Electronic Supplementary Information

Calculation of Apparent pK_a Values of Saturated Fatty Acids with Different Lengths in DOPC Phospholipid Bilayers

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Derivation of Equation (2)

p$K_{a(z)}$ can be calculated as follows, strictly using procedure followed in Ref 41 and thermodynamic cycle presented in Figure S1:

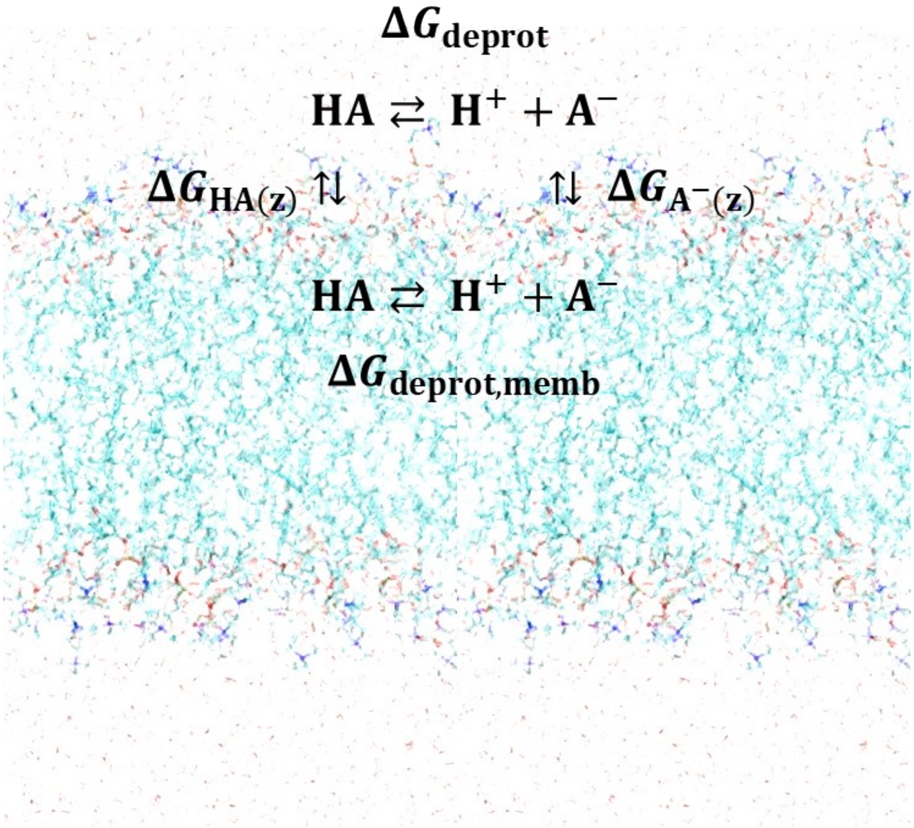


Figure S1. Thermodynamic cycle used to estimate the p$K_{a(z)}$ of fatty acid as a function of membrane depth *z*. The horizontal arrows correspond to deprotonation of fatty acid, while the vertical arrows correspond to the translocation of neutral or anionic fatty acid form along the membrane *z* axis. The proton is not explicitly taken into account and the pK_a in the bilayer is expressed as a shift vs. water due to membrane environment.

From the thermodynamic cycle, we get the expression:

$$\Delta\Delta G_{(z)} = \Delta G_{deprot,\,memb} - \Delta G_{deprot} = \Delta G_{A^-(z)} - \Delta G_{HA\,(z)}$$

Let's assume the following chemical equilibrium:

$HA \rightleftarrows H^+ + A^-$

where HA is fatty acid of interest with the corresponding dissociation constant K_a.

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

$$pK_{a,\,bulk} = -\log K_a = -\log \frac{[H^+][A^-]}{[HA]} = \frac{(G_{H^+} + G_{A^-}) - G_{HA}}{2.303RT} = \frac{\Delta G_{bulk}}{2.303RT}$$

$$\begin{aligned}\Delta G_{(z)} = (G_{H^+(z)} + G_{A^-(z)}) - G_{HA(z)} \\ + (G_{H^+bulk} + G_{A^-bulk}) - G_{HA\,bulk} \\ - (G_{H^+bulk} + G_{A^-bulk}) + G_{HA\,bulk}\end{aligned}$$

Here, we assume that the free energy associated with the deprotonation does not depend on the insertion depth indicating that the proton always ends up in bulk water. The difference in the free energy between the neutral state and the anionic state only depends on the difference between the free energy to solvate the fatty acid in bulk water and inside the membrane.

$$\begin{aligned}pK_{a(z)} &= \frac{\Delta G_{(z)}}{2.303RT} \\ &= \frac{1}{2.303RT}\left[(G_{H^+bulk} + G_{A^-bulk}) - G_{HA\,bulk} + \left(G_{A^-(z)} - G_{A^-bulk}\right) - (G_{HA\,(z)} - G_{HA\,bulk})\right] \\ &= \frac{1}{2.303RT}\left[\Delta G_{bulk} + \Delta\Delta G_{(z)}\right] \\ &= pK_{a,\,bulk} + \Delta pK_a\end{aligned}$$

The last expession is identical to the expression derived in Ref 41. However, we can rewrite this expression, taking into account deprotonation energy of fatty acid ΔG_{deprot} which is equal to 3.2 kcal mol^{-1} at 310 K at pH = 7 (ref 43):

$$\Delta G_{deprot} = 2.303RT(pK_{bulk} - pH) = 2.303RT(4.75 - 7) =- 3.2\ kcal\ mol^{-1}$$

$$\begin{aligned}pK_{a(z)} &= \frac{1}{2.303RT}\left[\Delta G_{bulk} + \Delta\Delta G_{(z)} + \Delta G_{deprot} - \Delta G_{deprot}\right] \\ &= \frac{1}{2.303RT}\left[\Delta G_{bulk} + (\Delta\Delta G_{(z)} - 3.2) - 2.303RT(pK_{bulk} - pH)\right]\end{aligned}$$

$$= \left[pK_{bulk} + \frac{\left(\Delta\Delta G_{(z)} - 3.2\right)}{2.303RT} - pK_{bulk} + pH) \right]$$

$$= pH + \frac{\Delta\Delta G'_{(z)}}{2.303RT}$$

In this way, pH is explicitly given in Equation (2). The difference between expression in Ref 41 and Equation (2) is that $\Delta G'$ in Equation (2) takes into account deprotonation energy of 3.2 kcal mol^{-1} ($\Delta G_{\mathrm{deprot}}$) for which the $\Delta G_{\mathrm{HA(z)}}$ curves of neutral forms were shifted in Figure 5:

$$\Delta\Delta G'_{(z)} = \Delta\Delta G_{(z)} - 3.2 = \Delta G_{A^-(z)} - \Delta G_{HA\,(z)} - 3.2 = (G_{A^-(z)} - G_{A^-bulk}) - (+ 3.2)$$

In total, the equation (2) and equation derived in Ref 41 are identical, but written in a slightly different way to better show the energy differences between neutral and anion FFA forms in Figures 5 and 6.

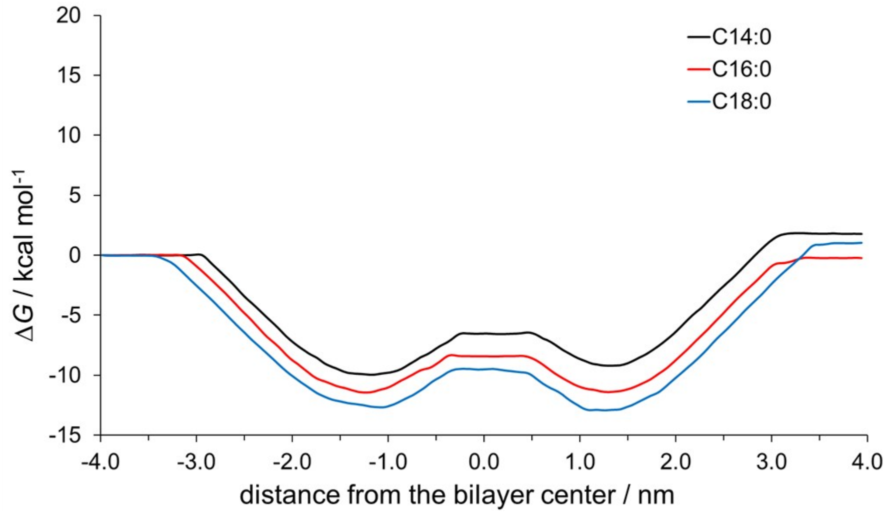


Figure S2. Non symmetrized free energy profiles for FFA translocation across DOPC bilayer for neutral forms of myristic (black), palmitic (red) and stearic acid (blue).

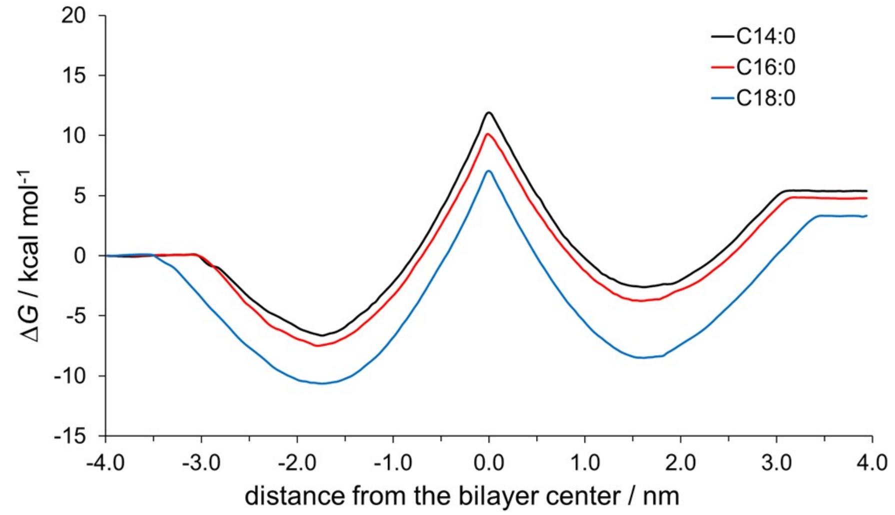
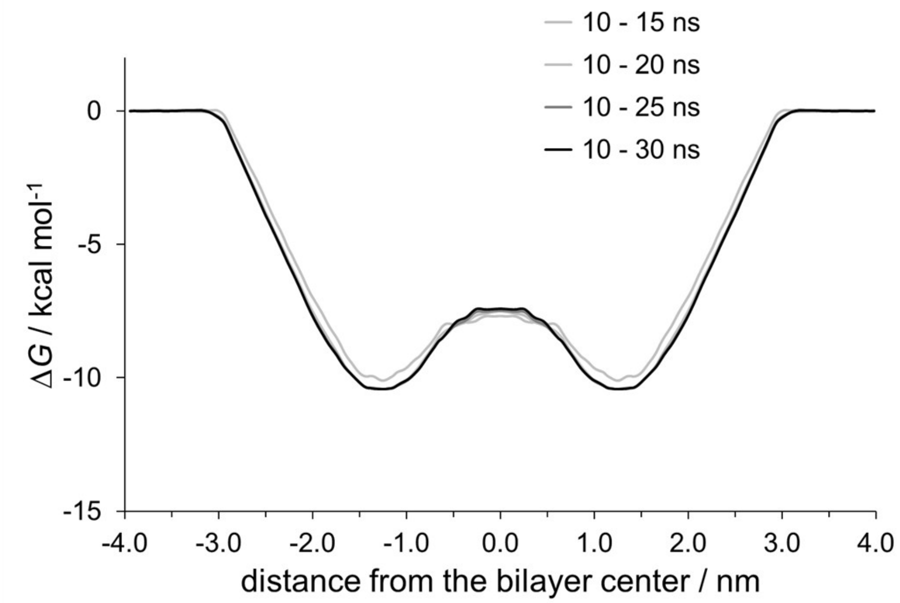


Figure S3. Non symmetrized free energy profiles for FFA translocation across DOPC bilayer for anionic forms of myristic (black), palmitic (red) and stearic acid (blue).

Myristic acid C14:0



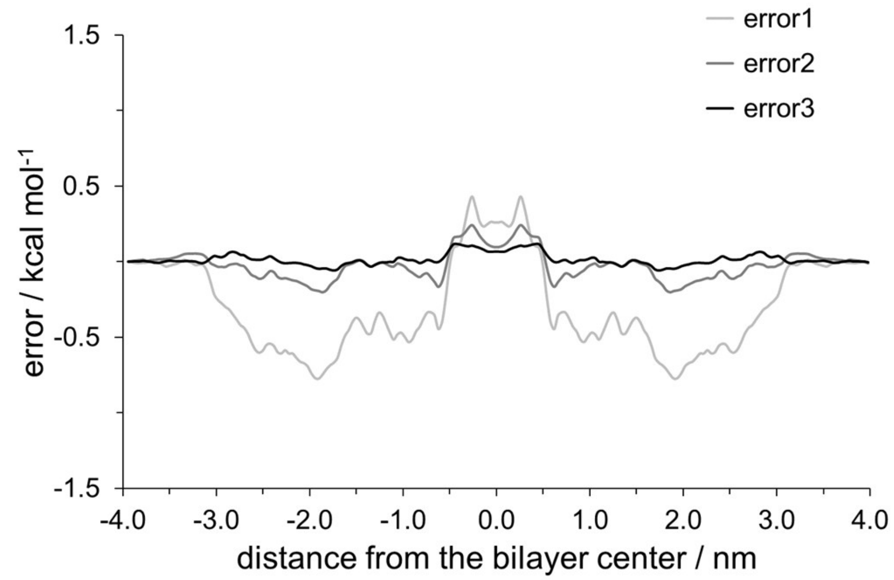
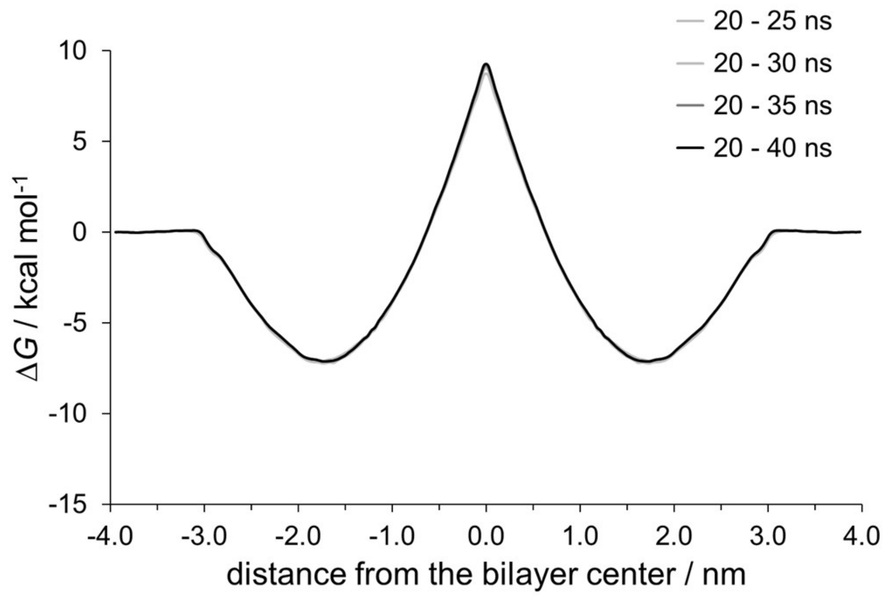


Figure S4. Free energy profiles for neutral myristic acid as a function of simulation time. Profiles are symmetrized between the leaflets. Errors in free energy curves are obtained by subtraction of free energy curve with the longest sampling time (10 – 30 ns) with free energy curves obtained for 10 – 15 ns (error 1), 10 – 20 ns (error 2) and 10 – 25 ns (error 3).



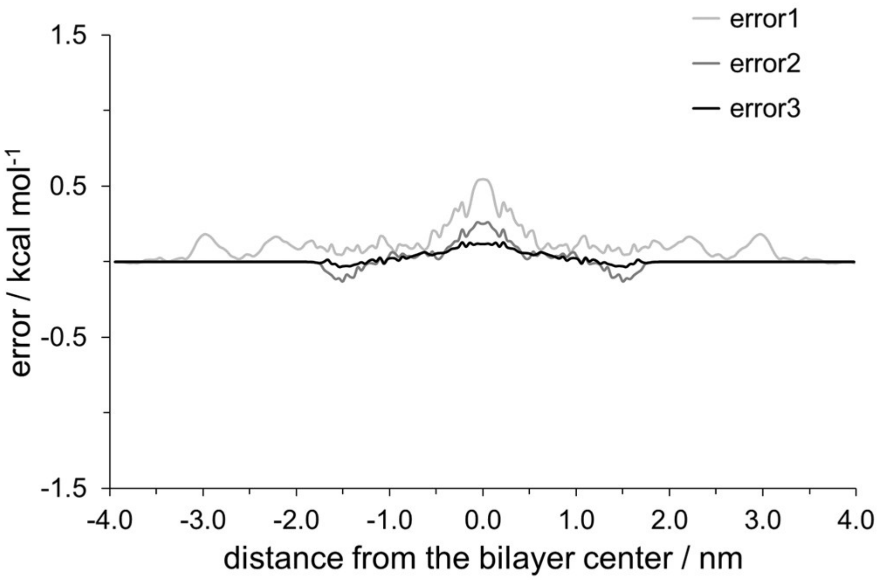


Figure S5. Free energy profiles for myristate as a function of simulation time. Profiles are symmetrized between the leaflets. Errors in free energy curves are obtained by subtraction of free energy curve with the longest sampling time (20 – 40 ns) with free energy curves obtained for 20 – 25 ns (error 1), 20 – 30 ns (error 2) and 20 – 35 ns (error 3).

Palmitic acid C16:0

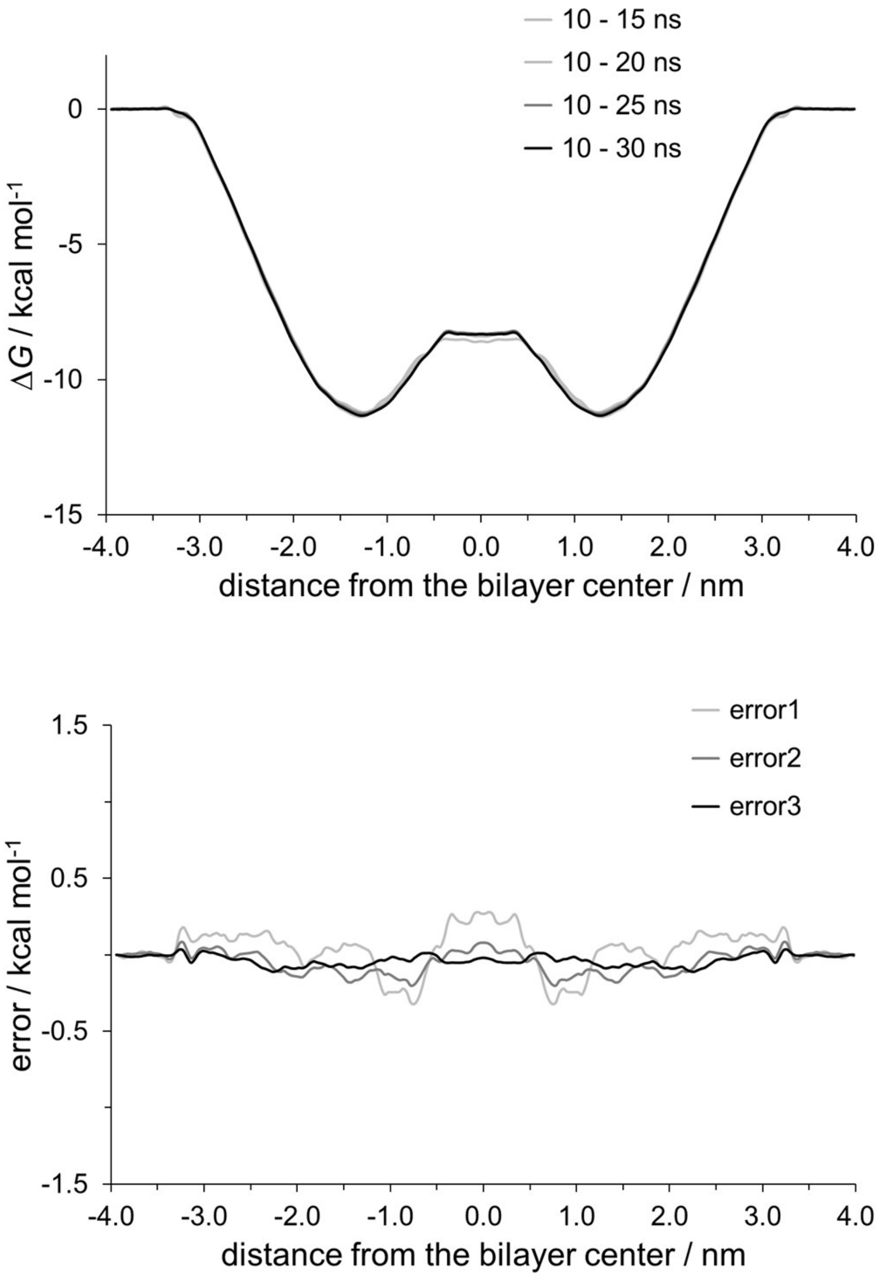
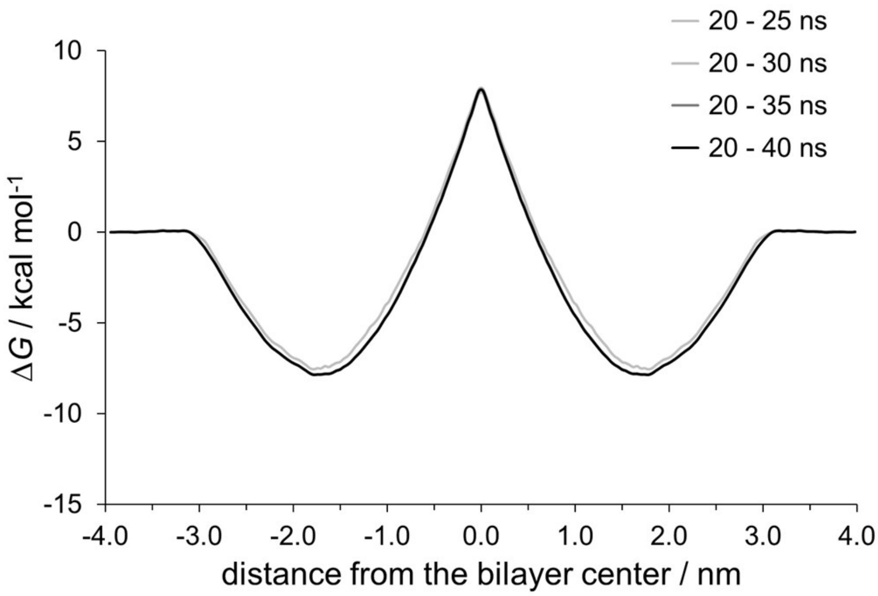


Figure S6. Free energy profiles for neutral palmitic acid as a function of simulation time. Profiles are symmetrized between the leaflets. Errors in free energy curves are obtained by subtraction of free energy curve with the longest sampling time (10 – 30 ns) with free energy curves obtained for 10 – 15 ns (error 1), 10 – 20 ns (error 2) and 10 – 25 ns (error 3).



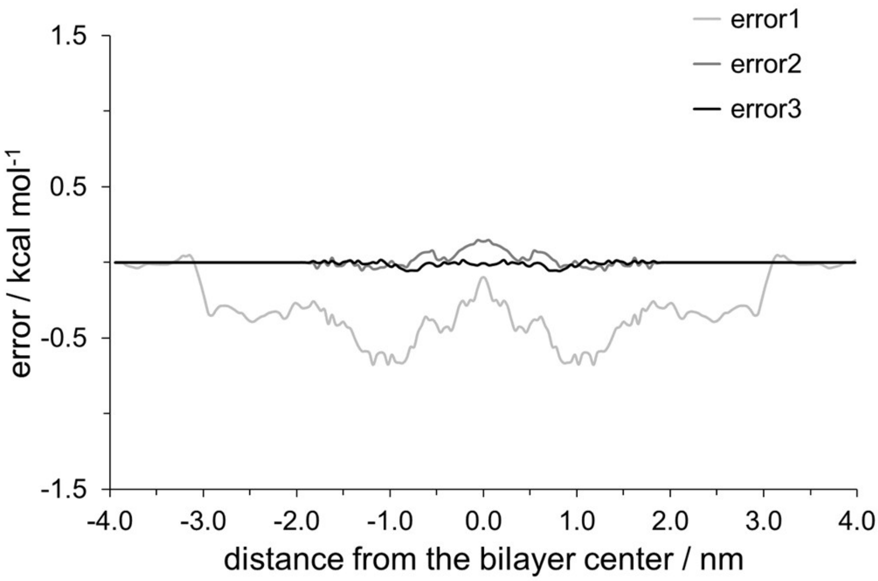


Figure S7. Free energy profiles for palmitate as a function of simulation time. Profiles are symmetrized between the leaflets. Errors in free energy curves are obtained by subtraction of free energy curve with the longest sampling time (20 – 40 ns) with free energy curves obtained for 20 – 25 ns (error 1), 20 – 30 ns (error 2) and 20 – 35 ns (error 3).

Stearic acid C18:0

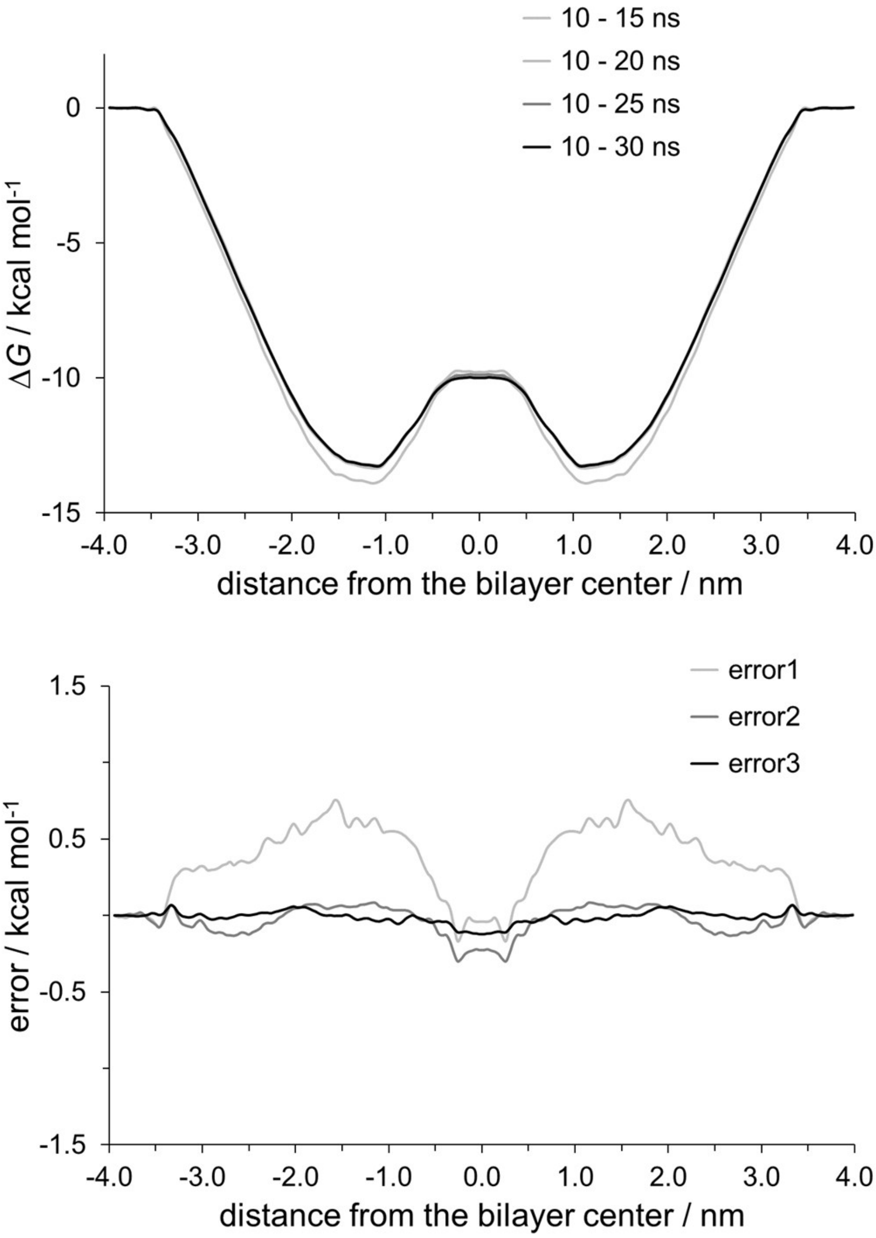
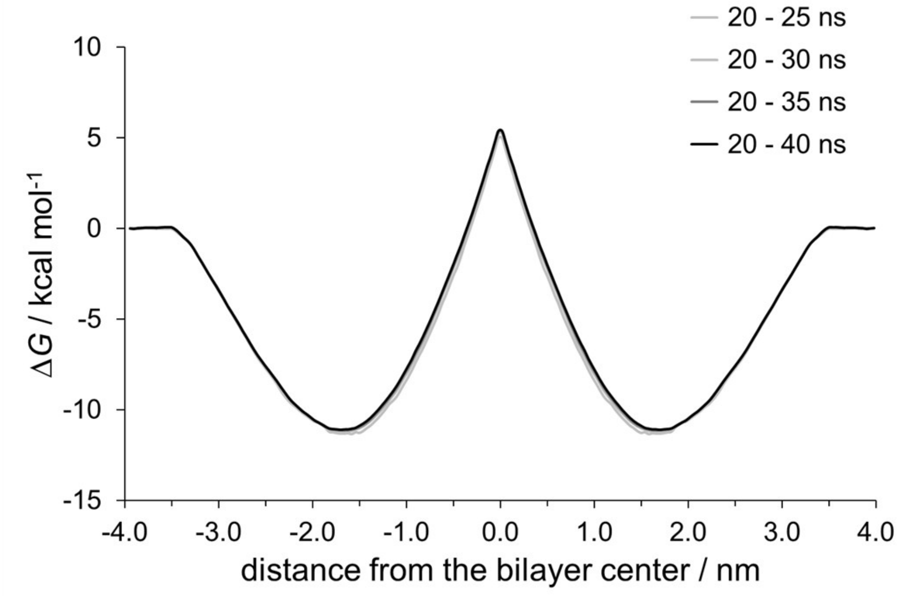


Figure S8. Free energy profiles for neutral stearic acid as a function of simulation time. Profiles are symmetrized between the leaflets. Errors in free energy curves are obtained by subtraction of free energy curve with the longest sampling time (10 – 30 ns) with free energy curves obtained for 10 – 15 ns (error 1), 10 – 20 ns (error 2) and 10 – 25 ns (error 3).



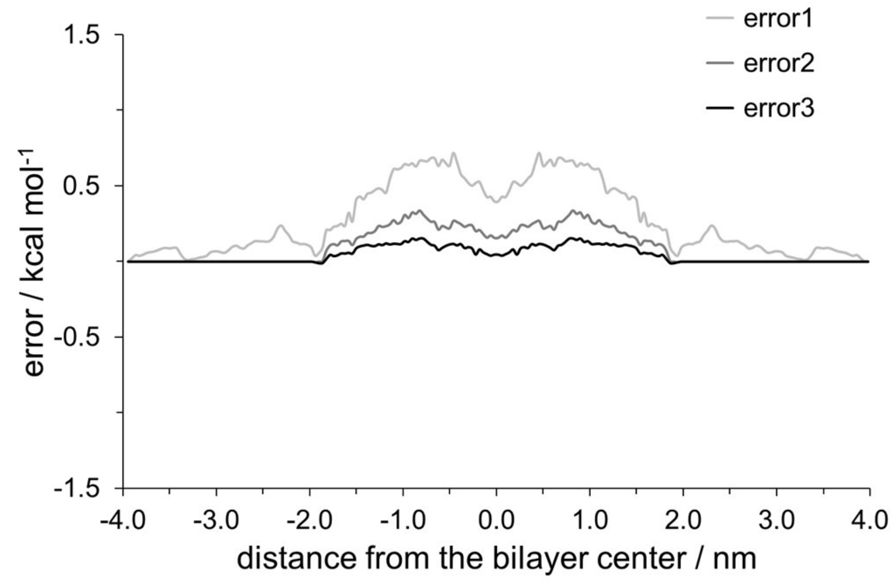


Figure S9. Free energy profiles for stearate as a function of simulation time. Profiles are symmetrized between the leaflets. Errors in free energy curves are obtained by subtraction of free energy curve with the longest sampling time (20 – 40 ns) with free energy curves obtained for 20 – 25 ns (error 1), 20 – 30 ns (error 2) and 20 – 35 ns (error 3).