EUROPEAN SYNCHROTRON RADIATION FACILITY
INSTALLATION EUROPÉENNE DE RAYONNEMENT SYNCHROTRON
ESRF

Application for Structural Biology BAG Beam Time at the ESRF

Proposal Summary

Title

Institut Pasteur BAG - Structural biology of infectious agents

Keywords
#1: Viruses #2: Actinobacteria #3: DNA processing enzymes #4: Drug targets

Abstract

The Institut Pasteur's BAG gathers the projects of the research units and platforms of the Institut Pasteur in Paris which are active in the field of structural biology: Structural Microbiology (P.M. Alzari), Structural Dynamics of Macromolecules (M. Delarue), Structural Virology (F. Rey) and the Platform of Crystallography (A. Haouz). A non-exhaustive list of ongoing projects includes:

- actinobacterial cell division proteins, central metabolic enzymes and their complexes;
- DNA gyrases, topoisomerases and regulators from mycobacteria or model organisms;
- eukaryotic and archeal DNA polymerases and replisome proteins;
- viral envelope proteins from pathogenic viruses, and their complexes with neutralizing antibodies;
- enzymes and structural proteins from other human pathogens, supported by evolving national and international collaborations.

- **This proposal is:**
  A new BAG proposal OR A continuation of BAG proposal reference : MX - 2410
- This proposal is:
  Fundamental Science ☑ % 90 Applied Science ☑ % 10 Industrial Science ☐ % 0

Societal Themes

- ○ Earth and Environment
- ◉ Health
- ○ Information and Communication Technology (ICT)
- ○ Other Functional Materials
- ○ Energy
- ○ Fundamental Science
- ○ Other

Scientific Area of the proposal

◉ MX - Macromolecular Crystallography

Main proposer (to whom correspondence will be addressed):
Laboratory Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS
Name Dr **BELLINZONI Marco** Phone 0145688608 Fax 0145688604
Email marco.bellinzoni@pasteur.fr

Principal Investigators: Please list ONLY crystallographers. (Laboratory if different from main proposer)
Name Dr. **ALZARI Pedro M.** Phone +33 (0)1 45 68 86 07 Email alzari@pasteur.fr
Name Dr **PETRELLA Stephanie** Phone 06 07 11 38 28 Email stephanie.petrella@pasteur.fr
Name Dr **WEHENKEL Annemarie** Phone 0145688606 Fax 0145688604
Email annemarie.wehenkel@pasteur.fr

Laboratory Institut Pasteur Biochemie et Biophysique des Macromolecules Dept de Biologie Structurale et Chimie 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15
Name Dr **RAYNAL Bertrand** Phone +33(0)144389475 Email bertrand.raynal@pasteur.fr

Laboratory Institut Pasteur Unité de Dynamique Structurale des Macromolec 25 rue du Dr. Roux FR - 75015 PARIS
Name Dr **DELARUE Marc** Phone 0145688605 Email delarue@pasteur.fr
Name Dr **SAUGUET Ludovic** Phone 0630085844 Email ludovic.sauguet@pasteur.fr

Laboratory Institut Pasteur Unité de Virologie Structurale 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15
Name Dr **DUQUERROY Stephane** Phone (0)145688266 Fax (0)145668993 Email sduquer@pasteur.fr
Name Dr **GUARDADO CALVO Pablo** Phone +330787192508 Fax +330145688287
Email pablo.guardado@gmail.com
Name Professo**REY Felix** Phone 45688563 Email rey@pasteur.fr

Société Civile
RCS Grenoble D 338 723 919
N° Siret 338 723 919 00027 - APE 7219 Z
Postal Address: ESRF User Office - CS 40220 - 38043 Grenoble Cedex 9 - France
Location: Polygone Scientifique Louis Néel - 71, avenue des Martyrs - 38000 Grenoble - France
Telephone (Switchboard): 33-(0)4 76 88 20 00 - Telefax: 33-(0)4 76 88 20 20

Name Dr **VANEY Marie-Christine** Phone 01 45 68 85 95 Fax 01 45 68 89 93
Email mcvaney@pasteur.fr

Laboratory Institut Pasteur Plateforme de Cristallographie 25 rue du Dr. Roux FR - 75015 PARIS
Name Dr **HAOUZ Ahmed** Phone 01 40 61 39 32 Fax 01 45 68 86 04 Email ahaouz@pasteur.fr

Summary of Beamtime Requested FOR SIX MONTHS

☑ Multi-wavelength	3	shifts
☑ Single-wavelength	3	shifts
☑ BM29 Bio-SAXS	3	shifts
☐ Cryo-EM		shifts
☐ ID29 SSX & TR-SSX		shifts
Total	9	shifts

Requests for specific beamlines or equipment:

For projects requiring screening of a large number of samples, it would be appropriate having 3 to 6 single-wavelength shifts allocated on the automated beamline ID30A-1. Thanks

Laboratory Support Facility

- ☐ BioMedical Facility
- ☐ Central Chemistry Lab (Science Building)
- ☐ PSCM Labs (Science Building)
- ☐ Protein Support labs
- ☐ icOS Lab (in crystallo Optical Spectroscopy)

Global Summary

Global Summary of this new BAG proposal including projects/experiments proposed and justification of the beamtime requested. Enter 8000 chars maximum (approximately 1000 words).

ALZARI

Bacterial signalling and cell division: The broader aim of the proposed experiment is to understand the molecular mechanisms of divisome and elongasome assembly and regulation in Corynebacteriales. In order to achieve this, we are using an integrative structural biology approach. High-resolution structures of individual components of the divisome/elongasome as well as sub-complexes formed of 2 or more proteins will be required. The samples that will be used are proteins of the actinobacterial divisome (FtsZ, FtsQ/B/L, SteA/B, RipA, Sip1, Sip2, DivIVA) and mutants thereof, as well as protein complexes whenever they can be isolated and crystallized. In parallel we will aim to identify new protein partners of the divisome using proteomic approaches, which will then be subjected to crystallization.

Actinobacterial central metabolism: we study the molecular basis of the fine regulation of central metabolism in actinobacteria, in the frame of an ANR/DFG grant with two groups in Germany (M. Bott, FZ Jülich, and B. Eikmanns, Univ. of Ulm). In particular, we are investigating protein-protein interactions and signal transduction along the GlnH-GlnX-PknG-GarA pathway, responsible, in Corynebacteriales, for the regulation of key metabolic enzymes located at the 2-oxoglutarate node (crossroad between carbon and nitrogen metabolism). Still part of this collaboration, we are performing structural studies on corynebacterial pyruvate-quinone oxidoreductase (PQO), an alternative way for pyruvate oxidation which physiological role is still to be determined.

DNA gyrases and topoisomerases: We solved the structure of the four individual Mtb DNA gyrase domains and of a full fusion version of this atypical protein. This permitted to dissect the structural aspects of the catalytic cycle of this gyrase. We are now working on its structural homolog from Corynebacterium glutamicum. We want to pursue the full structural characterization of the DNA gyrase in complex with its partners, e.g. solve the structures of these partners alone and in complex with the protein (individual domains but also entire enzyme) and inhibitors. These data are essential to understand the mechanisms of regulation of this family of topoisomerases.

Collaborative projects: in parallel to the main projects of the lab, described above, several collaborative projects on structural enzymology are ongoing, essentially on proven drug targets, or proteins involved in antibiotic resistance mechanisms. Noteworthy, we are part of an internal collaborative effort to develop new, highly active inhibitors of the SARS-CoV-2 protease (nsp5), which will involve extensive cocrystallization/soaking trials on nsp5 crystals for all promising hits (in collaboration with the crystallography platform, also part of this BAG). Another project concerns the structural characterization of the mycobacterial Bayer-Villiger monooxygenase EthA, known to be involved in the activation of several anti-tuberculosis prodrugs (collaboration J. Kordulakova, Univ. of Bratislava, Slovakia).

DELARUE

1) DNA/RNA polymerases project: we plan to solve the structure of the primer-independent PiPOLB polymerase in presence of DNA. In addition, we have crystals of a simplified multi-subunit RNA polymerase that we have phased and that diffract to 4.0 Angstrom. We plan to collect data at higher resolution and also to solve its complex with a helicase. Other RNA polymerases of viral origin are being cloned and expressed for crystallization tests.
Moreover, DNA polymerases of other phages that possess highly modified DNA are being studied to understand how they cope with this modified DNA as a template.

2) Archaeal replisome: Chromosomal DNA replication requires the complex interplay of a large number of protein factors in a coordinated manner. This multi-protein replication machine is named the replisome. As DNA replication depends on the coordinated interplay between many replication factors, Current trends are to determine the structures of complexes between different replication factors and DNA. Our model system is Pyrococcus abyssi, a hyperthermophilic archaea that was isolated next to ocean hot vents. The archaeal replisome is often considered as a simpler version of the eukaryotic one, and most archaeal replication factors express readily in E. coli and are good structural models. We have recently determined the first crystal and cryo-EM structures of the replication protein A, a key actor of the replisome that coats and protects single-stranded DNA from nucleases. We will now determine structures of the replication protein A in complex with other key actors of the replisome, including the DNA polymerase and the DNA primase.

HAOUZ

Several research teams at the Institut Pasteur have federated their expertise as a consortium to initiate drug discovery projects around the strategic axes of the Institut Pasteur, including antimicrobioal resistance, emerging infectious diseases, central nervous system pathologies and cancer. These teams offer complementary expertise in chemical library design and management, genetic screening (siRNA/gRNA), chemistry, virtual screening and chemoinformatics using Deep Learning, in vitro ADME-Tox profiling, and technologies available to support target identification/engagement. Many proteins targets are now included in this pipeline such as (SARS-CoV-2 NSP5 protease; epigenetic methyltransferases; Plasmodium vivax SUB1 protease; Mycobacterial histidine kinases; Cys-loops receptors; and others).

As a member of the drug discovery consortium at Pasteur, the crystallography platform will provide expertise in (i) HTS crystallization to generate crystals of drug-target complexes (ii) X-Ray data collection to solve the corresponding 3D structures. The final goal of these projects is to validate a potential hit to pave the way for the development of news drugs against human diseases.

REY
The group is still focused on four main objectives:
1) Viral envelope proteins from pathogenic viruses: ex. bunyaviruses, herpesviruses and their complexes with receptors, coronavirus (such as SARS-CoV-2) and flaviviruses.
2) Antigen/antibody complexes: by targeting epitopes by the most potent and broadly neutralizing human antibodies against envelope glycoproteins of pathogenic viruses such as bunyaviruses and flaviviruses (zika, dengue, yellow fever).
3) Viral replication: by understanding the genome transcription/replication on the respiratory syncytial virus.
4) Cellular membrane fusion proteins: such as class I cell-cell fusion syncytin and class II cellular eukaryotic membrane fusion proteins.

New project:
Poxviruses (variola, zoonotic monkeypox and ORF viruses) code for an “entry/fusion complex” (EFC) formed by 11 membrane proteins and several viral repressors. Little is known about the EFC organization on the viral surface or the mechanisms of activation and repression. The objective is to obtain high resolution structures of the entry-fusion complex embedded in membranes alone and in complex with fusion repressors. This project could help to better understand and develop treatments against poxviruses, but also for other related DNA viruses.

Samples and Datasets Summary

Sample Description(acronym)	Fixed #˜0.93Å	MAD # = 0.8Å - 1.3Å	MAD Other # Range	SAD # = 0.8Å - 1.3Å	SAD Other # Range	S-SAD	BioSAXS	Cryo-EM

Bunyavirus glycoproteins (BUNV)	20		0		0	0		
Cellular membrane fusion proteins (CELL)	10	20	0	20	0	0		
Corynebacterial dihydrolipoyl dehydrogenase (CgLpd)	20		0		0	0		
Dengue virus envelope proteins (DENV)	20		0		0	0		
FAD-dependent monooxygenase EthA (EthA)	20	5	0		0	0		
Flavivirus envelope proteins (POW,KFDV,OHFV) (FLAV)	20		0		0	0		
Corynebacterial cell division protein FtsB (FtsB)	15		0	15	0	0		
Corynebacterial cell division protein FtsL (FtsL)	15		0	15	0	0		
Corynebacterial cell division protein FtsQ (FtsQ)	20		0	10	0	0		
ABC transporter permease FtsX (FtsX)	10		0	10	0	0		
Mycobacterial glutamate dehydrogenase (GDH)	20	5	0		0	0		
Hantaan virus envelope proteins (HANT)	30		0	30	0	0		
Herpes virus envelope proteins (HSV)	20	10	0	10	0	0		
Kamiti River virus envelope proteins (KRV)	50		0		0	0		
M. tuberculosis DNA gyrase (MTGyr)	20		0		0	0		
Corynebacterial FHA regulator OdhI (OdhI)	20		0		0	0		
Pentameric ligand gated ion channel from bacteria (pLGIC)	15		0	1	0	0		
archaeal DNA polymerase D (PolD)			0		0	0		
Pyruvate:quinone oxidoreductase (PQO)	30		0		0	0		
Peptidoglycan hydrolase RipC (RipC)			0		0	0		
Respiratory syncitial virus ribonucleoprotein (RSV)	30	10	0	0	0	0		
Mycobacterial cell division protein Rv1698 (Rv1698)	50		0	10	0	0		
Coronavirus spike protein (SARS)	50		0		0	0		
Divisome protein Sip2 (Sip2)	20		0	5	0	0		
SteA/B/C complex (Ste)	20		0		0	0		
Corynebacterial cell division protein SteA (SteA)	10		0		0	0		
Corynebacterial cell division protein SteB (SteB)			0		0	0		
Tick-borne encephalitis virus envelope proteins (TBEV)			0		0	0		
Vaccinia virus envelope proteins (VACC)			0		0	0		
Yellow fever 17D/Asibi virus envelope proteins (YFV)	10		0		0	0		
Zika virus envelope proteins (ZIKV)	100		0		0	0		
Total:	665	50	0	126	0	0	0	0

Samples Scientific Justification

- BUNV (Bunyavirus glycoproteins)Bunyaviruses is a large family of viruses that infect humans and animals with great relevance in human health. The envelope glycoproteins are the sole target for neutralizing antibodies and antivirals and we are still working on their structures in order to develop novel immunogens for vaccination. We have already solved the structures of several bunyaviral glycoproteins both in prefusion and postfusion conformations and we are still working on glycoproteins of other bunyaviruses in complex or not with neutralizing human antibodies.
- CELL (Cellular membrane fusion proteins)We have determined the crystal structure of the C. elegans protein EFF-1, a member of the “fusion family” (FF). EFF-1 is responsible for a cell-cell fusion event during skin formation in the nematode. We will now focus in determining the crystal structure of the monomeric pre-fusion form of EFF-1, and of the intact trans-membrane post fusion trimer. IWe want also make use the experience accumulated over the years in crystallizing viral glycoproteins, to apply it to the conserved family of HAP2/GSC1 proteins involved in fusion of gametes during fertilization. These proteins exhibit a similar pattern of secondary structure elements in the ectodomain as class II proteins, but only a crystallographic analysis can identify a possible structural homology and provide the basis to understand the molecular mechanisms of cell-cell fusion.
- CgLpd (Corynebacterial dihydrolipoyl dehydrogenase)Conserved FAD-dependent dehydrogenase, essential to the PDH and ODH reactions and component of a mixed PDH-ODH supercomplex in C. glutamicum. The structure has been solved, but experiments are ongoing to clarify its regulation and protein-protein interactions within the supercomplex.
- DENV (Dengue virus envelope proteins)We solved several structures of envelope protein E from dengue virus from all the serotype (DENV-1-4) in complex with neutralizing antobody fragments Fab/scFv. We are still working on complexes of E with monoclonal human antibodies. In general we have small crystals and a microfocus beamline is essential to pursue the project.

- EthA (FAD-dependent monooxygenase EthA)Bayer-Villiger monooxygenase known to be involved in the activation of a number of prodrugs in Mycobacterium tuberculosis. As the enzyme has so far escaped all attempts to structural determination, we are working on its Mycobacterium thermoresistibile orthologue. Diffracting crystals have been obtained and their optimization is in progress (collab. Jana Kordulakova, Comenius Univ. Bratislava, Slovakia).
- FLAV (Flavivirus envelope proteins (POW,KFDV,OHFV))We are trying to crystallize the post-fusion form of sE from several flaviviruses with a longer stem in order to better understand the role of the stem during the fusion process.
- FtsB (Corynebacterial cell division protein FtsB)FtsB is part of the actinobacterial divisome for which we are trying to elucidate the molecular mechanisms of assembly and regulation. We are trying to co-crystallize this membrane protein in complex with its native partners, FtsQ and FtsL.
- FtsL (Corynebacterial cell division protein FtsL)FtsL is part of the actinobacterial divisome for which we are trying to elucidate the molecular mechanisms of assembly and regulation. We are trying to co-crystallize the membrane protein complex with its partners, FtsB and FtsQ.
- FtsQ (Corynebacterial cell division protein FtsQ)FtsQ is part of the actinobacterial divisome for which we are trying to elucidate the molecular mechanisms of assembly and regulation. The protein from two different actinobacterial species is currently undergoing crytsallization trials. We will eventually try to co-crystallize with its native partners, FtsB and FtsL.
- FtsX (ABC transporter permease FtsX)Part of a membrane transporter involved in cell division, proposed to promote cell separation with the peptidoglycan hydrolase RipC
- GDH (Mycobacterial glutamate dehydrogenase)Key protein for mycobacterial metabolism, inhibited by the FHA protein GarA as for the a-ketoglutarate decarboxylase KGD. However, the mechanism of inhibition by GarA on this enzyme is still unknown. Crystals diffracting to low resolution have been obtained and their optimization is in progress.
- HANT (Hantaan virus envelope proteins)Hantaviruses are important pathogens in humans and animals. We have obtained crystals of the viral envelope glycoprotein Gc. We are still working on glycoproteins of several hantavirus such as Andes virus.
- HSV (Herpes virus envelope proteins)We obtained a complete data set at 2.9 A resolution and solved the structure of pseudorabies gH fragment in complex with a Fab using MAD phasing. We still work on gH/gL complex from Herpes Simplex Virus 1 and pseudorabies virus.
- KRV (Kamiti River virus envelope proteins)Kamiti River virus (KRV) is a insect-specific flavivirus (ISF), which unlike the pathogenic flavivirus fails to replicate into mammalian cells. The envelope proteins of the ISFs have the characteristic to have a short-stem compared to the pathogenic flavivirus. We are interested in the interaction of the stem (before the trans-membrane region) with the fusion loops to better understand the fusion process. We solved the structure of soluble E glycoprotein of KRV in a post-fusion form with a short-stem. We have now crystals of the KRV sE protein with longer stems.
- MTGyr (M. tuberculosis DNA gyrase)We have solved the structure of the four individual Mtb DNA gyrase domains and of a full fusion version of this atypical protein. This permitted to dissect the structural aspects of the catalytic cycle of this gyrase. We want to pursue the full structural characterization of the DNA gyrase in complex with its partners, e.g. solve the structures of these partners alone and in complex with the protein (individual domains but also entire enzyme) and inhibitors. These data are essential to understand the mechanisms of regulation of this atypical topoisomerase. Crystals for a full length version of the gyrase are available, they diffract to 20A and have to be optimised (extensive screening)
- OdhI (Corynebacterial FHA regulator OdhI)OdhI is a small, FHA domain protein found in Actinobacteria and acting as a metabolic switch. We are characterizing complexes of this protein with the target enzymes in order to decipher the molecular basis of OdhI regulation.
- pLGIC (Pentameric ligand gated ion channel from bacteria)Pentameric ligand gated ion channel mediate signal transduction in the brain, for instance at the synaptic cleft. Bacterial homologues in this protein family are currently the only ones amenable to x-ray crystallography. Our group pioneered the discovery and structural characterization the the GLIC homologue (Bocquet et al., Nature 2009, Nury et al., PNAS 2010). We now try to further understand the gating mechanism of the protein by crystallizing it in several conformations. This implies mutagenesis, change of conditions, addition of ligands, etc... We need to test a lot of tiny crystals of this membrane protein that rarely diffract, Therefore we need regular access to beamlines with good automation and good vizualization/centering systems like the ESRF ones. We rarely need MAD or SAD for this project.
- PolD (archaeal DNA polymerase D)PolD is a novel DNA polymerase. It is essential in Archaea and have a great biotechnological potential
- PQO (Pyruvate:quinone oxidoreductase)Pyruvate:quinone oxidoreductase is a known metabolic alternative for pyruvate oxidation in actinobacteria, but its physiological role stays unknown. We therefore aim at studying the structure and the regulation of this enzyme from Corynebacterium glutamicum, which is closely related to homologous enzymes from other actinobacteria (but not Mycobacterium, where it is absent), enterobacteria and pseudomonads and more distantly related to pyruvate oxidases from “low GC Gram-positive bacteria” (lactic acid bacteria, bacillales). The enzyme is known to bind FAD and TPP.
- RipC (Peptidoglycan hydrolase RipC)
- RSV (Respiratory syncitial virus ribonucleoprotein)Human respiratory syncytial virus (hRSV) is the most common cause of lower respiratory tract infections in young children worldwide. It is a nonsegmented, negative-stranded RNA virus that belongs to the order Mononegavirales. The viral RNA of Mononegavirales is encapsidated by the nucleoprotein (N), making a ribonucleoprotein (RNP) complex of defined stoichiometry. This RNP complex serves as the template for viral RNA replication and transcription by the polymerase complex. When N is expressed in E. coli forms a ring-like structure, composed predominantly of 10 molecules of N and a short non-specific RNA of ¡Ö 70 bases. This structure will constitute the first atomic structure of the ribonucleoprotein of a Mononegavirales.
- Rv1698 (Mycobacterial cell division protein Rv1698)Protein involved in cell division and septation in Corynebacteriales, where it is supposed to form a complex with SteA. Our structural data on the Corynebacterium glutamicum orthologue, as well as the preliminary data on the mycobacterial Rv1698 argue against this protein being a porin as proposed in the literature. We are now pursuing the structural and functional characterization of this protein.
- SARS (Coronavirus spike protein)We study neutralizing and potentially broadly cross-reactive nanobodies against the S proteins of betacoronaviruses.
- Sip2 (Divisome protein Sip2)Sip2 is a newly identified divisome member from C. glutamicum and its function is unknown. It is a transmembrane domain and the full-length protein as well as fragments thereof will be crystallized alone or in complex with its partner proteins.
- Ste (SteA/B/C complex)This sample is a complex of 3 proteins involved in cell division in actinobacteria, two of which are of unknown function and transmembrane. We are currently carrying out crystallization trials of subcomplexes and the full 3 protein complex. 2 individual structures of the soluble domains are already available.
- SteA (Corynebacterial cell division protein SteA)Membrane protein proposed to form a complex with SteB/C and to play a role in the control of cell separation through the RipC pathway. The structure of its soluble domain has recently been solved by our group, and we are now aiming at solving the structures of its relevant protein complexes.
- SteB (Corynebacterial cell division protein SteB)
- TBEV (Tick-borne encephalitis virus envelope proteins)
- VACC (Vaccinia virus envelope proteins)Poxviruses (variola, zoonotic monkeypox and ORF viruses) code for an “entry/fusion complex” (EFC) formed by 11 membrane proteins and several viral repressors. The objective is to obtain high resolution structures of the EFC embedded in membranes alone and in complex with fusion repressors and how these complexes are organized on the viral surface. This project could help to better understand and develop treatments against poxviruses, but also for other related DNA viruses.

- YFV (Yellow fever 17D/Asibi virus envelope proteins)The crystal structure of Yellow fever glycoproteins in complex with neutralizing monoclonal antobodies.
- ZIKV (Zika virus envelope proteins)We solved several structures of ZIKV E protein in complex with Fab/scFv. We are working on different mutants and to obtain complexes with monoclonal human antibodies. In general we have small crystals and a microfocus beamline is essential to pursue the project.

ESRF BLOCK ALLOCATION GROUP: FULL 2-YEAR REPORT

BAG RESPONSIBLE: Marco Bellinzoni
PROPOSAL REF.: 93673 (continuation of MX-2410)

SHIFT USAGE SINCE LAST FULL 2-YEAR REPORT:

Allocated:	141	**Cancelled By Users:**	38	**Total Number Of Visits:**	32
Scheduled:	72	**Cancelled By ESRF:**	0	**Total Number Of Visitors:**	10
Used:	34				

COMMENT ON SHIFTS:

BAG PRINCIPAL INVESTIGATORS: (L) = no longer active in the BAG, (N) = new since last full 2-year report

- C. Mayer, Institut Pasteur 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15 (L)
- A. Wehenkel, Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS
- S. Petrella, Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS
- P. Alzari, Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS
- M. Bellinzoni, Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS
- B. Raynal, Institut Pasteur Biochemie et Biophysique des Macromolecules Dept de Biologie Structurale et Chimie 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15
- L. Sauguet, Institut Pasteur Unité de Dynamique Structurale des Macromolec 25 rue du Dr. Roux FR - 75015 PARIS
- M. Delarue, Institut Pasteur Unité de Dynamique Structurale des Macromolec 25 rue du Dr. Roux FR - 75015 PARIS
- M. Delarue, Institut Pasteur Unité de Dynamique Structurale des Macromolec 25 rue du Dr. Roux FR - 75015 PARIS
- M. Vaney, Institut Pasteur Unité de Virologie Structurale 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15
- P. Guardado Calvo, Institut Pasteur Unité de Virologie Structurale 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15
- F. Rey, Institut Pasteur Unité de Virologie Structurale 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15
- S. Duquerroy, Institut Pasteur Unité de Virologie Structurale 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15
- F. Rey, Institut Pasteur Unité de Virologie Structurale 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15
- A. Haouz, Institut Pasteur Plateforme de Cristallographie 25 rue du Dr. Roux FR - 75015 PARIS
- N. Reyes, Institut Pasteur Molecular Mechanisms of Membrane Transport Dept of Structural Biology and Chemistry FR - 75724 PARIS Cedex 15 (L)

FROM DATA COLLECTED ON ESRF BEAMLINES SINCE LAST FULL 2-YEAR REPORT:

Total number of PDB submissions:	Total number of publications:
15	11

FIVE MOST IMPORTANT PUBLICATIONS directly resulting from data recorded either wholy or partially on ESRF beamlines - (1): ESRF data only, (2): from more than one source

- Structural basis of synergistic neutralization of Crimean-Congo hemorrhagic fever virus by human antibodies | Mishra A.K., Hellert J., Freitas N., Guardado-Calvo P., Haouz A., Fels J.M., Maurer D.P., Abelson D.M., Bornholdt Z.A., Walker L.M., Chandran K., Cosset F.L., McLellan J.S., Rey F.A. | Science, vol.375, p.104-109, 2022 (2)

- The epitope arrangement on flavivirus particles contributes to Mab C10's extraordinary neutralization breadth across Zika and dengue viruses | Sharma A., Zhang X., Dejnirattisai W., Dai X., Gong D., Wongwiwat W., Duquerroy S., Rouvinski A., Vaney M.C., Guardado-Calvo P., Haouz A., England P., Sun R., Zhou Z.H., Mongkolsapaya J., Screaton G.R., Rey F.A. | Cell, vol.184, p.6052-6066, 2021 (2)

- Evolution and activation mechanism of the flavivirus class II membrane-fusion machinery | Vaney M.C., Dellarole M., Duquerroy S., Medits I., Tsouchnikas G., Rouvinski A., England P., Stiasny K., Heinz F.X., Rey F.A. | Nature Communications, vol.13, p.3718-1-3718-12, 2022 (2)

- Topoisomerase I (TOP1) dynamics: Conformational transition from open to closed states | Takahashi D.T., Gadelle D., Agama K., Kiselev E., Zhang H., Yab E., Petrella S., Forterre P., Pommier Y., Mayer C. | Nature Communications, vol.13, p.59-1-59-11, 2022 (2)

- Actinobacteria challenge the paradigm: A unique protein architecture for a well-known, central metabolic complex | Bruch E.M., Vilela P., Yang L., Boyko A., Lexa-Sapart N., Raynal B., Alzari P.M., Bellinzoni M. | Proceedings of the National Academy of Sciences of the USA, vol.118, p.e2112107118-1-e2112107118-11, 2021 (2)

SUMMARY OF THE RESULTS OBTAINED SINCE LAST FULL 2-YEAR REPORT:

ALZARI
1) Bacterial signalling and cell division: Several datasets have been collected and the structure of the N-terminal domain of Corynebacterium glutamicum DivIVA has been solved at around 1.5 Å resolution, from two different crystal forms (Martinez et al., in preparation).
2) Metabolic enzymes and structural enzymology: several datasets were collected from crystals of components of a mixed PDH/ODH supercomplex in C. glutamicum. Relevant results include the crystal structures of: catalytic domain of the dihydrolipoyl acetyltransferase (AceF) in complex with the N-terminal peptide of OdhA; dihydrolipoyl dehydrogenase (Lpd) in complex with the PSBD domain, at 1.6 Å resolution (Yang et al., in preparation); C. glutamicum pyruvate quinone oxidoreductase (collaboration with B. Eikmanns, Ulm); ligand binding domain of the chemoreceptor Htc10 from Halomonas titanicae (collaboration with M.N. Lisa, Rosario, Argentina); bovine glutamate dehydrogenase in complex with ADP and the allosteric regulator leucine (Aleshin et al., submitted).
3) DNA gyrases and topoisomerases: we are performing structural studies of Corynebacterial DNA gyrases (wild type, mutants and isolated domains) in complex with substrates, inhibitors, and DNA gyrase proteins partners involved in its regulation (GIP, EngA, MfpA/B).

DELARUE
1) DNA polymerases project: We have determined the structures of DNA polymerases of the PolB family, which had been evolved to accept synthetic (non-natural) nucleic acids (XNA), especially the structure of a functional HNA-incorporating mutant of the archaeal PolB from Thermococcus gorgonarius (Sansom et al, Biomolecules 2020). We also determined the structure of a primer-independent polymerase from PolB family, PiPOLB, which can both initiate and carry out an entire replication cycle of the circular DNA. We are trying to solve its structure as a complex with its various DNA substates.
2) Archaeal replisome: We have solved the structure of the archaeal replicative DNA polymerase PolD in complex with DNA and with its main replication factor, the proliferation cell nuclear antigen (PCNA) (Madru et al., Nature Commun. 2020) and recently the replication protein A (Madru et al., BioRxiv, 2022). This work included several new structures solved by cryo-EM and X-ray crystallography, as well as a wide range of biophysical and functional assays. More specifically, the structure of the complex between PCNA and the PolD-PIPbox was determined at ESRF (ID29) using X-ray crystallography at 2.7Å resolution (PDB: 6T7Y).
3) Incorporation of 2-aminoadenine into the DNA of the S-2L bacteriophage: crystals structures at high resolution (0.8 - 1.5 Å) of 4 different enzymes were solved that explain how 2-aminoadenine is synthesized and processed in atomic details (Czernecki et al., Nature Commun. 2021a and 2021b). We also solved the structure of the polymerase of a related phage that also have 2-aminoadenine in its genome (Czernecki et al., NAR 2021).

REY
We have published the structures of ectodomains of envelope proteins from human or veterinary pathogenic viruses concerning class II viral envelope proteins:
- X-ray structures of the tick-borne encephalitis virus (pr/sE)2 complex, revealing a new molecular switch that is used to control the processes of virus assembly, virus maturation and entry into new cells (Vaney et al, 2022),
- X-ray structures of Gc post-fusion trimers and complexes of mAbs/Gc from Crimean-Congo hemorrhagic fever virus, revealing the neutralization mechanism of antibodies against the virus (Mishra et al, 2022),
- X-ray structures of E from Dengue virus serotypes 1-4 and Zika virus in complex with neutralizing human monoclonal Ab C10, highlighting the importance not only of paratope/epitope complementarity but also the topological distribution for epitope-focused vaccine design (Sharma et al, 2021).

ALL PUBLICATIONS directly resulting from the use of data recorded on ESRF beamlines since last full 2-year report: (1): ESRF data only, (2): from more than one source

• Structural basis of synergistic neutralization of Crimean-Congo hemorrhagic fever virus by human antibodies | Mishra A.K., Hellert J., Freitas N., Guardado-Calvo P., Haouz A., Fels J.M., Maurer D.P., Abelson D.M., Bornholdt Z.A., Walker L.M., Chandran K., Cosset F.L., McLellan J.S., Rey F.A. | Science, vol.375, p.104-109, 2022 (2)

• The epitope arrangement on flavivirus particles contributes to Mab C10's extraordinary neutralization breadth across Zika and dengue viruses | Sharma A., Zhang X., Dejnirattisai W., Dai X., Gong D., Wongwiwat W., Duquerroy S., Rouvinski A., Vaney M.C., Guardado-Calvo P., Haouz A., England P., Sun R., Zhou Z.H., Mongkolsapaya J., Screaton G.R., Rey F.A. | Cell, vol.184, p.6052-6066, 2021 (2)

• Evolution and activation mechanism of the flavivirus class II membrane-fusion machinery | Vaney M.C., Dellarole M., Duquerroy S., Medits I., Tsouchnikas G., Rouvinski A., England P., Stiasny K., Heinz F.X., Rey F.A. | Nature Communications, vol.13, p.3718-1-3718-12, 2022 (2)

• Topoisomerase I (TOP1) dynamics: Conformational transition from open to closed states | Takahashi D.T., Gadelle D., Agama K., Kiselev E., Zhang H., Yab E., Petrella S., Forterre P., Pommier Y., Mayer C. | Nature Communications, vol.13, p.59-1-59-11, 2022 (2)

• Actinobacteria challenge the paradigm: A unique protein architecture for a well-known, central metabolic complex | Bruch E.M., Vilela P., Yang L., Boyko A., Lexa-Sapart N., Raynal B., Alzari P.M., Bellinzoni M. | Proceedings of the National Academy of Sciences of the USA, vol.118, p.e2112107118-1-e2112107118-11, 2021 (2)

• Structural dynamics and determinants of 2-aminoadenine specificity in DNA polymerase DpoZ of vibriophage VC8 | Czernecki D., Hu H., Romoli F., Delarue M. | Nucleic Acids Research, vol.49, p.11974-11985, 2021 (2)

• The antibacterial Type VII Secretion System of Bacillus subtilis: structure and interactions of the pseudokinase YukC/EssB | Tassinari M., Doan T., Bellinzoni M., Chabalier M., Ben-Assaya M., Martínez M., Gaday Q., Alzari P.M., Cascales E., Fronzes R., Gubellini F. | mBio, p.in press, 2022 (2)

• 3D architecture and structural flexibility revealed in the subfamily of large glutamate dehydrogenases by a mycobacterial enzyme | Lázaro M., Melero R., Huet C., López-Alonso J.P., Delgado S., Dodu A., Bruch E.M., Abriata L.A., Alzari P.M., Valle M., Lisa M.N. | Communications Biology, vol.4, p.684-1-684-8, 2021 (2)

• A tetratricopeptide repeat scaffold couples signal detection to OdhI phosphorylation in metabolic control by the protein kinase PknG | Lisa M.N., Sogues A., Barilone N., Baumgart M., Gil M., Graña M., Durán R., Biondi R.M., Bellinzoni M., Bott M., Alzari P.M. | mBio, vol.12, p.e0171721-1-e0171721-14, 2021 (2)

• A high-affinity calmodulin-binding site in the CyaA toxin translocation domain is essential for invasion of eukaryotic cells | Voegele A., Sadi M., O'Brien D.P., Gehan P., Raoux-Barbot D., Davi M., Hoos S., Brûlé S., Raynal B., Weber P., Mechaly A., Haouz A., Rodriguez N., Vachette P., Durand D., Brier S., Ladant D., Chenal A. | Advanced Science, vol.8, p.2003630-1-2003630-11, 2021 (2)

• Deciphering the unexpected binding capacity of the third PDZ domain of whirlin to various cochlear hair cell partners | Zhu Y., Delhommel F., Cordier F., Lüchow S., Mechaly A., Colcombet-Cazenave B., Girault V., Pepermans E., Bahloul A., Gautier C., Brûlé S., Raynal B., Hoos S., Haouz A., Caillet-Saguy C., Ivarsson Y., Wolff N. | Journal of Molecular Biology, vol.432, p.5920-5937, 2020 (2)

LIST OF BAG PARTICIPANTS (including Principal Investigators) during the reporting period:

- C. Mayer, Institut Pasteur 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15 (PI)
- A. Wehenkel, Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS (PI)
- S. Petrella, Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS (PI)
- P. Alzari, Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS (PI)
- M. Bellinzoni, Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS (PI)
- B. Raynal, Institut Pasteur Biochemie et Biophysique des Macromolecules Dept de Biologie Structurale et Chimie 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15 (PI)
- L. Sauguet, Institut Pasteur Unité de Dynamique Structurale des Macromolec 25 rue du Dr. Roux FR - 75015 PARIS (PI)
- M. Delarue, Institut Pasteur Unité de Dynamique Structurale des Macromolec 25 rue du Dr. Roux FR - 75015 PARIS (PI)
- M. Delarue, Institut Pasteur Unité de Dynamique Structurale des Macromolec 25 rue du Dr. Roux FR - 75015 PARIS (PI)
- M. Vaney, Institut Pasteur Unité de Virologie Structurale 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15 (PI)
- P. Guardado Calvo, Institut Pasteur Unité de Virologie Structurale 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15 (PI)
- F. Rey, Institut Pasteur Unité de Virologie Structurale 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15 (PI)
- S. Duquerroy, Institut Pasteur Unité de Virologie Structurale 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15 (PI)
- F. Rey, Institut Pasteur Unité de Virologie Structurale 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15 (PI)
- A. Haouz, Institut Pasteur Plateforme de Cristallographie 25 rue du Dr. Roux FR - 75015 PARIS (PI)
- N. Reyes, Institut Pasteur Molecular Mechanisms of Membrane Transport Dept of Structural Biology and Chemistry FR - 75724 PARIS Cedex 15 (PI)
- E. Bruch, Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS (PostDoc)
- J. Petit, Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS (Thesis Student)
- L. Yang, Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS (Thesis Student)
- Q. Gaday, Institut Pasteur Unité de Microbiologie Structurale 25 rue du Docteur Roux FR - 75015 PARIS (Thesis Student)
- A. Sharma, Institut Pasteur Unité de Virologie Structurale 25 rue du Docteur Roux FR - 75724 PARIS Cedex 15 (PostDoc)

BEAMLINE PERFORMANCE:

Our users confirm their overall satisfaction with the structural biology beamlines at the ESRF, and would like to thank the beamline managers and all the staff for the very successful upgrade of synchrotron facilities and beamline instrumentation. We are also grateful to all our local contacts and beamline scientists for their commitment to set up our experiments in remote access. It is worth to note that the new MXCuBE3 user interface contributed to make remote access work on all the MX beamlines significantly more fluent and user friendly, and was particularly appreciated.

Actinobacteria challenge the paradigm:

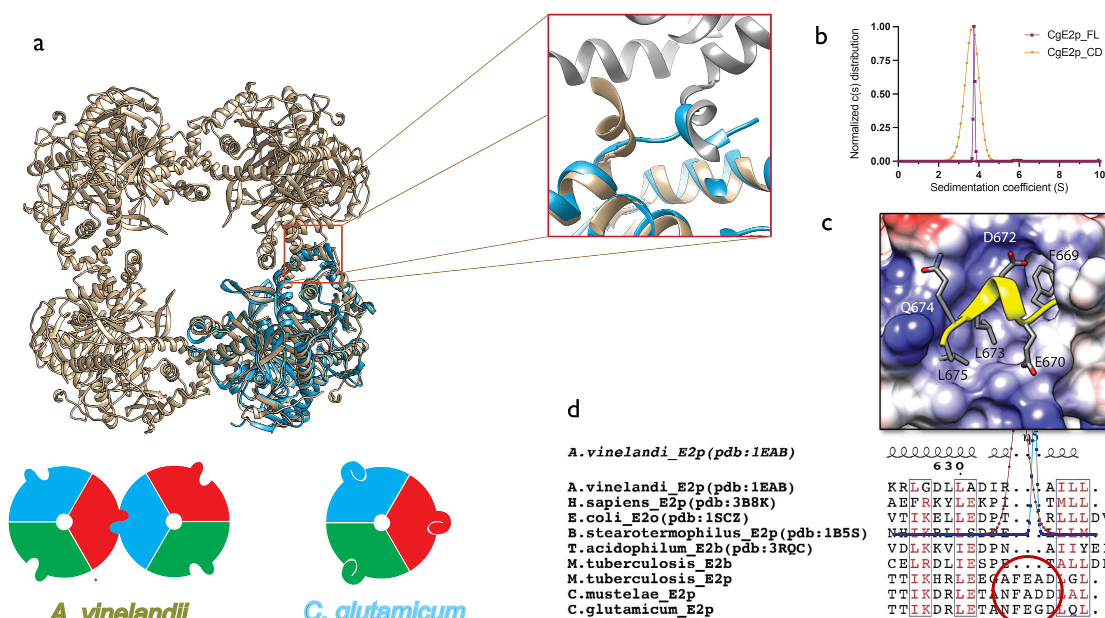
a unique protein architecture for a well-known central metabolic complex

Bruch, E.M., *et al.*, Proc. Natl. Acad. Sci. USA, 118, e2112107118, 2021 – doi: 10.1073/pnas.2112107118

Eduardo M. Bruch[1], Pierre Vilela[1], Lu Yang[1,2], Alexandra Boyko[1], Norik Lexa-Sapart[1], Bertrand Raynal[3], Pedro M. Alzari[1], Marco Bellinzoni[1]

[1] Unité de Microbiologie Structurale, Institut Pasteur, CNRS, Université de Paris, 75015 Paris, France; [2] Wuhan Institute of Biological Products Co. Ltd., 430207 Wuhan, People's Republic of China; [3] Plateforme de Biophysique Moléculaire, Institut Pasteur, 75015 Paris, France

α-ketoacid dehydrogenase are large, evolutionary conserved multi-enzymatic complexes that carry out key oxidative reactions in central metabolism. For decades, these complexes, of which the most known is pyruvate dehydrogenase (PDH), have been thought to share a similar organization in all aerobic organisms, structured around a hollow core resulting from the high oligomeric arrangement of the acyltransferase component. We show in this work that Actinobacteria, one of the largest eubacterial phyla, break this 'dogma' owing to a distinct trimeric acetyltransferase core. Specifically, using *Corynebacterium glutamicum* as a model, we show how a unique C-terminal helix in the acetyltransferase (AceF) catalytic domain, which bears an actinobacterial-specific insertion, precludes higher order protein oligomerization. This feature, together with the presence of an *odhA* gene that codes for a fusion enzyme carrying both the oxoglutarate decarboxylase and the succinyltransferase domains on the same polypetide, is spread over Actinobacteria and reflects the association of PDH and ODH into a single physical complex. Considering the central role of the pyruvate and 2-oxoglutarate nodes in central metabolism, and the importance of Actinobacteria for human welfare, our findings pave the way to both therapeutic and metabolic engineering applications.



(a) Cartoon representation of the superimposition of the dihydrolipoamide acetyltransferase (AceF) from the model Gram-negative *Azotobacter vinelandii* (PDB 1EAB; brown), in its canonical cubic assembly, and the corresponding enzyme from *C. glutamicum* (blue), that only assumes a trimeric state. On the right, zoomed view of the C-terminal 3_{10} helices from both enzymes, highlighting the different helix orientation in C. *glutamicum*. On the bottom, schematic representation of the intermolecular trimer-trimer interactions in *A. vinelandii vs.* intramolecular ones in *C. glutamicum*. (b) Sedimentation coefficient distribution from analytical ultracentrifugation experiments on corynebacterial AceF, both full-length protein and catalytic domain, consistent with a trimeric state. (c) View of the position of the amphipatic C-terminal helix, laying on the same AceF protomer. (d) Multiple sequence alignment of homologous acyltransferases with available structures, showing the actinobacterial specific C-terminal insertion (red circle).

Evolution and activation mechanism of the flavivirus class II membrane-fusion machinery.

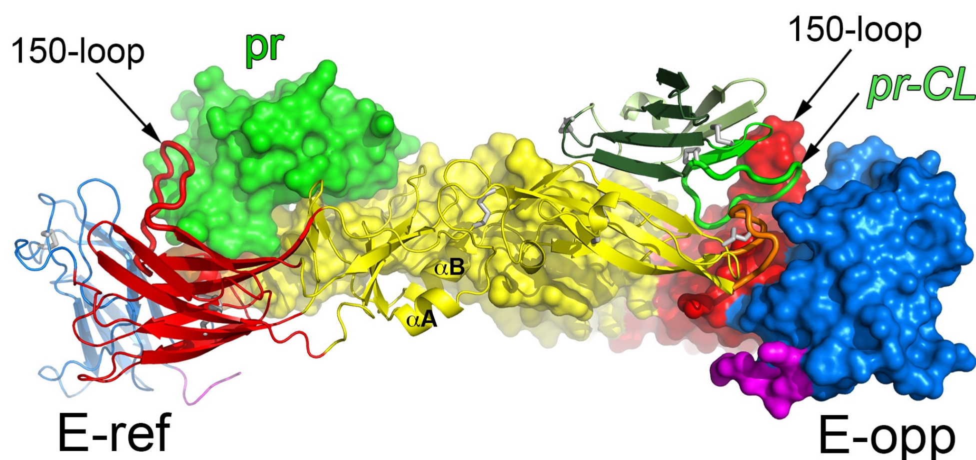
M.-C. Vaney [1,8], M. Dellarole [1,5,8], S. Duquerroy[1,2,8], I. Medits[3], G. Tsouchnikas[3,6], A. Rouvinski[1,7], P. England [4], K. Stiasny [3], F.X. Heinz [3] and F.A. Rey[1]. Nature Commun. 2022 Jun 28;13(1):3718.

[1]Institut Pasteur, Université Paris Cité, CNRS UMR 3569, Unité de Virologie Structurale, Paris, France. [2]Université Paris Saclay, Orsay, France. [3]Center for Virology, Medical University of Vienna, Austria. [4]Institut Pasteur, Université Paris Cité, CNRS UMR 3528, Ptf Biophysique Moléculaire, Paris, France. Present addresses : [5]CIBION, CONICET, Buenos Aires, Argentina.[6]HOOKIPA Pharma 19 Inc, Vienna, Austria. [7]Dpt of Microbiology and Molecular Genetics, Hebrew University of Jerusalem, Israel.

The flavivirus particle's life cycle includes four key steps:
1- assembly of glycoproteins prM and E into icosahedral spiky immature particles made of $(prM/E)_3$ into the neutral pH environment of the ER lumen;
2- maturation of virions across the mildly acidic pH of the trans-Golgi network (TGN) involves an acid-pH-driven rearrangement into smooth particles made of $(prM/E)_2$ dimers exposing a furin site for prM cleavage into "pr" and "M". "pr" stays still bound to the E dimers to protect the E fusion-loop to go into a premature fusion in the acidic TGN;
3- exit to the neutral pH extracellular environment where "pr" is released from the particle, leading to an infectious particle;
4- entry via receptor-mediated endocytosis into a target cell, and fusion of the viral and endosomal membranes at the mildly acidic endosomal pH.

All the mechanistic molecular understanding of these pH-driven particle transitions taking place during fusion, maturation and secretion of flavivirus particles was lacking.

We showed that "pr" is related to the family of DnaJ/ HSP40 cellular co-chaperons present in the ER, reflecting a specific adaptation to the unique features of flavivirus morphogenesis and entry into cells. We solved the x-ray structure of $(pr/sE)_2$ dimer of tick-borne encephalitis virus at acidic pH, and revealed the crucial role for the 150-loop of E in relaying the fusion-loop capping role of "pr" upon secretion. This new identified molecular switch, used to control the processes of virus assembly, virus maturation and entry into new cells, was found to be valid for all flaviviruses, including several pathogen mosquito-borne viruses (e.g., dengue, yellow fever, Zika, Japanese encephalitis and West Nile virus).



Structure of TBEV $(prM/E)_2$ dimer