

WATsite3.0 User Guide

A GPU-accelerated Hydration Site Prediction Program with PyMOL Plugin

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WATsite is a hydration site analysis program developed together with an easy-to-use graphical user interface (GUI) based on PyMOL. WATsite identifies hydration sites from a molecular dynamics simulation trajectory with explicit water molecules. The thermodynamic profile of each hydration site is estimated by computing the enthalpy and entropy of the water molecule occupying a hydration site throughout the simulation.

The latest version of WATsite requires an NVIDIA GPU workstation which utilizes the OpenMM [2] toolkit for GPU-accelerated molecular dynamics simulation. The current version is distributed in the form of a Docker image via Docker Hub. Only Linux OS (Redhat / Ubuntu) is supported.

WATsite2.0, based on Gromacs-4.6.5 simulations is available in case a

GPU workstation is not available. WATsite2.0 is available for download at <http://people.pharmacy.purdue.edu/~mlill/software/watsite/>.

When using WATsite please cite the following references:

1. Hu, B.; Lill, M. A., Watsite: Hydration Site Prediction Program with Pymol Interface. *J Comput Chem* 2014, 35, 1255-60.
 2. Yang, Y.; Hu, B.; Lill, M.A., WATsite2.0 with PyMOL Plugin: Hydration site prediction and visualization. *Protein Function Prediction: Methods and Protocols*, 2017.
 3. Yang, Y.; Abdallah, AHA.; Lill, M.A., Calculation of Thermodynamic Properties of Bound Water Molecules. *Computational Drug Discovery and Design*, 2018.
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I Changes and updates that have been made to WATsite3.0

- GPU-accelerated water energy calculation is implemented in OpenMM-WATsite based on OpenMM package [2].
- Option to place water for occluded binding site with 3D-RISM [1] and GAsol[3].
- Option to truncate the protein system to further speed-up the MD simulation.
- Option to compute desolvation free energy for discretized grid similar to GIST[4].
- Available force fields: Amber99SB, Amber99SBildn, Amber14SB.
- Available water models: SPC/E, TIP3P, TIP4P, TIP4PEW, OPC.

2 Installation of Docker and download the WAT-site image

In order to replace the tedious installation of multiple softwares, a pre-built docker image has been created by the authors.

Please check the detailed instructions at the reference website to install [docker](#) and [nvidia-docker](#). For the convenience, here, we only provide a list of commands for installation of docker-ce and nvidia-docker2 under ubuntu.

2.1 Install docker and nvidia-docker

1. Install docker-ce (Community Edition)

```
# Add the package repositories to install Docker
curl -fsSL https://download.docker.com/linux/ubuntu/gpg | sudo apt-key add -
sudo add-apt-repository "deb [arch=amd64] \
    https://download.docker.com/linux/ubuntu $(lsb_release -cs) stable"
sudo apt-get update

# Install Docker
sudo apt-get install -y docker-ce
```

2. Install nvidia-docker2

Make sure the NVIDIA driver (≥ 384.130) is installed.

```
# Add the package repositories
curl -s -L https://nvidia.github.io/nvidia-docker/gpgkey | sudo apt-key add -
curl -s -L https://nvidia.github.io/nvidia-docker/ubuntu16.04/amd64/nvidia-
docker.list | sudo tee /etc/apt/sources.list.d/nvidia-docker.list
sudo apt-get update

# Install nvidia-docker2 and reload the Docker daemon configuration
sudo apt-get install -y nvidia-docker2
sudo pkill -SIGHUP dockerd
```

2 *INSTALLATION OF DOCKER AND DOWNLOAD THE WATSITE IMAGE*⁶

3. Executing the docker command without sudo

By default, running the docker command requires root privileges. It can also be run by a user in the docker group, which is automatically created during the installation of Docker. To add the user to the docker group, you need to login as root and run the following command:

```
sudo usermod -aG docker <username>
```

2.2 Create a local watsite image from the Docker Hub

Upon successful installation of docker-ce and nvidia-docker, user can pull our ready-to-run docker image from the docker hub.

```
docker pull lilllab/watsite3
```

Currently, there are two ways the user can use the docker image for hydration site analysis with WATsite:

- a)** via PyMOL interface (go to section [3](#))
- b)** via command line (section [4](#))

The author recommends the easy-to-use PyMOL plugin. In case PyMOL won't start properly due to display driver issue or graphics engine conflict, WATsite can still be run via command line.

3 Running WATsite Analysis with PyMOL Plugin

- (1) In order to show the display within the docker container, user need to add user to the list which allows connections to the X server with the xhost command:

```
xhost +si:localuser:root
```

- (2) Next, run the watsite container with command:

```
docker run --runtime=nvidia -it --rm \
-e DISPLAY -v /tmp/.X11-unix:/tmp/.X11-unix \
-v /scratch/watsite:/opt/data \
lilllab/watsite3 bash
```

Users need to change /scratch6t/yang570 to the directory they want to access and work inside the container.

- (3) Finally, make sure PyMOL can be started properly:

```
pymol
```

Once PyMOL is started, the WATsite menu should be observed as shown in Figure 1. Following the workflow, the user will perform the preparation, simulation, and analysis with the PyMOL plugin.

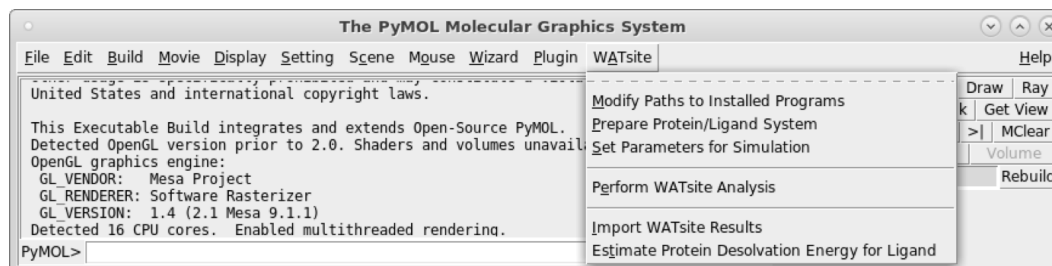


Figure 1: WATsite PyMOL plugin menu.

[Optional] Modify settings based on installation

Using the docker image, this step can be omitted since the paths have been set according to the authors' installation.

The location paths to the required molecular modeling programs need to be correctly specified. The settings only need to be modified for the first time, and will be automatically read in subsequent sessions.

1. Select the menu item “Modify Paths to Installed Programs” from the WATsite menu (Figure 1).
2. Specify or modify the paths to the programs according to their installation (Figure 2).
3. The location of WATsite directory should be defined for watsite_home. Similarly the python path associated with OpenMM, the path to AmberTools, PyMOL, and Reduce installation need to be specified.

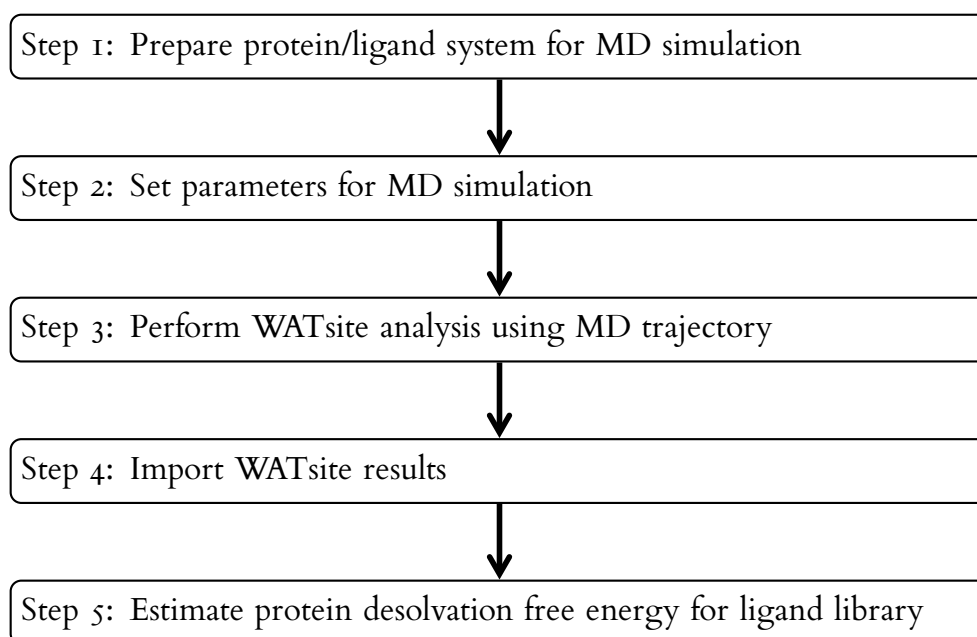
The screenshot shows a window titled "PyMOL WATsite Plugin". Below the title bar, there is a black header area with white text: "PyMOL WATsite Plugin: Hydration Site Prediction", "Ying Yang, Amr Abdallah, Bingjie Hu, Markus A. Lill", and "http://people.pharmacy.purdue.edu/~mlll/software/watsite/". Below this is a section titled "Modify Paths to Installed Programs" in blue. It contains five rows of input fields with labels and "Browse" buttons:

Label	Path	Action
python_dir (path to python associated with OpenMM installation):	/programs/miniconda/bin/	Browse
amber_home (main amber directory = \$AMBERHOME):	/programs/miniconda/	Browse
pymol_dir (directory containing pymol executable):	/usr/bin	Browse
watsite_dir (WATsite directory):	/programs/WATsite	Browse
reduce_dir (directory containing reduce executable):	/programs/WATsite/bin	Browse

At the bottom of the window are three buttons: "Exit", "Save", and "Reset to defaults".

Figure 2: specify the correct paths to the installed program

Work-flow of hydration site analysis with WAT-site PyMOL plugin



3.1 Step 1: Prepare protein/ligand system. Figure 3

PyMOL WATsite Plugin

PyMOL WATsite Plugin: Hydration Site Prediction
Ying Yang, Amr Abdallah, Bingjie Hu, Markus A. Lill
<http://people.pharmacy.purdue.edu/~mlll/software/watsite/>

System Preparation

Base directory: /opt/data/1hpx Browse

Project subdirectory: 1hpx_water

☐ Protein from file [PDB] Full path to protein file Search and Import

☒ Protein in current PyMol session ☒ 1hpx

☒ Use Reduce to optimize ASN, GLN, HIS conformations/protonation states

☐ Rename residues to be AmberFF compatible based on hydrogens in PDB

☐ Directly use AmberFF compatible residue names in PDB

Define binding site [PDB]: /opt/data/1hpx/lig.pdb Search and Import margin (Å): 3.0

☒ Initial water placement with 3D-RISM and GASol

☐ Truncate the protein from the center of the binding site by cutoff (Å): 20

☒ Prediction without ligand

☐ Prediction with ligand file [MOL2] Full path to ligand file Search and Import ligand charge: 0

☐ Predict AM1-BCC charge using Antechamber

☐ Take user's charge in mol2 file

Force Field: amber14SB

Water Model: SPC/E

PBC Box Minimal Distance (Å): 10.0

Prepare System Cancel

Figure 3: Prepare the protein or protein-ligand system.

- (Required) The user can specify a protein structure from a file or a structure already displayed in the current PyMOL session (Figure 3).
- (Required) Protonation states of ionizable residues in the protein binding site site may significantly affect the thermodynamic profiles of water molecules. Therefore, we provide three options for the user to choose from:
- use the Reduce^[7] program to adjust the protonation state for HIS based on the local environment, and ASN and GLN side chains may be flipped to optimize the hydrogen-bond network.

- b) user can provide protein PDB files with hydrogen added and protonation states predicted, for example by Propka[5].
- c) user can also provide protein PDB file with amber force field compatible residue names upon visual investigation.

(Required) To define the protein binding site, the user needs to provide a ligand molecule positioned within the binding site or a “pseudo-ligand” using binding site residues. A margin (in Å) will be used to define the binding pocket specifying a box surrounding the ligand/pseudo-ligand. The minimum distance between any ligand heavy atom to the edge of the box equals to the margin value.

(Optional) Fast convergence is not feasible for protein systems with occluded binding sites, especially when crystal water locations are not available. The authors recently added a new option for initial water placement with 3D-RISM [1] and GASol[3], which proved to be efficient and accurate in combination with WATsite refinement. The 3D-RISM prediction uses a solution of SPC water, Na⁺, and Cl⁻ ions with concentrations of 55.5M, 0.005M, and 0.005M respectively. Using the solvent distribution generated from 3D-RISM, GASol was used to identify the water network that optimally fits this distribution. A spatial filter was applied to GASol so only water molecules within a 10 Å radius of the binding site center were calculated.

User need to check the box if initial water placement is preferred. This option is recommended for protein systems with occluded binding site and/or no crystal water positions are available.

(Optional) For further speed-up of the MD simulation protocol, an option has been added to truncate the protein from the center of the binding site. This truncation is motivated by the fact that the protein is restraint in all WATsite simulations to achieve convergence in water occupancy and free-energy profiling. Capping acetyl (ACE) and amide (NME) groups will be added to the break points in the protein sequence. A minimum truncation radius of 15 Å is recommended.

(Optional) Hydration sites can be predicted for both ligand-free (apo) and ligand-bound (holo) protein structures. If the user intends to predict hydration sites at the interface between the protein and the ligand, the file location of the bound ligand is specified within the PyMOL plugin. The user also needs to

specify the net charge of the ligand, and choose the partial charge method. The atom types for the specified ligand will be assigned by antechamber[6] and will be included in the MD simulation and the following hydration site identification process. The current version does not have docking service for the user-specified ligand. Therefore, the provided ligand conformation needs to be a meaningful binding pose for the protein. In addition, the current version does not support initial water placement without ligand-bound protein structure.

(Required) The force field and water model for the system preparation need to be chosen. Currently, three choices of different amber force fields and five water models have been tested.

force fields:	amber99SB, amber99SBildn, amber14SB
water models:	SPC/E, TIP ₃ P, TIP ₄ P, TIP ₄ pEW, OPC

(Required) The system will be solvated in an orthorhombic water box. The user can also control the box size by specifying the minimum distance between any protein atom to the edge of the box. Lastly chloride and sodium ions will be added to neutralize the system.

The prepared protein system will then be loaded into PyMOL (Figure 4).

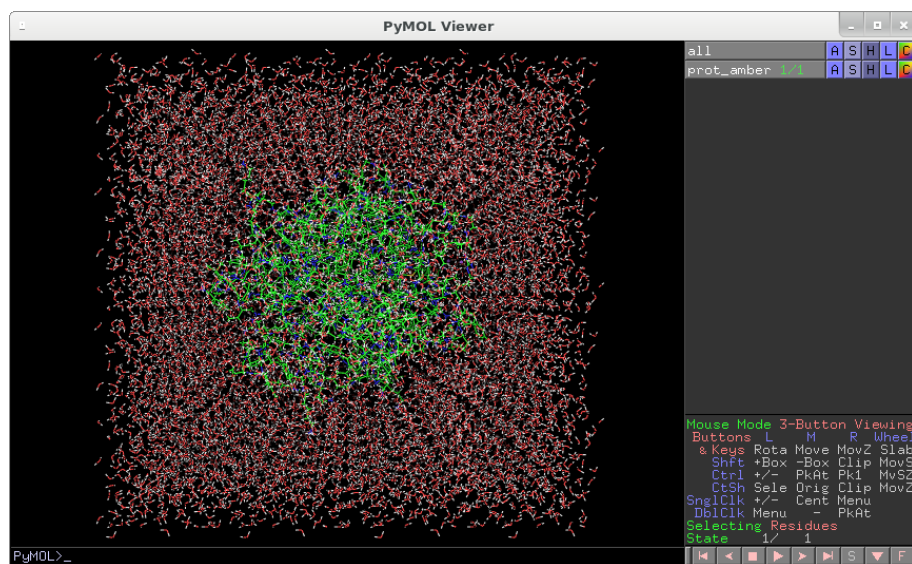
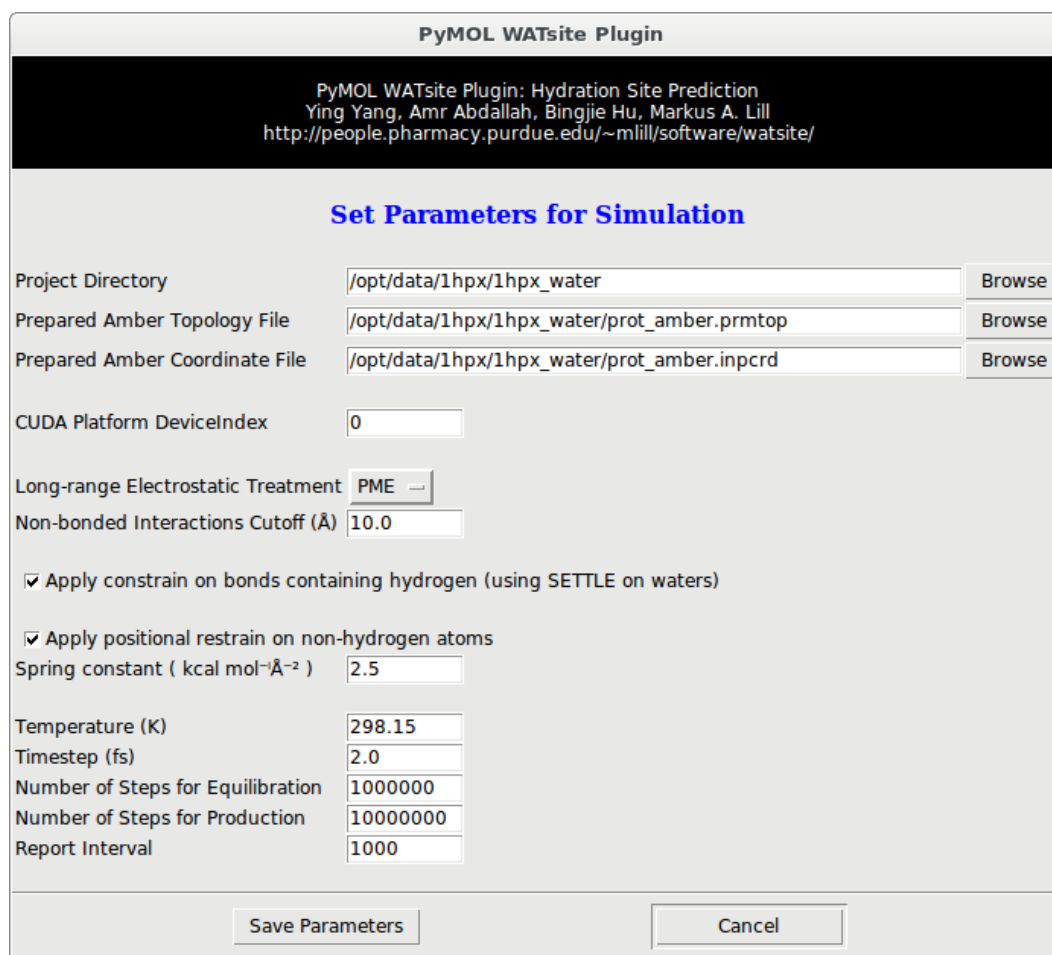


Figure 4: System preparation done and ready to run MD simulation.

3.2 Step 2: Set parameters for MD simulation. Figure 5



The image shows a dialog box titled "PyMOL WATsite Plugin". Below the title bar, there is a black header area with white text: "PyMOL WATsite Plugin: Hydration Site Prediction", "Ying Yang, Amr Abdallah, Bingjie Hu, Markus A. Lill", and a URL "http://people.pharmacy.purdue.edu/~mlill/software/watsite/". The main area of the dialog is titled "Set Parameters for Simulation" in blue. It contains several input fields and checkboxes. The "Project Directory" field is set to "/opt/data/1hpx/1hpx_water" with a "Browse" button. The "Prepared Amber Topology File" field is set to "/opt/data/1hpx/1hpx_water/prot_amber.prmtop" with a "Browse" button. The "Prepared Amber Coordinate File" field is set to "/opt/data/1hpx/1hpx_water/prot_amber.inpcrd" with a "Browse" button. The "CUDA Platform DeviceIndex" field is set to "0". The "Long-range Electrostatic Treatment" dropdown is set to "PME". The "Non-bonded Interactions Cutoff (Å)" field is set to "10.0". There are two checked checkboxes: "Apply constrain on bonds containing hydrogen (using SETTLE on waters)" and "Apply positional restrain on non-hydrogen atoms". Below the second checkbox, the "Spring constant (kcal mol⁻¹Å⁻²)" field is set to "2.5". The "Temperature (K)" field is set to "298.15". The "Timestep (fs)" field is set to "2.0". The "Number of Steps for Equilibration" field is set to "1000000". The "Number of Steps for Production" field is set to "10000000". The "Report Interval" field is set to "1000". At the bottom, there are two buttons: "Save Parameters" and "Cancel".

Figure 5: Set parameters for running the MD simulation via OpenMM.

In this step, users can change parameters for the MD simulation which subsequently will be used for hydration site analysis.

1. The default amber topology (prot_amber.prmtop) and coordinate (prot_amber.inpcrd) files are generated from the system preparation step. The users may also choose the amber files from their own preparation. The latter may be necessary for systems containing non-standard residues or membrane environment. For systems with restraints and truncation, membrane environ-

ment may only be necessary if the binding site is close to the membrane environment.

2. The CUDA device index needs to be entered based on the user's workstation.
3. Atom pairs within the user-specified cutoff will be used to calculate the direct non-bonded interactions. Long-range electrostatic interactions from atom pairs beyond the cutoff can be treated using one of the three methods (PME, Ewald, or NoCutoff). If NoCutoff is chosen, the cutoff distance will be ignored.
4. By default, we apply constraints on the length of all bonds involving a hydrogen atom, and make water molecules rigid. This will allow us to run simulations with an integration timestep of 2 fs. However, the user can disable the constrain and rigid water by uncheck the box, and change to a smaller timestep accordingly.
5. User can also change the strength of positional restraint on the non-hydrogen atoms during the simulation.
6. Temperature and number of steps for the equilibration and production can also be modified. For the choice of equilibration and production simulation length, our previous studies[8] showed that at least a 4 ns production simulation is required to obtain reliable prediction of the locations and thermodynamic properties of hydration sites.
7. Once the preparation has been finished, the user needs to change into the project directory within a terminal and start the OpenMM simulation:

```
nohup ./run_omm_watsite.sh &
```

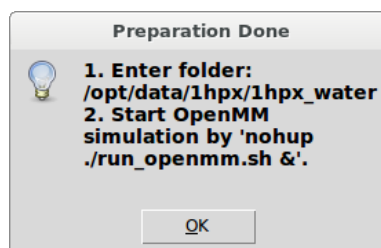


Figure 6: Message appear after run script has been successfully generated.

3.3 Step 3: Perform WATsite analysis with MD trajectories and water energy files. Figure 7

The image shows a PyMOL WATsite Plugin dialog box. At the top, it says "PyMOL WATsite Plugin" and "PyMOL WATsite Plugin: Hydration Site Prediction" by Ying Yang, Amr Abdallah, Bingjie Hu, and Markus A. Lill, with a URL. The main title is "Perform WATsite Analysis". It contains several input fields: "Prepared Amber Topology File" with a text box containing "/opt/data/1hpx/1hpx_water/prot_amber.prmtop" and a "Browse" button; "Prepared Amber Coordinate File" with a text box containing "/opt/data/1hpx/1hpx_water/prot_amber.inpcrd" and a "Browse" button; "Simulation Trajectory File" with a text box containing "/opt/data/1hpx/1hpx_water/sys_md_?.nc" and a "Browse" button; "Number of Steps for Production" with a text box containing "10000000"; "Report Interval" with a text box containing "1000"; "Water Model" with a dropdown menu showing "SPC/E"; and "Clustering Method" with a dropdown menu showing "QT". At the bottom, there are two buttons: "Perform Analysis" and "Cancel".

Figure 7: Perform WATsite analysis.

1. Upon the completion of MD simulations, we will perform WATsite analysis on the trajectory files and water energy files generated in the previous step (Figure 7). The amber topology (prot_amber.prmtop) and coordinate (prot_amber.inpcrd) files generated in 3.1, as well as the trajectory (sys_md.nc) file generated in 3.2 should be specified correctly.
2. The number of steps and water model used for WATsite analysis need to be identical to those used during the production simulation. Clustering algorithm used to predict hydration site locations from water density can also be chosen.
3. During the WATsite analysis step, the production trajectory will first be aligned to the reference which is the user input protein structure, and saved into pdb format. Then, WATsite analysis will be performed for predicting

hydration site location based on water density analysis, and for calculating entropy and enthalpy.

3.4 Step 4: Import WATsite results. Figure 8

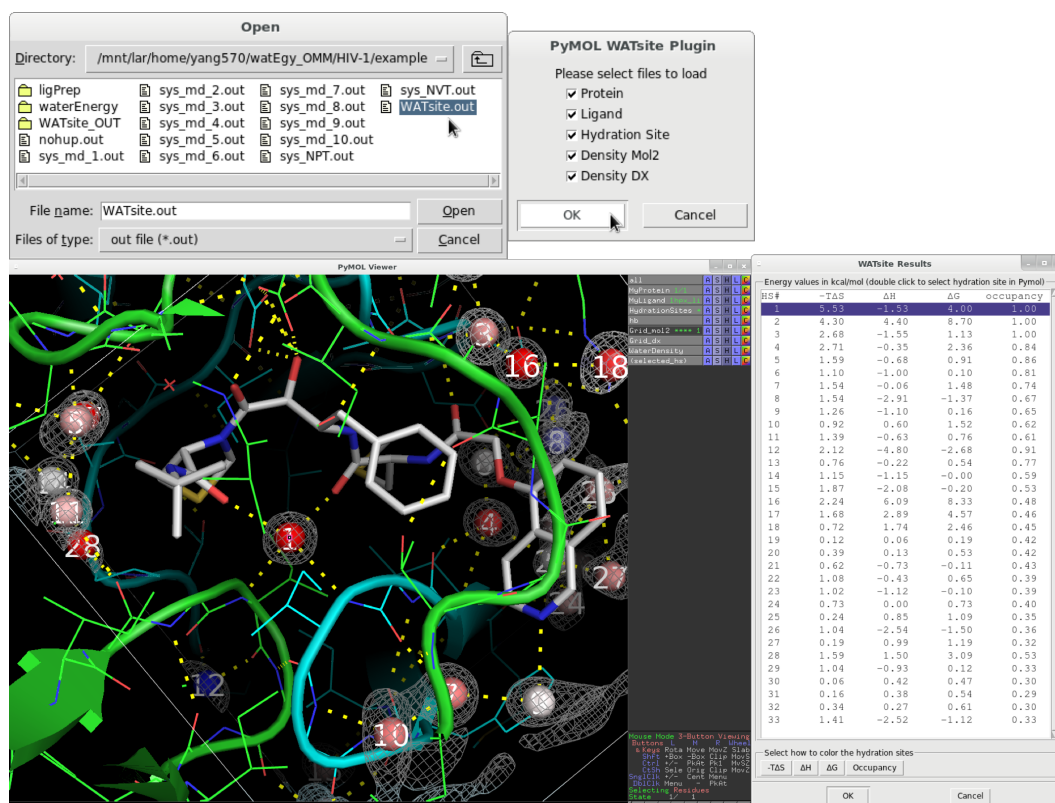


Figure 8: Perform WATsite analysis.

- After completion of WATsite analysis, we can import the results through the “Import WATsite Results” command under the WATsite menu, and select the “WATsite.out” file which stores the directory to the location of the prediction results.
- Here, we want to investigate water molecules at the binding interface between protein and ligand, so we select ‘Protein’, ‘Ligand’, and ‘Hydration Site’ to load into PyMOL.

- The result of the example case of HIV-1 protease are shown in Figure 8.
- The PyMOL viewer window shows the predicted hydration sites in the protein binding site. The hydration sites are shown as spheres and colored in this example based on their ΔG values in a blue-white-red spectrum where blue indicates relatively low ΔG values and red indicates relatively high ΔG values.
- A hydration site with a more positive ΔG value (darker red) indicates an unfavorable environment of the water molecule in the binding site. Therefore, a gain in free energy of binding can be expected if the water in that hydration site is replaced by a ligand.
- The “occupancy” values indicate the probability a water molecule is observed in the given hydration site during the MD simulation.
- The “WATsite results” window listing the estimated desolvation free energy (ΔG), enthalpy (ΔH), entropy ($-T\Delta S$), and occupancy for each hydration site. The user can also choose according to which descriptor the hydration sites are colored by clicking the corresponding ΔG , $-T\Delta S$, and ΔH , or “Occupancy” button.

3.5 Step 5: Estimate protein desolvation free energy for lig- and library. Figure 9

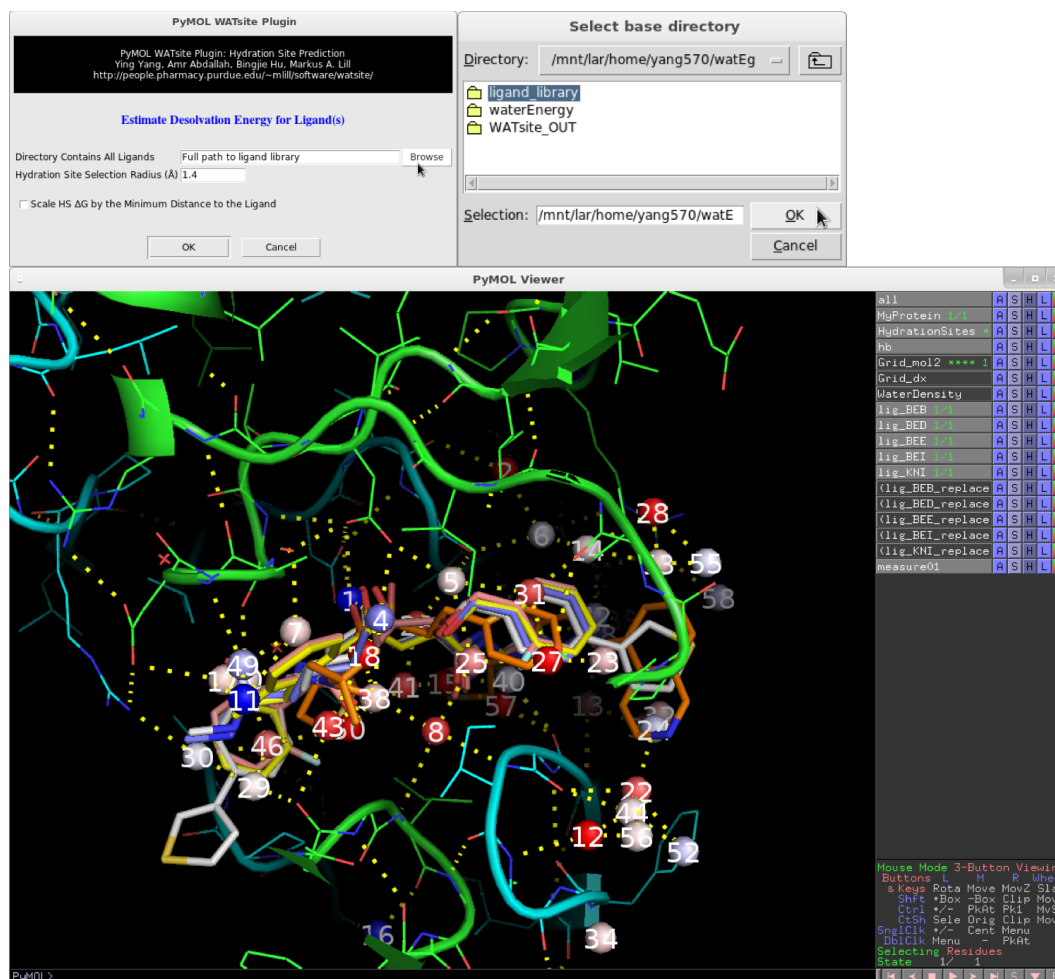


Figure 9: Hydration site result predicted without the ligand. Estimate protein desolvation free energy for a library of congeneric ligand series of HIV-1 protease.

- I. The user can perform hydration site prediction with the ligand removed from the protein binding site. This method can be useful to compare and evaluate the different protein desolvation free energies from a congeneric series of ligands.

2. The directory containing all ligands of interest as well as the radius/cutoff used to select the displaced hydration sites need to be specified.
3. For each ligand in the directory, the free energies of hydration sites that are within the user-specified distance to any of the ligand's heavy atoms are added up to estimate its protein de-solvation free energy. A more positive value means a more favorable contribution to the protein-ligand binding free energy.
4. The predicted desolvation energies (ΔG , $-T\Delta S$, and ΔH) are displayed in a new window, and the selection of displaced hydration sites is highlighted in the PyMOL viewer..

4 Running WATsite via command line

Users are expected to be familiar with AmberTools, OpenMM under Linux OS and comfortable to modify scripts depending on the requirement. Please refer to the corresponding step in section 3 for detail explanation.

Start Docker container

Run the following commands to start a docker container to run WATsite.

```
docker run --runtime=nvidia -it --rm \
-v /scratch/watsite:/opt/data \
lilllab/watsite3 bash
```

Users need to change /scratch6t/yang570 to the directory they want to access and work inside the container.

Use the RunScript.csh

A RunScript.csh has been provided to in /programs/WATsite/bin folder in order to prepare the system, run MD simulation, and WATsite analysis all at once. User can modify the environment variables to control how system is prepared e.g., whether water molecules will be placed in occluded binding site ('setenv occluded_bs o'); whether system truncation will be applied.

Step-by-step procedures

4.1 Prepare the Protein or Complex System

- (Required) Users need to provide a protein structure (named MyProtein.pdb) of interest and a ligand or pseudo-ligand (named MyBindingSite.pdb) to define the binding site. Also, the protonation states of ionizable residues have to be predicted and converted to amber force field compatible residue names (e.g., HIS → HIE, HID, HIP).
- (Optional) Use the following commands, if user intends to place water molecules in the occluded binding sites.

```
pymol -c /programs/WATsite/dat/remove_wat.pml
/programs/miniconda/bin/tleap -s -f /programs/WATsite/dat/tleap_rism3d.in
bash /programs/WATsite/bin/run_water_placement.sh
```

(Optional) Use the python script, if user intends to truncate the protein from the center of the binding site.

```
python /programs/WATsite/bin/truncate_prot.py --truncate 15 --pdb_amber
MyProtein.pdb --bs MyBindingSite.pdb
```

(Required) Use tleap to solvate the protein in a pre-equilibrated water box. Copy the tleap template from /program/WATsite/dat/tleap_MD.in to the working directory. Then, use vim or gedit to modify the tleap input file.

If user used water placement for occluded binding site, change prot = MyProtein.pdb into:

```
# for occluded binding site
prot = loadpdb prot_dry.pdb
wat = loadpdb gasol_wat.pdb
mol = combine {prot wat}
```

If user truncated protein, change prot = MyProtein.pdb into:

```
# for occluded binding site
mol = loadpdb prot_prep.pdb
```

Otherwise, keep the default. User can also change the water model and force field from the list described in section 3 Table 3.1.

Next is to run the tleap command in order to generate the amber topology and coordinate files.

```
/programs/miniconda/bin/tleap -s -f tleap_MD.in
/programs/miniconda/bin/ambpdb -p prot_amber.prmtop \
-c prot_amber.inpcrd > prot_amber.pdb
```

4.2 Run MD simulation

The next step is to run MD simulation for hydration site prediction. Copy the run script from `/programs/WATsite/bin/` to the working directory, and then run MD simulation. User can also change the length of simulation within the run script. If user has changed the water model in `tleap` input file, the default water model (SPC/E) also needs to be changed in the run script.

```
cp /programs/WATsite/bin/run_omm_watsite.sh .  
nohup ./run_omm_watsite.sh &
```

4.3 Hydration site prediction

The final step is to analyze the trajectory files and water energy files from the simulation for hydration site prediction.

```
pymol -c /programs/WATsite/dat/super.pml  
/programs/miniconda/bin/cpptraj < /programs/WATsite/dat/cpptraj.in  
/programs/WATsite/bin/watsite -c input.bcf -o WATsite_OUT
```

4.4 Analyze results

Lastly, user is recommended to use the PyMOL plugin to import the results for easy visualization. The PyMOL plugin can also be downloaded at <http://people.pharmacy.purdue.edu/~mlill/software/watsite/>. User can install it outside the docker container in case of graphics engine related issue.

References

- [1] Dmitrii Beglov and Benoît Roux. An integral equation to describe the solvation of polar molecules in liquid water. *The Journal of Physical Chemistry B*, 101(39):7821–7826, sep 1997.
- [2] Peter Eastman, Jason Swails, John D. Chodera, Robert T. McGibbon, Yutong Zhao, Kyle A. Beauchamp, Lee-Ping Wang, Andrew C. Simmonett, Matthew P. Harrigan, Chaya D. Stern, Rafal P. Wiewiora, Bernard R. Brooks, and Vijay S. Pande. OpenMM 7: Rapid development of high performance algorithms for molecular dynamics. *PLOS Computational Biology*, 13(7):e1005659, jul 2017.
- [3] Lucia Fusani, Ian Wall, David Palmer, and Alvaro Cortes. Optimal water networks in protein cavities with GASol and 3d-RISM. *Bioinformatics*, 34(11):1947–1948, jan 2018.
- [4] Crystal N. Nguyen, Tom Kurtzman Young, and Michael K. Gilson. Grid inhomogeneous solvation theory: Hydration structure and thermodynamics of the miniature receptor cucurbit[7]uril. *The Journal of Chemical Physics*, 137(4):044101, jul 2012.
- [5] Mats H. M. Olsson, Chresten R. Søndergaard, Michal Rostkowski, and Jan H. Jensen. PROPKA3: Consistent treatment of internal and surface residues in empirical pK_a Predictions. *Journal of Chemical Theory and Computation*, 7(2):525–537, feb 2011.
- [6] Junmei Wang, Wei Wang, Peter A. Kollman, and David A. Case. Automatic atom type and bond type perception in molecular mechanical calculations. *Journal of Molecular Graphics and Modelling*, 25(2):247–260, oct 2006.
- [7] J. Michael Word, Simon C. Lovell, Jane S. Richardson, and David C. Richardson. Asparagine and glutamine: using hydrogen atom contacts in the choice of side-chain amide orientation 1 redited by j. thornton. *Journal of Molecular Biology*, 285(4):1735–1747, jan 1999.
- [8] Ying Yang, Bingjie Hu, and Markus A. Lill. Analysis of factors influencing hydration site prediction based on molecular dynamics simulations. *Journal of Chemical Information and Modeling*, 54(10):2987–2995, oct 2014.