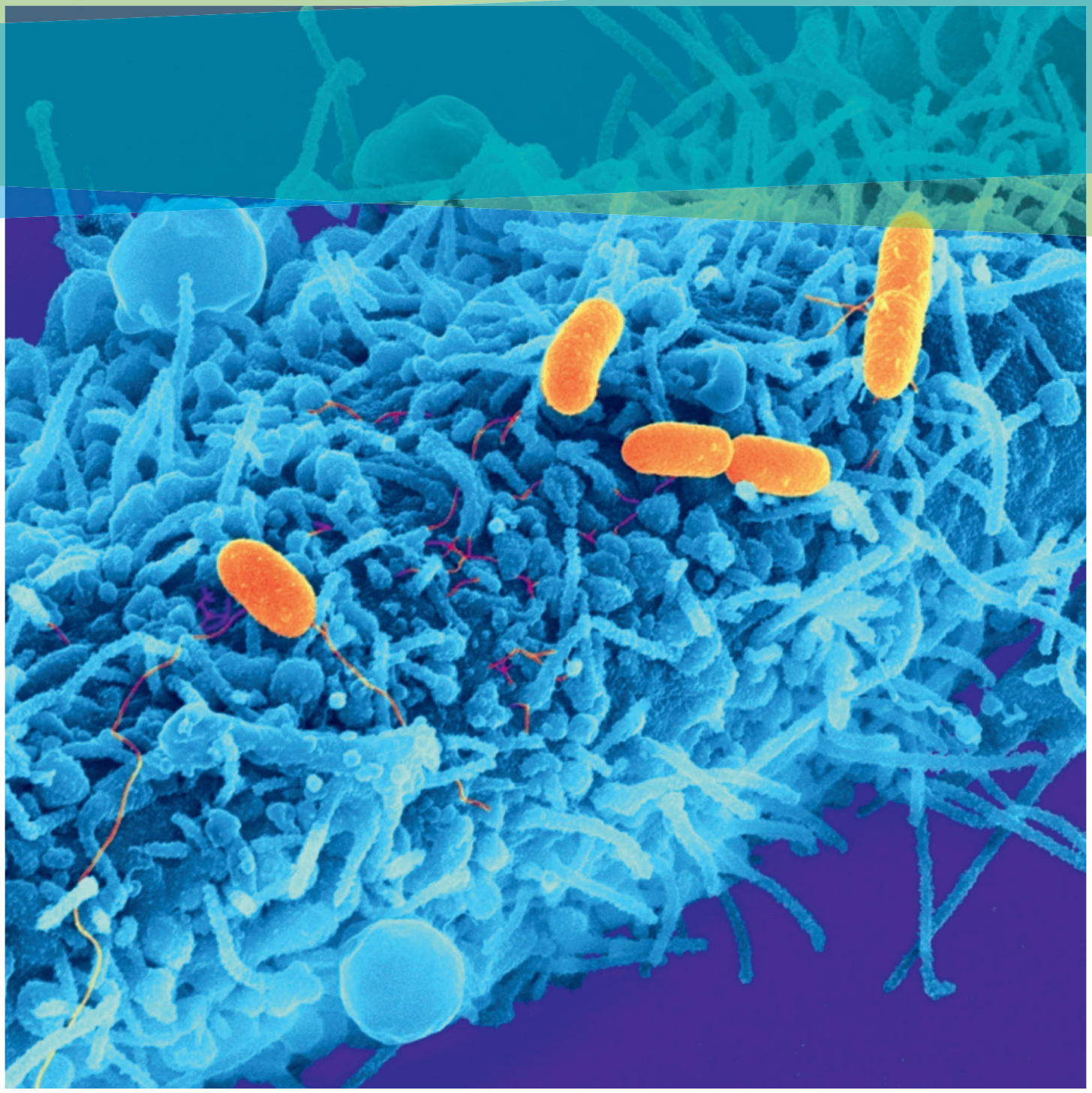
CSSB
Centre for Structural
Systems Biology

CSSB RESEARCH PROGRAMME



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EXECUTIVE SUMMARY

How does Salmonella manipulate host cells to cause disease? How do Plasmodia lead to Malaria? Why is tuberculosis a major cause of death in HIV-positive patients? What makes herpesviruses so 'successful' among human pathogens? These are key questions that touch on essential mechanisms in infection biology and medicine that must first be addressed by basic research before down-stream applications leading to novel and improved treatments for these infectious diseases can be developed.

The vision of the Centre for Structural Systems Biology (CSSB) is to develop a fundamental understanding of these mechanisms by investigating the molecular architecture of macromolecules from the host and pathogen as well as their functional interactions that trigger the infection process.

This knowledge is fundamental for developing new therapeutic strategies to combat these diseases. A cooperation of ten partner institutions, CSSB will use a novel approach which combines integrative structural biology with systems biology approaches to advance our understanding of the molecular mechanisms of some of the world's most widespread infections.

To achieve these goals, CSSB will make maximum use of the leading research infrastructures operated by the Deutsches Elektronen-Synchrotron DESY and the European X-ray Free Electron Laser. In parallel, CSSB will establish a state-of-the-art facility for electron cryo-microscopy. CSSB's ambition is to become an international leader by integrating these infrastructures with our in-house research expertise to address key questions regarding human health.

1. CSSB'S RESEARCH VISION

Obtaining a comprehensive and detailed understanding of the molecular interactions within living systems is both an overarching goal and one of the biggest challenges of basic biological research. To achieve this, the systematic analysis of the architecture of biological structures at all levels of resolution – ranging from molecules to cellular systems and entire organisms – is of fundamental importance. Structural biology and corresponding imaging approaches not only reveal the molecular details of proteins, nucleic acids, lipids and their functional complexes in living systems at atomic or near-atomic resolution but also help uncover the associated dynamics of these systems. This combination provides an unmatched research approach for unravelling the basic architecture and function of biological macromolecules.

Structural biology research strongly relies on access to appropriate research infrastructures, in particular on the use of highly collimated and intense X-ray beams. The Deutsches Elektronen-Synchrotron DESY in Hamburg, with facilities such as the PETRA III synchrotron storage ring and its contribution to the European X-ray Free Electron Laser, is a world-leading provider of such infrastructures and is consequently an ideal location for conducting cutting-edge research in structural biology.

The establishment of a life sciences research centre on the Hamburg-Bahrenfeld Research Campus will enable the optimal usage of these research infrastructures. The **Centre for Structural Systems Biology (CSSB)** will implement a state-of-the-art electron cryo-microscopy research facility, which complements the X-ray-based research infrastructures available on-site, thus establishing an integrated concept for structural biology at all scales of resolution and complexity **(Figure 1)**. CSSB's research focus is infection biology. CSSB aims to discover the structure and function of viral, bacterial and parasitic pathogens and to understand their interactions with the infected host by investigating the underlying processes of infection. This research theme ultimately aspires to improve the health of our global society by combating both existing and emerging infections with novel therapy concepts. The development of new interventions to combat infection is not possible without a profound understanding of the highly diverse molecular mechanisms of infections.

CSSB is presently a cooperation of ten research partners from Northern Germany including three universities and seven research institutions – three Helmholtz centres, three Leibniz institutes and the European Molecular Biology Laboratory. Several of these partners already have an internationally leading research track record in infection biology and medicine, which will be reinforced by their CSSB partnership.

CSSB partner institutions have already recruited leading experts **(Figure 2)** – the majority of whom have a research focus in either viral, bacterial or parasite infection biology. The recruited scientists have specific technical expertise in structural biology approaches such as X-ray/XFEL crystallography or electron cryo-microscopy. One of CSSB's future objectives is to further strengthen competencies in key complementary approaches such as computational modelling, systems biology, chemical biology, cell biology and genetics, as well as experimental '-omics', by both acquiring additional, strategic recruitments from partner institutions and by targeted cooperation with partners.

FIGURE 1: CSSB'S CONCEPT OF INTEGRATED STRUCTURAL BIOLOGY

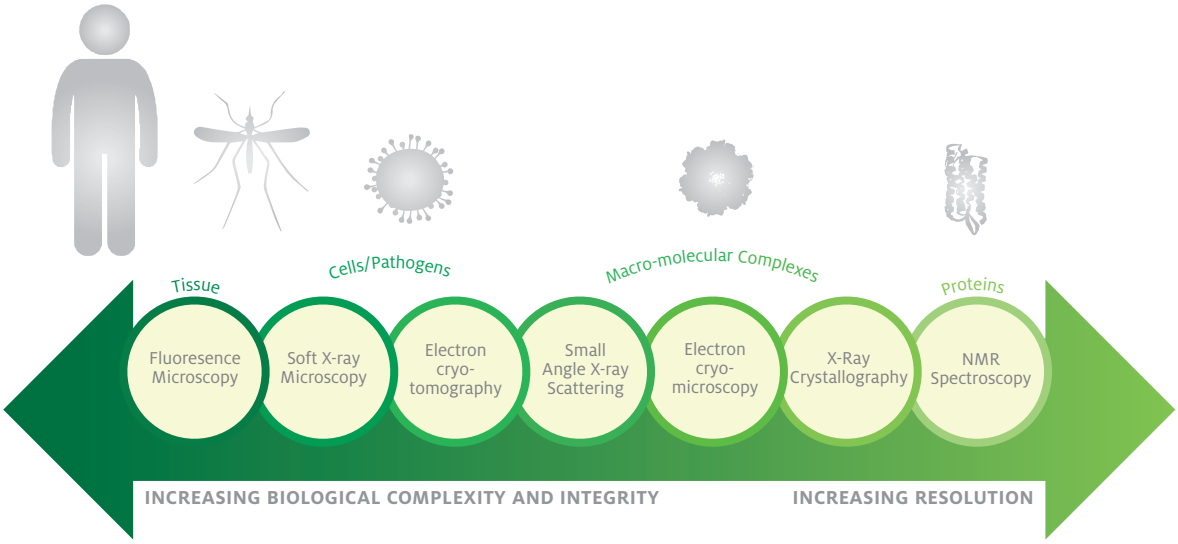


FIGURE 2: PRESENT CSSB PRINCIPAL INVESTIGATORS AND THEIR CSSB PARTNER HOME INSTITUTIONS

CSSB actively supports young independent researchers by providing them with attractive early-stage career opportunities. To accomplish this, CSSB has established a *Research Hotel* which provides young principle investigators with their own lab space as well as full integration into the CSSB environment, typically for a period of five years. The CSSB Research Hotel is open to scientists irrespective of CSSB affiliation and will therefore serve as an open gate between CSSB and international research institutions. Groups for the Research Hotel will be recruited based on their scientific excellence, scientific track record and future potential and will strategically fill existing gaps in target research areas and specific technical approaches.

A new building on the Hamburg-Bahrenfeld Research Campus, next to the synchrotron radiation beamlines at PETRA III, will enable all CSSB research groups to work together under one roof **(Figure 3)**. The CSSB building will be integrated into the 'science village' on the Hamburg-Bahrenfeld Research Campus **(Figure 4)** comprised of several different research centres such as the Center for Free Electron Laser Sciences (CFEL), Hamburg Advanced Research Centre for Bioorganic Chemistry (HARBOR), the Center for Hybrid Nanostructures (CHyN) and the Hamburg Unit of the European Molecular Biology Laboratory (EMBL) as well as the new Max Planck Institute for the Structure and Dynamics of Matter.

The four-story CSSB building will host more than 3000m² of laboratory space, at S2 biosafety level or higher; twenty percent of which is reserved for the CSSB Research Hotel. The building will provide space for up to 180 researchers and was designed to foster research collaboration and integration at all levels, thus supporting CSSB's vision of fostering joint research endeavours. A lecture hall for 180 individuals will allow CSSB to host international conferences. Larger meeting rooms are available for courses and smaller workshops and the communications areas are ideal for informal meetings. Dedicated research core facilities and areas for junior research groups will be associated with the laboratories of senior principal investigators to ensure efficient mentoring and the transfer of relevant research expertise. The basement of the CSSB building will contain a state-of-the-art electron cryo-microscopy and tomography facility. The construction of the building is in an advanced state and the building is expected to move into full operation in early 2017.

The CSSB Directorate is the centre's decision making body and is comprised of senior principal investigators appointed by the partner institutions. The Directorate meets every six weeks to make decisions in relation to CSSB's scientific strategy and overall operation. A *Scientific Advisory Board* (SAB) comprised of internationally recognised experts supports the CSSB Directorate by advising on its overall scientific strategy. The performance of CSSB's central leadership, individual principal investigators and scientific core facilities will be reviewed by an independent panel of experts every four years.

FIGURE 3: CSSB BUILDING CONCEPT: FOSTERING RESEARCH INTEGRATION AND COOPERATION

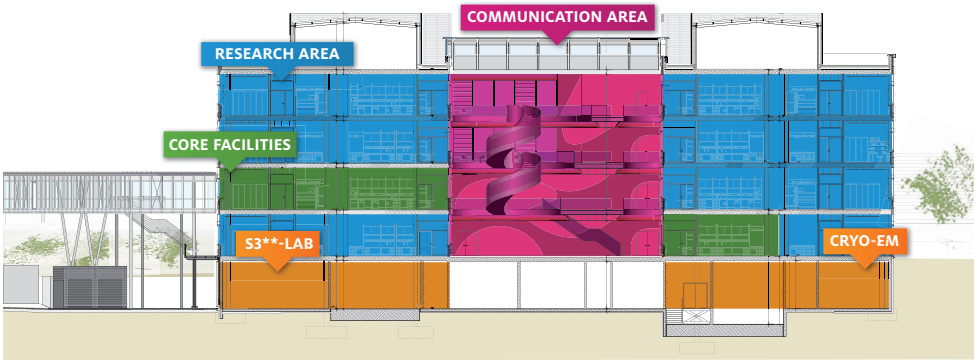
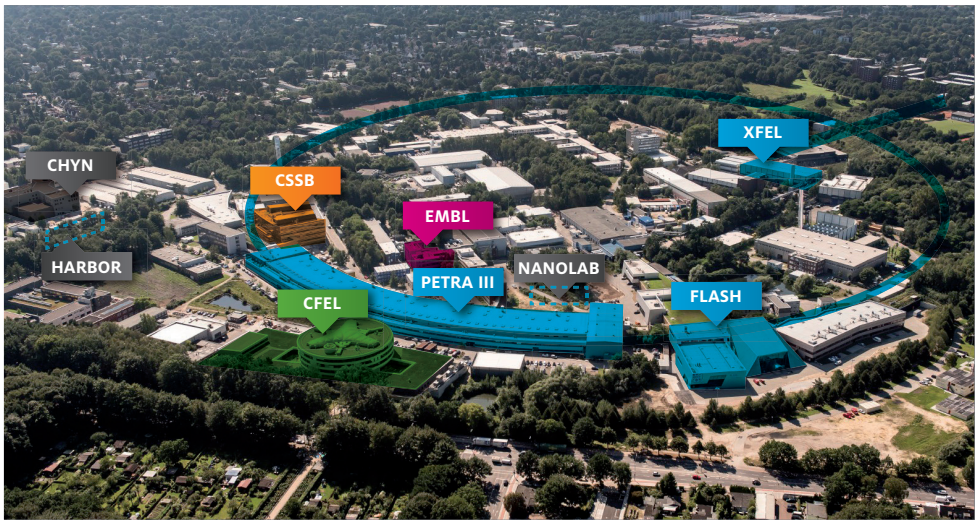


FIGURE 4: HAMBURG-BAHRENFELD RESEARCH CAMPUS / RESEARCH CENTRES



Overview of on-campus research infrastructures (blue), centres (green) and partners (magenta) relevant to CSSB (orange) research activities. Further on-campus research centres are also indicated (grey).

2. CSSB'S MECHANISTIC PERSPECTIVE OF THE INFECTION PROCESS

Responsible for 25% of global deaths, infectious diseases have a significant impact on society. Given the increasing antibiotic resistance of specific pathogens and the absence of effective and affordable vaccines for key diseases, infections are still considered a major threat to human health. Infection biology research addresses the huge challenges to human health posed by new pandemic outbreaks and persistent diseases such as malaria and tuberculosis as well as emerging infections and neglected tropical diseases.

Infectious disease research is a multifaceted and composite field encompassing various disciplines including epidemiology, clinical and basic research. However, insufficient knowledge of the molecular mechanisms underlying most infections presents a roadblock to the development of effective therapies. Molecular, structural and functional analysis, that uncovers and manipulates infection mechanisms, is essential for ensuring a rapid and effective response to current and future epidemics. CSSB is one of the first centres primarily focused on uncovering the molecular properties and ingenuities of infection mechanisms using a combined structural and functional approach.

Research at CSSB focuses on understanding the molecular interplay of the host and the invading pathogen. Examples include the investigation of the molecular architecture and underlying biophysical principles of bacterial secretion systems, infection by viruses such as Herpes simplex and HIV-1 as well as infection by other pathogens such as the malaria parasite. By providing insights into these processes, CSSB will help decode mechanisms of pathogenesis that may translate into molecular targets for novel intervention strategies.

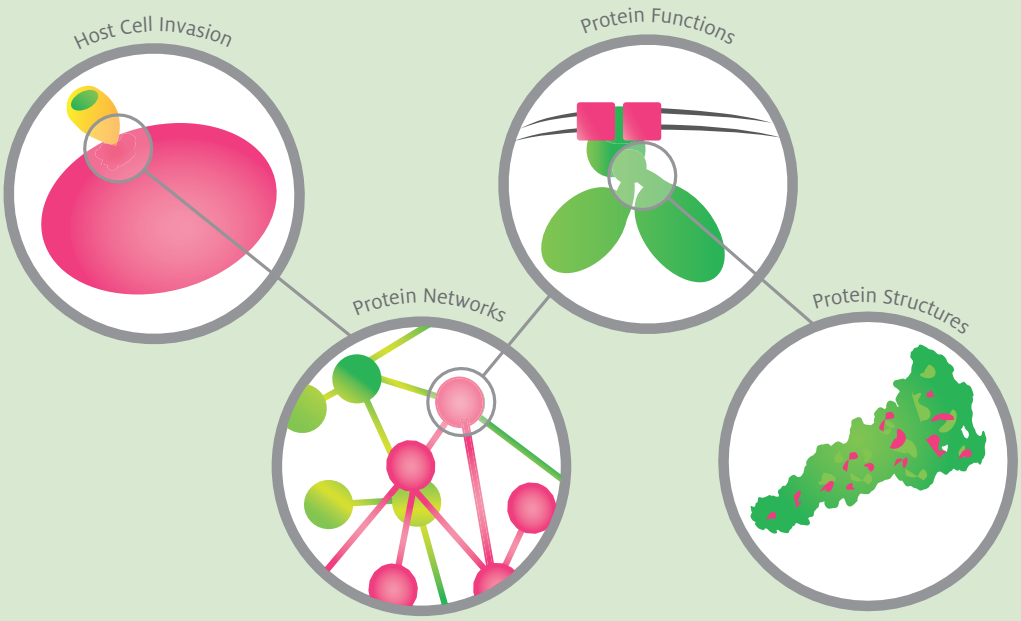
BOX 1: MOLECULAR MECHANISMS OF MALARIA INFECTION

Malaria represents a major global health burden, affecting an estimated 500 million people and resulting in over 500,000 deaths each year. It is caused by infection with the intracellular parasite *Plasmodium*, a unicellular pathogen transmitted by *Anopheles mosquitoes*. In spite of great advances in molecular medicine, a vaccine is not available and resistance to antimalarial drugs is widespread.

One of the key processes in the pathobiology of the malaria parasite is the invasion and subsequent modification of its specific host cell, the human erythrocyte. Understanding which and how proteins of the parasite operate in the context of invasion is critical to drive the next generation of vaccine and drug development to combat this devastating parasite.

CSSB scientists – in collaboration with research groups from the University of Toronto and the Sick Kids Research Institute (Toronto, Canada) – aim to dissect the molecular interactions occurring in the "invadome", the protein network responsible for red blood cell invasion. The integrated strategy will make use of complementary technical platforms and will combine systems biology, molecular and structural approaches. It includes a global screen for protein complexes in the malaria parasite, with the aim of capturing detailed physical interactions within and between the protein networks that are responsible for driving host cell invasion.

HOST CELL INVASION FROM CELLULAR TO MOLECULAR LEVEL



From left to right: (1) erythrocyte invasion by the malaria parasite (yellow) revealed a fast multi-step process. (2) It can be analysed using systems biology approaches that implicate a protein network of more than 400 parasite proteins. The precise function of individual members of the network can be elucidated using molecular (3) and structural (4) biology.

3. CSSB'S STRUCTURAL SYSTEMS BIOLOGY APPROACH TO INFECTION

The structural systems biology approach promoted by CSSB aims to reveal the structures and interactions of biological molecules in their native functional environment primarily covering a range from the macromolecular to cellular level. This encompasses capturing the dynamics of these structures – namely changes in composition, arrangement and conformation – over time. Within the context of infection biology, this specifically includes the dynamics of the molecular processes that lead to infection.

Structural systems biology will be implemented at CSSB by taking advantage of recent advancements in X-ray-based crystallography and electron cryo-microscopy. Together, these approaches extend the scope of high-resolution structural analysis to include very large protein complexes which are of particular interest when analysing the highly dynamic interactions between pathogens and their host. In addition, research at CSSB builds on the recent progress made in the assimilation and integration of large scale datasets arising from genomic, transcriptomic, proteomic and metabolomic analyses. By combining these results, CSSB's structural systems biology approach to infection biology enables a comprehensive mechanistic understanding of pathogenesis and infection at the atomic, cellular and multi-cellular level.

CSSB's research concept expands beyond traditional structural biology and aims to map the structural data of living systems and incorporate them into the spatiotemporal context of the cell or compartment, thus measuring and modelling/predicting its functional readout within the system. Conversely, such analyses aim to assess the impact of the system on the structures of specific proteins or protein complexes. These goals require the integration of vastly different types of data and the combination of structural biology approaches with experimental -omics and computational techniques used in structural modelling and systems biology. The interdisciplinary nature of CSSB provides an ideal setting to spearhead this research and to pursue ambitious structural systems biology projects.

Research in infection biology provides ideal systems of different complexities – the infecting pathogen, changes of the host cell and its compartments upon infection as well as the host/pathogen interactome during infection – that can be addressed by this approach and also have a high clinical relevance. A diverse range of interactions, such as the mimicking of endogenous host interactions by the pathogen and the controlled secretion/internalisation of specific molecules by both the pathogen and the host, play a central role in the infection process. Structural systems biology at CSSB holds the power to study these interactions mechanistically at all levels of structural complexity and to uncover the dynamics of molecules and protein assemblies in terms of their composition, arrangement and strength.

Finally, large parts of the proteomes of many pathogens have not yet been fully explored. Structural systems biology holds tremendous potential to identify the function of target proteins by designing structure-based protein variants that can be probed by systems biology approaches using experimental -omics methods or various forms of imaging. The resulting data could then be used to predict the role of a protein in complete biological systems. Overall, structural systems biology, as pursued at CSSB, is an attractive approach to quantitatively analyse and display the structure and function of molecular interactions between pathogens and their hosts, thus providing a holistic understanding of the mechanisms governing infection.

BOX 2: VESICLE COAT FORMATION AT THE INNER NUCLEAR MEMBRANE IN HERPESVIRUS NUCLEAR EGRESS DECIPHERED BY AN INTEGRATED STRUCTURAL CELL BIOLOGY APPROACH

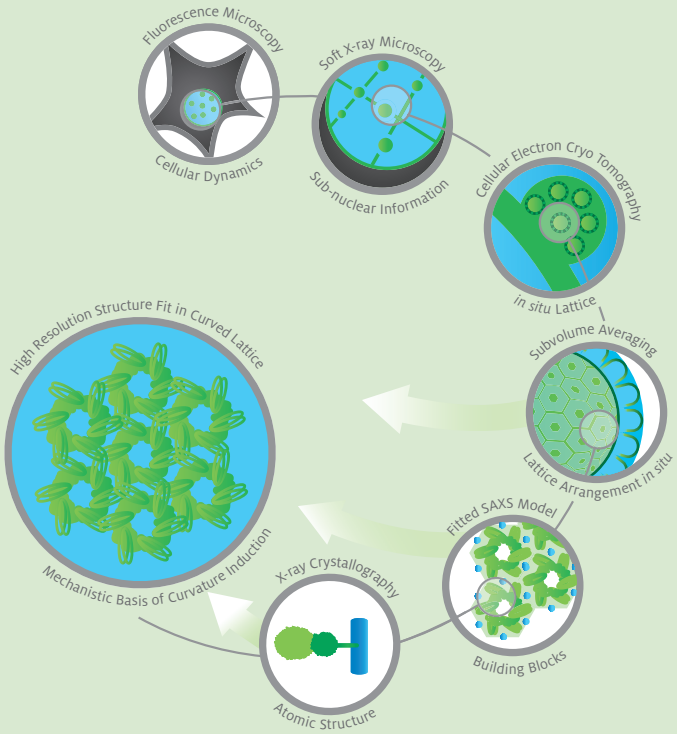
Herpesviruses are masters in hijacking cellular processes and have evolved a complex cellular 'life cycle'. Herpesvirus morphogenesis starts in the host cell nucleus where the viral DNA is replicated and packaged into newly assembled capsids. To exit the nucleus, capsids need to overcome the double membrane of the nuclear envelope. Herpesvirus capsids exit the nucleus in a two-step process via a unique vesicular pathway. First, capsids are enveloped at the inner nuclear membrane (INM). The resulting vesicles then fuse with the outer nuclear envelope and release their capsid cargoes into the cytosol.

Vesicle formation involves recruitment of specific coat proteins that function to locally deform the membrane. While vesiculation into and away from the cytosol is rather well characterised, the basis of vesicle formation at the inner nuclear envelope remained elusive. Previous studies had shown that the process is mediated by two conserved viral proteins pUL31 and pUL34 forming the heterodimeric nuclear egress complex (NEC).

Using an integrated structural biology approach (Figure 1) the Grünewald group and its partners from northern Germany, Tübingen, Princeton and Oxford revealed the structural underpinning for the nuclear membrane remodelling and this trafficking (Hagen et al., 2015, Cell 163: 1692–1701; Zeev-Ben-Mordehai et al., 2015, Cell Reports 13: 2645–2652). The in situ architecture of the NEC was characterised by applying an integrated multi-modal imaging approach covering variable scales and resolutions.

Integrative modelling combining information from live cell and different cryo-imaging modalities with small-angle X-ray scattering then allowed determination of the NEC coat lattice architecture that ensures a defined membrane curvature. Moreover, solving the crystal structure of the NEC heterodimer and fitting this into the curved *in situ* lattice allowed for establishing the mechanistic basis for budding of tailored vesicles at the INM.

STRUCTURAL BASIS OF HERPESVIRUS NUCLEAR EGRESS

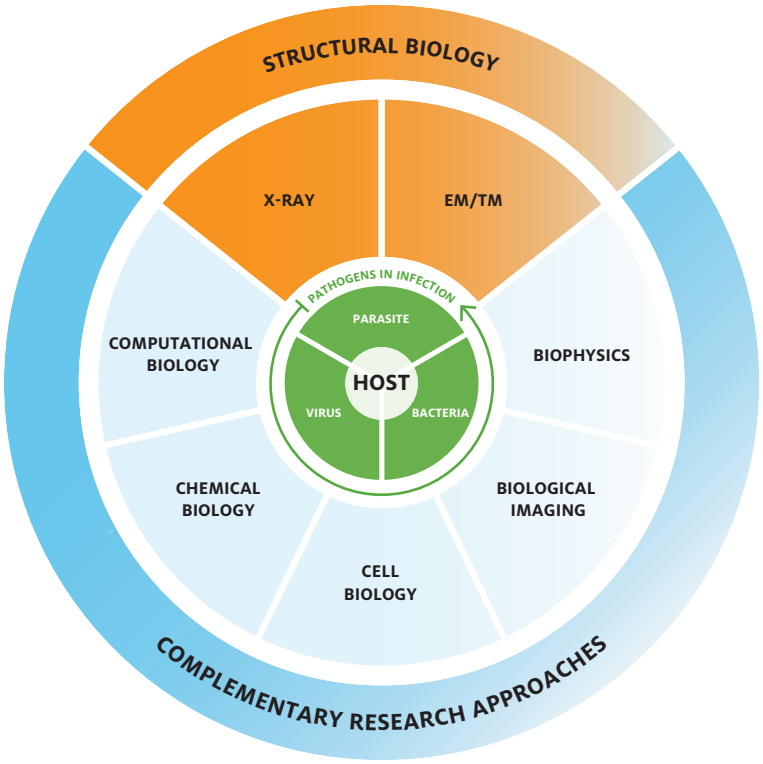


Mechanism of membrane remodelling of the inner nuclear envelope analysed using an integrated structural biology approach (individual approaches and insights indicated) bridging from the cell (dark grey in top circle) to sub-nuclear and atomic levels.

4. CSSB'S CORE COMPETENCE IN STRUCTURAL BIOLOGY

CSSB's core research competence is integrated structural biology and imaging **(Figure 5)**. This enables CSSB to address important research questions in infection biology, at various levels of complexity and multiple scales of resolution. CSSB researchers will have access to key technologies such as X-ray-based methods, electron cryo-microscopy and various light microscopy techniques. Some of these facilities will be hosted and operated by CSSB scientists. CSSB will enable the most efficient use of these technologies by expanding their overall capabilities through the development of integrative structural biology approaches. These will also include other techniques such as NMR spectroscopy, small angle X-ray scattering, various biophysical methods and structure-based mass spectrometry available at CSSB partner institutions.

FIGURE 5: CSSB CORE COMPETENCE AND COMPLEMENTARY RESEARCH APPROACHES



A. STRUCTURAL BIOLOGY

A.1 X-RAY CRYSTALLOGRAPHY

CORE COMPETENCE: M. KOLBE (HZI/UHH), J. LABAHN (FZJ/HHU), C. LÖW (EMBL)

X-ray crystallography remains a very powerful and versatile approach in structural biology that enables structural determination at the resolution of a single atom. When insight into atomic structures is required – for an active site, protein/inhibitor interactions, or protein/protein interfaces – X-ray crystallography is often the first method of choice. Several technological advances such as the availability of very purified samples using sophisticated eukaryotic expression systems, which have improved chances of crystallisation; the optimisation of automated pipelines from crystallisation to X-ray data collection; and the building of more powerful X-ray facilities (synchrotrons, free electron lasers) have resulted in the expansion of the approach. X-ray crystallography is now routinely applicable for high-resolution structure determination of up to Mega Dalton protein complexes, from very small crystals with micrometre to sub-micrometre dimensions. In the context of CSSB's research vision, X-ray crystallography provides excellent opportunities for the investigation of structural dynamics by applying rapid kinetics techniques.

Implementation: Several CSSB principal investigators have an expert knowledge of X-ray crystallography and are thus able to approach a wide range of challenges including the structural determination of integral membrane proteins and complicated protein/protein complexes. The work of CSSB principal investigators will be facilitated by direct access to advanced infrastructure and experimental beamlines at the PETRA III synchrotron storage ring and the European X-ray Free Electron Laser in Hamburg/Schenefeld. CSSB scientists will therefore have all the necessary technology and infrastructure available on-site to determine in a highly efficient manner significant 3D structures in the field of host/pathogen interactions.

A.2 ELECTRON CRYO-MICROSCOPY

CORE COMPETENCE: K. GRÜNEWALD (HPI/UHH), T. MARLOVITS (UKE/DESY)

Electron microscopy has been used to visualise the ultrastructure of cells for many decades. The development of new microscope designs, novel detectors, sophisticated image data processing software and ultrastructure-preserving sample vitrification techniques has enabled electron cryo-microscopy to become a highly attractive approach for resolving the structure and function of large macromolecular complexes to near atomic resolution, in a near-native or cellular environment. Using single particle and cryo-tomographic techniques, electron cryo-microscopy seamlessly unites and integrates classical high-resolution structural biology techniques with low-resolution cell biology approaches. Moreover, electron cryo-microscopy is able to capture the structural plasticity of macromolecular complexes in unprecedented detail which often provides important clues about their function.

Implementation: The electron cryo-microscopy research infrastructures within CSSB will be a novel addition to the Hamburg-Bahrenfeld Research Campus complementing existing X-ray-based research infrastructures. The design, layout and localisation of instruments for the new electron cryo-microscopy infrastructure is part of the overall CSSB building concept and serves to both optimise the infrastructure's technical performance and enhance its integration with other research approaches. Two senior CSSB principle investigators are leading experts and active developers in both single particle electron cryo-microscopy and electron cryo-tomography. The available infrastructure is expected to stimulate both thematic and technical collaborations with other CSSB principle investigators as well as other scientists on and near the Hamburg-Bahrenfeld Research Campus.

B. COMPLEMENTARY APPROACHES

B.1 CELL BIOLOGY

CORE COMPETENCE: T. GILBERGER (BNITM/UHH) , K. GRÜNEWALD (HPI/UHH)

Pathogens master the ability to communicate and interact with their host in a manner that not only leads to the host's colonisation but is also responsible for the onset and progression of disease. Although diverse in nature, pathogens often rely on an initial adhesion to the host cells for proliferation before manipulating cellular processes such as membrane trafficking or cytoskeletal architecture. Investigations of these diverse host-pathogen interactions rely on the use of experimental approaches that allow for the cultivation, manipulation and phenotypic dissection of the pathogen, either *in vitro, in situ* or *in vivo* using animal models. Methods include subcellular and cellular systems, genetic engineering and chemogenetics in conjunction with a broad range of phenotypic analysis tools such as imaging, 'omics and flow cytometry. The drastic expansion of the cell biology tool box to include CRISPR/CAS9 mediated genome editing, single cell transriptomics, optogenetics and humanised mouse models strengthens the central role of this approach in infectious disease research.

Implementation: The exploration of different pathogens requires custom-tailored cell biology approaches for pathogen propagation and manipulation. CSSB principle investigators are running dedicated Biosafety Level 2 facilities for the efficient and safe handling of different pathogens which enables experimental questions about a pathogen's biology and pathogenesis to be addressed. In the future, CSSB has the capacity for a Biosafety Level 3 laboratory which would enable investigators to work with pathogens that require a higher level of containment, like SARS and West Nile virus. CSSB has recruited two principal investigators with competence in this field and would like to further expand and diversify such expertise.

B.2 BIOLOGICAL IMAGING

CORE COMPETENCE: T. GILBERGER (BNITM/UHH), K. GRÜNEWALD (HPI/UHH)

Microscopy is an integral method for studying the function of virtually any cellular and macromolecular process. It provides quantitative data to determine how cellular organisation is related to its function. Advanced microscopy can provide molecular resolution and real-time and longitudinal information about complex biological processes at various levels, ranging from single cells, cell-cell contacts to entire tissues.

Most CSSB principle investigators require both light- and electron-based microscopy for their research. Fluorescent light-based imaging techniques offer powerful tools for multidimensional imaging *in vivo*, allowing the visualisation and quantification of biological events within their physiological environment through both time and space. In addition, advanced microscopy techniques with excellent time resolution such as 4D imaging and multiphoton microscopy will enable the examination of the dynamics of biological processes from a cellular perspective.

Combined and correlative imaging methods provide excellent tools to study biological processes and mechanisms across a wide scale of resolutions from the millimetre to the nanometre range. Correlative light and electron microscopy (CLEM) bridges the gap between live-cell imaging and electron microscopy (EM). This type of dual examination by imaging provides crucial information about cellular processes in the infection cycle.

Implementation: CSSB will focus on the development of correlative techniques for cryo-preserved specimens, in combination with other imaging approaches such as soft X-ray cryo-microscopy and super-resolution cryo-microscopy. For infection biology, these techniques will help bridge the resolution gap to electron cryo-microscopy as viral pathogens typically present objects that are smaller than amenable for diffraction limited light microscopy. State-of-the art instruments for advanced imaging will be offered via an in-house CSSB core facility.

B.3 BIOPHYSICS AND BIOCHEMISTRY

CORE COMPETENCE: K. GRÜNEWALD (HPI/UHH), M. KOLBE (HZI/UHH), J. LABAHN (FZJ/HHU), C. LÖW (EMBL), T. MARLOVITS (UKE/DESY)

Biophysical approaches are powerful tools to connect different high-resolution techniques within integrated structural biology and allow the independent validation and analysis of the biological significance of new structures. These approaches are essential to tackle major challenges such as determining the structure of large protein complexes that could be either soluble, membrane associated or membrane integrated and may only be partially folded. Key applications include technical cross-validation and the exploration of conformational space, both of which are often unattainable via high-resolution structural biology methods due to conformational flexibility or lack of folding of protein samples. Biophysical approaches are also essential prior to high-resolution structural biology experiments as a means of proper sample characterisation and quality control, thus ensuring the highest possible success rate and economical usage of high-end structural biology techniques.

For this purpose, various scattering techniques, such as small/wide angle X-ray and neutron scattering, static light scattering and dynamic light scattering as well as spectroscopic approaches such as circular dichroism, Raman and extended X-ray absorption fine structure are widely used. A second category of biophysical approaches exploits various non-structural properties that are generally used for sample purification and characterisation such as gel and chromatography separation methods and mass spectrometry. A third category allows for the assessment of binding affinities of protein complexes using techniques that are capable of measuring interactions quantitatively such as isothermal microcalorimetry, surface plasmon resonance, various types of fluorescence spectroscopy and thermophoresis.

Implementation: Several CSSB principal investigators have an excellent level of expertise in biophysical techniques. EMBL operates a dedicated Sample Protein Characterisation facility in the PETRA III experiment hall, next to CSSB. Within CSSB, the provision of appropriate instruments for biophysical experiments will play a crucial role in establishing a leading high-resolution structural biology research environment. Instruments and equipment will be offered centrally as part of CSSB's core facility concept. State-of-the-art SAXS beamline facilities will be offered via access to the PETRA III beamline P12, next to the CSSB building.

B.4 COMPUTATIONAL BIOLOGY

Many of the ground-breaking advances in biology have been enabled by the exponential growth in computing power and the simultaneous increase in the availability of computational algorithms, tools and resources. Computational approaches not only facilitate and accelerate analytical procedures, such as solving the structure of a protein or protein complex, but also increase the pace of experimental data acquisition and facilitate the generation of quantitative models with high predictive accuracy.

High-resolution structural biology has an unmatched power to predict structural models of homologous systems at various levels, including secondary structure, tertiary structure and quaternary architecture. Substrate specificities in enzymes, small molecule docking sites, folded/unfolded regions, binding motifs and protein interfaces can be predicted using computational approaches. Predictive approaches, such as energy minimisation, molecular dynamics or quantum chemistry techniques require adequate algorithms and highly demanding levels of computational power, data storage and data transfer. Searching large databases, as used in ligand docking approaches for instance, could generate additional computational requirements.

Systems biology-based experimental approaches that make use of structural biology techniques also require computational modelling approaches to develop a comprehensive understanding of biological systems. The information from fully sequenced genomes can, for example, be used to more accurately model homologous structures. Proteomics or metabolomics data can help to corroborate function with structure-based data.

Implementation: The establishment of computational approaches for the modelling of systems biology "omics" data is still in its infancy at CSSB and will therefore become a major focus of future recruitments. Some CSSB partner institutes such as DESY, EMBL, FZJ and HZI have leading records in using computational biology approaches and own state-of-the-art infrastructures, which will provide excellent leverage in creating a high-level CSSB research portfolio in structure-oriented computational approaches.

B.5 CHEMICAL BIOLOGY

Chemical biology includes the synthesis of non-natural chemical compounds to both interrogate and manipulate biology. Creating effective chemical compounds for infection biology purposes is particularly challenging as the compounds must not only be soluble and stabile under physiological conditions but must also travel across complex membranes and cell walls to accurately reach the target site of infection. Once compounds reach the proper target site, they can sense, report or alter the state and activity of a specific macromolecular target-structure and unlike most other approaches their effects are often reversible. Chemical biology approaches can also provide precise tools to alter the activity of specific components in the context of intact systems.

Pharmacological intervention for the treatment of human diseases is a natural extension of chemical biology, as both share the use of small molecules to manipulate biological activity as well as methods for drug discovery and optimisation. Given the strong medical research focus of several CSSB partner institutions, the development of a strong chemical biology portfolio within CSSB could foster follow-up clinical applications and allow for the effective exploitation of CSSB's fundamental and mechanistic research results.

Implementation: There are currently no CSSB principal investigators with expertise in chemical biology. However, this expertise does exist within several partner institutions and it is planned to make this accessible to CSSB via the establishment of a strategic partnership with HARBOR and potentially via the associated membership of selected principal investigators from partner institutions.

BOX 3: CHEMICAL BIOLOGY: STRUCTURE-BASED DEVELOPMENT OF DEOXHYPUSINE SYNTHASE INHIBITORS TARGETING A CELLULAR FACTOR USED FOR HIV-1 REPLICATION

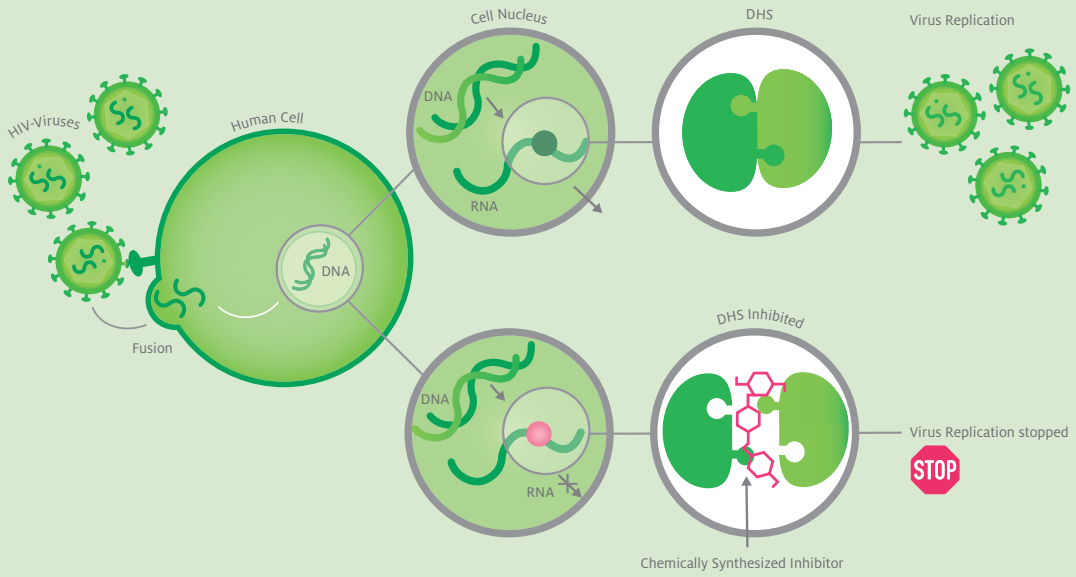
Great efforts have been made to treat HIV infections. The introduction of the combination antiretroviral therapy (cART) in the mid '90s, by which viral enzymes are targeted, was an important improvement. However, the occurrence of drug resistance and long-term side effects requires the search for new targets and novel drugs.

Cellular cofactors play important roles in the viral replication cycle. An example is the eukaryotic initiation factor 5A (eIF-5A), which is involved in the transport of unspliced viral mRNAs from the nucleus. A unique post-translational modification of a lysine residue to the amino acid hypusine is essential for the maturation of eIF-5A. Two cellular enzymes are involved in this process: the deoxyhypusine synthase (DHS) and the deoxyhypusine hydroxylase (DOHH). Both enzymes are, therefore, potential drug targets.

Compounds like GC-7 and CNI-1493, although unspecific, efficiently inhibit DHS, preventing the maturation of eIF-5A and thereby inhibiting HIV replication. Moreover, a crystal structure analysis showed that GC-7 binds to the active site of a tetrameric DHS cluster. However, CNI-1493 cannot have the same binding modes due to its size compared to GC-7, which is a low molecular weight inhibitor. A structure-based drug design approach for the discovery of novel, active site DHS inhibitors, was conducted in a collaborative approach involving scientists from the fields of bioinformatics, chemistry, virology and structural biology at different institutions in Hamburg. As a result, new inhibitors were discovered with marked inhibitory activity against DHS. Co-crystallisation studies of these new inhibitors with DHS are underway in collaboration with EMBL scientists. These studies seek to obtain more insights into their binding mode and to develop a structure-activity relationship.

In addition to understanding the mechanism of action, the project also hunts for inhibitors that may be used in clinics in the future, thus giving the project a translational aspect.

BINDING OF A CHEMICALLY SYNTHESIZED INHIBITOR TO THE ENZYME DHS



Inhibition of the viral RNA export by the deoxyhypusins-synthase (DHS) caused by chemically synthesized small molecules as a new target to combat HIV infections.

C. COLLABORATIVE, INTERDISCIPLINARY RESEARCH AT CSSB

The multi-institutional structure **(Figure 6)**, the breadth of available expertise and methodologies, and the innovative Research Hotel concept make CSSB an ideal platform for scientific exchange and a perfect incubator for interdisciplinary, collaborative work. The concentration of research activities in the new CSSB building and the implementation of a communication concept driven by internal openness will greatly enhance opportunities for collaboration. This is further supported by a recently implemented, central CSSB funding scheme entitled the Cooperation and Integration Fund that allows CSSB scientific leadership to provide financial incentives for targeted initiatives. This funding scheme will support endeavours that not only foster the integration of methods and insights from different disciplines, but also further our holistic understanding of host-pathogen systems.

CSSB has started to implement a collaboration-driven research concept at various levels. For instance, all CSSB principle investigators meet twice a year for a two-day retreat to exchange ideas regarding current research projects and to brainstorm regarding future project ideas and opportunities for collaboration. Several bottom-up collaborations have already emerged from this process **(Supplementary Table 1: Ongoing Collaborative Projects at CSSB)**. CSSB is very grateful to the Joachim Herz Stiftung for supporting our vision by contributing funds to three different collaborative research projects led by CSSB principal investigators **(see Box 4 for an example)**. CSSB endeavours to establish similar relationships with other funding bodies from both the public and private sector.

Other collaborative, multi-partner research initiatives include proposals to either support a CSSB-coordinated graduate school or to support CSSB's participation in an existing, on-site graduate school on the Hamburg-Bahrenfeld Research Campus. In parallel, several CSSB principle investigators are involved in planned national research excellence initiatives in the three CSSB-supporting states of Hamburg, Lower Saxony and Schleswig-Holstein, reflecting CSSB's emerging significance as a strategic research partner. Prominent engagement in these initiatives is seen as an important step towards our aspiration to establish CSSB as leader for future large-scale funding initiatives.

FIGURE 6: CSSB PARTNER COLLABORATION

BOX 4: UNDERSTANDING THE STRUCTURE AND FUNCTION OF THE TYPE III SECRETION SYSTEM

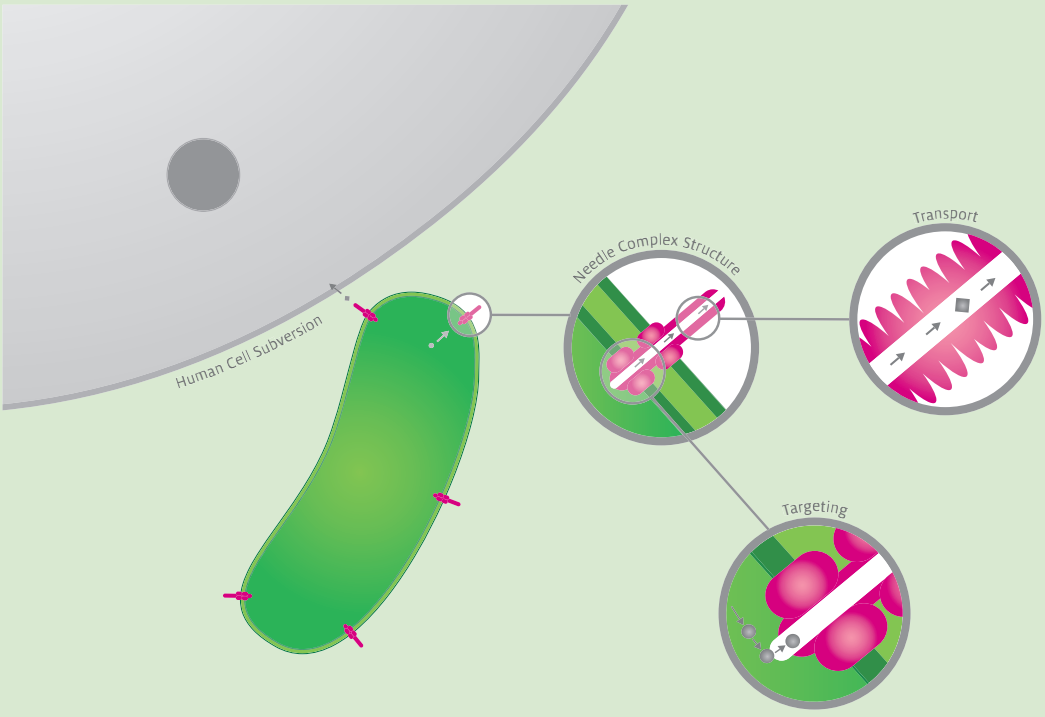
Gram-negative bacteria, including *Shigella*, *Salmonella* and *Yersinia* species, infect hundreds of millions of people worldwide and cause millions of deaths every year. A central structure in bacterial virulence is the Type III Secretion System (T3SS), which transports pathogenic bacterial proteins into the eukaryotic host cells to initiate infection. This system, highly conserved among bacteria and consisting of more than twenty different proteins, has its central element in the so-called needle complex (NC). By combining microbiology, advanced structural biology and computational approaches, CSSB scientists aim to elucidate the mechanistic details of T3SS function by identifying novel regulatory components of the NC and addressing their role in the dynamic process of protein transport across the T3SS. To pursue this, researchers will work together with colleagues from the Humboldt University in Berlin, the European XFEL and the Heinrich Pette Institute in Hamburg.

T3SS adopts different stages during protein translocation. To systematically study the molecular composition of these different stages during transport, the scientists will reconstitute the T3SS complex of *Shigella flexneri* in artificial lipid vesicles. Functional T3SS will be analysed by mass spectrometry to identify small ligands and regulatory elements bound to the complex. The information obtained regarding the novel molecules regulating T3SS activity at the different stages will lead to the generation of a detailed T3SS protein interaction network.

This experimental dataset will serve as a basis for computational modelling of the transport energetics and kinetics. The resulting models will allow researchers to test different hypotheses, for example regarding the effects of perturbing specific components of the T3SS, and to make predictions about other experimental scenarios.

The results of this work will help advance our understanding of protein translocation and thus of host cell invasion by Gram-negative pathogens. Importantly, the discovery of novel regulatory elements mediating this process could enable the design of new therapeutic drugs to control Shigellosis and other Gram-negative bacterial infections.

TYPE 3 DEPENDENT INVASION OF HOST CELLS



Gram negative bacteria (green) use the type 3 needle complex for invasion of host cells (grey). Delivery of effectors into host cells is a multi-step process including the targeting and transport of substrate molecules through the needle complex.

5. CSSB'S ACCESS TO ENABLING TECHNOLOGIES/FACILITIES

Modern research in life sciences is highly interdisciplinary and large-scale integrated structural biology projects require the combined use of highly diverse techniques. Consequently, scientists typically have expertise in key approaches central to their own research while performing further experiments in collaboration with other research groups or by using dedicated research services. Having access to a wide range of structural biology infrastructures and technologies is therefore essential to the success of CSSB. The infrastructure/technology access model for CSSB has four different categories **(Supplementary Table 2: Available Infrastructure and Technology)**:

- Access to large-scale infrastructures on and near the Hamburg-Bahrenfeld Research Campus
- Access to in-house research infrastructure for electron cryo-microscopy
- Access to in-house research core facilities
- Access to further research infrastructures at CSSB partner sites

LARGE-SCALE X-RAY-BASED RESEARCH INFRASTRUCTURES ON AND NEAR HAMBURG-BAHRENFELD RESEARCH CAMPUS

CSSB's research portfolio is directly linked to the available X-ray- and XFEL-based structural biology research infrastructures available on or near the Hamburg-Bahrenfeld Research Campus.

PETRA III is one of the most powerful high-brilliance synchrotron radiation sources worldwide. In partnership with DESY, the European Molecular Biology Laboratory (EMBL) provides three state-of-the-art synchrotron radiation beamlines for applications in life sciences using small angle X-ray scattering (P12) and X-ray crystallography (P13, P14). These beamlines are linked to complementary facilities for protein sample preparation and automated crystallisation as well as on-line data processing and interpretation. DESY operates one beamline for applications in macromolecular X-ray crystallography and X-ray imaging (P11), on behalf of the Helmholtz Association and the Max Planck Society.

The European X-Ray Free-Electron Laser (XFEL) is currently under construction and is expected to begin operation in 2017. Because of the long linear accelerator, the experimental stations will be situated outside the Hamburg-Bahrenfeld Research Campus in Schenefeld, Schleswig-Holstein. The European XFEL is expected to become the most powerful facility of its kind worldwide. Of particular interest for future life science applications will be the Single Particle Biology (SPB) instrument, complemented by an instrument for Serial Femtosecond X-ray crystallography (SFX) applications and by a state-of-the-art laboratory for sample preparation (XBI).

Initially, access to relevant synchrotron radiation beamlines at PETRA III and XFEL instruments will be by peer reviewed applications, including options for so-called Block Allocation Group (BAGs) proposals, which allow for a large number of projects to be clustered together. Depending on future access needs, the establishment of a Collaborative Research Group (CRG), an established concept for buying into privileged access, would be an option.

IN-HOUSE RESEARCH INFRASTRUCTURE FOR ELECTRON CRYO-MICROSCOPY

The CSSB building has been designed to operate a state-of-the-art electron cryo-microscopy research infrastructure in its basement, thus complementing already existing X-ray-based research infrastructures on the Hamburg-Bahrenfeld Research Campus. An initial investment of seven million Euros has already been made and CSSB is presently in the process of doubling this investment through external fundraising. This infrastructure enables CSSB to offer an entire range of leading and emerging structural biology techniques, thus effectively meeting the needs of scientists working on *integrated structural biology* projects. It will be operated as a multi-user facility, under the coordination of Prof. Kay Grünewald (HPI/UHH). Most projects will, therefore, occur in the context of research collaborations. Structured training opportunities will be offered and independent access may be granted for projects focused on initial characterisation, routine quality control or in established areas of single particle electron cryo-microscopy. Plans to expand CSSB's electron cryo-microscopy infrastructure into an external user facility are under consideration.

CSSB's electron cryo-microscopy infrastructure will consist of five instruments, of which two will be for high-end single particle and tomography applications and one will be used in S3** biosafety containment for work with pathogenic material. Sample preparation for cellular cryo-tomography will be supported by a dedicated dual beam cryo Focused Ion Beam-Scanning Electron Microscope (FIB-SEM). In addition, the infrastructure will host screening microscopes to analyse sample quality, to optimise imaging conditions for the subsequent collection of high-resolution imaging data. Various sample vitrification techniques and automated data collection regimes will be established to maximise microscopy performance and throughput. For long-term data storage and retrieval as well as efficient image processing, data storage routines and a direct connection to DESY's data storage and high-performance computing cluster will be provided. For correlative cryo-imaging approaches, fluorescence cryo-microscopes will be available, as part of the advanced light and fluorescence microscopy core facility at the CSSB (see below).

RESEARCH CORE FACILITIES AT CSSB

CSSB will have four core facilities within or nearby the building. These facilities will provide research services that are essential to support relevant CSSB research activities.

- High-throughput crystallisation (with direct pipelines) in close proximity to synchrotron radiation beamlines at PETRA III (coordinator: EMBL, extension of an already existing facility next to the CSSB building)
- Protein characterisation of protein samples (coordinator: EMBL, some instruments already available)
- Advanced light and fluorescence microscopy (coordinators: BNITM/HPI/UHH)
- Protein production (coordinator: HZI, in collaboration with a similar parent facility at HZI in Braunschweig)

Each core facility will be run by one or more CSSB partner institutions, which will be responsible for the implementation of a suitable operational plan and user concept. To allow for effective management, core facilities will generally be located close to the research area of a coordinating partner institution. The CSSB core facilities are expected to be available for at least 50% of the operational

time as research services for other CSSB research groups. Facility operational plans may include the recovery of cost via the implementation of user fees. To ensure the establishment of high-quality, competitive services during the initial operational phase, CSSB is currently seeking external funds to support the acquisition of additional equipment and the employment of qualified technicians.

RESEARCH INFRASTRUCTURES AT CSSB PARTNER INSTITUTIONS

CSSB scientists will have privileged access to decentralised research infrastructures located at partner institutions. More than 50 potential core facilities have been identified at partner institutions that could be relevant for CSSB research. These include, for instance, state-of-the-art mass spectrometry facilities and experimental screening facilities. CSSB will regularly evaluate its need for access to scientific core facilities and, if needed, may consider establishing additional in-house facilities.

6. CSSB'S RESEARCH HOTEL – AN OPPORTUNITY FOR YOUNG, INDEPENDENT RESEARCHERS

A major challenge exists within the scientific community to provide attractive research opportunities for independent junior investigators that effectively bridge the gap between postdoctoral research and independent senior investigator positions. In an effort to facilitate this transition and to uphold its mission of empowering the next generation of scientists, CSSB is implementing the concept of a *Research Hotel*. Approximately 20% of the CSSB building has been reserved for this purpose, with a capacity to host up to six research groups typically for five years.

The Research Hotel will be open to small research groups from CSSB partner institutions as well as other external institutions irrespective of country of origin. During their stay at CSSB, incoming groups will benefit from access to all infrastructure, instrumentation, training and funding opportunities open to CSSB researchers. The limited size of the research groups and their close vicinity to senior principle investigators will maximise integration and facilitate mentorship opportunities. Junior groups will be selected based on established research excellence criteria and a fit into the overall CSSB research strategy, with a particular focus on complementary expertise. The Research Hotel concept will add value to CSSB by ensuring a regular turnover of research groups, thus allowing for the rapid adaptation of new research directions and emerging technologies.

In addition to hosting external research groups, the CSSB Research Hotel will be accessible to visitors hosted by existing CSSB principal investigators. These visitors could be students, postdoctoral fellows or more experienced scientists who will benefit from the scientific environment, new technologies and cutting edge equipment available at CSSB while working with our scientists on collaborative projects.

7. CSSB'S FUTURE ROLE IN THE INTERNATIONAL RESEARCH LANDSCAPE

CSSB's aspiration is to achieve recognition in the fields of infection, structural and systems biology at the local, national and international level. To accomplish this, CSSB will benchmark itself against leading centres at the interface of these three fields. This will be measured using established criteria such as publication records, external fundraising, and CSSB's role in international projects and meetings. On the latter, a milestone was achieved with the first successfully organized international symposium "Systems in Infection Biology from Molecules to Organisms" in 2015. The next symposium will be held in 2017 in the new CSSB building and plans are in place to hold symposiums approximately every two years.

With the support of its ten renowned partners from across Northern Germany, CSSB is exceptionally well positioned to pursue an ambitious research portfolio. At the same time CSSB's philosophy is to remain open to accepting new partner institutions. CSSB will approach potential partners based upon criteria such as research excellence, complementary research interests, and the need to use in-house research infrastructures. The acquisition of new partners is supported by the modular CSSB building concept that allows for the addition of building extensions at a relatively low cost.

In parallel, CSSB will internally strengthen existing expertise and knowledge in specific research fields and technologies, such as systems biology, where there is a need for increased competency. One key tool will be the implementation of a CSSB Associate Membership concept. This will allow selected principal investigators from CSSB partner institutions to become a part of CSSB. CSSB will also continue to lobby for additional targeted recruitments to be made by its present partners in research areas if additional expertise is deemed to be essential.

With its central location on the Hamburg-Bahrenfeld Research Campus, CSSB is surrounded by other interdisciplinary research centres, such as: CFEL, HARBOR, and CHyN **(Figure 4)**. By fostering strategic partnerships with these centres, CSSB aims to take a leading role in defining the future portfolio and overall concept for life sciences on the campus. A central aspect of accomplishing this will be CSSB's aspiration to take on leadership in future national research excellence initiatives. During its inaugural phase, this will be accomplished by making contributions to applications for new excellence cluster initiatives supported by the three Northern German investing states, Hamburg, Lower Saxony and Schleswig-Holstein.

This will be complemented by a CSSB-coordinated research cluster or consortia initiatives at both the national and international level. The combination of these activities will place CSSB in an excellent position to become an internationally leading research centre renowned for its ability to further our understanding of the fundamental mechanisms of the infection process and to contribute to the development of novel therapeutic strategies for effectively combating infectious diseases.

SUPPLEMENTARY TABLE 1: ONGOING COLLABORATIVE PROJECTS AT CSSB

Collaboration Project Title	Group 1	Group 2	Group 3	Group 4	Funding
Use of sarposins for high-resolution structural biology of integrated membrane proteins	C. Löw (EMBL)	M. Kolbe (HZI/UHH)			internal
Molecular dissection of erythrocyte invasion using systems biology	T. Gilberger (BNITM/UHH)	C. Löw (EMBL)	J. Parkinson (Sick Kids Research Institute)	A. Emili (University of Toronto)	JHS
Reconstitution and modelling the process of protein transport across the bacterial Type III Secretion System of Gram-negative bacteria	M. Kolbe (HZI/UHH)	C. Uetrecht (HPI, European XFEL)	E. Klipp (HU-Berlin)		JHS
Eff2Score – A novel mathematical model to decode type-3 secretion signal sequences	T. Marlovits (UKE/DESY)	P. Dersch (HZI)	T. Rattei (University of Vienna)		JHS
Structure Type VII secretion system in mycobacteria	T. Marlovits (UKE/DESY)	M. Wilmanns (EMBL)			internal

SUPPLEMENTARY TABLE 2: AVAILABLE INFRASTRUCTURE AND TECHNOLOGY

Technology/Infrastructure	Type of Access	Partner
European XFEL	Peer Review	European XFEL GmbH
PETRA III	Peer Review	DESY
High-throughput crystallisation (with direct pipelines) in close proximity to synchrotron radiation beamlines at PETRA III	CSSB core facility	coordinator: EMBL, extension of an already existing facility next to the CSSB building
Protein characterisation of protein samples	CSSB core facility	coordinator: EMBL, some instruments already available
Advanced light and fluorescence microscopy	CSSB core facility	coordinator: BNITM/HPI/UHH
Protein production	CSSB core facility	coordinator: HZI, in collaboration with a similar parent facility at the HZI in Braunschweig
Electron cryo-microscopes	In-house infrastructure	coordinator: HPI/UHH
FLASH	Peer Review	DESY

LIST OF ABBREVIATIONS

BAG	Block Allocation Group
BNITM	Bernhard Nocht Institute for Tropical Medicine
cART	combination antiretroviral therapy
Cas9	CRISPR associated protein 9
CFEL	Center for Free-Electron Laser Science
CHyN	Center for Hybrid Nanostructures
CLEM	correlative light and electron microscopy
CRISPR	clustered regularly interspaced short palindromic repeats
CUI	Centre for Ultrafast Imaging
DESY	Deutsches Elektronen-Synchrotron
DHS	DNase I Hypersensitive Site
eIF	eukaryotic Initiation Factor
EMBL	European Molecular Biology Laboratory
EM	Electron Microscopy
FIB-SEM	Focused Ion Beam-Scanning Electron Microscope
FLASH	Free Electron Laser Hamburg
FZJ	Forschungszentrum Jülich
GC7	N1-Guanyl-1,7-diaminoheptane
HARBOR	Hamburg Advanced Research Centre for Bioorganic Chemistry
HHU	Heinrich Heine University Düsseldorf
HIV	Human Immunodeficiency Virus
HPI	Heinrich Pette Institut
HZI	Helmholtz Centre for Infection Research

INM	inner nuclear membrane
JHS	Joachim Herz Stiftung
KI	Karolinska Institutet
MHH	Hannover Medical School
NAD	Nicotinamide Adenine Dinucleotide
NC	Needle Complex
NEC	nuclear egress complex
NMR	nuclear magnetic resonance
P11 – P14	different PETRA beamlines
PDB	Protein Data Bank in Europe
PETRA	Positron-Elektron-Tandem-Ring-Anlage (electron-positron collider)
PETRA III	3rd Generation Synchrotron Radiation Source
RH	Research Hotel
SAB	Scientific Advisory Board
SARS	Severe Acute Respiratory Syndrome
SAXS	Small-Angle X-ray Scattering
SFX	Serial Femtosecond X-ray Crystallography
SPB	Single Particle Biology
T3SS	Type III Secretion System
UHH	Universität Hamburg
UKE	University Medical Center Hamburg-Eppendorf
XBI	XFEL-based Integrated Biology Infrastructure
XFEL	X-ray Free Electron Laser

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