

# User Guide for LeWater



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## 1. Hydrogen bonding penalty

Hydrogen bonds are very important for binding specificity, but can be made by the ligand either in the protein or in solution. In order to form hydrogen bonds between the ligand and the protein, donors and acceptors firstly have to break hydrogen bonds with water, and then form new hydrogen bonds. In this sense, the enthalpic gain by formation of new hydrogen bonds is marginal and the gain is mainly entropic due to releasing waters into bulk. However, if newly formed hydrogen bonds between the ligand and the protein are not in good geometry or desolvated donors/acceptors are not involved into new hydrogen bonds, an enthalpic loss arises due to uncompensated breakage of hydrogen bonds with waters.

The program is developed to calculate the hydrogen bonding penalty index in the nature of enthalpy by checking both the solvation and hydrogen bonding state of donors/acceptors at the protein-ligand interface. Further integrating the hydrogen bonding penalty into a scoring function, it is found that a value of 1 in penalty corresponds to about 1.7 kcal/mol in binding free energy, which is in good agreement with the experimental value, e.g., breakage of a neutral hydrogen bond resulting in loss of energy from 0.5 to 1.5 kcal/mol.

The program can also be used to generate a water map at the protein-ligand interface to facilitate hit-to-lead optimization.

## 2. Usage

`$LeWater`

Get "Help" information.

`$LeWater [input file] [protein PDB file] [ligand list]`

Calculate the hydrogen bonding penalty and fitness of hydrogen bonds specified at the protein-ligand interface.

`$LeWater [input file] [protein PDB file] [ligand list] -omap`

Generate a water map at the protein-ligand interface.

### 3. Input

#### 3.1. Protein PDB coordinate file

Explicit hydrogen atoms should be included in the PDB file.

#### 3.2. Ligand list

A list specifies the path of the ligands in PDB format such as

```
//  
L01.pdb  
L02.pdb  
L03.pdb  
.....
```

#### 3.3. Input file

In the input file, define the protein-ligand interface and specify donors/acceptors of the protein together with hydrogen bonding weight according to the specific atom type/environment. An example is as follows:

```
//  
Pocket  
Xmin  Xmax  Ymin  Ymax  Zmin  Zmax  
  
Donor  
A186  NH2   HH21  0  
B99   OH    HH  
....  
  
Acceptor  
157   OD1   0  
158   O  
69    OH    0.5  
....  
  
END
```

#### [Pocket]

Defines the size of the protein-ligand interface in a cubic box. It should be about 6 Å further away from the docked ligand. Recommended size: 6 Å further away from the binding site defined for docking. Too big an interface will increase the computational time.

[Donor]

A186 NH2 HH21 0

A: Chain Identifier. For more than one chain in the protein structure like HIV protease, please specify the chain identifier. If just one chain, the chain identifier can be omitted.

186: Residue number

NH2: donor heavy atom

HH21: donor hydrogen

0: weight for this atom type. Default weight: 0.5 if left blank.

[Acceptor]

157 OD1 0

157: Residue number. If more than one chain, please specify the chain identifier.

OD1: Acceptor

0: weight for this atom type. Default weight: 1 if left blank. For oxygen in a hydroxyl group of the side chain of Tyr/Ser/Thr, the recommended weight is 0.5, which need to be set manually.

69 OH 0.5

## 4. Output

The result is saved in a file named “filter”.

L01.pdb 0.8 P: 0.3 L: 0.5 Dpro: 1.0 0.2 0.0 Apro: 1.0 0.5 0.0

The first number is the overall hydrogen bonding penalty, the second is the penalty from the protein and the third is from the ligand. The following number indicates the fitness of hydrogen bonds formed with the specified donors/acceptors in the input file, with 0 indicates no hydrogen bond and 1 means a perfect hydrogen bond.

The water map is saved in a file “omap.pdb”.

## 5. Important Notes

- 1) In the input file, one has to specify all potential donors/acceptors at the protein-ligand interface that could form hydrogen bonds with the potential ligand. Otherwise, missing donors/acceptors could lead to artificial hydrogen bonding penalty.
- 2) Default weight for donor: 0.5. Side chains of residues such as Lys/Arg on the protein surface are rather flexible. Considering flexibility (which should be judged based on the structure), the weights for such hydrogen bonding donors could be set

to 0. So that those atoms have no penalty even they are desolvated by hydrophobic groups of the ligand, and function only as potential donors/acceptors for ligands

- 3) Default weight for acceptor: 1. However, Acceptor may have different weights depending on the specific atom type (e.g., 0.5 for hydroxyl oxygen). If the weight by the atom type is not 1, it should be set explicitly. Oxygen typically can accept two donors, if it has already been involved into a hydrogen bond, the recommended value is 0 (even the weight is 0, please do add it in the input file as it still could function as an acceptor to form a hydrogen bond with the potential ligand. A small weight of e.g., 0.1 can also be used).
- 4) The weights can be tuned by the users according to the specific requirement.

## Reference

1. Hongtao Zhao, Danzhi Huang; Hydrogen bonding penalty upon ligand binding. *PLoS One*, 6, e19923 (2011).