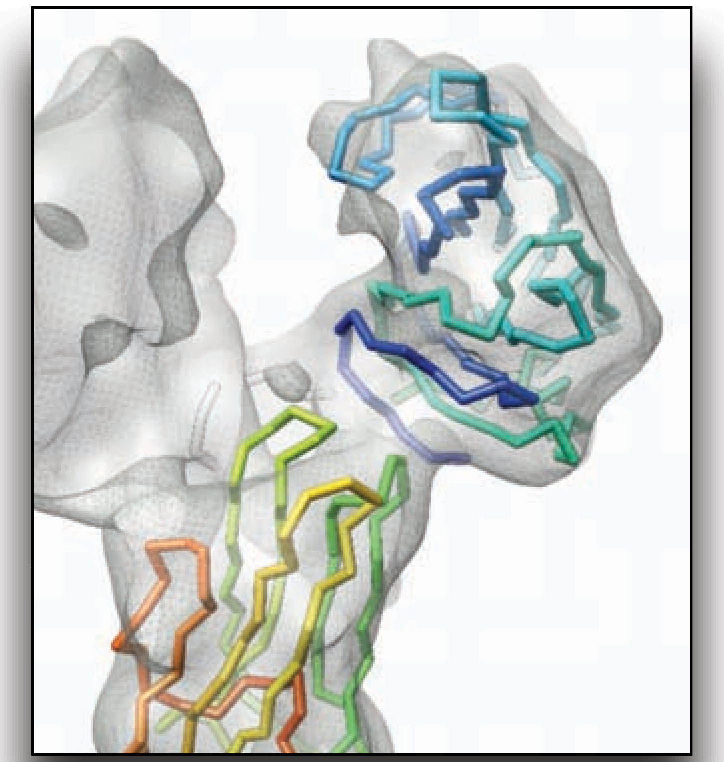


RESOLVABILITY MILESTONES

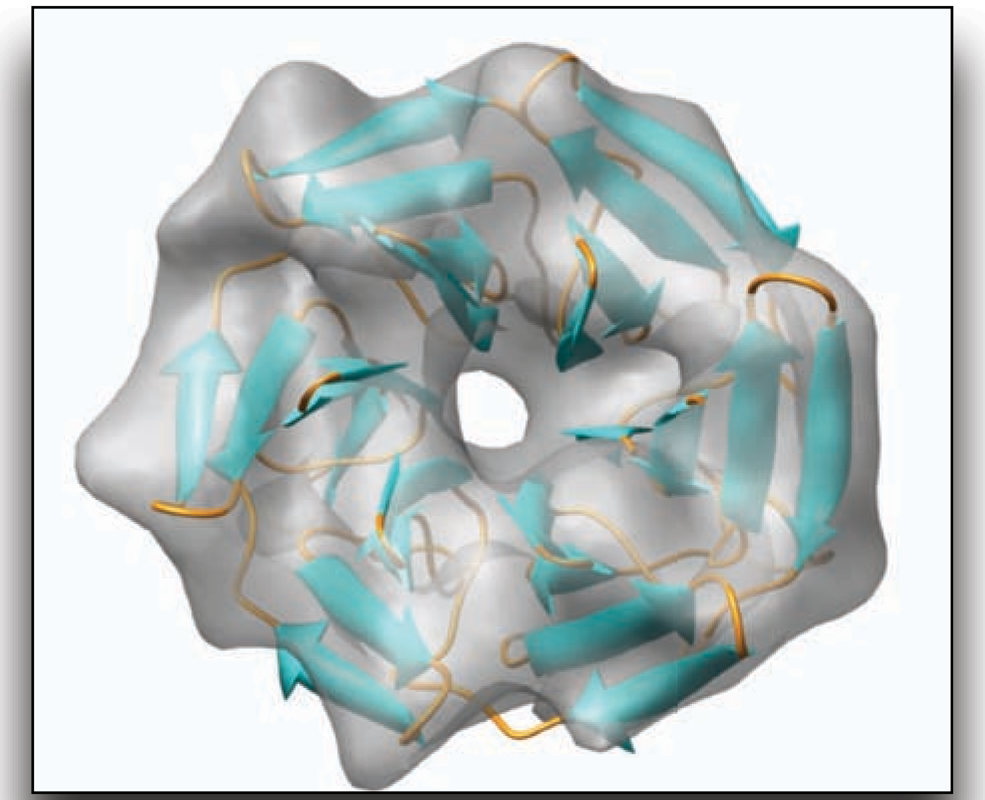
RESOLVABILITY MILESTONES



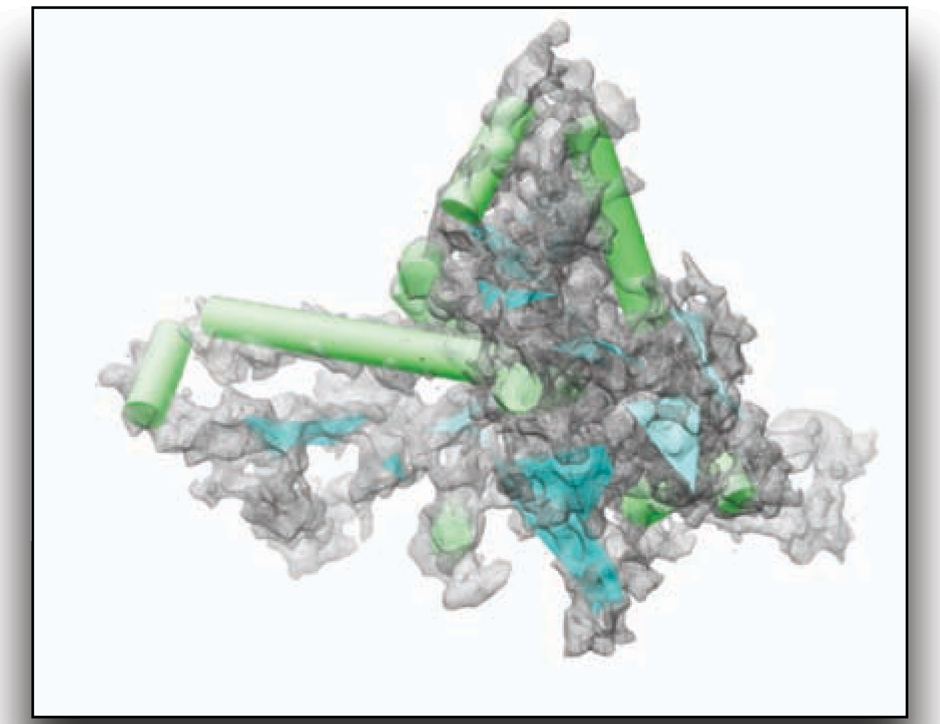
Density segmentation



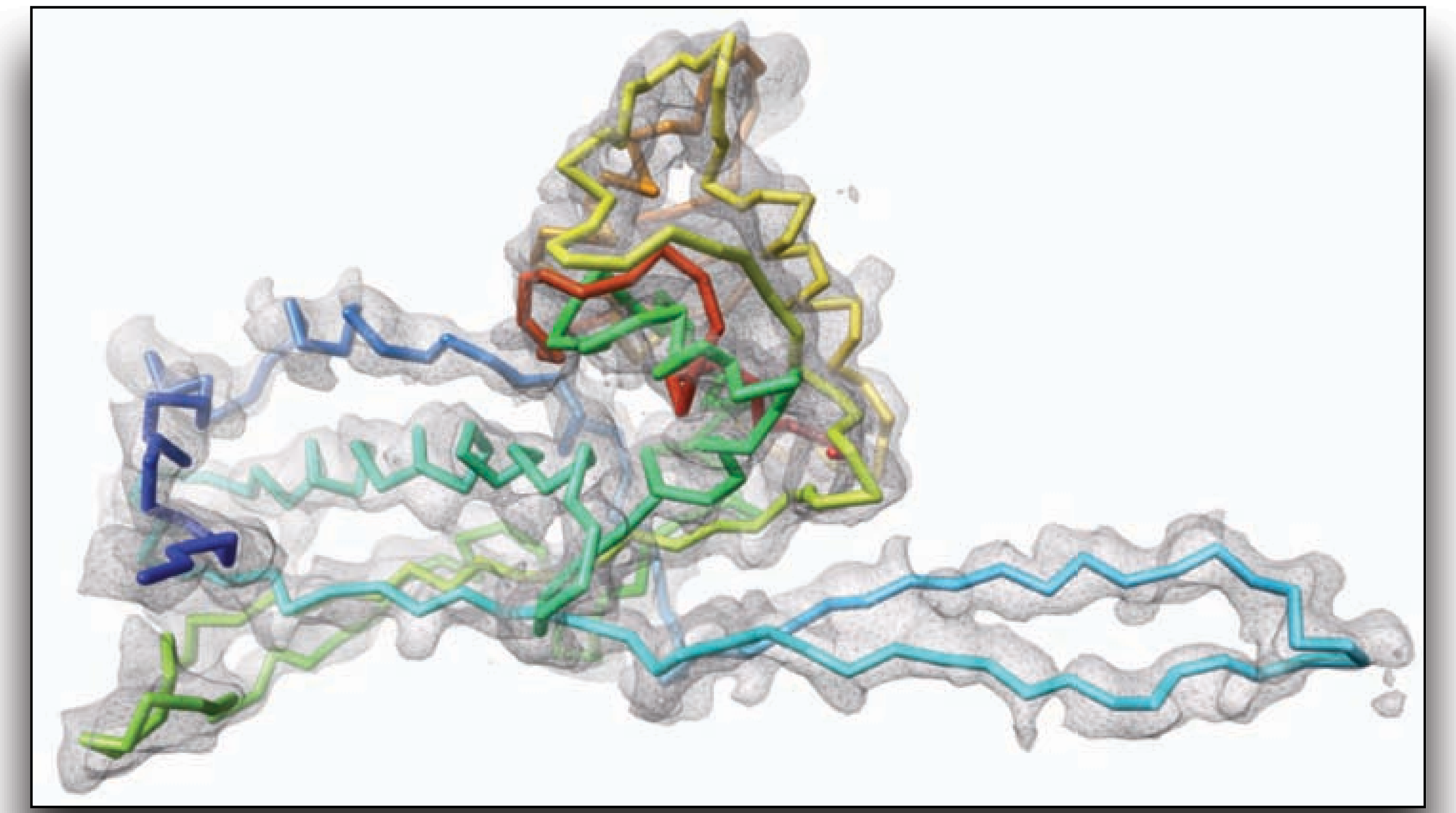
Domain fitting



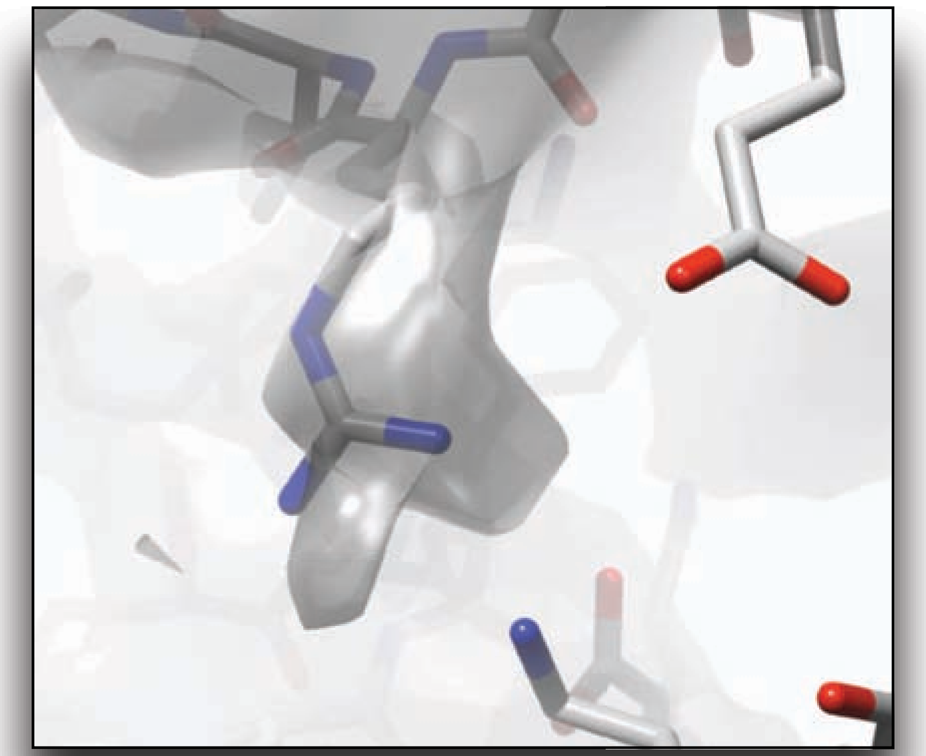
Constrained modeling



Secondary structure detection



C-alpha backbone tracing



All atom modeling

MODEL GENERATION, REFINEMENT AND VALIDATION: SUBNANOMETER RESOLUTION

- Models are generated primarily from a template structure
- Maps can serve as a constraint for fitting, constraining or selecting models
  - Rigid body fitting
    - Best “match” to the density map
  - Flexible fitting
    - Models are allowed to deform to fit the density map
    - Physical, chemical and geometric constraints can be used
  - Computational modeling
    - Density can serve as an envelope in improving comparative models
    - Density can be used to select best a best model from a set of decoys (ab initio)
- Agreement with density and observed structure usually serves as validation

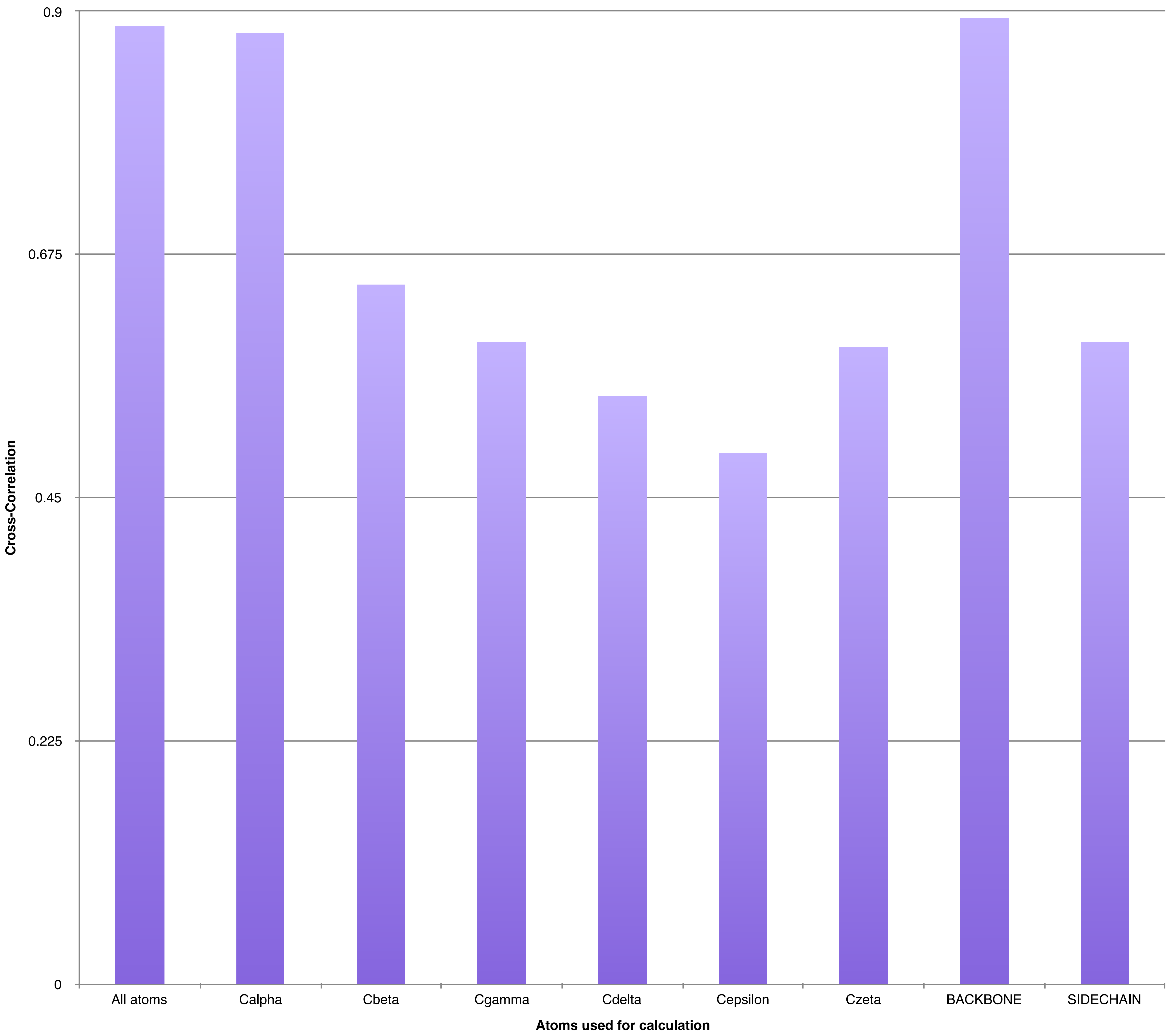
MODEL GENERATION, REFINEMENT AND VALIDATION: “ATOMIC” RESOLUTION

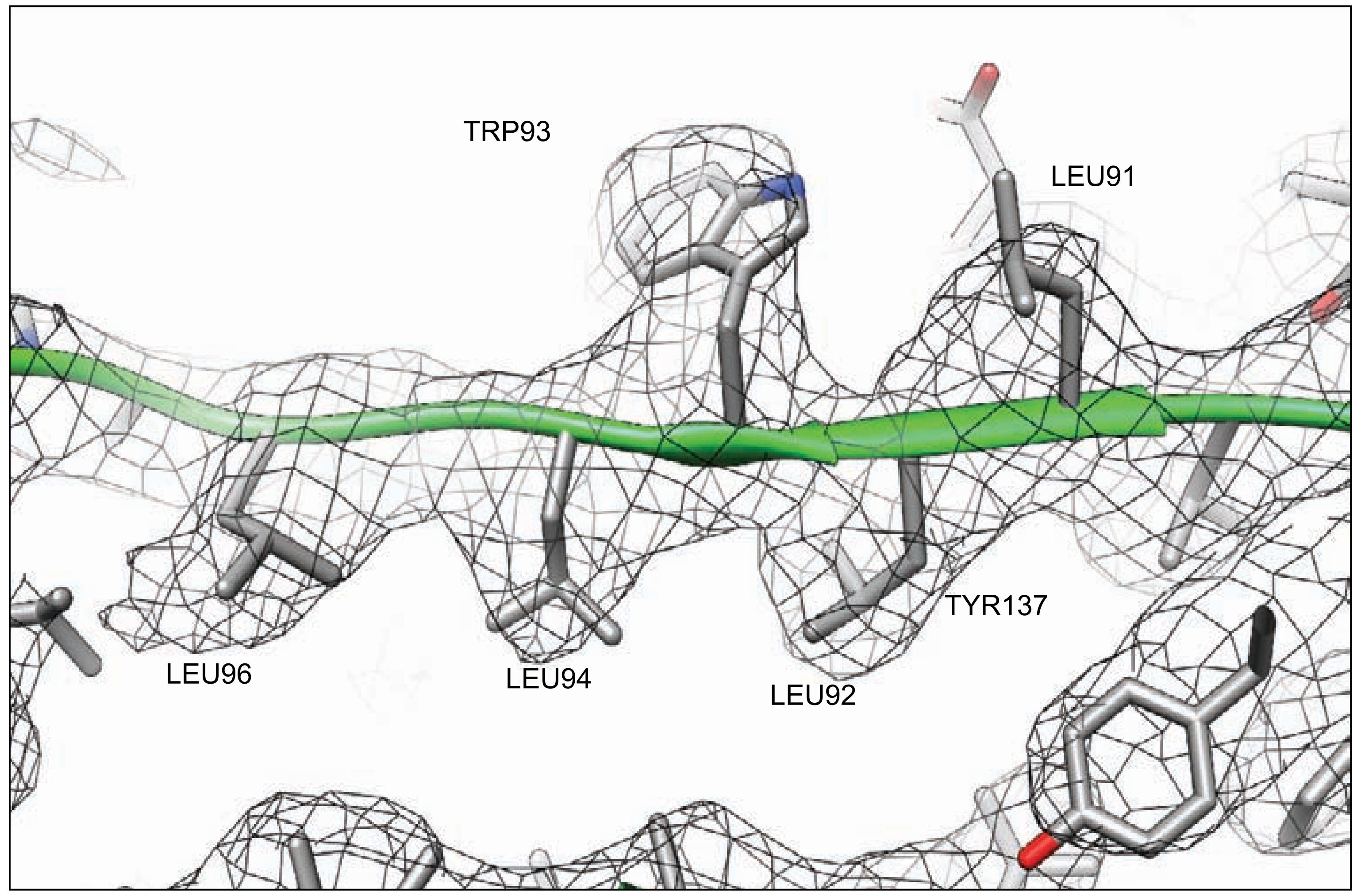
- Models can be generated directly from the density map with or without a template
- Density features allow for nearly unambiguous assignments
  - Complete density connectivity
  - Clear side chain density for the vast majority of residues
  - Rotamer identification is possible
  - Small molecules, ligands and waters can often be seen
- Real-space refinement optimizes fit to density and stereochemistry
- All atom models can use well established tools for model validation

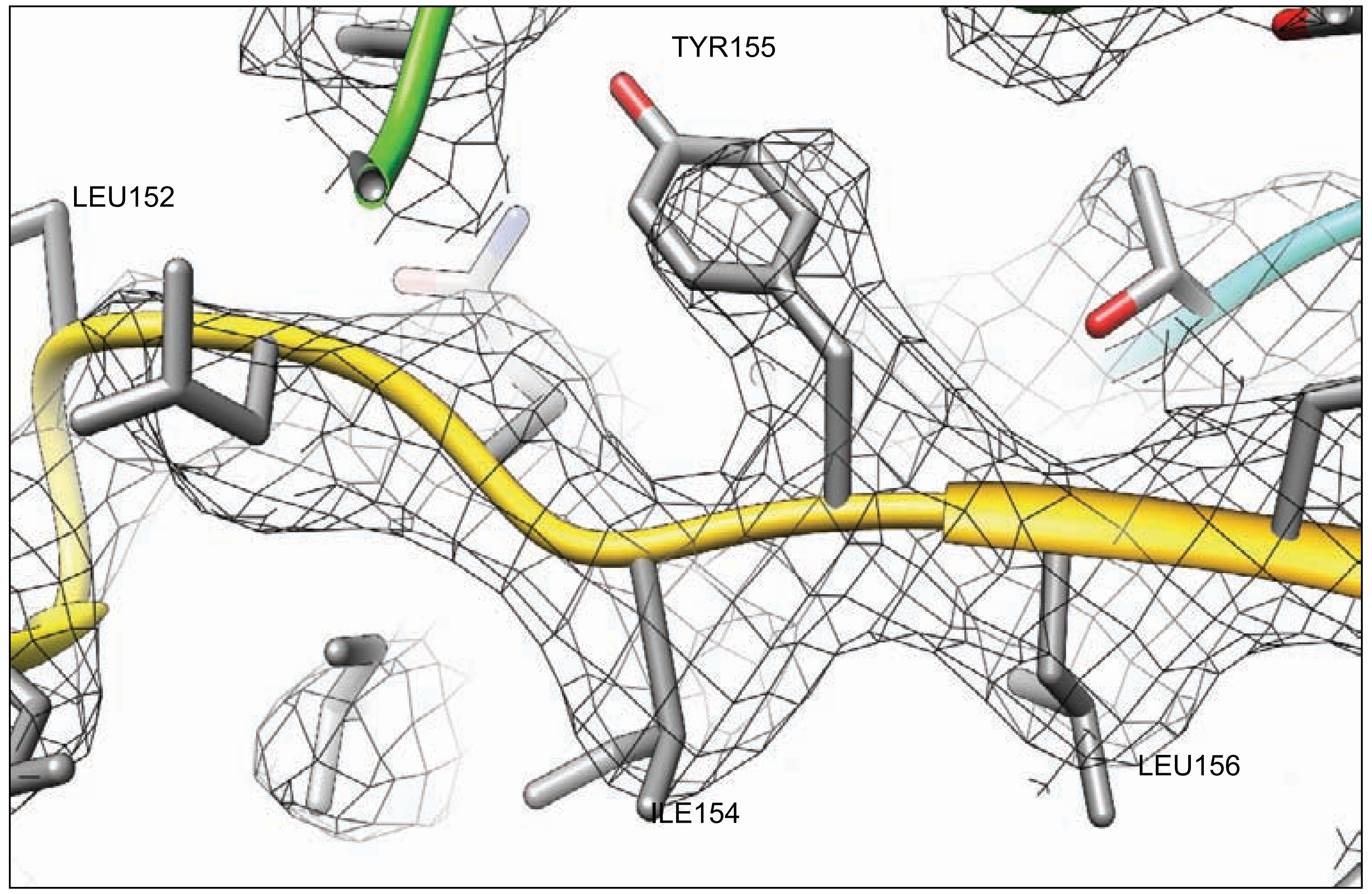
MODEL GENERATION, REFINEMENT AND VALIDATION: NEAR-ATOMIC RESOLUTION

- Models can be generated directly from the density map with or without a template
- Models type and accuracy depend on feature resolvability
  - C-alpha backbone and poly-A models can be built at ~5Å resolution
    - Pitch of alpha helices
    - Separation of strands in sheets
  - Full atom models require visible side chain density
    - Aromatic and large positively charged residues provide anchors
    - Chemistry provides constraints
- Refinement balances density and chemistry with respect to features
- All atom models can use well established tools for model validation

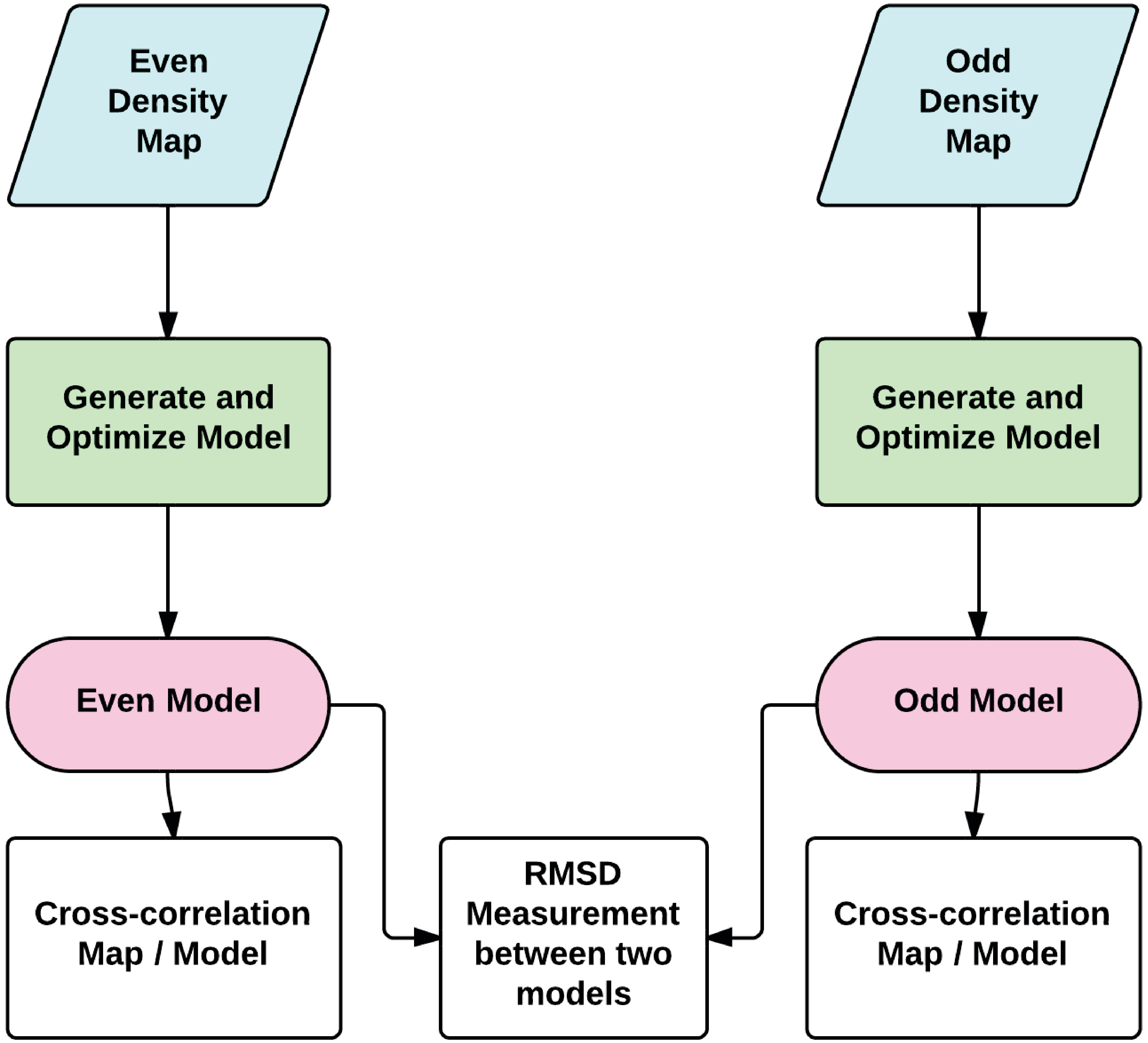
CHARACTERISTICS OF MODELS FROM NEAR ATOMIC RESOLUTION MAPS







MODEL RELIABILITY AT NEAR ATOMIC RESOLUTIONS


Even Density Map

Odd Density Map

Generate and Optimize Model

Generate and Optimize Model

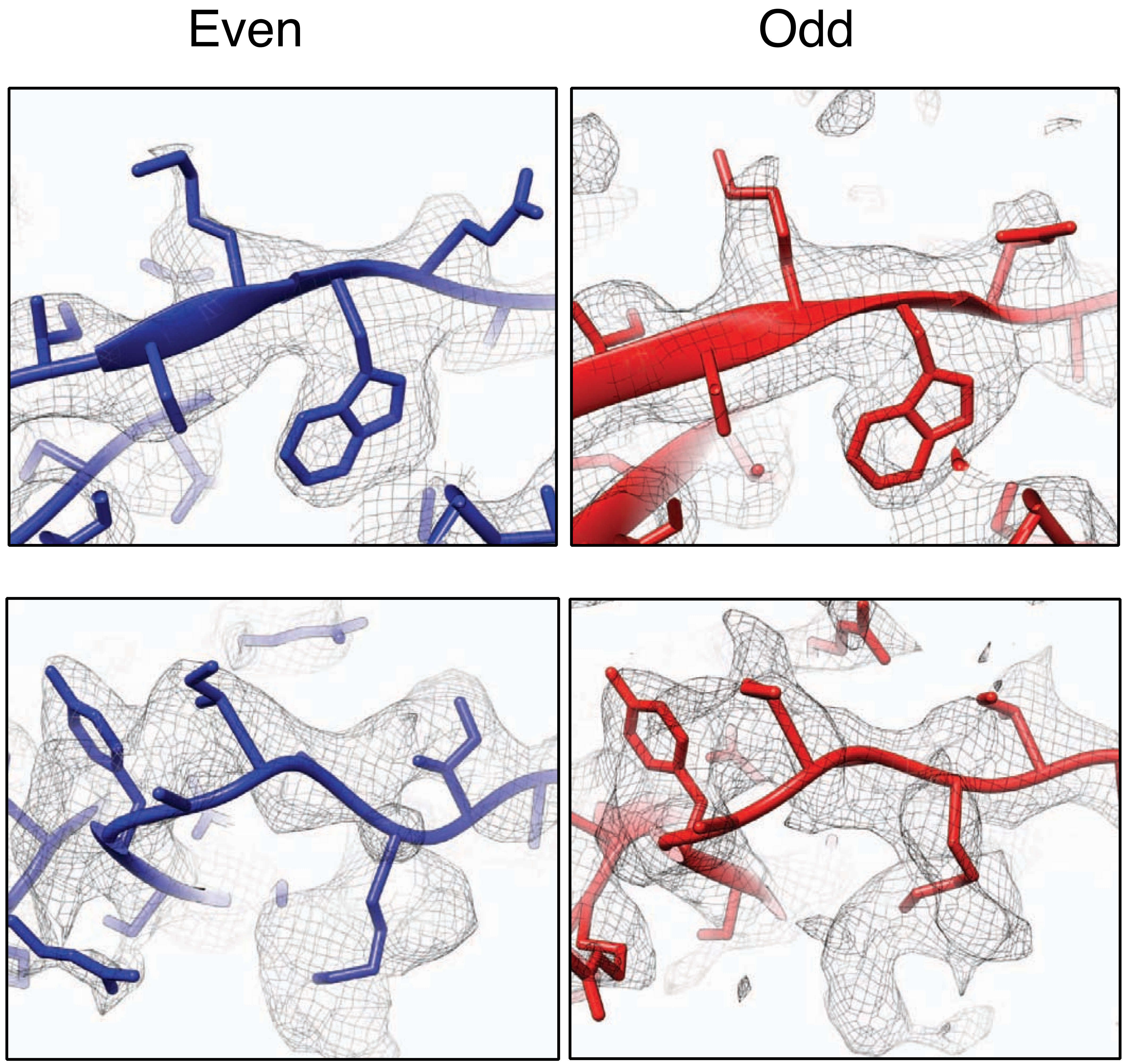
Even Model

Odd Model

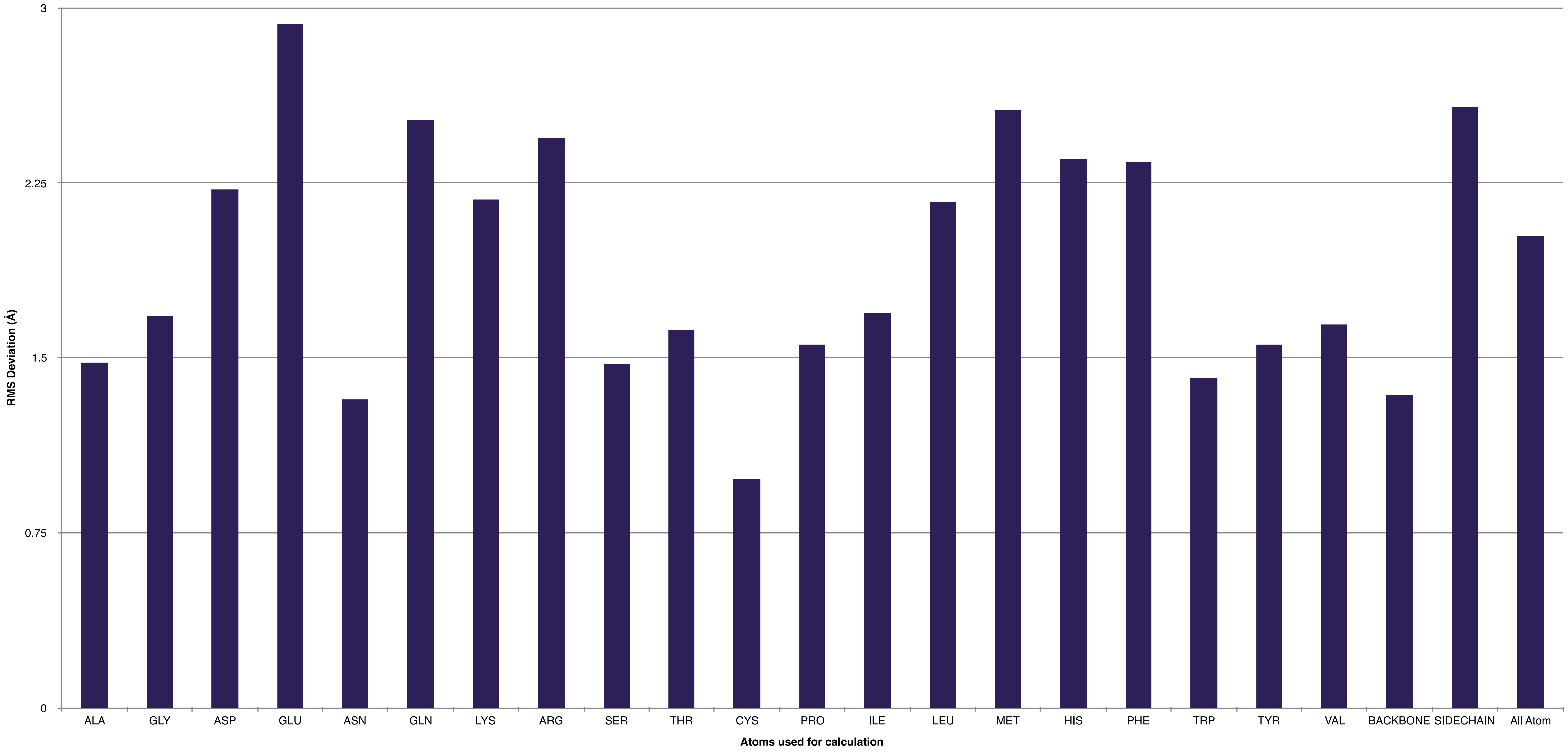
Cross-correlation Map / Model

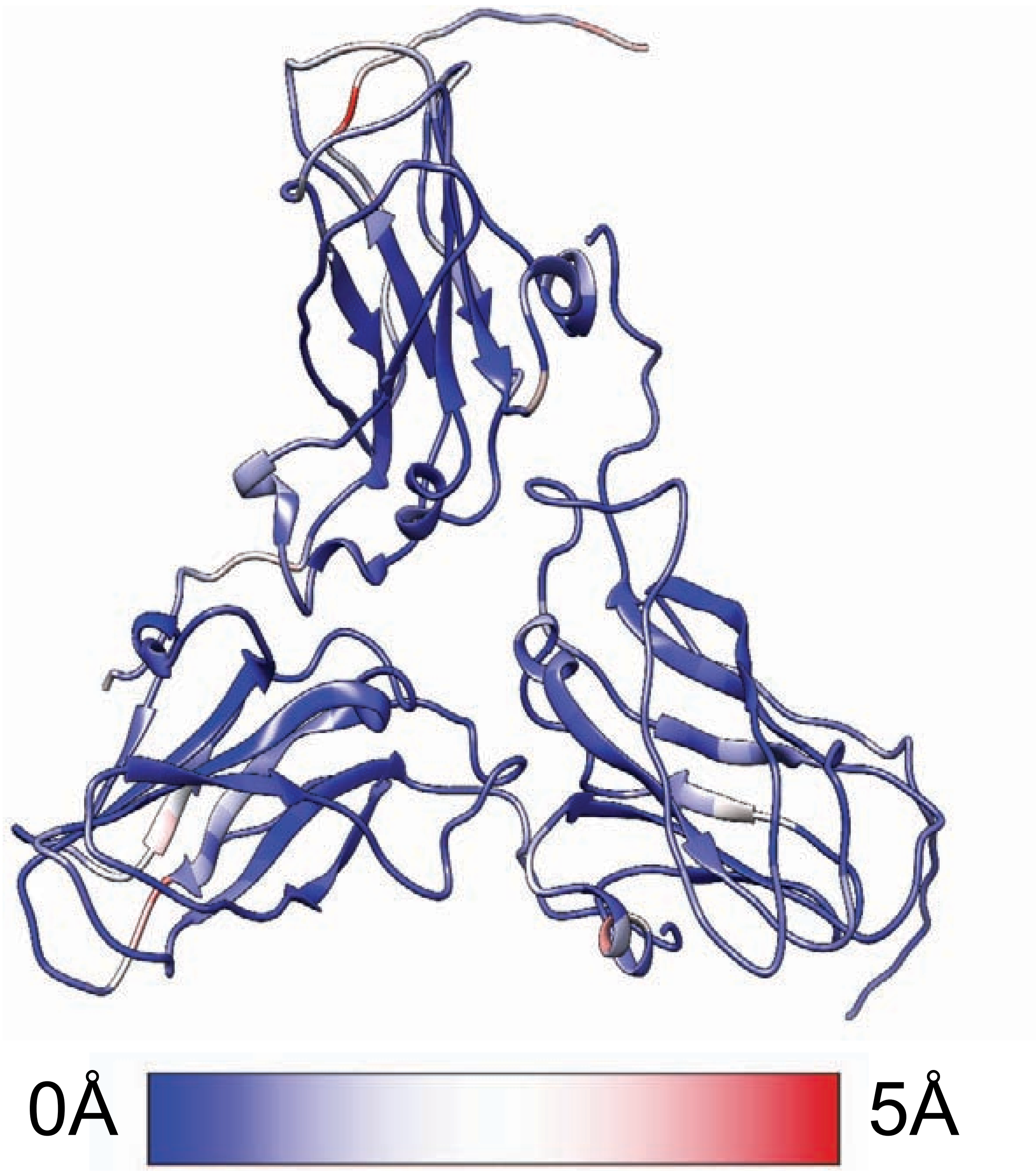
RMSD Measurement between two models

Cross-correlation Map / Model

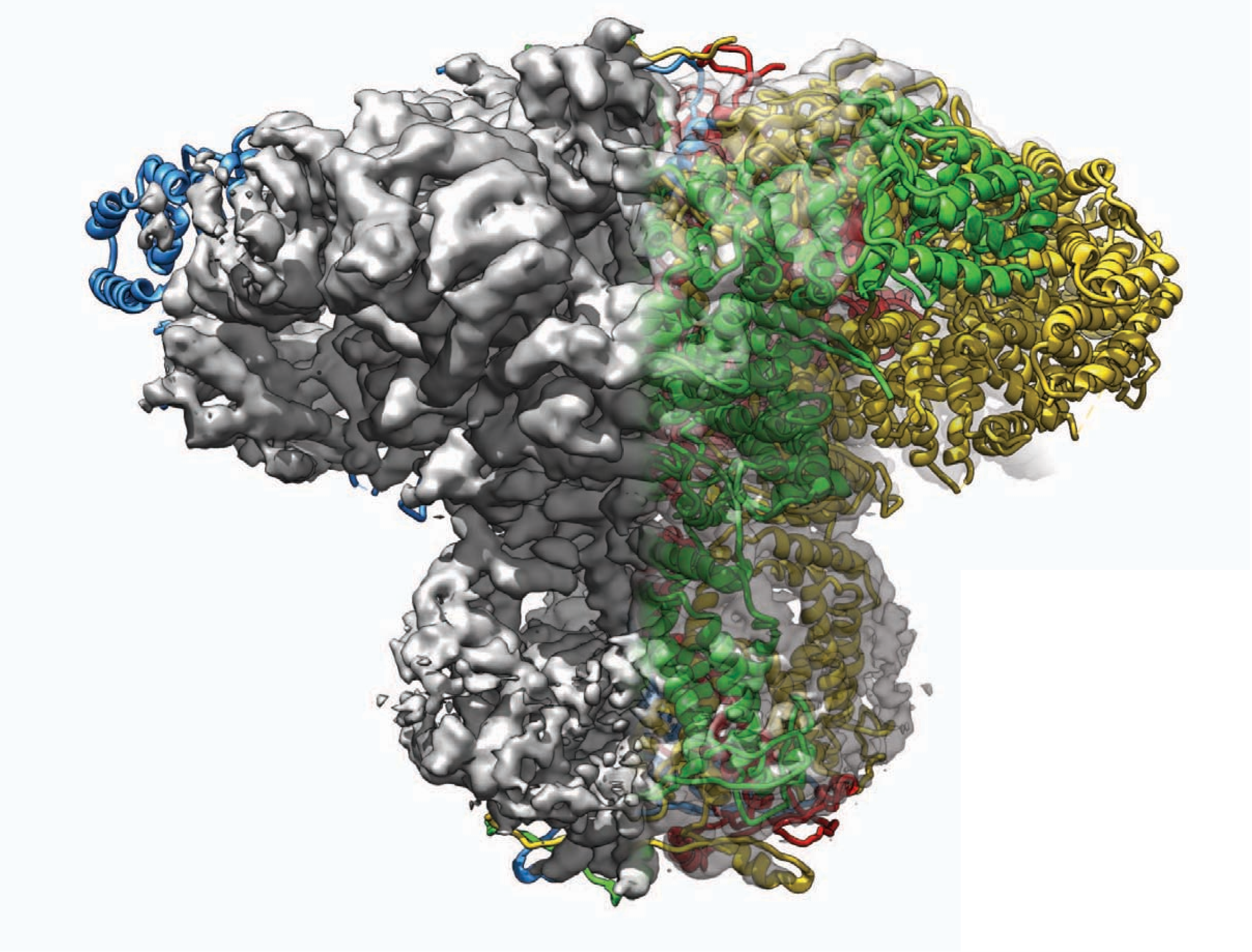


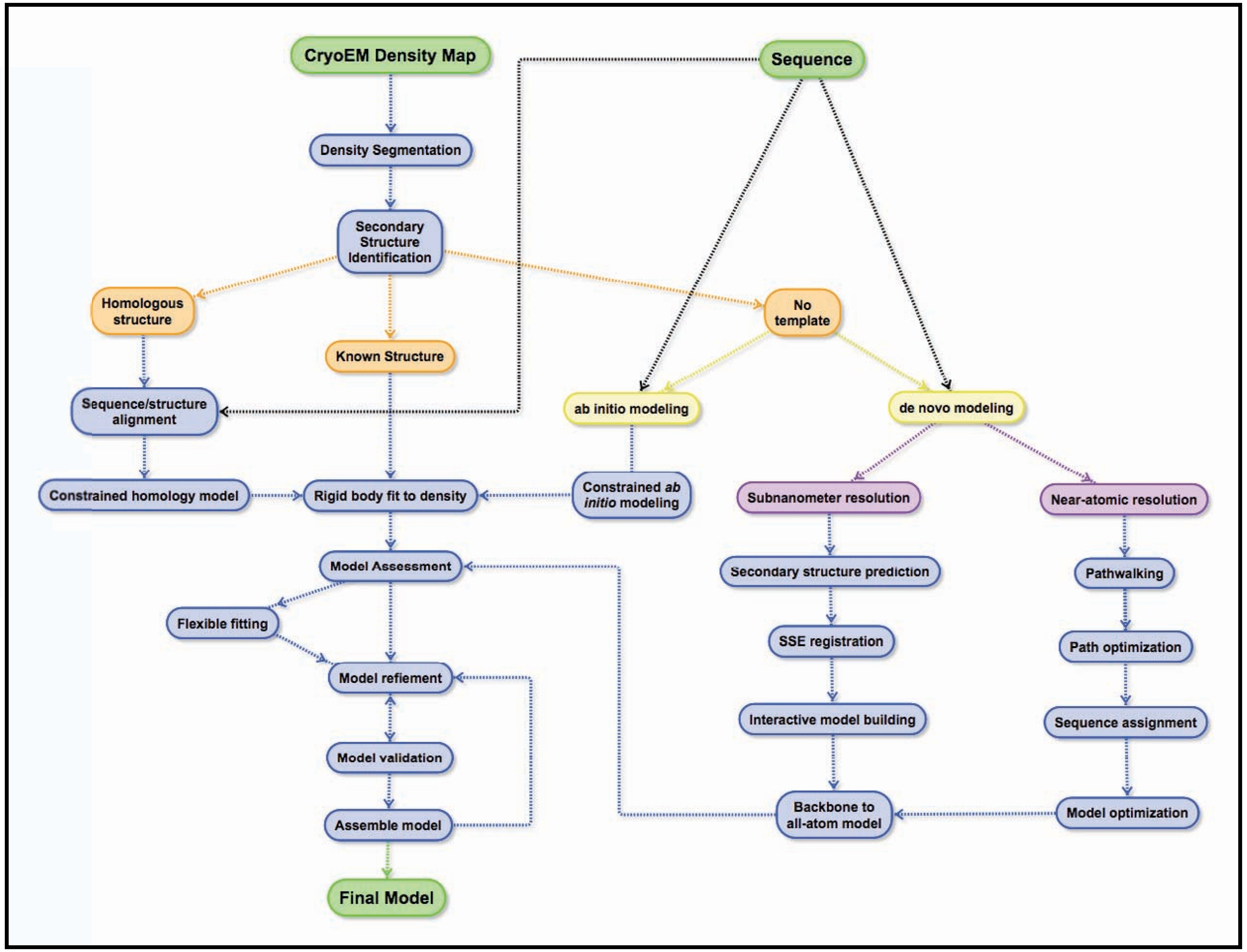
MODEL RELIABILITY AT NEAR ATOMIC RESOLUTIONS



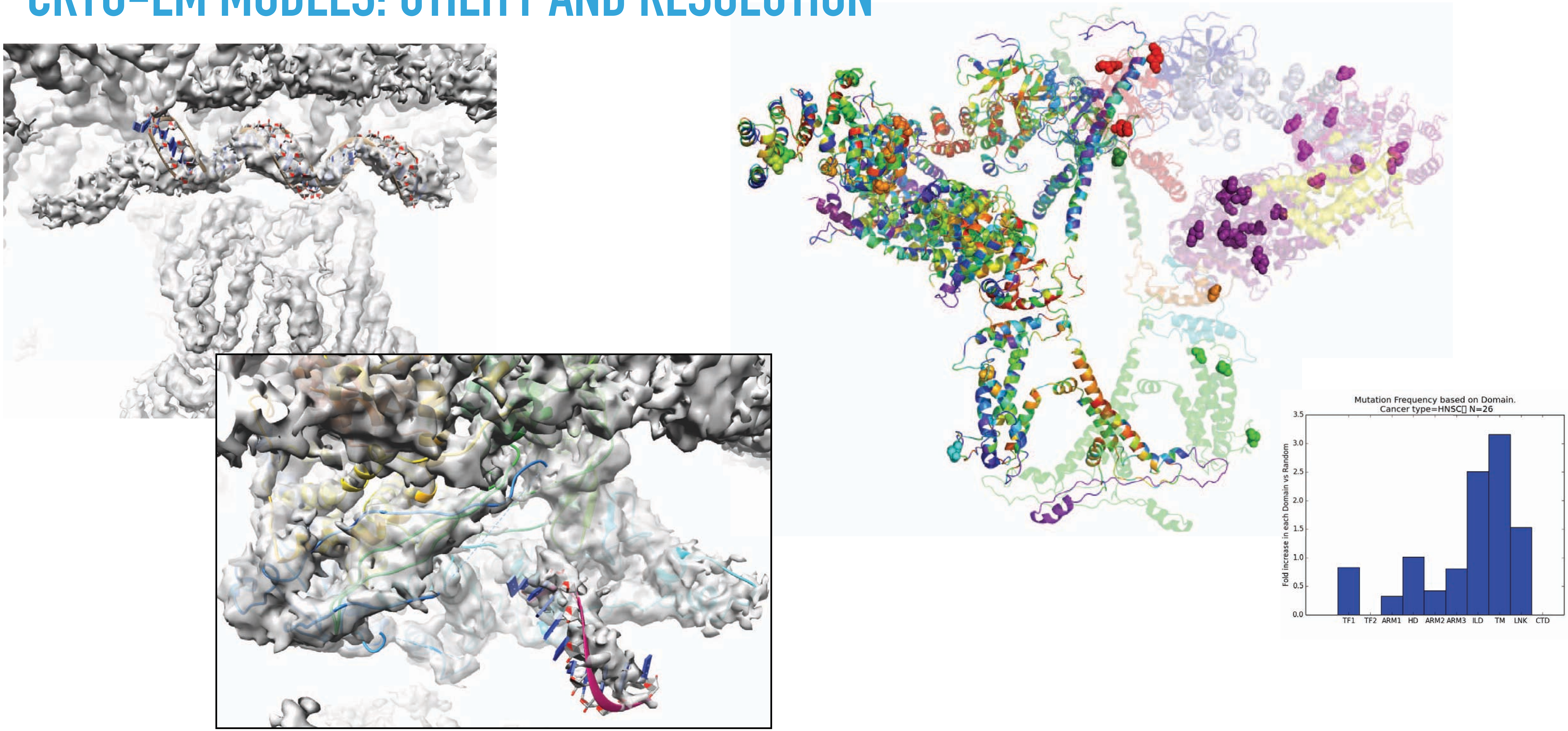


CRYOEM MODELING WORKFLOW

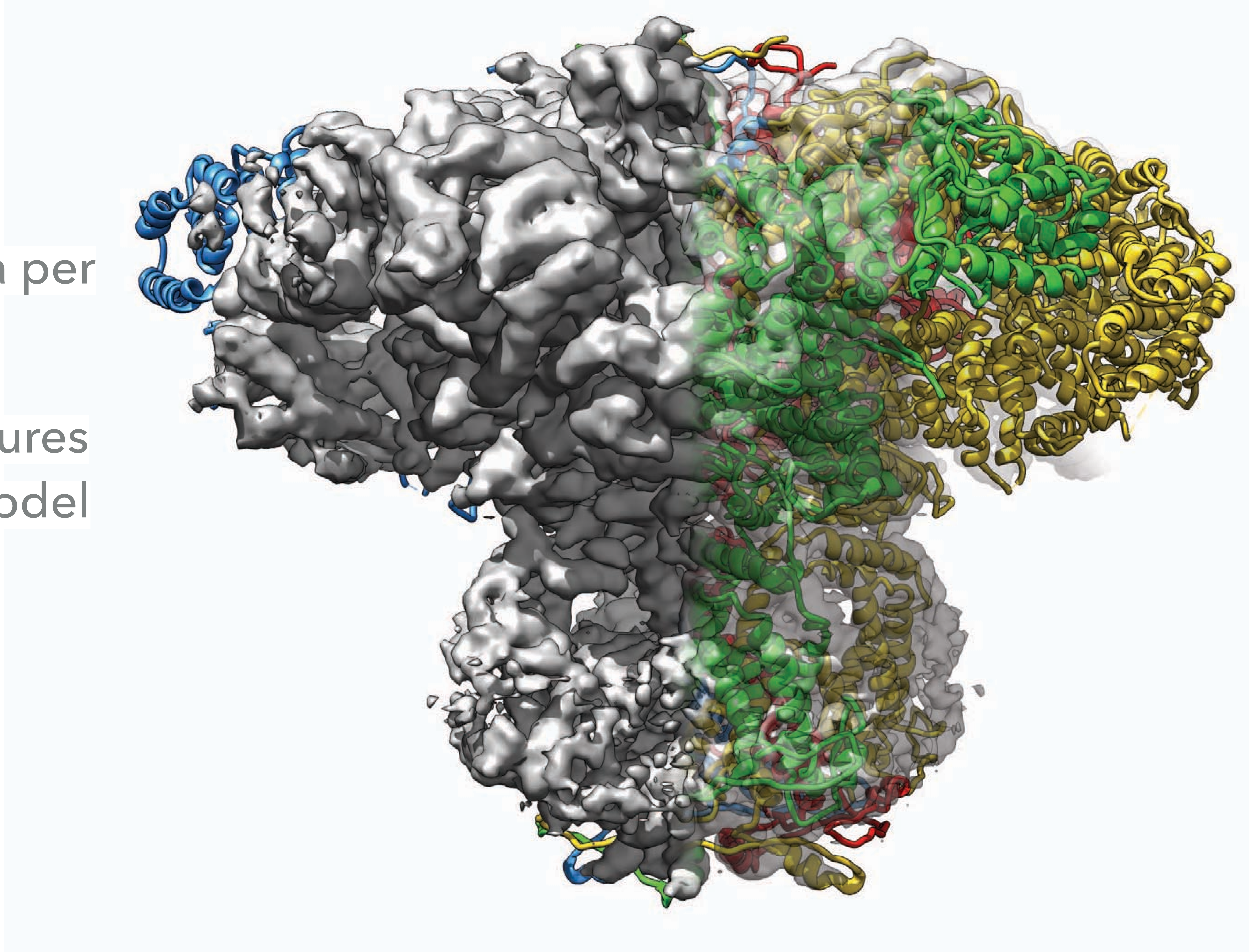




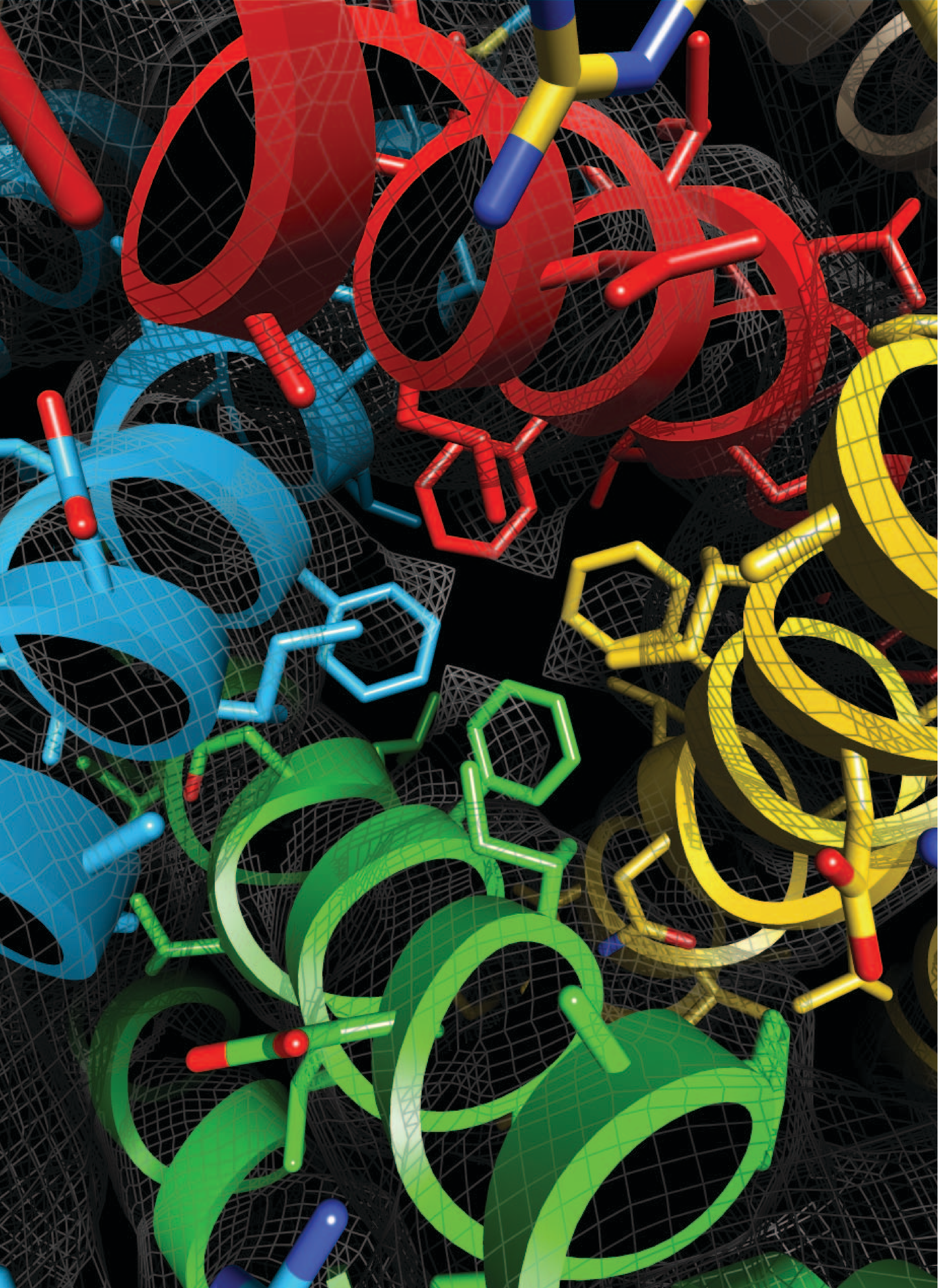
CRYO-EM MODELS: UTILITY AND RESOLUTION



CONCLUSIONS



- Resolution is only a guide
- Local resolution estimates provide a more detailed statistical analysis of resolution on a per voxel basis
- Visual inspection of a map for expected features is paramount in interpreting the map and model building
- Resolvability is key in model building and refinement
  - May contain different types of models



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