NANYANG TECHNOLOGICAL UNIVERSITY

School of Biological Sciences

Reg. No. 200604393R

Research Theme:
Research Project Title: **Mechanism of chaperone function of prostaglandin synthase for amyloid b-peptides**
Principal Investigator/Supervisor: **A/Prof Konstantin Pervushin**
Co-supervisor/ Collaborator(s) (if any): **NA**

Project Description

In many threatening neurodegenerative diseases, such as Alzheimer's, Parkinson and prion-related diseases which involve aggregation of specific proteins, Nature's quality control systems usually fend off these toxic protein aggregates or misfolded proteins by complex protein chaperones. In this proposal we aim to answer the question of how at the molecular level the major beta-chaperone for amyloid beta-peptides, the primary causative agent in Alzheimer's disease, does prevent malicious aggregation of the peptides and how this essential beta-chaperone function may be compromised by common drugs. In Alzheimer's disease the main component of the toxic amyloid deposits is a short, 42 amino acid long, amyloid beta-peptide or Abeta(1-42). Several extracellular proteins found in cerebrospinal fluid (CSF) in the brain exhibit protective function against aberrant toxic aggregation of Abeta(1-42)[1] by binding and keeping soluble amyloid beta-peptides from misfolding and inhibiting their aggregation and deposition. These protective proteins are generally designated as beta-chaperones and they are frequently found in the amyloid plaques or toxic deposits alongside Abeta and other associated peptides. Their malfunction or genetic mutation might be a primary cause for aberrant toxic protein aggregation leading to higher risk of neurodegenerative diseases. Up to date only limited structural data exist explaining with atomic details how these proteins interact with their substrates and prevent or even reverse toxic depositions of amyloid beta-peptides.

Recently it was discovered that the enzyme, Lipocalin-type Prostaglandin D Synthase (L-PGDS), in addition to its enzymatic activity, may function a major beta-chaperone in human cerebrospinal fluid (CSF)[2]. This protein, as many of the beta-chaperones, appears to be highly multifunctional with one of its primary activities being the direct catalysis of conversion of prostaglandin H2 to prostaglandin D2, an important inflammatory and nociception mediator, making it an important pharmaceutical target. L-PGDS is the most abundant protein in CSF and is predominantly expressed in the central nervous system rather than in the peripheral organs. The concentration of this protein is found to be decreased in CSF of AD patients and L-PGDS-knockout mice showed accelerated deposition of Abeta(1-42) fibrils in the brain. This protein inhibits amyloid beta-peptides aggregation in vitro and in vivo, tightly binds with nanomolar affinity to amyloid beta-peptides Abeta(1-40) and Abeta(1-42) and to the amyloid fibrils. The enzymatic activity of L-PGDS is conducted via its catalytically active Cys-65 residue. A mutant form of L-PGDS where Cys-65 is replaced with Alanine is catalytically inactive.

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Project Description (cont.)

A mutant form of L-PGDS where Cys-65 is replaced with Alanine is catalytically inactive. At the same time this mutant is unable to inhibit Abeta(1-42) aggregation, indicating strong structural and biochemical association between chaperoning and catalytic functions stipulating that chemical modulators or inhibitors of either function might compromise the other one. Since L-PGDS has also the ability to bind and transport lipophilic compounds like biliverdin, all-trans retinoic acid and bilirubin between cell membranes and is an important membrane associated transporter, it might exert its beta-chaperone function in association with lipid membranes where Abeta and other lipophilic compounds accumulate. In our preliminary research we addressed structural aspects of the prostaglandin D catalysis, substrate/ligand entry and egress routes using biophysical and biochemical methods, X-ray crystallography and NMR [3]. We solved the first structure of human L-PGDS in a complex with substrate analogues and in a ligand-free form revealing the mechanism of activation of the enzyme. To follow-up on our revealing results with human L-PGDS, we want to address here the challenging goal of establishing the mechanism of Abeta-chaperone activity at the atomic level. We aim to develop mechanistic models of the interactions of native amyloid beta-peptides 1-40 and 1-42 with L-PGDS, their entry and egress routes, the thermodynamics and kinetics of binding as well as the interaction of L-PGDS with variable order Abeta oligomers and amyloid filaments and potential de-aggregation of filaments by the chaperone. For this purpose, we will employ methods of high resolution NMR, in solution and condensed solid state, X-ray crystallography, state-of-the art methods of molecular dynamics simulations and biophysical techniques such as Surface Plasmon Resonance and Isothermal Calorimetry. This effort will evolve into a platform for studying the functional disturbance mechanism by natural L-PGDS substrates and inhibitors as well as a variety of FDA-approved drugs showing strong potential to interact with L-PGDS [4]. The set-up of a platform is an urgent task, since many of the mentioned drugs currently in clinical use exhibit sufficiently high affinity to L-PGDS with a potential of attenuating or even completely compromising its beta-chaperone function, thus exposing the patient to the risk of AD. Based on the mechanism of chaperone activity, we are going to describe with the atomic-level details how various exogenous and endogenous lipophilic substances can produce disturbance of the Abeta-chaperone function of L-PGDS. This may result into a novel drug-safety test critically important for the drugs in the long term use.

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