The winners of the 2017 CAP Best Student Presentation Competition at the CAP Annual Congress, 2017 May 29 - June 2, in Kingston are listed on pages 174-175. The extended abstracts of those winners of the CAP prizes who submitted them for publication are reproduced below. Ed.

CAFFEINE IN DRUG COCKTAILS: THE FORMATION OF "WATER POCKETS" IN MEMBRANES

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Caffeine is a small, hygroscopic molecule that we commonly associate with coffee. Although caffeine is the world's most prevalent psychostimulant [1], it is also one of the world's most common drug adjuvants – according to the DrugBank [2] it is included in more than 500 pharmaceutical preparations. In our research, we looked into the biophysical processes which govern the non-specific interactions between caffeine and primary drugs in pharmaceutical cocktails. We found that caffeine partitions in the hydrophilic-hydrophobic interface of the membranes and leads to changes in the membrane structure, which can substantiate the interaction between drugs and the membrane milieu [3].

Because of their simplicity, synthetic membranes are excellent model systems, both experimentally and computationally, to identify fundamental biophysical processes. In order to mimic a basic eukaryotic membrane, we prepared unsaturated/saturated zwitterionic lipid membranes of 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (POPC) with 3 mol% caffeine, a typical blood concentration of caffeine after drinking two cups of coffee. Multi-lamellar stacks of membranes were prepared on silicon wafers. The samples were then studied with X-ray diffraction and Molecular Dynamics (MD) simulations to probe the structural and kinetic biophysical processes. Experiments were conducted in-house using our high-resolution and high-intensity Biological Large Angle Diffraction Experiment (BLADE). MD simulations were conducted in-house using GROMACS on MacSim, a high performance GPU powered workstation.

SUMMARY

This article reports on the biophysical consequences of induced hygroscopic "water pockets" in cell membranes by the small molecule caffeine to understand membrane-mediated drug interactions.

As coffee is in particular known to have an impact on fluid balance in the body, the membranes were scanned at different relative humidities (RH) of 97%, 93%, and 85% RH equivalent to different osmotic pressures. All membranes were first transferred into a glove box and kept at 10% RH for 24–48 h to completely dehydrate the membranes prior to scanning. During the experiments, membranes were kept in a humidity chamber and the humidity was controlled through different saturated salt solutions.

In experiment and simulations, we observed that caffeine spontaneously partitioned in the membranes, between the lipid head and tail groups, as shown in Fig. 1. The

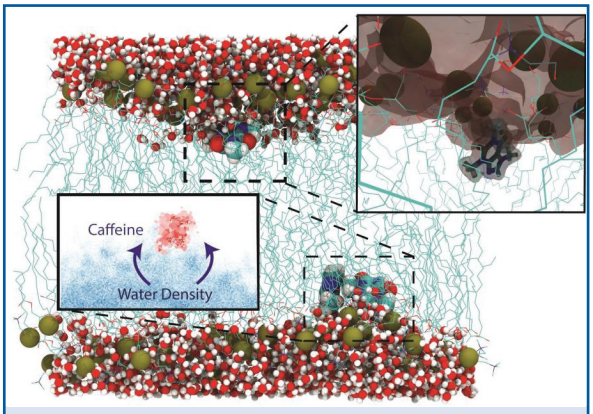

Fig. 1 Simulation snapshot of membrane system with embedded caffeine. Caffeine molecules spontaneously partition into membranes and locate in the interface between lipid head groups and lipid tails. Water molecules are then drawn to this interface. The subplot shows a zoomed-in image with the darker density representing the water molecules protruding into the membrane around the caffeine.

1. Adree Khondker tied for 2nd place in the CAP Best Student Oral Presentation competition at the 2017 CAP Congress at Queen's University in Kingston, ON.

amphiphilic caffeine molecule orients its relatively polar group toward the head groups, and the relatively nonpolar segment toward the lipid tails. However, it typically takes several hours in the experiments for the systems to reach equilibrium. In time-resolved diffraction experiments we found evidence that caffeine slowed down hydration of the membranes, i.e., the uptake of hydration water molecules, but at the same time led to an increased amount of hydration water molecules in the hydrophobic membrane core.

We used time-resolved X-ray diffraction to measure the evolution of the membrane width. Hydration in biology typically results in an increase in the number of hydration water molecules around a biological system. Interestingly, when the lamellar spacing, d_z, was decoupled into the thickness of the hydration water layer and the actual membrane thickness, we observed that caffeine led to an increase of membrane thickness, instead of increasing the hydration water layer. By fitting the increase in thickness (d_z) to an exponential curve, the corresponding time constants, τ, were determined (see Fig. 2a). By measuring τ at different relative humidities, we propose a modified Arrhenius equation $\left(\tau = \tau_0 \exp\left(\frac{\Delta E}{k_B T}\right)\right)$ to determine the corresponding hydration energy barrier, ΔE, for a water molecule to enter the interfacial layer of the membrane by using:

$$\Delta E = \alpha k_B T \ln\left(\frac{p}{p_0}\right).$$

A relative humidity $RH = p/p_0$ creates an osmotic pressure $\Pi_{osm} = (k_B T/v_w)\ln(p_0/p)$ (where v_w is the partial molar volume of a water molecule). The energy barrier to diffuse into the bilayer, ΔE, was assumed to be proportional to the change in chemical potential of the water molecules. The slope α to the logarithmic plots is fit in Fig. 2b). The two straight lines for POPC with and without caffeine are parallel to each other, suggesting that the effect of the relative humidity on the water diffusion is independent of the presence of caffeine. In summary, caffeine alters the membrane thickness by attracting greater amounts of water towards the caffeine, functionally dehydrating the rest of the bilayer.

To consolidate the experimental findings and investigate the mechanistic upbringing of this increase in membrane thickness, we used unified-atom molecular dynamics (MD) simulations. Typical simulation parameters and equilibrations were used [4], and production runs were performed for 200 ns. Measurements of membrane width, area-per-lipid, and caffeine partitioning in simulation agreed well with experiment such that the results can be directly compared. MD simulations can then provide atomistic resolution and allow for extrapolation of experimentally inaccessible parameters

As shown in the inset to Fig. 1, caffeine molecules spontaneously partition and interact with the lipid head groups and attract water molecules. The presence of caffeine in the head-tail interface of the membranes drew a significant amount of water molecules into the lipid tails, creating "water pockets". In doing so, the interaction began to affect the lipid tail structure, specifically perturbing the membrane's fluidity — a measure of the disorder of lipid tails. By measuring the proportion of kinks (i.e., gauche dihedrals) in the 16/18-carbon tails, we observed that the adsorption of caffeine increases the number of defects locally near the molecule while decreasing the number of defects away

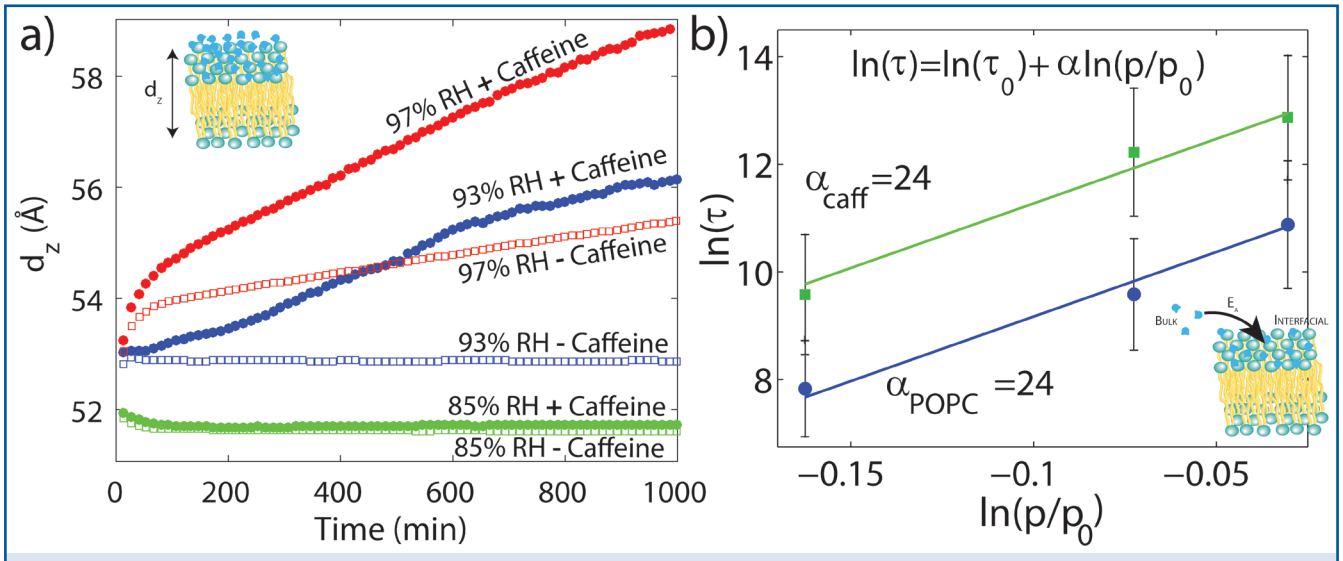

Fig. 2 The energy landscape for adding hydration water molecules to the membranes can be probed by measuring membrane swelling (a) as a function of relative humidity. The slope of this modified Arrhenius behaviour, a, was the same in membranes with and without caffeine (b). The derivation of this relationship is given in Khondker *et al.* 2017 [3].

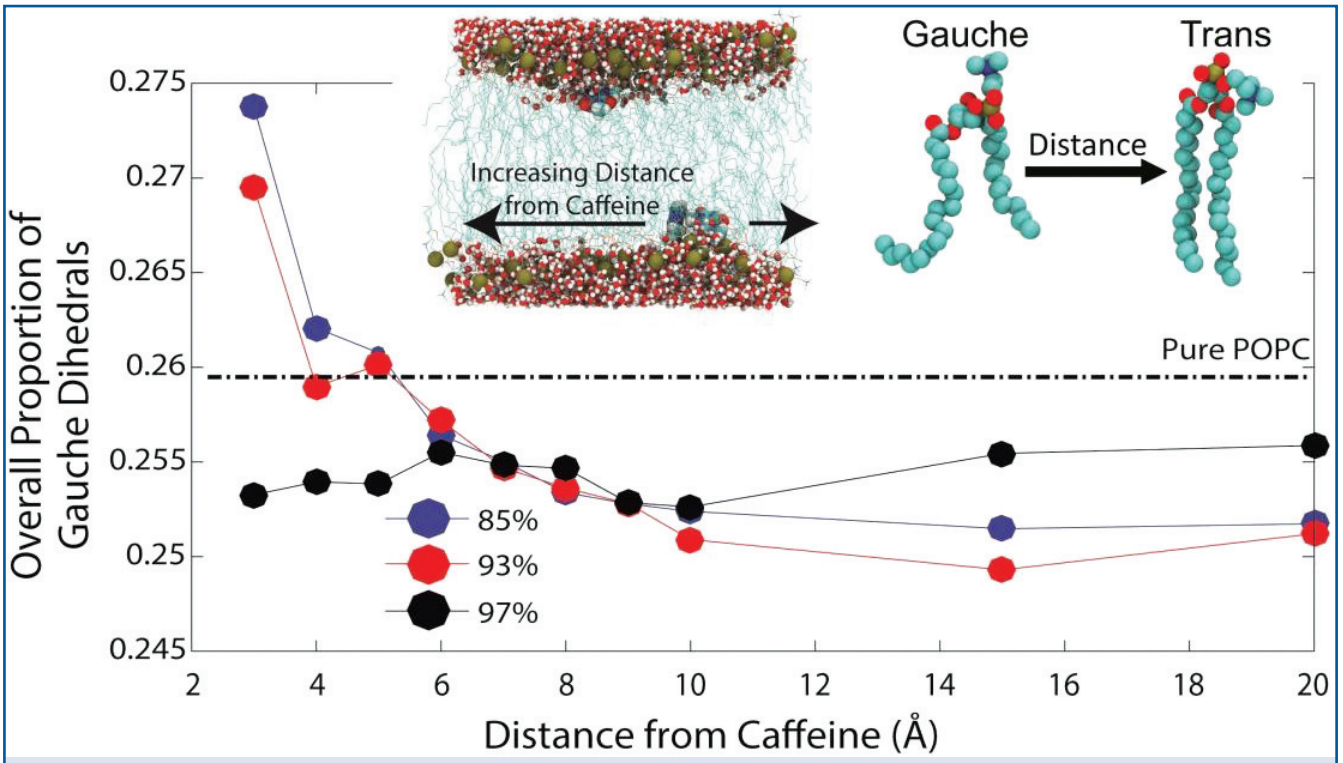

Fig. 3 Proportion of gauche dihedrals on lipid tail carbon atoms as a function of lipid distance from caffeine molecules at different hydration levels. The average gauche defect density of a pure POPC bilayer is plotted as dashed-dotted line for comparison. While the number of gauche defects is significantly increased near the caffeine molecules, the overall or averaged number of gauche defects is decreased as compared to pure POPC farther from the caffeine, indicative of a dehydration of the membranes.

from the molecule, as shown in Fig. 3. This led to the dehydration of membrane lipids farther from each caffeine molecule, which causes an overall decrease in membrane fluidity. A decrease in membrane fluidity — an increase in lipid tail order — is known to result in an increased membrane thickness [3], in agreement with the experiment.

In summary, we have gained an understanding of the effects of the hygroscopic molecule caffeine on lipid membranes by combining X-ray diffraction and MD computer simulations. We find that the formation of "water pockets", as a result of caffeine partitioning into the membranes, increases local membrane fluidity, while functionally dehydrating the rest of the membrane bilayer. This molecular dehydration precedes the increased intermembrane thickness and reduced bilayer swelling. Changes in membrane thickness and dynamic state of the lipid molecules lead in particular to changes of important membrane properties, such as diffusion and permeability. Such biophysical interactions could play a significant role in the rate of metabolism of analgesics [5]; therefore the presence of caffeine may make analgesics available in the body for longer periods of time than if administered independently. Our work thus provides a novel locus of interaction between bioactive molecules facilitated by a membrane surface, such as those found in the human body. To our knowledge, these are also the first experimental findings towards membrane surface bioenergetics with X-ray diffraction.

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