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Statistical Analysis, Optimization, and Prioritization of Virtual Screening Parameters for Zinc Enzymes Including the Anthrax Toxin Lethal Factor

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Abstract

The anthrax toxin lethal factor (LF) and matrix metalloproteinase-3 (MMP-3, stromelysin-1) are popular zinc metalloenzyme drug targets, with LF primarily responsible for anthrax-related toxicity and host death, while MMP-3 is involved in cancer- and rheumatic disease-related tissue remodeling. A number of *in silico* screening techniques, most notably docking and scoring, have proven useful for identifying new potential drug scaffolds targeting LF and MMP-3, as well as for optimizing lead compounds and investigating mechanisms of action. However, virtual screening outcomes can vary significantly depending on the specific docking parameters chosen, and systematic statistical significance analyses are needed to prioritize key parameters for screening small molecules against these zinc systems. In the current work, we present a series of chi-square statistical analyses of virtual screening outcomes for cocrystallized LF and MMP-3 inhibitors docked into their respective targets, evaluated by predicted enzyme-inhibitor dissociation constant and root-mean-square deviation (RMSD) between predicted and experimental bound configurations, and we present a series of preferred parameters for use with these systems in the industry-standard Surflex-Dock screening program, for use by researchers utilizing *in silico* techniques to discover and optimize new scaffolds.

Keywords

Anthrax; anthrax toxin lethal factor; docking and scoring; MMP-3; surflex-dock; virtual screening; zinc metalloproteinases

1. INTRODUCTION

Virtual screening methodologies such as docking and scoring are widely used in the drug design process, and have proven valuable for discovering new therapeutic scaffolds [1–3],

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

identifying chemical matter with potential biological activity against a specific target (or set of targets) [4,5], prioritizing compounds for synthesis [6], and investigating mechanisms of action [7]. However, the accuracy of virtual screening output varies widely between docking algorithms and scoring functions, and is often receptor-dependent. Metalloenzyme systems in particular have emerged as key drug targets in multiple disease modalities [1,8–16] but are especially challenging systems for virtual screening due to catalytic metal centers that are often not well represented by mechanics-based scoring functions designed for efficient computational throughput. Many studies have been conducted [17–21] evaluating the accuracy of various virtual screening programs and/or algorithms for biologically and therapeutically relevant ligand-receptor complexes; however, no systematic statistical analyses have yet been reported that recommend specific virtual screening options for relevant metalloprotein targets. Docking and scoring is often carried out using default parameters for the software of choice; however, variations in active-site dimensions and key tolerance thresholds involving electrostatic (Coulombic) energies and steric clashes strongly affect docking output and must be fine-tuned for the system(s) under consideration in order to yield accurate results. In the current work, we report a series of statistical significance analyses leading to a set of optimized parameters for docking and scoring a set of cocrystallized small molecules into two popular zinc metalloenzyme structures: all available X-ray coordinates of the anthrax toxin lethal factor (LF) that have been deposited in the Protein Data Bank (PDB), and one experimental structure of matrix metalloproteinase-3 (MMP-3, stromelysin-1, for the purpose of comparison), to be implemented as part of a virtual screening process to discover and optimize new leads targeting those receptors.

The LF enzyme is produced by the Gram-positive bacterium *Bacillus anthracis* as part of a tripartite exotoxin, and is primarily responsible for anthrax-related cytotoxicity. This zinc hydrolase functions by cleaving members of the mitogen-activated protein kinase kinase (MAPKK) family, resulting in inhibition of the host immune system [22] as well as disruption of key vascular barriers, leading to circulatory shock and death. While the anthrax bacilli are susceptible to antibiotics such as fluoroquinolones, treatment must take place in the earliest stages of the disease because antibacterial modalities have no effect on the rapidly secreted exotoxin. The LF enzyme has therefore attracted considerable attention as a drug target for effective postexposure anthrax countermeasures. [1,23–25] Matrix metalloproteinase 3 (MMP-3), or stromelysin-1, is another Zn^{2+} metalloenzyme that degrades extracellular matrix proteins, including those present in connective tissue, and plays a key role in tissue remodeling associated with metastatic cancer and inflammatory pathologies such as osteo- and rheumatoid arthritis. [8–10,26] MMP-3 has also been a highly popular drug target: although interest had waned somewhat over the last decade due to a series of pharmacokinetic and toxicity-related liabilities associated with MMP inhibitors, stromelysin-1 is re-emerging as an anticancer and antirheumatic therapeutic focus, with researchers seeking to identify novel, less toxic scaffolds that incorporate zinc-binding groups (ZBGs) with more favorable metabolic profiles than the traditional hydroxamic acid functionality.

It has been recognized that validating and optimizing key computer-aided drug design parameters based on experimental data is likely to facilitate the identification and

prioritization of new chemical matter in general, and specifically for LF and MMP-3 where the presence of catalytic transition metals pose a challenge to modeling. In the current study, a set of six virtual screening parameters for LF and MMP-3 were optimized based on experimental structural biology, namely X-ray coordinated for six enzyme-inhibitor complexes (PDB codes 1YQY [27], 1PWQ [28], 1PWU [28], 1PWP [29], and 1ZXV [23] for LF and PDB code 1SLN [26] for MMP-3). Taken together, these experimental systems constitute a suitable dataset for screening validation and optimization: the cocrystallized ligands are structurally diverse (a sulfonamide hydroxamate, a rhodanine derivative, a diquinoline urea analog, and two peptide hydroxamates); they demonstrate a range of biological activities against LF and MMP-3, from the nanomolar (1YQY: $IC_{50} = 60$ nM [27], 1PWP: $K_i = 0.5$ μ M [29], and 1SLN: $K_i = 230$ nM [26]) to the one- and two-digit micromolar range (1ZXV: $IC_{50} = 1.7$ μ M [23]; 1PWQ: $K_i^{app} = 2.1$ μ M [28]; and 1PWU: $K_i^{app} = 11$ μ M [28]); and they exhibit a variety of experimentally determined binding modes covering all key binding-area sub-sites in both receptors.

2. THEORETICAL APPROACH

The LF catalytic site comprises three binding regions: S1', which is strongly hydrophobic, sterically constrained, and occupies a relatively small volume; S1–S2, a solvent-exposed region with a combination of polar and hydrophobic residues; and the open-ended, less well characterized S2' subsite (1YQY.pdb, Fig. 1a). [1,23,27–29] The MMP-3 active site (1SLN.pdb, Fig. 1b) is a solvent-exposed groove with many ligand-receptor hydrogen-bonding interactions, featuring the sterically constrained, strongly hydrophobic S1' tunnel [8–10, 26] and the more electrostatically complicated S1–S2' region. Catalytic Zn^{2+} atoms are present in the active sites of both enzymes.

In this study, we focused on two sets of key parameters for Zn metalloenzyme virtual screening – protomol generation and docking variables – as implemented in the industry-standard Surflex-Dock package (Tripos, Inc.). [30–33] (Surflex-Dock was utilized in this work due to its wide use in computational drug discovery. protocols, as well as its demonstrated suitability for zinc metalloprotein docking [1].) This package applies an empirical scoring function based on the Hammerhead docking system, together with a surface-based molecular similarity algorithm set. In Surflex-Dock, the docking target area is represented by a “protomol,” a pseudomolecule representing an ideal ligand bound to the target. The protomol is constructed by modeling key interactions of small-molecule probes with selected protein residues, including hydrogen bond donor and acceptor functionalities and a diverse selection of hydrophobic and hydrophilic fragments. [30–33] The protomol location can be specified by identifying residues manually, by automatically selecting residues surrounding a cocrystallized ligand, or via an automatic detection algorithm. Our previous work [1] showed that ligand-based protomol generation resulted in more accurate active site identification than automatic identification via the detection algorithm, so only the former methodology is applied here.

The primary variable to be optimized in protomol generation is the threshold value (ranging from 0.01 to 1), a factor that determines the extent to which, i.e., what fraction of, the protomol may be “buried” within the protein. [30] As the S1–S2 pocket of the LF active site

and the main groove of the MMP-3 active site are solvent-exposed, it was expected that varying the protomol threshold value to accommodate this additional space might facilitate a more favorable docking outcome. Three threshold values were considered (0.25, 0.50, and 0.74, chosen to cover a range of commonly used values [30–33]), together with two ligand-based active site identification options (with or without hydrogens on the ligand), resulting in generation of six distinct protomols for each experimental X-ray structure, and a total of 36 protomols overall. All protomols were visualized in the SYBYL GUI to ensure coverage of the desired active site areas.

Four docking variables were then considered: the number of additional starting conformations per ligand; the number of Ångstroms by which to expand (bloat) the search grid; ring flexibility; and spin density of search. [30–33] Two options were explored for the number of additional starting conformations: zero (default value, meaning that only one, user-provided ligand conformation is used) and five additional conformations, generated by random perturbations of the position and conformation of the ligand in question, followed by bump perturbation. [30–33] The search grid bloat parameter increases the docking search (grid size) beyond the bounds of the protomol, and fine-tuning this parameter may be especially helpful when screening ligands that bind to target regions not completely enclosed by the protomol, e.g., surface-bound ligands. The values used for grid expansion were 6 Å (default) and 12 Å. The binary (on/off) ring flexibility parameter introduces conformational flexibility for five-, six-, and seven-membered ligand rings based on models of cyclopentane, cyclohexane, and cycloheptane, respectively. [30] The fourth parameter explored here, density of search for spin alignment, controls the extent to which ligand alignments to the protomol are sampled. Three options are available: 3 (rapid screening), 6 (higher accuracy), and 9 (exhaustive accuracy). Using these four parameters, 24 docking runs were designed for each protomol, for a total of 864 simulations. (Docking parameters not specified here were retained at default values.)

All virtual screening calculations were carried out within the SYBYL-X 1.0 (Tripos, Inc.) environment using Surflex-Dock 2.1 on a Dell Precision Workstation T7400 with 32GB RAM and dual quad-core Intel Xeon X5460 CPUs running at 3.16GHz, and operating under SUSE Linux Enterprise Desktop 11 (x86_64), SP1.

2. RESULTS AND DISCUSSION

Each cocrystallized ligand in the six Zn metalloenzyme X-ray structures of interest was docked into its corresponding receptor structure, varying the protomol and docking parameters as described above, with Zn located within the docking grid in each case. All four scoring functions in the Tripos CScore consensus scoring module [34] (G_SCORE, PMF_SCORE, D_SCORE and CHEMScore) were applied in order to represent the widest possible selection of ligand-receptor interactions taking place in the LF and MMP-3 active sites. Two measures of docking outcome were assessed: overall Surflex-Dock score, reported as predicted $-\log(K_d)$ (where a higher score indicates a lower dissociation constant and therefore tighter ligand-receptor binding), and the root-mean-square deviation (RMSD) in Ångstroms between the coordinates of each posed ligand and those of the respective cocrystallized ligand (experimental coordinates), where a lower RMSD indicates closer

agreement with experiment. Data analysis was executed based on three outcome criteria: score only, RMSD only, and pairs of equally weighted score and RMSD values. In each docking run, two results from the 20 outputs, one with maximum Surflex-Dock score (and its corresponding RMSD) and one with minimum RMSD (and its corresponding Surflex-Dock score), were retained for analysis. Results were analyzed using chi-square statistics, where the null hypothesis was that each of the specified parameter options would yield an equal number of favorable outcomes, which are defined in section 3.1 (except in chi-square tests for tri-valued parameters with one degree of freedom, where indicated below). Following the standard chi-square statistical calculation, a contingency table was constructed for each of the specified parameters using the observed frequencies, and the corresponding expected frequencies were calculated. Then the chi-square value was calculated by summing the squared difference between the observed frequency (O) and the expected frequency (E), divided by the expected frequency in all possible categories as shown in the following equation:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

In this study, two of the six parameters under consideration were tri-valued (threshold and density of search), and four were binary (hydrogens or no hydrogens on the ligand used to generate the protomol, number of additional ligand starting conformations, grid bloat, and ring flexibility). Chi-square tests were definitive for the two-value parameters, but were by necessity inconclusive for the tri-valued parameters. Additional tests were therefore designed (for both zinc metalloenzymes) for threshold and density: the three possibilities for each parameter were internally ranked as “A,” “B,” or “C,” with “A” referring to the parameter option resulting in the highest number of favorable outcome assessments as described above, “B” referring to the option yielding the next highest number of favorable outcomes, and “C” denoting the option resulting in the lowest number of favorable outcomes. In a more definitive (but less stringent) test than a chi-square test with two degrees of freedom, a chi-square test with one degree of freedom was implemented to evaluate whether the A option for each parameter was significantly superior to the B and C options. In this test, the null hypothesis was altered to expect a 33–67 distribution of good outcomes. An additional chi-square test with one degree of freedom assessed whether the A value was significantly superior to the B value.

All statistical analyses were carried out with respect to the sum of all threshold values (the primary variable of interest for LF and MMP-3) and with respect to each threshold value individually, although where the sample size within a single threshold was small ($n < 25$, as in the anthrax toxin LF analysis based on paired criteria, and in several of the MMP-3 analyses), type II statistical errors may be present. The analysis of the tri-valued parameters was carried out on the basis of all thresholds only. For all analyses reported here, “statistically significant” indicates $p < 0.05$ and “almost statistically significant” indicates that $0.05 \leq p \leq 0.10$.

3.1. Anthrax Toxin Lethal Factor (LF)

Two distinct scoring and RMSD ranges were used to determine whether a LF docking result was “favorable.” All of the docked poses of the fairly rigid 1 YQY.pdb cocrystallized ligand (MK-702/LF-1B) were very close to experiment, so the definition of a favorable docking outcome for that structure was somewhat more stringent than for the other four LF-inhibitor complexes. A favorable outcome for 1 YQY.pdb was defined as a Surflex-Dock score 10.00 and an RMSD value (between prediction and experiment) 0.50 Å. (In our previous work, MK-702/LF-1B docked into 1 YQY.pdb exhibited a predicted pK_d of 10, and the RMSD of that docked pose with respect to experimental coordinates was 0.54 Å [1]). For the other LF complexes, a favorable outcome was less strictly defined as a Surflex-Dock score 9.00 and an RMSD value 2.00 Å. Highest scoring poses for the 1YQY, 1PWQ, and 1PWU ligands more closely resembled experimental bound configurations (RMSD = 0.49 Å, 1.17 Å and 1.48 Å, respectively), than did the highest scoring poses for the 1PWP and 1ZXV ligands (RMSD = 3.30 Å and 4.97 Å, respectively), most likely because hydrophobic functionalities on the 1YQY, 1PWQ and 1PWU ligands bind strongly to the sterically constrained, hydrophobic S1' subsite, essentially “locking” the ligands in place, limiting the range of potential configurations available to the docking algorithm and increasing the probability of conformations close to experiment.

When considering RMSD values only, three of the six parameters of interest yielded statistically significant results. Protomol generation using ligands with or without hydrogens (hereafter, “PH” or “P” protomols, respectively) was a statistically significant variable across all threshold values, with the absence of hydrogens increasing the accuracy of results (see Table 1). Statistics for all other parameters for the LF system were therefore calculated solely with respect to “P” protomols. The second statistically significant parameter was the utilization of the ring flexibility functionality; including ring flexibility was significantly advantageous over all threshold values (Table 2). As expected, the threshold value itself was also statistically significant: applying the A (most favorable) value of 0.74 produced far superior results than did the aggregate B and C threshold values, and the A value was almost statistically significant when compared to the B threshold value of 0.50 (Table 3).

On the basis of Surflex-Dock score alone, only one parameter was found to be statistically significant: the density of grid search, which was significant at the level of the sum of all thresholds and at the threshold value of 0.74, and almost statistically significant at a threshold of 0.50 (Table 4). The A value ($d = 9$) was significantly better than the B and C values under all conditions tested (Table 5).

Using paired Surflex-Dock score and RMSD criteria, three parameters were found to be statistically significant. Using “P” protomols, and incorporating ring flexibility, were both distinctly preferable at the level of sum of all thresholds, and also at a threshold value of 0.25. The density of search (grid bloat) was statistically significant at all threshold values except for 0.50 (Table 6). The A value for search density ($d = 9$) was superior to the B and C values under all conditions tested (Table 5). Notably, there was no statistically significant difference in docking outcomes based on threshold value when paired (score and RMSD) criteria were considered; this is interesting, given that the A, B and C threshold values vary

depending on which outcomes criteria are used, and also given that the threshold value of 0.74 was strongly preferred when RMSD was used as the sole criterion (Table 3).

3.2. Matrix Metalloproteinase-3 (MMP-3, Stromelysin-1)

Favorable criteria for MMP-3 (1SLN.pdb) virtual screening were set at Surflex-Dock score 9.00 and RMSD value 2.00 (the same ranges used for the LF-inhibitor complexes 1PWQ and 1PWU). Three criteria for docking assessment were once again implemented: Surflex-Dock score, RMSD in Å between predicted and experimental bound configurations, and pairs of equally weighted Surflex-Dock scores and RMSD values.

Protomol generation with or without hydrogens was significant for two of these criteria options (Table 7). At all thresholds and a threshold value of 0.25, the presence of a “PH” protomol was statistically significant when analyzing with respect to RMSD values and pairs of score and RMSD values, but not with respect to scores only. Therefore, RMSD and paired RMSD/score analyses were based on the three protomols generated using ligands with hydrogens. No preference for protomol generation from ligands with hydrogens was evident when using Surflex-Dock scores as the sole criterion; therefore, all six protomols (incorporating all possible parameter combinations) were used to carry out the score-based statistical analyses.

Using RMSD values as docking criteria along with “PH” protomols, we noticed two statistically significant parameters and one almost statistically significant parameter (Tables 8 and 9). Using five additional ligand starting conformations was statistically significant at the level of all thresholds and at a threshold value of 0.50, while this variable was almost statistically significant at a threshold value of 0.74. Incorporation of ring flexibility was mildly disfavored in this analysis; when considering all thresholds or a threshold value of 0.25, turning ring flexibility off was almost statistically significant. Threshold value was statistically significant in the analysis using RMSD as the sole criterion (Table 9). The A value of threshold ($t = 0.25$) was significant when tested against the grouped B and C values, and it was almost statistically significant when tested against the B value alone.

When the same statistical analysis was done using Surflex-Dock score as the sole criterion, three of the four docking parameters exhibited statistically significant results (Table 10). At the level of all thresholds and at a threshold value of 0.74, using five additional starting conformations for the ligand(s) yielded a statistically significant difference in results, while the difference was almost statistically significant at threshold values of 0.25 and 0.50. Non-utilization of the ring flexibility option was statistically significant across all thresholds. The density of search parameter was also significant with respect to Surflex-Dock scores alone. The A value ($d = 9$) was significant when tested against the grouped B and C values of density (Table 11).

Statistical results based on pairs of score and RMSD values were quite similar to the results with respect to score only (Table 12). Using five additional starting conformations of the ligand was statistically significant at all thresholds and at threshold values of 0.50 and 0.74. The non-use of the ring flexibility utility was statistically significant across all thresholds. The A value for search density ($d = 9$) was almost statistically significant when considering

all thresholds in the chi-square test with two degrees of freedom, and it was statistically significant when tested against the aggregated lower two values in the test with one degree of freedom.

CONCLUSION

In this study, virtual screening protocols for two zinc metalloenzymes of interest – the anthrax toxin lethal factor and matrix metalloproteinase-3 – were optimized with respect to six key parameters, via docking a series of cocrystallized ligands into their respective active site coordinates obtained by X-ray crystallography and applying the Surflex-Dock and CScore scoring functions. The significance of these six parameters (threshold value, protomol generation with or without hydrogens, number of additional starting conformations per ligand, search grid expansion, ring flexibility, and spin density of search) varied depending on which criteria were used to assess docking favorability: Surflex-Dock score, RMSD value between predicted and experimental ligand bound configuration, or an equally weighted combination of the two. Note that a higher Surflex-Dock score for any one compound (or set of compounds) does not in itself reflect a more accurate docking protocol; for example, a virtual screen that returns high scores for inactive compounds is of questionable utility. However, in the current study, all six cocrystallized inhibitors demonstrated nanomolar or low-micromolar activity against their targets, so a lower predicted dissociation constant (=higher predicted pK_d) was deemed favorable in these cases, and the inclusion of RMSD as an additional criterion enables assessment of how well the docking algorithm reproduces experimental bound configurations.

For the anthrax toxin lethal factor systems we studied, applying a search density value of 9 (exhaustive accuracy) is encouraged, as this parameter value improved docking results when Surflex-Dock score and paired (score and RMSD) search criteria were used, despite a moderate increase in computation time. For LF virtual screening, we also suggest generating a protomol based on a cocrystallized ligand without hydrogens, incorporating a threshold value of 0.74. Turning the ring flexibility option “on” in Surflex-Dock was found to be significant when evaluating docking results by RMSD and by paired criteria. For MMP-3 virtual screens (as determined by ligand-receptor interactions and structural features in the 1SLN.pdb system), our results suggest that protomol generation based on a ligand with hydrogens will be preferable, perhaps due to the relatively large number of hydrogen-bonding interactions present in the MMP-3 binding groove. For the ligand-receptor complexes in this study, hydrogen bonding is expected to play a more significant role for MMP-3 than for LF; hydrophobic interactions generally figure more prominently in LF systems [1] while a number of hydrogen-bonding and polar interactions have been found to be essential for small molecule binding to MMP-3. [8–10] For stromelysin-1, we also suggest a threshold value of 0.25, a search density value of 9, and five additional ligand starting conformations. Note that while threshold value, protomol generation with or without hydrogens, search grid expansion, and spin density of search are chiefly receptor-dependent parameters (although of course they will be influenced by specific ligand-receptor interactions), the optimal number of ligand starting conformations and incorporation of ring flexibility for a system of interest will depend strongly on the bonding scheme and degrees of freedom exhibited by the small molecules screened. Here, our choice of 1SLN.pdb as the

sole representative for MMP-3 may have resulted in a bias toward a higher number of starting conformations: the *N*-carboxyalkyl peptide cocrystallized with MMP-3 in 1SLN.pdb has several rotatable bonds, and more exhaustive exploration of potential molecular conformations is likely to increase the accuracy of docking results in this case. Three of the five cocrystallized LF inhibitors we examined were relatively rigid by comparison, so the number of ligand starting conformations can be expected to play less of a significant role in those cases, which is borne out by the statistical analyses (Tables 6 and 12).

Note that preferred values for four of the six parameters tested differ between the two zinc hydrolases, emphasizing the need for systematic validation studies and experimental benchmarking when possible, to determine optimal docking parameters for each system of interest, even when targets are closely related and/or form part of an enzyme family. Our results strongly confirm that altering virtual screening parameters, even by slight amounts, may result in docking poses and scores that differ significantly, and that also vary in accuracy when compared to experiment. It is therefore critical to identify and implement docking parameters for a particular receptor molecule target, benchmarking against experiment when possible, prior to implementing a virtual screening protocol that will be used to predict binding modes or to prioritize compounds for synthesis or further prosecution in biological assays.

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Fig. (1).

(a) Catalytic site of the anthrax toxin lethal factor (1YQY.pdb [27]), with sulfonamide hydroxamate inhibitor MK-702/LF-1B, illustrating three key binding subsites (S1' with Leu677, S2' with Lys656, and S1-S2). (b) Catalytic site of matrix metalloproteinase-3 (MMP-3, stromelysin-1, 1SLN.pdb [26]), with *N*-carboxyalkyl peptide inhibitor L-702,842, illustrating the main solvent-exposed binding groove and the hydrophobic S1' pocket that accommodates the phenylethyl moiety of the cocrystallized inhibitor. Key ligand-receptor hydrogen bonds are shown as dashed yellow lines. Both active site surfaces are modeled using a MOLCAD Fast Connolly electron density surface; magenta sphere = catalytic Zn²⁺ (visualized in SYBYL-X 1.0, Tripos, Inc.)

Table 1

Chi-square statistical analysis for protomol generation using ligands with or without hydrogens in the anthrax toxin lethal factor (LF), across all five available cocrystallized LF-inhibitor systems (1YQY.pdb [27], 1PWP.pdb [29], 1PWQ.pdb [28], 1PWU.pdb [28], and 1ZXV.pdb [23]), classified by threshold value and outcome criteria applied.

Criteria	Protomol	All Thresholds		T = 0.25		T = 0.50		T = 0.74	
		X ²	P	X ²	P	X ²	P	X ²	P
RMSD Only ^a	P; PH ^b	48.4030	<0.0001	12.1620	<0.0001	12.2360	0.0005	24.4200	<0.0001
Score Only	P; PH	3.4530	0.0631	3.6570	0.0558	0.6210	0.4308	0.1700	0.6803
Both	P; PH	9.8940	0.0017	10.8000	0.0010	2.1300	0.1444	0.5000	0.4795

^a“RMSD Only” = using RMSD (Å) between experimental and predicted bound ligand conformations as the sole criterion; “Score Only” = using Surflex-Dock score (predicted ligand-receptor p*K_d*) as the sole criterion; “Both” = using equally weighted pairs of RMSD and Surflex-Dock scores as criteria.

^bP = protomols generated using ligands without hydrogens; PH = protomols generated using ligands with hydrogens.

Table 2

Chi-square statistical analysis for four key docking parameters applied to all five experimental structures of the anthrax toxin lethal factor (LF), classified by threshold value and using RMSD (Å) between experimental and predicted bound ligand conformations as the sole criterion.

Parameter	Values	All Thresholds (n = 197)		T = 0.25 (n = 52)		T = 0.50 (n = 61)		T = 0.74 (n = 84)	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P
Starting Conformations	0, 5 ^a	1.1420	0.2852	0.6920	0.4052	1.3280	0.2492	1.7140	0.1904
Grid Bloat	6, 12 ^b	0.0050	0.9432	0.0770	0.7815	0.0160	0.8981	0.0480	0.8273
Ring Flex	On, off ^c	21.4470	<0.0001	11.0770	0.0009	4.7380	0.0295	6.8570	0.0088
Search Density	3,6,9 ^d	2.8270	0.2433	1.6540	0.4374	2.9840	0.2250	2.5710	0.2765

^aNumber of additional starting conformations for the ligand were 0 (default) and 5. Acceptable range of values = 0–10.

^bPossible grid bloat values were 6 Å (default) and 12 Å.

^cConsideration of ring flexibility is a binary operator.

^dAvailable search density values were 3 (screening), 6 (accuracy), and 9 (exhaustive accuracy).

Table 3

Chi-square statistical analysis (with one degree of freedom) for the threshold value parameter, across all five experimental structures of the anthrax toxin lethal factor (LF), classified by outcome criteria applied.

Criteria	A ^d	B	C	All Thresholds		A vs. B and C ^a		Top Two Options ^b	
				X ²	P	X ²	P	X ²	P
RMSD Only	0.74	0.50	0.25	8.2940	0.0158	7.6780	0.0056	3.6480	0.0561
Score Only	0.25	0.50	0.74	3.5150	0.1725	3.2820	0.0701	1.6130	0.2040
Both	0.25	0.74	0.50	2.2100	0.3311	1.9740	0.1601	0.8570	0.3545

^aThe “A” value (parameter option resulting in the highest number of favorable outcomes) was tested against the grouped “B” and “C” values (parameter options resulting in the next highest and lowest, respectively, number of favorable outcomes) with an expected 33–67 distribution.

^bEvaluated whether the “A” value was significantly preferred over the “B” value.

^c“RMSD Only” = using RMSD (Å) between experimental and predicted bound ligand conformations as the sole criterion; “Score Only” = using Surflex-Dock score (predicted ligand-receptor pK_d) as the sole criterion; “Both” = using equally weighted pairs of RMSD and Surflex-Dock scores as criteria.

^dThe “A”, “B” and “C” values were determined by an internal comparison of the favorable docking outcome results using each threshold value.

Table 4

Chi-square statistical analysis for four key docking parameters applied to all five experimental structures of the anthrax toxin lethal factor (LF), classified by threshold value and using Surflex-Dock score (predicted ligand-receptor pK_d) as the sole criterion.

Parameter	Values	All Thresholds (n = 103)		T = 0.25 (n = 43)		T = 0.50 (n = 32)		T = 0.74 (n = 28)	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P
Starting Conformations	0, 5	1.1750	0.2784	1.1400	0.2858	0.0000	1.0000	0.5710	0.4497
Grid Bloat	6, 12	0.0870	0.7675	0.0230	0.8788	0.0000	1.0000	0.1430	0.7055
Ring Flex	On, off	0.4760	0.4904	2.8140	0.0934	0.5000	0.4795	0.0000	1.0000
Search Density	3,6,9	11.4370	0.0033	1.0860	0.5811	5.0000	0.0821	12.2860	0.0021

^a Number of additional starting conformations for the ligand were 0 (default) and 5. Acceptable range of values = 0–10.

^b Possible grid bloat values were 6 Å (default) and 12 Å.

^c Consideration of ring flexibility is a binary operator.

^d Available search density values were 3 (screening), 6 (accuracy), and 9 (exhaustive accuracy).

Table 5

Chi-square statistical analysis (with one degree of freedom) for the spin density of search parameter, across all five experimental structures of the anthrax toxin lethal factor (LF), classified by outcome criteria applied.

Criteria	A ^d	B	C	All Density Values		A vs. B and C ^a		Top two options ^b	
				X ²	P	X ²	P	X ²	P
RMSD Only ^c	9	3	6	2.8270	0.2433	1.7240	0.1891	0.3480	0.5555
Score Only	9	3	6	11.4370	0.0033	10.7230	0.0011	5.0000	0.0253
Both	9	3	6	41.7890	<.0001	6.6820	0.0097	3.8460	0.0499

^aThe “A” value (parameter option resulting in the highest number of favorable outcomes) was tested against the grouped “B” and “C” values (parameter options resulting in the next highest and lowest, respectively, number of favorable outcomes) with an expected 33–67 distribution.

^bEvaluated whether the “A” value was significantly preferred over the “B” value.

^c“RMSD Only” = using RMSD (Å) between experimental and predicted bound ligand conformations as the sole criterion; “Score Only” = using Surflex-Dock score (predicted ligand-receptor pK_d) as the sole criterion; “Both” = using equally weighted pairs of RMSD and Surflex-Dock scores as criteria.

^dThe “A”, “B” and “C” values were determined by an internal comparison of the favorable docking outcome results using each density of search value.

Table 6

Chi-square statistical analysis for four key docking parameters applied to all five experimental structures of the anthrax toxin lethal factor (LF), classified by threshold value and using equally weighted pairs of RMSD (Å) values between experimental and predicted bound ligand conformations and Surflex-Dock scores (predicted ligand-receptor pK_d values) as outcome criteria.

Parameter	Values	All Thresholds (n = 103)		T = 0.25 (n = 43)		T = 0.50 (n = 32)		T = 0.74 (n = 28)	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P
Starting Conformations	0, 5 ^a	0.4390	0.5078	0.0000	1.0000	0.6000	0.4380	0.2220	0.6374
Grid Bloat	6, 12 ^b	0.0180	0.8946	0.0000	1.0000	0.0670	0.7963	0.2220	0.6374
Ring Flex	On, off ^c	12.7890	0.0003	4.1670	0.0412	2.5790	0.1083	2.0000	0.1573
Search Density	3,6,9 ^d	41.7890	<0.0001	12.0000	0.0025	2.8000	0.2466	36.0000	<0.0001

^a Number of additional starting conformations for the ligand were 0 (default) and 5. Acceptable range of values = 0–10.

^b Possible grid bloat values were 6 Å (default) and 12 Å.

^c Consideration of ring flexibility is a binary operator.

^d Available search density values were 3 (screening), 6 (accuracy), and 9 (exhaustive accuracy).

Table 7

Chi-square statistical analysis for protomol generation using ligands with or without hydrogens in matrix metalloproteinase-3 (MMP-3, stromelysin-1, 1SLN.pdb [26]), classified by threshold value and outcome criteria applied.

Analysis Type	Values	All Thresholds		T = 0.25		T = 0.50		T = 0.74	
		X ²	P	X ²	P	X ²	P	X ²	P
RMSD Only ^a	P; PH ^b	6.7590	0.0093	19.6000	<.0001	0.7140	0.3980	0.6100	0.4349
Score Only	P; PH	0.0290	0.8658	0.0670	0.7963	0.1110	0.7389	0.0910	0.7630
Both	P; PH	3.8460	0.0499	8.0000	0.0047	1.2860	0.2568	0.0910	0.7630

^a“RMSD Only” = using RMSD (Å) between experimental and predicted bound ligand conformations as the sole criterion; “Score Only” = using Surflex-Dock score (predicted ligand-receptor pK_d) as the sole criterion; “Both” = using equally weighted pairs of RMSD and Surflex-Dock scores as criteria.

^bP = protomols generated using ligands without hydrogens; PH = protomols generated using ligands with hydrogens.

Table 8

Chi-square statistical analysis for four key docking parameters applied to matrix metalloproteinase-3 (MMP-3, stromelysin-1, 1SLN.pdb [26]), classified by threshold value and using RMSD (Å) between experimental and predicted bound ligand conformations as the sole criterion.

Parameter	Values	All Thresholds (n = 72)		T = 0.25 (n = 34)		T = 0.50 (n = 20)		T = 0.74 (n = 18)	
		X ²	P	X ²	P	X ²	P	X ²	P
Starting Conformations	0, 5 ^a	6.7220	0.0095	0.1180	0.7316	7.2000	0.0073	3.5560	0.0593
Grid Bloat	6, 12 ^b	0.0560	0.8137	0.0000	1.0000	0.0000	1.0000	0.2220	0.6374
Ring Flex	On, off ^c	3.5560	0.0593	2.9410	0.0863	0.8000	0.3711	0.2220	0.6374
Search Density	3,6,9 ^d	1.5830	0.4531	4.4710	0.1070	1.3000	0.5220	0.3330	0.8465

^a Number of additional starting conformations for the ligand were 0 (default) and 5. Acceptable range of values = 0–10.

^b Possible grid bloat values were 6 Å (default) and 12 Å.

^c Consideration of ring flexibility is a binary operator.

^d Available search density values were 3 (screening), 6 (accuracy), and 9 (exhaustive accuracy).

Table 9

Chi-square statistical analysis (with one degree of freedom) for the threshold value parameter, for matrix metalloproteinase-3 (MMP-3, stromelysin-1, 1SLN.pdb [26]), classified by outcome criteria applied.

Criteria	A ^d	B	C	All Thresholds		A vs. B and C ^a		Top two options ^b	
				X ²	P	X ²	P	X ²	P
RMSD Only ^c	0.25	0.50	0.74	6.3330	0.0421	6.2500	0.0124	3.6300	0.0568
Score Only	0.25	0.74	0.50	1.6000	0.4493	1.4290	0.2320	0.6150	0.4328
Pairs	0.25	0.74,0.50	0.74,0.50	1.0000	0.6065	1.0000	0.3173	0.6920	0.4054

^aThe “A” value (parameter option resulting in the highest number of favorable outcomes) was tested against the grouped “B” and “C” values (parameter options resulting in the next highest and lowest, respectively, number of favorable outcomes) with an expected 33–67 distribution.

^bEvaluated whether the “A” value was significantly preferred over the “B” value.

^c“RMSD Only” = using RMSD (Å) between experimental and predicted bound ligand conformations as the sole criterion; “Score Only” = using Surflex-Dock score (predicted ligand-receptor pK_d) as the sole criterion; “Both” = using equally weighted pairs of RMSD and Surflex-Dock scores as criteria.

^dThe “A”, “B” and “C” values were determined by an internal comparison of the favorable docking outcome results using each density of search value.

Table 10

Chi-square statistical analysis for four key docking parameters applied to matrix metalloproteinase-3 (MMP-3, stromelysin-1, 1SLN.pdb [26]), classified by threshold value and using Surflex-Dock score (predicted ligand-receptor pK_d) as the sole criterion.

Parameter	Values	All Thresholds (n = 103)		T = 0.25 (n = 43)		T = 0.50 (n = 32)		T = 0.74 (n = 28)	
		X ²	p	X ²	p	X ²	p	X ²	p
Starting Conformations	0, 5 ^a	10.3140	0.0013	3.2670	0.0707	2.7780	0.0956	4.4550	0.0348
Grid Bloat	6, 12 ^b	0.0290	0.8658	0.0670	0.7963	0.1110	0.7389	0.0910	0.7630
Ring Flex	On, off ^c	35.0000	<.0001	15.0000	0.0001	9.0000	0.0027	11.0000	0.0009
Search Density	3,6,9 ^d	6.2290	0.0444	0.4000	0.8187	12.6670	0.0018	5.6360	0.0597

^a Number of additional starting conformations for the ligand were 0 (default) and 5. Acceptable range of values = 0–10.

^b Possible grid bloat values were 6 Å (default) and 12 Å.

^c Consideration of ring flexibility is a binary operator.

^d Available search density values were 3 (screening), 6 (accuracy), and 9 (exhaustive accuracy).

Table 11

Chi-square statistical analysis (with one degree of freedom) for the spin density of search parameter for matrix metalloproteinase-3 (MMP-3, stromelysin-1, 1SLN.pdb [26], classified by outcome criteria applied.

				All Density Values		A vs. B and C ^a		Top Two Options ^b	
Criteria	A ^d	B	C	X ²	p	X ²	p	X ²	p
RMSD Only ^c	9	6	3	1.5830	0.4531	1.5630	0.2113	0.9610	0.3270
Score Only	9	6	3	6.2290	0.0444	5.1570	0.0232	1.6900	0.1936
Both	9	6	3	5.3330	0.0695	4.0000	0.0455	1.0000	0.3173

^aThe “A” value (parameter option resulting in the highest number of favorable outcomes) was tested against the grouped “B” and “C” values (parameter options resulting in the next highest and lowest, respectively, number of favorable outcomes) with an expected 33–67 distribution.

^bEvaluated whether the “A” value was significantly preferred over the “B” value.

^c“RMSD Only” = using RMSD (Å) between experimental and predicted bound ligand conformations as the sole criterion; “Score Only” = using Surflex-Dock score (predicted ligand-receptor pK_d) as the sole criterion; “Both” = using equally weighted pairs of RMSD and Surflex-Dock scores as criteria.

^dThe “A”, “B” and “C” values were determined by an internal comparison of the favorable docking outcome results using each density of search value.

Table 12

Chi-square statistical analysis for four key docking parameters applied to matrix metalloproteinase-3 (MMP-3, stromelysin-1, 1SLN.pdb [26]), classified by threshold value and using equally weighted pairs of RMSD (Å) values between experimental and predicted bound ligand conformations and Surflex-Dock scores (predicted ligand-receptor pK_d values) as outcome criteria.

Parameter	Values	All Thresholds (n = 103)		T = 0.25 (n = 43)		T = 0.50 (n = 32)		T = 0.74 (n = 28)	
		X ²	p	X ²	p	X ²	p	X ²	p
Starting Conformations	0, 5 ^a	10.8890	0.0010	2.0000	0.1573	5.0000	0.0253	5.0000	0.0253
Grid Bloat	6, 12 ^b	0.0000	1.0000	0.0000	1.0000	2.0000	0.6547	2.0000	0.6547
Ring Flex	On, off ^c	18.0000	<.0001	8.0000	0.0047	5.0000	0.0253	5.0000	0.0253
Search Density	3,6,9 ^d	5.3330	0.0695	1.0000	0.6065	5.2000	0.0743	5.2000	0.0743

^a Number of additional starting conformations for the ligand were 0 (default) and 5. Acceptable range of values = 0–10.

^b Possible grid bloat values were 6 Å (default) and 12 Å.

^c Consideration of ring flexibility is a binary operator.

^d Available search density values were 3 (screening), 6 (accuracy), and 9 (exhaustive accuracy).