

## MGMS and RSC MMG Young Modellers' Forum 2007

# POSTER PRESENTATIONS

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### *Poster 1*

#### **Class A $\beta$ -lactamases: QM/MM Study of Their Mechanism of Resistance to Antibiotics.**

Juliette J. Pradon, Dr. Jeremy N. Harvey and Dr. Adrian J. Mulholland.

*School of Chemistry, University of Bristol, Bristol BS8 1TS.*

$\beta$ -lactam compounds work by irreversibly inhibiting penicillin-binding proteins, which are enzymes responsible for the synthesis and repair of peptidoglycan, a major structural component of bacterial cell walls. The production of  $\beta$ -lactamase enzymes by many pathogenic bacteria makes them resistant to both naturally occurring and synthetically developed  $\beta$ -lactam compounds, such as penicillins, cephalosporins and carbapenems. These enzymes are able to hydrolyse the amide bond in the  $\beta$ -lactam ring. This represents a growing problem in current primary care medicine.

For class A  $\beta$ -lactamases, which are the most prevalent form in pathological strains, the overall catalytic mechanism is a two-step process: the enzyme is rapidly acylated at residue Ser70 by the  $\beta$ -lactam; the serine ester intermediate is then hydrolysed, leading to release of the cleaved, inactive antibiotic. Whilst the process of deacylation is widely accepted, there is no such consensus on the acylation step.

The acylation mechanism of a class A TEM-1  $\beta$ -lactamase by the anionic form of benzylpenicillin was studied with the combined Quantum Mechanics/Molecular Mechanics (QM/MM) methodology.

## **Understanding the Interaction Between Dye Molecules and Cellulose**

W.H. Hindy Mok\*, Jerry Winter<sup>†</sup>, Jonathan M. Goodman\*

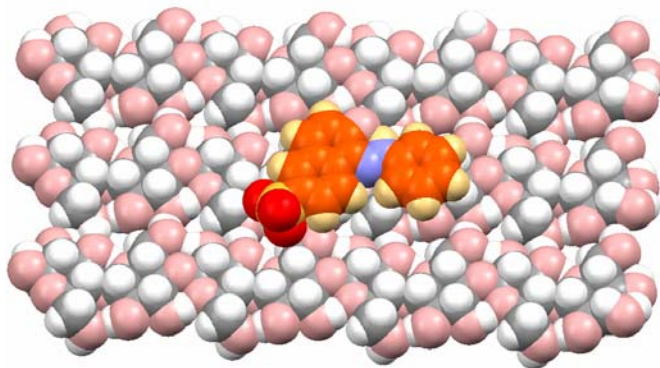
*\*Unilever Centre for Molecular Science Informatics, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK*

*<sup>†</sup>Unilever HPC, R&D Port Sunlight, Wirral, Merseyside, CH62 4ZD, UK*

The dyeing of cellulose, such as cotton fibres, is a widely used process dating back thousands of years. The global textile market is worth more than \$400 billion per annum and cotton is one of the most important fibres in the industry. However, the exact molecular interaction between the dye molecules and cellulose is not known. To help us to understand this, we used a molecular mechanics model and a genetic algorithm to search for possible conformations of dye molecules with respect to a cellulose chain.

Conformational searches were carried out in the gas phase using the Amber force field<sup>1</sup> within MacroModel<sup>2</sup>. New structures were generated and minimised. All possible ring closures and torsion rotations were applied to the dye molecule, which was allowed to be translated and rotated to find the lowest energy structure possible.

A Java program was written to evaluate the results from each conformational search. We identify specific interactions between the dye molecule and cellulose, thereby determining the preferred orientation of the dye in relation to the cellulose chain or surface.



### References:

1. Ferguson, D.M.; Kollman, R.A. *J. Comput. Chem.* **1991**, *12*, 620-626.
2. MacroModel 8.0, Schrödinger, Inc., Portland Oregon, 2000.

**Influence of Non-Nucleoside Inhibitors on the Conformation of Wild-Type and Mutant HIV-1 Reverse Transcriptase Studied by Molecular Dynamics**

Peerapol Nunriam<sup>1</sup>, Frederik Claeysens<sup>1</sup>, Jon I. Mujika<sup>1</sup>, Stephen Macrae<sup>1</sup>, Supa Hannongbua<sup>2</sup>, Jonathan W. Essex<sup>3</sup>, Mark Sansom<sup>4</sup> and Adrian J. Mulholland\*<sup>1</sup>

<sup>1</sup>*Centre for Computational Chemistry, School of Chemistry, University of Bristol, BS8 1TS, UK*

<sup>2</sup>*Department of Chemistry, Faculty of Science, Kasetsart University, 10900 Bangkok, Thailand*

<sup>3</sup>*Head of Chemical Biology, School of Chemistry, University of Southampton, SO17 1BJ, UK*

<sup>4</sup>*Department of Biochemistry, University of Oxford, OX1 3QU*

HIV-1 reverse transcriptase (RT) has continued to be an important target for anti-AIDS chemotherapy. Drugs known as non-nucleoside RT inhibitors (NNRTIs) appear to impair the structural and dynamical properties of RT which in turn inhibit RT's ability to DNA polymerization.<sup>1</sup> However, drug resistance is a key cause of failure for AIDS treatment. Particularly, substitution from lysine to asparagine at position 103 (K103N) is development of resistance to all NNRTIs. This mutation is also the most frequently observed mutation among patients failing highly active antiretroviral therapy (HAART). Molecular dynamics (MD) method is employed to explore the influence of NNRTI on the dynamics of RT and its interaction with the bound nevirapine, for both wild-type and K103N mutant. Additionally, the quantum mechanics and molecular mechanics (QM/MM) method is applied to study the effects of the mutation to drug-resistance especially around the non-nucleoside inhibitor binding pocket (NNIBP) with the bound nevirapine and PNU142721. We have seen that a bound nevirapine in the wild-type enzyme alters the motion of the p66 thumb subdomain, while the K103N mutation partially restores RT flexibility. The QM/MM dynamics have elucidated that the K103N mutation cause a loss of hydrophobic and electrostatic interactions between drug and the NNIBP by creating the openness of the putative entrance, which might increase the dissociation rate.<sup>2</sup> Therefore, the results from this study suggest that a good inhibitor should effectively interact with amino acids around the NNIBP and would be expected to have favourable interaction with the mutated side chain of Asn103.

References:

1. Das, K., J. Ding, Y. Hsiou, A. D. J. H. Moereels, L. Koymans, K. Andries, R. P. P. A. J. Janssen, P. L. Boyer, P. C. R. H. Smith, M. B. K. S. C. J. Michejda, S. H. Hughes and E. Arnold, *Journal of Molecular Biology*, 1996, **264**, 1085-1100.

2. Linberg, J., Snaevar, S., Seved, L., Hans, O.A., Christer, S., Rolf, N., Kerstin, F., Hong, Z. and Torster, U., *European of Journal Biochemistry*. 2002, **269**, 1670-1677.
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**Poster 4**

**Cryptic Chlorination by a Non-Heme Halogenase - a DFT Study**

Shanthi Pandian, Mark A. Vincent, Neil A. Burton and Ian H. Hillier

*School of Chemistry, University of Manchester, Oxford Road, Manchester, M13 9PL.*

Halogenation in natural product biosynthesis occurs on a diverse array of scaffolds, including olefinic centers, electron rich aromatic substrates and unactivated aliphatic carbon centers. Because of the difficulties associated with the functionalization of unactivated alkyl groups, these halogenations represent a substantial chemical challenge. A class of such halogenating enzymes (halogenases) which includes CmaB and SyrB2 has been recently characterized<sup>1,2</sup>. These mononuclear non-heme Fe(II)/ $\alpha$ -ketoglutarate ( $\alpha$ -kg) enzymes, which activate the alkane by hydrogen abstraction employing an Fe=O unit to yield a iron hydroxide species, have a Cl<sup>-</sup> ligand bound to the iron as the source of chlorine. Despite the presence of an OH group they yield only the chlorinated product, which differs from other enzymes in this group who have the ability to hydroxylate. The focus of this work is trying to understand the mechanism of exclusive chlorination by these enzymes. Our present theoretical investigation is based on density functional theory (DFT), employing the hybrid functional B3LYP in conjunction with 6-31G\*\* basis set. The quantum chemical program GAUSSIAN is used for geometry optimizations and for the calculation of final energies. The mechanistic cycle of these enzymes is believed to follow the same path as that of the  $\alpha$ -kg hydroxylases, which typically performs hydroxylation of unactivated carbons and oxidative cyclization. Our results for the isolated cluster indicate a small separation of the two competing rebound barriers (chlorination and hydroxylation) which may lead to a mixture, rather than the exclusive chlorinated product observed. The rate determining hydrogen abstraction step, by the iron (IV) oxo intermediate from the substrate in the halogenases is found to be higher than that in the hydroxylases. In a search for the chlorination pathway, the effect of neighbouring amino acid residues is investigated. In our models we observe the importance of neighbouring amino acid residues like arginine, glutamate and crystallographic water molecules in altering the reaction barriers of both halogenation and hydroxylation pathways.

References:

1. Blasiak, L. C., Vaillancourt, F. H., Walsh, C. T., Drennan, C. L., *Nature* (2006), **440**, 368-371.
2. Vaillancourt, F. H., Yin, J., Walsh, C. T., *Proc. Natl. Acad. Sci. USA* (2005), **102**, 10111-10116.

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**Poster 5**

**A QM/MM Investigation into the Biodegradation Mechanism of Chitinase B**

Heather Rowlands and *Adrian Mulholland*

*Centre for Computational Chemistry, School of Chemistry, University of Bristol,  
Cantock's Close, Bristol, BS8 1TS*

Chitinase is an important enzyme from the glycosyl hydrolase family of enzymes. Found in a diverse and numerous collection of organisms, it catalyses the break down of chitin, releasing dimers and trimers in a progressive manner. Due to its presence in such a wide range of organisms from yeast and bacteria to insects and crustaceans, it is a good target for inhibitors in the form of fungicides, insecticides and anti-malarials. An enzyme with chitinase activity has recently been found in humans and has been suggested to play a role in defence against chitin containing pathogens.

Better understanding of the mechanism of this enzyme could help in the design of novel inhibitors. A mechanism for chitinase B has been proposed from structural and mutational studies is, in some ways, similar to that of lysozyme and other amylases. The nucleophilic attack in particular differs from other glycosyl hydrolases in that the N-acetyl group of the sugar ring itself is the nucleophile, compared with lysozyme where the nucleophile is an aspartate side chain. Quantum Mechanics/Molecular Mechanics (QM/MM) techniques have been employed in this study to investigate the reaction profile of each step in the mechanism.

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**Poster 6**

**Preferential Solvation Behaviour of Manganese Ions**

Jens Rydén, *Hazel Cox*

*Chemistry Division, Department of Chemistry and Biochemistry, University of Sussex,  
Falmer Brighton, BN1 9QJ*

Many transition metal ions play a very important role in biological systems such as in proteins or in enzymes. One example of a transition metal ion with a specific role in biochemistry is the manganese (II)-ion in photosystem II in green plants, algae and cyano-bacteria. To understand the role of metal ions in biological systems is crucial for developing sustainable energy resources like artificial photosynthesis or when designing new drugs.

Experiments made by Kebarle<sup>1</sup> and co-workers on competitive gas-phase solvation for singly charged alkaline metal ions by water and methanol indicated a preference for

methanol as a ligand. Tony Stace<sup>2</sup> and co-workers have performed experiments on the manganese system in gas-phase, in which Mn(II)-water, Mn(II)-methanol as well as the Mn(II)-methanol/water system have been considered using mass-spectrometry. Several factors have been investigated such as ion intensity, which can reveal cluster sizes, unimolecular decay and fragmentation from collisional activation.

In order to explain the experimental results, theoretical investigations of the manganese ion in gas phase have to be performed. This can shed some light over the coordination with solvent molecules such as water in biological systems where the number of solvent molecules are limited. Initial structures were optimized and frequency analysed at the HF/6-31G(d) level. Re-optimisation were then performed at the MP2/6-311G(d,p) level. All calculations were done using the Gaussian-03 package.

Results so far indicate that having ligands in the first shell is more favourable than in a second coordination sphere and a coordination number of 4 (tetrahedral geometry) have shown to be particularly stable. Furthermore, there is a preference for methanol as a ligand over water. This is in agreement with experiments where fragmentation pathways and ion intensity shows a complex size of 4 to be stable and loss of water more favourable than loss of methanol.

Theories that can help explaining the results includes steric repulsion among ligands which makes a tetrahedral complex stable, differences in dipole moment and polarizability between water and methanol that makes ion-induced dipole interaction stronger for methanol on short distances.

References:

<sup>1</sup>**Competitive Gas-Phase Solvation of Alkali Metal Ions by Water and Methanol**  
Steen Brøndstedt Nielsen, Michael Masalla, and Paul Kebarle, J. Phys. Chem A , Vol. 103, No.48 9891-9898 (1999)

<sup>2</sup>**Solvent Coordination in Gas-Phase  $[\text{Mn}(\text{H}_2\text{O})_n]^{2+}$  and  $[\text{Mn}(\text{ROH})_n]^{2+}$  complexes: Theory and Experiment**  
Hazel Cox, Glen Aikbo-Betts, Rossana R. Wright, Nicholas R. Walker, Sharon Cutis, Bridgette Duncombe and Anthony J. Stace, Journal of the American Chemical Society, 125, 233-242, (2001)

## **Molecular Dynamics Simulations of Transport at Water-Silicate Interface**

Gren W<sup>1</sup>, Arrouvel C<sup>1</sup>, Marmier A<sup>2</sup>, Parker S C<sup>1</sup>

<sup>1</sup> *Department of Chemistry, University of Bath, Bath BA2 7AY, U.K.;*

<sup>2</sup> *School of Engineering, Computer Science and Mathematics, University of Exeter, Exeter, EX4 4QF, U.K.*

The aim of this work is to understand the effect of silicate-water interface on transport processes including those leading to adsorption and growth. We can achieve this by using Molecular Dynamics simulations. In order to obtain the best compromise between speed and reliability, we use the DL\_POLY code with shell model interatomic potentials that have demonstrated track record for modelling diffusion and surface properties of silicates. We will describe the average structure of the multi-layer hydration in calculating the Mean Squared Displacement (MSD) of water following the Einstein's relation to analyse the movement of the solvent.

The first step is to generate different silicate surfaces (e.g. quartz, pyrophyllite clay and the siliceous form of the zeolite LTA). The approach is to use the static lattice energy minimisation code METADISE, which will cleave a crystal of a given Miller index in different places such that there is no dipole perpendicular to the exposed surface. Excluding the (001) surface of pyrophyllite, all of the surfaces give rise to three-coordinate silicon, i.e. dangling bonds. In each case the surface was reacted with water such that hydroxide was added to the under-coordinated silicon and this was compensated by proton addition to unsaturated oxygen atoms. The comparison of each surface can give a relative order of stability. Following energy minimisation the lowest energy surface with lowest cleavage energy was placed into a DL\_POLY simulation cell, ensuring that the minimum surface cell dimension exceeded 20Å. Water was then added to the vacuum. In some cases bubbles formed in the simulation cell, these were then filled by the addition of further water molecules such that midway between the slab surfaces, water reached its bulk density.

We considered the hydroxylated (100) and (110) surfaces of  $\alpha$ -quartz. The surfaces could be considered to be negatively charged on the outer layer with various concentrations of OH groups. The (100) surface of quartz is more hydrophilic than the (110) surface, having only vicinal OH groups. The (110) surface have geminal and vicinal groups with geminal at the outer layer. The presence of siloxane increases the hydrophobicity whereas silanol groups increase the hydrophilicity which is consistent with our results.

Finally, we have investigated the relationship between water structure and its transport near the interface. We have also studied the influence of ionic strength and pH to ascertain whether there are generalised effects. We find a higher water ordering on the

(100) surface of quartz (See Figure) compared to the (110) surface. There is no water ordering on pyrophyllite at 300K. In addition, the diffusion of water on hydrophilic surface is slightly lower. On zeolites (LTA), the ordering and diffusion depend on the exposed surface i.e. there are two ways to cut the surface at the same surface energy level, a surface type ‘ $\alpha$ ’ exposing the  $\alpha$ -cage and one type ‘ $\beta$ ’ exposing the  $\beta$ -cage. Each demonstrates significantly different behaviour.

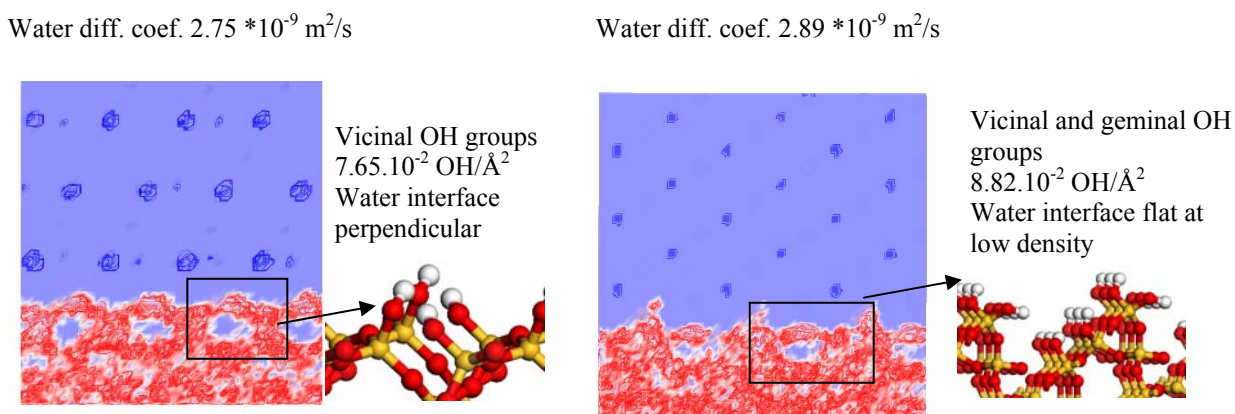


Figure: Water ordering (color in blue) on the a) (100) surface; b) (110) surface of  $\alpha$ -quartz (color in red)

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## Poster 8

### Identification of Allosteric Binding Sites in Proteins

Nadia Vahdati (1), Richard Ward (2) and Jonathan W. Essex (1)

(1) *School of Chemistry, University of Southampton, SO17 1BJ, United Kingdom*

(2) *AstraZeneca, Alderley Park, Macclesfield, Cheshire SK10 4TF, United Kingdom*

The primary aim of this research project is to develop a computational protocol to identify potential binding cavities that may appear as a result of protein flexibility, in particular allosteric motions.

Modelling the conformational flexibility of the protein correctly could lead to the opening of new binding sites that may have allosteric properties, for which suitable ligands could be designed. This concept is desirable as it offers better selectivity and the opportunity for discovering new chemotypes.

As large conformational changes are among the slow motions in the system, two computer simulation methods, molecular dynamics (MD) and normal mode analysis (NMA) have been adopted to model the protein flexibility in a fully atomistic

representation. The major motions from the MD simulation are extracted using principal component analysis (PCA). The principal modes are subsequently searched for new cavities that may have binding properties, which could have appeared during sampling of the slow motions.

The protocol will be discussed in the context of the allosteric enzyme, human glucokinase (GLK) which has an important role in the regulation of glucose metabolism. The enzyme undergoes a large conformational change upon glucose binding at the active site. Experimental studies have identified small synthetic molecules that bind to a remote site, ~20 Å away from the active site in the active form of glucokinase and act as activators<sup>1</sup>.

Reference:

[1] Kamata, K., Mitsuya, M., Nishimura, T., Eiki, J., and Nagata, Y., Structural basis for allosteric Regulation of the monomeric allosteric enzyme human glucokinase, *Structure*. (2004) **12**, 429-438.

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## Poster 9

### ILBP – Flexible Protein Scaffold for Multiple Ligand Transport

Mari Chikvaizde, Clare-Louise Evans, *Jonathan D. Hirst*

*School of Chemistry, University of Nottingham, University Park, Nottingham, NG7 2RD*

The native structure, equilibrium dynamics and cooperative binding behaviour of Ileal Lipid Binding Protein (ILBP) is explored using molecular dynamics (MD) simulations. ILBP is a member of family of bile acid transport proteins, consisting of ten anti-parallel  $\beta$ -strands capped by a small two-helix motif. The *O. cuniculus* ILBP is of interest, due to its unprecedented 3:1 binding stoichiometry that has not been observed for the *H. sapiens* form. The work is focused on studying the functional plasticity of this flexible protein scaffold in response to the cooperative binding of bile acid ligands. Since the NMR structure of *O. cuniculus* ILBP is not yet solved, CHARMM is being used, to build the structure based on the previously solved *H. sapiens* ILBP as a framework, by annotating point mutations at twenty two positions in silico. Ten replicas of this structure are built and solvated using explicit water model, to carry out all atom MD simulations for up to two nanoseconds. From these simulations, physical and time-dependent properties are calculated. In tandem, with our investigation of the equilibrium dynamics, the cooperative 3:1 binding stoichiometry of bile acid ligands to *O. cuniculus* ILBP is performed using AutoDock 4.

References:

1). Kouvatsos, N., Thurston, V., Ball, K., Oldaham, N. J., Thomas, N. R., and Searle, M. S., "Bile Acid Interactions with Rabbit Ileal Lipid Binding Protein and an Engineered

Helixless Variant Reveal Novel Ligand Binding Properties of a Versatile  $\beta$ -Clam Shell Protein Scaffold". *J. Mol. Biol.* (2007) **371**, 1365–1377.

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**Poster 10**

**Capturing Lipid Phases Using a Highly Simplified Lipid Model**

G D Chellapa, and *J. W. Essex*

*School of Chemistry, University of Southampton, Southampton SO17 1BJ*

A very simplified lipid model immersed in an explicit solvent has been developed using molecular dynamics methods.

The lipids are highly “coarse grained” and represented by single non symmetric rigid ellipsoids, the hydrophilic ends of lipids are represented by point dipoles embedded in spheres at one end of the ellipsoid with the other end representing the hydrophobic end [1]. The interactions between the lipids are captured through the Gay-Berne [2] and dipole potentials [3]. The solvent molecules are represented as dipolar spheres and were carefully parameterized to reproduce the dielectric constant of water using a reaction field method.

It will be shown how this simple model, in which much of the chemical detail has been lost, is still, with careful parameterization, able to capture the rich phase behaviour of lipids. It will be also shown how this simple lipid model is easily extended to represent several lipid species as well as their mixtures. This simple model allows us to run long simulations using large time steps enabling us to explore longer spatial and temporal limits inaccessible using conventional all atom simulations.

References:

[1] Ayton G. , Bardenhagen S.G. , Mc Murtry P., Sulsky D. and Voth G. A. Interfacing continuum and molecular dynamics: An application to lipid bilayers.

*J. Chem. Phys.* (2001) **114**, 6913-6924

[2] Gay J.G. and Berne B.J. Modification of the overlap potential to mimic a linear site-site potential. *J. Chem. Phys.* (1981) **74**, 3316-3319

[3] Price S.L. , Stone A.J. and Alderton M. Explicit formulas for the electrostatic energy, forces and torques between a pair of molecules of arbitrary symmetry. *Mol. Phys.* (1984) **52** , 987-1001

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**Poster 11****Self-Assembly and Dynamics of the Influenza A M2 Channel via Coarse Grain MD Simulations**

Timothy Carpenter, Peter Bond, *Mark S. P. Sansom*

*University of Oxford Biochemistry Department, South Parks Road, Oxford, United Kingdom, OX1 3QU*

Membrane protein folding occurs by peptides inserting into the bilayer and forming independently stable transmembrane helices, these then pack into the tertiary structure of the protein. The folding and assembly of small membrane peptides occurs on the  $\mu$ s timescale. This is a problem for conventional atomistic MD, as it is tenfold longer than the upper time limit. However, with CG MD the length of the simulation can be extended to tens of microseconds - long enough to observe the folding of the protein. As the Influenza A proton channel M2 is a relatively simple protein (a homo-tetramer of 97 amino acids), it will provide a good test case for the use of CG MD for protein folding/assembly. CG simulations have been used to produce a model of the M2 monomer inserted into a forming bilayer. Furthermore, with extended CG simulations, four copies of this inserted monomer are observed to tetramerise. The CG-produced tetramer has then been evaluated in terms of both its most occupied conformation and also its dynamics. This shows that the M2 tetramer appears to have dimer-of-dimers-like bundle packing, rather than a tetrameric 4-fold symmetry. Finally, the M2 structure is converted back into a fully atomistic model. Thus CG simulations can be used as a tool to construct simplified models for protein structures that can then be converted to an atomistic representation which can be used to resolve the finer detail of the conformation.

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**Poster 12****Modelling Interaction of the ITIH4 von Willebrand type A Domain with Fraction C4b of the Classical Complement Pathway**

A. Sebastián, M. A. Álava, M. Iturralde, F. Lampreave, A. Piñeiro

*Department of Biochemistry and Molecular and Cellular Biology. Faculty of Sciences. c/ Pedro Cerbuna, 12, 50009-Zaragoza. University of Zaragoza, Spain*

The inter-alpha-trypsin inhibitor (ITI) family constitutes a group of glycoproteins built up from one light chain (bikunin) and a variable set of heavy chains (HC1, HC2, HC3, H4). All these heavy chains contain two domains: the vault protein inter-alpha-trypsin (VIT) domain and a von Willebrand type A (vWA) domain. The function of the VIT domain is still unknown. The VWA domain is a well-studied domain involved in cell adhesion, in extracellular matrix proteins, and in integrin receptors.

We focus our study on the ITIH4, one member of the ITI family that is formed by only one heavy chain (H4). The homology with the other HC of the family is: 52% with HC1, 49% with HC2 and 57% with HC3. Nowadays, little is known about the physiological roles of this protein. Former studies proved that ITIH4 binds to the C4b fraction of the complement pathway (Hammer, et al., 1989). Thus, this protein appears to be a complement regulatory factor.

In the classical complement pathway, C4b binds C2a to form C3-convertase (C4b2a complex) in a similar way that C3b binds Bb fragment of factor B to form C3-convertase (C3bBb complex) in the alternative complement pathway.

C2a and Bb have in common a vWA domain that interacts with C4b and C3b respectively. We make use of this analogy to study ITIH4 vWA domain interactions with C4b. Using crystallographic structures of C3b and Bb we can predict C4b and ITIH4 vWA domain structures by homology modelling to perform computational studies of the interactions between both pairs of molecules.

#### References:

Hammer, C.H., Jacobs, R.M. and Frank, M.M. Isolation and characterization of a novel plasma protein which binds to activated C4 of the classical complement pathway, *The Journal of biological chemistry* (1989) **264**, 2283-2291.

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