Cryo-EM Map-Based Modeling

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NUS Cryo-EM Workshop
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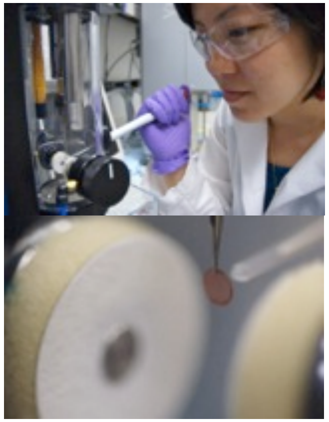
Pipeline in Single Particle Cryo-EM

Biochemical Preparation

→

Cryo-EM Sample Preparation



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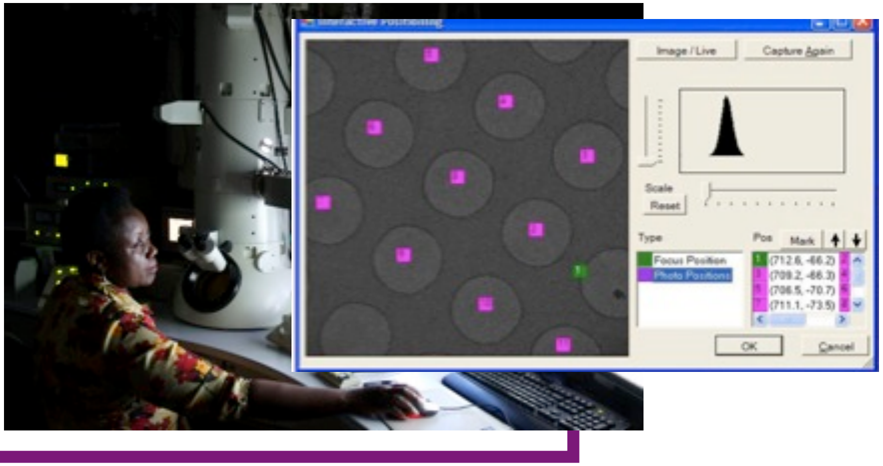
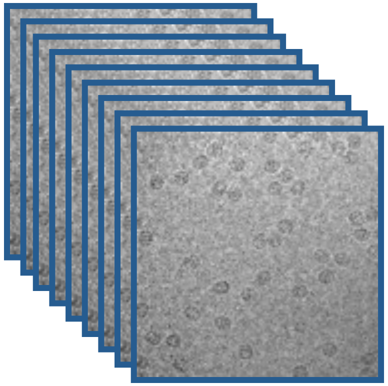
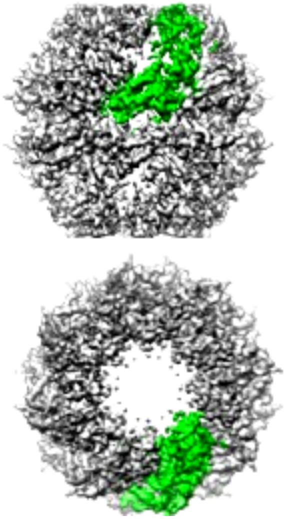
High Resolution Automated Data Collection
JADAS





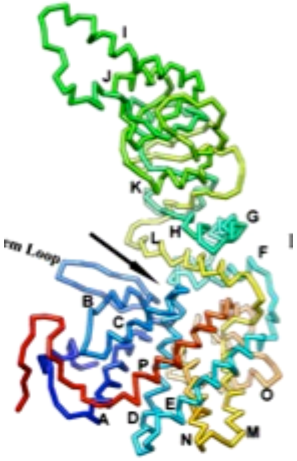
Data Archiving & Processing
EMEN

→



3D Reconstruction
EMAN

→



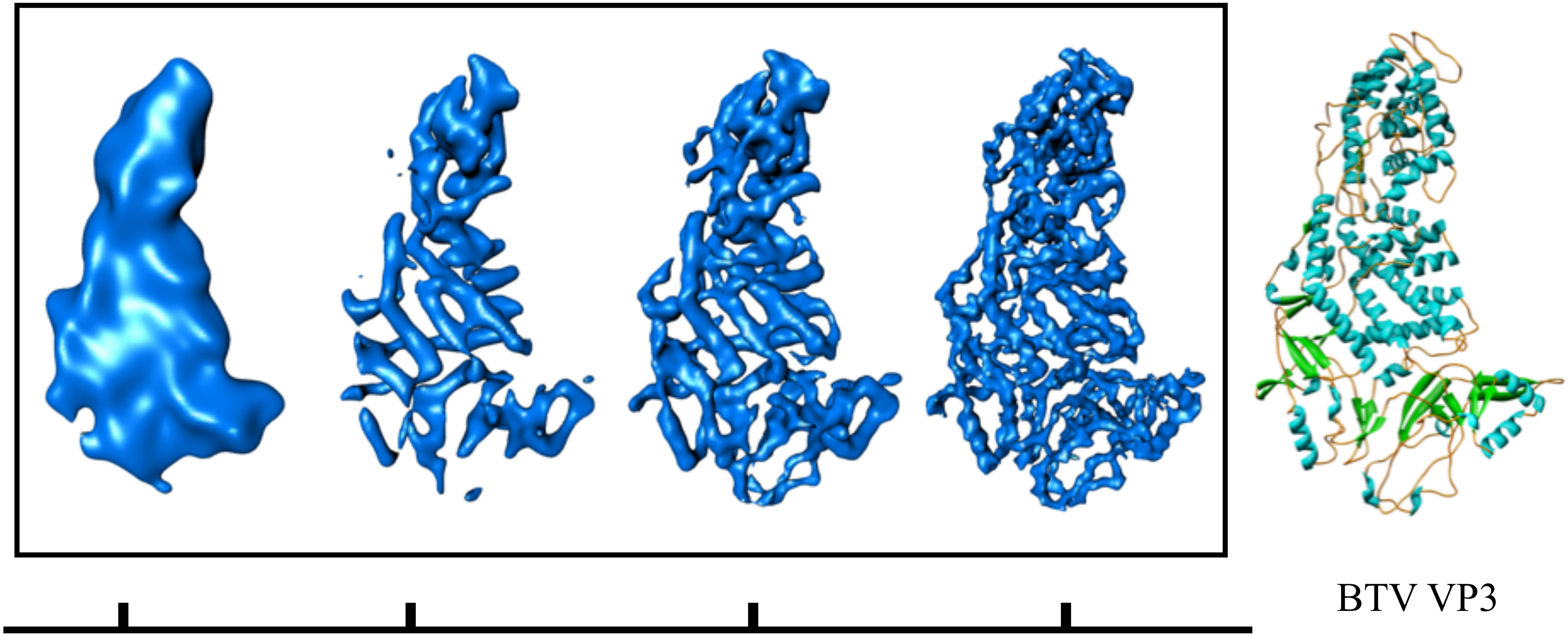
Model Building & Validation
Gorgon

→

WORLDWIDE PDB PROTEIN DATA BANK

Structure Deposition

Features as a Function of Resolution



BTV VP3

15+ Å

9 Å

6 Å

<4 Å

Size
Shape

Domains

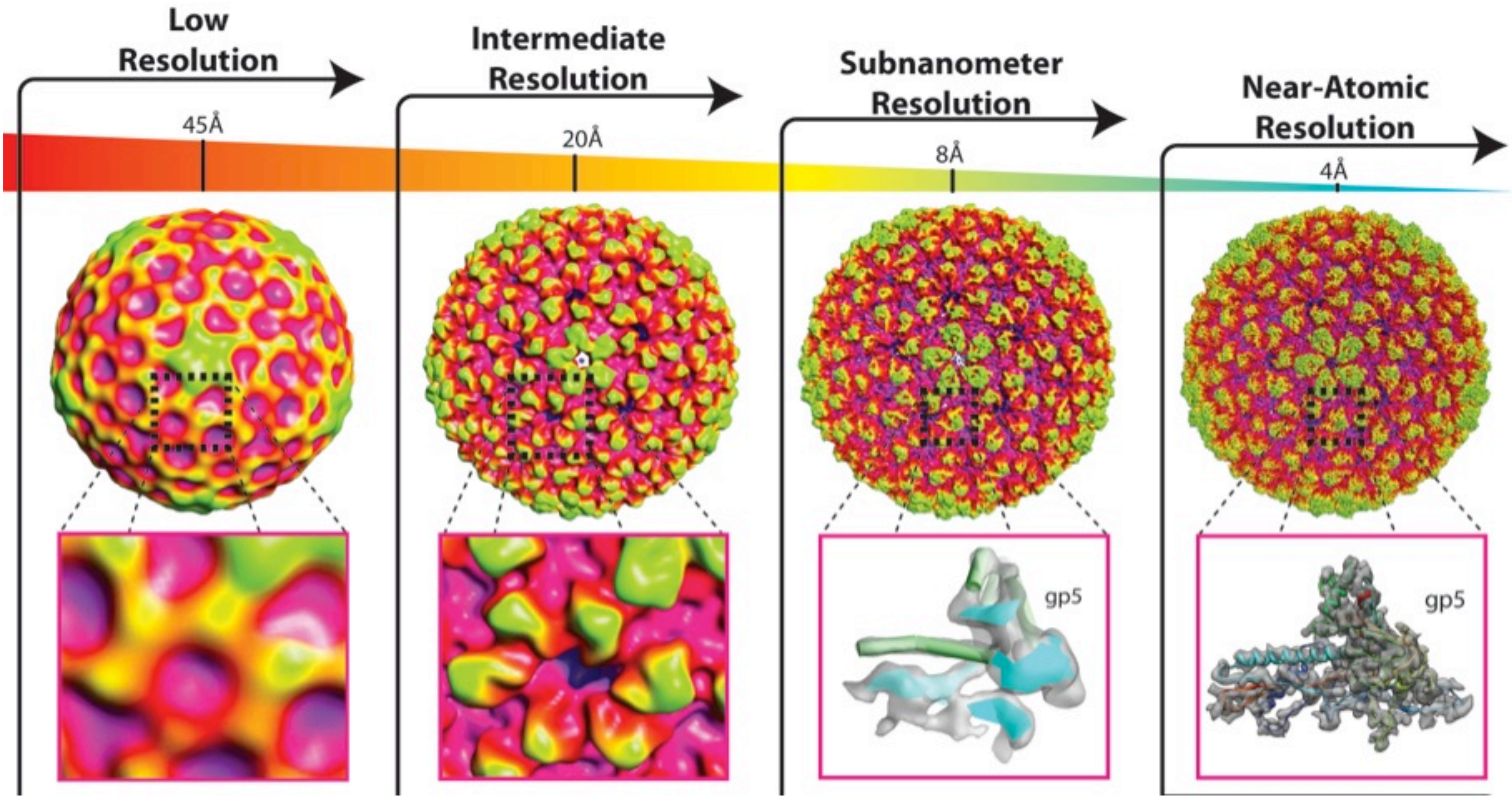
α-helices
β-sheets

Strands
Connectivity

Sidechains

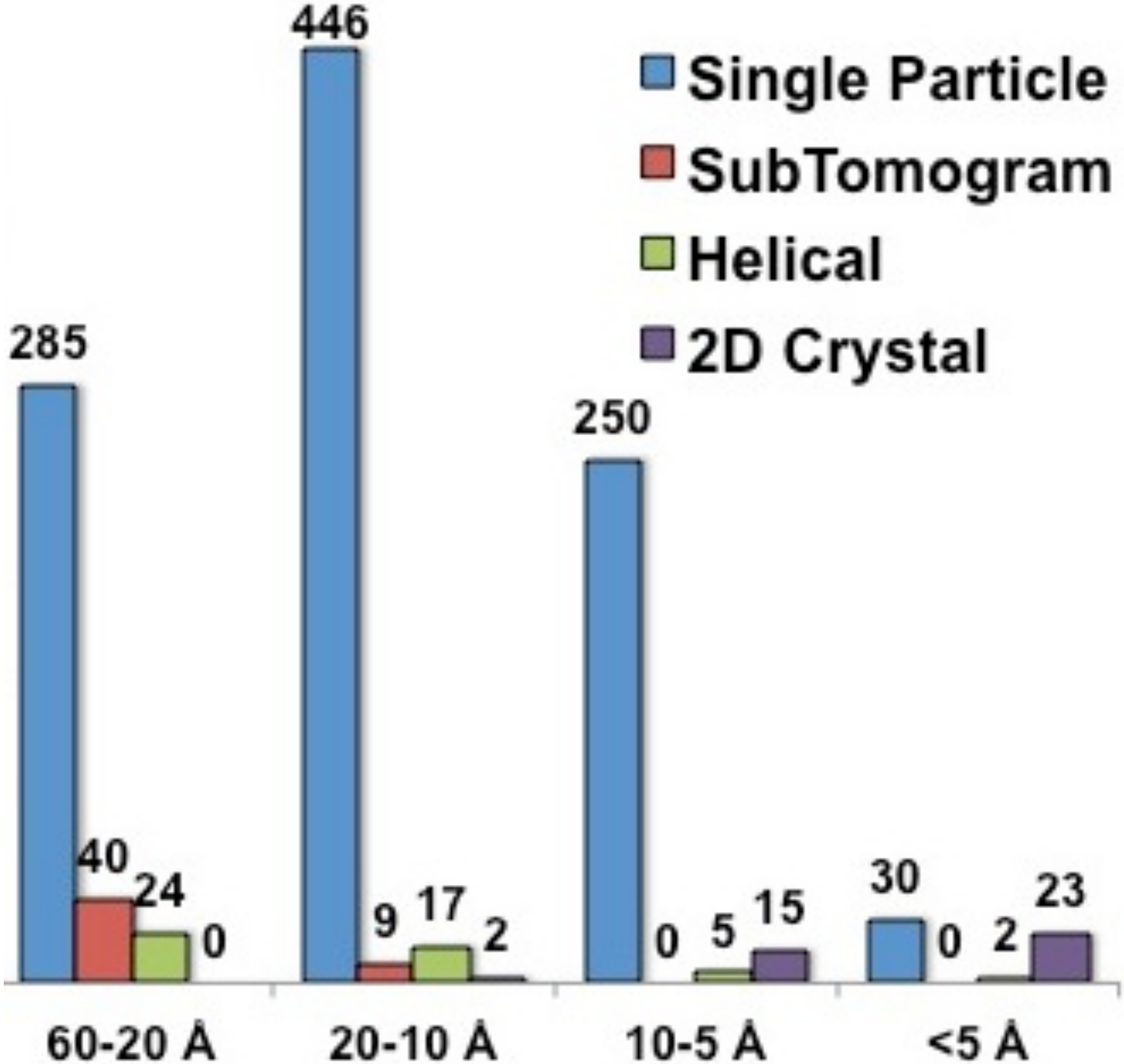
Courtesy of Matthew Baker

Structural Features at Different Resolutions



Courtesy of Ryan Rochat

EMDB Deposited Maps at Different Resolutions



Courtesy of
Cathy Lawson

Cryo-EM Map-Based Modeling

- Segmentation

  Identify locations of molecular components in a complex

- Feature Extractions

  Identify quaternary and secondary structure elements

- Rigid-body Docking

  Dock crystal or homology model into the density map

- Flexible fitting

  Deform model to better fit density map (and to discover new conformations as seen by Cryo-EM)

- *De Novo* modeling

  Build model with no template

References on Cryo-EM Based Modeling

- Pintilie, G. and Chiu, W. (2012). Comparison of Segger and other methods for segmentation and rigid-body docking of molecular components in Cryo-EM density maps. *Biopolymers* **97**: 742-760.
- Baker, M. L., Baker, M. R., Hryc, C. F., Ju, T. and Chiu, W. (2012). Gorgon and pathwalking: Macromolecular modeling tools for subnanometer resolution density maps. *Biopolymers* **97**:655-668.
- Baker, M. R., Rees, I., Ludtke, S. J., Chiu, W. and Baker, M. L. (2012). Constructing and validating initial Calpha models from subnanometer resolution density maps with pathwalking. *Structure* **20**: 450-463.
- Ludtke, S. J., Lawson, C. L., Kleywegt, G. J., Berman, H. and Chiu, W. (2012). The 2010 cryo-em modeling challenge. *Biopolymers* **97**: 651-654.

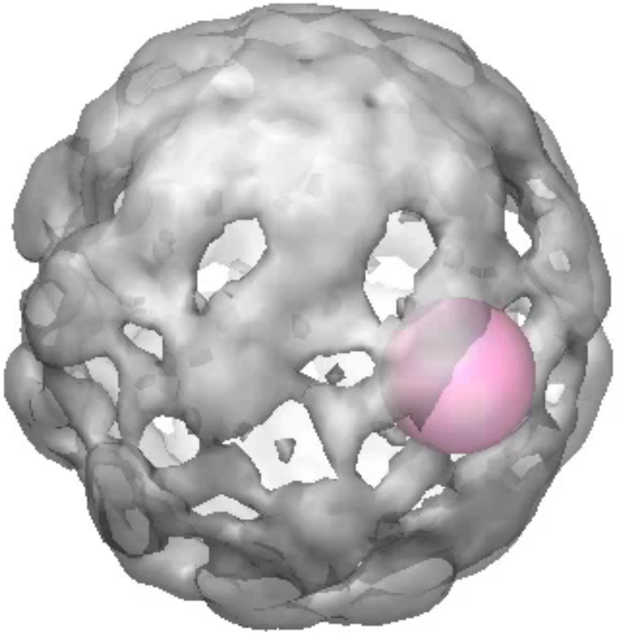
Segmentation

- Partition map into regions or segments that correspond to individual components such as proteins, subunits, DNA, etc.
- Methods:
  - Manual
    - By using "sphere eraser" in Chimera
    - By drawing boundary contours using Aviso^{TM}
  - Using docked model
    - Take all grid points close to atoms in a docked model
  - Semi-automated
    - **EMAN** (http://blake.bcm.edu/emanwiki/Segment3D)
    - **Segger** (http://ncmi.bcm.edu/ncmi/software/segger/docs)
    - **Volrover** (http://www.cs.utexas.edu/~bajaj/cvc/software/volrover.shtml)

Manual Segmentation – Sphere Eraser

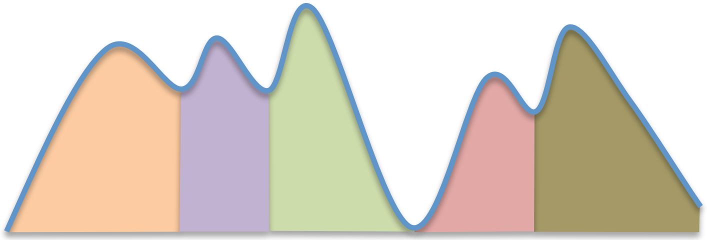
(http://www.cgl.ucsf.edu/chimera/)

- Erase parts of the map that are not in the region that corresponds to a component based on human judgment
- Very tedious, error-prone, and highly subjective



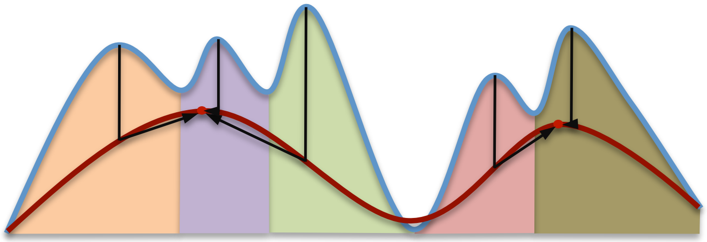
Semi-Automatic Segmentation - Segger

Example: 1-dimensional map

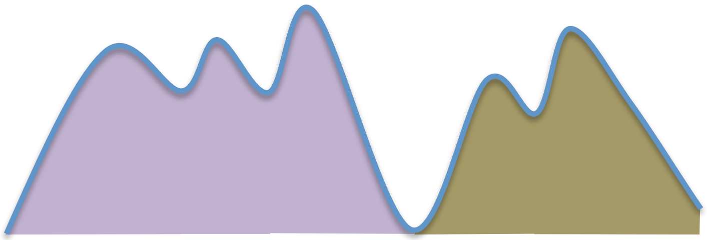


- Height proportional to density
- Watershed region produces 5 regions
- Each region corresponds to a peak in the density

Smoothing and Grouping



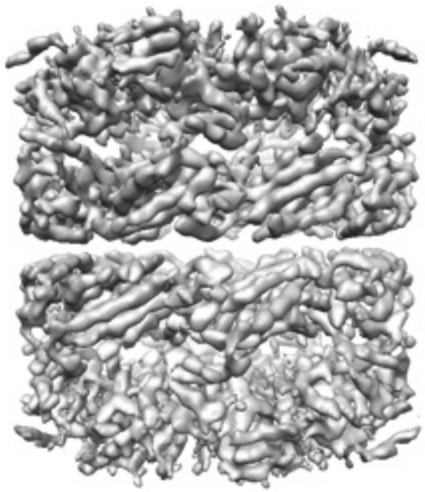
- Map is smoothed using Gaussian filter (red line)
- Regions are grouped based on which peak is reached after uphill climb (black lines)
- The result in this example is 2 regions, since there are two peaks in the smoothed map



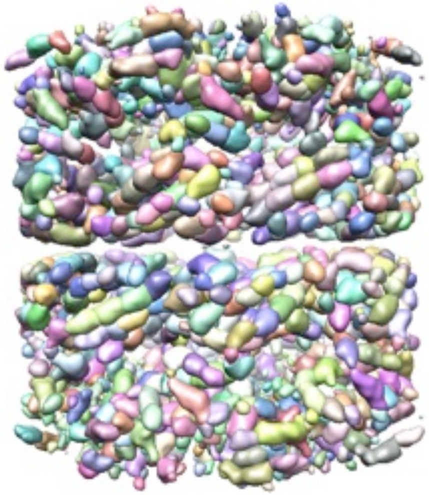
Pintilie et al (2010) *J Struct Biol* **170**(3):427-38.

Segmentation – Segger
A Case that Works

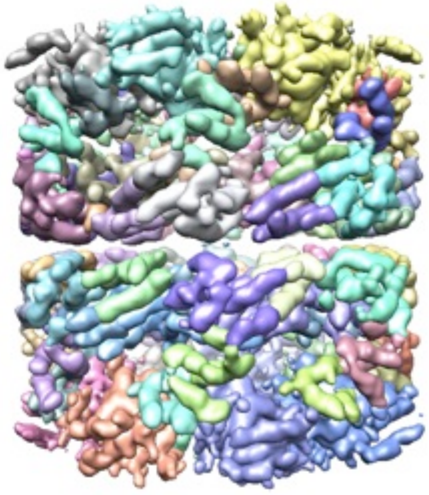
- CryoEM map of GroEL @4Å resolution
- After 3 steps of size 7 (Å), 14 regions are produced; each region corresponds to a protein



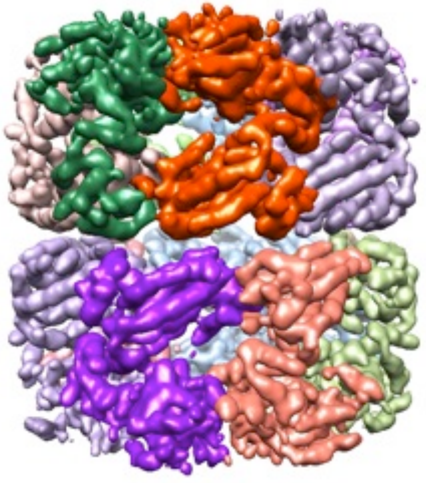
Cryo-EM map
EMDB:5001
GroEL @ 4Å



2124 watershed regions (too many)



After smoothing and grouping 1 step (42 regions)

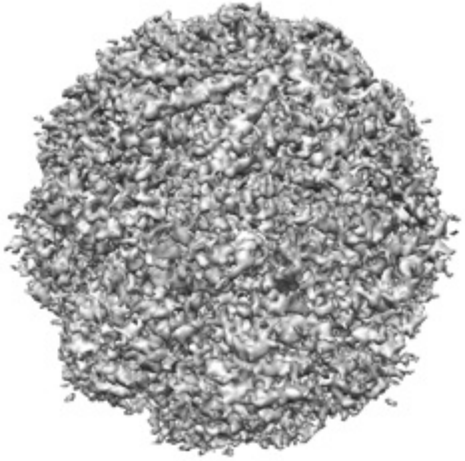


After smoothing and grouping 3 steps (14 regions)

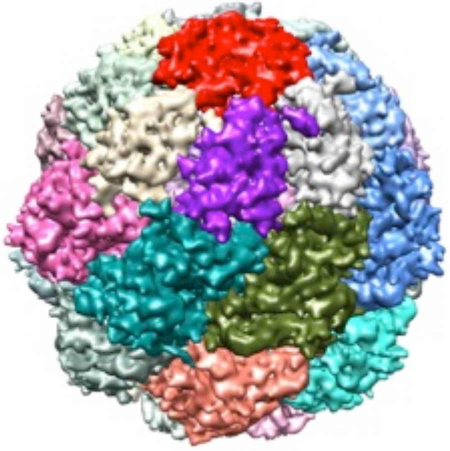
Pintilie et al (2010). *J Struct Biol* **170**(3):427-38.

User-Assisted Semi-Automatic Segmentation - Segger

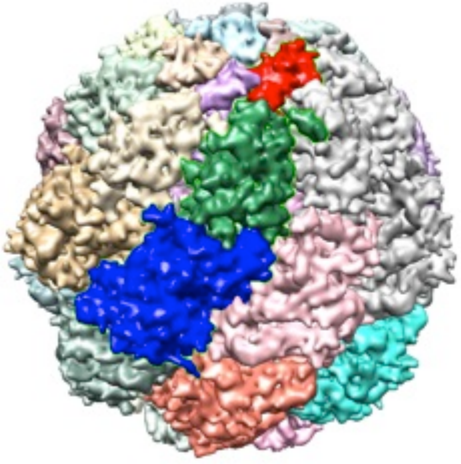
- In some cases, manual (subjective) grouping is needed as shown in the example below
- Use prior knowledge, or known homology models, for guidance



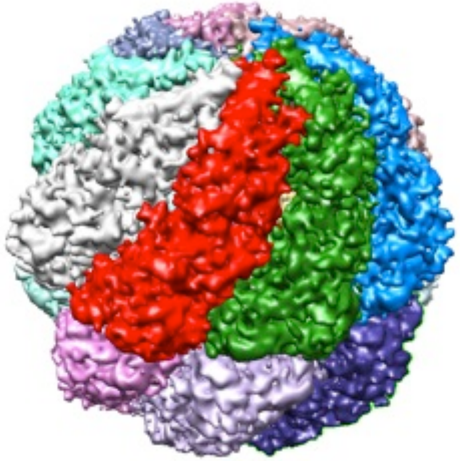
Cryo-EM map
EMDB:5137
Mm-cpn @ 4.3Å



Regions after smoothing and grouping – note that red region is incorrect as it spans multiple proteins

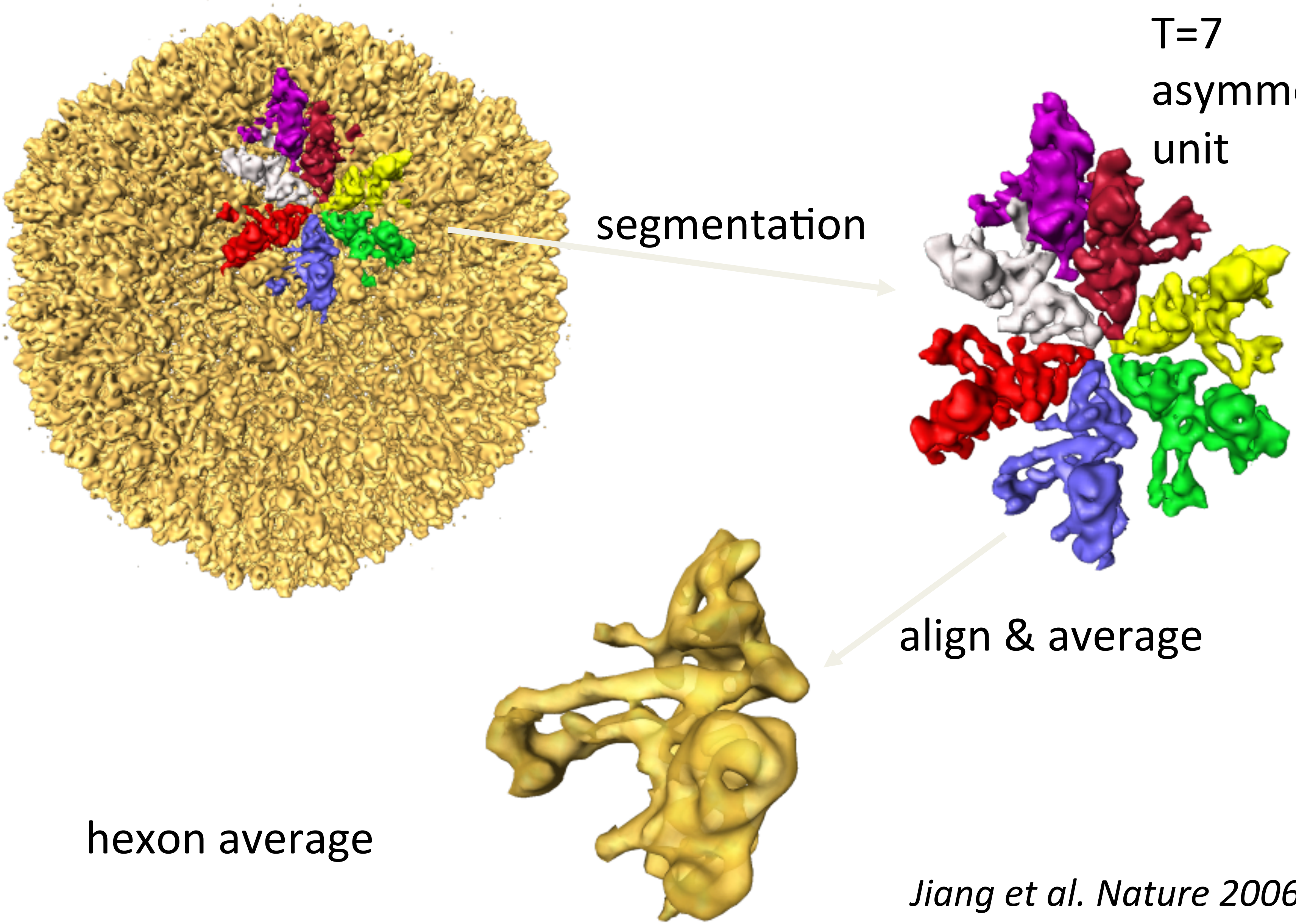


Regions can be selected and grouped interactively (the blue, green and red regions are selected above)



The correct segmentation, after manual grouping, contains 16 regions

Epsilon15 Phage at 9.5 Å Resolution



T=7 asymmetric unit

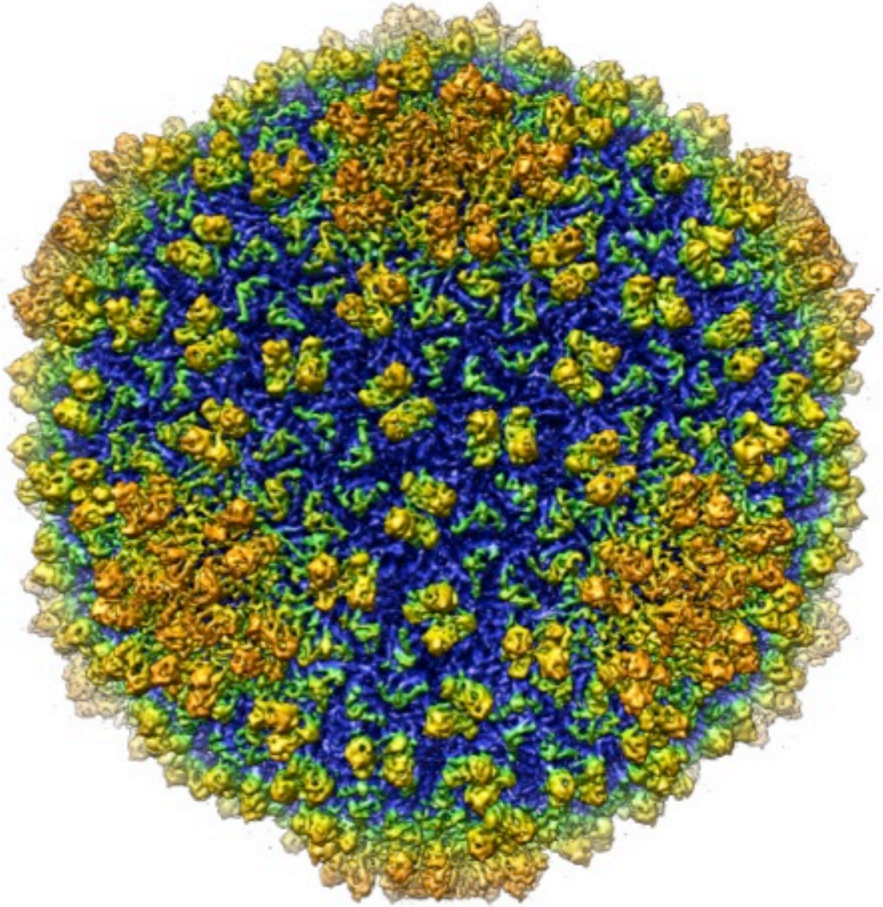
segmentation

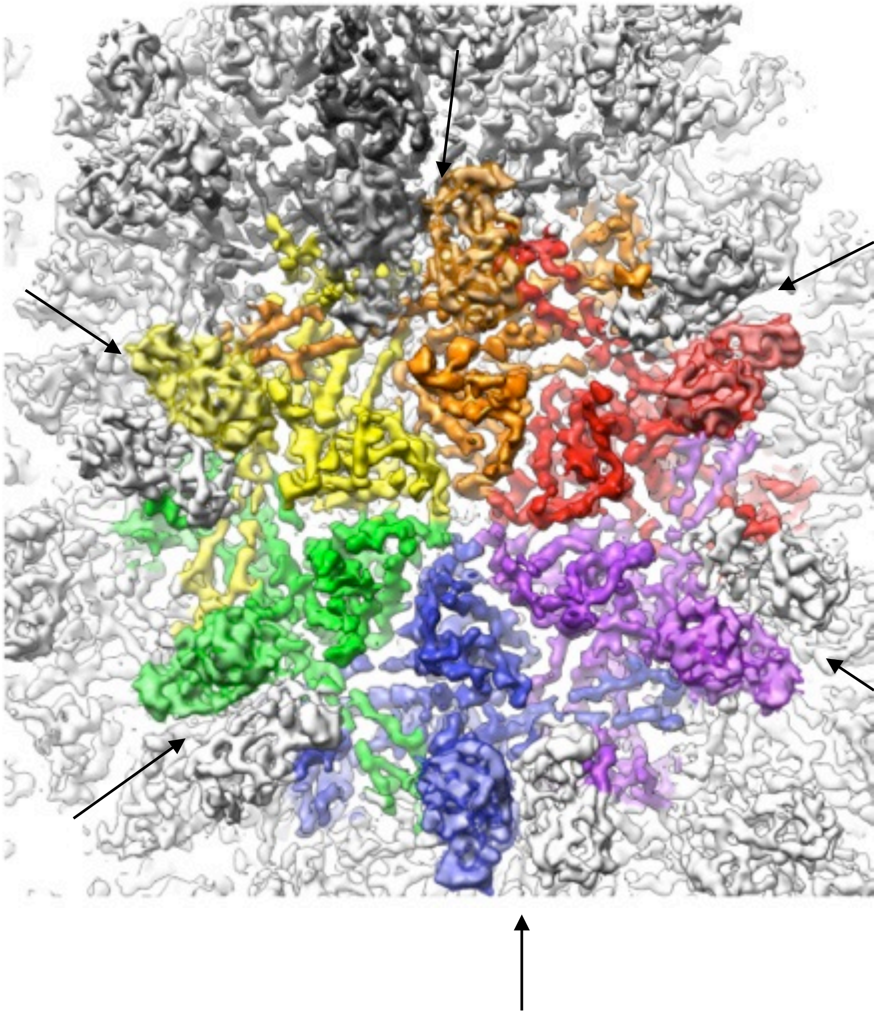
align & average

hexon average

Jiang et al. Nature 2006

Epsilon15 Phage at 4.5 Å Resolution



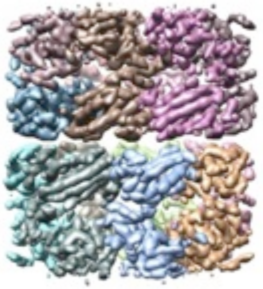


Jiang et al (2008) *Nature* **451**: 1130-4.

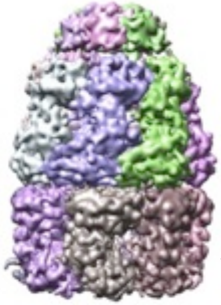
2010 Cryo-EM Challenge

Segger Segmentation Results

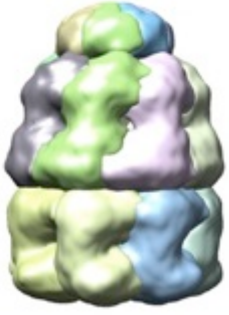
GroEL



4Å

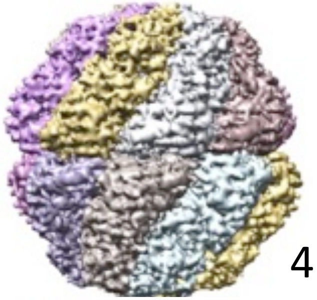


7.7Å

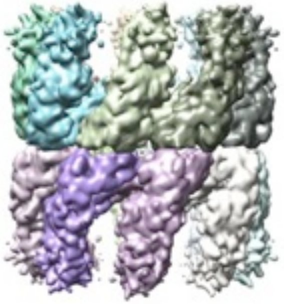


23.5Å

Mm-cpn

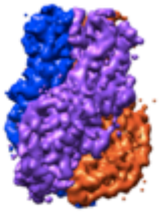


4.3Å



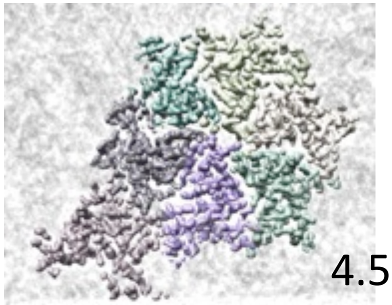
8Å

Rotavirus

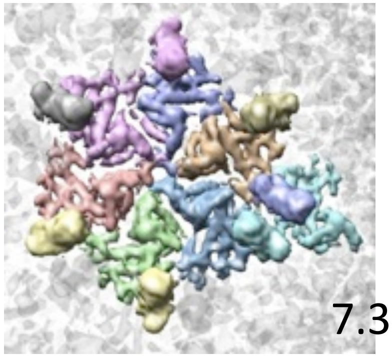


3.8Å

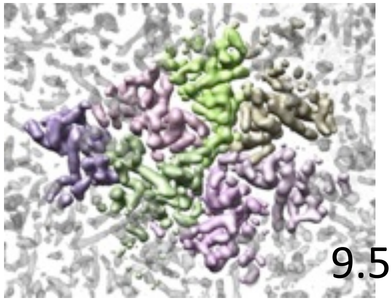
Epsilon15



4.5Å

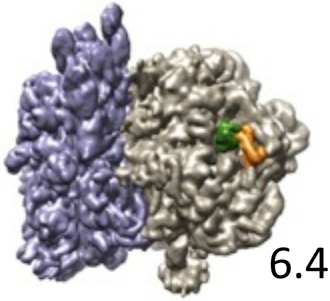


7.3Å

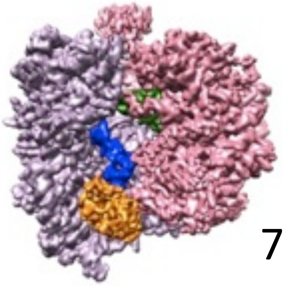


9.5Å

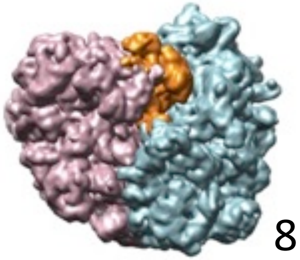
Ribosome



6.4Å



7.4Å



8.9Å

Segmentation Summary

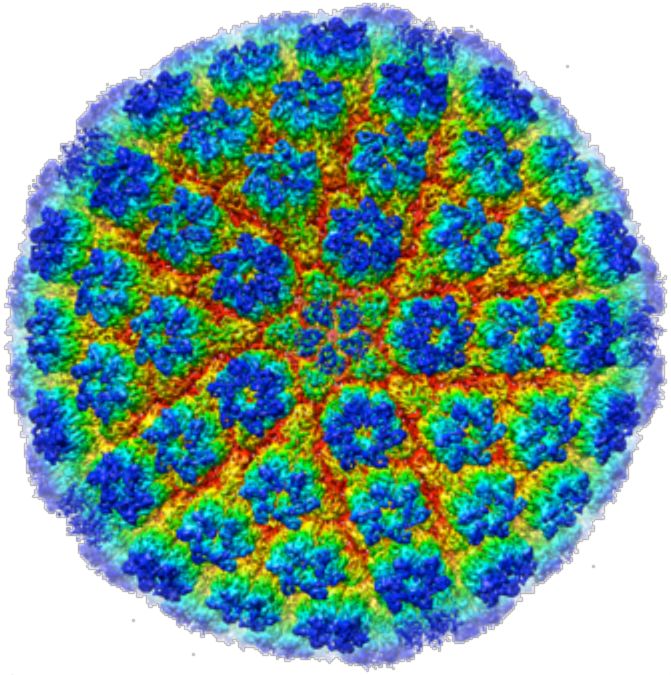
- **Segmentation is a difficult process. Why?**
  - Proteins have complex 3D shapes and contacts with other proteins
  - Complexes can consist of large numbers of proteins
  - Contact boundaries between different proteins can be as dense as intra-protein contacts; hence they are hard to identify accurately
- **In general:**
  - It tends to be easier in density maps where components are well-separated (e.g. GroEL)
  - It tends to be hard in density maps where there are many contacts between proteins, and proteins have long, narrow segments that contact adjacent proteins (e.g. in phages)
  - Knowing what the protein looks like (e.g. from homology model) helps a great deal

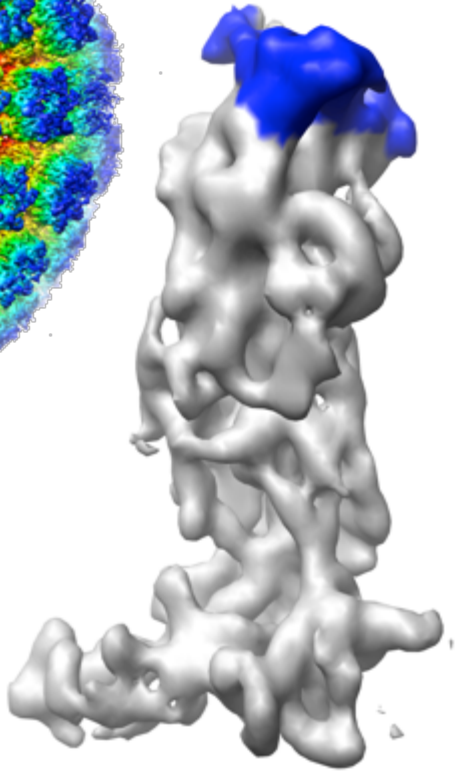
Feature Extraction

- Quaternary Structure
- Domains
- Helices and sheets

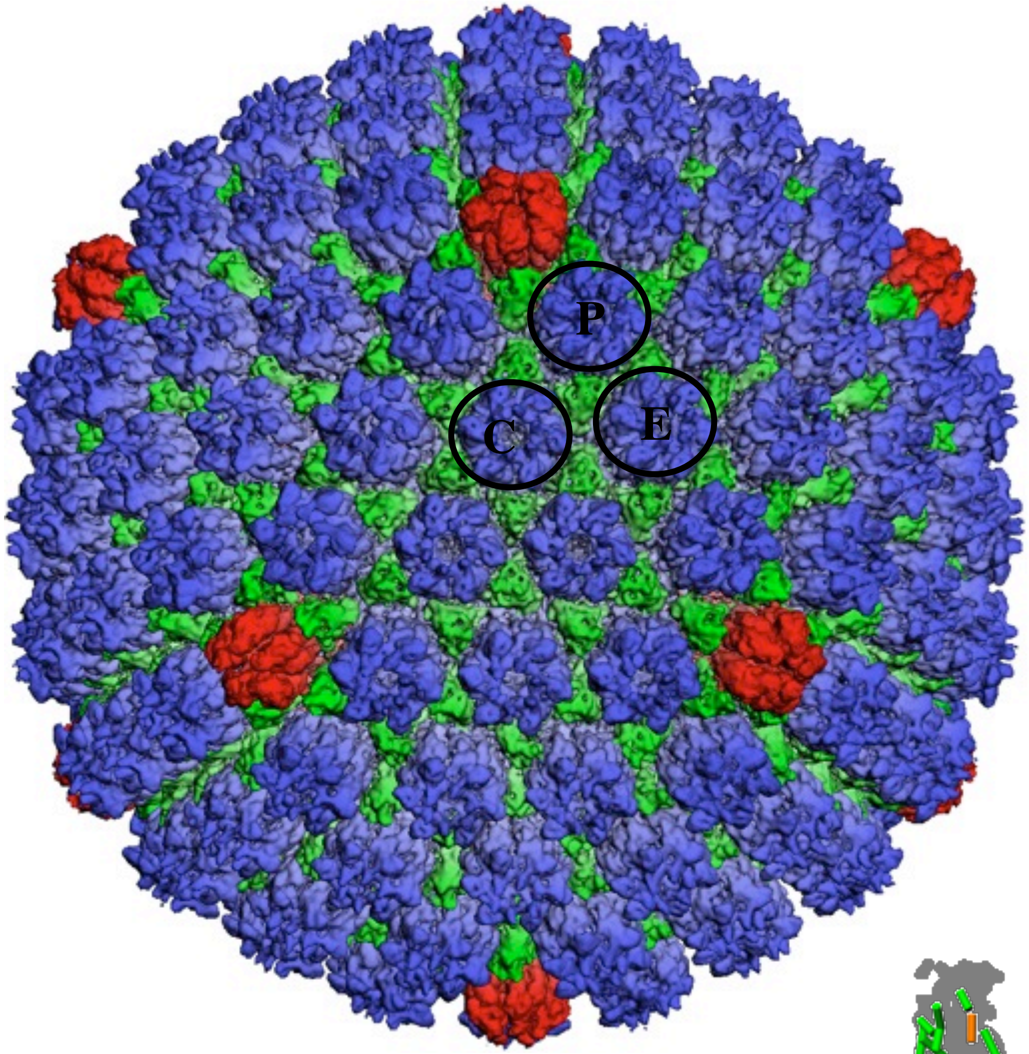
Herpes Simplex Virus-1 Capsid

- 8.5 Å resolution cryo-EM map (Zhou, *Science* 2000)
- 4 structural proteins
  - VP5: major capsid protein forms pentons and hexons
  - VP26: binds only VP5 at hexon specific positions
  - VP23 and VP19C form triplexes between hexons and pentons





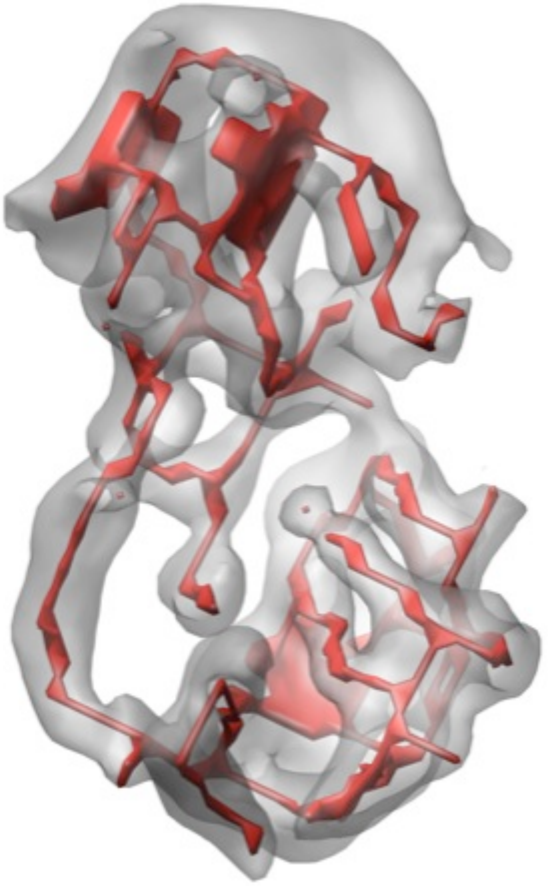
8.5 Å Map of HSV-1 Capsid



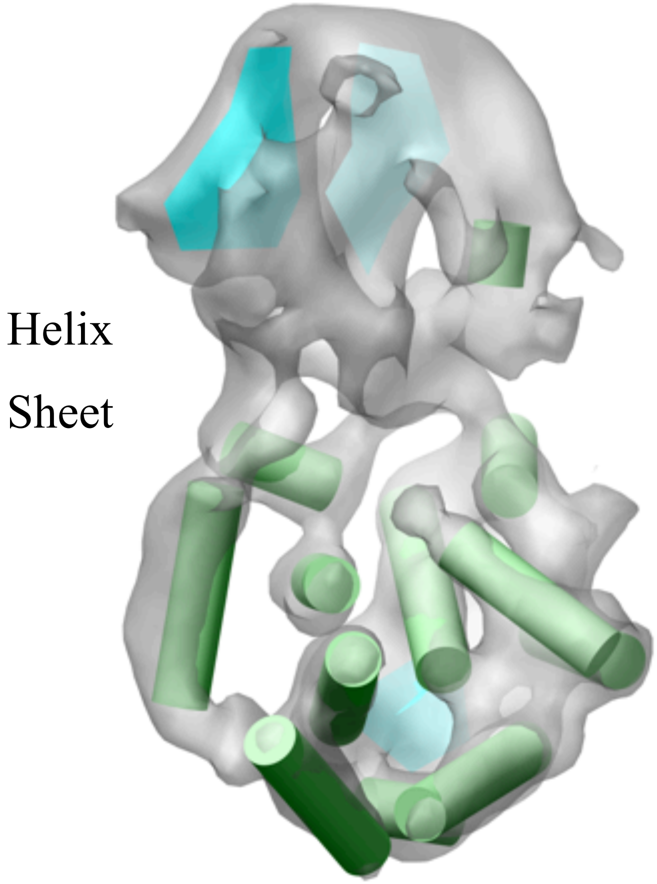
Manually identified helices

Zhou et al (2000) *Science* **288**: 877-880.

SSEHunter of a Cryo-EM Density

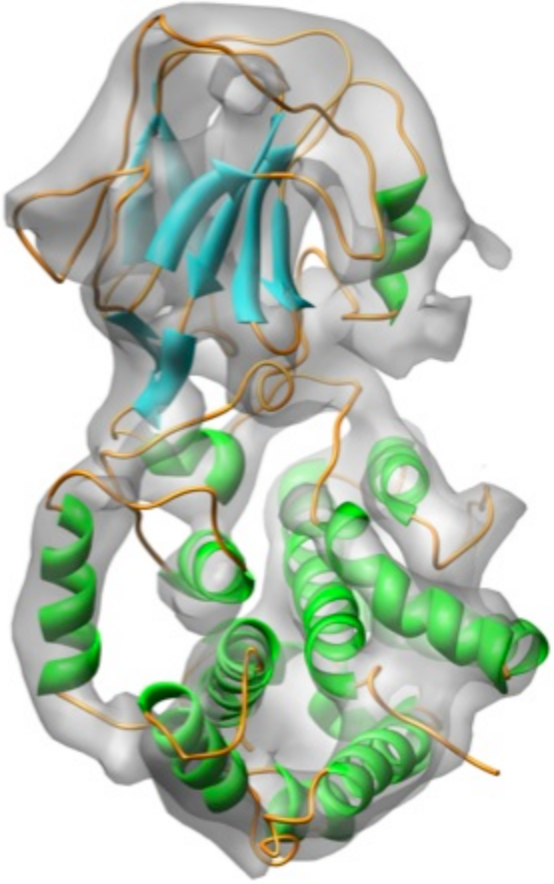


Skeleton



6.8 Å resolution cryoEM density map

Z.H. Zhou et al, 2001, Nature Struct Biol.



X-ray model

M. Baker et al. (2007) *Structure* **15**:7-19

Skeleton: Feature and Topology

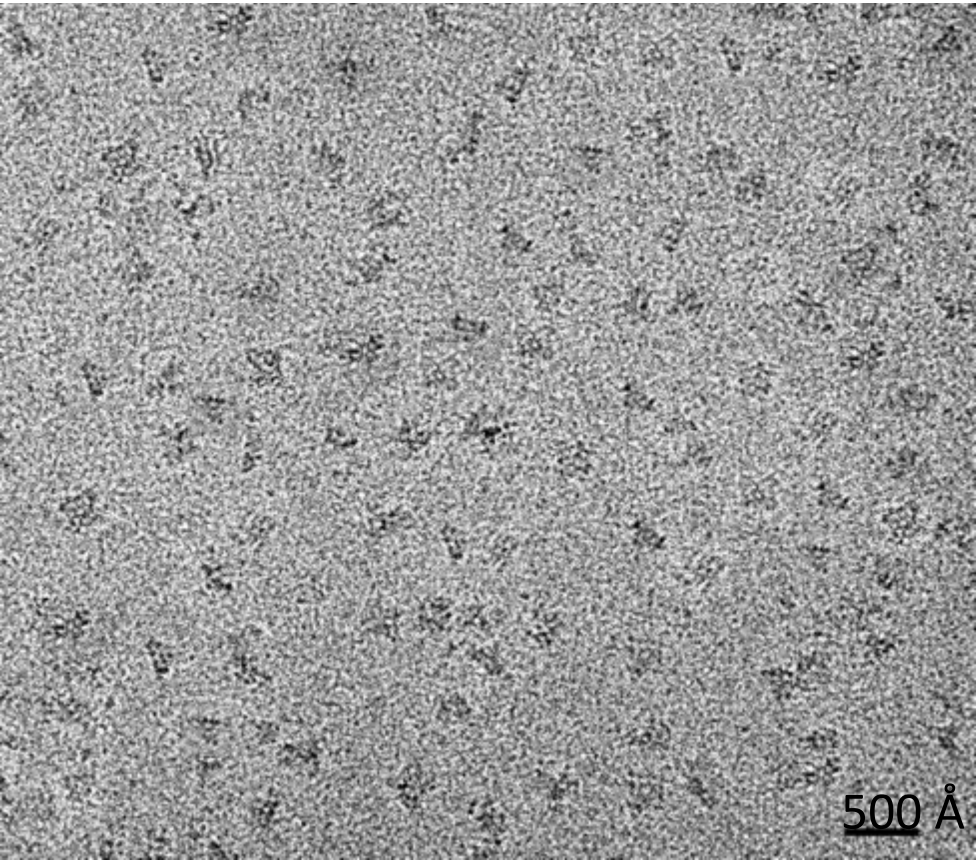
- Compact geometric representation of a volume

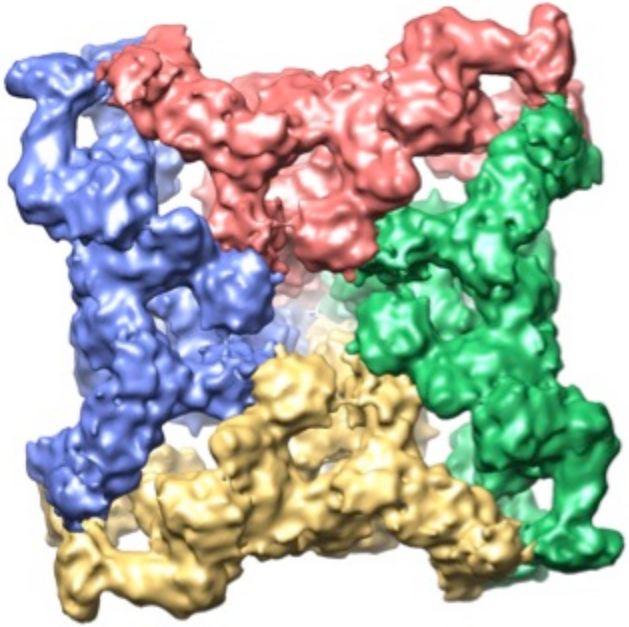
- Feature preserving
  - Sheets are represented as flat surfaces
  - Helices and loops are represented as curves

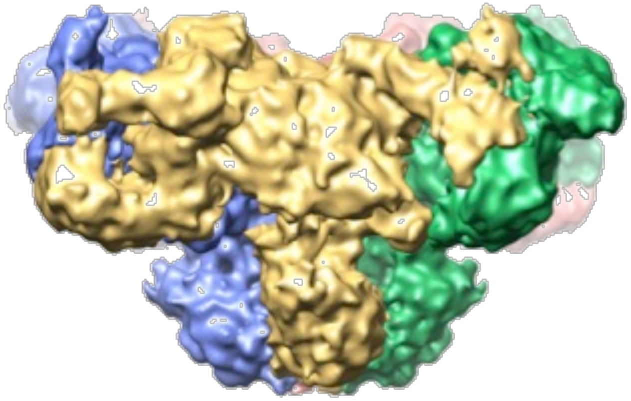
- Topology preserving
  - Maintains density connectivity while minimizing number of branches and breaks

Baker, M.L., Ju, T. and Chiu, W. (2007) *Structure*, **15**:7-19.

9.5 Å Cryo-EM Map of RyR1 (2.2 MDa)

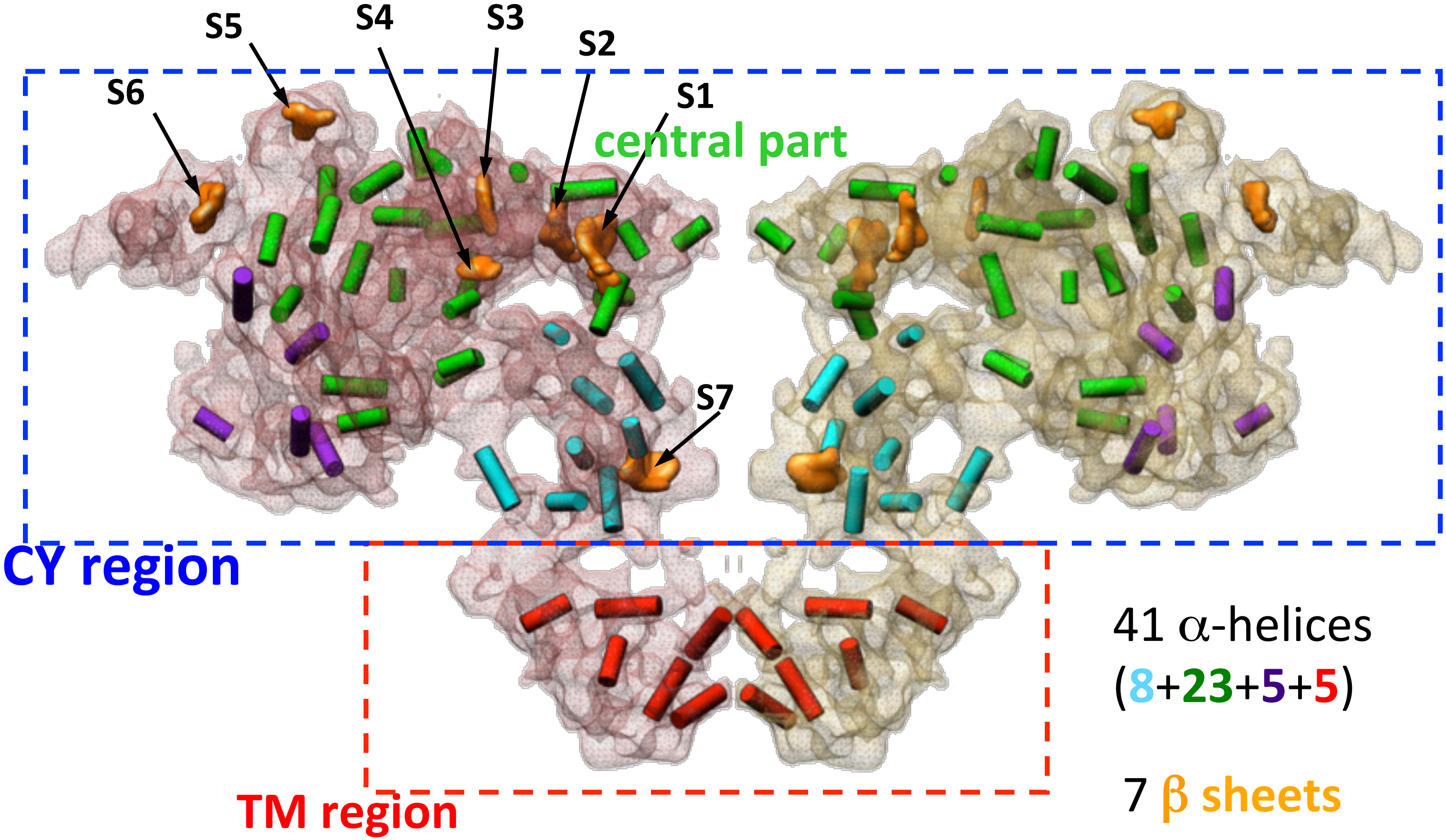






Ludtke et al (2005) *Structure* **13**: 1203-11.

Identified SSE in RyR1 Cryo-EM Map



41 α-helices
(8+23+5+5)

7 β sheets

Serysheva et al (2008) *Proc Natl Acad Sci U S A* **105**: 9610-5.

References on Fitting methods

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- Roseman AM. Docking structures of domains into maps from cryo-electron microscopy using local correlation. *Acta Crystallogr D Biol Crystallogr* 2000;56:1332–40. [PubMed: 10998630]
- Rossmann MG, Bernal R, Pletnev SV. Combining Electron Microscopic with X-Ray Crystallographic Structures. *J Struct Biol* 2001;136:190–200. [PubMed: 12051899]
- Jiang W, Baker M, Ludtke S, Chiu W. Bridging the information gap:Computational tools for intermediate resolution structure interpretation. *J Mol Biol* 2001;308:1033–1044. [PubMed: 11352589]
- Topf, M., Baker, M. L., John B., Chiu, W. and Sali, A. (2005). Structure characterization of components of protein assemblies by comparative modeling and electron cryo-microscopy. *J Struct Biol* **149**: 191-203.
- Pintilie, GD, Zhang, J, Goddard, TD, Chiu, W, and Gossard, DC (2010). Quantitative analysis of cryo-EM density map segmentation by watershed and scale-space filtering and fitting of structures by alignment to regions. *J Struct Biol* **170**:427-38.

Rigid-body Docking

- What is rigid-body docking:
  - Finding the translation and orientation that takes a known model and places it inside a density map so that it accurately overlaps the same component as it occurs in the density map
- Quantitatively speaking, we try to find the translation and orientation that maximizes the cross-correlation score between:
  - a simulated density map of the structure (S),
  - and the cryo-em density map (C)

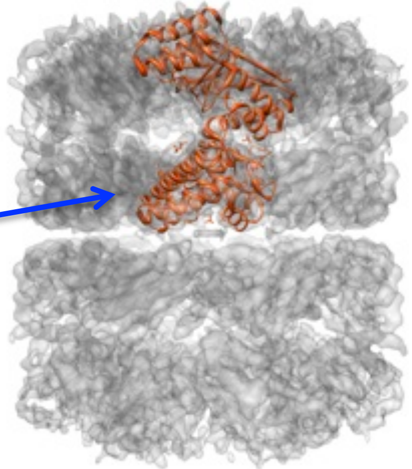
$$cc = \frac{\sum_{i,j,k \in (n_1 \times n_2 \times n_3)} d^C d^S_{i,j,k}}{\sum_{i,j,k \in (n_1 \times n_2 \times n_3)} d^C \sum_{i,j,k \in (n_1 \times n_2 \times n_3)} d^S_{i,j,k}}$$

Cross-correlation score

$d^S_{i,j,k}$ Density in S at grid point *i,j,k*

d^C (Interpolated) Density in C at grid point *i,j,k* in S

A known model (from PDB)

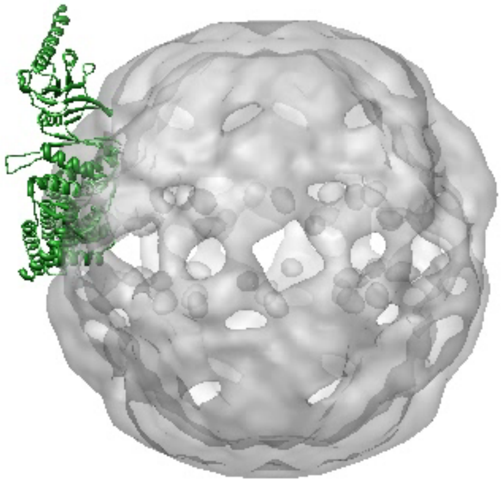


The model docked inside the density map

Rigid-body Docking I

Exhaustive search:

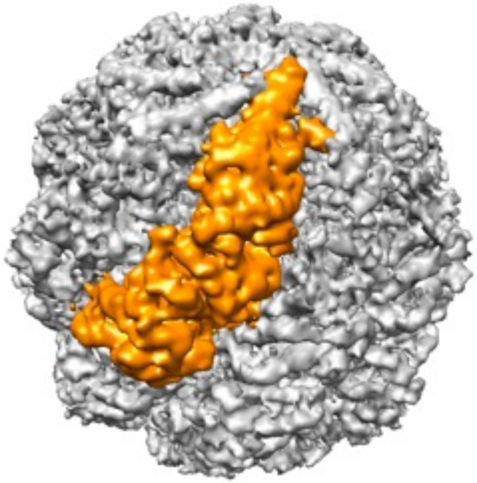
- Used by Situs, Foldhunter, ADP-EM
- The structure is placed at evenly-spaced positions and orientations
- The cross-correlation score is computed for each position/orientation
- This can take a long time in large maps (hours), but it can be accelerated using the Fourier transform, or by using multiple processors



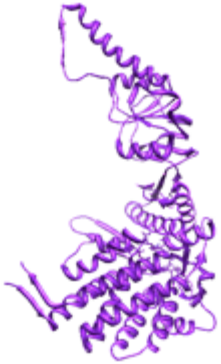
Rigid-body Docking II

Segger:

- Performs rigid-body docking by aligning a model to a segmented region
- Faster than exhaustive search, however user-guidance is required
- Steps:
  1. Segment map
  2. Choose a region and a model
  3. Align model to region using one of two methods

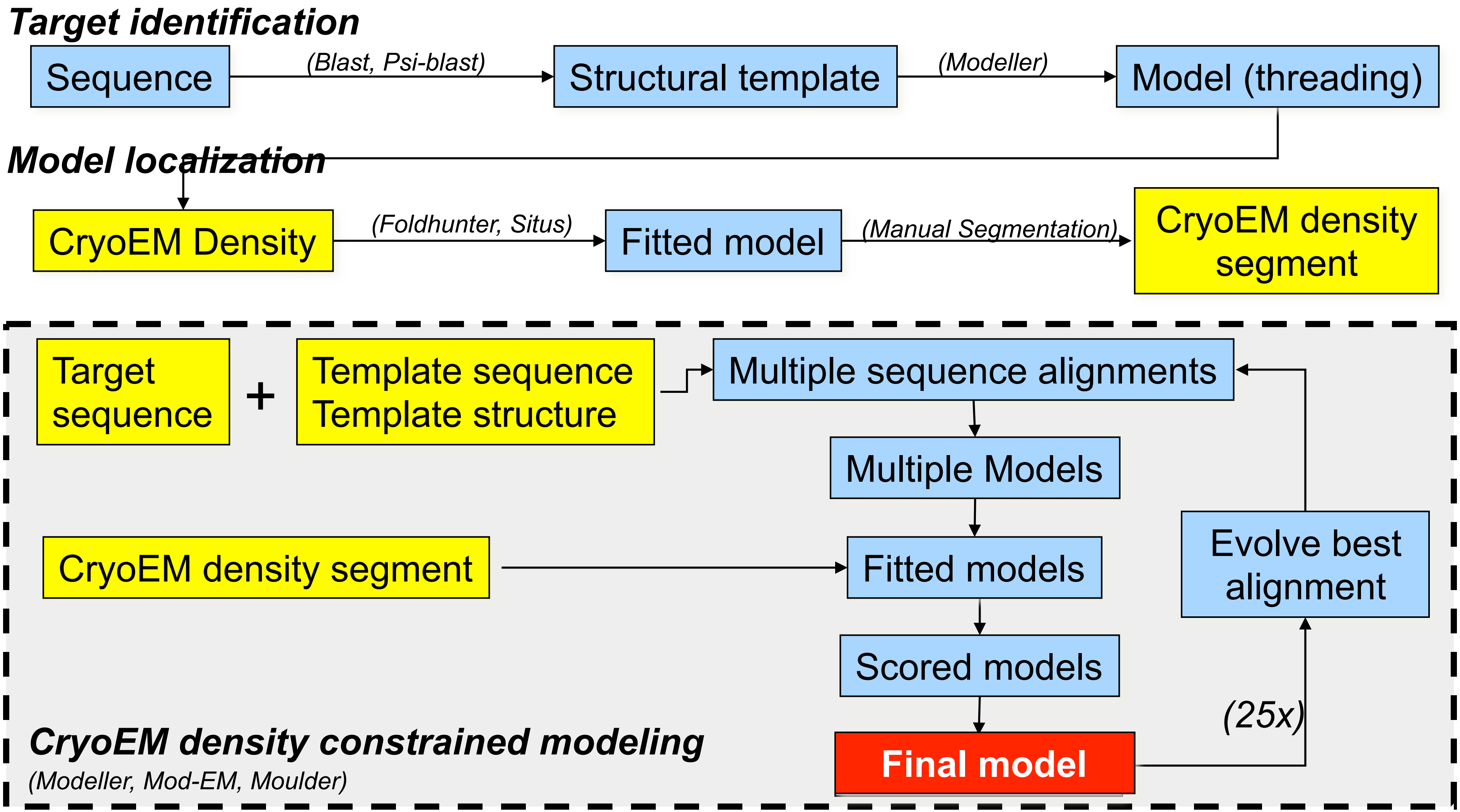


Mm-cpn @4.2Å
EMDB:5137



PDB:3kfb, chain A

CryoEM Restrained Comparative Modeling



Target identification

Sequence

(Blast, Psi-blast)

Structural template

(Modeller)

Model (threading)

Model localization

CryoEM Density

(Foldhunter, Situs)

Fitted model

(Manual Segmentation)

CryoEM density segment

Target sequence + Template sequence Template structure

Multiple sequence alignments

Multiple Models

CryoEM density segment

Fitted models

Scored models

Final model

Evolve best alignment

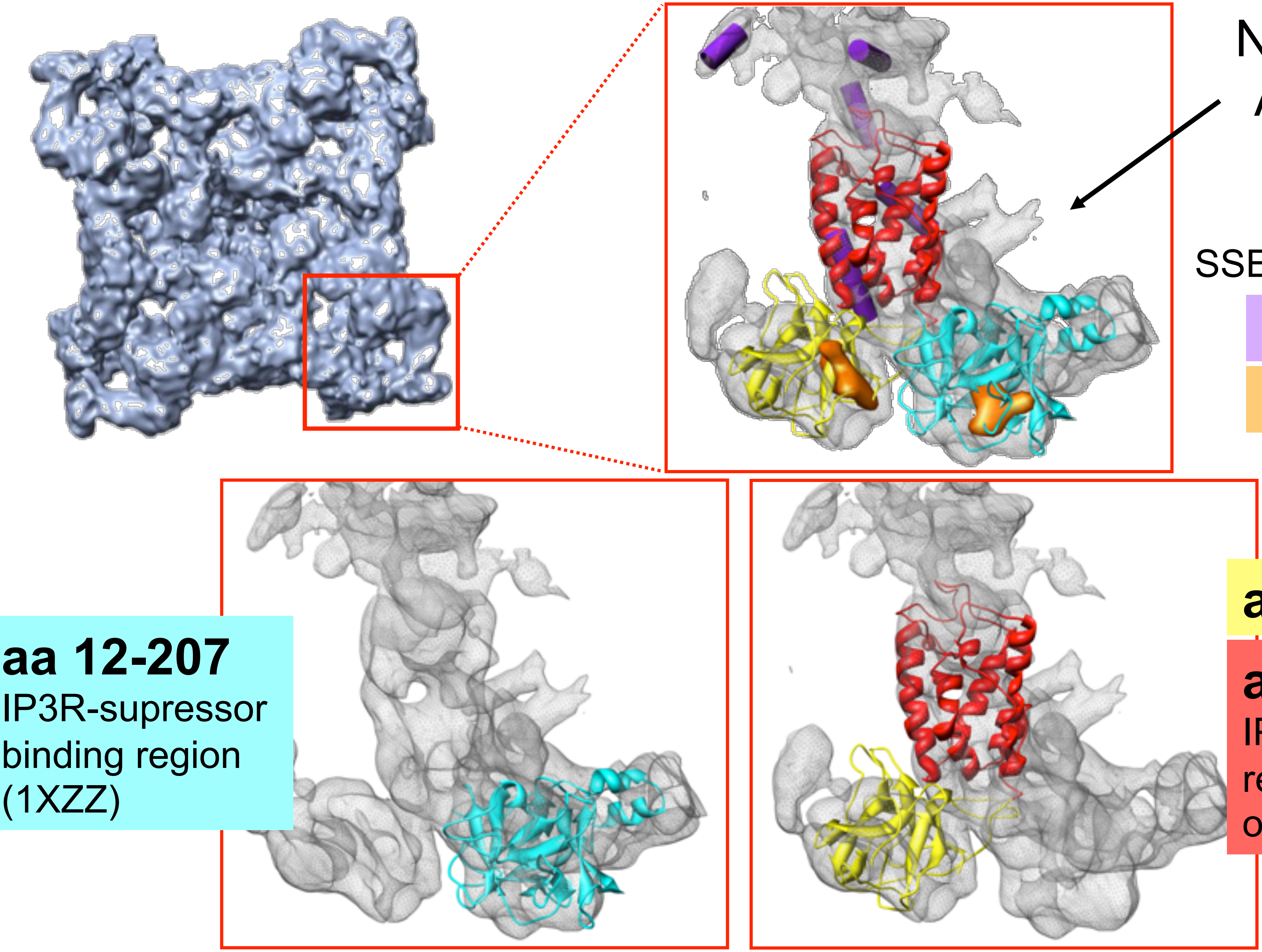
(25x)

CryoEM density constrained modeling

(Modeller, Mod-EM, Moulder)

Topf et al, *JMB* (2006)

CryoEM Restrained Modeling of the N-terminal of RyR1



N terminal Antibody binding

SSEhunter results:

α-helices

β-sheets

aa 12-207
IP3R-supressor binding region (1XZZ)

aa 215-420

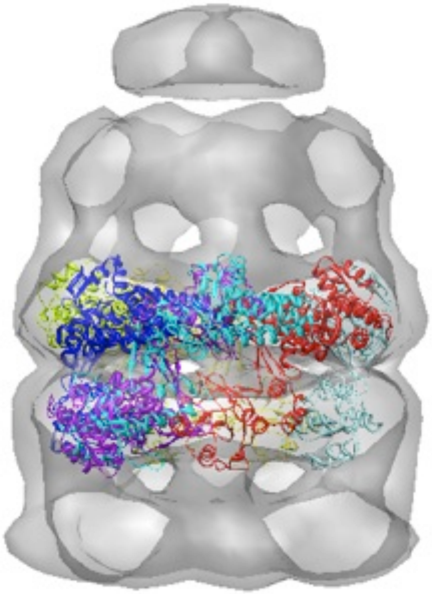
aa 418-564
IP3-binding region (1N4K) of IP3 Receptor

Rigid-body Docking

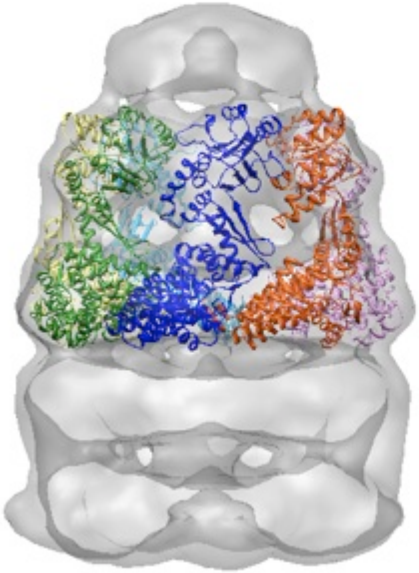
- Which alignment, produced by exhaustive search, or by Segger, or by other methods, is the right fit?
- It is typical to pick the fit with the highest score
  - Which score to use?
    - Cross-correlation tends to be the most reliable
      However this can fail, especially at lower resolutions

Vasishtan and Topf (2011). *J Struct Biol* 174:333-343.

Challenges in Rigid-body Docking



GroEL @23Å resolution
Alignments with highest cross-correlation are incorrect – they overlap the middle part of the map, which has higher densities, and hence give higher cross-correlation scores



The correct docking results are as shown. Can we compute other scores, or compute a confidence level in our docking results?

Rigid-body Docking

Q: How confident can we be that the alignment with the highest score is the correct fit?

A: Statistically, if the highest score is higher than scores of other alignments, this means the fit is significant.

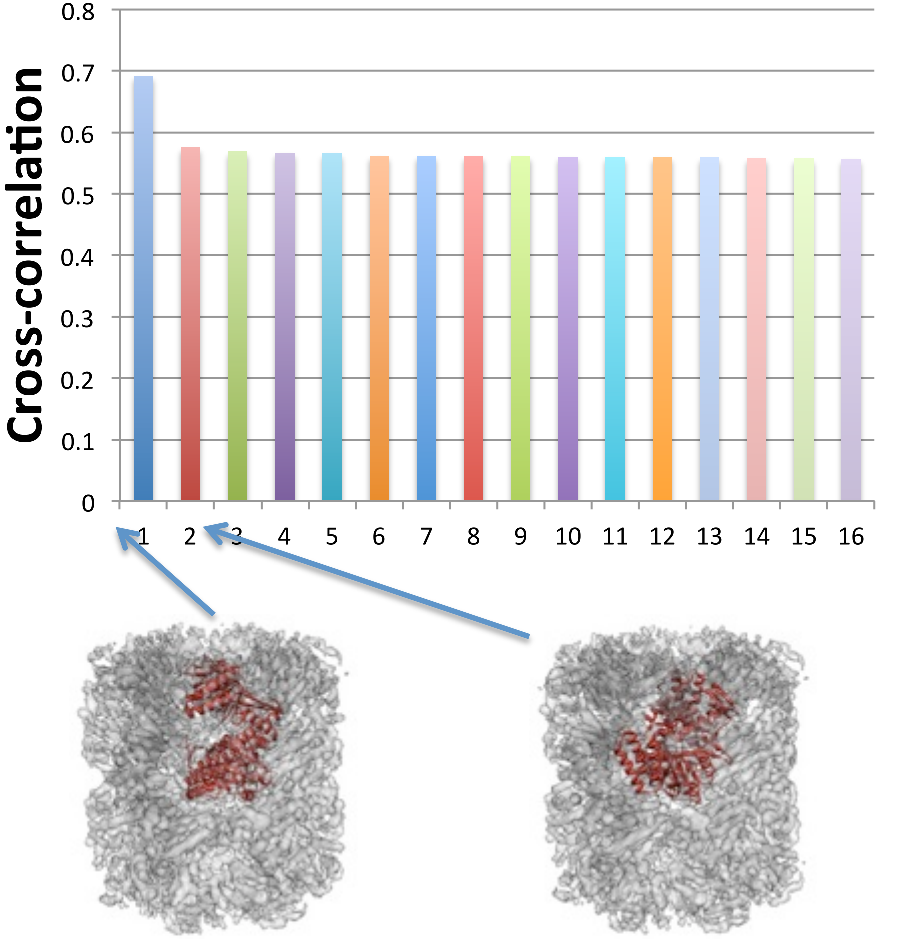
- *z-score:* indicates how much higher the score for the best alignment is compared to the mean score of the other alignments

Score Rigid-body Docking

- *z-score:*
  - How much higher is the best score compared to the mean of the other scores?
  - More precisely: how many standard deviations is the highest score above the mean?

$$z-score = \frac{S_1 - mean(S_{2..n})}{stdev(S_{2..n})}$$

S_1	Top score
$avg(S_{2..n})$	Mean of scores 2..n
$stdev(S_{2..n})$	Standard deviation of scores 2..n



Correct fit
(highest CC score)

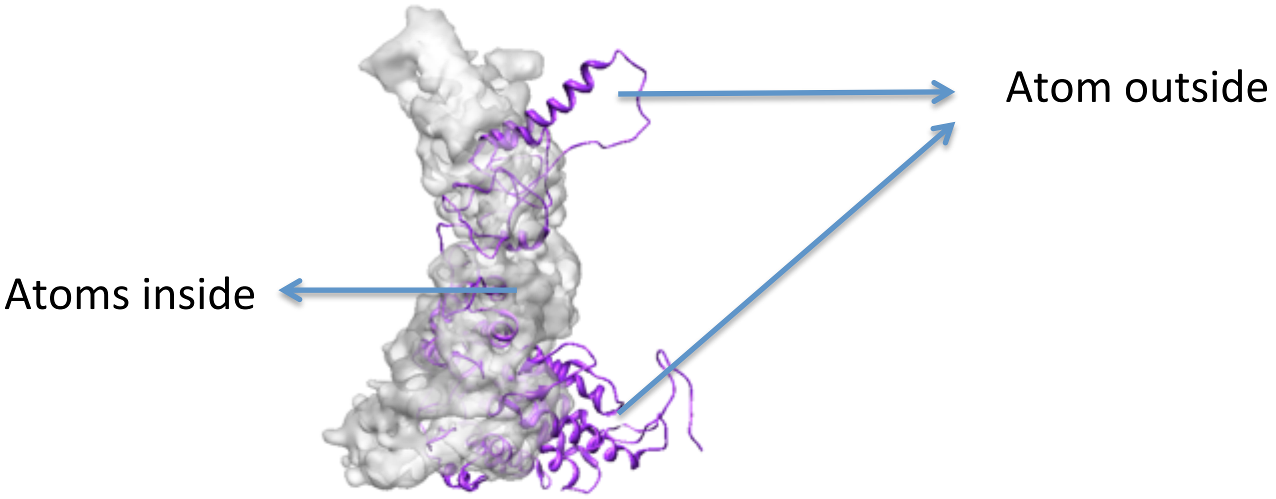
Incorrect fit
(significantly lower CC score)

Score Rigid-body Docking

- Other scores can be computed, to evaluate how confident we can be about a rigid-body docking result:
  - Atom inclusion:
    - How many atoms are inside the observed density?
    - If many atoms are outside, then the model may not be the best match for the map, or it may be incorrectly docked.
  - Density occupancy:
    - How many grid points with high density values are occupied by atoms
    - If many areas inside the density maps are un-occupied, then the docked models may not fully explain the experimental density map.
  - Clashes with other docked models, or with symmetric copies
    - Do multiple docked models clash
    - If there are many clashes, this could signify an unreliable docking result.

Rigid-body Docking

- Other scores can be computed, to evaluate how confident we can be about a rigid-body docking result:
  - Atom inclusion:
    - How many atoms are inside the observed density?
    - If many atoms are outside, then the model may not be the best match for the map, or it may be incorrectly docked.

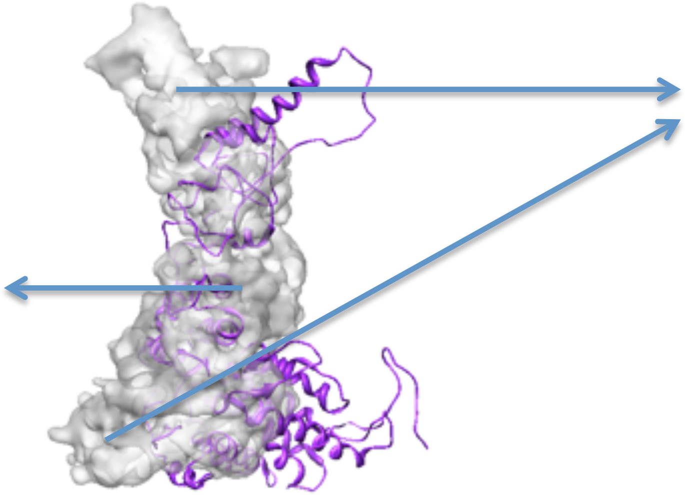


Atom outside

Atoms inside

Rigid-body Docking

- Other scores can be computed, to evaluate how confident we can be about a rigid-body docking result:
  - Density occupancy:
    - How many grid points with high density values are occupied by atoms
    - If many areas inside the density maps are unoccupied, then the docked models may not fully explain the experimental density map.

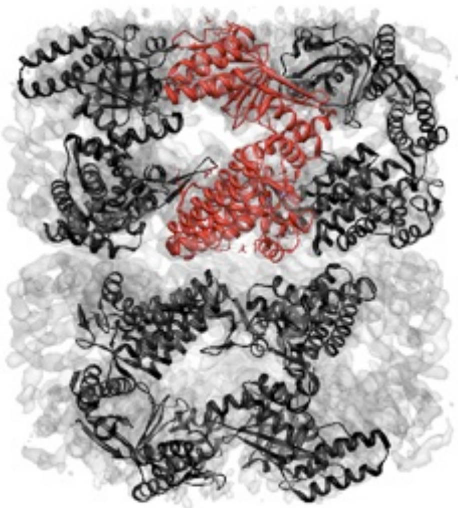


Unoccupied density

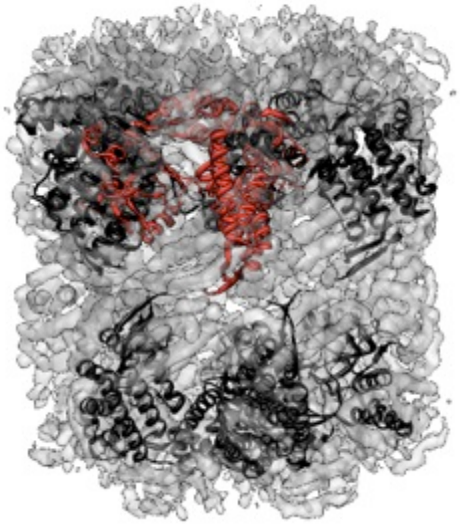
Occupied density

Rigid-body Docking

- Other scores can be computed, to evaluate how confident we can be about a rigid-body docking result:
  - Clashes with other docked models, or with symmetric copies
    - Do multiple docked models or symmetric copies clash?
    - If there are many clashes, this could signify an unreliable docking result.



Few clashes between symmetric copies: correct dock



Many clashes between symmetric copies: incorrect dock

Red ribbon:
docked model

Black ribbon:
symmetric copies

Rigid-body Docking Summary

- Exhaustive search
  - Can take a long time
  - Thoroughly searches for alignments of the model inside the map
  - Can produce unreliable results if a map is very heterogeneous (i.e. densities are higher in some regions - this makes the CC-score higher in those regions even if the model does not fit well there)
- Segger
  - Allows docking by aligning known models to segmented regions
  - Faster than exhaustive search
  - Allows more control of where the model is docked
  - Requires more prior-knowledge about where the model might be found inside the map
  - Allows cross-validation between segmentation and docking results
- The cross-correlation score is widely used as the score that determines the best fit
- Other scores can be used to assess confidence in fit (z-score, atom inclusion, density occupancy, etc.)

Flexible Fitting

- Typically, start by rigidly-docking a model
- Then assess whether the fit could be better
  - The conformation of the model comes from homology or X-ray crystallography
    - But, the conformation seen in the cryoEM map may be different
  - Molecules are inherently flexible – they can adopt different conformations; it is possible that the cryoEM density map shows a different conformation

Flexible Fitting

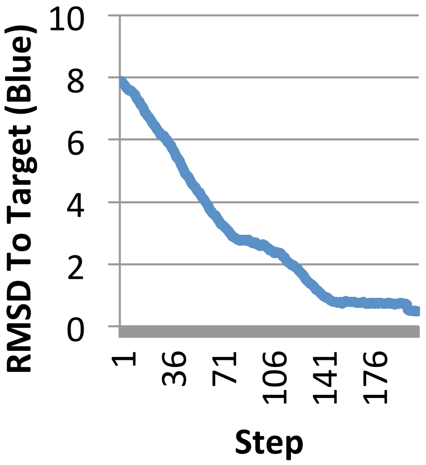
- How to do flexible fitting?
  - Assume atoms, or different subunits of the model (e.g. secondary structures), can move individually
  - Move each atom/secondary structure towards higher densities (in gradient direction)
  - Goals:
    - Increase fitting score (cross-correlation, atom inclusion, density occupancy, clashes, etc.)
    - Maintain a good structure – good bonds, good angles, good dihedrals, etc.

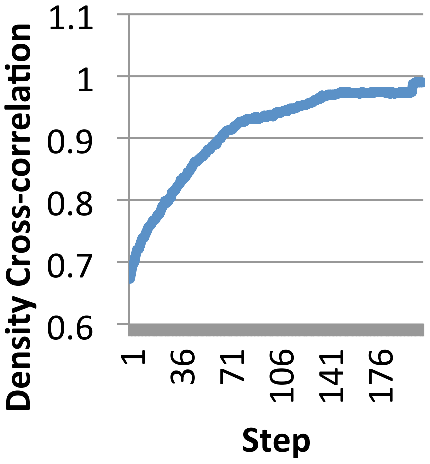
Flexible Fitting Methods

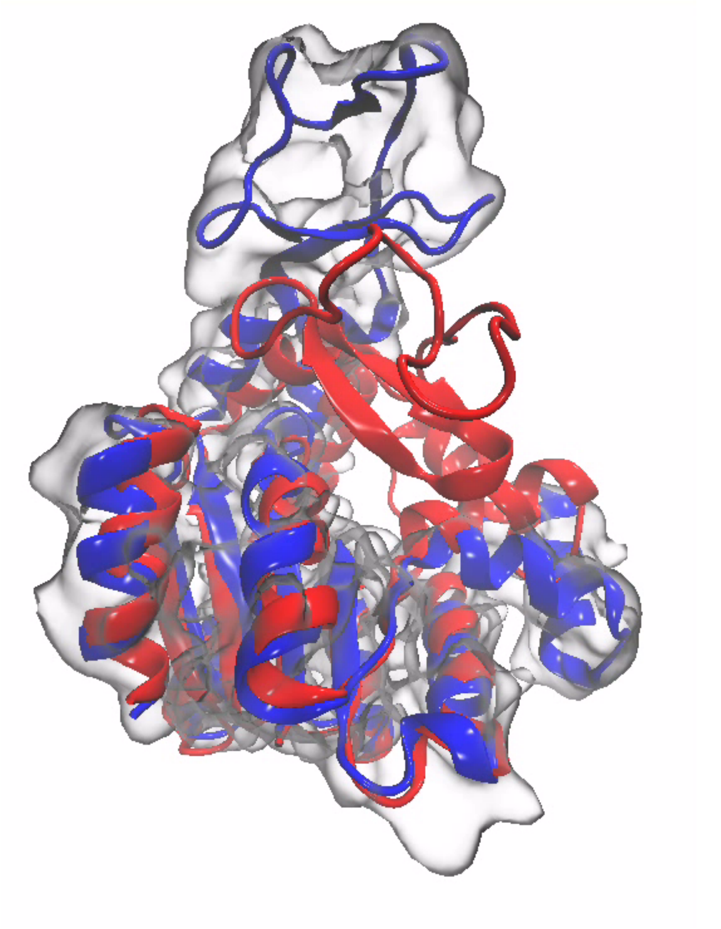
- MDFF (Molecular Dynamics Flexible Fitting)
  - Klaus Schulten lab
  - http://www.ks.uiuc.edu/Research/mdff/
- Direx
  - Gunnar Schröder lab
  - http://www.schroderlab.org/
- Flex-EM
  - Andrej Sali lab
  - http://salilab.org/Flex-EM/
- Rosetta
  - David Baker lab
  - http://www.rosettacommons.org/software/

Molecular Dynamics Flexible Fitting (MDFF)

- Apply force to each atom in the model to the high density of the map
- Apply full force field to keep good geometry (e.g. bonds, angles, dihedrals), and complementary charge interactions
- Use extra forces and bonds to maintain Secondary structures; H-bonds; Chirality; Cis-peptide bonds

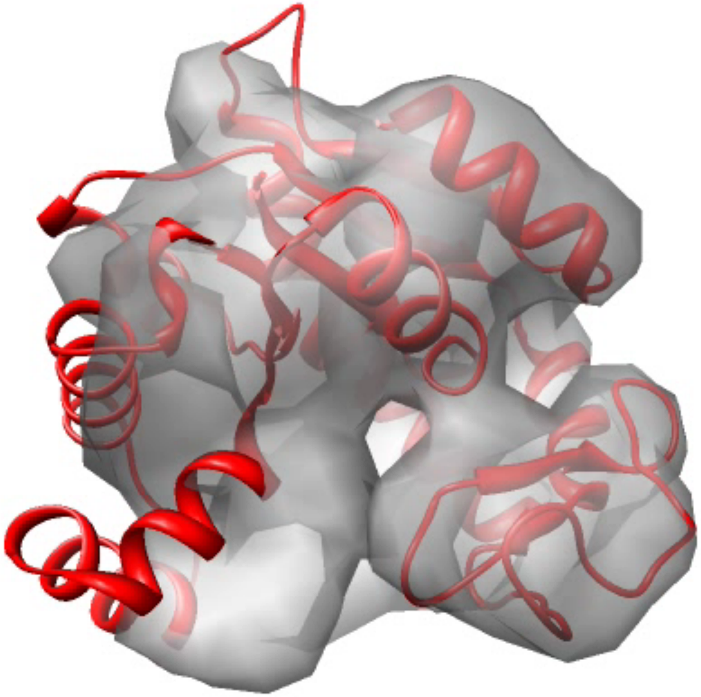


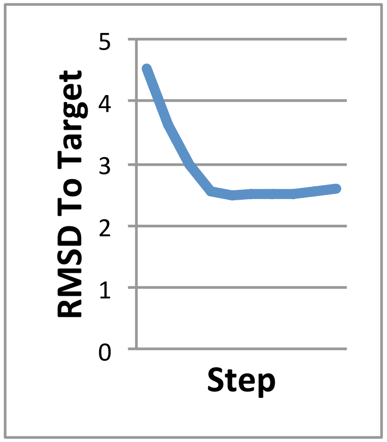


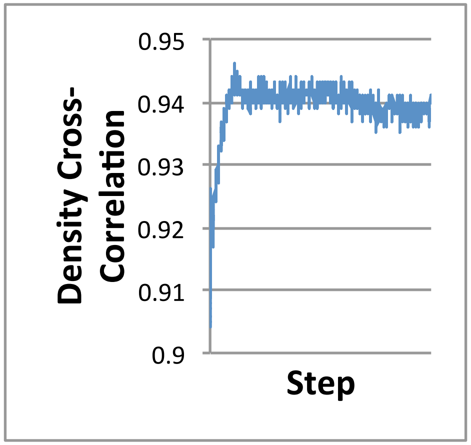


Flex-EM

- Also uses molecular dynamics, moving atoms to high density
- Secondary structures are rigid bodies
- Simulated annealing (temperature is cycled between low and high values)



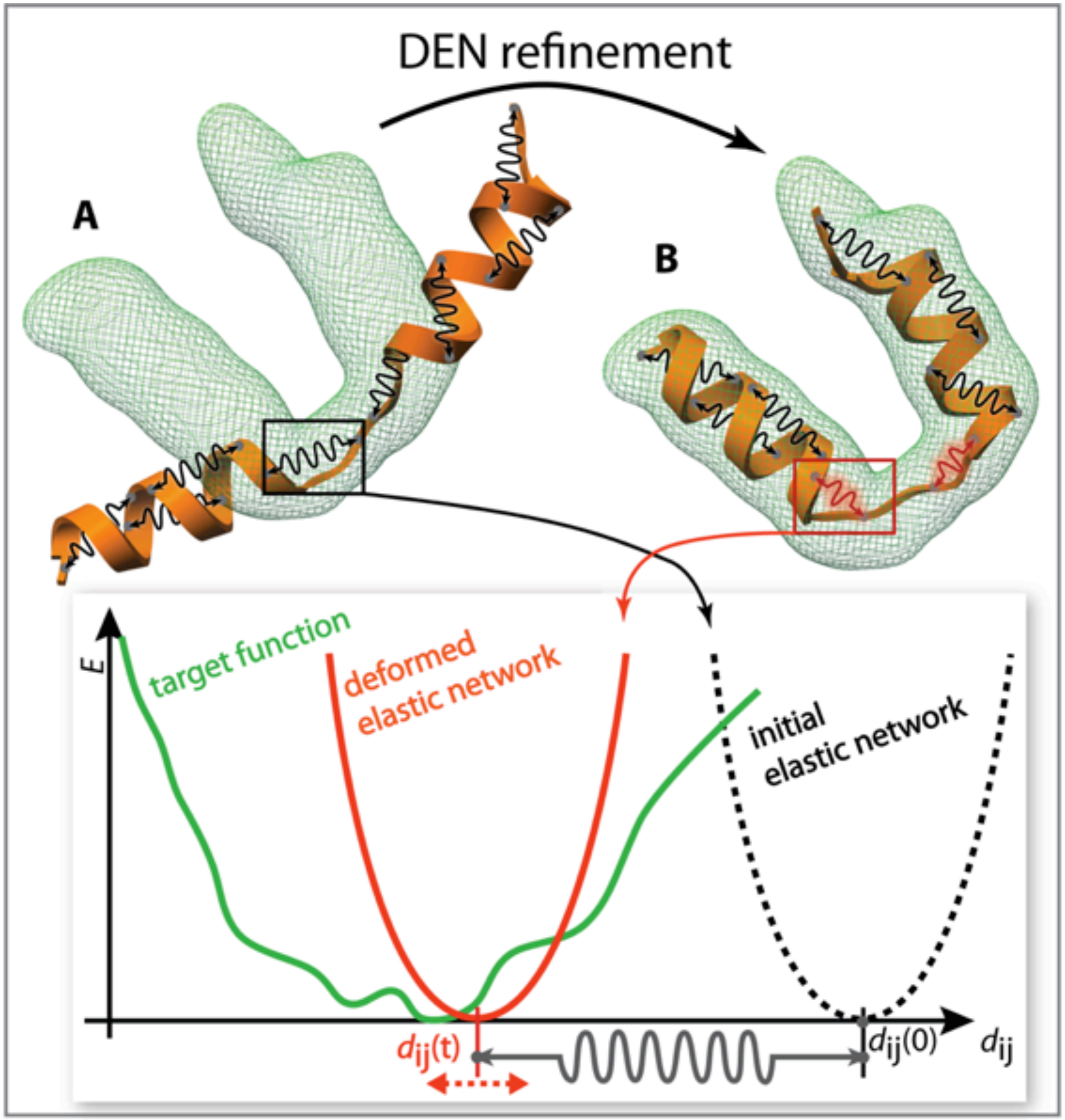




Deformable Elastic Network (DEN)

The network adapts itself to refine only those degree of freedom for which the experiment provides information.

. All-atom description
. Correct stereochemistry
. No atom clashes



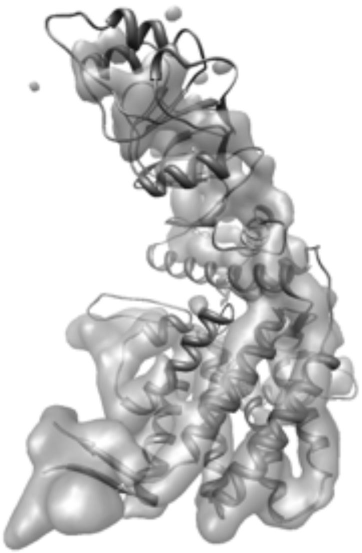
Schroeder et al (2007) *Structure*

Evaluation of Flexible Fitting Methods

- Does the model fit the density map better after flexible fitting?
  - Compute model-to-density scores
    - Density cross-correlation
    - Atom inclusion
    - Occupancy of high-density areas
    - Local density cross-correlation
- The model changes during flexible fitting; so we must also re-assess the quality of the model
  - Hence, we also calculate model-alone scores
    - Calculated using Molprobity
      - http://molprobity.biochem.duke.edu
    - Some of the scores it computes:
      - Clashes between atoms
      - Rotamer quality
      - Ramachandran outliers/favored
      - Bad bonds/angles

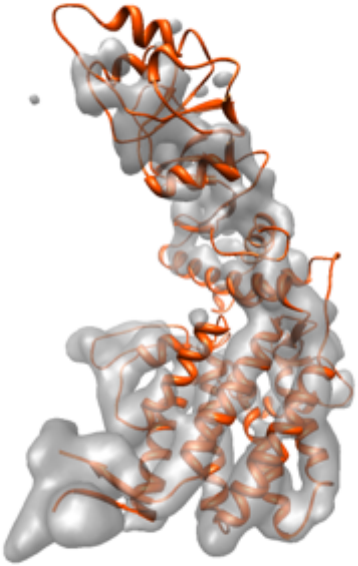
Flexible Fitting Test Case

Mm-Cpn @ 8Å (EMD-5140)

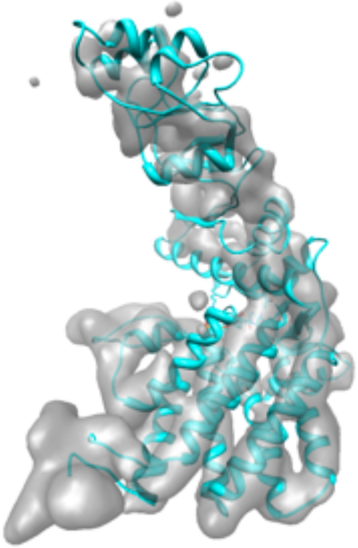


PDB:3KFE

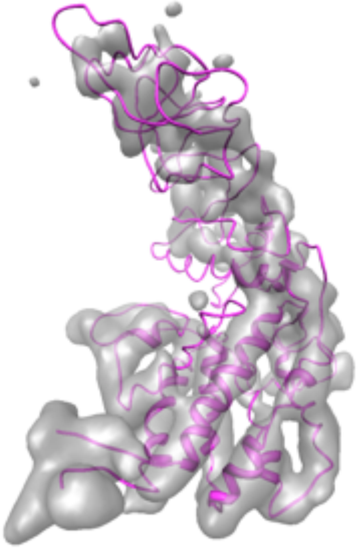
(crystal structure
closed state
rigid-body docked)



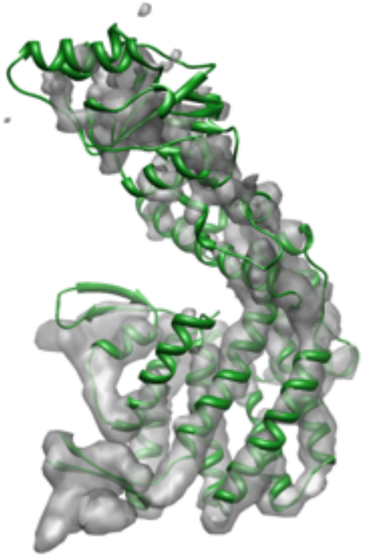
MDFF



Flex-EM



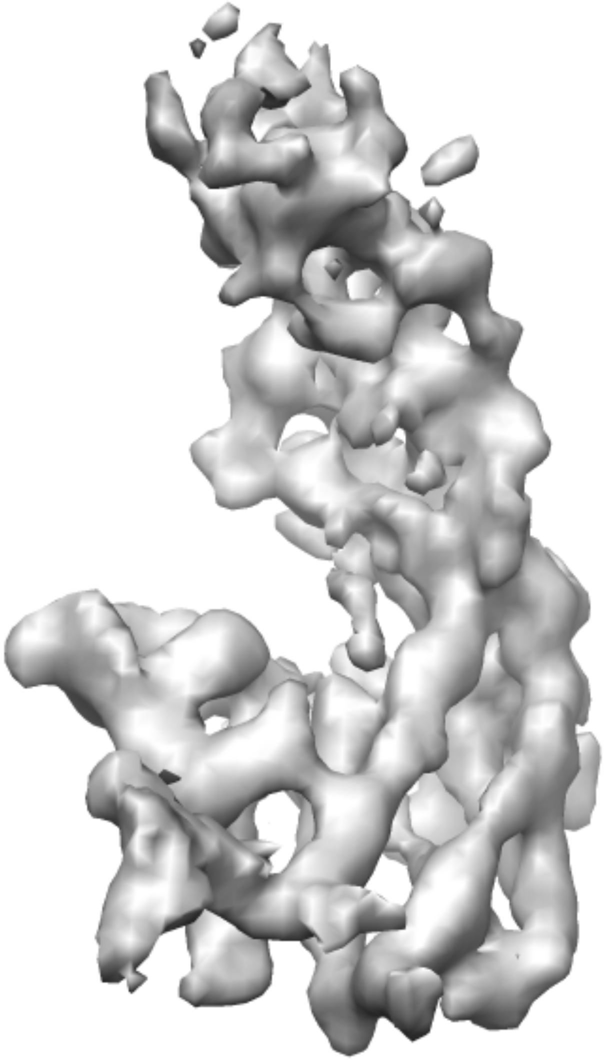
Direx

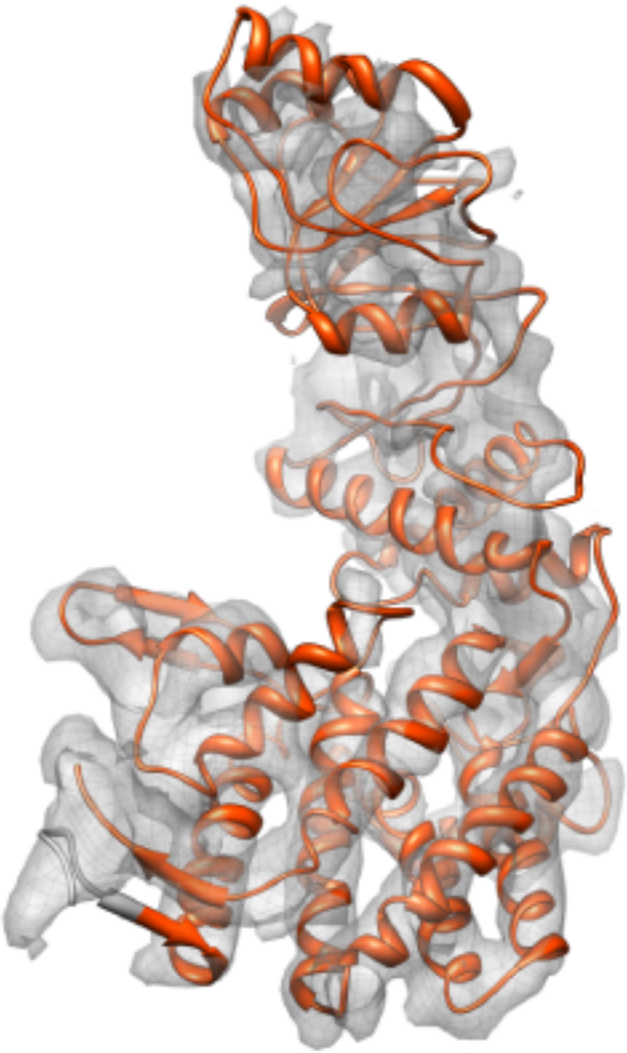


Rosetta

(from cryoEM
challenge)

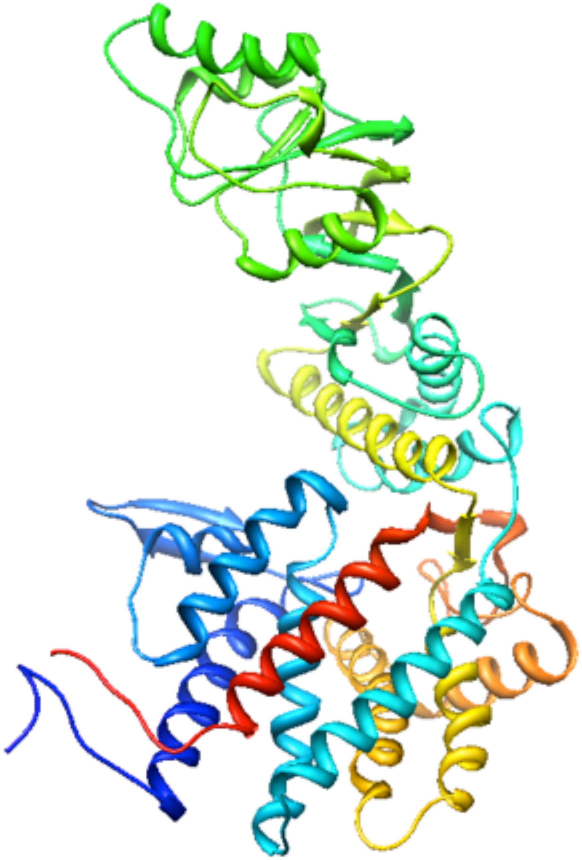
Modeling ∆lid Mm-Cpn Open State





Deformable Elastic Network (DEN)
Schroeder, Brunger and Levitt., *Structure*, 2007

Validate the 8 Å Resolution Cryo-EM Built Model for Δlid Mm-Cpn in the Apo State with 2 Modeling Tools



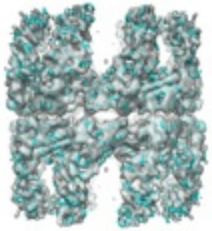
Rosetta-built Model

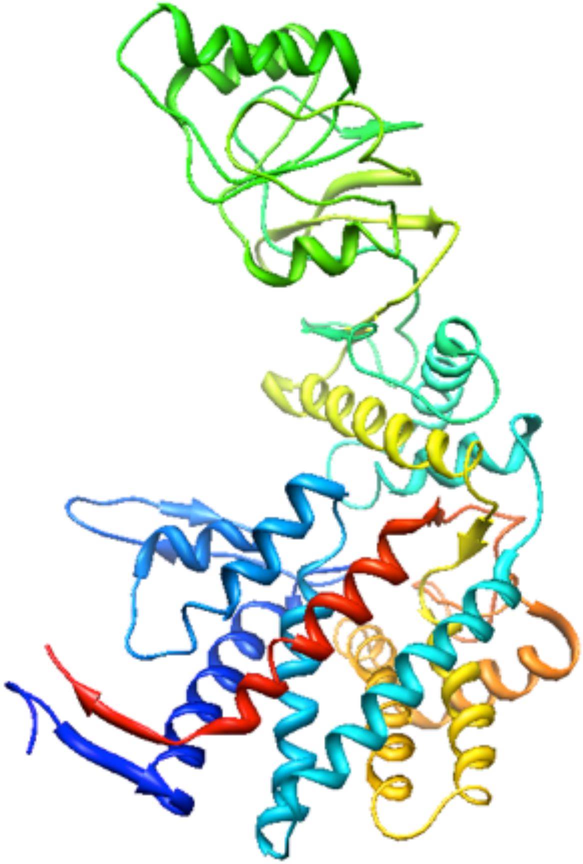
Overal Cα RMSD: 2.8Å

API Cα RMSD: 4.3Å

INT Cα RMSD: 2.3Å

EQU Cα RMSD:1.7Å



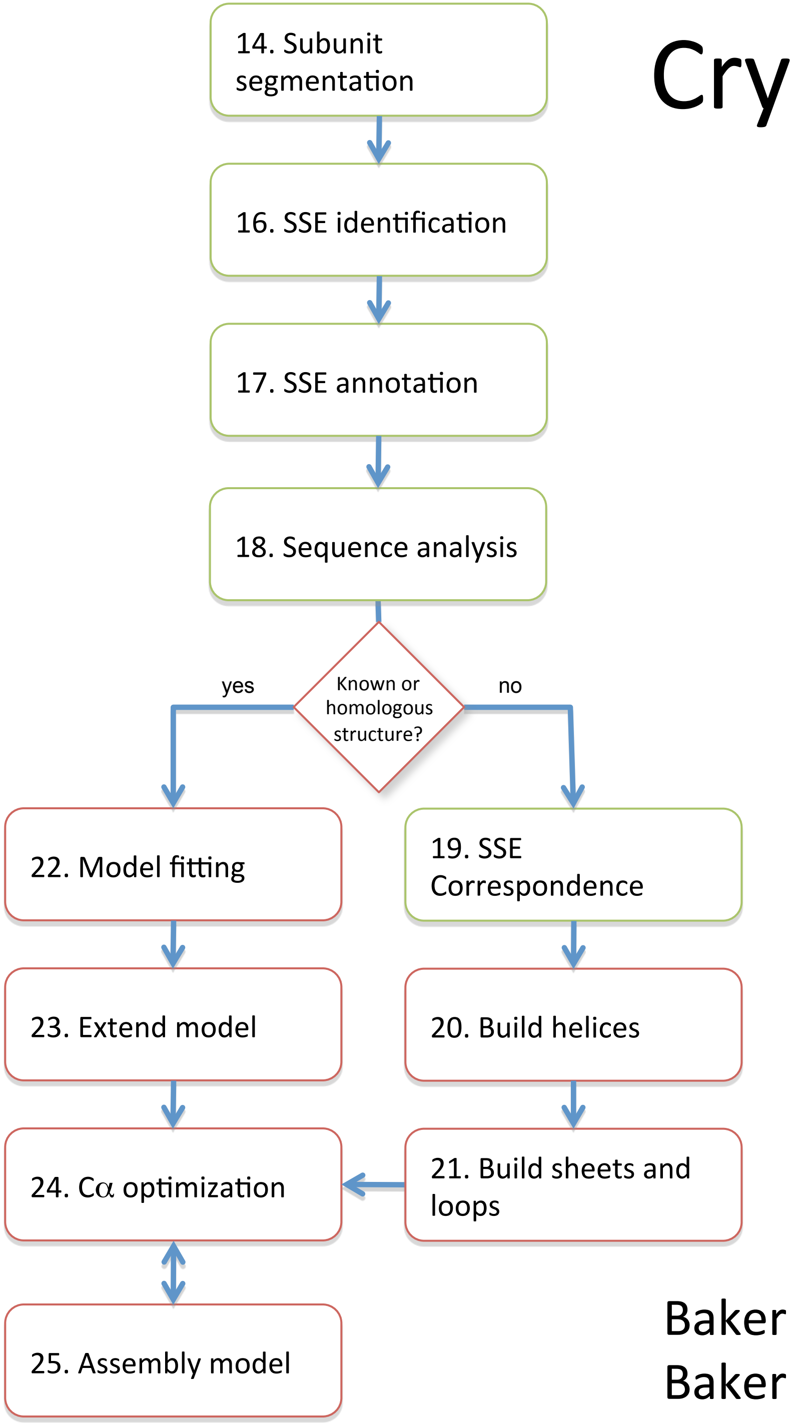


Direx-built Model

Data Statistics for Mm-Cpn + ATP/AlFx

- 110,000x effective camera magnification
- 637 CCD frames (Gatan 4k CCD)
- ~29,926 particle images used for final 3-D reconstruction
- Density map determined to 4.3 Å resolution based on the 0.5 criterion of Fourier Shell Correlation

Cryo-EM Structure Analysis



14. Subunit segmentation

16. SSE identification

17. SSE annotation

18. Sequence analysis

Known or homologous structure?

yes

no

22. Model fitting

23. Extend model

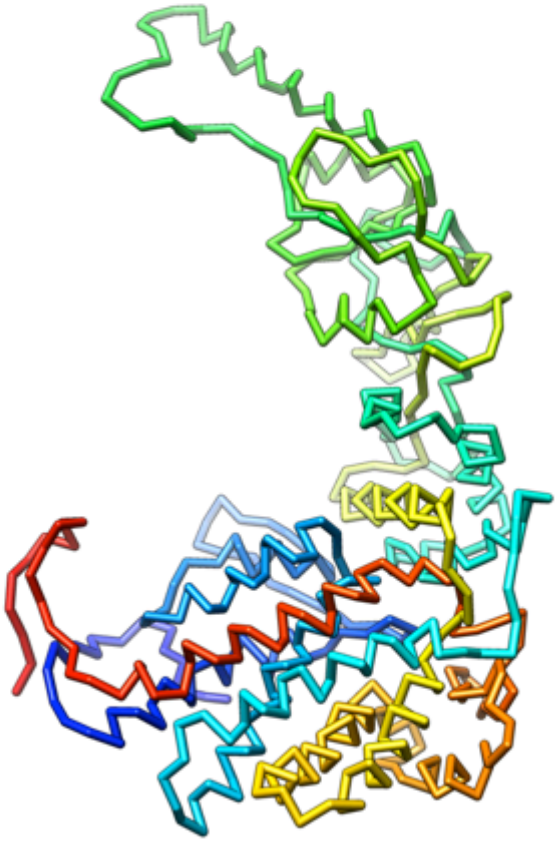
24. Cα optimization

25. Assembly model

19. SSE Correspondence

20. Build helices

21. Build sheets and loops



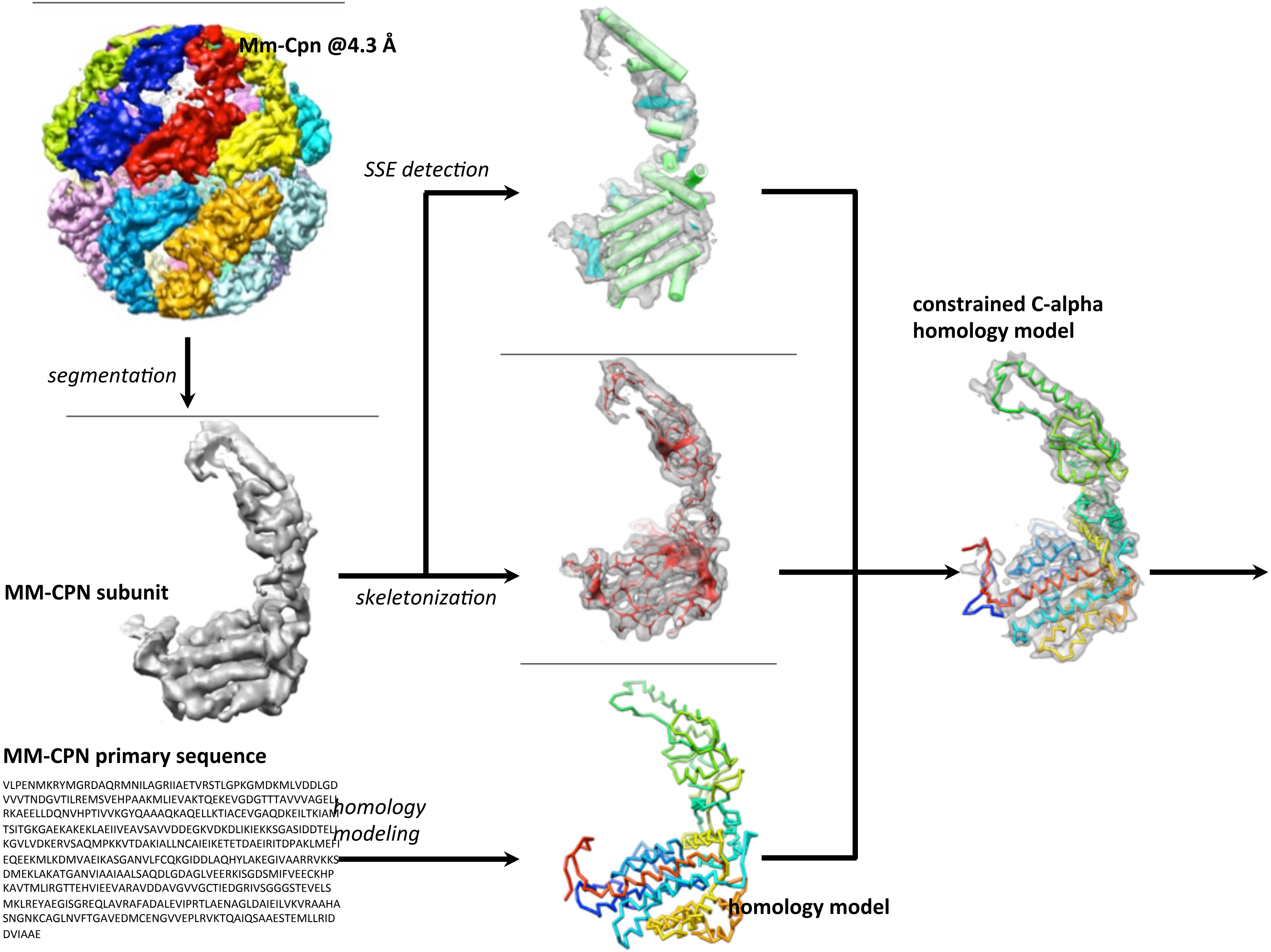
Baker et al. (2010) *Nature Protocols* **5**: 1697-1708

Baker et al. (2010) *Methods in Enzymology* **483**: 1-29

Mm-cpn
at 4.3 Å

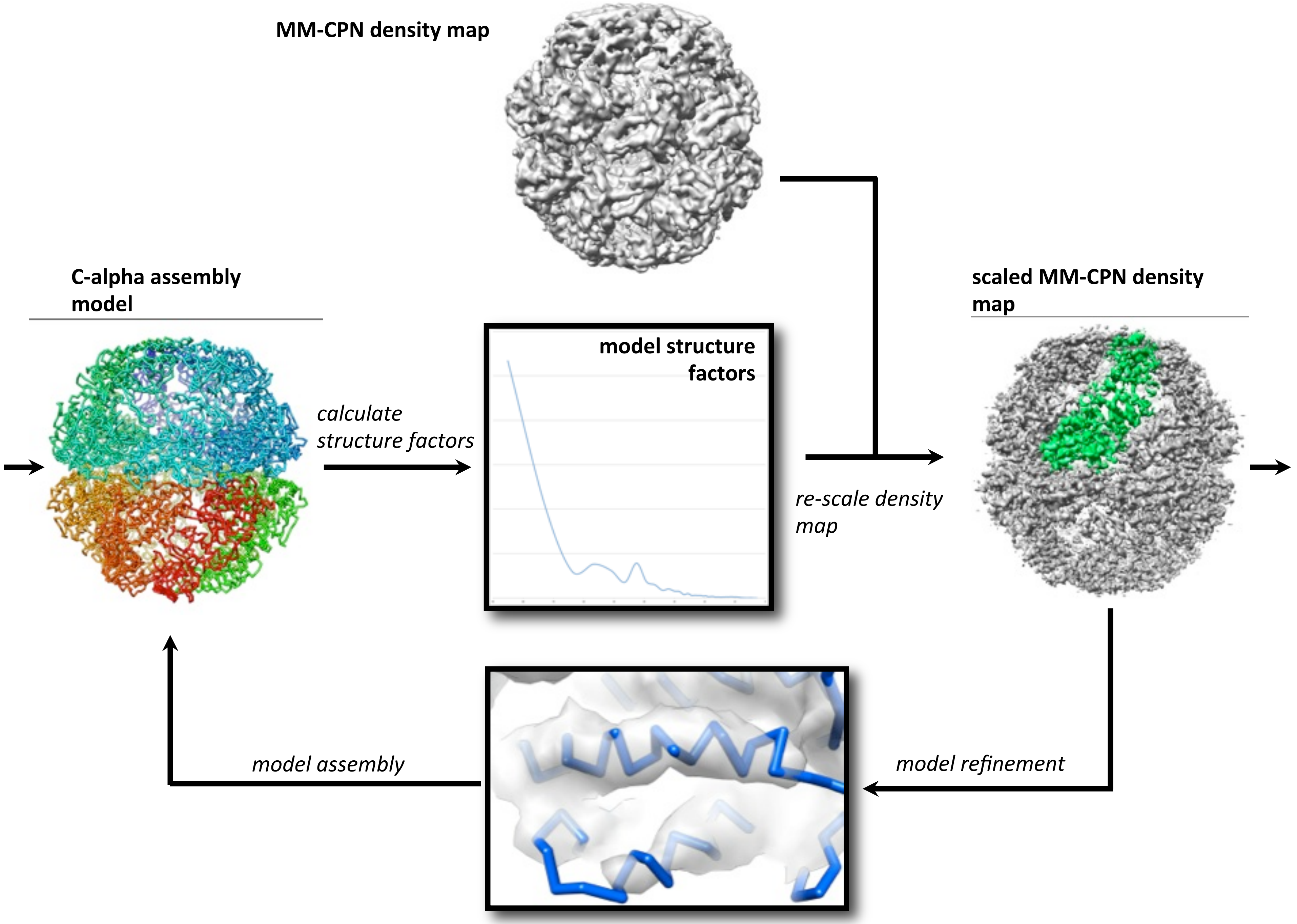
closed state

NCMI/A27D4

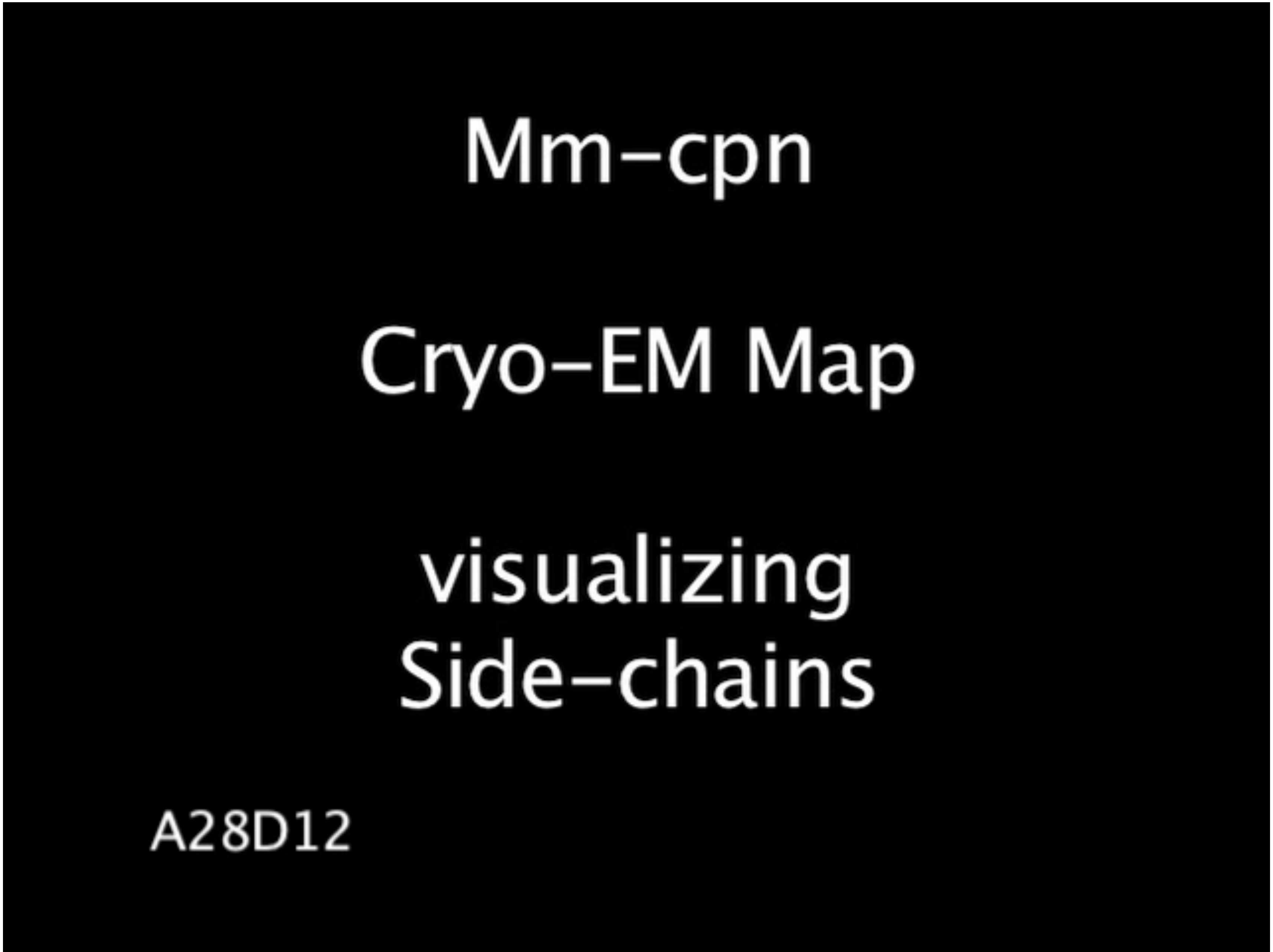


MM-CPN primary sequence

VLPENMKRYMGRDAQRMNILAGRIIAETVRSTLGPKGMDKMLVDDLGD
VVVTNDGVTILREMSVEHPAAKMLIEVAKTQEKEVGDGTTTAVVVAGELL
RKAEELLDQNVHPTIVVKGYQAAAQKAQELLKTIACEVGAQDKEILTKIAM
TSITGKGAEKAKEKLAEIIVEAVSAVVDDEGKVDKDLIKIEKKSGASIDDTELL
KGVLVDKERVSAQMPKKVTDAKIALLNCAIEIKETETDAEIRITDPAKLMEFI
EQEEKMLKDMVAEIKASGANVLFCQKGIDDLAQHYLAKEGIVAARRVKKS
DMEKLAKATGANVIAAIAALSAQDLGDAGLVEERKISGDSMIFVEECKHP
KAVTMLIRGTTEHVIEEVARAVDDAVGVVGCTIEDGRIVSGGGSTEVELS
MKLREYAEGISGREQLAVRAFADALEVIPRTLAENAGLDAIEILVKVRAAHA
SNGNKCAGLNVFTGAVEDMCENGVVEPLRVKTQAIQSAAESTEMLLRID
DVIAAE


MM-CPN density map
C-alpha assembly model
calculate structure factors
model structure factors
re-scale density map
scaled MM-CPN density map
model refinement
model assembly

Visualizing Side-Chains of Mm-cpn

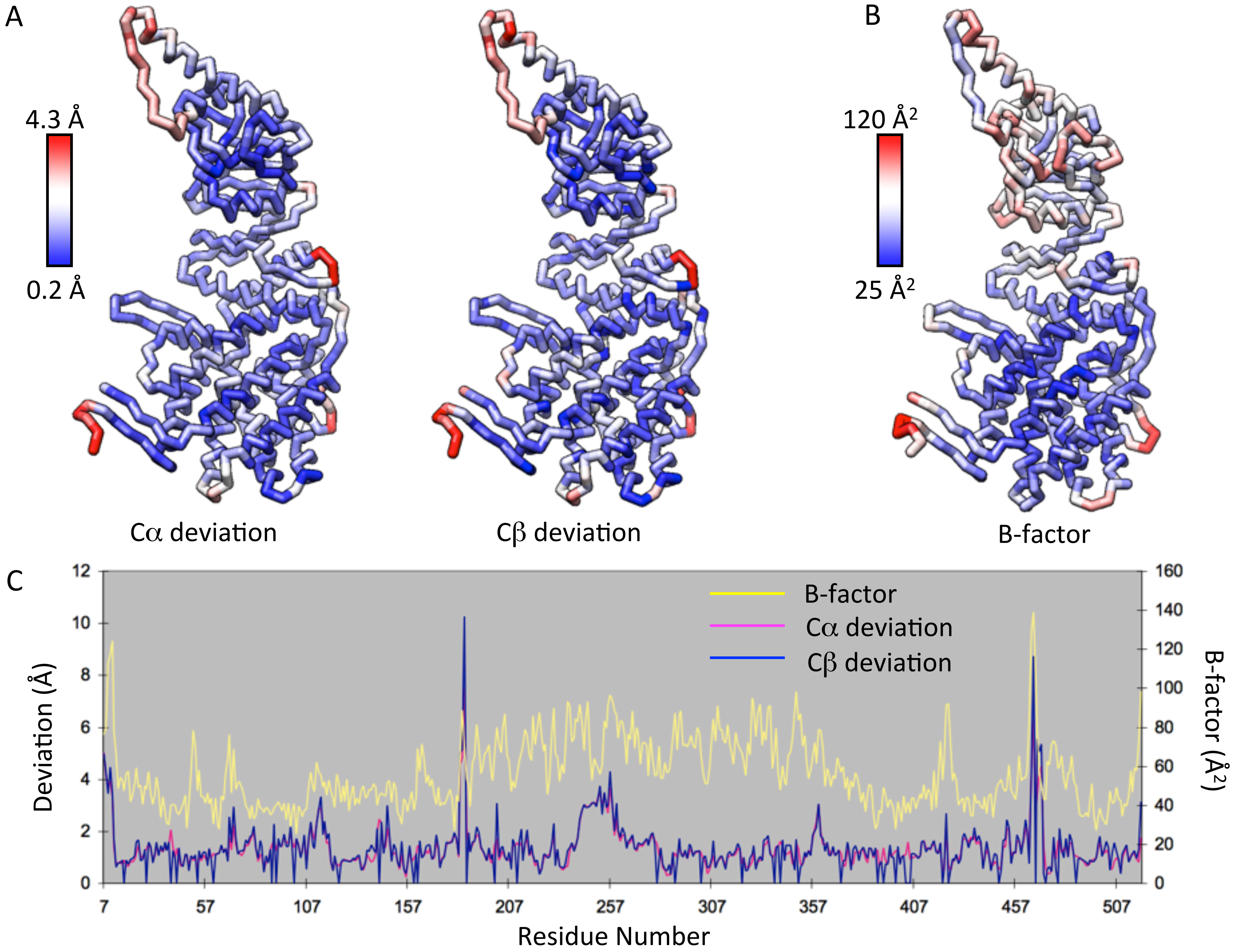


Mm–cpn

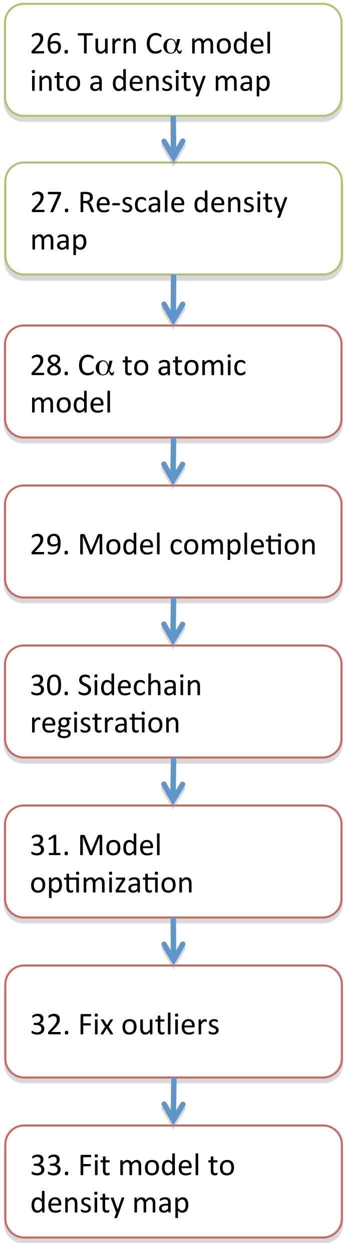
Cryo–EM Map

visualizing
Side–chains

A28D12


A
4.3 Å
0.2 Å
Cα deviation
Cβ deviation
B
120 Å^2
25 Å^2
B-factor
C
B-factor
Cα deviation
Cβ deviation
Deviation (Å)
0
2
4
6
8
10
12
B-factor (Å^2)
0
20
40
60
80
100
120
140
160
7
57
107
157
207
257
307
357
407
457
507
Residue Number

Cryo-EM Structure Analysis



26. Turn Cα model into a density map

27. Re-scale density map

28. Cα to atomic model

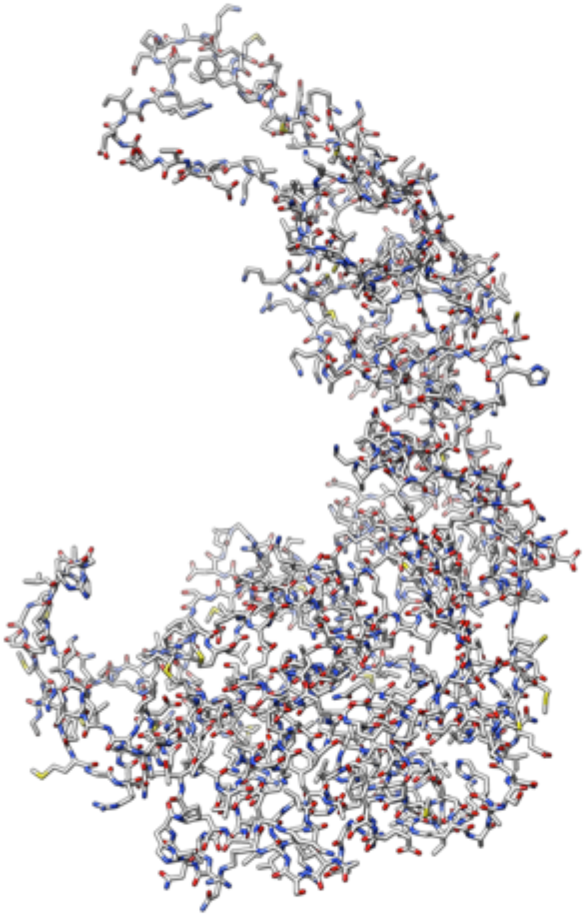
29. Model completion

30. Sidechain registration

31. Model optimization

32. Fix outliers

33. Fit model to density map

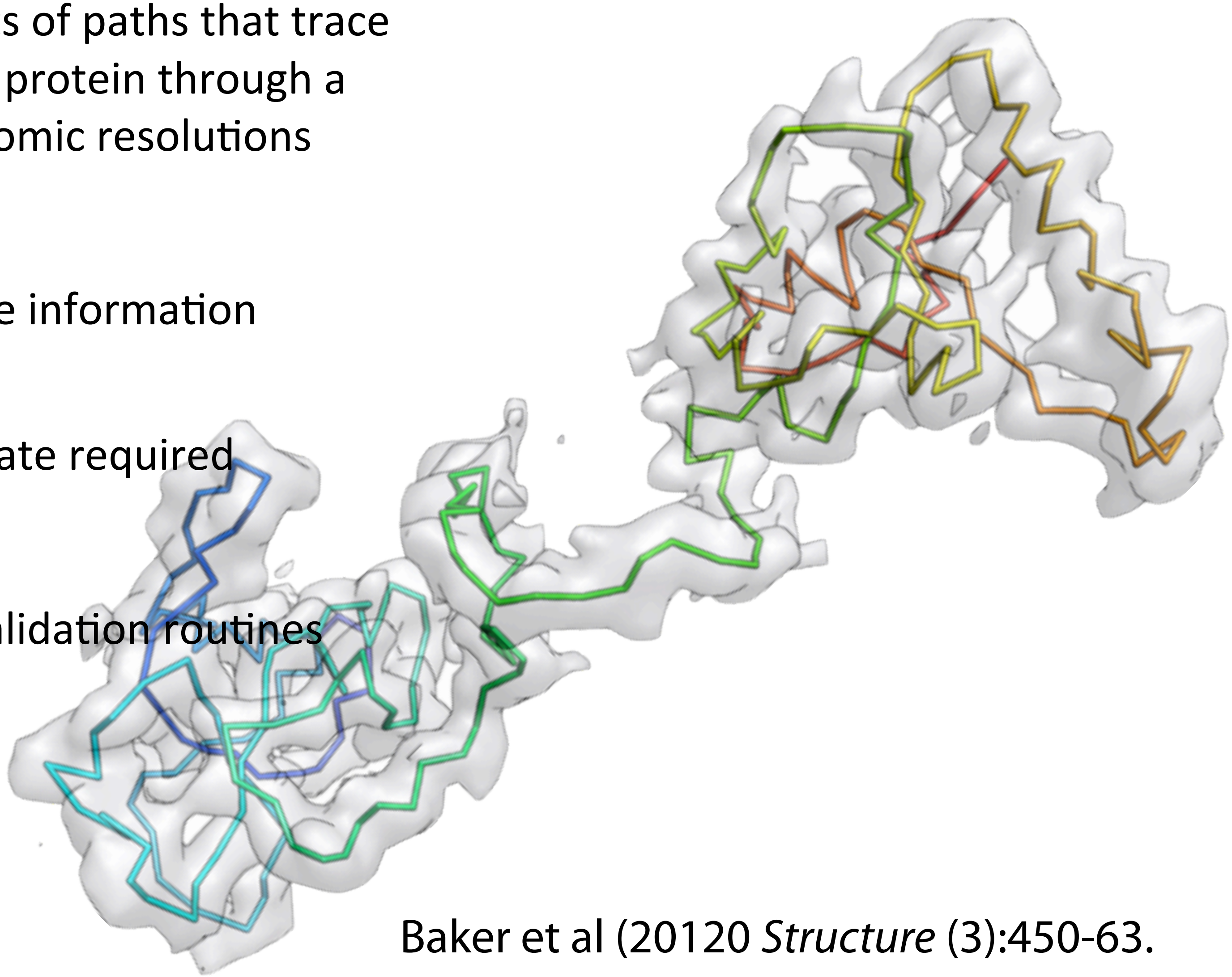


Baker et al. (2010) *Nature Protocols* **5**: 1697-1708
Baker et al. (2010) *Methods in Enzymology* **483**: 1-29

Automatic de Novo Modeling: Pathwalking

Goal: Find a path or sets of paths that trace the complete path of a protein through a density map at near-atomic resolutions

- No SSEs required
- No explicit sequence information required
- No structural template required
- Automated
- Optimization and validation routines



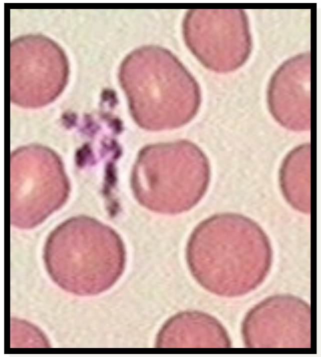
Baker et al (20120 *Structure* (3):450-63.

Flexible Fitting – Summary

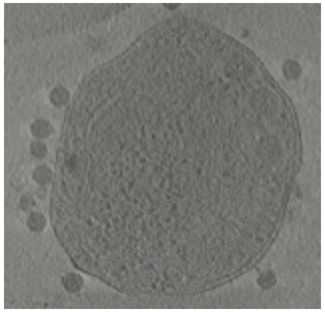
- MDFF
  - Con: Slower (since it moves each atom individually, using a full-force field); however it can be run on multiple processors
  - Pro: Good model-alone scores
- Flex-EM
  - Con: Secondary structures are kept rigid, so they are less able to deform to fit the density
  - Pro: Good model-alone score
- Direx
  - Pro: Fast, since full-force field is not used. Increases model-density scores the most.
  - Con: Poor model-alone scores, particularly in lower-resolution maps
- Rosetta
  - Pro: uses information from known crystal structures (fragment library) or initial de novo bulit; hence it gives good model-alone scores
  - Con: takes longer to change structure significantly, since only small parts are modified at one time

Structural Biology from Man to Atoms

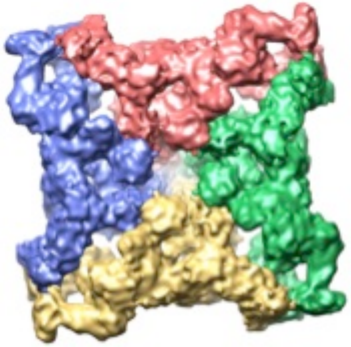
Optical microscopy

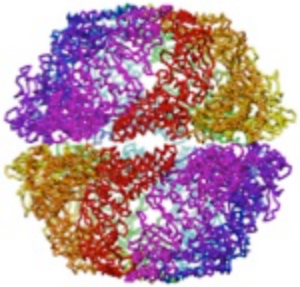


Electron cryotomography

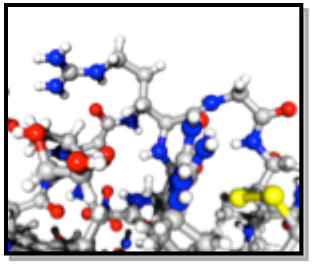


Electron cryomicroscopy





Crystallography



cm

200 nm

50nm

8nm

5nm

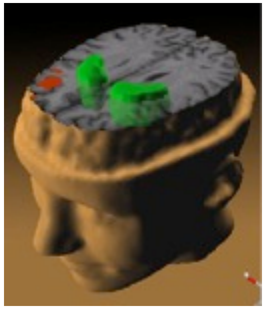
1nm

0.6nm

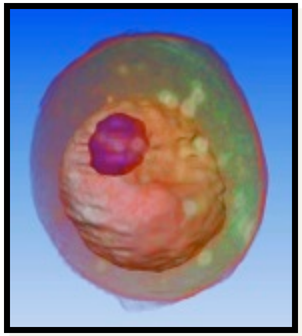
0.4nm

0.3nm

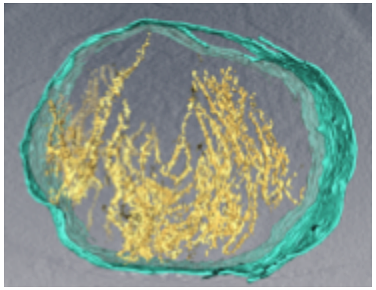
0.1nm



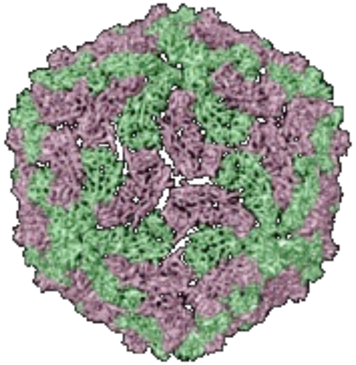
MRI



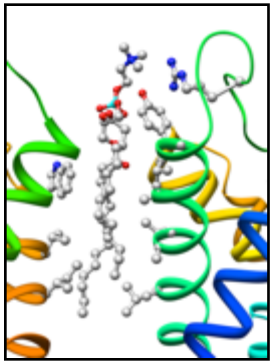
X-ray microscopy



Electron cryotomography



Electron cryomicroscopy



Electron & X-ray Crystallography NMR