This ia a postprint of *J. Phys. Chem. B*, 2008, 112 (38), pp 12148–12157. The original article can be found under http://pubs.acs.org/doi/abs/10.1021/jp801736k

COSMOmic: a mechanistic approach to the calculation of membrane-water partition coefficients and internal distributions within membranes and micelles

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Calculation of membrane-water partition coefficients

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ABSTRACT A new approach for the modeling of molecules in micellar systems and especially in bio-membranes, COSMOmic, is presented and its performance is validated on the example of the partitioning of molecules between water and biological membranes. Starting from quantum chemical calculations of the surfactant, solvent, and solute molecules, and being based on the COSMO-RS method for fluid phase thermodynamic properties, COSMOmic is essentially free of additional adjustable parameters. The inclusion of an elastic energy correction into the COSMOmic model did not turn out to yield any significant improvement. The novel COSMOmic method allows for the efficient prediction of distribution of molecules in micellar systems.

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Introduction

The partitioning into and distribution of molecules in biological membranes is of vital interest for drug design and environmental sciences. New insights into membranes at the molecular level make it possible to understand i) drug targeting, penetration, and permeation of cell membranes, ii) the interaction of drugs with membrane-bound receptors, and iii) the use of liposomes as smart drug-delivery systems.[1]

Therefore, a number of experimental methods using membrane lipids were developed over the last decades, with the majority based on liposomes, i.e. lipid bilayer vesicles. The partition coefficients between water and liposomes, referred to as K_{lipw} or K_{mw}, is the most popular experimental model to quantify the uptake into biological membranes as documented by several thorough reviews,[2-4] while immobilized artificial membranes[5,6] and solid-supported lipid membranes (SSLM)[7-9] are frequently used for high-throughput studies in life sciences. However, when it comes to computational chemistry applications in drug design or environmental sciences the partitioning into biological membranes is still predominantly approximated by K_{ow}, the octanol-water partition coefficient.

Two reasons explain the enormous success of octanol-water partition coefficients in computational chemistry. First, K_{ow} is the partition coefficient with largest number of experimental data and these data are easily accessible which explains the numerous empirical approaches.[10] On the other hand there is no established database of membrane-water partition coefficients, except a collection focused on potentiometric titration[11] and a recently published collection of data compiled from the open literature.[12] The second reason for the success of K_{ow} is that in many cases there is a good correlation between K_{mw} and K_{ow}. For a series of 121 compounds with experimental log K_{ow} between 1 and 5 a linear relationship was determined[12]

$$\log K_{mw} = 0.92\ (\pm 0.062)\cdot \log K_{ow} + 0.37(\pm 0.20) \qquad (1)$$

which had a good fit with $R^2 = 0.88$ and RMSE = 0.41. Admittedly, the correlation of log K_{mw} and log K_{ow} is surprisingly good and thus, if a measured K_{ow} is available it will on average lead to better predictions than any calculation method (an RMSE < 0.4 log units is very hard to beat by calculation methods). The homogeneous, water-saturated octanol phase appears to be a quite good model for the interior of biological membranes. But this must be considered as a lucky coincidence, because biological membranes are not homogeneous, and such a simplified description does not give any information about the specific locations and free energy barriers of the solutes. And most likely it will fail for molecules which are more bound in the outer region of the membrane. A first indication for a failure of the octanol model is the value of the slope of eq. 1 which points at lower log K_{mw} for highly lipophilic compounds as measured by Gobas *et al.* for a series of halogenated aromatic hydrocarbons with a log K_{ow} ranging from 3.0 to 8.4.[13] However, such results are controversial amongst experimentalists and recently two studies argue for a linear relationship even for highly lipophilic compounds with log K_{ow} up to 8.1.[14,15] Towards the lower end of the lipophilicity scale a similar situation exists, i.e. it is not unlikely that there is no linear relationship between log K_{ow} and log K_{mw} as the zwitterions of the polar head groups offer a much more polar environment than octanol (as indicated by the positive intercept of eq. 1).

The clearest evidence that compounds with low lipophilicity have more affinity towards membranes is the partition behaviour of ionic organic compounds. Both organic anions and cations have K_{mw} which

are orders of magnitude larger than their K_{ow}.[16-18] Thus, the lipophilicity of charged compounds is dramatically underestimated by approaches using K_{ow} as a measure for the partitioning into biological membranes and some tools even assume that only the neutral species partitions into the lipophilic phase, an assumption which leads to further underestimation of the true partition coefficient. The treatment of charged species is especially important for drug design considering that more than 30 000 compounds in the 1999 world drug index are acids or bases which corresponds to 63% of all drugs.[19]

Despite the good empirical performance of the log K_{mw} estimations based on log K_{ow} in the case of neutral compounds, such empirical correlations cannot provide any mechanistic insight into the partitioning of drugs between water and bio-membranes, and they specially cannot answer the advanced questions raised above, e.g. the treatment of ionic organic compounds. Mechanistic models with a more detailed description of the anisotropic membrane system are required for such answers. However, the precondition for mechanistic models of membrane-water partitioning of charged compounds is an advanced understanding of the partitioning of neutral compounds. Therefore this study will focus completely on neutral compounds while charged species will be treated in a forthcoming publication.

To date very few approaches for the calculation of membrane-water partition coefficients exist. Two structure-property relationships (QSPR) were published by Vaes *et al*.[20] and Patel *et al*.[21] and in both studies models based on log K_{ow} plus additional descriptors performed substantially better than models based only on calculated descriptors. The correlations in both studies were high ranging from 0.87 to 0.9 (for models based solely on calculated descriptors). The sizes of the datasets were $n = 28$ and $n = 49$, respectively, and cover a limited structural diversity which is a drawback for empirical approaches like QSPRs.

The only approaches based on a molecular description of the membranes are molecular dynamics simulations (MD). In MD studies it is possible to study the distribution of solutes in water-membrane systems with explicit treatment of both the solvent and the solute. While such computationally expensive calculations can be very useful to understand a large number of phenomena like effects of the solute on the membrane,[22] permeability,[23] lateral pressures,[24] and especially interesting the internal distribution within the membrane [22,23] they do not appear suited to calculate the absolute values of K_{mw}. Especially, it is questionable whether standard non-polarizable force-fields can handle the crossover of polar molecules from polar to non-polar regions of such systems with the accuracy which is required for a reliable description of the distribution of partially polar molecules in such systems. Most importantly, the MD simulation of solutes in biomembranes requires extremely time-consuming simulations for each solute and hence is not suited for a rapid screening of drug compounds.

The goal of the present work is a model which combines advantages of both approaches allowing the calculation of the absolute values of K_{mw} of organic compounds regardless of their chemical class, but also giving insights into the internal distribution over the membrane. The possibility to calculate internal distributions opens the door to describe vast number of processes like permeability,[23] toxic effects of protonophores,[12,25] and washout rates of drugs which determine their half-life.[26] Furthermore, insights into the internal distributions of drugs are of importance to understand why some reach membrane-bound receptors better than others, if, according to Herbette *et al.*, certain drugs exert their pharmacologic effects by interacting with membrane proteins from within the lipid milieu rather than from intracellular sites.[27,28]

The model referred to as COSMOmic (COSMO-RS for micelles) is an extension of COSMO-RS an approach based on quantum-chemical calculations and a statistical thermodynamics algorithm for independently pair-wise interacting surfaces[29-31] and is based on an earlier diploma thesis of Busalla.[32] As the diploma study, which was performed at Bayer AG, the present paper will focus on lipid bilayers,

because these most likely are the most important and best investigated self-organized micellar systems, with the largest amount of experimental partition data available. Nevertheless, the simulation method presented here is equally well applicable to other micellar systems composed of non-ionic or ionic surfactants.

Evaluation of experimental data

The composition of biological membranes differs among tissues which results in differing partition coefficients. Therefore, partition coefficients measured with liposomes are preferable because they can be produced with a well-defined chemical composition. Their common feature is that they consist of amphiphilic lipid molecules, typically phospholipids, with their hydrophobic acyl chains assembled in the bilayer core and the polar head groups facing towards the water phases inside and outside the vesicle. They differ by the type of phospholipids which affects the charge status (neutral, zwitterionic, charged) and the phase (liquid-crystal state, gel state). Liposomes of a given composition can be prepared with different techniques resulting in differ sizes and different lamellarity (uni-lamellar, multi-lamellar) and additionally there is a large number of methods for the determination of the partition coefficient (equilibrium dialysis, potentiometric titration, ultracentrifugation and ultra filtration). A good overview of all these experimental aspects is given by Krämer[2].

For the goals of these studies the following criteria were used to compile the experimental liposome-water partition coefficients: 1) determined with zwitterionic phospholipids, 2) in liquid crystal-state, 3) preferably unilamellar, 4) and preferably equilibrium dialysis, but also some data with ultracentrifugation and potentiometric titration if the latter fulfill the criteria given in ref [2].

Special attention has been paid to criterion 2) because in their active state biological membranes are generally in the liquid crystal-state. As liposomes with a defined composition have a well defined transition temperature this criterion directly defined which phospholipids could be used for the present study. All K_{mw} used here were determined with either 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), if measured above 25 °C (DMPC has a transition temperature of 24 °C [33]), or egg yolk lecithin.

The use of data determined with four different types of phospholipids still could be a considerable source of variability. In order to get an estimate of this variability compounds measured in more than one lab and fulfilling the criteria 1-4 above were compared using the studies cited in ref [12] and additionally refs. [16,34,35]. This resulted in a total of 35 partition coefficients (all for the neutral species) measured in 9 different laboratories with an average absolute difference of 0.18 and a root mean squared error of 0.22 which is a surprisingly low variability between labs compared to other physico-chemical properties like solubility. Finally, for partition experiments at fixed pH the pK_a of the compound was checked to make sure that the K_{mw} of the neutral species was measured. Note, that the data set of ref [12] also contains compounds which are not neutral at physiological pH, e.g., salicylic acid with a pK_a of 3. However, as the partition coefficients of these compounds were measured over a large pH range, it was possible to determine both the partition coefficients of the neutral and the charged species, respectively. Only the partition coefficient of the neutral species was used in the present study.

Two data sets were used in this study. The first data set is the previously mentioned collection of literature data [12] that was compiled with the criteria described above. This data set was split into a training set of 105 and a test set of 56 compounds by ordering the data according to their K_{mw} (within the chemical classes). The test set was only used after the final model had been established and will be referred to as test set 1.

The second data set, referred to as test set 2, with a total of 91 neutral drugs was taken from a previously published in-house collection of data from Bayer Healthcare.[9,36] These data were measured at pH 7.4 with egg lecithin SSLMs also known under the trade name TRANSIL.[8,9] Again, the pK_a values of the compounds were checked to make sure that the K_{mw} of the neutral species was measured. TRANSIL measurements showed a good correlation (r^2 = 0.92) with the expensive equilibrium dialysis method in the case of neutral compounds,[9] but the slope of the log-log plot differed (0.86) which makes a separate analysis of the data sets advisable.

Theoretical Calculations

In order to reach the goal of establishing COSMOmic as a mechanistic membrane model the essential difference between the partitioning process into a bulk solvent like octanol and an anisotropic solvent like phospholipid bilayers needs to be reflected in the model.

It should be possible to achieve this goal to a large extent by the basic assumption of the COSMOmic approach which is to consider the bilayer or micellar system as a layered liquid. These layers may be spherical shells, as in the case of spherical micelles, cylindrical shells as for cylindrical micelles, or just flat layers as for lamellar systems, e.g. biomembranes. Note, that in this section all statements using the term micelle also apply to membrane bilayers. In difference to a bulk, homogeneous liquid each layer has a specific composition with respect to the solvent system, i.e. with respect to atoms of the surfactant molecules and ions, which compose the micelle and its mostly aqueous solvent environment. This assumption is visualized in Figure 1 for the case of a phospholipid bio-membrane.

FIGURE 1 HERE

Let us assume that the detailed membrane composition is known either from experimental data or from other simulation techniques as MD/MC. For a COSMOmic simulation a table describing the probability distribution of the atoms with respect to the layers must be derived from such data as input for COSMOmic.

COSMOmic also requires DFT/COSMO files for all species composing the micellar system, as well as for the compounds to be treated as solutes in the micellar system. However, as we have the probability distribution of the atoms it is not necessary to calculate a whole membrane system, but it is sufficient to calculate one phospholipid and one water molecule (and salts if necessary). The theory and details for the calculation of such COSMO files is given elsewhere.[31] Briefly, the calculations consist of the following steps:

1) creation of start geometry, e.g. using CORINA[37],

2) full DFT geometry optimization with the Turbomole program package[38,39] using the B–P density functional[40,41] with TZVP quality basis set and applying the RI approximation[42] and applying the COSMO continuum solvation model in the conductor limit ($\varepsilon = \infty$)

3) analysis of tautomers and conformations if necessary.

4) Next we go beyond the dielectric continuum solvation models, following the concepts COSMO-RS.[28-30] In COSMO-RS the solute and solvent molecules are described by the histograms of the molecular surface with respect to the COSMO polarization charge density σ, the so-called σ-profiles $p^X(\sigma)$. A bulk solvent then is characterized by its normalized solvent σ-profile $p_S(\sigma)$, which is just the sum of the σ-profiles of the components multiplied by their mole fractions, and normalized by the average surface area.

5) Finally COSMO-RS expresses the molecular interactions as pair-wise surface interactions based on the polarization densities σ and σ' of the surfaces, and performs a statistical thermodynamics of the interacting surface pieces in order to end up with free energies and chemical potentials.

6) Next we make use of the fact, that the COSMO files, and in this case the COSMO files of the micelle molecules, hold all information about the COSMO surface polarization charges on small surface segments of the COSMO cavity. Because each surface segment in a COSMO file is associated to its underlying atom, the probability distribution of the atoms of the micellar system can be easily converted into a probability distribution for the individual surface segments. The probability distribution of the surface segments in the micellar system corresponds to a specific σ-profile $p_M(\sigma, r)$ for each layer, where M denotes the membrane system, e.g. aqueous DMPC, and r is the radial distance of the center of the layer from the symmetry center of the micellar system.

7) For the next step we treat each layer like a homogeneous liquid and convert the σ -profile $p_M(\sigma, r)$ of each layer into a layer specific σ -potential $\mu_M(\sigma, r)$, applying the same algorithm as used for the standard COSMO-RS model. With this σ-potential, i.e. the chemical potential of an effective surface segment of polarity σ in a layer, we now have an effective potential which tells us how much a certain layer of a micellar system likes surface of a certain polarity σ.

8) Now we can evaluate the chemical potential of a solute molecule X at fixed position and orientation in such micellar system M in the following way: Let us denote the radial coordinate of the center position of X by r^X, and the orientation of X with respect to the radial vector of the micellar system by a unit vector $\underline{d}^X$. The radial distance r_ν of each surface segment ν of the solute can then easily be evaluated. As in ordinary COSMO-RS, we now evaluate the chemical potential contribution arising from the COSMO-RS surface segment paring $\mu^X_{M,CRS}(r^X, \underline{d}^X)$ by summation of the individual segment chemical potentials, which in difference to the standard COSMO-RS now depend on the position of each segment ν with respect to the micellar system:

$$\mu^X_{M,CRS}(r^X, \underline{d}^X) = \sum_{\nu \in X} a_\nu \mu_\nu \left(r_\nu (r^X, \underline{d}^X) \right) \equiv \sum_{\nu \in X} a_\nu \mu_M \left(\sigma_\nu, r_\nu (r^X, \underline{d}^X) \right) \qquad (2)$$

where the second equality of eq. 2 holds for the simplified case of just σ -based interactions. This CRS part of the chemical potential usually is the dominating part, at least for polar compounds. By the summation of the local segment chemical potentials this part is responsible for favoring such positions and orientations of a molecule X in which the more polar parts are located in more polar regions of the membrane system and the less polar ones in less polar regions, respectively.

9) In general, we consider additional contributions to the total chemical potential of the solute X in a certain position and orientation:

$$\mu^X_M(r^X, \underline{d}^X) = \mu^X_{M,CRS}(r^X, \underline{d}^X) + \mu^X_{M,comb}(r^X, \underline{d}^X) + \mu^X_{M,elast}(r^X, \underline{d}^X) + \mu^X_{M,zeta}(r^X, \underline{d}^X) \qquad (3)$$

As in ordinary, bulk solvent COSMO-RS the chemical potential includes a small but significant combinatorial contribution, which takes into account size and shape ratios of solute and solvent molecules. In bulk, homogeneous liquids the combinatorial chemical potential of a solute X is based on the mole fractions of the different molecular species and their volumes and surface areas. In close analogy, we here evaluate the local combinatorial contribution $\mu^X_{M,comb}(r)$ of a solute X at a radial position r based on the local molar composition of the micellar system. Because the solute molecule is extended within the micellar system, we calculate the combinatorial contribution for a solute X at a certain center position r^X and orientation $\underline{d}^X$ as a surface average of its segments:

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$$\mu^X_{M,comb}(r^X,\underline{d}^X) = \sum_{\nu\in X}\frac{a_\nu}{A^X}\mu^X_{M,comb}\left(r_\nu(r^X,\underline{d}^X)\right) \qquad (4)$$

10) Contribution of the elastic deformation free energy: The third term in eq. 3 reflects the elastic deformation free energy of the micellar system by the solute X. Such deformation energy does not exist in a bulk liquid, but in an inhomogeneous system we must be aware that such contribution may exist and may be relevant. Unfortunately there is very little quantitative and qualitative knowledge available about the deformation energy of micellar systems. We analyzed a number of different publications concerning this aspect,[24,43-45] but we have not been able to extract any general and transferable information, and partly the results of the different studies even appear to be in contradiction. A major problem in the analysis of the elastic term is that in simulations the elastic term cannot be evaluated separately from the other energy contributions. Therefore we decided to construct a new functional form for the elastic deformation energy of micellar systems based on simple, plausible assumptions and consistency requirements. The starting point is the assumption that the elastic deformation energy shall in some way have a quadratic dependence on the local volume perturbation resulting from a solute, with a local elastic coefficient. In this way the elastic perturbation energy of a micellar system by a sphere would increase with the squared volume and it would depend on the position of the sphere by the elastic coefficient. As a necessary requirement, the elastic coefficient has to approach the limit of zero in the pure bulk solvent. It is expected to be most important in the highly ordered regions of the micellar system, which are most likely those regions with small water concentrations and a small number of alkyl chain ends. On the other hand, such expression would result in a large elastic deformation energy for a cylindrical perturbation penetrating through a micellar bilayer. But the elastic deformation of a bilayer by such cylinder should be zero, because the cylinder just pushes the micelle molecules outward, without any resulting stress or strain. From this example we learn that the elastic energy should not depend on the local perturbation volume, but on the variation of the local perturbation volume in the direction of the system normal vector. Hence we introduce here an empirical approximate expression for the elastic deformation energy of a micellar system by a solute X at a certain center position r^X and orientation $\underline{d}^X$ as

$$\mu^X_{M,elast}(r^X,\underline{d}^X) \cong \int_{-\infty}^{\infty} c^0_{M,el}\left(1 - x_S(r) - \lambda_{M,end}\frac{c_{end}(r)}{c_{end}(0)}\right)\left[\frac{\partial A^X_{cs}(r;r^X,\underline{d}^X)}{\partial r}\right]^2 dr \qquad (5)$$

Here $x_S(r)$ is the local solvent mole fraction, $c_{end}(r)$ is the local volume concentration of alkyl end groups, and $c^0_{M,el}$ and $\lambda_{M,end}$ are two adjustable parameters. $A^X_{cs}(r;r^X,\underline{d}^X)$ is the local cross section of the solute X .The derivative of $A^X_{cs}(r;r^X,\underline{d}^X)$ with respect to r can be relative easily calculated from a summation over the contributions of the surface segments of the solute X.

11) Role of electrostatic zeta potential: The final contribution in eq. 3 results from the electrostatic zeta potential $\Phi^{zeta}_M(r)$ of the micellar system. While in a normal, disordered bulk solvent no long range electrostatic potential exists, the self-organization order of a micellar system usually causes non-vanishing net charge densities in some of the layers of the system, resulting in a long range electrostatic potential, usually called zeta potential. Since the electrostatic interaction energy of such potential with a charge is just the product of potential and charge, and since each surface segment ν of a solute X just carries a charge of $q_\nu = a_\nu\sigma_\nu$, the contribution of the zeta potential to the total chemical potential of a solute X at a certain center position r^X and orientation $\underline{d}^X$ can be trivially calculated as

$$\mu^X_{M,zeta}(r^X, \underline{d}^X) = \sum_{\nu \in X} a_\nu \sigma_\nu \Phi^{zeta}_M\left(r_\nu(r^X, \underline{d}^X)\right) \qquad (6)$$

Unfortunately, our attempts to derive the zeta potentials of micellar systems from the results of MD simulations resulted in unrealistically strong zeta potentials of up to 5 V, which would result in completely unphysical localizations of ions in the membrane systems. Because for neutral solutes even the artificially strong zeta potentials are of small influence on the overall distribution, we neglect the zeta potential from now on, since only neutral solutes in micellar systems are considered. Nevertheless, for the simulation of ionic solutes the zeta potential cannot be neglected. Therefore the question of zeta potentials will be reconsidered in a forthcoming paper dealing with ionic solutes in micellar systems.

12) Chemical potential of solute in membrane layers: Based on eq. 3, COSMOmic then calculates the chemical potential of each solute centered in each of the layers and for a large number of evenly distributed orientation vectors. This sampling of all possible positions and orientations is schematically shown in Figure 1. Thus we yield the partition function of the solute X with respect to all positions n center positions and all m orientations.

$$Z^X_M = \sum_{i=1}^{n} Z^X_M(r_i) = \sum_{i=1}^{n}\sum_{j=1}^{m} \exp\left\{\frac{-\mu^X_M(r_i, \underline{d}_j)}{kT}\right\} \qquad (7)$$

Now the probability to find the solute X in layer i is given by

$$p^X_M(r_i) = Z^X_M(r_i) \Big/ Z^X_M \qquad (8)$$

and the free energy profile of the solute throughout the micellar system is given by

$$G^X_M(r_i) = -kT \ln p^X_M(r_i) \qquad (9)$$

If the simulation volume includes a sufficiently broad solvent layer (mostly water) around the micelle, so that the solute in the outmost layer n is entirely in bulk water, then the probability distribution of eq. 8 includes the information about the micelle-solvent (e.g. water) partition coefficient, i.e.

$$K^X_{M,S} = const. Z^X_M(r_n) \Big/ Z^X_M \qquad (10)$$

where the constant is just for unit conversion with respect to the different units used for partition coefficients. This expression is valid in the typical range of log K_{mw} > 0.5, as we find them in the experimental data sets. A more general expression holding also for hydrophilic compounds is discussed in Appendix A.

The COSMOtherm implementation of COSMO-RS is used throughout this paper, with the latest BP_TZVP_C21_0107 parameterization unless marked otherwise.[46]

Results

The depth dependent membrane composition used in COSMOmic was derived from an MD-study of Gurtovenko *et al.*[47] who simulated a phospholipid bilayer – water system with 128 DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) and 3655 water molecules. The pdb-file of the bilayer simulation was analyzed in order to get the probability distribution of each atom type over the membrane normal with a resolution of 1 Å, that is, the simulation box with a length of 62 Å in the direction of the membrane normal was split into 62 layers. As an example, the 128 DMPC-phosphorus-atoms of the bilayer showed quite some spread and could be found as close as 10 Å and as far as 23 Å

away from the center of the bilayer, but had a distinct peak in an area between 15 and 21 Å with almost 80% of all P-atoms in this region.

The properties of solutes and solvents can be visualized with σ-profile and σ-potential plots.[48,49] In the case of our membrane one obtains a σ-profile and σ-potentials for each layer reflecting the depth dependent changes of the membrane composition. These σ-profiles and σ-potential can be visualized in a 3D-plot as shown in Figure 2.

Figure 2 HERE

The narrow peak close to the membrane center, i.e. between 0 and 7 Å, corresponds to the predominance of the hydrocarbon chains in this region and the other smaller but wider peaks correspond to the carbonyl-groups, the phosphate and the choline-groups, respectively, as indicated on the plot. The 3D-plot including the water fraction in each layer underlines the importance of water in the membrane even if the fraction drops substantially below the head groups.

The influence of water is even more pronounced in the σ-potential plot where only the very first completely water-free layers resemble the known potential of non-polar solvents like hexane.

In a first comparison the difference between isotropic solvents and anisotropic solvents like the presented membrane model of COSMOmic were explored. Partition coefficients between water and (liquid) bulk phase 1 DMPC were calculated for the training set of 105 compounds using COSMO-RS. A relatively high correlation was observed as shown in Table 1 for this first model (M1). Fitting the experimental log K_{mw} against the calculated log K_{mw} resulted in surprisingly meaningful coefficients, with an intercept of almost zero and a slope of 0.84. This result is in good agreement with recent results by Mokrushina *et al.* who reported a very good agreement of experimental water-micelle partition coefficients with COSMO-RS predictions for bulk water-surfactant partition coefficients.[50]

Table 1 HERE

In the next step the original COSMOmic which did not yet include the combinatorial term $\mu^X_{M,comb}$ of eq. 4 was tested using the DMPC membrane composition shown in Figure 2. A look at Table 1 (M2) shows that correlation and RMSE did not improve compared to the bulk solvent model, while the slope had a lower value of 0.71 and the intercept also deviated from zero. However, when $\mu^X_{M,comb}$ was included in the calculation of the chemical potential (M3) the COSMOmic predictions were substantially better than a bulk solvent prediction. Both the slope and the intercept took physically meaningful values of 0.95 (±0.09) and -0.43 (±0.32).

The negative intercept means that on average the predictions in M3 were about a factor of 3 too high suggesting that other interactions exist which "drive" the solute out of the membrane, e.g., the influence of an elastic deformation free energy. Therefore, in a fourth model (M4) the adjustable parameters $c^0_{M,el}$ and $\lambda_{M,end}$ of eq. 5 were varied systematically from 10^{-4} to $5\cdot10^{-3}$ and 0 to 1, respectively. In all tests the predicted K_{mw} turned out to be rather insensitive $\lambda_{M,end}$ and it was therefore set to a constant value of 0.5, while $c^0_{M,el}$ had strong effects for values above 10^{-3}. While the correlation with the experimental data did not improve it was possible to reach an intercept of 0 and a slope of 1 for $c^0_{M,el} = 10^{-3}$, however at the cost of a slight decrease of R^2 from 0.82 to 0.79, from M3 to M4, respectively. Thus, although appearing physically sound, we did not use the elastic term further considering that we are dealing only

with a small shift of the intercept of 0.4 log units and furthermore considering that such rather small differences in slope and intercept of the fit equation can be strongly influenced by a few outliers. However, it may well be that in other micellar systems the elastic contribution is of significant importance, especially of phospholipids in the gel phase. Figure 3 shows a plot of the experimental versus the predicted log K_{mw} of M3 the best model so far, i.e., with the combinatorial term on and the elastic term off.

Figure 3 HERE

A total of 9 compounds deviated more than 1 log unit from the fit equation drawn as a dashed line with the largest deviation caused by p-methylbenzylheptylamine which is predicted 1.78 log units too high. As matter of fact, the whole data of these (p-methylbenzyl)akylamines measurements[51] were overestimated substantially. The largest underestimation was the prediction of diclofenac (1.57 log units too low). The three compounds with absolute deviations larger then 1.5 log units were all measured with potentiometric titration a method that can cause problems for more lipophilic compounds because very high solute concentrations might be needed and this might lead to lower partition coefficients than equilibrium dialysis.[2] However, this would not explain the much too low value of diclofenac. The predictions for this compound can be improved by 0.68 log units by accounting for different conformers. Twenty compounds were additionally evaluated for the influence of conformations. Apart from diclofenac no other compound had more than 0.2 log units difference in the predicted log K_{mw} when the energy weighted conformations and the lowest energy conformer was compared. Finally, an interesting observation is that the compounds causing large deviations were not the same as the compounds deviating strongly from the relationship with the octanol-water coefficient, i.e. from eq. 1.

It is difficult to estimate how strongly the results are influenced by the probability distribution of the solvent atoms. Therefore, two additional MD-simulations were evaluated with the training set: a POPC bilayer consisting of 200 lipids and 5483 water molecules[52] referred to as M5, and a more complex MD-study consisting of 512 DMPC at a mole fraction of 0.04 in a 1M NaCl solution[53] referred to as M6. POPC differs from DMPC in the acyl chain length by two methylene groups and also one acyl group has a double bond between fatty acid carbon 9 and 10. In our analysis of experimental data we found three compounds which have been measured with both DMPC and POPC: 1,3,5-trichlorobenzene (0.37), chlorobenzene (0.19), and dibutylphthalate (0.04) with the differences given in brackets. Similarly, in COSMOmic models M3 (DMPC) and M5 (POPC) differed only little and only small changes in the fit with the experimental data could be observed. However, about five compounds showed remarkable differences in the predicted values up to a factor of 5.

The third MD-study evaluated here[53] again modelled a DMPC-water system, but in the presence of NaCl at a high ionic strength of 1 M which makes it especially interesting for studies with charged compounds. The correlation of M6 was substantially lower with $R^2 = 0.73$ and RMSE = 0.74 and also the slope took a much lower value of 0.80. Currently we have no explanation for the weaker performance of this salt-containing model.

Test set 1 with 56 compounds was predicted with M3 the “final model”, i.e. with the probability distribution of DMPC from ref [47] and without elastic term and adjusted by the fit equation with a slope of 0.95 and an intercept of -0.43. Figure 3 shows that the predictions for the test set are in agreement with the training set predictions (with a slightly higher R^2 of 0.86 and an RMSE of 0.58). Fitting the test set data alone would result in a somewhat higher slope of 1.11 (±0.12) compared to 0.95 (±0.09) for the training set. The lower slope of the training set is due to the uneven division of the data into training and

test set. Other models showed similar trends for the test set, e.g. M1 the bulk lipid model with an R^2 of 0.77 and an RMSE of 0.71.

For drug design screening studies large data sets need to be processed. We therefore also evaluated the training set with the AM1-SVP parameterization of COSMOtherm, which replaces the full TZVP DFT/COSMO optimizations of the molecules by much less expensive AM1 geometry optimizations,[54] with subsequent single-point DFT/COSMO calculation with the smaller SVP basis set of TURBOMOLE. This level saves roughly a factor of 50 of computation time and it is regularly used in drug-design applications of COSMOtherm. No decrease of the predictive power and also no substantial change in the fit equation was observed (M7). Therefore, test set 2, i.e., the TRANSIL measurements from Bayer, including many very large drug molecules, was evaluated only on the AM1-SVP level.

The predictions for the Bayer data set resulted in a substantially lower R^2 and RMSE. As visible in Figure 4 there are some very large deviations especially D9-THC with an experimental log K_{mw} of 3.4 and a predicted log K_{mw} of 7.0.

FIGURE 4 HERE

We have no explanation for the extremely low experimental log K_{mw} especially considering that the experimental octanol-water partition coefficient is very high (log K_{ow} = 6.97).[55] If this extreme outlier is left out one obtains an RMSE which comes close to the value obtained for the other data sets. The slope also differs from the value obtained with liposomes, but this is in good agreement with experimental comparisons of TRANSIL measurements and partition coefficients measured with liposomes in which a slope of 0.86 and an intercept of +0.29 was determined.[9]

A big advantage of COSMOmic compared to simple empirical relationships with log K_{ow} (like eq. 1) is that one does not only obtain overall partition coefficients, but also information about the probability to encounter a compound in a certain depth and with a certain orientation. As an illustration we calculated the internal distribution of three drugs studied by Herbette *et al.* who determined the depth of the concentration maxima of three calcium channel blockers in sarcoplasmic reticulum membrane bilayer samples using X-ray and neutron diffraction techniques. [26] The internal distribution calculated with COSMOmic is in good qualitative agreement with their measurements.

FIGURE 5 HERE

Propranolol and amidiorone are both cations in aqueous solution at physiological pH and the partition coefficients of these cations are relatively high, e.g., propranolol where the K_{mw} of the cation is only a factor of 3 lower than the K_{mw} of the neutral species.[56] Therefore, one can expect that significant or even predominant fractions of these compounds are present as cations in the membrane as well. As a matter of fact, the internal distributions of the cationic species shown in Figure 5 are in better agreement with the experimental data than the neutral species. However, the curves of the cations must be considered as preliminary results as COSMOmic has not yet been tested extensively with cations.

Discussion and Conclusion

Starting from the general COSMO-RS theory we have developed a model for anisotropic solvents like micelles or bilayers. Its remarkable feature is that it contains no fitted parameters in addition to the general thermodynamics parameters of COSMO-RS, but instead builds on theoretical considerations.

The fact that the predictions with COSMOmic (M3, M5) are clearly better than the predictions for a bulk water-lipid system, which has the same composition (M1), is encouraging. The fit with experimental data is generally satisfying, although for compounds with available experimental octanol-water partition coefficients it is still advisable to use the empirical relationship of Eq. 1 (if the compound is uncharged and has a log K_{ow} between 1 and 5). The quality of the predictions (with M3) for the first test set is slightly better than for the training set. In QSPR approaches with large number of fitted parameters often the contrary is the case. However, in the case of M3 slope and intercept are the only additional adjusted parameters and therefore the result is sensible. The small differences must be due to different composition of training and test set. These differences also lead to differing estimates for slope and intercept, e.g., in the case of M3 with a slope of 0.95 for the training set and a slope of 1.11 for test set (if the 56 test set compounds are fitted separately). As can be seen in Figure 3 the split into training and test set is a little bit uneven towards higher values and all compounds with experimental Kmw > 5.5 are part of the training set and these values have a strong leverage on the slope. Furthermore, the training set contains some extreme values lowering the slope, e.g. leaving out the two (p-methylbenzyl)alkylamines (p-methylbenzylheptylamine and p-methylbenzylpropylamine) mentioned above would raise the slope from 0.95 to 1.0.

The lower prediction quality for the second test data set consisting of complex drugs could probably be further improved by more thorough evaluations of possible zwitterionic forms or tautomerisms. There are two other factors which influence the quality of the predictions. First, the composition of the solid-supported lipid membranes might play a role, that is, the egg lecithin membranes contain also a small percentage of head groups which are not phosphatidylcholines which might result in different interactions with the solutes. Second, the partition coefficients were measured with solid-supported lipid membranes which is a high-troughput method and a lower experimental quality than equilibrium dialysis data used in the first data set must be expected. These two factors might explain a large portion of the increase in RMSE from test set 1 to test set 2.

The sensitivity of the COSMOmic results with respect to the choice of different MD simulations was tested and showed almost no effect in the case of M3 and M5. However, the experience with the third MD-simulation tested (M6) shows that it is not advisable to take the pdb-File of any MD-study without further tests with experimental data. Furthermore, if COSMOmic is used for completely different types of bilayers, e.g. for charged instead of zwitterionic lipids or for phosphatidylcholines which are in gel phase, it is necessary to dispose of an appropriate MD-simulation. The inclusion of the COSMO-RS combinatorial contribution showed a strong improvement of the results (M3 versus M2), but the inclusion of an elastic energy term (M4) did not yield any significant improvement.

In addition to the calculation of global partition coefficients, the COSMOmic method can also be used to study the internal distribution of solutes in membranes and micellar systems. This may be of importance for a better understanding of drug behaviour in cell membranes and for the estimation of drug cell permeability. Permeability can be derived from the free energy profile of a compound in the membrane, which usually requires extensive MD-simulations.[23] The probability distribution shown in Figure 5 corresponds to the inverse of such a free energy profile, i.e. a compound with a very low probability of occurrence in the center has a high energy barrier and thus, a low permeability. Such free-energy barrier calculations have already been successfully applied to model toxic effects in energy-transducing membranes.[12]

A part from the barrier height also the location of the concentration maxima or even the full curve of the internal distribution might be of interest. Although the presented example with the three calcium channel blockers is more of qualitative nature, such calculations open the door for a vast number of

insights, as the specific location, orientation and conformation of the drug in the membrane bilayer might influence the intake of the drug into the active site of the protein.[26] COSMOmic could help to answer such questions which up to now required very complex experiments or computationally very expensive simulations.

Although we already have encouraging results for anion partitioning, further work is required for the treatment of charged species. We will discuss these in more detail in a forthcoming paper. Furthermore, applications to other micellar systems as tenside micelles will be published elsewhere.

ACKNOWLEDGMENT The authors thank Jung-Hwan Kwon and Beate Escher from the Swiss Federal Institute of Aquatic Science and Technology (EAWAG) and Stefanie Krämer from ETH-Zürich for helpful discussions.

APPENDIX A:

Based on the solute probability distribution, for solutes which have a high affinity to the membrane the definition of the membrane-water partition coefficient is relatively simple, if the outmost layer of the simulation can be considered as a representative of bulk water. In that case we only need to know the probability $p_M^X(r_{out})$ of the solute X in the outmost layer. Since the total probability of the solute in the simulated system is 1, and since the small probability to find the solute in one of the outer layers is negligible compared to 1, we have 1 molecule of solute bound to the $n_{surfactant}$ surfactant molecules in the simulation unit. The volume concentration X in bulk water is just $p_M^X(r_{out})$ divided by the volume of the outmost simulation layer. Dividing the two concentrations yields the desired micelle-water partition coefficient.

If the fraction of solute residing in water or in water-like outer layers of the simulation unit is significant, the definition becomes dependent on the question which layers are counted to the membrane and which to the water environment. Therefore we have introduced the concept of a bulk solute/solvent ratio k_S^X, where in general the solvent S is just water. This can be derived from the probability to find the compound in the outmost layer divided by the number of solvent molecules in the outmost layer. Now we can define a pseudo-water bound probability

$$p_{S,i}^X = k_S^X n_{M,i}^S$$

in each layer *i*, with $n_{M,i}^S$ being the number of water molecules in layer *i*. Subtracting this from the total probability $p_M^X(r_i)$ to find the compound in layer *i*, we have partitioned the probability of X in each layer into a micelle and a solvent contribution. By summation of the micelle-associated contributions and subsequent division by $n_{surfactant}$ one gets the micellar solute concentration, and by summation of the solvent-associated probabilities, multiplication by $n_{M,out}^S / n_{M,tot}^S$ and division by the volume of the outmost layer we yield the volume concentration in water. This definition coincides with the previous one for solutes with high micelle-affinity, and it is stable and robust even for very hydrophilic compounds.

This ia a postprint of *J. Phys. Chem. B*, 2008, 112 (38), pp 12148–12157. The original article can be found under http://pubs.acs.org/doi/abs/10.1021/jp801736k

FIGURE CAPTIONS

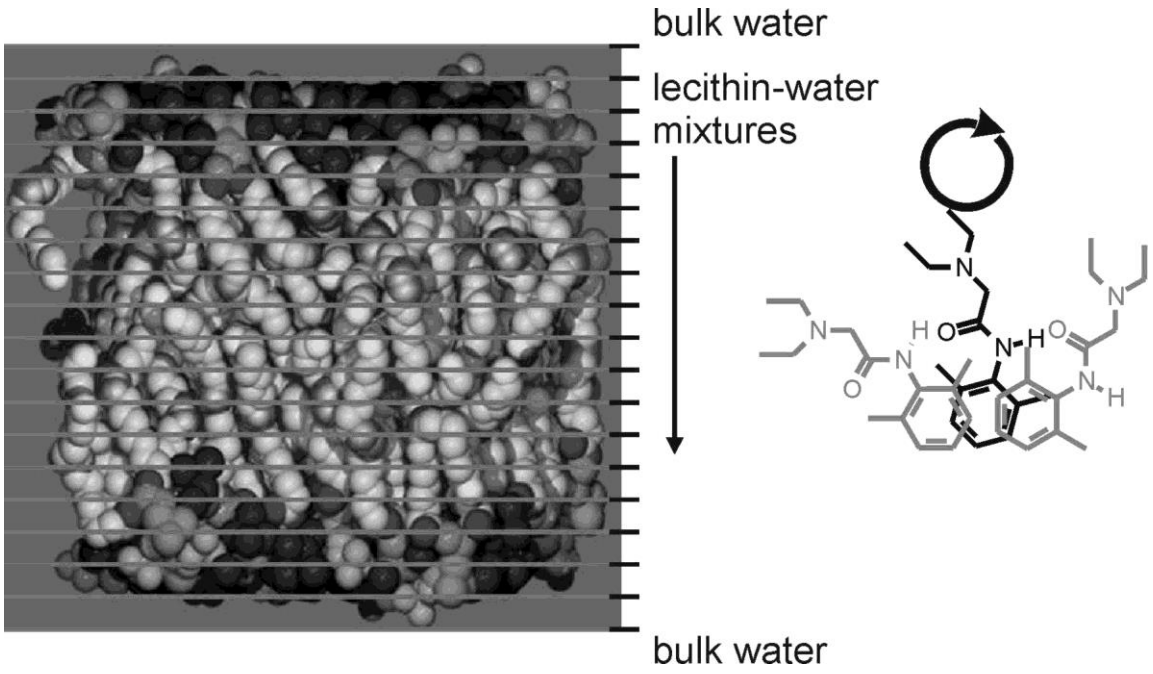

Figure 1. Lecithin distribution taken from MD-study.[57] The anisotropy of the solvent is accounted for in COSMOmic by calculating a depth-dependent composition of the lecithin-water mixture, i.e. by considering a membrane as a layered liquid. In order to calculate the distribution of a solute the solute is moved through each layer and rotated within each layer (right side of this figure). For each orientation at each depth the chemical potential is calculated according to eq. 7.

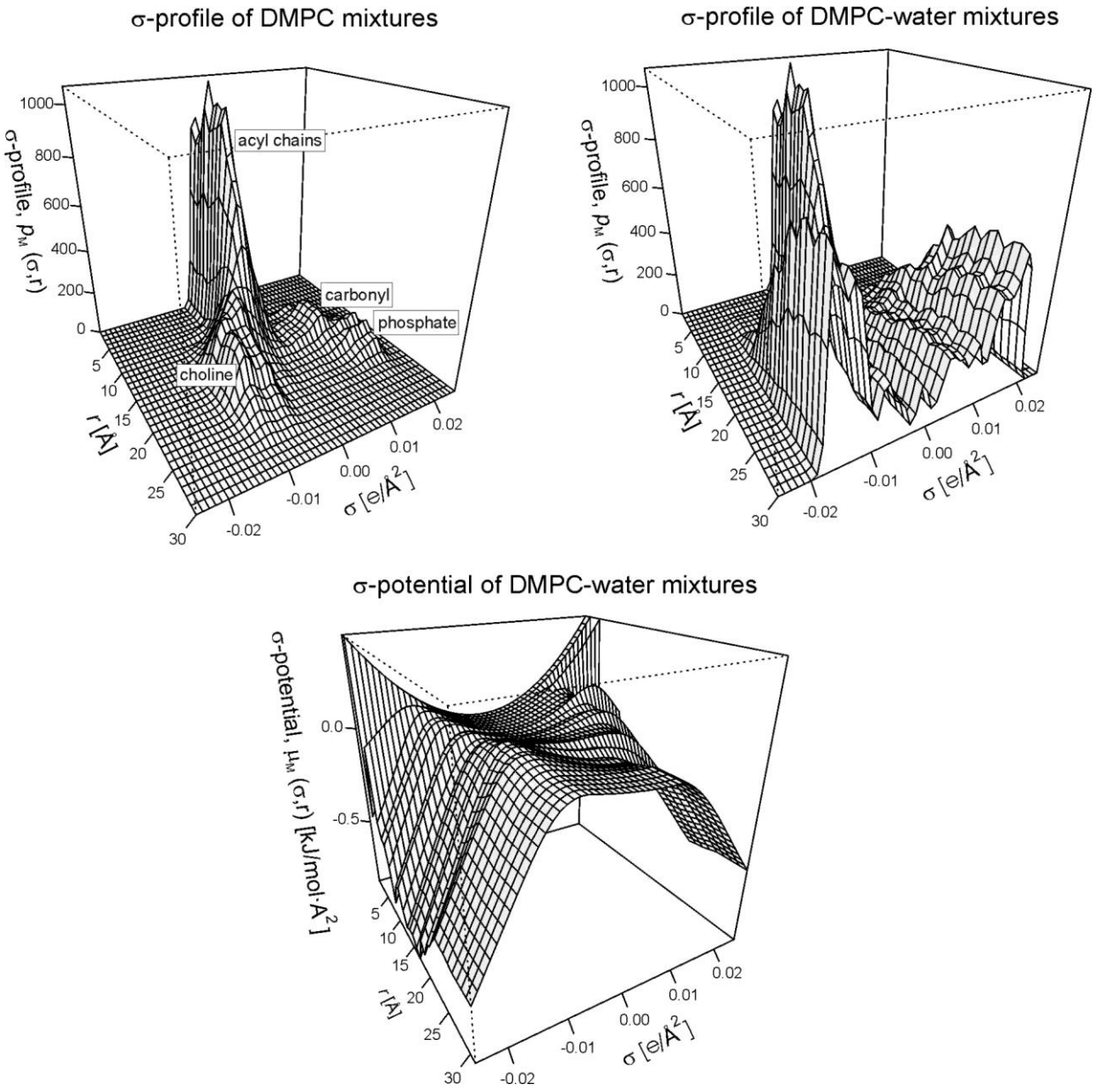

Figure 2. a) σ-profile, i.e., amount of surface with polarity σ, of DMPC-membrane ranging from the membrane center (r = 0) to the aqueous phase (r > 26 Å) the contributions of the hydrophobic acyl chains, choline, phosphorous, and carbonyl, respectively are visible b) σ-profile of DMPC-water mixtures with the composition used for COSMOmic calculation c) σ-potential, i.e., affinity for surface with polarity σ, of DMPC-water mixture. The layer thickness for the all calculations was set to 1 Å.

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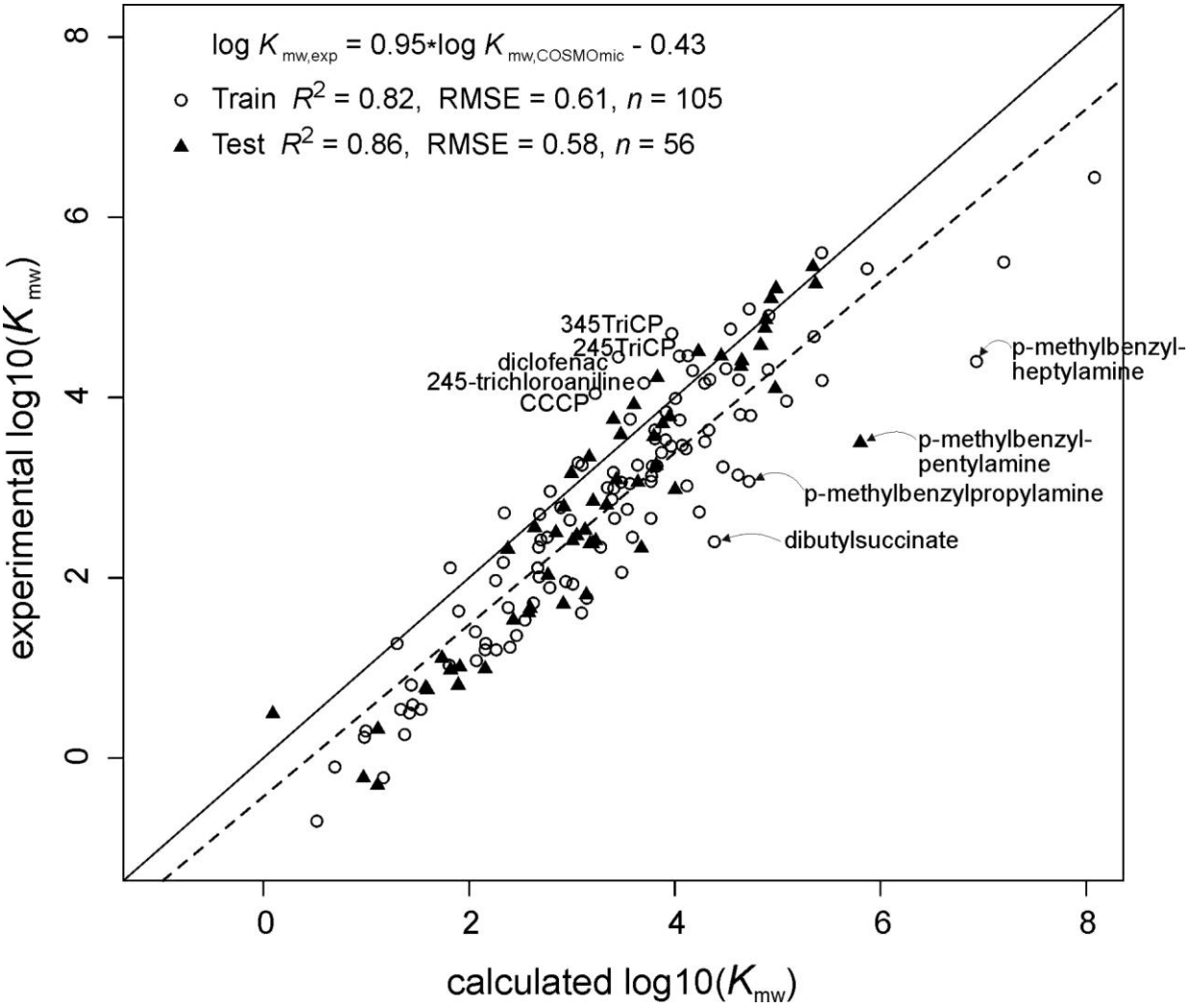

Figure 3. Experimental versus predicted log K_{mw} with the dashed line showing slope and intercept of the fit equation (of the training set).

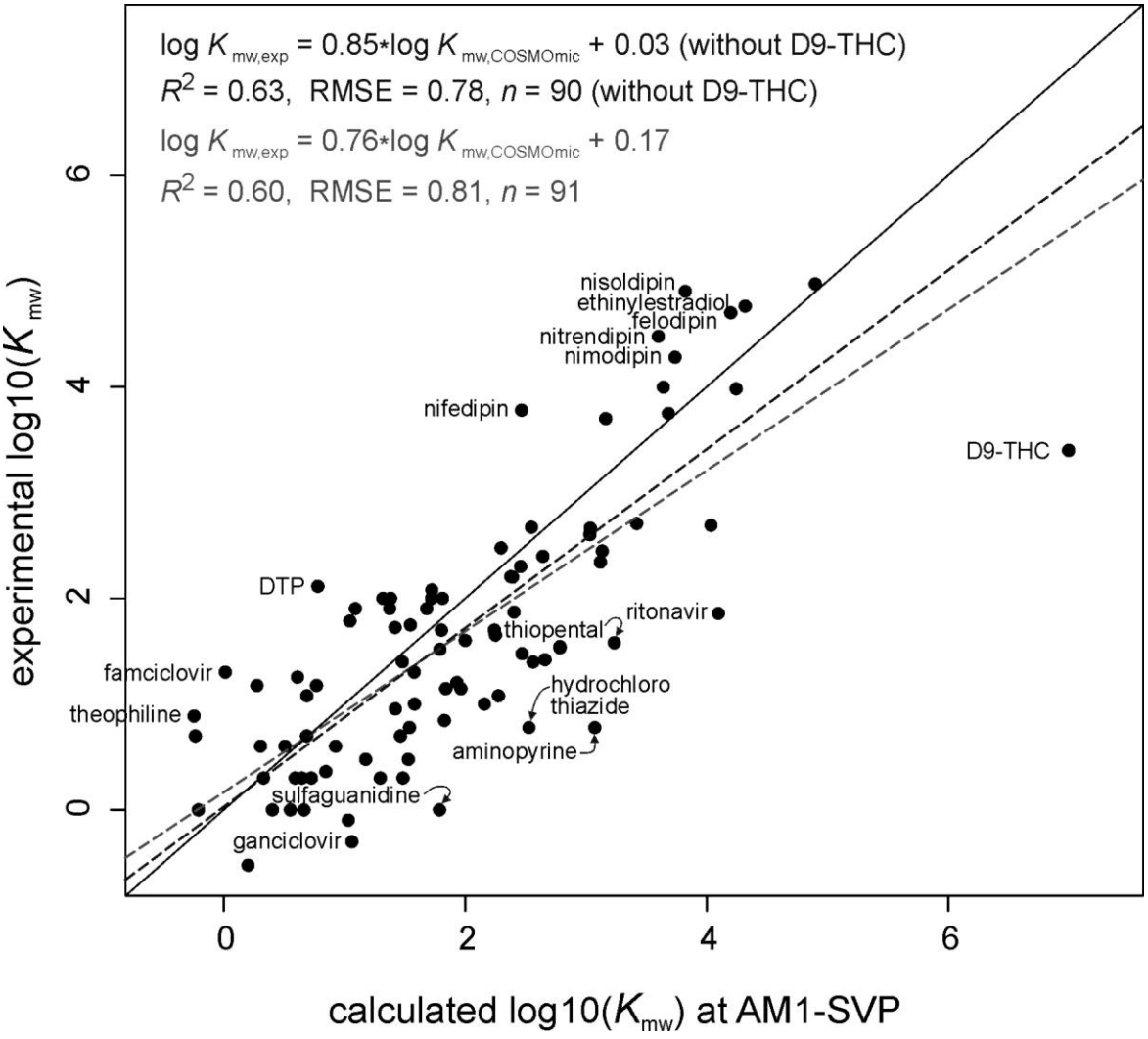

Figure 4. Experimental versus predicted log K_{mw} of test set 2. Numbers and dashed line in grey: extreme values of D9-THC included.

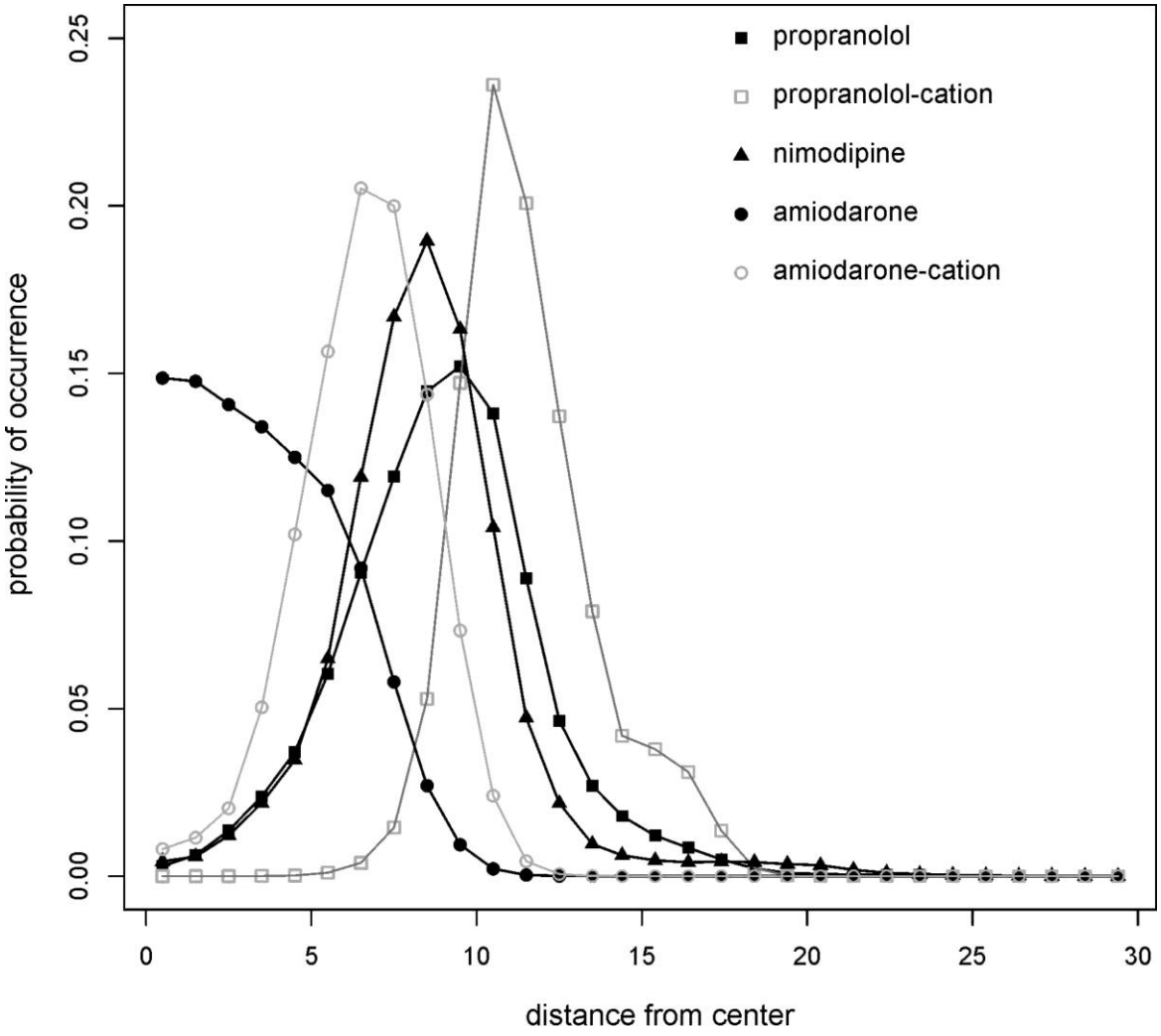

Figure 5. Internal distribution of propranol, nimodipine and amiodaron in neutral (black) or charged state (grey), respectively. The probability of occurrence is given as calculated for the center of the solute. The experimentally determined maxima are at a distance of 12 Å (propranolol, nimodipine) and 6 Å from (amiodorone) from the center of the membrane.[26]

TABLES

Table 1. Comparison of different models tested with the training set of n = 105 compounds. Q^2_{loo} is the explained variance in a leave-one-out cross-validation. In the text the models are referred to by the model number.

Number	Model	R^2	Q^2_{loo}	RMSE	slope[a]	intercept[a]
M1	bulk DMPC	0.76	0.75	0.70	0.84	+0.02
M2	COSMOmic (DMPC without $\mu^X_{M,comb}$, cf. eq. 4)	0.75	0.74	0.71	0.71	-0.38
M3	COSMOmic (DMPC)	0.82	0.81	0.61	0.95	-0.43
M4	COSMOmic (DMPC with elastic term on, $c^0_{M,el}$ = 1.5·10^{-3}, $\lambda_{M,end}$ = 0.5, cf. eq. 5)	0.79	0.78	0.65	1.01	0.00
M5	COSMOmic (POPC)	0.82	0.81	0.60	0.97	-0.53
M6	COSMOmic (DMPC with I = 1 M, ref [53])	0.74	0.73	0.72	0.80	-0.20
M7	COSMOmic (AM1-SVP)	0.82	0.81	0.61	0.99	-0.47

[a] determined as a linear regression of log K_{mw} (experiment) = β_1·log K_{mw} (predicted) + β_0·

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