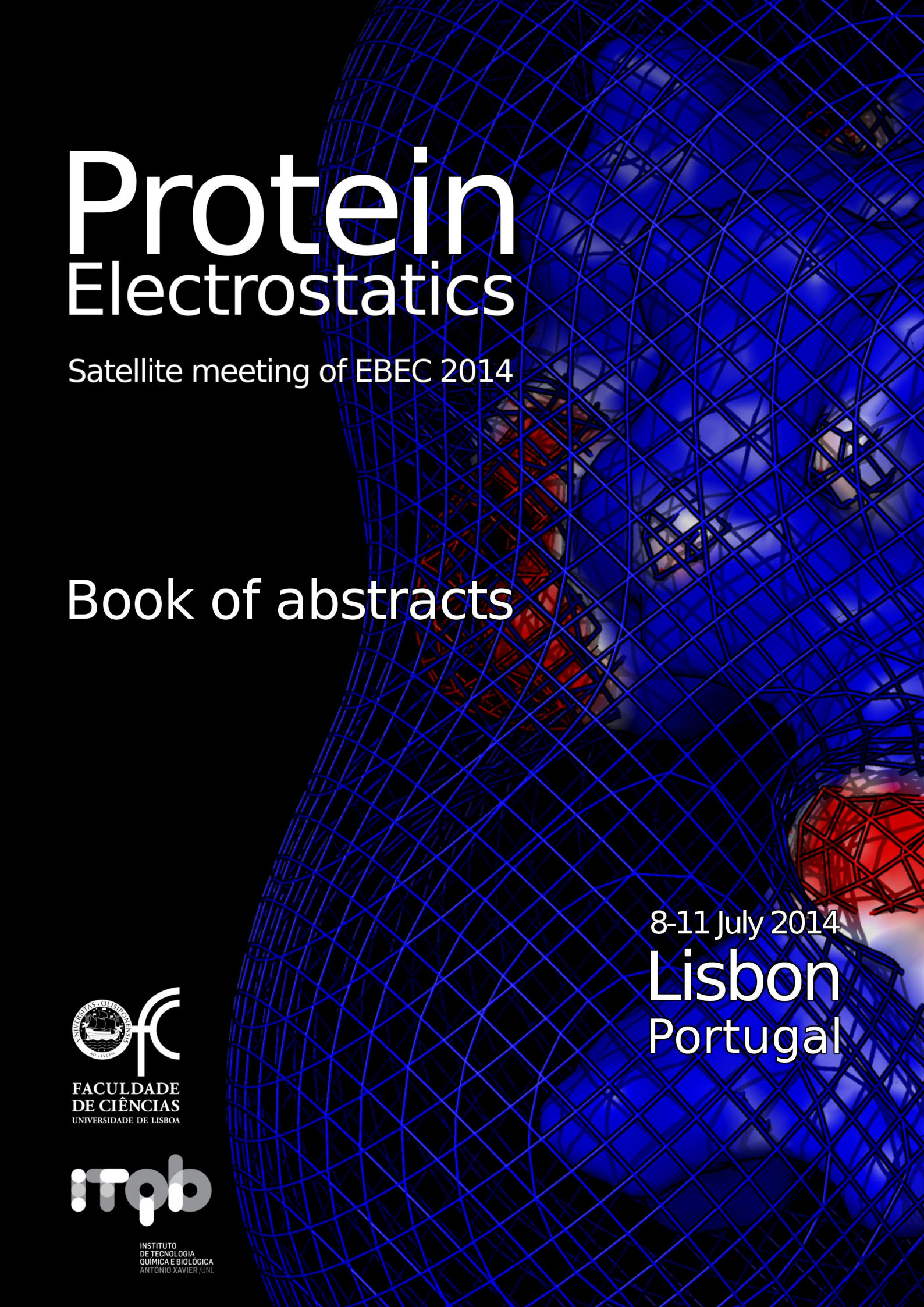

Protein Electrostatics

Satellite meeting of EBEC 2014

Book of abstracts

8-11 July 2014

Lisbon

Portugal

FACULDADE DE CIÊNCIAS
UNIVERSIDADE DE LISBOA

INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA ANTÓNIO XAVIER /UNL

Welcome Address

Electrostatics is a major determinant in the action of proteins, being involved in key processes from catalysis and folding to electron transfer and proton transport. Despite the progress over the last decades, a multitude of experimental, theoretical and computational studies continues to be pursued to further understand how such electrostatic effects are modulated at the molecular level through an interplay of desolvation, polarization, structural relaxation, charge-charge and charge-conformation couplings, water penetration, etc.

This Protein Electrostatics meeting, a satellite of the European Bioenergetics Conference (EBEC) 2014, is precisely aimed at those working on elucidating the molecular-level mechanisms behind electrostatic effects in proteins and other biomolecules. The goal is to gather experimentalists, theoreticians and simulators into a single focused event, in order to create synergies and make clearer what experimental physical data do we have or need, how well we are doing in terms of computational/theoretical predictions, how can we help each other, and whether we are overlooking any crucial aspects. The spirit is largely borrowed from the Telluride workshop on Protein Electrostatics, regularly organized by Bertrand García-Moreno and Marilyn Gunner.

http://protelec2014.org

Organizers:

António M. Baptista—ITQB/UNL, Portugal

Miguel Machuqueiro—FCUL, Portugal

G. Matthias Ullmann—University of Bayreuth, Germany

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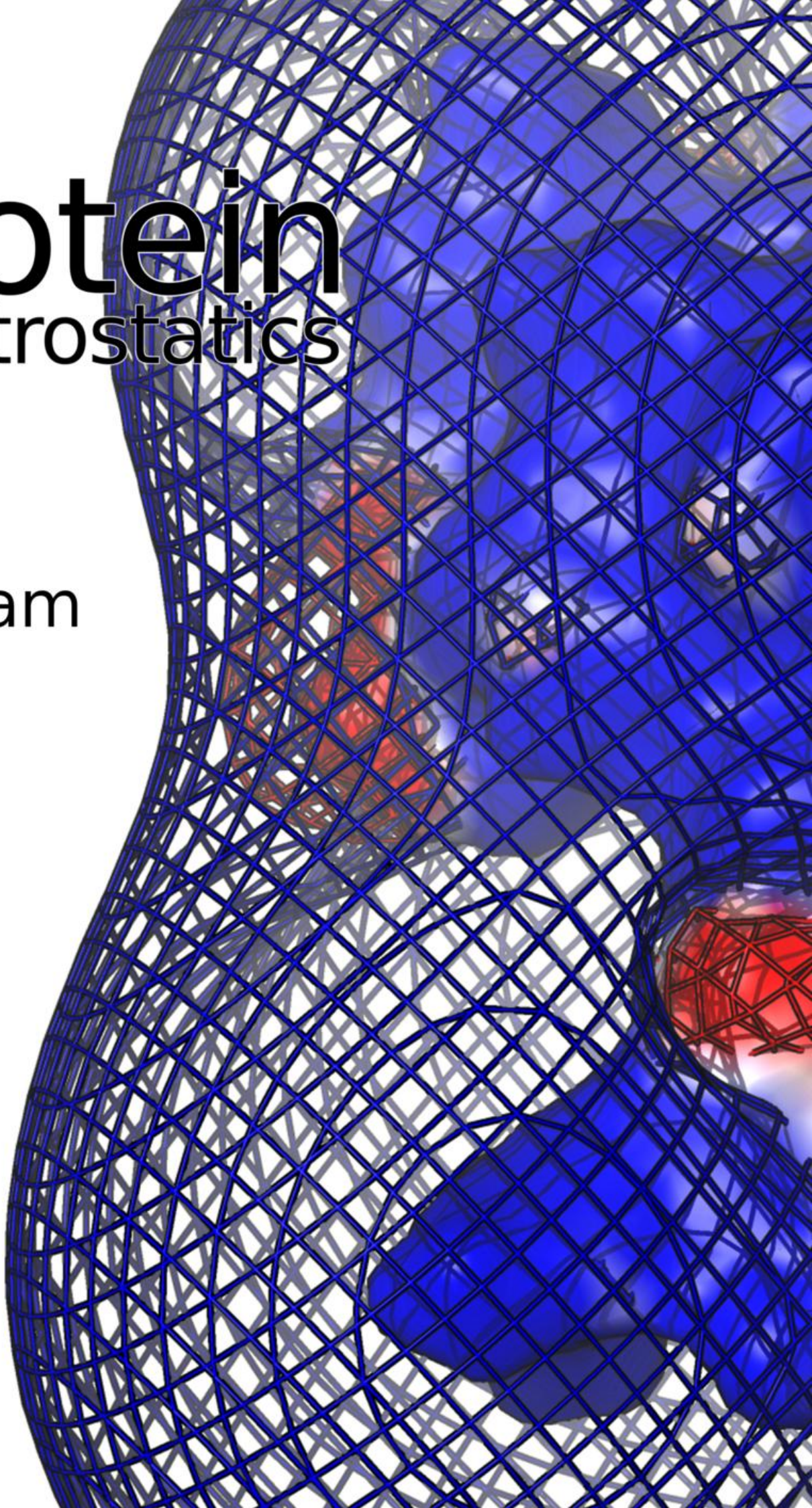

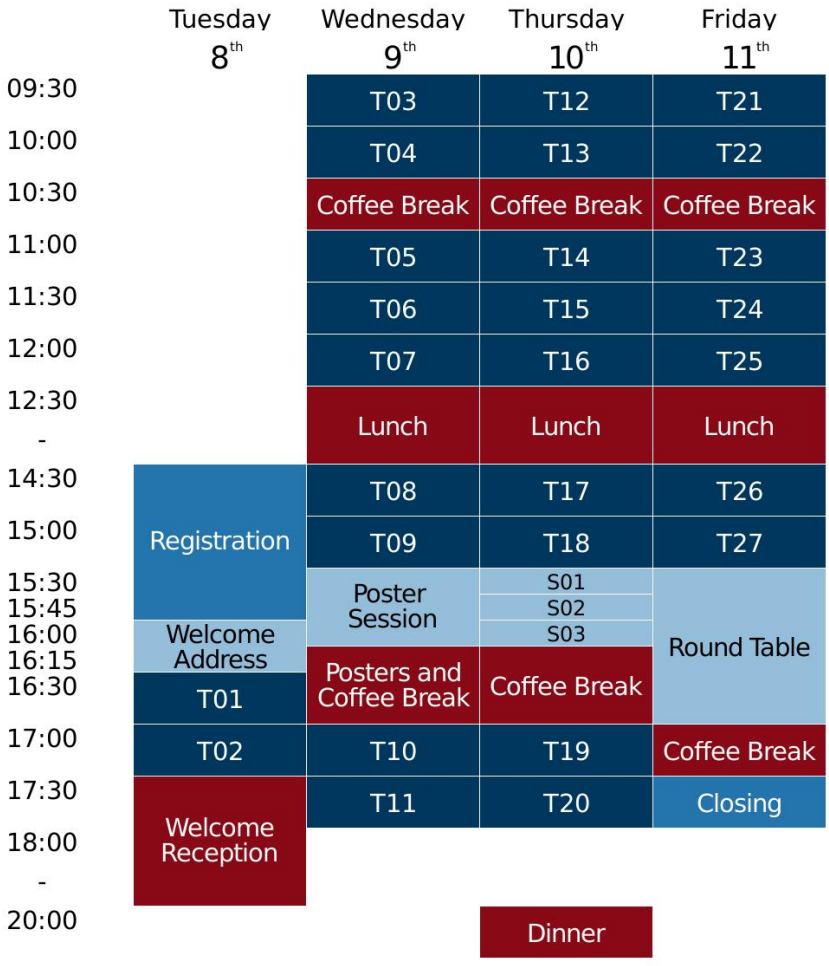

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Protein Electrostatics

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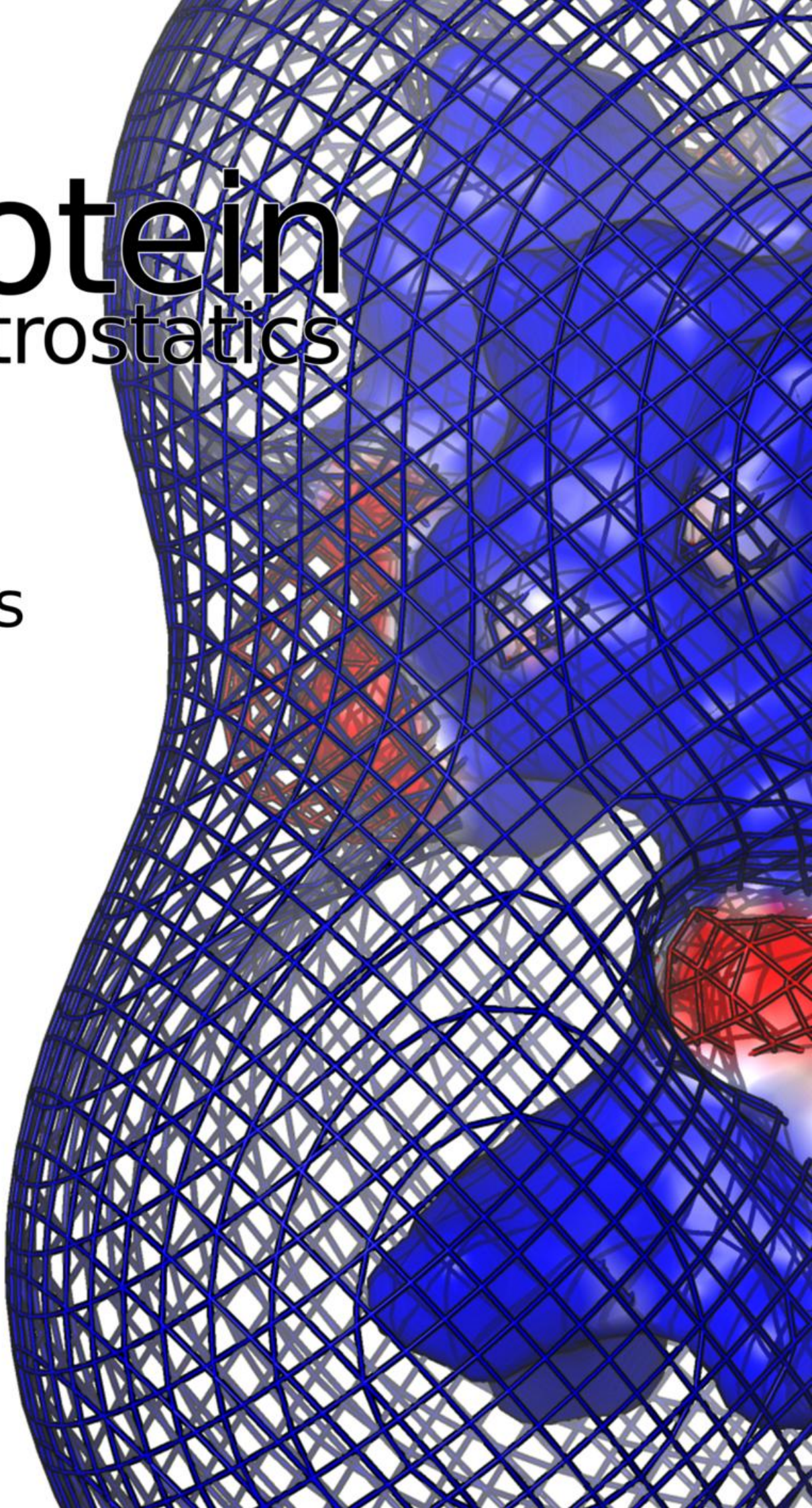

Electrostatics, quantum mechanics and studies of protein function

Donald Bashford

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Macroscopic electrostatic models, particularly in combination with quantum mechanics, can help elucidate protein structure/function relationships, including the catalytic mechanism of enzymes. The basic methods will be outlined and examples will be presented.

Understanding the active-site of thiol-disulfide oxidoreductases with molecular dynamics and pK_a calculations.

Nicolas Foloppe[a], Andrey Karshikoff[b,c], Lennart Nilsson[c], Alexios Vlamis-Gardikas[d]

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Molecular dynamics simulations and pK_a calculations provided new insights into the structure, dynamics and electrostatics of active sites of enzymes in the thioredoxin superfamilly (thioredoxin, glutaredoxins, DsbA, mycoredoxin, tryparedoxin, …). The variety of cellular functions performed by such enzymes relies on the wide range of redox potentials associated with their active site -Cys-X1-X2-Cys- motif, and the unusually low pK_a of a reduced catalytic cysteine. However, the reduced form of the -Cys-X1-X2-Cys- motif has proved difficult to characterize experimentally. This gap has been largely filled by simulations and electrostatic calculations which provided extensive information on a variety of -Cys-X1-X2-Cys- motifs in diverse proteins of the thioredoxin superfamily. The computational studies give a better understanding of the functional properties of these enzymes, including the factors modulating the pK_{aS} (and, indirectly, redox potentials) of key catalytic cysteines. The calculations were used as basis for predictions subsequently tested by experiments (mutagenesis, pK_a measurements). A unifying theme for the structural determinants of the thiol pK_a differences across -Cys-X1-X2-Cys- motifs has now emerged.

References:

[1] N. Foloppe, J. Sagemark, K. Nordstrand, K.D. Berndt, L. Nilsson, Structure, Dynamics and Electrostatics of the Active Site of Glutaredoxin 3 from Escherichia coli: Comparison with Functionally Related Proteins. J. Mol. Biol. 310 (2001) 449−470.

[2] N. Foloppe, A. Vlamis-Gardikas, L. Nilsson, The -Cys-X1-X2-Cys- Motif of Reduced Glutaredoxins Adopts a Consensus Structure That Explains the Low pKa of Its Catalytic Cysteine, Biochemistry 51 (2012) 8189−8207.

[3] A. Karshikoff, L. Nilsson, N. Foloppe, Understanding the −C–X1–X2–C– Motif in the Active Site of the Thioredoxin Superfamily: *E. coli* DsbA and Its Mutants as a Model System, Biochemistry 52 (2013) 5730–5745.

Electrostatic origin of protein halophilic adaptation

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I will discuss how simple continuum electrostatic arguments can help understand unusual stability of halophilic proteins at extreme salt concentrations. If time permits, I will also describe our latest work on improving continuum solvent models.

The role of buried residues in protein channel electrostatics

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Ionizable residues which are solvent accesible or located close to the permeation pathway are believed to control the ion transport through protein ion channels. However, increasing evidence shows that residues buried deep into the protein medium could affect the electrostatic properties of proteins in solution. Relevant examples (E. coli OmpF porin, S. aureus alpha-hemolysin, Neisseria M. PorA/C1) that emphasize this idea are analyzed and discussed.
By combining approaches with different level of detail (from mean field theories to all-atom MD simulations) we investigate the contribution of all charges present in the protein (ionized residues in our system) to the overall electrostatic potential, either when charges are buried into the protein medium or closer to the aqueous pore solution. We show that even in the more detailed models the influence of buried charges is not trivial to figure out. Electrostatic effects depend on different local factors like the permittivity of the protein medium or the actual location of residues in the protein hydrophobic core. Interestingly, these factors are not explicitly considered in most of the available theoretical models.

Investigating the Role of Sodium Ion in the Activation of Thrombin Protease, Using Molecular Dynamics Simulations

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[b] *Department of Biochemistry and Molecular Biology, Mathematical Biology Unit, Faculty of Sciences, Holon Institute of Technology, Holon, Israel*

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Thrombin is a protease playing a crucial role both in blood clotting and in thrombolysis. The enzyme exists in three forms which are in equilibrium: a state that has no catalytic activity and cannot bind Na^+; a slow activity state that is free from Na^+ and a fast turnover state that contains a single Na^+. The binding site is ~15 Å apart from the catalytic site, too far to be considered as a “co-factor". Molecular dynamics was used to characterize the slow and fast forms for clarifying how Na^+ enhances the rate of catalysis. The simulations were initiated from a crystal structure 1PPB.pdb [1]. A single Na^+ ion was placed in the location indicated by Zhang and Tulinsky [2].

In the simulations with the bound ion, the Na^+ did not remain in its initial location but shuttled towards a more stable site, mainly due to electrostatic interaction with the carboxylate of D189 that also participated in the binding of the substrate’s Arginine. During the simulations of Na^+ bound state, there were few distinct events where the bound Na^+ diffused out and simultaneously another Na^+ ion entered the protein at a remote location. We propose that there are two entering locations for the ion.

Kinetic analysis of the catalytic cycle revealed that the release of the product is the slowest event and the one most accelerated in presence of Na^+. Accordingly, it is very probable that the ion accelerates the catalysis by destabilization of the product-enzyme complex.

References:

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[2] E. Zhang, A. Tulinsky, The molecular environment of the Na^+ binding site of thrombin, Biophysical chemistry, 63 (1997) 185-200.

The role of electrostatics in protein adaptation to hypersaline environments

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Life on earth exhibits an enormous adaptive capacity and living organisms can be found even in extreme environments. The halophilic archaea are a group of microorganisms that grow best in highly salted lakes (with KCl concentrations between 2 and 6 molar). To avoid osmotic shock, halophilic archaea have the same ionic strength inside their cells as outside. All their macromolecules, including the proteins, have therefore adapted to remain folded and functional under such ionic strength conditions. As a result, the amino acid composition of proteins adapted to a hypersaline environment is very characteristic: they have an abundance of negatively charged residues combined with a low frequency of lysines. We are interested in the relationship between this biased amino-acid composition and protein stability as well as in enzyme activity. Our results show that salt induced stabilization of proteins share the same mechanism as the neutral osmolites. By using NMR spectroscopy, the relative contribution from the excluded volume, the electrostatic forces and the preferential (hydrophobic) interactions can be disentangled at the residue resolution level.

Polar and ionizable groups in hydrophobic environments in proteins

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Structural and equilibrium thermodynamic methods were used to study properties of polar and ionizable residues buried in the hydrophobic interior of staphylococcal nuclease. The effects of substitution of the wild type residues with Asn, Gln, Ser, Thr or Tyr on thermodynamic stability were measured at pH 7. The effects of substitution of internal positions with Lys, Arg, His, Asp and Glu under conditions of pH where the ionizable groups are normally neutral were also measured. Some of the pKa values measured for these internal ionizable groups are highly anomalous. Crystal structures of several dozen of these variants with internal ionizable groups have been deposited in the PDB. Systematic studies with NMR spectroscopy were used to examine how the structure of the protein is affected by ionization of the internal Lys residues. We have also measured **(a)** the magnitude of interactions between internal and surface ionizable groups, **(b)** the magnitude of interactions between basic and acidic moieties in many buried Lys-Glu and His-Glu pairs. **(c)** X-ray and NMR spectroscopy have been used to examine the properties of several excited states obtained by ionization of buried groups. Besides contributing novel insight into determinants of pKa values of internal residues in proteins, and into dielectric relaxation properties of proteins, these experimental data constitute a useful set for stringent benchmarking of computational methods. These cases are especially useful to examine the ability of computational methods to reproduce conformational changes driven by the ionization of groups buried in hydrophobic environments in proteins.

mFES: A robust molecular Finite Element Solver for electrostatic energy computations

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We present a robust method for the calculation of electrostatic potentials of molecular systems using tetrahedral finite elements (FE). Compared to the finite difference (FD) method using a regular simple cubic grid to solve the Poisson equation, the FE method can reach high accuracy and efficiency using an adaptive grid. Here, the grid points can be adjusted and are placed directly on the molecular surfaces to faithfully model surfaces and volumes. The grid point density decreases rapidly toward the asymptotic to reach very large distances with just a few more grid points. A broad set of tools are applied to make the grid more regular and thus robust, while reducing the number of grid points without compromising accuracy. The latter reduces the number of unknowns significantly and yields shorter solver times. The accuracy is further enhanced by using second order polynomials as shape functions. Generating the adaptive grid for a molecular system is expensive, but it pays off, if the same molecular geometry is used several times as is the case for pK_A and redox potential computations of charge variable groups in proteins. Application of the mFES method is also advantages, if the molecular system is too large reach sufficient accuracy when computing the electrostatic potential with conventional FD methods. The program mFES is free of charge and available at http://agknapp.chemie.fu-berlin.de/mfes.

Electrostatics models for Biology

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This presentation will describe aspects of developing and applying continuum electrostatics methods (including those based on Finite Difference solutions) to biology, and refer to current challenges. For example, from the 'field focussing' effect in enzyme active sites that follows directly from dielectric boundary conditions, to the lack of a robust predictive tool with which enzymologists can routinely characterise the role of charge interactions in catalysis. Successes in producing models capabable of predicting pKas and redox potential changes, through the combined efforts of several groups over many years, are balanced by the realisation that these are often insufficient for complex molecules with conformational change coupled to pH-dependence. In many cases our understanding of charge interactions for an isolated protein or biological complex may be completely transformed within a crowded biological environment. Thus far we have paid relatively little attention to the role of charges within intrinsically unstructured regions of proteins, common targets for post-translational modifications. Incorporating a proteomic approach presents opportunities as well as challenges, since large-scale measurements can reveal enrichments of specific protein properties with a particular experimental or physiological feature. It will be argued that the use of informatics methods to facilitate a combination of 'omics data and physics-based modelling is likely to increase. This point will be demonstrated with application to protein solubility, and the properties of protein therapeutic molecules.

Electrostatics Of The Protein-Water Interface

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The interface of the protein with water is highly heterogeneous both dynamically and statistically. I will discuss the interfacial properties of hydrated proteins as obtained from atomistic simulations. At the same time, a number of these observables can be extracted from solution experiments, including depolarized light scattering, dielectric spectroscopy, and absorption of radiation [1-3]. The connection between these techniques and simualtions is discussed.

References:

[1] D. V. Matyushov, J. Chem. Phys. 136 (2012) 085102.
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[3] M. Heyden, D. J. Tobias, and D. V. Matyushov, J. Chem. Phys. 137 (2012) 235103.

The Role of Electrostatics in DNA dynamics and Protein-DNA Binding

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As a polyelectrolyte, it is clear that electrostatics will play a major role in determining the behavior and the interactions of DNA. I will present some recent work using all-atom molecular dynamics simulations and free energy calculations to study the role of electrostatics in the conformational dynamics of the DNA double helix and its interactions with its ionic environment and also in the recognition mechanisms leading to the formation of sequence-specific interactions with proteins.

Acknowledgements: Some of the work presented involves simulations carried out by the ABC (Ascona B-DNA consortium) group of laboratories and ongoing collaborations with the groups of J.H. Maddocks (EPFL, Switzerland) and M. Orozco (IRB, Spain). The authors thank the Agence Nationale de la Recherche for funding through the projects ALADDIN and CHROME.

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[1] B. Bouvier, K. Zakrzewska, R. Lavery, Protein-DNA recognition triggered by a DNA conformational switch, Angew. Chem. Int. ed. 50 (2011) 6516-6518.
[2] R. Lavery, J.H. Maddocks, M. Pasi, K. Zakrzewska, Analyzing ion distributions around DNA, Nucleic Acids Res. (2014) Epub 10.1093/nar/gku504.
[3] P.D. Dans, I. Fautino, F. Battistini, K. Zakrzewska, R. Lavery, M. Orozco, Unraveling the sequence-dependent polymorphic behavior of d(CpG) steps in B-DNA, (2014) submitted.
[4] M. Pasi et al. μABC: a systematic microsecond molecular dynamics study of tetranucleotide sequence effects on the conformations and fluctuations of B-BNA, (2014) in preparation.

Electrostatic problems for membrane proteins: binding and transport

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I'll discuss a number of our research topics that touch upon the treatment of electrostatics involving membrane proteins. First, I'll discuss our recent analysis of hydration level change in cytochrome c oxidase and its impact on the pK_a of key groups and proton transport in this prototypical proton pump. Second, I'll discuss the treatment of electrostatics for the analysis of binding at anionic membrane surfaces. I'll compare results from implicit membrane and explicit membrane simulations and a simple way to estimate binding free energies at the membrane surface. Finally, if time permits, I'll briefly discuss our recent efforts in describing electrostatics in coarse-grained models for macromolecular solutions.

A (surprising) proton pathway in quinol-dependent nitric oxide reductase (qNOR)

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I will present the results of our recent studies (MD, pK_a and EVB calculations) which suggest that qNOR uses the water channel from the cytoplasmic side of the membrane for proton uptake. This is in sharp contrast to the cytochrome c-dependent NORs (cNOR) which have several pathways connecting to the outer side of the membrane. The proton pathway in qNOR is positioned equivalently to the K-channel in cytochrome c oxidases.

Can electrostatic help to decipher the mechanism of the Qi site of the b_6f complex?

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Our aim is to present questions concerning the quinone reducing site Qi of the b_6f complex that we are struggling to answer with the hope that electrostatics could help us to overcome them. The cytochrome b6f complex belong to the Rieske/cytchrome *b* family, it is a partial homologue of the mitochondrial and bacterial bc_1 complex. It is a complex with a mass of 2x100 kDa that comprises eight polypetide chains, 2 *b* haems, one *c* haems, one *c*' haem, one chlorophyll *a* and one carotene. Our aim here is to focus on the Qi site that in addition to the b_H haem, present in the bc_1 complexes, harbours a c' haem called c_i. The later has peculiar properties: It has only one thioether bridge, it has no proteic axial ligand. Indeed one side is protected by a relatively flexible Phe, while the other side possesses a water molecule or a hydroxyl ion as axial ligand, which is itself hydrogen bounded with a propionate from the b_H haem. Binding of external ligand like NQNO will induced a strong downshift of the apparent redox potential and modify the electromagnetic coupling oh the haem c_i with b_H.

New *in silico* methods to model biological membranes with increased realism

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The study of biological membranes has been for quite some time a challenge for researchers. In recent years, molecular modelling is probably the field of research that has more enthusiastically contributed with information at the atomic level. A detailed description of a lipid bilayer has to take in consideration all important factors that affect the membrane behavior and stability. pH is recognizably one of these factors even though it is usually ignored due to its high complexity.

The presence of negatively charged groups in the membrane gives rise to a surface electrostatic potential. The negative charges come mainly from anionic lipids which are prone to protonation under certain conditions. It is not so uncommon to find this fact neglected in the literature. Many studies, both experimental and theoretical, tend to "simplify" the problem by using zwitterionic phospholipids in their membrane models.

In this presentation, I will present the most recent results on the application of the new method that allows the inclusion of pH in molecular dynamics simulations of lipid bilayers.

Acknowledgements: Fundação para a Ciência e a Tecnologia for supporting projects PTDC/QUI-BIQ/113721/2009 and Pest-OE/QUI/UI0612/2013.

Proton pumping: lessons from cytochrome c oxidase and bacteriorhodopsin

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Proteins such as cytochrome c oxidase (CcO) and bacteriorhodopsin (BR) pump protons from the high pH, N-side to the low pH, P-side of cellular membranes. The resulting proton gradient stores the energy from sources such as a photon (BR) or reduction of O_2 (CcO). To pump protons across a protein, the proton affinity of key residues must change through the reaction cycle. In addition, the access of protons to the N- and P-sides of the membrane must be controlled so that protons are taken up from the side at low concentration and released to the side at high concentration.

CcO was investigated to show how the protein controls the proton affinity of E286, a key residue with an unusually high pK_a. In particular, a water filled cavity opens when the heme a_3 propionic acid is protonated, resulting a pK_a of E286 that is low enough that it can be protonated at pH7 [1]. In addition, the protonation in CcO was calculated through a series of redox and protonation sub-states in the reaction cycle. This provides the location of a proton loading site on the P-side of the protein and the stages in the reaction cycle when it loads and releases protons.

In bacteriorhodopsin the proton transfer between the central cluster (CC, the Schiff base, D85 and D212) and the P-side, extracellular cluster (EC, E194 and E204) must be closed in the M state where the proton is released from the EC. Later in the O intermediate a pathway opens to allow a proton from D85 to reprotonate the EC. The hydrogen bond networks were mapped in BR and the energy of proton hopping between the CC and EC calculated. The connections and energy barriers for proton transfers in BR structures trapped in different reaction cycle intermediates will be described.

Acknowledgements: We acknowledge helpful discussions with Qiang Cui and Puya Goyal.

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Constant pH simulations in explicit solvent with EDS-HREX method; Sampling with DR-pH-Rx method

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I will present a new method for molecular dynamics simulations at constant pH in explicit solvent (EDS-HREX) [1], and a new sampling method in constant pH simulations (DR-pH-Rex) [2]. The (EDS-HREX) method is based on a combination of the enveloping distribution sampling (EDS) and Hamiltonian replica exchange (HREX) methods. EDS generates a hybrid Hamiltonian of different protonation states. Replica exchange between EDS potentials with different *s* values allows for sampling of multiple protonation states with frequent state transitions. The accuracy and efficiency of this method is tested on aspartic acid, lysine, and glutamic acid, which have two protonation states, a histidine with three states, a four-residue peptide with four states, and snake cardiotoxin with eight states. The pK_a values estimated with the EDS-HREX method agree well with the experimental pK_a values. The double reservoir pH replica exchange method (DR-pH-Rex) method relies on pre-generation of two reservoirs of conformations that correspond to two end states at pH values where titratable groups are fully protonated and fully deprotonated. The end state conformations are coupled to simulations at intermediate pH values through the pH-Rex methodology. The method is tested on model compounds, a small peptide and V66K variant of Staphylococal nuclease and performs better than the pH-Rex method.

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New simulation techniques for studying proton-dependent processes in biology and material sciences

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Over the last decade, significant progress has been made in the development of constant pH molecular dynamics techniques. Here I discuss our recent development of the all-atom continuous constant pH molecular dynamics technique and how it enables atomically detailed knowledge of proton-dependent processes in biology and material sciences. The latter will be illustrated using application studies involving spider silk protein, proton channel as well as surfactant and peptide systems that undergo pH-dependent self-assembly and phase transitions.

Constant-pH MD in membrane environments: from mimetic solvents, to peptide internalization, to pH gradients

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The ionization behavior of (bio)molecules in membrane environments involves an interplay between polar and non-polar interactions, as well as membrane asymmetry, that is sometimes unexpected or non-straigtforward. I will illustrate the use of our constant-pH MD method to address membrane environments modeled in three different ways. The first study involves the ionization of the N-terminal amine of a dipeptide in a membrane bilayer, showing how a simplistic view of membrane electrostatics can be seriously misleading. The second study involves the study of a helical membrane peptide in a membrane-mimetic solvent, questioning whether the suitability of such solvents should be taken for granted. Finally, I will discuss some of our on-going work on the inclusion of membrane pH gradients in the constant-pH MD method

pK_a Values in Proteins – What do they mean and what can be measured?

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Because of their central importance for understanding enzymatic mechanisms, pK_a values are of great interest in biochemical research. It is common practice to determine pK_a values of aminoacid residues in proteins from NMR or FTIR titration curves by determining the pH at which the protonation probability is 50%. 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 non-linearly on pH. To account for this non-linear dependence, we show that it is required to introduce pK_a values for individual sites in proteins that depend on pH. The pH dependence of pK_a values may be an important concept in enzyme catalysis. By neglecting this concept, important features of enzymes may be missed and the enzymatic mechanism may not be fully understood.

Electrostatic free energies and ligand binding in GTPases

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I will use one (possibly two) universally-conserved GTPase(s) to illustrate the complexity of electrostatic interactions in proteins and some of the difficulties in modelling them. The first system is the archaeal Initiation Factor 2, or aIF2, involved in translation of the genetic code. It switches between an active, ON conformation and an inactive, OFF conformation under the control of GTP or GDP along with a co-bound Mg^{2+} ion. To understand the nucleotide-specific conformational selection in this system, we compute relative GTP/GDP binding free energies in both the ON and OFF states, and interpret them with the help of a dielectric continuum model and a free energy component analysis. We analyze the effect of a simple force field for phosphate:magnesium binding, and compare to the polarizable AMOEBA force field. We also consider possible free energy artefacts related to the periodic simulation model and the long-ranged Coulomb potential, and those related to Ewald summation with “tinfoil” boundary conditions. If time allows, I will show how a second translational GTPase, aIF5B, may follow a very unusual scheme for a GTPase, with its ON/OFF states defined by the binding of a single proton, without any conformational switching.

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The Combined Effect of Water Solvation and Electrostatic Interactions on General Base Catalysis in Serine Protease.

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We shall present the comparative analysis of the mechanism of general base catalysis in water solvated serine protease chymotrypsin and intarmembrane rhomboid serine protease, E. coli GlpG. The former contains classical catalytic triad, Asp-His-Ser. The latter contains the His-Ser catalytic dyad only. We shall analyze how the difference in the catalytic machinery between two families of proteases is reflected in the fact that many commonly used inhibitors of water solvated serine protease are inefficient in rhomboid proteases. The analysis is based on our MD-QM/SCRF(VS) approach combining molecular dynamics and QM calculations for the estimation of pKa of catalytic residues in enzyme active site.

Development and Applications of Polarizable Atomic Multipole AMOEBA Protein Electrostatics

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Protein electrostatic network optimization via the global optimization of protein side-chains using discrete rotamer libraries is a challenging problem due to the large number of rotamer permutations. To address this, rigorous inequalities have been described that eliminate high-energy rotamers, rotamer pairs, and so on (i.e. dead-end elimination), dependent on the assumption of a pairwise decomposable energy function. However, important biomolecular driving forces, including the hydrophobic effect and electronic polarization, are fundamentally many-body in nature. Neglect of these forces has unduly limited the accuracy of rotamer optimization and its applications to protein structure refinement and design. We will describe rotamer elimination criteria that facilitate provable global optimization of protein side-chains using a polarizable (many-body) force field. Our many-body elimination criteria are only modestly more complex than pairwise criteria, yet drastically reduce the error relative to truncation of a many-body potential at pairwise residue-residue interactions. This opens the door to the use of not only polarizable force fields, which are now widely available for protein simulations, but also quantum mechanical potentials and/or implicit solvents such as Poisson-Boltzmann, Generalized Born and Generalized Kirkwood. The power of this method is demonstrated on mid-resolution structures of proliferating cell nuclear antigen (PCNA), including insights into the physiochemical basis for the destabilizing effect of mutations.

Drude Polarizable Force Field for Proteins and Peptides: Development and Applications

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The polarizable force field based on Drude oscillators has been recently developed. Discussed is the optimization of the electrostatic parameters of the polypeptide backbone and the connection between the backbone and side chains. The specific nature of the force field, namely the interconnection between the interacting local dipoles, require the use of innovative approaches to determine electrostatic parameters. Optimization of the backbone electrostatic parameters targeted quantum mechanical conformational energies, interactions with water, molecular dipole moments and polarizabilities. Promising models were tested against experimental condensed phase data for short polypeptides (Ala_5). Additional optimization of the backbone phi, psi torsional were performed through empirical adjustment of the two-dimensional CMAP terms. Validation of the model included simulations of a collection of peptides and proteins. This first generation polarizable model is shown to maintain the folded state of the studied systems on the 500 ns time scale in explicit solvent MD simulations. The Drude model typically yields larger RMS differences as compared to the additive CHARMM36 force field (C36) and shows additional flexibility as compared to the additive model. Analysis of dipole moments associated with tryptophan side chains show the Drude model to have considerably larger values than those present in C36, reflecting the induced effects from neighboring residues and solvent. Although there are still some limitations, the presented model, termed Drude-2013, is anticipated to yield a molecular picture of peptide and protein structure and function that will be of increased physical validity and internal consistency in a computationally accessible fashion.

Polarizable molecular interactions in condensed phase and their equivalent nonpolarizable models

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Earlier, using phenomenological approach, we showed that in some cases polarizable models of condensed phase systems can be reduced to nonpolarizable equivalent models with scaled charges. Examples of such systems include ionic liquids, TIPnP-type models of water, protein force fields, and others, where interactions and dynamics of inherently polarizable species can be accurately described by nonpolarizable models. To describe electrostatic interactions, the effective charges of simple ionic liquids are obtained by scaling the actual charges of ions by a factor of 1/sqrt(eps_el), which is due to electronic polarization screening effect; the scaling factor of neutral species is more complicated. Here, using several theoretical models, we examine how exactly the scaling factors appear in theory, and how, and under what conditions, polarizable Hamiltonians are reduced to nonpolarizable ones. These models allow one to trace the origin of the scaling factors, determine their values, and obtain important insights on the nature of polarizable interactions in condensed matter systems.

The role of electrostatics in the prediction of binding hot-spots

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Although protein-based interfaces usually comprehend a high number of residues it has been proved that the majority of the binding energy can be accounted by the interaction of a small number of residues known as Hot-Spots (HS).[1] HS detection is still a difficult process. Although various types of methodologies have been proposed ranging from feature-based methods[2] to full atomistic approaches[3], we are still far from a 100% reliable protocol. While laborious, fully atomistic approaches allow a quantitative representation of the binding HS. However, one of their major limitations is the calculation of the electrostatic term, problem stressed out even more in protein-DNA interfaces, typical highly charged and polar. We studied the influence of the variation of several parameters in the calculation of the binding free energy values of a dataset of protein-DNA mutants: the ionic concentration; the energy terms included in the calculation; the various types of solvent representation; the force field used; the PB solvers used; the MM-PBSA vs MM-GBSA approach; and the internal dielectric constant. Our final formulation of the method consists in performing explicit solvent MD simulations and a post-processing treatment using the GB model with a 0.145 M salt concentration, which is the typical value inside the cell. We also noted that we could get more accurate values if we only considered the two major energetic terms at play $\Delta\Delta E_{ele}$ and $\Delta\Delta G_{polar\ solvation}$. Overall our method yields for the data set an average error $|((\Delta\Delta E_{ele}+\Delta\Delta G_{polar\ solvation}) -\Delta\Delta G_{experimental})|$ of 1.55 kcal/mol, 0.77 and 0.92 for Accuracy and Specificity, respectively, as well as 0.78 and 0.50 on Precision and Recall.[4] We have to highlight that the charged residues, the ones with higher importance for protein-DNA interfaces, are the ones with higher average errors. This type of behavior was already encountered for the HS determination in protein-protein interfaces[3] and demonstrates the need for more accurate methods to deal with the electrostatic contribution to the binding of protein-based complexes.

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Robustness of Poisson-Boltzmann Binding Free Energy Calculations

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The Poisson-Boltzmann (PB) equation is now widely used to obtain estimates of absolute and relative electrostatic (polar) binding free energies. The molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) protocol is now widely used for many applications such as drug design. The use of this protocol entails thousand or more PB binding free energy calculations for snapshots of molecular dynamics simulations of a biomolecular complex. Due to the widespread use of the MM-PBSA approach and other PB-based binding applications it is critical to obtain robust polar binding free energy predictions that are accurate, fast and reliable [1-2]. With this goal in mind we examine the polar binding free energies of a diverse set of biomolecular complexes.

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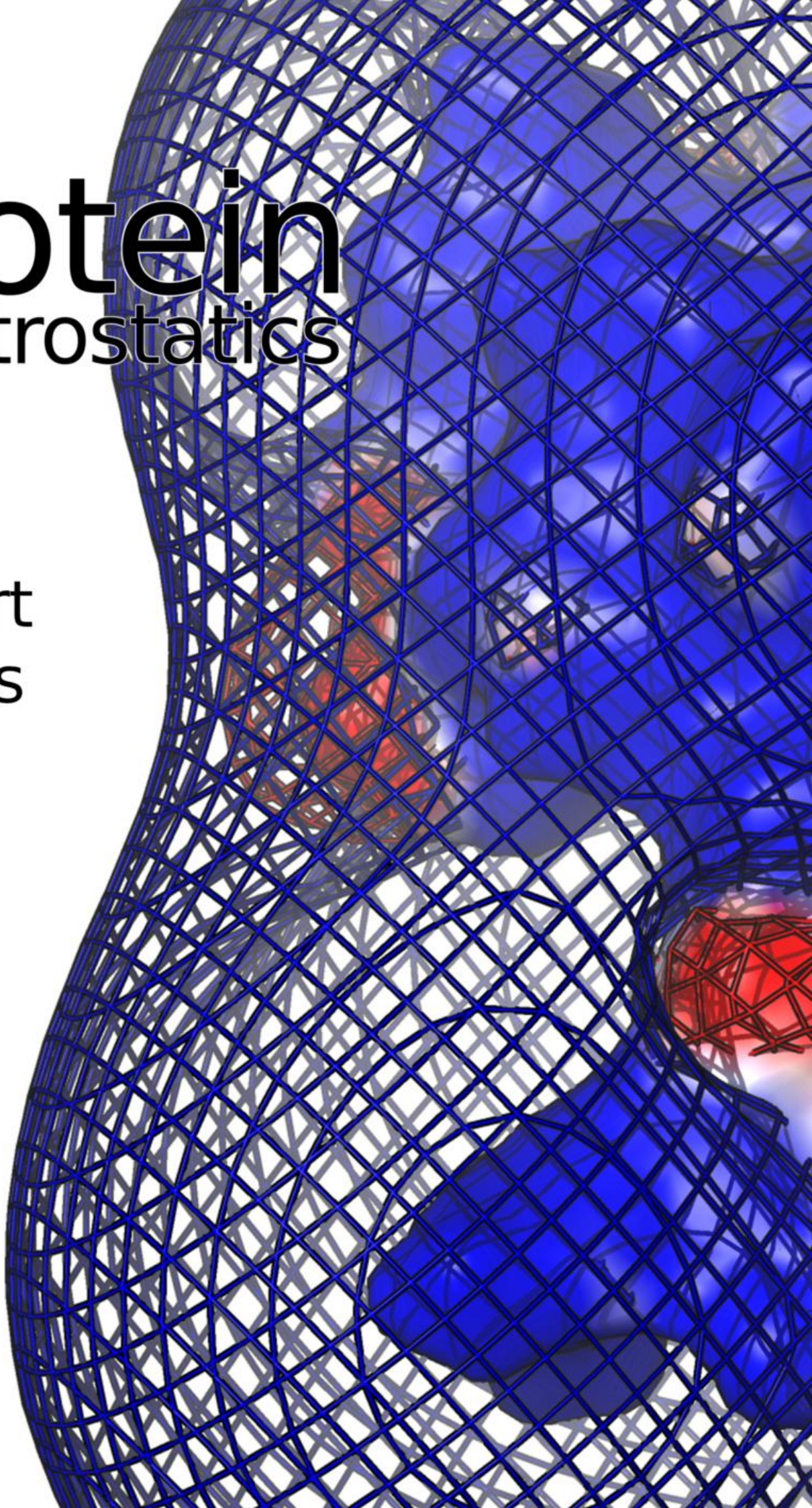
Protein Electrostatics

Short Talks



On the treatment of ionic strength effects close to charged surfaces

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Biological membranes are complex systems that have been broadly studied in recent years. Due to the presence of many different anionic lipids, these membranes are negatively charged and sensitive to pH. The resulting ionization and the ion distribution close to the bilayer are two of the main challenges in biomolecular simulations of these systems. Until now, the two problems have been circumvented by using ionized (deprotonated) anionic lipids and enough counterions to preserve the electroneutrality. In this work, we propose an alternative method based on the Poisson–Boltzmann equation to estimate the counterion and coion concentration close to a lipid bilayer that defies the need for neutrality at this microscopic level. The estimated number of ions was tested in a 25% DMPA/DMPC lipid bilayer at different ionizations and our results are in strong agreement with experiment. Our approach was the only one that correctly modelled the ionization dependent isothermal phase transition in the studied system.

Acknowledgements: We acknowledge financial support from Fundação para a Ciência e a Tecnologia through projects PTDC/QUI-BIQ/113721/2009 and PEst-OE/QUI/UI0612/2013 and grant SFRH/BD/81017/2011.

Role of Electrostatic and Hydrophobic Interactions in the Encounter Complex Formation of Plastocyanin and Cytochrome *f*

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Cytochrome *f* (cyt *f*) and plastocyanin (pc) are electron transfer proteins forming a transient complex. During complex formation, an initial encounter complex rearranges into an active complex. Both, electrostatic and non-covalent short-range forces have been reported to be important for the complex in *Phormidium laminosum* or *Nostoc* [1]. In this work, the association of cyt *f* and pc were studied using paramagnetic NMR spectroscopy, Monte Carlo simulations [2] and ensemble docking in order to get deeper insights into the dynamics of the electron transfer complexes. For this purpose, spin labels were attached to cyt *f*, and relaxation enhancements of pc nuclei were measured, demonstrating that a large part of the cyt *f* surface area is sampled by pc. The distribution of the encounter complex indicates that electrostatic interactions are important for the protein preorientation. The following gradual rearrangement increases the overlap of nonpolar surface areas leading to an electron transfer active complex.

In order to characterize the influence of different interaction contributions in detail, we studied a cross complex of *Nostoc* cyt *f* and *Phormidium* pc [3]. Our results indicated that this complex interacts with an affinity that is intermediate between those of the *Nostoc* complex and *Phormidium* complex. The lower net charge of pc in Phormidium decreases but not abolishes the attraction to cyt *f*, resulting in the formation of an encounter complex that is more diffuse than that of the *Nostoc* complex. The most affected amino acids of pc are located at its hydrophobic patch, indicating a direct interaction of this patch with the active site of cyt *f*. Thus, electrostatic interactions direct pc towards the active center of cyt *f*, but the final complex is predominantly stabilized hydrophobically.

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Combining molecular dynamic simulations with electrostatic energy calculations to evaluate pK_a values and study proton transfer pathways in proteins

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The accuracy of pK_a prediction in proteins using electrostatic energy computations depends sensibly on the quality of the protein structure used for the computation. Whenever a protein undergoes larger structural changes as a result of protonation or deprotonation of individual residues, a single structure - e.g. an x-ray crystal structure - is not sufficient to describe the protein for the whole range of pH, resulting in inaccurate pK_a predictions. So far this problem was addressed by the software Karlsberg+ [1] by modeling several protein conformations, each representing the protein in a certain pH range. This modeling was very simple and included the relaxation of hydrogen bonds and salt bridge interactions. While being already a significant improvement, this approach reaches its limit when structural changes become too large, e.g. by rearrangement of the protein backbone or the formation of water filled cavities. To overcome this limitation we generalized the method by sampling protein conformations for different protonation states using molecular dynamic (MD) simulations. Thereby each MD trajectory represents the protein in a certain pH range. One of the most crucial steps for the method is the correct choice of protonation states used for the titratable residues in the MD simulations. An advantage of electrostatic energy calculations is the possibility to get a detailed understanding how individual residues influence the total energy of the system as a result of changes in structure and protonation state. This information is used to adapt the conditions for MD simulations iteratively until the protonation states corresponding to a specific pH interval remain invariant. This procedure leads to improved pK_a prediction. In addition, the software Karlsberg+ has been extended with tools to analyze the results of the electrostatic energy calculations in a novel way in order to identify and study proton transfer pathways in proteins.

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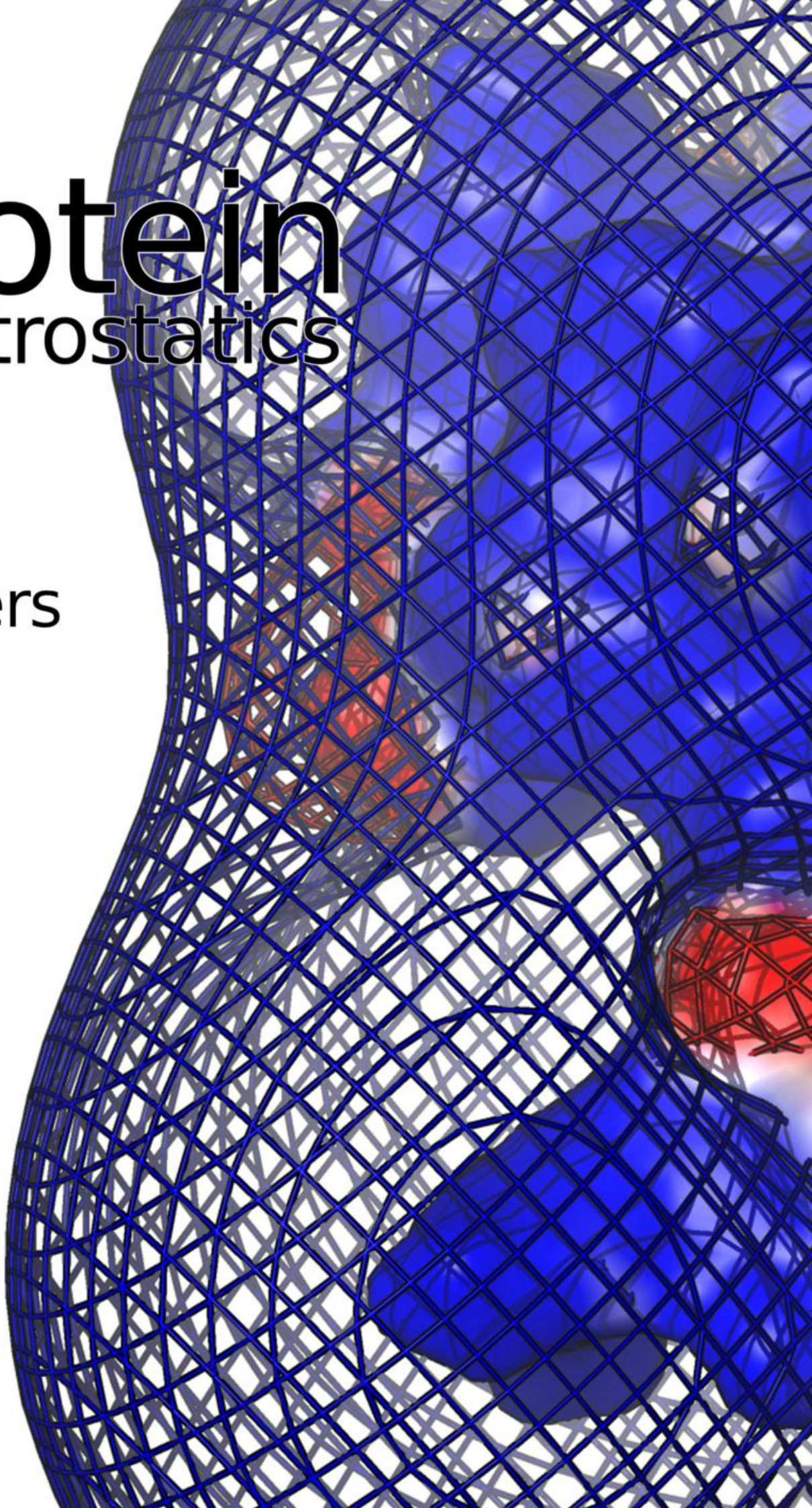

Proton Gating in Cytochrome *c* Oxidase via D- and K-channel

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The central chemical reaction of aerobic metabolism is the conversion of molecular oxygen to water catalyzed by cytochrome *c* oxidase (C*c*O). In a stepwise redox-reaction, four electrons and four chemical protons enter the enzyme and are consumed in the reaction, while four physical protons are pumped across the membrane establishing an electro-chemical gradient. While all four electrons are transferred via the same cofactors to the binuclear reaction center (BNC), the protons take two different routes in the A-type C*c*O. Two of the four chemical protons arrive via the D-channel in the oxidative first half starting after oxygen binding. The other two chemical protons enter via the K-channel in the reductive second half of the reaction cycle. In contrast, all four pumped protons are transferred via the D-channel. This branched conduction of protons requires specific constraints onto the proton delivery channels.

In this study, we use molecular dynamics simulation and electrostatic energy computations to analyze the proton channel properties and their gating elements that ensure unidirectional proton flux. The D- and the K-channel appear very different at first glance. The D-channel possesses an hydrogen bond network of water and several acidic groups connecting the aqueous phase with the BNC. In contrast, the hydrogen bond pattern of the K-channel is disconnected having only few titratable groups and little amount of water molecules in the crystal structure. Still, the channels share some similarities. Most impressively, both channels have in common a hydrophobic environment around their key residue – Glu286 (numbering from *R. sphaeroides*) in the D-channel and Lys362 in the K-channel. As a consequence, these residues are likely in the charge-neutral protonation state, while glutamate and lysine are usually charged in aqueous solution. Therefore, change of protonation of Glu286 and Lys362 may be induced by changes in the hydration level or the electric field in a regulated manner. The unique feature of these key residues enables them to control proton movement inside the input channels. But there are also contrasting properties; e.g. Glu286 must release a proton to render the D-channel conducting, while Lys362 takes up a proton to open the K-channel.

Our comprehensive analysis of these two residues may explain several experimental observations and elucidates also general features of regulated proton conductance.

Influence of H2 diffusion and proton transfer pathways in the high activities of a [NiFeSe] hydrogenase

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Hydrogenases are metalloenzymes that catalyze the reversible reaction H_2 <-> $2H^+ + 2e^-$. Their study can lead to the development of new catalysts that will enable the use of H_2 as an alternative fuel. Among the class of the [NiFe] hydrogenases, the subgroup of the [NiFeSe] hydrogenases is particularly interesting because of their higher activities [1]. However the structural determinants responsible for this property remain unknown. In hydrogenases, the H_2 and proton pathways are very important because of the deeply buried active site, but they have never been characterized in [NiFeSe] hydrogenases. Therefore we ask if the higher activities of [NiFeSe] hydrogenases are related with these pathways.

We used molecular dynamics simulations to study H_2 diffusion. The explicit solvent simulations, with 100 H_2 molecules added to protein, were performed with the software GROMACS and the GROMOS 43a1 force field. To identify residues involved in proton transfer we calculated the protonation thermodynamics of the protein using a combination of Poisson-Boltzmann calculations and Monte Carlo simulations. Additionally, we also used a distance based criteria.

The comparison with previous results for a standard [NiFe] hydrogenase [2,3] revealed the existence of an extra channel for the H_2 diffusion exclusive of the [NiFeSe] hydrogenase which may explain the higher activities of these hydrogenases [4]. In the proton pathways found for [NiFeSe] and [NiFe] hydrogenases only two residues near the active site are conserved, which may also be related to the difference in activity between the two groups of enzymes [4].

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Accuracy of electrostatic energy calculations using the finite difference and finite element method to define molecular shapes

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Finite difference (FD) solvers are widely used in solving electrostatic problems. Accuracy is eminent for algorithms, which e.g. calculate solvation energy or pK_a values of molecules. In this work we analyze the accuracy of electrostatic potential calculations which are computed by FD and alternatively by finite element (FE) methods. Providing insight into the molecular surface generated by different methods, we help the community to understand the influence of model discretization on regular and irregular grids. The later are used in the FE method. We look at surface shapes and analyze errors in electrostatic energy, volume and surface area by using small model systems and lysozyme (PDB id 2LZT) as an example for a more complex molecular shape. This analysis helps to understand possible systematic errors occurring in discretization of molecular systems. To obtain small errors in molecular solvation energies, effort is needed for molecular surface generation and also charge discretization. In examples it is demonstrated for the FD end FE method how the discretisation error of molecular surfaces is influenced by differences in the molecular shape in particular by concave and convex molecular surface regions.

Simulation of Charge and Exciton Transfer Kinetics in Proteins

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Capturing sunlight and converting it to chemical energy is a central process in nature. The complexity and the number of proteins taking part in this process vary strongly depending on the organism. Examples of such proteins are light harvesting complexes such as LH2 and LH1, which are part of purple bacterial photosynthetic membranes, or bacteriorhodopsin, a membrane protein which uses sunlight directly to generate a proton gradient. To fully understand the underlying mechanism of these proteins, the analysis and simulation of the exciton transfer as well as the proton and electron transfer processes are crucial.

We present here the application of a Dynamic Monte-Carlo (DMC) algorithm [1] to simulate this kind of transfer kinetics [2]. At each time step the analyzed system is represented by a microstate description [3]. Depending on the kind of reaction, transition rates between these states are taken either from the literature or are calculated based on continuum electrostatics and Marcus theory. To test the reliability of our method energy transfer in arrays of light harvesting antenna complexes (LH2 [4,5]), transfer kinetics in LH1-RC complexes and proton transfer in bacteriorhodopsin were investigated.

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Conformational determinants of peptidic tree-like molecules: insights from molecular dynamics simulations

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Peptide dendrimers are tree-like molecules formed by alternating functional amino acids with branching diamino acids such as lysine.[1] Unfortunately these molecules have not yielded to structural characterization and little is known about their molecular-level structure. Computational methods seem to be an adequate tool to address these issues.

Herein we present a comprehensive structural characterization of peptide dendrimers using molecular simulation methods.[2,3] Multiple long molecular dynamics (MD) simulations were used to extensively sample the conformational preferences of several third-generation peptide dendrimers, including some known to bind aquacobalamine.

The results clearly show that a trade-off between electrostatic effects and formation of hydrogen bonds controls structure acquisition in these systems. Moreover, by selectively changing the dendrimers charge we are able to manipulate the exhibited behavior.[3]

Our results are in accordance with the most recent experimental evidences and shed some light on the key molecular level interactions controlling structure acquisition in these systems.

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Continuum electrostatics calculations of C*c*ox in the presence of a pH gradient

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Cytochrome *c* oxidase (C*c*ox) is an integral membrane protein complex which couples the oxidation of cytochrome *c* to the reduction of O_2 to H_2O. C*c*ox is also a proton pump, generating an electrochemical gradient, which provides the energy to fuel different cellular processes.[1,2] As a proton pump, C*c*ox is negatively affected as the pH gradient that it generates across the membrane increases. In order to study the effect of the pH gradient on the titration behavior of several key sites, we have modified our continuum electrostatics method in order to include this gradient, similarly to what was done by Calimet *et al*.[3]

C*c*ox was inserted in an explicit DMPC membrane, and all titratable sites were considered to be connected to either the P- or N-side of the membrane, which were exposed to different pH values. We observed the effect of the pH gradient on the titration behavior of C*c*ox in its oxidized and reduced forms, particularly regarding the residues in the catalytic core and in the K- and D-channels. The effect of the pH gradient on key residues was analyzed by calculating the two-dimensional titration curves of these residues as function of the pH on each side of the membrane. In particular, we found that Lysine-362, in the K-channel, although located in the N-side of the membrane, was affected by the pH on both sides.

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A molecular view on the interaction of antibiotic deoxy glycosides with model phospholipid bilayers

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Sugar-based surfactants are known for their use in membrane protein crystallography and feature many other applications as a result of their biocompatibility properties and low toxicity when compared to traditional cationic detergents. We have developed a new family of alkyl deoxy glycosides with relevant antibacterial activity against *Bacillus spp.* and other pathogens.[1] Experimental data shows that deoxygenation of the sugar moiety leads to an increase on the surface activity of these molecules, which seems to modulate their antimicrobial properties. Many amphiphilic antibiotics are known to target bacterial membranes, destabilizing the thermotropic properties of lipid bilayers upon insertion. Thus, unraveling the molecular details of the interactions of these glycosides with model membranes is paramount to understand their mechanism of action.

In this work, we used atomistic molecular dynamics simulations to characterize the micelles formed by these glycosides in aqueous media and study their behavior at a model phospholipid bilayer interface. We also simulated phospholipid/glycoside binary mixtures to analyze the effect of partitioning increased molar fractions of glycosides into the bilayer on its structural features. The results herein presented provide valuable information with respect to the physical and biological properties of these molecules and may have implications on the design of new antibiotics with increased potency.

Acknowledgements: QREN-COMPETE program is gratefully acknowledged for the financial support of the project FACIB (QREN-SI I&DT in co-promotion – Project nr. 21547), and Fundação para a Ciência e a Tecnologia for supporting strategic project Pest-OE/QUI/UI0612/2013 and PhD grant SFRH/BD/81017/2011. COST is acknowledged for funding through action CMST/COST-Action/CM1102.

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Protonation behavior of peptidic scaffolds containing three close histidines studied by constant-pH MD

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C-Asp, O-Asp, C-Asn, and O-Asn are four peptidic scaffolds synthesized to mimic the metalloproteins coordination of Cu^{2+} [1, 2]. These decapeptides contain three histidine residues and are able of forming a main coordination species at neutral pH that seems to coordinate a single Cu^{2+} through the three neutral imidazole groups. The peptides are either cyclic (C-Asp and C-Asn) with two Pro-Gly β-turn inducer units, or open (O-Asp and O-Asn) with only one Pro-Gly unit. C-Asp and O-Asp contain a single aspartate residue that also coordinates the Cu^{2+} ion, contributing to a stronger binding. This residue is substituted by an asparagine in C-Asn and O-Asn. Besides cyclization and the presence of aspartate, Cu^{2+} binding depends on the protonation state of the histidines, for which reason a characterization of the protonation behavior of the peptides becomes relevant.

These four peptides were simulated at different pH values (2–11, C-Asp and O-Asp; 4–11, C-Asn and O-Asn) using the stochastic titration constant-pH MD method [3, 4]. The titration curves obtained from the simulations were in excellent agreement with the potentiometric titration curves [1, 2], with some deviation for O-Asp at pH < 4 explained by the frequent interactions between aspartate and nearby histidines slightly affecting the protonation sampling. The microscopic pK_a values were also calculated by fitting each residue's protonation curve to a Hill equation, confirming very close pK_a values for the histidines.

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Unraveling the coupling between protonation and conformation: Constant-pH MD simulations of Cytochrome *c* oxidase

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Cytochrome c oxidases (CCOX) are members of the heme-copper oxidase superfamily and are the terminal enzymes of the respiratory chain. These proteins are membrane-bound multi-subunit redox-driven proton pumps, which couple the reduction of molecular O_2 to water with the creation of a transmembrane electrochemical proton gradient.

Over the last 20 years, most of the CCOX research focused on the mechanisms and energetics of reduction and/or proton pumping but, until now, these mechanisms are still elusive. In particular, it is still not clear which are the functionally relevant conformational changes, nor how these rearrangements affect proton pumping. The main objective of this work is to identify the redox-induced structural changes and to study the dependence of protein's structure with the protonation state of certain residues. For that, we have performed extensive constant-pH MD simulations [1] of CCOX from *Rhodobacter sphaeroides* in two states (oxidized [2] and reduced [3]) inserted into a lipid bilayer. From our simulations, several residues with unusual titration behaviors highly dependent on the redox state of the metallic centers were identified.

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The complex protonation behavior of PEI polymers: a computational study

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As far as gene therapy is concerned, the development of new delivery agents is pivotal, since the commonly used viral vectors have many associated issues like toxicity, immunogenicity and even the relatively high costs. That is where the cationic polymers, polyethyleneimines (PEI) in particular, appear as efficient alternatives, mainly due to their capabilities of transfecting efficiently and consistently different types of cells, having also low immunogenicity, toxicity and costs. [1,2]

Despite all this, PEI knowledge is still in a very early stage, with limited comprehension about the complexation process between PEI and DNA and their internalization in cells. In order to complement experimental data, some information at the molecular level of these processes can be retrieved using computational methods. Constant-pH molecular dynamics simulations allow the inclusion of pH effects and the correct sampling of both protonation and conformation in a coupled way. With this approach, we can study the influence of pH in the conformation of PEI, both alone and complexed with DNA. Also, PEI has been synthesized and used in different forms, linear, comb-like branched and dendrimer-like hyperbranched structures. Therefore, in this work, we present the protonation behavior of PEI in three different degrees of branching, alone and in the presence of a DNA model.

Acknowledgements: We acknowledge financial support from Fundação para a Ciência e a Tecnologia through project PEst-OE/QUI/UI0612/2013 and grant SFRH/BD/81017/2011.

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Protein Electrostatics

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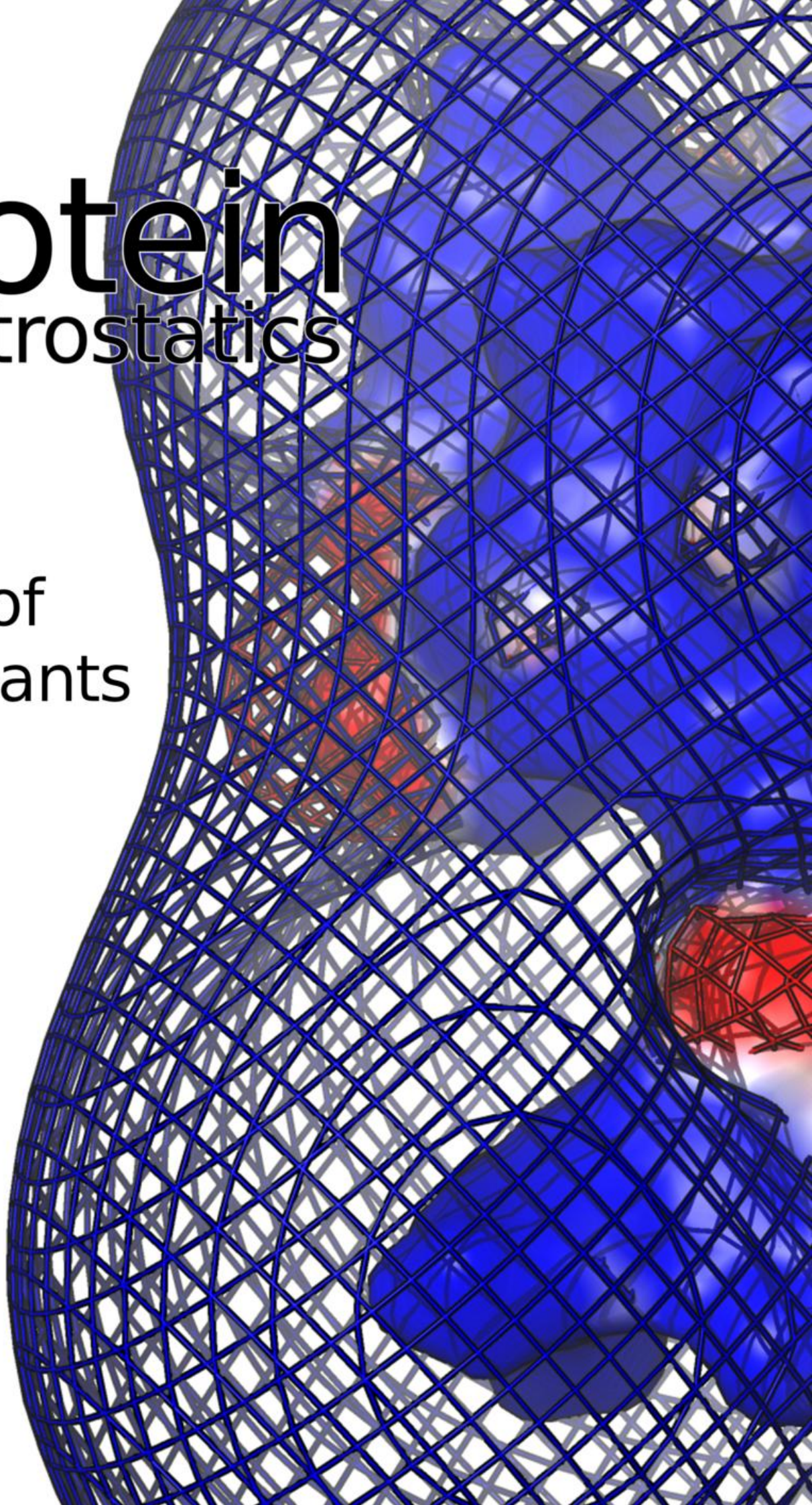

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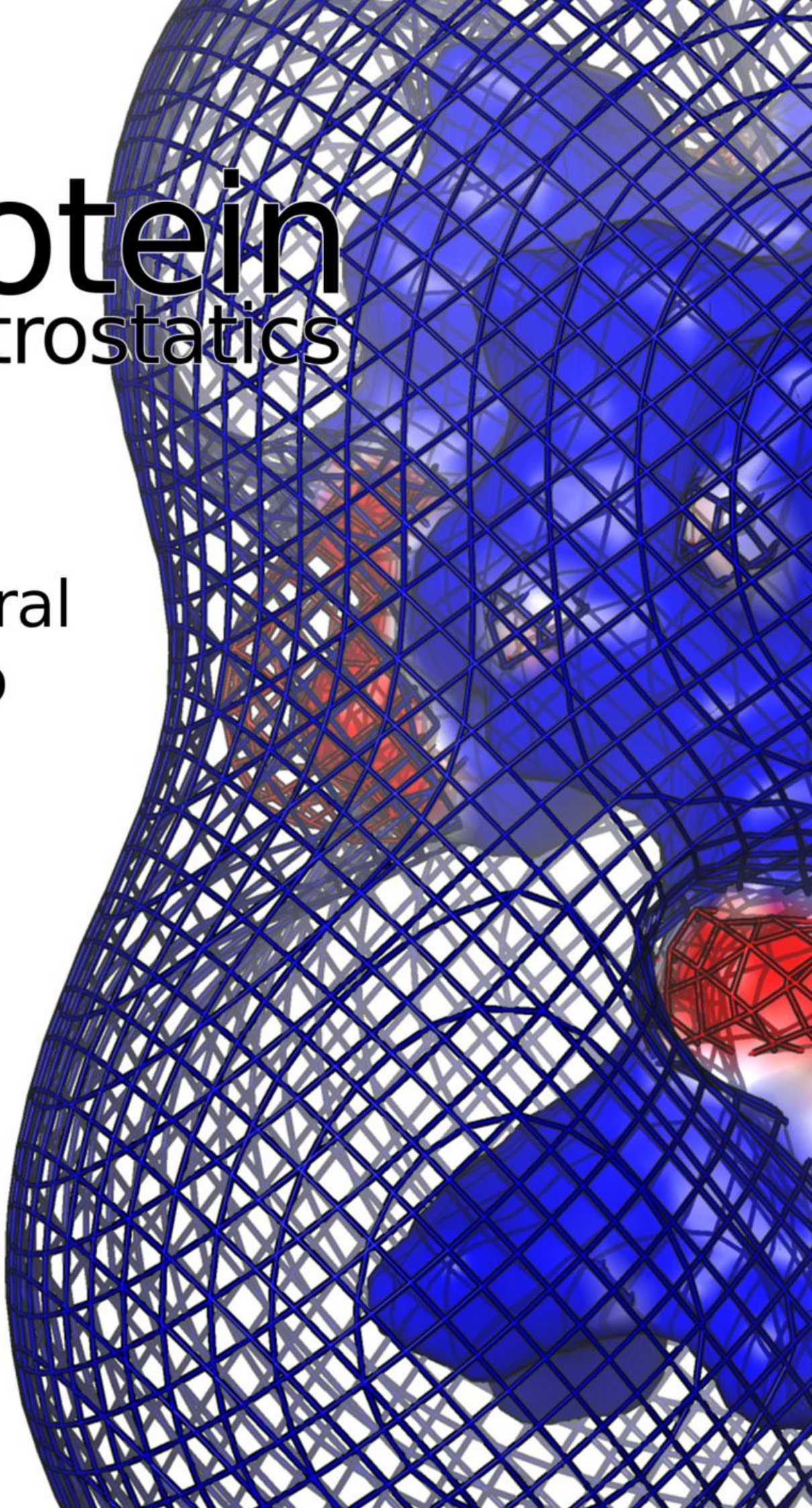

GENERAL INFORMATION

Passport and Visas
A valid passport (or identity card for European Community nationals) is required. Visas are not necessary for citizens of European Union countries, the U.S.A., Canada and most major countries. Please contact your local Portuguese Embassy, Consulate or your Travel Agency for further information.

Health Requirements
With the exception of vaccination certificates for persons coming from areas where yellow fever is endemic presently there are no special health requirements.

How to reach Lisboa by air
Lisboa is just a few hours flying away - 6/8 hours from North America and 2/3 hours from most European cities. TAP Air Portugal, with more than 60 offices around the world and daily flights to and from major cities, covering almost 50 destinations in four continents.

Lisboa Airport welcome desk
TA DMC own office, located right at the arrivals hall of Lisboa airport, provides personalized assistance to the incoming participants.

Car rental
Special arrangements are made with the main reliable rent-a-car outlets to grant special discounts to delegates and companions.

Accommodation
The Portuguese Hotel Industry has established a good reputation abroad basically due to the high standard of services rendered. Also good professional teams together with the Portuguese natural warmth and friendliness make a powerful combination that will certainly help you feel welcome.

Language
The official language is Portuguese. English and French are widely spoken.

Local time
Mainland Portugal is 1 hour behind European Standard Time.

Currency
As a member of the European Monetary System the € [euro] is the Portuguese monetary unit. At present, the bills in circulation are: € 5,00 ; € 10,00 ; € 20,00 ; € 50,00 ; € 100,00 ; € 200,00 ; and € 500,00. The coins are as follows: 1 cent ; 2 cents ; 5 cents ; 10 cents ; 20 cents ; 50 cents ; € 1,00 and € 2,00.

Tipping
Tipping is optional, but normally 10% is customary in taxis, restaurants and bars.

Exchange
Major credit cards are accepted in most hotels, shops and restaurants. Travellers cheques and currency can be changed at hotels or at a bank - these are open Monday to Friday from 08h30 to 15h00. Automatic changing and cash dispensing machines linked to international networks are also widely available.

Post and Telecommunications
Automatic direct dial telephone service is available to and from most countries in the world. Public phones accept either a pre-paid card, or coins (see signs on the booth). Credit systems such as AT&T are also available. Post offices are open Monday to Friday from 08h30 to 12h00 and 14h00 to 18h00.

Medical Care
Clinics and hospitals provide round the clock emergency service. The national emergency phone number is 112. Hotels have a doctor on call through the reception. Reciprocal European Union cover is available at out-patient departments, otherwise private consultation fees are charged.

Electrical Current
European type 2 pin sockets with 220 volts AC at 50 cycles are used. The phase 380 volt current is normally available in meeting and exhibition rooms.

Smoking
Smoking is forbidden by law in public transportation, and in closed public areas.

Shops
Shops are open from 09h00 to 13h00 and 15h00 to 19h00 Monday to Friday, and 09h00 to 13h00 on Saturdays. In major town centres and in many shopping malls, they stay open during lunch hours, and close later at night, including weekend.

Main Shopping Areas
The Pombaline section at downtown, bordered by the magnificent Praça do Comércio [Black Horse Square] facing the river Tejo, the Rua do Ouro, Rua Augusta and Rua da Prata finishing at Rossio Square, Avenida da Liberdade, and the "Chiado" leading to Bairro Alto. The new areas of Av. de Roma, Praça de Londres and Av. Guerra Junqueiro. Some of the main Shopping Centres are Colombo [one of the biggest in Europe], Vasco da Gama, Amoreiras, Imaviz, Monumental, Atrium Saldanha,Via Veneto, El Corte Inglês, etc...

Shopping
Fine leather goods, lead crystal ware, porcelain, vintage wines, golden and silver filigree, pottery and specialist textiles are considered excellent buys in Portugal.

Driving
Vehicles drive on the right side of the road. The use of safety belts is compulsory, and children under 12 must ride in the back seats. Portugal has actually a large freeway network crossing the whole country from the North to the South in Algarve, and from the Ocean front to the border with Spain. Valid driving licenses from European Union countries, the USA, Canada and other major countries are acceptable for use in Portugal up to 6 months stay.

Public transportation
There is a wide inexpensive network in all towns and cities. In Lisboa, city of the seven hills, you can choose between bus, underground metro, elevators, electric trams or railway, within the city or to the suburbs. Trains and express bus service also links the main towns of the country.

Meals
Breakfast is normally served between 07.30 and 10.00 hrs., lunch from 12.30 to 15.00 hrs., and dinner from 19.30 to 22.00 hrs.

Dinning
There are a wide variety of restaurants and cafés in Lisboa ranging through the elegant and sophisticated to the casual and inexpensive. Take-away and fast food is also available from many outlets. Restaurants, bars, some with living shows, and discos along the river Tagus, at Rocha Pier and at the Expo 98 site, become very popular, specially at weekend nights.

Entertainment
Lisboa has a variety of theatres and venues catering for most tastes. Opera, ballet, plays, concerts, etc. take place on a regular basis all year round. Portuguese Fado and Folklore shows are popular, and an international show at Casino Estoril [the largest in Europe], with glittering cabaret complements the many discos and other night spots.

Recreation
Excellent golf courses, tennis and squash courts, water sports and horse riding tempt the energetic delegates. The more relaxed might choose from many noteworthy museums and monuments, sunny beaches and interesting cities to explore.

Religious Services
Portugal is predominantly Catholic, but a wide number of other faiths [Protestant, Christian, Moslem, Jewish, and Hindu, among others] are also followed. Please inquire at hotel reception for times and places of services.

Security
Crime rates in Portugal are among the lowest in the world. Hotels have their own security staff, which is sufficient for most events. Specialist firms are also available if necessary, and the police provide special protection for visiting dignitaries and high risk individuals.

Check in / out policy
Should hotel rooms be required for guaranteed occupancy before 14.00 hrs, the previous night should be reserved. Check out time is 12.00 hrs. noon.

Accessibility
Lisboa International Airport located 2 kms from the congress venue, 6 kms from the city centre, and about 20 minutes driving time from Estoril, is served by all major international airlines from all over the world.

Climate
The climate in Lisboa is temperate, offering the best of both Atlantic and Mediterranean sea breezes all year round.

V.A.T.
Value added tax (I.V.A. in Portuguese) is included in prices quoted. For non EU residents, tax free shopping schemes are available at many shops.

Water
Tap water is safe to drink. Portuguese and imported bottled mineral water is also readily available.

Public Hospital
(Closest to Meeting Venue)
Hospital de Santa Maria Avenida Professor Egas Moniz 1649-035 Lisboa Tel: (+351) 217 805 000.

Museums
Most of the Museums are closed on public holidays and open Tuesdays to Sundays, from 10h00 to 17h00. Please check with the Secretariat of the Congress for further information. Some of the most important museums in Lisboa are:

Museu Nacional de Arte Antiga
Ancient Art.

Museu Arqueológico, Convento do Carmo
Archaeology.

Museu Nacional dos Coches
The world's best collection of this type, ranging from the 17th century coach of King Philip II to 19th century carriages.

Museu Calouste Gulbenkian
It houses the 17th and 18th century oil-billionaire Gulbenkian's remarkable collection containing Egyptian, Graeco-Roman, Oriental and European art, and a fine selection of works by René Lalique.

Museu de Arte Moderna
As part of the Calouste Gulbenkian Foundation Museum, it houses the best collection of 20th century Portuguese art.

Museu Militar
Collection of military weapons, armour, paintings and uniforms.

Museu de Marinha
Collection of vessels, paintings and ships rigging. Also the royal barges and sea planes.

Museu Nacional do Azulejo
Collection of decorative tiles (azulejos).

Museu de Arte Sacra
Religious art at São Roque Church

Museu do Chiado
Painting and sculpture collection from the romantic period.

Olissipona (at St. George Castle)
Lisboa's Interpretation Centre.

Ajuda Royal Palace
Furniture, tapestry and painting collection.

City Museum
It houses a large collection, including paintings, drawings, engravings, glazed sculpture, showing the evolution of Lisboa from prehistory until the beginning century.

Museu Ricardo Espirito Santo
Important decorative art collection in the ambience of an old Portuguese manor house.

Museu do Teatro
Collection of costumes and stage props, stage models and costume designs, photographs, posters, programmes, manuscripts, scores, portraits and cartoons. Next to Museu do Traje, amid a beautiful common park.

Museu do Traje
Its collection illustrates the history of court and folk clothing throughout the ages. The museum also mounts temporary exhibitions of fashion design, accessories and jewellery.

Vieira da Silva & Arpad Szenes
Painting Collection

Lisbon Oceanarium

Located at "Parque das Nações", the former Expo 98 site, is the largest in Europe and the second one in the world. Is a vast aquarium recreating four different ocean habitats [the Atlantic, the Pacific, the Indian and the Antarctic], with more than 16,000 living species from all over the world, offers the visitor a spectacular display of light and colour.

Parks and Gardens

Jardim Botânico
Alongside the Academy of Sciences, one of the most beautiful in Europe has an usual display of more than 20,000 exotic plant species.

Zoo
Set in the lovely Laranjeiras Park, one of the richest zoos with more than 2,000 animals and the best dolphinarium in Europe, is definitively one of the nicest in the world, also the Animatrix playground for children.

Parque Ecológico de Monsanto
Ecological park covering an area of 300,00 square meters, provides the perfect interplay between man and nature and offers visitors a close contact with wild animals and vegetal species.

Parque Eduardo VII
With its fascinating "Estufa Fria", a magnificent glass plant hot house with magnificent subtropical flora.

Remarkable Churches and Palaces

Sé Patriarcal (Cathedral)
Build in 1150, blends today a variety of succeeding architectural styles.

São Vicente de Fora (Alfama)
St. Vincent. Italianate, rebuilt in the XVI century.

Santa Engrácia (Alfama)
The National Pantheon, with its high dome.

São Miguel (Alfama)
Built in the 17th century, and restored in the18th.

São Roque (Bairro Alto)
Featuring the most lavishly decorated chapel (baroque) and holding a remarkable Sacred Art Collection.

Palácio da Ajuda
The largest in the city, brimming with art works and curiosities.

Palácio Marquês de Fronteira
Private estate with elegant gardens with remarkable tiles.

Palácio dos Condes de Óbidos
XVIII Palace rich in marble and fine tiles, housing the headquarters of the Portuguese Red Cross.

Palácio Nacional de Queluz
A "rococo" Royal Palace with beautiful gardens, just outside Lisboa.

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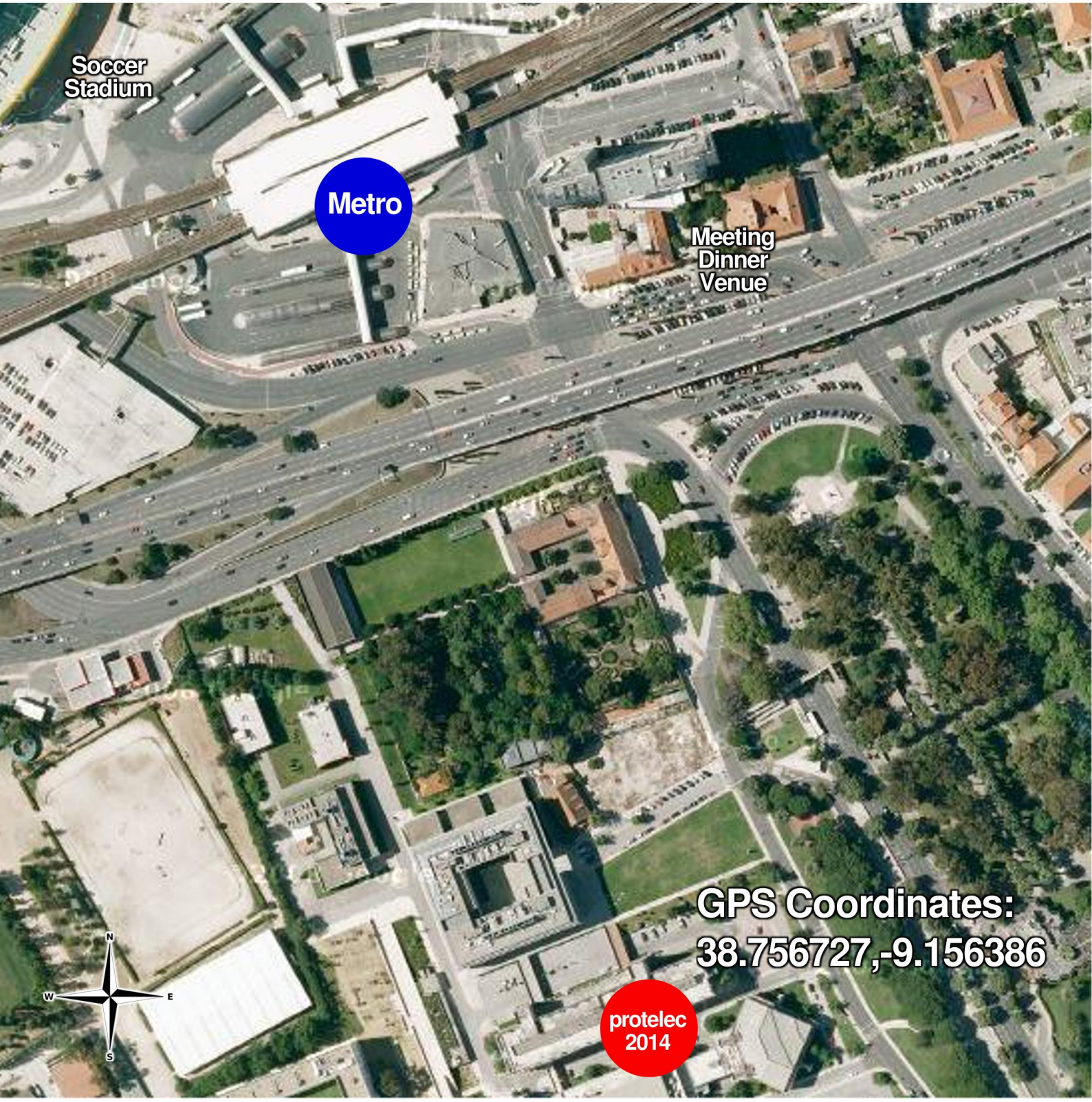
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