Analysis of a section of a hERG ion channel molecular dynamics trajectory

**Indroduction.**

The human Ether-a-go-go Related Gene (hERG) potassium ion channel is expressed in heart tissue and is involved in the control of your heart beat. Mutations in this protein can lead to genetic conditions such as long Qt syndrome, and accidental blocking of the channel by pharmaceutical agents can lead to arrhythmias and heart failure. Hence drug companies routinely screen new lead molecules for hERG blocking activity. Dr C. Dempsey’s group has recently generated many hundreds of nanoseconds of molecular dynamics simulation of this homo-tetrameric protein in a model lipid bilayer. In this exercise we will examine and analyse the behaviour of the system over a short period of a 500 ns simulation of the wild type protein.

**Simulation and trajectory details.**

The simulation comprised one hERG tetramer embedded in 1136 1-palmitoyl-2-oleoyl-3-glycerophosphocholine lipids and 130833 water molecules containing 0.15 mM potassium chloride at pH 7. The forcefield used was the OPLS/AA and Berger lipid combination with SPC water. The molecular dynamics program used was GROMACS and the simulations performed on Bristol’s HPC machine, BlueCrystal ([http://www.acrc.bris.ac.uk](http://www.acrc.bris.ac.uk/)). The simulation conditions were 330 K, NPT (constant pressure ensemble, 1 Barr) under PBC (periodic boundary conditions) and PME (particle mesh Ewalds) for long range electrostatics. The integration timestep was 2 fs and a structure (“frame”) was saved every 5000 steps.

**Q1** What is the time interval in ps between saved structures (frames)?

**Running VMD**

VMD is a molecular graphics program from the University of Illinois (<http://www.ks.uiuc.edu/Research/vmd/>) that is particularly good for the display of molecular dynamics trajectories. Click on the icon on your desktop to launch VMD

At the “VMD main” window select File-> New Molecule.. and Browse for the file called popc\_herg\_lip\_dry\_30.pdb and Load this file.

You should see a square of lipid with the hERG protein embedded in the bilayer. Note that the water and K Cl present in the simulation has already been removed to make the file sizes smaller, however, two potassium ions in the hERG selectivity filter are still present. Practice moving the set of molecules around with the left and right mouse buttons. These are set to perform rotations. You can change this by clicking on the “VMD Main” Mouse menu and choosing the appropriate option (Translate or Scale) keyboard equivalents are shown on the right of the menu. If you lose the molecules from the screen use Display -> Reset View to restore the display to its original state.

**Loading the trajectory**

In the “VMD main” window, click to highlight the “Molecule” line, then go to File -> Load Data Into Molecule and Browse for the file called popc\_herg\_lip\_dry\_100\_105.xtc and click OK. This should load about 500 frames from the trajectory file.

**Q2** Based on your answer to Q1, how many ns does this represent?

Next, we will us the text interface in VMD to show the PBC box. In the “VMD Main” window, select Extensions -> Tk Console and type pbc box into this console window and you should see the box boundaries drawn in blue.

Use the controls at the bottom of the “VMD Main” window and the mouse rotate, translate and scale functions to examine this section of trajectory.

**Q3** Can you see something different in the second half of the trajectory animation compared with the first half (Hint, look at the bilayer sideways on).

**Modifying what is displayed**

VMD has powerful ways of allowing you to control what is displayed. We will examine some of the possibilities here, using the “VMD Main” Graphics -> Graphical Representations window. The default representation is Style = Lines, Color = Name, Selection = all. You can alter these values to change what is displayed. You can also create new representations to display different parts of the structure in different ways. Finally, you can double-click on each representation to toggle its display on and off. For our purposes we will leave the lipids drawn as lines, the protein as a ribbon cartoon and the two potassium ions as vdW spheres, by: Click twice on Create Rep in the “Graphical Representations” window so that you have three representations. Highlight one representation and select the Drawing Method to New Cartoon and the Coloring Method By Chain, make sure Selected Atoms contains all and hit apply (Note that Selected Atoms is case sensitive and must be all, not All or ALL). Select the next representation and fill in Selected Atoms resname POP Coloring Method Name Drawing Method Lines. And the third representation, Selected Atoms resname K Coloring Method Name Drawing Method VDW.

The potassium ions are now represented as two pink spheres and you can see how the one nearest to the bilayer centre remains bound in the channel, while that nearest the solvent dissociates into free solution during the trajectory. (cf Q3). Our next task is to monitor, graph and quantitate this behaviour.

**Monitoring a distance**

In “VMD Main”, move the slider to the beginning of the animation. Use the mouse translate, rotate and scale options to zoom in on the two potassium ions so they are easy to pick. Next choose Mouse->Label-> Bonds and then pick both K ions, giving a white dotted line and a distance drawn between them (note that the software does not require a chemical bond between atoms, it really means “distance” not “bond” in this case). Play the trajectory animation and you will see how the distance is updated each at each frame. In order to graph these data we can return to “VMD Main” and select Graphics -> Labels and Bonds in the “Labels” window’s selection box. Choose the Graph tab and the two potassium ion labels should appear on one line, select this line and the option Graph... This graph shows a series of transitions.

**Q4** What is happening around time point 130, between 130 and 250, after 250 and about 440?

**Q5** Which of these transitions is an artefact?

When you have finished with the atom and distance labels you can delete them in the Graphics->Labels window.

**A closer look at the protein**

Double click on the representation showing the lipid molecules, in the “Graphical Representations” window to turn off the display of the lipids. In “VMD Main”, move the slider to the beginning of the animation. View the protein from the “top” with the dissociating K+ nearest to you. With the cartoon coloured by chain label it is clear that each subunit is composed of two domains. The channel region where the K+ ions are bound is the selectivity filter. One strand from each subunit with a sequence TSVGFG forms the selectivity filter. This conforms to the potassium-ion-channel families consensus sequence TVGYG for this structural motif. The more open intracellular face of the protein is lined with relatively hydrophobic residues like PHE and TYR, and this is the established site of drug block.

The central channel region is surrounded by four domains which provide the channel activation behaviour in response to an electrical potential difference applied across the membrane. Hence these are known as the voltage sensing domains. Exactly how the voltages sensing is coupled to opening and closing of the hERG ion channel is not completely understood, but clues have been afforded by modelling and simulation. For example, let’s look at the distribution of charged residues in this protein. In the “Graphical Representations” window click Create Rep and set Selected Atoms to protein and resname ARG LYS GLU ASP Coloring Method to Res Type and Drawing Method to CPK This will display all the charged amino acids in the protein, with positively charged ones blue and negatively charged ones red. The displayed residues are in Ball and Stick representations (CPK is a misnomer). Next, highlight the New Cartoon representation and change the Coloring Method to ColorID and choose 8 white. Have a good look at the distribution of charged residues in the protein.

**Q6** Is there anything unexpected in the distribution of charged residues? If so, how might this relate to voltage reponse?

**A closer look at the Lipid**

To get a sense of the dynamic behaviour of the lipid molecules over this time period, it is handy just to display a few of them. The POPC molecules have residue numbers between 676 and 1811. Pick a number N between 676 and 1811Create a representation with a Selected Atom resid N Coloring Method Name and Drawing Method VDW. You can enter a list of resid numbers to add more lipids to the display. You should observe that the lipid tails are pretty dynamic but little lateral diffusion of the lipid occurs over this short period of 5 ns.

**RMSD measurements**

VMD has other trajectory analysis tools. We will have a quick look at the RMSD Trajectory Tool. The RMSD (Root Mean Squared Deviation) between two sets of atoms provides a convenient, if crude, measure of structural similarity. An RMSD of 0.0 means the structures are the same (all the atoms are in the same relative positions in space), while the larger the RMSD the more structurally different are the two sets of atoms. In “VMD main” select Extensions-> Analysis->RMSD Trajectory Tool. Within that window, check the Selection Modifiers Backbone, check the Trajectory On/Off option and enter Frame ref 1. Next check the Plot option and then hit the RMSD button. You should see a graph of RMSD vs time starting about 1 Å levelling off around 3.5 Å. Next hit the ALIGN button, this superimposes each frame on the original, and hit RMSD again.

**Q7** Can you rationalise why the RMSD plot levels out to a lower value (around 1.5 Å) this time?

**Roundup**

These exercises have given you a flavour of the information contained within large MD simulations of a complex biological system. We have used a few of the analysis tools in VMD and its excellent graphics capabilities. Many of the MD programs like AMBER, CHARMM, NAMD and GROMACS have their own analysis programs and methods for more in depth structural and temporal investigations. This is just a taster of the delights of biomolecular simulation.