An Investigation on the Use of Constraints-Applied Voxels in the Search for Protein Surface Atoms

Ling Wei Lee, and Andrzej Bargiela, *Member, IEEE*

***Abstract*—The functionalities of proteins are attributed to atomic arrangements on surfaces which define the reactivity of regions that bind with external agents. A good extraction of protein surface atoms not only provides a ready list of dataset for surface studies, but it also reduces the amount of processing required in computer-aided drug design programs by displaying only the exterior portion of the protein. Software methods for molecular surface studies typically implement algorithms of a probe or geometrical nature, with the latter represented as alpha/beta shapes, Voronoi tessellations etc. Grid units and voxels were used in some of the earliest programs for dock sites identification; however the role and contribution of voxels in the extraction of surface atoms has not been investigated. We present here such a method with constraints applied to the voxels in the form of voxel occupancy and atomic membership, and the approach concludes with a 'peeling' method for the removal of internal atoms in the extracts. The obtained results are visualised and compared against output from the MSMS and Surface Racer programs for accuracy verification.**

***Index Terms*—constraints-filtering, protein-surface-atoms, space-voxelisation, voxel-based-analysis.**

I. Introduction

THE selective behaviour of proteins in binding to specific agents is attributed to the arrangement of atoms on the surface [1]-[3]. According to [4], "protein surface comparison is a hard computational challenge and evaluated methods allowing the comparison of protein surfaces are difficult to find". Past implementations for the representation and study of protein surfaces include approaches such as triangulation, Voronoi tessellations, lattice modeling, geometric hashing etc [5]-[8]. Another approach is the convex hull [9] – defined as the smallest convex polyhedron enclosing all atom centers and is a subset of the Delauney triangulation. Methods enlisting the use of polyhedrons calculate estimations of the protein's surface, and details may be sacrificed due to generalisation of the features. However significant regions are usually retained in the simplification process.

Probe-based methods for studying molecular surfaces can be traced back to some of the earliest implementations such as the Connolly algorithm [10]. A probe sphere – usually the size of a water molecule – is used to inspect molecular surfaces. Concepts such as Solvent Excluded Surface (SES) [11] and Solvent Accessible Surface (SAS) [12] are manifestations of the probe technique. Sanner [13] introduced a program called MSMS which integrates a reduced surface version of both SES and SAS for the fast examination of proteins. Users are given options to change the probe size as well as commands for generating different types of output files. The Surface Racer [14] program executes calculations for the exact accessible surface area, molecular surface area as well as the average curvature of molecular surface. Users are allowed to specify the size of the probe used and the algorithms they would like executed.

The use of grid spaces or voxels in the study of proteins has been used in early programs for locating cavities on protein surfaces [15, 16]. The POCKET program [17] uses grids and a test sphere to identify possible cavities with the surfaces of the cavities modeled using a variation of the marching cubes algorithm. Hendlich et al [18] presented a similar method termed LIGSITE with additional rigorous scanning to overcome the grid-space induced rotational problems in POCKET. Claimed to be fast LIGSITE is capable of locating potential sites to high precisions.

Grid spaces offer a robust environment for the study of proteins as proven in the discussed programs. As a universe, a grid space constitutes of subsets in the form of units or voxels. Objects in such spaces may be represented and defined in terms of the number of units occupied, the estimated total surface area and volume from the voxels, as well as the overall shape based on the voxels cluster. Partitioning can be carried out to divide a space into units of desired sizes. However the orientation problem associated with the rigidity of a grid space is inherent and remains a challenge. Different orientations of an object often result in varied sets of shortlisted voxels.

A good surface atoms extraction algorithm is capable of providing full atoms listings for the study of binding sites located on surfaces. When used in computer-assisted drug design (CADD) programs the exclusion of internal atoms lead to reduced processing time. In a recent study Kim et al [19] presented a real-time method for the locating of boundary surface atoms on a GPU. A previous implementation by [20] identified surface entries based on computed atomic contribution to the SAS area. In an attempt to investigate if voxels are capable of extracting

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Ling Wei Lee is with the University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia. (e-mail: leelingwei@yahoo.co.uk).

Andrzej Bargiela is with the University of Nottingham Jubilee Campus, Wollaton Road, Nottingham NG8 1BB, UK. (e-mail: Andrzej.Bargiela@nottingham.ac.uk).

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surface atoms and producing competitive results, we propose a method wherein constraints are introduced for units and atoms filtering. Using only spatial coordinates, atom type information and van der Waals radii we show that a combination of voxel properties and experimentally-derived constraints are capable of delivering promising results.

II. Surface Atoms and Data Preparation

A. Background

A surface atom can be defined as an atom lying on the outermost layer of a molecule and is exposed to the external environment. An atom may or may not be completely/partially occluded by neighbouring atoms, with the probability of the atom in participating in interactions dependent on its externally exposed area. A fully exposed atom is a definite surface entry; an occluded atom is considered as internal with a low or almost no chances of contributing to binding activity. However a partially occluded atom has equal chances of being accepted or rejected – the acceptance condition being that the exposed area must be sufficiently large for interaction with an external atom or for the probe to produce contact with in probe-based studies. [20] stated that a surface entry "must not only be exposed at the van der Waals surface...but must also be exposed at the so-called SAS of the macromolecule".

B. Pre-processing

In the search for all surface atoms of an input protein, the data sources must first be obtained. Three sets of data have been selected for this study. The first set contains a total of three FK506-binding proteins [PDB: 1YAT, 1BKF, 1FKF]. The second set contains experimental entries from [20] which are [PDB: 6CHA, 1RA2, 3FXN, 7TLN, 1TIM, 3RTA] and the third set are entries from [19] listed as [PDB: 2PLT, 1A19, 1Q3Y, 1QBS, 1EA1]. All files are downloaded from the RCSB Protein Data Bank in PDB format. The spatial coordinates and type for each atom are extracted and stored in a separate file and each atom is supplied its van der Waals radius. All coordinates are then checked and translated to the all-positive quadrant of which purpose is to facilitate image visualisations of the atoms.

III. Method

A. Preparing the Environment

Using the pre-processed information the protein is first projected into a 3D grid environment – the size of which is dependent on the protein. Van der Waals radii range from about 1.0 Å to 2.0 Å (diameter ~2.0 Å to 4.0 Å) with the exception of several elements. As such a value of 4.0 Å was chosen for each voxel unit – a value sufficiently large for a unit to fully encapsulate most atoms. To reduce processing time we targeted only the space enclosing the protein and discarded the rest. This optimal area is determined by locating the maximum and minimum coordinates of the protein for all axes and then identifying the upper and lower bound values. For example, a min-X of 6.8 and max-X of 18.9 leads to a lower bound of 4.0 and upper bound of 20.0. These values are dependent on the size of the units, i.e. 4.0 Å. The process is repeated for all axes until the enclosing space has been fixed.

Experiments have been conducted prior to the selection of 4.0 Å as the unit size to determine the optimum value. At a voxel size of 8.0 Å, crevices were overlooked whereas at size 2.0 Å the method picked up small existing spaces between atoms. Taking into consideration that most atoms are >2.0 Å in diameter, it is therefore unlikely that spaces of 2.0 Å contribute significantly to ligand-binding activity. A reasonable assumption can be made that a voxel of 4.0 Å accommodates most atoms with respect to the radii range.

B. Representing the Protein

The next stage is the identification of units occupied by the protein in the test space. As the 3-dimensional grid environment induces high processing complexities, an approach was taken to reduce the dimensionality. A 'slicing' process was used to split one of the three axes into a series of images. This is conceptually similar to the Z-buffer in 3D graphics. The number of images obtained is equivalent to the number of segments occupied in the chosen axis – of which the Z-axis was selected in the studies. Details of this method are documented in [21]. Simple image processing techniques were applied to the images to determine voxels containing parts of the protein. By default a voxel is selected as long as a single pixel belonging to the protein is detected. This is not feasible as only certain atoms are selected (Fig. 1). A condition for selecting voxels containing the optimum number of atoms is required and this is introduced in the form of a constraint termed the 'voxel occupancy'. A series of statistical studies have been carried out to determine the best value and a percentage of 40%-100% occupancy was found to produce highly consistent output from a range of test sets [22]. Fig. 2 shows a sample image for a 40%-100% occupancy applied to the voxels.

C. Selection of the Surface Voxels and Atoms

Referring again to Fig. 2 it can be seen that the surface atoms of the protein are enclosed within the outermost voxels. A set of definitions based on voxels properties can

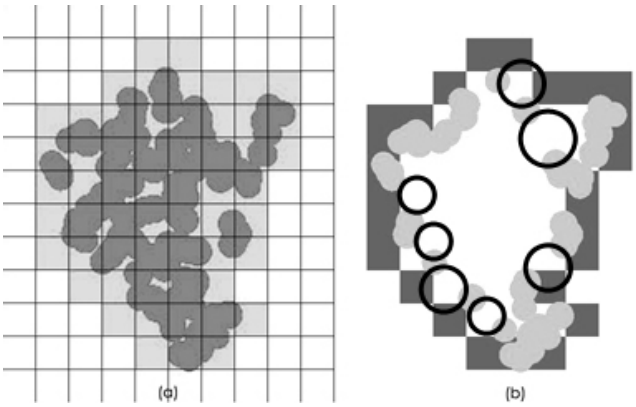

Fig. 1. Application of a >0% voxel occupancy criteria in the highlighting of protein occupied voxels. (a) Layer 400 of protein 1FKF, with all atoms visible. (b) Layer 400 of protein 1FKF, with only surface voxels selected and surface atoms visible. The circled areas show the regions where surface atoms have not been selected.

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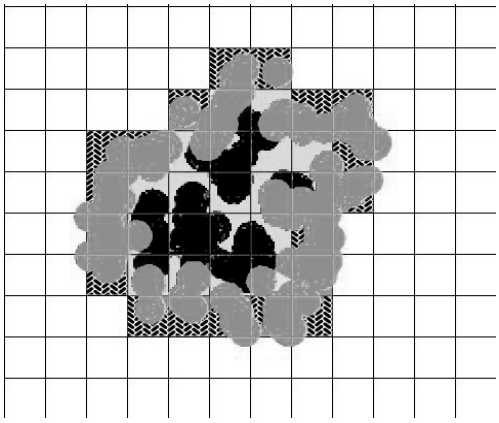

Fig. 2. A value of 40%-100% voxel occupancy applied to the units. Patterned units are the surface voxels, while the highlighted atoms are surface entries associated with the surface voxels. The dark-colored atoms are non-extracted internal atoms.

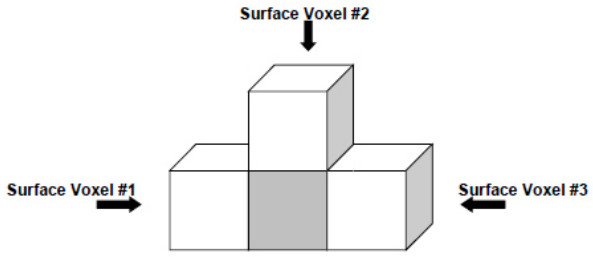

Fig. 3. The illustration shows three surface voxels surrounding an inner voxel. A voxel is defined as being 'surface' when one or more of its faces is/are externally exposed. However to the use of the slicing algorithm and projection into 2D images, the front and back of the voxels are excluded from consideration.

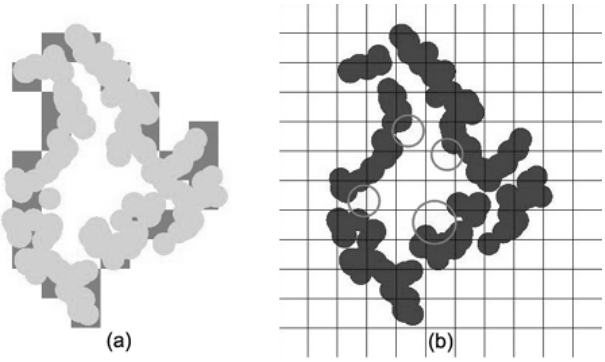

Fig. 4. (a) The original extracts from the protein. (b) Circled areas show where the atoms have been removed post-application of a >5% atomic membership setting.

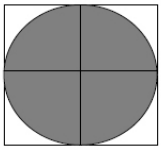

Fig. 5. Illustration of an atom contain in a voxel. The white area of the voxel is complementary to the atom occupied area.

be assigned to differentiate the surface units. The notion of 'voxel exposure value' is introduced which checks for the count of exposed surfaces for each voxel (Fig. 3). Only the units on the boundaries contain exposed faces to the external environment. Cases wherein empty units occur within the protein while their neighbouring voxels are labeled as surface entries suggest the possibility of an internal binding area or part of a cavity extended inwards. The rule applied for filtering of the surface voxels is such that if one or more faces of a voxel is/are exposed, then selection is made.

The following step is to extract all the surface atoms from the protein. By using the coordinate points of each atom together with its van der Waals radius the area size for the atom is calculated and checked for overlap with any of the selected surface voxels. The atom is selected if the overlap is positive. However it is possible for internal atoms to occupy part of the surface voxels. A visual inspection may aid in eliminating these internal entries but it is infeasible to carry out such checking on a large set of proteins. An approximated and fast method capable of improving the extractions is required.

D. Refining the Extracts

Similar to voxel occupancy, the notion of atomic membership is introduced. Defined as the percentage of an atom belonging to a voxel, a value greater than 5% was found to be most effective in filtering out internal atoms. When applied to the extracts improvements were shown (Fig. 4). Circled areas show the locations where internal atoms have been removed. The >5% membership does not eliminate all internal entries – increasing the value was found to remove some surface atoms as well. An additional checking is therefore introduced to determine the acceptability of each atom within the surface voxels. Internal entries with less exposure to the external environment are consequently 'peeled' off.

Secondary information derived from the surface atoms are used for the 'peeling' method. The algorithm first checks for the atom furthest from the averaged center of the protein within the domain of each host surface voxel. Consecutive iterations check for the exposure of each atom (in the host surface voxel and excluding the identified furthest atom) to the external environment. The atom is marked if external exposure was found to be higher than internal exposure. The implementation is given as follows.

1. Identify all surface voxels using a voxel occupancy of 40%-100%. Store the extracted atoms in lists such that voxel → list of atoms.
2. Determine the averaged center of the protein and 'color-fill' the internal environment in grey.
3. Identify all furthest atoms from the averaged center within the contexts of their voxels. The atoms are stored in a new list (hereon referred to as List X_c) in which the entries are unique.
4. Check for all other atoms within each surface voxel on consecutive iterations. Comparisons are made based on the following rules :-
   I. If the atom overlaps with entries from List X_c the overlapping portion is flagged. This flagged area is used for checking in case Condition II fails – the flagged area > internal area occupied.

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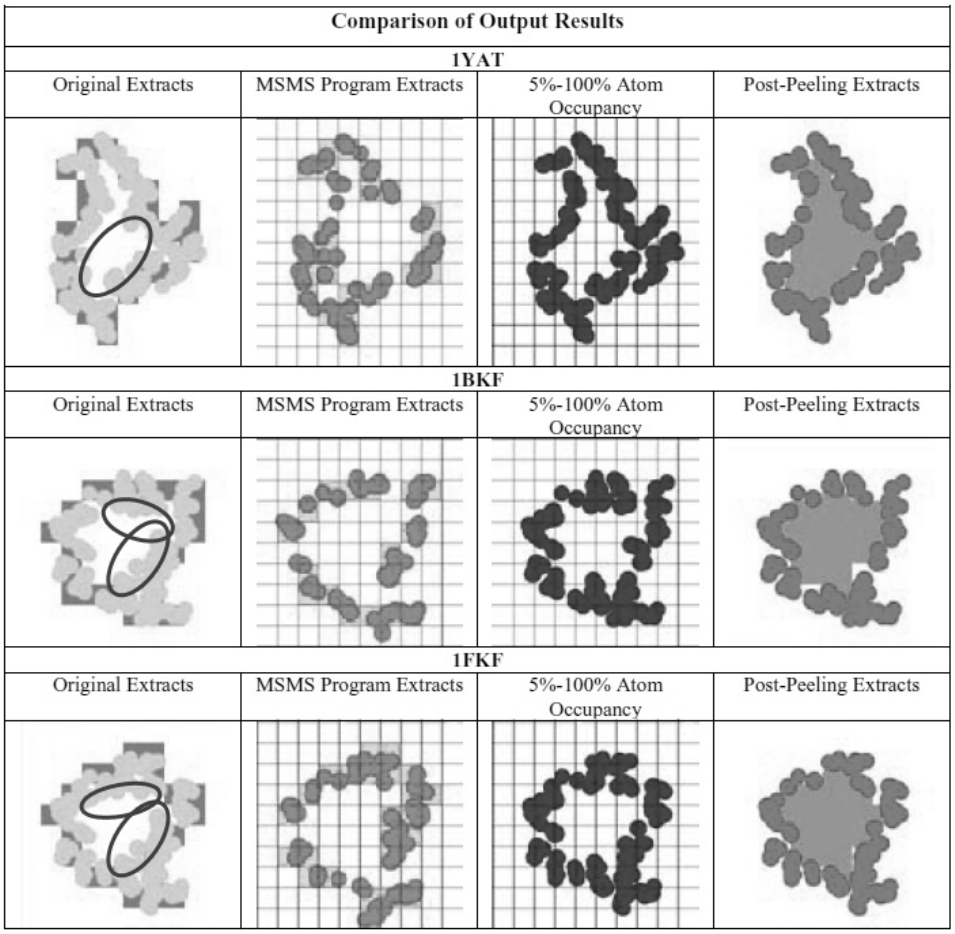

Fig. 6. The figure shows comparisons between the original extracts with voxel occupancy of 40%-100% against the results obtained from the MSMS program. Also presented are the results with constraints and 'peeling' applied. The probe radius for the MSMS program was set to 2.0 Å (diameter of 4.0 Å to ensure consistency in experimental environments). From the images, the original extracts contain a number of internal atoms as shown in the circled areas. Application of the atomic membership constraint and the internal 'peeling' algorithm successfully reduced the unwanted entries.

II. The complement of the atom in a voxel boundary is checked for external/internal environment overlap. An atom is considered a surface entry if the total overlap of the complement area to the external environment is larger than a quarter of the white area in Fig. 5.

After the final stage of 'peeling' the remaining atoms are visualised in images and compared against extractions from other available methods.

IV. Results

A. Experiment Set I

A visual comparison of the output obtained from the implemented voxel-based method and results from one of the test programs (MSMS) is presented in Fig. 6. The images illustrate the differences in the output for various stages of the algorithm as well as the output obtained from the program for all three proteins of 1YAT, 1BKF and 1FKF. Note that the MSMS program uses a reduced surface method for both SES and SAS areas. For comparison purposes both the results were merged together. The Surface Racer program returns output for SAS and molecular surface (MS) areas. These too are merged into a single list for each protein. The first column shows cross sectional images of the proteins using 40%-100% voxel occupancy with only surface voxels and their associated atoms shown. The second column gives the merged output from the MSMS program using a probe radius of 2.0 Å (diameter 4.0 Å) to ensure consistency with the voxel size. Images in the third column demonstrate how the atomic membership filtering reduces some internal atoms and the last column shows improvements in the final set of atoms post-application of the 'peeling' method.

Statistics for the extracts compared against MSMS and Surface Racer are shown in Tables I and II.

TABLE I
Comparison of Extractions from the Voxel-based Method and the Merged Output of MSMS Program.

Protein	T_A	Identified Surface Atoms		Unique Atoms*		Matches
		Voxel	MSMS	Voxel	MSMS	
1YAT	849	569	475	205	111	364
1BKF	827	514	489	172	147	342
1FKF	832	529	495	113	79	416

* Unique atoms which are identified as surface atoms by a method and not found in the other approach. All following tables are similarly defined.

† T_A – total number of atoms in the protein. All following tables are similarly defined.

†† The identified surface atoms for voxel are based on the final count after the filtering and 'peeling' processes were applied. All following tables are similarly defined.

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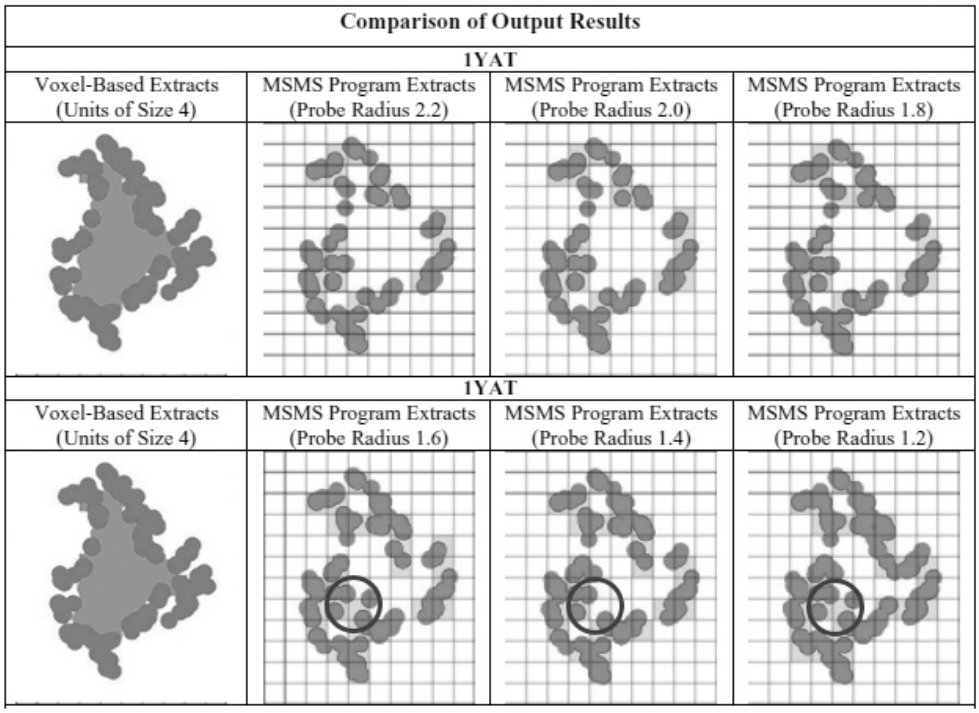

Fig. 7. Comparison between post-peeling extracts from the voxel-based method to the obtained range of output from applying different probe sizes in the MSMS program. The probe sizes range from 1.2 Å to 2.2 Å. The usage of a smaller probe radius leads to the presence of more internal atoms in the results (circled areas).

TABLE II
COMPARISON OF EXTRACTIONS FROM THE VOXEL-BASED METHOD AND THE OUTPUT OF SURFACE RACER (SR) PROGRAM.

Protein	T_A	Identified Surface Atoms		Unique Atoms		Matches
		Voxel	SR	Voxel	SR	
1YAT	849	569	485	150	66	419
1BKF	827	514	495	115	96	399
1FKF	832	529	498	114	83	415

B. *Experiment Set II*

The second experiment consists of 6 proteins obtained from the study by [20] namely 6CHA, 1RA2, 3FXN, 7TLN, 1TIM, and 3TRA. The results are not replicated in this study as differences were detected in the total number of atoms for the proteins. 4 out of 6 proteins display total atom values that are different from the total count obtained from RCSB PDB downloaded files. Comparisons are only carried out between the implemented method and the MSMS/SurfaceRacer programs. A probe radius of 1.4 Å (diameter 2.8 Å) was used for the programs, while the unit sizes were retained at 4.0 Å.

TABLE III
COMPARISON OF EXTRACTIONS FROM THE VOXEL-BASED METHOD AND THE MERGED OUTPUT OF MSMS PROGRAM.

Protein	T_A	Identified Surface Atoms		Unique Atoms		Matches
		Voxel	MSMS	Voxel	MSMS	
6CHA	3472	1814	1519	873	578	941
1RA2	1268	881	677	407	203	474
3FXN	1073	623	584	156	117	467
7TLN	2432	1302	1003	564	265	738
1TIM	3740	2015	1853	673	511	1342
3TRA	1374	1145	1116	343	314	802

TABLE IV
COMPARISON OF EXTRACTIONS FROM THE VOXEL-BASED METHOD AND THE OUTPUT OF SURFACE RACER (SR) PROGRAM..

Protein	T_A	Identified Surface Atoms		Unique Atoms		Matches
		Voxel	SR	Voxel	SR	
6CHA	3472	1814	1842	494	522	1320
1RA2	1268	881	730	254	103	627
3FXN	1073	623	584	145	106	478
7TLN	2432	1302	1117	388	203	914
1TIM	3740	2015	1841	631	457	1384
3TRA	1374	1145	1052	213	120	932

C. *Experiment Set III*

The third experiment consists of 5 proteins from the study carried out by [19] which are 2PLT, 1A19, 1O3Y, 1QB5, and 1EAI. The surface extracts are first compared as in the previous two sections followed by a comparison to the results reported by the authors. Probe radius was maintained at 1.4 Å (diameter 2.8 Å) for the programs, while the unit sizes were retained at 4.0 Å.

TABLE V
COMPARISON OF EXTRACTIONS FROM THE VOXEL-BASED METHOD AND THE MERGED OUTPUT OF MSMS PROGRAM.

Protein	T_A	Identified Surface Atoms		Unique Atoms		Matches
		Voxel	MSMS	Voxel	MSMS	
2PLT	727	406	387	176	157	230
1A19	1438	911	774	263	126	648
1O3Y	2664	1603	1277	774	448	829
1QB5	3750	2009	1595	913	499	1096
1EAI	4540	2854	2190	1142	478	1712

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TABLE VI
COMPARISON OF EXTRACTIONS FROM THE VOXEL-BASED METHOD AND THE OUTPUT OF SURFACE RACER (SR) PROGRAM.

Protein	T_A	Identified Surface Atoms		Unique Atoms		Matches
		Voxel	SR	Voxel	SR	
2PLT	727	406	373	133	100	273
1A19	1438	911	758	246	93	665
1O3Y	2664	1603	1398	451	246	1152
1QB5	3750	2009	-	-	-	-
1EAI	4540	2854	2296	905	347	1949

TABLE VII
COMPARISON OF EXTRACTIONS FROM THE VOXEL-BASED METHOD AND THE REPORTED OUTPUT BY THE AUTHORS.

Protein	Total Number of Atoms in Protein	Identified Surface Atoms	
		Reported	Voxel
2PLT	727	338	406
1A19	1438	681	911
1O3Y	2664	1261	1603
1QB5	3750	1482	2009
1EAI	4540	2151	2854

V. DISCUSSION

Based on Fig. 7 the constraint filtering of atomic membership was shown to improve the extractions through removal of unnecessary internal atoms. The 'peeling' method eliminated entries which bypassed the previous stage. In Fig. 8 a comparison is made between the post-peeling extracts and a series of probe-based output from the MSMS program with different probe radii applied. The extracts from the program display good results from a radius of 1.6 Å and below. However the cross sectional images show the inclusion of some internal atoms as depicted in the circled regions. Comparison of the images shows that the voxel-based method performed better at extracting boundary atoms.

Referring to the tables the voxel implementation classified a higher number of atoms as surface entries compared to both the MSMS and Surface Racer programs. However the higher count does not necessarily indicate that all the atoms have been identified correctly. Each method was shown to contain a number of unique entries not identified by the compared approach. A series of image layers were generated to show the different atoms extracted and are given in Fig. 8 and Fig. 9. In Table VI the entry 1QB5 has been highlighted due to the failure of Surface Racer in processing the protein.

The images from Fig. 8 and Fig. 9 show comparisons of the common atoms extracted between the voxel-based method and the compared programs. From the tables a number of unique atoms are identified for each of the method. These unique atoms are represented as patterned atoms in the images. For example, in the first two images of Fig. 8 – the left image shows extracts from the voxel-based method whereas the right shows atoms identified from the MSMS program – the MSMS program did not identify a number of atoms in the upper left and lower right sections of the protein which are picked up by the voxel-based method. The results produced by Surface Racer are much more concise. Extracts from the voxel-based method displayed higher similarities to those of Surface Racer.

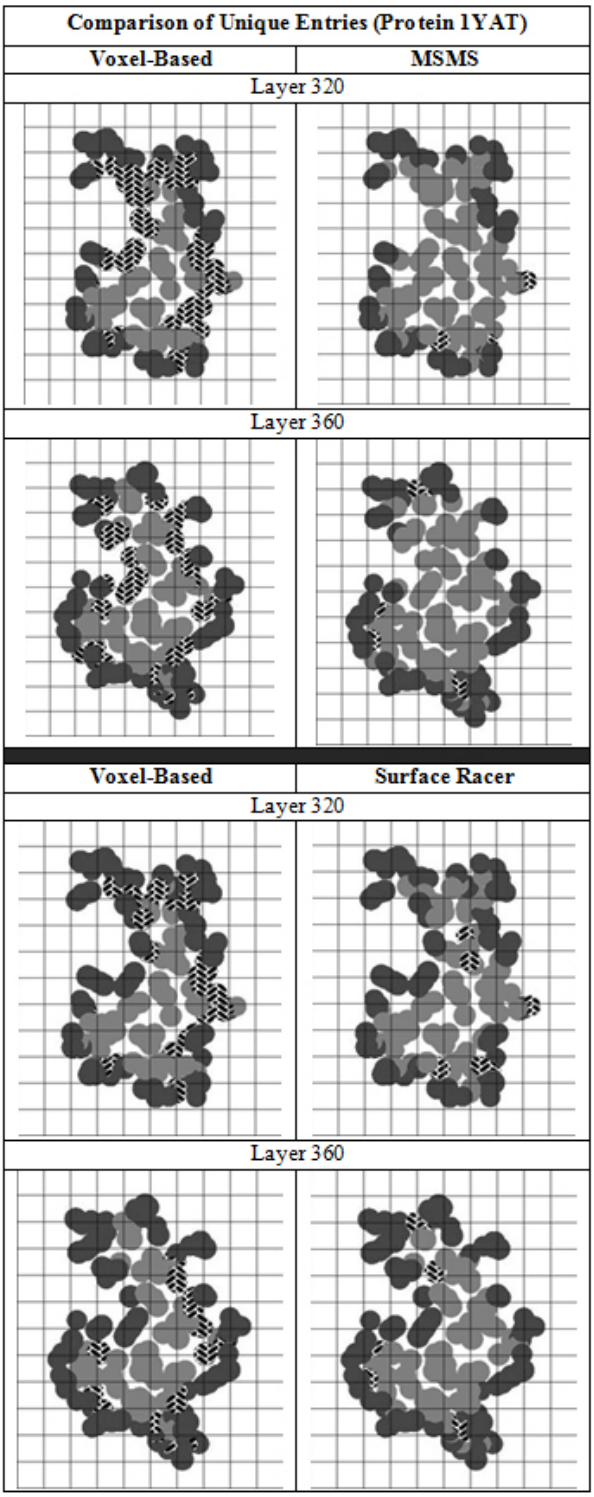

Fig. 8. Visualisations of the unique atoms found for each method for protein 1YAT. The upper four cells show comparisons between the voxel-based method and the MSMS program. The lower four cells show comparisons between the voxel-based method and the Surface Racer program. Two layers of cross-sectional projections are given for each comparison, i.e. layer 320 and 360. All identified common surface atoms are colored in dark gray. Each image shows a number of atoms filled with patterns. These are the atoms unique to each of the method which are not identified in the compared method. From the images it can be concluded that the voxel-based method showed better performance compared to the MSMS program and is highly competitive against the Surface Racer program.

Due to the size of the voxel used, a larger number of atoms are encapsulated within a single unit resulting in more atoms being selected. Removal of these entries may require

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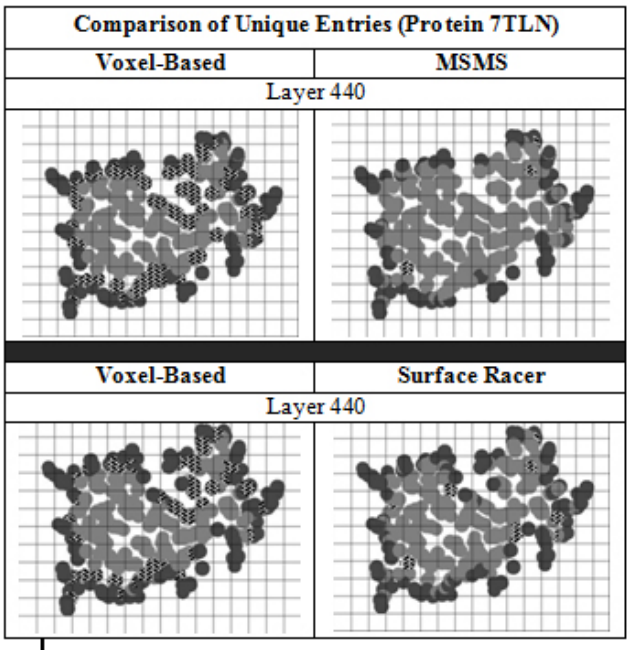

Fig. 9. Visualisations of the unique atoms found for each method for protein 7TLN. The upper two cells show comparisons between the voxel-based method and the MSMS program. The lower two cells show comparisons between the voxel-based method and the Surface Racer program. The cross-sectional projections of layer 440 are given for each comparison. All identified common surface atoms are colored in dark gray. Each image shows a number of atoms filled with patterns. These are the atoms unique to each of the method which are not identified in the compared method. Again, the voxel-based method picked up areas which have been overlooked by the MSMS program and showed high similarities with the Surface Racer program.

filters of higher complexities. Nevertheless the availability of these atoms may aid in the study of binding sites which consider both surface atoms and atoms close to the surface that contribute to the reactivity of the site. Different parameters settings have been tested for the proteins with the findings that smaller voxel sizes often lead to a higher number of internal atoms and an increase in execution time. Usage of a higher or lower voxel occupancy value resulted in varied sets of surface voxels being selected – extraction quality was found to follow a Gaussian distribution wherein the highest number of surface atoms corresponds to a 40%-100% voxel occupancy [22].

VI. Conclusion

The voxel-based method with applied constraints for extraction of protein surface atoms was implemented and compared against output from two programs – MSMS and Surface Racer. The investigation revealed that the use of experimentally determined filtering constraints lead to improvements in the extracts resulting in less interference of internal atoms. The results were shown to be promising with the voxel-based method achieving extracts that were not identified by the MSMS program while displaying high competitiveness against the Surface Racer program. This study proved that with proper constraints applied voxels can be a competitive tool for use in proteins analysis.

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