Mechanistic Understanding of PHD2 enzyme upon ligand interactions using SAXS and SANS

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Prolyl hydroxylasedomain proteins (PHDs) play an important role in the regulation of cellularhomeostasis in response to changes in cellular oxygen level.[1] In thepresence of oxygen, PHDs catalyse the oxygen-dependent hydroxylation of hypoxiainducible factor (HIF), which lead to its degradation. However, in the absenceof oxygen, HIF cannot be hydroxylated and can therefore trigger downstreamhypoxic responses including the formation of new blood vessels and red bloodcells.

PHDs catalyse thehydroxylation of HIF at two different proline residues.[2–3] We areinterested in understanding the structural basis in the substrate selectivityof PHDs. Using small angle X-ray scattering (SAXS), we examined the conformationalchanges of PHD2 particularly ?2 ?3 loop in the presence of CODD and NODD substrateswhere we see change in overall size and shape of PHD2. We obtained PHD2-NODDsolution structure as a confirmation to crystallographic structure[3].We would further confirm these conformational changes upon ligand interactionsusing Small angle neutron scattering. It would help us in understand ?2 ?3 loopand ?4 regions of PHD2 which is crucial in binding of CODD and NODD substrates. The contribution of ?2 ?3 loop and ?4 regions of PHD2 in protecting the active site is known from the

NMR data[2–3.]

Both SAXS and SANS would be complementary to NMR data in solution which would further help us in design PHD2 inhibitors for hypoxia related diseases like ischemic heart disease and anaemia.

References:

1. Schofield, C. J.;Ratcliffe, P. J. Oxygen sensing by HIF hydroxylases. *Nat. Rev. Mol. Cell. Biol.* **2004**,*5*, 343–354.

2. McDonough, M. A. *et al*. Cellular oxygen sensing: crystalstructure of hypoxia-inducible factor prolyl hydroxylase (PHD2). *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 9814–9819.

3. Chowdhury, R. *et al*. Structural basis for binding ofhypoxia-inducible factor to the oxygen-sensing prolyl hydroxylases. *Structure* **2009**, *17*, 981–989.