

SICI 6 month (July-December 2010)

Internal Progress Report

Group: Protein and Virus Crystallograph (Partner 16)

IP: Diego M. A. Guérin

i) Progress towards ongoing Deliverables and Milestones, indicating any achieved ones and the results obtained since last report (December 2009):

i.1) Crystallization assays with KcsA (*Streptomyces lividans*) mutant (1-125) in combination with lipids, using various techniques including detergents and Lipidic Cubic Phases (LCPs). We will continue with these assays by screening new precipitant solutions.

i.2) Interaction and Co-crystallization of human Calmodulin (CaM) and KcsA. This experiment intent to employ CaM as a crystallographic chaperone of KcsA. Using several experimental approaches we obtained experimental evidence (NMR, fluorescence, pull-down, DLS, etc.) on the positive interaction between the two proteins. We settled-up for the expression and purification protocol of recombinant CaM from *E. coli* strain BL-21. We already runned some crystallization assays and will continue in the future doing more crystallization experiments of the KcsA-CaM complex.

i.3) Culture assays of *Halobacterium halobium*: The aim of this experiment is to produce bacteriorhodopsin (Br) in enough amount to be used as reference system for the crystallization in LCP. We already succeed in maintaining the culture and to grow two bacteria strains (*) under adequate saline and high temperature (45°C) conditions. We already begun the purification procedure with the aim of to obtain Br in quantity and purity enough to run LCPs crystallization experiments.

* CECT395 and BR59, this last is a gift from Prof. Juan Manuel González Mañas, from the UPV/EHU.

i.4) Development of monoclonal antibodies (mABs) against His-tags: The company charged of this development (Abyntek SA) succeed in obtaining many clones, out of which we tested and selected three. We will now proceed with the next steps of this subproject. The final objective is to use these mABs as crystallization chaperone, not only for KcsA, but also with others recombinant proteins.

ii) Publications between January and June 2010: None

Congresses presentations: "**Preliminary studies of Calmodulin as crystallization chaperone of KcsA channels**". A. Rendón-Ramírez, J. Agirre, A. E. Mechaly, A. Alaimo,

C. Malo, A. Villaroel, A. M. Fernández, O. A. Millet, J. M. González-Ros, and D. M. A. Guérin. **“International Workshop on Membrane Proteins, Signal Transduction, and Disease”**, July 12-13, 2010, Hotel Indautxu, Bilbao

iii) Collaborations with other members of the Consortium, newly established or ongoing:

Project i.1 is in collaboration with P2, project i.2 is in collaboration with P2, P20, and P25., and project i.4 s in collaboration with Abyntek SA, P1, and P2.

**iv) Problems Detected, resolved and pending since last report (June 2010) .
Please identify any Deliverables/Milestones affected:**

Only one SICI partner (P.2) produce protein in adequate conditions (purity and sample in a milligram scale) to run crystallogenesi s experiments. Milestone and Deliverable affected: M3.1, D.56, 57, 58 and 59.

v) Please indicate research activity planned for the next 6 months:

We will continue to work in the same directions as indicated in points i.1) to i.4)

As a Training committee member, please indicate any activities undertaken to assist in knowledge management, including:

We organized and advanced course on Structure determination and analysis to be held in Bilbao on february 2011.

List Milestone and Deliverable

Milestone	Milestone Name	WPs Involved	date
M.3.2	Purified channel forming subunits for structural analysis	2,3,4	24

This milestone was achived by P2 through the purification of the KcsA 1-125 mutant.

Deliverable	Deliverable Name	WP number	Delivery date
D.59	Purified pore forming subunits	3	24

This Deliverable corresponds to the publication: Encinar, JA, *et al.*, FEBS Letter 2005 579(23):5199-204