Application for Macromolecular Crystallography BAG Beam Time at the ESRF

New proposal

Proposal Title (175 chars maximum.)

BAG Groups of CIC bioGUNE Bilbao

Keywords

#1: CBS domains #2: RNAP #3: GARP #4: GLTP

- **This proposal is:**

  A new BAG proposal ☐ OR A continuation of BAG proposal reference : MX - 1283

- This proposal is: Fundamental Science ☑ % 100 Applied Science ☐ % 0 Industrial Science ☐ % 0

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Summary of Beamtime Requested FOR SIX MONTHS

☑ Multi-wavelength	40	shifts
☑ Single-wavelength	40	shifts
☑ BM29 Bio-SAXS	1	shifts
Total	81	shifts

Laboratory Support Facility

Chemistry Lab. ☐ Biology Lab. ☑

Global Summary

Global Summary of this BAG project (enter 8000 chars maximum - approximately 1000 words) 0 0

CIC bioGUNE is a non-profit research center located near Bilbao, Spain. The center enjoys three buildings placed at the technological Park of Bizkaia, one of which is just focused on Structural Biology. The Macromolecular Crystallography Unit is formed by four groups headed by Drs. A. Martínez-Cruz, L. Malinina, A. Hierro and N. Abrescia who work in close collaboration with the X-Ray Platform manager (A. Rojas) and other crystallography groups at the University of the Basque Country, (Drs. Guerin and Viguera). The projects are devoted to human diseases and explore the structural principles of protein/ligand recognition for developing the means to effectively use and pharmacologically modulate protein specificity. Among them are:

- Proteins that participate in the processing of pre-mRNAs (splicing and export), tethering complexes and proteins such as the SNARE proteins, which are core components of the vesicle fusion machinery, human Glycolipid transfer protein (hGLTP), CNG_repeating RNAs, and complexes of CNG-repeating_siRNA with p19 and its mutants.

- Proteins containing Cystathionine Beta-Synthase (CBS) motifs: We aim to unravel the molecular mechanisms by which CBS motifs regulate the activity of: (i) the enzyme cystathionine beta synthase (first enzyme of the transsulfuration pathway (for which a unique full-legnth structure from D. melanogaster is available so far) and (ii) the less studied family of Mg2+ transporters known as cyclins M or CNNMs. Punctual mutations in the CBS domains of these proteins are linked to severe inherited diseases in humans.

-Molecular mechanisms of “gene expression”. This process is carried out by multi-subunit RNA polymerase (RNAP) enzymes that transcribe DNA into RNA. Transcription can be divided into three major steps: Initiation, Transcription/Elongation, Termination. Initiation and Termination are the less understood processes but relevant in many associate gene disorders. Eukaryotes have three different nuclear RNAPs whilst Archaea and Bacteria have single RNAPs. Archaeal

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transcription is homologous to that of eukaryotes but initiation only requires accessory factors: TFB and TBP. Our recent X-ray crystal structure of the archaeal 13-multisubunit RNAP complex from Sulfolobus shibatae (Korkhin Y et al., (2009) Evolution of Complex RNA Polymerases: the Complete Archaeal RNA Polymerase Structure. submitted in Plos Biology) has fully elucidated its architecture and revealed a new subunit, named Rpo13, whose location and topology suggests its role in the formation of the transcription bubble. We have continued our structural investigation on the archaeal RNA polymerase (RNAP) complex that is composed by 13-subunits with a total MW of ~400 kDa. We have obtained crystals of the apo-RNAP diffracting at 3.2Å (the highest resolution so far achieved for this archaeal enzyme) and of the DNA-RNAP complex diffracting at 4.3Å. This is the only structure available of the archaeal enzyme engaged with dsDNA depicting the initiation state in archaeal transcription.

- Structural characterization of major capsid proteins of novel archaeal viruses by X-ray crystallography.
This project is at the crystallization stage. Success in this phase will open up the possibility to collect diffraction data at the available synchrotrons, included ESRF at the beamlines ID14/ID23.

- The Retromer complex regulates the sorting of specific cargo with the physical process of membrane deformation. Retromer comprises two distinct subcomplexes, a dimer of still undefined combination of sorting nexins (SNXs) capable of sensing and driving membrane curvature for the formation of tubular vesicles and a heterotrimer composed of vacuolar protein sorting 26 (Vps26), Vps29 and Vps35 responsible for cargo recruitment, thus referred as the cargo recognition complex. Our objective is the structural characterization of the association between the cargo recognition complex and its Rab GTPase in order to determine the dynamics for specific membrane recruitment and cargo selection. We have succeeded in getting SeMet-cystals of the complex diffracting to a resolution of 4.5 Å at our home source and are in a good position to initiate SAD/MAD experiments. We will require a tunable micro-focus beam that allows data collection from relatively small crystals and large unit cells.

- GARP complex is associated with the cytosolic face of the TGN where it functions tethering retrograde vesicles derived from endosomes and promoting SNARE complex assembly. GARP depletion not only decreases the formation of Syntaxin-6/Syntaxin-16/Vti1a/Vamp4 SNARE complex but also blocks the transport of STxB in vivo.These findings establish a novel link between GARP and its interaction with the SNARE Syntaxin-6 as an essential step for STxB retrograde transport. Our objective is to elucidate the mechanism of interaction between the Vps51 subunit of the GARP complex and the SNARE Syntaxin-6 that provides the functional specificity exploited by the Shiga toxin entry. We have obtained crystals with dimensions 0.3x0.1x0.1 mm, therefore a microfocus beam is essential to accurately gather the best quality diffraction patterns. In addition, we have produced selenomethionine-sustituted protein.

- The proteins of the family Inhibitors of Growth (ING1-5) form part of chromatin remodeling complexes modulating the transcription levels of their target genes. Their primary biological function is the inhibition of cell growth and proliferation, and enhances apoptosis in response to genotoxic stress. Our collaborator, Dr P. Blanco has characterized the structure of ING4, which has a conserved N-terminal coiled-coil dimerization domain, a central flexible region, and a C-terminal PlantHomeoDomain (PHD). He has also studied the binding of the PHD to peptides from the N-terminal tail of histone H3 trimethylated at lysine 4. We have observed that the N-terminal domain of ING5 forms homodimers in solution and the full-length protein has a structural organization similar to that of ING4. As part of the process of the structural characterization of ING5 we have crystallized this dimerization domain. Future attempts will be oriented towards SeMet protein production and screening different heavy metal derivatives.

- Structural approaches for developing the potential means against human disorders, focusing the research on viral RNA silencing suppressor (RSS) p19 and human glycolipid transfer protein, hGLTP. We think that CNG-repetitive sequences cause human Trinucleotide Repeat Expansion Diseases (TREDs) due to formation of the double helical RNAs and that the pathology can be inhibited by means of the silencing suppressor p19. Our goal is to identify the p19 mutations which make it disease-repeat specific. We have co-crystallized 5 single/double p19 mutants with various CNG-repeating RNAs and constructed 6 p19 mutants with the insertions of different length. We are now focused on construction of the p19 mutants, specifically recognizing the CNG-repeats and will collect data from the crystal of the insertion-mutants complexed with RNA.

- Molecular Basis for Manipulating the Selectivity of Glycolipid Transfer. We aim to enhance the selectivity of human Glycolipid Transfer Protein (hsGLTP) for transfer of sulfatides using mutational and crystallographic approaches. We will study the role of the GLTP C-end and dimer-structure for transfer selectivity.

Among our collaborators are A. Accardi (Cornell Univ., NY), JP. Kraus (Univ. Colorado), V. Kozich (Inst. of Inherited Metabolic Disorders, Prague), V. Spiwok (Institute of Chemical Technology, Prague), R. Lathi (Univ. of Turku), A. Baykov (Moscow State University), D. Müller (Hospital Le Charite, Berlin), JL. Neira (Univ. Miguel Hernández, Elche, Spain), R. Herbst-Irmer (Univ. of Göttingen), DJ Patel (Sloan-Kettering Institute), JH. Hurley and J. Bonifacino (NIH, Bethesda), J. Navaza (CNRS) and S. Bell (Oxford University).

Proposal - WARNING

The section "A continuation of ..." is set with the proposal MX-1283 managed by:Alfonso Martinez (amartinez@cicbiogune.es)
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This information is very important for the processing of your proposal (report submission,...).So please check that MX-1283 is really the good one, otherwise, change it!.