PEB 2016

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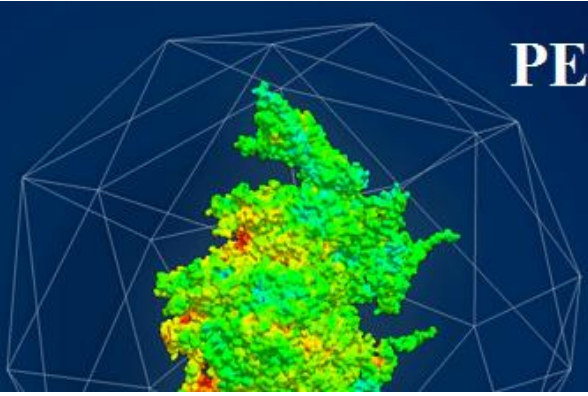

BOOK OF ABSTRACTS

POSTER PRESENTATIONS

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Titratable Macroions in Multivalent Electrolyte Solutions

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In the world of proteins one can find exotic electrostatic phenomena such as long-ranged attraction between two electro-neutral protein particles in an aqueous solution, stemming from thermal charge fluctuations of dissociable charge groups on their surface. It was first observed by Kirkwood and Schumaker, half a century ago, who described it in the framework of statistical mechanical perturbation theory and showed that this interaction scales different from standard van der Waals interaction. Recently we have formulated a theory of fluctuation interaction between macroions subject to charge regulation and showed how the Kirkwood-Schumaker (KS) interaction follows directly from charge regulation, thereby generalizing the KS perturbation approach. Nevertheless, it has been a challenge to broaden a theory to be valid in the regimes where the original KS results fails, which is the case in strongly coupled electrolyte solutions. The experiments showed that the presence of multivalent ions can even enlarge this type of attraction between electro-neutral proteins.

Here we present a theoretical description of the effect of polyvalent ions on the interaction between titratable macro-ions. The model system consists of two point-like macro-ions with dissociable sites, immersed in an asymmetric ionic mixture of monovalent and polyvalent salts. We formulate a dressed ion strong coupling theory, based on the decomposition of the asymmetric ionic mixture into a weakly electrostatically coupled monovalent salt, and into polyvalent ions that are strongly electrostatically coupled to the titratable macro-ions. The charge of the macroions is not considered as fixed, but is allowed to respond to local bathing solution parameters (electrostatic potential, pH of the solution, salt concentration) through a simple charge regulation model. The approach presented, yielding an effective polyvalent-ion mediated interaction between charge-regulated macro-ions at various solution conditions, and describes the strong coupling equivalent of the Kirkwood-Schumaker interaction.

P2

Computational QM/MM Studies of Protonation States of Conserved Histidine Residues in Phytochrome *Agp2*

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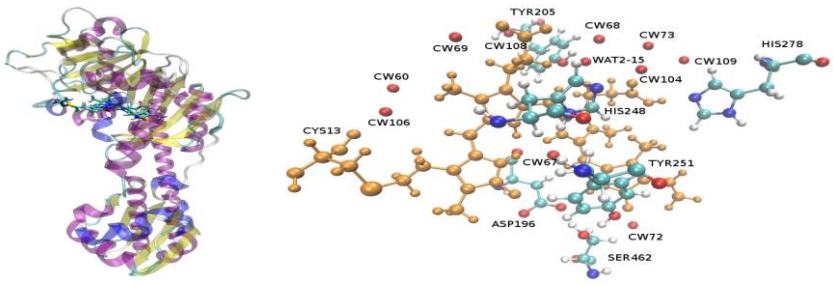
Phytochromes are important light sensitive biomolecular switches, used in nature for a variety of processes in various organisms, like bacteria and plants. Understanding the functionality of these proteins is helped by determination of structural elements, with the help of X-ray crystallography. Many functions in biomolecules are triggered by very small molecular events, such as hydrogen transfers, therefore it is necessary to determine the correct protonation states of potentially involved residues, especially for such as histidine (HIS). This unfortunately cannot be guaranteed by crystal structures alone, that is why theoretical approaches are of importance for filling the gaps. In all phytochromes in general and the one of *Agrobacterium Tumefaciens Agp2*, two conserved HIS (H248 and H278) can be found in near vicinity of the chromophore (tetrapyrolic biliverdine (BV)), which seem to be of utter importance for the photoconversion and stabilization of BV. Here, we report the use of an extended quantum mechanics/molecular mechanics (QM/MM) approach, combining the benefits of both methods (accuracy and moderate computational costs) for the merit of finding the correct protonation state of named residues. Several amino acids, as well as water molecules close to the cofactor were treated quantum mechanically. Further we developed two different models of BV, one with an additional proton (H^+) on the propionic side chain of ring C (PSC(C)) and one without (Model A and Model B respectively). Finally, we created nine diverse models with different protonation sides for the two HIS (either an H^+ on the ε-, δ- or both positions). Analyses of different structural parameters of the optimized structures yielded a clear picture of the most probable structure, which is the H248E/H278D case and is in best agreement to the crystal structure. Also, this is in accordance with recent RR-spectroscopic findings, which suggested, that the ε-position on H278 should be unoccupied in the Pfr ground state, since it becomes necessary for the thermal back conversion from Pr to Pfr and involved H^+transfer from PSC(C) to H278-ε position. Further confirmation was achieved by the computations of Model B, which yielded on one hand H248E/H278P as most stable model and showed on the other hand, a strong tendency of a H^+-transfer from ε-position of H278 to the unoccupied PSC(C), thus resulting again in the H248E/H278D structure. Conclusively, we present here an improvement of the crystal structure of Agp2 *via* QM/MM methods by determination of HIS protonation states, which may help future research of the photoconversion mechanism.



P3

Structural Rearrangements Preceding Dioxygen Formation by the Water Oxidation Complex of Photosystem II

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Water oxidation is catalyzed by the Mn_4CaO_5 cluster of photosystem II. Recent studies implicate an oxo bridge atom, O5, of the Mn_4CaO_5 cluster, as the 'slowly exchanging' substrate water molecule. D1-Val185 is part of the broad aqueous channel as it passes the Mn_4CaO_5 cluster and in the close vicinity of O5. The D1-V185N mutant is shown to retard both the lag and O_2 release phases of the $S_3^+ \rightarrow S_0$ transition. The pH dependence, D/H isotope effect, and temperature dependence on the O_2 release kinetics for this mutant were studied using time-resolved O_2 polarography and made comparison with wild-type and two mutants of the putative proton gate D1-D61. Both two kinetic phases in V185N are independent of pH and buffer concentration and have weaker H/D kinetic isotope effects. Each phase is characterized by a parallel or even lower activation enthalpy, but a less favorable activation entropy than the wild-type. The results indicate new rate-determining steps for both phases. It is concluded that the lag does not represent inhibition of proton release, but rather, slowing a previously unrecognized kinetic phase involving a structural rearrangement or tautomerism of the S_3^+ ground state as it approaches a configuration conducive to dioxygen formation. The parallel impacts on both the lag and O_2–formation phases suggest a common origin for the defects surmised to be perturbations of the H-bond network adjacent to O5.

This work was funded by the National Science Foundation (MCB-1244586)

P4

Portraying the Protonation Behavior of Close Titrating Sites in four Peptidic Scaffolds using Constant-pH MD

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C-Asp, O-Asp, C-Asn, and O-Asn are four peptidic scaffolds synthesized to mimic the coordination of Cu^{2+} in metalloproteins (Fragoso et al., *Chem. Eur. J.* **2013**, 19, 2076; Fragoso et al., *Dalton Trans.* **2013**, 42, 6182). These decapeptides contain three histidine residues and are capable of forming a main coordination species at neutral pH that binds a single Cu^{2+} exclusively through the 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), which is substituted by an asparagine in C-Asn and O-Asn. A detailed description of the protonation behavior of each histidine could help understanding the coordination species being formed in the pH range and eventually further optimizing the peptide's design. However, the standard current methods (NMR titrations) are not very suited for proximal groups titrating in the same pH range. In this work, we used the stochastic titration constant-pH MD method (Baptista et al., *J. Chem. Phys.* **2002**, 117, 4184; Machuqueiro et al., *Proteins* **2011**, 79, 3437) to calculate the protonation curves and pK_a of each titrable residue in the four decapeptides, in the absence of Cu^{2+} ions. The global protonation curves obtained in our simulations are in very good agreement with the existing potentiometric titration curves. (Fragoso et al., *Chem. Eur. J.* **2013**, 19, 2076; Fragoso et al., *Dalton Trans.* **2013**, 42, 6182). The histidines are titrating very closely, and the Asp forms abundant salt bridges with the basic residues, displaying an unusually low pK_a value. In addition, we could observe that the four peptides are very unstructured in the absence of copper, and not even the cyclic forms exhibit a significant β-sheet, unlike what could be expected from the presence of β-turn inducer units in this type of scaffold.

P5

Mechanism of Tungsten-containing Benzoyl-CoA Reductase

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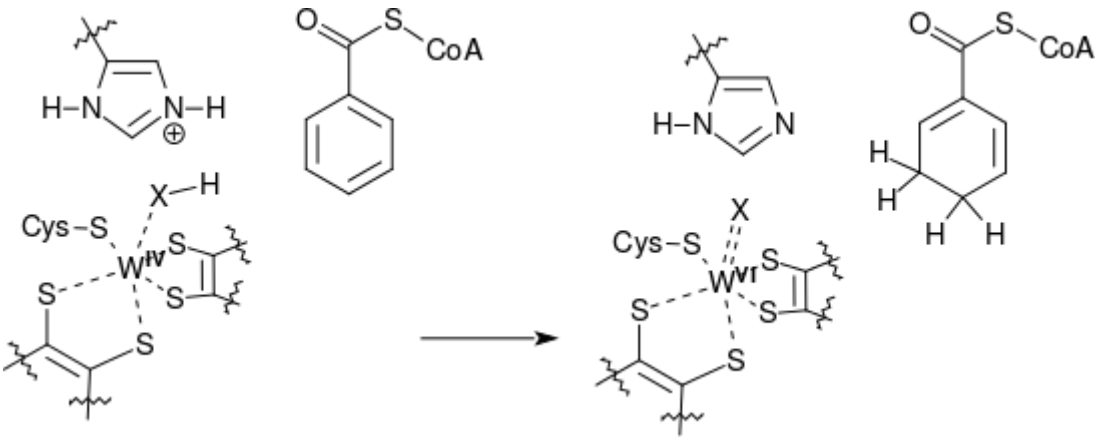
Benzoyl-Coenzyme A plays a central intermediate role in anaerobic metabolism of aromatic compounds in living organisms. First step towards aromatic ring degradation in anaerobic microorganisms is two-electron reduction of benzoyl-CoA to cyclohexa-1,5-dienoyl-1-carboxyl-CoA (dienoyl-CoA). Albeit facultative anaerobes utilize two molecules of ATP for this aromaticity disruption, in obligatory anaerobes, where ATP is more scarce, a multi-subunit ATP-independent enzyme complex BamB-I developed instead. In *Geobacter metallireducens* it is composed of eight subunits - BamBCDEFGHI, where BamB is the catalytic subunit, while the rest serve as electron carriers, providing electrons for the difficult reduction by so far unclear mechanism.
In our computational study, we started from crystal structure of BamBC part of the complex. It is a heterotetramer, composed of two big B subunits connected by two small C subunits, that probably link the tetramer to the rest of the complex. Catalytic subunit B contains tungsto-bispterin and [4Fe4S] cluster, each subunit C binds three [4Fe4S] clusters. The substrate is bound in the vicinity of the tungsten, the final electron donor. The tungsten is coordinated by four sulfurs of the pyranopterin cofactors, a sulfur of a cysteine and by a sixth ligand, that could not be reliably resolved in the crystal structure (donoted X). This uncertain ligand X could, however, play a significant role in catalysis, since it is supposed to be pointing towards the substrate.
In presented study, we first investigated the influence of tungsten oxidation state and ligand X identity on the protonation state of the protein using continuum electrostatic methods. The influence of the initial active site oxidation and protonation state as well as the ligand X identity on the catalytic mechanism was then investigated in the hybrid QC/MM environment.



P6

Exploring the Protonation Changes at the K-channel of Cytochrome c Oxidase

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Cytochrome c oxidase (CcO) is the final enzyme in the respiratory chain in mitochondria but also an integral part of the metabolism of many types of bacteria. In a complex, stepwise redox-reaction, CcO catalyzes the reduction of molecular oxygen to water and utilizes the resulting free energy to pump protons across the membrane thereby creating an electrochemical gradient. Despite intensive research, it is not understood how CcO achieves the unidirectional proton transport and avoids short circuit of the pump. Proton pumping is achieved via proton input channels, which can vary in number and role in different organisms.[1,2]

This study is focusing on exploring the protonation pattern at the entrance of the K-channel in *Paracoccus denitrificans*. To investigate proton pumping spectroscopically it is possible to label the entrance of the proton entrance channel with fluorescein, a pH sensitive dye, which allows determining local changes in proton concentration at the cytoplasmic CcO surface and related properties. Redox state of copper and heme centers affects such properties at the cytoplasmic surface.[3] This is a theoretical approach to investigate changes of pK_A values of the fluorescein label at the entrance of the K-channel in both oxidized and reduced CcO by performing molecular dynamics (MD) simulations. Further work is based on calculations of pK_A values of the fluorescein using software Karlsberg$^+$.[4,5] In addition, behavior of a couple of key histidine residues at the surface of the K-channel has been investigated.

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P7

Adsorption of Charged Patchy Proteins to Polyelectrolyte Brushes

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The interaction of proteins with polymer-coated surfaces and nanoparticles is of fundamental interest for applications in medicine, biotechnology, and soft functional material design [1]. As protein bear charges on their surface, one of the key driving forces for protein ad- or desorption is the electrostatic interaction. Often the protein surface charges are clustered into patches which gives rise to large multi-polarity and very local interaction effects [2,3]. Here, we present results on coarse-grained (implicit-solvent, explicit-salt) computer simulations of charged patchy protein models (CPPMs) [4] with like-charged polyelectrolyte (PE) brushes to study patch-effects in like-charge complexation. We indeed observe large binding affinities between the CPPM and the PE brush in the tens of the thermal energy, k_BT, in cases of low salt concentration and/or large charge density of the patch(es). Our analysis shows a clear correlation between the distance-resolved potentials of mean force, the number of ions released from the PE brush, local electrostatic potentials and fields, and CPPM orientation effects. In order to describe the salt-dependence of the binding affinity for mainly dipolar (one-patched) CPPMs, we introduce a phenomenological model that captures the essential physics of electrostatic complexation in our systems well. Here, the main driving forces for adsorption are identified to be the dipolar attraction, counterion-release effects, and favorable changes of the Born self-energy of the charged globule upon insertion into the salty brush.

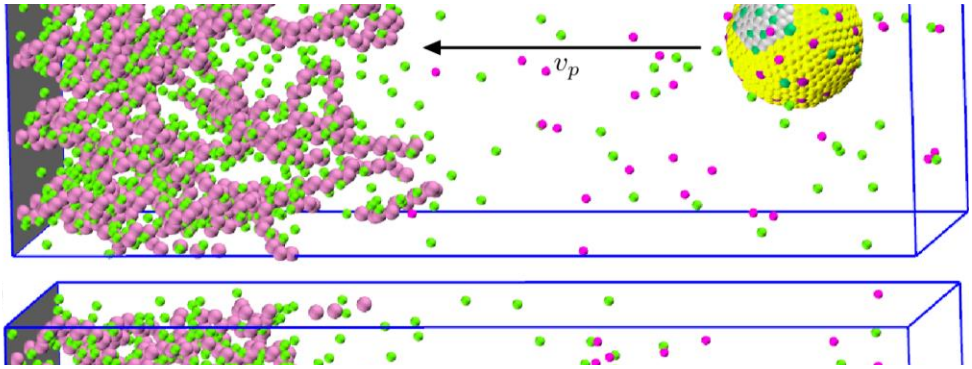

Figure 1. A illustration of the simulation of the uptake of a charged patchy particle model (yellow patchy globule) by a planar polyelectrolyte brush (magenta colored connected beads) in the presence of co- and counterions (small magenta and green beads, respectively).

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P8

Unraveling the mechanics of the proteasome
Molecular dynamics simulations and electrostatic calculations

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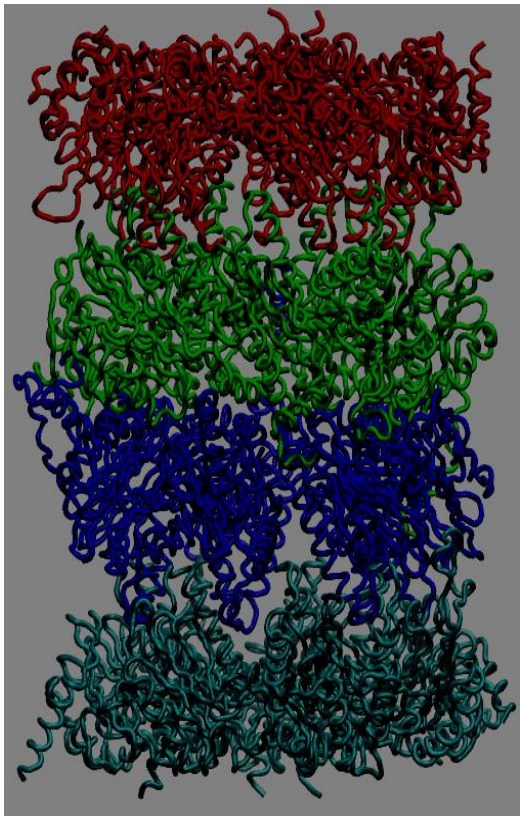
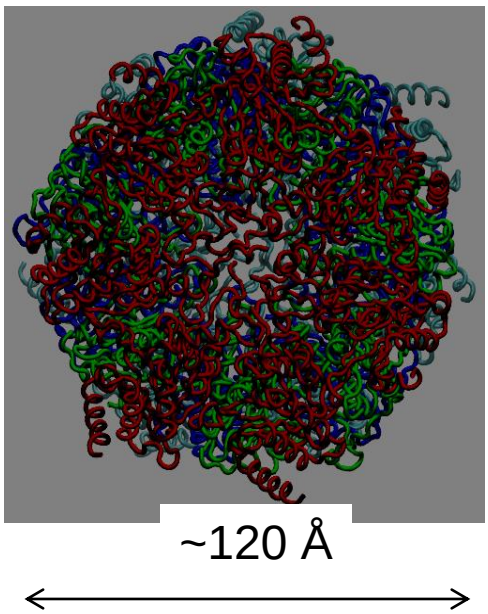
The core particle (CP) of the 20S proteasome is a hollow, barrel-shaped cylindrical structure that is composed of 28 subunits arranged as a stack of four heptameric rings. When the "gate" of the CP is open, protein substrates and peptide chains that enter the proteolytic chamber are hydrolyzed and cleaved, resulting in shorter fragments that are released to the outside. Although many high-resolution proteasome structures have been determined, the mechanisms governing the gating event, as well as the proteolysis, remain largely unknown. Large scale conformational changes in the protein, together with changes in protonation states, may be responsible for guiding the substrate to the activation site and initiating hydrolysis. Using a combination of molecular dynamics simulations and electrostatic calculations, we aim to unravel this highly complex puzzle.



Side view of proteasome CP



View down the barrel of the proteasome CP

P9

Modulating the reduction potential of copper proteins

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Redox-active copper proteins are of interest as potential electrodes for applications such as biofuel cells or artificial photosynthetic centers. Predicting how their reduction potential is fine-tuned would be useful to inform their design. Numerous computational methodologies have been published which attempt to predict the reduction potential shift of copper protein mutants – with mixed success. Continuum electrostatics is an attractive method due to its low computational cost, allowing for a wider range of mutants to be screened. However, they fail to quantitatively predict the change in reduction potential of azurin *P. aeruginosa* (a T1 copper protein) mutants. The discrepancy between computed and experimentally obtained reduction potentials seems to result from solvent exclusion effects, driven by the hydrophobicity of key residues near the copper. Plotting this discrepancy against the change in hydrophobicity of a mutated residue reveals a linear relationship which can be used to derive a correction factor. Combining continuum electrostatics calculations with this correction factor yields excellent agreement with experiment. Work is currently being undertaken in trying to better understand the physical basis of the correction factor and further test it experimentally. The hope is to then apply this method to predict the reduction potentials of more complex (and biotechnologically relevant) proteins, such as multicopper oxidases.

P10

The Dielectric Permittivity of Protein Solutions

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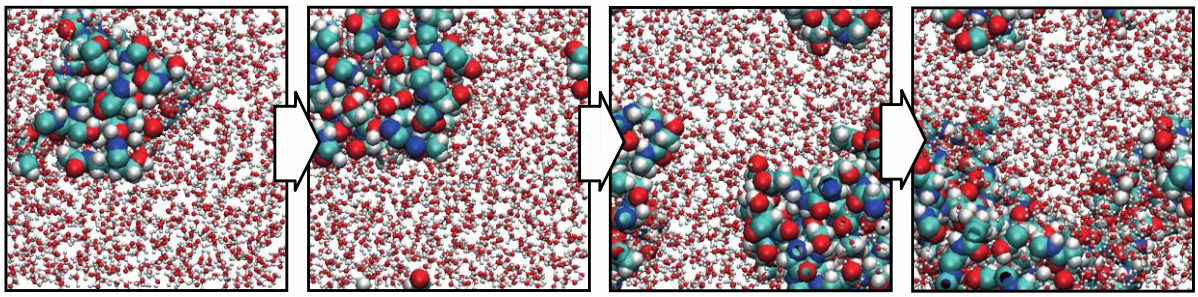
Electrostatic interactions are essential to understand the conformation and function of proteins. For a quantitative understanding of electrostatics of biological systems, for instance to describe the internal electric field of transmembrane proteins, the dielectric permittivity of proteins has to be known. We here present molecular dynamic simulations of protein solutions, which especially allows us to take the interaction of the protein an the hydration water into account. By studying the dielectric permittivity in dependence of the protein concentration, we can extrapolate our results to the limit of a pure protein and disentangle water and protein contributions to the electrostatic interaction.



P11

The Dielectric Properties of Water in Cylindrical Confinement

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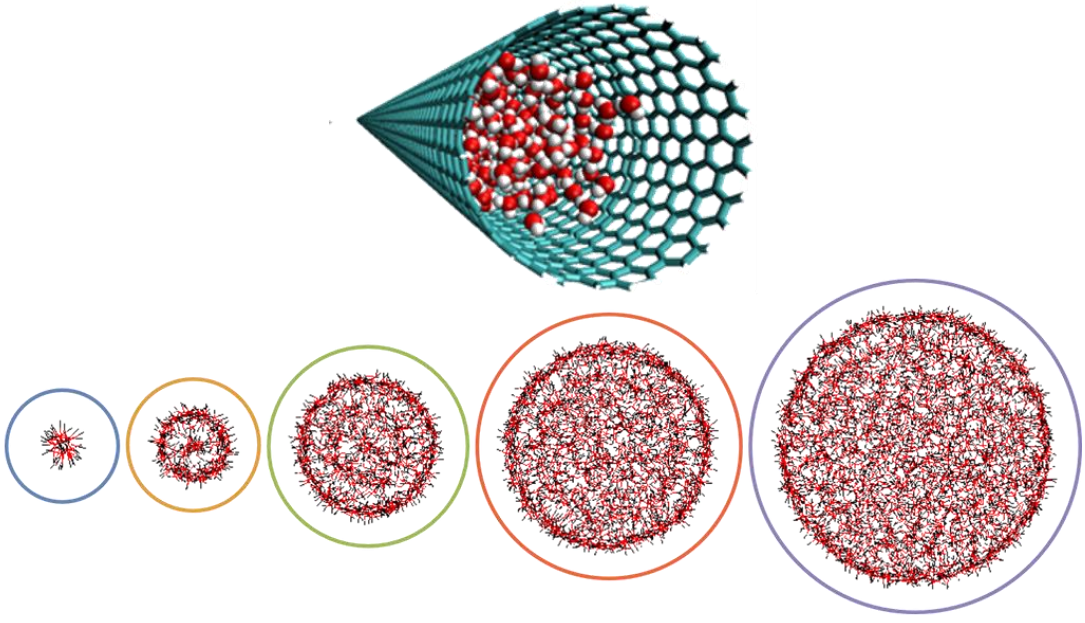
Dielectric properties of confined fluids are important for understanding elementary processes in nanofluids and nanochemistry. Especially cylindrical geometries are of great interest because their applications cover transport through membrane proteins, desalination via nanopores, or transport processes. We use molecular dynamics simulations to investigate the dielectric properties of SPC/E water confined in carbon nanotubes of several radii and lengths as a model for cylindrical cavities.

In cylindrical confinement the dielectric tensor splits into two independent components. Comparing various radii and lengths, our results show a high anisotropy between the axial and a radial component. The axial dielectric response is drastically increasing for confining water, whereas the radial one is decreasing. This observation can be explained by simple arguments of statistical mechanics and by an expansion of the well-known Langevin dipole model. These effects have major consequences for the mobility of ions or protons. Together with the viscosity one gains deep insight into the transport properties of a confined water.



P12

Critical Role of Cutoff Parameter to Calculate Effective Born Radii in Simulating Protein-Protein Interaction

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Molecular dynamics (MD) simulation has been extensively used to study the physical mechanism of association and dissociation of protein and ligand molecules. In particular, implicit solvent models have been developed to calculate the association-dissociation energetics and dynamics with lower computational cost compared to using the explicit solvent model.

The generalized Born (GB) model is an implicit solvent model widely used in MD simulations for electrostatic calculations. In the GB model, the heterogeneous dielectric environment inside and outside a protein molecule is taken into account by introducing the "effective Born radius" (R_{eff}). To make the calculation of R_{eff} convenient, the GB model usually adopts the so-called Coulomb field approximation (CFA) and employs cutoff parameters [2,3]. So far, the accuracy of the GB model, which is determined by the accuracy of R_{eff} calculation, has been improved so as to reproduce the stability of the native state of a single molecule of protein and a double-strand DNA. Therefore, it is not clear whether the current GB model can simulate the protein-ligand/protein/DNA interactions with sufficient accuracy. Indeed, it has been reported that the GB model fails to well reproduce the protein-ligand binding when the ligand is charged [1].

In this study, we investigated how accurately the GB model can simulate the protein-protein electrostatic interaction. To do this, we calculated the potential of mean force (PMF) between two small helices with a single intermolecular salt-bridge pair, and then examined the accuracy of PMF by reference to PMF obtained by the explicit solvent model MD simulations. We found that the cutoff parameter that is usually employed to reduce the computational cost to calculate R_{eff} largely affects the PMF even at long intermolecular distances. Interestingly, smaller cutoff values, which should deteriorate the calculation in principle, did improve the calculation accuracy, showing good agreement with PMF of the explicit solvent model at long distances. Moreover we clarified the reason for this peculiar result: CFA employed in the GB model intrinsically over-destabilizes the intermolecular electrostatic interaction, and the small cutoff alleviates the over-destabilization. Our results thus provide a possible way to improve the simulation accuracy, without increasing computational cost, of the GB model in simulating protein-protein interaction, shedding light on the potential usefulness of the GB model to study association-dissociation dynamics of biomolecules.

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P13

Piezoelectric Allostery of Protein

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Allostery is the molecular basis for biological functions of proteins. While chemical stimulus (such as ligand binding) is the most common input for allosteric proteins, mechanical stimulus also plays an important role in allosteric regulation of protein [1]. However, in contrast to the well-studied chemically-stimulated allostery, the underlying physical mechanism of the mechanically-stimulated allostery remains unclear.

In this study, we investigated the mechanically-stimulated allostery of myosin. Myosin is a molecular motor that exerts a mechanical force along an actin filament by using the free energy of ATP hydrolysis. Myosin is also a mechanosensor, changing the binding affinity with the actin filament and ATP-hydrolysis products in response to the mechanical stimulus [2]. We investigated the response of myosin to an externally-applied mechanical stimulus by conducting molecular dynamics simulations with the replica exchange umbrella sampling technique.

We found that the actin-binding and ATP-binding regions of myosin show significant electrostatic potential changes in response to the positional change of the "converter" domain (which is supposed to be involved in the force-generation) even though the two regions are well-separated from the converter (Fig. A). The electrostatic potential changes were large enough to alter the binding affinity for charged actin and hydrolysis products. Furthermore, we found that this response is induced by a rearrangement network of electrostatic bonds (ionic and hydrogen bonds) in myosin which successively grows from the converter (Fig. B). This novel allostery caused by the mechano-electrostatic coupling is reminiscent of "piezoelectricity", so that we call this "piezoelectric allostery" [3]. The piezoelectric allostery not only explains the force-generating and regulating mechanism of this motor protein but also provides a general explanation for the physical mechanism of mechanosensing and mechanotransduction.

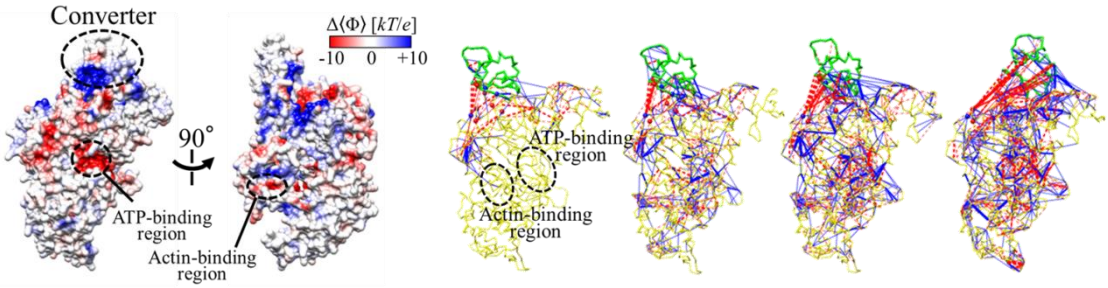

FIG. (A) Electrostatic potential change, $\Delta\langle\Phi\rangle$, associated with the positional change of the converter. (B) Electrostatic bond rearrangement, $\Delta\langle n\rangle$ (change in the number of bonds), which is successively extended from the converter (green) and grows as the converter position is moved to the force-generating direction (from left to right). Blue lines are drawn between the residues with $\Delta\langle n\rangle > 0$ and red (broken) lines with $\Delta\langle n\rangle<0$.

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P14

Modelling Proton Transfer Pathways in Cytochrome c Oxidase

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Cytochrome c Oxidase (CcO) catalyzes the reduction of molecular oxygen to water and utilizes the chemical energy to establish an electrochemical gradient by pumping protons across the membrane it is embedded in. The proton transfer occurs through two distinct channels (D- and K-) by the formation and cleavage of covalent bonds along a hydrogen bond network. By simplified proton transfer models, resembling the channel conditions, we analyzed the proton transfer using different quantum mechanical energy functions and the influence of several degrees of freedom (e.g rotation and translation of water molecules) on the proton transfer. Furthermore, optimal proton transfer pathways are determined from explicitly or semi-explicitly (water positions from MD simulations) sampled Transition Networks. In addition to the model calculations we investigated the proton transfer within CcO's D-channel, thereby elucidating its sterical and hydrational gating.

P15

Does the Ionic Strength Treatment Influence the Protonation/Conformational Space of Charged Biomolecular Systems?

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We have recently shown that the most common approach to deal with charged membrane systems, namely the full system neutralization, leads to excessively ordered lipid bilayers in a 30% DMPA/DMPC system.[1] However, when a significantly smaller number of ions, estimated from Poisson-Boltzmann calculations at a defined ionic strength value, is used, we were able to reproduce the correct isothermal pH dependent lipid phase transition.

An important conclusion of these findings is that, in charged membrane systems, full neutralization only takes place at several nanometers away from the lipid interface. Therefore, we are now raising the question of whether this issue of estimating and using the correct number of counter-ions in MD simulations is also determinant to correctly model charged globular systems. In these systems, like proteins, dendrimers, etc., it is possible to become significantly charged, depending on pH, and the amount of counter-ions added could influence its conformation space and protonation profile.

In this work, we used our Constant-pH MD method[2] to study the conformational space and titration profile of polyamidoamine (PAMAM) dendrimers (2^{nd} generation) with two different ionic strength treatments: an implicit approach (with no explicit counter-ions) using the generalized reaction field; and PME with explicit ions to approximate system neutrality.

The main question now is whether the most common approach used by the scientific community (PME/neutralization) is able to correctly describe highly charged globular systems. We already know the answer and will share it with you in our poster.

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

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P16

Computing pK_A values in different solvents by electrostatic transformation

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We introduce a method, which requires only moderate computational effort to compute pK_A values of small molecules in different solvents with an average accuracy of better than 0.7 pH units. With known pK_A value in one solvent the electrostatic transform computes the pK_A value in any other solvent, if the proton solvation energy is known in both considered solvents. To apply the electrostatic transform to a molecule the electrostatic solvation energies of the protonated and deprotonated molecular species are computed in the two considered solvents using continuum dielectric to describe the solvent. This is demonstrated for 30 molecules belonging to ten different molecular families considering 77 measured pK_A values in four different solvents: water, acetonitrile, dimethyl-sulfoxide and methanol.[1]

[1] *Computing pK_A values in different solvents by electrostatic transformation, Emanuele Rossini; Roland R. Netz; Ernst-Walter Knapp, Journal of Chemical Theory and Computation, 2016, accepted/in press*

P17

Water Dielectric Effects in Planar Confinement

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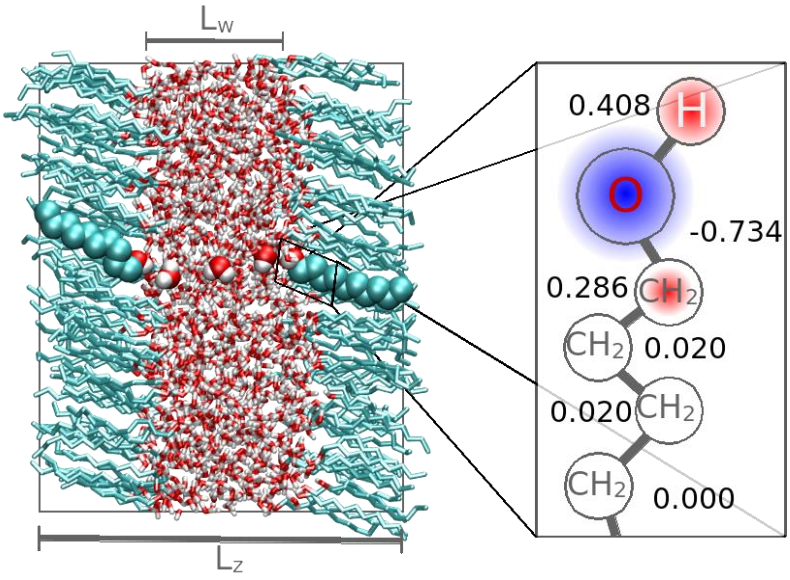
We investigate the dielectric profile of water confined between two planar polar walls using atomistic molecular dynamics simulations. For a water slab thickness below 1 nm the dielectric response is highly asymmetric: while the parallel component slightly increases compared to bulk, the perpendicular one decreases drastically due to anti-correlated polarization of neighbouring water molecules.
We demonstrate the importance of the dielectric contribution due to flexible polar head groups and derive an effective dielectric tensorial box-model suitable for coarse-grained electrostatic modelling. We show the significance of the detailed understanding of electrostatic interactions when disentangling solvation and hydration effects at biologically relevant surfaces like lipid membranes.



P18

Peptide-PDZ Recognition: Designing Peptide Ligands to Inhibit the Tiam1 Signaling Protein

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Small, modular protein domains often direct protein-protein interactions (PPIs) and form the building blocks of eukaryotic signaling pathways. PDZ domains are among the most widespread and best-studied, with over 250 in our genome. They specifically recognize the 4-8 C-terminal amino acids of their target proteins, as well as the corresponding peptides in isolation. We focus on the Tiam1 protein, a Rac GTP exchange factor involved in neuronal protrusion and axon guidance. Its activity modulates Rac signaling, whose dysregulation can cause cancer. Tiam1 can be inhibited by small peptides that bind to its PDZ domain and downregulate its interaction with its target proteins, including Rac1. Inhibition of PPIs is currently a difficult but recognized strategy for drug and bioreagent design.

To investigate the Tiam1-peptide binding mechanism and discover potential inhibitors, structural models were built and MD simulations done for over 40 Tiam1-peptide complexes, based on available Xray structures. The peptide sequences included experimentally-studied variants of the natural target ligand syndecan, sequences from a combinatorial library of peptide binders, and variants with new sidechain types at the main specificity positions (the last four positions of the peptide). Binding free energy differences were computed using both Poisson-Boltzmann and Generalized Born continuum electrostatics models, combined with a simple Linear Interaction Energy free energy function. For a few of the variants, more rigorous, alchemical MD free energy simulations were performed, providing reference data. All these simulations lead to simple but well-parameterized PB/GB models and datasets that represent an important benchmark for this level of theory. They also give detailed insights into the groups and interactions that determine protein-peptide binding, and should help us to discover very tightly binding peptides and peptidomimetics for use as bioreagents.

P19

Electrostatic Models for the Interpretation of FRET Experiments on Fluorescent-labelled Proteins

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Förster resonance energy transfer (FRET), introduced in the late forties of the last century, is one of the most important methods to measure distances in proteins. FRET is measured on proteins labelled with a donor and an acceptor chromophore. After optical excitation of the donor chromophore the electronic excitation is transmitted to the acceptor chromophore, which is initially in the ground state. The transfer efficiency is highly dependent on the inter-chromophore distance and orientation. To extract information about these quantities from FRET experiments two models are often used: i) chromophores, represented by point-dipoles, undergoing isotropic rotations and ii) point-dipole dyes with restricted rotations. We have developed a method that overcomes the limits of these standard approximations in two respects: (i) it goes beyond the point-dipole approximation; and (ii) it provides an improved description of screening and local field correction of the Coulomb coupling between chromophores. The approach combines MD simulations with a solution of the Poisson equation for the electrostatic potential of the chromophore's transition densities [1]. We present an application of the method to describe FRET experiments on a polyproline, labelled with Alexa dyes, in aqueous solution [2]. The results of the calculations demonstrate an improved description of the experimental FRET efficiencies, in particularly, for short inter-chromophore distances, where the point-dipole approximation fails and the screening/local field corrections depend on the mutual orientation of the chromophores.

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P20

QM/MM Calculations on Agp1 Phytochrome

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Phytochromes consist of family of photoreceptors that are found in plants bacteria and fungi. Their chromophore, a methine-bridged tetrapyrrole, acts as photo-switch between two stable parent states, the red light absorbing- (Pr) and the far-red light absorbing-state (Pfr). The transition between these two parent states is reversible. Upon light absorption, isomerization around the double bond of the methine bridge between the ring C and ring D takes place (figure 1). After this initial event, a thermal relaxation of the protein environment occurs over several intermediate states.

During the photocycle (figure 2), the chromophore is stabilized by electrostatic interactions with certain amino acids such as the H250 and H280, which are found in its close vicinity. Therefore, the protonation states of the considered histidines, and the changes in the hydrogen bond network around the phytochrome cofactor are of high interest. On the other hand, the spectral properties of the cofactor are most likely to be affected by the linkage arrangement of the cofactor. However, due to the highly conjugated π-electrons in the cofactor structure, the linkage configuration is not unambiguously defined by the crystal structure. Also crystal structures resolution is not high enough to define the protonation states of the amino acids, therefore, theoretical studies are of intrinsic importance to understand the nature of the cofactor linkage, the protonation dynamic and the related structural changes of phytochromes.

Here, MD-simulation combined with **Q**uantum **M**echanics/**M**olecular **M**echanics (QM/MM) calculations and frequency calculations were done on the Pr-state of the prototypical phytochrome Agp1 in order to calculate Raman spectra and in this way to identify the linkage arrangement of the cofactor and the protonation state of the conserved histidine residues (H250 and H280).

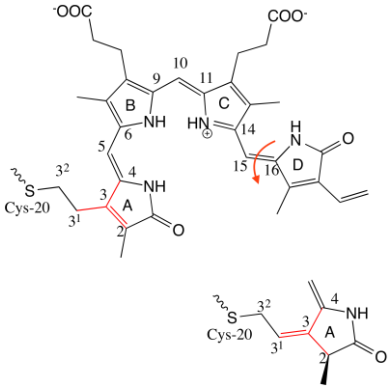

Figure-1: The biliverdin cofactor with an endo-cyclic configuration (top) and exo-cyclic configuration (bottom).

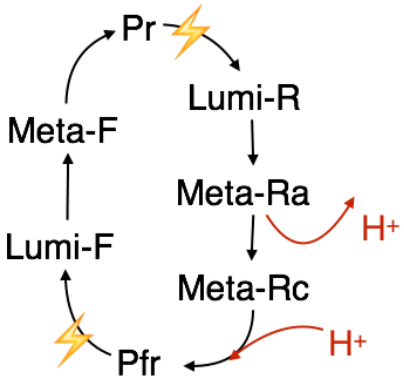

Figure-2: The photocycle of prototypical phytochrome

By comparing calculated and experimental spectra, we made two main observations. First, The Agp1 seems to have a linkage in endo-cyclic configuration. Second, H250 is most likely protonated at the Nδ-position, whereas the protonation of H280 remains unclear. Another interesting observation is the effect of the pyrrole water fluctuation on the calculated Raman spectra of biliverdin cofactor in Agp1, these effects are analyzed carefully in this work.

P21

Development of a Monte Carlo method for continuum- electrostatics energy calculations in Computational Protein Design

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Computational Protein Design techniques involve the exploration of huge ensembles of conformations and sequences. Even for small proteins, the number of degrees of freedom is enormous: the development of proper structural models, energy functions and sampling techniques is a stimulating and challenging problem.
The Simonson group is actively developing the *Proteus* suite [1], a meta-package for CPD calculations. In a standard simulation, the protein backbone is kept fixed; the solvent is modeled implicitly while side chain coordinates belong to sets of rotamers. Within this framework, the sequence/structure space is explored with a combinatorial approach. With a pairwise energy function, residue-residue interactions can be pre-computed and stored in lookup tables, allowing for all combinations of residue types and rotamers. Then a fast Monte Carlo sampling allows the search of preferred sequences and structures.
Continuum electrostatics implicit solvent models are intrinsically non-pairwise. Interactions between groups of protein charges depends on the entire solute shape: in principle, it is not possible to express the total energy as sum over residue pairs.
We developed a Monte Carlo scheme to manage solvation free energies with *Exact-GB*, a residue-pairwise Generalized Born scheme which allows to overcome this difficulty [2].
Screening energies are interpolated by a simple, parabolic function of *residue Born solvation radii*. Fitting coefficients are pre-computed for all residue pairs and tabulated together with the other energy terms. The information about the dielectric environment, captured by residue Born solvation radii, is carefully kept up-to-date during the Monte Carlo sampling. This is a significant improvement of the physical model: although the old implementation produces good results, only Exact-GB preserves the many-body character of the GB solvation free energy.
To validate our implementation in Proteus, we compute titration curves with constant-pH Monte Carlo simulations. It is particulary interesting to compare the new results with those obtained from previous calculations [3], where more approximations were assumed.
Furthermore, Exact-GB will be exploited by the whole group for its main research project, specifically focused in the study of PDZ domains.

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P22

Interaction of Human Serum Albumin with highly charged macromolecules: calorimetry versus computer simulations

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Using Isothermal Titration Calorimetry (ITC) and coarse-grained (implicit solvent/explicit salt) Langevin computer simulations, we study the interaction of Human Serum Albumin (HSA) with highly charged macromolecules, such as short polyelectrolytes like polyacrylic acid (PAA) and hyperbranched polyglycerol sulfate (PGS) nanoparticles. The binding affinity calculated from the simulations is found to be in semi-quantitative (for PAA) and qualitative (PGS) agreement with the ITC data. The simulation reveals explicitly the structural properties of the complexation, where for the HSA-PAA complex we observe only one PAA chain bound per HSA molecule, whereas bivalent binding is indicated for the HSA-PGS complex due to the highly branched, globular architecture of PGS. We demonstrate that the driving force of the complexation in both cases originates mainly from the release of condensed counterions from the macromolecule upon binding, supported by the thermodynamic signature of the ITC-data. We construct a phenomenological binding model in the framework of DLVO theory, accurately including charge renormalization and extended to consider multipolar and counterion-release effects, in an attempt to rationalize the size- and salt-dependence of the HSA-PGS binding affinity as determined in simulations and experiments.

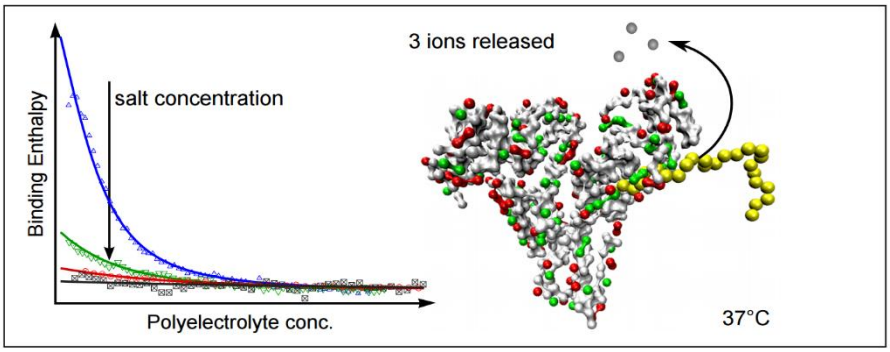

Figure 1. An illustration of the ITC data profile and HSA-PAA complex.

P23

Correlation of electrostatic potentials and energy of hydrogen bonds

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The electrostatic potentials can be used to explain and predict intermolecular interactions. There is a correlation of electrostatic potentials and distribution of the electron densities in molecules [1]. The electrostatic potential is a real physical property, an observable, which can be obtained experimentally by diffraction methods [2] as well as computationally. The sign of V(**r**) in any region is determined by whether the positive effect of the nuclei or the negative one of the electrons is dominant there. The most positive, $V_{s,max}$, and the most negative, $V_{s,min}$, values on the electrostatic potential surface can be correlated with calculated energy of the intermolecular interactions. It was shown that the values of electrostatic potential ($V_{s,max}$) are in good correlation with the hydrogen bond energies calculated between coordinated and noncoordinated water molecules [3]. Recently, the results on geometries of C-H/O interactions in protein structures were explained by calculated electrostatic potential maps [4].

In this work we present the results based on the analysis of data in the Protein Data Bank (PDB) and quantum chemical calculations of electrostatic potentials.

In order to understand the strength of noncovalent interactions in protein structures, electrostatic potential maps were calculated. We want to point out correlation of the most positive, $V_{s,max}$, and the most negative, $V_{s,min}$, values on the electrostatic potential surface with the geometric parameters and strength of noncovalent interactions in protein structures.

The results of calculated electrostatic potentials show correlation with the strength of noncovalent interactions.

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P24

Continuum Electrostatic Calculations of Redox Potential of Single Core Iron-Sulfur Complexes

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Iron-sulfur-proteins are often involved in electron transfer processes. These proteins show a wide range of redox potentials from -700 mV to +500 mV. The family of iron-sulfur-proteins include also the rubredoxins which are involved in the sulfur metabolism of bacteria and archaea. In contrast to other iron-sulfur-proteins, like ferredoxins or HiPIP, rubredoxins have no inorganic sulfur in the iron-sulfur-cluster. The rubredoxins from *Clostridium pasteurianum*, which was studied here in wildtype and mutated form, showed a range of redox potentials from -61 mV to 50 mV in experiments. In agreement with previous theoretical studies, the redox potentials were calculated by using continuum electrostatics calculations. The necessary point charges for the redox potential calculation were determined on the basis of the DFT optimized model compound $[Fe(SCH_3)_4]^{1-,2-}$. The large variation in the redox potentials were also seen in the calculations. In addition to the introduced point mutations, this variation was caused by several structural changes like the bond lengths within the iron-sulfur complex and the length of the hydrogen bonds of cysteine sulfur with the environment. The position of the protein backbone and the side chains to the iron-sulfur complex also played a role. And finally the accessibility of water to the iron-sulfur complex was important, too. Due to the nature of the calculations the redox potential is also affected by the certain point charges and the resultant surrounding potential. Therefore, the experimental data could be determined with a maximum error of ±20 mV.