Phytochromes are a major class of photoreceptors in plants, also found in bacteria and fungi. Bacterial and cyanobacterial phytochromes are class-divided into red-light (Pr) absorbing canonical and far red-light (Pfr) absorbing bathy phytochromes. We studied both types from the soil bacterium *Agrobacterium tumefaciens*, termed Agp1 (canonical) and Agp2 (bathy). Phytochromes are modular proteins comprising the highly conserved N-terminal photosensory core module (PCM) consisting of PAS, GAF and PHY domain and a variable C-terminal output module, usually a histidine-kinase.

We obtained in close collaboration with the Krauß lab (Queen Mary) and the Lamparter lab (KIT) a crystal structure of Agp1-PCM at 1.8 Å resolution [1]. In the structure, we observe a direct interaction between the “tongue” of the signal-transmitting PHY domain and the chromophore. This underlines a model that in canonical phytochromes the signal is communicated via the PHY-tongue as it undergoes structural changes from β-sheet to α-helix upon light-stimulation [3]. In our studies we analyse the role of the rearrangement of the tongue secondary structure during light-activation by introducing mutations that disallow helix formation and thereby hinder Pfr-state. For the bathy phytochrome Agp2-PCM we recently solved a crystal structure in Pfr-state at 2.5 Å resolution that shows a head to head overall organization of a homodimer. The chromophore-pocket shows new features that increase our understanding of the photocycle in bathy phytochromes [2].


---

**Figure 1.** PCM of (a) canonical phytochrome Agp1 in the Pr – ground state [1] and (b) bathy phytochrome Agp2 in the Pfr – ground state [2]. Chromophore Billiverdin is shown within 2Fo-Fc electron density map contoured at 1.5σ.
In *Arabidopsis* multistep phosphorelay pathway (MSP), the signal transfer from sensor histidine kinases to corresponding response regulators is performed by His-phosphotransfer proteins (AHPs). The previously reported ability of receiver domains of individual sensor histidine kinases to interact with only subset of AHPs indicates a certain level of interaction specificity in MSP. Here, for the first time in eukaryotic organism, we demonstrate how the specificity of MSP signaling is mediated on molecular level. Based on the newly solved 3D crystal structure of AHP2 and molecular dynamics modelling complemented by experimental approaches, we identified key residues necessary for molecular recognition of AHP2 by receiver domain of histidine kinase CKI1 (CKI1_RD). The comparison of the AHP2-CKI1_RD model with recently published crystal structure of the related AHP1-AHK5_RD complex revealed small number of amino acids in both AHPs and RDs that cause significant structural differences between two complexes. The importance of the identified residues for the specificity of interaction was experimentally confirmed by the ability of rewired AHP1 to interact with non-cognate partner CKI1_RD.

Supported by CZ.1.05/1.1.00/02.0068, ME ČR ME09016, GAČR 521/09/1699 and P305/11/0756, CZ.1.05/2.1.00/01.0024 and by the AS ČR AV0Z60870520.
TOWARDS THE STRUCTURE OF THE IMPORTIN $\beta$ – IMPORTIN 7 COMPLEX

Martje Kunz

Department of Molecular Structural Biology, Georg-August University Göttingen
mweidma1@gwdg.de

One of the most crucial transport systems in the cell is the nucleocytoplasmic transport. This machinery assures the import and export of ribosomal proteins, transcription factors and RNAs among others. To manage such a versatile process several transport receptors mediate the import and export. Most of these transport receptors belong to the Importin $\beta$ superfamily. For the members of this superfamily it is very characteristic that the single transport receptor binds its substrate directly via a specific transport signal and guide it through the nuclear pore complex (NPC). The most established member is the Importin $\beta$ (Imp$\beta$). It was crystallized with different substrates [1,2] and is well understood. Unlike the typical binding event Imp$\beta$ is known to be able to form a heterodimer with Importin 7 (Imp$\gamma$), another member of the $\beta$ superfamily. This heterodimer mediates the transport of histone H1 through the NPC. Both transport receptors interact with each other and the substrate. Until now this is the only known case where a binding like this takes place [3,4]. Because of the unknown mode of interaction between Imp$\gamma$ and Imp$\beta$ we are highly interested in the structure of Imp$\gamma$, the Imp$\gamma$-Imp$\beta$ heterodimer and the complex with bound histone. So far all crystallization trials were unsuccessful. Nevertheless we are trying to address this question by complementary methods. With the help of SAXS-, ITC- and cross-link-experiments we attempt to get insight into the structure and dynamics of this unique and complex binding event.


Figure 1. Scheme of nuclear transport [5].
The Human Cytomegalovirus (HCMV) is a ubiquitous pathogen with global infection rates between 60 and 100% of the adult population. While infection is generally asymptomatic in immunocompetent adults, congenitally infected children as well as immunocompromised and immunosuppressed adults suffer from various, potentially lethal symptoms. HCMV replicates and encapsidates its genome inside the host cell’s nucleus, thereby becoming too big to exit the nucleus via nuclear pore complexes; instead, the virus relies on a complex process involving viral effector proteins as well as host cell factors, resulting in budding through the inner nuclear membrane [1].

To destabilize the nuclear lamina and facilitate budding, a nuclear egress complex (NEC) is recruited by viral proteins pUL50 and pUL53 to the inner nuclear membrane [2]; The lamina is locally depleted by lamin phosphorylation via viral protein kinase pUL97 and cellular protein kinase PKC, also part of the NEC [3; Fig. 1]. The aim of this project is to analyze the interactions of the NEC components on an atomistic scale using X-ray crystallography, beginning with the pUL50/53 complex.


We would like to thank Prof. Dr. Clemens Steegborn and Dr. Michael Weyand (University of Bayreuth, Department of Biochemistry) for their help with testing SeMet-derived and heavy-atom-soaked crystals, and also the user support team of BESSY II beamline 14.1 and PETRA III beamline P14 for their help with data collection. We also thank Prof. Dr. Manfred Marschall and Dr. Jens Milbradt (FAU Erlangen-Nürnberg, Institute for Clinical and Molecular Virology) for the ongoing collaboration on this project.

Figure 1. Model of nuclear lamina depletion in HCMV-infected cells [4]. ONM: outer nuclear membrane; INM: inner nuclear membrane.
Salmonella enterica is an important food-borne pathogen with the ability to cause diseases ranging from diarrhoea and intestinal inflammation to typhoid fever, a life-threatening systemic condition. Salmonella is an invasive and facultative intracellular pathogen. Recently it has been shown that a type I secretion system (T1SS) in Salmonella is necessary for invasion of polarized epithelial cells. This T1SS is encoded by the Salmonella pathogenic island 4 (SPI4) and consists of the genetic information of the SiiAB-complex, an outer membrane pore (SiiC), a periplasmatic adaptor protein (SiiD), a giant nonfimbrial adhesin (SiiE) and the inner membrane ATP-binding cassette (SiiF) [1]. The function of the SiiAB-complex is not well understood. The detectable low sequence homology of SiiAB to MotAB suggests that SiiAB forms a proton channel through the inner membrane [2]. Although it is not known how SiiAB contributes to the T1SS, SiiAB has been shown to be required for the secretion of the adhesin SiiE [1].

Therefore it is our goal to get further insight into the structure and function of the SiiAB integral membrane complex. This would lead not only to additional information of Salmonella invasion but would also provide first experimental insights into the structure and function MotAB and homologous torque motors. So far, we succeeded in solving the structure of the C-terminal domain of SiiA (unpublished results) and in producing the SiiAB-complex in low amounts.

In order to purify SiiAB using fluorescence-assisted size exclusion chromatography (FSEC) [3], we designed a SiiAB-GFP-8xHis fusion construct, as well as point mutations to inactivate the channel. Here, I will present the structure of the C-terminal domain of SiiA and some preliminary results from the purification of the integral membrane protein complex SiiAB using FSEC.


We thank Prof. Michael Hensel, Universität Osnabrück, and Roman Gerlach, RKI Wernigerode, for the collaboration and for providing the plasmids. We further thank Prof. Samuel Wagner, Universitätsklinikum Tübingen, for introduction to the FSEC-Method and for providing the MT56 strain, a E.coli BL21(DE3) derived strain with increased membrane protein overexpression. We thank the DFG (RTG 1962) for funding.
PUSHING THE LIMITS OF EXPERIMENTAL PHASING WITH NEW HPC DETECTORS

A. Förster on behalf of DECTRIS Ltd.

DECTRIS Ltd., Neuenhoferstrasse 107, 5400 Baden, Switzerland
andreas.foerster@dectris.com

With the recently introduced EIGER, hybrid photon counting (HPC) enters a new dimension of spatial and temporal resolution and expands the field of X-ray experimentation. Besides enabling new ways of doing X-ray crystallography, continuous read-out with frame rates up to 3000 Hz and a pixel size of 75 µm open HPC detectors to new techniques like imaging in scanning configuration and various time-resolved and high-throughput experiments. EIGER R 1M and 4M are mega-pixel HPC detector for the laboratory. The absence of any detector noise in combination with an image bit depth of 32 bit and high spatial resolution turn them into versatile platforms for virtually any laboratory application.

A short outline of the differences between EIGER and PILATUS3 will highlight key aspects of the new detector technology. The main focus of the presentation will be on native single anomalous dispersion with highly redundant low-dose data [1] and data collected from multiple crystals [2]. These novel approaches to experimental phasing, made possible by concerted upgrades to radiation sources, beamline optics and detector instrumentation, take crystallography to the next level, beyond the previous state of the art set by fine phi slicing on PILATUS [3].


AUTOMATION IN CRYSTALLIZATION

J. Mueller-Dieckmann

Formulatrix
jochen@formulatrix.com

Formulatrix provides automated solutions for the entire crystallization process, including pipetting robotics, drop setters, imagers and a comprehensive software suite to design and score experiments and manage the entire data administration.

The reliable detection of protein crystals of frequently small sizes (5 µm or less) under difficult conditions like precipitation and their discrimination from unwanted crystals, originating from precipitants, constitutes a serious bottleneck in crystallography. To overcome this hurdle, Formulatrix combines automated high resolution imaging with a variety of innovative imaging techniques, like UV, FRAP, SONICC (Second Order Nonlinear Imaging of Chiral Crystals) and trace fluorescence labeling.

An overview over the different imaging technologies together with real-life results is presented.