Scientific Computing

Model Validation

Agnel Praveen Joseph
CCP-EM STFC

Why are structural details important?

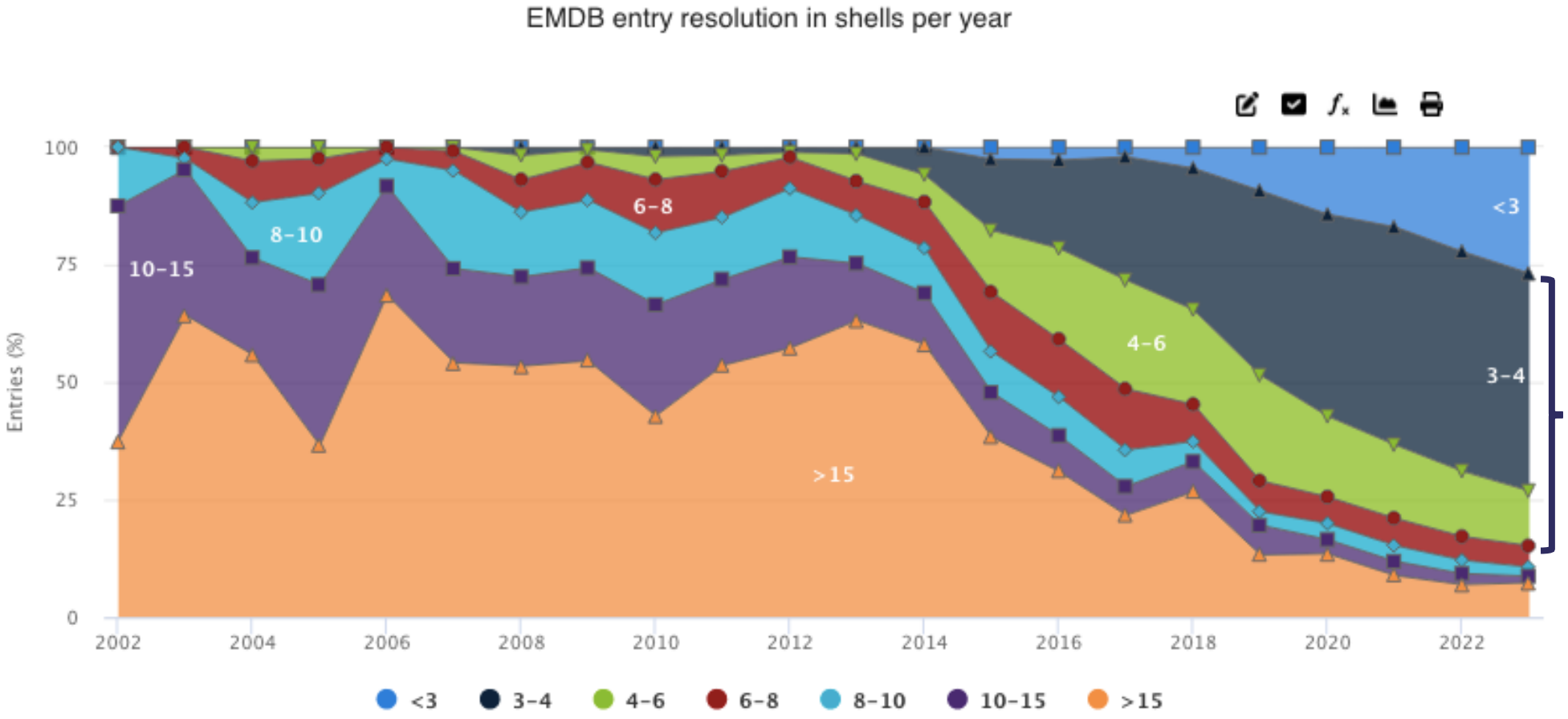
Atomic model gives a more interpretable representation of the structure.

Finer details of function, interaction and dynamics.

Structure based drug-design.

EM DataBank: map resolution

>28k cryo-EM reconstructions in EMDB



Last 3 years
3-6 Å: **~60%**

https://www.ebi.ac.uk/emdb/statistics/emdb_resolution_year

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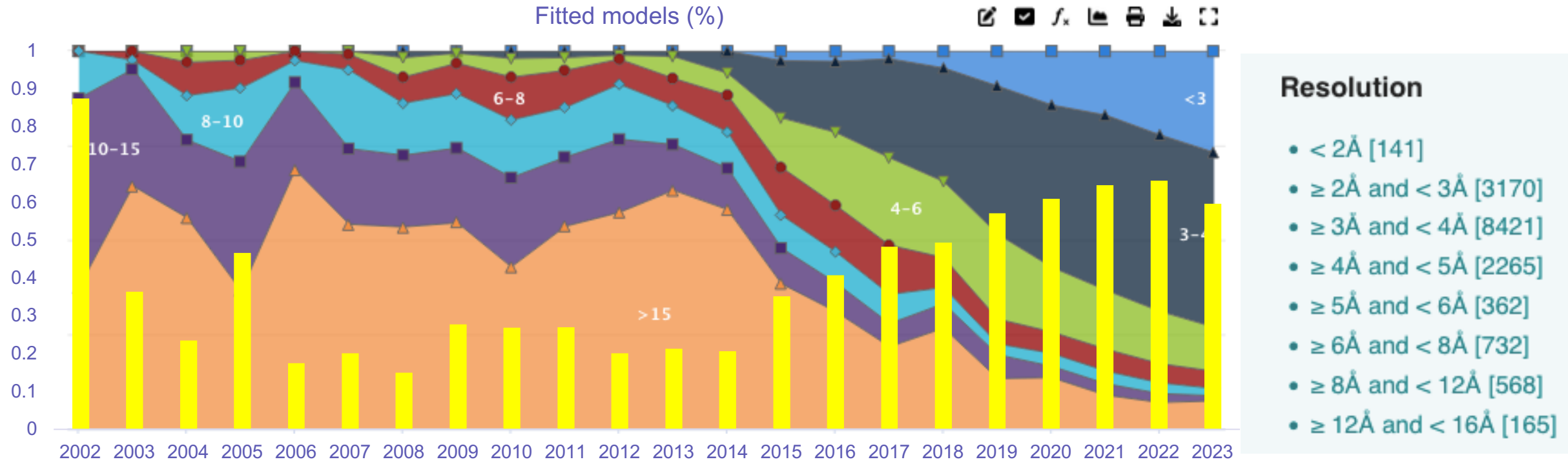
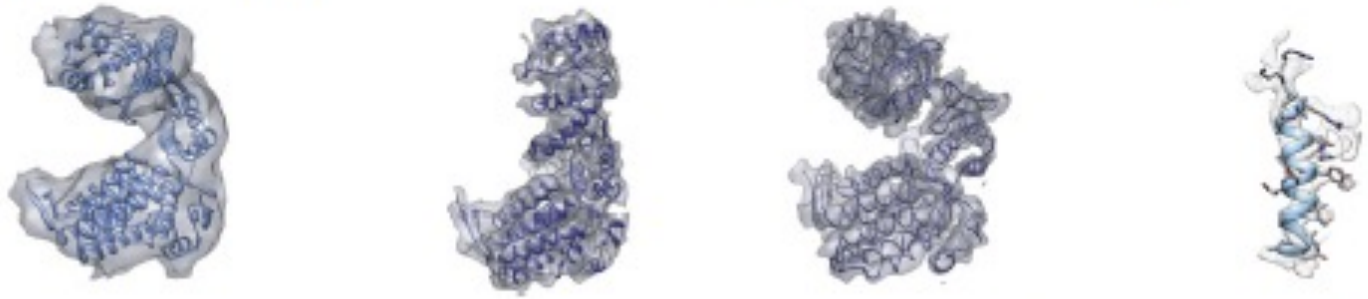
EMDB: maps with fitted models



https://www.ebi.ac.uk/emdb/statistics/emdb_entries_pdb_models

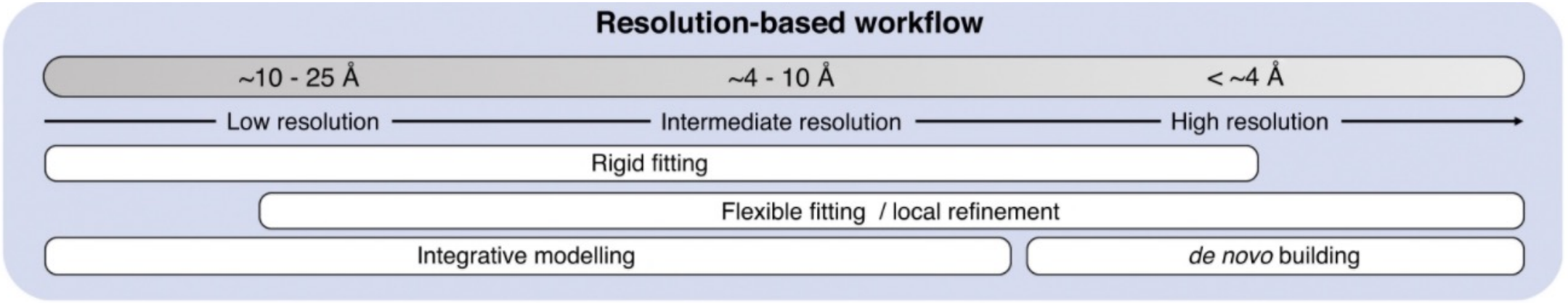
Features interpretable at different resolutions



Villa et al. 2014

Up to 20 Å	Up to 9 Å	Up to 6 Å	Up to 4 Å
Conformational changes	Conformational changes	Conformational changes	Conformational changes
Domain boundaries	Domain boundaries	Domain boundaries	Domain boundaries
		Beta sheets	Beta sheets
			Individual beta strands
	Alpha helices	Alpha helices	Alpha helices
			Pitch of alpha helices
		Pitch of RNA helices	Pitch of RNA helices
			Phosphate "bumps"
			Side chains

Malhotra S. et al., COSB 2019



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Model validation
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Aspects of validation

Fit to data (Global and Local)

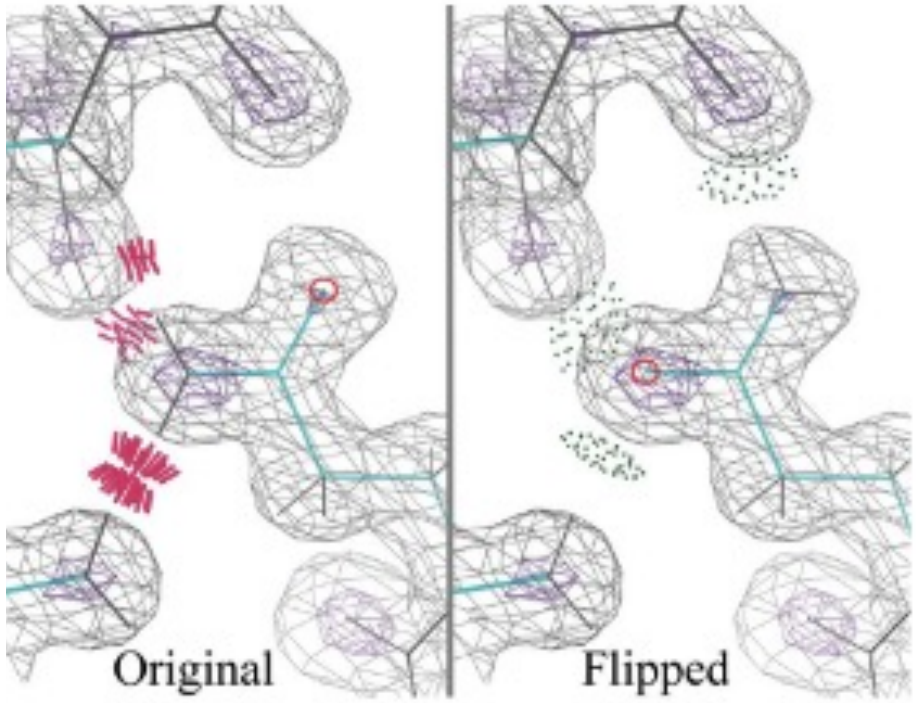
Model quality (geometry)

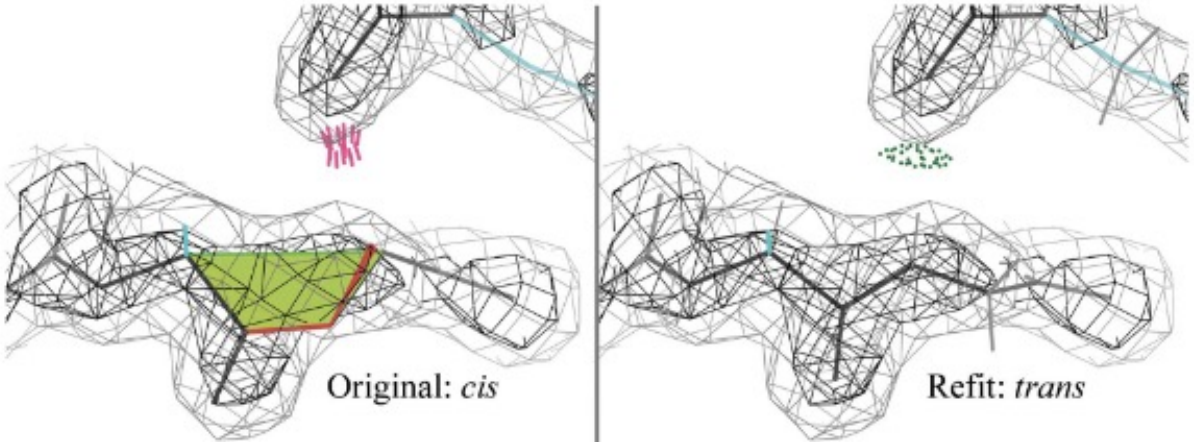
Overfitting

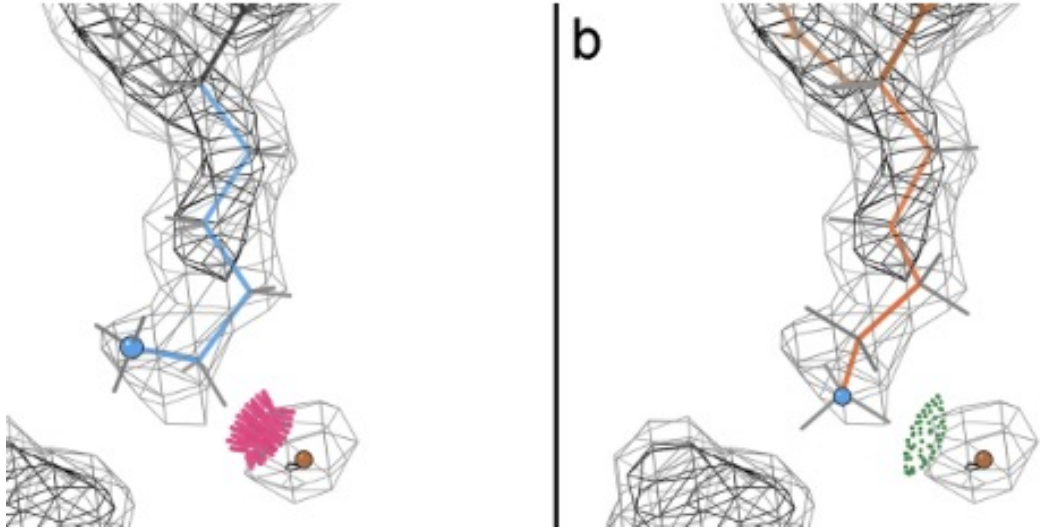
Model bias

Model geometry

Clashes







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Richardson et al. *Acta D*, 2018
Prishant et al. *Prot Sci*., 2020

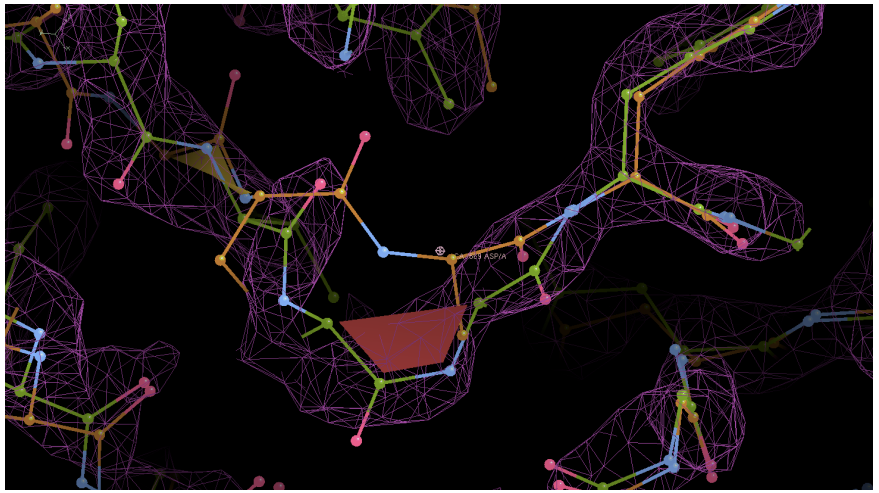
Model geometry

Cis-peptides

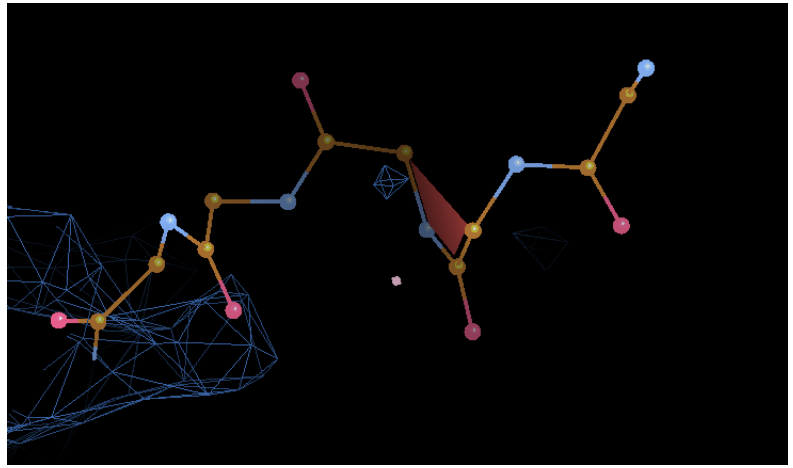
Cis-Pro : 5-6%

Cis-nonPro: ~0.05%

genuine



?



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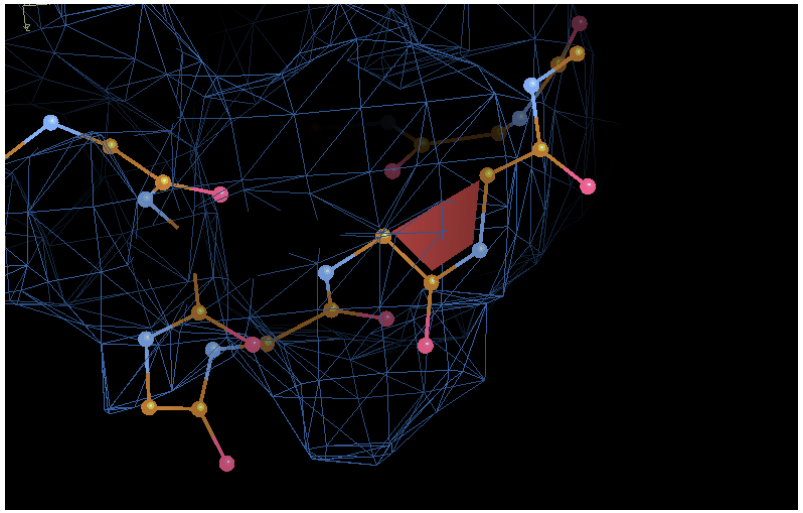

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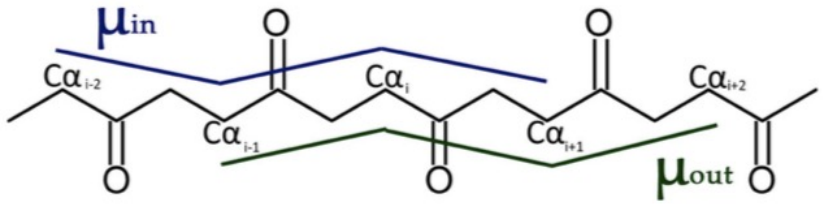
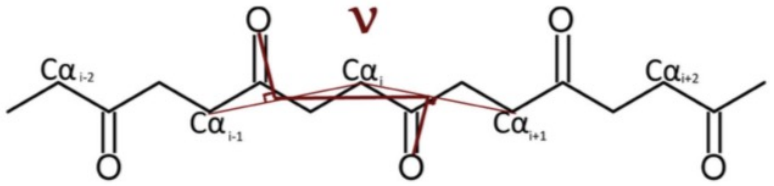
Richardson et al. *Acta D*, 2018

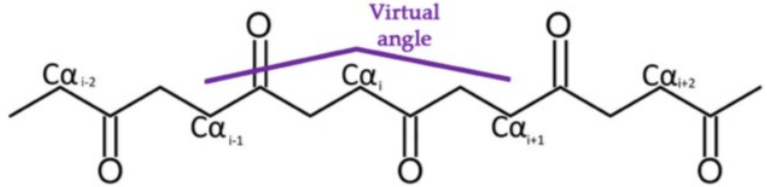
Prishant et al. *Prot Sci.*, 2020

Model geometry

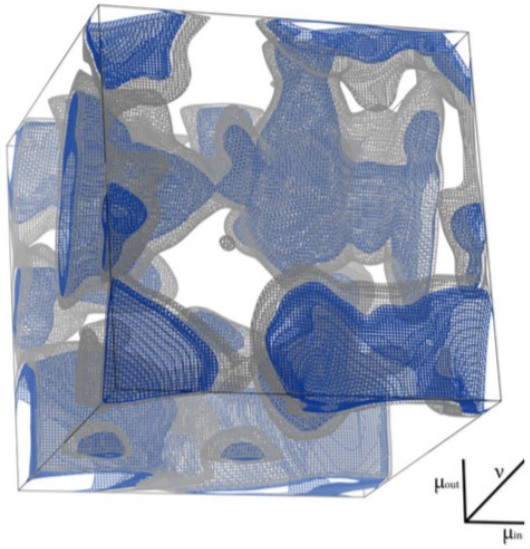
CaBLAM

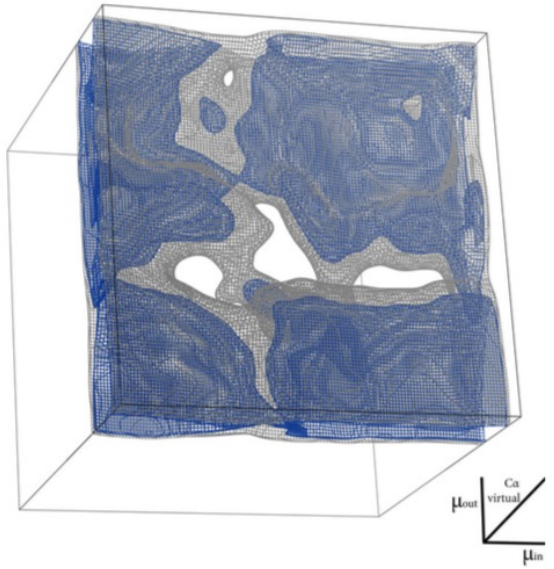


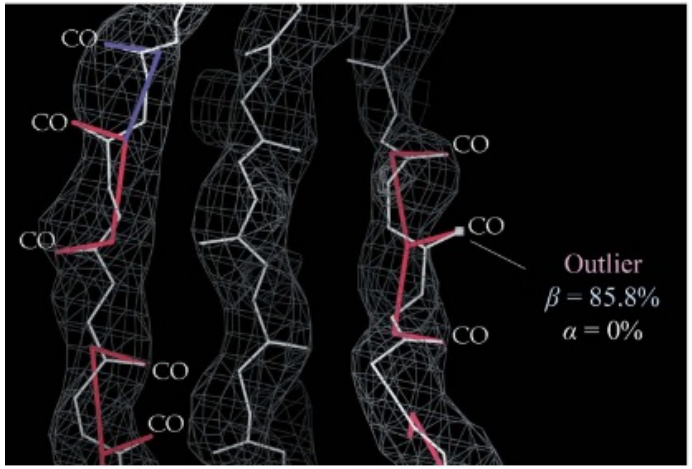




Backbone geometry check







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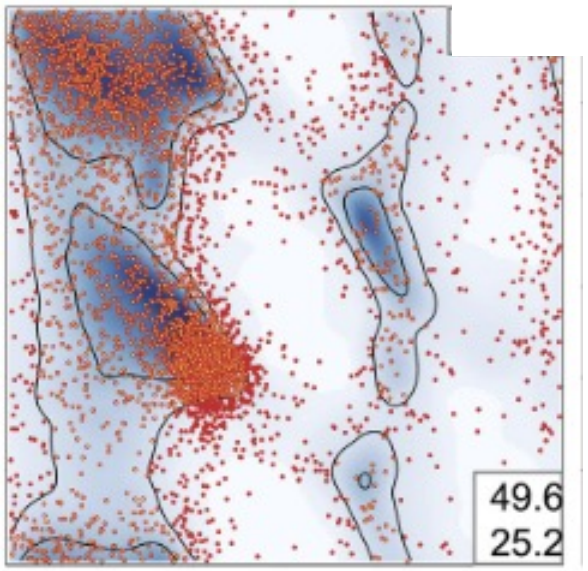
Williams et al. *Prot Sci*., 2018

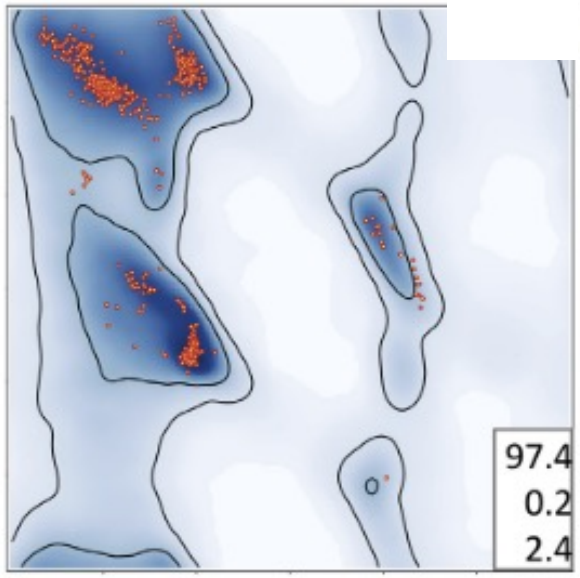
Prishant et al. *Prot Sci*., 2020

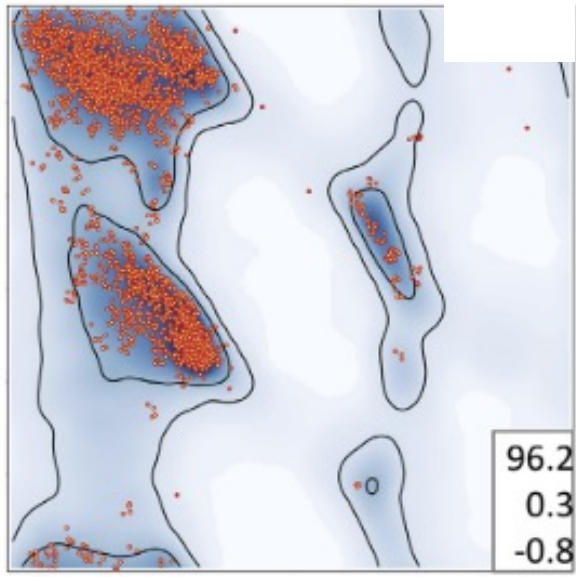
Model geometry

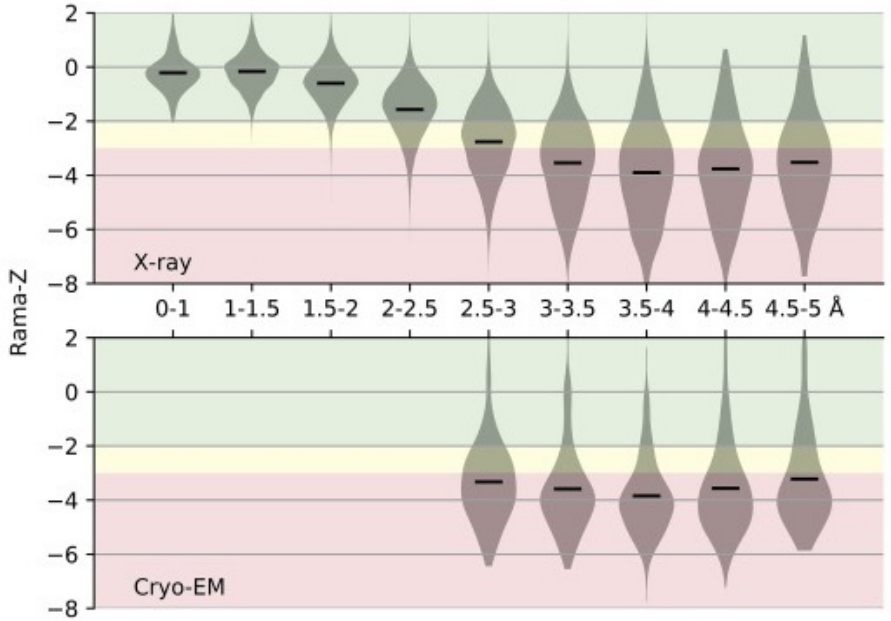
Ramachandran outliers



Ramachandran z-scores







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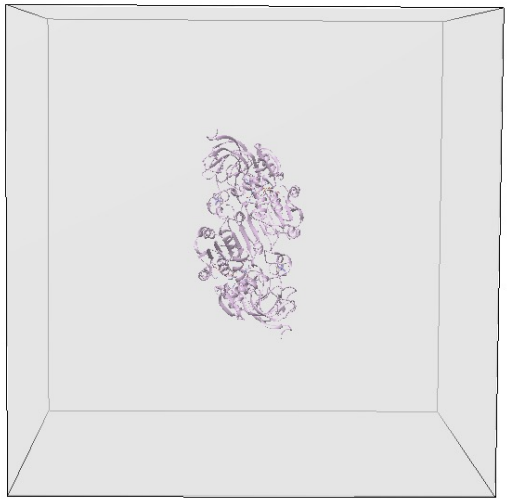
Sobolev et al. *Structure*, 2020

Global agreement with map

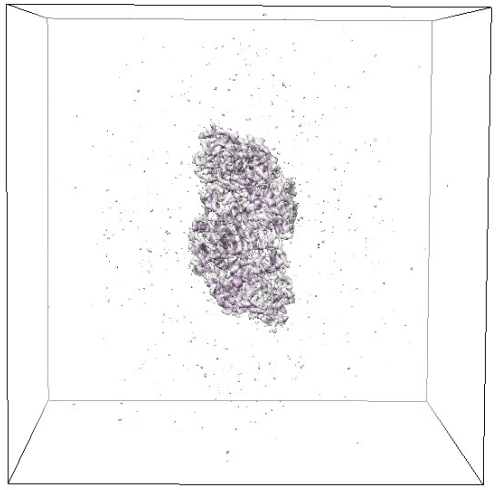
Cross-Correlation Coefficient

Values vary depending on map processing, resolution

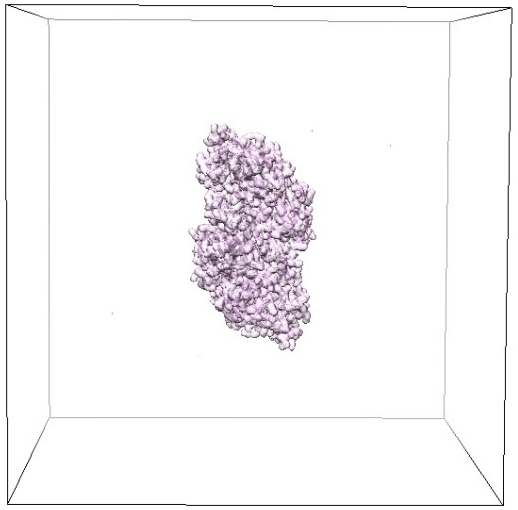
2.9Å full map: 0.32



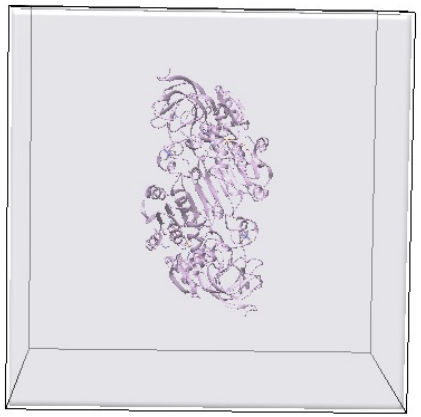
Contoured map: 0.59



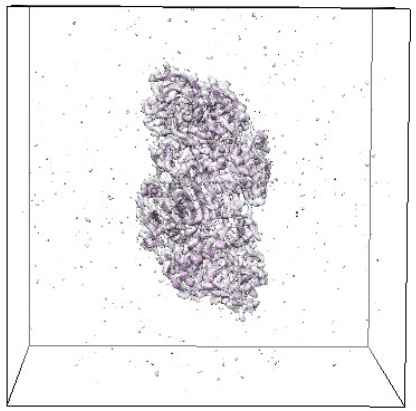
Map-model overlap mask: 0.42



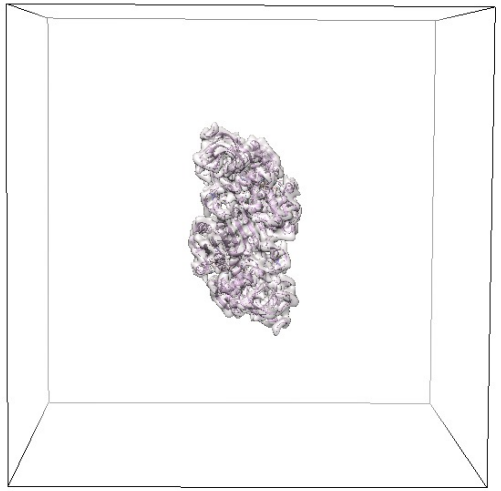
Cropped full map: 0.37



Cropped,contoured map: 0.55



5Å contoured map: 0.76



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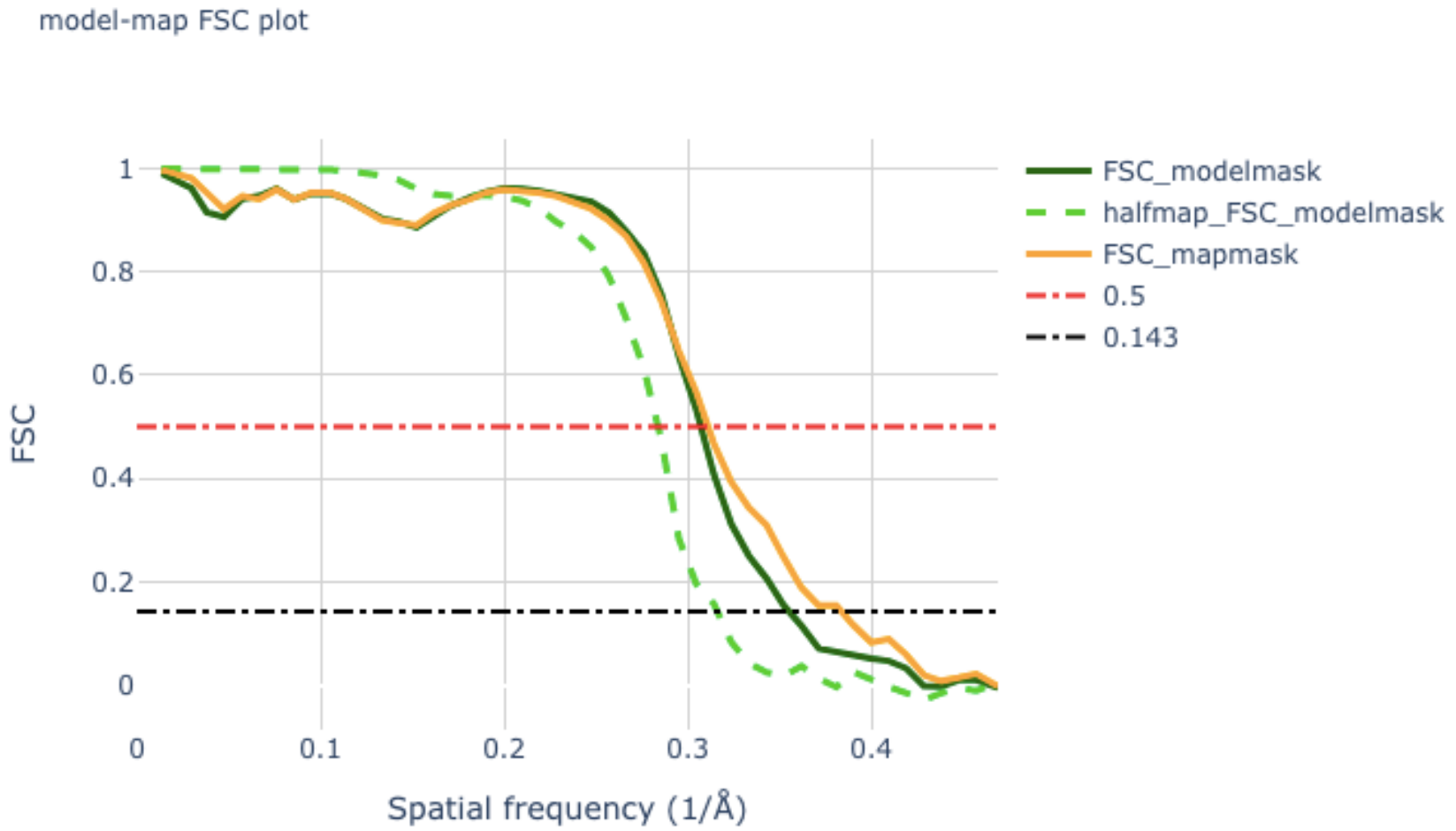
Global agreement with map

Model-map FSC



Brown et al. Acta D, 2015
Yamashita et al. Acta D, 2021

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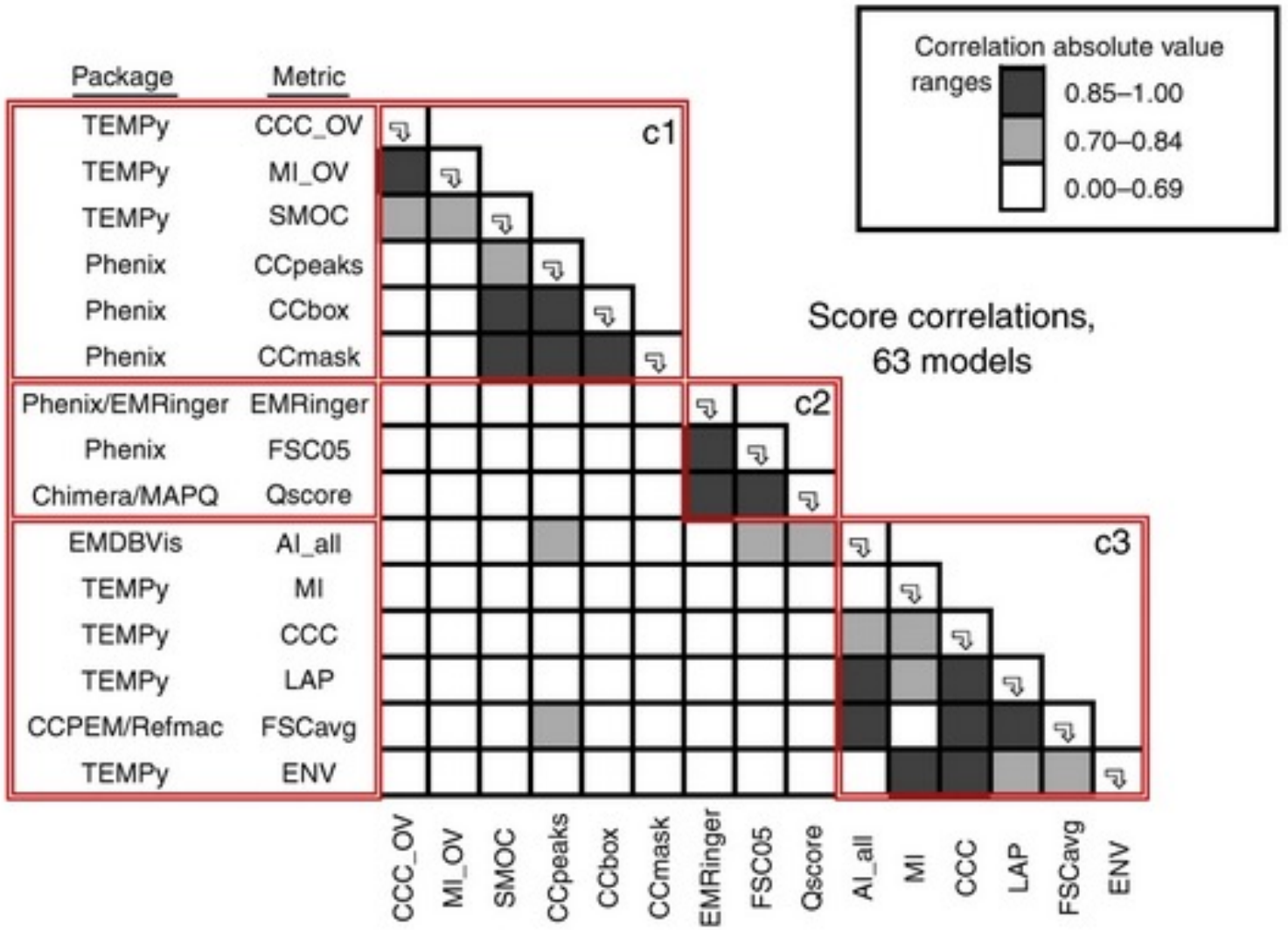
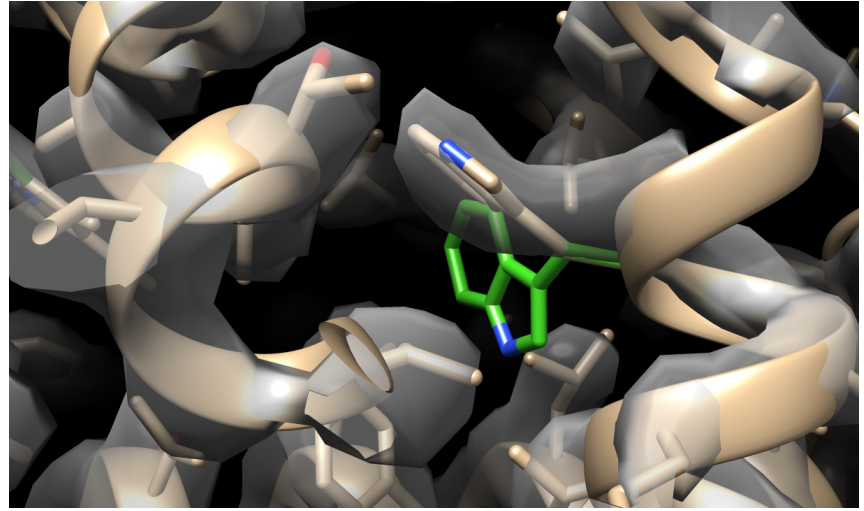
Global agreement with map



Lawson et al. *Nature Methods*, 2021

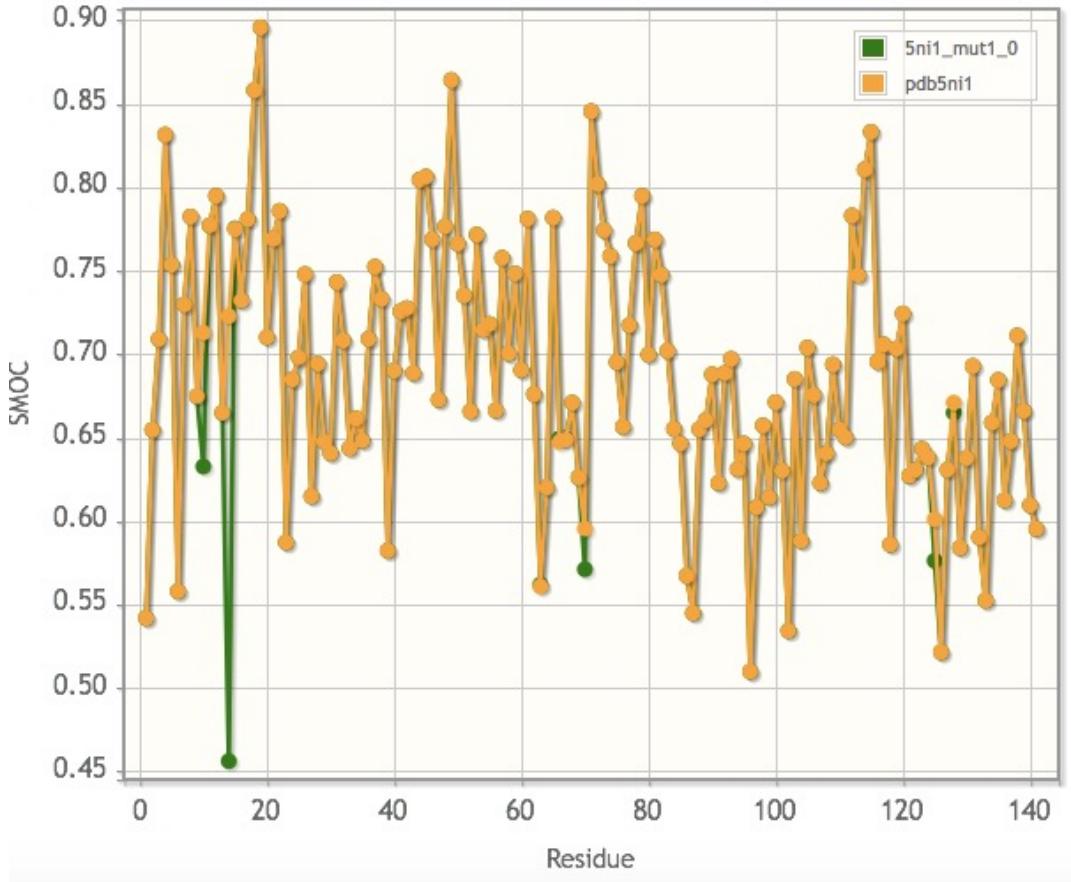
Local agreement with map

Segment based Manders' Overlap Coefficient (SMOC)



An overlap coefficient is calculated over voxels covered by each residue (and the local neighborhood)

$$SMOC = \frac{\sum_{i=1}^{n} X_i Y_i}{\sqrt{\sum_{i=1}^{n} X_i^{\,2}} \sqrt{\sum_{i=1}^{n} Y_i^{\,2}}}$$



Joseph *et al. Methods* 2016 , Farabella *et al. Acta D* 2015

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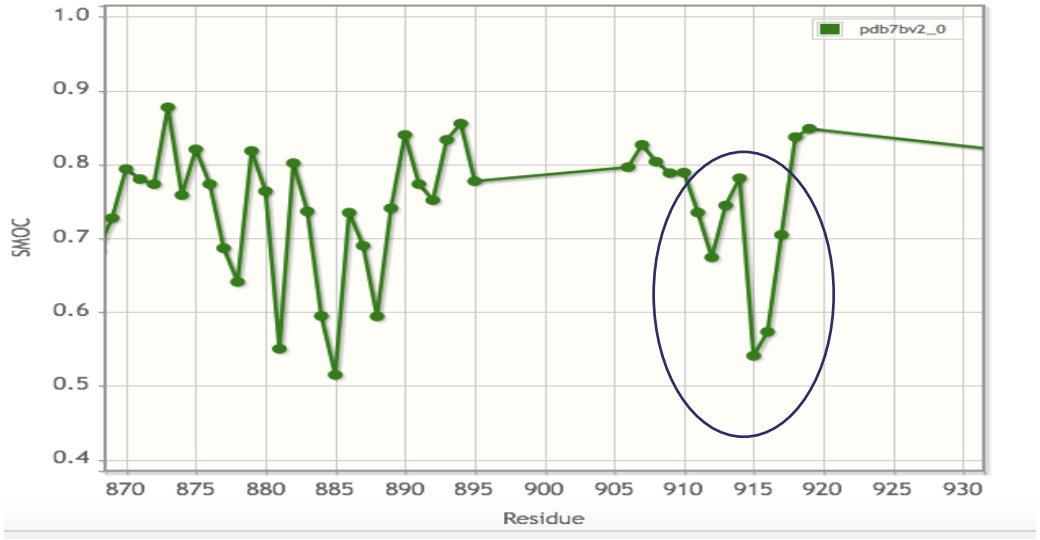
Local agreement with map

2.5Å SARS-CoV2 RNA pol

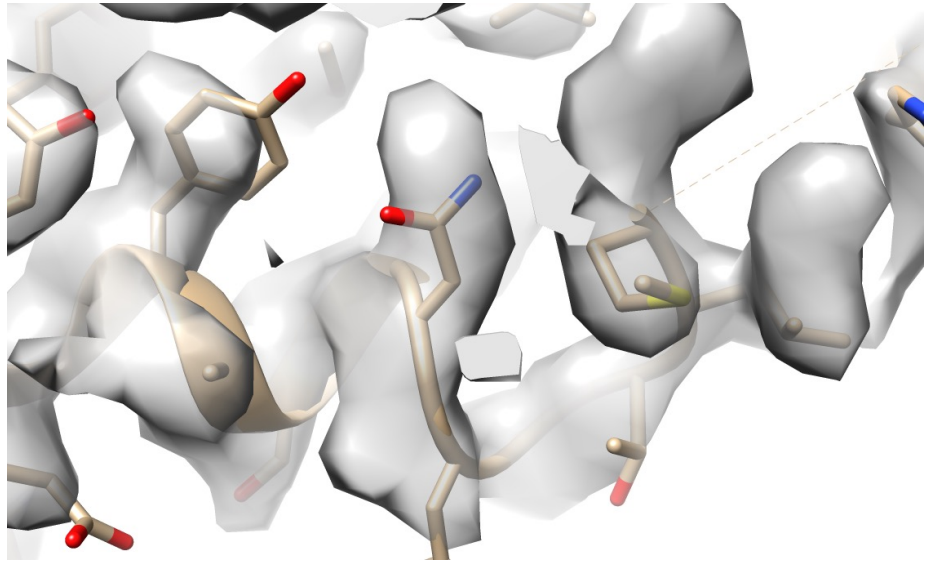


Chojnowski G. Acta Cryst 2022

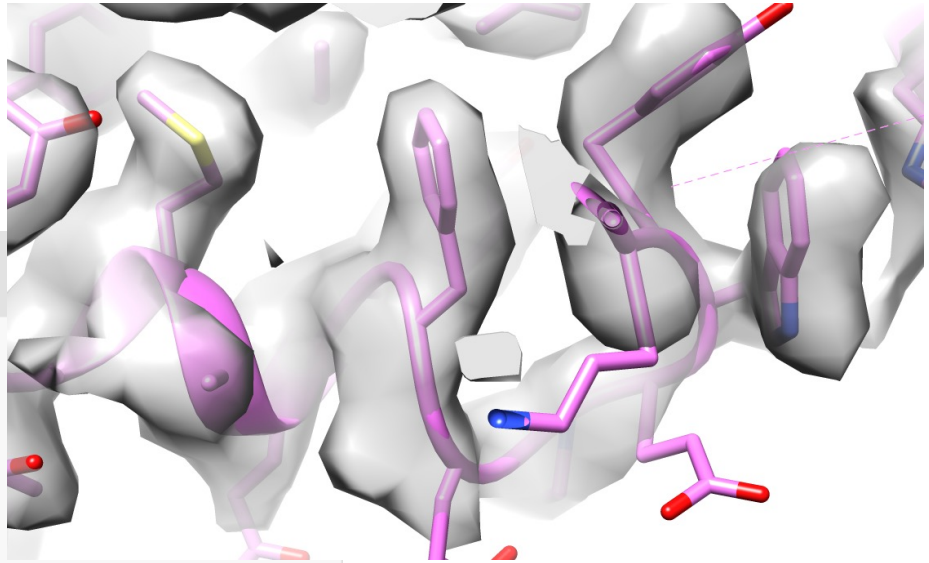
CheckmySequence Chain: A

```
*** Register shift:

 protein start: 906 end: 919 start_new: 915 end_new: 928 -log(pvalue): 1.0068789995424352 si: 100.0

model seq: dmysvMLTNDNTSRYWEPEfyeamytphtvlqgg
new seq  : dmysvmltndntsrYWEPEFYEAMYTPHtvlqgg
```

Deposited

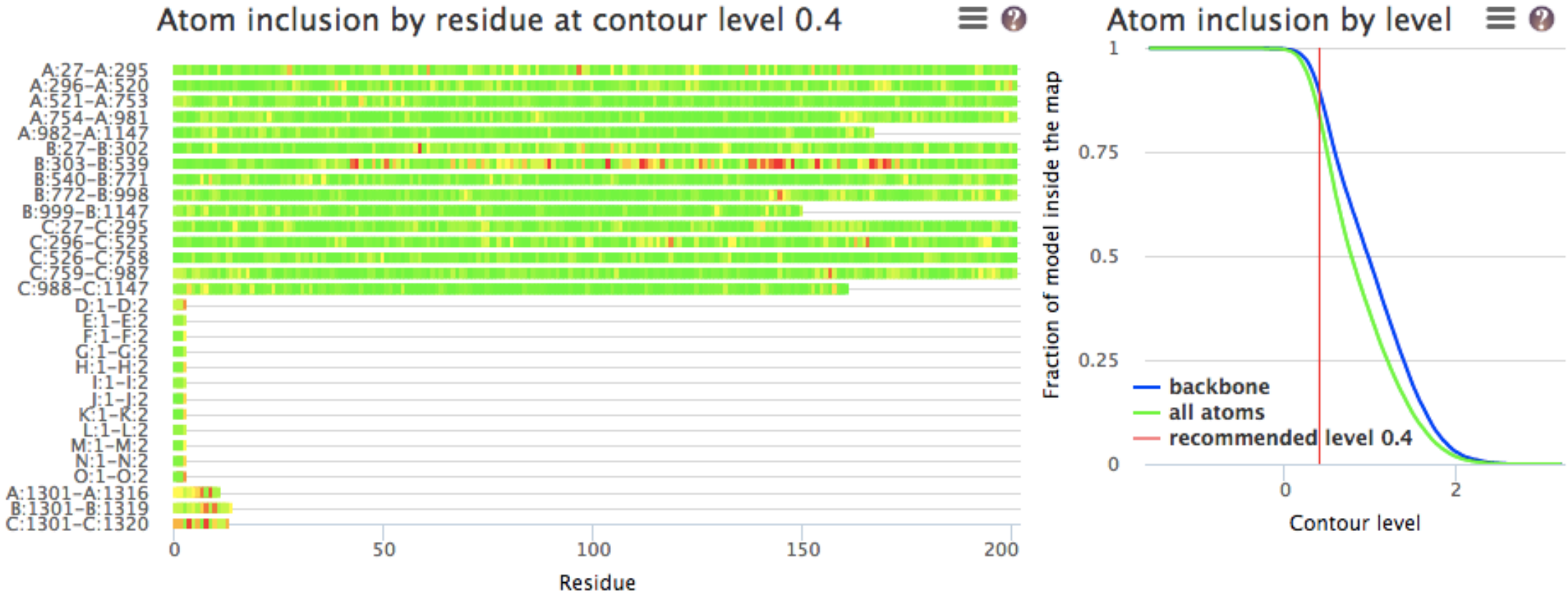


Remodelled



Local agreement with map

Atom inclusion score



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https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-21457/analysis

Local agreement with map

Lawson et al. *Nature Methods*, 2021

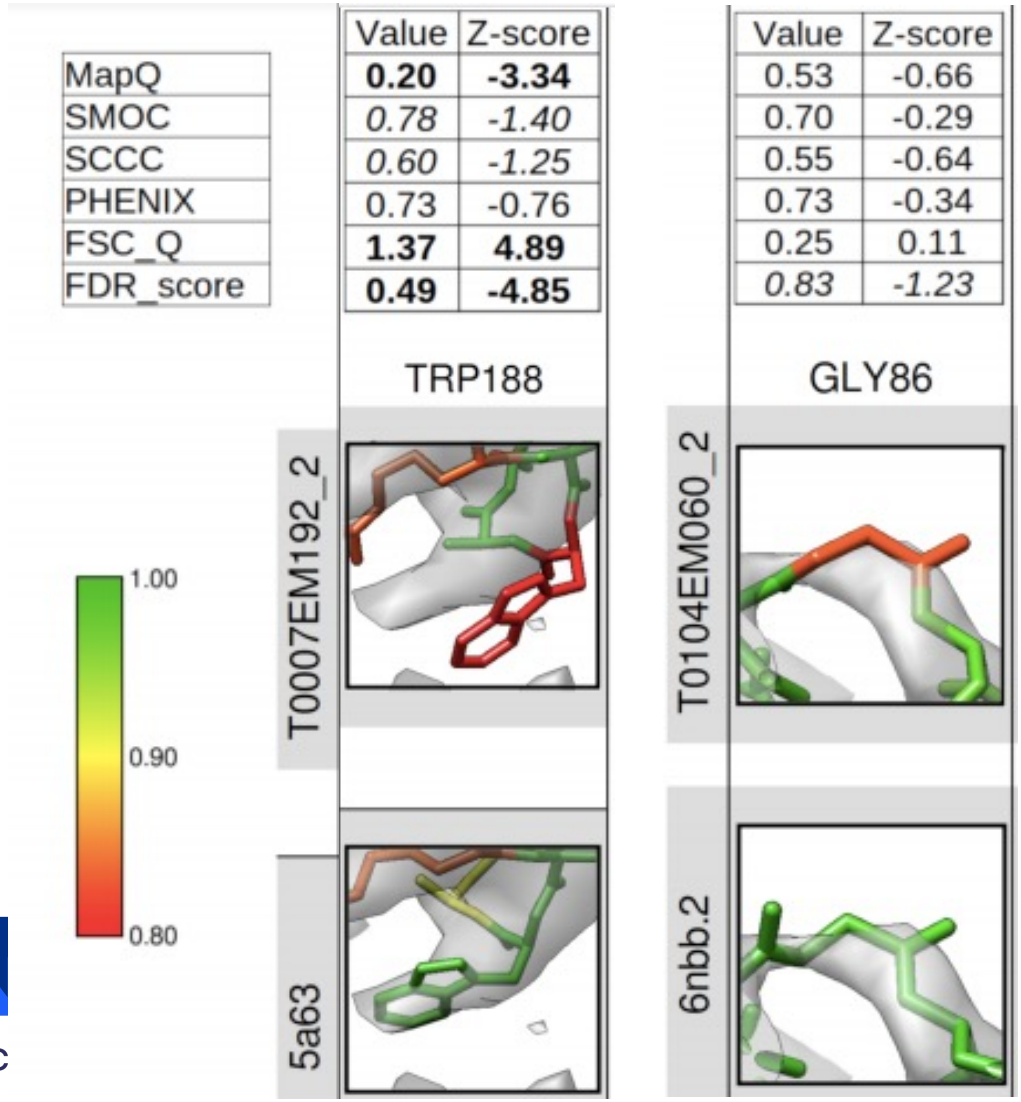
FDR validation score

	Value	Z-score
MapQ	**0.20**	**-3.34**
SMOC	*0.78*	*-1.40*
SCCC	*0.60*	*-1.25*
PHENIX	0.73	-0.76
FSC_Q	**1.37**	**4.89**
FDR_score	**0.49**	**-4.85**

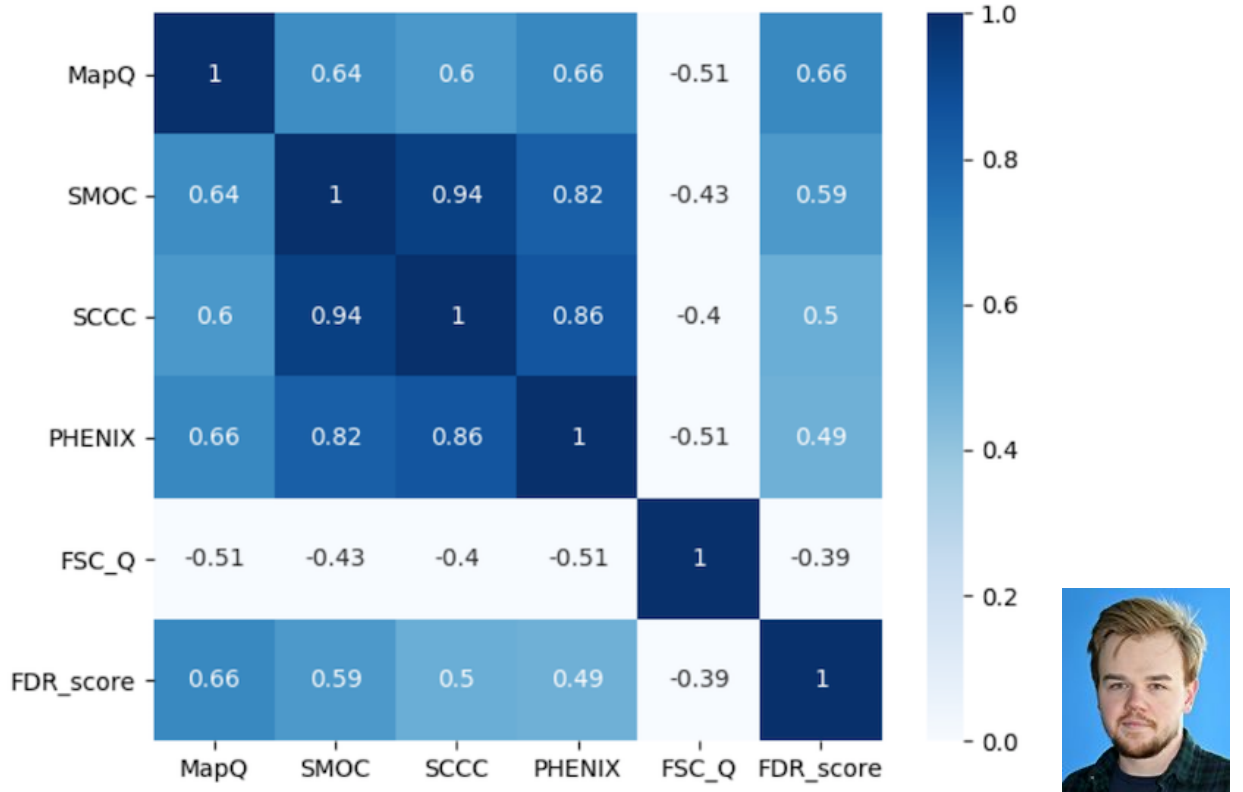
TRP188

	Value	Z-score
	0.53	-0.66
	0.70	-0.29
	0.55	-0.64
	0.73	-0.34
	0.25	0.11
	0.83	*-1.23*

GLY86



T0104EM060_2



Maxmillian Beckers, Arjen Jakobi and Carsten Sachse. 2016

Olek Mateusz
Kevin Cowtan

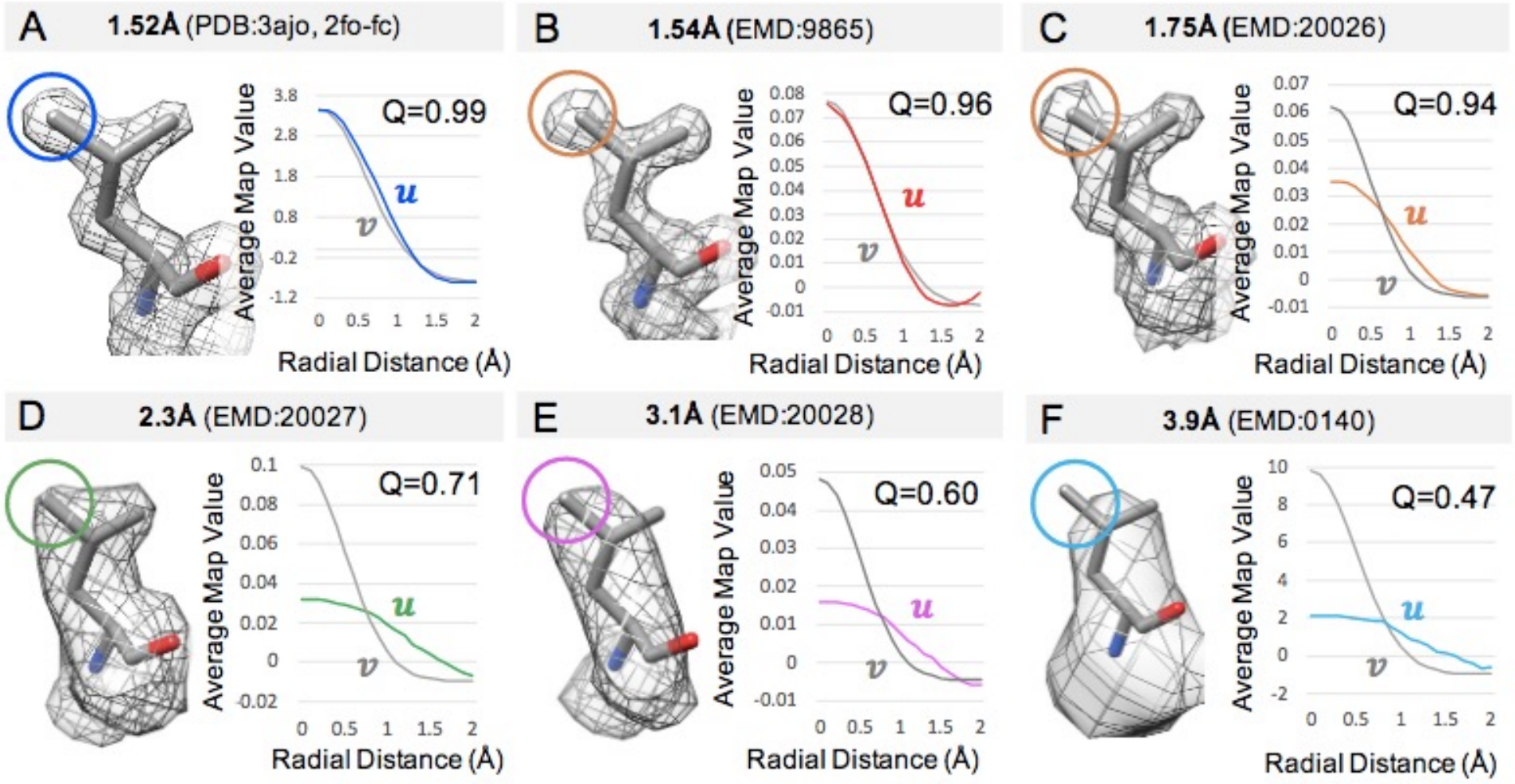
UKRI Scientific

University of York

eBIC diamond

Local agreement with map: resolvability

Q score



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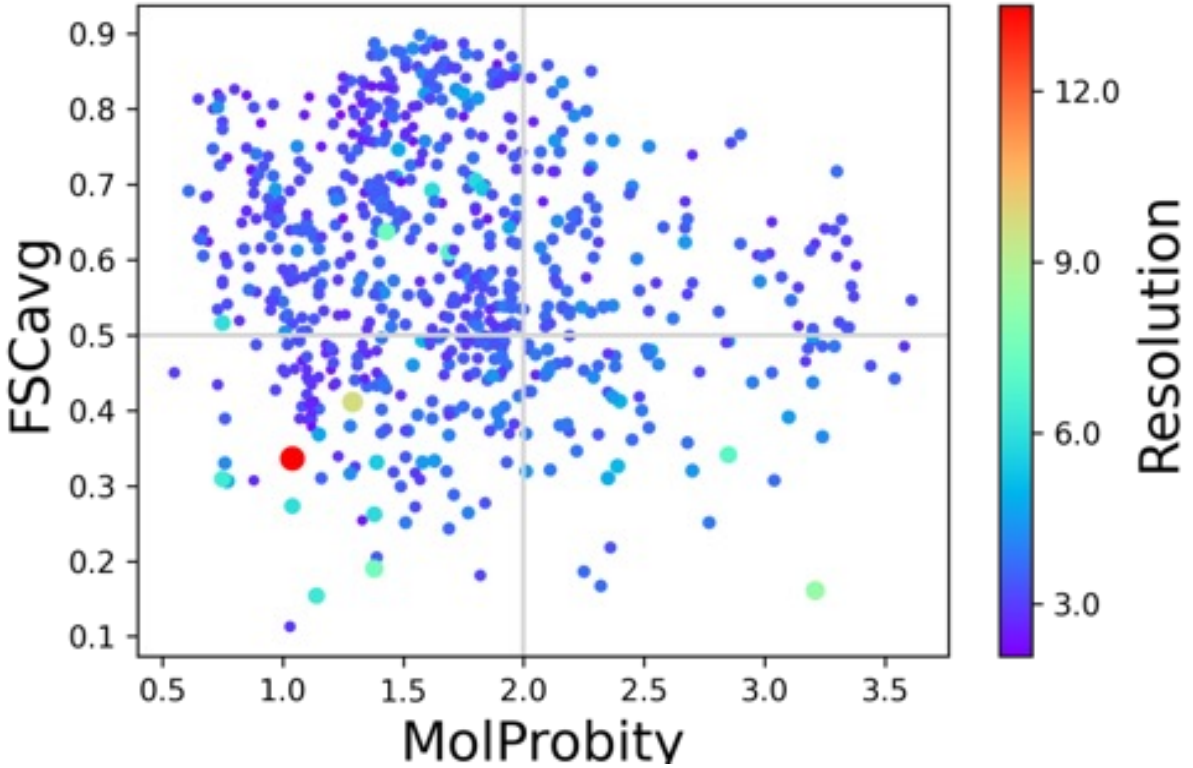
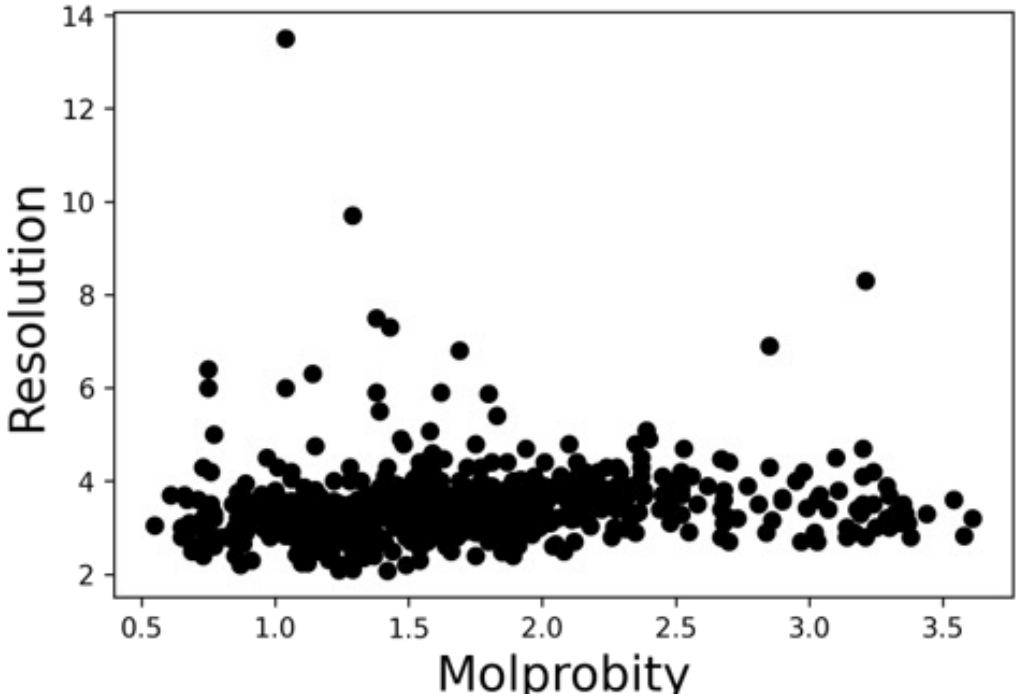
Pintile et al. 2020

Model geometry vs fit-to-data

Do the models represent the map data well?

Do we need more validation metrics for publication?





Mean score of structures worse than 3.5Å resolution is 1.8 (< 3.5Å is 1.6).

31.2% of the structures had FSCavg scores worse than 0.5.

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Model geometry vs fit-to-data

Geometry ✓

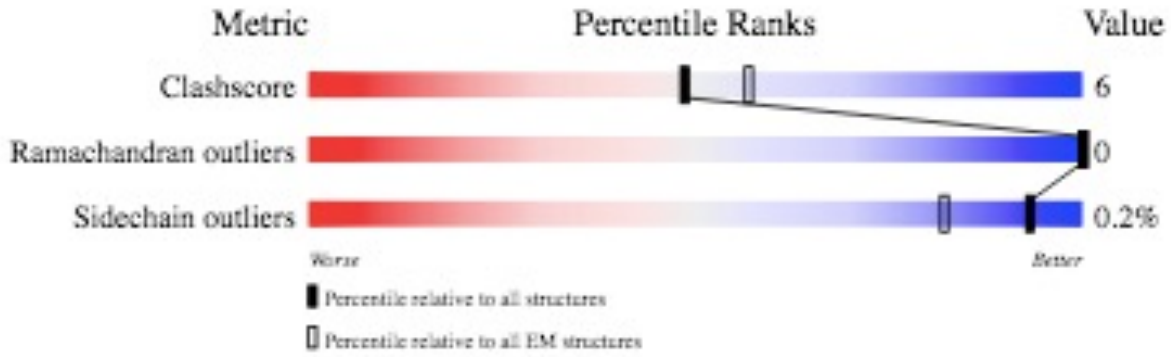
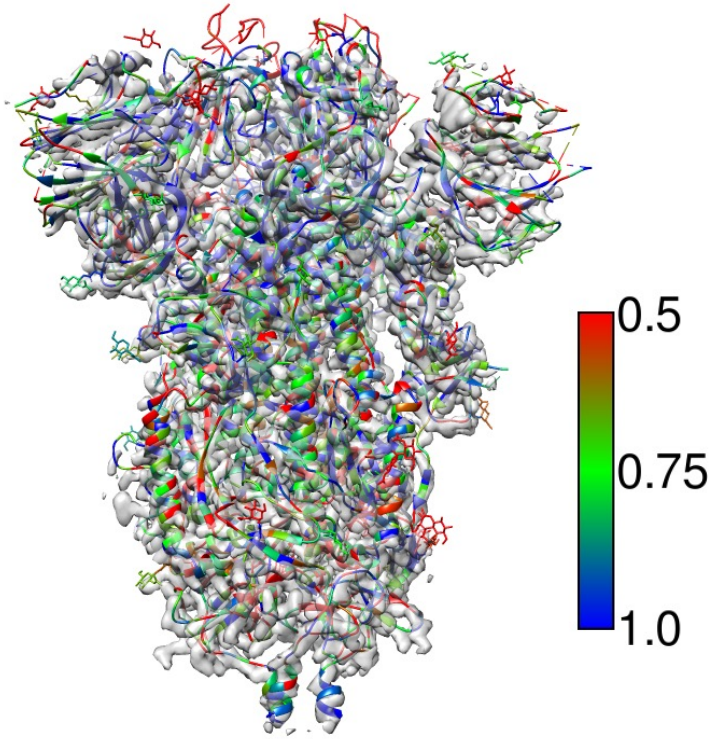


Image:
PDBe/wwPDB

Map agreement ?

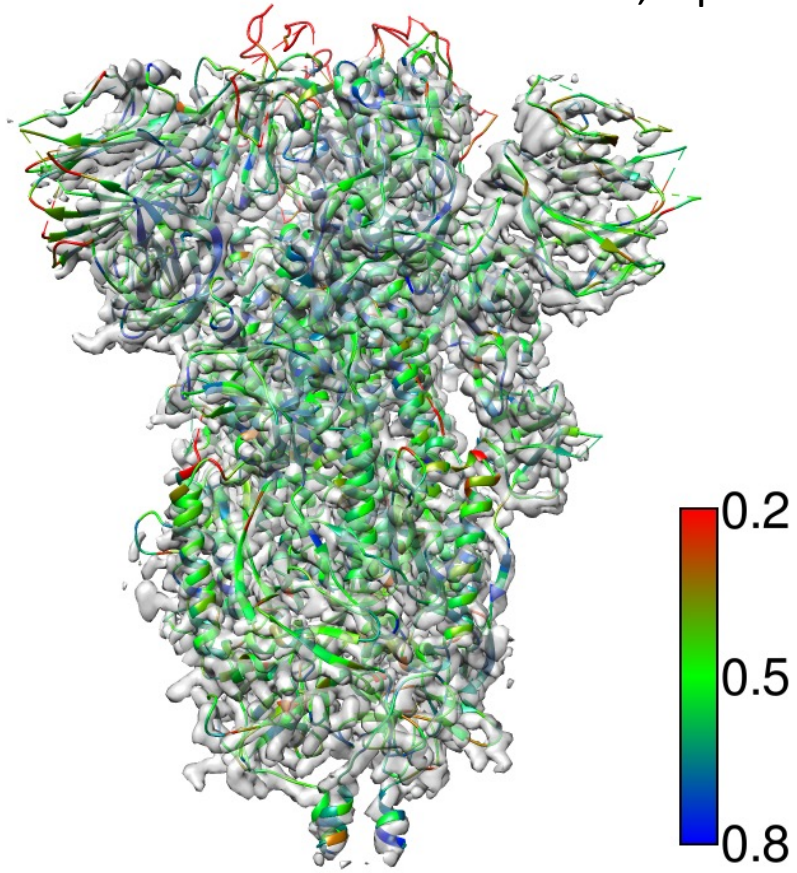


Overfitting ?

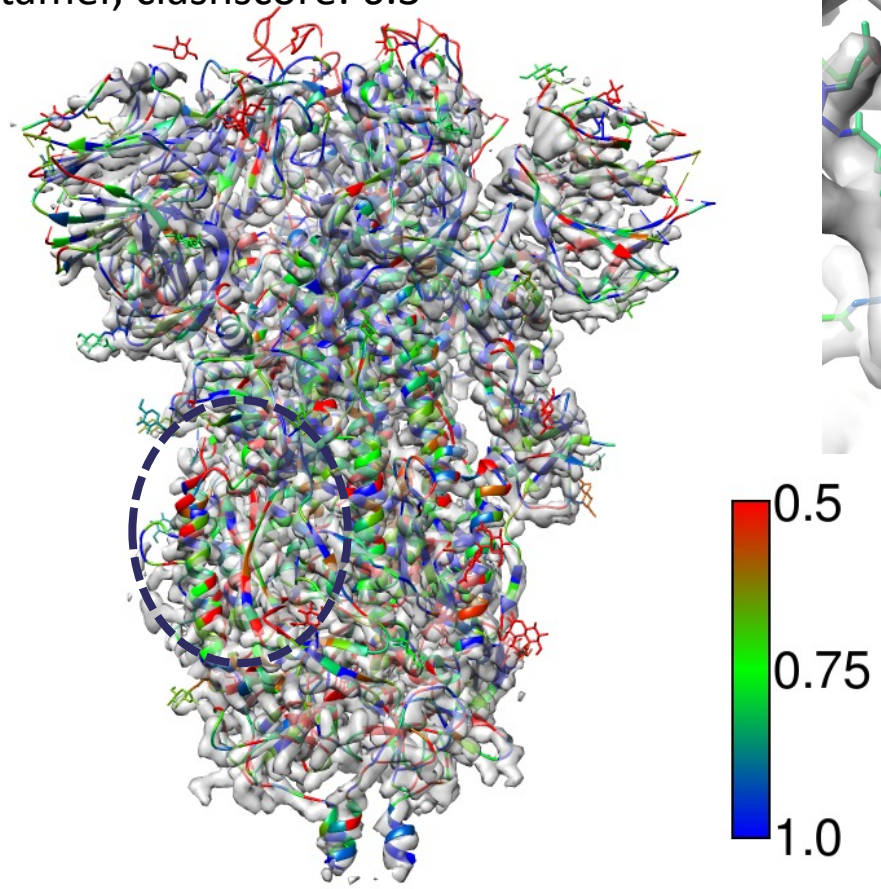
Poor fit

~28% of structures have potential issues with backbone trace (FDR-backbone score)

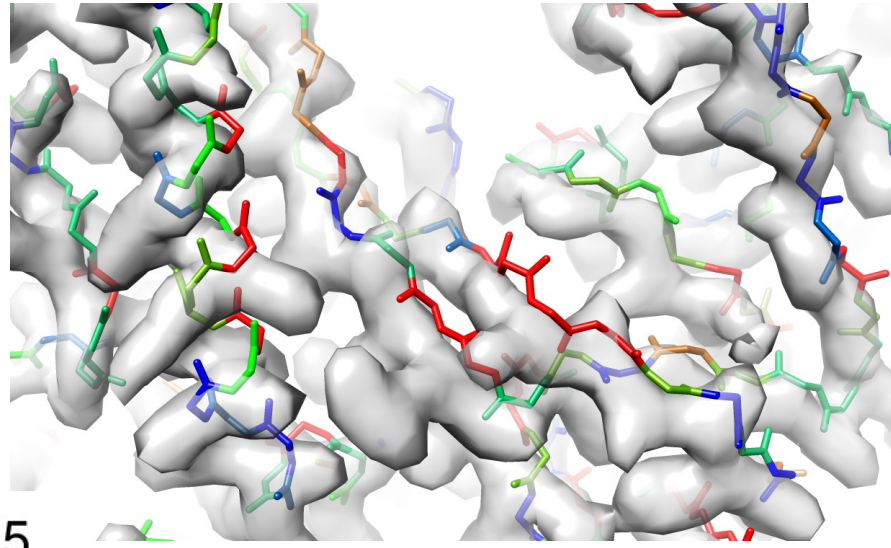
Molprobity score : 1.39
0 Ramachandran outlier, 1 poor rotamer, clashscore: 6.3



SMOC score
(Joseph et al. 2016)



FDR-backbone score
(Olek et al. 2021)



3.4Å

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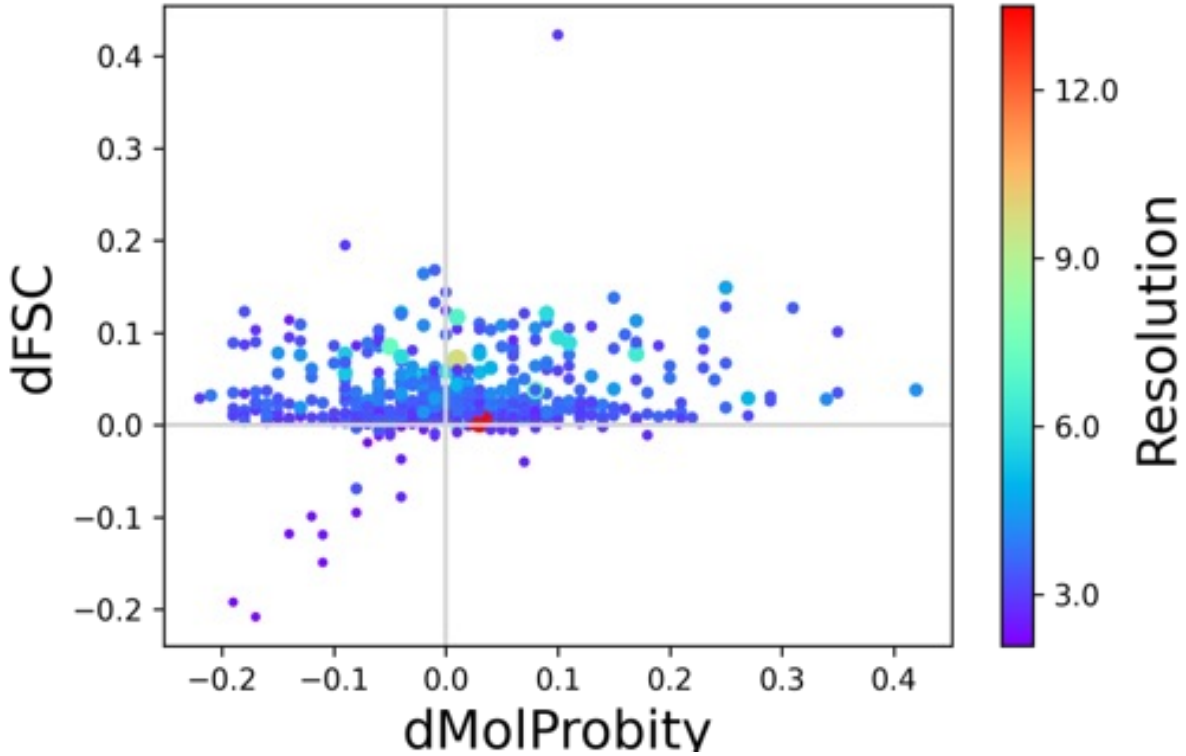
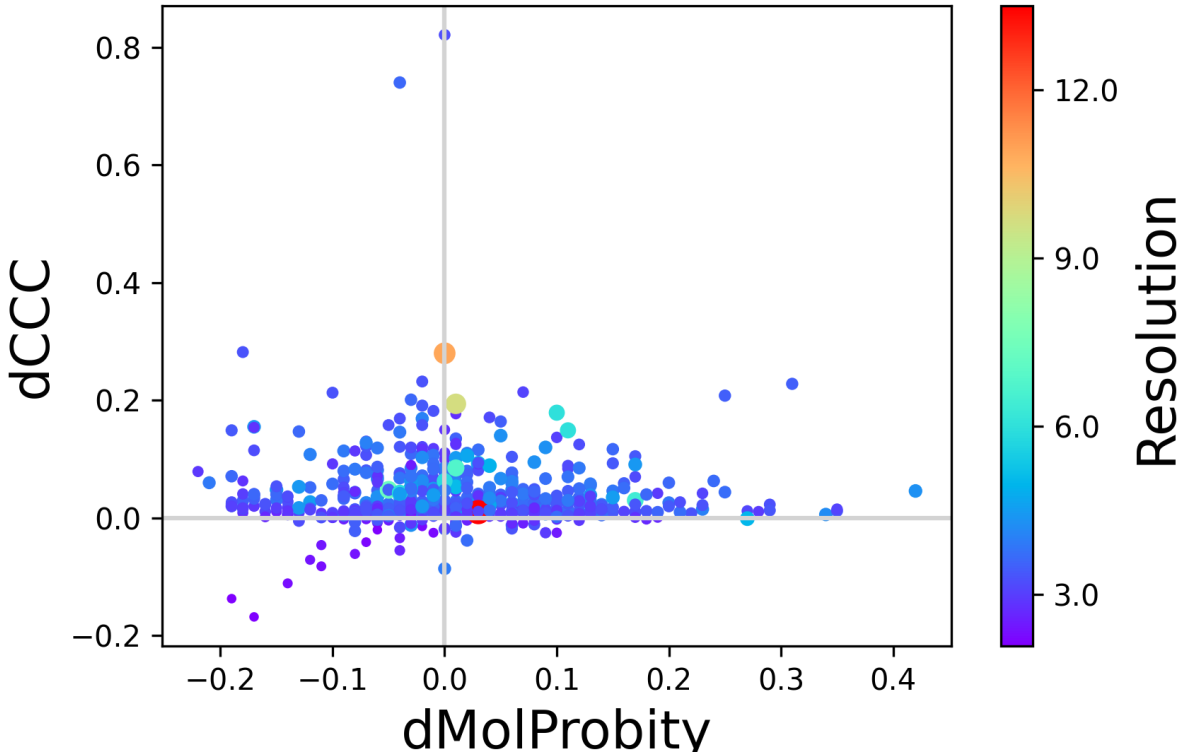
Model geometry vs fit-to-data

Can the map fit / representation be improved ?





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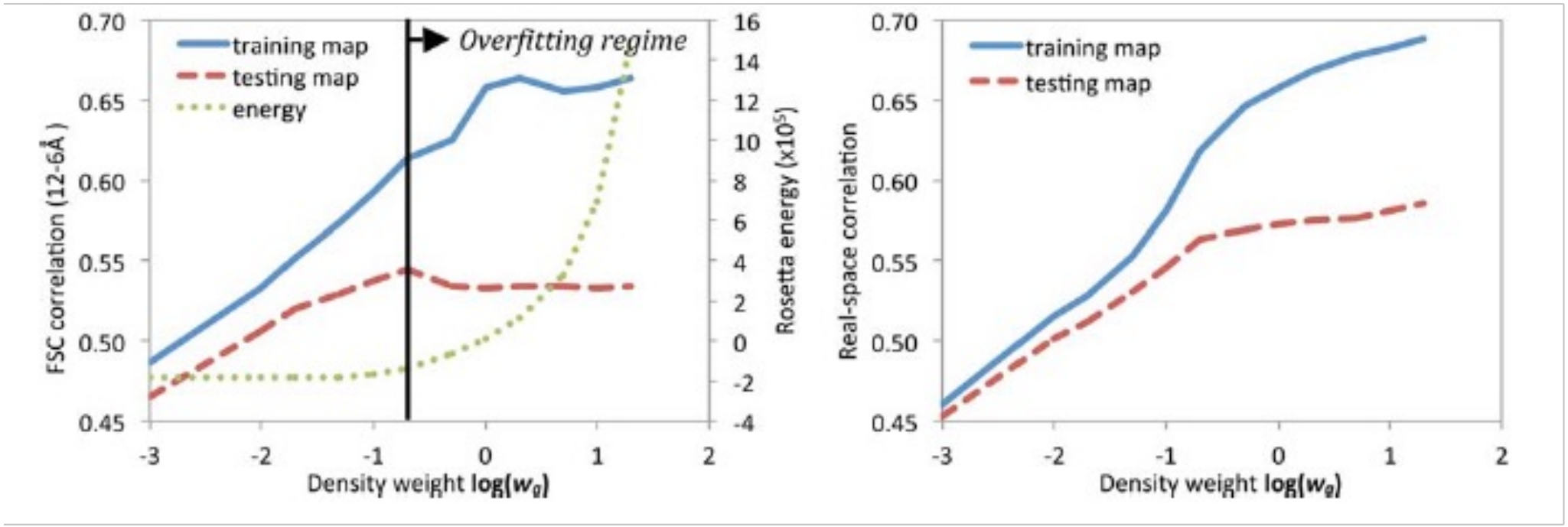
FSCavg of 94% of structures in the dataset improved with further refinement

~45% of the dataset had improved MolProbity scores as well

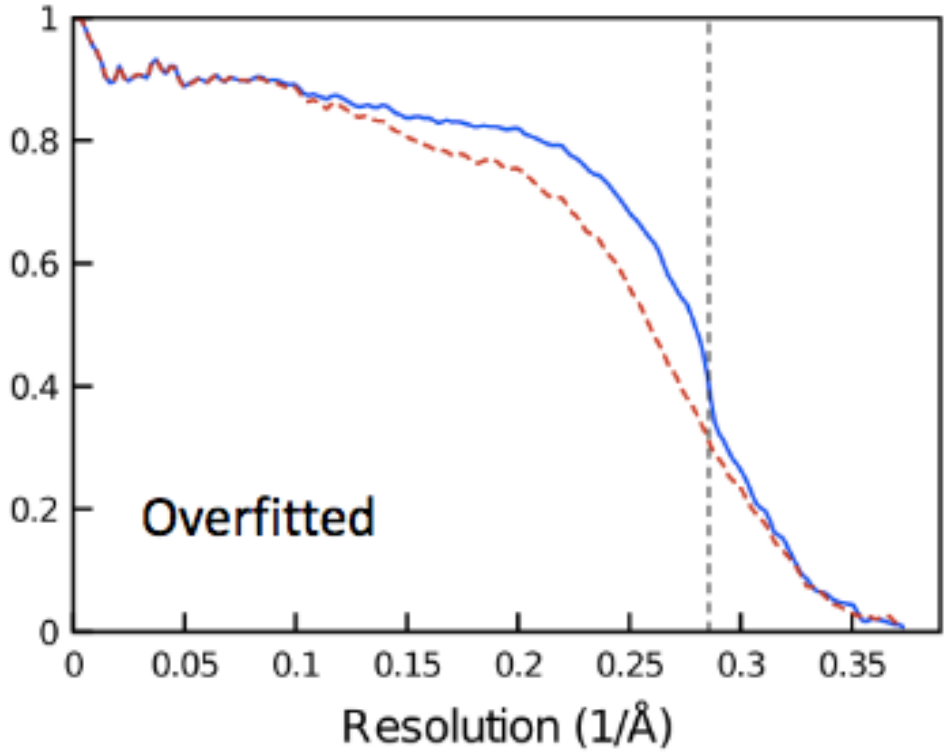
Test against equivalent but independent data

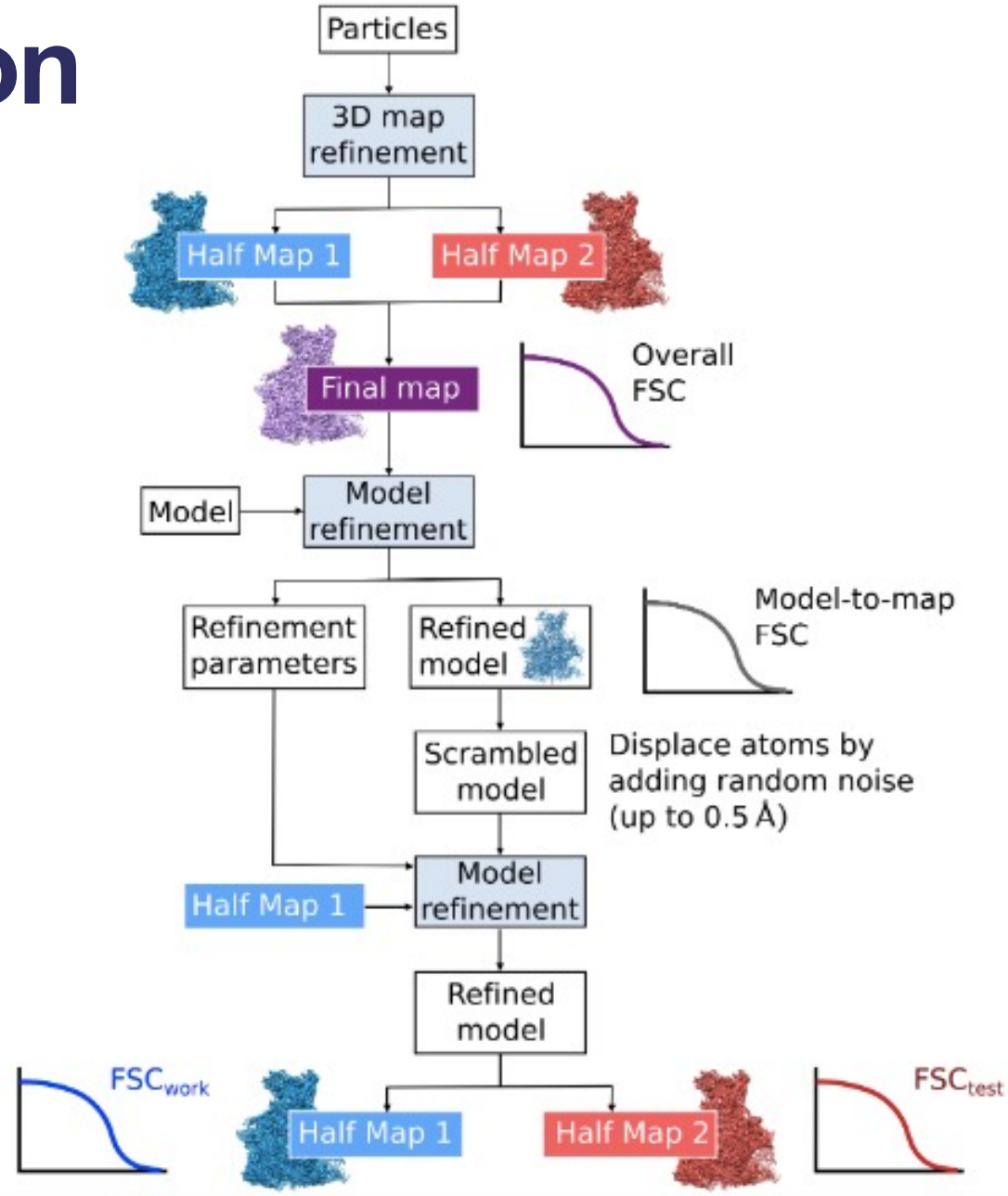


Mm-cpn in the ATP/AlFx induced closed state
4.3 A resolution

DiMaio et al 2013; DiMaio and Chiu 2016

Half-map cross-validation





Brown et al. 2015

Goodness of fit

TEMPy, Coot, Refmac, Phenix,
Emringer, SMOC,
Q-score, FSC-Q

Model geometry

Molprobity, Coot, CaBLAM, Tortoize
What-check
…

Secondary structure

Molprobity, Coot, CaBLAM, Qmean,
Jpred, …

Tertiary structure

Verify-3D, ProQ2 (Rosetta),
Prosa, DOPE (Modeller), ModFold, ..

Quarternary structure

PI-score, PISA,
Docking scores

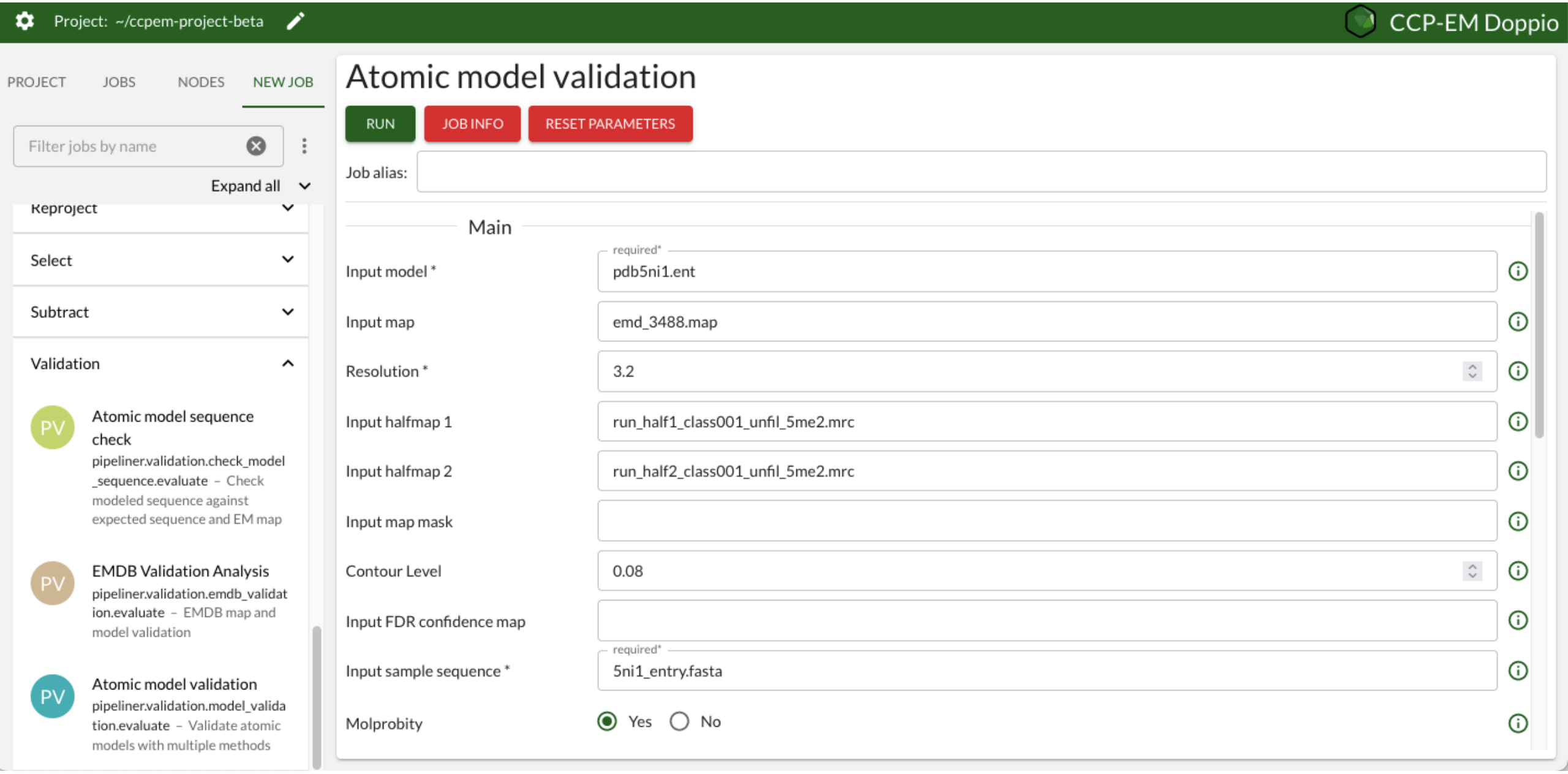
Cross-Validation

Half map (Refmac, Rosetta)
Resolution shells (Direx)

Ensemble assessment (TEMPy)

Experimental validation

CCP-EM model validation



CCP-EM model validation

Molprobity	(•) Yes ( ) No
Servalcat FSC	(•) Yes ( ) No
TEMPy global scores	(•) Yes ( ) No
TEMPy SMOC	(•) Yes ( ) No
FDR backbone score	(•) Yes ( ) No Confidence map not provided - will be calculated.Requires unmasked input map, preferrably raw-map
TORTOIZE	(•) Yes ( ) No 'tortoize' from CCP4 is currently broken on Mac
CheckMySequence	(•) Yes ( ) No

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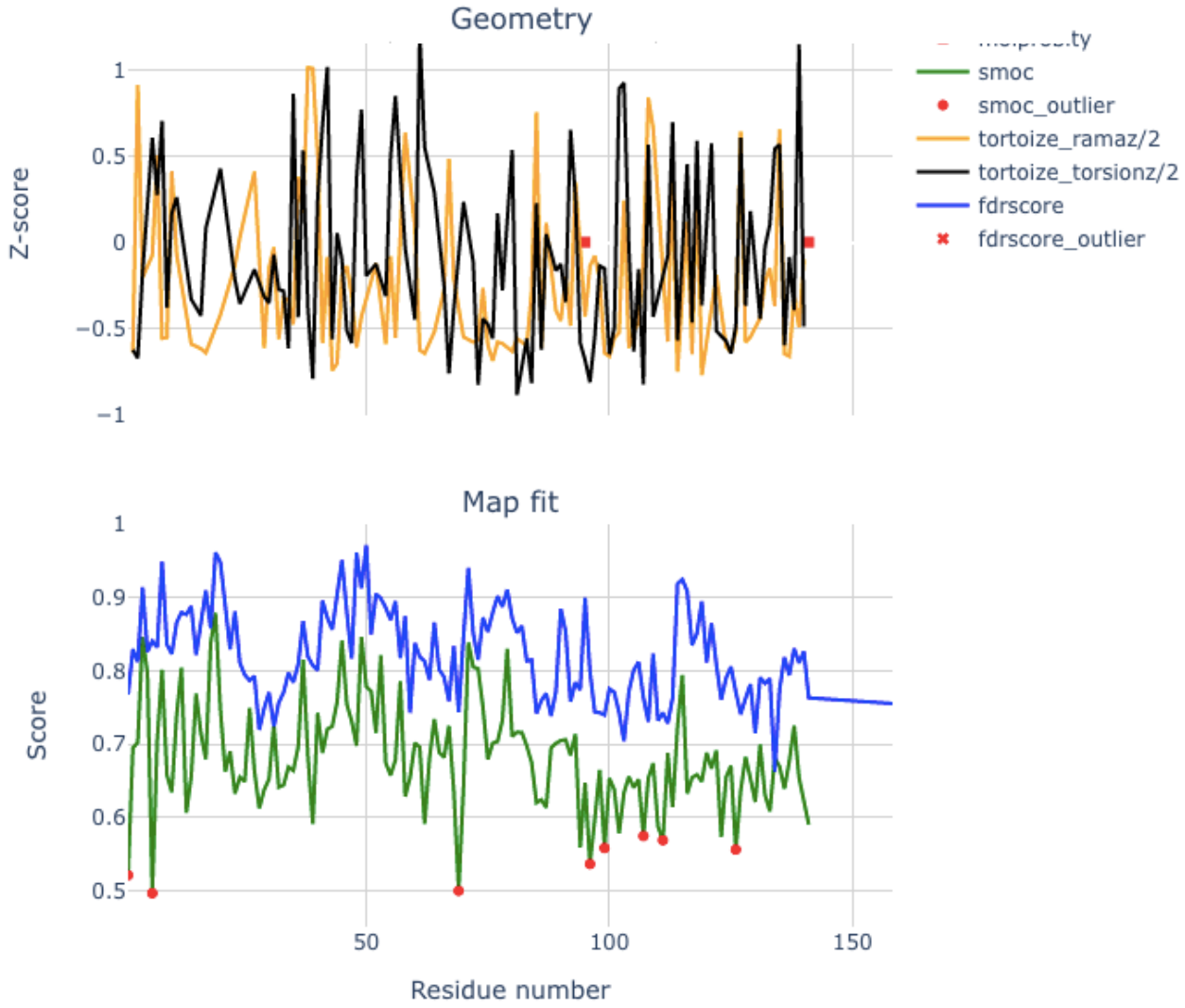
Willams et a. 2018, Chen et al. 2015, Brown et al. 2015,
Joseph et al. 2016, Olek and Joseph 2021, Chojnowski G 2022, Cragnolini et al. 2021

CCP-EM model validation

Outlier clusters

Cluster	Chain_Residue	Outlier Type(s)
6	B34	smoc (raw:0.514;Z:-1.88)
6	B301	fdrscore (raw:0.558;Z:-2.2)
6	B305	fdrscore (raw:0.525;Z:-2.826)
7	B104	smoc (raw:0.559;Z:-1.981)
7	B303	fdrscore (raw:0.6;Z:-2.221)
7	B304	fdrscore (raw:0.548;Z:-2.6)
8	C95	dihedral_angles (1_C_94_CA-1_C_94_C-1_C_95_N-1_C_95_...
8	C96	smoc (raw:0.536;Z:-1.854)
8	C99	smoc (raw:0.558;Z:-1.66)
0	A95	dihedral_angles (1_A_94_CA-1_A_94_C-1_A_95_N-1_A_95_...
0	A96	smoc (raw:0.511;Z:-2.218)
1	A63	smoc (raw:0.562;Z:-1.596)
1	A61	torsion_z (2.438)
2	A86	smoc (raw:0.569;Z:-1.772)
2	A87	smoc (raw:0.542;Z:-2.165)
3	A102	smoc (raw:0.535;Z:-1.909)
3	A302	fdrscore (raw:0.584;Z:-2.035)
4	A38	rama_z (2.007)
4	A39	rama_z (2.014)

CCP-EM model validation



CheckmySequence Chain: C

```
*** Sequence mismatch:

 protein start: 1 end: 141   evalue: 2.1e-63      seq2ref_si: 98.54014598540147



                123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
  model         ----------VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHGKKVADALTNAV
                          ||||||| |||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
  refseq        AAAAAAAAAAVLSPADK-NVKAAWGKVGASAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHGKKVADALTNAV

  model         AHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKYR---------
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  refseq        AHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKYRAAAAAAAAA

  model         -

  refseq        A
```

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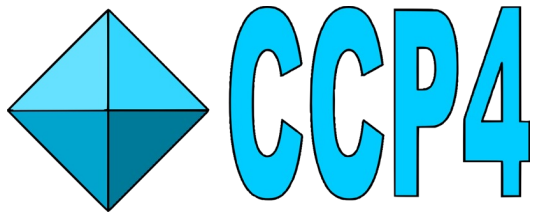
Acknowledgements



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Questions?

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Thank you

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