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Blind Pose Prediction, Scoring, and Affinity Ranking of the CSAR 2014 Dataset

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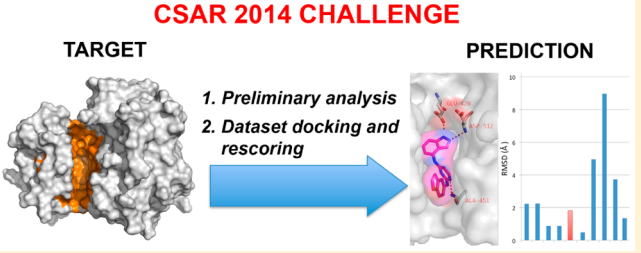
Supporting Information

ABSTRACT: The 2014 CSAR Benchmark Exercise was focused on three protein targets: coagulation factor Xa, spleen tyrosine kinase, and bacterial tRNA methyltransferase. Our protocol involved a preliminary analysis of the structural information available in the Protein Data Bank for the protein targets, which allowed the identification of the most appropriate docking software and scoring functions to be used for the rescoring of several docking conformations datasets, as well as for pose prediction and affinity ranking. The two key points of this study were (i) the prior evaluation of molecular modeling tools that are most adapted for each target and (ii) the increased search efficiency during the docking process to better explore the conformational space of big and flexible ligands.



INTRODUCTION

The Community Structure−Activity Resource (CSAR) Benchmark Exercises are unique opportunities for the molecular modeling community to evaluate, in "blind" conditions, the performance of the computational chemistry tools and methods that are currently available, in a continuous quest for improving the existing computational methods, with a special emphasis on docking and scoring.

Previous CSAR challenges took place in 2010 (343 diverse protein−ligand complexes with binding data in Binding MOAD or PDB bind),[1,2] 2011−2012 (six protein targets: CDK2, CDK2-cyclinA, urokinase, Chk1, ERK2, and LpxC; 647 compounds with biological affinities; and 82 crystal structures),[3,4] and 2013 (proteins designed to bind a steroid).

In 2014, the CSAR Benchmark Exercise was focused on three protein targets (Figure 1): coagulation factor Xa (FXA),[5−12] spleen tyrosine kinase (SYK),[13−23] and bacterial tRNA methyltransferase (TRMD).[24−29] In Phase 1, the participants were asked to score several datasets containing 200 pre-generated decoys, whereas in Phase 2 they had to dock five datasets containing small molecules in the corresponding target protein and provide the coordinates and the scores for each docked small molecule.

METHODS

Protein Structures. All ligands, ions, and solvent molecules that were present in the original structures downloaded from the Protein Data Bank (PDB)[30] were manually removed, and hydrogen atoms were added using Hermes, the graphical interface of Gold[31] software. The binding sites for docking were defined as follows: (i) for FXA, a sphere with a radius of 15 Å centered on the center of mass of the native ligand of structure FXA_gtc000401_2.07; (ii) for SYK, a sphere with a radius of 15 Å centered on the CE atom of residue Met448; (iii) for TRMD, a sphere with a radius of 15 Å centered on the backbone O atom of residue Tyr136. More generally, these binding sites were defined to cover all interactions with the ligands from existing X-ray crystal structures (see Supporting Information for a complete list).

Ligands. The structures used for Phase 2 were provided in SMILES format, and they were converted into three-dimensional MOL2 files using CORINA (Molecular Networks, http://www.molecular-networks.com/). The protonation state for all compounds was adjusted at physiological pH using LigPrep (Schrödinger, http://www.schrodinger.com/). Some ligands were provided as salts, and the counterion was removed during the LigPrep's ligand preparation protocol.

Docking. In the preliminary analysis step, several docking programs and scoring functions were evaluated for their ability to reproduce the existing protein−ligand complexes of FXA, SYK, and TRMD (see Supporting Information for a complete list of PDB structures that were used for this purpose): Glide (Schrödinger, http://www.schrodinger.com/), with the standard precision (SP) scoring function; Gold,[31] with the GoldScore, ChemScore, ChemPLP, and ASP scoring functions; Autodock;[32] and Autodock Vina.[33] For the pose prediction step, Gold with the GoldScore scoring function gave globally the best results, whereas for the rescoring step different scoring functions were found to be more suitable for each of the three targets. In most

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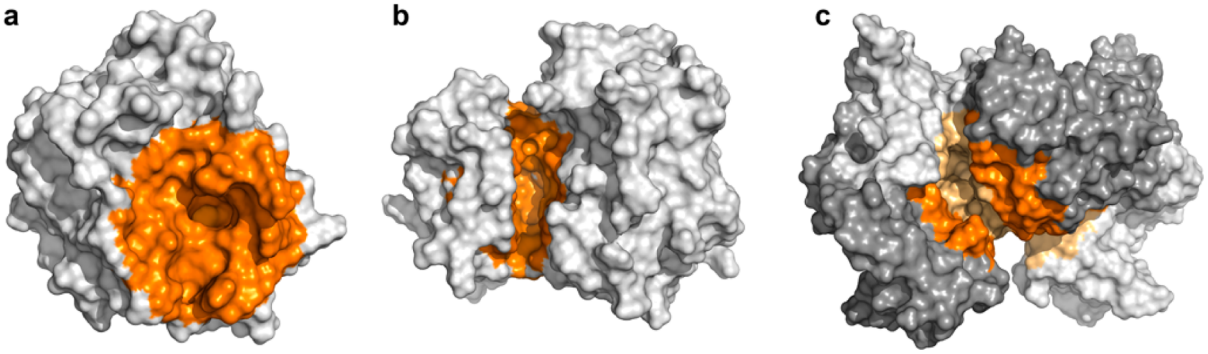


Figure 1. The three protein targets included in the CSAR 2014 challenge: FXA (a), SYK (b), and TRMD (c). In (a) and (b) the surface of the protein is colored in gray, and the binding site, as defined for our docking studies, is colored in orange. In (c) the structure is a homodimer, with the monomers colored in gray and dark gray, and their respective contributions to the binding site colored in light orange and orange.

Table 1. Overview of Docking Programs and Scoring Functions That Gave the Best Results in the Preliminary Analysis, with Each of the Three Proteins[a]

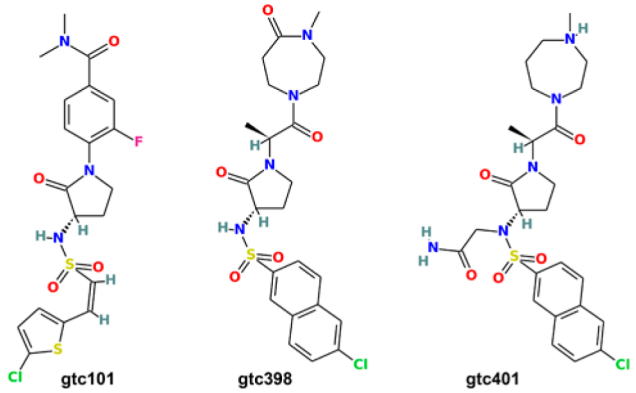
Protein	FXA	SYK	TRMD
Rescoring	Gold	Gold	Gold
	GoldScore, ChemScore, ChemPLP, ASP	ChemScore	ChemScore, (ChemPLP)
Docking	Gold	Gold	Gold
	GoldScore	GoldScore	GoldScore

[a]The scoring functions that were effectively used in Phases 1 and 2 are colored in red.

cases the selected docking conformations were ranked in the first position. However, in a few instances, the expected binding modes, according to the representative interactions identified in the preliminary analysis step, were found ranked in the second position. Thus, these ones were considered as correct answers and selected for the final submission. Default parameters were used in all cases for docking, except with Gold, where a search efficiency of 200% was used. Prior calculations showed that a search efficiency of 100% is not high enough to completely explore the large conformational space of ligands proposed for the CSAR 2014 challenge (data not shown).

Graphics. Chemical structures were depicted using CACTVS Chemoinformatics Toolkit (Xemistry, http://www.xemistry.com/), and images for protein structures were generated using PyMol (Schrödinger, http://www.pymol.org/).

Chart 1. Chemical Structures of the Three FXA Ligands Included in Phase 1



RESULTS AND DISCUSSION

The protocol that we followed for the CSAR 2014 benchmark is globally similar to the approach that we used for the SAMPL3 (2011)[34] and SAMPL4 (2013)[35] virtual screening challenges, which proved to be highly successful. It involves the preliminary analysis of the existing structural data for all protein targets and identification of the most appropriate combination of docking software and scoring function for each target, which is then used for the prediction. Another key point was the use of normal docking (not virtual screening) parameters, as our previous studies[34,35] showed that these parameters provided better results, due to improved conformational sampling within the binding site. In this work, given the size and conformational flexibility of ligands, a search efficiency of 200% was used to further improve the conformational sampling of docking conformations.

Preliminary Analysis. We started by identifying the structural data available for the three targets—FXA, SYK, and TRMD—in the PDB,[30] which was used to evaluate several different docking software and scoring functions. In this way, we found those that are the most adapted for the given targets in positioning the ligand in the binding site (useful for Phase 2) and scoring (or rescoring) the docking poses (useful for Phase 1). From the analysis of the existing protein−ligand complexes from the PDB we could also identify the representative interactions (mainly hydrogen bonds) and key residues of the protein responsible for the stability of the complexes, which could be used as additional criteria to evaluate the pertinence of pose predictions. The structures of co-crystallized ligands were also compared to the sets of ligands provided for the CSAR challenge in order to identify possible common structural patterns that should get a special attention in the analysis of the docking results.

FXA. PDB contains 125 structures of human coagulation factor Xa and one structure of the corresponding bovine protein. All of

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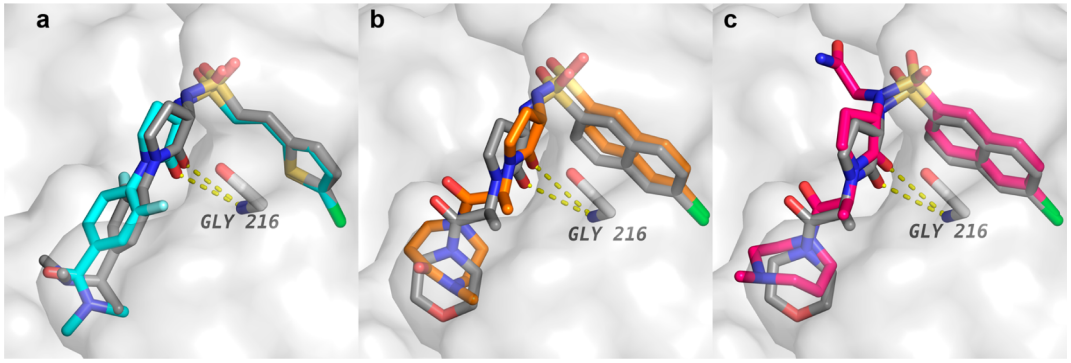
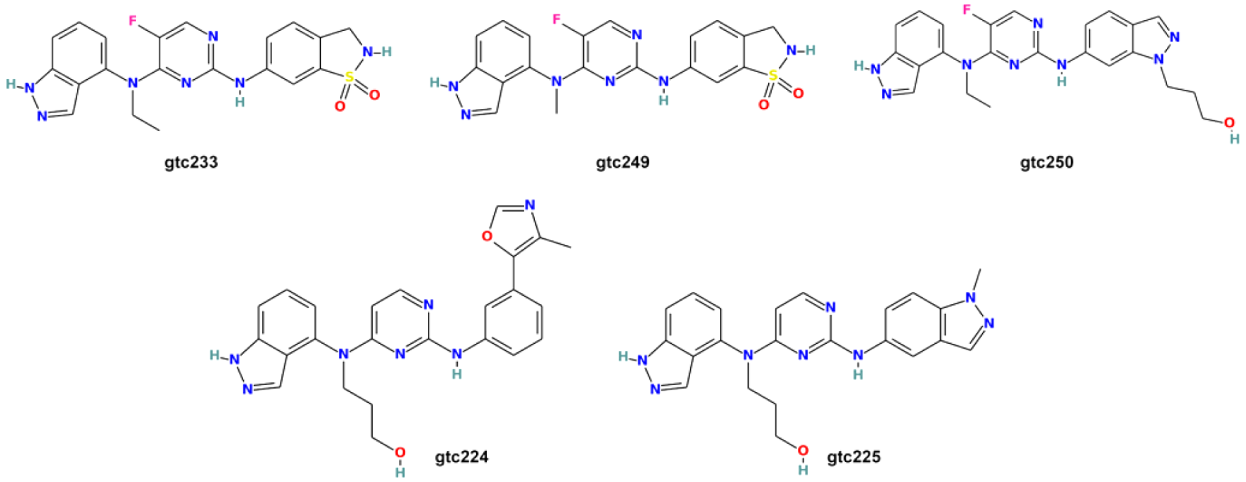


Figure 2. Predicted binding modes of the three FXA ligands (**gtc101**, **gtc398**, and **gtc401**) in Phase 1. The ligands are colored in cyan (a), orange (b), and purple (c), respectively. The reference compounds, ligands 461 (a) and GSK (b,c) from the PDB structures 2WYG and 2CJI, respectively, are colored in gray.

Chart 2. Chemical Structures of the Five SYK Ligands Included in Phase 1



them are obtained by X-ray diffraction. The analysis of the protein−ligand interactions in these complexes evidenced a number of residues that are important for binding, especially Gly126 which is involved in hydrogen bonds with heteroatoms from the ligands. All four scoring functions implemented in Gold (GoldScore, ChemScore, ChemPLP, and ASP) performed equally well for the rescoring step, whereas GoldScore scoring function was selected for docking (Table 1).

SYK. Thirty-one structures were identified in the PDB for SYK, 24 of them presenting a ligand in the binding site (see Supporting Information for more details). Gold with the GoldScore scoring function was found to be the most appropriate to perform the docking, whereas ChemPLP and ChemScore were the best for the rescoring, with a slight advantage for the latter, which was therefore used to evaluate the SYK series in Phase 1 (Table 1).

TRMD. Two groups of protein-ligand complexes, with different binding motifs, were identified from the TRMD structures available in the PDB (see Supporting Information for the complete list of structures): (i) in the first group, the ligands are adenosine derivatives that interact with the binding site through 4 hydrogen bonds with a consensus motif IGxYxL; (ii) in the second group, the ligands feature a 4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-5-carboxamide fragment, whose interaction with the binding site takes place through four hydrogen bonds with a consensus motif SIxxYxL. The calculations involving this protein were carried out with Gold, using ChemScore and GoldScore for the rescoring and docking steps, respectively (Table 1).

Phase 1. The Phase 1 dataset of the 2014 CSAR Benchmark Exercise consisted of 22 crystal structures of the three target proteins (3 for FXA, 5 for SYK, and 14 for TRMD), each structure being associated with a set of 200 docking poses of different ligands. Among the docking poses, one had an RMSD ≈ 1 Å compared to the experimentally determined conformation of the ligand. The participants were asked to identify this conformation, by providing an ordered list of the 200 docking poses, including the rank and score.

We rescored the docking poses that were provided using the most efficient docking software and scoring function identified in the previous step, and then we used the knowledge gathered from the analysis of the PDB complexes to check the pertinence of the rescoring result (in all cases we found a good agreement). The score submitted in the answer in addition to the rank comes directly from the rescoring, with the exception of FXA, for which we found in the PDB ligands very similar to those from the Phase 1 dataset. In this case the score submitted is the difference between an arbitrary value (50 in our case) and the RMSD compared with the ligands found in the PDB (the rankings based on rescoring and on RMSD were very similar). This approach allowed us to submit homogeneous answers, with the higher value for the best-ranked pose.

FXA. The chemical structures of the three FXA ligands included in Phase 1 are shown in Chart 1.

Compound **gtc101** is very similar to ligand 461 from the PDB structure 2WYG, which makes a unique hydrogen bond between

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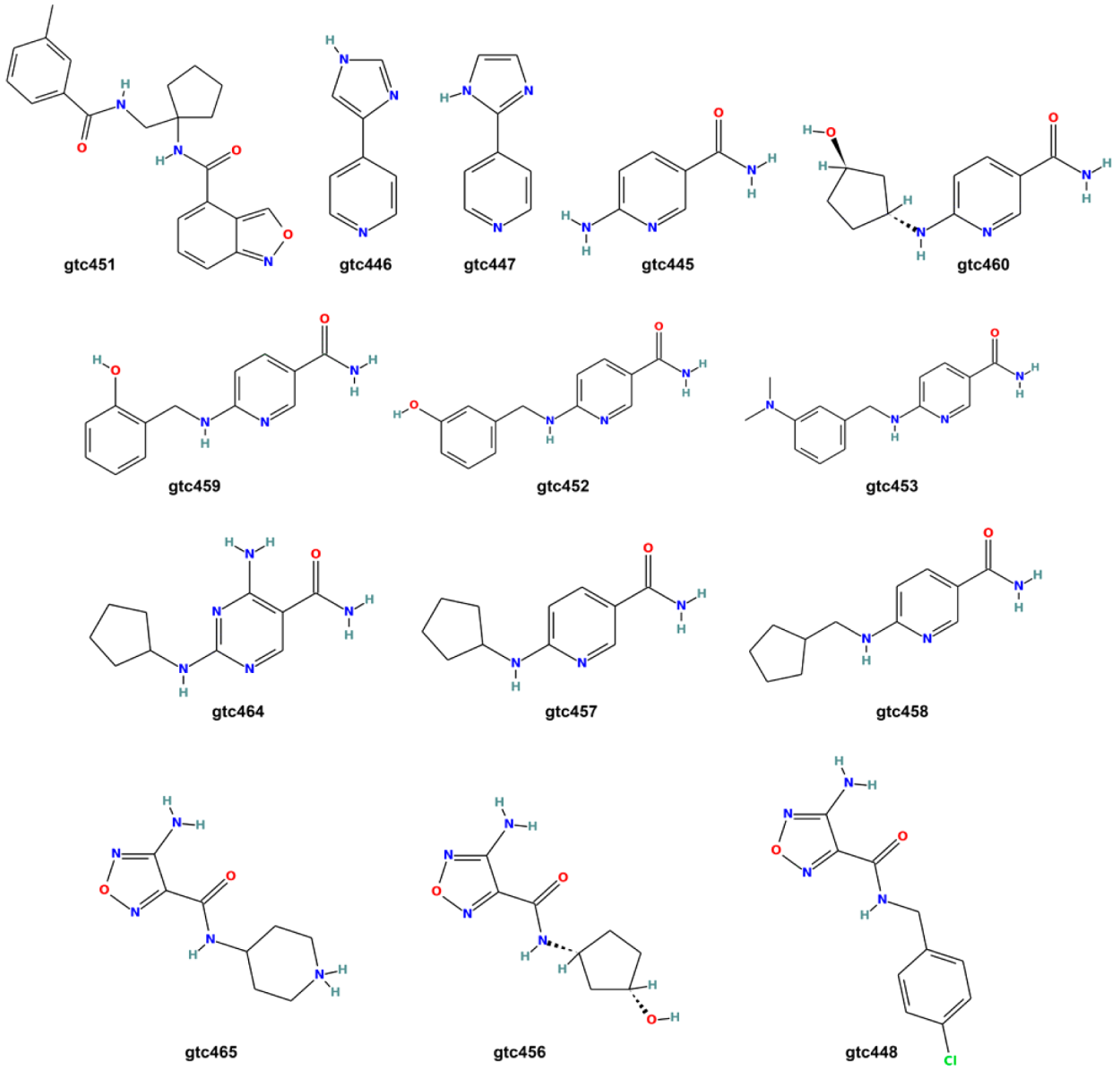
Chart 3. Chemical Structures of the 14 TRMD Ligands Included in Phase 1



Gly216 and the oxygen atom bound to the pyrrole ring. Among the 200 docking poses of **gtc101**, only two of them (conformations 76 and 146) have an oxygen atom at the same position. Conformation 146 is the closest compared to the one from the crystal structure 2WYG, and therefore it was used as reference to calculate the RMSD for the remaining 199 conformers. Compounds **gtc398** and **gtc401** are very similar to ligand GSK from the PDB structure 2CJI, which makes a hydrogen bond between Gly216 and the oxygen atom bound to the pyrrole ring. The closest conformations compared to the crystal structure are 190 and 37, respectively, which were also used as reference structures for RMSD calculations. It is worthy to note that for all ligands rescoring of the 200 poses with any of the four scoring functions implemented in Gold (GoldScore, ChemScore, ChemPLP, and ASP) provided the selected poses (146, 190, and 37) always ranked at the first position (Figure 2).

SYK. The chemical structures of the five SYK ligands included in Phase 1 are shown in Chart 2. Each set of 200 conformations corresponding to individual ligands was rescored using the ChemScore scoring function implemented in Gold, and the top-ranking conformations presented the conserved interactions with the binding site that were identified in the preliminary analysis.

TRMD. The chemical structures of the 14 TRMD ligands included in Phase 1 are shown in Chart 3. Rescoring of the corresponding datasets, containing each one 200 conformers, with the four scoring functions implemented in Gold (GoldScore, ChemScore, ChemPLP, and ASP) confirmed the superiority of ChemScore, and to a lesser extent ChemPLP, in providing top-ranking conformations containing the conserved interactions with the binding site that were identified in the preliminary analysis step.

The subsequent release of experimental data related to Phase 1 showed that *we have correctly predicted the top conformation for all of the 22 protein−ligand complexes (100% success rate)*. This result emphasizes the importance of the preliminary analysis step, in order to choose the most suitable tool that is able to provide the correct answer for a given problem.

Phase 2. In Phase 2 of the 2014 CSAR Benchmark Exercise, the participants were asked to dock five ligand datasets (three for

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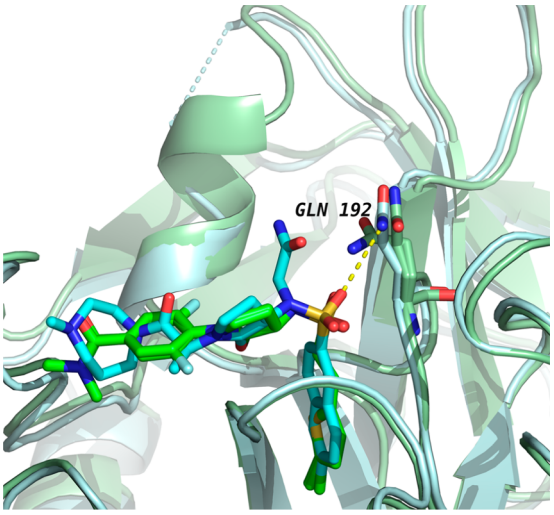

Figure 3. Superposition of the crystal structures FXA_gtc000401_2.07 (cyan) and FXA_gtc000101_1.61 (green) with their native ligands, **gtc401** and **gtc101**, respectively.

FXA, one for SYK, and one for TRMD) using one or several crystal structures of the target proteins that were used in Phase 1 and to provide a ranked list of ligands from each dataset, as well as the coordinates of the best pose for each ligand.

We used the docking software and scoring function identified previously, and a number of preliminary calculations were carried out, as described below, to find which protein conformer from those provided is the most appropriate for docking the datasets, which size of the binding site should be considered for docking, etc. An important point was the use of a search efficiency of 200% in Gold in order to improve the sampling of the ligands, which generally were big and rather flexible. Again, the knowledge gathered from the PDB complexes was used to evaluate the pertinence of the docking results, in addition to the score.

FXA. Three protein structures (FXA_gtc000101_1.61, FXA_gtc000398_1.86, and FXA_gtc000401_2.07) were provided, as well as three ligand datasets (set1, set2, and set3), containing 45, 67, and 51 molecules, respectively (see Supporting Information for the chemical structures of all these ligands). A careful analysis evidenced two main differences

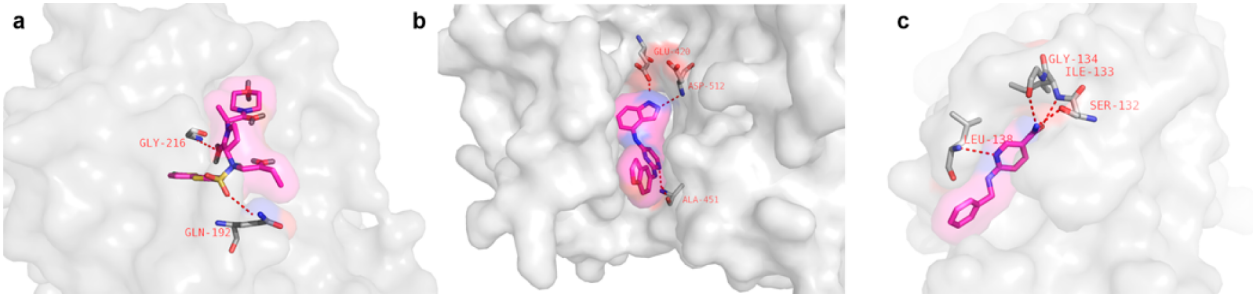

Figure 4. Representative interactions between the proteins FXA (a), SYK (b), and TRMD (c) and the ligands GTC000010A, GTC000111A, and GTC000444A, respectively. Protein surfaces are colored in gray, and the ligands are colored in magenta. Hydrogen atoms were omitted for clarity.

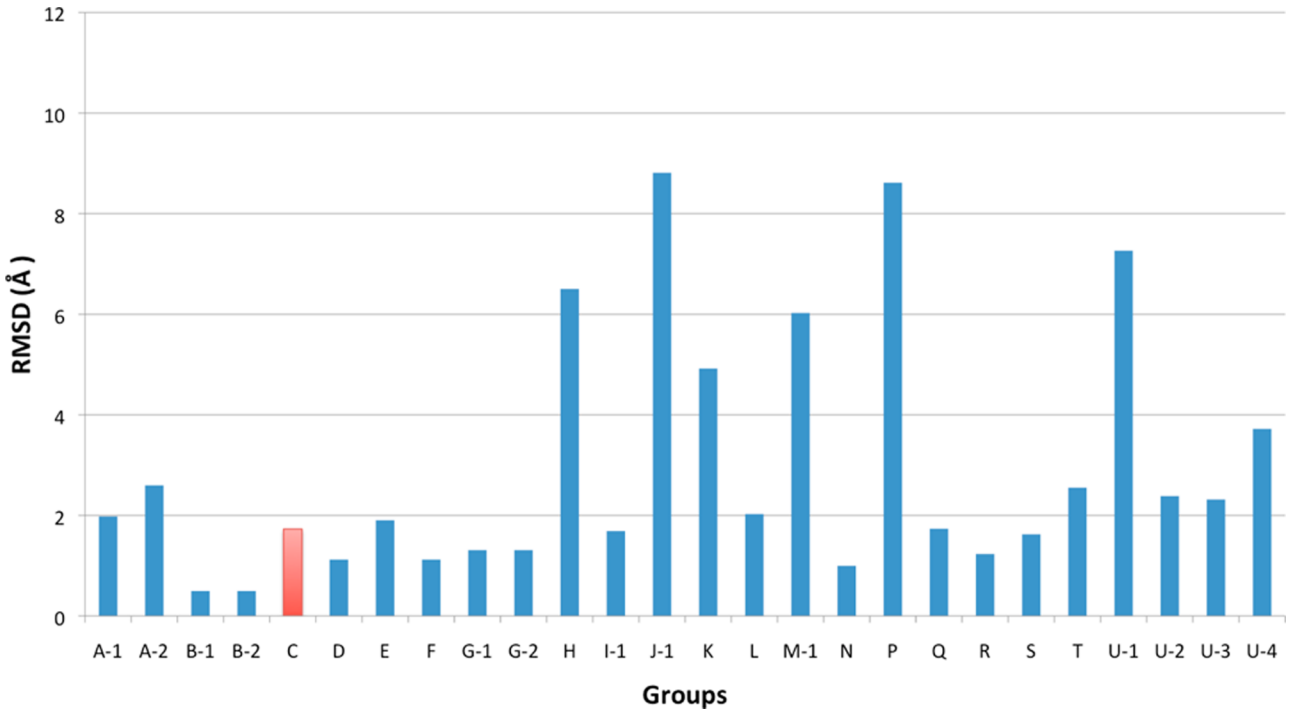

Figure 5. Mean RMSD of our prediction for FXA (1.728 Å) compared to the other predictions from CSAR 2014 Phase 2 (values calculated from one structure).

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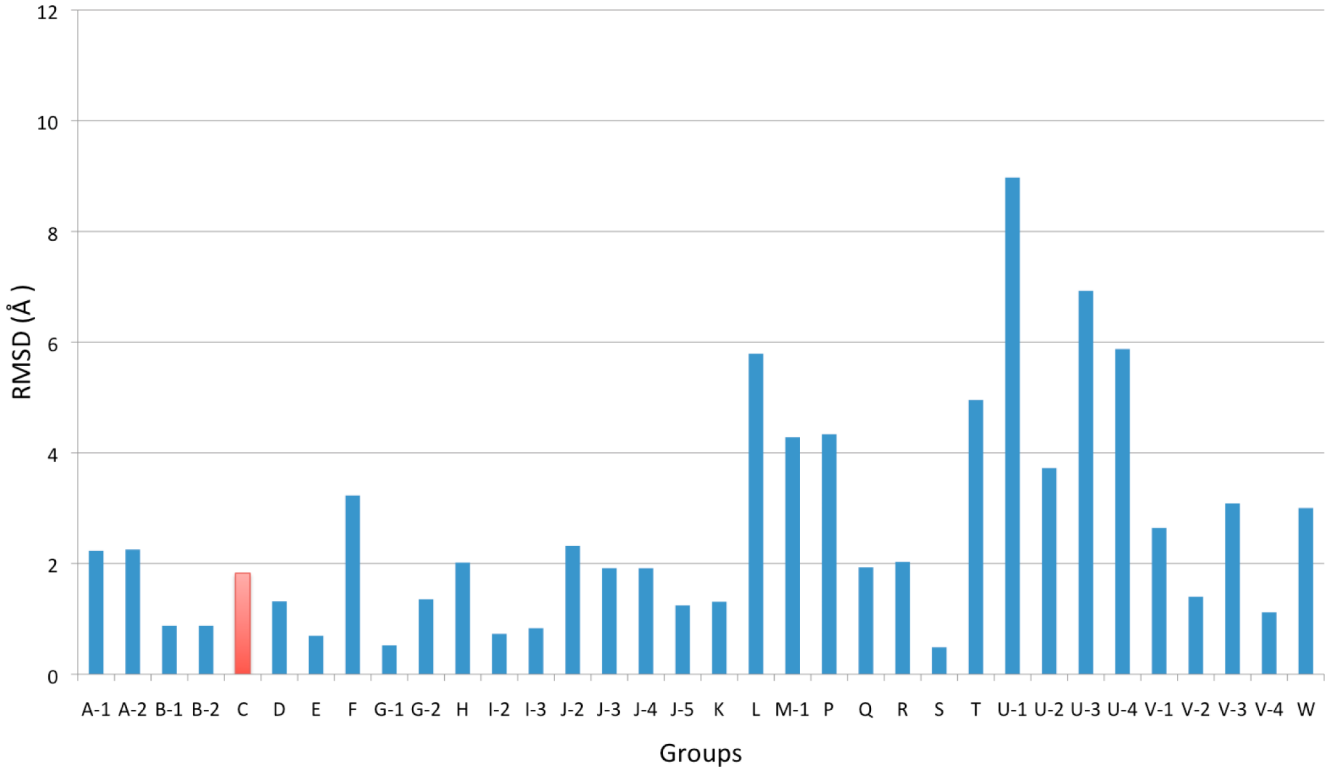

Figure 6. Mean RMSD of our prediction for SYK (1.826 Å) compared to the other predictions from CSAR 2014 Phase 2 (values calculated from five structures).

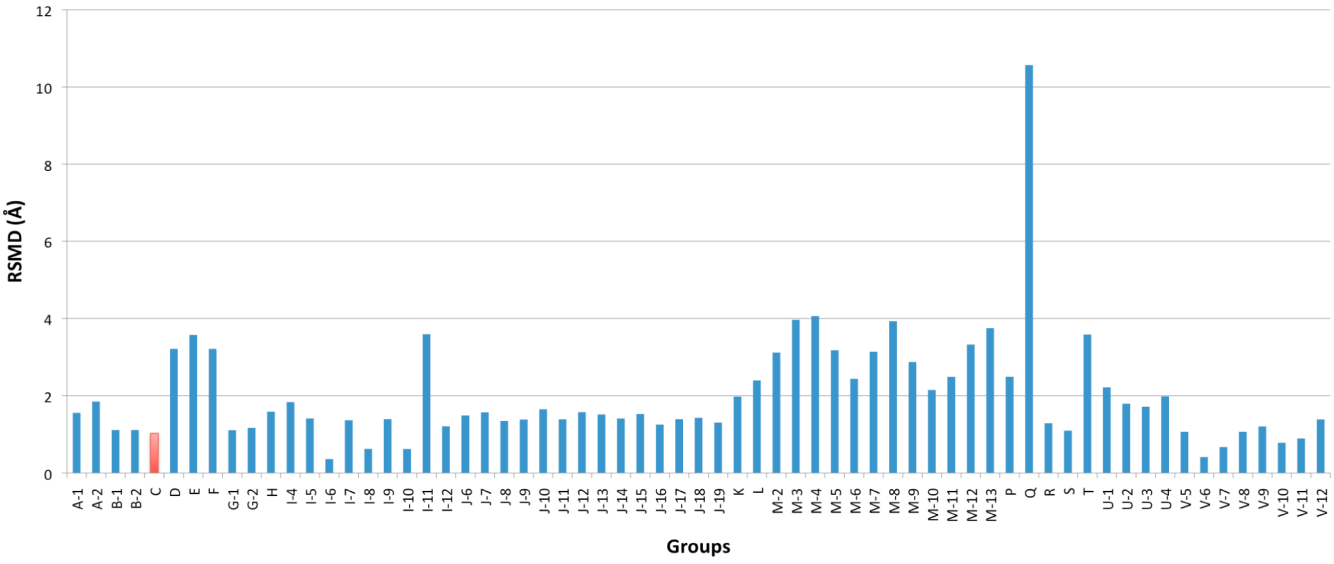

Figure 7. Mean RMSD of our prediction for TRMD (1.027 Å) compared to the other predictions from CSAR 2014 Phase 2 (values calculated from 14 structures).

among the protein structures: (i) the structure FXA_gtc000-401_2.07 had a missing loop, but this loop was not located in the vicinity of the binding site, and its absence would not affect the interaction with the ligands; (ii) the residue Gln192 shows two different conformations in all these structures. Analysis of the corresponding complexes with their native ligands showed similar interactions except a hydrogen bond between Gln192 and an oxygen atom from the ligand that is present only in the structure FXA_gtc000401_2.07, which was therefore used for docking the three ligand datasets (Figure 3).

The analysis of docking results showed in many cases two conserved hydrogen bond interactions, (i) between the backbone N of Gly216 and an amide carbonyl from the ligand and (ii) between the NE2 atom of Gln192 and a sulfonamide oxygen from the ligand (Figure 4a).

SYK. Five protein structures were provided (gtc000224_SYK, gtc000225_SYK, gtc000233_SYK, gtc000249_SYK, and gtc-000250_SYK) and a single ligand dataset containing 275 molecules (see Supporting Information for the chemical structures of these ligands). In order to choose the most appropriate protein

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Table 2. Number of Contacts and RMSD for our CSAR 2014 Phase 2 Pose Prediction

			no. of contacts submitted			no. of reference contacts		
protein	ligand	RMSD	hetero−hetero	carbon−carbon	total	hetero−hetero	carbon−carbon	total
FXA	GTC000101A	1.728	12	42	54	12	31	43
SYK	GTC000224A	3.502	10	38	48	12	37	49
	GTC000225A	1.724	8	33	41	9	35	44
	GTC000233A	2.169	8	32	40	16	22	38
	GTC000249A	0.676	6	32	38	9	27	36
	GTC000250A	1.062	8	44	52	9	32	41
TRMD	GTC000445A	0.394	7	16	23	6	15	21
	GTC000446A	0.749	3	23	26	2	20	22
	GTC000447A	0.548	3	22	25	2	22	24
	GTC000448A	0.936	13	25	38	12	21	33
	GTC000451A	1.686	8	52	60	4	37	41
	GTC000452A	1.652	7	29	36	6	25	31
	GTC000453A	1.681	8	38	46	6	25	31
	GTC000456A	0.797	8	16	24	8	10	18
	GTC000457A	0.893	5	24	29	6	26	32
	GTC000458A	1.111	8	27	35	6	27	33
	GTC000459A	0.968	9	25	34	8	20	28
	GTC000460A	0.866	8	19	27	7	25	32
	GTC000464A	1.339	7	17	24	8	21	29
	GTC000465A	0.758	7	11	18	6	12	18

Table 3. Performance of the CSAR 2014 Phase 2 Affinity Ranking Prediction

dataset	N	R^2 [a]	ρ [b]
FXA_1	45	0.001487	0.0954
FXA_2	67	0.005863	−0.1382
FXA_3	51	0.015903	−0.0734
FXA_all	163	0.003506	−0.1647
SYK	276	0.070026	0.3153
TRMD	31	0.349394	0.4698

[a] R^2 is the squared Pearson linear-regression correlation coefficient. [b] ρ is the Spearman-rho rank-order correlation coefficient.

structure for docking, those presenting atoms with occupancies lower than 0.5 in the vicinity of the binding site were discarded, and the remaining were compared (RMSD) with the structures containing 24 ligands that were identified in the preliminary analysis step. Both SYK_233 and SYK_250 correspond to these criteria (see Figure 4b for a representative example). Redocking of the native ligands with Glide (SP scoring function) and Gold (GoldScore, ChemScore, ChemPLP, and ASP scoring functions) showed that generally all scoring functions behaved well on SYK_233, but the GoldScore scoring functions provided the best results: less than 1 Å RMSD for SYK_233 and about 4 Å RMSD for SYK_250. Therefore, the docking calculations were carried out with SYK_233 and the 275 ligands from the dataset, using Gold and GoldScore scoring function.

TRMD. Fourteen protein structures were provided, together with a ligand dataset containing 30 molecules. Preliminary docking studies showed that only the structure TRMD_447_ dimer, which has a more open binding site conformation, was able to accommodate the rather bulky molecules from this dataset (Figure 4c).

Overall, our approach afforded docking pose predictions with average RMSD values less than 2 Å for FXA (Figure 5) and SYK (Figure 6), and about 1 Å for TRMD (Figure 7), which corresponds to a position in the first third of the predictions submitted for the Phase 2 of the 2014 CSAR Benchmark Exercise. The detailed information corresponding to each individual structure is provided in Table 2.

However, the ranking of ligands from the five datasets (Table 3) was more problematic. Only the TRMD dataset showed acceptable values (0.35 and 0.47, respectively) for the squared Pearson linear-regression correlation coefficient (R^2) and for the Spearman-rho rank-order correlation coefficient (ρ). The factors that would explain this important discrepancy between scoring, pose prediction, and affinity ranking are currently under investigation.

CONCLUSIONS

The protocol that we used for the 2014 CSAR Benchmark Exercise involved a preliminary analysis of the structural information available in the PDB for the protein targets of interest and the rescoring of several docking conformations datasets, as well as pose prediction and affinity ranking. The two key points for this study were (i) the prior evaluation of docking software that are most adapted for each target and (ii) the increased search efficiency during the docking process to better explore the conformational space of big and flexible ligands, at the cost of extra computational time.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jcim.5b00337.

PDB structures that were used for the preliminary analysis, as well as the chemical structures of all compounds included in the five data sets from Phase 2 (PDF)

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Notes

The authors declare no competing financial interest.

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