Tools and methods used in Discovery Studio® for the visualization, characterization and analysis of the electrostatic effects on the alkali-activated K⁺ channel, TASK-2

Modules used:
- DS Visualizer Pro Enterprise
- DS Biopolymer
- DS CHARMM

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Introduction

It is well known that proteins are subject to molecular forces that affect their fold, stability and mechanism (1,2). These forces can be categorized as either covalent or non-covalent. Non-covalent forces include: salt-bridges, hydrogen bonds, hydrophilic/ hydrophobic interactions, van der Waals, and electrostatics/solvation (1,2). Electrostatic analysis looks at the overall charge distribution of molecules, which depends on the fold and molecular composition of the system; this charge distribution is unique to every protein, much like a fingerprint (1 – 5). In this application note, we use Delphi (6) and CHARMM (7) in Discovery Studio to visualize, characterize and analyze the electrostatic effects of the alkali-activated K+ channel, TASK-2. This application note is based on the work reported by Niemeyer et al (8), who report that a single arginine residue (R224) might be responsible for blocking the channel through electrostatic effects. Our analysis verifies their hypothesis, and in addition we propose the existence of what we believe to be another key player involved in keeping the channel at an open state, this amino acid is aspartic acid 208.

Summary of Steps Performed

Step 1: Preparation of the system: assignment of atoms charges and radii
Step 2: Running of Delphi experiments and Visualization
Step 3: Further Analysis of results

Delphi analysis of the alkali-activated K+ channel: TASK-2

Step 1: Preparation of the system: assignment of atoms charges and radii
Before running a Delphi calculation one needs to assign the proper charges to all the atoms in the system. This can be done easily in Discovery Studio by using one of the following four strategies: 1) Use of CHARMM or Discover (9) force-field parameters; 2) Use of PARSE charges (6); 3) Use of AMBER charges (10); and 4) Use of Delphi default charges. All the Delphi calculations done in this application note were carried out using the fourth option, which is with Delphi default charges.

In the work reported by Niemeyer et al., the authors performed electrostatic calculations using the PBEQ module in CHARMM, version c31b1 (7). In their calculations a low dielectric planar slab was used to represent the membrane. In our case, we decided to first use an explicit membrane to show that the effects of the membrane have a relatively small effect on the potentials at the entrance of the pore which is exposed to the solvent (Figure 1 and Figure 2). Larger electrostatic disturbances are usually observed at the interface between the protein and the membrane (11), but we suspected that a much lesser effect would occur at the center.
To show the magnitude of the effects that the explicit membrane had on the calculated potentials of the pore as a function of internal distance, we compared the potentials with and without the membrane (Figure 2). We noticed a variation of potentials to be within $-1.6$ and $1.6 \, kT$ (about $-1$ to $1 \, \text{kcal/mol}$) at its highest. Since this variation is relatively small and all subsequent calculations were done with the intent to analyze the difference in charge potential between a charged and uncharged residue, the membrane was removed.

All the other electrostatic parameters used by Niemeyer et al were replicated, except for the assignment of the atomic charges for which we used the Delphi default charges. Delphi calculations with the charmm27 parameters gave a very similar analytical description of the charge distribution on a solvent accessible surface (Figure 4b), but using Delphi charges gave a much cleaner view.
Once the charges had been assigned, we used the settings shown in Figure 3 for running the Delphi calculation.

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<thead>
<tr>
<th>Electrostatic Potential</th>
<th>Parameter Name</th>
<th>Parameter Value</th>
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<td>Advanced</td>
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</table>

Figure 3: Settings used for all of the Delphi calculations described in this application note.

**Step 2: Running Delphi experiments and visualization**

After all the parameters have been entered, running Delphi is trivial and a series of fast experiments were done. However, it is important to make sure the protein system is well defined, and any corrections are made prior to running the calculations. In the case of the homology model built by Niemeyer et al (TASK-2PoreMod.pdb), we noticed that the histidine residues were listed as HSD. In order to run the necessary experiments within Discovery Studio, we made modifications to the residue nomenclature and bond connectivity, and generated new hydrogens. These changes were easily done using the client interface and tools within.

**Experiment 1: Looking at the overall charge distribution with default Delphi charges.**

The goal of this first experiment was simply to map the Delphi potentials on a solvent accessible surface and get a general overview of the charge distribution of the TASK-2 model.

![Delphi potentials](image)
In Figure 4a and 4b, we clearly see that the center section of the pore is negatively charged, indicating that the pore is at an open state. The location of R224 is masked by the blue coloring on the right and left sides of the picture.

**Experiment 2: Isolating the charges.**
The TASK-2 channel is a homodimer made of chains A and B, therefore for all charge analysis studies we will assign charges to residues on both chains. In this experiment the goal was to visualize the effect of the charges from only R224, and look at their contribution to the overall electrostatic field. For this we set all the charges to zero, except for the charges on the two R224 (A and B chains) which were set to +1.0, giving a system net charge of +2 (Figure 5).

![Figure 5: a) Solvent accessible surface colored by Delphi with charges on A:R224 and B:R224, and with zero charges for the rest of the pore. b) Mean potential distribution of phosphates and waters at the pore. The dots on the left are from molecules at the entrance of the pore (top) and the molecules on the right are at from molecules at the bottom of the pore.](image)

From this calculation we can see that the electrostatic effect of both arginines is sufficient to positively polarize the entrance of the pore. Interestingly we have only modified the charges, and not any other properties like hydrophobicity or hydrophilicity of the system. Niemeyer et al, explain that the hydrophobic environment might be responsible for lowering the pKa and removing the protons on the arginines, thereby neutralizing their effect on the pore. Our calculations show that hydrophobic effects are sufficient to accomplish charge quenching, and therefore, we suspected that other residues might be involved in the gating mechanism.

**Experiment 3: Finding other residues involved in the gating of the pore.**
Based on the observations made in experiment 2, we set out to search other charged groups in the vicinity. By performing a search of all charged residues within 18 Å of the arginines 224, we found three ionizable residues: lysine 107 (K107), glutamic acid 186 (E186) and aspartic acid 208 (D208). Since we’re looking for residues with a negative charge, which could mitigate the effects of the positively charged arginines 224, we decided to only focus on the effects that E186 and D208 have on the charge of arginines.

To test our hypothesis, we assigned a negative charge on E186 (A and B chains) first and left the positive charges on R224 (A and B chains) (Figure 6).
From the experiment shown in Figure 6a, we noticed a significant suppression of the charges at the pore. However, Figure 6b shows a plot of positive mean potentials for phosphates and waters at the pore. From this observation, we expect the K⁺ channel to remain blocked at this point, which implies that a negatively charged E186 is not sufficient to open the pore.

The next step is to isolate the effect of having a negatively charged D208. For this step we have set the charges on E186 (A and B chains) to zero and left the R224 positively charged (Figure 7).
In this calculation, we observed a complete shift in the pore potential. In figure 7a, the charge at the pore has gone from positive to negative, thereby neutralizing the effects of R224 and opening the pore. Figure 7b shows the same plot as in Figure 6b, except that the mean potential of the phosphates and waters at the pore is now negative.

**Step 3: Further Analysis of results**

**Finding additional players through symmetry**

The homology model built by Niemeyer et al. for TASK-2 was done using the Kv1.2 K+ channel (PDB ID code 2A79), which has a 4-fold symmetry. TASK-2 is a homodimer built mainly of 6 alpha helices for each monomer (13), with each domain being an integral part of the pore. By splitting each monomer and creating 2 subunits based in the 4-fold symmetry from the original template, and by performing structural superposition of each subunit, we were able to identify an additional arginine, which may also play an important role in pH gating of the channel. The arginine that we found by structural superposition was arginine 112 (R112) which sits at a 4-fold symmetry position to R224. By assigning zero charges to all the atoms and leaving R112 positively charged, we ran a new Delphi calculation to determine if this residue alone affects the overall charge of the pore (Figure 8).

Similarly to the analysis shown in figures 5a and 5b, in figures 8a and 8b we noticed that R112 also plays a significant role as it also contributes to the positive ionization of the pore.

So the question remains, if after having both residues R112 and R224 positively charged, are the two negatively charged D208 (from A and B chains) capable of changing the potential and opening the pore? In order to answer this question we used a system with all atom charges set to zero, and assigned charges to R112, R224 and D208.
Our calculations show in Figure 9a and 9b, a set of two negatively charged D208 (one per monomer) alone are capable of keeping the pore at an open conformation, i.e. negatively charged, even after considering two sets of positively charged arginines, R112 and R224.

We also noticed through structural superposition, that the 4-fold equivalent residue to E186 was tyrosine 39 (Y39), and the equivalent residue to D208 was asparagine 103 (N103). Only tyrosine may be titratable under the pH ranges that Niemeyer et al. studied, i.e. pH 6.0, pH 7.5 and pH 9.0, therefore; we expected the charge of Y39 to be neutral, and if ionized, it’s charge contribution to be relatively minor in comparison to that of D208. Since we have shown that D208 alone is capable of opening the pore (figure 7a), we did not consider Y39 to have a major role in the gating of the pore.

**Calculation of pKa shifts**

Delphi calculations can also be used to estimate the pKa shift (ΔpK) of an amino acid titratable group due to the presence of another residue (14). For the case of TASK-2, we decided to use the charge to charge complementarity property (reciprocity, reference 14) to estimate the ΔpK on R224 and R112 due to the presence of a charged D206 and E186. We also calculated the ΔpK on Asp208 and Glu186 due to the presence of a charged R224 and R112.

For the method used for estimating the change in pK, please refer to reference 14.

<table>
<thead>
<tr>
<th>Case</th>
<th>Salt Concentration</th>
<th>Estimated ΔpK shift (A:B) (from residue)</th>
<th>Published pKa for side chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>R224</td>
<td>0.145M</td>
<td>1.44/1.47 (A:B:D208+E186)</td>
<td>12.5</td>
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<tr>
<td>R112</td>
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<td>1.40/2.02 (A:B:D208+E186)</td>
<td>12.5</td>
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<tr>
<td>E186</td>
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<td>1.13/1.41 (A:B:R224+R112)</td>
<td>4.5</td>
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<td>D208</td>
<td>0.145M</td>
<td>1.67/2.02 (A:B:R224+R112)</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Table 1. Estimated pKa shift using Delphi and the reciprocity law of charges

Figure 9. a) Solvent accessible surface colored by Delphi with +1 positive charge on R112 and R224, and -1 negative charge on D208. b) Mean potential distribution of phosphates and waters at the pore.
The Delphi estimated ΔpK shifts of around 2 units for D208 correlated well with the experimental data reported by Niemeyer et al (8). In our estimate, we believe that at pH 6.0 the Asp208 residue has not yet lost its proton, and therefore, the net potential of the channel is positive due to R224 and R112. As the pH increases, say to pH 9.0, D208 loses its proton and becomes negatively charged, thereby inducing a net negative potential on the channel as shown on Figures 4, 7 and 9. From our calculations, we conclude that the pKa change of D208 might be more relevant to the gating mechanism of the pore than the lowering of the pKa of R224.

Conclusion:

In the work reported by Niemeyer et al., electrostatic effects are shown to have a major role in the overall function of the potassium ion channel TASK-2. Our results show that D208 might be a key residue responsible for neutralizing R224 and keeping the ion channel at an open state. The estimated pKa shifts of Arg224, and especially the pKa shift of D208, further support the pH dependency of the opening and closing mechanism of the channel. We predict that mutation of D208 to alanine or any other neutral residue might cause the channel to stay in a closed conformation, and hence, abolishing the flow of potassium ions across the TASK-2 channel. However, it is important to remember that the conclusions made in this application note have been drawn from a homology model, which may have inherited inaccuracies. Once the experimental structure is elucidated, a more conclusive analysis may be made.

In this application note we have shown how Delphi, CHARMm and supporting tools in Discovery Studio can be used to visualize, characterize and analyze important electrostatic effects in proteins. The analysis and methods performed in this application note may be used for other molecular systems in which electrostatic forces are suspected of having a significant functional role.

References