which is activated by the catalytic histidine. Histidine substitution impairs hydrolysis step leading to the formation of stable protein ligand complex [1]. In this study, we have developed a protocol for specific labeling of the tunnel opening and elimination of all unbound and non-specifically bound coumarin molecules.

Acrylamide quenching and time-resolved anisotropy experiments confirmed the selective labeling of enzyme by coumarin and complete removal of unbound molecules of coumarin. Steady-state and time-resolved emission spectra measurements showed significant differences in the polarity, accessibility and mobility of the dye and its microenvironment for both studied haloalkane dehalogenases. Coumarin bound in haloalkane dehalogenase DbjA is more flexible and more hydrated in comparison with coumarin bound in DhaA. Microenvironment displays higher polarity and lower viscosity than in DhaA. The obtained experimental data showed good agreement with the results obtained by molecular dynamics calculations. These results reflect geometry of the tunnel mouths evident from the crystal structures [2].

Solvent dynamics in the tunnel mouth will be further studied in other natural haloalkane dehalogenases and their variants. Comparison of solvent dynamics for various constructs will help us to better understand how this dynamics influences functional properties of the enzymes with buried active sites.

1. HaloTag™ Interchangeable Labeling Technology, Technical Manual, Promega Corporation, Madison, USA; 2006.
2. A. Jesenska, J. Sykora, A. Olzynska, J. Brezovsky, Z . Zdrahal, J. Damborsky & M. Hof, *J. Am. Chem. Soc.* (*in press*).

Lectures - Friday, March 13, Session III

L10

ELECTRONEGATIVITY EQUALIZATION METHOD – FAST METHOD FOR CHARGE CALCULATION

Z. Jiroušková, R. Svobodová Vařeková, J. Vaněk, J. Koča

[1]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, xjirousk@chemi.muni.cz

The progress which appeared in the latest decades in the computational field established computational chemistry methods to be of comparable quality to experimental methods. The number of cases in which computational chemistry methods can be successfully applied is still increasing and these methods are nowadays used with profit for modeling molecular systems and detailed studies of various structural and functional properties.

Partial atomic charge is a molecular property which is very often used in chemistry, particularly for clarification of differences in structure or reactivity between molecules. Unfortunately, partial charges are not obtainable from experiment, but they can be derived using the quantum chemistry methods. In the quantum chemistry, there are more approaches, how to solve this task, but none of them can be considered to be the best one and what is more, these methods are on one hand very precise, but also quite time-demanding. For some types of calculations it is not acceptable to wait for the results for such long time and for that reasons also some alternative approaches appeared. These approaches are based mostly on the semi-empirical principles and the Electronegativity Equalization Method is one of them.

The Electronegativity Equalization Method was developed as a semi-empirical method based on the Density Functional Theory [1] and it is a fast way how to obtain appropriate partial charges for arbitrary molecule. The methodology is based on the Sanderson's Electronegativity Equalization Principle [2] which is applied to the Density Functional Theory. Due to its semi-empirical character, it is necessary to parameterize the Electronegativity Equalization Method before the first usage and the parametrization process influences the quality of resulting charges. We have already parameterized the Electronegativity Equalization Method on very large sets of organic, organohalogen and organometal molecules from the Cambridge database of crystallographic structures (CSD) and the National Cancer Institute 3D structure database (NCI DIS). Based on these training sets, very robust parameterization was performed and the number of so far parameterized elements was increased [3, 4]. The obtained parameters were carefully validated and resulting partial atomic charges were in a very good agreement with quantum mechanically calculated partial atomic charges.

1. R. G. Parr, W. Yang, Density-Functional Theory of Atoms and Molecules, *Oxford University Press,* (1989).
2. R. T. Sanderson, *J Am Chem Soc*, **105**, (1983), 2259.
3. R. Svobodová Vařeková, Z. Jiroušková, J. Vaněk, Š. Suchomel, J. Koča, *Int. J. Mol. Sci.*, **8**, (2007), 572.
4. Z. Jiroušková, R. Svobodová Vařeková, J. Vaněk, J. Koča, *J Comput Chem, electronically published ahead of print*.

L11

INTERACTION OF "PIANO-STOOL" RUTHENIUM COMPLEXES WITH DNA; QM/MM STUDY

Zdenek Futera, Jaroslav V. Burda

Department of Chemical Physics and Optics, Charles University in Prague, Czech Republic

Ruthenium(II) "piano-stool" complexes $[Ru^{II}(\eta^6\text{-arene})(en)Cl]^+$ (en = ethylendiamine) were reported by Sadler's group [1,2] as promising anticancer drugs. Their behavior in biological environment is similar to well known chemotherapeutic cisplatin, i.e. first the chlorine is replaced by water molecule and after this hydration reaction the aqua ligand is exchanged by nucleic base.

We have shown in our previous computational study that the most preferable site in DNA for bonding Ru is N7 position on guanine which is well accessible from major groove of DNA. Guanine replacement reaction passes complex two-step mechanism. DFT/MP2 calculations on this system were performed both *in vacuo* and in COSMO regime. Thermodynamic parameters and rate constants were determined and compared with experimental results.

Possibility of intrastrand cross-link formation was investigated by QM/MM methodology. We use model where Ru complex is bound to 6-base-pair DNA oligomere. QM part of system is evaluated by program Turbomole while the MM surrounding is treated by Amber program package. These programs are governed by script-based interface called ComQum [3,4]. AIM and NBO analysis were calculated. Investigated process of the cross-link forming is exothermic and the piano-stool structure is lost during the reaction.

1. Fernandez, R.; Sadler, P. J. et al. *J. Inorg. Biochem.* **2003** *96*(1), 130.
2. Guichard, S. M.; Sadler, P. J. et al. *Biochem. Pharm.* **2006** *71*(4), 408-415
3. Ryde, U. J. Comput.-Aided Mol. Design **1996**, 10, 153-164
4. Ryde, U.; Olsson, M. H. M. *J. Quant. Chem.* **2001**, *81*, 335-347.

L12

METACENTRUM - E-INFRASTRUCTURE FOR SOLVING STRUCTURAL BIOLOGY RESEARCH CHALLENGES

M. Kuba[1], V. Žáková[2], S. Licehammer[2] and J. Kmuníček[1]

[1]CESNET, z.s.p.o., Zikova 4, 160 00 Praha 6, Czech Republic
[2]Faculty of Informatics, Masaryk University, Botanická 68a, 620 00 Brno, Czech Republic
kmunicek@ics.muni.cz

Current structural biology research comprises from many distinct areas creating huge amount of research problems as engineering of enzymatic structures, QM/MM studies of catalysis, gaining insight into the binding process, protein structure prediction, protein-protein docking, multidimensional parametric studies, genome/genome evolution analysis and many others. To be able to tackle them on appropriate level one needs mature environment and corresponding tools. Within the Czech Republic, project MetaCentrum [1] is currently providing such support and tools through its idea of shared distributed resources. The sharing of resources – including computing capacities, storage capacities and various scientific instruments – is one of the fundamental elements of the e-Infrastructure concept.

MetaCentrum, the project of CESNET association, therefore represents a production infrastructure (grid) composed from facilities throughout the Czech Republic. MetaCentrum production services are provided to the whole Czech academic community with applications covering wide range of domains from chemistry, life sciences through engineering applications based on finite element modeling up to physics and astronomy. Current research in MetaCentrum covers virtualisation, security, job scheduling and the utilization of advanced network features provided by the CESNET2 network. Besides the computational support the researchers can address through MetaCentrum issues concerning storage and regular backup of vast amount of data generated during long biomoleculer simulations. Similarly, they can benefit from available videoconferencing facilities to routinely utilize them for remote cross-border day-to-day collaboration.

[1] http://meta.cesnet.cz/.

L13

RANDOM WALK MODEL OF DISPERSION IN BIOREACTORS AND ITS APPLICATION TO MODELLING OF MICROBIAL GROWTH

V. Štumbauer, Š. Papáček, D. Štys

Institute of Physical Biology, University of South Bohemia, Academic and University Center, Zámek 136, 373 33 Nové Hrady, Czech Republic
stumbav@gmail.com

The paper deals with microbial growth modelling in the bioreactor. Our approach is based on random walk models of turbulent dispersion instead of much more common approach based on raw CFD (Computational Fluid Dynamics) based on finite volume method. Biochemical reactions are described using the stochastic approach. Finally, the simulation of photosynthetic organisms growth in a Couette-Taylor photobioreactor is presented.

1. Bezzo, F., Macchietto, S. & Pantelides, C.C. (2003) General Hybrid Multizonal/CFD Approach for Bioreactor Modelling, AIChE Journal, 49, pp 2133-2148.
2. Eilers, P.H.C. & Peeters, J.C.H. (1993) Dynamic behaviour of a model for photosynthesis and photoinhibition. Ecological Modelling, 69, pp. 113-133.
3. Saxton, M.J. (2007) Modeling 2D and 3D Diffusion, Methods in Molecular Biology, 400, pp. 295-321.

Lectures - Friday, March 13, Session IV

L14

QUANTUM CHEMICAL DESCRIPTION OF THE PROPERTIES AND REACTION MECHANISMS OF SELECTED ANTICANCER METALLODRUGS WITH BIOMOLECULES

J. V. Burda

Department of Chemical Physics and Optics, Charles University in Prague, 121 16 Prague 2,Czech Republic

Pt(II)/Pt(IV) complexes

Cisplatin (diammine-dichloro-platinum(II) complex) and its analogues are known for their high activity in the anticancer treatment. The physico-chemical background of the activation of these drugs in the hydration process of replacing chloro-ligards by water molecules was examined. Thermodynamic and kinetic parameters were determined for this hydration reaction. Comparing with experimental data it can be seen very good agreement of both characteristics. The process of cisplatin activation can be understand purely on the thermodynamical footings as formation of less stable Pt-complexes under the LeChatelier-Braun-van Hoff's principle of chemical equilibrium.

Ru(II)-piano-stool complexes

Detachment of the chloro-ligand in [Ruthenium(II)(Arene)(en)Cl]$^{+}$ is studied in connection with cisplatin activation. Similarly, transition state for process of the water replacement was searched and both thermodynamical and kinetical data for activation and interactions with purine nucleobases bases were estimated. Comparison with measured data demonstrates a power of such computational tools for further investigations.

Dinuclear Rh(II) complexes

Preference for coordination of diaqua-tetrakis- -acetatodirhodium to purine DNA bases is examined. Higher thermodynamic affinity of the Rh-complex to guanine was found despite the larger stability of the $(Rh)_2$-adenine structure (in accord with HSAB principle). Possible reaction mechanism for the DNA base substitution is explored using simple model molecules in the both gas phase and PCM approaches.

L15

SOLVATION OF NUCLEIC ACID BACKBONE: A DFT STUDY

Ladislav Benda[1,2], Bohdan Schneider[3], Vladimír Sychrovský[1]

[1]*Inst. of Organic Chemistry and Biochemistry AS CR, Flemingovo nám. 2, 166 10 Praha 6, Czech Republic*
[2]*Faculty of Mathematics and Physics, Charles Univ. Prague, Ke Karlovu 3, 121 16 Praha 2, Czech Republic*
[3]*Institute of Biotechnology AS CR, Vídeňská 1083, 142 20 Praha 4, Czech Republic*
ladislav.benda@marge.uochb.cas.cz

A negatively charged phosphate group of nucleic acid backbone interconnecting two (2-deoxy)ribose units represents one of the most important solvation sites in nucleic acids. An impressive amount of work has been done on characterizing the structure of the solvation shell of canonical DNA as well as of other backbone patterns found in RNA. Surprisingly narrow regions of water occurrence in the direct contact (H-bond) with phosphate group have been observed in crystals. The presence of physiological monovalent and divalent cations in the phosphate first solvation shell was also confirmed [1].

The X-ray identification of 3rd period alkali metal ions (Na^+, Mg^{2+}) is not a straightforward task since these ions and the water molecule possess the same number of electrons. In many cases, the methods of molecular spectroscopy can be used for metal ion recognition [2-4]. We investigated the possibility of characterizing the specific interactions of metal ions with nucleic acids by NMR spectroscopy. Ab-initio computational methods were applied to selected nucleic acid structural patterns including explicit solvent molecules. We outline several options for monitoring the presence of metal ions in contact with nucleic acids.

1. B. Schneider, M. Kabeláč, *J. Am. Chem. Soc.*, **120**, (1998), 161.
2. Y. Tanaka, K. Taira, *Chem. Commun.*, (2005), 2069.
3. B. Halle, V. P. Denisov, *Proc. Natl. Acad. Sci. USA*, **97**, (2000), 629.
4. E. Freisinger, R. K. O. Sigel, *Coord. Chem. Rev.*, **251**, (2007), 1834.

L16

THE INFLUENCE OF BACKBONE AND SOLVENT DYNAMICS ON ^{31}P CHEMICAL SHIFT TENSORS IN DICKERSON DODECAMER: A COMBINED MD/DFT STUDY

J. Přecechtělová[1], P. Novák[1], Martin Kaupp[2], M. L. Munzarová[1], and Vladimír Sklenář[1]

[1]*National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, CZ-611 37, Brno, Czech Republic*
[2]*Institut für Anorganische Chemie, Universität Würzburg, Am Hubland D-97074, Germany*
janap@ncbr.chemi.muni.cz

^{31}P chemical shift tensors ($_{ii}$) can aid nucleic acid structure determination [1]. Due to the lack of experimental data, theoretical calculations are a valuable method of choice to obtain $_{ii}$. Previous results of such calculations on static models of phosphate groups proved to provide useful, yet rather limited information, as they do not account for dynamical effects [2,3]. Internal conformational motion as well as the continuous breaking and forming of hydrogen bonds between solvent molecules and phosphate oxygens influence ^{31}P chemical shift tensors considerably. Therefore, we have performed classical molecular dynamics (MD) simulation of $[d(CGCGAATTCGCG)]_2$ and used the snapshots from the MD trajectory for chemical shift tensor calculations. Small cluster models consisting of dimethyl phosphate and water molecules within the first solvation shell have been employed. Calculations were carried out at the density functional level (DFT) of theory, applying gradient-corrected BP86 functional and IGLO-III basis set. Changes in chemical shift tensors introduced 1) by extending the explicit solvent beyond the first solvation shell and 2) by adding implicit solvent or partial point charges to the small cluster models have been analysed. In order to assess the direct effect of hydrogen-bonding, the results obtained are also compared to chemical shift tensors calculated for dimethyl phosphate without any coordinated water molecules.

1. Z. Wu, N. Tjandra, A. Bax, *J. Am. Chem. Soc.*, **123**, (2001), 3617.
2. J. Přecechtělová, Markéta L. Munzarová, P. Novák, V. Sklenář, *J. Phys. Chem. B*, **111**, (2007), 2658.
3. J. Přecechtělová, P. Padrta, Markéta L. Munzarová, V. Sklenář, *J. Phys. Chem. B,* **112**, (2008), 3470.

This work was supported by the Grants MSM0021622413 and LC06030 of the Ministry of Education, Youth, and Sports of the Czech Republic. Deutscher Akademischer Austausch Dienst (DAAD) is acknowledged for providing a scholarship to Jana Přecechtělová for her research stay at Universtät Würzburg, Germany.