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## ANALYSIS OF HETEROGENEOUS HINGE-REGION O-GLYCOSYLATION OF HUMAN IGA1 USING MALDI-TOF/TOF MASS SPECTROMETRY

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Changes in the glycosylation patterns of various glycoproteins are associated with several diseases. Hence determining disease-associated glycosylation patterns and heterogeneity provides a better understanding of disease mechanisms. This work focuses on the *O*-glycosylation of immunoglobulin A1 (IgA1), where aberrant glycosylation plays a key role in the pathogenesis of IgA nephropathy (IgAN). IgA1 hinge region carries 3-6 *O*-glycans consisting of *N*-acetylgalactosamine (GalNAc) with galactose (Gal); both glycans may be sialylated. In IgAN patients, some *O*-glycans on a fraction of IgA1 molecules are Gal-deficient. Here we describe a sample preparation protocol with optimized cysteine alkylation of a Gal-deficient polymeric IgA1 myeloma protein prior to in-gel digestion

and analysis of hinge-region glycopeptides by MALDI-TOF/TOF mass spectrometry (MS) as a novel strategy. IgA1 hinge-region glycopeptides were fractionated by reversed-phase liquid chromatography using a microgradient device and identified by MALDI-TOF/TOF tandem MS (MS/MS). The acquired MS/MS spectra were interpreted manually and by means of our own software, which allowed assigning up to six *O*-glycosylation sites and suggested possible isomeric *O*-glycoforms. The most abundant Gal-deficient *O*-glycoforms were GalNAc<sub>4</sub>Gal<sub>3</sub> and GalNAc<sub>5</sub>Gal<sub>4</sub> with one Gal-deficient site and GalNAc<sub>5</sub>Gal<sub>3</sub> and GalNAc<sub>4</sub>Gal<sub>2</sub> with two Gal-deficient sites. The most frequent Gal-deficient sites were at Ser230 and/or Thr236.

Thursday, March 14, Session II

L5

## EVOLUTION OF GENETIC DIVERSITY OF REPETITIVE EXTRAGENIC PALINDROMIC ELEMENTS (REPS): A COMPARATIVE STUDY

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Repetitive extragenic palindromic elements (REPs) constitute a group of bacterial genomic repeats known for their high abundance and several functions of importance for host cells' physiology. We analyzed the phylogenetic distribution of particular classes of REP elements in genomic sequences of sixty-three bacterial strains belonging to the *Pseudomonas fluorescens* species complex and ten strains of *Stenotrophomonas* sp., in order to assess intraspecific REP diversity and to gain insight into long-term REP evolution.

Based on proximity to RAYT (REP-associated tyrosine transposase) genes, twenty-two and thirteen unique REP classes were determined in fluorescent pseudomonads and stenotrophomonads, respectively. REPs were generally occurring in hundreds or even over a thousand of perfect

copies of particular REP class per genome. REP sequences showed highly heterogeneous distribution. The abundances of REP classes roughly followed host strains' phylogeny, differing markedly among phylogenetic clades. High abundances of particular REP classes appeared to depend on the presence of cognate RAYT gene, and deviations from this state could be attributed to recent or ancient mutations of *rayt*-flanking REPs, or RAYT loss. RAYTs of both studied bacterial groups are monophyletic, and their cognate REPs show species-specific characteristics, suggesting shared evolutionary history of REPs, RAYTs and their hosts.

Our results show that REP elements constitute intriguingly dynamic components of genomes of fluorescent pseudomonads and stenotrophomonads, and indicate that REP diversification and proliferation are



ongoing processes. High numbers of REPs have apparently been retained during the entire evolutionary time since the establishment of these two bacterial lineages, probably because of their beneficial effect on host long-term fitness. REP elements in these bacteria represent suitable platform

to study interplay between repeated elements, their mobilizers and host bacterial cells.

L6

## STRUCTURE AND DYNAMICS OF BIOMOLECULES IN NON-AQUEOUS IONIC SOLUTIONS

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Biomolecules such as enzymes can be solvated in organic solvents and ionic liquids, leading either to conformational changes in their enzyme structure, or changes in their enzymatic activity. The use of enzymes in organic media can bring benefits for pharmaceutical industries by exploiting its potential for the asymmetric synthesis of drugs because a majority of these compounds are insoluble in water and organic solvent can improve their solubilities. Although organic solvents and ionic liquids can improve the solubility of such non-soluble compounds, solutions containing organic solvents can not be used at all concentrations for enzymatic reactions thus limiting the usage of organic solvents in industrial applications. Non aqueous solutions are not only changing the native structure of enzymes or their catalytic activity, but they may lead even to denaturation of biomolecules. In order to understand the solvation structure of biomolecules in non-aqueous media and ionic liquids surface structure and bulk properties of them must be studied both experimentally and theoretically.

Surface and bulk properties of such non-aqueous media can be studied by different experimental methods such vibrational sum frequency generation (VSFG) spectroscopy, second harmonic spectroscopy as surface sensitive methods and X-ray and neutron diffraction methods as

techniques to study bulk properties. As theoretical method, classical molecular dynamics (MD) simulations have been used to study both bulk and surface properties of pure non-aqueous media and aqueous solutions of organic solvents and ionic liquids.

Due to special properties of ionic liquids such as low vapor pressure, high thermal stability, electrochemical stability and low volatility they can serve as better solvents than usual organic solvents.

Non-aqueous media can have different effect of the catalytic reaction such as lowering the energy of activated complex or stabilization of enzyme in solution. Also using solution of organic solvents can improve the solubility of some reactant and stabilize the structure of biomolecules both by hydrophobic interactions and hydrogen bonding.

In this talk surface and bulk properties of some protic and aprotic ionic liquids will be presented both theoretically and experimentally. Moreover, the structure and dynamics of model protein in non-aqueous media such as pure and aqueous solution of protic ionic liquid which is studied by means of classical molecular dynamics (MD) simulations will be presented.

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## INTERDOMAIN INTERACTION AND COMMUNICATION IN THE MOTOR SUBUNIT OF *E. COLI* IN RESTRICTION MODIFICATION SYSTEM ECOR1241

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Type I restriction modification system are intriguing multifunctional multisubunit molecular motors that can catalyze both restriction and modification activity. These enzymes binds to its target sequence and its activity as an endonuclease or methyltransferase is determined by the methylation state of the target sequence. If the target sequence is unmodified, the enzyme while bound to its target site is believed to translocate or pull the DNA towards itself

simultaneously in both directions in an ATP dependent manner.

The crystal structure of the motor subunit R has been determined by our group but the molecular mechanism by which these enzymes translocate and cleave the DNA is not fully understood.

The type I motor subunit has a square planar arrangement of globular domains with a prominent cleft that can accommodate DNA extending between two canonical

helicase domains to the endonuclease active site. ATP binding is proposed to play a major role in coupling the both the activities as the crystal structure shows an unexpected contact of endonuclease LYS220 to the translocase bound ATP. We believe this contact play a major role in signal transfer.

To explain the underpinning molecular mechanisms of coupling ATP-dependent DNA translocation and DNA cleavage and the communication pathway through the motor subunit, we carried out molecular dynamics simulations with selected mutations on the endonuclease 220 and 180 loop, that could be potentially engaged in conformational changes that occur once translocation is stalled and a signal is transmitted to the endonuclease.

Additional *in silico* mutants and their simulations demonstrate the importance of inter domain interactions be-

tween helical-helicase2 domain and at the helical-endonuclease interface close to the proposed DNA path for DNA translocation and consequent restriction activity.

*We gratefully acknowledge support from the Czech Science Foundation (project number GACR P207/12/2323), and the Grant Agency of the University of South Bohemia (grant no. 170/2010/P). Some computations were performed in MetaCentrum SuperComputer facility.*

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**L8**

## ANALYSIS OF PROTEIN/DNA INTERACTIONS BY NOVEL BIOINFORMATIC TOOLS

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We analyzed interactions between proteins and DNA from over a thousand crystal structures of their complexes. To this end, we built a database of more than 50 thousand protein/DNA contacts that can be sorted by a wide range of criteria such as the identity of the interacting atoms and residues, type of contacts including water-mediated ones, crystallographic resolution, and protein Pfam classification.

Novelty of the analysis lies in our fine-grained categorization of protein and DNA local conformations. Protein structures were classified into “peptide blocks” [de Brevern et al. *Proteins* 41, 271 (2000)] and DNA structures into dinucleotide conformers [Svozil et al. *NAR* 36, 3690 (2008)]. We determined how distributions of peptide blocks and dinucleotide conformers differ at and outside the protein/DNA interface and discussed variability of the distributions between various groups of structures (DNA

complexes of enzymes, transcription factors, structural proteins) overall and also for contacts to the DNA minor and major grooves and phosphates. We examined how different are occurrences of peptide blocks and dinucleotide conformers at the interface and whether they are statistically over- or under-represented. We concentrated on analysis of direct polar contacts (mostly hydrogen bonds) and water-mediated contacts and observed e.g. a unique behavior of the water-mediated contacts in the DNA complexes of transcription complexes. Analysis of temperature displacement factors (“B-factors”) of the analyzed complexes showed the fundamental difference between behavior of proteins and DNA at the interface.

*This work is supported by the Czech Science Foundation (P305/12/1801). BS and JC are supported by the institutional grant AV0Z50520701.*