New and Notable

Protein-Protein Diffusional Encounter

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Protein-protein association is clearly a very important process in biology, including events such as signal transduction, immune response, and transcription. Protein-protein association rates are in the range of 10^3 to 10^9 M^{-1} s^{-1}, with those in the diffusion-limited regime having rates of $\geq 10^6$ M^{-1} s^{-1}. One well-characterized protein-protein system, barnase-barstar, associates at a rate of $\sim 10^8$ M^{-1} s^{-1}, falling well into the diffusion-limited regime (Schreiber and Fersht, 1996, and references therein). There are other factors that help to identify a system as being diffusion limited, which are that the association rates are influenced by solvent viscosity, ionic strength, temperature, and diffusional environment. The barnase-barstar system has such a high rate of association because of the contribution of electrostatics (Schreiber and Fersht, 1996). Wild-type barnase has a net charge, at pH 8, of +2, and that of barstar is −6.

In this issue, Gabdoulline and Wade (1997) describe the application of their effective charge method (Gabdoulline and Wade, 1996) in a simulation of the diffusional encounter of barnase and barstar. Protein-protein association still represents a significant challenge for computational chemistry, even though substantial advances have been made in the development of theory for these problems. Molecular dynamics methods are generally too time consuming and demanding, especially if explicit solvent is included. Most computational work on protein-protein association has been accomplished with the use of Brownian dynamics (BD) methods, which treat the solvent as a continuum. These methods, because they generally use the Poisson-Boltzmann (PB) equation for the forces due to electrostatics effects, also allow for the treatment of dissolved ions (ionic strength). Earlier work in this area focused on very simple models for the proteins, typically low-dielectric spheres with one or more embedded charges (Nambi et al., 1991; Northrup et al., 1993; Zhou, 1993a). The spheres were decorated with reactive patches, and reactions were defined to occur when the patches satisfied prescribed geometric or energetic conditions. In most of the work, the diffusing protein was treated as a "test charge" moving in the field generated by the fixed target protein (Northrup et al., 1993). This means that the diffusing protein does not perturb the electrostatic potential field of the target protein, or vice versa. This clearly represents a potentially severe assumption. Other work in this area did extend the methodology to allow for more exact treatments of the charged dielectric volumes, but they are currently too slow to be of use in BD simulations (for an example see Zhou, 1993b).

The work by Gabdoulline and Wade (1997) describes the application of an efficient method for including the effects of the diffusing, charged, low-dielectric volumes of both proteins. This is done by fitting a set of "effective charges" that reproduce the electrostatic potential fields of each protein independently. The procedure is very similar to that used for fitting partial charges to electrostatic potentials generated from quantum mechanical wave functions. A cavity term is added that corrects for the effects of the low dielectric cavities on the interaction of the effective charges. In other words, this corrects for the effect of the dielectric cavity of one molecule on the potentials of the other (Gabdoulline and Wade, 1996). The procedure is quite computationally efficient, with no more cost than the "test charge" methods, once the effective charges are computed.

A key result of Gabdoulline and Wade's work concerns the definition of the reaction criteria for a protein-protein encounter. Defining general reaction criteria has been a difficult issue in protein-protein association. The enforcement of simple geometric criteria (i.e., root mean deviation, RMSD, between the coordinates in the docked structure and those from the x-ray structure) did not work well. This may be peculiar to this protein system, inasmuch as the key contact residues in barstar make contacts with residues in barnase that are in a concave cavity. Thus it is possible to have a large RMSD while still maintaining contact with residues that are part of the cavity in barnase. In addition, the use of an interaction energy criterion did not work well, primarily because differences in rates between barnase mutants are influenced by changes in surface area and topology as well as electrostatics. However, reaction criteria that depended on a certain number of correct residue-residue contacts (2–3) did work well. This may be because this method represents the real association event, which generally consists of two phases, namely, the diffusion up to the target protein to form a weak diffusional encounter complex (with zero to one specific interactions), which then goes on to form a high-affinity complex after restricted diffusion.

Remaining areas for improvement include getting the error down as much as possible (it is currently ~0.5 kcal/mol), finding some way of ensuring that the potentials inside of the proteins are represented properly, increasing the speed of doing the BD computations, and identifying some alternative way of representing the exclusion grid so that particles can get closer than twice the radius of the exclusion probe. The last point is especially relevant to

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the problem of docking small molecules with a concave binding pocket, particularly when the opening to the site is narrow.

The use of a probe to define the exclusion grid implies that all of the atoms of the molecule being excluded have the same radii (i.e., that of the probe), which the authors point out. This is not ideal, although it is difficult to judge the impact of this very commonly made assumption. The use of a grid for the mutual exclusion of the proteins is a problem in itself. Gabdoulline and Wade use a standard method for defining it, which is that individual grid points are assigned a value of 1 or 0 to indicate whether that grid point is accessible by the probe. This results in a jagged exclusion surface that can be in error by up to a full grid spacing (half a grid spacing for each exclusion grid, i.e., one grid for each protein). On the other hand, they do not note any significant effect of reducing the exclusion grid spacing on their rates, although the encounter times do systematically decrease. This exclusion grid problem presents a difficulty in applying these methods to small molecule docking with a mostly buried binding pocket, although, in that case, one might be able to get away with treating the small molecule as a collection of test charges.

The advancements applied in the work of Gabdoulline and Wade, along with subsequent refinements, will be useful in helping to elucidate the kinetic and structural aspects of other protein-protein diffusional processes, such as signal transduction (G-proteins), immune response (antibody-antigen), and transcription (the transcription complex).

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