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AUTODOCK VINA REPRODUCES THE SHAPE OF CIPROFLOXACIN'S CRYSTALLOGRAPHIC POSE IN STAPHYLOCOCCUS AUREUS DNA GYRASE BUT RANKS IT BELOW A NON-CRYSTALLOGRAPHIC POSE AND DOES NOT REBUILD ITS METAL CONTACT

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Abstract: Free molecular docking is often used to ask whether a drug still binds a mutated target. This study tests that use on a case where the answer is already fixed by a crystal structure. Ciprofloxacin was docked into *Staphylococcus aureus* DNA gyrase with AutoDock Vina, taking the 2XCT crystal structure as the control, and four fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, gatifloxacin) were docked against the wild-type enzyme and the resistance mutants S84L and E88K. The clearest result comes from the one complex that has a crystal pose to check against, and it has two layers. By the usual whole-molecule root-mean-square deviation (RMSD) benchmark, Vina reproduced the crystal pose of ciprofloxacin in all three independent runs (1.44, 1.48 and 1.47 Å), yet it ranked that pose third, fourth and eighth, while first place went in every run to the same non-crystallographic pose about 5.9 Å away with a better score (−9.7 kcal/mol). But the RMSD match is only a match of shape. The contact that actually holds the drug, both keto-acid oxygens chelating the noncatalytic metal ion, was not rebuilt even in the RMSD-matched pose (its oxygens sit 3.37 to 3.40 Å from the ion, outside the 2.6 Å bidentate distance), and across all 333 docked poses only one made that contact. A second metal ion that sat inside the earlier, larger grid box was measured and then removed by shrinking the box, and the top-ranked pose did not change, so the box is not the cause; this points to the scoring function as the most likely remaining cause. Scores for the mutants did not separate from the wild type by more than the method's own noise, so the known resistance was not reproduced. On this target, Vina recovers the ligand geometry but misses both top-1 ranking and the defining bidentate metal contact. Consequently, standard top-score docking screens cannot reliably detect resistance mutations here.

Keywords: molecular docking; AutoDock Vina; DNA gyrase; fluoroquinolone resistance; scoring function; metal coordination; pose ranking; ciprofloxacin.

1. Introduction

Fluoroquinolones such as ciprofloxacin kill bacteria by jamming DNA gyrase, a type II topoisomerase, while it is cutting and rejoining DNA [1, 4]. The drug does not grip the enzyme on its own. It bridges to the protein through a divalent metal ion and water molecules: the C3 carboxyl and C4 keto oxygens of the drug chelate the metal, and the metal is held to the enzyme by residues Ser84 and Glu88 of the GyrA subunit [4]. This drug-stabilising ion is a noncatalytic ion, separate from the metal ions the enzyme uses to cut DNA [4]. In the 2XCT structure it is resolved with Mn^{2+}

[1]; the physiological ion is Mg^{2+} [4]. The two most common resistance mutations in *S. aureus*, S84L and E88K, change exactly these two anchor residues [4], both of which help coordinate the metal-and-water bridge that holds the drug [4]. So a second question, alongside reproducing the crystal pose, is whether free docking can register that these mutations weaken drug binding at all.

Molecular docking is attractive here because it is free and runs on a laptop. A natural question is whether free docking can reproduce this known resistance, that is, whether it scores the drug worse against the mutant enzymes than against the wild type. But before trusting docking to answer that, it has to pass a simpler check. On the one complex where the correct answer is fixed by a crystal structure, can docking put the drug where the crystal says it sits, and with the contact the crystal shows?

The control is 2XCT, *S. aureus* DNA gyrase with ciprofloxacin and DNA, solved at 3.35 Å resolution with four copies of the drug and eight metal ions [1]. At this resolution the bridging water molecules are not resolved [1], which already limits what any docking into this structure can rebuild. A recent crystal structure of a related fluoroquinolone (delafloxacin) on the same enzyme shows the same drug class trapping the cleavage complex and supports the general picture of a metal-and-water bridge, although delafloxacin is a different, more acidic drug and its exact contacts are its own [6].

This paper reports a benchmark built around that control. The question is not only "does docking reproduce resistance" but, more basically, "where does free docking break on this target." The answer separates two things that are usually reported as one number: whether docking can reproduce a pose at all, and whether it ranks it first, and it adds a third, whether the pose it calls a match actually carries the interaction that defines the real pose.

2. Materials and methods

Structure and preparation. The receptor was taken from the 2XCT crystal structure [1] downloaded from the RCSB Protein Data Bank [2]. The docked receptor kept the cleavage complex intact: two copies of the GyrB-GyrA fusion chain (chains B and D, residues 417 to 1491 in the deposited numbering, in which GyrA Ser84 and Glu88 appear as Ser1084 and Glu1088), the cleaved DNA duplex (chains E, F, G and H, 37 nucleotides and 838 atoms) and four Mn^{2+} ions, among them the noncatalytic ion of the drug-binding site. The bridging water molecules are not resolved in 2XCT at 3.35 Å [1] and so were not present for a pose to coordinate against. The four fluoroquinolones were ciprofloxacin, levofloxacin, moxifloxacin and gatifloxacin. The wild-type GyrA sequence and the two single-point mutants S84L and E88K were used. Receptor and ligand preparation (hydrogens, charges, conversion to PDBQT) and all docking runs were carried out by the author on a personal laptop. The mutants were built as single-point changes to the wild-type structure, not from mutant crystals.

Docking. Docking used AutoDock Vina 1.1. The search box was an 18 Å cube centred at coordinates (2.8, 43.7, 68.7) on the drug-binding site. Each run used exhaustiveness 32 and produced nine poses (num_modes 9). To separate the random search from the scoring, each complex was docked three times with different random seeds (42, 7 and 2024) rather than once.

Two transparency notes apply to the settings. First, AutoDock Vina 1.1 does not print the grid box into its output files, so the box could not be read back from a log. For every run the box was instead reconstructed by measuring the coordinate range covered by the nine output poses; across all files the poses fill the 18 Å cube above (for example, the mutant runs reach the cube walls at z from 59.70 to about 77.70 Å and y up to 52.47 Å, which overshoots a 16 Å box and matches the 18 Å box). Second, the exhaustiveness value of 32 is taken from the command line reported by the author and cannot be recovered from the output files; it is stated here as reported.

Why the box was shrunk. One earlier ciprofloxacin wild-type run used a larger 22 Å box. In that box a second metal ion of the structure sits inside the grid, and two poses of that run docked onto this extra ion (closest oxygen 2.45 and 2.58 Å). To remove this confound the box was shrunk to 18 Å, which still holds ciprofloxacin with room to spare (the drug stays within 5.7 Å of the box centre) but places the second ion about 1 Å outside the grid (nearest y wall at 52.7 Å, ion at 53.458

Å). The 22 Å run is reported only to document why the box was changed; all other runs use the 18 Å box.

Pose evaluation. Three measures were kept for every ciprofloxacin wild-type run, because they answer three different questions.

1. *Did Vina rank the right pose first?* This is the standard way Vina is used and a fair test of the tool as people actually run it: look only at the top-scored pose.

2. *Could Vina find the right shape at all?* All nine poses were checked, and the closest to the crystal by whole-molecule heavy-atom RMSD was recorded even if it was not ranked first. The RMSD was computed against the ciprofloxacin molecule resolved in 2XCT, in the 2XCT coordinate frame, using the usual 2 Å cutoff for a correct prediction [3].

3. *Did any pose rebuild the metal contact?* For each pose the distance from each keto-acid oxygen to the noncatalytic metal ion was measured. A pose was counted as making the crystal contact only if both oxygens sat within 2.6 Å of the ion at the same time (the bidentate contact seen in the crystal) and the pose had not landed on the second, stray metal ion. This is a stricter test than RMSD: a pose can match the crystal by shape and still miss this contact.

For the mutants there is no crystal to measure RMSD against, so the wild-type/mutant comparison used the top-ranked score and, as a second read, the score of the pose closest in geometry to the wild-type crystal pose. The strict two-oxygen contact test above is reported as a separate diagnostic across the whole dataset, not as a filter that selects mutant poses, because almost no pose passes it (see Results).

Significance threshold. A score difference between wild type and mutant was treated as meaningful only if it was larger than 0.6 kcal/mol. This cutoff was fixed before the wild-type/mutant comparison, at about the method's own run-to-run and pose-to-pose score scatter: the nine poses of a single run span about 1 kcal/mol, so a wild-type/mutant gap smaller than this is inside the noise and is not reported as resistance.

Scope of the data. In total 37 unique runs were analysed: 27 for levofloxacin, moxifloxacin and gatifloxacin (three protein versions, three seeds each), 6 for ciprofloxacin against S84L and E88K (three seeds each), 1 earlier ciprofloxacin wild-type run on the 22 Å box, and 3 ciprofloxacin wild-type runs on the 18 Å box. Nine poses per run gives 333 poses in all.

3. Results

3.1 Vina reproduces the crystal shape but ranks it below a wrong pose

Table 1 is the central result, because ciprofloxacin in the wild-type enzyme is the one complex with a crystal pose to check against. By whole-molecule RMSD, all three seeds produced a pose almost on top of the crystal pose (1.44, 1.48 and 1.47 Å, all well under the 2 Å cutoff). In all three seeds that pose was not ranked first. It came third, fourth and eighth. First place in every seed went to the same wrong pose, about 5.9 Å from the crystal, and that wrong pose had the better (more negative) score, −9.7 kcal/mol.

Table 1. Ciprofloxacin, wild-type DNA gyrase, 18 Å box: shape found, ranking missed, across three seeds.

Seed	Rank of the closest-RMSD pose	RMSD of that pose to crystal, Å	Score of that pose, kcal/mol	Top-ranked pose RMSD to crystal, Å	Top-ranked pose score, kcal/mol
42	3rd	1.44	−9.2	5.88	−9.7
7	4th	1.48	−9.2	5.88	−9.7
2024	8th	1.47	−8.7	5.90	−9.7

The scores sit in a narrow band. For seed 42 the nine poses score −9.7, −9.4, −9.2, −9.2, −8.9, −8.9, −8.8, −8.6 and −8.6 kcal/mol, a spread of only 1.1 kcal/mol from first to last. So for seed 42 the shape-matched pose loses first place by 0.5 kcal/mol, and across the three seeds the gap is 0.5 to 1.0 kcal/mol, inside a crowd of poses that Vina treats as almost equally good. The search is not the limit on shape: the right overall shape is there every time. The ordering is wrong.

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3.2 The box is not the cause

To rule out grid artifacts from the preliminary 22 Å box, where a second Mn^{2+} ion attracted two poses, we reduced the grid to 18 Å, placing that ion ~1 Å outside the boundary. All poses stayed at least 4.35 Å away from it, yet the same non-crystallographic top-1 pose persisted across all seeds. With the grid excluded and receptor parameters fixed, the most likely cause is the scoring function, which treats metal ions as simple hydrogen-bond donors without a coordination term [5]. Other settings were held fixed and not varied here (see Limitations).

3.3 The metal contact that defines the real pose is almost never rebuilt

The RMSD match in Table 1 is a match of shape only. In the crystal both keto-acid oxygens of ciprofloxacin sit on the noncatalytic metal ion, about 2.1 Å away (measured at 2.10 and 2.11 Å for the two oxygens). This bidentate contact is what pins the drug in place [1, 4]. In the shape-matched docked pose the same oxygens stand 3.37 to 3.40 Å from the ion: this pose is outside the 2.6 Å bidentate distance, so by the stricter contact test it does not reproduce the binding contact even though its RMSD is 1.44 to 1.48 Å. The top-ranked pose reaches closer to the ion, 2.48 to 2.51 Å, but with only one oxygen, so it does not make the two-oxygen contact either.

This held across the whole dataset. Of all 333 docked poses (37 runs times 9 poses), exactly one made the bidentate contact of both oxygens within 2.6 Å of the metal at once: gatifloxacin in the wild-type enzyme, seed 7, pose 4, scored −8.0 kcal/mol. For ciprofloxacin, no pose in any run made this contact. So the contact that physically holds these drugs is essentially never rebuilt, in the RMSD-matched pose or the top-ranked pose alike. "Finding the pose" is therefore only half true on this target: the shape is found, the interaction is not.

3.4 The mutants: no resistance reproduced

For ciprofloxacin the top-ranked score was −9.7 kcal/mol for the wild type and for both mutants, in every seed. On the number a docking screen normally reads, there is no difference at all between the sensitive and the resistant enzymes: the gap is zero, well inside the method's own run-to-run scatter. The scoring did not reproduce the known resistance of S84L or E88K.

Two cautions sit on this flat result. First, as Section 3.3 showed, no ciprofloxacin pose rebuilds the metal contact that these mutations act on, so the model never forms the interaction a resistance effect would have to change; on this target the method is, in that sense, blind. Second, fluoroquinolone resistance is not only a matter of how tightly the drug binds at equilibrium; much of it works through the kinetics of trapping the cleavage complex [4]. A flat binding score is therefore weak evidence either way, and even a metal-aware method might not show S84L or E88K as a simple binding-energy gap. The honest reading is not that these mutations leave the drug binding, but that this score cannot tell. Flat Vina scores therefore do not imply preserved affinity; rather, the scoring function lacks the sensitivity to resolve S84L and E88K resistance effects.

4. Discussion

The results separate three things that are usually collapsed into a single docking "success." First, whether the search can reproduce the pose by shape: on this target, yes, in every run the closest pose was within 1.5 Å of the crystal. Second, whether the top-ranked pose is that pose: no, in every run a non-crystallographic pose scored better. Third, whether the reproduced pose actually carries the interaction that defines the crystal pose: no, the bidentate metal contact was absent in every ciprofloxacin pose and in all but one pose of the whole set. The honest one-line summary is that Vina reproduces the shape of the pose but not its metal contact, and does not rank even the shape-matched pose first.

These results point to one factor they are likely to share. The real pose is held by a bidentate metal contact and by waters bridging the metal to Ser84 and Glu88 [4]. Vina has no energy term for metal coordination and scores the metal ion, which was kept in the receptor, as an ordinary hydrogen-bond donor, and it is known to be weak on metalloproteins [5]. On top of that, at 3.35 Å the bridging waters are not present in the control structure at all [1], so there is nothing for a pose to coordinate against even geometrically. This is consistent with what the poses do: the oxygens settle about 1.3 Å too far from the ion, the shape-matched pose is not held in place, and a differently packed pose scores slightly better and takes first place. The same missing term would also explain

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why the mutants do not separate, since the model never forms the contact that S84L and E88K disturb. Because other receptor factors were held fixed rather than varied (Section 5), this is the most likely shared cause, not a proven one.

This has a direct consequence for the original goal. A free-docking resistance screen reads off the top score and compares it between wild type and mutant. Here the top-ranked pose is not the crystal pose, the binding contact is never formed, and the wild-type/mutant scores do not separate at all. The honest conclusion is not that S84L and E88K leave the drug binding, but that this method cannot tell on this target. That is worth stating for anyone planning to use a laptop docking run to argue that a mutation does or does not break a drug.

The value of running the control is exactly this. Without the crystal, the top-ranked pose would have looked like a plausible answer and the flat wild-type/mutant scores would have read as "no resistance predicted." The crystal shows that the top-ranked pose is not the crystal pose, that even the shape-matched pose misses the key contact, and that the flat scores are better read as the method being unable to tell here than as proof that resistance is absent.

5. Limitations

This study uses one enzyme, one crystal structure and one scoring function, so the finding is specific to fluoroquinolones on *S. aureus* DNA gyrase with AutoDock Vina 1.1, not a general claim about docking. The docking runs were generated by the author rather than taken from a public benchmark, which is a departure from a pure secondary-data design; they were re-measured against the crystal to check the numbers. Section 3.2 rules out the grid box as the cause of the ranking but does not test every alternative: receptor protonation, partial-charge assignment, ligand flexibility, and the absence of the bridging waters (not resolved at 3.35 Å) were held fixed, so the scoring function is identified as the most likely remaining cause, not the only possible one. The noncatalytic metal ion was kept in the docked receptor, so the non-rebuilt contact is not simply the result of a missing ion. The DNA of the cleavage complex was kept in the docked receptor (Section 2), so the top-ranked pose is the optimum of the same complex the crystal pose belongs to, and its disagreement with the crystal pose is not an artefact of a stripped receptor. Grid box sizes were reconstructed from pose coordinates because Vina 1.1 does not record them, and the exhaustiveness setting is taken from the reported command line rather than a file. The mutant structures were single-point models, not mutant crystals. The significance threshold of 0.6 kcal/mol is a practical cutoff set from score scatter, not a statistical test. Three seeds per complex sample the search but do not fully map its variability, so statements such as "in every run" describe these three runs, not a proof over all seeds. The one pose that made the metal contact was gatifloxacin, not ciprofloxacin, so even that single success is outside the control complex.

6. Conclusion and further work

On the one *S. aureus* DNA gyrase complex that has a crystal pose, AutoDock Vina reproduces the shape of ciprofloxacin's crystallographic pose in every run (RMSD 1.44 to 1.48 Å) but ranks it third, fourth or eighth rather than first, and even that shape-matched pose does not rebuild the bidentate metal contact that defines the real pose. The grid box was shown by measurement not to be the cause, which points to the scoring function, consistent with its lack of a metal term [5]. The metal contact was rebuilt by one pose out of 333, and the top scores against the S84L and E88K mutants did not separate from the wild type at all. Finding a shape, ranking it, and reproducing its key contact are three different things, and a free-docking resistance screen that trusts the top score inherits all three failures.

The natural next step is a metal-aware scoring function such as Lin_F9, which adds terms for metal coordination [5], and, where a higher-resolution structure exists, explicit bridging waters. Both go beyond a laptop and a free tool and were kept out of scope here. For lightweight docking workflows on metalloproteins, validating against a co-crystal control and verifying key coordination distances rather than relying solely on top-1 Vina scores or heavy-atom RMSD is essential to avoid false negative resistance predictions.

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Author contributions

Mustafaryza Shakhin carried out all 37 docking runs, including receptor and ligand preparation, the choice of the three random seeds, the change from the 22 Å to the 18 Å box, and the pose-by-pose inspection of the output, and reported the per-run scores and pose RMSD values. The scientific supervisor, Kurakbay Myrzabek (the author's mentor at the ADMIT programme), re-measured these values against the 2XCT crystal structure (scores, RMSD values, box sizes from pose coordinates and metal-oxygen distances), checked the pose counts, and worked with the author on the interpretation and on preparing and editing the manuscript.

AI-use disclosure

All 37 docking runs, including receptor and ligand preparation, the search box and the random seeds, were carried out by the author. The English text of this manuscript was drafted and edited with a generative AI tool (Claude, Anthropic) under the supervisor's direction, and the re-measurements against the 2XCT crystal structure (scores, RMSD values, metal-oxygen distances, grid boxes reconstructed from pose coordinates, and pose counts) were made with the same tool and checked by the supervisor. No docking run, score, RMSD value, distance, count or citation in this paper was produced by an AI model: every number comes from an AutoDock Vina output file or from a measurement against 2XCT, and the numbers and references were checked in two rounds of review by separate AI agents before the supervisor read the final text. The supervisor is responsible for the analysis and for the content of this manuscript.

Data availability

The 37 AutoDock Vina output files (nine poses each), the three ciprofloxacin wild-type 18 Å runs, the six ciprofloxacin mutant runs, and the per-pose measurements against 2XCT are held by the author and are available on reasonable request. The crystal structure 2XCT is public at the RCSB Protein Data Bank [2].

Conflicts of interest

The authors declare no conflict of interest.

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