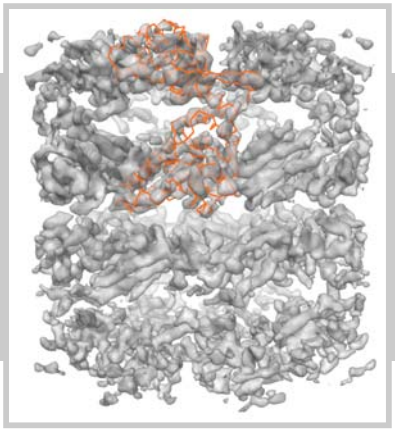
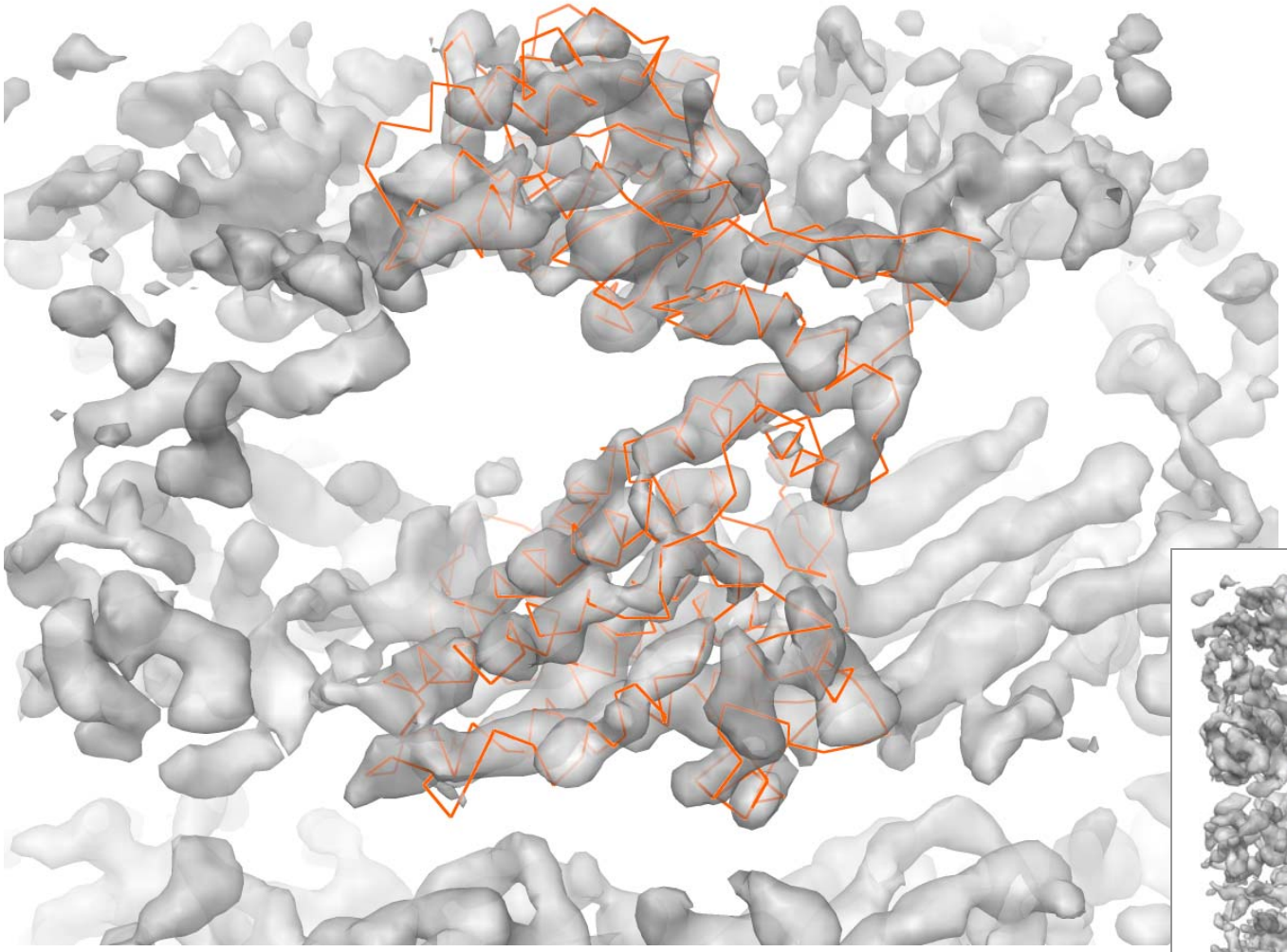
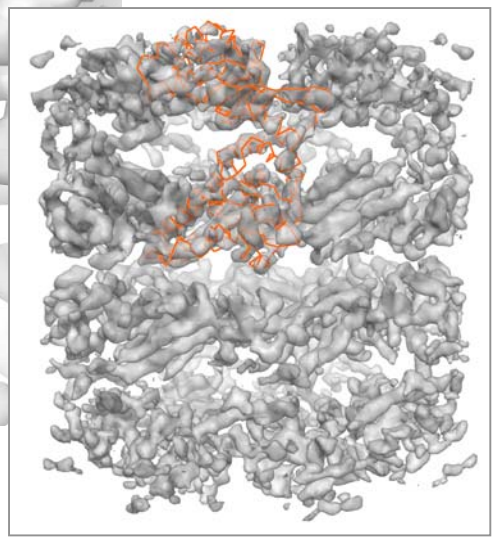


Modeling Structures into Density Using Rosetta

Frank DiMaio
Baker Lab
RosettaCon 2009
dimaio@u.washington.edu

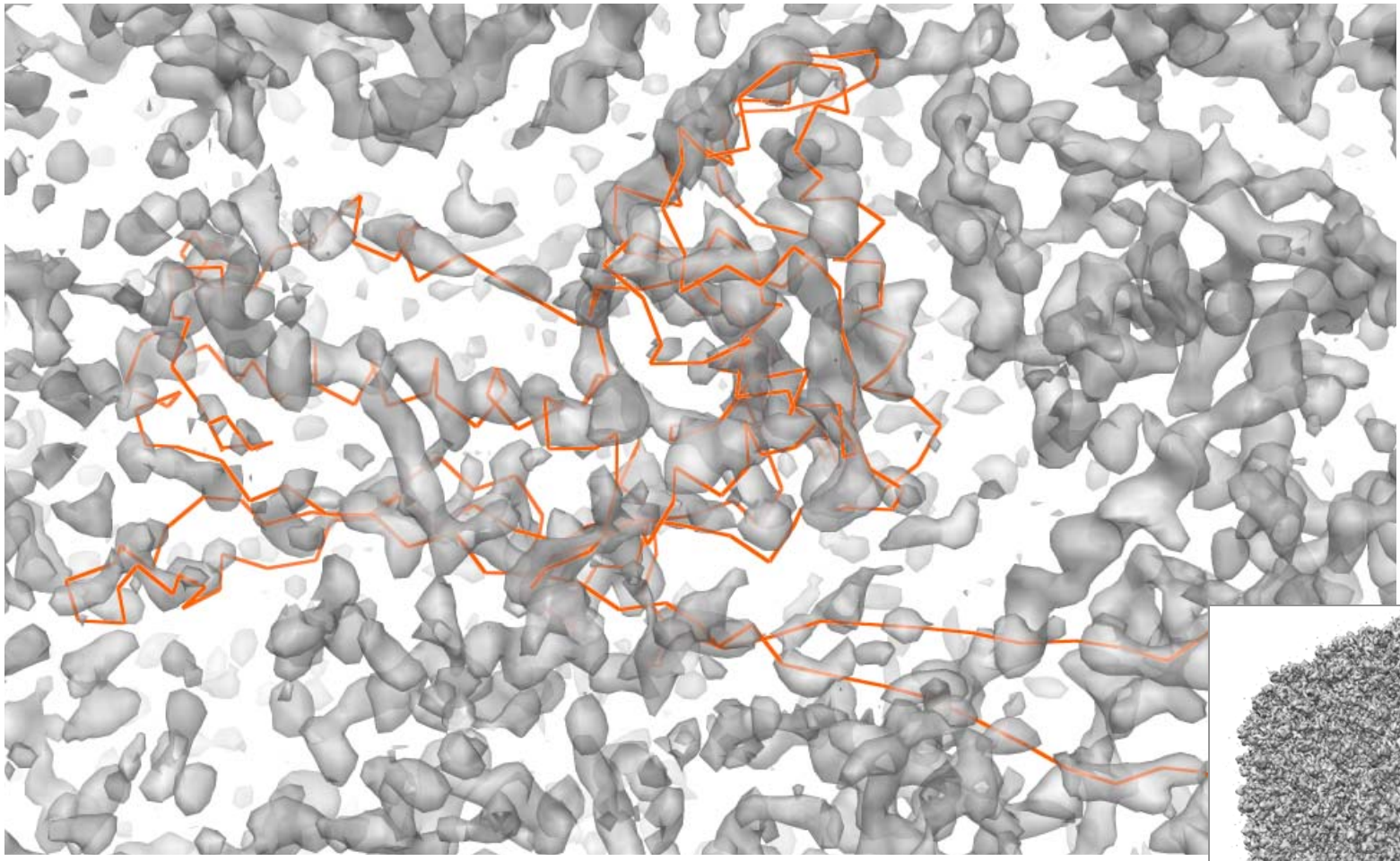
Electron Cryomicroscopy

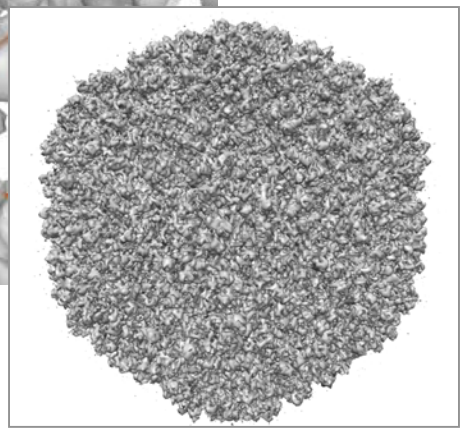




4.7Å GroEL

Electron Cryomicroscopy





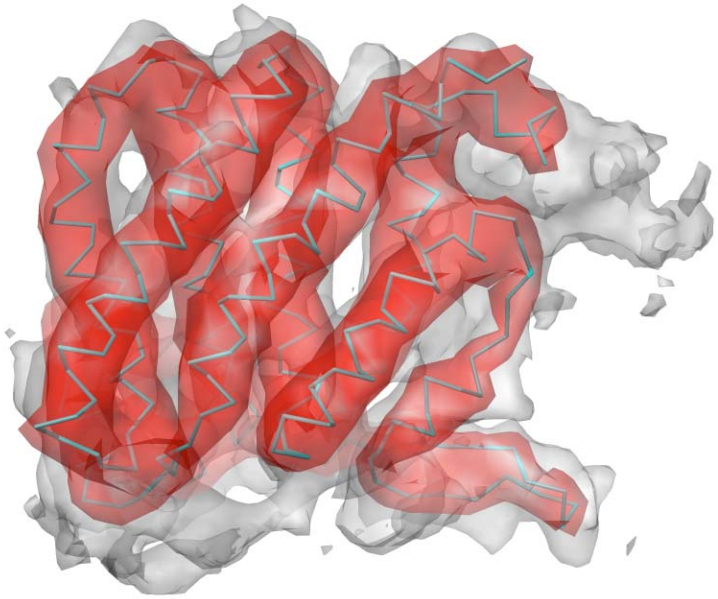
4.5Å Bacteriophage Epsilon 15

Outline

- Adding a fit-to-density energy function to Rosetta
- Comparative modeling into density
- Refining a low-resolution Cα-only model into density
- Density in Rosetta: A (very) brief user's guide
- Refining symmetric complexes into density

Scoring Structures With Density

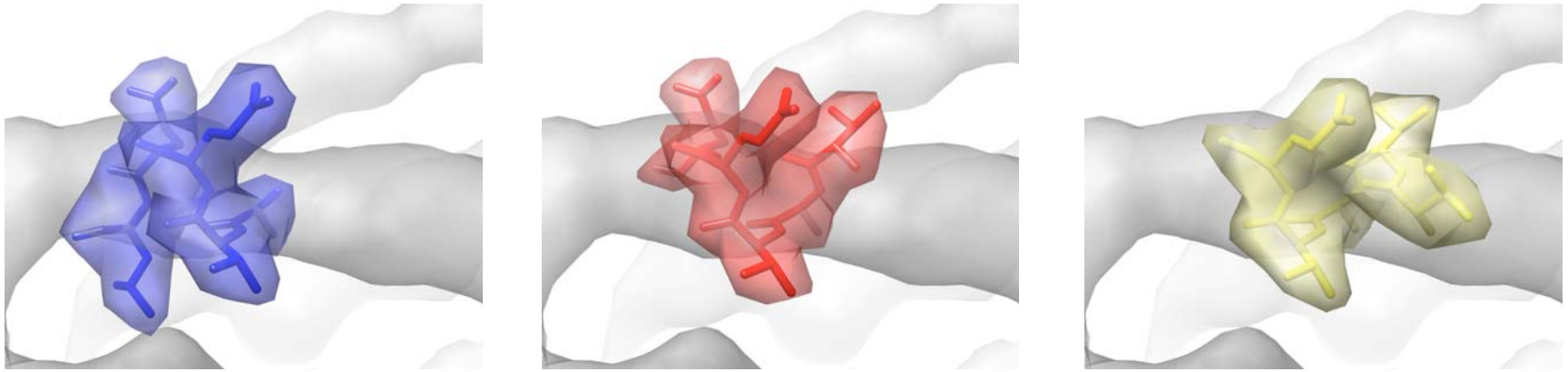
- Use correlation coefficient between expected and observed density
- **Low-resolution score** places a single Gaussian at each Cα/heavyatom and computes masked correlation



- Very quick to compute
- Density not used in sidechain repacking

Scoring Structures With Density

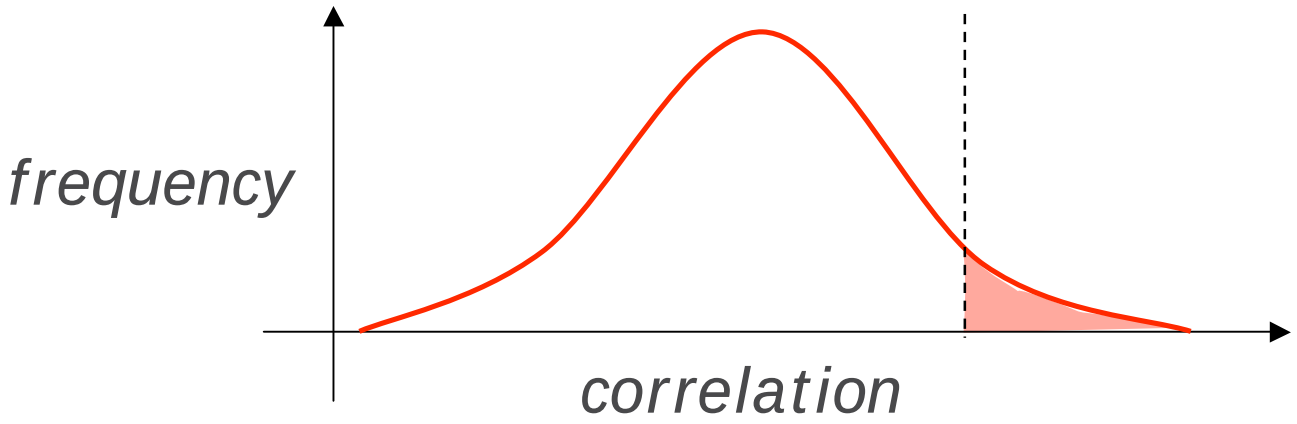
- Use correlation coefficient between expected and observed density
- **High-resolution score** places a Gaussian at every heavy atom; scores computed in a sliding window



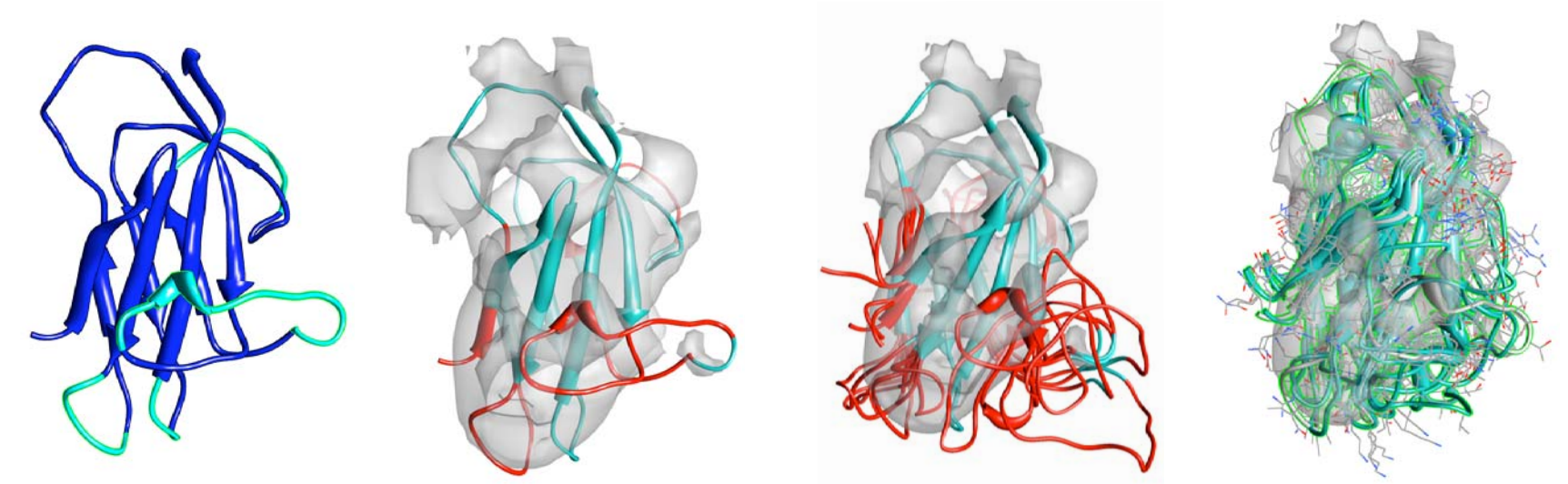
Scoring Structures With Density

- Given a correlation *CC* between map and model, *fit-to-density energy* based on probability of *seeing that correlation by random chance*

$$score_{density} = \log\left(0.5 \cdot \left(1 - \mathrm{erf}\left(Z_{CC}\right)\right)\right)$$



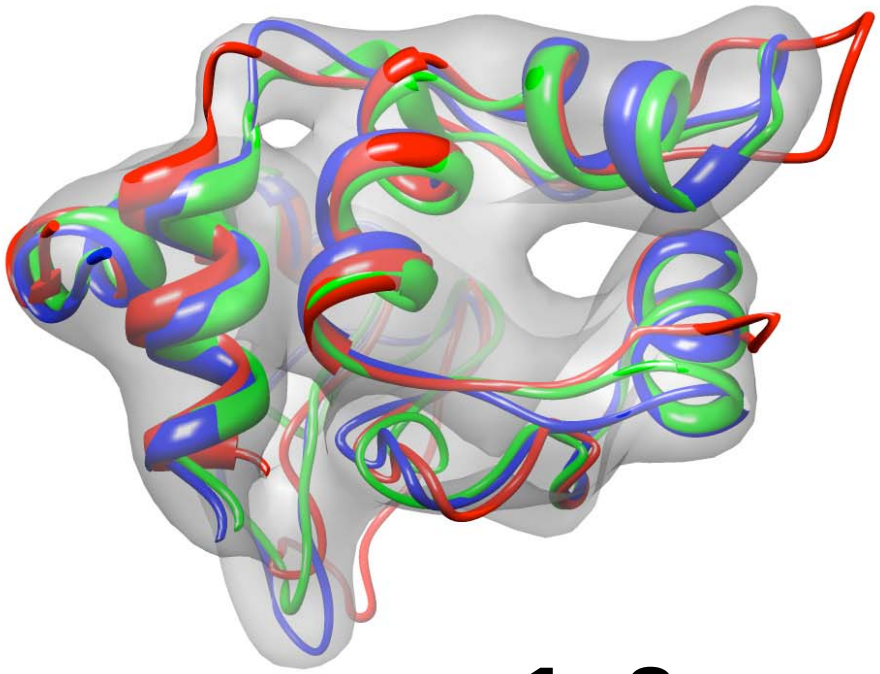
Comparative Modeling using Electron Density



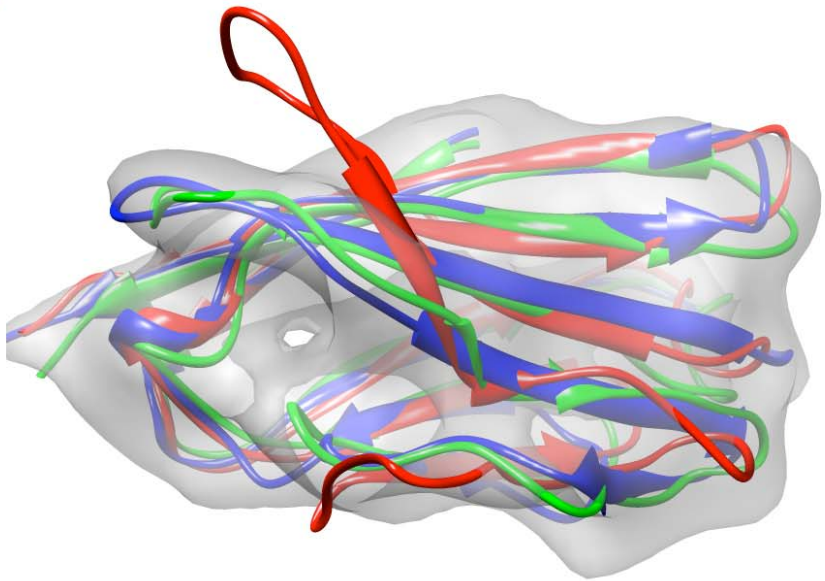
Results on synthetic maps

	nres	lowest-RMS starting model	lowest-energy refined 5A structures	lowest RMS of 10 lowest-energy (5A)	lowest-energy refined 10A structures	lowest RMS of 10 lowest-energy (10A)
1bbh	127	2.48 / 3.41	1.76 / 2.47	1.60 / 2.31	2.31 / 2.98	1.78 / 2.57
1c2r	115	3.45 / 4.15	0.54 / 1.12	-	1.61 / 2.43	1.37 / 2.40
1cid	109	3.34 / 4.33	1.82 / 2.99	1.66 / 2.79	1.97 / 3.24	1.88 / 3.30
1dxt	143	2.02 / 2.78	0.50 / 1.14	-	1.12 / 1.88	-
1lga	279	3.16 / 3.77	2.27 / 2.83	-	2.40 / 3.07	2.24 / 2.91
1mup	152	3.49 / 4.47	2.19 / 3.25	1.35 / 2.68	2.67 / 3.77	1.99 / 3.23
1onc	101	2.23 / 2.97	0.81 / 1.92	0.53 / 1.47	1.31 / 2.09	1.09 / 1.91
2cmd	310	2.50 / 3.42	1.80 / 2.63	1.43 / 2.31	2.21 / 3.36	2.02 / 3.09

Results on synthetic maps



1c2r



1cid

RDV upper domain at 6.8Å cryoEM data

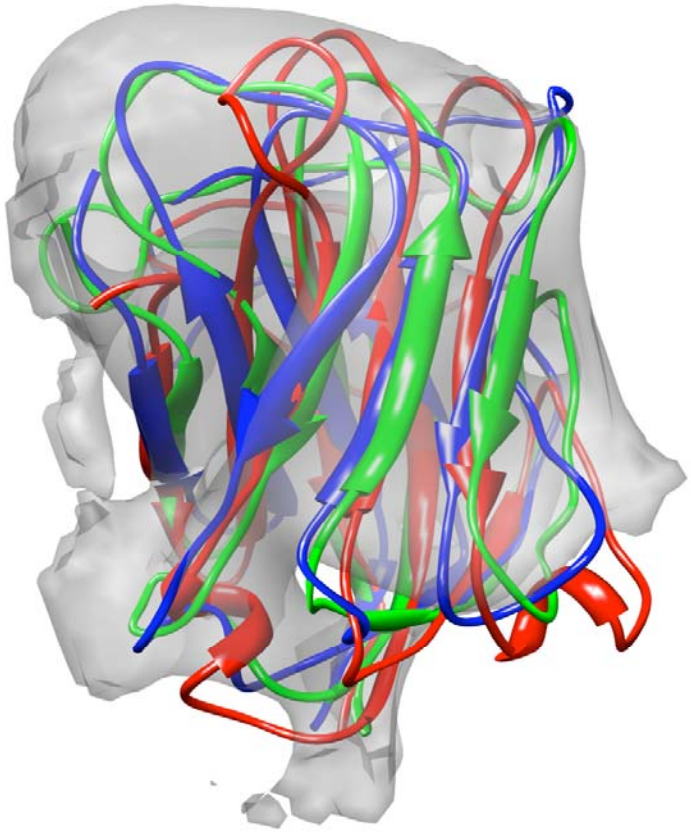
Homology Template

- 5.6Å RMS

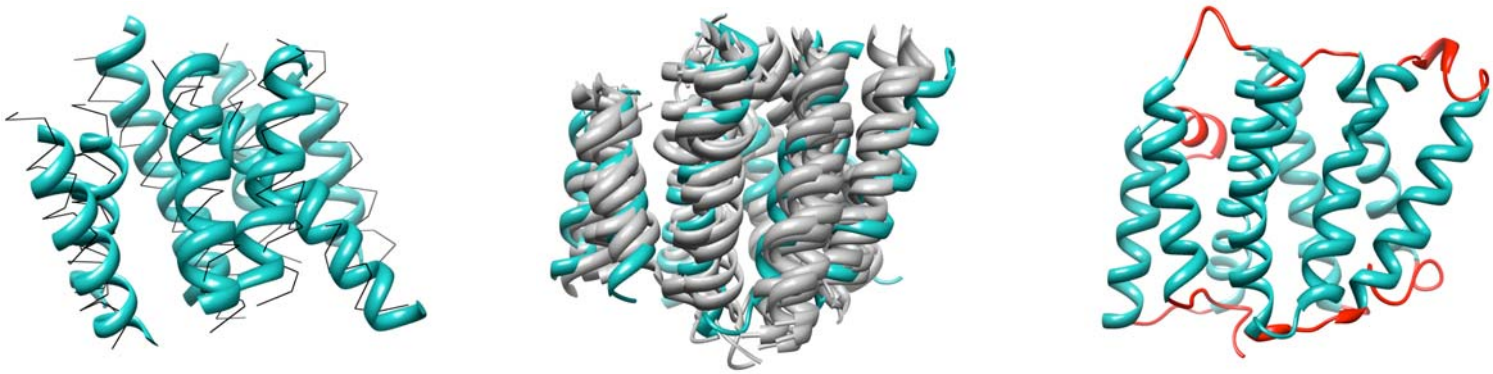
Rosetta prediction

- 3.7Å RMS

Native structure



Refining a Cα only model



Insert random fragments using Cα constraints

Rigid-body perturb secondary structure elements

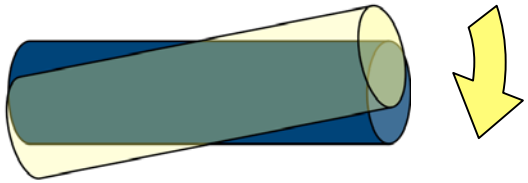
Rebuild loops using CCD loop closure

Refinement into density

Rigid-body moves

Helix twisting

Helix sliding

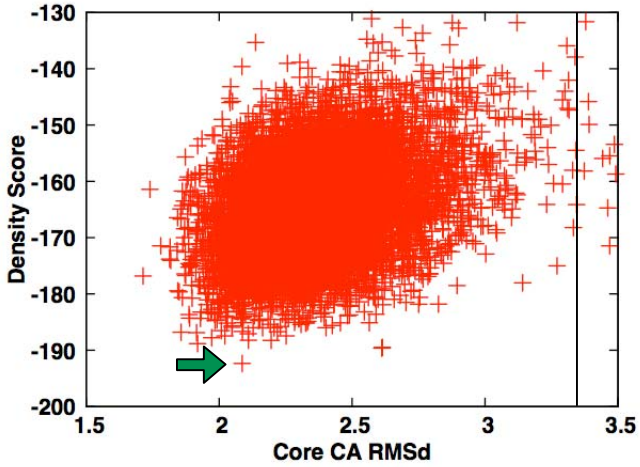


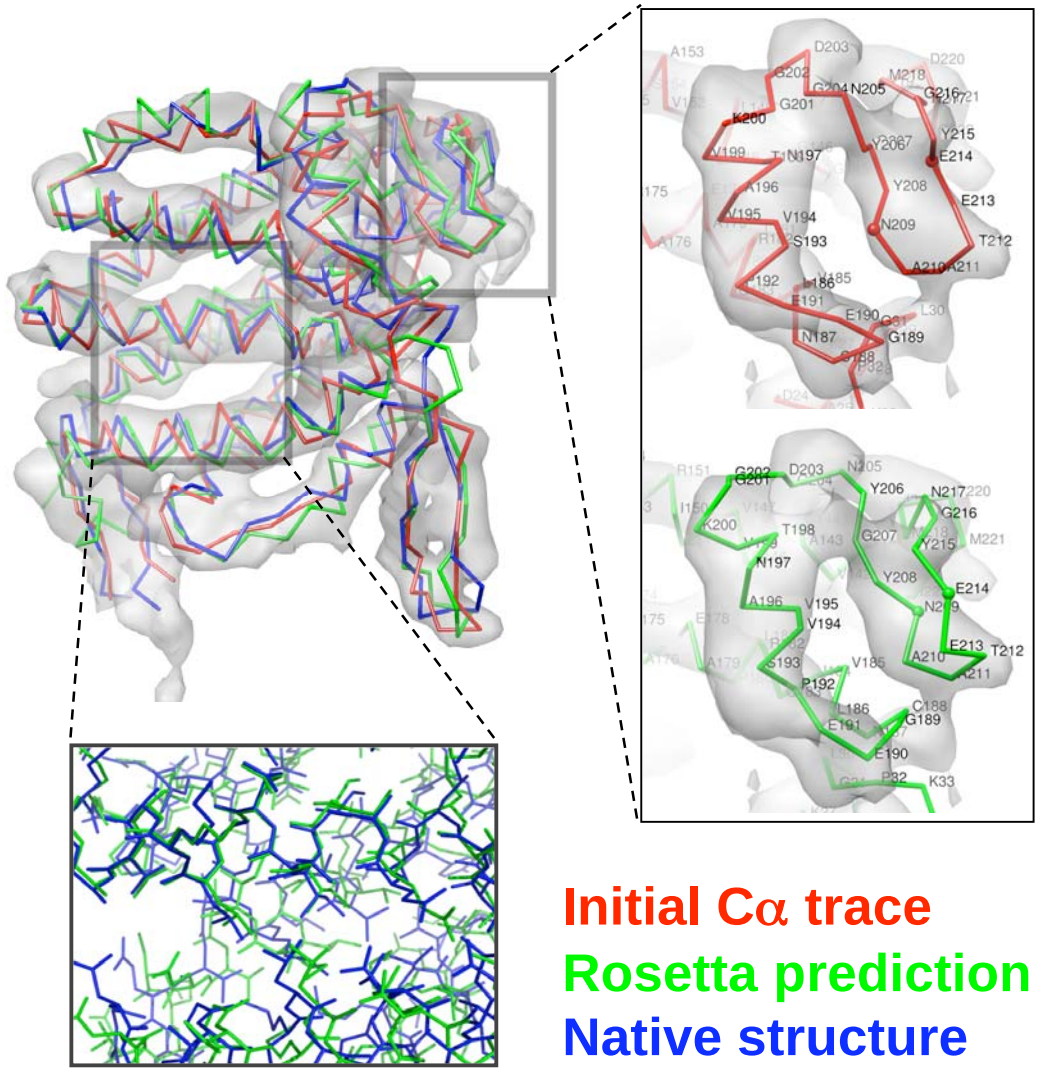
Small off-axis helical moves

Sequence shifting

Small backbone perturbations in strands

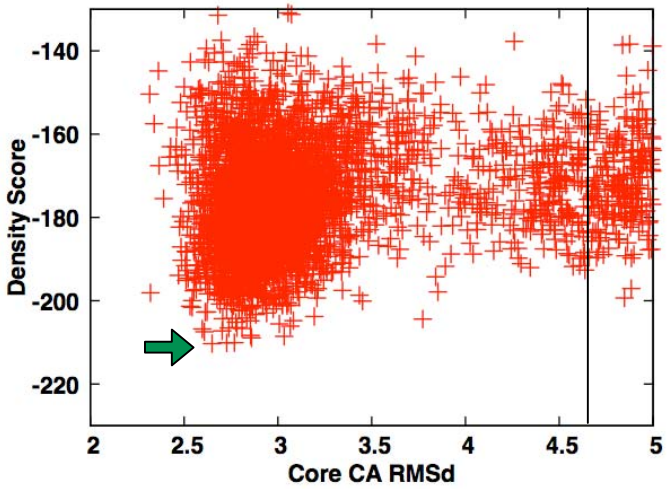
High-resolution GroEL model from 4.2Å cryoEM data

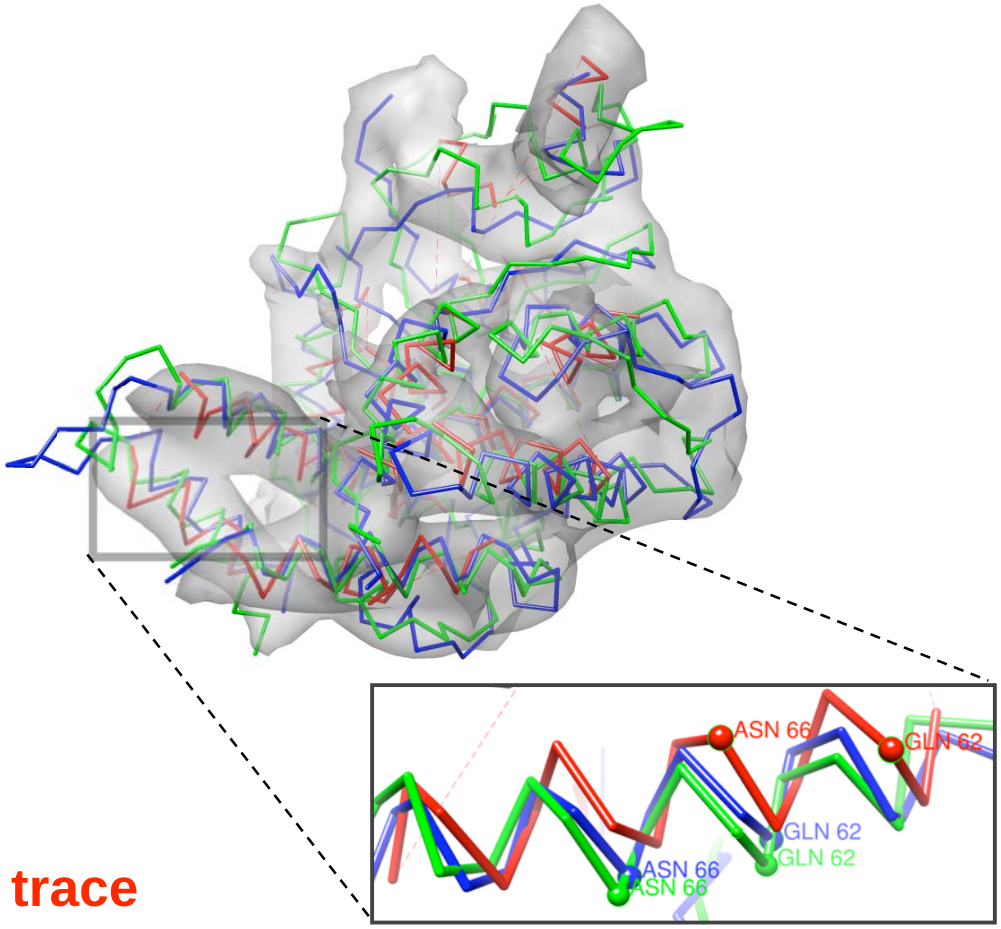




Initial Cα trace
Rosetta prediction
Native structure

High-resolution model of RDV from 6.8Å cryoEM data





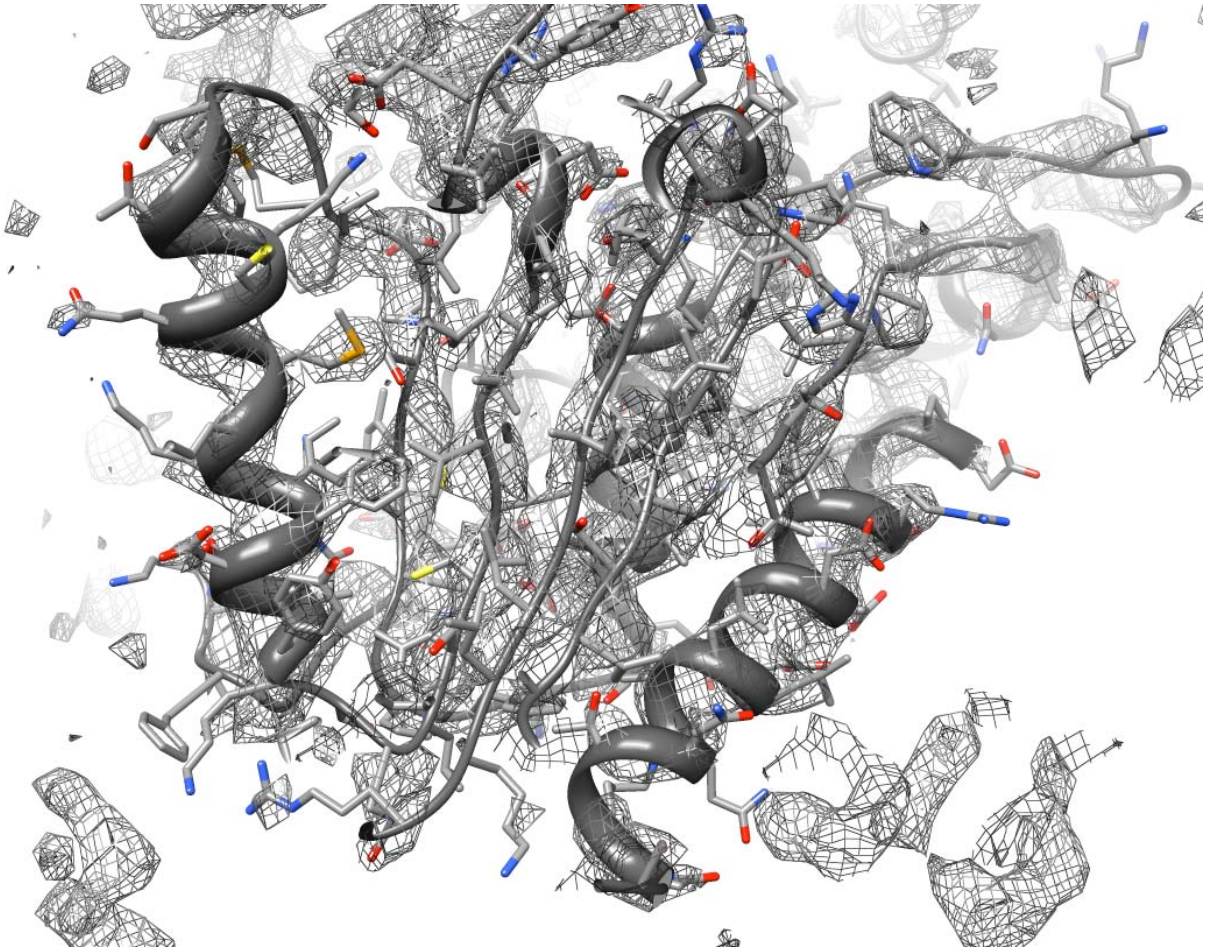
Initial Cα trace

Rosetta prediction

Native structure

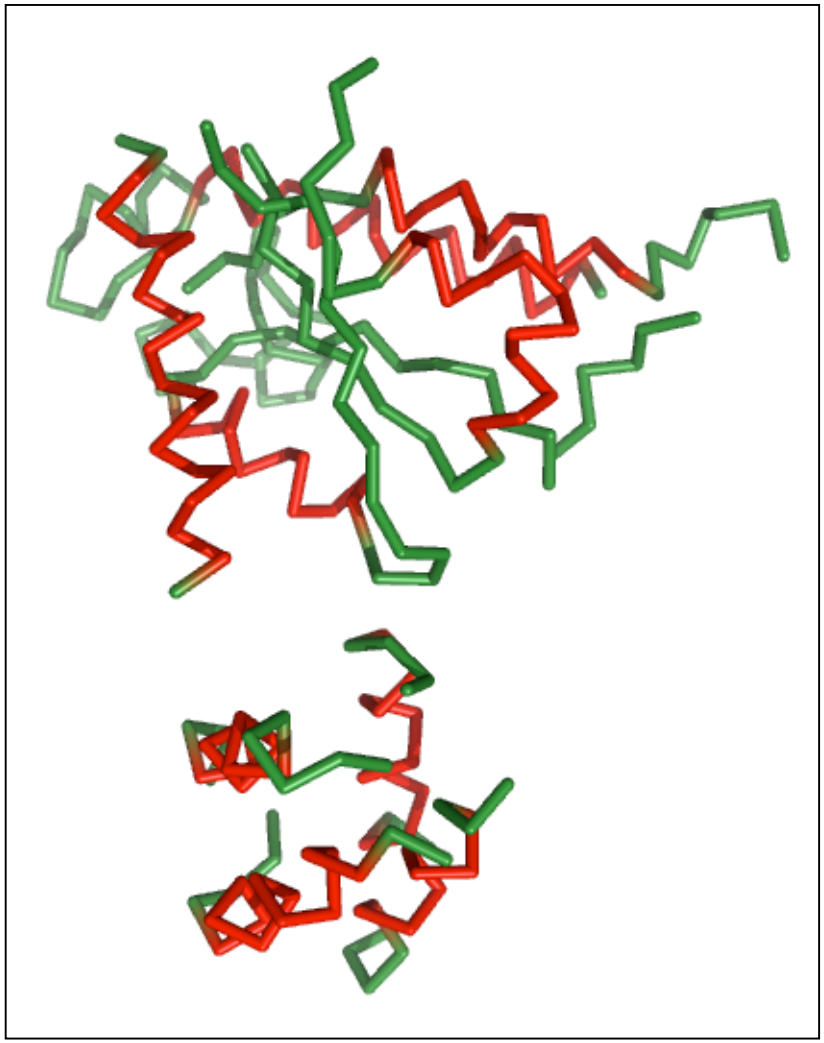
Improving an autobuilt model in 4Å crystallographic data

- **1NSF** autobuilt into density using data up to 4Å resolution
- Autobuilt model 1.12Å from native



1NSF at 4A

- Jumping ab initio
- Per-residue fit-to-density scores identified helices as well-resolved
  - Fix helices, introduce cuts
  - Fragment insertion outside of helices
  - Ramping chainbreak score
  - Density score (whole structure allatom) in final stage



with Oliver Lange

Improving an autobuilt model in 4Å crystallographic data

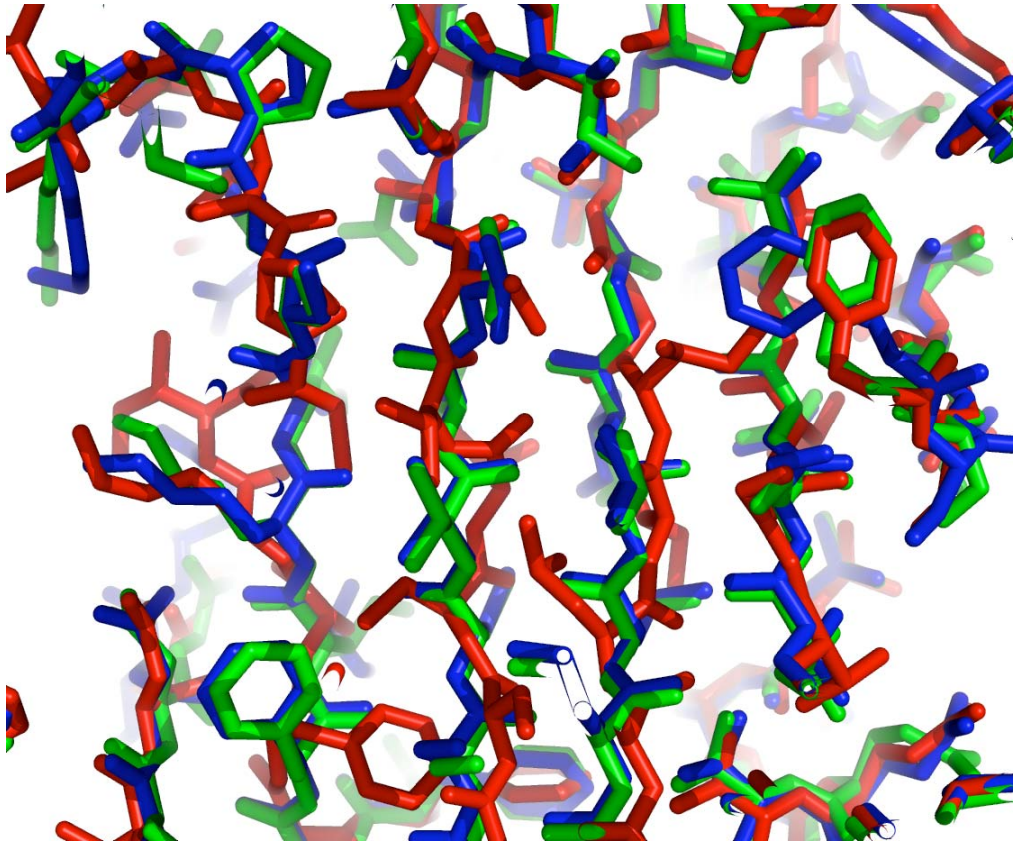
Autobuilt model

- 1.12Å RMS
- 85% Cα within 1Å of native

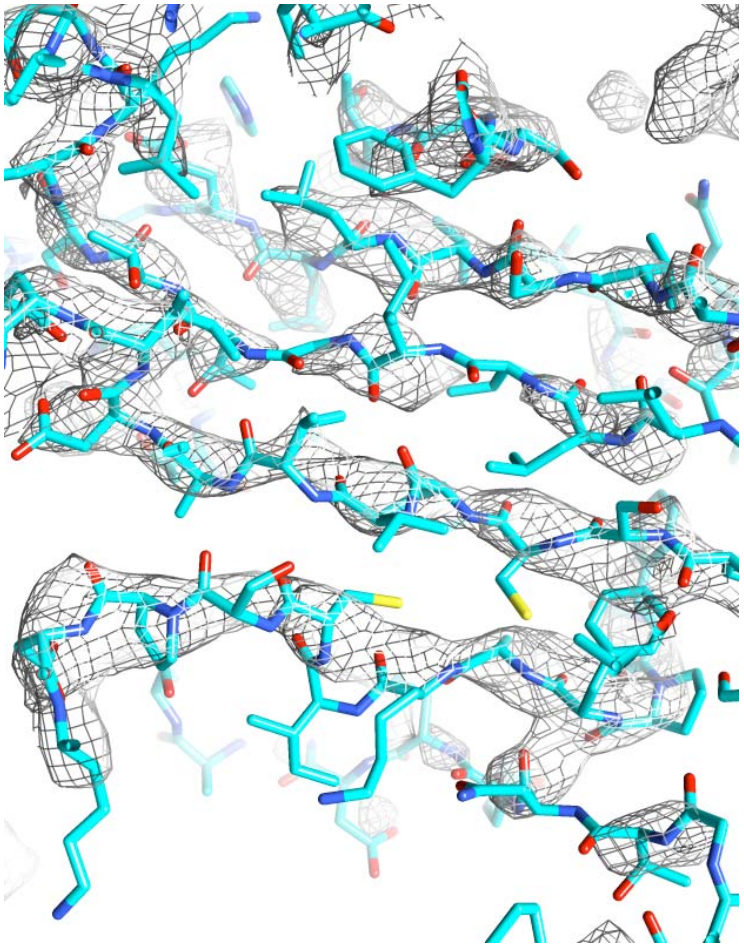
Rosetta prediction

- 0.88Å RMS
- 92% Cα within 1Å of native

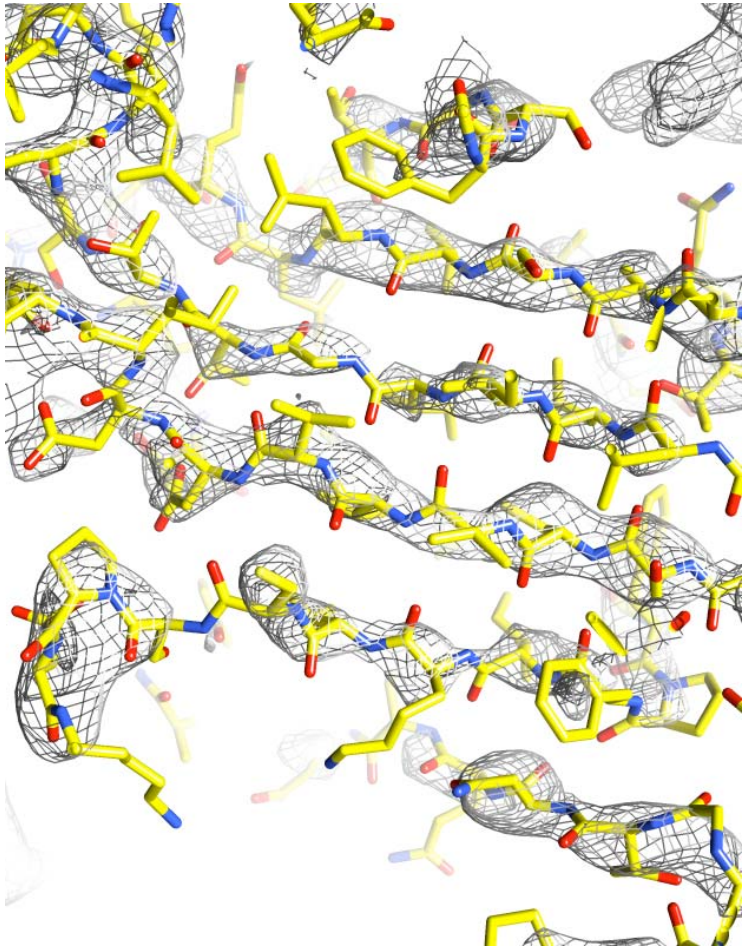
Native structure



Phase improvement in 4Å crystallographic diffraction data

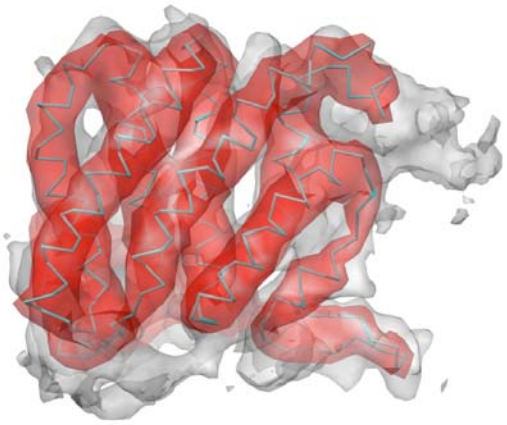


Autobuilt (resolve) model
R_{work}/R_{free} = 0.23/0.37



Refined with Rosetta into density
R_{work}/R_{free} = 0.21/0.34

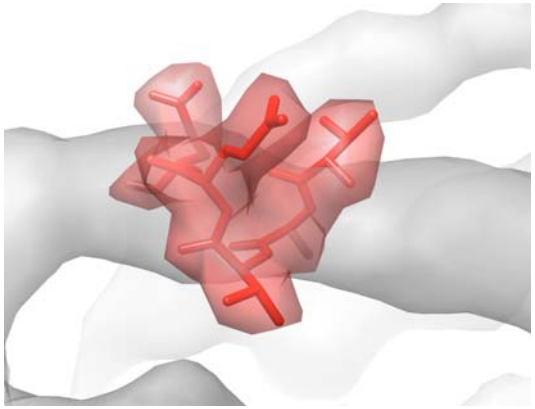
Three fit-to-density scoring functions



elec_dens_whole_structure_ca

elec_dens_whole_structure_allatom

- Score uses whole-structure masked correlation with density data
- Density computed using all heavyatoms / CAs only
- Score not used in repacking



elec_dens_window

- Score uses sum of sliding-window fit-to-densty scores computed for each residue
- Used in repacking; very slow

Using fit-to-density scoring from the command line

-edensity::mapfile gp7.mrc
-edensity::mapreso 4.0
-edensity::grid_spacing 2.0

Input map in CCP4/MRC format covering asymmetric unit. Compute rho_c using *mapreso* resolution, resampling map to *grid_spacing* per voxel.

-edensity::sliding_window_wt 0.5
-edensity::whole_structure_ca_wt 0.0
-edensity::whole_structure_allatom_wt 0.1

IF SUPPORTED BY THE PROTOCOL, set the weight on the three scoring functions.

Using fit-to-density scoring from the command line

- Supported protocols
  - score app
  - relax
  - loopmodel
  - RBrelax
  - CM
  - ab initio (through the topology broker)
    density scores must be set through patches
  - (viewer)
  - Several density-specific protocols

Adding fit-to-density scoring to a protocol

protocols::electron_density::SetupForDensityScoringMover

- Ensures pose is rooted on VRT
- Uses -edensity::realign flag value to dock pose to dens map
- [OPTIONALLY] Use only a subset of residues to initially dock pose

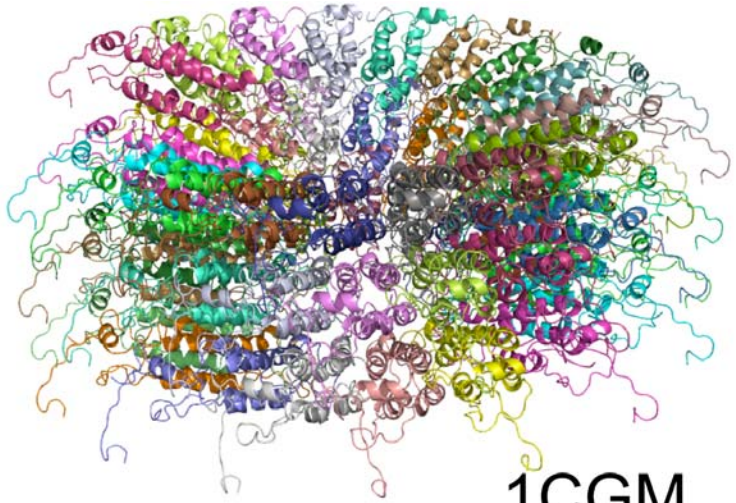
core::scoring::electron_density::
add_dens_scores_from_cmdline_to_scorefxn(ScoreFunction&)

- Uses values of -edensity::**sliding_window_wt**, -edensity::**whole_structure_ca_wt** and -edensity::**whole_structure_allatom_wt** to update score function

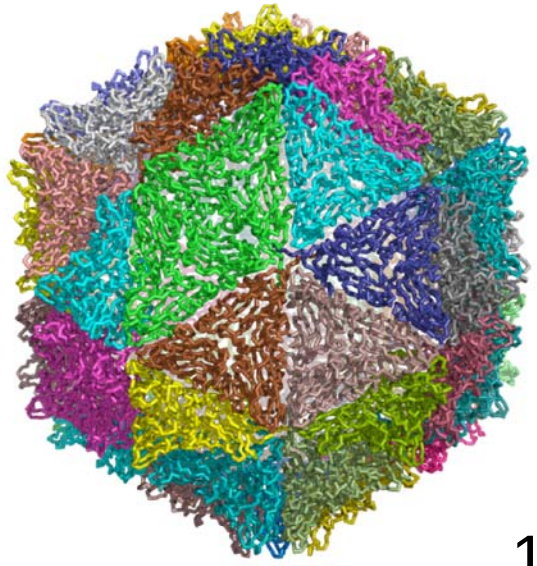
Refining Symmetric Complexes into Density

- Symmetric modeling code allows refinement of various symmetries

1EI7



1CGM



1F2N

with Ingemar Andre

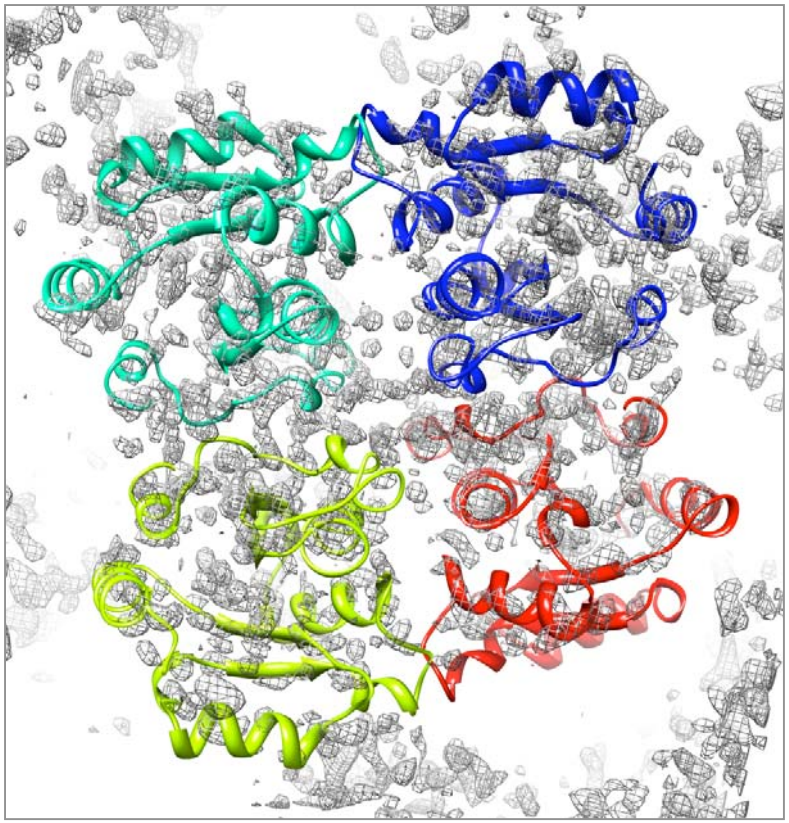
Refining Symmetric Complexes into Density

-symmetry::symmetry_definition

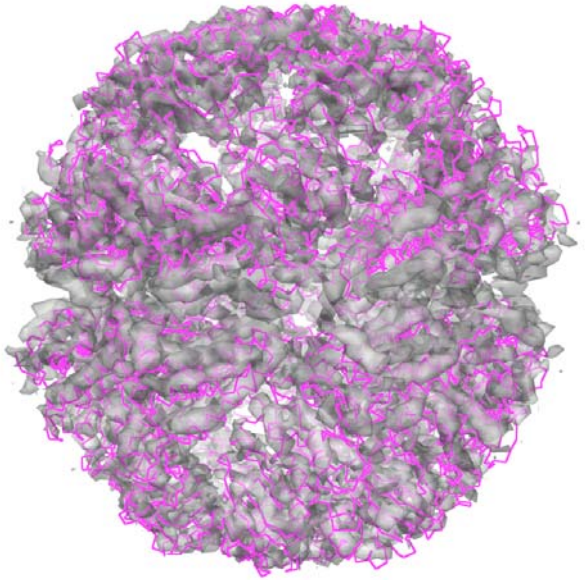
- Define the symmetric DOFs & master/slave subunits
- Typically generated by a script (see Doxygen!)

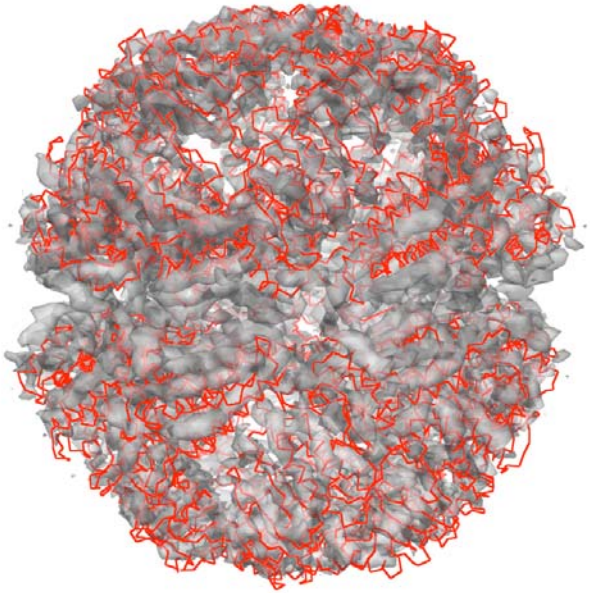
-edensity::score_symm_complex

- Score a symmetric pose's fit to density over the entire structure
- Maps asymmetric scores/derivatives to the master subunit
- Derivatives remapped at each symmetric DOF



with Ingemar Andre





Ongoing: MM-CPN

Dock monomers into density

↓

Symmetrize model using
‘**apps/pilot/frank/make_NCS.pl**’

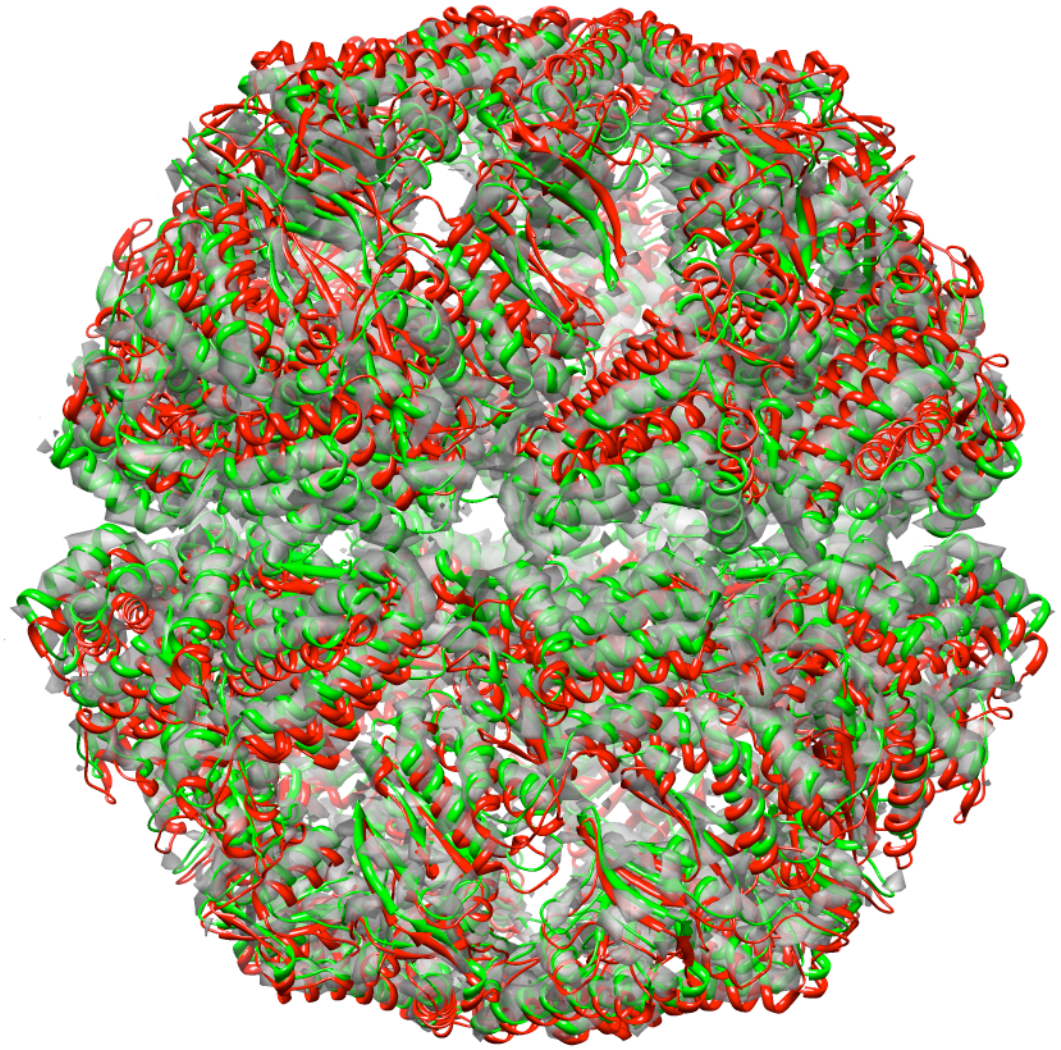
↓

Rigid-body minimization
along symmetric DOFs

↓

Symmetric refinement into density

MM-CPN



Docked Homology Model

Corr. (backbone): 0.43

Corr. (allatom): 0.31

Symmetrized

Corr. (backbone): 0.36

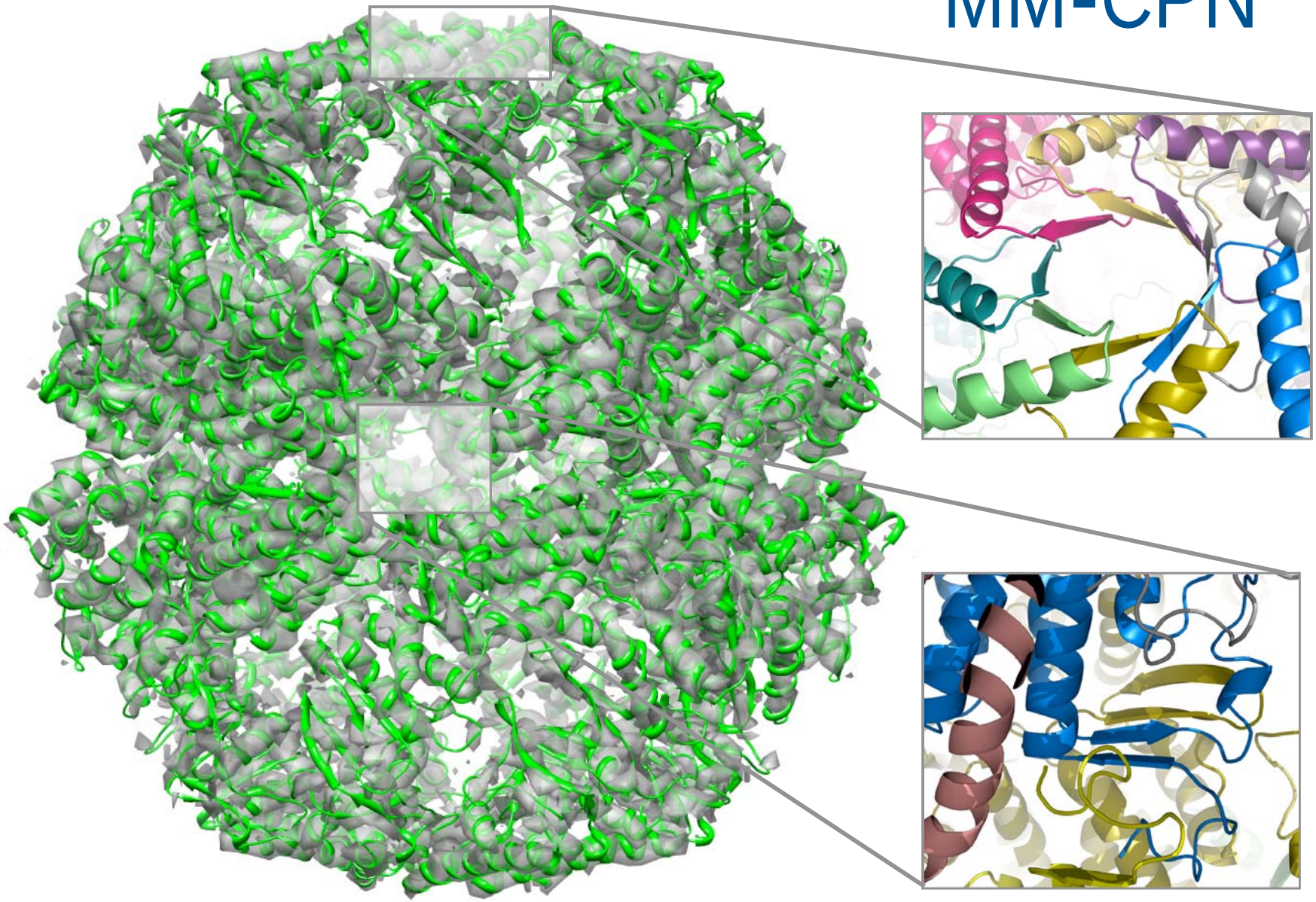
Corr. (allatom): 0.27

Rosetta prediction

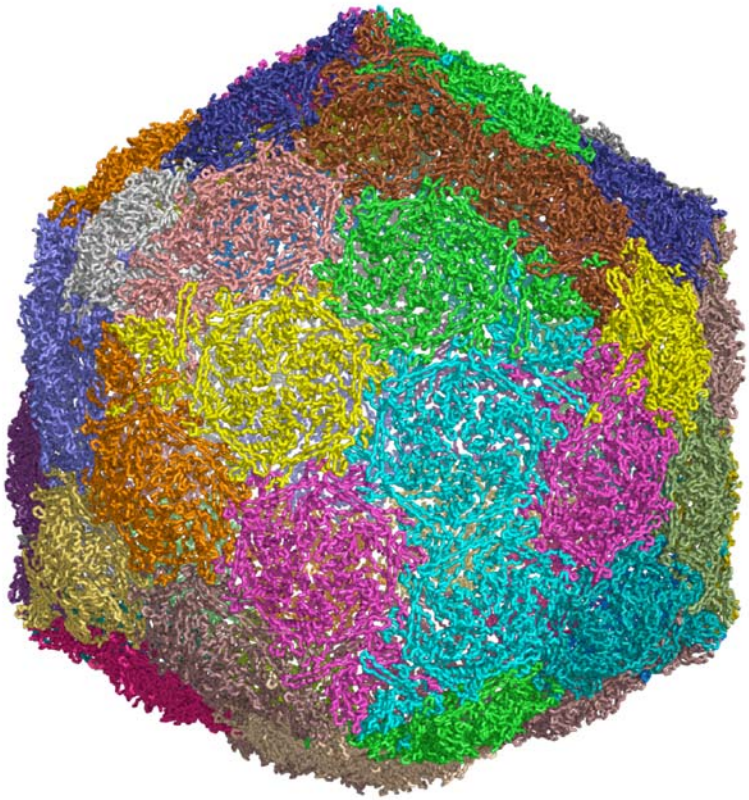
Corr. (backbone): **0.71**

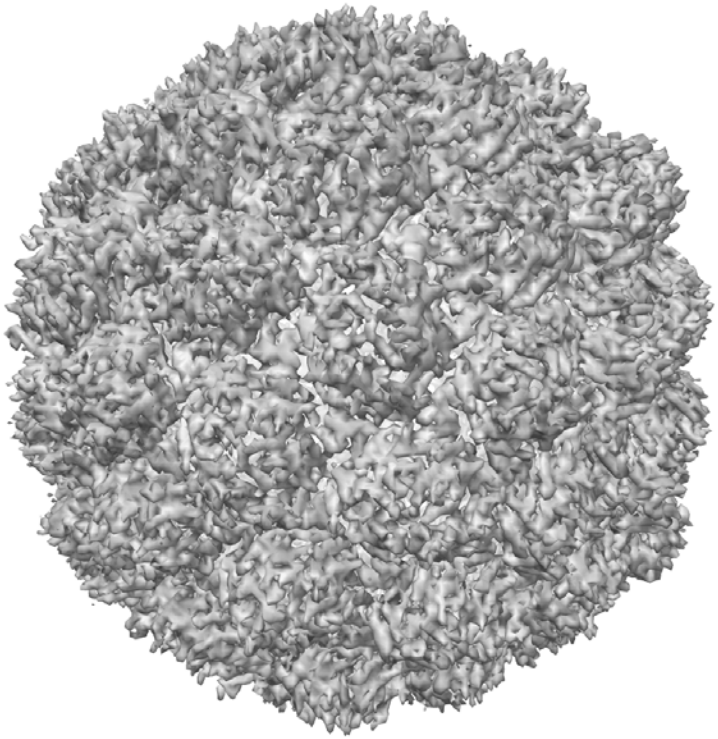
Corr. (allatom): **0.54**

MM-CPN



Ongoing: Capsid modeling





1OHG

Acknowledgements

- Baker Lab
  - Oliver Lange, Mike Tyka
  - Ingemar Andre
- NCMI (Baylor)
  - Wah Chiu
  - Matt Baker
  - Junjie Zhang