

Model-building with PHENIX and Rosetta

*ICSG 2011
Toronto, Canada*

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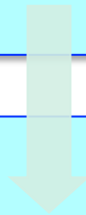
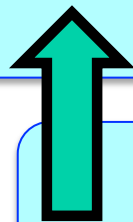
*Iterative density modification, model-building and refinement
with phenix.autobuild*

Experimental data, sequence, phase
information or starting model

Model-building and refinement

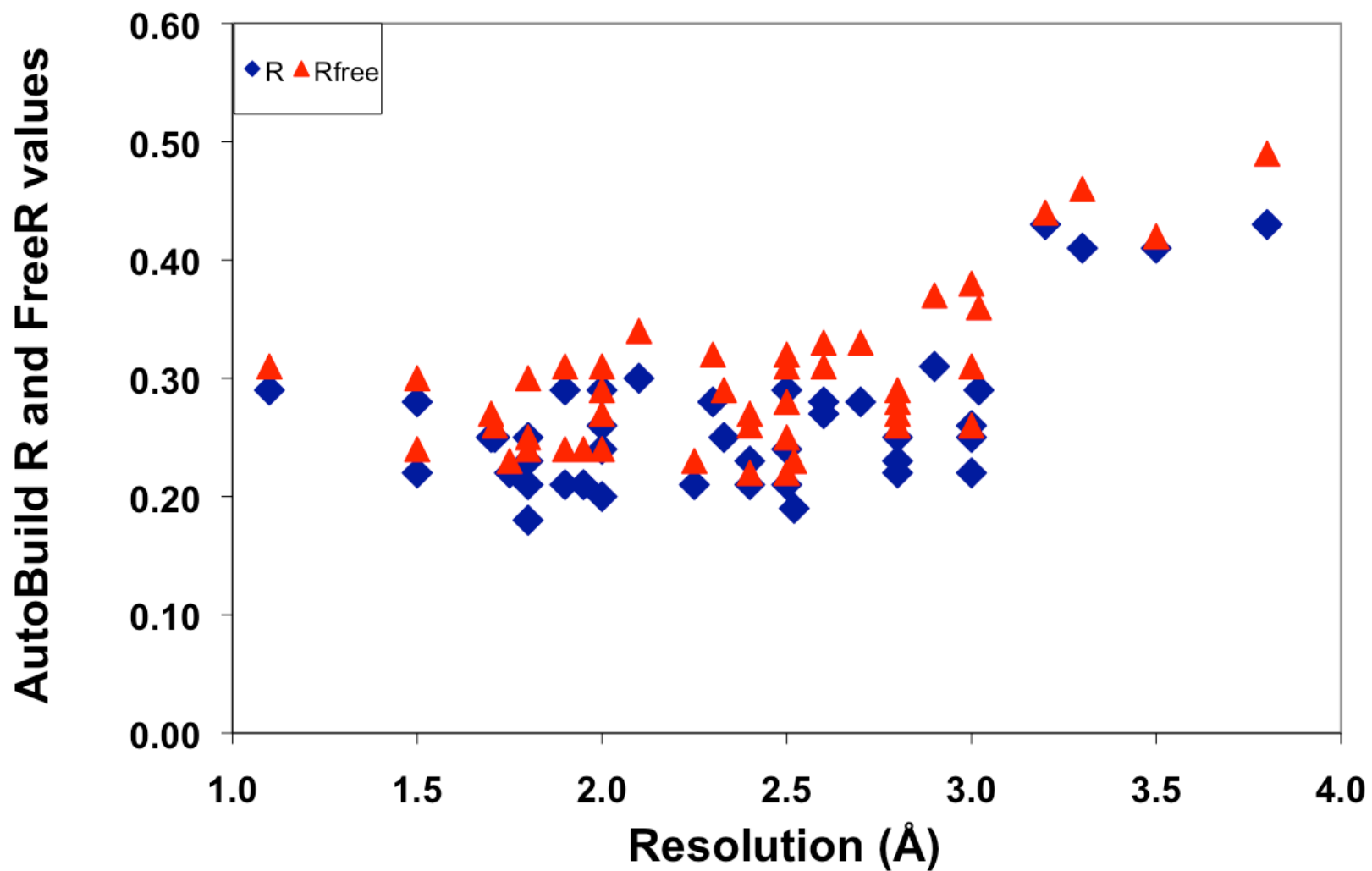
*Resolve building
Secondary-structure only
Connect chains
Fit loops
Build outside model*

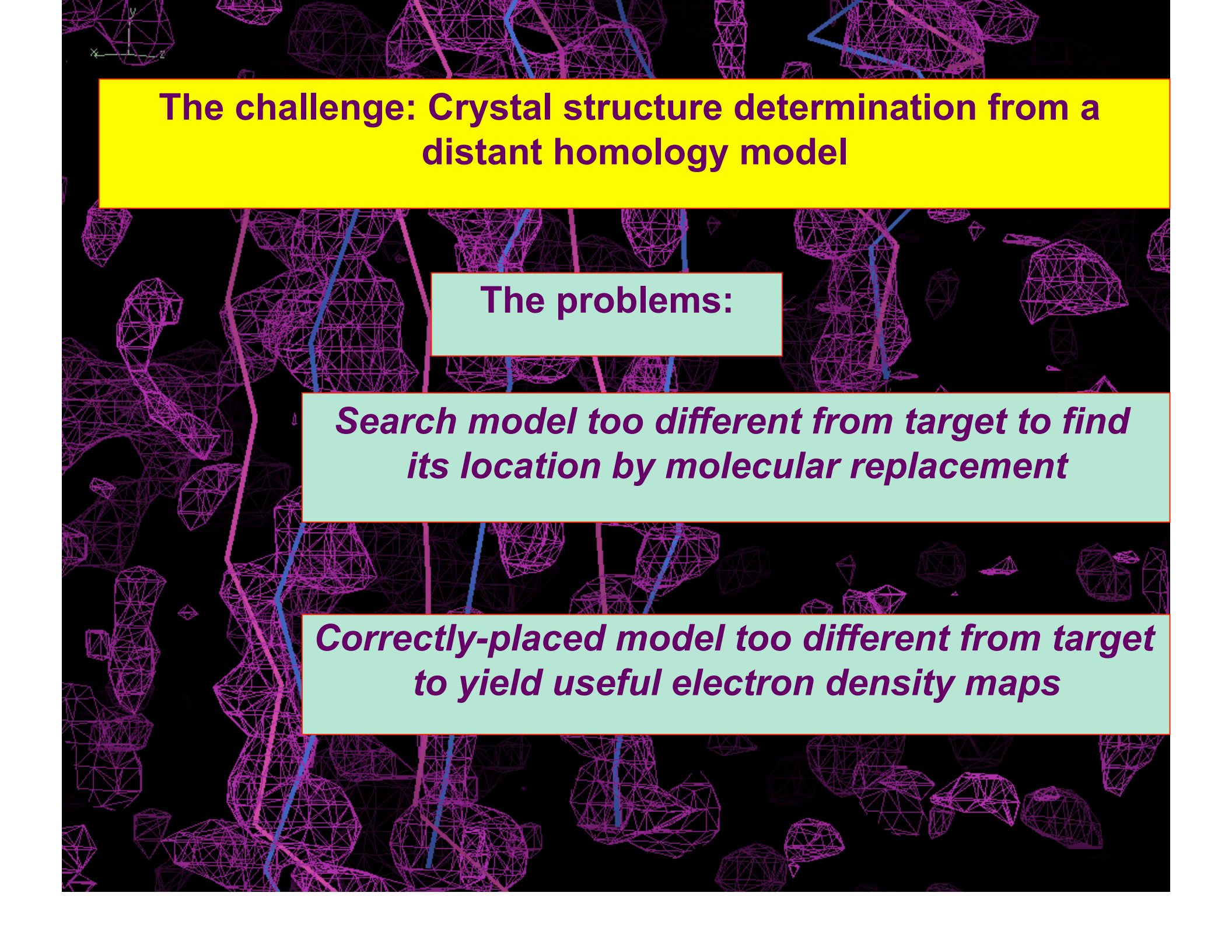
Density modification



AutoBuild – tests with structure library

Fully automated iterative model-building, final R/Rfree



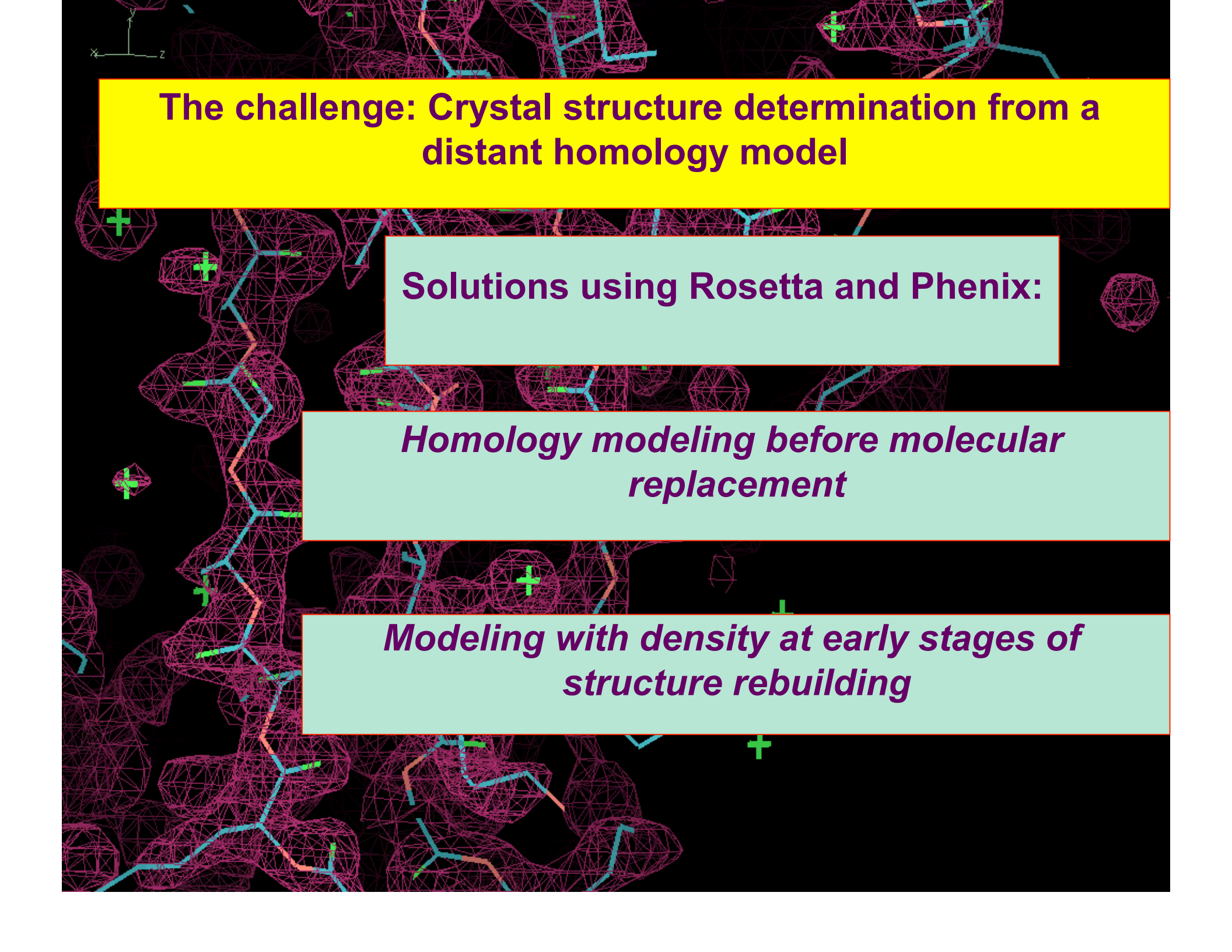


The challenge: Crystal structure determination from a distant homology model

The problems:

Search model too different from target to find its location by molecular replacement

Correctly-placed model too different from target to yield useful electron density maps



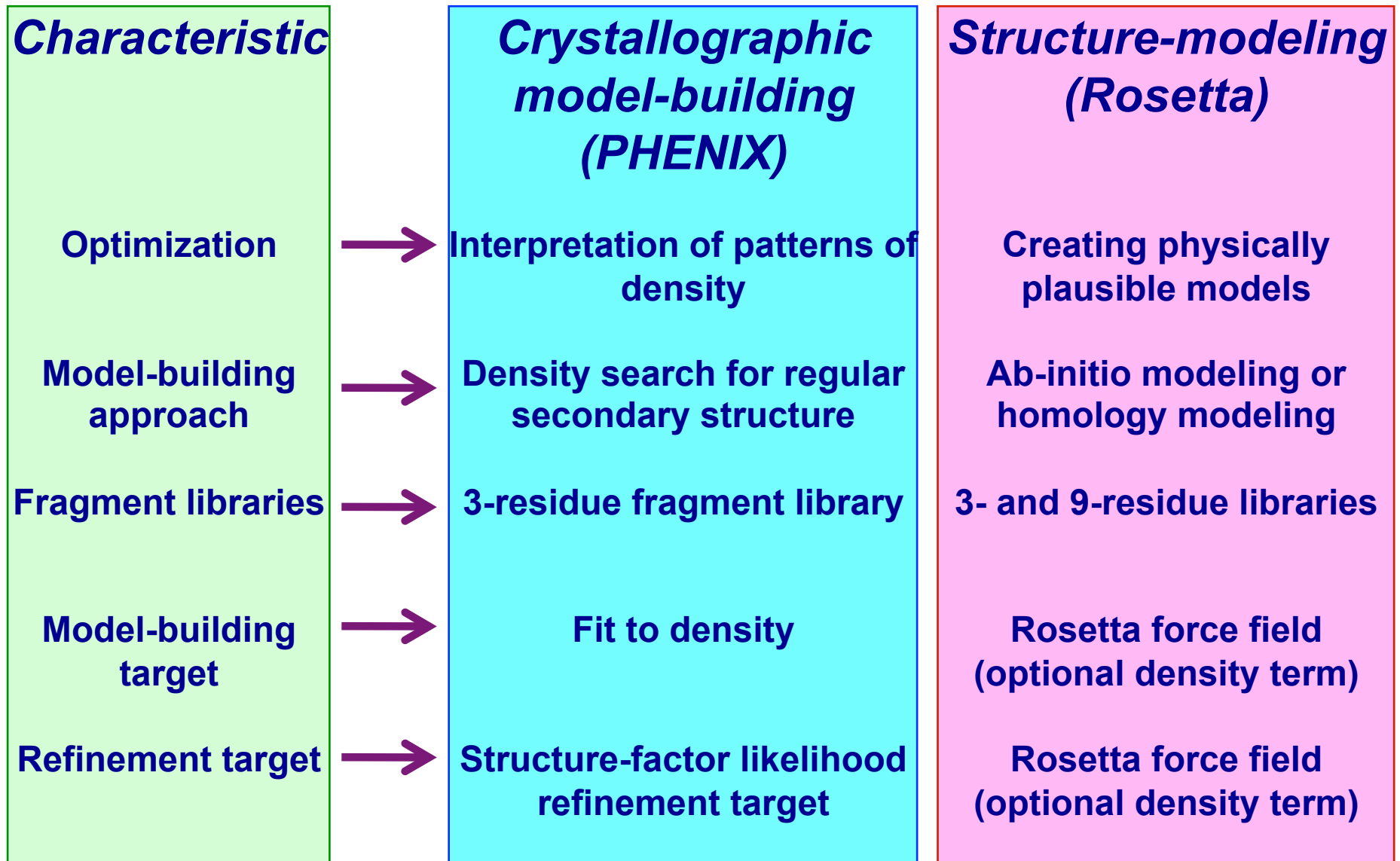
The challenge: Crystal structure determination from a distant homology model

Solutions using Rosetta and Phenix:

Homology modeling before molecular replacement

Modeling with density at early stages of structure rebuilding

Complementarity of PHENIX and Rosetta model-building



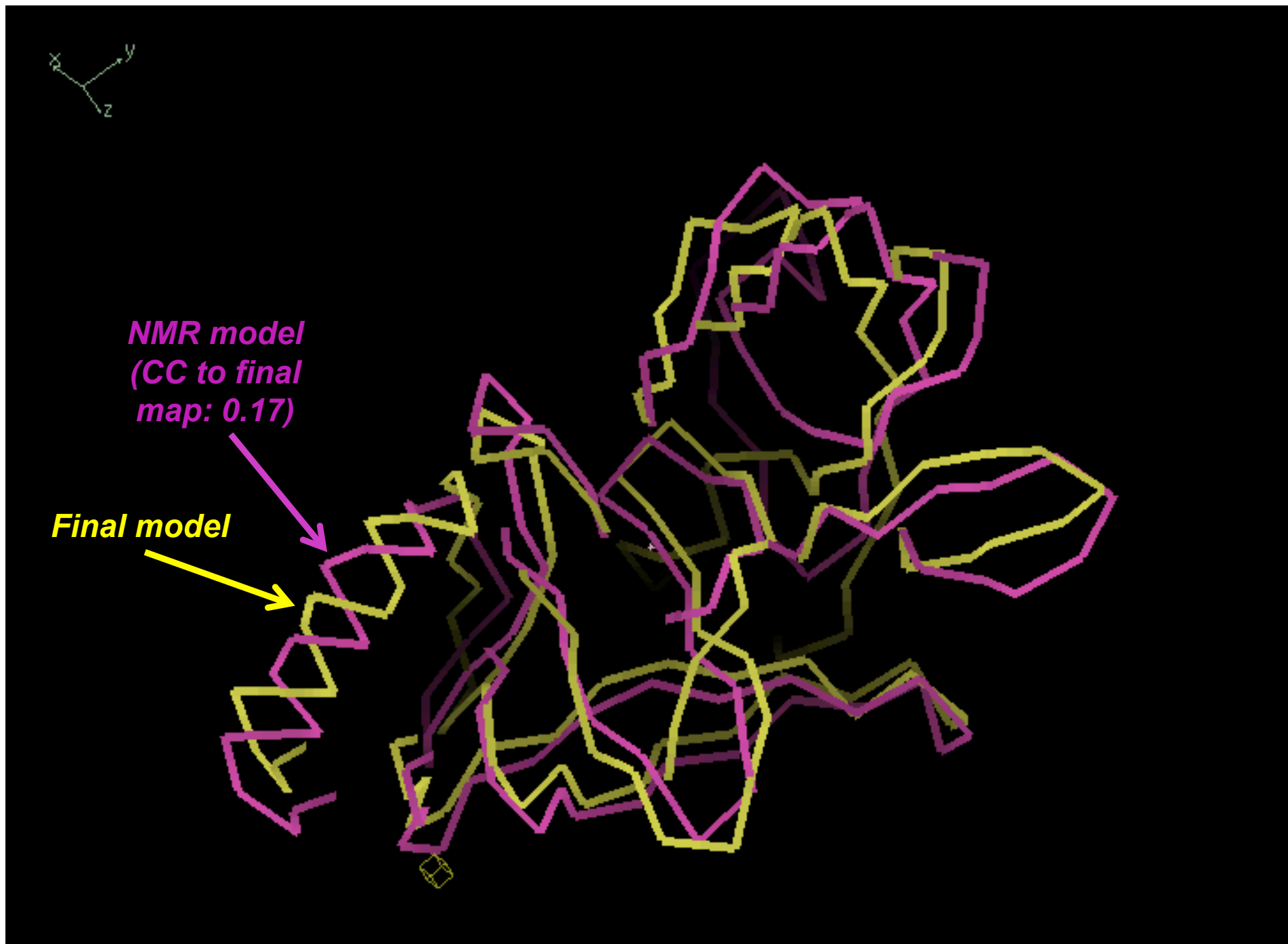
Generating improved homology
models for molecular replacement
with Rosetta

ag9603

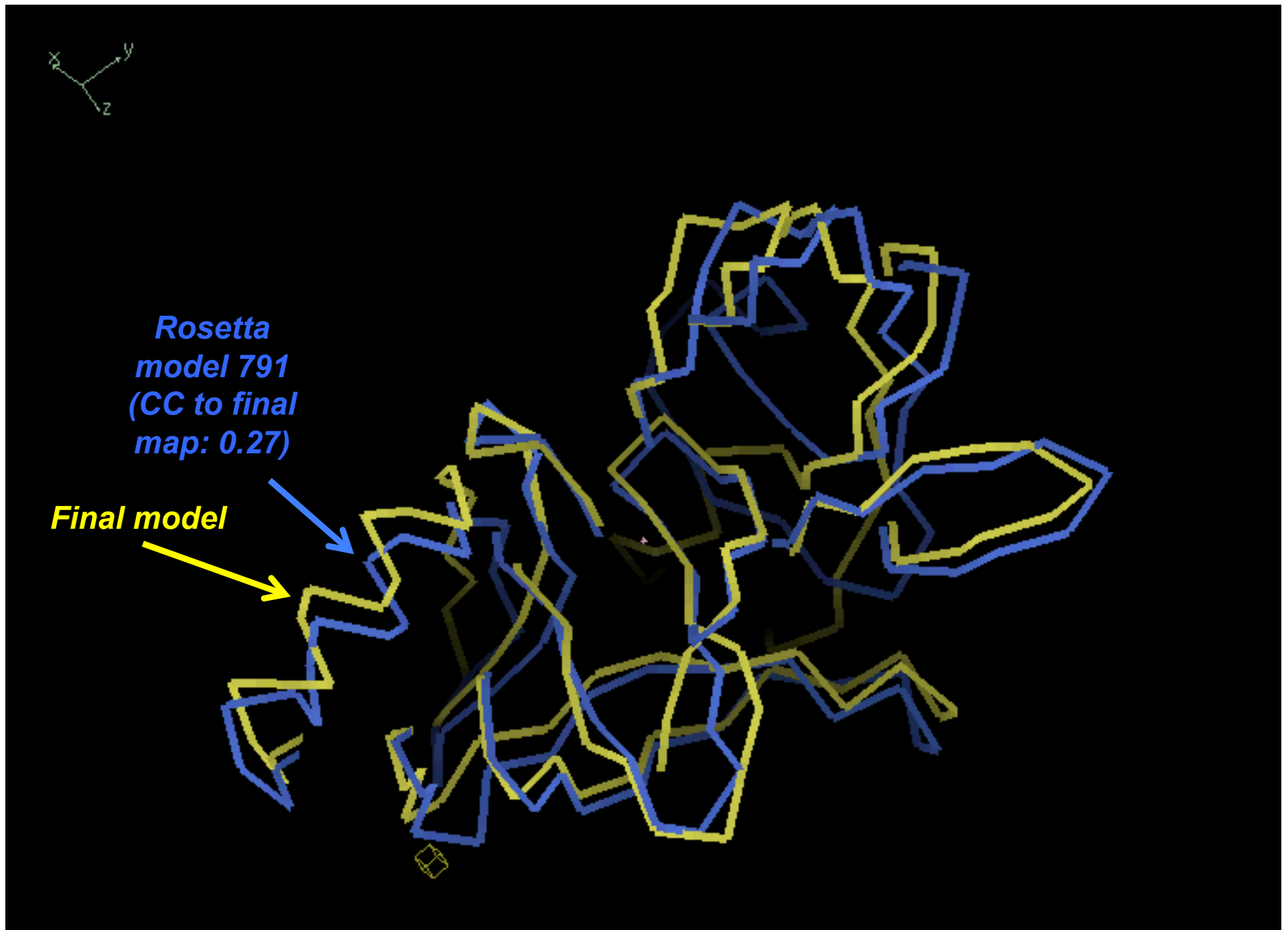
NMR model, 100% identity

1.7 Å data

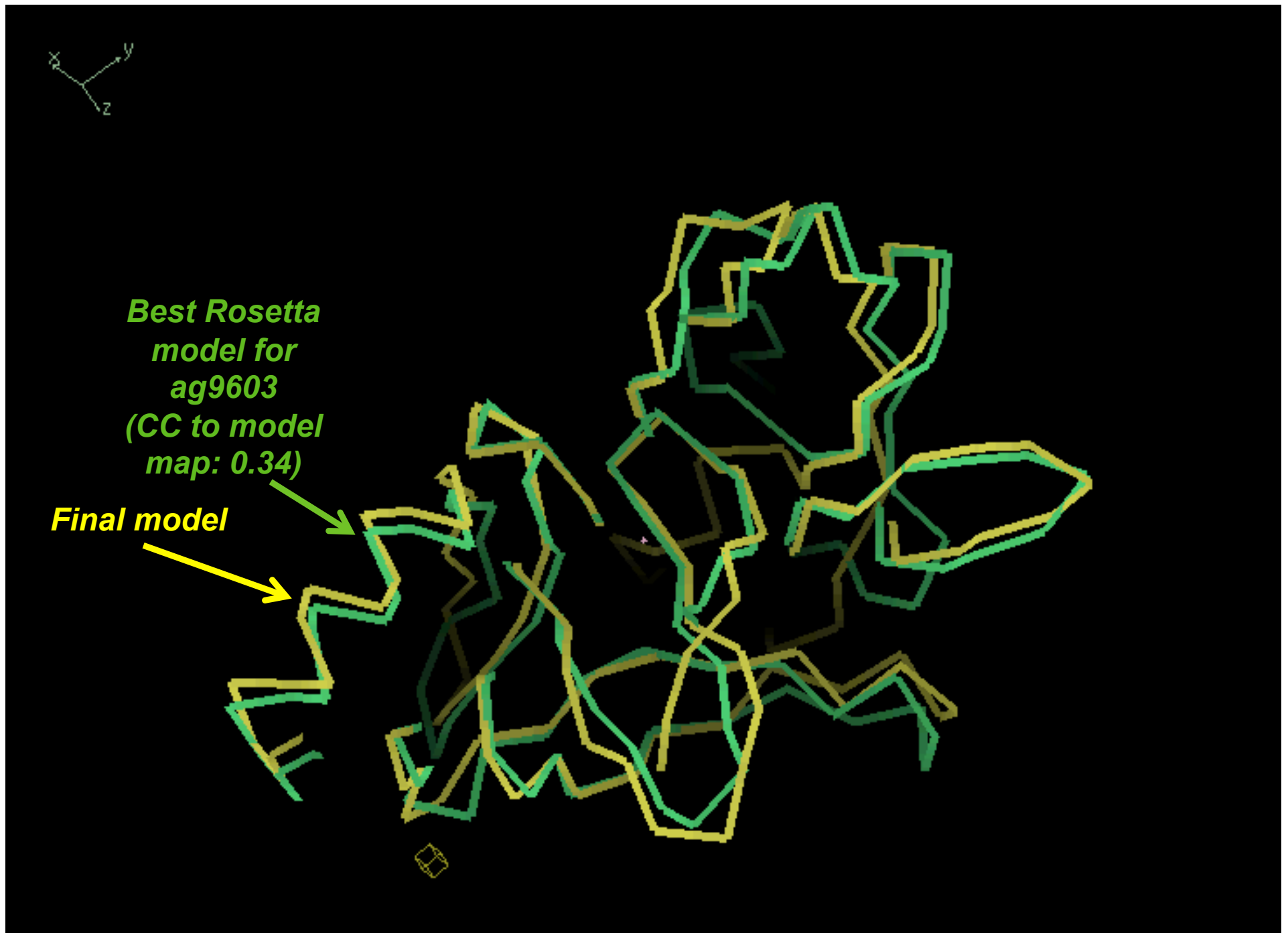
NMR model of of ag9603



Typical Rosetta model of of ag9603



Best Rosetta model of of ag9603

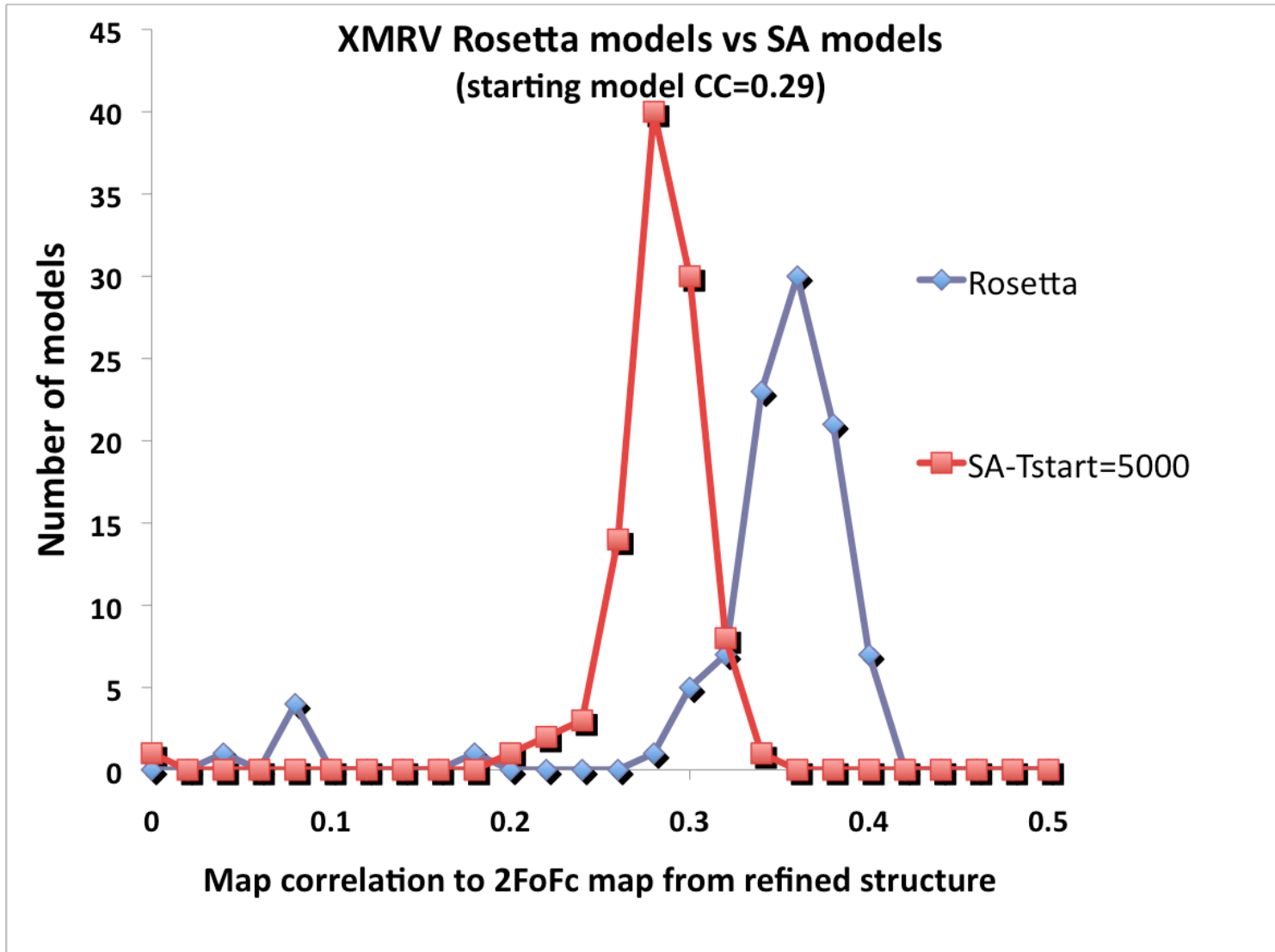


Improving models with Rosetta using density

Density fit as part of optimization target
Modeling segments not in template

Comparison with simulated annealing

SA vs Rosetta models



Integrating Rosetta modeling in Phenix

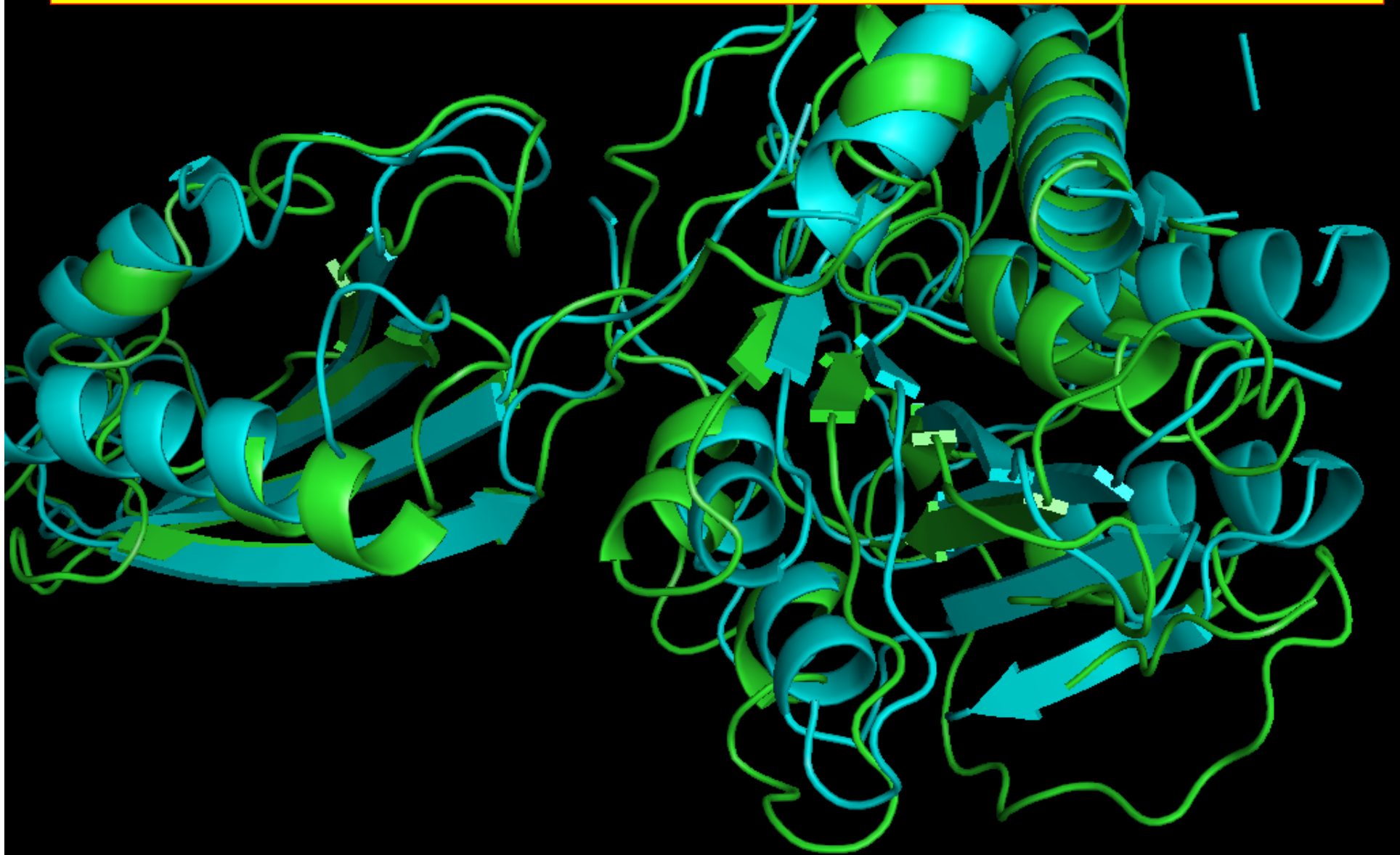
phenix.mr_rosetta

Molecular replacement

Model rebuilding

Single machine or a cluster

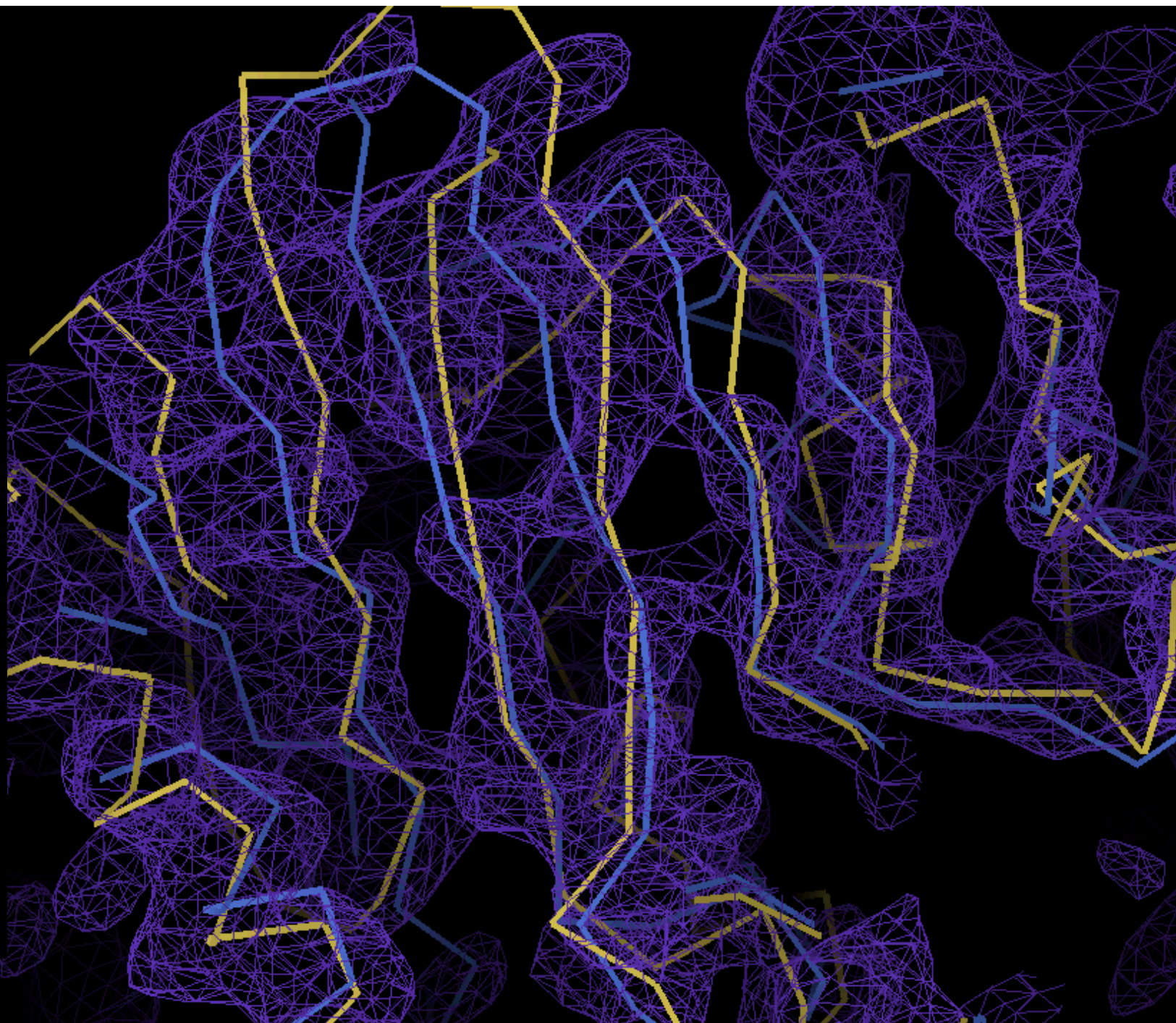
**mr_rosetta example:
HP3342, 22% identity template, 3.2 Å data, B=87 Å²**



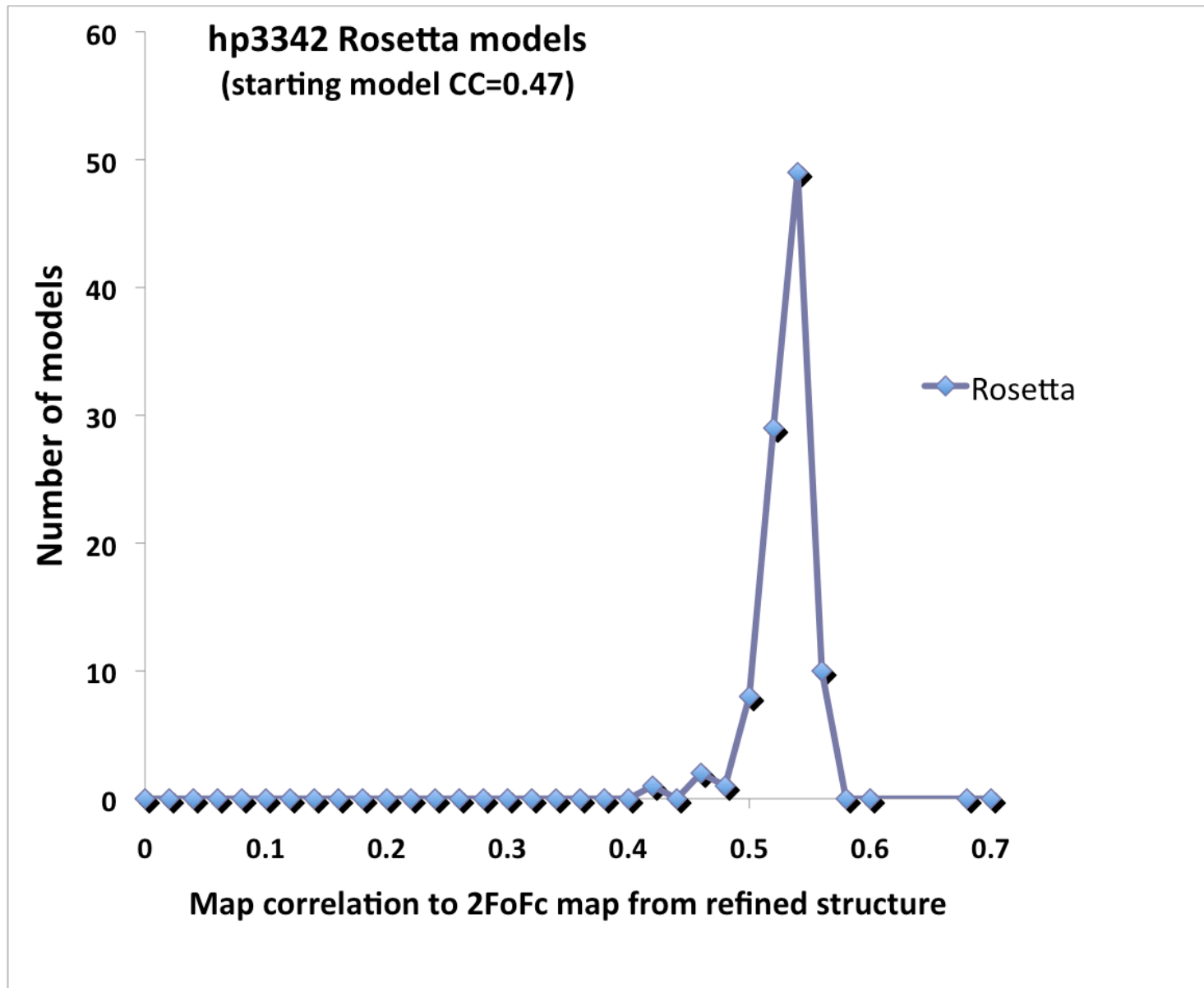
hp3342, 22%
identity
template,
3.2 Å data

Density-
modified map
based on
1vgy

Yellow: final
model
Blue:
template
(1vgy)



MR_rosetta example: hp3342



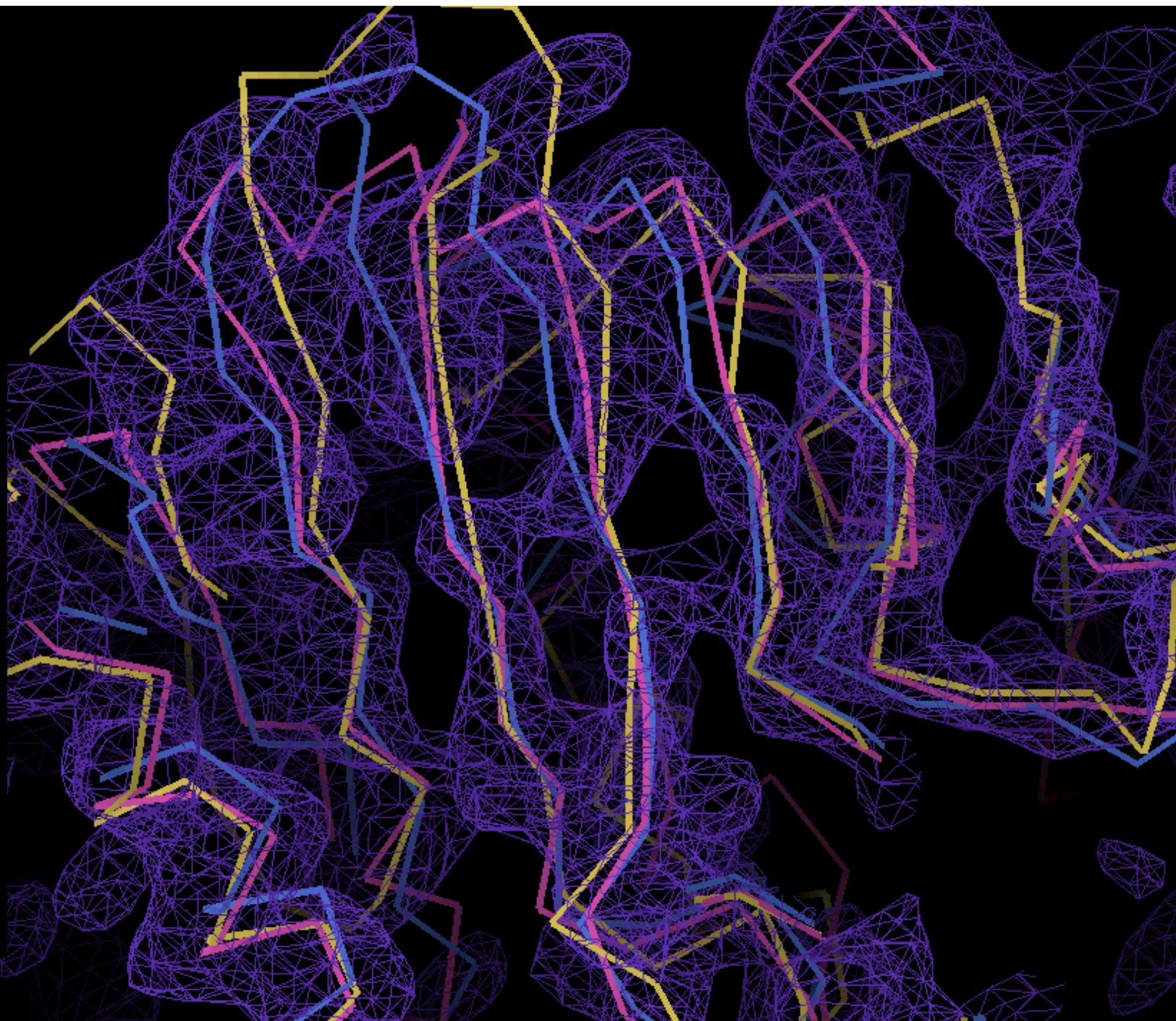
hp3342, 22%
identity
template,
3.2 Å data

Density-
modified map
based on
1vgy

Yellow: final
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template
(1vgy)

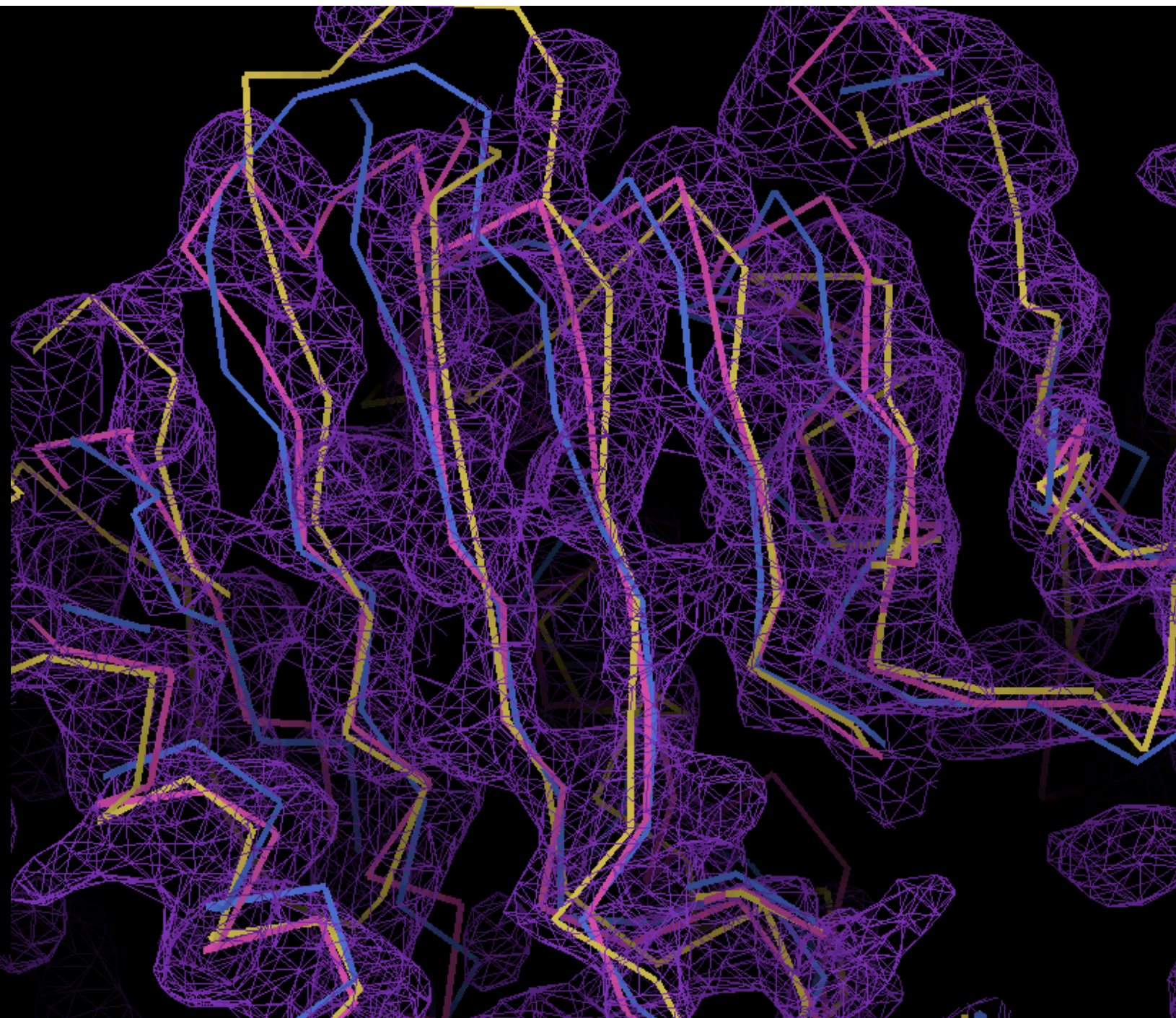
Pink: Highest-
scoring
Rosetta model



hp3342, 22%
identity
template,
3.2 Å data

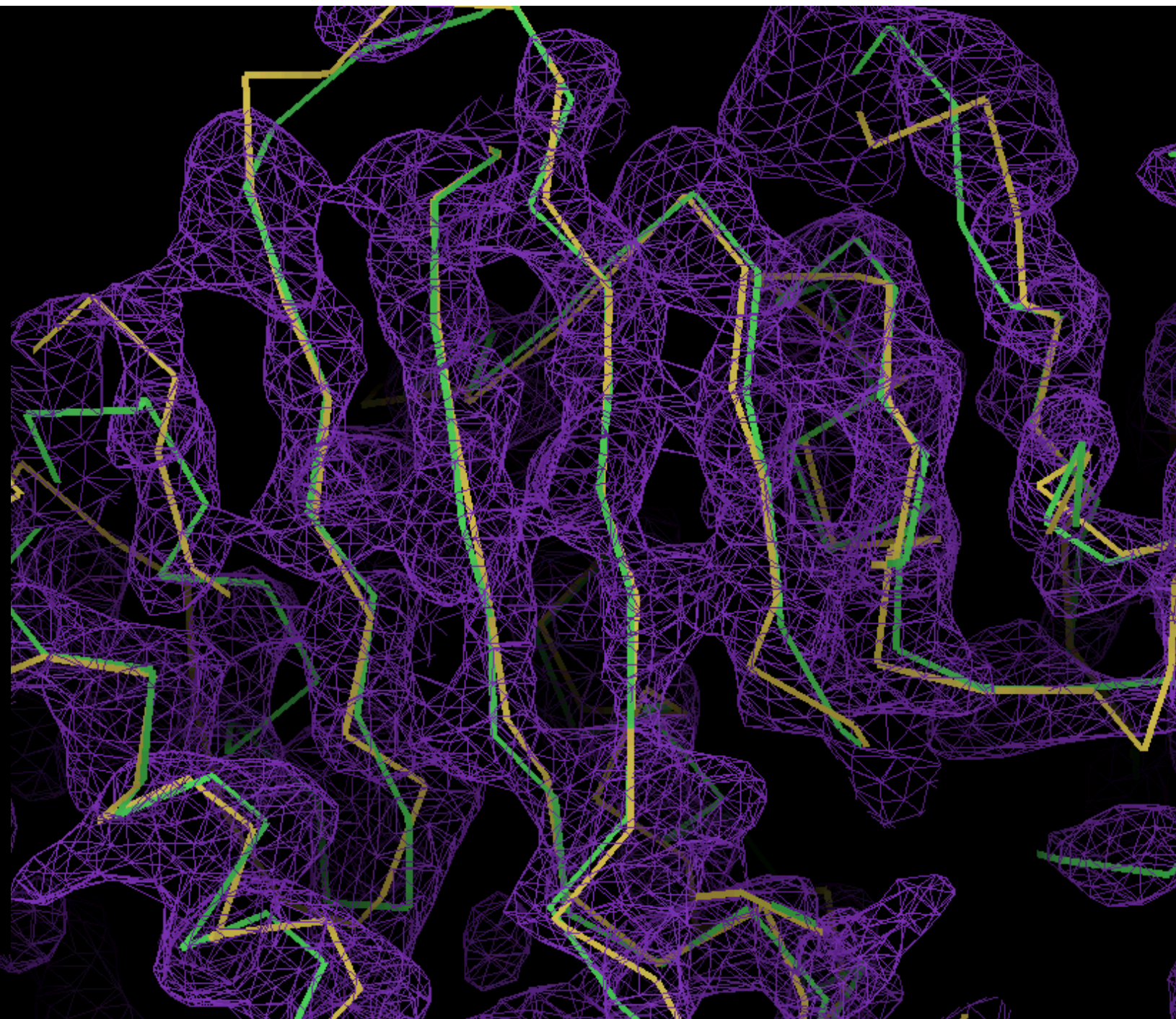
Density-
modified map
based on
Rosetta
model
Yellow: final
model
Blue:
template
(1vgy)

Pink: Highest-
scoring
Rosetta model

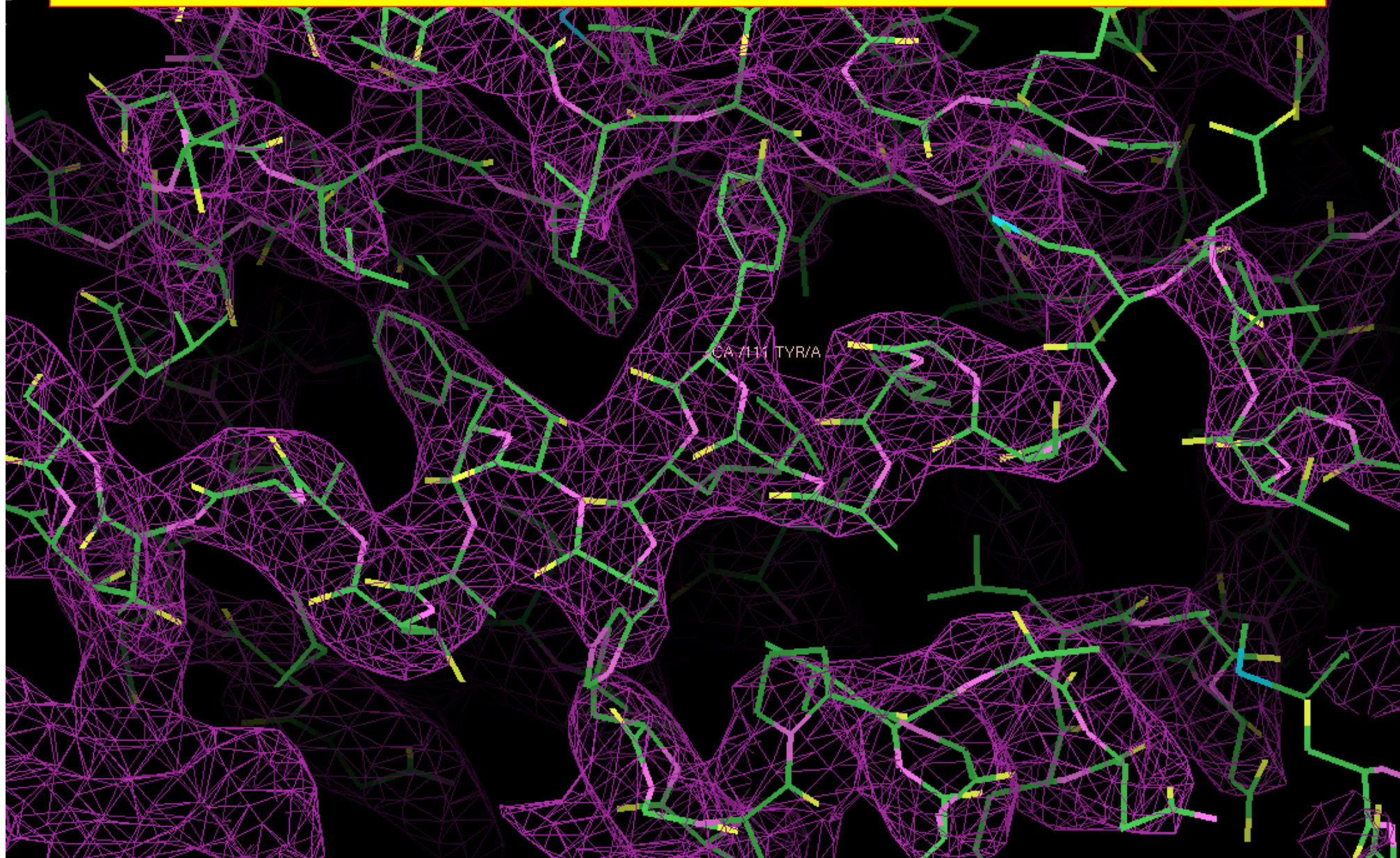


hp3342, 22%
identity
template,
3.2 Å data

Density-
modified map
based on
Rosetta
model
Yellow: final
model
Green:
autobuild
model

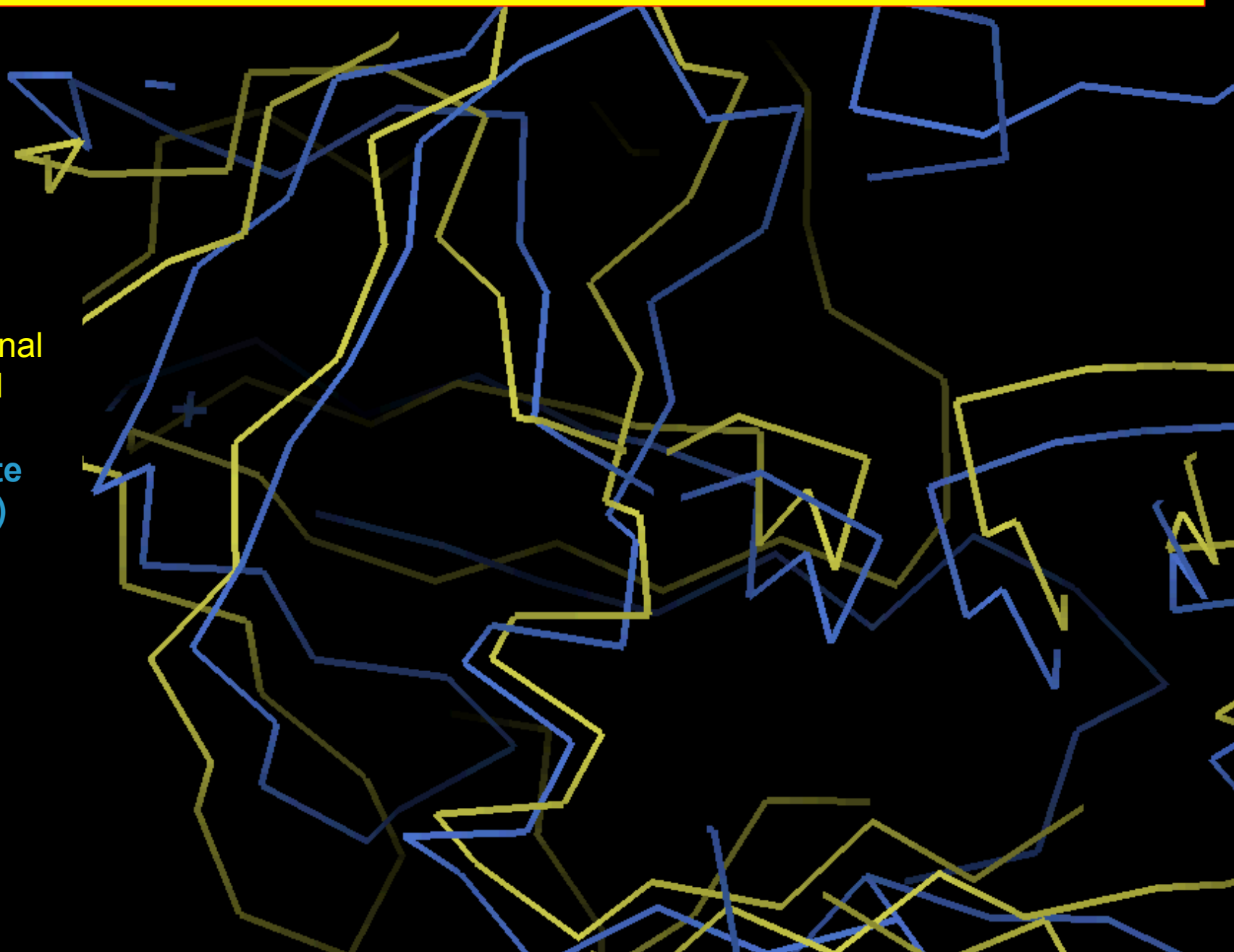


HP3342, 22% identity template, 3.2 Å data,
R/Rfree=0.34/0.41 B=87 Å²



**mr_rosetta example II:
xmrsv, 30% identity template (2hs1), 2.0 Å data**

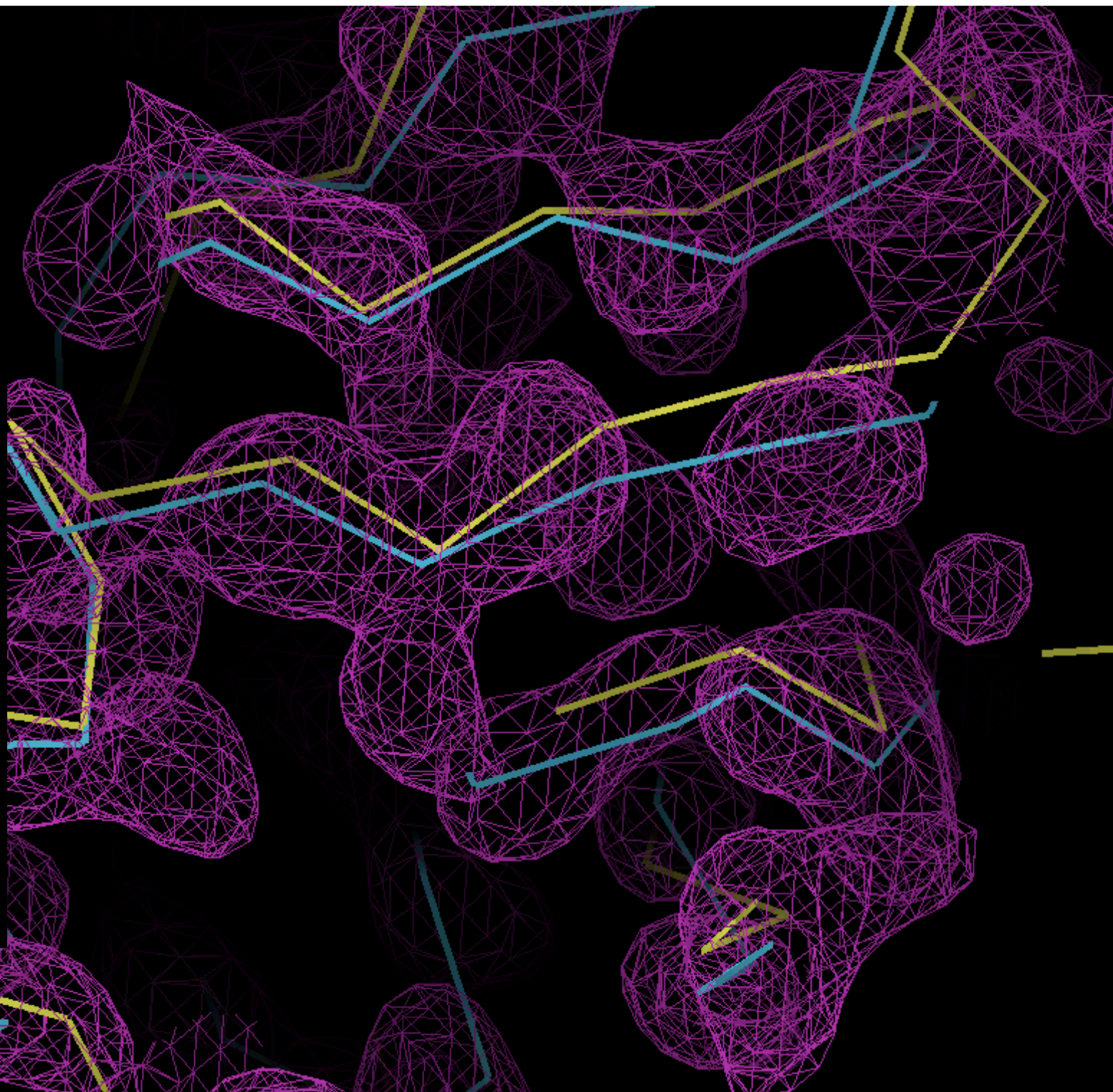
Yellow: final
model
Blue:
template
(2hs1)



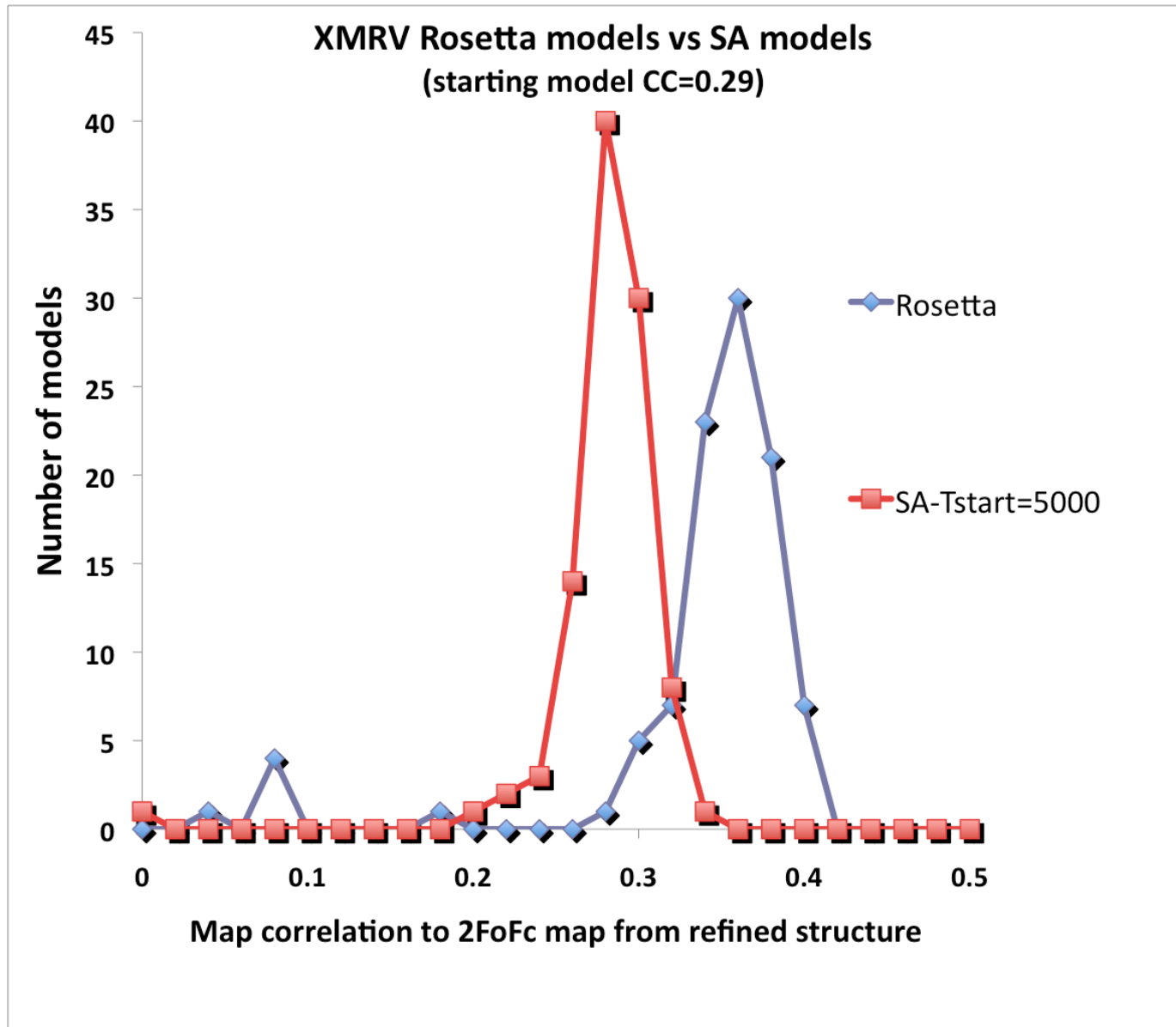
xmrv, 30%
identity
template,
2.0 Å data

Density-
modified map
based on
2hs1

Yellow: final
model
Blue:
template
(2hs1)



MR_rosetta example: xmr



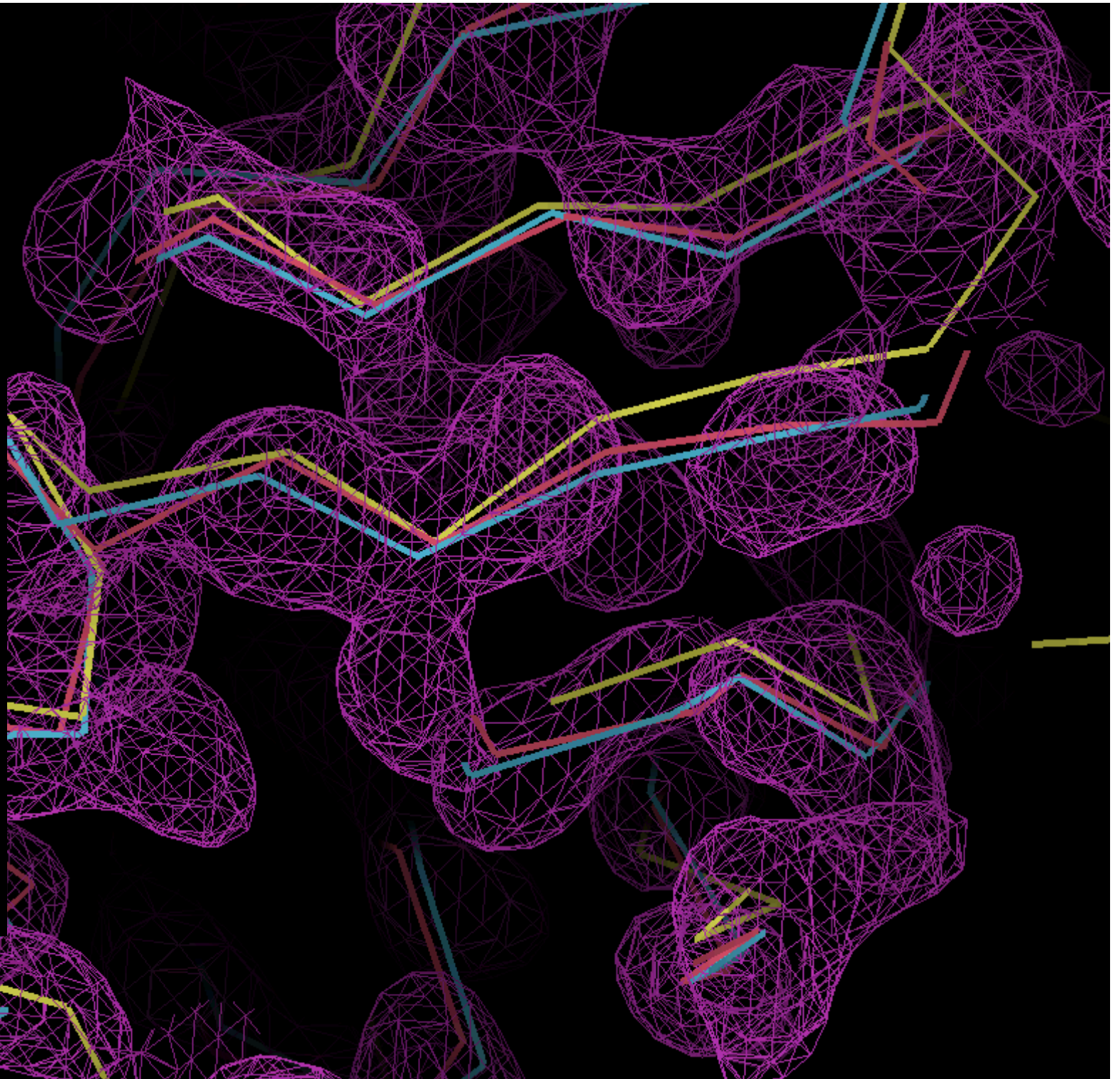
xmrv, 30%
identity
template,
2.0 Å data

Density-
modified map
based on
2hs1

Yellow: final
model

Blue:
template
(2hs1)

Red: Highest-
scoring
Rosetta model



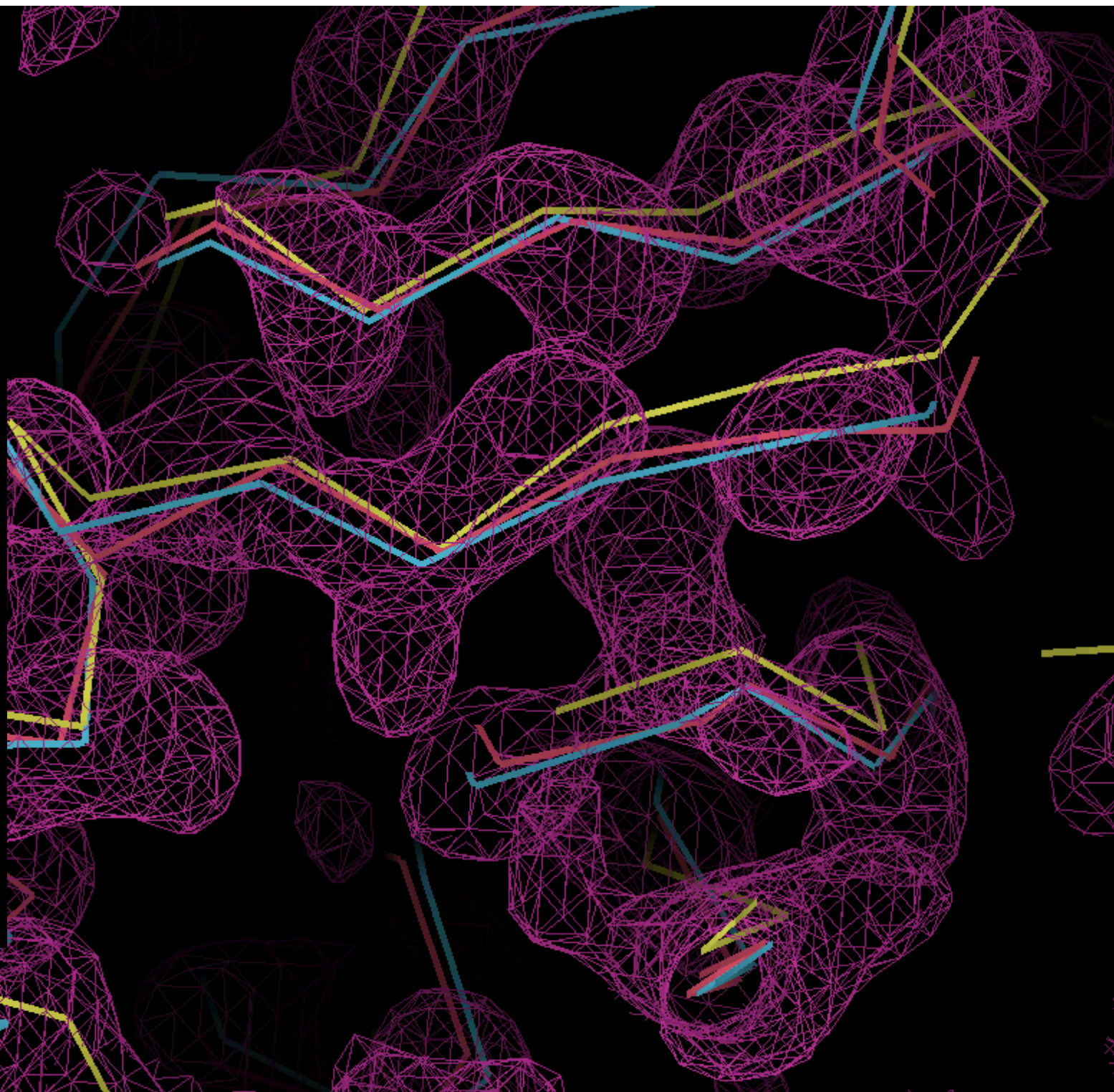
xmrv, 30%
identity
template,
2.0 Å data

Density-
modified map
from Rosetta
model

Yellow: final
model

Blue:
template
(2hs1)

Red: Highest-
scoring
Rosetta model



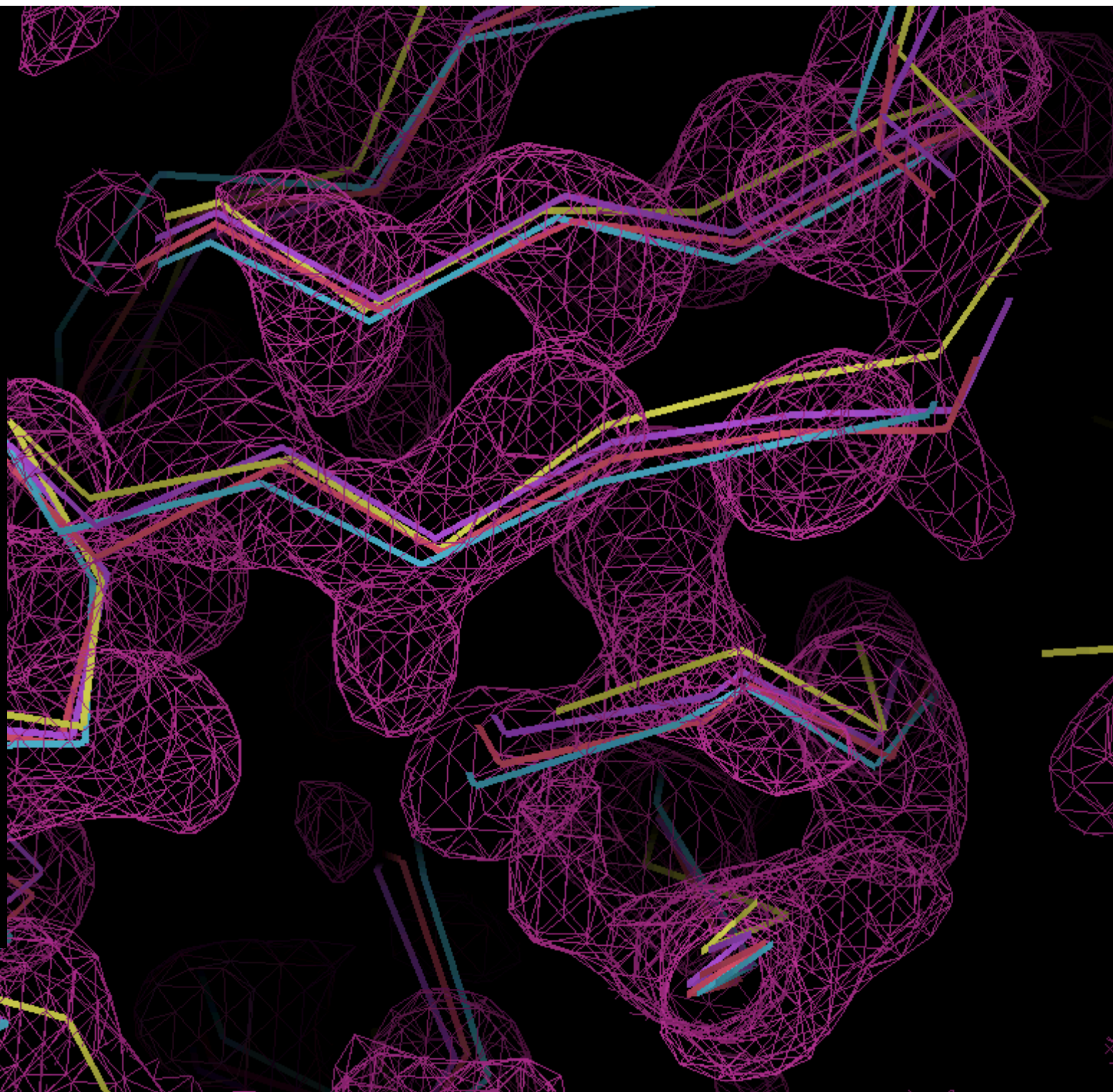
xmrv, 30%
identity
template,
2.0 Å data

Density-
modified map
from Rosetta
model

Yellow: final
model

Blue:
template
(2hs1)

Purple:
Relaxed
Rosetta model



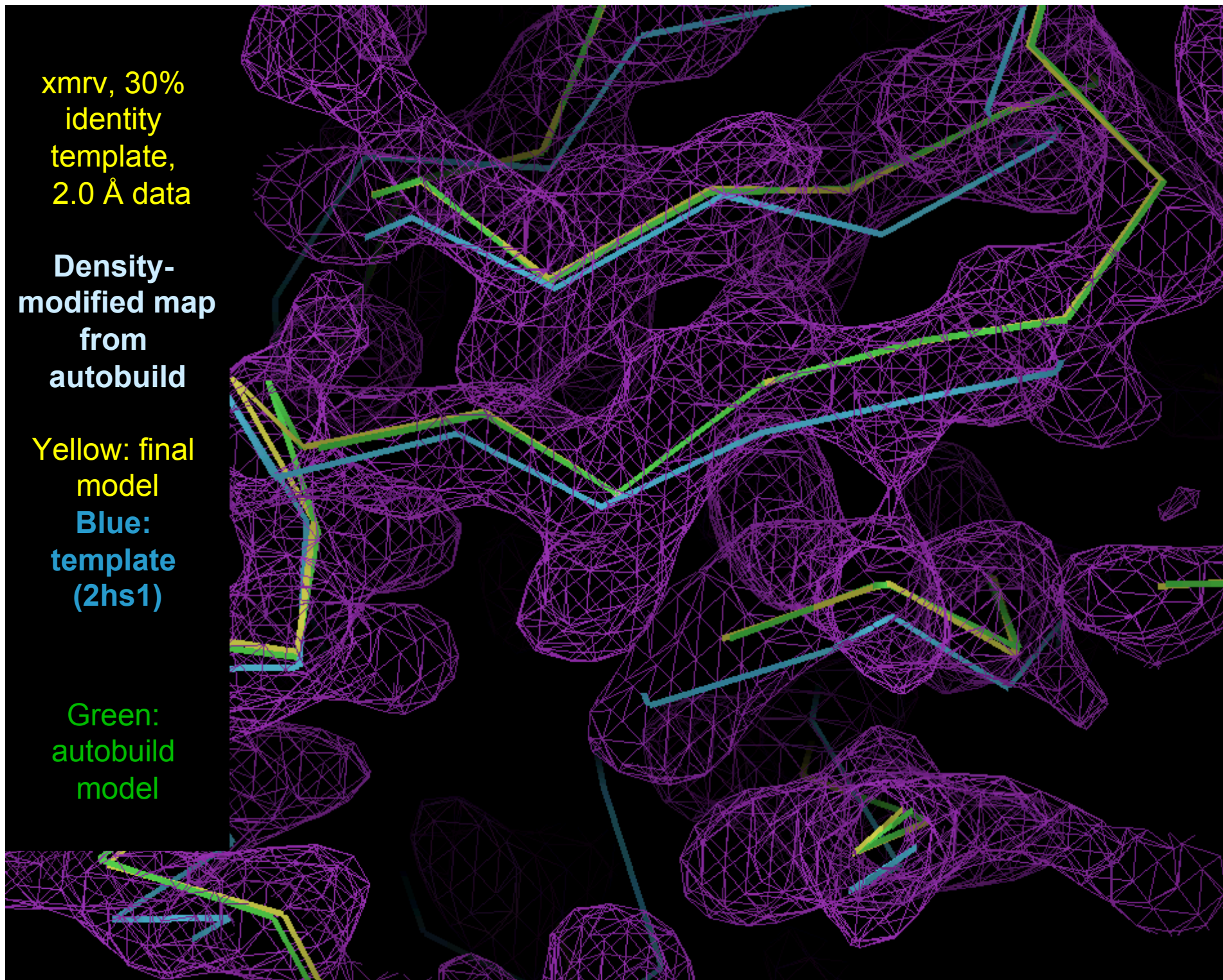
xmrv, 30%
identity
template,
2.0 Å data

Density-
modified map
from
autobuild

Yellow: final
model

Blue:
template
(2hs1)

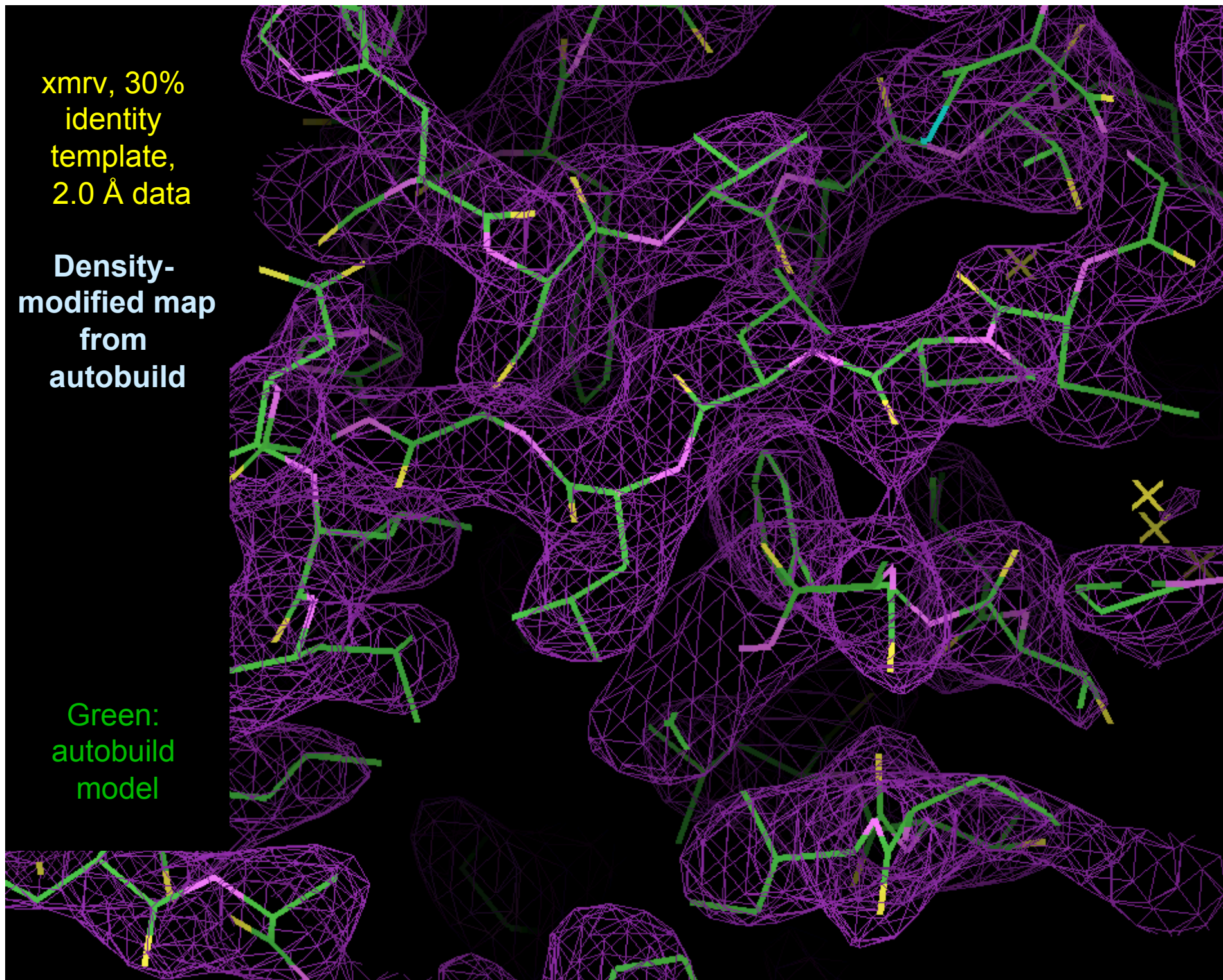
Green:
autobuild
model



xmrv, 30%
identity
template,
2.0 Å data

Density-
modified map
from
autobuild

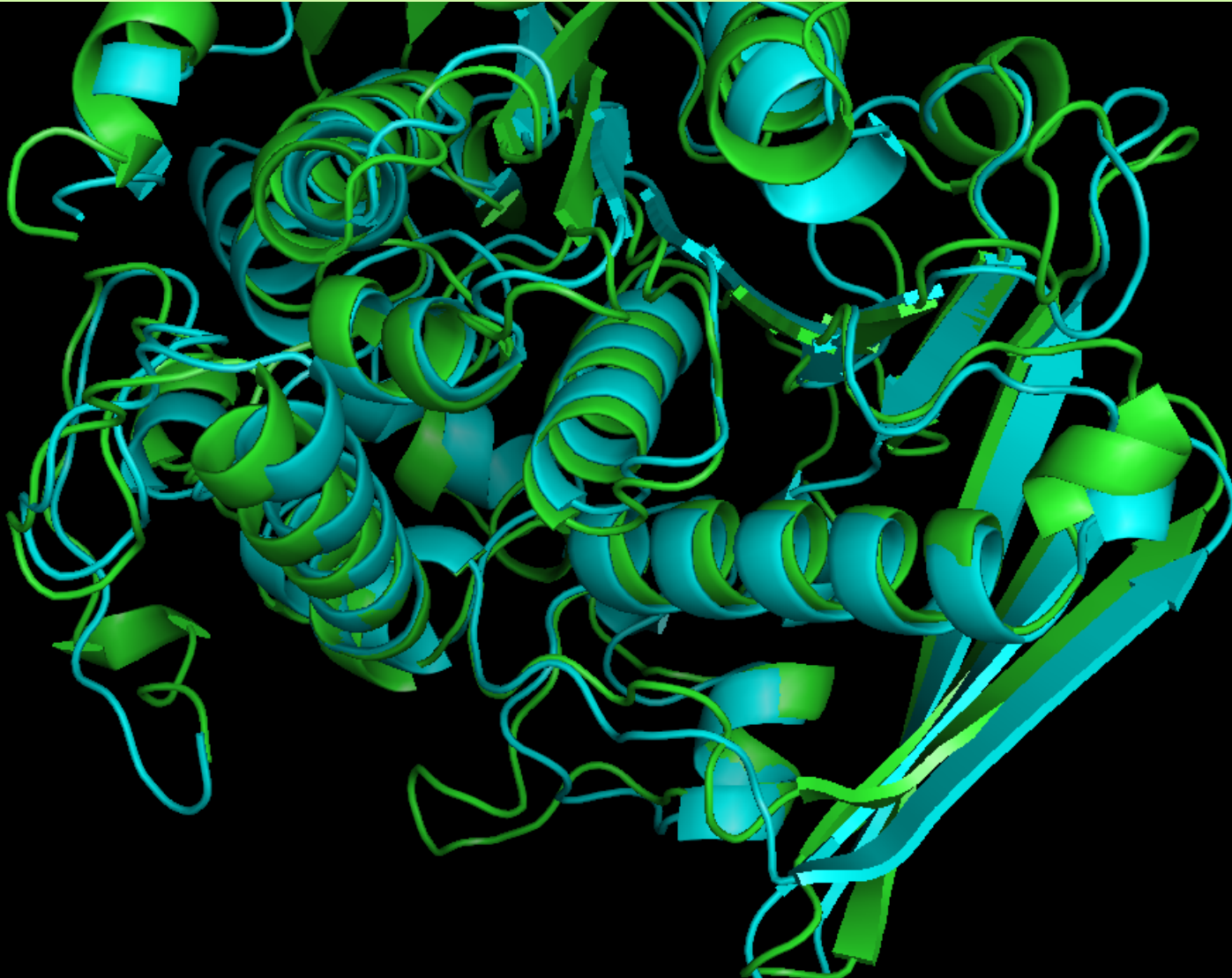
Green:
autobuild
model



Structure determination of cab55348 (using template supplied by user)

1.9 Å, 28% sequence identity (AutoMR alone fails with R/Rfree=0.47/0.53)

MR model: blue, Final model: green



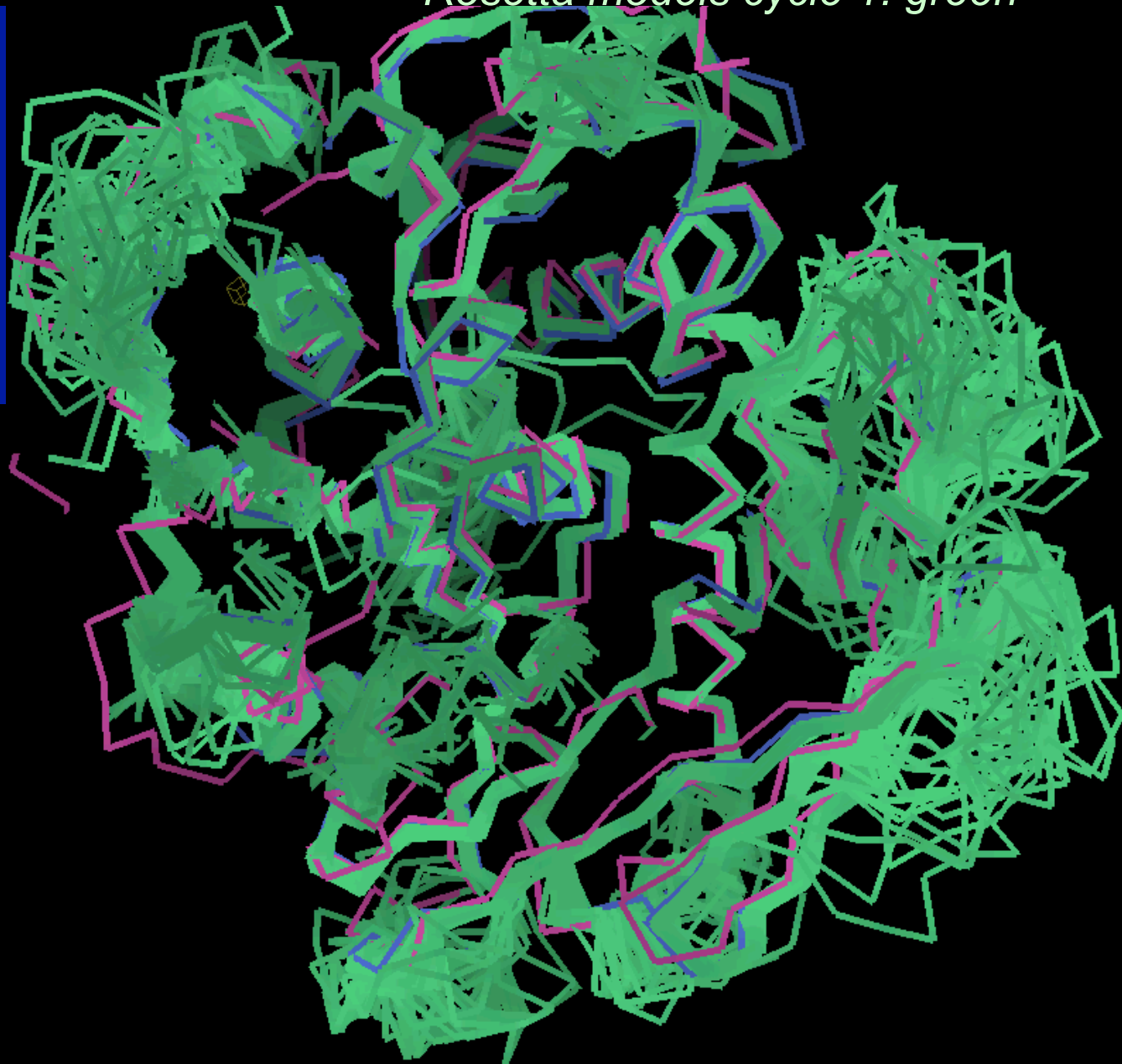
MR model : blue

Final model: pink

Rosetta models cycle 1: green

cab55348

*Sample
Rosetta
models in
cycle 1*



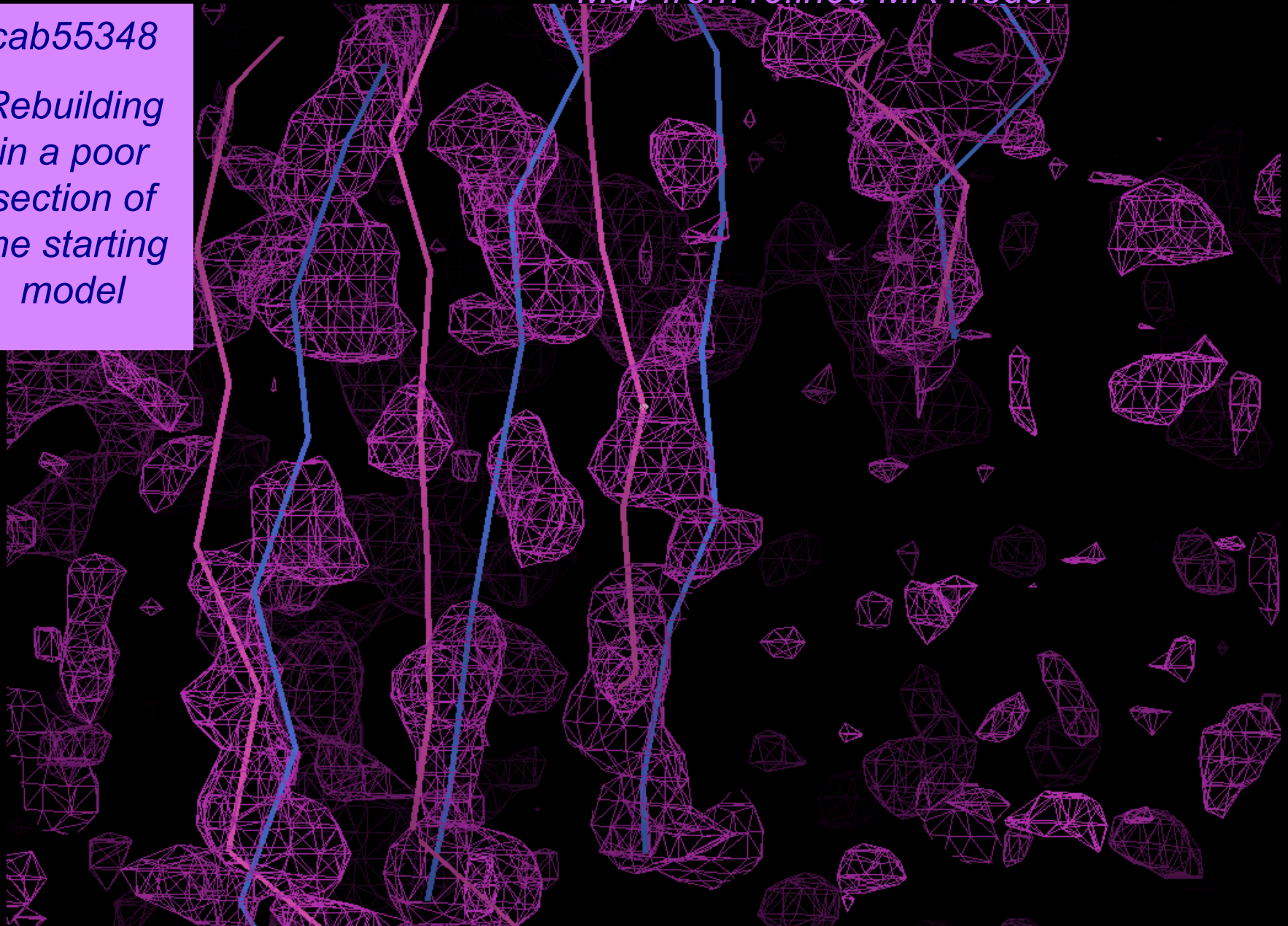
MR model : blue

Final model: pink

Map from refined MR model

cab55348

*Rebuilding
in a poor
section of
the starting
model*



MR model : blue

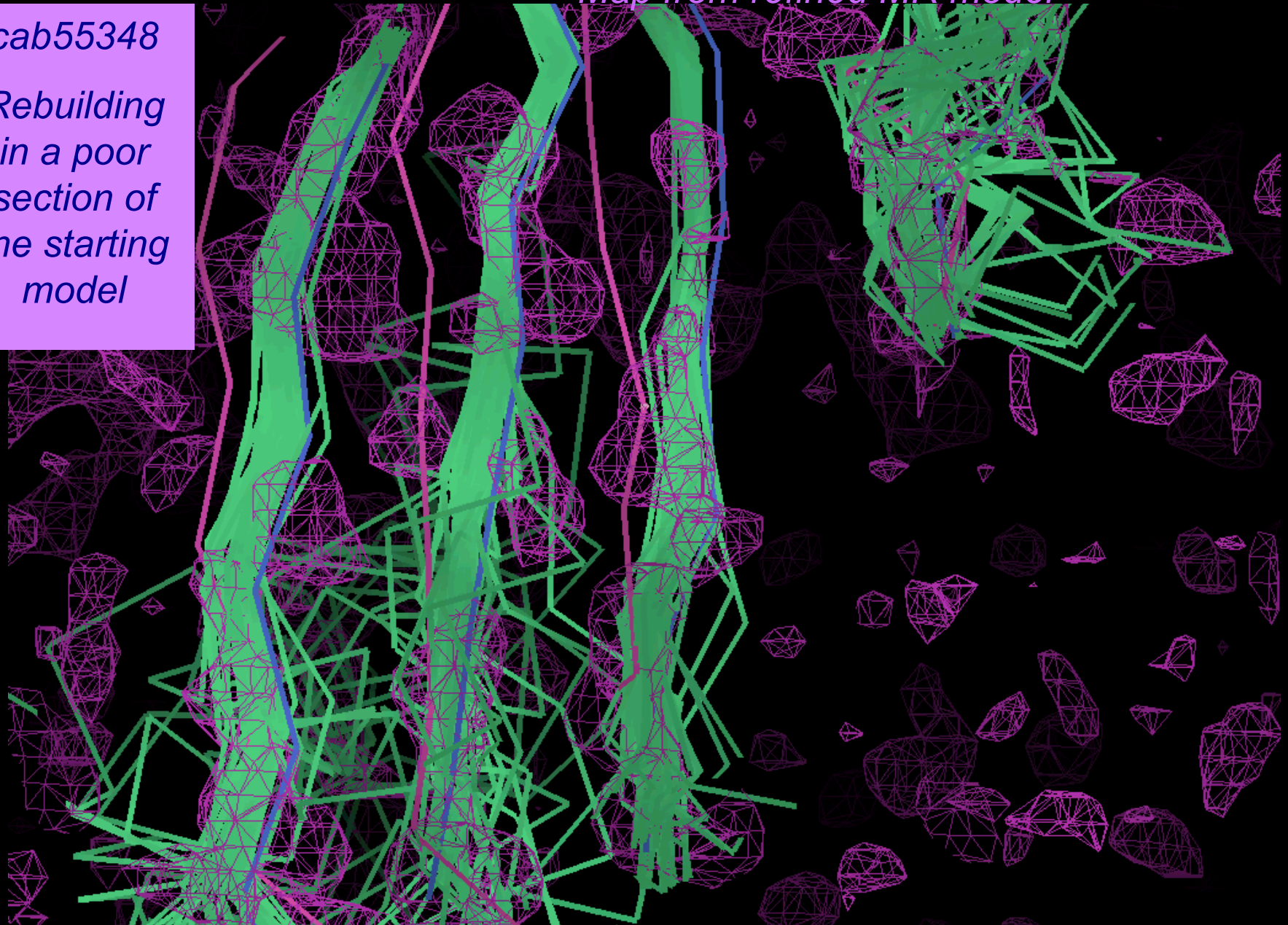
Final model: pink

Rosetta models cycle 1: green

Map from refined MR model

cab55348

*Rebuilding
in a poor
section of
the starting
model*



MR model : blue

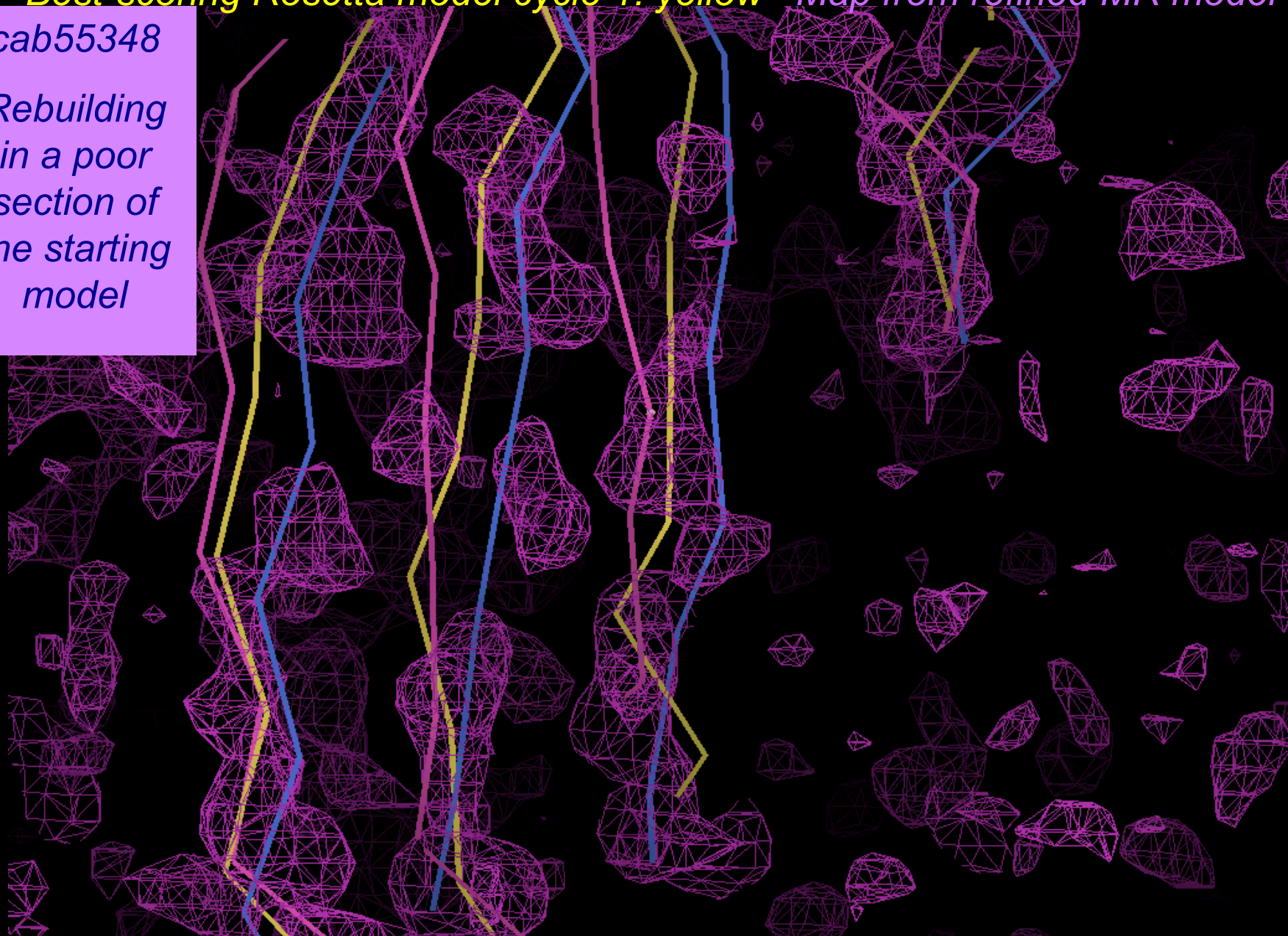
Final model: pink

Best-scoring Rosetta model cycle 1: yellow

Map from refined MR model

cab55348

*Rebuilding
in a poor
section of
the starting
model*



MR model : blue

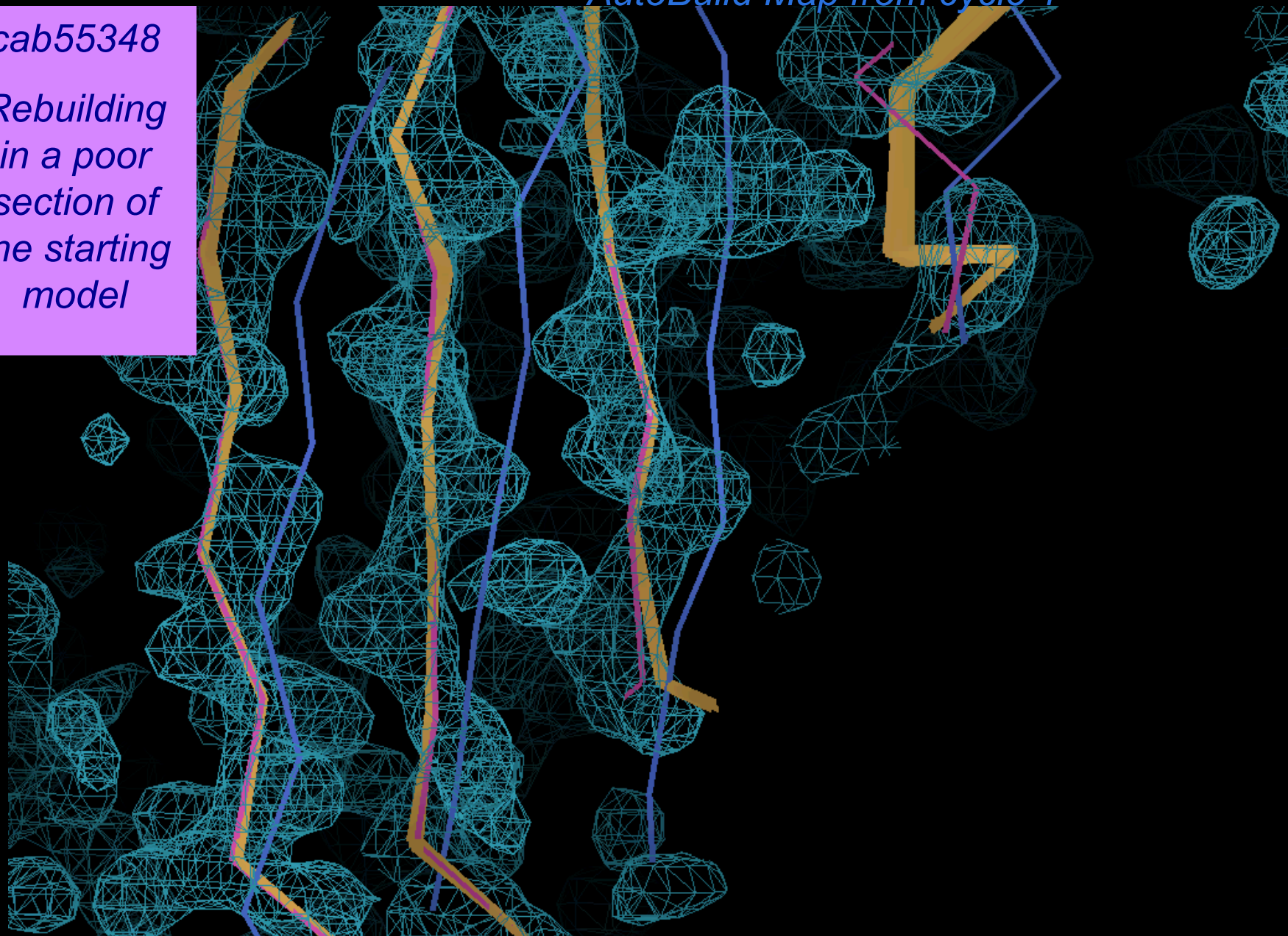
Final model: pink

Rosetta models cycle 2: yellow

AutoBuild Map from cycle 1

cab55348

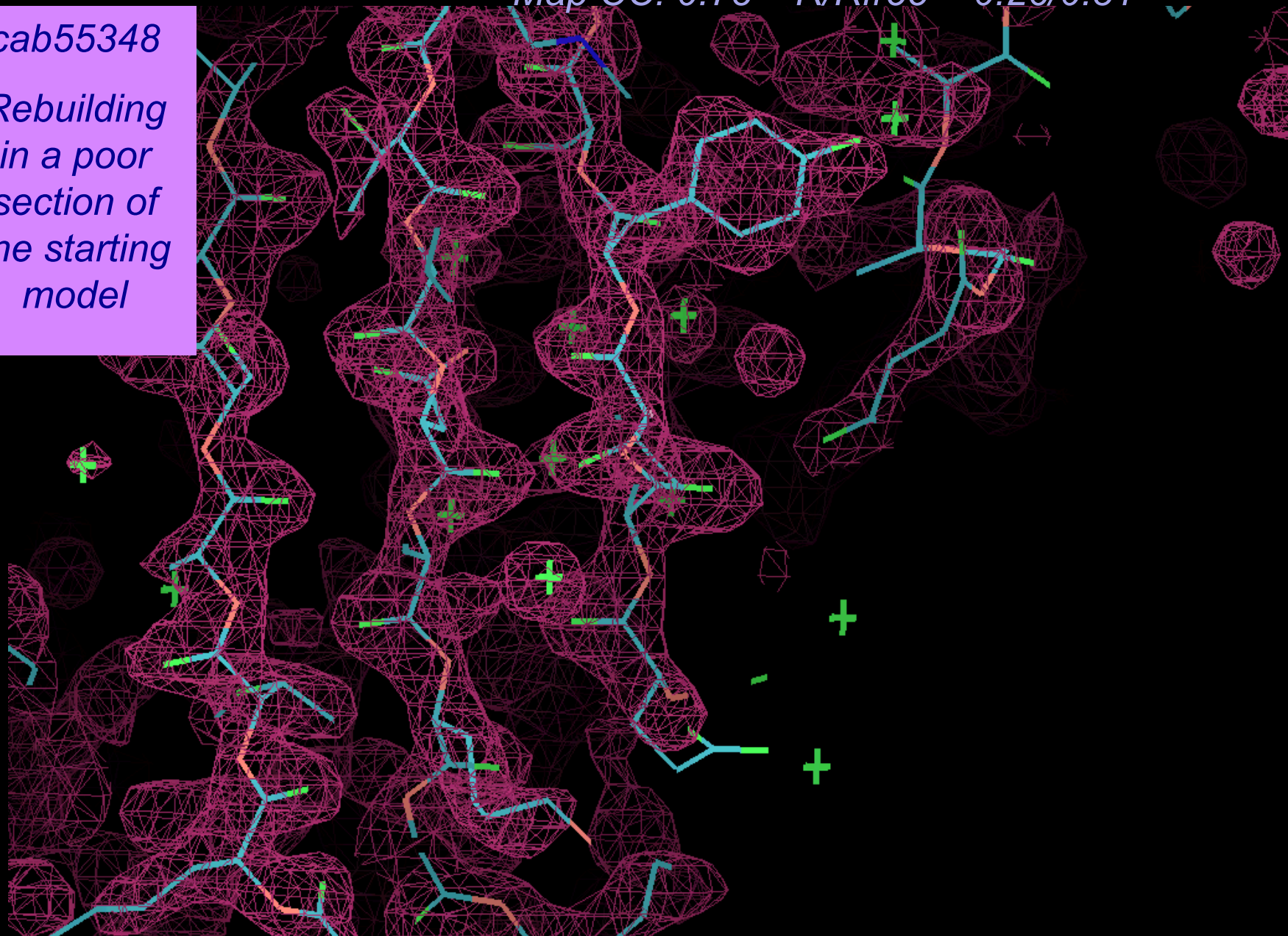
*Rebuilding
in a poor
section of
the starting
model*



AutoBuild model cycle 2

Map CC: 0.78 R/Rfree = 0.26/0.31

cab55348
Rebuilding
in a poor
section of
the starting
model



FreeR values starting with placed templates

| structure | dmin | % ident | ncs | AutoBuild | mr_rosetta |
|------------------|-------------|--------------------|------------|------------------|-------------------|
| ag9603a | 1.7 | 100 | 2 | 0.51 | 0.27 |
| cab55348 | 1.9 | 31 | 1 | 0.52 | 0.23 |
| xmrv | 2.0 | 30 | 2 | 0.57 | 0.34 |
| fk4430 | 2.1 | 22 | 1 | 0.31 | 0.29 |
| thiod | 2.1 | 22/15 | 1 | 0.56 | 0.30 |
| bfr258e | 2.2 | 19 | 2 | 0.29 | 0.28 |
| niko | 2.5 | 27 | 2 | 0.34 | 0.31 |
| estan | 2.5 | 18 | 1 | 0.55 | 0.25 |
| fj6376 | 2.7 | 21 | 4 | 0.30 | 0.30 |
| pc02153 | 2.8 | 29 | 1 | 0.54 | 0.44 |
| pc0265 | 2.9 | 29 | 2 | 0.46 | 0.39 |
| tirap | 3.0 | 22 | 1 | 0.46 | 0.42 |
| hp3342 | 3.2 | 20 | 1 | 0.50 | 0.42 |

Thanks for data to...

Alex Wlodawer, NCI (xmrv)

Herb Axelrod, Debanu Das, JCSG (hp3342)

Gustav Oberdorfer, Ulrike Wagner, Univ. of Graz

Eugene Valkov, Univ. of Cambridge

Assaf Alon, Deborah Fass, Weizmann Institute of Science

Sergey M. Vorobiev, NESG

Hideo Iwai, Univ. of Helsinki

P. Raj Pokkuluri, Argonne National Laboratory



Data and scripts for phenix.mr_rosetta
are available at...

[http://www.phenix-online.org/phenix_data/terwilliger/
rosetta_2011/](http://www.phenix-online.org/phenix_data/terwilliger/rosetta_2011/)

The PHENIX Project



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Paul Adams, Ralf Grosse-Kunstleve, Pavel Afonine, Nat Echols, Nigel Moriarty, Jeff Headd, Nicholas Sauter, Peter Zwart



Los Alamos National Laboratory

Tom Terwilliger, Li-Wei Hung



Randy Read, Airlie McCoy, Gabor Bunkoczi, Rob Oeffner

Cambridge University



Duke University

Jane & David Richardson, Vincent Chen, Chris Williams, Bryan Arendall, Swati Jain, Bradley Hintze



*An NIH/NIGMS funded
Program Project*