## Biological interpretation of kinome generated data by Metacore pathway analysis

*COST report*
*Host lab: Unit of human nutrition, INRA*
*Host supervisor: Dr. Dragan Milenkovic*
*Visitor: Chandra Sekhar Chirumamilla*

Human genome encodes for more than 500 different kinases that regulates a complex array of intracellular signal transduction cascades in cell (1). Despite the plethora of available knowledge in gene expression data of phytochemicals beneficiary effects on cardio protection (2), low levels of endogenous kinase mRNA levels seriously limit our understanding phytochemical compounds complex behavior on protein kinase signalling. With the aim to characterize kinase networks of Immune cells (T and B lymphocytes) followed by withaferin treatment and endothelial cells treated with Epicatechin/Anthocyanin's (Ongoing experiments) in understanding complex behavior of phytochemicals on protein kinase signaling. To investigate kinome changes after treatment with phytochemicals we have used recently developed chemical proteomics techniques like high throughput peptide array technologies like Pamgene (3) (Pamgene bvba, The Netherlands). Our preliminary results of kinase peptide array platform have revealed that withaferin A had significant impact on the phosphorylation of peptide substrates by different upstream kinases ($P < 0.05$), suggesting its capacity to modulate several serine-threonine kinases (STK's). We found that key kinases that are modulated by withaferin in resting T cells are mainly cyclin dependent kinases (CDK2 and CDKL5), Homeodomain interacting protein kinase (HIPk1), dual specificity kinase (DYRK1B), intestinal ser/thr kinases ICK, calmodulin (CALM), and tyrosine-protein kinase (SYK). In order to obtain more meaningful interpretation of the kinase centric data obtained from Pamgene experiment, bioinformatics analyses have been performed. The pathway analysis software tool Metacore (https://portal.genego.com) was used to overcome the redundancy in terminology of biological process from pamgene derived kinase centric data. Metacore pathway analyses have shown RelA (p65 of NFkB subunit), CREB proteins as a key transcription factors and PKC, AKT as potential kinases were significantly affected by WA treatment, **Figure 1** (Full details are not given). Our findings demonstrate that applying pathway analysis to kinome data can provide insight into mechanistic pathways involved in disease intervention by phytochemicals regulated signalling pathways.

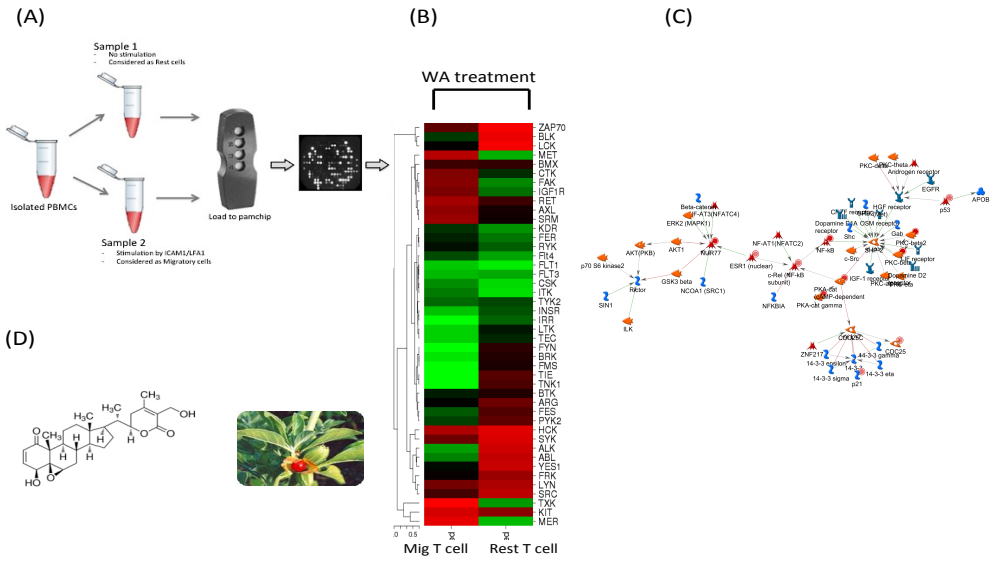



Figure 1. The serine threonine kinase activity profile on pamChip microarrays and their network analysis using metacore. (A) General outline of the experimental setup using pamgene peptide array of both Migratory and Rest T cells treated with plant metabolite Withaferin A. (B) The Ser/Thr kinse activity profile obtained by pamchip microarrays are represented in heatmap in

which WA treatment related downregulation of kinase activity is represented as red and upregulated kinase activity of kinases are represented in green (C) Statistical significant ($P < 0.05$) ser/thr kinase substrate modulation by withaferin A were analysed by using Metacore (GeneGO) (D) Structure of withaferin A

**Experiments done during short visit**:

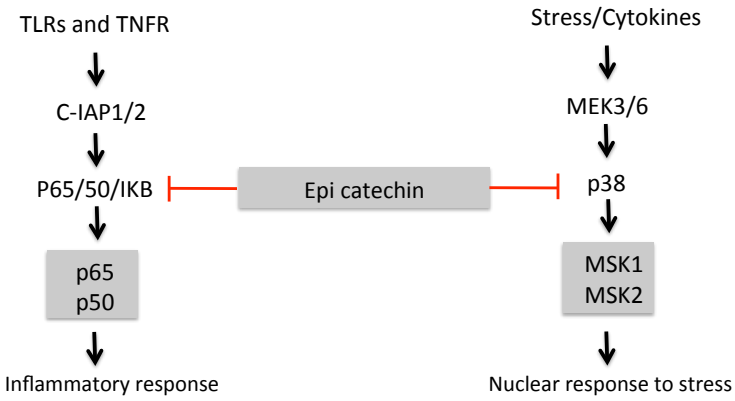
Earlier findings from INRA's group have shown that Epicatechin metabolites modulates the expression of genes involved in regulation of transendothelial migration and cell adhesion (4,5). This transcriptional regulation of anti-inflammatory genes by epicatechines was mediated by blocking of p38-MAPK and P65 (P50-NFkB complex) phosphorylation events suggesting Epicatechin is effective in cardio protection (6). However, direct effects epicatechin on protein kinase signalling have not been completely investigated. Therefore, aim of our current study was to investigate the impact of epicatechin and its metabolites on the cellular kinase signalling paways by applying high-throughput novel peptide array based (profiling of both tyrosine and Ser/Thre kinase activity) Pamgene technology.

**Experimental design**: Human umblical vein endothelial cells (HUVECs) are pretreated with Epicatechin metabolites before cell activation using TNF. Proteins will be extracted to map changes at the "kinome" scale. Cellular fingerprints of phosphopeptide phosphorylation are then converted to a cellular Tyr/Ser/Thr kinome activity profile by Bionavigator software (Pamgene) followed by biological interpretation using pathway analysis software Metacore.

During my stay, I also started the work on investigation on the modulation of p38MAPK and NFkB signalling pathway using molecular docking simulation of epicatchine and its metabolites on p38MAPK pathway (Figure 2). In current study, three epicatechin derivatives have stronger binding affinities towards the ATP binding pockets but less effect on the binding affinities on activation loop suggesting inhibitory effects on the phosphorylation of p38 results of inhibition of upstream kinase inhibition. It is recommended that to study upstream kinase analysis can be useful for comprehensive understanding of epicatechin role in theses two pathways. Three-dimensional structure of the p38 MAPK was downloaded from the www.rcsb.org (PDB id: 3EFK) followed by cleaning up of protein structure to proceed to docking. We have used three metabolites of the epictechin (methylated, glucorynated, and sulfated) downloaded from the pubchem www.pubchem.com (See Figure 2) for performing docking along with the FMK as a control. Autodock vina was used to perform molecular docking of the prepared receptor and ligand by targeted docking method by setting grid box (x=16, y=12, z=12) for docking at ATP binding site and grud box of (x=14, y=14,z=16) for P+1 site. The docking simulation results were categorized based on their binding affinity in Kcal/mole. Two-dimensional representations of the docking results were shown by discovery studio visualizer. Where as interactions between amino acid residues and the ligands were represented in the form of hydrogen and hydrophobic interactions ( Complete details are not given ).

Three metabolites of the Epicatechin have shown binding affinity of around -7.3 Kcal/mole with O-sulfonated Epicatechin ($\Delta G$= -7.9 Kcal/mol), glycorylated ($\Delta G$= -7.9 Kcal/mol), and methylated Epicatechin ($\Delta G$ -7.8 Kcal/mole). The binding site of the O-sulfonated Epicatechin includes hydrogen bond of 1.95 AU with Met-109 and six residues with observed hydrophobic interactions. The binding affinity of Epicatechin binding at the ATP binding pocket scores more than the binding at P+1 site (Thr-180, Gly-181 and Tyr-182). This strong binding affinity observed at the ATP site could be due to the volume of catalytic pocket binding cavity (Area: 873.3 and Volume: 1531.4 of 3FMK determined by http://sts.bioe.uic.edu/castp/ server) and also observed hydrogen bond between Met-109 (Bond length of 1.93Au). All three metabolites show binding site similarity up to 60% with the crystalized ligand FMK. Molecular docking simulations of the Epicatechin and its metabolites exhibited higher

Binding affinity at the ATP binding Pocket than the dual phosphorylation sites of TGY motif suggests protein kinase modulation can occur via allosteric binding or direct binding to kinase catalytic domain. The results raises a question that role of Epicatechin directly at the level of kinases or at the level of upstream kinases further need to be investigated.



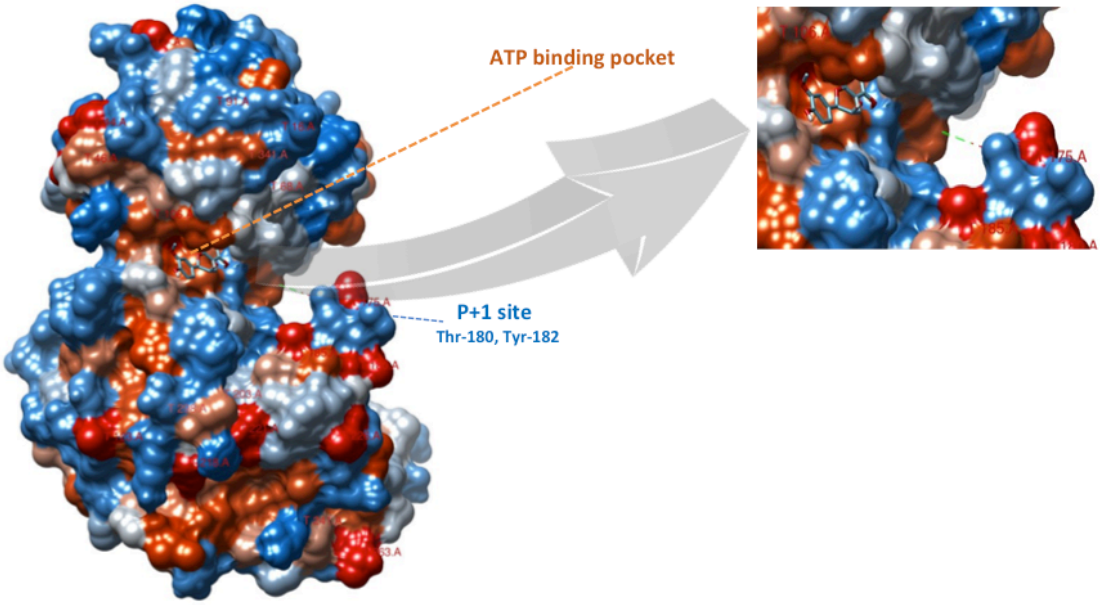




Figure 2: Out line of the Epicatechin role in inhibition of down stream kinase regulation of P38 MAPK and P65 signalling pathway , Three dimensional structure of p38 MAPK (PDB id: 3FMK) docked with epicatechin in catalytic binding pocket (ATP binding site) of p38-MAPK.

**How this COST-STSM contributed towards my personal learning**

My stay at INRA allowed me to learn more about interpretation of the data generated from high throughput technologies like Pamgene peptide array in the context of relevant biological process, networks and pathways by using pathway analysis software **Metacore®**. In addition to pathway analysis software I learnt to predict biotransformation of the plant derived compounds in vivo conditions by predicting *in silico* tool **Nexus meteor®** (Lhasam limited) software. This could be very helpful in understanding the fate of plant compounds when there is not much experimental metabolism data is available. Since understanding biotransformation of our in house plant derived compounds could help us to understand fate of plant metabolites in *in vivo* condition compared with parent compounds. My stay in INRA not only limited to learn new *in silco* techniques, but also enabled me to perform some *in vitro* experiments to explore molecular mechanisms behind the cardioprotective nature of plant flavanol compounds like epicatechin and its metabolites at the level of protein kinase signaling (Follow up studies to be done in Pamgene kinome platform in University of Antwerpen). This short term research opportunity at INRA exposed me to solve some practical problems that our research group had experienced for biological interpretation of data and also provided me with a steep learning curve of new platforms.

## References

1. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The Protein Kinase Complement of the Human Genome. Science (80- ). 2002;298(5600):1912–34.
2. Manach C, Hubert J, Llorach R, Scalbert A. The complex links between dietary phytochemicals and human health deciphered by metabolomics. Molecular Nutrition and Food Research. 2009. p. 1303–15.
3. Hilhorst R, Houkes L, Mommersteeg M, Musch J, van den Berg A, Ruijtenbeek R. Peptide microarrays for profiling of serine/threonine kinase activity of recombinant kinases and lysates of cells and tissue samples. Methods Mol Biol. 2013 Jan;977:259–71.
4. Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong C-P, Nettleton JA, et al. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am J Clin Nutr. 2007;85(3):895–909.
5. Buijsse B, Weikert C, Drogan D, Bergmann M, Boeing H. Chocolate consumption in relation to blood pressure and risk of cardiovascular disease in German adults. Eur Heart J. 2010;31(13):1616–23.
6. Claude S, Boby C, Rodriguez-Mateos A, Spencer JPE, G??rard N, Morand C, et al. Flavanol metabolites reduce monocyte adhesion to endothelial cells through modulation of expression of genes via p38-MAPK and p65-Nf-kB pathways. Mol Nutr Food Res. 2014;58(5):1016–27.