Proposition de thèse à l'Ecole Doctorale INTERFACES

Modeling of the signaling pathways of the native receptor tyrosine kinase KIT and its mutant

Modélisation des voies de signalisation du récepteur à activité tyrosine kinase KIT sous forme sauvage et mutée

INTRODUCTION

Functions: The receptor tyrosine kinase (RTK) KIT is cell-surface transmembrane protein that controls multiple intracellular signaling pathways [1]. As the other RTKS, KIT acts as sensor for extracellular specific ligand, the binding of which triggers receptor dimerization, activation of the cytoplasmic domain and intermolecular autophosphorylation of specific tyrosine residues. These intra-receptor processes lead to the recruitment, phosphorylation and activation of multiple downstream signaling proteins, which eventually govern various aspects of the cell physiology [2]. Aberrant signaling, mutation in RTKs or in downstream signaling pathways have been involved in many diseases and pharmacological modulation of RTKs activation has been successfully used in the treatment of a wide range of cancer [3]. With regards to KIT, the receptor has been found mutated in several human malignancies, including cutaneous mastocytosis (CM), systemic mastocytosis (SM), acute myeloid leukemias (AML) and gastrointestinal stromal tumors (GIST) [4] Depending on the disease, the KIT mutation may occur in the kinase domain (KIT D816V; in SM and AML), in the juxtamembrane domain (JMD; in GIST) or in the extracellular domain (ECD; in CM). All these various KIT mutations lead to the constitutive activation of the receptor in the absence of its ligand, the stem cell factor (SCF) [5, 6].

The study of the transduction processes evoked by KIT (and by other RTKs) by experimental techniques is highly restricted. The theoretical structure-based description of KIT is also limited since to date, despite a considerable number of published structures, no data describing a full-length KIT has been collected. The reported structures characterize the non-complete cytoplasmic content [7] in which the kinase insert domain (KID) has not been resolved (**Fig 1**). Of note, in KIT, a unique RTK having as functional sites simultaneously the Tyr and Ser residues, the KID (79 aas) contains five potential phosphorylation sites – Y703, Y721, Y730, S741 and S746.

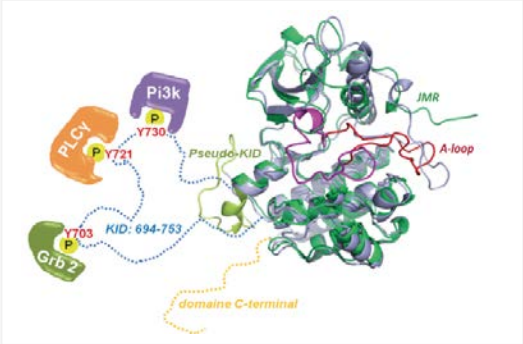

Fig. 1. Structure of the native receptor tyrosine kinase KIT. Superimposed structures of the cytoplasmic domain in inactive (1T45, grey and pink) and active states (1PKG, green and red). The missing KID and C-terminal domains are traced by doted lines. The tyrosine residues in KID and the proteins that specifically recognize the phosphotyrosine sites are schematized.

In wild-type KIT, the Y703, when phosphorylated, becomes a binding site for SH2 domain of Grb2, the adaptor protein initiating the Ras/MAP kinase signaling pathway induced mitogenesis. Besides, Y721 and Y730 have been identified as the recognition sites of PI3K, and phospholipase C (PLCδ), respectively. In addition, phosphorylated S741 and S746 are able to bind PKC (protein kinase C) inducing mitogenesis and cellular motility and are involved in re-control of PKC activity under the receptor stimulation. Phosphorylation of S746 mediates by PKC and conducts to a global decreasing of KIT tyrosine phosphorylation. The significant contribution of the KIT deregulated activity to cancer requires a careful study of this receptor pointing to the post-transduction events. Alternative phosphorylation of the five functional tyrosine and serine residues promotes an adaptation of the binding sites for selective recognition of PI3K, Grb2 and PLCδ, initiating at least three different signaling pathways.

Besides, the involvement of the JAK2-STAT5 pathway in growth and survival of normal mast cells (MCs) with wild type KIT (KIT WT) is well known [8]. Consequently, several teams have studied the implication of STAT5 in KIT D816V+ neoplastic MC or AML cell growth, survival and transformation, and some light has been shed on its implication in tumor growth downstream of the mutant receptor. A first study showed that pSTAT5 is found in the cytoplasm of malignant cells from patients with KIT D816V SM or AML [9]. Another study further emphasized the molecular interactions between STAT5 and PI3K *via* the GAB2 scaffold protein interaction bridging p85 and pSTAT5 interaction. Moreover, knockdown of STAT5 led to cell growth inhibition [10]. Furthermore, the same team showed that KIT D816V promotes direct STAT5-activation, by bypassing the canonical JAK2-STAT5 pathway evoked usually by KIT WT, and that pSTAT5 contributes to growth of neoplastic MCs [11]. Finally, the expression levels of STAT5 seem to be critical for transcriptional regulation in KIT mutant HMC-1 and ROSA MC lines, and for neoplastic cell growth and survival [12, 13].

Altogether, these results strongly suggest that STAT5 is one major cellular effector below KIT D816V by controlling the mutant KIT-mediated aberrant growth signaling. However, if STAT5 inhibitors are able to decrease proliferation and to induce apoptosis in KIT D816V+ neoplastic human MCs or AML cells [13,14], these effects are observed at drug concentrations above 20 mM, and thus new STAT5 inhibitors active at pharmacological doses on both indolent and aggressive forms of SM are still needed.

Our preliminary results: In series of our papers [15-19], we studied the structural and dynamical features of the native and mutated kinase domain (without KID) of KIT, yielding a detailed description of its mechanisms of activation, ligand-depend for the native proteins and constitutive for the distinct mutants. The mechanisms of KIT activation are described in terms of allosteric regulation between coupled regulating fragments of the protein, juxta-membrane region (JMR) and activation (A-) loop [16,18,20]. As some mutations promote resistance to the clinically used drugs, we analyzed the affinity of imatinib to these therapeutic targets [21]. The computationally obtained (*in silico*) data were correlated with *in vivo* and *in vitro* observations, thus validating our numerically based accounts. Recently, we generated a model of the native full-length cytoplasmic domain of KIT [22] that opens a way for prediction of the transduction processes in KIT. Moreover, we published the structural models of STAT5 proteins [23], a KIT signaling protein [24]. The paper [23] was choice "to be featured in the *World Biomedical Frontiers*, because of its innovation and potential for significant impact" [25].

PROJECT

In this PhD project, we plan to obtain the models of KIT interacting with its cellular partner(s) in two different context – KIT is the native protein (wt-KIT) and KIT having the highly oncogenic mutation D816V (m-KIT). For the modelling, we will use the previously developed structural models – of the native and mutated kinase domain (without KID) of KIT [15,18] of the native full-length cytoplasmic domain of KIT [22], of the STAT5 [23] and the crystallographic data of JAK2 from PDB [4].

***Protein-protein interactions*:** The proteins partners (PP= JAK3 and STAT5) of KIT will be docked (HADDOCK [Bonvin], ATTRACT [Zacharias], ROSETTADock [Gray] and REVIDYM [the haptic docking, CMLA, ENS Paris-Saclay] into each phosphorylated model (wt-KIT, m-KIT). The obtained KIT-PP models will be studied using the multi-step protocol published in [15, 18]. Detailed statistical analysis of MD simulation data of each KIT-PP complex completed by calculation of the binding free energy will be used for identification of the binding site of each cellular partner. The main output is a delivering of the noncovalent bound KIT-PP models, representing the functionally relevant protein-protein complexes, which may be formed during the transduction processes of the native and mutated KIT.

Data analysis: MD data will be analyzed by the widely-used routines (RMSDs, RMSFs, PCA, clustering, …) and the more rigorous statistical techniques '*in-house-made*' [20, 23, 26] or adapted from literature. In particular, the conformational space explored by each KIT-PP complex during MD simulations will be analyzed with *ConfigScan* [26] that use a population estimate (density) of conformations in reduced space. The energy landscape will be presented as a 3D surface that display the evolution of dynamics as a random walk in this landscape. The energy landscape is useful for the tracing of visited main and secondary basins and for

calculating the locations of energy minima. The interaction interface characterization will performed with *Functional Mode Analysis* [27].

The PP interactions of each KIT-PP complex will be carefully analyzed (I) to identify the sequences of residues, which contribute form the protein-protein interaction interface (PPII), and (ii) to estimate a degree of flexibility of this polypeptide. The sequence composed of the partner's residues contributing to PP interactions will be considered as a prototype of a novel molecule enable to be (i) specific to KIT mutant similarly to functional partner and (ii) competitive with a partner.

Next to KIT-PP interaction, other questions remain open for the KIT functions, the deepest of which relates to the activation mechanisms. Is the KID phosphorylation does influence or not the structure and dynamics of kinase domain? How is allosteric communication transmitted from the phosphotyrosine residues to the other regions engaged in KIT and PP activation?

The communication pathways will be analyzed with MONETA [20], the surface pockets search will be performed with FPockets [28] and TRAPP [29]. This study will open the door to different applications – the structure-based drug design or a search for other KIT interactions, opening the door to description of KIT 'interactome'.

Equipment: The PhD student will generate the MD simulation data on HPCs of GENCI (TGCC-Curie, IDRIS) and FUSION (ENS P-S & CentraleSupelec). The data processing and analysis will be performed using the local CMLA cluster TopDyn and performant personal working station. The immersive visualization platform SHIVA [30] of ENS Paris-Saclay (Cachan), a unit of DIGISCOPE (http://www.digiscope.fr/en/platforms/shiva), will be used for a modelling at conception level and for the results representation. The PhD student will used the REVIDYM (Virtual Reality in coupling with MD Simulation), which we developed in the SHIVA environment as a system to used haptic graphical interfaces for 3D interactive modelling of proteins, which have enabled the tangible interactions with force haptic feedback and the interfaces ensuring the exchange with the DM simulation tools (AMBER, GROMACS).

This interdisciplinary project – a modeling of the biological process represented by mathematical models with using bioinformatics technics – will be supervised by L. Tchertanov (Directeur de Recherche au CNRS, CMLA ENS Paris-Saclay) and A. Trouvé (Professeur ENS Paris-Saclay, CMLA). The project will be realized in a close collaboration with Michel Arock, professor at ENS Paris-Saclay and performing his research work at Centre de Recherche des Cordeliers (15 rue de l'Ecole de Médecine, 75006 PARIS), who will supply the experimental data to guide a modeling of protein-protein interactions and validate the obtained predictions.

References: (our papers are denoted in blue)

1. Downward J. The ins and outs of signalling (2001) Nature 411, 759–762 doi:10.1038/35081138.
2. Hubbard SR, Miller WT. Receptor tyrosine kinases: mechanisms of activation and signaling. (2007). Curr Opin Cell Biol; 19: 117-123.
3. Zwick E, Bange J, Ullrich A. (2001) Receptor tyrosine kinase signalling as a target for cancer intervention strategies. Endocr Relat Cancer;8(3):161-73.
4. Boissan M, Feger F, Guillosson JJ, Arock M. c-Kit and c-kit mutations in mastocytosis and other hematological diseases.J Leukoc Biol. 2000 Feb;67(2):135-48.
5. Longley BJ, Tyrrell L, Lu SZ, et al. Somatic c-KIT activating mutation in urticaria pigmentosa and aggressive mastocytosis: establishment of clonality in a human mast cell neoplasm. Nat Genet. 1996;12(3):312-4.
6. Bodemer C, Hermine O, Palmerini F, et al. Pediatric mastocytosis is a clonal disease associated with D816V and other activating c-KIT mutations. J Invest Dermatol. 2010;130(3):804-15.
7. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) The Protein Data Bank. Nucleic Acids Research 28: 235-242.
8. Shelburne CP, McCoy ME, Piekorz R, Sexl V, Roh KH, Jacobs-Helber SM, et al. Stat5 expression is critical for mast cell development and survival. Blood. 2003;102(4):1290–99.
9. Harir N, Pecquet C, Kerenyi M, Sonneck K, Kovacic B, Nyga R, et al. Constitutive activation of Stat5 promotes its cytoplasmic localization and association with PI3-kinase in myeloid leukemias. Blood. 2007;109(4): 1678–86.

10. Harir N, Boudot C, Friedbichler K, Sonneck K, Kondo R, Martin-Lanneree S, et al. Oncogenic Kit controls neoplastic mast cell growth through a Stat5/PI3-kinase signaling cascade. Blood. 2008;112(6):2463–73.
11. Baumgartner C, Cerny-Reiterer S, Sonneck K, Mayerhofer M, Gleixner KV, Fritz R, et al. Expression of activated STAT5 in neoplastic mast cells in systemic mastocytosis: subcellular distribution and role of the transforming oncoprotein KIT D816V. Am J Pathol. 2009;175(6):2416–29.
12. Chaix A, Lopez S, Voisset E, Gros L, Dubreuil P, De Sepulveda P. Mechanisms of STAT protein activation by oncogenic KIT mutants in neoplastic mast cells. J Biol Chem. 2011;286(8):5956–66.
13. Peter B, Bibi S, Eisenwort G, Wingelhofer B, Berger D, Stefanzl G, Blatt K, Herrmann H, Hadzijusufovic E, Hoermann G, Hoffmann T, Schwaab J, Jawhar M, Willmann M, Sperr WR, Zuber J, Sotlar K, Horny HP, Moriggl R, Reiter A, **Arock M**, Valent P. Drug-induced inhibition of phosphorylation of STAT5 overrides drug resistance in neoplastic mast cells. Leukemia. 2018 Apr;32(4):1016-1022.
14. Wingelhofer B, Maurer B, Heyes EC, Cumaraswamy AA, Berger-Becvar A, de Araujo ED, Orlova A, Freund P, Ruge F, Park J, Tin G, Ahmar S, Lardeau CH, Sadovnik I, Bajusz D, Keserű GM, Grebien F, Kubicek S, Valent P, Gunning PT, Moriggl R. Pharmacologic inhibition of **STAT5** in acute myeloid leukemia. Leukemia. 2018 Feb 2
15. Laine E., Chauvot de Beauchêne I., Perahia D., Auclair C. and **Tchertanov L**. (2011). Mutation D816V Alters the Internal Structure and Dynamics of c-Kit Cytoplasmic Region: Implications for Dimerization and Activation Mechanisms. *PLOS Comput Biol* 7(6): e1002068. doi:10.1371/journal.pcbi.1002068.
16. Laine, E., Auclair, C. and **Tchertanov, L**. (**2012**). Allosteric Communication across the Native and Mutated KIT Receptor Tyrosine Kinase. *PLoS Comput Biol.* 8(8):e1002661, doi:10.1371/journal.pcbi.1002661.
17. Vita M, Tisserand J C, Chauvot de Beauchêne I, Panel N, **Tchertanov L**, Mescam-Mancini L, Agopian J, Fouet B, Fournier B, Dubreuil P, Bertucci F, and De Sepulveda P. (**2014**). Characterization of S628N, a novel KIT mutation found in a metastatic melanoma. *JAMA Dermatology*, doi:10.1001/jamadermatol.2014.143.
18. Chauvot de Beauchêne I., Alain A., Panel N., Laine E., **Trouvé A**., Dubreuil P. and **Tchertanov L**. (**2014**). Oncogenic mutations of KIT receptor differentially modulate tyrosine kinase activity and drug susceptibility. *PLOS Comput. Biol*.;10(7):e1003749. doi: 10.1371/journal.pcbi.1003749.
19. Chauvot de Beauchêne I. and **Tchertanov L**. (**2016**). How Missense Mutations in Receptors Tyrosine Kinases impact Constitutive Activity and alternate Drug Sensitivity: Insights from Molecular Dynamics Simulations. Invited Research highlight for *Receptors & Clinical Investigation*. Vol.3, e1372. doi: 10.14800/rci.1372.
20. Allain A., Chauvot de Beauchêne I., Langenfeld F., Guarracino Y., Laine E., and **Tchertanov L**. (**2014**). Allosteric Pathway Identification through Network Analysis from Molecular Dynamics Simulations to Interactive 2D and 3D Graphs. *Faraday Disc.,* DOI:10.1039/C4FD00024B. 169, 1-18.
21. Da Silva Figueiredo Celestino Gomes P., Chauvot de Beauchêne I., Panel N., Pascutti P., Solary E., and **Tchertanov L**. Insight on Mutation-Induced Resistance from Molecular Dynamics Simulations of Wild-type and mutated CSF-1R and KIT. (**2016**) *PLoS ONE.*11(7):e0160165. doi: 10.1371/journal.pone.0160165. eCollection 2016.
22. Inizan F., G. Delevoye, Chauvot de Beauchêne I., **Tchertanov L**. (**2018**). The First 3D Structure of the KIT cytoplasmic domain reveals a New Look for an Old receptor. *Paper in preparation*
23. Langenfeld F., Guarracino Y., Arock M., **Trouvé A.** and **Tchertanov L**. (**2015**). How intrinsic molecular dynamics controls intramolecular communication in Signal Transducers and Activators of Transcription Factor STAT5. *PLOS ONE*. Dec 30;10(12):e0145142. doi: 10.1371/journal.pone.0145142. E-Collection 2015.
24. Bibi S., Arslanhan M. D., Langenfeld F., Jeanningros S., Cerny-Reiterer S., Hadzijusufovic E., **Tchertanov L.,** Moriggl R., Valent P., Arock M. (**2014**). Cooperating STAT5 and AKT signaling pathways in Chronic Myeloid Leukemia and Mastocytosis: Possible new Targets of Therapy. *Haematologica.* **99**(3): 417-429.
25. Langenfeld F., Guarracino Y., Arock M., **Trouvé A**. and **Tchertanov L**. (**2016**). How Intrinsic Molecular Dynamics Control Intramolecular Communication in the Signal Transducers and Activators of Transcription Factor STAT5. Invited Research highlight for *World Biomedical Frontiers*. http://biomedfrontiers.org/cancer-2016-6-9/.
26. Chatron N., Chalmond B., **Trouvé A**., Benoît E., Caruel H., Lattard V., **Tchertanov L**. (2017). Identification of the Functional States of Human Vitamin K Epoxide Reductase from Molecular Dynamics Simulations. RSC Advances, 7, 52071 – 52090.
27. Hub J S., de Groot Bert L (2009) Detection of Functional Modes in Protein Dynamics. *PLOS Comp. Biol*. V5, 5, e1000480
28. Le Guilloux V, Schmidtke P and Tuffery P, (2009) Fpocket: An open source platform for ligand pocket detection, *BMC Bioinformatics*, 10:168.
29. Kokh D B., Richter S, Henrich S, Czodrowski P, Rippmann F, Wade R C.(2013). TRAPP: a Tool for Analysis of Transient Binding Pockets in Proteins *J Chem Inform Modeling*, 53:1235-1252.
30. De Vuyst F., Guillas S., Kendira A., Labourdette C., **Tchertanov L**. (**2014**). L'ENS Cachan voit grand avec le mur d'image SHIVA. *HPC Today*. 26 sept. 2014. http://www.hpctoday.fr/regional/lens-cachan-voit-grand-avec-le-mur-dimages-shiva/.