PEB 2016

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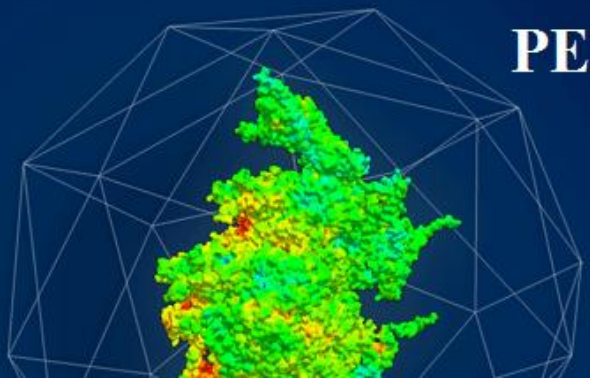

BOOK OF ABSTRACTS

ORAL PRESENTATIONS

Table of contents

Couplings Involving Protonation and Conformation in Cytochrome *c* Oxidase: Insights from Constant-pH MD and PB/MC Simulations

António M. Baptista, A. Sofia Oliveira, Sara R. R. Campos, Pedro R. Magalhães, Cláudio M. Soares

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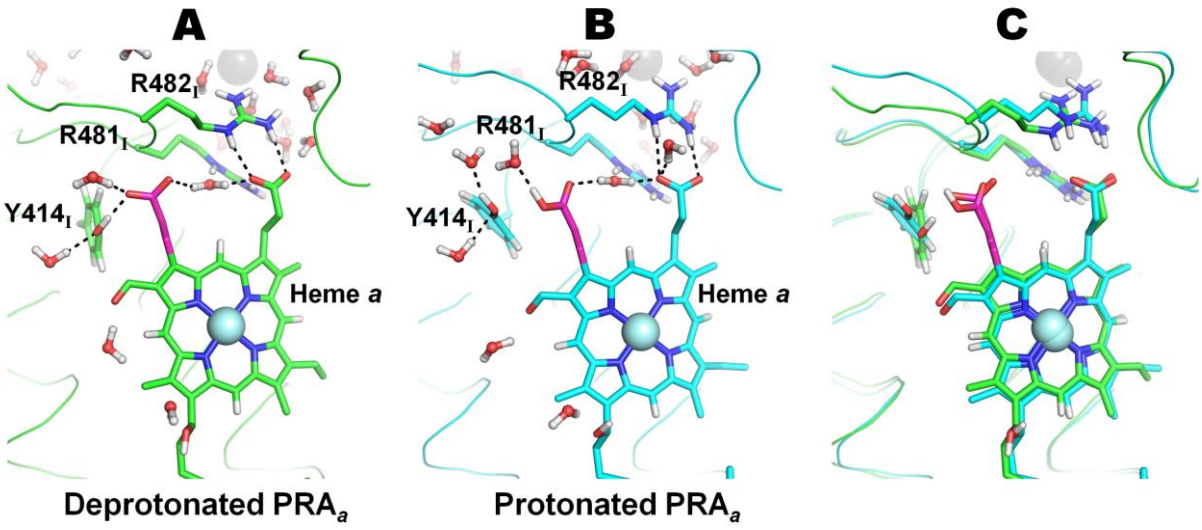
Cytochrome *c* oxidases (CcOs) are the terminal enzymes of the respiratory chain in mitochondria and most bacteria, reducing O_2 to water while generating a transmembrane electrochemical proton gradient. Despite their importance in the aerobic metabolism and the large amount of structural and biochemical data available for the A1-type CcO family, there is still no consensually accepted description of the molecular mechanisms operating in this protein.

We present a constant-pH MD simulation study of the protonation–conformation coupling in CcO of *R. sphaeroides* inserted into a lipid bilayer in two redox states (oxidized and reduced) at physiological pH. This revealed several groups with unusual titration behavior that are highly dependent on the protein redox state, including the A-propionate from heme *a* and the D-propionate from heme a_3, two key groups possibly involved in proton pumping. The protonation state of these two groups is heavily influenced by subtle conformational changes in the protein (notably of $R481_I$ and $R482_I$) and by small changes in the hydrogen bond network.

The effect of a transmembrane pH gradient in this system was also investigated, using a Poisson-Boltzmann/ Monte Carlo method with a single (rigid) structure. We find three residues whose titration behavior depends on the pH on both sides of the membrane, namely $E286_I$, $Y288_I$ and $K362_I$, previously identified as key residues in the proton transfer process, playing critical roles for the catalytic or proton pumping functions of CcO. Our results suggest that when the pH gradient increases, these residues may be part of a regulatory mechanism to stem the proton flow.



Interfacial Waters of Protein-Protein Complexes: an Exploration by MD Simulations and Continuum Electrostatics

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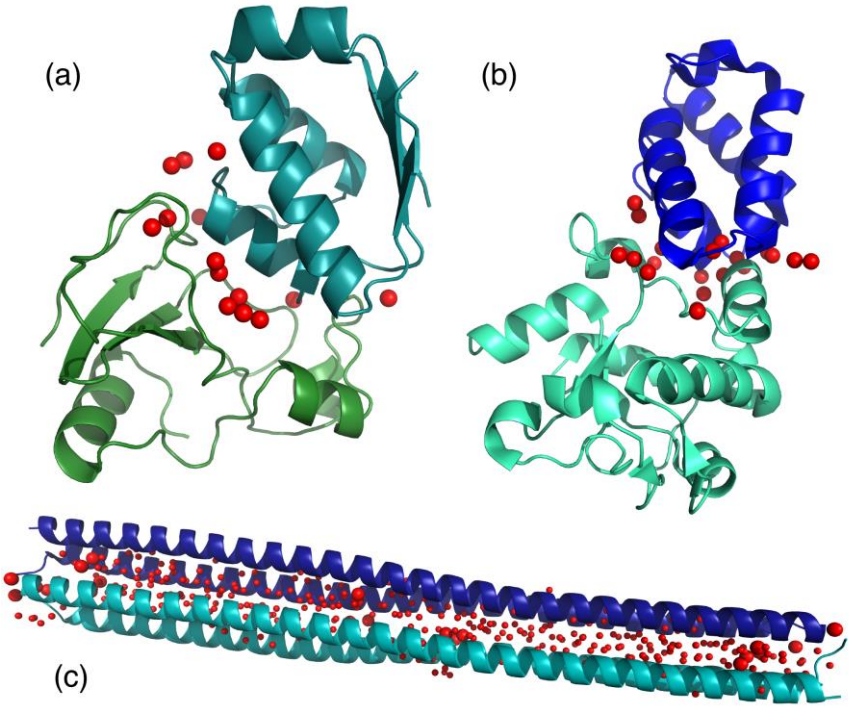
Interfacial waters are increasingly appreciated as playing a key role in protein-protein interactions. We report on a study of the prediction of interfacial water positions by both Molecular Dynamics and explicit solvent-continuum electrostatics based on the Dipolar Poisson-Boltzmann Langevin (DPBL) model, for three test cases: a) the barstar-barnase complex, b) the complex between the DNase domain of colicin E2 and its cognate Im2 immunity protein and c) the highly unusual anti-freeze protein Maxi which contains a large number of waters in its interior. We characterize the waters at the interface and in the core of the Maxi protein by the statistics of correctly predicted positions with respect to crystallographic water positions in the PDB files as well as the dynamic measures of diffusion constants and position lifetimes. Our approach provides a methodology for the evaluation of predicted interfacial water positions through an investigation of water-mediated interchain contacts. While our results show satisfactory behaviour for molecular dynamics simulation, they also highlight the need for improvement of continuum methods.



Constant pH Molecular Dynamics: Probing pH-mediated Processes from Viral Capsids to Membranes

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In this talk I will review our methodology for probing pH-mediated processes with constant pH molecular dynamics (CpHMD). I will discuss applications ranging from protein and peptide function in solution and membranes to nucleic acids and viral capsids. Finally, I will describe our recent advances of moving these methods to GPU accelerated platforms and the promise we believe these approaches hold for routine application of CpHMD in biological applications.

A Continuum Electrostatic Approach Applicable to Biomacromolecules using All-atom or Coarse-grain Representations

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We present a revised version of CVCEL (Circular Variance Continuum Electrostatics) model, an analytical approach for evaluating electrostatic potential and field distributions as well as the electrostatic energy of biomacromolecules in a continuum solvent/salt environment as a function of their conformation and their interactions.

The approach models both the self and cross terms of the reaction field energy by fitting to all-atom Poisson-Boltzmann calculations. In contrast to the Generalized-Born (GB) approach that calculates an effective Born radius by solving the integral of the energy density of the Coulomb field, here the distance of a charge centre from the solute-solvent boundary is modelled in terms of circular variance (CV), a measure of the distribution of the neighbours around the charge of interest whose calculation only requires simple vector operations. CVCEL describes the self energy as a function of CV and the cross term as a function of the self contributions and requires a small set of parameters that depend only on the chemical identity of the atoms constituting the macromolecule (H, O, N, C, S, P). CVCEL notably outperforms the GB approach in predicting the energy differences between conformations involving the close approach of charges (exemplified by the "collapse" of certain nucleic acid conformations).

The CVCEL model is also transferable to a coarse-grain model such as our PaLaCe protein representation (2). As already demonstrated in our earlier approach (1), this model represents a significant step forward from the distance-dependent dielectric or charge-scaled methods that are commonly used with coarse-grain approaches. Coupling with a CV-based non-polar solvation energy term under development will provide a complete implicit solvation model applicable to arbitrary biomolecules and their assemblies.

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Electrostatics for Protein-Membrane and Nanoparticle-Membrane Interactions

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Electrostatic interactions are important to many problems that involve lipid membranes. In this presentation, I'll first discuss analysis of protein-membrane interactions, which play an important role in processes such as cellular localization of proteins. I'll illustrate the approach with an application to RecA-membrane interaction, which highlights the interplay of electrostatics, hydrophobic insertion and lipid packing defects in determining the orientation, insertion depth and binding affinity of proteins at membrane surface. If time permits, Ill also briefly discuss the interaction between functionalized nanoparticle and membrane, for which an understanding of the charge (titration) state of the ligands is important. We have adopted a hybrid Monte Carlo and Molecular Dynamics approach that sample the titration and conformational states, respectively. Application of the approach will be presented.

Constant pH Simulations with the EDS-HREX Method

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We have recently developed a new computational approach for constant pH simulations in explicit solvent based on the combination of the enveloping distribution sampling (EDS) and Hamiltonian replica exchange (HREX) methods (EDS-HREX). The method is based on discrete protonation states. EDS generates a hybrid Hamiltonian of different protonation states. A smoothness parameter is used to control the heights of energy barriers of the hybrid-state energy landscape, for easy transitions between the states. Replica exchange between EDS potentials with different smoothness parameters allows us to readily obtain a thermodynamically accurate ensemble of multiple protonation states with frequent state transitions. I will talk about recent progress in further method development and applications involving the EDS-HREX method.

Implicit Formalisms for Describing Membrane Environments

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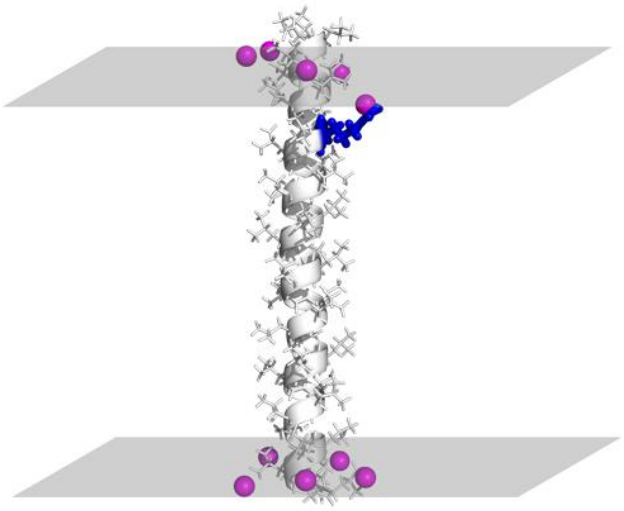
Continuum electrostatics models are well-established for describing the energetics of biological membranes. The Poisson-Boltzmann equation and related Generalized Born approximations provide a starting point for capturing the characteristics of membranes via a varying dielectric profile along the membrane normal. However, the flexibility of membranes and interactions within the membrane are more difficult to capture in this manner. Improved generalized Born treatments based on the heterogeneous dielectric generalized Born (HDGB) model are presented that allow membrane deformations to be considered by coupling the generalized Born model to elasticity theory (DHDGB) and by adding an implicit van der Waals formalism (HDGBvdw) to improve the description of intra-membrane interactions of membrane-embedded molecules. Recent applications of these models to describe the dynamics of membrane-bound biomolecules, estimates of membrane permeation for small molecules, and as a scoring function for membrane protein structure decoys are presented.



Redox Tuning of Quinone in the Respiratory Complex I

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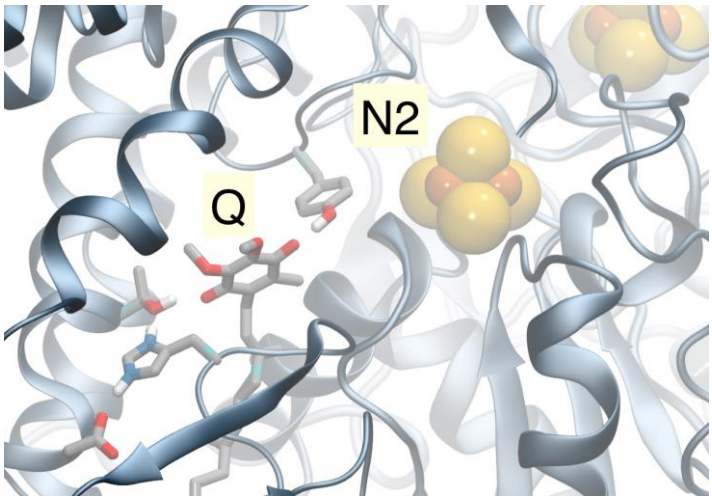
The respiratory complex I (NADH:ubiquinone oxidoreductase) functions as the entry point for electrons in aerobic respiratory chains. It couples the transfer of protons across the mitochondrial membrane with the electron transfer from nicotinamide adenine dinucleotide (NADH) through a chain of iron sulfur clusters (ISC) to a quinone (Q) which has a characteristic location in complex I [1]. Recent electrochemical studies [2] indicate that the redox potential of Q is with -300 mV, unusually low in comparison with the redox potential of Q in the membrane and the terminal ISC, N2. By using models based on the experimentally resolved structure of complex I from *Thermus thermophilus* [3], we performed first-principles quantum chemical calculations, hybrid quantum mechanics/classical mechanics (QM/MM) simulations, as well as classical Poisson-Boltzmann electrostatics considering the whole protein to show how the conformational dynamics of the bonded Q modulates the rate of the terminal electron transfer. We find that formation of semiquinone is unfavored by a large non-adiabatic electron transfer barrier.



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Determinants of pKa Values of Ionizable Residues Buried in the Hydrophobic Interior of Proteins

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I will discuss the roles of internal hydration, hydrogen bonding, Coulomb interactions and structural reorganization as determinants of the anomalous pK_a values of ionizable residues buried in the hydrophobic interior of a protein. The presentation will summarize results from extensive and systematic studies involving X-ray crystallography, NMR spectroscopy and equilibrium thermodynamic measurements.

Dissecting the Ion Atmosphere: Understanding the Electrostatics of Nucleic Acids

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DNA and RNA, biological macromolecules with central roles in information storage, gene expression, and function, are polyelectrolytes. All aspects of nucleic acid behavior, including their folding, condensation, and binding to proteins and other ligands, are profoundly influenced by a cloud of mobile ions that surrounds nucleic acids, referred as an ion atmosphere.[1] Thus, the ion atmosphere is a critical structural and energetic component of nucleic acids and dissection of its properties and behavior is required to understand nucleic acid structure, dynamics, and function. Given the complex nature of the ion atmosphere, synergy between theory and experiment is needed. Although numerous theoretical models have been proposed, there is a dearth of tests of these models. Indeed, only a limited number of experimental studies can critically address the properties of the ion atmosphere, as it is a dynamic and disordered sheath of ions and hence challenging to study. Therefore, the development of straightforward and rigorous experimental methods, and application of these methods to test predictions from theoretical models, is critical to better understand the ion atmosphere.

An experimental approach that has been particularly effective for testing theoretical predictions concerning the ion atmosphere is "ion counting". We will present an improved version of this method, using buffer-exchange atomic emission spectroscopy (BE-AES)[2] to accurately "count" the number of cations associated with and anions excluded from nucleic acids. Importantly, this method provides a unique opportunity for testing existing models, since the number of ions within the ion atmosphere can be readily computed using theoretical methods that calculate ion densities around nucleic acids solutes.[3,4] We will discuss how ion size and charge affect the ionic atmosphere occupancy and its energetics around simple, well-defined DNA and RNA molecules. We will show, for the first time, an experimental evidence of strong ion-ion correlation effects leading to formation of ion-pairs within the ion atmosphere.[5] In addition, we will provide a comparison of the experimental results with Poisson Boltzmann theory and other computations models.

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The Effect of Regulating Molecules on the Structure of the PPAR-RXR Complex

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The PPAR-RXR complex is one of the most significant and prevalent regulatory systems that control lipid metabolism by gene expression. Both proteins are members of the nuclear hormone receptor family (NRs); each subunit consisting of a ligand binding domain (LBD), a hinge and a DNA binding domain (DBD). The PPAR and RXR form a nuclear heterodimer. When free of activating ligands and silenced by co-repressor proteins, the heterodimer is inactive. Upon binding of an activating ligand and co-activator to each subunit, the heterodimer becomes active. The active complex binds DNA and initiates gene expression. The present study aimed at clarifying the main structural transformations associated with the different PPAR's isomers coupled with RXR at the active and inactive states, we implemented model-independent all atom molecular dynamics simulations, using different program packages and force fields. These simulations were carried out at three levels of complexity: (1) The LBD of PPAR; (2) A complex composed of the two LBDs of PPAR-RXR; (3) A complex of PPAR-RXR-DNA. While, in the simulations in the active state, in both proteins helices H10-H11 fused into one helix, serving as a pivot for the mutual motions. In the case of the non-active states, H12 of the PPAR contacted the co-repressor and was shifted towards the H10-H11 of the RXR, thus a rupture in this continuity appeared. The partial unfolding of the helixes exposes more residues for interactions with the other protein, leading to a more rigid structure. The transition from tight to relaxed conformations was observed for the three levels of complexity simulated in this study.

The PPAR-RXR complex is one of the most significant and prevalent regulatory system that control lipid metabolism by gene expression; appears in the nucleus in two states: active and non-active. The active complex binds to the DNA and initiates gene expression. The main structural transformations associated with the different PPAR's isotopes coupled with the RXR were studied by molecular dynamics, at three levels of complexity: a whole active complex of PPAR-RXR-DNA, the two ligand binding domains of PPAR-RXR and PPAR by itself, in active or non-active states. In both proteins, helices H10-H11 fused into one helix, serving as a pivot for the mutual motions. When H12 of the PPAR contacted the co-repressor and shifted towards the H10-H11 of the RXR, a rupture in this continuity appeared. The partial unfolding exposes residues which interacted with the other protein, leading to a rigid structure.

Application of MCCE to Proton Pumping Proteins

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Continuum electrostatics is a well-developed method for analysis of the relative stability of different charge states in proteins. As is well known the main barrier to applying this method to analysis of proteins is the difficulty in analysing the conformational responses to changes in charge of the protein and solvent in an efficient manor. We will briefly compare and contrast various methods developed by others to add implicit flexibility including changing the dielectric boundary description or the radius of the probe and compare this to results with explicit conformer sampling in MCCE.

We will then describe how the Monte Carlo sampling in MCCE can help determine the pathways for proton pumping in Cytochrome c Oxidase. In addition, we will explore how the ability to compute the energy of any charge state in the protein allows the energy of proton hopping through the protein to be estimated.

Interplay of Protonation State, Conformation, and Reactivity

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In many enzymatic reactions the chemical step of interest, i.e. a certain bond breaking or formation, is often only one step of a cascade of transitions and elementary steps. Local conformational changes precede or accompany the "chemical step" thereby allowing the reaction partners to be poised in a more favourable conformation. Similarly, proton transfer between several residues of an enzymatic active site may provide an electrostatic configuration that facilitates the actual reaction, as known e.g. from acid-base catalysis. Furthermore, the protonation state of a titratable residue and its conformational dynamics are tightly coupled.
We present here a discrete sampling approach to explore the interplay and most likely order of conformational, proton transfer, and "chemical" transitions in an enzymatic reaction.

Proton Transfer Reactions in Water Oxidizing Enzyme Photosystem II

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In protein environments, proton transfer reactions occur via H-bond networks. Knowledge of energetics of H-bonds is essential to understand the mechanism of the proton transfer reactions in protein environments. When the pK_a values of the H-bond donor and acceptor moieties are equal (1-5), significantly short, symmetric H-bonds can be formed between the two (6), and proton transfer reactions can occur in an efficient manner. However, such short, symmetric H-bonds are not necessarily stable when they are situated near the protein bulk surface (3-5), because the condition of matching pK_a values is opposite to that required for the formation of strong salt bridges, which play a key role in protein–protein interactions. To satisfy the pK_a matching condition and allow for proton transfer reactions, proteins often adjust the pK_a by using redox changes of the cofactors (7,8).

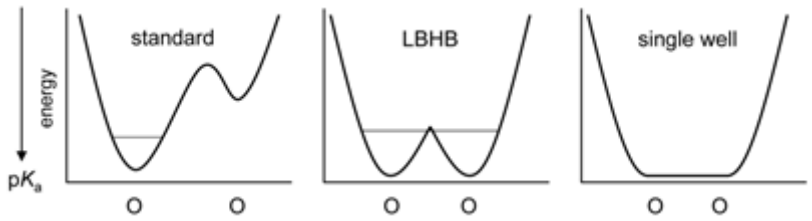

Fig 1. Potential-energy profiles of standard H-bond (left), low-barrier H-bond, LBHB (middle), and single-well H-bond (right).

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Protein Binding Sites Dynamics in Drug Discovery

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We have developed new methodological solutions for prediction and study of protein binding sites, based on graph theoretical approaches, combined with molecular dynamics simulations. Protein binding sites have been subject of intensive research, due to their key role in binding of drugs, and also, because they allow us to understand biochemical processes of a cell. For efficient drug development, one should know structure of the binding site and its dynamics, that is, one should be able to predict how the process of binding a molecule will change the binding site. The research of protein binding sites is also driven by scientific field named structural genomics, whose goal is to determine three-dimensional structures of representatives of all known proteins and their biochemical functions. Because biochemical functions of proteins are closely related to protein binding sites, further development of computational approaches to predict protein binding sites is needed.

We have developed a freely available ProBiS web tools enabling the discovery of molecules relevant to pharmaceutical research. This new tools will enable researchers to predict molecules that will bind to their investigated proteins using ProBiS, and molecular dynamics will provide a quantitative measure of how firmly the predicted molecules will bind to a protein. Here, we review these algorithms and their use in pharmaceutical discovery.

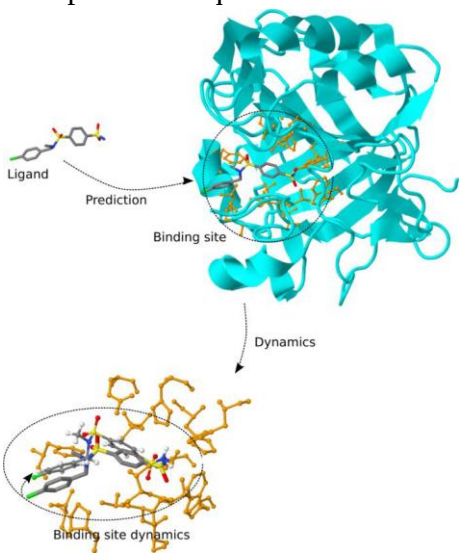

Fig. 1. *Prediction of the protein binding site, the ligand, and their binding dynamics. Depicted here is a small-molecule ligand (CPK colors, sticks) binding to the predicted binding site (orange, balls and sticks) in human carbonic anhydrase protein (cyan, cartoon).*

In particular:

• **ProBiS Algorithm**: for detection of structurally similar protein binding sites by local structural alignment (J. Konc and D. Janežič, *Bioinformatics* 26 (**2010**) 1160-1168). ProBiS enables binding sites & ligands prediction based on detection of similar evolutionary patterns in proteins.

• **ProBiS-CHARMMing Web Server** @ http://probis.nih.gov for prediction and energy optimization of ligands (J. Konc, B.T. Miller, T. Štular, S. Lešnik, H.L. Woodcock, B.R. Brooks, D. Janežič, *J. Chem. Inf. Model.* 55 (**2015**) 2308-2314). ProBiS-CHARMMing predicts & minimizes ligands for any protein and can be used to generate holo protein structures from apo proteins (or prepare ligand-receptor complex for molecular dynamics simulation).

COSMO Polarization Charge Densities: A Highly Efficient and Accurate Alternative Representation of Molecular and Protein Electrostatics

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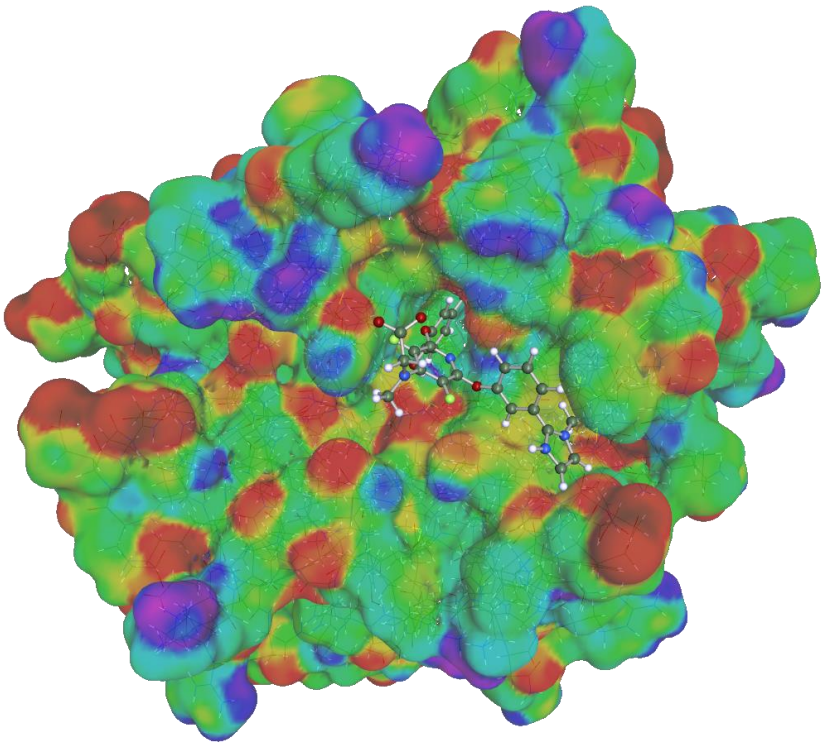
Conductor polarization charge densities, i.e. the polarization charge densities arising on the surface of a molecule, if the molecules would be embedded in a perfect conductor, have been proven to be highly efficient descriptors for the quantification of electrostatic and hydrogen bond interactions of molecules in pure and mixed solvents. Such polarization charge densities, usually denoted by σ, can easily calculated by the combination of quantum chemical, mostly DFT calculations with the solvation model COSMO (COnductor-like Screening MOdel). With this description of molecular interactions in combination with a highly efficient statistical thermodynamics treatment of pair-wise surface contacts, the COSMO-RS method has become the most accurate method to predict all kinds of fluid phase equilibrium constants in chemical engineering and many other areas of computational chemistry. Several broad comparisons and blind tests have proven that the COSMO-RS predictions of chemical potentials and free energies of transfer have about half of the stand deviation from experiment compared to predictions made with force field approaches.

The increase of available compute power in combination with linear scaling quantum codes nowadays enable the calculation of COSMO polarization charge densities for complete proteins. In this talk we will discuss the potential of using polarization charge densities as an alternative to electrostatic potentials in biochemistry, medicinal chemistry, and drug design.



Accurate and Efficient Calculations of Histidine Residue pKa in Ensembles of Protein Conformations using Molecular Dynamics

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pH-Dependent protein conformational change or structure unfolding occur in a variety of situations in protein functioning, as well as in pathology. We have recently described such pH-dependent structure transformation of a water-soluble form of the Diphtheria Toxin Translocation (T) domain [1]. Acidification of the environment solution leads to the diphtheria toxin conformational reorganization and insertion into a cellular membrane. Partial unfolding and refolding of the T-domain in water with protonated histidine residues was observed in microsecond-long molecular dynamics simulations and supported by experiments [1]. Whether a specific protonatable residue drives modification of the structure depends on the difference in pKa values of such group between different conformations of a protein (for example, in a folded and unfolded state). Even in a stable protein that remains in a single conformation, fluctuations of the structure near equilibrium may affect a pKa of titratable residues. In order to compute pKa values of the residues in different protein conformations it is necessary to account for structural fluctuations. In this work, Free Energy Perturbation (FEP) method was implemented to sample from long Molecular Dynamics trajectories generated by high-performance GPU-based pmemd.cuda module of AMBER with AMBER99SB force-field. FEP was used for computing free energy change due to protonation of histidine groups for variety of proteins in different conformations. The method convergence data and selection of the reference compounds will also be presented. We calculated the pKas of the histidine groups in several pH-stable proteins and compare our results with experimental data. We will also present pKa value evolution in different conformations that occur during the T-domain unfolding in MD simulations.

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Electrostatics: Computing and Applications to Electro-Diffusion Processes

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In equilibrium, the electrostatics of a molecular system is usually described by the Poisson-Boltzmann equation, whereas in non-equilibrium, Poisson-Nernst-Planck equations can be applied to describing electrodiffusion of charged particles in solution. I will talk about some computational approaches developed in our group, including mesh generation, fast boundary element and finite element methods. A webserver is designed to help users avoid most of the technical difficulties encountered in setting up and simulating complex systems. One of our application is to study the effect of charged substrate and product species on the reaction rate of a diffusion-controlled substrate-enzyme reaction process. The results show that charged substrate and product together contribute like a non-reactive species to the overall electrostatic steering in diffusion-reaction processes. This explains why in the theory of Debye-Hückel limiting law the charged substrate and product particles are not specifically and explicitly considered except for using an overall ionic strength. Another application is to perform the PNP calculation to study the current-voltage curves of ion channels and nanopores. The current signals of a DNA-nanopore system for gen sequencing are predicted for different nanopore geometries.

Association of Like-Charged Bio-Molecules

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The free energy of interaction between bio-molecules is often strongly influenced by electrostatic interactions due to titratable amino acid residues that can be modulated by solution pH, salt concentration and salt type. Although like-charged macro-molecules may naively be expected to repel, electrostatic anisotropy can lead to significant attractive interactions that give rise to a non-monotonic variation of i.e. the osmotic second virial coefficient with respect to salt concentration (doi:10/xqw). Using Metropolis Monte Carlo simulations and experimental scattering techniques (SLS/SAXS) we study the self-association of the milk protein lactoferrin as well of a small peptide and show how like-charged bio-molecules may attract each other due to electrostatic anisotropy and stacking interactions between arginine side-chains.

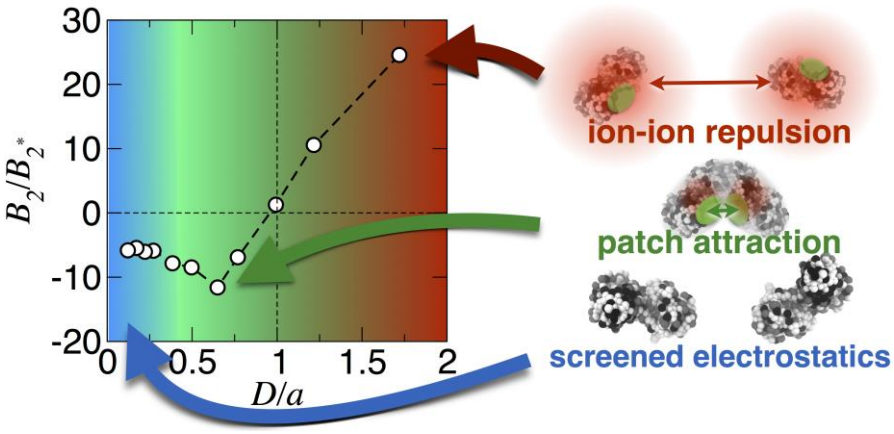

Figure caption: Non-monotonic variation of the osmotic second virial coefficient, B_2, for the milk protein lactoferrin at different Debye screening lengths (D), normalized by protein radius, a. At intermediate salt concentrations, the interaction free energy is dominated by a patch interaction, shown in green.

Computational Methods to Calculate pK_a Values at the Water/Membrane Interface

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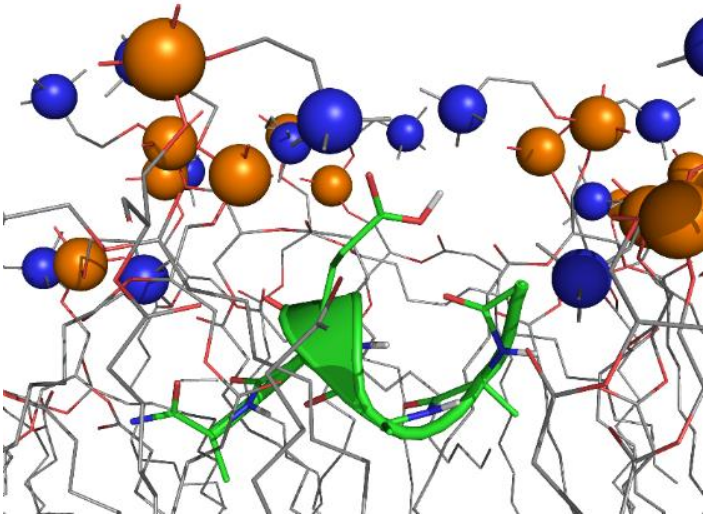
pH is a crucial physicochemical property that affects most biomolecules. Changes in protonation equilibrium of susceptible sites will modify the electrostatic environment and, consequently, have an effect on the molecular structure, stability and catalysis.[1] The pK_a values of the typical titrable amino acids can be significantly influenced by changes in solvent mixture or due to insertion in a lipid bilayer.[2-3]
In this work, we present a methodology that allows us to calculate pK_a values of both peptides/proteins and lipid headgroups at the water/membrane interface. We take advantage of the recently developed CpHMD-L methodology[4] and apply it to different systems, namely, the model Ala-based pentapeptides that have already been well characterized in water by Pace and co-workers,[5] and the pHLIP peptide, a 36 amino acid peptide derived from bacteriorhodopsin that is able to insert the membrane at acidic pH.[6] Our most recent results with these systems will be presented.



We acknowledge financial support from FCT through projects PTDC/QEQCOM/5904/2014, UID/MULTI/00612/2013, and Incentivo/QUI/UI0612/2014.

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Elucidation of Protonation States of Key Residues in Phytochromes: a Combined Computational and Spectroscopic Approach

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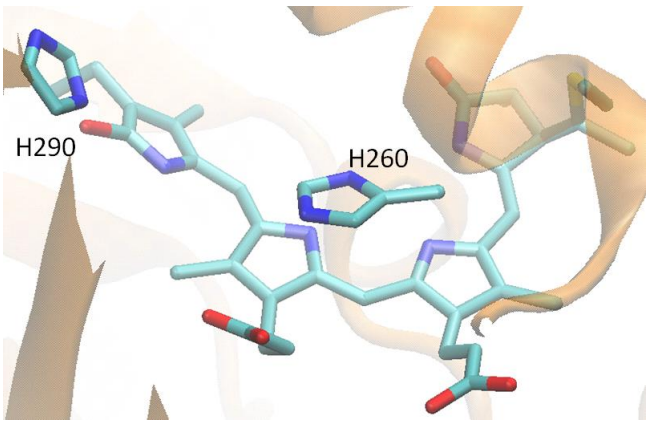
Phytochromes are biological red-light photoreceptor found, in plants, bacteria and fungi. Light sensing is done by an open-chain-tetrapyrrole chromophore which is covalently attached to the protein matrix via a thioether linkage (see figure). Upon light absorption, the bilin chromophore is capable to switch between two relatively stable states: a red-light absorbing (Pr) state and a far-red light absorbing (Pfr) state. Despite the large amount of structural and spectroscopic data available for these photoreceptors a detailed and consistent description of the ground state structural properties of the chromophore binding pocket is still lacking. An important factor veiling these studies is the intrinsic heterogeneity of the chromophore structure in the dark state.
The first attempt to characterize the structural heterogeneity of the PCB chromophore of *Cph1* was achieved by means heteronuclear magic spinning NMR spectroscopy. These studies, proposed the existence of two conformers, named as the Pr-I- and Pr-II- forms, which differ basically in the protonation state of the His260 and His290. However, contradictory spectroscopic evidence followed this initial observation.
This presentation is focused on a computational-spectroscopic approach, based on QM/MM calculations and resonance Raman spectroscopy, used to identify the protonation state of histidines in the chromophore binding pocket and investigate in detail the molecular basis for the structural heterogeneity in phytochromes.



Three-dimensional structure of the Phycocyanobilin (PCB) chromophore in Cph1-phytochrome, together with neighboring histidine residues.

Dielectric Effects of Electrolytes, Charged Surfaces and Proteins

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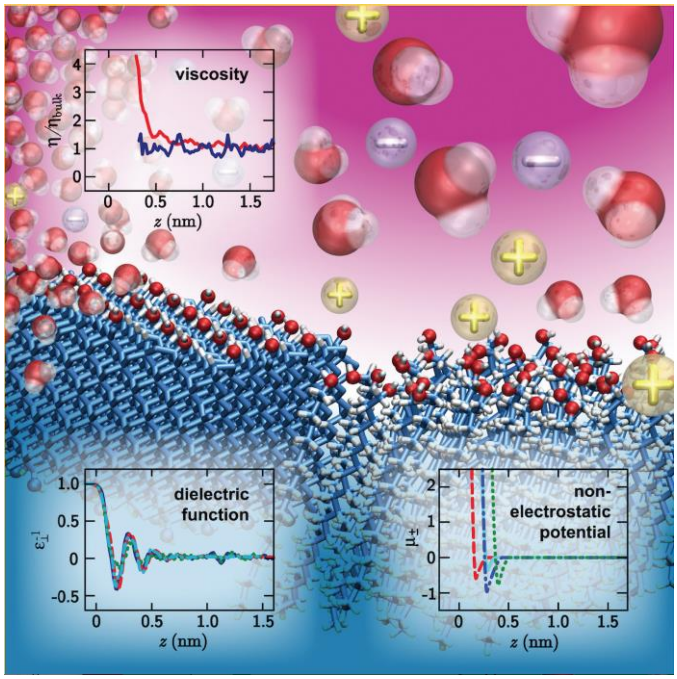
The layer of water molecules on surfaces and around polymers and ions, the so-called hydration layer, is crucial for the properties of all charged biological and technological systems. Insight is gained from all-atomistic simulations in conjunction with appropriate continuum interpretation and modelling. We discuss three different scenarios: i) The dielectric properties of hydration water at surfaces are different from bulk water and exhibit pronounced anisotropy effects. As a consequence, ions accumulate into a highly condensed interfacial layer, causing the well-known reduction of the surface capacitance. ii) Infra-red spectroscopic studies suggest that solvation water next to ions is generally slowed down while dielectric spectroscopy experiments in the GHz range demonstrate a speed-up (blue-shift) of the water relaxation with rising electrolyte concentration. We show that this blue shift is due to the attenuation of collective dynamic effects, i.e., the decoupling of the dynamics of one water molecule to its neighbours. iii) In proteins and hydrogels, partial charges on the polymeric backbone significantly contribute to the dielectric constant. We discuss how an effective dielectric constant inside a protein can be defined.



Electrostatics of DNA and RNA: Small Differences and their Large Consequences

Alexey Onufriev

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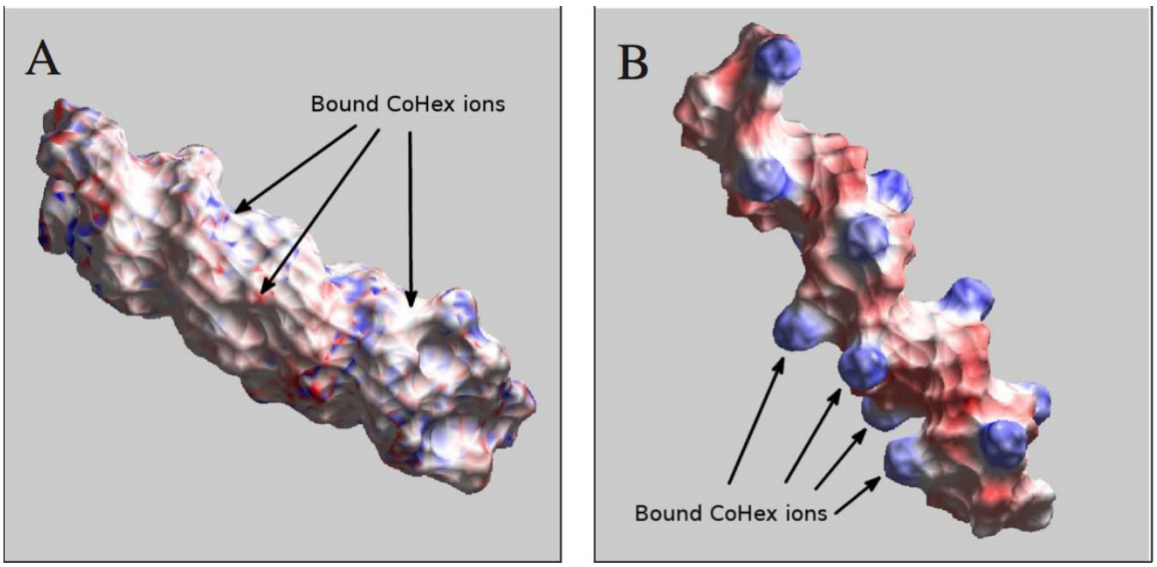
From the basic physics standpoint, double-stranded DNA and RNA look very much alike: highly charged cylinders of almost identical dimensions. Unexpectedly, relatively small differences in electrostatic properties of the two helices can lead to dramatically different, and sometimes opposite behavior in electrostatically-driven phenomena:
(1) DNA can be condensed by certain multivalent ions into structured aggregates. In contrast, recent experiments demonstrate that double-stranded RNA helices resist condensation under conditions where short DNA duplexes condense readily. Why?
(2) It is well known that DNA becomes softer as ionic strength of the solution is increased, because ions screen out long-range electrostatic interactions along the polymer. In contrast, we show that multivalent ions make RNA stiffer, not softer.

We combine experiment, theory, and atomistic simulations to explain these novel phenomena.



Charge neutralization patterns of DNA (A) and RNA (B) duplexes by bound $CoHex^{3+}$ ions.

Electrostatics of Excited Electronic States of Chromophores in Proteins

Thomas Renger, Julian Adolphs, Marcel Schmidt am Busch, Florimond Collette, Alexander Klinger, Dominik Lindorfer, Frank Müh

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We have developed electrostatic methods to parametrize the Hamiltonian of pigment-protein complexes, in order to investigate excitation energy transfer and optical spectra of these systems. Three classes of parameters are needed: (I) the local optical excitation energies of the pigments in their binding sites in the protein, termed site energies, (II) the excitation energy transfer (excitonic) couplings, and (III) the spectral density of the exciton-vibrational coupling that describes the modulation of the site energies and excitonic couplings by protein dynamics. We use a combination of quantum chemical/electrostatic/normal mode analysis methods for the calculation of these parameters. I will give an overview about the methods and present applications to the study of energy transfer and optical spectra of photosynthetic light-harvesting proteins and the identification of functional states of the BLUF photoreceptor, that is based on electrochromic shift calculations of the wild-type and various site-directed mutants.

Coupling Continuum Electrostatics with Integral Equations Theory to Include High Field Effects in the Study of Redox-active Proteins

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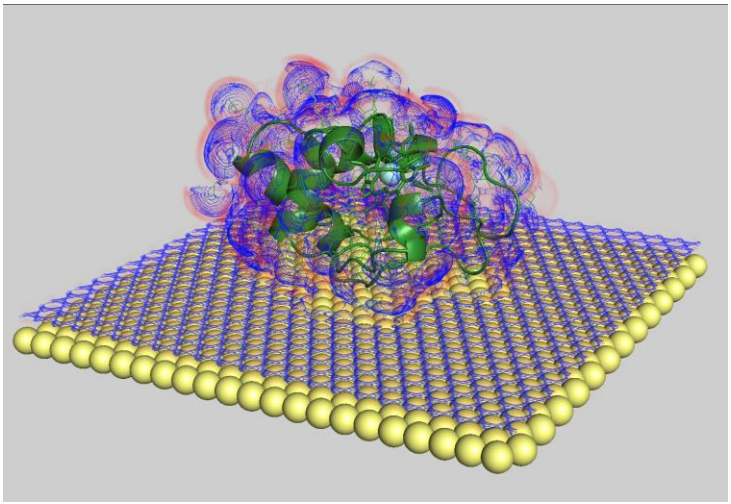
The electrochemical investigation of redox-active proteins at electrodes is very relevant from both the theoretical and the applicative standpoints. Its computational analysis is however a particularly challenging task, especially if the considered fields are so strong to induce high ionic concentrations where ion-ion correlations cannot be neglected. This makes mean field approaches such as Poisson-Boltzmann equation less predictive of experimental outcomes. Therefore in these cases a better description of the short-range interactions, which means also incorporating the granularity of the solvent, including the ionic strength, becomes unavoidable.

Integral equations theories (IET) of liquids can provide statistical-mechanics-based alternatives or integrations to mean field approach. In particular, we focus on 3D-RISM. Taking as an input partial charges and Lennard-Jones parameters (or other short-range potentials) 3D-RISM equations output detailed description of the water and ionic density surrounding solutes, e.g. biomolecules, nanoparticles, walls, etc. These densities can be used per se or as an input for Poisson equation for further analysis of the electrostatic interactions. Such treatment is hampered, for instance, in areas where the electrostatic potential is externally controlled, such as in scanning tunneling microscopy, organic electronics, electrochemistry etc. Here, we present the coupling of 3D-RISM and Poisson equation in an iterative scheme aiming to include the electrostatic potential control in the integral equations based simulations. The object of the study is the Gold/Cytochrome-C field effect transistor, which is has been extensively investigated in recent times [1-2].



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Space and Time Evolution of the Electrostatic Potential During the Photoisomerization in Rhodopsin

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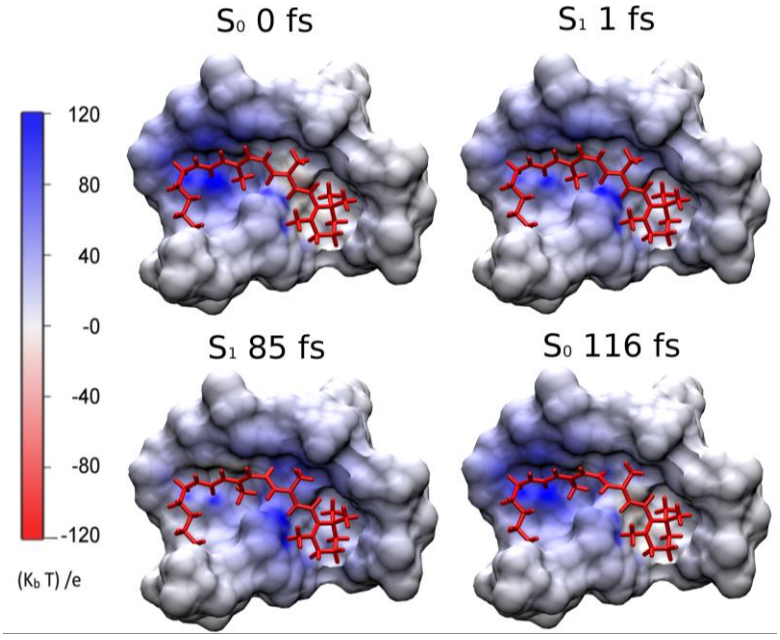
The primary event of vision in the vertebrate eye is the highly selective and efficient photoisomerization of 11-*cis*-retinal protonated Schiff base (pSb) bound to the visual protein rhodopsin (Rh). With its high selectivity and efficiency (65% quantum yield), and the formation of the first photoproduct within 200 fs, this photoreaction is considered the archetype of a chemical reaction optimized by nature to achieve a specific molecular response. Recently, we have resolve the isomerization mechanism for the chromophore in rhodopsin by means of combined *ab inito* multiconfigurational QM/MM molecular dynamics simulations at the CASPT2/CASSCF//AMBER level of theory.[1]
In addition, we investigated the possible consequences of the charge translocation associated with the photoisomerization.[2] We show that the evolution of the electrostatic potential projected by the chromophore onto the surrounding protein displays intense but topographically localized sudden variations in proximity of the decay region. pKa calculations carried out on selected snapshots and used as probes, indicate that the only residue which may be sensitive to the electrostatic potential shift is Glu181. Accordingly, our results suggest that the frail Tyr191/268-Glu181-Wat2-Ser186 hydrogen bond network may be perturbed by the transient variations of the electrostatic potential.



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Mechanisms of pH Activation in Enzymes and Membrane Transporters

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pH-triggered processes are ubiquitous in biology; however, detailed mechanisms are often unknown due to the limited resolution of experimental measurements. Towards this goal, in recent years a special molecular dynamics technique called constant pH molecular dynamics has been developed. In this presentation I will discuss the most recent applications that offered new insights into the pH dependent activation and inhibition of BACE1 enzyme, a major drug target for Alzheimer's disease, and the pH-regulated sodium-proton exchange process of NhaA, an antiporter principally responsible for cellular sodium and proton homeostasis in E. coli.

Stepwise Versus Concerted Mechanisms in General-Base Catalysis by Serine Proteases

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General-base catalysis in serine proteases still poses mechanistic challenges despite decades of research. Whether proton transfer from the catalytic Ser to His and nucleophilic attack on the substrate are concerted or stepwise is still under debate, even for the classical Asp-His-Ser catalytic triad. To address these key catalytic steps, the transformation of the Michaelis complex to tetrahedral complex in the covalent inhibition of two prototype serine proteases was studied: chymotrypsin (with the catalytic triad) inhibition by a peptidyl trifluoromethane and GlpG rhomboid (with Ser-His dyad) inhibition by an isocoumarin derivative. The sampled MD trajectories of averaged pKa values of catalytic residues were QM calculated by the MD-QM/SCRF(VS) method on molecularclusters simulating the active site. Differences between concerted and stepwise mechanisms are controlled by the dynamically changing pKa values of the catalytic residues as a function of their progressively reduced water exposure, caused by the incoming ligand.

Electron Transfer in bc1 Complex

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Recent calculations from our group of electron transfer reactions in bc1 complex will be presented. The work has been recently published in a series of papers that include:

1. Quantum Calculations of Electron Tunneling in Respiratory Complex III, Muhammad A. Hagras, Tomoyuki Hayashi, and Alexei A. Stuchebrukhov, J. Phys. Chem. B 2015, 119, 14637−14651.

2. Internal Switches Modulating Electron Tunneling Currents in Respiratory Complex III, Muhammad A. Hagras, Alexei A. Stuchebrukhov, BBA Bioenergetics, Doi: 10.1016/j.bbabio.2016.02.005

New Twist in the Theories on the Electrostatic Vibrational Response of Peptides and Related Systems

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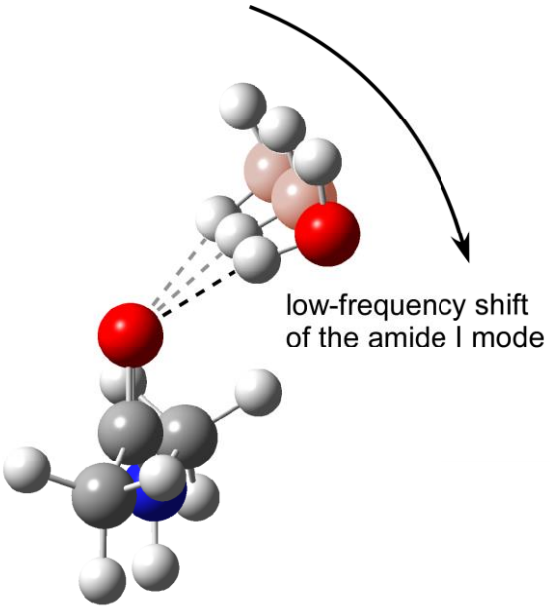
There are a few characteristic vibrational modes known for the peptide groups in proteins, among which the amide I mode is the most well known. It is regarded as a useful marker of the secondary structures and the electrostatic situations. However, there still remain some important points yet to be clarified. One of them would be the structural properties of the so-called hydrated helix, appearing at ~20 cm^{-1} lower than a typical α-helix in the amide I spectral region. This is related to the question as to whether a water molecule with a weak (less stable) out-of-plane hydrogen bond really induces a large low-frequency shift of the amide I mode.

To clarify this point, theoretical analyses have been carried out for peptide–water complexes [1]. It is found that the frequency shift strongly depends on the C=O...H angle, with a larger low-frequency shift as the C=O...H becomes more bent, even if the hydrogen bond is relatively weaker, challenging our vague expectation on the correlation between the hydrogen-bond strength and the frequency shift. It is also found that, although this low-frequency shift cannot be correctly represented by any of the electrostatic interaction models that have been previously proposed for this vibrational mode, a carefully constructed new electrostatic interaction model can correctly represent it.

In fact, this strong angular position dependence of the hydration-induced frequency shift is found in a few characteristic vibrational modes. For those cases also, carefully constructed electrostatic interaction models can correctly represent the strong angular position dependence [2]. A unified understanding of the hydration-induced high-frequency shift of the C≡N stretch of acetonitrile and the low-frequency shift of the S=O and C=O stretches is also possible by using such electrostatic interaction models.
The use of these properties for examining the electrostatic situations in proteins, as well as the relation to the phenomenon called vibrational Stark effect, will also be discussed.



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The Role of Electrostatic Attraction Forces in the Reconstruction and Stabilization of the Binding Site of a Homo-trimer Enzyme

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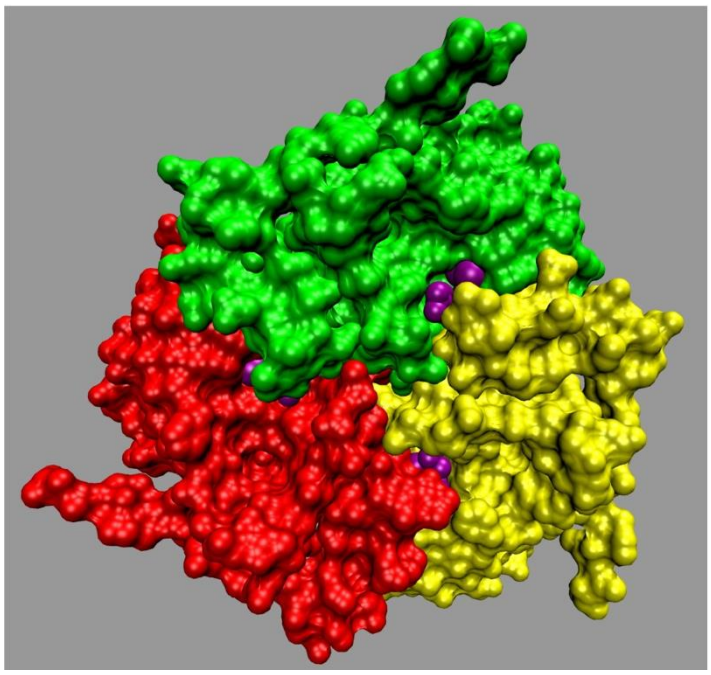
This study evaluates the role of electrostatic interactions in the attraction and stabilization of homo-trimeric structures. The protein under study is a homo-trimer where the ligand-binding site is located at the interface of two subunits. One subunit contributes a substrate-binding pocket, while the other donates the catalytic residues and some other binding residues. The structure was manipulated *in vivo* and *in silico* by fragmenting the protein chain into two: an N-terminal fragment containing a β-strand followed by an α-helix and keeping it in close vicinity to C-terminal fragment that form trimer by a long flexible linker attached to the C-terminus of the same subunit. *In vivo* experiment revealed that the manipulated protein retained its enzymatic activity demonstrating that the detached fragments regained the original structure. The molecular dynamics reproduced in time the convergence of the truncated N-terminal fragment into its native location.

The reassembly of the native state was stepwise where the helical section of the detached N-terminal fragment was the first to assume its position, a process dominated by specific electrostatic interaction of specific arginine and histidine moieties from one fragment with three carboxylates on the other fragment. The last residues to restore their structure are located at the site where the sequence was split, thereby catalysis is recovered.



pH-dependent Insertion of pHLIP Peptide into Lipid Bilayers: pK_a Values of Key Residues

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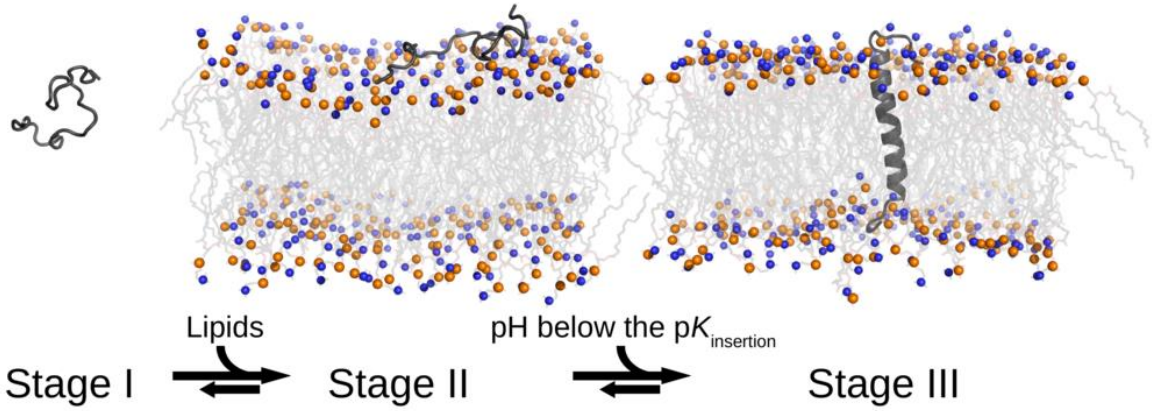
The pH (low) insertion peptide (pHLIP)[1-3] is a family of peptides that are able to insert into a lipid bilayer at acidic pH. These peptides are based in a transmembranar sequence of bacteriorhodopsin that is unstructured in solution (stage I), interacts with lipid bilayers remaining unstructured at neutral pH (stage II) and inserts into the bilayer with a significant α-helical content at acidic pH (stage III). This family of peptides have already been used to target tumor cells in vivo since acidosis is an hallmark of these tissues.[4] These events are difficult to study at the molecular level, in particular, the relation between the pK of insertion of pHLIP peptides and the pK_a of some key residues is yet to be clarified. In this work, we used a linear response approximation to determine the pK_a of these residues. We studied four different pHLIP variants to understand the importance of the ASP positions and its mutation to GLU. For these variants, there are experimental data available that we used to validate our approach. Finally, we also propose the addition of a HIS residue to the sequence, which we expect to turn off the pHLIP peptide insertion into the membrane (stage III) at too low pH values.



We acknowledge the financial support from FCT (PTDC/QEQCOM/5904/2014 and UID/MULTI/00612/2013).

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Electrostatics Models for pH-dependence and Solubility

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Recent work in our group has included a large collaborative element with academic and industrial groups interested in bioprocessing, around a common interest in protein solubility and pH- / ionic strength-dependent properties. The roles of charges in protein solubility and pH-dependence are in part very simple and widely recognized, for example the susceptibility of proteins to aggregate at pH around their isoelectric points. Details beyond this are yet to be firmly established, but charge engineering for solubility is now common in many groups, including in our collaborations. This area will be discussed, with reference to emerging data from high throughput analyses of protein solubility, and our own work in looking for sequence and structural features that correlate with higher solubility. It is hoped that such observations will be helpful to bioprocessing pipelines (e.g. for biopharmaceutical drug development), and also to the developing field of synthetic biology.

Some of the most interesting applications of electrostatics are evident where nature has evolved pH-dependence for a particular purpose, and we will touch on this area. There is, in fact, a cross-over with biopharmaceuticals, since some of these drugs are being developed to use a naturally occurring pH-dependent recycling system.