2015-2016

Internship proposal (Master or final project engineering school) at the LMGP

Unravelling the physiological role of ERM proteins using biomimetic lipid membranes

Abstract

Cytoskeletal biopolymers such as actin and tubulin are the main actors in regulating cell shape and dynamics. In turn, numerous proteins control their polymerization and stabilization at the cell membrane. The members of the ezrin/radixin/moesin (ERM) family insure the link between the cell cytoskeleton and the cell membrane and play a key role in a large number of physiological processes, including immune synapse formation, morphogenesis and cell motility (*1*) (*2*). Moesin is involved in the budding of viruses as well as immunological synapse formation. However, the precise molecular interactions between the ERM proteins, the plasma membrane and the cell cytoskeleton are still unknown.
The aim of this project is to use biomimetic lipid membranes and recombinant proteins to study in well-defined environments the role of ERM proteins in an *in vitro* reconstituted system.

Detailed internship proposal

Moesin is known to switch dynamically between the cytosol, where it is in a dormant state (or inactive state), and the membrane, where it is activated (open conformation). Once activated, moesin can bind actin and tubulin filaments at its C-terminal domain. Two events are believed to induce Moesin activation: membrane binding and/or phosphorylation at specific sites. The *in vivo* sequence of these two events is not known. Our project aims to: 1) unravel the molecular events occurring during moesin binding to the plasma membrane; 2) understand the role of specific phosphorylation sites; 3) study the subsequent binding of cytoskeletal components
To this end, we employ recombinant proteins (moesin, fluorescently tagged proteins, and mutant proteins mimicking the phosphorylation) as well as biomimetic membranes: large unilamellar vesicles (LUVs) (*3*) (*4*) and supported lipid bilayers (SLBs).
In the framework of this master training, we will employ supported lipid bilayers (formed by fusion of the LUVs onto a substrate) to investigate moesin/membrane interactions as well as moesin/membrane/cytoskeletal polymer interactions. The interactions at the nanometer scale will be investigated by sensitive techniques including quartz crystal microbalance, fluorescence microscopy and atomic force microscopy (AFM).

Location

The candidate will be working at the LMGP in the IMBM team. For more information about the lab and team : http://www.lmgp.grenoble-inp.EN/

Profile & requested skills

5th year engineering school with an interest for biophysics and biochemistry. This internship is at the interface between engineering and biochemistry. Aptitude for teamwork, good spoken and written English are requested.

Stipend: a “gratification” will be provided following the French law.

Application : please send a CV + a cover letter (including names/contact email of 2 referees) + the record of your grades of the 2 past academic years (2013/2014 & 2014/2015) to **catherine.picart@grenoble-inp.fr**

Bibliographic References

1. J. K. Burkhardt, Cytoskeletal function in the immune system. *Immunological reviews* **256**, 5 (2013).
2. R. G. Fehon, A. I. McClatchey, A. Bretscher, Organizing the cell cortex: the role of ERM proteins. *Nat Rev Mol Cell Biol* **11**, 276 (2010).
3. G. Blin, E. Margeat, K. Carvalho, C. A. Royer, C. Roy, C. Picart, Quantitative analysis of the binding of ezrin to large unilamellar vesicles containing phosphatidylinositol 4,5 bisphosphate. *Biophys J* **94**, 1021 (2008).
4. O. Maniti, N. Khalifat, K. Goggia, F. Dalonneau, F. Guérin, L. Ramos, L. Blanchoin, C. Picart, Binding of Moesin and Ezrin to membranes containing phophatidylinositol (4,5) bisphosphate: a comparative study of the affinity constants and conformational changes. *accepted with minor revisions*, (2012).