



Lectures - Friday, March 19, morning, Session III

L13

COMPUTATIONAL METHODS AS A COMPONENT OF MODERN STRUCTURAL BIOLOGY

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Computational methods and their real computer implementation have always been a part of structural biology. At the beginning, mainly computational protocols involved in experimental data refinement and interpretation in X-ray crystallography and NMR spectroscopy were used to obtain final 3-D structure.

Later on, when computers became more powerful and theoretical models more reliable, computational methods started to form an independent part of structural biology. Nowadays, computational methods are often used for, e.g., *ab initio* 3-D structure prediction. One of the unique features, that make computational methods very important, is their ability to see single atoms and to follow their mutual displacement in space as a function of time. As theoretical models are of increasing quality, computational protocols based on them are more and more reliable and robust and, consequently, more trusted by the users. This has, besides

all the good implications, also some bad effects. The key one is that the user sometimes expects to obtain more information from a computational method than such method can provide, sometimes the quality and reliability of the obtained information is overestimated.

Based mainly on research projects of the National Centre for Biomolecular Research in Brno, it will be shown in the lecture, in which areas computational methods can successfully be used and, *vice versa*, where pitfalls may be expected. Examples will be given in the field of software development as well as protein complexes modeling and simulations.

The research presented here has been supported by Ministry of Education of the Czech Republic (MSM0021622413, LC06030, ME08008) and the European Community's Seventh Framework Program under grant agreement n° 205872.

L14

FREE ENERGY MODELLING OF SUBSTRATE DISTORTION IN THE ENZYME ACTIVE SITE: AN EXAMPLE OF INFLUENZA NEURAMINIDASE

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Protein-ligand interactions are often associated with conformational changes of the molecule of protein. However, there are several interesting examples of induced fit in the molecule of ligand. For example, numerous X-ray structures of glycosidases show that the substrate (pyranose) is bound in a high-energy boat or skew-boat conformation, instead of the energetically favourable chair conformation. This substrate distortion probably facilitates the catalysis. Such distortion was also observed for -N-acetylneuraminic acid (Neu5Ac) bound to the active site of influenza neuraminidase (sialidase).

Stabilization of the boat conformation of Neu5Ac in the active site of neuraminidase was studied using molecular dynamics simulation and metadynamics [1]. The results allowed us to predict thermodynamics and kinetics of the studied conformational change in a good agreement with available experimental data. We show that the distortion is

likely to be stabilized by the conserved arginine cluster, but also by the "150-loop". This loop (mainly Asp151) has been proposed to play important role in catalysis, but, as far as our knowledge goes, its involvement in substrate distortion has not yet been studied. The results help to elucidate molecular mechanism of the reaction catalyzed by this medicinally important enzyme.

1. Laio A., Parrinello M., *Proc. Natl. Acad. Sci. USA*, **99**, (2002) 12562.

This investigation was supported by the Czech Ministry of Education, Youth and Sports (MSM6046137305). Computational resources were funded by the Science and Technology Assistance Agency, Slovakia, under the Contract APVV-0607-07 and the Centres of Excellence program of the Slovak Academy of Sciences (COMCHEM, Contract No. II/1/2007).

L15

SITEBINDER – SOFTWARE TOOL FOR SUPERIMPOSING MULTIPLE STRUCTURAL MOTIVES

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A lot of information about 3D structure of proteins (including metalloproteins, lectines etc.) is nowadays available in the Protein Data Bank database. This large amount of data allows us to analyze biochemically significant motives in these molecules. For example, we can study binding sites of metals in metalloproteins, surroundings of sugars in lectines, or other biochemically important motives (such approaches were used for example in [1] and [2]).

To perform these analyses a software tool is needed, which is capable of superimposing many structural motives at once (minimizing their mutual root mean square deviation - RMSD) and computing a model of the particular motive. The structural motives often exhibit partial symmetry; therefore, even for two motives, there exist several valid pairings of atoms of the structures, each yielding a good value of RMSD. Nevertheless, when superimposing multiple structures at once, the chosen pairing plays a vital role and different pairings might result in different values of RMSD. Unfortunately, currently available tools for superimposing of molecules (VMD, UCSF Chimera, etc.) are mainly focused on whole molecules and assume the pairing of atoms is given a priori. There are more sophisticated tools such as PyMol that optimize pairing; nevertheless, they automatically neglect poorly fitting atoms, which is an undesirable feature when trying to compute a model of the motive.

Therefore, a software package called SiteBinder was designed and implemented. This software applies currently published algorithms for alignment using quaternion math [3] and for superimposing of multiple structures [4] (both algorithms were adapted for our needs). Furthermore, SiteBinder provides algorithms for searching for optimal pairing of atoms (which utilizes the information about residues, atomic names, and chemical symbols contained in

PDB file format) and calculating a model from multiple structures. SiteBinder allows the user to select subset of atoms on each structure which are then used for superimposing. This is achieved through user friendly and intuitive interface with features such as real-time highlighting of atoms of interest and automatic selection of atoms on multiple structures at once. Furthermore, detailed information about RMSD is provided including average RMSD to the other structures in the ensemble (this feature is very useful for example for classification of subclasses of a given structural motives). Finally, SiteBinder is able to export the superimposed structures in PDB format as well as in a rendered image.

We tested the software on several sets of biochemically important motives (i.e. about 500 Zinc finger motives, about 1000 Ca binding sites in lectines etc.) and compared RMSD values with other software (VMD, UCSF Chimera, and PyMol). SiteBinder provided better results for most of the tested motives.

1. F.P. Casey, N.E. Davey, I. Baran, R. Svobodová Vařeková, D.C. Shields: Web Server To Identify Similarity of Amino Acid Motifs to Compounds (SAAMCO), *J. Chem Inf Model*, 10 (2008) 1524 - 1529.
2. I. Baran, R. Svobodová Vařeková, L. Parthasarathi, S. Suchomel, F. Casey, D.C. Shields: Identification of Potential Small Molecule Peptidomimetics Similar to Motifs in Proteins. *J Chem Inf Model*, 47 (2007) 464 - 474.
3. E.A. Coutsias, C. Seok, K.A. Dill: Using Quaternions to Calculate RMSD. *J Comp Chem*, 25 (2004) 1849 - 1857.
4. X. Wang, J. Snoeyink: Defining and Computing Optimum RMSD for Gapped and Weighted Multiple-Structure Alignment. *IEEE/ACM Trans Comput Biology Bioinform*, 5 (2008), 525 – 533.



L16

IMPROVING PROTEIN BINDING, IN SILICO APPROACH

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The ligand engineering group at the Institute of Biotechnology develops peptide/protein ligands for high affinity and high specificity binding of different cytokine molecules. Powerful experimental tools of molecular biology such as ribosome display are successfully combined with theoretical and computational tools including bioinformatics, homology modeling, and molecular dynamics. For example, analysis of available crystal structures, computational energy calculations, and preliminary screening are necessary for suggesting residues that should be randomized in the first round of screening by the ribosome display.

This step of computer-aided analysis is then followed by the rational improvement of high affinity mutants from ribosome display. Theoretical protocols for choosing the most probable residue candidates for in vitro mutagenesis will be presented. We will also compare roles of different theoretical methods and their interlocking with the experimental procedures that led to ligand improvements in our laboratory.

The support of the Institute of Biotechnology AS CR, v.v.i. (AV0Z50520701) is acknowledged.

Lectures - Friday, March 19, morning, Session IV

L17

COMPUTATIONAL ANALYSIS OF THE STABILIZATION EFFECTS IN SUCCESSIVE U-HG-U BASE PAIRS

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Metal-DNA interactions represent one of the key topics in nucleic acid research. The idea of mercury complexing with DNA bases appeared already in early 1960's [1]. Hg^{II} was shown to possess a very high affinity towards nitrogen atoms of nucleic acid bases [2]. Recently, a very stable structural pattern with mercury stabilizing a T-T mismatch inside double-strand DNA oligonucleotide has been revealed by means of NMR spectroscopy [3-5]. Furthermore, mercury binding to successive T-T mismatches inside DNA was also reported [3-4]. Theoretical studies of T-Hg^{II}-T base-pair properties followed the original experimental work [6-7].

The present study uses computational tools to question the stabilization effects in successive T-Hg^{II}-T base pairs. By applying the RI-MP2 *ab initio* method we analyzed the nature of the attractive interaction between two U-Hg^{II}-U units, where uracil base was introduced instead of thymine in order to reduce the computational costs. By decomposing the interaction energy we identified the role of mercury in stabilizing the successive U-Hg^{II}-U base pairs. Significant interaction energy contributions arised

from the charge redistribution on uracil rings induced by mercury, mercury through-space interaction with uracil of the other U-Hg^{II}-U unit, and Hg-Hg metallophilic attraction.

1. T. Yamane, N. Davidson, *J. Am. Chem. Soc.*, 83, (1961), 2599.
2. L. D. Kosturko, C. Folzer, R. F. Stewart, *Biochemistry*, 13, (1974), 3949.
3. Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami, A. Ono, *J. Am. Chem. Soc.*, 126, (2006), 2172.
4. Y. Tanaka, H. Yamaguchi, S. Oda, Y. Kondo, M. Nomura, Ch. Kojima, A. Ono, *Nucleosides, Nucleotides, and Nucleic Acids*, 25, (2006), 613.
5. Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, Ch. Kojima, A. Ono, *J. Am. Chem. Soc.*, 129, (2007), 244.
6. A. A. Voityuk, *J. Phys. Chem. B*, 110, (2006), 21010.
7. A. Bagno, *J. Am. Chem. Soc.*, 129, (2007), 11360.

L18

FAST METHODS OF ATOMIC CHARGE CALCULATION: PARAMETERIZATION OF EEM FOR APPLICABILITY TO METAL CONTAINING PROTEINS

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Atomic charges, although not physical observables, are used to explain many molecular properties and are needed in many molecular packages. The quantum chemical approach to calculating various types of atomic charges can be very precise, but extremely time demanding; in any case, its applicability to biomolecules is restricted by the size of the systems. One of the already available alternative solutions is the Electronegativity Equalization Method (EEM), which allows for the fast calculation of partial atomic charges with remarkable precision [1], provided that the proper parameters have been previously determined.

Previous studies in this respect have made great progress since the original development of EEM by improving the formalism [2], increasing the number of covered atom types [3], testing the amenability of various atomic charge schemes [4], implementing the EEM formalism in modeling software [5] etc. However, none of them has dealt with system sizes of more than 200 atoms, a number which is hardly relevant at a biomolecular level.

We have obtained EEM parameters for the elements commonly found in proteins (C, H, N, O, S) and the Ca ion

that may appear as a ligand, for systems whose size is around 1000 atoms. All these systems are parts of very large proteins, and therefore the parameters we have obtained should be able to predict partial atomic charges on full-sized real proteins to a good approximation. We present the complete process of generating these EEM parameters.

1. W.J. Mortier, S. K. Ghosh, S. Shankar, *J. Am. Chem. Soc.*, **108**, (1986), 4315.
2. Z.-Z. Yang, C.-S. Wang, *J. Phys Chem. A*, **101**, (1997), 6315.
3. R. Svobodova Vařekova, Z. Jirouskova, J. Vanek, S. Suchomel, J. Koca, *Int. J. Mol. Sci.*, **8**, (2007), 572.
4. P. Bultinck, W. Langenaeker, P. Lahorte, F. De Proft, P. Geerlings, C. Van Alsenoy, J. P. Tollenaere, *J. Phys. Chem. A*, **106**, (2002), 7895.
5. K. S. Smirnov, B. van de Graaf, *J. Chem. Soc., Faraday Trans.*, **92**, (1996), 2469.

L19

EMPLOYMENT OF FREE ENERGY CALCULATIONS TO ESTIMATION OF CARBOHYDRATES AFFINITIES TOWARDS PA-IIL SUPERFAMILY LECTINS

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Binding free energy is the important thermodynamic property used for description of affinity of ligand to receptor. It is used in drug design for fast qualitative ranking of drug-like compounds. Experimental laboratory procedure leading to acquirement of binding free energy is multi step approach, which is long and possibly without results. Problems with purification and obtaining of efficient concentration of sample for measurement of thermodynamic parameters are most common. Thus free energy calculations are convenient supply laboratory measurements. When 3D structure of ligand receptor complex is available, then binding free energies can be calculated. Moreover affinities of structurally similar ligands can be then easily calculated. Most demanding objective in this field is

preparation of universal procedure for calculations of carbohydrate affinities to lectins.

Potential of free energy calculations will be shown on project from glycobiochemistry field, particular on lectins from bacteria *Pseudomonas aeruginosa* and *Chromobacterium violaceum*. Lectins are group of proteins of non-immune origin specifically binding carbohydrates with high affinity. They play crucial role in cell recognition, signaling and adhesion. Bacterial lectins are known as very important participants in process of bacterial infiltration of host organism.

Lectin PA-IIL is a tetrameric protein where dimer is functional unit with two calcium ions in each binding site. This lectin mediates adhesion of bacteria *Pseudomonas aeruginosa* to tissues of respiratory tract of patients suffer-



ing from cystic fibrosis. PA-IIL shows highest affinity to L-fucose and binds also other carbohydrates [1]. *Chromobacterium violaceum* lectin CV-IIL is member of PA-IIL lectin superfamily and shows similar function. In silico designed and prepared mutant structures of this lectin were used for molecular dynamics simulations. Their affinity to α -L-fucose and α -D-mannose was evaluated to elucidate binding contributions of residues within binding pocket.

The binding free energies of complexes of PA-IIL with α -L-fucose, Me- α -L-fucose and Me- α -L-galactose and complexes of CV-IIL and CV-IIL mutants with α -L-fucose α -D-mannose were estimated by thermodynamic integration method. This method is available in Amber 9 package [3]. It is based on slow alchemical transformation of one molecule to another. This method allows using explicit solvent model and parallelization of computations. Computed binding free energies of PA-IIL, CV-IIL and various monosaccharides were compared with isothermal titration microcalorimetry data. Computed relative binding free energies were used for compilation of series of ligand affinity to receptor which was in agreement with experimentally obtained data. Affinities computed by thermodynamic integration differ from experimental values in range of 0.1 to 1.0 kcal/mol.

L20

MOLECULAR AND STRUCTURAL MECHANISMS OF CELL DIVISION SITE RECOGNITION IN *Bacillus subtilis*

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Bacillus subtilis is an internationally-recognised model organism, whose physiology, biochemistry and genetics has been studied for many years. Our research is oriented toward studying the proteins involved in basic processes in *Bacillus subtilis* as cell division, sporulation and programmed cell death.

Probably the most controversial question regarding cell division of rod-shaped bacteria concerns the mechanism that ensures correct placement of the division septum. At least two distinct mechanisms contribute to placement of the division machinery: the Min system and nucleoid occlusion. The fluid mosaic model of membrane structure has been revised in recent years as it has become evident that domains of different lipid composition are present in eukaryotic and prokaryotic cells. Using membrane binding fluorescent dyes, we demonstrate the presence of lipid spirals extending along the long-axis of cells of the rod-shaped bacterium *B. subtilis*. These spiral structures

This work has been supported by Ministry of Education of the Czech Republic (MSM0021622413, LC06030). The access to the METACentrum supercomputing facilities provided under the research intent MSM6383917201 is highly appreciated. The research leading to these results has received funding from the European Community's Seventh Framework Programme under grant agreement no. 205872. This work has been supported by Czech Science Foundation (301/09/H004, doctoral program).

1. J. Adam, M. Pokorná, C. Sabin, E. P. Mitchel, A. Imberty & M. Wimmerová, BMC Structural Biology, 7 (2008), Engineering of PA-IIL lectin from *Pseudomonas aeruginosa* - Unravelling the role of the specificity loop for sugar preference.
2. M. Pokorná, G. Cioci, S. Perret, E. Rebuffet, N. Kostlánová, J. Adam, N. Gilboa-Garber, E. P. Mitchell, A. Imberty & M. Wimmerová, Biochemistry, 45 (2006) 7501-7510, Unusual entropy-driven affinity of *Chromobacterium violaceum* lectin CV-IIL towards fucose and mannose.
3. D. A. Case, T. A. Darden, T. E. Cheatham, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo, K. M. Merz, D. A. Pearlman, M. Crowley, R. C. Walker, W. Zhang, B. Wang, S. Hayik, A. Roitberg, G. Seabra, K. F. Wong, F. Paesani, X. Wu, S. Brozell, V. Tsui, H. Gohlke, L. Yang, C. Tan, J. Mongan, V. Hornak, G. Cui, P. Beroza, D. H. Mathews, C. Schafmeister, W. S. Ross, P. A. Kollman, University of California, San Francisco, 2006, Amber 9.

are absent from cells in which the synthesis of phosphatidylglycerol is disrupted suggesting an enrichment in anionic phospholipids. Green fluorescent protein fusions of the cell division protein MinD from *B. subtilis* also form spiral structures and these were shown by fluorescence resonance energy transfer (FRET) to be coincident with the lipid spirals. These data indicate a higher level of membrane lipid organization than previously observed and a primary role for lipid spirals in determining the site of cell division in bacterial cells. Little is known however of the origin of these spiral structures. In our current work we have focused on analyzing these lipid structures in correlation with other previously observed helical structures in the cell membrane or its close proximity.

This work was supported by the grant APVT-51-027804, No. ESF-EC-0106, LPP-0218-06 and VEGA grant 2/7007/27 from the Slovak Academy of Sciences and The Wellcome Trust Grant 082829/Z/07/Z.