Supporting Information

Initial structure preparation.

The initial test structure of β-galactosidase was obtained by subjecting the reported structure (Singharoy et al., 2016) to a 4-ns MD simulation at a temperature of 300 K in vacuum and using secondary structure restraints. Trajectory frames recorded at 2-ps intervals were evaluated for backbone RMSDs with respect to the reported structure. A frame with lowest global cross-correlation with respect to the reported map was picked to be the initial test structure, and subsequently ReMDFF was performed with CHARMM36 force field as reported elsewhere (Singharoy et al., 2016), and with CHARMM36m reported here.

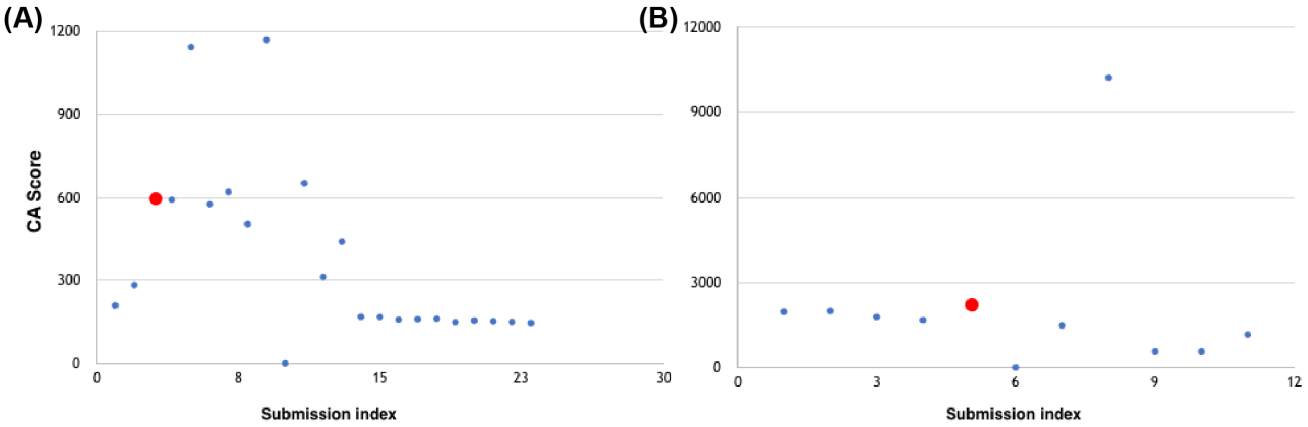

Fig. S1 CA scores for the 2015-2016 Cryo EM Model Challenge for **(A)** TRPV1 and **(B)** β-galactosidase. Revealing structural closeness of the submitted models to those of the targets, CA score exhibits three clear clusters for TRPV1 entries. Indicated in red, the ReMDFF model belongs to the middle cluster suggesting some structural difference with respect to the target. This difference is lower for the ReMDFF model of β-galactosidase, where a much larger number of Challenge entries furnished structures closer to the target.

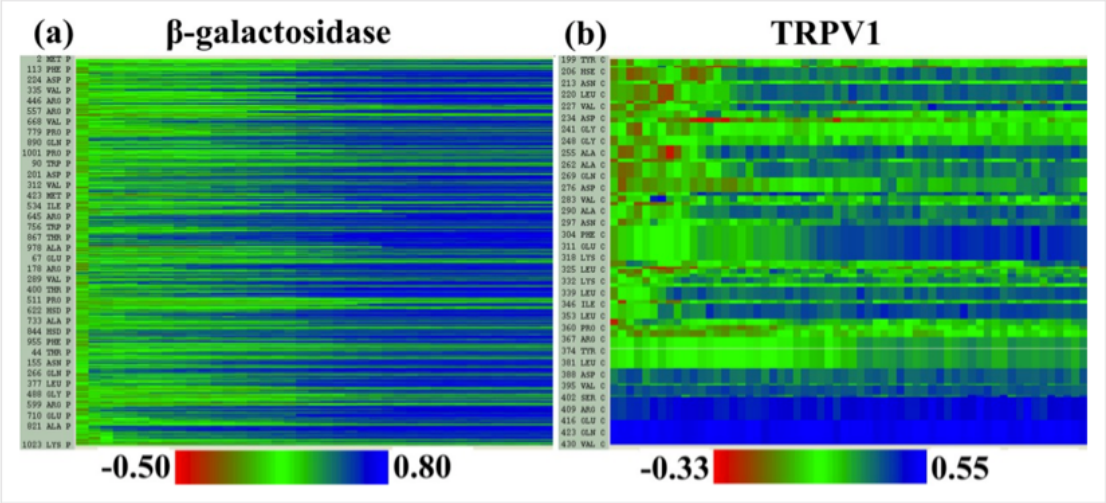

Fig. S2 Local cross-correlations during cMDFF. Local cross-correlations of residues within the fitted regions of **(A)** β-galactosidase and **(B)** TRPV1 plotted over the course of the cMDFF fitting show improvement over the successive MDFF refinement

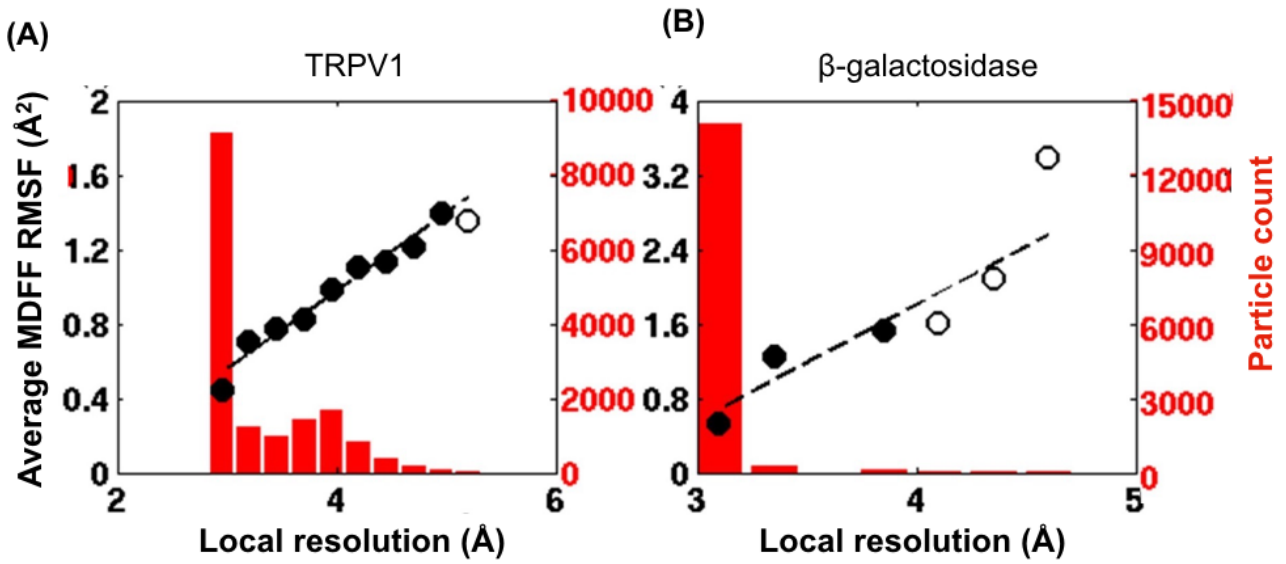

Fig. S3 Root Mean Square Fluctuation (RMSF) *vs.* local resolution plots. For each test case shown, atoms in the MDFF-refined structure are classified by local resolution of the map regions they are fitted into. The average RMSF value of atoms (during MDFF simulation) in each resolution bin is calculated and plotted against the local resolution in the cases of **(a)** TRPV1 at 3.4 Å and **(b)** β-galactosidase at 3.2 ÅThe numbers of atoms in the resolution bins are displayed as a histogram (in red) spanning a system-specific range of resolutions. The lowest resolution bins contain low (< 20) populations and visual inspection consistently revealed the atoms to be on the edges of the density or were otherwise located inside map noise, and were therefore ignored during further analysis. A clear linear correlation between RMSF and local resolution is observed in each case, showing higher resolutions produce lower RMSF.

Table S1 Table depicting the effect of GSCALE parameters on MolProbity scores of the ReMDFF models for β-galactosidase. Models resulting from a range of GSCALE values (0.3–0.6) are presented. Atrributes of the poor-quality starting structure are presented under the Initial column. Additionally, the scores for an explicit solvent ReMDFF model derived at GSCALE 0.3 are provided in the last column. The numbers reported are in percentages (%). For a GSCALE of 0.4 the overall MolProbity score is minimum implying a model satisfying most of the geometry criteria.

MolProbity Parameters	Initial	GSCALE 0.3	GSCALE 0.4	GSCALE 0.5	GSCALE 0.6	Explicit
Poor rotamers (%)	6.24	2.5	2.12	3.0	2.75	2.75
Favored rotamers (%)	83.4	92.76	92.38	91.51	93.01	92.76
Ramachandran outliers (%)	2.17	0.98	1.08	0.98	1.08	1.19
Ramachandran favored (%)	90.56	93.17	93.71	93.6	93.93	93.6
C_β deviations (%)	8.6	0.55	0.58	0.45	0.68	0.89
Bad bonds (%)	4.56	0.01	0.01	0.01	0.01	0.02
Bad angles (%)	5.69	0.27	0.29	0.3	0.27	0.3
Cis prolines (%)	6.45	6.45	6.45	6.45	6.45	6.45
CaBLAM outliers (%)	4.71	3.63	3.73	3.34	3.24	3.43
C_α Geometry outliers (%)	1.28	0.88	1.28	1.18	1.08	0.88
Overall MolProbity score	1.64	1.24	1.16	1.28	1.24	1.25
EMRinger	2.37	2.97	3.19	3.25	3.25	3.14

References

Singharoy, A., I. Teo, R. McGreevy, J. E. Stone, J. Zhao, and K. Schulten, 2016. Molecular dynamics-based refinement and validation for sub-5 Å cryo-electron microscopy maps. eLife 10.7554/eLife.16105.