

A THEORETICAL STUDY ON THE PARTITIONING OF PERFLUORINATED ALKYLATED SUBSTANCES BETWEEN WATER AND A BI-LAYER MEMBRANE

Van Roon A, Den Besten C, De Voogt, P

Earth Surface Processes and Materials, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV, Amsterdam, The Netherlands

Introduction

Perfluorinated alkylated substances (PFAS) are fully fluorinated, fatty acid analogues that are characterized by a perfluoroalkyl chain and a terminal functional group. PFAS are used in a wide variety of applications and products including sealants, fire fighting foams and surface coatings.¹ Due to emissions, their persistence and transport properties, PFAS are now globally distributed in the environment. Especially perfluorooctane carboxylate (PFOA) and perfluorooctane sulfonate (PFOS) are widely distributed. Although many toxicological studies have been carried out,^{1,2} little do we know about the mechanisms underlying their modes of action. One possible mechanism is the partitioning of PFAS in biological membranes, which causes a change in membrane structure and function.³

In this theoretical study the partitioning of PFOA and PFOS between water and an 1,2-Dilauroyl-DL-phosphatidyl ethanolamine (DLPE) membrane model was studied using molecular mechanics force field (FF) calculations. The water to membrane transfer enthalpy of PFOA and PFOS was calculated by subtracting the energy of interaction between the contaminant and surrounding water molecules from the energy of interaction between this contaminant and the DLPE membrane. Through this study we would like to find the sites in the membrane, which are preferred by contaminants from an energetic point of view.

Materials and Methods

The AMBER FF⁴ updated with parameters for the perfluorinated chain available from literature⁵ was used. The AMBER FF is based on a relatively simple function to evaluate the energy of a system. This makes it possible to apply it to large systems such as membranes.

The structures of the protonated and ionized states of PFOA and PFOS were constructed and optimized in the AMBER FF (Figure 1). PM3 based Mulliken atomic charges were used. These charges were found to reproduce the experimental enthalpy of vapourization of perfluorohexane and perfluorooctane via a method described elsewhere.⁶

For calculations in the water phase, a molecule was placed at the center of a standard periodic water box available in the software. The water box was adopted from Jorgensen's equilibrated box of 216 H₂O (dimensions $x, y, z = 18.70 \text{ \AA}$) described by the TIP3P potential function.⁷ Atomic charges of $-0.595 e$ and $+0.2975 e$ were used for the H₂O oxygen and hydrogen atoms

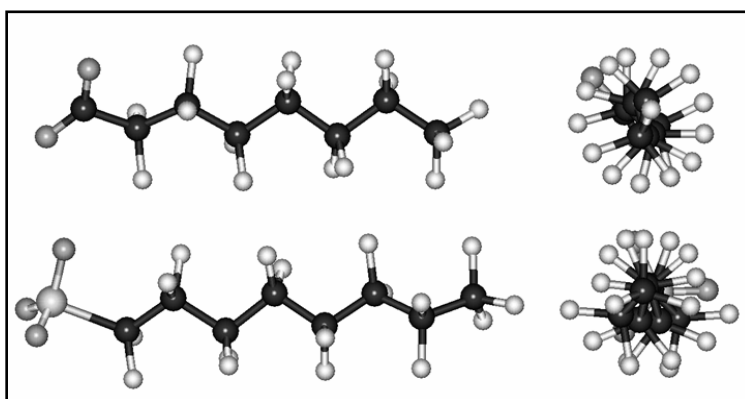


Figure 1 Molecular structures of the ionized form of PFOA (upper) and PFOS (lower) as found by geometry optimization in the AMBER FF. Black = carbon, white = fluorine, large gray = sulfur, smaller darker gray = oxygen. The views of PFOA and PFOS on the right show the helical conformation of the perfluorinated tail.

because they were previously found to reproduce the experimental enthalpy of vapourization.⁶ In the periodic box calculations, summation cut-offs of 9/5 Å for non-bonded interactions were applied.

The construction of the DLPE membrane was described previously.⁸ Briefly, the DLPE membrane bi-layer was constructed using crystallographic data.⁹ The monoclinic unit cell contained $Z = 4$ DLPE-Acetic acid molecules with molecular formula of $C_{29}H_{58}O_8NP(C_2H_4O_2)$. The acetic acid was cut from the DLPE molecule. A bi-layer of 64 DLPE molecules, two opposite layers of 8×4 molecules, was built preserving the molecular structures and positions as found in the crystal. To these water layers were added. This water had the standard equilibrated structure as used in the software's periodic water box option. No cut-offs for non-bonded interactions were used. To reduce computational time, two selections were made, one to model the apolar region and one to model the polar region in the membrane. Selected atoms were allowed to change position during calculations whereas non-selected atoms were fixed to their pre-optimized positions. Only non-bonded interactions were possible between selected and non-selected atoms. The selections are shown in Figure 2.

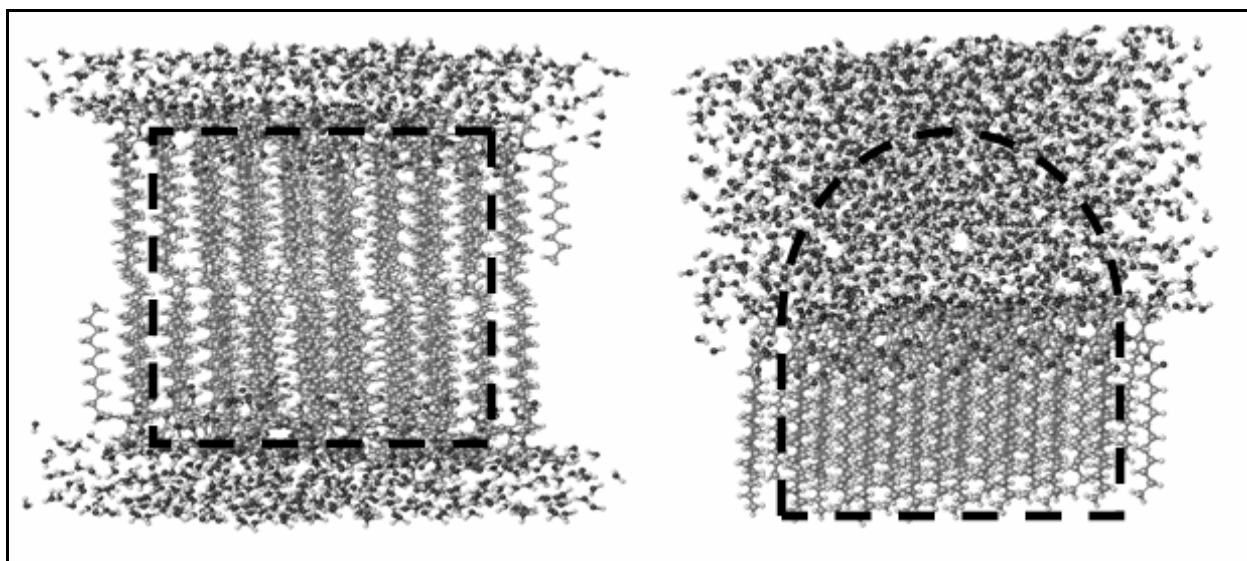


Figure 2 DLPE membrane structure with indicated the apolar (left side) and polar (right side) selections.

Since volume changes in condensed phases are small, we assume the calculated energy changes (ΔE) to be equal to the enthalpy changes (ΔH). The enthalpy of transfer (ΔH_{mw}) was calculated as $\Delta H_{mw} = H_w - H_m$ where H_w and H_m are the enthalpy of the PFAS in water and the membrane, respectively. The enthalpy values are the sum of the intra-molecular (conformational) energy of the PFAS and the inter-molecular (interaction) energy. The calculations started by manually placing a PFAS molecule in bulk water and in the membrane. These structures were then geometry optimized and the energies were calculated. We have shown before,^{6,11} that energy differences of geometry optimized (zero Kelvin) structures are similar to energy differences obtained by time consuming simulations at 300 K. A problem of geometry optimizations is that the calculation may converge in a local (high energy) minimum. To reduce the problem of local minima, we applied simulated annealing: The structure was heated in a molecular dynamics calculation to 300 Kelvin allowing structural hotspots to relax, and was subsequently cooled slowly to zero Kelvin.

All calculations were performed on a 1.10 GHz, 256 MB PC using the Hyperchem® version 5.1 software package.¹⁰ A simulated annealing calculation took approximately 1 day of computational time.

Results and Discussion

Figure 3 shows thermodynamic cycles for PFOS and PFOA partitioning between water and the DLPE membrane. The cycles include both the protonated and ionized states of the PFAS. The arrows of favourable energetic change suggest the ultimate accumulation of the ionized PFOS and PFOA in bulk water. This agrees with their low pKa values.¹ The transfer of PFOS and PFOA into the membrane was energetically not favourable, but less unfavourable for the (neutral) protonated states. In the membrane, PFOS had the lowest energy in the polar region with the protonated state having the lowest energy. In the membrane PFOA had the lowest energy in the polar region in its ionized state. The energy of the protonated state of PFOA was somewhat higher and was similar in the polar and apolar membrane regions. As an example, the optimized position and orientation of the ionized state of PFOA in the polar and apolar regions are shown in Figure 4. The positions and orientations are similar for the protonated state and for PFOS.

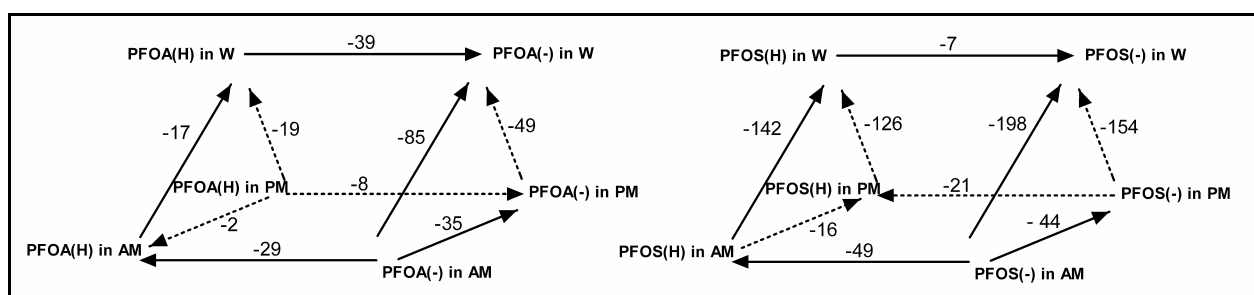


Figure 3 Thermodynamic cycles for PFOA (left side) and PFOS (right side) partitioning into the DLPE membrane. W indicates the bulk water phase, PM and AM indicate the polar and apolar parts of the membrane, respectively. (H) indicates the protonated and (-) indicates the ionized state of a compound. The arrows indicate the direction of energetically favourable change (negative enthalpy values). Energies are in kcal/mol.

The results raise the following discussion points and recommendations:

- Although the calculation results suggest a low affinity of PFOA and PFOS for the membrane, we stress that only enthalpy effects were calculated and entropy effects were ignored. It has been argued, however, that entropy might strongly influence the water-membrane partitioning process.¹¹
- The calculations were carried out for non-buffered pure water and the DLPE membrane, whereas in reality both water and the membrane will be different. Although we do not expect this to change the conclusions of the current calculations, the calculations should be repeated using different types of membranes, for example a bi-layered membrane composed of dipalmitoylphosphatidylcholine (DLPE) molecules. In addition, mono-layered membranes as found in liver should be included in the study, because PFOS distributes mainly into the liver.^{1,2}
- Although experimental data was used in the calibration of the FF method, we interpreted the calculated energies only in a qualitative way. For a thorough validation of the calculations, membrane-PFAS interaction enthalpies (ΔH) should be known, at least for a few compounds. These can be derived from the temperature dependence of measured membrane-PFAS partitioning constant (K_{mw}).
- The method could be developed further to calculate K_{mw} via the free enthalpy of transfer ($-RT \ln K_{mw} = \Delta G_{mw}$, with R as gas constant and T as absolute temperature), provided the entropy of transfer (ΔS) can be quantified. The pertinent partitioning constant could be applied as a descriptor for the bio-concentration factor and toxicity parameters instead of the *n*-octanol-water partition constant.
- Another mechanism in membrane toxicity is the interaction of contaminant with functional proteins in the membrane. The interaction between contaminants and enzymes can also be studied using force field calculations.¹²

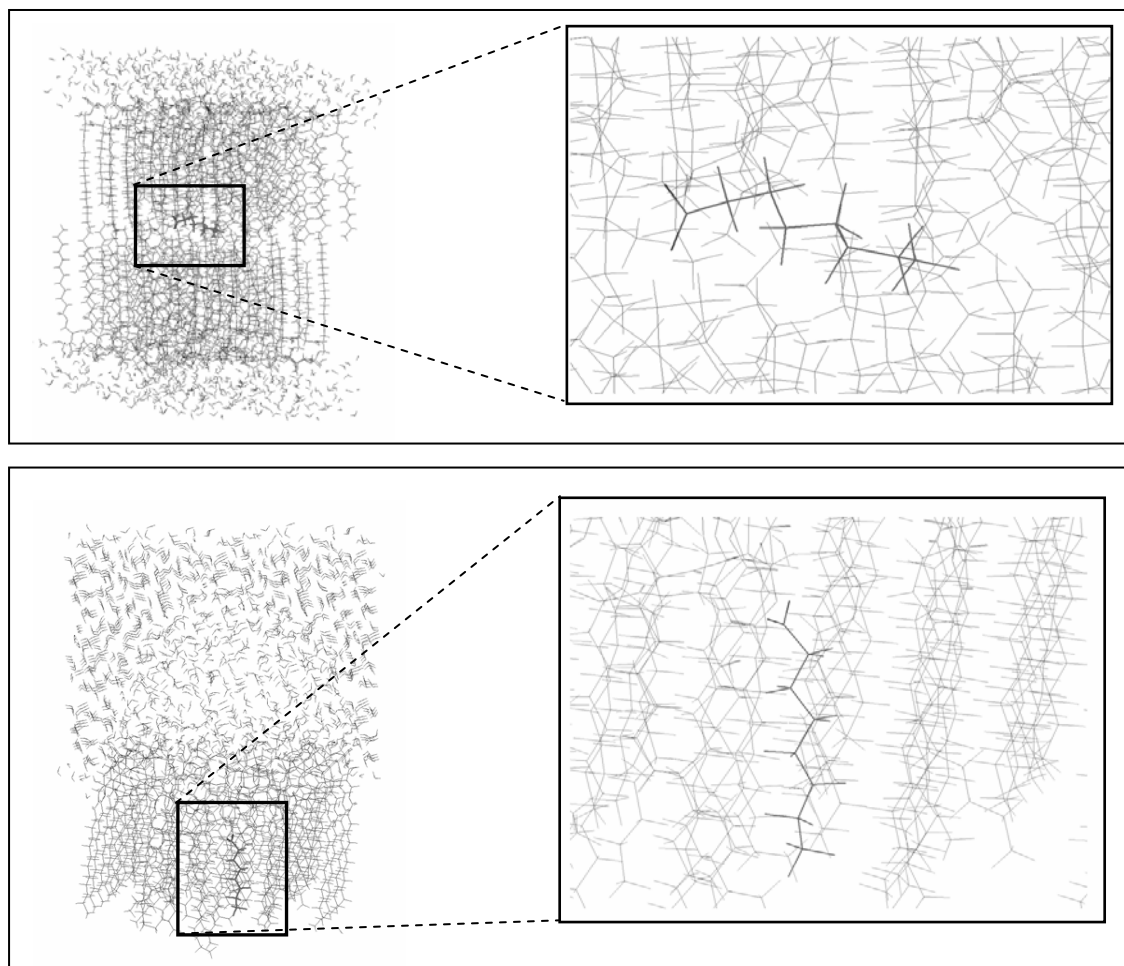


Figure 4 The optimized positions of PFOA(-) in the apolar (above) and polar (below) parts of the DLPE membrane. PFOA(-) is indicated with thicker lines. The boxes in the membrane structure on the left indicate the selections which were blown up on the right-hand side of the Figure.

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