ESRF	**Experiment title: Studies of structure-function relationship of proteins investigated at the MPI Dortmund**	**Experiment number**: LS-1815
Beamline: BM14	**Date of experiment**: from: 28-Sep-00 to:29-Sep-00	**Date of report**: 22.08.01
Shifts: 3	**Local contact(s)**: Dr. Vivian Stojanoff	*Received at ESRF:*
Names and affiliations of applicants (* indicates experimentalists): Holger Rehmann*, Eva Wolf*		

Report:
The following projects were investigated:

IIGP1

IIGP1 is a representative of the 47kda–family of interferon-γ-inducable GTPases. We have obtained two crystal forms of SeMet-substituted full length IIGP1, which belong to space groups P422 and P212121 with almost identical unit cells. On BM14 we collected MAD data sets on both crystal forms. We have collected a 98.5% complete 2.4Å SeMet-MAD-dataset on a P212121 crystal, using three wavelengths corresponding to peak (λ1=0.9786 Å), inflection point (λ2=0.9789 Å) and high energy remote above the Se-edge (λ3=0.9184 Å). We also collected a 96% complete 2.8 Å SeMet-MAD-dataset on a P422 crystal using the same three wavelengths. 19 of 22 possible Se-positions could be identified by Patterson analysis of the P212121 data set and provided phases resulting in an interpretable experimental map. We are currently refining the structure.

ESRF	**Experiment title: Studies of structure-function relationship of proteins investigated at the MPI Dortmund**	**Experiment number**: LS-1815
Beamline: **ID14-3**	**Date of experiment**: from: 29-Sep-2000 to: 2-Oct-2000	**Date of report**: **22.8.2001**
Shifts: 6	**Local contact(s)**: Steffi Arzt	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Ingrid Vetter, Michael Seewald*, Dr. Ilme Schlichting*, Dr. Axel Scheidig*, Dr. Eva Wolf*, Holger Rehmann*

Report:

The following projects were investigated:

Proteins involved in nuclear transport

RanGAP and RanBP1 are regulators of nuclear transport. They end one cycle of nuclear import by triggering the hydrolysis of the GTP which is bound to Ran. Since the structure of RanGAP itself (which is known) does not resemble any other GAP of a small GTP binding protein, the mechanism of acceleration of GTP hydrolysis was already thought to be different from the other GAPs. The function of RanBP1 is a further enhancement of the GTP hydrolysis rate which is already accelerated by RanGAP. We have solved the structure of the ternary complex of Ran with RanGAP and RanBP1, the publication has been submitted to Nature. RanBP1 was shown to stimulate the GTPase activity indirectly by increasing the binding affinity of Ran for RanGAP. On ID14-3 we collected a 98 %-complete 3.4 Å dataset of the transition-state mimic complex with GDP and aluminum fluoride which helped to corroborate that the mechanism of RanGAP-stimulated GTP-hydrolysis does not involve an arginine, in contrast to all other GAP complex structures known to date.

Photosystem II

Photosystem II (PS II) is a complex dimeric membrane protein consisting of over 20 different subunits. It is located in the thylakoid membrane of higher plants, algae and cyanobacteria where it functions as a light-driven electron pump. This intramolecular electron transport is coupled to the splitting of water by a manganese cluster which yields molecular oxygen and is the basis of the oxygenic atmosphere on earth. Due to the large unit cell and the weak diffraction evaluation of crystals on the home source rotating anode is not possible. During this beamtime we have tested 23 different crystals (native and potential heavy atom derivatives). No usefull heavy atom derivative could be detected.

Rab geranylgeranyl transferase (RabGGTase) from yeast

The small regulatory Rab GTPases are attached to lipid membranes via the addition of two geranylgeranyl moieties, a post-translational modification carried out by Rab geranylgeranyl transferase (RabGGTase). In contrast to farnesyl transferase (Ftase) and geranylgeranyl transferase type I, RabGGTase specifically prenylates Rab proteins. During this beamtime we have tested five different crystals. None of them diffracted better than 7 Å and no data set was collected.

DAM:DNA complex

The adenine methyltransferase (DAM) from *E.coli* was crystallized in complex with a specific double stranded DNA. We have tested six different crystals and could collect one complete data set with maximum resolution of 2.2 Å. Molecular replacement using known DNA methyltransferases as search model were unsuccessful indicating major structural changes for this MTase (which is indicated as well by biochemical results). Further MIR or MAD data sets are needed.

Tryptophan Synthase

The bifunctional enzyme tryptophan synthase (TRPS) is a paradigm for substrate channelling. The α- and β-subunits of TRPS are arranged in a linear αββα form and catalyze the last two steps in the biosynthesis of tryptophan. In the α-subunit indole glycerole phosphate (IGP) is cleaved; subsequently indole is channeled through a 25 Å long tunnel to the β-active site where it is condensed with serine that has been activated by a pyridoxal phosphate cofactor. The two reactions remain in phase by an extensive network (including a Na^+ ion) of allosteric interactions that are mediated by a rigid but movable domain of the β-subunit. We determined the structure of the TRPS complex with the true a-substrate IGP to 1.7 Å resolution. In contrast to the complex with the inhibitor IPP, the catalytically crucial aE49 rotates to the active conformation where it can interact with the 3'hydroxyl group of IGP. We collected a 1.7 Å resolution dataset of the complex with indolacetyl-valine (an inhibitor of the α-reaction but allosteric activator of the β-reaction) but the inhibitor was not bound. We also tried to determine the structure of the quinonoid intermediate of the β-reaction TRPS. Unfortunately there was no electron density for the intermediate calculated from 1.7 Å data.

Heatshock protein ClpB

ClpB is a heatshock protein (MW 96kD) that dissolves aggregated proteins in an ATP dependent fashion. In addition to its role in protein folding it is also involved in the activation of pre-prion like proteins in yeast and the disassembly of multimeric protein complexes. We obtained several crystal forms from different nucleotide complexes and mutants of ClpB under various conditions. Unfortunately none of the many crystals tested diffracted to a resolution higher than 7 Å.

Nucleoside monophosphate kinases:

Herpes Simplex Virus Thymidine Kinase (HSV-TK) is a bifunctional nucleoside monophosphate kinase that catalysis the reversible phosphoryl transfer reactions: ATP + thymidin <--> ADP + TMP and ATP + TMP <--> ADP + TDP. In order to investigate the catalytic mechanism we crystallized HSV-TK with the bisubstrate inbihibitor TP5A (mimiking

the second reaction). The dataset is 98.4 % complete to 1.7 Å resolution. Refinement showed TP5A bound to one of the two molecules in the A.U. but no Mg2+ . This is due to the high concentration of ammonium sulfate used for crystallization. Since the presence of Mg2+ is essential for catalysis we are now looking for a new crystal form grown from PEG.
In contrast to the bifunctional HSV-TK, human thymidylate kinase (hTmpK) catalysis only the second reaction listed above. In addition to the physiological substrate TMP, hTMPK can also phosphorylate the monophosphorylated HIV prodrug AZT (AZTMP). Since hTmpK forms the bottleneck in the 3 step activation of AZT to its triphosphate form, we designed mutant enzymes that are better catalysts. In principle these could be used in genetherapeutic approaches to the treatment of AIDS. One of these mutants is a chimeric human enzyme containing the “LID” region of E.coli TMPK. To elucidate why this mutant is faster we cocrystallized it with the ATP analog ß,γ-imido-ATP and AZTMP. A dataset has been collected to 1.7 Å resolution and refined. The mutant is faster because it stabilizes the active, closed conformation.

ESRF	**Experiment title:** Studies of structure-function relationship of proteins investigated at the MPI Dortmund	**Experiment number**: LS-1815
Beamline: ID14-1	**Date of experiment**: from: 10-Nov-2000 to: 13-Nov-2000	**Date of report**: 22.8.2001
Shifts: 6	**Local contact(s)**: Dr. Hassan Belrhali	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Ilme Schlichting, Dr. Ingrid Vetter, Dr. Eva Wolf, Dr. Axel Scheidig*, Michael Seewald*, Dr. Michael Weyand*, Dr. Alexey Rak*

Report:

Proteins involved in nuclear transport

During this beamtime we could improve the quality of our previously collected data set at ID14-3. On ID14-1 we collected a 98 %-complete 3.1 Å dataset of the transition-state mimic complex with GDP and aluminum fluoride. The improved electron density map further helped to corroborate that the mechanism of RanGAP-stimulated GTP-hydrolysis does not involve an arginine, in contrast to all other GAP complex structures known to date.

Rap guanine-nucleotide exchange factor directly activated by cAMP (Epac II)

We have crystallized the 45kda regulatory domain of Epac II, which contains two cAMP-binding domains and a DEP domain for membrane targeting. The crystals belong to space group P212121. Experimental phases obtained from SeMet-MAD phasing allowed building of an initial model at 3.2Å resolution. At ID14-1 we collected a 93.5% complete 2.5Å dataset, which was used for refinement to higher resolution.

Photoactive membrane proteins

We have tested four different crystals of the sensory rhodopsin pSRII. None of them diffracted better than 9 Å (the same, but smaller crystalls diffracted at beamline ID13 during microfocus test-beamtime up to 3.2 Å).

Five crystals of photosystem PSII were tested. One derivative data set could be collected up to 4.5 Å resolution. Due to time limitation the data set could only be collected up to 60% completeness.

Tryptophan Synthase

The bifunctional enzyme tryptophan synthase (TRPS) is a paradigm for substrate channelling. The α- and β-subunits of TRPS are arranged in a linear αββα form and catalyze the last two steps in the biosynthesis of tryptophan. In the α-subunit indole glycerole phosphate (IGP) is cleaved; subsequently indole is channeled through a 25 Å long tunnel to the β-active site where

it is condensed with serine that has been activated by a pyridoxal phosphate cofactor. The two reactions remain in phase by an extensive network (including a Na^+ ion) of allosteric interactions that are mediated by a rigid but movable domain of the β-subunit. We tried again to determine the structure of the quinonoid intermediate of the β-reaction TRPS. Due to the extreme light-sensitivity of the quinonoid complex we cannot use a microspectrophotometer to test whether the crystals contain the intermediate. Unfortunately no indication for the intermediate could be found in the electron density calculated from 1.9 Å data. We collected a 1.9 Å resolution dataset of the complex with indolacetyl-valine (an inhibitor of the α-reaction but allosteric activator of the β-reaction) and identified the structural basis for the allosteric activation. This finding is being submitted to JBC. The ßS178P mutant of TRPS has α,β activities that are close to the wildtype, but is deficient in the α<-->β communication. We determined the structures of the uncomplexed ßS178P-TRPS, and of complexes with the the α-ligands indol-acetyl-glycine (IAG) and glycerol phosphate (GP) to 1.7, 1.7, and 1.5 Å resolution, respectively. We identified the structural basis for the lack of allosteric communication and submitted the paper to JBC.

Heatshock protein ClpB

ClpB is a heatshock protein (MW 96kD) that dissolves aggregated proteins in an ATP dependent fashion. In addition to its role in protein folding it is also involved in the activation of pre-prion like proteins in yeast and the disassembly of multimeric protein complexes. We obtained several crystal forms from different nucleotide complexes and mutants of ClpB under various conditions. Unfortunately none of the many crystals tested diffracted to a resolution higher than 7 Å

ESRF	**Experiment title: Studies of structure-function relationship of proteins investigated at the MPI Dortmund**	**Experiment number**: LS-1815
Beamline: **ID14-3**	**Date of experiment**: from: 11-Dec-2000 to: 12-Dec-2000	**Date of report**: 22.8.2001
Shifts: 3	**Local contact(s)**: Dr. Hassan Belrhali	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Ilme Schlichting, Dr. Ingrid Vetter, Dr. Eva Wolf, Dr. Axel Scheidig, Marc Saric*, Michael Weyand*

Report:

Proteins involved in nuclear transport

Importin-β and Ran are proteins involved in nuclear transport. They form a complex with importin α and the substrate to achieve the directional diffusion through the nuclear pore. The structure of the complex has been solved by our group, but it did not yield sufficient information about the mechanism of nuclear transport. Especially the conformational changes of importin-β mutant proteins in complex with Ran will give insights into the function of the various region of importin-β, e.g. the function of the protruding "stalk" or the acidic loop which is important for Ran binding. The structures of the Importin β mutants in complex with Ran (together with their biochemical characterization) would mean a great progress in solving the puzzle of nuclear transport. Synchrotron radiation is essential since at a rotating anode the crystals diffract only to less than 3 Å. Two datasets were collected on ID14-3, one of the mutant importin-β (D338A)-Ran to 2.7 Å resolution and one of the double mutant importin-β (D338A,D339A) to 3.4 Å resolution. The mutants showed changes in the acidic loop region and in the interface to Ran which can explain the biochemical data. The correlation of the results of nuclear transport assays with the x-ray structures is still in progress.

Tryptophan Synthase

The bifunctional enzyme tryptophan synthase (TRPS) is a paradigm for substrate channelling. The α- and β-subunits of TRPS are arranged in a linear αββα form and catalyze the last two steps in the biosynthesis of tryptophan. In the α-subunit indole glycerole phosphate (IGP) is cleaved; subsequently indole is channeled through a 25 Å long tunnel to the β-active site where it is condensed with serine that has been activated by a pyridoxal phosphate cofactor. The two reactions remain in phase by an extensive network (including a Na^+ ion) of allosteric interactions that are mediated by a rigid but movable domain of the β-subunit. We tried again to determine the structure of the quinonoid intermediate of the β-reaction TRPS. Due to the extreme light-senistivity of the quinonoid complex we cannot use a microspectrophotometer to

test whether the crystals contain the intermediate.Two datasets of differently prepared crystals were collected to1.8 Å and 2.0 Å resolution. Unfortunately no electron density for the intermediate could be found.

ESRF	**Experiment title:** Studies of structure-function relationship of proteins investigated at the MPI Dortmund	**Experiment number**: LS-1815
Beamline: ID14-1	**Date of experiment**: from: 2-Feb-2001 to: 5-Feb-2001	**Date of report**:
Shifts: 9	**Local contact(s)**: Dr. Laurence Dumon	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Ilme Schlichting*, Dr. Ingrid Vetter, Dr. Eva Wolf, Dr. Axel Scheidig*, Dr. Michael Weyand*, Dr. Nicolas Thomä*, Dr. Alexey Rak*

Report:

APC

The Wnt signaling pathway activates the transcription of many important genes, which themselves regulate cell proliferation. The knowledge of these genes and their roles in the regulation of cell proliferation is a prerequisite for the understanding of the function of the Wnt signaling pathway during tumor development. We were able to identify APC, a gene which is transcriptionally activated by Wnt. The APC protein fulfills many important intracellular functions. These include the regulation of level of the proto-oncoprotein β-catenin. The knowledge of the exact three-dimensional structure of the APC protein will help to understand the biochemical function of the APC protein as a part of the Wnt signaling cascade and thus the exact role of the APC protein during tumorigenesis.

On ID14-1 we collected datasets from three cystals, one wild type and two selenomethinine-labelled crystals to 2.0 Å (wild type and one SeMet crystals) and 2.5 Å, respectively. The SeMet data allowed the solution of the structure by SAD and gave excellent electron density. The phases were the used to refine the wild type structure since its data quality was better. The preparation of the publication is in progress.

Rap guanine-nucleotide exchange factor directly activated by cAMP (Epac II)

We have crystallized the 45kda regulatory domain of Epac II, which contains two cAMP-binding domains and a DEP domain for membrane targeting. Experimental phases were obtained from SeMet-MAD data sets, which were collected in space group P212121 and allowed building of an initial model at 3.2Å resolution. Refinement against a 2.5Å dataset was problematic and suggested the actual space group to be P1 with P212121 pseudosymmetry. We therefore collected a 84.4% complete 2.5 Å P1-data set at ID14-1 for further refinement.

IIGP1

IIGP1 is a representative of the 47kda–family of interferon-γ-inducable GTPases. We have solved the structure of IIGP1 alone and refined it to 2.3 Å resolution. We have now obtained cocrystals of the IIGP1-GDP complex, which belong to space group P212121. At ID14-1 we collected a 90% complete 2.7Å dataset of the IIGP1-GDP complex crystals for structure determination by molecular replacement and refinement.

Photosystem II

Photosystem II (PS II) is a complex dimeric membrane protein consisting of over 20 different subunits. It is located in the thylakoid membrane of higher plants, algae and cyanobacteria where it functions as a light-driven electron pump. This intramolecular electron transport is coupled to the splitting of water by a manganese cluster which yields molecular oxygen and is the basis of the oxygenic atmosphere on earth. Due to the large unit cell and the weak diffraction evaluation of crystals on the home source rotating anode is not possible. During this beamtime we have tested 15 different crystals (native and potential heavy atom derivatives). No usefull heavy atom derivative could be detected.

Rabaptin and complex of Rabaptin:Rab4

Rabaptin was the first described effector protein of Rab5 and Rab4, Rab proteins associated with early endosomes. Rabaptin has a very high percentage of coiled coil secondary structure and exists as a rod like shape homodimere. At its N-terminus it interacts with Rab4(GTP) and with its C-terminal domain it interacts with Rab5(GTP). A huge number of effector proteins associated with vesicular transport are proteins with more or less extented coiled coil regions causing the question how these regions are specifically recognized by the cognate Ypt/Rab proteins. The structures of Rab4(GTP) complexed with the N-terminal domain of Rabaptin will give insight into this specific question and into the question of microdomain formation at the membrane surface of vesicles. We obtained very small crystals and used this beamtime to evaluate their diffraction quality. Overall we have tested 13 crystals. Their spacegroup is P1 with around 30 molecules per unit cell. Since the best resolution obtained was around 3.5 Å we will try to obtain different crystals with better properties using new protein constructs.

DAM:DNA complex

The adenine methyltransferase (DAM) from *E.coli* was crystallized in complex with a specific double stranded DNA. Recently we collected at ID14-3 a complete data set with maximum resolution of 2.2 Å. Since molecular replacement using known DNA methyltransferases as search model was unsuccessful we used this beamtime to search/collect for potential heavy atom derivatives. We collected one data set with a completeness of around 85% and maximum resolution of 3.4 Å. This data set was not enough to solve the phase problem.

MTaqI in complex with DNA and cofactor analogue

The methyltransferase MTaq I is a model system for studying the base-flipping mechanism of DNA MTases. We have determined the ternary structure of MTaqI:DNA in complex with a non-reactive substrate analogue. In followup studies we analyse the influence of different DNA substrates and cofactor analogues on the enzyme structure and mechanism. During this beamtime we have tested eight new crystals of a ternary complex and collected one complete

data set with a maximum resolution of 2.4 Å. The refinement of the structure is finished and will be published soon.

OxyB

The heme protein Oxy B belongs to the cytochrome P450 superfamily. It is involved in the biosynthesis of the medicinally important antibiotic vancomycin, which is derived from a linear heptapeptide. OxyB catalyzes one of the ring formations. We crystallized oxyB in two different space groups, an orthorombic one from PEG and a monoclinic one from ammonium sulfate. We collected native datasets of both crystal forms to 2.0 Å and 1.7 Å resolution, respectively, and a 2. 5 Å resolution anomalous dataset of a $HgCl_2$ derivative (monoclinic crystal form). We determined the structure by SAD in the monoclinic form and used the model for molecular replacement in the orthorombic form. The structures have been refined and the manuscript describing them is being written.

Heatshock protein ClpB

ClpB is a heatshock protein (MW 96kD) that dissolves aggregated proteins in an ATP dependent fashion. In addition to its role in protein folding it is also involved in the activation of pre-prion like proteins in yeast and the disassembly of multimeric protein complexes. We obtained several crystal forms from different nucleotide complexes and mutants of ClpB under various conditions. Unfortunately none of the many crystals tested diffracted to a resolution higher than 7 Å

E924K mutant of Myosin-S2 fragment

Myosin is a key motor protein in muscle contraction. Recently a family of myosin-binding proteins MyBP, MyBP-C and MyBP-H have been identified that seem to regulate myosin function. MyBP-C binds to the N-terminal 126 residues of the myosin rod S2 segment. Human mutations have been described in both MyBP-C and the 126 amino acid long region of S2, which result in familial hypertrophic cardiomyopathy (FHC). We crystallised the S2 fragment carrying the E924K FHC-mutation and collected a first native dataset to 2.7 Å resolution (2-fold redundancy, R_{sym} 6.0) in a triclinic crystal form (a=33.5, b=41.8, c=111.5, a=90.1, ß=95.4, γ=109.6). The self rotation indicates two molecules per a.u. Since molecular replacements attempts with coiled coil fragments were not successful we plan to collect derivative data next time.