BAG MPI-Dortmund, Germany

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The following publications resulted from our beamtime at the ESRF:

Crystal structures of a new class of allosteric effectors complexed to tryptophan synthase,
Michael Weyand, Ilme Schlichting, Anna Marabotti, Andrea Mozzarelli, JBC in press

Crystal structure of the βSer178Pro mutant of tryptophan synthase: a "knock-out" allosteric enzyme.
Michael Weyand, Ilme Schlichting, Petra Herde, Anna Marabotti, Andrea Mozzarelli, JBC in press.

RanGAP mediates GTP hydrolysis without an arginine finger.
Michael Seewald, Carolin Körner, Alfred Wittinghofer and Ingrid R. Vetter, Nature 415, 662-666 (2002)

Structure of the nuclear export domain of the APC tumor suppressor protein and its interaction with crm-1.
Lara Tickenbrock, Janina Cramer, Ingrid R. Vetter and Oliver Müller, EMBO J. submitted

ESRF	**Experiment title: Studies of structure-function relationship of proteins investigated at the MPI Dortmund**	**Experiment number**: LS-2082
Beamline: 14-1	**Date of experiment**: from: 26-Sept-2001 to: 27-Sept 2001	**Date of report**:
Shifts: 3	**Local contact(s)**: Dr. Cecile Jamin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Axel Scheidig*, Dr. Ilme Schlichting*, Dr. Ingrid Vetter, Dr. Eva Wolf, MPI Dortmund

RabGGTase:REP-1 complex

The Ypt/Rab proteins are members of the Ras superfamily of small GTP-binding proteins and have been implicated in the mechanisms by which transport vesicles identify and fuse with their target compartment. Crucial for their functionality is the posttranslational prenylation of C-terminal cysteins by the action of the Rab geranylgeranyltransferase. We have cocrystallized RabGGTase, REP-1 and a stable analogue of the substrate geranylgeranly-phyrophosphate. The crystals (space group $P2_1$) are extremely thin, and many had to be tested to identify a single one. A complete data set was collected to 2.8 Å resolution.

NO Synthase

Nitric Oxide (NO), is one of the most important signalling molecules in biology. It is synthesised by NO synthases (NOS), a family of three isozymes that contain minimally a reductase domain, and a heme domain with a pteridin cofactor. Uncontrolled generation of NO leads to pathology. While NO overproduction by nNos and iNOS is directly linked to the pathogenesis of stroke and shock, respectively, NO generated by eNOS is crucial for angiogenesis and blood pressure regulation. It is therefore highly desirable to design isozyme specific inhibitors. We collected two datasets of iNOS (spacegroup $P6_122$) complexed with the inhibitors N-ω-propyl-arginine and ARL-R17447 to 2.35 and 2.9 Å resolution, respectively. For comparison we collected a dataset of nNOS (spacegroup $C222_1$) complexed with N-ω-propyl-arginine to 2.05 Å resolution.

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. We have collected one complete native data set to 3.8 Å resolution.

ESRF	**Experiment title: Studies of structure-function relationship of proteins investigated at the MPI Dortmund**	**Experiment number**: LS-2082
Beamline: ID29	**Date of experiment**: from: 27-Sept-2001 to:28-Sept-2001	**Date of report**:
Shifts: 3	**Local contact(s)** Dr. Germaine Sainz	*Received at ESRF:*

Names and affiliations of applicants (*indicates experimentalists)

Dr. Axel Scheidig*, Dr. Ilme Schlichting*, Dr. Ingrid Vetter, Dr. Eva Wolf, MPI Dortmund

Report:

General Remark:

There were a lot of problems with the beamline and the detector. We lost a lot of time and about 50% of our datasets were useless (horrible N(Z) statistics, high Rsym values).

The following projects were investigated successfully:

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 ID29 we collected a complete 2.0Å data set of the IIGP1-GDP complex crystals for structure determination by molecular replacement and refinement.

ß-catenin

β-catenin is a protein containing an ARM-repeat domain which mediates its binding to APC. β-catenin is an important component of the Wnt-signalling pathway that leads to a certain kind of colon cancer if permanently activated. We are investigating its ligand binding properties to find the determinants of APC-binding to catenin. Therefore, we have co-crystallized β-catenin with different ligands under different conditions and collect high-resolution datasets to determine the conformation of a crucial loop. The final goal would be an interruption of the β-catenin interaction with the other proteins. On ID29, we could collect a complete dataset from a crystal soaked in a new compound to 2.2 Å resolution. No ligand was found in the structure, contrary to our expectations. We plan to repeat the experiment with different buffer conditions to facilitate binding. The crystals are too small to test the presence of the ligands at the home source.

LOV domain

Phototropin, a major blue-light receptor for phototropism, exhibits blue-light-dependent autophosphorylation and contains two LOV (Light, Oxygen or Voltage) domains and a Ser/Thr kinase domain. The LOV domains belong to the PAS superfamily of sensor proteins. They contain a noncovalently bound FMN and exhibit a reversible photocycle that allows them to function as signalling switch. The lifetime of the photo-product depends strongly on pH. We photolyzed a crystal (spacegroup $P6^522$) of the LOV1 domain of *Chlamydomonas* at pH 6.0 and collected a dataset to 2.5 Å resolution. Refinement indicates that the photoproduct has only very low occupancy.

NO synthase

Nitric Oxide (NO), is one of the most important signalling molecules in biology. It is synthesised by NO synthases (NOS), a family of three isozymes that contain minimally a reductase domain, and a heme domain with a pteridin cofactor. Uncontrolled generation of NO leads to pathology. While NO overproduction by nNos and iNOS is directly linked to the pathogenesis of stroke and shock, respectively, NO generated by eNOS is crucial for angiogenesis and blood pressure regulation. It is therefore highly desirable to design isozyme specific inhibitors. We co-crystallized the nNOS heme domain with the nNOS specific inhibitor ARL-R17477 in spacegroup $C222_1$. A dataset was collected to 1.94 Å resolution and refined.

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. We have collected one complete derivative data set to 4.1 Å resolution.

DAM:DNA complex

The adenine methyltransferase (DAM) from *E.coli* was crystallized in complex with a specific double stranded DNA. Molecular replacement using known DNA methyltransferases as search model for a native data set to 2.2 **Å** was unsuccessful indicating major structural changes for this MTase (which is indicated as well by biochemical results). Therefore, we have introduced five bromine atoms into the dsDNA and performed a Br-SAD experiment at the white line. The crystals are very thin needles (200 x 20 x 20 μm^3) and are very radiation sensitive. Due to severe detector problems (see above) we could use the collected data set for phasing. Further experiments are needed.

ESRF	**Experiment title:** Studies of structure-function relationship of proteins investigated at the MPI Dortmund	**Experiment number**: LS-2082
Beamline: ID29	**Date of experiment**: from: 7-Dec-2001 to: 8-Dec-2001	**Date of report**:
Shifts: 3	**Local contact(s)**: Dr. Germaine Sainz	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Axel Scheidig*, Dr. Ilme Schlichting*, Dr. Ingrid Vetter, Dr. Eva Wolf, MPI Dortmund

Report:

14-3-3-H^+-ATPase-Fusicoccin complex

Fusicoccin is a fungal phytotoxin, which binds to the complex between 14-3-3 proteins and the cytoplasmic C-terminus of the H^+ATPase of infected plants. It thereby irreversibly arrests the H^+ATPase in an activated state, which leads to a loss of water and consequently to the death of the plant. We have collected a 2.7 Å data set of cystals of the ternary complex between 14-3-3c, fusicoccin and a phosphorylated H^+-ATPase C-terminal peptide, as well as 2.3 Å and 2.7 Å datasets of crystals of the complexes between 14-3-3c and one of both ligands. All datasets are complete. The structures were solved by molecular replacement and revealed electron density corresponding to all expected molecules. Refinement is in progress.

Rho complex with a fragment of the ROCK kinase

Rho is a small GTP/GDP-binding protein that binds in a GTP-dependent fashion to its effector molecules - e.g. protein kinases - and thus regulates important cellular processes like actin cytoskeleton rearrangements. The binding and regulation of effector molecules by Rho proteins is poorly understood, and the structure of the Rho complex with the corresponding kinase will help to understand those mechanisms. We collected a complete dataset to 2.6 Å resolution which allowed the solution of the structure by molecular replacement. Refinement is in progress. A dataset to higher resolution should be collected in the future from improved crystals.

Cytochrome P450cam

P450cam is a heme containing mono-oxygenase that splits O_2 to hydroxylate its substrate camphor to 5-exo-hydroxy-camphor with the concomitant production of a water molecule. Some aspects of the reaction mechanism of this important class of enzymes are still poorly understood. The mechanistically important double mutant D251N-T252A is unstable and we

could only obtain very small crystals (5 x 5 x 30 μm). We collected a dataset of a cyanide complex to 2.6 Å resolution.

Oxy C

Oxy A,B and C belong to the heme containing cytochrome P450 superfamily. They are part of a gene cluster involved in the biosynthesis of the medicinally important antibiotic vancomycin, which is derived from the same heptapeptide as the antibiotic balhimycin. The Oxy proteins catalyze the ring-formations in vancomycin. The occurrence of numerous vancomycin-resistant bacteria has demonstrated the need for new glycopeptide antibiotics. In principle, these can be derived enzymatically by modifing the synthesising enzymes such as the Oxy proteins. We crystallized oxyC in a monoclinic spacegroup in the presence of its presumable product the aglycon of vancomycin. A dataset was collected to 1.8 Å resolution. The structure was determined by molecular replacement. The data suggest that the aglycon is present, although at low occupancy. We plan to repeat the exeriment with higher product concentration.

LOV domain

Phototropin, a major blue-light receptor for phototropism, exhibits blue-light-dependent autophosphorylation and contains two LOV (**L**ight, **O**xygen or **V**oltage) domains and a Ser/Thr kinase domain. The LOV domains belong to the PAS superfamily of sensor proteins. They contain a noncovalently bound FMN and exhibit a reversible photocycle that allows them to function as signalling switch. The lifetime of the photoproduct is strongly pH-dependent. We collected a dataset to 2.45 Å resolution of a crystal ($P6_522$) illumited at pH 5.0.

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 seleno-methionne derivative of the S2 fragment in order to determine the structure by Se-MAD. Unexpectedly these crystals are very radiation sensitive and died after half a dataset.

RabGGTase:REP-1 complex

The Ypt/Rab proteins are members of the Ras superfamily of small GTP-binding proteins and have been implicated in the mechanisms by which transport vesicles identify and fuse with their target compartment. Crucial for their functionality is the posttranslational prenylation of C-terminal cysteins by the action of the Rab geranylgeranyltransferase. We have cocrystallized RabGGTase, REP-1, peptide, and a stable analogue of the substrate geranlygeranly-phyrophosphate. The crystals (space group $P2_1$) are extremely thin, and many had to be tested to identify a single one. A complete data set was collected to 3.2 Å resolution.

NO synthase

Nitric Oxide (NO), is one of the most important signalling molecules in biology. It is synthesised by NO synthases (NOS), a family of three isozymes that contain minimally a reductase domain, and a heme domain with a pteridin cofactor. NOS utilizes L-arginine to form citrulline and NO. Two major steps are involved in the arginine-to-citrulline conversion that require electrons and dioxygen: (1) a N-oxidation reaction to produce N-hydroxy-arginine, and (2) a C-N bond cleavage to release NO. The first reaction is believed to be catalyzed in a similar way as the ones by the P450 enzymes. We crystallized inducible NOS from rat in the presence of L-arginine and cyanide, an analogue for oxygen, in spacgroup $P6_122$. A dataset was collected to 2.5 Å resolution.

DAM:DNA complex

The adenine methyltransferase (DAM) from *E.coli* was crystallized in complex with a specific double stranded DNA. Molecular replacement using known DNA methyltransferases as search model for a native data set to 2.2 Å was unsuccessful indicating major structural changes for this MTase (which is indicated as well by biochemical results). Therefore, we have introduced five bromine atoms into the dsDNA and performed a Br-SAD experiment at the white line. The crystals are very thin needles (200 x 20 x 20 μm^3) and are very radiation sensitive. We could collect one complete data set to 3.2 Å resolution with average redundancy of 7.2. However, we do observe significant radiation damage (loss of diffraction power and non-isomorphism) and could not deduce enough anomalous signal for phasing. During the fluorescence scan for the bromine edge we could detect another fluorescence signal which might be caused by bound zinc. We will evaluate the zinc binding potential of the crystals for alternative phasing strategies.

ESRF	**Experiment title:** Studies of structure-function relationship of proteins investigated at the MPI Dortmund	**Experiment number**: LS-2082
Beamline: ID14-2	**Date of experiment**: from: 8-Dec-2001 to: 9-Dec-2001	**Date of report**:
Shifts: 3	**Local contact(s)**: Dr. Stephanie Monaco	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

NO synthase

Nitric Oxide (NO), is one of the most important signalling molecules in biology. It is synthesised by NO synthases (NOS), a family of three isozymes that contain minimally a reductase domain, and a heme domain with a pteridin cofactor. Uncontrolled generation of NO leads to pathology. While NO overproduction by nNos and iNOS is directly linked to the pathogenesis of stroke and shock, respectively, NO generated by eNOS is crucial for angiogenesis and blood pressure regulation. It is therefore highly desirable to design isozyme specific inhibitors. We collected datasets of nNOS (spacegroup $C222_1$) complexed with the inhibitors W1400 and ARL-R17338 to 2.4 and 2.5 Å resolution, respectively. For comparison we collected a dataset of iNOS (spacegroup $P6_122$) complexed with W1400 to 3.2 Å resolution.

Cytochrome P450cam

P450cam is a heme containing mono-oxygenase that splits O_2 to hydroxylate its substrate camphor to 5-exo-hydroxy-camphor with the concomitant production of a water molecule. Some aspects of the reaction mechanism of this important class of enzymes are still poorly understood. We collected a dataset of a cyanide complex of the catalytically impaired D251N mutant to 1.95 Å resolution.

Oxy B

Oxy A,B and C belong to the heme containing cytochrome P450 superfamily. They are part of a gene cluster involved in the biosynthesis of the medicinally important antibiotic vancomycin, which is derived from the same heptapeptide as the antibiotic balhimycin. The Oxy proteins catalyze the ring-formations in vancomycin. The occurrence of numerous vancomycin-resistant bacteria has demonstrated the need for new glycopeptide antibiotics. In principle, these can be derived enzymatically by modifing the synthesising enzymes such as

the Oxy proteins. We crystallized oxyB in the presence of its substrate the linear heptapeptide. A dataset was collected of the monoclinic crystalform to 2.2 Å resolution. The data suggest that the peptide is present, although at low occupancy. We plan to repeat the exeriment with the orthorombic crystal form.

Sulfur-oxidation

For the lithotrophic oxidation of sulfur to sulfuric acid of *Paracoccus pantotrophus* a mechanism is suggested which consists of four protein complexes: SoxXA, SoxYZ, SoxB, and SoxCD. The sulfur-oxidizing system has been reconstituted *in vitro* and accepts hydrogen sulfide, sulfur, thiosulfate or sulfite as electron donors. We collected a preliminary native data set of SoxCD (very long and thin needles, 600 x 50 x 50 μm^3) to 3.6 Å resolution. This data set with very anisotropic diffraction could be used to determine the space group and cell dimensions.

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. We have collected one complete derivative data set to 4.0 Å resolution.

Period

Period is a central component of the circadian master clock, which controls the organism's 24h sleep-wake cycle. The Period molecule contains two PAS domains, which mediate its interaction with other clock proteins. To establish the correct space group of our Period crystals and attempt structure determination by molecular replacement, we have collected a 4 Å data set, which was complete in space group C2.

VASP

VASP is involved in actin cytoskeleton dynamics. Tetramerisation of VASP enhances F-actin bundling and interactions of VASP with other proteins. In order to attempt structure determination by molecular replacement or direct methods, we have collected a complete 1.3 Å data set (space group I4) from a native VASP crystal.