Proposition du Projet FARMAN

- Nom du projet, titre : "Development of ***MONETA - MOdular NEtwork Analysis of Protein Structures"*** (**MONETA**)
- Durée du projet : **1-2 years**
- Responsables scientifiques : **Luba Tchertanov (BiMoDyM, LBPA)**

  Frederic Pascal (CMLA)

- Membres de l'équipe-projet avec la proportion de temps consacré au projet :

BiMoDyM, LBPA :

L. Tchertanov (DR CNRS), 15%

E. Laine (Post Doctorante ENSC), 20%

S. Demarest (MS1 de Bioinformatique à Paris 7, Université Diderot), stage de 5 mois de 5 Mars à 31 Juillet 2012

J. Abécassis (ENS, L3), stage de 2 mois de 1 Juin à 31 Juillet 2012 ?

CMLA

F. Pascal (Professeur) , 15%

Etudiants L3 (pour la 2eme année du projet)

- Description de la problématique scientifique :

The experimental evidence of allosteric regulation taking place in proteins that are not allosteric according to the classical definition calls for the development of computational tools able to rationally guide an understanding of information transmission throughout protein structures. We propose development of **a novel** and **original approach** which permits to localize and visualize communication across a protein three dimensional space via a network of residues. This approach allows the description of the distortion of such communication induced by a perturbation, particularly by a point mutation, phosphorylation events, the substrate/ligand/inhibitor binding. The principle of the method consists in the use of dynamical correlations computed from a conformational ensemble generated by Molecular Dynamics (MD) simulations, to build a modular network representation of the protein composed of clusters of flexible residues whose atomic fluctuations represent the most striking features of the protein local dynamics - *the independent dynamic segments*, linked by chains of interacting residues that mediate conformational changes - the communication pathways (Figure 1).

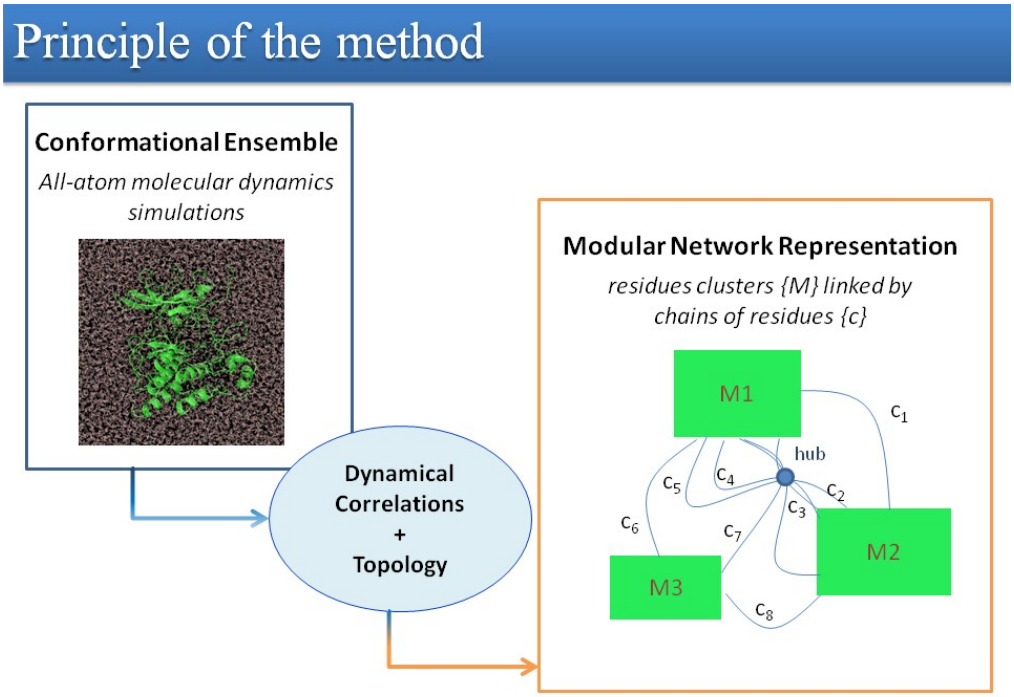

Figure 1

The prototype of this approach has been developed and implemented by BiMoDyM in the form of a package called MONETA (MOdular NETwork Analysis). We used MONETA to study of a particular problem, the oncogenic mutation D816V effects of the receptor tyrosine kinase KIT (Laine et al. 2011; Laine et al 2012).

Receptor tyrosine kinases (RTKs) are a family of proteins that act as ligand/substrate-dependent activators which contribute to the regulation of cell signaling pathways. RTKs catalyze the phosphorylation of specific tyrosine sites, thereby switching on multiple signaling pathways by interacting with enzymes and adaptor proteins. Hence, they can be considered as nodes in a complex interaction network that regulates physiological processes

critical for cell survival, growth, proliferation and differentiation. Their functions are ensured by a remarkable conformational plasticity. In the native state, RTKs interconvert between various conformational states ranging from the inactive auto-inhibited to the fully active phosphorylated forms. This equilibrium can be displaced by the binding of the extra-cellular ligand or by an inhibitor, phosphorylation events or point mutations. In particular, mutations are mainly responsible for the deregulation of activity/functions of RTKs, causing various forms of cancer, inflammatory diseases (e.g. arthritis) and neuronal disorders(e.g. Alzheimer's pathology). Therefore, deciphering the molecular mechanisms of oncogenic activating mutations represents an important biological challenge.

We have recently reported the structural, dynamic and thermodynamic effects of D816V mutation of the RTK KIT (Laine et al., 2011). We have shown that D816V mutation induces double effects – a local, partial unfolding of activation loop (A-loop) at proximity of mutation site; and a long range event resulting in an important structural reorganization of the juxta-membrane region (JMR) – distant by more than 15 Å. We have established the biological significance of these two effects for the activation and functions of D816V KIT mutant.

We applied MONETA to decipher the molecular determinants of the propagation of the mutational effects throughout KIT structure, which we described as a disruption in the communication between two spatially distinct sites, positioned in two regulatory regions crucial for the receptor activation/deactivation process. Finally, based on this knowledge, an in silico mutagenesis was performed to introduce a counter balancing/neutralizing second mutation that re-established the communication observed in the native protein.

Briefly, our study points to three new elements, (i) **a novel and rational approach** for the **analysis of a perturbation effects** in proteins; (ii) a detailed description of how D816V mutation could enable allosteric conformational transition in KIT protein; (iii) an *in silico* design of a counter-balancing/neutralizing mutation, which **validated the robustness** of the **proposed approach**. The computational approach we deliver constitutes a powerful and efficient tool for analyzing MD conformational ensembles and for comprehensive visualization of the results.

Preliminary results were reported at international and national meetings where they were very well received by the community [8th European Biophysical Congress EBSA, 23-27 Aug. 2011, Budapest and MG2SB (coarse-grained modeling of biological systems) 2011 workshop 23-24 Nov., 2011, IBPC-Paris].

We are able to describe a communication (sub-)network between two (or more) distinct sites of a protein, representing only a portion of a communication pathway established between different proteins in the context of a complex cellular signaling network. **Perspectives for improving the reported method** would consist in (i) using the representation for studying **other kinds of perturbation** (phosphorylation event, the binding of a ligand), (ii) using the representation in **a dynamic way** to investigate **multiple states** (inactive, intermediate, active) and/or **conformational transitions** of a protein, (iii) **extending** the representation to consider the **communication established between proteins** implicated in cellular signaling. Tools derived from MONETA should pave the way for an integrative framework for the modeling of information transmission across different spatio-temporal scales, from intra-protein residue networks to cellular protein networks.

Both partners (BiMoDyM-LBPA et CMLA) will participate in development/improvement/ generalization of MONETA, that will be capable to study any kind of perturbation effects in a protein. Further development will include the extensions of the method to study of perturbation effects transmission over the proteins involved in interaction network (cascade) responsible for cellular signalling. Our project will include three parts or steps : (i) one dealing with the theoretical/mathematical aspects of the method – conception level – (CMLA), (ii) one aiming at improving the algorithm efficiency – optimization – (CMLA and BiMoDyM), and (iii) one consisting in the computational validation of the method by applying it to the analysis of given cellular physiological signalling pathways (BiMoDyM).

Partner BiMoDyM needs:

- 2 100 € per year for the student gratification (5 months every year);
- 3 300 € Work Station

Partner CMLA needs:

-2 100 € the second year for the student gratification (5 months);
-3 000 € laptop and documentation (books, article, thesis,...)

References:

- Laine E., I. Chauvot de Beauchêne, C. Auclair and L. Tchertanov (**2011**) Propagation of D816V/H mutation effects across KIT receptor. **8th European Biophysics Congress, 23-27 août 2011**, **Budapest, HN,** published in *Eur. Biophys. J. Biophys. Lett.* 40:S109
- Laine E., I. Chauvot de Beauchêne, D. Perahia, C. Auclair, L. Tchertanov (**2011**) Mutation D816V alters the internal structure and dynamics of KIT cytoplasmic region: implications for dimerization and activation mechanisms. ***PLOS Comput. Biol.*** **7**:e1002068 (and references herein)
- Laine E., C. Auclair and L. Tchertanov. A Modular Network Analysis of Protein Structures: Propagation of Mutation D816V Effects across KIT. *Biophys. J.* (*submitted*) (and references herein)