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Gatekeepers of Prolyl-4-Hydroxylase 2 and mechanistic studies

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G. Fiorini, S. Marshall, W. Figg, M. Kibria, F. Arif, P. Rabe, M. A. McDonough, C. J. Schofield

In humans, three HIF prolyl-hydroxylases (PHD1-3) play key roles in hypoxia sensing. The PHDs are 2-oxoglutarate (2OG)-dependent dioxygenases that catalyse trans-4-prolyl hydroxylation of the hypoxia-inducible factors (HIFs). Prolyl-hydroxylation enables the proteasomal degradation of HIF, causing the suppression of the hypoxic response. The aim of this work is to use time-resolved crystallography and spectroscopical techniques to elucidate the mechanism of the PHDs, by characterising the reaction intermediates. High-resolution structures of an anoxic PHD2.Fe.2OG.HIF2αCODD complex were obtained and exposure to O2, validated the possibility of monitoring the reaction in crystallo leading to the first high-resolution structures of a PHD2:product complex. One of PHDs peculiarities is their slow reaction with O2. It is unclear how O2 diffuses towards the active site, but of particular interest is residue Thr387. PHD2.substrate crystal structures show Thr387 to be involved in hydrogen bond networks with water molecules in the first and second coordination sphere of the active site. Mutations of the Thr387 to less polar residues cause an increase in enzyme activity. Crystal structures of these mutants showed a lack of water molecules near the active site when compared with the wild-type PHD2, providing a possible explanation for the increased turnover. Additionally, it was possible to spectroscopically identify a potential “ferryl”intermediate not yet reported for PHDs. Overall, there results elucidate key binding interactions between PHD2, product and cofactor, confirm the stereoselectivity of the hydroxylation, and identify a gate keeper residue in PHD2.

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Primary authors: Prof. SCHOFIELD, Christopher (University of Oxford); FIORINI, Giorgia; Dr MCDONOUGH, Micheal (University of Oxford); Dr RABE, Patrick (University of Oxford); Dr FIGG, William (University of Oxford)

Presenter: FIORINI, Giorgia

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