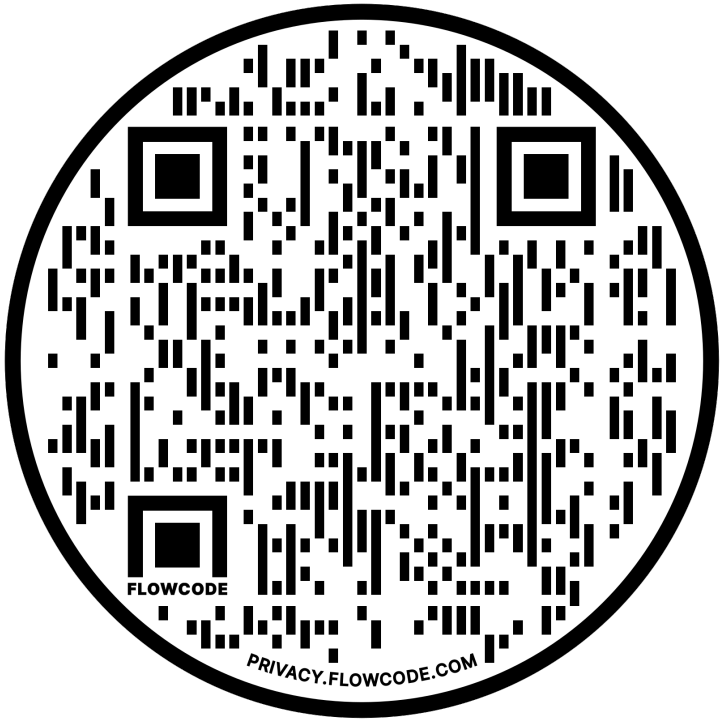
Model building tutorial



Tutorial PDF: https://bit.ly/2XPsiox

Data: https://bit.ly/3ASQ41I

AlphaFold add on: https://bit.ly/3KTo6qX

Model building and validation for cryoEM

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COLUMBIA UNIVERSITY
MEDICAL CENTER

“Is my map buildable??”

An atomic model is a compact interpretation of the density map in light of prior knowledge (both specific and general).

- Aim is to build a model that is consistent with **both** the density map and everything we independently know about the structure & composition of the macromolecule of interest, both specifically and in terms of our general knowledge of protein structure and chemistry.

- At medium resolution (3-5 Å), this still requires manual building (yes, even if you start from an AlphaFold prediction…🙃). Even the best autobuilt model still requires manual inspection and correction in most cases. (generates many fragments which need inspection, correction, merging)

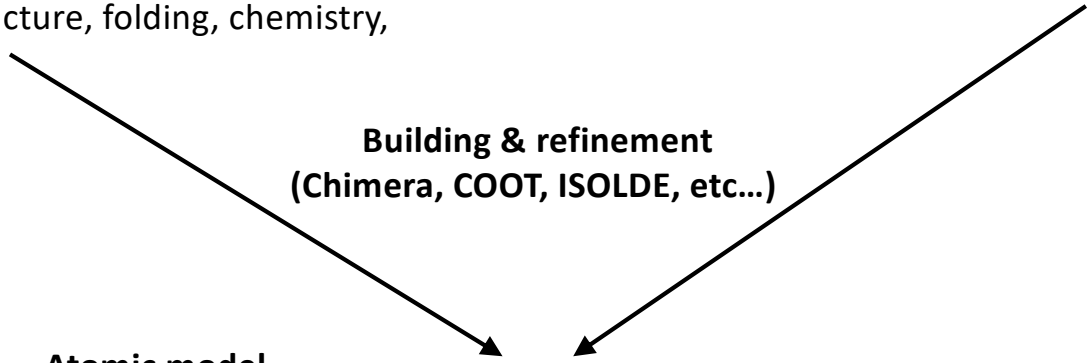
- Tradeoff between available prior knowledge and required resolution for atomic modelling – at the extremes, if a complete crystal structure is already available, 10Å data may be sufficient, while if no sequence/composition data is available even 3Å may not suffice.

Prior knowledge

- Protein sequence and derived info (secondary structure predictions, covariation/conservation, patterns of large/aromatic residues), disorder & contact prediction
- Crystal structures (+ homology & **ML-derived models**)
- Knowledge of protein structure, folding, chemistry, geometry.

Density map

- Resolution (+ local resolution, + map modification/sharpening)
- Patterns of large/small/absent sidechains
- Sharpening and density modification
- Conformational/compositional heterogeneity

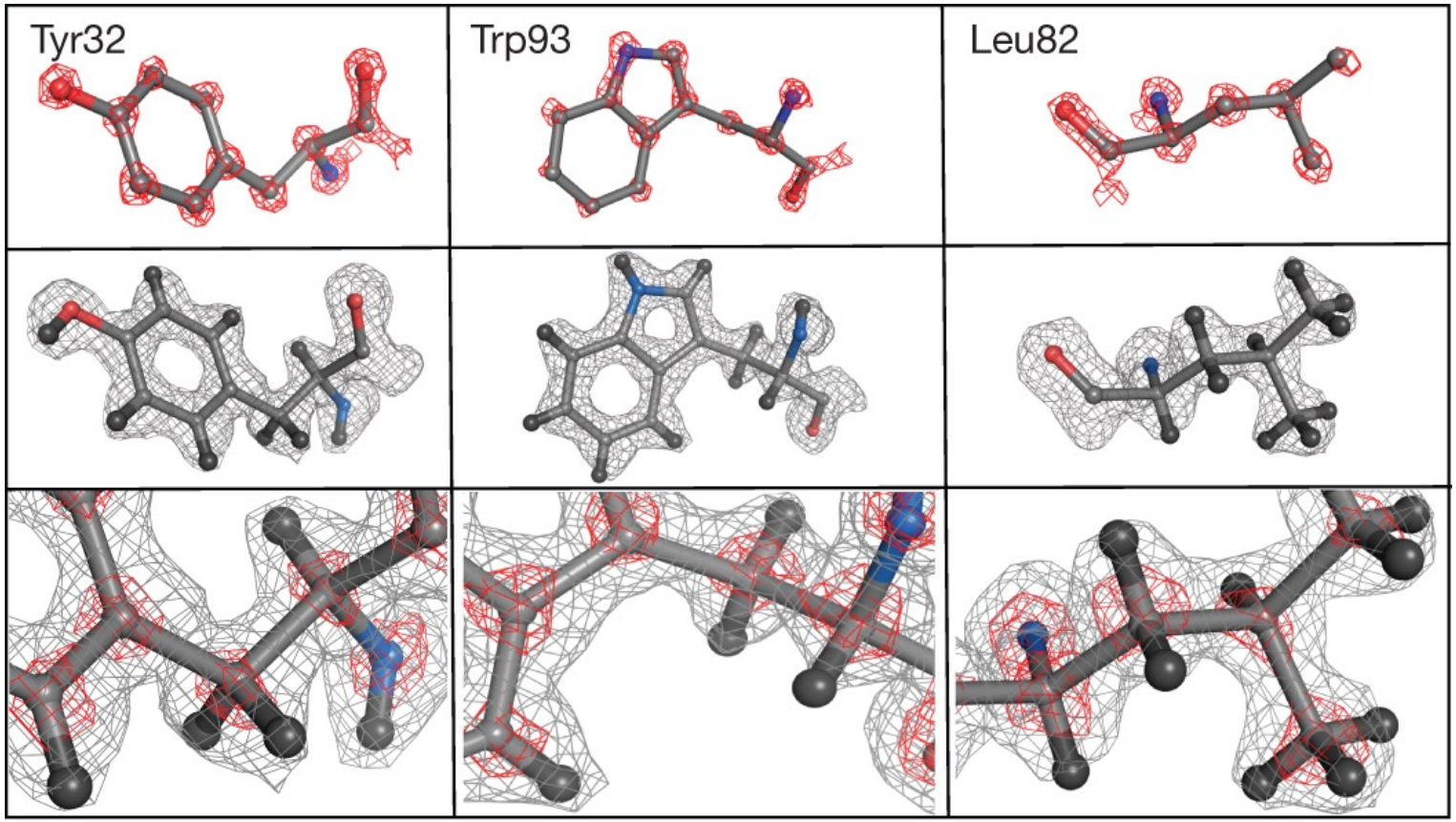


Building & refinement
(Chimera, COOT, ISOLDE, etc…)

Atomic model

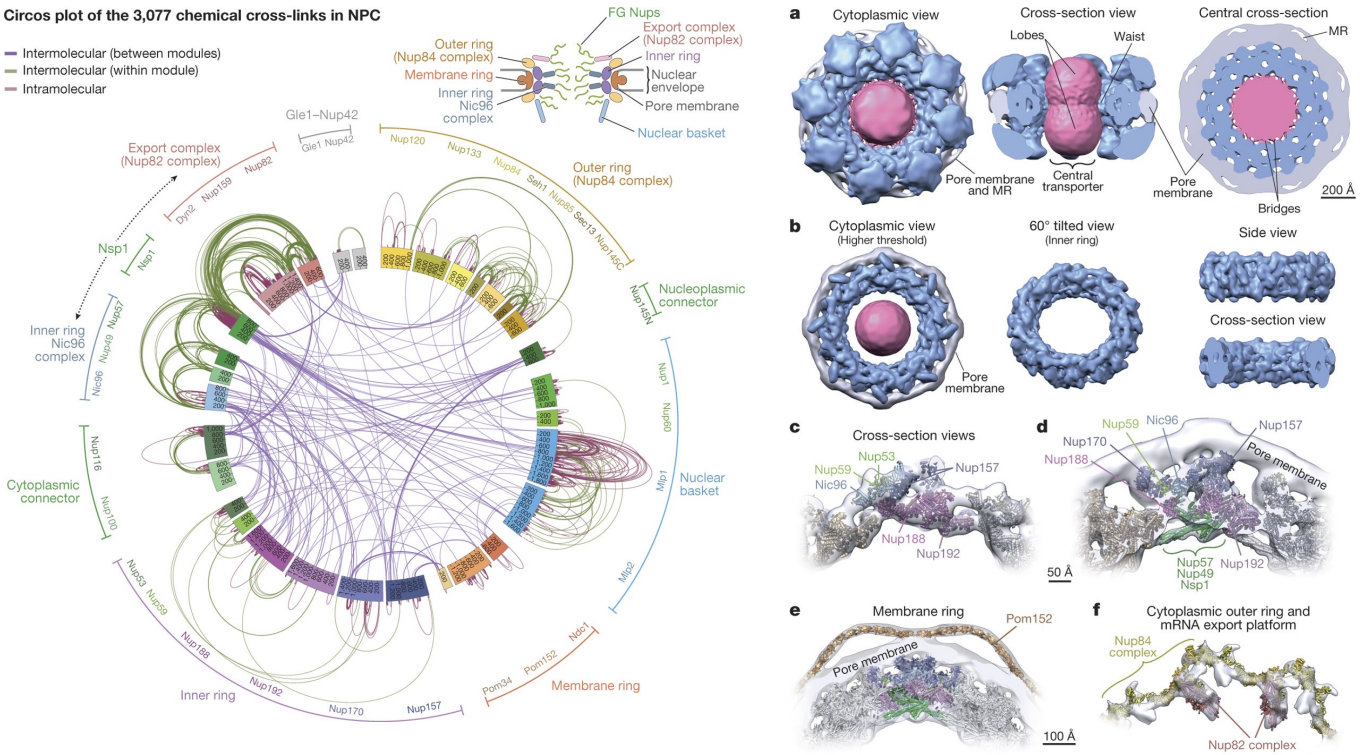
- If possible, unique model that agrees with both density map and priors
- Otherwise (and per region), specify ambiguity (w/UNK residues and numbering or Ca only model)
- Validation not just (or even mostly) about overfitting.
- Identify, analyse, fix errors.
- Direction and register of sequence fit.
- Ligand identification/assignment.
- No model is or ever will be perfect. That’s okay.

One extreme – at atomic resolution, the position of many atoms can be inferred without prior knowledge of the sequence



Yip, K.M., Fischer, N., Paknia, E. et al. Atomic-resolution protein structure determination by cryo-EM. Nature ***587,*** *157–161 (2020)*

At 20 Å (here using cryoET), an informative model can be generated by taking advantage of external information – crystal structures, connectivity from crosslinking & MS, even when de novo building is not possible.



Kim, S., Fernandez-Martinez, J., Nudelman, I. et al. Integrative structure and functional anatomy of a nuclear pore complex. Nature ***555,*** *475–482 (2018)*

Usually, we are somewhere in between the two – combining prior knowledge with inferences made from analyzing the density map.

To build a better/more reliable model, we can either get additional/better priors, or improve our density map (or part of it).

Before you start – make sure your maps are appropriately sharpened and low pass filtered! (and consider whether building is justified or whether further improvement of the reconstruction is required first)

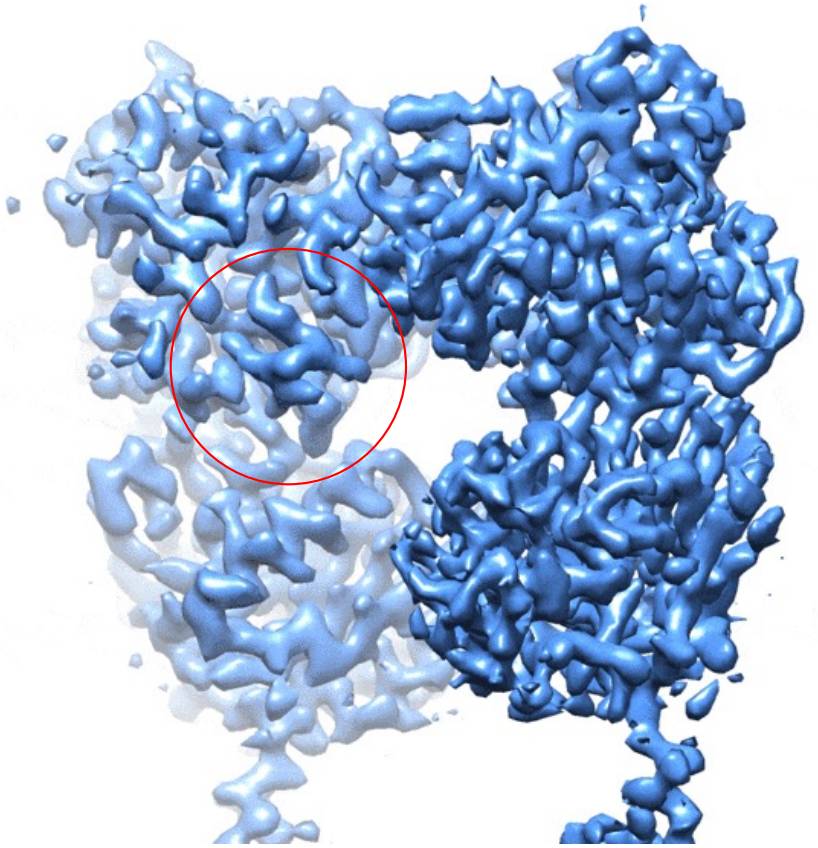
- **Often it is helpful to build using multiple maps**. Assuming 3-3.5Å global res, I would suggest using a map filtered to the global resolution, one filtered to the best local resolution, and one filtered to ~4-4.5 Å (to better visualize connectivity and mobile ligands/lipids).

- Try both simple B-factor sharpening and the approach used by *`phenix.resolve_cryo_em`*, which incorporates anisotropy removal and statistical density modification. In cases of **severe** anisotropy, deepEMhancer can be useful to assist map interpretation (**approach with caution**).

- Also, if your map doesn't "look like" 4 Å, trust your eyes! If it is nominally 4Å and there are no sidechains visible, or your helices look "stretched", assess orientation bias (3D-FSC server: *https://3dfsc.salk.edu*), local resolution variation, and double check sharpening and masking parameters (are you *sure* you're looking at the sharpened map? Is the mask used for FSC calculation sensible?)

Example of map anisotropy mitigated by masked refinement

- Map anisotropy hinders interpretation, even when resolution in “good” direction is high
- Can derive from either preferred orientation, or interdomain mobility (or combination).
- In latter case, masked refinement can improve local map quality to aid model building and map interpretation. **Always better to improve the map than build in marginal density**
- If anisotropy derives from preferred orientation, it is best to address this by improving the sample or data collection (tilt). If all else fails, ML-based map improvement using deepEMhancer can improve map interpretability.



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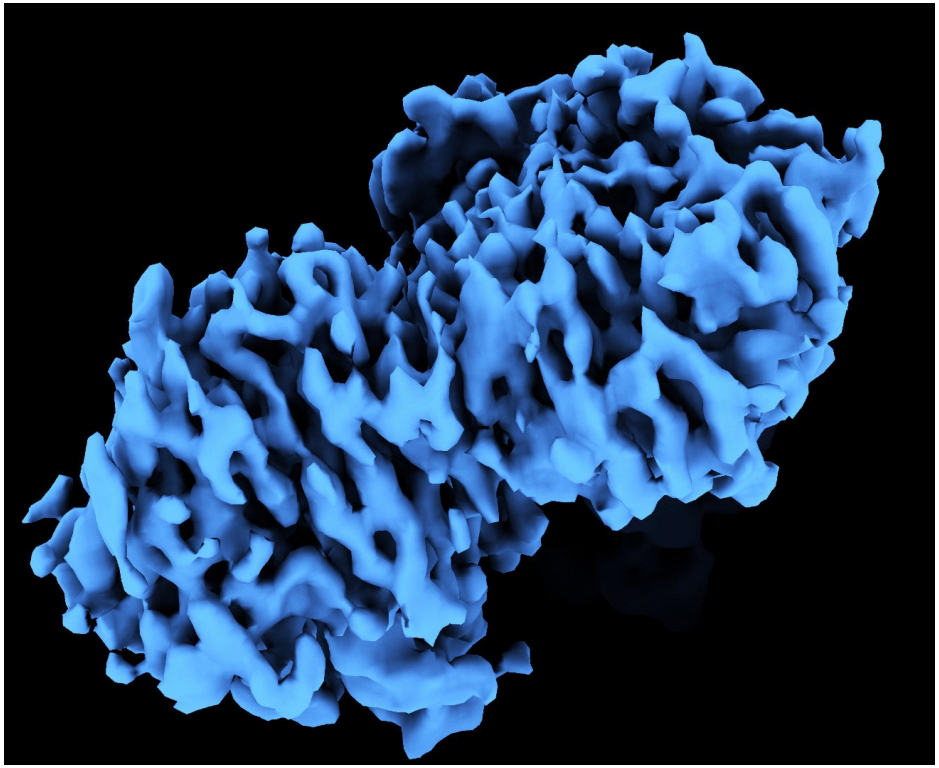

DeepEMhancer

(Sanchez-Garcia R., 2021 Comm. Biol.)

Prep for model building - what can we learn from the sequence alone?

Your protein sequence contains a lot of useful information which you can use to aid model building:

- Start by identifying boundaries of conserved domains (NCBI CDD: https://www.ncbi.nlm.nih.gov/Structure/cdd/; DELTA-BLAST also performs CD-search by default)
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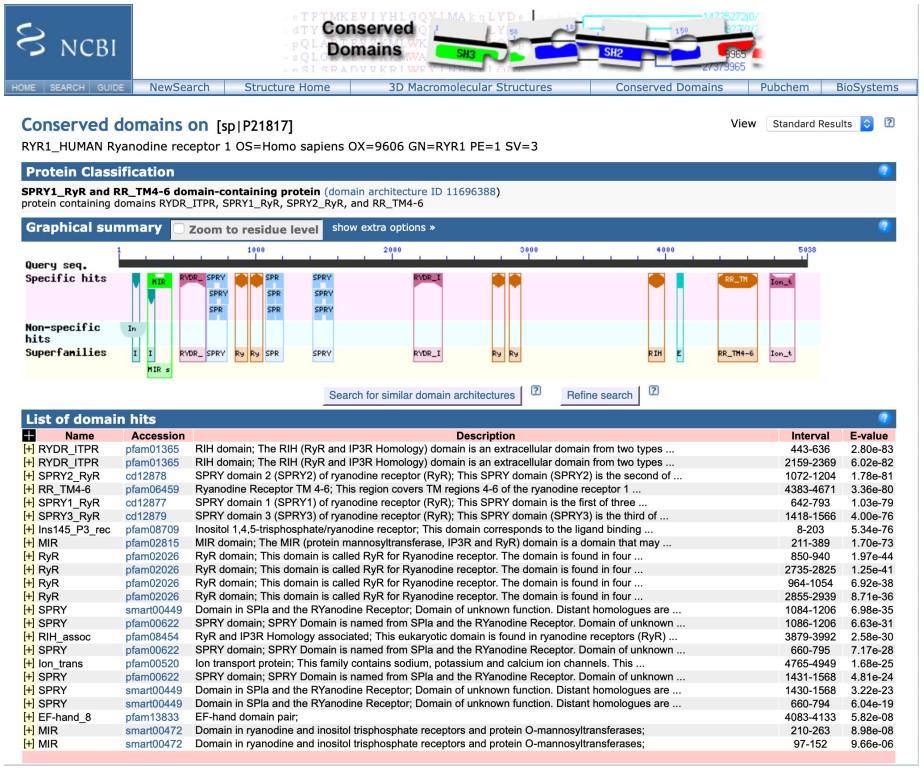
CDD provides a guide to domain level architecture, including sequence alignments & representative structures.



Name	Accession	Description	Interval	E-value
[+] RYDR_ITPR	pfam01365	RIH domain; The RIH (RyR and IP3R Homology) domain is an extracellular domain from two types ...	443-636	2.80e-83
[+] RYDR_ITPR	pfam01365	RIH domain; The RIH (RyR and IP3R Homology) domain is an extracellular domain from two types ...	2159-2369	6.02e-82
[+] SPRY2_RyR	cd12878	SPRY domain 2 (SPRY2) of ryanodine receptor (RyR); This SPRY domain (SPRY2) is the second of ...	1072-1204	1.78e-81
[+] RR_TM4-6	pfam06459	Ryanodine Receptor TM 4-6; This region covers TM regions 4-6 of the ryanodine receptor 1 ...	4383-4671	3.36e-80
[+] SPRY1_RyR	cd12877	SPRY domain 1 (SPRY1) of ryanodine receptor (RyR); This SPRY domain is the first of three ...	642-793	1.03e-79
[+] SPRY3_RyR	cd12879	SPRY domain 3 (SPRY3) of ryanodine receptor (RyR); This SPRY domain (SPRY3) is the third of ...	1418-1566	4.00e-76
[+] Ins145_P3_rec	pfam08709	Inositol 1,4,5-trisphosphate/ryanodine receptor; This domain corresponds to the ligand binding ...	8-203	5.34e-76
[+] MIR	pfam02815	MIR domain; The MIR (protein mannosyltransferase, IP3R and RyR) domain is a domain that may ...	211-389	1.70e-73
[+] RyR	pfam02026	RyR domain; This domain is called RyR for Ryanodine receptor. The domain is found in four ...	850-940	1.97e-44
[+] RyR	pfam02026	RyR domain; This domain is called RyR for Ryanodine receptor. The domain is found in four ...	2735-2825	1.25e-41
[+] RyR	pfam02026	RyR domain; This domain is called RyR for Ryanodine receptor. The domain is found in four ...	964-1054	6.92e-38
[+] RyR	pfam02026	RyR domain; This domain is called RyR for Ryanodine receptor. The domain is found in four ...	2855-2939	8.71e-36
[+] SPRY	smart00449	Domain in SPla and the RYanodine Receptor; Domain of unknown function. Distant homologues are ...	1084-1206	6.98e-35
[+] SPRY	pfam00622	SPRY domain; SPRY Domain is named from SPla and the RYanodine Receptor. Domain of unknown ...	1086-1206	6.63e-31
[+] RIH_assoc	pfam08454	RyR and IP3R Homology associated; This eukaryotic domain is found in ryanodine receptors (RyR) ...	3879-3992	2.58e-30
[+] SPRY	pfam00622	SPRY domain; SPRY Domain is named from SPla and the RYanodine Receptor. Domain of unknown ...	660-795	7.17e-28
[+] Ion_trans	pfam00520	Ion transport protein; This family contains sodium, potassium and calcium ion channels. This ...	4765-4949	1.68e-25
[+] SPRY	pfam00622	SPRY domain; SPRY Domain is named from SPla and the RYanodine Receptor. Domain of unknown ...	1431-1568	4.81e-24
[+] SPRY	smart00449	Domain in SPla and the RYanodine Receptor; Domain of unknown function. Distant homologues are ...	1430-1568	3.22e-23
[+] SPRY	smart00449	Domain in SPla and the RYanodine Receptor; Domain of unknown function. Distant homologues are ...	660-794	6.04e-19
[+] EF-hand_8	pfam13833	EF-hand domain pair;	4083-4133	5.82e-08
[+] MIR	smart00472	Domain in ryanodine and inositol trisphosphate receptors and protein O-mannosyltransferases;	210-263	8.98e-08
[+] MIR	smart00472	Domain in ryanodine and inositol trisphosphate receptors and protein O-mannosyltransferases;	97-152	9.66e-06

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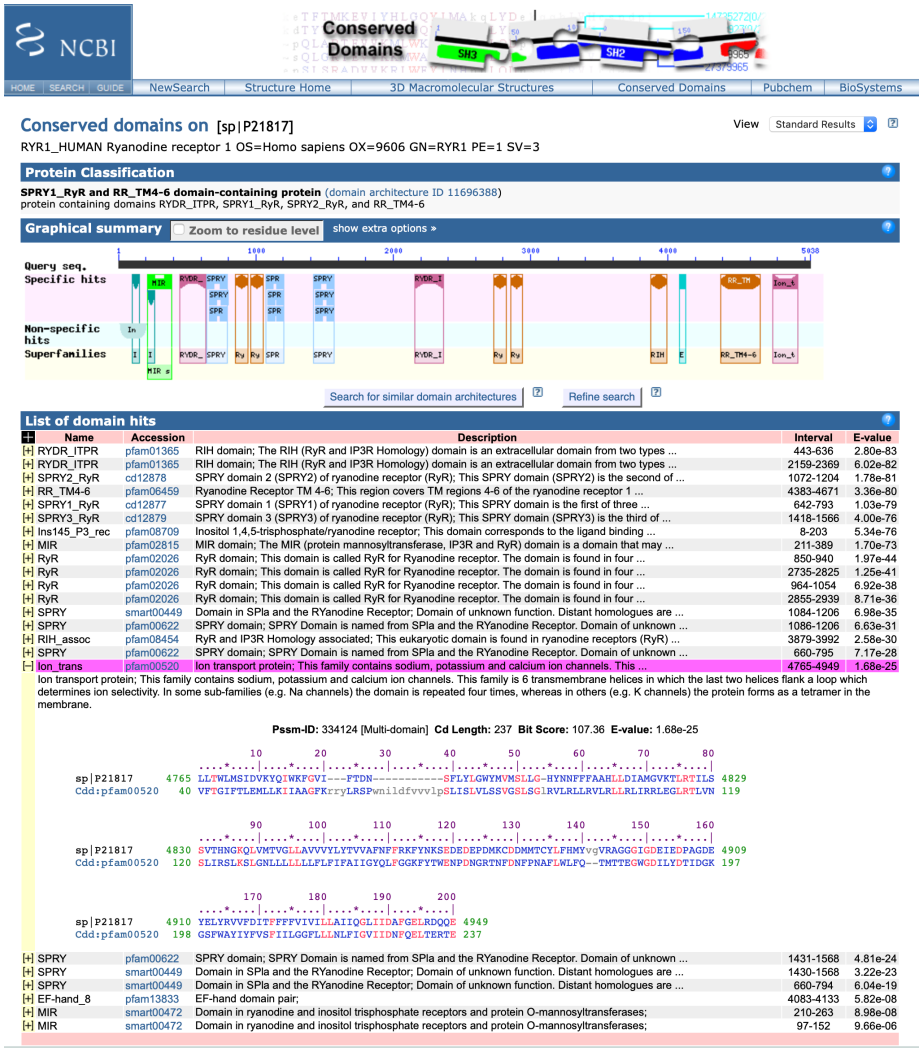

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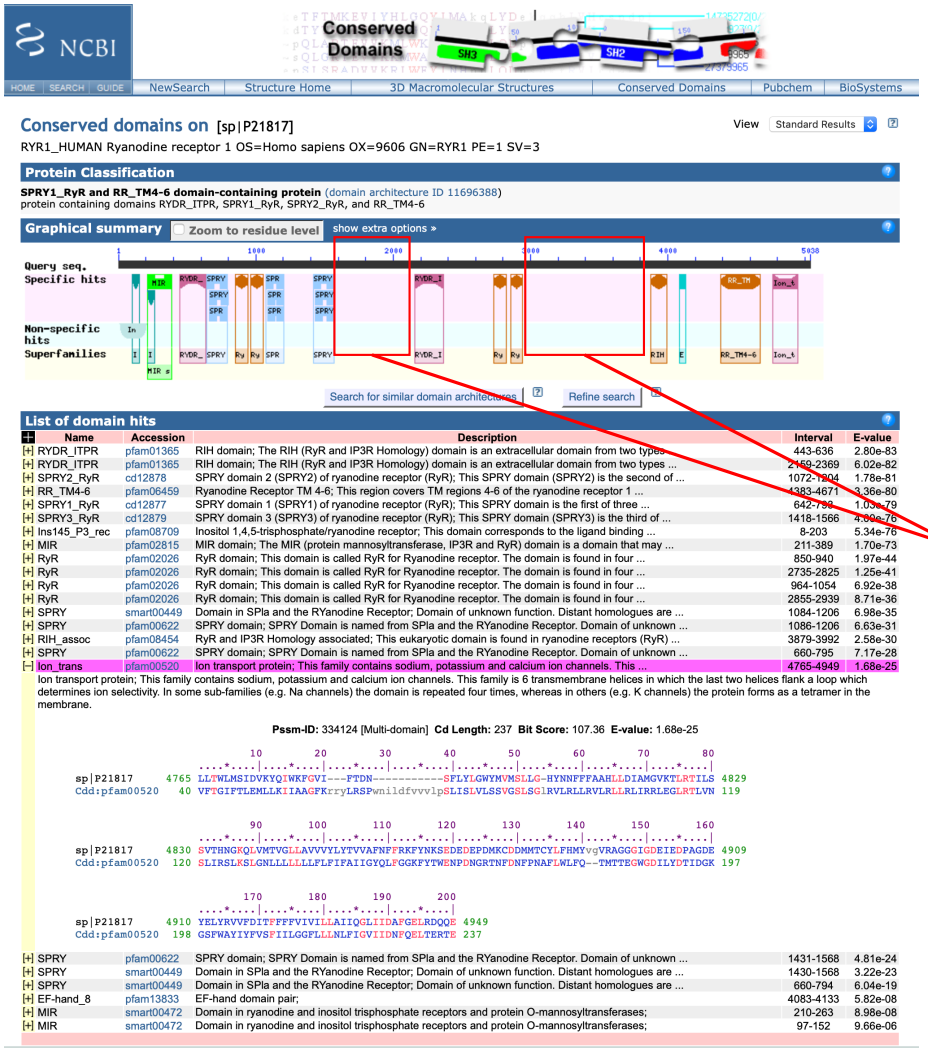

Once an initial trace is obtained for these regions, use DALI or PDBeFold to identify structural homologs that could not be identified by sequence alone.

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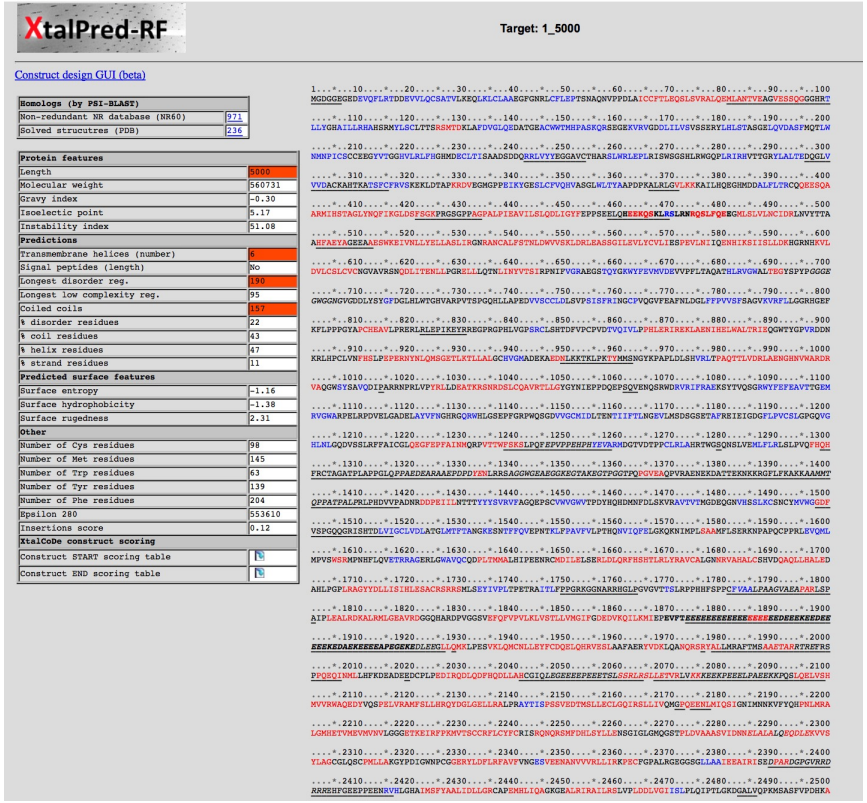
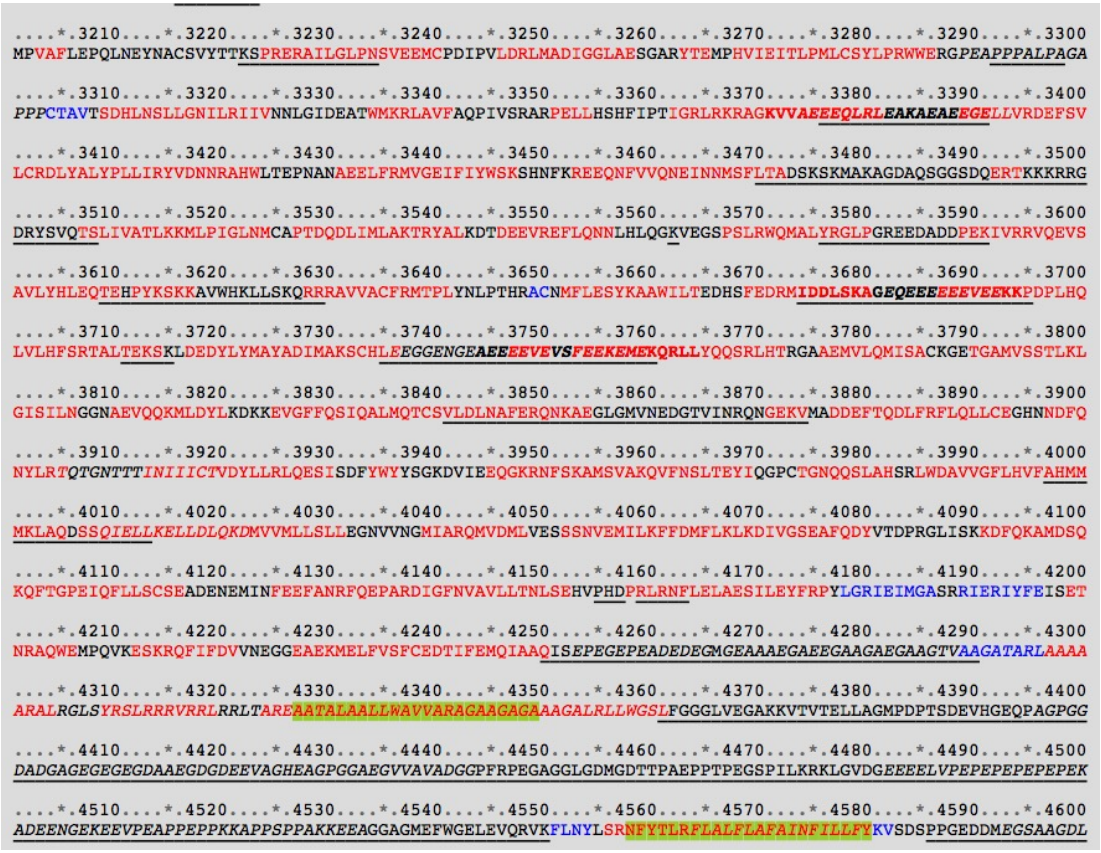
XtalPRED is a great tool for summarizing predicted sequence properties.

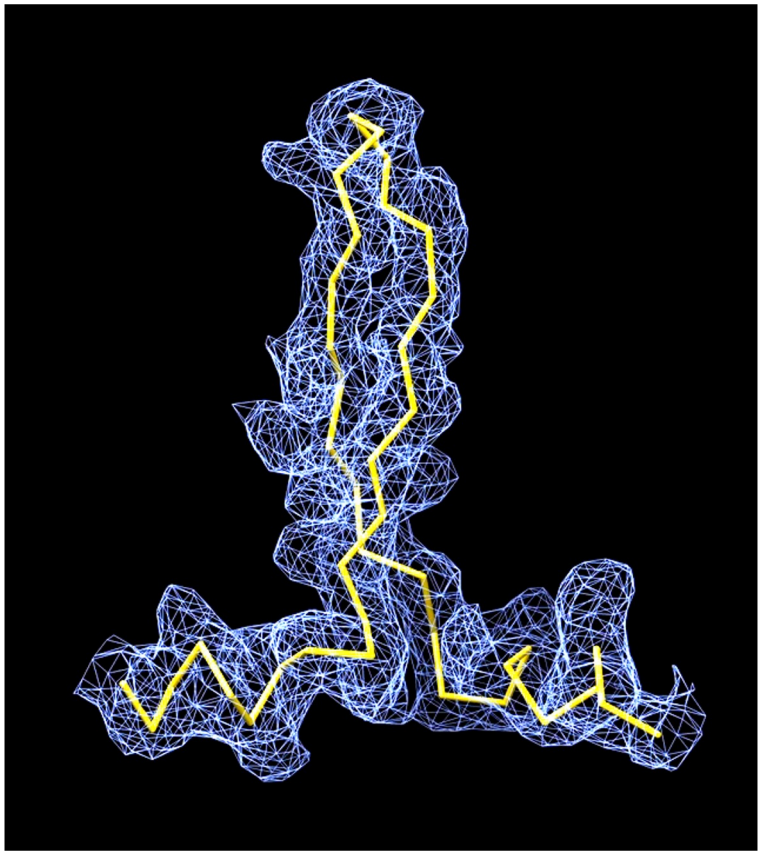


Highlights predicted secondary structure, disorder, low complexity regions on sequence in an easily digestible format. Useful to print and consult while building. Also provides list of structural homologs. (http://ffas.burnham.org/XtalPred-cgi/xtal.pl)

(Also consider using some of the newer single purpose neural-network based classifiers; e.g. SPIDER-3 & SPOT-DISORDER-SINGLE from Yaoqi Zhou lab: http://sparks-lab.org/index.php/Main/Services)

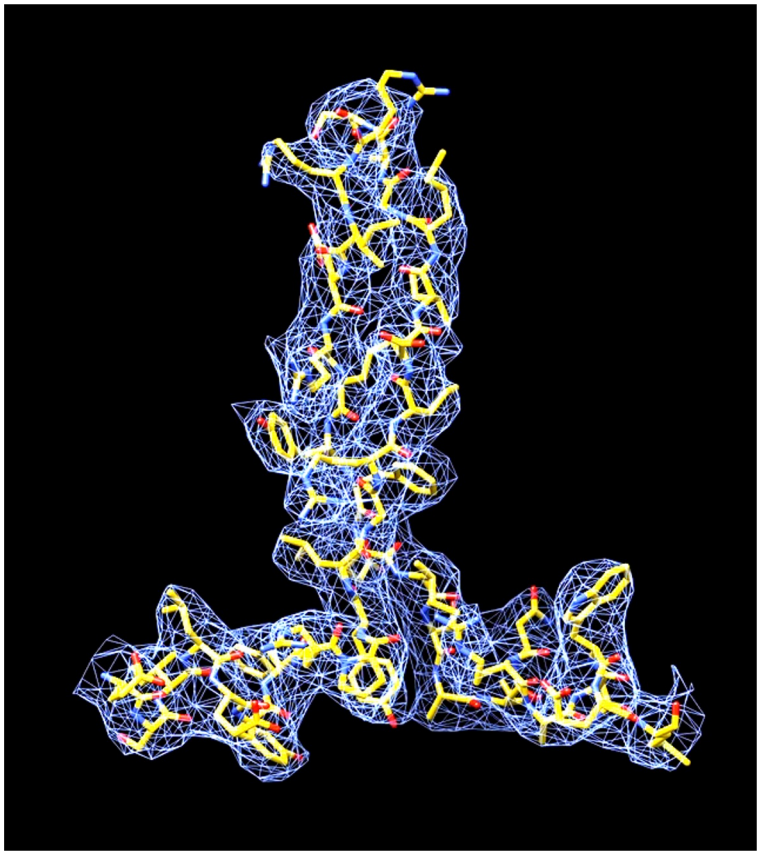
Secondary structure prediction is a very useful guide when building.





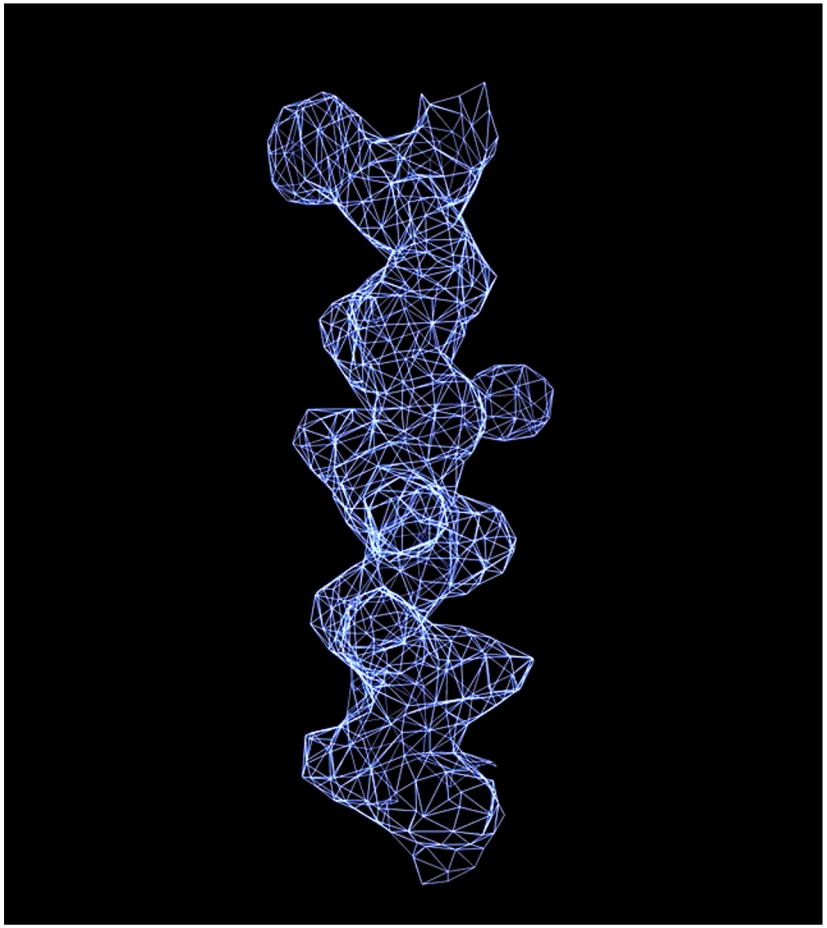
Where is this motif in the sequence?

Secondary structure prediction is a very useful guide when building.



Secondary structure prediction is ~80% accurate. So if your model consistently disagrees with predicted secondary structure, look at it very closely!

What can we learn from the map alone?



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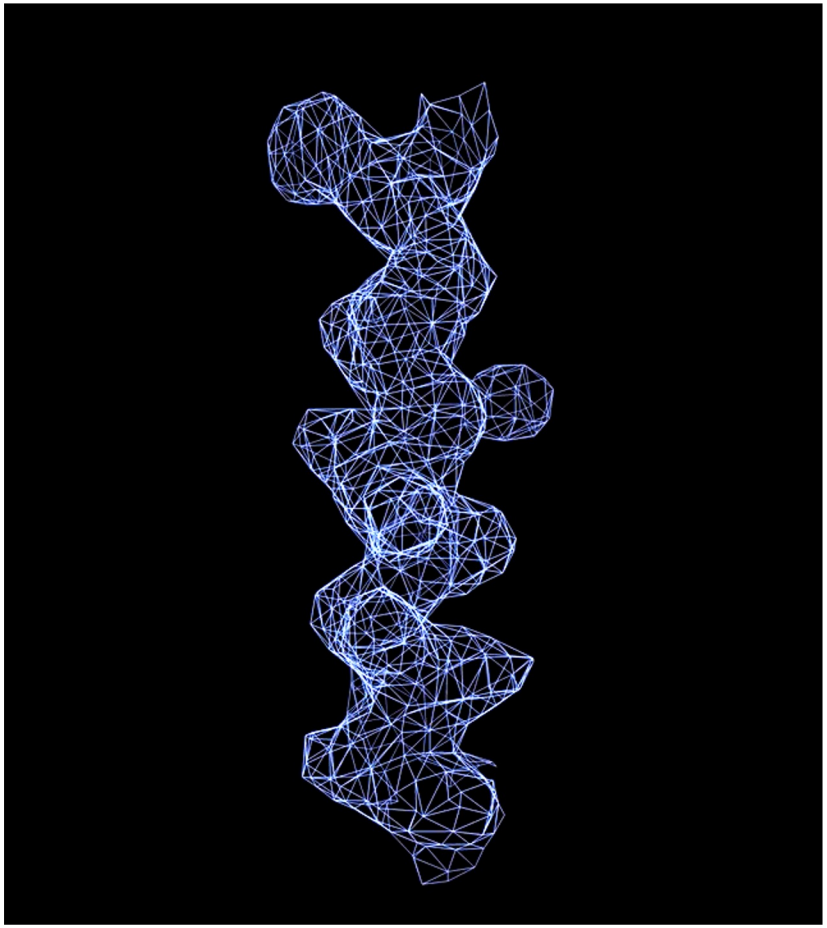
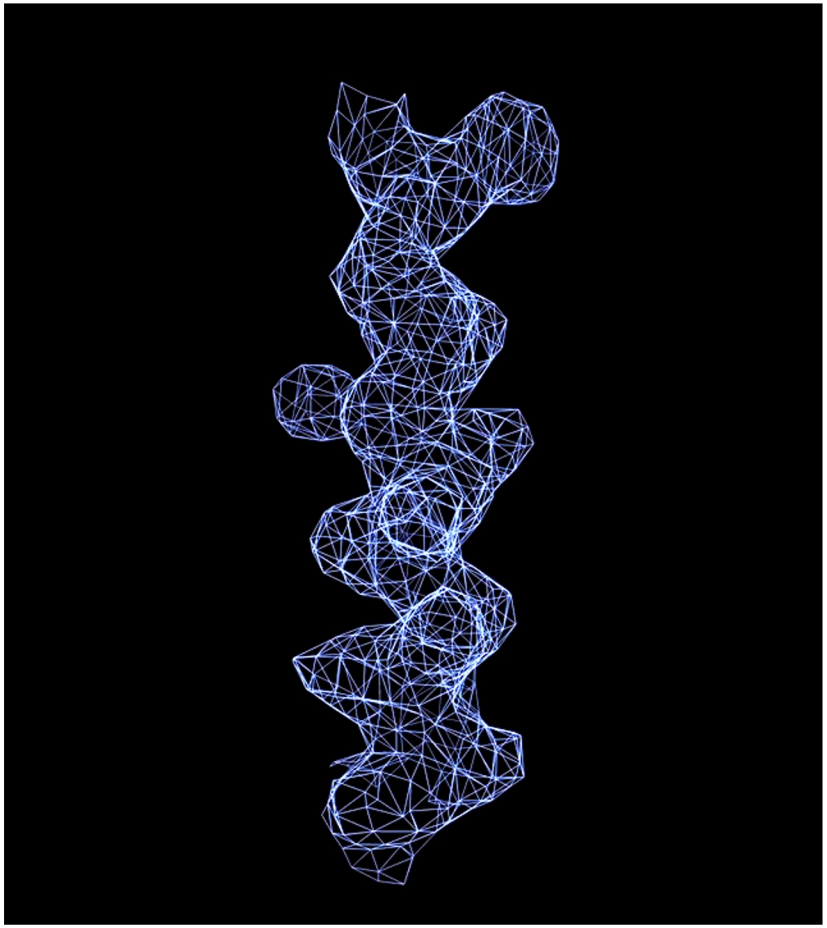
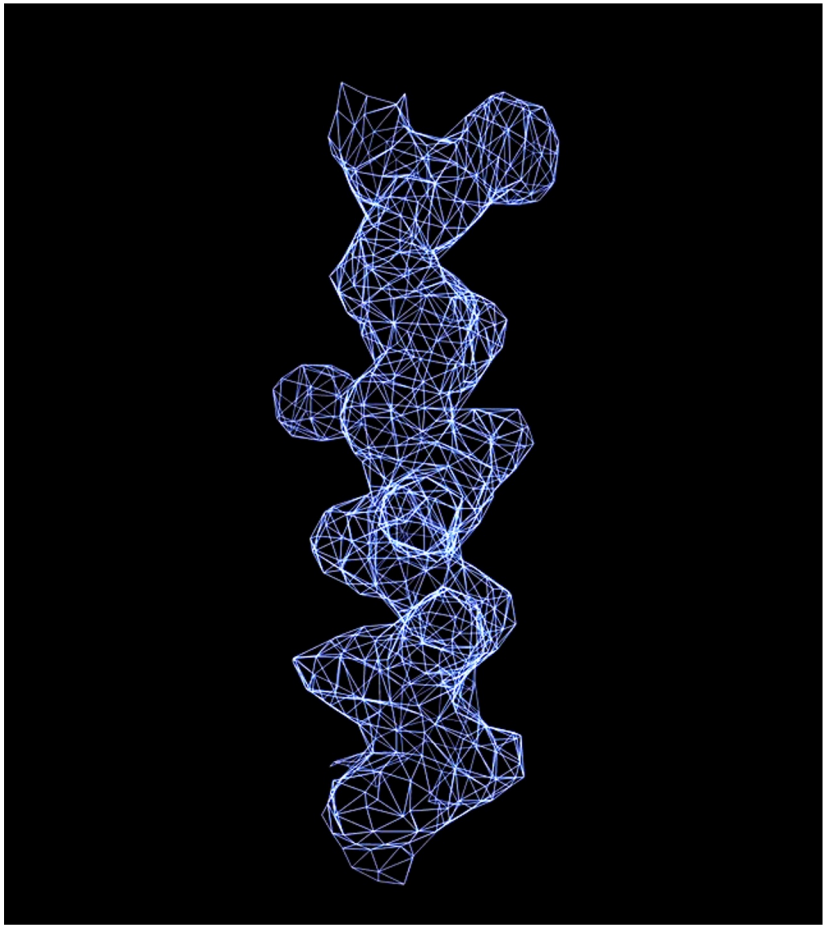


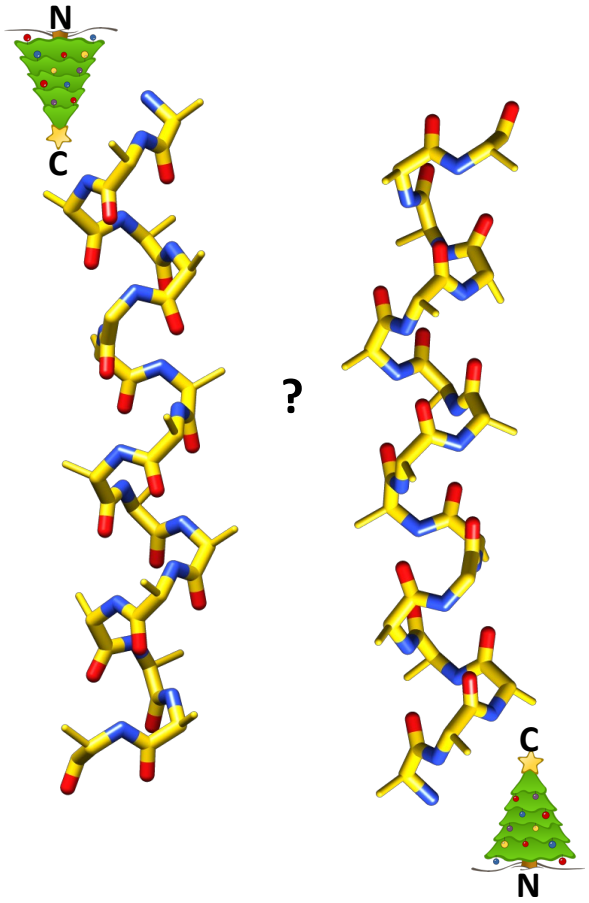
Left handed! Obvious here – can be less clear at lower res, so be careful.

OK, that's better! What can we learn from the map alone?



Which direction does the helix point?





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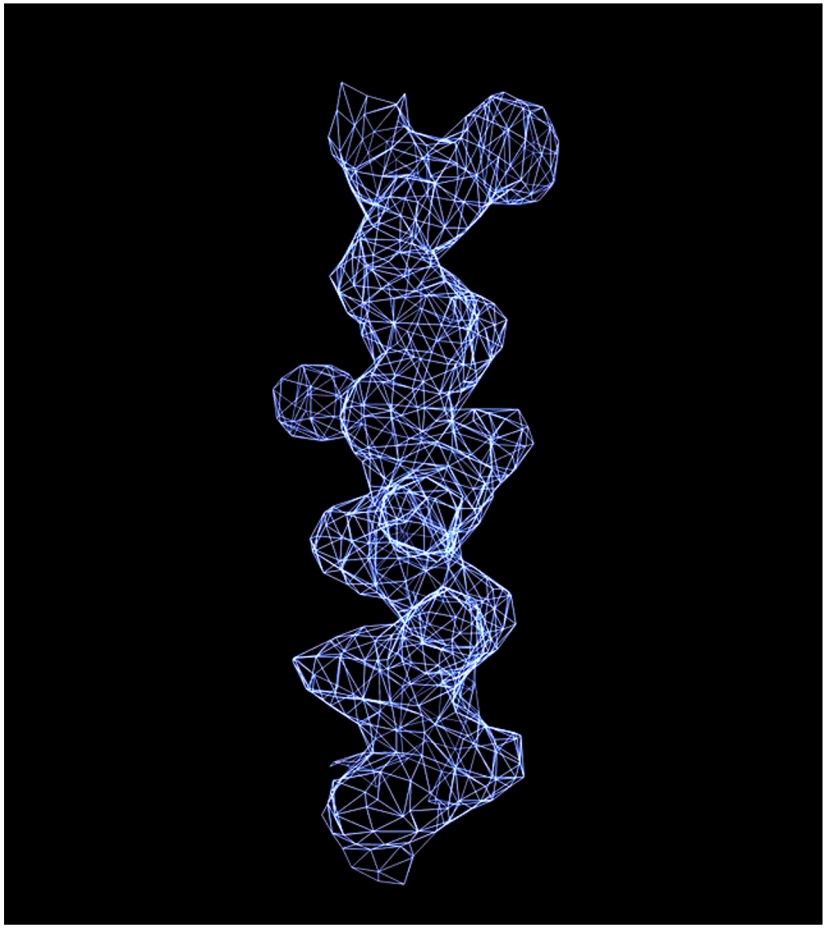

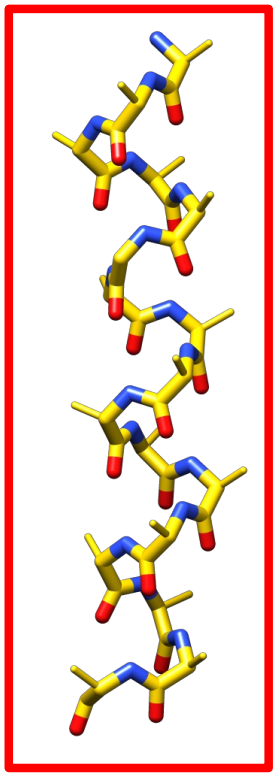

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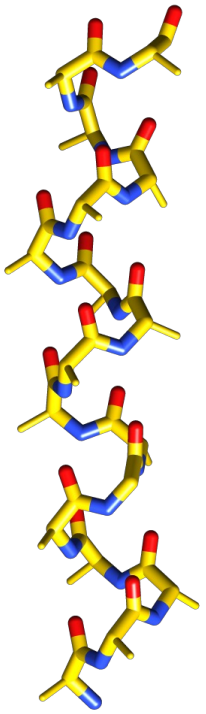
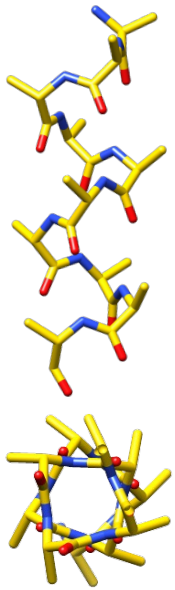
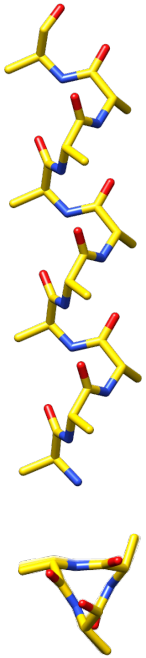


Helices – alpha and 3_{10}



Alpha

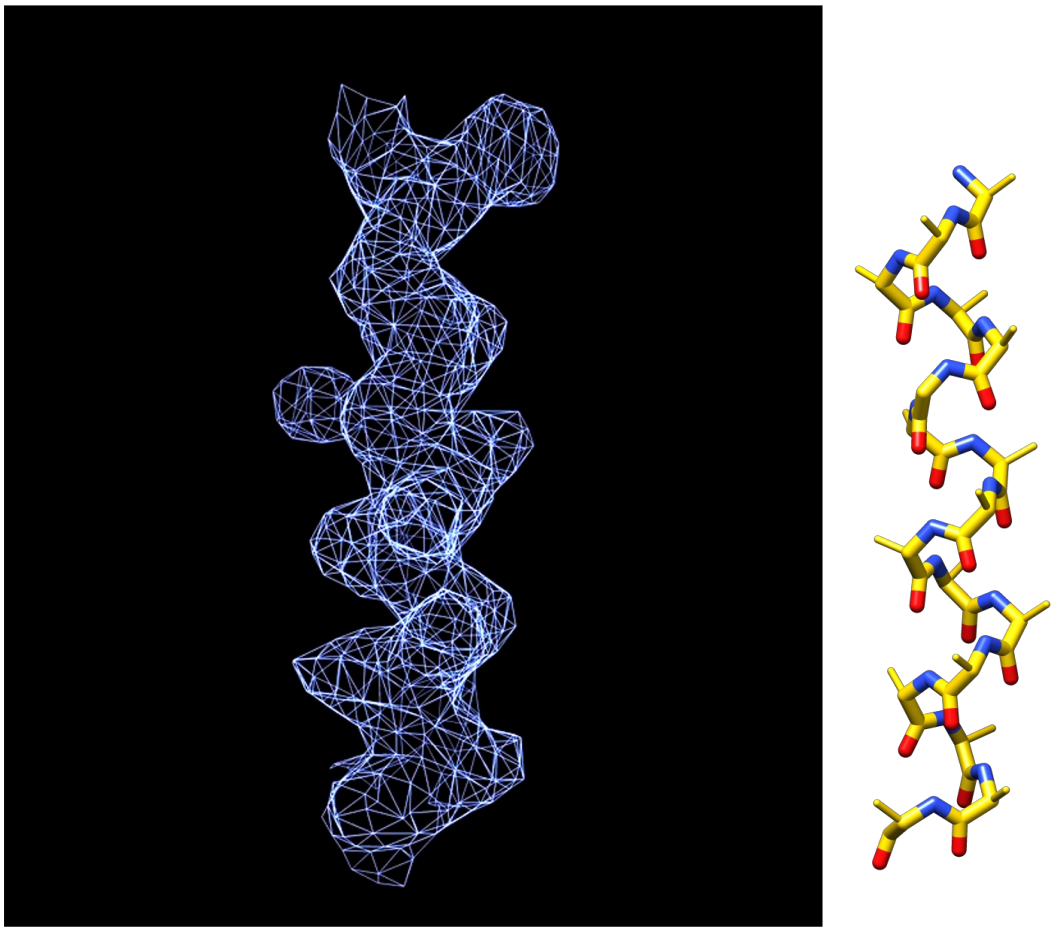
- ~90%
- 3.6 residues per turn
- Fat



3_{10}

- ~10%. More common in TM? (e.g. S4 of VSD)
- 3 residues per turn. Triangular cross section.
- Skinny
- Can be tricky to identify at low resolution, can lead to register errors.

Can we identify any probable sidechains from the density?



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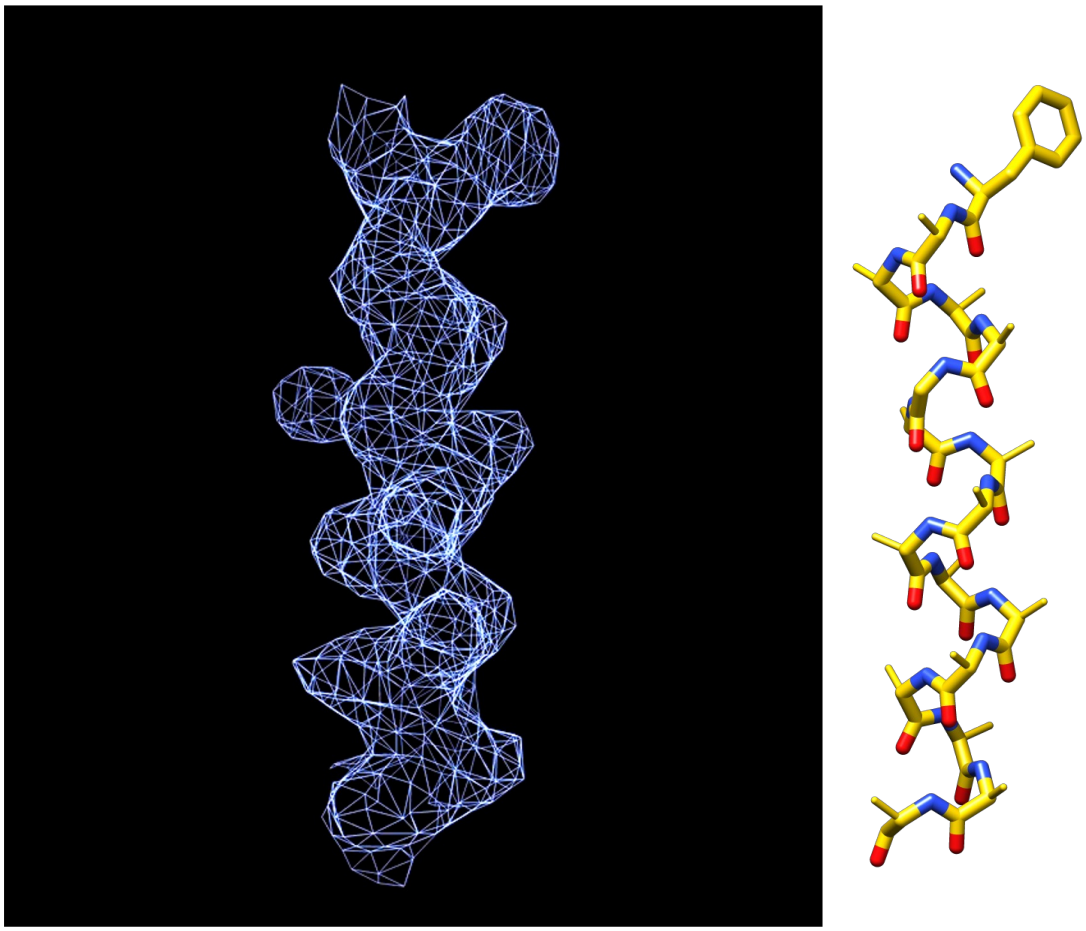

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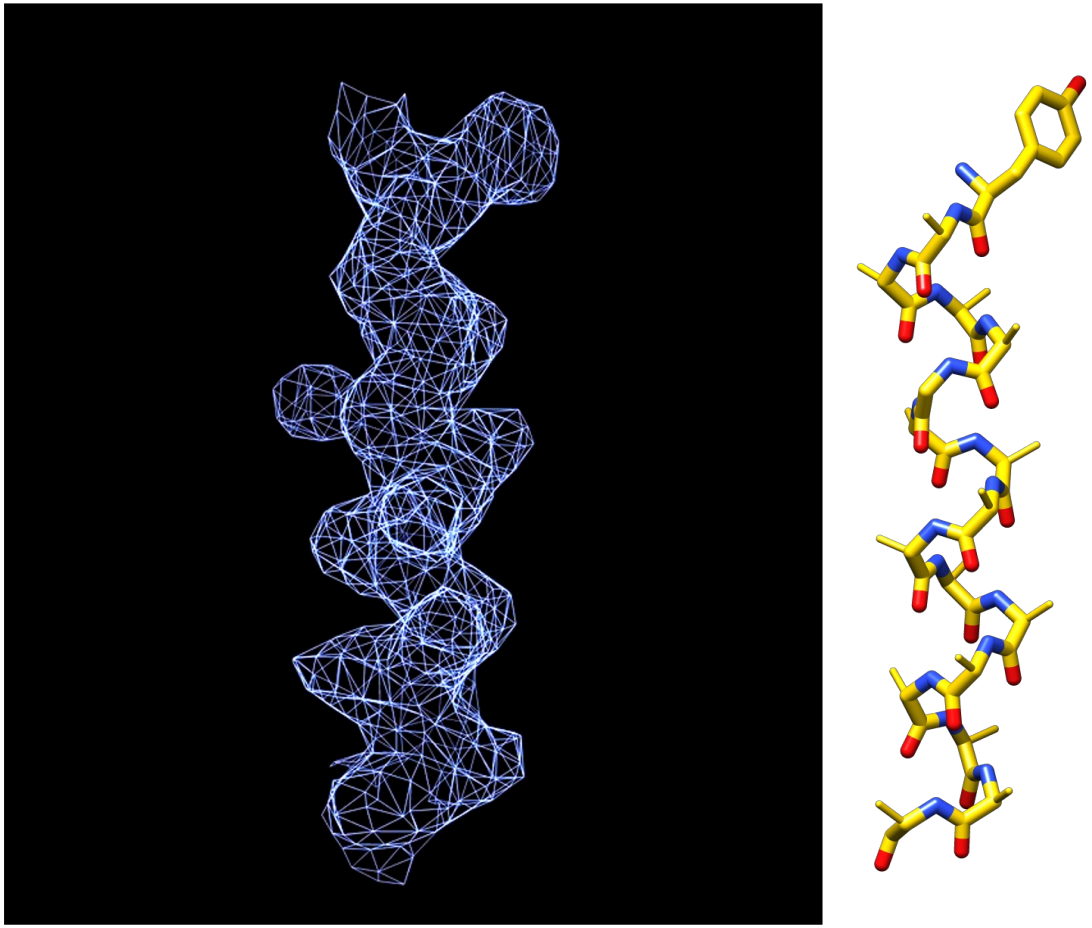

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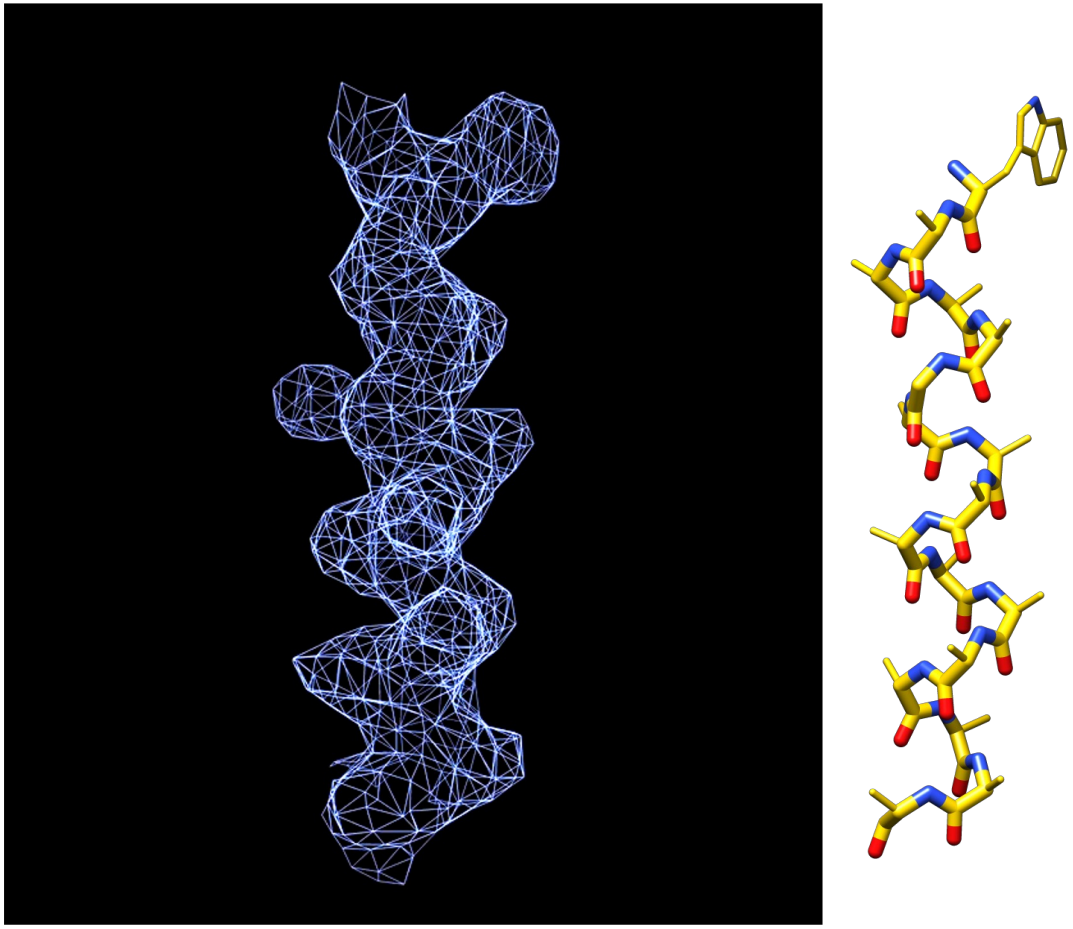
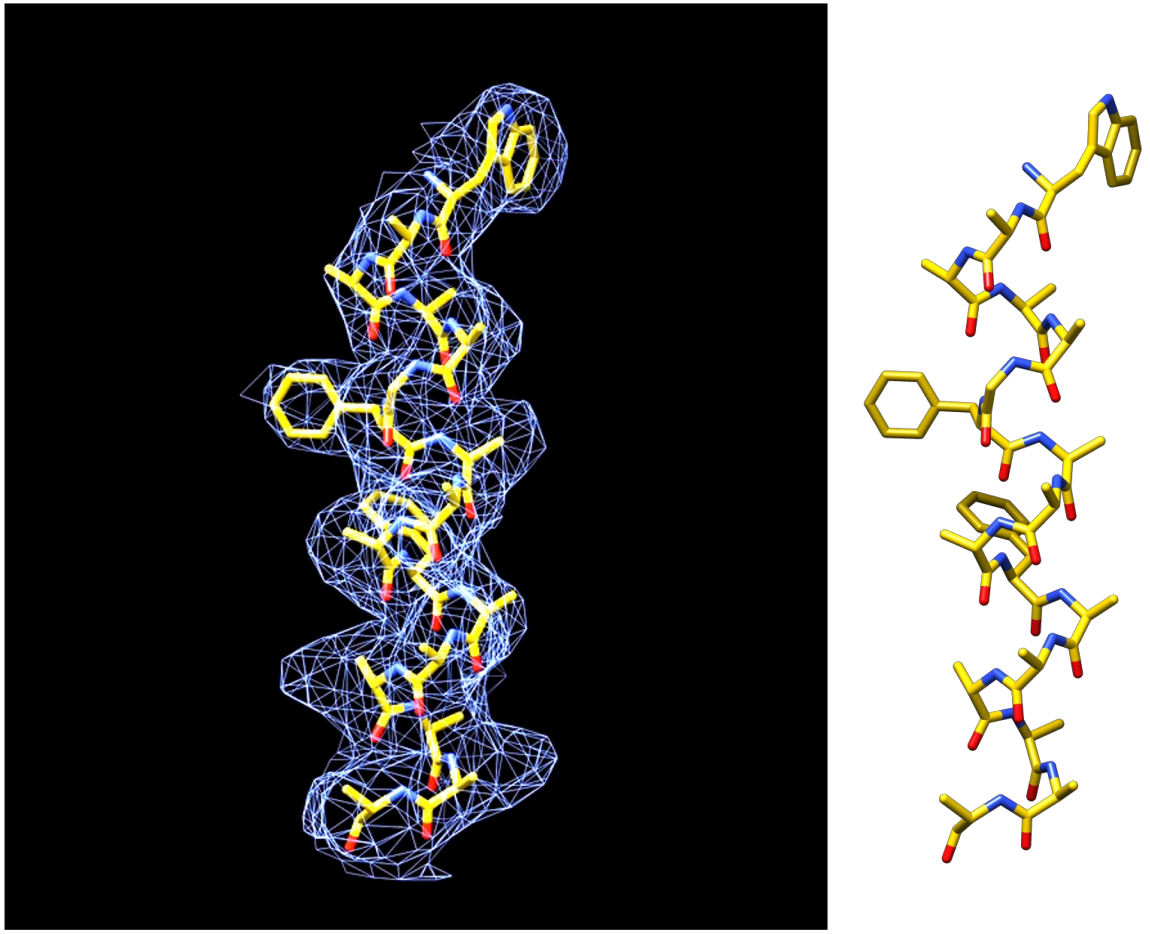


Test the initial hypothesis by extending sequence assignment along the chain.



…VFNSLTEYIQGPCTGNQQSLAHSRL**W**DAVVG**F**LHV**F**AHMMMMKLAQDSSQIELLKELLDLQ…

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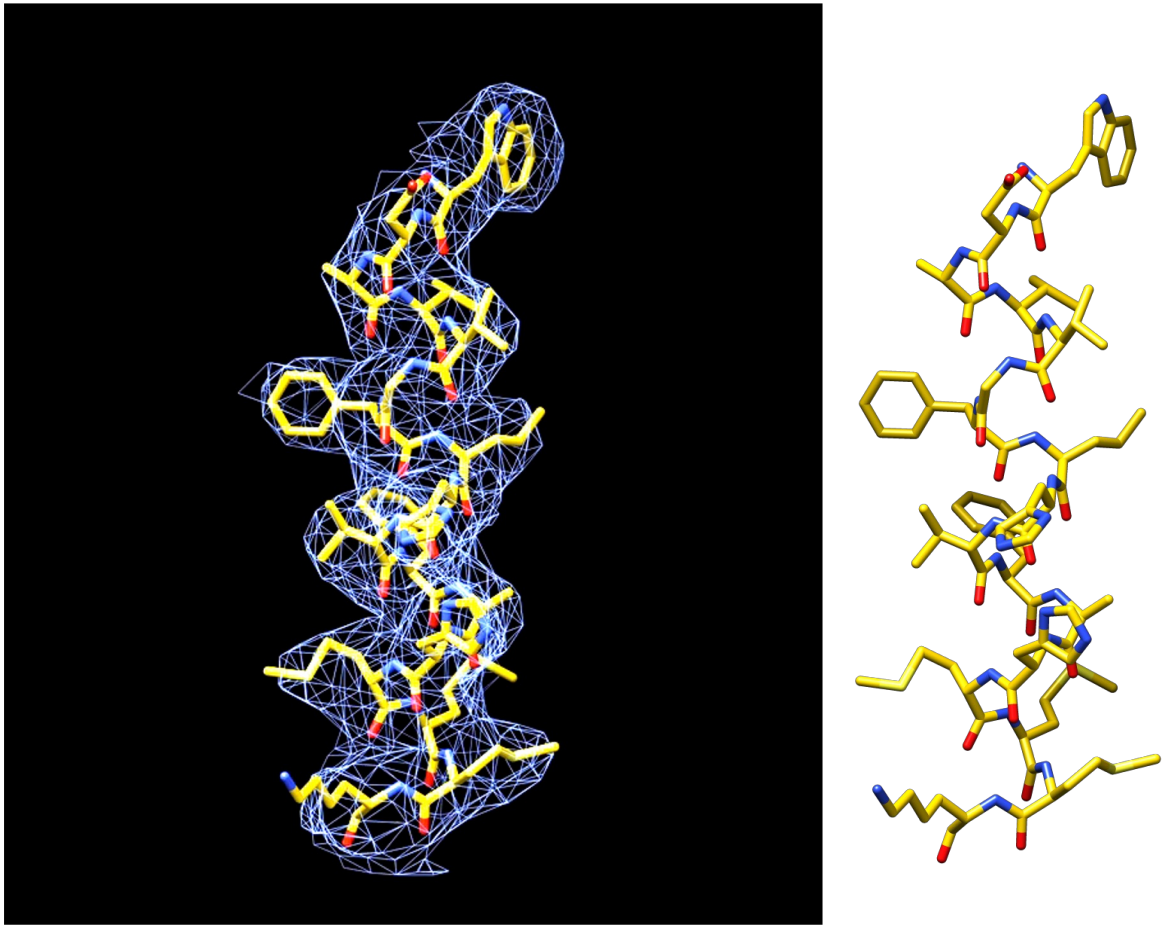

…VFNSLTEYIQGPCTGNQQSLAHSRL**WDAVVGFLHVFAHMMMK**LAQDSSQIELLKELLDLQ…

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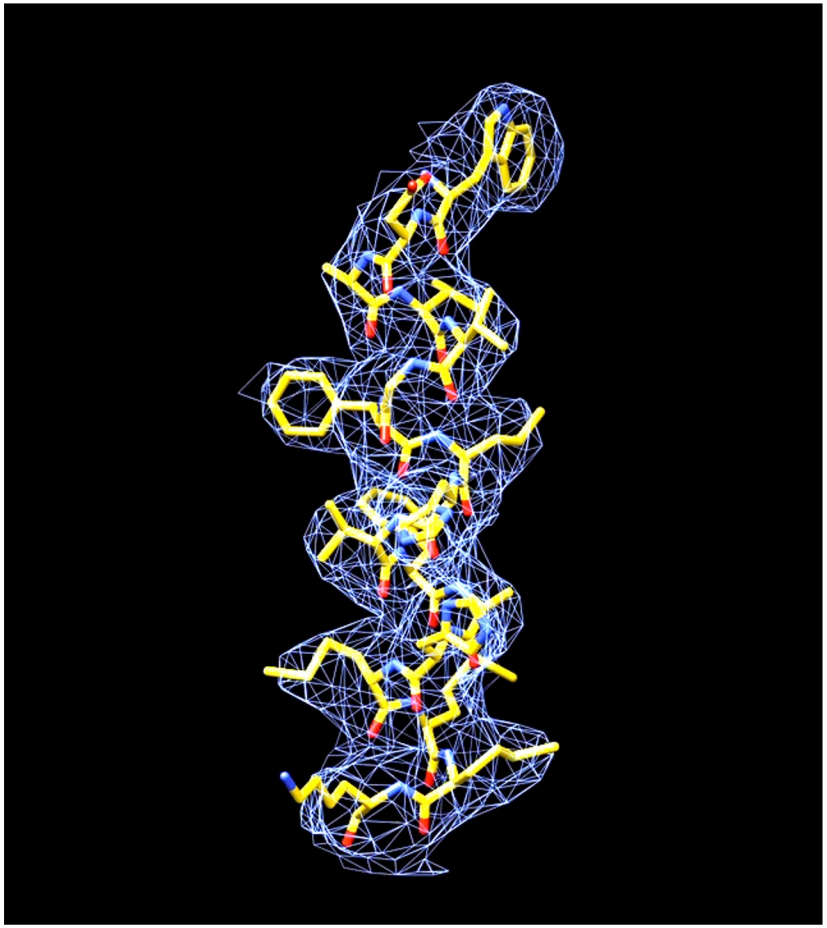
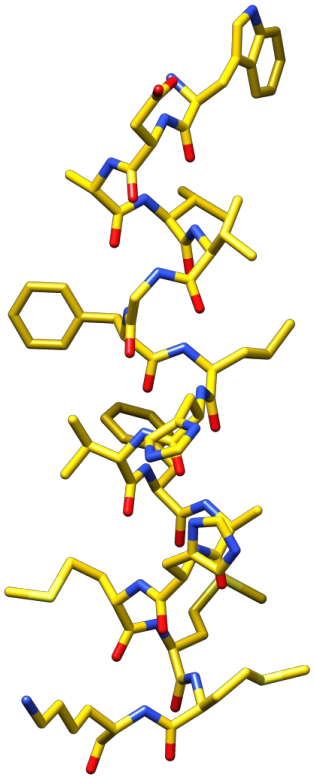
Notice that the **absence** of large sidechain densities at small residue positions is just as valuable in validating the fit as the fit of large sidechains to the density.

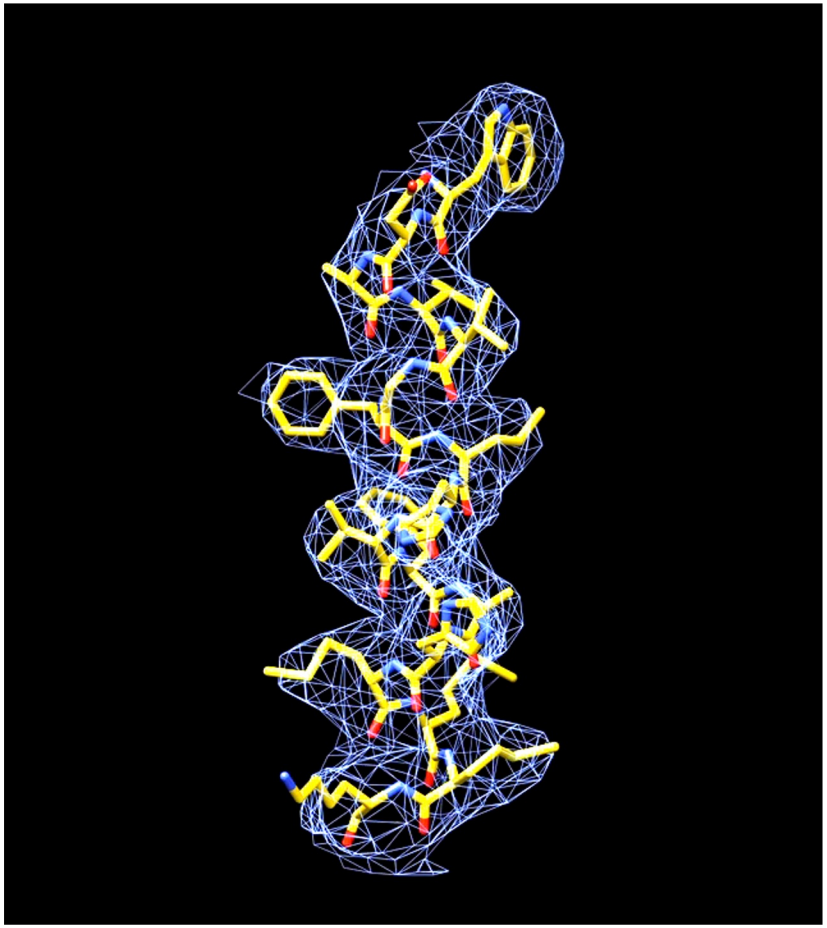


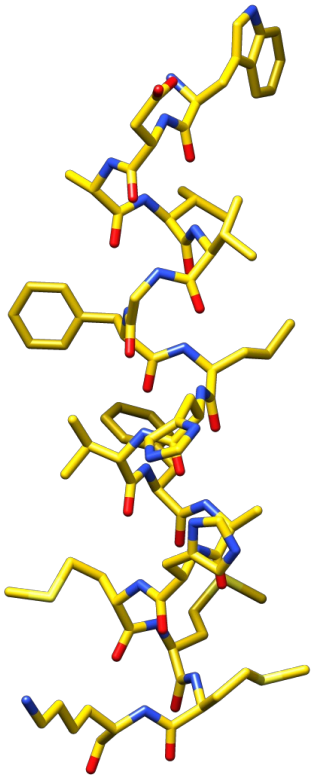


…VFNSLTEYIQGPCTGNQQSLAHSRL**WDAVVGFLHVFAHMMMK**LAQDSSQIELLKELLDLQ…

Test the initial hypothesis by extending sequence assignment along the chain.

Also, note that the information content of local regions varies. Consider "VTVVAASSTVV" vs "FGAAYWVTRA" – which is more likely to be uniquely identifiable from the map?

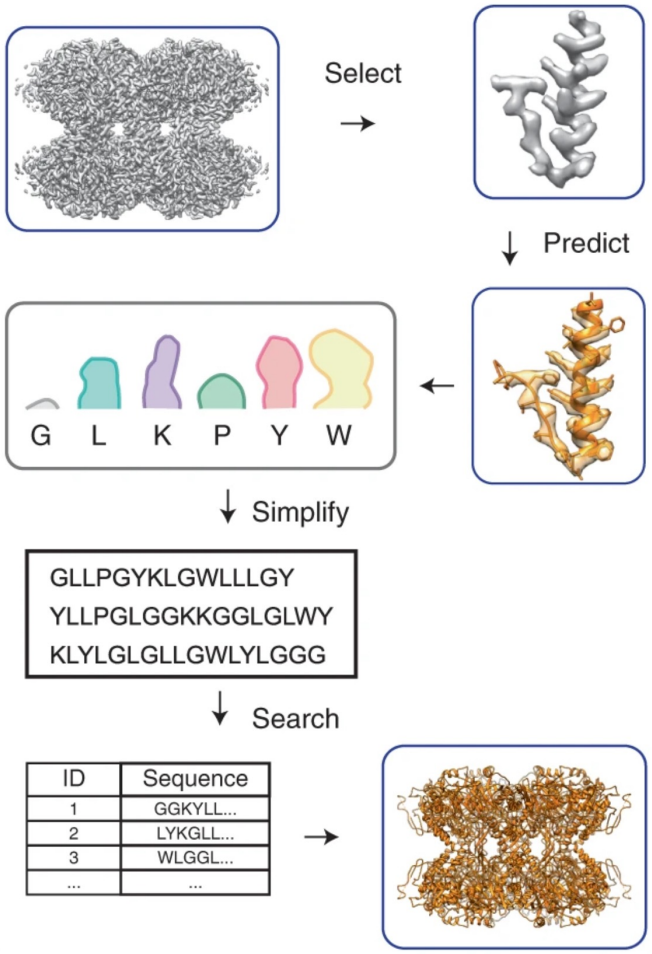




…VFNSLTEYIQGPCTGNQQSLAHSRL**WDAVVGFLHVFAHMMMK**LAQDSSQIELLKELLDLQ…

CryoID can help when you don't even know the sequence!

- Similar approach codified and automated in the "cryoID" program – but in this case, starting from the density, with no sequence input!
- Split map into fragments
- Use reduced complexity pseudo-sequence to convert map fragments into motifs which can be used to search sequence database.
- Identify most likely candidate sequence, combine fragments and rebuild.
- Useful when purifying from endogenous sources, where composition may not be known.



(Ho et al., Nature Methods, 2020)

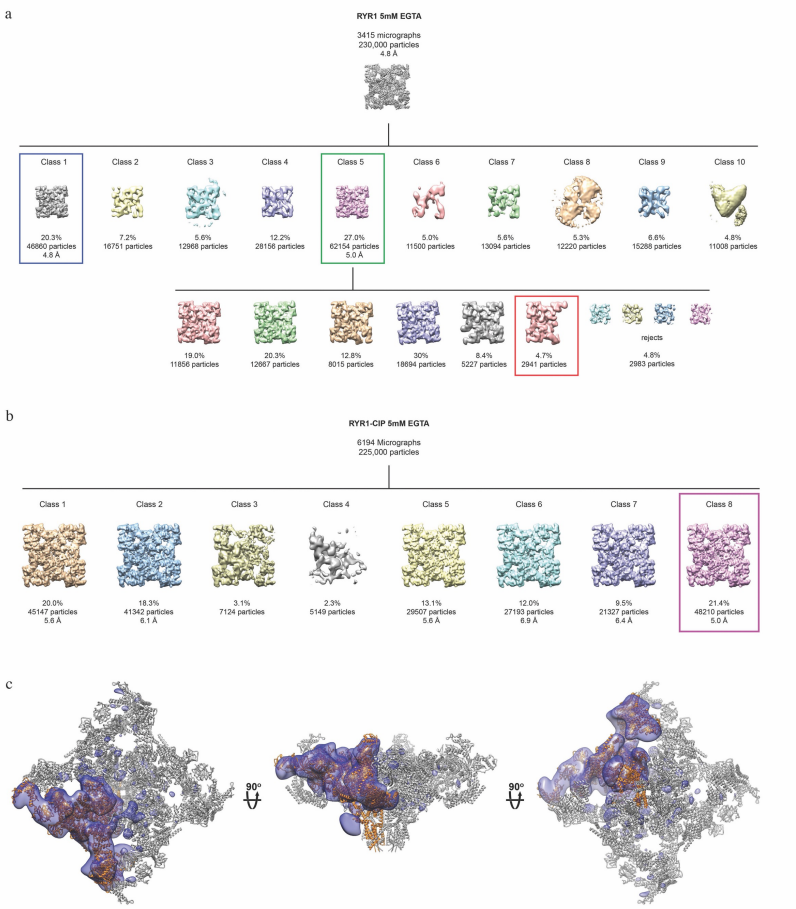
How to deal with uncertainty in sequence assignment and sidechain placement

- You will likely encounter situations where you cannot be certain of the local sequence register – what to do?

- No clear consensus, but I suggest assigning residue code as “UNK” and numbering to “best guess” value. A more granular way to quantify/convey uncertainty would be helpful!

- Sidechain placement – two main camps – trim sidechains to density vs place them all (+/- zero occ.). The former may sound more conservative, but it can hide errors during validation (during analysis of clashes). Either is acceptable, just be consistent, and preferably outline the approach taken when writing up the structure.

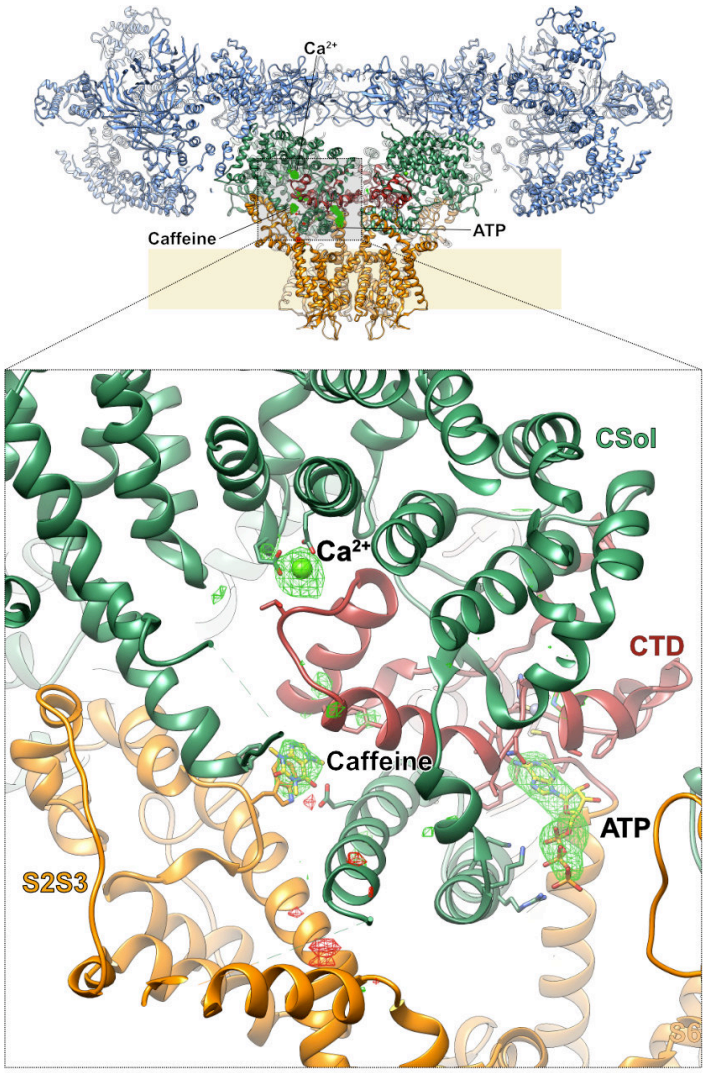
Prior knowledge can come in many forms – use any and all available info to guide model building.



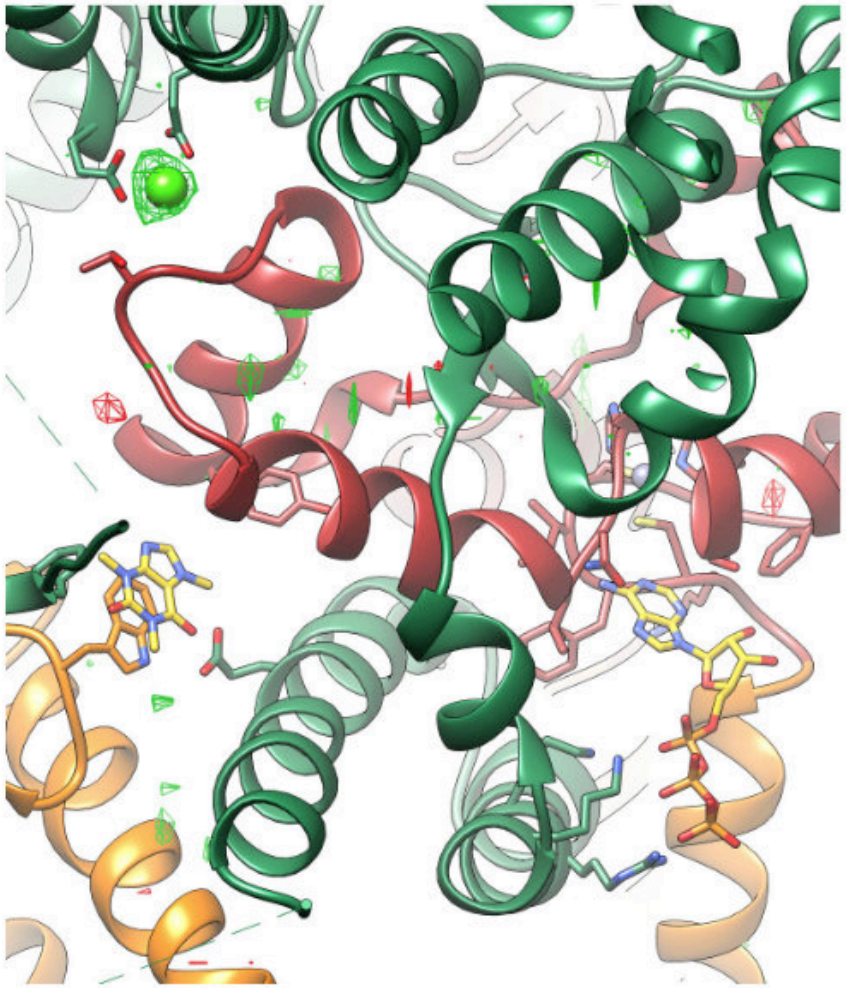
Here, serendipitous identification of a conformational class of RyR1 lacking density for one subunit aided identification of protomer boundaries. In other cases, cross-linking data or NS data on subcomplexes or Fab-complexes may be helpful.

(Zalk et al, Nature 2015)

In a similar manner, we can use locally aligned difference maps between holo and apo structures to locate ligands.

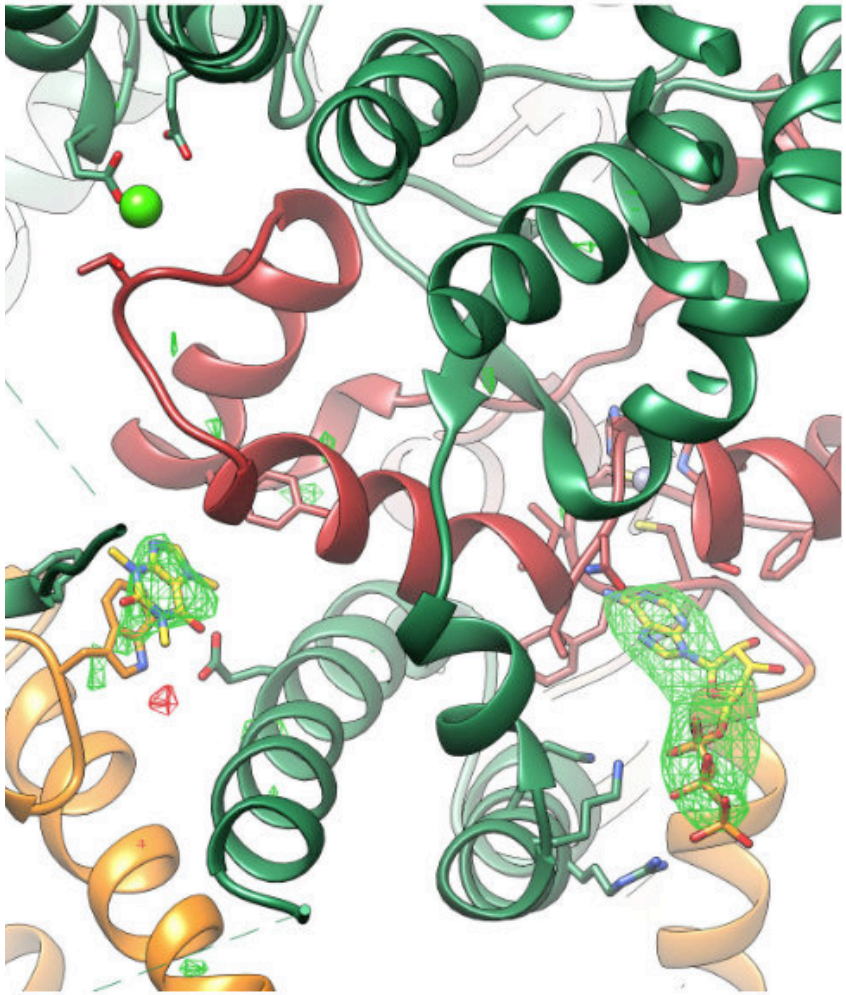


The three ligands are clustered around the C-terminal domain.



(Ca^{2+} only) minus (EGTA only)

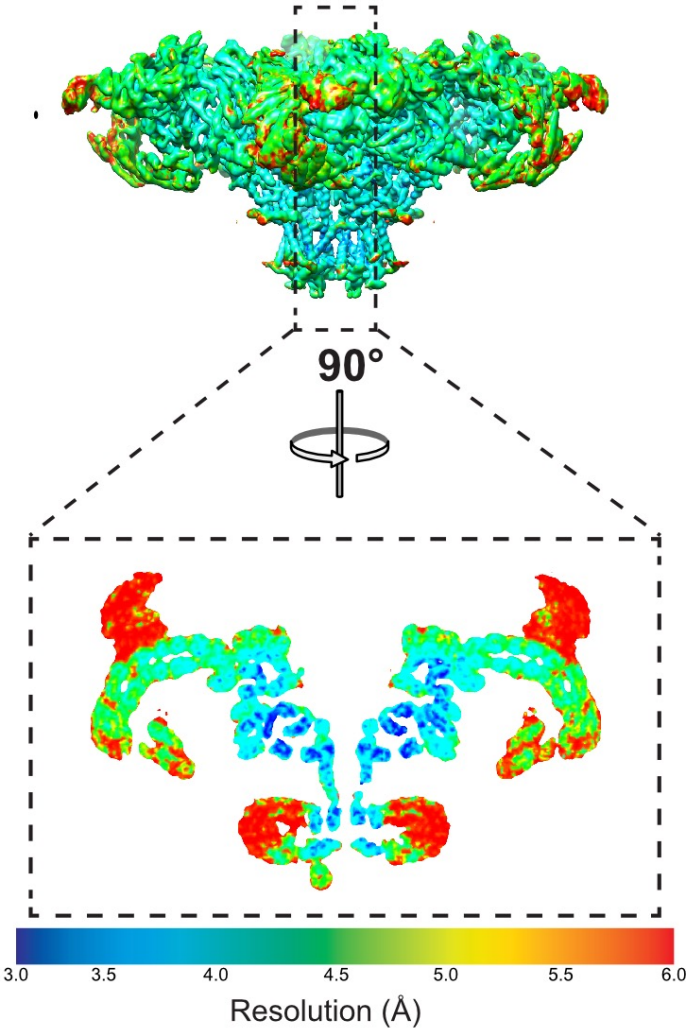
The three ligands are clustered around the C-terminal domain.



(ATP/Caffeine) minus (EGTA only)

EM-specific considerations

- No unambiguous sequence markers at low resolution (no equivalent of SeMet).
- No feedback from phase improvement, but also no model bias – WYSIWIG.
- Often substantial variation in local resolution – different strategies and levels of detail required for different regions. Map sharpening essential.
- ”Medium” resolution (4-6Å) much more common than for crystallography.
- Often have more than one map, with different composition or conformation (may be convenient to combine focused refinements in Chimera by taking max value at each voxel after alignment, e.g.: `vop maximum #1,2 ongrid #1`)

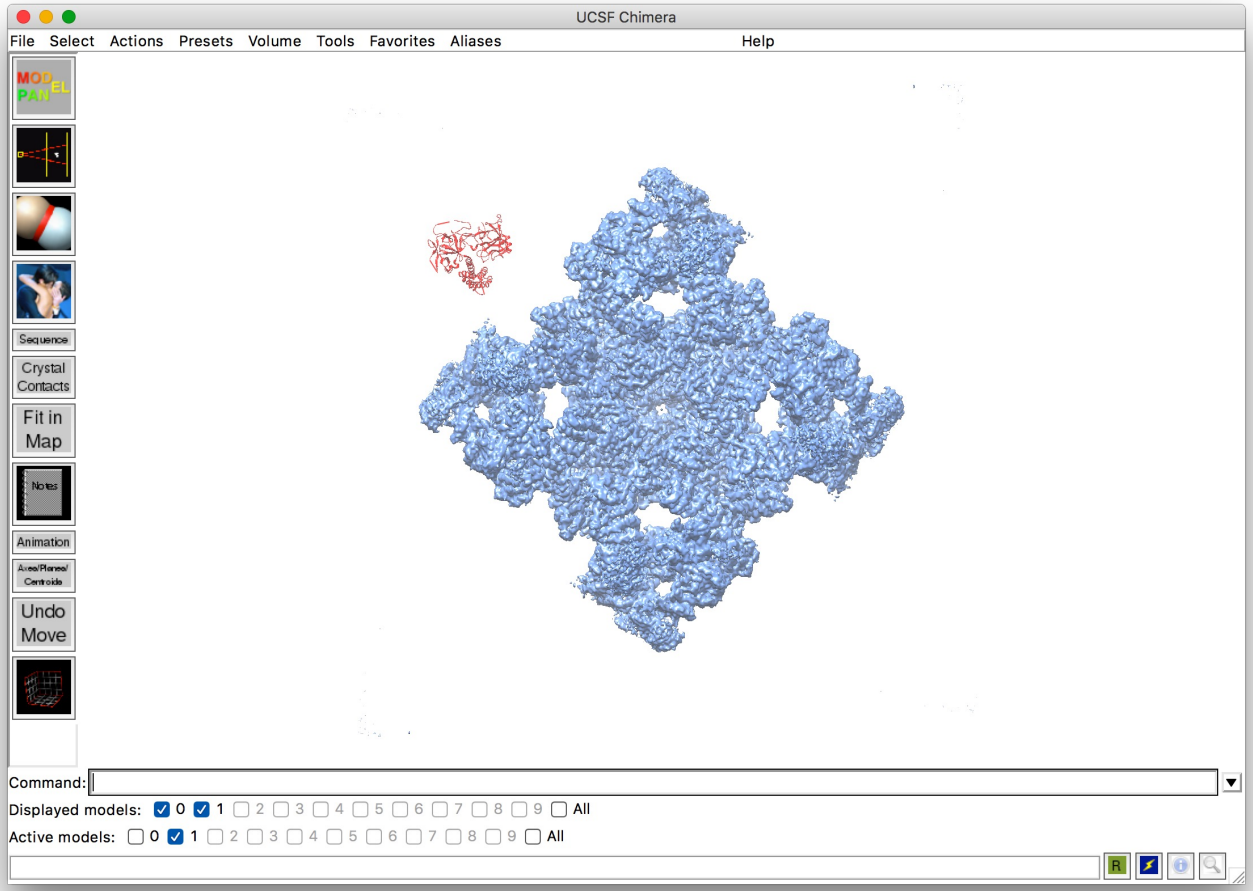


Building an initial model - where to start?

- If you have a crystal structure, of a fragment or a homology model of a domain, place it, and extend into density. (***Now, Alphafold & Rosettafold mean this is almost always the case****)*

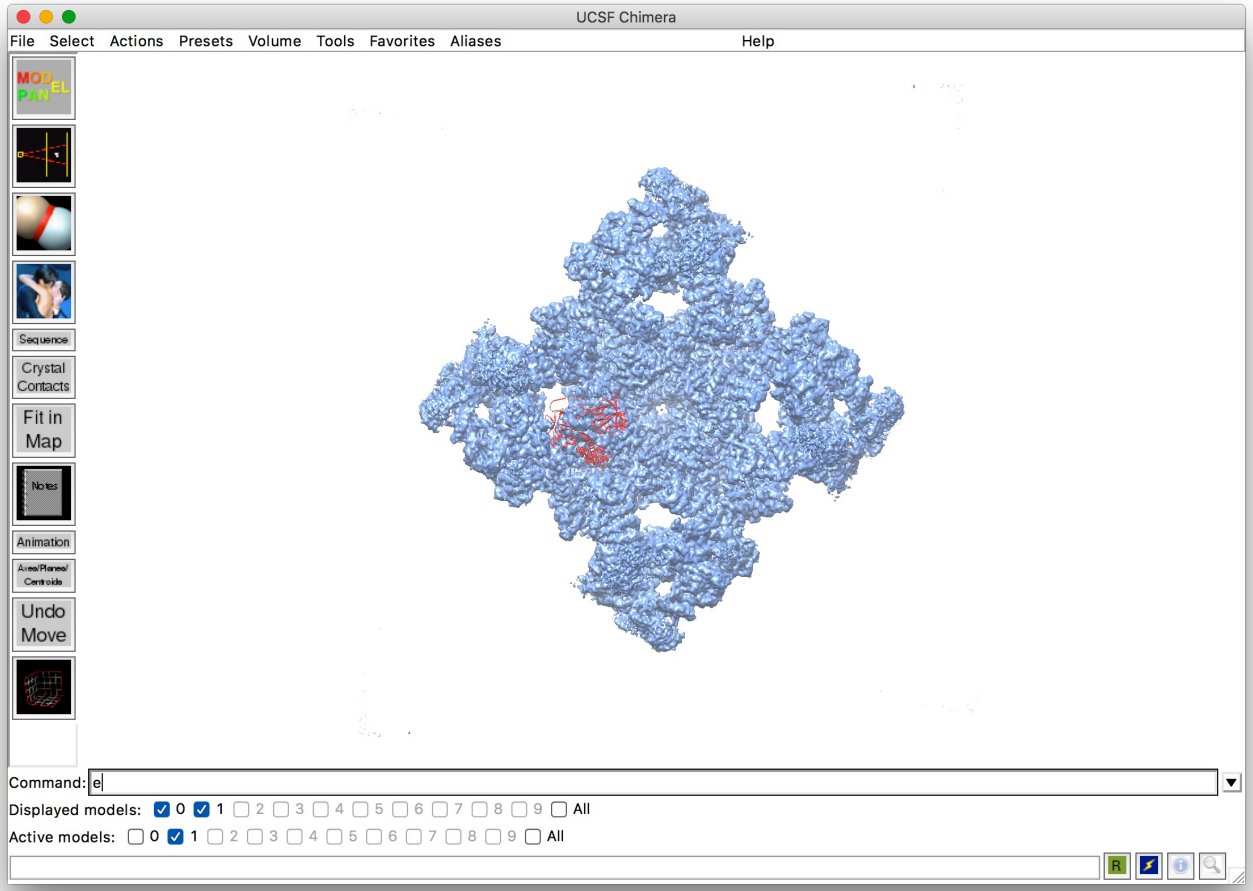
- Otherwise, identify structurally distinctive motifs in the sequence – for example, a strongly predicted helix with three aromatic residues near the N-term end – and identify candidate locations in the density map. Extend and see if hypothesis still holds.

Using UCSF Chimera to fit solved domains



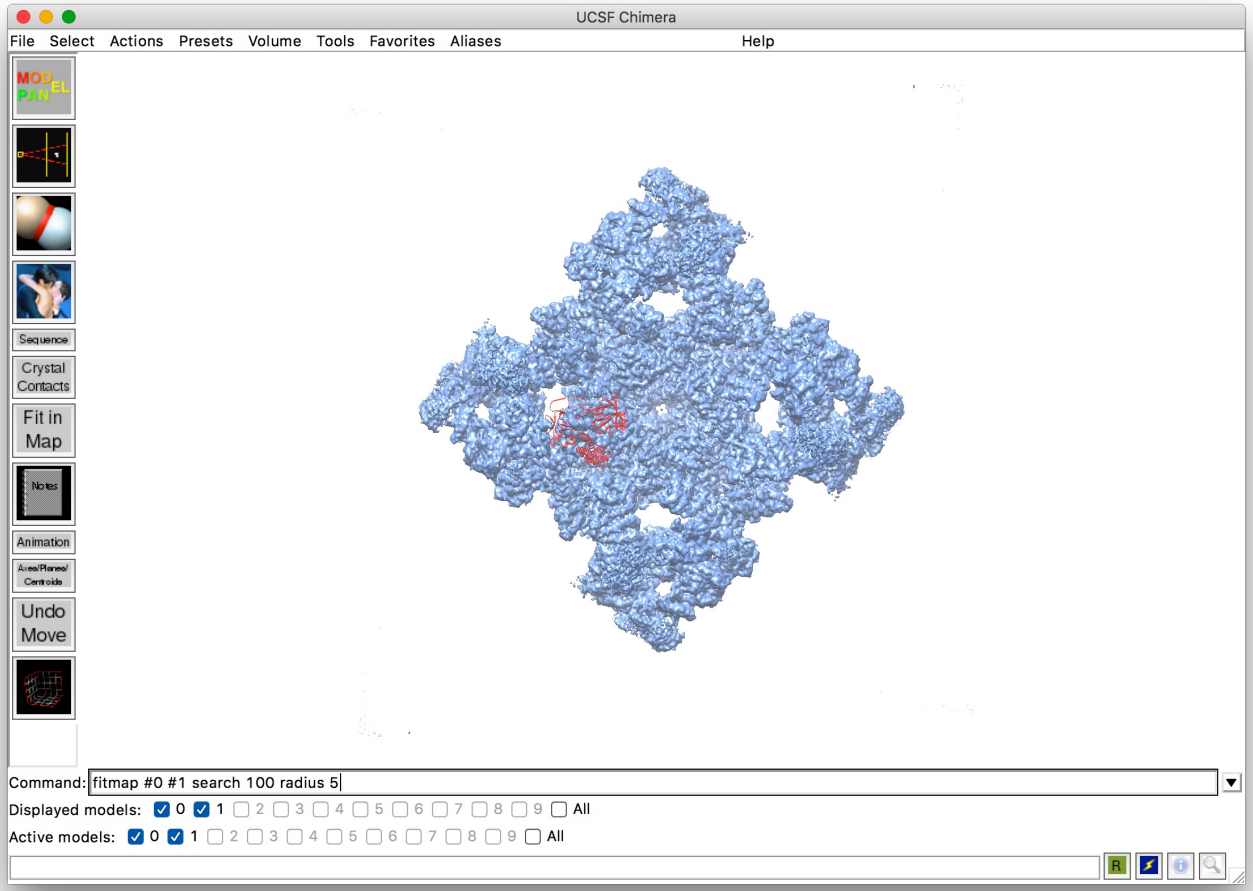
Start with map and model.

Using UCSF Chimera to fit solved domains



Move model to approximate position (if known, to save computation)

Using UCSF Chimera to fit solved domains



Run fitmap with ‘search’ (here 100 orientations) and ’radius’ (here 5 Å)

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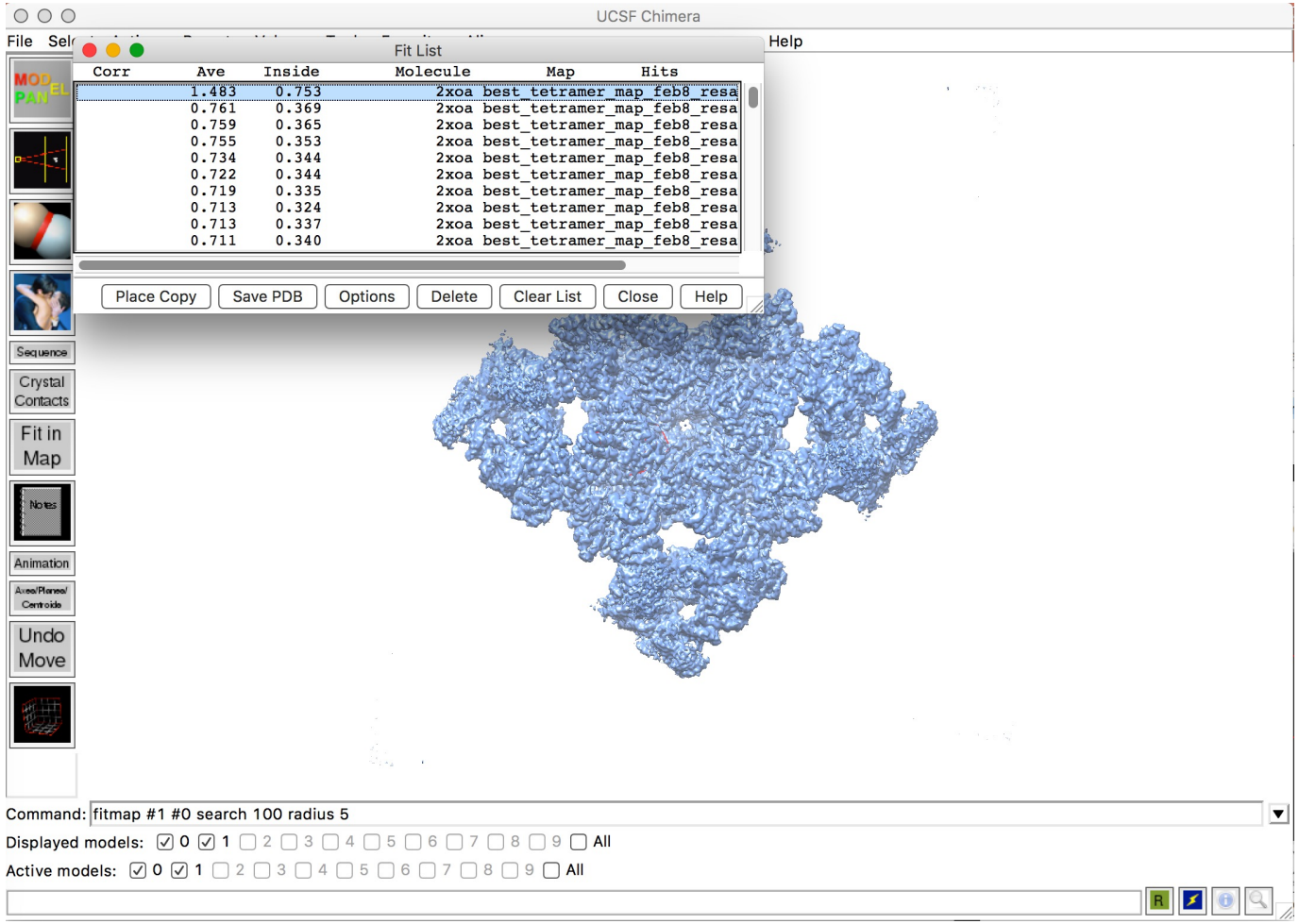

Chimera will return a list of candidate orientations, ranked by agreement with the map. Hopefully there will be a clear separation between the correct and incorrect solutions.

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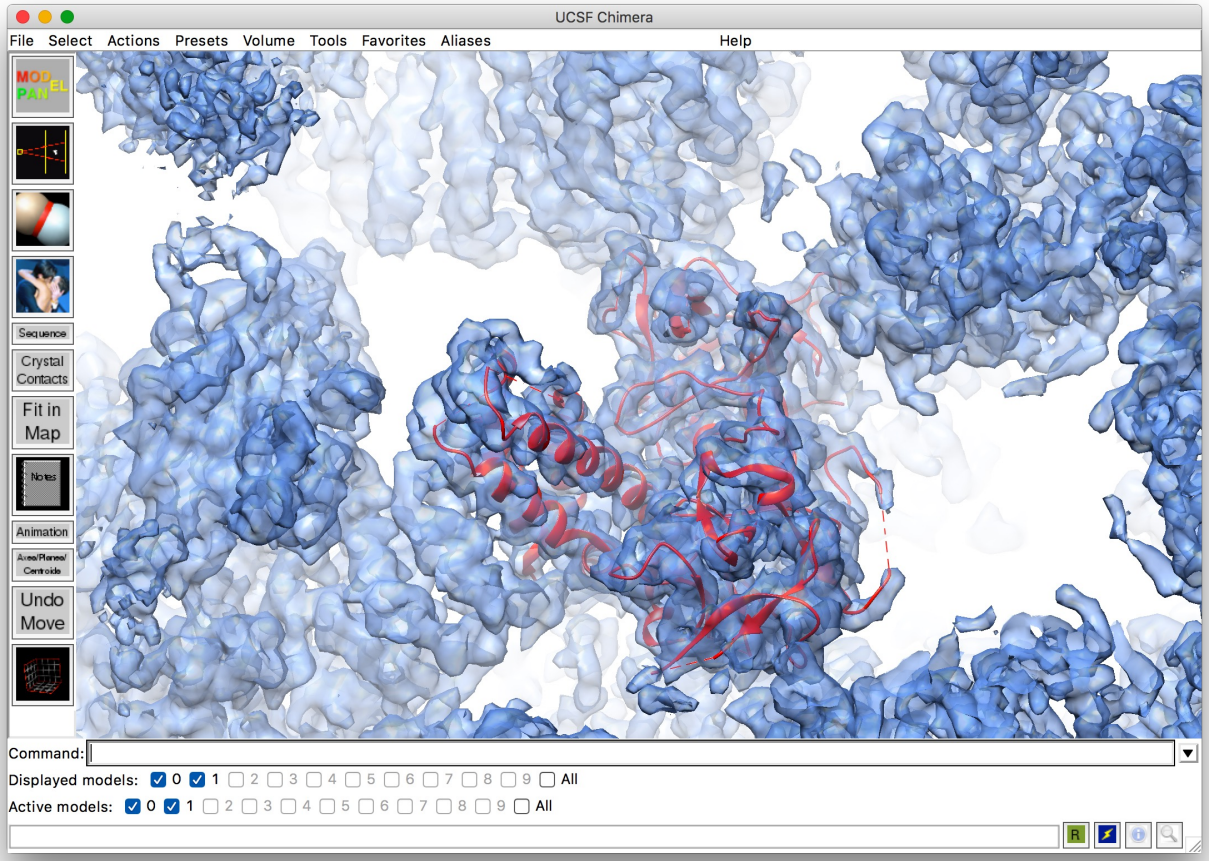

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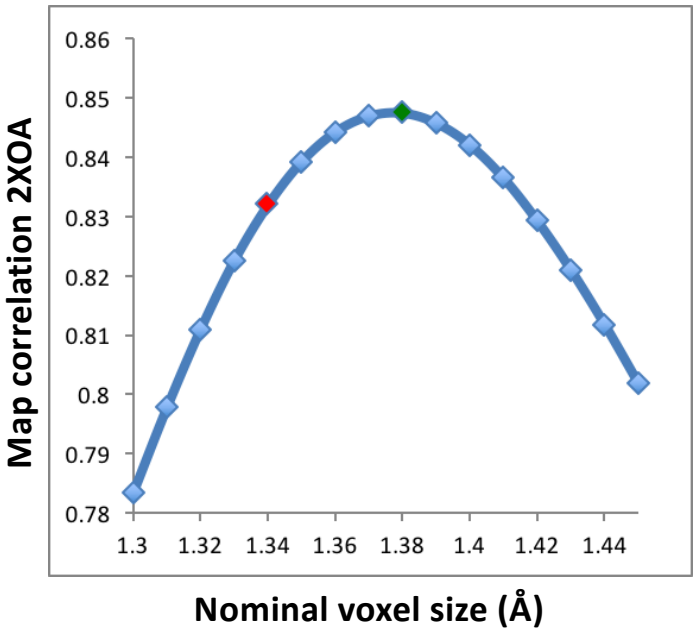
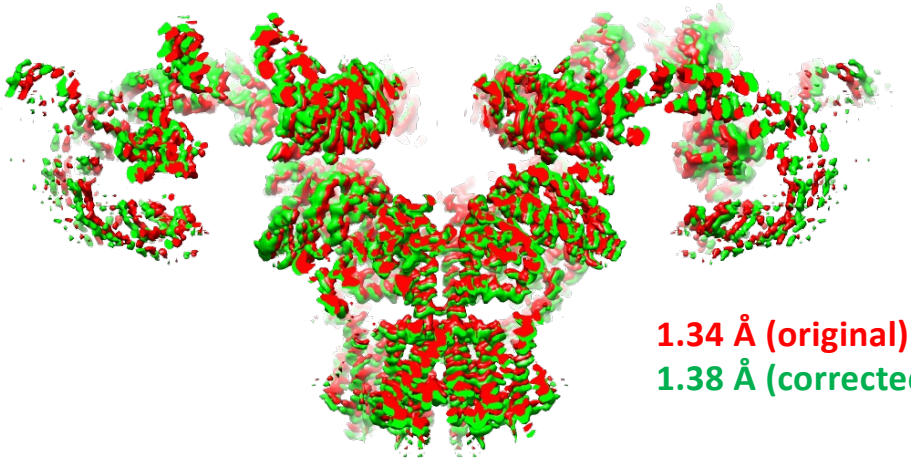
Using UCSF Chimera for voxel size calibration (of your map and others)

- Voxel size generally requires calibration against a crystal structure.
- Once calibrated, generally stable between samples/datasets at same magnification.
- Can calibrate by fitting in Chimera at range of nominal voxel sizes and measuring correlation.
- Incorrect voxel sizes are common in deposited maps - **be aware of this when comparing structures**. E.g. here there is a 3% difference – affects structural alignment, reported resolution (3.8 vs 3.9Å).





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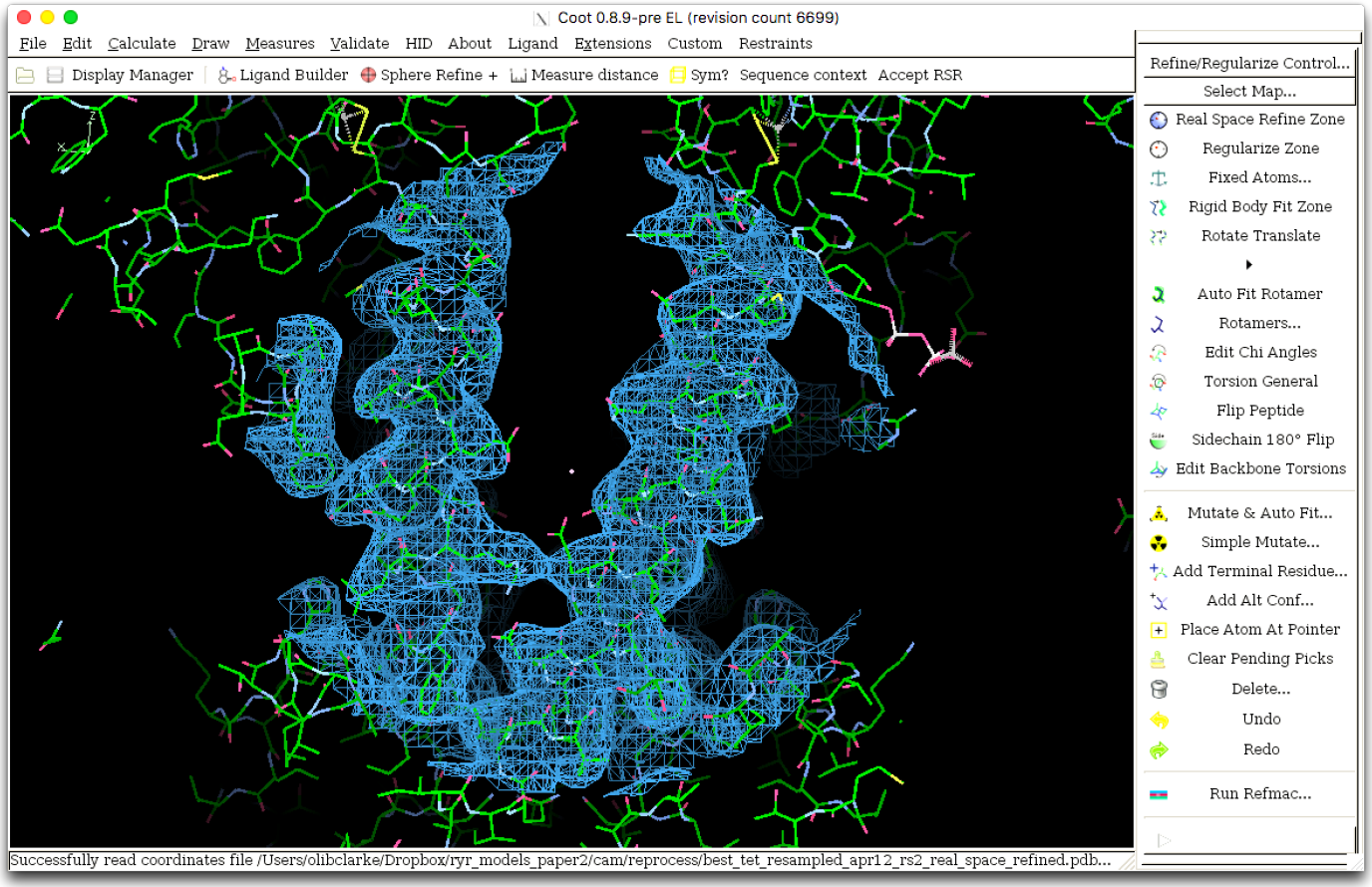
- Simple, intuitive interface for building and manipulating atomic models in density maps.
- Low computational requirements
- Extensive API – easy to script or modify (using simple Python code)
- On-the-fly sharpening and low pass filtering (for MTZ).



(Try the latest nightly with new features for EM, improved RSR: http://www.ccpem.ac.uk/download.php)

(*Emsley P. 2004, Acta Cryst. D; Casañal A. et al. 2020, Protein Science)*

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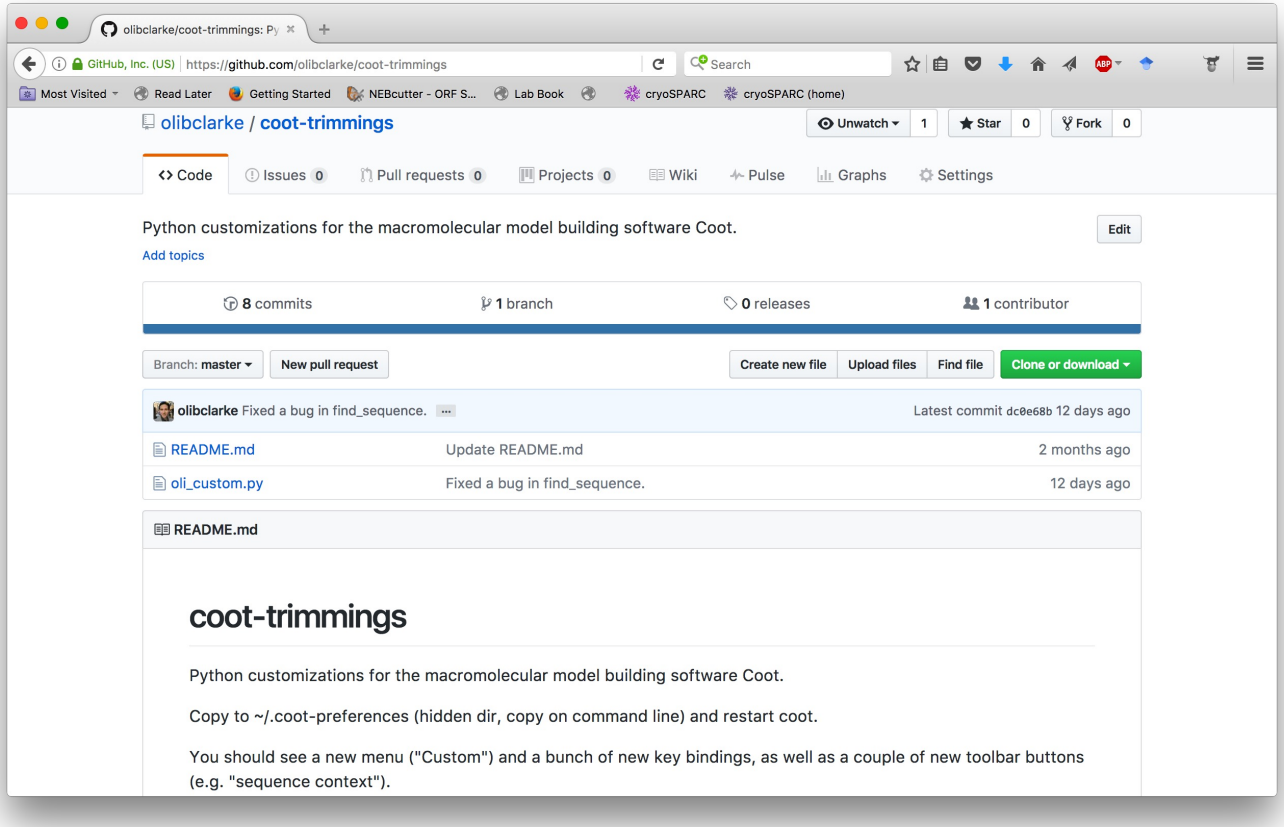

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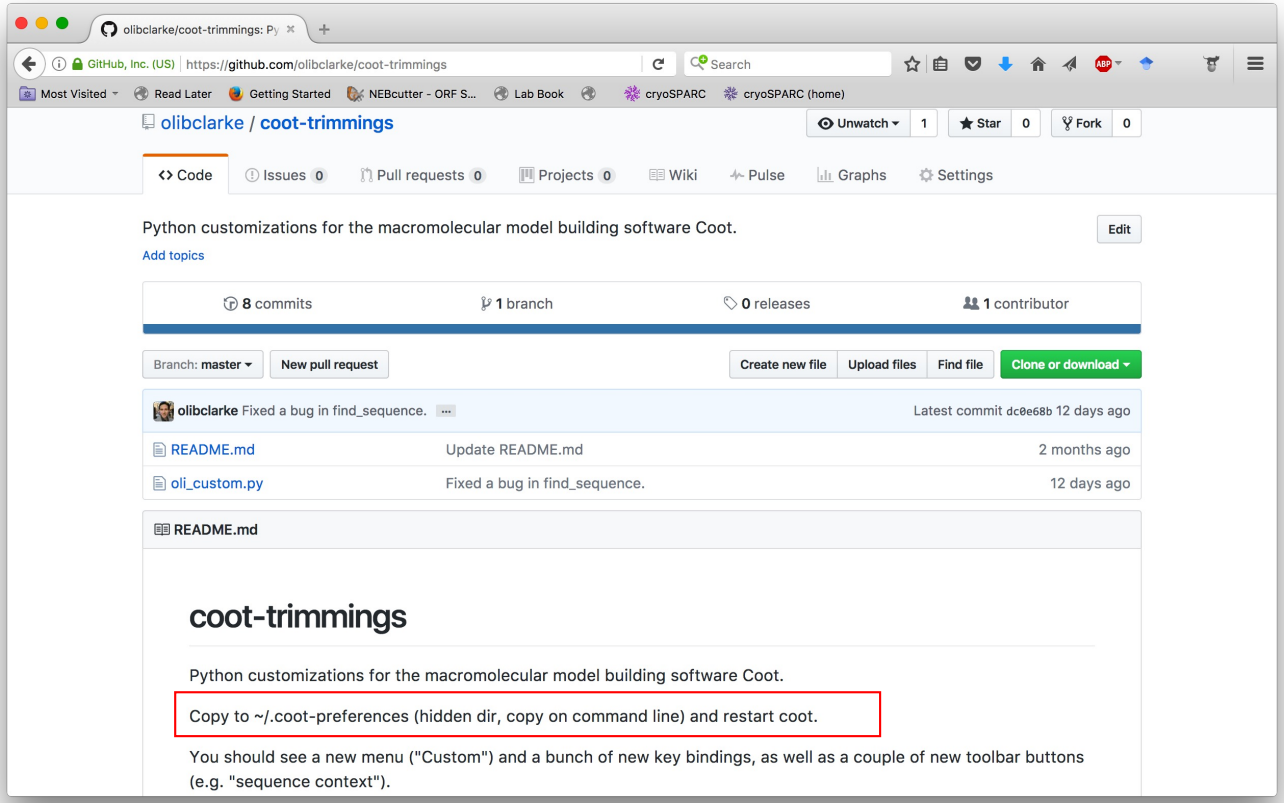

Any Python (or Scheme) file you put in ~/.coot-preferences will be executed when starting Coot. Can use this for extra key bindings, scripts, custom functions.

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```
def mutate_by_entered_code():
  def mutate_single_letter(X):
    entry=str(X).upper()
    mol_id=active_residue()[0]
    ch_id=active_residue()[1]
    resno=active_residue()[2]
    ins_code=active_residue()[3]
    resname=residue_name(mol_id,ch_id,resno,ins_code)
    map_id=imol_refinement_map()
    aa_dic={'A':'ALA','R':'ARG','N':'ASN','D':'ASP','C':'CYS','E':'GLU','Q':'GLN','G':'GLY','H':'HIS','I':'ILE','L':'LEU','K':'LY
    nt_list=['A','C','T','G','U']
    if (resname in aa_dic.values()) and (aa_dic.get(entry,0)!=0):
      mutate(mol_id,ch_id,resno,ins_code,aa_dic.get(entry,0))
    elif (resname in nt_list) and (entry in nt_list):
      mutate_base(mol_id,ch_id,resno,ins_code,entry)
    else:
      info_dialog("Invalid target residue! Must be protein or nucleic acid, and entered code must be single letter.")
  generic_single_entry("New residue? (single letter code)","A","Mutate by single-letter code",mutate_single_letter)
```

```
#mutate active residue to entered residue code (upper or lower case single-letter)
add_key_binding("Mutate by single letter code","M",
lambda: mutate_by_entered_code())
```

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Many pre-packaged functions available in COOT API. Mostly documented in online manual. Very easy to write your own! Useful e.g. for scripting domain-wise rigid body refinement.

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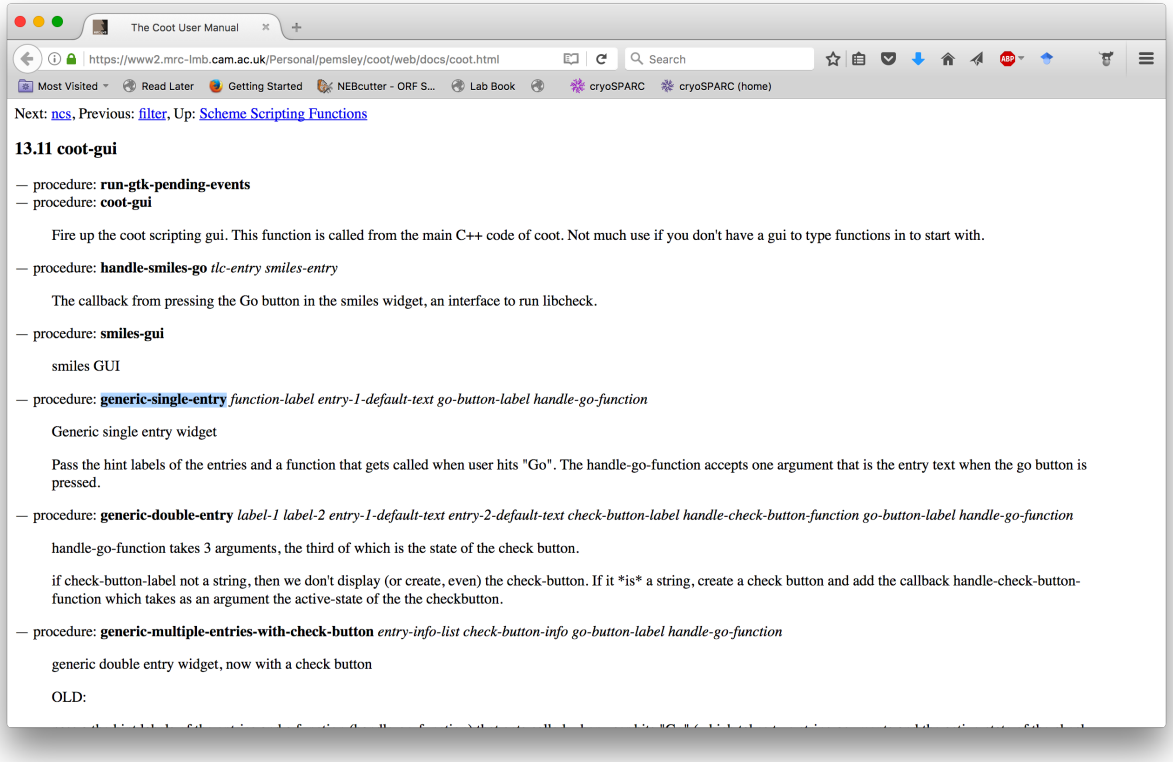

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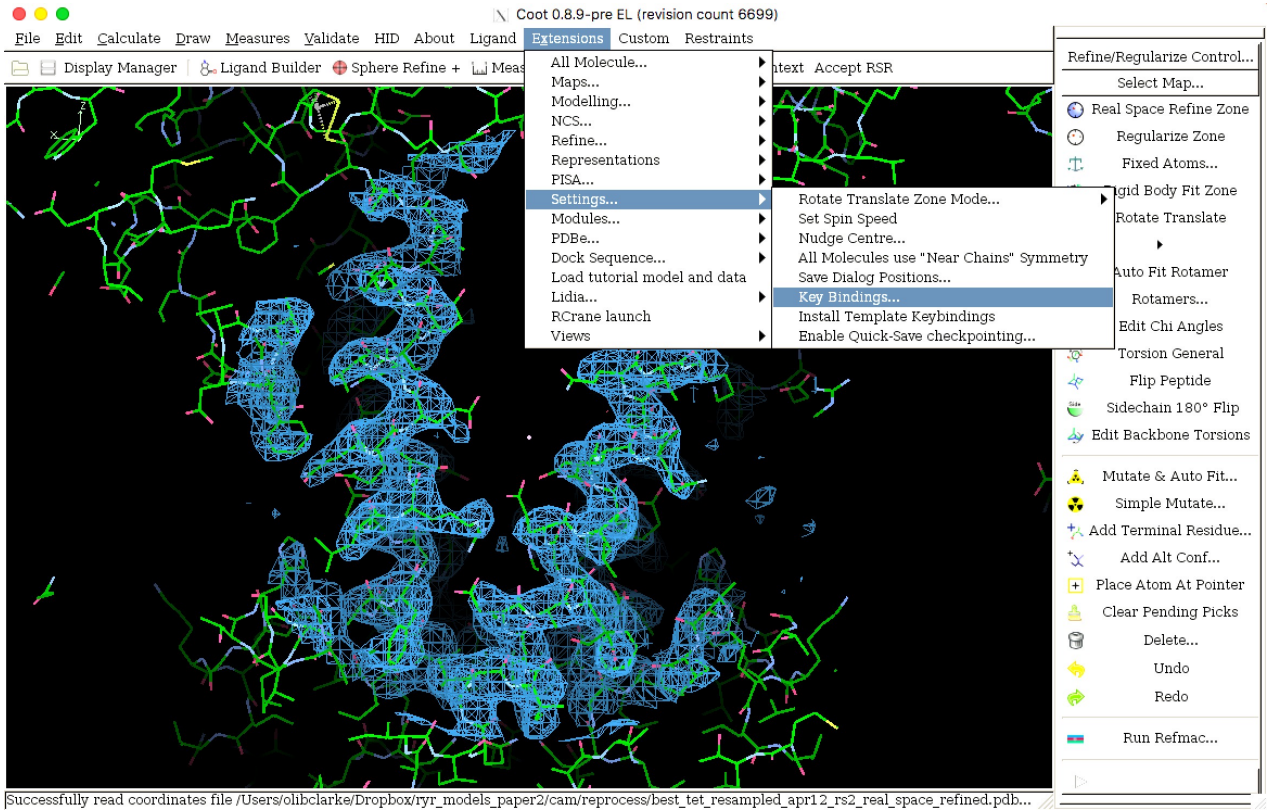

Lots of key bindings, and easy to define custom keys. Learn them. They make everything much faster.

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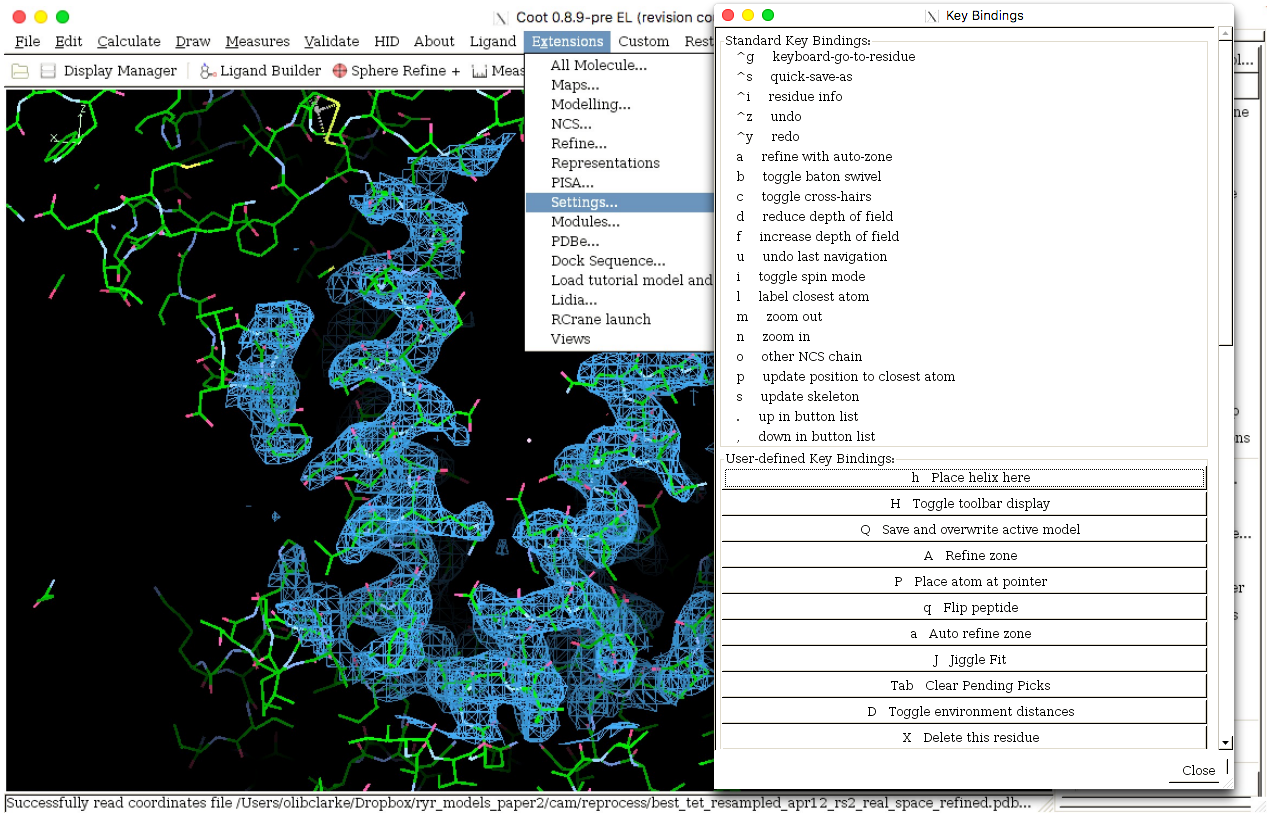

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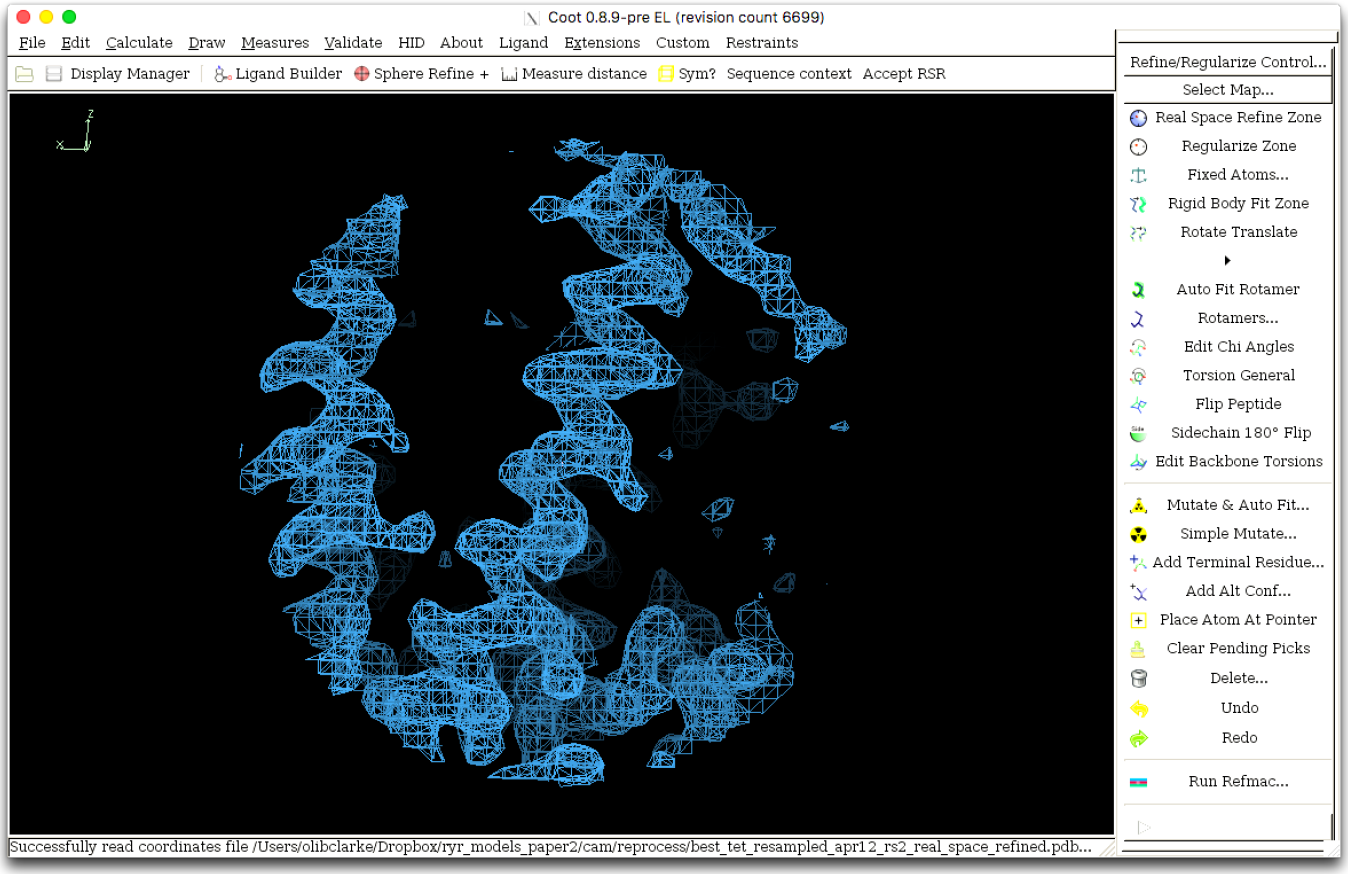
- Semi-automated helix placement
- Place cursor at the center of the helix and trigger ”Place helix here” (I suggest via a key binding - “h” with *`coot-trimmings`*)
- Coot will attempt to automatically determine the length and direction of the helix.
- Trim/extend, adjust weights, then refine using real-space refine zone. Drag into density to adjust fit.



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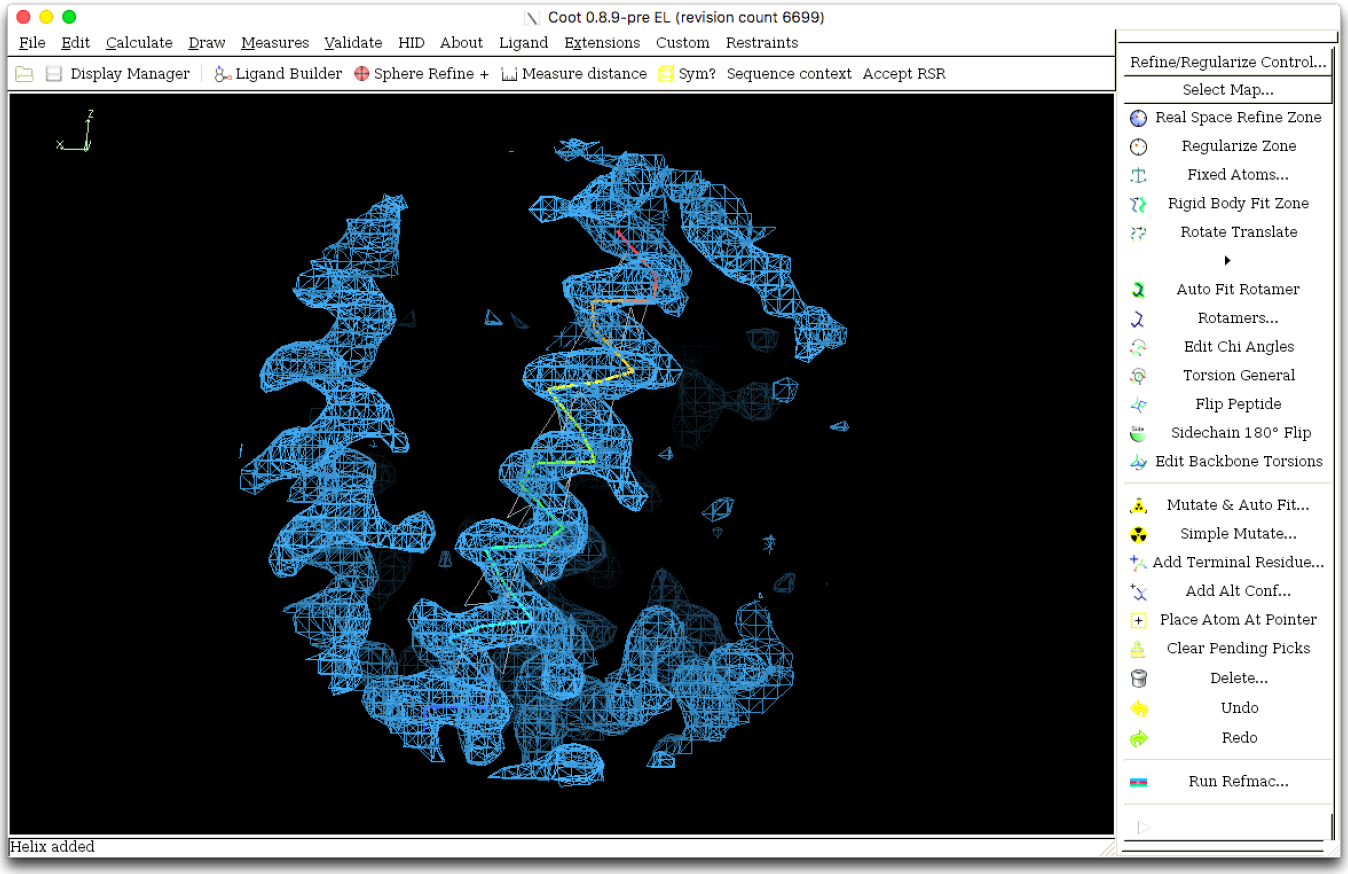

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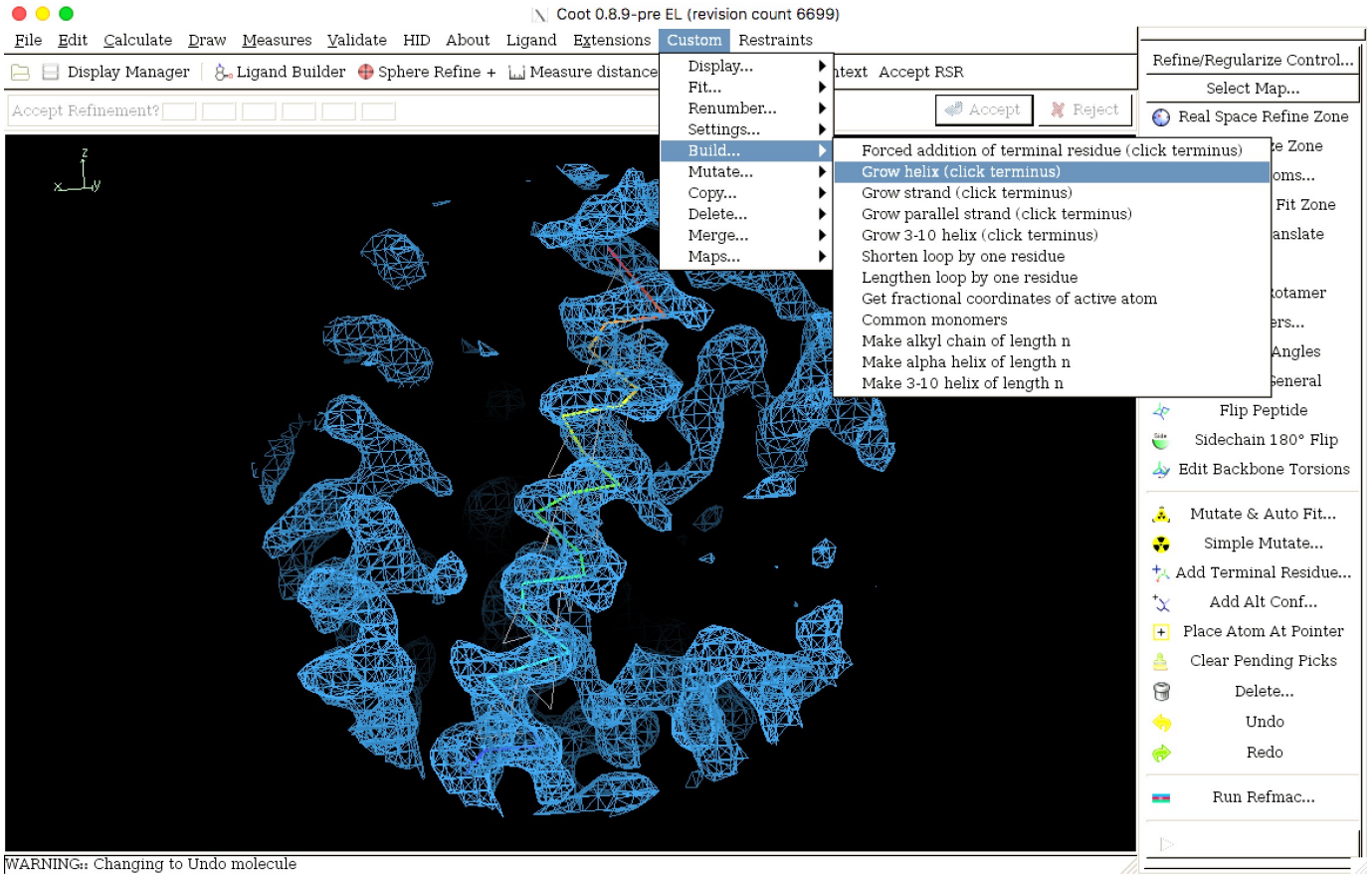

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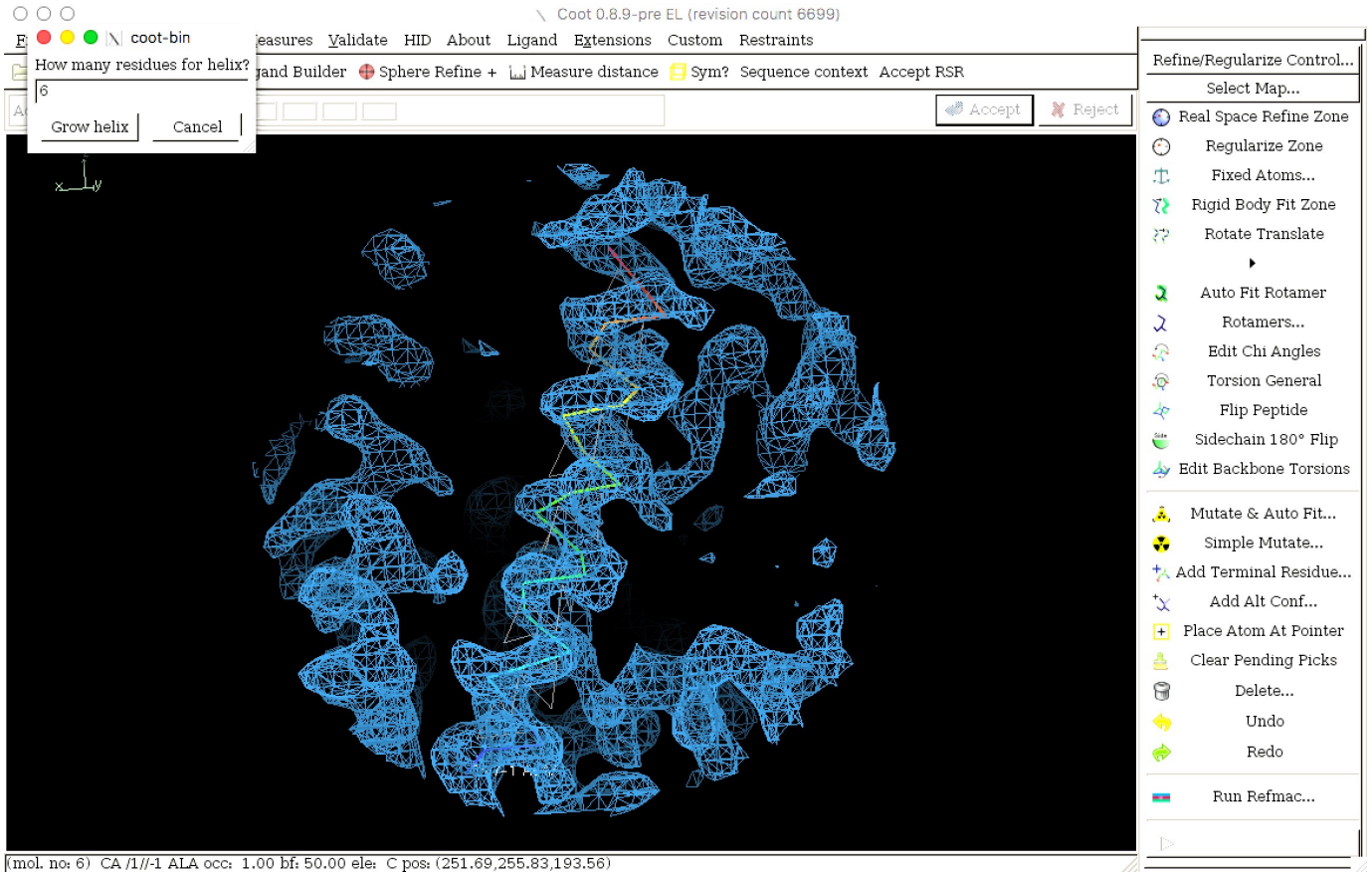

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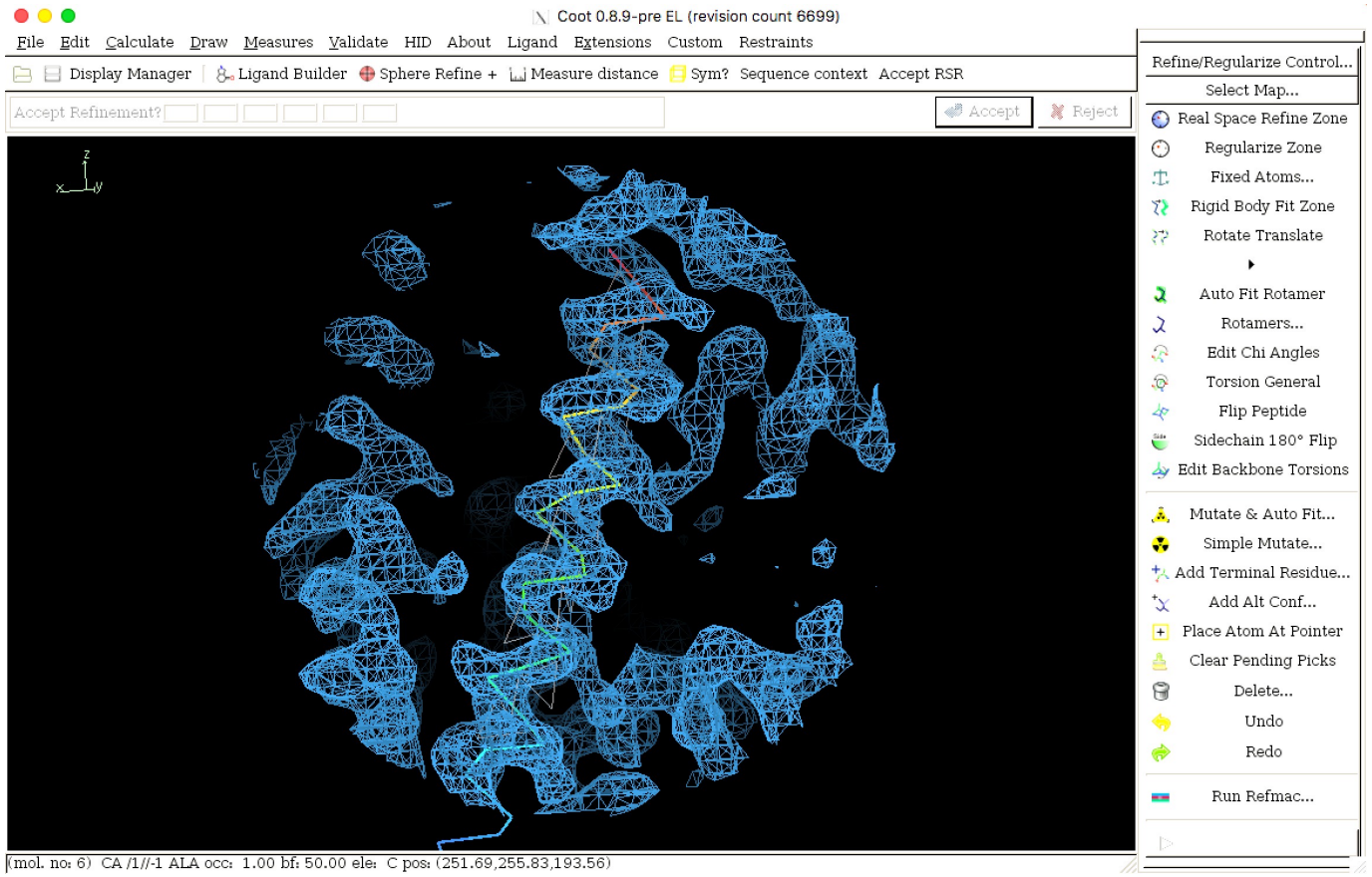

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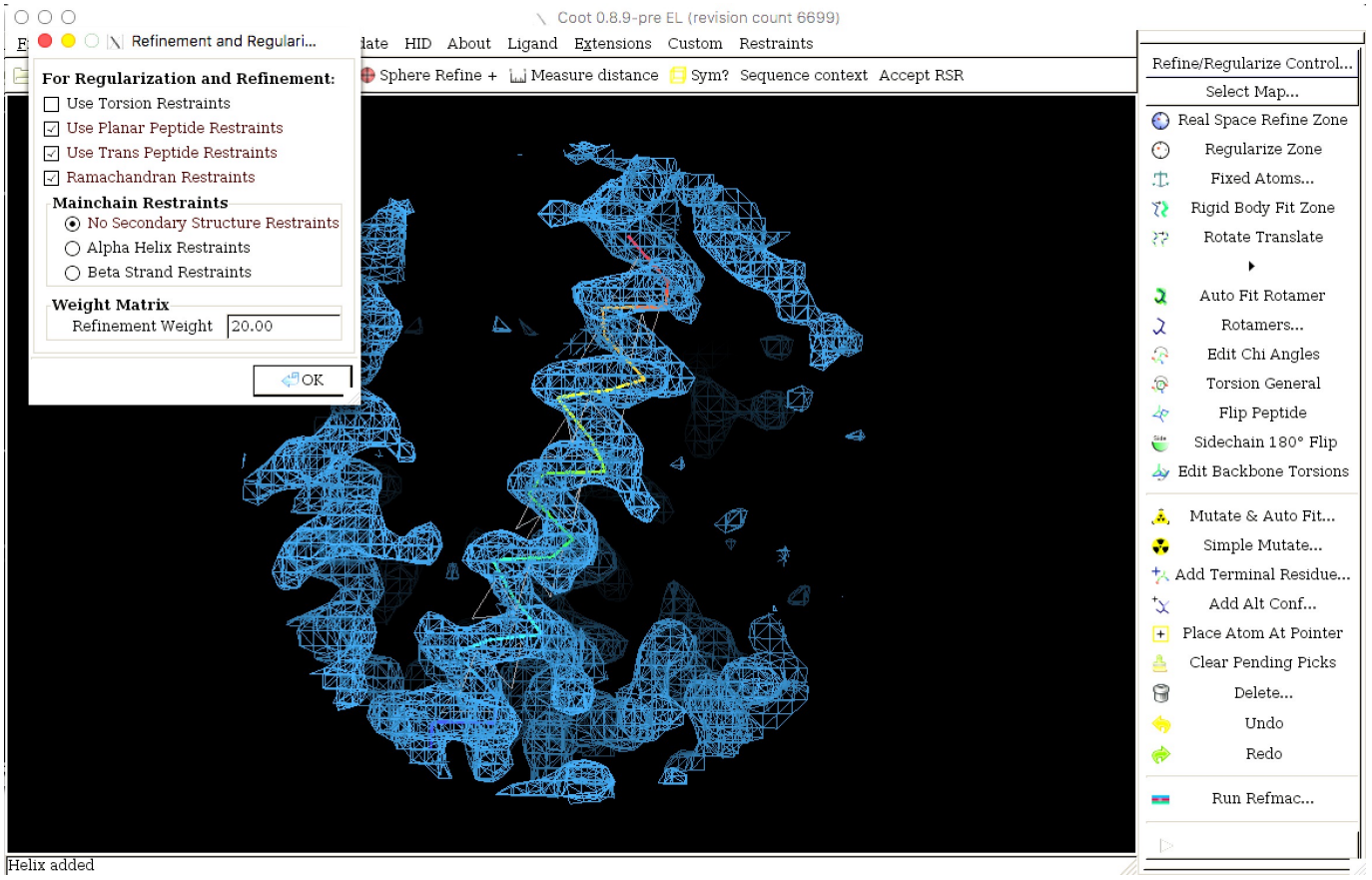

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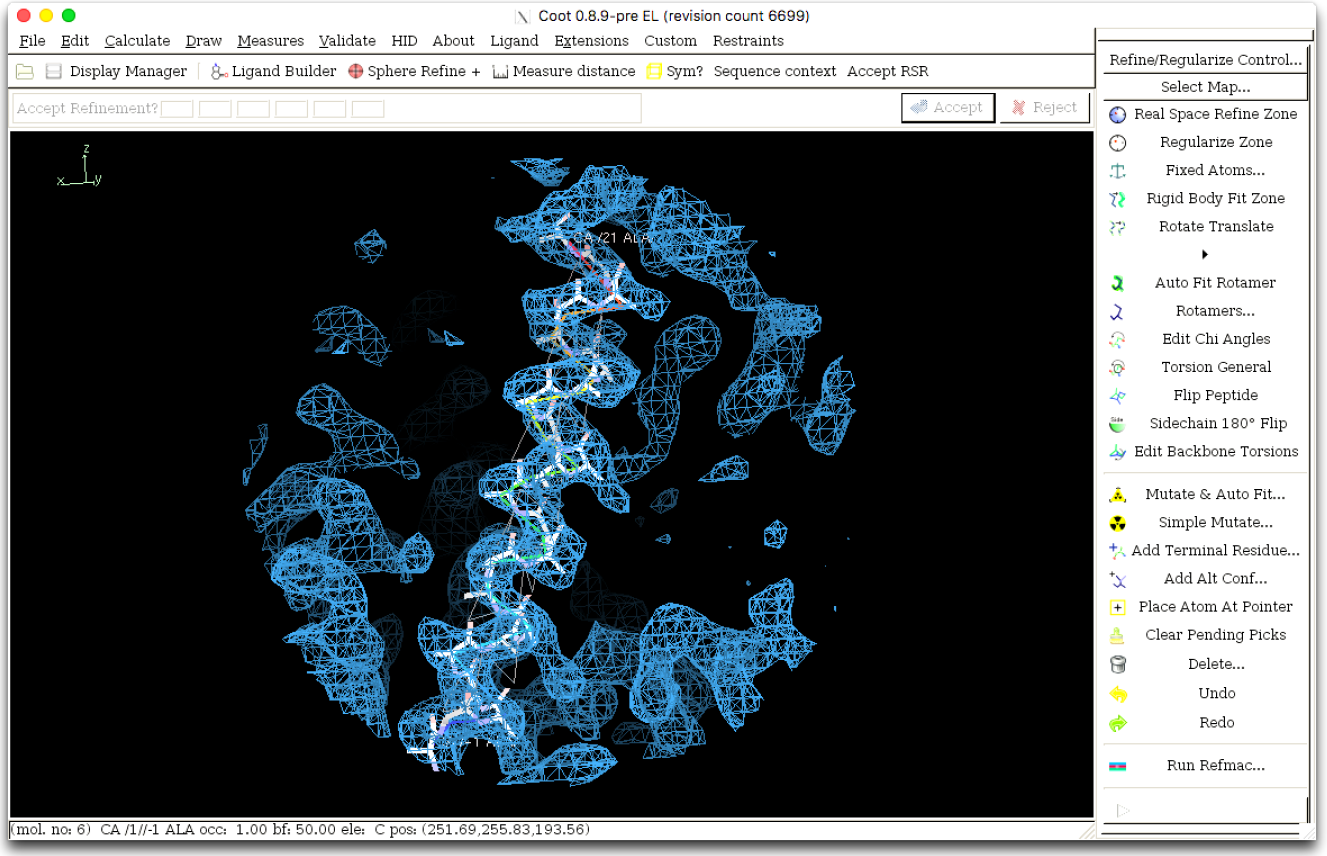

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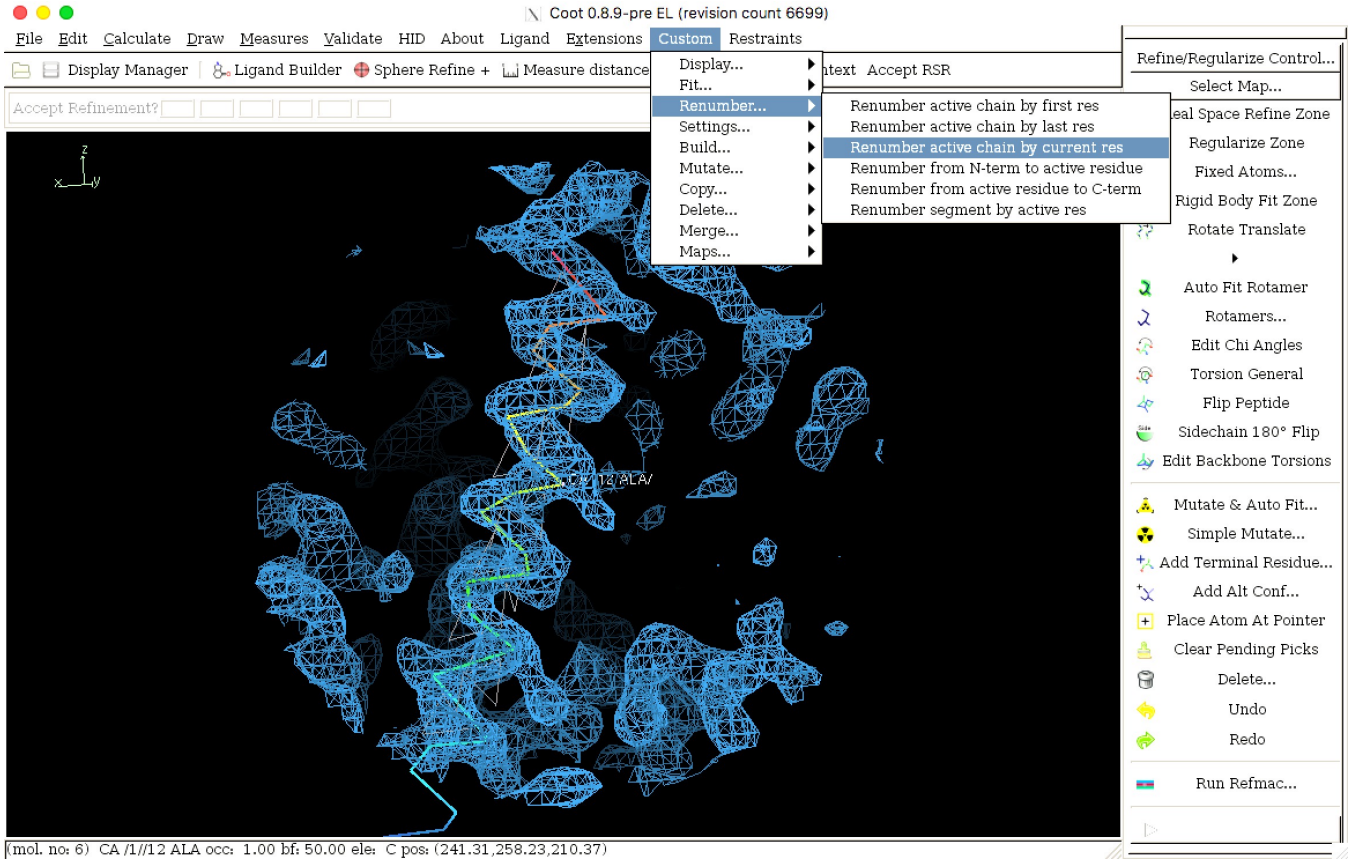
- Mutate to match sequence
- Fill sidechains manually.
- Adjust sequence register to optimize local fit to sidechain densities.



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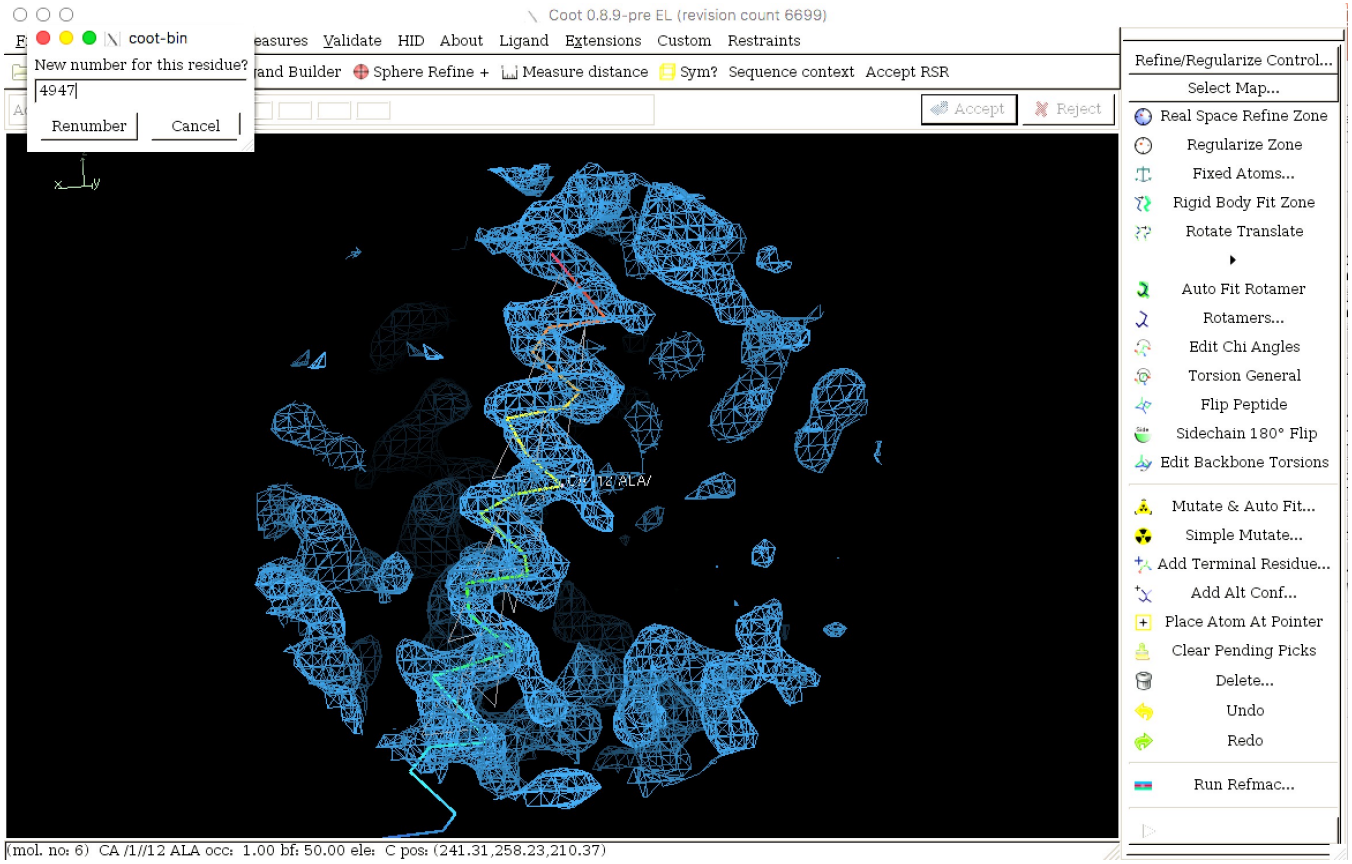

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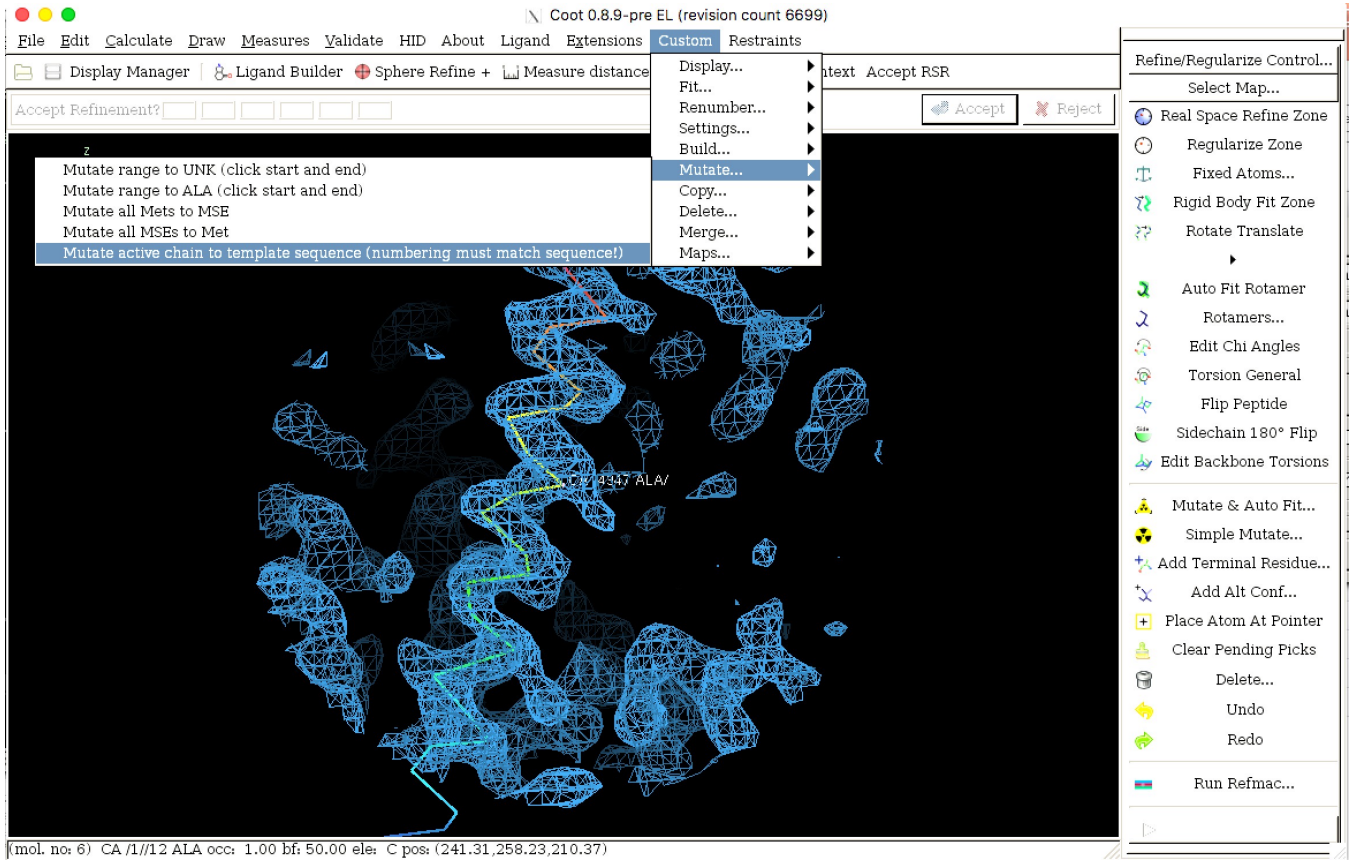

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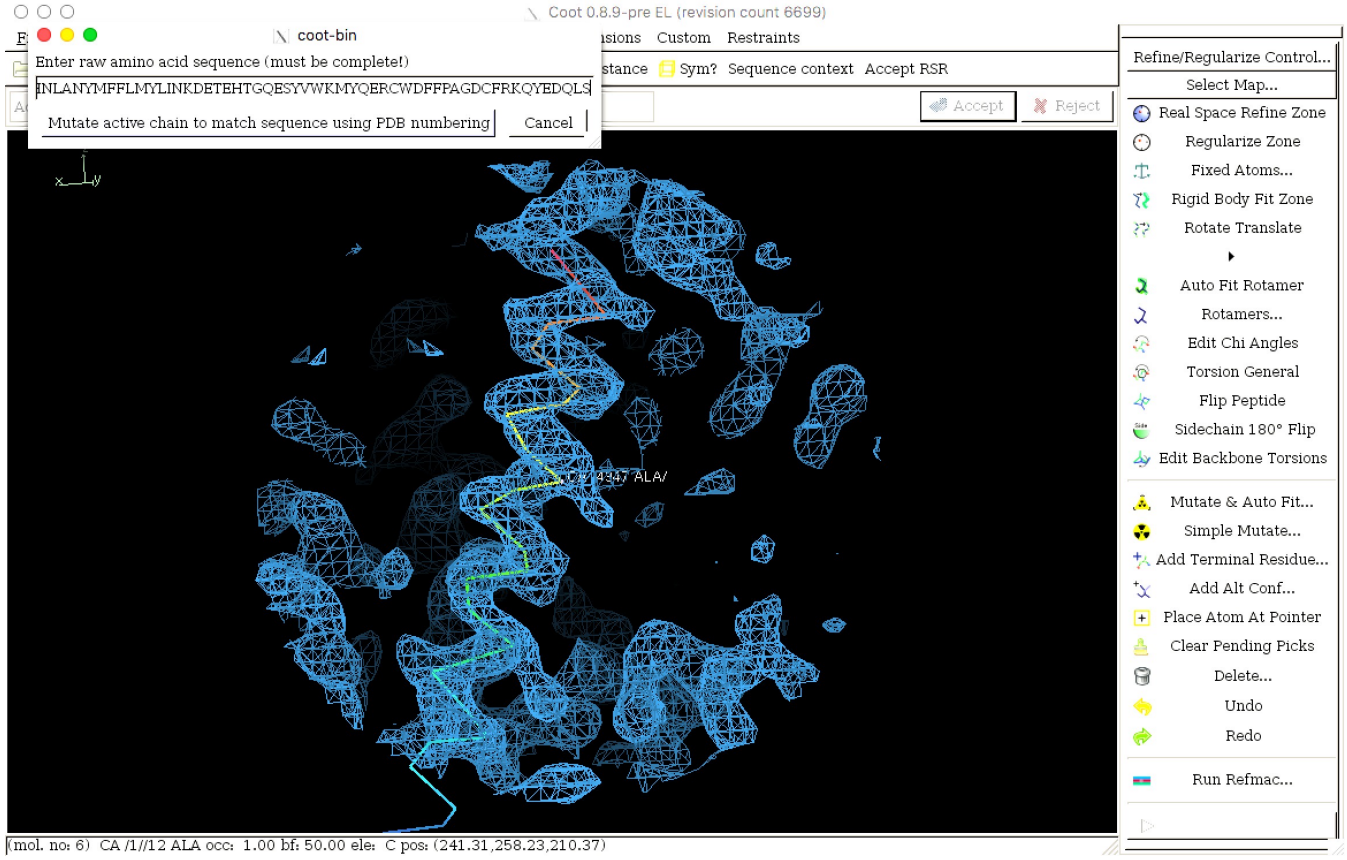

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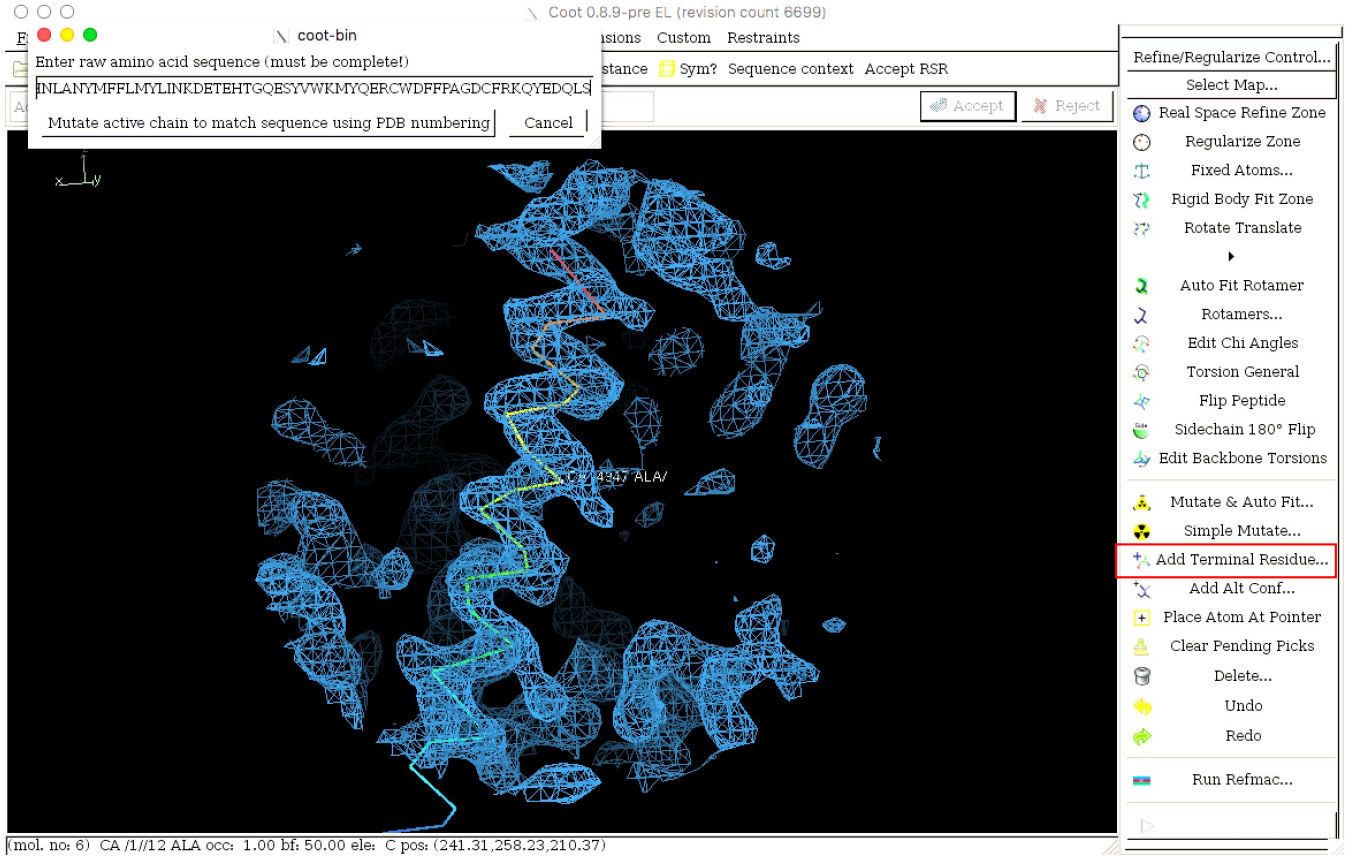
- Fill sidechains manually.
- Adjust sequence register to optimize local fit to sidechain densities.



Use 'Add Terminal residue' to extend chain.

ISOLDE

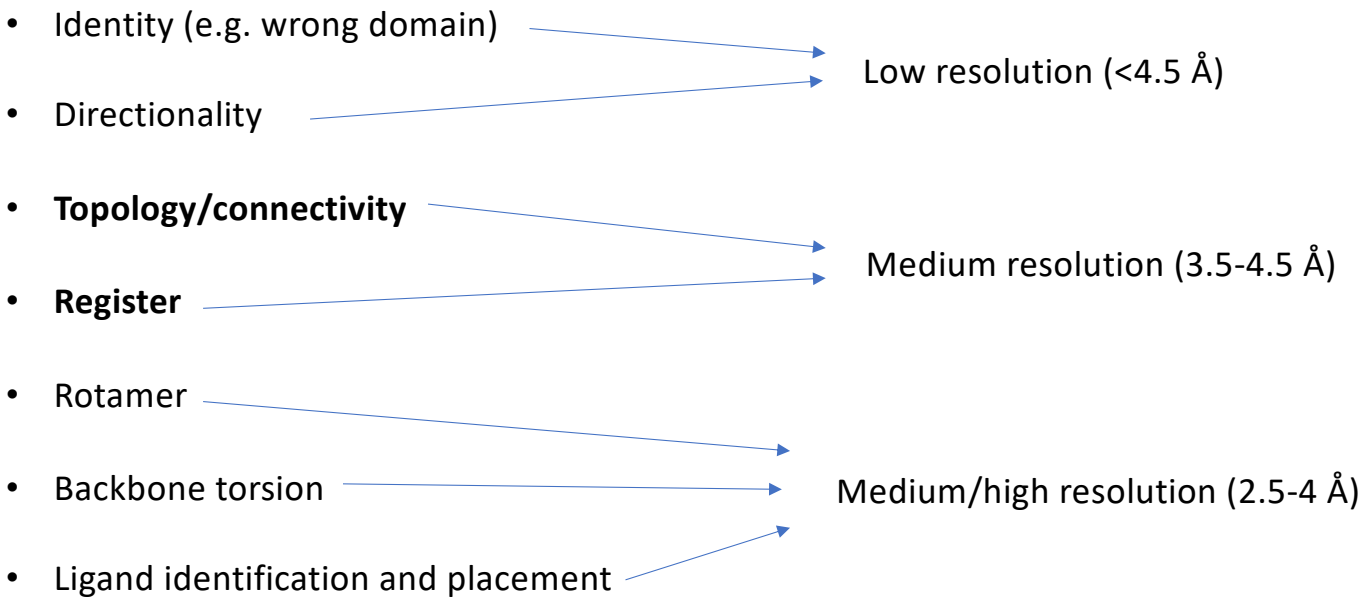
- Interactive molecular dynamics flexible fitting, implemented as plugin for ChimeraX
- Useful during “polishing” stage of generating a final model, identifying and fixing otherwise difficult to correct errors in geometry, non-bonded contacts. Physically realistic simulation guided by map, user input.
- Complementary to COOT – COOT better for de novo building and assembly, ligand placement, ISOLDE very useful for final round of real space fitting.

(Croll, 2018, Acta. Cryst. D)

Types of errors in macromolecular models

- Identity (e.g. wrong domain)
- Directionality
- Topology/connectivity
- Register
- Rotamer
- Backbone torsion
- Ligand identification and placement

Types of errors in macromolecular models



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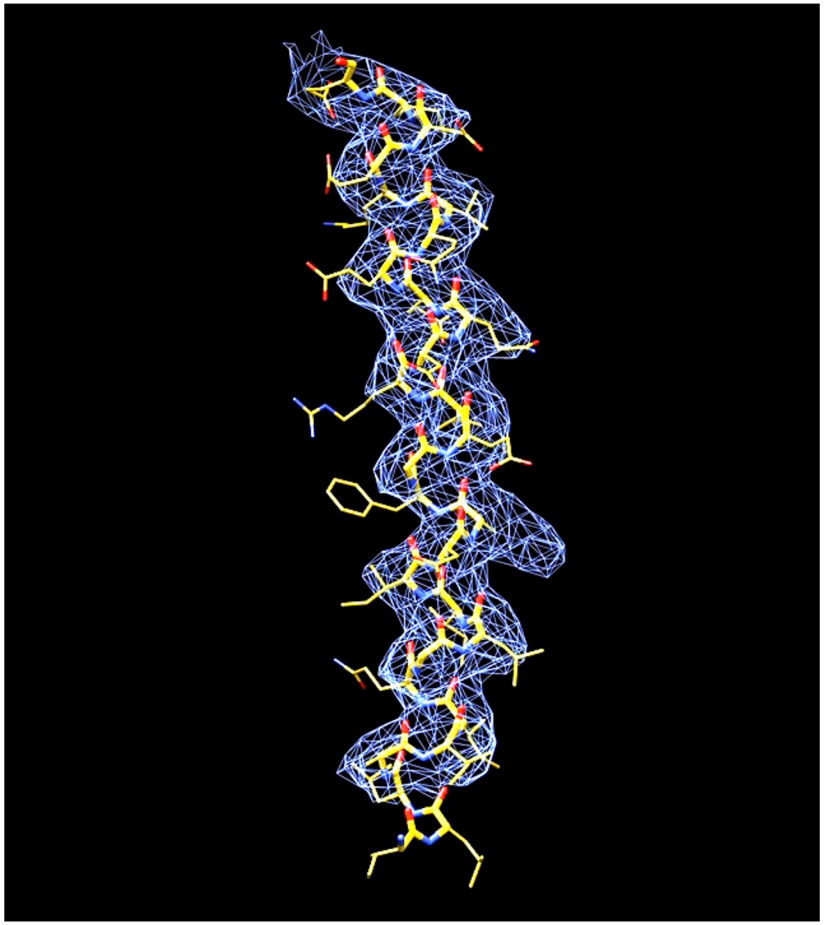

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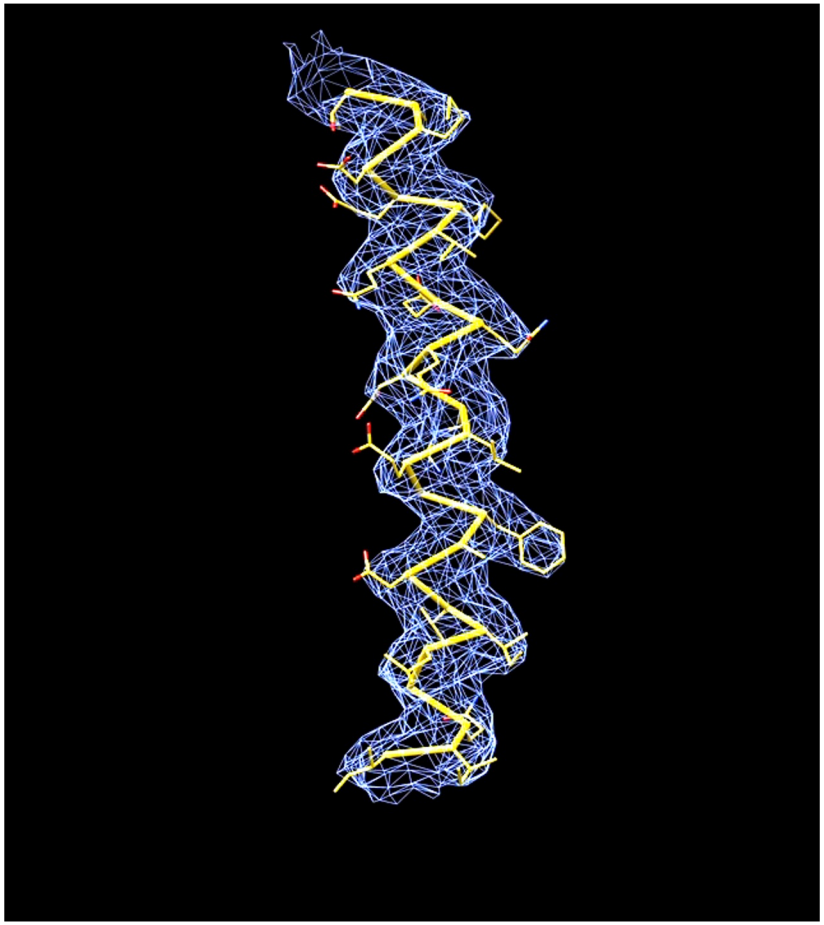


Strategy for identifying and correcting errors.

- Analyse as you go – “sanity checks” on chemistry, nonbonded interactions, surface composition. Use Molprobity for clashes, Chimera or pymol to check e.g. for buried polars, exposed hydrophobics. Monitor agreement with secondary structure, disorder predictions.

- Use EM-ringer (or Q-scores) to identify errors in backbone and rotamer geometry.

- **Look at everything! Manually check and recheck the fit of every residue. Tedious but necessary.**

- Sometimes, you just can’t tell the right answer. Don’t be afraid to specify sequence ambiguity (use UNKs).

- Half-map FSCs are only really useful to analyse overfitting – they tell you little about the local quality or correctness of the model.

Finally…

“ALL MODELS ARE WRONG, BUT SOME ARE USEFUL” – *George P. Box*

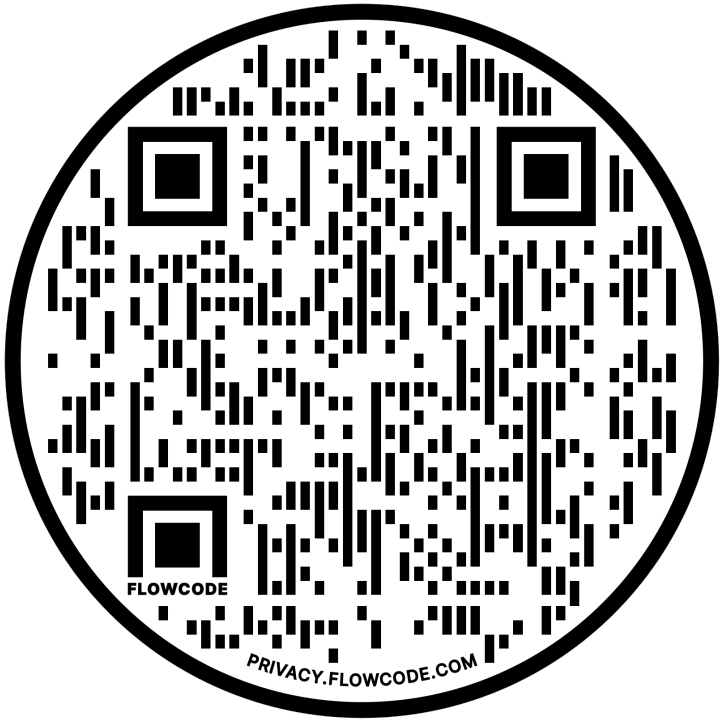
* It should be remembered that just as the Declaration of Independence promises the pursuit of happiness rather than happiness itself, so the iterative scientific model building process offers only the pursuit of the perfect model. For even when we feel we have carried the model building process to a conclusion some new initiative may make further improvement possible. Fortunately to be useful a model does not have to be perfect.

George P. Box, “Robustness in the Strategy of Scientific Model Building”, 1979

Thank you for listening!

COLUMBIA UNIVERSITY
MEDICAL CENTER

Model building tutorial



Tutorial PDF: https://bit.ly/2XPsiox

Data: https://bit.ly/3ASQ41I

AlphaFold add on: https://bit.ly/3KTo6qX