3.4. Report on the research activity of Team 2.
Structural Biology and Protein Engineering

1. Focus of the team

The main objective of the team is to mechanistically explain fundamental biological processes with potential applications of the results in the diagnosis and treatment of diseases. Our aim is to understand the mechanisms underlying intermolecular interactions in biomolecular systems and, in a broader sense, the relationship between the biomolecular structure and function. The studied molecular systems are produced by advanced techniques of molecular biology and protein engineering; their dynamics, structure, stability, and activity are determined by the state-of-the-art structural and biophysical experimental and computational methods.

Our primary research efforts have been directed into the following five main areas. Our achievements in these fields demonstrate that the team plays an internationally recognized role in basic as well as oriented biomedical research.

Structure-assisted drug design. Aimed at the development of small molecules targeting human glutamate carboxypeptidase II (GCPII) and histone deacetylases (HDACs) that could be used as imaging or therapeutic modalities (Kopka et al., 2017) (Novakova et al., 2016) (Shen et al., 2019).

Protein engineering. Combining techniques of *in vitro* protein evolution and of computer modeling for targeted modulation of protein properties such as binding affinity to medically important proteins (Cerny et al., 2015) (Hlavnickova et al., 2018) (Petrokova et al., 2019) (Kosztyu et al., 2019) (Zahradnik et al., 2019).

Structure-function studies of proteins. *De novo* structures of receptors, enzymes or complexes with biomedical or technological potential, employing modern methods of biophysical and structural molecular analysis including x-ray crystallography (Skalova et al., 2015) (Koval et al., 2016) (Koval et al., 2019) (Kovaľová et al., 2019).

Structural bioinformatics. Bioinformatics as a tool to better understand biomolecular structures, large scale comparison of structures, description of general structural behavior of nucleic acids and proteins, discovery of structural motifs (Biedermannova & Schneider, 2016; Cerny et al., 2016; Schneider et al., 2018; Schneider et al., 2017).

Dynamics of biomolecules represent a new line in our research established in 2015. We use single molecule imaging and force measurement techniques to uncover how individual structural elements of the cytoskeleton mechanically cooperate to drive diverse cellular processes (Siahaan et al., 2019) (Schmidt-Cernohorska et al., 2019).

2. Research activity and characterization of the main scientific results

The primary focus of the team is high-quality basic research in the field of structural biology and protein engineering with potential applications of the results in the diagnosis and treatment of diseases. Over the evaluated period, the team published in total 93 publications in peer-reviewed journals, 2 chapters in scientific books and obtained 17 applied results. The team comprises 6 laboratories, whose research activity over the evaluation period is detailed below. The overview of the publications is in the Appendix 3.6.

2.1. Laboratory of structural biology

Our research revolves around recombinant proteins, their production, engineering, structural and functional characterization from the single molecule level to their physiological functions in cellular and organism environments (Fig. 1). A platform for heterologous expression and purification of recombinant proteins implemented in our laboratory is extensively used not only by us but also by a

wide network of our collaborators and in our commercially oriented on-demand protein production. We are primarily interested in two major protein families as elaborated below:

(i) A family of peptidases homologous to **glutamate carboxypeptidase II (GCPII), a.k.a. prostate specific membrane antigen (PSMA).**

The human enzyme is implicated in several (patho)physiological processes. In the nervous system, GCPII exerts its peptidase activity by hydrolyzing a peptidic neurotransmitter. Accordingly, GCPII-specific inhibitors have been reported to be neuroprotective in multiple preclinical models of neurodegeneration. Furthermore, over-expression of GCPII in prostate carcinoma makes the enzyme a prime marker for prostate cancer imaging in clinics and a promising target of future therapeutic interventions.

In a series of eight manuscripts we contributed significantly to our understanding of structural features of human GCPII that could be exploited for the rational design of small-molecule GCPII-specific inhibitors. We described an unprecedented binding mode of hydroxamate-based compounds in the internal cavity of GCPII (Novakova et al., 2016) and these inhibitors were later converted into prodrugs improving thus their bioavailability (Rais et al., 2017). In collaboration with our partners, we designed and characterized GCPII-specific compounds of different chemistries (phosphoramidates, carboranes, ureas) and physicochemical characteristics. Additionally, our long-term contribution to the field was recognized by an invitation to co-author a review summarizing 20 years of the development of urea-based radioligands targeting GCPII (Kopka et al., 2017).

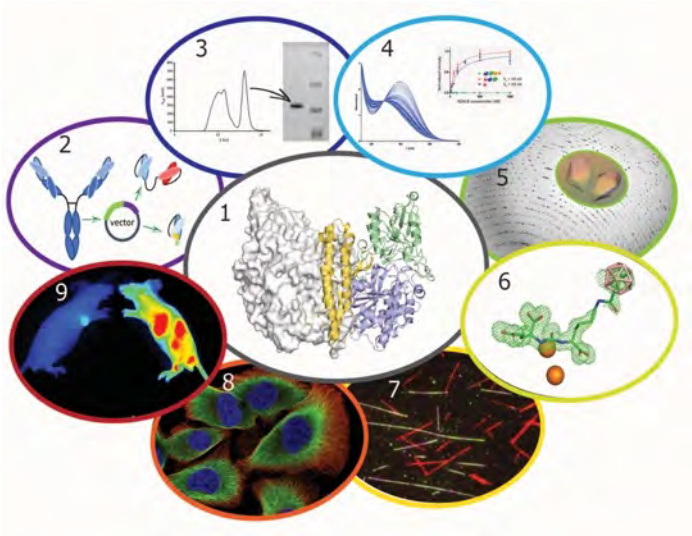


***Fig. 1**. Our research revolves around proteins (1) and includes protein engineering (2), expression/purification (3), biochemical and biophysical characterization (4), X-ray crystallography (5), structure-assisted drug design (6), in vitro (7), cells-based (8) and in vivo (9) assays.*

Protein engineering efforts resulted in three manuscripts describing the development of (i) protein scaffolds that offer a viable alternative to antibodies for biomedical applications (collaboration with A. Skerra, TUM); and (ii) GCPII-specific monoclonal antibodies with exquisite specificity and sub-nanomolar affinity (Banerjee et al., 2019; Novakova et al., 2017). These results have also been patented (US1040624; patent application WO2018129284-A1) and further efforts are aimed at their translation as diagnostics and therapeutics into human medicine.

Several of our projects were/are aimed at the understanding of role(s) of GCPII and its orthologs/paralogs in physiological processes, including uncovering a physiological function of NAALADase L (Tykvart et al., 2015), defining the role of GCPII for angiogenesis (Conway et al., 2016), and elucidating the functional significance of the calcium ion for GCPII stability and hydrolyzing activity (Ptacek et al., 2018).

(ii) A family of zinc dependent **histone deacetylases (HDACs 1 – 11)** that remove acyl/acetyl groups from the lysine side chain, a major post-translational modification found on >10% of proteins of the human proteome. Here we aim at (i) deciphering the structure-function relationship, (ii) defining physiological functions, and (iii) designing specific inhibitors of HDAC6 and HDAC11 isoforms.

Previously, we have established methodology for facile production of all eleven HDAC isoforms that become a cornerstone for our current research. Here developed and implemented *in vitro* assays for profiling isoforms specificity of HDAC inhibitors (Kutil, Mikesova, et al., 2019) (Zessin et al., 2019), established several cell-based assays, an ADMET pipeline, and a structural (X-ray) platform to

evaluate HDAC6/inhibitor complexes. This methodological portfolio is exploited in a series of ongoing projects within a network of our academic and corporate collaborators as it complements their expertise in medicinal chemistry and biological (*in vivo*) studies. Within the evaluation period we published total seven manuscripts aimed at the development of HDAC6-selective inhibitors for different applications (neurological disorders, melanomas), with several more manuscripts pending. Furthermore, several of our HDAC-related products (recombinant proteins/antibodies, assays) found their way into a commercial sector revealing thus the commercial outreach of our research.

Our "basic-research oriented" projects are focused on characterization of physiological functions and substrate preferences of HDAC6 and HDAC11. We have discovered that HDAC11 serves as a proficient fatty-acid deacylase and these findings can facilitate the uncovering of additional biological functions of the enzyme ((Kutil et al., 2018); 26 citations in two years). Using human acetylome microarrays and peptide libraries we mapped the substrate specificity of HDAC6, and these data can point out unknown physiological substrates of the enzyme (Kutil, Skultetyova, et al., 2019). We provided a detailed analysis of deacetylation of tubulin, the most prominent physiological substrate of HDAC6, and determined that free tubulin dimers are preferential form deacetylated by HDAC6 (Skultetyova et al., 2017). We discovered the unstructured N-terminus of HDAC6 to be the microtubule-binding domain critical for efficient tubulin deacetylation (Ustinova et al., 2020). We currently follow-up and broaden our research focus by implementing of methods of genetic code expansion to obtain more detailed understanding of substrate recognition by the HDAC family.

2.2. Laboratory of structure and function of biomolecules

Our structure-function studies of enzymes with biomedical and biotechnological potential have yielded results in three main directions: (i) non-specific nucleases, (ii) bilirubin oxidase, and (iii) novel glycosidases.

(i) S1-P1 nucleases have a broad range of biotechnological utilizations and there are many questions regarding their mechanism and applicability in treatment of cancer and other diseases. Continued from previous structure-function work we have finalized characterization of tomato nuclease TBN1 by determining 3D structure of its mutant N211D in complex with phosphate in its active center (Stransky et al., 2015) and determining the influence of glycosylation on its function (Podzimek et al., 2018). Eukaryotic nucleases of the S1-P1 type display a strong dependence of their activity and specificity on the level of glycosylation. Artificial increase of glycosylation leads to changes of enzyme specificity. We have determined the first three-dimensional structure of S1 nuclease from *Aspergillus oryzae*, together with functional and ligand binding details (Koval et al., 2016). Here, rules of its interactions with nucleic acids and new enzyme functionality have been discovered for this enzyme, known well for its biotechnological applications. S1-P1 nuclease from the causative agent of the Legionnaires' disease – *Legionella pneumophila* has been expressed and characterized in detail (Trundová et al., 2018). It has been shown to be an RNase with high temperature stability. The first study of this type of nuclease related to a human pathogen has indicated that this nuclease with extreme properties very likely binds to cell membrane. Our further structure-function analysis has yielded new results regarding the role of the surface binding sites of this nuclease (paper in preparation). Our review *Characteristics and application of S1–P1 nucleases in biotechnology and medicine* (Kovaľ & Dohnálek, 2018) was motivated by our growing experience and interest in this field and by the lack of such comprehensive work. This first review dealing with the properties and application of these enzymes concluded that this biotechnologically essential enzyme type has also an interesting potential in treatment of diseases caused by pathogens and also of some types of cancer.

(ii) Bilirubin oxidase is used in medicine for determination of bilirubin blood level and in industry, biofuel cells, and biosensors for its universal oxidation activity and efficient electron transfer. The enzyme employs a new and unique chemical bond between two amino acids. Using crystallography and mutagenesis we have shown that this Trp–His covalent bond is important for interaction with substrate, depending on the particular substance and environment (Koval et al., 2019). The results have implications for biotechnology and nanomaterials.

(iii) Our studies of new **glycosidases** are focused on proteins with unexplained structural properties and with expected application potential. We have determined the first crystal structure of α-L-

fucosidase of GH29 from *Paenibacillus thiaminolyticus* with a unique hexameric arrangement (6 molecules forming one functional unit) and active site complementation (Kovaľová et al., 2019). Its active site is complemented by a tryptophan residue from a neighbour protein chain within the hexamer and its mutation influences its fucosidase and transglycosylation activities. Also, a new type of carbohydrate binding domain has been identified. The results are important for applications of oligosaccharide-modifying enzymes. We have also contributed to determination of X-ray structures of two new, heavily glycosylated, β-D-glucosidases of family GH3 from *Aspergillus* sp. (Agirre et al., 2016), which provided new insights into fungal β-D-glucosidases and a platform for new enzyme variants for industrial application.

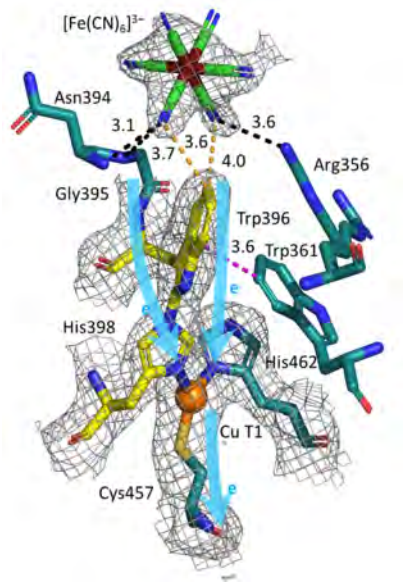


***Fig. 2**. Active site of enzyme bilirubin oxidase with unique covalent bond between amino acids tryptophan and histidine (sticks model with carbon in yellow) as observed in our crystal structure.*

In **natural killer cell receptors** we have explained the structural behavior (X-ray crystal structures) of the human cell surface protein LLT1 – a C-type lectin-like ligand of the human receptor NKR-P1 (Skalova et al., 2015). This was the first study with structural insights into LLT1. Our continued effort in collaboration with the team of O. Vaněk (Charles University, Faculty of Science) has resulted in determination of structures of several other complexes of mammalian cell surface receptors and ligands, awaiting completion of functional analysis.

Intracellular **heme-dependent signal proteins** lack detailed structure-function understanding of their function. In collaboration with the team of M. Martínková (Charles University, Faculty of Science) we have determined the first crystal structures of the globin domain of intracellular oxygen sensor AfGcHK (globin coupled histidine kinase) from soil bacterium *Anaeromyxobacter* sp. Fw109-5 with 5-coordinated and 6-coordinated heme states. By combination of X-ray diffraction and H/D exchange, some characteristics of signalling were explained for the first time (Stranava et al., 2017). Further studies have increased our understanding of the signal transfer and our monomeric crystal structure of the AfGcHK globin domain and mutational analysis of Tyr15 have shown the importance of Tyr15 for globin domain dimerization and its necessity for phosphorylation activity of the enzyme (Skalová et al., 2019).

Our structure-function studies of the **transcription machinery in Gram-positive bacteria** have been focused on new protein partners/factors involved in transcription regulation. Using small angle X-ray scattering and transcription experiments, we have determined new details regarding the domain structure of HelD, an interaction partner of *Bacillus subtilis* RNA polymerase (Kovaľ et al., 2019). The N-terminal domain is required for ATP-dependent transcription stimulation but not for binding to the RNA polymerase core. The study laid foundations for further work on HelD. We have also contributed to a complex study of conformational behavior of the intrinsically disordered region of the delta protein interacting with RNA polymerase from *Bacillus subtilis*, employing NMR, small-angle X-ray scattering and other biophysical methods for verification of interactions (Kuban et al., 2019). The results have increased our understanding of the features of the charged intrinsically disordered part of the delta protein important in regulation of bacterial transcription.

2.3. Laboratory of ligand engineering

One of the most important research activities of the Laboratory of Ligand Engineering during the last five years was dedicated to the development of high-affinity binding proteins targeting paratopes of broadly neutralizing antibodies that mimic epitopes recognized by these antibodies due to shape/charge complementarity. By screening of highly complex combinatorial libraries we identified “protein prints” that can be used as strong immunogens for the stimulation of protective antibodies similar to those ones originally used as molecular targets (Fig. 3). This concept, which has never been

used before for the generation of protective vaccines, has recently been demonstrated by us as a novel strategy in the reverse vaccinology. We generated "non-cognate ligands" of HIV-1 broadly neutralizing VRC01 antibody paratope that were identified from our in-lab-designed albumin-binding domain (ABD)-derived combinatorial library. These proteins called VRA ligands mimic gp120/Env epitope located in CD4 binding site and induce gp120-specific and HIV-1-neutralizing antibodies in serum of immunized mice (Kosztyu et al., 2019). As a high-efficacy preventive HIV vaccine is still not available, mainly due to extraordinary mutability of the Env gene, efficient glycan shielding of antigenic epitopes and low immunogenicity of HIV Env glycoprotein, we provided a radical concept for generation of vaccines via small-protein mimetics (see comment by P.J. Klasse *"Non-cognate ligands of Procrustean paratopes as potential vaccine components"*, EBioMedicine, 47, 6-7, 09-2019). As VRA proteins generated in our laboratory possess a promising commercial potential, we submitted Czech patent application (Maly, 2017), which will be extended to a PCT application before September 2020.

Another long-term research activity of the Laboratory is focused on the development of non-immunoglobulin inhibitors of cytokines and their receptors based on small binding proteins. A pivotal role in this effort plays IL-23/IL-17 pro-inflammatory axis which is responsible for several autoimmune disorders including psoriasis and inflammatory bowel disease (IBD). After a successful introduction of our first protein blockers, IL-23 receptor-specific REX protein antagonists (Kuchar et al., 2014) (Krizova et al., 2017) and an international patent by Malý et al. 2017, we streamlined other players in this cascade, IL-23 cytokine and IL-17 receptor A (IL-17RA). We generated a collection of human IL-23-targeted proteins called ILP binders that substantially diminished binding of recombinant p19 protein, alpha subunit of the IL-23, to IL-23 receptor on human THP-1 cells. The most promising candidates inhibited IL-23-driven expansion of IL-17-producing primary human CD4+ T-cells. These novel binders represent unique IL-23-targeted probes useful for IL-23/IL-23R epitope mapping studies and could be used for designing novel p19/IL-23-targeted anti-inflammatory biologics (Krizova et al., 2017; Maly, 2019). We also targeted human IL-17 receptor A (IL-17RA), a downstream member of the IL-23-stimulated/Th-17 cell-mediated pro-inflammatory cascade. We generated a unique collection of human IL-17RA-specific inhibitors called ARS ligands that blocked binding of IL-17 cytokine to recombinant IL-17RA-IgG chimera, inhibited binding of IL-17 cytokine to human keratinocyte HaCaT cells and THP-1 cell line, and suppressed secretion of Gro-alpha (CXCL1) by normal human skin fibroblasts CCD1070Sk upon IL-17A stimulation in vitro (Hlavnickova et al., 2018).

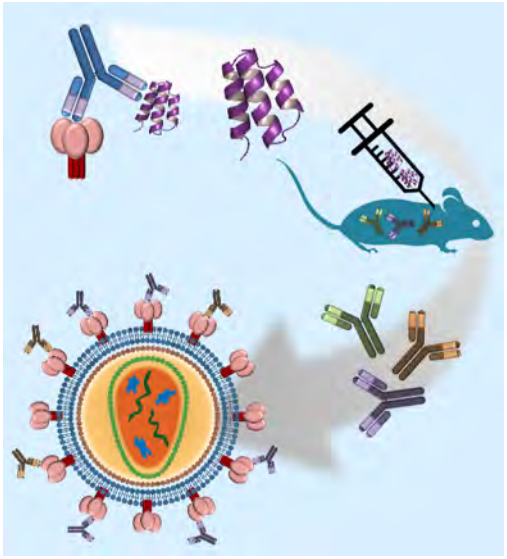


Fig. 3*. Cartoon summarizing the experimental approach of the Laboratory of ligand engineering.*

As lactic acid bacteria have recently been demonstrated as promising non-toxic and by host tolerable delivery vehicles useful for transport of per-orally administrated therapeutics into the gut, we established collaboration with Dr. Aleš Berlec, PhD. from the Department of Biotechnology at the Jožef Stefan Institute in Ljubljana, Slovenia, for experimental verification of REX, ILP and ARS binders in treatment of intestinal inflammation. In this collaboration, supported by the Czech Academy of Sciences and Slovenian Academy of Sciences and Art (SAZU) by bilateral mobility project (Reg. No. SAZU-16-01, 2016-2018), we developed unique strains of *Lactococcus lactis* that secrete, or display via AcmA peptidoglycan anchor, ILP proteins and retain IL-23-cytokine binding specificity and inhibitory function (Skrlec et al., 2018). In a similar study, we generated a collection of *L. lactis* strains secreting variants of IL-23 receptor-specific REX binding proteins (Plavec et al., 2019). As IL-23/Th17 inflammatory axis plays a substantial role in IBD and Crohn´s disease, all these ILP- and REX-secreting bacteria should be tested in vivo for their anti-inflammatory potential in mouse model of colitis. In a separate project, we developed ABD-derived protein binders targeting Shiga Toxin 1B Subunit and generated recombinant *L. lactis* secreting Shiga Toxin 1B-specific

binders (Zadravec et al., 2016). Infections with Shiga toxin (Stx)-producing bacteria may develop into a life-threatening hemolytic uremic syndrome (HUS) with acute renal failure. The developed *L. lactis* strains secreting Stx-binding proteins could serve as tools for removal of Shiga toxins from the intestine.

In another part of our research we developed protein binders useful for in vitro or in vivo imaging. We generated binders targeting human serum prostate cancer oncomarkers including Prostate Specific Protein 94 (PSP94) (Mareckova et al., 2015). In a collaborative project, focused on the development of nanoliposomal systems for rapid diagnosis of thrombi by MRI, we generated a fibrin-specific protein which binds to fibrin fibers of human thrombus and, if immobilized to the liposomal surface, delivers fluorescently labelled protein-liposome complexes under dynamic flow conditions to human thrombus in 3D model of middle cerebral artery (Petrokova et al., 2019). In another project, we collaborated on the development of microfluidic chips based on high-affinity ABD proteins for serum or urine proteins detection (Maly et al., 2016; Semeradtova et al., 2018).

2.4. Laboratory of biomolecular recognition

We focus on understanding the interactions driving specific recognition between biomolecules with potential diagnostic, medical or biotechnological use. In our research, we take advantage of experimental and computational methods of protein engineering, structural biology, bioinformatics, and molecular modeling. The central technique of most projects running in the lab is protein engineering: (i) cytokines and their receptors (ii) time-resolved biomolecular dynamic of light-inducible proteins, and (iii) a bacterial transposase RAYT and the related REP DNA. In close collaboration with Laboratory of Dr. Cerny, we work on (iv) structural bioinformatics of nucleic acids. More information about the lab work and results can be found at the website biorecognition.structbio.org.

(i) Proteins of the innate immune system. Cytokines are small signaling proteins essential for proper immune responses during inflammation, infection, trauma or cancer. Any error in their regulation causes serious autoimmune and/or allergic health disorders. We concentrate on the structural aspects of interactions between cytokines from the group of interleukin 10 (family FIL-10) and extracellular parts of their receptors. In the last few years, we have worked on interferon-ɣ and its two receptors. Specifically, we increased the affinity of human IFN-ɣ receptor 1 to its cognate IFN-ɣ (Cerny et al., 2015) and solved a crystal structure of IFN-ɣ receptor 2 (PDB ID 5eh1 (Mikulecky et al., 2016)). Our interest in the system has further spurred the study of evolution of interferons of type II which concluded in a comprehensive overview of evolution of the IFN-ɣ system in fish; the study included the first structure of non-mammalian interferon (PDB ID 6f1e (Zahradnik et al., 2018)).

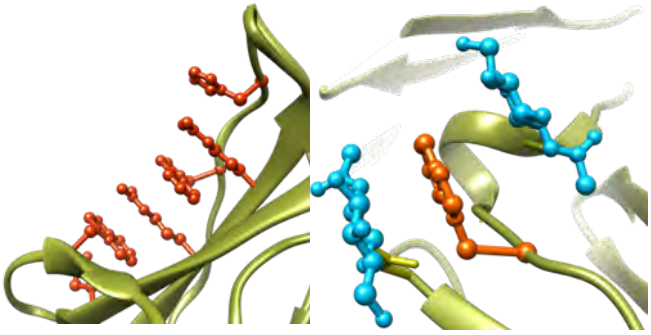


***Fig. 4**. Structure of human IFN-ɣ receptor 2 (PDB 5eh1 (Mikulecky et al., 2016)). Figure on the left shows the stacking motif stabilizing the fold, figure on the right the surface-exposed tryptophan residue sandwiched between two sugar residues and shielded thus from the solvent*

We use techniques of *in vitro* protein evolution, ribosome and yeast display to *de novo* development of specific proteins binding the FIL-10 cytokines and receptors to block or modify their signaling. We have developed a new small protein scaffold that has been trained to bind to interleukin 10 (Pham, Huliciak *et al*. in preparation 2020) and this and other scaffolds have been trained against other FIL-10 cytokines and receptors. By combining modeling approaches, mainly application of the PROSS algorithms, we succeeded in producing stable and non-signaling (PDB ID 6gg1) as well as signaling variants of IL-24 (Zahradnik et al., 2019). The IL-24 related project continues in collaboration with several research groups at the Weizmann Institute of Science and has been extended to protein engineering of other FIL-10 proteins.

(**ii) Time-resolved biomolecular dynamic of light-inducible proteins**. In 2017, two labs of the IBT Team 2 opened a project "Dynamics of biological processes" with the infrastructure ELI-Beamlines in neighboring Dolni Brezany. The goal is to decode the structural dynamics of photo-controlled transcription factors and other proteins. The main workhorse is the light-oxygen-voltage (LOV)-containing protein EL222 but we work also with CarH, coenzyme B12-binding, and CooA, heme-binding transcription factors. We have been approaching the lit-state structural ensemble of EL222 by an integrative structural biology approach combining crystallography, NMR, and small-angle neutron and X-ray scattering (SANS, SAXS). Scattering and fluorometric data indicate light-induced oligomerization of EL222 beyond the dimeric state and possibly large but reversible aggregates. The molecular mechanism of EL222 photoactivation is being approached by time-resolved vibrational spectroscopy methods, particularly femtosecond-stimulated Raman (FSRS) and infrared spectroscopy. As an important step for the understanding of EL222 dynamics, we unveiled the ultrafast (femtosecond-to-nanosecond) dynamics of flavin mononucleotide (FMN), the light-absorbing cofactor of EL222) by advanced quantum chemistry calculations and FSRS experiments (Andrikopoulos *et al.*, PCCP, January 2020, doi: 10.1039/c9cp04918e). The group has collaborated in the commissioning of two high-end optical spectroscopy set-ups at the IBT Centre of Molecular Structure: a time-resolved FTIR instrument coupled to a nanosecond UV/Visible/NIR laser, and a picosecond time-resolved spectrofluorometer. Of a great value for the whole Team 2, IBT and the center BIOCEV has been implementation of protein engineering tools, like site-specific isotopic labeling (SSIL) and genetic code expansion (GCE) technology, in order to introduce non-canonical amino acids carrying spectroscopy-sensitive probes and chemical handles for FTIR, FSRS and fluorescence-based techniques.

(**iii) Transposase RAYT and the related REP DNA oligonucleotides**. We just mention currently a smaller project dealing with the RAYT proteases and REP-related oligonucleotides and (Charnavets et al., 2015). Crystal structures of three REP-related oligoes have been solved and refined (Kolenko et al., to be submitted 2020) with help of our know how of the NA conformational space as described below.

(**iv) Structural bioinformatics** of nucleic acids is a long-standing research interest of the group recently in a close collaboration with Dr. Cerny lab (established 2017) of the IBT Team 2. Nucleic acids are structurally plastic molecules, and their biological functions are enabled by adaptation to their binding partners. We study structural aspects of their recognition by other molecules. To identify structural polymorphisms of nucleic acids we analyzed hundreds of thousands of dinucleotide structures in hundreds of crystal structures. The work is summarized in several publications and the results are available on the website dnatco.org. We have further developed and fundamentally improved our older approaches to the structural analysis of nucleic acids. The main step was a comprehensive description of the structures of DNA dinucleotides by so called Nucleotide Conformers, NtC and the derived Conformational Alphabet of Nucleic Acids, CANA (Schneider et al., 2018). The methodology has been extended to both DNA and RNA (Cerny *et al*., published in Nucleic Acids Research May 2020, doi: 10.1093/nar/gkaa383). The DNA-limited CANA and NtC classification set was applied to analysis of DNA structures; comparison of structural features of transcription factors and histone-bent DNA revealed significant structural differences between the specific and non-specific interactions between proteins and DNA (Schneider et al., 2017).

A part of our bioinformatic studies focuses on the structure of the solvation shell around biomolecules. The structures of the first hydration shell of amino acids residues in proteins and of DNA dinucleotides are reconstructed by analysis of solvation of large numbers of crystal structures. The results show how hydration depends on the amino acid (Biedermannova & Schneider, 2015) or dinucleotide (Biedermannova et al. to be published) structure and sequence. The results have been summarized in our review (Biedermannova & Schneider, 2016) and are available on the website dnatco.org/wataa (Cerny et al., 2017). We are currently working on the DNA hydration atlas.

2.5. Laboratory of structural bioinformatics of proteins

Activities covering structural bioinformatics and molecular modeling of biomolecules or small drug-like molecules resulting from numerous collaborations within the Team II as well as with the Team I are partially discussed in the corresponding parts of the report.

We continued with the development and application of our unique level of description of nucleic acid conformations which resulted in a series of publications (Cerny et al., 2016; Schneider et al., 2018; Schneider et al., 2017). Initially, concentrating still only on DNA, we developed an automated protocol assigning 44 distinct conformational classes called NtC (Nucleotide Conformers) and the DNA structural alphabet CANA (Conformational Alphabet of Nucleic Acids) to describe the DNA structural polymorphism. The NtC classification was used to define a validation score called confal, which quantifies the conformity between an analyzed structure and the geometries of NtC. A novel projection of CANA and confal onto the DNA structure was also developed for simple annotation and validation of nucleic acid structures by non-experts. The structural alphabet was also successfully applied to study the differences and true mechanism of bending of DNA complexed with regulatory proteins and in the nucleosome core particle (NCP). We have identified the specific role of two DNA structural forms, A-DNA, and BII-DNA, discriminating the specific and non- specific binding of DNA to proteins. A-DNA conformations are avoided in non-specific NCP complexes, where the wrapping of the DNA duplex is explained by the periodic occurrence of BII conformation every 10.3 steps. The automated assignment of the NtC classes and CANA codes is freely available for all interested users at dnatco.org and provides a powerful tool for unbiased analysis of nucleic acid structures by structural and molecular biologists. The web service based on our unique automated protocol assigning conformational classes currently allows quantitative assessment and validation of NA conformations and their subsequent analysis by means of pseudo-sequence alignment. The method also allows search for nontrivial structural patterns of nucleic acids. The description of implementation and example use cases for the second version of the DNATCO (dnatco.org/v2) web service were published.

Recently, we have significantly extended and at the same time simplified the structural alphabet which now combines both DNA and RNA and allows analyses of nucleic acid conformations at a unified level. We derived 96+1 NtCs, which describe the geometry of RNA and DNA dinucleotides. NtC classes are grouped into 15 codes of the structural alphabet CANA to simplify symbolic annotation of the prominent structural features of NAs and their intuitive graphical display. Our analysis employing the new extended and universal structural alphabet revealed two surprising results. Notably, over 30% of the nearly six million dinucleotides in the PDB cannot be assigned to any NtC class but we demonstrate that up to a half of them can be re-refined with the help of proper refinement targets. A statistical analysis of the preferences of NtCs and CANA codes for the 16 dinucleotide sequences showed that neither the NtC class AA00, which forms the scaffold of RNA structures, nor BB00, the DNA most populated class, are sequence neutral but their distributions are significantly biased.

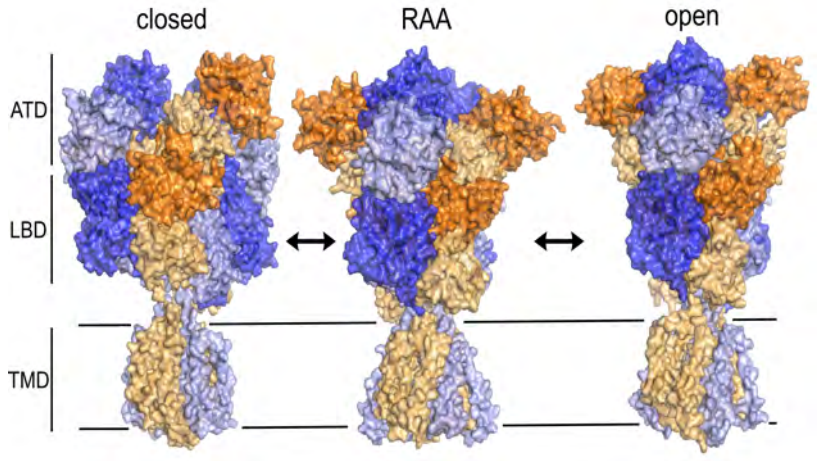


***Fig. 5**. The NMDAR is shown during the structural transition from the closed to the open state of the receptor as captured by molecular dynamics simulations. The snapshots of geometry from the MD simulation were structurally superimposed using the transmembrane domain (TMD) M3 helices as a reference.*

Our development in the field of enhanced sampling simulations of biomolecules resulted in two new theoretical techniques which can significantly accelerate the sampling rate and at the same time improve the accuracy of simulated structural and conformational ensembles. We have developed an enhanced sampling method based on a hybrid Hamiltonian which combines experimental distance restraints with a multiple path-dependent bias (Peter & Cerny, 2019). This simulation method determines the bias-coordinates on the fly and does not require a priori knowledge about reaction coordinates. The method accelerates the sampling of proteins as tested using experimental NMR-restraint data and distance restraints from chemical crosslinking/mass spectrometry experiments. The second enhanced sampling method for molecular dynamics simulation of protein and DNA systems (Peter & Cerny, 2018) is based on a potential of mean force (PMF)-enriched sampling, determined from DNA and protein structures in the PDB. Our results show that the simulation technique enriches the conformational space of biomolecules, provides improved distributions of conformations compared to the underlying forcefield, while we also observe a considerable speed increase in the sampling by factors ranging from 13.1 to 82. Both methods can be combined for further acceleration and higher accuracy of simulations.

As a part of our long-standing collaboration with the Department of Cellular Neurophysiology at the Institute of Physiology studying the N-methyl-D-aspartate (NMDA) receptor we have revealed details of the activation mechanism of the receptor. It is now well established that NMDARs play a crucial role in rapid excitatory synaptic transmission in the mammalian central nervous system and promote persistent changes in synaptic strength. NMDAR states associated with excessive receptor activation, as well as receptor hypo- or hyper-function, are clinically relevant. In our recent publication we report the first complete description of the molecular mechanisms of the NMDA receptor transition from the state where the ion channel is in the open configuration to the unliganded state where the channel is closed (Cerny et al., 2019). Using MD simulations we identified distinct structural states of the NMDA receptor and revealed functionally important residues. The activated (RAA) and open states of the receptor are structurally similar to the liganded crystal structure, while in the unliganded receptor the extracellular domains perform surprisingly large rearrangements leading to a clockwise rotation of up to 45 degrees around the longitudinal axis of the receptor, which closes the ion channel (Fig. 5). The ligand-induced rotation of extracellular - amino-terminal (ATD) and ligand binding (LBD) - domains, transferred by LBD-TMD linkers to the membrane-anchored ion channel is responsible for the opening and closing of the transmembrane ion channel, revealing the properties of NMDA receptor as a finely tuned molecular machine. The study can stimulate the development of new potential drugs modulating NMDA receptor with the necessary functional state specificity.

2.6. Laboratory of structural proteins

This junior laboratory was established in the 2015. During the initial five years of the lab existence, the lab research activities comprised three main areas: (i) Regulatory roles of intrinsically disordered microtubule-associated proteins, (ii) Organization principles of higher order microtubule assemblies and (iii) Collective effects in functioning of molecular motors.

(i) Intrinsically disordered microtubule-associated proteins, such as tau, are essential cytoskeletal regulators important especially in neurons. Their malfunctioning is associated with a number of neurodegenerative diseases, such as the Alzheimer's disease. Recent results suggest that many intrinsically disordered proteins can form compartment by liquid-liquid phase separation. These compartments locally facilitate specific reactions by establishing microenvironments different form the cytoplasm. It is not known whether intrinsically disordered microtubule proteins can phase separate. Consequently, what would be the roles of these compartments is an open question.

We found that microtubule associated protein tau can form liquid-like drops through liquid-liquid phase separation. We found that soluble tubulin partitions into these drops, which locally increases tubulin concentration and enables nucleation of microtubules from the drop and their bundling. Our results thus suggest that condensed compartments of intrinsically disordered microtubule-associated proteins could promote the local formation of microtubule bundles in neurons, acting as non-centrosomal microtubule nucleation centers (Hernandez-Vega et al., 2017).

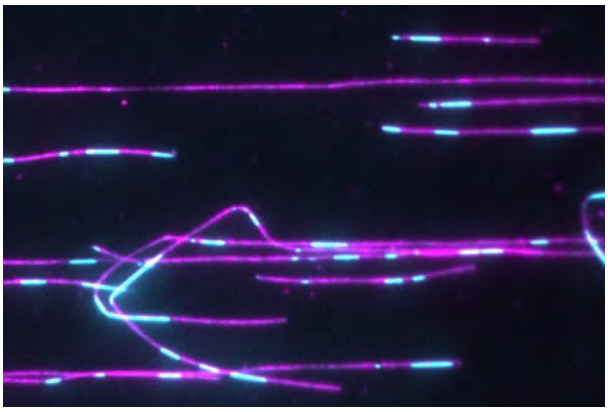


***Fig. 6**. Multichannel fluorescence micrograph showing cohesive islands of intrinsically disordered protein tau (cyan) on microtubules (magenta).*

Knowing that tau can form liquid-like compartments in solution, we next investigated tau condensation on the surface of microtubules. We found that tau molecules on microtubules cooperatively form cohesive islands that are kinetically distinct from tau molecules that bind to microtubules individually (Fig. 6). Importantly, the islands exhibit regulatory qualities distinct from a comparably dense layer of individually bound tau. The islands shield microtubules from particular molecular motors, such as kinesin-1, and microtubule-severing enzymes, such as katanin. Our results thus reveal a microtubule-dependent phase of tau that differentially regulates the access of other microtubule-associated proteins to the microtubule surface (Siahaan et al., 2019).

(ii) The cilium, composed of a **higher order microtubule assembly,** is an organelle crucial for motility, as well as for sensing environmental cues such as signaling molecules, light, and mechanical stimuli. The core structure of the cilium is characterized by nine microtubule doublets, consisting of an incomplete "B-microtubule" coupled to the surface of a complete "A-microtubule". Despite the fundamental role of microtubule doublets, the molecular mechanism governing their formation is unknown. Here we found, using an in vitro assay, a crucial inhibitory role of the C-terminal tail of tubulin in the assembly of the microtubule doublets. Removal of the C-terminal tail of an assembled A-microtubule allowed for the nucleation of a B-microtubule on its surface. We characterized the dynamics of the B-microtubule nucleation and we uncovered a distinctive isotropic elongation of the B-microtubule. We thus found that inherent interaction properties of tubulin provide a structural basis driving flagellar microtubule doublet assembly (Schmidt-Cernohorska et al., 2019).

(iii) Remodeling of microtubule networks underpins essential cellular processes, such as cell division or cell motility. **Microtubule-crosslinking and sliding molecular motors**, such as the members of the kinesin-14 family, are essential regulators of the microtubule network remodeling. In cells, these motors function predominantly in teams. Although these motors have been extensively characterized on the single molecule level, their ensemble dynamics and their collective functioning are much less understood.

Members of the kinesin-14 family typically interact with one microtubule via their non-processive motor domains and with another via their diffusive tail domains. The influence of the tail domains on the performance of the motors is not understood. Here, we showed that diffusive anchorage of the kinesin-14 tail domains considerably impacts velocity and force generated by the motor ensemble. Our data rather suggest a role of kinesin-14 as a flexible element, pliantly sliding and crosslinking microtubules to facilitate remodeling of the mitotic spindle (Ludecke et al., 2018).

Kinesin-14 motors have been shown to collectively slide microtubules at a constant velocity until no overlap remains between them, leading to the breakdown of the initial microtubule geometry. We showed that the sliding velocity of microtubules, driven by ensembles of human kinesin-14 HSET, decreases when microtubules start to slide apart. This results in the maintenance of finite-length microtubule overlaps. We quantitatively explained this feedback using the local interaction kinetics of HSET with overlapping microtubules, which leads to the generation of an entropic force that antagonizes the force exerted by the motors. We thus demonstrated that the spatial arrangement of microtubules can locally regulate the collective action of molecular motors (Braun et al., 2017).

3. Cooperation within international research area

The team has numerous strong collaborative links throughout the world with leading scientists and institutions, within infrastructural pan-European projects and also individually. Here, we list the most prominent ones.

3.1. Infrastructural pan-European projects

We have been involved in the design, construction, development and management of the Centre of Molecular Structure (CMS), which belongs to the **Czech Infrastructure for Integrative Structural Biology** (CIISB), together with our partner laboratories of CEITEC, Masaryk University in Brno (https://www.ciisb.org/). The collaboration in setting up, securing funding and managing the research infrastructure since 2012 has resulted in provision of state-of-the-art technologies for structural biology research and beyond (2013-now) and inclusion as one of the core centers of the European infrastructure for structural biology **Instruct-ERIC** (https://instruct-eric.eu/). The international evaluation of CIISB had one of the highest scores among the Czech research infrastructures. So far, our activities in CMS have attracted funding from the ERDF sources and from the national support of large research infrastructures of more than 100 million CZK in years 2015-2019.

In collaboration with the Laboratory of O. Vaněk, Charles University, we have also contributed to the realization of the events of the **ARBRE-MOBIEU** – a research network focused on development and application of biophysical methods in molecular and cellular biology (Cost Action CA15126, Lead partner, Institut Pasteur, Paris). This collaboration enabled our successful application to the newly emerging European consortium of facilities providing access to biophysical methods (to participate in the EC Infraia call in 2020).

3.2. Individual international collaborations

Prof. David Ehrhardt, **Carnegie Institution for Science, Stanford, CA, USA** - plant cytoskeleton remodeling

Prof. Paul Guichard, **University of Geneva, Switzerland** - dynamics of microtubule doublet formation

Prof. Stefan Diez, **TU Dresden, Germany** - 3D particle tracking

Prof. Richard McKenney, **UC Davis, CA, USA** - unstructured microtubule associated proteins

Prof. Kassandra Ori-McKenney, **UC Davis, CA, USA** - unstructured microtubule associated proteins

Prof. Pieter Rein ten Wolde**, AMOLF, Amsterdam, The Netherlands** - cytoskeletal networks

Mihail Sarov, head of the **Genome Engineering Facility, MPI-CBG, Dresden, Germany** - iPS cells production

Prof. Stephan Grill, director of the **MPI-CBG, Dresden, Germany** - actin networks chirality

Carsten Janke, team leader **Institut Curie, Paris France** - posttranslational modifications of microtubules

Barbara Borgonovo, head of PEPC **MPI-CBG, Dresden, Germany** - protein production

Prof. Darren Hart, **Institut de Biologie Structurale - Grenoble, France** - robotically driven system of evaluation of random mutations in E. coli expression systems).

Prof. Aleš Berlec, **Jozef Stefan Institute in Ljubljana**, **Slovenia** - small binding protein engineering

Prof. Gideon Schreiber, Prof. Joel Sussman, Drs. Yoav Peleg, Tamar Unger, **Weizmann Institute of Science, Rehovot, Israel** - protein engineering & structural biology

Prof. Helen Berman, Prof. Stephen Burley, Prof. Cathy Lawson, Dr. Brinda Vallat, Institute for Quantitative Biomedicine, **Rutgers University, USA** - the PDB team of the Research Collaboratory for Structural Bioinformatics

Prof. B Slusher, director, **Johns Hopkins Drug Discovery, Johns Hopkins School of Medicine, Baltimore, MD, USA** - GCPII inhibitors

Prof. M. G. Pomper, division director, **Department of Radiology, Johns Hopkins Medical Institutions, Baltimore, MD, USA** - GCPII inhibitors/antibodies

Prof. A. Skerra, Lehrstuhl für Biologische Chemie, **Technische Universität München, Freising, Germany** - Anticalins, protein engineering

Prof. M. Schutkowski, Institute for Biochemistry and Biotechnology, **Charles-Tanford-Proteinzentrum, Martin-Luther-Universität Halle-Wittenberg, Halle, Germany** - HDACs, substrates

Prof. A. Kozikowski, CEO, **StarWise Therapeutics LLC, Madison, WI, USA** - HDAC inhibitors

Prof. C. Berkman, Department of Chemistry, **Washington State University, Pullman, WA, USA** - GCPII inhibitors

Novozymes A/S, Copenhagen, Denmark - structural analysis of novel enzymes

Phenix and **CCP4** - nucleic acid structure annotation, validation, refinement and modeling

3.3. National collaborations

Within our research activities we have collaborated with the following teams in the **Czech Republic**: Laboratory of Libor Krásný, Institute of Microbiology of the Czech Academy of Sciences (long term collaboration, transcription in Gram positive bacteria, joint research grants from the Czech Science Foundation), Laboratory of Petra Lipovová, University of Chemistry and Technology in Prague (long term collaboration, glycosidases and nucleases), Laboratory of Ondřej Vaněk, Faculty of Science, Charles University in Prague (long term, natural killer cell receptors, joint research grants from the Czech Science Foundation), Laboratory of Markéta Martínková, Faculty of Science, Charles University (sensor proteins), Laboratory of Jaroslav Matoušek, Institute of Plant Molecular Biology of the Czech Academy of Sciences, České Budějovice (plant nucleases), Laboratory of Lukáš Žídek, Masaryk University Brno (bacterial RNA polymerase), group of Marek Piliarik, Institute of Photonics and Electronics of the Czech Academy of Sciences, group of Milan Raška, Medical Faculty of University Palacky in Olomouc, group of Jaroslav Turánek, Veterinary Research Institute in Brno, the group of Robert Mikulík, St. Anne´s University Hospital in Brno, group of Petr Arenberger, Faculty Hospital Královské Vinohrady in Prague and Ladislav Pažout, Czech producer and exporter of veterinary vaccines DYNTEC s.r.o., prof. Pavel Martásek, I.LF UK Praha

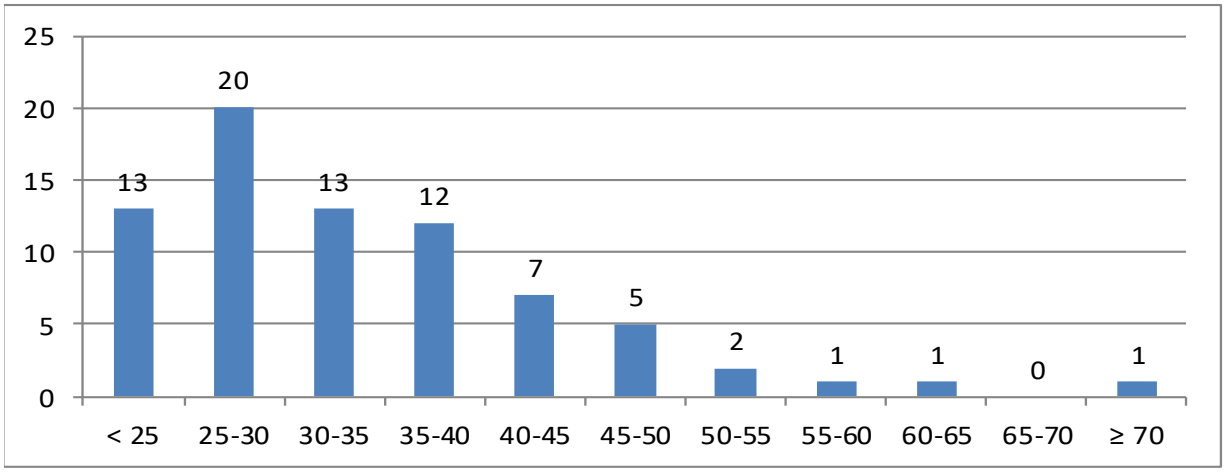
4. HR policy of the team

The HR policy of our team is based on open position calls and the selection of most qualified candidates in an unbiassed and fair selection process, resulting in recruitment of talented researchers and students on all levels. As of the end 2019, there were total 80 people (62.025 FTE) in the team, including 21 staff scientist, 11 postdocs, 22 PhD students, 14 technicians, and 12 undergraduate students. The team is currently composed of 56% female and 44% male scientists, reflecting the overall trend in biological sciences in the Czech Republic. The team is composed of biologists, physicists and chemists with foreign nationals comprising 26% of the team, creating a stimulating interdisciplinary and international environment. Supervision of undergraduate and graduate students is distributed among researchers of the team according to their specialization and training capabilities. Both students and researchers are encouraged to apply for national and international funding, participate in advanced training activities on national and also international level and present their results regularly in an event of an international character. Majority of the graduate and undergraduate students in the team study at one of the three major technical / life science universities in Prague, Charles University in Prague, Czech Technical University in Prague and University of Chemistry and Technology Prague. Within the individual laboratories and within the whole team, weekly and monthly meetings, respectively, are organized and aimed especially for students, providing ample opportunities to discuss current results, network and solve organization issues. The positive impact of our HR policy is reflected in our scientific results and in the high level of competitiveness of our alumni in applying for their next position.

5. Age structure of the team

Related to 31 December 2019

Age category	< 25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65-70	≥ 70
Number of members	13	20	13	12	7	5	2	1	1	0	1



6. Strengths and weaknesses

6.1. Strengths

Stabilized team and broad expertise. The main asset of our team is its consolidated structure with a solid base of experienced staff with physics, biophysics and chemistry background for complex structure-function studies and capable of mentoring new generations of scientists. This core is supported by highly motivated junior scientists and students. The team is fully focused on young generation – each research project involves multiple students. The human resources are well complemented by the institute infrastructure and core facilities of the institute and the BIOCEV research center, such as the Centre of Molecular Structure or Imaging Methods Infrastructure, which provide state of the art equipment enabling the team to perform world-class research. This is reflected in our publications in top specialized journals as well as interdisciplinary journals with broad audience, including *Science, Nature Cell Biology, Nature Chemical Biology, Journal of the American Chemical Society, Molecular Cell, Nucleic Acid Research, Nature Communications, Cell Reports, Journal of Medicinal Chemistry, eLife, Journal of Physical Chemistry, Acta Crystallographica* etc.

External funding. A key asset of our team, facilitating the team's steady scientific output, is its success in securing external funding (higher success rate compared to the National average) from various sources, such as the Czech Science Foundation, Czech Health Foundation and Ministry of Education Youth and Sports, totaling approximately 9.7 million EUR within the evaluation period. The level of external funding was steadily growing over the last five years providing ample funding for the individual laboratories.

International and national collaborations. An important asset of our team is its embeddedness in the international scientific community. As detailed above and as evidenced by our publications, members of our team have multiple strong international collaborative links throughout the world leading to large scale synergies across disciplines. We are actively participating in the organization of conferences, workshops and training events. We are furthermore strongly connected to the European structural biology community through large scale infrastructures collaborations (for details see below) providing networking opportunities and access to state of the art instrumentation across Europe.

6.2. Weaknesses

Location. The location of the institute outside of Prague is arguably its biggest weakness. Especially for students, who often have to attend lectures in Prague city center, it is inconvenient to commute back and forth. We are however working hard to overcome this weakness by making the institute more attractive for students by giving lectures at the universities, providing world class mentoring capacities and stimulating research environment and offering state of the art instrumentation. We are furthermore discussing with universities the possibility of dedicating the last year of their master's programs solely to experimental and theoretical work on the student's thesis project at our institute, which would drastically decrease the commuting inconvenience for the students.

Administrative overload. Another weakness might stem from possible overload of administrative work concerning mostly, but not being restricted to, the principal investigators of the individual laboratories within the team. At the moment, the Financial and Administrative Department of the institute is doing a great work to minimize the administrative workload on the researchers. We have to be aware of this possible threat and work together with the Academy of Sciences to reduce the administrative workload on the researchers.

Potential lower funding of science, loss of external funding. Finally, obtaining external funding for the individual groups and for running, upgrading and replacing the instrumentation, which worked very well during the evaluation period, might potentially become a threat in the future. We are aware of this and we work towards world class excellence of our research, providing more competitiveness for our researchers within the national and international funding schemes.

7. Assessment of the activity plan of the team for the period 2015-2019

The previous evaluation period (2011-2015) could be characterized as the time of team instituting; our major tasks within that period were to establish, develop, stabilize, and sustain our research activities. The goal has been to design and build a strong, internationally recognized research group with synergy between individual Research Laboratories. The plan for the years 2015-2019 was to further build on these foundations, expanding the achieved expertise, mastered methods and techniques, consolidating the team and the research infrastructure. During this period, we planned to have reached the critical mass of human potential and technical means to conceive and perform large and ambitious projects of scientific excellence, further strengthening our collaborations and involvement in the international community. As evidenced in detail in the chapters above, **we have succeeded in implementing all of these planned features**. The institute personnel as well as the institute research infrastructure is consolidated, which enabled us to generate multiple exciting results over the evaluation period of 2015-2019. Consequently, our team and the individual Laboratories comprising the team are now well established within the international scientific community leading to higher international visibility of the institute, the BIOCEV research center, and the Czech Academy of Sciences as a whole.

8. Implementation of recommendations from past evaluation

During the last evaluation of the period 2010-2014 by an international committee in the year 2015, our team has received very positive assessment. The recommendation of the committee was to continue in the planned activities, which according to the committee will lead to even higher publication activities. The committee further noted that the elaboration and execution of joint projects with ELI should be encouraged and supported. In the 2015-2019 period **we have fully implemented these recommendations**. All the individual research laboratories continued along the research lines drawn in the previous evaluation period and we have indeed increased both in quantity and quality of our scientific outputs. The joint project with ELI was executed, as described above, and led to the establishment of a new exciting research direction and to new results. Moreover, the infrastructure-oriented activities have been broadened to application to the newly arising consortium of facilities providing access to biophysical techniques. The application was evaluated and several facilities of the Centre of Molecular Structure were accepted as a unit of the new consortium called MOSBRI, with local responsible scientist Jan Dohnálek. The consortium is applying for support in the INFRAIA call of the E.C. for support of starting infrastructures, under the leadership of Dr. Patrick England, Institut Pasteur.

9. Activity plan of the team for the period 2020-2024

The activity plan of the team stems from the projects listed above in Chapter 2. The plan of the individual Laboratories is detailed below.

9.1. Laboratory of structural biology

1. We will invest most of our resources into projects aimed at HDACs. We plan on implementing synthetic biology (orthogonal expression) to prepare “natively acetylated” HDAC substrates (HSP90, STAT3, survivin, cortactin) and characterize in detail their interactions with HDACs to understand structural determinants of HDAC specificity at the protein level. In collaboration with several academic and commercial partners we will use our established workflow for the identification and development of isotype-specific HDAC inhibitors. Finally, we will focus on the description of HDAC6 involvement in transport systems inside the cell, namely structural and functional description of interactions with molecular motors.

2. In the GCPII research we will focus on the protein engineering of monoclonal antibodies with the ultimate goal of translation into the clinic. As for the basic research projects, we are interested in understanding physiological functions of GCPII orthologs in non-mammal species as the proteins are ubiquitously expressed in virtually all higher organisms, but their function(s) unknown.

9.2. Laboratory of structure and function of biomolecules.

Research in the field of immune system signaling, especially in innate immunity and surface receptors responsible for recognition and lysis of cancer cells or cells affected by viral infection, focus on complexes and derivation of the therapeutic value of the basic research outputs. Structure-function studies of unknown territories in bacterial transcription in Gram-positive bacteria responsible for infections and diseases. Search for new targets and research into proteins involved in the life cycle and viability of organisms (mostly bacteria) responsible for nosocomial diseases and representatives of the multidrug-resistance classes of pathogens. Expansion of the research infrastructure for biomolecular research towards electron-based methods (cryoelectron microscopy, electron diffraction, cryoelectron tomography). Establishment and operation of the new European network for access to biophysical techniques. Involvement in methods development both on the level of experimental work in biophysics and structural biology and on the level of computational data processing, structure determination/refinement algorithms, statistical data processing, data formats standardization and centralized data storage for users and research public.

9.3. Laboratory of ligand engineering

Laboratory of Ligand Engineering will continue in the development of novel collections of binding proteins targeted to HIV-1 broadly neutralizing antibodies as novel immunogens and will test their HIV-1 neutralizing potential in collaboration with the University Palacky in Olomouc. Currently, we summarize new results demonstrating that the proposed “non-cognate ligand strategy” might be valuable and promising for vaccine development and transferable to other R&D vaccine projects. We also might use this concept for SARS-Cov-2 research leading to a preventive COVID-19 vaccine if the support is provided from the governmental as well as private sphere. We will also generate and characterize inhibitory binding proteins targeting human interleukins and their receptors that were selected from combinatorial libraries of protein domain scaffolds. We plan to verify the efficacy of lactic acid bacteria secreting our developed REX, ILP and ARS protein blockers in suppression of experimentally induced inflammation in mouse model of colitis. In a collaborative project with two Czech companies DYNTEC s.r.o. and FAGOFARMA s.r.o., supported by Ministry of Trade of the Czech Republic and TAČR agency, we will develop and test products of phage endolysin genes specific for several human pathogens developing biofilms as a potential human drug surpassing the current burden of omnipresent antibiotic resistance. We will support DYNTEC s.r.o. to finish testing SUIVAC CCR and HORNED CCR vaccines to enter the European market.

9.4. Laboratory of biomolecular recognition

In the protein engineering, we now concentrate on combining two methods of directed evolution, ribosome and yeast displays to train high affinity and specificity binders against cytokines and their receptors. It allows parallel testing of more scaffolds (we currently use four) and speeds up the process of selecting the promising binders. We mostly work on newer, more recently discovered cytokines. In some cases just their production, as was the case of IL-24 represent a research project. We work on IL-26, some of IFN-lambda proteins and the related receptors. We achieved encouraging results in development of artificial binders that mimic the function of some of these cytokines, either signal or block the signaling. As we are well aware of application potential of the best binders as diagnostics and therapeutics we plan to direct a part of the lab activities to more development-oriented projects. We believe that some of our know-how, mainly ability to produce difficult proteins in form of various tailored constructs, opens ways to collaborate with Team I on new ways to increase the effectivity anticancer therapy by linking conventional anticancer drugs to immunity boosting proteins.

Research of the RAYT/REP bacterial systems will continue by studying the sequence specificity of all (currently 4-5) RAYT transposases that we know how to express. A paper describing crystal structures of three REP-related 18-mers (PDB IDs 6ror, 6ros, 6rou) is about to be sent to Acta Cryst. D and we will continue with crystallization trials of more REP-like oligoes as well as of RAYT/REP protein/DNA complexes.

We have developed unique tools to analyze nucleic acid structures, the last paper was just published in Nucleic Acids Research [Cerny et al. 2020]. The focus on structural bioinformatics of nucleic acids

will therefore remain one of the research directions of the laboratory. In collaboration with Laboratory of 3D bioinformatics (IBT Team II), we will further develop the formal classification of nucleic acid structures by the dinucleotide conformers NtC and the related structural alphabet CANA and work on the tools to model, refine, validate, and classify nucleic acid structures. This will include improvements and enhancement of the web services currently available at the address dnatco.org. We also plan to use up the potential of sequence-like identification of structural motifs of especially large RNA molecules by searching for recurrent motifs of NtC or CANA sequences.

The laboratory has a long tradition in structural characterization of the hydration around biomolecules. We plan to finish description of the structure of the first hydration layer of DNA dinucleotides where we take advantage of the structural classification of DNA structures by the NtC classes. We will build an unified atlas for both nucleic acids and proteins (amino acids) by expanding dnatco.org/wataa at a new address watlas.structbio.org.

The research direction into short to medium time dynamics of biomolecules (from femtoseconds to milliseconds) will continue by measuring femtosecond Raman (FSRS), transient absorption, and time resolved infrared spectra as well as time resolved SANS and NMR data of light-sensitive proteins. Currently, the most advanced are studies of the EL222 transcription factor but we work on alternative protein and nucleic acid targets as well. This project is carried out in collaboration with the ELI-Beamlines infrastructure in Dolni Brezany, Czech Republic.

9.5. Laboratory of structural bioinformatics of proteins

We will continue our work on bioinformatics of proteins and nucleic acids. As the founding members of the 3DBioInfo ELIXIR Structural Bioinformatics Community, we want to further develop our unique structural alphabets for biomolecules into a set of tools and standards for annotation, validation, model building, and refinement of crystallographic and cryoEM structures. We will also continue with design, development and utilization of methods for molecular modeling of biomolecules.

9.6. Laboratory of Structural Proteins

We plan to follow up the promising research directions initiated during the years 2015-2019. We will further explore the regulatory roles of intrinsically disordered microtubule associated proteins, the molecular mechanism governing the formation of complex microtubule assemblies and the ensemble dynamics of molecular motors. Furthermore, we have recently initiated a new project exploring the roles of actin-microtubule crosstalk in neuronal pathfinding during development. Mechanical forces driving the pathfinding are generated in the growth cone, a distinct structure at the tip of neuronal axons. The progression of the growth cone is propelled by actin dynamics, while the direction of growth is governed by microtubules, implying that crosstalk between microtubules and actin filaments is essential for the growth cone steering. Although vital, this crosstalk and its impact on the steering is not understood. We will elucidate the steering by focusing on the proteins that couple actin filaments and microtubules. These crosslinkers constitute the hubs of cytoskeletal activity, coupling the driving force to directionality. We will employ live cell imaging, in vitro reconstitutions, together with microsurgical and single molecule perturbations and mathematical modeling. We will provide mechanistic explanation of the actin-microtubule crosstalk enabling neuronal pathfinding - the fundament required for the development of diagnostic or therapeutic tools addressing neurodevelopmental disorders.

10. Pedagogical activity

University	Lectured by	Title of the lecture	Number of semestrial lectures, seminars and courses 2015-2019		
			Bachelor	Master	Doctoral
Czech Technical University in Prague	J. Dohnálek	Diffraction Methods of Structural Biology		4	
University of Chemistry and Technology, Prague	J. Dohnálek	Biophysical chemistry (part of course)		5	
Czech Technical University in Prague	P. Kolenko	Structure and Function of Biological Macromolecules (Bc), Seminar (MSc., 3), Laboratory in Macromolecular Crystallography (8, MSc.)	5	11	
Czech Technical University in Prague	L. Švecová	Structure and function of biological macromolecules (part of laboratory practicals)	1		
University of Chemistry and Technology, Prague	K. Adámková	Advanced Biochemistry: Laboratory course		2	
Charles University, Prague	Z. Lansky	Single molecule microscopy and manipulation	3	3	3
Charles University, Prague	M. Braun	Single molecule microscopy and manipulation	3	3	3
Charles University, Prague	V. Henrichs	Single molecule microscopy and manipulation	3	3	3
Charles University, Prague	I. Zhernov	Single molecule microscopy and manipulation	3	3	3
Charles University, Prague	Z. Lansky	Advances in Cell Biology	3	3	3
Charles University, Prague	Z. Lansky	Cellular Machines	1	1	1
Charles University, Prague	M. Braun	Cellular Machines	1	1	1
Charles University, Prague	B. Schneider	Structural Bioinformatics		5	5
South Bohemian University Ceske Budejovice	B. Schneider	X-ray crystallography		5	5

Supervision of students

Type of study	No. of supervisors	No. of consultants	Theses defended 2015-2019
Bachelor	18	4	16
Master	23	4	13
Doctoral	26	6	7

11. Participation of PhD students in the outputs

One of our main priorities is to mentor PhD students. The students are thus participating in all running projects on all levels of the project, including experimental design, employing state of the art methodology and equipment, interpreting the results and writing up the manuscripts. Students furthermore regularly actively participate in international conferences. Students are also encouraged to apply for student funding available within the Charles University in Prague and master thus also the skills of proposal writing. Natural outcome of this effort is that students participate on majority of our scientific outputs (totaling 35 papers within the evaluated period), often on a prominent position as first authors (15 papers).

12. Involvement in the research centers with universities

The team is an integral part of the research center BIOCEV, a joint research center of the Academy of Sciences and the Charles University in Prague. Research performed in BIOCEV is focused on the selected areas of biotechnologies and biomedicine. The scientific scope of BIOCEV has been divided into five research Programs, each of them dealing with a number of separate research projects. Our team is responsible for Program 3 “Structural Biology and Protein Engineering”. The scientific output and mentoring of students generated within this Program is discussed above in detail.

13. Participation of the team members in activities of scientific community

Memberships at National and International scientific bodies

Jan Dohnálek - 2010–now Founding chairman of the Czech Society for Structural Biology

Jan Dohnálek - 2015-now Vice-chair, SIG1 Macromolecular crystallography, European Crystallography Association

Jan Dohnálek - 2017-now Member of the Regional committee of Czech and Slovak Crystallographers of the International Union of Crystallography (IUCr)

Jan Dohnálek - 2018-now Member of the Executive committee of the European Crystallographic Association

Jan Dohnálek - 2019-now Scientific Council for PhD studies in Biochemistry and Bio-organic Chemistry, University of Chemistry and Technology in Prague

Jindřich Hašek - 2015-now Chairman of the Czech and Slovak Crystallographic Association

Jindřich Hašek - 2015-now Councillor of the European Crystallographic Association

Jindřich Hašek - 2015-2017 Auditor of the European Crystallographic Association

Jindřich Hašek - 2019-2021 Chairman of the National Advisory Board of the 25th Congress of the International Union for Crystallography, Prague Congress Center, 14-22 August 2021, expected 2 500 participants, https://iucr2020.org/organization/#nab

Cyril Bařinka - Member of the Academy Assembly of the Czech Academy of Sciences (CAS).

Bohdan Schneider - member of the IUCr Commission on Biomolecules

Bohdan Schneider - Member of the Academy Assembly of the Czech Academy of Sciences and a member of its Oversight commission.

Bohdan Schneider - The Czech government evaluation 17+ and others („Hodnoceni výzkumných organizaci")

Bohdan Schneider - CSSB Board member

Active in European infrastructural projects

Jan Dohnálek - 2008–now Czech representative in the Integrated Infrastructure for Structural Biology in Europe Instruct-ERIC

Jan Dohnálek - 2015-now Member of the Scientific advisory board of infrastructure Elixir-CZ

Jan Dohnálek - 2015-now Member of the executive committee of the Czech infrastructure for integrative structural biology

Petr Pompach - 2018-now Committee for training activities of Instruct-ERIC.

Bohdan Schneider - Evaluation of potential key resource sites for ELIXIR

Journals and peer review

Jindřich Hašek - 2015-now Editor of the journal Materials Structure (ISSN 1211-5894)

Jan Dohnálek - Peer review of ~20 research publications for scientific journals included in the Science Citation Index.

Zdeněk Lánský - peer review of ~10 papers for Nature Chemical Biology, Current Biology, Biophysical Journal, Journal of Cell Science, eLife, Nature Communications etc.

Bohdan Schneider - peer reviews in NAR, Acta Cryst, others.

Reviewing National and International grant proposals

Jan Dohnálek - Peer review of about 10 research grant applications (national and international).

Jindřich Hašek - Peer review of 8 research grant applications MSMT, GACR

Petr Kolenko - Peer review of 8 national research grant applications.

Zdeněk Lánský - Grant reviewer for Agence Nationale de la Recherche

Bohdan Schneider - Grant review for the Dutch grant agency NWO, program VICI

14. Organized conferences and workshops

Petr Malý - Organizing Committee Member - 15th Asia-Pacific Biotechnology Congress, July 20-22, 2017, Melbourne, Australia

Cyril Bařinka - Organizer of the EMBO Young Scientists Forum (EYSF) 2019, Prague. Over 140 participants, focused on PhD students.

Heart of Europe bio-Crystallography meeting, Kutná Hora, 24-26 September 2015, main organizer J. Dohnálek, co-organizers from the team, 130 participants, international.

Several members of the team are the main organizers of the annual Discussions in Structural Molecular Biology in Nové Hrady, several co-organizers from the team. 2015-2017, 2019, about 120 participants each year, mostly national.

International workshop Computational approaches in macromolecular crystallography – Structure refinement at low and atomic resolution, Nové Hrady, 17-19 March 2015, 30 participants, international, J. Dohnálek main organizer, co-organizers from the team.

Seminar 301. Meeting of the Czech and Slovak Crystallographic Association, 26 April 2017, IBT Biocev, Vestec, main organizer J. Hašek and co-organizers from the team, 50 participants.

Training course SAXS, 5 April 2017, IBT Biocev Vestec, 20 participants, organizers.

International workshop Proteins for Life – the 12th P4EU Meeting, 11-12 December 2017, IBT Biocev, Vestec, 2 co-organizers from the Team, 50 participants.

Seminar CMS Update, 31 October 2018, IBT Biocev, Vestec, co-organizer.

International workshop Instruct/CIISB course on fragment screening using crystallography laboratory equipment, 5-6 April 2018, IBT Vestec, co-organizer, 25 participants.

Biocev Infrastructure Open Day, 3 April 2019, Biocev, Vestec, co-organizer, national.

Instruct-ERIC workshop Computational Approaches in Integration of Structural Biology Techniques, 8-10 October 2019, IBT Biocev, Vestec, main organizer J. Dohnálek, co-organizers from the team, international, 20 participants, lectures – 50 participants.

International course SAXS – Advanced Data Analysis & Evaluation Training, 26-27 November 2019, IBT Biocev, main organizer J. Stránský, 20 participants.

Course CCP4i2: Introduction to 21st century, 11 February 2019, 12 participants, IBT Biocev, Vestec, J. Stránský organizer.

Course on Coot, 25 February 2019, 14 participants, IBT Biocev, Vestec, J. Stránský organizer.

5x Czech Mass Spectrometry Conference 2015-2019. Annual conference, 120 participants, co-organizer P. Pompach. 15-17 April 2015, Hradec Králové, 13-15 April 2016 České Budějovice, 29-31 March 2017, Olomouc, 11-13 April 2018 Prague, 27-29 March 2019 Olomouc.

International conference APERIODIC 2015, Prague Břevnov, 30.8 – 4. 9. 2015. 60 main lectures, 90 posters, published http://www.xray.cz/ms/bul2015-4.htm, J. Hašek organizer.

15th International Conference on the Crystallisation of Biological Macromolecules, Prague, Pyramida, July 1-5, 2016, http://www.xray.cz/iccbm/, http://www.xray.cz/ms/bul2016-2.htm. J. Hašek organizer.

15. Invited lectures and earned awards

Malý P., invited speaker at 4th International Conference on Influenza and Zoonotic Diseases, July 02-03, 2018, Vienna, Austria;

Malý P., invited speaker at 10Th Asia-pacific Biotech Congress, July 25-27, 2016, Bangkok, Thailand

Malý P., invited speaker at 10th PepCon-2017, March 22-24, 2017, Fukuoka, Japan;

Malý P., invited speaker at 7th World Gene Convention, November 3-5, 2016, Shanghai, China;

Malý P., invited speaker at 9th World Gene Convention, November 13-15, 2018, Singapore;

Malý P., invited speaker at International Biotechnology Congress-2017, October 25-27, 2019, Singapore;

Malý P., invited speaker at International Drug Delivery Science and Technology (IDDST-2019), October 25-27, 2019, Kyoto, Japan),

Malý P., keynote speaker at 2nd International Conference on Advances on Biotechnology, July 23-25, 2018, Kuala Lumpur, Malaysia

Dohnálek, J. invited speaker at ECM 30, 28 August – 1 September 2016.

Dohnálek, J. invited speaker at Novozymes A/S, Copenhagen, 5 October 2017.

Koval, T. invited speaker at BioTech 2017 and 7th Czech-Swiss Symposium with exhibition, Prague, 13-17 June 2017.

Skálová, T. invited speaker at International school of crystallography, Erice, Italy, 2-11.6. 2017.

Dohnálek, J. invited speaker at FEBS Congress Prague, 7-12 July 2018, Invited scientific talk for the Instruct-Ultra section of research infrastructures.

Dohnálek, J. invited speaker at ELI Beamlines workshop on Laser-driven X-ray Sources and Applications, ELI Beamlines, Dolni Brezany, 24-25 October 2019.

Dohnálek, J. invited speaker at the Institute of Microbiology lecture series, Prague, 3 October 2019.

Hašek, J. invited speaker at MedeA® Users Group Meeting. Sofitel Grande Ile, Strasbourg, France 19 –21 September 2017

Awards

Students of the team are successful in poster and talk prizes at National and International conferences:

Leona Švecová, 1st prize in category Biocrystallography and chemical crystallography, talk Bilirubin oxidáza: Strukturní analýza komplexů s ligandy v aktivním místě a studie aktivit, at conference Struktura 2017, by the Czech and Slovak Crystallographic Association.

Martin Malý, 2nd prize in category Biocrystallography and chemical crystallography, talk Diffraction limit in macromolecular crystallography, at conference Struktura 2017, by the Czech and Slovak Crystallographic Association.

Martin Malý, 2nd prize in category Theoretical physics, biophysics and physics of molecular systems, talk Structural analysis of complexes of oxidase with ligand, at the 9th Czech-Slovak student scientific conference in Physics 2018 by the Faculty of Mathematics and Physics, Charles University.

Martin Malý, 1st prize in category Biocrystallography and chemical crystallography, talk Optimization of computational procedures in macromolecular crystallography, at conference Struktura 2019, by the Czech and Slovak Crystallographic Association.

Lucie Marečková, “Best Poster Award” at 12th Euro Biotechnology Congress, November 07-09, 2016 in Alicante, Spain

Lucie Marečková, young prospect for the 17th FEBS Young Scientists‘ Forum with the financial support at the 42th FEBS Congress in Jerusalem, Israel, September 10-14, 2017

16. Participation in large collaborations

The team has collaborated by provision of instruments, knowledge, expertise and financial contribution in provision of specialized services of the **Centre of molecular structure** in structural biology within the **Czech Infrastructure for Integrative Structural Biology** and within the **European infrastructure for structural biology Instruct-ERIC**, resulting in 57 publications from the team members and from external users, e.g. (Kovaľ & Dohnálek, 2018) or Zeman, J., et al. (2019). *Nucleic Acids Research 47(15)*: 8282-8300.

The team has collaborated by provision of instruments, knowledge, and expertise to conceive, formulate, and submit the operational project Excellent teams ELIBIO provided by the Czech Ministry of Education, Youth, and Sports (CZ.02.1.01/0.0/0.0/15_003/0000447). The project explores new frontiers in photon physics and optics to create breakthrough science in biology, chemistry and physics. The goal is to establish an Interdisciplinary Centre of Excellence to exploit the photon beams of the European Extreme Light Infrastructure (ELI-BL Dolni Brezany) in life sciences and to establish collaborations with other large infrastructures such as free-electron lasers, including the European XFEL in Hamburg and the LCLS at Stanford. An essential goal of the project is to understand photon-material interactions in extremely intense X-ray or photon fields where new physics can be expected. Our experiments explore fundamental questions in the physics of photoemission and electron dynamics of biomolecules. We use the new knowledge and methods enabled in ELI-Beamlines and IBT BIOCEV to study the structure, function and dynamics of biomolecules. We develop new methods and technologies to enable such measurements and answer key questions in health and disease. The project has been awarded in November 2016 and brought together experts from complementary fields of photon physics at ELI-Beamlines infrastructure (8 researchers) and in biophysics and protein engineering at IBT (8 researchers from the IBT Team 2). The team has so far (at the beginning of its 4th year) published 34 papers.

17. Outreach activities

Yearly, Open days of the IBT. Every year 20 to 80 visitors from high schools, individuals.

Collaboration with Weizmann Institute of Science: workshop Frontiers in targeted modulation of protein function with researchers from the Weizmann Institute of Science on November 28-29 2016. The first part of the specialized workshop was open to a broad public.

Project Open Science of the Czech Academy of Sciences, ~8 students

Week of Science (Týden vědy na Jaderce), Introduction to scientific work to students of secondary education in a form of mini-conference, with small laboratory projects, co-organizers from the team, 19-26 June 2016, Czech Technical University and IBT.

Week of Science (Týden vědy na Jaderce), Introduction to scientific work to students of secondary education in a form of mini-conference, with small laboratory projects, co-organizers from the team, 16-21 June 2019, Czech Technical University and IBT.

Science night (Noc na Jaderce), Introduction to protein crystallography in lectures and practical demonstration of results, co-organizers from the team, Czech Technical University, 15 November 2016.

Science Festival (Festival Vědy), popularization lecture, P. Kolenko, DDM of Prague, Czech Technical University in Prague, Prague 6, 6 September 2017.

Excursion for secondary school students (Gymnázium Jaroslava Heyrovského, Praha 5) focused on Methods of studies of biomolecules – lecture and visit of laboratories with practical demonstrations, October 2016.

Excursion for secondary school students (Karlínské gymnasium, Praha 8) focused on Methods of studies of biomolecules – lecture and visit of laboratories with practical demonstrations, 7 November 2018.

Popularization lecture for secondary school students (Gymnázium Omská, Praha 10) focused on protein crystallography, 2018.

Street Exhibition organized by Academy of Sciences (2015) - demonstration of the development of anti-inflammatory binding proteins as a non-immunoglobulin alternative for drug research.

Týden, 29.10.2018, Vnitřní vesmír v našich buňkách – Z. Lánský

Pokroky matematiky, fyziky a astronomie, 4/2016, Kráčející proteiny v nitru živých buněk – Z. Lánský

Information about IBT collaboration with the Weizmann Institute of Science in the Czech TV and social media – B. Schneider, June 26 2017.

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