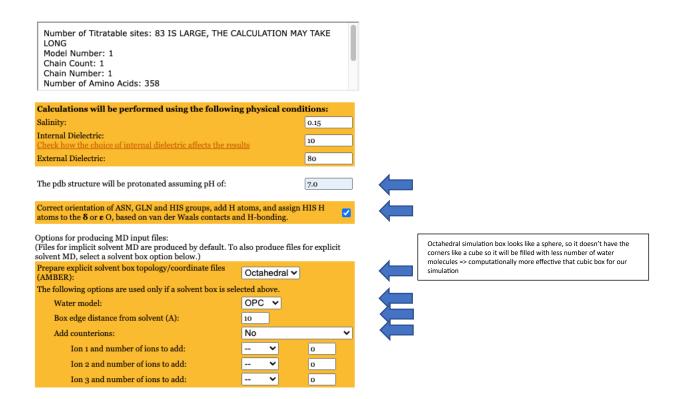
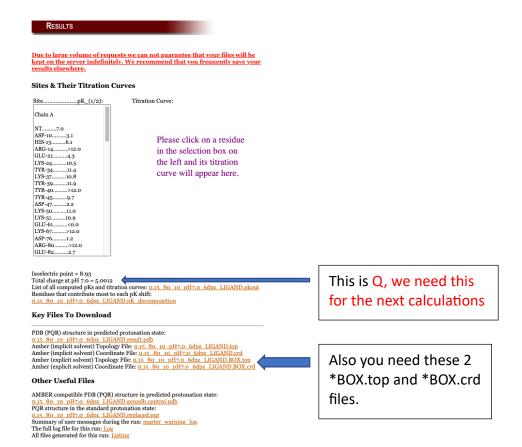
## H++ Tutorial for Tim

At first we run the complex.pdb (protein\_LIGAND.pdb) file through H++ without adding any ions. But we put this adjustment as follow:



Once it processed, we need to get 2 informations:

- 1) Total charge of the complex (Q)
- 2) Number of the water molecules inside the box



To count the number of WATER molecules inside the simulation box we need to make a .pdb file from those \*BOX.top and \*BOX.crd files using AMBER.

Here is the command that we use:

ambpdb -p <u>0.15 80 10 pH7.0 6dpz LIGAND.BOX.top</u> <<u>0.15 80 10 pH7.0 6dpz LIGAND.BOX.crd</u>> complex.pdb

```
[[fatemeh@strugatsky HHH]$ ls
0.15_80_10_pH7.0_6dpz_LIGAND.BOX.crd 0.15_80_10_pH7.0_6dpz_LIGAND.BOX.top
[[fatemeh@strugatsky HHH]$ ambpdb -p 0.15_80_10_pH7.0_6dpz_LIGAND.BOX.top <0.15_80_10_pH7.0_6dpz_LIGAND.BOX.crd> complex.pdb
[[fatemeh@strugatsky HHH]$ ls
0.15_80_10_pH7.0_6dpz_LIGAND.BOX.crd 0.15_80_10_pH7.0_6dpz_LIGAND.BOX.top complex.pdb
```

Using the generated .pdb file, you can calculate the number of water molecules. Here in this example is = 936 WAT molecules

Then we use the equations of the previously sent paper:

$$N_0 = \frac{N_w M_w c_0}{\rho_w} \sim \frac{N_w c_0}{56}$$

 $N_0 = 936*0.15/56 = 2.5$ \*\* 0.15 is the salt concentration that we usually use in explicit solvent simulations \*\*

Where Nw is the number of water molecules, c0 is the salt concentration in Molar units and 1/56 is the conversion factor.

Then:

$$N_{\pm} = N_0 \sqrt{1 + \left(\frac{Q}{2N_0}\right)^2} \mp \frac{Q}{2}$$

CI- = 2.5 
$$\sqrt{1 + (\frac{5}{2*2.5})}$$
 + 5/2 = 3.5+2.5=6  
Na+ = 3.5 - 2.5 = 1

Now we go back to the H++ for the final set up:

| Number of Titratable sites: 83 IS LARGE, THE OLONG Model Number: 1 Chain Count: 1 Chain Number: 1 Number of Amino Acids: 358                          | CALCULATION M            | IAY TAKE        |   |
|---|--------------------------|-----------------|---|
| Calculations will be performed using the following  | ing physical cor         | ditions:        |   |
| Salinity:   | 01 7                     | 0.15            |   |
| Internal Dielectric:  |                          | 10              |   |
| Check how the choice of internal dielectric affects the results   |                          |                 |   |
| External Dielectric:  |                          | 80              |   |
| The pdb structure will be protonated assuming pH of:  |                          | 7.0             |   |
| Correct orientation of ASN, GLN and HIS groups, add I atoms to the $\delta$ or $\epsilon$ O, based on van der Waals contacts a                        |                          | n HIS H         |   |
| Options for producing MD input files:<br>(Files for implicit solvent MD are produced by default. T<br>solvent MD, select a solvent box option below.) | o also produce file      | es for explicit |   |
| Prepare explicit solvent box topology/coordinate files (AMBER):   | Octahedral >             | •               |   |
| The following options are used only if a solvent box is se  | elected above.           |                 |   |
| Water model:  | OPC 🕶                    |                 |   |
| Box edge distance from solvent (A):   | 10                       |                 |   |
| Add counterions:  | Add the following ions V |                 |   |
| Ion 1 and number of ions to add:  | Na+ 🕶                    | 1               |   |
| Ion 2 and number of ions to add:  | CI- 🕶                    | 6               |   |
|   |                          |                 | 1 |

We need these 2 new files to start Molecular Dynamics Simulation:

## 0.15 80 10 pH7.0 6dpz LIGAND.BOX.top 0.15 80 10 pH7.0 6dpz LIGAND.BOX.crd

If you open .top file, you'll see the desired ions are added correctly.

-Done, Let me know if you have any questions. Fatemeh22@vt.edu (540)558-3061