RESEARCH STRATEGY OF THE CENTER

BRIDGING MOLECULAR AND CELLULAR RESOLUTION: FROM ATOMIC INSIGHT INTO PROTEIN-PROTEIN INTERACTIONS TO NETWORKS IN AND ACROSS CELLS

At the VIB-VUB Center for Structural Biology (CSB), we study the structure and dynamics of macromolecular complexes in health and disease to explain their mode of action. We integrate our structural biology work with genetic and cellular studies, aiming to bridge molecular and cellular resolution. We excel in translating our discoveries into biotechnological and medical applications.

TURNING EXCELLENCE IN STRUCTURAL BIOLOGY INTO VALUE FOR SOCIETY

Structural biology is undergoing a major leap forward in defining three-dimensional submicroscopic structures because of the development of cryo-electron microscopy (cryo-EM) and from novel approaches to imaging in cell biology. We envision a structural biology that is increasingly closer to the cellular context, working on in situ or ex vivo samples, with minimal manipulation and as close as possible to the physiological processes under study. We aspire a further integration of time as an integral dimension of the phenomena that we study.

We conceive a structural biology that transcends the explanatory power of the technique, into an exploratory instrument that leads to the discovery of novel biological pathways.

CREATING THE FLANDERS BIOLOGICAL ELECTRON MICROSCOPY CENTER

Recent breakthroughs in both hard- and software transformed single-particle cryo-microscopy. This enables an increased understanding of complex biomolecules and their functions at atomic level. To contribute significantly to this new level of scientific enquiry, CSB invested in cryo-EM. A competitive FWO-HERCULES grant allowed us to set up the first Belgian cryo-EM facility equipped with 300 kV FEG microscope and direct electron detector.

SHARING UNIQUE AND DIFFERENTIATING PLATFORMS WITH THE RESEARCH COMMUNITY

Our center provides Belgian and European scientists access to cryo-EM. Nanobodies4INSTRUCT ensures worldwide access to nanobody-enabled structural biology.

Han Remaut & Jan Steyaert, Science Directors

EXCELLENCE IN TECH TRANSFER

In accordance with VIB’s entrepreneurial spirit, we at CSB have an excellent track record in turning science into value for society, even though it has a strong focus on fundamental research. The prime example is the development of Nanobody® technology, which gave rise to three established spin-off companies: Ablynx, Biotalys (formerly Agrosafe) and Confo Therapeutics. In addition, the center’s expertise in nanopore technology has led to collaborations with leading nanopore sequencing providers. All this was achieved by promoting a healthy mix of scientific excellence and entrepreneurship, complemented by the strengths of VIB’s valorization team.

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VIB is a non-profit institute with multiple sites, which unites world-class researchers in teams and research centers that are embedded in five universities in Flanders. VIB’s scientists conduct groundbreaking biomolecular research in life sciences, leading to sustained scientific progress and contributing to a better world.

All VIB centers consist of several research groups with a specific expertise. At VIB, researchers are stimulated to collaborate across disciplines beyond the borders of their own center or even institute.

The VIB centers perform world-leading basic research on some of the most poignant biological questions of our time. It is VIB’s strong conviction that fundamental scientific research – often guided by serendipity – can result in major, sometimes unforeseen, breakthroughs. However, scientific research should benefit society as a whole. That is why each research group is also encouraged and supported in framing their work in a socially relevant applicable context. As such, each group has a substantial interest in a translational research line, where they aim to develop their basic findings in a way that can make a big impact on various societal challenges.

In short, the VIB centers aim to do cutting-edge basic research and translate results into advances for the benefit of everyone.

In the presentation of the research groups below, each group in the VIB-VUB Center for Structural Biology performs trailblazing basic research that leads to significant translational potential. Through a fundamental focus on unraveling the structural details of life’s building blocks, CSB discoveries pave the way towards groundbreaking insights into biological mechanisms responsible for both health and disease in a wide variety of organisms, including humans and crops.

The Vorobieva lab interest lies at the interface of computational and experimental biochemistry and biophysics. We recently showed the feasibility of designing transmembrane beta-barrel proteins (TMBs) from first principles. We now aim to develop new computational design methods and experimental high-throughput assays to further study the fundamentals of membrane protein folding and enable the custom design of TMBs with a function.

Sequence determinants of membrane protein folding

De novo protein design has greatly contributed to the understanding of basic protein folding. It provides an integrative approach for the investigation of the protein sequence/structure relationship. Advances in high performance computing, and DNA synthesis and sequencing, have enabled the generation of massive design/testing feedback cycles that have yielded more robust design methods and allowed detailed exploration of sequence determinants of protein folding. The computational design of membrane proteins, however, is still challenging because of the poor understanding of folding pathways and limited biophysical and structural data. We aim to address these shortcomings by developing new computational design methods and experimental screening assays in biological and synthetic membranes. We analyze the resulting datasets to investigate basic questions about membrane protein biogenesis that are otherwise hidden by evolutive history.

Stability/function trade-offs in membrane proteins

Membrane proteins have a tremendous potential for biotechnology, and the engineering of functional membrane proteins able to fold into lipid membranes of different properties would enable the development of molecular machines, such as enzymatic nanoreactors or nanopores for single-molecule analytics. However, little is known about the constraints that a specific function places on the stability and folding of membrane proteins. We apply a de novo design/high-throughput testing approach to probe the trade-offs between stability, folding, and function in TMBs. We develop computational methods to generate libraries of TMB sequences simultaneously optimized for folding into lipid bilayers and for binding small molecules that we screen to identify the sequences that fold and function as designed.

Synthetic biology

In eukaryotic cells, TMBs fold in the outer membrane of mitochondria (MOM), which is a cross-road for cellular signaling pathways and apoptosis. We design TMBs that specifically bind cell-permeable and non-toxic fluorescent dyes and use the fluorescent protein/dye complexes to study TMB folding in mitochondria. Our transmembrane fluorescent proteins will be engineered into modular synthetic biology tools to study MOM biological processes by super-resolution microscopy.
Mechanisms of molecular machines

Many proteins and protein complexes in the living cells function as molecular machines. Such machines are involved in energy conversion, synthesis of biopolymers, molecular transport and many other processes. While molecular machines resemble human-made macroscopic machines, at the atomic scale the mechanisms of these machines are completely different from their human-made analogues and they are often non-intuitive. The Efremov lab is undertaking efforts to reveal fundamental principles behind mechanisms of such proteins. Activity of protein-based molecular machines involves transitions between specific conformations that enable them to direct processes against their chemical potential and entropy using chemical energy of fuel. The laboratory uses tools of structural biology, in particular single particle cryo-EM, to solve the structures of the proteins in the functionally important conformations. In order to advance beyond state of the art, the research team works on method development. New approaches towards preparation of cryo-EM samples that enable trapping short-living intermediates in the cells are being explored using microfluidics.

Membrane protein complexes

The molecular machines studied in the laboratory include membrane protein complexes involved in cell bioenergetics, transport and signaling. These proteins are purified from the cells using biochemical approaches. Further time-resolved cryo-EM is used to trap the short-living intermediates of functional cycles of the corresponding proteins with a goal to solve their high-resolution structures using cryo-EM. Structural biologists, biochemists and engineers work together to understand principles governing protein functioning and to move forward the methodology of cryo-EM. The long-term ambition of the laboratory is to develop a deep understanding of the structural organization and functional principles of the protein-based molecular machines such that they enable designing and making completely new machines with functions not existing in nature. Such designed proteins will have new biotechnological applications.

Intrinsic disorder in prokaryotes

The first line of research in the Loris group deals with intrinsic disorder in prokaryotes, where toxin-antitoxin systems (TA systems) are used as model system. These are operons encoding a stable toxin and a labile antidote. They play a major role in bacterial stress physiology by temporarily halting cell division when nutrients are scarce. They are proposed to be involved in multidrug resistance. Many different families of TA modules exist, differing in the cellular target of the toxin and the nature of the DNA binding domain employed by the antitoxin.

In TA systems, the antitoxin is either fully or partially disordered. This disorder is functional and allows the antitoxin to mediate different functions that would be difficult to combine in a classic folded protein. These include folding upon binding with strong negative cooperativity, conditionally enhancing or reducing affinity for the operator and rejuvenation mechanisms to restore growth in inhibited cells.

Intrinsic disorder in plant cell cycle regulation

The second major focus of the group is on intrinsic disorder in plant cell cycle regulation. Here the team focuses on NAC transcription factors and in particular SOG1, which is a likely candidate for the role of mammalian p53 in plants. SOG1 is central in the DNA Damage Response. In collaboration with the VIB team of Prof. Lieven De Veylder (VIB-UGent Center for Plant Systems Biology), a combination of in vivo and in vitro work including integrated structural biology is used to unravel the SOG1 regulatory network and to understand how it selects its target genes.

From a fundamental science point of view, we aim to learn how specific and non-specific DNA binding can be discriminated in the context of so-called fuzzy recognition events. From the more applied point of view, our team is interested in how aluminum resistance relates to cell cycle regulation and can be enhanced in order to increase crop yields.

Intrinsic disorder in plant cell cycle regulation

The interests of the Loris group encompass the broad area of structure-function relationships in proteins. The group uses a variety of structural biology and biophysical techniques and is involved in different projects dealing with protein structure and molecular recognition. In particular, the lab members are interested in mechanisms of action of intrinsically disordered proteins and in mechanisms of transcription regulation.

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Molecular imaging tools based on protein conformational changes.

Here, the Joris Messens lab strives to make the subcellular metabolite flux visible. To investigate intracellular signaling by metabolites, it is important to have proper tools to visualize their trafficking in real time in living cells at subcellular resolution, which current mass spectrometry ‘omics’ methods cannot offer.

In response to this need, we design protein-based biosensors. They consist of a transcription factor that, by nature, has evolved to be specific for a metabolite, and into which we genetically introduce a fluorescent protein as read-out. The Joris Messens lab uses these biosensors in combination with the latest tools for \( \text{H}_2\text{O}_2 \) detection and manipulation to probe the crosstalk between \( \text{H}_2\text{O}_2 \) and metabolic pathways.

In gram-negative bacteria, these adhesions are displayed on the outer membrane as single proteins or are incorporated into filamentous polymers. Adhesion-mediated attachment is involved in several aspects of the infection process. In an era of increased antibiotic resistance and difficulties in controlling hospital-acquired infections, it is essential to gain a better understanding of the fundamental principles governing the infection process. The lab studies the structural molecular biology of bacterial adhesions and cell-surface filaments with respect to their function in bacterial pathogenesis, with the aim of developing a new generation of virulence-targeted antimicrobials. The team pursues this goal via three main projects.

Helicobacter pylori adhesion - Countering a life-long attachment to the host.

Due to its extreme persistence in the host and the known involvement of a complex adherence profile in maintaining infection, Helicobacter forms the ideal proof-of-principle case for the development of anti-adhesion drugs. Mounting evidence shows that the presence of only a selected number of virulence factors is associated with disease-causing HP strains, responsible for peptic ulceration and an increased risk for gastric cancers. The development of anti-virulence therapies targeting HP adhesins has the potential for the broad-scale selective clearance of pathogenic HP strains only.

Chaperone/ usher pilus assembly - Towards selective disarmament of adhesive fibers.

P and type 1 pili are responsible for the early onset and persistence of UPEC-caused urinary tract infections (UTIs) by mediating attachment to the kidney epithelium or attachment and invasion of the bladder epithelium cells. They are assembled by the conserved chaperone/ usher (CU) pathway, responsible for the biogenesis of more than 100 surface organelles in many other important human pathogens (including Yersinia, Salmonella, Shigella, Hemophilus). Two strategies are investigated for countering pili-mediated disease processes: anti-adhesive compounds targeted against the adhesive sub-units, and pili biogenesis inhibitors.

Curli - Structural biology of controlled amyloid deposition.

Curli are proteinaceous filaments found on the surfaces of \( \text{E. coli} \) and Salmonella species where they mediate biofilm formation and have been shown to bind a range of human plasma and contact-phase proteins. Contrary to what is seen in human pathogenic amyloid depositions, curli formation follows a controlled biosynthetic pathway involving several protein co-factors. The structural molecular biology of curli biosynthesis is used as a model system for controlled amyloid deposition and as a route towards future nanobiotechnological applications.

New technologies, tools, and approaches, often spanning several disciplines, are revolutionizing biology and providing unprecedented opportunities to advance the frontiers of bioscience knowledge. Fascinated by the power of new technologies on the progress of science, Jan Steyaert focuses on the development of a generic molecular toolbox to study the conformational changes in proteins. We knew that many proteins are flexible and that their function is tightly connected to conformational changes but for many years, these different conformations were intractable for structure determination by any general method.

‘Freezing’ proteins to study their structure.

The Steyaert lab pioneered the use of Nanobodies as exquisite tools to freeze dynamic proteins into single functional conformations. Nanobodies are the variable domains of heavy-chain only antibodies that naturally occur in camels. X-ray crystallography or single particle cryo-EM can then be used to determine the structures of different stills of the same moving biomolecule. The power of this approach compares to the series of photographs that Eadweard Muybridge made in 1878 to uncover if a horse that trots or gallops does ever become fully airborne.

Expanding the Nanobodies’ utility.

Jan Steyaert discovered nanobodies to study the dynamics of the highest hanging fruits in structural biology including amyloidogenic proteins, membrane proteins, and transient multiprotein complexes. And he is expanding the utility of...
such conformational Nanobodies for integrative structural biology. Conformational Nanobodies can be introduced inside living cells (intra-bodies) as conformational biosensors. His lab uses protein engineering to make such Nanobodies amenable to single particle cryo-EM (Megabodies). The Steyaert lab also extensively explored the benefit of locking targets in druggable conformations for better-focused drug discovery (Confobodies).

Most of this work is performed in collaboration with top scientists worldwide, aiming to validate his toolbox on their most challenging projects. Jan Steyaert also (co)founded 3 companies (Ablynx, Biotalys and Confo Therapeutics), providing a living example of how basic research can be translated into value for the society.

The focus of the Tompa lab’s research is the structural disorder of proteins. Regions of proteins, or even full-length proteins, exist and function without well-defined 3D structures, which challenges the classical structure-function paradigm. Structural disorder is prevalent in eukaryotic proteomes and disordered proteins carry out unique functions. Due to their frequent involvement in regulatory and signaling functions, structural disorder also plays important roles in serious diseases, such as cancer and neurodegeneration. Detailed experimental and theoretical characterization of the structural ensemble of disordered proteins in isolation and in interactions hold the key to understanding these proteins and extending the structure-function paradigm to the disordered state. In this spirit, the team undertakes three different lines of research to push the frontiers of the field of disorder.

Extend the paradigm of disordered chaperones to cellular conditions.

The team suggested some time ago that fully disordered proteins or disordered regions of classical chaperones can have chaperone function on their own. Now, they seek to elucidate details of the underlying mechanism through proteomic studies to determine the physiological partners of this disordered chaperone, in-cell NMR studies to see the structure and interactions of ERD14 in live plant cells, and detailed structure-function studies to see which sequence elements are involved in transient binding to the partner and how the interplay of induced folding and disorder in the bound state contribute to chaperone activity.

Structure-function relationship of very large proteins.

Structural biology has traditionally addressed the structure of small folded proteins, whereas the field of structural disorder has focused on either fully disordered proteins/regions or short disordered elements that undergo induced folding in the presence of their partner. Here the team will probe into the structure of the very large transcriptional co-activator CREB-binding protein (CBP). CBP has about seven domains and disordered linker regions connecting them, the topology of which will be outlined by a combination of high-resolution (NMR, X-ray) and low-resolution (MS, EM, AFM) techniques.

Structure-function relationship of protein disorder and drug development

Although intrinsically disordered proteins play key roles in the regulation of cellular function and in disease, and they represent an important group of therapeutic targets, members of this 'disorderome' have not yet been successfully targeted by drugs. The primary reason is that traditional design principles cannot be applied to their highly dynamic, heterogeneous structural states. The team’s goal is to combine an ensemble description and selection of small-molecule binders into a rational design of targeted, specific inhibitors of therapeutic importance.

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Proteins are some of the most fascinating and complex macromolecules in living systems that play extraordinarily diverse roles in sustaining life. These roles are mediated through the interactions that proteins establish both with other proteins and with different molecular constituents of the cell.

The rules of protein interactions

The continuing focus of the Wodak lab’s research is on investigating the principles (structural, energetic and dynamic) that govern protein interactions. To that end the researchers employ molecular simulation and modeling techniques to study protein-protein, protein-DNA, and protein-ligand interactions at the atomic scale. A complementary research strand is the analysis of the properties of known protein structures and sequences in order to gain insight into how evolution has shaped the functional specificity of proteins.

Computational analysis

In parallel to these molecular-level investigations, the team is actively engaged in developing quantitative and integrative computational approaches for analyzing physical and functional interactions between proteins, protein complexes and biochemical pathways at the cellular level.

These research activities closely combine the development of analysis methods and software tools with their application to understand important biological problems with relevance to human health.
Membrane proteins mediate diverse processes as electrical excitability, solute transport and signaling in the cell. Structural biology of these proteins is still demanding, yet it is a necessity for a complete understanding of the protein’s inner workings. As membrane proteins are major targets for small molecule drugs, in-depth insights into the atomic structure and conformational landscape is of prime importance for the treatment of life-threatening diseases. The Brunner lab focuses on two major research topics within membrane biology and applies mainly biophysical, structural and biochemical methods but is also striving to integrate these results in a cellular context.

**Ion channels**

We are interested in ion channels of the endomembrane system, in particular the lysosome. The lysosome is the cellular recycling machinery and is required for the turnover of autophagosomes, membrane structures that engulf cytosol and proteins in bulk for subsequent fusion with the lysosome. This interplay makes lysosomes thus also pivotal for the removal of protein aggregates from the cytosol and connects them to neurological diseases like Parkinson’s disease. Ionic gradients across the lysosomal membrane are crucial for the function of the organelle making ion channels important regulators of lysosomal transport and fusion as well as the degradation of lysosomal contents. Consequently, modulation of ion channel activity in these organelles holds great prospects for medical applications. In the Brunner lab, we seek to understand how lysosomal ion channels achieve selectivity and how they are gated to open or close. We further investigate the significance of ion gradients across the lysosomal membrane for the turnover of autophagosomes and maintenance of the luminal pH.

**Lipid transporters and membrane asymmetry**

The second major research line is related to membrane lipid asymmetry and its regulated breakdown as a signaling cue. In our research activities we focus on membrane proteins that impact the organization of the lipid bilayer, in particular lipid transporters that contribute to membrane lipid asymmetry and scramblases that randomize the distribution of lipids between the membranes. We investigate how certain lipid species reach their destination in a distinct leaflet of the bilayer, why lipid asymmetry in membranes is important and which functions are elicited through the concerted breakdown of this asymmetry by scramblases. Our efforts aim to contribute to a deeper understanding of the involved proteins in lipid trafficking and signaling in healthy and pathological conditions.

Besides our basic research activities, we develop methodologies to greatly reduce the required biomass and sample consumption for the determination of membrane protein structures by cryo-EM.

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**Drug discovery**

In parallel of the fundamental part that aims at a better understanding of the resistance and virulence capabilities of *A. baumannii*, medium to high-throughput screens are undertaken. The aims are the identification of new antibiotic, anti-resistance or anti-virulence compounds that are active against most of the current multidrug-resistant clinical isolates of *A. baumannii*. Efficient screening approaches using population and single-cell levels analyses, with and without a priori, are implemented directly on the bacteria themselves, or using a host-pathogen interaction context.

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**Bacterial resistance**

We decipher the molecular mechanisms governing bacterial resistances by combining phenotypic characterization with whole genome sequencing data using reference strains and recent clinical isolates. The main targeted mechanisms studied are involved in desiccation, disinfectant, serum and phagocytic resistances. The factors identified represent new targets for a priori screening of anti-resistance compounds.

**Bacterial virulence**

Another part of our research studies *A. baumannii* virulence, with an emphasis on the polysaccharide capsule. We compare the levels of capsule production in *A. baumannii* using in vitro and in vivo infection models. The virulence factors identified represent new targets for the discovery of new anti-virulence molecules.
While VIB invests strongly in socially beneficial translational research, this could not be possible without the world-class fundamental research done in its centers. Investigating the basic biological mechanisms of life, across the great diversity of organisms, lies at the core of scientific progress and the societal and technological advances that flow from it. The VIB-VUB Center for Structural Biology aims to contribute to progress in molecular technology. Below is a brief selection of impactful papers detailing their work. Many more can be found on the center’s website.

**DEVELOPING AN ULTRASENSITIVE FLUORESCENT H₂O₂ BIOSENSOR**

H₂O₂ is a reactive oxygen species that needs to be tightly controlled in cells. Crystallographic, biochemical, and biophysical insights in the H₂O₂-sensing mechanism of a transcription factor led to the design of an ultrasensitive fluorescent H₂O₂ biosensor by the Messens lab.


**DEVELOPING MEGABODIES**

To overcome two major obstacles that limit the resolution of single-particle cryo-EM reconstructions: particle size and preferential orientation at the water-air interfaces, the Steyaert lab developed and characterized novel constructs, termed Megabodies. By grafting nanobodies onto selected protein scaffolds they could increase their molecular weight while retaining the full antigen binding specificity and affinity. Moreover, we have demonstrated that megabodies can be used to obtain 3D reconstructions for (membrane) proteins that suffer from severe preferential orientation or are otherwise too small to allow accurate particle alignment. The first megabodies were used to solve the high-resolution cryo-EM structure of the human synaptic GABAA receptor and uncover its signaling mechanisms.


**ANTIMICROBIAL MONOCLONAL ANTIBODIES TARGETING BACTERIAL CELL SURFACE STRUCTURES**

The human pathogen Bacillus anthracis is covered by a paracrystalline protein layer or S-layer. We solved the structure of the Sap S-layer and demonstrated that S-layer disrupting nanobodies induce extensive cell surface defects that result in a bactericidal activity that proved therapeutic in a mouse model of lethal anthrax disease.

LIQUID-LIQUID PHASE SEPARATION IN ALS

Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease which is marked by pathological protein deposits in motor neurons of affected individuals. The Tompa lab is investigating the molecular mechanisms of protein deposition, and provided evidence that a leading cause is the formation of protein aggregates via the intermittent demixing of ALS-linked RNA-binding proteins (e.g. hnRNPA2) and dipeptide repeats (e.g. PR30) by liquid-liquid phase separation (LLPS) that leads to the formation of dynamic liquid droplets. This mechanistic insight opens novel ways of drug development against ALS, and possibly other neurodegenerative diseases.


In 2020 Jan Steyaert was elected as EMBO member, one of the most highly prized recognitions of excellence in the life sciences for individual researchers.

For his outstanding work, Tomasz Uchanski received the 2020 B&KD cross-disciplinary PhD award and the 2020 Solvay PhD award.

In 2020 Antonella Fioravanti received the EOS Pipet, a prize awarded to the most promising young researcher in Flanders.

Both Joris Messens and Han Remaut have been awarded and ERC Consolidator Grant, a prestigious grant from the European Research Council that allows its laureates to consolidate their successful research career.

Jan Steyaert has been a Francqui Research professor at VUB since 2015.

From 2019 on, Jan Steyaert and Els Pardon are recognized as Highly Cited Scientist by Clarivate Analytics, which means they belongs to the 1% of scientists in the world whose papers are most frequently cited in the fields of Biology and Biochemistry.

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CSB researchers are also active in many European networks and consortia:

- CAPRI (2002-present): CAPRI is hosted by the Protein Data Bank in Europe group and organizes community-wide experiments on the comparative evaluation of protein-protein docking for structure prediction

- ELIXIR (2019-present): the ELIXIR 3DBioInfo Community plays a crucial role in validating and developing bioinformatics methods and tools to analyze, predict, archive and validate the three-dimensional (3D) structure data of biomacromolecules.

- ISCStoresearch (2017-present): the International Society for Computational Biology is a scholarly society for advancing understanding of living systems through computation and for communicating scientific advances worldwide.

- IDPfun (2018-22): IDPfun is an H2020 funded Marie Skłodowska-Curie Research and Innovation Staff Exchange project for creating a collaborative environment for research on novel ways to detect and characterize different IDP phenomena in their evolutionary context.

- PhaseAGE (2020-2025): PhaseAGE is an H2020-funded Marie Skłodowska-Curie ITN, designed to develop a new platform for rapid detection and characterization of pathologically relevant intrinsically disordered protein aggregates.

- CAID (2018-present): CAID is a community-wide experiment to determine and advance the state of the art in the detection of intrinsically disordered residues forming the amino acid sequence.

- Nanobodies4Instruct (2016-present): we host Nbs4INSTRUCT, a flagship service platform of Instruct-ERIC, one of the first ESFRI projects that provides cutting-edge technology, scientific expertise and training to the European research community. We provide conformational Nbs and now also megabodies to facilitate the structural analysis of proteins that are notoriously difficult to purify, crystallize or study by any other method.

- IDPput (2018-22): IDPfun is an H2020 funded Marie Skłodowska-Curie Research and Innovation Staff Exchange project for creating a collaborative environment for research on novel ways to detect and characterize different IDP phenomena in their evolutionary context.

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CSB
IN NUMBERS

185 PAPERS
OF WHICH
67 TIER 5
>1850 CITATIONS
IN THE LAST THREE YEARS

2.57 M
TOTAL INDUSTRIAL INCOME

3.3 M
TOTAL INTERNATIONAL INCOME

3 SPIN-OFFS
Ablynx, confo, Biastyn

2 ERC GRANTS

150 PDBs

3 YEAR AVERAGE
33 PhDs
12 BELGIAN
21 NON-BELGIAN

24 POSTDOCS
7 BELGIAN
17 NON-BELGIAN

33PhDs
AWARDED IN THE PAST THREE YEARS

EXPERTISE

At CSB, detailing the structure of proteins and protein complexes is a focal point of the research. As such, significant expertise is available among its members including X-ray crystallography, NMR, cryo-EM. The strong structural biology know-how at the center has even led to the initiation of the Nanobody Core Facility, embedded within CSB, that collates this expertise and makes it available to potential collaborators within and outside of VIB.

The center also founded Nanobodies4Instruct as part of Instruct-ERIC, one of the first ESFRI projects that provides cutting-edge technology, scientific expertise and pioneering training. Any scientist from an Instruct member state can submit a proposal for access to the Nanobodies4Instruct center for the generation of conformational nanobodies and now also megabodies to facilitate the structural analysis of proteins that are notoriously difficult to purify, crystallize or study by any other method.
NANOBODY CORE
Developed at the CSB, the powerful Nanobody® technology based on llama antibodies is now available to both academic and non-academic researchers through the VIB Nanobody Core.

Nanobodies® are an extremely useful class of molecules for research, diagnostics and therapeutics in human and animal health. They can be used in a variety of fields, such as immunohistochemistry, immunomodulation, intracellular expression (intrabodies) and biosensor-applications. As proteomics tools, they can be deployed in expression profiling, physical mapping of proteins, protein-protein interaction studies, functional analysis and (in)activation of genes.

In addition to the delivery of standard Nanobodies®, additional services can be offered, such as determination and ranking of affinity, and engineering towards higher stability and/or affinity. Expression vectors can be made available to express modified recombinant constructs: Nanobodies® can be cloned in front of a human or mouse IgG domain (or any other animal/isotype), tagged with Flag, c-myc, His, Cy-dyes, FITC, GFP, lactamase, etc. Nanobodies® can also be biotinylated for use in immunoprecipitation applications with streptavidin beads or engineered into bivalent or bispecific antibody constructs.

For any questions, feel free to contact expert technologist Gholamreza Hassanzadeh Ghassabeh (reza.hassanzadeh@vub.vib.be).

BECM
VIB-VUB facility for Bio Electron Cryogenic Microscopy

CRYO-EM FACILITY:
SEEING LIFE IN UNPRECEDENTED DETAIL
CSB recently opened the ‘international facility for Bio Electron Cryogenic Microscopy’ (BECM) at the VUB Campus in Brussels. This facility houses a new kind of cryo-electron microscope (cryo-EM), one of just three in the world. The microscope pushes the boundaries of research into active proteins for the development of medicines and, thanks to its unique properties, will be operating on a 24/7 basis.

The microscope, which is unique in Europe and was installed thanks to a 4 M€ grant from Research Foundation - Flanders (FWO) allows images of the building blocks of the human body to be produced with atomic precision. Despite the crucial importance of proteins for the efficacy of medicines, our knowledge of the function of human proteins extends to only one-third of these, of which the structure of only one in ten is known. This is why scientists have spent years searching for suitable imaging techniques that can create images at an atomic level of how these molecules function and the way in which they interact.

The new microscope will allow researchers to study these proteins, which they had never before been able to see with such precision using any other method.

The device is so unique that other universities as well as biotechnology companies are lining up to use it for measurement purposes.

The new microscope will run be run as a multi-user facility under the expert supervision of Prof. Rouslan Efremov. The BECM was officially opened in 2018 by the then Flemish Minister of Labor, Economics, Innovation and Sport Philippe Muyters, an event that was also joined by Nobel Prize winner Professor Richard Henderson (Medical Research Council, Cambridge, UK), recipient of the 2017 Nobel Prize for. According to the Nobel Committee, his research into the ingenious operation of the cryo-electron microscope, which was conducted jointly with Professors Jacques Dubochet and Joachim Frank, was a breakthrough on which CSB will continue to build with the help of the BECM.
more technology

CSB aims at integrated structural biology from the atomic to the cellular scale. For this, we combine high-end in-house equipment in cryo-EM, X-ray crystallography and NMR that is open for external collaboration with both academic and industrial partners. In-house screening of crystals allows efficient use of our frequent access to the macromolecular crystallography beamlines of the Diamond, ESRF, Soleil and Petra-III synchrotrons, where we also co-ordinate several block allocation groups.

The Jean Jeener NMR Center houses two state-of-the-art Bruker 600 and 800MHz spectrometers. These instruments, equipped with different probes for applications in organic chemistry and biochemistry, support a wide range of NMR experiments on biological macromolecules, not only for complete structure determination projects but also for characterization of dynamics and most important for interaction studies in the context of drug discovery.

We further extend the three high resolution techniques with low resolution structural characterization using SAXS, CD spectroscopy and SEC-MALS. These structural techniques are further complemented with a range of methods to study protein-ligand interactions that include isothermal titration calorimetry, bio-layer interferometry, microscale thermophoresis and stopped flow fluorescence.

In order to advance towards a next generation structural biology, CSB is setting up a new strategic technology platform at the interface of Nanobody technology, microfluidics, next-generation sequencing and mass-spectrometry. This platform will serve fundamental and translational projects and drive different innovation tracks. The focus of this platform is to develop methods that allow to study biological complexes as close as possible to their biological context. For this, miniaturizing sample requirements, minimizing sample manipulation and working on in situ or ex vivo samples are key. We further work towards time-resolved structural biology and towards conformational or phenotypically selective isolation of macromolecular complexes to discover new biology and novel therapeutic approaches in human and animal disease processes.

Technology transfer

With the support of VIB’s Innovation & Business team, the center has catalyzed the use of VHHs as research tools in biotechnology, and the exploration of VHHs as marketable products in medicine and crop protection. VHHs or Nanobodies®, the small and stable antigen-binding fragments derived from a special type of antibody present in camels such as lamas, have been discovered by VUB and VIB in the nineties. Further research proved that VHHs are an extremely versatile class of molecules for research, diagnostics and therapeutics in human and animal health.

The VHH technology continues to be the subject of intensive research at VIB. The ongoing research of Jan Steyaert’s lab continuously contributes to new discoveries, which have sparked multiple collaborations with other institutes and industry players.

So far, the success of these tiny antibody fragments has given rise to three VIB spin-offs: Ablynx (°2001), Biotalys (°2013) and Confo Therapeutics (°2015). Ablynx, now a Sanofi company, is focused on the discovery and development of Nanobodies® as therapeutics for a range of serious life-threatening human diseases. The company currently has more than 40 drugs in the pipeline and launched the first Nanobody®-based medicine in 2018: caplacizumab to treat aTTP, a rare blood clotting disorder. Ablynx’s successful track record convinced Sanofi to acquire the Ghent-based company in 2018 for 3.9 BE.

Biotalys explores the potential of VHHs (fittingly named Agrobodies®) to control plant diseases in agriculture. Crop protection products with Agrobodies® as formulation agents can be used at lower dosages, and have a longer lasting effect without repeat applications and thereby contribute to improved and more sustainable agricultural production.

Confo Therapeutics is building a portfolio of first-in-class programs based on its proprietary Confo® technology. This technology makes use of VHHs named ConfoBodies™ to stabilize G-protein coupled receptors (GPCRs) in a particular conformation of interest as a superior starting point for drug discovery. The company was nominated as ‘one of the Fierce 15 Biotech companies of 2019’.

Moreover, the VHH technology lies at the basis of four other VIB spin-offs: Orionis Biosciences (°2015), Oncurious (°2015), Animab (°2020) and ExeVir Bio (°2020).

Beyond startups, CSB has developed several enabling technologies. A great example of such technology is the ‘Megabody’ technology originating in Jan Steyaert’s lab. Such Megabodies are research tools that enable the high-resolution structure determination of proteins previously ‘intractable’ to cryo-EM, hence advancing structure-based drug discovery on these targets. In 2019, Confo Therapeutics signed an agreement with VIB for an exclusive, worldwide license to VIB’s ‘Megabody’ technology for applications on GPCRs.

In 2016, VIB concluded a deal with Oxford Nanopore Technologies, a company that uses biological nanopores to sequence DNA. Nanopore sequencing is a technology in which DNA strands are led through tiny pore proteins, called ‘nanopores’, to determine the genetic code of the strands. The structure of the nanopore protein significantly influences many features of this sequencing process.
While good scientists are specialists on the specific topic they work on, great scientists are able to survey a broader field of knowledge within their domain.

Backed by its research facilities and scientists, VIB offers a range of training courses in the life sciences for all (VIB and non-VIB) doctoral and postdoctoral researchers. The driving force for taking off now with this open cross-disciplinary training course is that in this 21st century it is no longer enough to stay in the lab and to stick to a specific discipline of interest to become a topnotch scientist. Success as a scientist increasingly hinges upon a combination of wet-lab activities with professional training in additional scientific and so-called parascientific disciplines. The availability of such broad training allows VIB scientists and students to develop into multi-disciplinary scholars.

To expose its students to cutting-edge research from across the broad field of structural biology, the VIB-VUB Center for Structural Biology contributes to the education of aspiring structural biologists that seek to unravel the mysteries of life and contribute to technological breakthroughs.

The center has a well-established tradition of teaching a wide range of courses on topics related to structural and molecular biology, as well as the theory and applications of bioinformatics. The following courses are taught in the context of the bioengineering, biology and chemistry curricula at the Vrije Universiteit Brussel: Biochemistry, Protein engineering, Molecular design.

OUTREACH

Science is not just for scientists. Quite the contrary, it is for everyone. To do their work, scientists often receive funds from governmental sources, which, in turn, derive from the public. Scientific researchers are well-aware of this money trail and most of them are eager to engage with the public to show what they are doing, and why it is worthwhile.

Beyond the technological and scientific developments and the benefits that flow from these, public outreach is another way for scientists to give back to the community and show their appreciation for the support they receive.

Many members of the VIB-VUB Center for Structural Biology engage in outreach events for the public at large, such as the Dag van de Wetenschap (day of science), Biotechdag (biotechnology day), etc. Several researchers of the center also take part in the education project from VIB called Wetenschap op stap (science on the move), where scientists visit primary schools to answer questions of the pupils and convey enthusiasm for science. This project is one of the STEM initiatives to encourage youngsters to choose an education in one of the STEM disciplines.
“The VIB-VUB Center for Structural Biology provided a unique environment to develop my scientific and soft skills. Thanks to its excellent facilities, state-of-the-art techniques and a great collaborative network, this center fostered an exceptional atmosphere to do research.”

Brandan Pedre - Postdoctoral fellow, German Cancer Research Center, Heidelberg, Germany

“As an early-career postdoc, I was supported by many opportunities for skill development and academic growth at the the VIB-VUB Center for Structural Biology. Much of my present ability to effectively communicate my research findings stems from experience I gained at one of VIB’s many free training courses. Finally, the umbrella of VIB serves to strengthen connections between researchers at CSB making for a socially healthy working environment”

David Young - Postdoctoral Training Fellow, Molecular Structure of Cell Signalling Laboratory, The Francis Crick Institute, UK

“During my time at CSB I was very fortunate to witness the initiation and set up of the first cryo-EM facility in Belgium up-close. This facility is a great addition to the high-end research being performed at VIB. The CSB was a very inspiring scientific environment that made feedback, collaboration and networking very easy through organized internal and external seminars, team-building and social events. Despite the distance, I am still in close contact with a lot of former colleagues, who during my PhD were a great source of information, inspiration and mentorship”

Katrien Willegems - Postdoctoral researcher, University of British Columbia, Vancouver, Canada

“I am very grateful for the inspiring PhD research experience at the CSB. CSB provides an optimal and stimulating research environment. The unique combination of excellent facilities, support of specialists and the great team atmosphere, allowed me to pursue my own scientific questions and to develop as a researcher.”

An Vandemeulebroecke - Biochemistry Group leader, Roche Pharma Research and Early Development, Basel, Switzerland

“CSB has not only the state-of-the-art technologies but also an ensemble of world class experts in the field. It is an absolute heaven for any structural biologist. My time at CSB taught me to think and work as an independent researcher while maintaining close co-operations with others in the group at the same time”

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Basic research in life sciences is VIB's raison d'être. VIB is an independent research institute where some 1,500 top scientists from Belgium and abroad conduct pioneering basic research. As such, they are pushing the boundaries of what we know about molecular mechanisms and how they rule living organisms such as human beings, animals, plants and microorganisms.

Based on a close partnership with five Flemish universities – Ghent University, KU Leuven, University of Antwerp, Vrije Universiteit Brussel and Hasselt University – and supported by a solid funding program, VIB unites the expertise of all its collaborators and research groups in a single institute.

VIB's technology transfer activities translate research results into concrete benefits for society such as new diagnostics and therapies and agricultural innovations. These applications are often developed by young start-ups from VIB or through collaborations with other companies. This also leads to additional employment and bridges the gap between scientific research and entrepreneurship.

VIB also engages actively in the public debate on biotechnology by developing and disseminating a wide range of science-based information. More information is available at [www.vib.be](http://www.vib.be)