AQuaRef: Machine learning accelerated quantum refinement of protein structures

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ABSTRACT

Cryo-EM and X-ray crystallography provide crucial experimental data for obtaining atomic-detail models of biomacromolecules. Refining these models relies on library-based stereochemical restraints, which, in addition to being limited to known chemical entities, do not include meaningful noncovalent interactions relying solely on nonbonded repulsions. Quantum mechanical (QM) calculations could alleviate these issues but are too expensive for large molecules. We present a novel AI-enabled Quantum Refinement (AQuaRef) based on AIMNet2 neural network potential mimicking QM at substantially lower computational costs. By refining 41 cryo-EM and 30 X-ray structures, we show that this approach yields atomic models with superior geometric quality compared to standard techniques, while maintaining an equal or better fit to experimental data. Notably, AQuaRef aids in determining proton positions, as illustrated in the challenging case of short hydrogen bonds in the parkinsonism-associated human protein DJ-1 and its bacterial homolog YajL.

INTRODUCTION

While advances in predictive modeling, such as AlphaFold3[1] or RoseTTAFold[2,3], have provided powerful tools for structural biology, they remain limited while experimental methods, including protein crystallography and cryo-EM, are still cornerstones of structural biology and drug development[4]. Experimental data allow for the discovery of new structures emerging in life evolution, potentially exhibiting previously unseen features. These discoveries require unbiased information provided by experiments to explore the unknown[5]. Atomic model refinement is a crucial near-final stage in crystallographic or cryo-EM structure determination aimed at producing molecular models that meet standard validation criteria while optimally fitting the experimental data[6]. Refinement heavily relies on stereochemical restraints to maintain the correct geometry of the atomic model while fitting to the experimental data[7]. These restraints originate from standard libraries that tabulate the topology and parameters of known chemical entities[8,9],

which are universally employed across popular software packages, such as CCP4[10] and Phenix[11].

The limitations of library-based restraints are manifold. Firstly, they only include terms for maintaining covalent bond lengths, bond angles, torsion angles, planes and chirality while preventing clashes through non-bonded repulsion[12]. However, it has been demonstrated that at low resolution, these restraints are insufficient to maintain realistic, chemically meaningful macromolecular geometries, making it essential to include additional restraints on protein main chain φ/ψ angles, side chain torsion χ angles, as well as hydrogen bond parameters and π-stacking interactions to stabilize protein or nucleic acid secondary structure[12–18]. These additional restraints cannot be reliably inferred from the atomic model alone and thus require manual error-prone annotation and curation using additional sources of information, such as homologous high-resolution models. Secondly, library-based restraints parametrize only known chemical entities, such as standard amino and nucleic acids as well as previously defined ligands. Consequently, any nonstandard entities or interactions, such as novel ligands or covalent cross-chain links, require manual annotation and definition, without which refinement may fail to proceed correctly or at all. Finally, deviations from standard covalent geometry due to local chemical interactions are not uncommon[19–21]. While these deviations are valid, restraints may interpret them as violations requiring 'correction'.

The advantage of using simple restraints[7] is the minimal computational cost they add to the refinement workflow. A possible next step is to use a classical force field to account for geometric elements[22]. However, these force fields have their own set of limitations: they require parametrization for new chemical species and cannot distinguish between chemically equivalent bonds in different chemical environments.

Quantum refinement is a fundamentally different approach, balancing the fitting to experimental data with a term related to the quantum mechanical energy of the system[23,24]. It has been demonstrated that the entire atomic model can benefit from a full QM treatment[25–27]. Figure 1 presents a timeline showcasing the evolution of quantum mechanics calculations for proteins, highlighting four key stages of progress and advancements in technology and methodology. Traditionally, quantum refinements were

deemed impractical for macromolecules due to the computational requirements. Methods often focused solely on the macromolecular region of interest, such as a ligand-binding pocket or enzyme active site, while employing a classical approach for the rest of the molecule[28,29]. Numerous approaches and implementations have been reported over time[30], with GPU-accelerated codes enabling QM calculations for peptides and small proteins of a few hundred atoms being one of the most prominent milestones[31]. Interaction-based model partitioning into chemically meaningful fragments[32] solved the scalability issue in quantum calculations[26], which in turn enabled the refinement of larger proteins. However, this approach remained computationally demanding.

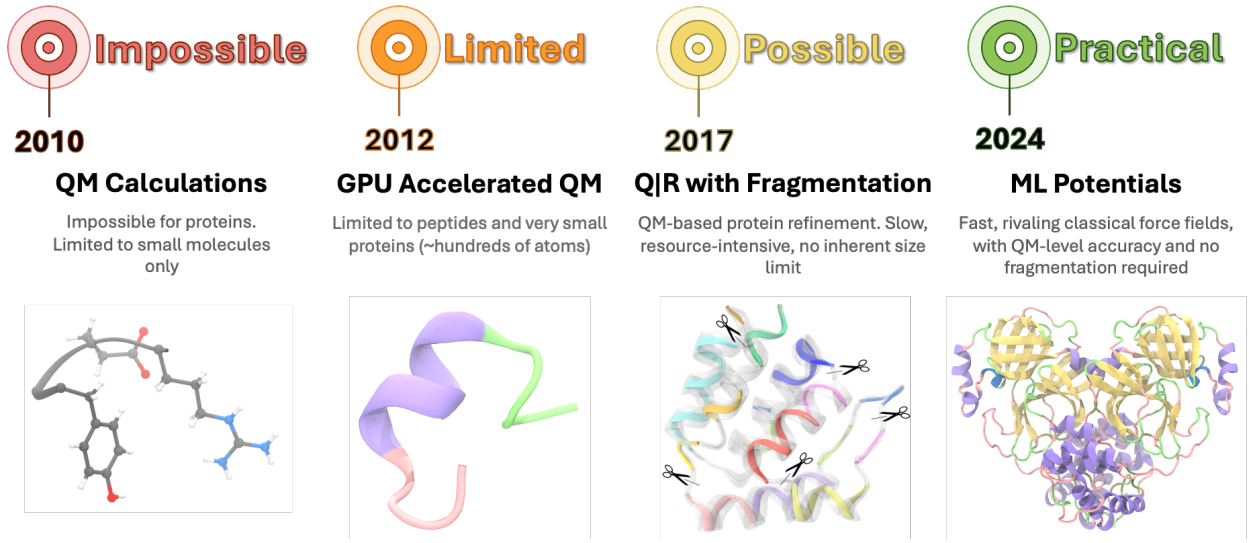

Figure 1. Timeline illustrating the progression of quantum mechanics calculations applied to proteins, emphasizing four critical stages marked by advancements in technology and methodology.

Refinement of selected cryo-EM and X-ray atomic models across various resolutions demonstrates the AQuaRef's ability to produce atomic models with superior geometric quality compared to conventional techniques while maintaining or improving agreement with experimental data. This work represents the first example where machine learning (ML) potentials have been adopted to perform quantum refinement of the entire protein,

in contrast with a recent approach where ML potentials have been combined with the ONIOM-like QM/MM partitioning[38].

RESULTS

Conceptually, quantum-based atomic model refinement is very similar to classic refinement wherein atomic model parameters are iteratively adjusted in order to minimize the residual, $T = T_{data} + w * T_{restraints}$. Here, T_{data} describes the fit of the model to the data and $T_{restraints}$ incorporates chemical restraints with an *a-priori* unknown weight, w[39].

However, there are four fundamental differences. First, in QM refinement, restraints are derived from quantum-mechanical calculations for the specific macromolecule in consideration. Second, the requirements for the initial atomic model in QM refinement are stricter compared to standard refinement: the atomic model must be correctly protonated, atom-complete and free of severe geometric violations such as steric clashes or broken covalent bonds[23,24]. Third, while crystallographic software packages inherently account for crystal symmetry, QM codes generally do not. Fourth, crystallographic software is capable of handling static disorder that is modeled with alternative conformations, whereas QM codes typically lack this capability. All these nuances specific to quantum refinement (except handling static disorder, which is a current limitation) are addressed in the Quantum Refinement package (Q|R)[23,26,27,40], which is being developed as part of this work and provides the necessary procedures to enable quantum refinement within the Phenix software.

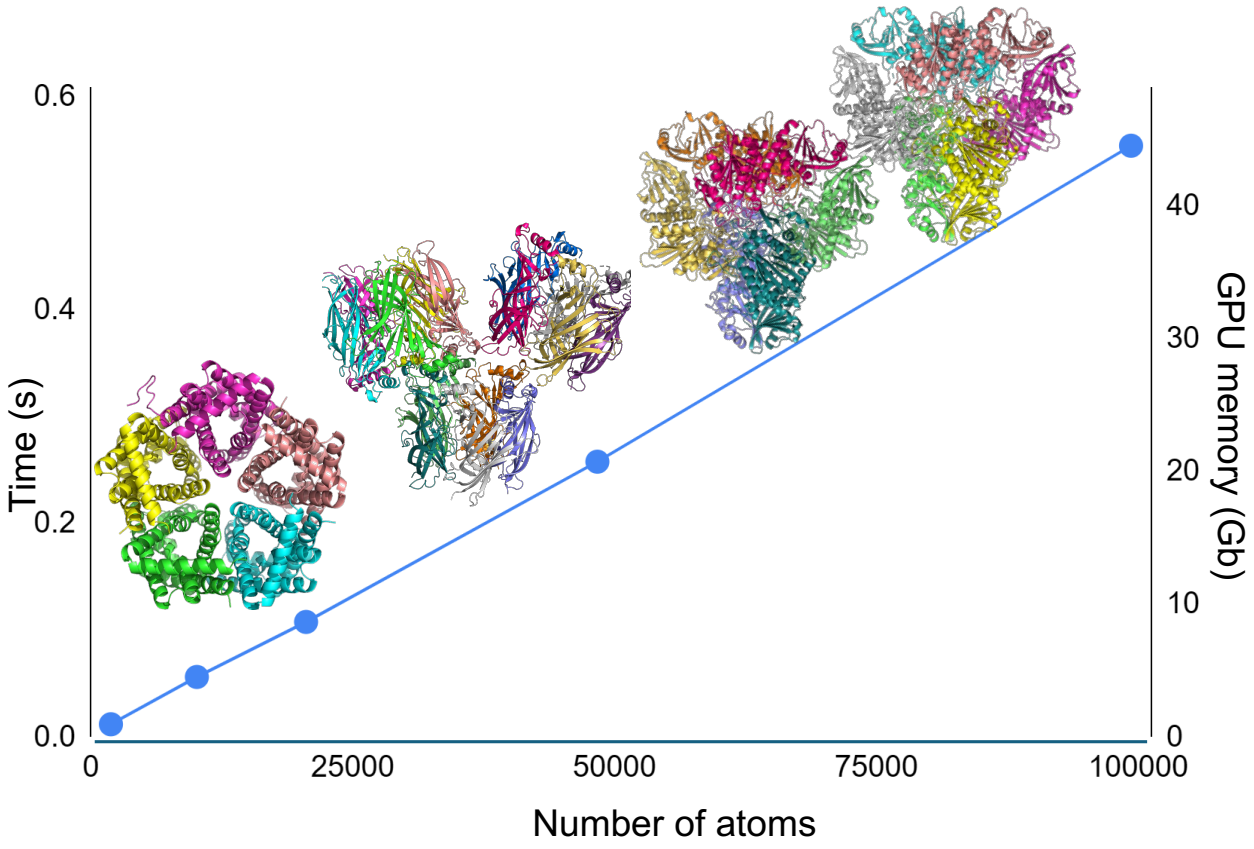

Figure 2. Computational scaling of the AIMNet2 neural network model in AQuaRef. Time to compute energy and forces (left axis) and peak GPU memory usage (right axis) versus the number of atoms in the system. Calculations are performed on a single Nvidia H100 PCIE 80GB GPU.

Conventional QM methods like density functional theory (DFT) for *N*-electron systems require $O(N^2)$ storage and $O(N^3)$ arithmetic operations. This $O(N^3)$ complexity is a critical bottleneck that limits the ability to study large realistic biological systems like proteins. Figure 2 shows the computational scaling of the AIMNet2 model, where both energy and force calculations, as well as peak GPU memory usage, scale linearly ($O(N)$) with system size. For a large protein system of 100,000 atoms, single-point energy and forces can be computed in 0.5 seconds. Overall, an atomic model consisting of approximately 180,000 atoms can fit into the 80GB memory of a single NVIDIA H100 GPU.

We tested the new quantum refinement procedure on 41 cryo-EM atomic models, 20 low-resolution and 10 very high-resolution X-ray atomic models. Standard stereochemistry[41,42] and model-to-data fit criteria[43–45], MolProbity validation tools[46] along with newly developed metrics to evaluate hydrogen bond quality[18] were used to assess the atomic models. Typically, the time needed for quantum refinement is about twice as long as standard refinement, and often shorter than the standard refinement with additional restraints such as the Ramachandran plot, secondary structure and side-chain rotamer restraints[47–50]. Quantum refinement takes under 20 minutes in about 70% of models considered in this work, with a maximum of about 1 hour (Supplementary Data: Table 6). These computations can be performed on GPU-equipped laptops, with the only limitation being available GPU memory.

Quantum refinement

The AQuaRef refinement procedure begins with a check for the completeness of the atomic model, followed by the addition of any missing atoms. This may result in steric clashes, particularly if the model was previously refined without hydrogen atoms. Models with missing atoms that cannot be trivially added (e.g., missing main chain atoms) cannot be used for quantum refinement. If clashes or other severe geometric violations are detected, quick geometry regularization is performed using standard restraints, ensuring that atoms move as little as necessary to resolve the clashes. For crystallographic refinement, to account for interactions arising from crystallographic symmetry and periodicity of unit cells, the model is expanded into a supercell by applying appropriate space group symmetry operators[25]. Subsequently, it is truncated to retain only parts of the symmetry copies within a prescribed distance from atoms of the main copy[40]. This step is unnecessary for refinement against cryo-EM data. The atom-completed and expanded model then undergoes the standard atomic model refinement protocol as implemented in Q|R package[23].

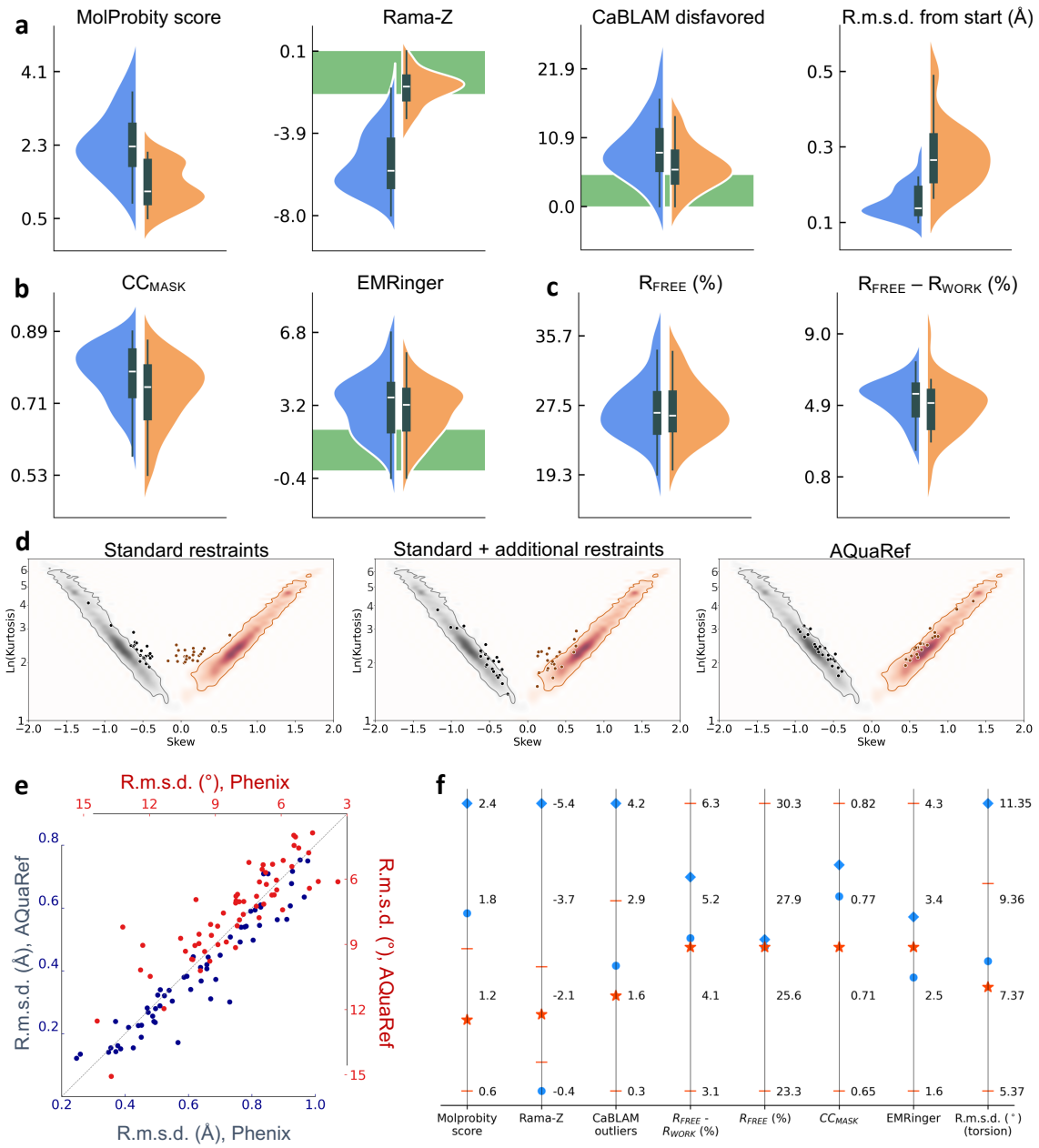

Figure 3. a-d: Summary of refinements of 41 low-resolution cryo-EM models and 20 low-resolution X-ray models using standard stereochemistry (blue) and AQuaRef (orange) restraints (Supplementary Data: Table 1). **a:** MolProbity score, Ramachandran plot Z-score, CaBLAM disfavored and r.m.s. deviation of refined model from initial model. **b:** cross-correlation between experimental and model-generated maps (CC_{mask}), and EMRinger score for cryo-EM models. **c:** R_{free} and R_{free}-R_{work} for X-ray models

178 (Supplementary Data: Table 3). Green band indicates favored range of corresponding
179 values. **d:** skew-kurtosis plots for hydrogen bond parameters (Hydrogen(H)...Acceptor(A)
180 distances and Donor-H…A angles) for refinements using (left-to-right): standard
181 restraints; standard restraints with addition of Ramachandran plot, secondary-structure
182 and side-chain rotamer restraints; and AQuaRef restraints. **e:** r.m.s. deviations between
183 refined and high-resolution homology models, refinements using standard versus
184 AQuaRef restraints, calculated using matching Cartesian coordinates (blue, lower-left)
185 and matching torsion angles (red, upper-right) (Supplementary Data: Tables 2,4). **f:**
186 summary of mean values, for all test refined models: MolProbity score, Ramachandran
187 Z-score, CaBLAM outliers, r.m.s. deviation of matching torsion angles between refined
188 and high-resolution homologous models, as well as R_{free}-R_{work} and R_{free} for X-ray models
189 and CC_{mask} and EMRinger score for cryo-EM models for refined models with standard
190 restraints (blue rhombi), standard restraints with addition of Ramachandran plot,
191 secondary-structure and side-chain rotamer restraints (blue circles); and AQuaRef
192 restraints (red stars). Red bars show standard deviations for starred values.

193

194 **Application of the new refinement procedure to a set of deposited atomic models**

195 To evaluate the performance of the new QM-based refinement, we refined 41 low-
196 resolution cryo-EM atomic models, 20 low-resolution and 10 ultra-high-resolution X-ray
197 atomic models, which contain only proteins. All selected 61 low-resolution atomic models
198 have high-resolution homologs, which were used as the ground truth for comparison
199 (Supplementary Data: Tables 2,4). Refinements were carried out using three sets of
200 restraints: QM restraints from AIMNet2 (AQuaRef refinement); standard restraints; and
201 standard restraints plus additional restraints on hydrogen bonds and angles involved in
202 maintaining secondary structure, main-chain φ/ψ angles (Ramachandran plot restraints)
203 and side-chain torsion χ angles (rotamer restraints).

204 Overall, low-resolution atomic models after quantum refinement exhibit systematically
205 superior geometry quality compared to those obtained using standard restraints, as
206 indicated by their MolProbity scores[51], Ramachandran Z-scores[52], CaBLAM disfavored[46]
207 (Fig. 3a), and skew-kurtosis plots for hydrogen bond parameters[18] (Fig. 3d). They also

208 systematically deviate more from the initial coordinates. These atomic models
209 demonstrate a very similar fit to the experimental data (Fig. 3b,c), with slightly less data
210 overfitting for X-ray atomic models, as evidenced by a smaller R_{work}-R_{free} gap and similar
211 R_{free}[53,54]. Since there is no equally efficient control over overfitting in cryo-EM as there is
212 with R_{free} in crystallography, the slightly lower cross-correlation between experimental and
213 model-calculated masked maps (CC_{mask})[43] and essentially the same EMRinger scores[55],
214 together with significantly improved atomic model geometry, likely indicate a reduction in
215 overfitting. Augmenting standard restraints with secondary structure, Ramachandran plot
216 and side-chain rotamer restraints expectedly improves the geometry (Fig. 3d,f), yet using
217 AQuaRef still produces superior atomic model geometries. With a few exceptions, atomic
218 models refined with quantum restraints are systematically closer to their higher-resolution
219 homologs compared to those using standard restraints alone or complemented with
220 additional restraints (Fig. 3e,f). In some of the most remarkable cases, the local structure
221 obtained with AQuaRef restraints closely matches the high-resolution homologs and
222 differs from those obtained using standard restraints by up to two Angstroms (Fig. 9a-c).
223

224 **Comparison with alternative state-of-the-art approaches**

225 To further evaluate the performance of AQuaRef refinement compared to other major
226 refinement methods and software, we refined selected low-resolution X-ray models using
227 the AMBER force field as a source of geometric restraints[56], the Rosetta all-atom force
228 field combined with its powerful sampling methods[57], and standard refinement as
229 implemented in REFMAC5[58]. For cryo-EM, there are fewer refinement alternatives, with
230 Servalcat[59] being the most popular, which we also used in this analysis.

231 For X-ray models, AQuaRef produced slightly better overall R_{free} values (Fig. 4a) and
232 substantially less data overfitting, as indicated by the R_{free}-R_{work} gap (Fig. 4b). For cryo-
233 EM models Servalcat lead to notably better CC_{mask} (Fig. 4c) and both scored the same
234 by EMRinger method (Fig. 4d). Models refined using AQuaRef and Rosetta performed
235 similarly well in terms of Rama-Z scores, achieving excellent results in most cases, while
236 REFMAC5 and Servalcat had the worst scores, and AMBER fell somewhere in between
237 (Fig. 4e). In terms of MolProbity scores and CaBLAM outliers (Fig. 4f,g), AQuaRef and

Rosetta also performed similarly well, significantly outperforming REFMAC5 and Servalcat. Rosetta-refined models were closest to the high-resolution reference models, followed by AQuaRef (Fig. 4h). This is likely due to Rosetta's use of non-gradient optimization techniques, such as sampling and local model repacking, which have a larger convergence radius compared to the gradient-driven minimization used in other programs. Finally, AQuaRef and Rosetta both produced models that fit the expected distribution of hydrogen bond parameters (Fig. 4i, Fig. 3d), followed by AMBER. REFMAC5 and Servalcat largely failed to produce models fitting this distribution, with Servalcat performing the worst.

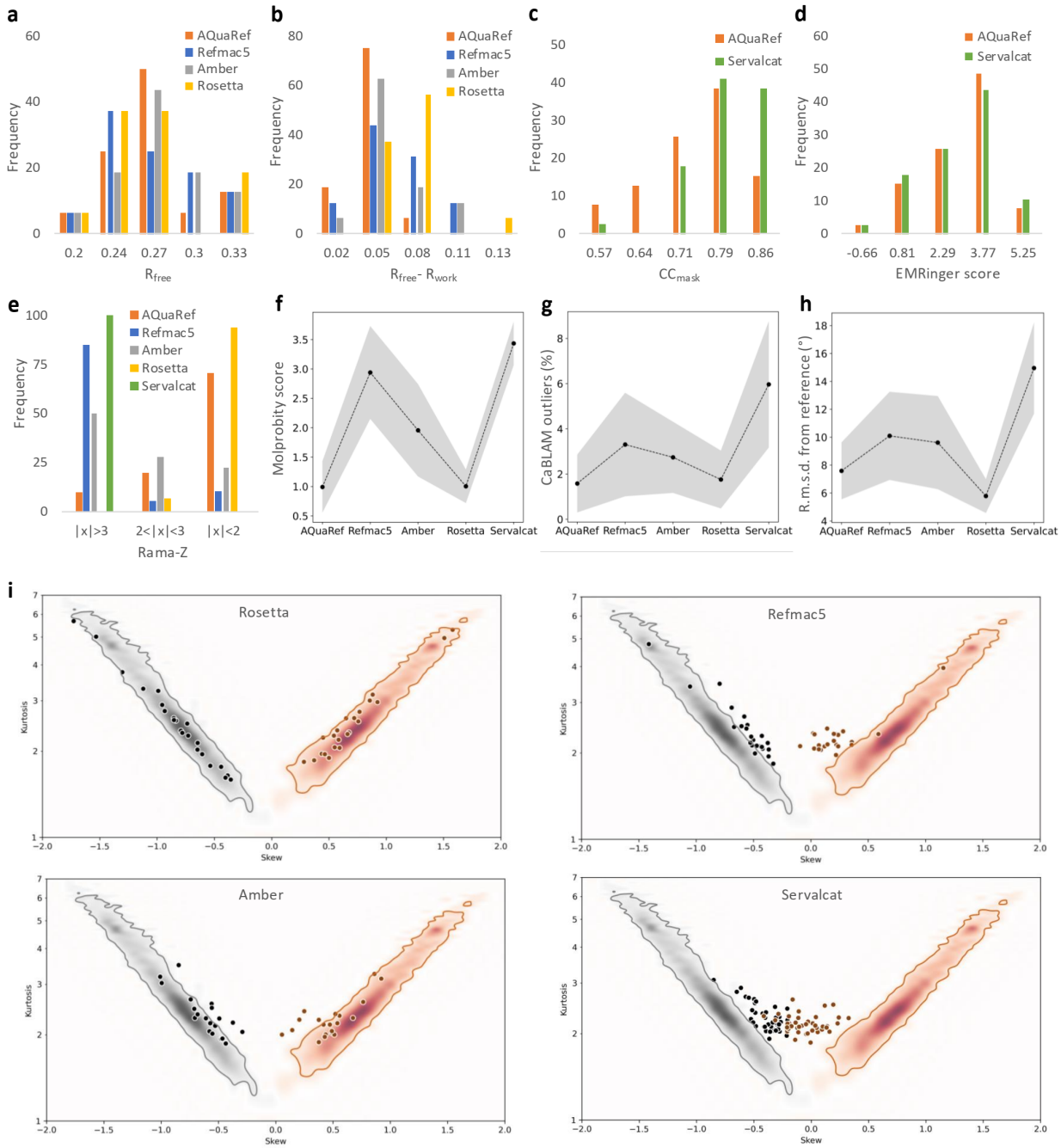

Figure 4. Summary of refinements for 41 low-resolution cryo-EM models using AQuaRef and Servalcat, and 20 low-resolution X-ray models using AQuaRef, REFMAC5, AMBER, and Rosetta. **a-e:** Distributions of R_{free}, R_{free}-R_{work}, CC_{mask}, EMRinger score, and Rama-Z, respectively. **f-h:** Mean values of MolProbity score, CaBLAM outliers, and r.m.s. deviation from the reference model, calculated across all refined models; gray bands represent the standard deviation. **i:** Skew-kurtosis plots for hydrogen bond parameters

255 (Hydrogen(H)...Acceptor(A) distances and Donor-H…A angles) for refinements
256 performed using REFMAC5, AMBER, Rosetta and Servalcat.

257

258 **Case study: Short hydrogen bonds in human DJ-1 and its bacterial homologue**
259 **YajL**

260 Short hydrogen bonds play a key functional role in proteins, and determining the
261 protonation states of involved residues is critical. However, accurate location of proton
262 positions experimentally remains challenging at resolutions near 1 Å. Lin *et al* [60] analyzed
263 high-resolution X-ray crystal structures of human DJ-1 and its bacterial homolog YajL to
264 determine the protonation states of carboxylic acids involved in dimer-spanning hydrogen
265 bonds. Their approach combined bond length analysis, leveraging the distinct lengths of
266 C=O and C–OH bonds, with qualitative interpretation of difference map peaks to identify
267 potential evidence of protons.

268 This method is complicated by stereochemical restraints applied during coordinate
269 refinement, which can bias bond lengths. For example, in E/D residues, bond length
270 restraints for COOH groups depend on whether a hydrogen atom is explicitly modeled
271 (Fig. 5c). To minimize this bias, Lin et al. performed final rounds of conjugate gradient
272 least-squares refinement in SHELXL[61] without applying restraints to the residues of
273 interest. In contrast, QM-based refinement avoids such biases entirely.

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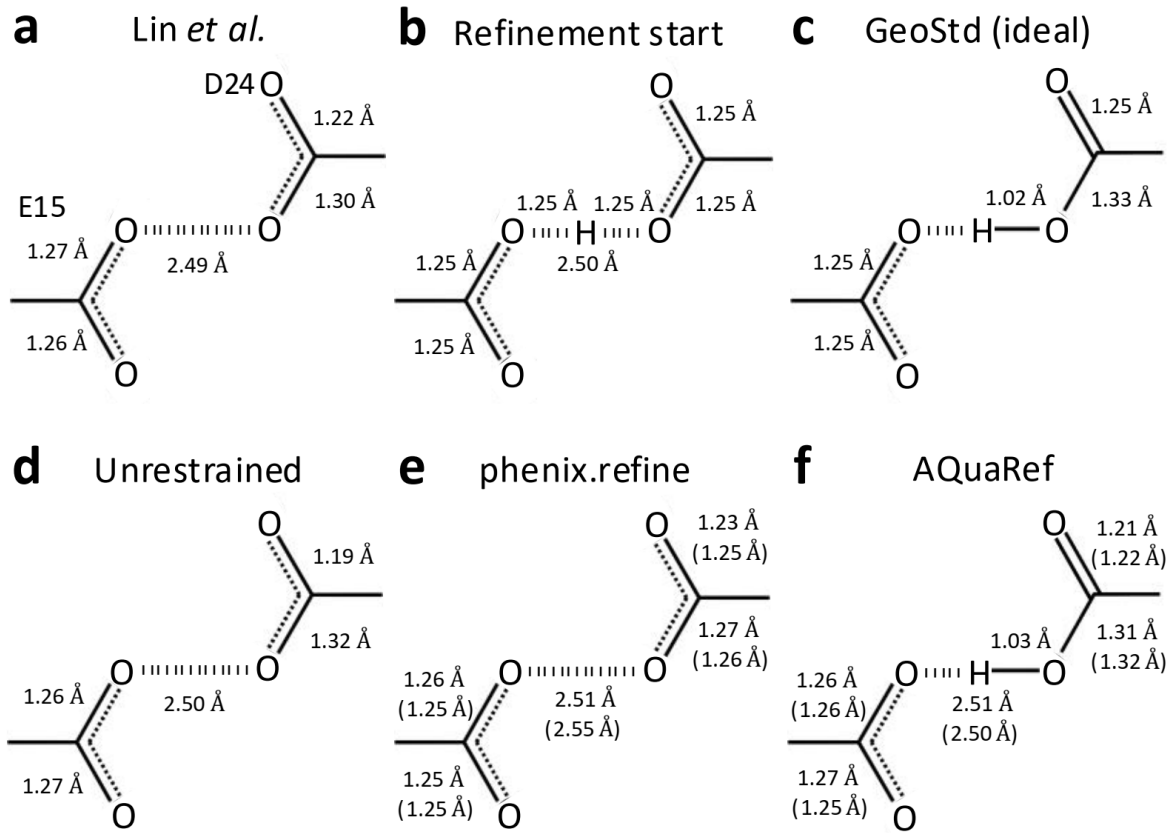

Figure 5. Wild-type DJ-1 (PDB code: 5SY6). Bond distances in the moiety of hydrogen bond between Oε2 (E15) and Oδ2 (D24), **a:** as measured in downloaded from PDB model, **b:** starting geometry for all refinements (H is present only in AQuaRef refinement), **c:** ideal library values in Phenix; geometry of –COOH or –COO groups is the same for Asp and Glu residues, **d:** unrestrained and **e:** restrained refinement with phenix.refine, **f:** refinement with AQuaRef. Distances in parentheses correspond to refinement using resolution-truncated data at 2 Å. H atom is shown only if it was explicitly modelled (present in the PDB model file).

AQuaRef refinement of DJ-1, starting with E15/D24 in an unprotonated state (Fig. 5b), produced proton positions and bond geometries (Fig 5f) consistent with Lin et al.'s findings (Fig. 5a) and unrestrained refinement using phenix.refine (Fig. 5d). However, restrained refinement with phenix.refine (Fig. 5e) yielded bond geometries that matched

library values assuming no proton on either COO group, highlighting the impact of restraint bias.

To test the robustness of AIMNet2 restraints in preserving accurate geometries, the same refinements were performed using experimental data truncated at 2 Å resolution. This truncation removed atomic-level details that could resolve bond lengths and hydrogen positions. AQuaRef produced results nearly identical to those obtained using the original 1.1 Å atomic resolution data, whereas restrained refinements with phenix.refine further biased oxygen-carbon distances toward idealized values for the unprotonated state (Fig. 5e-f, values in parentheses).

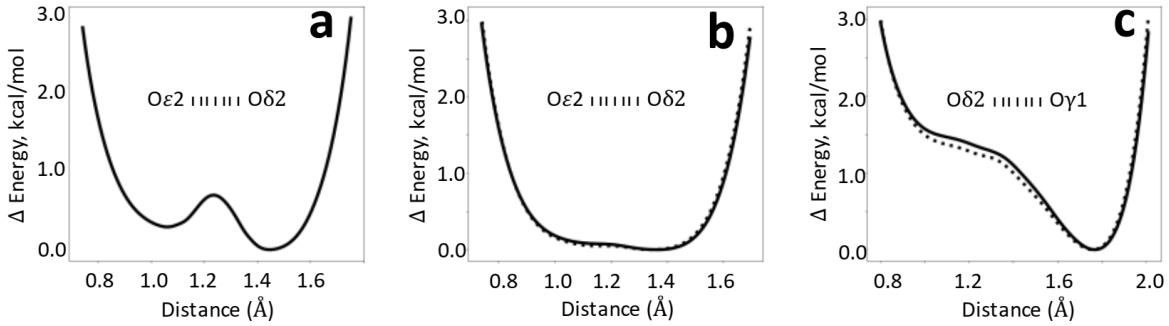

Figure 6. AIMNet2 energy values relative to their minimum as a function of hydrogen position between corresponding oxygen atoms, **a:** Oδ2 (D24) and Oε2 (E15) in DJ-1, **b:** Oδ2 (D23) and Oε2 (E14) in YajL, and **c:** Oδ2 (D23) and Oγ1 (T16) in YajL. Solid and dashed lines represent two instances of the bond in the YajL model.

Starting from an idealized symmetric arrangement (Fig. 5b), the refinement could, in principle, place the proton on either E15 or D24. To explain why the proton ultimately settled on Oδ2 of D24, two independent sources of evidence were considered. First, sampling the hydrogen position along the Oδ2–Oε2 bond vector and computing the AIMNet2 energy profile revealed a slight preference for D24 protonation (Fig. 6a).

Second, while the resolution and R-factors were insufficient for definitive proton identification in the difference map, the difference map values along the Oε2-Oδ2 axis showed elevated positive values near Oδ2, close to the prospective hydrogen position (Fig. 7a). This, together with the energetic preference from AIMNet2, may have guided the refinement to move the hydrogen toward D24.

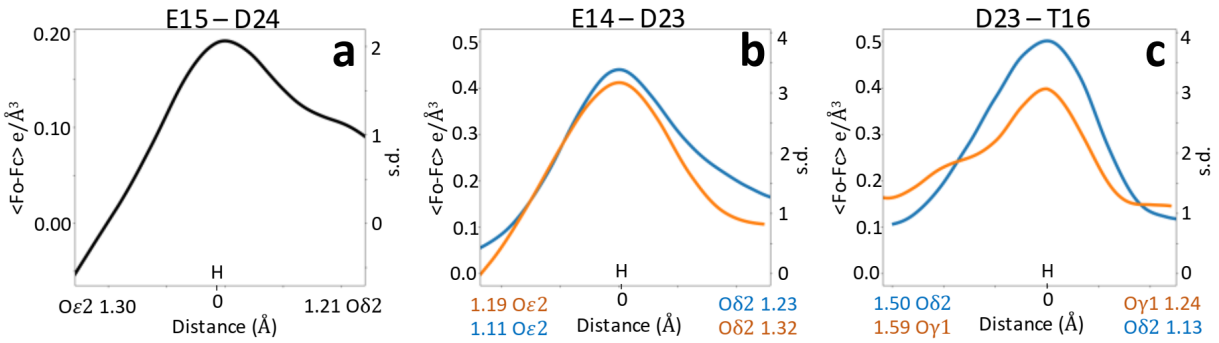

Figure 7. Mean values of the difference density map, shown in absolute units (e/Å³) and as standard deviation values along the O-H vector for the analyzed bonds for: **(a)** DJ-1 and **(b-c)** E. coli YajL models. All peak centers are aligned to the origin. Atoms belonging to chains A and B are shown in blue and orange, correspondingly.

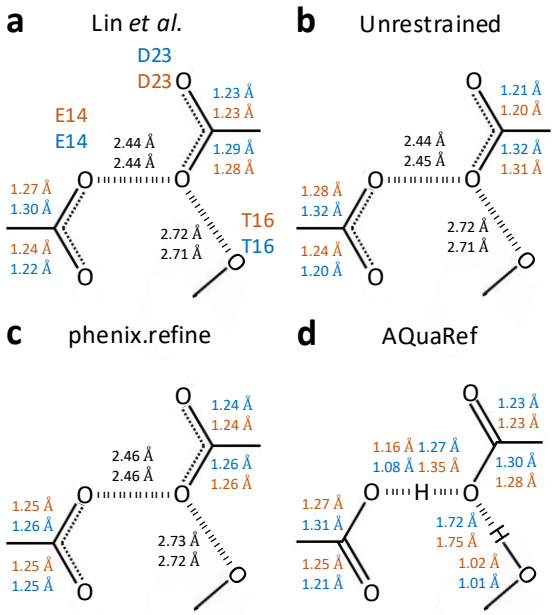

Figure 8. E. coli YajL (PDB code: 5SY4). Bond distances in the moiety of hydrogen bond between Oε2 (E14) and Oδ2 (D23) across chains A (blue) and B (orange), **a:** as measured in downloaded from PDB model, **b:** unrestrained and **c:** restrained refinement using phenix.refine, **d:** refinement with AQuaRef. H atom is shown only if it was explicitly modelled (present in the PDB model file).

Bacterial DJ-1 homologue, the YajL structure, contains two copies of the molecule in the asymmetric unit, resulting in two instances of the E14/D23 interaction. Similar to DJ-1, unrestrained refinement of YajL (Fig. 8b) yielded results consistent with Lin et al. (Fig. 8a). As with DJ-1, restrained refinement introduced significant bias in bond lengths (Fig. 8c) for both instances of the E14/D23 interaction.

Results from AQuaRef refinement aligned with Lin et al. and unrestrained phenix.refine refinement, suggesting that both D23 and E14 are protonated. However, in contrast to DJ-1, the proton in YajL does not appear to be fully associated with either of the two oxygen atoms. Instead, it seems to be shared between Oε2 and Oδ2, consistent with a Low Barrier Hydrogen Bond.

The AIMNet2 energy profile between Oε2 and Oδ2 supports this interpretation, showing a relatively flat energy landscape (Fig 6b). This indicates that the hydrogen's position could be entirely guided by the experimental data while staying within the flat region of the AIMNet2 energy well. Indeed, there is a significant difference map peaks above 3 s.d. and well above mean solvent density of 0.25 e/Å^3 very close to the position of hydrogens in the refined model in both instances of the E14/D23 interaction (Fig. 7b).

Further evidence that C-Oδ2 elongation is due to Oε2···H···Oδ2 LBHB is provided by the analysis of another hydrogen bond involving D23 and T16. All three, AQuaRef refinement (Fig 8d), the AIMNet2 energy profile (Fig. 6c), and difference map density values along the Oδ2 of D23 and Oγ1 of T16 (Fig 7c), confirm the protonation of T16 and rule out the D23 protonation in the "anti" configuration.

DISCUSSION

Here, we present AQuaRef, a novel approach to the quantum refinement of entire protein structures, made possible by using ML-accelerated quantum mechanical calculations with AIMNet2. For the first time, this allows for the refinement of full atomic models of realistic protein structures using stereochemical restraints derived from quantum mechanical calculations.

Test refinements using 61 low-resolution X-ray and cryo-EM atomic models show systematic improvements in geometric validation criteria by using QM restraints while maintaining a similar fit to the experimental data and reducing overfitting. The presence of high-resolution homologous atomic models, which are expected to better represent the actual true structures than low-resolution atomic models, allowed us to assess whether these improvements are associated with refined structures becoming closer to the true ones. With a few exceptions, we find that atomic models refined with AQuaRef restraints are systematically closer to their high-resolution matches. This indicates that QM-based refined atomic models not only improve standard validation metrics but also provide more realistic representations of the true structures compared to atomic models refined with standard restraints. Expectedly, refining 10 very high-resolution atomic models did not

369 significantly alter the atomic coordinates but did lead to improved *R*-factors for all ten
370 models (Supplementary Data: Table 5). The most notable differences compared to
371 refinement with standard restraints were observed in the position of hydrogen atoms,
372 specifically those with rotational degrees of freedom (Fig. 9 d-g), where some of these
373 atoms reoriented during refinement to better fit the data and, at the same time, form
374 favorable hydrogen bonds. Another notable difference is the increased r.m.s. deviations
375 from ideal (library) bond and angle values in the case of AQuaRef refinement
376 (Supplementary Data: Table 5), which together with improved hydrogen positions is likely
377 to contribute to improved *R*-factors.

378 An extended comparison with popular state-of-the-art software packages and refinement
379 methods, including the use of AMBER and Rosetta force fields as refinement restraints,
380 as well as REFMAC5 and Servalcat from the CCP4 software suite, shows that for crystal
381 structure refinement, only Rosetta approaches AQuaRef in terms of the quality of refined
382 atomic model geometries. However, AQuaRef produces slightly improved R_{free} values
383 and significantly better R_{free}-R_{work} gaps, indicating reduced data overfitting. It is also worth
384 noting that Rosetta-based refinement is only available for crystal structures using X-ray
385 data, and refinement times with Rosetta are up to an order of magnitude slower. Although
386 Servalcat achieved superior CC_{mask} values compared to AQuaRef (Fig. 4d), this suggests
387 that Servalcat overfits the map, producing higher CC_{mask} values at the cost of significantly
388 poorer model geometry.

389 The case study of short hydrogen bonds in human DJ-1 and its bacterial homolog YajL,
390 as well as the protonation states of carboxylic acids involved in these hydrogen bonds,
391 highlights the feasibility of AQuaRef in determining proton positions consistent with
392 experimental evidence across diverse scenarios. This process is fully automated and
393 unbiased by the choice of restraints. Additionally, AIMNet2 energy profiles provide further
394 information about the characteristics of hydrogen bonds and protonation states, which
395 can be used to support specific hypotheses.

396 The method has been implemented in the quantum refinement software (Q|R), which is
397 built upon the CCTBX library[62] and optionally utilizes tools from Phenix. Q|R is accessible

398 within Phenix, thereby making these methods readily available to the broader community
399 of structural biologists.

400 Currently, AQuaRef is trained using commonly known amino acid residues, which means
401 the method can only be applied to protein-only structures. Another main limitation is that,
402 at present, static disorder (alternate conformations) is not handled in Q|R. Removing both
403 limitations is the subject of future work.

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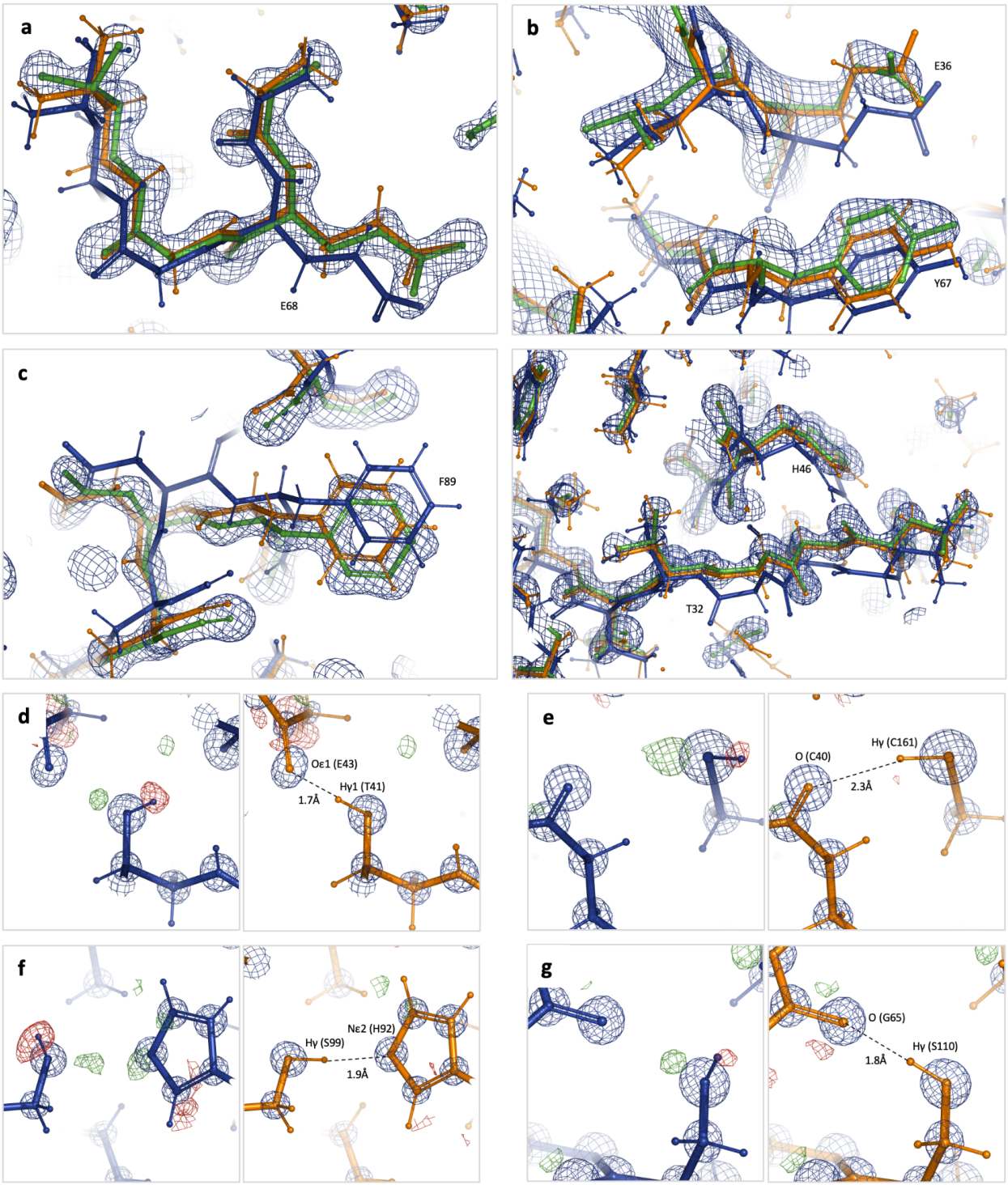

Figure 9. **a-c:** Close-up showing models refined with standard restraints (blue) and AQuaRef restraints (orange) superposed onto their higher-resolution homologous models (green) with their corresponding 2mFo-DFc Fourier maps contoured at 2σ; for PDB 5YI5, 8R1G, and 6XMX, respectively. **d-g:** Refinement with standard AQuaRef restraints

(orange) overlaid with their corresponding 2mFo-DFc and mFo-DFc Fourier maps, contoured at 5σ (blue) and ±2σ (green, red), respectively (PDB 4O8H). The focus is on hydrogen atoms with rotational degrees of freedom that re-orient during refinement with AQuaRef restraints to satisfy the residual map and participate in hydrogen bonding.

METHODS

AIMNet2 training dataset and AQuaRef model

Since our goal was the parametrization of ML potential for polypeptides, our training dataset needed to cover chemical (amino acid sequence and protonation states), conformational, and intermolecular degrees of freedom. We began by creating a library of small peptides as SMILES strings. We used all 20 standard amino acids, 11 alternate protonation forms, three options for sequence start (ACE, NH3+, NH2), and four options for the end (NME, NHE, CBX, CBA). We enumerated all possible mono- and di-peptides and selected a random subset for tri- and tetra-peptides. Additionally, we generated SMILES for peptides linked by the cysteine-cysteine disulfide bond and their selenium counterparts. Molecular conformations were generated with OpenEye Omega[63] software using dense torsion sampling. No restrictions were applied to the configurations of the chiral centers, ensuring that the dataset and resulting model should work equally well for D-, L-, and mixed stereochemistry peptides. Intermolecular interactions were modeled by generating intermolecular complexes of 2 to 4 peptides with random orientations. No prior knowledge of preferred types of secondary structure for polypeptides was used. To manage the size of the dataset and the training process, we limited the size of peptides and complexes to less than 120 atoms, including hydrogens.

Non-equilibrium conformations of peptides and complexes were sampled with molecular dynamics simulations using the GFN-FF[64] force field. Cartesian restraints were added to keep structures near the input structure, with random torsion and intermolecular degrees of freedom. Molecular configurations for labeling (DFT calculations) and inclusion into the training dataset were selected using Query-By-Committee active learning (AL)

439 approach[35]. We started with a random selection of 500k samples, used an ensemble of
440 4 models, and performed a total of 4 iterations of AL adding new samples with high
441 uncertainty of energy and atomic forces prediction. In the final iteration of AL, we
442 performed uncertainty-guided optimization of the structures, minimizing the weighted
443 difference of energy prediction and its uncertainty. This type of active sampling finds
444 structures that balance low predicted forces and high energy uncertainty. The entire
445 procedure resulted in a training dataset containing about one million samples, with a
446 median number of 42 atoms per sample.

447 DFT calculations were performed with the B97M-D4/def2-QZVP[65-68] method using ORCA
448 5.0.3 software[69]. Since the Q|R does not use periodic boundary conditions, and usually
449 not all ions and solvent molecules are resolved in the refinement, we used implicit
450 treatment of solvent effects with CPCM[70] method using parameters for water as solvent.

451 The core architecture of the AQuaRef model matches the base AIMNet2 model[33], with
452 few modifications. First, we did not use explicit long-range Coulomb and dispersion
453 interactions, we trained to total DFT-D4 energy instead. With CPCM treatment, the
454 Coulomb term could not be estimated using interactions between partial atomic charges,
455 and also long-range interactions are effectively screened with a polarizable continuum.
456 Long range dispersion interactions beyond the local cutoff of 5 Å have little effect on
457 atomic forces, which are important in Q|R refinement. We also added explicit short-range
458 exponential repulsion terms to make the potential more robust for the structures with
459 clashes. The model was trained to reproduce DFT-D4 energies, forces, and Hirshfeld
460 partial atomic charges.

461

462 **Experimental data and atomic models for test cases**

463 Protein-only, single-conformation high-to-low resolution X-ray crystallography and Cryo-
464 EM models, along with their corresponding experimental datasets, were selected from
465 RCSB and EMDB based on multiple criteria. These criteria include model size (between
466 1,000 and 10,000 non-hydrogen atoms), resolution (between 2.5 and 4 Å), geometric
467 model quality (MolProbity clashscore better than 50, with no covalent bonds deviating by

468 more than 4 r.m.s.d. from ideal library values), goodness of fit between the model and the
469 experimental data (Cryo-EM: CC_{mask} > 0.6, X-ray: R_{work} < 0.3), and the availability of a
470 higher-resolution (better than 2 Å) homologous model (main chain superposition r.m.s.d.
471 < 1 Å, sequence identity greater than 95%) for each considered model. Additionally, 11
472 ultra-high resolution single-conformation X-ray models were selected that contained only
473 protein and ordered water atoms.

474

475 **Comparison of models**

476 All atoms were used to calculate coordinate r.m.s. deviations between models before and
477 after refinement, as shown in Figure 3a. Coordinate r.m.s. deviations between models
478 used for test refinements and their high-resolution homologues were calculated using the
479 Phenix tool phenix.superpose_pdbs, which included all non-hydrogen backbone atoms
480 plus Cβ and Cγ atoms where present. R.m.s. deviations in torsion angle space were
481 calculated using CCTBX[62], with matching torsion angles selected as described by Headd
482 et al.[15].

483

484 **Atomic model preparation for refinement**

485 Model preparation for refinement (e.g., adding any missing atoms) was done using
486 qr.finalise program of Q|R, which uses the Reduce program[71] to add hydrogen atoms at
487 geometrically predicted positions. Model geometry regularization was done using the
488 Phenix tool phenix.geometry_minimization.

489

490 **Model refinement**

491 The exact same input models were used for all trial refinements. Real-space refinement
492 in Phenix was performed using the phenix.real_space_refine program[12]. Four refinement
493 runs were performed independently, starting with the same input maps (cryo-EM) or
494 reflection data (X-ray) and models. The runs included: 1) standard restraints consisting

of restraints on bond lengths, bond angles, torsion angles, planes, chirality, and non-bonded repulsion; 2) standard restraints with the addition of secondary-structure restraints; 3) standard restraints with the addition of Ramachandran plot restraints; and 4) standard restraints with the addition of secondary-structure and Ramachandran plot restraints.

Quantum-based real- and reciprocal-space refinement was performed using the qr.refine program of Q|R, using all default settings except for the source of QM restraints (AQuaRef).

Reciprocal-space refinement in Phenix was performed using phenix.refine[72] with the exact same four choices of restraints as in real-space refinement.

Software and availability

Phenix software is available at: phenix-online.org. Quantum refinement (Q|R) software is available at qrefine.com. AQuaRef refinement is available in Phenix starting dev-5395 version. CCTBX-based Python scripts and the data (atomic models, cryo-EM maps, X-ray diffraction data) used in this study are available at: https://phenix-online.org/phenix_data/afonine/qr_aimnet2_2024/. Refinement parameters are documented in README files, as well as in the Python scripts used to run the refinements. Input data for deposited models were obtained from the Protein Data Bank[73] and Electron Microscopy Data Bank[74], either by using the Phenix tool phenix.fetch_pdb or from the CERES server[75].

Graphics software

Map and model images were prepared using PyMOL[76]. Routine inspection of maps and models was performed using Coot[77]. Plots were generated using Matplotlib[78].

Acknowledgements

PVA, NWM, and PDA acknowledge funding from the National Institutes of Health (grants R01GM071939, P01GM063210, and R24GM141254), as well as support from the Phenix Industrial Consortium and the US Department of Energy under Contract No. DE-AC02-05CH11231. OI acknowledges support from the US National Science Foundation (NSF CHE-2154447). AER acknowledges support from the US National Science Foundation (NSF CHE-1802831 and OAC-2311632). MB acknowledges support from the COST Action CA21101 “Confined molecular systems: from a new generation of materials to the stars’ (COSY)” supported by COST (European Cooperation in Science and Technology) and computer resources provided by Wroclaw Centre for Networking and Supercomputing (http://wcss.pl).

This work used Expanse at SDSC and Bridges-2 at PSC through allocation CHE200122 from the Advanced Cyberinfrastructure Coordination Ecosystem: Services & Support (ACCESS) program, which is supported by NSF grants #2138259, #2138286, #2138307, #2137603, and #2138296. This research is part of the Frontera computing project at the Texas Advanced Computing Center. Frontera is made possible by the National Science Foundation award OAC-1818253. This research, in part, was done using resources provided by the Open Science Grid, which is supported by the award 1148698 and the U.S. DOE Office of Science.

Author contributions

Conceptualization: P.V.A., A.E.R., O.I., M.B.; Methodology: R.Z., H.G., K.R.; Software: P.V.A., N.W.M., R.Z., M.P.W, H.K.; Validation: M.B., K.R., H.G., P.D.A.; Formal analysis: M.B.; Data Curation: R.Z., H.G., K.R., P.V.A.; Writing (original draft): P.V.A., M.B., R.Z.; Writing (review & editing): all authors; Visualization: P.V.A., M.B., H.G., R.Z.; Supervision, P.V.A., A.E.R., O.I.; Project administration: P.V.A., A.E.R., O.I., M.B.; Funding Acquisition: P.D.A., P.V.A., A.E.R., O.I.

549

550 **Competing Interests**

551 The authors declare no competing interests.

552

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741

742 SUPPLEMENTAL METHODS

743 **Case study: Short hydrogen bonds in human DJ-1 and its bacterial homologue**
744 **YajL**

745 Atomic models and experimental data for human DJ-1 and the bacterial homologue YajL
746 were downloaded from the Protein Data Bank (PDB) using accession codes 5SY6 and
747 5SY4, respectively. Reflection intensities were used in all calculations. Both atomic
748 models were protonated using the Reduce program from Phenix suite, as the deposited
749 models lacked hydrogen atoms.

750 To address uncertainty in refined atomic model parameters due to intrinsic refinement
751 properties, particularly in the coordinates, interatomic distances are reported as averages
752 with standard deviations, based on 100 refinement runs. Each refinement run (restrained
753 and unrestrained with phenix.refine as well as AQuaRef) used identical settings but began
754 with models where coordinates were randomized with an r.m.s.d. of 0.1 Å.

755 To avoid biases in assumptions about the protonation states of E/D and D/T residues,
756 these residues were modeled as unprotonated with idealized geometries in the input
757 refinement models. For AQuaRef refinements, the proton was positioned exactly between
758 the Oε2 and Oδ2 atoms for DJ-1 (Fig. 5b) and YajL, as well as between the Oδ2 and Oγ1

759 atoms for YajL. In phenix.refine, this proton was not explicitly modeled to avoid assuming
760 its association with a particular residue.

761 Restrained phenix.refine refinements were performed with 10 macro-cycles to ensure
762 convergence. Unrestrained refinements used the same settings, except geometric
763 restraints affecting hydrogen bond interaction moieties in E/D and D/T residues were
764 eliminated. Excluded restraints were covalent bond and angle restraints for COO moieties
765 and repulsion restraints between Oε2, Oδ2, and Oγ1 atoms. AQuaRef refinements were
766 performed with all default settings.

767 The AIMNet2 energy profiles between corresponding oxygen atoms were calculated by
768 sampling the proton positions along the line connecting the oxygens at 100 equally
769 spaced points.

770 The sigma-A weighted Fo-Fc difference map value profiles were interpolated along the
771 Oδ2-H, Oε2-H, and Oγ1-H lines using tri-cubic interpolation and averaged across 100
772 difference maps from corresponding refinements. The mean bulk-solvent density value
773 was estimated using the flat bulk-solvent model, as implemented in Phenix.

774 All Python scripts using Q|R and CCTBX libraries to perform all the above calculations
775 are available in Supplemental Data.

776