Programme

Wednesday 13th September

11.00 Registration opens

12.45 Lunch

13.45 Opening remarks

Session Theme: Combining Experiment and Simulation

Session 1 (chair: Natasha Rhys)

14.00 Michele Vendruscolo “Principles of protein structural ensemble determination”

14.50 Peter Bond “Multiscale Simulations Help Map Flavivirus Fusion Dynamics”

15.15 Jordi Juarez-Jimenez “Combining molecular simulations and NMR for the detailed characterisation of protein minor states”

15.40 Coffee / Tea

Session 2 (chair: Sarah Harris)

16.10 Sylvia McLain “The essential role of water in biomolecular function”

17.00 Emma Sutton “Complementary use of SAXS and MD to determine structure-function relationships of anti-CD32b antibodies”

17.25 Eric Lang “Studying computationally the selective binding of a fluorescent dye to de novo designed α-helical barrels”

17.50 Poster pitches 1 (odd numbers)

18.30 Poster session 1 (odd numbers) and drinks

20.00 Dinner
Thursday 14th September

Session Theme: Enhanced Sampling

Session 3 (chair: Julien Michel)

9.00 Professor Francesco Gervasio “Investigating allosteric regulation and cryptic pocket formation through enhanced sampling simulations”

9.50 Nicolae-Viorel Buchete “Replica Exchange Molecular Dynamics Simulations of Peptide Folding and Binding Kinetics”

10.15 Hannah Bruce Macdonald “Predicting water networks and ligand binding free energies in proteins using grand canonical Monte Carlo”

10.40 Coffee / Tea

Session 4 (chair: Federico Comitani)

11.10 Bettina Keller “Girsanov reweighting for path ensembles and Markov state models”

12.00 Charlie Laughton “CoCo-MD: Enhanced Sampling with Hyperspace Jumps”

12.25 Bogdan Iorga “Towards in silico prediction of β-lactamase-mediated antibiotic resistance using free energy calculations”

12.50 Lunch

Session Theme: Structure, Function, and Assembly of Biomolecules

Session 5 (chair: Maria Musgaard)

14.00 Lucy Forrest “Using asymmetry to predict protein conformational change”

14.50 Richard Henchman “Entropy of Liquid Mixtures of Flexible Molecules from Computer Simulation”

15.15 Anna Duncan “Nanoscale Protein Interactions Determine the Mesoscale Dynamic Organisation of Biomembranes”

15.40 Coffee / Tea

Session 6 (chair: Syma Khalid)

16.10 Franca Fraternali “Unraveling the Good and the Bad in Protein Networks: Functional versus Dysfunctional Interactions”
17.00 Carlo Guardiani “Permeation and selectivity mechanism in NaChBac channel and its mutants”

17.25 Richard Sessions “Atomistic simulations of self-assembled nanocages (SAGEs)”

17.50 Poster pitches 2 (even numbers)

18.30 Poster session 2 (even numbers) and drinks

19.00 Barbecue Dinner
Session Theme: Reactions and Interactions

Session 7 (chair: Reynier Suardiaz)

9.00 Jean-Phillip Piquemal “Scalable polarizable molecular dynamics using Tinker-HP: millions of atoms on thousands of cores”

9.50 Christof Jaeger “Towards Engineering Radical Enzymes - Thermodynamic Reaction Profiling and Mechanistic Insights into QueE”

10.15 Silvia Gómez Coca “A conserved Mg-pincho motif in NTP processing enzymes and its importance in the polarisation of the cleaved phosphate”

10.40 Coffee / Tea

Session 8 (chair: Nicolas Foloppe)

11.10 CCPBioSim Showcase – Julien Michel and Chris Woods

11.30 Vicent Moliner “Revealing the origin of enzyme catalysis from computational studies”

12.20 Miguel Soler “Computational design of binders for protein molecular recognition”

12.45 Daniel Cole “Quantum Mechanics Based Potentials for Computer-Aided Drug Design”

13.10 Poster prizes and closing remarks

13.20 Lunch and depart
Posters:

Poster 1. Interaction of Pleckstrin Homology Domains with Phosphatidylinositol Phosphate Lipids: Structures and Energetics by Simulation

Poster 2. From topological constraints to the function: a case of tRNA methyltransferase

Poster 3. Towards understanding the unbound state of drug compounds: Implications for the intramolecular reorganization energy upon binding

Poster 4. Environment effects on a potential trans-cis molecular switch for opening the ion channel of the serotonin-activated 5-HT<sub>3</sub> receptor

Poster 5. On the Role of Hydration in the Folding of Ordered Structures in Peptides

Poster 6. β2-microglobulin interaction with hydrophobic surfaces: A molecular dynamics study

Poster 7. Oligomeric Structure of Pulmonary Surfactant Protein B Causes Lateral Lipid Reorganization and Perturbations in Surfactant Membranes and Monolayers

Poster 8. De Novo Peptide Self-Assembly for

Poster 9. Multi-scale coarse-graining for the self-assembly of two dimensional shapes from single stands of DNA

Poster 10. Modelling Self-Association of Sterols and Sterol Esters in Food Organogels

Poster 11. Unlocking Nicotinic Selectivity via Direct C–H Functionalisation of (−)-Cytisine

Poster 12. Identifying Cryptic Pockets in FGFR with Enhanced Sampling Simulations

Poster 13. Binding Selectivity/Specificity in the minor groove of DNA Sequences: A Metadynamics Study

Poster 14. Optimal Clustering of Markov State Models

Poster 15. Mutating active site residue changed the reaction mechanism of Calb: new insight for rational engineering of CalB from QM/MM calculations

Poster 16. The reaction mechanism of isopentenyl phosphate kinase: a QM/MM study

Poster 17. MD simulations with membrane-bound pyrophosphatases

Poster 18. AMP-membrane interaction characterized with atomistic MD simulation: a study on Cecropin B

Poster 19. Intramolecular parameters for protein molecular mechanics force

Poster 20. Towards cholesterol condensation using ELBA coarse graining

Poster 21. Computational Investigation of i-motif Quadruplex DNA

Poster 22. Investigating the flexibility of myosin 7a using experimental and computational approaches

Poster 23. Understanding protein allostery: Developing analysis methods for molecular dynamics

Poster 24. Towards a molecular understanding of phosphomimetic substitutions in Huntingtin 1-19

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TALK ABSTRACTS
The biological functions of protein molecules are intimately dependent on their conformational dynamics. This aspect is particularly evident for disordered proteins, which constitute perhaps one-third of the human proteome. Therefore, structural ensembles often offer more useful representations of proteins than individual conformations. Here, we describe how the well-established principles of protein structure determination should be extended to the case of protein structural ensembles determination. These principles concern primarily how to deal with conformationally heterogeneous states, and with experimental measurements that are averaged over such states and affected by a variety of errors. We first review the growing literature of recent methods that combine experimental and computational information to model structural ensembles, highlighting their similarities and differences. We then address some conceptual problems in the determination of structural ensembles and define future goals towards the establishment of objective criteria for the comparison, validation, visualization and dissemination of such ensembles.
The positive single-stranded RNA flaviviruses are primary causative agents of infectious disease. Members include dengue virus (DENV), responsible for >400 million infections per year, along with emerging pathogens such as Zika. The viral nucleocapsid core is encapsulated by a phospholipid bilayer, within which are embedded envelope and membrane proteins. Low pH conditions trigger conformational changes in the envelope protein and exposure of the highly conserved fusion peptide (FP) region; the latter is critical for membrane fusion and represents a significant target for antiviral drugs and antibodies. Here, I will give an overview of our recent progress in understanding the role of the FP in governing virus-host interactions, via a combination of integrative modelling, multiscale simulations, and biophysical experiments.

Initially, we combine atomic-resolution simulation sampling totalling >0.1 milliseconds with spectroscopic measurements to characterize FP dynamics. The FP sequence is shown to encode a “funnelled landscape” that spans NMR and X-ray crystallographic structures and favours conformations optimal for selective anchoring to host endosomal membranes. This is emphasized by a non-fusogenic mutant, in which the funnelled landscape is abolished. These observations help to rationalize the extraordinary sequence conservation in flavivirus FPs, and suggest that reconsideration of the peptide structure as an ensemble of states may open up novel routes to antiviral therapeutics. Subsequently, multiscale modelling combined with cryo-electron microscopy and hydrogen/deuterium exchange mass spectrometry are used to investigate the relationships between FP exposure, maturation, and infectivity. Simplified “toy” membrane models containing varying densities of envelope proteins reveal the intrinsic capacity of the FP to remodel lipid bilayers. Reconstruction of the complete flavivirus envelope particle in different states of maturation provides structural insights into how host-pathogen interactions govern exposure of the FP along the viral life cycle. In particular, simulations of the “spiky” fusogenic form of the virion show how multiple FPs may simultaneously embed themselves within the late endosomal membrane, prior to formation of the hemifused intermediate, challenging traditional mechanistic views of flaviviral fusion and resultant infective states.
Combining molecular simulations and NMR for the detailed characterisation of protein minor states

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Historically, it has been difficult to characterize protein conformational ensembles in a quantitative fashion and with atomic detail. One of the major impediments lies in the difficulty to combine the high-resolution, albeit static information gathered from X-ray crystallography with the dynamic low-resolution data obtained from biomolecular NMR. Due to recent advances in hardware, software and analysis algorithms, computational models have attained a stage where they may be able to bridge the long lasting gap between these two powerful techniques. The optimum approach to integrate computational models and experimental structural information remains, to date, unclear.

In this work we present an adaptive sampling approach that combines accelerated Molecular Dynamics simulations (aMD), equilibrium MD trajectories and Markov State Models (MSMs) to explore conformational transitions from “visible” (major) to “invisible” (minor) conformational states of the prolyl-peptide isomerase cyclophilin A (Cyp A). We have put our ensemble model to the test by comparing back-calculated NMR observables with experimental values. Moreover, we prospectively leveraged our model to design a previously unreported Cyp A mutant with altered dynamics.

Altogether, we propose a multidisciplinary framework that blends computational models, X-ray and NMR information to deliver a highly detailed model of the dynamic ensemble of Cyp A.
Abstract: Understanding how biological molecules interact with physiologically relevant environments is crucial for understanding their function. From proteins folding into their functional forms to ligand-protein binding and membrane formation, understanding how nature engineers self-assembled biomolecules is fundamental to understanding the development of life itself. Water is the physical milieu in which these interactions occur, yet there is relatively little understanding of the role that water plays, especially on the atomic scale where an interplay between hydrophilic and hydrophobic motifs gives rise to more complex functions.

Using experimental techniques that probe on the atomic scale - predominately neutron scattering and NMR - in combination with computational techniques, we have investigated the interactions between a variety of naturally-occurring and synthetic biomolecules and water in solution. This research has provided insights into a variety of phenomenon, where specifically hydration in both protein folding and psycho-active drug-delivery will be discussed. In both of these systems, water has been found to play a more active role than it has previously been thought, where protein folding appears to be actively initiated by site specific hydration around the protein backbone. Similarly, drug-conformation has also been linked to hydration, where specifically understanding water-mediated drug interactions appear to be the key to understanding both their delivery and function in vivo.
Complementary use of SAXS and MD to determine structure-function relationships of anti-CD32b antibodies

Emma Sutton1,2,4, Richard Bradshaw2, Christian Orr1,4, Björn Frendéus3, Gunilla Larsson3, Ingrid Teige3, Ivo Tews4, Mark Cragg1 and Jonathan Essex2

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Therapeutic monoclonal antibodies (mAb) can be used to train the host immune system to target and clear cancerous cells. Antibody structure and conformation are critical for function, but the large, flexible nature of the proteins can make structure determination by X-ray crystallography, or nuclear magnetic resonance spectroscopy, challenging. Small angle X ray scattering (SAXS) is becoming a popular method to study the conformation of flexible proteins in solution. As SAXS is a low-resolution technique, high-resolution crystallographic structures are routinely used to interpret the solution data. However, often the crystal structure of a protein does not accurately represent the conformations observed in solution. As computational simulations can incorporate both the buffer environment of SAXS experiments and the atomistic detail observed in crystallographic models, atomistic molecular dynamics (MD) simulations offer the potential to determine physiologically relevant atomistic structures from SAXS data.

We are interested in understanding the structure-function relationship of antibodies targeted against the human inhibitory Fc gamma receptor, CD32b. The crystal structure of an antibody binding fragment (F(ab)) of an anti-CD32b mAb in complex with CD32b has been determined. However, there are differences between the crystallographic structure and SAXS data for the protein. Using long-timescale MD simulations with Principal Component Analysis (PCA) we are able to determine the principal conformations observed for the F(ab) in solution both alone and in complex with CD32b. Comparison of MD structures from similar regions of PC space, allow both global and localised motions that influence the agreement of atomistic models to our SAXS data to be identified.

This method was used to determine the structure of two anti-CD32b antibodies with differing activities; one able to activate the receptor, and one that blocks CD32b activation. From this structural comparison we are able to better understand the structure-function relationship of these antibodies, which will aid in the design of increasingly effective therapeutic antibodies for the treatment of cancer.
Studying computationally the selective binding of a fluorescent dye to de novo designed α-helical barrels

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Recently, a reliable approach to design α-helical barrels consisting of coiled-coil monomers which self-assemble into parallel pentamers, hexamers and heptamers has been developed[^1-4]. These water-soluble α-helical barrels reveal a central pore aligned by hydrophobic residues that makes them prospective candidates for lipophilic small-molecule carrier systems.

Herein, we present the application of molecular modelling to shed light on the molecular mechanisms that govern the binding of environment sensitive fluorescent dye 1,6-diphenylhexatriene (DPH) to a set of de novo designed α-helical barrels. In particular, we use extensive molecular dynamics simulations as well as analysis methods borrowed from the computational study of membrane transport proteins to explain the experimentally observed binding specificity of DPH to these coiled coils.

We confirm the stability of the DPH-barrel complexes in solution and identify the preferred binding cavities inside the barrels. We show that the structure of some of the α-helical barrels adopt different conformations in solution compared to their X-ray crystal structures, thereby changing the diameter and shape of the pores and thus their ability to bind specific small molecules. We discuss the effect of the presence and absence of DPH on the size of the channels and provide some insights into the induced circular dichroism band observed experimentally for DPH in complex with the barrels. We conclude that, because of their relative simplicity, the study of these de novo engineered α-helical barrels reveals valuable information directly transferable to ligand recognition in natural proteins and provides an ideal playground to compare modelling and experimental results.

**References**
Investigating allosteric regulation and cryptic pocket formation through enhanced sampling simulations

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Allosteric regulation plays such a fundamental role in biology to be described by Jacques Monod as 'the second secret of life. In signalling proteins such as protein kinases, ligand binding to allosteric sites have been shown to be able to up- or down-regulate the catalytic activity. Understanding the molecular mechanisms underlying the observed allosteric effects is of great importance for the rational design of novel biologically active allosteric regulators. One major challenge and opportunity in computer-aided drug design is the accurate description of the conformational landscape prior to and upon the binding of the allosteric regulator. In particular, cryptic allosteric pockets, that is, sites on protein targets that only become apparent when a drug bind, provide a promising alternative to orthosteric sites, when discovered. To this aim we have developed and successfully applied various enhanced sampling algorithms together with atomistic simulations. Here we show how we these methods were successfully used to understand allosteric activation mechanisms in GPCRs and find known and unknown cryptic sites. The structural model of a previously unknown cryptic site in a bio-medically relevant drug target was used to design novel potent and selective inhibitors affecting protein-membrane interactions.

Replica Exchange Molecular Dynamics Simulations of Peptide Folding and Binding Kinetics

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We show how accurate rates of formation and dissociation of peptide dimers can be calculated using a direct transition counting (DTC) method for analyzing temperature replica exchange molecular dynamics (REMD) simulations.[1] In this approach, continuous trajectories corresponding to system replicas evolving at different temperatures are used to assign conformational states. In a second step, we analyze the entire REMD data to calculate the corresponding rates at each temperature directly from the number of transition counts. Finally, we compare the kinetics extracted directly, using the DTC method, with indirect estimations based on our previous trajectory likelihood maximization method, using short-time propagators, and with estimations from the decay rates of state autocorrelation functions. The DTC method is simpler to implement than previous approaches, and leads to similarly accurate temperature-dependent rates as the alternative, indirect methods.[2,3] We apply the DTC rate-extraction method to all-atom REMD simulations of dimerization of amyloid-forming NNQQ tetrapeptides in explicit water,[1,2] and to the conformational dynamics of FF dipeptides.[4] We assess the corresponding REMD efficiency, and we obtain remarkably good agreement with theoretically predicted errors for calculating the parameters of the coarse master equation that corresponds to the underlying kinetic processes.

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Predicting water networks and ligand binding free energies in proteins using grand canonical Monte Carlo

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 uncertainties shown in brackets, cycle closures shown in centre.

Understanding the location and energetics of water molecules is helpful for rational drug design. We present our grand canonical Monte Carlo (GCMC) method for predicting water locations and binding free energies in proteins\cite{Ross2015}. Further, we can calculate binding free energies of ligands with different active-site water networks by combining ligand alchemical perturbations and GCMC in a single simulation (GCAP).

The energetics of an active site water molecule can be vital to drug binding, through either stabilising the bound complex, or releasing entropy upon water displacement. Rationalising how water molecules should be treated is difficult – whether a water molecule should be retained in a bound structure, or if it should be displaced to recover entropy and allow for direct protein-ligand interactions, is unclear\cite{Barillari2007}. GCMC is able to calculate the free energy of multiple water networks in a single simulation, and as the water location is predicted automatically as part of the simulation, no experimental knowledge of hydration site location is required.

While it is important to understand the hydration thermodynamics in protein systems, the most important property remains the ligand binding free energy. Although conventional free energy simulations can calculate relative binding free energies of ligands, issues can arise when the water network changes with the ligand, particularly in the case of occluded cavities\cite{Michel2009}. We show that GCMC can now be performed in conjunction with ligand binding free energy simulations. This allows for direct calculation of relative free energies between ligands with different water occupancies within a single simulation. We refer to this combination of GCMC and thermodynamic integration as GCAP. Experimental data for Scytalone Dehydratase (SD) for congeneric ligands, where displacement of a water molecule results in a large increase in affinity, have been reproduced using the GCAP method. GCAP is therefore able to directly provide the free energy difference between ligands while automatically accounting for changing hydration effects.

References
Many molecular processes, such as protein-protein interactions or protein-ligand binding events, are not accessible with direct MD simulation - even on a supercomputer. Enhanced sampling techniques, such as metadynamics or umbrella sampling, in which a biasing potential $U(x)$ is added to the unbiased force field $V(x)$ increase the sampling of rare events. However, the distortion of the timescales in the system due to the biasing potential is not uniform. The resulting biased trajectories can hence not be used to estimate models of the molecular dynamics, e.g. Markov state models.

I will present the Girsanov reweighting method with which one can estimate the the expected path ensemble average of an unbiased dynamics for a set of biased paths. The method is based on the concept of path probability measure and the Girsanov theorem, a result from stochastic analysis to estimate a change of measure of a path ensemble.

Since Markov state models of molecular dynamics can be formulated as a combined phase-space and path ensemble average, the method can be extended to reweight these models by combining it with a reweighting of the Boltzmann distribution. I will explain how to efficiently implement the Girsanov reweighting in a molecular dynamics simulation program by calculating parts of the reweighting factor “on the fly” during the simulation, and I will demonstrate the construction of Markov state models from biased simulations for several test systems. Besides its use in enhanced sampling simulations, the Girsanov reweighting can also be used to test slow dynamic processes to perturbations of the potential energy surface and might therefore be useful in the development of new force fields.

References:

CoCo-MD: Enhanced Sampling with Hyperspace Jumps

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There are many different approaches to the enhanced sampling of conformational space in biomolecular simulation. Broadly speaking they may be divided into those that are appropriate when you have some idea of the destination for the advanced sampling, or at least the route, and those that are appropriate when you have no a priori knowledge of the space to be explored. Over the last three years the ExTASY consortium (www.extasy-project.org), an EPSRC and NSF-funded UK-US collaborative project, has been exploring new approaches to tackling the second type of problem.

The ExTASY approach is based on a) using large numbers of replicate simulations to parallelise the process of conformational sampling and b) the use of adaptive collective coordinates, updated on-the-fly, to direct sampling towards the most interesting and/or challenging regions.

I will describe the development and application of one of the ExTASY approaches, CoCo-MD, where the adaptive collective coordinates come from the application of the PCA-based CoCo (‘Complementary Coordinates’) method originally developed to enrich ensembles derived from NMR data [1]. A unique feature of the CoCo approach is that includes ‘hyperspace jumps’ where new simulations are started from regions of conformational space beyond any that have been sampled up to that point.

Figure 1: Application of CoCo-MD to cyclosporin A. Left: conventional MD (cMD), right: an equivalent amount (20 ns) of CoCo-MD. The black dot marks the starting conformation; crosses mark the positions of centroids identified by Witek et al. [2] from their 10 microsecond cMD simulation.

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Towards *in silico* prediction of β-lactamase-mediated antibiotic resistance using free energy calculations

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The main mechanism of resistance to β-lactam antibiotics is represented by the appearance of β-lactamases. A representative example is the class A KPC-2 β-lactamase that in a few years became one of the most menacing β-lactamase currently spreading worldwide [1].

We have developed a protocol using the PMX software [2] that allows the evaluation of the energetic cost associated with a mutation in the active site of a β-lactamase, in the presence of the β-lactam substrate. The identification of the most favourable mutations will guide the development of new β-lactamase inhibitors, which will be active not only against the currently known mutants, but also against those that are likely to appear in the near future.

This protocol include three steps: i) conformational space sampling; ii) *in silico* mutation (morphing) and iii) free energy estimation. The molecular dynamics simulations are carried out using Gromacs and the OPLS-AA force field. The force field parameters of ligands are taken from Ligandbook [3] and the experimental data from the Beta-Lactamase DataBase (BLDB) [4]. This protocol was validated by comparison with various experimental data. The difficulties associated with the mutations involving a change in the overall charge will be discussed.

A variant of this protocol involves mutations on a ligand in complex with the protein of interest. The use of this variant for our participation to the D3R Grand Challenge 2 [5] will also be presented.

**References**

Membrane-embedded proteins are essential components of all cellular organisms, allowing cells to communicate with their surroundings. Secondary active transporters constitute one of the largest classes of membrane proteins, and serve to capture the chemical potential energy of preexisting electrochemical gradients so as to facilitate active uptake of nutrients or neurotransmitters, or extrusion of toxins. A 50-year-old hypothesis posits that transporters must adopt two alternate conformations in order to achieve coupled transport and to avoid dissipating the electrochemical gradient. From the perspective of structure determination, this property poses a particular challenge, however, since the proteins must adopt multiple alternate conformations without any direct mechanical or chemical intervention from e.g. ATP hydrolysis. We have studied this problem using structural modeling strategies that take advantage of the asymmetry inherent in many of these states. Through close collaboration with experimental groups, we have shown how an unusual, evolutionarily-unexpected transmembrane topology present in the majority of active transporters, provides an elegant solution to the alternating access requirement.
A fundamental property of any system is its entropy. It quantifies the total molecular flexibility and, together with enthalpy, determines molecular stability. It is particularly important in biomolecular systems because of the prevalence of dynamic, weak interactions and large amounts of disorder. However, the difficulty of calculating entropy restricts its widespread use in understanding structure and stability. Studies are limited to qualitative and partial measures of structure, differences in entropy or free energy, or entropies from the distribution functions of small, rigid molecules. Building on previous work for liquids [1,2,3,4] and isolated flexible molecules [5], we present new theory to calculate the entropy from a trajectory generated in a molecular dynamics simulation for the significant and poorly explored case of liquid mixtures of flexible molecules which are prototypical of biomolecular systems. The vibrational entropic term is derived from the covariance matrix of forces and torques for sets of atoms of each molecule. The mixture and conformational entropic term is derived from the probabilities of molecular coordination states defined using the parameter-free RAD algorithm [6]. The theory is tested on a range of simple liquids and organic liquids and results are compared with values from perturbation methods.

Figure 1: A representative organic liquid, 1-propanol.

References

Nanoscale Protein Interactions Determine the Mesoscale Dynamic Organisation of Biomembranes

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Recent advances in experimental biophysical techniques, such as super-resolution microscopy, have given new insights into the complex organization of membrane proteins for length scales of hundreds of nanometers and timescales of milliseconds. Likewise, progress has been made in term of computational models, allowing the creation of biomembrane systems containing molecular detail at the 100 nanometer lengthscale. Thus, it has recently been shown that protein crowding underpins the turnover of bacterial outer membrane proteins, a process that is vital for the adaptation of certain bacteria to new environments1. Nevertheless, experiments as well as computational models have been devoid of a full understanding of protein crowding in both molecular detail and at experimentally observable time and length scales. In the current study, we describe how these clusters may be generated for the Outer Membrane Proteins (OMPs) BtuB and OmpF using coarse-grain (CG) Molecular Dynamics (MD) simulations. However, a mesoscale model is necessary to assess the dynamic behaviour of these OMPs at the mesoscale (hundreds of nanometers and millisecond timescales) and to bridge MD simulations and single molecule fluorescence microscopy. From the dynamics and protein interactions observed in our CG-MD simulations, we generate such a mesoscale model, and are able to directly compare in vitro and in silico results by using single molecule tracking analysis on both. Simulations using the mesoscale model reveal that bacterial outer membranes are comprised of protein clusters that present a mesh of moving barriers that can act to confine newly inserted proteins into OMP ‘islands’.

References

Unraveling the Good and the Bad in Protein Networks: Functional versus Dysfunctional Interactions

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In the last years, protein interactome comparisons have highlighted conserved modules that might represent common functional cores of ancestral origin. However, recent analyses of protein-protein interaction networks (PPINs) have led to a debate about the influence of the experimental method on the quality and biological relevance of these interaction data. It is crucial to know to what extent discrepancies between the networks of different species reflect sampling biases of the respective experimental methods, as opposed to topological features due to biological functionality. This requires new, precise and practical mathematical tools to quantify and compare the topological structures of networks at high resolution. To this end, we have studied the relationship between structured random graph ensembles and real biological signaling networks, focusing on the number of short loops in networks, which represent complexes in PPINs. We have developed methods to map common genomic variation and disease-related mutations to functional PPIN, so to highlight specific interactions that may be targetable in novel drug-design strategies.

Nevertheless, one must keep in mind that not all the interactions between proteins result in a functional role that benefits the cell. One example is protein aggregation, resulting in neurotoxic assemblies that lead ultimately to cell death. We investigate in detail the case of interactions between fragments of the Prion protein (PrP) constituted by only the helices H2 and H3 of the entire protein. We have investigated the molecular mechanisms of the self-assembly process in solution by Molecular Dynamics. Our simulations show that this process occurs by assembly of small modules of four monomers that precede the creation of a “base” of six to eight H2H3 monomers; starting from this “base”, other H2H3 units attach to it in various configurations, assembling short filaments.


Permeation and selectivity mechanism in NaChBac channel and its mutants

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NaChBac was the first discovered sodium voltage dependent channel, yet computational studies are still limited due to the lack of a crystal structure. In this work a pore-only construct built using the NavMs template was investigated using unbiased Molecular Dynamics and Metadynamics. The Potential of Mean Force (PMF) from the unbiased run features four minima, three of which correspond to sites IN, CEN and HFS discovered in NavAb. During the run the Selectivity Filter (SF) is spontaneously occupied by two ions and frequent access of a third one is often observed. Metadynamics simulations biasing one and two ions, show an energy barrier in the SF that prevents single-ion permeation. An analysis of the permeation mechanism was performed both computing minimum energy paths in the axial-axial PMF and through a combination of Markov State modeling and Transition Path Theory. Both approaches reveal a knock-on mechanism involving at least two, but possibly three ions.

When the channel is simulated in CaCl₂ the SF becomes occupied by a single ion that remains blocked inside. There are two reasons for the inability of Ca²⁺ to permeate NaChBac. First of all, the free energy of binding of Ca²⁺ to the SF, that we computed through the free energy perturbation approach, is 3.5 times higher than that of Na⁺, leading to a stronger attraction to the glutamates of the EEEE-ring. Second, using metadynamics simulations with a fixed ion and a mobile ion, we showed that a Ca²⁺/Ca²⁺ knock-on mechanism cannot occur due to the high repulsion energy that the resident ion exerts on a second, potentially incoming ion. Our work thus, provides an alternative scenario with respect to the traditional view of the Ca-exclusion model that postulates the inability of Ca²⁺ to bind to the Selectivity Filter of sodium channels.

A remarkable feature of NaChBac is the switch in selectivity from Na⁺ to Ca²⁺ when the number of glutamates in the SF is increased. Our equilibrium simulations of TLEDWAS and TLDDWAD mutants in NaCl and CaCl₂ provide an interpretation of the differential selectivity of these mutants consistent with the charge/space competition model. Since the mutants are negatively charged, they tend to draw into the SF as many cations as are necessary to neutralize the excess of negative charge. Since sodium carries a charge +1 while calcium carries a charge +2, the mutants need to accommodate a larger number of sodium ions. Thus, due to space availability reasons, calcium ions can all be accommodated in the top region of the SF, while some sodium ions need to occupy also the bottom part of the channel. However, the simulations show that as soon as a sodium ion reaches the Leucine ring, the lower part of the SF is closed, which possibly results in a decreased sodium current. The simulations show that this effect is more pronounced in TLDDWAD than in TLEDWAS explaining the differential selectivity of the two channels.
“Atomistic simulations of self-assembled nanocages (SAGEs)”

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An Archer Leadership Award has enabled us to run atomistic molecular dynamics simulations on the self-assembling nanocage structures (SAGEs) from the Woolfson laboratory. SAGEs are built in the laboratory by mixing two types of hub (acidic and basic) each comprised of six 25 residue peptides (Figure 1 A). The experimental data are consistent with these objects being hollow spheres around 100 nm in diameter with a different exposure of the N and C termini of the peptides at the SAGE surface¹.

An idealised model of a SAGE particle was constructed from 3720 25-mer peptides (Figure 1 B) and MD simulations performed in explicit solvent. Simulations of the parent SAGE and two derivatives were carried out in the presence of other globular proteins and a range of small molecules (Figure 1 C). The talk describes the evolution of SAGE structure over the microsecond timescale and the behaviour of the solutes. Both adhesion to, and permeation through, the surface SAGE peptide network by solutes is observed and discussed.

Figure 1: A, each peptide trimer (upper left) is coupled to one half of the heterodimer (lower left) by a disulphide link. Mixing the ensuing pair of six-peptide hub constructs leads to polymerisation to a largely hexagonal lattice that closes to give SAGE particles. B, the idealised SAGE molecule generated as the initial model. C, time frame at 50 ns showing the simulation box with SAGE an solute molecules, water and salt not shown.

References

Scalable polarizable molecular dynamics using Tinker-HP: millions of atoms on thousands of cores

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Tinker-HP (http://www.ip2ct.upmc.fr/tinkerHP) is a CPU based, double precision, massively parallel package dedicated to long polarizable molecular dynamics simulations and to polarizable QM/MM. Tinker-HP is an evolution of the popular Tinker package (http://dasher.wustl.edu/tinker) that conserves its simplicity of use but brings new capabilities allowing performing very long molecular dynamics simulations on modern supercomputers that use thousands of cores. The Tinker-HP approach offers various strategies using domain decomposition techniques for periodic boundary conditions in the framework of the (N)log(N) Smooth Particle Mesh Ewald or using polarizable continuum simulations through the new generation ddCosmo approach. Tinker-HP proposes a high performance scalable computing environment for polarizable force fields giving access to large systems up to millions of atoms. I will present the performances and scalability of the software in the context of the AMOEBA force field and show the incoming new features including the advanced SIBFA polarizable molecular mechanics approach and the density based GEM force field as well as newly available "fully polarizable" QM/MM capabilities. Various benchmarks and examples on biomolecular systems will be provided on several architectures showing that the approach is competitive with GPUs for small and medium size systems but allows addressing larger molecules on modern supercomputers. As the present implementation is clearly devoted to petascale applications, the applicability of such an approach to future exascale machines will be exposed and future directions of Tinker-HP discussed.


iii) A QM/MM approach using the AMOEBA polarizable embedding: from ground state energies to electronic excitations. D.Loco, E. Polack, S. Caprasecca, L. Lagardère, F. Lipparini,
Towards Engineering Radical Enzymes - Thermodynamic Reaction Profiling and Mechanistic Insights into QueE

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Radical S-adenosylmethionine (SAM) dependent enzymes [1] are a relatively recently discovered enzyme family with great prospects for biotechnological applications. Their key catalytic communality is to deal with highly reactive radical intermediates during catalysis. The way the enzymes control these intermediates, which are particularly hard to control in classical synthesis, makes them essential in a broad set of challenging biosynthetic pathways that often lead to products of potential use in anti-viral, anti-cancer and antibiotic treatments.

A thorough knowledge of the reaction mechanisms involved in the biocatalysis of these enzymes presents the foundation for rational enzyme engineering and one of the bottlenecks for a more rapid access to rational enzyme design simultaneously. In the best case scenario everything from the catalytic reaction mechanism, the kinetics, to substrate binding, and dynamic effects is known in detail. Still, individual steps of the catalysis can be addressed by quicker methods, in order to get a first qualitative picture of how these steps can be influenced and can feed into the enzyme design process. This work focuses on potential transferable approaches to reduce complexity and computational effort and to focus on key features related to rSAM enzyme catalysis for their use in enzyme design.

Radical stabilization energies (RSEs) [2] are an example that offers an attractive possibility to assess the overall thermodynamics of radical reactions and rearrangements as central steps in radical SAM enzyme catalysis. Through the example of the bacterial 7-carboxy-7-deazaguanine (CDG) synthase (QueE), [3] we will highlight key features and details of the biocatalytic radical rearrangement mechanism involved [4] and will discuss the potential of using radical stabilization energies for thermodynamic reaction profiling directly from enzyme substrate complexes. Further, we will provide insights into other challenges of radical SAM enzymes addressed in the context of enzyme engineering.

References
A conserved Mg-pinch motif in NTP processing enzymes and its importance in the polarisation of the cleaved phosphate

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Phosphate processing enzymes are crucial to most of the biochemical processes that all living cells depend on, including signal transduction, gene regulation, metabolism and energy transfer [1]. Obtaining a detailed description of the mechanism of action of these biomolecules, and understanding the prominent role of phosphate groups and metal ions in biological systems, will enable fundamental advances to be made in our knowledge of the molecular basis of life. It will also create opportunities for the rational development of better treatments for combating disease and improving welfare.

Herein we focus on the role of Mg²⁺ ions in the active site of enzymes involved in phosphate processing. We analyzed the coordination geometry in over 800 PDB structures in 14 EC categories. In most EC categories, it was possible to identify a unique active site geometry with one or two magnesium ions bound with a specific coordination to the phosphate groups. The reaction can be fully characterized by identifying the phosphate at which the attack takes place, and the one that represents the leaving group. We found that in all consensus structures both the attacked phosphate and the leaving phosphate groups were coordinated to a magnesium ion. This coordination corresponds to a ring resembling a Mg²⁺-pinch. Thereby we found that the chemistry carried out by the enzyme determined the features of the Mg²⁺ coordination with respect to the triphosphate groups [2].

To assess the quantitative effects of this coordination geometry on the bond strength, we carried out QM and QM/MM calculations. Here we propose that the role of the Mg²⁺ ion in phosphate processing enzymes is to assist in the charge transfer from the H acceptor to the leaving group via a stabilized transition state. This stabilization is possible due to wave function polarization and not merely by pure coulombic effect of the divalent ion [2].

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Revealing the origin of enzyme catalysis from computational studies

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Theoretical and computational estimations of kinetic isotope effects (KIEs) represent valuable tools to derive conclusions on the action of enzymes, including the role of tunnelling, electrostatic and dynamic effects. This knowledge can be used as a guide for the design of new biocatalysts where the inclusion of transition state stabilizing interactions either in a pre-existing protein structure (enzymatic re-design) or in a completely new designed enzyme (de novo design) must be taken into account.

We will show in this communication recent studies on different enzyme catalyzed processes where the Transition State Theory is demonstrated to be a proper framework to understand the origins of enzyme catalysis. We will focus on aspects such as the controversial debate on whether protein dynamics are linked to the chemical reaction step,[1,2] the role of the quantum tunnelling and the electrostatic effects contributions to catalysis,[2-4] or the relevance of compression effects in enzymatic methyl transfer reactions.[5]

References


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Computational design of binders for protein molecular recognition

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The ability to design biomolecules that binds proteins with high affinity is essential in nanomedicine and nanoengineering, as they can be used as probes for the molecular recognition of biomarkers, for the oriented immobilization of target proteins, and for the inhibition or modulation of the enzyme activity [1]. Typical binders are antibodies due to their high performance in the molecular recognition of target proteins [2]. However, the common antibody engineering tools based on the maturation and in vitro selection of binder candidates are in general expensive, time consuming, and present difficulties to specify the target binding site to which the antibody will bind to [3]. Thus, different methods and binders have arisen as an alternative. One of the most promising approaches is the use of computational protocols for the design of small- and medium-size protein binders, as it offers minimum costs and optimal speed [4]. Here, we present a novel stochastic evolutionary algorithm for the design of binders that interact specifically in a chosen target binding site [5]. Along these years, we have optimised peptides and single domain antibodies of camelid origin (also known as nanobodies) to bind proteins with different structural properties: β-2-microglobulin, lysozyme and HER-2. Our engineered probes have shown capable of recognising their target in solution, and have been further employed for protein oriented immobilisation.

Figure 1: (a) Optimisation kernel of the design algorithm. (b) Evolution of the scoring energies of peptide-β-2-microglobulin complexes during a typical run.

References
Computational prediction of protein-ligand binding affinity is an invaluable technique in structure-based drug design [1,2]. Despite significant recent progress, the accuracy of these simulations is still affected to some extent by limitations in the underlying molecular mechanics force field. These limitations include the assumption of parameter transferability, the neglect of large-scale polarisation effects in biomolecular assemblies, and the fixed functional form of the force field. Here, I give an overview of some of our efforts to overcome these force field limitations, including i) the derivation of force field charges and Lennard-Jones parameters directly from the quantum mechanical (QM) electron density of small molecules or entire proteins [3]; ii) a new method for the accurate derivation of bond and angle force field parameters directly from the QM Hessian matrix [4]; and iii) a machine learning based intramolecular potential for the simulation of flexible organic molecules [5]. These techniques represent promising steps towards our goal of accurate and automated quantum mechanics based potentials for computer-aided drug design applications.

\[ Q_A = \int n_A(\mathbf{r}) d^3 \mathbf{r} \]
\[ C_6 = \left( \frac{V_A}{V_0} \right)^2 C_0^6 \]

Figure 1: Derivation of quantum mechanics based potentials for computer-aided drug design

References

The targeted binding of peripheral membrane proteins to cell membranes is vital for many processes within the cell, including many signalling pathways; disruptions in these interactions are often linked to disease. Targeted binding is achieved through lipid-binding domains which interact with specific membrane lipids. Pleckstrin Homology (PH) domains are among the most common lipid-binding domains, and are known to associate with phosphatidylinositol phosphate (PIP) lipids. Different PH domains bind PIPs with differing specificity and affinity. The structural and energetic details that lead to these differences may be difficult to determine experimentally, but can be investigated using Molecular Dynamics (MD) simulations. We previously demonstrated use of MD with the Umbrella Sampling (US) free-energy calculation method and a coarse-grained representation, where groups of around 4 heavy atoms are represented by a single bead, to allow feasible calculation of energy profiles for the binding of the GRP1-PH domain to membranes containing PIP2 and PIP3 [1]. We now expand this analysis to a further 12 PH domains. Experimentally determined selectivities and mutational effects were reproduced. Several distinct binding modes were identified, revealing a greater degree of PIP interactions at a secondary binding site than previously suggested. Additional simulations and comparison with experimental results further suggested PH domains may simultaneously bind multiple PIP lipids to achieve high-affinity membrane association. This work demonstrates how a computational simulation approach can allow investigation and comparison of protein-membrane interactions in order to improve understanding of targeted membrane binding.

References

TrmD is a bacterial enzyme with crucial function – it methylates tRNA assuring correct codon-anticodon pairings during translation. Lack of this modification leads to a malfunction in translation, leading to the death of the cell. The protein’s cavity for the ligand is formed by a trefoil knot [1], which shows the involvement of the knotted region in the catalytic process.

Using a series of mutations within the knotted region of TrmD, we show that this region is involved in signal transduction [2]. Several mutations were done using both experimental and theoretical approach, in order to show molecular mechanisms lying behind them. In particular, Y115A mutation in TrmD causes large decreases in rate of both tRNA binding and methyl transfer, not seen in this extent in any other mutant. Using molecular dynamics simulations with explicit solvent, we show that the mutation impairs protein-tRNA interaction network and causes conformational changes within distant parts of the enzyme.

Moreover, we found that Y115A mutation changes also the global motions of the TrmD [2]. As a homodimeric protein, TrmD has two identical knotted regions, which, without the mutation, exhibit different, uncorrelated motions. It was shown earlier that TrmD can bind only one tRNA per dimer [3], which explains the asymmetry we found in the complex. In simulations Y115A mutation affects this asymmetry and equalizes the subunits. This is in agreement with experimental data, which shows that mutated TrmD binds two tRNA molecules, one per each subunit.

Our results are the first step to answering a fundamental question – how can TrmD’s mechanism of function be efficient, with only one subunit catalytically active at a time. Understanding this process is of a great importance, since proper level of TrmD’ activity is essential for the protein synthesis, and thus growth of the cell.

References

Poster 3. Towards understanding the unbound state of drug compounds: Implications for the intramolecular reorganization energy upon binding

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Abstract text [1]. Characterization of the unbound state of pharmaceutical compounds is essential to better understand their recognition by biological macromolecules, including the much debated conformational intramolecular reorganization energy of a compound upon binding ($\Delta E_{\text{Reorg}}$). That is also important for ligand-based drug discovery, e.g. with pharmacophore modelling. These questions were addressed with molecular dynamics (MD) simulations of 26 diverse compounds (including 7 approved drugs) unbound in explicit solvent. The MD-simulated free compounds are compared to their bioactive X-ray structure and conformers generated with ad hoc sampling in vacuo or with implicit generalized Born (GB) aqueous solvation models. The GB conformational models depart from those obtained in explicit solvent, and suffer from conformational collapse almost as severe as in vacuo. Thus, the global energy minima in vacuo or with GB are poor representations of the unbound state, which can instead be extensively sampled by MD simulations. The notion of conformational pre-organization for binding was investigated by comparing the MD-simulated compounds to their bioactive X-ray structure. The ligand-protein complexes were also simulated in explicit solvent to estimate $\Delta E_{\text{Reorg}}$. This approach yielded low to moderate values of $\Delta E_{\text{Reorg}}$ for 18 out of 26 compounds. For three particularly polar compounds, $\Delta E_{\text{Reorg}}$ was substantial ($\geq 15$ kcal/mol). Those large $\Delta H_{\text{Reorg}}$ values correspond to a redistribution of electrostatic interactions upon binding. Overall, the study illustrates how MD simulations offer a promising avenue to characterize the unbound state of medicinal compounds.

References

Poster 4. Environment effects on a potential trans-cis molecular switch for opening the ion channel of the serotonin-activated 5-HT₃ receptor

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Pentameric ligand-gated ion channels (pLGICs), embedded in the membrane of nerve cells, are important neuroreceptors that mediate fast synaptic transmission, are involved in several neurological disorders and are target sites for drugs. For example, the serotonin-gated 5-HT₃ receptor is the target for anti-vomit drug for patients undergoing chemotherapy. Working on a model of the 5-HT₃ receptor based on a recently resolved x-ray structure, we have investigated a potential molecular switch for opening the ion channel, consisting of a proline at the interface between the extra-cellular and the transmembrane domain undergoing a trans-cis isomerization, as suggested by electrophysiology mutagenesis experiments. Our study is based on molecular dynamics and free energy calculations with the enhanced sampling method metadynamics, and aims at elucidating the effect of the receptor environment.
Proteins with the same sequence are found to fold into the same 3D structure reliably in solution, yet the exact processes underpinning the interactions between peptide and solvent in the folding process are not well understood. Previous study on the glycyl-prolyl-glycinamide (GPG-NH$_2$) tripeptide has indicated that hydrogen bonding between backbone amide groups and solvent water molecules may mediate protein folding processes. This study focuses on a longer peptide; tyrosyl-prolyl-glycyl-serinamide (YPGS-NH$_2$), which is a sequence designed to have beta-turn forming propensity. Experimentation on this peptide with and without an acetyl cap on the N-terminus reveal differences in overall folding propensity and equilibrium ratio of cis- to trans- conformers around the YP peptide bond. Whole-molecule and specific interactions between water and hydrogen bonding groups on these peptides are compared using complementary experimental and computational techniques: solution nuclear magnetic resonance spectroscopy, molecular dynamics and empirical potential structure refinement modelling. This multi-faceted approach aims to produce a unified and experimentally consistent model of the system. Most probable nearest-neighbour water molecules indicate a bridging water relationship for the trans conformer not observed in cis-(Figure 1). Solvent composition also affects the peptide conformation and hydration, as shown by complementary simulation data in bulk DMSO, bulk water, and in a 1:1 molar DMSO-water binary mixture.

References
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Poster 6. β2-microglobulin interaction with hydrophobic surfaces: A molecular dynamics study

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In this work we explore the interactions of β2-microglobulin [1], a paradigmatic protein model for amyloidogenic pathologies, with a model cubic hydrophobic box. A system made up with 27 copies of the protein is surrounded by the united atoms representation of methane hydrocarbons and simulated using the OBC-GBSA [2] implicit solvent molecular dynamics. The results show that most proteins adsorb on the walls of the box without major distortions of local geometry, whereas free molecules maintain proper structure and fluctuations as observed in explicit solvent molecular dynamics simulations. Thermodynamic analysis suggests that van der Waals interactions are much larger than all other contributions to the free energy of binding. Loss of secondary structure and local unfolding could have important physiological consequences suggesting that hydrophobic surfaces could thus act in vivo as promoters of partial unfolding and local clustering that are essential for seeding the formation of amyloid fibrils. Owing to the emerging role of biological interfaces occurring in vivo in general and in protein aggregation and fibril formation in particular, we believe our findings afford a general outlook complementary to the biochemical studies on this protein.

Figure 1: Snapshot at 61 ns. Only one face of the bounding box (green) is shown for the sake of clarity, but it is evidently suggested by the proteins adsorbed at the interface. Red and blue cartoon representations distinguish between free and adsorbed chains in the simulated timescale respectively while red licolire representation highlights some binding residues with the surface.

References

Poster 7. Oligomeric Structure of Pulmonary Surfactant Protein B Causes Lateral Lipid Reorganization and Perturbations in Surfactant Membranes and Monolayers

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Pulmonary surfactant is a protein-lipid complex covering the air-liquid interface in the alveoli of mammalian lungs [1]. Pulmonary surfactant consists of 90\% lipids and 10\% surfactant-associated proteins. The unique lipid composition and the surfactant-associated proteins are together responsible for the main function of pulmonary surfactant, that is surface tension reduction during breathing cycles, which prevents the alveoli from collapsing and also optimizes structural reorganizations in the surfactant during breathing [1,2].

Surfactant protein B (SP-B), a 79-residue polypeptide of the saposin-like protein family, is an essential part of the pulmonary surfactant. It facilitates surfactant adsorption upon contact with the pulmonary surfactant interface, and mediates transfer of surface active molecules from the aqueous phase to the air-liquid interface during the compression-expansion cycles by promoting membrane-membrane contacts, lipid fusion, and formation of multilayer lipid structures [2,3,4]. The lack of or deficiency in SP-B leads to lethal respiratory pathologies at birth. Due to its high hydrophobicity and the presence of charged residues SP-B attains a permanent superficial binding with both bilayers and monolayers [2]. However, despite its importance, the structure and the molecular mechanism of SP-B are poorly understood, though a recent experimental study suggests a major leap forward as it demonstrated ring-shaped oligomeric SP-B assemblies to be stable and functional structures in pulmonary surfactant [5].

We refined the SP-B model suggested by Olmeda et al. [5], and explored it through coarse-grained model simulations to study the interplay of SP-B oligomers with model surfactant lipid membranes. Our results showed clear specificity of certain lipid types in the protein structure. SP-B was also found to cause lateral lipid reorganization in both lipid monolayers and bilayers. The results are discussed in terms of functions maintained by the pulmonary surfactant, in particular considering how SP-B could be involved in lipid transfer between adjacent surfactant assemblies.

References

Antimicrobial resistance has massively increased in the last decades qualifying itself as a threat to human health. In the last decade, the lack of newly discovered antibiotics have pointed out the emerging potential of antimicrobial peptides (AMP): short fragments of larger proteins naturally occurring in the mammal organisms exerting a broad spectrum of weak antibiotic actions. AMPs attack selectively the bacterial membrane and not the mammalian one due to the different charge and structure between them, and have a low potential for resistance development as any mutation reducing the binding affinity of AMPs is likely to impact negatively on the life related functions of the membrane.

AMPs potential can be enhanced when many copies are collected together: recently engineered units including a short antimicrobial sequence derived from the bovine lactoferricin (1LFC) have been shown to self-assemble into hollow spherical capsules viable for drug and gene delivery [1]. The structure of such self assembled system, as well as the precise antimicrobial mechanism of these artificial units, is not entirely accessible to current experiments. Molecular dynamics (MD) simulations can uncover the structure and processes at the atomistic scale describing the dynamics of the system studied in [1]. We use a hierarchical approach to test small crucial entities involved in the recognition mechanism between units and in shaping their geometry, identifying the role of hydrogen bonds and of hydrophobic segregation in the stability of the structure. In parallel, simulations on model membranes mixtures resembling the ones used in experimental conditions, highlight the relation of such structure with the antimicrobial activity observed. The atomistic simulations of larger assemblies are possible including stronger hypotheses in building its putative geometry, thus many of these are being tested to identify the features which lead to a stable system. Once characterised the structures, a Coarse Grain approach will be followed to monitor the self-assembly itself.

References

Poster 9. Multi-scale coarse-graining for the self-assembly of two dimensional shapes from single stands of DNA

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Inspired by recent successes in using single-stranded DNA tiles to produce complex structures [1,2], we develop a two-step coarse-graining approach that uses detailed thermodynamic calculations with OxDNA[3], a nucleotide based model of DNA, to parametrise a coarser kinetic model that can reach the time and length scales needed to explicitly describe self-assembly. We test the model by performing a detailed study of the assembly of the 2D structures made up of 334 unique strands of 42 bases each from ref. [1,4]. Without using adjustable parameters, we find good agreement with the experimental rate-of-assembly curves as a function of temperature (fig. 1 – left). Furthermore, we perform detailed thermodynamic calculations on nucleation barriers and distribution of nucleus shapes. Although nuclei can be quite heterogeneous in shape, classical nucleation theory (CNT) provides nevertheless a good fit for the nucleation curves close to the critical temperature (fig. 1 – right).

Figure 1: Left – Assembly rate as a function of temperature for a cooling ramp with rate 3h/°C. Simulations on the canonical ensemble (200 replicas). Maxima at T=50.75°C (kinetic model) and T=53°C (experiments). Right: free energy as a function of nucleus size for single-replica assembly (with concentration of strand types fixed at 100nM).

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The solution to obesity is complicated and will require changes in eating habits and medical interventions. Food manufacturers have responded to this challenge by developing reduced or non-fat alternatives to conventional foods. These have not always been well received by the consumer as they are perceived as having inferior taste and texture [1]. A particular problem occurs with oily food products such as margarines and spreads which are solidified by including a network of small crystallites of triglycerides, which is rich in saturated fatty acids. Saturated fatty acids in food raise blood cholesterol level, which in turn has been identified as a risk factor for heart disease. An alternative method for structuring unsaturated triglyceride oils is the formation of so-called oleogels [2]. Several potential oleo-gelators have been identified including mixtures of phytosterols and sterol esters. The use of phytosterols as oleogelators is particularly appealing as these have also been linked to cholesterol lowering properties, and have been incorporated into some spreads for these reasons. A mixture of β-sitosterol and γ-oryzanol is an example of an oleo-gelating system. In triglycerides, mixtures of the sterol β-sitosterol and sterol ester γ-oryzanol self-associate to form elongated hollow tubules. These interact to form a network structure that traps oil in the structure thus forming a gel.

A thorough understanding of the physico-chemical energy balances involved and the structure-function relations of oleogelling compounds would allow a more rational selection of oleogelator candidate molecules, screening their properties and interactions before any de novo synthesis of alternative improved gelators is required. To this end, we have used docking methods and molecular dynamics simulations to investigate the self-association of β-sitosterol and γ-oryzanol in order to understand better the mechanism of self-association at a molecular level. Molecular simulations are also used to study the molecular features that determine fibril crosslinking.

Figure 1: In-silico model of β-sitosterol (blue) and γ-oryzanol (silver)
References

Poster 11. Unlocking Nicotinic Selectivity via Direct C–H Functionalisation of (−)-Cytisine

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Cigarette smoking is considered, nowadays, to be a major public health problem. Recent estimates indicate that approximately 1/4th of the world's population smokes [1] and that smoking is the second most prevalent cause of death in the world [2]. Every year, more than five million people die prematurely from smoking-related diseases, such as lung cancer and pulmonary diseases. Currently, the FDA-approved anti-smoking compounds, such as varenicline, are only moderately effective in reducing the symptoms of nicotine withdrawal and may cause undesirable side effects. Consequently, there is a growing need to develop new smoking cessation agents with improved effectiveness and tolerability.

Nicotine is the major biologically psychoactive agent in tobacco and it binds to the nicotinic acetylcholine receptors (nAChRs) [3]. The binding of nicotine at the interface between two subunits of the receptor promotes the opening of an ion channel, thereby allowing the flow of cations across the cell membrane [3]. In this work, we have used a combination of synthetic and computational chemistry to generate novel nAChRs modulators with improved sub-type specificity. Furthermore, we also characterized, at the molecular level, the agonist’s interactions with several human receptor sub-types in order to identify the molecular determinants that modulate binding in each case.

Figure 1: X-ray structure of the Human α4β2 nAChR [4]

References

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Fibroblast Growth Factor Receptors (FGFRs) are a family of membrane proteins involved in a wide variety of functions, from neuronal development to angiogenesis and cell division. Their delicate role in cell growth and differentiation makes them an attractive therapeutical target for the development of anti-cancer drugs. In this project, we helped design allosteric inhibitors of FGFR, targeting the protein-membrane interactions that drive their activation [1]. Here, enhanced sampling simulations were crucial in disclosing the presence of cryptic pockets, sites that only become apparent after an initial binding of the drug. The hidden nature of this sites makes them difficult to spot and characterise in detail: in most cases, they are discovered by chance. The molecular mechanism behind their formation is still unclear, an interplay between induced fit and conformational selection seems to be present to various degrees in different biological systems. Enhanced sampling methods such as Parallel Tempering Metadynamics and SWISH (Sampling Water Interfaces through Scaled Hamiltonians) a novel approach combining Hamiltonian Replica Exchange with small molecular probes [2], can provide effective tools to investigate the molecular mechanism of cryptic pocket formation in pharmacologically relevant targets.

Figure 1: Example of conformational change induced in the FGFR3c receptor by the interaction with hydrophobic molecular probes.
References

DNA-drug interactions are mainly important because most of the anticancer and antitumor drugs show their biological activities upon binding to the nucleic acids. So, all these drug like molecules are used as a target for the development of new anticancer drugs. There are few drugs that binds selectively to the DNA minor groove, predominantly to the A…T sites which are known as the minor groove binders. In this study, we picked the Hoechst 33258 dye which shows very high affinities upon binding to different A…T sequences in the minor groove region. Here we have provided a comprehensive study on the binding affinities of Hoechst 33258 dye to the different selective binders such as ATTC\(^1\), AATT\(^2\), ATTT\(^3\) and ATAT\(^4\) sequences through all atom well balanced Metadynamics simulation. Our calculation also predicts the sequence binding specificities within the helix if the drug can span its binding specificity to the G…C pair next to the A…T pair. Overall, the metadynamics simulation is able to explore all the possible binding motifs of the dye Hoechst 33258 binding to the different DNA sequences, therefore, a complete free energy landscape of the whole binding/un-binding events has been thoroughly explained. At the end, all the calculated binding data were thoroughly compared with the available experimental binding affinities. Finally, a fruitful understanding of the interactions between drug Hoechst 33258 and DNA, further the binding/un-binding mechanism of DNA-drug system, also the binding sequence specificity will help for searching new drug like molecules for anticancer therapy.

Figure 1: Selective binding of the Hoechst 33258 dye to different ATTC, AATT, ATTT, ATAT B-DNA sequences.

References

Poster 14. Optimal Clustering of Markov State Models

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Following from recent work [1] which demonstrates the existence of a variational principle for the projection of Markov state models, we present a method for the optimal and automatic identification of transition and metastable states [2] by projecting the model such as to preserve the slowest relaxation process within the system. In addition we present analytic results which provide an interpretation of this result in terms of the flux passing through the transition state boundaries. We hope that by providing an optimal definition of a transition state in terms of the flux, this optimal projection methods can be more readily extended to higher dimensional cases.

References

Poster 15. Mutating active site residue changed the reaction mechanism of Calb: new insight for rational engineering of CalB from QM/MM calculations

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Candida antarctica lipase B (CalB) is an industrially important enzyme. The binding modes of a substrate p-nitrophenyl benzoate in the WT CalB were investigated by MD and the hydrolysis reaction mechanism was studied by QM cluster and QM/MM methods. We also investigated the binding and reaction mechanism of a CalB variant which exhibits high catalytic activity. Our results show that the reaction mechanism is dependent on the relative stability of the ionic pair formed between two catalytic residues Ser105 and His224 in relation to the neutral form. We elucidated that the reaction catalyzed by the WT enzyme occurs via a concerted mechanism. Interestingly, we found that the reaction occurs via a sequential two step mechanism to form the tertiary intermediate in the CalB variant. This is the first study on the reaction mechanisms of ester hydrolysis reaction catalyzed by the CalB. The reaction mechanism is altered by mutating the catalytic site residue. This research would provide novel strategy on engineering the enzyme to improve its catalytic efficiency.
Poster 16. The reaction mechanism of isopentenyl phosphate kinase: a QM/MM study

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Mevalonate kinase (MK) and mevalonate diphosphate decarboxylase (MDD) are members of the GHMP kinase superfamily which catalyze crucial steps of the mevalonate pathway via Mg2+-ATP dependent phosphorylation reactions to ultimately produce isopentenyl diphosphate, an important precursor in the synthesis of isopentols used in the production of biofuels. It has been postulated that both MK and MDD utilize a catalytic base for proton abstraction of the substrate prior to a phosphorylation reaction[1-2], however ambiguity surrounds the exact mechanism of these enzymes since the position of metal ion in the crystal structures of these kinases in complex with their nucleotides and respective substrates have not been unambiguously resolved. Here we report their catalytically competent structures in complex with the metal ion Mg2+, substrate and nucleotide on the basis of a data study on the metal coordination sphere in similar enzymes, molecular dynamic simulations and QM/MM calculations with the aim to shed light on the reaction mechanisms of these GHMP enzymes. Further, our results have revealed important conformational changes in these enzymes for substrate binding mediated by a molecular switch mechanism. The results provided here lay the basis for further engineering of these kinases, valuable in the production of biofuels.

Diseases caused by protozoan parasites, ranging from malaria (*Plasmodium sp.*) and toxoplasmosis (*Toxoplasma gondii*) to visceral leishmaniasis (Kala-azar, caused by *L. donovani*), have a huge impact on human health worldwide. Plasmodium and Leishmania alone are responsible for 230 million cases and 700,000 deaths per year [1]; half of the world’s population is at risk from malaria and two thirds are affected by toxoplasmosis, which is correlated with the occurrence of schizophrenia [2]. Finally, the opportunistic anaerobic pathogen *Bacteroides vulgatus* is one of the causes of brain abscesses and is a reservoir for antibiotic resistance [3].

What links these organisms is the presence of membrane integral pyrophosphatase (mPPase) protein, which uses pyrophosphate (PPi) hydrolysis to generate an ion gradient. mPPases are essential under conditions of low-energy stress and are required for virulence in both *Leishmania major* [4] and *Toxoplasma gondii* [5]. Recently, a structure of inhibitor-bound bacterial mPPase (from *Thermotoga maritima*; TmPPase) was solved in complex with PPi mimicking inhibitor imidodiphosphate (IDP) [6], and we utilized the structure for computational drug design, which led to several hit compounds.

Here we present atomistic molecular dynamics simulations with TmPPase both in the apo form and in complex with inhibiting ligands. The results will not only give us understanding of the binding of the inhibitors—to be utilized for further compound design—but also the dynamics of the whole system.

References

Poster 18. AMP-membrane interaction characterized with atomistic MD simulation: a study on Cecropin B

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Antimicrobial peptides (AMPs) are part of the innate defence system [1]. They demonstrate antimicrobial, anticancer, anti-HIV, etc. activities, and thus pose as prominent future drug candidates. Understanding the mechanism behind the activity is certainly vital for designing AMPs into drugs of the right efficacy. There are many proposed modes of action that are mostly addressing the pathogen membrane disruption by AMPs. However, the underlying mechanisms at atomistic level are still incomplete to support hypotheses and clarify experimental observations. In this work, an all-atomistic study on the cecropin B (CecB) using molecular dynamics simulation is presented. Through analysing the free energy profiles of this peptide permeating the membrane, its behavioural changes due to different initial configurations, and the comparisons to its derivatives, an addition to the commonly accepted mechanisms is suggested.

References

Poster 19. Intramolecular parameters for protein molecular mechanics force fields

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Density functional theory (DFT) allows us to carry out electronic structure calculations for large biological systems, but cannot tell us about biological processes which take place on timescales longer than picoseconds due to the computational expense. To retain some of the accuracy of DFT calculations, whilst being able to access longer timescales, we use the electron density from DFT calculation to parameterise the intermolecular terms of a biomolecular force field [1]. Intramolecular terms also need to be parameterised for use with the new intermolecular terms, and we have developed a parametrization approach which derives accurate harmonic bond and angle parameters directly from the quantum mechanical Hessian matrix. This involves a modification to the Seminario method [2] and reduces the average error in the reproduction of quantum mechanical normal mode frequencies of a benchmark set of 70 molecules from 117 cm⁻¹ using the original method, to under 50 cm⁻¹. The modified Seminario method is fully automated and all parameters are computed directly from quantum mechanical data, thereby avoiding interdependency between bond and angle parameters and other components of the force field. Interdependencies therefore only occur in the torsional parameters, which are also being parameterised for use in our force field.

References


Poster 20. Towards cholesterol condensation using ELBA coarse graining

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It is known that, upon addition of cholesterol to a phospholipid bilayer, the area per lipid decreases more than one would expect from ideal mixing; an effect known as 'cholesterol condensation'. The classic demonstration of cholesterol condensation is to plot a simple graph of “area per bilayer molecule” against “mole fraction of cholesterol” and find condensation if the resulting plot deviates below the ideal mixing line. However, difficulty lies with the definition of the ideal mixing line - what should be used as the area per lipid of a hypothetical pure cholesterol bilayer?

A more quantitative definition of cholesterol condensation uses partial molar area calculations¹ which allows both calculation of the extent of the effect, but also quantitative comparison between different forcefields.

Dual resolution simulations using the ELBA (Electrostatics BAse coarse graining) forcefield² for both water and phospholipid species, together with atomistic (CHARMM36) cholesterol molecules have been conducted. Analysis of these using the partial molar area technique produces quantitatively comparable condensation results to those obtained from analysing wholly atomistic trajectories. These contrast with the results obtained through analysis of simulations using the coarse grained MARTINI forcefield, which although demonstrating deviation from an ideal mixing curve, do not demonstrate condensation as calculated by partial molar area technique.

Two new "ELBA-type” coarse grained parameterisations of cholesterol are also described. The first following the standard ELBA ~3:1 mapping scheme with a point dipole embedded in the cholesterol head bead. The second parameterisation is similar, but with the inclusion of 2 additional beads to represent the methyl groups projecting from the rigid ring system. This reduction in abstraction allows closer reproduction of the contrast between the 'rough' and 'smooth' faces of cholesterol in a coarse grained model. The effects of these 2 different parameterisations in wholly coarse grained simulations on cholesterol condensation is also discussed.

References

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The i-motif, first reported in 1993, is the only known DNA structure, which consists of parallel stranded duplexes held together through intercalated cytosine base pairs\(^1\). This quadruplex dna structure attracts a lot of attention not only for its biological role but also for its use in nano-biotechnology\(^2\). In this work, computational simulation methods are employed to study the dynamics, confimation and loop stability of i-motif quadruplexes. The reliability of molecular simulations of DNA complexes is directly related to the careful choice of the most appropriate force field. To identify the best choice, we have compared three recent DNA-specific force fields and compared with available experimental data. Of particular interest are the loop regions between adjacent cytosine stretches, as these have an impact on conformations and stability\(^3\). In order to investigate verify the conformational stability of the loop regions, simulations of fully protonated and fully deprotonated systems are performed and analysed. The loop length and composition was also considered. The unfolding/folding of i-motif DNA depends on the number of protonated base pairs and composition of the loop. The results presented within this study are a first step towards understanding the functional effects of loop sequences and length on the thermal stability of folding/unfolding kinetics and binding small molecule ligands.

References

Poster 22. Investigating the flexibility of myosin 7a using experimental and computational approaches.

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Myosin 7a is a molecular motor found in hair cells of the inner ear and the photoreceptor cells of the eye. Myosin 7a is able to regulate its activity through a head-tail interaction (1). However, this mechanism is not completely understood. We have investigated the flexibility of the various subdomains of the lever region within myosin 7a. Fluctuating Finite Element Analysis (FFEA), a novel coarse-grained simulation approach that treats proteins as viscoelastic continua subject to thermal noise (2) was used to investigate the flexibility of myosin 7a. FFEA allows sufficiently long time simulations to allow myosin 7a to explore its full range of configurations and was used to probe the necessary flexibility of the lever in order to form the regulated structure. Preliminary results suggest that, as a static structure, the lever would need to be extremely flexible in order to form the regulated structure. Experimental techniques were used in conjunction with the modelling, including electron microscopy and pull down assays. These techniques were utilised to aid in parameterising the coarse-grained model. Electron microscopy of full-length myosin 7a in both its regulated and extended structures reveal the lever is much shorter than expected. Three-dimensional reconstruction of the regulated structure revealed that only 3IQ motifs could reliably fit within the density. We found myosin 7a only binds calmodulin in vivo, indicating the shorter than expected lever is not a product of missing the correct accessory proteins.

References

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Although allostery was first discovered over 50 years ago, the molecular determinants underlying signal transduction are not yet completely understood. The ability to predict the activity of allosteric small molecules could have a huge therapeutic impact, as targeting allosteric sites in proteins potentially presents significant benefits over active site inhibitors, in both selectivity and efficacy. While some systems undergo fairly well understood structural changes, there is no overall model that satisfactorily describes how allostery works. Molecular dynamics (MD) simulations provide a tool to study protein dynamics at the atomistic level, however traditionally employed analysis methods have been proved inadequate to deliver a mechanistic description of allostery.

In this work, we present our approach to tailor MD simulation analysis methods to identify motions which may be significant to signal transmission in the case study of PDK1 (Phosphoinositide-dependent kinase-1). Long MD trajectories were run for PDK1 in complex with covalent activator and inhibitor small molecules, using the software Sire/Somd\(^1\). A geometrical analysis using the Kullback-Leibler divergence allowed comparison of probability distributions of various descriptors. Subsequently, an energetic comparison was performed using a per-residue decomposition of the interaction energy between the protein and the substrate. Mutual information was then used to determine whether particular structural changes correlate with changes in energetics, to identify motions which are important for the allosteric signal.

References

Poster 24. Towards a molecular understanding of phosphomimetic substitutions in Huntingtin 1-19

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Huntington’s disease is a progressive neurodegenerative disease and a member of the polyglutamine diseases, characterised by CAG repeats in the genome, which are translated into a poly-glutamine tract, leading to protein aggregation and subsequent neuron death1. Huntington’s polyglutamine tract is preceded by a 17-residue fragment (HTT 1-17), which is intrinsically disordered in aqueous environments, but forms an amphipathic helix in the presence of TFE or DPC micelles in vitro.2 HTT 1-17 is subject to phosphorylation, acetylation and other post-translational modifications in the cell, which modulate its structure and, ultimately, the subcellular localisation of Huntingtin. Phosphorylation at Thr3 plays a key role in stabilising the helical structure of HTT 1-193. In this project, we used Molecular Dynamics simulations and SWISH4, a recently developed enhanced sampling method, to study the similarities and differences between the phosphorylated peptide and its phosphomimetic counterparts, as well as effects of acetylation and multiple phosphorylations. Our results show interesting patterns of helix stabilisation and are in agreement with recently published experimental findings3.

References

Poster 25. MacA gating ring or how an efflux pump prevents the backflow of drugs

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Bacterial efflux pumps play a major role in driving antibiotic resistance. Recently, a cryo-EM map revealed the stoichiometry (3:6:2) and the quaternary structure of the E. coli efflux pump MacAB-TolC. It spans through both membranes of the cell envelope and extrudes several substrates, including macrolide antibiotics (e.g. erythromycin) and larger polypeptides. While MacB energetically drives export, a hexamer of the periplasmic protein MacA bridges between a TolC trimer in the outer membrane and a MacB dimer in the inner membrane, forming an extended conduit for substrate export.

The periplasmic duct displays a conserved constriction site that obstructs the channel such that substrates are seemingly prevented from passing. An inter-protomer hydrogen-bonding network formed by a conserved glutamine residue stabilizes this site. We studied the transport of erythromycin through this constricted zone combining equilibrium molecular dynamics and enhanced sampling simulations, Our results suggest that it forms a gating ring that acts as a one-way valve under non-equilibrium efflux conditions. It recognises the substrate on the internal face and facilitates its translocation in the outward direction without disrupting the gating ring, requiring minimal work. In contrast, flux in the inward direction causes the gating ring to distort more drastically, which requires a higher input of work. We suggest that this gating ring arrangement could be a molecular mechanism to prevent backflow of substrates in bacterial transporter proteins linked to ABC-transporters.

References

Poster 26. The first steps of building a complicated structure of glycocalyx - ab initio calculation of raman optical activities of fragments of hyaluronic acid

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Glycocalyx is a highly charged layer of biomacromolecules that surrounds the cells membranes. Its structure and functionality are dominated by glycosaminoglycans, glycoproteins, and glycolipids. This thick layer of macromolecules takes part in cell-cell recognition, acts as a selective filter between surroundings and the interior of the cell. It also serves as protection against direct action of stress. Therefore, the microscopic structure of glycocalyx is of great importance. To the date, its structural details are mostly unknown because its fragile and dynamic nature complicates its study experimentally. We believe computer simulations could be of enormous help in this case. Glycosaminoglycans, which are large sugar polymers, link all components in this fluid and dynamic layer resulting in a highly entangled network. In this work, we study the structure of one glycosaminoglycan, hyaluronan, in solution. Hyaluronic acid is a negatively charged glycosaminoglycan comprising of repeating dimeric units of glucuronic acid (GlcA) and N-acetylglucosamine (GlcNAc). In the glycocalyx, it takes the form of a long extended polymer with up to tenths of thousands repeating units. Contrary to proteins, experimental techniques to access structural features of this glycosaminoglycans are rather limited. To study his structure in solution, we combine ROA (Raman optical activity), and VCD (Vibrational circular dichroism) experimental techniques, together with computer simulations to interpret their complex spectra and relate them to structural features. Previous attempts to study this molecule without the computational aid provided very limited insights about its structure¹. We studied this glycosaminoglycan using molecular dynamics to generate structures in combination with quantum mechanics calculations to compute their ROA and VCD spectra over several small fragments of hyaluronic acid, e.g. GlcA, GlcNAc, and hyaluronic acid dimer (both GlcA beta-(1→3) GlcNAc and GlcNAc beta-(1→4) GlcA). On these fragments we also probe inclusion of explicit water molecules into quantum chemical calculations of the spectra of these relatively strongly solvated molecules. They prove to be important, especially for charged glucuronic acid moiety. Further, we study whether changes in secondary structure of hyaluronic acid dimer cause any detectable changes in the spectra. With all this information, we can verify the accuracy of used force field by comparing the obtained results with the in-house acquisition of the correspondent ROA and VCD spectra on similar fragments. This approach should allow us to test and refine, if needed, the sugar force fields that we want to use in the construction of larger systems relevant to the glycocalyx layer to study its properties microscopically.

References

Poster 27. Exploring the druggability of hPNMT with the JEDI collective variable

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Human Phenylethanolamine N-Methyl Transferase (hPNMT) is the enzyme that catalyses the terminal step of adrenaline synthesis and is known to be involved in a wide range of diseases. Different hPNMT inhibitors bind different conformations of the flexible noradrenaline binding site with binding affinities that can differ in the order of magnitude\cite{1,2}. The druggability (i.e. the ability to bind a drug-like molecule) of protein cavities often depends on factors such as the volume and the hydrophobicity of those, a fact that has led to the development several methods for druggability estimation.

In the present work, biased molecular dynamics simulations of hPNMT have been performed using the structure that binds a milimolar affinity inhibitor as a starting point. A taboo-search protocol based in the JEDI druggability function\cite{3} and the RMSD of the residues in the binding site has been implemented in order to find different conformations with higher druggability scores. The results have been subsequently tested with docking calculations performed using known inhibitors as ligands.

References

Poster 28. Electronic correlation in biology: advances and application of linear scaling DFT, DFT+U, and DMFT

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Many interesting biological systems --- such as haemoglobin and the oxygen-evolving complex of photosystem II --- contain transition metal ions in a wider protein environment. Such systems present two contrasting challenges for accurate electronic structure calculations. Firstly, the calculations must be capable of routinely handling thousands of atoms, but at the same time, the transition metal atoms require very accurate treatment due to electronic correlation. This work explores how to address these two very different challenges simultaneously.

To meet the first requirement linear-scaling density functional theory (DFT) codes [1] are required (that is, if one is to operate on a quantum-mechanical level), while the transition metals atoms can be handled using higher-level approaches such as Hubbard-augmented DFT (DFT+U) and dynamical mean field theory (DMFT). This work will introduce the ONETEP + TOSCAM interface, which combines the power of ONETEP, a linear-scaling DFT package capable of routinely performing quantum-mechanical calculations on tens of thousands of atoms [2], with TOSCAM (a DMFT toolkit). Calculations of the binding and spectroscopic properties of haem will be presented [3].

However, DMFT remains a computationally demanding approach, and for some tasks (such as geometry optimisation) one must rely on less complicated theories such as DFT+U. However, DFT+U still has its complications: perhaps most notably, how to accurately determine the Hubbard parameter $U$. This parameter can be calculated from first principles via linear-response [4], but such approaches (a) typically overestimate $U$ and (b) are ill-suited to electronic structure codes that operate via total-energy-minimisation. This work will demonstrate how to address both of these concerns, using the example of hexahydrated transition metal complexes.

References
Poster 29. Effects of Lipids on the Regulation and Behaviour of TLR4 Involved in the Immune System

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Toll Like Receptors (TLRs) are proteins expressed on the surface of sentinel and dendritic cells. Their activation constitutes the first response of the innate immune system, triggering signalling cascades and providing immediate and efficient pro-inflammatory and antimicrobial responses [1,2]. Thirteen TLRs (named simply TLR1 to TLR13) have been identified in humans and mice together. TLR4 is activated by the presence of specific bacterial lipids found on the outer membrane of Gram-negative bacteria, but the exact mechanisms of the activation are unclear [3]. TLR4 plays many roles in different diseases, such as in regulating DNA damage responses and provoking of neuroinflammation in Alzheimer’s disease [4], which is why understanding the activation mechanisms of TLR4 is of prime importance to develop efficient drugs targeting TLR4 and help cure those diseases. It has been discovered experimentally that glycolipids play a role in TLR4 activation, though the mechanism remains a mystery. To understand TLR4 conformational changes and dynamics associated with activation, and the role that glycolipids play in these processes, we performed large-scale atomistic molecular dynamics simulations of the active TLR4 dimer complex in a lipid bilayer, with and without glycolipids, making sure that the complex atomistic models matched the systems studied in experiments. The key results and their implications will be discussed in the poster presentation.

Figure 1: Snapshot of the TLR4 dimer complex inserted in a lipid bilayer.

References

Poster 30. Modelling Ligand Binding to Adenosine A2a Receptor

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The adenosine A₂a receptor, a member of the GPCR family, is a promising target that is being investigated for its possible role in the treatment of Parkinson’s disease, ADHD and for suppressing the adenosine-mediated reduction of the immune response in the tumoral environment. As with other GPCRs, the process of ligand binding is thought to be influenced by the interaction with various regions of the protein before entering the binding pocket. In this study the binding free energy landscape of ZM241385 and four derivatives is being evaluated in order to identify the details of these interactions. A previous study¹ on this series found that the salt bridge between Glu169 and His264 is an important determinant in the process and that can significantly influence the residence time through a stacking interaction between the phenyl moiety of some derivatives and the histidine ring. Moreover, we seek to confirm and further analyse the crucial role of the extracellular vestibule in directing the ligand towards the binding site, as suggested by previous research.² Parallel-tempering well-tempered metadynamics is being coupled with a funnel-shaped potential that constrains the sampling in order to focus the exploration in the vicinity of the orthosteric site and therefore achieve faster convergence of the free energy surface. Preliminary data show that the interactions with ECL2 and the extracellular vestibule have an important role in driving the ligand to the binding site, with the most likely binding path passing between ECL2 and ECL3. The Glu168-His264 salt bridge hinders the entrance of the ligand and discourages the dissociation of the molecule, when bound; stabilising the ionic interaction amplifies its effect on the binding process.

Figure 1: ZMA241385 interacting with ECL2. Glu169 and His264 can be seen at the entrance of the binding site

References


Poster 31. Atomistic simulations of immunostimulatory single stranded bacterial DNA

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Among immunostimulators of microbial origin, oligodeoxynucleotides (ODNs) represent the most advanced potential adjuvants1. ODNs are unmethylated single stranded DNA sequences with CpG-motifs, which are able to activate the innate immunity and also responsible for mounting acquired responses by binding to their cognate TLR9 receptors2. Adjuvant effects are optimized by maintaining ODNs and vaccine antigens in close proximity, which can be achieved by loading the immunostimulator and the antigen cargo to an appropriate carrier. We propose inorganic nanoparticles as carriers, such as hydroxy-apatite, which is a bioactive and biocompatible material used in current medical applications. The designed drug delivery system should provide a stable attachment of the ODNs to the carrier HAP surface, while not obstructing the multivalent presentation of the ODN molecules to their cognate receptors.

Incorporation of ODN immunostimulators into drug delivery systems requires knowledge of their conformational properties, however, the solution structure of ODN immunostimulators has not been experimentally assessed so far. We carried out atomistic simulations on ODN immunostimulators using the AMBER force field. The conformations of the ssDNA showed transient local secondary structure elements interspaced with random coil conformations.

It has been observed that dsDNA and ssDNA with strong secondary structure tendencies has stronger binding affinity to HAP surfaces, while for TLR9 binding, it has been suggested that only ssDNA can act as an agonist, while dsDNA containing the CpG motif have greatly reduced affinity. Thus, we compared the conformations with the structure of agonistic ssDNA as observed in TLR9 bound states and analysed the potential for optimal alignment of immunostimulatory DNA strands on HAP NPs.

References


The dynamics of proteins and other large biomolecules that are key in driving biological processes often involve the evolution of various orthogonal degrees of freedom over timescales that cannot be feasibly simulated with conventional all-atom MD, and thus the use of enhanced sampling methods such as metadynamics is required. The following study describes an attempt to model the folding of a Tryptophan cage (Trp-Cage) miniprotein from an extended conformation using only standard and well-tempered metadynamics. This follows from a previous attempt to explore the free energy surface of Trp-Cage during folding using bias-exchange metadynamics. Comparisons are also drawn between free energy profiles for the folding process obtained by performing this enhanced sampling with a fixed charge force field and the polarisable AMEOBA force field. The relative performance of the AMEOBA and fixed charge force fields in comparison with experiment is discussed.

References

Poster 33. Kinetic profile of drug transport across the membrane using Umbrella Sampling enhanced sampling simulations in combination with Markov State Model analysis.

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It is well known that a drug, to be effective, not only has to bind strongly to its target, but also is required to have a good ADME (Absorption, Distribution, Metabolism, Excretion) profile. An important factor for absorption is the lipophilicity of the molecule, as the drug must transport across the cell membrane to reach its target, which is particularly difficult for crossing the blood brain barrier. For this reason, here we demonstrate a new method to predict the kinetic behaviour of small ligands to pass across the phospholipid membrane based on mean first passage times (MFPT).

MFPT is the mean time to first reach a defined target state "B", starting from an initial state "A". Here we focus on the MFPTs for a ligand to enter the lipid barrier, flip across the lipophilic membrane and exit from the other side of the membrane.

In this project, we analysed the behaviour of seven approved drugs, in collaboration with Novartis Institutes for Biomedical Research groups. We will use the established unbiased long MD simulations that provide benchmarking results for the molecular kinetics of membrane permeation, together with converged free energy profiles using Umbrella Sampling (US) biased simulations [1].

We have analysed the US simulation data [1] to calculate kinetic rates for the entry into the membrane, flipping rates, and exit from the membrane, and compare these to values obtained for long unbiased MD simulations. We found an excellent agreement between calculated kinetics from biased simulations, and the rates obtained from unbiased MD simulations, also in agreement with experimental permeation measurements, demonstrating that these calculations provide accurate in silico kinetic rates for these important dynamical processes.

In conclusion, using our method DHAM [2], we can obtain fast estimation of the MFPTs from Markov state model analysis, gaining directly useful kinetic information for small ligand ADME properties.

![Figure 1](image.png)

Figure 1: Comparison between kinetic rates using DHAM/US calculations.

References
Poster 34. Infrared Spectra at the interface between water and protein: A molecular dynamics study

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The interaction between water and biomaterial interfaces is of great interest in biological systems [1-3]. Understanding the interaction provides information about how the water hydrogen bond network has been modified. However, the nature of the hydrogen-bonded network formed in this interface remains in controversy [4]. In hydrated proteins, water molecules in contact with, or close to, the protein surface have properties quite different than in the bulk-water environment. Molecules in the interface from a so-called “hydration layer”; they are not able to crystallise to form ice, and are usually still in a liquid state below the melting point of water, moving in a short glassy amorphous state [5]. The water molecules near the interface have a confined environment that manifests itself in variations of their vibrational properties, and in the resultant infrared spectrum. In this communication, we explain our theoretical approach based on molecular dynamics simulation to characterise the water-protein interaction and obtain this infrared spectrum.

All Molecular Dynamics simulations were performed using GROMACS [6] package with OPLS [7] and Ferguson flexible model for Ubiquitin and water, respectively. Simulations were carried out with a constant temperature (using a velocity rescaling) and constant pressure (Parrinello-Rahman method). The smooth particle-mesh Ewald (SPME) method was used to handle the long-range electrostatic interaction. The integration time step was 0.4 fs because any constraint algorithm for hydrogen vibration was not used. The trajectories were sampled every 2 ps for analysis. Fourier transformation was applied to calculate the velocity auto-correlation function, which allows the power spectra to be obtained. IR spectra for all systems were obtained via the electrical flux approach [8]. Power spectra and IR spectra for the ubiquitin water interface have been compared to ice and liquid water structure simulations using the same water model in order to find similarities.

References
β2-microglobulin (β2-m) is a protein responsible for the Dialysis Related Amyloidosis that affects patients suffering from renal failure, producing fibrillar deposits on osteoarticular tissues. The structural and dynamical features of the β2-m aggregation are still unclear, but following recent studies [1,2,3] we investigated β2-m wild type (WT) and its mutants in the most likely first steps of fibrillation process.

In the first part of the work, β2-m WT and amyloidogenical mutants ΔN6 [2] and D76N [4] were studied in protein-protein interactions (e.g. dimeric form) by full atomistic molecular dynamics (MD) simulations. With the aid of various analysis tools, including Essential Dynamics [6], we monitored the behavior of the dimer interface patches and the internal structural rearrangements. For ΔN6 we found a “zipping” mechanism of closure driven by formation of salt-bridges, further explored by Temperature Replica Exchange MD.

In the second part of the research, the focus is on Proline32 cis-trans isomerization [7], considered one of the amyloidosis triggering factor. We are currently investigating the effect of this process on the monomeric forms of β2-m WT, D76N amyloidogenical mutant and W60G aggregation-resistant mutant. Using the PLUMED plug-in [8], we apply the Metadynamics method [9] to study the proline isomerization free energy landscape of each system, to determine the effects of mutations on the relative stability of the two isomers and on the height of the free-energy barrier that separate them.

References

Poster 36. Investigating enzyme dynamics using simulations – the role of heat capacity in catalysis

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Enzymes are efficient natural catalysts that facilitate chemical reactions in biological processes. They are vital for life and play a large role in combatting or causing many diseases in humans, and are also potentially useful as industrial catalysts. Recent experimental work has attempted to understand the role of dynamics in enzyme catalysis by studying the change in heat capacity of activation [1-2]. This unique view suggests that enzyme dynamics play a large role in their catalytic properties, and could provide an explanation as to why enzymes are such large proteins, why they have such complex and varied structures, and how the optimum temperature of each enzyme is “tuned” by evolution. This has been termed macromolecular rate theory (MMRT), and provides a theoretical justification of the curvature of enzyme rate profiles. By measuring thermodynamic properties of enzyme-catalysed reactions using classical molecular dynamics (MD) simulations it may be possible to identify and investigate the physical origins of enzyme catalysis, and to examine the role of protein dynamics in enzyme function.

References

The role of water in the formation of proteins is not well understood at the atomic level where these interactions occur \textit{in vivo}. While folding necessarily results from the complex interplay between hydrophobic and hydrophilic groups in close proximity, the details how water contributes to this process, especially in the early stages of folding, remains unclear. Amphipathic helices have a defined secondary structure, whereby the peptide folds in such a way that the hydrophobic amino acid side chains are aligned on one side of the helix and the hydrophilic residues on the other. It is unclear as to whether or not these secondary structures are formed in aqueous solution or rather they require contact with a hydrophobic surface to nucleate folding. The present study explores the solvation of amphipathic helices with repeating residues of lysine and leucine (KLL) in amphiphilic solutions using a combination of Molecular Dynamics, Nuclear Magnetic Resonance spectroscopy and Circular Dichroism measurements. By investigating these model peptides in amphiphilic solutions the folding state can be controlled and the details of these interactions revealed.
Poster 38. Estimating Free Energy Changes in Molecular Dynamics with Direct Entropy Calculations

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Evaluating solvation entropies directly and combining with direct energy calculations is one way of calculating free energies of solvation and is used by Inhomogeneous Fluid Solvation Theory (IFST) [1]. The configurational entropy of a fluid is a function of the interatomic correlations and can thus be expressed in terms of correlation functions. The entropies in this method are directly calculated from a truncated series of integrals over these correlation functions. Many studies truncate all terms higher than the solvent-solute correlations. This poster discusses the inclusion an additional solvent-solvent correlation term and assesses the associated free energy when IFST is applied to a fixed Lennard-Jones particle solvated in neon where the strength of the central potential is varied to imitate larger solutes [2]. Average free energy estimates with both levels of IFST are able to reproduce the estimate made using Free energy Perturbation (FEP) to within 0.16 kcal/mol. We find that the signal from the solvent-solvent correlations is very weak but should be stronger in simulations with water solvents. We also show how the theory may be applied to more general chemical and biological systems. A preliminary conclusion is that for monatomic fluids simulated by pairwise classical potentials the correction term is relatively small in magnitude. This study shows it is possible to reproduce the free energy from a path based method like FEP, by only considering the endpoints of the path and can potentially skip the problems associated with path based methods like FEP and thermodynamic integration, when applied to larger more complex systems such as protein ligand absolute binding free energy calculations.

References

The goal of this research is to contribute towards new methodologies to integrate experimental results of biomolecular NMR measurements with molecular dynamics (MD) simulations. The study involves understanding the differential dynamics of binding of cyclosporin A (CsA) and alisporivir (DEB025) to cyclophilin A (CypA) using MD simulation studies. Experimental studies in the literature have shown that the two cyclophilin A ligands CsA and DEB025 have similar structure and dissociation constant ($K_D$ 11 nM and 7 nM respectively). However, the dissociation rate ($k_{off}$) parameter is ca. 10 fold slower for CsA than for DEB025 ($k_{off}$ 27 +/- 3 10^{-4} s^{-1} and 2.4 +/- 0.1 x 10^{-4} s^{-1} respectively). Therefore, to understand the binding and unbinding mechanisms of both the cyclic peptides MD simulation approaches involving differential dynamics of the CsA-CypA and DB025-CypA complexes, Markov State Models (MSMs) and conformational dynamics of free CsA and DEB025 in aqueous solutions will be used. The study aims to understand the major and minor conformational preferences of CsA and DEB025 in solution and characterisation of their rate of exchange. The initial study involved force field parameterization to create a new residue template for 7 non-standard amino acid residues in both the peptides. The atomic partial charges were derived using R.E.D. (RESP and ESP charge Derive) software and backbone torsion parameters were fitted using Paramfit. The derived parameters were able to reproduce free energy plots for standard amino acids similar to the AMBER force field parameters. Finally, AMBER force field libraries were generated for non-standard amino acid residues of both the peptides. The results of MD simulations will be analysed to understand the binding mechanism.

References

Poster 40. Active Site Dynamics and Substrate Permissiveness of Hydroxycinnamoyltransferase

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The active site dynamics of Hydroxycinnamoyltransferase (HCT) are studied using multiple 1-μs long molecular dynamics (MD) simulations. The native function of HCT is to catalyse the transfer of hydroxyl-cinnamoyl-moiety (p-coumaroyl moiety) to shikimate, i.e. synthesizing the p-coumaroylshikimate. Yet, this protein is found to be promiscuous and can transfer the associated moiety to various non-native substrates [1, 2]. Our simulations reveal the difference in binding and in catalysing strategies between the native substrate shikimate and the non-native substrate 3hydroxyaceptophenone. The knowledge obtained here helps one to understand how the promiscuous reaction can take place and hence sheds a new light on the interrelation between the reaction promiscuity and the enzyme evolution [3, 4]. Finally, we present a systematic MD study over 5 different HCT proteins, which reveals an unexpected CoA precondition effect [5].

References

Poster 41. The conformation of proline rich segment of neuronal protein tau studied by the X-ray crystallography, molecular dynamics simulations and biophysical methods

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Introduction: Protein tau, which is implicated in Alzheimer’s disease and other tauopathies, is an intrinsically disordered protein (IDP). In contrast to folded globular proteins, IDPs use for their interactions short segments called linear motifs, instead of tertiary structures. Despite their short length and lack of stable structure, linear motifs may have considerable structural propensities, which often resemble bound-state conformations [1].

Method: The monoclonal antibody Tau5 was crystallized with tau peptide from its proline rich region [2]. The dynamics of the X-ray observed conformation was probed by 1 μs unrestrained MD simulations of tau peptide Ace\textsuperscript{218}PPTREPKKV\textsuperscript{226}NH\textsubscript{2} and its T220A mutant. The simulations were run in NAMD program with CHARMM36m force field that is suitable for modelling of IDPs [3]. The simulations were started from either the X-ray observed conformation or an unrelated conformation produced by simulated annealing protocol. The simulation results were compared with the biophysical measurements.

Results: In the structure of Tau5 Fab fragment with tau peptide we were able to observe a 16 amino acid long tau peptide. Its conformation is stabilized by an intrachain hydrogen bond that creates a ST-turn motif. The mutated peptide, which is not able to form this hydrogen bond, has shown decreased affinity to Tau5 in biophysical measurements (SPR, ELISA, and ITC). During the simulation time, the wild type tau peptide has occupied the bound-like conformation four times longer than the T220A mutated peptide.

Conclusion: The intrachain hydrogen bond is stabilizing a short structural motif in the proline rich region of protein tau and its loss leads to a 2-fold affinity reduction in the affinity to the Tau5 antibody. This supports a suggestion that local and global conformation of IDP tau chain can be efficiently regulated by local structural propensities.

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References

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Poster 42. Pharmacological diversity of nicotinic acetylcholine receptor positive allosteric modulators revealed by mutagenesis and a revised α7 structural model.

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Nicotinic acetylcholine receptors (nAChRs) are a diverse family of pentameric ligand gated ion channels (pLGIC) located within the central and peripheral nervous system. Of particular interest for pharmaceutical drug discovery is the α7 nAChR subtype, which has been implicated in various neurological and psychiatric disorders. Whereas most nAChRs are heteromeric complexes of more than one type of transmembrane (TM) subunit, the α7 nAChR contains five copies of a single subunit subtype.

A large number of positive allosteric modulators (PAMs) have been identified that potentiate nAChR agonist responses and display selectivity for the α7 nAChR. Several of these α7-selective PAMs have been proposed to bind at the TM region of the ion channel. In part, the identification of a binding site for these α7-selective PAMs has relied on an α7 nAChR homology model that is based on the three dimensional structure of the nAChR from the electric organ of the marine ray Torpedo. With a growing number of high-resolution, atomic pLGIC models, it is now thought an error in register of amino acids in the TM domain is present in the Torpedo nAChR structure. Despite this error, the Torpedo nAChR remains to be the only pLGIC structure to have been determined in a lipid membrane environment. We believe this is likely to be vital to preserving the native structure of the TM domain.

Here, a homology model of the α7 nAChR has been constructed, based on a model of the Torpedo nAChR structure, where the supposed error has been adjusted using a hierarchical refinement of the structure into its electron cryo-microscopy density map. A consensus docking protocol has been developed and docking of known PAMs performed using this α7 nAChR model. Clustering of docking solutions, reveals that these compounds are likely to bind at an inter-subunit cavity, as opposed to a previously postulated intra-subunit cavity. The predicted binding modes agree with and tie together experimentally obtained data showing diversity of α7 nAChR PAMs.
Poster 43. Linking proton sensors and channel motions in acid-sensing ion channels

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Acid-sensing ion channels (ASICs) are proton-gated sodium channels found throughout the central and peripheral nervous system and e.g. involved in pain signalling. ASICs open upon acidification of the synaptic cleft and at least three states are involved in the functional cycle, namely the closed (deprotonated) state, the open (protonated) state and the desensitised (protonated, closed) state. Several crystal structures for the two protonated states have been solved, showing a trimeric channel with a large extracellular domain (ECD) and a smaller transmembrane domain (TMD), Figure 1 [e.g. 1]. The structure of the deprotonated state is, however, still unknown. Thus, we lack structural understanding of the coupling mechanism between protonation/deprotonation and channel opening/closure. Two ECD proton-sensing regions have been suggested [2], around 20 Å and 60 Å from the TMD, respectively, and our aim is to understand how these proton sensors couple to channel gating.

We have studied the influence of different protonation states, corresponding to different pH values, on the overall protein dynamics by performing atomistic molecular dynamics simulations. Our initial results, including only the ECD, illustrate that the isolated ECD is structurally stable at the given time scale, and that deprotonation promotes motions in the ECD-TMD linker regions which would cause channel closure in a full-length model with linker distances corresponding to those in crystal structures of the desensitized state. On the contrary, for the protonated state the linker regions remain separated, corresponding to the open state crystal structures. We are currently investigating whether the full channel responds similarly to deprotonation, and thus whether we can explain the coupling mechanism linking deprotonation to channel closure.

Figure 1: The trimeric structure of ASICs [1]. The three chains are shown in different shades of grey. Two clusters of acidic residues, potential proton sensors, are shown in black vdW representation.

References
Poster 44. The role of water in adhesion of fibronectin III 9-10 and its relation to network assembly

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Fibronectin (FN) is a large extracellular matrix glycoprotein that affects many cell processes including differentiation, migration and proliferation. FN’s function requires its quaternary structure to transition from a compact to an extended form, a process that is integrin dependant and leads to FN network formation (Figure 1). Recently much research was carried out on an alternative to this integrin dependant process - a material driven approach, with the significant portion of the work carried out on poly (methyl/ethyl) acrylates by Prof. Manuel Salmeron-Sanchez’s group in Glasgow [1]. FN on poly(ethyl acrylate) assumes the extended form, leading to network assembly, whereas the chemically similar surface poly(methyl acrylate) shows FN aggregation and no network formation. To gain further insight into how such a small difference in the surface chemistry has has such drastic consequences in FN’s structure, we used molecular dynamics to simulate the crucial domains FN III 9-10 on self-assembled monolayers (SAMs) that were functionalised using the previously mentioned polymer’s side chains. Our simulations indicate that a) water hydration plays an important role in the adhesion of the two domains by creating dense solvation layers around the functional groups, and b) the residues that are particularly important in the FN-integrin binding, the RGD motif and the PHSRN synergy region, do not have any significance on this adhesion. These results are consistent with the latest mutational analysis and atomic-force microscopy experiments (unpublished).

Figure 1: On the substrate made up of poly(ethyl acrylate), or PEA, fibronectin extends and subsequently self-associates forming a characteristic network, as shown on the right. An almost identical polymer poly(methyl acrylate), or PMA shows a very different behaviour. The pictures used with permission from Salmerón-Sánchez’s lab, University of Glasgow [1].

References
Poster 45. Allosteric effects in the Cyclophilin A S99T mutant are driven by changes in fast dynamics of active site residues

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Cyclophilin A (CypA) is a member of the Cyclophilin family of peptidyl-prolyl isomerases which catalyzes the isomerization of proline peptide bond. Previous biophysical studies have suggested that CypA wild type (WT) active site interconverts between a ‘major’ catalytically active conformation and a ‘minor’ catalytically impaired conformation on millisecond timescales.[1] In the same study they have used S99T mutation to stabilize the minor conformation of CypA, which caused a substantial drop in the catalytic activity of the protein. Although such example is frequently cited in support of the importance of millisecond dynamics for enzymatic function, the details of how the S99T mutation reduces catalytic activity are still unclear.

In this research, molecular dynamic (MD) and biased MD simulations were carried out to investigate the link between conformation changes and catalysis in wild type (WT) and S99T mutant. In our study, the conformation changes between ‘major’ and ‘minor’ active site conformations on nanosecond timescales is observed. Yet in agreement with previous previous experimental data1, free energy profiles from our simulations showed that S99T catalyzed isomerization reactions have a larger energy barrier than in WT CypA. Further analysis shows that this is a result of weakened hydrogen bonding interactions between S99T and the transition state substrate. Additional simulations of enzyme-substrate complexes revealed that these weakened transition state stabilization is caused by an overall increase in fast(nanosecond) dynamics of active site residues due to poorer side-chains packing in the S99T mutant.

In conclusion, our results disputes literature claims of a link between slow (millisecond) protein dynamics and catalysis,1 and suggests instead that changes in fast (nanosecond) dynamics are sufficient to explain the reduced catalytic power of the S99T Cyclophilin A mutant.

References

Poster 46. Optimisation of a Coarse-Grained Force Field

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Computational tools have become important in revealing the driving forces in bio-molecular processes, such as the dynamics of proteins and their interactions with their surroundings. Molecular dynamics simulations use the basis of Newton’s equations of motion to provide this picture, giving us a visual and mathematical understanding of important processes, e.g. protein-protein interactions and protein aggregation. Classical atomistic models have the advantage of giving us a detailed representation of the system at the expense of the computational effort that requires, but are inadequate for simulations of large systems over long timescales.

Coarse-grained (CG) simulations currently represent one of the most important approximations for the construction and simulation of larger systems. By subsuming groups of atoms into single interaction sites, much faster calculations can be realised. However, a disadvantage of CG models is the loss of accuracy associated with reducing the number of interacting particles. Moreover, coarse-graining typically smooths the energy landscape compared to classical atomistic models, diminishing the energy barriers between different states and reducing trapping in energy minima. This can greatly affect dynamic events, such as the rates of conformational changes.

The SIRAH CG force field \cite{1} is a promising alternative to conventional atomistic protein force fields. Unlike MARTINI, the SIRAH force field does not use elastic networks to overcome the problem of secondary structure stability. The use of a higher resolution backbone representation produces hydrogen bond-like interactions, which fully stabilise the secondary structure. Moreover, SIRAH models long-ranged electrostatic interactions using a dielectric constant of unity, which is an advantage compared with the MARTINI model where a dielectric constant of 15 is used.

We will show that the SIRAH model is unable to fully reproduce conformational changes for different protein systems and the free energies of hydration of amino acid side chains, probably due to issues in the parameterisation process. We have started to address this problem with a full optimisation of the SIRAH water model WT4, using the ForceBalance \cite{2} method, an automated process to derive accurate force field parameters from experimental and theoretical data. Our revised model is able to fully reproduce experimental condensed phase properties of water at 298 K. In addition, we have begun to optimise the amino acid side chains using their equivalent liquid-phase properties as target data. We also propose a mathematical expression for the future implementation of hydration free energies as targets for force field optimisation in ForceBalance.

\textbf{References}
Poster 47. Identification of an Arginine Finger for the Pyrophosphatase dUTPases

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The dUTPase enzymes catalyse the hydrolysis of dUTP to dUMP. In this way dUTPase removes dUTP from the deoxynucleotide pool, avoiding U to be misincorporated in DNA, and produces dUMP that is a precursor of dTTP. Inhibition of dUTPase produces an increment of the dUTP/dTTP ratio in the nucleotide pool resulting in increased uracil content of DNA that activates a hyperactive futile cycle of DNA repair. dUTPase is a trimer of identical subunits containing one active site each. The C-terminal arm of each monomer determines the catalytic efficiency and contribute to selectivity of the enzyme. This segment contains a conserved Arg directly preceding a glycine-rich P-loop-like motif. Numerous studies demonstrated that the presence of this conserved arginine together with the P-loop-like motif are critical for optimal catalytic efficiency. In the present study, we focus on the role of the conserved Arg in the mechanism of the trimeric dUTPase from Mycobacterium tuberculosis. As this Arg governs interprotomer catalysis while being located on a distant loop, we propose that it conceivably meets the requirements established for Arg fingers. We address its contribution to the catalytic mechanism of Mycobacterium tuberculosis dUTPase by investigating constructs of with either deletion of the P-loop-like motif or exchange of the conserved arginine. The structural data from crystallography and molecular dynamics simulations together with kinetic and ligand binding analyses reveal the unique role for the Arg in active site organization and providing optimal ligand geometry for catalysis. QM/MM calculations are performed to quantitatively assess the structural and electrostatic contributions of the Arg in catalysis.1

Figure: Arg fingers from various NTP hydrolase/transferase enzymes. (a) Arg residues occupy clusters in specific positions around the γ-phosphate in NTP cleaving enzymes. The nucleoside group is shown for dUTPase. (b) Analogous positions of the Arg fingers around the nucleotide for dUTPase (PDB: 2PY4) (c, d) Analogous positions of the Arg fingers from a GTPase (PDB: 2QTV, c) and an ATPase (PDB: 2JIZ)

References
Poster 48. Reaction profiling of a set of acrylamide-based human tissue transglutaminase inhibitors

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The major function of the enzyme human tissue transglutaminase (TG2) is the crosslinking of proteins via a transamidation between the γ-carboxamide of a glutamine and the ε-amino group of a lysine. Overexpression of TG2 can lead to undesirable outcomes and has been linked to conditions such as fibrosis, celiac disease and neurodegenerative diseases. Accordingly, TG2 is a tempting drug target. The most effective TG2 inhibitors to date are small-molecule peptidomimetics featuring electrophilic warheads that irreversibly modify the active site catalytic cysteine (CYS277). In an effort to facilitate the design of such TG2 inhibitors, we undertook a quantum mechanical reaction profiling of the Michael reaction between a set of six acrylamide-based known TG2 inhibitors [1] and the TG2 CYS277 (Figure 1). The inhibitors were docked into the active site and the coordinates were refined by MD simulations prior to modelling the covalent modification of the CYS277 thiolate. The results of QM/MM MD umbrella sampling applied to reaction coordinates driving the Michael reaction are presented for two approximations of the Michael reaction: a concerted reaction (simultaneous thiolate attack onto the acrylamide warhead and pronation from the adjacent HIS335) and a two-stage reaction (consecutive thiolate attack and protonation). The two-stage approximation of the Michael reaction gave the better results for the evaluation of acrylamide-based potential TG2 inhibitors in silico. Good correlations were observed between the experimental TG2 IC50 data and the calculated activation energies over the range 0.0061 - 1.625 µM (approximately 2.5 orders of magnitude). We propose that this approach may be used to evaluate acrylamide-based potential TG2 inhibitors.

References
Peripheral membrane proteins associate with lipids in the plasma membrane in many important cell signalling processes. Type I phosphatidylinositol phosphate kinase A (PIP5K1A) is one such example, which phosphorylates the head group of the lipid phosphatidylinositol 4-phosphate (PI4P) to generate phosphatidylinositol (4,5)-bisphosphate (PIP2)[1].

The dynamic interactions of peripheral proteins with their target membranes is difficult to capture on experimentally. Whilst molecular dynamics (MD) simulations are a valuable tool for the investigation of protein-lipid interactions[2], the PIP5K1A kinase has not been studied computationally. A recent crystal structure[1] at 3.3 Å resolution enables the further investigation of the structure and dynamics of PIP5K1A and its interactions with lipids in the plasma membrane.

Here we use coarse-grained (CG) and atomistic (AT) MD simulations of the PIP5K1A protein kinase to investigate the nature of monomer and dimer binding to the plasma membrane and their effects on lipid clustering. The AT simulations suggest that whilst dimerisation of the kinase results in an increase in the area of the binding surface, the monomers may not bind concurrently. This in turn suggests that an allosteric mechanism for stabilisation of the productive binding conformation is more likely. We have also used simulations to investigate the relationship between conformational dynamics of the activation loop and interactions with lipids in the cell membrane. Furthermore, large-scale CG simulations (1 million particles) reveal how lipid composition and membrane curvature relate to the interactions of an ensemble of 25 kinases with a model membrane.

PIP kinases are implicated in many cancers as a result of their influence on cell processes. Studies in mice and human tissue samples suggest that inhibition of PIP5K1A is a potential target for treatment[3]. Simulations of the interactions of PIP5K1A with realistic models of cell membranes will aid design of drugs targeted at these and other related lipid kinases.

References
