

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:

Number of Titratable sites: 83 IS LARGE, THE CALCULATION MAY TAKE LONG
Model Number: 1
Chain Count: 1
Chain Number: 1
Number of Amino Acids: 358

Calculations will be performed using the following physical conditions:
Salinity: 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 H atoms, and assign HIS H atoms to the δ or ϵ O, based on van der Waals contacts and H-bonding. ☒

Options for producing MD input files:
(Files for implicit solvent MD are produced by default. To also produce files for explicit solvent MD, select a solvent box option below.)

Prepare explicit solvent box topology/coordinate files (AMBER): Octahedral ▾

The following options are used only if a solvent box is selected above.

Water model: OPC ▾

Box edge distance from solvent (Å): 10

Add counterions: No ▾

Ion 1 and number of ions to add:	-- ▾	0
Ion 2 and number of ions to add:	-- ▾	0
Ion 3 and number of ions to add:	-- ▾	0

Octahedral simulation box looks like a sphere, so it doesn't have the corners like a cube so it will be filled with less number of water molecules => computationally more effective than cubic box for our simulation

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

RESULTS

Due to large volume of requests we can not guarantee that your files will be kept on the server indefinitely. We recommend that you frequently save your results elsewhere.

Sites & Their Titration Curves

Site.....	pK _a (1/2):	Titration Curve:
Chain A		
NT.....	7.0	
ASP-10.....	3.1	
HIS-13.....	6.1	
ARG-14.....	>12.0	
GLU-21.....	4.3	
LYS-24.....	10.5	
TYR-34.....	11.4	
LYS-37.....	10.8	
TYR-39.....	11.9	
TYR-40.....	>12.0	
TYR-45.....	9.7	
ASP-47.....	2.2	
LYS-50.....	11.0	
LYS-51.....	10.9	
GLU-61.....	<0.0	
LYS-67.....	>12.0	
ASP-76.....	1.2	
ARG-80.....	>12.0	
GLU-82.....	2.7	

Please click on a residue in the selection box on the left and its titration curve will appear here.

Isoelectric point = 8.93
 Total charge at pH 7.0 = 5.0012
 List of all computed pK_as and titration curves: [0.15_80_10_pH7.0_6dpz_LIGAND.pkout](#)
 Residues that contribute most to each pK shift:
[0.15_80_10_pH7.0_6dpz_LIGAND.pK_decomposition](#)

This is Q, we need this for the next calculations

Key Files To Download

PDB (PQR) structure in predicted protonation state:
[0.15_80_10_pH7.0_6dpz_LIGAND.result.pdb](#)
 Amber (implicit solvent) Topology File: [0.15_80_10_pH7.0_6dpz_LIGAND.top](#)
 Amber (implicit solvent) Coordinate File: [0.15_80_10_pH7.0_6dpz_LIGAND.crd](#)
 Amber (explicit solvent) Topology File: [0.15_80_10_pH7.0_6dpz_LIGAND.BOX.top](#)
 Amber (explicit solvent) Coordinate File: [0.15_80_10_pH7.0_6dpz_LIGAND.BOX.crd](#)

Also you need these 2 *BOX.top and *BOX.crd files.

Other Useful Files

AMBER compatible PDB (PQR) structure in predicted protonation state:
[0.15_80_10_pH7.0_6dpz_LIGAND.genpdb.cpntr.pdb](#)
 PQR structure in the standard protonation state:
[0.15_80_10_pH7.0_6dpz_LIGAND.replaced.pqr](#)
 Summary of user messages during the run: [master_warning_log](#)
 The full log file for this run: [Log](#)
 All files generated for this run: [Listing](#)

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 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
[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 N_w is the number of water molecules, c_0 is the salt concentration in Molar units and $1/56$ is the conversion factor.

Then:

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

$$\begin{aligned} \text{Cl}^- &= 2.5 \sqrt{1 + \left(\frac{5}{2 \times 2.5} \right)^2} + 5/2 = 3.5 + 2.5 = 6 \\ \text{Na}^+ &= 3.5 - 2.5 = 1 \end{aligned}$$

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

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 Add counterions:

Ion 1 and number of ions to add:

Ion 2 and number of ions to add:

Ion 3 and number of ions to add:

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

o.15 80 10 pH7.0 6dpz LIGAND.BOX.top
o.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.

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