National Centre for Biomolecular Research

/ Faculty of Science / Masaryk University

CEITEC central european institute of technology
BRNO | CZECH REPUBLIC

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THEMATIC RESEARCH FOCUS

RESEARCH AREA

- Computational chemistry and molecular modelling
- NMR spectroscopy of biomolecules
- Glycobiochemistry
- RNA/protein interactions
- RNA processing and degradation
- DNA reparation

EXCELLENCE

Research of structure-function relationships of biomolecular systems that integrates computational and experimental methods. It includes methods for structure characterization (NMR, protein crystallization), thermodynamic and kinetic measurements (ITC, SPR), and nanobiotechnology (AFM) which is complemented with a strong computational background for theoretical studies (molecular dynamics, free energy calculations, quantum chemistry, protein-ligand docking).

MISSION

We conduct research in the field of structural biology and biomolecular chemistry utilizing methods of computational chemistry, NMR spectroscopy, glycobiochemistry, protein engineering, nanobiotechnology and nucleic acid research. Theoretical and experimental research focuses on the structural characterization of biologically interesting molecules, especially proteins, nucleic acids, carbohydrates and their complexes. Top methods are employed in order to describe structural and functional properties of the molecules that may be interesting, for example, for drug design and development. Individual research projects focus on molecular recognition and host/pathogen interactions, RNA quality control, DNA repair mechanisms and cholinesterases in relation to neural diseases and chemical weapons, and other topical subjects.

DEVELOPED TECHNOLOGIES

CONTENT OF RESEARCH

Computational Chemistry and Molecular Modelling

- Computational studies of structure, dynamics and function of catalytic RNA
- Molecular interactions in nucleic acidsComputational studies of lectin-carbohydrate interactions and in silico protein engineering
- Activation and inhibition of cyclin-dependent kinases
- Acetylcholinesterase and reactivation
- Structure and dynamics of restriction endonuclease HINC II
- Development of TRITON software for protein engineering, docking and enzymatic reactions modelling
- Development of software for conformational analysis
- Charge calculation and study of electrostatic interactions
- Methods for free energy calculations implementation and application

NMR Spectroscopy

- Ab initio calculations of NMR parameters
- Novel experimental techniques in nuclear magnetic resonance of biomolecules
- Data and structure validation
- Protein structure and dynamics
- Structure and dynamics of nucleic acids
- Studies of purine derivatives, proton transfer processes, complexations
- Structural studies of isoquinoline alkaloids

Glycobiochemistry

- Structure-functional studies of prokaryotic and eukaryotic glycosyltransferases
- Structure-functional studies on lectins from pathogenic organisms and their interactions with carbohydrates

Nanobiotechnology

- Development of a novel sensing technique based on nanomechanics for the rapid detection of bioagents
- Nanotechnological and bioanalytical detection of the DNA damage

DNA/RNA Research

» Homologoues recombination and repair of DNA DSB breaks
» Molecular basis of RNA quality control and degradation in cell nucleus
» Structural basis for poly(A) independent transcription termination and processing

FIELDS OF RESEARCH RESULTS APPLICATION

» Medicine and pharmacology – study of protein targets for the rational design of chemotherapeutic agents against M. tuberculosis, anti-tumour therapeutics, design of protein molecules for drug delivery and innovative methods for testing quality of materials for implants
» Military defence technologies – development of acetylcholinesterase reactivators for treatment of organophosphates (nerve agents and pesticides) intoxication

ALUMNI PROFILE

Graduates have knowledge of NMR techniques for the study of biomolecules, computational chemistry methods for the study of proteins, nucleic acids and carbohydrates (molecular dynamics, QM calculations, molecular docking), experimental techniques for the study of protein-ligand complexes (ITC, SPR), experimental methods for protein and nucleic acid isolation and analysis, experimental techniques for nanotechnology (AFM), bioinformatics and scientific software development.
Graduates are then applied primarily as experts in biomolecular and medical research, molecular modelling, bioinformatics, drug R&D and scientific software development.

NUMBER OF RESEARCH POSITIONS ↘

SENIOR RESEARCH STAFF

18

JUNIOR RESEARCH POSITIONS (INCL. PH.D. STUDENTS)

82

COMPUTATIONAL CHEMISTRY AND MOLECULAR MODELLING ↘

COMPUTATIONAL STUDIES OF STRUCTURE, DYNAMICS AND FUNCTION OF CATALYTIC RNA

The project is focused on study of ribosomal protein-RNA complexes, and other protein-NA complexes with the aim to better characterize the catalytic centre of the ribozymes. State-of-the-art computational techniques are used, including explicit solvent molecular dynamics simulations, advanced ab initio quantum chemical methods and modern bioinformatics methods. Understanding the structure and dynamics of these complexes can be useful for development of new therapeutic agents based on blocking proteosynthesis of pathogenic microorganisms.

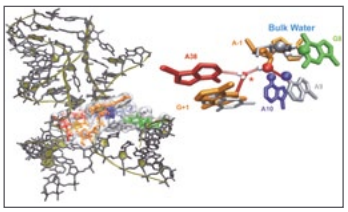

Figure 1 3D structure of Hairpin ribozyme with highlighted catalytic cavity

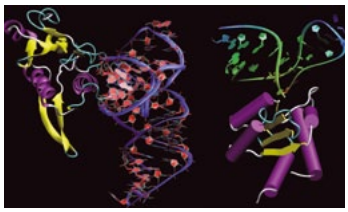

Figure 2 Detail of active site

COMPUTATIONAL STUDIES OF LECTIN-CARBOHYDRATE INTERACTIONS AND IN SILICO PROTEIN ENGINEERING

Lectin-saccharide interactions are related to the virulence of several bacteria that are capable of acting as opportunistic human pathogens or fytopathogens. The project employs methods of molecular docking and molecular dynamics to study lectin-saccharide interactions. The aim of the project is a development of a reliable in silico based method for prediction of binding affinity between ligand and lectin molecule.

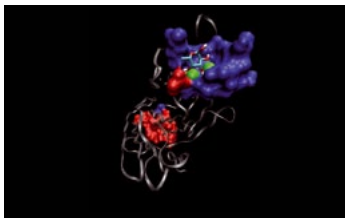

Figure 3 The structure of PA-IIL saccharide binding site

ACTIVATION AND INHIBITION OF CYCLIN-DEPENDENT KINASES

The enzymes from the Cyclin Dependent Kinases (CDK) group play an important role in controlling the eukaryotic cell division cycle. Their deregulation was proven in a series of tumours. For the synthesis of the selected inhibitors, detailed knowledge about all the interactions in the active site of the protein is important. We use the molecular dynamics method, for studying the conformational behaviour of proteins and also for studying interactions between proteins and their substrates or solvent molecules.

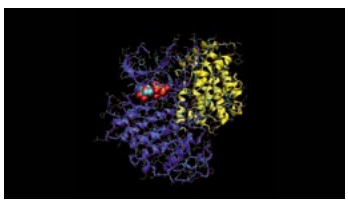

Figure 4 Complex of cyclin A (yellow) with cyclin dependent kinase 2. Molecule ATP is highlighted in the active site of the CDK2

ACETYLCHOLINESTERASE AND REACTIVATION

Acetylcholinesterase is responsible for regulation of nerve signal transmission. Organophosphates such as nerve agents and pesticides are able to inhibit this enzyme by covalent modification of serine residue in the active site.
In the case of nerve agents, this inhibition is lethal. Substances called reactivators are able to attack the covalently bonded organophosphate and liberate acetylcholinesterase. This project contributes to the search for better reactivators by providing structural information. Methods used so far are molecular dynamics and protein-ligand docking.

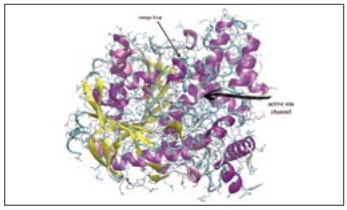

Figure 5 The structure of acetylcholinesterase enzyme.

STRUCTURE AND DYNAMICS OF RESTRICTION ENDONUCLEASE HINC II

Restriction endonuclease HincII cleaves DNA at GTPyPuAC sequence. Magnesium atom is an essential cofactor for this enzyme. Molecular dynamics is used as a tool to describe reaction partners or intermediates. We want to describe the structure of the complex to provide detailed view of the active site and relationships in it and to bring some ideas about the structure of the active site and possible role of the ions in it.

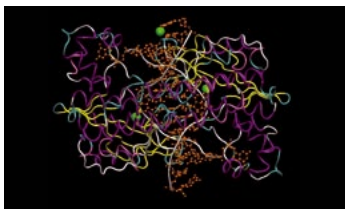

Figure 6 Dimeric biological unit of restriction endonuclease

DEVELOPMENT OF SOFTWARE FOR COMPUTATIONAL CHEMISTRY

Projects focused on software development include the program CICADA (for conformational analysis), EEM solver and ABEEM solver (charge calculation and study of electrostatic interactions), the graphical program TRITON (visualization of scientific data) and MOLE (location and characterization of channels in protein structures).

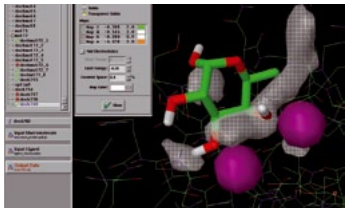

Figure 7 Graphical interface of the TRITON program

NMR SPECTROSCOPY ↘

AB INITIO CALCULATIONS OF NMR PARAMETERS

Research interests focus on computing and understanding the NMR parameters of building blocks of nucleic acids by means of ab inito quantum mechanics. Isotropic chemical shielding and spin-spin coupling constants in nucleosides is studied for a series of anhydrodeoxythymidines. and genuine deoxyribonucleosides.

NOVEL EXPERIMENTAL TECHNIQUES IN NUCLEAR MAGNETIC RESONANCE OF BIOMOLECULES

A general goal of NMR studies of biomolecules is to obtain as accurate as possible data that can characterize the structure and/or dynamics of the studied system. Efficient new methods are studied to measure small changes in spin-spin couplings induced by oriented media and to obtain accurate values of chemical shifts of as many nuclei in the molecule as possible.

Photo 1 600 MHz NMR spectrometer Bruker AVANCE

PROTEIN STRUCTURE AND DYNAMICS

Mouse major urinary protein I (MUP-I) is studied using NMR techniques and molecular dynamic simulations. Results indicate that the pheromone binding does not rigidify the MUP-I structure. On the contrary, several regions of increased flexibility have been identified in the protein-pheromone complex. Other studied proteins include mammalian lectin-like receptor domains, plant lipid-transfer proteins, bacterial RNA polymerases, retroviral proteases etc. The main goal is to provide a complex description of the systems and thus help to understand their biological roles.

STRUCTURE AND DYNAMICS OF NUCLEIC ACIDS

Although the general structural features of regular DNAs and RNAs are well known, there exist a plethora of structural motifs different from the regular double helix. The structures of d(C4G4) and d(G4C4) duplexes and d(GCGAAGC) hairpin were among those solved in our laboratory. Measurement of nuclear spin relaxation by NMR spectroscopy is a powerful approach for studying intramolecular motions at atomic resolution on the nanosecond to picosecond time scale.

GLYCOBIOCHEMISTRY ↘

STRUCTURE-FUNCTIONAL STUDIES OF PROKARYOTIC AND EUKARYOTIC GLYCOSYLTRANSFERASES

The project is focused on structure-function studies of proteins, which participate on oligosaccharide synthesis (glycosyltransferases) using bioinformatic tools and molecular biology experimental methods.
Studied proteins are mycobacterial glycosyltransferases which are involved in biosynthesis of mycobacterial cell wall. They are potential and attractive targets for the rational design of novel chemotherapeutic agents against M. tuberculosis.

Photo 2 Instrumentation for ITC (isothermal titration calorimetry)

Photo 3 Instrumentation for SPR (surface plasmon resonance)

STRUCTURE-FUNCTIONAL STUDIES ON LECTINS FROM PATHOGENIC ORGANISMS AND THEIR INTERACTIONS WITH CARBOHYDRATES

Research is focused on studies of carbohydrate binding proteins (lectins) from opportunistic human pathogen Pseudomonas aeruginosa (and some other organisms) as they can play a key role in host-pathogen interactions. Advanced functional analysis methods (isothermal titration microcalorimetry, surface plasmon resonance, differential scanning microcalorimetry) are used to obtain a wide range of kinetic and thermodynamic data of protein-carbohydrate interactions. The aim is to develop methods of the rational design of carbohydrate-based drugs directed against adhesion and virulence of pathogenic bacteria and fungi.

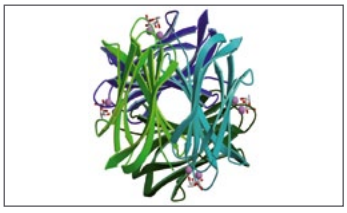

Figure 8 Structure of PA-IIL lectin complex with fucose

NANOBIOTECHNOLOGY ↘

DEVELOPMENT OF A NOVEL SENSING TECHNIQUE BASED ON NANOMECHANICS FOR THE RAPID DETECTION OF BIOAGENTS

The goal of the research is to develop a new sensing device capable of the rapid detection of bioagents in an ambient environment. The project addresses the detection of three classes of bioagent: toxins, viruses, and bacteria. For each class of bioagents, suitable bioreceptors will be used which will be immobilized on the cantilever surface.

NANOTECHNOLOGICAL AND BIOANALYTICAL DETECTION OF THE DNA DAMAGE

The aim of the project is the development of novel methods for detection of DNA damage resulting from exposure to polyaromatic hydrocarbons (PAH). The localization of DNA damage is studied using atomic force microscopy, nanoparticle-labelled antibodies serve for visualization of the point of damage. Newly developed detection methods are tested on real samples and validated with alternative approaches.

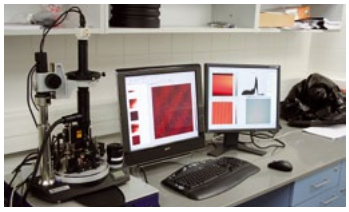

Photo 4 Atomic force microscope (AFM)

DNA/RNA RESEARCH ↘

HOMOLOGOUES RECOMBINATION AND REPAIR OF DNA DSB BREAKS

Homologous recombination plays a vital role in DNA metabolic processes and its defects can lead to pathological outcomes, including genetic diseases and cancer. Mutations in the BRCA2 gene represent the cause of a significant portion of familial breast cancers. The goal of this project is to demonstrate and characterize the recombination mediator function of BRCA2 and also to define the molecular basis for its function.
Another project is focused on the SRS2 gene product (from the yeast Saccharomyces cerevisiae) which participates on the regulation pathway of homologous recombination. The goal is to identify the role of SRS2 as a molecular switch during recombination and DNA repair.

MOLECULAR BASIS OF RNA QUALITY CONTROL AND DEGRADATION IN CELL NUCLEUS

The project aims to characterize in detail molecular mechanisms that assure that aberrant RNA species in the nucleus are efficiently recognized and destroyed. We use a combined approach of molecular biology, biochemistry, structural biology and genetics.

STRUCTURAL BASIS FOR POLY(A) INDEPENDENT TRANSCRIPTION TERMINATION AND PROCESSING

RNA Polymerase II produces not only messenger RNA but also a set of functional RNAs that are essential for the proper function of a cell. The biogenesis of these RNAs remains poorly understood and involves many dynamical processes mediated by protein-RNA and protein-protein complexes that assemble at the site of transcription. We focus on determining the structures at the atomic resolution of such complexes using Nuclear Magnetic Resonance (NMR) spectroscopy.

KEY RESEARCH EQUIPMENT

LIST OF DEVICES

NMR spectroscopy:
600 MHz NMR spectrometer Bruker AVANCE
500 MHz NMR spectrometer Bruker AVANCE III
300 MHz NMR spectrometer Bruker AVANCE

Advanced instrumentation:
Surface plasmon resonance instrument Biacore3000
Isothermal titration microcalorimeters VP-ITC
Diferential scanning calorimeter VP-DSC
Crystallisation robot Mosquito
Optical stereoscope Leica with CCD camera
Automatic liquid handling system with vacuum manifold Tecan Evo 150
Automatic Colony picker PM-1s
AKTApurifier and several AKTAfplc chromatographs
Liquid phase AFM Ntegra
CD spectrometer Jasco J-815

Computational hardware
Computational cluster with 320 processor cores (Xeon E5620, 2.4 GHz)
Computational cluster with 72 processor cores (Opteron 8431, 2.4 GHz, 3x99GB RAM)
Computational cluster with 72 processor cores (Opteron 2218, 2,66 GHz)
3D visualization equipment
Access to Academic Supercomputer Centre (METAcenter)

Computational chemistry and molecular modelling software
Quantum chemistry programs (Gaussian, Gamess, Mopac, Spartan, deMON, CPMD)
Molecular mechanics and dynamics programs (Amber, X-PLOR, PME-MD)
Molecular visualization packages (WHAT IF, VMD, ICM, GRASP, RasMol, gOpenMol, MOLMOL, Gromacs, Spartan, Chimera, Midas Plus, MOIL-View, CCP4, Pymol, SPDB viewer, MolScript)
Docking software (AutoDock, DOCK, ICM)
Protein modelling tools (MODELLER, GRID, DelPhi, Promotif)
X-ray software and databases (CSD, O)
„In-House" software for protein engineering, potential energy (hyper) surfaces and flexibility analysis: TRITON, CICADA, EEM and ABEEM solver, MOLE, MULDER, PANIC, DRIVER, COMBINE, ANALYSE, VADER, ECSTASY, AIDA, PEGAS

BUDGET

TOTAL (MIL. CZK/ MIL. EUR)

80 / 3.2

PART OF THE TOTAL BUDGET FROM PRIVATE RESOURCES (%)

5

PART OF THE TOTAL BUDGET FROM FOREIGN RESOURCES (%)

25

MAIN PROJECTS

2009–2013: EAST-NMR: Enhancing Access and Services to East European users towards an efficient and coordinated Pan-European pool of NMR capacities to enable global collaborative research & boost technological advancements (Contract No. RII3-026145EU-NMR, EC FP7 R&D programme, European Commission)

2009–2012: Compact Training Centre in Structural Biology and Biomolecular Chemistry (Operation programme Education for Competitiveness)

2008–2011: POSTBIOMIN: Program developing interdisciplinary research potential for the studies of biomolecular interactions (REGPOT-2007-1, EC FP7 R&D programme, European Commission)

2005–2011: Proteins in metabolism and interaction of organisms with the environment (MSM0021622413, Long term research plan, Ministry of Education, Youth and Sports)

2006–2010: Biomolecular centre (LC06030, Government programme of basic research centres, Ministry of Education, Youth and Sports)

MAIN COLLABORATING PARTNERS

COLLABORATION WITH ACADEMIC PARTNERS

Bowling Green State University (Bowling Green, Ohio, US)
University of Louis Pasteur (Strasbourg, FR)
Jacobs University Bremen (Bremen, DE)
University of Utah (Salt Lake City, Utah, US)
Swiss Federal Institutes of Technology (Lausanne, CH)
University of Barcelona (Barcelona, ES)
University of Arizona (Tucson, Arizona, US)
Cermav-CNRS (Grenoble, FR)
Norwegian University of Science and Technology (Trondheim, NO)
University of Kentucky College of Pharmacy (Lexington, Kentucky, US)
IFOM (Milan, IT)
Columbia University (New York, US)
Yale University (New Haven, Connecticut, US)
University of California (US)
University of Florence (IT)
University of Frankfurt (DE)
University of Lund (SE)
Institute of Bioorganic Chemistry (Poznan, PL)
University of Warsaw (PL)
Institute of Organic Chemistry and Biochemistry (Prague, CZ)
Charles University (Prague, CZ)
Institute of Chemical Technology (Prague, CZ)
Institute of Molecular Genetics (Prague, CZ)
Institute of Scientific Instruments (Brno, CZ)
Palacky University (Olomouc, CZ)
University of Defence (Hradec Králové, CZ)

COLLABORATION WITH COMPANIES

ANF Data – Siemens (Brno, CZ)
Biovendor (Brno, CZ)
Bruker (DE)
Polymer Institute (Brno, CZ)
Contipro Group (Dolní Dobrouč, CZ)
Synthon (Blansko, CZ)
I.Q.A. (Praha, CZ)

EXPECTATIONS

REQUIREMENTS

» To attract world researchers in the field, stability of funding

OFFERS

» Competitive research in a young team

04 / 2011