then composed of patches whose areas can be precisely integrated. van der Waals surfaces consist of convex spherical triangles whose areas can be estimated by the Gauss–Bonnet theorem. Re-entrant surfaces are comprised of concave spherical triangles whose areas can be similarly estimated and toroidal saddle-shaped patches whose areas can be calculated by analytical geometry and calculus.

22.1.2.2.2. *Lee & Richards planar slices*

The first method for calculating the accessible surface area overlaid the molecule on a regular stack of finely spaced parallel planes (Lee & Richards, 1971). The advantage of this method was the ease with which the area could be calculated. The intersection of the atomic surfaces with the planes were circular arcs whose lengths were readily calculated and multiplied by the planar spacing to give an approximation to the surface area. Programs that are currently distributed use more sophisticated methods.

22.1.2.2.3. *Connolly dot surface algorithm*

A molecular dot surface is a smooth envelope of points on the molecular surface. A probe sphere is placed at a set of approximately evenly spaced points so that the probe and van der Waals surfaces of a given atom are tangential. If the probe sphere does not overlap any other atom, the point is designated as surface. To define the re-entrant surface, sphere centres are also sampled that are tangential to both van der Waals spheres of a pair of neighbouring atoms and are equidistant from the interatomic axis. Arcs are then drawn between surface points and the arcs are subdivided into a set of finely spaced points to define the re-entrant surface. Similarly, spheres contacting triplets of neighbouring atoms are tested, and approximately evenly spaced points within the concave triangle defined by the three contact points are added to the re-entrant surface.

22.1.2.2.4. *Marching-cube algorithm*

This is conceptually the simplest method and is used in the program *GRASP* (Nicholls *et al.*, 1991). First, grid points of a cubic lattice overlaid on the molecule are segregated into 'interior' and 'exterior' as follows. All points farther from an atom than the sum of the van der Waals radius and a probe radius are flagged as external. External points with an internal neighbour are flagged as an approximate 'accessible surface'. All grid points falling within probe spheres centred at each surface point now join the set of exterior points. Points that remain 'interior' define the volume enclosed by the molecular surface.

All that remains is to contour the molecular surface that lies between interior and exterior grid points. It is a little complicated in three dimensions and is achieved by the marching-cube algorithm. Cubes containing adjacent grid points that are both interior and exterior are used to define potential polyhedral vertices. Triangles are defined by joining the midpoints of unit-cell edges that have one interior and one exterior point. The triangles are joined at their edges in a consistent manner to create a polyhedral surface.

22.1.2.2.5. *Complete and connected rolling algorithms*

Several algorithms start by dividing the surface into regions within which the surface is smooth and continuous. The surface can be efficiently described in terms of a set of arcs and their start and end points. In complete rolling, the probe is placed in all possible positions at which it contacts the van der Waals spheres of three neighbouring atoms. Those surrounding the same atom are paired as the start and end points of an arc. The complete rolling algorithm does not distinguish outer and inner (cavity) surfaces. In the connected rolling algorithm, the process starts at a triple contact point that is far from the centre of mass and therefore likely to be external. The probe is then rolled only along crevices between two atoms, pursuing all alternatives, stopping each pathway only when the probe returns to a place that has already been probed. This algorithm therefore produces only the outer surface.

22.1.2.2.6. *Analytic surface calculations and the Gauss–Bonnet theorem*

An analytical method was also proposed for calculating approximate accessible areas (Wodak & Janin, 1980). It assumed random distributions of neighbouring atoms, but this can be a sufficient approximation when calculating the area of an entire molecule. The areas of spherical and toroidal pieces of surface can be calculated exactly by analytic and differential geometry (Richmond, 1984; Connolly, 1983). An advantage of analytical expressions over the prior numerical approximations is that analytical derivatives of the areas can be calculated, albeit with significant difficulty. This then provides the opportunity to optimize atomic positions with respect to surface area. Pseudo-energy functions that approximate the hydrophobic contribution to free energy with a term proportional to the accessible surface area (Richards, 1977) can therefore be incorporated in energy-minimization programs. Although rigorous, these methods are computationally cumbersome and are not used in all energy-minimization routines. Incorporation of solvent effects may become more universal with the Gaussian atom approximations discussed below.

22.1.2.2.7. *Approximations to the surface*

The methods discussed above are computationally quite cumbersome, especially if they need to be repeated many times. Thus, they are not well suited to comparisons of many structures. They are also not well suited to the calculation of surface-area-dependent energy terms during dynamics simulation or energy minimization, which require the calculation of the derivatives of the surface area with respect to atomic position. It has been argued by several (including A. Nicholls and K. Sharp, personal communications) that simplifying approximations to the surface-area calculations are in order, because the common uses of surface area already embody crude *ad hoc* approximations, such as non-integer numbers of spherical solvent molecules.

In the treatments discussed earlier, the volume of the protein is (implicitly) described by a set of overlapping step functions that have a constant value if close enough to an atom, or zero if not. Several authors have replaced these step functions with continuous spherical Gaussian functions centred on each atom (Gerstein, 1992; Grant & Pickup, 1995) in treatments reminiscent of Ten Eyck's electron-density calculations (Ten Eyck, 1977). This speeds up the calculation and also facilitates the calculation of analytical derivatives of the surface area. A surface can be calculated for graphical display by contouring the continuous function at an appropriate threshold. The final envelope can be modified by using iterative procedures that fill cavities and crevices that are (nearly) surrounded by protein atoms (Gerstein, 1992).

22.1.2.2.8. *Extended atoms account for missing hydrogen atoms*

Structures of macromolecules determined by X-ray crystallography rarely reveal the positions of the hydrogen atoms. It is, of course, possible to add explicit hydrogen atoms at the stereochemically most likely positions, but this is rarely done for surface-area calculations. Instead, their average effect is approximately and implicitly accounted for by increasing the heteroatom van der Waals radius by 0.1 to 0.3 Å. (It is not usual to smear atoms to account for thermal motion.)

references