Prediction of Phospholipid-Water Partition Coefficients of Ionic Organic Chemicals using the Mechanistic Model COSMOmic

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KEYWORDS: partition coefficients, lipid membranes, ions, membrane dipole potential, COSMO-RS

ABSTRACT

The partition coefficient of chemicals from water to phospholipid membrane, K_{lipw}, is of central importance for various fields such as biophysics, pharmaceutical science and environmental chemistry. It has been repeatedly demonstrated in the literature that log K_{lipw} correlates with the log of bulk solvent-water partition coefficients such as the octanol-water partition coefficient, K_{ow}, for neutral molecules. However, this is not the case for charged compounds, for which a mechanistic modelling approach is highly necessary. In this work, we extend the model COSMO*mic*, which has been shown to reliably predict K_{lipw} for neutral compounds, to the use of ionic solutes. COSMO*mic* adapts the COSMO-RS theory for anisotropic phases by representing the structure of membranes and micelles as many stacked layers of liquids. In order to make the model applicable for the calculation of K_{lipw} of ions, we implemented the internal membrane dipole potential in COSMO*mic*. We empirically optimized the potential with experimental K_{lipw} data of 161 neutral and 75 ionic compounds yielding potential shapes that are in good agreement with experimentally determined potentials from the literature. Implementing the potential in the model has no negative effect on the prediction accuracy of neutral compounds (root mean square error, RMSE = 0.62 log units), while it highly improves the prediction of ions (RMSE = 0.70 log units). The refined COSMO*mic* is, to the best of our knowledge, the first mechanistic model that is able to predict the membrane affinity of both ionic and neutral species with accuracies better than 1 log unit.

INTRODUCTION

Membrane lipids are the major lipid component of several human tissues, e.g., they constitute around 70% (v/v) of red blood cells, liver, and kidneys on the dry volume basis.[1] A realistic

description of partitioning into lipids is essential for various fields of science ranging from biophysics to pharmaceutical and environmental sciences.[2–4] Traditionally, membrane affinity is approximated by the partitioning between water and a bulk organic solvent like octanol,[5] implying that the logarithm of the octanol-water partition coefficient, log K_{ow}, correlates well with that of the membrane-water partition coefficient of biological membranes.

Alternatively, liposomes which are artificial lipid bilayer vesicles of defined composition and size have been used as an experimental system approximating cell membranes since around 1960[6] and are now well-established, because they proved to be easy-to-handle and robust. Typically, liposomes consist of zwitterionic phospholipids with a negatively charged phosphate group and a positively charged choline structure; the former is esterified with two long-chain fatty acids. From their composition and structure, it appears obvious that liposomes are a much more realistic experimental approximation of cell membranes than any bulk organic solvent.[5,7,8] Nevertheless, in the case of neutral chemicals, the logarithm of the liposome-water partition coefficient, log K_{lipw}, and log K_{ow} agree fairly well with each other. In the most comprehensive collection of publicly available experimental data, log K_{lipw} of 156 neutral compounds were compared with experimental log K_{ow} and a correlation coefficient R^2 of 0.95 and a standard deviation of 0.43 log units were observed.[2] The slope (1.01) and the intercept (0.12) of the regression indicate that the two partition coefficients are generally in agreement. For charged chemicals, however, the situation is completely different: first, the K_{ow} values of an ionic species strongly depend on the coexisting ions[8] and second, the K_{lipw} values are up to several orders of magnitude higher than the respective range of K_{ow} values. Thus, the lipophilicity of charged compounds is dramatically underestimated by approaches using K_{ow} as a measure for the partitioning into biological membranes.[8–11]

There are major differences between liposomes and bulk organic solvents, and with regard to the partitioning of ions, two structural differences may be important: the much larger surface to volume ratio of liposomes and the ordered structure which results in an internal dipole potential (Ψ_d) of lipid bilayers. As a consequence of the high surface to volume ratio (with a typical mean diameter of 0.27 μm for liposomes),[12] sorption of charged species can be electrically neutralized by counterions from the electrolyte solution (diffuse double layer), while bulk media have to maintain electrical neutrality either by the partitioning of ion pairs or by the partitioning of free ions together with counterions. This explains the high sensitivity of measured K_{ow} of an ionic chemical to the ionic strength while K_{lipw} data show very little ionic-strength dependence.[8] The internal dipole potential can be caused by several factors: charge separation in the head groups (this is not a necessary condition as also neutral glycerylmonooleate bilayers reveal a positive membrane dipole),[13] alignment of dipolar residues of the lipids and/or oriented water dipoles in the region between the aqueous phases and the hydrocarbon-like interior of the membrane.[13,14] The height of the hill-shaped Ψ_d in the center of zwitterionic phosphatidylcholine bilayers has been indirectly determined with several experimental approaches and ranges from 227 mV for DPPC bilayers[14] to 280 mV for egg phosphatidylcholine bilayers,[15] positive in the membrane interior.

One approach to predict K_{lipw} based on a molecular description of the membrane is molecular dynamics (MD) simulation of lipid bilayers in the presence of solutes. MD simulations can reproduce a large number of effects and properties related to the membrane-solute interactions and can also yield an internal membrane dipole potential distribution.[14,16,17] However, we did not find a sufficient number of studies predicting absolute values of K_{lipw} for lipophilic ions to evaluate the accuracy of predictions based on MD simulation. This may be due to the fact that

the computational costs for MD simulations of membranes including a solute at a specific position are extremely high.[18]

A computationally much more efficient alternative to such MD simulations has been proposed by Klamt et al. in form of the COSMO*mic* (i.e. COSMO-RS for MICells) approach.[19] COSMO*mic* requires as input the structural composition of a micelle or membrane, usually derived from one or a series of snapshots from a MD simulation of the respective micellar system. The micelle, i.e. in our application a phospholipid membrane is then virtually split into layers of approximately 1 Å thickness, and the probability to find each of the atoms of the phospholipid and of water in each of the layers is derived from analyzing the MD snapshots. DFT/COSMO calculations are performed in order to yield the surface polarities, i.e. the conductor surface polarization charge densities σ on the molecular and thus also on the atom surfaces of the phospholipid and water molecules. Combining these with the atom distribution taken from MD leads to a polarity profile, i.e., a σ-profile, for each layer. Then COSMO-RS (i.e., COnductor-like Screening Method for Real Solvents) in its COSMO*therm* implementation is used in order to derive the affinity of each layer for a certain molecular surface polarity σ, shortly called σ-potential of each layer. With this information, the free energy of any solute, which is also represented by its DFT/COSMO surface polarization charge densities, can be evaluated at each position and orientation in the membrane system. An integration over all possible orientations for each position leads to a free energy profile of the solute throughout the membrane system and finally to predictions of the membrane-water partition coefficient. The COSMO*mic* approach has recently been demonstrated by two independent groups to yield results of comparable, if not slightly superior quality, with respect to the distribution of neutral solutes in phospholipid membrane systems,[16,17] but at computational costs which are several orders of

magnitude lower than for the respective MD simulations. While the calculation of a free energy profile with COSMOmic takes a few minutes on a single core (given that all input files are ready-to-use), the same calculation conducted as MD simulation would take 15 to 48 h on super computers with more than 100 cores.[20] COSMO*mic* has been used to predict partition coefficients and free energy profiles of anions previously.[21] For the studied 35 anions a reasonably low root mean square error (RMSE) was observed, but it was necessary to empirically fit the predicted values to experimental data, as apparently some relevant mechanism for the prediction of ions was not accounted for yet.[21]

The first goal of this work was to give an overview of published data on liposome-water partition coefficients of organic anions and cations and an appraisal of the quality of the existing data. In order to systematically increase data diversity, own measurements were conducted, leading to a more thorough validation of the modelling approaches. The second goal was to apply the existing COSMO*mic* to the available data on liposome-water partition coefficients, identify the areas where adaptations are needed, and refine the model in accordance. We newly implemented a membrane potential in COSMO*mic* to achieve an improved computation of interaction energy between phospholipid membrane and ions. Finally, the refined COSMO*mic* model was used for calculations of K_{lipw} of anionic, cationic and neutral species to evaluate the performance of the model.

METHODS

Experimental Section

Chemicals

The solutes tested here (fenamic acid, 2,4,6-tribromo-phenol, 4-octylbenzene-1-sulfonate, flufenamic acid and 5-nitro-2-trifluoromethyl-benzimidazole) and additional chemicals (see SI) were purchased of the highest purity available (≥98%) and used as received from the following companies: Fluorochem, Roth, Sigma Aldrich, Fluka, and Merck (see SI). The synthetic POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine) came from Avanti Polar Lipids (>99%).

Determination of Membrane-Water Distribution Coefficients

All liposome-water partition coefficients were determined at 20-22°C via equilibrium dialysis experiments and HPLC analysis. The experimental details are described in the SI, and thus the description here is brief. Salt concentration (100 mM KCl) was constant for all experiments. Buffer (MOPS, pK_a=7.2 or CHES, pK_a=9.3) was chosen so that the pH in the experiments was at least 3 pH units higher than the pK_a of the investigated chemicals (to be sure that only the anionic species is considered). POPC liposomes were prepared with a membrane extruder (Lipex Biomembranes, Vancouver, BC, Canada with Whatman polycarbonate filter membrane, pore size 0.1µm) as described elsewhere.[22] Custom made glass dialysis cells consist of two chambers that were separated by a dialysis membrane made of regenerated cellulose with a cutoff of 10 000-20 000 Da (Thomapor, Reichelt Chemie Technik, Heidelberg). One chamber was filled with buffer solution and the other with liposome suspension. The latter received the test anion. The liposome free side of every dialysis cell was sampled twice (i.e, on the 4th and 6th day) and the

samples were subjected to the HPLC analysis. Mass recovery of every chemical was tested accordingly in control experiments without liposome where both dialysis chambers were filled with buffer solution (revealing that losses were less than 5%). Each dialysis cell experiment was conducted at least in triplicates. All experiments were conducted with a liposome load below 0.08 mol(substance)/mol(lipid), which has been shown to be within the linear part of the sorption isotherm.[23]

Data collection and evaluation

All data collected from the literature were measured with phosphatidylcholine liposome. Overall neutrality of the phophatidylcholine membrane as a sorption phase is important to note, since a charged membrane would have significant impact on the sorption of charged chemicals.[24] The experiments in the literature have been conducted at different ionic strengths, which should not be crucial for the modelling of ion partitioning since the ionic strength does not significantly influence the membrane partition coefficients of ionic compounds.[8] Only partition coefficients measured above the main phase transition temperature of the membrane were considered, ensuring that the membrane is in its natural condition, the liquid crystalline state. The state of the membrane has been shown to be an essential parameter for the partition coefficient of neutral chemicals.[25]

All experiments considered here were conducted with unilamellar vesicles, preferably using the equilibrium dialysis method, but also other experimental methods are considered (see SI for details). This results in a total of 51 experimental values for anions (from which 5 are our own measurements in this work) and 24 experimental values for cations, representing the largest publicly available collection of K_{lipw} values for ions we know of. When multiple experimental data for the same ion were found, the arithmetic mean of the log K_{lipw} values was used (see SI).

The difference of the single reported values from the corresponding mean value was between 0.02 and 0.21 log units for the anions (with a total of 6 repeatedly measured ions) and 0.03 to 0.28 log units for the cations (with a total of 4 repeatedly measured ions), with the exception of two reported values for atenolol that differ by 0.50 log units from their mean.

Theoretical Section

COSMO-RS and COSMOmic

To run COSMO*mic*, as outlined in the introduction, a detailed membrane structure is required which is taken out of MD simulations. Care was taken to use time averaged atomic distributions which are furthermore centered in the middle of the simulation box.[26] The atom distributions were kindly simulated (CHARMM36 force field) and provided by Sven Jakobtorweihen.[26] In addition, TZVP[27–30] cosmo files are needed for all involved relevant conformers of all the solutes in the partitioning process. Therefore COSMOconfX13 (version 3.0, COSMOlogic) templates, based on Turbomole version 6.5[31], have been used for full energy minimization and conformer generation.[32]

Estimation of membrane potentials

In addition to the depiction of the membrane anisotropy, the membrane dipole potential may need to be described in the model, when the model is used for the prediction of ions. There is no direct experimental method to measure the membrane dipole potential, but several indirect approaches allow the quantification of Ψ_d for different types of bilayers. For egg phosphatidylcholine bilayer vesicles the values in the membrane interior range from 0.24 V (deduced with a combination of kinetic and binding data of lipophilic ions)[33] to 0.28 V (electron paramagnetic resonance spectroscopy in combination with nitroxide spin-labeled hydrophobic

ions).[15] For DPPC bilayers (dipalmitoylphosphatidylcholine), two values of 0.227 and 0.24 V are given in the review of Wang.[14]

We initially tried to derive a profile of Ψ_d from the available MD simulations. Using the fundamental equations of electrostatics, the electrostatic potential of a planar membrane can either be derived from the charge density in each layer:

$$\Psi_d^c(z) = \frac{1}{4\pi\varepsilon_0}\int_0^z du \int_0^u dv \rho(v) \qquad (3)$$

where $\rho(v)$ is the charge density (charge per area) in layer v, or from the dipole moment density:

$$\Psi_d^c(z) = \frac{1}{4\pi\varepsilon_0}\int_0^z du\ D(u) \qquad (4)$$

where $D(u)$ is the z-component of the dipole moment density in membrane layer u. Here $\Psi_d^c(z)$ is the value of the dipole potential with respect to the center of the membrane. The default definition of the dipole potential $\Psi_d(z)$ with respect to the bulk water phase can easily be found from $\Psi_d(z) = \Psi_d^c(z) - \Psi_d^c(\infty)$. Using eq. (3) together with the DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) snapshot from Gurtovenko et al.,[34] which was used for predicting K_{lipw} of neutral compounds previously,[19] and with the partial charges applied in the respective MD simulation results in a potential which is by 0.99 V higher at the center of the membrane than in water. This value is too high by more than a factor 3 compared to the experimentally expected value of ~0.3 V. The value of 0.99 V originates from a contribution of -4.55 V caused by the charges on the DMPC atoms and an overcompensation of 5.55 V by the water molecules. Thus, the MD-derived membrane potential is the difference of two large numbers and is highly sensitive to any inaccuracy in the potential contributions from both molecules. It is furthermore surprising and counterintuitive that the net potential is opposite to the potential generated by the

phospholipid molecules themselves. If we calculate the membrane potential using partial atomic charges from BP-TZVP-COSMO files instead of the charges used in the MD force field, then the water molecules produce a potential of 5.2 V, i.e. very similar to the result from the MD charges, but the DMPC contribution is only -0.6 V, compared to -4.55 V using the MD charges. This demonstrates that two plausible representations of the electrostatics of the phospholipid molecules can result in very large differences in the membrane potential, suggesting general difficulty to obtain a precise consensus for the potential distribution from MD simulations.

Wang reviewed the membrane potentials from 10 different MD simulations of phospolipid bilayers reported in literature, all using different combinations of force-fields, partial charges and electrostatic summation techniques.[14] The three DMPC simulations had values of 0.9 V, 0.9 V, and 0.77 V, while all results (including diphytanoyl-, dipalmitoyl- and diphytanyl phosphatidylcholine) range from 0.3 to 1.0 V, with a mean value of 0.7 V. This means that, even if optimistically analyzed, the variability of the membrane potentials derived from MD simulations is at least 0.2 V, and they seem to have the tendency to be about 0.4 V higher than the experimental value of ~0.3 V.[13] Wang mentions one promising MD simulation using polarizable force fields,[35,36] yielding dipole potentials closer to the experimental estimate, but it is at present not clear whether such force fields are generally more accurate.

At this point, it may be worth noting the theoretical maximum of the membrane potential that a DMPC double layer with a typical density of one DMPC molecule per 33Å³ would produce, if all zwitterionic dipoles pointed outward. Simple calculus yields a dipole moment of 25 D for the stretched zwitterion, and that would yield a membrane potential of roughly -17 V. In addition, each DMPC molecule has the dipole moments of the two ester groups, each being in the order of 2.5 D, which could add positively or negatively to the zwitterion dipoles. Obviously, such an

arrangement would have a completely unrealistic high electrostatic energy, and thus nature and the thermodynamic equilibration of the MD simulation always care for a strong reduction of the average net dipole in DMPC membranes. As a result, the zwitterionic dipoles seem to be orientated essentially parallel to the surface. Given the enormous maximum value of 17 V, it is already a remarkable achievement that the different MD simulations seem to agree within 0.2 V in their calculations of the membrane potential, i.e. within ~1.2% of the theoretical maximum value, and that they deviate from the experimental values only by about 0.4 V - 0.7 V, i.e. by 2.5% – 4%.

Nevertheless, despite this remarkable success, an error of 0.4 V still causes a shift of the energy of a singly charged ion by almost 10 kcal/mol, and thus a shift of K_{lipw} by almost 7 log units. This means that, for the prediction of K_{lipw} with a desired minimum accuracy of say 0.7 log units (which is a typical RMSE of COSMO-RS predictions for homogeneous phases),[37] we need even 10 times higher accuracy in the description of the potential, i.e. we can only tolerate errors of 0.04 V, which corresponds to 0.25% of the theoretical maximum of the DMPC potential.

Optimization of a model membrane potential

Given the large uncertainties involved in the derivation of the membrane dipole potential from MD simulations, we decided to use an empirical model potential with a small number of adjustable parameters. In order to achieve a physically most plausible shape of the membrane potential, we assume that the net dipole density of the membrane and of water can be represented by one or two Gauss distributions. As a result, the shape of the model potential is either a Gaussian-type error function, or the sum of two such functions. Each Gaussian has three adjustable parameters: The height h, the center position p and the width w:

$$D(u) = h \exp\left\{ -\left(\frac{x - p}{w} \right)^2 \right\} \qquad (5)$$

In the optimization algorithm, we iteratively searched for the values of these three or six parameters (*h, p* and *w*) that minimize the error in the prediction of the 75 ionic and 161 neutral K_{lipw} values. As shown in Figure 1, this approach leads to a plateau value in the membrane center when using one Gaussian distribution (red and green curves). With two Gaussian distributions this approach even allows for membrane potentials which may have a minimum or maximum in the head group region (dotted blue curve).

The robustness of the empirical fitting approach for adjusting the internal membrane potential Ψ_d with experimental K_{lipw} values was evaluated by dividing the data set into a training and a test set. Both ionic and neutral compounds were ordered by their K_{lipw} within chemical classes and then distributed roughly in a ratio of 2:1. Ionic compounds were categorized into a training set of 50 and a test set of 25 compounds (see SI), neutral compounds into a training set of 105 and a test set of 56 compounds (see Klamt et al.).[19] The 161 neutral K_{lipw} values from the original publication[19] were included in the process of adjusting Ψ_d, in order to check whether the adaptions of the model made with the addition of a membrane dipole affect the prediction accuracy for neutral compounds. The experimental values are averaged experimental K_{lipw} for temperatures up to 40°C and were taken from Endo et al.[2] They should therefore better represent the currently available data than the ones used in the original COSMO*mic* publication.[19]

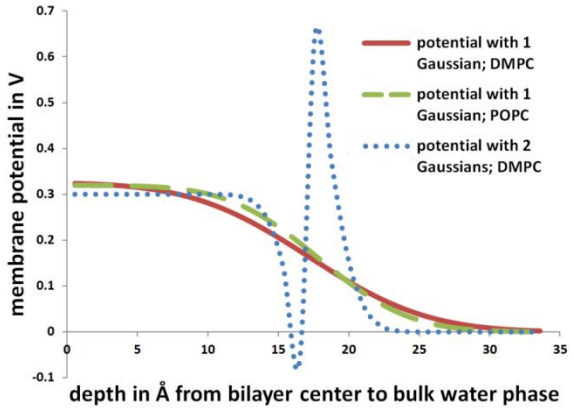

Figure 1. Three different profiles of the adjustable membrane potential for two different bilayers (DMPC and POPC) resulting with different height, position and width parameters of one (solid red and dashed green) and two (dotted blue) Gaussian-type dipole moment distributions. The depth is given along the membrane normal, starting in the membrane interior.

RESULTS and DISCUSSION

Predicting K_{lipw} using COSMO-RS with phosphatidylcholine lipid as bulk solvent

For a first comparison between isotropic and anisotropic solvents as depicted in COSMO*mic,* we calculated the partitioning from water to DMPC as bulk solvent. As shown before,[19] simple neutral compounds are surprisingly well predicted by considering phospholipid as bulk solvent (RMSE = 0.70, n = 161). Only for some bi-functional compounds the consideration of the bilayered structure plays a decisive role.[2] In contrast to these findings for neutral compounds, we see that the prediction of anions into bulk DMPC solvent is 2.4 to 15.7 log units lower than the experimental K_{lipw} values (resulting in RMSE = 9.51, n = 51), while the cations are predicted 4.3 to 9.6 log units too high (RMSE = 6.22, n = 24). Using a bulk solvent of POPC lipid for the prediction gives the same picture.

As expected, bulk solvent lipids are not anywhere near to being an appropriate model for membranes when it comes to the prediction of ion partitioning. While anisotropy can be neglected for most neutral compounds – being one reason for the good correlation between their K_{ow} and K_{lipw} – this simplification is not suitable for ions. Here, it seems that the orientation and location in the membrane is of crucial importance when it comes to the description of the sorption behavior of ions.

Predicting K_{lipw} using COSMO*mic* without considering the membrane potential Ψ_d

In a next step, the partition coefficients were calculated using COSMO*mic* but without accounting for the internal dipole potential. Calculations were made using trajectory averaged membrane structures over 80 ns simulation time with 128 DMPC and 3919 water molecules (which corresponds to a mole fraction of 0.032 and 0.968, respectively).[26] The simulation box was split into 30 layers giving a resolution of 1.13 Å for each layer. For every compound 162 different orientations in each layer were calculated. Predicting K_{lipw} of the above introduced 51 anions, 24 cations and 161 neutral compounds using COSMO*mic* as introduced previously[19] – i.e. not considering the membrane potential – results in Figure 2. The calculation without considering the membrane dipole potential leads to big systematic deviations for ionic compounds but not for neutral compounds.

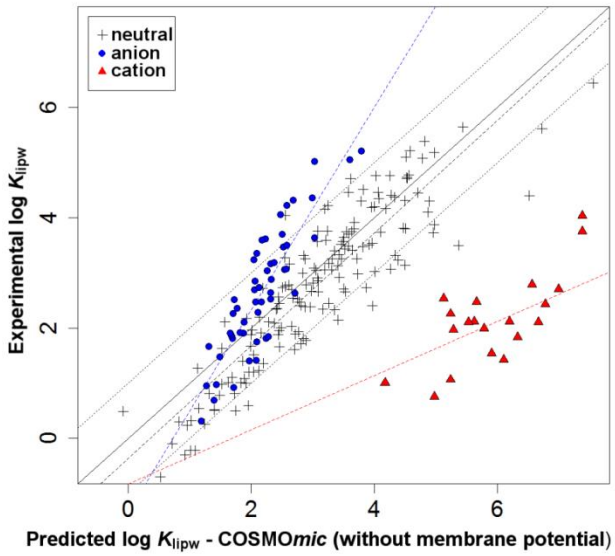

Figure 2. Experimental K_{lipw} of 161 neutral (black crosses), 51 anionic (blue circles) and 24 cationic (red triangles) compounds into a DMPC membrane against predicted values. The COSMO*mic* calculation here does not consider the membrane potential. The identity line (1:1 line) is indicated as solid line; deviations of +/- 1 log units are shown as dotted lines. For the dashed regression lines for neutral compounds, anions and cations, a least square regression has been used.

A plot of the σ-profile and the σ-potential reveal the anisotropic nature of the DMPC bilayer used for the calculations as shown in Figure 3. The probability distribution for the head group phosphorous and nitrogen atoms peaks at a distance of 18.7 and 19.8 Å, respectively, from the bilayer center, while the outermost bulk water layer is at 33.4 Å.

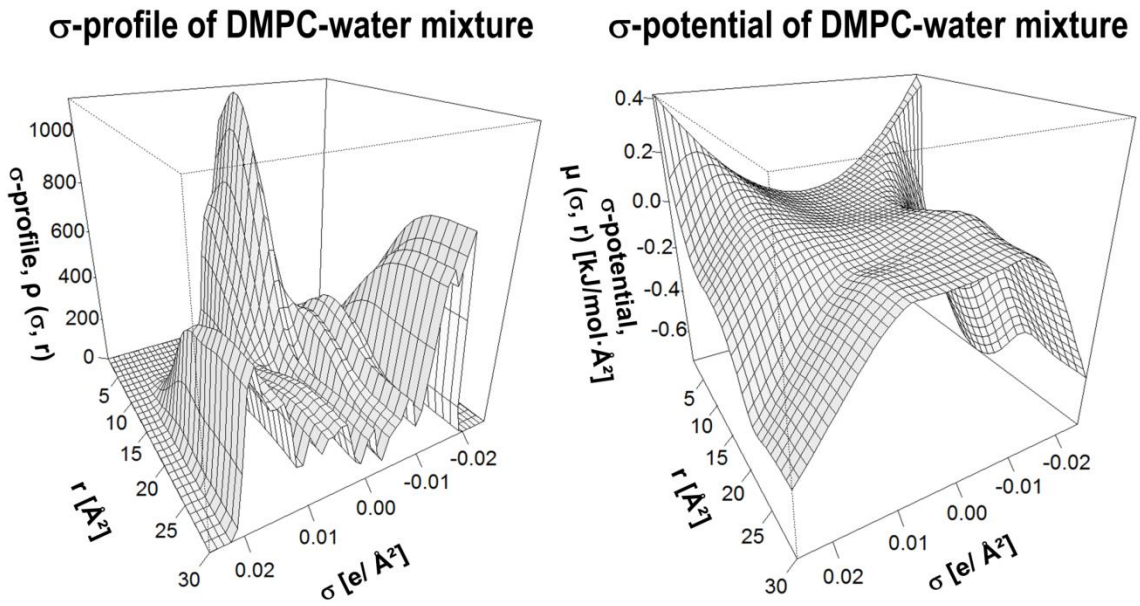

Figure 3. σ-profile (left) and σ-potential (right) of the DMPC-water system used for models M2, 2a and 3 as summarized in Table 2. These figures show the slicing of the membrane into consecutive liquids as done in COSMO*mic* (here no membrane dipole potential is additionally accounted for, yet). It can be seen how the DMPC lipids span from the first layer (representing the membrane bilayer center) to layer 27 (at 30 Å), where the bulk water phase begins. Each layer has a thickness of 1.13 Å.

The neutral compounds are as well predicted as expected. A linear equation of the regression line appears as follows:

$\log K_{lipw}(exp) = 1.02\ (\pm 0.04) * \log K_{DMPC/w}(calc) - 0.37\ (\pm 0.13)$; RMSE = 0.70, n = 161

Note that assuming errors in both experimental and calculated values in the regression analysis would result in slightly different slopes and intercepts. The predictions of the ions give a more heterogeneous picture. While all of the K_{lipw} values for cations are 0.9 to 2.3 log units overestimated, most of the K_{lipw} values for anions are underestimated (up to 1.9 log units) for the DMPC membrane shown in Figure 2. Using the POPC membrane yields the same result with

marginally different numbers. Fitting a least square regression through both differently charged groups separately gives the following equations for the DMPC membrane:

$\log K_{lipw}(exp) = 0.49\ (\pm 0.12) * \log K_{DMPC/w}(calc, cation) - 0.83\ (\pm 0.76)$; RMSE = 0.68, n = 24

$\log K_{lipw}(exp) = 1.84\ (\pm 0.15) * \log K_{DMPC/w}(calc, anion) - 1.34\ (\pm 0.33)$; RMSE = 0.55, n = 51

Here, the RMSEs are given with respect to the regression lines. One could use the regression equations for semi-empirical predictions as it has been done previously.[21] However, this would not be a satisfying approach, especially when considering the initial aspiration for a mechanistic model that is not limited to any kind of compound class or charge. We would not recommend using this fit, because the improvements presented in the next chapter render a fit unnecessary.

Using COSMO*mic* with an optimized membrane potential Ψ_d

Empirical membrane potentials have been optimized as outlined above for different membrane types (DMPC und POPC) and different salt concentrations (0 und 0.1 M KCl). The center positions, heights and widths as defined in equations 5 and 6 of the resulting Gauss curves are summarized in Table 1.

Table 1. Comparison of position, width and height of Ψ_d derived for different membrane structures based on time-averaged atom distributions

number	model	center position [Å]	height [mV]	width [Å]
M1	POPC (1 gauss)	17.891	320	7.138
M2	DMPC (1 gauss)	17.080	326	8.866
M2a	DMPC training (1 gauss)	15.948	357	9.332

M3	DMPC (2 gauss curves)	Pos1: 17.131 Pos2: 17.663	Height1: -996 Height2: 1296	Width1: 0.198 Width2: 2.813
M4	DMPC 0.1 M KCl (1 gauss)	16.258	340	10.796

Center position, height and width are the three adjustable parameters in the Gauss-type error function as defined above. For each model given here, all 161 K_{lipw} values for neutral and 75 values for ionic compounds have been used, except for model M2a, which has its potential optimized based on 56 neutral and 27 ionic K_{lipw} values.

There are only marginal differences in height and position of Ψ_d for all optimization runs with one Gaussian. The width differs slightly for the MD simulation including salt (0.1 M KCl), but this hardly has an influence on the predictive power as shown in Table 2. For the DMPC membrane, two different potentials have been optimized based on one and two Gaussian distributions (i.e. three and six adjustable parameters, respectively). The double Gaussian model did perform only marginally better than the single Gaussian distribution. Furthermore, the extreme fluctuations of the dipole potential fitted based on two Gaussians appear to be rather unlikely (see Figure 1). Hence, we consider the single Gaussian model as recommended.

To further evaluate the dependence of the potential optimization on the compound selection, the 75 ionic and 161 neutral species were divided into a training and test set (see SI) as described above. The potential was optimized for the same DMPC membrane as in model M2, but for model M2a the optimized Gaussian potential is based only on the training set. The performance of the resulting model M2a has been tested with the compounds of the test set, in order to evaluate how sensitive the predictions are in respect to the data set used for deriving the potential curves (see Tables 2 and 3). Although there are slight differences in the model M2 and model M2a potentials, the predictions of K_{lipw} values differ less than 0.3 log units between the two models for the 27 test compounds. The RMSEs and also the slopes and intercepts have very

similar values within the range of error, which indicates that the chosen approach results in robust predictions despite slightly different shapes of potentials. However, we do not recommend using the model M2a potential because a potential optimization based on all available experimental data should yield the most reliable potential shape.

Table 2. Comparison of the calculation of log K_{lipw} values for neutral and ionic compounds with different membranes, salt concentrations, forms of potential distribution (1 or 2 Gaussians) and different datasets underlying the potential optimization

num-ber	model	slope	intercept	offset	n	RMSE (ions)	RMSE (neutr.)
M1	POPC	0.94 ± 0.04	-0.11 ± 0.11	0.30 ± 0.04	236	0.71 (n=75)	0.63 (n=161)
M2	DMPC	0.96 ± 0.04	-0.21 ± 0.12	0.32 ± 0.04	236	0.70 (n=75)	0.62 (n=161)
M2a	DMPC training set	1.04 ± 0.06	-0.35 ± 0.19	0.25 ± 0.07	83	0.68 (n=27)	0.59 (n=56)
M3	DMPC (2 gauss curves)	0.97 ± 0.03	-0.34 ± 0.11	0.43 ± 0.04	236	0.66 (n=75)	0.60 (n=161)
M4	DMPC 0.1 M KCl	0.96 ± 0.04	-0.24 ± 0.12	0.37 ± 0.04	236	0.71 (n=75)	0.63 (n=161)

All models are based on the optimization of one Gauss curve for the membrane potential, except model M3. Slope, and intercept are given with the respective standard errors and are derived with a least square regression for neutral and ionic compounds together for the regression equation log K_{lipw} (experimental) = slope * log K_{lipw} (calculated) + intercept. The offset describes the non-weighted average of predicted minus experimental values for the calculated ionic and neutral species. The RMSE is obtained separately for n neutral and ionic compounds after subtracting the offset from the calculated value.

On average, COSMO*mic* predicts the K_{lipw} values roughly 0.3 log units too high, as shown by the different offset values in Table 2. It is important to note that during the potential optimization procedure, this systematic overprediction has not been minimized. The offset is significantly different from zero (two-tailed P values are not bigger than 0.0005 for the different models) and might be explained by remaining simplifications in the model like the assumption of structureless liquids for each of the membrane slices. Also, a possible contribution of the membrane deformation energy caused by the sorbing solutes is not considered. Accounting for such kind of 'volume work' would make the partitioning into the membrane less favorable and, therefore, reduce the absolute value of the offset. First attempts using an elastic term as introduced previously[19] showed this trend at the cost of an increased scatter in the prediction, indicating that the empirical expression for the deformation energy should be reinvestigated in further refinement. Up to this end, we can assess that the offset is fairly constant for different membranes (see Table 2) as well as for differently charged species. For model M2, for example, the predictions of the neutral species have an offset of 0.30, the anions of 0.37 and the cations of 0.40 log units, resulting in an average of 0.32 log units. Therefore, the RMSE in the predictions can be decreased by simply subtracting the average offset from the predicted K_{lipw} values as done in Table 2. The RMSEs of the ions were reduced by 0.09 to 0.13 log units by subtracting the offset values, except for model M3, having its RMSE reduced by 0.17 log units. Considering the remaining simplifications in COSMO*mic* as discussed above, the average overprediction of K_{lipw} as expressed in the offsets appears to be rather small.

The membrane potentials optimized for the DMPC and POPC membrane of course have a slightly different shape (Table 1), but lead to the same quality in the prediction (Table 2 and 3). This is in accordance with experimental results, which do not show significant differences in the sorption behavior of DMPC and POPC membranes either.[2] Similarly, the inclusion of a 0.1 M KCl concentration in the DMPC-water system (model M4) does not result in a big difference of the derived membrane potential and partition coefficients. It has experimentally been demonstrated that different salt concentrations (0.001 – 0.1 M KCl) have only marginal influence on K_{lipw} of ions.[8]

A good example of the influence of the membrane potential on the Gibbs free energy profiles and resulting calculated K_{lipw} is given by the experimental and calculated sorption behavior of the two oppositely charged tetraphenyl-analogs TPB and TPP (Figure 4). Although the negatively charged TPB is structurally very similar to the positively charged TPP, K_{lipw} of TPB is 4 orders of magnitudes higher than that of TPP.[38,39] Deviating almost exclusively by the sign of the surface charge (but not the charge density), this difference can only be explained by the influence of the membrane potential. The resulting attractive interactions between the positive inner potential of the membrane and negative TPB are reflected by a descending calculated ΔG profile. In contrast, the inclusion of the repulsive interactions between membrane potential and the positively charged TPP elevate the calculated ΔG profile.

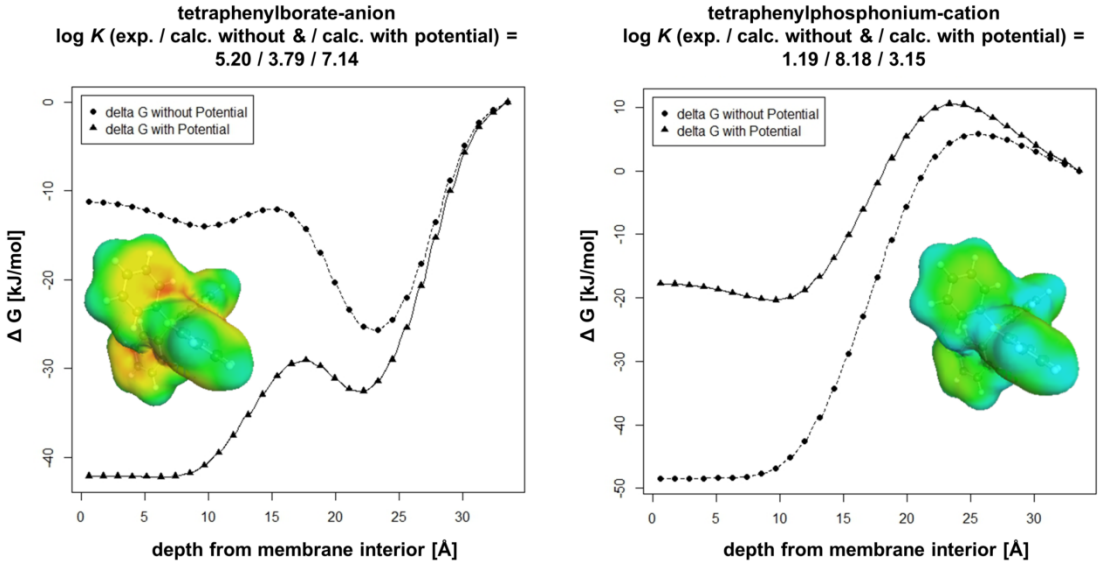

Figure 4. Influence of the membrane potential on the ΔG profiles of TPB and TPP in the DMPC membrane (model M2). Experimental data are from Flewelling and Hubbell[38] and Demura et al.[39] ΔG = 0 in the bulk water phase.

For model M2, on average, the ΔG values at the membrane center are 30.63 kJ/mol more negative for the anions, while the values for the cations are 30.58 kJ/mol more positive in comparison to the values without considering the membrane potential (see SI for ΔG profiles of all ions). For most of the anions, a local ΔG minimum can be found under the influence of the membrane potential in the area around 10 Å, while the global minima are mostly around the head group region at 22 Å from the bilayer center. In contrast, the ΔG minima for the cations are located around 11-13 Å from the bilayer center, i.e. deeper in the membrane despite the repulsive forces of the potential (only TPP has its ΔG minimum even deeper in the membrane). While 13 out of the 51 anions yield a ΔG profile that is negative throughout the whole expansion of the membrane, all of the cations do have an energy barrier in the membrane-water interface that might be explained by unfavorable interactions with the positively charged choline.

Reflecting the ΔG profiles, the peak maxima for the relative solute distributions are further away from the membrane bilayer center for the anions (mostly around 23 Å for M2) than for the cations (mostly around 13 Å for M2). Plots of the relative solute distribution of all ions are shown in the SI. The membrane potential has only little influence on the maximum peaks of the relative distribution for most ions: for most anions in model M2 it gets shifted one layer further towards the membrane center, while for most cations it gets shifted one layer towards the head groups. Unexpectedly, almost all presented cations can be found closer to the membrane center than the anions, despite the positive membrane potential in the membrane interior. It cannot be unambiguously concluded whether this is due to the present selection of ions or a generalizable finding.

Figure 5 shows the experimental values against the overall satisfying predictions of the model M2. Looking at cations and anions separately reveals that the predictions for anions (RMSE = 0.68) are slightly better than for cations (RMSE = 0.74). Note, however, that there are considerably less K_{lipw} data for cations (n = 24) than for anions (n = 51). In addition, some of the experimental K_{lipw} data from Fruttero et al.[40] for cationic secondary amines show an unusual sorption behavior, i.e., K_{lipw} decreases with increasing chain length for relatively short-chain amines.

The strong outliers which are more than 1.2 log units off in the prediction are mainly big molecules with a molecular weight above 300 except for p-methylbenzyl-methylamine cation, which is one of the secondary amines measured by Fruttero et al.[40] A reason for the inaccurate prediction of these big molecules might be changes in the membrane provoked by the sorbing molecules that are not accounted for in COSMO*mic*, like the possible membrane perturbation caused by salmeterol that may have an influence on the fluidity as proposed in Lombardi et al.[41]

The implementation of the membrane potential leads to a contrariwise shift of the calculated K_{lipw} values for anions and cations, as expected. The impact of the potential on the calculation of K_{lipw} is different for each ion, but leads to an improved prediction for almost all ions. Not only the potentials absolute height is of importance but also the position and the width matter because most ions have their maximum probability of presence in the head group area, where the potential levels off to zero. Within the model M2, 5-chloro-3-tert-butyl-2'-chloro-4'-nitrosalicylanilide (S-13) and TPB show the biggest increase amongst the anions of more than 3 log units, while 4-octylbenzene-1-sulfonate exhibits the lowest change with 0.52 log units. The changes for the cations are overall larger than those for anions, going from a decrease of 3.06 (amlodipine) to 5.03 log units (TPP).

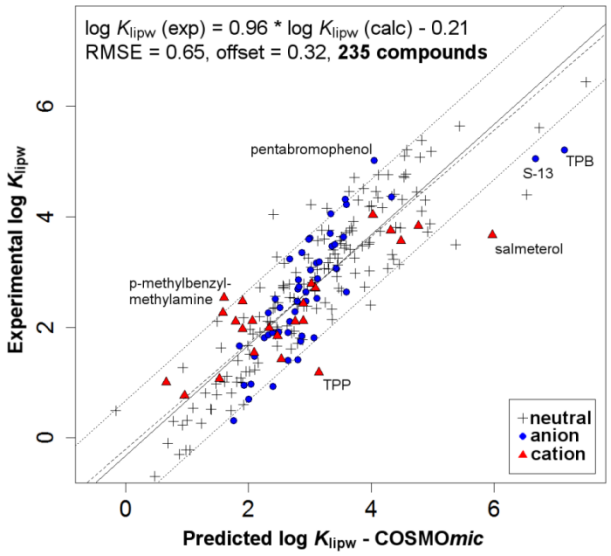

Figure 5. Prediction of neutral (black cross), anionic (blue circles) and cationic (red triangles) compounds with COSMO*mic* including the membrane potential using one Gaussian potential for a DMPC membrane (model M2). The identity line (solid) as well as the deviations of +/- 1 log units (dotted) are shifted by 0.32 log units according to the offset of model M2. The linear equation describes the least square regression (dashed line). The RMSE is calculated for all 235

neutral and ionic compounds after subtracting the offset. All ions that are predicted more than 1.2 log units off are annotated.

The influence of the membrane potential on the prediction of neutral compounds

The prediction of K_{lipw} values for neutral compounds is better than for the ionic compounds according to the lower RMSE (if no constant offset was subtracted from the calculated values, the RMSEs would be 0.05 to 0.11 log units higher than given in Table 2). Note that the dataset for neutral compounds comprises disproportionally more values and spans over more orders of magnitude than the dataset of ionic compounds. The membrane potential has only a marginal influence on the calculation of K_{lipw} for neutral compounds – for model M2 (Figures 2 and 5) the largest change due to the implementation of membrane potential is 0.23 log units for carbonyl cyanide p-methoxyphenylhydrazone. Note that in the case of PAHs, there seems to be a trend to underestimate their partition coefficients from water to both octanol and the membrane lipid phase, as has been observed previously.[2] As the data set of 161 neutral compounds used in this work contains no PAHs, this effect does not show up in the present study.

The observed insensitivity of K_{lipw} predictions of neutral compounds with respect to the membrane potential is as expected and an affirmative result, confirming the presumed considerations of the model. If the membrane potential had a crucial impact on the sorption of neutral compounds, the bulk phase partitioning between octanol and water could not be expected to correlate so well with neutral K_{lipw} values.

CONCLUSIONS

In order to predict how strongly ions sorb to phospholipid membranes, the membrane potential has to be adequately accounted for, although it cannot be deduced directly from the membrane

structure. In the presented enhancement of COSMO*mic,* the membrane potential has been implemented as a Gaussian-type error function that is optimized with the experimental sorption data, yielding satisfying K_{lipw} predictions for neutral and ionic compounds. In principle, this extension of COSMO*mic* should also be applicable for multivalent ions, but experimental values for only two di-cations were found in the literature:[42] trifluoperazine is reported to have a log K_{lipw} of 3.76 and quinine of 1.33, respectively (measured at a pH of 2 in l(water) over kg(lipid)). The COSMO*mic*-calculated log K_{lipw} values with model M2 are 4.34 and 1.15, respectively, which are in good agreement with the experimental data. Further investigations on multivalent ions are desirable.

The overall prediction accuracy of the revised COSMO*mic* model presented in this work is well within the expected accuracy of COSMO*therm*, which is reported to be 0.65 to 0.93 log units for the prediction of the partitioning between different liquid/liquid systems for highly diverse datasets.[37] Although there seems to be still some scatter in the prediction especially for cations, the presented enhancement of COSMO*mic* is, to the best of our knowledge, the first mechanistic model that is able to predict the sorption of both ions and neutral species in such a complex anisotropic phase as membranes are.

In future research, the energy profiles derived with COSMO*mic* could be used to predict the permeability of ions through membranes, as the main resistances in the membrane is strongly related to the Gibbs free energy profiles. This is specifically important when it comes to the toxicity of uncouplers, which involves the transfer of ions through energy transducing membranes.[21] The presented improvement of COSMO*mic* for the use with ions may also have implications in drug design, where the ”lipophilic efficiency” of ionogenic drugs is still often quantified by using an empirically estimated octanol-water partition coefficient (i.e. log D_{ow}).

Furthermore, we expect an improvement of the assessment of the bioaccumulation potential of charged or partially charged compounds which may be important for industry as well as regulation authorities.

ASSOCIATED CONTENT

Supporting Information

Details on the experimental conditions and values used, definition of the estimation of variance (RMSE), Gibbs free energy and relative probability profiles for all ions with and without the implementation of the membrane potential (for model M2) and all predicted values of the different models. 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.

ACKNOWLEDGMENTS

We thank Sven Jakobtorweihen for the membrane structures that have been calculated according to the procedure suggested in Jakobtorweihen et al.[26] and Pavel Smejtek, Stefanie Krämer and Beate Escher for fruitful discussions.

REFERENCES

(1) Schmitt, W. General Approach for the Calculation of Tissue to Plasma Partition Coefficients. *Toxicol. In Vitro* **2008**, *22*, 457–467.

(2) Endo, S.; Escher, B. I.; Goss, K.-U. Capacities of Membrane Lipids to Accumulate Neutral Organic Chemicals. *Environ. Sci. Technol.* **2011**, *45*, 5912–5921.

(3) Loidl-Stahlhofen, A.; Hartmann, T.; Schöttner, M.; Röhring, C.; Brodowsky, H.; Schmitt, J.; Keldenich, J. Multilamellar Liposomes and Solid-Supported Lipid Membranes (TRANSIL): Screening of Lipid-Water Partitioning toward a High-Throughput Scale. *Pharm. Res.* **2001**, *18*, 1782–1788.

(4) Honig, B. H.; Hubbell, W. L.; Flewelling, R. F. Electrostatic Interactions in Membranes and Proteins. *Annu. Rev. Biophys. Biophys. Chem.* **1986**, *15*, 163–193.

(5) Avdeef, A. Structure of Liposomal Membranes in Relation to Permeation. In *PHARMACOKINETIC OPTIMIZATION IN DRUG RESEARCH*; 2001.

(6) Bangham, a. D.; Standish, M. M.; Watkins, J. C. Diffusion of Univalent Ions across the Lamellae of Swollen Phospholipids. *J. Mol. Biol.* **1965**, *13*, 238–252.

(7) Krämer, S. D.; Wunderli-Allenspach, H. Physicochemical Properties in Pharmacokinetic Lead Optimization. *Farm.* **2001**, *56*, 145–148.

(8) Escher, B. I.; Sigg, L. Chemical Speciation of Organics and of Metals at Biological Interphases. In *Physicochemical Kinetics and Transport at Biointerfaces*; v. Leeuwen, H. P.; Köster, W., Eds.; John Wiley & Sons: Chichester, UK, 2004; pp. 205–269.

(9) Smejtek, P.; Wang, S. Distribution of Hydrophobic Ionizable Xenobiotics between Water and Lipid Membranes: Pentachlorophenol and Pentachlorophenate. A Comparison with Octanol-Water Partition. *Arch. Environ. Contam. Toxicol.* **1993**, *25*, 394–404.

(10) Escher, B. I.; Snozzi, M.; Schwarzenbach, R. P. Uptake, Speciation, and Uncoupling Activity of Substituted Phenols in Energy Transducing Membranes. *Environ. Sci. Technol.* **1996**, *30*, 3071–3079.

(11) Avdeef, A.; Box, K.; Comer, J.; Hibbert, C.; Tam, K. Y. pH-Metric logP 10. Determination of Liposomal Membrane-Water Partition Coefficients of Lonizable Drugs. *Pharm. Res.* **1998**, *15*, 209–215.

(12) Olson, F.; Hunt, C. A.; Szoka, F. C.; Vail, W. J.; Papahadjopoulos, D. Preparation of Liposomes of Defined Size Distribution by Extrusion through Polycarbonate Membranes. *Biochim. Biophys. Acta - Biomembr.* **1979**, *557*, 9–23.

(13) Clarke, R. J. The Dipole Potential of Phospholipid Membranes and Methods for Its Detection. *Adv. Colloid Interface Sci.* **2001**, *89-90*, 263–281.

(14) Wang, L. Measurements and Implications of the Membrane Dipole Potential. *Annu. Rev. Biochem.* **2012**, *81*, 615–635.

(15) Franklin, J. C.; Cafiso, D. S. Internal Electrostatic Potentials in Bilayers: Measuring and Controlling Dipole Potentials in Lipid Vesicles. *Biophys. J.* **1993**, *65*, 289–299.

(16) Ingram, T.; Storm, S.; Kloss, L.; Mehling, T.; Jakobtorweihen, S.; Smirnova, I. Prediction of Micelle/water and Liposome/water Partition Coefficients Based on Molecular Dynamics Simulations, COSMO-RS, and COSMOmic. *Langmuir* **2013**, *29*, 3527–3537.

(17) Paloncýová, M.; Devane, R.; Murch, B.; Berka, K.; Otyepka, M. Amphiphilic Drug-Like Molecules Accumulate in a Membrane below the Head Group Region. *J. Phys. Chem. B* **2014**, *118*, 1030–1039.

(18) Paloncýová, M.; Fabre, G.; DeVane, R. H.; Trouillas, P.; Berka, K.; Otyepka, M. Benchmarking of Force Fields for Molecule–Membrane Interactions. *J. Chem. Theory Comput.* **2014**, 140708151005004.

(19) Klamt, A.; Huniar, U.; Spycher, S.; Keldenich, J. COSMOmic: A Mechanistic Approach to the Calculation of Membrane-Water Partition Coefficients and Internal Distributions within Membranes and Micelles. *J. Phys. Chem. B* **2008**, *112*, 12148–12157.

(20) Jakobtorweihen, S.; Zuniga, A. C.; Ingram, T.; Gerlach, T.; Keil, F. J.; Smirnova, I. Predicting Solute Partitioning in Lipid Bilayers : Free Energies and Partition Coefficients from Molecular Dynamics Simulations and COSMOmic. *J. Chem. Phys.* **2014**, *141*.

(21) Spycher, S.; Smejtek, P.; Netzeva, T. I.; Escher, B. I. Toward a Class-Independent Quantitative Structure--Activity Relationship Model for Uncouplers of Oxidative Phosphorylation. *Chem. Res. Toxicol.* **2008**, *21*, 911–927.

(22) Kaiser, S.; Escher, B. I. The Evaluation of Liposome-Water Partitioning of 8-Hydroxyquinolines and Their Copper Complexes. *Environ. Sci. Technol.* **2006**, *40*, 1784–1791.

(23) Escher, B. I.; Schwarzenbach, R. P.; Westall, J. C. Evaluation of Liposome−Water Partitioning of Organic Acids and Bases. 1. Development of a Sorption Model. *Environ. Sci. Technol.* **2000**, *34*, 3954–3961.

(24) Thomae, A. V; Koch, T.; Panse, C.; Wunderli-Allenspach, H.; Krämer, S. D. Comparing the Lipid Membrane Affinity and Permeation of Drug-like Acids: The Intriguing Effects of Cholesterol and Charged Lipids. *Pharm. Res.* **2007**, *24*, 1457–1472.

(25) Wezel, A. van; Cornelissen, G.; Miltenburg, J. van; Opperhuizen, A. Membrane Burdens of Chlorinated Benzenes Lower the Main Phase Transition Temperature in Dipalmitoyl-Phosphatidylcholine Vesicles: Implications for Toxicity by Narcotic Chemicals. *Environ. Toxicol. Chem.* **1996**, *15*, 203–212.

(26) Jakobtorweihen, S.; Ingram, T.; Smirnova, I. Combination of COSMOmic and Molecular Dynamics Simulations for the Calculation of Membrane-Water Partition Coefficients. *J. Comput. Chem.* **2013**, 1–9.

(27) Becke, A. D. Density-Functional Exchange-Energy Approximation with Correct Asymptotic Behavior. *Phys. Rev. A* **1988**, *38*, 3098–3100.

(28) Perdew, J. Density-Functional Approximation for the Correlation Energy of the Inhomogeneous Electron Gas. *Phys. Rev. B* **1986**, *33*, 8822–8824.

(29) Schäfer, A.; Huber, C.; Ahlrichs, R. Fully Optimized Contracted Gaussian Basis Sets of Triple Zeta Valence Quality for Atoms Li to Kr. *J. Chem. Phys.* **1994**, *100*, 5829.

(30) Eichkorn, K.; Treutler, O.; Öhm, H.; Häser, M.; Ahlrichs, R. Auxiliary Basis Sets to Approximate Coulomb Potentials. *Chem. Phys. Lett.* **1995**, *242*, 652–660.

(31) TURBOMOLE V6.5 2013, a development of University of Karlsruhe and Forschungszentrum Karlsruhe GmbH, 1989-2007, TURBOMOLE GmbH, since 2007; available from http://www.turbomole.com.

(32) Vainio, M. J.; Johnson, M. S. Generating Conformer Ensembles Using a Multiobjective Genetic Algorithm. *J. Chem. Inf. Model.* **2007**, *47*, 2462–2474.

(33) Flewelling, R. F.; Hubbell, W. L. The Membrane Dipole Potential in a Total Membrane Potential Model. Applications to Hydrophobic Ion Interactions with Membranes. *Biophys. J.* **1986**, *49*, 541–552.

(34) Gurtovenko, A. a; Patra, M.; Karttunen, M.; Vattulainen, I. Cationic DMPC/DMTAP Lipid Bilayers: Molecular Dynamics Study. *Biophys. J.* **2004**, *86*, 3461–3472.

(35) Chowdhary, J.; Harder, E.; Lopes, P. E. M.; Huang, L.; MacKerell, A. D.; Roux, B. A Polarizable Force Field of Dipalmitoylphosphatidylcholine Based on the Classical Drude Model for Molecular Dynamics Simulations of Lipids. *J. Phys. Chem. B* **2013**, *117*, 9142–9160.

(36) Harder, E.; Mackerell, A. D.; Roux, B. Many-Body Polarization Effects and the Membrane Dipole Potential. *J. Am. Chem. Soc.* **2009**, *131*, 2760–2761.

(37) Stenzel, A.; Goss, K.-U.; Endo, S. Prediction of Partition Coefficients for Complex Environmental Contaminants: Validation of COSMOtherm, ABSOLV, and SPARC. *Environ. Toxicol. Chem.* **2014**, *33*, 1537–1543.

(38) Flewelling, R. F.; Hubbell, W. L. Hydrophobic Ion Interactions with Membranes. Thermodynamic Analysis of Tetraphenylphosphonium Binding to Vesicles. *Biophys. J.* **1986**, *49*, 531–540.

(39) Demura, M.; Kamo, N.; Kobatake, Y. Binding of Lipophilic Cations to the Liposomal Membrane: Thermodynamic Analysis. *Biochim. Biophys. Acta - Biomembr.* **1987**, *903*, 303–308.

(40) Fruttero, R.; Caron, G.; Fornatto, E.; Boschi, D.; Giuseppe, E.; Gasco, A.; Carrupt, P.-A.; Testa, B. Mechanisms of Liposome/Water Partitioning of (p-Methylbenzyl)alkylamines. *Pharm. Res.* **1998**, *15*, 1407–1413.

(41) Lombardi, D.; Cuenoud, B.; Krämer, S. D. Lipid Membrane Interactions of Indacaterol and Salmeterol: Do They Influence Their Pharmacological Properties? *Eur. J. Pharm. Sci.* **2009**, *38*, 533–547.

(42) Pallicer, J. M.; Krämer, S. D. Evaluation of Fluorescence Anisotropy to Assess Drug-Lipid Membrane Partitioning. *J. Pharm. Biomed. Anal.* **2012**, *71*, 219–227.