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ORIGINAL PAPER

Drug permeability prediction using PMF method

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Abstract Drug permeability determines the oral availability of drugs via cellular membranes. Poor permeability makes a drug unsuitable for further development. The permeability may be estimated as the free energy change that the drug should overcome through crossing membrane. In this paper the drug permeability was simulated using molecular dynamics method and the potential energy profile was calculated with potential of mean force (PMF) method. The membrane was simulated using DPPC bilayer and three drugs with different permeability were tested. PMF studies on these three drugs show that doxorubicin (low permeability) should pass higher free energy barrier from water to DPPC bilayer center while ibuprofen (high permeability) has a lower energy barrier. Our calculation indicates that the simulation model we built is suitable to predict drug permeability.

Keywords DPPC · Molecular dynamics · Permeability · Potential of mean force (PMF)

Introduction

Drug permeability refers to the capability that a drug molecule can cross a lipid bilayer. A drug must have fine permeability to be administered orally. Drug permeability is the major factor to decide whether a drug candidate could make the development continue. Therefore, it is important to predict drug permeability before investment. Caco-2 cells are often used in vitro model to predict the intestinal transport of drugs [1]. Although the current experimental methods are quite perfect to determine drug permeability, they are often slower and more expensive when compared with computational methods. So it is still necessary to develop computational methods to predict drug permeability in drug lead generation and optimization.

There are many studies focusing on drug permeability prediction, but in most of them QSAR method is employed [2–6]. This type of prediction model seriously depends on the experimental outcomes and it is only accurate when the predicted molecule has the same scaffold or parent pharmacophore as those used to construct models. The free energy change of barrier that drug crosses could be estimated using the difference in implicit solvent chloroform and water, respectively [7, 8]. Chloroform was used to simulate the inner membrane, while water was used to simulate outer membrane. Swift and Amaro [9] have calculated the free energy difference of drugs in water and chloroform using solvent effects and they found that the experimental Caco-2 permeability was correlated well with the free energy difference between water and chloroform solvent. Orsi et al. [10, 11] have studied the permeability of several small molecules and drugs with hybrid method. They adopted coarse-grain models to simulate the lipid and water molecules, while the atomistic model for small molecules and drugs, and the calculated permeability coefficients were generally consistent with experimental data available.

Due to the large system and complex computational work, there are few using molecular dynamics method. Tieleman [12] et al. have performed MD simulations with potential of mean force (PMF) method and they have found that tryptophan preferred to partition to 11–13 Å from the

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DOPC bilayer center and the PMF profile had a deep interfacial minimum at this site. This calculation strongly supported that the minimum of PMF profile corresponds to the stable interaction of ligand with bilayer. Boggara and Krishnamoorti [13] have studied partitioning of two charge states (neutral and anionic) of two nonsteroidal anti-inflammatory drugs (aspirin and ibuprofen) in lipid membranes using PMF method. The DPPC bilayer used in these investigations is considered symmetrically and the solvent is water. They found that both drugs had higher partition coefficients in the lipid bilayer than in water. In our MD model we also use DPPC bilayer to model membrane, water to simulate the environment, and the process that drug molecule passes from water into membrane center is studied.

Calculation details

Drug permeability was modeled using molecular dynamics simulation method. DPPC was used to simulate the membrane. Three drugs, viz doxorubicin (low permeability), atenolol (moderate permeability) and ibuprofen (high permeability) were selected to construct three systems with DPPC. The structures of these three drugs are shown in Fig. 1. All the MD simulations were performed using GROMACS program, version 4.5.4 [14].

System building

The Berger lipids [15, 16] containing 128 DPPC lipids and 3655 water molecules was used as the initial lipid bilayer structure. Then one water box with the same x and y length as the bilayer and z length with 0.7 nm were added to the two ends of z axis. The system was equilibrated for 500 ps. The coordinates and the partial charges of drugs (Fig. S1 and Table S1) were generated using Dundee PRODRG server (http://davapc1.bioch.dundee.ac.uk/programs/prodrg/) [17]. The structures of drugs (see Fig. 1) were submitted to PRODRG and reduced charges were selected. Then the obtained coordinate file and topology file were downloaded to further use. GROMOS96 53a6 force field was used here. The drug center was set at the center of xy plane with the 0.7 nm z-axis. The water molecules with the distance to drug molecule less than 2 Å were deleted. Then three drug-DPPC-water systems were obtained.

Computation of potential of mean force

The free energy profile of drugs which cross the DPPC bilayer was calculated using potential of mean force (PMF) method. The drug molecule was placed in bulk water, and then it was pulled into the DPPC bilayer center along the z-axis using umbrella method. A harmonic restraint of 1500 kJ mol^{-1} nm^{-2} and a pulling rate of 0.01 nm/ps were applied to distance z between the center of mass (COM) of drug molecule and DPPC bilayer. Several pulling rates have been tested and this pulling rate can pull the drug into the DPPC center without disturbing the bilayer structure. Then the configuration at different z locations was sampled in the direction normal to the DPPC bilayer. The drug molecule was constrained at the z distance between the COM of the drug and DPPC bilayer, and allowed to move freely in the x-y plane.

The reference temperature and pressure were set at 310 K and 1 atm, respectively. Nose-Hoover coupling method was used for temperature coupling while the pressure was controlled semi-isotropically to keep the total pressure constant. The time constant for temperature and pressure are 0.1 ps and 1.0 ps, respectively. Particle mesh Ewald (PME) method was used to calculate the long range Coulombs interactions. The cut-off distance for Coulombs interactions and van der Waals interactions were set to be 1.4 nm. Every configuration was simulated 10 ns and the atom coordinates were saved every 2 ps. The last 2 ns of each MD run was used to calculate the free energy profile using weighted histogram analysis method [18] embedded in GROMACS. Statistical errors were estimated using Bayesian bootstrap analysis (N=50). The obtained free energy profile was regarded symmetric through the DPPC bilayer center.

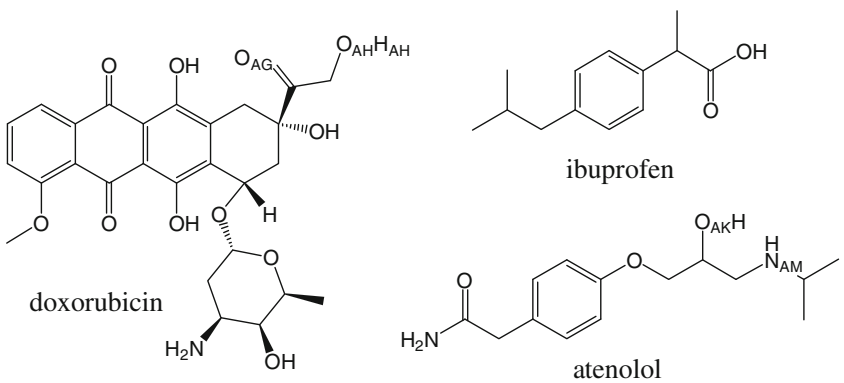

Fig. 1 Chemical structure of three drugs

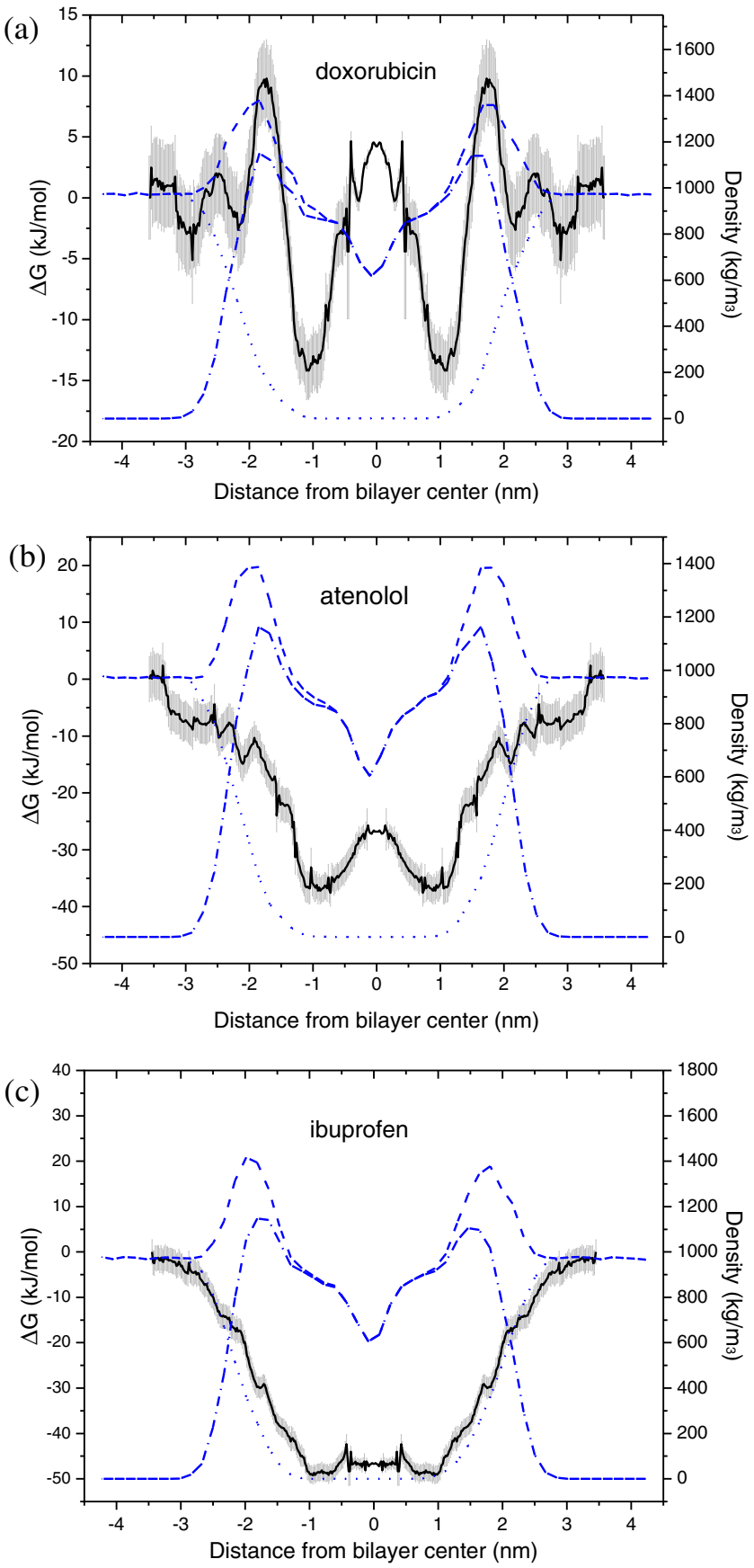

Fig. 2 Free energy profiles for **a** doxorubicin, **b** atenolol and **c** ibuprofen. Profiles are assumed to be symmetric across the bilayer center. Density values are obtained from one of the MD simulations. The free energy profile is drawn in black line and the standard deviation derived by bootstrap analysis is shown in gray areas. Density of system, DPPC and water are shown in blue dash line, dash dot line and dot line, respectively

Analyses

g_hbond and g_rdf were used to analyze hydrogen bonds and radial distribution function, respectively. The nonbonded interaction energies (Lennard-Jones and Coulomb terms) were calculated using g_energy. All analyses were performed using the last 2 ns MD trajectory of each umbrella sampling simulation.

Results and discussion

Potential of mean force (PMF)

The free energy profiles of the three drugs are shown in Fig. 2. The free energy was set to be zero in bulk water ($z<-3.5$ nm or $z>3.5$ nm). As can be seen from Fig. 2, doxorubicin has two free energy barriers when entering DPPC bilayer center: one occurs at the $z=\pm1.6$ nm (at which DPPC has the largest density), and the other occurs at the bilayer center (at which DPPC has the smallest density). The relative energies of these two maxima are about 7.0 kJ/mol and 4.0 kJ/mol, respectively. The free energy has a minimum of −14.4 kJ/mol at $z=\pm1.1$ nm. The shape of free energy profiles for ibuprofen and atenolol are different from that for doxorubicin. There only exists one obvious free energy barrier near the bilayer center in their free energy profiles. For these two drugs, the free energy profile firstly decreases as coming from bulk water to DPPC bilayer until it reaches a minimum, then it increases as approaching bilayer center. The free energy minimum for ibuprofen occurs at $z=-0.6\sim-0.9$ nm, which is deeper into bilayer center than that for doxorubicin. This is because ibuprofen is a hydrophobic molecule and tends to distribute in the hydrophobic region of bilayer. The free energy profile of atenolol is somewhat similar to that of ibuprofen, though it has several local maxima in the water and bilayer region. As atenolol has a polar group, it has a minimum near $z=\pm1.0$ nm. From Fig. 2 we can see that doxorubicin should overcome resistance (there exists a higher free energy barrier between DPPC bilayer center and bulk water) when entering DPPC bilayer, while ibuprofen tends to stay in membrane (the free energy barrier is quite low). In the case of ibuprofen, the drug entering from water to bilayer hardly has resistance and it can come into membrane easily.

Figure 2 also illustrates the statistic error of PMF. The largest error for doxorubicin is 5.51 kJ/mol, and the rest are all less than 3.61 kJ/mol. The statistic errors for atenolol are less than 4.15 kJ/mol, while that for ibuprofen are less than 3.08 kJ/mol. The obtained PMF errors are in moderate size as compared with other PMF results for DPPC system [18, 19], i.e., our PMF results are reliable.

The free energy barrier is determined by the minimum and maximum of free energy profile. From Table 1, it is observed that the free energy barrier for doxorubicin, atenolol and ibuprofen are 18.4 kJ/mol, 10.8 kJ/mol and 6.4 kJ/mol, respectively. Therefore, it is difficult for doxorubicin to pass through DPPC membrane but it is much easier for ibuprofen. The partition of the drug depends on the relative energy, while the permeability is decided by the free energy barrier that the drug should overcome in the whole transmembrane process. Our calculation suggests that the permeability of these three drugs is doxorubicin<atenolol< ibuprofen, which agrees well with the experimental results (Table 1), suggesting that the model we built to predict drug permeability is practicable and PMF method could be used to predict drug permeability.

Table 1 The calculated free energy barrier and experimental permeability

	Minimum (kJ/mol)	Maximum (kJ/mol)	ΔG (kJ/mol)	Permeability[a] (cm/s)
Doxorubicin	−14.4	4.0	18.4	0.16E-6
Atenolol	−38.1	−27.3	10.8	2.70E-6
Ibuprofen	−49.3	−42.9	6.4	52.5E-6

[a] The intestinal transcellular permeability P_m measured across Caco-2 cell were taken from reference [20].

The free energy profile of ibuprofen is somewhat different from reference [13]. This is due to different partial charges used (Table S2). As is well known, a potential energy profile for pulling a solute molecule across a bilayer depends seriously on solute atomic charges. For example, Paloncýová et al. [21] have used RESP, Mulliken and PRODRG charges for coumarin to calculate the free energy profiles for coumarin across DOPC bilayer, and then found the variation range of the three charge schemes are different.

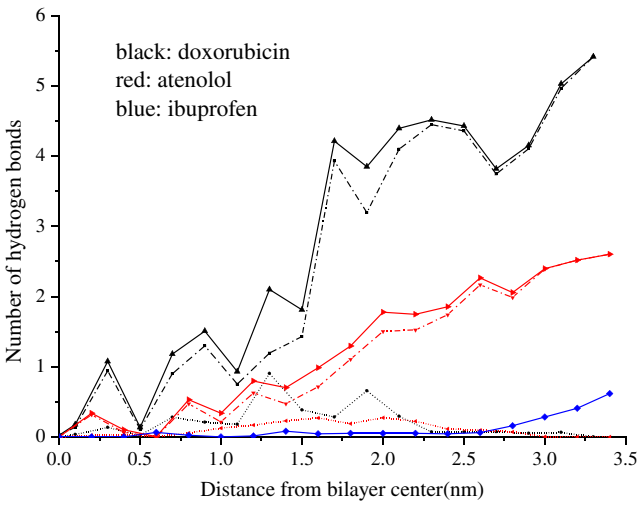

Fig. 3 Number of hydrogen bonds formed across DPPC bilayer

Hydrogen bonding

The hydrogen bonds were calculated using default criteria of GROMACS, i.e., the distance between the donor and the acceptor is less than 0.35 nm and the angle of donor, hydrogen and acceptor atom is less than 30°. The total number of hydrogen bonds between drug and water was estimated as the average number of hydrogen bonds each 10 ps over the last 2 ns molecular dynamics simulation. In Fig. 3, the H-bonds are formed between the three drugs and the remaining system. We can see from Fig. 3 that the H-bond between doxorubicin and DPPC has the largest number of 0.9 at z ~ 1.3 nm and this value greatly affects the total number of all H-bonds, while the number of H-bond between drug and DPPC at other distance does not have significant effects on the number of all H-bonds. That is to say, the H-bonds between drug and water play a major role. So we only consider the H-bonds between drug and water in the following content. After comparing the H-bonding profiles of the three drugs, one can see that the number of H-bonds also decreased with decreasing of the distance between drug and DPPC bilayer center (drug entering bilayer). The free energy minima of the three drugs occur at about 1 nm from bilayer center, but the number of H-bonds does not have the largest value at z ~ 1 nm. The location of maximum H-bonds does not correlate with free energy minima, which coincides with the ibuprofen outcome of Boggara et al. [13]. The number of H-bonds formed between doxorubicin and water is larger than that of atenolol and ibuprofen, as doxorubicin has more H-bond donor and acceptor as well as more polar surfaces.

Figure 4 shows the snapshots of the three drug-DPPC systems at z ~1 nm. From Fig. 4 we can see that with the movement of drug molecule, polar water molecules also enter into the hydrophobic region of DPPC bilayer, serving to hydrate the polar group of drug. With the presence of water molecules in DPPC hydrophobic region, the surrounding lipid molecules tilt to decrease the energy barrier. The extrusion of drug and water molecules makes the depth of local bilayer thinner. Such phenomena have been observed in several studies, including peptide molecule [22] and polar molecule [19] partitioning in lipid bilayer. It can be seen from Fig. 4a doxorubicin forms three H-bonds with water molecules. The carboxyl atom O_{AG} forms an H-bond with water molecule (the $O_{AG}\ldots H_w$ distance is 1.813 Å). The hydroxyl group O_{AH}–H_{AH} forms two H-bonds with two water molecules (The $O_{AH}\ldots H_w$ and $H_{AH}\ldots O_w$ distances are 2.157 Å and 2.253 Å, respectively). The hydroxyl group O_{AK}–H and the amine group N_{AM}–H of atenolol form two H-bonds with the same water molecule (the two H-bond distances are 2.277 Å and 2.480 Å). Ibuprofen does not form H-bond with surrounding water molecules.

In order to better interact with DPPC polar region, doxorubicin takes a conformation normal to the DPPC molecules. As for ibuprofen, it almost parallels to DPPC bilayer and keeps the carboxyl group facing the polar group of

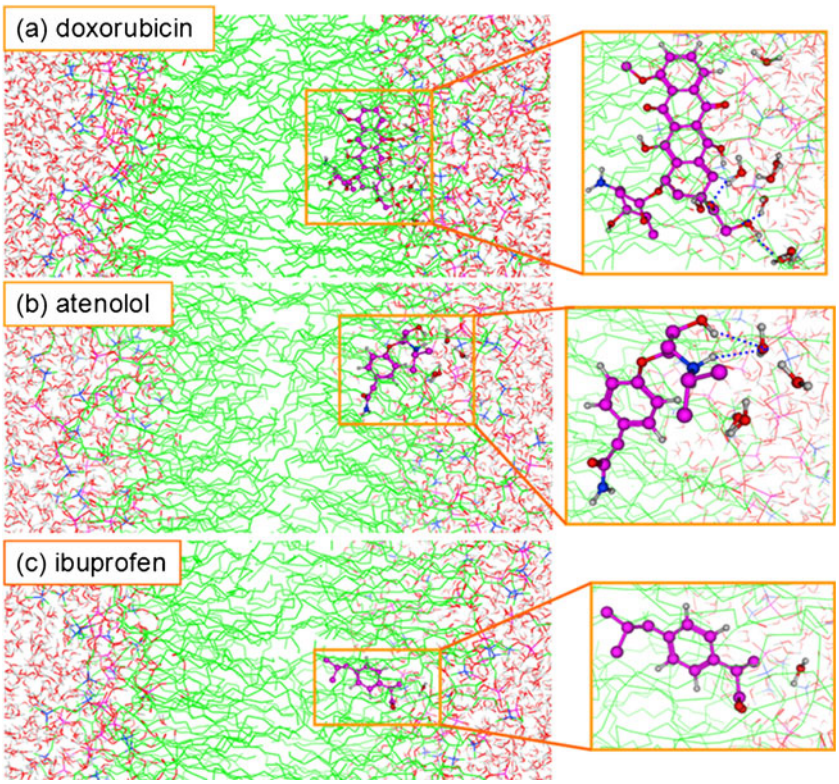

Fig. 4 Example of MD umbrella sampling of drugs in DPPC bilayer at z ~ 1.0 nm. Drugs and water molecules within the range of 3.5 Å are shown in ball and sticks model, while others are shown in lines. Drugs are colored in pink carbon schedule. Hydrogen bonds are shown in blue dashed line

DPPC bilayer, which makes it enter the hydrophobic region deeply and the whole system more stable.

The COM movements of the three drugs in x-y plane of bilayer are alike and all have separated domains (Fig. S3). The separated domains indicate that the drug cross the DPPC bilayer through hopping mechanism, as reported by Boggara et al. [13].

Drug hydration (radial distribution function)

The hydration of drugs was quantified by estimating the total number of water molecules in the first hydration shell of radial distribution function (RDF) between the COM of the drug and water as reference [13], and this was calculated using the g_rdf program of GROMACS. The RDF was calculated for one representative case (z∼1.0 nm from the bilayer center), in which the system has lowest energy. From Fig. S5 we can see that the maximum of hydration occurs in the sequence of atenolol, doxorubicin, ibuprofen. The maximum of ibuprofen is very small as compared with the other two drugs. The maximum of doxorubicin occurs later than atenolol, because it has larger volume than atenolol.

Energy analysis

Figure 5 shows the nonbonded energy contribution (including Lennard-Jones and Coulomb contribution) of the three drugs with DPPC and water, respectively. From Fig. 5, it can be seen that the Lennard-Jones energy between the drug and DPPC decreases from z=0 to 1 nm and has minima at 1 nm or so, and then it increases and approaches about 0 kJ/mol in bulk water (meaning that the interaction almost does not exist if the drug is far away from DPPC). The Lennard-Jones energy between the drug and water is about 0 kJ/mol in DPPC bilayer center and decreases as the distance between drug and bulk water turns shorter and shorter. As for ibuprofen these two terms cross each other at about z=2.2 nm (the interface of DPPC and water, see Fig. 2). Compared with Lennard-Jones energy, the Coulomb energy contributes less as to the whole interaction energy. It is concerned with the polarity of drugs. The coulomb energy between drug and water decreases as the drug passes from DPPC bilayer center to bulk water, and the smallest value is about −100, −40, −10 kJ/mol for doxorubicin, atenolol and ibuprofen, respectively. This is in accordance with the drug polarity, i.e., the larger the drug polarity is, the stronger the coulomb interaction between drug and water will be.

Because doxorubicin and atenolol are polar molecules, they have stronger interaction with water and polar groups of DPPC, which causes larger energy barriers to overcome when passing from water to DPPC bilayer center. However, ibuprofen is a hydrophobic molecule, so it prefers to stay in hydrophobic bilayer center rather than stay in bulk water. Consequently, ibuprofen has low energy barrier and high permeability.

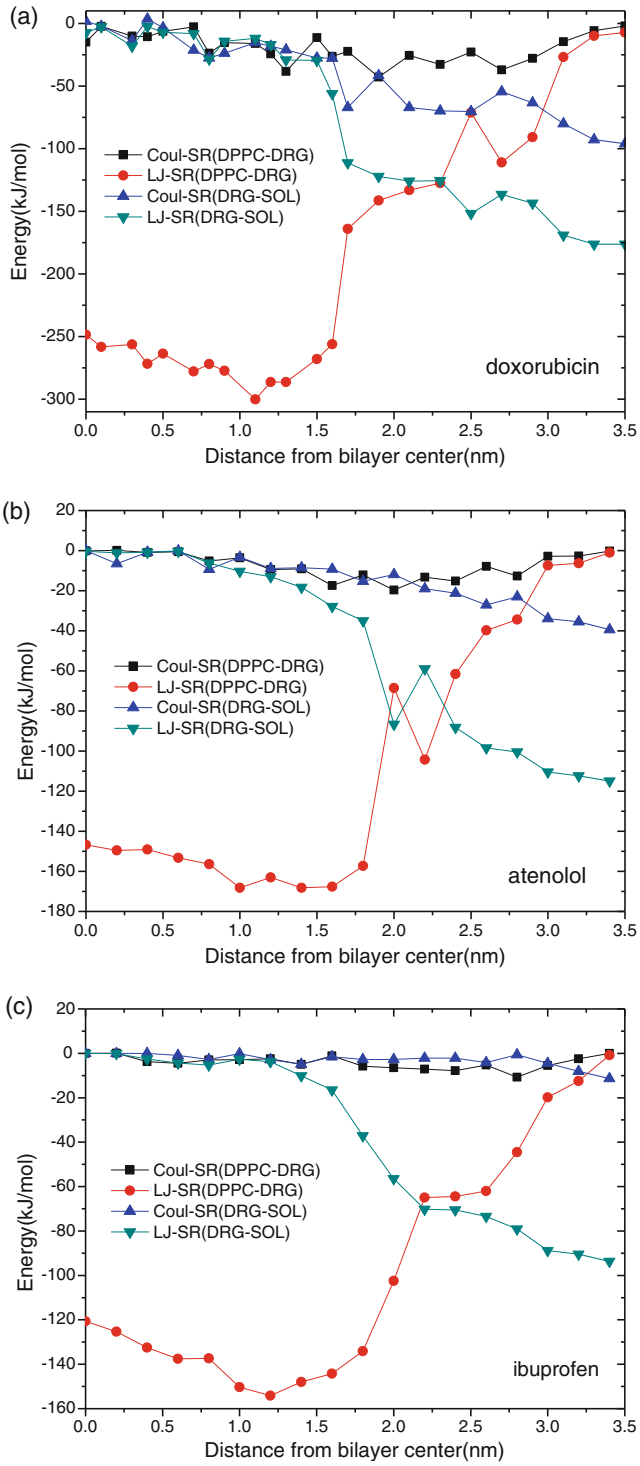

Fig. 5 Nonbonded energy contribution including Lennard-Jones (LJ) and Coulombic (Coul) energies between drug (DRG) and DPPC or water (SOL) from all the frames of umbrella sampling simulations

Conclusions

In this paper we present a new theoretical method to predict drug permeability. The advantage of our method lies in that unlike other methods such as QSAR, our model is based on all atoms of the whole drug and does not depend on the pharmacophore. Molecular dynamics calculations on three drugs with different permeability have been performed using this model. The results show that low permeability drug has high free energy barrier while high permeability drug has low free energy barrier. The drug with hydrophobic group tends to pass membrane quickly but it is difficult for drug with hydrophilic group to pass membrane due to strong interactions with DPPC polar groups and surrounding water molecules. The model we used is suitable to predict drug permeability.

Drug permeation has lots of mechanism including passive diffusion, active uptake, efflux, endocytosis, paracellular, and so on, among which passive permeation is a fundamental mechanism for the transport of molecules across biological membranes. A large number of drugs are absorbed via passive diffusion from the gastrointestinal tract. PMF method could be employed to predict drug permeability when passive diffusion is the major permeation mechanism as our model only considers the membrane structure and outer water environment.

Finally, although our model can obtain reasonable correlation with experiment, the calculation is expensive and time-consuming, and requires a high performance machine. Therefore, a new method to predict drug permeability with simple and inexpensive calculation should be proposed in the future.

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