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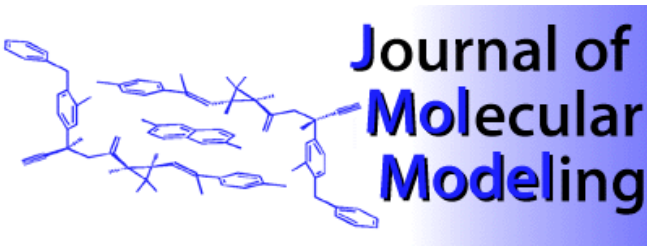

SHORT COMMUNICATION

Characterisation of Protein-Ligand Interfaces: Separating Surfaces

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Abstract A new method for characterising protein-protein complexes is presented wherein the interface is modelled as a separating surface. This surface is defined by a set of points located halfway on the shortest distance vectors between surface points of the two molecular partners. The surface is generated using a grid-based algorithm. The distance to the nearest atom is stored on the grid points and an isosurface is generated forming the separating surface. Size and shape of the surface characterises the complex interface. Distances, forces, and other physicochemical properties can be mapped onto the surface and are used to study the intermolecular interactions. This is demonstrated with the systems lysozym-antibody, p53-DNA and trypsin-BPTI.

Keywords Molecular recognition, Binding site, Interface, Molecular surface

Introduction

Macromolecular complexes are essential for biological activity and specificity, such as signal transduction, cell-cycle control, the regulation of transcription and translation, DNA repair, and many other processes. The question of how molecules in particular proteins recognize each other is of central importance. An answer to this question can be given, if one has information about the size and shape of the interface and physical properties in the interface region of the protein-ligand complexes.

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The concept of molecular surfaces [1] has been widely used to characterize the physicochemical properties and interactions of molecules. Tsai et. al related binding properties of protein-protein complexes to the solvent-accessible surface area of the interfaces [2]. Many authors used the visualization and interactive inspection of the interface regions in order to get insight in the molecular recognition process [1]. Various methods and programs exist for visualizing cavities and surface grooves. The SURFNET program developed by Laskowski is able to visualize gap regions between molecules [3]. It generates a three-dimensional grid of density values by fitting spheres in the void space between the molecules and mapping the positions of the spheres onto the grid using gaussian distributions. An isosurface of this density encloses the gap regions of the complex interface. Varshney described a parallel, analytic approach for defining and computing the inter- and intramolecular interfaces of molecules [4]. Duncan and Olson used a special

surface generation procedure for the interfacial surfaces and analysed protein-protein interactions on this basis [5]. Their interfacial surface is defined as the locus of points "halfway" between two molecular surfaces and is computed straightforward by searching for every point of the first surface the closest point of the second. The midpoints between these pairs of points form the interfacial surface where the physical properties of each subunit can be displayed. A similar approach was introduced by Gabdoulline and Wade [6]. They also generated a surface, which is defined by an exponential distance function and is obtained using a growing ring procedure. Then a projection of this surface onto a plane was used as a basis for the comparison of physical properties of the complex interface like interprotein distances, hydrophobicity, electrostatic potential etc.

In this paper a new generation method for separating surfaces is described which follows a very simple but effective algorithm.

Computational Methods

The separating surface proposed in this paper is defined by a set of points located halfway on the shortest distance vectors between surface points (CPK-model) of the two molecular partners. The surface is generated by a grid based algorithm similar to the approach of Voorintholt [7]. The molecules of the complex are placed in a regular three-dimensional grid. For each of these molecules a pseudo-density is defined by a simple linear function of the distance between the grid points and the nearest atoms (see Equation 1) . If the distance is less than the sum of the van-der-Waals radius R_j of atom j and the cutoff radius R_V, the pseudo-density value is linearly decreasing with the distance (see figure 1). Otherwise it is set to 0.

$$dens(p_i) = \begin{cases} \max_j \left(\dfrac{R_V + R_j - d_{ij}}{R_V} \right) & d_{ij} < R_V + R_j \\ 0 & d_{ij} \geq R_V + R_j \end{cases} \quad (1)$$

where:

$dens(p_i)$	is the pseudo-density at grid point i
R_V	is the cutoff radius
d_{ij}	is the distance between grid point i and atom j
R_j	is the van-der-Waals radius of atom j

If a grid point is located at equal distance from both complex partners, the difference of the pseudo-densities becomes zero. An isosurface (contour value = 0) of the difference is generated using a marching cube algorithm [8] connecting all these points and forming a triangulated surface. This isosurface is the separating surface.

The size and shape of the separating surface characterizes the complex interface. Mapping of the atom-atom distance on the separating surface can be used to identify overlapping atoms or gaps in the interface. Forces and other properties (e.g. electrostatic potential or local lipophilicity) can also be mapped onto this surface to characterize the interaction between the complex partners. Very useful for the identification of recognition sites are the product of the electrostatic potentials of both molecules or similarity indexes (like the Hodgkin index [9]).

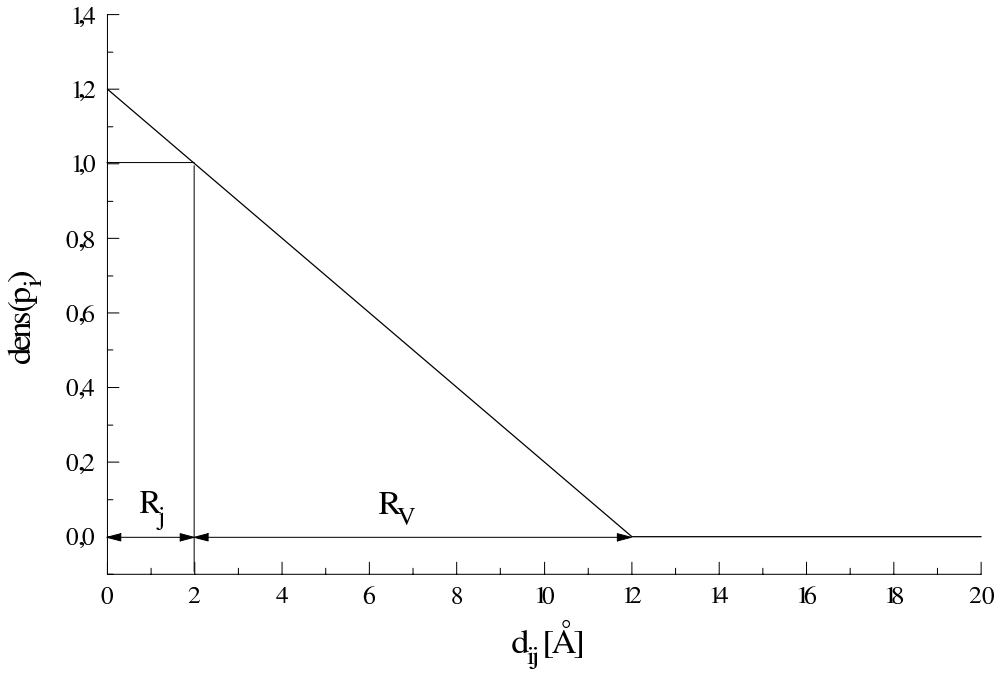

Figure 1 *Pseudo-density function* dens(p_i) *versus distance* d_{ij} *(see Equation 1)*

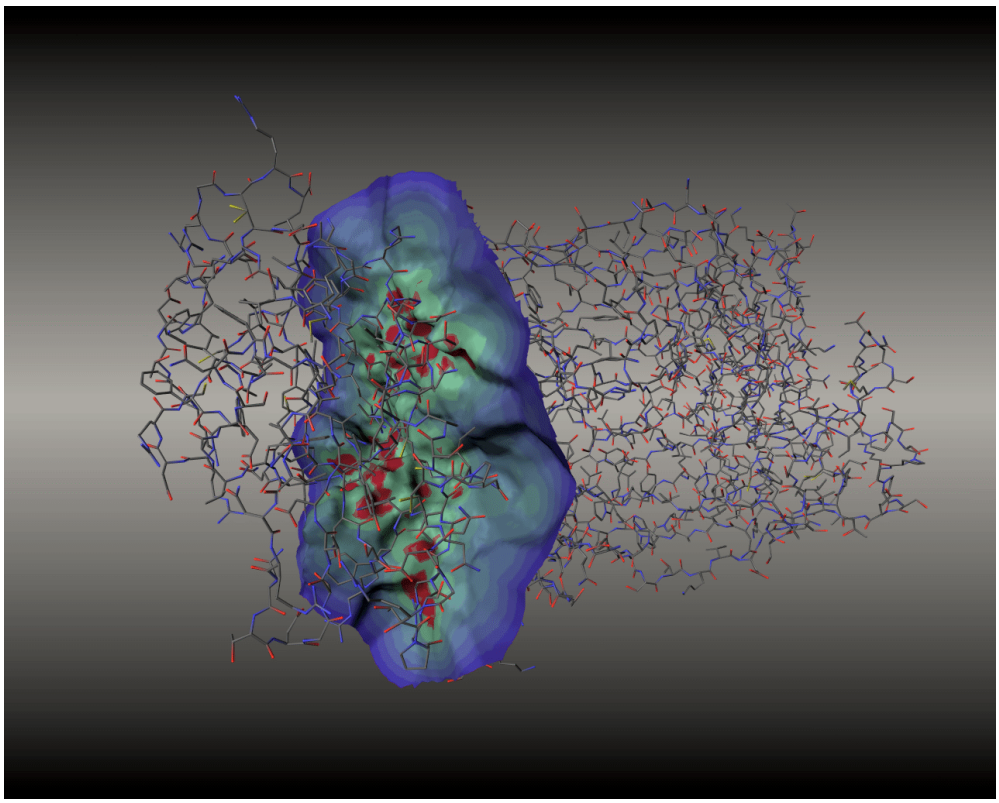

Figure 2 *Separating Surface of the lysozym-antibody interface color coded according to the distances to the nearest atoms (red: overlapping atoms)*

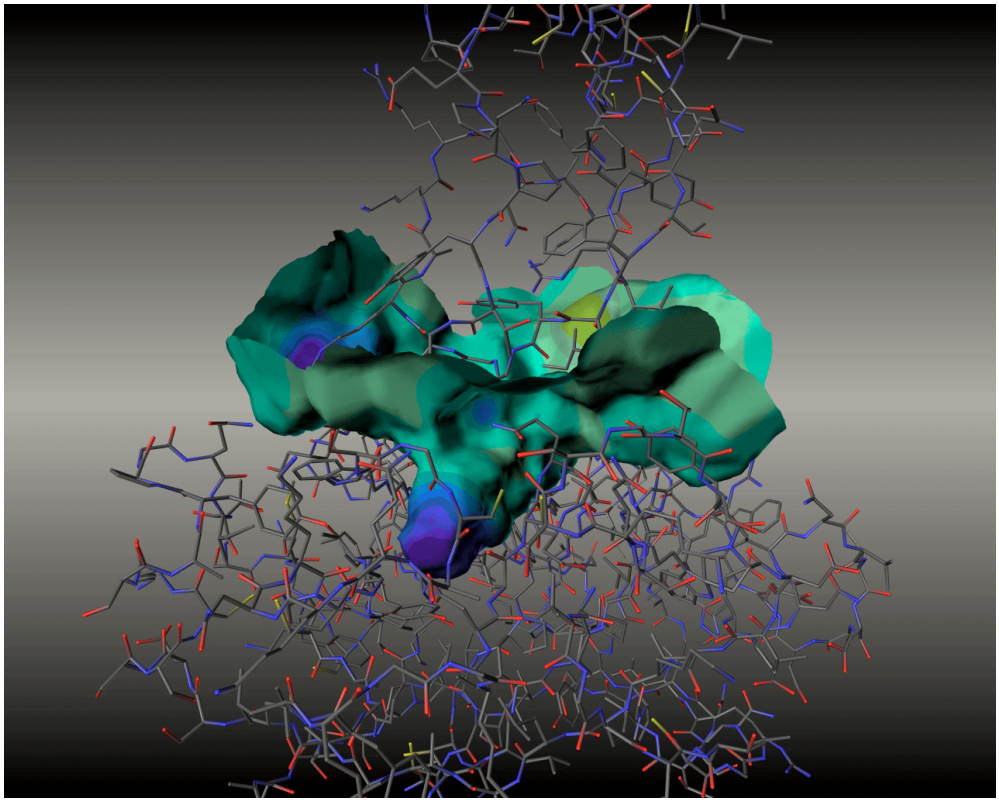

Figure 3 *Separating Surface of the trypsin-BPTI complex color coded according to the product of the electrostatic potential of both molecules (blue: attraction; red: repulsion)*

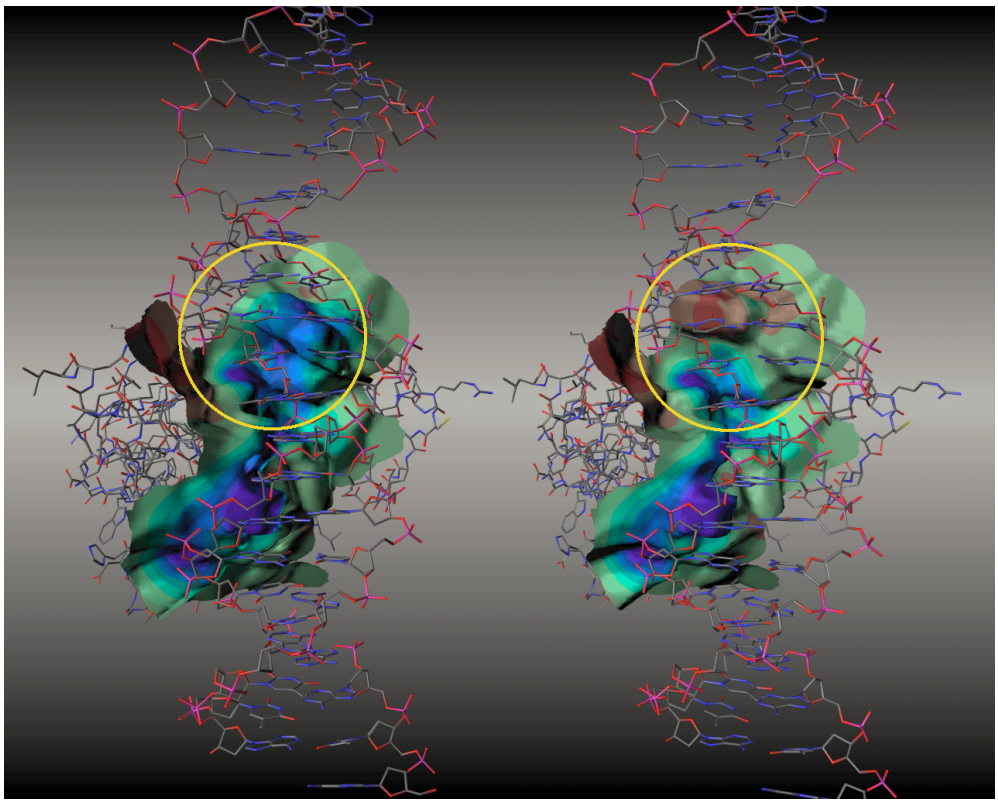

Figure 4 *Comparison of the wild type p53-DNA (left) and the Arg248Gln-DNA complex (right). The Separating Surface is color coded according to the product of the electrostatic potential (blue: attraction; red: repulsion). The mutation changes the electrostatic interaction significantly (yellow circle)*

Results and Discussion

The density method has some advantage over the direct implementation of Duncan and Olson [5]. It was mentioned by the authors that the direct algorithm of searching the nearest pairs of points on both surface is asymmetrical. Interchanging the two surfaces yields a slightly different interface. The algorithm described above does not suffer from this disadvantage. It is independent of the order of the molecules. The accuracy of the separating surface depends only of the step width of the grid. Various calculations showed that a step width of 0.5 Ångstrøm is sufficient for almost all applications.

In figures 2-4 several example applications of the separating surface method are shown. Figure 2 presents the separating surface of the lysozyme-antibody complex. Clearly visible is the big, planar interface between lysozyme and its antibody. The distance mapping reveals the tight binding with some overlapping atoms but no gaps in the interface. The trypsin-BPTI complex is shown in figure 3. In contrast to the planar lysozyme-antibody interface a knoblike part of the BPTI binds in a pocket of the trypsin. The product of the electrostatic potential of both molecular partners is mapped onto the separating surface, clearly indicating the attractive interaction (blue) at the center of the interface.

The comparative analysis of *wild type* and mutated p53 complexed with DNA demonstrated the influence of single amino acid substitutions [10]. The electrostatic potential mapped onto the separating surface show significant differences at the positions of the mutated amino acids and explain the loss of the tumor suppressor function (see figure 4).

We have presented a new, very effective algorithm for the generation of separating surfaces of molecules. The chosen examples demonstrate that the separating surface concept can be applied to different types of complexes. It can be used to investigate planar or crooked protein-protein interfaces and protein-DNA complexes as well.

Supplementary Material Available Statement The lysozyme-antibody, trypsin-BPTI, and p53-DNA complex are available in PDB- and VRML-format as supplementary materials. The separating surfaces of the complexes are included in the VRML-files and can be examined interactively with a VRML browser.

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