Calculation of hydration structures of molecular surfaces and interfaces

Version 1.0

User's Manual

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NOTE ON FUTURE VERSIONS

ANALYSIS MODE OF THE PROGRAM IS UNDER DEVELOPMENT AND ITS DETAILS WILL BE RELEASED AND/OR MODIFIED IN FUTURE PUBLICATIONS.

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Scope and applications

MobyWat is a program for analysis and prediction of hydration structure of molecular surfaces and interfaces. The program uses a series of frames sampled from molecular simulations for calculation of positions of structural water molecules. MobyWat has been thoroughly tested on protein surfaces and interfaces, and can be recommended for experimental or theoretical investigations dealing with hydration problems. Possible applications may include but are not restricted to the following projects.

- Refinements and analyses of hydration structure assigned by crystallography. Prediction of hydration structure at problematic (overlapping, non-defined) regions of the density map.
- Prediction of hydration structure of solute molecules such as proteins or their complexes measured by nuclear magnetic resonance spectroscopy.
- Building hydration structure around homology modeled proteins and other modeled molecules or surfaces.
- Selection of structural water molecules for calculation of binding strength between molecular partners of complexes.
- Selection of surface-bound water molecules stabilizing protein structure.
- Selection of conserved water molecules.
- Estimation of local density and mobility of the hydration structure.

1 Background

1.1 Determination of hydration structure

Hydration is involved in most biological processes. It is remarked in a prominent text book on protein structure and function (Petsko and Ringe 2009) that "… waters in fixed positions should be considered as part of the tertiary structure, and any detailed structure description that does not include them is incomplete." Surface hydration is key determinant of solubility and aggregation of solute molecules (Israelachvili and Wennerström 1996). Protein-ligand interactions are also largely affected by interfacial water molecules (Baron et al. 2012), and therefore, knowledge of their location is of primary importance during structure-based drug design. Whereas resolving hydration structure is important, it is also a very difficult task and there is no ultimate method for determination of hydration structure at atomic level.
The difficulties come from mobility and complexity of interactions of water molecules located on a molecular surface. Residence of a water molecule on the surface is affected not primarily by the strength of its protein-water interaction. It is "rather a topography that prevents the water molecule from exchanging by a cooperative mechanism" (Halle 2004a). Importantly, such a cooperative mechanism of exchange also includes several water-water interactions often detected (Finney 1977) between surface or interface water molecules. Thus, it is very problematic to predict the residence of water molecules in the hydration layer of a protein using merely thermodynamic or kinetic approaches (Halle 2004a).
A brief outlook is provided in the forthcoming text on available experimental and theoretical methods for determination of hydration structure placing an emphasis on their limitations.

1.2 Experimental methods

1.2.1 Crystallography

Arrays of hydrated protein molecules arranged in a regular, repeating manner can form a crystal which acts as a diffraction grating and scatters radiation ending up in a diffraction pattern. The diffraction pattern can be converted into electron density maps and used for three-dimensional structural fits of the protein molecule and the surrounding hydration layer. X-ray and neutron crystallography are primary, indispensable, and direct methods for determination of atomic coordinates of hydrating water molecules (Savage 1986). Functionally important water molecules generally reside on the surface or at interface positions. Their determination is possible at resolutions of at least 2 Å (Carugo 1999, Finney 1977). There are more than eighty thousand crystallographic structures deposited in the Protein Data Bank (PDB, Berman et al. 2003) containing atomic level information on water structure of molecular surfaces and interfaces. Despite the large number of PDB entries, there are limitations of crystallographic determination of the hydration structure − few of which listed below.
1) Whereas the number of crystallographic structure refinement techniques is increasing (e.g. Afonine et al. 2013), assignation of electron density peaks to possible interface water positions is still not a routine job due to inherent mobility of water and high number of degrees of freedom (Badger 1997).

2) Quality of a solved structure depends on molecular size (Finney 1977). For small proteins assignation of electron density peaks to atomic positions is easier than it is for larger macromolecules.
3) Electron density peaks of water are generally smaller than those of the surrounding (protein) interface as measured by X-ray diffraction. Small electron density peaks are consequences of small X-ray scattering of the oxygen atom compared to the surrounding group of atoms in the interface, and very low scattering power of hydrogen atoms. Thus, X-ray crystallography of water structure is focusing on a difficult task of identification of a small effect from water hidden among large effects from the surrounding molecules (Finney 1977).
4) Protein hydration in the crystal is not the same as in solution (Halle 2004a). For small proteins, 30–40% of the solvent-accessible surface is usually buried at crystal contacts (Islam & Weaver 1990), where water molecules often mediate protein–protein interactions.
5) Assignation of electron densities to water molecules is often performed to improve the fit of data during structural refinement. Misleading identification of water sites at this stage was found to be a bad practice (Ladbury 1996).
6) Cryocrystallography used for protection of the protein molecules from damages caused by high energy synchrotron beams suffer from structural cryo-artefacts (Halle 2004b).
etc.

1.2.2 Nuclear magnetic resonance (NMR)

While crystallographic methods measure long-time-averaged occupancy of a water positions and provide direct information on hydration structure of protein surfaces, NMR detects only water molecules with residence time of the same magnitude of tumbling time of the molecule in solution (Schoenborn et al. 1995). However, NMR and related techniques can provide useful information on instantaneous time behavior of structural water molecules, such as their residence time on protein surfaces (Halle 2004a).

1.3 Theoretical methods

1.3.1 Static approaches

There are various rapid methods for prediction of hydration sites on molecular surfaces or in interfaces. A common feature of these methods is that they are focused on the protein molecule or on protein-water interactions and completely neglect water-water interactions and co-operations. Notably, these interactions largely determine residence of water molecules in the hydration network (see Section 1.2 for explanation). Most rapid methods use a static picture not considering dynamic exchange (mobility) between surface and bulk water molecules and focusing on protein(ligand)-water interactions. Prominent examples of static algorithms are described below.
Knowledge-based. Using structural reference data sets distilled from crystal structures of the PDB early methods were published for detection of hydration sites (Pitt and Goodfellow 1991).
Structural. It was shown that using e.g. directionality of hydrogen bonds can be applied for systematic solvation of proteins (Vedani and Huhta 1991).

Scoring. A method based on docking of water molecules to the protein binding sites and subsequent use of a scoring scheme for the selection process was recently introduced (Ross et al. 2012).

Thermodynamics. A force field-based approach (Schymkowitz et al. 2005) used free energy calculations in combination with the knowledge-based method of Pitt and Goodfellow (1991). A statistical mechanics-based approach with Monte Carlo sampling of possible hydration site configurations (Michel et al. 2009) was also developed. The method starts with definition of the binding site and fills up a grid covering the site with water molecules. Dynamic exchange of water molecules between the binding site and the bulk is not performed explicitly: an idealized particle concept is used to calculate exchange thermodynamics between bulk and the site.

1.3.2 Dynamic approaches

Molecular dynamics (MD) has long been applied (Rossky and Karplus 1979, van Gunsteren et al. 1983, Pettitt and Karplus 1987) for investigation of hydration of peptides and proteins. All-atom MD with explicit water models is an invaluable source of mobility information of any hydrated biological systems. During the simulation time, movements (trajectory) and all interactions of water molecules can be followed at atomic level including not only protein-water, but also water-water contacts and exchanges of primary importance (Section 1.2). Two main branches of approaches applying MD for prediction of hydrate structure are discussed below.

Density-based calculations. Several studies (Virtanen et al. 2010, Makarov 1998, Lounnas 1994) have dealt with MD-based calculation of average solvent density and construction of proximal radial distribution function (pRDF) of hydration shells for different atom types occurring in proteins. The aim of these studies is to use the constructed, generalized pRDFs for the reconstruction of hydration shell density of any protein without MD simulation. In other words, this approach applies MD for calculation of solvent density and construction of pRDFs. Positions of individual water molecules can be obtained from fits to densities. Limitations of the radial distribution function-based approaches were discussed in details (Henchman and McCammon 2002).

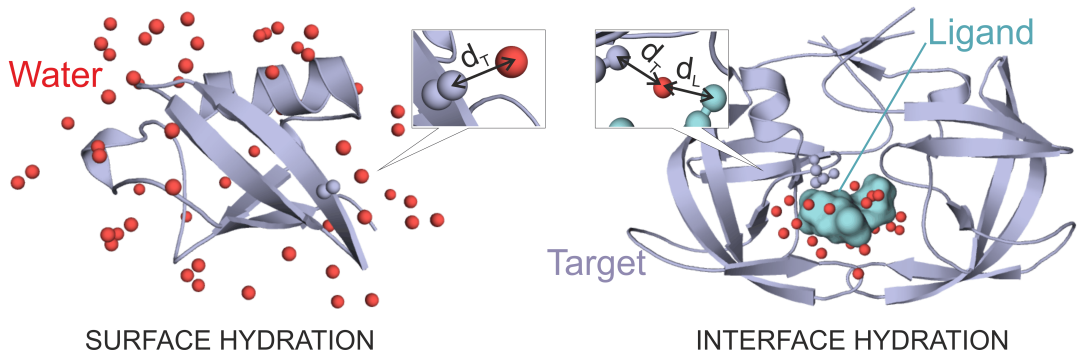
Occupancy-based calculations. With advancement of computational infrastructure and theory speed of MD calculation have increased in the past decades (Dror et al. 2012). It has become a real alternative to perform atomic level MD with explicit water molecules for analysis (Schoenborn et al. 1995) and direct prediction of hydration structure of a protein or its complex. Whereas there are numerous analysis studies, there are much fewer studies on testing the usefulness of direct MD approaches for obtaining hydration sites (Huang et al. 2008, Henchman and McCammon 2002, Madhusudhan 2001). Direct MD approaches use individual positions of hydrating water molecules (instead of average densities) and apply various occupancy-based evaluation schemes to obtain hydration sites. For example Henchman and McCammon (2002) define time averaged positions for this purpose. MobyWat also works with occupancy values and uses water mobility for prediction or analysis of the hydration structure.

2 Glossary

Frame. Mobility of water molecules can be followed at an atomic scale by calculation methods such as molecular dynamics. As a result of a molecular dynamics run, spatial positions of all atoms are recorded at regular time steps. The set of coordinates of all atoms recorded after a time step is called a frame.

Hydration types. MobyWat predicts hydration of the surface (SF) of a single solute molecule (target) or the interface (IF) of a target-ligand complex. Tests of SF predictions were recently published (Jeszenői et al. 2015a). Results of experimenting on extra tricks for "filling" the IF region with water and the corresponding methodology will be submitted for publication soon (Jeszenői et al. 2015b). Fig. 2.1 shows molecular situations for both hydration types.

Fig. 2.1



Hydration types

Reference structure. Experimental structure of a target or a target-ligand complex with the surrounding water molecules. Most of reference structures are produced by crystallography where water molecules are represented by their oxygen atoms. See also water pools.

Solute. A target (usually a large molecule) and optionally a ligand (usually a small molecule) surrounded by water or other solvent.

Trajectory. . A joint collection (log-book) of a time-series of frames. A trajectory includes all four dimensional mobility information on water molecules including three Cartesian coordinates along the time dimension. Trajectories can be stored as separate or NMR-type PDB files or, preferably, in portable binary files (see Section 4.1 for details).

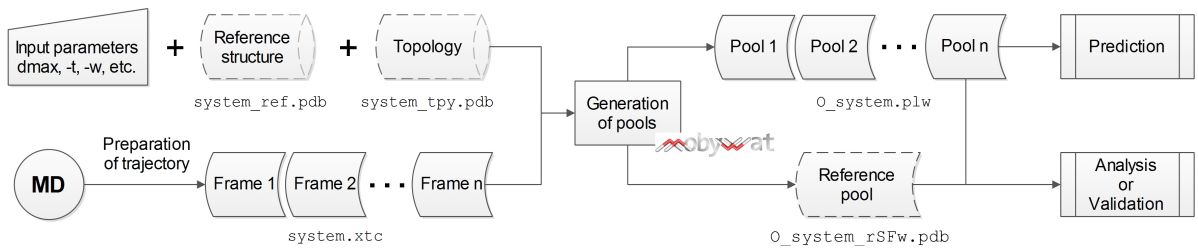
Water pools. Frames and reference structures include all water molecules at various distances from the solute. Importantly, during the generation of the frames molecular dynamics does not distinguish between bulk waters and others close to the solute molecule allowing continuous exchange of any water molecules with each-other in the system. For MobyWat evaluations it is reasonable to separate a pool of water molecules with possible structural role to distinguish them from bulk waters of no use. A maximal distance limit (dmax) is used for the distinction. In the case of surface hydration, a water molecule is selected for the reference or candidate pools if a distance (d_T in Fig. 2.1) measured between its oxygen atom and the closest heavy atom of the target satisfies Eq. 2.1.

Eq. 2.1

$$d_T \leq dmax$$

MobyWat generates pools from experimental data (reference pool in analysis mode and validation sub-mode) or from frames of an MD trajectory (candidate pool in prediction mode and validation sub-mode) according to Fig. 2.2.

Fig. 2.2

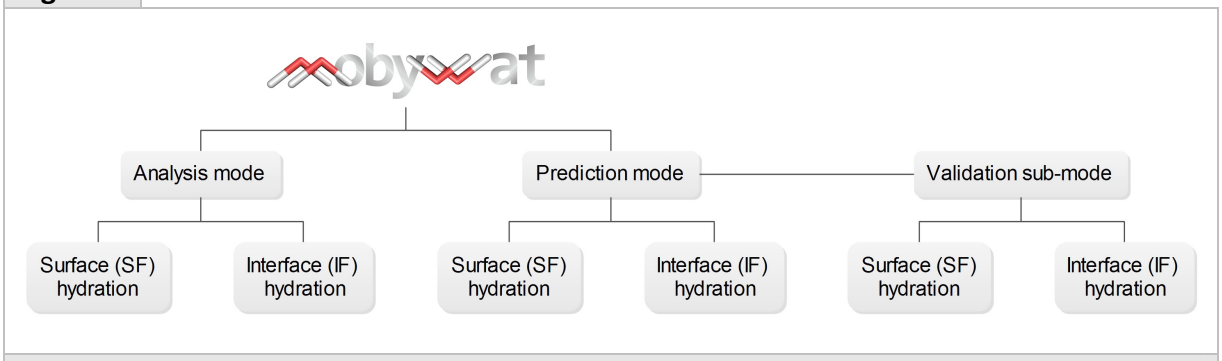


Generation of water pools from trajectory or reference structure

3 Program modes

MobyWat can work either in analysis (Section 3.1) or in prediction (Section 3.2) mode. The program has been tested for surface (Jeszenői et al. 2015a) and interface hydration (Section 2). Results on interface hydration will be fully documented in a forthcoming publication (Jeszenői et al. 2015b). Prediction mode is accompanied by a validation sub-mode (Section 3.3) for test, scan and calibration of working parameters. Default program mode is prediction.

Fig. 3.1



Program modes and hydration types

3.1 Analysis mode

3.1.1 Overview

In analysis mode, MobyWat compares the positions of water molecules of a reference structure (see Section 2 for definition of reference and frames) with positions of water molecules from a molecular dynamics calculation. Commutability, mobility, and occupancy values are calculated for all water molecule helping an assessment of quality and stability of their experimental positions. Success rates are also calculated to estimate the quality of water positions from simulations.

3.1.2 Inputs

Analysis mode of MobyWat requires reference and frame structural files (Table 3.1) of the system. The solute molecules and the surrounding waters may be shifted to different spatial positions in different frames. Thus, superimposition of solute atoms of all frames to solute atoms of the reference structure is necessary to bring all molecules into the same coordinate system in the beginning of prediction process. Importantly, the position of water molecules relative to their interacting solute partners must not change during superimposition. Such superimposition or structural fit can be accomplished by most programs (GROMACS, PyMOL, LSQMAN, etc.). See also Section 9.2 for practical details of fitting frames of a trajectory.
MobyWat can print the per-frame series of root mean squared deviations (RMSD, Eq. 3.1) of a total of N backbone C_α atomic vectors. This can be useful for checking the quality of fit between target molecules in the reference (R) and in the nth frame (F_n). And RMSD < 1.0−1.5 Å can be recommended for a meaningful analysis.

Eq. 3.1

$$\mathrm{RMSD}_n = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left|\vec{F}_{n,i} - \vec{R}_i\right|^2}\ ,\quad n = 1,2,\ldots,\text{number of frames}$$

Processing of structural reference inputs ends with separation of **reference pool** using a distance tolerance (Section 2, dmax) and a B-factor limit (bmax, Table 3.1). This step allows exclusion of reference water molecules with large B-factors and also those which are located at a distance larger than dmax from the molecular surface analyzed (see also Section 2 for the role of dmax in the definition of surface and interface water molecules). Filtered reference water molecules are printed into a PDB file (Table 3.1).

3.1.3 Algorithm

MobyWat identifies water molecules in each frame closest to the selected reference waters. Distances between a reference and all water oxygen atoms in the frame are calculated and water molecules with the lowest distances are selected. The procedure is repeated for each frame and for all reference waters. Atom and residue serial numbers of the selected, closest frame oxygen atoms, as well as the corresponding distances are collected as matrices and printed into separate text files as diagnostic output (Table 3.1). Commutability, mobility, occupancy and success rate values are calculated from the matrices according to definitions of Section 3.1.4.

Table 3.1

ANALYSIS MODE		
INPUT FILES & DEFAULTS		
-f	system.xtc	Trajectory file (xdr binary, optional)
-f	system.plw	Pool waters file (MobyWat binary, optional)
-f	system_mdl.pdb	Trajectory file (PDB NMR, optional)
-f	system_i.pdb	Trajectory file (PDB separate, i=1,2,…,#frame, optional)
-pli	system.pli	Pool information file (required if *.plw file is used)
-r	system_ref.pdb	Reference file (required)
-tpy	system_tpy.pdb	Topology file (required if *.xtc file is used)
INPUT PARAMETERS & DEFAULTS		
-bmax	75.000	B-factor limit, $Å^2$
-dmax	3.500	Distance limit, Å
-m	Prediction	Program mode, Analysis/Prediction
-mtol	1.500	Match tolerance, Å
-n	0-10	Frame range, x-y
-v	Silent	Verbosity, Silent/Verbose/Diagnostic
INPUT RANGES		
-l	x-y/[xy...]	Ligand range, atom serial numbers/chain IDs
-t	x-y/[xy...]	Target range, atom serial numbers/chain IDs
-w	x-y/WAT/Auto	Waters range, atom serial numbers/residue name/automatic
OUTPUT FILES		
Silent output (default)		
O_system.log		Log file
O_system.pli		Pool information file (generated if not *.plw was used as input)
O_system.plw		Pool waters file (generated if not *.plw was used as input)
O_system_NoEX.txt		Commutability values
O_system_asEX.txt		- Atom serial numbers (closest replacing waters)
O_system_rsEX.txt		- Residue serial numbers (closest replacing waters)
O_system_diEX.txt		- Distances (between reference and closest replacing waters)
O_system_ocEX.txt		Mobility values
O_system_occy.txt		Occupancy values
O_system_rSFw.pdb		Reference water pool (surface evaluation)[t]
O_system_succ.txt		Success rate values
O_system_tpy.pdb		Topology file (generated if *.pdb was used as input)
Verbose output		
O_system_rmst.txt		RMSD values (target-target C_α)
O_system_reft.pdb		Reference target coordinates
O_system_refw.pdb		Reference waters coordinates
Diagnostic output		
O_system_aser.txt		Atom serial numbers (closest frame waters)
O_system_rser.txt		Residue serial numbers (closest frame waters)
O_system_dist.txt		Distances (reference vs. closest frame waters)
O_system_frft.pdb		Target coordinates of the first frame
O_system_frfw.pdb		Water coordinates of the first frame
O_system_fSFw.pdb		Pool waters in the first frame
O_system_NoSF.txt		Number of pool waters per frame (surface evaluation)

3.1.4 Outcomes

3.1.4.1 Commutability

During a time period, a water molecule can either remain at its starting position or be replaced by other water molecules. Commutability tells how many water molecules **of different identity** would be capable for such a replacement by coming close to the position of the reference water molecule during the simulation time and the result is printed for each reference water molecule into a text file (Table 3.1). Note that in the present version of MobyWat there is no maximal distance limit defined for the closest water molecules, the distance matrices (Table 3.1) include all distance values calculated.

3.1.4.2 Mobility

For each replacing water molecules, the frequency of their occurrence during the simulation time is calculated, expressed in trajectory % and printed into a file (Table 3.1). High frequency values correspond to low mobility of a replacing water molecule.
Low commutability and mobility values may hint that a reference water is conserved.

3.1.4.3 Occupancy (residence, match)

The occupancy value shows how often any water molecule approached the spatial position of a reference water molecule during the time period of the trajectory. The user can set a maximal distance tolerance (mtol, Table 3.1) to define a successful approach. Occupancy values are listed as trajectory % for all reference water molecules into a separate text file (Table 3.1). Note, that in contrast with commutability and mobility, occupancy does not account for the identity of the water molecules selected from a frame. Large occupancy value of a reference water position shows that the position is well-defined and its spatial location is probably correct and/or conserved.

3.1.4.4 Success rate

Success rate quantifies the match between water positions produced by a simulation method and reference positions. The calculation is performed for all (n^{th}) frames of the trajectory (Eq. 3.2) without clustering of the frames.

Eq. 3.2

$$SR_n = 100\frac{\text{Number of matches in the } n^{th} \text{ frame}}{\text{Number of water molecules in the reference pool}}\%$$

The user can set a match tolerance (mtol, Table 3.1) as a maximum value between the oxygen atoms of the frame and reference water molecules. Success rate values are printed for all frames into a separate text file (Table 3.1). Notably, success rates are also calculated in Validation sub-mode for the prediction lists, i.e. not for the raw frames (Section 3.3.4).

3.1.5 Usage

3.1.5.1 Sample command

Specification of trajectory file name is not necessary, if a file named **system.xtc** is placed in your working directory. The program expects that a **system_ref.pdb** file including the reference coordinates and a topology file **system_tpy.pdb** also exist or the user can specify an arbitrary file name using **–r** and **–tpy** (see Section 4.1 for detailed description of file types). Specification of ranges of target and waters in the trajectory frames is an obligatory part of the command (see Section 4.3 for details on input ranges).

```
$ mobywat –t [A] –w Auto –n 0–100 –m Analysis –v Diagnostic
```

3.1.5.2 Reference ranges

MobyWat requires definition of reference ranges of target and waters in the header of the reference coordinate file as a **REMARK** section. This is necessary as ranges in the reference file may be different from those of the trajectory defined in the command line. The entry has the following sample syntax with fixed key words **REMARK mobywat_reference_XXXXXX** succeeded by user-defined values as used in command line (see Section 4.3 for details).

```
REMARK mobywat_reference_target [A]
REMARK mobywat_reference_waters Auto
```

3.2 Prediction mode

3.2.1 Overview

MobyWat converts mobility information of water molecules into prediction of hydration structure. MobyWat applies various prediction schemes using clustering by identity information of individual water molecules or by spatial positions during the prediction process. Mobility information can be recorded from molecular dynamics calculations for all atoms of a system during a time period. Thus, the movements (trajectories) of all water molecules can be used in MobyWat. The predictions have been validated and tested for reproduction of experimentally determined water positions of protein surfaces.

3.2.2 Inputs

Prediction mode of MobyWat requires the trajectory (frames) of a system in binary or PDB file formats (Table 3.2). Similarly to Section 3.1.2 superimposition of solute molecules is necessary as a preparatory step of prediction. In contrast with Section 3.1.2 the frames are superimposed on and RMSD values are calculated with the first frame (F_x) instead of R in Eq. 3.1 before prediction (x is specified by **–n x-y**, Table 3.1). See also Section 9.2 for practical details of fitting frames of a trajectory.

3.2.3 Algorithm

MobyWat applies two approaches of clustering for conversion of mobility information into structural predictions.

Identity-based clustering. This type of clustering identifies a candidate water molecule by its identity (ID) numbers such as atom and residue serial numbers and uses the history of residence of each molecule on solute surface for mobility calculations.

Position-based clustering. In contrast with ID-based clustering, position (POS) -based clustering accounts for the history of the spatial positions occupied by water molecules irrespective of their identities. Technically, position-based clustering uses only Cartesian (x,y,z) coordinates for representation of candidate water positions.

3.2.3.1 Separation of candidate pools

Prediction mode of MobyWat starts with separation of candidate pools of water molecules from bulk waters using dmax according to the definition given in Section 2. Plausibly, water molecules positioned far from the surface/interface region are of no interest for our algorithm. For each frame of the trajectory, a candidate pool of water molecules is stored (one pool per frame) in a binary file for further steps of the prediction process, and can be printed into files for program diagnostics, as well.

3.2.3.2 Calculation of occupancy lists

In this step, the candidate pools created in Section 3.2.3.1 are transformed into occupancy lists (Fig. 3.3). According to the two types of clustering, two definitions for producing occupancy lists are given. Both clustering types accounts for all members of all pools. Thus, the number of frames in the trajectory is a natural upper limit of occupancy numbers in both types of occupancy lists.

Identity-based occupancy list. A list of water molecules occurring in at least one candidate pool of the trajectory is created, one row for each different ID. Occurrence of a molecule in the pools with the same ID is counted during the whole trajectory and the count is registered in the list as an occupancy number corresponding to the ID. That is, the value of

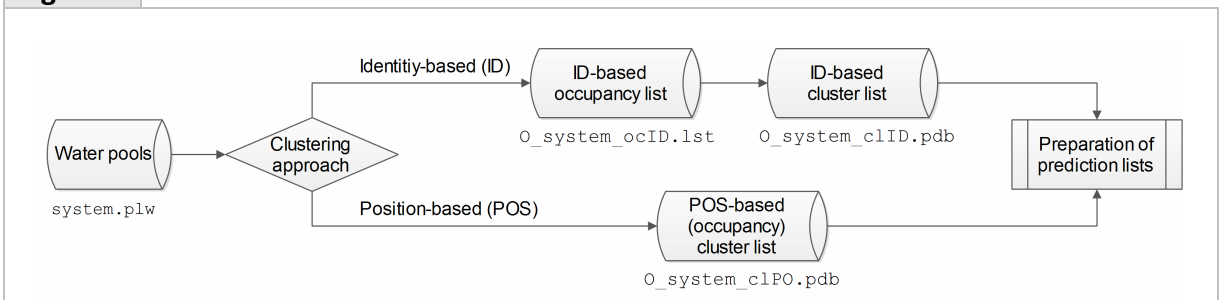
an occupancy number in the list is increased if a pool includes the water with the ID in question. After evaluating all candidate pools, occupancy lists are sorted by decreasing occupancies. An identity-based occupancy list can be considered as a result of clustering along the time dimension.

Position-based (occupancy) cluster list. A list of positions of water molecules occurring in the first candidate pool of the trajectory is created, one row for each different position. During evaluation of all pools, the occupancy number is increased by one, if the position of a water molecule of a pool is located closer to a previously listed position than a pre-defined clustering tolerance (ctol, Table 3.2). If such a position is found, an average of the previously listed and newly found positions will be calculated and used for comparison with the next pool, providing a dynamic (averaged) position definition. Notably, a close water position in a pool can increase occupancy of only one position of the occupancy list per frame (very large ctol values could allow increase of more than one list members, which is not desirable). If the distance between the position of a water molecule of a pool and a previously listed position is larger than or equal to ctol, then a new position (row) of the occupancy list is created. After evaluating all pools, occupancy lists are sorted by decreasing occupancies. A position-based occupancy list can be considered as a complete cluster list, based on time-dependent spatial mobility information.

3.2.3.3 Completion of identity-based clustering

Identity-based occupancy list represents distillation of the candidate pools of a trajectory along time dimension. A further, spatial clustering step was introduced to complete this type of clustering. Water molecules of different pools with the same ID (belonging to the same row of the occupancy list) are collected into a cluster using a pre-defined ctol value. That is, all water molecules in the cluster must have a maximal distance of ctol from each-other. In this way, all members of a row of the occupancy list are clustered, and the procedure is repeated for all rows of the list. Finally, the clusters are ordered by the count of their members and the average of the coordinates of the members is calculated for each cluster resulting in a representing entry for the identity-based cluster list.

Fig. 3.3



Preparation of cluster lists

3.2.3.4 Calculation of prediction lists

As a final step, MobyWat creates prediction lists from the cluster lists described in the previous Sections. Prediction lists contain the atomic coordinates of water positions and the corresponding mobility (M) values as final outcomes of the prediction process. A mobility (M) value is calculated for each row of the prediction lists from normalized occupancy (O) values (Eq. 3.3). Normally, M values scale between 0 and 100 and zero corresponds to the least mobile, conserved predicted water position. Predicted water positions are listed in order of increasing mobility in the prediction list. MobyWat produces four types of prediction lists (Fig. 3.4).

Eq. 3.3

$$O_i = \frac{\text{Occupancy of cluster}}{\text{Number of pools}}, \quad \text{where } i = 1,2,\ldots,\text{number of rows of prediction list.}$$

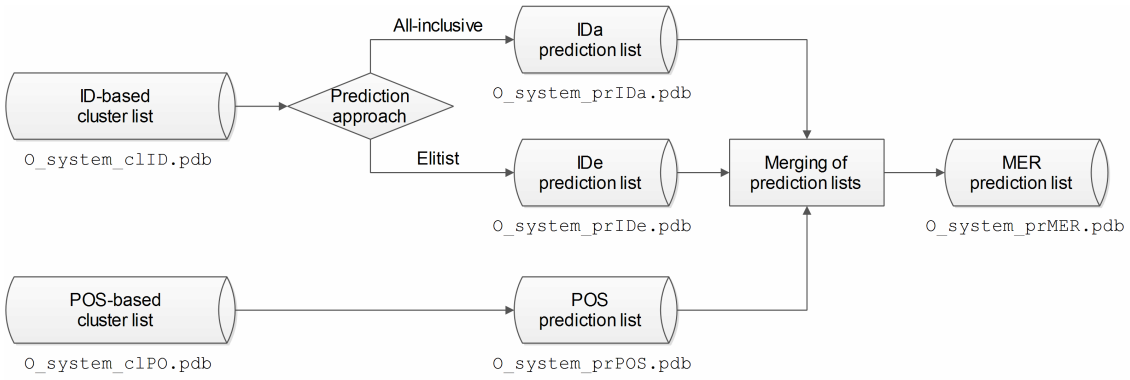
$$M_i = 100\begin{cases} \dfrac{O_{max} - O_i}{O_{max} - O_{min}}, & \text{if } O_{max} > O_{min}, \\ 1 - O_i, & \text{if } O_{max} = O_{min}, \end{cases} \quad \text{where } O_{max/min} = \max_i/\min_i O_i.$$

Elitist prediction list (IDe). This prediction list is produced from identity-based occupancy and cluster lists. The first clusters of each row of the occupancy list are used in the first round of the evaluation process. The largest first cluster is selected from among all rows of the occupancy list and placed at the top of the prediction list. Other first clusters are checked if the distance between their representing water position and that of the largest cluster is smaller than a pre-defined prediction tolerance (ptol) value. If the distance is smaller than ptol then the cluster is disqualified. Notably, this tolerance ensures to keep a minimal distance between the resulted members of the prediction list to avoid close contacts.

In the next step, the largest first cluster is selected again from among the clusters qualified in the first step and it is placed at the next position of the prediction list. The above procedure is repeated comparing prediction list members with available first clusters until first clusters of all rows of the occupancy list were either placed on the prediction list or disqualified. In the case if the first cluster of a row of the occupancy list was not placed on the prediction list, the procedure goes on with the second and higher clusters until all rows of occupancy list contributed a cluster to the prediction list.

This prediction list is called elitist as it uses up the first clusters of each row of the occupancy list first to fill up the prediction list.

Fig. 3.4



Preparation of prediction lists

All-inclusive prediction list (IDa). This prediction list is produced from list of identity-based clusters irrespective of their location on the occupancy list. The largest cluster is selected from among all clusters and placed on the top of the prediction list. The other clusters are checked if the distance between their representing spatial water position and that of the largest cluster is smaller than ptol. If the distance is smaller than ptol then the cluster is disqualified.

In the next step, the largest cluster is selected again from among the clusters qualified in the first step and it is placed on the next position of the prediction list. The above procedure is repeated comparing prediction list members with available clusters until all clusters were either placed on the prediction list or disqualified.

Position-based prediction list (POS). This prediction list is produced from position-based occupancy list, using the procedure detailed at “All-inclusive prediction list”.

Merged prediction list (MER). Merging (∪) of prediction lists is performed in a pair-wise manner. Two prediction lists are simply copied into the merged prediction list one after the other. As a final step, the merged prediction list is cleaned up by removing entries with a distance smaller than ptol with the procedure described at “All-inclusive prediction list”.

MobyWat creates merged prediction list from the three available prediction lists in the following order (Eq. 3.4).

Eq. 3.4

$$MER = (IDa \cup IDe) \cup POS$$

Table 3.2

PREDICTION MODE		
INPUT FILES & DEFAULTS		
-f	system.xtc	Trajectory file (xdr binary, optional)
-f	system.plw	Pool waters file (MobyWat binary, optional)
-f	system_mdl.pdb	Trajectory file (PDB NMR, optional)
-f	system_i.pdb	Trajectory file (PDB separate, i=1,2,…,#frame, optional)
-pli	system.pli	Pool information file (required if *.plw file is used)
-tpy	system_tpy.pdb	Topology file (required if *.xtc file is used)
INPUT PARAMETERS & DEFAULTS		
-cls	IDa	Clustering algorithm, IDa/IDe/POS/MER
-ctol	1.000	Clustering tolerance, Å
-dmax	3.500	Distance limit, Å
-m	Prediction	Program mode, Analysis/Prediction
-n	0-10	Frame range, x-y
-ptol	2.500	Prediction tolerance, Å
-top	50.000	Top cut of short prediction list, %
-v	Silent	Verbosity, Silent/Verbose/Diagnostic
INPUT RANGES		
-l	x-y/[xy...]	Ligand range, atom serial numbers/chain IDs
-t	x-y/[xy...]	Target range, atom serial numbers/chain IDs
-w	x-y/WAT/Auto	Waters range, atom serial numbers/residue name/automatic
OUTPUT FILES		
Silent output (default)		
O_system.log		Log file
O_system.pli		Pool information file (generated if not *.plw was used as input)
O_system.plw		Pool waters file (generated if not *.plw was used as input)
O_system_prXXX.pdb		Long (full) prediction list (XXX = IDa/IDe/POS/MER)
O_system_prXXX_top.pdb		Short (top-cut) prediction list (XXX = IDa/IDe/POS/MER)
O_system_tpy.pdb		Topology file (generated if *.pdb was used as input)
Verbose output		
O_system_rmst.txt		RMSD values (target-target C_α)
O_system_clID.pdb		Identity-based cluster list (for IDa/IDe/MER)
O_system_clPO.pdb		Position-based cluster list (for POS/MER)
O_system_ocID.lst		Identity-based occupancy list (for IDa/IDe/MER)
O_system_NoSF.txt		Number of pool waters per frame (surface evaluation)
Diagnostic output		
O_system_cSFw.pdb		List of clustered waters (surface evaluation)
O_system_frft.pdb		Target coordinates of the first frame
O_system_frfw.pdb		Water coordinates of the first frame
O_system_fSFw.pdb		Pool waters in the first frame

3.2.4 Outcomes

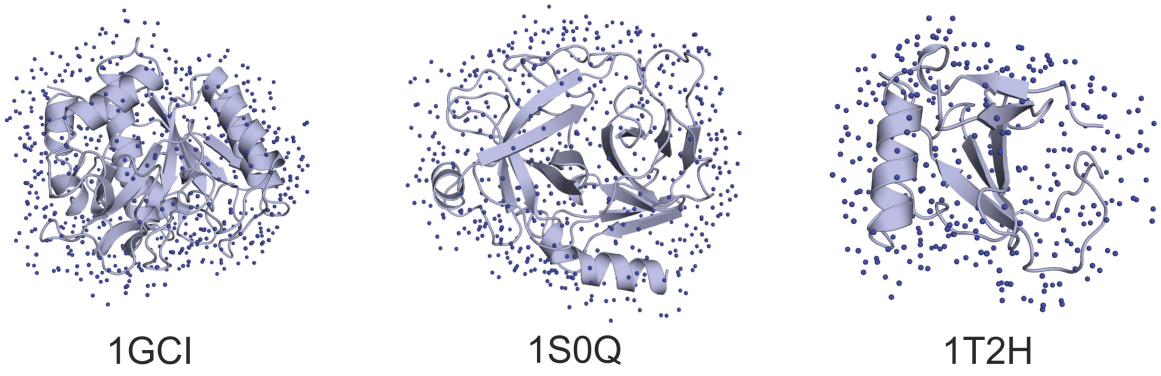
Primary outcomes of Prediction mode of MobyWat are prediction list. The lists are printed in standard PDB format and contain coordinates of oxygen atoms of predicted water positions (Fig. 3.5) in increasing order of mobility. Both types of mobility values of Eq. 3.3 are printed in crystallographic occupancy and B-factor columns, respectively.

The type of requested prediction lists can be specified by the user with the **–cls** switch. Notably, in the case of **–cls MER** all four (IDa, IDe, POS, MER) prediction lists are calculated and printed. Main parameters ctol and ptol can be also specified by the user at the command line.

Short (top-cut) version of the prediction lists are also printed including the top part (the least mobile) waters from the full prediction lists. The length of a short list can be specified with a cut switch **–top**. For most applications, the use of short prediction lists including the top 50 % (default in Table 3.2) and top 25 % of the predicted water molecules is recommended for SF prediction of the entire surface of protein molecules and of the binding (active) sites, respectively. For details on scoring performance of short-lists of water molecules, please refer to Section 3.3.4.

Other files listed in Table 3.2 such as occupancy and cluster lists, number of pool waters, etc. can be also printed by setting **–v** to **Verbose** or **Diagnostic**.

Fig. 3.5



Visualization of water positions of top 50 % prediction lists of three protein systems (PDB codes are shown). Detailed comparisons are shown in Fig. 3.7. Results on additional protein systems were published (Jeszenői et al. 2015a).

3.2.5 Usage

Sample commands

Specification of trajectory file name is not necessary, if a file named **system.xtc** is placed in your working directory. The program expects that a topology file **system_tpy.pdb** also exist or the user can specify an arbitrary file name using **–tpy** (see Section 4.1 for detailed description of file types). Program mode is **Prediction** by default, and therefore, use of **–m** is not necessary here. Specification of ranges of target and waters in the trajectory frames is an obligatory part of the command (see Section 4.3 for details on input ranges). The following commands can be used for different input situations.

Default trajectory and topology input files (**system.xtc** and **system_tpy.pdb**), frames between 0 and 100, target is specified by its chain ID, merged prediction list is requested:

```
$ mobywat –t [A] –w Auto –n 0–100 –cls MER
```

Default trajectory and topology input files (**system.xtc** and **system_tpy.pdb**), frames between 0 and 100, target is specified by range of atom serial number, merged prediction list is requested:

```
$ mobywat –t 1–925 –w Auto –n 0–100 –cls MER –m Prediction
```

Pool waters input file with corresponding pool information file (**O_system.pli**), merged prediction list is requested, other settings are read from pool information file:

```
$ mobywat –f O_system.plw –cls MER
```

NMR-type PDB file (**2FMA_mdl.pdb**), other options are as above:

```
$ mobywat –f 2FMA_mdl.pdb –t [A] –w Auto –n 1–100 –cls MER
```

Separate PDB files with root name "**2FMA**", other options are as above:

```
$ mobywat –f 2FMA.pdb –t [A] –w Auto –n 1–100 –cls MER
```

For certain types of IF water predictions range of the ligand molecule can be defined using an additional –l switch. A publication on IF hydration (Jeszenői et al. 2015b) is on the way. Corresponding examples on the usage of MobyWat for IF predictions will be provided here.

3.3 Validation sub-mode

3.3.1 Overview

Validation sub-mode of MobyWat can be used for test and calibration of clustering algorithms implemented in Prediction mode. Validation is not discussed as a separate mode as it is mostly based on the algorithms of Prediction mode. A comparison with a reference file allows calculation of the rate of successful predictions for different clustering schemes and tolerances.

3.3.2 Inputs

Similarly to Analysis mode, both reference frame file and trajectory file are required. From the reference file, a reference water pool is separated and used for comparison with the results of predictions. The reference file is usually a crystallographic structure from the PDB with several water molecules assigned.

3.3.3 Algorithm

A detailed description of prediction algorithms were given in Section 3.2. In addition, validation sub-mode uses a match tolerance (mtol) for comparison of the location of water oxygen atoms in the reference pool and in the prediction lists. A match is defined in the prediction list if the distance between the reference and predicted oxygen atoms is smaller than mtol. Validation sub-mode takes all members of each prediction lists and checks if a member has a match with the reference pool or not. All members of the reference pool can be used only once for each prediction list during identification of matches.

3.3.4 Outcomes

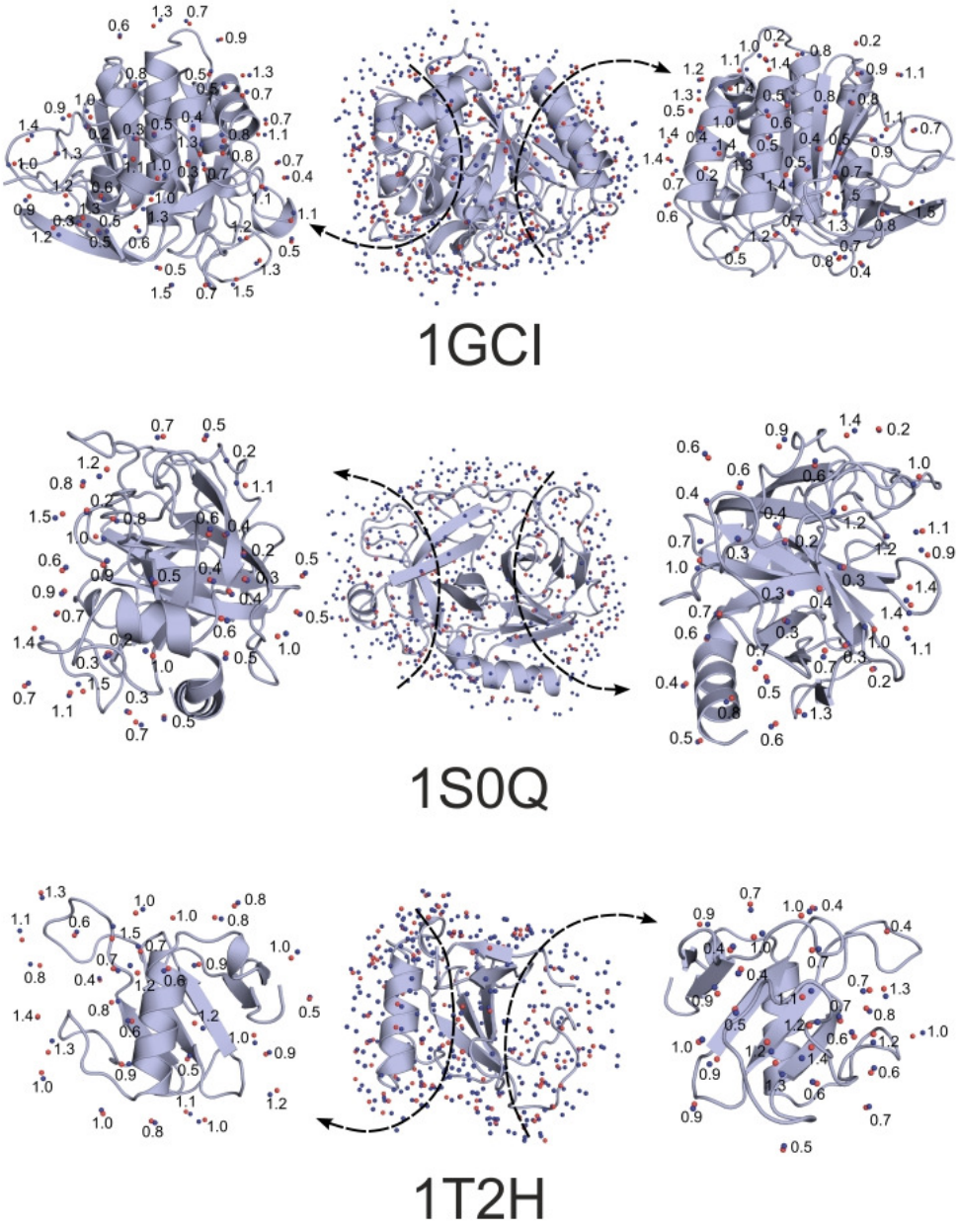
Match lists. The results are saved in match lists (text files, Fig. 3.6) including water serial numbers, occupancy counts and the distances used for identification of a match, i.e. comparison with mtol. In the last column “M” of the match list “x” marks a match (Fig. 3.7).

Fig. 3.6

```
Match List between ID-all-inclusive Prediction List and Reference List
First frame #             :        0
Last frame #              :    10000
Match tolerance (A)       :    1.500
B-factor limit            :   30.000
Distance tolerance (A)    :    3.500
Prediction tolerance (A)  :    2.500
Clustering tolerance (A)  :    1.000
=============================================================================================================
Water(pl)#     Water(ol)#    Cluster(ol)#     Count |  Ref.# Ref.atom# Ref.res.#  B-factor  Distance | M
-------------------------------------------------------------------------------------------------------------
         1              1               1      9385 |     13      1706       614      7.64     0.144 | x
         2              2               1      9187 |     16      1709       617      8.62     0.352 | x
         3              4               1      8913 |     20      1713       621      9.18     0.796 | x
         4              5               1      5645 |     36      1731       639     11.76     0.672 | x
         5              3               1      4156 |     82      1779       687     16.50     7.437 | -
         6              7               1      3305 |     42      1737       645     12.13     0.225 | x
         7              8               1      1424 |     49      1744       652     12.50     0.798 | x
         8             11               1      1131 |     24      1717       625     10.48     0.171 | x
✂·····································································✂
       649           3040             176         1 |    122      1831       739     21.82     6.217 | -
       650           3106             176         1 |     39      1734       642     12.05     3.804 | -
       651           4884              56         1 |     54      1749       657     13.77     1.508 | -
=============================================================================================================
SR       78.981
=============================================================================================================
```

Match list of PDB system 1T2H (abridged)

Fig. 3.7



Matching water positions of prediction lists of three protein systems (PDB codes are shown). Success rates of 83.9, 83.7, and 79.0 % were obtained, respectively. Blue and red spheres represent the prediction list and reference pools, respectively. Matching pairs of match lists and distances (Å) are shown for clarity. Results on additional protein systems were published (Jeszenői et al. 2015a).

Table 3.3

VALIDATION SUB-MODE		
INPUT FILES & DEFAULTS		
-f	system.xtc	Trajectory file (xdr binary, optional)
-f	system.plw	Pool waters file (MobyWat binary, optional)
-f	system_mdl.pdb	Trajectory file (PDB NMR, optional)
-f	system_i.pdb	Trajectory file (PDB separate, i=1,2,…,#frame, optional)
-pli	system.pli	Pool information file (required if *.plw file is used)
-r	system_ref.pdb	Reference file (required)
-tpy	system_tpy.pdb	Topology file (required if *.xtc file is used)
INPUT PARAMETERS & DEFAULTS		
-cls	IDa	Clustering algorithm, IDa/IDe/POS/MER
-ctol	1.000	Clustering tolerance, Å
-dmax	3.500	Distance limit, Å
-m	Prediction	Program mode, Analysis/Prediction
-mtol	1.500	Match tolerance, Å
-n	0-10	Frame range, x-y
-ptol	2.500	Prediction tolerance, Å
-top	50.000	Top cut of short prediction list, %
-v	Silent	Verbosity, Silent/Verbose/Diagnostic
INPUT RANGES		
-l	x-y/[xy...]	Ligand range, atom serial numbers/chain IDs
-t	x-y/[xy...]	Target range, atom serial numbers/chain IDs
-w	x-y/WAT/Auto	Waters range, atom serial numbers/residue name/automatic
OUTPUT FILES		
Silent output (default)		
O_system.log		Log file
O_system.pli		Pool information file (generated if not *.plw was used as input)
O_system.plw		Pool waters file (generated if not *.plw was used as input)
O_system_prXXX.pdb		Long (full) prediction list (XXX = IDa/IDe/POS/MER)
O_system_prXXX_top.pdb		Short (top-cut) prediction list (XXX = IDa/IDe/POS/MER)
O_system_tpy.pdb		Topology file (generated if *.pdb was used as input)
O_system_mtXXX.lst		Match list (XXX = IDa/IDe/POS/MER)
O_system_i_XXX.mat		Success rate matrix (XXX = IDa/IDe/POS/MER, i=1,2,…,num_mtol)
O_system_rSFw.pdb		Reference water pool (surface evaluation)[t]
Verbose output		
O_system_rmst.txt		RMSD values (target-target C_α)
O_system_clID.pdb		Identity-based cluster list (for IDa/IDe/MER)
O_system_clPO.pdb		Position-based cluster list (for POS/MER)
O_system_ocID.lst		Identity-based occupancy list (for IDa/IDe/MER)
O_system_NoSF.txt		Number of pool waters per frame (surface evaluation)
O_system_mtIDc.lst		Cluster match list (for IDa/IDe/MER)
O_system_reft.pdb		Reference target coordinates
O_system_refw.pdb		Reference waters coordinates
Diagnostic output		
O_system_cSFw.pdb		List of clustered waters (surface evaluation)
O_system_frft.pdb		Target coordinates of the first frame
O_system_frfw.pdb		Water coordinates of the first frame
O_system_fSFw.pdb		Pool waters in the first frame

Success rate (SR). From the match lists SR values are calculated for each prediction (list) according to Eq. 3.5. The higher the SR value, the more successful a prediction is in comparison with crystallographic water positions (Fig. 3.7).

Eq. 3.5

$$SR_{xxx} = 100\frac{\text{Number of matches in the XXX prediction list}}{\text{Number of water molecules in the reference pool}}\%,$$

where XXX = IDa/IDe/POS/MER.

SR matrices. Besides evaluation of matches on a single prediction list at fix (mtol, ptol, ctol) tolerance values, validation sub-mode of MobyWat can scan user-defined ranges of all three tolerance values and print the resulted SR values in (ptol,ctol) matrices for any mtol values. For this, MobyWat re-generates cluster and prediction lists as many times as required by the user and collects the resulted SR values into matrices. This option of the program is useful for the optimization of ptol and ctol values of the prediction algorithm (Section 3.2) and can be used as a development tool.

Score performance (SP). For many applications a short, top-cut version of the prediction lists (Section 3.2.4) can be useful excluding e.g. loosely bound, bulk-like waters and keeping only essential, structural ones. The quality of predicted water molecules at the top of the prediction list is expressed by the score performance (SP_x, Eq. 3.6). SP values are not calculated directly by MobyWat, but they can be easily obtained from the match lists (Fig. 3.6).

Eq. 3.6

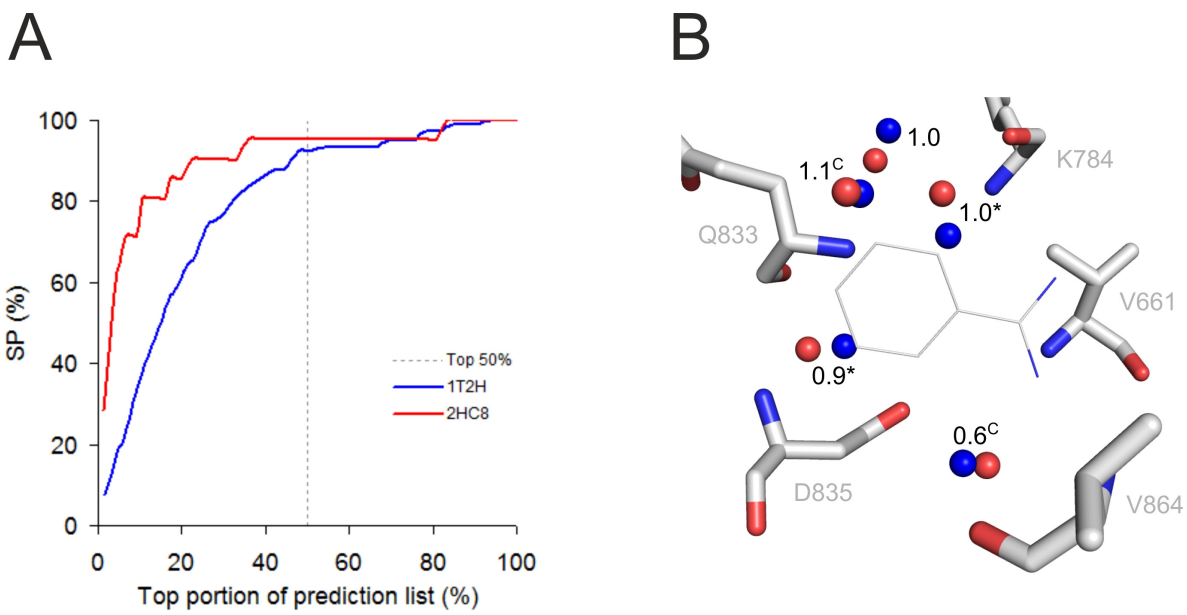
$$SP_X = 100\frac{\text{Number of matches in the top x \% of the prediction list}}{\text{Number of matches in the full prediction list}}\%.$$

MobyWat provides water positions in an increasing order of their calculated M (Eq. 3.3) value in the prediction list. In other words, M is used as a score of the predicted water positions. SP quantifies the performance of M in placing matched positions to the top of the prediction list.

Characteristic SP curves (Fig. 3.8A) are useful for estimation of the top cut-off value for production of the short version of prediction lists. Fig. 3.8A shows that in cases of PDB systems 1T2H and 2HC8, the default short prediction list (top 50%, Table 3.3) contains more than 90 % of the matched positions, indicating that the full prediction list can be shortened to its top half without a big loss of valuable water positions. SP curves of other systems were also calculated (Jeszenői et al. 2015a).

It was found (Jeszenői et al. 2015a) that SP values are remarkably larger in the vicinity of the ligand binding sites than for the entire protein surface. For example, in the case of the benzamidine binding site of bovine pancreatic trypsin, an SP_{25}=100 % was achieved (Fig. 3.8B), whereas an SP_{25}=70.6 % was obtained for the entire protein surface. Thus, MobyWat score shows an excellent performance for active sites of protein surfaces. Thus, the use of a reduced prediction list including the top 25 % of the full prediction list can be used for prediction of hydration structure active sites. Other examples of the performance of MobyWat for active site hydration are included in the MobyWat publication (Jeszenői et al. 2015a). Notably, the use of MobyWat for hydration of the protein-ligand binding interface will be described in a forthcoming publication (Jeszenői et al. 2015b).

Fig. 3.8



(**A**) Characteristic SP curves of PDB systems 1T2H and 2HC8 for MobyWat predictions on the entire surface of the protein molecules. (**B**) Active site of apo form of bovine pancreatic trypsin (PDB code 1S0Q) for a 3.5 Å vicinity of the benzamidine ligand. Match distances between crystallographic (red spheres) and predicted (blue spheres) water oxygen atoms are given in Å. Conserved and replaceable (i.e. replaced by the ligand during binding) water molecules are marked with C and asterisk at the distance values, respectively. Notably, conserved and replaceable water molecules could be found equally well at the ligand binding site. All five matches were located in the top 25 % of the prediction list (SP_{25}=100 %, calculated for the binding site). Benzamidine was inserted from superimposed ligand-bound enzyme structure (PDB code 1J8A) for comparison with predicted water positions.

3.3.5 Usage

3.3.5.1 Sample command

MobyWat automatically distinguishes between prediction and validation. Validation sub-mode is switched on if MobyWat finds a reference file in the working directory.

Specification of trajectory file name is not necessary, if a file named **system.xtc** is placed in your working directory. The program expects that a **system_ref.pdb** file including the reference coordinates and a topology file **system_tpy.pdb** also exist or the user can specify an arbitrary file name using **–r** and **–tpy** (see Section 4.1 for detailed description of file types). Specification of ranges of target and waters in the trajectory frames is an obligatory part of the command (see Section 4.3 for details on input ranges).

```
$ mobywat –t [A] –w Auto –n 0–100 –m Prediction –cls IDa
```

3.3.5.2 Reference ranges and SR matrix settings

MobyWat requires definition of reference ranges of target and waters in the header of the reference coordinate file as a **REMARK** section. This is necessary as ranges in the reference file may be different from those of the trajectory defined in the command line. The entry has the following sample syntax with fixed key words **mobywat_reference_XXXXXX** succeeded by user-defined values as used in command line (see Section 4.3 for details).

The user can also request calculation of SR matrices described in Section 3.3.4. For this, additional lines are required in the **REMARK** section starting with **mobywat_** defining the number of matrices (**num_mtol**) and the number of data of the matrices (**num_ptol** × **num_ctol**). All these **num_XXX** values have to be at least 1 to get the matrices requested. Entries **min_XXX** and **step_XXX** define the minimum value and step size of a parameter (mtol, ptol, or ctol).

```
REMARK mobywat_reference_target [A]
REMARK mobywat_reference_waters Auto
REMARK mobywat_min_ctol   1.0
REMARK mobywat_num_ctol   4
REMARK mobywat_step_ctol  0.50
REMARK mobywat_min_ptol   2.50
REMARK mobywat_num_ptol   1
REMARK mobywat_step_ptol  0
REMARK mobywat_min_mtol   1.500
REMARK mobywat_num_mtol   1
REMARK mobywat_step_mtol  0
```

4 File types, ranges, parameters

4.1 Input files

The present version of MobyWat accepts four types of structural input files. File types are automatically recognized by extensions of file names.

4.1.1. Binary trajectory file (*.xtc)

The xtc format is a portable binary format which can store trajectories in an efficient, compressed form. It uses the xdr routines for writing and reading data. Xtc files can be produced by MD package GROMACS. Note that xtc files contain only spatial coordinates x,y,z of all atoms of all frames of the trajectory. Similarly to other programs reading binary trajectories, MobyWat also requires the definition of atom types in a separate PDB-type topology file. The topology file must contain the same number of atoms in the same order as one frame. Production of such a topology file can be easily done along with the production of the xtc file in GROMACS (see Section 4.2 for details).

4.1.2 Separate PDB files (*.pdb)

For compatibility with any MD packages, MobyWat can also read a trajectory as a series of individual frames stored in standard PDB files. Obviously, the frames must have the same number of atoms in the same order. The files must have a sequential naming such as **system_i.pdb**, where the use of **_i.pdb** part of the file names is mandatory (i=x, x+1,x+2,…,y in MobyWat command line switch **–n x-y**, see also Section 4.3). Note, that topology file is not used for PDB files as they have all necessary information for MobyWat. For additional information on PDB files, please visit

http://www.wwpdb.org/docs.html

4.1.3 NMR-type PDB files (*.pdb)

Instead of a series of PDB files a trajectory can be also stored in a single PDB file and frames can be handled as NMR models within the same file. MobyWat automatically recognizes NMR-type PDB files if the file name contains the token mdl in a form such as system_mdl.pdb. Note, that in NMR-type PDB files the models (=frames) are defined using **MODEL** and **ENDMDL** tokens and model serial numbers specified after **MODEL** must be within the x-y range of switch **–n x-y** of MobyWat command line (see also Section 4.3). For additional information on NMR-type PDB files, please visit

http://www.wwpdb.org/documentation/format33/sect9.html#MODEL

It is important to note that storage of trajectories in PDB files may require much disk space, and therefore, xtc binary files can be recommended for routine use. However, MobyWat can also read PDB inputs described above to ensure compatibility with any MD software packages. When reading PDB input files MobyWat automatically converts the trajectories into xtc format and also creates a topology file used for subsequent calculations.

4.1.4 Binary pool waters file (*.plw)

MobyWat stores pool waters (Section 2) of the trajectory in a non-portable binary file which is automatically created as a first step of processing input files described in Sections 4.1.1-3. The file contains pool waters only from frames specified by the x-y range of switch **–n x-y** of MobyWat command line (see also Section 4.3). MobyWat also produces a pool information file (*.pli) along with the plw file for future use. The pli file contains information such as dmax specifying the pool. As creation of water pools from the trajectory file may take considerable time for large systems, pool waters file can be useful if re-running of a prediction/analysis is required by the user e.g. for experimenting with various clustering schemes, tolerances, etc. Importantly, dmax cannot be changed in such repeated runs as it defines the pool, itself (Section 2). For information on the structure of pool waters file, please refer to Section 5.

4.2 Output files

MobyWat produces binary files and text files and all of them are marked with **O_** in the beginning of the file name. Files xtc, plw, and pdb were described in Section 4.1. In other text files such as lst and txt additional information on e.g. clustering, RMSD, number of interface waters per frame, etc. are printed. MobyWat also creates a log file with details on input/output information.

4.3 Input ranges

4.3.1 Frame range

The user can specify the minimal (x) and maximal (y) serial numbers of frames with the use of switch **–n x-y** at MobyWat command line. Please, be sure that the frames exist in your trajectory input files (Section 4.1).

4.3.2 Molecular ranges

MobyWat requires definition of target and waters in the frames using **–t** and **–w** switches of the command line, respectively. There are three possibilities of definition.

1) Ranges of atom serial numbers can be given using minimal (x) and maximal (y) serial number in a form of **–t x-y** or **–w x-y**.

2) In the case of target, a list of one-letter chain IDs such as xy… can be also used in a form of **–t [xy…]**. Note, that use of **[…]** brackets is obligatory. This option is especially useful if atom list of target is non-continuous in the frame, and the target is stored in several chains.

3) In the case of waters, definition is also possible by specification of a single residue name such as **WAT** in the form **–w WAT**. MobyWat can automatically detect and assign water molecules in the frame using the **–w Auto** command line entry, as well. During automatic detection residue names **SOL**, **WAT** and **H2O** can be identified.

Besides their use for frames in the command line, the above specifications of molecular ranges can be used for the reference structure in Analysis mode and Validation sub-mode.

4.4 Input parameters

Required formats and default values of numerical and alphabetical parameters are listed e.g. in the quick help which can be printed to the screen by typing the name of the program at the command prompt (Fig. 4.1).

Fig. 4.1

```
$ mobywat
_____________________________________________________________________________

MobyWat
Calculation of hydration structures of molecular surfaces and interfaces
_____________________________________________________________________________
                                                              ===Ver=1.0=01=09=2014===
Usage
$ mobywat -x <value_x> -y <value_y> ...

Input files
-f       system.xtc      Trajectory file with frames
-pli     system.pli      Pool information file
-r       system_ref.pdb  Reference file
-tpy     system_tpy.pdb  Topology file

Input ranges
-l       x-y/[xy...]     Ligand range, atom serial numbers/chain IDs
-t       x-y/[xy...]     Target range, atom serial numbers/chain IDs
-w       x-y/WAT/Auto    Waters range, atom serial numbers/residue name/automatic

Parameters & defaults
-bmax    75.000          B-factor limit, A^2
-cls     IDa             Clustering algorithm, IDa/IDe/POS/MER
-ctol    1.000           Clustering tolerance, A
-dmax    3.500           Distance limit, A
-m       Prediction      Program mode, Analysis/Prediction
-mtol    1.500           Match tolerance, A
-n       0-10            Frame range, x-y
-ptol    2.500           Prediction tolerance, A
-ptop    50.000          Top cut of short prediction list, %
-v       Silent          Verbosity, Silent/Verbose/Diagnostic
_____________________________________________________________________________

For detailed instructions, please visit the web site at http://www.mobywat.com !
_____________________________________________________________________________
                                                                ===by=C=Hetenyi===
```

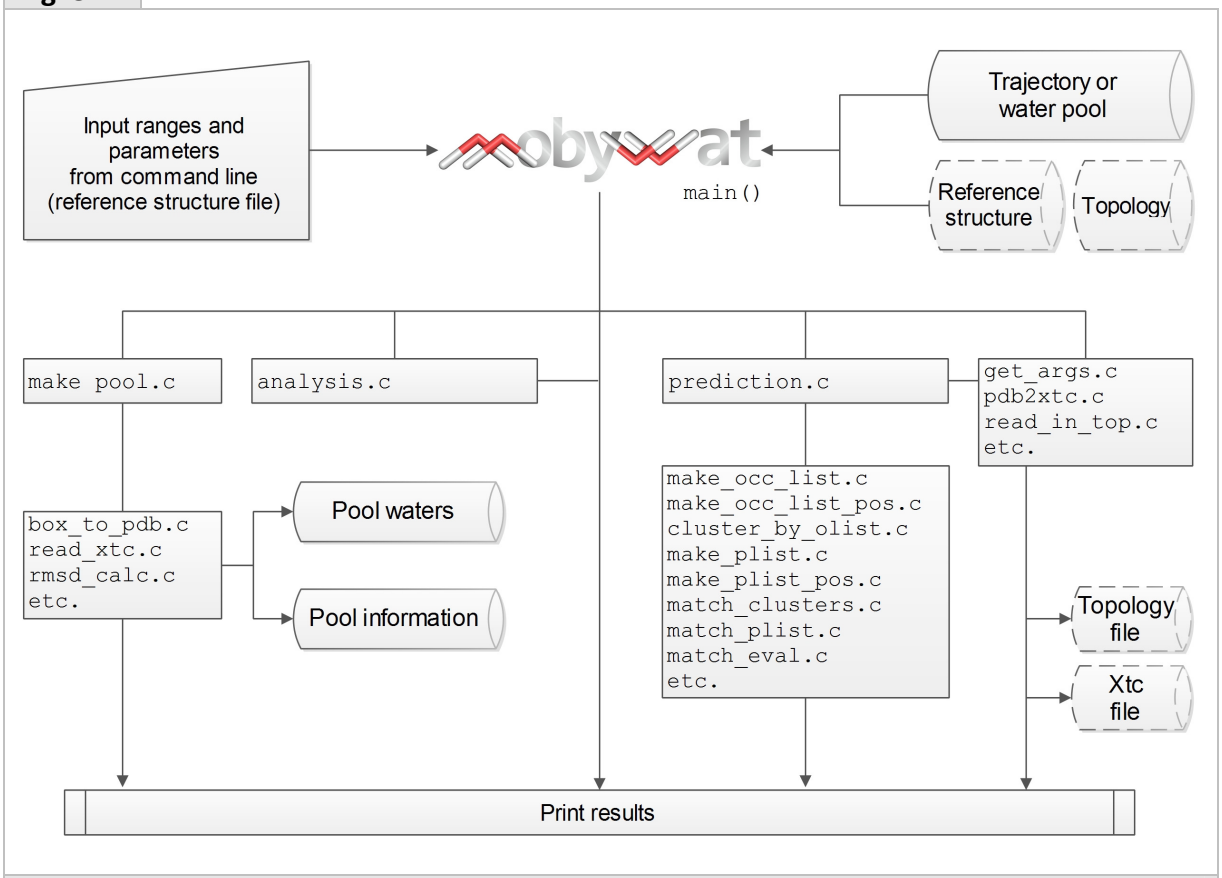
Quick help

5 Program details

5.1 Organization of code

MobyWat was written in standard C, and therefore, the source code shares known benefits of the language such as portability and efficient dynamic memory allocation. Thus, introduction of theoretical limit on system size was not necessary, which is beneficial for atomic systems of large target molecules of various sizes and up to tens of thousands of water molecules surrounding them in the simulation box. A schematic organization chart of the program is given in Fig. 5.1 as an overview of the main connections and I/O data flow in the code.

Fig. 5.1



Main connections and I/O flow in MobyWat source code

5.2 Water pool data type

MobyWat uses a pool list with spatial coordinates, atom- and residue serial numbers of oxygen atoms for identification of a water molecule in the pool (Fig. 5.2). The binary pool waters (plw, Section 4.1.4) file stores pool lists of all selected waters and all frames specified at command line. Besides the benefits of shorter repeated runs, creation of plw files allows efficient use of disk space and memory.

Fig. 5.2

```
struct pool_list {
      float x_coord;
      float y_coord;
      float z_coord;
      int aser;        // atom serial number
      int rser;        // residue serial number
      } ;

typedef struct pool_list pl;
```

Water pool data type

5.3 Binary trajectory files

At present, MobyWat handles xtc files. GROMACS xtc files are based on extended xdr libraries written using an algorithm where precision can be set by scale factor, typically 1000 used for multiplication of the coordinates in nm and the values are rounded as integers.
The modified xdr libraries were adopted by MobyWat for reading and writing xtc files, and the precision was increased to 10000 to handle PDB coordinates in Å from any source.
The modified xdr libraries and their documentation can be found at the following link.

http://www.gromacs.org/Developer_Zone/Programming_Guide/XTC_Library

6 Installation and tests

MobyWat is available free of charge for the scientific community. On the web page of MobyWat we provide a precompiled executable and test packages with detailed instructions which can be used without any further installation steps.
The User is encouraged to compile the program for his/her own machine. MobyWat is an open source program written in C, and therefore, it can be easily compiled and installed on practically any platforms.
For a standard GNU environment, some simple steps can be followed and the program is ready for use. First download the **mobywat.tgz** file from the program's web site into a directory called e.g. **$HOME/download** and type the following text at the command prompt.

```
$ cd $HOME/download
$ tar –xvf mobywat.tgz
$ cd mobywat/src
$ make
```

Now, you have the executable file named **mobywat** in **mobywat/src**. You can also use command **make install** to copy the executable file into **mobywat/bin** and **$HOME/bin**. Binary and object files can be removed from **mobywat/src** by typing **make clean**.

```
$ make install
$ make clean
```

7 Version history

7.1 MobyWat version 1.0

- I/O and other core functions
- Prediction mode
- SR calculation in analysis mode

7.2 Future plans

- Completion and test of analysis mode
- Coordinate-independent definition of water positions, generalization of ID-based approach

8 How to cite?

Jeszenői N, Horváth I, Bálint M, van der Spoel D, Hetényi C. (2015)
Mobility-based prediction of hydration structures of protein surfaces.
Bioinformatics, in the press.

9 Production of a trajectory

Trajectory files including all mobility information necessary for MobyWat analysis or prediction can be produced by any MD program package. In the following section, steps of preparation of trajectory of a target molecule called **system.pdb** are described using MD program package GROMACS ver. 5.0 Here, only short descriptions are given. For specific information on the contents on input files, and procedures, please refer to GROMACS web site. All example files specified in the following sections can be downloaded from the web page of MobyWat.

http://www.gromacs.org

9.1 Running MD calculations on a target protein

9.1.1 Preparation of a simulation box

As a first step target molecule is placed in a box and the box is filled up with water molecules. Coordinates of the box are stored in a file named **b4em.gro**. **Tip3p** water model, **Amber99sb-ildn** force field and a **cubic** box with 10 Å (=**1** nm) spacing were specified.

```
gmx pdb2gmx –water tip3p –ff amber99sb-ildn –ignh –f system.pdb
gmx editconf –o –d 1 –bt cubic –f conf.gro
gmx solvate –cp out –cs –o b4em –p topol
```

If it is necessary, neutrality of the system can be achieved by adding **X** copies of positive (**Na^+**) or negative (**Cl^-**) ions to the box.

```
gmx grompp –v –f steep –c b4em –o em –p topol
gmx genion –s em.tpr –o ion_b4em –p topol –pname NA –np X
gmx genion –s em.tpr –o ion_b4em –p topol –nname CL –nn X
```

9.1.2 Energy minimization of the system

Before launching productive MD calculations it is advisable to energy minimize the content of the box. Here, commands of a two step minimization are shown including steepest descent and a conjugated gradient runs. Minimizations are performed by the **mdrun_d** (double precision executable) program and the binary inputs are produced by **grompp**. Note, that **–c ion_b4em** can be specified instead of **–c b4em** if you have added neutralizing ions to your box (Section 9.1).

```
gmx grompp –v –f steep –c b4em –o st –p topol.top
gmx mdrun_d –v –s st –o st –c after_st –g st
gmx grompp –v –f cg –c after_st –o cg –p topol.top
gmx mdrun_d –v –s cg –o cg –c after_cg –g cg
```

9.1.3 Producing trajectory file

Final inputs are produced from the energy minimized system **after_cg** and MD calculations can be launched using **mdrun**. Trajectory of the system is stored in an **md.trr** file specified at switch **–o**.

```
gmx grompp –f md –o md –c after_cg –r after_cg –p topol.top –maxwarn 1
```

```
gmx mdrun -v -s md -e md -o md -c after_md -g md.log
```

9.2 Preparation of the trajectory for MobyWat

9.2.1 Preparation of the trajectory for prediction

Once you have your trajectory in an **md.trr** file fast conversions are recommended using **trjconv**. Such conversions handle periodic boundary effects, center the system in the box and fit target molecules in subsequent frames on the top of the first frame (Section 3.2.2).

```
gmx trjconv -f md.trr -s md.tpr -o pbc_1.xtc -pbc whole <<EOF
0
EOF
```

```
gmx trjconv -f pbc_1.xtc -s md.tpr -o pbc_2.xtc -pbc cluster <<EOF
1
0
EOF
```

```
gmx trjconv -f pbc_2.xtc -s md.tpr -o pbc_3.xtc -center -pbc mol -ur
compact <<EOF
1
0
EOF
```

```
gmx trjconv -f pbc_3.xtc -s md.tpr -o system.xtc -fit progressive <<EOF
3
0
EOF
```

In the last command line, instead of **–o system.xtc**, a switch **–o system_mdl.pdb or –sep –o system_.pdb** can be specified for an NMR-type PDB file or separate PDB files (Section 4.1), respectively.
A topology file system_tpy.pdb can be easily produced for MobyWat by **trjconv**.

```
gmx trjconv -f pbc_3.xtc -s md.tpr -o system_tpy.pdb -b 0 -e 0 -fit
progressive <<EOF
3
0
EOF
```

The **system.xtc** (or the corresponding pdb files) and **system_tpy.pdb** can be used as input for MobyWat.

9.2.2 Preparation of the trajectory for analysis or validation

If you want to compare the trajectory in **md.trr** to a reference crystallographic structure (Sections 3.1 and 3.3) named as e.g. **system_ref.pdb**, you will need to fit your trajectory onto the initial structure with waters using **confrms** (Section 3.1.2). The following command specifies multi-frame fit using C_α atoms of the protein backbone.

```
gmx confrms -label -one -f1 system_ref.pdb -f2 md.tpr -o fit.pdb <<EOF
3
3
EOF //fit to alpha carbons
```

A chain ID “**A**”can be added using **editconf**.

```
gmx editconf -label A -f fit.pdb -o fit.pdb
```

```
gmx trjconv -f md.trr -s md.tpr -o pbc_1.xtc -pbc whole <<EOF
0
EOF
```

```
gmx trjconv -f pbc_1.xtc -s md.tpr -o pbc_2.xtc -pbc cluster <<EOF
1
0
EOF
```

```
gmx trjconv -f pbc_2.xtc -s md.tpr -o pbc_3.xtc -center -pbc mol -ur
compact <<EOF
1
0
EOF
```

```
gmx trjconv -f pbc_3.xtc -s fit.pdb -o system.xtc -fit progressive <<EOF
3
0
EOF
```

Preparation of topology file is also similar to Section 9.2.1.

```
gmx trjconv -f pbc_3.xtc -s fit.pdb -o system_tpy.pdb -b 0 -e 0 -fit
progressive <<EOF
3
0
EOF
```

10 References

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