Standard Project

Experimental Report template

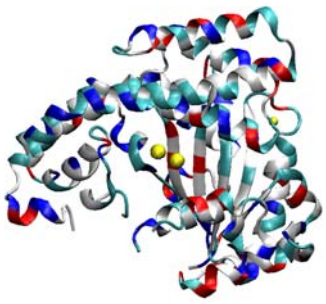
Proposal title: **Mimivirus and Megavirus proteins of interest**			Proposal number: 20110582
Beamline: BM30	**Date(s) of experiment: 2011** from:11/18/2011 from:11/07/2010 from: 02/28/2011	 to: 11/19/2011 to: 11/08/2011 to: 02/29/2011	**Date of report:** February 2012
Shifts: 9	**Local contact(s): F. Borel – JL Ferrer**		*Date of submission:* *2009*

Objective & expected results (less than 10 lines):

We selected a set of proteins involved in Mimivirus transcrption. The Megaviridae signals are unique, very different to prokaryotic or eukaryotic signals, and the proteins sharing homologies with known transcription enzymes either present extra domains of unknown function or specific features responsible for the virus specificity. Understanding these determinants may open new windows on general processes highjacked by mimivirus. For example, the R341 protein corresponds to a polyadenylate synthase sharing some homologies with the Poxvirus one while the L544 corresponds to an ORFan protein for which we have evidences of its involvement in the viral transcription.. Their 3D structures shoukd provide some insights on their molecular function at the atomic level.

Results and the conclusions of the study (main part):

L544: Acanthamoeba polyphaga Mimivirus.

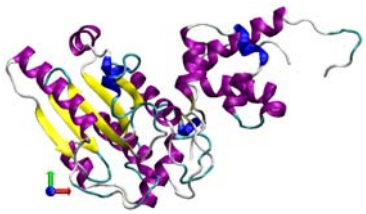


Mimivirus is a giant virus with a particle size of 750nm and a genome size of 1.2 Mb. This virus reaches the size and complexity of parasitic cellular organisms. The transcriptomic analysis of Acanthamoeba cells infected by Mimivirus highlighted a unique early promoter conserved in front of 50% of Mimivirus genes, a late transcript governing the expression of late Mimivirus genes and a unique hairpin signal at the 3' end of Mimivirus genes governing the transcription arrest and polyadenylation of all Mimivirus mature transcripts. These features necessitate specific recognition machinery for transcription still to be discovered. We decided to focus our interest on these proteins potentially involved in the virus transcription. The L544 gene product was predicted as a transcription factor. We collected numerous datasets to solve its structure taking advantage of the 3 selenomethionines present in the crystals [Ciaccafava et al., Acta Crystallogr Sect F 2011]. The structure was finally solved and reveals an unexpected polymerase domain. Some parts of the structure are still disordered and since we use a dehydratation protocol to improve their diffraction, native datasets are routinely collected to recover information on these disordered segments. One dataset at 2.4 A was collected in may 2011 at ESRF on a crystal produced in the presence of dAMP-CPP. Unfortunately, the nucleotide is not visible in the active site of the L544 structure. Before publishing the 3D structure of the L544 we need to perform enzymatic assays measurement to elucidate the functional role of this protein during the infectious process.

R355: A predicted Sumo protease from Mimivirus.

Mimivirus possesses a gene coding for a cysteine protease (R355) that displays sequence similarity with the SUMO-protease family. Since this enzyme family is known to play an important role in regulation and in

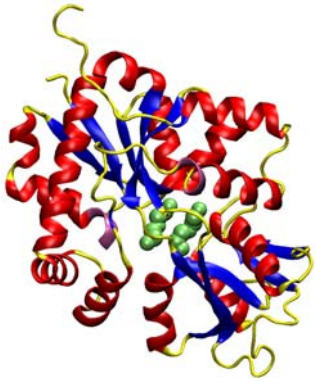
protein targeting to the nucleus, we have studied its 3D structure by X-ray crystallography. The structure has been solved at 1.5 Å by the method MAD using 7 substituted seleno-methionines. We validated the sumo-protease activity of R355 gene product on various test proteins expressed with a fused N-terminal SUMO domain. The R355 gene is expressed in the late phase of the infection cycle. In order to investigate its role within the infected amoeba, we propose to study its activity on proteins extracted from infected amoeba (at 4h), with or without purified R355. An article is in preparation.



We also published this year the 3D structure of a Candida Albicans conserved protein.

Candida albicans CA3427 structure

We selected this protein in search of new antifongique targets. The 3D-structure of the Candida albicans CA3427 protein was solved at 2.1 Å resolution. The combined analysis of its sequence and structure reveals a structural fold originally associated with periplasmic binding proteins. The CA3427 structure highlights a binding site located between the two protein domains, corresponding to a sequence segment conserved among fungi. Two crystal forms of CA3427 were found, suggesting that the presence or absence of a ligand at the proposed binding site might trigger a "Venus flytrap" motion, coupled to the previously described activity of bacterial periplasmic binding proteins. The conserved binding site defines a new subfamily of periplasmic binding proteins also found in many bacteria of the bacteroidetes division, in a choanoflagellate (a free-living unicellular and colonial flagellate eukaryote) and in a placozoan (the closest multicellular relative of animals). A phylogenetic analysis suggests that this gene family originated in bacteria before its horizontal transfer to an ancestral eukaryote prior to the radiation of fungi. It was then lost by the Saccharomycetales which include Saccharomyces cerevisiae.



Justification and comments about the use of beam time (5 lines max.):

We share the beam time between prospection and collects: Prospection is performed in 96 well plates to identify the best crystallization conditions in terms of diffraction. The results are them used to optimize the next run. We have to use dehydratation process for most of our projects which is performed directly on the Beam line and each time we get enough diffraction we collect the data set and process it on the spot to find out if more data are needed. We generally collect 4 to 6 data sets for a 3 shift beam allocation.

Publication(s):

- Ciaccafava A, Lartigue A, Mansuelle P, Jeudy S, Abergel C. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2011 67:922-5.
- Santini S, Claverie JM, Mouz N, Rousselle T, Maza C, Monchois V, Abergel C. PLoS One. 2011 6:e18528.
- Jeudy S, Lartigue A, Mansuelle P, Ogata Y, Abergel C. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2011 67:169-72.