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SURFNET: A program for visualizing molecular surfaces, cavities, and intermolecular interactions

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The SURFNET program generates molecular surfaces and gaps between surfaces from 3D coordinates supplied in a PDB-format file. The gap regions can correspond to the voids between two or more molecules, or to the internal cavities and surface grooves within a single molecule. The program is particularly useful in clearly delineating the regions of the active site of a protein. It can also generate 3D contour surfaces of the density distributions of any set of 3D data points. All output surfaces can be viewed interactively, along with the molecules or data points in question, using some of the best-known molecular modeling packages. In addition, PostScript output is available, and the generated surfaces can be rendered using various other graphics packages.

Keywords: Molecular surfaces, cavities, binding sites, molecular interactions

INTRODUCTION

Protein surfaces and internal cavities are of interest for the understanding of many aspects of protein structure and function. The shape and chemical properties of the surface govern interactions with other molecules, such as DNA,[1] ligands,[2,3] and other proteins,[4] and also form the basis of quaternary structure in multimeric proteins.[5] The size and shape of indentations in the surface are particularly important in ligand binding, and hence crucial in structure-based drug design.[6,7] Internal cavities affect protein stability[8] and play an important role in the close packing of amino acid side chains, particularly in the core of the protein.[9–11]

A number of programs are available for visualizing molecular surfaces and cavities. Molecular surfaces are most usually displayed as dot-surfaces[12] by the commonly used molecular modeling programs, such as *O*[13] and QUANTA (Molecular Simulations, Inc., Burlington, MA). The dots are placed at the van der Waals radius of each atom, at a given density and coloring, and provide an indication of the molecular surface. Where two molecules interact, their dot-surfaces can show their complementarity at the interface.

Solid renderings of surfaces can also be generated by these and other packages (Ref. 14 and references therein), with appropriate texturing and reflections.[15] For large molecules, such as proteins, these representations are difficult to manipulate interactively on the graphics, although graphics packages exist that can do this well, such as AVS (Advanced Visual Systems, Inc., Waltham, MA). One excellent program designed specifically for visualizing molecular surfaces, and in particular their electrostatic potentials, is GRASP,[16] which has quickly become very popular because of the striking images it generates.

Various methods and programs exist for visualizing cavities and surface grooves. Both types of region are difficult to perceive from just viewing a molecular surface alone. A given cavity, as defined by the convex surface of the atoms surrounding it, is a concave region that, because the atoms cannot fit together without leaving gaps, branches into myriads of channels and grooves in all directions and is impossible for the mind to grasp. Programs that best depict cavities, therefore, show them as convex surfaces, often built up by fitting probe spheres into the cavities in some manner, and then defining a surface around the resultant collection of interpenetrating spheres. By defining a minimum sphere size, the myriads of small gaps and crevices between the atoms are excluded and only the significant gaps are depicted.

Internal cavities, which are completely closed off from the outside world, are relatively easy to detect. The program of Ho and Marshall,[17] for example, "flood-fills" a cavity

Color Plates for this article are on pages 307–308.

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Journal of Molecular Graphics 13:323–330, 1995

655 Avenue of the Americas, New York, NY 10010

0263-7855/95/$10.00
SSDI 0263-7855(95)00073-9

from a given start point. The VOIDOO program[18] detects cavities, and certain invaginations as well, by an "atom-fattening" procedure that progressively closes off small channels both between cavities and also between cavities and the outside world by gradually increasing the atomic radii. The HOLE program of Smart et al.[19] is specifically geared to identifying, measuring, and visualizing ion channels in proteins, stepping through the channel from a given start point and filling it with spheres.

Some programs locate internal cavities as a by-product of generating the molecular surface. For example, the GRASP program, mentioned above, locates internal cavities on the basis of the connectivities of the surface elements. Similarly, the algorithm of Voorintholt et al.,[20] which is used by the WHAT IF molecular modeling packing,[21] can show the molecular surface that is accessible to different probe sizes as a density map, and so can detect the internal cavities of the molecule.

Surface grooves and indentations, on the other hand, are much more difficult to depict—principally because of the difficulty of knowing how far into open space to extend the groove region; where does the "sea level" of that part of the surface lie? The program of Delaney[22] uses a pattern recognition technique to identify the limits for the flood-filling procedure. The POCKET program[23] is an interactive cavity-filling program that detects pocket regions in the structure by placing a trial sphere of a given radius at points on a three-dimensional (3D) grid. At each grid point the sphere either makes, or does not make, contact with atoms from the molecule. Regions of no contact that are bounded by contact regions in either the x, y, or z directions are deemed to be cavities or pockets.

Other cavity-detection algorithms concern themselves primarily with the detection and analysis of cavities rather than with their visualization (e.g., Refs. 24–26). These usually involve moving a probe sphere about the surface to detect completely or partially buried voids.

Here we describe a new program, SURFNET, which generates both molecular surfaces and surfaces that delineate cavities, surface grooves, and pockets. The main advantages of the program are that the cavity and pocket-detection algorithm is very fast and simple, and that the program output is not tied to any one molecular graphics program; it can generate density maps for several different packages, namely FRODO,[27] *O*, QUANTA, and SYBYL (Tripos Associates, Inc., St. Louis, MO). Additionally, each of the surfaces can be output as either PostScript or Raster3D[28,29] files, meaning that they can be imported into various other graphics software packages. Crude measures of the volumes of the molecules and gap regions are also provided in the output.

The program has several applications. First, it can depict the internal cavities and surface pockets of large molecules such as proteins. The pockets are particularly useful in molecular modeling and drug design, where the regions of space available for binding ligands are clearly delineated. Second, the gap regions between two or more molecules can be visualized. Thus, for example, one can investigate the specificity of different inhibitors for a given binding site by viewing how closely the surfaces of the two molecules complement one another. Similarly, the interfaces between molecular subunits can be investigated (e.g., helix–helix packing). Here again, the size and shape of the gap regions between the molecules or subunits gives an indication of the closeness of their fit.

As a by-product of the surface-generation routines, SURFNET can also be used for contouring 3D density distributions of any general set of 3D data points. Examples of all these applications are presented.

SURFACE GENERATION

The program generates the following types of surfaces from coordinate data supplied in Protein DataBank (PDB)[30] format:

- van der Waals surfaces
- Gap regions between two or more molecules
- Cavities and grooves within a single molecule
- 3D density distributions of data points

Related programs PLANE, CURVE, and GENBOX can generate surfaces representing planes, paraboloids, cubic surfaces, or boxes.

Each surface is output as a 3D grid of density values, using a simple Gaussian function applied to each atom in the input PDB file. This approach is similar to the contouring method of Murray-Rust and Glusker,[31] which employs a spherical atomic electron density function with a characterizable temperature factor. The SURFNET function, depicted in Figure 1, is of the form

$$\rho = \rho_0 e^{-kr^2}$$

where ρ is the density assigned to a given grid point at a distance r from the center of the atom, ρ_0 is the density at the center of the atom (i.e., at $r = 0$), and k is a constant. Only grid points within three times the radius of the atom are updated as the density values become negligible beyond this distance (Figure 1).

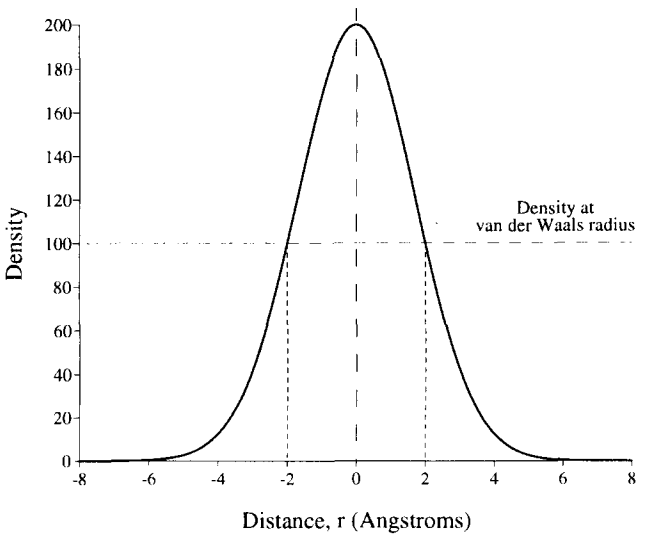

Figure 1. The Gaussian function used for representing the density ρ *of an atom at a distance* r *Å away. The function shown is for an atom with a van der Waals radius,* r_{vdW}*, of 2.0 Å; the density at* $r = \pm r_{vdW}$ *is 100.0.*

The constant k is chosen so that the density at the van der Waals radius of the atom, $r = r_{vdW}$, is half the central value ρ_0; hence

$$k = (\ln 2)/r_{vdW}^2$$

The different van der Waals radii, r_{vdW}, that are used are as follows:

Atom	r_{vdW}	Atom	r_{vdW}
C (Carbon)	1.87 Å	0 (Oxygen)	1.40 Å
Fe (Iron)	1.10 Å	P (Phosphorus)	1.90 Å
H (Hydrogen)	1.20 Å	S (Sulfur)	1.85 Å
N (Nitrogen)	1.65 Å	X (Other)	1.50 Å

To make contouring simpler, the density at the center of each atom, ρ_0, is chosen to be 200.0 and hence the van der Waals surface, being where the density is half this value, is defined by a contour level of 100.0 (Figure 1). Figure 2d shows the density values generated by a single atom on its nearby grid points. When these density values are contoured at a value of 100.0, the surface of the original sphere is regenerated as a set of polygons, as shown in Figure 2e. The smaller the grid spacing the closer the final surface to the original sphere.

When generating the surface of a whole molecule, the covalently bonded atoms will have their van der Waals radii overlapping and their surfaces interpenetrating. Thus simply summing their individual density contributions would give a distorted surface wherever these overlaps occur. To counteract this, only the largest density contribution is stored at each grid point. That is, each grid point is assigned the value ρ from a given atom only if this is larger than the current value of the grid-point. This ensures that the overall surface of the whole molecule is accurately represented by a single contour level of 100.0.

As well as generating molecular surfaces, SURFNET can also be used to generate a density distribution from a set of data points (much as in Ref. 31). In this case, the density values are summed together. Another difference is that the value of the parameter ρ_0 is empirically determined by the program at each run such that the net contribution of each data point to the total density, when smoothed cross all grid points, is unity. This ensures that the density values in the grid reflect the density of data points everywhere.

DEFINING GAP REGIONS, GAP VOLUMES, AND BINDING SITES

One of the most useful applications of the program is in delineating gap regions, as cavities, within a molecule or between two or more molecules. Like the surfaces, the gap regions can be viewed interactively using one of several common molecular modeling packages.

The algorithm for detecting gaps is particularly straightforward, and is illustrated in Figure 2. The gap regions are built up simply by fitting spheres into the spaces between atoms. This is similar to the approach of Kuntz et al.,[32] whereby each sphere is generated tangent to surface points i and j with its center on the surface normal at point i; and in the POCKET program of Levitt and Banaszak,[23] in which spheres are placed on a regular lattice and adjusted in size until they just touch the nearest atom. SURFNET differs in that it considers all relevant pairs of atoms in turn and places a sphere midway between each pair, reducing its size if it clashes with any neighboring atoms.

For example, if one is interested in the gap regions between an enzyme and its inhibitor, each atom of the enzyme is considered in turn with each atom of the inhibitor. For each pair of atoms a trial sphere is placed midway between their surfaces, just touching each one, as shown in Figure 2a. The radius of the sphere is then reduced whenever any neighboring atom is found to penetrate it, until all neighboring atoms have been considered, and one is left with a final gap sphere as shown in Figure 2b. If this sphere is still above some minimum size (usually 1.0 Å), its position and radius are stored.

The procedure is repeated until all possible pairs of atoms have been considered and one is left with a set of gap spheres filling the region between the molecules (Figure 2c). The surface encompassing these spheres is then generated as described above for van der Waals surfaces. Figure 2d and e illustrates the procedure for a single sphere.

The net result is a contour surface (Figure 2f) that delineates the gap regions between the two molecules. When viewed on a graphics terminal, together with the molecules in question, the gap regions clearly show where the molecules are not in contact with one another. Conversely, the absence of gaps shows where the molecules are packing closely against one another.

This procedure can be applied to visualizing the internal cavities and surface grooves in a single molecule. Here all pairs of atoms from the one molecule are considered. This gives a natural way of defining the "sea level" of the surface; wherever part of the molecule protrudes out from the surface, the use of all atom pairs to define gaps ensures that any grooves around the protrusion are included.

The approach is particularly useful for locating and defining binding sites in proteins. The binding site will usually by the largest of the gap regions, as found for a few examples by Kuntz et al.[32] A program called MASK enables you to pick out the required gap region from all those found by SURFNET. This region, by clearly delineating the binding site, is then a valuable aid in drug design as it shows the space available for docking new ligands or modifying existing ones.

In addition, SURFNET provides a crude measure of the volume of each gap region by counting the numbers of grid points within its surface. The volume of any molecular surfaces is computed in the same manner, and hence provides a means of obtaining the volume of the molecule(s) in question.

PROGRAM OUTPUTS

SURFNET outputs its surfaces a density maps in one of several formats: QUANTA (Molecular Simulations, Inc.), SYBYL (Tripos Associates, Inc.), and CCP4 format.[33] The last of these can be used to convert into FRODO[27] and *O*[13] formats.

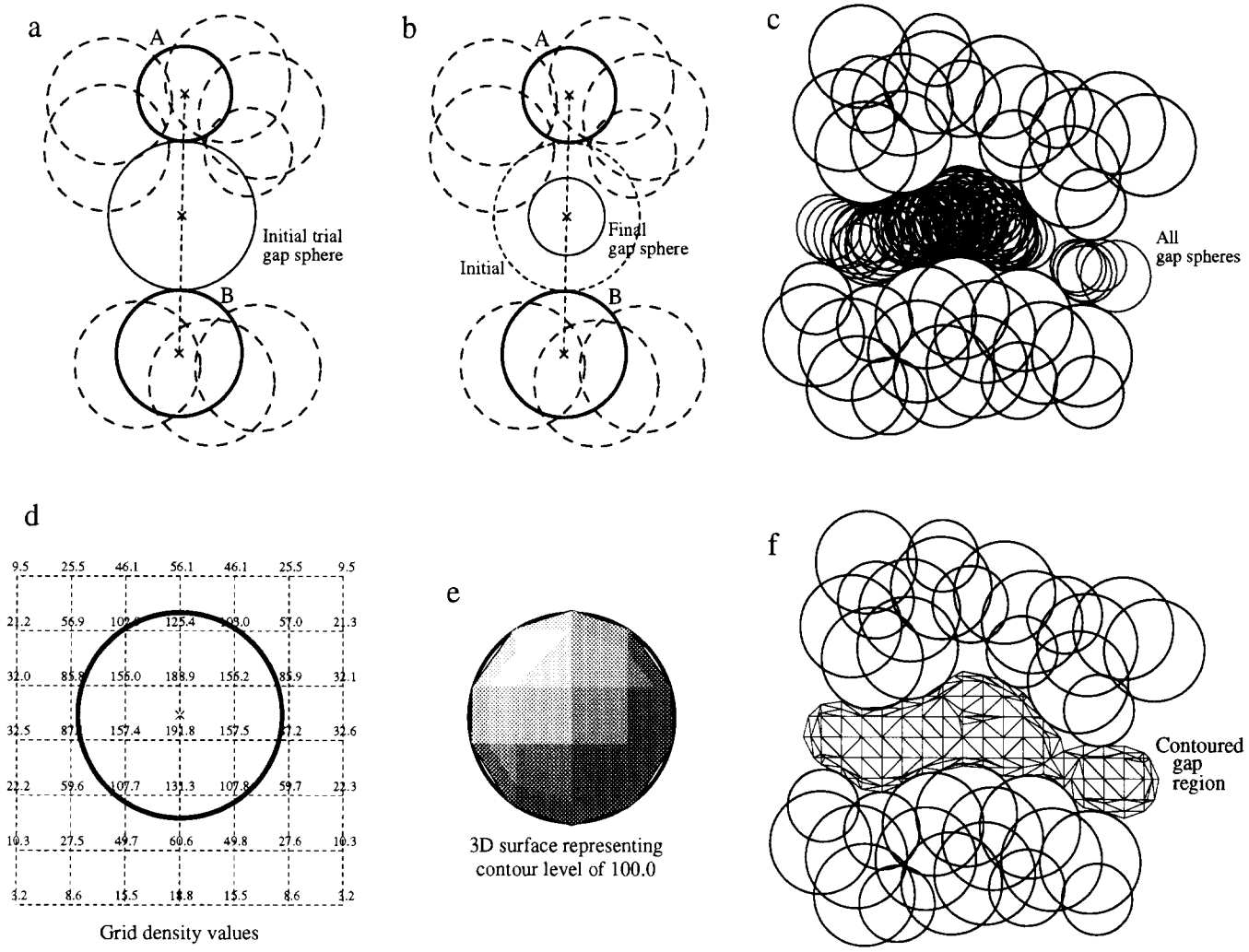

Figure 2. Generation of gap regions between two molecules. Gap regions are defined by first filling the region between the two molecules with "gap spheres" and then computing a 3D surface around them. (a) Each gap sphere is placed between a pair of atoms, as shown for atoms A and B, midway between their van der Waals surfaces and just touching each one. If any neighboring atoms penetrate this gap sphere its radius is reduced until it just touches the intruding atom. If the radius of the sphere falls below some predetermined minimum limit (usually 1.0 Å) it is rejected. Otherwise, (b) the final sphere is saved. (c) When all pairs of atoms have been considered the saved gap spheres fill the gap region. (d) Each sphere is used to update points on a 3D grid (a 2D slice of which is shown here), using a Gaussian function as in Figure 1. (e) When contoured, the densities in the array give a 3D contour surface made of polygons that approximates the original sphere. (f) The contour surface surrounding all the interpenetrating gap spheres in (c) gives a boundary that clearly delineates the size and shape of the gap region.

These surfaces can also be contoured and output in PostScript format[34] and by the related programs SURFACE and SURFPLOT. SURFPLOT can plot the surfaces either a wire-cage diagrams, or as rendered, solid surfaces. In Figures 4b, for example, both representations are included.

The program can also produce "box plots," like that shown in Figure 5, wherein the objects in the scene are back-projected onto the three walls of a box to give a better idea of their 3D disposition.

As well as outputting in PostScript format, SURFPLOT can also output Raster3D-format files, for rendering the surfaces using the program Raster3D.[28,29] It is also possible to import the surfaces into AVS (Advanced Visual Systems, Inc.) for rendering and interactive manipulation.

EXAMPLES

A number of examples of SURFNET applications are given in Figures 3–5 and Color Plates 1–5 and described below.

Binding sites

The first example is shown in Figure 3 and illustrates how SURFNET can be used to delineate the shape of the binding sites of a protein. Figure 3a shows the surface of the aspartic protease endothiapepsin (EC 3.4.23.6) with the inhibitor H-189 in its binding site, held in place by the binding flap. The structure is taken from the PDB entry 3er5.[35] Figure 3b shows the surfaces of all the gap regions generated

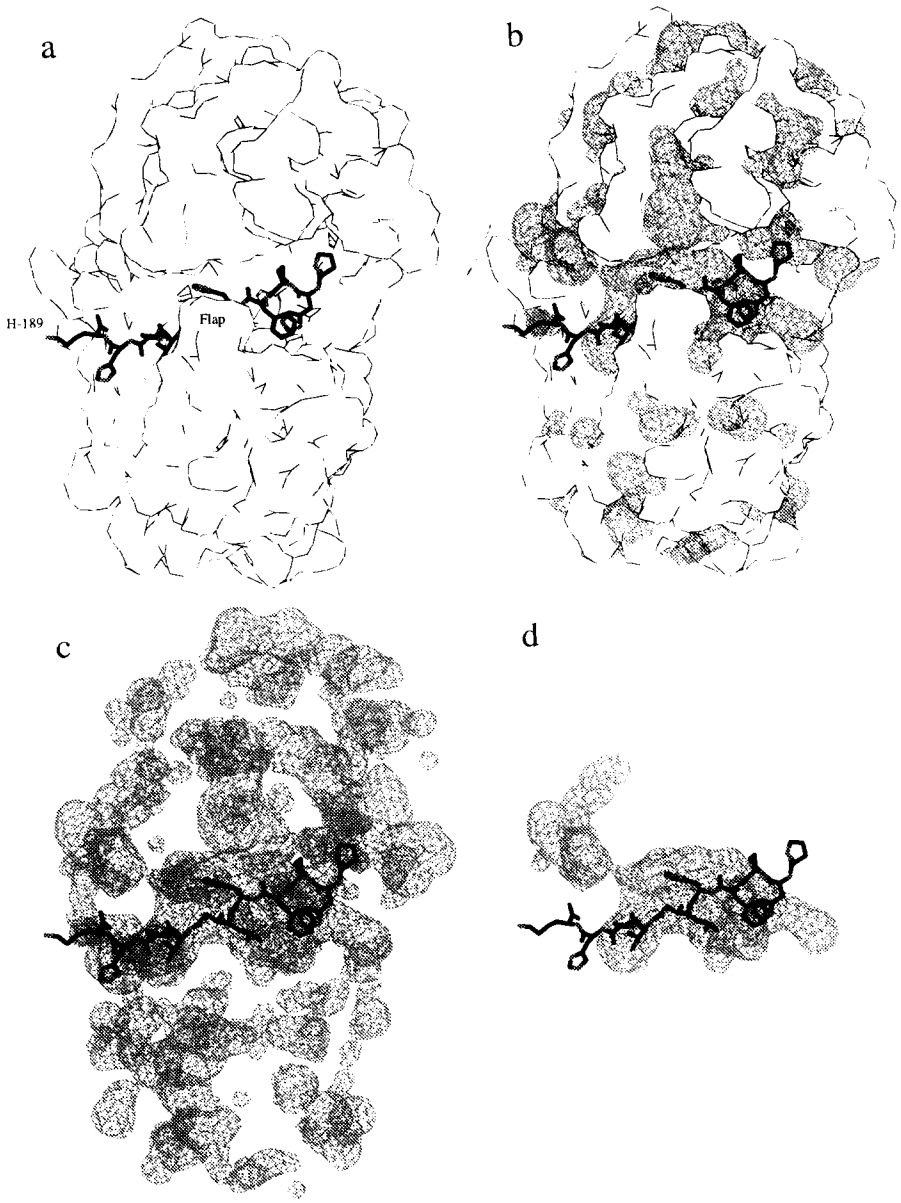

Figure 3. The surface, cavities, and binding site of the aspartic protease endothiapepsin (EC 3.4.23.6) as computed by SURFNET on PDB code 3er5. (a) The surface of the protein is shown as an unshaded sketch, with the thick lines representing the bonds of the H-189 inhibitor; the flap of the protein can be clearly seen holding the inhibitor in place. (b) The protein gap regions (which include all internal cavities and surface indentations) are shown as gray wirecage surfaces. (c) The gap regions and inhibitor, with the protein removed. The inhibitor can be seen within the largest of the gap regions, which corresponds to the protein binding site. (d) The binding site region shown on its own, with the H-189 inhibitor inside.

by SURFNET, encompassing not only internal cavities, but also grooves and channels in the protein surface. In Figure 3c the gap regions and inhibitor are shown on their own, minus the protein. Here it can be seen that the inhibitor is sitting in the largest of the gaps. This largest gap region, which corresponds to the active site of the enzyme can be extracted by MASK and is shown separately in Figure 3d. In fact, as the illustration shows, only the middle part of the inhibitor is located within the active site, its two ends poking out into free space on either side. It can also be seen that the binding site is connected by a thin channel to a nearby groove in the surface.

A similar use has been made of the program by Newman et al.[36] both to visualize the substrate-binding pocket of the aspartic proteinase from *Mucor pusillus*, and also to identify buried waters in the same structure.

Another binding site, that of thermolysin (EC 3.4.24.4), is shown in Color Plate 1, with three different inhibitors superimposed inside. The protein structure, and one of the inhibitors, come from PDB code 4tmm[37] while the other two inhibitors come from PDB codes 1tmm[38] and 1tlp.[39] Color Plate 1a was obtained using QUANTA, while Color Plate 1b gives a rendered view using AVS.

Gap regions and complementarity

Color Plate 2 shows the application of SURFNET to locating gaps between two different molecules. Again endothi-

apepsin (PDB code 3er5) is used as an example, but this time we are interested in the gap regions between the enzyme and its inhibitor, to see how well the two molecules fit together, and to visualize the complementarity of their surfaces. The surfaces of the inhibitor and gap regions are shown in different colors. The representation in Color Plate 2a comes from SURFPLOT, with the inhibitor surface shown as a wire-cage contour, while that in Color Plate 2b has been rendered by Raster3D, with solid surfaces for all the objects. The view is from the protein side, which means that all the visible parts of the inhibitor, not in contact with a gap region, are in close contact with the enzyme. This gives an indication of how specific the interaction between the two molecules is.

As mentioned above, the delineation of the cavities provides a means of computing their volumes. The cavity volumes computed by SURFNET have been used by Jones and Thornton[5] to define a "gap-volume index" for comparing the complementarity of surfaces in different protein–protein complexes. SURFNET has also been used to visualize cavities computed using a different algorithm, namely that of the PRO_ACT program of Williams et al.[26] The PRO_ACT algorithm concerns itself only with internal cavities, and not with surface grooves. It, too, locates cavities by inserting gap spheres into empty regions, although the exact method differs from the one described here.

Contouring three-dimensional distributions

Figure 4 shows one application of SURFNET to computing and contouring 3D distributions. In Figure 4a a box plot shows the distribution of carboxyl O^- atoms around arginine side chains, as extracted from a data set of protein structures. Each carboxyl O^- in contact with the guanidinium group of an arginine has been transformed onto a common reference frame defined by the arginine side chain. The contacts were extracted by a separate program, DISTRIB (R.A Laskowski, unpublished results), and are deemed to occur where atom centers are within the sum of the van der Waals radii of the two atoms, plus 1 Å. For clarity, only a representative subset of the oxygen atom is shown, each being represented by a gray sphere. The wire-cage contours show the clustering of the oxygen atoms into three favorable regions relative to the arginine, two regions being particularly heavily populated. Figure 4b shows just the arginine side chain and the contours, the higher-level contour being rendered by SURFPLOT as a shaded surface. The highly populated regions are those that are particularly favorable for hydrogen bond formation.[40]

The contouring of distributions by SURFNET has been used by Walshaw and Goodfellow[41] to highlight high-density regions in observed solvent distributions around apolar side chains in protein structures.

Box plots

The box plots produced by SURFPLOT can be useful for showing the 3D disposition of different objects relative to one another. For example, Figure 5 shows the 3D conformation of the three residues making up the His-Asp-Ser catalytic triad in the serine protease α-chymotrypsin (EC 3.4.21.1), PDB code 1cho.[42] It shows how the Asp-102 hydrogen bonds to the catalytic His-57 both to help correctly orientate it and reduce its pK_a to enable its N^{ϵ_2} to act as a proton donor or acceptor. The Ser-195, which donates its hydrogen to the histidine, performs the cutting of the

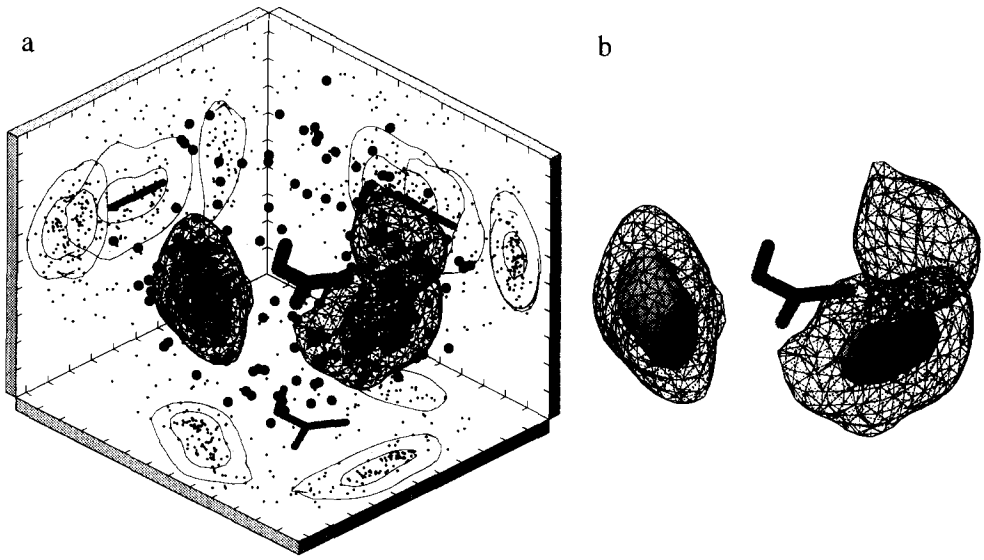

Figure 4. Three-dimensional distribution of carboxyl O^- atoms around arginine side chains, extracted from a data set of protein structures. (a) A box plot shows the distribution and density contours. The reference arginine side chain is shown by the thick bond lines, which are also projected down and back onto the three walls of the open box. The spheres in the foreground show the locations of the carboxyl O^- atoms; their back projections are shown as dots. The wire-cage contours, whose outlines are projected as continuous lines onto the back walls, show the favorable regions where clustering of the oxygen atoms occurs. (b) Only the reference arginine side chain and contour regions are shown, the inner contours being rendered as a shaded solid surface.

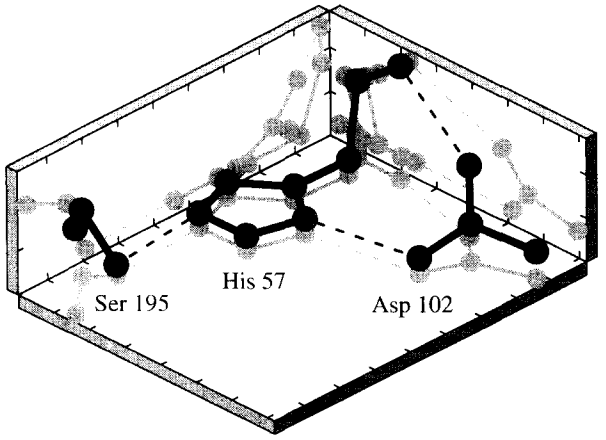

Figure 5. A box plot showing the conformation of the His-Asp-Ser catalytic triad in the serine protease α-chymotrypsin, PDB code 1cho. The gray shadows behind the side chains are orthographic projections onto the three back walls of the box, showing the three-dimensional conformation of each side chain. The dashed lines represent hydrogen bonds.

relevant bond in the substrate by attacking the scissile bond electrophilic carbonyl carbon. Color Plate 3 shows the same triad, but rendered using Raster3D.

Another box-plot example is shown in Color Plate 4. This shows the conformation of the two most favorable side chain–side chain interactions between arginine and aspartate side chains. Color Plate 4a shows the most commonly observed interaction, with the two aspartate carboxyl oxygens each hydrogen bonded to the arginine, one to the N^{η_2} and the other to the N^{ϵ}. This side-on interaction corresponds to the large favorable region for carboxyl oxygens depicted by the contours in Figure 4. Color Plate 4b shows the same interaction as Color Plate 4a, but with the van der Waals surfaces of the two side chains added in. Color Plate 4c and d shows the second most commonly observed interaction, corresponding to a head-on meeting of the arginine and aspartate side chains. These illustrations come from the electronic version of the *Atlas of Protein Side-Chain Interactions*.[43] The atlas, which analyzes all 400 possible pairwise interactions between the 20 side chains in proteins, can be accessed via the URL http://www.biochem.ucl.ac.uk/bsm/sidechains/index.html.

The box plots have also been used by Flanagan et al.[44] to depict both the calculated and observed distributions of water molecules around phenylalanine side chains.

Other surfaces

Finally, an example is given from one of the related programs to SURFNET. This is shown in Color Plate 5. The surface, generated by the program CURVE, shows a hyperbolic paraboloid that has been fitted to the β sheet in acylphosphatase (EC 3.6.1.7), (C. Mason, unpublished results), for PDB code 1aps.[45] This gives a clear indication of the curvature of the sheet. The CURVE program can also display planes and cubic surfaces, which are particularly useful in visualizing overall shapes of interfaces between molecules.

AVAILABILITY

SURFNET, and the related programs mentioned here, are freely available for academic use. The programs are written in Fortran and can be obtained by anonymous ftp from 128.40.46.11. E-mail enquiries can be sent to roman@bsm.bioc.ucl.ac.uk.

ACKNOWLEDGMENTS

The author thanks Parke Davis Pharmaceutical Research for financial support. Many thanks to Janet Thornton and Juswinder Singh for ideas, advice, and encouragement. Thanks also to Tim Slidel for the Raster3D-to-AVS conversion routines.

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