SAD Phasing and automated structure determination

***Phenix* workshop**

Shanghai, China
Jan. 14, 2016

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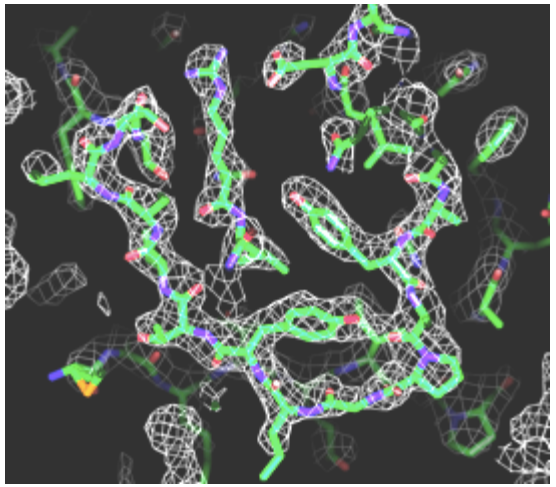
Los Alamos National Laboratory

Steps in Single Wavelength Anomalous Diffraction (SAD) Structure Determination



- Plan the experiment
- Measure the data
- Scale the data
- Evaluate the accuracy of the anomalous differences
- Find the anomalous sub-structure
- Identify hand of sub-structure
- Calculate experimental phases and a map
- Improve the map with density modification
- Build and refine a model

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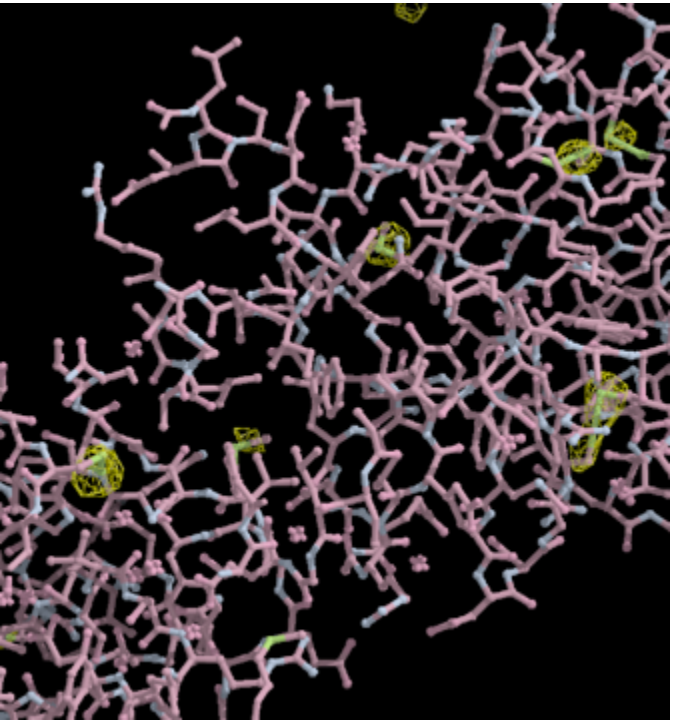
Planning a SAD experiment

Will I find the sites of anomalously-scattering atoms?



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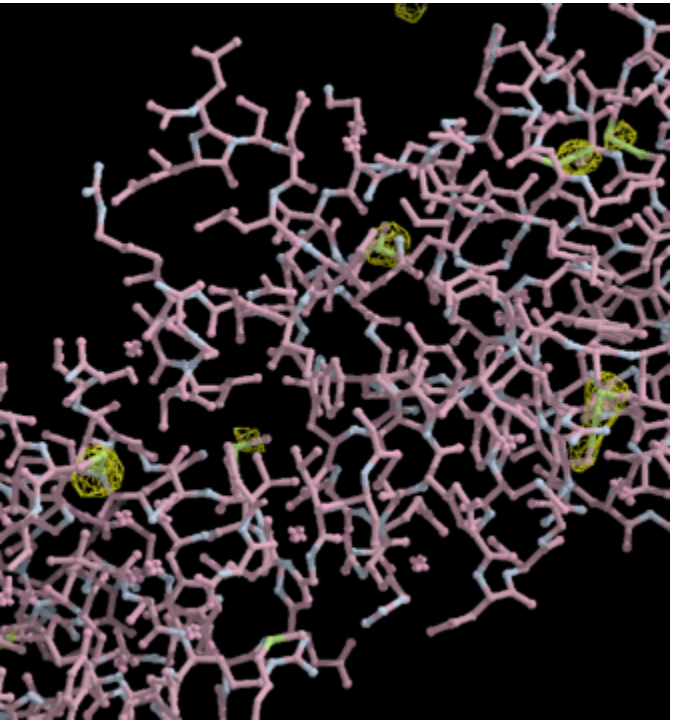
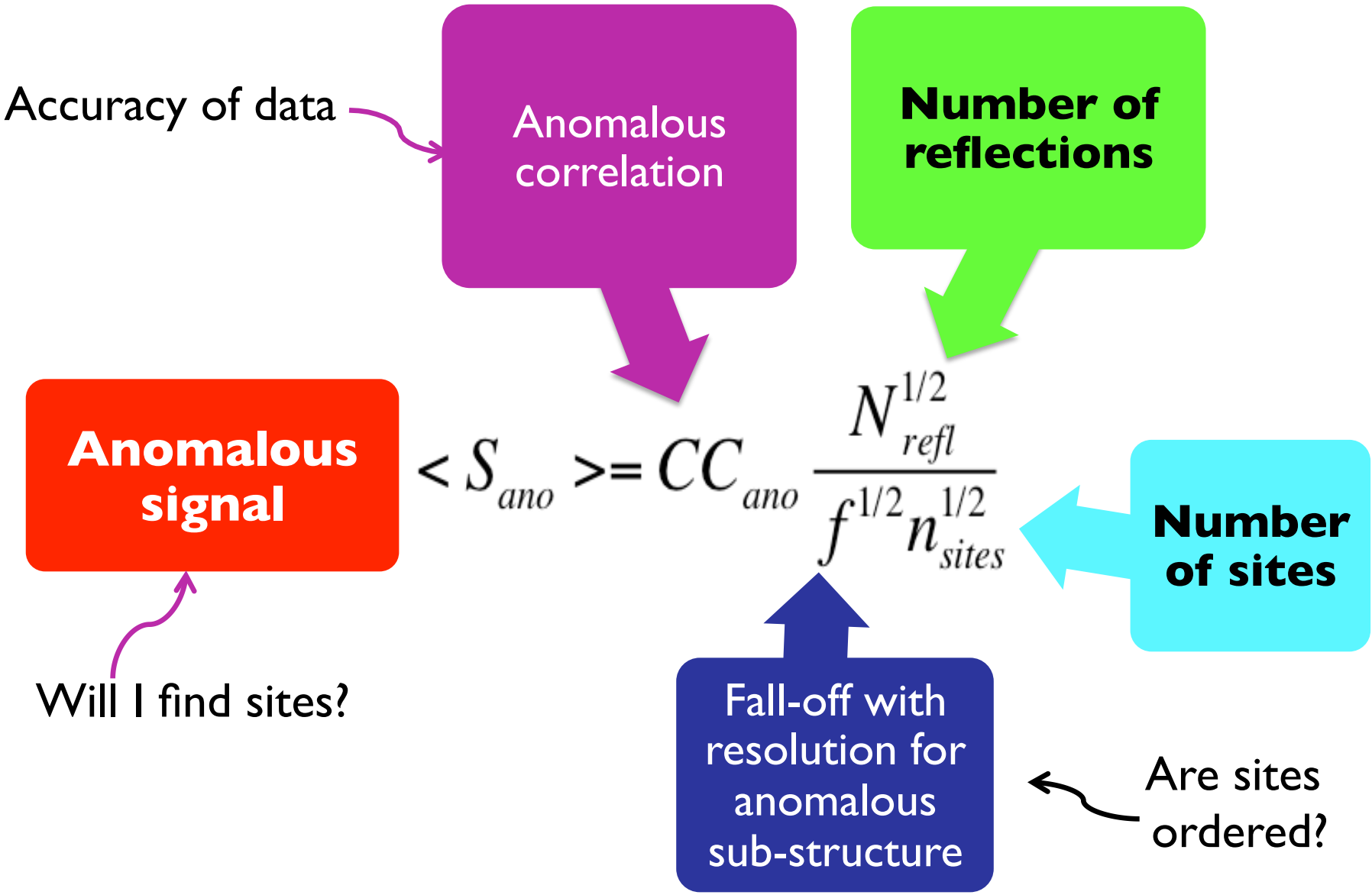
Planning a SAD experiment



How many sites?
How many reflections?

Are the sites (on average) well ordered?
Are the data well-measured?

What determines if I will find the sites?



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Maximizing the anomalous signal and the anomalous correlation

*The **anomalous correlation** is a measure of the accuracy of each anomalous difference*

*The **anomalous signal** is a measure of how much total information is present in the anomalous differences*

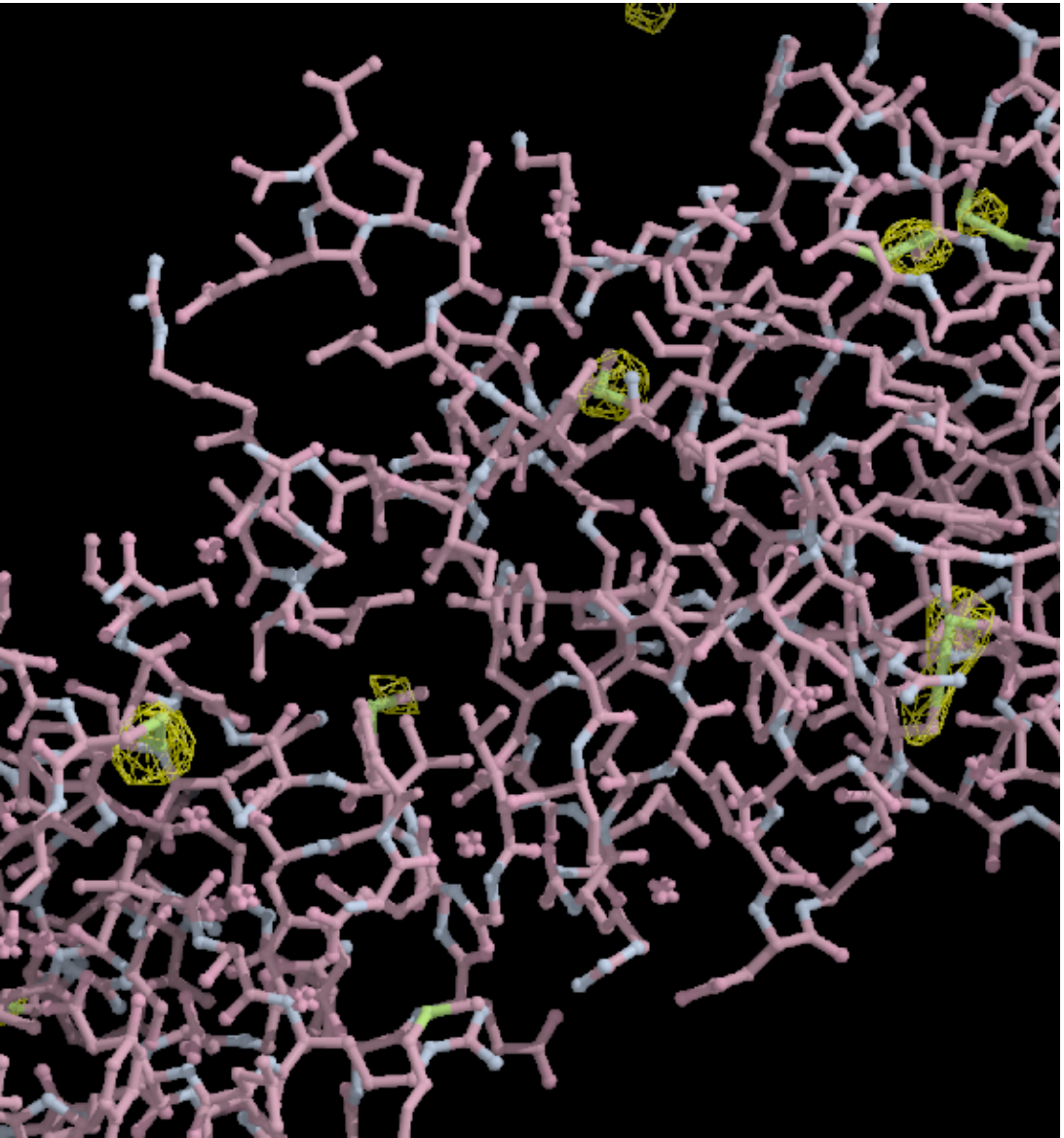
Anomalous correlation: accuracy of anomalous differences

Correlation of observed and sub-structure anomalous differences

$$CC_{ano} \equiv \frac{<\Delta_{ano,j}\Delta^{obs}_{ano,j}>}{<\Delta^{2}_{ano}>^{1/2}<\Delta^{2,obs}_{ano}>^{1/2}}$$

CC_{ano} indicates how much of each anomalous difference is useful (on average)

Anomalous signal: peak height in anomalous difference Fourier at coordinates of anomalously-scattering atoms



$$S_{ano} = \frac{< \rho_{ano}(x_j) >}{< \rho_{ano}^2 >^{1/2}}$$

Typical values of S_{ano} for solved datasets: 10-20

Anomalous difference Fourier with observed data and model phases

How big will my anomalous signal be?

Expected value of anomalous signal S_{ano}

$$< S_{ano} >= CC_{ano} \frac{N_{refl}^{1/2}}{f^{1/2} n_{sites}^{1/2}}$$

f is 2nd moment of the anomalous scattering factor
(accounts for weak high-resolution data)

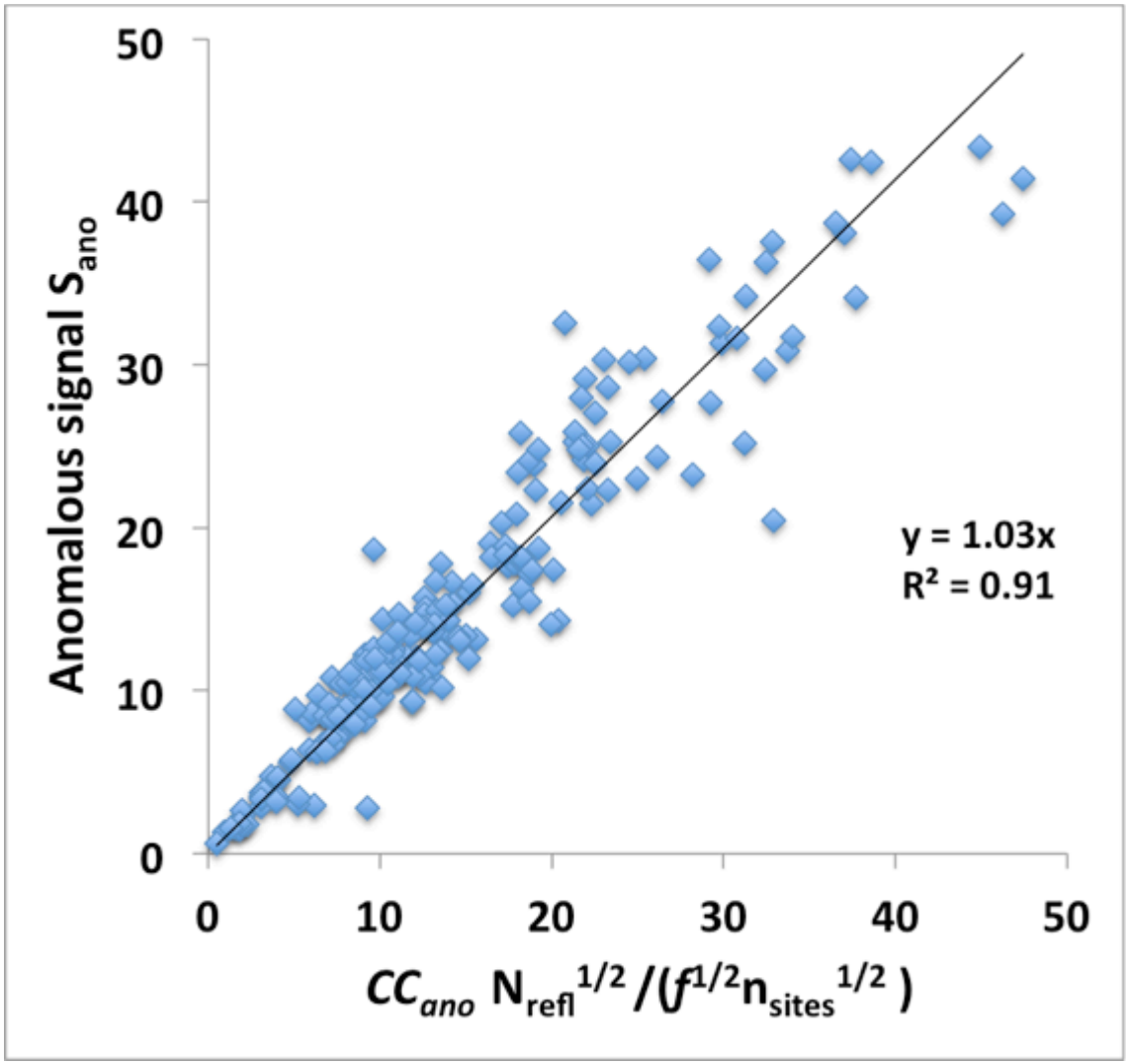
$$f = \frac{< (f^h)^2 >}{< f^h >^2}$$

Anomalous scattering factor

$$f^h \equiv f'' e^{-B\ (\sin^2 \theta_h / \lambda^2)}$$

Perfect data (20,000 reflections, 8 sites): S_{ano}= (20000/8)$^{1/2}$ = 50
Good data (overall CC_{ano}=0.36 f=2.0): S_{ano}= 12.6

Checking our simple model for anomalous signal



$$< S_{ano} >= CC_{ano} \frac{N_{refl}^{1/2}}{f^{1/2} n_{sites}^{1/2}}$$

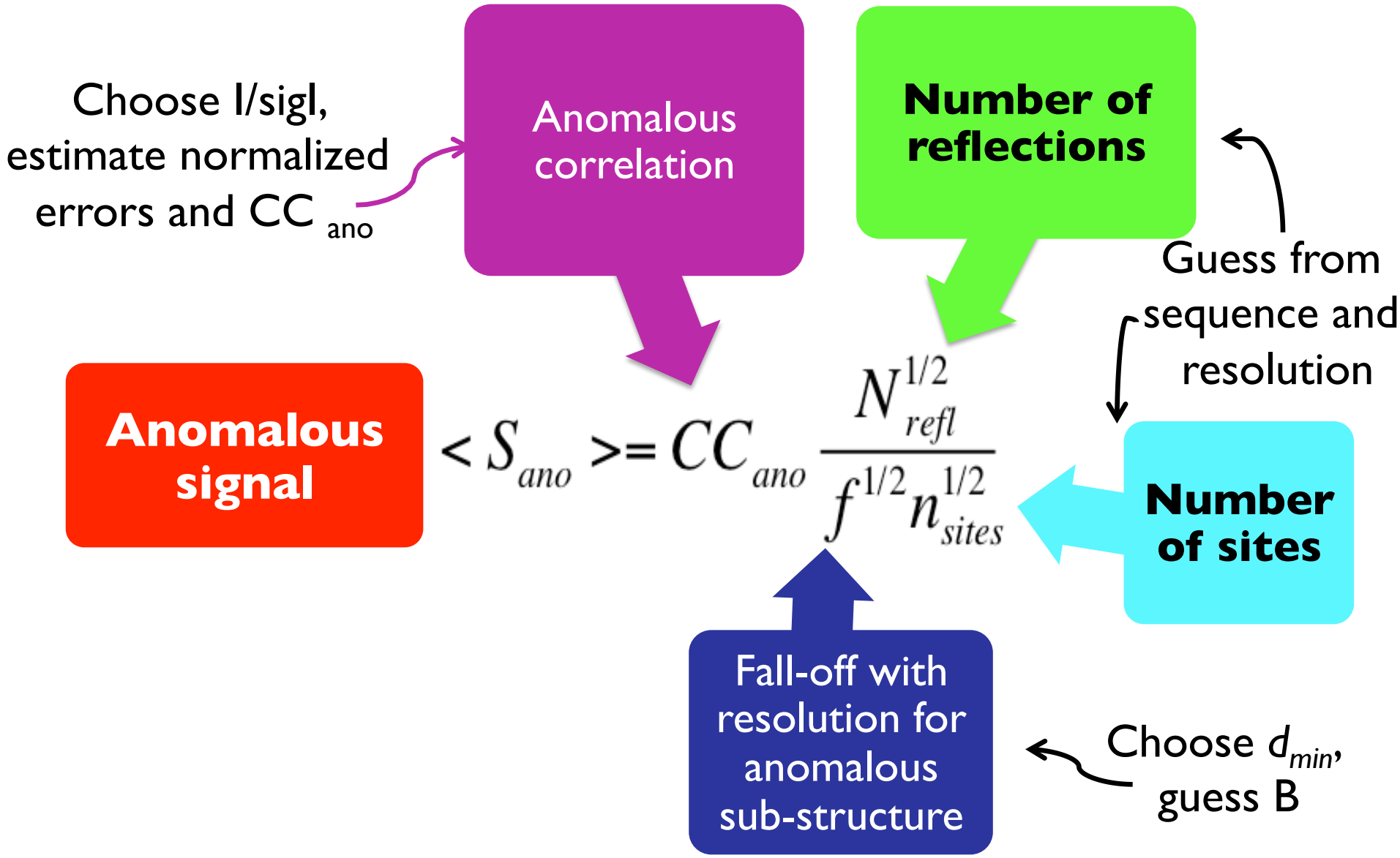
CC_{ano}: Correlation of anomalous differences with model differences

S_{ano}: Peak height in model-phased difference Fourier

218 SAD datasets 1.2 – 4.5 Å

phenix.plan_sad_experiment

Design an experiment that will give you enough anomalous signal



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Finding the anomalous sub-structure

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Using the SAD likelihood function to find sites

"The likelihood of measuring the observed

anomalous data

given

a potential sub-structure"

Using the SAD likelihood function to find the anomalous sub-structure

Start with guess about the anomalous sub-structure

From anomalous difference Patterson

Random

Any other source

Find additional sites that increase the likelihood

LLG completion based on log-likelihood gradient maps*

Iterative addition of sites

Related to using an anomalous difference Fourier—but better

*La Fortelle, E. de & Bricogne, G. (1997). Methods Enzymol. 276, 472-494
McCoy, A. J. & Read, R. J. (2010). Acta Cryst. D66, 458-469.

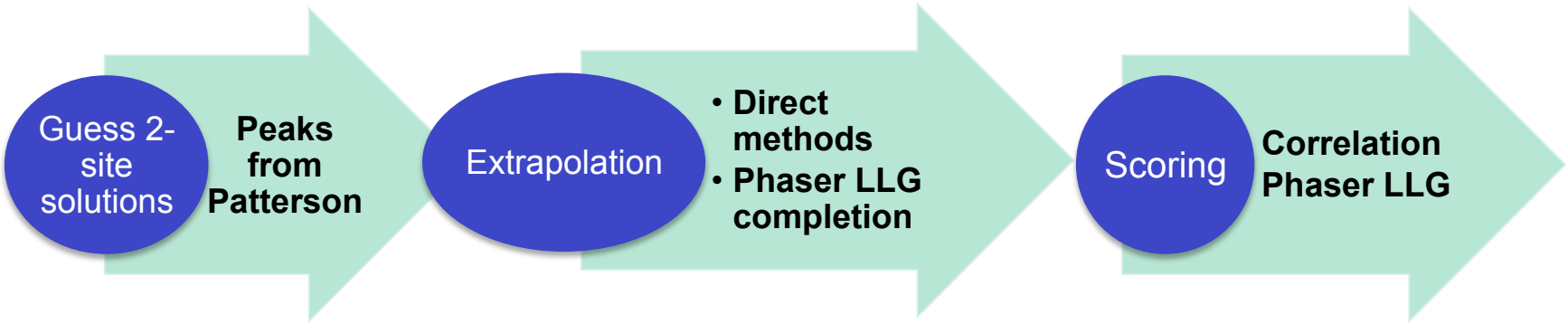
LLG sub-structure searches in Phenix

Test cases

164 SAD datasets from PDB (largely JCSG MAD data)

Using peak, remotes, inflection as available to include data with low anomalous signal

Finding anomalous substructure with LLG completion



- **Range of resolution**
  Variable number of Patterson solutions

Adjustable LLGC_SIGMA

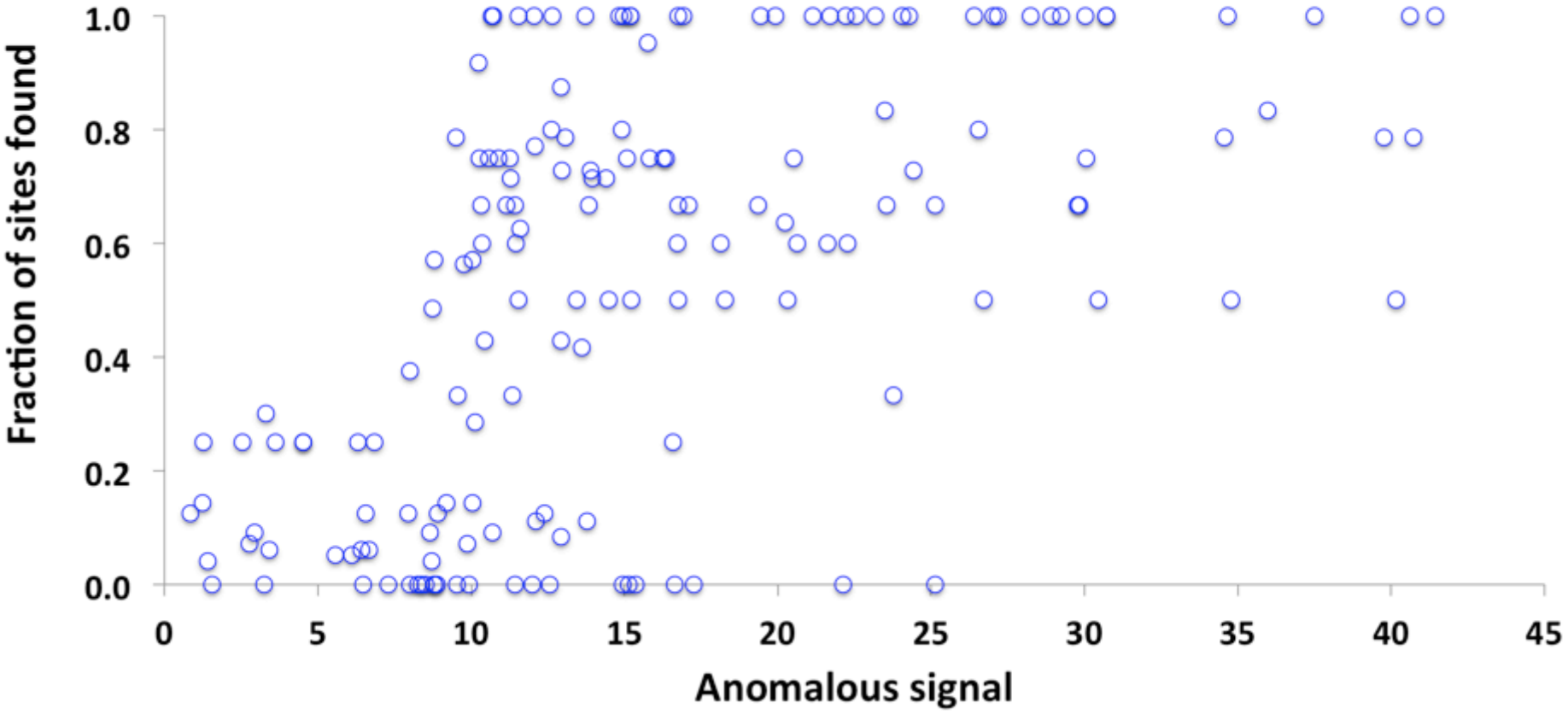
(cut-off for peak height)

Use LLG score to compare solutions

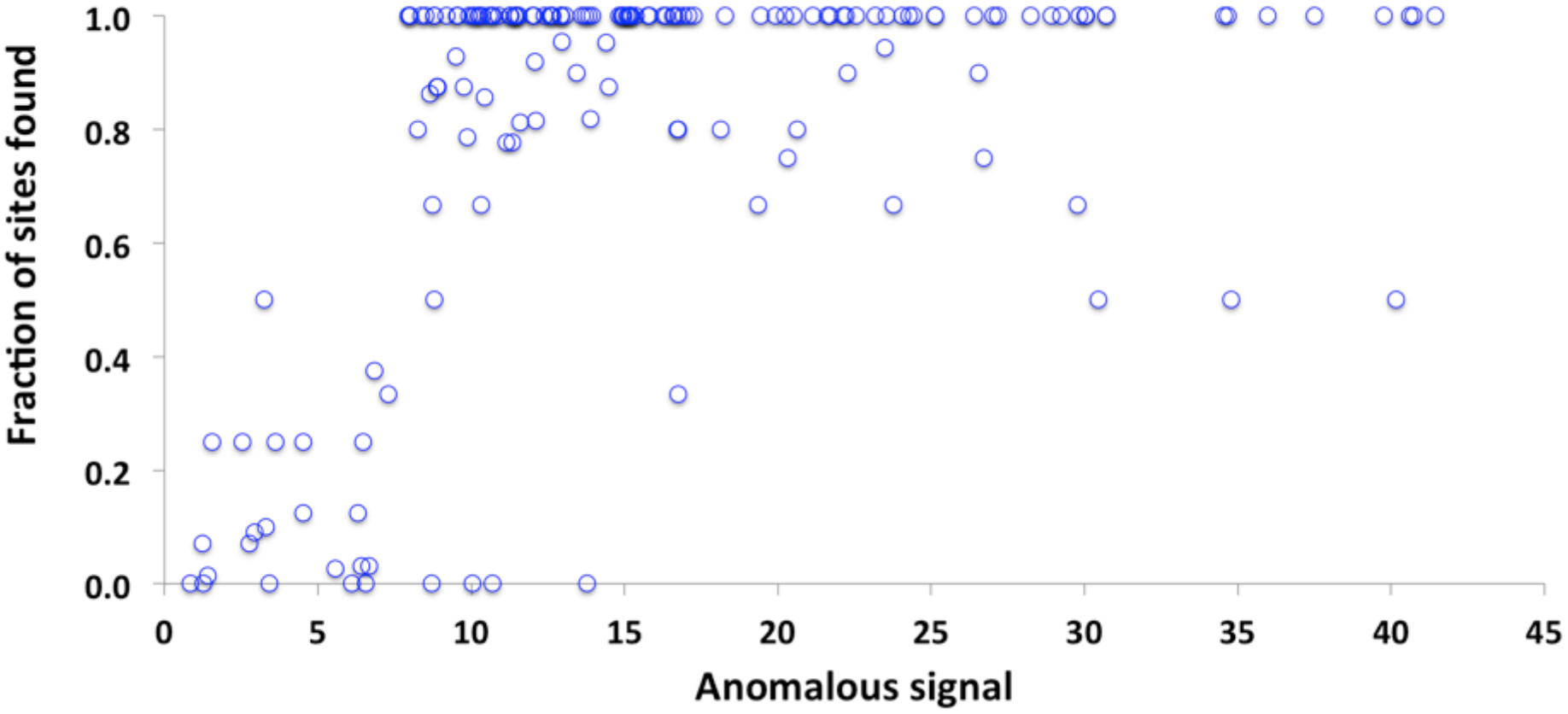
Terminate early if same solution found several times

Run quick direct methods first

Dual Space Sub-structure Completion

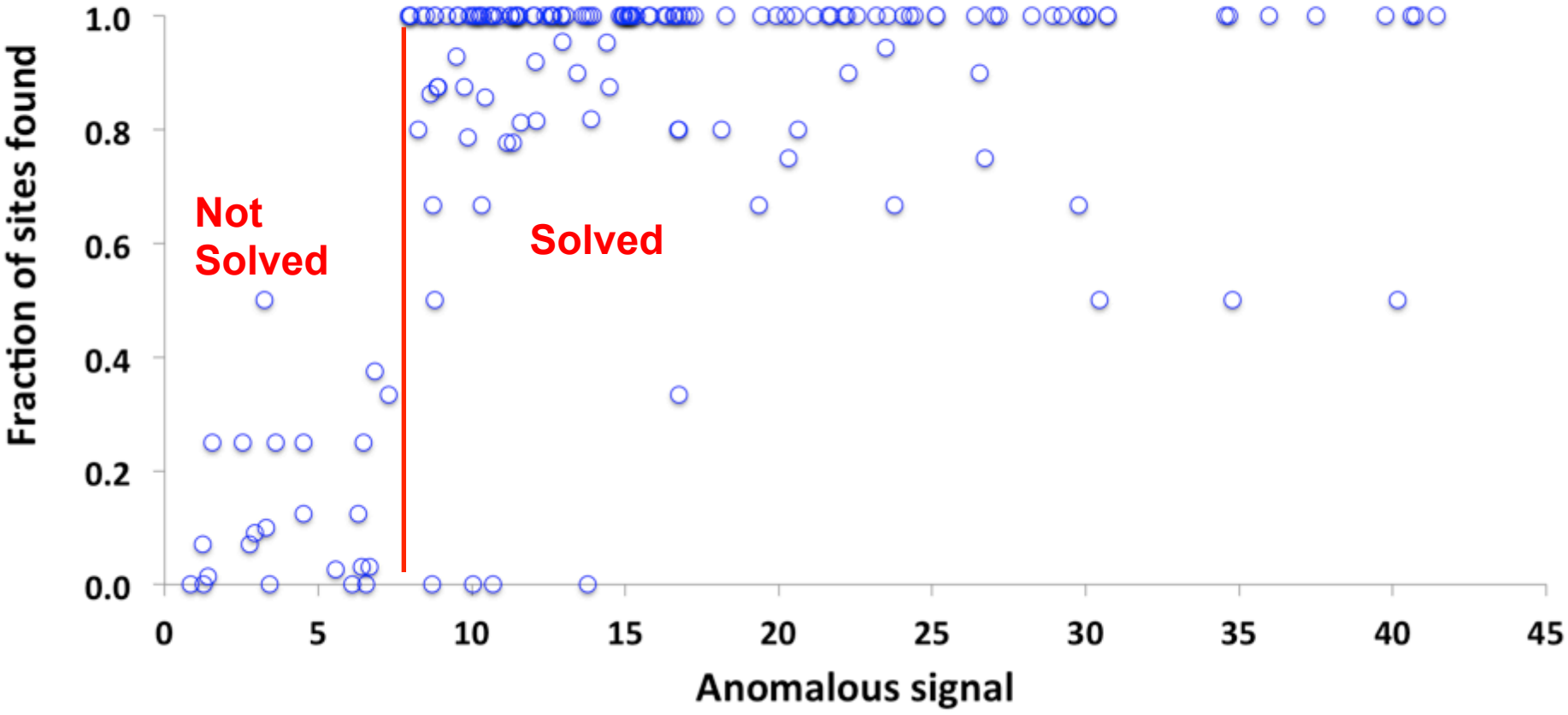


LLG Sub-structure Search



Bunkóczi et al., Nature Methods 12, 127–130 (2015).

Anomalous signal indicates if a dataset can be solved



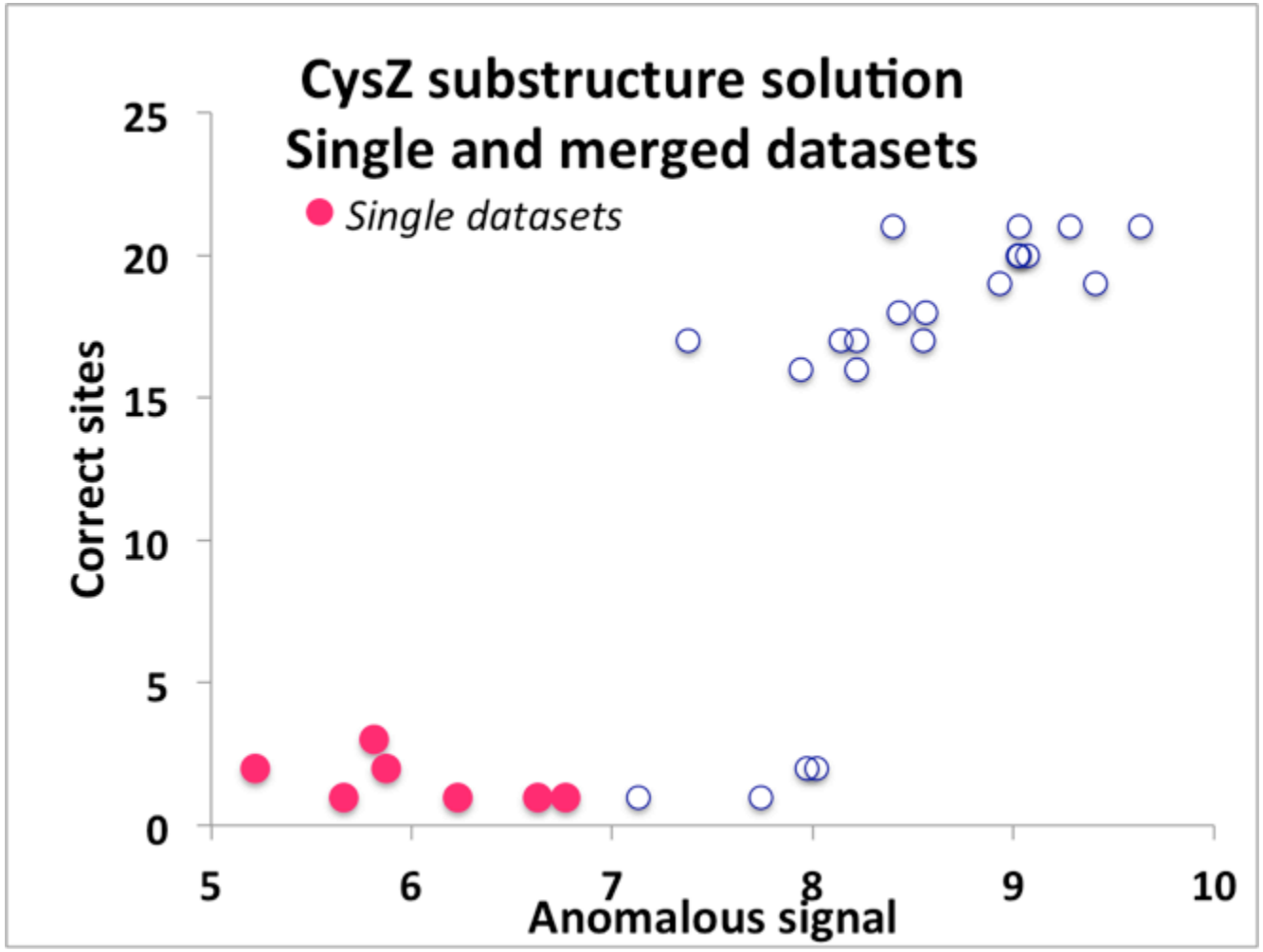
CysZ multi-crystal sulfur-SAD data

Qun Liu, Tassadite Dahmane, Zhen Zhang, Zahra Assur, Julia Brasch, Lawrence Shapiro, Filippo Mancia, Wayne Hendrickson (2012). Science 336, 1033-1037

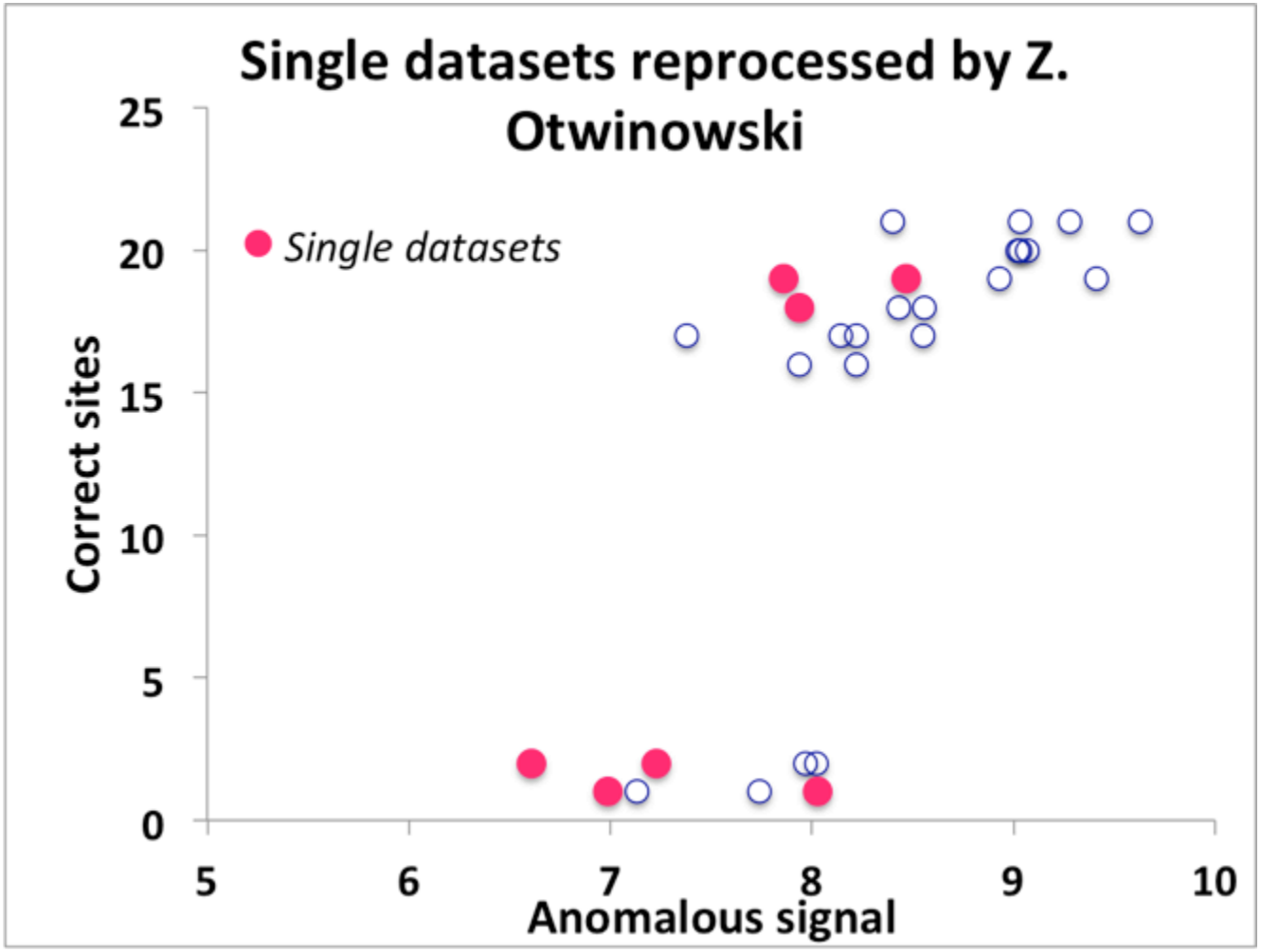
Data from 7 crystals collected at wavelength of 1.74 Å to resolution of 2.3 Å

Can anomalous signal tell us which merged datasets will be solved?

CysZ multi-crystal sulfur-SAD data

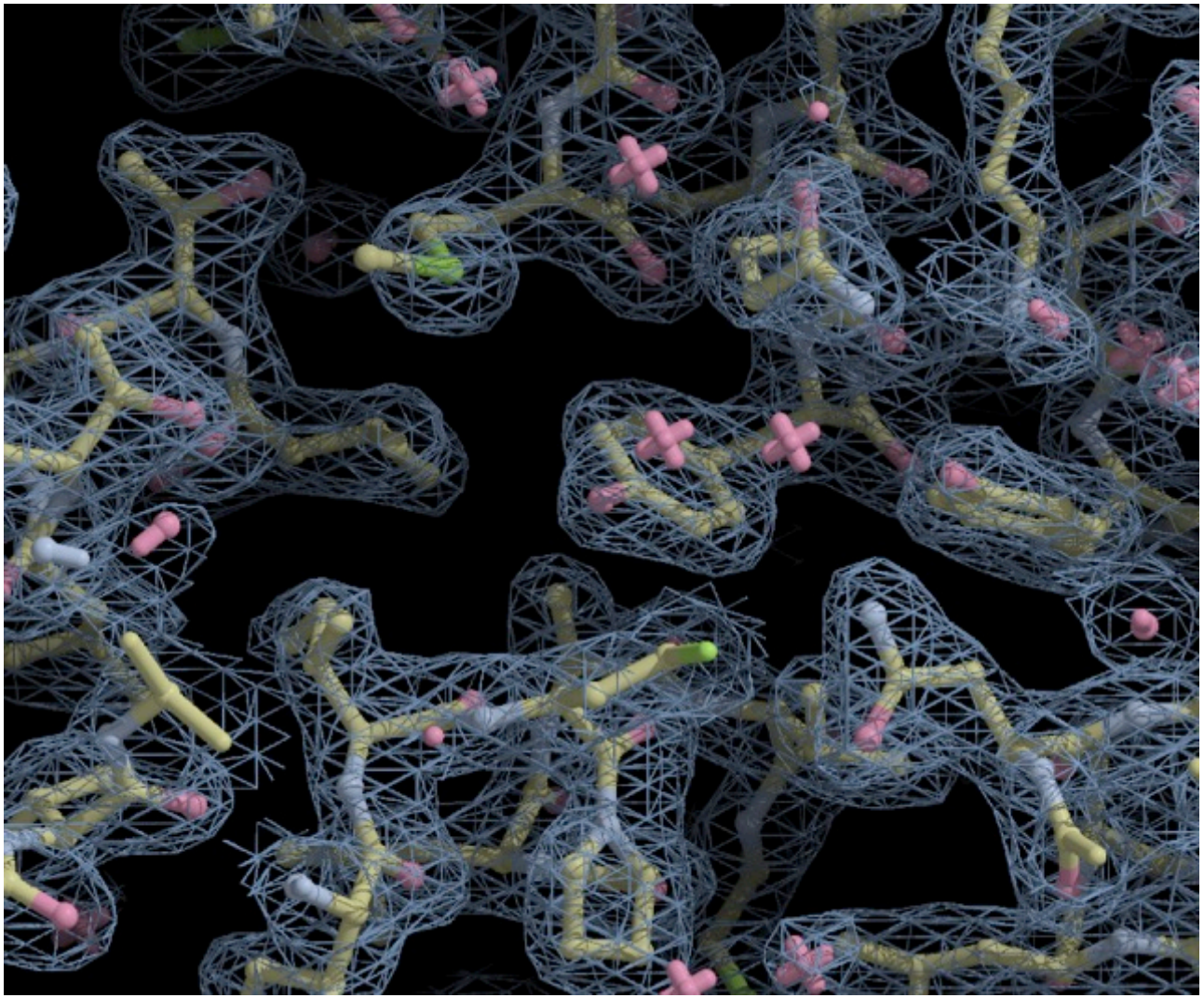


CysZ multi-crystal sulfur-SAD data



CysZ single-crystal sulfur-SAD data

Crystal 6 *AutoSol R/Rfree=0.24/0.27*



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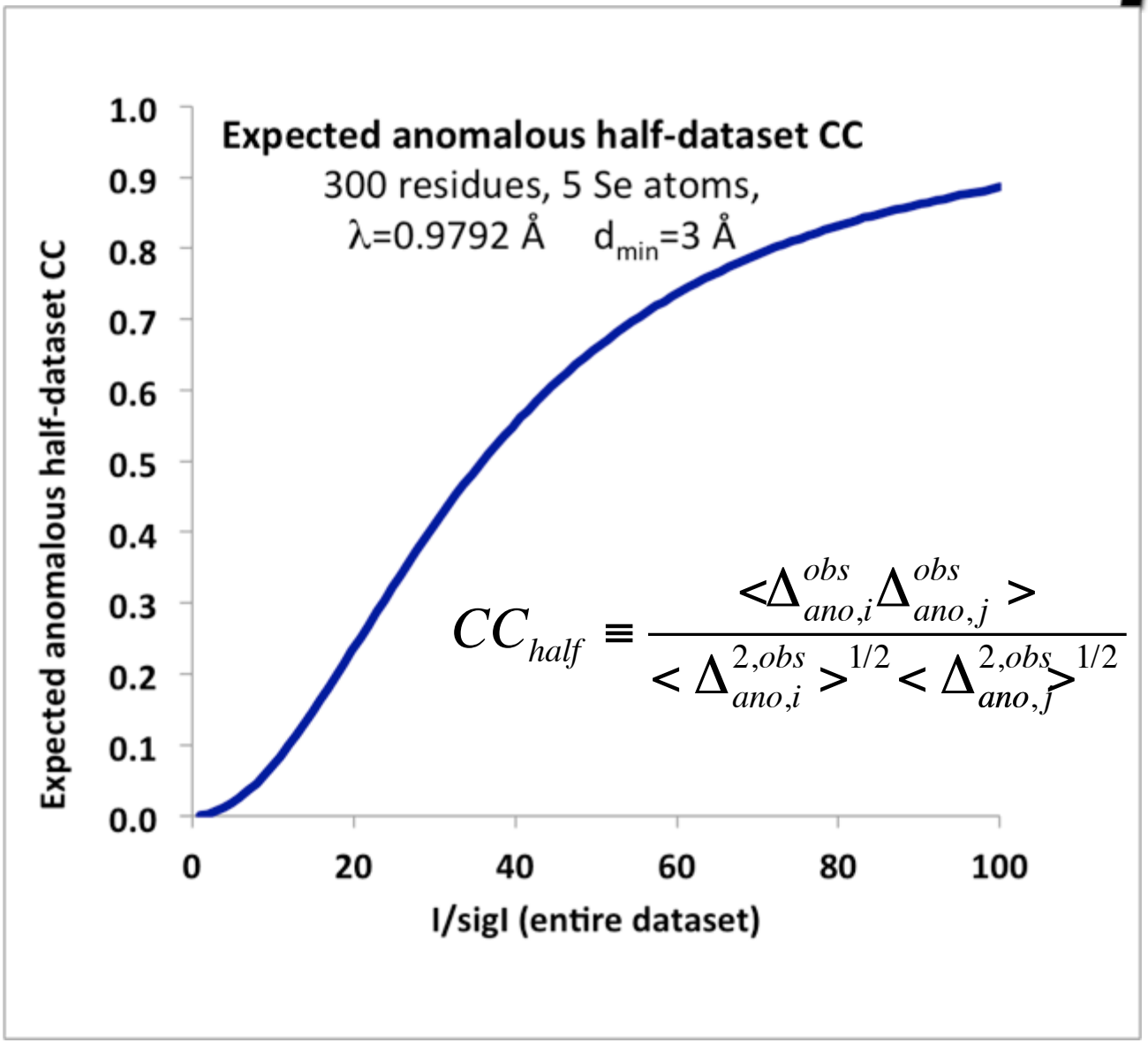
Will I solve my structure?

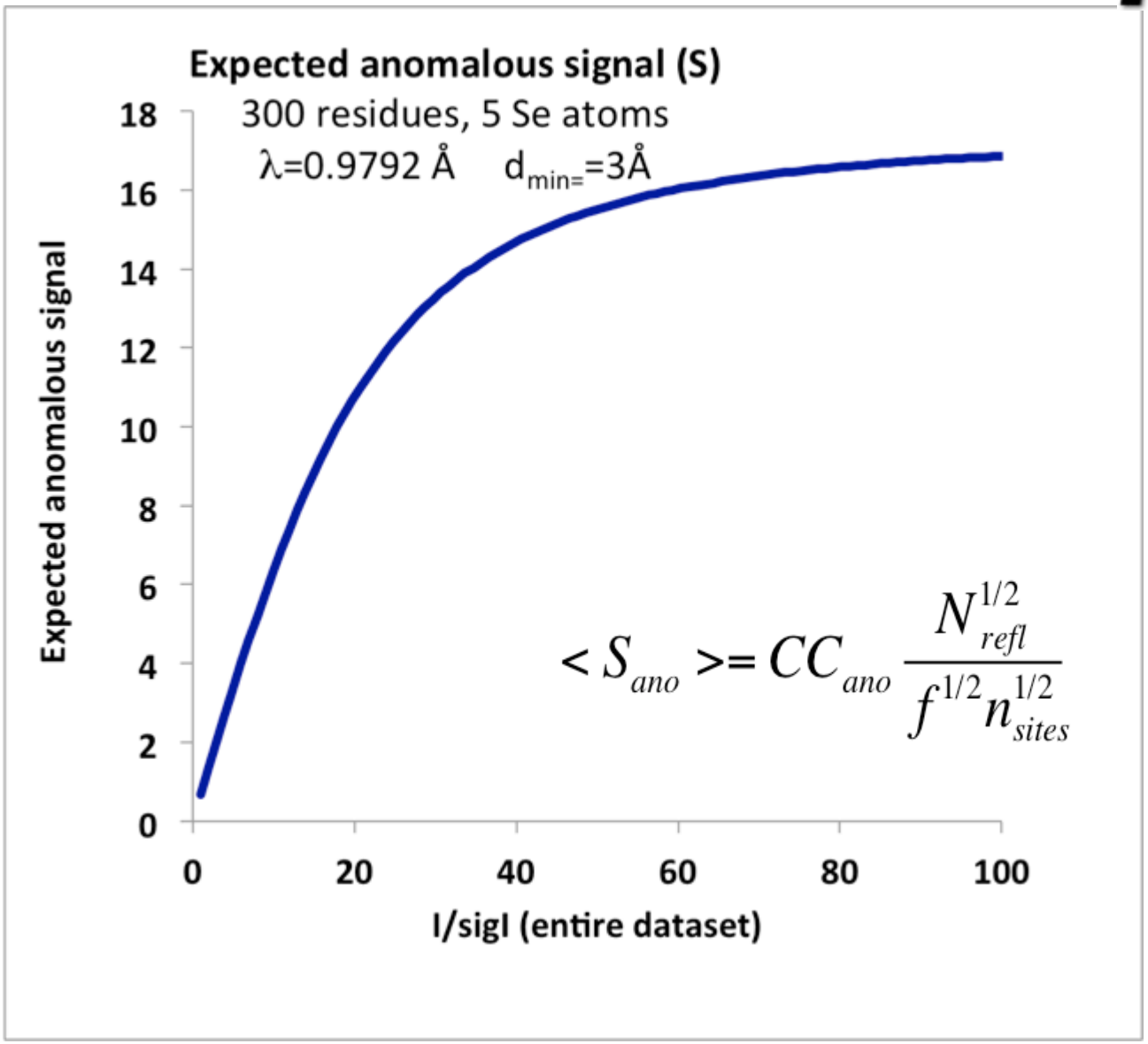
Simulate experiment with
phenix.plan_sad_experiment based on:

- I/sigma (errors in measurement)
- Anomalously-scattering atom (f")
- Sequence (other atoms)
- Resolution of data
- Number of sites

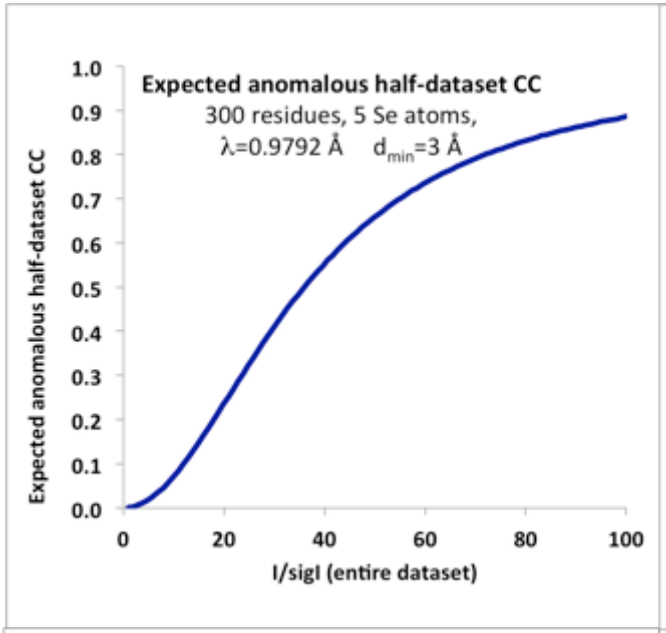
Anomalous data quality depends on I/sigI

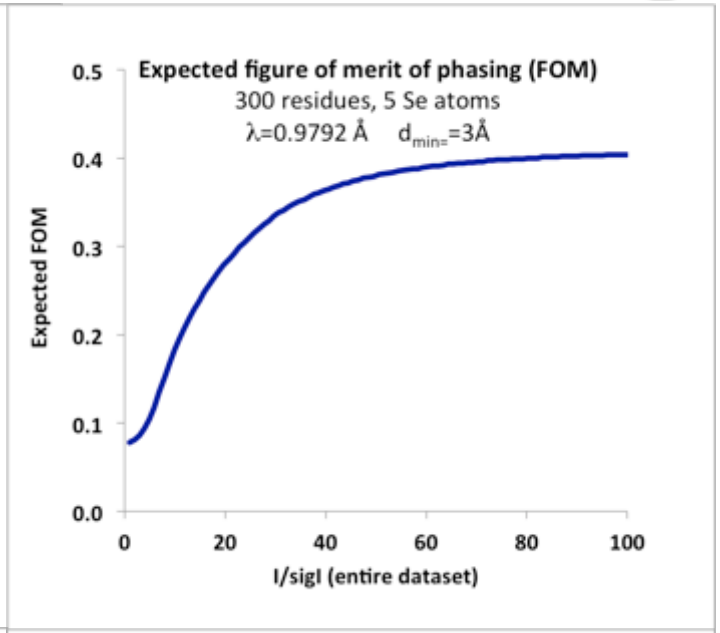


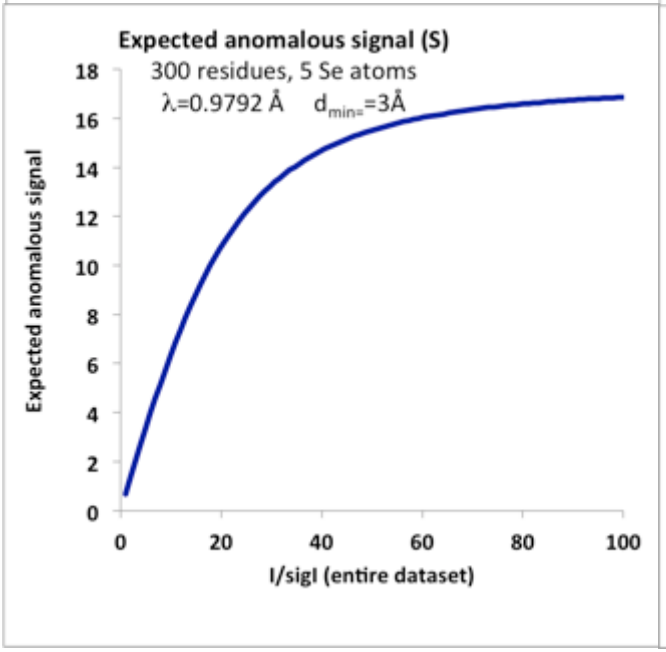
Anomalous data quality depends on I/sigI

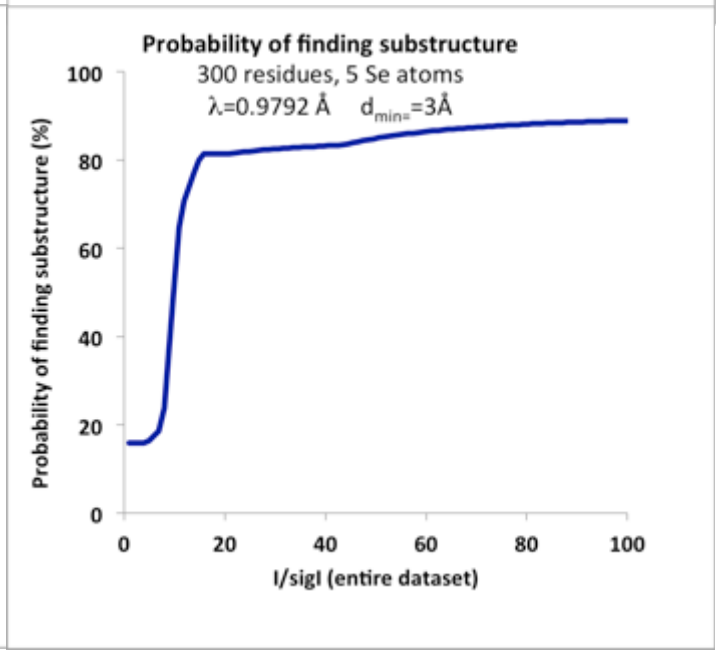


Anomalous data quality depends on I/sigI

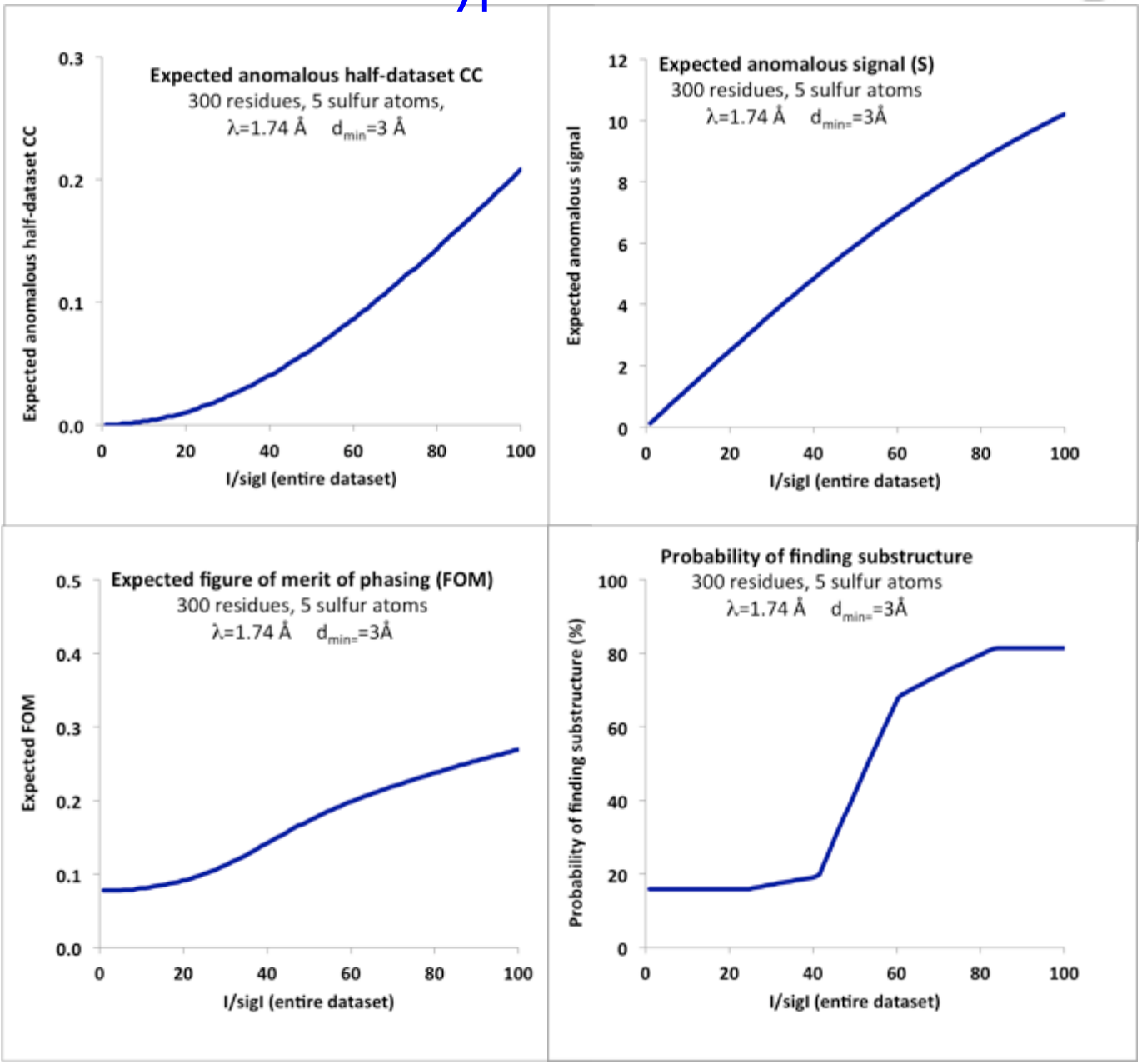




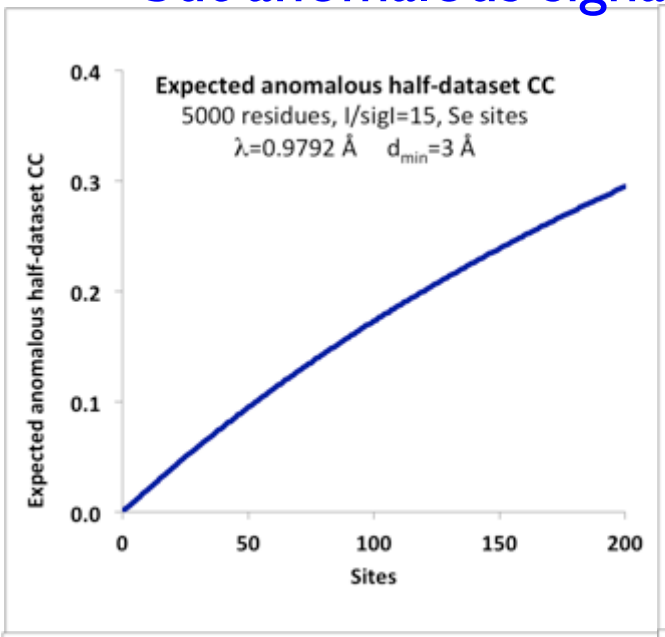


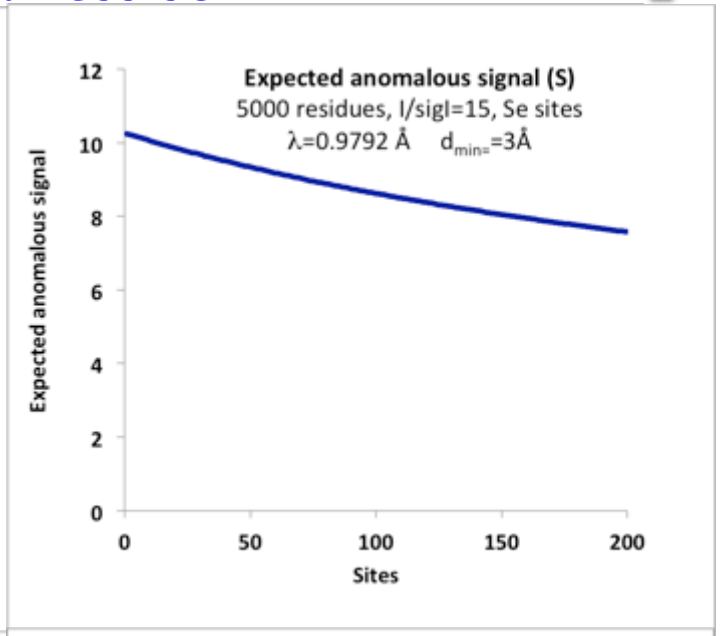


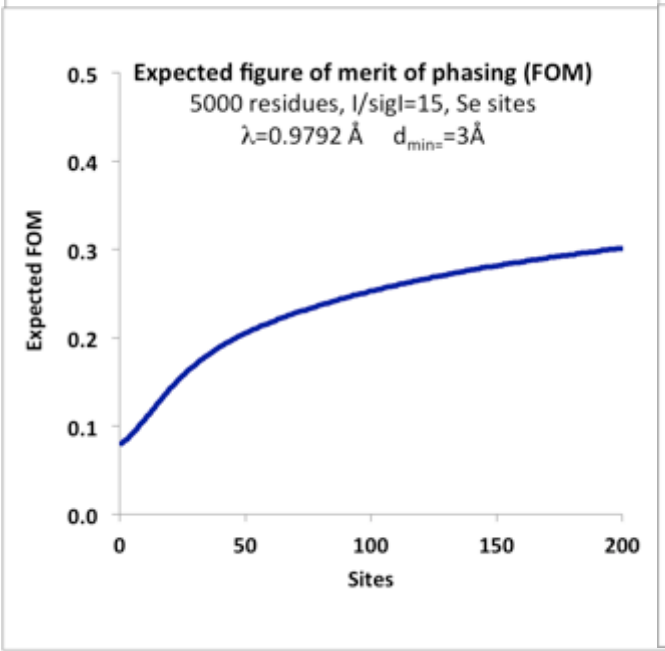
Anomalous data quality depends on I/sigI … and atom type

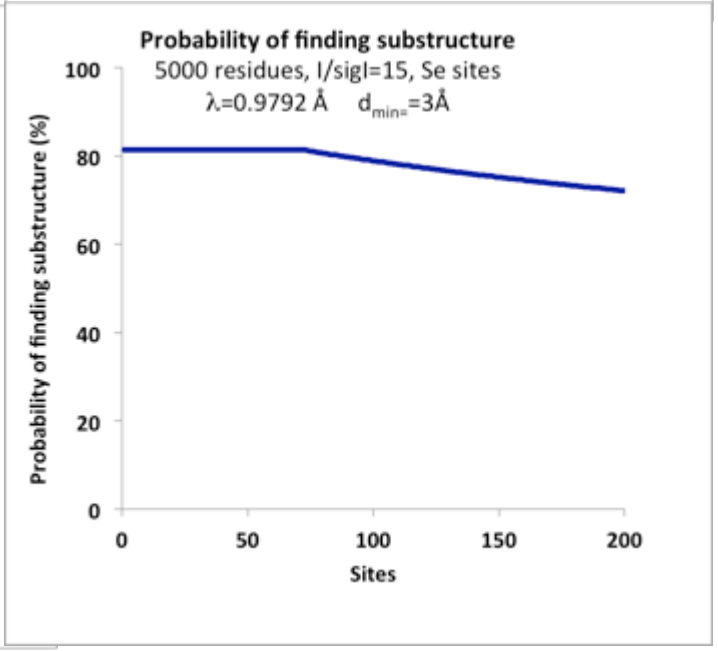


Phasing quality depends a lot on number of sites… but anomalous signal less so



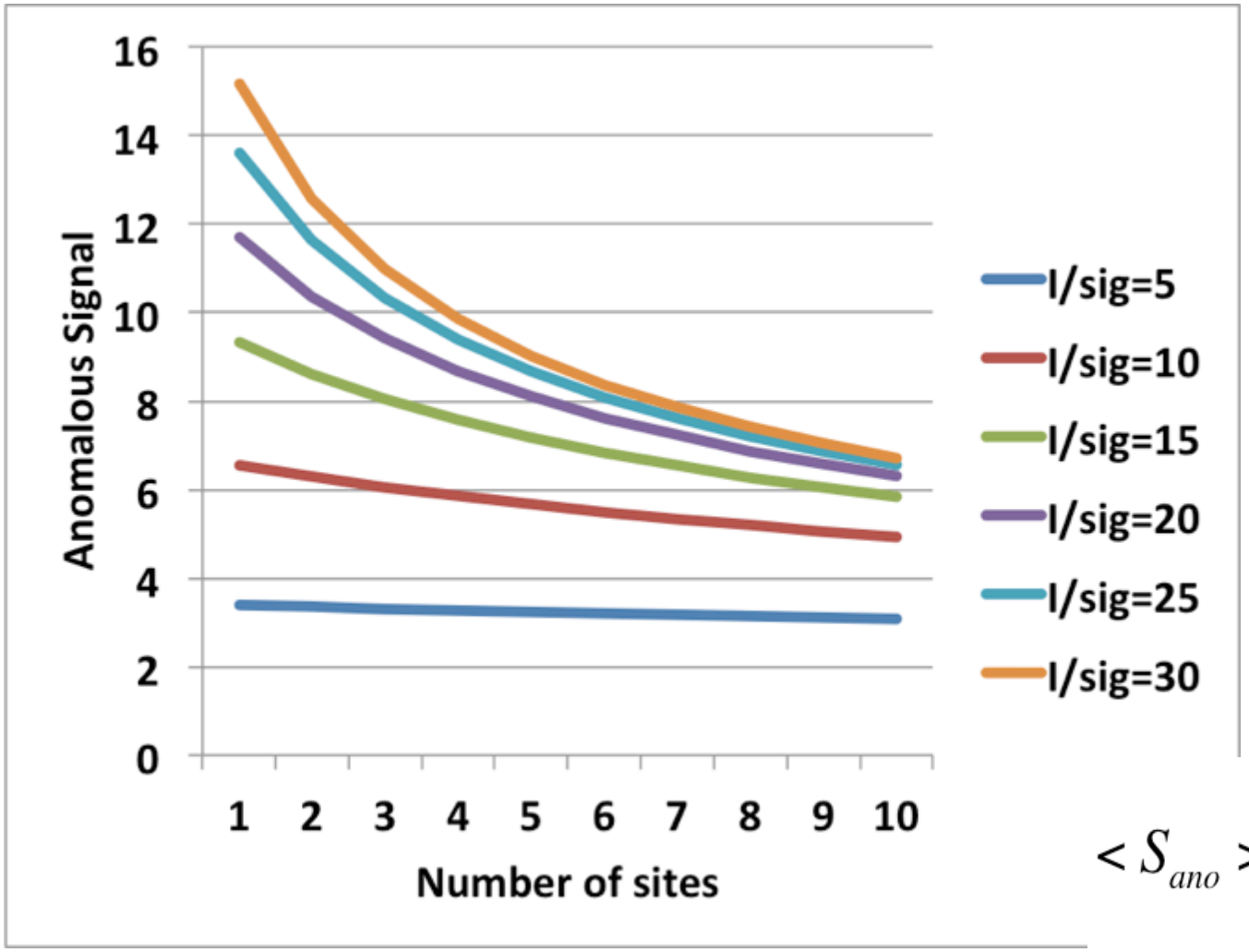






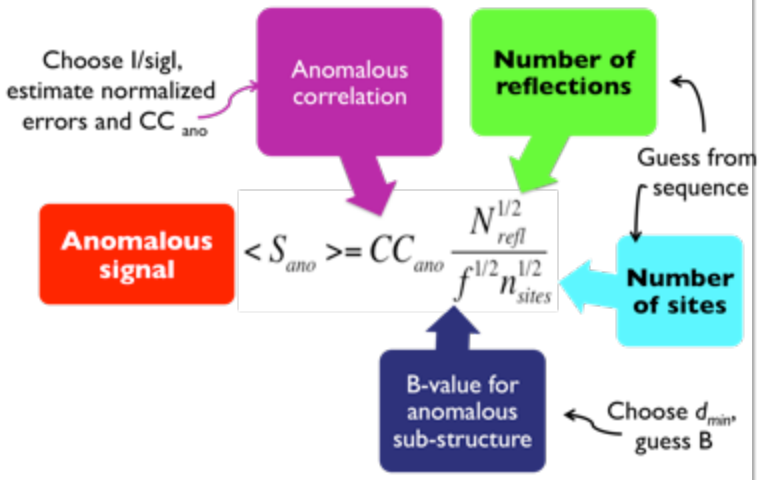
Anomalous signal vs I/sigI and sites

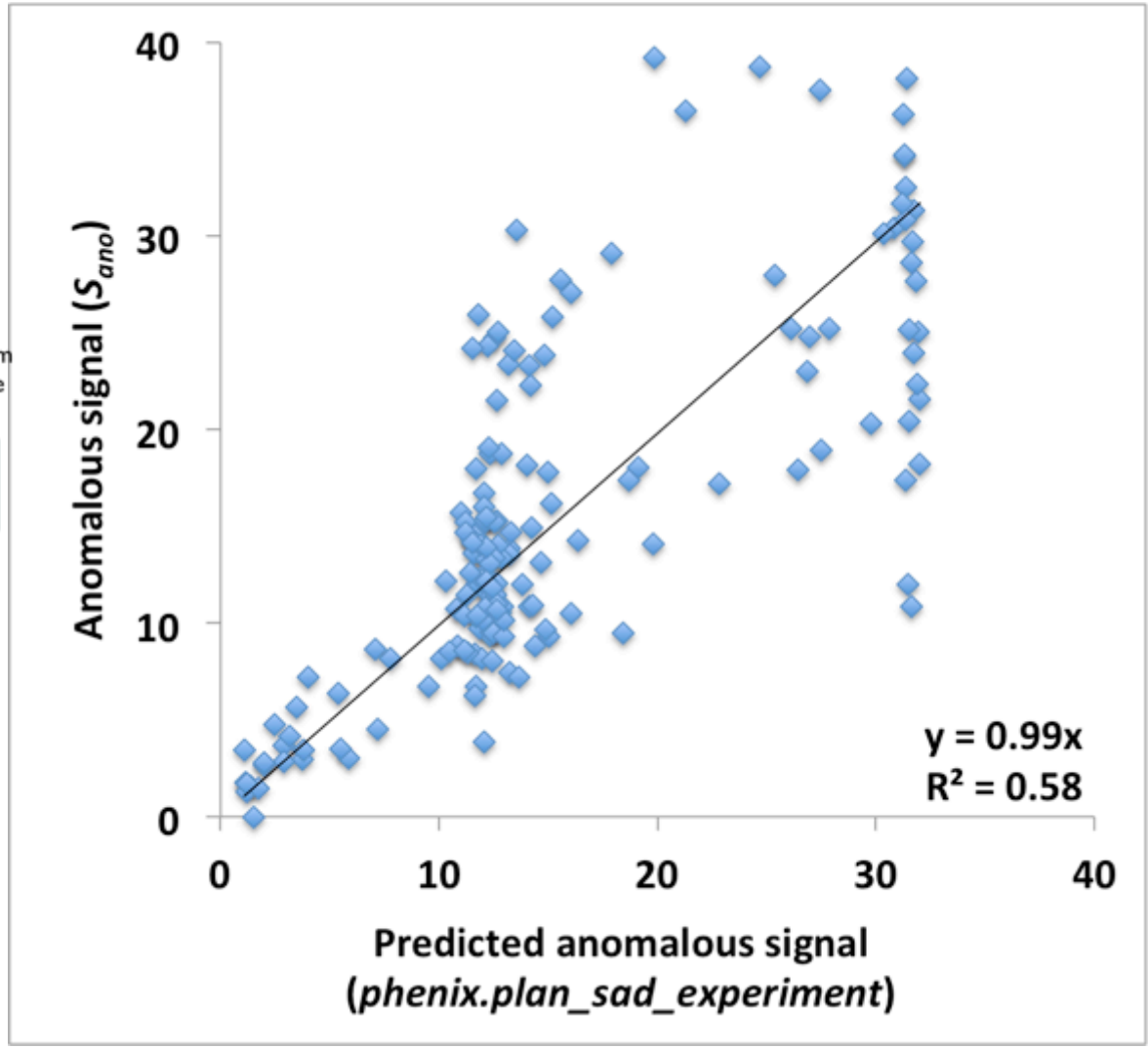
100 residues, varying Se, varying I/sigma



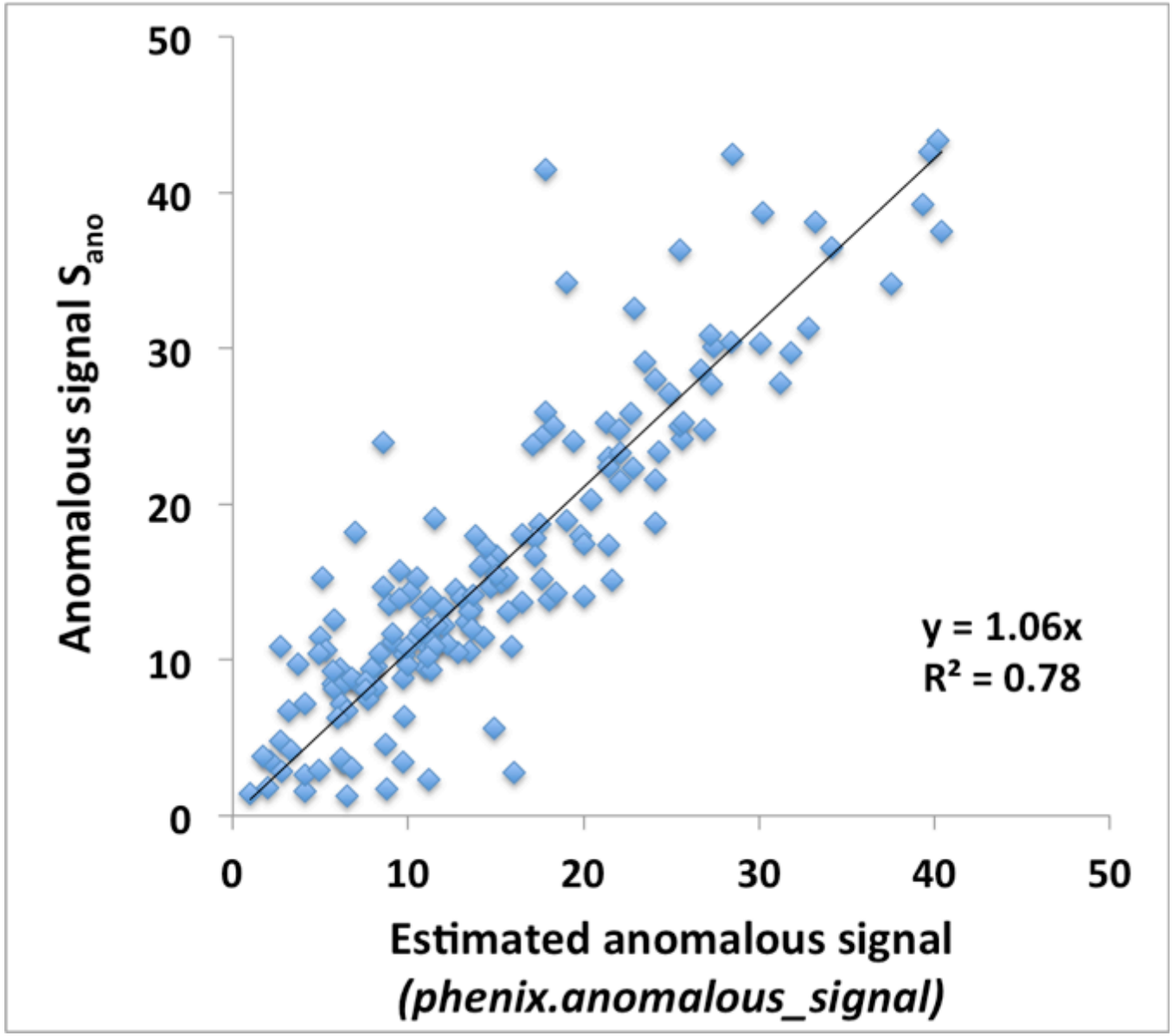
$$< S_{ano} >= CC_{ano} \frac{N_{refl}^{1/2}}{f^{1/2} n_{sites}^{1/2}}$$

Estimating the anomalous signal before collecting the data



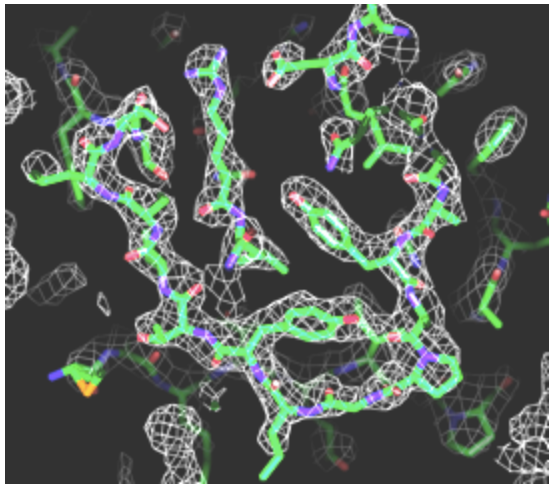


Estimating the anomalous signal after collecting the data



Planning an experiment: Summary

- **Plan the experiment:** *What overall I/sigI do I need to solve this structure? (phenix.plan_sad_experiment)*
- **Measure the data:** *Make sure I/sigI is high enough*
- **Scale the data:** *(phenix.scale_and_merge)*
- **Evaluate the accuracy of the anomalous differences** *(phenix.anomalous_signal)*
- **Find the anomalous sub-structure** *(phenix.hyss, phenix.autosol)*



Automation of structure determination

Automation…

makes straightforward cases accessible to a wider group of structural biologists

makes difficult cases more feasible for experts

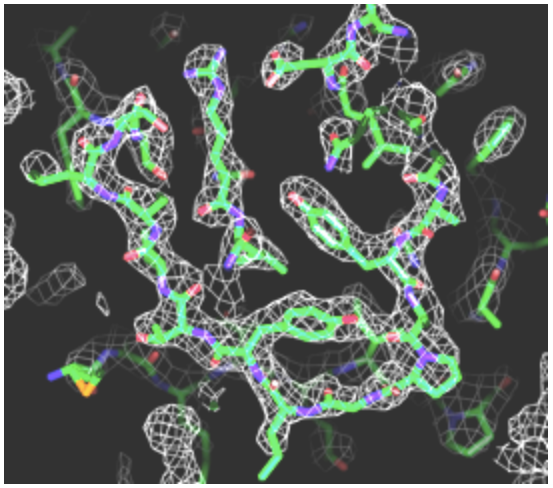
can speed up the process

can help reduce errors

Automation also allows you to…

try more possibilities

estimate uncertainties

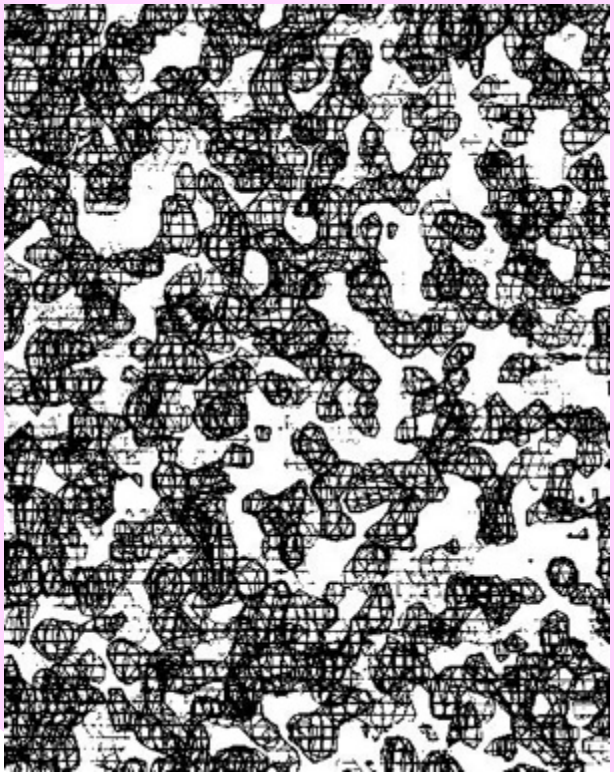


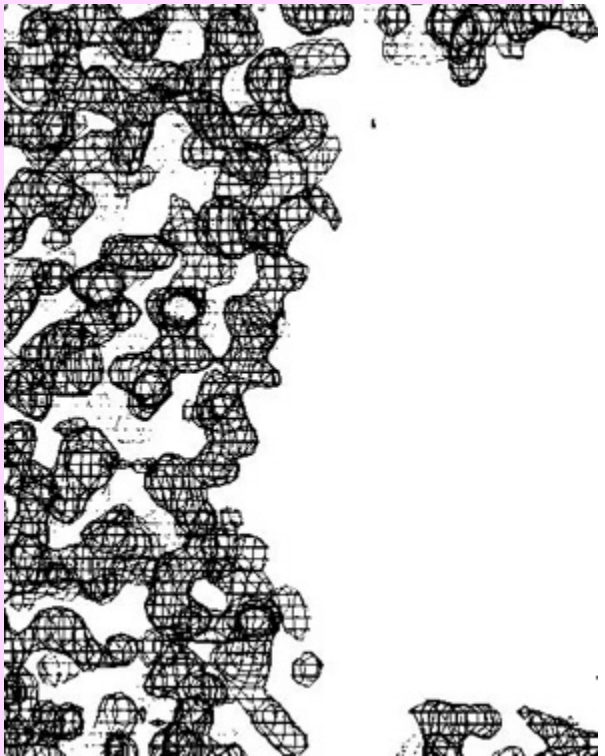
Deciding what is good:

Measures of the quality of an electron-density map:

Which solution is best?

Are we on the right track?

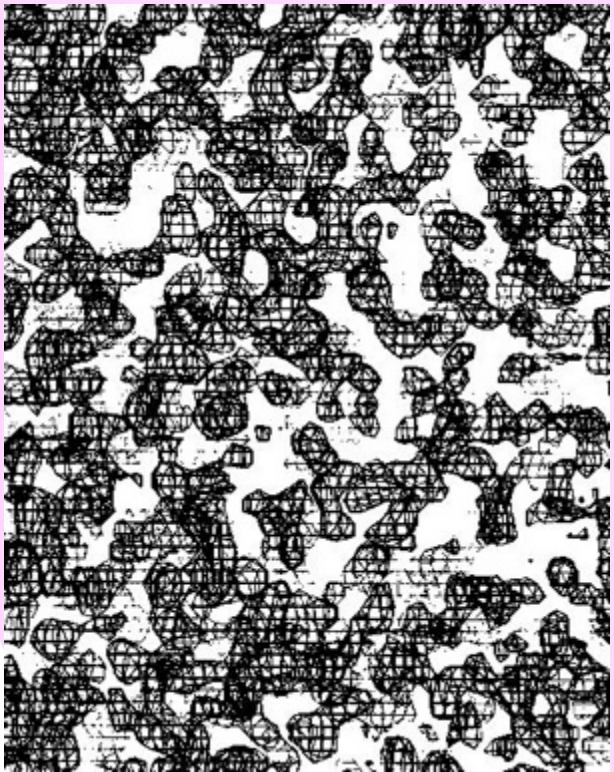


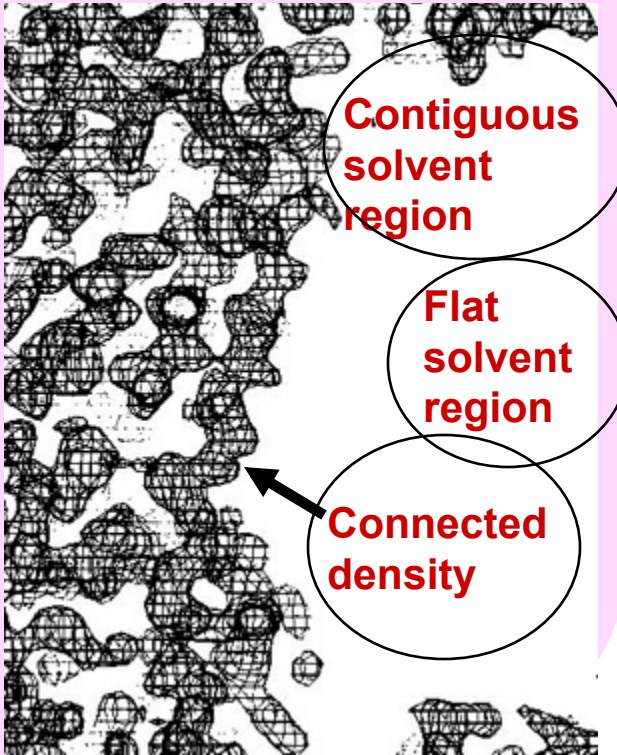


Why we need good measures of the quality of an electron-density map:

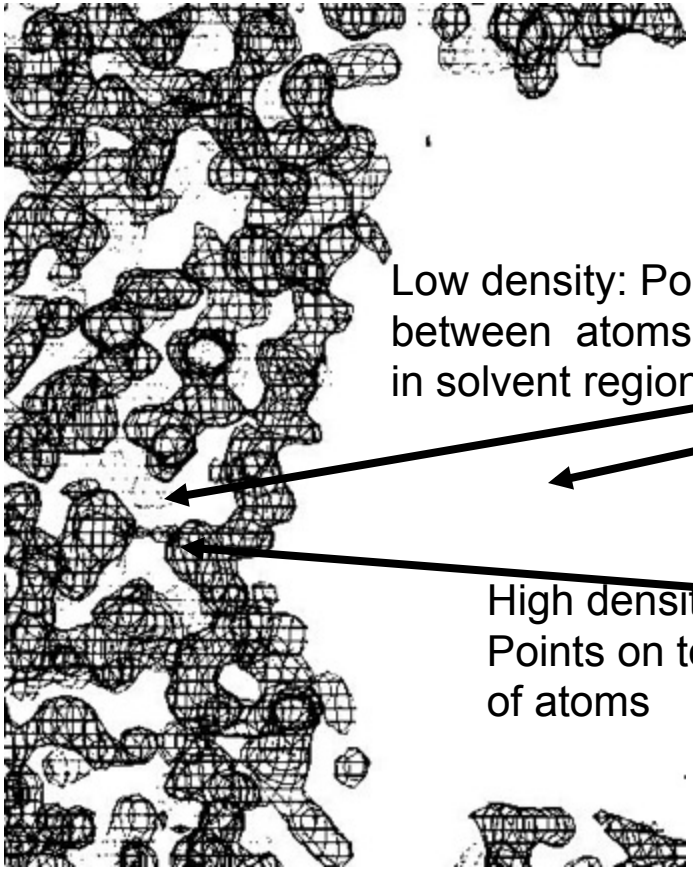
Which solution is best?

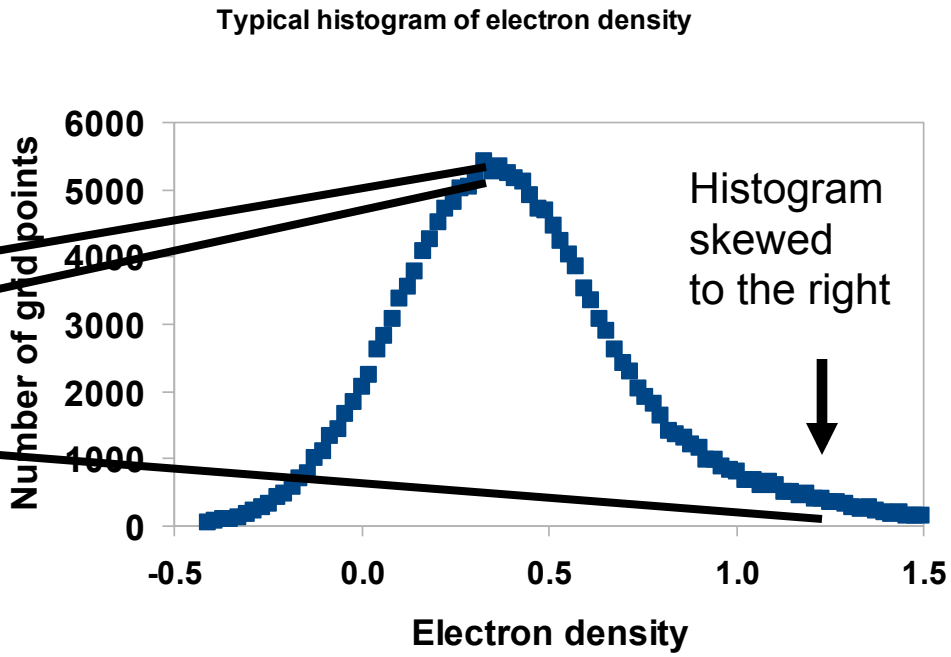
Are we on the right track?



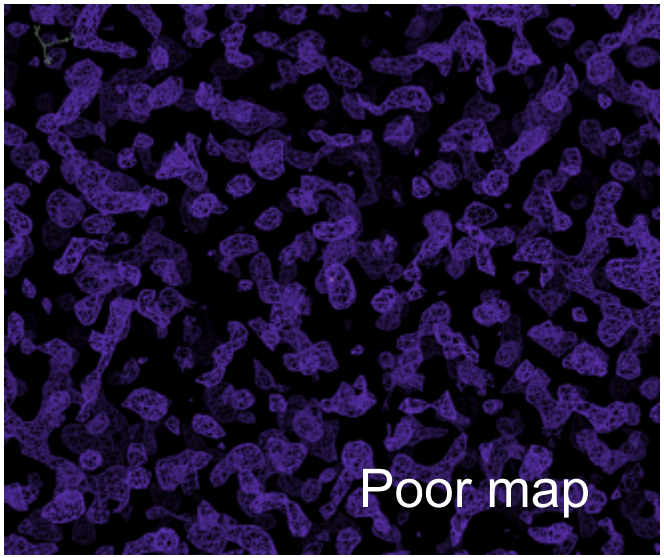


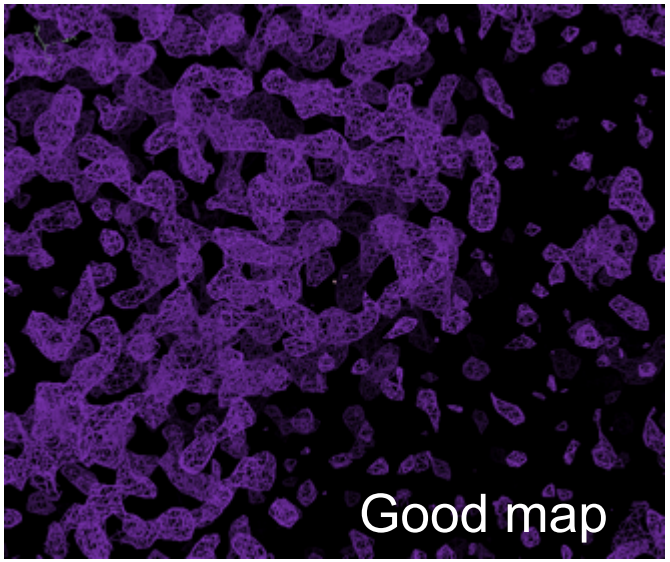
Histogram of electron density values has a positive “skew”

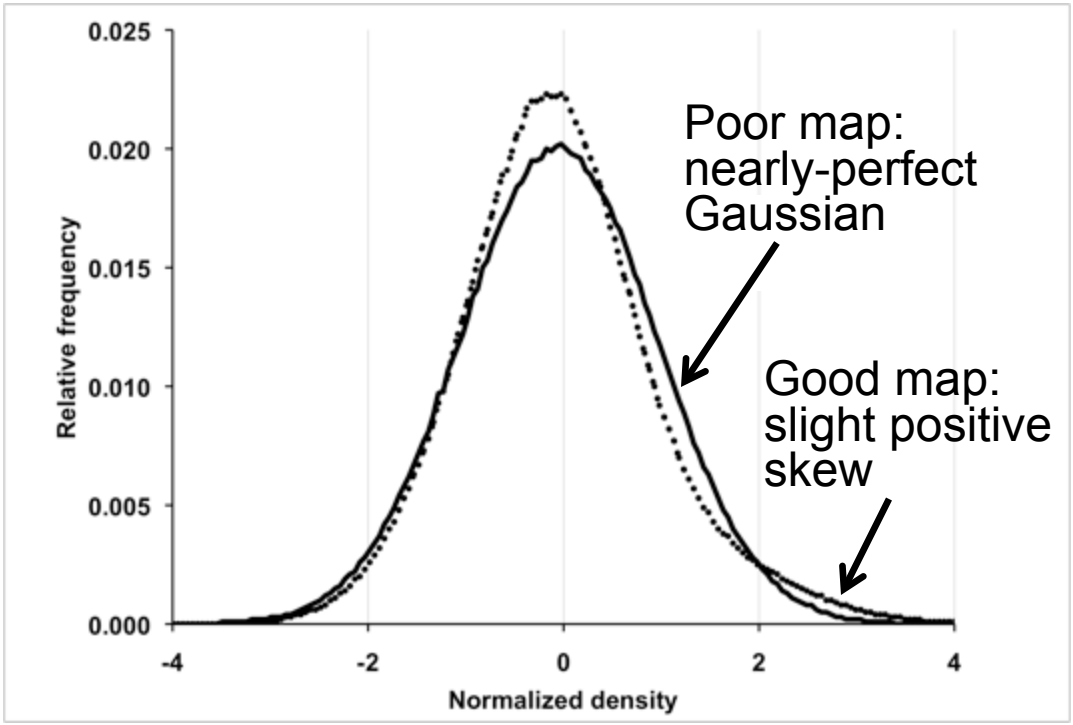




Skew of electron density for poor and good maps







Evaluating electron density maps

Basis	*Good map*	*Random map*
Skew of density (Podjarny, 1977)	Highly skewed (very positive at positions of atoms, zero elsewhere)	Gaussian histogram
Connectivity of regions of high density (Baker, Krukowski, & Agard, 1993)	A few connected regions can trace entire molecule	Many very short connected regions
Correlation of local rms densities (Terwilliger, 1999)	Neighboring regions in map have similar rms densities	Map has uniform rms density
R-factor in 1st cycle of density modification (Cowtan, 1996)	Low R-factor	High R-factor

How well does the skew reflect map quality?

Create real maps

Score the maps based on skew

Compare the scores with the actual quality of the maps

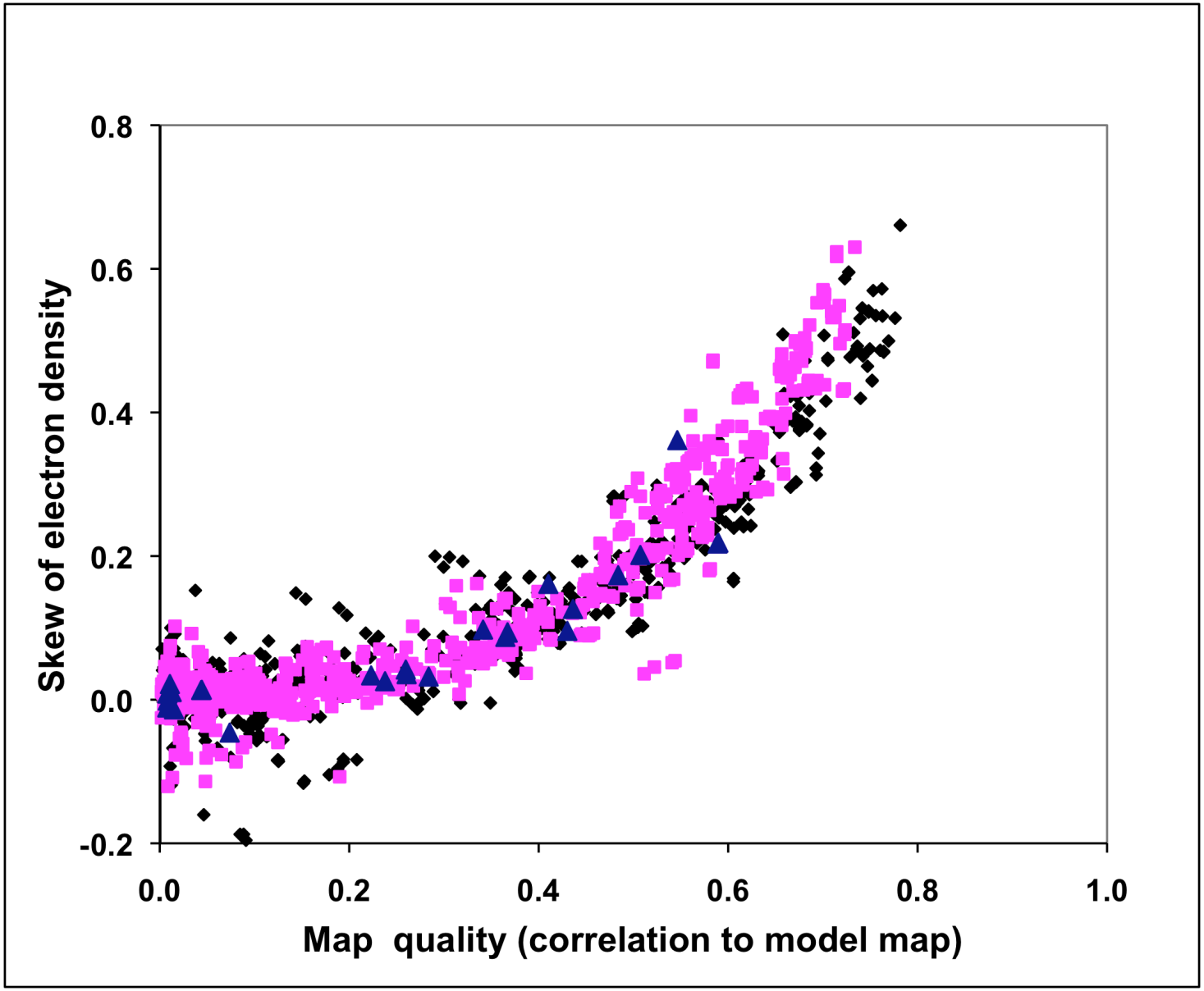
Creating real maps

247 MAD, SAD, MIR datasets with final model available
(PHENIX library and JCSG publicly-available data)

Run AutoSol Wizard on each dataset.

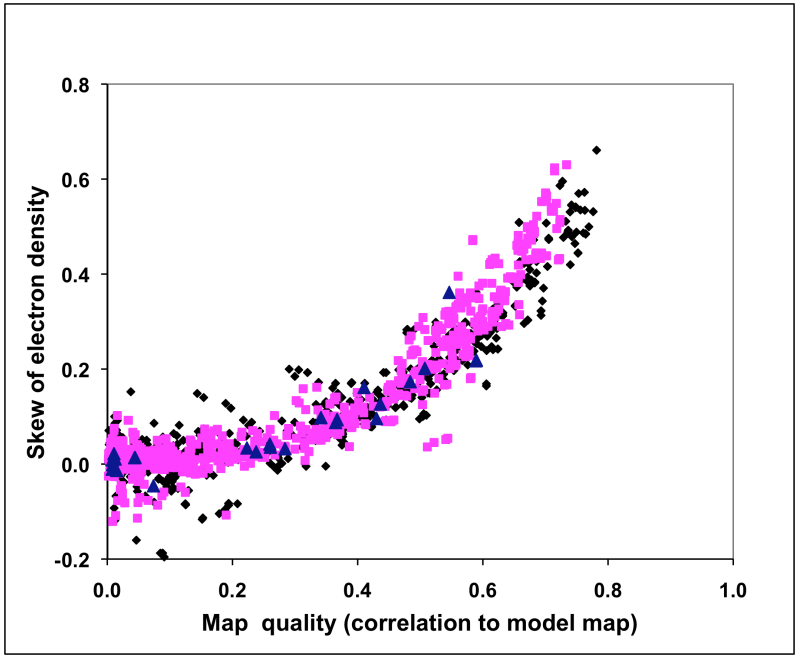
Calculate maps for each solution considered
(opposing hands, additional sites, including various derivatives for MIR)

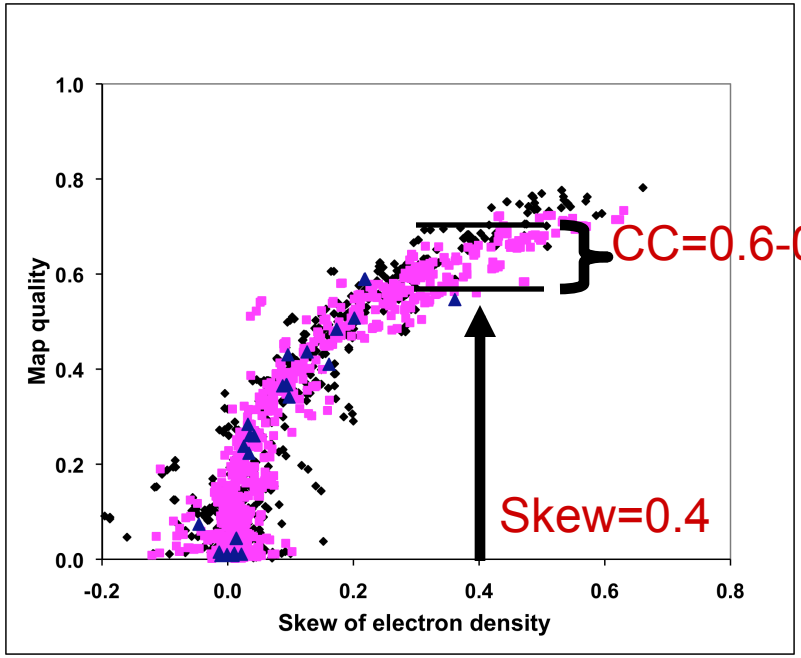
Skew of electron density – positive skew of density values



Using scoring criteria to estimate the quality of a map

Skew depends on map quality → Estimate map quality from skew





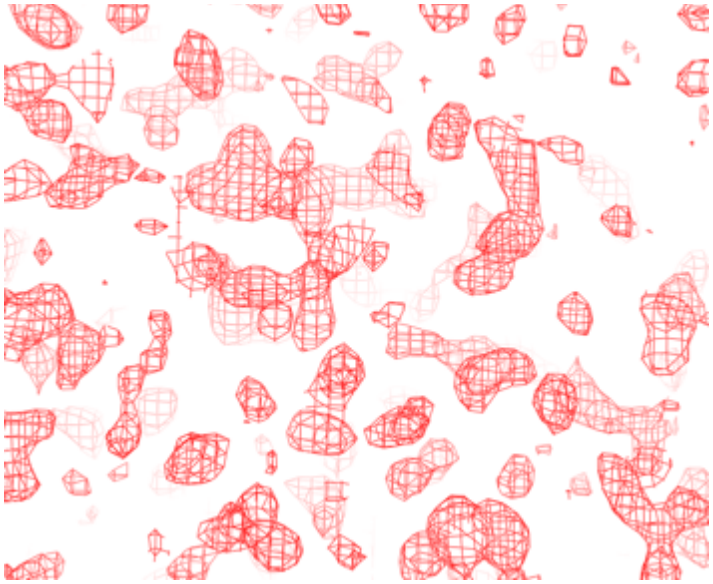
Estimated map quality in practice

Evaluating solutions to a 2-wavelength MAD experiment (JCSG Tm3681, 1VPM, SeMet 1.6 Å data)

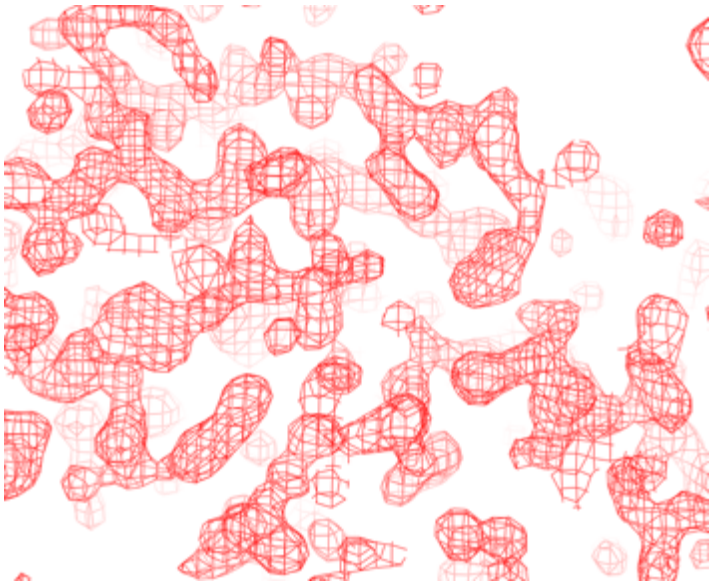
Data for HYSS	Sites	Estimated CC ± 2SD	Actual CC	
Peak	12	0.73 ± 0.04	0.72	←
Peak (inverse hand)	12	0.11 ± 0.43	0.04	
F_A	12	0.73 ± 0.03	0.72	
F_A (inverse)	12	0.11 ± 0.42	0.04	
Sites from diff Fourier	9	0.70 ± 0.17	0.69	

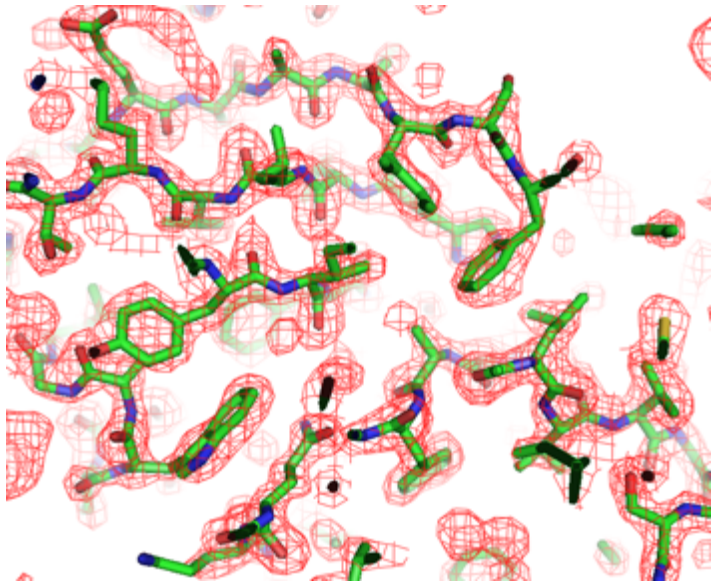
Improving map quality with density modification
SAD map , 2Å, no NCS, 50% solvent)

Phaser SAD map
(CC=0.43)

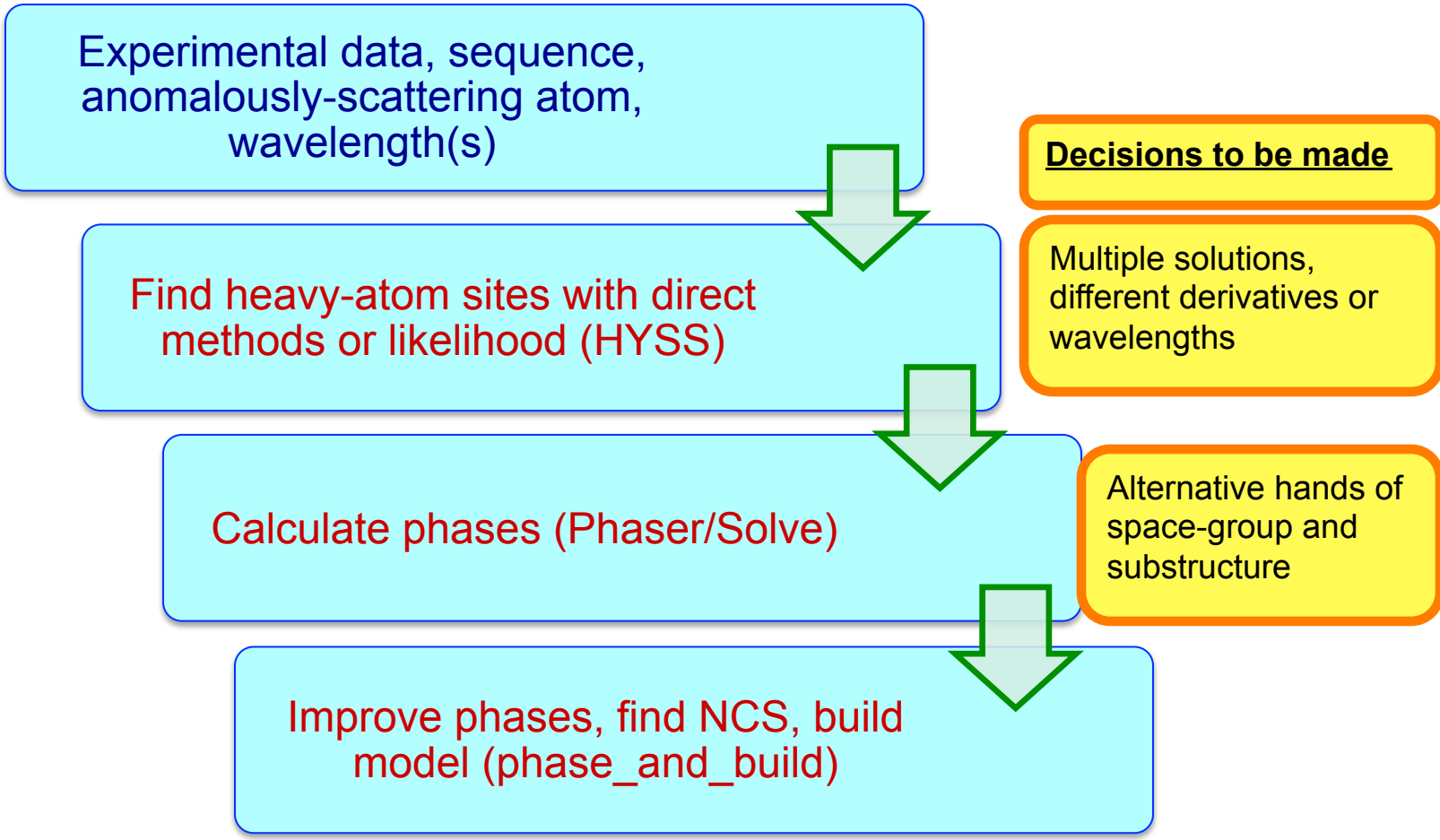


Phaser +RESOLVE
(CC=0.79)

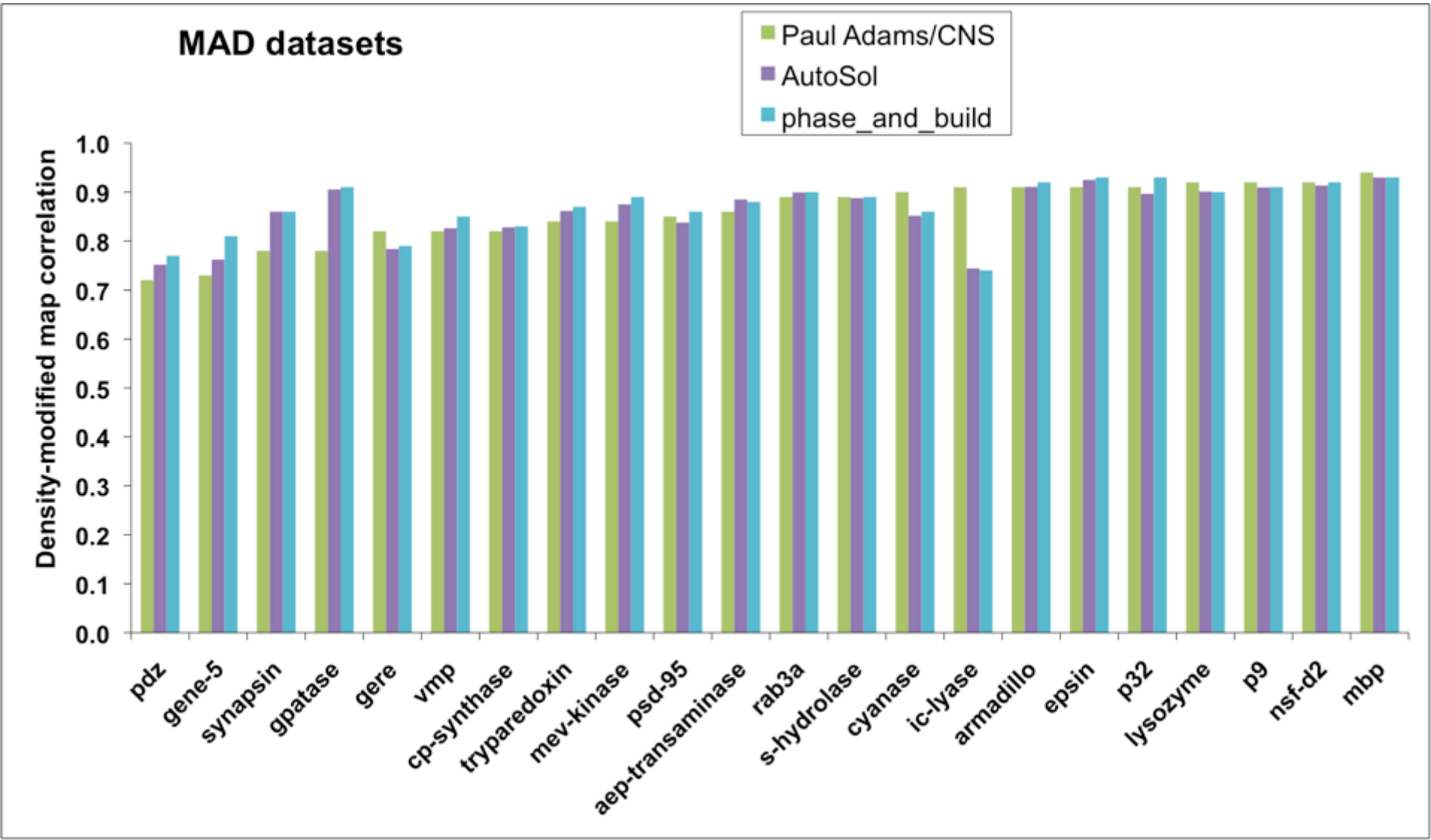




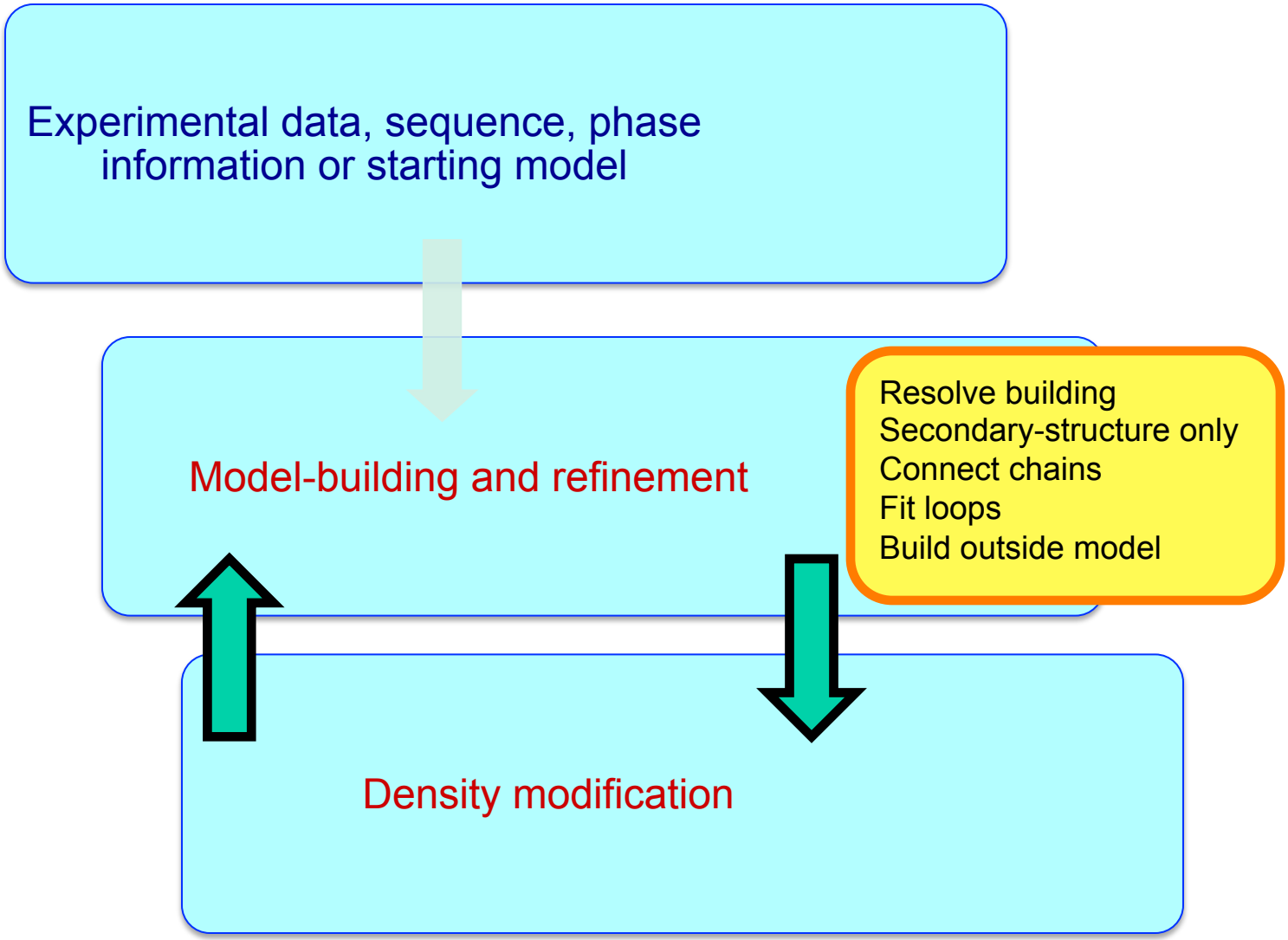
Structure solution with phenix.autosol



AutoSol – fully automatic tests with structure library
(MAD datasets, HYSS search, SOLVE)
RESOLVE/ phase_and_build maps



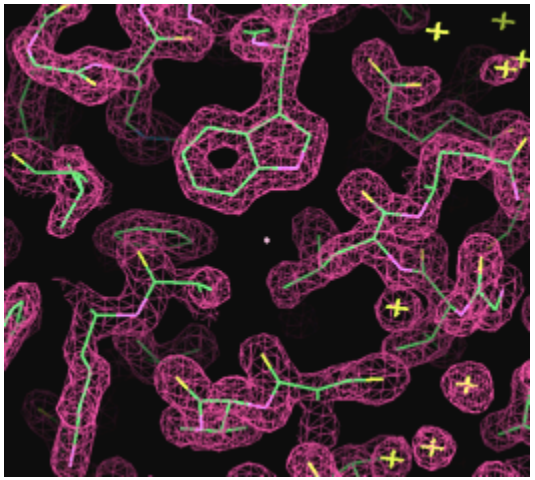
Iterative density modification, model-building and refinement with phenix.autobuild

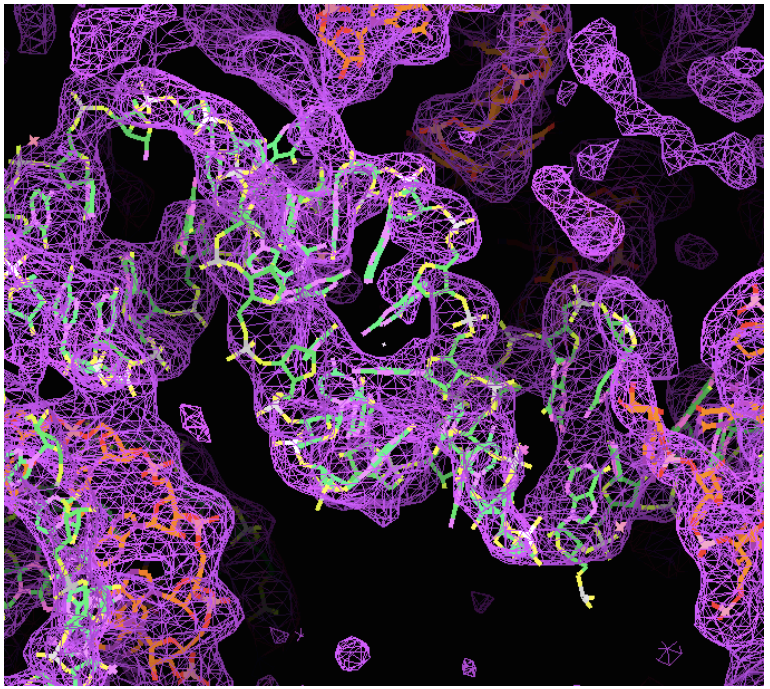


Model-building at moderate or high resolution

Phenix

- FFT-based identification of regular secondary structure
- Extension with short fragments from high-resolution structures
- Probabilistic sequence alignment





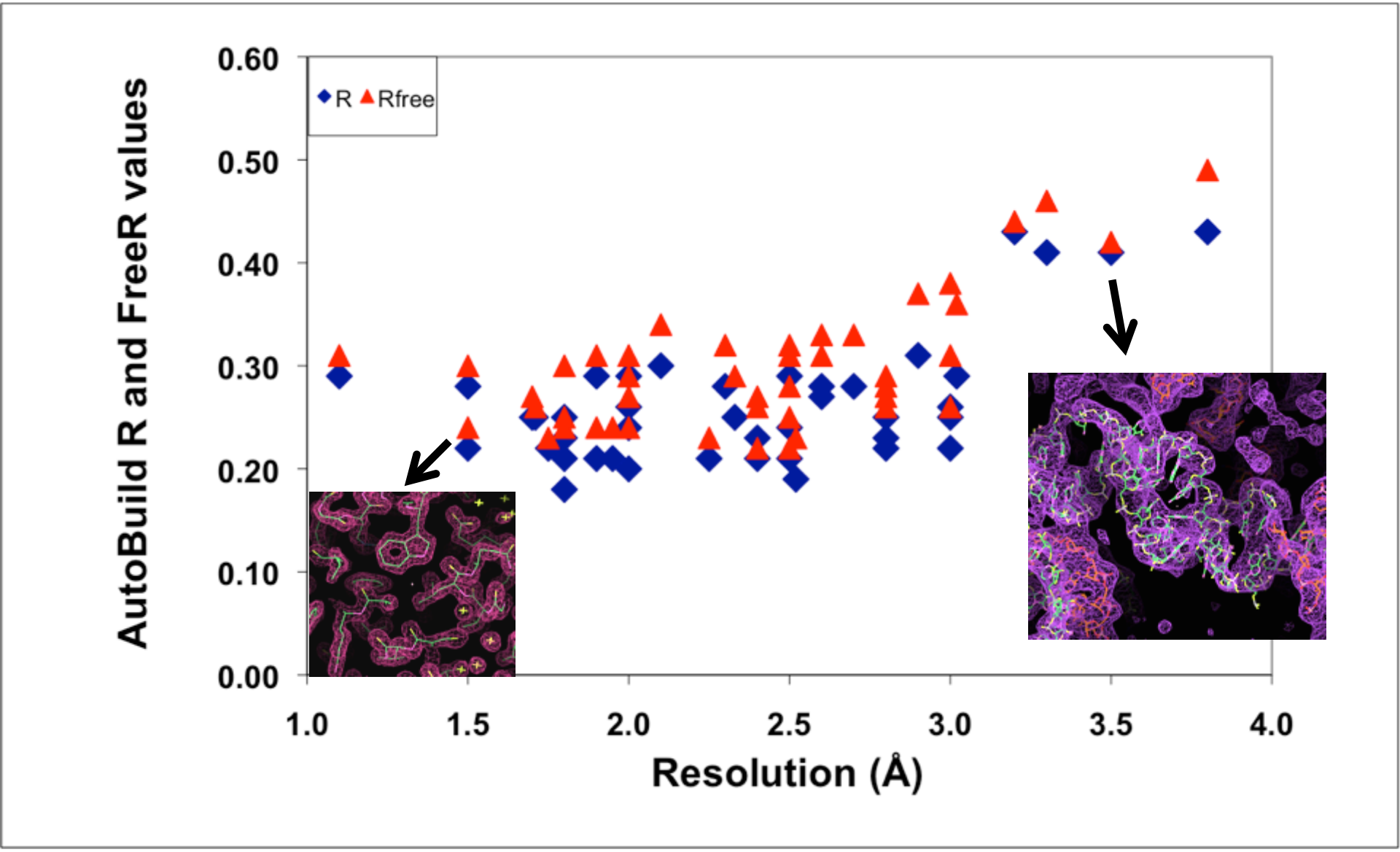
Los Alamos NATIONAL LABORATORY

BERKELEY LAB

UNIVERSITY OF CAMBRIDGE

AutoBuild – tests with structure library

Fully automated iterative model-building, final R/Rfree



The Phenix Project

Lawrence Berkeley Laboratory

Paul Adams, Pavel Afonine, Nigel Moriarty, Nicholas Sauter, Oleg Sobolev, Billy Poon

Los Alamos National Laboratory

Tom Terwilliger, Li-Wei Hung

Cambridge University

Randy Read, Airlie McCoy, Gabor Bunkoczi, Rob Oeffner

Duke University

Jane & David Richardson, Chris Williams, Bryan Arendall, Bradley Hintze

An NIH/NIGMS funded Program Project

Model-building and Density Modification

***Phenix* workshop**

Shanghai, China
Jan. 14, 2016

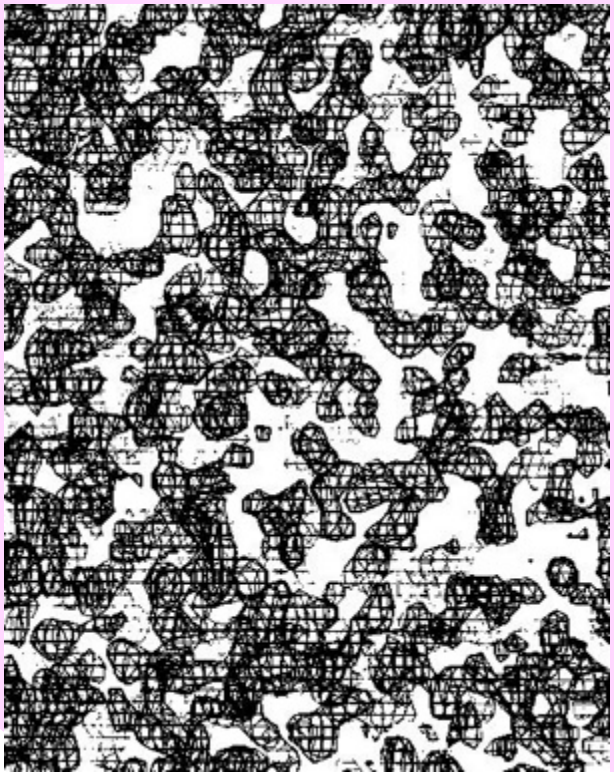
Tom Terwilliger
Los Alamos National Laboratory

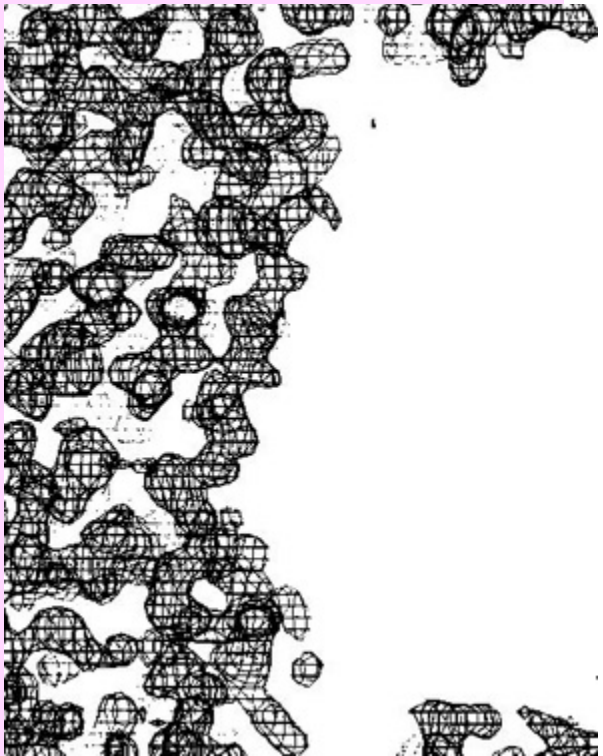
Deciding what is good:

Measures of the quality of an electron-density map:

Which solution is best?

Are we on the right track?

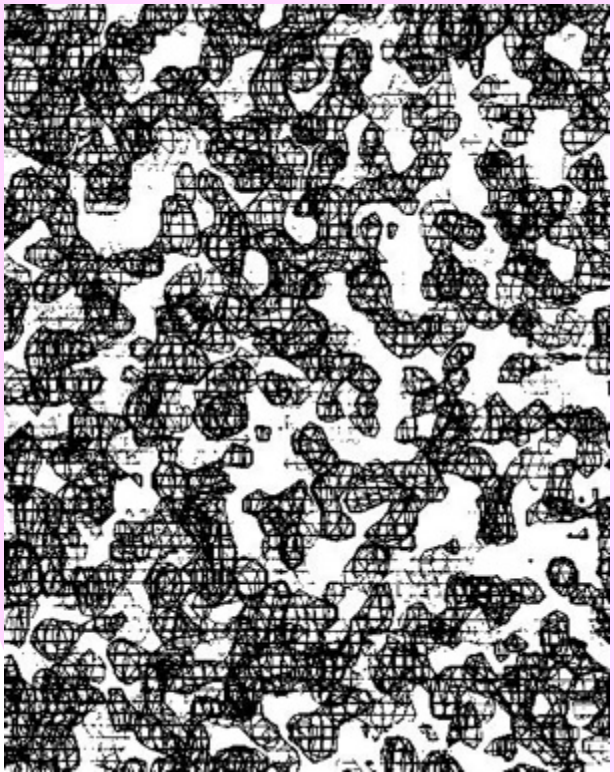


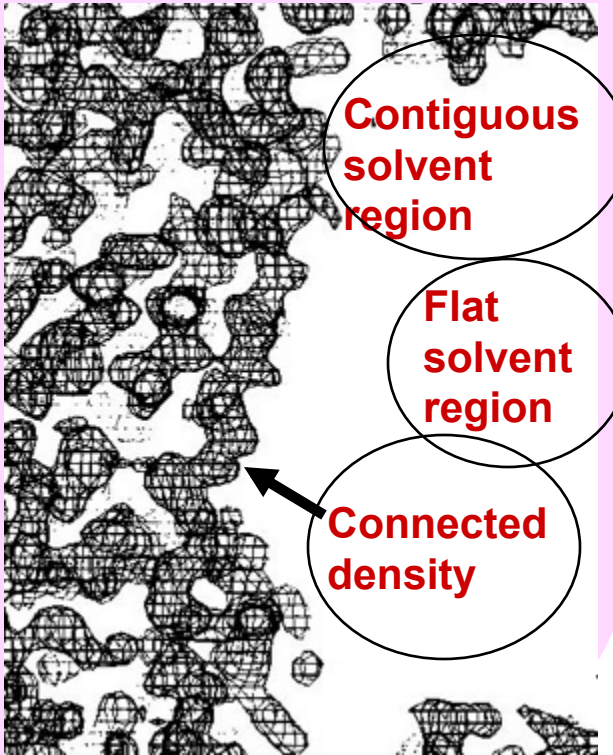


Why we need good measures of the quality of an electron-density map:

Which solution is best?

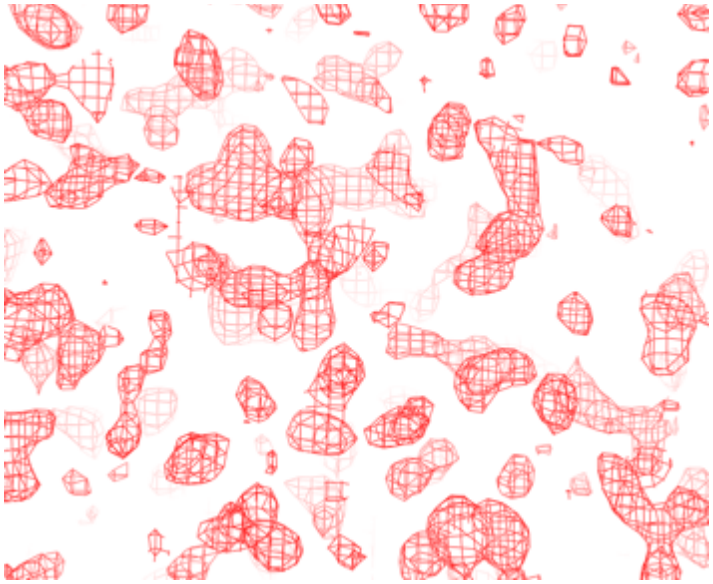
Are we on the right track?



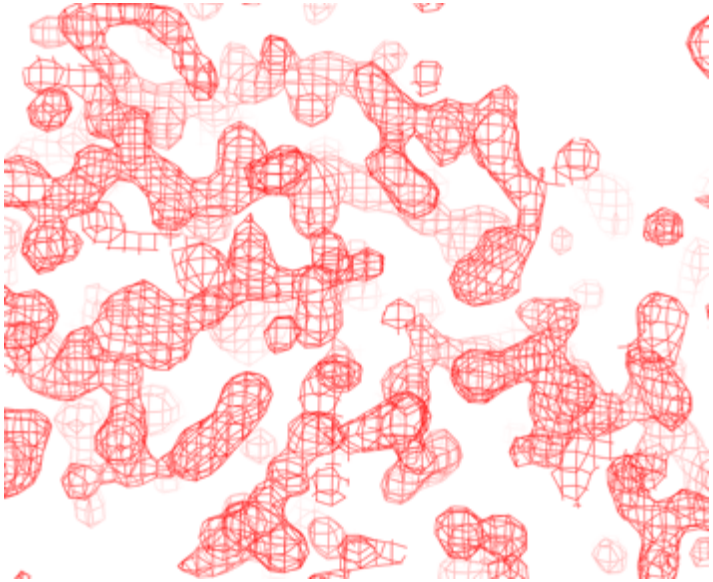


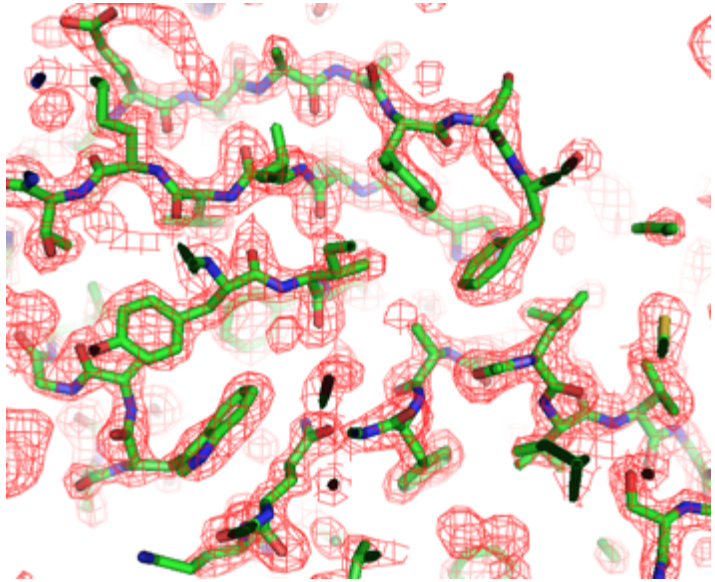
Improving crystallographic map quality with density modification (SAD map , 2Å, no NCS, 50% solvent)

Phaser SAD map
(CC=0.43)



Phaser +RESOLVE
(CC=0.79)





Statistical density modification

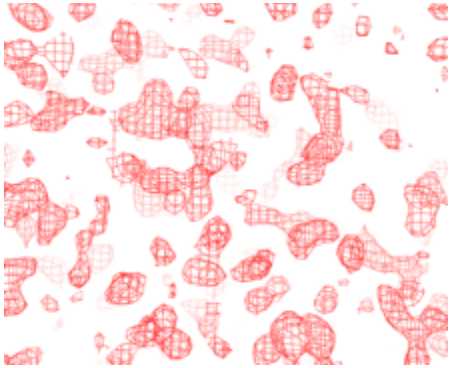
•Principle: phase probability information from probability of the map and from experiment:

•$P(\phi) = P_{\text{map probability}}(\phi)\, P_{\text{experiment}}(\phi)$

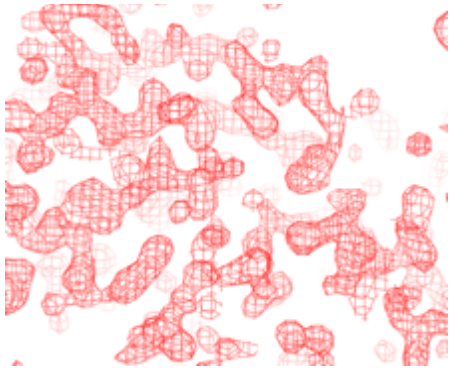
•"Phases that lead to a believable map are more probable than those that do not"

•A believable map is a map that has…
•a relatively flat solvent region
•NCS (if appropriate)
•A distribution of densities like those of model proteins

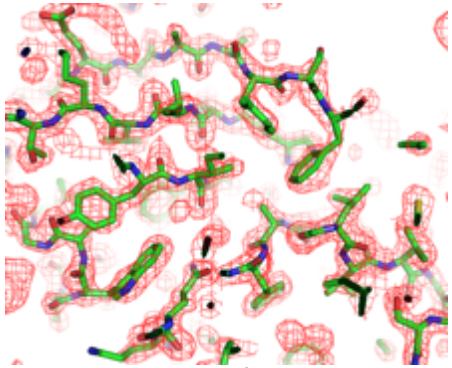
•Method:
•calculate how map probability varies with electron density ρ
•deduce how map probability varies with phase ϕ
•combine with experimental phase information



Experimental map

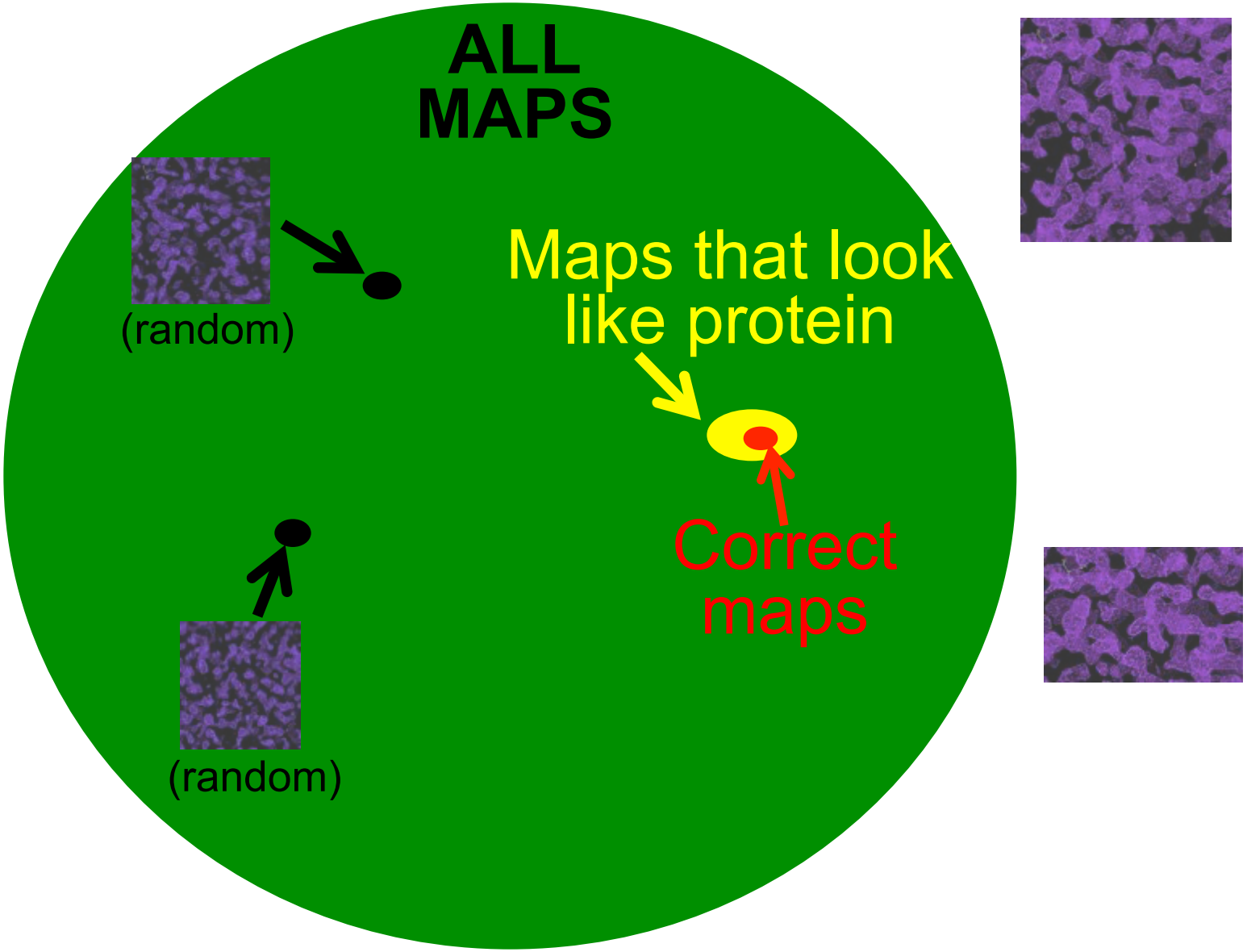


Density-modified



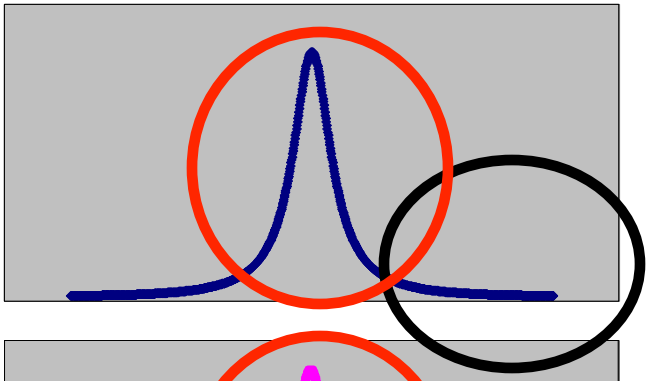
Interpreted

Maps that look like proteins are MUCH more likely to be correct than ones that do not

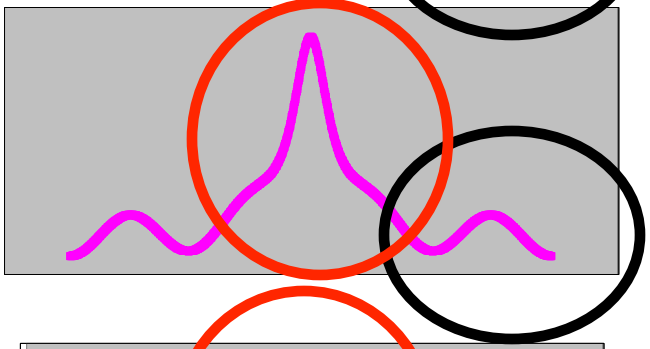


Map probability phasing: Getting a new probability distribution for each phase given estimates of all others

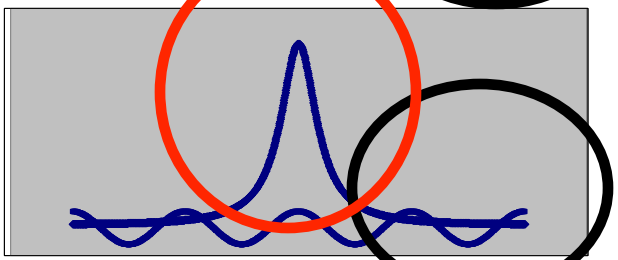
1. Identify expected features of map (flat far from center)
2. Calculate map with current estimates of all structure factors except one (k)
3. Test all possible phases ϕ for structure factor k (for each phase, calculate new map including k)
4. Probability of phase ϕ estimated from agreement of map with expectations
5. Phase probability of reflection k from map is independent of starting phase probability because reflection k is omitted from the map



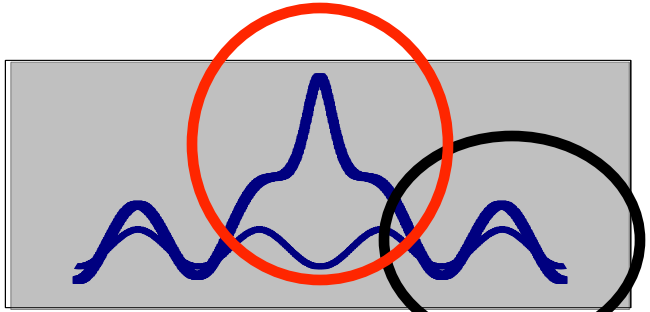
A function that is (relatively) flat far from the origin



Function calculated from estimates of all structure factors but one (k)



Test each possible phase of structure factor k. P(ϕ) is high for phase that leads to flat region



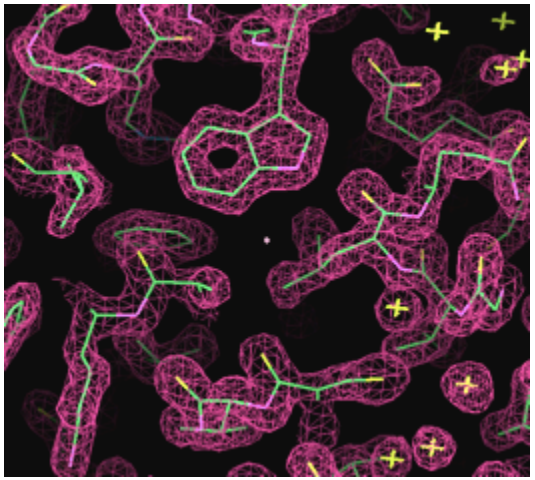
Model-building at moderate or high resolution

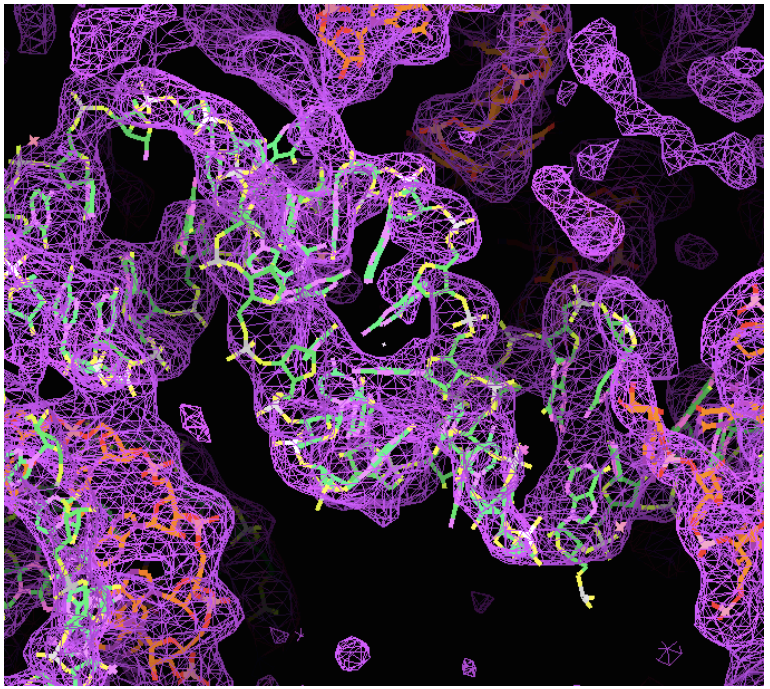
Phenix

•FFT-based identification of regular secondary structure

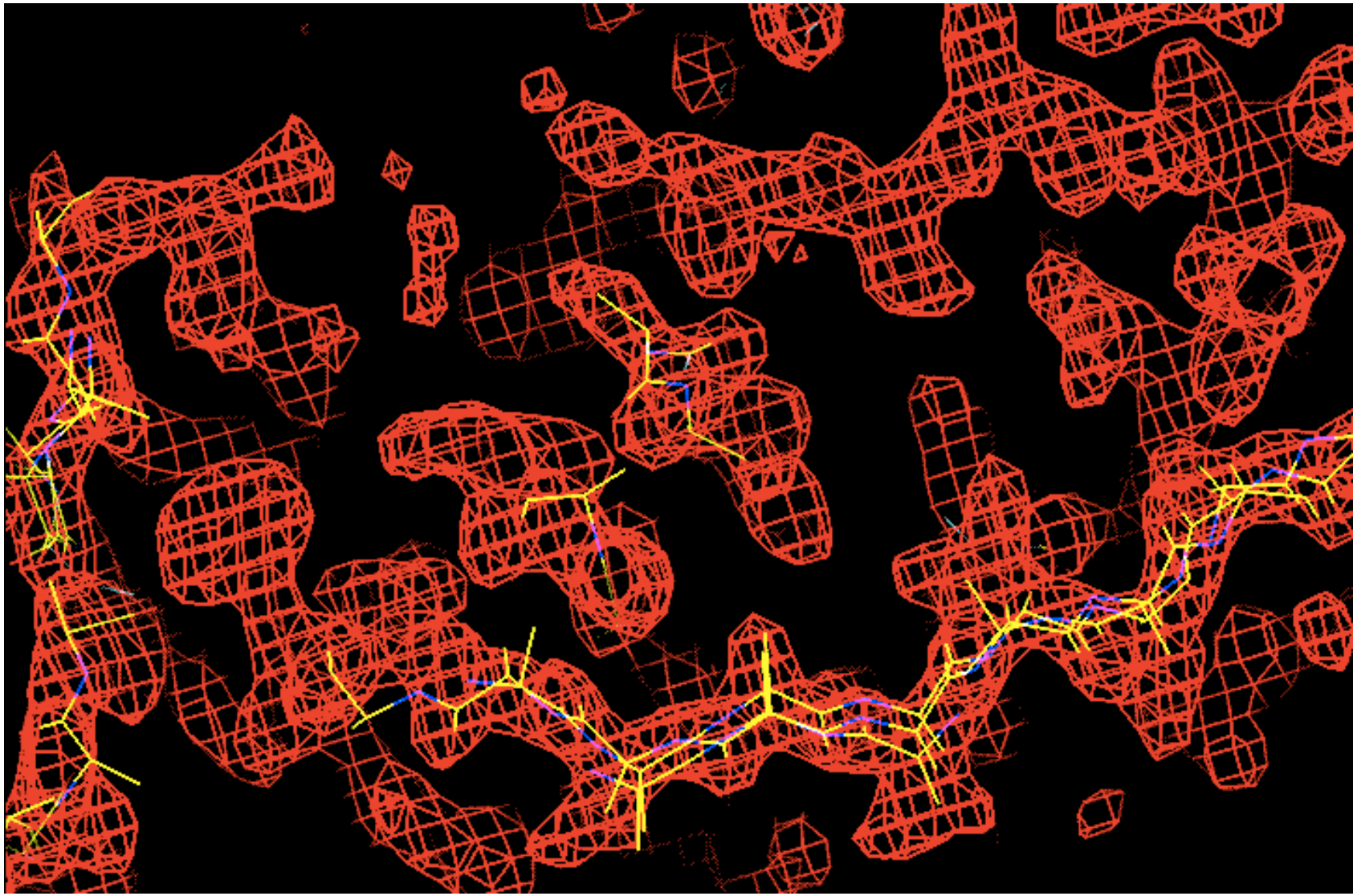
•Extension with short fragments from high-resolution structures

•Probabilistic sequence alignment

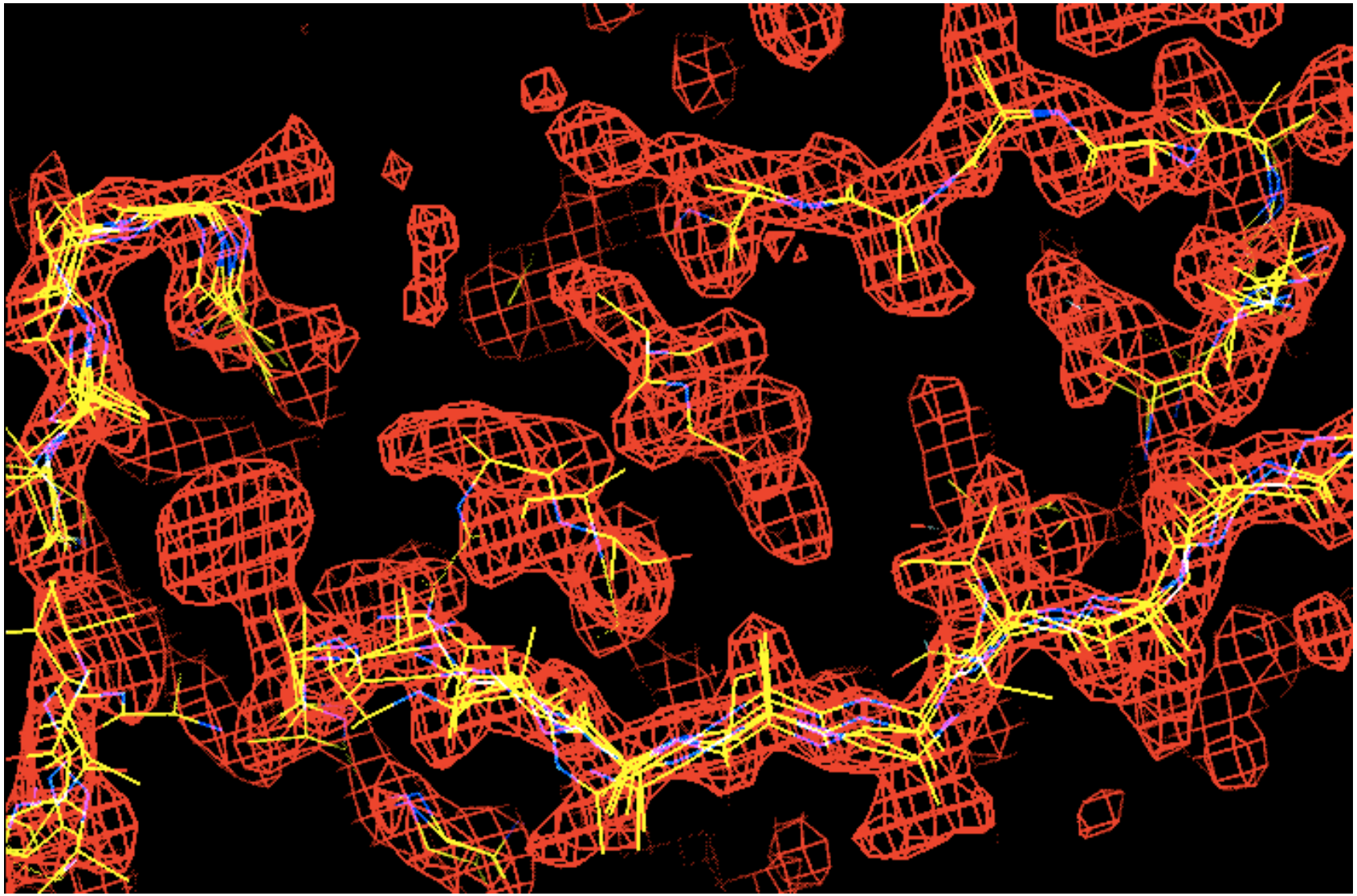




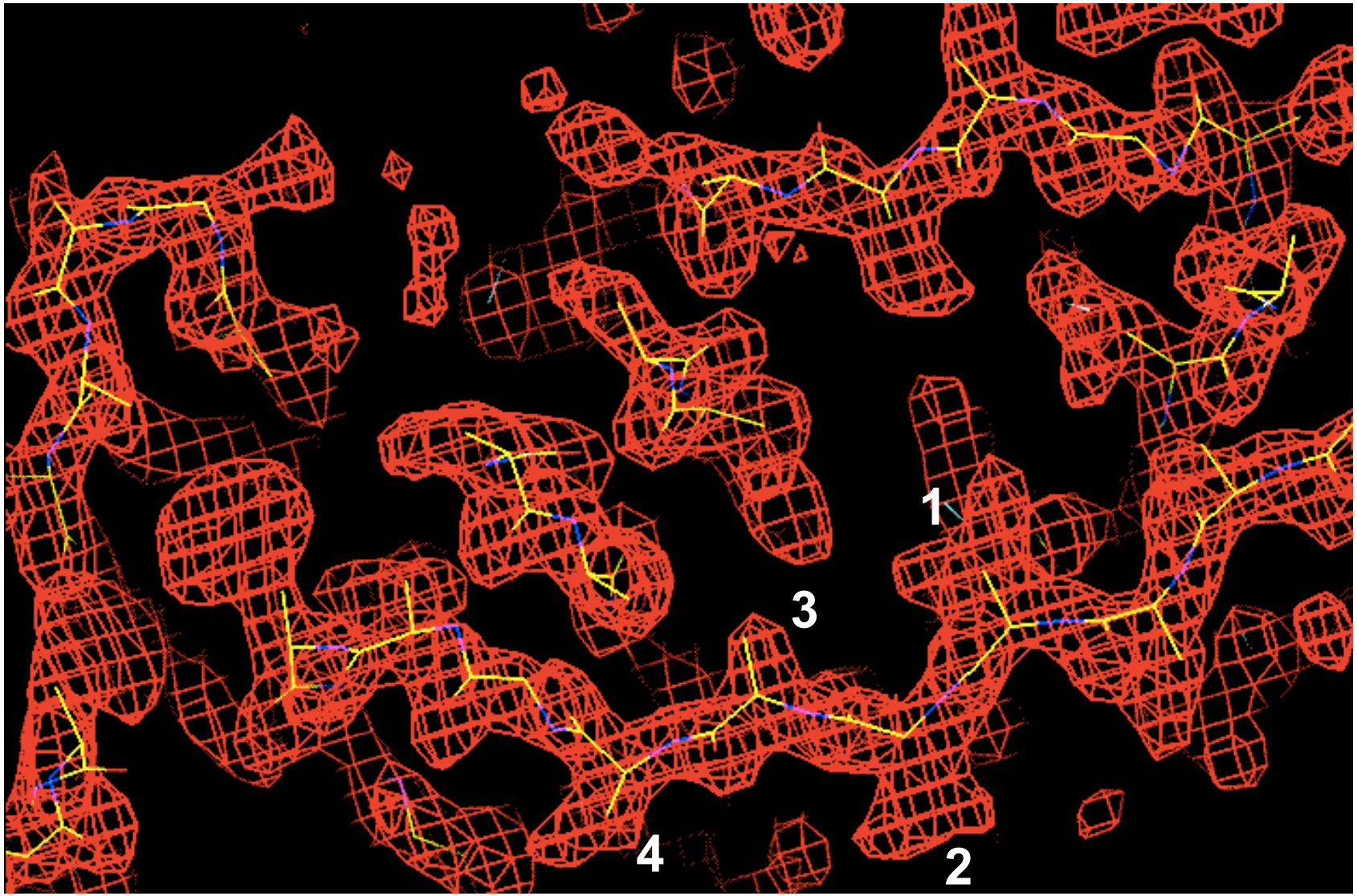
Initial model-building – strand fragments



Chain extension
(result: many overlapping fragments)



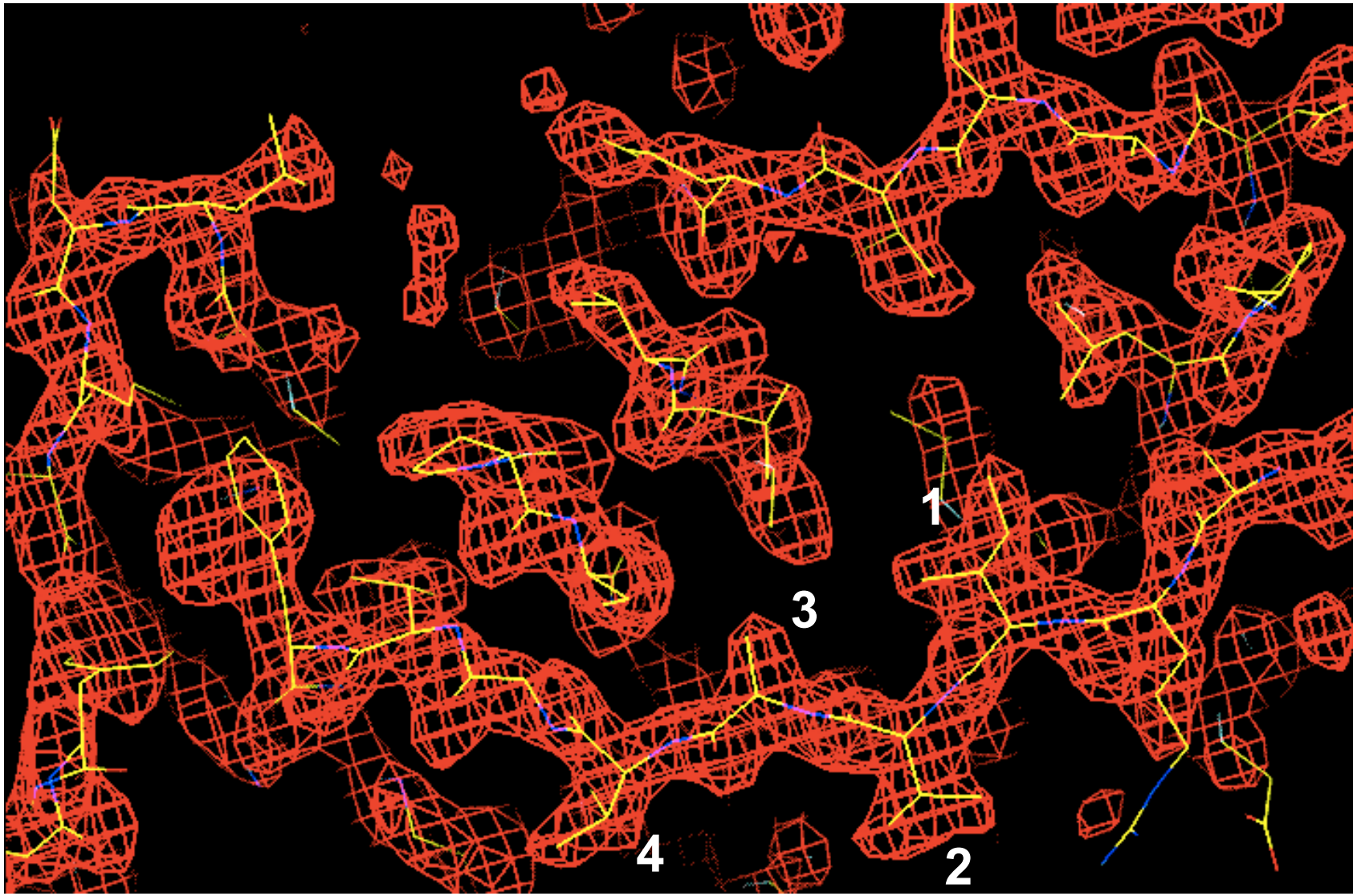
Main-chain as a series of fragments
(choosing the best fragment at each location)



Side-chain template matching to identify sequence alignment to map (IF5A data)
Relative probability for each amino acid at each position
(Correct amino acids in bold)

#	G	A	S	V	I	L	M	C	F	Y	K	R	W	H	E	D	Q	N	P	T
1	6	5	4	18	**18**	6	1	1	1	2	6	2	2	1	9	6	1	0	1	4
2	4	11	14	**37**	5	2	0	2	0	0	2	3	0	0	1	2	0	0	0	6
3	11	**23**	5	12	5	3	2	0	1	3	7	3	1	0	5	3	2	0	2	2
4	7	9	6	**16**	8	5	2	0	1	3	8	4	1	0	7	6	2	0	3	4
5	**31**	7	3	7	4	2	1	0	1	3	5	4	1	0	6	2	2	0	11	1
6	1	3	3	**41**	14	8	0	0	0	0	2	1	0	0	2	4	0	0	1	9
7	0	0	0	0	0	0	0	0	**15**	**63**	1	0	**17**	1	0	0	0	0	0	0
8	2	3	6	23	10	6	2	1	0	1	4	3	0	0	5	**16**	1	0	1	6
9	**96**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Addition of side-chains to fixed main-chain positions



A map-probability function – allowing different weighting of information from different parts of the map

Log-probability of the map is sum over all points in map of local log-probability

$$LL^{MAP}(\{\mathbf{F_h}\}) \approx \frac{\mathrm{N_{REF}}}{\mathrm{V}} \int_{\mathbf{V}} LL(\rho(\mathbf{x}, \{\mathbf{F_h}\}))\mathrm{d}^3\mathbf{x}$$

A map with a flat (blank) solvent region is a likely map

Local log-probability is believability of the value of electron density (ρ(x)) found at this point

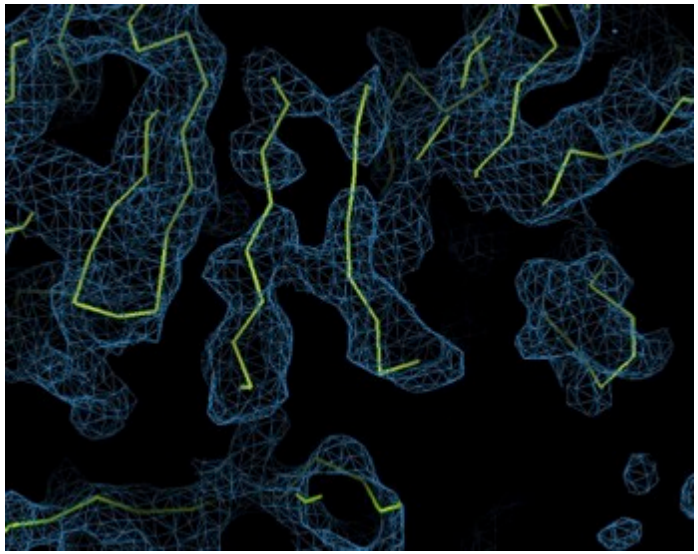
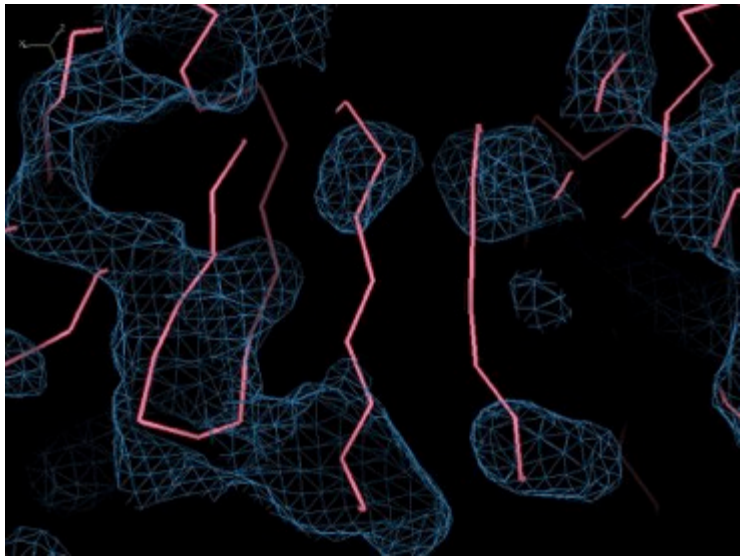
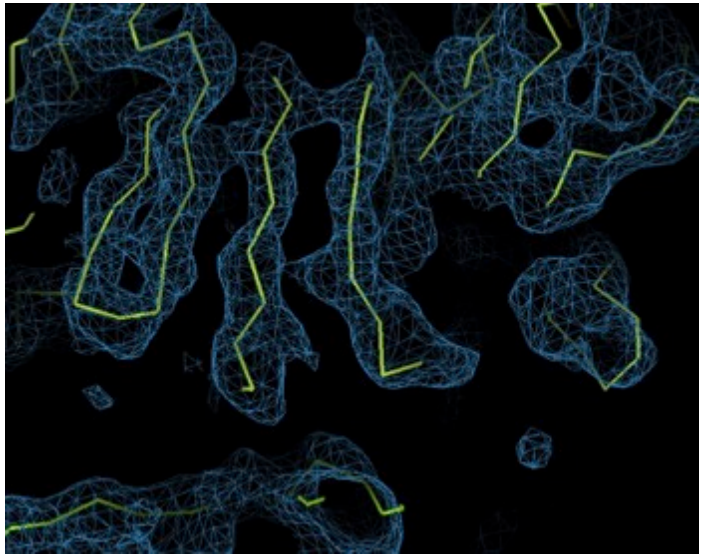
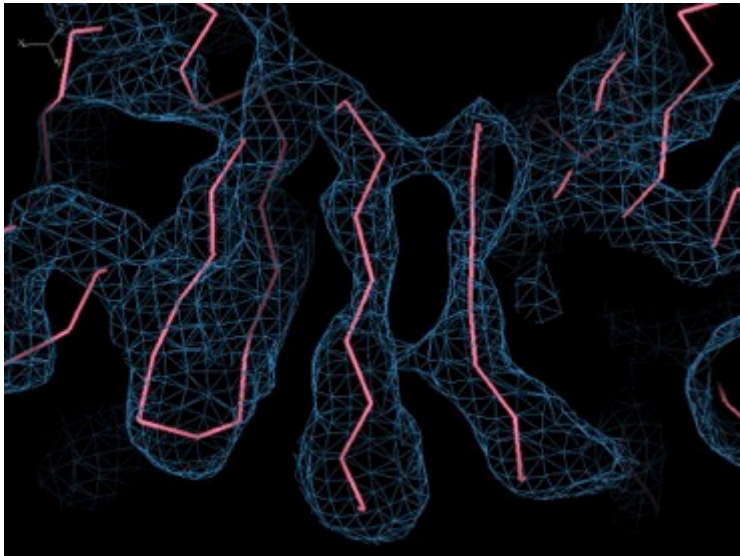
$$LL(\rho(\mathbf{x}, \{\mathbf{F_h}\})) = \ln[p(\rho(\mathbf{x})|PROT)p_{PROT}(\mathbf{x}) + p(\rho(\mathbf{x})|SOLV)p_{SOLV}(\mathbf{x})]$$

If the point is in the PROTEIN region, most values of electron density (ρ(x)) are believable

If the point is in the SOLVENT region, only values of electron density near zero are believable

Statistical density modification with cross-crystal averaging

Cell receptor at 3.5/3.7 Å. Data courtesy of J. Zhu

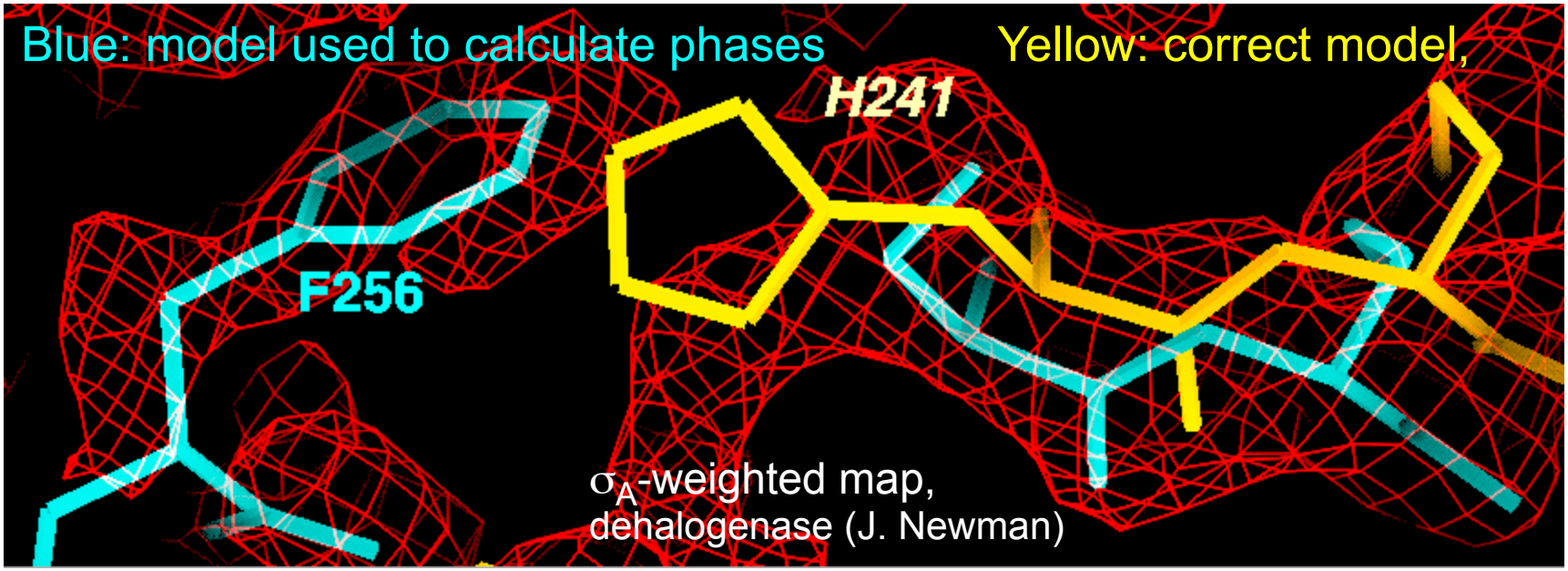
	Crystal 1 (4 copies)	Crystal 2 (2 copies)
RESOLVE density modification		
PHENIX Multi-crystal averaging		

Removing model bias with prime-and-switch phasing

The problem:

Atomic model used to calculate phases -> map looks like the model

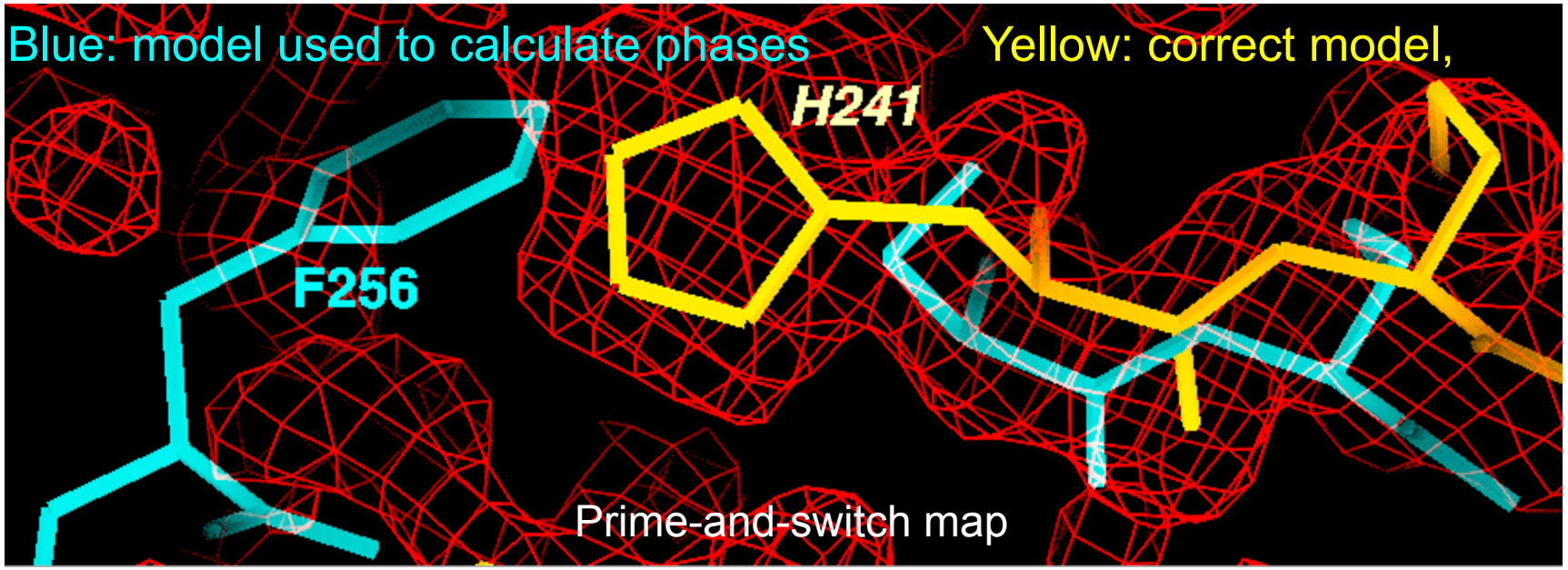
Best current solution: σ_A-weighted phases



Prime-and-switch phasing

A solution:

Start with σ_A-weighted map
Identify solvent region (or other features of map)
Adjust the phases to maximize the probability of the map – without biasing towards the model phases

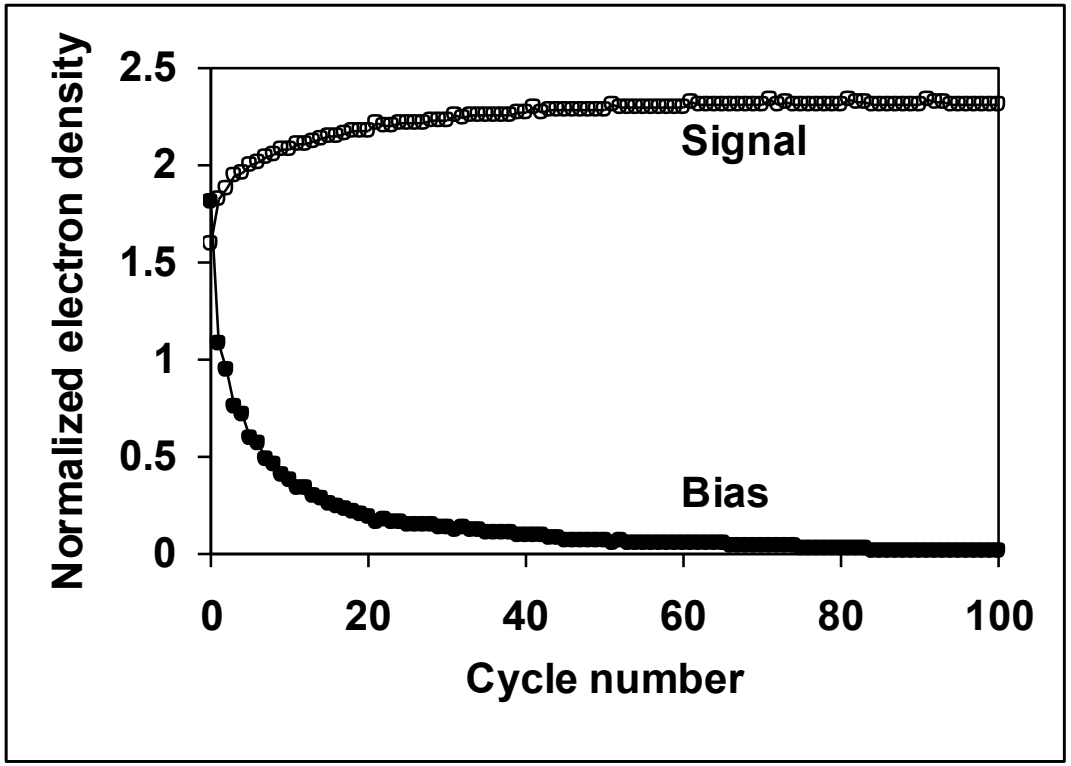


Prime-and-switch phasing

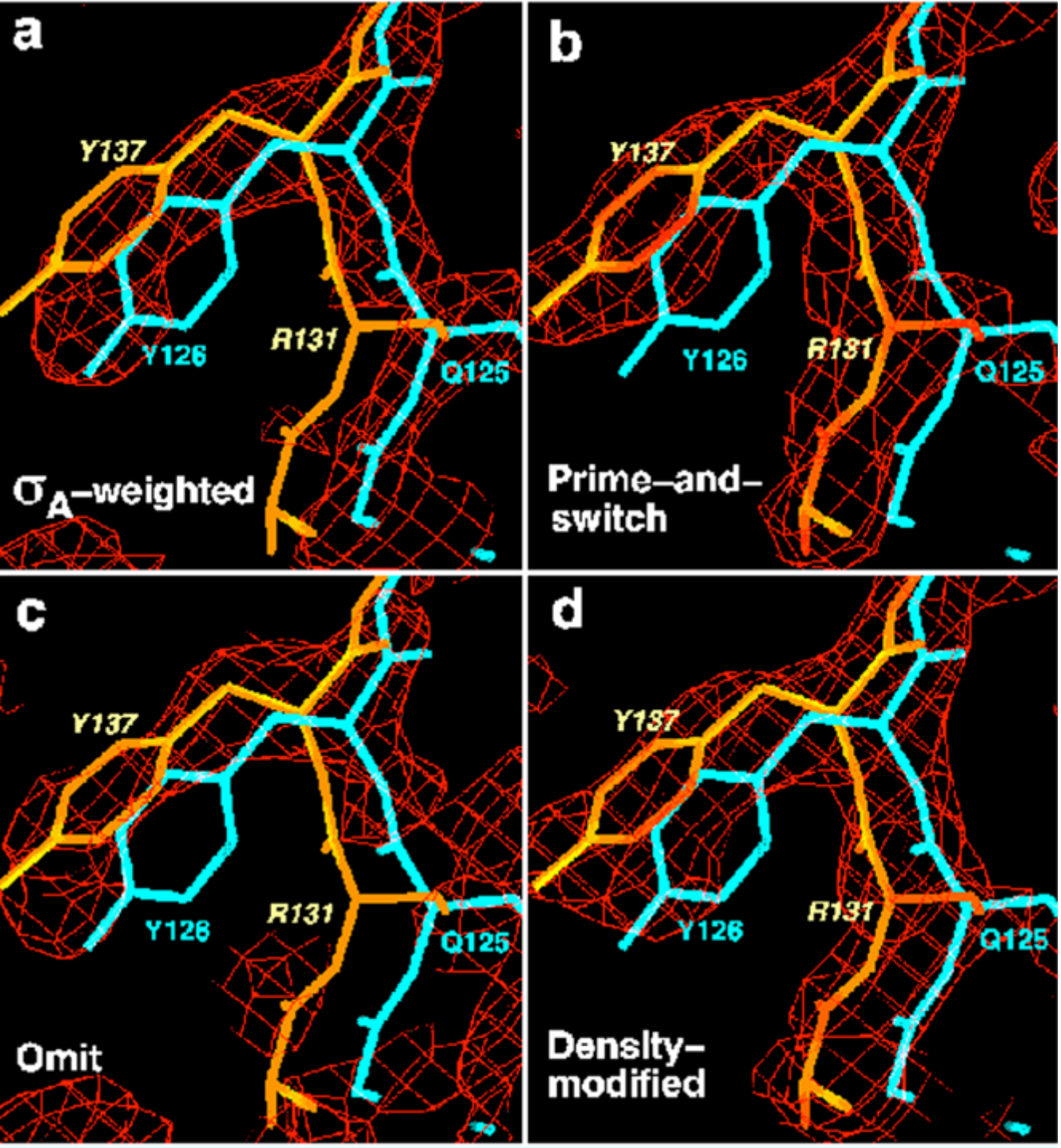
Why it should work…

Priming: Starting phases are close to correct ones…but have bias towards misplaced atoms

Switching: Map-probability phase information comes from a different source…which reinforces just the correct phase information



Signal: peak height at correct atomic positions
Bias: peak height at incorrect atoms in starting model



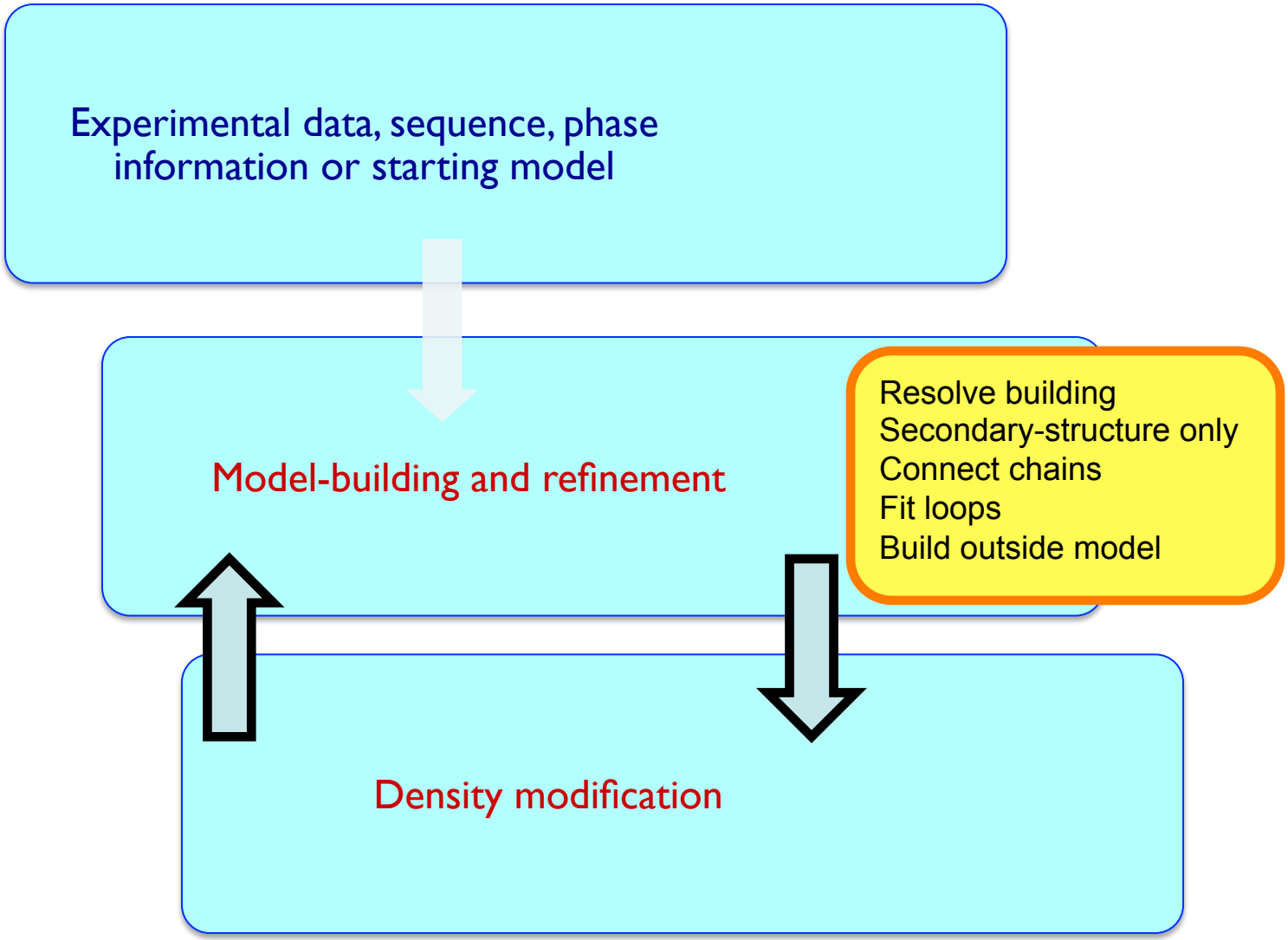
Prime-and-switch example

(IF5A, T. Peat)

Orange:
correct model

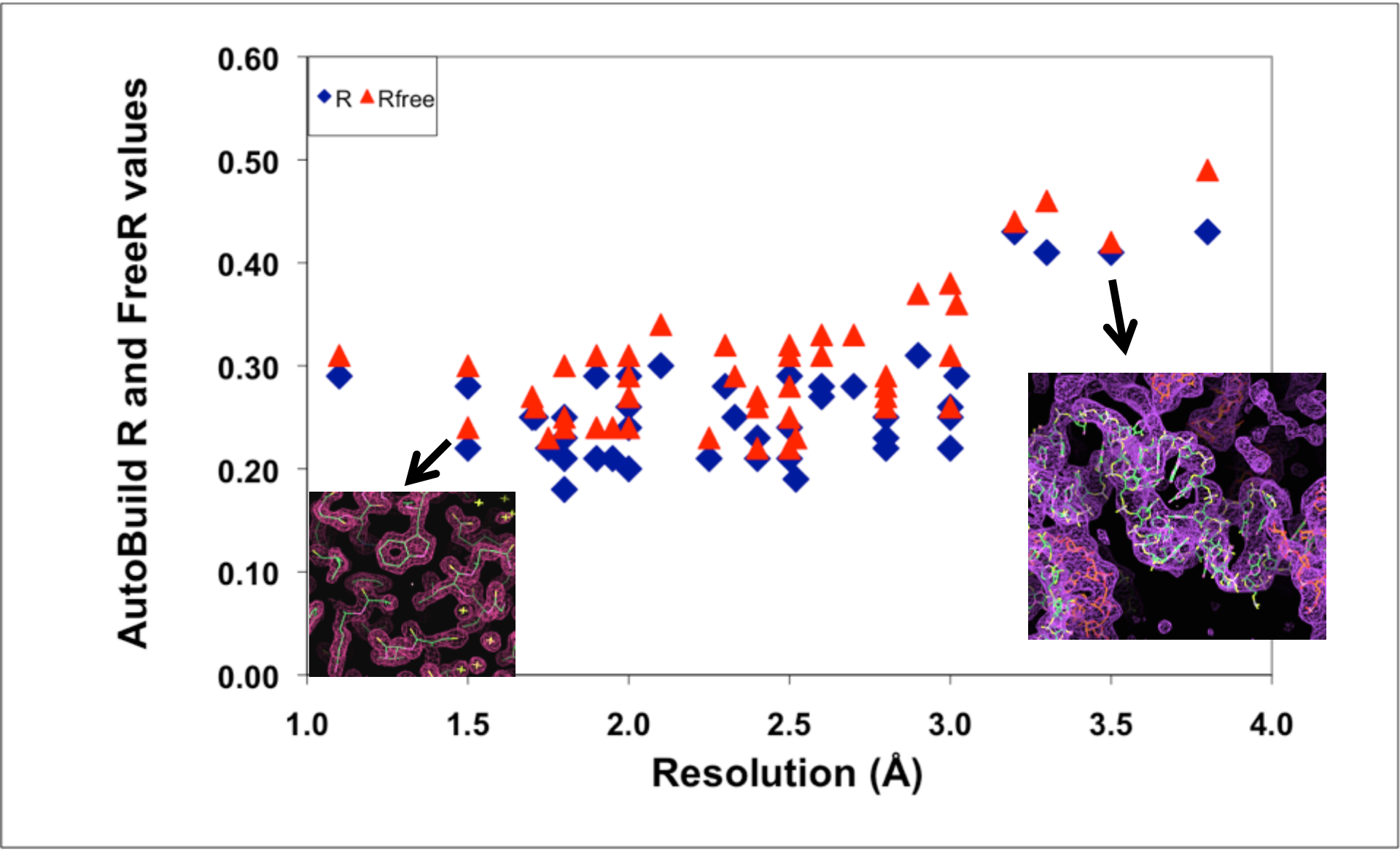
Blue: model used to calculate phases

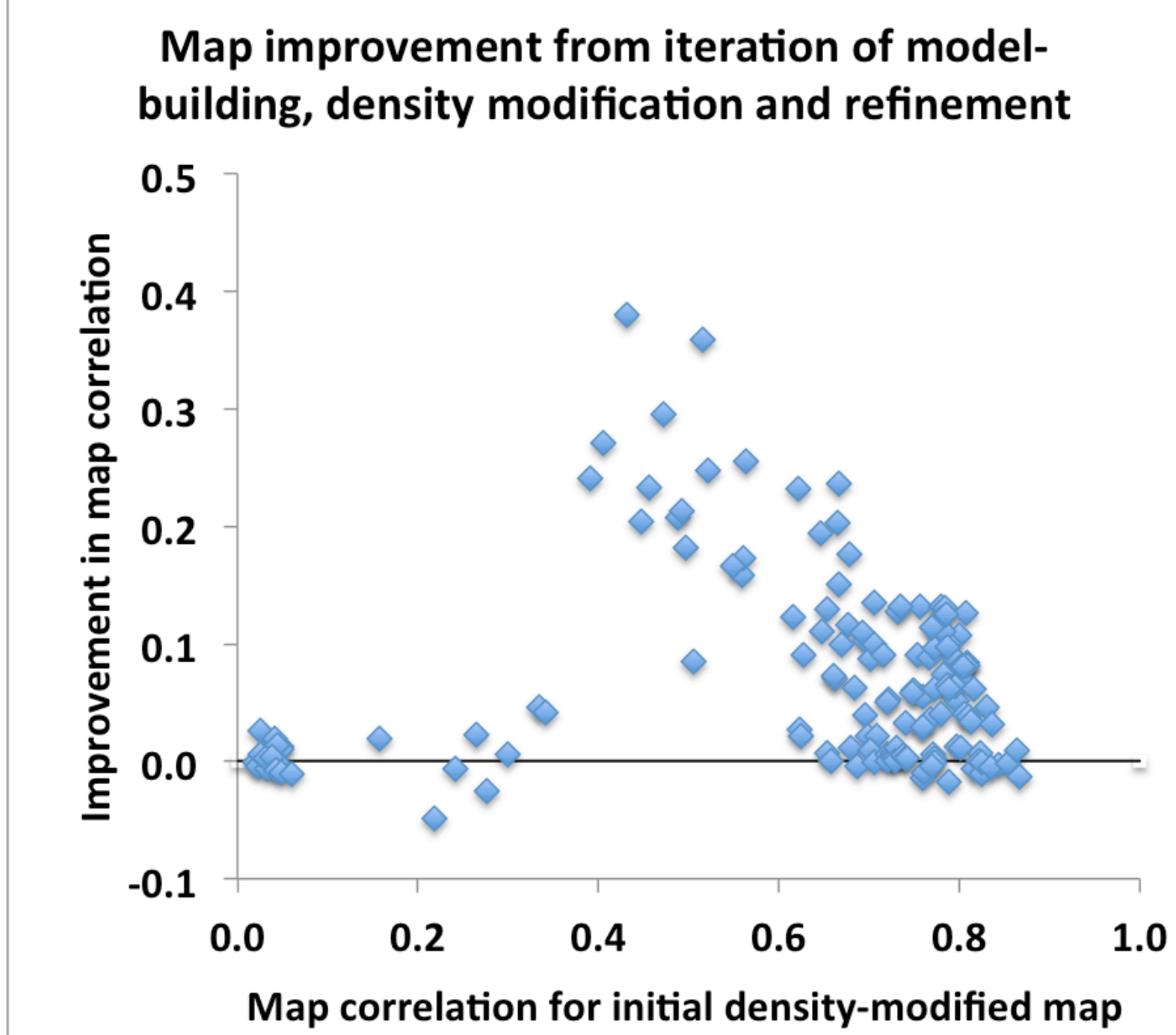
Iterative density modification, model-building and refinement with phenix.autobuild



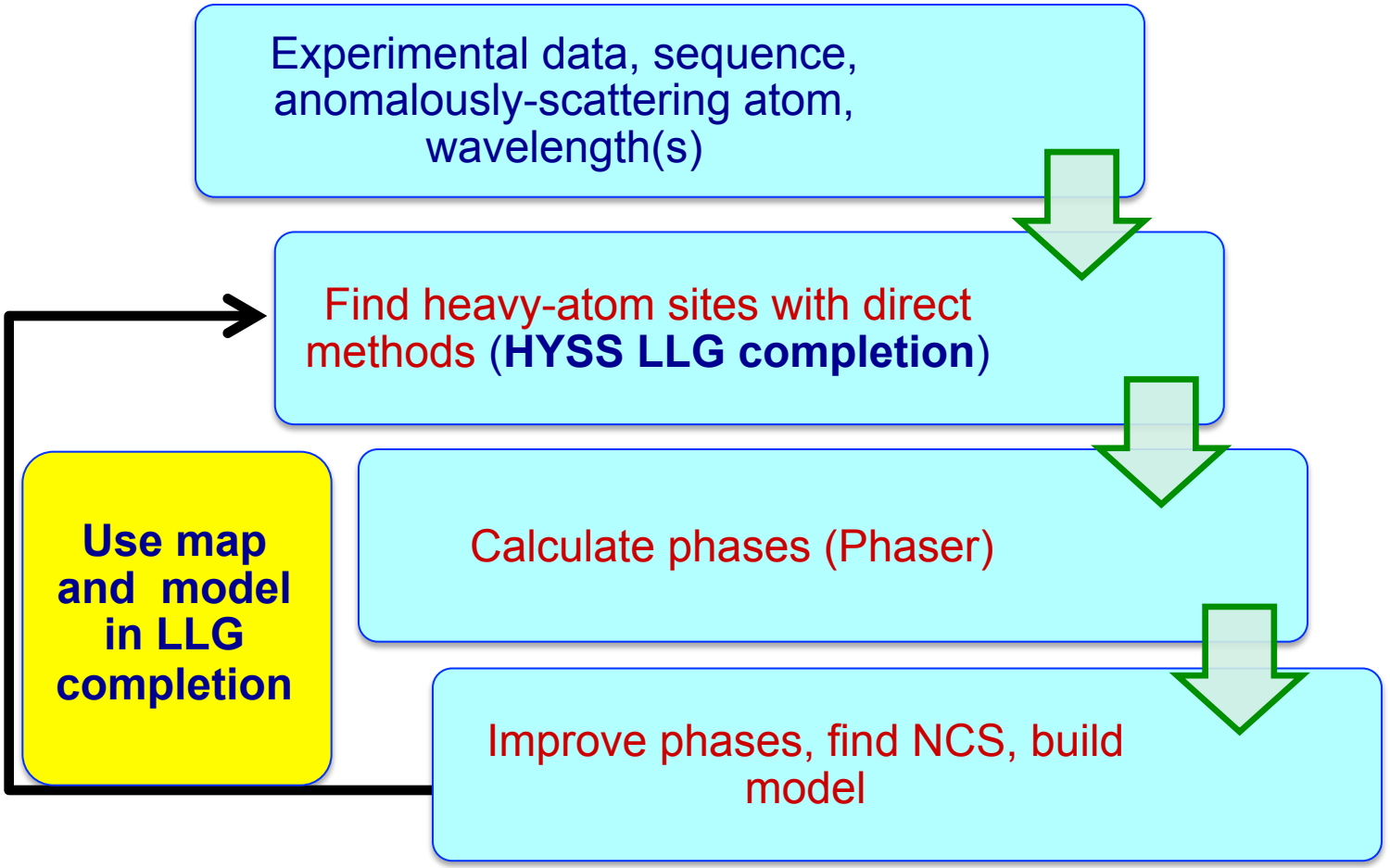
AutoBuild – tests with structure library

Fully automated iterative model-building, final R/Rfree

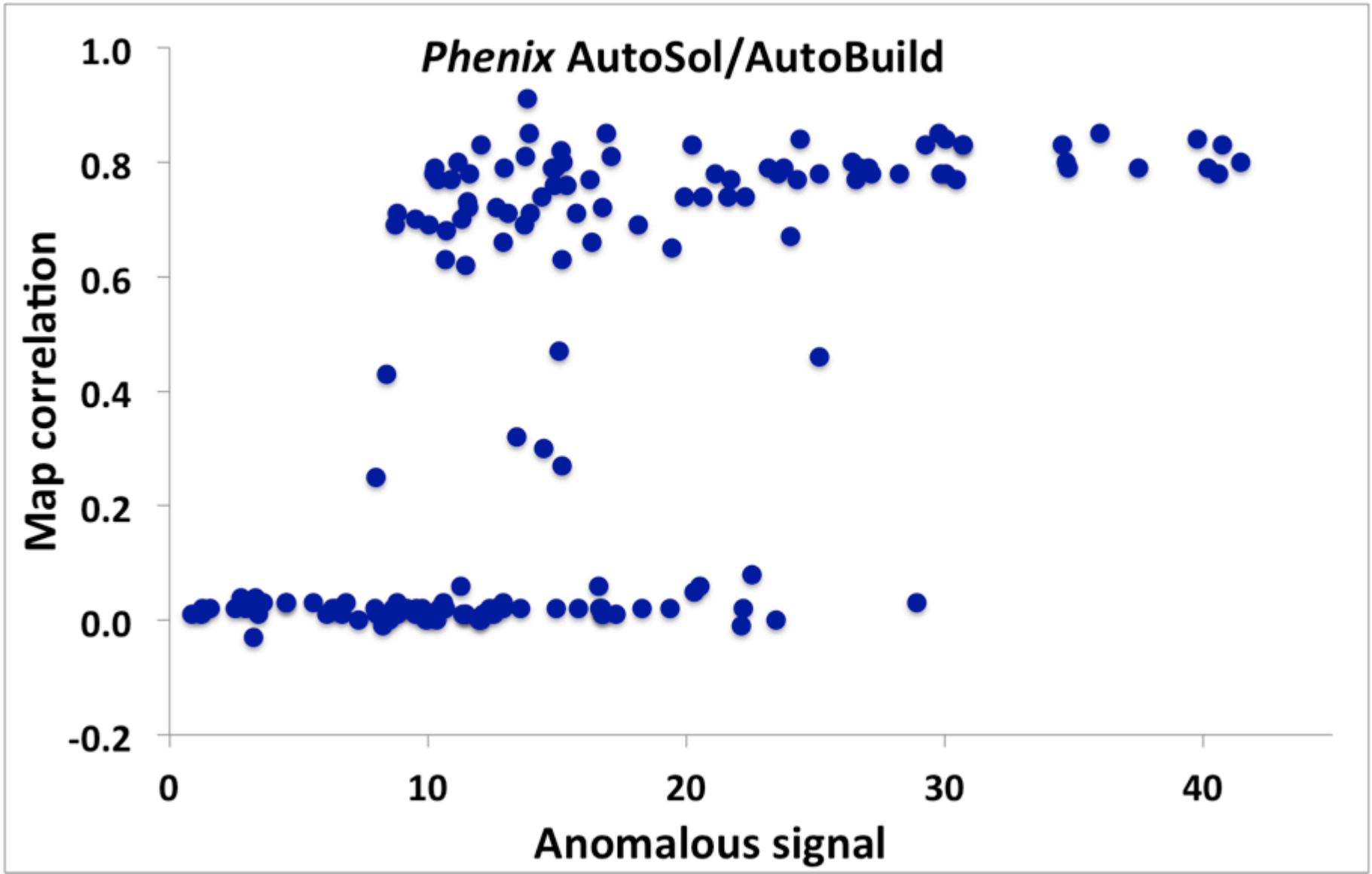



Map improvement from iteration of model-building, density modification and refinement
Improvement in map correlation
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0.0
0.2
0.4
0.6
0.8
1.0
Map correlation for initial density-modified map

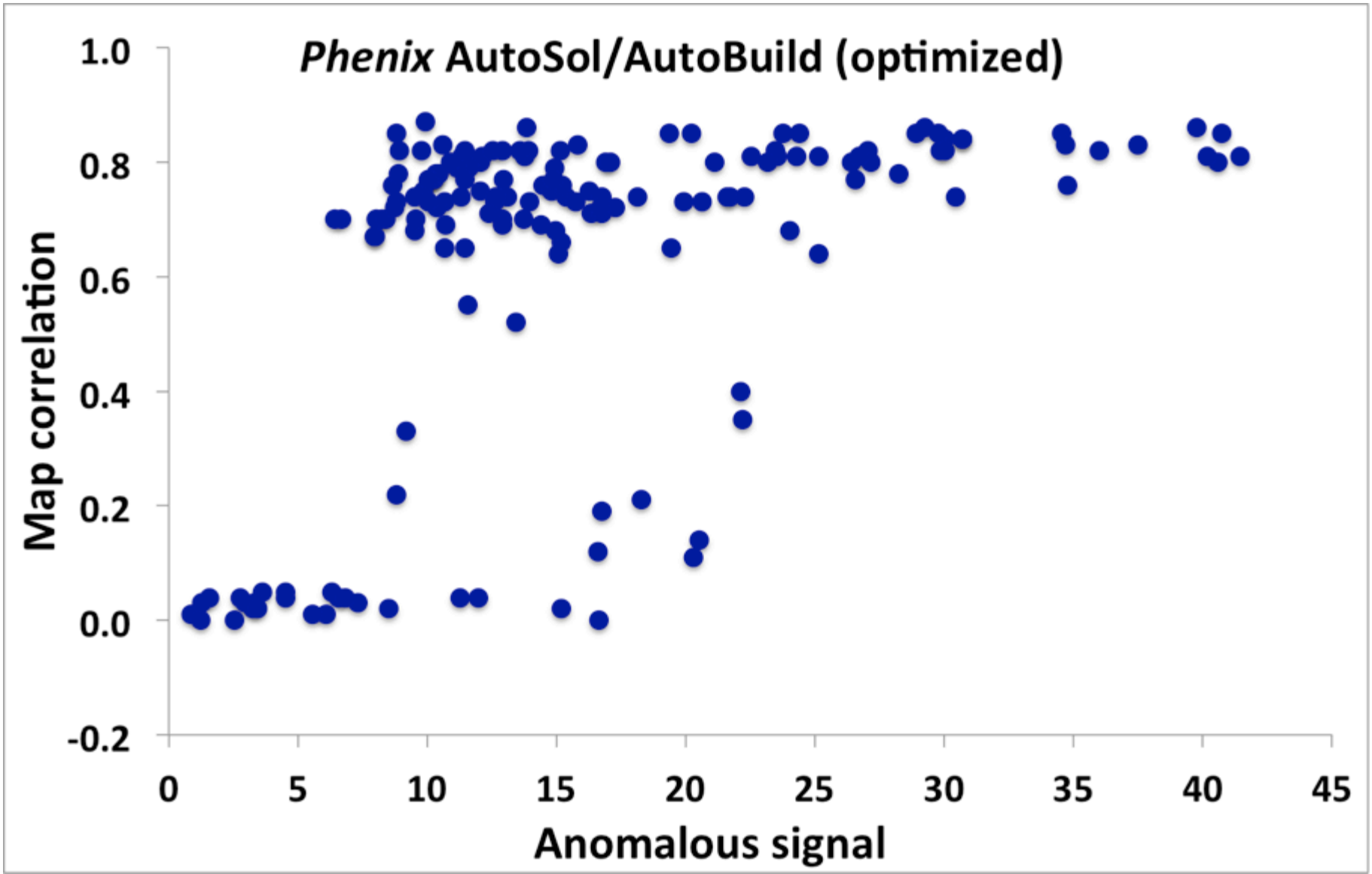
Structure solution with *Phenix*: enhancements for weak SAD data



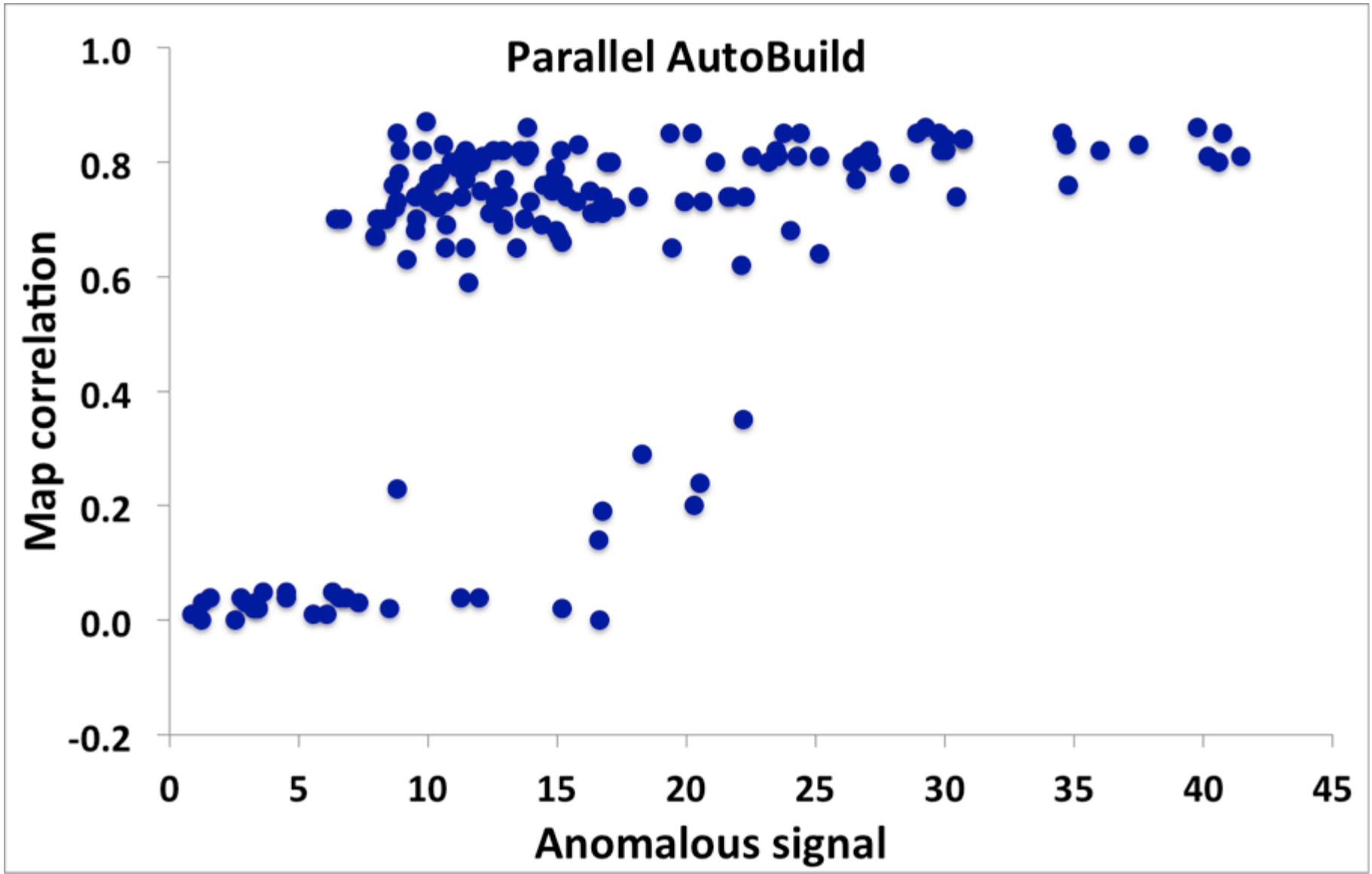
AutoSol structure solution
164 SAD datasets from PDB
(including inflection/remote datasets not previously used as SAD data)



AutoSol structure solution
164 SAD datasets from PDB



AutoBuild model-building
164 SAD datasets from PDB



What can you do with automated procedures for structure solution and model-building?

If a task is modular and automated…

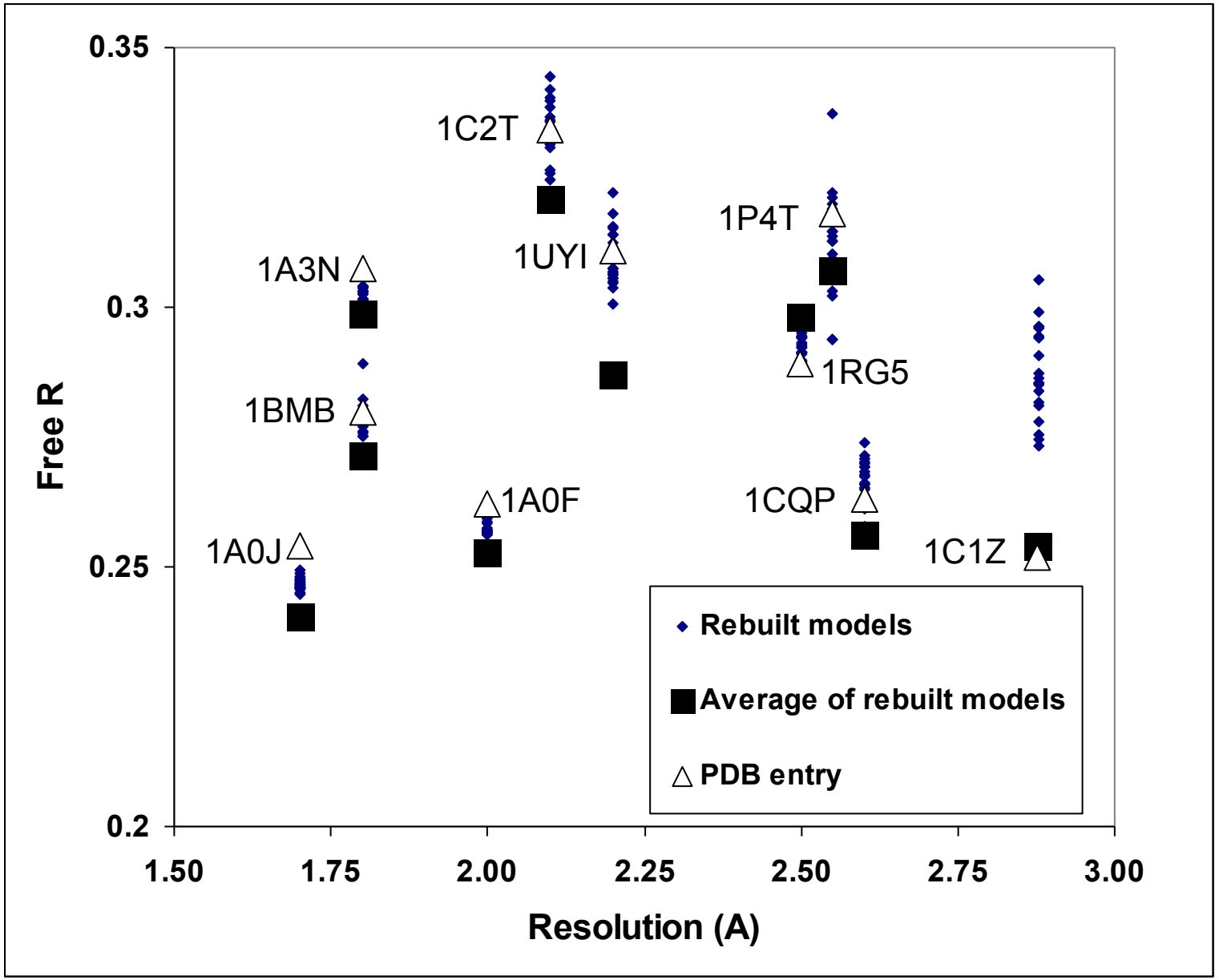
you can run it many times

…checking different space groups, datasets to use

…checking if your model is biasing your map

…checking if you always get the same model

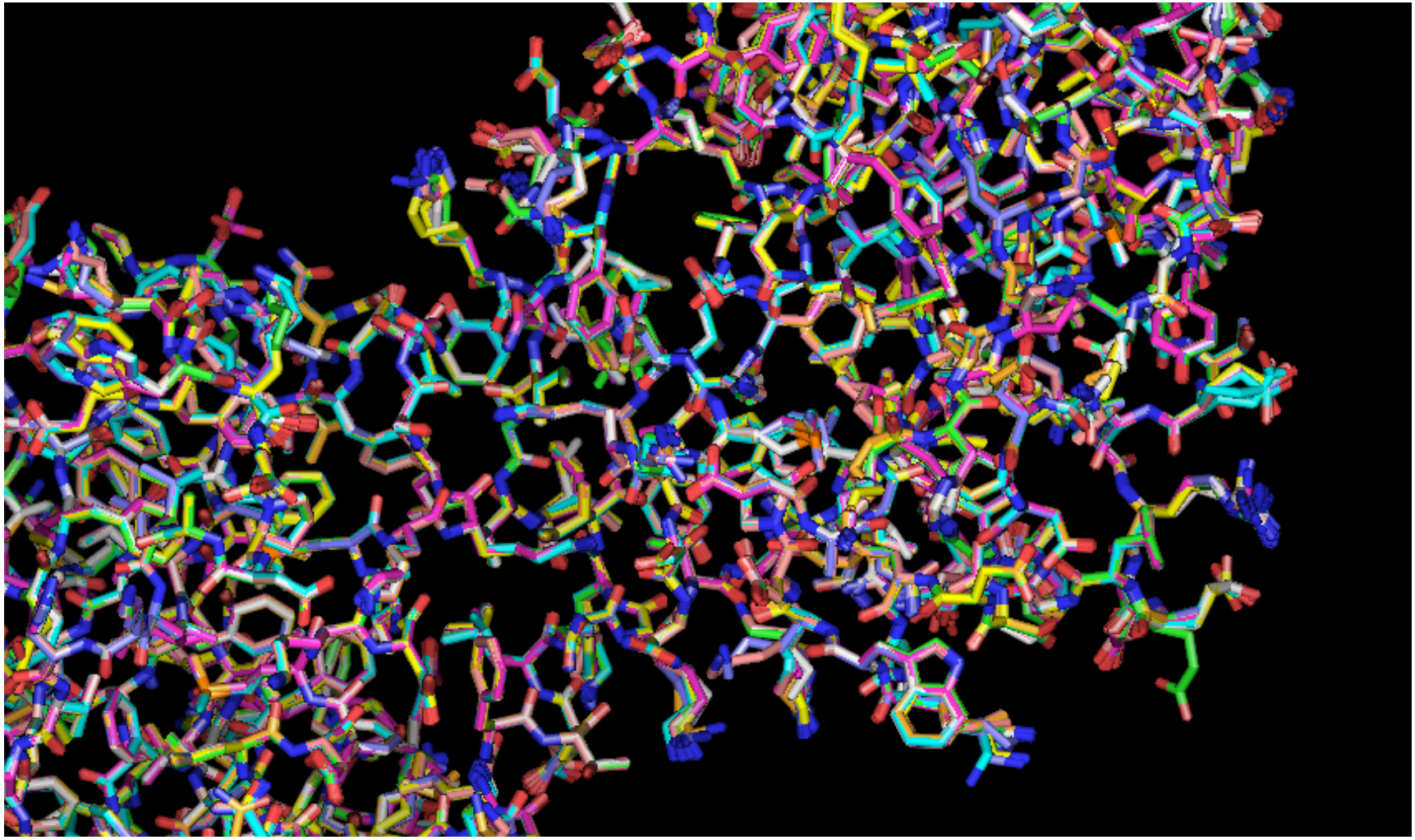
Building 20 models for each of 10 structures



Multiple-model representation of uncertainties

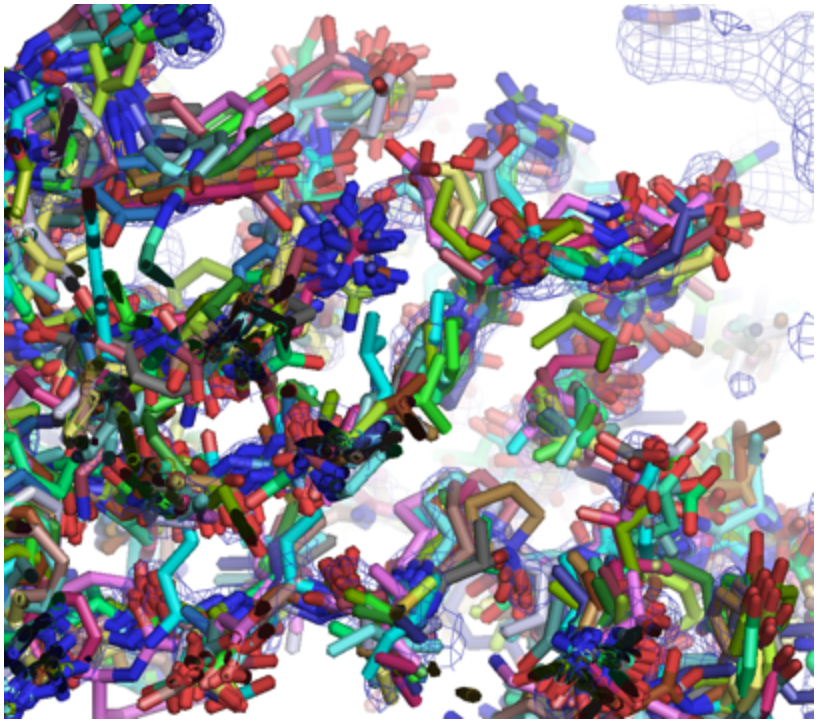
20 models built for 1CQP, no waters, Dmin=2.6 A R=0.19-0.20; Rfree=0.26-0.27

The variation among models is a lower bound on their uncertainty

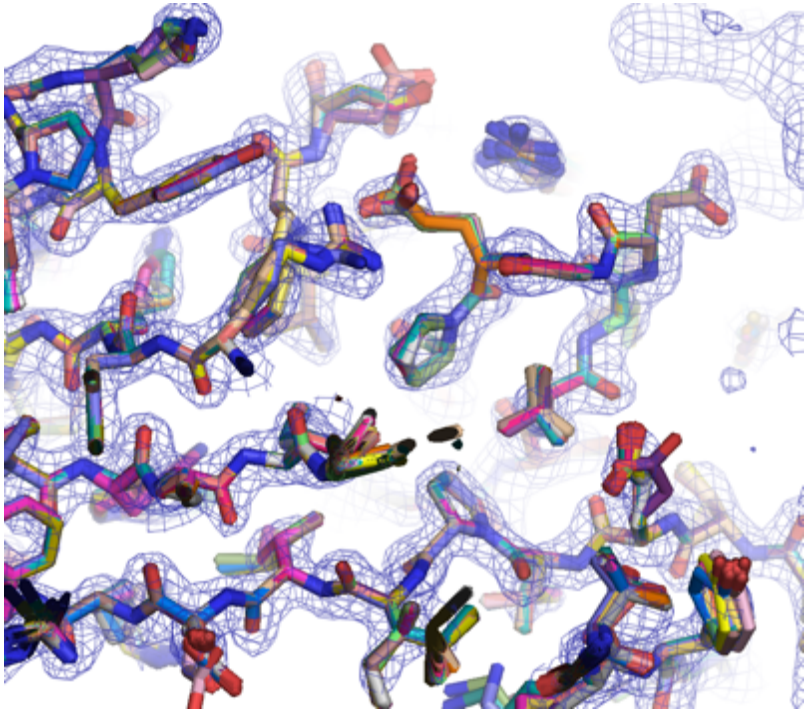


->The RMSD among models tells us (a lower bound on) the uncertainty in our models

(It is not the RMSD of true structures in the crystal)



Rebuild with 4.5 Å data



Rebuild with 1.75 Å data

The Phenix Project

Lawrence Berkeley Laboratory

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An NIH/NIGMS funded Program Project

Phenix

Low-Resolution Model-building

***Phenix* workshop**

Shanghai, China
Jan. 14, 2016

Tom Terwilliger
Los Alamos National Laboratory

Rapid building of models for regions containing regular secondary-structure

Helices:

Identification: rods of density at low resolution

Strands:

Identification: β structure as nearly-parallel pairs of tubes

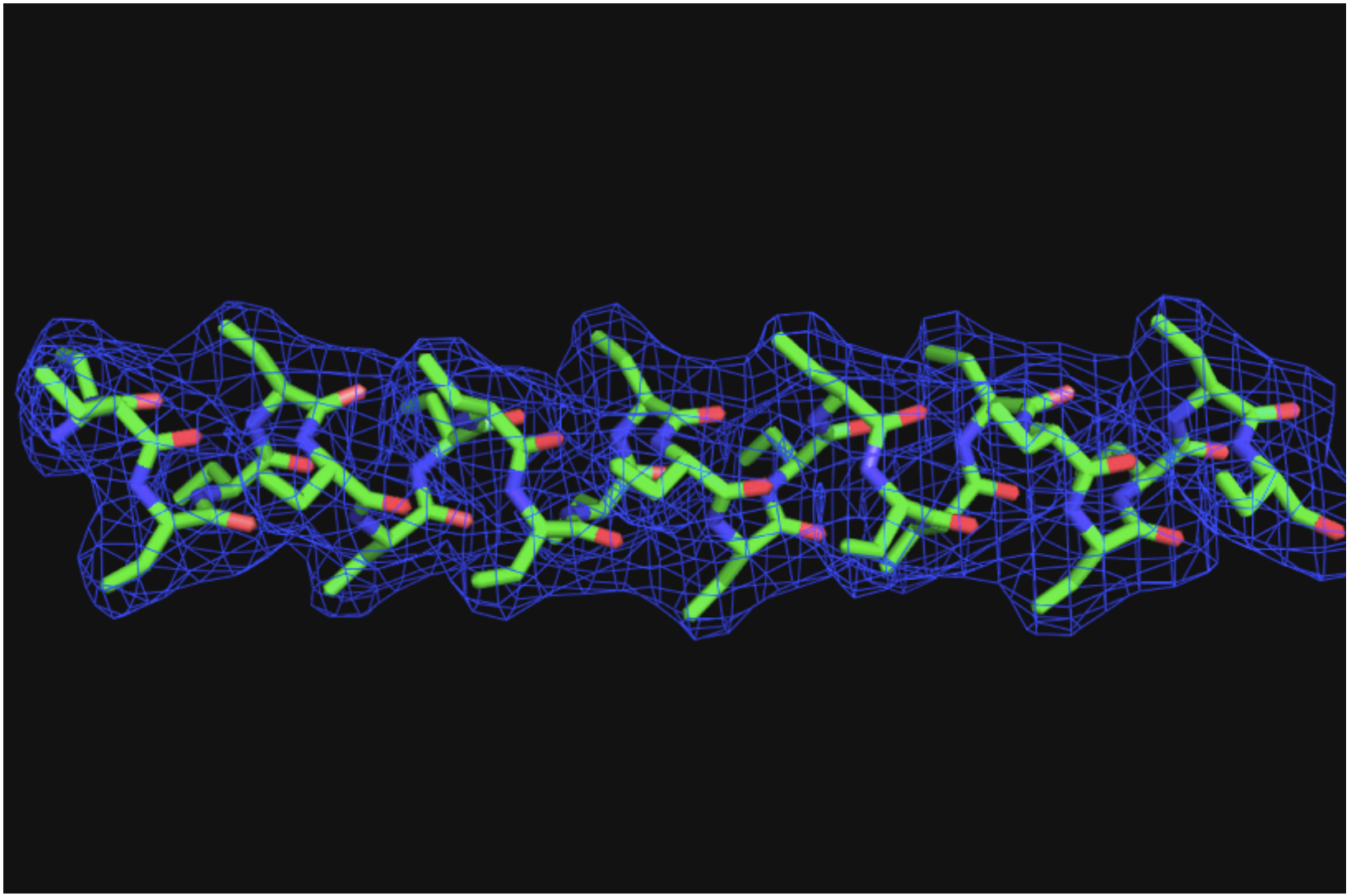
Any protein chains (trace_chain):

Identification: Cα positions consistent with density and geometry of protein chains

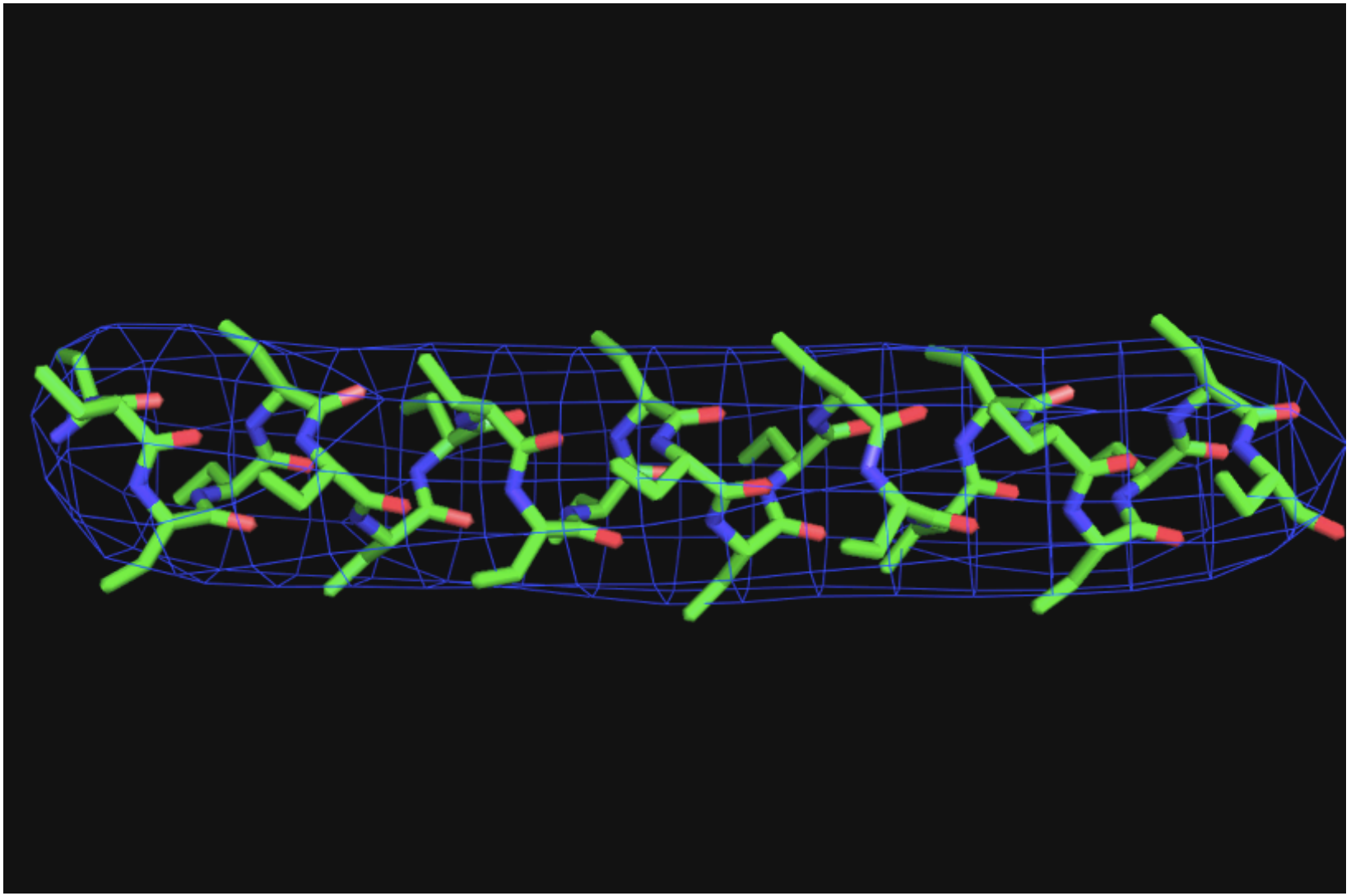
RNA/DNA:

Identification: match of density to averaged A or B-form template

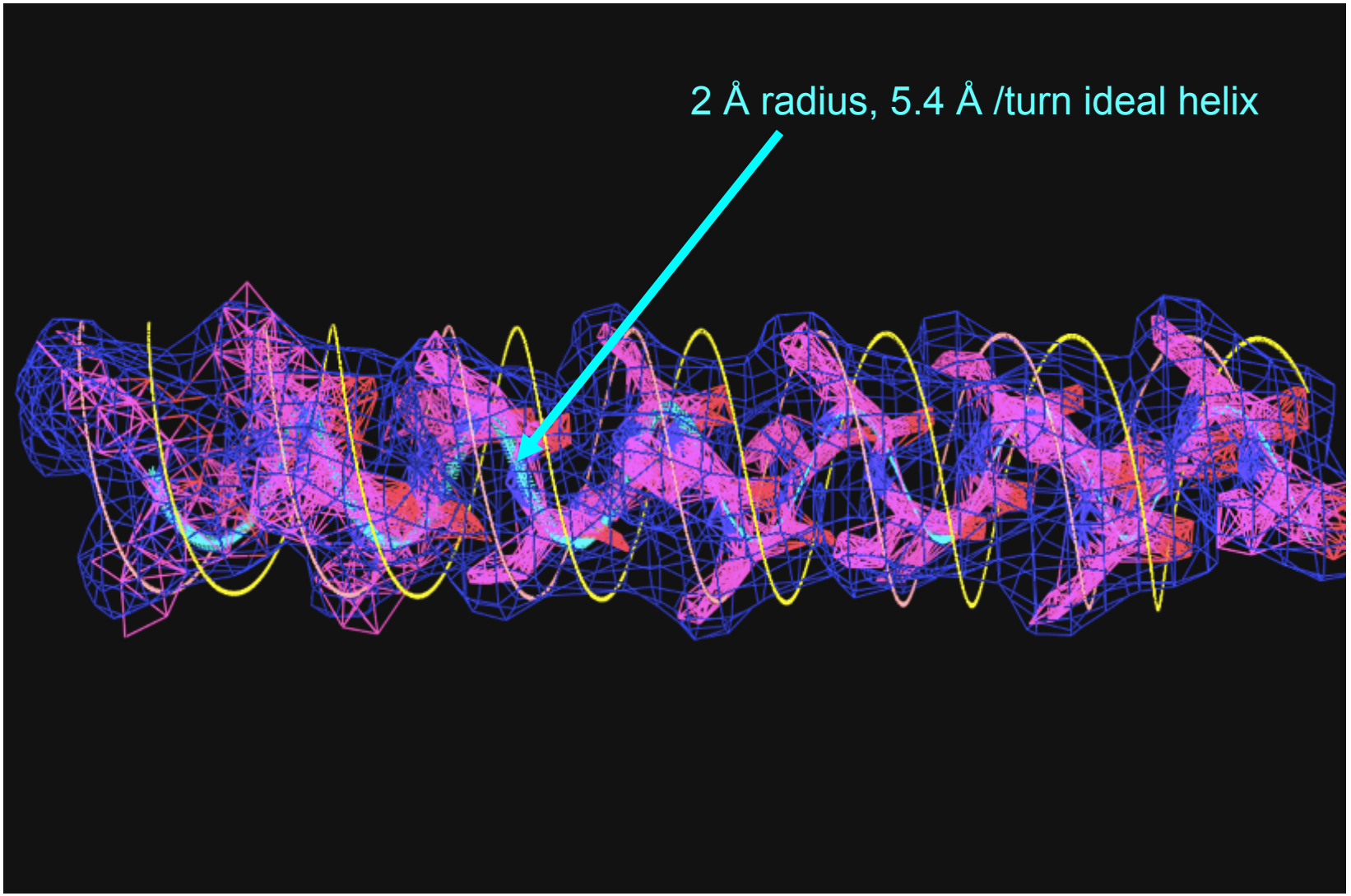
Model α-helix; 3 Å map



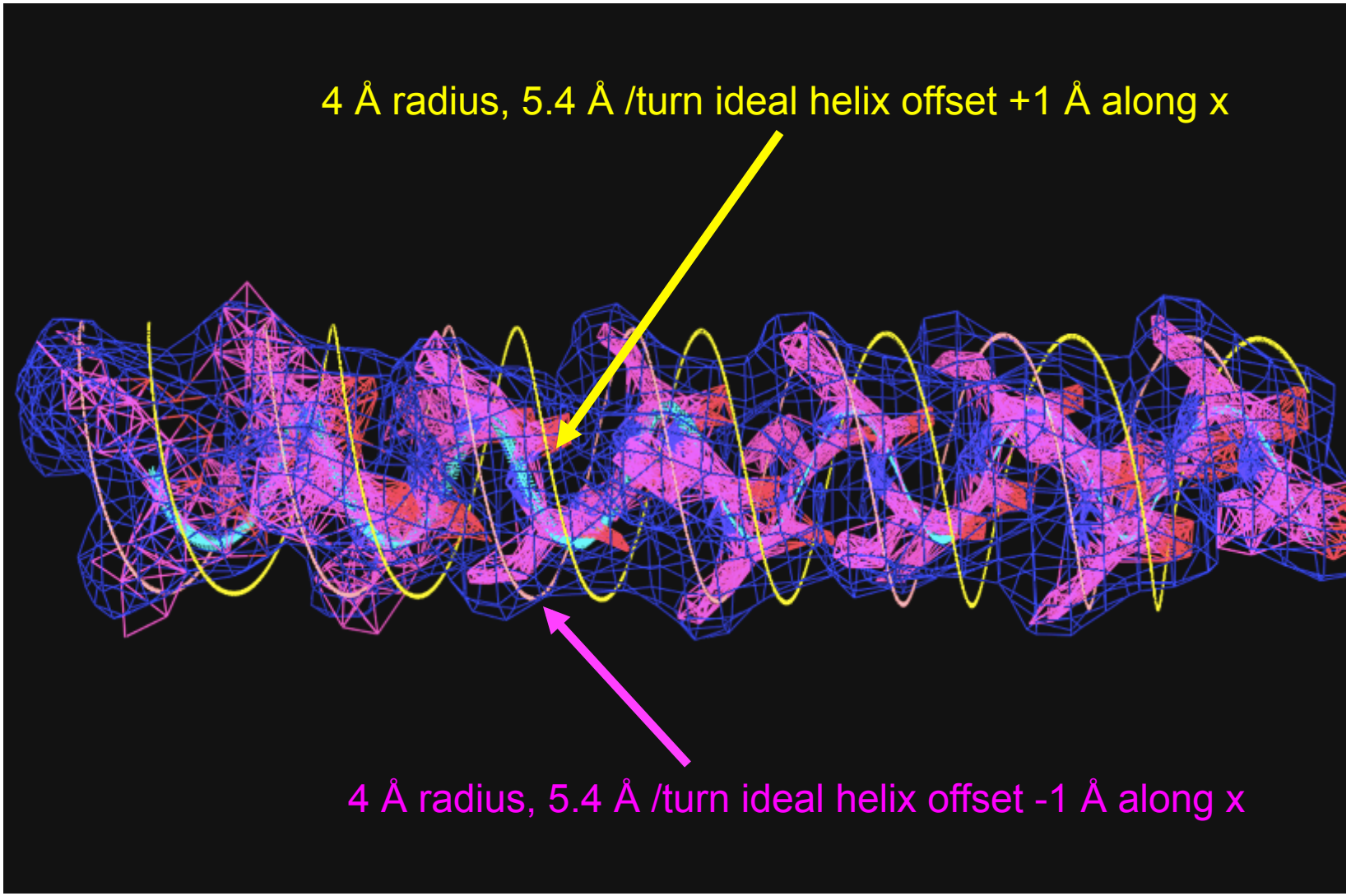
Model α-helix; 7 Å map



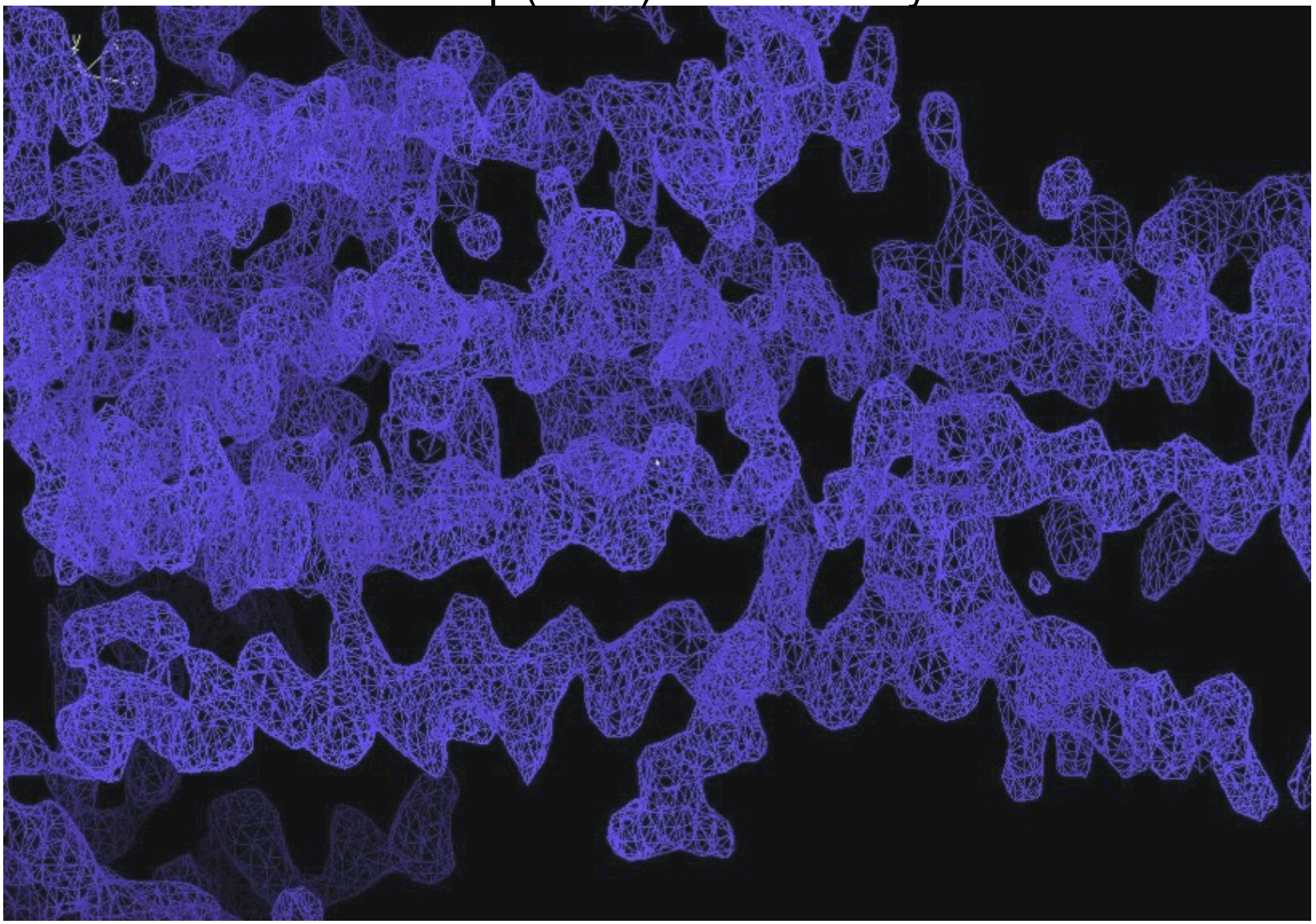
Trace main-chain with ideal helix, allowing curvature



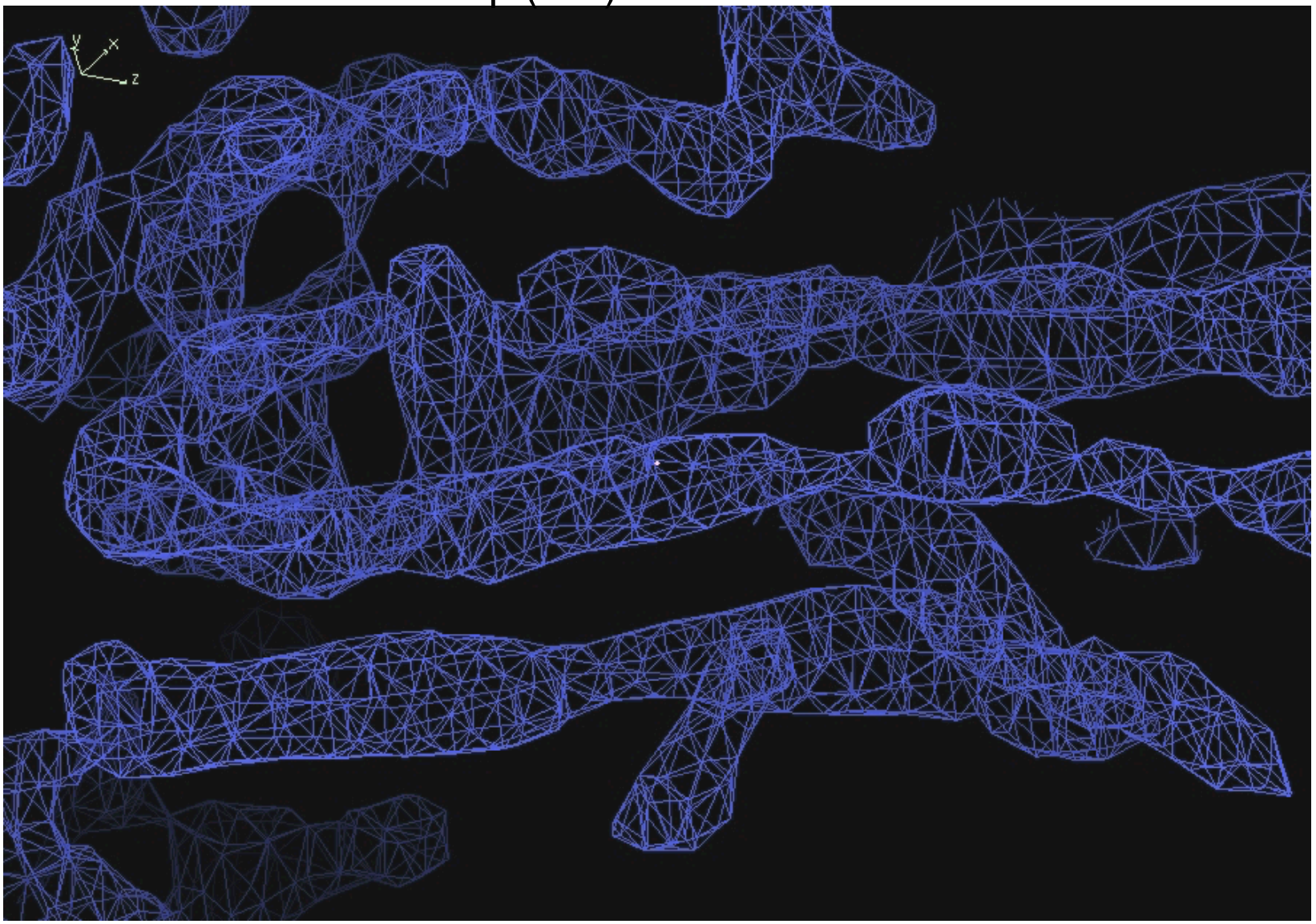
Identify direction and Cα position from overlap with 4 Å radius helices offset +/- 1 Å from main-chain



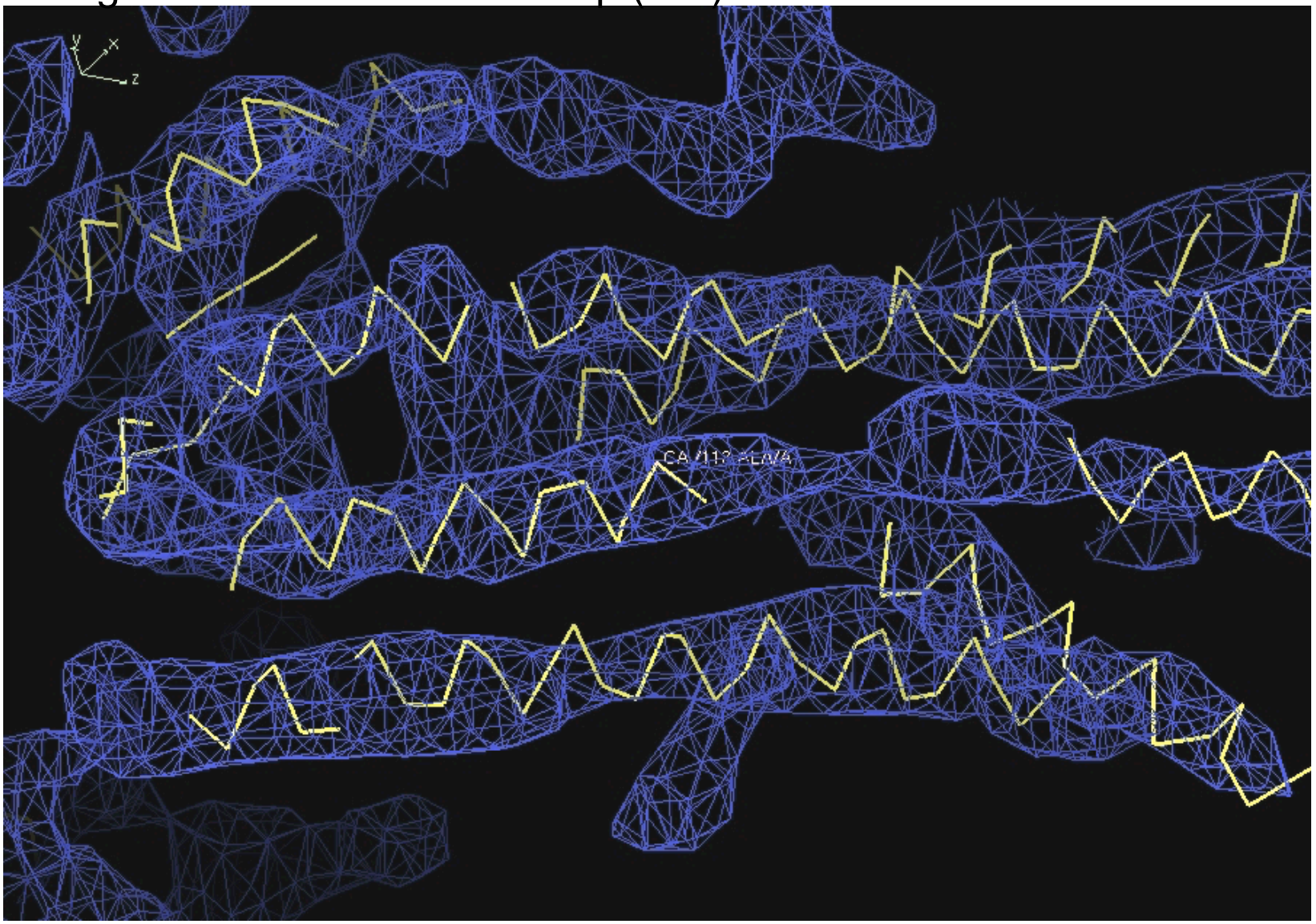
A real case: 1T5S SAD map (3.1 Å) Data courtesy of P. Nissen



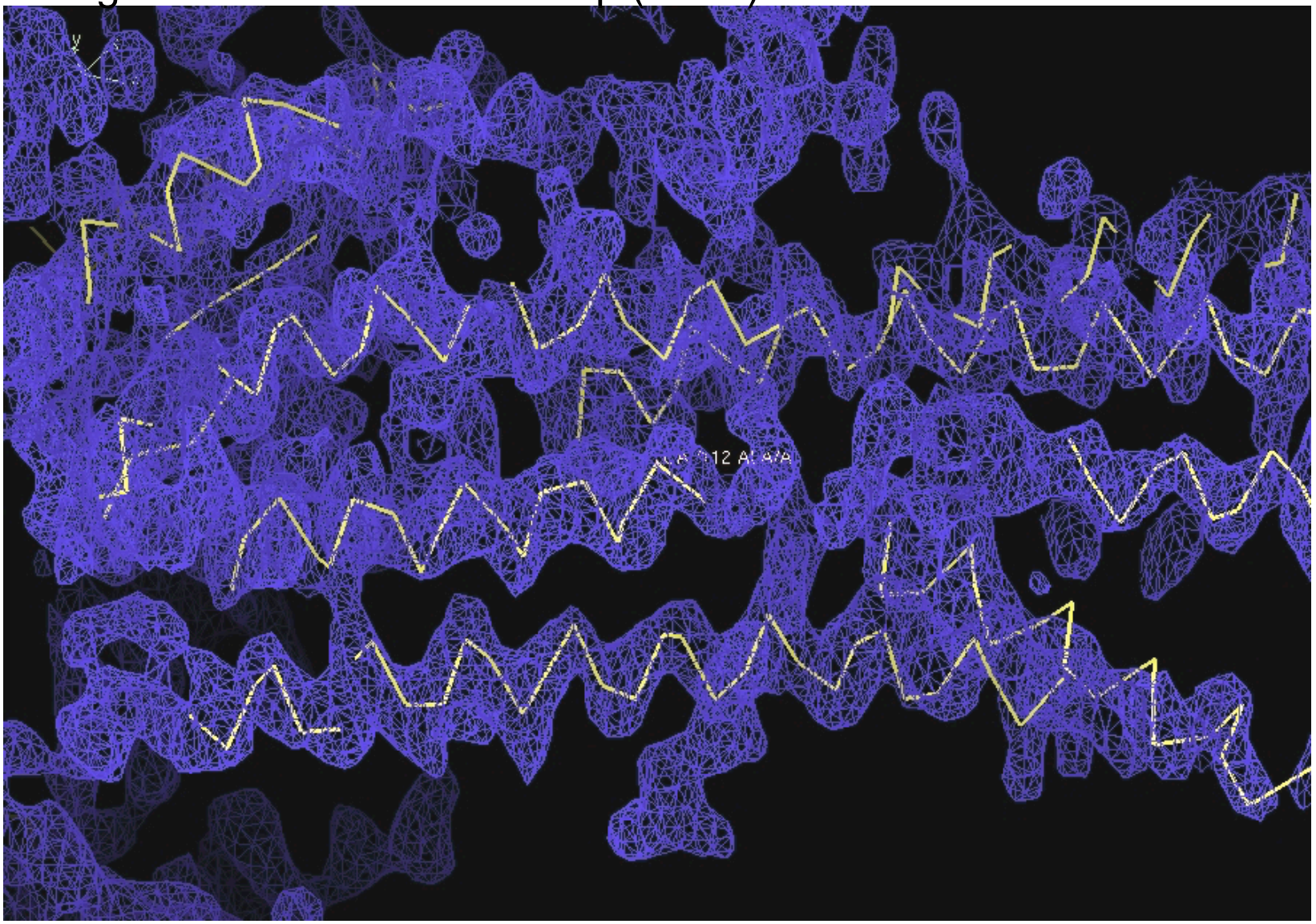
A real case: 1T5S SAD map (7 Å)



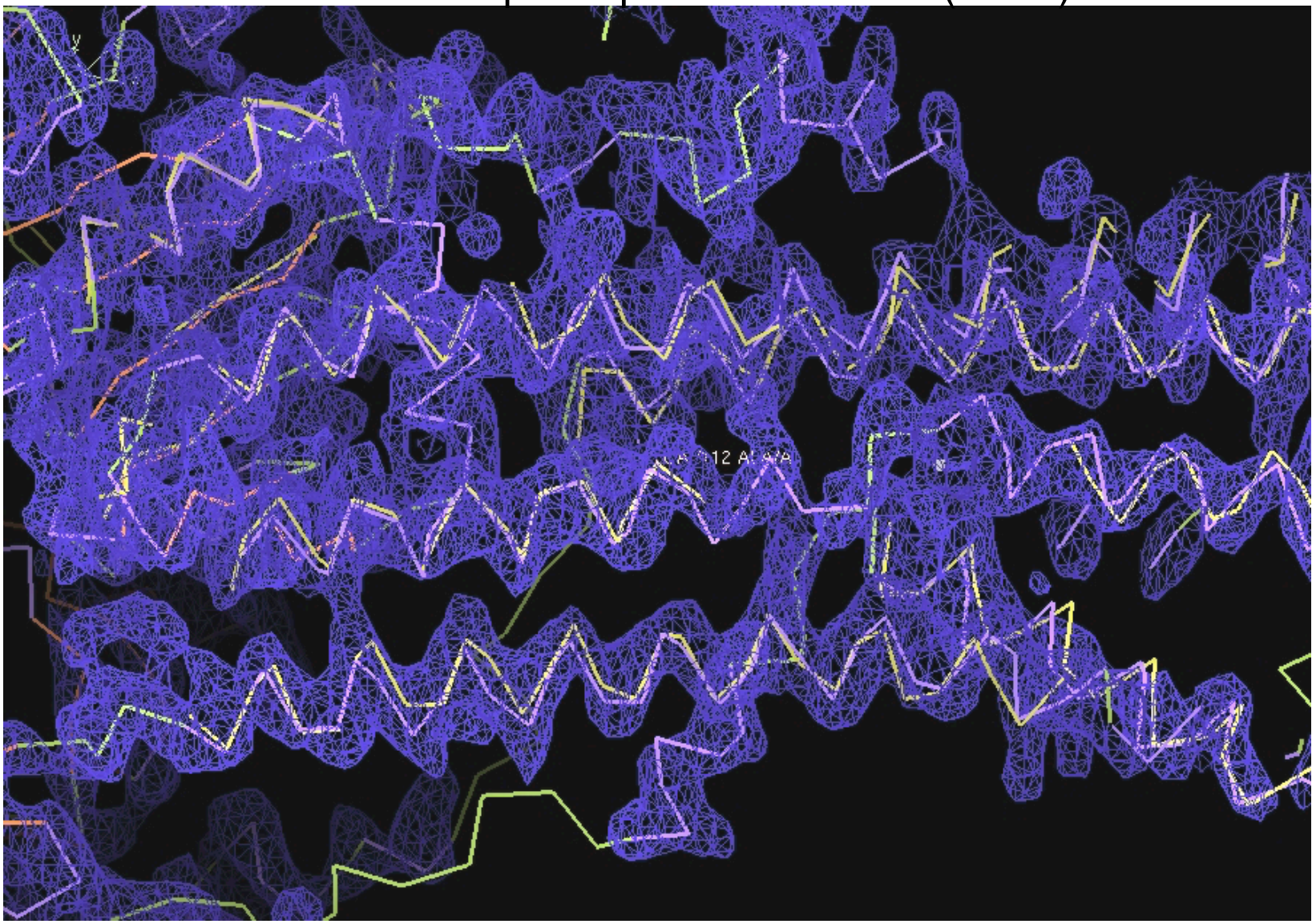
Finding helices in 1T5S SAD map (7 Å)



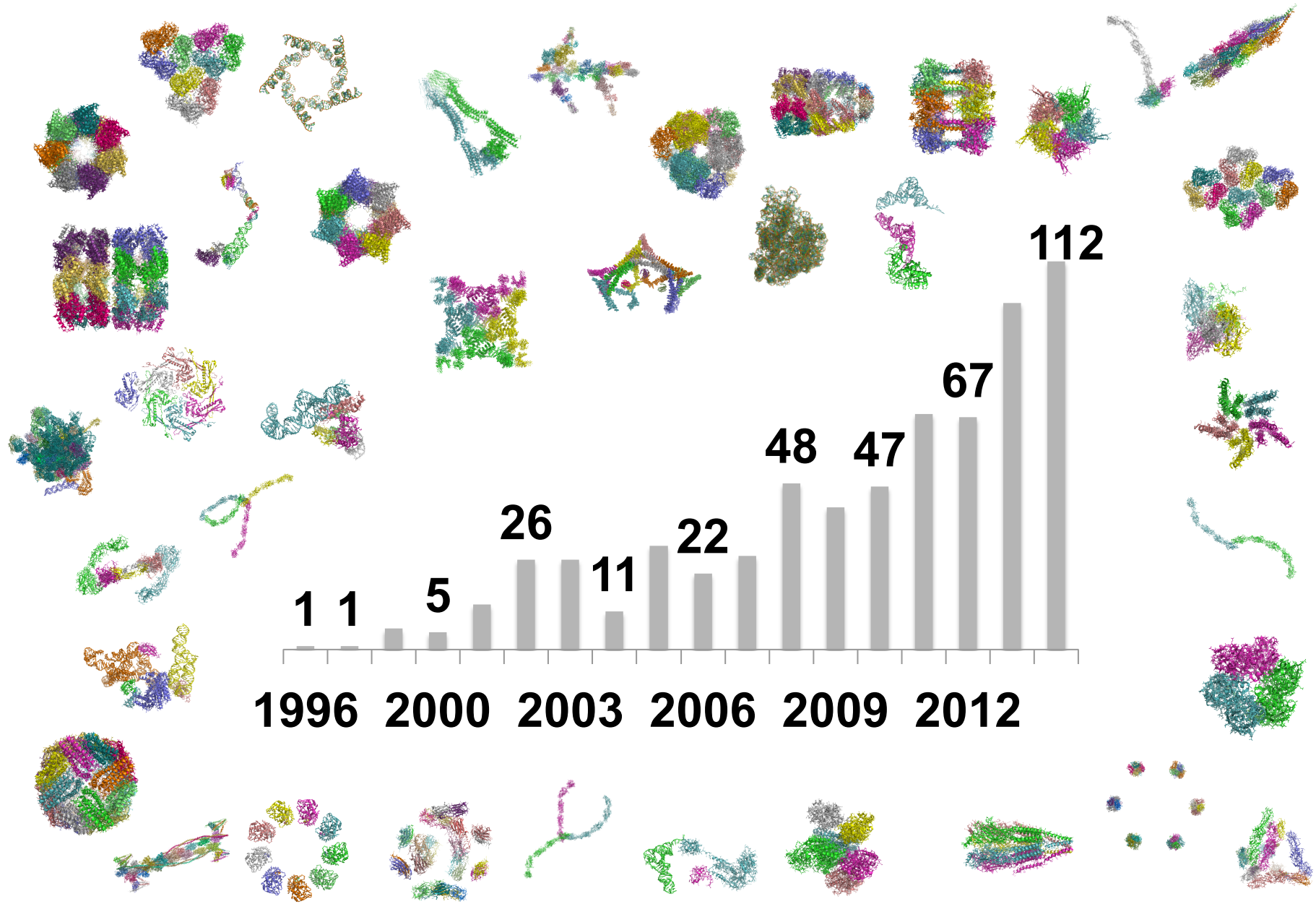
Finding helices in 1T5S SAD map (3.1 Å)



Helices from 1T5S SAD map compared with 1T5S (3.1 Å)



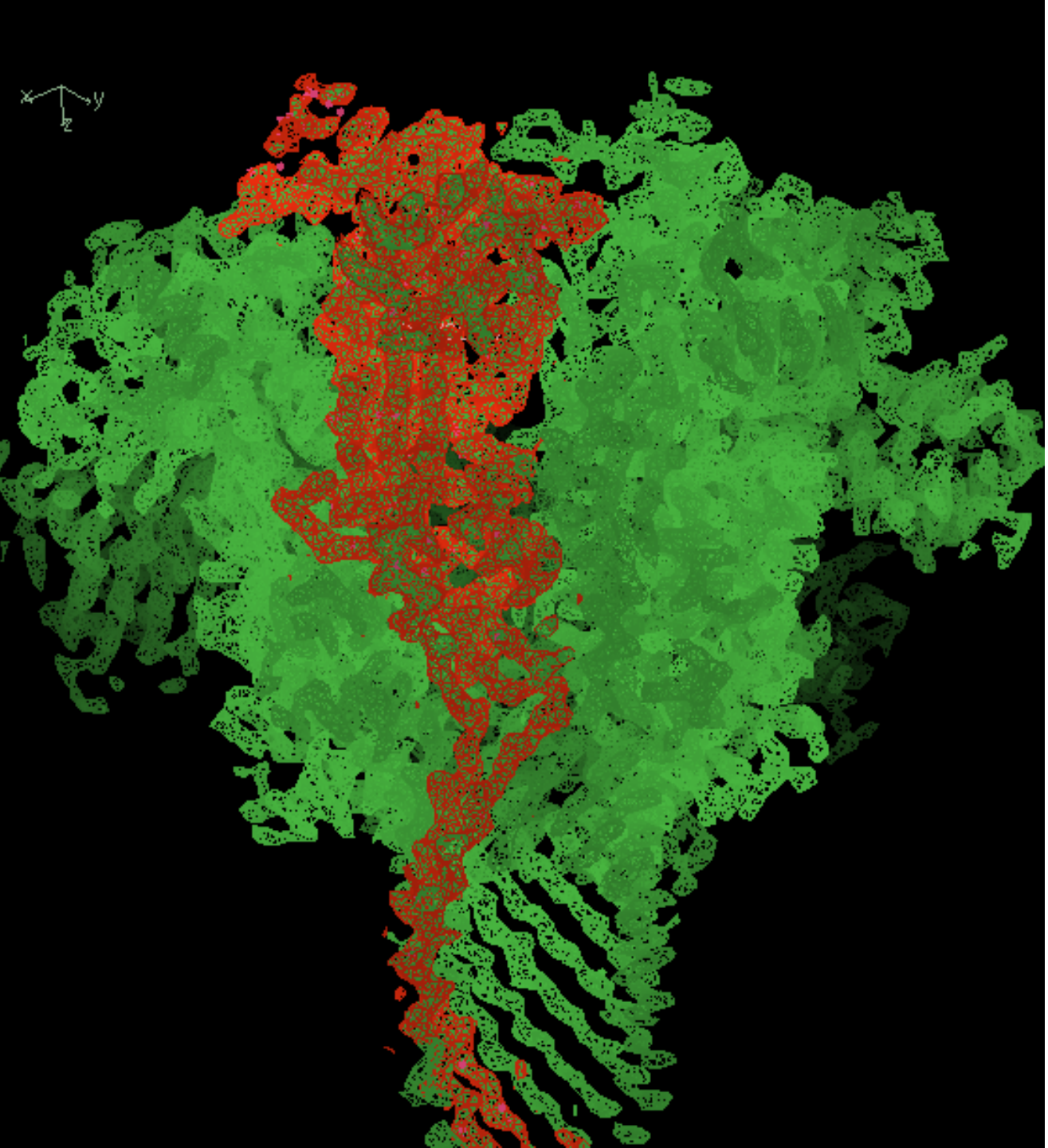
About 750 Cryo-EM structures in PDB



Building into cryo-EM maps

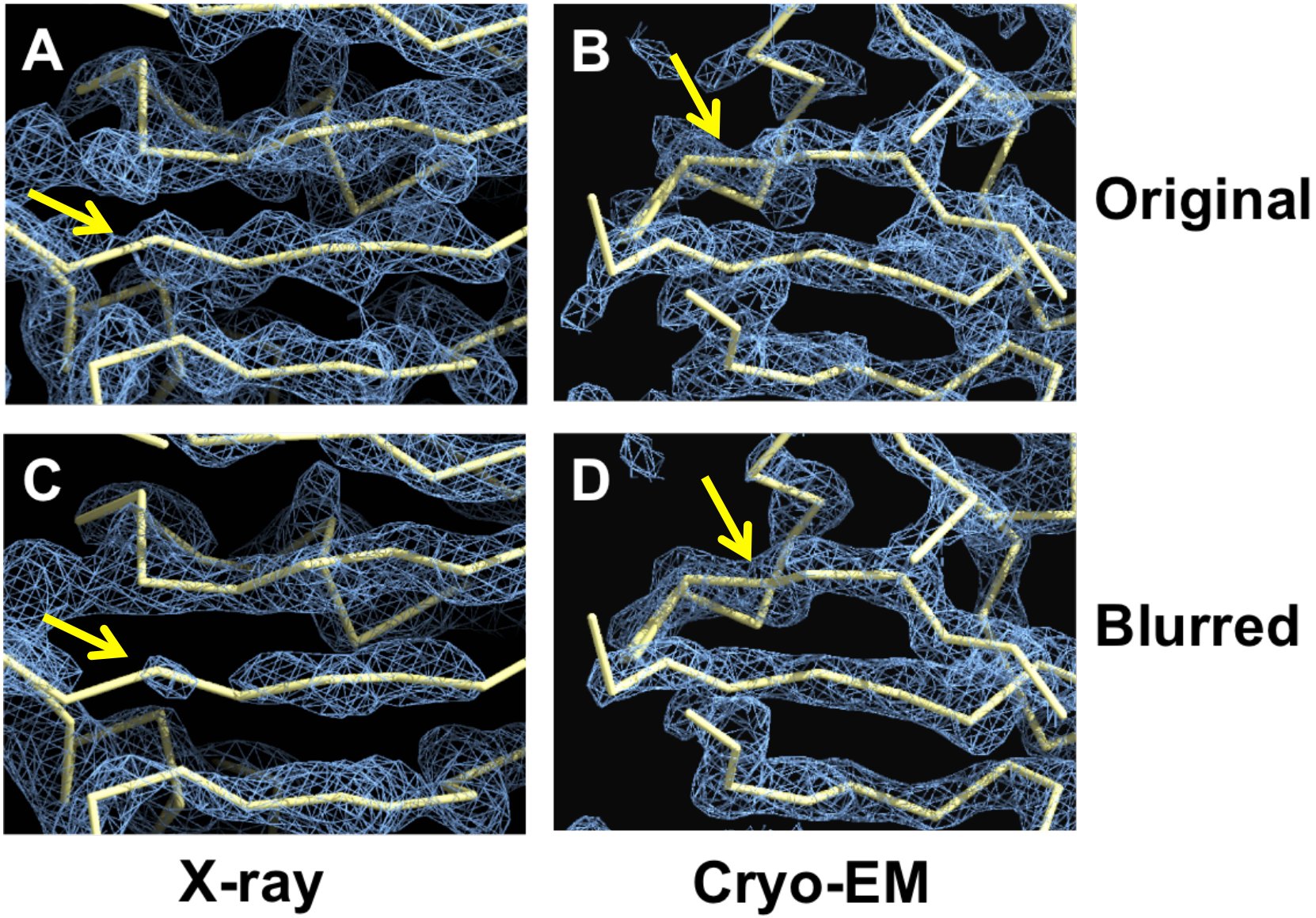
- Automatically segment maps and extract asymmetric unit of reconstruction
- Create maps emphasizing information at various resolutions by variable map sharpening
- Trace protein main chain using nearly-constant Cα-Cα-Cα distances and angles
- Identify direction of main-chain in models by fit to density

Automated segmentation of emd_6224 (anthrax toxin protective antigen pore at 2.9 Å; Jiang et al 2015)



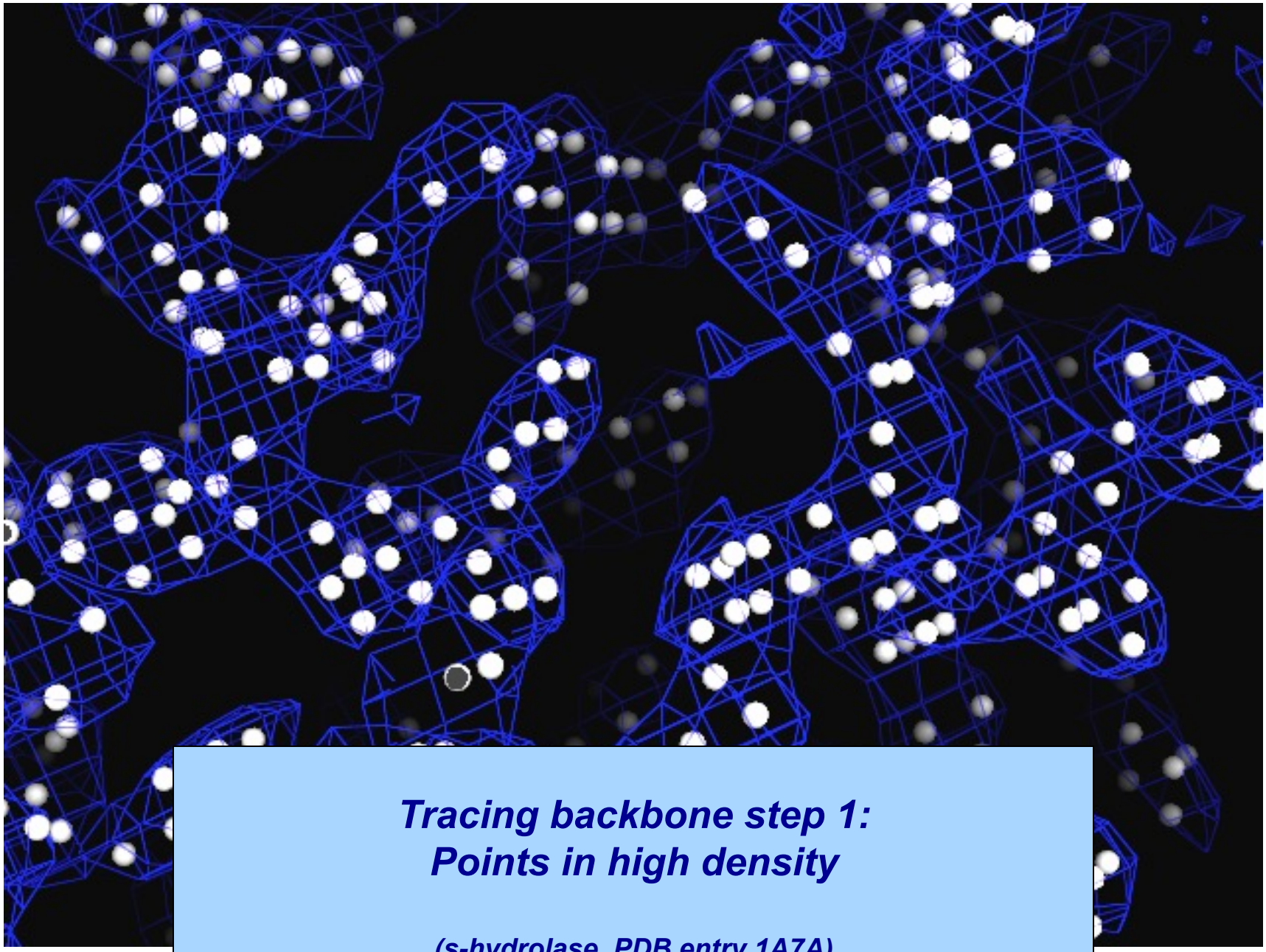
Automated segmentation of emd_6224 (anthrax toxin protective antigen pore at 2.9 Å; Jiang et al 2015)

Accurate low-resolution information in cryo-EM maps



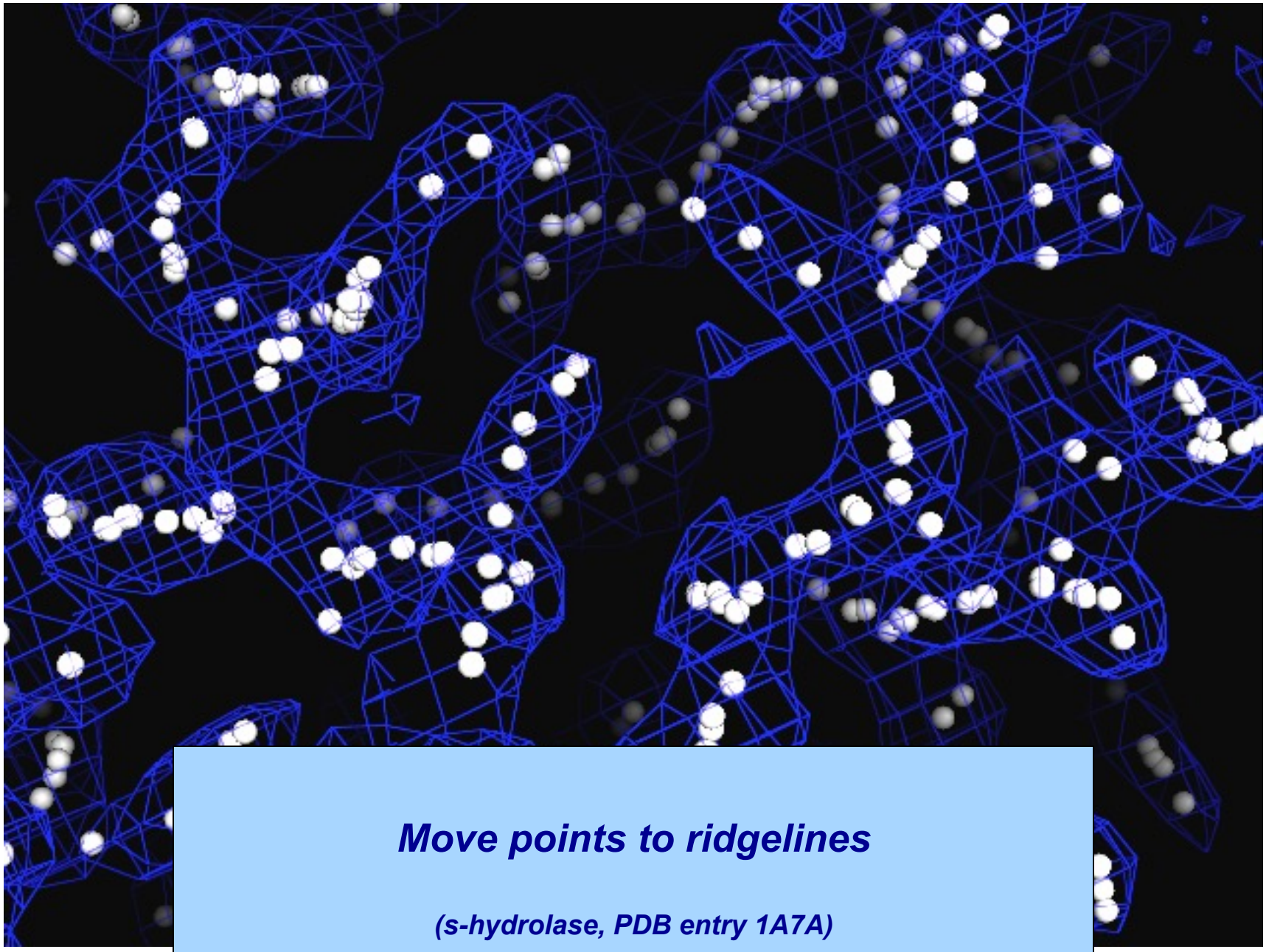
Tracing polypeptide backbone in a map

- Alternative to finding helices/strands
- Can be rapid
- Suited for lower-resolution maps where the backbone is clear but not side chains



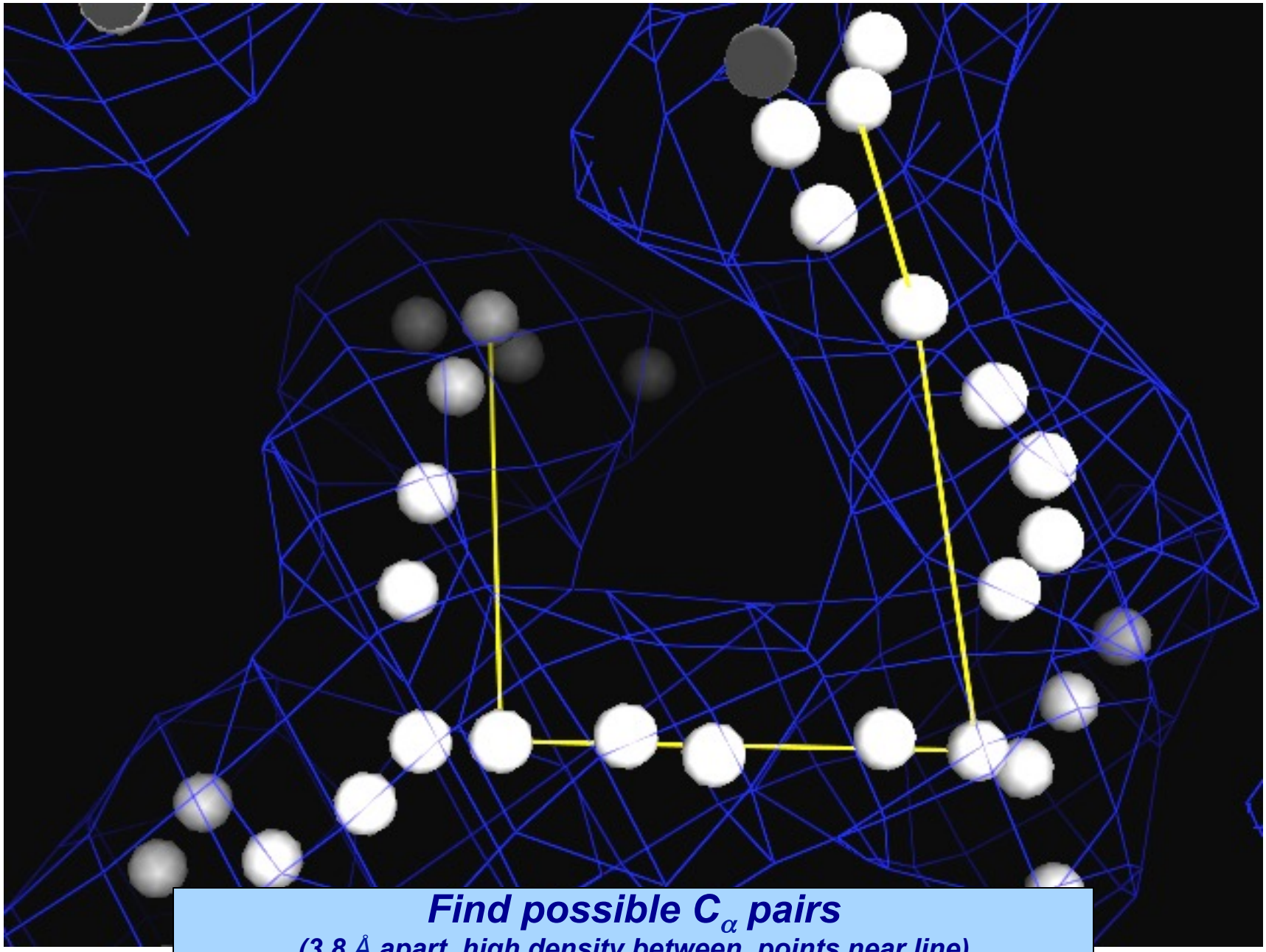
Tracing backbone step 1:
Points in high density

(s-hydrolase, PDB entry 1A7A)



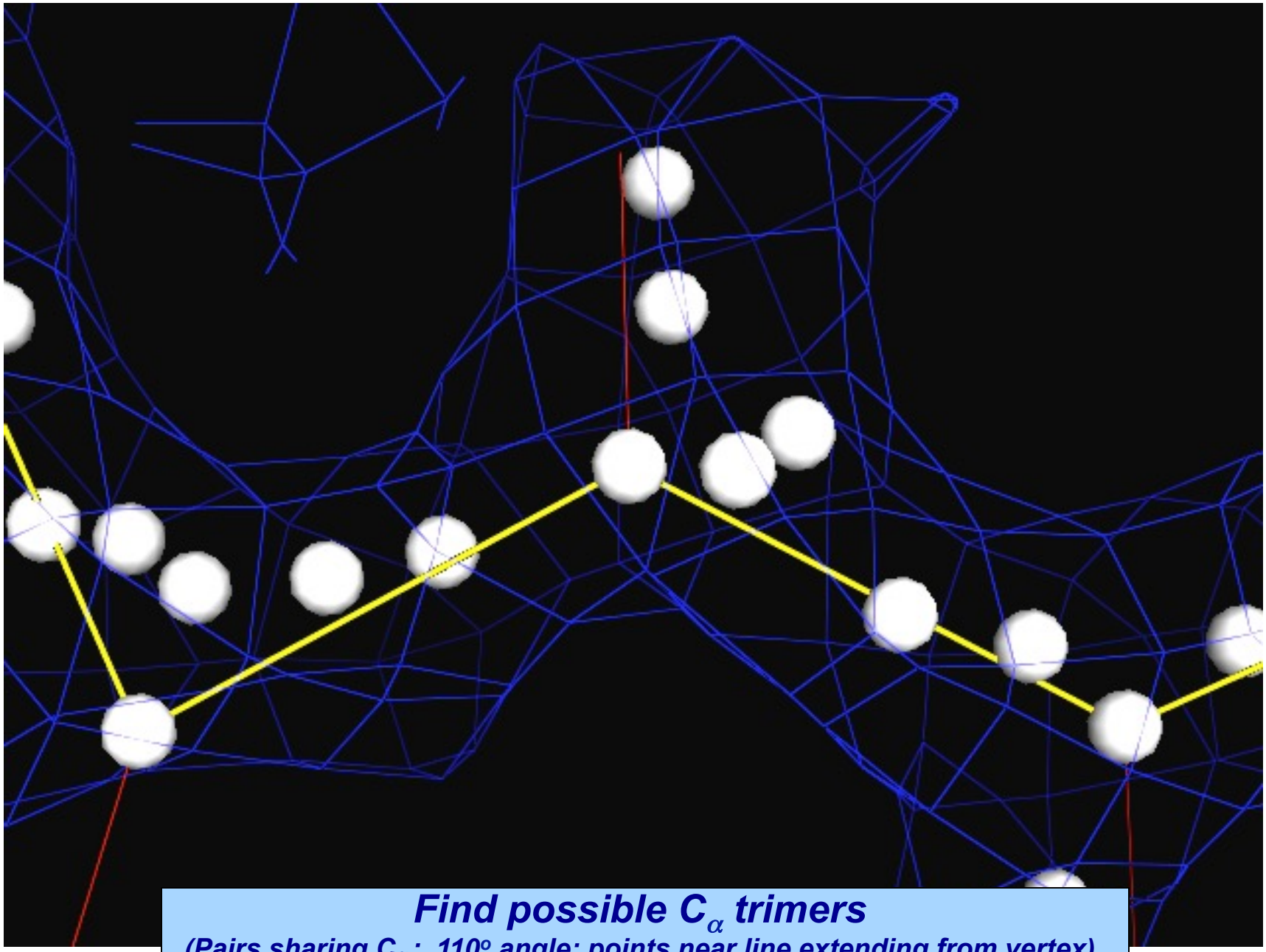
Move points to ridgelines

(s-hydrolase, PDB entry 1A7A)



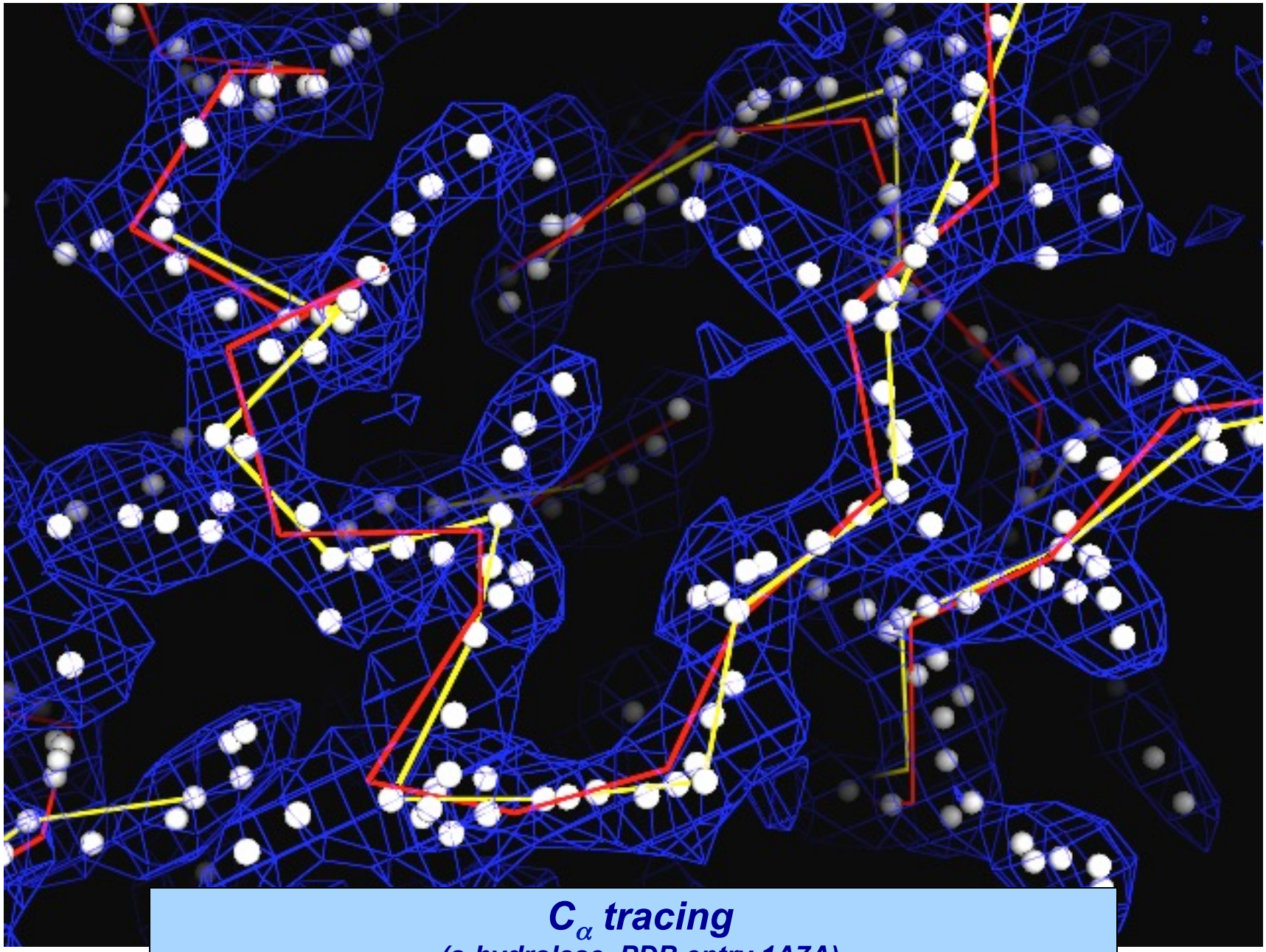
Find possible C_α pairs

(3.8 Å apart, high density between, points near line)



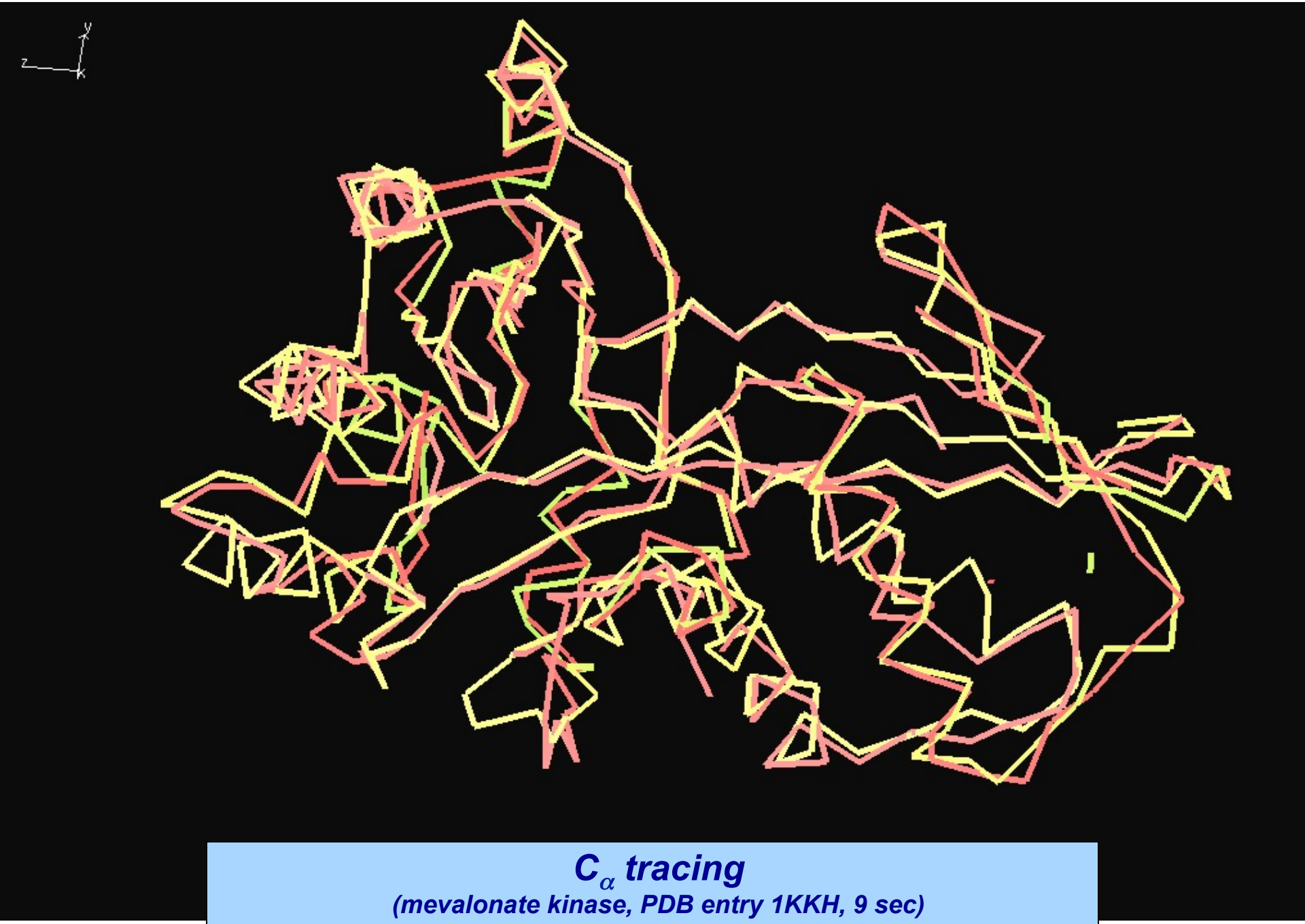
Find possible C_α trimers

(Pairs sharing C_α; 110^o angle; points near line extending from vertex)



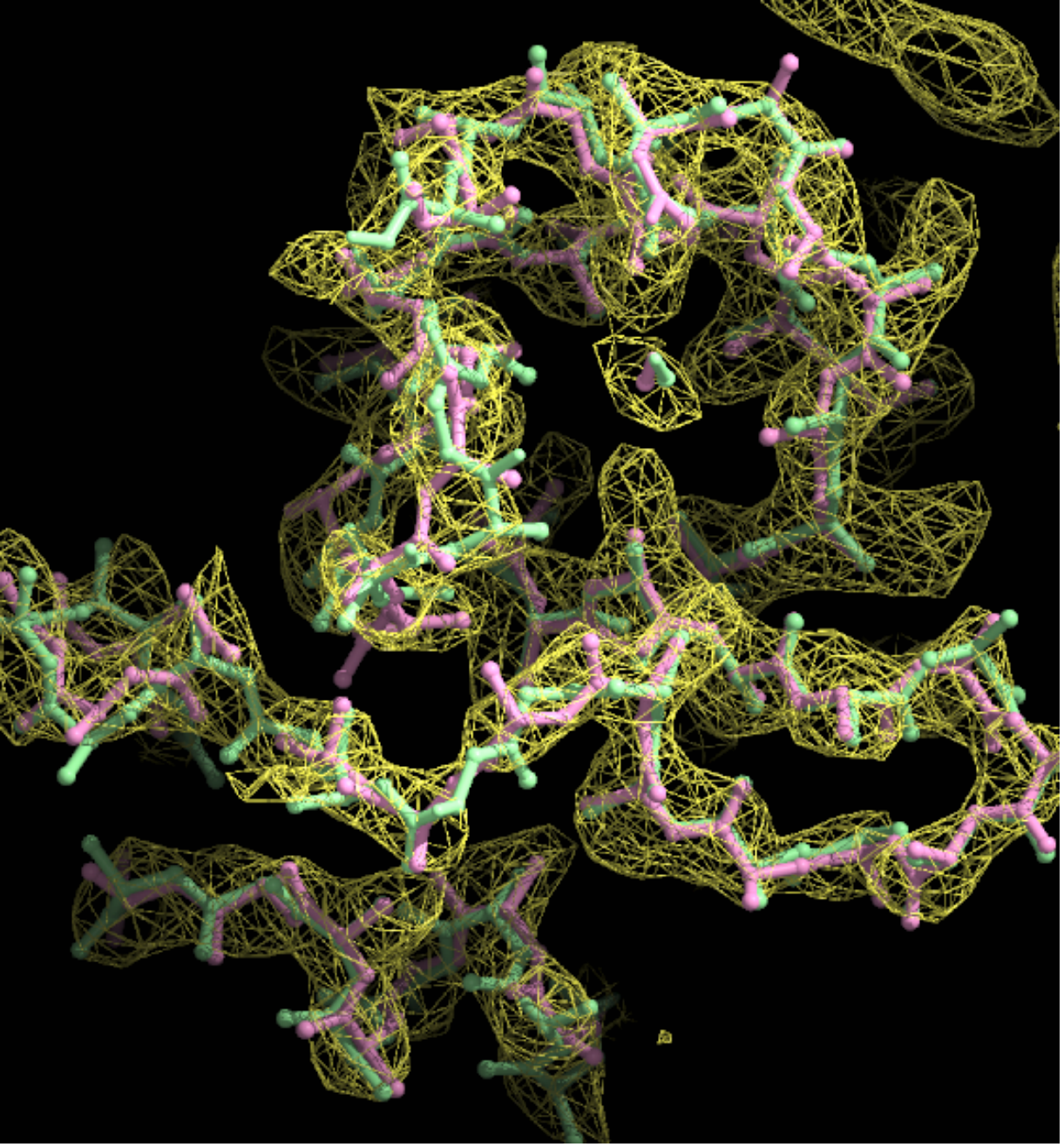
C_α tracing

(s-hydrolase, PDB entry 1A7A)



C_α tracing

(mevalonate kinase, PDB entry 1KKH, 9 sec)



Cryo-EM map from yeast mitochondrial ribosome (chain I of large subunit, 3.2 Å, Amunts et al., 2014)

Autobuilt model (pink)
Deposited model (green)
(main-chain and C^{β} atoms)

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