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Molecular Docking Using the Molecular Lipophilicity Potential as Hydrophobic Descriptor: Impact on GOLD Docking Performance

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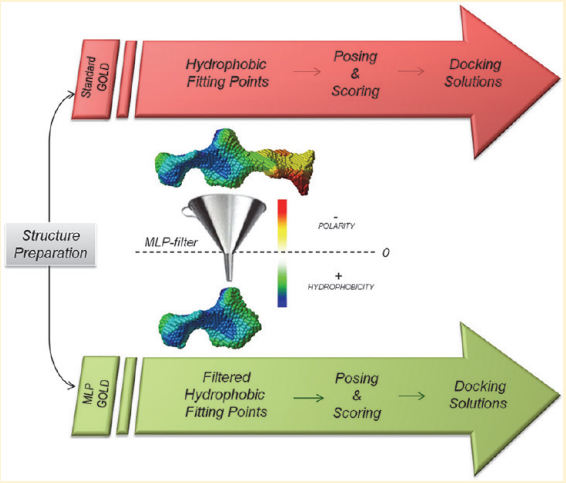
Supporting Information

ABSTRACT: GOLD is a molecular docking software widely used in drug design. In the initial steps of docking, it creates a list of hydrophobic fitting points inside protein cavities that steer the positioning of ligand hydrophobic moieties. These points are generated based on the Lennard-Jones potential between a carbon probe and each atom of the residues delimitating the binding site. To thoroughly describe hydrophobic regions in protein pockets and properly guide ligand hydrophobic moieties toward favorable areas, an in-house tool, the MLP filter, was developed and herein applied. This strategy only retains GOLD hydrophobic fitting points that match the rigorous definition of hydrophobicity given by the molecular lipophilicity potential (MLP), a molecular interaction field that relies on an atomic fragmental system based on 1-octanol/water experimental partition coefficients (log P_{oct}). MLP computations in the binding sites of crystallographic protein structures revealed that a significant number of points considered hydrophobic by GOLD were actually polar according to the MLP definition of hydrophobicity. To examine the impact of this new tool, ligand−protein complexes from the Astex Diverse Set and the PDB bind core database were redocked with and without the use of the MLP filter. Reliable docking results were obtained by using the MLP filter that increased the quality of docking in nonpolar cavities and outperformed the standard GOLD docking approach.



INTRODUCTION

The binding of small molecules to macromolecular targets is central to many biological processes. Additionally, reliable predictions of ligand binding modes with biological targets, the so-called docking problem, are of major importance in drug design.[1−6] An analysis of crystallized ligand−protein complexes indicated that high-affinity ligands fit intimately in binding cavities by maximizing intermolecular nonbonded forces. Thus, a correct modeling of these forces prior to, during, and after the posing process is fundamental for a precise depiction of the binding mode of ligands in protein cavities, i.e., to find the so-called docking solutions.

Docking engines make use of several different strategies to characterize intermolecular interactions linked with binding site properties, although with some approximations.[6,7] Hydrophobicity is a prominent example of a drastically approximated property often described in molecular modeling by means of van der Waals forces favorable only when good steric complementarity between atoms is achieved. This description ignores the hydrophobic nature of residues involved in putative interaction areas (Figure S1, Supporting Information). The traditional van der Waals Lifshitz theory may not be sufficiently rigorous to accurately and precisely quantify hydrophobicity. Thus, a more comprehensive parameter describing the subtle equilibrium between steric and weak electrostatic forces that characterizes hydrophobicity is needed.[8,9] There have been several attempts to quantify hydrophobicity. For example, in silico attempts have tried to translate the effects of hydrophobicity to molecular mechanic energies (kJ mol^{-1}) or to hydrophobic surface areas (A^2).[10,11] Experimental attempts have measured the force produced between two molecular surfaces (mN m^{-1}) or have evaluated the distribution of a compound between two immiscible phases, such as water and 1-octanol (log P_{oct}, no unit). The latter method allows for indirect estimation of the hydrophilic−hydrophobic (lipophilic) properties of a compound.[12−15]

By dividing lipophilicity in two main terms, hydrophobicity and polarity,[16,17]

$$\text{lipophilicity} = \text{hydrophobicity} - \text{polarity} \quad (1)$$

one can assume the presence of two different regions in a protein pocket: one in which polar interactions overcome hydrophobic interactions and one in which hydrophobic

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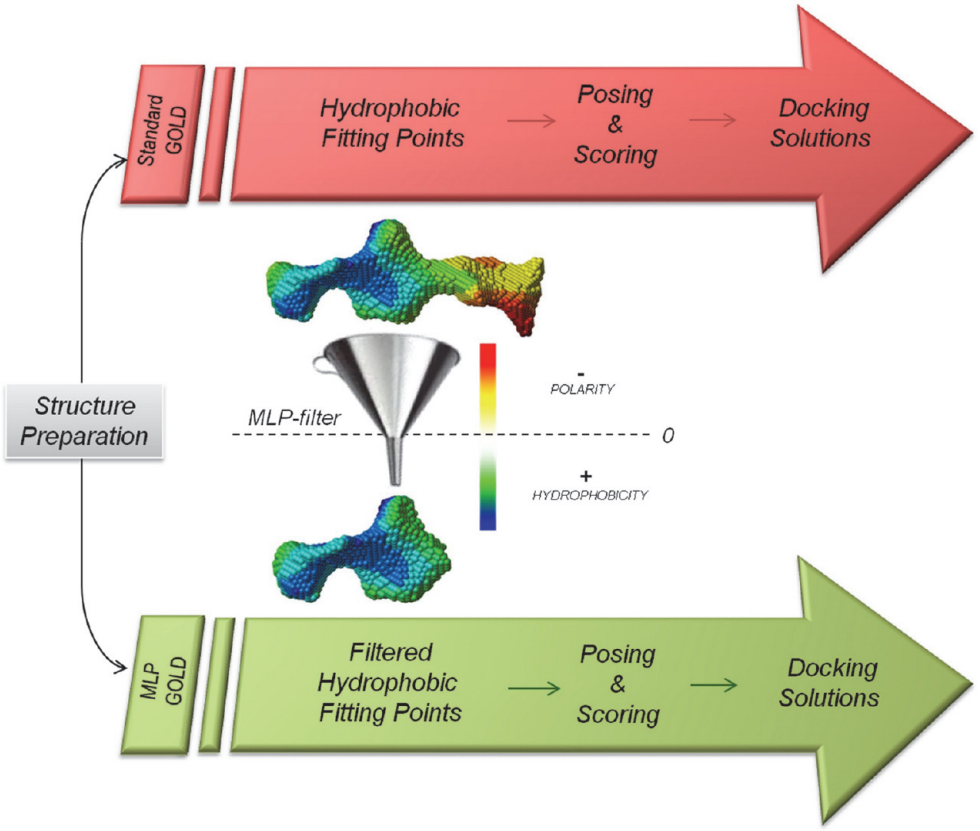

Figure 1. Scheme representing the application of the MLP filter to the standard GOLD docking protocol. The standard GOLD pathway follows the red arrow, whereas the MLP-GOLD pathway follows the green arrow.

interactions are the dominant intermolecular forces. According to eq 1, polar regions are characterized by negative lipophilicity, whereas hydrophobic regions are characterized by positive lipophilicity.

The present work introduces a novel hydrophobic descriptor of protein pockets in docking engines based on the molecular lipophilicity potential (MLP). The MLP is an in-house molecular interaction field that describes the lipophilic properties of chemical identities based on experimental 1-octanol/water partition coefficients (log P_{oct}) of small chemical compounds.[18,19] At a given point in space, k, the MLP indicates the cumulative lipophilic contributions of all atoms in a given chemical compound as

$$\mathrm{MLP}_K = \sum_{i=1}^{N} F_i \times f(d_{ik}) \qquad (2)$$

where N is the number of atoms in a molecule; F is the lipophilic fragmental value assigned to each atom that potentially interacts with k, as derived from an enriched atomic fragmental i database that largely relies upon the database of Broto and Moreau;[20] and f is the Fermi distance function, which defines how the MLP decreases with the distance d between the point k and a fragment i. MLP values are in the referential of 1-octanol/water partition coefficients. Negative values represent polar potentials, whereas positive values represent hydrophobic potentials.[19−21]

The MLP can be a useful descriptor of lipophilicity for small drug-like molecules and protein structures.[19,21−24] Thus, a new computational tool, the MLP filter, which contains MLP-based lipophilic descriptor machinery, was developed and herein applied to the docking program GOLD.[25,26] Among other docking engines, GOLD was chosen because of the simplistic method by which it evaluates the hydrophobicity of protein pockets. Before the posing phase, GOLD generates a list of points by building a grid over the binding site. At each grid point, hydrophobicity is estimated by calculating the Lennard-Jones potential between the atoms in the protein pocket and a sp^3 carbon probe. This information is stored in a user accessible map in which the hydrophobic points that influence the standard GOLD ligand positioning are listed. The role of the MLP filter in this protocol is to process this map by calculating the MLP at each of the listed points. Only those points with positive MLP values (thus strictly hydrophobic) steer the apolar parts of ligands toward MLP-wise favorable hydrophobic zones.

Is the MLP a relevant hydrophobic descriptor for GOLD molecular docking studies? To answer this question, the impact of the MLP filter is evaluated and compared with the standard GOLD docking approach through redocking experiments using a large collection of ligand/protein cocrystals derived from the Astex Diverse Set and the PDB bind core database.[27,28]

MATERIALS AND METHODS

Molecular Modeling. *Retrieving Complexes from Data Sets.* The MLP filter approach was tested on a high-quality test set, the Astex Diverse Set, which was previously used to evaluate GOLD docking performance.[27] Another data set, recently used for benchmarking docking studies involving GOLD and other docking programs, the PDB bind core database, was also considered.[28] A total of 65 and 163 drug discovery oriented complexes were isolated from the Astex

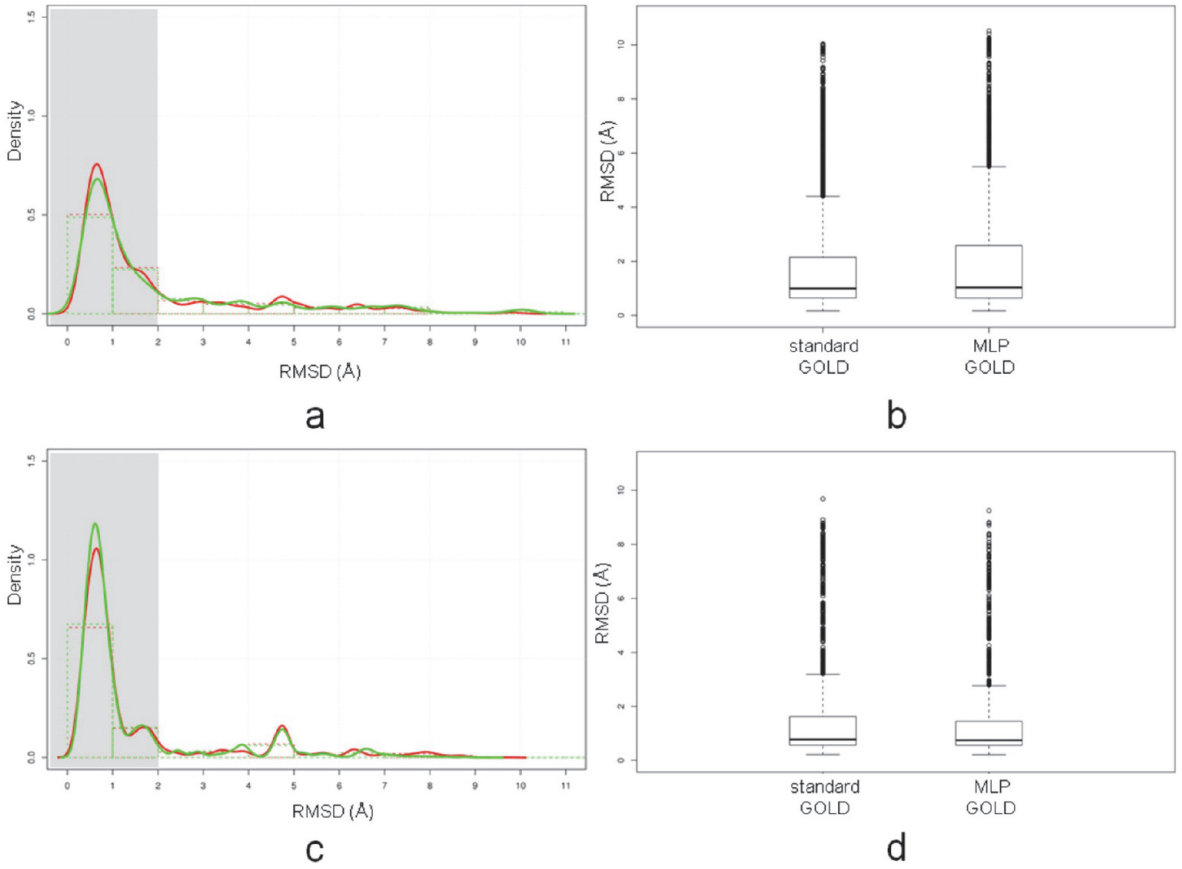

Figure 2. Distribution (a) and boxplot (b) of the 6500 RMSD values obtained by applying the standard GOLD (red) and the MLP-GOLD (green) protocols on the Astex Diverse Set. Distribution (c) and boxplot (d) of the 3200 RMSD values obtained by applying the standard GOLD (red) and the MLP-GOLD (green) protocols on the complexes of the Astex Diverse Set characterized by LI > 10%. The threshold for a docking success, fixed at 2 Å, is highlighted in gray.

Diverse Set and PDB bind core database, respectively, with a total of 227 complexes (one overlap between the two data sets).

Preparation of Complexes for GOLD Docking Runs: the Ligands. The chemical structures of the ligands were carefully checked within SYBYL 8.0 (Tripos Inc., St. Louis, MO), focusing on the Tripos force field atom and bond types.[29] The ionization state was defined as appropriate for pH of 7.4. To avoid geometrically biasing the docking solutions by a unique input, five starting conformers were generated for each ligand through the random search conformational search tool in SYBYL 8.0. Further translations and rotations in three-dimensional space were then performed, and the coordinates were saved in MOL2 files.

Preparation of Complexes for GOLD Docking Runs: the Receptors. The protein structures were prepared without cocrystallized water molecules by following the standard Wizard workflow implemented in the three-dimensional visualizer Hermes.[30]

Standard GOLD Docking Protocol. Automated docking was carried out by the program GOLD, version 4.0.[31] At the beginning of a docking run, GOLD detects binding site cavities and fills them with a grid made of points spaced by 0.25 Å. At each grid node, hydrogen bonding and hydrophobic properties are evaluated. In particular, at each point of the grid, van der Waals interaction energies between a carbon sp^3 probe and atoms of the protein are calculated. In a single MOL2 file, *x,y,z* coordinates of the points, whose interaction energies are more negative than −2.5 kcal/mol (the hydrophobic fitting points, HFPs), are stored. Preset options for the genetic search algorithm were used in calculations in which only ligand torsions were free to move. The GoldScore was used as an empirical scoring function because it has been shown to outperform the other GOLD scoring schemes in crystallographic pose retrievals.[32] Twenty docking solutions were retrieved for each ligand conformation, yielding 100 solutions for each ligand−protein complex.

MLP-GOLD: MLP Filter and Application to the Standard GOLD Docking Protocol. The MLP filter is a computational tool developed to map the hydrophobic zones of protein pockets within the GOLD docking methodology by using the definition of hydrophobicity enclosed in the MLP.[16,17] The MLP is a well-known molecular field that quantitatively describes the lipophilicity of small molecules as a function of connectivity and conformations.[33] The same capability is maintained when the MLP is applied to amino acids that characterize the binding sites of biological targets.[16,23−25] At the beginning of each docking run, before the searching and scoring docking steps, GOLD creates a MOL2 file in which the *x,y,z* coordinates of the HFPs are listed. The MLP filter tool can be successively applied by submitting these points to MLP calculations (eq 2). Only the points bearing MLP positive (hydrophobic) values are stored in a separate MOL2 file that is input into GOLD by replacing the original file. These points can be visualized by common molecular modeling environments and colored according to the MLP scale range from red

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Table 1. Summary of the Statistical Results Obtained From GOLD Redocking Runs on Complexes From the Astex Diverse Set and PDB Bind Core Database

	Astex diverse set		PDB bind core		Merged databases	
	GOLD	MLP-GOLD	GOLD	MLP-GOLD	GOLD	MLP-GOLD
Docking results <2 Å (%)	74.6	72.2	49.6	47.7	56.9	54.9
Lowest RMSD pose <2 Å (%)	81.5	80.0	54.6	54.0	83.7	82.4
Best ranked poses <2 Å (%)	81.5	80.0	54.6	54.0	62.6	61.7
	Astex diverse set LI		PDB bind core LI		Merged databases LI	
	GOLD	MLP-GOLD	GOLD	MLP-GOLD	GOLD	MLP-GOLD
Docking results <2 Å (%)	80.1	82.2	57.1	59.3	66.6	68.7
Lowest RMSD pose <2 Å (%)	87.5	90.6	53.7	59.3	81.2	87.1
Best ranked poses <2 Å (%)	87.5	90.6	53.7	61.1	67.1	72.9

(the most polar points) to blue (the most hydrophobic points).[19]

Standard GOLD vs MLP-GOLD: Handling of Docking Solutions. To assess the impact of the MLP filter, two series of redocking simulations were performed: one with the original HFPs (standard GOLD run) and one with the filtered HFPs (MLP-GOLD run) (Figure 1). The docking performance of both workflows was evaluated by calculating the heavy atom root-mean-square deviation (RMSD) between the predicted and crystallographic ligand binding pose. RMSD values were herein computed by using the Smart_RMS tool included in the GOLD suite package. Because large deviations from the experimental binding pose are symptoms of docking failure, only RMSD values lower than 2 Å were considered as a docking success. Statistical analyses were performed by considering: (i) the distribution of all the RMSD values; (ii) the distribution of the minimal RMSD values retrieved from each docking run; and (iii) the distribution of the RMSD values characterizing the best pose of each docking run according to the GoldScore scoring function.

Molecular dockings were carried out using PVM protocols on a high end server consisting of a 4-time AMD, 64-bit, 2.7 GHz quadcore Opteron running the Scientific Linux 5 operating system. Three-dimensional pictures and graphs were generated by SYBYL X-1.3 (Tripos Inc., St Louis, MO) and the R statistics package, respectively.

RESULTS AND DISCUSSION

The MLP filter was developed, implemented in GOLD, and tested on different crystallographic data sets known to the scientific community for their high quality and diversity.

MLP Filter and Astex Diverse Set. The Astex Diverse Set was first used to test the MLP filter because of its structural quality and its documented high GOLD performance.[27] As explained in the Material and Methods Section and depicted in Figure 1, two parallel docking runs were set up for each ligand conformation.

In the standard GOLD docking procedure, the original HFPs were used to define hydrophobic regions, whereas the hydrophobic regions in MLP-GOLD docking simulations were defined by using the HFPs obtained after applying the MLP filter (Table S1, Supporting Information).

The distribution of the RMSD values, calculated for each docking solution with respect to the corresponding crystal for both GOLD and MLP-GOLD methodologies, provides a global view of the impact of the MLP filter on GOLD docking runs. The Mann−Whitney U-test revealed that the two distribution curves, superimposed in Figure 2a, were significantly different (p-value of 0.01992). Box-and-whisker diagrams evaluated the spread of these data (Figure 2b). Information retrieved from these graphs, summarized in Table S2, Supporting Information, showed that the RMSD values of the MLP-GOLD docking poses were slightly more spread out, with an increased third quartile, higher whisker value, and larger percentage of outliers. Moreover, if docking success is defined by a RMSD value lower than 2 Å,[27] 74.6% of the docking results were successful with the standard GOLD calculations, whereas 72.3% were successful with the MLP-GOLD docking approach.

The docking solutions with the lowest RMSD value with respect to the corresponding crystallographic pose, $RMSD_{min}$, were retrieved from each complex, and the $RMSD_{min}$ distribution curves are displayed in Figure S2a, Supporting Information. Standard GOLD and MLP-GOLD docking processes were able to generate binding poses relatively similar to the native ones, with docking success rates of up to 80.0%. The performance of both methodologies was similar in this case (0.8826 is the p-value obtained from the Mann−Whitney U-test). The degree of dispersion values for the solutions most similar to the crystal structure was also comparable, as shown in the box-and-whisker diagrams (Figure S2b and Table S3, Supporting Information).

To evaluate the indirect impact of the MLP filter on the fitness assessment, the RMSD value of the pose with the best score with respect to the crystallographic one was calculated ($RMSD_{best}$) for each complex in the database. The MLP filter does not attempt to alter the scoring scheme. However, slight variations in fitness values were found for different positions of ligand moieties influenced by the filtered HFPs. The standard GOLD docking approach succeeded ($RMSD_{best}$ < 2 Å) in 81.5% of cases, whereas 80.0% of cases were successful by employing the MLP-GOLD procedure (Figure S3a,b, Supporting Information).

Statistical analyses on the docking results are summarized in Table 1. They revealed that the MLP filter did not drastically alter the standard GOLD docking performance even though a large number of HFPs, from 14.5 to 100%, were removed from each protein pocket before starting a docking job (Table S1, Supporting Information).

Lipophilic Index and MLP Filter: Two Key Concepts That Affect GOLD Docking Performance. The common experience reveals that GOLD, because of its hydrogen-bonding driven engine, is less effective when docking is guided by hydrophobic interactions.[26] Accordingly, Perola et al. suggested that the performance of GOLD is to some extent binding site dependent, with less precise results obtained when binding is mediated by hydrophobic interactions.[34] Thus, the

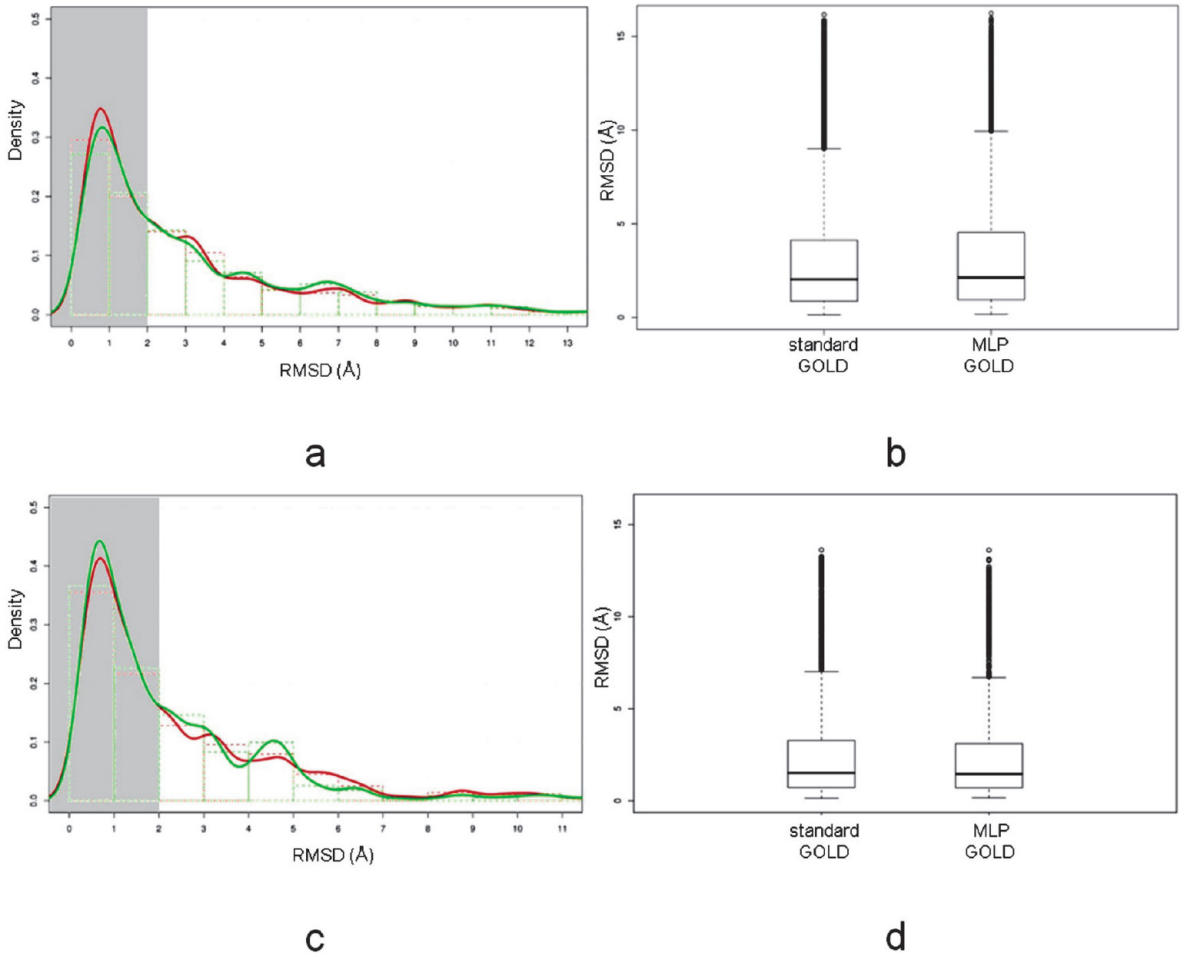

Figure 3. Distribution (a) and boxplot (b) of the 16 300 RMSD values from the PDB bind core database by applying the standard GOLD (red) and the MLP-GOLD (green) protocols, respectively. Distribution (c) and boxplot (d) of the 5400 RMSD values obtained by applying the standard GOLD (red) and the MLP-GOLD (green) protocols on the complexes of the PDB bind core database characterized by LI > 10%. The threshold for a docking success, fixed at 2 Å, is highlighted in gray.

link between lipophilicity of cavities, as defined by the MLP and GOLD's docking performance, was also investigated. A MLP-based parameter called the lipophilic index (LI), able to evaluate the lipophilicity of protein pockets, was introduced.

$$\mathrm{LI} = \frac{|\sum \mathrm{MLP}^{+}|}{|\sum \mathrm{MLP}^{+}| + |\sum \mathrm{MLP}^{-}|} \times 100 \qquad (3)$$

LI takes into account two parameters: the sum of the MLP values assigned to each HFP bearing a hydrophobic potential ($\sum \mathrm{MLP}^{+}$) and the sum of the MLP values assigned to each HFP bearing a polar potential ($\sum \mathrm{MLP}^{-}$).

The 65 complexes from the Astex Diverse Set were distributed into two groups: one containing proteins with purely polar binding sites (LI < 10%), one containing protein pockets mostly characterized by an apolar contribution (LI > 10%). Complexes satisfying the LI > 10% rule were selected to further investigate the benefits of the MLP definition of hydrophobicity in nonpolar pockets (Table S4, Supporting Information). The statistical protocol applied for the entire data set was herein employed to treat the docking results of the selected complexes. First, the RMSD values of all docking poses were considered (3200 solutions). Again, two non-normal distributions were obtained for both standard GOLD and MLP-GOLD protocols, as shown in Figure 2c. Significant differences between these distributions were confirmed by the U-test (p-value of 0.01726) and by the box-and-whisker plots (Figure 2d). RMSD values obtained through the MLP-GOLD procedure were less spread out than those from the standard GOLD protocol. In the first case, the third quartile and the highest whisker were 1.4 and 2.8 Å, respectively, compared with 1.6 and 3.2 Å obtained with the standard GOLD protocol (Table S2, Supporting Information). By considering an RMSD threshold of 2.0 Å for defining a successful docking, 80.6% of the results obtained with the standard GOLD procedure matched this criterion. The MLP-GOLD simulations performed better with 82.7% of dockings considered successful $\mathrm{RMSD}_{\mathrm{min}}$ value distributions were also analyzed (Figure S2c, Supporting Information). The two curves followed the same non-Gaussian distributions (U-test p-value of 0.8546). However, a slight discrepancy in docking success rate was observed, with 87.5 and 90.6% success rates for the standard GOLD and MLP-GOLD protocols, respectively. Box-and-whisker analyses also revealed a more extended spread of the $\mathrm{RMSD}_{\mathrm{min}}$ data for the standard GOLD procedure compared with the MLP-GOLD procedure (Table S3 and Figure S2d, Supporting Information). Consistent with previous observations, the $\mathrm{RMSD}_{\mathrm{best}}$ value distributions show that MLP-GOLD slightly outperformed the standard GOLD protocol (Table 1). The MLP-GOLD procedure succeeded in 90.6% of cases, whereas

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the standard GOLD docking method succeeded in 87.5% of dockings.

MLP Filter, LI, and Their Impact on GOLD Performance: the PDB Bind Core Database. To acquire a larger consensus on the robustness of our in-house tool, the standard GOLD and MLP-GOLD procedures were compared by analyzing a large test set composed of 163 protein−ligand complexes from the PDB bind core database.[28] The emphasis on diversity and on reliable binding affinity data renders this large data set ideal for testing molecular docking programs. Moreover, and in contrast with the Astex Diverse Set, this database was not explicitly built for evaluating GOLD docking performance.[27] The capability of both standard and MLP-GOLD protocols to reproduce crystallographic binding poses was compared by the same statistical protocol adopted for the Astex Diverse Set. Distribution curves and box-and-whisker boxes were generated to evaluate (i) the RMSD values of all of the docking solutions (Figure 3a−d); (ii) the solution with the lowest RMSD ($RMSD_{min}$) from each docking run (Figure S4a-d, Supporting Information); (iii) the RMSD of the solution with the best score from each docking run ($RMSD_{best}$) (Figure S5a-d, Supporting Information). Consistent with the results from the Astex Diverse Set, redocking results from the PDB bind core database revealed an insignificant decrease in GOLD docking performance (Table 1), even though an average of 77.2% of HFPs were withdrawn from each protein pocket by the MLP filter (Table S5, Supporting Information). Furthermore, the LI was used to classify the PDB bind core database to evaluate if a gain in docking performance occurred in the 54 nonpolar pockets characterized by an LI >10% (Table S6, Supporting Information). Indeed, statistical analyses of the 5400 retrieved docking poses revealed a general improvement in the docking results by using the MLP-GOLD method. RMSD value distributions and spreads explained this trend (Tables S2 and S3, Supporting Information; Figure 3c,d).

Benefits of the MLP Filter in the GOLD Docking Approach. Reliable docking results were obtained when the MLP filter was applied to redock complexes from the Astex Diverse and the PDB bind core databases by using the GOLD program (Table 1). This technique drastically diminished the number of HFPs in all protein pockets (Tables S1−S5, Supporting Information). From this observation, one would expect a detrimental effect on the proper guidance of hydrophobic ligand moieties toward favorable areas of interaction due to a lack of points. However, the filtering procedure yielded results comparable to those obtained with the standard GOLD procedure, confirming that the description of the hydrophobicity of protein cavities based on the MLP is applicable to GOLD.

A noteworthy finding is that the calculation of the MLP in binding pockets was not only useful for removing illegitimate HFPs that do not match the MLP definition of hydrophobicity (Figure S1, Supporting Information) but also for obtaining information about the hydrophobic−hydrophilic balance (lipophilicity) of protein binding sites through the so-called LI. Because this parameter enables a useful evaluation of lipophilicity, it was used to classify protein pockets according to this crucial property in molecular recognition. The docking success rate with the standard GOLD method was lower than that of the MLP-GOLD procedure whenever cavities with low polar contributions (LI > 10%) were considered. This evidence is consistent with the data reported in the literature. The original GOLD algorithm was primarily directed to find hydrogen-bond networks.[26] Only in later versions of the program hydrophobic interactions were detected through HFPs, in which van der Waals potentials should favor close contacts between hydrophobic groups.[35,36]

It should be noted that the LI does not take into account the repartition of polar/hydrophobic regions in space, reducing the lipophilic information to a single value. In few cases, the presence of certain HFPs in specific areas is fundamental for correct ligand positioning, even though the hydrophobic contribution is much lower than the polar contribution. In complexes such as 1sq5 or 1tow, which are classified as polar by the LI, a few but important hydrophobic regions that were properly identified by the MLP-GOLD approach influence the correct positioning of ligands in the binding site. In these cases, the MLP-GOLD approach outperformed the standard GOLD docking protocol, which failed in these cases as also reported in the literature[27] (Figure S6, Supporting Information). However, it has been shown in this work that the introduction of a 10% LI threshold ensures the MLP filter to not negatively affect the performance of GOLD. Thus, a certain degree of hydrophobicity in binding pockets can be considered an ideal condition for MLP filter users.

Because no individual parameters for metals exist in the MLP fragmental system, metalloproteins were not retained in this work. However, to test the influence of the MLP filter in presence of metals, 10 metalloproteins from the Astex Diverse Set were considered, and a unique negative MLP fragmental value was used for describing such elements. The negative fragmental value assigned to metals aims to emphasize their hydrophilic features[37] (Figure S7, Supporting Information). By using this approach, pockets from metalloproteins were classified as polar, with a LI lower than 10. That means that the inclusion of metalloproteins, by adding an empirical negative value for treating metals, would not alter the conclusions of the study: The MLP filter is a useful tool (1) for describing hydrophobic features of protein pockets and (2) for increasing the quality of docking in apolar cavities.

Table 1 summarizes the benefits of using the MLP filter as a GOLD add-on, which is also highlighted when the complexes from the Astex Diverse Set and the PDB bind core database are merged to form a database of 227 complexes from which 85 are selected according to the predefined LI hydrophobic threshold.

Successful Examples. As stated, GOLD docking performance can be affected by using the MLP-GOLD methodology. Two examples are reviewed in detail below.

For the case of 1nav, which is the human thyroid hormone receptor α in complex with the endogenous thyroid hormone, is of great interest from a pharmaceutical point of view because of its key role in growth, development, and homeostasis of mammals. Development of hormone agonists can lead to safe therapies for common metabolic disorders while avoiding cardiotoxicity.[38] The original cocrystallized ligand primarily binds to its receptor through two direct hydrogen bonds within the protein pocket. The backbone amide of Ser277 forms a hydrogen bond with the carboxylate functional group, and the side-chain of His381 makes polar contacts with the 4-hydroxyl group. Hydrophobic interactions involving halogens and the aromatic rings of the ligand also occur in two highly hydrophobic niches composed mainly of nonpolar residues: Phe385, Met388, Phe401, Phe405, Leu 292 and Ile222, Ile226, Met256, Met259, Leu287, Ile299.[38]

HFPs were generated (5695 points) in the pocket of the thyroid hormone receptor α (LI of 44.0%) and filtered (3434

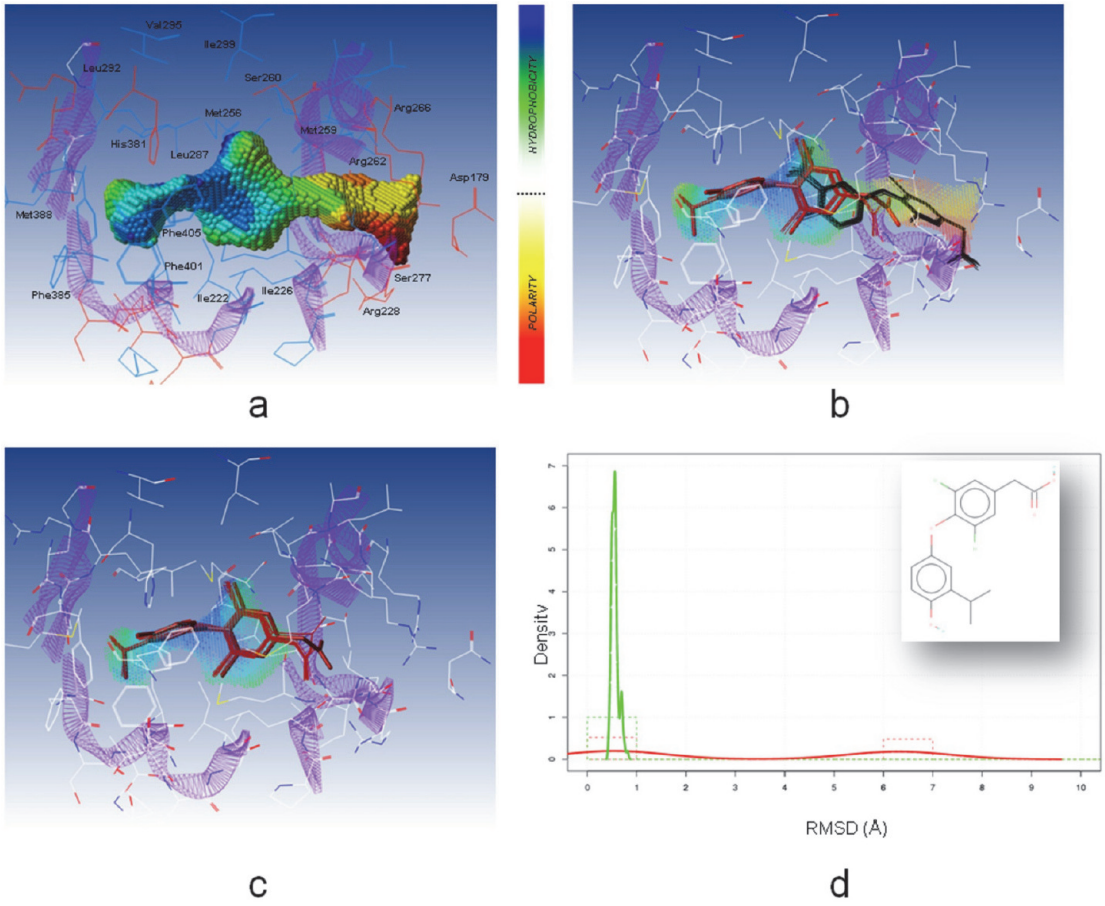

Figure 4. Binding site of the thyroid hormone receptor α from *Homo sapiens* (1nav PDB code). The HFPs are shown in the pocket, color-coded according the MLP scale range. Polar and apolar aminoacid side chains are colored in red and blue, respectively (a). The cocrystallized endogenous thyroid hormone is shown in red sticks (hydrogen atoms are hidden). GOLD docking solutions are visualized into the protein pocket and represented as red (cluster with RMSD values < 2 Å) and black (cluster with RMSD values > 2 Å) lines (b). Filtered HFPs leaded to 100.0% of docking solutions close to the crystallographic source (RMSD values < 2 Å), represented in the protein pocket as red lines (c). Distributions of the RMSD values calculated with respect to the endogenous thyroid hormone X-ray pose are represented in red (standard GOLD) and in green (MLP-GOLD). The 2D structure of the ligand (3,5-dichloro-4-[(4-hydroxy-3-isopropylphenoxy)phenylacetic acid) is also reported (d).

remaining points) through the MLP filter before proceeding with two parallel docking runs using the standard GOLD and MLP-GOLD methodologies, respectively. Among the 100 docking solutions retrieved by the standard GOLD approach, 48.0% were characterized by RMSD values higher than 2 Å with respect to the crystallographic binding pose (Figure 4d). GOLD predicts these solutions to be shifted into a zone that is rich in polar residues in which the carboxylate functional group is accommodated by creating a stable salt bridge with Arg228 (Figure 4b). The reason for this failure is related to a misleading definition of hydrophobicity in the binding site. The HFPs generated at the beginning of the docking simulation covered an extended area of the cavity, including a clearly polar region in the vicinity of Arg288 (Figure 4a). The MLP filter was able to recognize that polar region and remove points that guided the ligand in the wrong direction into the pocket (Figure 4c). Thus, 100% of the docking solutions were able to reproduce the experimental binding mode with the MLP-GOLD procedure, with RMSD values lower than 2 Å, demonstrating a strong improvement in the quality of docking (Figure 4d).

The hepatitis C virus RNA-dependent RNA polymerase is a key enzyme in virus replication. For this reason, understanding the binding site structural features is essential for developing antiviral compounds against the hepatitis C virus. The structure of this enzyme was solved in complex with non-nucleoside analog inhibitors, revealing that the nature of the interactions is primarily hydrophobic. The cyanophenyl thiophene group of the specific cocrystallized inhibitor CCT (PDB code 2d3u) establishes extensive van der Waals contacts in a zone that is rich in nonpolar residues (Val485, Leu489, Val494, Pro495, Pro496, Trp500), whereas the 2-methylphenyl moiety fills the hydrophobic binding cavity of the enzyme through van der Waals and stacking interactions (Leu419, Met423, Trp528). The carboxylate group also provides two stable hydrogen bonds with Tyr477 and Ser476 in the binding pocket.[39]

The cavity of the RNA polymerase, characterized by an LI of 11.8%, was filled with GOLD HFPs (6245 points) that were successively processed by the MLP filter (1970 remaining points) (Figure S8a, Supporting Information). Standard GOLD and MLP-GOLD procedures were applied, and the results were analyzed to examine the benefits of the MLP filter. The standard GOLD protocol reproduced the experimental binding mode with RMSD values lower that 2 Å in 48.0% of the docking poses (Figure S8d, Supporting Information). However, 52.0% of the docking poses were accommodated in a zone of the pocket in which the carboxyl group and the thiophene of the ligand can strongly interact with the Arg501 and Lys533

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side chains, respectively. Moreover, this binding mode is stabilized by the formation of a stacking interaction between the 2-methylphenyl ring and the side chain of Trp528 (Figure S8b, Supporting Information). As observed in the 1nav case, the HFPs considered as hydrophobic were in a zone of the pocket characterized by polar and positively charged amino acids. The MLP filter greatly improved the docking results because 100% of docking solutions had an RMSD value of lower than 2 Å with respect to the X-ray pose (Figure S8d, Supporting Information). The rigorous depiction of binding site hydrophobicity demonstrated by the removal of 68.5% of HFPs not only allowed for correct positioning of the hydrophobic rings but also led to the correct description of the anchoring polar interactions with the Tyr477 and Ser476 main chain amides (Figure S8c, Supporting Information).

CONCLUSIONS

The hydrophobicity term in GOLD, as in many other docking engines, is considered a purely steric property.[26,40,41] However, it has been demonstrated that hydrophobicity should take into account a subtle equilibrium between steric and weak electrostatic forces, as enclosed, for example, in experimentally determined log P_{oct} values. For this reason, a novel computational tool, the MLP filter, was developed to modify the GOLD docking process by restricting the search space of ligand hydrophobic moieties in binding site areas matching the accurate and experimentally-based MLP definition of hydrophobicity. Hence, the impact of such a description was evaluated in terms of docking accuracy by using crystallographic ligand−protein complexes from the Astex Diverse and the PDB bind core databases. The original and MLP-modified workflows showed comparable docking results. The standard GOLD methodology succeeded, especially in pockets in which the hydrogen-bonding contribution to the molecular recognition is essential, because hydrogen binding is the core of the GOLD docking algorithm. The hydrophobic contribution is simply evaluated by applying the definition of hydrophobicity enclosed in the Lennard-Jones potential between a carbon probe and each atom of the residues delimitating the binding site. This improper definition of hydrophobicity appears to decrease the ability of the program to predict ligand poses in cavities in which hydrophobic interactions dominate. In these specific cases, the inclusion of the MLP filter methodology was shown to more accurately reproduce protein−ligand binding modes, outperforming the classic GOLD approach.

In summary, the use of the MLP filter in GOLD can be beneficial for any kind of protein−ligand target because it uses a more detailed and experimentally-based definition of hydrophobicity in protein pockets and because higher docking success rates are obtained for proteins in which hydrophobic interactions with ligands are the main driving force. These findings suggest that the same machinery can be adapted to other widely used docking programs or used as a protein hydrophobic descriptor in drug design projects.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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