

MGMS and RSC MMG Young Modellers' Forum 2008

POSTER PRESENTATIONS

Poster 1

Discarding Functional Residues from the Substitution Table Improves Predictions of Active Sites within Three-Dimensional Structures

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Substitutions of individual amino acids in proteins may be under very different evolutionary restraints depending on their structural and functional roles. The Environment Specific Substitution Table (ESST) describes the pattern of substitutions in terms of amino acid location within elements of secondary structure, solvent accessibility and the existence of hydrogen-bonds between side-chains and neighbouring amino acid residues. Clearly amino acids which have very different local environments in their functional state compared to those in the protein analysed will give rise to inconsistencies in the calculation of amino acid substitution tables. Here, we describe how the calculation of ESSTs can be improved by discarding the functional residues from the calculation of substitution tables. Four categories of functions are examined in this study: protein-protein interactions, protein-nucleic acid interactions, protein-ligand interactions and catalytic activity of enzymes. Their contributions to residue conservation are measured and investigated. We test our new ESSTs using a program CRESCENDO designed to predict functional residues by exploiting knowledge of amino acid substitutions and compare the benchmark results with proteins whose functions have been defined experimentally. The new methodology increases the Z-score by 98% at the active site residues and finds 16% more active sites compared with the old ESST. We also find that discarding amino acids responsible for protein-protein interactions helps in the prediction of those residues although they are not as conserved as the residues of active sites. Our methodology can make the substitution tables better reflect and describe the substitution patterns of amino acids which are under structural restraints only.

Poster 2

A Computational Method for Examining the Conformational Stability of Family A GPCRs

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Obtaining crystal structures of GPCRs is extremely difficult, but very important to increasing our understanding of the structure and function of this family of proteins. Recently important progress has been made by using point mutations to stabilise certain conformations of a receptor (1-3). These mutational experiments are time consuming and expensive, here we propose a novel method for computationally determining the stability of GPCRs given a set of point mutations.

The recently crystallized B₁-adrenergic receptor mutant (B₁-AR-m23) (2) served as a good structure to test our method. The B₁-AR-m23 contained six key thermostabilizing point mutations (R68S, M90V, Y227A, A282L, F327L and F338M). We created a homology model of the wild type B₁-AR and ran molecular dynamics simulations on this and the mutant crystal structure, in order to assess the difference in stability. In addition, we also created mutant receptors of the B₁-AR and B₂-ARs with the mutations suggested by Roth *et al.* that have also been said to increase receptor stability (3), to further test our method.

The method for determining the stability of a receptor comprised of three key stages. In the first stage we carried out rigid core identification by means of the plug-in Bio3d in the program R. We define the “core” of a protein as the atoms that have the lowest structural variation, typically with root mean square deviations (RMSDs) less than 0.5 Å. In the second stage we use the method of principal components analysis to distinguish the key movements between the native and mutant states. Finally in the third stage we calculated the RMSD of each individual atom by analysing the frames of the trajectories produced from molecular dynamics of the proteins in CHARMM.

Rigid core identification in R showed a significantly bigger core in all mutant receptors than their wild-types. Interestingly for the crystallized B₁-AR mutant the core was not only significantly larger in terms of the number of atoms that comprise the core, but its location had also changed. We are hoping that these simple stability methods will help establish which combinations of mutations may stabilize other Family A GPCRs, which could potentially help guide experimentalists in developing mutant GPCR models that could be successfully crystallized.

References:

1. Serrano-Vega, M., Magnani, F., Shibata, Y., and Tate, C. Conformational thermostabilization of the β_1 -adrenergic receptor in a detergent-resistant form, *Proc. Natl Acad. Sci. USA* (2008) **105**, 877-882
2. Warne, T., Serrano-Vega, M., Baker, J., Moukhametzianov, R., Edwards, P., Henderson, R., Leslie, A., Tate, C., and Schertler, G. Structure of a β_1 -adrenergic G-protein-coupled receptor, *Nature* (2008) **454**, 486-491
3. Roth, C., Hanson, M., and Stevens, R. Stabilization of the Human β_2 -Adrenergic Receptor TM4-TM3-TM5 Helix Interface by Mutagenesis of Glu 122^{3,41}, A Critical Residue in GPCR Structure, *J. Biol. Chem.* **376**, 1305-1319

Poster 3

Protein Dynamics and Networks of Dynamically-Conserved Residues

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Protein conformational changes and dynamics are important for their biological function, such as enzymatic activity. The integration of computational methods in the fields of normal mode analysis (NMA), and the analysis of residue-specific evolutionary information using perturbation as a mimic of point mutation, is useful to understand the dynamics of proteins. NMA is based on the harmonic approximation to the potential energy function, where the first derivative is zero at a minimum energy conformation and terms higher than quadratic are ignored. NMA for large biomolecules can be performed with a simplified representation of potential energy. We use an elastic network model of NMA to calculate pairwise interactions between all atoms that are within a cut-off distance.

We studied the ATP-binding cassette (ABC) maltose transporter. It has a periplasmic maltose-binding protein (MBP), two integral membrane proteins: MalF and MalG, and two copies of cytoplasmic ABC MalK. MalK is an ATP-hydrolase responsible for energy coupling to the transport system. We used the distinct conformations of MalK: open, semi-open, closed, and in-complex. The closed and in-complex conformations correspond to the ligand-bound ones. We calculated the collectivity (the number of atoms affected in a mode), the NMA-derived B-factors, and conformational changes overlap. From the evolutionary information analysis, we obtained a network of evolutionarily-important residues. A network of dynamically-important residues was deduced from NMA. We also discovered correlations between a network of evolutionarily-important residues and a network of dynamically-important residues. These results suggest additional understandings on the mechanism of MalK maltose transporter

Reference:

Q. Cui and I. Bahar. Normal Mode Analysis. CRC Press, 2006

Poster 4

Use of Reduced Dimensionality Representation and Molecular Dynamics Techniques to Investigate PDZ Domain Similarity and Ligand Specificity

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PDZ domains are one of the most common protein interaction modules, found in organisms from bacteria to humans. One of the hot debates of today's science is classifying the PDZ domains and finding a way to predict protein-ligand interactions¹. The present work attempts to tackle this problem.

139 proteins from PDZ domains were carefully chosen to form a representative test set. Protein-protein similarity was evaluated using a reduced dimensionality representation method². For each protein a two dimensional map was created. We have considered the following criteria to assess protein similarity: amino acids' hydrophobicity, residue-replacement ability, three-dimensional structural and conformational similarity of the side chains. Similarity between two proteins was computed based on the similarity of their respective two-dimensional maps. The results show that physico-chemical properties of the amino acids enter with different weights in protein comparison. Based on the similarity scores we proposed a new classification of PDZ domains.

The electrostatic nature of the protein-ligand interactions is an important factor in the binding specificity. A rigorous description of the electrostatic effect is based on the Poisson-Boltzmann equation. We calculated the electrostatic contribution to the solvation free energy using two different numerical solvers for the Poisson-Boltzmann equation: Molecular Mechanics Poisson-Boltzmann Surface Analysis (MM-PBSA) and Adaptive Poisson-Boltzmann Solver (APBS). A set of 20 PDZ domains complexed with different ligands were modelled. We used energy decomposition to assess the importance of each residue in the binding affinity. Also, we used the information from the free energy calculation to get a deeper understanding of the actual binding specificity. Based on our findings we tried to re-organize and structure the PDZ-domain interactions.

References:

1.Chen, J.R., Chang, B.H., Allen, J.E., Stiffler, M.A., MacBeath, G., Predicting PDZ domain-peptide interactions from primary Sequences, *Nat. Biotech.* (2008) **26**, 1041-1045.

2. Albrecht, B., Grant, G.H., Richards, W.G., Evaluation of Structural Similarity Based on Reduced Dimensionality Representations of Protein Structure. *Prot. Eng. Des. Sel.* (2004) 17, 425-432.

Poster 5

Computational Studies of Water-Mediated Interactions in Ionotropic Glutamate Receptors

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Ionotropic glutamate receptors (iGluR) are ligand-gated ion channels which mediate excitatory synaptic transmission. These receptors are of great interest as they are central to fast synaptic neurotransmission in the brain and are thought to be essential in memory and learning. Furthermore, they have been associated with abnormal neuronal activity leading to a wide range of diseases, including Parkinson's, epilepsy, and ischemic stroke. Crystal structures of the ligand-binding domain not only reveal the manner in which ligands interact with iGluRs, but also highlight the importance of water molecules in ligand-receptor binding. Despite the wealth of structural information, the precise role of water molecules in mediating receptor-ligand interactions remains unclear. For example, although the ligands glutamate and AMPA are structurally similar, crystal structures revealed that AMPA adopts a slightly different binding-mode with a water molecule assuming a position that would normally be occupied by the γ -carboxyl group of glutamate [1]. It is possible water could play a significant role in the binding of other ligands as well. Thus, an understanding of the stability of water molecules within the binding site is critical to improved drug design.

This poster will describe a study carried out using MD simulations followed by a theoretical DFT investigation. The study focuses on the interaction energy of the protein-water-ligand complex with the aim of understanding the nature of its stabilization and quantification of the role of dispersion contribution. Interaction energy is determined as a sum of interaction energies of representative fragments containing protein amino acid residue(s), water molecules and ligand, as well as directly for the whole protein-water-ligand complex. This information will be extremely useful not only in the design of novel drugs for iGluRs but more generally in the field of drug design.

References:

- [1] Armstrong, N.; Gouaux, E. Mechanisms for activation and antagonism of an AMPA Sensitive glutamate receptor: Crystal structures of the GluR2 ligand binding core. *Neuron* **2000**, 28 (1), 165-181.

Poster 6

Dynamics of Phosphate Transport by the Anion-Specific Outer Membrane Protein OprP

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Outer membrane protein P (OprP) from *Pseudomonas aeruginosa* forms a water-filled channel which has an enhanced selectivity for anions, especially phosphates. This homotrimeric protein uniquely has three positively charged loops (L3, L5, and T7) folded into the lumen and serves to funnel the anions into the pore. Steered molecular dynamics simulations (SMD) have been performed to understand the mechanism of the phosphate transport by applying an external force to pull the phosphate from the extracellular to periplasmic side and vice versa. These SMD results have been supplemented by unbiased molecular dynamics simulations (MD). The force profiles and the phosphate trajectories show that there are at least five energy wells close to L5, L3, and T7 regions. The two dominant wells are identified at the centre and the lower of the L3 or constriction region, while the rest are at extracellular L5 (2 wells) and periplasmic T7 region (1 well). Both SMD and MD simulations also suggest four major interactions from the side chains of R59, R60, K121, and R133 that enable the formation of the phosphate-protein binding site. Moreover, hydrogen-bonding analysis and the relocation of K121 side chain demonstrate that K121 may not only play a role in the transfer of a phosphate from the lower L5 to the L3 region, but also control the passage from the upper to the lower L3 region. The results of our studies suggest a full possible pathway for phosphate transport.

References:

Moraes, T. F., Bains, M., Hancock, R. E., and Strynadka, N. C. An arginine ladder in OprP mediates phosphate-specific transfer across the outer membrane, *Nat. Struct. Mol. Biol.* (2007) **14**, 84-87.

Sukhan, A. and Hancock, R. E. The role of specific lysine residues in the passage of anions through the *Pseudomonas aeruginosa* porin OprP, *J. Biol. Chem.* (1996) **271**, 21239-21242.

Poster 7

Unsatisfied Hydrogen Bond Donors/Acceptors at Buried Protein-Ligand Interfaces

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Hydrogen bonding is the most important directional interaction underpinning protein structure (Baker and Hubbard, 1984). Proteins tend to maximize their hydrogen bonding potential upon folding and therefore their buried core regions have extremely low incidence of unsatisfied hydrogen bonding groups, as shown in previous surveys (Fleming and Rose, 2005). In a similar fashion, protein-ligand binding is governed by several inter-molecular interactions including hydrogen bonding. Protein-ligand docking methods aim to predict ligand geometries that fit sensibly into a protein active site and assess their affinity by emphasizing energy terms that represent stabilizing interactions. However, it has been shown experimentally and by the ongoing development of scoring functions that destabilization terms such as unsatisfied buried donors and acceptors should also be taken into account (Reulecke *et al*, 2007).

Here we survey a set of 75 high-resolution protein-ligand complexes from CCDC astex dataset (Nissink *et al*, 2002) to study the occurrence of unsatisfied hydrogen bond donors and acceptors at buried protein-ligand interfaces. The percentage of unsatisfied buried groups was studied as a function of resolution and normalized *B*-values. Ligands in the same dataset were then re-docked into their native binding partners and the distribution of unsatisfied groups was studied in the docked poses.

The identification of unsatisfied donors and acceptors is complicated by factors such as positioning of hydrogen atoms, disordered solvent and experimentally indistinguishable side-chain orientations. Based on our methods, we show that the presence of unsatisfied hydrogen bond potential is very unlikely in protein-ligand complexes with a percentage of 1.46% and 1.06% for main-chain NH and CO groups, respectively and 1.47% for ligand polar atoms. The percentage decreases with higher resolution suggesting more accurate results for high quality structures. The satisfied and unsatisfied hydrogen bonding groups show similar *B*-values which suggests that unsatisfied groups do not reflect any particular structural arrangement.

An analysis of protein-docked ligand complexes shows that non-native poses with RMSD values more than 3.0Å, from the crystallographically observed pose, frequently contained unsatisfied donors and acceptors. A clear trend between RMSD and unsatisfied hydrogen bonds could not be seen because of factors such as the very low number of total donor/acceptors in the ligand and interface water molecules. A more detailed treatment of such groups may lead to improvement in docking protocols and provide an additional filter to improve the performance of virtual screening campaigns.

References:

Baker EN, Hubbard RE. Hydrogen-Bonding In Globular-Proteins. *Progress in Biophysics & Molecular Biology*, (1984) **44(2)**, 97-179.

Fleming PJ, Rose GD. Do all backbone polar groups in proteins form hydrogen bonds? *Protein Science*, (2005) **14(7)**, 1911-1917.

Nissink JWM, Murray C, Hartshorn M, Verdonk ML, Cole JC, Taylor R. A new test set for validating predictions of protein-ligand interaction. *Proteins-Structure Function and Genetics*, (2002) **49(4)**, 457-471.

Reulecke I, Lange G, Albrecht J, Klein R, Rarey M. Towards an Integrated Description of Hydrogen Bonding and Dehydration: Decreasing False Positives in Virtual Screening with the HYDE Scoring Function. *Chem Med Chem*, (2007) **3(6)**, 885-897.

Poster 8

Molecular Dynamics Simulations of Mesophilic and Thermophilic Citrate Synthase

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An extremeophile is a broad term classifying organisms adapted to thrive in physically or geochemically extreme environments. These organisms are capable of existing in conditions which, from the human perspective, are clearly extreme, including high or low pH and temperature, high pressure and high salinity. The work presented focuses on the behaviour of the mesophilic and thermophilic counterparts of the enzyme citrate synthase.

A pressing question in the field of extremophiles is how, mechanistically, do extremozymes achieve stability? A number of theories have been put forward, of which the most widely accepted, for temperature extremozymes, is an increasing rigidity on progression from mesophilic through to hyperthermophilic extremozymes.

The aim of this study is to compare the adaptation of citrate synthase to mesophilic, thermophilic and hyperthermophilic temperatures in order to identify how the stability of enzymes is related to activity.

The first 2 ns of molecular dynamics simulations, performed with the parallel computing package NAMD, are presented for the open form of the mesophilic and thermophilic

citrate synthase. Essential dynamics have been performed on the 2 ns trajectories in order to determine and compare the significant global motions of the citrate synthase enzymes.

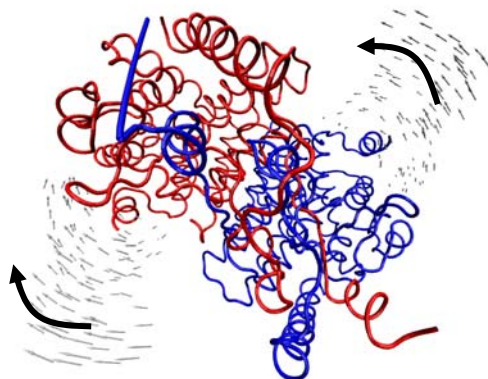


Figure 1 – A significant motion identified by essential dynamics showing the small domains of the mesophilic citrate synthase moving anti-symmetrically suggesting cooperativity.

Poster 9

QM/MM Study of the Sulfoxidation of Dimethyl Sulphide by Cytochrome P450

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The cytochromes P450 are ubiquitous enzymes involved in key biochemical processes. Their primary oxidant has never been isolated experimentally, but there is indirect evidence suggesting that it is the oxo-iron species, Compound I (Cpd I). However, recent experimental studies have cast this into doubt based on the sulfoxidation by P450_{BM3} that implicated the ferric-hydroperoxide species, also known as Compound 0 (Cpd 0), as the more likely oxidant [1].

We have performed a quantum mechanical/molecular mechanical (QM/MM) study on the sulfoxidation of dimethylsulfide (DMS) by both Compound I and Compound 0 on two systems: P450_{cam} and P450_{BM3}.

The study focused on the elucidation of the primary oxidant involved in the reaction and in particular on the oxidative capabilities of the two species. The sulfoxidation reaction involves a direct oxygen atom transfer from Compound I via a transition state leading to

the dimethylsulfoxide (DMSO) product. The reaction with Compound 0 similarly involves a single transition state.

In both enzyme systems studied, significantly higher reaction barriers were obtained for Compound 0, which implies that this candidate cannot act as an oxidant along side Compound I. The experimentally observed rate constants are more likely to be the result of two-state-reactivity patterns of competing doublet and quartet spin state surfaces of Compound I rather than a two-oxidant mechanism where both Compound I and Compound 0 oxidise the substrate.

References:

[1] Cryle, M. J.; De Voss, J. J. Is the Ferric Hydroperoxy Species Responsible for Sulfur Oxidation in Cytochrome P450s? *Angew. Chem. Int. Ed.* (2006), **45**, 8221-8223.

Poster 10

Specific Ion Effects on Structurally Persistent Micelles – Guiding Experiments with MD Simulations

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Long time-scale molecular-dynamics simulations have been used to investigate the structure and dynamics of structurally persistent micelles that consist of seven or twelve specifically designed T-shaped amphiphilic calix[4]arene derivatives.^[1] Experimental investigations by NMR and cryo-TEM techniques with subsequent 3D-reconstruction^[2] confirmed a highly developed topological arrangement of the micelles in water. However, other experiments such as ultrasonification of the micelles together with hexane and water led to micelles with a different topology and that consist of twelve monomers.

The results obtained allow a detailed interpretation of the cryo-TEM 3D-reconstructions of the micelles and several fascinating details of the structure of the solvent around the

micelles and of the factors that affect the structure of the micelles. Moreover, we want to emphasize the interplay between hydrophobic interactions in the interior of the micelle and specific ion effects on its surface.

Our calculations predict that different ions in the micelle-water interphase effect the stability and shape of the amphiphilic micelles as well as the presence of hexane molecules in the center of the dodecameric micelles determines the structure of the micelles and favors the formation of larger structures than without hexane.

Our predictions made on the basis of the simulations have been tested and verified experimentally and further simulations and experiments will cast light on the influence of different ion solutions as well as other encapsulated lipophilic molecules. These simulations and experiments help us to understand and guide the forces responsible for the defined structures of these persistent micelles and will be used to develop structured micelles for nanotechnological applications, such as the development of high-performance drug-delivery capsules.

References:

- [1] M. Kellermann, W. Bauer, A. Hirsch, B. Schade, K. Ludwig and C. Böttcher, *Angew. Chem. Int. Ed Engl.* **2004**, *42*, 2959-2962.
- [2] K. Ludwig, B. Baljinnyam, A. Herrmann and C. Böttcher, *EMBO J.* **2003**, *22*, 3761-3771.

Poster 11

The Role of Complex Formation between Substrates and Efflux Pump Inhibitors (EPIs) in Multidrug-Resistant (MDR) Bacteria

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Introduction: Resistance to antibiotics occur via three major mechanisms: receptor alteration, antibiotic modification and by drug efflux. Efflux pumps can be either specific, where they assist the efflux of only one compound or a class of compounds (as with TetK) or non-specific, where they assist the efflux of a broad range of compounds that are structurally unrelated (as with NorA) (Nelson 2002). It is believed that efflux pump inhibition occurs via binding of the inhibitor to the hydrophobic regions of the efflux pump, or via binding of the inhibitor to the substrate (Zloh et al 2004). Here, the role of binding of the inhibitor to the substrate in efflux pump inhibition is examined.

Method: A set of selected molecules from SuperDrug database were docked with the antibacterial (norfloxacin/tetracycline) using GRID. VegaZZ was used to study the nature of the interactions between the docked molecules and to determine molecular and lipophilicity properties of complexes. Minimum inhibitory concentration (MIC) and modulation assays were performed to analyse the antibacterial activity of the antibiotics, drugs and to observe if any of them had any potentiating activity on norfloxacin/tetracycline activity. NMR, ESI-MS, UV and fluorescence spectroscopy were used to examine the possibility of complex formation between the ligand and antibacterial.

Results: *In silico* screening was utilized to search for molecules that are similar to known efflux pumps inhibitors and a set of 89 possible known drugs were identified from the SuperDrug database. The result of docking all drugs to norfloxacin has indicated that 10 molecules might potentially form a complex with norfloxacin and these were selected for further studies. Apomorphine, bergapten and chlorpromazine exhibited the greatest binding energies (with norfloxacin) as calculated by Glue Apomorphine and chlorpromazine had exhibited some potentiating activity on the MIC of norfloxacin and tetracycline, indicating a greater uptake of antibiotics. Spectroscopic analysis found strong evidence of complex formation between apomorphine and the antibacterials and bergapten and antibacterials. Some evidence of complex formation between chlorpromazine and antibacterials was also seen.

Conclusion: This study provides evidence that complex formation occurs between antibacterials and efflux pump inhibitors. Molecules with the greatest predicted binding energies to an antibiotic also exhibited greatest effect on activity of antibiotics, thus examination of interaction between antibiotics and ligands may be used as a tool in the search for efflux pump inhibitors.

References:

Nelson M.L. (2002), "Modulation of Antibiotic Efflux in Bacteria", *Current Medicinal Chemistry* **1**, 35-54

Zloh, M., Kaatz, G.W. & Gibbons, S. (2004), "Inhibitors of multidrug resistance (MDR) have affinity for MDR substrates", *Bioorganic & Medicinal Chemistry Letters*, **14**, 881-885.

Poster 12

Easy and Rapid Method for Dendrimer 3D Structure Generation.

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Dendrimers are hyper-branched macromolecules with many end groups. Mono-saccharide modified polyamidoamine (PAMAM) dendrimers have been reported to have immuno-modulatory and antiangiogenic activity that can prevent the progress of LPS (lipopolysaccharide) triggered inflammatory response (Shaunak *et al*, 2004). The saccharide loading of these dendrimers has been estimated but the position and number of units on these molecules has yet to be determined. The current synthetic strategy for these saccharide modified PAMAM dendrimers does not allow for control of the level and location of saccharide conjugation. Hence it is not possible to determine detailed structure-activity correlations.

Molecular modelling studies are now underway to understand effects of 3D structure on glycosylation. In our first studies we have been developing an easy and rapid method that allows the generation of 3D models of these dendrimers *in silico*.

Xplor-NIH 2.18 was chosen to generate the 3D models from a starting sequence and for its initial refinement while NAMD 2.6 was used for solvation and dendrimer simulation. The force field for which the parameterisation was developed was Charmm22 and Charmm27. Extensive previous work with this force field provides a great diversity of atom types and parameters that are helpful for the development of novel monomers in macromolecules (http://mackerell.umaryland.edu/MacKerell_Lab.html).

Initially the monomeric units were defined for the saccharide modified dendrimers. Due to the absence of experimental data on exactly defined dendrimer structure, a set of *ab initio* and molecular mechanics calculations were carried out on various fragments of the dendrimers. The results of these computed 3D models were used for the determination of the different force field parameters. Bond length, bond angles and torsion angles was determined by conformational search using a precursor dendrimer known as a 2.5 generation dendrimer glucosamine. Charges of different atom types were determined as an average value of atom type charge from the *ab initio* calculation of residues and selected fragments of the dendrimer.

This methodology provides a powerful tool for generating 3D models of dendrimers and their saccharide modified derivatives. These models are now being used to study the interactions between the target proteins and the modified dendrimers. These important

interactions may provide information on the molecular mechanism for the interesting biological activities shown by these saccharide modified PAMAM dendrimers.

Reference:

Shaunak, S., Thomas, S. et al. (2004). "Polyvalent dendrimer glucosamine conjugates prevent scar tissue formation". *Nature biotechnology* **22**: 977-984.