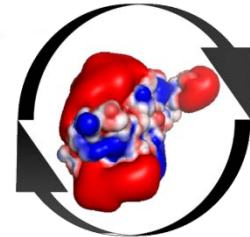


RotaMol Instructions:

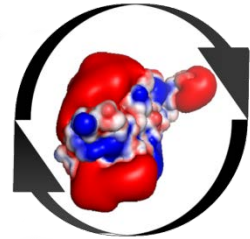


Overview.

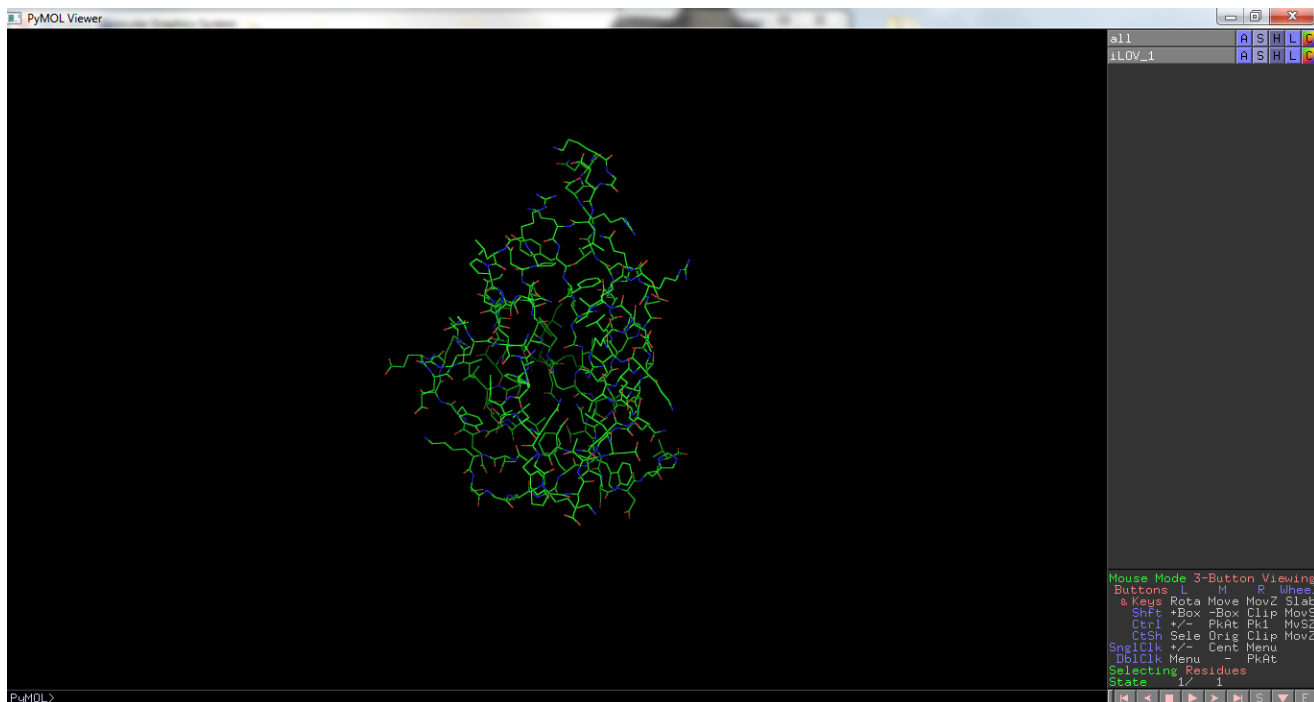
The screenshot shows the RotaMol v1.09 interface. At the top is a 'Timer' progress bar. Below it is 'Step 1: Define variables' with sliders for 'Pixel Skip' (set to 20), 'Angle of rotation' (set to 30), and 'Manual Zoom' (set to 30). A 'Load surface' button is at the bottom of Step 1. Below Step 1 are two tabs: 'Area Measurement' and 'Electrostatics'. The 'Area Measurement' tab shows 'Step 2: Measure protein' with a 'Count Pixels' button and input fields for 'Min area' (Min), 'Max area' (Max), 'Asymmetry' (0), and 'Average area' (Final Result). The 'Electrostatics' tab shows 'Step 2: Measure protein' with a 'Count Electrostatics' button and input fields for 'Positive area' (+), 'Negative area' (-), 'Charge Asymmetry' (0), and 'Combined Average' (Final Result). Brackets below the tabs group them as 'Area measurement' and 'Electrostatics measurement'. Three callout boxes provide context: 'Progress/Timer' points to the timer bar; 'Step 1: User defined variables. Default is sufficient for most Analyses.' points to the Step 1 sliders; 'Min/Max observed area for given User defined variables.' points to the 'Min' and 'Max' area input fields. Below the interface, three boxes describe the results: 'A Measure of Protein Asymmetry' (under Asymmetry), 'Collision Cross section' (under Average area), and 'External positive/negative area and Combined result.' (under Positive area, Negative area, and Combined Average).

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

RotaMol Instructions:



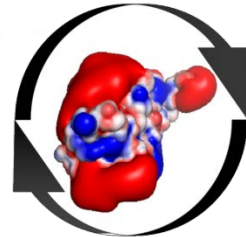
- **Open RotaMol**
- **Open PyMOL(1.3) and load PDB file**
(ensure only one copy of PyMOL is open)
- **Step 1:**
- **Load the relevant surface.**
use “Load surface” button in RotaMol for CSA predictions, or load electrostatics {See APBS tools plugin}
- **Make the protein fit well inside the PyMOL viewer – 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)



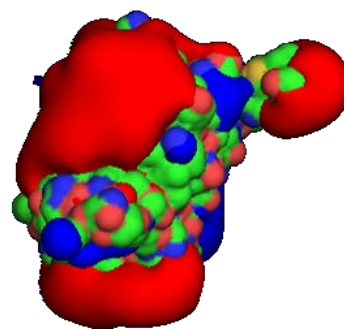
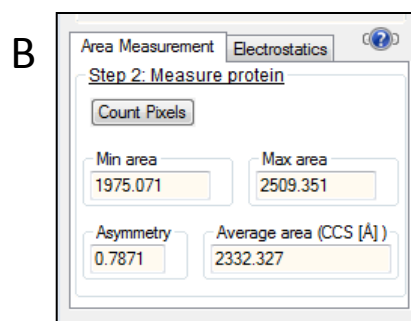
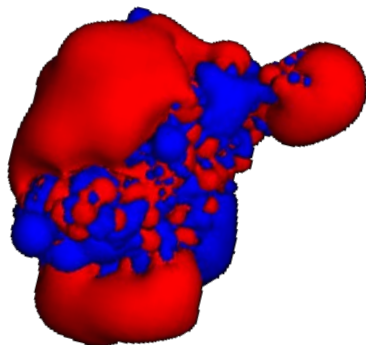
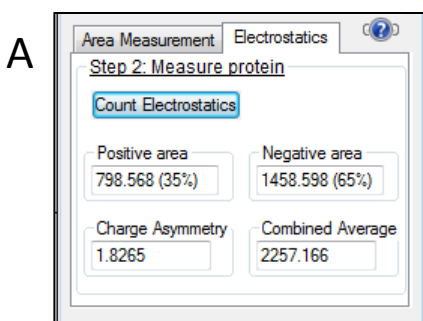
Step 2:

- **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)



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 Å²**