

1 **Outcomes of the 2019 EMDDataResource model challenge: validation of cryo-** 2 **EM models at near-atomic resolution**

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1 **Abstract**

2 This paper describes outcomes of the 2019 Cryo-EM Map-based Model Metrics Challenge
3 sponsored by EMDataResource (www.emdataresource.org). The goals of this challenge were (1)
4 to assess the quality of models that can be produced using current modeling software, (2) to
5 check the reproducibility of modeling results from different software developers and users, and
6 (3) compare the performance of current metrics used for evaluation of models. The focus was on
7 near-atomic resolution maps with an innovative twist: three of four target maps formed a resolution
8 series (1.8 to 3.1 Å) from the same specimen and imaging experiment. Tools developed in
9 previous challenges were expanded for managing, visualizing and analyzing the 63 submitted
10 coordinate models, and several novel metrics were introduced. The results permit specific
11 recommendations to be made about validating near-atomic cryo-EM structures both in the context
12 of individual laboratory experiments and holdings of structure data archives such as the Protein
13 Data Bank. Our findings demonstrate the relatively high accuracy and reproducibility of cryo-EM
14 models derived from these benchmark maps by 13 participating teams, representing both widely
15 used and novel modeling approaches. We also evaluate the pros and cons of the commonly used
16 metrics to assess model quality and recommend the adoption of multiple scoring parameters to
17 provide full and objective annotation and assessment of the model, reflective of the observed
18 density in the cryo-EM map.

1 Introduction

2 Electron cryo-microscopy (cryo-EM) has emerged as a key method to visualize and model a wide
3 variety of biologically important macromolecules and cellular machines. Researchers can now
4 routinely produce structures at near-atomic resolution, yielding new mechanistic insights into
5 cellular processes and providing support for drug discovery¹⁻³. Many academic institutions and
6 pharmaceutical companies have invested in modern cryo-EM facilities, and multi-user resources
7 are opening up worldwide⁴.

8 The recent explosion of cryo-EM structures raises important questions. What are the limits of
9 interpretability given the quality of the maps and resulting models? How do we quantify model
10 accuracy and reliability under the simultaneous constraints of map density and chemical rules?

11 The EMDDataResource Project (EMDR) was formed in 2006 as a collaboration between scientists
12 in the UK (EMDDataBank at the European Bioinformatics Institute) and the US (the Research
13 Collaboratory for Structural Bioinformatics and the National Center for Macromolecular Imaging).
14 Part of EMDR's mission is to derive validation methods and standards for cryo-EM maps and
15 models through community consensus⁵. We created an EM Validation Task Force⁶ analogous to
16 those derived for X-ray crystallographic and NMR structures^{7,8} and have sponsored Challenges,
17 workshops and virtual conferences to engage cryo-EM experts, modellers, and end-users^{5,9-13}.
18 During this period, cryo-EM has evolved rapidly (Figure 1).

19 This paper describes outcomes of EMDR's most recent Challenge, the 2019 Model "Metrics"
20 Challenge. The goals were three-fold: (1) to assess the quality of models that can be produced
21 using established as well as newly implemented modeling software, (2) to check the
22 reproducibility of modeling results from different software developers and users, and (3) to
23 compare the performance of model evaluation metrics, particularly fit-to-map metrics. Map targets
24 were selected in the near-atomic resolution regime (1.8-3.1 Å) with an innovative twist: three form
25 a resolution series from the same specimen/imaging experiment (Figure 2). The results lead to
26 several specific recommendations for validating near-atomic cryo-EM structures directed towards
27 both individual researchers and the Protein Data Bank (PDB) structure data archive.

1 Results

2 We describe here the pipeline and outcomes of the EMDR 2019 Model Metrics Challenge (Figure
3 3). Four maps representing the state-of-the-art in cryo-EM single particle reconstruction were
4 selected as the Challenge targets (Figures 2, 3a). Three maps of human heavy-chain apoferritin
5 (APOF), a 500 kDa octahedral complex of 24 α -helix-rich subunits, formed a resolution series
6 differing only in the number of particles used in reconstruction (EMDB entries [EMD-20026](#), [EMD-](#)
7 [20027](#), [EMD-20028](#))¹⁴. The fourth map was horse liver alcohol dehydrogenase (ADH), an 80 kDa
8 α/β homodimer with NAD and Zn ligands ([EMD-0406](#))¹⁵.

9 A key criterion of target selection was availability of high quality experimentally determined model
10 coordinates to serve as references. A 1.5 Å X-ray structure¹⁶ (PDB id [3ajo](#)) served as the
11 reference for all three APOF maps, since no cryoEM-based model was available at the time. The
12 X-ray model provides an excellent fit to each map, though not a fully optimized fit, owing to
13 method/sample differences. The ADH reference was the model deposited by the original authors
14 of the cryo-EM study (PDB id [6nbb](#))¹⁵.

15 Thirteen teams from the US and Europe submitted 63 models in total, yielding 15-17 submissions
16 per target (Figure 3b, Table I). The vast majority (51) were created *ab initio*, sometimes supported
17 by additional manual steps, while others (12) were optimizations of publicly available models.

18 Submitted models were evaluated as in the previous Challenge¹² with multiple metrics in each of
19 four tracks: Fit-to-Map, Coordinates-only, Comparison-to-Reference, and Comparison-among-
20 Models (Figure 3c, Table II). The selected metrics include many already in common use, as well
21 as several introduced via this Challenge.

22 Metrics to evaluate global Fit-to-Map included Map-Model Fourier Shell Correlation (FSC)¹⁷ as
23 encoded in Phenix¹⁸, Refmac FSC average¹⁹, EMDB atom inclusion²⁰, EMRinger²¹, multiple Map
24 vs. Model density-based correlation scores from TEMPy²²⁻²⁵, Phenix¹⁸, and the recently
25 introduced Q-score to assess atom resolvability¹⁴.

26 Metrics to evaluate overall Coordinates-only quality included Clashscore, Rotamer outliers, and
27 Ramachandran outliers from MolProbity²⁶, as well as standard geometry measures (bond, bond
28 angle, chirality, planarity, and dihedral angle RMSDs) from Phenix²⁷. PDB currently uses each of
29 these validation measures, based on community recommendations⁶⁻⁸. New in this round was
30 MolProbity CaBLAM, which evaluates protein backbone conformation across multiple residues
31 using novel virtual dihedral angle definitions²⁸.

1 Metrics assessing the similarity of a model to a reference structure included Global Distance Test
2 total score²⁹, Local Difference Distance Test³⁰, CaRMSD from OpenStructure/QS³¹, and Contact
3 Area Difference³². Davis-QA was used to measure similarity among submitted models³³. All of
4 these measures are widely used in CASP competitions³³.

5 Several metrics were also evaluated at the per-residue level: Fit-to-Map: EMRinger, Q-score,
6 EMD atom inclusion, TEMPy SMOC, and Phenix CCbox; Coordinates-only: Clashes,
7 Ramachandran outliers, and CaBLAM.

8 Evaluated metrics are tabulated with brief definitions in Table II; extended descriptions are
9 provided in Online Methods.

10 An evaluation system website with interactive tables, plots and tools (Figure 3d) was established
11 in order to organize and enable analysis of the Challenge results and to make the results
12 accessible to all participants (model-compare.emdataresource.org).

13

14 Overall and local quality of models

15 The vast majority of submitted models scored well, landing in “acceptable” regions for metrics in
16 each of the evaluation tracks, and in many cases performing better than the associated reference
17 structure which served as a control (Supplementary Figure 1). For teams that submitted *ab initio*
18 models, additional manual adjustment was beneficial, particularly for models built into the two
19 lower resolution targets. In general, the best scoring models were produced by well-established
20 methods and experienced modeling practitioners.

21 Evaluation exposed four fairly frequent issues: mis-assignment of peptide-bond geometry,
22 misorientation of peptides, local sequence misalignment, and failure to model associated ligands.
23 Sidechain model quality was not specifically assessed in this round.

24 Two-thirds of the submitted models had one or more peptide-bond geometry errors
25 (Supplementary Figure 2).

26 At resolutions near 3 Å or in weak local density, the carbonyl O protrusion disappears into the
27 tube of backbone density (Figure 2), and *trans* peptide bonds are more readily modeled in the
28 wrong orientation. If ϕ, ψ values are explicitly refined, adjacent side chains can be pushed further
29 in the wrong direction instead of fixing the underlying problem. Such cases are not flagged as
30 Ramachandran outliers but they are still recognized by CaBLAM³⁴.

1 Sequence misthreadings misplace specific chemical groups over very large distances. The
2 misalignment can be recognized by local Fit-to-Map criteria, with ends flagged by CaBLAM, bad
3 geometry, *cis*-nonPro peptides, and clashes (Supplementary Figure 3).

4 The ADH map contains tightly bound ligands: an NADH cofactor as well as two zinc ions per
5 subunit, with one zinc in the active site and the other in a spatially separate site where the metal
6 coordinates with four cysteine residues¹⁵. A number of models lacking these ligands had
7 considerable local modeling errors, sometimes this even mistracing the backbone (Supplementary
8 Figure 4).

9 Although there was evidence for ordered water in the higher resolution APOF maps¹⁴, only two
10 groups elected to model water oxygen atoms in their submissions. Model submissions were also
11 split approximately 50:50 for the following practices: (1) inclusion of predicted hydrogen atom
12 positions and (2) refinement of isotropic B-factors. Although near-atomic cryo-EM maps do not
13 have a sufficient level of detail to directly identify hydrogen atom positions, inclusion of predicted
14 H-atom positions can be useful for identifying model steric properties such as H-bonds or
15 clashes²⁶. Where provided, refined B-factors modestly improved Fit-to-Map scores against the
16 highest resolution map target (APOF 1.8 Å) but had little to no benefit against lower resolution
17 map targets.

18 **Evaluating Metrics: Fit-to-Map**

19 Fit-to-Map metrics (Table II, red section) were systematically compared using score distributions
20 of the submitted models (Figure 4a-d). For APOF targets, subunit models were evaluated against
21 masked subunit maps, whereas for the ADH target, dimeric models were evaluated against the
22 full sharpened cryo-EM map (Figure 2d). To control for the impact of hydrogen atom inclusion or
23 isotropic B-factor refinement on different subsets of Fit-to-Map metrics, all evaluated scores were
24 produced with hydrogen atoms removed and with B-factors set to zero.

25 Score distributions were first evaluated *for all 63 models across all four Challenge targets*.
26 Unexpectedly, a wide diversity in performance was observed, with poor correlations between
27 most pairs of metrics (Figure 4a). This means that a model that scored well relative to all 62 others
28 using one metric may have a much poorer ranking using another metric. A hierarchical cluster
29 analysis identified three distinct clusters of similarly performing metrics (Figure 4a, boxes 1-3).

30 The observed sparse correlations and clustering of the Fit-to-Map metrics can be readily
31 understood by considering their per target score distribution ranges, which differ substantially

1 from each other (Figure 4c). The three clusters identify sets of metrics that share similar trends
2 (Fig. 4c, panels 1-3).

3 *Cluster 1* metrics (Figure 4c, panel 1) share the trend of decreasing score values with increasing
4 map target resolution. The cluster consists of six correlation measures, three from TEMPY²²⁻²⁵
5 and three from Phenix¹⁸. Each evaluates a model's fit to the map in a similar way: by correlating
6 a calculated model-map density with the experimental map density. In most cases (5 of 6),
7 correlation is performed following model-based masking of the experimental map. The observed
8 trend arises at least in part because as map resolution increases, the level of detail that a model-
9 map must faithfully replicate in order to achieve a high correlation score must also increase.

10 *Cluster 2* metrics (Figure 4c, panel 2) share the inverse trend: score values improve with
11 increasing map target resolution. Cluster 2 metrics consist of Phenix Map-Model FSC=0.5¹⁸,
12 Qscore¹⁴, and EMRinger²¹. The observed trend is expected: by definition each metric assesses a
13 model's fit to the experimental map in a manner that is sensitive to map resolution.

14 *Cluster 3* metrics (Figure 4c, panel 3) share a different trend: score values are significantly lower
15 for ADH relative to APOF map targets. These measures include three unmasked correlation
16 functions from TEMPY²²⁻²⁵, Refmac FSCavg¹⁹, EMDB Atom Inclusion²⁰ and TEMPY ENV²². All of
17 these measures consider the full experimental map without masking, so can therefore be sensitive
18 to background noise. Background noise was substantial in the unmasked ADH map and minimal
19 in the masked APOF maps (Figure 2d).

20 Score distributions were also evaluated for how similarly they performed *per target*, and in this
21 case most metrics were strongly correlated with each other (Figure 4b). This means that within
22 any single target, a model that scored well relative to all others using one metric also fared well
23 using nearly every other metric. This situation is illustrated by comparing scores for two different
24 metrics, CCbox from Cluster 1 and Q-score from Cluster 2 (Figure 4d). The plot's four diagonal
25 lines demonstrate that the scores are tightly correlated with each other within each map target.
26 But as described above in the analyses of Clusters 1 and 2, the two metrics each have different
27 sensitivities to map-specific factors. It is these different sensitivities that give rise to the separate
28 and parallel spacings of the four diagonal lines, indicating score ranges on different relative scales
29 for each target.

30 One Fit-to-Map metric showed poor correlation with all others in the per target analysis: TEMPY
31 ENV (Figure 4b). ENV scores were poorly distributed with most models very close to the maximum
32 possible value (1.0). ENV evaluates atom positions relative to a density threshold that is

1 determined from the sample molecular weight. At near-atomic resolution this threshold is overly
2 generous and tends to include all modelled atoms. TEMPy Mutual Information (MI) and EMRinger
3 also diverged somewhat from the other metrics (Figure 4b). Within each target, all MI scores were
4 essentially identical to each other. This behavior may reflect a strong influence of background
5 noise, since MI_OV, MI's masked version, yielded distributed scores that correlated well with
6 other measures. As noted previously²¹, EMRinger follows similar trends with other measures but
7 yields distinct distributions owing to its focus on backbone placement.

8 Collectively these results reveal that multiple factors such as experimental map resolution,
9 presence of background noise, and density threshold selection can strongly impact Fit-to-Map
10 score values, depending on the chosen metric.

11 **Evaluating metrics: Coordinates-only and vs-Reference**

12 Metrics to assess model quality based on Coordinates-only (Table II, blue section), as well as
13 Comparison-to-Reference and Comparison-among-Models (Table II, green and grey sections)
14 were also evaluated and compared (Figure 4e-f).

15 Most of the Coordinates-only metrics were poorly correlated with each other (Figure 4e), with the
16 exception of bond, bond angle, and chirality RMSD, which form a small cluster. Interestingly,
17 Ramachandran outlier score, which is widely used to assess protein backbone conformation, was
18 poorly correlated with all other Coordinate-only measures, including the novel CaBLAM scores²⁸.
19 Score distributions explain this in part: more than half (33) of submitted models had zero
20 Ramachandran outliers, while only four had zero CaBLAM Conformation outliers (we note that
21 Ramachandran statistics are increasingly used as restraints^{35,36}). These results support the
22 concept of CaBLAM as a new informative score for validating backbone conformation²⁸.

23 The CaBLAM Conformation and C-alpha measures, while orthogonal to other Coordinate-only
24 measures, were unexpectedly found to perform very similarly to Comparison-to-Reference
25 metrics; several Fit-to-Map metrics also performed somewhat similarly to Comparison-to-
26 Reference metrics (Figure 4f). The similarity likely arises because the worst modeling errors in
27 this Challenge were sequence and backbone conformation mis-assignments. These errors were
28 equally flagged by CaBLAM, which compares models against statistics of high-quality structures
29 from the PDB, and the Comparison-to-Reference metrics, which compare models directly against
30 a high-quality reference. To a somewhat lesser extent these modeling errors were also flagged
31 by Fit-to-Map metrics.

1 Evaluating metrics: local scoring

2 As part of the evaluation pipeline, residue-level scores were calculated in addition to overall
3 scores. Five Fit-to-Map metrics either considered masked density for both map and model around
4 the evaluated residue (Phenix CCbox¹⁸, TEMPy SMOC²⁴), density profiles at non-hydrogen atom
5 positions (Qscore¹⁴), density profiles of non-branched residue C γ -atom ringpaths (EMRinger²¹),
6 or density values at non-hydrogen atom positions relative to a chosen threshold (EMDB Atom
7 Inclusion²⁰). In two of the five, residue-level scores were obtained as sliding-window averages
8 over multiple contiguous residues (SMOC: 9-residues; EMRinger: 21-residues).

9 Residue-level correlation analyses similar to those described above showed that local fit-to-map
10 scores diverged more than their corresponding global scores. Residue-level scoring was most
11 similar across the evaluated metrics for high resolution maps. This observation suggests that the
12 choice of method for scoring residue-level fit becomes less critical at higher resolution, where
13 maps tend to have stronger density/contrast around atom positions.

14 A case study of a local modeling error in one of the APOF 2.3 Å models (Supplementary Figure
15 3) showed that EMD Atom Inclusion²⁰, Phenix CCbox¹⁸, and Qscore¹⁴ measures produced
16 significantly lower (worse) scores within a 4-residue α -helical misthread relative to correctly
17 assigned flanking residues. In contrast, the two sliding-window-based metrics were largely
18 insensitive (a more recent version of TEMPy offers single residue analysis (SMOCd) and
19 adjustable window analysis based on map resolution (SMOCf³⁷). At near-atomic resolution,
20 single residue fit-to-map evaluation methods are likely to be more useful than windowing methods
21 for identifying local modelling issues.

22 Residue-level Coordinate-only metrics (Supplementary Figure 3), Comparison-to-Reference
23 metrics and Comparison-among-Models metrics (not shown) were also evaluated for the same
24 modeling error. The MolProbity server^{26,28} flagged the problematic 4-residue misthread via
25 CaBLAM, cis-Peptide, clashscore, bond, and angle scores, but all Ramachandran scores were
26 either favored or allowed. The Comparison-to-Reference LDDT and LGA local scores and the
27 Davis-QA model consensus score also strongly flagged this error. The example demonstrates the
28 value of combining multiple orthogonal measures to identify geometry issues, and further
29 highlights the value of CaBLAM as a novel, orthogonal measure for validation of backbone
30 conformation.

1 Group performance

2 Group performance was examined by modeling category and target by combining Z-scores from
3 metrics determined to be meaningful in the analyses described above (see Methods and
4 Supplementary Figure 5).

5 For *ab initio* modeling, lower resolution targets were more challenging for some groups. For the
6 higher resolution APOF 1.8 Å and 2.3 Å targets, six groups (10, 28, 35, 41, 73, 82, see Table I
7 ids) did very well ($Z \geq 0.3$), and a seventh (54, models 2) was a runner-up. For the lower
8 resolution APOF 3.1 Å and ADH 2.9 Å targets, a slightly different six groups (10, 27, 28, 35, 73,
9 82) did very well and another two (41, 90) were runners-up. A wide variety of map density features
10 and algorithms to produce a model, and most were quite successful yet allowing a few mistakes,
11 often in different places (see Supplementary Figures 2-4). For practitioners, it might be beneficial
12 to compare/combine models from several *ab initio* methods to come up with a better initial model
13 for subsequent refinement. Note that the performance results are specific to the Challenge task
14 and may not be directly applicable to other modeling scenarios.

15 As for optimization-based modeling, all made improvements, but sample size was too small to
16 produce a rating.

17 Discussion

18 This 3rd Model Challenge round has demonstrated that cryo-EM maps with resolution ≤ 3 Å and
19 from samples with limited conformational flexibility, have excellent information content, and
20 automated methods are able to generate fairly complete models from such maps, needing only
21 small amounts of manual intervention to be finalized (but some is always needed). Modeling could
22 readily be accomplished within a month, the time-period of this challenge. This outcome
23 represents a great advance over the previous challenges.

24 Inclusion of three maps in a resolution series enabled controlled evaluation of metrics by
25 resolution. Inclusion of a completely different map as the fourth target provided a useful additional
26 control. These target selections enabled observation of important trends that otherwise could
27 have been missed. In a recent evaluation of predicted models against several ~ 3 Å cryo-EM maps
28 in the CASP13 competition, TEMPy and Phenix Fit-to-Map correlation measures performed very
29 similarly³⁷. In this Challenge, because the chosen map targets covered a wider resolution range
30 and had more variability in background noise, the same measures were found to have distinctive,
31 map target feature-sensitive performance profiles.

1 The majority of submitted models were overall either equivalent to or better than the reference
2 model in terms of the fit of their atomic coordinates to the target map. This achievement reflects
3 significant advances in the development of modeling tools relative to the state presented a decade
4 ago in our first Model Challenge⁹. However, several factors beyond atom positions that become
5 important for accurate modelling at near-atomic resolution were not uniformly addressed: only
6 half of the submitted models included refinement of atomic displacement factors (B-factors), and
7 a minority of modellers attempted to fit water or bound ligands.

8 Fit-to-Map measures were found to be sensitive to different physical properties of the map,
9 including experimental map resolution and background noise level, as well as input parameters
10 such as density threshold. Coordinates-only measures were found to be largely orthogonal to
11 each other, while Comparison-to-Reference measures were generally well correlated with each
12 other.

13 The cryo-EM modeling community as represented by the Challenge participants have introduced
14 a number of metrics to evaluate cryo-EM models with sound biophysical basis. We find that some
15 of them are correlated to each other and to the resolution of the map, while others are not. Based
16 on our careful analyses of these metrics and their relationships, we make four recommendations
17 regarding validation practices for cryo-EM models of proteins determined at near-atomic
18 resolution as studied here between 3.1 Å and 1.8 Å, a rising trend for cryo-EM (Figure 1).

19 Recommendation 1: For researchers optimizing a model against a single map, nearly any of the
20 evaluated global fit-to-map metrics (Table II) can be used to evaluate progress because they are
21 all largely equivalent in performance. Exception: TEMPy ENV is more appropriate for medium to
22 low resolution (>4 Å).

23 Recommendation 2: To flag issues with local (per residue) fit to a map, metrics that evaluate
24 single residues such as CCbox, Qscore, and EMD Atom Inclusion are more suitable than those
25 using sliding window averages over multiple residues.

26 Recommendation 3: The ideal Fit-to-Map metric for *archive-wide ranking* will be insensitive to
27 map background noise (appropriate masking or alternative data processing can help), will not
28 require input of estimated parameters that affect score value (*e.g.*, resolution limit, threshold). and
29 will yield overall better scores for maps with trustworthy higher-resolution features. The three
30 Cluster 2 metrics identified in this Challenge (Figure 4a) meet these criteria.

- 31 • Map-Model FSC^{17,18} is already in common use¹³, and can be compared with the
32 experimental map's independent half-map FSC curve.

- 1 • Global EMRinger score²¹ can assess non-branched protein side chains.
- 2 • Q-score is a relatively new correlation metric that can be used both globally and locally for
- 3 validating non-hydrogen-atom x,y,z positions.¹⁴.

4 Other Fit-to-map metrics may be rendered suitable for archive-wide comparisons through

5 conversion of raw scores to Z-scores over narrow resolution bins, as is currently done by the PDB

6 for some X-ray-based metrics^{7,38}.

7 Recommendation 4: CaBLAM statistical measures and MolProbity cis-peptide detection²⁸ are

8 useful to detect protein backbone conformation issues. These are valuable new tools for cryo-EM

9 protein structure validation, particularly since maps at typical resolutions (2.5 - 4.0 Å; Figure 1)

10 may not resolve backbone carbonyl oxygens (Figure 2).

11 The 2019 Model “Metrics” Challenge was more successful than previous challenges because

12 more time could be devoted to analysis and because infrastructure for model collection,

13 processing and assessment is now established. EMDR plans to sponsor additional model

14 challenges in order to continue promoting development and testing of cryo-EM modeling and

15 validation methods. Future challenge topics are likely to cover medium resolution (3 to 4 Å),

16 particle heterogeneity, membrane proteins, ligand modeling, nucleic acids, and models derived

17 from tomograms.

18

1 Online Methods

2 Challenge Process and Organization

3 Informed by previous Challenges^{9,10,12}, the 2019 Model Challenge process was significantly
4 streamlined in this round. In March, a panel of advisors with expertise in cryo-EM methods,
5 modeling, and/or model assessment was recruited. The panel worked with EMDR team members
6 to develop the challenge guidelines, identify suitable map targets from EMDB and reference
7 models from PDB, and recommend the metrics to be calculated for each submitted model.

8 The Challenge rules and guidance were as follows: (1) *Ab initio* modeling is encouraged but not
9 required. For optimization studies, any publicly available coordinate set can be used as the
10 starting model. (2) Regardless of the modeling method used, submitted models should be as
11 complete and as accurate as possible (*i.e.*, equivalent to publication-ready). (3) For each target,
12 a separate modeling process should be used. (4) Fitting to either the unsharpened/unmasked
13 map or one of the half-maps is strongly encouraged. (5) Submission in mmCIF format is strongly
14 encouraged.

15 Members of cryo-EM and modeling communities were invited to participate in mid-April 2019;
16 details were posted on the challenges website (challenges.emdataresource.org). Models were
17 submitted by participant teams between May 1 and May 28, 2019. For apoferritin (APOF) targets,
18 coordinate models were submitted as single subunits at the position of a provided segmented
19 density consisting of a single subunit. Alcohol dehydrogenase (ADH) models were submitted as
20 dimers. For each submitted model, metadata describing the full modeling workflow were collected
21 via a Drupal webform, and coordinates were uploaded and converted to PDBx/mmCIF format
22 using PDBextract³⁹. Model coordinates were then processed for atom/residue ordering and
23 nomenclature consistency using PDB annotation software (Feng Z.,
24 <https://swtools.rcsb.org/apps/MAXIT>) and additionally checked for sequence consistency and
25 correct position relative to the designated target map. Models were then evaluated as described
26 below (Model Evaluation System).

27 In early June, models, workflows, and initial calculated scores were made available to all
28 participants for evaluation, blinded to modeler team identity and software used. A 2.5-day
29 workshop was held in mid-June at Stanford/SLAC to review the results, with panel members
30 attending in person. All modeling participants were invited to attend remotely and present
31 overviews of their modeling processes and/or assessment strategies. Recommendations were
32 made for additional evaluations of the submitted models as well as for future challenges. Modeler

1 teams and software were unblinded at the end of the workshop. In September, a virtual follow-up
2 meeting with all participants provided an overview of the final evaluation system after
3 implementation of recommended updates.

4 **Modeling Software**

5 Modelling teams created *ab initio* models or optimized previously known models available from
6 the PDB. *Ab initio* software included ARP/wARP⁴⁰, Buccaneer^{41,42}, Cascaded-CNN⁴³, Mainmast⁴⁴,
7 Terashi 2020, Pathwalker⁴⁵, and Rosetta⁴⁶. Optimization software included CDMD⁴⁷, CNS⁴⁸, DireX⁴⁹,
8 Phenix²⁷, REFMAC¹⁹, MELD⁵⁰, MDFF⁵¹, and reMDFF⁵². Participants made use of VMD⁵³,
9 Chimera⁵⁴, and COOT³⁵ for visual evaluation and/or manual model improvement of map-model
10 fit. See Table I for software used by each modeling team.

11 **Model Evaluation System**

12 The evaluation system for 2019 Challenge (model-compare.emdataresource.org) was built on the
13 basis of the 2016/2017 Model Challenge system¹², updated with several new evaluation
14 measures and analysis tools. Submitted models were evaluated for >70 individual metrics in four
15 tracks: Fit-to-Map, Coordinates-only, Comparison-to-Reference, and Comparison-among-
16 Models. A detailed description of the updated infrastructure and each calculated metric is provided
17 as a help document on the model evaluation system website.

18 For brevity, a representative subset of metrics from the evaluation website are discussed in this
19 paper. The selected metrics are listed in Table II, and are further described below. All scores were
20 calculated according to package instructions using default parameters.

21 Fit-to-Map

22 The evaluated metrics included several ways to measure the correlation between map and model
23 density⁵⁵, as implemented in TEMPy²²⁻²⁵ v.1.1 (CCC, CCC_OV, SMOC, LAP, MI, MI_OV) and the
24 Phenix²⁷ v.1.15.2 map_model_cc module¹⁸ (CCbox, CCpeaks, CCmask). These methods
25 compare the experimental map with a model map produced on the same voxel grid, integrated
26 either over the full map or over selected masked regions. The model-derived map is generated to
27 a specified resolution limit by inverting Fourier terms calculated from coordinates, B-factors, and
28 atomic scattering factors. Some measures compare density-derived functions instead of density
29 (Mutual Information, Laplacian²²).

30 The newly introduced Q-score (MAPQ v1.2¹⁴ plugin for UCSF Chimera⁵⁴ v.1.11) uses a real-space
31 correlation approach to assess the resolvability of each model atom in the map. Experimental

1 map density is compared to a Gaussian placed at each atom position, omitting regions that
2 overlap with other atoms. The score is calibrated by the reference Gaussian, which is formulated
3 so that a highest score of 1 would be given to a well-resolved atom in a map at ~ 1.5 Å resolution.
4 Lower scores (down to -1) are given to atoms as their resolvability and the resolution of the map
5 decreases. The overall Q-score is the average value for all model atoms.

6 Measures based on Map-Model FSC curve, atom inclusion, and protein side chain rotamers were
7 also compared. Phenix Map-Model FSC is calculated using a soft mask and is evaluated at
8 $FSC=0.5$ ¹⁸. REFMAC FSCavg¹⁹ (module of CCPEM⁵⁶ v1.4.1) integrates the area under the Map-
9 Model FSC curve to a specified resolution limit¹⁹. EMD Atom Inclusion determines the
10 percentage of atoms inside the map at a specified density threshold²⁰. TEMPY ENV is also
11 threshold-based and penalizes unmodeled regions²². EMRinger (module of Phenix) evaluates
12 backbone positioning by measuring the peak positions of unbranched protein C_γ atom positions
13 versus map density in ring-paths around C_α-C_β bonds²¹.

14 Coordinates-only

15 Standard measures assessed local configuration (bonds, bond angles, chirality, planarity,
16 dihedral angles; Phenix model statistics module), protein backbone (MolProbity Ramachandran
17 outliers²⁶; Phenix molprobity module) and side-chain conformations, and clashes (MolProbity
18 rotamers outliers and clashscore²⁶; Phenix molprobity module).

19 New in this Challenge round is CaBLAM²⁸ (part of MolProbity and as Phenix cablam module),
20 which employs two novel procedures to evaluate protein backbone conformation. In both cases,
21 virtual dihedral pairs are evaluated for each protein residue *i* using C_α positions *i*-2 through *i*+2.
22 To define CaBLAM outliers, the third virtual dihedral is between the CO groups flanking residue *i*.
23 To define Calpha-geometry outliers, the third parameter is the Calpha virtual angle at *i*. The
24 residue is then scored according to virtual triplet frequency in a large set of high-quality models
25 from PDB²⁸.

26 Comparison-to-Reference and Comparison-among-Models

27 Assessing the similarity of the model to a reference structure and similarity among submitted
28 models, we used metrics based on atom superposition (LGA GDT-TS and GDC scores²⁹
29 v.04.2019), interatomic distances (LDDT score³⁰ v.1.2), and contact area differences (CAD³²
30 v.1646). HBPLUS⁵⁷ was used to calculate nonlocal hydrogen bond precision, defined as the
31 fraction of correctly placed hydrogen bonds in residue pairs with > 6 separation in sequence.

1 DAVIS-QA determines for each model the average of pairwise GDT-TS scores among all other
2 models³³.

3 Local (per residue) Scores

4 Residue-level visualization tools for comparing the submitted models were also provided for the
5 following metrics. Fit-to-Map: Phenix CCbox, TEMPy SMOC, Qscore, EMRinger, EMDB Atom
6 Inclusion; Comparison-to-Reference: LGA and LDDT; Comparison-among-Models: DAVIS-QA.

7 **Metric Score Pairwise Correlations and Distributions**

8 For pairwise comparisons of metrics, Pearson correlation coefficients (P) were calculated for all
9 model scores and targets (N=63). For average per-target pairwise comparisons of metrics, P
10 values were determined for each target and then averaged. Metrics were clustered according to
11 the similarity score (1-|P|) using a hierarchical algorithm with complete linkage. At the beginning,
12 each metric was placed into a cluster of its own. Clusters were then sequentially combined into
13 larger clusters, with the optimal number of clusters determined by manual inspection. In the fit-to-
14 map evaluation track, the procedure was stopped after three divergent score clusters were formed
15 for the all-model correlation data (Figure 4a), and after two divergent clusters were formed for the
16 average per-target clustering (Figure 4b).

17 Score distributions are represented in box-and-whisker format in Figure 4c. Each box represents
18 the interquartile range (IQR) and is drawn between Q1 (25th percentile) and Q3 (75th percentile)
19 values. The inner horizontal line represents the median value (excluding outliers). Whisker lines
20 extend out to the highest and lowest measured scores that are within 1.5*IQR of each box end.
21 Scores falling outside the 1.5*IQR limits are considered outliers and are separately plotted as
22 dots.

23 **Controlling for Model Systematic Differences**

24 As initially calculated, some Fit-to-Map scores had unexpected distributions, owing to differences
25 in modeling practices among participating teams. For models submitted with all atom occupancies
26 set to zero, occupancies were reset to one and rescored. In addition, model submissions were
27 split approximately 50:50 for each of the following practices: (1) inclusion of hydrogen atom
28 positions and (2) inclusion of refined B-factors. For affected fit-to-map metrics, modified scores
29 were produced excluding hydrogen atoms and/or setting B-factors to zero. Both original and
30 modified scores are provided in the web interface. Only modified scores were used in the pairwise
31 metric comparisons described here.

1 Evaluation of Group Performance

2 Rating of group performance was done using the Model Compare Pipeline/Comparative
3 Analyses/Model Ranks (per target) tool on the Challenge evaluation website. The tool permits
4 users, for a specified target and for all or a subcategory of models (e.g., *ab initio*), to calculate Z-
5 scores for each individual model, using any combination of 47 of the evaluated metrics with any
6 desired relative weightings. The Z-scores for each metric are calculated from all submitted models
7 for that target. The metrics (weights) used to generate individual-model Z-scores were as follows:

8 Coordinates-only: CaBLAM outliers (0.5), Calpha-geometry outliers (0.3), and Clashscore (0.2).
9 CaBLAM outliers and Calpha-geometry outliers had the best correlation with match-to-target
10 parameters (Figure 5b), and clashscore is an orthogonal measure. Ramachandran and rotamer
11 criteria were excluded since they are often restrained in refinement and are zero for many models.

12 Fit-to-Map: EMringer (0.3) and Q-score (0.3), Atom Inclusion-backbone (0.2), and SMOC (0.2).
13 EMringer and Q-score were among the most promising model-to-map metrics, and the other two
14 provide distinct measures.

15 Comparison-to-Reference: LDDT (0.9) and GDC_all (0.9) and HBPR>6 (0.2). LDDT is
16 superposition-independent and local, while GDC_all requires superposition; H-bonding is distinct.
17 Metrics in this category are weighted higher, because although the target models are not perfect,
18 they are a reasonable estimate of the right answer.

19 Individual Z-scores for each model were then averaged across each group's models on a given
20 target, and further averaged across T1+T2 and across T3+T4, yielding overall Z-scores for high
21 and low resolutions. The scores consistently showed 3 quite separate clusters: a good cluster at
22 $Z > 0.3$, an unacceptable cluster at $Z < -0.3$, and a small cluster near $Z = 0$ (see Supplementary
23 Figure 5). Other choices of metrics were tried, with very little effect on clustering.

24 Group 54 models were rated separately because they used different methods, their 2nd model
25 versions were much better. Group 73's second model on target T4 was not rated because the
26 metrics are not set up to meaningfully evaluate an ensemble.

27 Molecular Graphics

28 Molecular graphics images were generated using UCSF Chimera⁵⁴ (Figure 2 and Supplementary
29 Figure 3: maps with superimposed models) and KiNG⁵⁸ (Supplementary Figures 2 and 4: maps
30 with superimposed models and validation flags).

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21

1 **Author Contributions**

2 PDA, PVA, JF, FDM, JSR, PBR, HMB, WC, AK, CLL, GDP, MFS: expert panel that selected
3 targets, reference models and assessment metrics, set challenge rules; attended face-to-face
4 results review workshop. KZ generated the APF maps for the challenge. MAH provided the
5 published ADH map. CLL: designed and implemented the challenge model submission pipeline,
6 drafted initial manuscript. Authors as listed in Table I: Built and submitted models; presented
7 modeling strategies at review workshop. AK: designed and implemented evaluation pipeline and
8 website, calculated scores. AK, CLL, BM, MAH, JSR, CJW, PVA, JF: analyzed models and
9 model scores. AP, ZW, MT, ADJ, GDP, PVA, CJW: contributed software, advice on use and
10 scores interpretation. CLL, AK, GDP, JSR: drafted figures. AK, HMB, GDP, WC, MFS, MAH,
11 JSR: contributed to manuscript writing. All authors: reviewed and approved final manuscript.

12

13 **Competing Interests**

14 The authors declare no competing interests.

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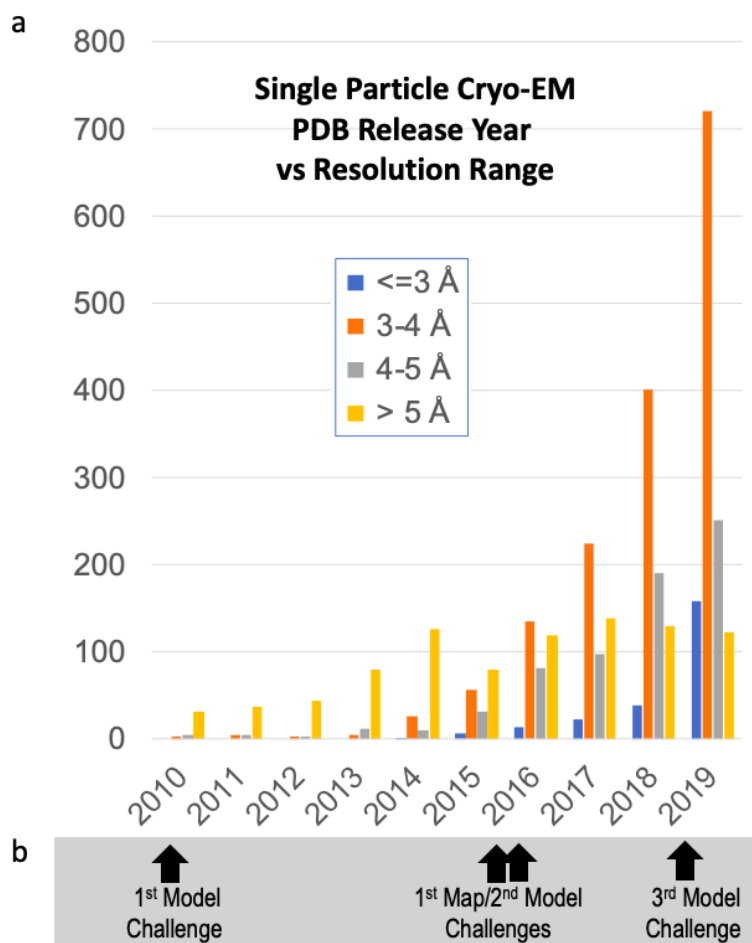
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11

1 Figure Legends/Figures

2 Figure 1. Single particle cryo-EM models in PDB

3 The availability of models in the Protein Data Bank⁵⁹ derived from single particle cryo-EM maps
4 has increased dramatically since the “resolution revolution” circa 2014⁶⁰. (a) Plot shows that the
5 steepest increase is for structures with reported resolution in the 3-4 Å range (orange bars).
6 Higher resolution structures (blue bars), the topic of the Challenge presented here, are also
7 beginning to trend upward. (b) EMDDataResource Challenge activities timeline.



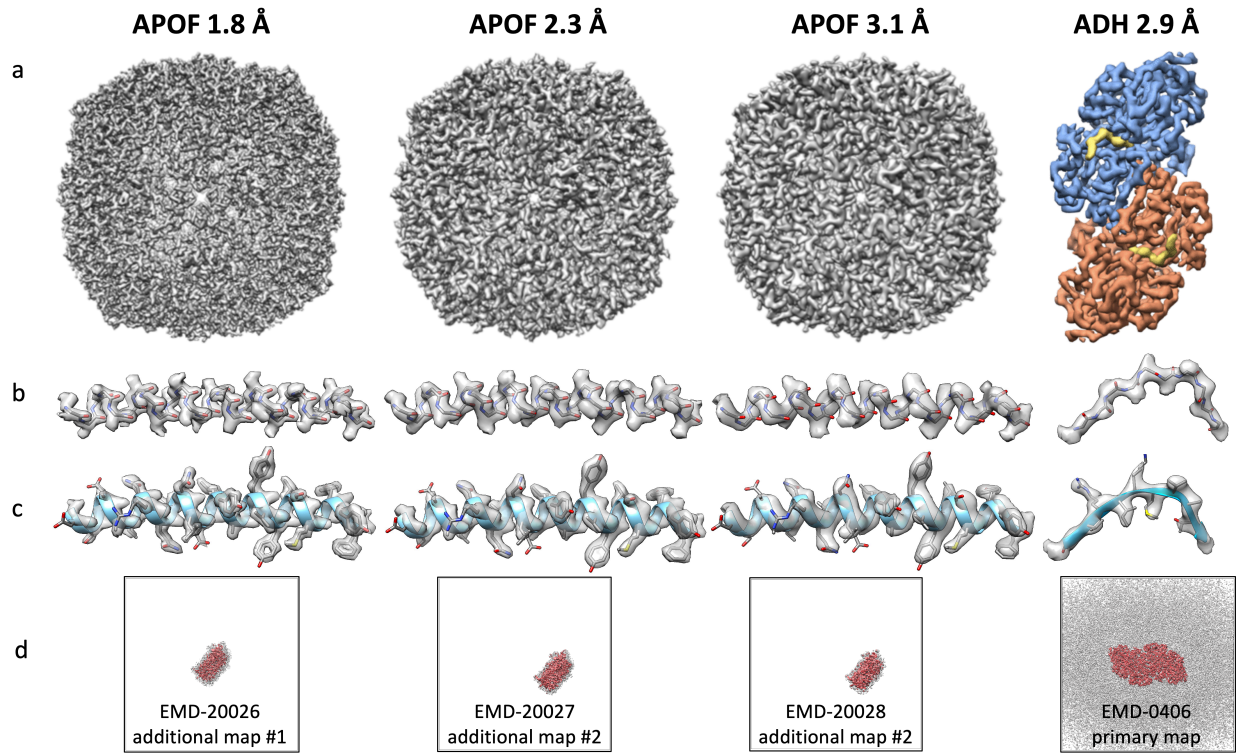
1 **Figure 2. Challenge targets: cryo-EM maps at near-atomic resolution**

2 Three density maps of α -helix rich apoferritin (APOF) at 1.8, 2.3, and 3.1 Å form a resolution
3 series differing only in the number of particles averaged (EM Data Bank entries EMD-20026,
4 EMD-20027, EMD-20028). The fourth density map, alcohol dehydrogenase (ADH) at 2.9 Å,
5 contains both α -helices and β -sheets, as well as ligands (yellow density bound to blue and red
6 subunits is NAD; EMDB entry EMD-0406). (a) Full map for each target. (b,c) Representative
7 secondary structural elements (APOF: residues 14-42; ADH: residues 34-45) with masked density
8 for protein backbone atoms only (b), and for all protein atoms (c).

9 Over the 1.8-3.1 Å resolution range represented by the four target maps, visible map features
10 transition from near-atomic to secondary structure-dominated. At 1.8 Å (APOF), most protein
11 atom positions are well defined by the map density: backbone protrusions delineate carbonyl
12 oxygen positions and holes appear inside aromatic rings. At 2.3 Å (APOF), most protein atom
13 positions are within the contoured map density; carbonyl oxygen atom “bumps” in the map help
14 to define direction of backbone trace. At 2.9 Å (ADH) and 3.1 Å (APOF), secondary structure
15 features begin to predominate. Notably, carbonyl oxygen atoms “bumps” are absent in the map,
16 making it harder to identify the direction of backbone trace.

17 (d) EMDB maps used in model Fit-to-Map analysis (APOF targets: masked single subunits; ADH:
18 unmasked sharpened map). The molecular boundary is shown in red (EMDB recommended
19 contour level), background noise is represented in grey ($1/3^{\text{rd}}$ of EMDB recommended contour
20 level), and the full map extent is indicated by the black outline.

1



2

1 **Figure 3. Challenge pipeline**

2 *Setup* (a): A panel of experts selected four target maps in the 1.8-3.1 Å resolution range as well
3 as reference models.

4 *Submissions* (b): Methods involving *ab initio* and optimization, both with and without additional
5 manual steps, were represented by 63 models submitted by 16 modeling teams.

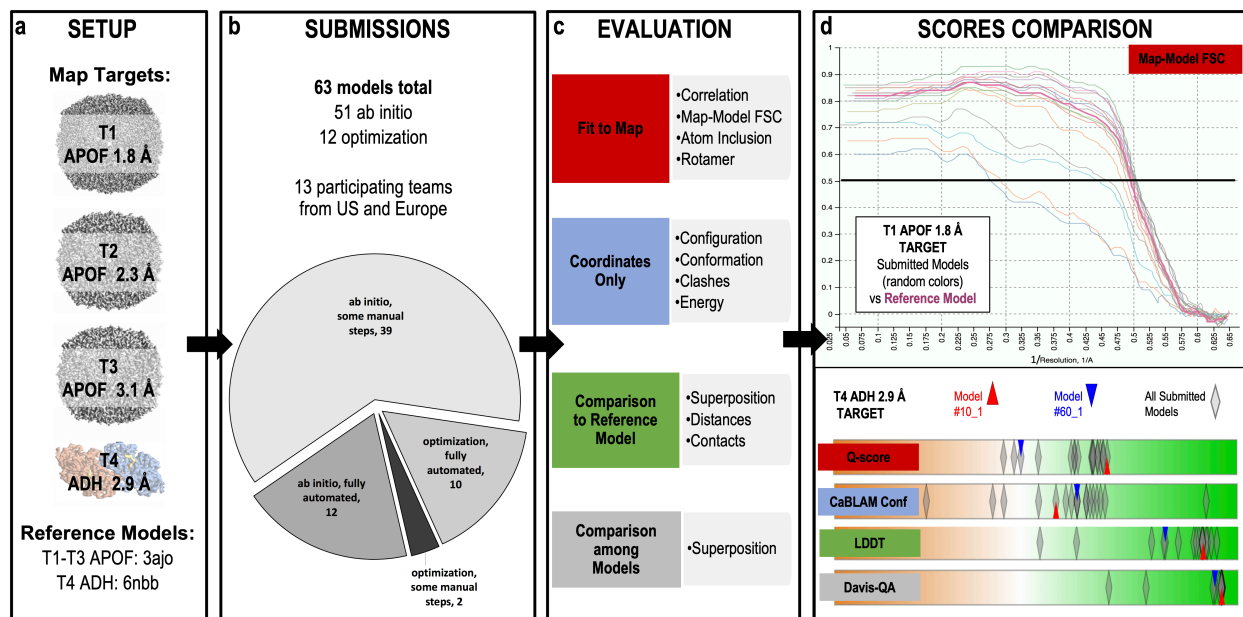
6 *Evaluation* (c): Building on the previous model challenge round¹², the evaluation system was
7 organized in four tracks (coordinates only, fit-to-map, comparison to reference model, and
8 comparison among models), each with its own set of software tools for generating scores.

9 *Scores Comparison* (d): Multiple interactive tabular and graphical displays enable comparative
10 evaluations on the model-compare website (model-compare.emdataresource.org).

11 *Top*: Map-Model Fourier Shell Correlation (FSC) curves for models submitted against the APOF
12 1.8 Å target (random light colors), versus curve for the reference model (bold cherry red). Map-
13 Model FSC measures the overall agreement of the experimental density map with a density map
14 derived from the coordinate model (model map)¹⁷. Curves are calculated from the Fourier
15 coefficients of the two maps and plotted vs. frequency (resolution⁻¹). Visualization of the full curve
16 is useful to ensure that it follows the expected sigmoidal shape with the tail decaying
17 exponentially. The resolution value corresponding to FSC=0.5 (black horizontal line) is typically
18 reported, with smaller values indicating better fit. In this example, some overlaid curves indicate
19 poor agreement between submitted model and the APOF 1.8 Å experimental map, but the
20 majority indicate equivalence to or even improvement over the X-ray reference model (PDB entry
21 3ajo was rigid-body fitted to the cryoEM target map without further refinement).

22 *Bottom*: scores comparison tool for ADH 2.9 Å target models. Interactive score distribution sliders
23 reveal at a glance how well the submitted models performed relative to each other. Parallel lanes
24 display score distributions for each evaluated metric. They are conceptually similar to the
25 graphical display for key metrics used in wwPDB validation reports^{7,38}. Displayed here are the
26 score distributions for all models submitted against the ADH target for four representative metrics,
27 one from each evaluation track, and scores for two individual models are also highlighted. Model
28 scores are plotted horizontally (semi-transparent diamonds) with color-coding to indicate worse
29 (left, orange) and better (right, green) values. Darker, opaque diamonds indicate multiple
30 overlapping scores. The interactive display enables scores for individual models to be identified
31 and compared (red and blue triangles). The model indicated by red triangles scored better than
32 the model indicated by blue triangles for Fit-to-Map: Q-score, Comparison-to-Reference: LDDT,

- 1 and Comparison-among-Models: Davis-QA, but the same model scored lower for Coordinates-
- 2 only: CaBLAM Conformation. The example demonstrates the importance of evaluating models in
- 3 each track: a high score in any one metric/track does not guarantee full optimization as judged by
- 4 another metric.



5

6

1 **Figure 4. Evaluation of metrics**

2 Model metrics (Table II) were compared with each other to assess how similarly they performed
3 in scoring the Challenge models.

4 (a-d) Fit-to-Map metrics were compared with each other to assess how similarly they performed
5 in scoring the Challenge models. Their similarity was evaluated in two ways: (a) Pairwise
6 correlation coefficients were calculated for all models across all map targets (n=63); (b) Average
7 correlation per target was calculated (separate correlation coefficients for each map target were
8 averaged). In both, pairs of metrics that are strongly similar in performance are indicated by dark
9 shading (high correlation/black: 0.85-1.0; moderately high correlation/grey: 0.7-0.84). Those that
10 perform very differently (poor correlation) are indicated with no shading. Correlation-based
11 metrics are identified by bold labels.

12 The ordering of metrics in (a) is based on hierarchical cluster analysis of all-model correlation
13 values (see Methods). Three red-outlined boxes along the table diagonal correspond to identified
14 clusters (#1-3); labels at left are also boxed in red according to these clusters. For ease of
15 comparison, the ordering of metrics in (b) is identical to (a). The red-outlined box in (b) identifies
16 the single cluster identified by hierarchical cluster analysis of the per-target correlation averages.
17 This one cluster includes all metrics but ENV.

18 In (c), representative score distributions for nine metrics, three from each cluster in (a), are plotted
19 for each map target. These plots illustrate the systematic differences in scoring per map target
20 that are responsible for the division of the evaluated metrics into the three clusters. In Cluster 1,
21 score distributions are lowest for the highest resolution target and increase as map resolution
22 decreases. Cluster 2 metrics have the opposite trend: score distributions are highest for the
23 highest resolution target and decrease as map resolution decreases. Cluster 3 metrics have a
24 mixed trend with respect to map resolution, but uniformly have lower score distributions for ADH
25 map target models relative to all three APOF map target models. See the main text for discussion
26 of the most likely factors behind these trends.

27 In (d), scores for one representative pair of metrics that belong to different clusters in (a) but to
28 the same cluster in (b) are plotted against each other. As highlighted by the diagonal lines
29 representing linear fits to scores for map target, both metrics (CCbox from Cluster 1 and Qscore
30 from Cluster 2) perform very similarly to each other within any one map target. Different
31 sensitivities of the two metrics to map-specific factors give rise to the separate and parallel
32 spacings of the four diagonal lines, with score ranges on different relative scales for each target.

- 1 (e) Coordinates-only and (f) Comparison-to-Reference metrics comparisons. Analogously to (a)
- 2 and (b), the Pearson correlation coefficient was determined for each metric pair for all submitted
- 3 model scores (n=63) across all map targets. In (f), Comparison-to-reference metrics are
- 4 contrasted with each other as well as with several Fit-to-Map and Coordinates-only metrics.

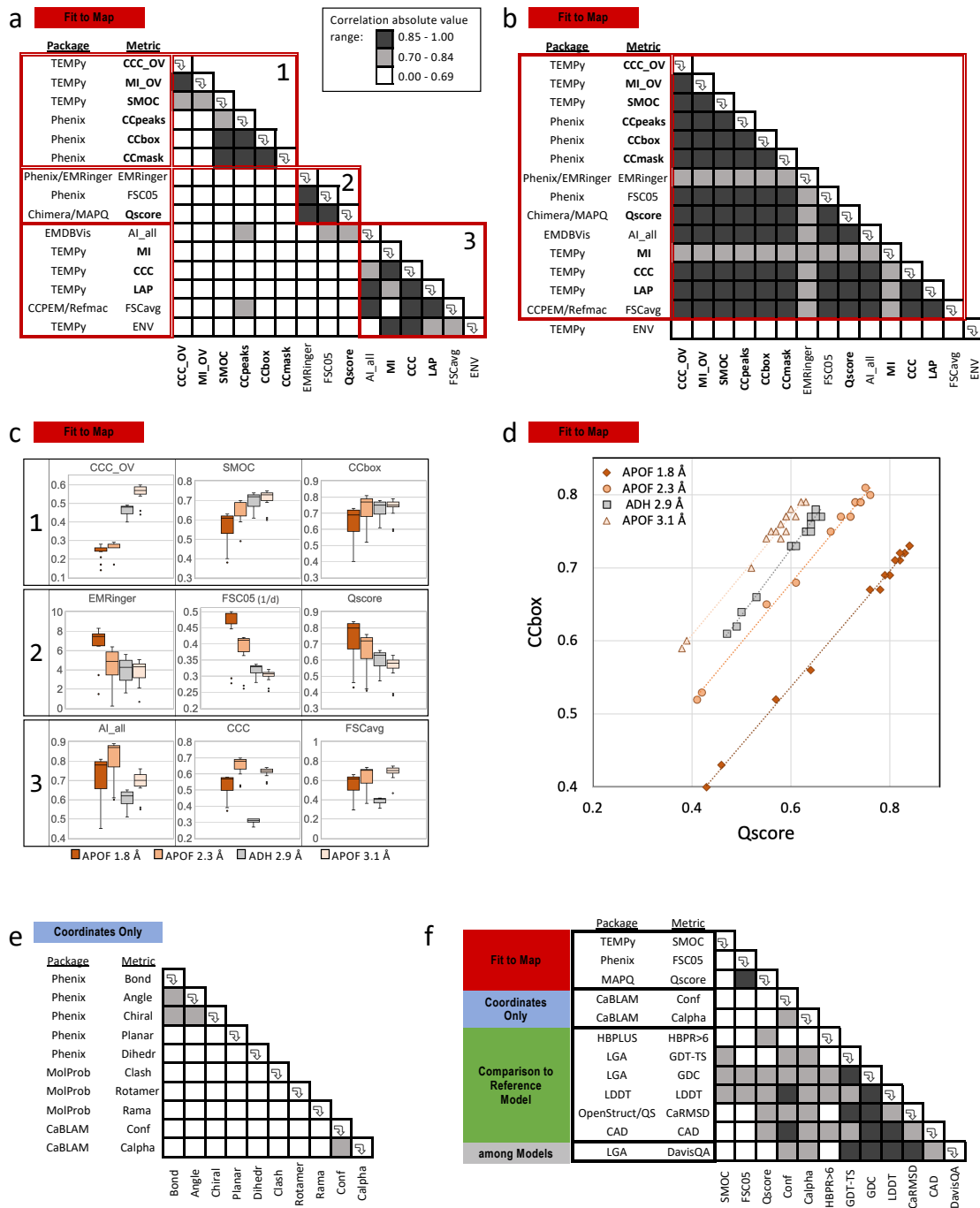


Table I. Participating Modeling Teams

Team ID, name	Team Members	# of Submitted Models	Effort Type(s)	Software
10 yu	Xiaodi Yu	4	ab initio+manual	Phenix, Buccaneer, Chimera, Coot, Pymol
25 cdmd	Maxim Igaev, Andrea Vaiana	4	optimization automated	CDMD
27 kumar	Dilip Kumar	1	ab initio+manual	Phenix, Rosetta, Buccaneer, ARP/wARP, Coot
28 ccpem	Soon Wen Hoh, Kevin Cowtan, Agnel Praveen Joseph, Colin Palmer, Martyn Winn, Tom Burnley, Mateusz Olek, Paul Bond, Eleanor Dodson	4	ab initio+manual	CCP-EM, Refmac, Buccaneer, Coot, TEMPy
35 phenix	Pavel Afonine, Tom Terwilliger, Li-Wei Hung	4	ab initio+manual	Phenix, Coot
38 fzjuelich	Gunnar Schroeder, Luisa Schaefer	3	optimization automated	Phenix, Chimera, DireX, MDFF, CNS, Gromacs
41 arpwarp	Grzegorz Chojnowski	8	ab initio automated, ab initio+manual	Refmac, ARP/wARP, Coot
54 kihara	Daisuke Kihara, Genki Terashi	8	ab initio+manual	Rosetta, Mainmast, MDFF, Chimera
60 deeptracer	Liguo Wang, Dong Si, Renzhi Cao, Jianlin Cheng, Spencer A. Moritz, Jonas Pfab, Tianqi Wu, Jie Hou	10	ab initio automated, ab initio+manual	Cascaded-CNN, Chimera
73 singharoy	Mrinal Shekhar, Genki Terashi, Sumit Mittal, Daipayan Sarkar, Daisuke Kihara, Ken Dill, Alberto Perez, Abishek Singharoy	5	ab initio+manual, optimization automated	reMDFF, MELD, VMD, Chimera, Mainmast
82 rosetta	Frank DiMaio, Dan Farrell	8	ab initio automated, ab initio+manual	Rosetta, Chimera
90 mbaker	Matt Baker	2	ab initio+manual	Pathwalker, Phenix, Chimera, Coot
91 chiu	Greg Pintilie, Wah Chiu	2	optimization+manual	Phenix, Chimera, Coot

Table II. Evaluated Metrics

	Metric Class	Package <u>Metric</u> Definition
Fit-to-Map	Correlation Coefficient, all voxels	Phenix <u>CCbox</u> full grid map vs model-map density correlation coefficient ¹⁸ TEMPy <u>CCC</u> full grid map vs model-map density correlation coefficient ²³
	Correlation Coefficient, selected voxels	Phenix <u>CCmask</u> map vs model-map density, only modelled regions ¹⁸ Phenix <u>CCpeaks</u> map vs model-map density, only high-density map and model regions ¹⁸ TEMPy <u>CCC_OV</u> map vs model-map density, overlapping map and model regions ²⁵ TEMPy <u>SMOC</u> Segment Manders' Overlap, map vs model-map density, only modelled regions ²⁵
	Correlation Coefficient, other density function	TEMPy <u>LAP</u> map vs model-map Laplacian filtered density (partial 2 nd derivative) ²² TEMPy <u>MI</u> map vs model-map Mutual Information entropy-based function ²² TEMPy <u>MI_OV</u> map vs model-map Mutual Information, only modelled regions ²⁵
	Correlation Coefficient, atom positions	Chimera/MAPQ <u>Qscore</u> map density at each modeled atom vs reference Gaussian density function ¹⁴
	Fourier Shell Correlation	Phenix <u>FSC05</u> Resolution (distance) of Map-Model FSC curve read at point FSC=0.5 ¹⁸ CCPEM/Refmac <u>FSCavg</u> FSC curve area integrated to map resolution limit ^{19,56}
	Atom Inclusion	EMDB/VisualAnalysis <u>AI_all</u> Atom Inclusion, percentage of all atoms inside depositor-provided density threshold ²⁰ TEMPy <u>ENV</u> Atom Inclusion in envelope corresponding to sample MW; penalizes unmodeled regions ²²
	Side Chain Density	Phenix <u>EMRinger</u> evaluates backbone positioning by sampling map density around C γ -atom ring-paths for non-branched residues ²¹
Coordinates-only	Configuration	Phenix <u>Bond</u> Root-mean-square deviation (RMSD) of bond lengths ²⁷ Phenix <u>Angle</u> RMSD of bond angles ²⁷ Phenix <u>Chiral</u> RMSD of chiral centers ²⁷ Phenix <u>Planar</u> RMSD of planar group planarity ²⁷ Phenix <u>Dihedral</u> RMSD of dihedral angles ²⁷
	Clashes	MolProbity <u>Clashscore</u> Number of steric overlaps $\geq 0.4\text{\AA}$ per 1000 atoms ²⁶
	Conformation	MolProbity <u>Rotamer</u> sidechain conformation outliers ²⁶ MolProbity <u>Rama</u> Ramachandran ϕ, ψ mainchain conformation outliers ²⁶ MolProbity <u>CaBLAM_3D</u> outliers of CO-CO virtual dihedral incompatible with 2 surrounding Ca-Ca virtual dihedrals ²⁸ MolProbity <u>Calpha</u> 3D outliers of 2 Ca virtual mainchain dihedrals plus Calpha virtual bond angle ²⁸
vs. Reference Model(s)	Atom Superposition	LGA <u>GDT-TS</u> Global Distance Test Total Score, average % of model Ca that superimpose with reference Ca, multiple distance cutoffs ²⁹ LGA <u>GDC</u> Global Distance Calculation, average % of all model atoms that superimpose with reference, multiple distance cutoffs ²⁹ OpenStructure/QS <u>CaRMSD</u> Root-Mean-Square Deviation of Ca atoms ³¹
	Interatomic Distances	LDDT <u>LDDT</u> Local Difference Distance Test, superposition-free comparison of all-atom distance maps between model and reference ³⁰
	Contact Area	CAD <u>CAD</u> Contact Area Difference, superposition-free measure of differences in interatom contacts ³² HBPLUS ⁵⁷ <u>HBPRC</u> >6, Hydrogen bond precision, nonlocal. fraction of correctly placed hydrogen bonds in residue pairs with > 6 separation in sequence.
	Atom Superposition, Multiple	<u>DAVIS QA</u> average of pairwise LGA GDT-TS scores among submitted models ³³