Understanding the relative contributions of individual protonation states in PIP_3 and PIP_2 lipid towards free energy of binding of AKT1 protein with membrane

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The concentrations of individual states of PIP_3 are represented using [345], [34′5], [345′], [3′45], [34′5′], [3′45′], [3′4′5] and [3′4′5′]. Similarly, the concentrations of individual protonation states of PIP_2 are represented using [45],[4′5],[45′] and [4′5′].

We adopted the equations from reference[1] and re-wrote them to get the functional forms of fraction of individual protonation states of PIP_3 and PIP_2. Using equations set 1, 3 in 2 and 4 respectively, we could reproduce the fraction of each protonation state of PIP_3 and PIP_2 respectively as function of pH. We further quantified the uncertainity of each of these fractions using the error on the pK_as as reported in [1].

PIP_3

$$
\begin{aligned}
[3'45] &= [345]10^{pH-pK_{a1}} \\
[34'5] &= [345]10^{pH-pK_{a2}} \\
[345'] &= [345]10^{pH-pK_{a3}} \\
[3'4'5] &= [345](10^{2pH-pK_{a1}-pK_{a7}} + 10^{2pH-pK_{a2}-pK_{a9}}) \\
[3'45'] &= [345](10^{2pH-pK_{a1}-pK_{a8}} + 10^{2pH-pK_{a3}-pK_{a11}}) \\
[34'5'] &= [345](10^{2pH-pK_{a2}-pK_{a10}} + 10^{2pH-pK_{a3}-pK_{a12}}) \\
[3'4'5'] &= [345](10^{3pH-pK_{a1}-pK_{a7}-pK_{a16}} + 10^{3pH-pK_{a2}-pK_{a9}-pK_{a16}} \\
&\quad + 10^{3pH-pK_{a1}-pK_{a8}-pK_{a17}} + 10^{3pH-pK_{a3}-pK_{a11}-pK_{a17}} \\
&\quad + 10^{3pH-pK_{a2}-pK_{a10}-pK_{a18}} + 10^{3pH-pK_{a3}-pK_{a12}-pK_{a18}})
\end{aligned}
\tag{1}
$$

$$
\begin{aligned}
f_{3'45} &= \frac{[3'45]}{[345]+[3'45]+[34'5]+[345']+[3'4'5]+[3'45']+[34'5']+[3'4'5']} \\
f_{34'5} &= \frac{[34'5]}{[345]+[3'45]+[34'5]+[345']+[3'4'5]+[3'45']+[34'5']+[3'4'5']} \\
f_{345'} &= \frac{[345']}{[345]+[3'45]+[34'5]+[345']+[3'4'5]+[3'45']+[34'5']+[3'4'5']} \\
f_{3'4'5} &= \frac{[3'4'5]}{[345]+[3'45]+[34'5]+[345']+[3'4'5]+[3'45']+[34'5']+[3'4'5']} \\
f_{3'45'} &= \frac{[3'45']}{[345]+[3'45]+[34'5]+[345']+[3'4'5]+[3'45']+[34'5']+[3'4'5']} \\
f_{34'5'} &= \frac{[34'5']}{[345]+[3'45]+[34'5]+[345']+[3'4'5]+[3'45']+[34'5']+[3'4'5']} \\
f_{3'4'5'} &= \frac{[3'4'5']}{[345]+[3'45]+[34'5]+[345']+[3'4'5]+[3'45']+[34'5']+[3'4'5']} \\
f_{345} &= 1-(f_{3'45}+f_{34'5}+f_{345'}+f_{3'4'5}+f_{3'45'}+f_{34'5'}+f_{3'4'5'})
\end{aligned}
\tag{2}
$$

PIP_2

$$
\begin{aligned}
[4'5] &= [45]10^{pH-pK_{a1}} \\
[45'] &= [45]10^{pH-pK_{a2}} \\
[4'5'] &= [4'5]10^{pH-pK_{a4}} + [45']10^{pH-pK_{a5}} \\
&= [45](10^{2pH-pK_{a1}-pK_{a4}} + 10^{2pH-pK_{a2}-pK_{a5}})
\end{aligned} \tag{3}
$$

$$
\begin{aligned}
f_{4'5} &= \frac{[4'5]}{[45] + [4'5] + [45'] + [4'5']} \\
f_{45'} &= \frac{[45']}{[45] + [4'5] + [45'] + [4'5']} \\
f_{4'5'} &= \frac{[4'5']}{[45] + [4'5] + [45'] + [4'5']} \\
f_{45} &= 1 - (f_{4'5} + f_{45'} + f_{4'5'})
\end{aligned} \tag{4}
$$

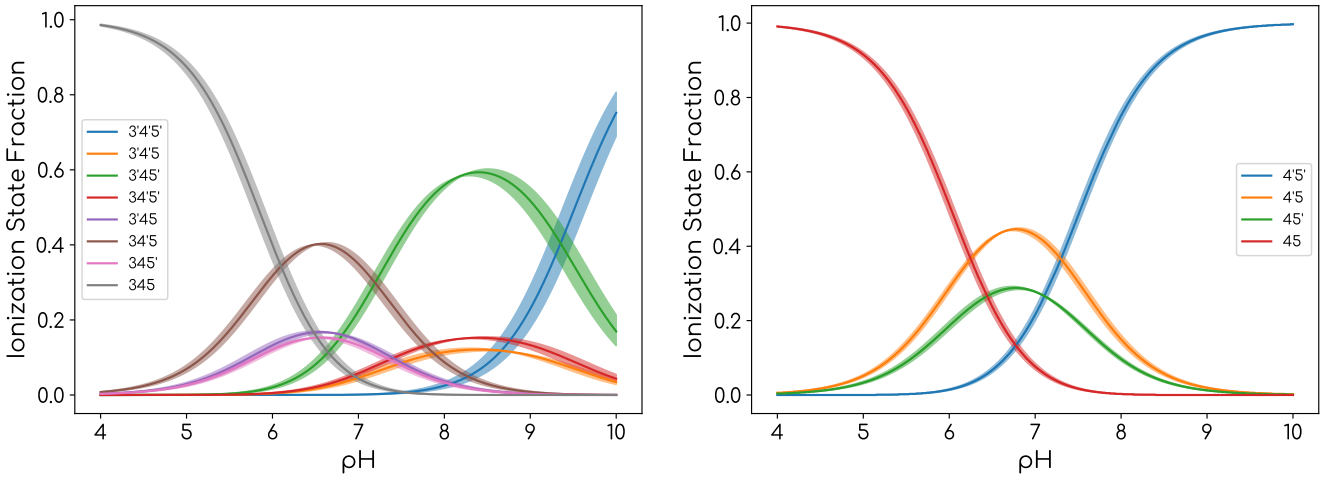

Figure 1: Fraction of each protonation state of PIP_3 (left) and PIP_2 (right) as a function of pH

These analytical forms were used along with the free energy of binding obtained from umbrella sampling simulations to evaluate the free energy of binding as a function of pH ($\Delta G^{sim}_{binding}(pH)$) using eqn.5 (shown in figure 2). The uncertainity (ΔG_{error}) on these curves were evaluated using primitive error propagation relations (eqn.6), accounting for (i) Uncertainity on fraction of each protonation state($f_{i,error}(pH)$) using error evaluated on pK_as of the above model[1]. (ii) Uncertainity quantified using bootstrap analysis from pmf profiles ($\Delta G^{sim}_{i,error}$) obtained from umbrella sampling simulations.

$$\Delta G^{sim}_{binding}(pH) = \sum_i f_i(pH)\Delta G^{sim}_i \tag{5}$$

$$\Delta G_{error}(pH) = \sum_i (f_i(pH)\Delta G^{sim}_{i,error} + f_{i,error}(pH)\Delta G^{sim}_i) \tag{6}$$

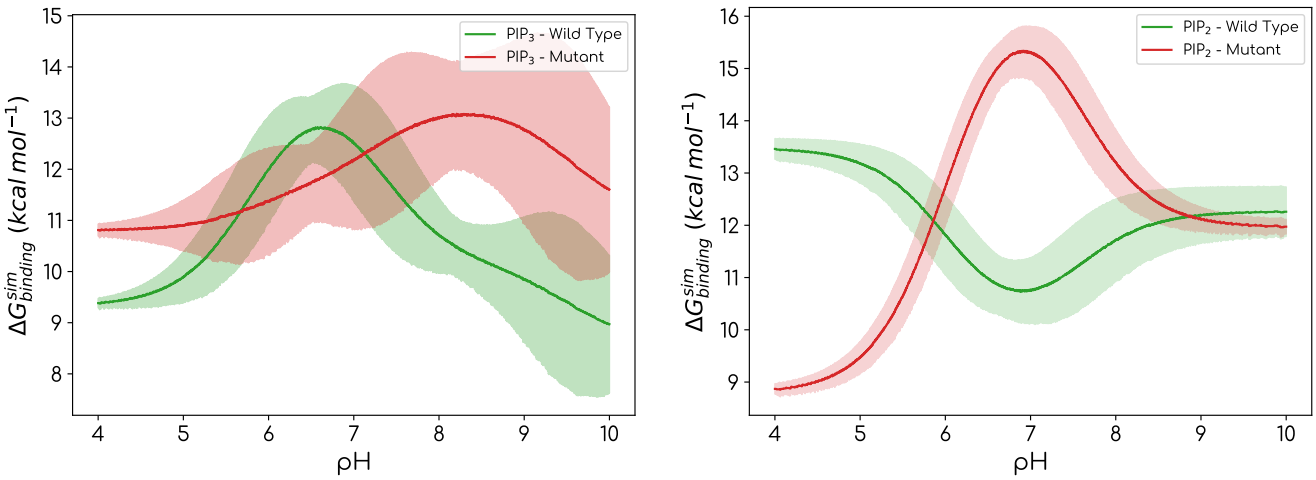

Figure 2: Free energy of binding as a function of pH for AKT1-WT(green) and AKT1-E17K mutant(red) in PIP_3(left) and PIP_2(right) systems respectively

References

[1] Zachary T Graber, Joseph Thomas, Emily Johnson, Arne Gericke, and Edgar E Kooijman. Effect of h-bond donor lipids on phosphatidylinositol-3, 4, 5-trisphosphate ionization and clustering. *Biophysical Journal*, 114(1):126–136, 2018.