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PERFLUOROOCTANOIC ACID VS. PERFLUOROOCTANESULPHONIC ACID: DIFFERENTIAL EFFECTS ON MEMBRANE INTERFACIAL DYNAMICS

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Abstract

Perfluorinated alkyl substances (PFAS), such as perfluorooctanoic acid (PFOA) and perfluorooctanesulphonic acid (PFOS), are persistent water contaminants known to interact with biological membranes. In this study, the effects of PFOA and PFOS on membrane interfacial properties were investigated using 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) large unilamellar vesicles (LUVs) and Laurdan generalized polarization (GP) as a probe of membrane dipolar relaxation. Under control conditions, POPC vesicles exhibited a liquid-disordered phase (negative GP values). While PFOA did not induce significant changes in GP, PFOS caused a significant decrease, indicating enhanced interfacial hydration and dipolar relaxation. These differences are attributed to structural features of PFAS, with PFOS exhibiting stronger interfacial anchoring and membrane perturbation. Overall, the results demonstrate that PFOS more effectively disrupts membrane interfacial properties, suggesting a greater potential to affect membrane function and associated cellular processes.

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Introduction. Perfluorinated alkyl substances (PFAS), including perfluorooctanoic acid (PFOA) and perfluorooctanesulphonic acid (PFOS), are environmentally persistent amphiphilic compounds of increasing global concern [1, 2]. In recent years, significant attention has been directed toward understanding their sources, environmental transport pathways, and residual concentrations. As aquatic systems represent a major reservoir for PFAS, numerous studies have focused on assessing contamination levels in surface waters [3]. Their widespread environmental distribution poses a serious threat to both ecosystems and human health. In line with their extensive environmental presence, a growing body of evidence indicates that PFOA and PFOS exert harmful effects across a broad range of organisms [2]. Due to their exceptional chemical stability and long environmental half-lives, often spanning several decades, these compounds can accumulate in living organisms and biomagnify along the food chain, ultimately posing long-term risks to human health. Exposure to PFOA and PFOS has been associated with various adverse health outcomes, including developmental abnormalities, reduced birth weight, impaired fertility, and compromised immune function and antibody production [4, 5]. Despite extensive toxicological evidence, the molecular mechanisms underlying PFAS-induced cellular effects remain incompletely understood. In particular, their interactions with biological membranes and the subsequent activation of cellular signalling pathways are considered key initial steps in mediating their biological activity [6, 7]. The amphiphilic nature of PFAS, characterized by fully fluorinated hydrophobic chains and polar headgroups, enables their partitioning into lipid bilayers and interaction with the lipid-water interface, thereby modulating membrane structure and dynamics [8].

To better elucidate these mechanisms, model lipid membranes provide a well-defined experimental platform. Large unilamellar vesicles (LUVs) composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) are widely used as a model of fluid, zwitterionic bilayers representative of biological membranes in the liquid-disordered (L_d) state. Within such model systems, membrane properties can be effectively probed using Laurdan, a polarity-sensitive fluorescent probe, whose emission spectrum depends on the extent of dipolar relaxation of surrounding water molecules. The Laurdan generalized polarization (GP) parameter reflects changes in the local dielectric environment at the lipid–water interface and is therefore sensitive to membrane hydration and dipolar dynamics rather than lipid packing alone [9].

Based on this framework, the aim of this study was to quantitatively evaluate and compare the effects of PFOA and PFOS on the interfacial dipolar environment of POPC LUVs, using Laurdan GP as a sensitive reporter of membrane hydration and dipolar relaxation, and to assess how these changes are partially related to alterations in membrane lipid order.

Materials and methods. ***Reagents.*** The synthetic monounsaturated lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from Avanti Research (Alabaster, AL, USA). The perfluorinated alkyl substances (PFAS), the organic solvents (methanol and chloroform) and the fluorescence probe 6-dodecanoyl-N, N-dimethyl-2-naphthylamine (Laurdan) were obtained from Sigma-Aldrich (Darmstadt, Germany).

Preparation of large unilamellar vesicles (LUVs). Large unilamellar vesicles (LUVs) composed of POPC were prepared using a standard extrusion method [10]. Prior to vesicle preparation, POPC and Laurdan, dissolved in an organic solvent, were mixed at a 200:1 lipid-to-probe molar ratio at a total lipid concentration of 1 mM. After solvent evaporation, lipid films were hydrated in 0.5 mM HEPES buffer (pH 7.4) and subsequently subjected to repeated extrusion through polycarbonate membranes (800 and 100 nm) to obtain vesicles of uniform size. Stock solutions of PFOA and PFOS were prepared by dissolving each compound in the same buffer. An aliquot of the solutions was then added to the diluted LUV suspensions (200 µM) to achieve a final PFAS concentration of 10 µM. Thus, the lipid/PFAS molar ratio was 20:1. The samples were incubated for 2 h at 37 °C.

Laurdan fluorescence measurements. Membrane properties were assessed using Laurdan generalized polarization (GP). Laurdan is an environment-sensitive fluorescent probe that partitions into the lipid–water interface of membranes. Its emission spectrum depends on lipid packing and hydration: tightly packed membranes with limited water penetration exhibit a blue shift (440 nm), whereas more disordered and hydrated membranes show a red shift (490 nm). These changes are quantified by the GP parameter, where higher GP values (+1) indicate greater lipid order and lower hydration, while lower GP values (−1) reflect increased disorder and water penetration (L_d phase) [9]. Measurements were performed for control vesicles and vesicles treated with PFOA or PFOS. For each condition, a total of 30 measurements were obtained, derived from two independent experiments with 15 measurements each.

Statistical analysis. All data are presented as distributions of individual measurements. Statistical analysis was performed using unpaired two-tailed Student's *t*-tests to compare groups. Comparisons were made between: control vs. PFOA, control vs. PFOS, and PFOA vs. PFOS. A p-value < 0.05 was considered statistically significant. Effect sizes were calculated using Cohen's d, defined as the difference between group means divided by the pooled standard deviation. Effect sizes were interpreted as small ($d \approx 0.2$), medium ($d \approx 0.5$), or large ($d \geq 0.8$). Data visualization was performed using violin plots, where the width of each distribution represents data density and individual measurements are shown as points.

Results and discussion. The initial Laurdan GP value of −0.227 indicates that the POPC vesicles are predominantly in a liquid-disordered (L_d) phase under

control conditions (Fig. 1). Such negative GP values are characteristic of membranes with low lipid packing and increased interfacial hydration, reflecting a fluid and loosely organized bilayer.

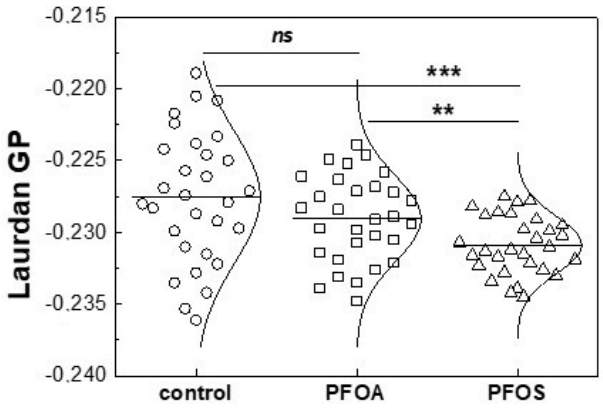


Fig. 1. Effect of PFAS (PFOA and PFOS) on Laurdan GP in POPC LUVs. POPC large unilamellar vesicles (LUVs) were incubated with PFAS at a PFAS/lipid molar ratio of 1:20. Each point represents an individual measurement; distributions are shown as violin plots with overlaid data points. Statistical analysis indicates no significant difference (ns) between control and PFOA-treated samples, while PFOS treatment results in a significant decrease in GP values compared to both control (***, $p < 0.001$) and PFOA (**, $p < 0.007$). Lower GP values reflect increased membrane fluidity or reduced lipid order

Violin plots in Fig. 1 represent the distribution of GP values measured in POPC LUVs in the absence (control) and presence of PFOA or PFOS ($n = 30$ per group, derived from two independent experiments with 15 measurements each). Individual measurements are shown as dots, and violin width reflects data density. No statistically significant difference was observed between control and PFOA-treated vesicles (Student's t-test, $p = 0.138$), indicating that PFOA does not significantly affect membrane packing under the tested conditions. In contrast, PFOS treatment resulted in a statistically significant decrease in GP compared to control ($p < 0.001$), suggesting increased membrane disorder and/or enhanced interfacial hydration. Additionally, PFOS differed significantly from PFOA ($p < 0.007$), exhibiting lower GP values and thus a stronger effect on membrane organization. PFOS induces a ~ 2.4-fold greater decrease in GP compared to PFOA, indicating a substantially stronger perturbation of the membrane interfacial environment. Given the zwitterionic nature of POPC, this effect is likely driven primarily by the preferential partition of PFOS into the lipid bilayer and its ability to dis-

rupt lipid packing, rather than by strong electrostatic interactions. The bulky perfluorinated chain of PFOS may induce local defects and increase water penetration at the membrane interface. Effect sizes were quantified using Cohen's d, indicating a small-to-moderate effect for PFOA ($d = -0.39$) and a large effect for PFOS ($d = -0.94$). It also confirms PFOS causes an approximately 2.4-fold larger reduction in GP than PFOA, reflecting a markedly stronger disruption of the membrane interfacial environment.

The observed differences in Laurdan GP values between PFOA and PFOS can be rationalized in terms of their distinct chemical structures and membrane interaction profiles. Both molecules possess a fully fluorinated hydrophobic chain, which promotes partitioning into lipid bilayers [11]. However, their headgroups differ significantly, leading to distinct interfacial behaviours [12]. PFOS, containing a sulphonate headgroup, is more strongly hydrated and exhibits a higher degree of charge delocalization compared to the carboxylate group of PFOA. This structural feature enhances its ability to remain anchored at the lipid-water interface while its perfluorinated chain penetrates into the hydrophobic core of the membrane [13]. Such an orientation facilitates deeper insertion and stronger perturbation of lipid packing, resulting in increased interfacial water penetration and enhanced dipolar relaxation, as reflected by the significant decrease in GP values. In contrast, PFOA, with its carboxylate headgroup, is more dynamic and less strongly anchored at the interface, which may limit its residence time and depth of insertion within the bilayer. As a result, its effect on membrane organization is weaker and does not lead to statistically significant changes in GP. The smaller effect size observed for PFOA supports this interpretation. Importantly, the differences in GP values do not solely reflect changes in lipid order, but rather alterations in the interfacial dielectric environment and water dynamics. The stronger effect of PFOS therefore indicates a more pronounced disruption of the membrane interfacial region, likely due to its combined hydrophobicity and interfacial stability.

In addition to molecular structure, parameters such as pH can further modulate PFAS-membrane interactions [12, 14]. The interaction of PFAS with lipid membranes is strongly influenced by pH, primarily through its effect on the ionization state of their headgroups and, consequently, on their amphiphilic balance and interfacial behaviour. PFOA ($pK_a < 0.5$ to 3.8) and PFOS ($pK_a < 1$) [12, 15, 16] exist predominantly in their deprotonated, anionic forms under physiological conditions as it is in our experimental design. However, under acidic conditions, partial protonation, particularly in the case of PFOA, can significantly alter their physicochemical properties and membrane affinity [17]. At neutral pH, both PFOA and PFOS are negatively charged and tend to localize at the lipid-water interface. In this state, their interactions with membranes are governed by a balance between hydrophobic insertion of the perfluorinated chain and electrostatic interactions involving the charged headgroup, surrounding water molecules,

and counterions. The charged headgroups favour interfacial positioning, limiting deep penetration into the hydrophobic core of the bilayer [18]. As a result, PFAS primarily perturb the membrane interfacial region, influencing dipolar relaxation dynamics and hydration, as reflected by changes in Laurdan GP. A decrease in pH leads to increased protonation of PFAS, most notably for PFOA due to its higher pK_a. The neutral (protonated) form exhibits increased hydrophobicity and reduced electrostatic repulsion, which facilitates deeper insertion into the lipid bilayer. This shift in partitioning behaviour can enhance disruption of lipid organization, promote local defects in the membrane, and increase water penetration at the bilayer interface. Consequently, larger changes in dipolar relaxation and Laurdan GP values would be expected under acidic conditions. In contrast, PFOS, due to its very low pK_a and highly delocalized sulphonate charge, remains predominantly ionized even at moderately acidic pH [12]. This makes its membrane interaction less sensitive to pH changes compared to PFOA. Instead, PFOS maintains a strong interfacial anchoring, with its perfluorinated chain inserting into the membrane while the sulphonate group remains hydrated at the interface. This configuration supports persistent perturbation of the interfacial region, contributing to its stronger overall effect on membrane properties. Importantly, pH dependent effects are further modulated by the lipid composition of the membrane. In zwitterionic systems such as POPC, the impact of pH is largely mediated through changes in PFAS ionization rather than alterations in membrane charge. However, in membranes containing charged lipids, pH-induced changes in lipid headgroup protonation could further influence PFAS binding, electrostatic interactions, and membrane insertion. Overall, these considerations indicate that pH plays a critical role in modulating PFAS-membrane interactions by altering the balance between electrostatic interactions and hydrophobic partitioning. This has important implications for understanding PFAS behaviour in biological systems, where local pH variations such as those found in endosomes, lysosomes, or inflamed tissues may significantly influence their membrane activity and associated biological effects.

Conclusion. PFOS induces a significantly stronger perturbation of the membrane interfacial environment compared to PFOA, suggesting a greater potential to disrupt membrane-associated processes in biological systems. These findings highlight the importance of PFAS structure in determining their impact on membrane function and potential cellular effects.

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