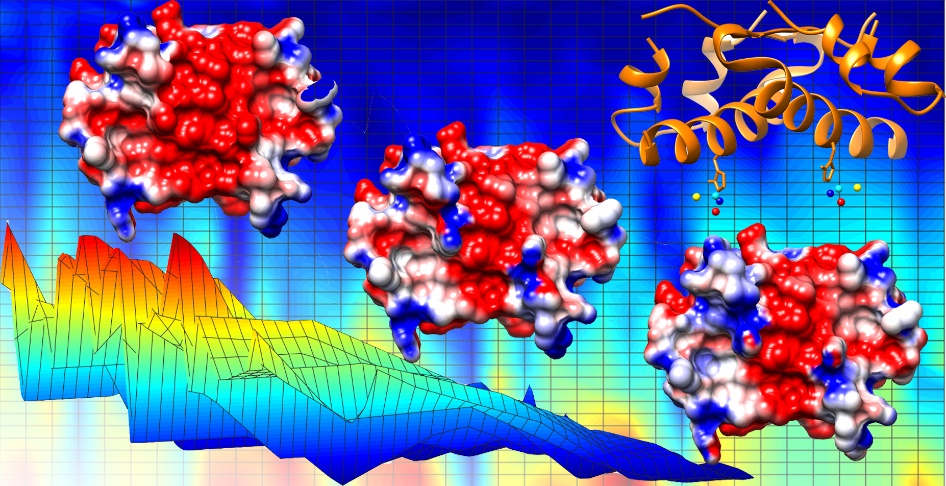
**HIT-2 Manual**



The HIT-2 is a tool to calculate the position of bound ions for highly charged biomolecule system. The result can be output in PDB format. In download/bin folder, there are several files:

HIT2\_Linux.o

HIT2\_MAC.o

HIT2cpp\_Linux.o (Recommended).

The simple usage of HIT-2 is:

1. Open vmd, load your simulations, go [Extentions] -> [RMSD Trajectory Tool in VMD] / [RMSD Visualizer Tool in VMD] -> [ALIGN] and save your protein and ions position -> [myprecious.pdb]. (If there is no significant rotation on your protein, alignment is not necessary. Just save the positions of biomolecules and ions from all frames in a file.)
2. Run HIT-2: ~$ ./HIT2\_Linux.o [myprecious.pdb] [target\_frame\_index]” (In MAC please use HIT2\_MAC.o)

The advanced usage of HIT-2 is:

1. Open vmd, load your simulations, go [Extentions] -> [RMSD Trajectory Tool in VMD ] / [RMSD Visualizer Tool in VMD] -> [ALIGN] and save your protein and ions position -> [myprecious.pdb]. (If there is no significant rotation on your protein, alignment is not necessary. Just save the positions of biomolecules and ions from all frames in a file.)
2. Run HIT-2: ~$ ./HIT2\_Linux.o [myprecious.pdb] [target\_frame\_index] [threshold of filling ratio] [step size]” (threshold of filling ratio should be bigger than 0.5 but smaller than 1; Step size should be smaller than 2 to generate enough iterations to produce accurate results)
3. For compiling HIT-2, remember to link math lab. Which is gcc HIT2.c -o HIT2.o -lm
4. If the ions name is special, not in our dataset, you can add your ions the names in two headers files. HIT2\_pre.h and HIT2\_alg.h. like the following.

char cation\_string[]="POT SOD COP ZIN CAL Na+ CA \0";

char anion\_string[]="CLA Cl-\0";

char IONS[]=" POT SOD CLA ZIN COP CAL Na+ Cl- CA ";

If you are using HIT2cpp version, please add your ions name into the amino\_nucleic\_acid.h and compile the HIT2.cpp

Output:

by HIT2\_Linux/MAC.o

1. HIT2\_biomolecules.pdb
2. HIT2\_boundions.
3. HIT2\_complex.pdb

by HIT2cpp\_Linux.o

1. HIT2\_(filename)\_biomolecules.pdb

2. several files: HIT2\_bound\_(ion\_name).pdb.

3. HIT2\_(filename)\_complex.pdb

The HIT2\_biomolecules.pdb is the protein/DNA/RNA structure in certain frame you selected (target\_frame\_index).

The HIT2\_boundions.pdb is the relative positions of bound ions, which represents the bound ions when the mass center of biomolecules are in (0.00, 0.00, 0.00).

The HIT2\_complex.pdb is the target biomolecules with bound ions. It is the one for further analysis.

**Tutorial on an example**

Here is an example of using HIT2 on a kinesin-tubulin complex on Linux system.

Once HIT.zip is downloaded, the example can be found in the Ex\_download/example/ folder.

This example is the kinesin-tubulin complex. To run the example, go to the example folder and run the following commands.

Open terminal and run the following command:

~$ ./HIT2\_Linux.o 1zeh10ns\_DO\_NOT\_OPEN\_IN\_CHIMERA.pdb 1000

After the run, you will get such files:

1. HIT2\_biomolecules.pdb is the 1ZEH structure in certain frame you selected (target\_frame\_index).
2. The HIT2\_boundions.pdb is the relative positions of bound Zn2+ and Cl- when the mass center of biomolecules1ZEH are in (0.00, 0.00, 0.00).
3. The HIT2\_complex.pdb is the final target biomolecules with bound ions (Figure 1).

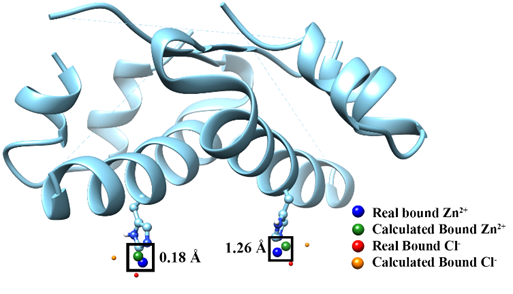


Figure 1. The results from bound Zn2+ testing from the protein (PDB: 1ZEH) simulations.

Reference: