Model building and validation for cryoEM

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“Is my map buildable??”

🤷‍♂️

General principles to keep in mind…

- Be skeptical of your own data – always consider the null hypothesis
  - E.g. particles ***not*** protein, not ***my*** protein, ligand ***not*** bound, etc…

- Test, test, & test again…
  - E.g. do different reconstruction/refinement algorithms give same result?
  - Confirm presence of ligands/subunits using difference maps, labels, mass spec..

- Trust your eyes!
  - If 3Å reconstruction has no sidechains, retrace your steps – postprocessing? Masking? Initial model?
  - Remember that many metrics (e.g. FSC) measure **self-consistency**, not accuracy/reality. No substitute (yet!) for human judgement and careful attention to detail.

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 in **all**, and correction in *most* cases. (generates many fragments which need inspection, correction, merging)

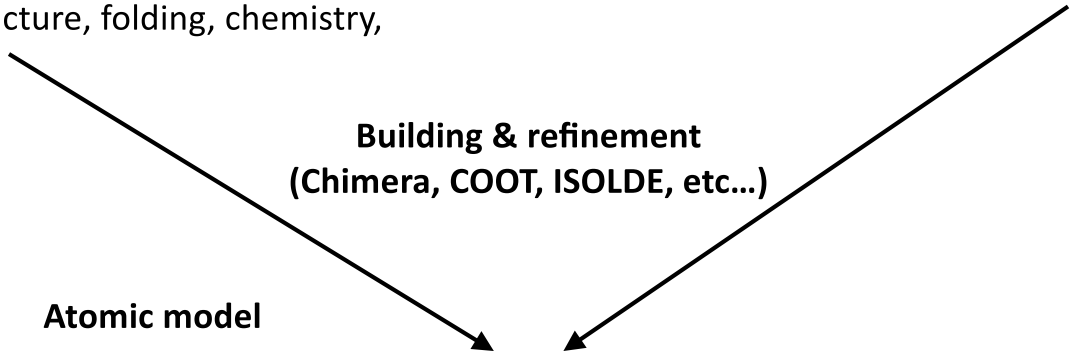
- Tradeoff between available prior knowledge and required resolution for atomic modelling – at the extremes, if a complete crystal structure is already available, 10Å data may be sufficient, while if no sequence/composition data is available even 3Å may not suffice.

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 – AlphaFold, Modelangelo**)
- 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

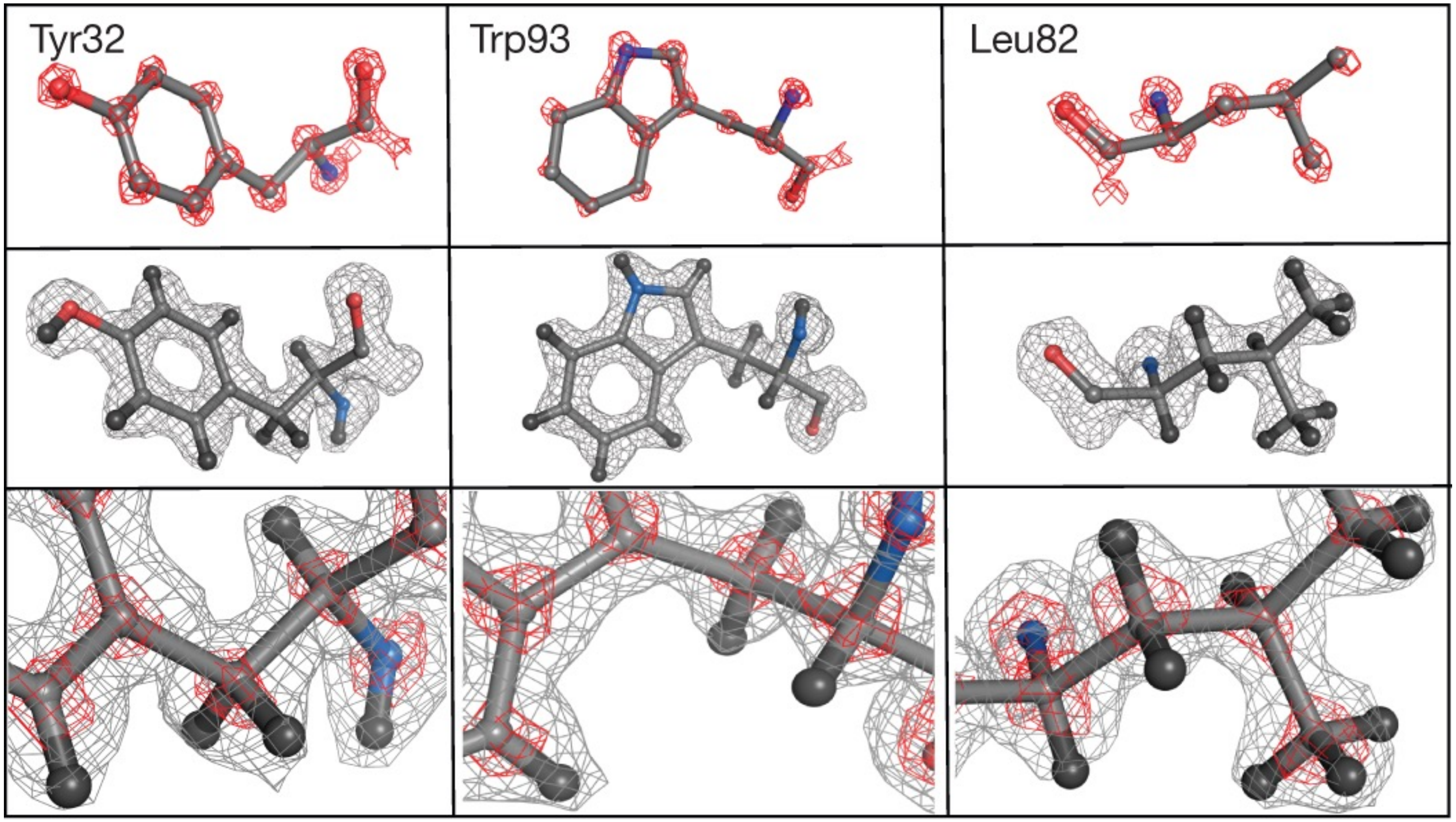


Building & refinement (Chimera, COOT, ISOLDE, etc…)

Atomic model

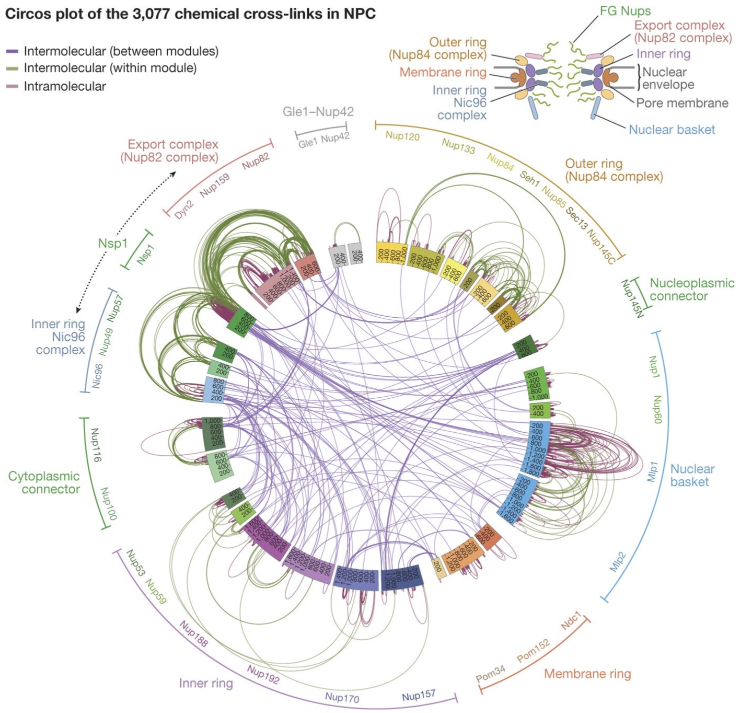
- If possible, unique model (or ensemble) that agrees with both density map and priors
- Otherwise (and per region), specify ambiguity (w/UNK residues and numbering or Ca only model)
- Validation not just (or even mostly) about overfitting.
- Identify, analyse, fix errors.
- Direction and register of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

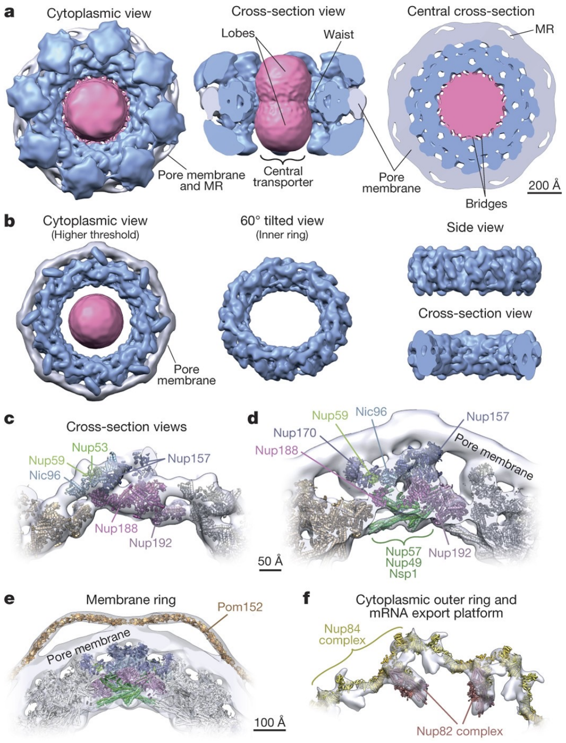
One extreme – at atomic resolution, the position of many atoms can be inferred without prior knowledge of the sequence



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)

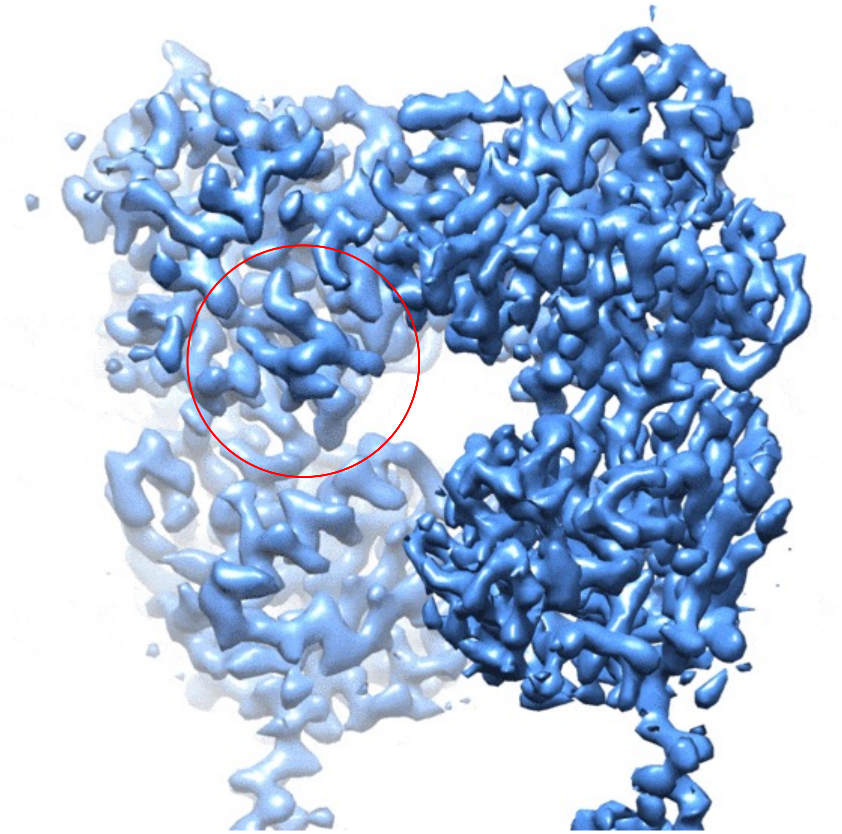
- **Often it is helpful to build using multiple maps**. Assuming 3-3.5Å global res, I would suggest using a map filtered to the global resolution, one filtered to the best local resolution, and one filtered to ~4-4.5 Å (to better visualize connectivity and mobile ligands/lipids).

- 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/EMReady/spIsoNet 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 (additives, constructs, substrates) or altering data collection strategy (tilt). If all else fails, ML-based map improvement using deepEMhancer can improve map interpretability.



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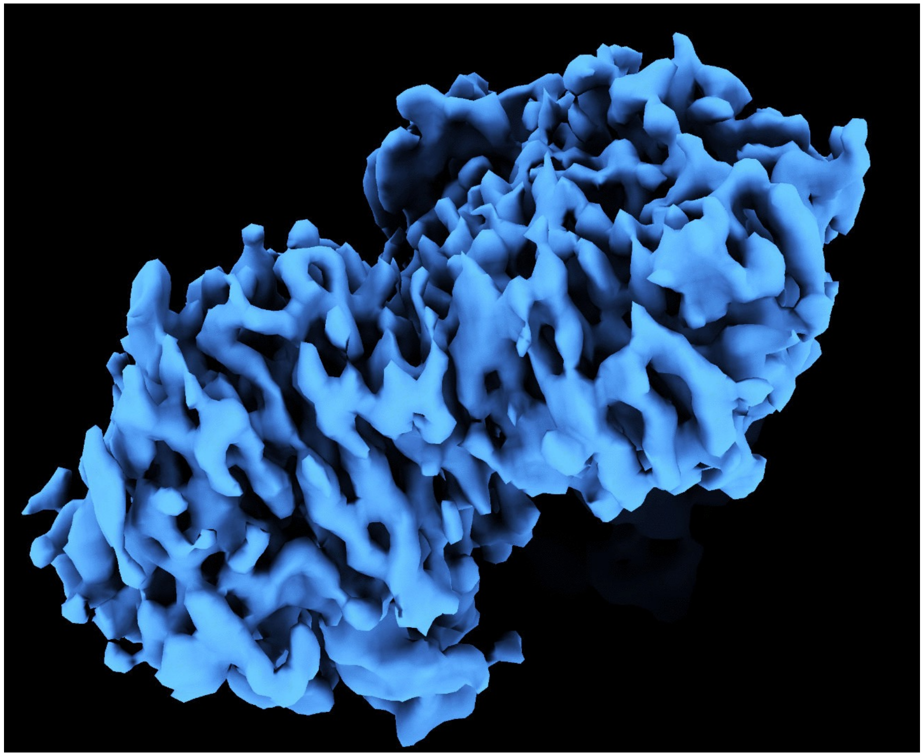
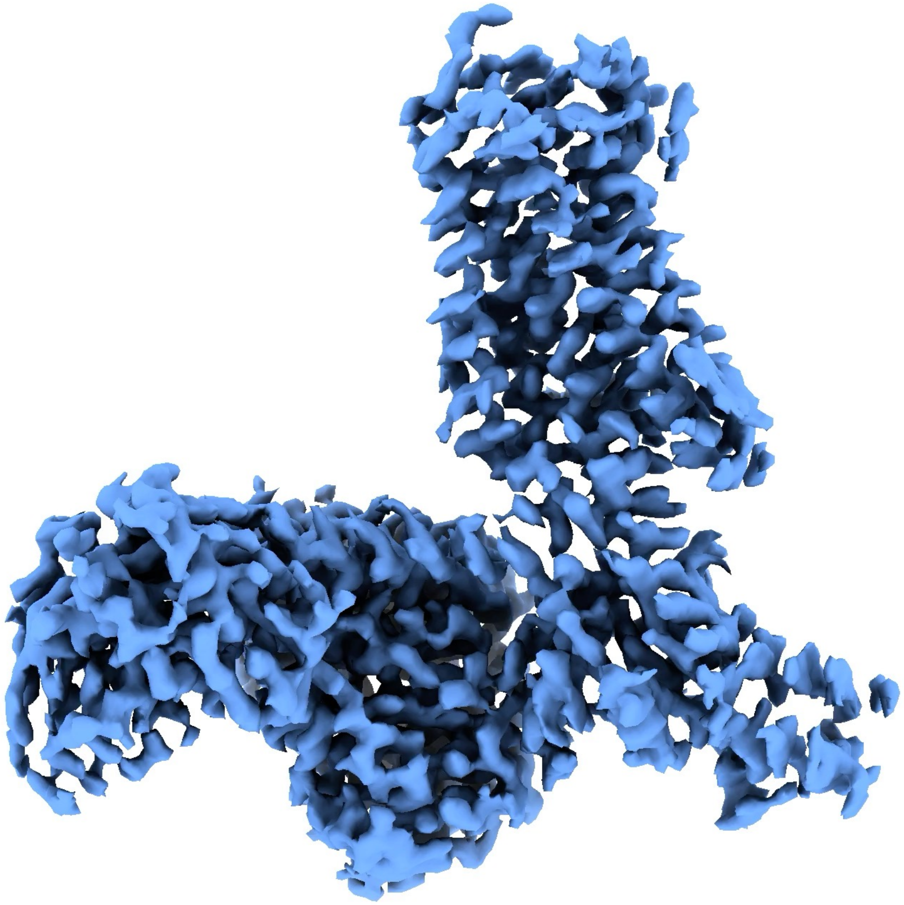
- Also check out new approach from Yifan Cheng’s lab – non-ML based map deconvolution, AR-Decon



DeepEMhancer

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

Example from the literature



Good direction – looks okay

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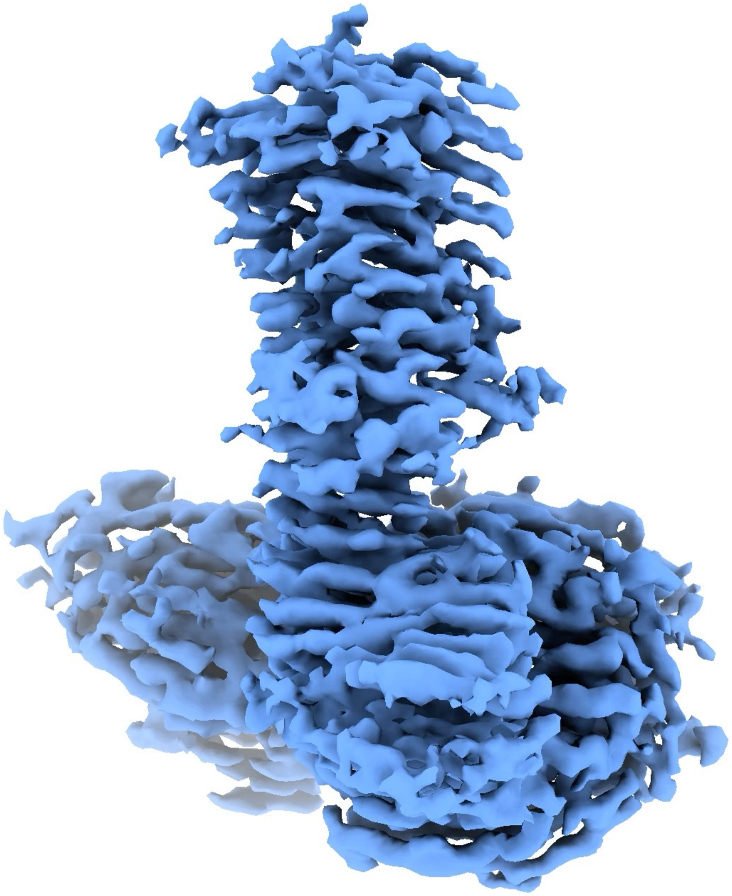

Bad direction – ”ropy”, stretched, hard to interpret

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)
- Then identify and/or generate suitable structural templates for building known domains: Predominantly alphafold, unless a better experimentally determined model is available. Modelangelo also useful for generating initial model, especially if sequence unknown.
- Secondary structure, TM & disorder prediction (XtalPRED for overall summary; specific tools such as SPOT-DISORDER, SPIDER3 for best accuracy).
- Contact prediction from evolutionary couplings: EVFOLD & GREMLIN.
- Conservation analysis: Use favorite MSA algorithm (MUSCLE & CLUSTAL-OMEGA work well; TM-COFFEE, PRALINE-TM useful for membrane proteins) to create a sequence alignment of your protein with a few orthologs; gaps & insertions most commonly occur in loops/disordered regions. Useful as a guide during building.

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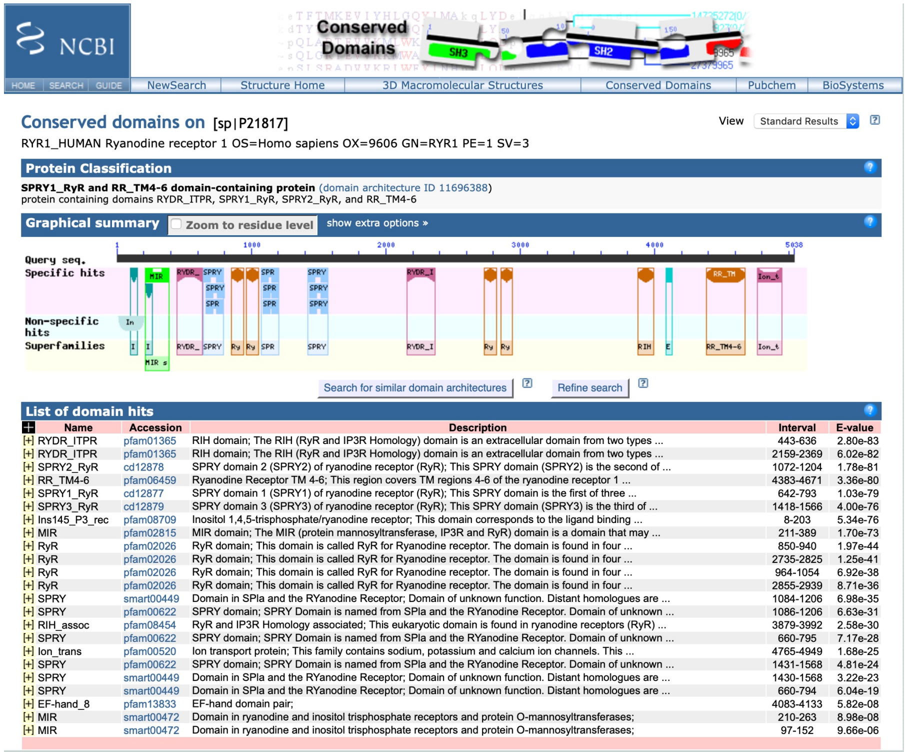

Conserved domains on [sp|P21817]

View Standard Results

RYR1_HUMAN Ryanodine receptor 1 OS=Homo sapiens OX=9606 GN=RYR1 PE=1 SV=3

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SPRY1_RyR and RR_TM4-6 domain-containing protein (domain architecture ID 11696388)
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Graphical summary ☐ Zoom to residue level show extra options »

Search for similar domain architectures

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TED (The Encyclopedia of Domains) also a good option for this.

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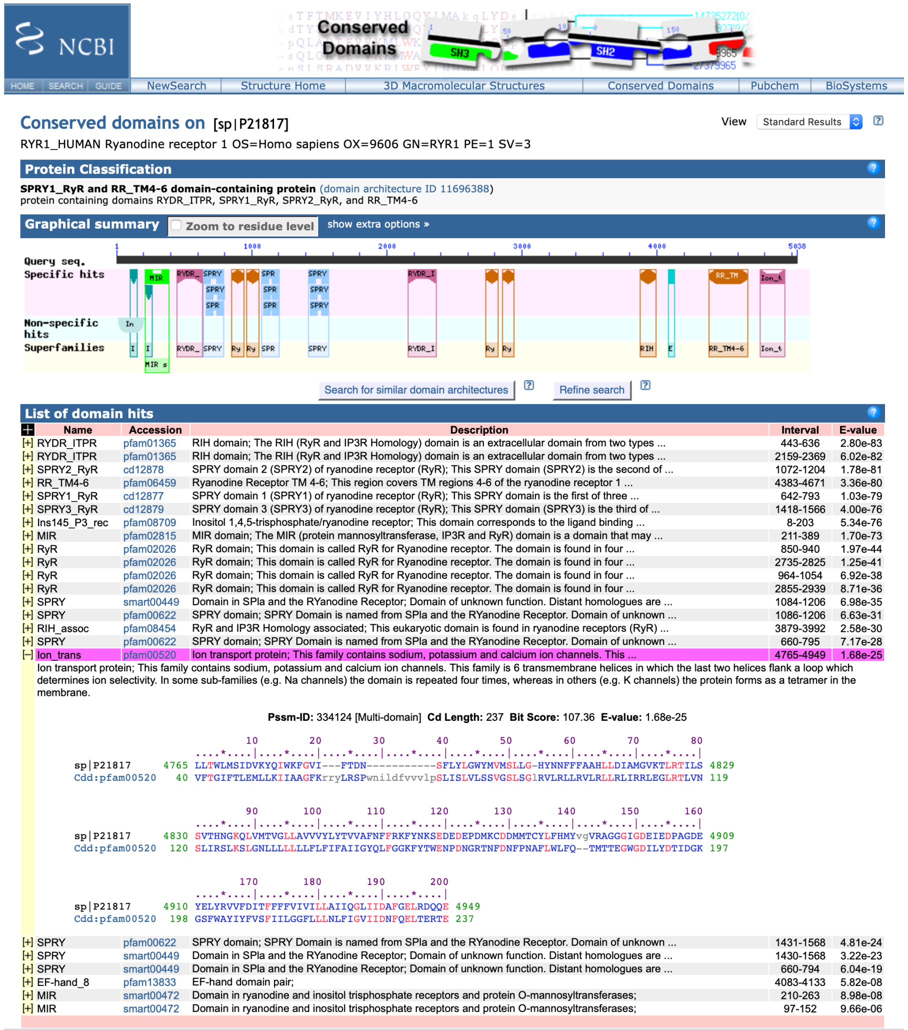

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Pssm-ID: 334124 [Multi-domain] **Cd Length:** 237 **Bit Score:** 107.36 **E-value:** 1.68e-25

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                         90       100       110       120       130       140       150       160
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Cdd:pfam00520  120 SLIRSLKSLGNLLLLLLLFLFIFAIIGYQLFGGKFYTWENPDNGRTNFDNFPNAFLWLFQ--TMTTEGWGDILYDTIDGK 197

                        170       180       190       200
                 ....*....|....*....|....*....|....*....|
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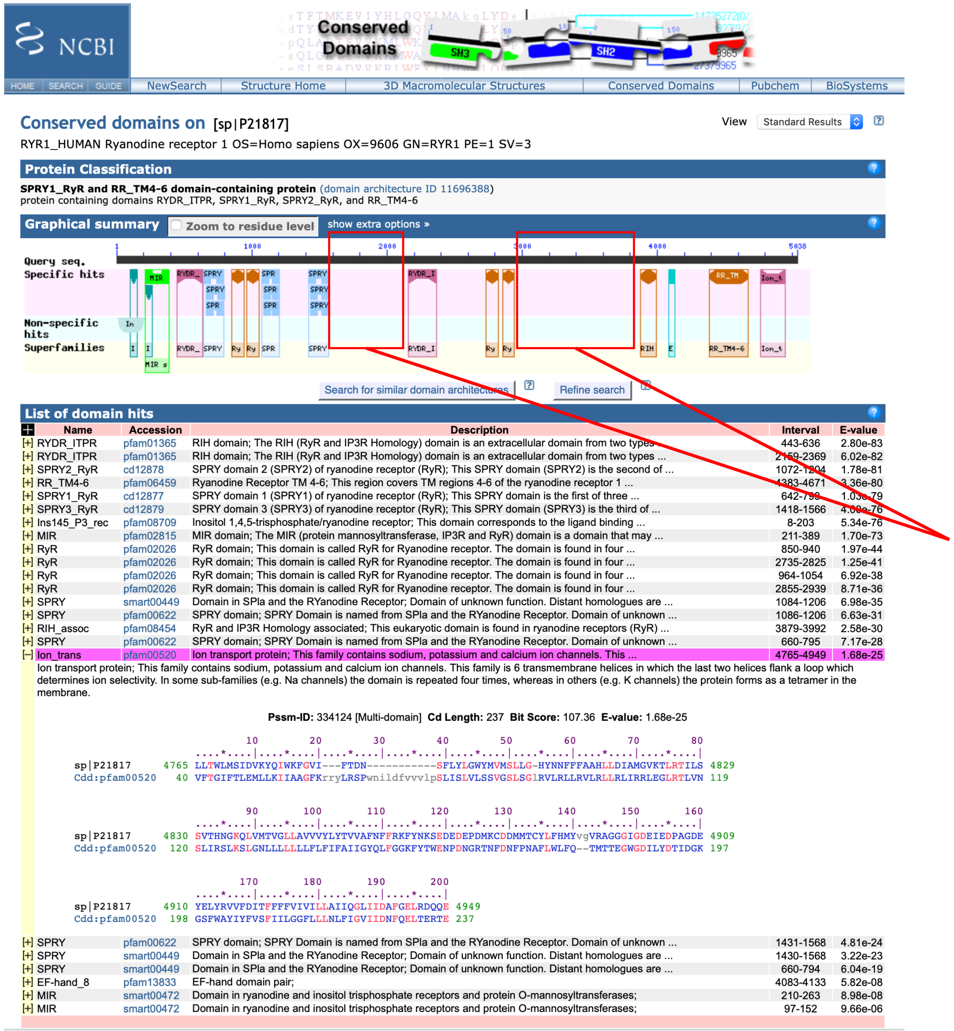

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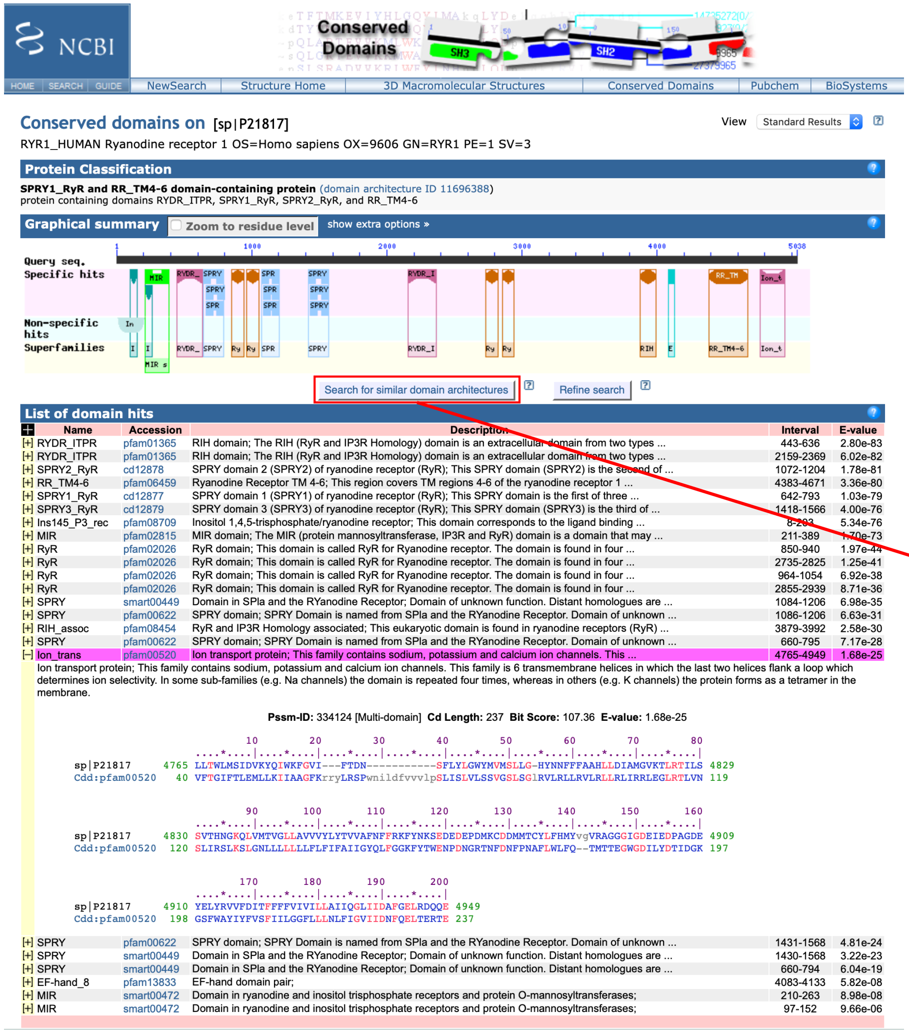
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
sp|P21817      4830 SVTHNGKQLVMTVGLLAVVVYLYTVVAFNFFRKFYNKSEDEDEPDMKCDDMMTCYLFHMYvgVRAGGGIGDEIEDPAGDE 4909
Cdd:pfam00520   120 SLIRSLKSLGNLLLLLLLFLFIFAIIGYQLFGGKFYTWENPDNGRTNFDNFPNAFLWLFQ--TMTTEGWGDILYDTIDGK 197

                           170       180       190       200
                    ....*....|....*....|....*....|....*....|
sp|P21817      4910 YELYRVVFDITFFFFVIVILLAIIQGLIIDAFGELRDQQE 4949
Cdd:pfam00520   198 GSFWAYIYFVSFIILGGFLLLNLFIGVIIDNFQELTERTE 237
```

	Name	Accession	Description	Interval	E-value
[+]	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

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

CDD provides a guide to domain level architecture, including sequence alignments & representative structures.



Conserved domains on [sp|P21817]

RYR1_HUMAN Ryanodine receptor 1 OS=Homo sapiens OX=9606 GN=RYR1 PE=1 SV=3

Protein Classification

SPRY1_RyR and RR_TM4-6 domain-containing protein (domain architecture ID 11696388)
protein containing domains RYDR_ITPR, SPRY1_RyR, SPRY2_RyR, and RR_TM4-6

List of domain hits

	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

Ion transport protein; This family contains sodium, potassium and calcium ion channels. This family is 6 transmembrane helices in which the last two helices flank a loop which determines ion selectivity. In some sub-families (e.g. Na channels) the domain is repeated four times, whereas in others (e.g. K channels) the protein forms as a tetramer in the membrane.

Pssm-ID: 334124 [Multi-domain] **Cd Length:** 237 **Bit Score:** 107.36 **E-value:** 1.68e-25

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                              10        20        30        40        50        60        70        80
                      ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
sp|P21817        4765 LLTWLMSIDVKYQIWKFGVI---FTDN-----------SFLYLGWYMVMSLLG-HYNNFFFAAHLLDIAMGVKTLRTILS 4829
Cdd:pfam00520      40 VFTGIFTLEMLLKIIAAGFKrryLRSPwnildfvvvlpSLISLVLSSVGSLSGlRVLRLLRVLRLLRLIRRLEGLRTLVN 119

                              90       100       110       120       130       140       150       160
                      ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
sp|P21817        4830 SVTHNGKQLVMTVGLLAVVVYLYTVVAFNFFRKFYNKSEDEDEPDMKCDDMMTCYLFHMYvgVRAGGGIGDEIEDPAGDE 4909
Cdd:pfam00520     120 SLIRSLKSLGNLLLLLLLFLFIFAIIGYQLFGGKFYTWENPDNGRTNFDNFPNAFLWLFQ--TMTTEGWGDILYDTIDGK 197

                             170       180       190       200
                      ....*....|....*....|....*....|....*....|
sp|P21817        4910 YELYRVVFDITFFFFVIVILLAIIQGLIIDAFGELRDQQE 4949
Cdd:pfam00520     198 GSFWAYIYFVSFIILGGFLLLNLFIGVIIDNFQELTERTE 237
```

	Name	Accession	Description	Interval	E-value
[+]	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
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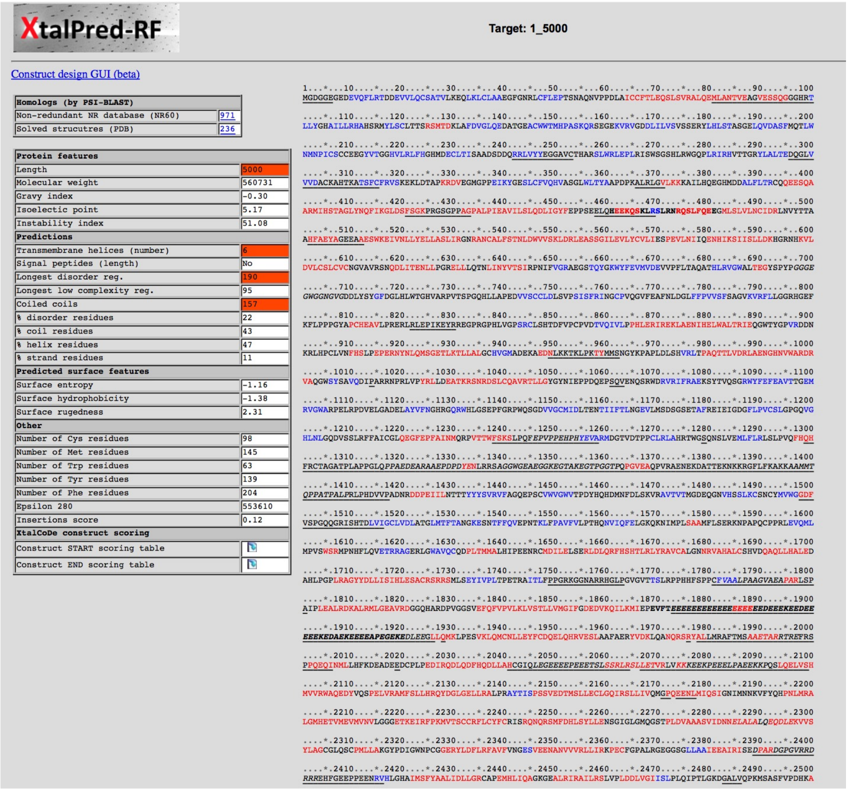
[+]	MIR	smart00472	Domain in ryanodine and inositol trisphosphate receptors and protein O-mannosyltransferases;	210-263	8.98e-08
[+]	MIR	smart00472	Domain in ryanodine and inositol trisphosphate receptors and protein O-mannosyltransferases;	97-152	9.66e-06

Useful for identifying related proteins – which one can then investigate further using Alphafold.

XtalPRED is a great tool for summarizing predicted sequence properties.



XtalPred-RF

Target: 1_5000

Construct design GUI (beta)

Homologs (by PSI-BLAST)	
Non-redundant NR database (NR60)	971
Solved strucutres (PDB)	236

Protein features	
Length	5000
Molecular weight	560731
Gravy index	-0.30
Isoelectic point	5.17
Instability index	51.08
Predictions	
Transmembrane helices (number)	6
Signal peptides (length)	No
Longest disorder reg.	190
Longest low complexity reg.	95
Coiled coils	157
% disorder residues	22
% coil residues	43
% helix residues	47
% strand residues	11
Predicted surface features	
Surface entropy	-1.16
Surface hydrophobicity	-1.38
Surface rugedness	2.31
Other	
Number of Cys residues	98
Number of Met residues	145
Number of Trp residues	63
Number of Tyr residues	139
Number of Phe residues	204
Epsilon 280	553610
Insertions score	0.12
XtalCoDe construct scoring	
Construct START scoring table	
Construct END scoring table	

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1...*...10....*...20....*...30....*...40....*...50....*...60....*...70....*...80....*...90....*..100
MGDGGEGEDEVQFLRTDDEVVLQCSATVLKEQLKLCLAAEGFGNRLCFLEPTSNAQNVPPDLAICCFTLEQSLSVRALQEMLANTVEAGVESSQGGGHRT

....*..110....*..120....*..130....*..140....*..150....*..160....*..170....*..180....*..190....*..200
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....*..210....*..220....*..230....*..240....*..250....*..260....*..270....*..280....*..290....*..300
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....*..310....*..320....*..330....*..340....*..350....*..360....*..370....*..380....*..390....*..400
VVDACKAHTKATSFCFRVSKEKLDTAPKRDVEGMGPPEIKYGESLCFVQHVASGLWLTYAAPDPKALRLGVLKKKAILHQEGHMDDALFLTRCQQEESQA

....*..410....*..420....*..430....*..440....*..450....*..460....*..470....*..480....*..490....*..500
ARMIHSTAGLYNQFIKGLDSFSGKPRGSGPPAGPALPIEAVILSLQDLIGYFEPPSEELQHEEKQSKLRSLRNRQSLFQEEGMLSLVLNCIDRLNVYTTA

....*..510....*..520....*..530....*..540....*..550....*..560....*..570....*..580....*..590....*..600
AHFAEYAGEEAAESWKEIVNLLYELLASLIRGNRANCALFSTNLDWVVSKLDRLEASSGILEVLYCVLIESPEVLNIIQENHIKSIISLLDKHGRNHKVL

....*..610....*..620....*..630....*..640....*..650....*..660....*..670....*..680....*..690....*..700
DVLCSLCVCNGVAVRSNQDLITENLLPGRELLLQTNLINYVTSIRPNIFVGRAEGSTQYGKWYFEVMVDEVVPFLTAQATHLRVGWALTEGYSPYPGGGE

....*..710....*..720....*..730....*..740....*..750....*..760....*..770....*..780....*..790....*..800
GWGGNGVGDDLYSYGFDGLHLWTGHVARPVTSPGQHLLAPEDVVSCCLDLSVPSISFRINGCPVQGVFEAFNLDGLFFPVVSFSAGVKVRFLLGGRHGEF

....*..810....*..820....*..830....*..840....*..850....*..860....*..870....*..880....*..890....*..900
KFLPPPGYAPCHEAVLPRERLRLEPIKEYRREGPRGPHLVGPSRCLSHTDFVPCPVDTVQIVLPPHLERIREKLAENIHELWALTRIEQGWTYGPVRDDN

....*..910....*..920....*..930....*..940....*..950....*..960....*..970....*..980....*..990....*.1000
KRLHPCLVNFHSLPEPERNYNLQMSGETLKTLLALGCHVGMADEKAEDNLKKTKLPKTYMMSNGYKPAPLDLSHVRLTPAQTTLVDRLAENGHNVWARDR

....*.1010....*.1020....*.1030....*.1040....*.1050....*.1060....*.1070....*.1080....*.1090....*.1100
VAQGWSYSAVQDIPARRNPRLVPYRLLDEATKRSNRDSLCQAVRTLLGYGYNIEPPDQEPSQVENQSRWDRVRIFRAEKSYTVQSGRWYFEFEAVTTGEM

....*.1110....*.1120....*.1130....*.1140....*.1150....*.1160....*.1170....*.1180....*.1190....*.1200
RVGWARPELRPDVELGADELAYVFNGHRGQRWHLGSEPFGRPWQSGDVVGCMIDLTENTIIFTLNGEVLMSDSGSETAFREIEIGDGFLPVCSLGPGQVG

....*.1210....*.1220....*.1230....*.1240....*.1250....*.1260....*.1270....*.1280....*.1290....*.1300
HLNLGQDVSSLRFFAICGLQEGFEPFAINMQRPVTTWFSKSLPQFEPVPPEHPHYEVARMDGTVDTPPCLRLAHRTWGSQNSLVEMLFLRLSLPVQFHQH

....*.1310....*.1320....*.1330....*.1340....*.1350....*.1360....*.1370....*.1380....*.1390....*.1400
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....*.1410....*.1420....*.1430....*.1440....*.1450....*.1460....*.1470....*.1480....*.1490....*.1500
QPPATPALPRLPHDVVPADNRDDPEIILNTTTYYYSVRVFAGQEPSCVWVGWVTPDYHQHDMNFDLSKVRAVTVTMGDEQGNVHSSLKCSNCYMVWGGDF

....*.1510....*.1520....*.1530....*.1540....*.1550....*.1560....*.1570....*.1580....*.1590....*.1600
VSPGQQGRISHTDLVIGCLVDLATGLMTFTANGKESNTFFQVEPNTKLFPAVFVLPTHQNVIQFELGKQKNIMPLSAAMFLSERKNPAPQCPPRLEVQML

....*.1610....*.1620....*.1630....*.1640....*.1650....*.1660....*.1670....*.1680....*.1690....*.1700
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....*.1710....*.1720....*.1730....*.1740....*.1750....*.1760....*.1770....*.1780....*.1790....*.1800
AHLPGPLRAGYYDLLISIHLESACRSRRSMLSEYIVPLTPETRAITLFPPGRKGGNARRHGLPGVGVTTSLRPPHHFSPPCFVAALPAAGVAEAPARLSP

....*.1810....*.1820....*.1830....*.1840....*.1850....*.1860....*.1870....*.1880....*.1890....*.1900
AIPLEALRDKALRMLGEAVRDGGQHARDPVGGSVEFQFVPVLKLVSTLLVMGIFGDEDVKQILKMIEPEVFTEEEEEEEEEEEEEEEEEEDEEEKEEDEE

....*.1910....*.1920....*.1930....*.1940....*.1950....*.1960....*.1970....*.1980....*.1990....*.2000
EEEKEDAEKEEEEAPEGEKEDLEEGLLQMKLPESVKLQMCNLLEYFCDQELQHRVESLAAFAERYVDKLQANQRSRYALLMRAFTMSAAETARRTREFRS

....*.2010....*.2020....*.2030....*.2040....*.2050....*.2060....*.2070....*.2080....*.2090....*.2100
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....*.2110....*.2120....*.2130....*.2140....*.2150....*.2160....*.2170....*.2180....*.2190....*.2200
MVVRWAQEDYVQSPELVRAMFSLLHRQYDGLGELLRALPRAYTISPSSVEDTMSLLECLGQIRSLLIVQMGPQEENLMIQSIGNIMNNKVFYQHPNLMRA

....*.2210....*.2220....*.2230....*.2240....*.2250....*.2260....*.2270....*.2280....*.2290....*.2300
LGMHETVMEVMVNVLGGGETKEIRFPKMVTSCCRFLCYFCRISRQNQRSMFDHLSYLLENSGIGLGMQGSTPLDVAAASVIDNNELALALQEQDLEKVVS

....*.2310....*.2320....*.2330....*.2340....*.2350....*.2360....*.2370....*.2380....*.2390....*.2400
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....*.2410....*.2420....*.2430....*.2440....*.2450....*.2460....*.2470....*.2480....*.2490....*.2500
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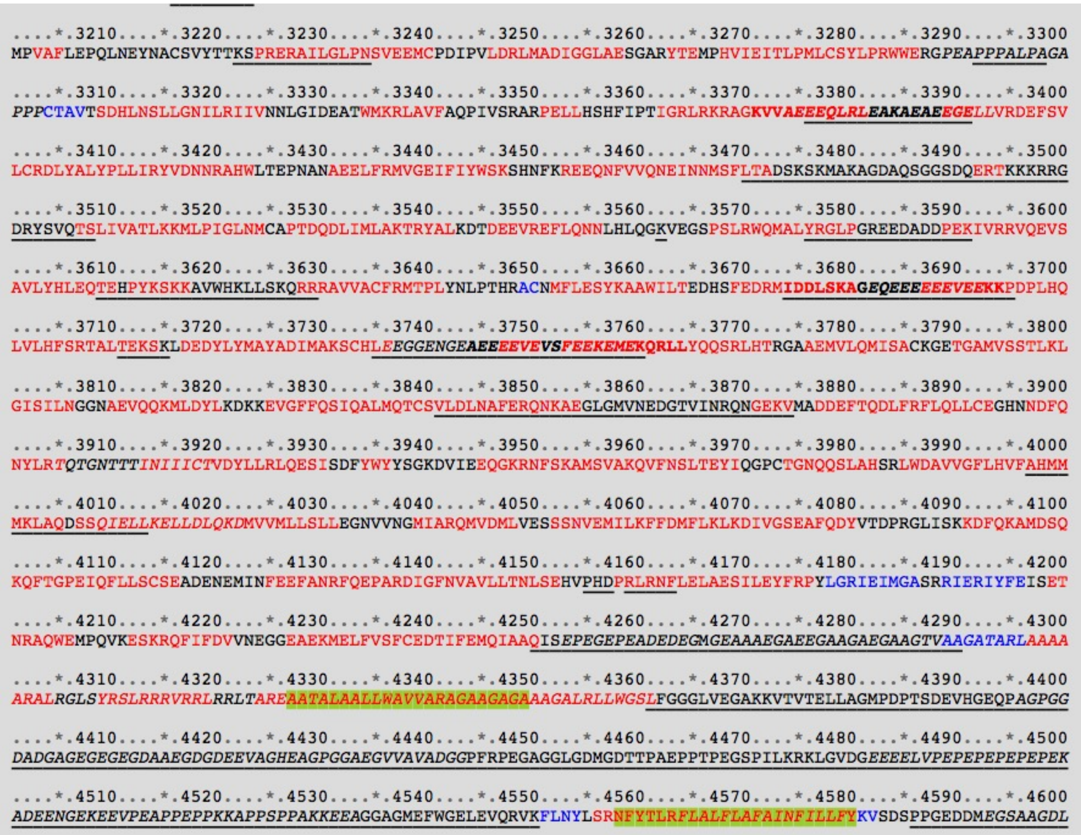
```

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)

Secondary structure prediction is a very useful guide when building.



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....*.3310....*.3320....*.3330....*.3340....*.3350....*.3360....*.3370....*.3380....*.3390....*.3400
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....*.3410....*.3420....*.3430....*.3440....*.3450....*.3460....*.3470....*.3480....*.3490....*.3500
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....*.3510....*.3520....*.3530....*.3540....*.3550....*.3560....*.3570....*.3580....*.3590....*.3600
DRYSVQTSLIVATLKKMLPIGLNMCAPTDQDLIMLAKTRYALKDTDEEVREFLQNNLHLQGKVEGSPSLRWQMALYRGLPGREEDADDPEKIVRRVQEVS

....*.3610....*.3620....*.3630....*.3640....*.3650....*.3660....*.3670....*.3680....*.3690....*.3700
AVLYHLEQTEHPYKSKKAVWHKLLSKQRRRAVVACFRMTPLYNLPTHRACNMFLESYKAAWILTEDHSFEDRMIDDLSKAGEQEEEEEEVEEKKPDPLHQ

....*.3710....*.3720....*.3730....*.3740....*.3750....*.3760....*.3770....*.3780....*.3790....*.3800
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....*.3810....*.3820....*.3830....*.3840....*.3850....*.3860....*.3870....*.3880....*.3890....*.3900
GISILNGGNAEVQQKMLDYLKDKKEVGFFQSIQALMQTCSVLDLNAFERQNKAEGLGMVNEDGTVINRQNGEKVMADDEFTQDLFRFLQLLCEGHNNDFQ

....*.3910....*.3920....*.3930....*.3940....*.3950....*.3960....*.3970....*.3980....*.3990....*.4000
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....*.4010....*.4020....*.4030....*.4040....*.4050....*.4060....*.4070....*.4080....*.4090....*.4100
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....*.4110....*.4120....*.4130....*.4140....*.4150....*.4160....*.4170....*.4180....*.4190....*.4200
KQFTGPEIQFLLSCSEADENEMINFEEFANRFQEPARDIGFNVAVLLTNLSEHVPHDPRLRNFLELAESILEYFRPYLGRIEIMGASRRIERIYFEISET

....*.4210....*.4220....*.4230....*.4240....*.4250....*.4260....*.4270....*.4280....*.4290....*.4300
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....*.4310....*.4320....*.4330....*.4340....*.4350....*.4360....*.4370....*.4380....*.4390....*.4400
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....*.4410....*.4420....*.4430....*.4440....*.4450....*.4460....*.4470....*.4480....*.4490....*.4500
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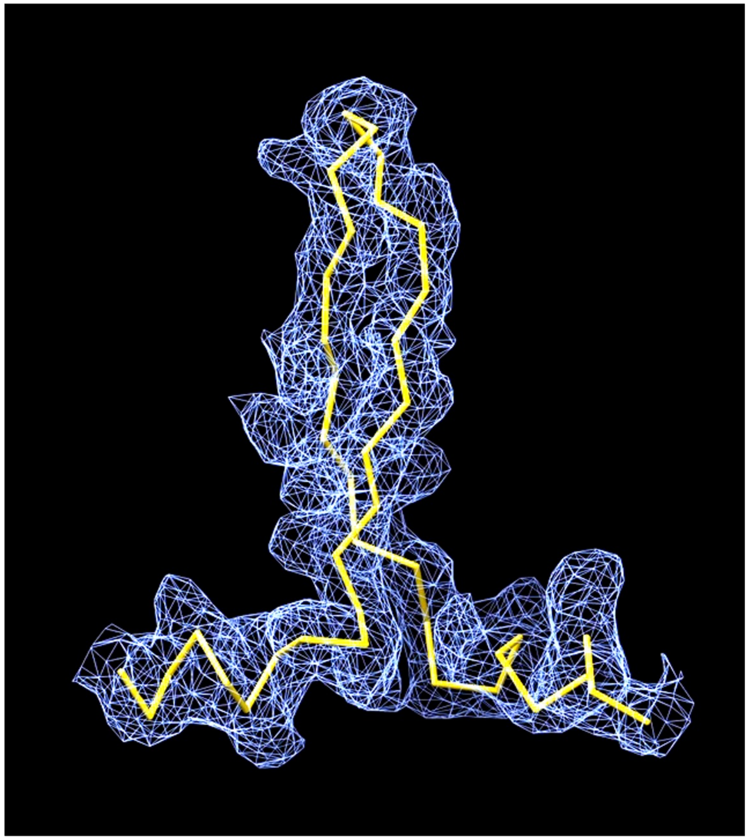
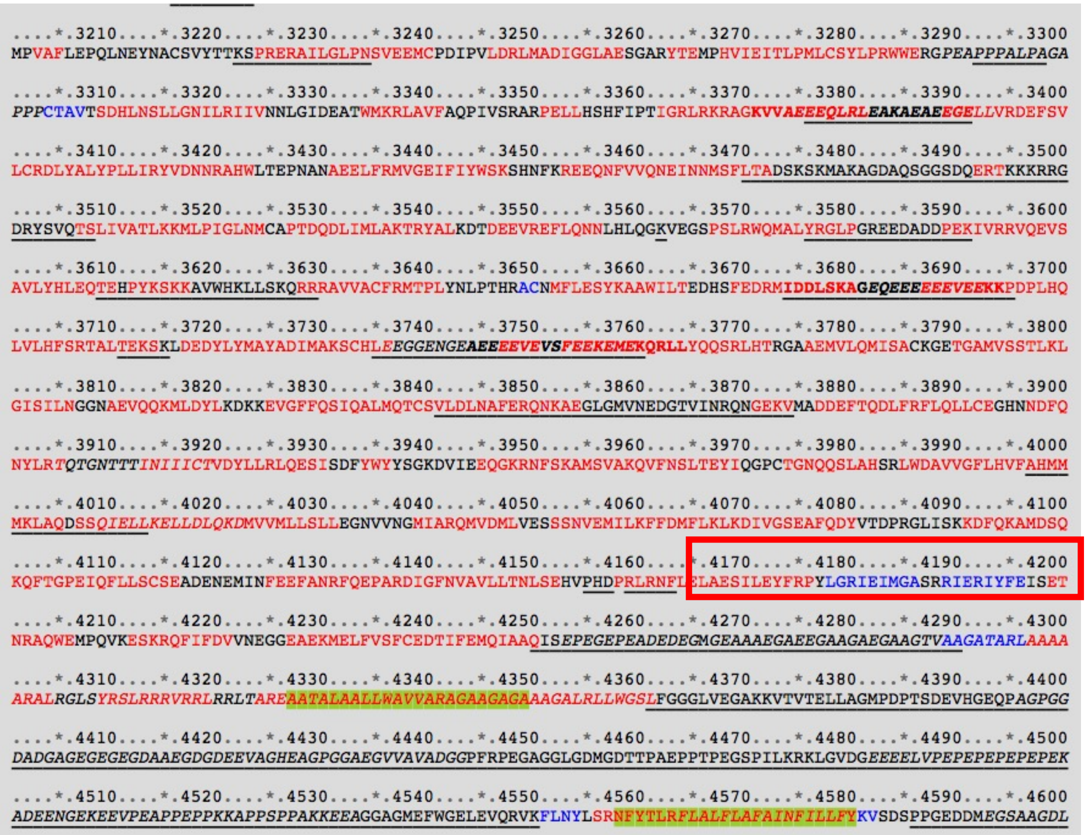


Where is this motif in the sequence?

Secondary structure prediction is a very useful guide when building.



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....*.3210....*.3220....*.3230....*.3240....*.3250....*.3260....*.3270....*.3280....*.3290....*.3300
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....*.3310....*.3320....*.3330....*.3340....*.3350....*.3360....*.3370....*.3380....*.3390....*.3400
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....*.3410....*.3420....*.3430....*.3440....*.3450....*.3460....*.3470....*.3480....*.3490....*.3500
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....*.3510....*.3520....*.3530....*.3540....*.3550....*.3560....*.3570....*.3580....*.3590....*.3600
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....*.3610....*.3620....*.3630....*.3640....*.3650....*.3660....*.3670....*.3680....*.3690....*.3700
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....*.3710....*.3720....*.3730....*.3740....*.3750....*.3760....*.3770....*.3780....*.3790....*.3800
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....*.3810....*.3820....*.3830....*.3840....*.3850....*.3860....*.3870....*.3880....*.3890....*.3900
GISILNGGNAEVQQKMLDYLKDKKEVGFFQSIQALMQTCSVLDLNAFERQNKAEGLGMVNEDGTVINRQNGEKVMADDEFTQDLFRFLQLLCEGHNNDFQ

....*.3910....*.3920....*.3930....*.3940....*.3950....*.3960....*.3970....*.3980....*.3990....*.4000
NYLRTQTGNTTTINIIICTVDYLLRLQESISDFYWYYSGKDVIEEQGKRNFSKAMSVAKQVFNSLTEYIQGPCTGNQQSLAHSRLWDAVVGFLHVFAHMM

....*.4010....*.4020....*.4030....*.4040....*.4050....*.4060....*.4070....*.4080....*.4090....*.4100
MKLAQDSSQIELLKELLDLQKDMVVMLLSLLEGNVVNGMIARQMVDMLVESSSNVEMILKFFDMFLKLKDIVGSEAFQDYVTDPRGLISKKDFQKAMDSQ

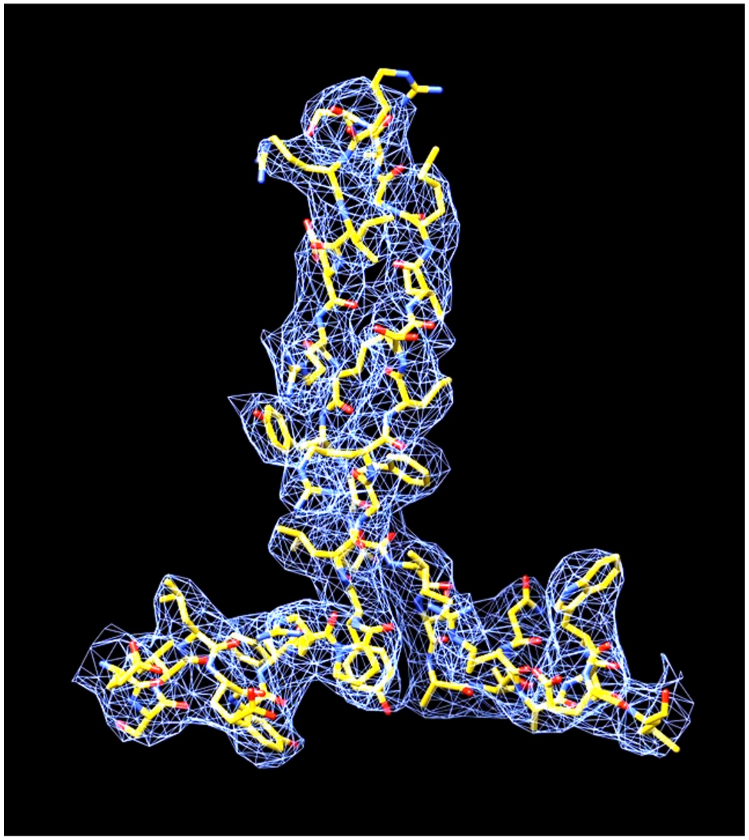
....*.4110....*.4120....*.4130....*.4140....*.4150....*.4160....*.4170....*.4180....*.4190....*.4200
KQFTGPEIQFLLSCSEADENEMINFEEFANRFQEPARDIGFNVAVLLTNLSEHVPHDPRLRNFLELAESILEYFRPYLGRIEIMGASRRIERIYFEISET

....*.4210....*.4220....*.4230....*.4240....*.4250....*.4260....*.4270....*.4280....*.4290....*.4300
NRAQWEMPQVKESKRQFIFDVVNEGGEAEKMELFVSFCEDTIFEMQIAAQISEPEGEPEADEDEGMGEAAAEGAEEGAAGAEGAAGTVAAGATARLAAAA

....*.4310....*.4320....*.4330....*.4340....*.4350....*.4360....*.4370....*.4380....*.4390....*.4400
ARALRGLSYRSLRRRVRRLRRLTAREAATALAALLWAVVARAGAAGAGAAAGALRLLWGSLFGGGLVEGAKKVTVTELLAGMPDPTSDEVHGEQPAGPGG

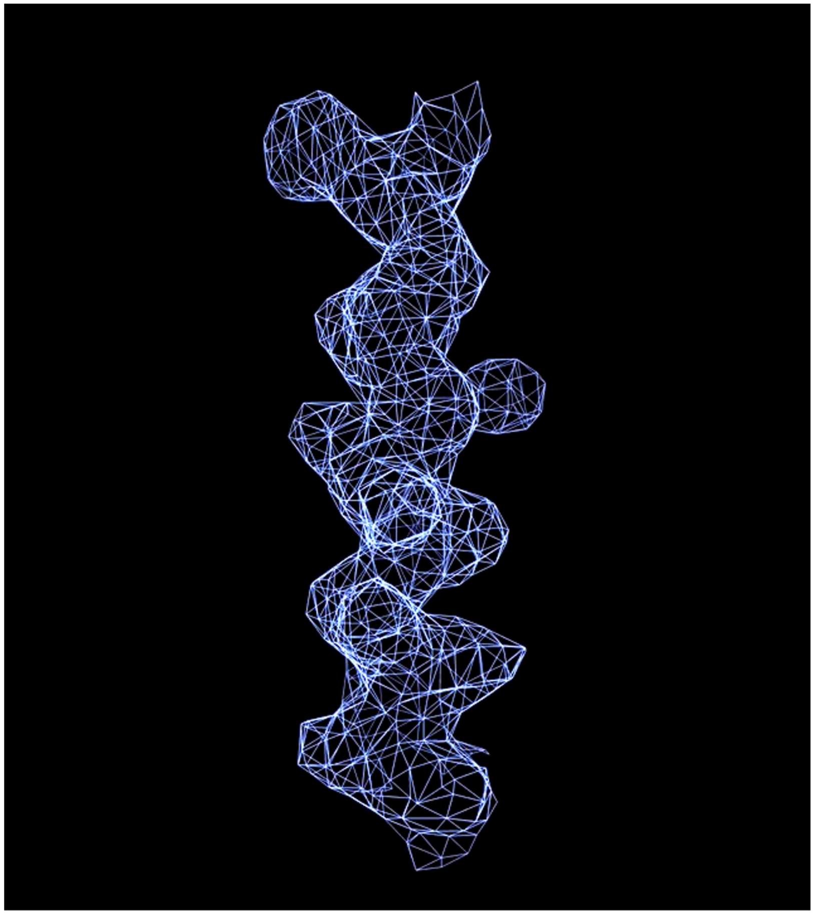
....*.4410....*.4420....*.4430....*.4440....*.4450....*.4460....*.4470....*.4480....*.4490....*.4500
DADGAGEGEGEGDAAEGDGDEEVAGHEAGPGGAEGVVAVADGGPFRPEGAGGLGDMGDTTPAEPPTPEGSPILKRKLGVDGEEEELVPEPEPEPEPEPEK

....*.4510....*.4520....*.4530....*.4540....*.4550....*.4560....*.4570....*.4580....*.4590....*.4600
ADEENGEKEEVPEAPPEPPKKAPPSPPAKKEEAGGAGMEFWGELEVQRVKFLNYLSRNFYTLRFLALFLAFAINFILLFYKVSDSPPGEDDMEGSAAGDL
```

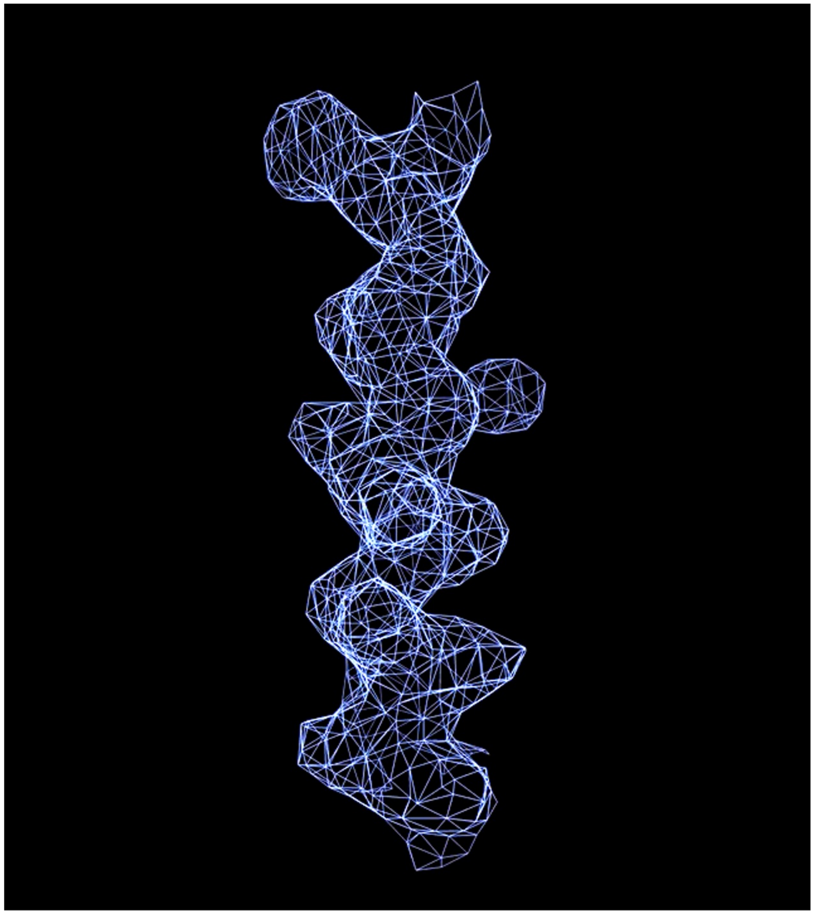


Secondary structure prediction is ~80% accurate. So if your model consistently disagrees with predicted secondary structure, look at it very closely!

What can we learn from the map alone?

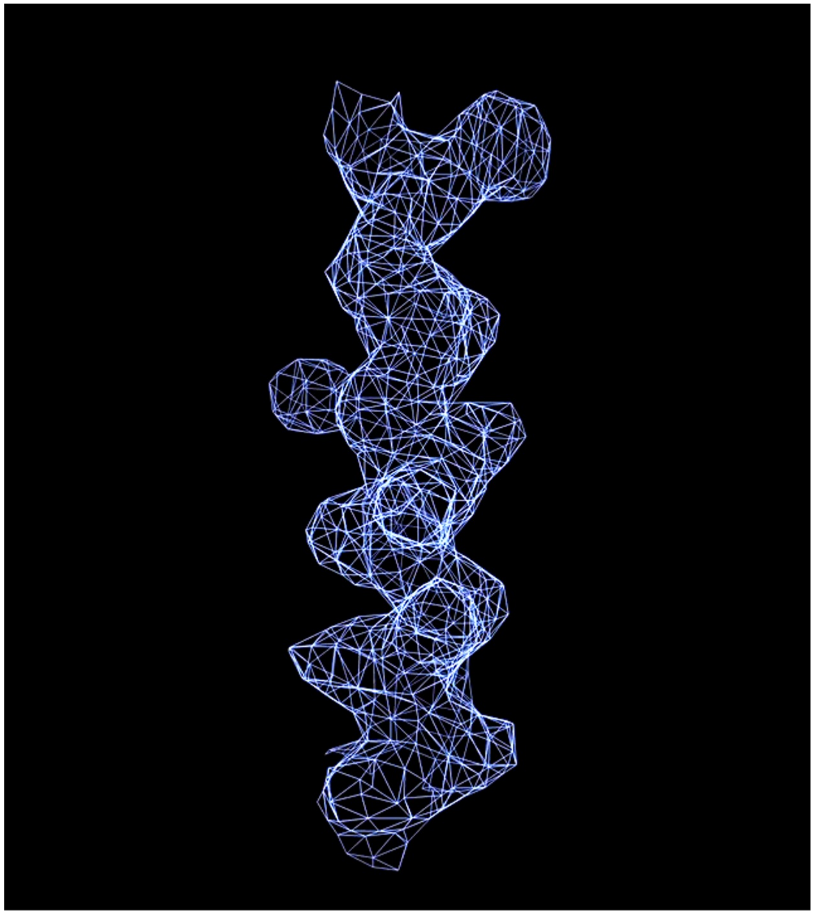


What can we learn from the map alone?

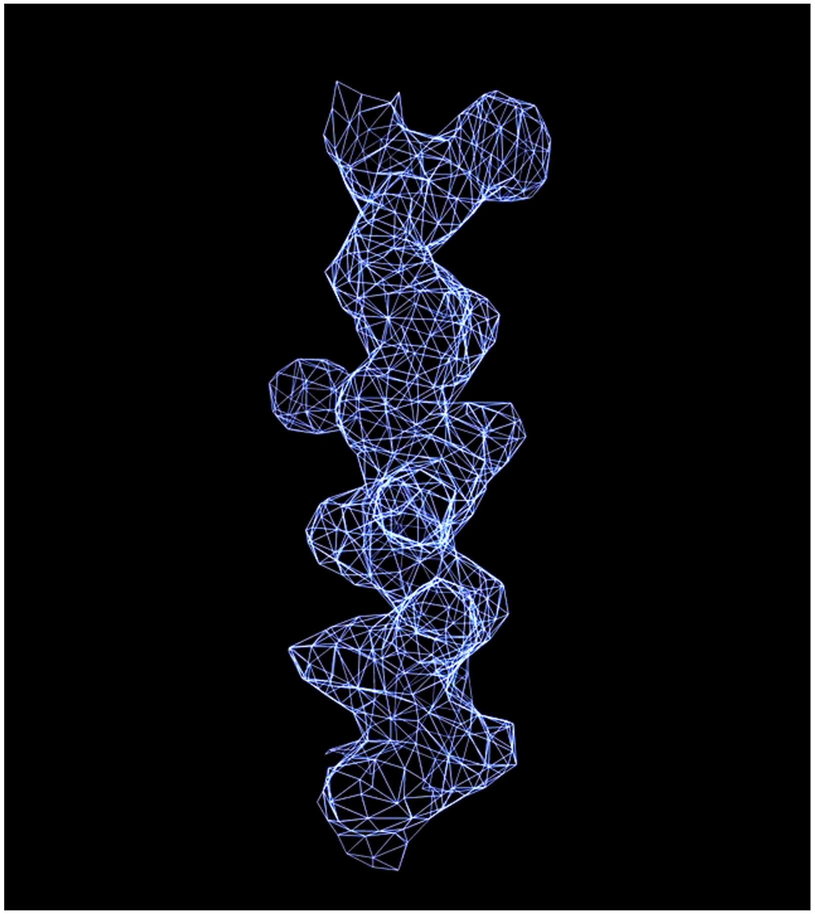


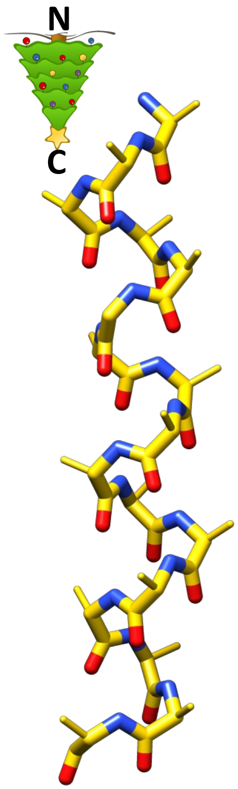
Left handed! Obvious here – can be less clear at lower res, so be careful.

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

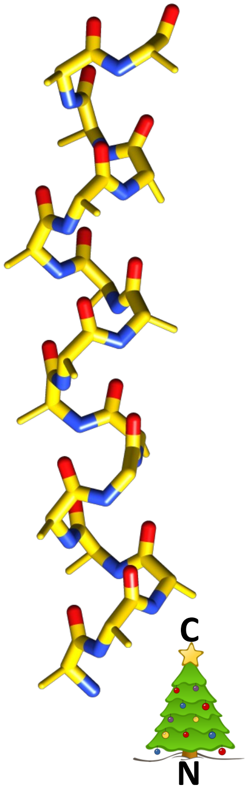


Which direction does the helix point?

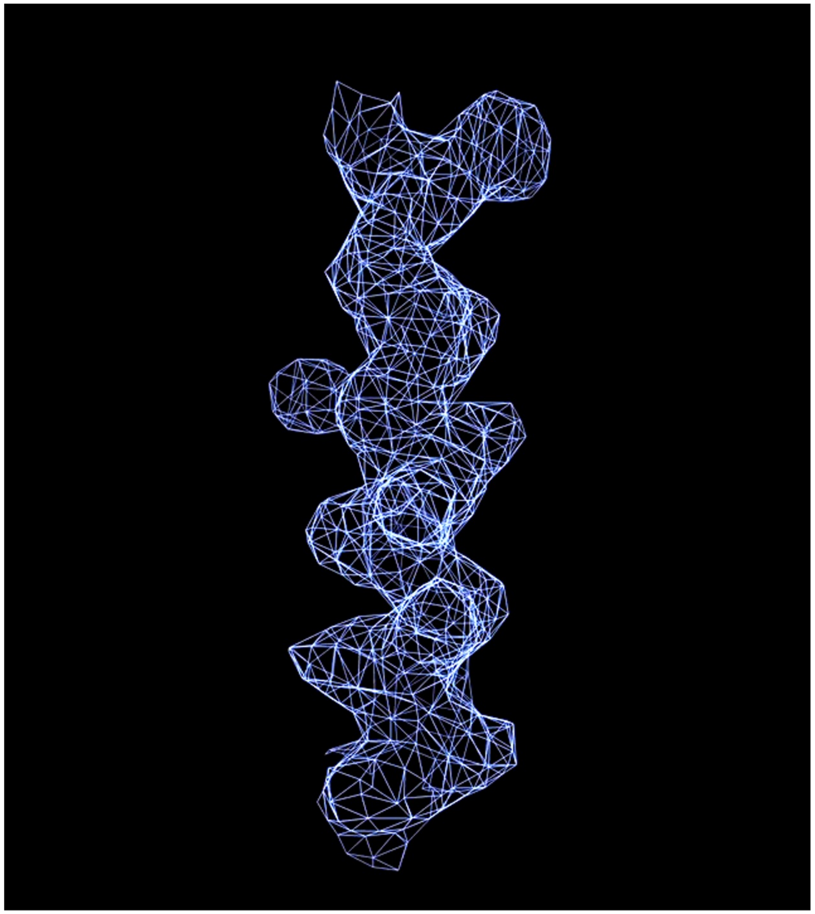


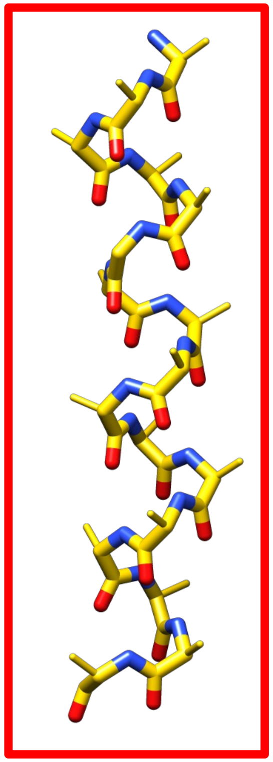


?

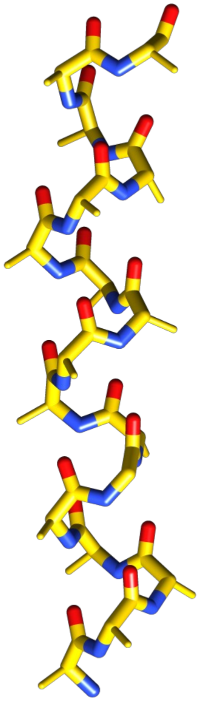


Which direction does the helix point?

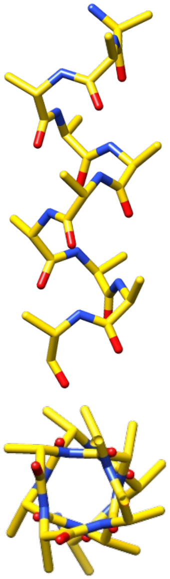




?

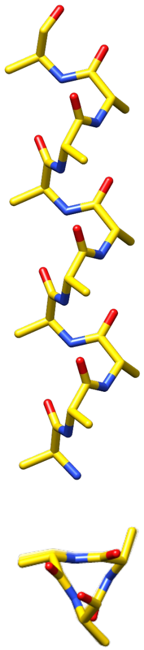


Helices – alpha and 3_{10}



Alpha

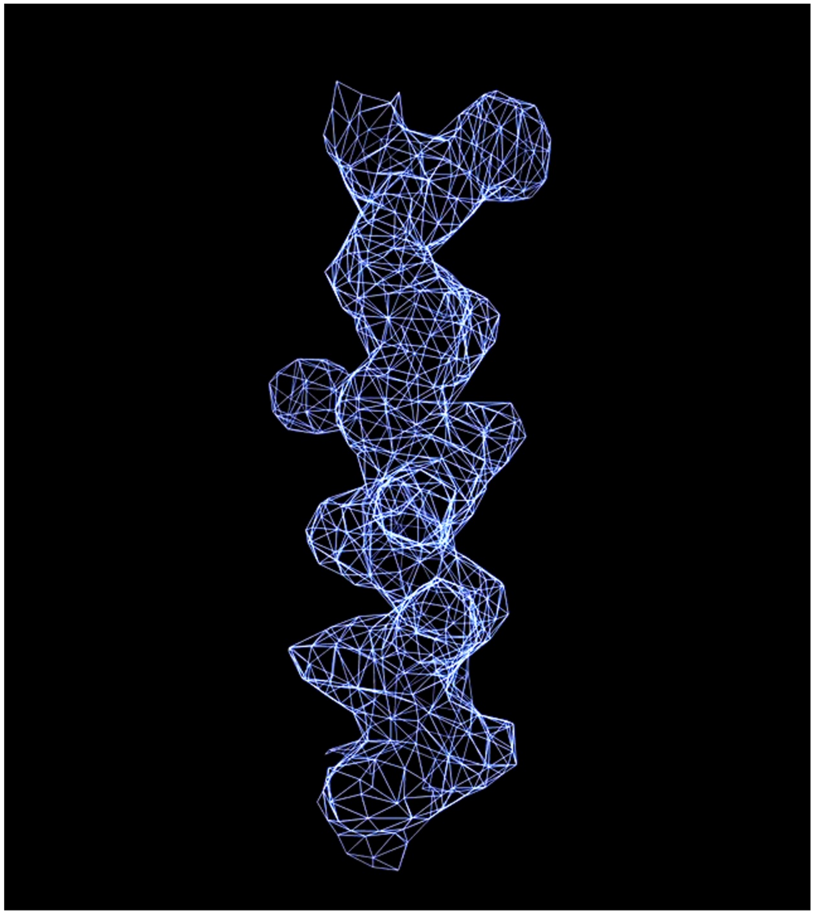
- ~90%
- 3.6 residues per turn
- Fat

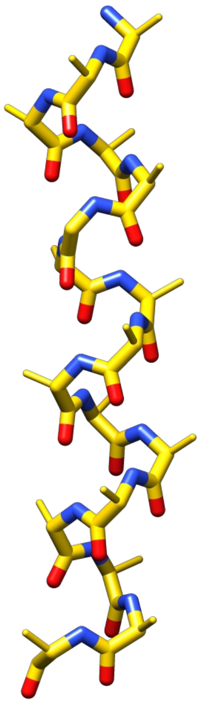


3_{10}

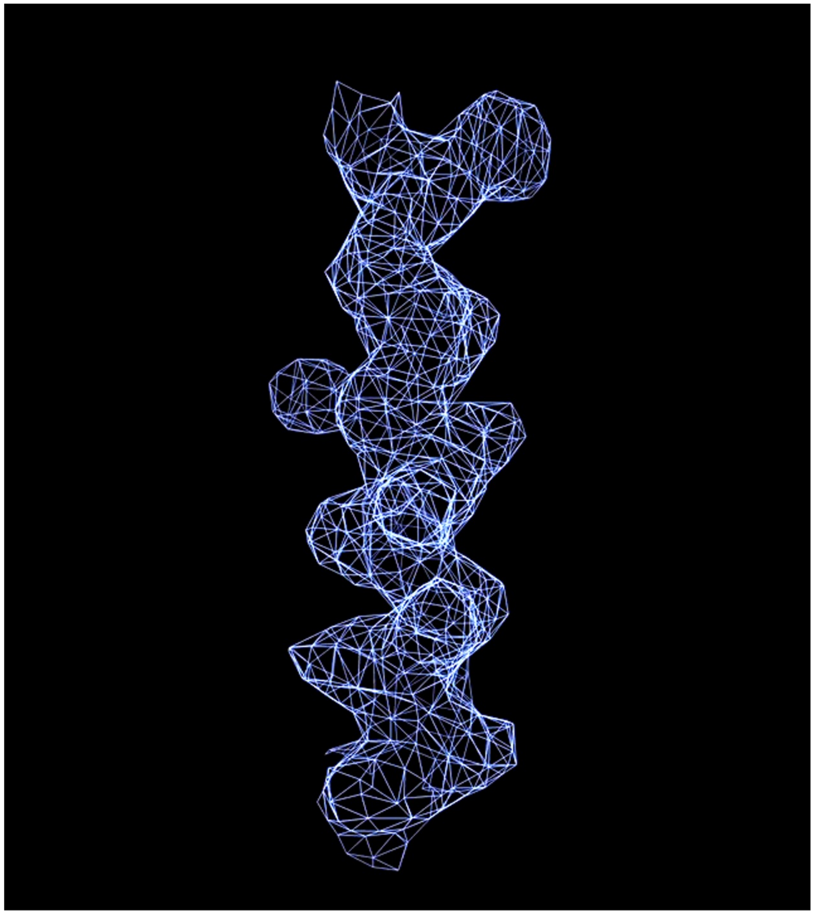
- ~10%. More common in TM? (e.g. S4 of VSD)
- 3 residues per turn. Triangular cross section.
- Skinny
- Can be tricky to identify at low resolution, can lead to register errors.

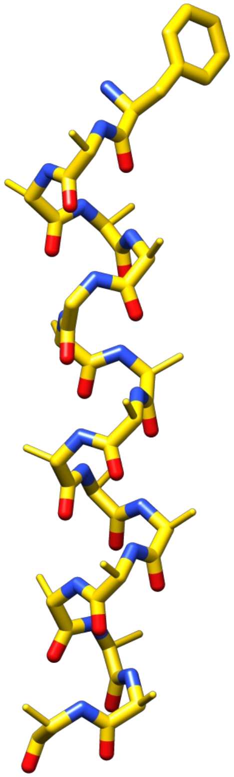
Can we identify any probable sidechains from the density?



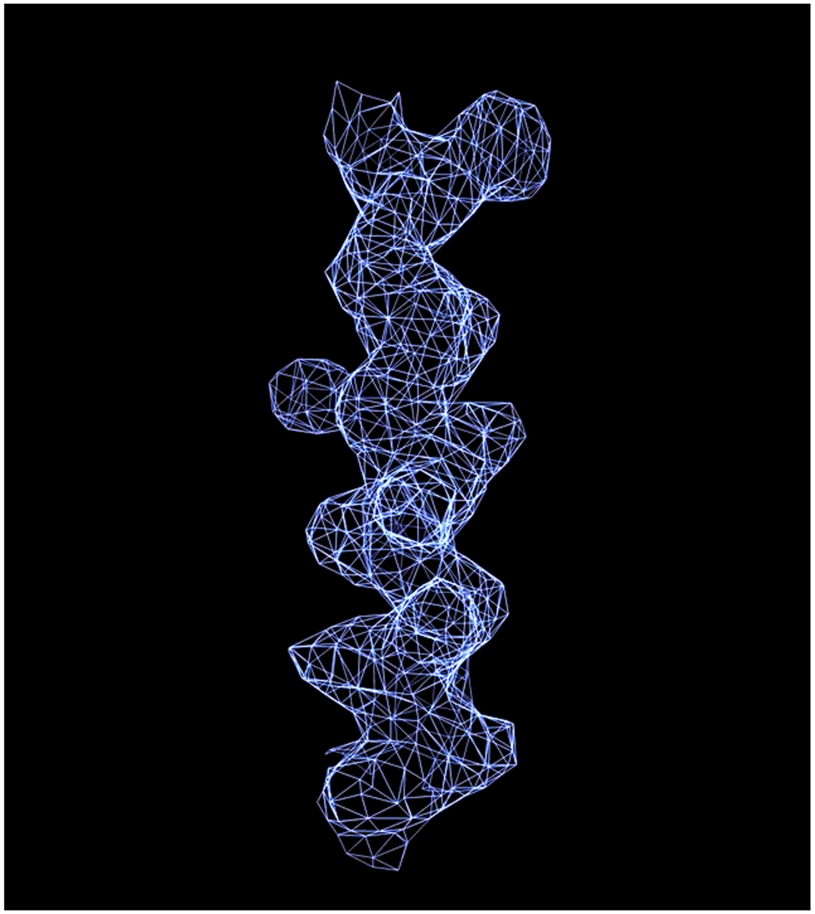


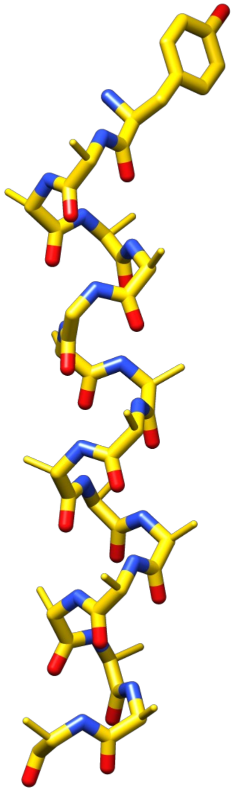
Can we identify any probable sidechains from the density?



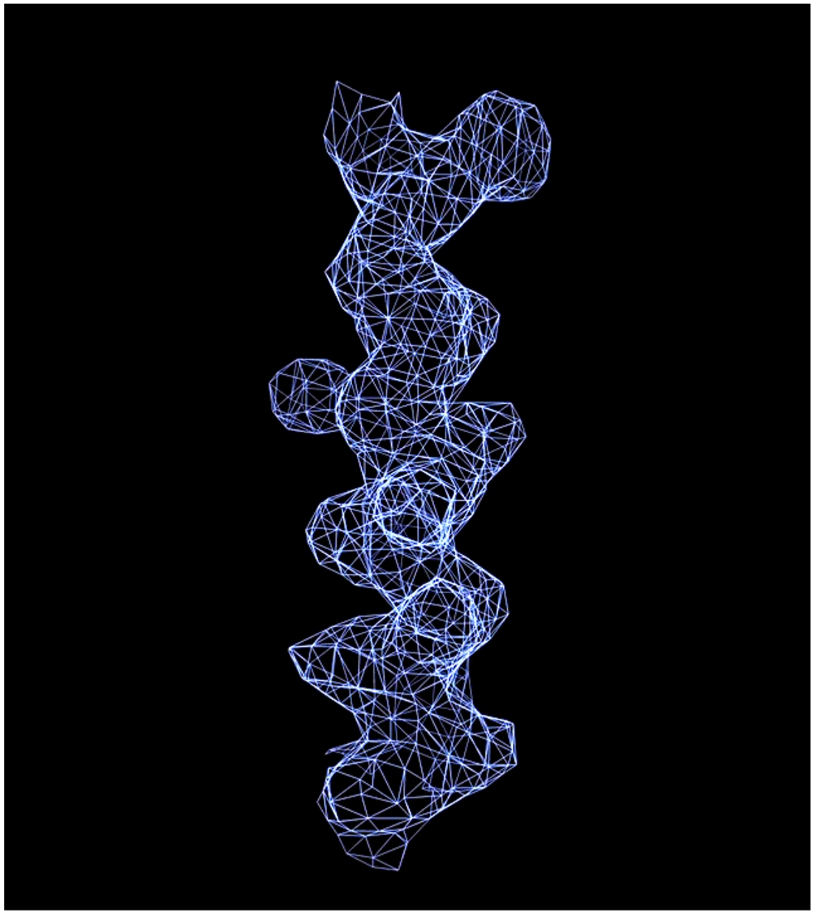


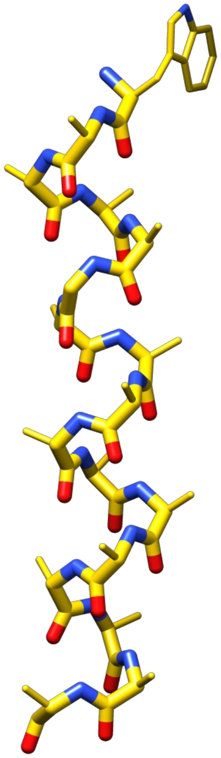
Can we identify any probable sidechains from the density?



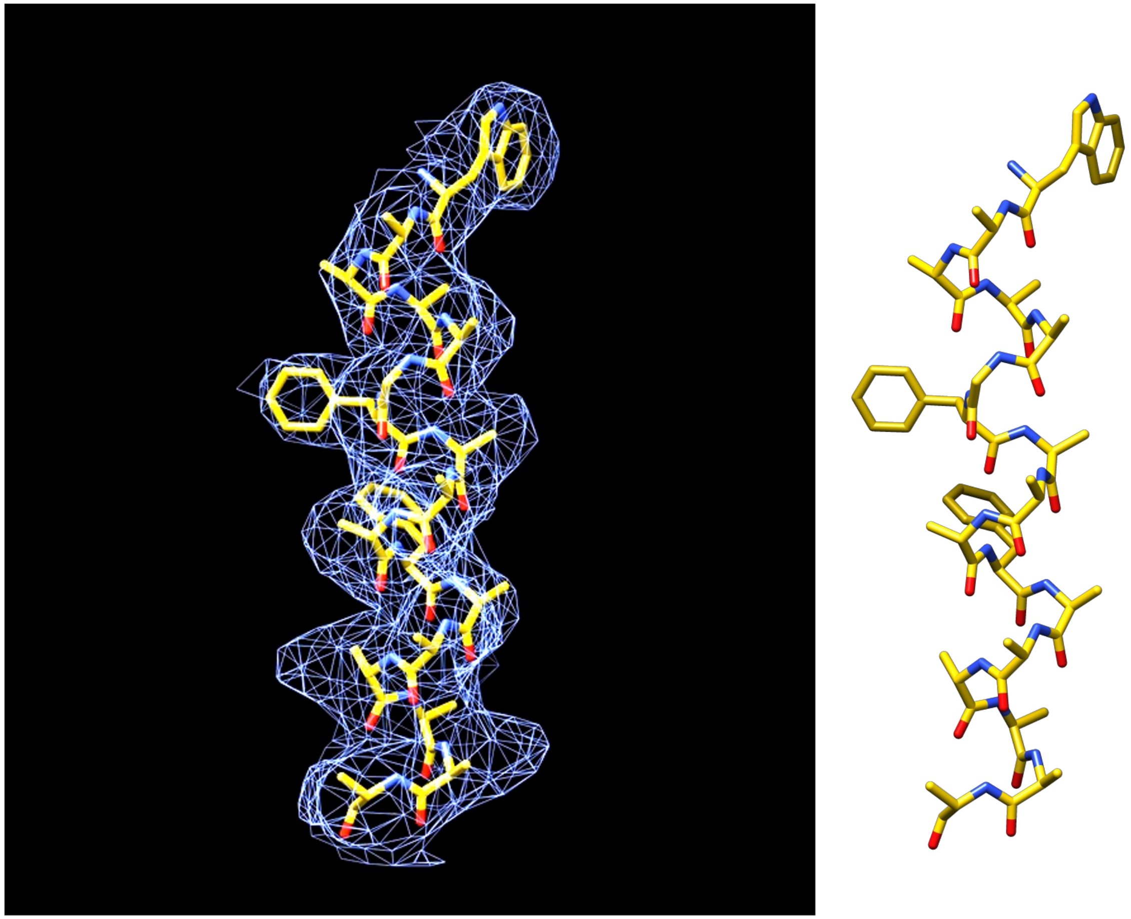


Can we identify any probable sidechains from the density?



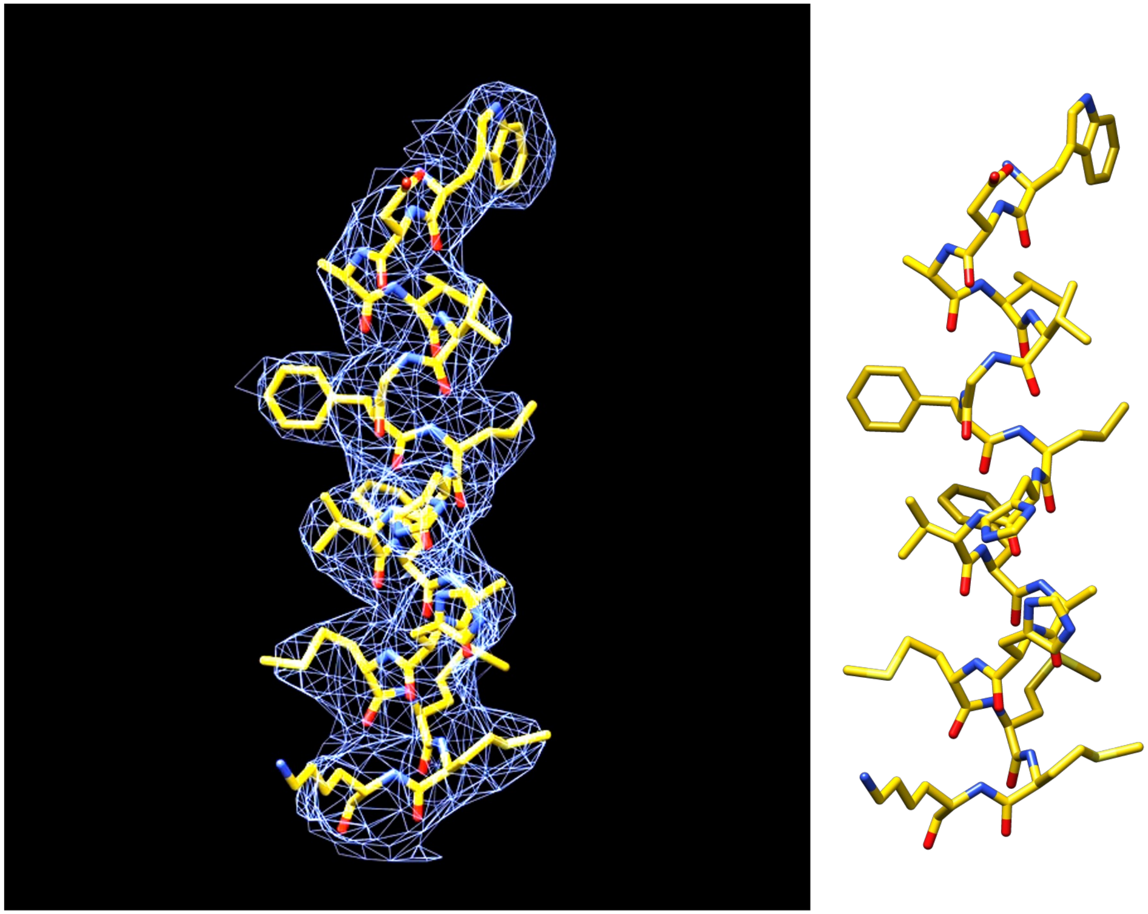


Test the initial hypothesis by extending sequence assignment along the chain.



…VFNSLTEYIQGPCTGNQQSLAHSRL**W**DAVVG**F**LHV**F**AHMMMKLAQDSSQIELLKELLDLQ…

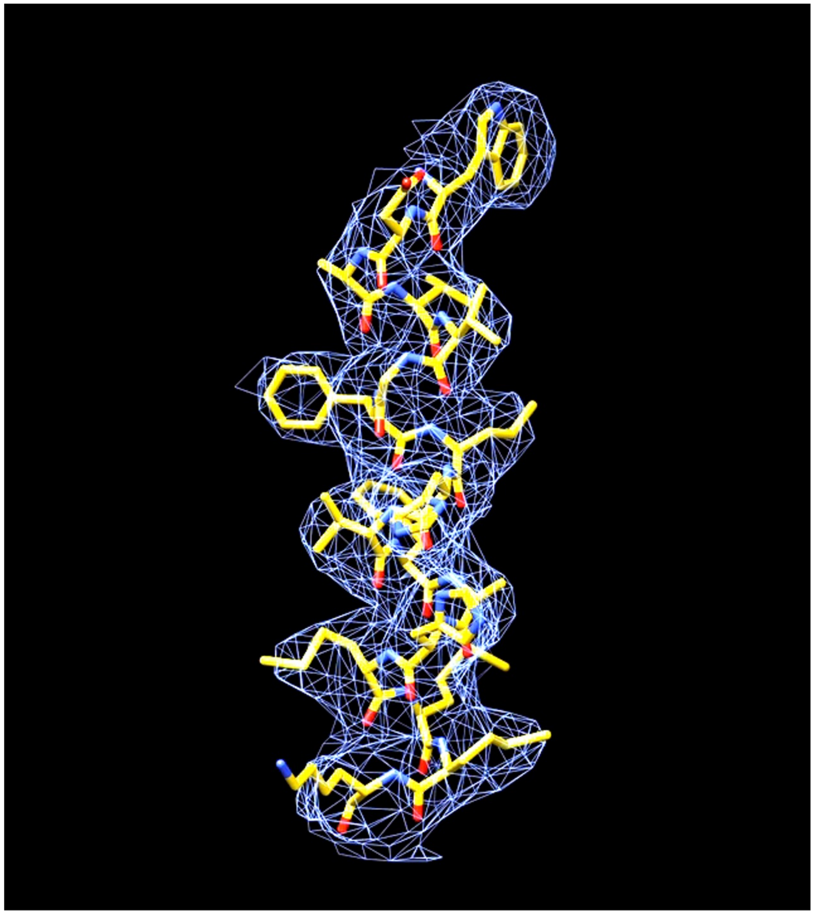
Test the initial hypothesis by extending sequence assignment along the chain.

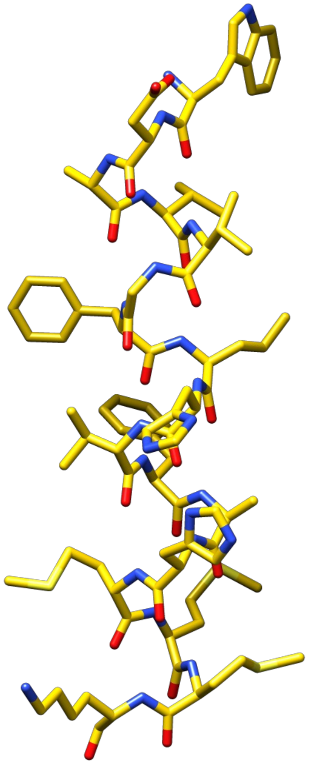


…VFNSLTEYIQGPCTGNQQSLAHSRL**WDAVVGFLHVFAHMMMK**LAQDSSQIELLKELLDLQ…

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

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.

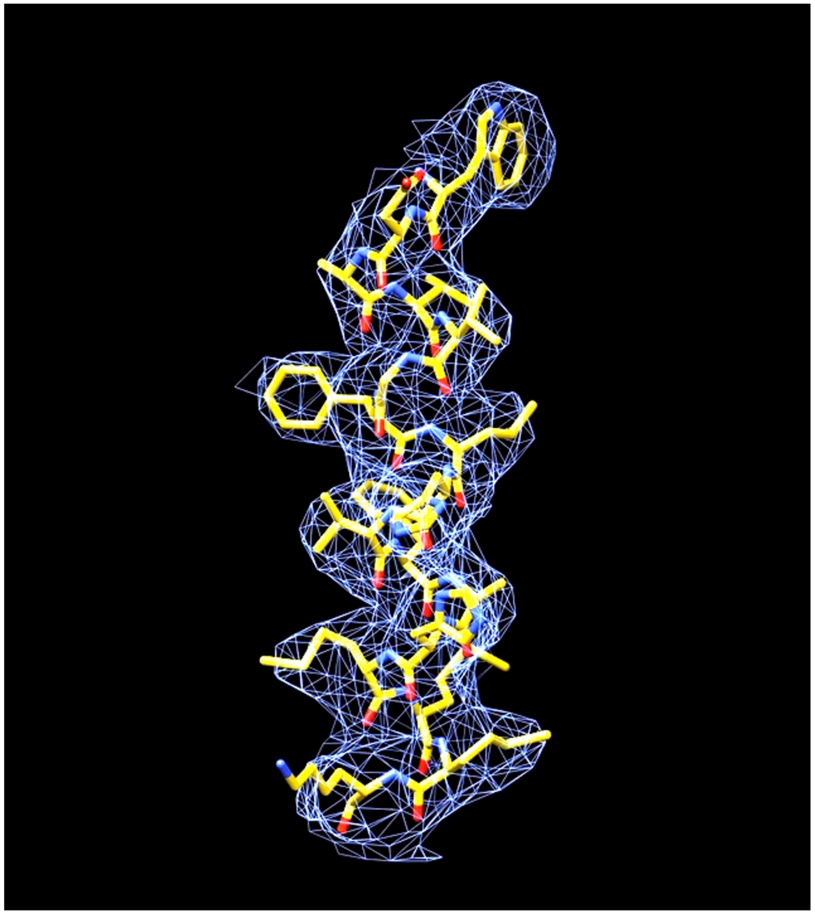


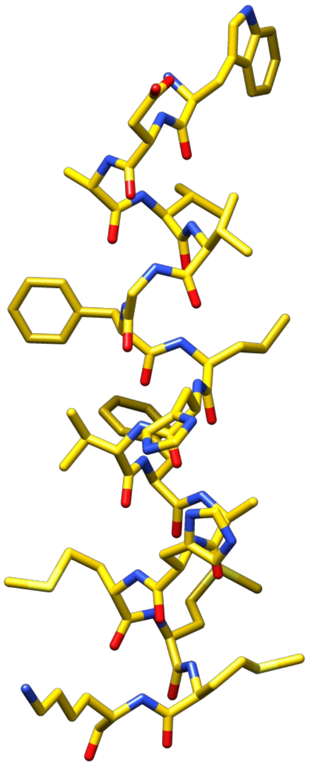


…VFNSLTEYIQGPCTGNQQSLAHSRL**WDAVVGFLHVFAHMMMK**LAQDSSQIELLKELLDLQ…

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?

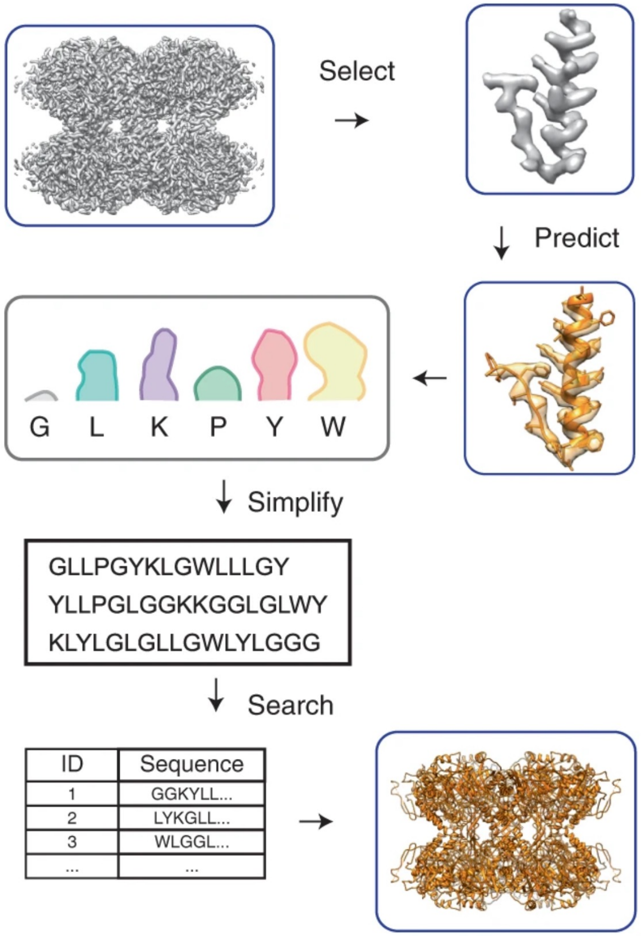




…VFNSLTEYIQGPCTGNQQSLAHSRL**WDAVVGFLHVFAHMMMK**LAQDSSQIELLKELLDLQ…

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.



GLLPGYKLGWLLLGY
YLLPGLGGKKGGLGLWY
KLYLGLGLLGWLYLGGG

ID	Sequence
1	GGKYLL...
2	LYKGLL...
3	WLGGL...
...	...

(Ho et al., Nature Methods, 2020)

What if we don’t have sidechains?

- CryoID requires sidechains – what if we don’t have them? E.g. sub-nm reconstructions from cryoET
- Still a lot of information encoded in arrangement of secondary structural elements.
- Can use COLORES (in SITUS package) to query Alphafold database using segmented densities as query
- Requires caution and biochemical validation in interpreting results (and subsequent fitting).

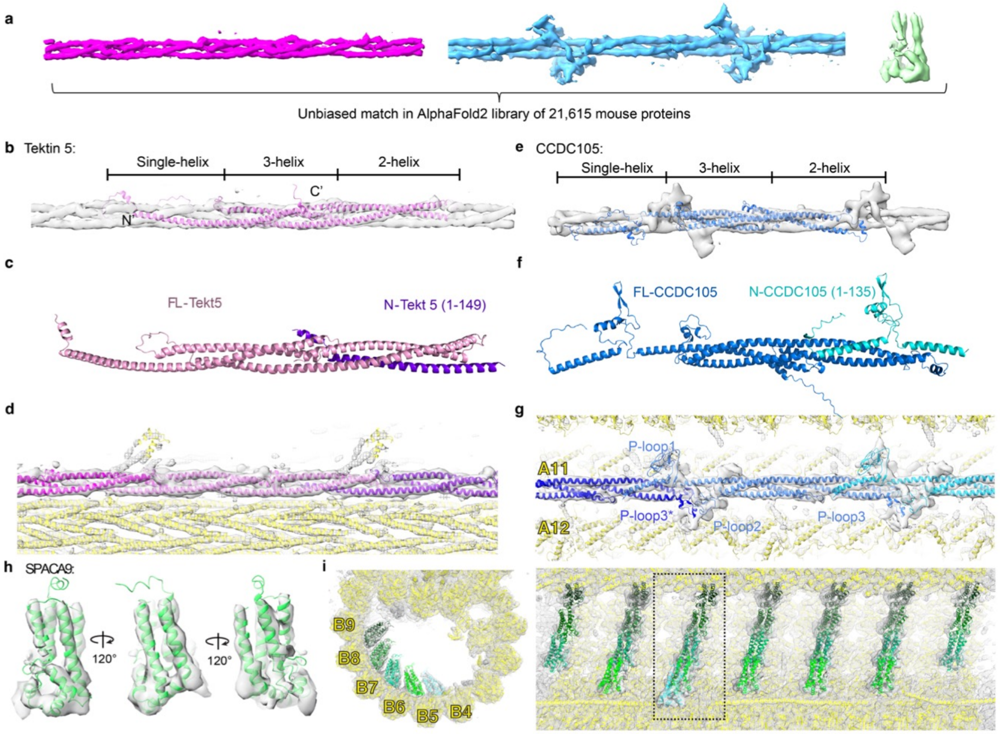
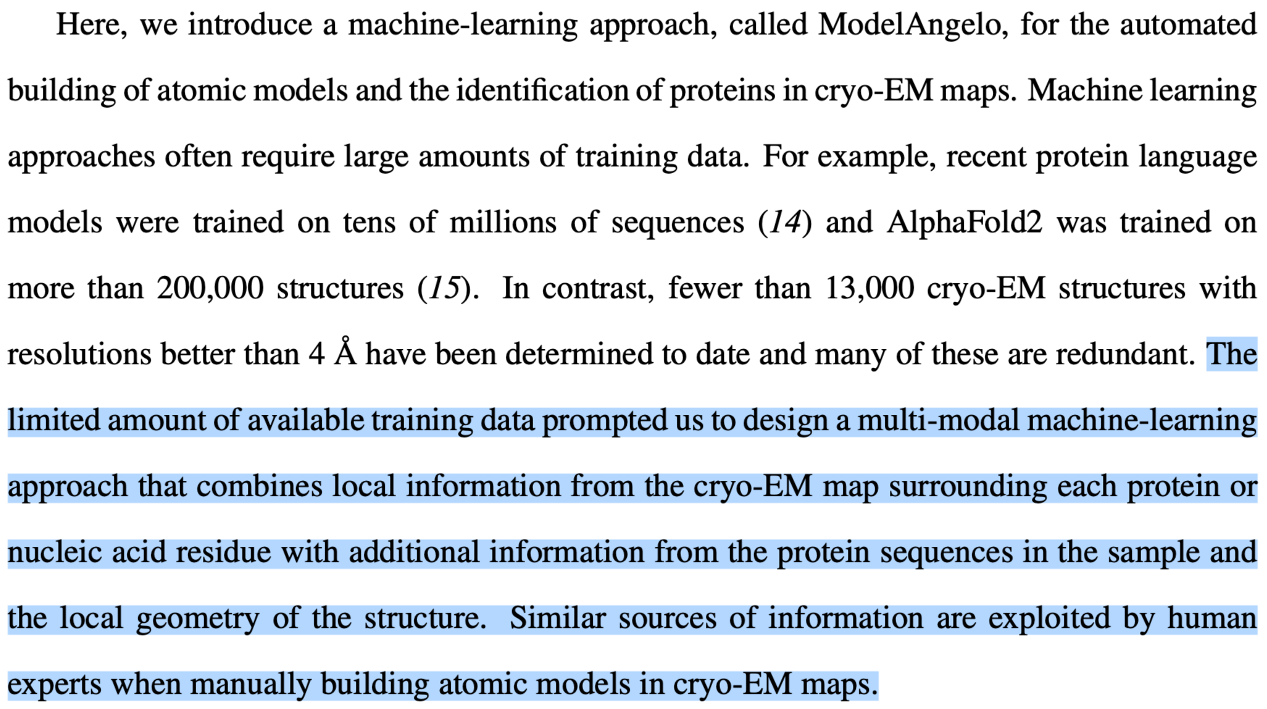


Figure 2. Unbiased matching of target densities from *in situ* cryoET reconstructions with structure models of 21,615 mouse proteins predicted using AlphaFold2.

(Zhen et al., BiorXiV, 2023)

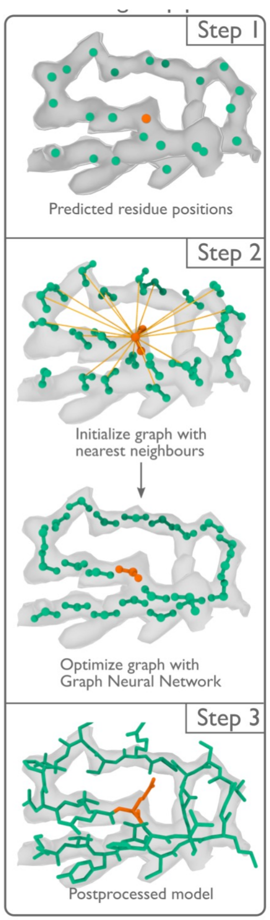
Modelangelo – applying neural networks to map interpretation



Here, we introduce a machine-learning approach, called ModelAngelo, for the automated building of atomic models and the identification of proteins in cryo-EM maps. Machine learning approaches often require large amounts of training data. For example, recent protein language models were trained on tens of millions of sequences (*14*) and AlphaFold2 was trained on more than 200,000 structures (*15*). In contrast, fewer than 13,000 cryo-EM structures with resolutions better than 4 Å have been determined to date and many of these are redundant. The limited amount of available training data prompted us to design a multi-modal machine-learning approach that combines local information from the cryo-EM map surrounding each protein or nucleic acid residue with additional information from the protein sequences in the sample and the local geometry of the structure. Similar sources of information are exploited by human experts when manually building atomic models in cryo-EM maps.

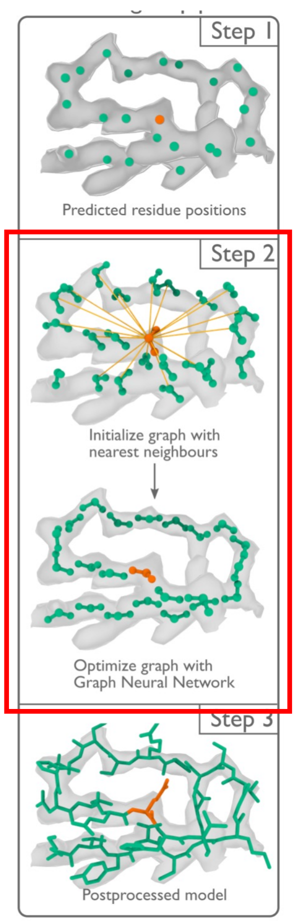
(Jamali et al., BiorXiv, 2023)

Modelangelo – applying neural networks to map interpretation



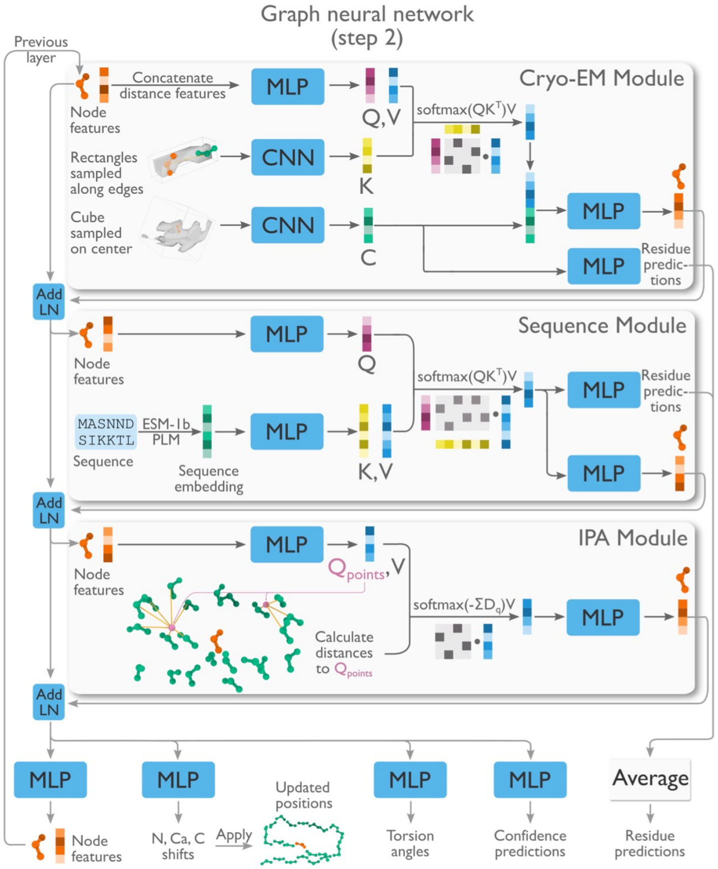
(Jamali et al., BiorXiv, 2023)

Modelangelo – applying neural networks to map interpretation


Step 1
Predicted residue positions
Step 2
Initialize graph with nearest neighbours
Optimize graph with Graph Neural Network
Step 3
Postprocessed model

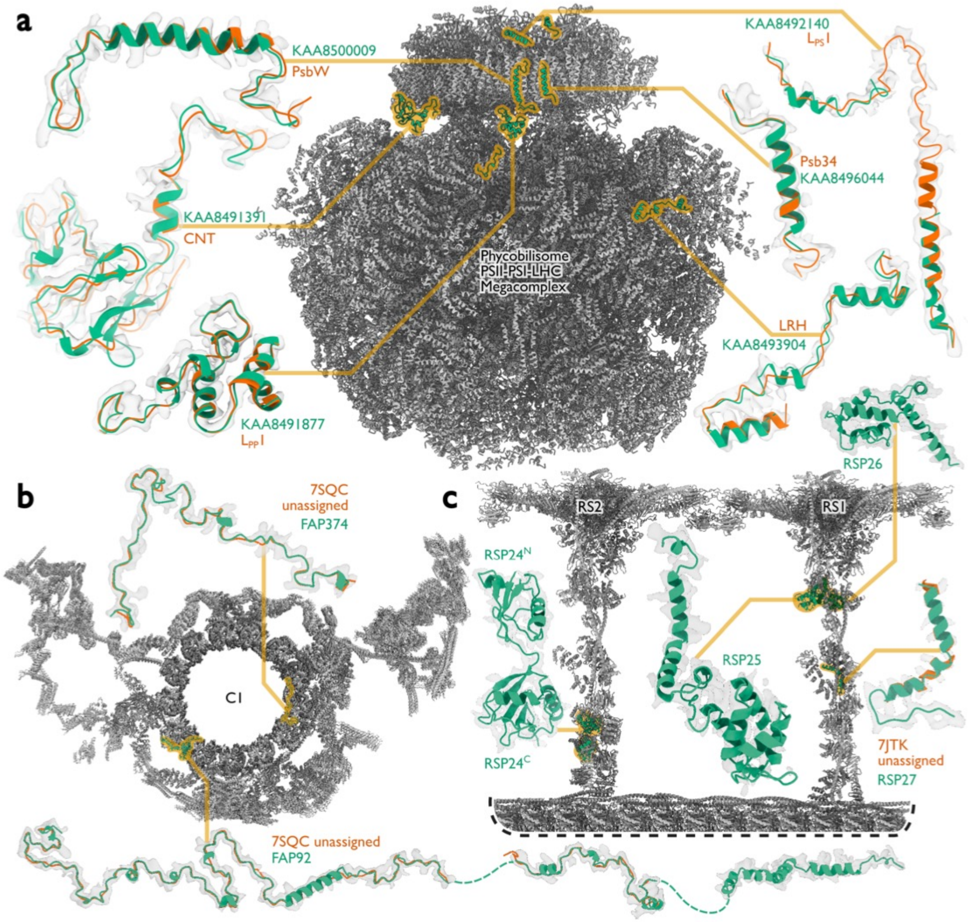
(Jamali et al., BiorXiv, 2023)

Modelangelo – applying neural networks to map interpretation



(Jamali et al., BiorXiv, 2023)

Modelangelo – applying neural networks to map interpretation



(Jamali et al., BiorXiv, 2023)

Modelangelo – applying neural networks to map interpretation

- ***Fantastic*** starting point for model building – generates near-complete models with good geometry in favorable areas.

- Difficult regions (e.g. flexible, anisotropic) still require manual building (for now!)

- Model still requires manual inspection and analysis (both for completion/correction/validation, and understanding!)

- Can't build waters/ions/ligands (yet!)

- ***Allows us to build better, faster, and focus on difficult/important regions.***

(Jamali et al., BiorXiv, 2023)

How to deal with uncertainty in sequence assignment and sidechain placement

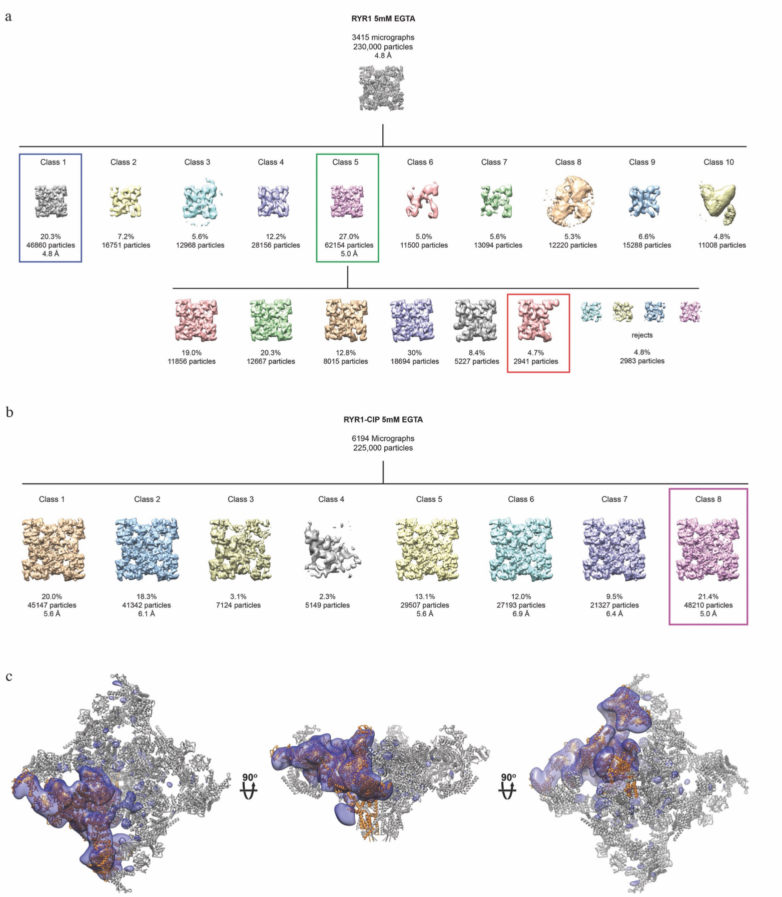
- You will likely encounter situations where you cannot be certain of the local sequence register – what to do?

- 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 document the approach taken when writing up the structure. You can read long discussions of the merits of both approaches on the CCP4bb should you so desire 🙃

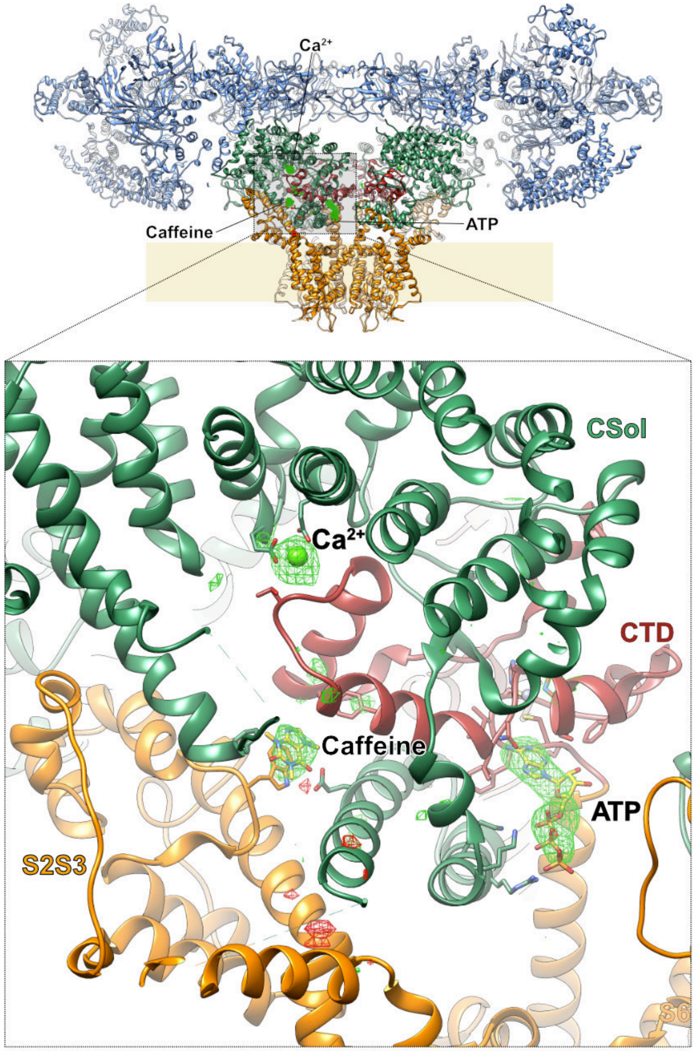
Prior knowledge can come in many forms – use any and all available info to guide model building.



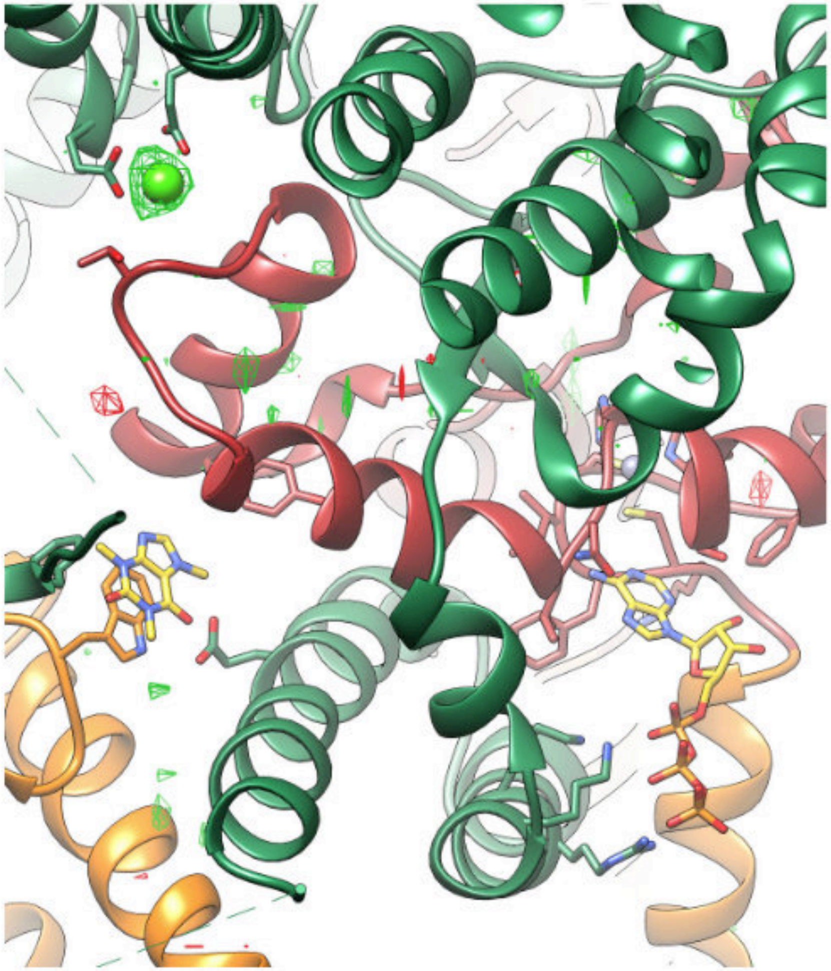
Here, serendipitous identification of a conformational class of RyR1 lacking density for one subunit aided identification of protomer boundaries. In other cases, cross-linking data or NS data on subcomplexes or Fab-complexes may be helpful.

(Zalk et al, Nature 2015)

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

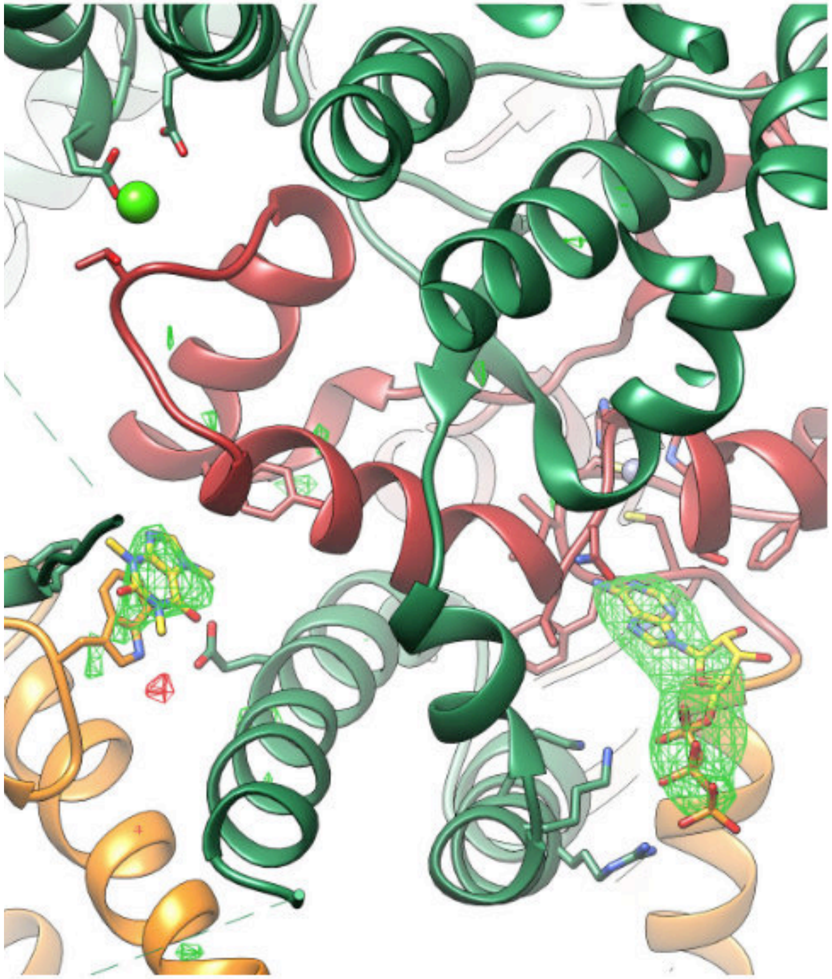


The three ligands are clustered around the C-terminal domain.



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

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



(ATP/Caffeine) minus (EGTA only)

EM-specific considerations

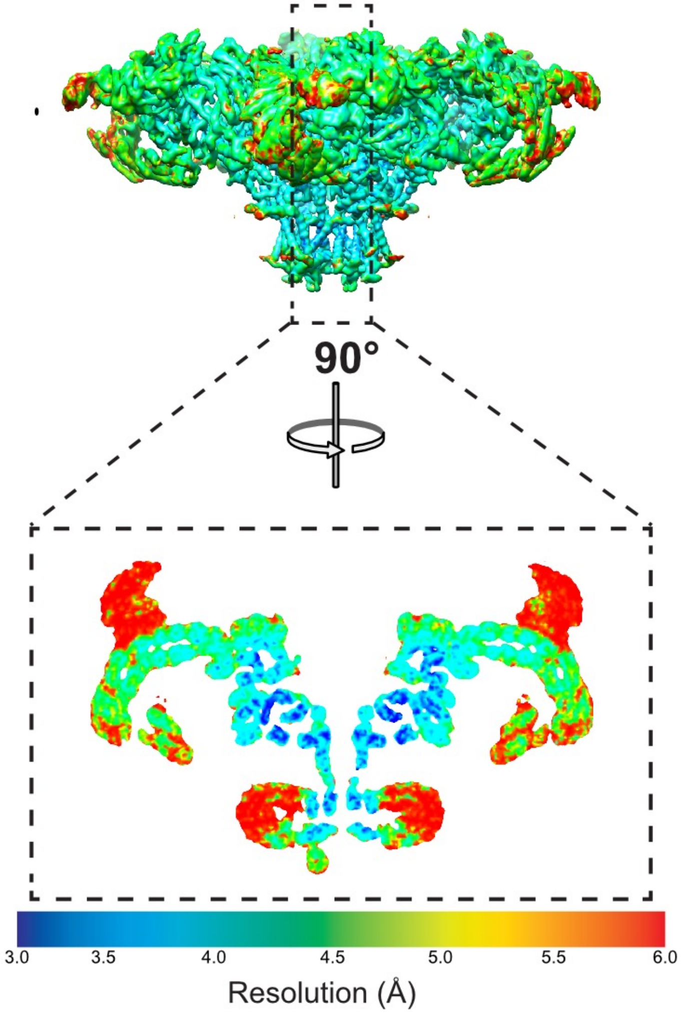
- No unambiguous sequence/elemental markers at low resolution (no equivalent of SeMet yet).

- 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`)

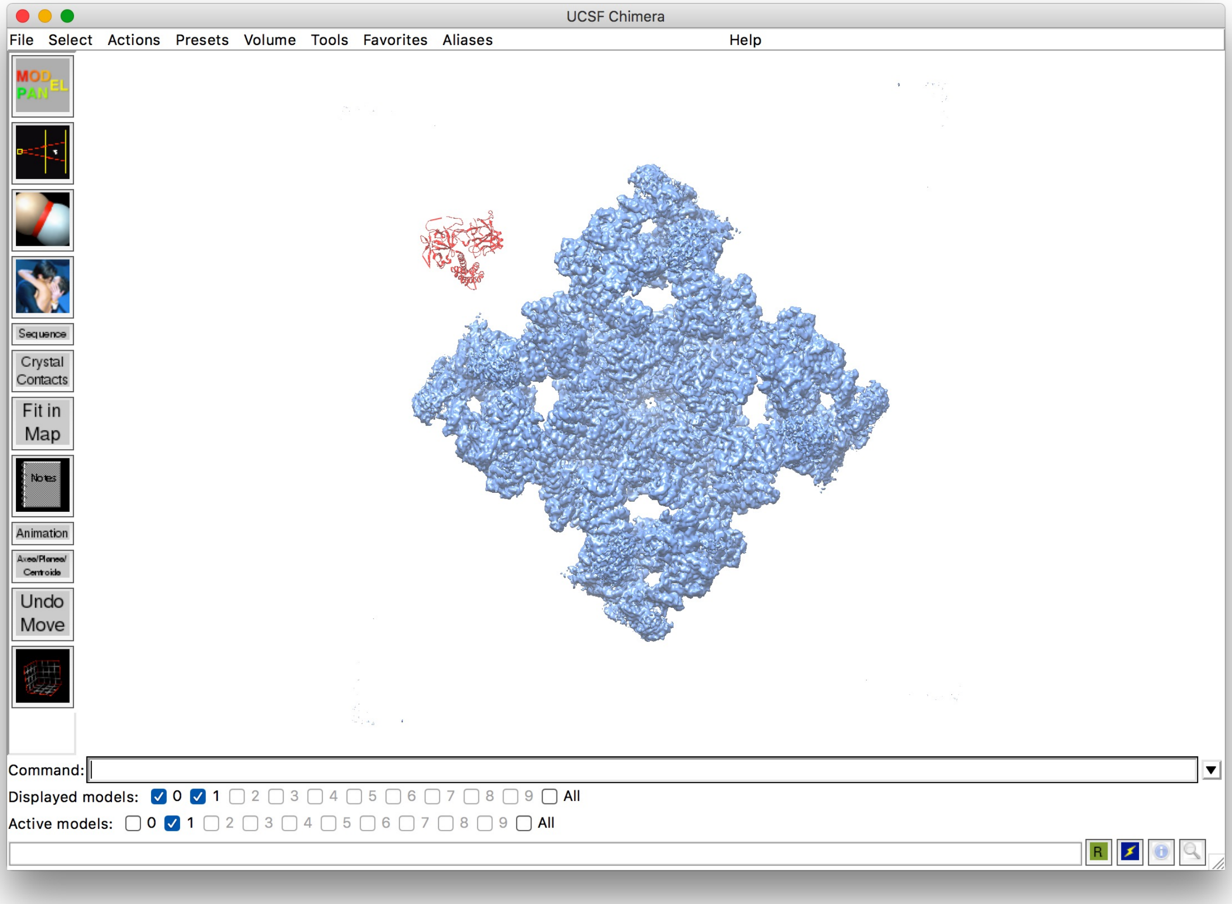


Building an initial model - where to start?

- 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



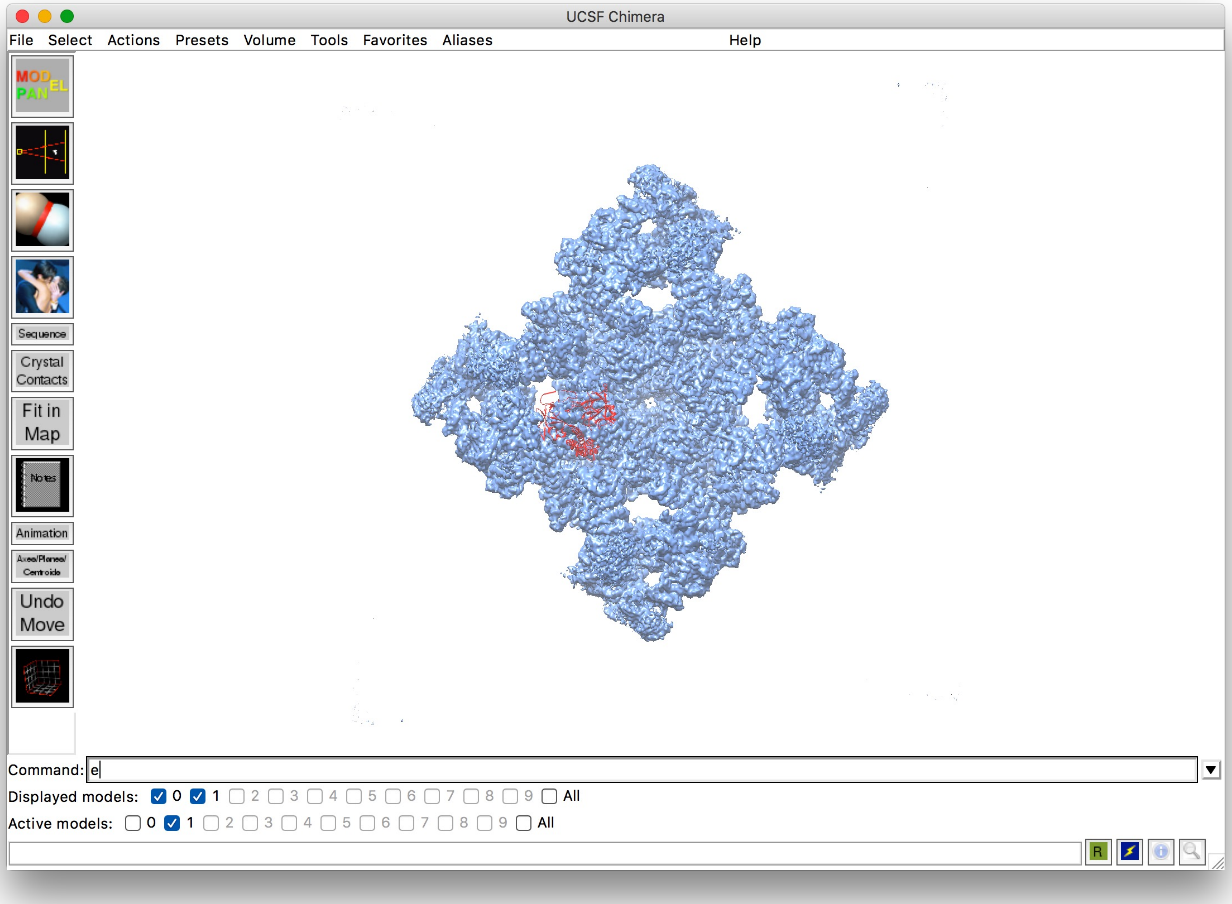
Command:

Displayed models: ☑ 0 ☑ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 □ 9 □ All

Active models: □ 0 ☑ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 □ 9 □ All

Start with map and model.

Using UCSF Chimera to fit solved domains



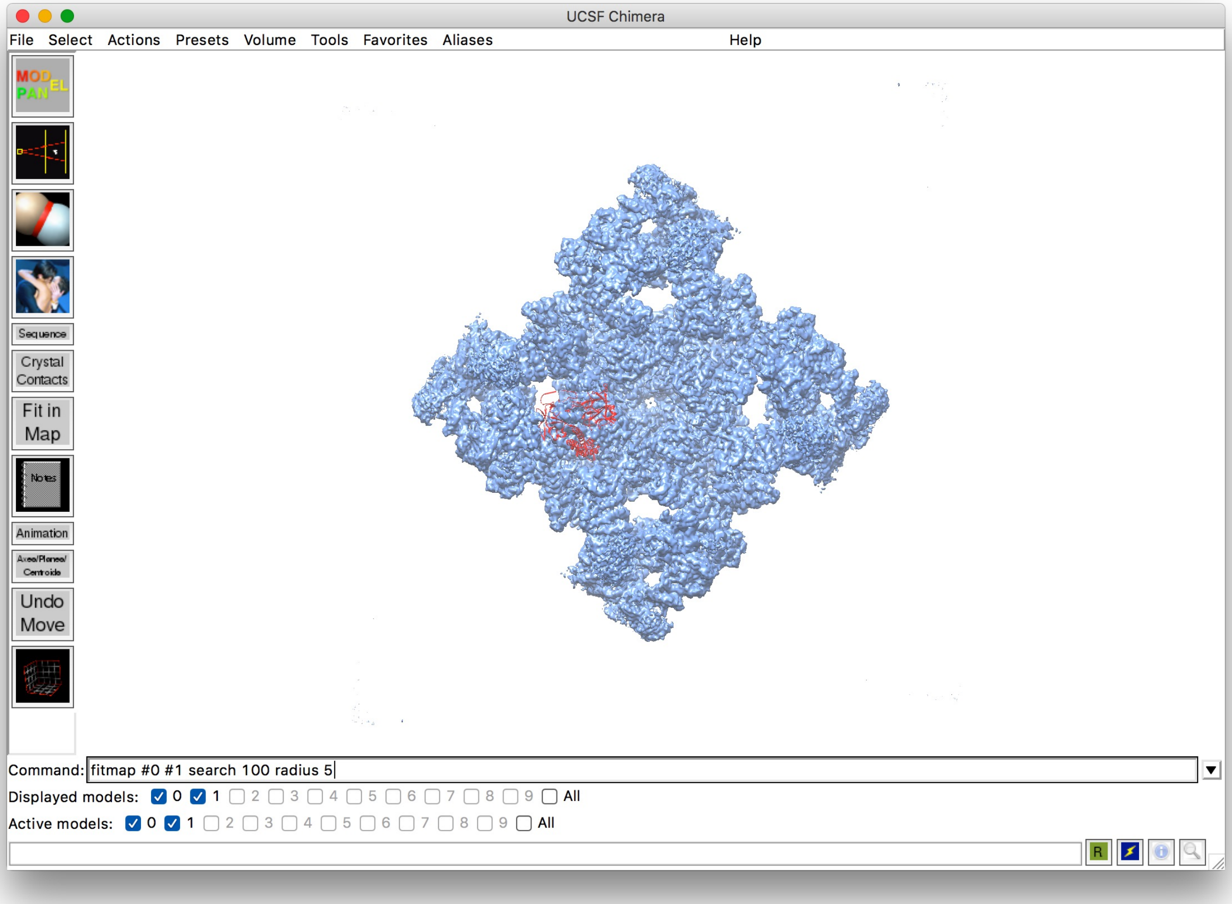
Command: e

Displayed models: ☑ 0 ☑ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 □ 9 □ All

Active models: □ 0 ☑ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 □ 9 □ All

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

Using UCSF Chimera to fit solved domains



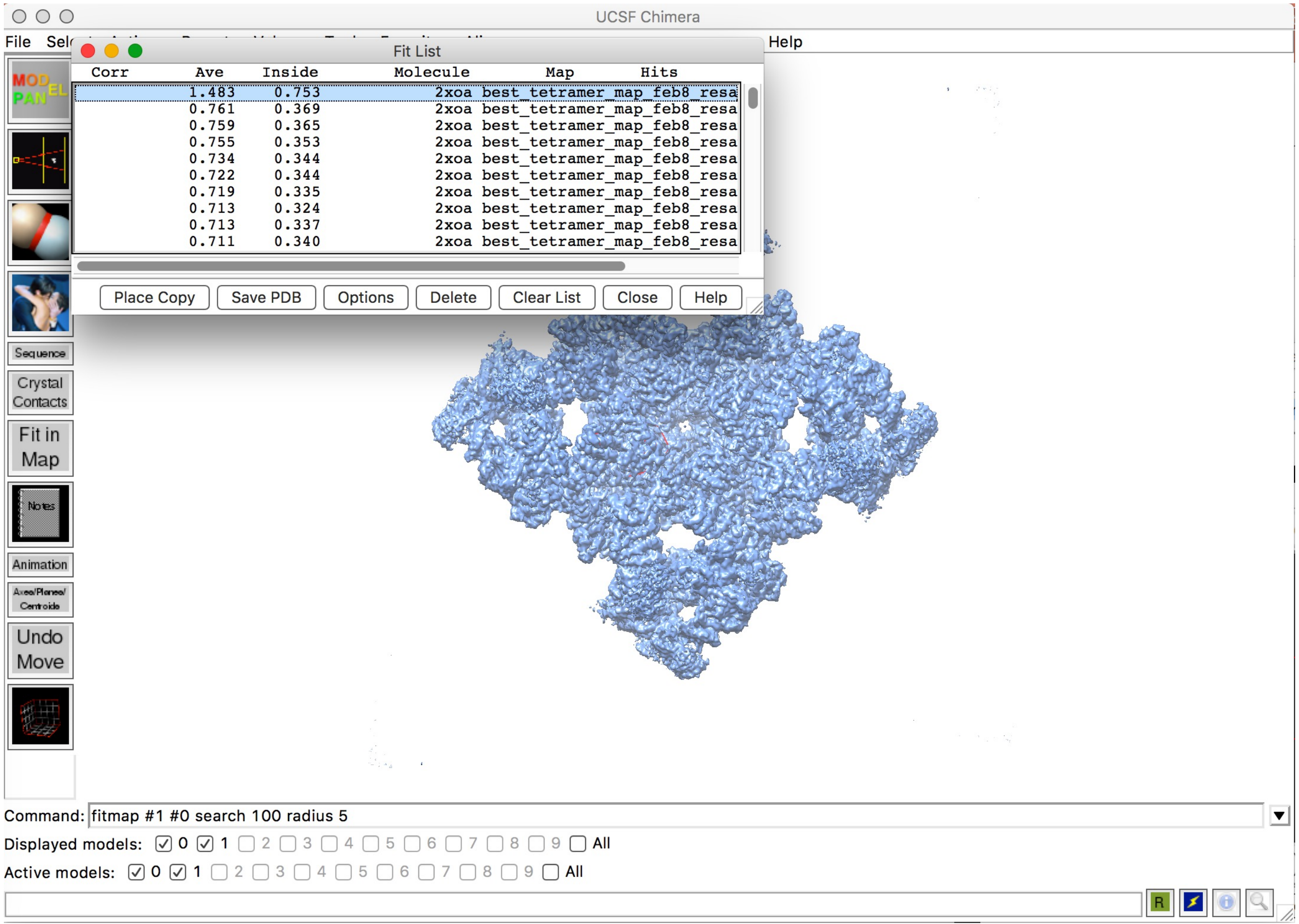
```
Command: fitmap #0 #1 search 100 radius 5
```

Displayed models: ☑ 0 ☑ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 □ 9 □ All

Active models: ☑ 0 ☑ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 □ 9 □ All

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

Using UCSF Chimera to fit solved domains



```
 Corr        Ave     Inside          Molecule          Map         Hits
                1.483     0.753           2xoa best_tetramer_map_feb8_resa
                0.761     0.369           2xoa best_tetramer_map_feb8_resa
                0.759     0.365           2xoa best_tetramer_map_feb8_resa
                0.755     0.353           2xoa best_tetramer_map_feb8_resa
                0.734     0.344           2xoa best_tetramer_map_feb8_resa
                0.722     0.344           2xoa best_tetramer_map_feb8_resa
                0.719     0.335           2xoa best_tetramer_map_feb8_resa
                0.713     0.324           2xoa best_tetramer_map_feb8_resa
                0.713     0.337           2xoa best_tetramer_map_feb8_resa
                0.711     0.340           2xoa best_tetramer_map_feb8_resa
```

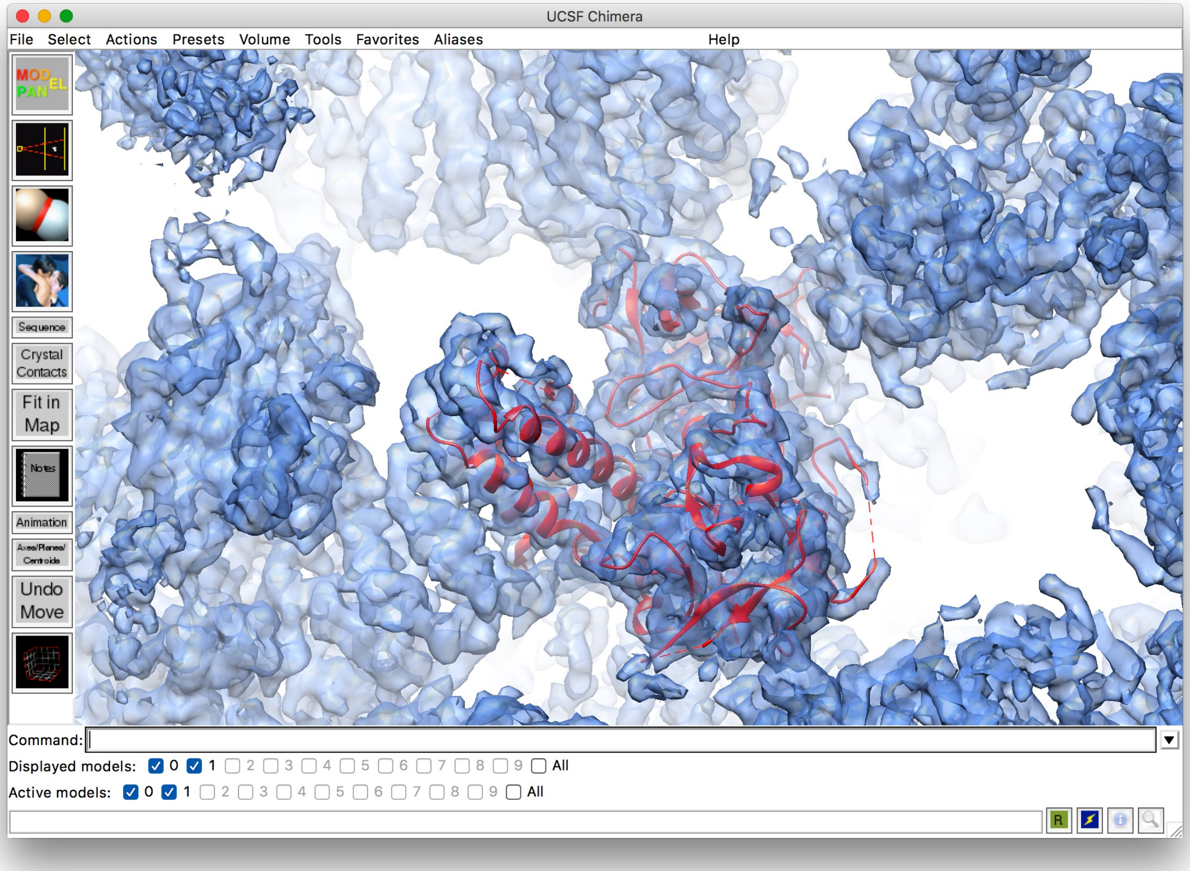
Command: fitmap #1 #0 search 100 radius 5

Displayed models: ☑ 0 ☑ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 □ 9 □ All

Active models: ☑ 0 ☑ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 □ 9 □ All

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.

Using UCSF Chimera to fit solved domains



Command:

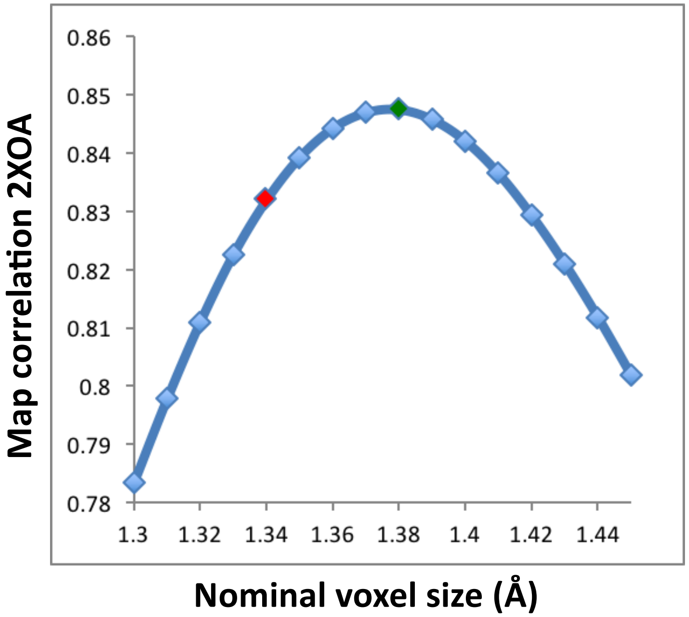
Displayed models: ☑ 0 ☑ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 □ 9 □ All

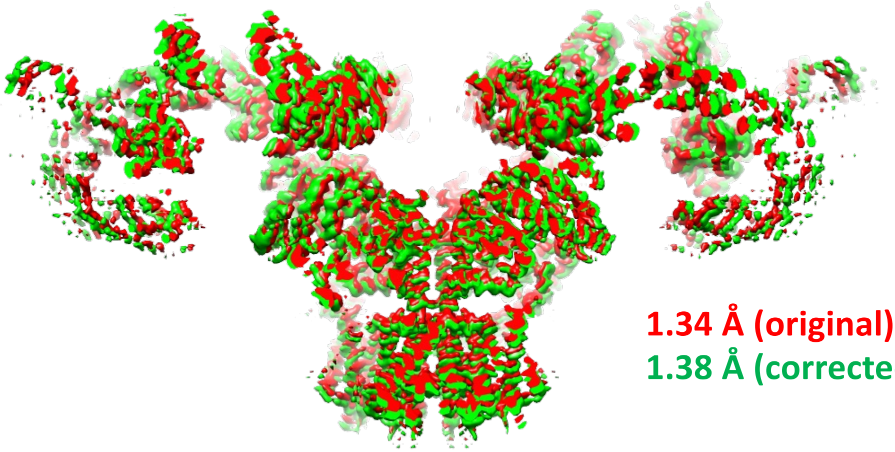
Active models: ☑ 0 ☑ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 □ 9 □ All

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.

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).





1.34 Å (original)
1.38 Å (corrected)

COOT – Crystallographic Object Oriented Toolkit

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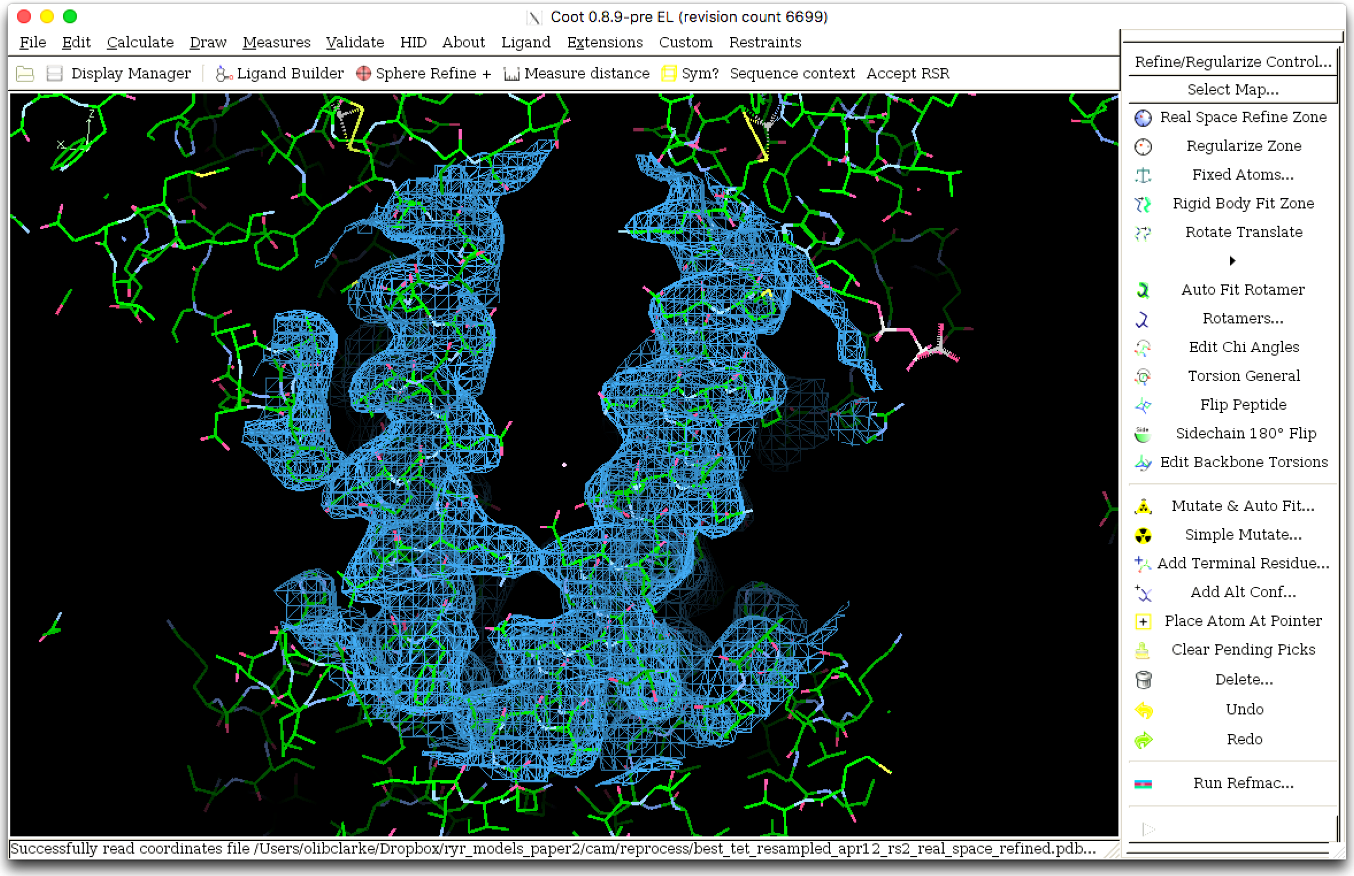

Successfully read coordinates file /Users/olibclarke/Dropbox/ryr_models_paper2/cam/reprocess/best_tet_resampled_apr12_rs2_real_space_refined.pdb...

(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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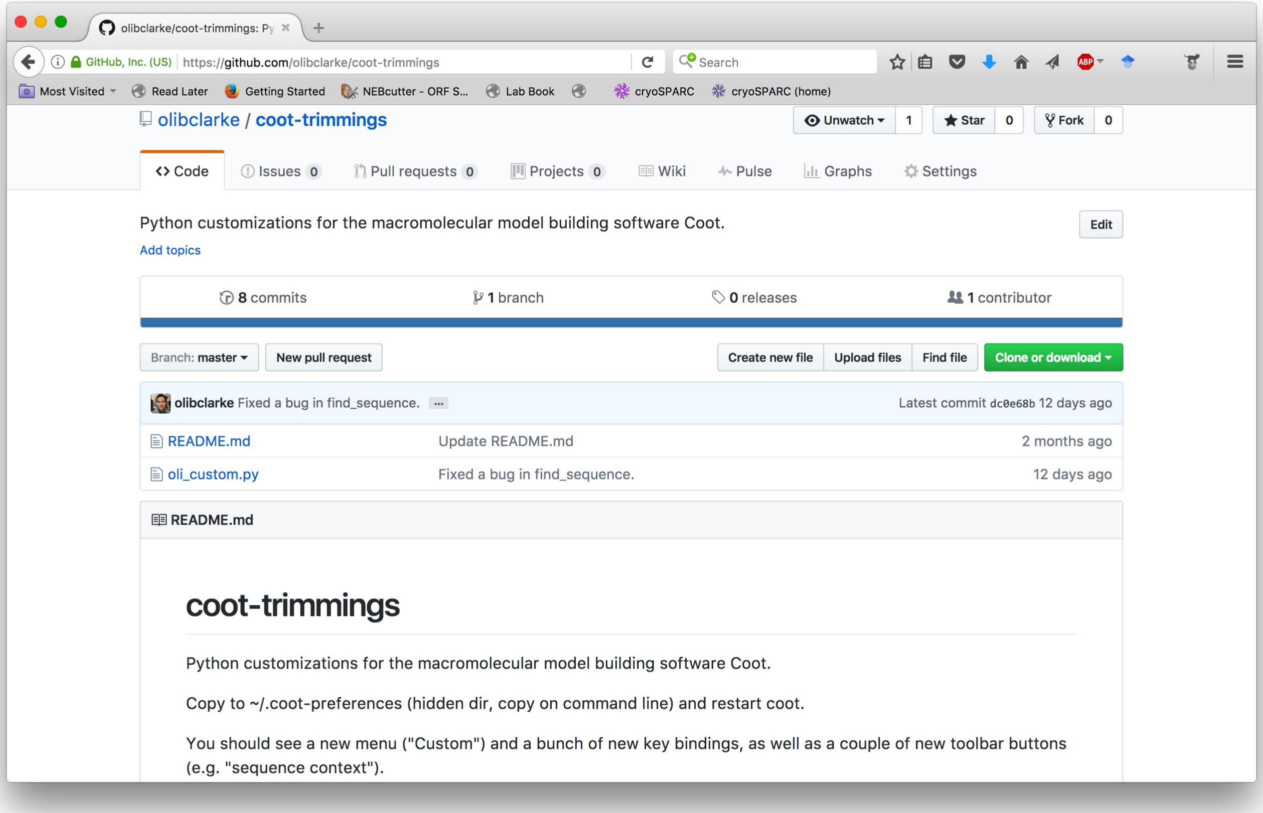

olibclarke / coot-trimmings

Python customizations for the macromolecular model building software Coot.

8 commits | 1 branch | 0 releases | 1 contributor

olibclarke Fixed a bug in find_sequence. … Latest commit dc0e68b 12 days ago

README.md	Update README.md	2 months ago
oli_custom.py	Fixed a bug in find_sequence.	12 days ago

README.md

coot-trimmings

Python customizations for the macromolecular model building software Coot.

Copy to ~/.coot-preferences (hidden dir, copy on command line) and restart coot.

You should see a new menu ("Custom") and a bunch of new key bindings, as well as a couple of new toolbar buttons (e.g. "sequence context").

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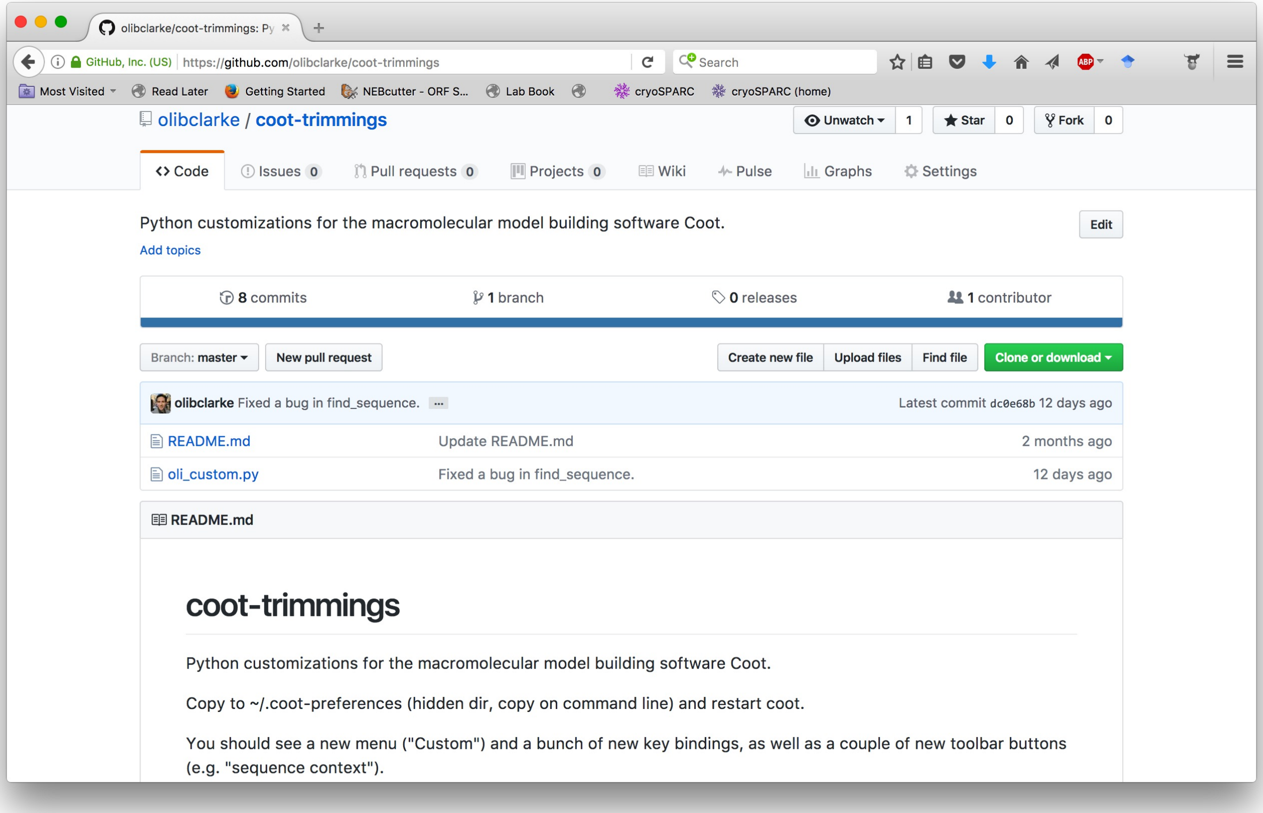

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README.md	Update README.md	2 months ago
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README.md

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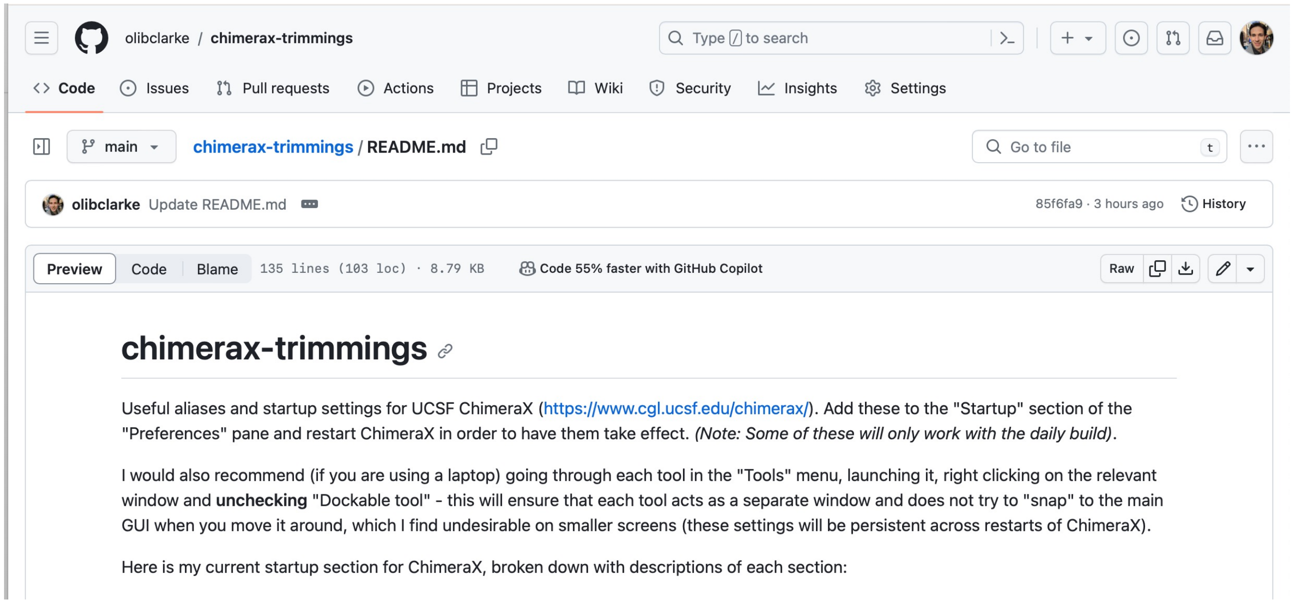
Sidenote – trimmings for ChimeraX:

- Added some shortcuts and a bunch of aliases that I find helpful for using ChimeraX with maps/models.
- Use and modify as you like!


olibclarke / chimerax-trimmings
Type / to search
Code
Issues
Pull requests
Actions
Projects
Wiki
Security
Insights
Settings
main
chimerax-trimmings / README.md
Go to file
t
olibclarke Update README.md
85f6fa9 · 3 hours ago
History
Preview
Code
Blame
135 lines (103 loc) · 8.79 KB
Code 55% faster with GitHub Copilot
Raw

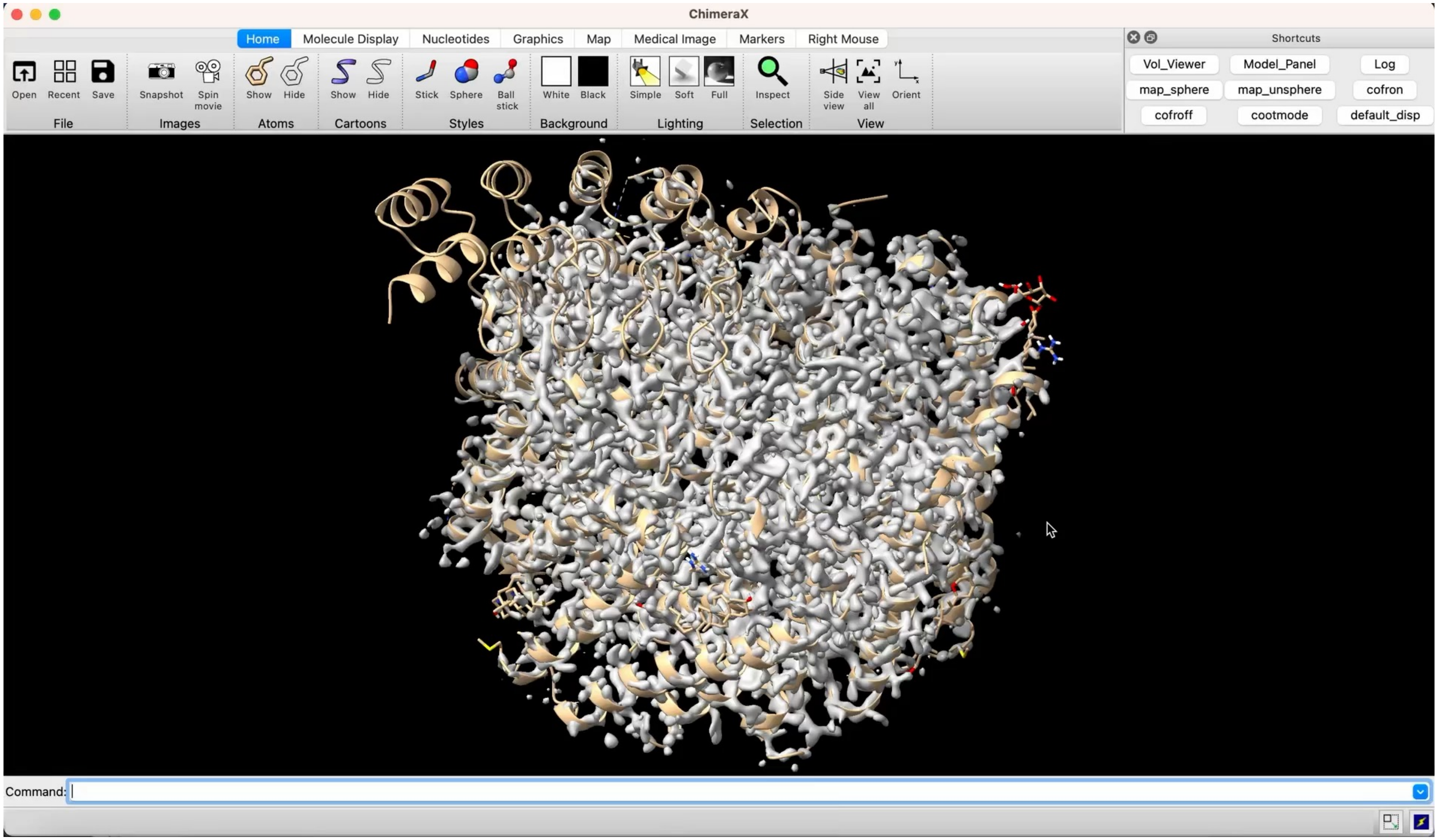
chimerax-trimmings

Useful aliases and startup settings for UCSF ChimeraX (https://www.cgl.ucsf.edu/chimerax/). Add these to the "Startup" section of the "Preferences" pane and restart ChimeraX in order to have them take effect. *(Note: Some of these will only work with the daily build)*.

I would also recommend (if you are using a laptop) going through each tool in the "Tools" menu, launching it, right clicking on the relevant window and **unchecking** "Dockable tool" - this will ensure that each tool acts as a separate window and does not try to "snap" to the main GUI when you move it around, which I find undesirable on smaller screens (these settings will be persistent across restarts of ChimeraX).

Here is my current startup section for ChimeraX, broken down with descriptions of each section:

Sidenote – trimmings for ChimeraX:



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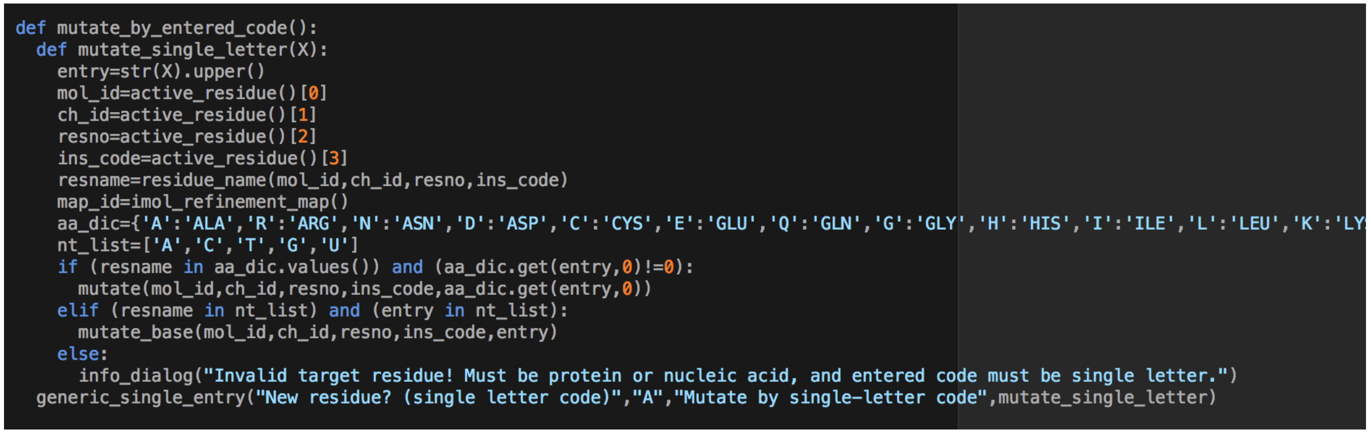
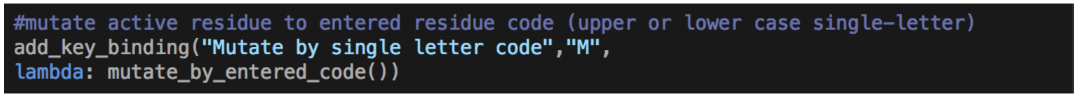


```
def mutate_by_entered_code():
  def mutate_single_letter(X):
    entry=str(X).upper()
    mol_id=active_residue()[0]
    ch_id=active_residue()[1]
    resno=active_residue()[2]
    ins_code=active_residue()[3]
    resname=residue_name(mol_id,ch_id,resno,ins_code)
    map_id=imol_refinement_map()
    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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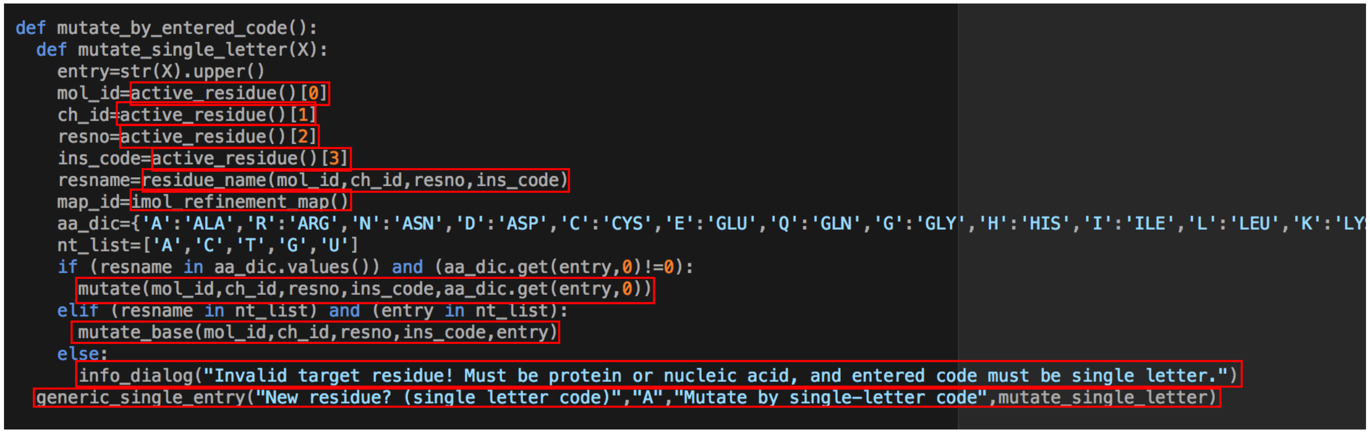

```
def mutate_by_entered_code():
  def mutate_single_letter(X):
    entry=str(X).upper()
    mol_id=active_residue()[0]
    ch_id=active_residue()[1]
    resno=active_residue()[2]
    ins_code=active_residue()[3]
    resname=residue_name(mol_id,ch_id,resno,ins_code)
    map_id=imol_refinement_map()
    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:
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  generic_single_entry("New residue? (single letter code)","A","Mutate by single-letter code",mutate_single_letter)
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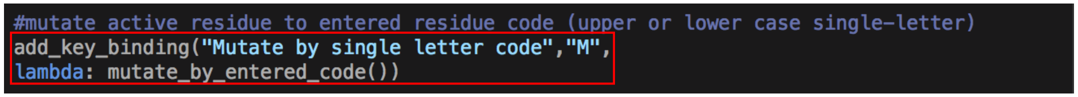

```
#mutate active residue to entered residue code (upper or lower case single-letter)
add_key_binding("Mutate by single letter code","M",
lambda: mutate_by_entered_code())
```

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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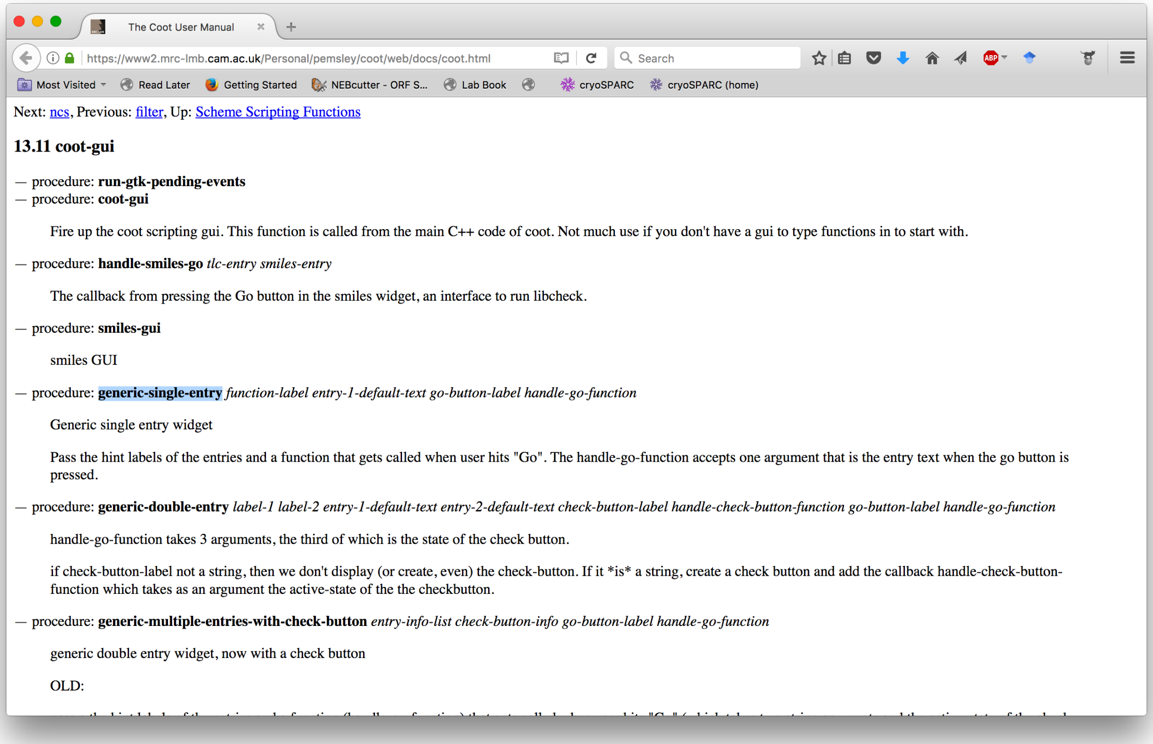

Next: ncs, Previous: filter, Up: Scheme Scripting Functions

13.11 coot-gui

— procedure: **run-gtk-pending-events**
— procedure: **coot-gui**

Fire up the coot scripting gui. This function is called from the main C++ code of coot. Not much use if you don't have a gui to type functions in to start with.

— procedure: **handle-smiles-go** *tlc-entry smiles-entry*

The callback from pressing the Go button in the smiles widget, an interface to run libcheck.

— procedure: **smiles-gui**

smiles GUI

— procedure: **generic-single-entry** *function-label entry-1-default-text go-button-label handle-go-function*

Generic single entry widget

Pass the hint labels of the entries and a function that gets called when user hits "Go". The handle-go-function accepts one argument that is the entry text when the go button is pressed.

— procedure: **generic-double-entry** *label-1 label-2 entry-1-default-text entry-2-default-text check-button-label handle-check-button-function go-button-label handle-go-function*

handle-go-function takes 3 arguments, the third of which is the state of the check button.

if check-button-label not a string, then we don't display (or create, even) the check-button. If it *is* a string, create a check button and add the callback handle-check-button-function which takes as an argument the active-state of the the checkbutton.

— procedure: **generic-multiple-entries-with-check-button** *entry-info-list check-button-info go-button-label handle-go-function*

generic double entry widget, now with a check button

OLD:

Many pre-packaged functions available in COOT API. Mostly documented in online manual.

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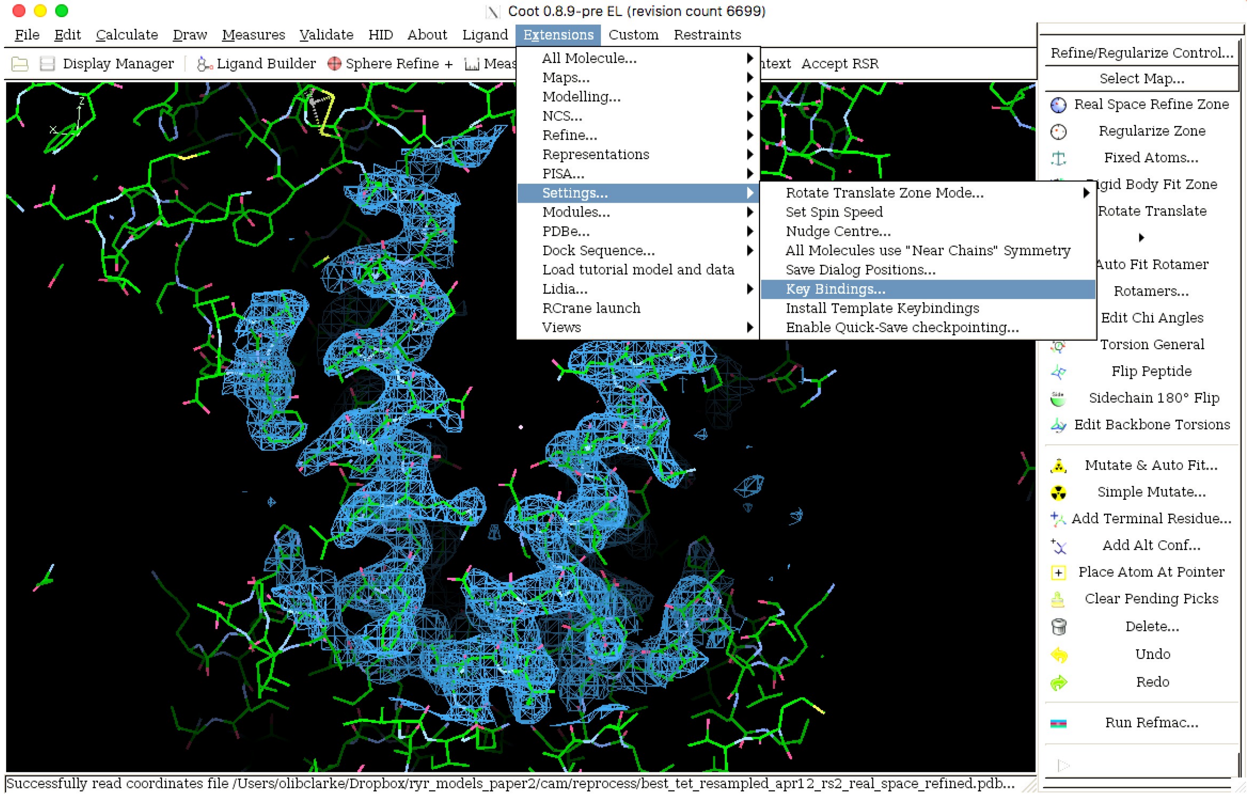

Successfully read coordinates file /Users/olibclarke/Dropbox/ryr_models_paper2/cam/reprocess/best_tet_resampled_apr12_rs2_real_space_refined.pdb...

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

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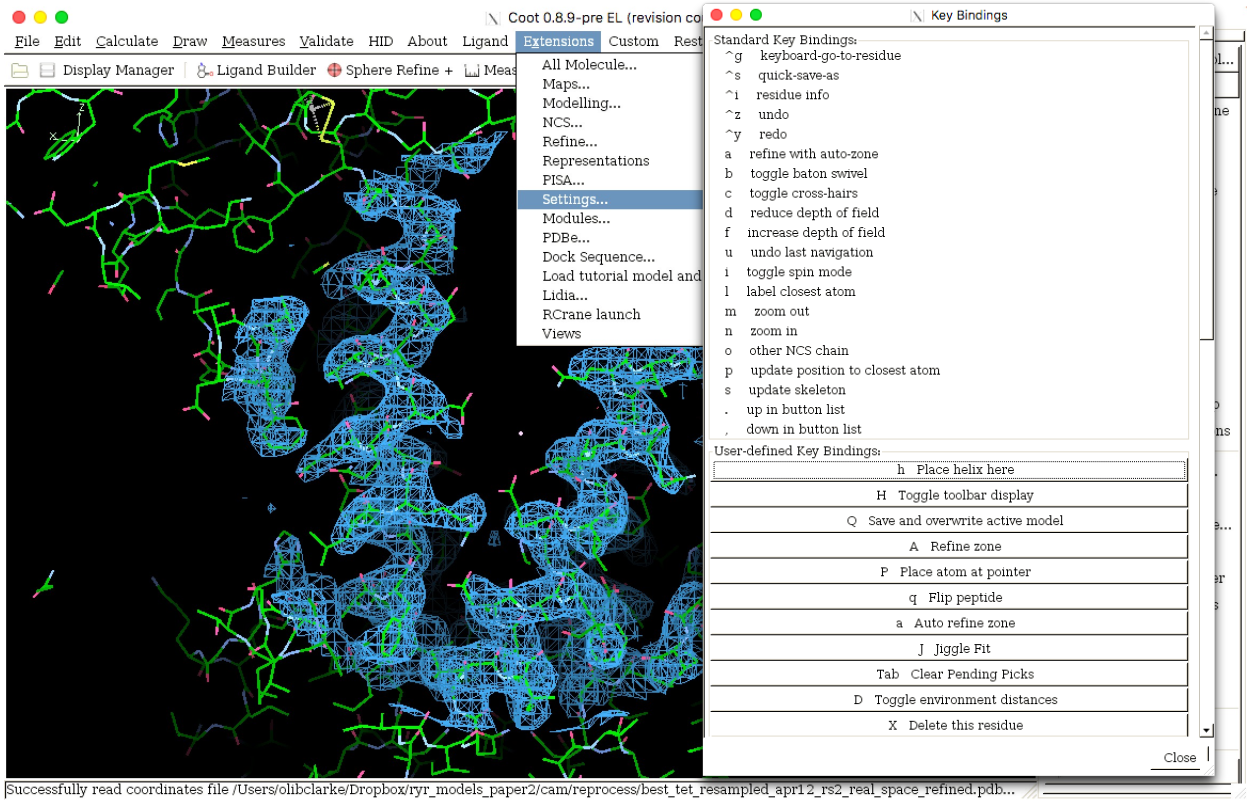

Standard Key Bindings:

^g	keyboard-go-to-residue
^s	quick-save-as
^i	residue info
^z	undo
^y	redo
a	refine with auto-zone
b	toggle baton swivel
c	toggle cross-hairs
d	reduce depth of field
f	increase depth of field
u	undo last navigation
i	toggle spin mode
l	label closest atom
m	zoom out
n	zoom in
o	other NCS chain
p	update position to closest atom
s	update skeleton
.	up in button list
,	down in button list

User-defined Key Bindings:

h	Place helix here
H	Toggle toolbar display
Q	Save and overwrite active model
A	Refine zone
P	Place atom at pointer
q	Flip peptide
a	Auto refine zone
J	Jiggle Fit
Tab	Clear Pending Picks
D	Toggle environment distances
X	Delete this residue

Successfully read coordinates file /Users/olibclarke/Dropbox/ryr_models_paper2/cam/reprocess/best_tet_resampled_apr12_rs2_real_space_refined.pdb...

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

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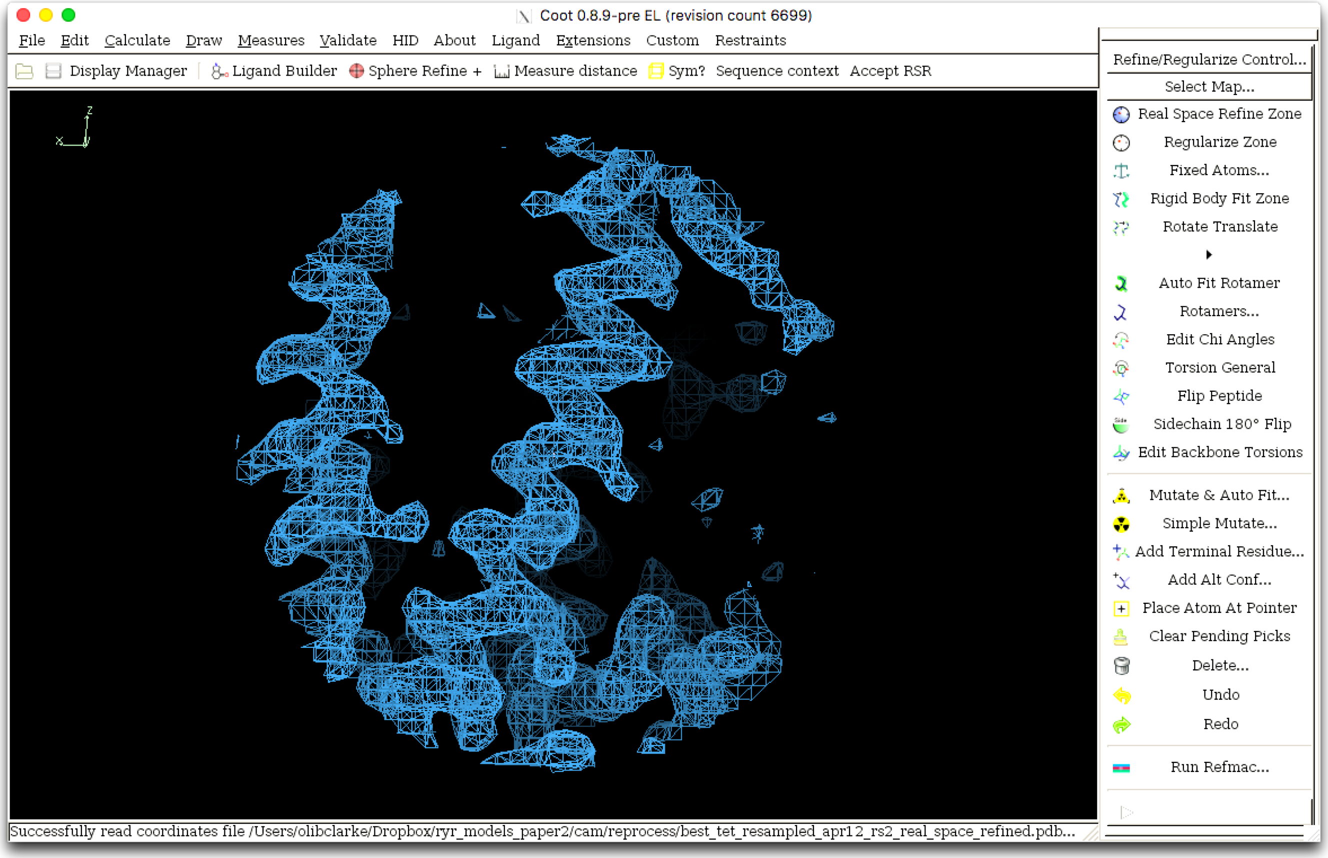
- Semi-automated helix placement
- Place cursor at the center of the helix and trigger ”Place helix here” (I suggest via a key binding - “h” with `coot-trimmings`)
- Coot will attempt to automatically determine the length and direction of the helix.
- Trim/extend, adjust weights, then refine using real-space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



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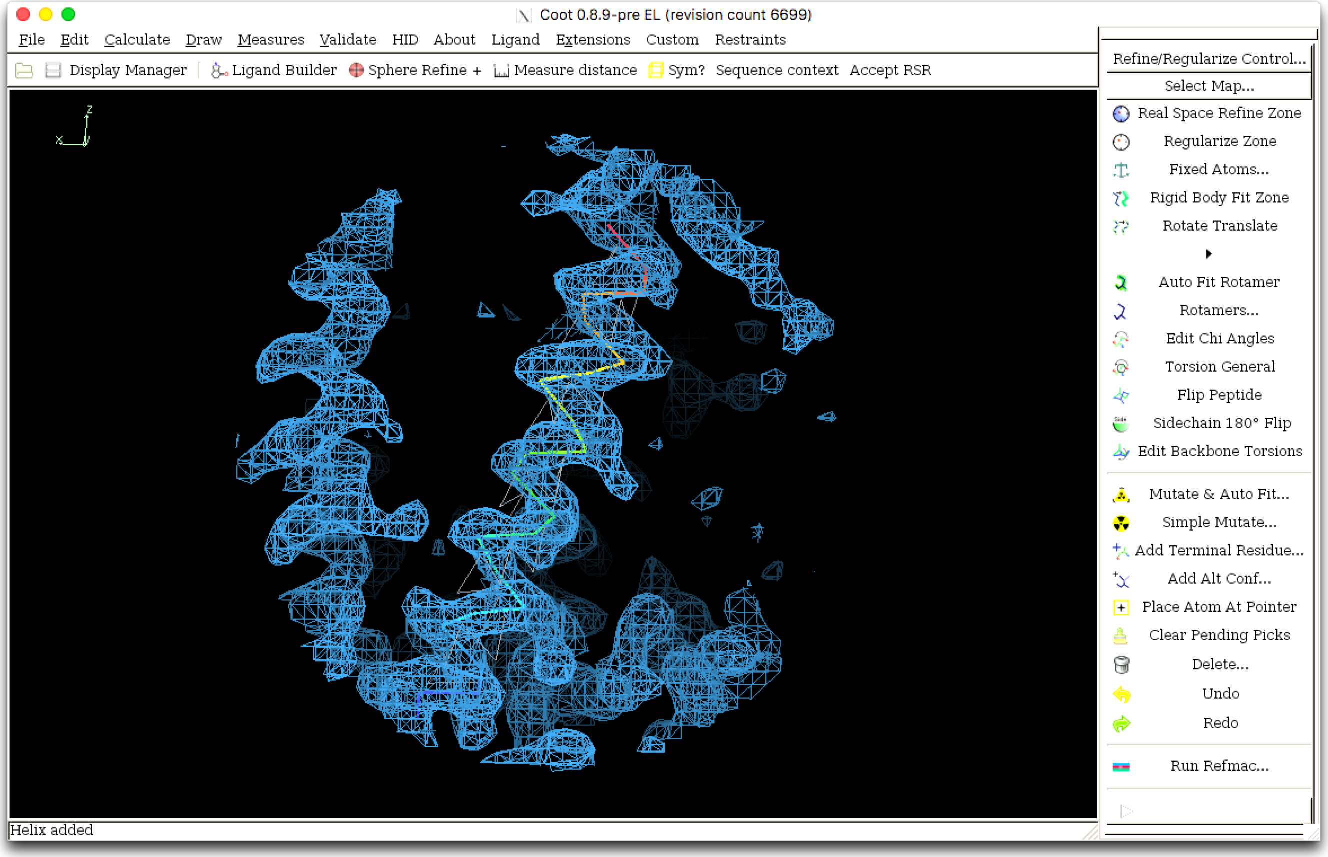

Helix added

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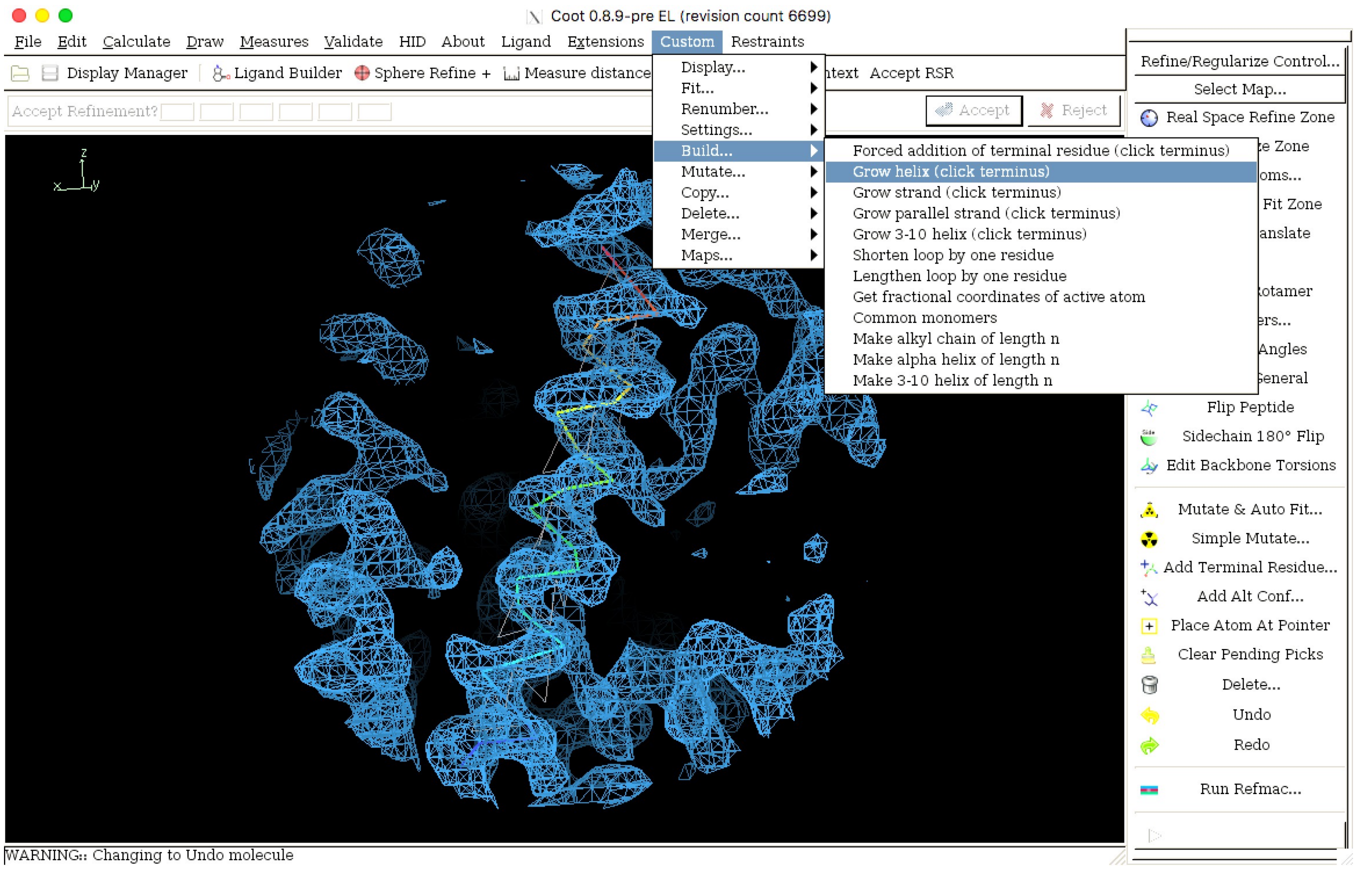
Coot 0.8.9-pre EL (revision count 6699)
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Custom Restraints
Display Manager
Ligand Builder
Sphere Refine +
Measure distance
ntext Accept RSR
Accept Refinement?
Accept
Reject
Display...
Fit...
Renumber...
Settings...
Build...
Mutate...
Copy...
Delete...
Merge...
Maps...
Forced addition of terminal residue (click terminus)
Grow helix (click terminus)
Grow strand (click terminus)
Grow parallel strand (click terminus)
Grow 3-10 helix (click terminus)
Shorten loop by one residue
Lengthen loop by one residue
Get fractional coordinates of active atom
Common monomers
Make alkyl chain of length n
Make alpha helix of length n
Make 3-10 helix of length n
Refine/Regularize Control...
Select Map...
Real Space Refine Zone
e Zone
oms...
Fit Zone
anslate
otamer
ers...
Angles
eneral
Flip Peptide
Sidechain 180° Flip
Edit Backbone Torsions
Mutate & Auto Fit...
Simple Mutate...
Add Terminal Residue...
Add Alt Conf...
Place Atom At Pointer
Clear Pending Picks
Delete...
Undo
Redo
Run Refmac...
z
x
y

WARNING:: Changing to Undo molecule

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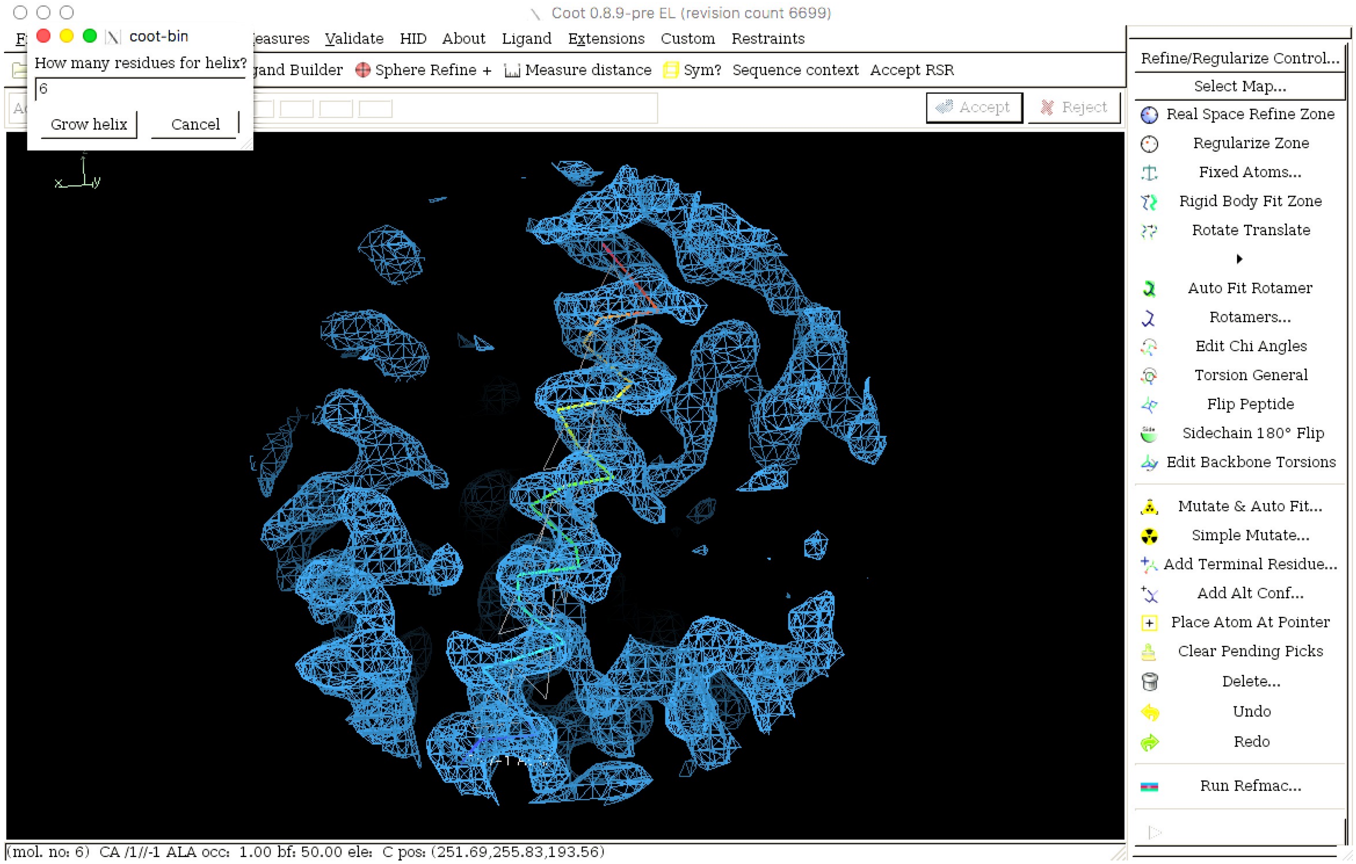
Coot 0.8.9-pre EL (revision count 6699)

coot-bin

How many residues for helix?

6

(mol. no: 6) CA /1//-1 ALA occ: 1.00 bf: 50.00 ele: C pos: (251.69,255.83,193.56)

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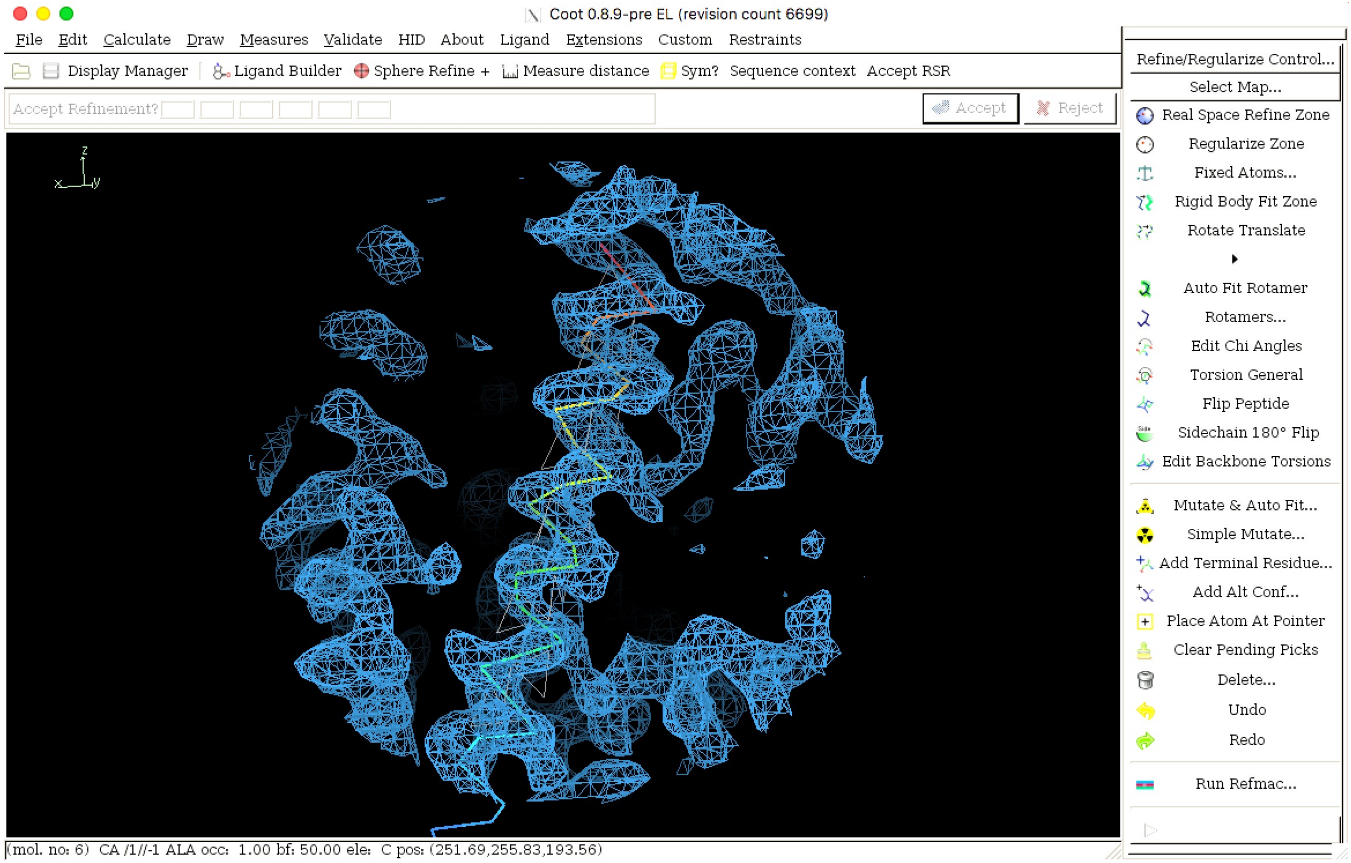

```
(mol. no: 6)  CA /1//-1 ALA occ:  1.00 bf: 50.00 ele:  C pos: (251.69,255.83,193.56)
```

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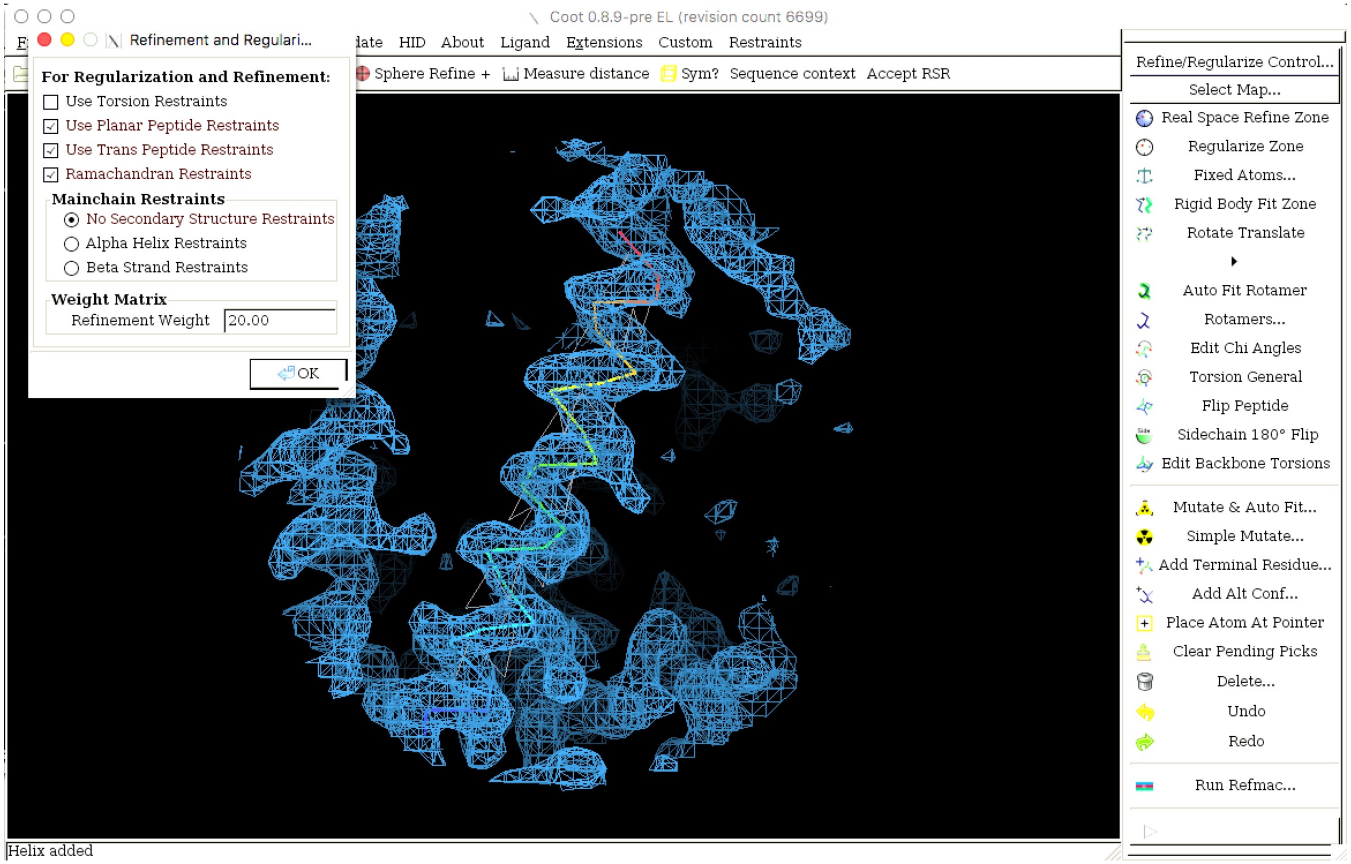

For Regularization and Refinement:

☐ Use Torsion Restraints
☑ Use Planar Peptide Restraints
☑ Use Trans Peptide Restraints
☑ Ramachandran Restraints

Mainchain Restraints

● No Secondary Structure Restraints
○ Alpha Helix Restraints
○ Beta Strand Restraints

Weight Matrix

Refinement Weight 20.00

Helix added

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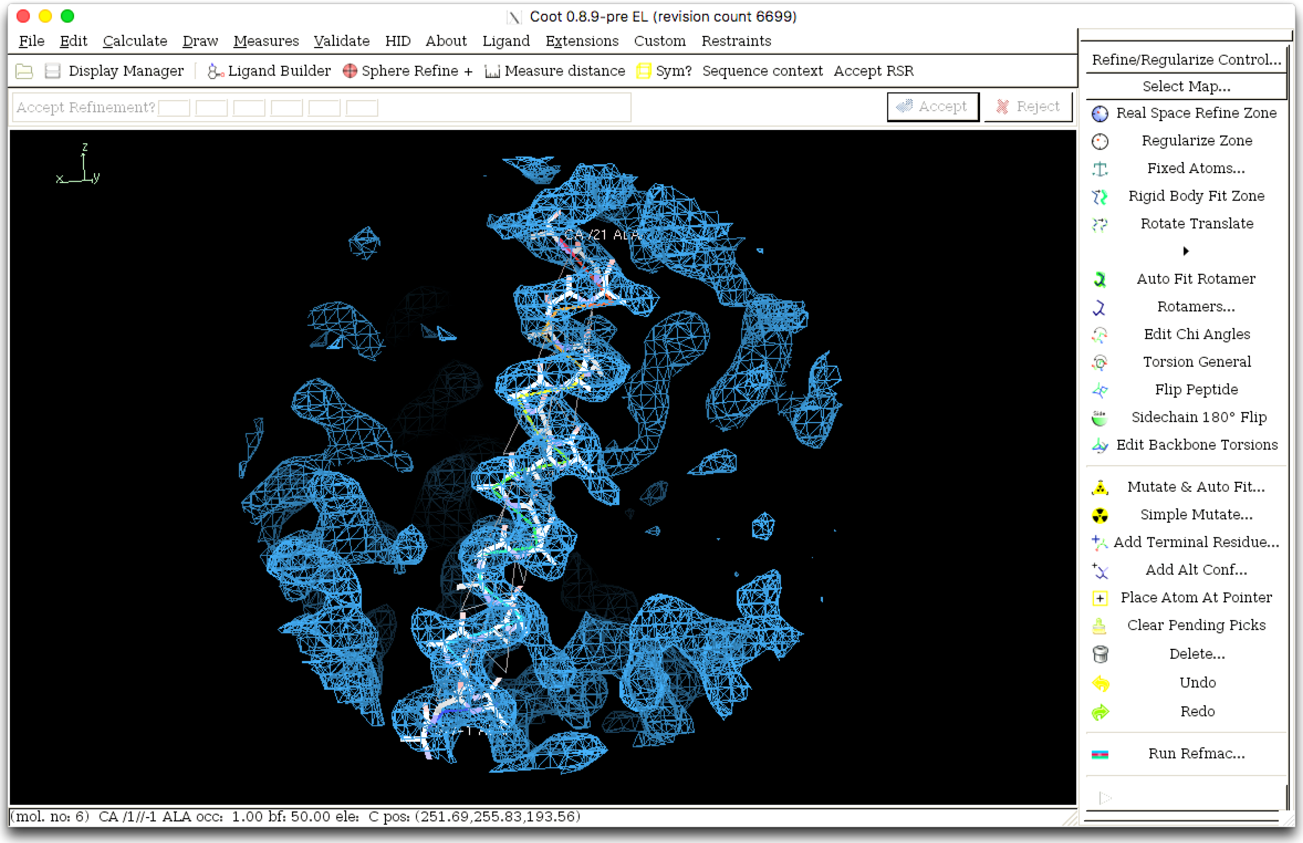
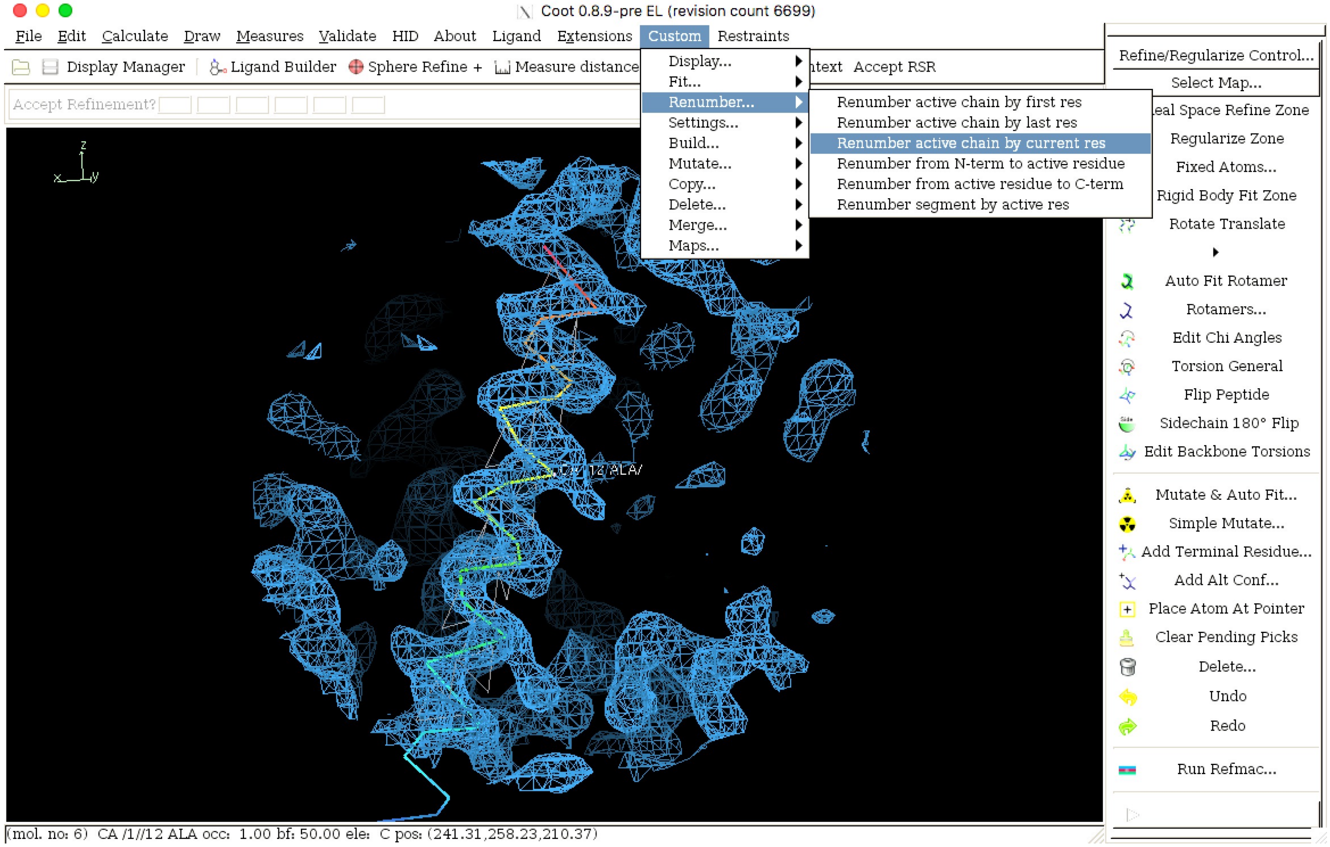


(mol. no: 6) CA /1//-1 ALA occ: 1.00 bf: 50.00 ele: C pos: (251.69,255.83,193.56)

COOT – Crystallographic Object Oriented Toolkit

- Sequence assignment.
- **Adjust numbering to match expected position in sequence.**
- Mutate to match sequence
- Fill sidechains manually.
- Adjust sequence register to optimize local fit to sidechain densities.



(mol. no: 6) CA /1//12 ALA occ: 1.00 bf: 50.00 ele: C pos: (241.31,258.23,210.37)

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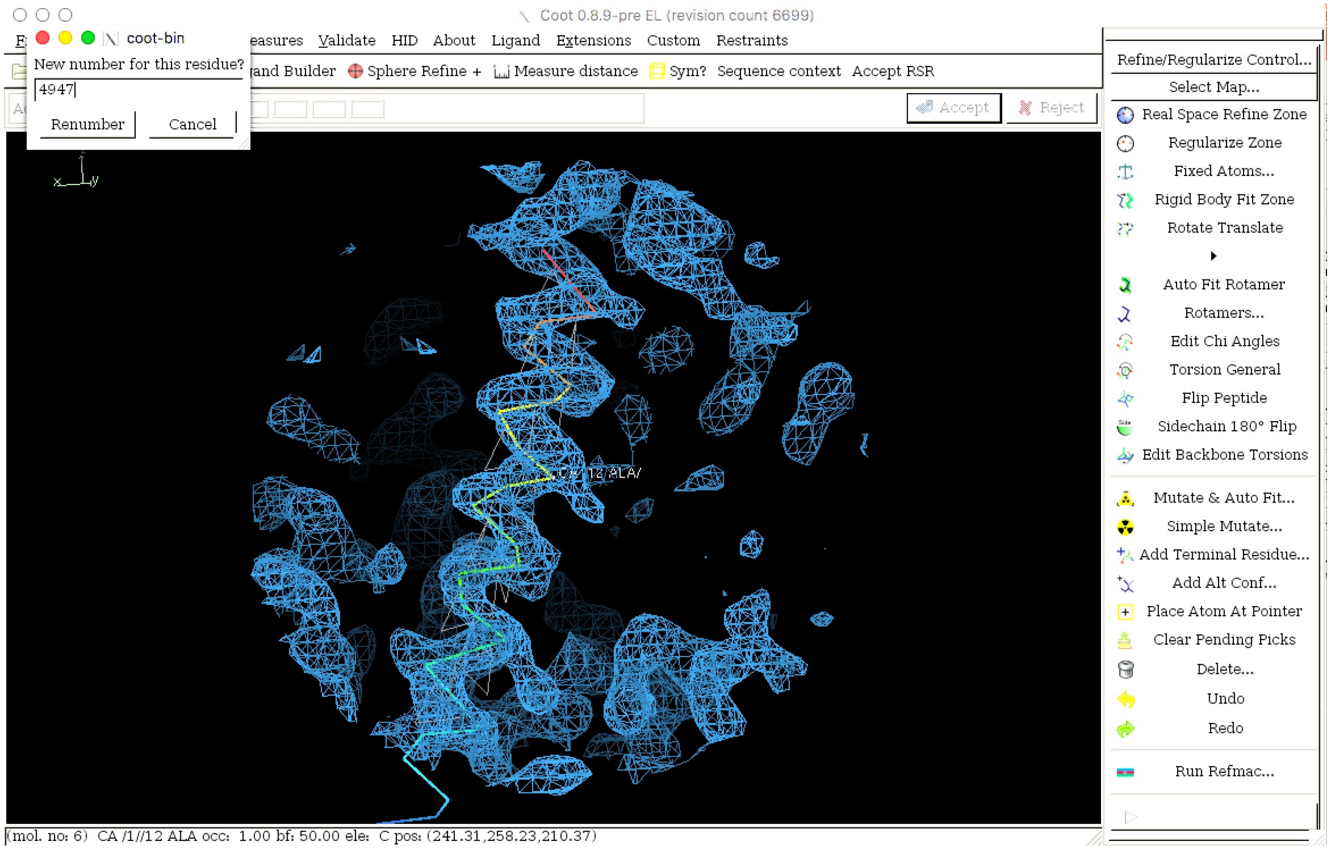

Coot 0.8.9-pre EL (revision count 6699)

coot-bin

New number for this residue?

4947

(mol. no: 6) CA /1//12 ALA occ: 1.00 bf: 50.00 ele: C pos: (241.31,258.23,210.37)

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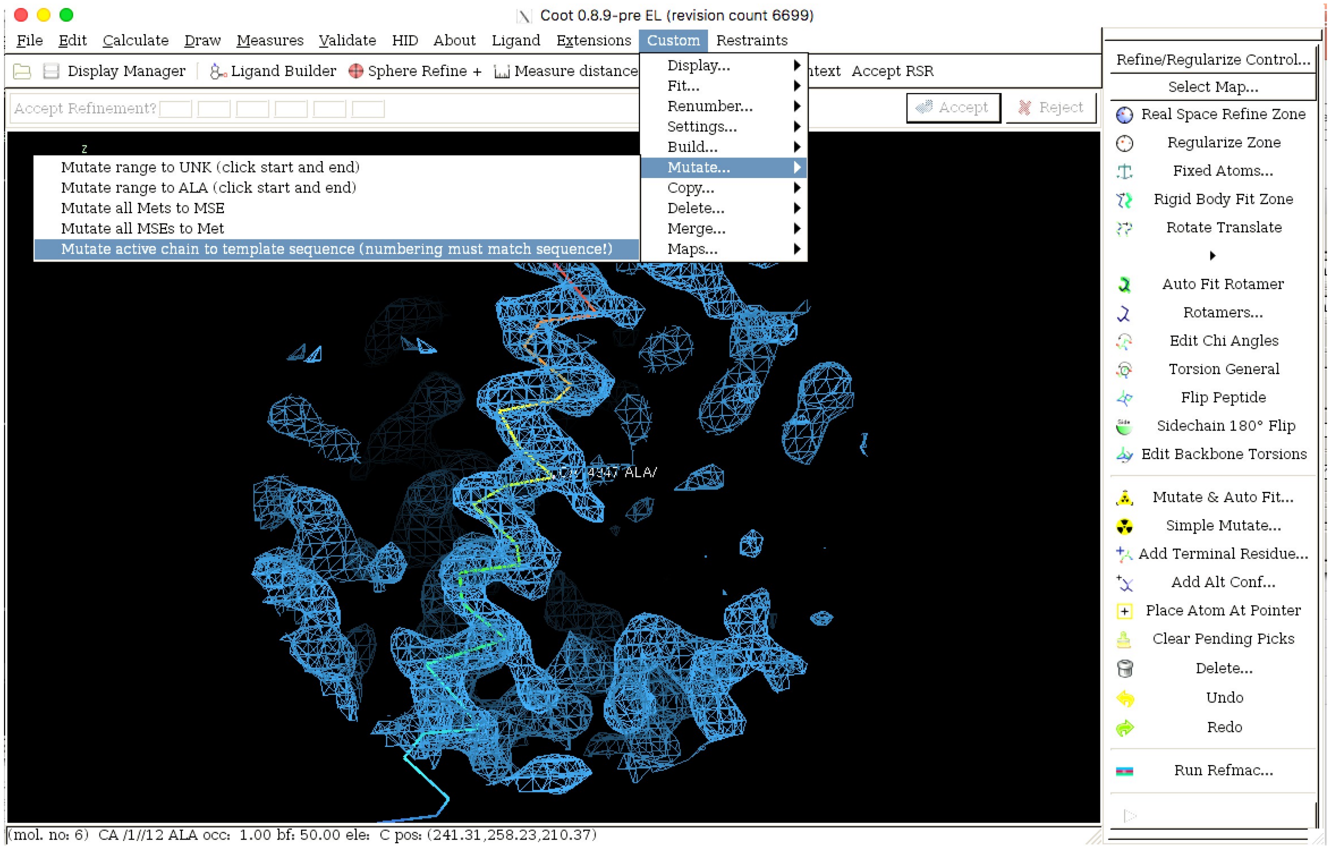

```
(mol. no: 6)  CA /1//12 ALA occ:  1.00 bf: 50.00 ele:  C pos: (241.31,258.23,210.37)
```

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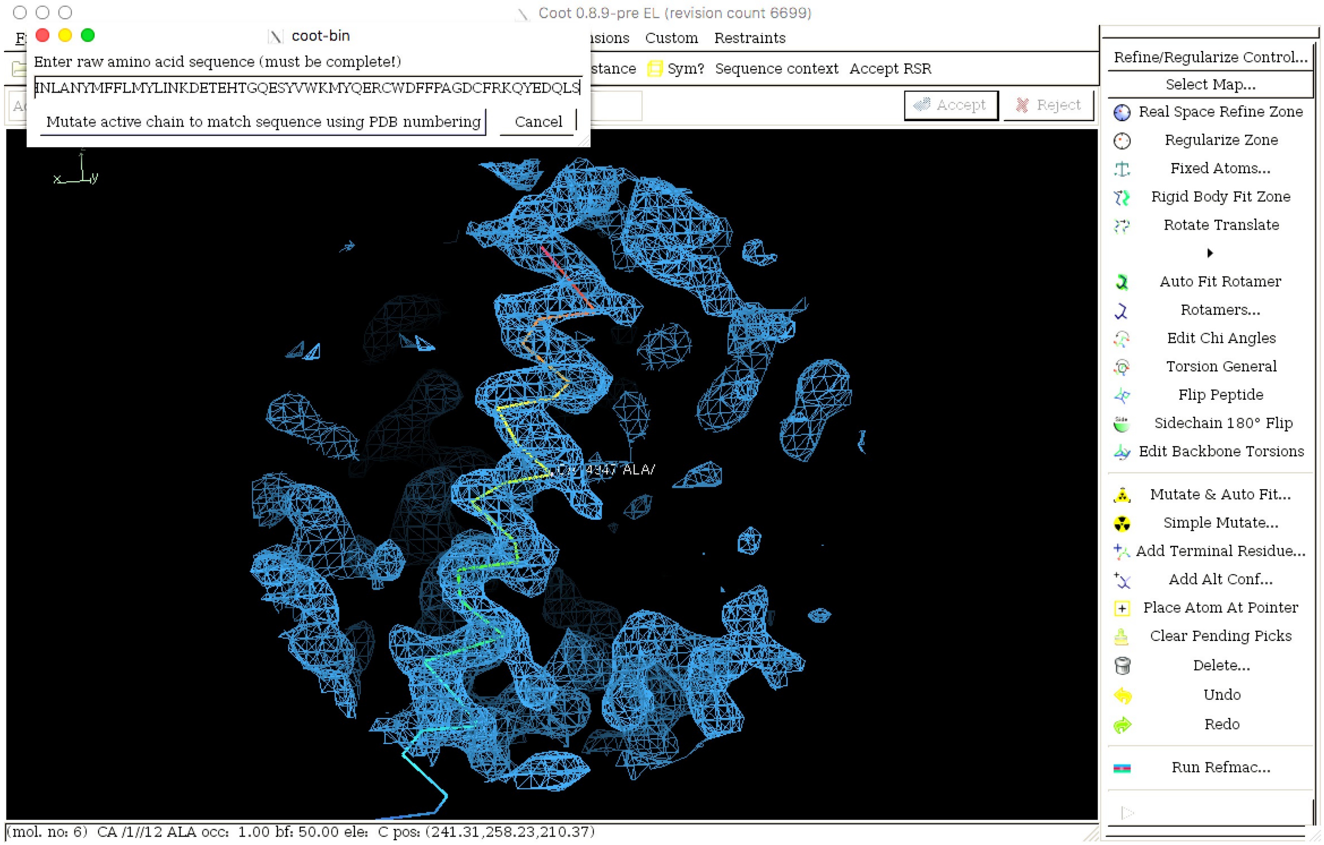

coot-bin

Enter raw amino acid sequence (must be complete!)

```
INLANYMFFLMYLINKDETEHTGQESYVWKMYQERCWDFFPAGDCFRKQYEDQLS
```

(mol. no: 6) CA /1//12 ALA occ: 1.00 bf: 50.00 ele: C pos: (241.31,258.23,210.37)

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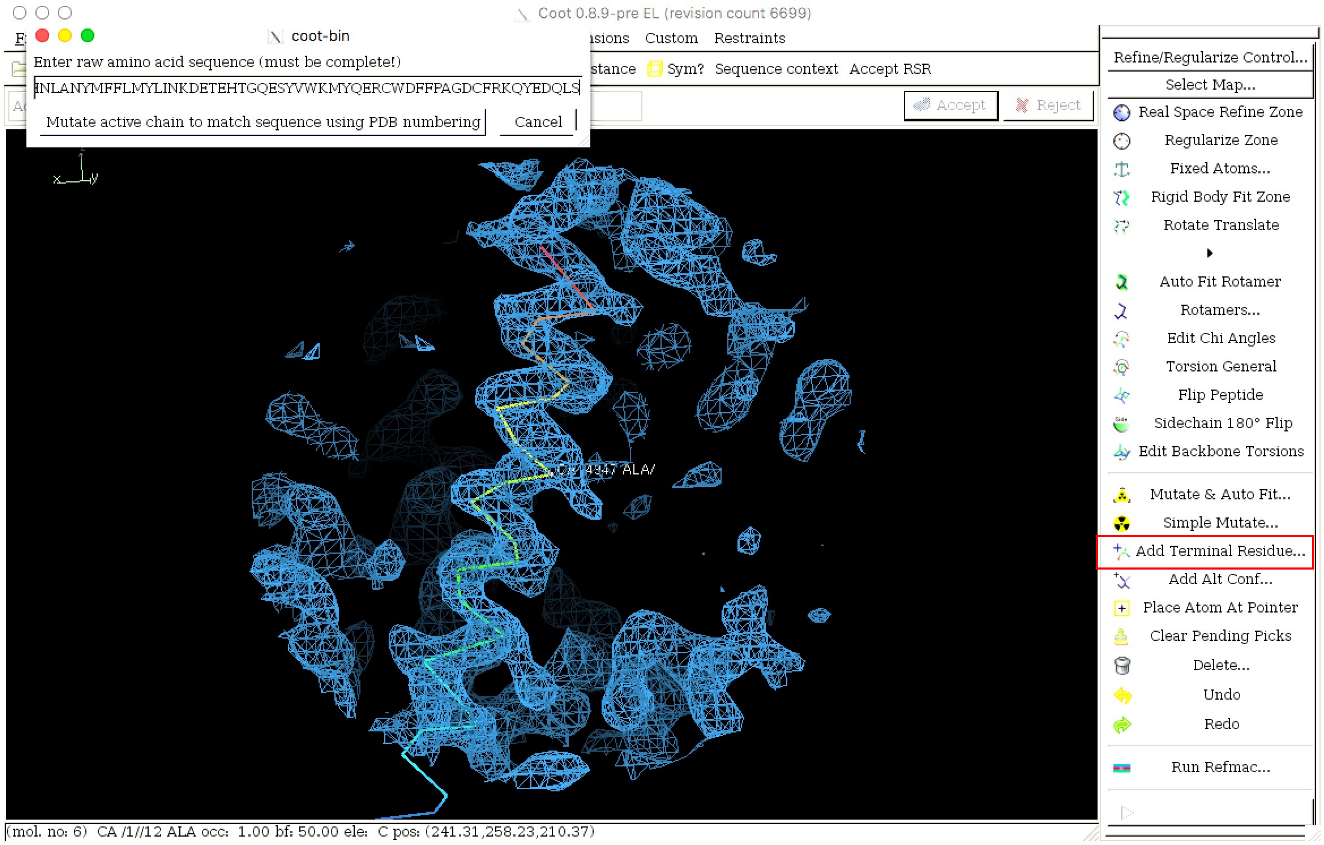
- Fill sidechains manually.
- Adjust sequence register to optimize local fit to sidechain densities.



Coot 0.8.9-pre EL (revision count 6699)

coot-bin

Enter raw amino acid sequence (must be complete!)

INLANYMFFLMYLINKDETEHTGQESYVWKMYQERCWDFFPAGDCFRKQYEDQLS

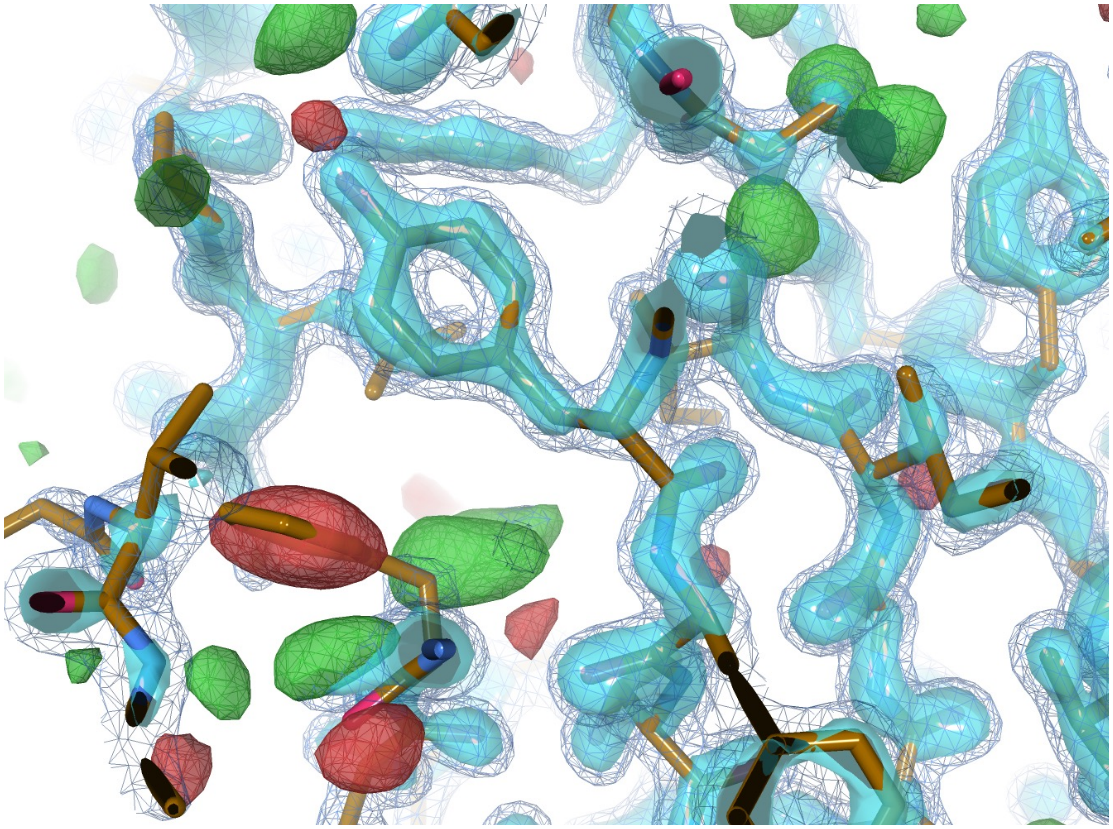
Mutate active chain to match sequence using PDB numbering

Cancel

(mol. no: 6) CA /1//12 ALA occ: 1.00 bf: 50.00 ele: C pos: (241.31,258.23,210.37)

Use 'Add Terminal residue' to extend chain.

COOT 1.0 coming soon! Improved visualization, refinement, modern GUI



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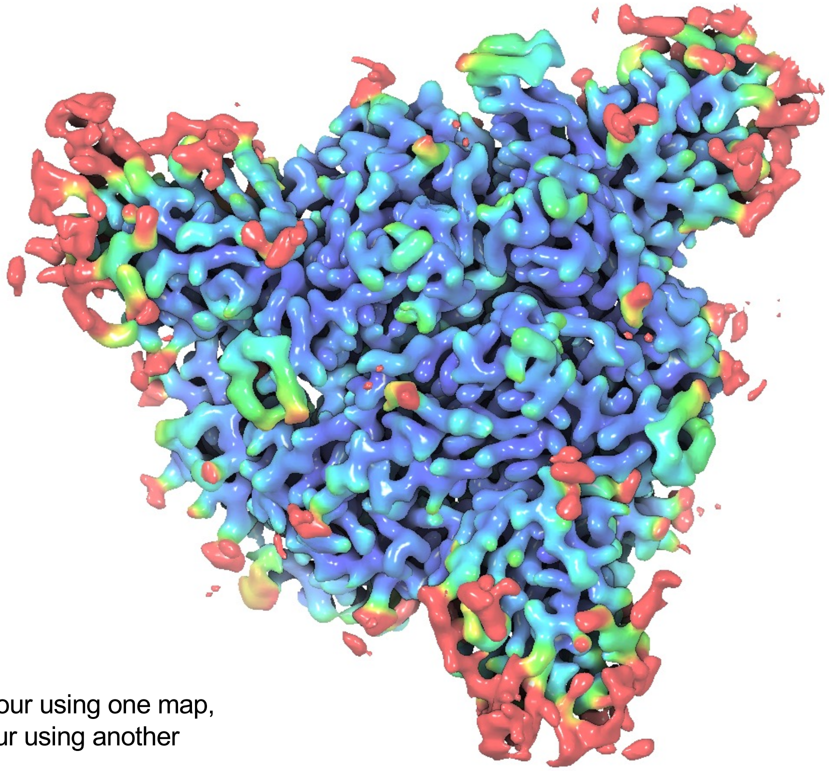

Contour using one map,
Colour using another

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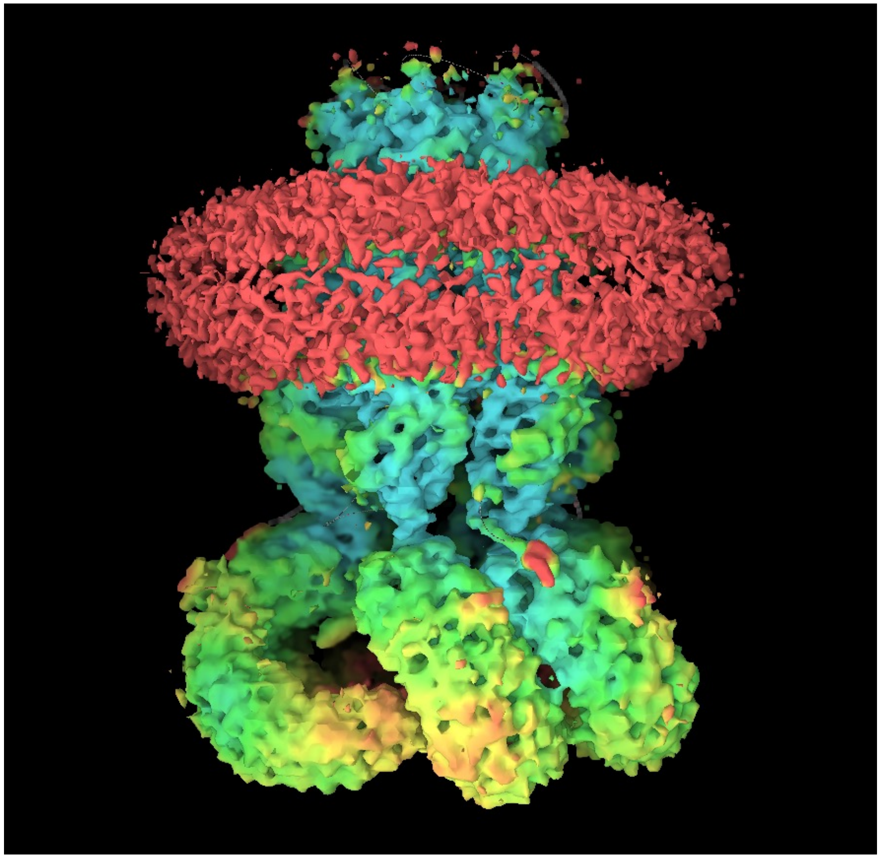

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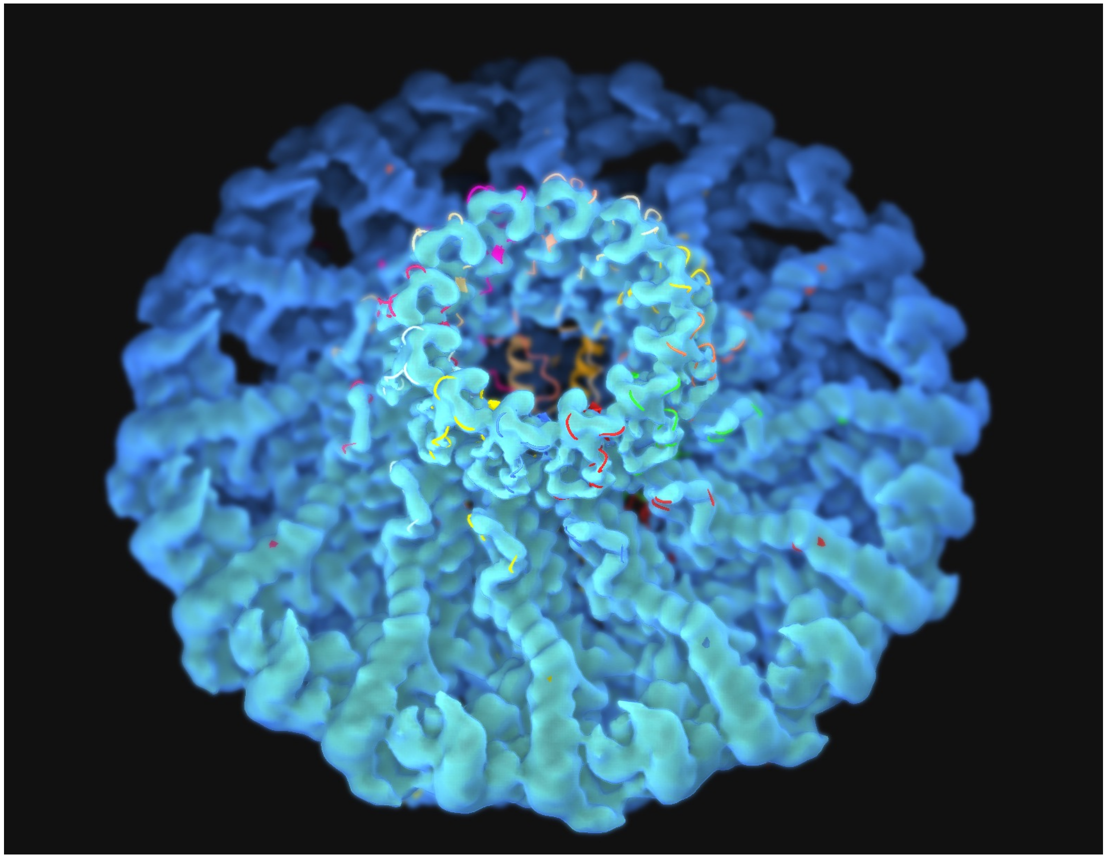

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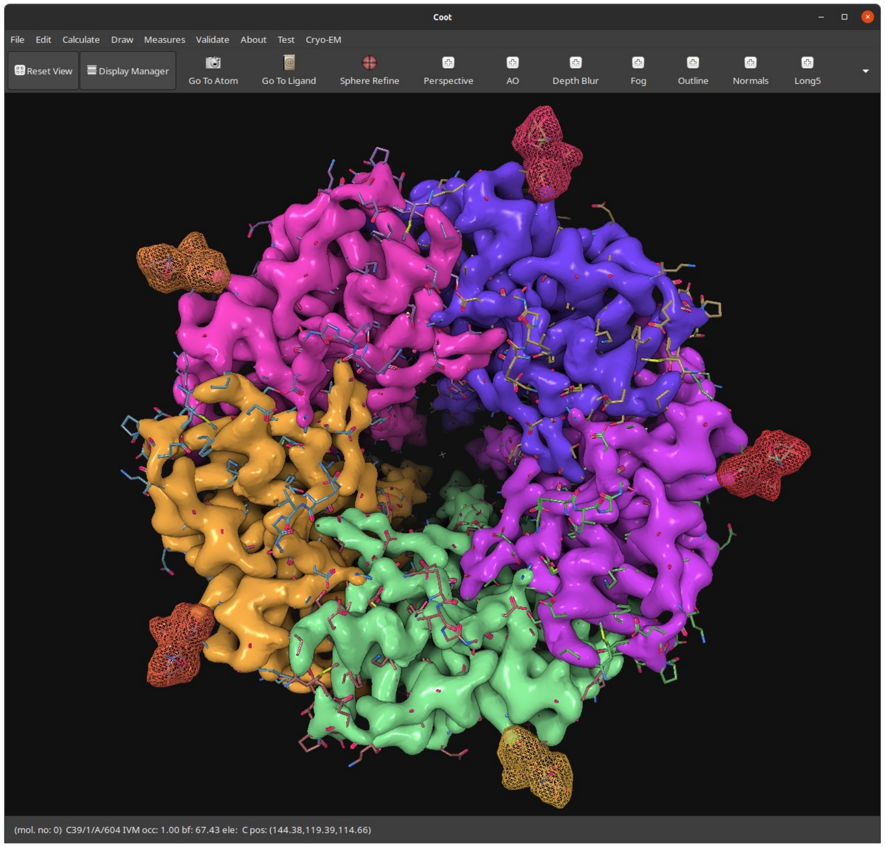
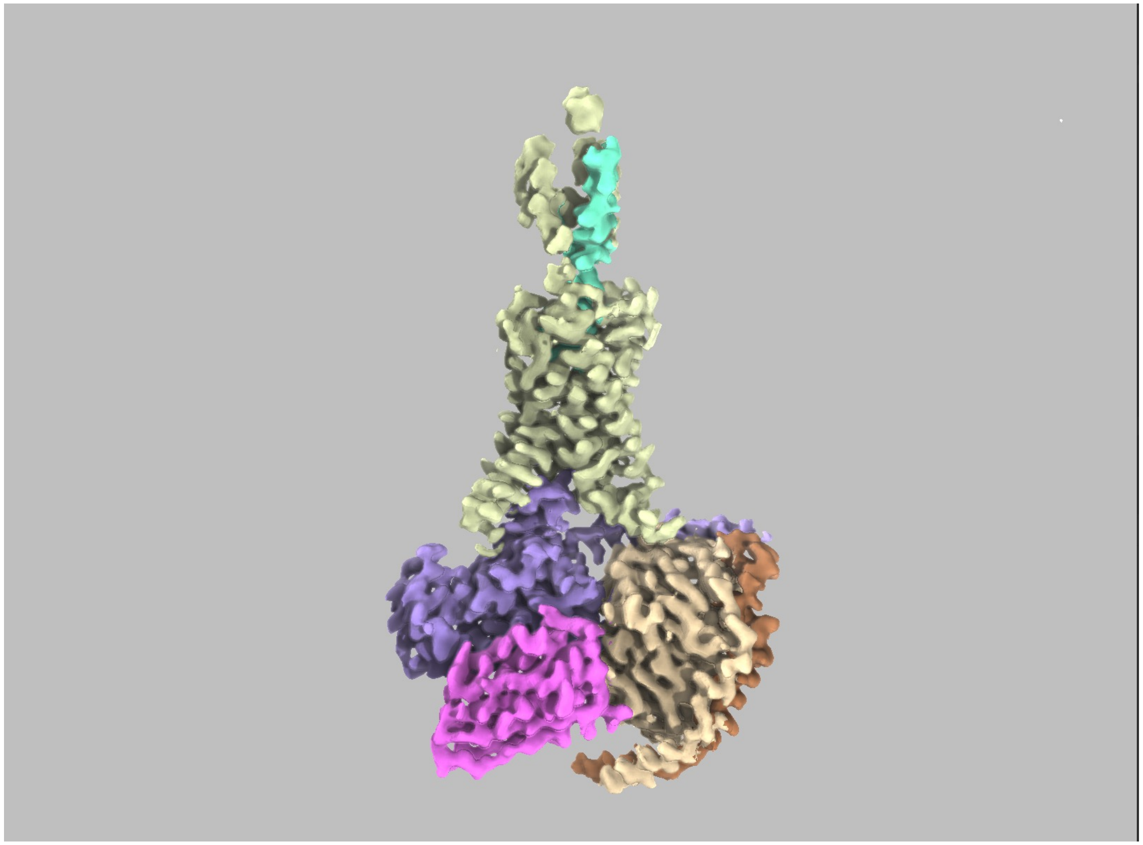


```
(mol. no: 0)  C39/1/A/604 IVM occ: 1.00 bf: 67.43 ele:  C pos: (144.38,119.39,114.66)
```

COOT 1.0 coming soon! Improved visualization, refinement, modern GUI



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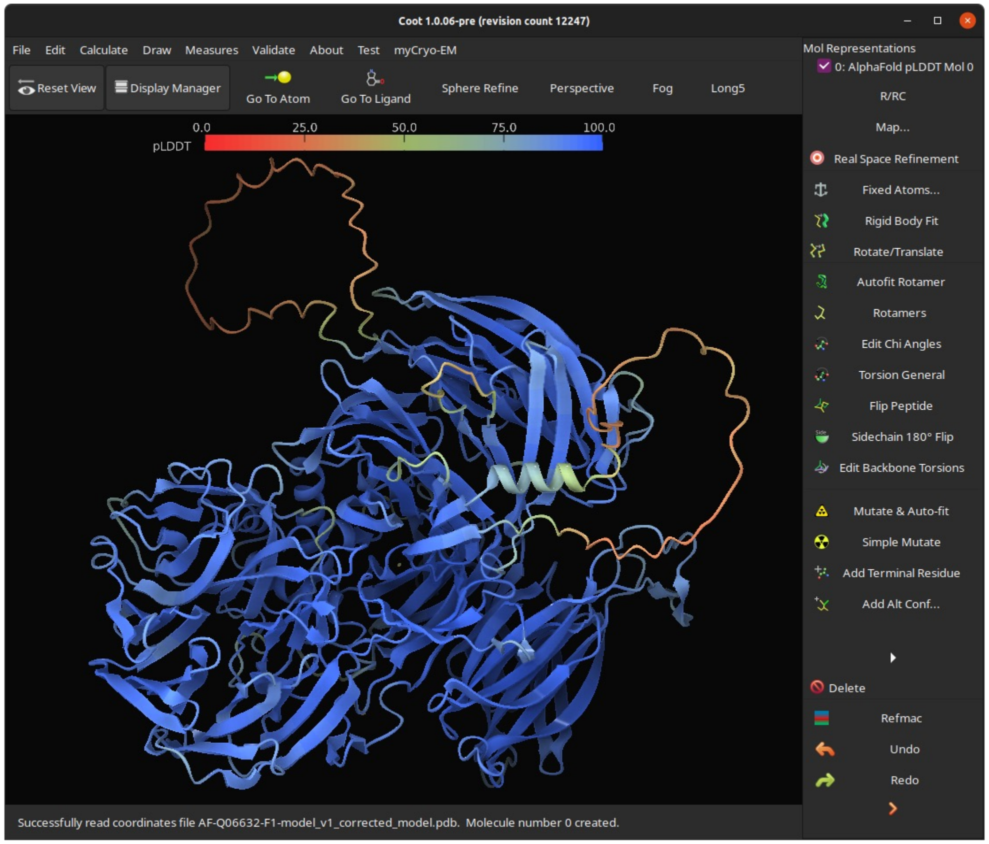

Successfully read coordinates file AF-Q06632-F1-model_v1_corrected_model.pdb. Molecule number 0 created.

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

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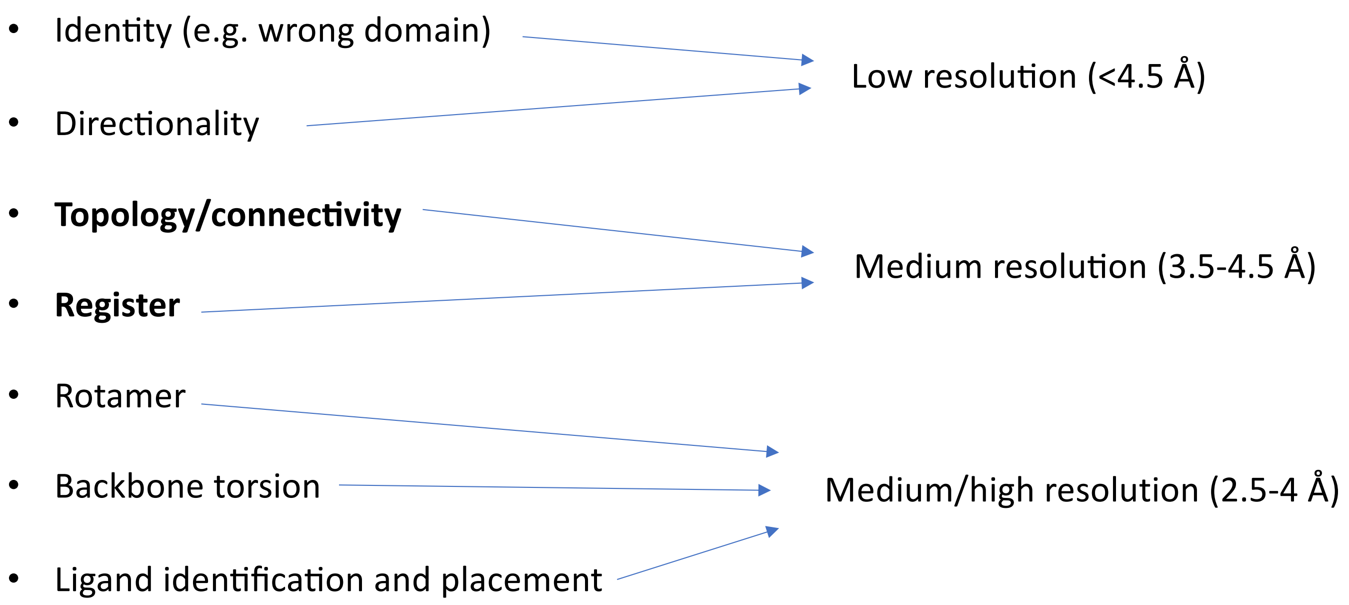

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Low resolution (<4.5 Å)

Medium resolution (3.5-4.5 Å)

Medium/high resolution (2.5-4 Å)

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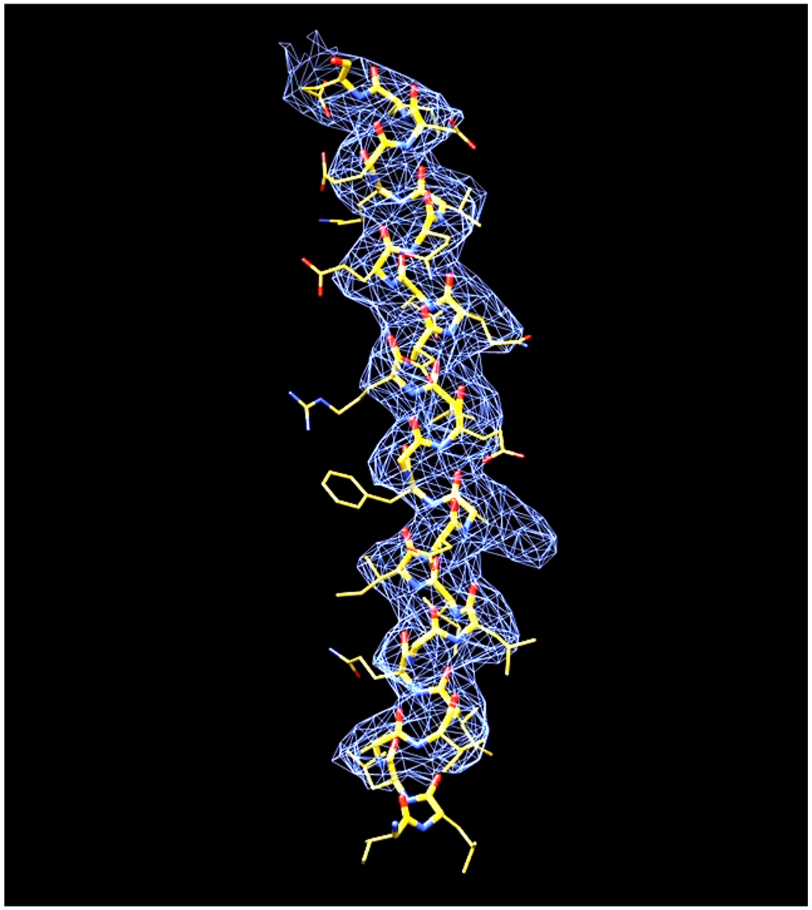

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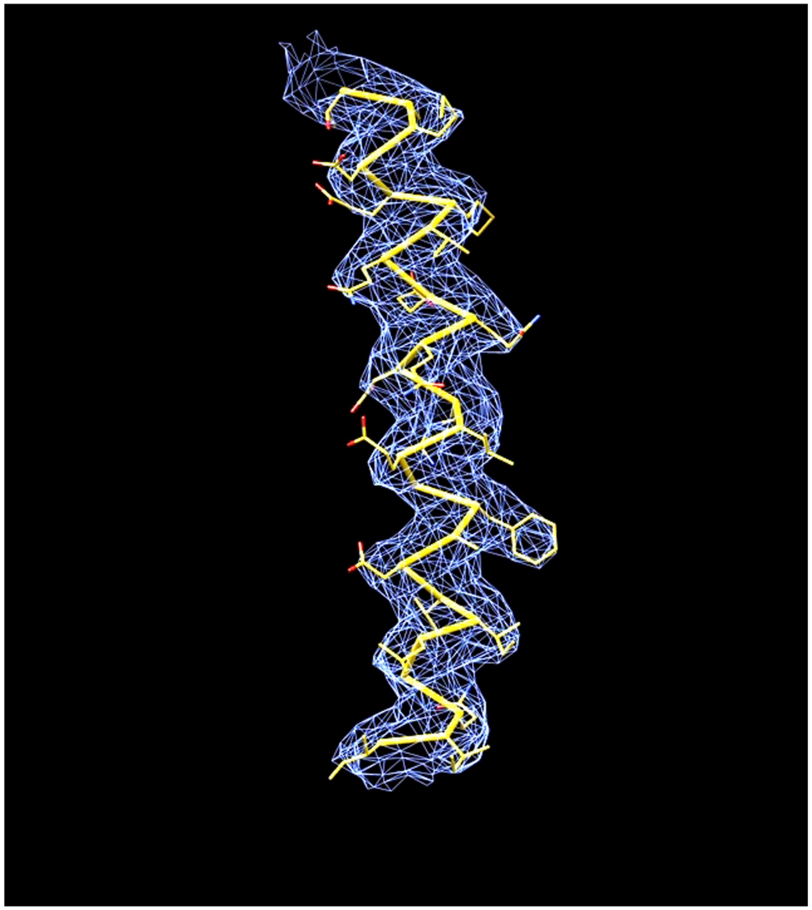

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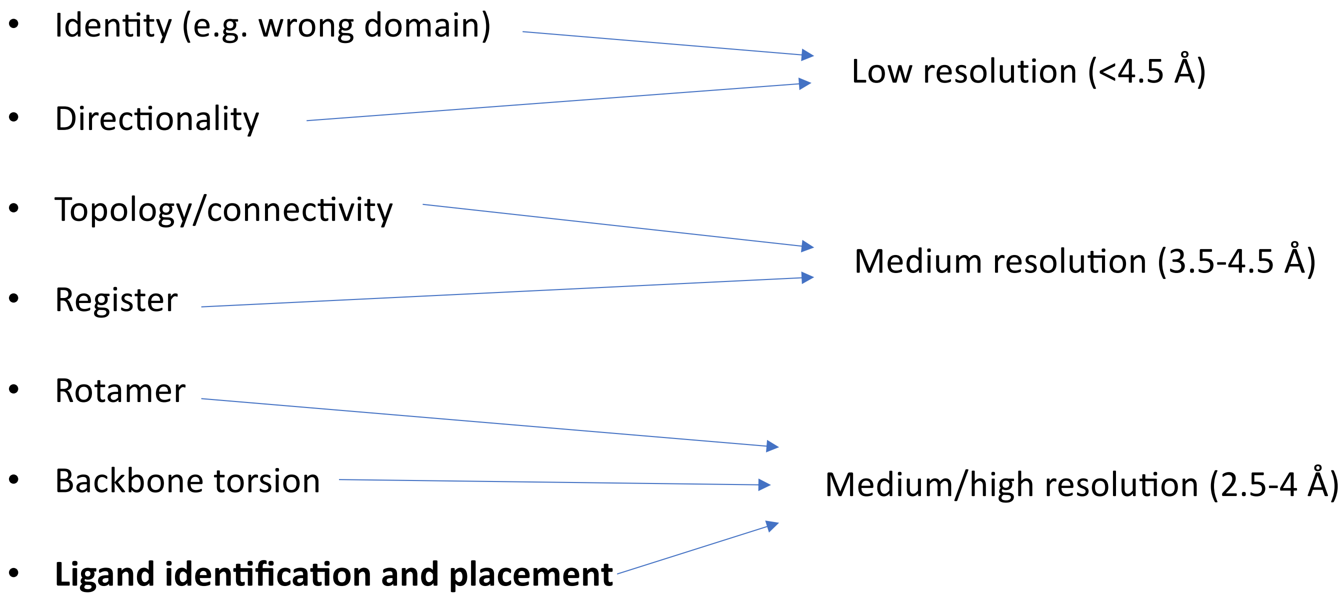

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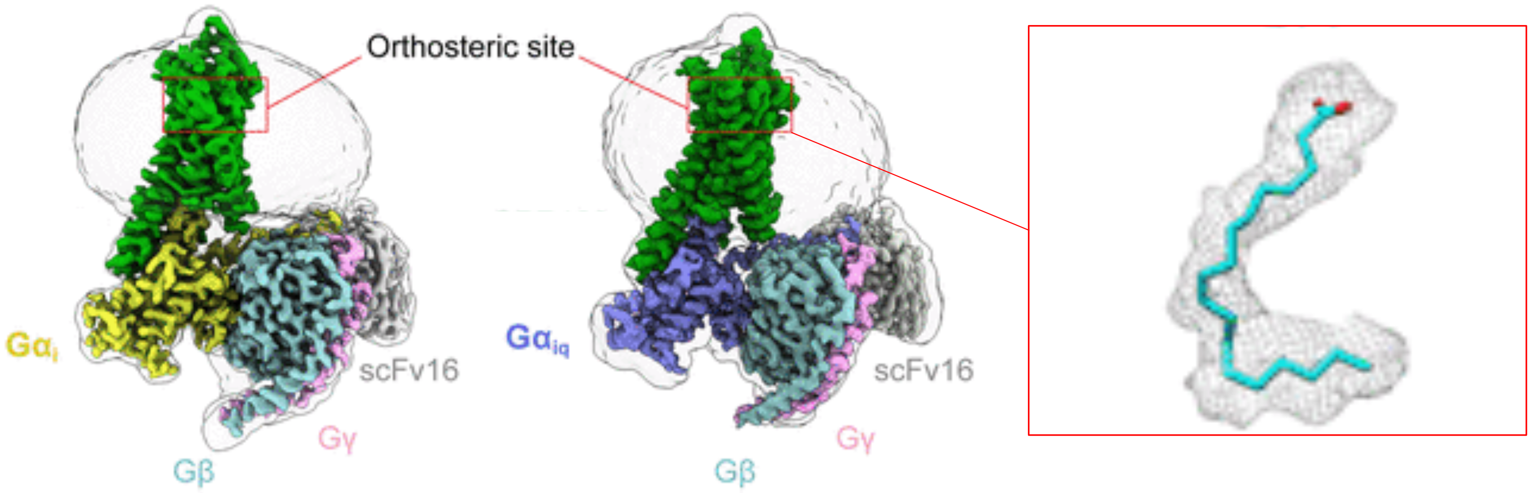
Cautionary note – don’t believe everything you read in the (Nature/Science/Cell) paper…



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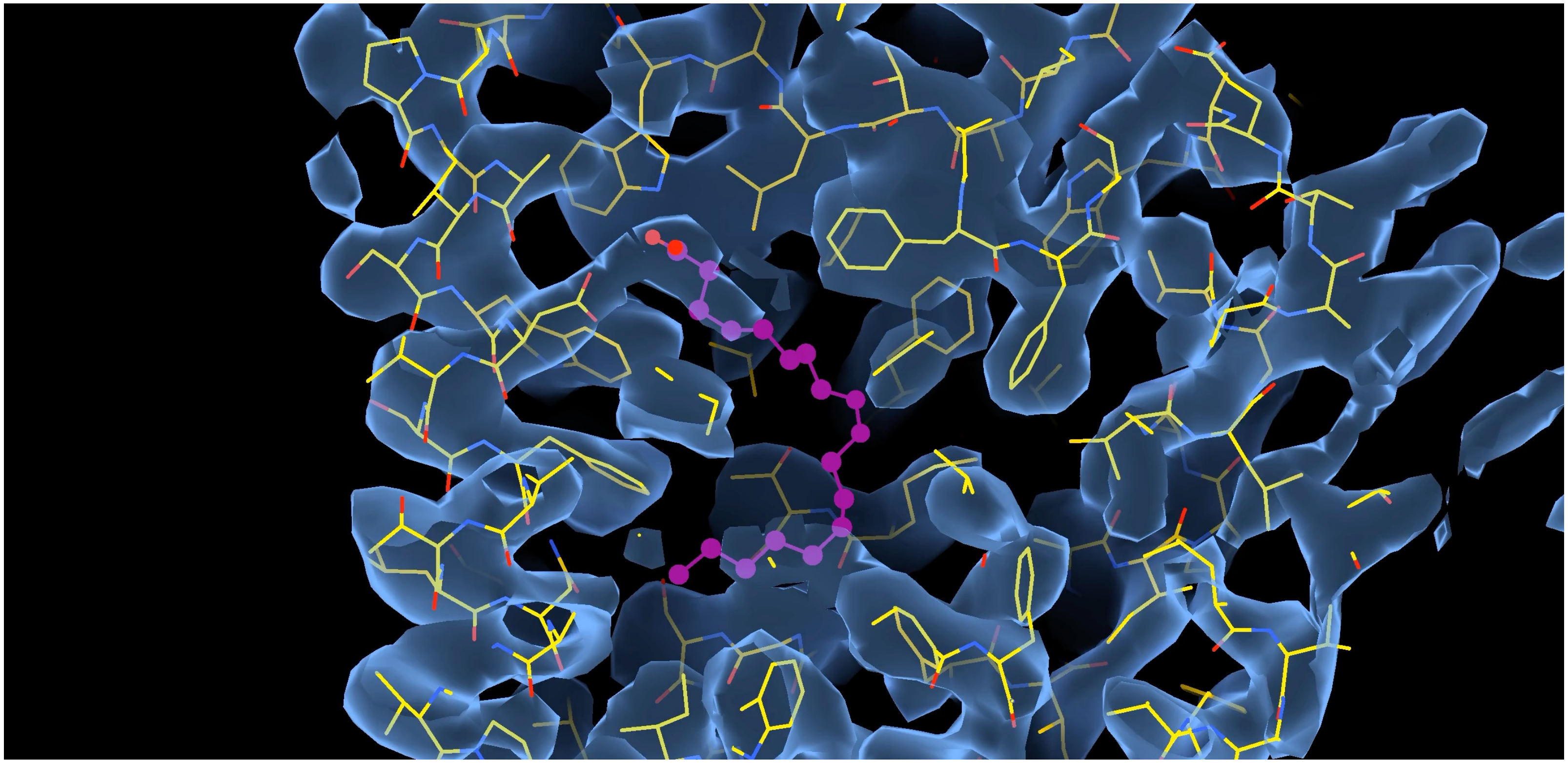

Ligand invisible at any reasonable contour; protein density anisotropic and broken.

So how was nice density figure for ligand made? By carving the map around the ligand. Please don’t do this!

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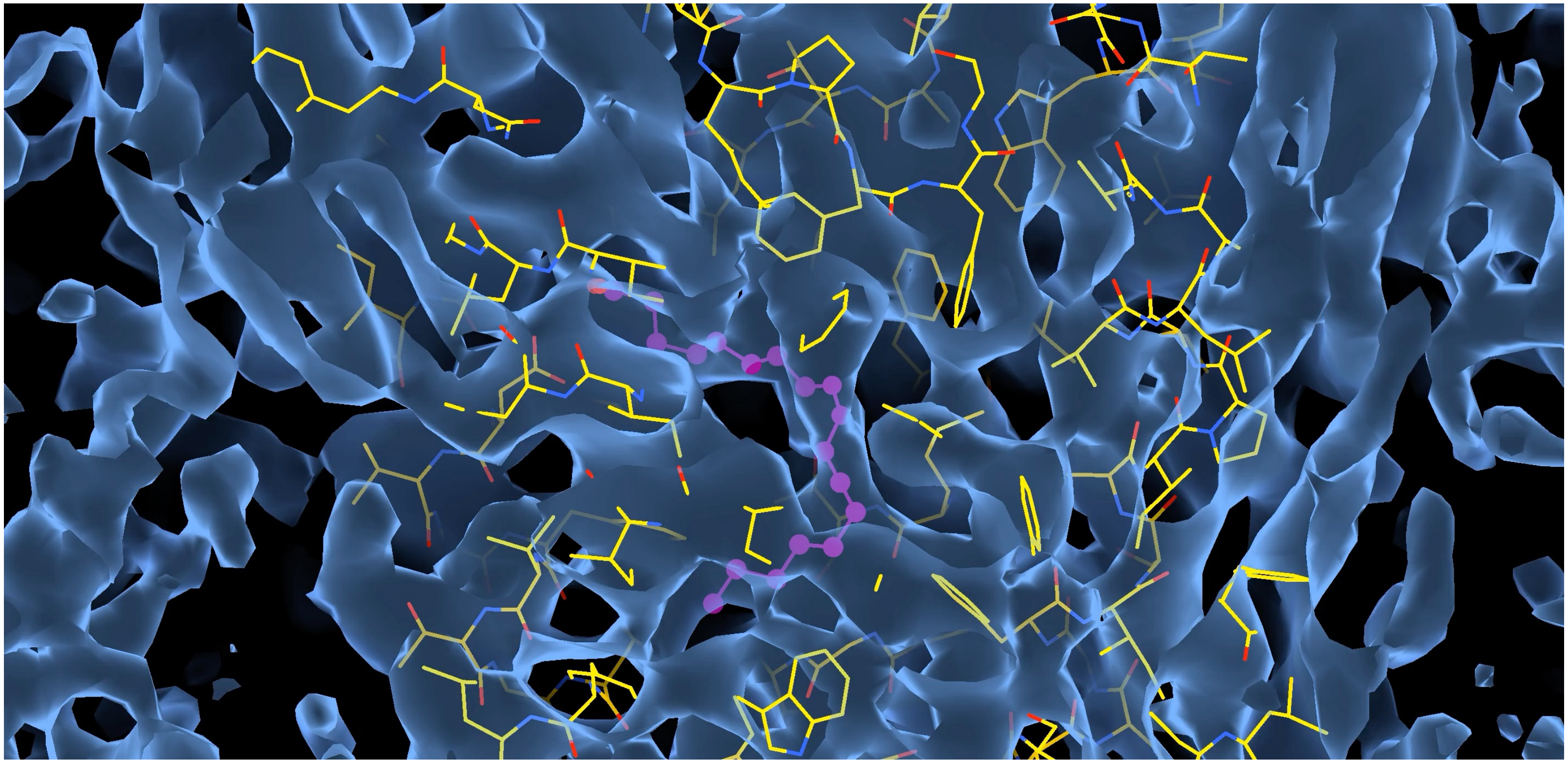

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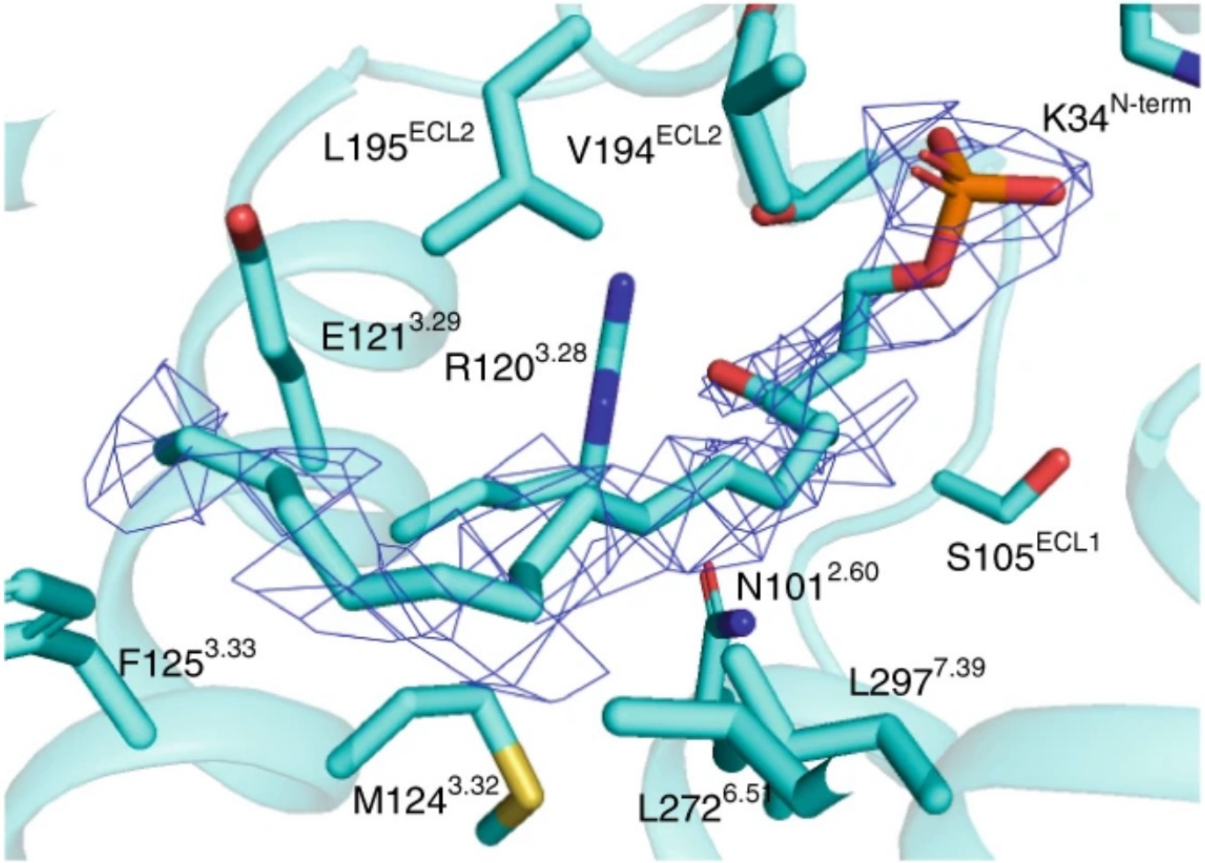

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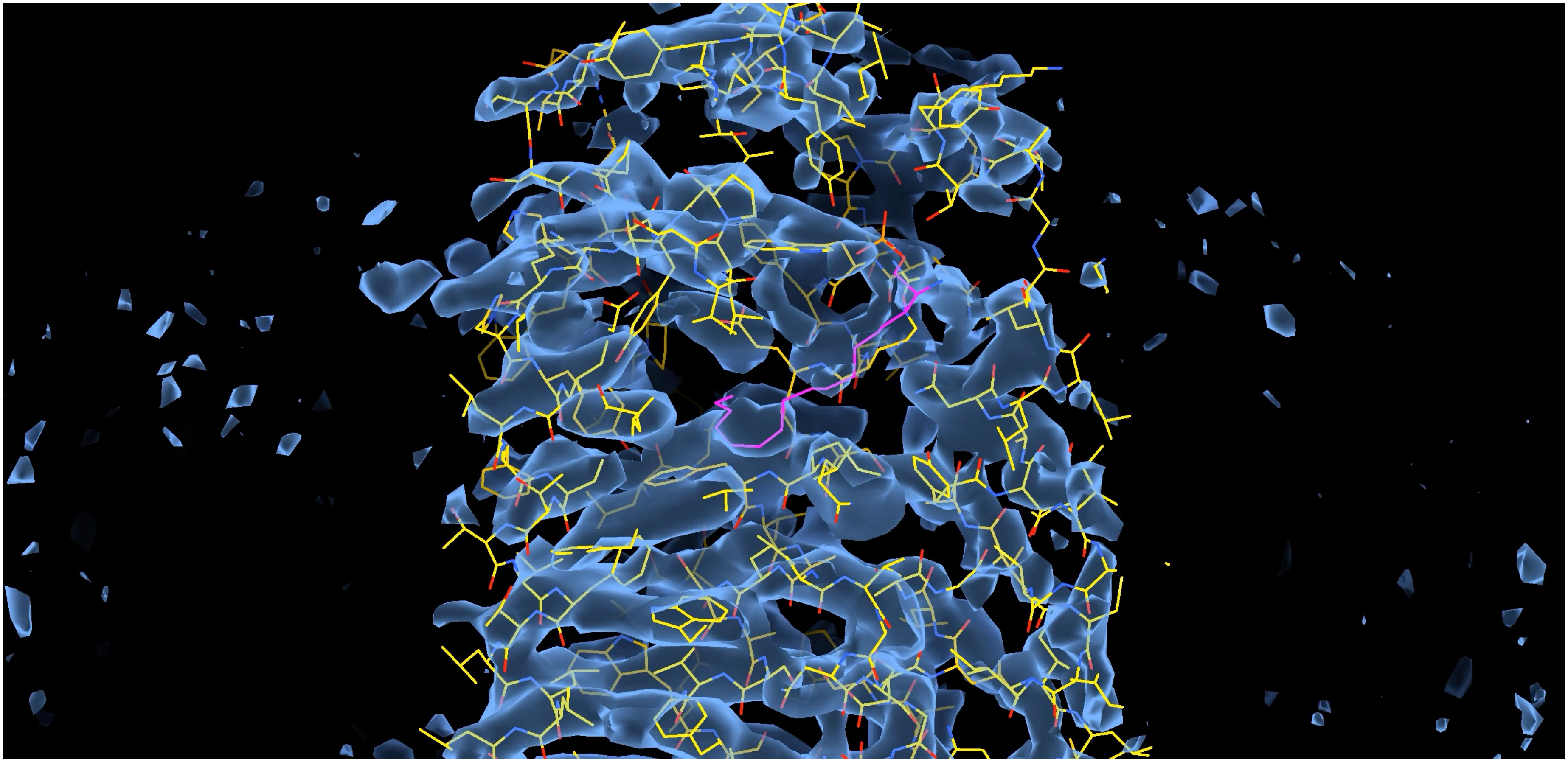


Strategy for identifying and correcting errors.

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

- Use EM-ringer (or other local metrics – Q-score, Strudel score, MEDIC all useful) to identify errors in backbone and rotamer geometry.

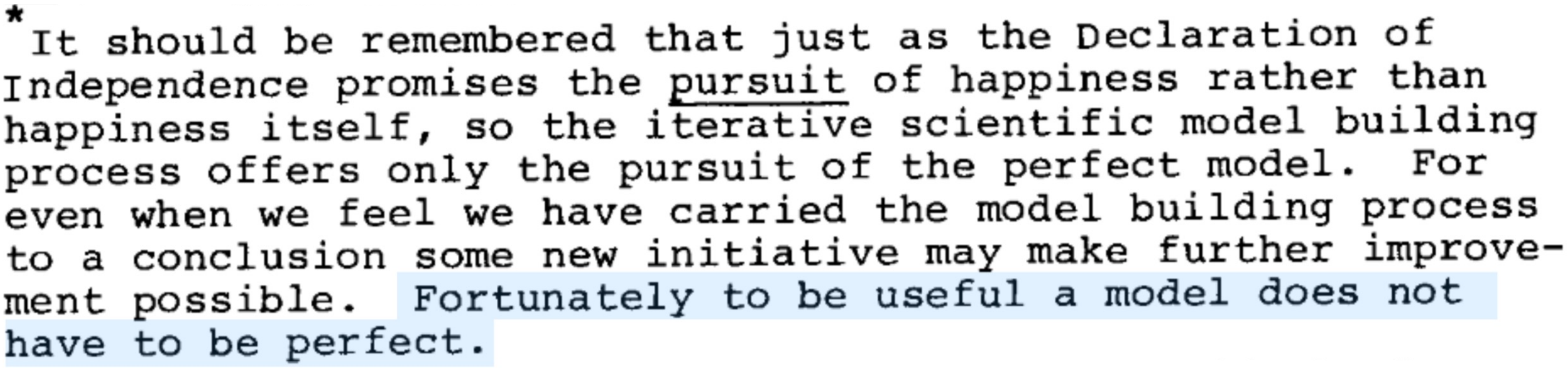
- **Look at everything! Manually check and recheck the fit of every residue. Tedious but necessary.**

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

- Half-map FSCs are useful to analyze/identify overfitting during refinement, but 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