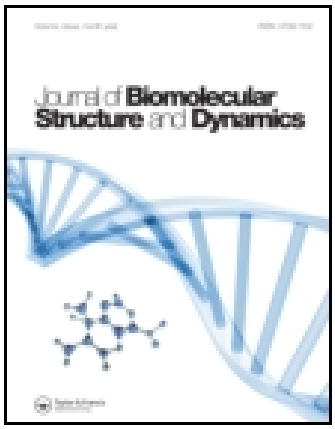

Journal of Biomolecular Structure and Dynamics
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbsd20

Deducing Hydration Sites of a Protein from Molecular Dynamics Simulations

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Published online: 15 May 2012.

To cite this article: M. S. Madhusudhan & S. Vishveshwara (2001) Deducing Hydration Sites of a Protein from Molecular Dynamics Simulations, Journal of Biomolecular Structure and Dynamics, 19:1, 105-114, DOI: 10.1080/07391102.2001.10506724

To link to this article: http://dx.doi.org/10.1080/07391102.2001.10506724

Journal of Biomolecular Structure & Dynamics, ISSN 0739-1102
Volume 19, Issue Number 1, (2001)

Deducing Hydration Sites of a Protein from Molecular Dynamics Simulations

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Abstract

Invariant water molecules that are of structural or functional importance to proteins are detected from their presence in the same location in different crystal structures of the same protein or closely related proteins. In this study we have investigated the location of invariant water molecules from MD simulations of ribonuclease A, HIV1-protease and Hen egg white lysozyme. Snapshots of MD trajectories represent the structure of a dynamic protein molecule in a solvated environment as opposed to the static picture provided by crystallography. The MD results are compared to an analysis on crystal structures. A good correlation is observed between the two methods with more than half the hydration sites identified as invariant from crystal structures featuring as invariant in the MD simulations which include most of the functionally or structurally important residues. It is also seen that the propensities of occupying the various hydration sites on a protein for structures obtained from MD and crystallographic studies are different. In general MD simulations can be used to predict invariant hydration sites when there is a paucity of crystallographic data or to complement crystallographic results.

Introduction

It has been well documented that water molecules play a pivotal role in the structural stability and functionality of a protein [1]. In several cases, such key water molecules have been identified and their roles characterized [2]. The identification procedure makes use of a static picture provided by structure solution by X-ray crystallographic methods. Though individual structures give the location of the water molecules, their importance is deduced only after verifying their existence in the same locality in several other structures of the same protein or related proteins. Some studies have focussed on removing the artifacts that crystal environments impose on the hydration pattern [3]. The presence of water in the same spatial location, interacting with the same or equivalent residues in similar proteins is usually construed to be an "invariant" water molecule. To these invariant water molecules are then ascribed structural or functional importance.

In this study we examine hydration sites of a protein as detected from an analysis of Molecular Dynamics (MD) simulation structures. The results of the analysis on Ribonuclease A (RNase A), Hen egg white lysozyme (HEWL) and Human Immuno-deficiency Virus 1-protease (HIV-1 protease) are compared with an analysis done on their respective crystal structures. The aim is to establish whether hydration sites remain invariant even when we analyze structures that are snap shots from a dynamic process (MD) in a fully solvated environment. A preliminary implementation of our method has been reported earlier [4]. It is further intend to

Abbreviations:
Molecular Dynamics (MD), bovine pancreatic ribonuclease A (RNase A), hen egg white lysozyme (HEWL), accessible surface area (ASA), root mean square (RMS), picosecond (ps)

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use such a comparison to evaluate whether this process of identifying invariant hydration sites can be applied to a general case where there is insufficient crystal structure data to ascribe importance to particular hydration sites. MD simulations studies have been carried out on three systems, as listed above. While two of them (RNase A and HEWL) are monomers, the HIV-1 protease is a dimer and in this case has been taken along with a tripeptide ligand. The choice of the systems was made because of the abundance of crystal structures available for comparison, besides earlier analyses on hydration sites from these structures [5,6,7]. A comparison of our procedure for hydration site detection with the many crystal structures of RNase A and HEWL and with earlier studies serves as a benchmark. Several studies have also been carried out using both Xray Crystallography [8] and NMR spectroscopy [9] to elucidate the structure of HIV-1 protease and the role played by water molecules in the reaction it catalyses. This system serves as a good test case for our method.

Methods

Simulation Protocol All simulations reported in this study have been carried out using the AMBER 4.1 suite of programs [10]. The starting structures for the simulations of RNase A, HEWL and HIV-1 protease were their respective crystal structures whose PDB codes are 7rsa, 1hel and 1ytg respectively. The all-atom models [11] of these structures were solvated using a box of TIP3P [12] water molecules. The amino acids Asp and Glu carried net negative charge while the residues His, Arg and Lys all carried a net unit positive charge. A constant dielectric value of 1 was used. The extent of solvation was to account for at least two hydration shells of water. Most importantly, waters of crystallization were not used in the simulation systems. The system sizes in the 3 simulations were 9062 atoms (2525 waters) in RNase A, 8616 atoms (2348 waters) in HEWL and 11369 atoms (2930 waters) in HIV-1 protease. The systems were then energy minimized. The first 200 steps of minimization made use of the steepest descent algorithm while the rest of the 1000 steps utilized the conjugate gradient method.

The minimized system was then subjected to 1.02 nanoseconds of MD in two stages. In the first stage, 20 picoseconds of MD was carried out in the NVT ensem-

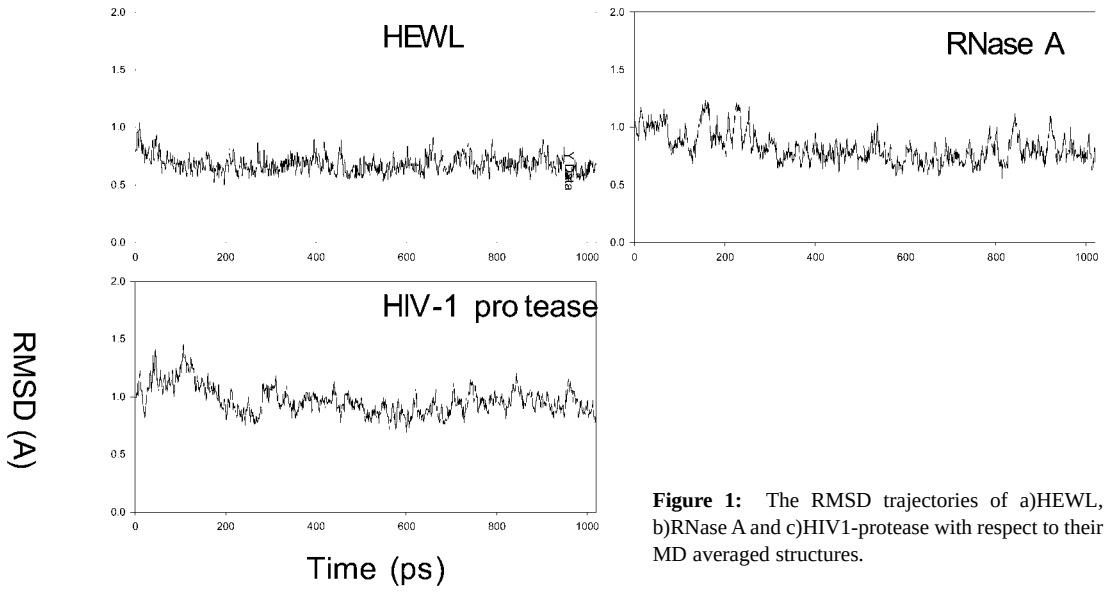

Figure 1: The RMSD trajectories of a)HEWL, b)RNase A and c)HIV1-protease with respect to their MD averaged structures.

ble. The system was coupled to a heat bath using Berendsen's coupling [13], with a coupling constant of 0.1ps. the temperature of the system was kept at 100K for the first 4ps, then raised to 200K for the next 4ps and finally raised to 300K for the

next 12ps. At the end of these 20ps, the coupling of the heat bath was removed and 1 nanosecond of dynamics in the NVE ensemble marked the second stage. SHAKE [14] was used to constrain all covalent bonds. A time step of 2 femtoseconds was used throughout the simulation. Particle Mesh Ewald sum (PME) [15,16] method was used to compute long range interactions. During the simulation, trajectory snapshots were stored every 1ps.

Analysis

The analyses of all MD trajectories were carried out using programs coded in FOR-

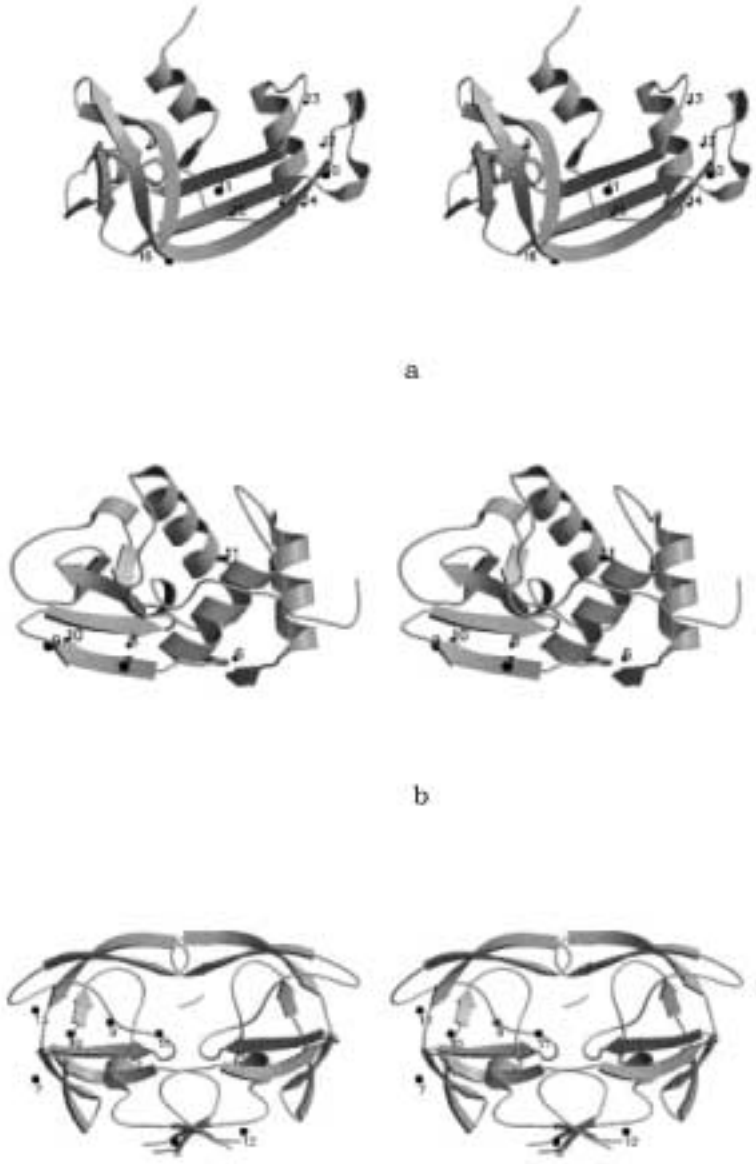

Figure 2: Stereo views of cartoons (MOLSCRIPT[22]) of a) RNase A, b) HEWL and c) HIV1-protease. The water molecules that are invariant in both the crystal structures and MD structures analysis are shown and numbered according to their appearance in tables 1,2 and 3 respectively

TRAN. For the simulations the Root Mean Square Deviation (RMSD) from its starting and time-averaged structures, residue wise RMS fluctuation were calculated. Along with these analyses, the acceptor-donor distance of potential hydrogen bonding pairs was also monitored. The criteria for hydrogen bond formation are, a donor acceptor distance of less than 3.6Å and a proton-acceptor distance of less than 2.6 Å.

Accessible Surface Area The surface accessible areas were calculated using the algorithm of Lee and Richards [17]. A probe sphere of 1.4 Å was used. The average and RMSD of the ASA were found using snapshots from the MD simulation.

Conserved hydration sites from crystal structures. For the 3 systems RNase A, HEWL and HIV-1 protease, high resolution (< 2Å) crystal structures of their native or mutants were taken for analysis. The PDB codes of the structures are:- RNase

Table I

Residues in RNase A that are bridged by 'invariant' water molecules in Crystal and MD snapshot structures. The waters marked with an "*" in are those that are invariant in the low hydration structures.

water number	interactions in crystal structures	interactions in MD structures
1*	Glu49Oε1,Oε2; Ser50N,Oγ; Asp53Oδ2	
2*	Ala5O; Pro117O	
3*	Asp83O,Oδ1,Oδ2; Lys98O; Thr100N,Oγ1	
4*	Gln60Oε1,Nε2; Tyr76N; Ser77N,Oγ	
5*	Gln69Oε1,Nε2; Asn71Oδ1,Nδ2; Glu111Oε2	
6	Ala4O; Val118O	
7*	Glu9Oε2; Gln55Nε2,Oε1	
8*	Asn27N,Oδ1,Nδ2; Tyr97O,N; Thr99Oγ1,O	Asn27Oδ1,Nδ2; Tyr97O,N; Thr99Oγ1
9	Lys66N; Asn67N,Oδ1,Nδ2; Asp121Oδ1,Nδ2	Lys66Nζ, N; Asn67N; Asp121O,Oδ1
10	Asn27O,Oδ1,Nδ2; Cys95O	Asn27Oδ1,Nδ2; Cys95O
11	Thr17O; Ser18O; His48O	Ser18O; His48O; Ser80Oγ1
12	Cys40O,N; Glu86Oε1,Oε2	Cys40O; Glu86Oε1,Oε2
13*	Thr36 Oγ1,O; Pro93O	Thr36 Oγ1,O; Pro93O
14*	Ser23O; Thr99N	Ser23O; Thr99N; Gln101N
15	Thr45Oγ1; Asp83Oδ2,Oδ1; Ser123Oγ	Thr45Oγ1,N; Asp83Oδ2,Oδ1; Ser123Oγ
16	Thr78Oγ1; Asn103Nδ2,Oδ1	Thr78Oγ1,O,N; Asn103Nδ2,Oδ1
17		Ala20O,N; Ile81N,O; Gln101O,Nε2,Oε1
18		Arg10NH1,NH2; Asn34O,Oδ1
19		Gln11Nε2,Oε1; His12Nε2; His119Nδ1
20		Asp14Oδ2; Thr25OH; Met29Sδ
21		Lys41O; Tyr97OH
22		Lys37N,O; Asp38Oδ1,Oδ2; Arg39NH1,NH2; Tyr92OH

A (1a5p, 1rbx, 1rnd, 1rnx, 1rpg, 3rn3, 3rsd, 3rsk, 3rsp, 4rsd, 4rsk, 7rsa), HEWL (132l, 193l, 194l, 1at5, 1at6, 1bvx, 1bwh, 1bwi, 1bwj, 1rks, 1lsm, 1lsn, 1lsy, 1hel) and HIV-1 protease (1a30, 1ask, 1a94, 1ajv, 1ajx, 1d4y, 1dif, 1hpv, 1htg, 1hxw, 1mtr, 1odx, 2aid, 7upj, 1ytg). All these structures were superimposed on the crystal structure taken for the simulation. If in more than half the structures taken there were water molecules that occupied the same spatial location (within 2Å of the corresponding water molecule in any other structure) and had at least one water-protein interaction in common with the corresponding water molecules in other structures, it was taken to be a conserved hydration site.

Invariant water analysis from MD structures. Structures are extracted from the simulations at intervals of 10ps. (Water in the bulk will diffuse about 2.8 Å in 10ps). This way there are about 100 structures extracted from each simulation.

These structures are all superimposed [18] on a reference structure. Only waters that are within hydrogen bonding distance of any protein atom are retained for analysis. The proteins and the water molecules are then enclosed in a box of dimensions 160 X 160 X 160 $Å^3$ (radius of gyration of the protein ~15 Å). This box is further compartmentalized into a million cubes each of side 1.6 Å. The longest distance, along the diagonal of the box is 2.8 Å, which is the minimum separation between 2 water molecules (from radial distribution calculations). This choice of box dimension ensures single occupancy per structure. On an average the occupancy of these boxes by first hydration shell waters is 2.6. By contouring for boxes that have an occupancy of 15 or more we pick out the most occupied boxes. Since the positioning of the boxes is arbitrary, the waters in the neighbouring boxes are also considered while contouring if the water molecules in these boxes are within 2.8 Å of those in the original. The interactions of the water molecules that fill in the most occupied boxes are studied. The water molecules that "invariantly" bridge two or more residues are taken to be of interest. The water molecules that have interactions with single residues are not considered.

Analysis similar to that described here has been carried out earlier on simulation data of proteins [19] and nucleic acids [20].

Table II

Residues of HEWL that are bridged by 'invariant' water molecules identified from an analysis of Crystal and MD snapshot structures. The waters marked with an "*" in are those that are invariant in the low hydration structures.

Water number	interactions in crystal structures	interactions in MD structures
1	Lys6O,N; Arg128O,N; Arg5 N	
2	Lys1N,O; Asn39Oδ1,Nδ2; Gln41Nε2,O	
3	Thr40O; Ile55N; Leu83 O; Ser91Oγ	
4	Tyr53O; Ile55N; Leu56N; Ser91Oγ	
5	Asn65Nδ2,Oδ1; Asp66N,Oδ1; Ser72Oγ	
6*	Lys1N,Nz; Ser86Og,O	Lys1N, Nζ; Ser86Oγ
7*	Asp52Oδ2,Oδ1; Gln57Oε1,O	Glu35O,N; Asn44Nδ2,Oδ2; Asp52Oδ1,Oδ2; Gln57Oε1,O
8*	Ala107O; Val109N	Gln57 Oε1,O; Ala107O,108; Val109O
9*	Arg45NH2; Gly49O; Arg68O,NH2,Nε	Arg45 Nε,NH2; Gly49O; Arg68Nε,NH2
10	Gly49O; Thr51N,Oγ1; Asp66Oδ2; Arg68Nε; Thr69Oγl	Gly49O; Thr51Oγ1;Asp66 Oδ1,Oδ2; Thr69
11	Asn27Oδ1; Gly117N; Thr43Oγl	Asn27Oδ1;Trp111N,O
12		Asn46O,Nδ2; Asp52Oδ1,Oδ2
13		Asp48Oδ1,Oδ2,O; Arg61Nε,NH2,N
14		Asp65Oδ1,Oδ2; Asn74Oδ1,Nδ2,O
15		Gly16 O; Lys96Nζ,O,N
16		Glu35Oε1,Oε2; Val109N
17		Tyr53O,OH; Asp66Oδ1,Oδ2; Arg68N,Nε

Results and Discussion

Figure 1 shows the RMSD fluctuations of the three systems RNase A, HEWL and

HIV-1 protease during the course of dynamics about their <MD> structure. The flat trajectory in all three simulations indicates that the systems were well equilibrated.

1. Invariant water molecules in RNase A and HEWL In tables 1 and 2 are listed the invariant water molecules as identified by both an analysis on crystal

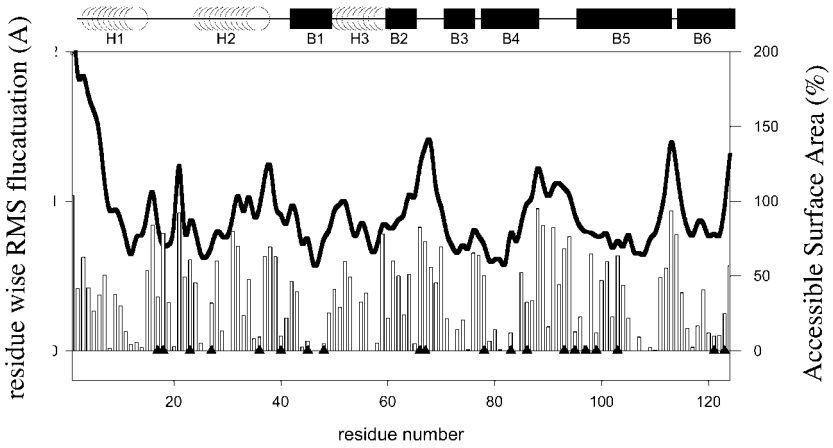

Figure 3: The residue wise RMS fluctuation (dark lines) in RNase A is co-plotted with the average accessible surface area (vertical bars) in the simulation against the residue number. Shown in dark triangles are the residues that interact with the invariant water molecules that are common to both the crystal structures and MD structures. The shaded histograms represent residues that interact with the waters that are found invariant only in the MD structure analysis

structures and on the MD simulation structures on RNase A and HEWL. This list segregates water molecules that are identified as invariant in only the crystal structure analysis, invariant in both the MD and crystal structure analyses and those that are identified only from the analysis on the MD structures. Figure 2a and 2b are cartoon representations of these two proteins and the water molecules that invariantly solvate them in both the crystal and MD structures.

1a. Comparison of the MD and crystallographic analysis From the comparison of MD and crystal structures, not all water molecules that are picked up as

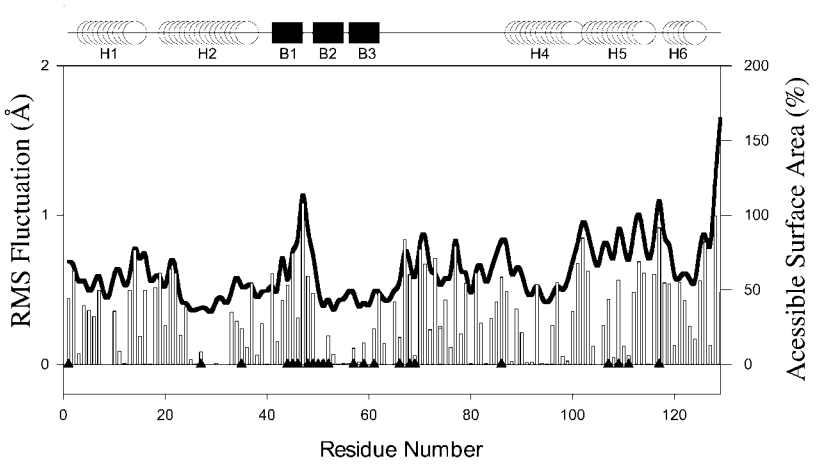

Figure 4: A plot similar to figure 3 for HEWL

invariant in one set feature in the other. The waters identified in the two analyses belong to three broad categories, a) those that are common to MD and crystallography, b) those present only in the crystallographic analysis and c) those present only in the MD analysis. Between the two sets however there is good correlation (~55% in common). Of the 16 waters identified as invariant from the crystal structures, 9 are in common to those identified by the MD structures analysis on RNase A. A similar analysis on HEWL yields 6 waters common to the 11 identified as invariant in the crystal structures. These common waters interact with residues important in catalysis. Residues 45, 123 and 83 in RNase A, that interact with water number 15 form a part of the substrate binding site [21]. It is also known that a water in this location plays a crucial role in imparting substrate specificity to RNase A. In HEWL, water numbers 7 and 8 interact with residues 52, 57, 107 and 109 that

are part of the active site cleft [7]. However, from the common list (tables 1 and 2) it can be observed that the residues being bridged are not always identical. This is because the dynamic picture obtained from the MD analysis allows for water molecules to sample other interactions depending on the local potential and structure of the protein.

Water molecules that feature as invariant in the crystallographic list only interact

Table III
Residues of HIV-1 Protease that are bridged by 'invariant' water molecules identified from the crystal and MD snapshot structures.

water number	interactions in crystal structures	interactions in MD structures
1.	Pro39O; Arg41O; Asp 60 N	
2.	Cys95A O; Leu5B N	
3.	Arg87A NH2,NH1; Trp6B O; Leu5B O	
4.	Thr31 N,O; Thr74 O; Asn88 Oδ1,Nδ2	
5.	Lys70 O; Gln92 O	
6.	Ile50A N; Ile50B N	
7.	Gly16 N; Leu63 O	Gly16 N, Leu63 O
8.	Gly94A O; Thr96A Og1; Asn98B O	Thr96AO,Oγ1, Asn98BOδ2
9.	Glu21 O,Oε2,N; Asn83 N,Nδ2	Lys20N,Nζ; Glu34O,Oδ2; Asn83N,O
10.	Gln61 N,Nε2; Thr74 N,Oγ1	Asp30Oδ2; Asp60Oδ2,Oδ1,O; Thr74,O,Oγ1
11.	Thr26A O; Gly27A O; Asp29A O,Oδ1; Arg87A Nε; Arg8B Nε	Gly27A O; Asp29AO,Oδ1; Arg87A Nε,NH2; Lys7B Nζ; Arg8B O,NH2
12.	Phe99A O; His69B Nε2; Ile93B O	Phe99AO; His69B N,Nε2
13.	Leu38 O; Gly40 O; Tyr59 OH	Trp42O,N; Tyr59OH
14		Glu35Oε1,Oε2; Arg57NH2,O,N
15		Trp42 N; Arg57Nε,NH2
16		Gly48O; Val201O,N
17		Asp25A Oδ1,Oδ2; Asp25B Oδ1,Oδ2; Pro199O

with residues with which water in the simulation do not have predominant interactions. In some cases this is due to the high RMSD of the residues that the water bridges. Residue 69, 71 and 111 in RNase A are typical examples of this case. The RMS fluctuation of the main chain of these three residues in the MD simulation is 0.48Å. This is in comparison with an RMSD of 0.1Å in the Xray structures used in the analysis. There could be a network of water molecules that bridge these residues when they go farther apart, as they do in MD simulations. Our method is however not equipped to compute the presence of such water clusters.

Another category of invariant water molecules are those that are identified only by MD simulation analysis and not by an analysis on X-ray structures. These water molecules are present in only some of the crystal structures. They do not feature as invariant on the X ray crystallographic list since they do not feature in most structures

1b. Correlating hydration sites with ASA, secondary structure and RMS fluc-

tuations From figures 3 and 4, it is apparent that there is no cognizable pattern that links the surface accessible area of residues to the location on invariant waters. The residues that interact with the 'invariant' waters are not all buried (a buried residue has an ASA of 40% or less of the maximum). There also appears to be no particular preference of secondary structure to which these invariant waters will interact. In the case of RNase A and its ligand bound complexes it was earlier noted [5] that invariant waters predominantly interact with residues that belong to helices. From the MD simulation on lysozyme and RNase A this trend is not observed. There is also no apparent correlation between the RMSDs of the residues interacting with the invariant waters. Hydration appears to be dependent on local potential, which differs from one fold to another.

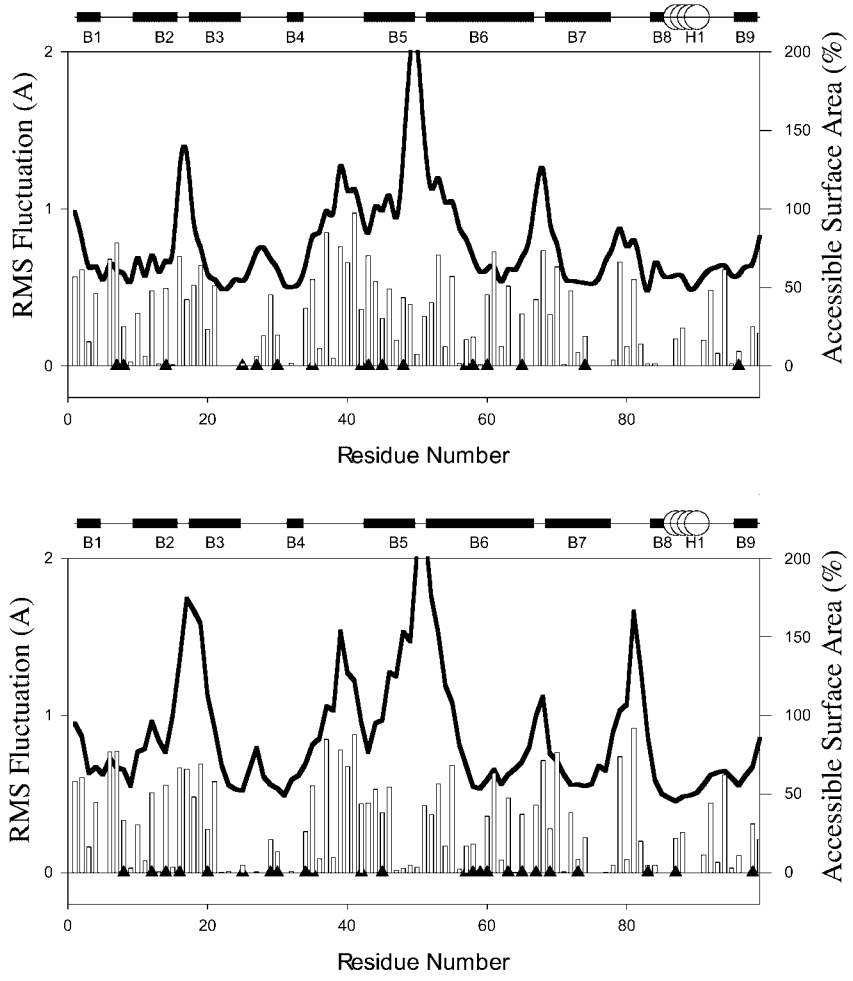

Figure 5: A plot similar to figure 3 and 4 for the two monomers (a) and (b) of HIV1-protease.

1c. Comparing with low hydration crystal forms For RNase A and lysozyme there have been crystallographic studies carried out at low hydration levels [6,7]. The aim of these studies is to deplete the protein structures of water and then attribute structural/functional significance to those that remain. It is found that correlation between MD structure data and these studies is poor though the low hydration crystal structures are almost identical to the structures at normal hydration. One reason could be the absence of some functional waters (which may not have structural importance) from the low hydration structures because of crystallization conditions like low pH. For instance, water 15 in table 1 is of functional importance to RNase A as discussed earlier and is common to both the MD and crystal structure analysis. This water is however absent in most low-hydration crystal forms. It should be noted that a relaxation in the cut-off criteria for choosing 'invariant' water molecules from MD simulations increases the correlation with both low hydration and regular crystal structures. This however enormously increases the number of invariant waters

leading to the inclusion of several less important waters.

2. Hydration sites on HIV-1 protease This water site prediction procedure has been used to locate invariant hydration sites on the homo-dimeric system of HIV-1 protease that has a tripeptide product attached to it. The results of our analysis are represented in table 3 and pictorially in figures 5a and 5b. 7 of the 13 waters identified as invariant by the x-ray structure analysis forms part of the MD simulation analysis list. Some key interactions like those made by water number 6 in table 3 [8] have not been picked up by the MD simulation analysis. The reason for this is apparent in figures 5a and 5b, which show a very high RMSD for the residues Ile50 from each monomer, residues bridged by this water in the X-ray structure. This high RMS value is of biological significance as these residues form part of a flap that is postulated to open and close to admit substrates for proteolysis. The flap region in one of the monomers also has a lower average ASA when compared to the other. This is because of the binding of the ligand more towards one of the monomers than the other. This is brought out in table 3 where 2 water molecules bridge residues of the ligand to one of the monomers of the proteins. All other interactions where water bridges are formed are symmetrical with respect to the two monomers. The location of the water molecules invariant to both the crystallographic and MD analysis are shown in figure 2c.

Conclusions

An algorithm to identify "invariant' Water molecules from structures obtained from MD simulations have been tested on three proteins (RNase A, HEWL and HIV-1 protease) on which extensive crystallographic data is available. Several hydration pockets and sites dot the surface of the protein structure. The occupancy of these sites depends on the environment. From the analysis of MD structures to select invariant hydration sites, there seems to be good correlation with similar analyzes done on crystal structures. Though there are several key residues that are bridged by these 'invariant' water molecules that feature in both analyses, there are some that feature in only one of the two lists. These sites are picked up in either one of the analyses with a lower priority to be considered 'invariant'. In that sense the MD simulation data analysis seems to reorganize the propensities of occupancy of hydration sites as detected by crystallography. Even amongst the residues that are bridged by waters common to both analyses, there are instances where only one residue is in common while the other bridged residues could vary. Water molecules, which are very mobile entities in MD simulations could sample other local interactions and favour one over many others depending on the relative strengths of the hydrogen bonding potentials. Our procedure can be utilized to find hydration sites in cases where there is a dearth of crystal structure data.

Acknowledgements

We wish to thank the Supercomputer Education and research Centre for computation facilities. J.V. Pratap is thanked for useful discussions. Support from the Department of Science and Technology (DST grant NO: SP/SO/D-107/98) is acknowledged.

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Date Received: September 30, 2000

Communicated by the Editor Manju Bansal