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Drug Distribution. Part 1. Models to Predict Membrane Partitioning

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Abstract

Purpose—Tissue partitioning is an important component of drug distribution and half-life. Protein binding and lipid partitioning together determine drug distribution.

Methods—Two structure-based models to predict partitioning into microsomal membranes are presented. An orientation-based model was developed using a membrane template and atom-based relative free energy functions to select drug conformations and orientations for neutral and basic drugs.

Results—The resulting model predicts the correct membrane positions for nine compounds tested, and predicts the membrane partitioning for $n = 67$ drugs with an average fold-error of 2.4. Next, a more facile descriptor-based model was developed for acids, neutrals and bases. This model considers the partitioning of neutral and ionized species at equilibrium, and can predict membrane partitioning with an average fold-error of 2.0 ($n = 92$ drugs).

Conclusions—Together these models suggest that drug orientation is important for membrane partitioning and that membrane partitioning can be well predicted from physicochemical properties.

Keywords

drug distribution; microsomal partitioning; molecular modeling

INTRODUCTION

Partitioning of drugs into membranes is an important component of both *in vivo* and *in vitro* drug distribution. With the possible exception of adipose tissue, cellular membranes are the largest contributor to lipid partitioning, and play a key role in tissue distribution. In addition, some cellular processes are driven by membrane concentrations (e.g. passive diffusion and efflux by P-gp (1)). For *in vitro* experiments, it is also necessary to know the unbound fraction of a drug, since this fraction interacts with drug targets. Many compounds such as hydrophobic amines partition into membranes, and in the presence of cells or membrane fractions, the unbound drug concentration can be much lower than total drug concentration.

Since microsomes are essentially unsorted phospholipid membranes and microsomal partitioning data is usually readily available, microsomal partitioning should provide the experimental input needed to represent general membrane partitioning during the process of drug distribution.

Many values of microsomal membrane binding are being reported, since membrane partitioning can impact both cytochrome P450 inhibition constants and microsomal clearance values (2–11). Work by Austin *et al.* showed a strong correlation between hydrophobicity and the microsomal membrane binding constant (6). This suggests that non-specific binding to microsomes is not associated with protein binding but instead is associated with the substrate partitioning into the microsomal membrane (12). Also, the general lack of species differences for this non-specific binding is consistent with partitioning into membranes rather than binding to microsomal proteins (2,13).

While the fraction unbound in microsomal membranes (f_{um}) can be measured experimentally, several models to predict f_{um} have been developed. Austin developed a linear relationship between LogP or LogD and f_{um} (6). Hallifax reported improved predictability with a quadratic relationship between Log P/D and f_{um} (14). More recently, Poulin *et al.* (15,16) have developed models to predict drug partitioning with microsomal or hepatocyte composition models. Like the tissue partitioning models in physiologically based pharmacokinetic (PBPK) modeling, the authors state that these models are mechanistic since they are derived from assumptions about microsomal lipid content and the interactions between drugs and these lipids. As with many PBPK modeling approaches (17–19), only bases are proposed to partition into acidic phospholipids and only neutral molecules are proposed to partition in to net-neutral phospholipids. Finally, this method also requires both experimental fraction unbound in plasma (f_{up}) and blood-to-plasma ratio (BP) in addition to LogP and pKa.

Most of the current models to predict f_{um} use LogP as a primary input. Although octanol does provide a good surrogate for general hydrophobic interactions in biological systems, the unique features of a lipid bilayer cannot be fully represented by a single solvent (20). Ideally, one could take advantage of the phospholipid composition and orientation to model drug-membrane interactions. For example, the polar head groups of phosphatidylcholine bilayers contain ionized choline and phosphate groups, the linkers consist of H-bond acceptors, and the hydrophobic core is essentially devoid of polar functionalities.

The goal of the present work was to use computational approaches to explore drug-membrane interactions and to develop a simple model with minimal chemical descriptors and standard physicochemical information (pKa, LogP) to predict f_{um} . Ideally this model would obviate the need for additional experimental data. In Part 2 of these manuscripts, experimental or predicted f_{um} values are used to construct models for volume of distribution (V_{ss}) prediction.

METHODS

Predictive Model for Membrane Orientation and Fraction Unbound in Microsomes (f_{um})

The compounds used for all models were restricted to monoprotic acids and bases, and neutral compounds (zwitterions were excluded). Experimental f_{um} values were available for 124 drugs (2,6,9,10,21–25). When more than one experimental value was available, either a consensus value across studies, or the value based on the best experimental design was selected. A microsomal protein concentration such that both sides of the dialysis apparatus had similar concentrations at equilibrium was considered to be the best experimental design. The physicochemical values and experimental f_{um} values are provided in Table S1. The experimental f_{um} values were normalized to 1 mg/ml microsomal protein by assuming linearity and calculating an average binding constant and predicting the value at 1 mg/ml. Linearity is assumed since membrane partitioning is expected to exceed protein binding (2,13). Molecules were divided into training and validation sets using the diverse subset selection routine in MOE software package (version 2014.09), with a set of descriptors that included molecular weight, charge, LogP, dipole, acceptors, donors, and polar surface areas.

For the orientation model, acidic compounds were excluded since it is possible that only the neutral fraction of these compounds partitions significantly into membranes. Since basic drugs likely bind as cations, only bases highly ionized at physiological pH were modeled. Lipid bilayer regions similar to those proposed by Balaz (20) were used. These regions included a polar head group region, a soft polymer region, and a hydrophobic core. We used the dimensions from a molecular dynamics simulation study of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine lipid bilayers by Heller *et al.* (26) to define the regions. With the bilayer oriented parallel to the xz plane, the polar head group regions extended from -2.5 to $+2.5$ Å, the soft polymer region extended from 2.5 to 7.5 Å, and the hydrophobic core was > 7.5 Å. Free energy functions were then constructed for atoms that reside in these three regions. These functions were constructed with combinations of logistic functions and are shown in Fig. 1. The negative free energy values of the plateau regions for each function were optimized to predict the observed value of LogK_L (see below).

Since it would be unreasonable to evaluate all drug conformations in all membrane orientations, a rational filtering method was developed. We took advantage of the molecular alignment routines in MOE by designing an artificial membrane template against which drug conformations could be aligned. For this template, we used a 10 phospholipid cluster extracted from a phosphatidylcholine lipid bilayer model (26). In order to use the similarity matching routine, ester groups (hydrogen bond acceptors) were changed to amidines (hydrogen bond donors/acceptors), phosphates were changed to protonated amines, phosphodiester oxygens to amines (hydrogen bond donors/acceptors), and quaternary amines were changed to carboxylates. Some choline groups were held constant to represent phosphatidyl serine. The resulting template (Fig. 2, coordinates in Supplementary Material) was used with the flexible alignment routine in MOE to align different molecular conformations of the drug molecule to the membrane template. The flexible alignment routine was modified with weightings of 2.0 for volume, H-bond donor, and hydrophobe atoms, 25 for acid/base, 0 for hydrogen bond acceptors, and default values for all other

parameters. An alpha value (SD of the Gaussian) of 1.0 Å was used. These modifications allowed the molecules to be aligned with hydrogen bond donors, acid/base groups, and hydrophobes, while ignoring hydrogen bond acceptors. Three hundred conformations for each drug in the gas phase were aligned to the template and stored in a MOE database. The best conformation and orientation was selected using svl code for a descriptor that incorporates the logistic-based free energy functions shown in Fig. 1. Using the selected conformations for the neutral and basic drugs, the plateaus of the logistic functions were then optimized within Mathematica to provide the best fit to the Log K_L values. This model was then transferred back to the svl descriptor code in MOE, and the molecules were reselected. The model was considered to be converged when R^2 values showed no further improvement.

Descriptor-Based Model for f_{um}

The model used to derive the equations for microsomal membrane partitioning is shown in Fig. 3.

The lipid binding constant (K_L) times the lipid concentration (L) can be calculated as $(1-f_{um})/f_{um}$. In terms of drug concentrations (see Fig. 3), the association constant for drug binding to lipid, K_L multiplied by the lipid concentration L, can be written as

$$LK_L = \frac{D_{u,L} + D_{i,L}}{D_u + D_i} \quad (1)$$

Rearrangement of Eq. 1 gives Eq. 2

$$LK_L = \frac{K_{unionized} + \frac{D_{i,L}}{D_u}}{1 + \frac{D_i}{D_u}} \quad (2)$$

Where $K_{unionized} = \frac{D_{u,L}}{D_u}$

From the Henderson-Hasselbach equation for acids, we know that

$$\frac{D_i}{D_u} = 10^{pH - pKa} \quad (3)$$

Substituting Eq. 3 into 2, and converting to log,

$$\text{Log} LK_L = \text{Log} \left(K_{unionized} + \frac{D_{i,L}}{D_u} \right) - \text{Log} \left(1 + 10^{pH - pKa} \right) \quad (4)$$

The term $\frac{D_{i,L}}{D_u}$ in Eq. 4 can be multiplied and divided by D_i and rewritten as

$$\frac{D_{i,L}}{D_u} = K_{ionized\ acid} 10^{pH-pKa} \quad (5)$$

Where $K_{ionized\ acid} = \frac{D_{i,L}}{D_i}$

Substituting Eq. 5 into 4,

$$LogLK_L = Log \left(K_{unionized} + K_{ionized\ acid} 10^{pH-pKa} \right) - Log \left(1 + 10^{pH-pKa} \right) \quad (6)$$

A similar equation can be derived for bases:

$$LogLK_L = Log \left(K_{unionized} + K_{ionized\ base} 10^{pKa-pH} \right) - Log \left(1 + 10^{pKa-pH} \right) \quad (7)$$

The expressions for $K_{unionized}$, $K_{ionized\ acid}$ and $K_{ionized\ base}$ were optimized with partial least squares (PLS) analysis with the software Moe as described under Methods. The following PLS expressions were used:

$$K_{unionized} = 10^{const1 + a1\ LogP + d1\ dipole + e\ SO + f\ NO2} \quad (8)$$

$$K_{ionized\ acid} = 10^{const3 + a3\ LogP + b3\ acc + c3\ don + e\ SO + f\ NO2} \quad (9)$$

$$K_{ionized\ base} = 10^{const2 + a2\ LogP + b2\ acc + c2\ don + e\ SO + f\ NO2} \quad (10)$$

When the acid, base and neutral models were combined, LogP and donors became statistically insignificant for the acid model and were removed. Finally, combining Eqs. 6 and 7 for modeling neutral, acidic and basic drugs simultaneously, and substituting in Eqs. 8–10, we get

$$\begin{aligned} \text{Log} (LK_L) = & \text{Log} \left(10^{\text{const}1+a1 \text{LogP}+d1 \text{dipole}+e \text{SO}+f \text{NO}_2} + 10^{\text{const}2+a2 \text{LogP}+b2 \text{acc}+c2 \text{don}+e \text{SO}+f \text{NO}_2+pK_{a,b}-7.4} \right. \\ & \left. 10^{\text{const}3+b3 \text{acc}+e \text{SO}+f \text{NO}_2+pK_{a,a}+7.4} \right) - \text{Log} \left(1+10^{pK_{a,b}-7.4} \right) - \text{Log} \left(1+10^{7.4-pK_{a,a}} \right) \end{aligned}$$

(11)

Where, const, a, b, c, d, e and f are optimized constants for the intercept, LogP, number of acceptors (acc), number of donors (don), dipole, number of S=O groups (SO), and number of NO₂ groups (NO₂), respectively. The pK_a values for acids and bases are pK_{a,a} and pK_{a,b}, respectively. This equation uses the pK_a values of acids and bases to model the partitioning of all species (neutral and ionized) simultaneously (see derivation above). This derivation does not simply use the fraction unionized but instead considers the equilibrium distribution of all species.

Three basic steps were used for the descriptor model development. First, a conformational analysis was performed using the MOE software package. The conformations were calculated in the gas phase to simulate a non-polar environment. Descriptors calculated for each conformation included total energy, polar surface area, dipole, energy of solvation, and amphiphilic moment (VsurfA (27)). Ultimately, it was found that sorting first on total energy (<3 kcal/mol above the lowest energy conformation) followed by the highest amphiphilic moment consistently provided the best models. For the second step, PLS was used to select the best set of descriptors for each class of molecules (neutrals, acids, and bases). The third step involved developing a combined equation that includes neutral and ionized species and the fraction ionized for all drugs. Equation 11 includes terms for unionized species (neutral drugs and the unionized fractions of acids and bases), and the ionized forms of acids and bases. The final descriptors are listed in Table S2. Use of a single equation for all neutral and ionized forms allows for the simultaneous optimization of parameters. This prevents solutions that have different models for the neutral fractions of each class of drugs.

Since the form of Eq. 11 is not amenable for PLS analysis, the coefficients for each of the terms were optimized within Mathematica (Mathematica 10.0.1) using the NonlinearModelFit function. The log of the binding constants was fit, and no further weighting was used. Goodness-of-fit was determined with R², parameter errors, and analysis of the correlation matrix.

RESULTS

For the orientation model, the optimized functions relating the atomic contributions to Log K_L versus membrane position are shown in Fig. 1. The function for hydrophobic atoms (Fig. 1a) shows an increasing contribution to partitioning with increasing distance from the aqueous phase. As expected, the greatest contribution to partitioning occurs when the hydrophobic atom resides in the hydrophobic core of the bilayer. For H-bond acceptors (Fig. 1b), all regions have negative coefficients. This is consistent with a general lack of H-bond

donors in all regions of the membrane. Lukacova *et al.* suggest that even the water molecules in the polar headgroup region maintain H-bond acceptor capacity but have decreased H-bond donor capacity (28). The optimized plateaus for H-bond donors (Fig. 1c) are zero for the polar headgroup region, slightly positive for the soft polymer region, and negative for the hydrophobic core. A positive coefficient in the soft polymer region may be expected since the polar functionalities in this region are H-bond acceptors (ester linkages). For cations (Fig. 1d), a positive coefficient of 0.43 in the polar headgroup region is the only truly optimized parameter in this function, since the alignment procedure forced cationic amines into this region. The large negative free energy values for the more hydrophobic regions were fixed to prevent the selection of orientations that place cations in these regions. A combination of these functions was able to correctly predict the orientation for nine compounds for which experimental orientations have been reported (20). These include compounds that reside in the polar headgroup, in the interface between the soft polymer region and the hydrophobic core, and within the hydrophobic core (Fig. 4). The resulting model was used to fit experimental f_{um} values for $n = 67$ neutral and basic drugs (neutral and basic from training set, Table S1). One consistent outlier (zidovudine) was removed. This compound was also an outlier for the subsequent descriptor-based model as well as in the V_{ss} models in Part 2 of these manuscripts. The resulting f_{um} predictions for the orientation model had an average absolute fold error (AAFE) of 2.4 and are shown in Fig. 5.

The next model developed for f_{um} uses LogP, pKa and five simple, structure-based descriptors. Although a plethora of structure-based descriptors is available, only descriptors that had a mechanistic justification were considered. Mechanistic considerations included terms that could represent hydrophobic interactions (LogP, polar surface area, solvation energy), hydrogen-bond donors and acceptors, descriptors that could describe favorable alignment in an ordered membrane (dipole moment and hydrophobic moment), and pKa values for acids and bases. During model development, it was found that the f_{um} for compounds containing nitro groups was under-predicted, while that for compounds containing sulfoxides and sulfones was over-predicted. This led us to incorporate descriptors for the number of these groups into the final model. The final model for partitioning into microsomal membranes is given in Eq. 11. Four parameters were selected by PLS for neutral drugs: the experimental LogP, dipole moment, the number of S=O groups, and the number of NO₂ groups. For ionized bases, selected parameters were experimental LogP, H-bond acceptors, H-bond donors, the number of S=O groups, and the number of NO₂ groups. The selected parameters for ionized acids were H-bond acceptors, the number of S=O groups, and the number of NO₂ groups. Partitioning of the unionized acid or base is modeled by the neutral term in Eq. 11.

The optimized f_{um} model for 92 molecules (94–2 outliers, zidovudine and cortisol) in the training set is given in Table I, and the plot of predicted *versus* experimental f_{um} is shown in Fig. 6a. The R^2 value for the fit including outliers was 0.80 with an AAFE of 2.1, and excluding 2 outliers R^2 was 0.85 with AAFE of 2.0. For the validation set ($n=30$), the R^2 value of the fit was 0.74 with 4 outliers and 0.87 without outliers (Fig. 6b), suggesting that optimization outside of PLS did not result in substantial over fitting. For the training set, the f_{um} values for acids, neutrals, and bases were predicted with similar accuracy with AAFEs

of 2.0, 2.2 and 1.8, respectively. For the validation set, these values were 2.4, 2.7, and 1.8, respectively.

DISCUSSION

In vivo drug distribution is determined by the balance between plasma protein binding and partitioning into membranes and tissue lipids. Although f_{up} is difficult to predict from structure due to the complexities of heterogeneous proteins with multiple binding sites, f_{um} prediction should be relatively straightforward due to interactions of drugs with ordered phospholipid membranes. In order to investigate drug membrane interactions, we developed models for f_{um} based on the assumption that acids, bases, and neutral drugs interact differently with the membranes. As described previously by Balaz (20), drugs can interact with three distinct regions in the membrane: the polar head group, the soft polymer region, and the hydrophobic core. The hydrophobic groups of neutral drugs will likely partition into the hydrophobic interior of the membrane. The cationic groups of basic drugs are assumed to interact directly with the negatively charged phosphate group of the phospholipid bilayer, and possibly acidic groups of phospholipids such as the carboxylate of phosphatidylserine. The lack of hydrogen bond donors in membranes makes it unlikely that ionized acids will significantly partition into membranes.

In order to investigate the three dimensional orientation of various drugs in the lipid bilayer, we first developed a drug-membrane orientation model. This model is parameterized with experimental microsomal membrane partitioning data. Microsomes are assumed to consist of unsorted phospholipids from the endoplasmic reticulum (13). The computational model for a modified phospholipid bilayer does not consider possible interactions with membrane associated proteins or sterols such as cholesterol. The importance of these interactions for membrane partitioning is unknown.

The membrane orientations of many molecules have been compiled and discussed by Balaz (20). Molecules were classified as residing primarily in the polar head group region, interface region, or hydrophobic core. Alignment with the template in Fig. 2 and sorting on the free energy functions in Fig. 1 accurately reproduced the observed orientation of several drug-like molecules (Fig. 4). Furthermore, the energy functions in Fig. 1 were optimized to predict the experimental f_{um} values for 67 neutral and basic drugs and the results are shown in Fig. 5. The accurate alignment of molecules within the lipid bilayer and the reasonable predictions of f_{um} suggest that the bilayer regions described by Balaz provide an adequate representation of the physiological membrane environment, and orientation effects are an important determinant of drug-membrane partitioning. In addition to supporting a three-region membrane model, these results also suggest that interactions with these membrane regions can be predicted *in silico*, and in turn be used to predict drug partitioning. Although future models may be more accurate, currently the orientation-based model is complex, requires specialized software, and cannot easily incorporate both ionized and neutral species for the same drug. Therefore, we developed a descriptor-based model for f_{um} .

The descriptor-based model described by Eq. 11 simultaneously considers both the ionized and neutral fractions of drugs. The free energy relationships shown in Fig. 1 suggest that

hydrophobicity, hydrogen bond donors, and hydrogen bond acceptors are important descriptors of f_{um} . For neutral compounds and ionized bases, LogP is the most important descriptor. This is consistent with previous models that have used LogP to predict f_{um} for neutral and basic drugs, and LogD to predict f_{um} for acids (6,14). In our model, the dipole moment is a significant descriptor with a negative coefficient (Table I) for neutral molecules, but not significant for ionized bases. Dipole moments for neutral compounds likely correspond to their molecular polarity, whereas ionized bases will exhibit high dipole moments due primarily to the cationic group. For bases, the important descriptors are LogP, H-bond donors, and acceptors. Since phospholipid membranes contain no H-bond donors, negative coefficients for acceptors and positive coefficients for donors are observed as expected (Table I). The observation that H-bond donors and acceptors are important descriptors for ionized bases but not for neutral drugs may be due to more specific membrane orientations for bases. The protonated amine of hydrophobic bases will likely be anchored to the polar head group, with the hydrophobic groups extending into the hydrophobic core. For acids, primarily the unionized form is expected to partition into membranes. For the ionized fraction of acids, LogP is not a significant descriptor, and acceptors have a positive coefficient. This is consistent with minimal partitioning of ionized acids into membranes.

Although NO₂ and SO descriptors were incorporated into the model in order to correct for a general under and over prediction of f_{um} , respectively, the reasons for the apparent systematic deviation of these compounds are unclear. Sulfoxide and sulfone groups are considered to be H-bond acceptors whereas NO₂ groups are not by most formalisms. The under prediction of nitro containing molecules may suggest that the nitro group is hydrophobic or interacts favorably with some part of the membrane. The over prediction of the S=O containing molecules suggests that there may be unfavorable interactions with these groups in the polar head group region. Since these groups were not evenly distributed into the various classes of compounds (acids, bases, neutrals) the descriptors were included for all classes. Additional data may suggest that these descriptors be modified or removed. Certainly, the present model does not provide sufficient justification to modify NO₂ and SO groups during lead optimization.

The training set for model development had two outliers, cortisol and zidovudine, and the validation set had four outliers, amobarbital, bosentan, cinoxacin, and losartan. Errors in the predictions could be due to either inaccurate experimental data or deficiencies in the model. Inter-laboratory variability in reported f_{um} values would suggest that both sources of error are likely. In fact, a comparison of V_{ss} predictions using predicted and experimental f_{um} values indicates that both are involved (see Part 2). For example, zidovudine was an outlier both for the present work and when experimental f_{um} values were used to predict V_{ss} . Also, use of predicted f_{um} for zidovudine resulted in accurate V_{ss} prediction (see Part 2).

Overall, the descriptor based model appears to be simple and useful for predicting f_{um} . Although a number of models to predict f_{um} have been reported (6,14,15,29), a commonly used model proposes a quadratic relationship between f_{um} and LogP or LogD (14). A comparison of f_{um} predictions using the quadratic relationship and Eq. 11 is shown in Fig. 7. The data for this figure is limited to those compounds for which experimental f_{um} , and LogP

or LogD are available ($n=112$). Using Eq. 11, 75% of the LogLK_L values were predicted within 3-fold, and 54% were predicted within 2-fold error with an $R^2 = 0.79$. In comparison, the quadratic equation provides 70% prediction within 3-fold error in LogLK_L , 47% prediction within 2-fold error and an $R^2=0.73$. For these data, the AAFE values are 1.7 vs. 2.5 for acids, 3.5 vs. 2.5 for neutrals, 2.1 vs. 1.8 for bases, and 2.5 vs. 2.3 for all compounds, for quadratic relationship and Eq. 11, respectively. Although the quadratic relationship has a lower AAFE for acids, if two outliers for the descriptor model are removed, the AAFE becomes 1.9. Outliers in these plots have not been removed, but zidovudine is highlighted and is poorly predicted by both models. The residual plots for the f_{um} predictions clearly show non-random residuals for the quadratic method but not for Eq. 11.

A recently reported composition-based model by Poulin and Haddad (15,16) uses membrane composition to predict f_{um} in a manner similar to that used to predict partition coefficients for PBPK models (17–19). The reported AAFE values cannot be compared since the authors used the actual f_{um} values rather than binding constants in the analysis. Converting the reported experimental and predicted f_{um} values to binding constants ($\text{LK}_L=(1-f_{um})/f_{um}$) and excluding experimental values of 0.99 and 1.0, the AAFE for that model is 4.0. If predicted f_{um} values > 0.98 are excluded, the AAFE is 2.4. Therefore, the descriptor-based model described above (AAFE = 2.1) compares favorably to previously reported models.

In summary, we have explored factors that determine the orientation and affinity of compounds in phospholipid membranes. Using membrane alignment routines and an atom-based free energy model, we can accurately predict the orientation of several drugs in the membrane. This model can also predict the affinity of neutral and basic compounds with an AAFE of 2.4. A descriptor-based model that considers neutral and ionized fractions of acids, neutrals, and bases can predict the microsomal membrane binding constants with an AAFE of 2.1. The results of this study suggest that orientation effects are an important component of membrane partitioning and that binding constants can be accurately predicted from simple descriptors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

acc	Number of acceptors
don	Number of donors
f_{um}	Fraction unbound in microsomal incubation
f_{up}	Unbound fraction in plasma
K_L	Association constant for drug binding to the lipid

K_p	Tissue partition constant
L	Amount of lipid in the tissue
NO₂	Number of NO ₂ groups
PBPK	Physiologically based pharmacokinetic models
pKa, a and pKa,b	pKa values for acids and bases are respectively
PLS	Partial least squares
SO	Number of S=O groups
V_{ss}	Steady-state volume of distribution of a drug

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Fig. 1.

Free energy functions for the orientation model. Functions of atom-based relative free energy *versus* vertical position across a leaflet of the lipid bilayer for (a) hydrophobe, (b) H-bond acceptor, (c) H-bond donor, and (d) cation are shown.

Fig. 2.

Template for the orientation model. Inverse membrane template used to align molecules for efficient selection of membrane-specific conformations and orientations. Ester groups (hydrogen bond acceptors) were changed to amidines (hydrogen bond donors/acceptors), phosphates were changed to protonated amines, phosphodiester oxygens to amines (hydrogen bond donors/acceptors), and quaternary amines were changed to carboxylates. This template was used with the flexible alignment routines in MOE (Chemical Computing Group). The lines for the different regions approximate the middle of the transition areas between regions in Fig. 1.

Fig. 3.

Model for predicting f_{um} . D_u – unionized drug concentration, D_i – ionized drug concentration, $D_{u,L}$ – unionized drug concentration in lipid, $D_{i,L}$ – ionized drug concentration in lipid.

Fig. 4. Predicted orientation of compounds in phospholipid membranes. Placement was predicted with the orientation-based model, and compared with known orientations (20). *A*: propranolol (interface), *B*: Ciprofloxacin (polar head group region), *C*: Ibuprofen (interface), *D*: O-CH₃- 8 -tetrahydrocannabinol (hydrophobic core), *E*: 4-C(CH₃)₃-[3-(CH₂)₂Cl-ureido] benzene (interface) *F*: bisphenol A (interface), *G*: Amioradone, *H*: 2-[4-N(CH₃)₂-phenyl]-3-OH-flavone (hydrophobic core), *I*: Fluoxetine (interface).

Fig. 5. Predictions of f_{um} with the orientation model. Predicted *versus* experimental Log LK_L values are plotted for $n=67$ neutral and basic drugs. AAFE is average fold error.

Fig. 6. Predictions of f_{um} with the descriptor-based model. Predicted *versus* experimental $\text{Log L} \cdot K_L$ values are plotted for A) $n=92$ drugs in the training set, and B) $n=30$ drugs in the validation set. See drug list in Table S1. Red: acidic drugs, blue: basic drugs, green: neutral drugs. Open circles represent outliers. The dashed and dotted lines represent 2-fold and 3-fold error, respectively. R^2 values including and excluding outliers are listed.

Fig. 7.

Comparison of f_{um} models. Predictions of f_{um} were compared with the present model, Eq. 11 (left panels, **a** and **c**), and the quadratic equation by Hallifax *et al.* (14) (right panels, **b** and **d**). Predicted *versus* experimental $\log L \cdot K_L$ values are plotted (**a** and **b**; $n=112$; zidovudine is *circled*), along with residual plots (**c** and **d**). Red: acidic drugs, blue: basic drugs, green: neutral drugs. The *dashed* and *dotted* lines represent 2-fold and 3-fold error, respectively.

Table I

Optimized Model Parameters and Parameter Errors for the Descriptor Based f_{um} Model (Eq. 11) with the Training Set (Table S1)

Model parameter	Including outliers ($n=94$)	Excluding outliers ($n=92$) ^a
R ²	0.80	0.85
Average fold error	2.1	2.0
Const1	-0.92±0.24	-1.36±0.23
a1	0.52±0.063	0.63±0.059
d1	-0.63±0.17	-0.55±0.15
Const2	-1.57±0.34	-1.55±0.29
a2	0.53±0.069	0.53±0.060
b2	-0.12±0.084	-0.12±0.073
c2	0.21±0.17	0.21±0.15
Const3	-1.56±0.36	-1.56±0.31
b3	0.27±0.092	0.26±0.080
e	-0.45±0.11	-0.43±0.091
f	0.76±0.22	0.77±0.19

Data are presented as parameter±error

$$\begin{aligned} & \text{Log} (L * KL) = \\ & \text{Log} \left(10^{\text{const1}+a1 \text{ LogP}+d1 \text{ dipole}+e \text{ SO}+f \text{ NO2}} + 10^{\text{const2}+a2 \text{ LogP}+b2 \text{ acc}+c2 \text{ don}+e \text{ SO}+f \text{ NO2}+pKa,b-7.4} \right. \\ & \quad \left. + 10^{\text{const3}+b3 \text{ acc}+e \text{ SO}+f \text{ NO2}-pKa,a+7.4} \right) - \text{Log} \left(1+10^{pKa,b-7.4} \right) - \text{Log} \left(1+10^{7.4-pKa,a} \right) \end{aligned}$$

^aOutliers were cortisol and zidovudine