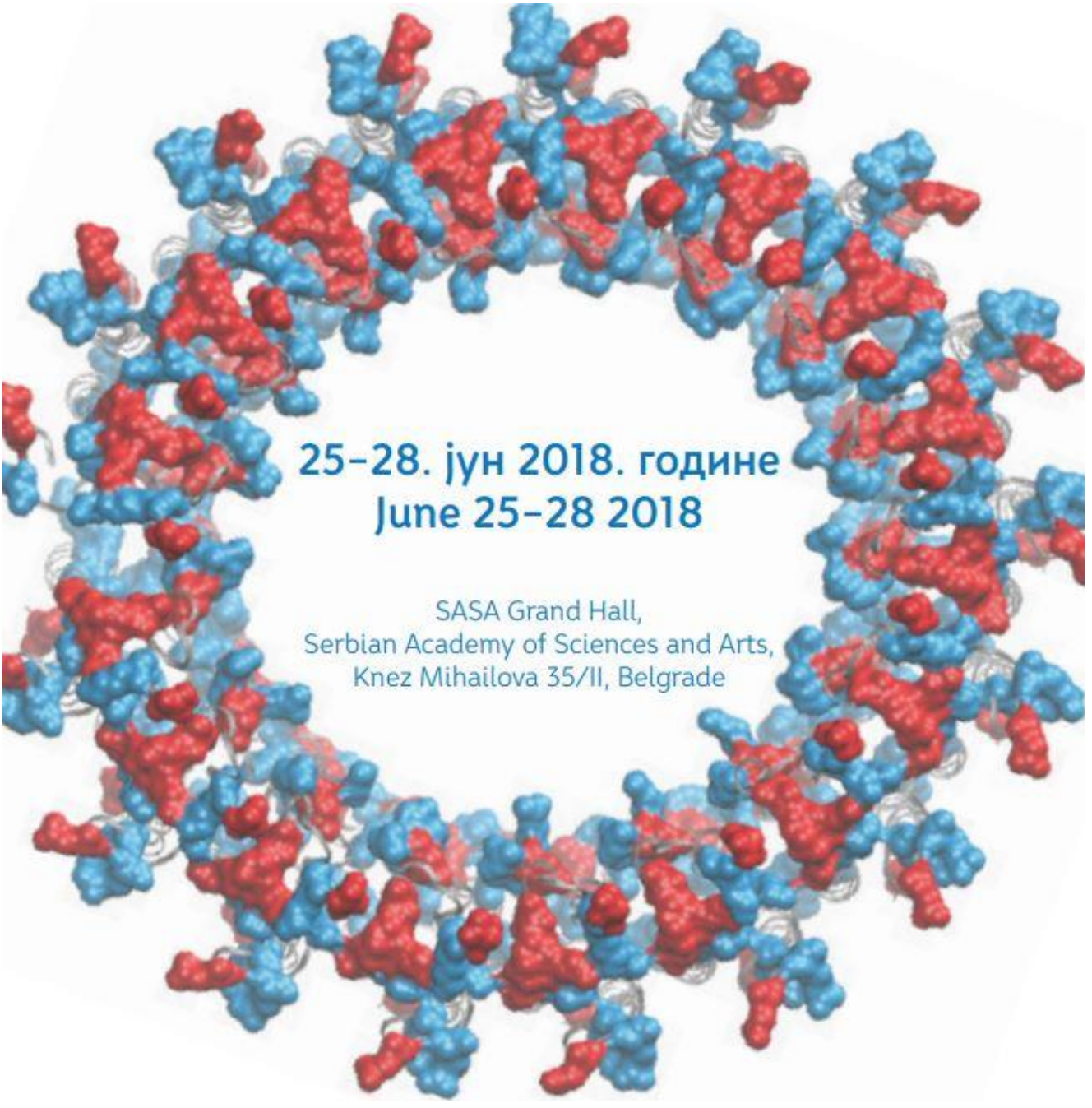
Protein Electrostatics Meeting

BOOK OF ABSTRACTS



Protein Electrostatics Meeting
Abstracts

Organizers:
Serbian Academy of Sciences and Arts
University of Belgrade - Faculty of Chemistry
The Center of Computational Chemistry and Bioinformatics

Publisher:
University of Belgrade - Faculty of Chemistry
Studentski trg 12-16, 11000 Belgrade, Serbia
phone/fax: 381-11-2184-130

For the publisher:
Snežana Zarić

ISBN 978-86-7220-093-5

Printing:
DISK
Vaska Pope 47
26300 Vršac

Vršac
2018

This publication is financially supported by Ministy of Education, Science and Technological Development of the Republic of Serbia

Protein Electrostatics Meeting

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Table of Contents

ORAL PRESENTATIONS 1

Tracing hydrogen bonding pathways for proton transfer 2
Interactions of specific modulation radiofrequencies with cellular microtubules predicted by biological surrogates in humans 3
Energetics in both electron transfer pathways in photosynthetic reaction centers 5
Free energy calculations of ligand binding to RNA's 6
Electrostatic Mechanism of the Nucleosome Function 7
Dielectric constant of hydrated proteins. Kirkwood-Onsager-Froehlich theory revisited 8
Generalized Continuum Theory for Protein Ion Channels 9
Accurate and individual side chain titrations curves measured by NMR spectroscopy 11
pH-dependent conformational change in dengue and other flaviviruses 12
Decomposing the free energy of pH titration 13
The role of water interior in protein translocation by the SecYEG channel 14
A posteriori error estimates for the linear Poisson and the fully nonlinear Poisson-Boltzmann equations: reliable adaptive finite element method 15
Continuum electrostatics models for mining 16
Reaction Path Prediction in Proton Transfer Systems 17
Progresses and first results in the coupled approach between continuum electrostatics and integral equations theory 18
Electrostatic Effects on Optical Spectra of the Photosystem II Reaction Center 20
Constant pH study of a sodium channel 21
Electrostatics of peptide dendrimers 22
Ab-initio/electrostatic/molecular dynamics description of FRET experiments on fluorescent-labeled proteins 23
Electrostatic interaction effects in the kinetics of tri-N-acetylglucosamine binding to lysozyme 25
Rhomboid protease substrate selectivity originates in combined effect of membrane environment and pKa of catalytic residues 26
How well does the AMOEBA polarisable force field reproduce protein electrostatics? 28
Development of a pH replica exchange scheme within the stochastic titration CpHMD method 30
Dynamics equilibria of short-range electrostatic interactions at protein-DNA interfaces: Insight from NMR spectroscopy 31
Electrostatics and Electrodynamics in Lipid Bilayer Membranes 32
Hydrogen-bonded network and water dynamics in the proton transfer channels of Cytochrome c Oxidase 33
Computational Study of Coronaviruses Interactions with Receptors and Antibodies 34
pKa computations in different environments: proteins and liquids 35

ProBiS Tools at the PDB scale for prediction of protein binding site, the ligand, the sequence variant, and their binding dynamics 36
Coupling enhanced sampling and biased MD simulations with CpHMD 37
How Post-Translational Modifications of C-Termini of Microtubules Impact Intra-Cellular Traffic by motor proteins 38
How tubulin's electrostatic properties determine the conductivity of microtubules 39
Long-range electrostatic interactions of E-hooks provide guidance and a soft landing for the microtubule binding domain of dynein 40
Molecular determinants of glutamine synthetase deactivation by tyrosine nitration: Impact of a pKa shift 41
Continuum modeling of selective ion permeation in potassium channel 43
Accurate PDZ: peptide binding specificity with additive and polarizable free energy simulations 44
Free Energy from Implicit Solvent End-Point Simulations 45
Niemann-Pick Type C proteins: Insights from molecular dynamics and QM/MM energy calculations 46

POSTER Abstracts 47

Replica Exchange CpHMD simulations of pHLIP peptide 48
Combining the Polarizable Drude Force Field with a Continuum Electrostatic Poisson-Boltzmann Implicit Solvation Model 50
DelPhi4Py, a python wrapper for the Poisson–Boltzmann solver DelPhi: Application to fast pKa calculations of biomolecules 51
Extending a CpHMD method to estimate the encapsulation-induced pKa shifts in drugs 52
The membrane electrochemical potential in Poisson–Boltzmann/Monte Carlo approaches 53
Molecular modeling study of pH effects on β-lactoglobulin 54
Proton Loading Site in Cytochrome c Oxidase 55
pH effects on PG/PC and PS/PC lipid binary mixtures: a CpHMD study 56
Analysis of sulfur-sulfur interactions in proteins from the Protein Data Bank 57
pH-dependent changes in Aβ42 aggregation behavior 58
On the importance of π-π stacking and hydrogen bonding cooperativity on aqueous uracil aggregation5963 59
Upshift of pKa values of alpha-synuclein during amyloid formation 60

ORAL PRESENTATIONS

Tracing hydrogen bonding pathways for proton transfer

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Grand Canonical Monte Carlo sampling with a Continuum Electrostatics force field for electrostatic interactions has well documented strengths and weaknesses. The MCCE program imposes limitations of a rigid backbone but is able to robustly and (relatively) rapidly find the equilibrium distribution of protonation states. Here we will describe using MCCE to trace out the Boltzmann distribution of hydrogen bond connections that can be used for proton transfers through proteins via waters and polar residues. The behavior of MCCE explicit waters will be compared with that found in Molecular Dynamics simulations. Methods to evaluate the multiplicity of pathways as well as the energy to move the proton by transient protonation/deprotonation of residues and waters in the protein will be described.

Interactions of specific modulation radiofrequencies with cellular microtubules predicted by biological surrogates in humans

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We report on two prospective clinical studies involving 124 individuals divided into patients with advanced cancer and healthy controls. Each individual was exposed to radiofrequency electromagnetic fields (RF EMF) using 27.12 MHz carrier frequency amplitude-modulated to 194 cancer-specific frequencies (ranging from 100 Hz to 20 kHz). The RF EMF generator used a spoon -shaped antenna applicator placed intrabucally and delivered a whole-body SAR of 13.5 mW/kg [1,2].

Using a high precision non-invasive hemodynamic monitor and a proprietary artificial intelligence computing process, we demonstrated that the human body reacts to specific amplitude-modulated frequencies differently between patients with cancer and health controls ($p<0.001$). This autonomic reaction to RF EMF had consistent and reproducible pattern of heart rate variability (HRV) among individuals with same diagnosis (advanced hepatocellular carcinoma vs advanced breast cancer vs healthy controls) ($p<0.001$). Hence, we hypothesize these effects represent a specific biological surrogate with both diagnostic and therapeutic implications. Additionally, those 194 cancer-specific frequencies caused pronounced disruption of the mitotic spindle, influx of calcium and antiproliferative effects in cancer cells in experiments conducted in humans, xenograft animal models and cell culture studies [3-7]. We propose an mechanism connecting the biological surrogate reaction to specific RF EMF

and electrical oscillations in cytoplasmic microtubules (MTs). Cell morphology differences and MT length variability could explain different cellular responses. We further postulate that coherent resonant modulation disrupts MTs in cancer cells but not in corresponding normal cells. The cell-level mechanism of action of these RF EMF additionally involves ion channel activity, ionic conduction along MTs and possibly actin filaments consistently with earlier computational simulations [8-11].

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Energetics in both electron transfer pathways in photosynthetic reaction centers

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We report redox potentials (Em) for one-electron reduction for all chlorophylls in the two electron-transfer branches of, water-oxidizing enzyme photosystem II (PSII), photosystem I (PSI), and purple bacterial photosynthetic reaction centers (PbRC). The Em values were calculated using the crystal structures, solving the linear Poisson-Boltzmann equation, and considering the protonation states of all titratable sites in the entire proteins. In PSI, Em values for the accessory chlorophylls were similar in both electron -transfer branches. In PbRC, the corresponding Em value was 170 mV less negative in the active L-branch (BL) than in the inactive M-branch (BM), favoring BL●– formation. This contrasted with the corresponding chlorophylls, ChlD1 and ChlD2, in PSII, where Em(ChlD1) was 120 mV more negative than Em(ChlD2), implying that to rationalize electron transfer in D1-branch, ChlD1 would need to serve as the primary electron donor. Residues that contributed to Em(ChlD1) < Em(ChlD2) simultaneously played a key role in (i) releasing protons from the substrate water molecules and (ii) contributing to the larger cationic population on the chlorophyll closest to the Mn4CaO5 cluster (PD1), favoring electron transfer from water molecules. These features seem to be the nature of PSII, which needs to possess the proton-exit pathway to use a protonated electron source—water molecules.

Free energy calculations of ligand binding to RNA's

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We will discuss recent metadynamics simulation studies of proteins, peptide and small molecules binding to a variety of RNA's . These studies, which turn out to be consistent with available experimental data, have allowed to design new ligands with possible beneficial effects against diseases such as cancer and neurodegeneration. These examples suggest that, in spite of the current limitations of force fields for RNA, molecular simulation may greatly help design ligands targeting these fascinating molecules.

Electrostatic Mechanism of the Nucleosome Function

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The nucleosome -- a complex of 147 base-pairs of DNA with eight histone proteins -- is the fundamental unit of chromatin compaction in the living cell, which controls access to its genetic information. The exact mechanism of the control remain unclear. We analyze the DNA wrapping/unwrapping transition in the nucleosome by a simplified physical model that reveals possible general principle of the accessibility control -- alteration of the nucleosome core charge via post-translational modifications. An all-atom model confirms the basic idea, but reveals many counter-intuitive trends, and makes further predictions. Connection to transcription regulation in-vivo is made, and a model of transcription modulation via charge-altering PTMs in the histone core is proposed. Stability of the nucleosome complex is predicted to depend strongly on pH of the environment in the physiologically relevant range.

Dielectric constant of hydrated proteins. Kirkwood-Onsager-Froehlich theory revisited

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We have recently developed a simple protein hydration program, called Dowser++, which is based on a popular program Dowser, but has significant modifications in both energy evaluations and the search algorithm. The new program predicts a significant increase of hydration of proteins, which is usually not seen in experimental crystal structures, unless taken at sub-angstrom resolution and low temperatures. Using Dowser++, we studied hydration of respiratory complex I and complex IV. The internal water present in proteins raises the question about its contribution to dielectric constant of the hydrated protein structures. This question led us to revise the theory of dielectric constant evaluation from molecular dynamics simulations, usually done by studying fluctuations of the dipole moment of a sample. We show that the previous calculations on popular water models TIPnP (n=3,4,5) are in error, and propose a new scheme. The new results suggest that the actual value of the dielectric constant of TIPnP water models is significantly lower than it was thought before; it is only the inclusion of the electronic polarization correction, missing in the previous theories, that provides reasonable values of the static dielectric constant of TIPnP models.

Generalized Continuum Theory for Protein Ion Channels

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Ion channels in cell membranes play important roles in cell physiology. Such channels are formed by integral membrane proteins. Ion channels conduct ions driven by electric potential or a difference in ionic concentrations across the membrane. Ion current through the channel can often be described by the continuum theory of drift-diffusion (known as a Nernst-Plank (NP) equation) supplemented by a description of the electrostatic field in the system via the Poisson Equation (PE) [1,2]. In the absence of a current the Poisson-Nernst-Plank theory results in the Poisson-Boltzmann equation. To account for ion interaction with the protein forming the channel we have recently introduced a soft repulsion (SR) model [3]. The model has been applied to compute current-voltage characteristics of an alpha-hemolysin channel. We have also investigated model dependency on the choice of the diffusion constant distribution. The PNP -SR algorithm is implemented in a new efficient parallel Poisson, Poisson-Boltzmann, and PNP equation solver, also incorporated in a graphical molecular modeling package HARLEM. We have also developed a graphical processing unit (GPU) PE solver [4]. The dependency of current-voltage characteristics of the α-hemolysin channel on the channel position within the membrane was also studied [5]. The presence of the membrane environment also influences protonation state of the residues at the boundary of the water-lipid interface. We predict that Asp and Lys residues at the protein rim change their protonation state upon penetration to the lipid environment. Free energies of protein insertion in the membrane for different penetration depths was estimated using the Poisson-Boltzmann/solvent accessible surface area (PB/SASA) model. The results show that rectification and reversal potentials are very sensitive to relative position of channel in the membrane, which, in turn, contributes to alternative protonation states of lipid penetrating ionizable groups. The prediction of channel position based on the matching of calculated rectification with experimentally determined rectification is in good agreement with recent neutron reflection experiments. Based on the results we conclude that α-hemolysin membrane position is determined by a combination of several factors and

not only by the pattern of the surface hydrophobicity as is typically assumed.

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Accurate and individual side chain titrations curves measured by NMR spectroscopy

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Nuclear Magnetic Resonance (NMR) spectroscopy has the unrivalled potential to quantify electrostatic interactions in proteins experimentally. However, NMR chemical shifts are affected by several sources that need to be disentangled: (i) the protonation state of a residue itself, (ii) charges developing in its direct environment, and (iii) conformational changes. We have made significant progress over the years in developing new NMR methodology that allows unambiguous access to the first term for all amino acids in proteins, using 13C and 15N chemical shifts as strategic reporters [1]. This improved methodology has allowed accurate insights into the microscopic charge states [2,3] and nature of electrostatic coupling in small proteins [4,5]. In addition, we developed an effective approach for modelling electrostatics in unfolded and intrinsically disordered proteins [6], to help in predicting pH-dependent protein stability..

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pH-dependent conformational change in dengue and other flaviviruses

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Dengue virus is a mosquito-borne, single-stranded RNA virus infecting over 390 million people per year with symptoms ranging from mild fever to severe haemorrhagic fever and shock syndrome [1]. The viral envelope consists of a lipid bilayer with 90 embedded E -protein dimers covering the surface in a tightly packed herringbone pattern. Mature virus enters the cell by endocytosis and remains contained in an endosome while the vesicle is gradually acidified. The acidic environment causes a great conformational change in the viral envelope: E-protein dimers dissociate and subsequently form trimeric spikes on the viral surface, which enables the virus to fuse with the endosomal membrane and to enter the cellular cytoplasm. We used molecular dynamics simulations and Finite difference Poisson-Boltzmann/Debye-Hückel method (FD/DH) to calculate pKa values in both smooth and spiky conformations in order to detect potential pH-sensing and pH-coupled residues [2]. Previous research mostly focused on conserved histidines as residues that potentially contribute to the conformational change [3]. However, our research has shown that other residues, notably conserved aspartate and arginine, also play an important role as pH-coupled residues. We detected a group of four conserved residues and the N-terminus clustered in the vicinity of a fusion loop in the mature smooth form. The cluster is consistently present in mature and immature forms of other flaviviruses, but comes apart during the formation of trimeric spikes. In the fusogenic spiky form most of the residues contribute to the stabilization of the newly-formed domain I-domain III interface.

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Decomposing the free energy of pH titration

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Because of their central importance for understanding enzymatic mechanisms, pKa values are of great interest in biochemical research. It is common practice to determine pKa values of amino acid residues in proteins from NMR or FTIR titration curves by determining the pH at which the protonation probability is 50%. The pH dependence of the free energy required to protonate this residue is then determined from the linear relationship $\Delta G_{prot} = RT \ln 10 (pH - pKa)$, where R is the gas constant and T the absolute temperature. However, this approach neglects that there can be important electrostatic interactions in the proteins that can shift the protonation energy. Even if the titration curves seem to have a standard sigmoidal shape, the protonation energy of an individual site in a protein may depend nonlinearly on pH. To account for this nonlinear dependence, we show that it is required to introduce pKa values for individual sites in proteins that depend on pH. We demonstate how this pH dependence can be decomposed in entropic and entalipic contributions in the frame work of a grand canonical partition function.

The role of water interior in protein translocation by the SecYEG channel

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Many bacterial proteins, including most secretory proteins, are transported across the plasma membrane through a channel that is formed from a conserved heterotrimeric membrane protein complex, called the SecYEG complex [1]. This channel provides a lateral exit into the bilayer for membrane proteins, while simultaneously offering a pathway into the aqueous interior for secreted proteins. The molecular mechanisms of the choice made by translocon between these two pathways and driving forces of the translocation are not comprehensively understood. Important contribution to both may come from the properties of water inside the channel. Firstly, the water is highly sensitive to the hydrophobicity of the peptide. Furthermore the presence of water molecules near titratable amino acid residues can change their protonation state, affecting the electrostatic interactions and the free energy landscape. It was proposed that pKa shifts of the translocated peptide residues alter the probability of being charged for basic and acidic residues that determines the driving force of the translocation [2]. In our work we analyse the interplay of water distribution, hydrophobicity of the peptide and pKa shifts. Molecular dynamics simulations and electrostatic calculations have been used to monitor: (i) the water diffusion in the channel, depending on hydrophobicity of the translocated peptide (ii) the formation of H-bonds between water and SecY, water and peptide and (iii) the protonation state of titratable residues.

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A posteriori error estimates for the linear Poisson and the fully nonlinear Poisson-Boltzmann equations: reliable adaptive finite element method

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In numerical methods for partial differential equations, a posteriori error estimates are used for efficient global and local error control. In contrast to the finite difference methods, the finite element method allows for local refinement of the underlying mesh and a better approximation of the protein geometry with much less degrees of freedom. Reliable a posteriori error estimates are the basis for constructing well justified adaptive finite element solvers. We show how to derive reliable a posteriori error estimates for the approximate solutions of the linear Poisson equation and the fully nonlinear Poisson-Boltzmann equation which describe the electrostatic potential in a system of biomolecules. We have derived two types of error estimates for the Poisson equation – functional a posteriori estimates for the error in energy norm and goal oriented error estimates. For the first type, we obtain tight lower and upper bounds for the relative error and efficient estimation of the distribution of the local errors which we use for adaptive mesh refinement (AMR). The mesh is adapted until we meet a predefined error tolerance for the relative error. The purpose of the second type of error estimates is, as the name suggests, to steer the adaptive refinement in such a way that we minimize the error measured in terms of some goal functional. For example, if the goal is to obtain an accurate value for the electrostatic interaction energy between two polypeptides, then the goal functional is just a linear combination of pointwise evaluations of the electrostatic potential with weights given by the corresponding point charges.

For the fully nonlinear Poisson-Boltzmann equation, we have derived functional type a posteriori error estimates for the error in energy norm which give guaranteed bounds on the global error and an efficient error indicator, used in our adaptive finite element solver. We also show numerical experiments and comparison with other solvers.

Continuum electrostatics models for mining

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Computational biology, structural bioinformatics, and simulation have developed hierarchies of model complexity to fit system size. The same philosophy can be applied to system multiplicity e.g. measurements across families of proteins or proteomics datasets. Our group has been exploring various types of datasets, and whether their readouts correlate with biophysical calculations. One simple and well-known example is the enrichment for phosphorylation in intrinsically disordered regions of proteins, so that a simple sequence-based calculation is the basis of a powerful prediction tool. We have been looking at the reactivity of cysteine and lysine sidechains with regard to biologically important modifications. In a specific case, cysteine that serves as a target for covalent drug binding in some protein kinases, is located at the analogous helix amino-terminal position to reactive cysteines in the thioredoxin family. More generally, modified cysteines occur in a surprisingly high proportion of sites that would be inaccessible in native protein structure, leading to a hypothesis in which cysteine modification may couple to protein degradation. Related work will also be discussed, including our continuing development of web-accessible tools for the prediction of protein solubility and the influence of ionic strength and pH on protein stability. Overall, we conclude that high-throughput 'omics data, whilst perhaps a challenge for the structurally-minded computation scientist, is a valuable and fruitful link to modern biology.

Reaction Path Prediction in Proton Transfer Systems

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The translocation of protons through solvated protein channels is a high dimensional process, usually involving several consecutive proton transfer events paired with re-arrangements of the underlying water network and conformational changes of residues at the channel boundaries. Defining a reaction coordinate in steered simulations, which accounts for all these events, is problematic and will most likely bias the determined transition pathways to individual mechnaisms or sequences of events. An alternative approach is provided by Transition Networks. A challenging aspect in calculating TNs is their exponential increase with increasing system size. Therefore Transition Network calculations require in most cases a limitation of the sampled degrees of freedom, which renders several re-calculations of the Transition Network for individual configurations of the unsampled degrees of freedom a necessity. A method is presented which uses the Minimum Spanning Tree of the Transition Network for an initial configuration of the unsampled degrees of freedom and its sensitivity to determine a coarse grained Transition Network for an altered configuration of the unsampled degrees of freedom, thereby reducing the re-calculation costs significantly, up to 50 % on average, while important network porperties, e.g.the minimax transition barrier, are maintained.

Progresses and first results in the coupled approach between continuum electrostatics and integral equations theory

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Electrified interfaces are ubiquitous, including metals in contact with electrolytes, disperse systems and biological membranes, and are relevant in a wide range of physical, chemical and biological systems. Their study is pursued by researchers with different scientific backgrounds and using different methodological approaches. Modeling phenomena occurring in the presence of electrified surfaces is a relevant and challenging task. One traditional approach that can be used is based on the Poisson-Boltzmann equation, which, however has some inherent limitations, due mainly to the fact that it considers only the electrostatic contribution to the interactions and that it neglects, in the treatment of the electrolytic solution, finite size and ion-ion correlation effects.

In the search for a viable alternative, we started considering, a few years ago, the possibility of using Integral Equations of liquids and, more specifically, the 3D-RISM (Reference Interaction Site Model) technique. In this approach, solute-solvent interaction is treated at the molecular mechanics level, while solvent -solvent interaction is derived from known bulk correlation, providing a greater detail than the Boltzmann-like description. However, while the 3D -RISM theory is routinely applied to the treatment of biomolecular solvation in free-space boundary conditions, it was not devised to deal with charged solid-liquid interfaces, giving rise to several challenges:

- in electrochemical experiments the controlled variable is the potential, whereas integral equations take the charge density as input;
- polarization of the electrode must be taken into account;
- the natural result of RISM are densities, which must be further integrated to obtain the overall electrostatic potential;
- considering a charged interface which is infinite in two dimensions, one has to deal with a divergent potential, making impossible the use of the Fourier transform, which is normally adopted by the 3D-RISM solvers;

• more generally, long-range interactions induced by the 2D-periodic boundary conditions must be properly renormalized in the real space;
• 3D-RISM often makes use of the hypernetted chain approximation, which leads to the divergence of the differential capacitance, requiring the introduction of a bridge function to permit considering high voltages.

In this work we show how to overcome these problems and illustrate the results obtained applying 3D-RISM and 3D-Ornstein-Zernike equations to the classical example of the bare 111 gold electrode with and without cytochrome proteins bound.

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Electrostatic Effects on Optical Spectra of the Photosystem II Reaction Center

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Conversion of solar energy in natural photosynthesis involves two major steps: (i) Light-harvesting, where specialized pigment-protein-complexes (PPCs) absorb light and transfer the excitation energy (excitons) to distinct sites, referred to as reaction centers (RCs), and (ii) the actual charge separation that takes place in the RCs. An example is photosystem II (PSII), a membrane protein complex responsible for the production of atmospheric oxygen. To understand, how the molecular structures of PPCs determine their function, a method has been developed that combines quantum chemistry (QC) with electrostatics based on the Poisson-Boltzmann (PB) equation (PBQC method) to calculate the influence of the protein environment on the optical transition energies of individual pigments in their binding sites (site energies) [1]. The site energies enter the simulation of optical spectra and exciton transfer [2, 3]. This contribution focuses on recent refinements of the PBQC method and applications to the RC in PSII [4]. It will be demonstrated that the exciton Hamiltonian of this RC can be understood on the basis of protein electrostatics.

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Constant pH study of a sodium channel

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The conductivity of sodium and calcium channels is pH dependent, but the mechanism of pH dependence is not known. The selectivity filter of bacterial sodium channels consists of four glutamate residues, however, it is not clear how the direct interaction between the four residues, and interaction with sodium ions affects the protonation states of this tetrade, and thus implicitly the conductivity. We use constant pH simulations, as well as free energy perturbation to address this question. Previous molecular dynamics studies have found that on average 1.8 ions are present in the selectivity filter when all four residues are charged. We find that the number of ions present strongly affects the protonation state of the tetrade. While we cannot yet obtain the pKa values for the tetrade due to insufficient sampling, we find that the fully charged protonation state, and the singly protonated state are both very likely at physiological pH.

Electrostatics of peptide dendrimers

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Peptide dendrimers are tree-like synthetic molecules, composed of "normal" and branched amino acids, whose periphery can be "decorated" with any convenient functional groups, providing a good multivalent scaffold that can be tailored to fulfill various roles.

Furthermore, they are very interesting for biomedical use because they tend to have high biocompatibility, high biodegradability, high protease resistance and low toxicity, being often used for catalysis, binding, drug delivery, etc. Despite their widespread use, the conformation of peptide dendrimers in solution remains unknown but is likely disordered (x - ray and NMR fail), and their design is usually based on the two-dimensional representation of its structural formula, ignoring potentially relevant three-dimensional features. This talk presents a series of (mostly) molecular simulation studies aimed at clarifying some of the structural determinants of peptide dendrimers, where electrostatics seems to play a major role. A systematic study of several dendrimer variants, using a combination of standard MD and constant-pH MD simulations, reveals a highly disordered structure whose compactness depends strongly on the net charge, in agreement with existing data for negative dendrimers and corroborated by us with subsequent experiments for positive dendrimers. Furthermore, constant-pH MD simulations of a series of poly-His dendrimers reveal elongated titration curves and substrate-induced charging, as observed experimentally.

Ab-initio/electrostatic/molecular dynamics description of FRET experiments on fluorescent-labeled proteins

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Foerster resonance energy transfer (FRET) has been introduced as a "spectroscopic ruler" for the estimation of intermolecular distances by Stryer and Haugland 50 years ago [1] and since then has found wide-spread applications, e.g., in protein folding studies [2]. The standard interpretation of FRET experiments with Foerster theory relies on the following approximations: (i) a point-dipole approximation (PDA) for the coupling between the transition densities of the chromophores, (ii) a screening of this coupling by the inverse optical dielectric constant of the medium, and (iii) a fast isotropic sampling over the mutual orientations of the chromophores. In the literature anyone of these approximations has been proven to become invalid under certain conditions as, e.g., short interchromophore distances. The present study goes beyond all of these approximations and, hence, allows to investigate their interplay. The Poisson-TrEsp method [3] for the ab-initio/electrostatic calculation of excitonic couplings in a dielectric medium is combined with all-atom molecular dynamics (MD) simulations to calculate FRET efficiencies. The method is applied to analyze single-molecule experiments on a polyproline helix of variable length labeled with Alexa dyes [4]. Our method provides a quantitative explanation of the overestimation of FRET efficiencies by the standard Förster theory for short interchromophore distances for this system. A detailed analysis of the di fferent levels of approximation that connect the present Poisson-TrEsp/MD method with Foerster theory reveals error compensation effects, between the PDA and the neglect of correlations in interchromophore distances and orientations on one hand and the neglect of static disorder in orientations and interchromophore distances on the other. Whereas the first two approximations are found to decrease the FRET efficiency, the latter two overcompensate this decrease and are responsible for the overestimation of the FRET efficiency by Foerster theory [5].

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Electrostatic interaction effects in the kinetics of tri-N-acetylglucosamine binding to lysozyme

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To probe electrostatic interaction effects in the kinetics of tri-N-acetylglucosamine binding to lysozyme, we registered, in a stopped-flow spectrofluorimeter, progress curves representing time changes of fluorescence after mixing a solution of lysozyme with solutions of tri-N-acetylglucosamine in glycine buffers containing different amounts of added monovalent salt.

Progress curves obtained from stopped-flow fluorimetry experiments were analyzed numerically in terms of a two-step binding mechanism, composed of four elementary processes: formation of an encounter complex, dissciation of the encounter complex, conformational transition of the encounter complex, and its reverse transformation to the form of the encounter complex. The considered binding model is related to experimental data assuming that at any given moment of time the fluorescence of the solution can be represented as a sum of contributions from distinguishable molecular species in the mixture i.e. free protein and ligand molecules, and possible forms of their complexes. We determined ionic strength dependences of these rate constants for solutions of two different pH values, 3 and 11.

Rhomboid protease substrate selectivity originates in combined effect of membrane environment and pKa of catalytic residues

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Rhomboid proteases constitute a family of intramembrane serine proteases ubiquitous in all forms of life. They differ in many aspects from their soluble counterparts. We applied molecular dynamics (MD) computational approach to address several challenging issues regarding their catalytic mechanism: How does the exosite of GlpG rhomboid protease control the kinetics efficiency of substrate hydrolysis?

What is the mechanism of inhibition by the non-competitive peptidyl aldehyde inhibitors bound to the GlpG rhomboid active site (AS)? What is the underlying mechanism that explains the hypothesis that GlpG rhomboid protease is not adopted for the hydrolysis of short peptides that do not contain a transmembrane domain (TMD)?

Two fundamental features of rhomboid catalysis, the enzyme recognition and discrimination of substrates by TMD interactions in the exosite, and the concerted mechanism of non-covalent pre-catalytic complex to covalent tetrahedral complex (TC) conversion, provide answers to these mechanistic questions [1]. Conclusions are derived from structural analysis of the enzyme-substrate complexes generated by MD simulations.

The analysis is based on our previously formulated analytical expression for the pKa dependence of the enzyme catalytic residues on the degree of their water exposure and on the total charge of the ionizable groups in the enzyme active site.2 Another source of the analysis is our MD-QM/SCRF(VS) computational protocol that allows to rationalize and predict the trend of pKa change caused by the decrease of water exposure of the enzyme active site due to ligand binding [2,3].

The method quantitatively estimates the dynamically changing pKa values of the catalytic residues as a function of their progressively reduced water exposure, caused by the incoming ligand. By this way we demonstrated that in serine proteases the proton transfer from the catalytic Ser to His (general-base catalysis) and nucleophilic attack on the substrate is concerted in rhomboid protease with Ser-His catalytic dyad and stepwise in proteases with classical Ser-His-Asp catalytic triad [4].

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How well does the AMOEBA polarisable force field reproduce protein electrostatics?

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Force field quality is a critical factor in determining the accuracy of protein molecular simulations. With the increase in computational resources there has been a growing interest in moving away from conventional fixed -charge force fields and towards force fields including a more complete description of the electrostatic interactions, typically through the inclusion of multipoles and explicit polarisation. Of these models, the AMOEBA force field is one of the most popular [1]. It incorporates atom-centred multipoles up to quadrupoles, and induced dipoles for polarisation. We have recently explored the accuracy of AMOEBA in reproducing small molecule free energies of hydration [2] and solvation [3].

In this presentation, we will describe the application of AMOEBA to modelling the electric fields in protein systems, first in the case of protein-ligand binding free energy calculations, and second in the context of electric-field moderated enzymatic processes.

Considering protein-ligand binding, it has previously been observed in fixed-charge calculations that the binding free energies of ligands to cytochrome c peroxidase are sensitive to the level of ligand polarisation [4]. We have performed rigorous binding free energy calculations on this dataset using AMOEBA, to determine whether the inclusion of explicit rather than effective polarisation is better able to reproduce the experimental binding free energies.

To measure the electric field in protein systems, the vibrational Stark effect observed in infra-red spectroscopy is commonly used. To determine whether AMOEBA is required to reproduce these fields, or whether conventional fixed-charge force fields are sufficient, we have investigated two systems. It has previously been reported that the enzymatic activity of the peptidylprolyl isomerase cyclophilin A is driven by the alignment of the protein electric field with the dipole moment of the C=O of the X-Pro peptide bond in the transition state. This has been referred to as an electrostatic handle [5], with the nearby Arg55 being considered to be

particularly important for both magnitude and orientation of the environmental field supplied by the enzyme. We have performed molecular dynamics simulations with AMOEBA, and post-processed configurations from those simulations using the ONETEP linear-scaling DFT code [6], performing DFT calculations on about ~20,000 atoms with near-complete basis set accuracy. We find excellent numerical agreement between the whole-protein DFT electric fields and those of AMOEBA, while the AMBER fixed-charge force field performs markedly less well, validating the electrostatic representation of AMOEBA in this context. In the case of ketosteroid isomerase, the vibrational Stark effect suggests very high electric fields in the ligand binding site of the order of 120 MV cm-1 [7]. Here we first calculate solvatochromic shifts for a range of AMOEBA solvents to calibrate the experimental frequency shifts to field strengths. We then show that AMOEBA is able to quantitatively reproduce these fields, and furthermore, the simulations identify alternative, low-field, bound ligand conformations which may not be catalytically productive. These simulations suggest that AMOEBA is able to reproduce the detail of protein electrostatics in catalytic processes.

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Development of a pH replica exchange scheme within the stochastic titration CpHMD method

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pH is a crucial physicochemical property that affects most biomolecules. Changes in protonation equilibrium of susceptible sites will modify the electrostatic environment and, consequently, have an effect on the molecular structure, stability, and catalysis. However, the protonation behavior of pH sensitive biomolecules is difficult to study using experimental techniques and can strongly benefit from using computational approaches. In this context, we have successfully studied several systems using the stochastic constant-pH molecular dynamics (CpHMD) method. In these studies, we were able to obtain titration curves for proteins, membranes, and peptides at the membrane water interface. In the later case, it was observed that, when the titrable groups are deeply inserted in the membrane, the conformational/protonation sampling becomes very limited. In this project, we extended the stochastic CpHMD method to introduce enhanced protonation sampling. We implemented a pH replica exchange scheme and applied it to ethylenediamine, a simple molecule with two strongly coupled macroscopic pKa values, and to hen egg white lysozyme (HEWL), a typical test system for pKa prediction methods. In the future, we will use this method to study challenging pH dependent phenomena in complex biological systems.

We acknowledge financial support from FCT through grant SFRH/BPD/110491/2015 and projects PTDC/QEQCOM/5904/2014, UID/MULTI/04046/2013, and UID/MULTI/00612/2013.

Dynamics equilibria of short-range electrostatic interactions at protein-DNA interfaces: Insight from NMR spectroscopy

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Short-range electrostatic interactions via ion pairs of protein Lys/Arg side chains and DNA phosphates are important for DNA recognition by proteins. Over the past decade, we have developed NMR methods for investigating ion pairs involving basic side chains of proteins. Our NMR studies revealed the dynamic aspects of the intermolecular ion pairs at protein-DNA interfaces. These interfacial ion pairs undergo dynamic transitions between the contact-ion-pair (CIP) state and solvent-separated ion -pair (SIP) state, although the crystal structures show only either one of the states for each ion pair [1,2]. We analyzed the free energy landscape of the CIP-SIP equilibria using computational approaches in collaboration with Prof. Montgomery Pettitt, and assessed the energy barriers for the equilibria using NMR data on temperature-dependence of internal motion of NH3+ group [2]. Using NMR, isothermal titration calorimetry (ITC), and approaches of chemical biology, we also showed that the ion-pair dynamics can make significant entropic contribution to binding free energy for protein-DNA association [3,4]. The highly dynamic nature of the ion pairs became more evident when internal motions of all basic side chains for proteins in the free state and DNA -bound states were compared [5,6]. In my presentation, I will show some of our recent data on the dynamic nature of the macromolecular ion pairs.

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Electrostatics and Electrodynamics in Lipid Bilayer Membranes

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Lipid bilayer membranes are complex, dynamic, and functional structures composed of a wide diversity of lipids, proteins, small molecules, and water organized in heterogeneous domains through noncovalent interactions. The structure and motion of these molecules generate large electric fields within the interior of the membrane that are critical to membrane structure and function. Here, we describe how vibrational spectroscopy of unnatural nitrile chromophores places throughout the membrane structure is used to measure electrostatic fields in peptides intercalated in free-standing lipid bilayer membranes of increasing chemical complexity. In combination with electrodynamics simulations, these experiments highlight how common small molecules such as cholesterol dramatically affect membrane structure and dynamics through large changes to membrane electric fields.

Hydrogen-bonded network and water dynamics in the proton transfer channels of Cytochrome c Oxidase

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Proton transfer in Cytochrome c oxidase (CcO) from the cellular inside to the binuclear redox centre (BNC) as well as proton pumping through the membrane takes place through proton entrance via two distinct pathways, the D- and K-channel. Both channels show a dependence of their hydration level on the protonation states of their key residues, K362 for the K -channel, and E286 or D132 for the D-channel. In the oxidative half of CcO's catalytic cycle the D -channel is the proton conducting path. For this channel an interplay of protonation state of the D-channel residues with the water and hydrogen-bond dynamics has been observed in molecular dynamics simulations of the CcO protein, modelled in different protonation states. Protonation state, hydrogen-bonded network and hydration level are furthermore coupled to the conformational dynamics af the Asparagine gate in the D-channel, leading to an autoregulation with respect to proton transport. Communication of the D-- and the K-channel, as revealed by our network analyses, suggests a further regulation by the protonation state, hydrogen-bond networks, and hydration level in the K-channel which is of particular importance in the reductive phase of CcO's catalytic cycle.

Computational Study of Coronaviruses Interactions with Receptors and Antibodies

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The SARS Corona Virus (SARS-CoV) and the MERS Corona Virus (MERS-CoV) are two members of the Coronavirus family known to cause fatal respiratory illnesses. In this study, we wish to investigate the interaction of the Receptor Binding Domaine (RBD) of the Spike glycoprotein (S protein) coating the viruses, with their cellular receptors as well as neutralizing monoclonal antibodies (nmAbs). In addition, we will compare the WT systems to information obtained for known RBD mutants: the D480A SARS-CoV RBD mutant and the E513A MERS-CoV RBD mutant.

In the present research we shall introduce a stepwise in silico approach that follows the pathway taken by proteins interacting in solution. Molecule Dynamics calculations of the complexes between the RBD of SARS and MERS will be presented. We will follow after the mutual structural adjustments necessary for complex stabilization, and their effects on the dissociation processes.

These preliminary results used special protocols developed in our lab to characterize the interaction between the RBDs of each virus and their counterpart. Consequently, we will show the interaction “fingerprint” of the RBD/receptor complexes to the interaction pattern of the RBD/nmAbs complexes, as well as comparing the WT interactions to the mutated systems.

pKa computations in different environments: proteins and liquids

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pKa values of titratable compounds vary with the environment. The environment can be interior or surface of a protein or a liquid. In a protein the micro -environment of a titratable residue consists of mutual influence of the protonation states of other titratable residues, distance from the protein surface and subtle dependencies on protein conformations. The influence of protein conformations can be taken care using molecular dynamics data. An unspecific background interaction in protein and solvent is considered using an electrostatic continuum. In the protein volume containing the atomic partial charges of the protein atoms dielectric constant (e=4) is small. Outside of the protein a high dielectric constant (e=80) describes the structureless aqueous solution. pKa values in proteins are computed by evaluating the difference of electrostatic solvation energies of the protonated and deprotonated titratable compound in protein and aqueous environment and adding the resulting double energy difference to the known pKa value in aqueous solution. Thus, pKa values in aqueous solution are transformed to the corresponding values in protein environment. With the same scheme pKa values known in one type of liquid can be transformed to corresponding values in another liquids. Here, the liquids are represented by a dielectric continuum with appropriate dielectric constants. However, in this case protic and aprotic solvents need to be discriminated by adjusting atomic radii with a multiplicative factor and the appropriate proton solvation energies are needed. The resulting RMSD values between transformed and measured pKa values in different liquids is around 0.6 pH units. This value is much lower that what so far is obtained for pKa computations in proteins using essentially the same procedure. Hence, there is still space for improving results of pKa computations in proteins.

ProBiS Tools at the PDB scale for prediction of protein binding site, the ligand, the sequence variant, and their binding dynamics

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Proteins interact with other molecules through their protein binding sites, which are functionally important regions on the protein surface. Each binding site usually binds one or a few specific molecules, the ligands. Detection of binding sites gives insight in protein functionality and is hence essential for drug design. Sequence variants that occur in coding regions of genes may alter protein's amino acids and presumably affect protein function. It was found that disease-causing sequence variants are preferentially located at protein-protein interfaces rather than in noninterface regions of protein surfaces. Binding site sequence variants are of great interest to drug development.

We have developed new methodological solutions for prediction and study of protein binding sites on the PDB scale, based on graph theoretical approaches, combined with molecular dynamics simulations. In particular, we have developed computational tools – ProBiS tools - which enable drug discovery based on protein structures (ProBiS, ProBiSCHARMMing, GenProBiS and ProBiS H2O web servers). GenProBiS web server implements a novel approach to the discovery of sequence variants that have potentially deleterious effect on protein function and ligand binding through gain or loss of the binding site. A novel ProBiS H2O approach uses existing experimental structural data to identify conserved water sites on the interfaces of protein complexes, for example protein–small molecule interfaces, and elsewhere on the protein structures.

Our newly developed approaches are particularly useful in the context of precision medicine. Our tools enable joining several otherwise disconnected areas of research, for example genome sequence studies, protein structures, and MD simulations.

Coupling enhanced sampling and biased MD simulations with CpHMD

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The macroscopic properties of many biomolecules are pH dependent. Changes in protonation equilibrium of susceptible groups will modify the electrostatic environment and, consequently, have an effect on the molecular structure, stability and reactivity. The pKa values of common titrable sites in proteins, peptides or other simple organic molecules are usually coupled with their conformation and the observed electrostatic environment. In more complex systems, this coupling requires slow kinetics equilibration, which creates an insurmountable difficulty to constant pH MD (CpHMD) methodologies [1].

In this work, we present our latest attempts to couple CpHMD with replica exchange and umbrella sampling schemes in order to overcome aforementioned sampling limitations. We performed pKa calculations of peptides at the water/membrane interface and known acetylcholinesterase (AChE) inhibitors bound to their receptor. We take advantage of the recent extensions to the CpHMD methodology [1,2] and apply it to these different systems, namely, the model Ala-based pentapeptides [1,3], the pHLIP peptide [4] and to donepezil and galantamine, two commercially available drugs that are inhibitors of AChE [5].

Acknowledgements
We acknowledge financial support from FCT through grant SFRH/BPD/110491/2015 and projects PTDC/QEQCOM/5904/2014, and UID/MULTI/00612/2013.

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How Post-Translational Modifications of C-Termini of Microtubules Impact Intra-Cellular Traffic by motor proteins

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Recent experimental evidences revealed that the hotspots for chemical and functional diversity of microtubules (MTs) are the tubulin C-terminal tails (CTTs). These CTTs of α-and β-tubulin are the sites of main sequence variations among tubulin isotypes. The most important post-translational modifications are tyrosination and detyrosination of α-tubulin CTTs, tubulin poly-glutamination and poly-acetilation.

For example, tyrosinated α -tubulin is dominant in dynamic MTs with pertaining lifetimes of the order of a few minuts, while detyrosinated tubulin is present in long-living MTs with lifetimes of the order of days.

We here offer an original biophysical model which addresses the mechanisms underlying these conspicious changes in MT function caused by the change of a single amino acid tyrosin present in CTTs of MTs. The model is based on the fluctuation dissipation theorem in the context of Langevine dynamics.

It includes system of three ingreedients; motor protein stronger interacting with stepping on tubulin dimer of MT and weaker interacting with pertining CTT. Our model indicates that weak motor-CTT interaction serves to keep motor close to the protofilament enabling it to mentain proper orientation in processive advancing.

How tubulin's electrostatic properties determine the conductivity of microtubules

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We provide an overview of the modeling performed at both atomistic and coarse-grained levels in order to gain insight into electrostatic and electroconductive properties of microtubules. Computer simulations carried out for microtubules will be presented. Electric charge and dipole values for monomers and dimers as well as polymerized forms of these proteins are summarized. Continuum approximations for cable equations of ionic flows along microtubules compare favorably to measurements in buffer solutions showing soliton waves and transistor-like amplification of ionic signals. Conductivity and capacitance of tubulin and microtubules have been measured and modeled. A dramatic change in conductivity occurs when tubulin forms microtubules. In living cells, this signals a conductive phase transition coinciding with mitosis. Finally, we provide estimates of the forces, energies and power involved in the action of Tumor Treating Fields (TTFields) on microtubules.

Long-range electrostatic interactions of E-hooks provide guidance and a soft landing for the microtubule binding domain of dynein

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Macromolecular binding is a complex process that involves sensing and approaching the binding partner, adopting the proper orientation, and performing the physical binding. We computationally investigated the role of E-hooks, which are intrinsically disordered regions (IDRs) at the C-terminus of tubulin, on dynein microtubule binding domain (MTBD) binding to the microtubule as a function of the distance between the MTBD and its binding site on the microtubule. Our results demonstrated that the contacts between E-hooks and the MTBD are dynamical; multiple negatively charted patches of amino acids on the E-hooks grab and release the same positively charged patches on the MTBD as it approaches the microtubule. Even when the distance between the MTBD and the microtubule was greater than the E-hook length, the E-hooks sensed and guided MTBD via long-range electrostatic interactions in our simulations. Moreover, we found that E-hooks exerted electrostatic forces on the MTBD that were distance dependent; the force pulls the MTBD toward the microtubule at long distances but opposes binding at short distances. This mechanism provides a “soft-landing” for the MTBD as it binds to the microtubule. Finally, our analysis of the conformational states of E-hooks in presence and absence of the MTBD indicates that the binding process is a mixture of the induced-fit and lock-and-key macromolecular binding hypotheses. Overall, this novel binding mechanism is termed “guided-soft-binding” and could have broad-reaching impacts on the understanding of how IDRs dock to structured proteins.

Molecular determinants of glutamine synthetase deactivation by tyrosine nitration: Impact of a pKa shift

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Tyrosine nitration is a covalent post-translational protein modification mediated by reactive nitrogen species [1]. Levels of nitrated tyrosine residues have been established as a biomarker for nitroxidative stress. Moreover, tyrosine nitration in proteins has been linked to structural and functional changes and is suggested to play a crucial pathophysiological role in human diseases [2, 3]. Human glutamine synthetase (GS) is highly sensitive to the nitration of Tyr336, causing GS deactivation [4, 5], which has been linked to hyperammonemia and cerebral ammonia intoxication [6]. However, the molecular mechanism how Tyr336 nitration deactivates GS has remained elusive.

Here, we provide evidence that suggests that nitrated Tyr336 in its deprotonated form hampers adenosine triphosphate (ATP) binding to GS. By means of unbiased molecular dynamics simulations as well as binding and configurational free energy computations, we observed that, first, Tyr336 nitration weakens the direct interaction with ATP, and, second, Tyr336 nitration introduces structural and energetic barriers along the ATP binding path. Both results indicate a reduced binding affinity of ATP if Tyr336 is nitrated. Furthermore, we computed a marked decrease in the pKa of nitrated Tyr336 in its protein environment, suggesting that only the negatively charged variant is relevant for GS deactivation under physiological conditions. The suggested pH sensitivity of GS function may be of clinical importance, as a reduced GS activity leads to hyperammonemic conditions, which, in turn, may then completely abolish GS activity.

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Continuum modeling of selective ion permeation in potassium channel

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The Poisson-Nernst-Planck (PNP) model describing electrodiffusion processes can qualitatively capture some macroscopic properties of certain ion channel systems such as current-voltage characteristics, conductance rectification, and inverse membrane potential. But as a continuum mean-field model it has no or underestimates the discrete ion effects, in particular the ion solvation effect, which makes it not applicable to selective permeation simulations. Potassium channels are much more permeable to potassium than sodium ions, although potassium ions are larger and both carry the same positive charge. This puzzle cannot be solved based on the traditional PNP model because it treats all ions as point charges and has no any ion size information, therefore cannot discriminate potassium from sodium ions. It is known that the dehydration effect (closely related to ion size) is crucial to selective permeation in potassium channels. We incorporated Born solvation energy into the PNP model to account for ion hydration/dehydration effects when passing through the inhomogeneous dielectric channel environments. A variational approach was adopted to derive a Born-energy-modified PNP (BPNP) model. The model was applied to study a cylindrical nanopore and a realistic KcsA channel, and three-dimensional finite element simulations were performed. The BPNP model can distinguish different ion species by ion radius and predict selectivity for K+ over Na+ in KcsA channels. Furthermore, ion current rectification in the KcsA channel was observed by both the PNP and BPNP models. The I -V curve of the BPNP model for the KcsA channel indicated an inward rectifier effect for K+ (rectification ratio of ∼3/2) but indicated an outward rectifier effect for Na+ (rectification ratio of ∼1/6). These phenomena can be properly explained by the electrostatic energy landscape of the permeative ion along the channel resulted from the BPNP model.

Accurate PDZ: peptide binding specificity with additive and polarizable free energy simulations

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PDZ domains are domains of 80--100 amino acids that bind short C-terminal sequences of target proteins. Their specificity is essential for cellular signaling pathways. We studied the binding of the Tiam1 PDZ domain to peptides derived from the C-termini of its Syndecan-1 (Sdc1) and Caspr4 targets. 18 complexes were characterized using rigorous, alchemical free energy perturbation simulations, or FEP. FEP is a powerful tool to understand protein:ligand binding. It depends, however, on the the accuracy of molecular dynamics force fields and conformational sampling. Both aspects require continued testing, especially for mutations that alter the net charge. For six mutations that did not modify the net charge, we obtained excellent agreement with experiment using the additive, Amber ff99SB force field, with an rms deviation of 0.37 kcal/mol. For six mutations that modified the net charge, agreement was also good, with one large error (3 kcal/mol) and an rms deviation of 0.9 kcal/mol for the other five. The large error arose from the overstabilization of a protein:peptide salt bridge by the additive force field. Four of the ionic mutations were also simulated with the polarizable Drude force field, which represents the first test of this force field for protein:ligand binding free energy changes. The large error was eliminated and the rms error for the four mutations was reduced from 1.8 to 1.2 kcal/mol. The overall accuracy of FEP indicates it can be used to understand PDZ:peptide binding. Importantly, our results show that for ionic mutations in buried regions, electronic polarization plays a significant role. Ongoing work will allow us to simulate the effect of peptide phophorylation on binding, thanks to our recent development of Drude force field parameters for phospho-tyrosine.

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Free Energy from Implicit Solvent End-Point Simulations

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Here we consider the calculation of free energy, enthalpy and entropy from end-point molecular dynamics simulations. Implicit solvent models are crucial for this task as they encode enthalpic and entropic contributions to free energy due to the solvent in model parameters.

The estimation of free energy using implicit solvent models is typically considering only solute and solvation energy, neglecting or treating in approximate ways solute entropic terms.

Here we provide two programs to estimate the rotational-translational and conformational entropy of the solutes based on the nearest neighbour method, enabling the calculation of free-energy from implicit solvent end-point simulations.

The programs are available through the github repository (https://github.com/federico-fogolari/pdb2entropy and https://github.com/federico-fogolari/pdb2trent).

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Niemann-Pick Type C proteins: Insights from molecular dynamics and QM/MM energy calculations

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The Niemann Pick type C (NPC) proteins, NPC1 and NPC2, have received a great deal of attention in the recent years, not only as they are involved in the lethal hereditary NPC disease, but also as the NPC1 protein has been identified as being necessary for Ebola and Marburg virus infection. Specifically, viral infection occurs when the virus glycoprotein (GP) binds to the NPC1 protein. Cells deficient in the NPC1 protein are protected from infection. On the other hand, mutations in either NPC1 or NPC2 can lead to an accumulation of cholesterol and lipids in the late endosomal(LE)/lysosomal(Lys) compartments, the primary phenotype of the NPC disease. Understanding structural features of the NPC1 and NPC2 binding domains thus is the first step in developing therapeutic treatments for the NPC disease as well as designing inhibitors against Ebola and Marburg viruses. Here, we analyze protein -protein docking as well as cholesterol binding and transfer between the binding pockets of NPC1 and NPC2 using a combination of quantum mechanical/molecular mechanical (QM/MM) energy calculations and molecular dynamics (MD) simulations. Free energies of cholesterol binding and transfer are computed and analyzed.

POSTER Abstracts

Replica Exchange CpHMD simulations of pHLIP peptide

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The "pH (low) insertion peptide"(pHLIP) is an alpha-helical transmembrane peptide with the ability to insert in membranes in a pH-dependent manner [1]. The insertion process can be divided into two components: thermodynamic and kinetic. The thermodynamic component dictates the populations for each state of the peptide and is mainly regulated by the protonation equilibrium of the Asp14 residue at the membrane interface [2]. The kinetic component relates to the speed at which these state transitions occur, which is dependent on the full protonation of the C-terminus domain acidic residues [3]. The stochastic constant-pH molecular dynamics (CpHMD) method [4] was previously used to study wt-pHLIP and it allowed a detailed molecular description of the inserted configuration in this system. However, the method suffers from long simulation times and sampling issues at deep membrane regions where ionized states are hard to sample, thus obtaining less precise estimates of pKa values.

In this work, we present our study of the pHLIP system using a replica-exchange (RE) method, coupled to our CpHMD methodology, in order to solve our sampling limitations. We performed pKa profiles for several key residues of pHLIP using different system parameters for both the CpHMD and RE simulations. By varying the number of replicates and pH values, it allowed us to make a fair comparison between simulations' speeds, sampling quality and the predictive ability of the two methodologies, comparing them with the experimental pKa values.

Acknowledgments:
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Combining the Polarizable Drude Force Field with a Continuum Electrostatic Poisson-Boltzmann Implicit Solvation Model

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We have combined the polarizable force field based on the classical Drude oscillator with a continuum Poisson-Boltzmann/Solvent Accessible Surface Area (PB/SASA) model. In practice, the positions of the Drude particles experiencing the solvent reaction field arising from the fixed charges and induced polarization of the solute must be optimized in a self-consistent manner. We parameterized the model to reproduce experimental solvation free energies of a set of small molecules. The model reproduces well experimental solvation free energies of 70 molecules, yielding a root mean square difference of 0.8 kcal/mol versus 2.5 kcal/mol for the CHARMM36 additive force field. The polarization work associated with the solute transfer from the gas-phase to the polar solvent, a term neglected in the framework of additive force fields, was found to make a large contribution to the total solvation free energy, comparable to the polar solute-solvent solvation contribution. The Drude PB/SASA also reproduces well the electronic polarization from the explicit solvent simulations of a small protein, BPTI. Model validation was based on comparisons with the experimental relative binding free energies of 371 single alanine mutations. With the Drude PB/SASA model and protein dielectric constant of one the root mean square deviation between the predicted and experimental relative binding free energies is 3.35 kcal/mol, lower than 5.11 kcal/mol computed with the CHARMM36 additive force field. Overall, the results indicate that the main limitation of the Drude PB/SASA model is the inability of the SASA term to accurately capture non-polar solvation effects.

DelPhi4Py, a python wrapper for the Poisson–Boltzmann solver DelPhi: Application to fast pKa calculations of biomolecules

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DelPhi4Py is a python module for electrostatic potential calculations of single structures using DelPhi [1], a popular electrostatics simulation program written in Fortran 77. DelPhi is known for its good performance in accurate and robust calculations of electrostatic based properties using a finite difference grid-based method. However, the usage of DelPhi with other programs/scripts requires an additional I/O parsing effort as input and output files have to be written and read. In this work, we present a module that can easily be used by any python script to calculate the electrostatic potential and solvation energy of a system. DelPhi4Py can be used in either single or double precision and supports CPU and GPU parallel computing [2].

DelPhi4Py was used to write a python library for pKa calculations of biomolecules. This object-oriented module follows the same rationale used by Teixeira et al.[3] and it has been applied to anisotropic (membrane) and isotropic (protein) systems. The development of both modules is still in progress and they will be made available to the scientific community in the near future.

Acknowledgments:
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Extending a CpHMD method to estimate the encapsulation-induced pKa shifts in drugs

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Molecular machines have recently been associated with the development of molecular carriers to enhance drug properties, such as solubility or bioavailability. One possible approach is through drug encapsulation by a host molecule, such as cucurbituril (CB) rings, which modifies the environment of the guest molecule. CB rings are able to encapsulate guest molecules providing a hydrophobic cavity and several carbonyl groups that stabilize cationic hosts that interact with this region. This results in significant pKa shifts for drugs with titrable (cationic) groups that can be exploited in order to improve drug bioavailability, whether by enhancing their solubility, stabilizing their active form or by protecting them against external agents. The aforementioned approach can be used for medical targeting, such as cancer therapy, by designing carriers that deliver guest molecules at specific conditions, knowing the specific target properties [1]. Computational tools are a powerful way to help the rational design of CB-guest complexes. In particular, the stochastic titration constant -pH MD (CpHMD) method allows a molecular dynamics simulation to have the pH value as an external parameter and, consequently, obtain full titration curves and pKa values. Our main goal is to develop a strategy to model benzimidazole (BZ) pKa shifts, a «proof-of-concept» molecule, and then extrapolate this process to other host-guest complexes. BZ has a well-known shift of ~3.5 pKa units when encapsulated by a CB ring and, with a CpHMD method, it is possible to elucidate the molecular details of these host-guest interactions.

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The membrane electrochemical potential in Poisson–Boltzmann/Monte Carlo approaches

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The membrane electrochemical potential, central in Mitchell's chemiosmotic theory, is composed of two parts: a chemical gradient (pH gradient) and an electrostatic potential difference across the membrane (membrane potential). Despite its physiological importance, it is often difficult to include the electrochemical potential in experimental setups, which justifies the relevance of developing theoretical approaches that deal with this potential.

A set of methods was developed by Ullmann and co-workers [Calimet, N, Ullmann, GM, J Mol Biol 2004, 339, 571; Bombarda, E, Becker, T, Ullmann, GM, J Am Chem Soc 2006, 128, 12129] to include the treatment of the electrochemical potential in rigid-structure Poisson– Boltzmann/Monte Carlo (PB/MC) calculations. The pH gradient is treated by assigning the residues to a proton reservoir on either side of the membrane, whereas the membrane potential can be included in the PB equation and treated with an additional PB calculation step, namely solving the PB equation for a set of uniformly distributed charges on the solvent around the neutral protein-membrane system.

The effect of the pH gradient can be easily tested by producing 2D titration profiles combining a range of pH values on the two sides of the membrane. However, it is not obvious how to choose relevant combinations of the membrane potential and the pH gradient since, physiologically, they do not seem to relate in a straightforward manner, existing some discrepancy in the literature concerning a possible linear relation [Schuster, S, Ouhabi, R, Rigoulet, M, Mazat, J-P, Bioelectrochem Bioenerg 1998, 45, 181]. These approaches have been tested on the proton pumps cytochrome c oxidase and bacteriorhodopsin and the results will be compared.

Molecular modeling study of pH effects on β-lactoglobulin

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Milk and its derivatives are an important worldwide food source, particularly for infant nutrition, but their use faces a major health complication: some of their proteins are allergens, especially β-lactoglobulin (BLG), a major component of the bovine milk. The fate of BLG upon human ingestion remains unsettled, being unclear how extensive BLG proteolysis is and how it relates to allergenicity. The fact that its proteolytic resistance and antigenic response remain related even in the case of non-oral administration [1] suggests that they are not causally related but rather reflect an underlying common feature. This feature may be the formation of dimers, which can hinder proteolysis and seems to facilitate the binding of protein allergens to IgE antibodies; indeed, BLG is dimeric when complexed with IgE Fab fragments [2] and shows lower antigenicity when in the monomeric form [3]. As shown in experimental studies, this form is predominant at pH below 3 and above 8 and between these there's the formation of a reversible dimer at a moderate ionic strength [4]. The changes in pH are also associated to the Tanford transition, that is, a change in the conformation in a loop near the binding site, allowing or inhibiting the binding of ligands, regulated by the protonation of Glu89 [5].

Previous studies have shown that the dimerization involves electrostatic interactions, for which a better understanding at a molecular-level is essential. In this study, we intended to analyse the effect of the pH in conformational alterations on the monomer and dimer and its dissociation process. For that, Constant pH molecular dynamics (CpHMD) simulations were performed for the monomer and dimer, which allows us to treat pH as an explicit parameter and couples the MM/MD and Poisson-Boltzmann/Monte Carlo (PB/MC) algorithms.

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Proton Loading Site in Cytochrome c Oxidase

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Cytochrome c oxidase (CcO) is an integral part of the respiratory chain found in mitochondria and in many types of bacteria. CcO catalyzes the reduction of molecular oxygen to water and utilizes the resulting free energy to pump protons across the membrane against an electrochemical gradient [1,2]. As a proton pump, CcO must satisfy several conditions in order to maintain it's function. Those are: input channels for protons (D- and K-channel), gating residues to prevent back-leaks [3,4], a place where chemical reaction takes place and a Proton Loading Site.

The Proton Loading Site (PLS) in the center of CcO should briefly store a pumped proton and then release it by changing its pKA value depending on the redox-state of the enzyme cofactors. The identity of the PLS is still not clear. Several candidates are proposed: the propionates of heme a3 [5,6] or histidine His334 coordinated to the Cub [7], an unspecified cluster of residues surrounding hemes [8] or a nearby water cluster [6]. In this work is we provide more insight on the identity and function of PLS by focusing on histidines and propionates of heme a and heme a3, mainly by performing electrostatic energy computations combined with MD simulations for estimating the corresponding pKA values.

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pH effects on PG/PC and PS/PC lipid binary mixtures: a CpHMD study

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Membranes are vital components of biological systems, fulfilling a myriad of roles in the cell [1]. Despite their highly diverse composition, they are primarily comprised of zwitterionic and anionic lipids which, in some cases, makes them sensitive to changes in pH. In computational methods, the inherent complexity of these systems is often simplified via the use of model membranes normally composed of a single lipid type or, in some cases, of binary or ternary mixtures. While these approximations are generally adequate, there are particular instances where the pH, and consequently, the titration of lipid headgroups, plays a key role in membrane stability and function, meaning that the development of more realistic membrane models is extremely important.

Previously, we reported in simulations of a 25% PA/PC (phosphatidic acid/phosphatidylcholine) mixture a pH-dependent phase transition from gel to fluid [2]. In this work, we assembled binary mixtures of either phosphatidylglycerol (PG) or phosphatidylserine (PS) in phosphatidylcholine (PC) with different molar fractions (10%, 25%, 50% or 75% of PG or PS) and studied the effect of pH using the latest implementation of our constant-pH MD method with lipid titration (CpHMD-L) [2].

Acknowledgments:
We acknowledge financial support from FCT through grant SFRH/BPD/110491/2015 and projects PTDC/QEQCOM/5904/2014, UID/MULTI/00612/2013 and UID/MULTI/04046/2013.

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Analysis of sulfur-sulfur interactions in proteins from the Protein Data Bank

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Sulfur-sulfur interactions in proteins have been studied by analyzing the data from the Protein Data Bank (PDB). The S···S interactions were studied between sulfur-containing functional groups of cysteine and methionine. According to the statistical analysis for the obtained data, three types of nonbonded S···S interactions have been characterized: cysteine-cysteine, cysteine-methionine and methionine-methionine. Proteins represent greater variety of geometrical arrangements of S···S interactions than small crystals found previously in the Cambridge Structural Database [1].

Quantum-chemical calculation of electrostatic potential of a model system for cysteine (CH3SCH) and methionine (CH3SCH3), and its spacial alignment with the interacting amino acids in PDB revealed the electrostatic pattern of these interactions. Based on the analysis of the values of potentials on the Van der Waals surface of the two interacting amino acids, we estimated the importance of electrostatics in the sulfur-sulfur interactions in proteins.

These results can be important for recognizing role of electrostatic component in the S···S interaction of sulfur-containing amino acids in the proteins.

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pH-dependent changes in Aβ42 aggregation behavior

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One of the hallmarks of Alzheimer's disease (AD) is the formation of dense plaques chiefly consisting of fibrillar deposits of the peptide amyloid beta (Aβ). The aggregation of the Aβ peptide follows a complex mechanism which is highly sensitive to solution conditions. This work uses thioflavin T fluorescence, solution NMR spectroscopy and cryo-electron microscopy to investigate how this mechanism changes as a function of pH in a phosphate buffered solution. The observed trend is an overall faster rate of aggregation with decreasing pH as could be expected. However below neutral pH there are additional changes in the rates of fibril formation and monomer depletion which are not well described by existing models for amyloid formation. These are accompanied by the transient appearance of non-fibrillar aggregates in the samples. Further investigations into the nature of these phenomena will give us a more complete understanding of this highly complex process.

On the importance of π-π stacking and hydrogen bonding cooperativity on aqueous uracil aggregation

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Nucleobases spontaneously aggregate in water by forming stacked dimers and multimers. The stabilization of these structures comes from base-base and solvent-mediated forces. The former forces consist of exchange-repulsion, dispersion, electrostatic and induction interactions, whereas the latter forces are due to the hydrogen-bonding capability of water and excluded solvent volume. By studying the uracil monomer and dimer in bulk water with the density functional based molecular dynamics, we provide evidence that stacking increases the uracil-water hydrogen bonding strength and alters the hydration structure of uracil [1]. The contribution of this cooperativity between π-π stacking and hydrogen bonding will be compared with dipole-dipole, dispersion and excluded solvent volume forces. Our findings provide insight into the mechanism of heteroaromatic association in water and the importance of electronic structure theory in understanding of the forces which govern this process.

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Upshift of pKa values of alpha-synuclein during amyloid formation

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Background: Alpha-synuclein is a 140 residue long amyloidogenic protein. The formation of alpha-synuclein amyloid fibrils and their accumulation into Lewy bodies is associated with Parkinson´s disease [1]. The last 40 residues of alpha-synuclein - referred to as the C-terminal tail - are of great interest in this project. The tail is highly acidic, consisting of 15 acidic groups and it has been found to be unstructured both in the monomeric and fibrillar form [2,3]. The mechanism of aggregation of alpha-synuclein into amyloid fibrils is highly dependent on pH, which is believed to be linked to the high acidity of the C-terminal tail [4]. Further studies on the effect of the acidic residues in the tail on the amyloid formation are important in order to gain a deeper understanding of the pH dependence of alpha-synuclein aggregation and the role of electrostatic interactions in alpha-synuclein amyloid formation.

Questions addressed: The effect of amyloid formation on the pKa values of the acidic residues in the C-terminal tail of alpha-synuclein was examined.

1. Do the pKa values of the acidic residues become upshifted during amyloid formation?
2. If so, is that a result of the high density of acidic residues in the unstructured C-terminal tail?

Results and discussion: A significant increase in pH was detected during fibril formation of alpha-synuclein. The increase in pH suggests that the affinity of alpha-synuclein for protons increases during fibril formation, thus indicating an upshift in pKa values. Calculations of the average pKa value of the acidic residues of alpha-synuclein indicated a pKa-shift during fibril formation.

The pH increase during fibrillation was also measured for an alpha-synuclein mutant, in which five acidic residues in the C-terminal tail had been substituted for five non-charged polar residues. The Increase in pH during amyloid formation was significantly lower for the mutant. A smaller pKa shift was calculated for the mutant upon fibril formation. This suggests that fewer protons were taken up during the aggregation of the

mutant (whose tail is less acidic) in comparison with the wild-type alpha-synuclein (whose tail is more acidic). These results indicate that the pH increase during amyloid formation, and therefore, the upshift of the pKa values is linked to the high density of acidic residues in the C-terminal tail. The pH increase during amyloid formation has also been detected by NMR spectroscopy and in a weak buffer system using a pH sensitive molecule.

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