Steering Protein−Ligand Docking with Quantitative NMR Chemical Shift Perturbations

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Lead optimization benefits from including structural knowledge of the target. We present a new method that exploits quantitatively NMR amide proton chemical shift perturbations (CSP) on the protein side for protein−ligand docking. The approach is based on a hybrid scoring scheme consisting of a weighted sum of DrugScore, describing protein−ligand interactions, and Kendall's rank correlation coefficient, which scores ligand poses with respect to their agreement with experimental CSP data. For back-calculating CSP for a ligand pose, an efficient empirical model considering only ring-current effects is applied. The hybrid scoring scheme has been implemented in AutoDock. Compared to previous approaches, the rank correlation provides a measure that is more robust against the presence of outliers in back-calculated CSP data. Furthermore, our methods exploit CSP information at docking time and not for postfiltering, resulting in an enhanced generation of native-like solutions. As we exploit CSP information quantitatively, the experimental information effectively contributes to orient the ligand in the binding site. When applied to 70 protein−ligand complexes with computed CSP reference data, the docking success rate increases from 71%, if no CSP information is used, to 99% at the highest CSP weighting factor tested. Global optimization, thus, performs satisfactorily on the hybrid docking energy landscape. We next applied the approach to three test cases with experimental CSP reference data. Without CSP information, neither of the complexes is successfully docked. Including CSP information with the same CSP weighting factor, as determined above, leads to successful docking in all three cases. Only then native-like ligand configurations are generated at two of the three complexes. Binding site movements of up to 2 Å are found to not deteriorate the docking success. The approach will be particularly important for protein−ligand complexes that are difficult to predict computationally, such as ligands binding to flat interface regions.

INTRODUCTION

Incorporating structural information of the target into lead optimization efforts results in superior success rates, particularly when it comes to enhancing lead potency.[1] X-ray crystallography and NMR are the key techniques to obtain protein−ligand complex structures, but their applicability is strongly case dependent. Even more, traditional uses of any of these techniques incur high costs in terms of time and resources.[2] Advances in X-ray crystallography and NMR techniques have expanded the range of tractable targets along with improving the overall throughput.[3] Of particular interest are developments in the field of NMR for cases where the structure of the target protein is already known. These developments have been fuelled by an improved theoretical understanding of the underlying physical phenomena and by increasingly available computer power. Depending on target sizes, labeling schemes, and binding regimes, one can choose from a variety of methods where different observables from the ligand and/or protein side (e.g., (CSP), nuclear Overhauser effects (NOEs), residual dipolar couplings, and cross-correlation rates) feed sophisticated algorithms to provide information about protein−ligand complexes. The derived information ranges from interacting surfaces, ligand conformations, and binding epitopes to detailed complex structures.[4,5] Chemical shifts, or, in the case of protein−ligand complexes, CSP are the most ubiquitous observables under any condition, serving as a starting point to assign and interpret spectra. However, paradoxically, applications directly exploiting them are comparatively underrepresented among these new developments.[6,7] Historically, the first remarkable use of CSP in structure-based drug design is the SAR-by-NMR technique.[8] Here, low affinity, small molecular fragments are used to explore the binding site of the target protein. By monitoring which fragments produce the largest CSP on which protein residues, fragments can be matched with favorable binding regions. The data are then used to combine fragments into new compounds, which are potentially better binders.

Even more, the quantitative use of CSP information for structure elucidation is only starting to be reported.[9−11] Two main reasons can be identified. On the one hand, CSP are extremely sensitive markers of noncovalent interactions; on the other hand, they provide comprehensive information about an atom's complex chemical environment[12] and, as such, are rather difficult to interpret structurally. Phrased differently, it is not as straightforward to infer the orientation of interacting partners from CSP as it is, e.g., in the case of NOEs, which can be directly translated into interatomic

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distances.[13] However, an increased theoretical understanding and an improved modeling of the phenomena leading to CSP by quantum and empirical methods have opened an avenue for exploiting quantitative CSP information.[7]

The so-called ring current effect generated by aromatic rings in ligands accounts for the largest contribution of the observed CSP on the protein, if no dramatic protein rearrangements occur upon binding. Aromatic rings occur frequently in known drugs, with benzene being the most common.[14] For this reason, aromatic rings are valuable chemical fragments for deducing a ligand orientation with respect to the protein, by means of analyzing CSP quantitatively. To the best of our knowledge, McCoy and Wyss have been the first to follow this route for the case of protein−ligand complexes.[15] (Studies of other types of supramolecular complexes, such as host−guest complexes, exploiting quantitative CSP have been recently reviewed by Hunter et al.[10]). The authors used the program SHIFTS[16] for predicting CSP of calmodulin protons that a tryptophane probe would induce from different pregenerated orientations. Comparing the predictions with reference experimental values, they found that the largest agreement occurred for orientations closest to the native one of the original complex. They successfully applied the same idea in a subsequent study for developing new hepatitis C virus NS3 protease inhibitors.[17] Recently, a similar approach has been implemented in a program called SDILICON,[18] which was applied in a structure-based design of allosteric inhibitors of leukemia-associated proteins Runx1 and CBFβ.[19]

Encouraged by these studies, we anticipated that it is possible to go beyond current uses of CSP information in terms of qualitative analysis[8,20] and/or post filtering of already generated protein−ligand configurations.[15,20] Instead, we intend to actually exploit quantitative amide proton (HN) CSP information to guide protein−ligand docking. We expect a 2-fold benefit in this case: (i) Considering experimental restraints will lead to an increased sampling of native-like conformations during the docking. Failure to sample near-native conformations has been recently identified to be a major cause for failure of scoring functions to identify native-like conformations.[21] (ii) Augmenting a scoring/docking function with experimental restraints will help to overcome deficiencies even present in the latest scoring schemes,[22] thus, improving their predictive power with respect to native-like ligand poses. Our goal has been to devise a new hybrid scoring function for docking (E_{hybrid}, eq 1) that combines standard scoring by the DrugScore function[23] (E_{DS}) with a new energy term that maximizes the quantitative agreement between experimental and computed (eq 2) CSP (E_{QCSP}):

$$E_{\text{hybrid}} = E_{\text{DS}} + \omega_{\text{QCSP}} E_{\text{QCSP}} \quad (1)$$

E_{QCSP} is the negative Kendall's rank correlation coefficient[24] (eq 3) between experimental and computed quantitative CSP (QCSP) for a given ligand pose. The rank correlation is a robust statistic measure that compares favorably to scoring schemes based on minimizing squared deviations between experimental and computed CSP[9,15] that are highly sensitive to the presence of outliers in the back-calculated CSP data. That way we also circumvent the necessity to account for the degree of protein saturation by a bound ligand, which may vary from complex to complex and influence the magnitude of CSP. ω_{QCSP} is a weighting factor yet to be determined. The computation is done considering a model where ring-current effects are the only source, as it performed best in our hands in preliminary tests. Additionally, it is also known that ring currents account for the largest effect on CSP and produce a well-defined pattern of perturbations.[25]

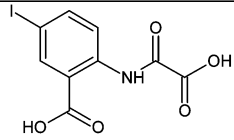
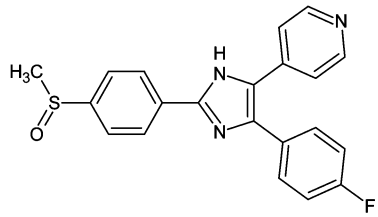
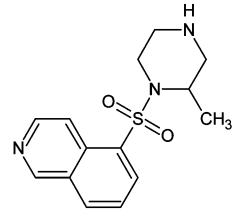
To determine the most appropriate weighting factor ω_{QCSP} and to assess the predictive power of our approach, we followed a three-step procedure: (i) Docking based on DrugScore only fails if solutions with more than 2.0 Å rmsd from the native structure[26] are better scored than the native or a native-like geometry. These solutions are called "geometric decoys" or simply decoys. To "rescue" such dockings, the QCSP-based scoring term must favor native-like configurations over decoys. To be more specific, the DrugScore energy gap between native-like and decoy conformations must be compensated by the E_{QCSP} contribution. Consequently, we first set out to determine the magnitude of the energy gaps between native-like and decoy configurations for both the DrugScore and the QCSP terms. We based this analysis on those 70 out of 85 complexes from the Astex diverse set[27] that contain ligands with aromatic rings. Since no experimental QCSP information for these complexes is available, we computed the CSP reference data from the native complex structures in analogy to the study of Stark and Powers.[20] (ii) From the analysis of the energy gaps, we propose three different values for ω_{QCSP}, considering the maximum gap in the E_{DS} term and tentative cut-offs for the E_{CSP} contribution. (iii) Finally and more importantly, we evaluate the applicability of the results obtained with the computed CSP reference data to cases for which experimental CSP reference data are available. For this, we consider three protein-ligand complexes described in the work of Schieborr et al.[28] (Tables 1 and 2). Moving from computed to experimental CSP reference data, furthermore, allowed us to assess the impact of limitations of the model used to compute CSP, the receptor flexibility, and the extent and distribution of the CSP assignment on the docking success.

RESULTS AND DISCUSSION

Analyzing Energy Gaps between Native-Like Configurations and Decoys. DrugScore is one of the most reliable scoring functions for identifying[23] and generating[21] native-like docking solutions,[22,29] with success rates of about 75%. Still, docking based on only DrugScore may fail due to, e.g., not considering interactions to structural waters, wrongly assigned protonation states or missed entropy terms. Docking decoys then obtain a more favorable DrugScore score E_{DS} than native-like solutions, leading to an "inverse" E_{DS} gap. Incorporating experimental information in terms of a QCSP-based score is expected to lead to a successful docking if the E_{QCSP} contribution compensates the "inverse" E_{DS} gap. Determining an appropriate and generally applicable weighting factor ω_{QCSP} is, thus, key to the success of the hybrid scoring function (eq 1).

We anticipated that analyzing the differences in E_{DS} and E_{QCSP} contributions between decoys and native-like solutions, respectively, will lead to appropriate bounds for the weighting factor. We, therefore, studied the distribution of E_{DS} and E_{QCSP} gaps in a comprehensive data set, consisting of the 70

Table 1. Data Set Used for Validation

Ligand	PDB code[a]	Rmsd[b]
	1ecv (PTP1b)	0.73 (1pty)
	1a9u (p38)	1.01 (1p38)
	1ydr (PKA)	2.11 (1cmk)

[a] The protein name is given in parentheses. [b] Rmsd between the holo and apo structure, computed for all atoms of residues within 7 Å from the ligand in Å. The PDB code of the apo structure is given in parentheses.

Table 2. Summary of HN CSP Considered in the Calculations with Experimental CSP Reference Data

PDB code	exptl. CSP[a]	significant CSP[b]	CSP set to average[c]	discarded CSP[d]	CSP finally considered[e]	binding site[f]
1ecv	82	20	62	Asp-22, Phe-30, Val-34, Asp-53, Lys-58, Ile-57, Asn-68, Leu-204, Asp-236, Ser-242	72	7/21
1a9u	193	20	173	Gln-11, Phe-42, Arg-57, Asn-100, Gly-219, Ile-297	187	17/22
1ydr	137	14	123	Glu-64, Gln-84, Lys-111, Ala-304, Glu-346	132	11/30

[a] Total number of experimental HN CSP available. [b] Number of experimental HN CSP that deviate by more than one standard deviation from the average CSP over all HN nuclei. [c] Number of experimental HN CSP whose value was set to the average CSP over all HN nuclei. [d] Experimental HN CSP that were not considered in the calculation, although they deviate by more than one standard deviation from the average CSP over all HN nuclei. See Materials and Methods section for further explanation. [e] Number of CSP finally considered in the docking. [f] Number of HN within 7 Å of the ligand in the native structure that have CSP assigned, and total number of HN within 7 Å of the ligand in the native structure.

protein−ligand complexes containing aromatic rings out of 85 complexes of the Astex diverse set.[27] As detailed in the Materials and Methods section, for each complex we performed 100 independent docking runs, using only DrugScore as a scoring function. Geometrically similar ligand configurations were clustered together. If the solution with the lowest energy in the largest cluster had an rmsd to the native structure of <2.0 Å, then we considered the docking successful. Independent of this success/failure classification, we considered a docking a "decoy generator" if cluster(s) of solutions were generated with a, on average, better DrugScore score than the native-like solutions. Accordingly, we divided the 70 complexes into three groups: (i) those which did not generate decoys (i.e., the first ranked cluster of solutions consists of native-like configurations); (ii) those which generated decoys and native-like solutions; and (iii) those which generated only decoys. These results are summarized in the Supporting Information, Table S1.

For analyzing the E_{DS} gap between decoys and native-like solutions, we can only study the group of 13 cases (Supporting Information, group II, Table S1) for which both species were generated. Figure 1 shows the distribution of energy differences between the average energy of the best-scored cluster in each case containing decoys and the average energy of the cluster with the best average rmsd in the whole run. Interestingly, 46% of the cases have a low energy gap (<0.5 DrugScore units) with an average energy gap of 0.61 units and a maximum gap of 1.31 units.

For analyzing the E_{QCSP} gap, we needed to compute the CSP of HN protons induced by the bound ligand because no experimental CSP is available for the Astex diverse set (see Materials and Methods section). Given that the E_{QCSP} contribution is represented by the negative Kendall's rank correlation coefficient, native-like solutions should score approximately −1, whereas decoys should score 0 or more. Instead of an expected gap of the E_{QCSP} contribution of about 1 unit, however, an analysis of the group II cases for which both native-like solutions and decoys were generated revealed an average gap of only 0.36 with standard deviations for both decoys and native-like solutions of 0.20 and 0.29,

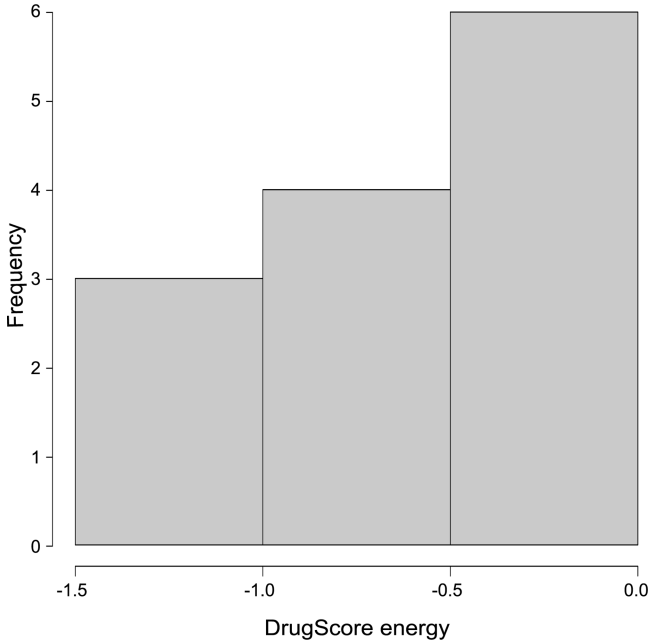


Figure 1. Distribution of DrugScore energy gaps between decoys and native-like solutions for the 13 cases where DrugScore-only docking generated both native-like solutions and decoys. In 46% of the cases, the energy gap is <0.5 DrugScore units with a maximum gap of 1.31 units.

respectively (Supporting Information, Table S2). A more representative analysis can be performed by expanding this sample to those cases where either no decoys or native-like solutions were generated (Supporting Information, groups I and III, Table S1). The distributions of the E_{QCSP} scores for these cases are shown in Figure 2. While 36% of the native-like cases scored above -0.8, only 7% of the decoy cases obtained E_{QCSP} contributions below -0.8. This underlines two points: (i) native-like solutions show poorer scores than expected; and (ii) E_{QCSP} scoring is more specific than sensitive; that is, it is better at rejecting decoys than at favoring native-like solutions, which is acceptable for our purposes. Finally, the fact that some native-like poses show poor E_{QCSP} scores reflects a "hard" scoring term character,[30] where slight changes in the orientation of a ligand, with respect to the native state, translate into large changes of the CSP pattern.

Weighting of the QCSP Contribution. The analysis of both the E_{DS} and E_{QCSP} contributions for decoys and native-like solutions leads us to suggest three alternative weighting factors:

(i) As E_{QCSP} gap, we consider the difference of the median values of decoys (0.26) and native-like solutions (0.85), which results in $\Delta E_{QCSP} \approx 0.6$. Since the sample for establishing the E_{DS} gap is much smaller, we take the maximum E_{DS} gap (1.31 units) plus one standard deviation (0.45), which results in $\Delta E_{DS} \approx 1.80$. The weighting factor to compensate an "inverse" E_{DS} gap by E_{QCSP} then results in $\omega_{QCSP} = 3$.

(ii) We consider different cut-offs for the E_{QCSP} gap in order to include 75% of the decoys (lowest scores; third quartile: 0.54) and 75% of the native-like cases (best scores; first quartile: 0.73), which results in $\Delta E_{QCSP} \approx 0.2$. Considering the same E_{DS} gap as in (i), we then set the weighing factor to $\omega_{QCSP} = 10$.

(iii) For the sake of completeness, we explore also an intermediate weighting factor of $\omega_{QCSP} = 5$.

Docking with Computed CSP Reference Data. Table 3 shows the percentage of docking successes for the 70 complexes from the Astex diverse data set using the hybrid scoring function (eq 1) and considering each of the weighting factors proposed above. Again, CSP of HN protons computed for the native ligand configurations were used as reference to determine E_{QCSP}. We considered docking results successful when the lowest energy solution in the largest cluster of poses has a rsmd to the native ligand pose of <2.0 Å. Compared to DrugScore-only docking (71% successful dockings), a large improvement in the docking accuracy is already achieved when $\omega_{QCSP} = 3$ is used for weighting the E_{QCSP} contribution (93% successful dockings). Thus, for the additional 15 cases that can now be docked successfully, the "inverse" E_{DS} gap between decoys and native-like solutions is sufficiently small, so that a small E_{QCSP} contribution suffices to compensate for it. An additional 6% of the cases is recovered when $\omega_{QCSP} = 10$ is applied. Importantly, no deterioration of cases is observed that already could have been docked successfully by DrugScore-only. Thus, the scoring optima coincide for both the E_{DS} and E_{QCSP} contributions in these cases. Only in one case docking was not successful (PDB code: 1gm8), even though hybrid scoring with $\omega_{QCSP} = 10$ now generated some native-like solutions for this complex.

Docking with Experimental CSP Reference Data. For the above results, CSP of HN protons computed from ligand-bound complexes were used. That way, conformational changes of the protein structure upon complex formation are neglected, which may influence the comparison between experimental and computed CSP. Likewise, the obtained CSP are not prone to experimental uncertainties and so can be perfectly reproduced with the empirical model. Finally, all HN CSP in the binding site region are available and used during docking, an unlikely situation in a real experimental setting. Thus, the high docking success rate when including this CSP information should not be surprising.

In order to investigate the behavior of the hybrid scoring function in "real-life" cases, we used three protein−ligand complexes for which there is experimental CSP data available (PDB codes: 1ecv, 1a9u and 1ydr; see Table 1). Of these three cases, only for 1ecv native-like docking solutions could be generated in a DrugScore-only docking. In addition, in all three cases, DrugScore-only docking tends to lead to poses that are maximally buried in the binding site. This behavior can be understood if one takes into account that the DrugScore lacks contributions from configurational and vibrational entropy changes upon binding, such that poses that maximize the number of contacts with the protein usually lead to better scores (see Figure 3A, D, G).

The above docking experiments with the computed CSP reference data suggest that $\omega_{QCSP} = 10$ should be an appropriate weighting factor, in general. In fact, when redocking the three complexes with experimental CSP reference data using $\omega_{QCSP} = 10$, average rmsd values of poses of the largest cluster, with respect to the native structure, of 1.36 and 1.13 Å were obtained for 1ecv and 1a9u, respectively (Table 4). In contrast, for 1ydr, successful docking with rmsd $\approx$ 0.8 Å required lower ω_{QCSP} values of 3 or 5. This indicates that overweighting experimental CSP reference data can lead to a deterioration of docking success, in contrast to what we found for docking with computed

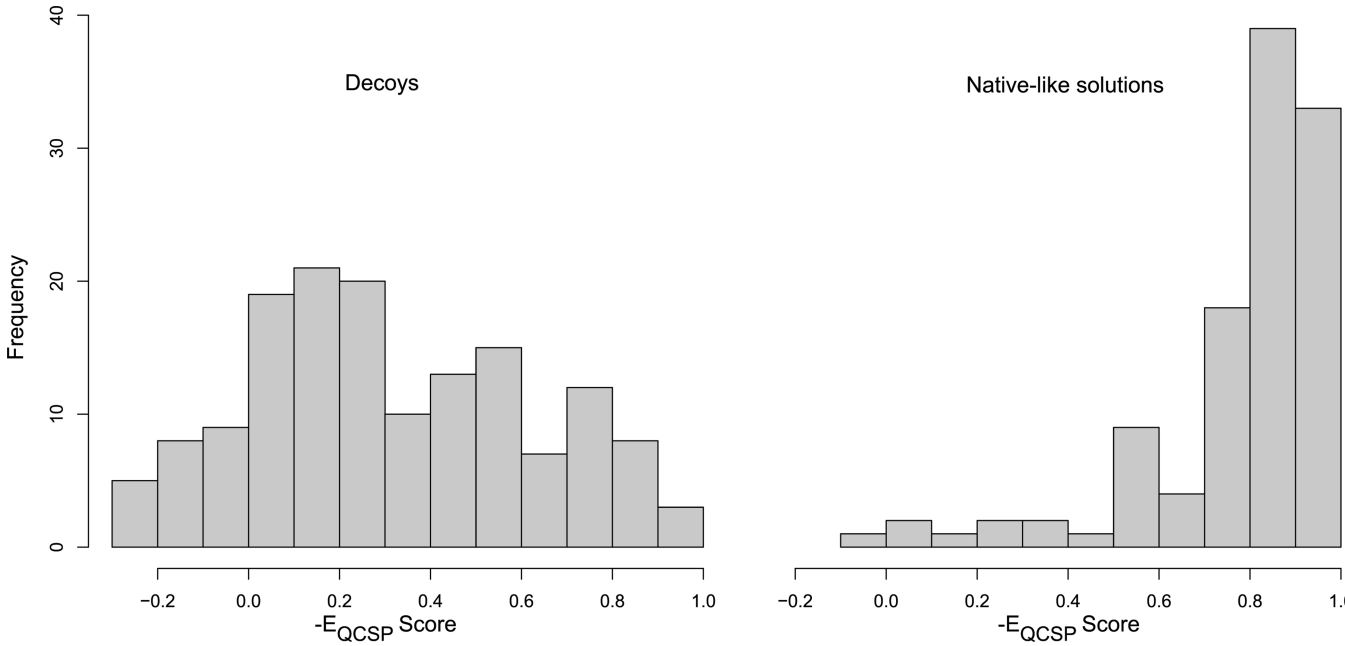


Figure 2. Distribution of the E_{QCSP} contributions for decoys and native-like poses, considering the subset of the ligands with aromatic rings. The CSP data for the bound ligand configuration were computed. Even if only 64% of the native-like solutions show very good E_{QCSP} contributions (<-0.8), it is reassuring to observe that only 7% of the decoy cases do so too, which yields E_{QCSP} as a very specific scoring scheme for rejecting decoys.

Table 3. Docking Results for the 70 complexes of the Astex Diverse Set Containing Ligands with Aromatic Rings as a Function of the Weighting Factor ω_{QCSP}

	successful[a]	unsuccessful, native-like[b]	unsuccessful, no native-like[b]
No QCSP	50 (71%)	13 (19%)	7 (10%)
$\omega_{QCSP} = 3$	65 (93%)	3 (4%)	2 (3%)
$\omega_{QCSP} = 5$	67 (96%)	2 (3%)	1 (1%)
$\omega_{QCSP} = 10$	69 (99%)	1 (1%)	0

[a] A case is considered successful if the solution with the lowest energy in the largest cluster has an rmsd to the native structure of <2.0 Å. Values in brackets indicate the percentage of such cases. [b] For the unsuccessful cases, we also distinguish those that at least generate a native-like solution from those that do not.

CSP data. The requirement to use lower ω_{QCSP} values points to the problem of overemphasizing the influence of imperfect experimental CSP reference data and/or neglecting shortcomings in the computational model used for back-calculating CSP from a given ligand pose. We, thus, set out to investigate in more detail the factors that influence the performance of the hybrid scoring scheme in the case of experimental CSP reference data. We identified as the most important determinants the hydrogen bond formation, the extent and distribution of the CSP assignment, and the flexibility of the target. The latter also includes structural differences of the NMR- and X-ray-determined protein structures.

Hydrogen-Bond Effect. In both the 1a9u and 1ydr cases, the ligand forms a hydrogen bond to a HN proton in the protein for which experimental CSP is available. The effect of a hydrogen bond formation cannot be modeled accurately with current empirical methods.[6,16,31,32] Accordingly, following preliminary tests, we decided to omit this effect when back-calculating CSP. In the case of 1a9u, omitting the hydrogen bond formed between the pyridine N and HN of Met-109 did not deteriorate the final docking result (Table 4). This is because back-calculating the CSP of HN of Met-109 is successful (Figure 3E, F) even with the ring current-only model, if the pyridine ring of the ligand adopts a slightly tilted and displaced conformation compared to that of the native structure. A similar observation was already described by McCoy and Wyss,[33] who pointed out that the typical downfield shift of a proton in a hydrogen bond can also be generated by a properly oriented aromatic ring in the proton's vicinity. We note, however, that such a fortuitous effect could not have been expected if the interaction had occurred in a narrower crevice, which does not allow ring tilting and displacement. This then would certainly compromise the docking accuracy, as demonstrated for 1ydr. Here, the ligand is forming a hydrogen bond with the HN proton of Val-123 (Figure 3G). At the same time, it is tightly constrained in the binding pocket. Thus, no evasive movements of the quinoline ring are possible that could allow for a compensation of the missing hydrogen-bond term when computing the CSP. As a result, a low E_{QCSP} score is obtained even for native-like solutions. Following the idea that no CSP contribution should be better than a wrong one, we repeated the docking without considering HN of Val-123 (Table 4). With an average E_{QCSP} score decreasing modestly but significantly from -0.07 to -0.11 for native-like solutions, a successful docking was now achieved with $\omega_{QCSP} = 10$. Thus, all three complexes with experimental CSP reference data were successfully docked using a uniform weighting factor $\omega_{QCSP} = 10$.

The limitation in describing CSP due to hydrogen-bond formation is well-known, and more work is needed on the theoretical side to improve on this. In the meantime, we propose to handle this limitation by omitting CSP induced by hydrogen-bond formation from the hybrid scoring scheme. These CSP can be identified by visually inspecting the experimental CSP pattern. Typically, a hydrogen bond is characterized by a large downfield perturbation, which can be of a similar magnitude as the one due to a nearby aromatic

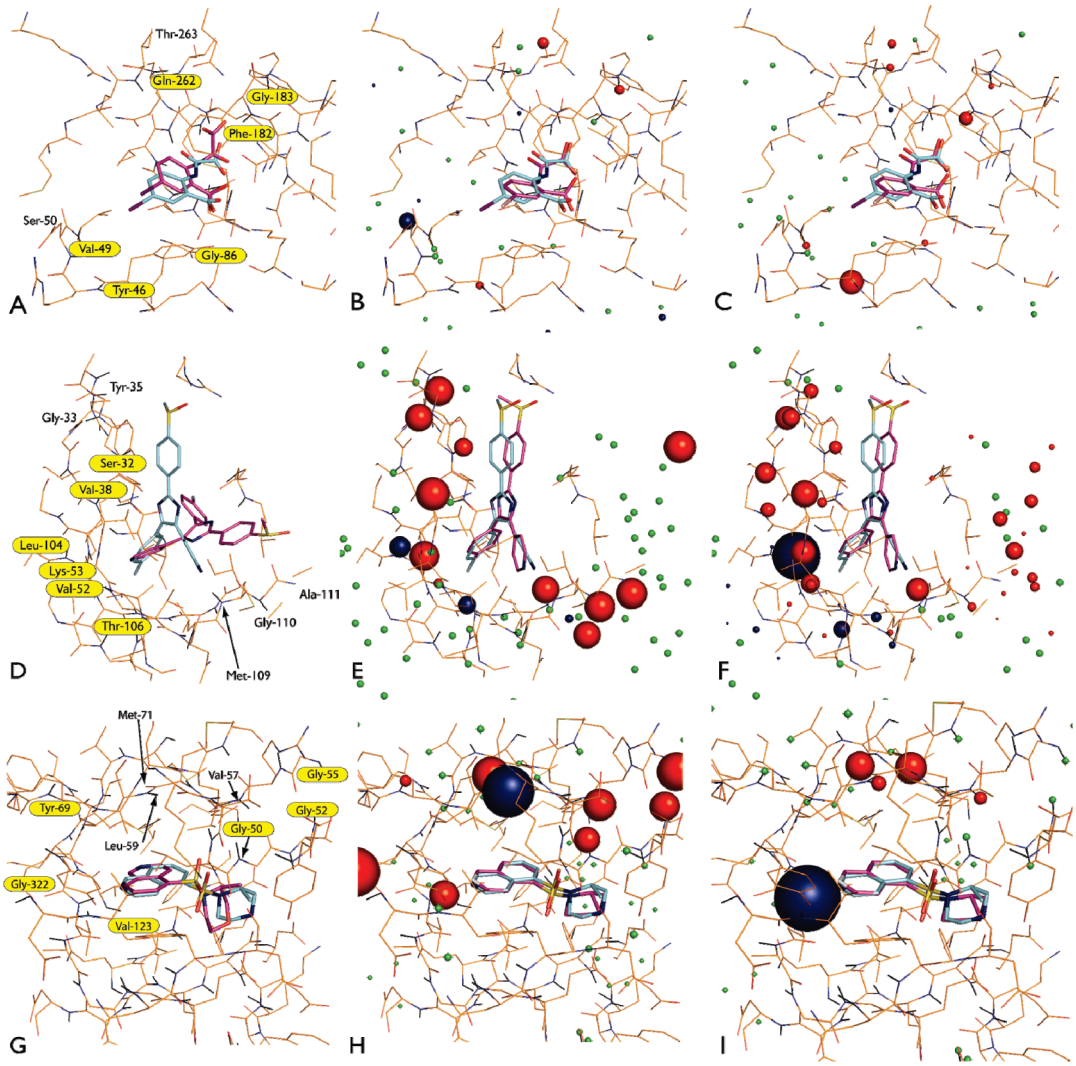


Figure 3. Comparison of docking solutions (magenta) with the crystal structure (cyan). In the first column (A, D, G), results using only DrugScore for scoring are presented. The second and third columns (B, C, E, F, H, I) display the best solutions obtained with QCSP-steered docking. Results for the complex 1ecv is given in panels A, B, C, 1a9u is in panels D, E, F, and 1ydr is in panels G, H, I. In the second column, experimental CSP are mapped to the protein, whereas in the third column, calculated CSP corresponding to the docked pose using the ring currents-only model are displayed. Blue balls represent upfield shifts (positive CSP), red balls represent downfield shifts (negative CSP), and green balls are used to mark CSP that have been assigned but do not experience any perturbation. The size of the blue and red balls is proportional to the magnitude of the CSP.

ring. However, a hydrogen-bond-induced CSP usually appears isolated and does not show the typical pattern of CSP on neighboring atoms that an aromatic ring would induce.

Extent and Spatial Distribution of CSP Assignment. The extent of CSP assignment of HN protons in the binding site area of the three complexes is 33 and 37% for 1ecv and 1ydr, respectively and 75% for 1a9u. Despite the comparable extent of HN CSP assignments, the highest E_{QCSP} values found for native-like solutions of the first two complexes differ considerably (1ecv: −0.34; 1ydr: −0.11, omitting the HN involved in a hydrogen bond). This difference can be explained by the spatial distribution of the CSP assignment. In the case of 1ecv, the CSP distribute uniformly around the binding site and so capture the traits of the ring-current pattern (Figure 3B). On the contrary, for 1ydr, the assignment excludes a large part of the adenosine-binding pocket surface,[34] which leads to increased minimal E_{QCSP} scores, as the ring-current pattern lacks key reference points in space (Figure 3H). Thus, in the case of sparse experimental CSP data, it is the distribution of the CSP rather than the amount of data per se that determines the success of QCSP-steered docking.

Flexibility of the Target. In all three studied cases, the proteins undergo rearrangements upon ligand binding with rmsd of the binding sites between bound and unbound conformations ranging from 0.73 Å in the case of 1ecv and 1.0 Å in the case of 1a9u to 2.1 Å in the case of 1ydr. Protein rearrangements are challenging for properly back-calculating CSP in a rigid protein docking scheme because aromatic ring movements in the protein can produce CSP as large as those induced by a ligand. Rearrangements of solvent molecules contribute to CSP, too. At present, our hybrid scoring scheme assumes that most of the observed effects can be directly attributed to the ligand as a source, and that this proportion suffices to orient the ligand in the native-like position. If the ligand is the major source of CSP, E_{QCSP} scores should be approximately −1 for native-like poses. In turn, an increase of the minimal E_{QCSP} score is expected with

Table 4. Summary of Docking Results for Three Complexes Where Experimental CSP Reference Data Were Available[a]

weight[b]	LC size[c]	rmsd[d]	τ[e]	% native-like[f]
		1ecv		
0	37	2.13	0.10	57
3	60	1.84	0.13	70
5	37	1.80	0.14	78
10	51	1.36	0.34	87
		1a9u		
0	96	4.86	0.00	0
3	76	4.80	0.06	1
5	59	4.80	0.07	1
10	42	1.13	0.25	42
		1a9u - no H-bond		
3	81	4.83	0.04	0
5	70	4.82	0.04	1
10	67	1.06	0.22	67
		1ydr		
0	100	3.12	−0.16	0
3	72	0.75	0.07	72
5	61	0.81	0.07	61
10	56	10.10	0.24	0
		1ydr - no H-bond		
3	80	0.76	0.11	80
5	98	0.81	0.12	98
10	54	0.84	0.12	54

[a] Results are presented for the proposed weighting factors ω_{QCSP} = {3,5,10} in addition to DrugScore-only docking. [b] The weighting factor ω_{QCSP}. DrugScore-only docking corresponds to weight 0. [c] Size of the largest cluster of solutions. [d] Average rmsd of the poses of the largest cluster with respect to the native crystal structure in Å. [e] Average Kendall's correlation coefficient of the poses in the largest cluster. [f] Percentage of native-like solutions among the whole pool of 100 generated solutions.

increasing protein movement, and this is what is observed for the three experimental cases studied here (Table 4). Too large protein movements will, thus, render the model for back-calculating CSP insufficient. Nonetheless, even movements up to 2.1 Å rmsd are still tolerated in the case of 1ydr. In general, we note that target flexibility is not a limitation for the hybrid scoring scheme described here. We believe that using CSP information can be very helpful in a fully flexible protein−ligand docking scenario because protein refinement against such data has been shown to improve structural quality,[35] and one ought to expect the same for a complex structure.

It is also interesting to note that not only protein movements upon ligand binding can deteriorate the accuracy of CSP computation but also structural differences of protein structures originating from different experimental sources or conditions. We illustrate this point by the HN proton of Lys-53 of 1a9u. The HN proton experiences a downfield shift in experiment, whereas our approach predicts an upfield shift for the native-like solution. The latter corresponds to a position of the proton in or close to the plane of the aromatic ring. In the native structure, we find that the proton is almost coplanar to the ring, too. Accordingly, the computed CSP for this structure also shows an upfield shift. The experimental downfield shift can be reproduced properly if an alternative crystal structure of the same complex (PDB id: 2ewa) is used, which differs slightly in the mutual orientation of the ring and the HN of Lys-53 (Figure 4). Again, as in the case of the hydrogen bonds, local disagreements between

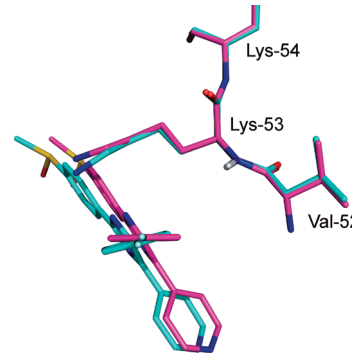


Figure 4. Superimposition of p38 complexes 2ewa (model 1) (magenta) and 1a9u (cyan). The angle between the ring plane and a vector from the ring center to the HN of Lys-53 differ by 12° (2ewa: 31°; 1a9u: 23°). This difference is sufficient to predict a downfield shift for 2ewa (CSP: −0.18) and an upfield shift for 1a9u (CSP: 0.07), using the empirical model implemented in our scoring function.

experimental and computed CSP can be compensated by a complete and/or a well-distributed CSP assignment. It is the global CSP pattern then that still allows the finding of native-like solutions (Figure 3E vs 3F).

Comparison to Related Methods. Recently, much attention has been turned toward using CSP for complex structure elucidation. Methods similar in spirit to ours that exploit protein CSP upon ligand binding are SDILICON,[18] an implementation of the seminal work of McCoy and Wyss,[15] LIGDOCK by Schieborr et al.,[28] and AutoDock-Filter by Stark et al.[20] NMRScore by Wang et al.[36] uses CSP information observed at the ligand instead, but otherwise shares similar ideas and goals. In the following, we compare these methods to ours and discuss their advantages and disadvantages, which eventually will define their scope and limitations.

The method by McCoy and Wyss has been already successfully applied in structure-based drug discovery programs.[17,19] Here, a CSP postfilter is applied to generated docking solutions. The filtering is done according to the correspondence between experimental and computed CSP for every configuration using SHIFTS[16] and considering only ring current effects. The main difference to our approach is that we use experimental information already during docking time, which furthers the generation of native-like poses by the docking engine. The authors also suggest using HA instead of HN protons because CSP of the latter are more difficult to predict. On the other hand, the experimental assignment of HA shifts is more demanding. Although we have not tested it because no experimental data was available, we see no reason why our approach could not satisfactorily work with HA data either. Receptor flexibility is neglected in the approach by McCoy and Wyss. A further refinement of this kind of approach has been presented recently by Cioffi et al.,[9] using GOLD[37] together with only a van der Waals scoring term for generating shape-complementary ligand poses. These poses serve as starting points for further optimization by minimizing the rmsd between experimental and back-calculated CSP, using an empirical model similar to the one described here. CSP information is, thus, used as a sophisticated postfilter. Unfortunately, the authors did not discuss the superiority of the method to standard GOLD docking.

LIGDOCK borrows ideas from the protein−protein docking program HADDOCK.[38] Ambiguous restraints[39] are defined between residues supposed to be in the interface of each interacting counterpart, based on CSP information. This reduces considerably the search space, favoring the convergence of the docking algorithm to successful solutions. In order to put the ideas into the protein−ligand context, LIGDOCK defines ambiguous restraints between the ligand atoms and the most perturbed protein residues. This can be seen as a semiquantitative use of CSP information; it ensures that the ligand is placed in the binding site. However, it was found difficult to correctly orient symmetric ligands, as was the case with the MAP kinase p38 complex (1a9u) also studied here. The quantitative use of CSP information pursued in our approach solved this problem because it leads to more specific orientation requirements of the chemical groups of the ligand. LIGDOCK can consider flexibility of residues in the binding site. Arguably, LIGDOCK calculations are more time-consuming and require a larger computational effort than our approach.

AutoDockFilter is based on the same principles as LIGDOCK because it follows the idea of applying ambiguous restraints. Here, however, a linear relationship between the ligand distance to an amide group and the observed CSP is assumed. This is a rather crude approximation because − although there is a linear dependency for each kind of perturbation source − different perturbation sources show different ranges. In terms of efficiency, AutoDockFilter shows an improvement over LIGDOCK because it uses AutoDock for generating ligand conformations, which is the most remarkable advantage. However, CSP information in AutoDockFilter is used for postfiltering leading to the same limitation as the approach by McCoy and Wyss: the postfiltering will fail if no native-like poses are generated upfront. Initially, the authors successfully tested the approach with artificially generated CSP data, as we did in the first part of this study. Then, they corroborated the results with experimental data for a single test case. It is noteworthy that only CSP on the protein surface are considered in this approach, which might be a limitation in cases where only a limited assignment is available and/or a ligand is symmetric.

NMRScore, unlike the previous approaches and the one described here, is based on the computation of CSP observed on the ligand side. The computation of the ligand CSP is done at the semiempirical level, which is more accurate but also computationally much more demanding. Hence, NMRScore can be used efficiently only in postfiltering. For some of the investigated cases, the CSP did to not suffice to distinguish between two possible orientations. In this case, additional experiments, e.g., NOE measurements, would have been needed to solve the ambiguity. In that sense, the problem is similar to the one arising for symmetric ligands in LIGDOCK but now originating from the ligand side. In our opinion, a combination of NMRScore with our approach would have solved the ambiguity, too. Thus, considering CSP from both the protein and ligand sides will have synergistic effects.

Comparing similar methods is not straightforward because small differences may determine the respective scope. Clearly, the goals of the project, e.g., higher throughput docking versus detailed structure refinement, and the data in hand will determine the choice of one method over another. Nonetheless, we believe that the method presented in this study will be appealing in most of the cases: (i) only NMRScore needs less experimental data; (ii) we expect our approach to be more accurate compared to methods exploiting CSP only qualitatively, as seen from a direct comparison to LIGDOCK; (iii) in terms of computational demand, it is probably the most efficient approach because it uses an empirical model; and (iv) from an operational point of view, it is highly integrated and automated.

CONCLUSION

We have developed a new method that exploits quantitatively experimental HN CSP on the protein side to effectively steer protein−ligand docking. To that end, we developed a hybrid scoring scheme consisting of the weighted sum of DrugScore (E_{DS}), describing protein−ligand interactions, and the Kendall's rank correlation coefficient (E_{QCSP}) for scoring ligand poses with respect to their agreement with experimental CSP data. Using the rank correlation, we circumvent the necessity to account for the degree of protein saturation by a bound ligand, which may vary from complex to complex and influence the magnitude of CSP. In addition, the rank correlation is a robust statistic measure, which compares favorably to scoring schemes based on minimizing squared deviations between experimental and computed CSP.[9,15] The problem with these scoring schemes is their high sensitivity to the presence of outliers in the back-calculated CSP data, which occur due to inaccuracies and simplifications in the empirical model used. The advantage of this can be seen, in particular, in the case of 1ecv, where the range of experimental CSP reference data values is only about half of the ranges observed for 1a9u and 1ydr (Figure 3B, E, H), yet docking using the CSP reference data still provides a native-like solution.

Here, an empirical model considering only ring-currents effects[16] was applied, as our method relies on the efficiency of back-calculating CSP for candidate ligand poses during docking. Compared to approaches at higher levels, the model shows a limited accuracy. Computations of HN chemical shifts are accurate to about 0.5 correlation coefficient as tested for multiple proteins.[16,40] We note that still higher inaccuracies are to be expected if chemical shift perturbations are considered, as this requires taking the difference between two numbers that are, on average, an order of magnitude larger than the expected result. By targeting the detection of patterns of CSP and the relative rank among them rather than predicting individual CSP values, we strived to accommodate the limited accuracy of the empirical model at the benefit of computational efficiency.

Initially, we determined an appropriate weighting factor ω_{QCSP}, using 70 protein−ligand complexes for which we computed CSP reference data. Since, in this case, no inaccuracies occur upon back-calculating the CSP and all amide protons in the binding site have a CSP value assigned, this represents an ideal case for testing the influence of the CSP contribution on the docking outcome. At the smallest weighting factor tested ($\omega_{QCSP} = 3$) a docking success rate of 93% was found, already 22% higher than DrugScore-only scoring. This rate improved to 99% at the largest weighting factor tested ($\omega_{QCSP} = 10$). In total, this part of the study revealed that, despite the E_{QCSP} contribution being a "hard"

scoring term, global optimization performs satisfactorily on the combined E_{DS}/E_{QCSP} docking energy landscape.

We then applied the hybrid scoring scheme to three cases for which experimental CSP reference data was available. Using DrugScore only, neither of the complexes could be successfully docked. Including the E_{QCSP} contribution with a uniform weighting factor $\omega_{QCSP} = 10$ led to successful docking in all three cases, provided that the HN involved in hydrogen bonds with the ligand are not considered in the hybrid scoring scheme. It is also worth noting that only when E_{QCSP} was included, native-like solutions were generated for 1a9u and 1ydr. This demonstrates the importance of CSP information for guiding the docking algorithm: had the E_{QCSP} contribution not been considered during docking but only as part of a postfiltering approach,[15,20] no native-like conformations would have been identified. When analyzing additional factors that influence the docking success, it was encouraging to see that binding site movements of up to 2 Å did not deteriorate the docking success when considering CSP information. Furthermore, an extent of CSP assignments of HN protons in the binding site region below 40% can still be tolerated, if the CSP are rather uniformly distributed.

Using only three cases with experimental CSP reference data is insufficient to statistically demonstrate the advantage of one approach over another. Still, we believe that our hybrid scoring scheme is advantageous compared to that of other recently published approaches. On the one hand, our approach uses the experimental CSP information at docking time and not as a postfilter. Our approach, thus, results in an enhanced generation of native-like solutions. On the other hand, as our method uses CSP quantitatively, the experimental information effectively contributes to orient the ligand in the binding site. In contrast, methods that use CSP only qualitatively during docking are capable to place the ligand in the binding site but then need to rely on the underlying docking function to identify a native-like pose.

There are limitations that need to be addressed in future work. First, protein flexibility is not considered, although this limitation is due to the underlying docking engine rather than the hybrid scoring scheme. Second, our approach is limited to ligands where aromatic rings are present, which is the case for >95% of the compounds in the MDL Drug Data Report database.[41] Finally, our method is expected to benefit from ongoing research in at least three areas: the improved empirical methods for predicting CSP of HN involved in hydrogen bonds,[32] the more efficient quantum chemical methods to calculate chemical shifts,[42] and the development of techniques for high-throughput assignment of proton chemical shifts,[43] including C_α atom protons, which can be more accurately computed by current empirical methods.

Finally, it is our opinion that our approach will be particularly relevant when it comes to dealing with protein−ligand complexes that are difficult to predict computationally. This is given if the ligand binds to a rather flat binding site,[44] e.g., a protein−protein interface or for docking of small fragments,[45] which are also known to be a challenge for classical docking functions.

MATERIALS AND METHODS

QCSP-Steered Docking. Our approach relies on AutoDock 3.0.5 as a generator of protein−ligand configurations using DrugScore potentials (E_{DS}) as a scoring function, as described before.[46] The original AutoDock code was extended to include the CSP information (E_{QCSP}) in a hybrid scoring scheme (eq 1). Practically, the docking proceeds as a standard AutoDock run, generating and evaluating potential protein−ligand configurations. For efficiency, only if a generated configuration has a favorable E_{DS} interaction energy, the E_{QCSP} contribution is computed. The total time required for a docking with our hybrid scoring scheme increases linearly by a factor of ∼3 with the number of aromatic rings considered. As an example, a single docking run of 1a9u (four aromatic rings) takes 14 min on an Intel Pentium D 2.80 GHz CPU, whereas docking without the CSP information requires ∼1 min.

Each docking experiment consisted of 100 independent runs with the following parameters: initial population: 150; termination criterion: 1×10^6 energy evaluations; mutation and crossover rates: 0.8 and 0.02, respectively; elitism: 1; local search frequency: 0.06; maximum iterations: 300. At the end, standard AutoDock clustering of solutions was performed using a rmsd tolerance of 1.0 Å. Clusters comprising less than three elements are discarded in order to account for some convergence in the solutions. Docking is considered successful if the solution with the lowest energy in the largest cluster has an rmsd of <2.0 Å to the native conformation. Additionally, unsuccessful dockings according to this criterion are further divided into two groups: those that generated native-like solutions and those that did not.

Computation of Protein HN CSP. CSP of amide protons in the protein were computed using the same empirical model used and implemented in other popular programs.[16,47−49] This model divides the observed chemical shifts of an atom into through-space and through-bond contributions.[6,7] Through-bond contributions originate from local conformation effects and play a major role for chemical shifts of ^{15}N and ^{13}C. Such effects are less dominant in the case of HN and HA chemical shifts, as these atoms are less buried in the structure. When a ligand binds noncovalently to a protein, the expected perturbation on the protein chemical shifts from the ligand is through-space in nature. Through-space effects contribute most to the observed CSP of hydrogen atoms, among which HN protons are the least buried ones and, accordingly, the most sensitive ones.[6] At the same time, HN protons are the easiest to assign in an NMR spectrum, overall making the HN proton the most valuable probe for our purpose. Assuming no conformational rearrangements of the protein due to binding, the CSP caused by the ligand on the protein protons can be calculated as:

$$\mathrm{CSP} = \Delta\delta_{rc} + \Delta\delta_{ef} + \Delta\delta_{m} \quad (2)$$

Here, $\Delta\delta_{rc}$ is the ring current contribution, $\Delta\delta_{ef}$ is the electric field contribution, and $\Delta\delta_{m}$ accounts for other magnetic contributions from anisotropy generating groups, such as double or triple bonds.[16] If aromatic rings are present, then the ring current usually contributes most to the observed CSP. It is also the most studied and best understood and parametrized effect. Previously, others relied only on the ring current contribution to orient ligands to the protein.[15] Here, the ring current contributions are computed using the Haigh−Mallion model as described in ref 16. Intensity parameters for the aromatic systems were taken from

Abraham et al.[48,50] However, since our CSP scoring scheme is based on a rank correlation, the intensity parameter is only critical when more than one ring is present. Preliminary tests showed that including the electrostatic contribution, as in SHIFTS (i.e., as a function of the projection of the electric field generated by the ligand in vacuum onto the N−H bond, neglecting (de)solvation effects[16,51]), compromised the accuracy of the method in some cases and, thus, was discarded. The magnetic contribution arising from anisotropy-generating chemical groups requires identification of such groups and corresponding parametrizations. Recently, Packer reported transferable parameters for some of these chemical groups.[52] Since these parametrized groups did not occur in our experimental data set, we did not consider this contribution, thus, leaving only the ring-current contribution.

Hybrid Scoring Function. The score of each ligand pose is the weighted sum of E_{DS} plus an E_{QCSP} score. The latter accounts for the agreement between the experimentally determined and computed CSP originating from a ligand pose. In a direct approach, the sum of squared differences between both the experimental and computed CSP can be used as an error function. Such a scheme is applied, e.g., by McCoy and Wyss.[15] However, this strategy is sensitive to the presence of outliers in the data. Such outliers are likely to occur in our case, given the assumptions on which the CSP computation is based and the inherent inaccuracy in computing CSP. In particular, we expect the method to perform poorly in cases where solvent effects play a role or a HN proton is involved in a hydrogen bond.[32] For this reason, we resort to the more robust Kendall's rank correlation coefficient for assessing the agreement between experimental and computed CSP.

Kendall's rank correlation coefficient (or Kendall's τ) is defined as[24]

$$\tau = \frac{4P}{n(n-1)} - 1 \tag{3}$$

where n is the number of experimental and computed CSP pairs, and P is the number of concordant pairs. A pair of experimental (x_i, y_i) and computed values (x_j, y_j) is concordant if $\mathrm{sign}(x_j - x_i) = \mathrm{sign}(y_j - y_i)$. The values of τ go from -1 (perfect ranking disagreement) to $+1$ (perfect ranking agreement) and pass through 0, which denotes an independence of rankings. Finally, we set $E_{QCSP} = -\tau$ to account for the fact that better agreement between experimental and computed CSP should result in more negative scores.

Data Set Description. Two different data sets were used in this study, the first one for analyzing E_{DS} and E_{QCSP} gaps and the second one for validation with experimental CSP reference data. As the first data set, a subset of the Astex diverse set[27] was chosen that contains ligands with aromatic rings (70 out of 85 complexes). The Astex data set has been assembled considering high quality structures from the PDB. Ligands are all drug-like and proteins are all drug-discovery or agrochemical targets. No target is represented more than once.

The experimental set comprises three protein−ligand complexes already studied by Schieborr et al.[28] (PDB: 1ecv, 1a9u and 1ydr) in the context of the development of a CSP-based method for solving protein−ligand complexes. The extent of CSP assignments in the binding site varies from 30−40% for 1ydr and 1ecv to 75% for 1a9u. This rather limited set results because we required three criteria to be fulfilled: (i) a crystal structure is available in the PDB for each case as a reference; (ii) the protein experiences only some rearrangement upon ligand binding. In fact, the rmsd between free and bound structures ranges from 0.73 Å in the case of PTP1b to 2.11 Å in the case of PKA, where the enzyme changes between "open" and "closed" forms (Table 1); and (iii) DrugScore-only docking fails to provide native-like solutions. Only in this case, including the E_{QCSP} score will allow improving the results.

Protein and Ligand Preparation for Docking. The Astex diverse set was obtained directly from http://www.ccdc.cam.ac.uk/products/life_sciences/gold/validation/astex_diverse/. Proteins in mol2 format were converted to PDB using Openbabel. The ligand-bound protein complexes used for the experimental validation were obtained from the PDB. In both cases, proteins were protonated with REDUCE.[53] This step is needed to determine the position of amide protons for the evaluation of E_{QCSP}; DrugScore only considers heavy atoms. DrugScore grids were calculated using a grid spacing of 0.375 Å. The potential values were then scaled as previously described.[46] Grids are centered on the binding site and covered a volume that extends at least 7 Å beyond any ligand atom in the native bound conformation. Ligands were converted to mol2 format with PRDRG.[54] Atom types and AM1-BCC partial charges were calculated and assigned using ANTECHAMBER.[55] In the case of 1ydr, an iodine atom in the ligand was substituted by bromine, as no potentials for iodine atoms were available in DrugScore. All rotatable bonds were defined as active torsions in the AutoDock context.

Ligands were visually inspected to detect aromatic rings. These groups were listed together with their corresponding intensities (see empirical model for CSP computation above) in an additional input file by referring to the atom numbers in the final AutoDock ligand file.

CSP Data Preparation. From the set of available HN CSP for each complex, values within ± 1 standard deviation from the average were considered as noise arising from the digital resolution of the spectrometer. Yet, these signals still provide some relevant information with respect to the orientation of the ligand because they indicate areas not affected by the binding. Thus, they were not discarded but reset to the average CSP value. Visual inspection of the location of large CSP was used to delimit the binding site area, thus, discarding large isolated CSP presumably originating from protein rearrangements. Table 2 displays the finally used experimental data for each complex.

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a patch file to be applied to AutoDock 3.0.5 by H.G. The DrugScore software is available from G. Klebe, Philipps-University, Marburg, upon request. For individual trials, an online version of DrugScore is available at http://www.agklebe.de/drugscore.

Supporting Information Available: Classification of cases according to the solutions generated by DrugScore-only docking, and E_{QCSP} scores for decoys and native-like solutions for complexes of the Astex data set. This material is available free of charge via the Internet at http://pubs.acs.org.

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