A New Concept for Multidimensional Selection of Ligand Conformations (MultiSelect) and Multidimensional Scoring (MultiScore) of Protein−Ligand Binding Affinities

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In this work, eight different scoring functions have been combined with the aim of improving the prediction of protein−ligand binding conformations and affinities. The obtained scores were analyzed using multivariate statistical methods to generate expressions, with the ability (1) to select the best candidate between different docked conformations of an inhibitor (MultiSelect) and (2) to quantify the protein−ligand binding affinity (MultiScore). By use of the docking program GOLD, 40 different inhibitors were docked into the active site of three matrix metalloproteinases (MMP's), yielding a total of 120 enzyme−inhibitor complexes. For each complex, a single conformation of the inhibitor was selected using principal component analysis (PCA) for the scores obtained by the eight functions SCORE, LUDI, GRID, PMF_Score, D_Score, G_Score, ChemScore, and F_Score. Binding affinities were estimated based on partial least-squares projections onto latent structures (PLS) on the eight scores of each selected inhibitor conformation. By use of this procedure, $R^2 = 0.78$ and $Q^2 = 0.78$ were obtained when comparing experimental and calculated binding affinities. MultiSelect was evaluated by applying the same method for selecting docked conformations for 18 different protein−ligand complexes of known three-dimensional structure. In all cases, the selected ligand conformations were found to be very similar to the experimentally determined ligand conformations. A more general evaluation of MultiScore was performed using a set of 120 different protein−ligand complexes for which both the three-dimensional structures and the binding affinities were known. This approach allowed an evaluation of MultiScore independently of MultiSelect. The generality of the method was verified by obtaining $R^2 = 0.68$ and $Q^2 = 0.67$, when comparing calculated and experimental binding affinities for the 120 X-ray structures. In all cases, LUDI, SCORE, GRID, and F_Score were included as important functions, whereas the fifth function was PMF_Score and ChemScore for the MMP and X-ray models, respectively.

Introduction

The ability to position a ligand in the active site of a protein (docking) and calculate the binding affinity (scoring) is a very important step in the process of structure-based drug design. The methods have to be fast, reliable, and accurate to be of any value in the screening of large databases. Docking programs based on various principles have been developed over the years, and while not yet perfect, their performance is improving.[1] Early docking programs treated both the receptor and ligand as completely rigid structures, which of course is a severe limitation of the method because the receptor-bound ligand conformation often is not known. In more recently developed docking programs, flexibility is taken into account in various ways. Some programs rely on the principle of building the ligand into the binding site by incrementally positioning fragments of the ligand. Others use simulated annealing to obtain the optimal ligand conformations, and still others use genetic algorithms.[1] These programs generally allow conformational flexibility of the ligand, while flexibility of the protein is more difficult to take into consideration. The importance of this feature is highlighted by the various examples of protein conformational changes upon ligand binding.[2] In short, the optimal docking procedure has to be fast, generate reliable ligand geometries, rank the ligand conformations correctly, and thereby, estimate the binding energy.

The concept of predicting protein−ligand binding affinities has been approached by different methods. Some of the methods are relatively simple and fast, while others are more computationally demanding but often more accurate in their predictions. The choice of scoring function relies on the amount of information one needs to generate. If the purpose is virtual screening of large databases, it is necessary to use fast methods, whereas accurate methods like free energy perturbation (FEP) would be of no use in that context.[3,4] In contrast, the knowledge-based methods are generally fast and perfectly suited for this purpose. However, these functions are generally less accurate, and therefore, the

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results obtained are often a compromise between speed and accuracy.

This work was performed as part of a project aiming to identify strong and selective inhibitors of matrix metalloproteinases (MMP's). MMP's belong to a very important enzyme family, which has been found to participate in various disease states. In the past few years, these enzymes have been the targets for extensive research concerning arthritis, cancer, and osteoporosis.[5] In these diseases, an imbalance is observed between MMP's and their natural inhibitors (TIMP's). Presently, 17 human MMP's are known, and they are believed to have different functions in relation to both health and disease states. It is not quite clear, though, which of these enzymes are responsible for the observed disease states and how they interact with each other biologically. Design of selective inhibitors for individual MMP's would provide a pharmacological tool to help understand the involvement of different members of this enzyme family in various diseases. In this context, the structural knowledge of the MMP's is very useful,[6] and in the search for selective inhibitors, it would be of outmost importance to be able to predict both ligand conformation and binding affinity. Besides the demonstration of the ability to select a reliable inhibitor conformation (MultiSelect), the selected MMP inhibitors are used for development of a method to quantify protein−ligand interactions (MultiScore) using multivariate statistical analysis. Most of this work was done with MMP's, but to evaluate the generality of the methods, other protein−ligand complexes with known three-dimensional structures were considered as well.

Background for the Development of Multivariate Selection

The main purpose of docking programs is to identify favorable conformations of the ligand, but the scoring functions implemented in the docking programs do seldomly perform satisfactorily to make quantitative predictions of the binding affinity.[7] Therefore, different scoring functions, based on various principles, have been developed in order to predict the binding affinity more precisely. Still, their performance relies on the ligand being correctly positioned in the active site, and so they strongly depend on the performance of the docking algorithm. Although the ranking of conformations could not be used directly as a measure of the binding affinity, it has been found that a ligand conformation corresponding closely to the bioactive conformation very often is found among the solutions suggested by the docking program.[7,8] Therefore, the challenge is to identify the most favorable ligand conformation and to estimate a reliable value for the binding to the protein.

The docking of a ligand results in a series of possible ligand conformations from which the bioactive conformation has to be selected. Hoffmann et al.[9] have introduced a two-stage method in an attempt to rerank the inhibitor conformations generated by a docking program. The reranking includes energy minimization, which is quite a time-consuming procedure, and it is preferable to circumvent this step. Very recently, Vieth et al. have described an approach that relies on the statistical determination of the docking mode (DoMCoSAR), which also involves docking in two stages.[10] The MultiSelect method described in this paper circumvents the second docking step and is able to select a single ligand conformation. The method was implemented using a set of MMP-inhibitor complexes,[11,12] where the three-dimensional structure of the complexes could be deduced from experimentally determined structures of MMP-inhibitor complexes (presently approximately 40 structures in the RCSB Protein Data Bank).[13] Because no structures of the complexes are available, it was not possible to evaluate directly the selection procedure based on this experiment. Therefore, 18 protein−ligand complexes of known three-dimensional structures were used for evaluation. The conformation selected as the bioactive conformation by MultiSelect was compared to the ligand conformation found in the X-ray structures.

Background for the Development of Multivariate Scoring

The free energy of binding can be described thermodynamically by the equation

$$\Delta G = \Delta H - T\Delta S$$

It is known that ΔH and ΔS may vary due to different experimental conditions (pH, temperature, ionic strength). This is a problem if the experimental values for binding affinity are determined using different experimental conditions. However, these variations in experimental conditions are most often ignored when scoring functions for estimating ΔG are considered.[14,15]

The free energy of binding, ΔG, relates to the binding constant K_i by the equation

$$\Delta G = -RT \ln K_i$$

Several different methods for estimating the binding affinity between a ligand and a protein are available.[14,15] All scoring functions encountered in this work rely on additivity of terms to obtain a measure for the free energy of binding. The intention is not to fully review the scoring functions, but a short overview of their different underlying principles will be presented. Only fast-performing scoring functions are considered, and therefore, no attention is paid to the scoring functions relying on more computational-intensive principles.[14,15] The scoring functions considered in this work can all be described as either force-field-based or knowledge-based scoring functions. Of these, the empirical functions are especially very useful for fast screening. The major drawback is the uncertainties associated with these—one could in general only expect the functions to perform well on complexes similar to those used for development of the functions. MultiScore was developed using a specific example with 120 MMP−inhibitor complexes, and in an attempt to develop a more generally applicable method, a diverse set of 120 protein−ligand complexes was considered. Thus, the aim of this work is to obtain an improved estimate for binding affinity by combining results from different scoring functions using multivariate statistical analysis.

Several of the scoring functions considered in this work are based on the work of Böhm,[16,17] who introduced the possibility of estimating binding affinities by summing up parameters that describe different types

of protein−ligand interactions. The scoring functions LUDI,[16,17] SCORE,[18] ChemScore,[19] and F_Score[8] rely on this principle. Other methods like GOLD,[7] G_Score,[7] D_Score,[20] and GRID[21−24] are force-field-based functions. The use of GRID as a scoring function is based on describing the ligand as consisting of different probes that each resemble an atom or a functional group. The interaction of each probe with the protein is calculated, and the binding energy is the sum of the probe interaction energies. The PMF_Score[25] is a knowledge-based function that is not dependent on experimental K_i values but relies on derivation of distance-dependent Helmholtz free interaction energies of protein−ligand atom pairs. The scoring functions estimate the free energy of binding by division into contributions from several terms (hydrogen bonds, hydrophobic interactions, ionic interactions, etc.). The main distinction between the functions is due to the different weighting of these terms, and especially regarding the empirical functions, these would presumably be most appropriate for application to problems similar to those used for development of the function. In an attempt to overcome this problem, the principle of consensus scoring has been introduced, which aims to improve the predictability by a combination of scoring functions.

Consensus Scoring

The concept of combining scoring functions to perform a ranking of inhibitors has previously been described. The CScore (Consensus Score) implementation in SYBYL, version 6.6.2,[26] uses a combination of five different scoring functions to rank inhibitors. These functions are F_Score (FlexX-Score), G_Score (based on the principles of the GOLD scoring function), D_Score (based on the principles of the DOCK scoring function), PMF_Score, and ChemScore. All of these functions are considered in this work. The concept of CScore is to identify those ligand conformations that have the highest score in most of the scoring functions. This is a rather simplistic approach because all five scoring functions are assigned the same importance and the ligands that generate good scores in most of the scoring functions are considered to be the best ligands. No attempt to quantitatively estimate binding affinity is done in this context, and often more than one solution is obtained.

An extensive review of the use of consensus scoring has been performed by Charifson et al.[27] The purpose of their study was to examine whether the use of several scoring functions could reduce the number of false positives obtained by individual scoring functions. They conclude that a combination of scoring functions significantly enhances hit rates. Apparently, no attempts have been made to combine the scoring functions in a quantitative manner. Our approach has been inspired by the concept of consensus scoring, but we decided to proceed and replace the consensus principle with data treatment using multivariate statistical methods.

The prediction of ligand conformation and binding affinity is a general problem in the drug design process, and having a reliable method to perform these tasks would be of incredible value in all cases where the target structure is known. Therefore, we have developed a generally applicable method that can be used for a diversity of protein−ligand complexes. It is likely that a single precise scoring function could be developed for a series of similar complexes, but it is a problem that one never knows which scoring function to use for a specific problem. However, when more scoring functions are introduced in a concept like the one presented here, it is possible to select the most appropriate functions for a specific problem and thereby add value to the predictions. Thus, the two main goals of this study were (1) to develop a statistical method to select the bioactive conformation between different docking results and (2) to improve the estimation of binding affinity of ligands to proteins. Both features are obtained by a combination of scoring functions and use of multivariate statistical analysis; a concept that will be referred to as multivariate selection (MultiSelect) and multivariate scoring (MultiScore), respectively.

Methods

1. Development and Evaluation of MultiSelect. 1.1. MMP Enzyme Structures. Three MMP X-ray structures were retrieved from the RCSB Protein Data Bank:[13] MMP1 (PDB entry 1HFC),[28] MMP2 (PDB entry 1QIB),[29] and MMP3 (PDB entry 1HFS).[30] The structures were energy-minimized by a previously described approach[31] using the SYBYL, version 6.6.2, molecular modeling software.[26] The force field used was AMBER, version 4.1,[32,33] as implemented in SYBYL. The 1−4 nonbonded interactions were scaled by a factor of 0.5, and a convergence criterion of 0.005 kcal/(mol Å) was applied. The nonbonded cutoff distance was 8 Å. All hydrogen atoms were included, considering a neutral pH for charged residues. The partial charges on protein residues were AMBER95 all-atom charges,[32] and a full charge representation of both calcium and zinc ions (+2) was used. The dielectric constant was set to 4, representing an intermediate value between water and vacuum conditions. A nonbonded approach was undertaken for ion representation, and nonbonded parameters for zinc (van der Waals (vdW) radius of 0.69 Å; $\epsilon = 0.014$) and calcium (vdW radius of 1.6 Å; $\epsilon = 0.1$) were added to the force field.[31,34,35] Distance constraints (2.045 Å and 100 kcal/(mol Å^2) between zinc and coordinating nitrogen atoms were applied in the first steps of the energy-minimization procedure. A model substrate (Pro-Leu-Ala-Leu-Phe-Ala) and a catalytically important water molecule were modeled into the active site to avoid collapse of the active-site pockets.[31]

1.2. MMP Inhibitor Structures. The inhibitors to be docked in the MMP structures were taken from Porter et al.[11] and Morphy et al.[12] (see Figure 1 and Table 1) and built by combining standard fragments from the fragment database in SYBYL. The structures were subjected to a short energy minimization using the Tripos force field[36] (200 iterations) to relieve any strain occurring in the structures.

1.3. Docking of MMP Inhibitors. Forty inhibitors were docked into energy-minimized X-ray structures of MMP1, MMP2, and MMP3, resulting in 120 different complexes. The docking program GOLD, version 1.0,[7] was used to position the MMP inhibitors in the active sites of the proteins. GOLD is based on a genetic algorithm, which has been shown to be very effective in positioning ligands correctly into the active site of a protein.[7] GOLD takes protein flexibility into account to some degree, but full side chain flexibility is not allowed. The active site, into which the MMP inhibitors were docked, was defined as a sphere ($r = 25$ Å) around the catalytic zinc ion. The default setup was used except for the torsional angles, where the MIMUMBA torsional distributions were applied.[37] The MMP inhibitors are all right-hand side inhibitors (i.e., binding in the primed side of the active site),[38] and the binding mode was anticipated to be very similar in all cases. Constraints were applied on zinc coordinating atoms (distance between 1.5 and 2.5 Å and a force constant of 100 kcal/(mol Å^2). In addition, hydrogen bond constraints were applied for the two carbonyl oxygens and two amide nitrogens binding to the backbone of the enzymes (see Figure 2). Early termination was allowed if

Table 1. Inhibitor Constants for MMP's[a]

inhibitor	R_1	R_2	MMP1	MMP2	MMP3	ref
1a	$CH_2CH(CH_3)_2$		7.8	0.33	25.3	11
1b	$(CH_2)_2C_6H_5$		618	2.24	77	11
1c	$(CH_2)_3C_6H_5$		203	0.062	8.3	11
1d	$(CH_2)_4C_6H_5$		2 500	0.254	177	11
1e	$(CH_2)_3C_6H_4$-4-CH_3		921	0.08	8.24	11
1f	$(CH_2)_3C_6H_4$-4-OCH_3		1 530	0.06	4.16	11
1g	$(CH_2)_3C_6H_4$-4-Cl		2 440	0.03	7.31	11
1h	$(CH_2)_3C_6H_4$-4-F		296	0.06	13.5	11
1i	$(CH_2)_3C_6H_4$-4-C_2H_5		5 120	0.10	5.44	11
1j	$(CH_2)_3C_6H_4$-4-CF_3		5 000	0.30	90.3	11
2a	Cl	$(CH_2)_2CO_2H$	193	0.01	0.8	11
2b	Cl	$(CH_2)_2CO_2CH_3$	135	0.01	0.96	11
2c	H	$(CH_2)_2C_6H_4$-4-SO_3H	942	0.59	81.5	11
2d	CH_3	$(CH_2)_2X$	455	0.01	6.26	11
2e	Cl	$(CH_2)_4X$	313	0.03	2.39	11
2f	CH_3	$(CH_2)_4X$	642	0.02	2.61	11
2g	Cl	$(CH_2)_4N(CH_3)_2$	1 050	0.05	22.2	11
2h	CH_3	$(CH_2)_4N(CH_3)_2$	313	0.02	5.87	11
2i	Cl	$(CH_2)_2COX$	319	0.02	1.38	11
2j	CH_3	$(CH_2)_2C_6H_4$-4-SO_2NH_2	329	0.01	2.98	11
2k	Cl	$(CH_2)_2C_6H_4$-4-SO_2NH_2	382	0.03	6.35	11
2l	CH_3	$(CH_2)_2NHSO_2X$	385	0.01	2.75	11
2m	Cl	$(CH_2)_2NHSO_2X$	302	0.01	1.73	11
3a	H	$(CH_2)_2C_6H_5$	25 000	18.9	701	12
3b	H	$(CH_2)_2CO_2H$	22 100	46.5	952	12
3c	H	$(CH_2)_2X$	100 000	812	8400	12
3d	CH_3	$(CH_2)_2C_6H_5$	17 000	2.5	277	12
4a	H (*RS*)	$(CH_2)_2C_6H_5$	21 100	10	834	12
4b	H (*SS*)	$(CH_2)_2C_6H_5$	20 000	210	20000	12
4c	H	$(CH_2)_2CH_4$-4-SO_2NH_2	40 200	6.4	952	12
4d	H	$(CH_2)_2CO_2CH_3$	57 800	6	1470	12
4e	H	$(CH_2)_3CONH_2$	28 400	3.06	1090	12
4f	CH_3	$(CH_2)_2C_6H_5$	22 100	1	472	12
4g	CH_3	$(CH_2)_2C_6H_4$-4-SO_2NH_2	47 800	0.9	384	12
4h	OCH_3	$(CH_2)_2C_6H_4$-4-SO_2NH_2	100 000	1.83	1110	12
4i	Cl	$(CH_2)_2C_6H_4$-4-SO_2NH_2	55 000	1.03	358	12
4j	CH_3	$(CH_2)_2CO_2CH_3$	41 400	1.17	353	12
4k	CH_3	$(CH_2)_2CO_2H$	100 000	3.82	321	12
4l	CH_3	$(CH_2)_4X$	100 000	10.3	1600	12
4m	H	H	100 000	123	3120	12

[a] All data are K_i values in nM. See Figure 1 for formulas.

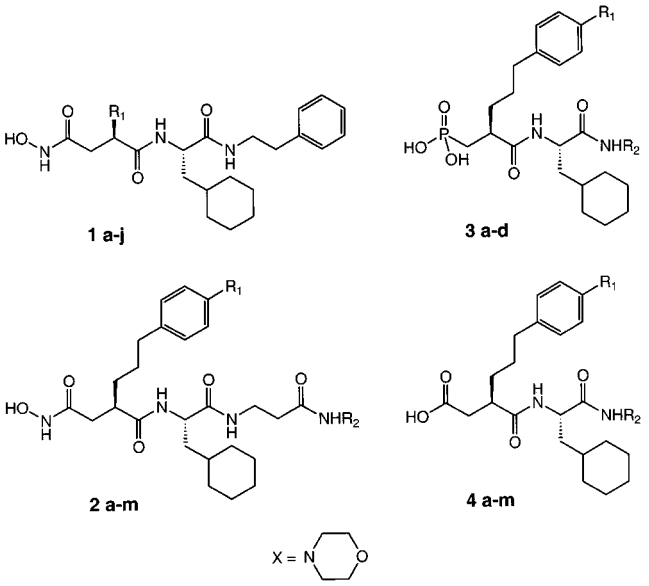

Figure 1. Structures of inhibitors docked into the active sites of MMP1, MMP2, and MMP3. See Table 1 for description of substituents R1 and R2.

the top three solutions were within a root-mean-square deviation (rmsd) of 1.5 Å, and a maximum of 10 solutions were retrieved from each docking.

1.4. Docking of Ligands from X-ray Structures. Evaluation of the selection procedure was done by docking ligands from the structures 1BRA,[39] 1CBX,[40] 1CPS,[41] 1DBB,[42] 1ETT,[43] 1FKF,[44] 1MDQ,[45] 1MNC,[46] 1NNB,[47] 1TNG,[48] 1TNH,[48] 1TNI,[48] 1TNJ,[48] 1ULB,[49] 2DRI,[50] 2IFB,[51] 5P21,[52] and 6TIM[53] into their corresponding X-ray structures using the docking program FlexX.[8] FlexX relies on a principle of building the ligand from fragments into the active site. A maximum of 30 different conformations were retrieved from each docking. The default setup for docking by FlexX in SYBYL was used.

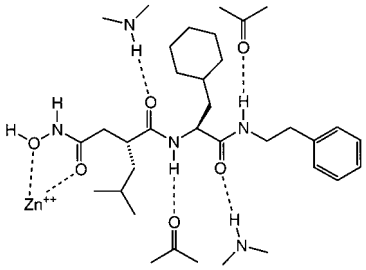

Figure 2. Schematic representation of the binding of a hydroxamate inhibitor **1a** to an MMP. The zinc-inhibitor contacts and hydrogen bond interactions are depicted as dotted lines.

1.5. Scoring of Inhibitors. Eight different scoring functions were applied to estimate the binding affinity of the inhibitors to the protein. The scoring was performed using a combination of SPL (SYBYL programming language) scripts and Perl scripts. The scoring functions are the five functions implemented in the CScore module of SYBYL, version 6.6.2.[26] These functions are F_Score,[8] D_Score,[20] PMF_Score,[25] G_Score,[7] and ChemScore.[19] In this context, Gasteiger charges were applied to both proteins and ligands, as default in CScore. In addition, the scoring function SCORE[18] was used together with the LUDI[16,17] scoring function. The inhibitors were scored by the GRID program, version 17,[21−24] by summing up interac-

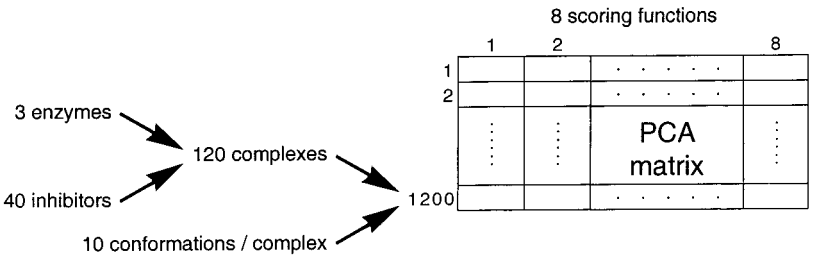

Figure 3. Procedure used to build the *X* matrix for MultiSelect. For each enzyme−ligand complex (MMP data set, 3 enzymes and 40 inhibitors) a number of dockings were performed (MMP data set, 10 conformations/complex), yielding the 1200 enzyme−ligand conformations. Each row represents the scoring with the eight scoring functions, resulting in a total of 1200 × 8 data points.

tion energies for the individual inhibitor atoms. GRID allows protein side chain flexibility to be taken into account, and both approaches have been used. However, no significant differences were observed. The results presented here were obtained with the nonflexible approach. The LUDI scoring function has been directly implemented in SYBYL using SPL.[54] SCORE and GRID are external programs, which were automated using SPL and Perl scripts, respectively. The scores obtained directly from GOLD were also considered to examine if this figure correlates with binding affinity, which of course would be preferable. However, the GOLD score was not included in the MultiScore concept.

1.6. Principal Component Analysis of Docking Results. A single conformation of each MMP inhibitor was selected by analyzing the obtained scores from the eight scoring functions by principal component analysis (PCA) (see Figure 3). For this purpose, the SIMCA-P, version 8.0, statistical software was used.[55] All variables were scaled to unit variance and centered. The model quality was expressed by the parameters R^2 (the explained variation) and Q^2 (the predicted variation). The obtained model was inspected for the presence of strong outliers, and if present, these would be excluded from the data set. An initial PCA model based on all scoring functions was derived. This model was improved by excluding scoring functions of minor importance, until a final model with one principal component was derived. The PCA model was used to predict scores by projection on the first principal component for all ligand conformations. Because the highest predicted score could be anticipated to correspond to the bioactive conformation of the ligand, a single ligand conformation was selected in each case. In addition, the PCA would reveal, if different scoring functions were contributing, similar information.

1.7. Equations Used. Formulas for R^2 and Q^2 are given below:

$$R^2 = 1 - \frac{\sum(y_{exp} - y_{pred})^2}{\sum(y_{exp} - y_{mean})^2}$$

$$Q^2 = 1 - \frac{\sum(y_{exp} - y_{pred})^2}{\sum(y_{exp} - y_{mean})^2}$$

Here, y_{exp} is the experimental value and y_{mean} is the average y value.

When R^2 was calculated, y_{pred} is the value predicted by the model. When Q^2 was calculated, y_{pred} is the value predicted when the data have been grouped and partial models from the reduced data sets (one group omitted at a time) obtained. The omitted data were used as a test set, and Y values were predicted. Several parallel models were obtained, and these were used for the calculation of Q^2.

The same procedure was performed for evaluation of PCA models except that y was substituted by x in the equations.

2. Development of MultiScore. 2.1. Partial Least-Squares Projection to Latent Structures. The eight scores for each of the MMP complexes selected by MultiSelect were extracted and correlated to the experimentally determined binding affinities,[11,12] using the SIMCA-P software[55] to perform a PLS analysis. All the results from the scoring functions were defined as X values, and the experimental pK_i value was defined as the Y variable. Similar to the PCA, the values were autoscaled and the model quality expressed by R^2 and Q^2. Selection of variables was performed using the VIP value (variable influence on projection parameter). This parameter takes into account the amount of explained Y variance of each dimension. A cutoff value of 0.8 was defined for discrimination between important and unimportant predictors. In addition, the information obtained from the PCA was used to identify variables contributing similar information, and on this background, the final variables important for the model were selected. The statistical significance of the estimated predicted power was described by the response permutation testing procedure as implemented in SIMCA-P. In this procedure, the X data were left intact while the Y data were permuted to appear in a different order. A PLS model was fitted to the permuted Y data, and R^2 and Q^2 values were computed for the derived model. This procedure was repeated 10 times, resulting in 10 different models. The obtained R^2 and Q^2 values were plotted as a function of the correlation coefficient between original and permuted response data. A valid model would have an intercept for R^2 below 0.3 and an intercept for Q^2 below 0.05.[56]

2.2. Protein−Ligand Complexes from the Protein Data Bank. A diverse training set consisting of 120 protein−ligand complexes was retrieved from the RCSB Protein Data Bank.[13] Each of the complexes were divided into the protein part and the ligand part. The atom types of the ligands were manually checked. No geometry optimization was performed because the ligand conformation found in the complex was assumed to be the bioactive conformation. All water molecules were deleted from the protein structures except for structurally important water molecules. Metal ions were retained in all cases. All hydrogen atoms were included considering a neutral pH for the charged residues. The binding affinities of these complexes were estimated by use of the eight scoring functions and correlated to the experimentally determined values.[18] The results were analyzed using PLS as described above. A subset of these complexes was used for evaluation of MultiSelect.

Results and Discussion

Selection of a single ligand conformation, which is believed to correspond most closely to the bioactive conformation, was the first challenge of this study. The selection was performed assuming that the best inhibitor conformation corresponds to the structure with the most favorable binding energy. This is a common anticipation in the scoring functions, and because this concept relies on the performance of these, this anticipation will also be used in this context. As previously mentioned, the docking programs generate several ligand conformations, but often the ranking of these, and thus the identification of the correct ligand conformation, is not satisfying. In the past years several new scoring functions have been introduced that claim to be

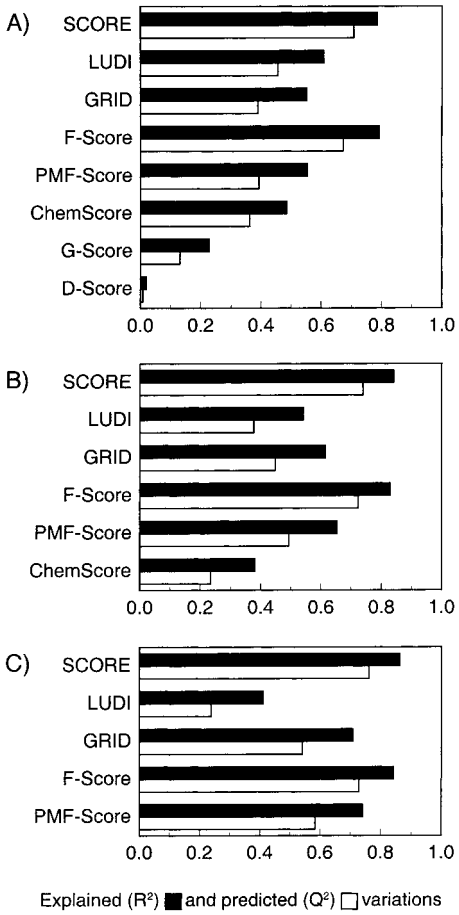

Figure 4. Explained (R^2) and predicted (Q^2) variation by the first principal component: (A) initial model with all eight scoring functions; (B) model after exclusion of D_Score and G_Score; (C) final model after exclusion of ChemScore.

able to handle this problem. In addition, these scoring functions should be able to make improved predictions of binding affinities, a feature that is also considered in this context. Instead of developing new scoring functions, we have used already available scoring functions in a novel way.

Selection of Bioactive Ligand Conformation. The docked MMP inhibitors were scored using the eight scoring functions mentioned above. The output from these functions was analyzed using PCA, and initially, a model with three principal components was obtained. No strong outliers were detected, and therefore, no observations were excluded from the data set. The possibility of obtaining a one-component model by excluding two of the scoring functions from the initial model was examined by excluding D_SCORE and G_SCORE from the model because these were not well accounted for by the first principal component (see Figure 4). A second PCA was therefore performed using the remaining six scoring functions, resulting in a two-component model. This led to exclusion of the ChemScore function, and a third model was derived on the basis of five scoring functions (see Figure 4). The exclusion of the three scoring functions mentioned above was also supported by the finding that these functions had the weakest correlation to experimental data. The PCA of the results from the remaining five scoring functions (GRID, LUDI, SCORE, F_Score, and PMF_Score) resulted in the desired one-component model with $R^2 = 0.73$ and $Q^2 = 0.59$. The contribution of the five scoring functions to this model is shown in the lower part of Figure 4. Using this model, the binding of the docked conformations was predicted on the basis of a quantitative combination of the scoring functions. As the data were normalized, the output from the model did not correspond directly to either the binding energy or the pK_i, but still, the highest predicted value indicated the best binding and was therefore assumed to correspond to the bioactive conformation. In contrast to consensus scoring, this approach identifies a single solution as the bioactive conformation. Comparing the results from this study with the results obtained using the CScore function as implemented in SYBYL, version 6.6.2, it was found that in 46% of the cases CScore identified solutions different from those found by MultiSelect, and in only 25% of the cases CScore identified a single conformation that was identical to the conformation found by MultiSelect. This is most likely due to CScores' emphasis on five scoring functions, of which three are excluded from MultiSelect. At least in this case, these three functions were not useful for predictions. From analysis of the results obtained from the consensus scoring method (CScore), it was revealed that in 48% of the cases CScore suggested more than one solution.

To evaluate the selection procedure, 18 inhibitors with known binding conformation were docked into their corresponding protein structures. The selection procedure was evaluated, comparing the selected conformation with the ligand conformations in the X-ray structures. The ability to select a docked conformation, which resembles the conformation in the X-ray structure, would be the ultimate goal of this procedure. The evaluation was performed on various protein−ligand complexes to show the generality. The structures considered were 1BRA, 1CBX, 1CPS, 1DBB, 1ETT, 1FKF, 1MDQ, 1MNC, 1NNB, 1TNG, 1TNH, 1TNI, 1TNJ, 1ULB, 2DRI, 2IFB, 5P21, and 6TIM (see Figure 5). The docking procedure produced a maximum of 30 solutions (conformations), and the selection of conformations was done using the PCA model derived for the MMP's. By comparison with the ligand conformations present in the X-ray structures, an indication of the reliability of the selection procedure was obtained. The rmsd's between the ligand conformation in the X-ray structure and the different solutions suggested by the docking program are depicted in Figure 6. It is observed that the selection procedure in all cases selected a conformation with a low deviation from the conformation in the X-ray structures (see Figure 6 and Table 2). The order of the conformations reflects the F_Score (FlexX) ranking. From Figure 6 it is also revealed that the poor correlation between conformations selected by CScore and conformations selected by MultiSelect described above could be a special case for the MMP's. The only case where CScore and MultiSelect generated very different results was for 1MNC, which is an MMP structure. In the other proteins, CScore suggested at least one reasonable ligand conformation among the possible solutions. Still, the identification of several possible solutions is a severe problem when using the CScore method, and in this context, MultiSelect has a major advantage.

Correlation to Experimental Data. Using a single scoring function to quantify ligand binding is often not enough. This is illustrated when considering the MMP

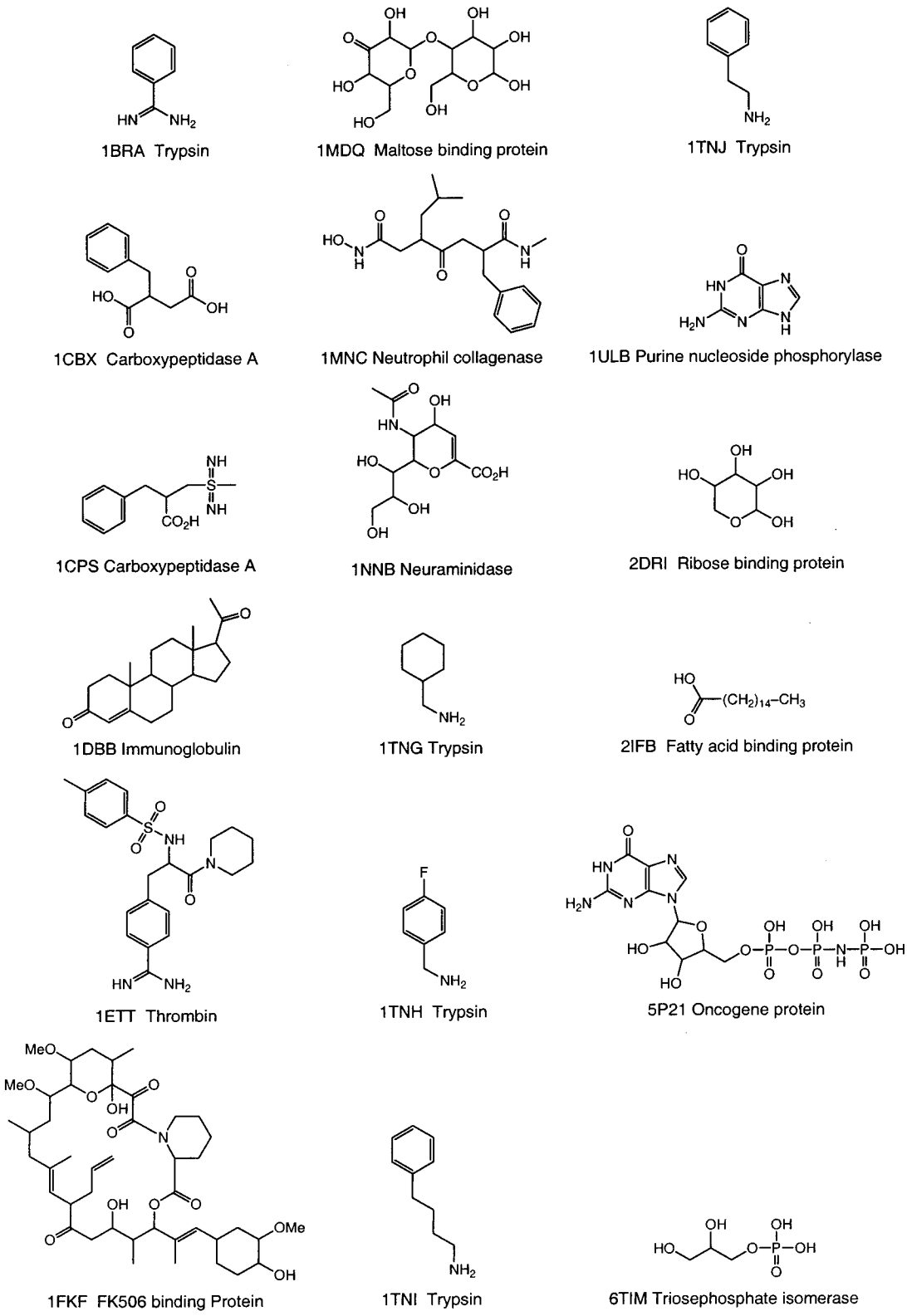

Figure 5. Ligands from X-ray structures of 18 different protein−ligand complexes used for evaluation of MultiSelect.

inhibitor conformations obtained by docking. Selecting the conformation that each individual scoring function regards as the best solution and correlating the corresponding scores with the experimental values result in correlation coefficients i between 0 and 0.7 for the different scoring functions (see Table 3). Therefore, we examined whether a combination of the results from the scoring functions could clarify the picture. The solutions selected by MultiSelect were analyzed using PLS analysis of the results from the scoring functions. A model including all scoring functions was developed, but three of the scoring functions (D_Score, G_Score, and ChemScore) did not contribute significantly to the model (see Figure 7). A new PLS analysis was performed using the five remaining scoring functions. A one-component model was obtained with $R^2 = 0.78$ and $Q^2 = 0.78$ (see upper part of Table 4). This implies both a good correlation and, more importantly, a very good predic-

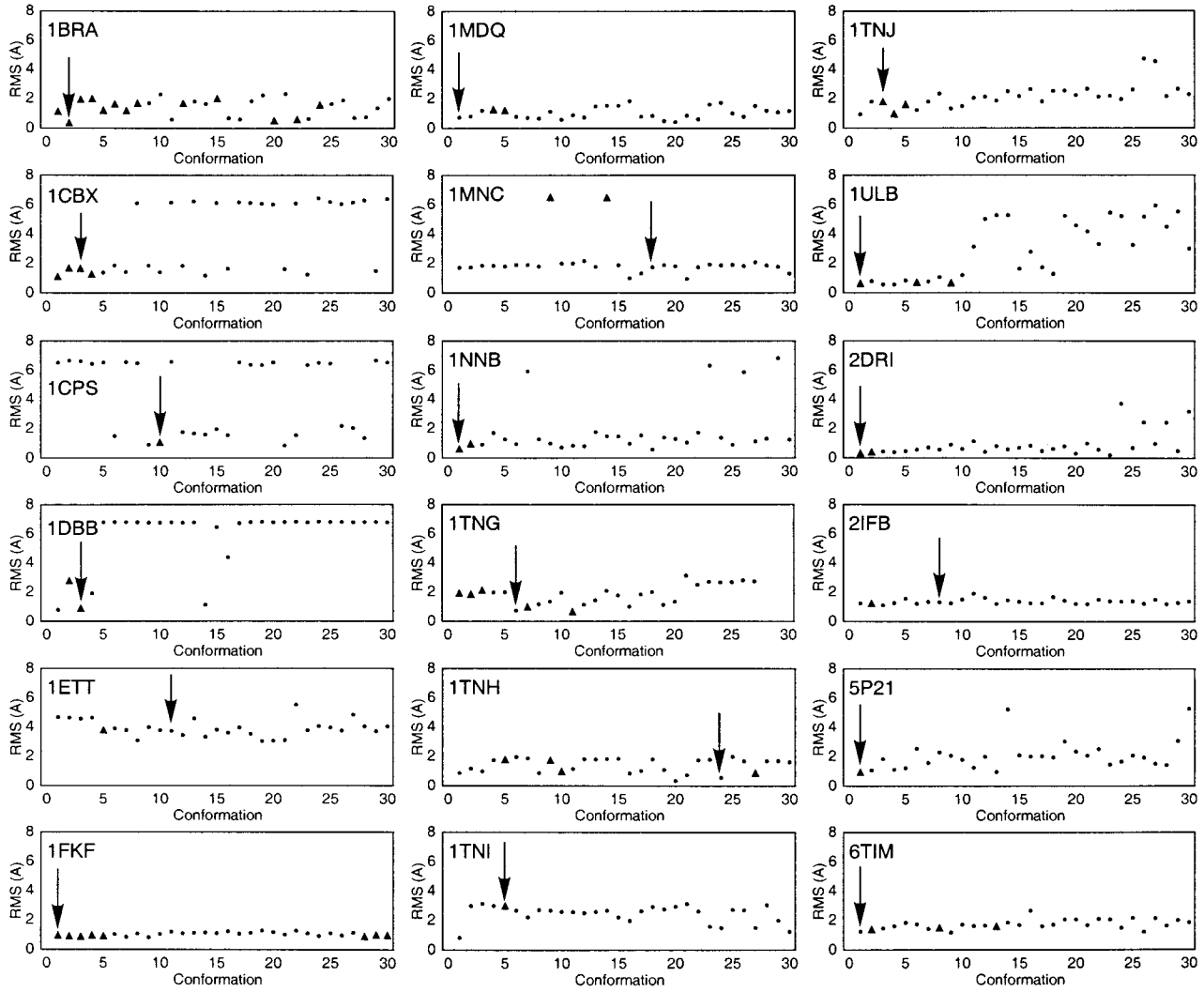

Figure 6. Result of the MultiSelect evaluation. The rmsd values between X-ray ligand conformations and conformations obtained by docking. Conformations selected by MultiSelect are indicated by arrows, and conformations selected by CScore are depicted as triangles.

Table 2. The rmsd between X-ray Ligand Structures and the Conformations Selected by MultiScore, F_Score, and CScore[a]

	MultiSelect	F_Score	CScore
1BRA	**0.36**	1.13	1.13; 0.36; 1.95; 1.99; 1.21; 1.65; 1.19; 1.70; 1.68; 2.01; 0.51; 0.59; 1.58
1CBX	1.66	**1.11**	1.11; 1.68; 1.66; 1.27
1CPS	**1.08**	6.52	1.08
1DBB	0.88	0.77	2.77; 0.88
1ETT	3.71	4.66	3.78
1FKF	0.98	0.98	0.98; 0.91; 0.89; 0.97; 0.92; 0.92; 1.00; 0.98
1MDQ	**0.72**	0.72	1.28; 1.21
1MNC	1.74	1.70	6.50; 6.48
1NNB	0.61	0.61	0.61; 0.97
1TNG	0.72	1.93	1.93; 1.86; 2.13; 1.00; 0.66
1TNH	0.56	0.86	1.80; 1.75; 0.99; 0.88
1TNI	3.01	**0.82**	3.01
1TNJ	1.79	**0.93**	1.79; 0.96; 1.61
1ULB	0.65	0.65	0.65; 0.71; 0.68
2DRI	0.33	0.33	0.33; 0.42
2IFB	1.32	1.22	1.24
5P21	0.94	0.94	0.94
6TIM	1.23	1.23	1.40; 1.51; 1.60

[a] Bold-faced numbers indicate where considerable differences are observed between conformations selected by F_Score and by MultiSelect.

Table 3. Regression Coefficients Obtained by Individual Use of Scoring Functions for the 120 MMP-Inhibitor Complexes (See Text for Details)

scoring function	R^2
SCORE	0.66
LUDI	0.41
GRID	0.57
F_Score	0.73
PMF_Score	0.55
ChemScore	0.12
G_Score	0.12
D_Score	0.00

Table 4. Statistical Quality of the MMP and X-ray Models[a]

model	no. of components	R^2 (overall)	Q^2 (overall)	R^2 (intercept)	Q^2 (intercept)
MMP	1	0.779	0.778	−0.04	−0.06
X-ray	2	0.678	0.667	−0.03	−0.06

[a] R^2 (overall) is the explained variation of the model, while Q^2 (overall) is the predictive power. R^2 and Q^2 (intercept) are the intercepts with the y axis after permutation of the Y data (experimental data) (see Methods for further details).

tivity of the model. In addition, the internal evaluation also implies a valid model. The correlation between the observed experimental binding affinities and the predicted binding affinities (pK_i's) is shown in Figure 8.

The reason for the poor performance of three of the scoring functions is not clear. The G_Score is mainly

Table 5. PDB Codes for Complexes Used for Development of the X-ray Model[a]

1AAQ, 1APT, 1APU, 1APV, 1APW, , 1CLA, 1CPS, 1DBB, 1DBJ, 1DBK, 1DBM, 1DR1, 1DRF, 1DWB, 1DWC, 1DWD, 1ETR, 1ETS, 1ETT, 1FKB, 1FKF, 1L83, 1LGR, 1MCB, 1MCF, 1MCH, 1MCJ, 1MCS, 1MDQ, 1MNC, 1NNB, 1PGP, 1PPC, 1PPH, 1PPK, 1PPL, 1PPM, 1RBP, 1RNE, 1SNC, 1TLP, 1TMN, 1TMT, 1TNG, 1TNH, 1TNI, 1TNJ, 1TNK, 1TNL, 1ULB, 2CGR, 2CTC, 2DBL, 2DRI, 2GBP, 2IFB, 2PK4, 2R04, 2SNS, 2TMN, 2XIS, 3CLA, 3CPA, 3FX2, 3PTB, 3TMN, 4CLA, 4FAB, 4TIM, 4TLN, 4TMN, 5ACN, 5ENL, 5TIM, 5TLN, 5TMN, 6APR, 6CPA, 6ENL, 6TIM, 6TMN, 7DFR, 7EST, 7TLN, 8CPA, 8XIA, 1DHF, 1FBP, 1HSL, 1LYB, 1RUS, 1THA, 1XLI, 2AK3, 2YPI, 3GAP, 4DFR, 4XIA, 5XIA, 6GST, 7TIM, 9AAT, 9ABP

[a] The last 17 complexes in the table are dimers

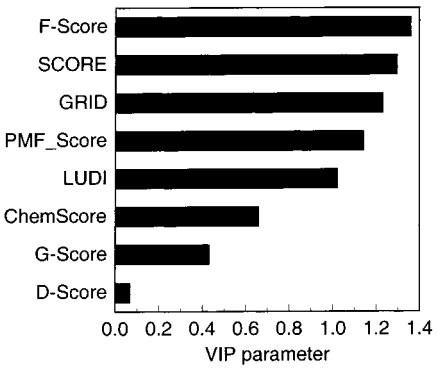

Figure 7. The variable influence on projection (VIP) parameter shown for the initial MMP model including all eight scoring functions.

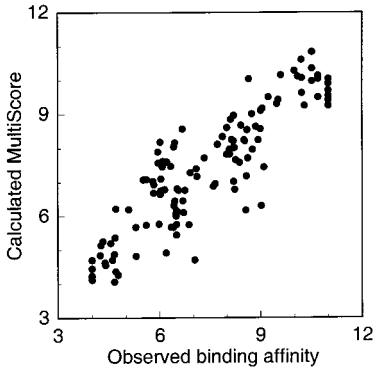

Figure 8. Calculated MultiScore as a function of the observed binding affinity for 120 MMP-inhibitor complexes.

useful for estimating binding affinities for systems when there are possibilities of hydrogen bonding between ligand and protein.[7] This is certainly the case for the MMP-inhibitor complexes, but the result could be impaired by the fact that most of the inhibitors contain similar possibilities for hydrogen bonding, and the differences between them do not rely on this feature. With respect to D_Score, no reason for the poor performance is obvious. The last scoring function to be excluded from the model is ChemScore. ChemScore is based on the same principles as other empirical scoring functions in this study (e.g., LUDI, F_Score, and SCORE), and the performance of the method has been evaluated in a similar manner. The scoring functions, which contribute to the model, are the scoring functions SCORE, LUDI, F_Score, PMF_Score, and GRID. The coefficients are depicted in Figure 9.

To evaluate the general performance of the MultiScore concept, 120 different complexes were selected from the RCSB Protein Data Bank (see Table 5) and a model for prediction of the binding affinities was derived in a similar manner as for the MMP's. The data set was split into a training set and a test set each consisting of 60 complexes. A model was derived on the basis of five scoring functions because three of the scoring functions were excluded due to a VIP value less than

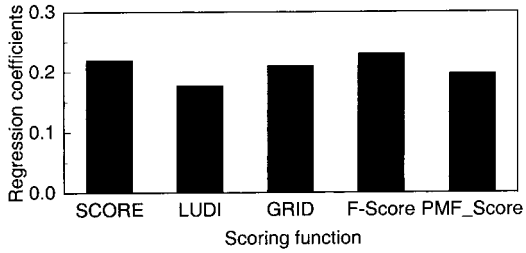

Figure 9. Regression coefficients of scaled and centered variables (scoring functions) for the MultiScore MMP model.

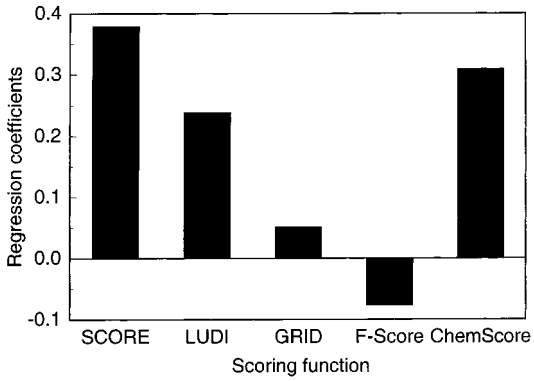

Figure 10. Regression coefficients of scaled and centered variables (scoring functions) for the MultiScore X-ray model.

0.8. Use of this model for prediction of the binding affinities for the complexes in the remaining 60 complexes (the test set) resulted in a correlation to experimentally determined data with $R^2 = 0.68$. A model based on all 120 complexes was then derived, and in this context, the only scoring function to be excluded due to a low VIP value was the PMF_Score. However, the presence of G_Score and D_Score did not improve the model, and these scoring functions were excluded too, leading to a model that was based on the same scoring functions as the model derived from 60 of the complexes. The model was then based on five scoring functions, of which four were identical to the scoring functions contributing to the MMP model. In this context ChemScore was included in the model in exchange for the PMF_Score function. The reason for this is not obvious; the PMF_Score function should not be less generally valid than the ChemScore function. The model data are listed in the lower part of Table 4, and the coefficients are depicted in Figure 10. The negative coefficient for F_Score should not be interpreted as if this scoring function has a negative influence on the model. The scores from this function have a tendency to be shifted toward positive values, and therefore, the sign is reverted. It can be seen that the model derived for the MMP's is more specific and accurate, but it can also be concluded that a general model that is better than any single scoring function has been derived for the diverse protein set. The correlation between experimental data and predicted

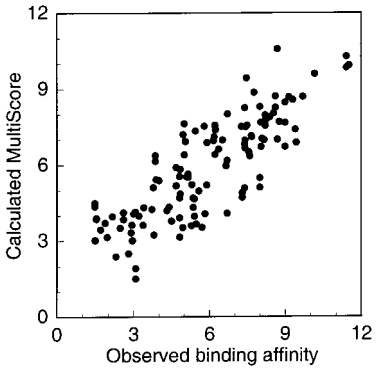

Figure 11. Calculated MultiScore as a function of the observed binding affinity for 120 different complexes with known X-ray structure.

Table 6. Regression Coefficients Obtained by Individual Use of Scoring Functions for 120 X-ray Protein−Ligand Complexes (See Text for Details)

scoring function	R^2
SCORE	0.56
LUDI	0.56
GRID	0.35
F_Score	0.25
PMF_Score	0.10
ChemScore	0.53
G_Score	0.44
D_Score	0.34

Table 7. MultiScore Results for the MMP Inhibitors[a]

	MMP model	X-ray model
$pK_i \pm 1$ (%)	73	49
$pK_i \pm 2$ (%)	97	85

[a] Predictions within ±1 and ±2 pK_i units are given for application of the MMP model and the X-ray model, respectively.

values with $R^2 = 0.68$ and $Q^2 = 0.67$ is depicted in Figure 11. The corresponding correlations between experimental data and the scores obtained by the individual scoring functions are listed in Table 6. The scoring functions have been derived on the basis of different principles and sets of data, and thereby their predictions will be most accurate when predicting something that the functions are trained to predict. Regarding the differences in important scoring functions in the two cases examined, this could of course be a result of different data sets.

To examine whether the model based on the X-ray structure complexes could be used in general, this model was used for prediction of the binding energy for the MMP ligands to the MMP's. The correlation was still very good using this more generally applicable model ($R^2 = 0.60$). The prediction of the MMP ligand binding using the two different models is summarized in Table 7. This indicates that in many cases where sufficient data are not available to develop a specific model, the general model can generate meaningful results. This is a major strength of this principle because it is not necessary to develop a new model for each new series of complexes when a rough estimation of the binding is the primary intention. However, if a more precise estimate is wanted when estimating binding data for ligands of a specific enzyme family, this work has shown that a more specific model could solve this problem. The models are very fast to derive, and therefore, it is easy to develop new models for different proteins. However, this work has also shown that a general model could be used as a first approximation, improving the estimating power compared to the individual scoring functions.

Conclusion

A new method for selecting inhibitor conformations obtained from docking studies is described. This method could be used for the selection of a single ligand conformation, which most probably will resemble the bioactive conformation. The method was developed using 120 MMP complexes and evaluated by 18 complexes selected from the RCSB Protein Data Bank. Extending the principle of consensus scoring in a quantitative statistical manner has led us to introduce the MultiSelect principle. This method relies on PCA and is able to identify a single ligand conformation among different docked conformations that resembles the bioactive conformation. In the second part of this article, we have used PLS analysis to combine different scoring functions in the MultiScore concept, and in that way we have improved the prediction of binding affinity. It can be concluded that it is possible to develop a model encompassing a variety of different complexes and still be able to obtain an improved score relative to the scores obtained by individual scoring functions. However, if more accurate predictions are desired, a more specific model could easily be derived. When the principles of MultiScore are used, this is a very fast and feasible task, and therefore, this approach should have a great potential to be included in the process of database screening.

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