# Journal Scan

## Conformational analysis of the DFG-out kinase motif and biochemical profiling of structurally validated type II inhibitors

Structural coverage of the human kinome has been steadily increasing over time. The structures provide valuable insights into the molecular basis of kinase function and also provide a foundation for understanding the mechanisms of kinase inhibitors. There are a large number of kinase structures in the PDB for which the Asp and Phe of the DFG motif on the activation loop swap positions, resulting in the formation of a new allosteric pocket. We refer to these structures as "classical DFG-out" conformations in order to distinguish them from conformations that have also been referred to as DFG-out in the literature but that do not have a fully formed allosteric pocket. We have completed a structural analysis of almost 200 small molecule inhibitors bound to classical DFG-out conformations; we find that they are recognized by both type I and type II inhibitors. In contrast, we find that nonclassical DFGout conformations strongly select against type II inhibitors because these structures have not formed a large enough allosteric pocket to accommodate this type of binding mode. In the course of this study we discovered that the number of structurally validated type II inhibitors that can be found in the PDB and that are also represented in publicly available biochemical profiling studies of kinase inhibitors is very small. We have obtained new profiling results for several additional structurally validated type II inhibitors identified through our conformational analysis. Although the available profiling data for type II inhibitors is still much smaller than for type I inhibitors, a comparison of the two data sets supports the conclusion that type II inhibitors are more selective than type I. We comment on the possible contribution of the DFG-in to DFG-out conformational reorganization to the selectivity.

### Comment

Human kinome consists of 518 protein kinases. Authors selected 257 kinases from the PDB and divided into classical DFG out, non-classical DFG out and DFG in forms based on D1 < 7.2 and D2 > 9Å where D1 is distance between Cá atom and Asn of HRD motif, and D2 is distance between conserved Glu belonging to áC helix and Phe of the DFG motif. Authors concluded that structurally validated type II inhibitors are generally more selective than type I inhibitors. Authors also suggested the overall importance of free energy conformations of DFG-in to DFG-out compared with binding energy in the selectivity of inhibitors for individual kinases remains to be determined.

*Vijayan RSK, He P, Modi V., Duong-Ly KC, Ma H, Peterson JR, Dunbrack, Jr. RL, Levy RM. Conformational analysis of the DFG-out kinase motif and biochemical profiling of structurally validated type II Inhibitors. J of Medicinal Chem 2015;58:466-79.*

## Enzymatic characterization of *Chlamydophila pneumoniae* phospholipase D

*Chlamydophila pneumoniae*, an aetiological agent of respiratory infection, is also thought to play an immuno- pathogenetic role in atherosclerosis by contributing to inflammation and plaque

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http://svimstpt.ap.nic.in/jcsr/Jul-Sep15_files/js15.pdf

instability. Phospholipase D (PLD) is an enzyme involved in lipid metabolism and may have a direct or indirect impact on virulence and the inflammatory response. Some aspects of the developmental cycle of C. pneumoniae suggest a direct implication of its PLD (CpPLD) in the pathogenesis, specifically by affecting the regulation of lipid metabolism and lipid exchange between C. pneumoniae and host cells. Our previous studies disclosed a specific anti-CpPLD antibody response in patients with acute coronary syndromes chronically infected with C. pneumoniae, and demonstrated that this antigen is a factor able to drive the inflammatory process in atherosclerosis. Due to the intriguing aspects of the CpPLD, the present study investigated CpPLD enzymatic activity of the protein and the two domains that include one HKD motif each polypeptide. Our results showed that CpPLD was able to synthesize the cardiolipin (CL) but unable to hydrolyze phospholipids. It was also observed that each single HKD motif has an independent CL synthetase activity. This enzymatic activity of CpPLD could be important in the inflammatory process within the atherothrombotic events.

**Comment**

CpPLD has two domains and each domain has HKD motif with independent cardiolipin (CL) synthetase activity. CL is one of the principle phospholipids in the mammalian heart, a tissue particularly sensitive to oxidative stress and mitochondrial dysfunction. Oxidative mechanisms of CL are related with cardiac disorders including ischemia and heart failure. CpPLD could play an important role in the pathological remodeling of CL in mitochondrial cardiac dysfunction and subsequent heart injury. The enzymatic activity of CpPLD may be important in the progression of atherothrombotic events, and can be used as a therapeutic target for the prevention and treatment of the disease.

*Mancini F, Ciervo A. Enzymatic characterization of Chlamydophila pneumoniae phospholipase D. New Microbiol 2015;38:59-66.*

## Increased inflammatory response in cytomegalovirus seropositive patients with Alzheimer's disease

Alzheimer's disease (AD) has been associated with increased local inflammation in the affected brain regions, and in somestudies also with elevated levels of proinflammatory cytokines in peripheral blood. Cytomegalovirus (CMV) is known topromote a more effector-oriented phenotype in the T-cell compartment, increasing with age. The aim of this study was toinvestigate the inflammatory response of peripheral blood mononuclear cells (PBMCs) from AD patients and non-demented (ND) controls. Using a multiplex Luminex xMAP assay targeting GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IP-10 and TNF-α, cytokine profiles from PBMCs were analyzed after stimulation with anti-CD3/CD28 beads, CMV pp65 peptide mix or amyloid β (Aβ) protofibrils, respectively. CMV seropositive AD subjects presented with higher IFN-γ levels after anti-CD3/CD28 and CMV pp65 but not after Ab stimulation, compared to CMV seropositive ND controls. When analyzing IFN-γ response to anti-CD3/CD28 stimulation on a subgroup level, CMV seropositive AD subjects presented with higher levelscompared to both CMV seronegative AD and CMV seropositive ND subjects. Taken together, our data from patients withclinically manifest AD suggest a possible role of CMV as an inflammatory promoter in AD immunology. Further studies of AD patients at earlier stages of disease, could provide better insight into the pathophysiology.

**Comment**

In this article, authors investigated the functional capacity and cytokine release profile of peripheral blood mononuclear cells (PBMCs) in response to CMV, Aâ antigen challenge as well as the correlation to ApoE

genotype and systemic inflammatory biomarkers. CMV infection increases the local inflammatory response against wide variety of antigens including Aâ by inducing the production of amyloid precursor protein (APP) and formation of neurotoxic protofibrils, thereby enhancing the cognitive decline which raises the AD pathogenesis. The article provided information about immune response in AD subjects with CMV infection. Studies with a prospective design, CMV serostatus analysis, systemic inflammation and cellular immune response, in subjects at prodromal disease stages could be better estimate of the clinical implications.

*Westman G, Berglund D, Widen J, Ingelsson M, Korsgren O, et al. increased inflammatory response in cytomegalovirus seropositive patients with Alzheimer's Disease. PLoS ONE 2014;9:e96779.*

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