MM and QM/MM Dynamics Simulations of the Tetrahedral Intermediate in the Deacylation Step of the Catalytic Mechanism in Serine Proteases.

Maya Topf, Peter Varnai and W. Graham Richards

Central Chemistry Lab, University of Oxford, South Parks Rd, Oxford, OX1 3QH
maya@bellatrix.pcl.ox.ac.uk

MM and ab-initio QM/MM dynamics simulations were used to simulate the formation of the tetrahedral intermediate and its characteristics during the deacylation step of serine protease catalysis. The model is based on recent high-resolution crystallographic data for the acyl-enzyme intermediate at pH 5. PMF of the reaction in water and in the enzyme was calculated using umbrella sampling. The QM/MM dynamics reveals a weakly bonded tetrahedral system, which is stabilized by short hydrogen bonds to the backbone amide groups of Gly-193 and Ser-195 in the oxyanion hole. Other unusual short hydrogen bonds were observed in the active site, as well as a proton transfer with a low barrier between the imidazole ring of His-57 and the carboxylate group of Asp-102. Movement of loop 216-223 and P2-P4 residues support the results of a recent crystallographic time-resolved study on the deacylation step, suggesting that this movement is related to product release.

Simulating the Induced Fit: A Combined QM/MM Docking Approach

Frank Beierlein, Harald Lanig, Gudrun Schuerer, Anselm Horn and Tim Clark

Computer-Chemie-Centrum, Friedrich-Alexander-Universitaet Erlangen-Nuernberg, Naegelsbachstr. 25, D-91052 Erlangen, Germany, Tel. +49 9131 85 26581, Fax +49 9131 85 26565, E-mail: frank.beierlein@chemie.uni-erlangen.de

A combined QM/MM docking approach for the investigation of protein-inhibitor complexes will be presented. A docking algorithm, which uses a rigid protein environment, first explores the binding position, the orientation and the internal degrees of freedom of a small organic molecule within an enzyme. The subsequent semiempirical AM1 QM/MM optimization of the complex obtained by docking gives a more detailed description of the binding mode and the electronic properties of the ligand. As we use a flexible protein environment in the QM/MM optimizations, we are able to simulate the structural changes of the enzyme upon binding a ligand. The method was validated using a set of structurally known protein-inhibitor complexes, whose crystallographic data were taken from the Protein Data Bank. The scoring function of AutoDock 3.0 was compared with the results of the QM/MM calculations. Structures of uncomplexed HIV-1-protease and thrombin, which were also taken from the Protein Data Bank, were used successfully for QM/MM docking experiments. By comparing the resulting structures with those obtained using protein structures from crystallized protein-inhibitor complexes, we could show that the method is able to simulate the effect of the induced fit.

QM/MM STUDIES OF THE CITRATE SYNTHASE REACTION MECHANISM

Francesca Perruccio and Adrian J. Mulholland
School of Chemistry, University of Bristol, Bristol BS8 1TS, UK
Francesca.Perruccio@bristol.ac.uk
Adrian.Mulholland@bristol.ac.uk

Enzymes, biological catalysts, accelerate and co-ordinate a large number of chemical reactions necessary to develop and sustain life. An important aim in biochemistry is to describe fully enzyme-catalysed reactions. Computer simulation can be a powerful tool to allow insight into the reaction mechanism: with this kind of study we can calculate the energy profile for the reaction within the enzyme directly, find information on unstable species and transition states, that are often difficult to analyze experimentally. Citrate synthase is an important and well characterized enzyme: it has been studied intensively by a variety of techniques (a number of high resolution crystallographic structures of inhibitor and substrates complexes have been solved [1], and there is a large amount of kinetic data available [2]). It plays a key role in a central metabolic pathway, catalysing the entry of a carbon atom into the citric acid cycle according to the scheme:

oxaloacetate + acetyl-CoenzymeA + H_2O → citrate + coenzymeA

QM/MM (quantum mechanics/molecular mechanics) methods are a promising approach to study enzyme mechanism: the most interesting aspect is the possibility to combine the versatility of a QM method with the simplicity and speed of a molecular mechanics force field. QM/MM calculations [3] have been carried out at *ab initio* and semiempirical molecular orbital QM levels for the citrate synthase mechanism. The goal is to examine the formation of intermediates and the role of catalytic residues, and to identify important interactions within the enzyme. Both the first step (deprotonation of acetyl-CoA) and the subsequent nucleophilic attack on oxaloacetate have been studied. Approximate transition state structures and reaction pathways have been calculated. Important residues have been identified as targets for site-directed mutagenesis. The results have been compared with experimental studies. The findings shed light on the reaction mechanism of citrate synthase.

References:

[1] Karpusas, M., Holland, D., Remington, S. J. *Biochemistry* 1991, 30, 6024-6031.
[2] Lenz, H., Buckel, W., Wunderwald, P., Biederman, G.; Buschmeier, V., Eggeger, H., Cornforth, J.W., Mallaby, R. *Eur. J. Biochem.*1971, 24, 207-215.
[3] Mulholland, A. J., Lyne, P.D. & Karplus, M., *J. Am. Chem. Soc.*, 122, 534-535, 2000

Asymmetry of LysU demonstrated though molecular dynamics simulations and free energy calculations.

Samantha J. Hughes

Department of Chemistry, Imperial College of Science, Technology and Medicine, Exhibition Road, London SW7 2AY. email: s.j.hughes@ic.ac.uk

Molecular dynamics simulations of the homodimeric lysyl-tRNA synthetase from *E.coli*. (LysU) show that the enzyme exhibits asymmetry both in terms of its overall structure and in the binding of MgATP. This is particularly interesting as the crystal structure of the MgATP bound LysU is symmetric. Isothermal titration calorimetry experiments, carried out to further explore this asymmetry, have revealed a binding stoichiometry of only one MgATP per LysU dimer. We have further examined this apparent half-of-the-sites binding behaviour using the recently developed MM-PBSA[1] method for determining absolute and relative binding free energies. The calculated binding affinities show a clear difference between the two sites, in good agreement with experiment.

In this talk, the development of asymmetry will be demonstrated. The MM-PBSA method will be described and its application to determining the absolute and relative free energies of MgATP binding at each site will be discussed. A pathway for the transmission of information between the remote active sites will be proposed, along with possible implications of the half-the-sites activity for the correct functioning of LysU and other aminoacyl-tRNA synthetases.

---

[1] P.A. Kollman et al, *Acc. Chem. Res*., **2000**, 33, 889-897

Bivariate Multicanonical WHAM: An efficient and accurate Route to Relative Binding Free Energies and Entropies.

Christopher J. Woods(i)(a), Michael A. King(ii), Jonathan W. Essex(i)(b)

(i) University of Southampton, Highfield Campus, Southampton, SO17 1BJ, UK

(ii) Celltech Chiroscience plc., 216 Bath Road, Slough, SL1 4EN, UK

(a) cjw196@soton.ac.uk

(b) jwe1@soton.ac.uk

The rapid and accurate calculation of relative binding free energies is one of the great challenges of computational chemistry. The traditional methods of free energy perturbation (FEP) and thermodynamic integration (TI) suffer from poor convergence due to thier sampling from a Boltzmann distribution. To overcome this, a new method is proposed that uses adaptive umbrella WHAM to sample from an arbitrary distribution - in this case an even-energy / even-lambda distribution. This generates a multicanonical ensemble which improves convergence through enhanced sampling of transition states. In addition, this combination of adaptive umbrella WHAM(1) with the bivariate multicanonical ensemble(2), here called Bivariate Multicanonical WHAM (BMW), also allows the efficient and accurate calculation of relative binding entropies and internal energies.

This talk will detail the theory and development of BMW. It will discuss the results of its application to two test systems, and further, its comparison with adaptive umbrella WHAM and FEP.

(1) Bartels, C, Shaefer, M, Karplus, M, 'Determination of equilibrium properties of biomolecular systems using multidimensional adaptive umbrella sampling', J. Chem. Phys., 111:17, 8048-8067, 1999

(2) Higo, J, Nakajima, N, Shirai, H, Kidera, A, Nakamura, H, 'Two-Component Multicanonical Method for Effective Conformational Sampling', J. Comp. Chem., 18:16, 2086-2092, 1997

A Target Selection Informatics Resource for Structural Genomics

Ana Rodrigues *[Supervisor: Prof. Roderick E. Hubbard]*

York Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York YO10 5DD. rodrigues@ysbl.york.ac.uk

The availability of the complete genomic sequences from a variety of organisms, including that of the human species, has resulted in radical changes for many areas of biological research. Structural biology is no exception, spinning off a new breed of research programs, which have become known as structural genomics projects. These endeavours aim at obtaining a complete structural description of a defined set of molecules, such as those encoded by a genome.

Unfortunately, determining a protein structure is still significantly more time-consuming and expensive than determining its sequence. Thus, the success of the upcoming structural genomics projects depends on the development and improvement of high-throughput methods for structure determination, as well as intelligent selection and prioritisation of targets.

The aim of my work is to develop an informatics resource as well as the bioinformatics methodology to support a structural genomics research program. It should be capable of selecting and prioritising candidate proteins for structural determination from the raw genomic data the user has an interest in. The work is being developed at three distinct levels. The first focuses on the identification of the kinds of annotations that can be devised for a nucleotide or protein sequence and the assessment of the usefulness of such information for structural genomics. The second aims at the establishment of an informatics framework to support these calculations and data through a relational database. The third envisages the refinement of the system and its application to a real structural genomics program.

The proposed talk would focus on presenting, discussing and establishing the context of the work carried out in the past year. This work reflects each of the strands of research described above, thus ranging from bioinformatics problems to user interface usability considerations.

References:

Structural Genomics Supplement - Nature Structural Biology 7, 2000.

Computational Modelling of Molecular Motor Proteins

Barry Grant [Supervisors: Dr. Leo Caves (York) & Dr. Rob Cross (MCRI, Oxted)]

York Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York, YO10 5DD
Molecular Motors Group, Marie Curie Research Institute, The Chart, Oxted, Surrey RH8 0TL
grant@ysbl.york.ac.uk

Molecular motors allow cells to set up complex structure, and then continuously to maintain and adjust it, by directing packets of molecular components to localised, and distant, reaction sites. For example they shuffle chromosomes around during cell division, move organelles and neurotransmitters around inside brain cells, help microbes move and cause the motion of muscles.

Asking questions about the mechanism of these processes leads quickly down to the molecular level and the necessity to elucidate the mechanism of conformational changes in motors. Over the last few years, a general but somewhat incomplete picture of how motor proteins move and generate force has emerged: small conformational changes initiated in the nucleotide binding pocket of the motor domain are amplified into large structural changes in the protein. It is these conformational changes which produce forceful stepping of the motor along its track, and which the cell must control and harness to organise itself.

To elucidate further molecular motor's mechanism of motion, my investigations have concentrated largely on kinesin, the smallest and potentially the simplest known motor. The focus of these investigations being the implementation of computational simulations (that incorporate the available biochemical, biophysical, and structural data), to explore mechanisms for conformational change, and then to experimentally test the mechanisms using optical trap techniques (which are capable of applying and measuring force at the single molecule level).

EVALUATION OF K^+ CHANNEL MODELS USING MD SIMULATIONS

Charlotte E. Capener and Mark S. P. Sansom

Laboratory of Molecular Biophysics, University of Oxford, Oxford OX1 3QU

Contact: charlc@biop.ox.ac.uk

K channels are a large family of integral membrane proteins that selectively allow the movement of K ions across bacterial and mammalians cells and are involved in numerous cellular processes.

Kir6.2 is one of the family of inwardly rectifying K channels (Kirs) and provides the pore-forming domain of the ATP-sensitive K channel that plays an important role in controlling insulin release in β-pancreatic cells. Kirs share a topology with the bacterial K channel KcsA (PDB code 1BL8). However, whilst KcsA shows highest identity to the pore-forming of domain of the eukaryotic voltage-gated K channels, it appears to be more distantly related to the Kirs with sequence ~15%. Consequently there is still some debate over whether the structural architecture of KcsA is also common to Kirs.

Here, two homology models of a Kir channel are generated from different sequence alignments of the second transmembrane helix with KcsA. A series of molecular dynamics simulations show that general properties of models such as stability (measured by structural drift) are robust to changes in simulation protocol. Simulations were run to compare the dynamic properties of the two homology models. The transmembrane domain of the model with helical packing closest to that of KcsA was identified as significantly more stable than the other model. A shared architecture between KcsA and the Kirs may be further supported by a recently discovered bacterial Kir homologue.

Homology Modelling of the Novel Cytochrome P450 CYP4X1

Lorna Gillett

Cancer Research Laboratories,School of Pharmaceutical Science, University of Nottingham

lorna@holmes.cancres.nottingham.ac.uk

Recently a novel human cytochrome P450 (CYP) has been cloned, which is highly and specifically expressed in certain tissues. With clear homology to the CYP4 class of enzymes, it has been named CYP4X1.It has been hypothesised that this novel P450 may be involved in eicosanoid metabolism, and so have an important physiological role in the kidney and other organs. However, the true natural substrate for this enzyme remains unknown.

A homology model for CYP4X1 has been constructed from the bacterial crystal structure CYP102. This has been compared with a model of the closely related CYP4A1.Investigation of the putative active sites of the CYP4X1 with the additional experimental knowledge from CYP4A1 has revealed the residues and sites expected to be important in substrate binding. Thus, possible substrates for CYP4X1 have been predicted.

Gillett LC [1],O'Brien SE [1],Laughton CA [1],Barrett DA [2],Shaw PN [2],Bell DR [3]

[1]Cancer Research Laboratories,School of Pharmaceutical Science

[2]Separation Science Group,School of Pharmaceutical Science

[3]School of Biological Sciences

University of Nottingham

References

1.Laughton C.A.(1994) Prediction of Protein Side-chain Conformations from Local Three dimensional Homology Relationships, J. Mol. Biol., 235, 1088-1097

2.Case D.A. et al (1999) AMBER 6, University of California, San Francisco

3.Laskowski R.A., MacArthur M.W., Moss D.S. & Thornton J.M.(1993) PROCHECK: a program to check the stereochemical quality of protein structures. J. Appl. Cryst., 26, 283-291

4.Laughton C.A., Unpublished CYP4A1 model, Personal Communication

GENERATION AND DISPLAY OF ACTIVITY-WEIGHTED CHEMICAL HYPERSTRUCTURES

Nathan Brown [n.brown@shef.ac.uk], **Peter Willett, David J. Wilton**
Krebs Institute for Biomolecular Research, Department of Information Studies, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK.

Richard A. Lewis
Eli Lilly & Company, Erl Wood Manor, Windlesham, Surrey, GU20 6PH, UK.

ABSTRACT

The chemical hyperstructure is a single graph representing a library of 2D molecular structures, and is generated by sequentially overlapping every molecule in the library to the hyperstructure so as to maximise the number of node and edge correspondences thereby limiting structural redundancy in the resultant hyperstructure [3]. However, this mapping procedure – defined in graph theoretic terms as the discovery of the maximum overlap set [MOS] or the maximum common edge subgraph [MCES] – suffers from combinatorial explosion for all but the most trivial of problems. To limit the computational intractability of the mapping stage, a non-deterministic optimisation technique, inspired by observations of genetic evolution in nature, is applied; referred to as a Genetic Algorithm [GA]. Adapting the canonical GA to the problem of discovering the maximum overlap between two graphs permits the generation of a suitably reduced chemical hyperstructure in far less CPU cycles than would be feasible with more traditional deterministic methods [1, 2].

By applying the chemical hyperstructure generation process with input structures that are known to be either active or inactive in a particular assay of interest, we aim to provide an approach to substructural analysis that maintains full topological information obviating the need to fragment the molecules that are being analysed. This presentation will describe the algorithm we have developed for the generation of hyperstructures and the results of preliminary experiments which suggest that the approach is able to identify structural features that are correlated with [in]activity.

[1] Brown, R. D.; Downs, G. M.; Jones, G. and Willett, P. Hyperstructure Model for Chemical Structure Handling: Techniques for Substructure Searching. *J. Chem. Inf. Comput. Sci.* **1994**, *34*, 47–53.

[2] Brown, R. D.; Jones, G. and Willett, P. Matching Two-Dimensional Chemical Graphs Using Genetic Algorithms. *J. Chem. Inf. Comput. Sci.* **1994**, *34*, 63–70.

[3] Vladutz, G. and Gould, S. R. Joint Compound/Reaction Storage and Retrieval and Possibilities of a Hyperstructure-Based Solution. In *Chemical Structures. The International Language of Chemistry*; Warr, W. A., Ed.; Springer Verlag: Berlin, 1988, pp 371–384.

A QSAR for all Sweet Tasting Compounds

By Michael G.B.Drew and Channa K.Hattotuwagama.

Department of Chemistry, University of Reading, Whiteknights,

Reading, RG6 6AD, UK. c.k.hattotuwagama@reading.ac.uk

We have successfully created Quantitative Structure Activity Relationships (QSARs) for separate families of sweet-tasting compounds including isovanillate [1], sulfamate [2], guanidine and sucrose derivatives. These QSARs can be used to help explain the sweet-taste of known molecules in the families and also to predict the taste of molecules as yet untasted or unsynthesised.

However it is well known that QSAR methods work best with families of molecules and it is a major challenge to develop QSARs for a variety of sweet-tasting molecules. If there is only one sweet-taste receptor then it ought to be possible to achieve this goal, but it could be argued that the existence of more than one sweet-taste receptor would make this goal impossible to achieve, although it might be possible to distinguish between the mode of action for different groups of families.

We have carried out preliminary statistical analyses for several sweet-tasting families including isovanillates, sulfamates, guanidine and sucrose derivatives. Relative Sweetness values range from 0.2 to 205,000. Conformational analyses have been carried out on these molecules to obtain the lowest energy conformations which were subsequently used to generate the molecular descriptors. Principal Components Analysis, Discriminant Analysis and Tree Analysis methods have all been used to distinguish between the tastes of the various families and preliminary results suggest that these techniques are likely to be successful in the development of a complete QSAR.

[1] Bassoli, A, Drew, M.G.B, Hattotuwagama, C.K, Merlini, L, Morini, G, Wilden, G.R.H. *Quant.Struct.Act.Rel.* 2001, 20, 1.

[2] Drew, M.G.B, Wilden, G.R.H, Spillane, W.J, Walsh, R.M, Ryder, C.A, Simmie, J.M. *J.Agric.Food Chem.* 1998, 46, 3016.

The Application of Neural Networks to Molecular Recognition

Thomas Kabir

Department of Biochemistry & Molecular Biology,
University College London. thomas@biochemistry.ucl.ac.uk

In the work that is presented here a neural network to improve upon an existing technique known as Patch Analysis to study protein interactions. Given the structure of a protein alone we aim to determine where on the surface of a protein are its binding sites for various classes of protein-protein complexes such as homodimers and antibody-antigens. It should be possible to extend this approach to other protein-ligand complexes and to determine (as far as possible) any characteristic properties of the binding sites of these complexes. So we aim to see for instance, if a small ligand-binding site can be distinguished from a DNA binding site or a protein-protein-binding site. In the results presented here a neural network based approach is used to predict the location of the protein-protein interfaces of two separate datasets of 28 and 72 non-homologous homodimers and a dataset of 26 homo-trimers. The structural characteristics non-homologous datasets of homo-dimeric, trimeric, tetrameric and hexameric proteins are also discussed. The approach that is used to predict the location of protein-protein binding sites is based upon a method known as Patch Analysis (Jones & Thornton, 1997). The Patch Analysis technique defines a series of contiguous patches over the surface of a protein. Physical and chemical information about each patch is encoded in the form of six parameters. One of these parameters is hydrophobicity. Another parameter that is used is accessible surface area (ASA). This parameter gives a measure of the extent to which proteins may form contacts with other molecules in solvent. All six parameters for every patch that is defined on the surface of a protein together with the its actual overlap with known protein-protein interface are then presented to a feed-forward neural network. The network then attempts to make a prediction as to what extent each patch overlaps with the known protein-protein interface using the six parameters alone. The patches are then ranked according to their predicted overlap with the known interface. Seventy six percent of the dimer interfaces were correctly located as compared to sixty six percent using the original Patch Analysis technique. Sixty five per cent of the protein-protein interfaces of the dataset of homo-trimeric proteins are also correctly located. A prediction is defined as being correct if any of the three highest ranking patches contains seventy percent of the residues that are to be found in the patch which best covers the protein-protein interface. In many of the cases for which the dimer interface is not located a known ligand-binding site is located instead. A neural network has the ability to adjust its weights so as to more efficiently use the data that is presented to it. It is this ability that accounts for the improved performance of the neural network over the original Patch Analysis technique. An analysis of the weights of the neural network can yield information as to which of the six parameters are being considered to be most important in the predictive process. Such an analysis of the weights of the network when tested on both datasets of homodimers and the dataset of homo-trimers reveals that hydrophobicity and surface accessibility appears to be major factors that discriminate between binding and non-binding regions on a protein surface. Further work is being carried out on datasets of hetero-proteins.

Reference

Jones, S & Thornton, J. Prediction of Protein-Protein Interfaces Using Patch Analysis.
J. Mol. Biol. Vol. 272 p132-143 (1997)

The 11β-hydroxysteroid dehydrogenase isozymes - challenges in drug design

Amber Seigel and P. M. Dean

Amber.Seigel@ddg.phar.cam.ac.uk
Drug Design Group, University of Cambridge, Department of Pharmacology, Tennis Court Road,
Cambridge, CB2 1QJ, United Kingdom

Two 11β-hydroxysteroid dehydrogenase (11β-HSD) isozymes have been described: type I and type II. 11β-HSD type I is responsible for regenerating active cortisol in vivo and has been implicated in the pathology of non-insulin dependent diabetes mellitus, neurodegeneration, hypertension, obesity and fertility. This isozyme has therefore been identified as a good candidate for drug design. 11β-HSD type II inactivation of excess cortisol is essential to prevent inappropriate stimulation of the mineralocorticoid receptors in the kidney. It is therefore not desirable to inhibit this isozyme. Designing inhibitors selective for type I is most likely to be successful using structure-based methods.

These techniques require knowledge of the 3D structure of the binding sites of the isozymes which have not yet been experimentally elucidated. Fortunately, these enzymes belong to the short chain dehydrogenase (SDR) family which is characterised by a highly conserved domain structure despite low pairwise sequence identities (<20%). The 3D structure of several of these homologous SDR proteins has been determined by X-ray crystallography.

The work presented here describes the challenges faced and the lessons learnt in predicting the 3D structure of these isozymes, in characterising their binding sites and finally in designing novel chemical inhibitors selective for 11β-HSD type I. A brief description of the computational methods employed is included. These include the homology modelling program MODELLER[1,2], protein structure assessment methods[3,4,5], the flexible docking program AFFINITY[6,7], site analysis algorithms developed in-house and the structure generation algorithm SKELGEN[8,9].

[1] Sali, A. and Blundell, T. L., (1990) *Journal of Molecular Biology*, **212**, 403-428.
[2] Sali, A. and Blundell, T. L., (1993) *Journal of Molecular Biology*, **234**, 779-815.
[3] Laskowski, R. A. (1993) *Journal of Applied Crystallography*, **26**, 283-291.
[4] Lüthy, R., Bowie, J. U. and Eisenberg, D., (1991) *Proteins*, **10**, 229-239.
[5] Ramachandran, G. N., Ramakrishnan, C. and Sasisekharan, V., (1963) *Journal of Molecular Biology*, **7**, 95-99.
[6] Luty, B. A., Wasserman, Z. R., Stouten, P. F. W. *et al.*, (1995) *Journal of Computational Chemistry*, **16**, 454-464.
[7] Stouten, P. F. W., Froemmel, C., Nakamura, H. *et al.*, (1993) *Molecular Simulation*, **10**, 97-120.
[8] Todorov, N.P. and Dean, P.M., (1998) *Journal of Computer-Aided Molecular Design*, **12**, 335-349.
[9] Todorov, N.P. and Dean, P.M., (1997) *Journal of Computer-Aided Molecular Design*, **11**, 175-192.

Residue pairing preference in beta sheet proteins

Helen Fooks

School of Animal & Microbial Sciences
The University of Reading
Whiteknights
PO Box 228
Reading
RG6 6AJ
H.Fooks@reading.ac.uk

An essential factor in understanding how the structure of a protein is dictated by its amino acid sequence is to comprehend which amino acids are most favoured in each type of secondary structure. A major problem in modelling protein structures is obtaining the correct alignment of the sequence onto the three-dimensional structure. In this research, we concentrate on residue pairing preferences in **parallel** beta-sheets in order to score the threading of a sequence onto a structure. This is the first full analysis of pairing preferences in parallel beta-sheets using an up-to-date non-homologous data-set.

In parallel beta sheets, where each pair has a main chain hydrogen bonded and a main chain non-hydrogen bonded residue, negative-positive charge pairs are significantly favoured **if** the negatively charged residues is positioned at the hydrogen bonded position but **not** the non-hydrogen bonded position. Other favoured pairs include Asn_{HB}-Asn_{nHB}, Asn_{HB}-Thr_{nHB} and Arg_{HB}-$Thr_{nHB.}$

Detailed conformational analysis of **Asn-Asn pairs** (significantly favoured interaction at $p<0.0001$) shows that most of the side-chains of the two residues are orientated into their most preferred conformations and that 56% of pairs form side-chain hydrogen bonds.

In **Asn-Thr** and **Arg-Thr** pairs, **Asn_{HB}-Thr_{nHB}** and **Arg_{HB}-Thr_{nHB}** interactions were significantly favoured whilst the reverse pairs (**Thr_{HB}-Asn_{nHB}** and **Thr_{HB}-Arg_{nHB}**) were not.

Detailed conformational analysis showed that sidechain hydrogen bonds form between **Asn_{HB}-Thr_{nHB}** when both sidechains are orientated in their preferred conformations whilst **Thr_{HB}-Asn_{nHB}** pairs only form side-chain hydrogen bonds if they are orientated into their second-most preferred conformations. **Arg_{HB}-Thr_{nHB}** pairs form sidechain hydrogen bonds **if** the Thr residue has its' sidechain orientated into its most favourable conformation. However, in **Thr_{HB}-Arg_{nHB}** pairs, sidechain hydrogen bonds only form **if** the sidechain of Thr residues is orientated into its second-most preferred rotamer position. In both cases, Thr seems to dictate the assymetric favourability of these pairs.

Future work will derive potentials from the statistical analysis for scoring alignments of sequence onto structure.

Modeling and Molecular Dynamics Studies of Human Aquaporin – 1.

Richard J. Law *[Supervisor – Prof. Mark S. P. Sansom]*
Laboratory of Molecular Biophysics, South Parks Road, Oxford University, Oxford, OX1 3QU
Tel: +44 - (0)1865 - 275380 Fax: +44 - (0)1865 - 275182

Email:rlaw@biop.ox.ac.uk WWW: http://indigo1.biop.ox.ac.uk/rlaw/newhtml/

Abstract

Until about ten years ago it was assumed that all water passage between cells occurred via diffusion through the membrane, but since then, many water specific channels have been found (*Marples, 2000*). Aquaporins selectively transport water, even to the point of excluding protons and hydroxyls. The NPA residues in the two loops are considered the most important for function but other residues are highly conserved and many give non-functional channels and can cause certain disease on mutation. The most widely studied member of the aquaporin family is Human Aquaporin 1 (Aqp-1). Molecular dynamics simulations of two medium resolution structures of Aqp-1 (*Ren et al 2001, Murata et al, 2000*), as well as a homology model of Aqp-1 based on the GlpF structure (*Fu et al, 2000*), in the monomeric form, and simulation of the tetramer of the Aqp-1 homology model, were performed. The stability of the protein in an octane slab membrane mimetic, were analysed, as well as the properties of water diffusion within the channel. A single file column of water was stable within the channel for certain positions of the NPA loop but the lack of rigidity of these motifs made it difficult to confirm any model for water selection using these structures.

Refs.

Marples, D. (2000). **Water Channels – who needs them anyway?** *Lancet 355: (9215): 1571 – 1572*

Ren G, Cheng A, Reddy V, Melnyk A, Mitra AK (2001). **Three-dimensional fold of the human AQP1 water channel, determined at 4Å resolution by electron crystallography of two-dimensional crystals embedded in ice.** *J Mol. Biol. 301: 369 – 387*

Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, Engel A (2000). **Structural determinants of water permeation through aquaporin-1.** *Nature 407: 599 – 605*

Fu D, Libson A, Miercke LJW, Weitzman C, Nollert P, Krucinski J, Stroud RM (2000). **Structure of a glycerol-conducting channel and the basis of its selectivity.** *Science 290: 481 – 486.*

A MOLECULAR SURFACE-BASED METHOD FOR OPTIMISATION OF PROTEIN-LIPID INTERFACES IN MEMBRANE PROTEIN SIMULATIONS

José D. Faraldo-Gómez, Graham R. Smith, Mark S.P. Sansom

Laboratory of Molecular Biophysics, University of Oxford.
The Rex Richards Building, South Parks Road.
Oxford OX1 3QU, U.K.
josed@biop.ox.ac.uk

Membrane proteins are of great importance in current biology, particularly with respect to discovery of new drug targets [1]. They constitute *ca.* 30% of known genes [2] and experimental studies are starting to yield a substantial number of membrane protein structures. Molecular dynamics simulations (MD) play an important role in helping us to fully understand the conformational dynamics of membrane proteins, and hence the relationship between their structure and biological function. These simulations must include a lipid bilayer or other membrane mimetic to model the hydrophobic environment in which these proteins exist; however, given the slow dynamics of lipid molecules in fluid-phase bilayers [3], and the irregular shape of most membrane proteins, obtaining a correctly configured initial system is a non-trivial task, and yet the reliability of the subsequent simulation may depend on how this is performed.

In order to build these protein-lipid bilayer systems, two approaches have been reported in the literature. The first [4] consists of building a bilayer around the protein lipid by lipid, each individual molecule being selected from a library of lipid conformations; unfavourable lipid-lipid and protein-lipid contacts are removed during a second stage using a rigid-body conformational search. The second approach [5] uses a previously equilibrated lipid bilayer, in which a cylindrical hole to accommodate the protein is created by the application of weak repulsive radial forces on the lipid atoms.

In this abstract we report a method that has been developed from this second approach, but that allows the cavity in the lipid bilayer to have an arbitrary shape, thus generalising the method to any protein geometry. The method aims to generate this suitable cavity using the solvent-accessible surface of the protein as a template, during the course of a short, steered molecular dynamics simulation of a solvated lipid membrane. This is achieved by a two-stage process: firstly, lipid molecules whose headgroups are inside a cylindrical volume equivalent to that contained by the protein surface are removed; then the protein-lipid interface is optimised by applying repulsive forces perpendicular to the protein surface, and of gradually increased magnitude, to the remaining lipid atoms inside the volume occupied by the protein surface until it is emptied. The protein itself may then be inserted. Using the bacterial membrane proteins KcsA and FhuA as test cases, we show how the method achieves the formation of an optimised cavity in the interior of a dimyristoyl phosphatidylcholine (DMPC) lipid bilayer without perturbing the configuration of the non-interfacial regions of the previously equilibrated lipid bilayer, even in cases of membrane proteins with irregular geometrical shapes.

1. Terstappen GC, Reggiani A (2001) *In silico* research in drug discovery. Trends Pharmacol Sci 22:23-26
2. Wallin E, von Heijne G (1998) Genome-wide analysis of integral membrane proteins from eubacterial, archean, and eukaryotic organisms. Prot Sci 7:1029-1038
3. Gennis RB (1989) Biomembranes: molecular structure and function, 1st edn. Springer-Verlag, New York
4. Woolf TB, Roux B (1994) Molecular dynamics simulation of the gramicidin channel in a phospholipid-bilayer. Proc Natl Acad Sci USA 91:11631-11635
5. Shen L, Bassolino D, Stouch T (1997) Transmembrane helix structure, dynamics, and interactions: multi-nanosecond molecular dynamics simulations. Biophys J 73:3-20

Molecular Dynamics Simulations of Biomembranes and Small Molecule Permeation

Daniele Bemporad and Jonathan W. Essex

Chemistry Department, University of Southampton, Highfield Campus, SO17 1BJ Southampton, UK

E-mail contact: danibemp@soton.ac.uk

In order to reach their biological target, drugs have to cross cell membranes, and understanding passive membrane permeation is therefore crucial for rational drug design. Molecular dynamics simulations offer a powerful way of studying permeation at the single molecule level, yielding detailed dynamic and thermodynamic data. Biological membranes have a very inhomogeneous character and a highly anisotropic behaviour. A computer model of such a complex system is proposed. Static and dynamic properties have been calculated and compared with available experimental data, suggesting that the model is able to reproduce membrane physical properties. A method to study the permeation of small organic molecules across the above membrane model is also proposed. Free energy profiles and diffusion coefficients along the bilayer normal have been calculated for small organic molecules, and both properties are shown to depend strongly on the chemical nature of the permeant, the position in the membrane interior and the size, shape and conformational flexibility of the permeant. These data also allow for the calculation of permeability coefficients, the results for which are compared with the available experimental data.

Investigating The Mechanism of Dihydrofolate Reductase By Molecular Dynamics Simulations

<u>Paul Shrimpton and Rudolf K. Allemann</u>

School of Chemical Sciences
The University of Birmingham

Dihydrofolate Reductase (DHFR) catalyses the nicotinamide adenine dinucleotide phosphate (NADPH) dependent reduction of 7,8-dihydrofolate (H2F) to 5,6,7,8-tetrahydrofolate (H4F). H_4F is an active form of the vitamin folic acid and a precursor of cofactors required for the production of purines, pyrimidines and several amino acids. DHFR is therefore an essential enzyme in these biosynthetic pathways and as such a target for antitumor and antimicrobial drugs such as methotrexate and trimethoprim, respectively. Despite extensive studies many aspects of the DHFR mediated reaction are still unclear. It is know that the reaction occurs by way of hydride transfer to the 6 position of H2F and protonation of N5. Based on crystallographic data a water molecule is thought to protonate N5 although such a water molecule has never been seen in a crystal structure representing the active complex. It is generally accepted that protonation precedes hydride transfer although there is no direct evidence for this. Here we present the results of a series of 1ns Molecular Dynamics (MD) simulations on the *E. coli* form of DHFR. We show that water can gain access to the relevant position in the active site in the Michaelis complex to protonate N5 and find evidence which indicates that hydride transfer may precede protonation rather than the other way round.

E-mail contact: pjs862@bham.ac.uk

The School of Chemical Sciences
The University of Birmingham
Edgbaston
Birmingham
B15 2TT

Active and inactive conformations of the β_2-adrenergic receptor

Nathan J Kidley, Paul R Gouldson, Robert P Bywater and Christopher A Reynolds

Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, Essex, CO4 3SQ and Biostructure Department, Novo Nordisk A/S, Novo Nordisk Park DK-2760 Måløv, Denmark
njkidl@essex.ac.uk

Molecular models, derived from biochemical and biophysical data, for an active and inactive conformation of the β_2-adrenergic receptor are presented. Distance restraints have been derived from substituted cysteine accessibility methods, site-directed spin labelling, site-directed cross-linking, engineered zinc binding studies and photoaffinity labelling experiments conducted on class A seven transmembrane G protein coupled receptors. Restrained molecular dynamics simulations in the presence of the restraints were used to generate two distinguishable receptor models, one corresponding to the inactive structure and one corresponding to the active structure. Examination of these two models reveals that pharmacological characteristics of ligands are consistent with the stabilisation of either active or inactive receptor conformations. The results provide new insights and explanations for the conflicts observed between the biochemical data in the literature and the crystal structure of Rhodopsin. Based upon the two conformational models, a mechanism for the structural rearrangements that occur during activation of the β_2 adrenergic receptor is presented.

Molecular Modelling of Delta-class Glutathione S-Transferases

Mike Nagle

The New Chemistry Laboratory,
University of Oxford,
South Parks Road,
Oxford,
OX1 3QT,
UK
naglem@bellatrix.pcl.ox.ac.uk

Abstract

Malaria is one of the greatest threats to the health of the population of the developing world. One of the most successful preventative strategies used in recent years aims to control the population of mosquitoes responsible for the spread of the disease using bednets soaked in pyrethroid insecticides. Currently a test is in development that is based on the fact that pyrethroids bind to the active site of Glutathione S-Transferase (GST) proteins and inhibit their catalytic mechanism. However, if the test is to be adapted for use in the field, it will be necessary to increase the binding affinities of the pyrethroids for the GST. As crystal structures of delta class GSTs are not yet available, a model of *Anopheles gambiae* GST1-6 was built based on the structure of the blowfly enzyme, *Lucilia cuprina* GST. This model will provide the detailed information on the active site of *ag*GST1-6 needed to understand the pyrethroid's ability to inhibit the enzyme and will show how protein engineering can optimise the interaction between enzyme and inhibitor.