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Interatomic contacts in viral capsids*

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Spherical viral capsids possess icosahedral symmetry and are made of a large number of densely-packed protein subunits, each consisting of thousands of atoms. Similarly, a large number of close interactions contribute to packing in crystals of virus particles. A computational method, based on the representation of the three-dimensional shape of the subunits in the icosahedral asymmetric unit as a binary map for the fast evaluation of all short interatomic contacts between subunits within the capsid as well as between particles in the crystal lattice is presented. This method might be useful in the examination of the spatial relations of three-dimensional objects. Its application to sesbania mosaic virus reveals subunit packing dominated by polar interactions, in consistence with observed properties.

THE principles of helical and spherical virus architecture were enunciated forty years ago by Casper and Klug[1]. These principles have been revisited and reviewed in

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the light of the three-dimensional structures of spherical viruses determined in the 1980s[2] and 1990s[3]. It has been found that the protein shells or capsids of most ss-RNA icosahedral viruses consist of 180 identical protein subunits ($T = 3$ class of Casper and Klug[1]). The length of these polypeptide chains is of the order of 200–300 residues, accounting for 2,00,000–3,00,000 atoms in the capsid. The subunits are usually tightly packed in the capsid and held together by a large number of electrostatic, van der Waals and hydrogen-bonding interactions. Enumeration of these atomic interactions is essential for understanding the chemical basis of virus stability. Similarly, in the crystalline state, virus particles are tightly packed and are held together by a large number of interatomic interactions. Listing these interactions involves evaluation of N^2 distances, N being the number of atoms in the virus particle. It is shown here that a representation of the protein subunit shape as a binary map reduces the computational task, such that it could be performed on any small machine.

Sesbania mosaic virus (SeMV)[4–6] is a small, isometric plant virus that infects *Sesbania grandiflora*. It is comprised of a monopartite, single-stranded, positive-sense RNA genome of size 4149 nucleotides, encapsidated in an icosahedral protein shell made up of 180 identical coat protein subunits, each consisting of 268 amino acids. The crystal structure of the SeMV has been determined at 3 Å resolution[5,6]. Evaluation of the interatomic distances between subunits within a particle and between particles in SeMV using the procedure described in this communication reveals the dominant polar character of interactions.

The atomic coordinates of protein subunits in the asymmetric unit of the virus particle are usually specified in a Cartesian system parallel to three mutually-perpendicular, two-fold axes of the icosahedral particle.

Let X_i, $i = 1, N$ be the coordinates of atoms in the asymmetric unit of the virus particle. Positions X' of atoms in symmetry-related subunits could be derived from

$$X' = [S]X,$$

where $[S]$ is one of the sixty 3×3 rotation matrices representing icosahedral symmetry in the Cartesian system. Similarly, the positions Y of atoms in other virus particles in the crystal are given by

$$Y = [C]\,X' + [T],$$

where $[C]$ is a 3×3 matrix representing the rotational part and $[T]$ is a column vector representing the translational part of a crystal symmetry transformation expressed in the Cartesian orthogonal system. We need to evaluate all distances less than a specified limit (contact limit) between atoms X and X' (intra-viral, inter-subunit), and X and Y (inter-viral or crystal contacts).

A binary map is defined in a box surrounding the protein subunits in the icosahedral asymmetric unit, such that it extends in all three directions by at least the desired contact limit. This map is sampled at intervals of the

order of 1 Å. All bits representing the binary map are initially set to 0. As the atomic coordinates are read, the bits representing the cells around the atomic positions, which are within the contact distance, are set to 1.

Once the binary map is created, the atomic coordinates are transformed by the icosahedral symmetry to generate the coordinates of atoms in the other subunits. If the symmetry-related atom does not fall in a cell with bit on, it will not be at a distance less than the contact limit to any atom of the subunits in the reference asymmetric unit, and hence could be immediately discarded. Only the symmetry-related atoms that fall in cells with bit on are retained. Once the symmetry elements are exhausted, all potential contact-making atoms would have been generated. The next task is to evaluate the distance from these atoms to those of the reference subunits. Even here, if the ith atom of reference subunit makes contact with jth atom of a symmetry-related subunit, then closed symmetry requires that the jth atom of the reference subunit makes contact with ith atom of some other symmetry-related subunit. Therefore, atoms of the reference subunit are relevant only if they also occur in the list of atoms retained in symmetry mates. Hence, only these atoms of the reference subunit are retained resulting in a relatively small number of atoms, both in the reference and symmetry-related subunits. Therefore, evaluation of distances between these sets of atoms using the usual distance formula will be fast.

A similar procedure is also adopted for evaluating crystal contacts. Here, further enhancement of speed is achieved by noting that only the atoms in the exterior surface of the virus could be involved in contacts. Therefore, only atoms at distances greater than a specified limit from the viral centre need to be examined. This distance depends on the viral radius and crystal cell parameters.

SeMV particles consist of 180 identical protein subunits. The icosahedral asymmetric unit consists of three subunits designated as A, B and C (Figure 1). The amino terminal 72, 72 and 46 residues of A, B and C subunits, respectively are disordered in the electron-density map calculated at 3.0 Å resolution. The capsid consists of two distinct dimeric associations of protein subunits, 30 copies of $C/C2$ and 60 copies of $A/B5$. The additionally ordered segment at the amino terminus of C subunits is inserted in the inter-subunit region between two C subunits related by the icosahedral two-fold axis (C and $C2$ in Figure 1). The other distinct association is made by A and B subunits (A and $B5$ in Figure 1) related by a quasi two-fold axis of symmetry. The additional disorder found at the amino termini of A and B subunits results in a 'flatter' association when compared to the $CC2$ dimers. Figure 2 illustrates these interactions in SeMV. The three polypeptides constituting the icosahedral asymmetric unit contain a total of 4512 ordered atoms. The total number of atoms in the viral capsid is, therefore, 2,70,720.

The coordinates of the three subunits in the icosahedral asymmetric unit of SeMV have been described in a Car-

tesian system *XYZ* defined by three mutually-perpendicular icosahedral two-folds[4]. In this system, the three subunits extend from -48 to 43 Å, -11 to 75 Å and 101 to 146 Å, respectively along *X*, *Y* and *Z* directions. The size of the binary map representing the molecular envelope sampled at grid intervals of 1 Å exceeded those of the molecules in the asymmetric unit by a few Å. It is
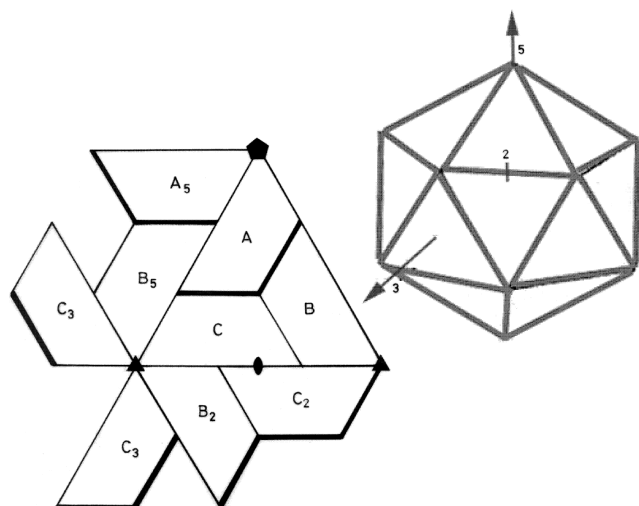
essential to examine $\sim 4512 \times 4512 \times 60$ distances to list intra-viral interactions within the specified limit by direct distance calculations. In the current procedure, application of icosahedral symmetry brought coordinates of only 771 atoms into cells with bits on. Hence the final distance calculation yielding all distances below the specified limit was obtained by computing only 771×771 distances.

Computation of inter-viral contacts usually requires examination of one cell step in the positive and negative directions (-1 to $+1$ along crystal *a*, *b* and *c* directions). This implies examination of 26 neighbours (three along *a*, three along *b* and three along *c*; excluding the molecule related by 000 translation), involving a formidable $\sim 2,50,000 \times 2,50,000 \times 26$ ($\sim 10^{12}$) distance computations. However, with the present procedure, the task is several orders of magnitude diminished by limiting the search to atoms near the outer surface of the virus, and using the binary map to select only those that have a potential to make contacts. The entire intra- and inter-capsid calculations could be completed within a few seconds on a Pentium processor.

Table 1 compares the composition of some of the amino acids that occur at least ten times in the coat protein with the composition of residues that make up the inter-subunit interfaces. At least one atom of these residues is at a distance less than 3.3 Å from an atom of another subunit. These contacts mostly correspond to hydrogen-bonding interactions. It is easily seen that the interfaces are dominantly polar in character. Serine is the most abundant residue of the capsid protein (12.8%). It is also the most frequent residue at the interface, and its interactions constitute 29% of all contacts within 3.3 Å. The other residue that occurs frequently in the interface is aspartate. Ile, Leu and Val are severely suppressed at the interface. Even when the distance criterion for deducing the residues at the interface is increased to 3.8 Å, a limit that accounts for most of the non-bonded van der Waals interactions, the interface remains essentially polar. At this distance limit, the polar residues that occur significantly more frequently than expected from the amino acid composition are Asp, Asn, Gln and Ser. The nature of

Figure 1. Organization of protein subunits in SeMV capsids. The icosahedral asymmetric unit contains three subunits designated as *A*, *B* and *C*. *A* subunits cluster as pentamers at the twelve five-fold, while *B* and *C* subunits cluster as hexamers at the twenty icosahedral three-fold axes. Capsid can also be visualized as constructed from 90 protein dimers, 30 of type *C/C*2 at the exact two-fold and 60 of type *A/B*5 at quasi two-fold symmetry axes of the icosahedral particle. (Inset) Symmetry axes of an icosahedron.
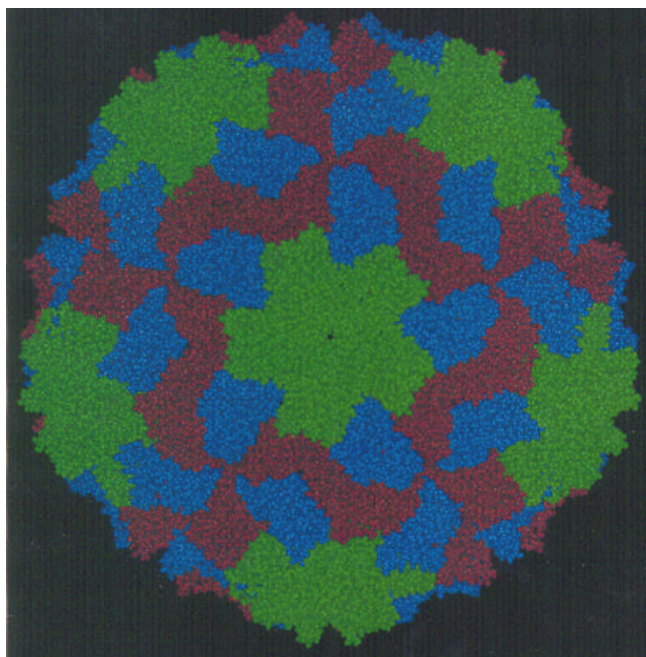

Figure 2. A hemisphere of SeMV structure shown as a space-filling model illustrating the close spatial packing of protein subunits. *A*, *B* and *C* subunits are coloured green, blue and red respectively. The figure was created using insightII.

Table 1. Comparison of amino acid composition in protein subunits and interfaces in SeMV. Residues with at least one atom within 3.3 Å from an atom of another subunit are included in the interface. The last column shows the ratio of columns 3 and 2

Amino acid	Composition (%) in the polypeptide	Number (%) of contacts	Ratio
Ala	9.2	3.0	0.33
Asp	5.6	10.8	1.92
Gly	7.1	5.7	0.80
Ile	6.1	0.6	0.10
Ser	12.8	29.0	2.30
Thr	9.7	3.0	0.31
Val	10.2	4.1	0.41
Tyr	5.6	5.4	0.96
Leu	8.2	2.4	0.29

these contacts is consistent with the observed biophysical and biochemical characteristics of the capsid[7]. The virus particles are more stable at pH 5 than at pH 8. It is possible to dissociate the virus and reassemble the subunits without denaturation, reflecting the polar nature of individual subunits. Trypsin treatment of intact particles leads to truncation of the amino-terminal segment by about 70 residues. The remaining protein domain remains soluble and folded, and assembles into $T = 1$ (ref. 8) particles containing 60 subunits. In contrast to the dense packing of protein subunits within the capsid, virus particles do not make extensive contacts in the crystals. Therefore, no statistical analysis of these contacts is presented.

The method presented here of representing the shape of a three-dimensional object as a binary map and using this map to simplify the evaluation of contacts between protein molecules, might find application in other analyses of protein structure and architecture such as complementarity of surface features and analysis of van der Waals volumes. A similar approach has been used for protein-ligand docking[9]. This method is also useful for excluding spurious solutions that lead to penetration of neighbouring molecules in molecular replacement[10], a technique for protein structure determination based on homology to a previously known structure.

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Application of molecular sexing to free-ranging Asian elephant (*Elephas maximus*) populations in southern India*

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Selective poaching of Asian elephant (*Elephas maximus*) males for ivory has resulted in highly female-biased adult sex ratios, necessitating regular monitoring of population structure and demography. We demonstrate that molecular sexing from dung-extracted DNA, based on ZFX–ZFY fragment amplification and ZFY-specific *Bam*HI site restriction, can be applied to estimate sex ratios of free-ranging Asian elephants, in addition to or instead of field demographic methods. The adult sex ratios using molecular sexing in Nagarahole and Mudumalai–Bandipur reserves during May 2001 were 1 : 3.1, matching the demography-based sex ratio for the same month, and 1 : 9.4, respectively.

MOLECULAR sexing is the process of sexing individuals based on variation in DNA between sexes. Methods include amplification of Y-specific fragments, usually based on the *SRY* gene[1], and amplification of homologous fragments (the amelogenin gene or *ZFX–ZFY* genes in mammals/CHD-CHD-W in non-ratite birds) on both X and Y chromosomes, using length polymorphism[2] or restriction fragment length polymorphism (RFLP)[3] to differentiate between the sexes. Molecular sexing has been widely used to sex foetuses in humans, other primates and livestock[2,4,5], and to a lesser extent in sexing birds[3,6,7], whales[1,8,9], seals[10] and fish[11], which are difficult/impossible to sex visually.

Embryonic fluid, blood or tissue samples are generally used as a source of DNA in these instances. Since most large terrestrial mammals are sexually dimorphic, there have been only a few field studies of molecular sexing in such species, for example apes[12] and bears[13]. However, molecular sexing can be a useful tool to sex juveniles, which lack dimorphism, or to estimate population sex ratios by carrying out noninvasive sampling. Here, we demonstrate the applicability of molecular sexing to free-ranging populations of the Asian elephant (*Elephas maximus*).

Poaching for ivory in the Asian elephant began assuming threatening proportions during the 1970s, the average number of elephants poached over the last decade in