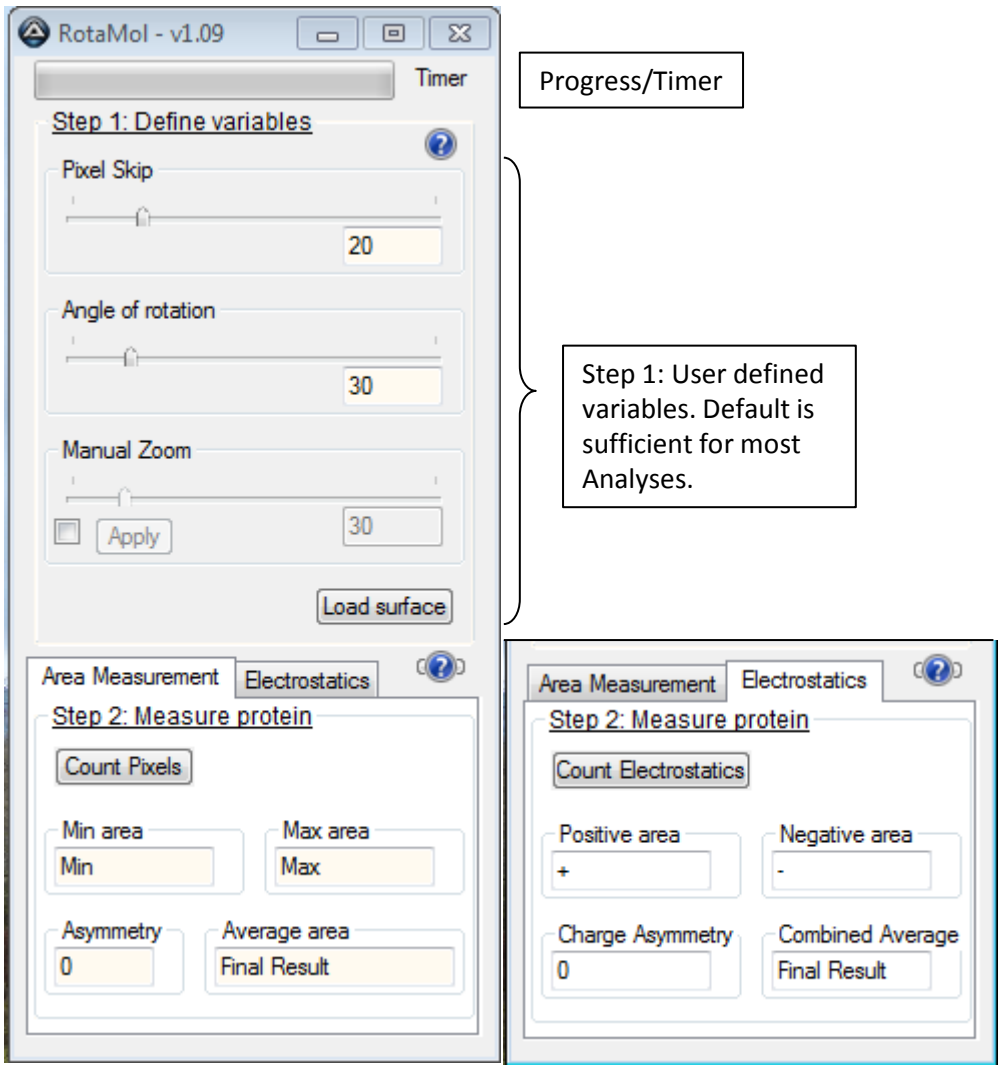
RotaMol Instructions:

Overview.



Progress/Timer

Step 1: User defined variables. Default is sufficient for most Analyses.

Min/Max observed area for given User defined variables.

Area measurement

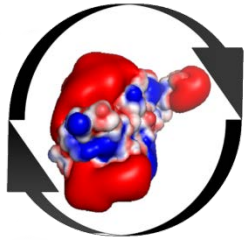
Electrostatics measurement

A Measure of Protein Asymmetry

Collision Cross section

External positive/negative area and Combined result.

Questions, comments, or suggestions email RotaMol@gmail.com



RotaMol Instructions:

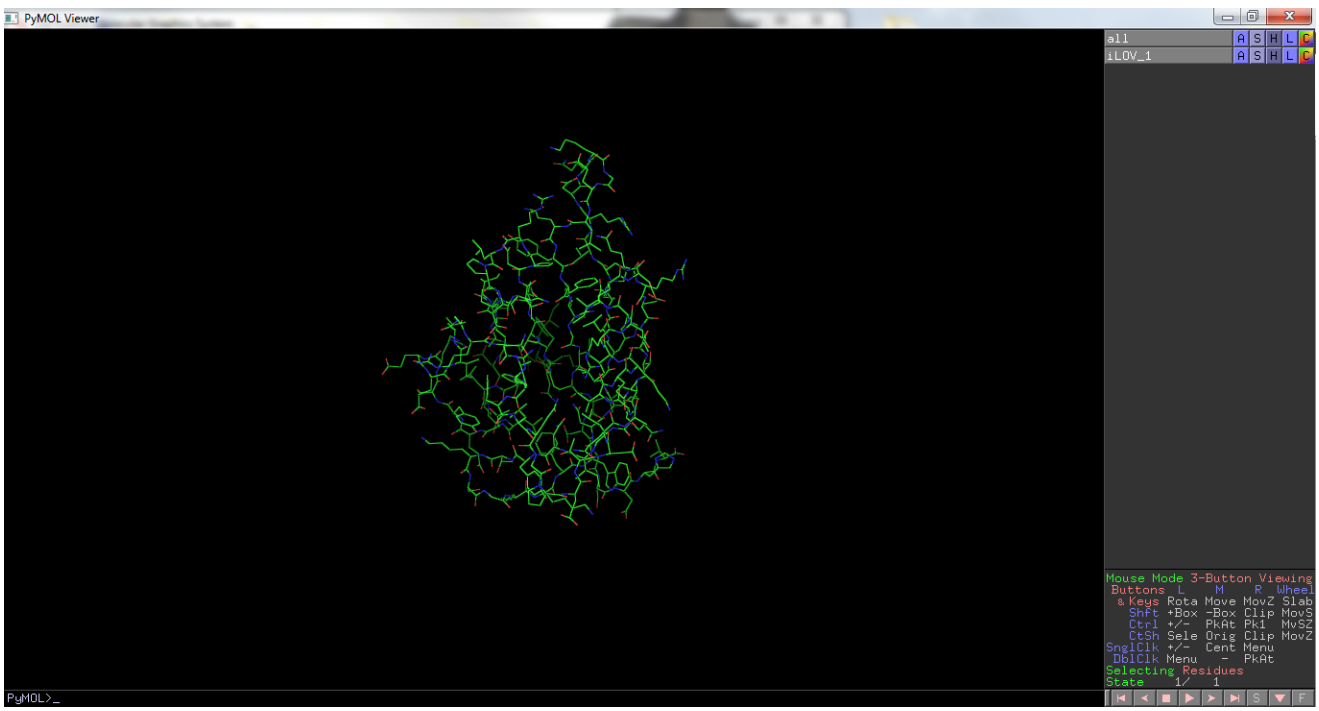
- **Open RotaMol**

- **Open PyMOL(1.3) and load PDB file**
  (ensure only one copy of PyMOL is open)

- *Step 1:*
- **Load the relevant surface.**
  use “Load surface” button in RotaMol for CSA predictions, or load electrostatics {See APBS tools plugin}

- **Make the protein fit well inside the PyMOL viewer – use Manual zoom**
  Zoom-out using the manual zoom in RotaMol is highly recommended. Rotate the protein to ensure it fits well in the window as shown below leaving plenty of room for rotations {take care with asymmetric proteins that they remain inside the viewer when rotated}: (you can do this with manual zoom in RotaMol or normally with mouse click drag in PyMOL)

- **Define your Pixelskip and angle of rotation**
  (Default is sufficient for most applications)

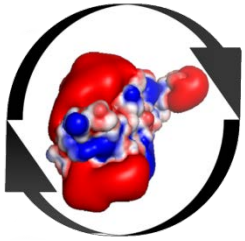


Step 2:

- **Press “Count Pixels” in RotaMol:**
  Leave it alone and don’t move anything over the PyMOL Viewer, wait until it’s finished. (depending on the computer <10 seconds at default, progress bar will tell you when it’s done

- **Results are given as average cross sectional area (CSA) and Minimum/Maximum observed areas for the given angle of rotation.**

RotaMol Instructions: Electrostatics



- **Open RotaMol**

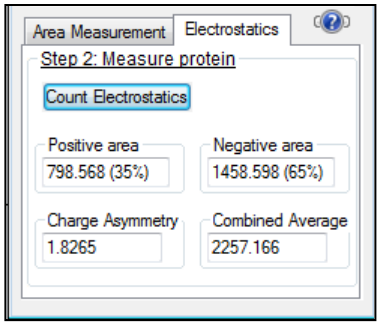
- **Open PyMOL(1.3) and load PDB file**
  (ensure only one copy of PyMOL is open)

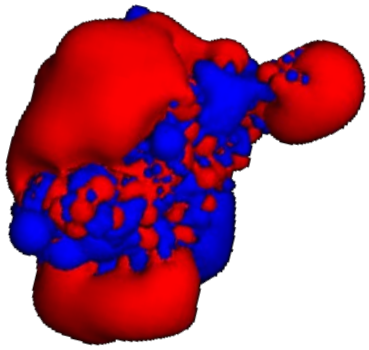
- *Step 1:*
- **Load the positive and negative Isosurfaces using ABPS tools2**
  Do NOT use "Load surface" button in RotaMol for electrostatics if you want only the percentage area of positive and negative external charges (Figure A below) {RotaMol electrostatics works by measuring colours}.
  If you want a combination of size and charge, use the "Load surface" in RotaMol. (Figure B below) and use the normal area measurement tab after loading the Isosurfaces not the electrostatics tab {electrostatic percentages won't work with the 1.4 Å solvent accessible surface}.

- **Make the protein fit well inside the PyMOL viewer – use Manual zoom**
  As normal.

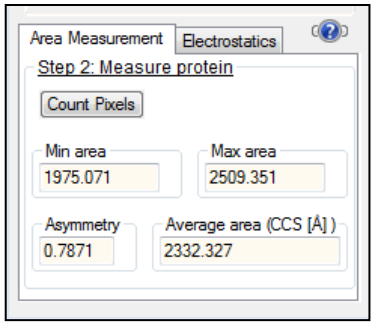
- **Define your Pixelskip and angle of rotation**
  (Default is sufficient for most applications)

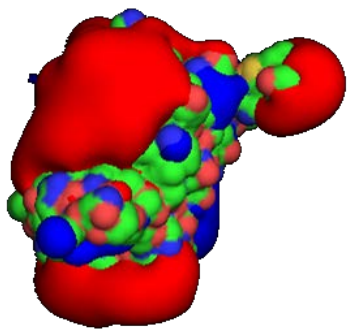
A





B





Step 2:

- **Depending on step 1 press either "count pixels" or "count electrostatics"**
  Leave it alone and don't move anything over the PyMOL Viewer, wait until it's finished. (depending on the computer < 1 minute at default, progress bar will tell you when it's done

- **Results are given as percentage coverage of Positive area and Negative area of the molecule, and the combined total area in Å^2**