Wade Group

Molecular modeling, simulation and design

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The main focus of this research group is the study of biomolecular interactions using computational techniques. Modelling and simulation can provide a microscopic picture of events that may be difficult to investigate experimentally and can assist the identification of features that are important in determining molecular recognition. This information can be used for predictions of ligand-receptor complex formation and to design of ligands and protein mutations to produce desired ligand-receptor interactions. Our studies involve methodological developments and applications. The main topics of study this year are summarized below. Further information can be obtained from our Web pages at http://www.embl-heidelberg.de/~ExternalInfo/wade.

Protein-protein interactions

Protein-protein association

The rate of protein-protein association limits the response time due to protein-protein interactions. The bimolecular association rate may be diffusion-controlled or -influenced, and in such cases, Brownian dynamics simulations of protein-protein diffusional association may be used to compute association rates. Extending earlier work (Gabdoulline & Wade, 1997, 1998, Elcock *et al*., 1999), we Improved the computational model of the protein Interaction forces used In our Brownian dynamics simulations, and this has enabled us to perform comparative computations of diffusional association rates for diverse protein-protein pairs (Gabdoulline & Wade, 2001). For all protein pairs investigated, the effects of mutations can be well reproduced by the simulations, even though the degree of the electrostatic translational and orientational steering varies widely between the cases (see Figure 1). Comparison of computed and experimental rates points to Important effects of conformational gating due to protein flexibility on the association rates for some protein pairs. Our BD simulation method has most recently been applied, along with a variation of our PIPSA (Protein Interaction Properties Similarity Analysis) tool (Blomberg *et al*., 1999; De Rienzo *et al*., 2000) to computing the effects of mutations on the rates of electron-transfer between plastocyanin and cytochrome f (with F. De Rienzo, C. Menziani, P. de Benedetti, University of Modena).

Protein-protein docking

When two proteins bind, there Is usually some structural rearrangement. However, In most computational techniques for docking two proteins to predict the structure of their complex, the proteins are considered as rigid bodies or else only side-chain mobility is allowed for. We carried out an Investigation of the Impact of protein flexibility on the ability to dock two proteins that display small-scale changes In conformation upon binding. Considering the complexation of barnase and barstar as a well characterized example, we found that It was essential to consider small backbone readjustments at the same time as side-chain rearrangements for accurate docking. Further, we developd a new approach for flexible protein docking that employs the techniques of multiple copy simulation and ensemble enrichment to enhance conformational sampling (Ehrlich & Wade, 2001).

Protein-ligand binding and design

Quantitative structure-activity relationships

In COMBINE (Comparative Binding Energy) analysis (Wade *et al*., 1998), quantitative structure-activity relationships (QSARs) are derived by chemometric analysis of the results of molecular mechanics calculations

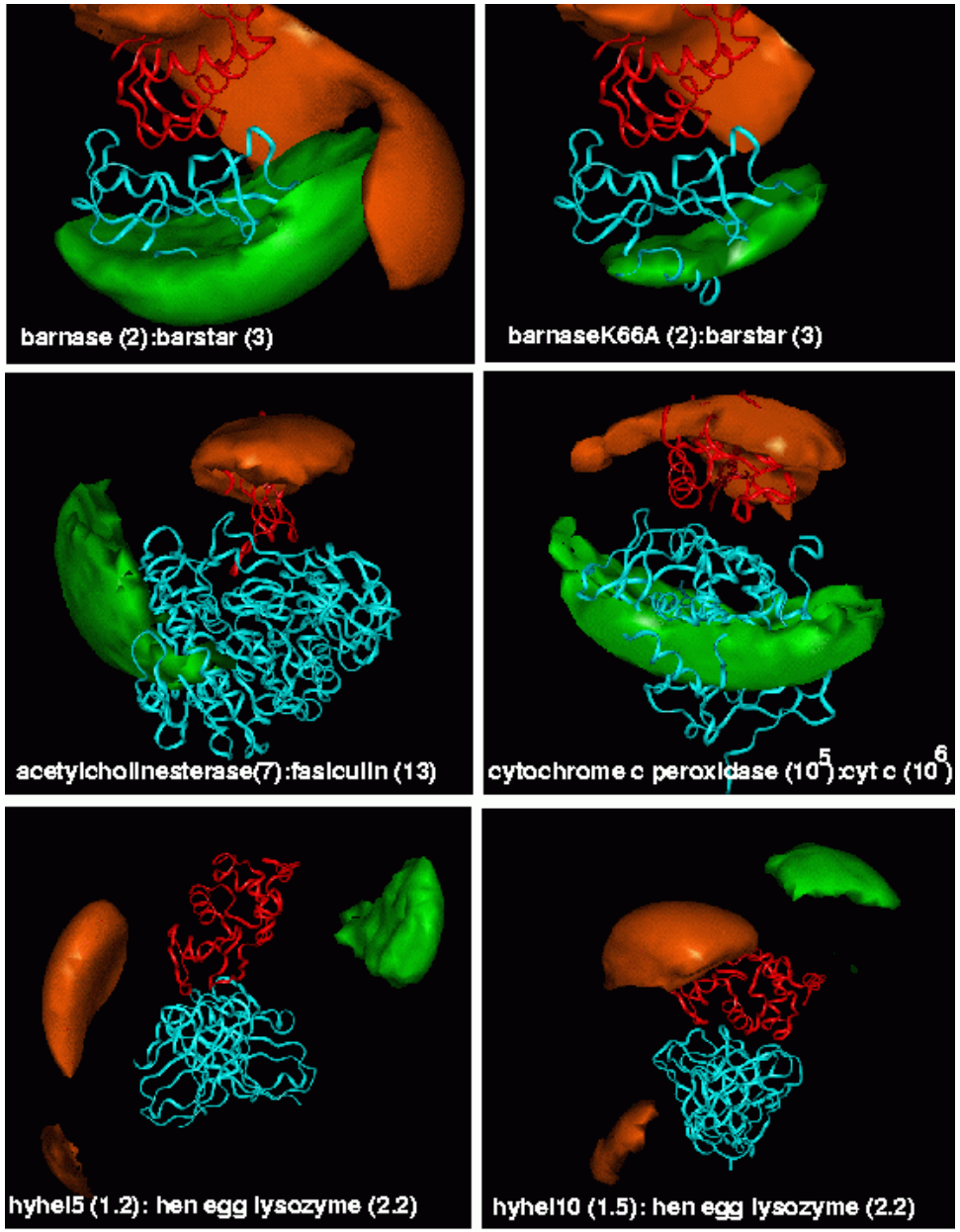

Figure 1. *Electrostatic steering for protein-protein complexes. 6 protein-protein complexes (5 wild-type and 1 mutant) are shown in ribbon representation as arranged In crystal structures of their complexes. The contours show the most energetically favourable regions for each protein to reside in around the other as derived from computation of Boltzmann factors of the protein interaction energies. Only electrostatic and exclusion interactions are considered to act between the two proteins. Green contours are for the distribution of the protein shown in blue (named first) around the protein shown in red (named second). Orange contours are for the distribution of the protein shown in red around the protein shown in blue. The contour level is indicated in parentheses after the protein name and is a probability. The strongest steering and electrostatic attraction is present for the cytochrome c peroxidase-cytochrome c interaction. Orientational but less perfect steering towards the bound complex is seen for the acetylcholinesterase-fasciculin and barnase-barstar complexes. The single point mutation for barnase demonstrates how its orientational steering could be improved. However such a mutation does not significantly alter the association rate of the proteins, which in both cases is enhanced by electrostatic interactions. For the two antibody-lysozyme complexes, the bound complexes are far from being the most electrostatically favorable although electrostatic interactions do weakly enhance the rate of association of these protein pairs.*

for 3D structures of protein-ligand complexes. This year, we have applied COMBINE analysis to several diverse problems:

- Influenza neuraminidase Inhibition. Neuraminidase Inhibitors have recently become available In the clinic for treatment of Influenza. By studying a diverse set of Inhibitors and their binding to different subtypes and mutants of Influenza neuraminidase, we derived a predictive QSAR model that highlights Interactions Important for Inhibition. This QSAR model provides guidelines for structural modification of current Inhibitors and the design of novel Inhibitors In order to optimize Inhibitory activity (Wang & Wade, 2001ab)

- Nuclear receptor-DNA binding specificity. Following our COMBINE analysis of a dataset of 320 mutant glucocorticoid receptor DNA-binding domain - DNA response element complexes (Tomic *et al.*, 2000ab), we studied a second set of different mutants Is better characterized experimentally. Models of better predictive ability were obtained, despite a much smaller dataset size (collaboration with S. Tomic, Ruder Boskovic Institute, Zagreb).

- Haloalkane dehalogenase substrate specificity. Haloalkane dehalogenases are microbial enzymes that catalyze dehalogenation reactions, which are important for the degradation of environmental pollutants. COMBINE QSAR models have been derived to predict substrate binding specificity and the effects of point mutations, and will be used to guide site-directed mutagenesis to engineer enzymes with modified substrate specificity for environmental clean-up purposes (collaboration with J. Damborsky & J. Kmunicek, Masaryk Univ, Brno; A. Ortiz, Mount Sinai School of Medicine, New York; & F. Gago, Univ. of Alcala de Henares, Madrid).

Enzyme inhibitor design

Thymidylate synthase is involved in DNA synthesis and, because of Its role In the cell cycle, Is considered a design target for drugs against hyperproliferative diseases such as cancer and, more recently, against bacterial and viral Infections. We employed structure-based drug design techniques to design new compounds based on lead anti-cancer and anti-bacterial agents. We also Investigated the basis of species specificity for a new class of non-folate compounds, and proposed a mechanism dependent on Inter-species differences In protein dynamics. The proposals from modeling studies are currently being tested experimentally (collaboration with S. Ferrari, M. Ingrami & M. P. Costi, Univ. Modena).

Cytochrome P450-ligand binding

Cytochrome P450s play important roles in the synthesis and metabolism of endogenous and xenobiotic compounds. They are targets for redesign for large-scale biosynthesis and for development as biosensors. They are also Important In drug design, In particular as they are key to the breakdown of many drugs and a common cause of drug-drug Interactions. We have continued studies Into the mechanisms by which substrates access and products exit the buried active sites of cytochromes P450 by our Random Explusion Molecular Dynamics (REMD) approach (Luedemann *et al.*, 2000ab). Recent simulations of cytochrome P450eryF have led to Identification of a novel mechanism for controlling ligand channel opening In the protein Involving a buried arginine residue. We have also developd our MolSurfer visualization tool (Gabdoulline *et al.*, 1999, 2001) to assist visualization of simulated ligand explusion trajectories. Recently, we have performed modeling studies of a mammalian microsomal cytochrome P450 that have provided the basis for mutagenesis studies to Investigate Its function, Induction and degradation (collaboration with A. Banerjee, Wayne State Univ, Detroit).

Biochemical pathways

We have started to develop software for modelling and visualization of the structures of protein-ligand complexes In the context of biochemical pathway analysis (R. Gabdoulline, A. Gonzalez, European Media Laboratory, Heidelberg In collaboration with P. Bork, EMBL, R. Herrmann, ZMBH, E. Minch, Lion Biosciences, I. Rojas, EML, Heidelberg).

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