Supporting Information for:

Structure and elasticity of genistein and daidzein in lipid membranes using X-ray scattering and MD simulations

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Ratio of genistein to daidzein concentrations in gA experiments

Lundbaek et al.[7] estimated the ratio of the volume of the lipid and the decane annulus V_H to the aqueous volume V_A as $V_H/V_A \approx 0.001$. The hydrocarbon/aqueous partition coefficient K gives the ratio of concentration of bioflavinoid in hydrocarbon c_H to its aqueous concentration c_A as $c_H/c_A=K$. Therefore, the ratio of the amount of bioflavinoid in the hydrocarbon region B_H to the amount in the aqueous solution B_A is $B_H/B_A = K(V_H/V_A) \equiv x$; using the values of the partition coefficients[31] $K_G \approx 1100$ and $K_D \approx 330$, the ratio x_G is close to 1 for genistein and $x_D \approx 1/3$ for daidzein. Therefore, for equal total amounts of bioflavinoid added to the gA experimental apparatus, half the genistein resides in the hydrophobic region compared to only 25% of the daidzein. Consequently, the concentration of genistein in the membrane is twice that of daidzein when both are added to the gA experiment in equal amounts. This alone accounts for genistein increasing the gA channel lifetimes twice as much as daidzein.

Comparison of concentrations

It is of interest to compare the concentration of bioflavinoids in the membrane for the gA experiments to our experiments. In the gA experiments, the largest reported concentration[9] was 40□M. Half the genistein partitioned into the hydrocarbon region whose volume was 1000 times smaller than the aqueous volume, so the concentration in the membrane was 20mM. Similarly, the daidzein concentration was 10mM. For our experiments the maximum molarity of genistein was 1/4 the molarity of lipid. The volume of DOPC is 1303 $Å^3$ per molecule which is 780ml/mole which gives a lipid molarity of 1.28 M/liter. Therefore, the maximum molarity of genistein in our experiments was 320mM. Similarly, taking into account the solubility limit, our maximum molarity of daidzein in DOPC was 210mM. This latter estimate indicates that the gA experiments would not have encountered the membrane solubility limit that we found for daidzein.

Fully hydrated D-spacings in MLVs are shown in Fig. S1. In the MLV samples used for the D-spacing measurement, there was no evidence of crystal formation in contrast to our oriented samples. Apparently, some of the bioflavinoid partitioned into excess water in the MLV samples.

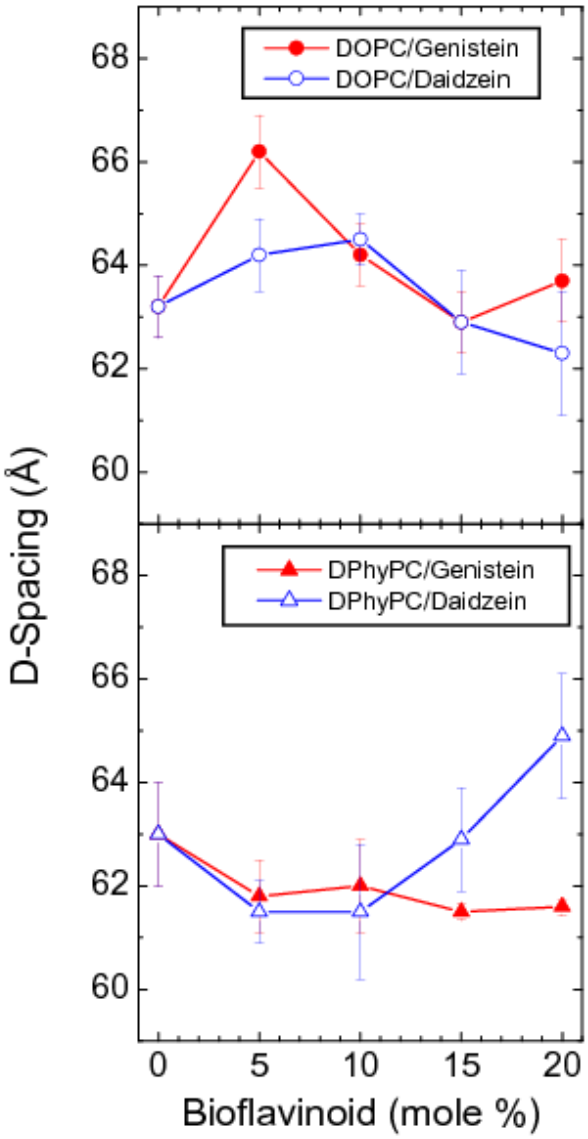
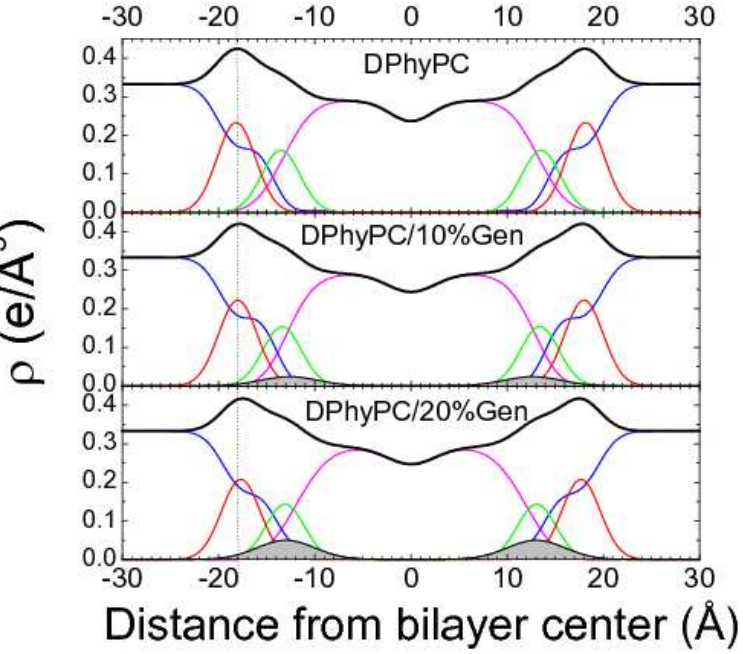


Figure S1. D-spacings of DOPC (top) and DPhyPC (bottom) with increasing concentration of bioflavinoid in excess water in x-ray capillaries at 30 ^{o}C. Genistein is shown with red solid symbols and daidzein with blue open symbols.

Supplementary electron density profiles (EDP) with component groups for bioflavinoids in DPhyPC are shown in Figure S2 and S3. These EDP were obtained by model fitting using the SDP program to the form factor data as described in Materials and Methods in the main paper. The genistein position was nearly constant at ~13.0 Å, while the daidzein position increased marginally from ~13 Å (5,10 mole%) to 13.4 Å (12 mole%).

Figure S2. Electron density profiles (EDP) of DPhyPC and increasing genistein content. Component groups are phosphate (red), carbonyl-glycerol (green), methylenes and terminal methyl group (magenta), water (blue), bioflavinoid (filled grey) and total (black).



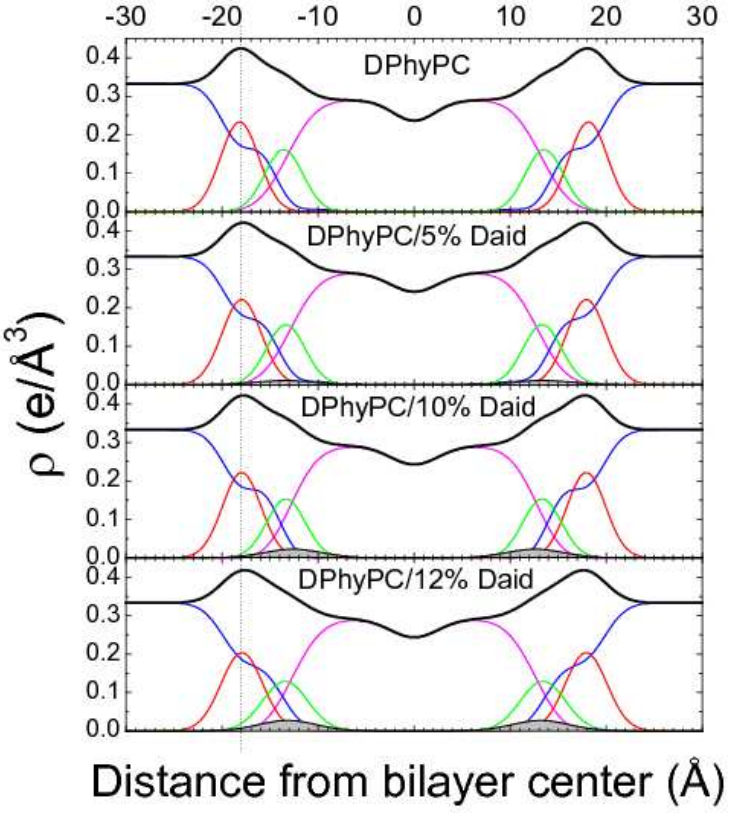


Figure S3. Electron density profiles (EDP) of DPhyPC and increasing daidzein content. Component groups are colored as in Fig. S2.

Electron density profiles from the MD simulations (non-symmetrized) are shown in Fig. S4, without the component groups for ease of comparison. The profiles were constructed for the following fixed areas: DOPC (75 Å^2), 20 mole% genistein/DOPC (83 Å^2) and 14 mole% daidzein/DOPC (79 Å^2).

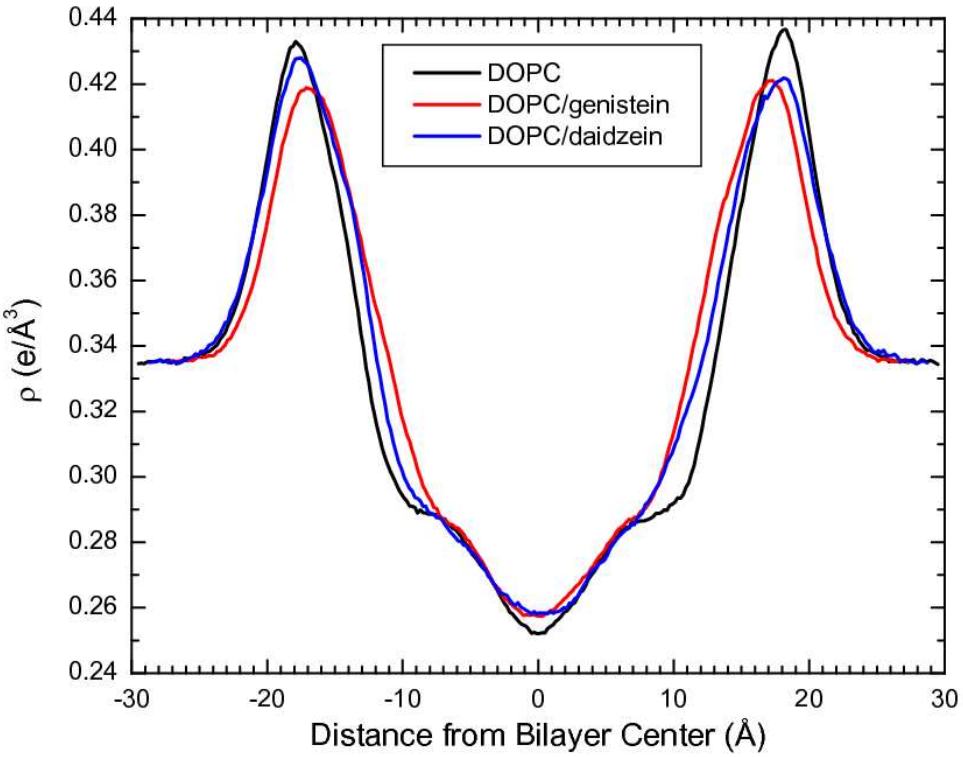


Figure S4. Electron density profiles (EDP) of DOPC (black) with 20 mole% genistein (red) and 14 mole% daidzein (blue).

Figure S5 shows a histogram of the bioflavinoid positions determined using the last 25 nanoseconds of the simulations of bioflavinoids and DOPC. 20 mole% genistein is located at 11.8 Å, while 14 mole% daidzein is located at 12.3 Å from the bilayer center. The error in the calculation is +/- 0.2 Å. The inset is a zoom on the baseline in order to clarify that the outlier positions for genistein are towards the center of the bilayer, while the outlier positions for daidzein are towards the interfacial region. These results are listed in Table 1.

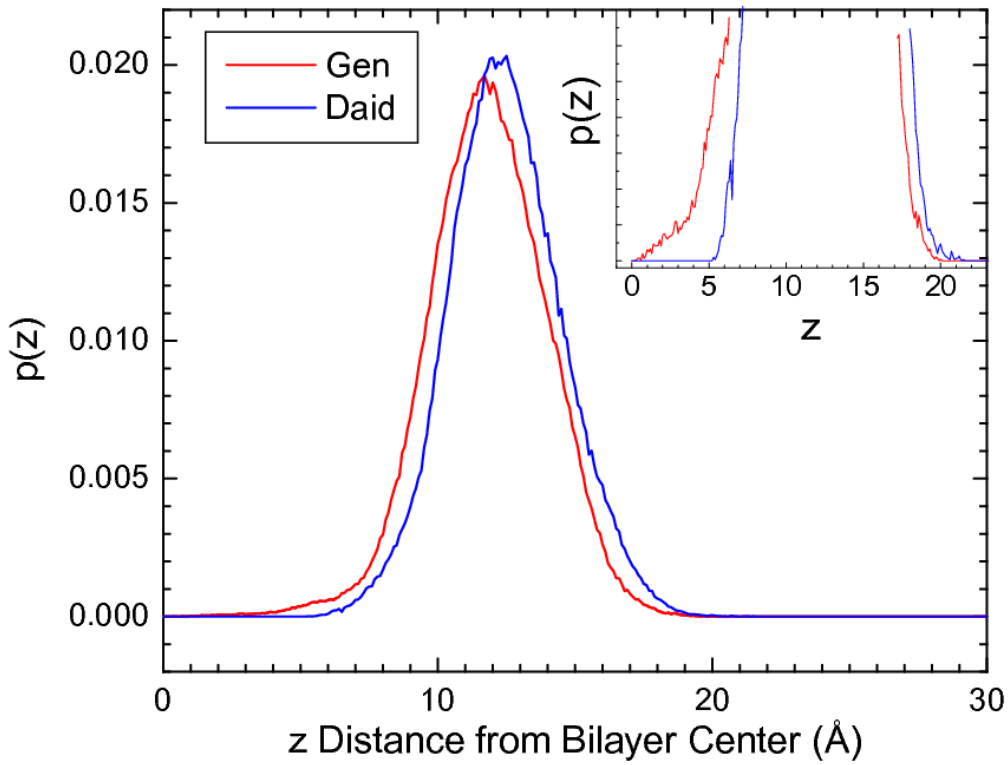


Figure S5. Histograms of bioflavinoid positions in DOPC calculated from MD simulations. Colors are: 20 mole% genistein (red) and 14 mole% daidzein (blue). Inset is a zoom on the baseline of data in main plot.