Structural Bioinformatics

Prediction of the permeability of neutral drugs inferred from their solvation properties

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Abstract

Motivation: Determination of drug absorption is an important component of the drug discovery and development process in that it plays a key role in the decision to promote drug candidates to clinical trials. We have developed a method that, on the basis of an analysis of the dynamic distribution of water molecules around a compound obtained by molecular dynamics simulations, can compute a parameter-free value that correlates very well with the compound permeability measured using the human colon adenocarcinoma (Caco-2) cell line assay.

Results: The method has been tested on twenty-three neutral drugs for which a consistent set of experimental data is available. We show here that our method reproduces the experimental data better than other existing tools. Furthermore it provides a detailed view of the relationship between the hydration and the permeability properties of molecules.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

The study of drug absorption is of critical importance in the development of effective drugs. The path of a drug from the site of administration to its target cells or compartments implies the crossing of several semipermeable cell membranes, therefore it is relevant to be able to predict whether and to which extent a molecule can pass through the cell membranes. Passive permeation of drugs through the biological cell membranes is obviously strongly dependent on the molecule physicochemical properties (Meanwell, 2011). It has been established that the acid-base character of the molecule (which influences the charge of the molecule at the specific pH), its lipophilicity (which affects its partition between aqueous and lipid environments) and solubility are the most relevant parameters to take into account. These parameters are well described by the molecule hydrophathy profile (Siew, et al., 2012; Smith, et al., 2010). A more lipophilic drug is more likely to effectively cross the hydrophobic phospholipid bilayer. On the other hand, extremely hydrophobic molecules, insoluble in aqueous body fluids, might be poorly absorbed (Frenkel, et al., 2005). In summary, there should be an appropriate balance between the hydrophobicity and hydrophilicity of a molecule (Ghuman, et al., 2005; Seelig, et al., 1994; Waring, 2009).

From an experimental point of view, data on permeability can be obtained by in situ and/or in vivo animal studies, but these are time consuming and expensive experiments and therefore only performed towards the end of the drug development process. Efforts have therefore focused on the development of in vitro permeability assays that can mimic the relevant characteristics of in vivo absorption. Among these, there are the parallel artificial membrane permeability assay (PAMPA) (Avdeef, et al., 2007), the human colon adenocarcinoma (Caco-2) cell line assay (Artursson, et al., 2001), the Madin-Darby canine kidney (MDCK) cell assay (Irvine, et al., 1999), the rat duodenal immortalized cell line assay (2/4A1 cell) (Tavelin, et al., 2003), and the rat everted gut sac assay (Bohets, et al., 2001). All of them are routinely used for the preliminary assessment of drug permeability. In particular, the Caco-2 cell is probably the most extensively characterized cell-based model and the most popular both in the pharmaceutical industry and in academia (Balimane, et al., 2006). It has been shown that this model can effectively predict the human initial drug absorption (Artursson and Karlsson, 2005).
The membrane permeability for a given compound is usually estimated from its partition coefficient, logP, defined as the logarithm of the relative concentration of the molecule when it partitions between a two-phase system, usually water and octanol, where the latter is assumed to have a lipophilicity comparable to that of a cell membrane (Artursson, et al., 2001; Seddon, et al., 2009).

From the theoretical point of view, many computational approaches have been developed to infer drug properties, such as bioavailability, aqueous solubility, initial absorption, plasma-protein binding and toxicity (van de Waterbeemd and Gifford, 2003). These are often related to features such as molecular size, hydrophobicity, or number of hydrogen bonds established by the compound with water molecules (since these bonds need to be broken to allow the molecule to pass the membrane) (Hou, et al., 2004).

Other popular methods are the QSAR (Quantitative Structure-Property Relationship) analysis (Yu and Adedoyin, 2003), Multiple Linear Regression (MLR), Partial Least Square (PLS), Linear Discriminant Analysis (LDA), Artificial Neutral Networks (ANNs), Genetic Algorithms (Gas), Support Vector Machines (SVMs) and the "Lipinski rule of five" (Lipinski, 2000). In particular, the Lipinski’s rule takes into account different features to assess whether a compound is likely to be cell membrane permeable and easily absorbed by the body on the basis of the following criteria: molecular weight of the compound lower than 500; logP lower than 5; number of hydrogen bond donors (usually the number of hydroxyl and amine groups in a drug molecule) lower than 5; number of groups that can accept hydrogen atoms to form hydrogen bonds (estimated by the number of oxygen and nitrogen atoms) lower than 10.

In this work we describe a new method based on an estimate of the hydrophobicity and charge distribution of a compound deduced from the distribution and orientation of the water molecules around it. We have already successfully used a similar approach to estimate the hydrophobicity of the twenty natural amino acids (Bonella, et al., 2014). Here we show that, when applied to a set of 23 drugs, neutral at physiological pH, to compute their hydrophobicity and charge distribution, the method can effectively predict their ability to cross the plasma membrane.

Our dataset only includes neutral compounds since these are well known to mainly use passive transport to cross the phospholipid bilayer of the cell membrane (Neuhoff, et al., 2003; Neuhoff, et al., 2005; Seelig, 2007) and therefore their diffusion and permeability is essentially related to their chemico-physical properties that is what our method can infer.

2 Methods

We analysed thehydration of small solutes by investigating the changes in the structure of the dynamic hydrogen bond network formed by the water molecules surrounding them as well as their orientation as obtained by molecular dynamics simulations.
We can define the angles related to hydrogen bond orientations ($\theta_1$) lies along the bisectrix of the angle formed by the oxygen and the first component of the hydrophilic index related to the two peaks in the first hydration shell. The green arrows show the first and the second component of the hydrophobic peaks that are localized in the second hydration shell. In the P($\theta_2$) histogram, the blue arrow indicates the contribution of positive charge distribution. The pink arrow indicates the contribution of the negative charge distribution.

Fig. 2. Histograms of $P(\theta_1(R))$ and $P(\theta_2(R))$ for Diazepam. In both histograms the cells highlighted in grey are used to calculate the sum of the conditional probability densities at each given angle and distance. In the P($\theta_2(R)$) histogram, the yellow arrows indicate the first and second component of the hydrophilic index related to the two peaks in the first hydration shell. The green arrows show the first and the second component of the hydrophobic peaks that are localized in the second hydration shell. In the P($\theta_2(R)$) histogram, the blue arrow indicates the contribution of positive charge distribution. The pink arrow indicates the contribution of the negative charge distribution.

gen bond vector (for clarity, only one of the four angles is represented in blue in Figure 1). Similarly, we can define the angle $\theta_d$ related to the orientation of the dipole vector as the angle formed by the straight line connecting a solute atom (S in Figure 1) to the oxygen atom of the closest water molecule (in black) and the dipole vector of the molecule itself (in red). The different orientations of the water molecules around a solute can be used to analyse the compound hydrophilicity and hydrophobicity. In fact a water molecule in the vicinity of a hydrophobic solute positions one of the faces of the tetrahedron toward the solute. On the other hand, for a hydrophilic solute, a water molecule reorients to point toward the compound with one of its vertices. We need to take the dipole vector into account because the four vertices of the tetrahedron representing the waters are equivalent in our model and therefore it would be impossible to distinguish between positive and negative partial charges without considering $\theta_d$.

At each step of the molecular dynamics simulation, we can measure the values of the five angles ($\theta_{1A}$, $\theta_{1B}$, $\theta_{2A}$, $\theta_{2B}$ and $\theta_d$) and the distance R (Å) between each water molecule and the nearest solute atom and compute the probability of finding a water molecule with a given orientation and around at a given distance from the solute atoms. The hydrophathy and charge distribution properties are computed from the conditional probability density of the waters in the appropriate intervals of the angles and distances described before. We can build two three-dimensional histograms for each simulation; the first reports the conditional probability density $P(\theta_{ik}(R))$ (for $i = 1, 2, 3, 4$), the second is...
the conditional probability density $P(\theta, R)$. $R$ is defined as the distance between each solute atom and the oxygen atom of the nearest water molecule. The histogram distance and angle bins were set to 0.05 Å and 1°, respectively (Bonella et al., 2014).

2.4 Molecular descriptors

The analysis of the conditional probability density distributions allows us to compute four indices, named $I_p$, $I_n$, $I_s$, and $I_c$, obtained by summing the intensity of the peaks in the appropriate angle and distance range.

As described in more detail in our previous work (Bonella et al., 2014) the distribution $P(\theta, R)$ permits to distinguish between the hydrophilicity and hydrophobicity of a compound on the basis of the probability values observed in the first and second hydration shell, respectively. Intuitively, this is justified by the fact that a polar solute will establish Coulomb interactions with the closest water molecules and this situation will contribute to the peaks observed in the first hydration shell of the hydrogen bond histogram, while a hydrophobic (or apolar) solute will cause the waters to orient themselves as to maximize the number of hydrogen bonds with neighboring waters, forming a cage around the solute, and will contribute to peaks in the second hydration shell in the hydrogen bond histogram.

The dipole probability density $P(\theta, R)$ in the first hydration shell takes into account which of the vertices of the tetrahedron representing the waters (all equivalent in our model) is oriented towards the solute and therefore provides information about the electric charge (positive or negative) of the interacting solute atoms.

We define the compound hydrophilicity $I_p$ and hydrophobicity $I_n$ as the sum of the hydrogen bond probability densities, computed over the appropriate distance and angle range ($\Delta \theta$ and $\Delta R$) in the first and second shell of hydration, respectively. The charge indices $I_s$ and $I_c$ are defined as the sum, in the appropriate range, of the probability densities in the first shell of the distribution related to dipole moment (see Figure 2). For more details, see ref. (Babiaczyk et al., 2010; Bonella et al., 2014).

As shown in Figure S3a-c, the length of the MD simulation (1.5 ns) is sufficient to ensure convergence of the indices.

The scheme used to select the boundaries of the region ($\Delta \theta$ and $\Delta R$) is based on Gaussian fits. In particular, we performed a Gaussian fit of the probability distribution for both the first and second hydration shell along the $\theta$ axis (see Figure S4) and determined the average and standard deviation of the Gaussian distributions for each of the compounds. The average of these values is used to compute the volume of each peak. A similar approach has been used to determine the range of integration along the $R$ axis.

The analytical details of the scheme used to select the boundaries of the region ($\Delta \theta$ and $\Delta R$) are described in the Supporting information. The scripts for running the simulations and perform the analysis are available at: http://arianna.med.uniroma1.it/neutraldrugs/.

2.5 Statistical analysis and comparison with other methods

The program used to analyse the molecular dynamics trajectories and to build the histograms was written in Fortran90. The R package (Ihaka and Gentleman, 1996) [http://www.R-project.org] was used to analyse the histograms. The same package was used to calculate the indices, perform the Gaussian fitting and the Multiple Regression Analysis (MRA), compute the Pearson’s correlation coefficient, r and perform the cross validation analysis. The clustering analysis was performed using the Euclidean distance and via the "hclust" function from the "stats" package of R (in particular, the "average" method of the "hclust" function was used). We compared our results with those of several other methods. In particular we computed, for each of the 23 compounds, the predicted permeability values according to the two methods described in ref. (Fujiiwara et al., 2002), based on a linear combination of molecular descriptors (Fui_1), or including quadratic terms (Fui_2). We also compared our results with those obtained by a linear regression (Hou) and a multiple linear regression (Guangli and Yiyu, 2006) (Gua_1) method. Finally we also used for comparison the Support Vector Machine based method (Gua_2) described in ref. (Guangli and Yiyu, 2006).

![Fig. 3. Scatter plot correlating the predicted permeability values in the cross validation (P$_\text{pred \_CV}$) and their experimental Caco-2 values. For each compound the average predicted value and the standard deviation are reported.](image-url)

3 Results

In silico permeability prediction is consistent with available published data. We computed four indicators ($I_p$, $I_n$, $I_s$, and $I_c$) described in the Methods section for each of the drugs in our dataset. As explained in detail in the Methods section, these indices are derived from the conditional probability of finding a water molecule with a given orientation around the solute atoms estimated from the results of molecular dynamics simulations. In particular, the first two ($I_p$ and $I_n$) provide information about the hydrophilic and hydrophobic properties of the compound and are computed from the probability values of finding water molecules in the first and second hydration shells, respectively. $I_s$ and $I_c$ are related to the dipole orientation of the water molecules surrounding the analysed compound and therefore to the effect of its positive and negative charges.

The values of the indices for the analysed molecules are reported in Table S1. Three of these parameter-free indicators ($I_s$, $I_n$, and $I_c$) correlate remarkably well with the permeability data while the $I_p$ index shows a lower level of correlation.

We tested whether a combination of these indices can represent a good proxy for estimating the permeability of a molecule. To this end, we used a multiple linear regression algorithm as implemented in the R function "lm" (Ihaka and Gentleman, 1996) to find the weights providing the best
correlation with the Caco-2 experimental data. The tool also provides the probability p-value of a computed coefficient to be different from 0. We tested both linear and quadratic terms in the regression. The best correlation is obtained by a linear fit of the \( I_y \) and \( I_+ \) indices (p-value < 0.001), while \( I_y \) and \( I_+ \) were found to contribute very little to the overall correlation (p-value > 0.05). This is consistent with the values of their correlation coefficients (see Table 1).

We compared our results with those of several other methods (as described in the Methods section) and the results are reported in Table 3 and Figure S5a-e. It can be appreciated that the correlation between predicted and experimental values is higher for our method. The average error is lower than all other tested methods, but for the Gua_2 method (Guangli and Yiyu, 2006) that shows a very similar value.

### Table 1. Correlation between the values of the indices in our dataset. Also the correlation value between each index and Caco-2 experimental value is reported.

| \( I_y \) | \( I_n \) | \( I_+ \) | \( I_- \) | Caco-2 |
|---|---|---|---|---|
| 1 | -0.05 | -0.39 | 0.89 | 0.28 |
| \( I_y \) | -1 | -0.76 | 0.33 | 0.85 |
| \( I_+ \) | - | - | 1 | -0.76 | -0.81 |
| \( I_- \) | - | - | - | 1 | 0.59 |

The regression model corresponding to the best fit is:

\[
P_{\text{pred}} = (a \cdot I_y) + (b \cdot I_+) + c\quad \text{Eq. (1)}
\]

where \( a = 3.06 \) (p-value = \( 4.7 \times 10^{-7} \)), \( b = 0.04 \) (p-value = \( 2.6 \times 10^{-4} \)) and \( c = -3092 \) (p-value = \( 4.0 \times 10^{-4} \)).

In Table 1 we also report the correlation between each index and the Caco-2 permeability values. As the highest linear correlation value is between \( I_n \) index (hydrophobic index) and Caco-2 permeability value because a more lipophilic drug is more likely to effectively cross the hydrophobic phospholipid bilayer. More interesting is the correlation linked to positive charge distribution index \( I_+ \). It can be observed that the index with the highest value of negative correlation is \( I_- \), indicating that most likely positive groups prevent uptake of compounds more than negative ones (see also Figure S5).

Table 2 reports the predicted \( P_{\text{pred}} \) permeability values using equation 1 for all the drugs considered and shows that they reproduce very well the experimental Caco-2 permeability values (Pearson’s correlation coefficient, \( r = 91\% \)). We also performed a cross validation analysis by repeatedly leaving out 20% of the compounds (testing sets) and re-computing the coefficients of Eq. (1) on the remaining ones (training sets) as described in the Methods section. We iterated this procedure 10,000 times, randomly choosing the training set at each step. The predicted average values (\( P_{\text{pred, CV}} \)) obtained for each drug in the test set are reported in Table 2. Once again, the correlation between prediction and experiment is very satisfactory (88 %) (Figure 3).

Table 3. Comparison of the results of the \( P_{\text{pred}} \) method with those obtained by a number of other predictors (described in Experimental section). \( P_{\text{pred, CV}} \) (using the test set data) also has been reported. The goodness of fit parameters (\( r^2 \) and error) are also shown.

| Ref. | Fuj_1 | Fuj_2 | Hou | Gua_1 | Gua_2 | \( P_{\text{pred}} \) | \( P_{\text{pred, CV}} \) |
|---|---|---|---|---|---|---|---|

| \( r \) | 0.66 | 0.62 | 0.80 | 0.85 | 0.79 | 0.91 | 0.88 |
| \( R^2 \) | 0.43 | 0.39 | 0.64 | 0.72 | 0.63 | 0.83 | 0.78 |
| Average error | 9.9 | 4.8 | 6.2 | 4.8 | 4.3 | - | - |

### 4 Conclusion

We have shown here that an approach based on the simultaneous analysis of molecule hydrophobicity and charge distribution has the potential to accurately predict the passive plasma membrane permeability of neutral drugs. This method may be useful for investigating the mechanism of passive permeation of small neutral compounds since it can easily pro-
vide information on the role that every single atom plays on the hydration process.

Our P_mol indicator correlates very well with the experimentally determined Caco-2 permeability values and performs better than other available methods. Furthermore, it only requires the knowledge of the chemical structure of the compound. Given the cost and impact of late stage failures in drug development we believe that the relatively high computational cost of running the molecular dynamics simulations (an average of 48 hours on a 20 CPU server for each molecule) is not necessarily a relevant drawback of the approach.

As is the case also for several in vitro methods, our method cannot estimate the permeability of drugs that use an active uptake system. In these cases, additional techniques, such as docking the compounds to efflux/influx protein models, should be explored.

Acknowledgements

The authors would like to thank Prof. Antonello Mai for critical comments and discussion, Dr. Claudio Graziani and Dr. Jacopo Falleti for helping with the graphical representations.

Funding

KAUST Award No. KU1K-11-012-43 made by King Abdullah University of Science and Technology. Progetto di Ricerca di Università, anno 2014 - prot. G26A14RFYP, EPKEN flagship project and PRIN 20100XYTH5

Conflict of Interest:

None declared.

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