CONFORMATIONAL SEARCH TUTORIAL

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UMDNJ Structural Bioinformatics I

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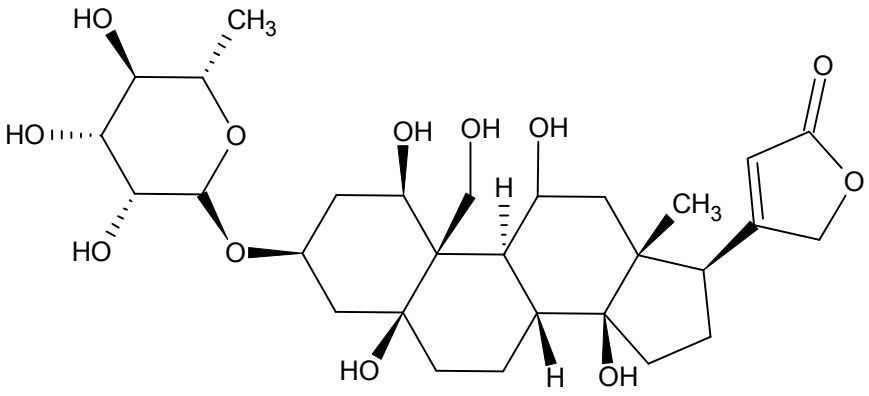
Inhibition of the Sodium Potassium (Na, K-) ATPase by cardioglycosides such as digoxin and digitoxin has been the mainstay clinical treatment for congestive heart failure and as anti-arrhythmics since the cardiovascular effects of *Digitalis purpurea* were first described in 1785. Unfortunately, digoxin, digitoxin, and other cardioglycoside drugs have narrow therapeutic indices resulting in severe toxic effects including patient death. While much effort has been directed toward the discovery of novel therapeutic agents, the lack of three-dimensional structural coordinates for the receptor has markedly limited success. Our research group has modeled the Na, K-ATPase from the high-resolution crystal structure of SERCA1a (skeletal muscle sarcoplasmic recticulum/endoplasmic recticulum Ca_2^+-ATPase) and in doing so has developed a pharmacophore, which, for the first time, will allow for the *de novo* design of drug candidates based on receptor structure.

Ouabain and digoxigenin are members of a class of glycosylated steroids collectively known as cardiac glycosides due to their therapeutic efficacy in the treatment of congestive heart failure. Ouabain achieves this effect by binding to the catalytic subunit of Na^+/K^+-ATPase and inhibiting its transport of Na^+ across the plasma membrane.



OUABAIN

In this tutorial we will explore the conformation space of ouabain using systematic conformational search technique and compare its output with one of the possible binding modes of ouabain to Na^+/K^+-ATPase.

Create directory "**conf**" and enter it.

In this tutorial, to save time we will use a molecular structure of ouabain already created. Download mol2 file (Sybyl format) from Internet

ww2.umdnj.edu/~kholodvl
ouabain.mol2

Alternatively, you can draw the structure in Sybyl or MOE. Make sure that all chiral centers are set correct.

MOE

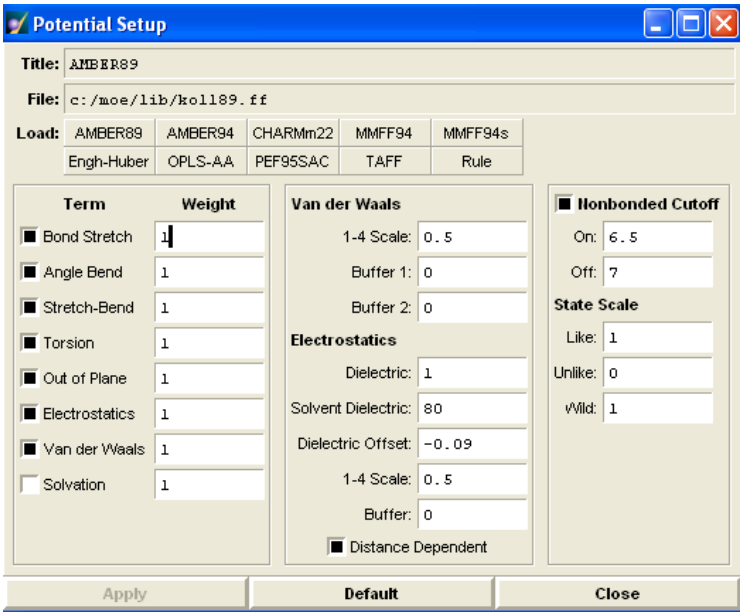
type **moe**

From **File** menu open **ouabain.mol2** file.

In main window go to Potential Control:
Window->Potential Setup

Set force field to **MMFF94**

Close **Potential Setup.**



In **Compute** menu choose

Conformations -> Systematic Search

In **Rotation Bonds** section different type of rotatable bonds and step angles are listed. Such exhaustive conformational search requires great amount of time and lies beyond the goal of this tutorial. We will focus only on four bonds shown in the **Figure 1**.
These are the bond connected a steroid and lactone parts of ouabain, two rotatable bonds between sugar component and steroid and ethoxy group of steroid.

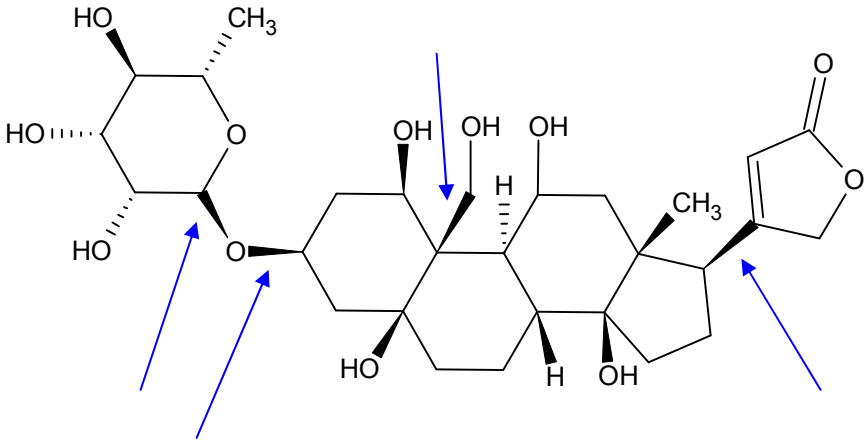
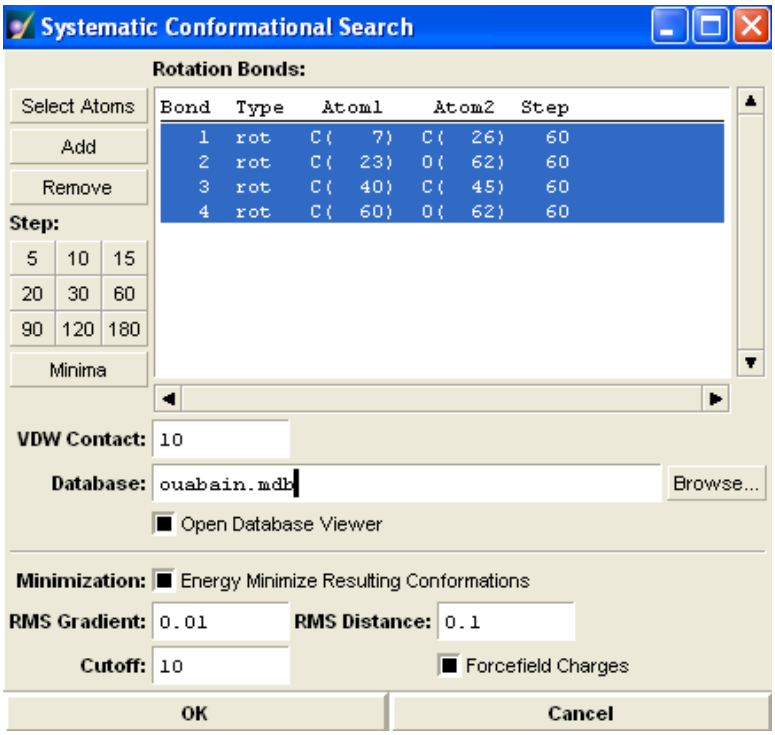


Figure 1.

In **Systematic Conformational Search** window in **Rotational Bond** section, select all entries and press **Remove** button on the left.

In the main window select atoms that form four bond described earlier. Use Shift for multiple selection.

Return to the **Systematic Conformational Search** and click on **Add**. Four entries should appear in the **Rotation Bonds** section. Select all entries and change Step to 60 (click on 60 button in Step section on the right when all entries are selected).



Change the name of the database to **ouabain.mdb**

Mark **Open Database Viewer**, **Energy Minimize Resulting Conformations** and **Force Field Charges.**

Press **OK.**

MOE starts to generate conformers and put them into the **Database Viewer.**
After all possible conformations are generated, the minimization process will start.
Notice that not all conformers are energetically favorable and after minimization, all structures with RMSD 0.1 or less will be united to one low-energy representative in Database Viewer.

It is always useful to clean your directory and keep as much free space as you can by deleting all unnecessary or bulky files. Because further we will work only with minimized structures, there is no reason to keep all generated conformers that are still hidden in the database. Before proceed to the next step save the database as **ouabain_min.mdb**. Close all open databases.

Open new terminal console.
Compare size of oubain.mdb and ouabain_min.mdb.
Delete database **ouabain.mdb**.

Return to MOE and open **ouabain_min.mdb.**

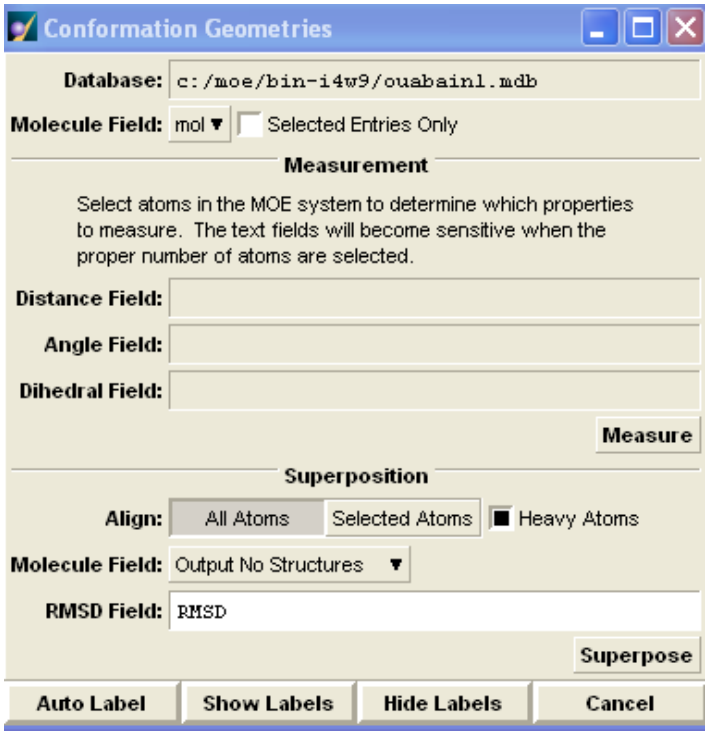
File->Open

Choose **ouabain_min.mdb** from the list and click on **Open in Database Viewer** button in the right side **Operations** menu.

Open original file **ouabain.mol2** which you downloaded from Internet. This structure is the one of binding conformation of ouabain to sodium potassium (Na, K-) ATPase.
Compare all structure from MOE search with "binding" structure based on RMS deviation of all heavy atoms.

In Database Viewer go to

Compute-> Conformation Geometry



Choose **mol** for **Molecular Field**. Make sure **Selected Entries Only** checkbox is unmarked.
In Superposition section click on All Atoms and check Heavy Atoms.
Molecular Field: Output No Structure
Name **RMSD Field** as **RMSD** and press **Superpose** button.

New field with RMSD values will be created for each entries in Database Viewer.

Sort entries by this filed.

Create plot of Energy vs. RMSD

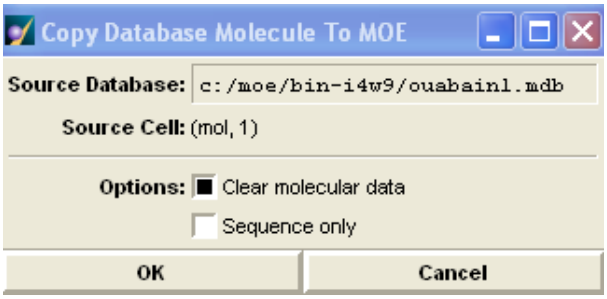
In Database Viewer
Compute-> Analysis-> Correlation plot

click on the header of RMSD field to select it as a field to plot along X –axis then click on U (energy) field to plot its values along Y-axis.

The new plot window will appear.
Analyze the plot. Find the closest structure to "binding" conformational mode. Is the binding mode of ouabain is the lowest energy conformer? How far from the global energy minimum could the ligand be to effectively bind to the target protein?

You can evaluate the rotation along which rotatable bond causes the maximum change in Potential Energy profile and plot Potential Energy versus Change in Torsion Angle. This procedure known as "Torsion Driving" could be very helpful for the first, very rough estimation of potential energy surface of the compound and the contribution of each pair of atoms to the global change of potential energy. Let us check if we achieved the global minimum along four defined previously rotatable bonds.

In the **Database Viewer** click on the first molecule to bring it to the screen.

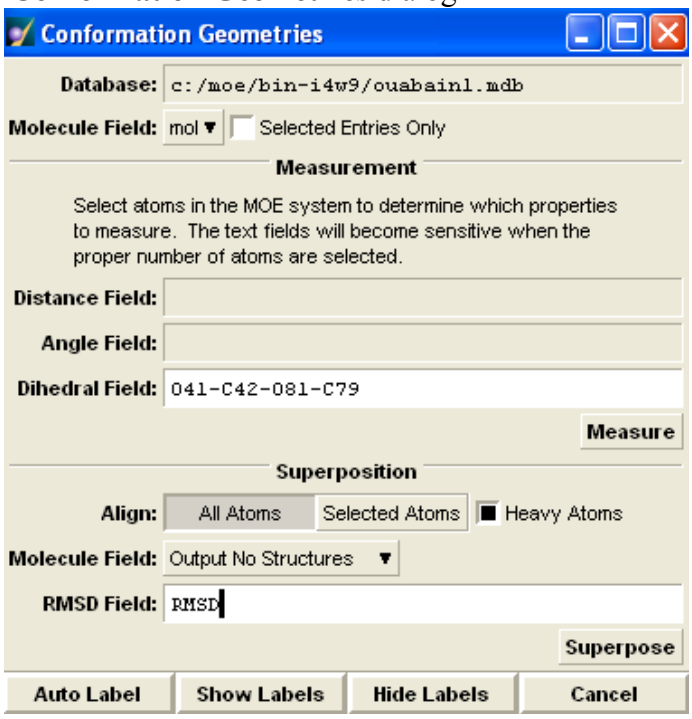


Make sure to check **Clear molecular data** box and click **OK**.

In the **Database Viewer** choose **Compute ->Conformation Geometry**

In main window choose 4 atoms that form dihedral angle along first rotatable bond **O41-C42-O81-C79** (See diagram on the previous page)

Press **Measure** button in **Conformation Geometries** dialog

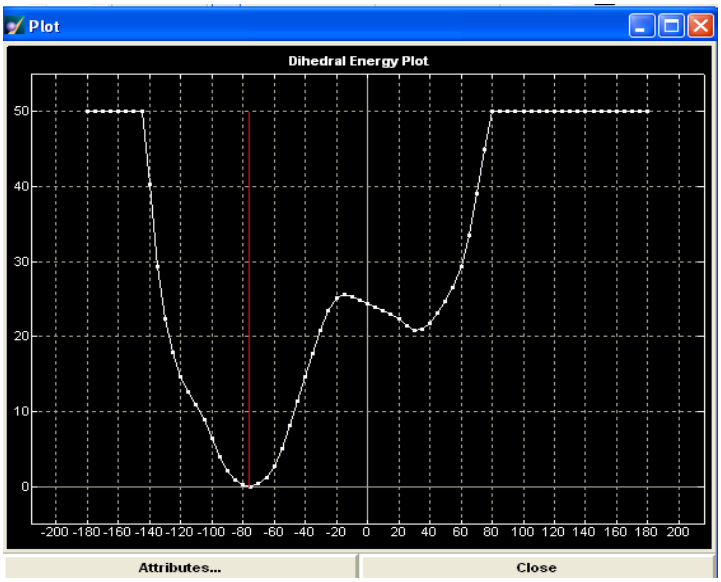


New field will be added to Database Viewer with values of dihedral angles for each entry.

Return to main window

Compute->Mechanics-> Dihedral Energy Plot

Choose the same 4 atoms that you picked for dihedral measuring O41-C42-O81-C79.
New plot window should pop up when you finish selection of 4th atom.



Click on **Attributes** and in the following window change title of plot to **O41-C42-O81-C79**
From the shape of the curve, you can estimate how steep is a well to the global minimum and how many local minima (stable conformations) molecule can adopt. Find the global minimum on the curve (approximately -56 degree). Minimize plot window.

In main window four atoms formed dihedral angle should be still highlighted. If they are not, select them again.

Click on **Builder** in the right side menu and type in the dihedral angle value in the **New Dihedral Angle (Deg)** section. Click **Apply** next to filed box.

Repeat the same procedure starting from computing the **Dihedral Energy Plot** for other dihedrals.

C42-O81-C79-C71
O48-C45-C15-C95
C16-C64-C59-C68

After you assign all dihedral angles to the structure on the screen, **MINIMIZE** structure with **MMFF94** force field.

Save it as
ouabain_min.mol2
Clean the screen by clicking on **Close** button in the right side menu.

Open just saved molecule again: **ouabain_min.mol2**

Return to the **Database Viewer**.
calculate RMSD between the “ideal” minimum structure and conformers from the database.

Compute->Conformation Geometry

Choose **mol** for **Molecular Field**. Make sure **Selected Entries Only** checkbox is unmarked.
In Superposition section, click on **All Atoms** and check **Heavy Atoms**.
Molecular Field: Output No Structure
Name **RMSD Field** as **RMSD_min** and press **Superpose** button.

New field with RMSD values will be created for each entry in **Database Viewer**.

Sort entries by this field (RMSD_min)

The first entry in the table should be the conformer with the lowest energy.
Click on the name of this entry to bring molecule onto the screen. This time uncheck box **Clear molecular data.**
Two molecules should have almost the same shape.

DO NOT FORGET TO CLEAN YOUR DIRECTORY WHEN TUTORIAL IS COMPLETED.