

HIF-prolyl-hydroxylase 2 clinical inhibitor complex structure and XChem fragment-based screen obtained with succinate co-product used as a crystallisation tool

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The hypoxia mediated prolyl-hydroxylase isoforms 1-3 (PHD1-3) are members of the Fe(II)- α -ketoglutarate (2OG)-dependent oxygenase superfamily of enzymes [1]. PHD1-3 catalyse *trans*-4-prolyl-hydroxylation of the labile hypoxia-inducible factor- α subunit (HIF α) in the presence of Fe(II), 2OG, ascorbic acid, and dioxygen [1]. The hydroxylation of the oxygen degradation domain (ODD) of the HIF1-3 α substrates marks the transcription factor for degradation *via* the ubiquitin-E3 ligase-28S proteasome pathway [1]. In hypoxic conditions, PHD1-3 are less active and the HIF α subunits translocate into the nucleus and form an α , β -heterodimer with the stable HIF β subunit that subsequently leads to the genetic cascade in response to hypoxia, *e.g.* upregulation of erythropoietin (*EPO*) and vascular endothelial growth factor (*VEGF*) [1]. Inhibition of the PHDs, and specifically PHD2, the most commonly expressed isoform, has been the focus for small-molecule based therapies to treat individuals with anaemia and ischaemia-related disorders [2]. Recently, several PHD inhibitors are approved; Roxadustat (Japan, Chile, and China), Daprodustat (Japan), Molidustat (Japan), Enarodustat (Japan), and Vadadustat (Japan) [3]. Co-crystal structures of some of the clinical inhibitors have been challenging to obtain due to conformational heterogeneity induced through ligand complexation [2-3]. A clinical inhibitor complex structure with PHD2 has been obtained using a novel crystallisation system utilising succinate, co-product of the PHDs reaction, as a crystallisation tool to stabilise the active site during incubation with Molidustat, the related compound IOX4, and monodentate binding inhibitor Takeda-17 (Fig. 1A-B) [3-4]. The novel crystal form was then further used for inhibitor soaking and scale-up of crystal growth (~500 crystals) for an XChem fragment-based screen (I04-1, Diamond Light Source) in search for allosteric binding sites. To date, the crystallisation system yielded 3 inhibitor co-crystal structures, 8 complex structures through soaking, and 5 hits from the fragment screen.

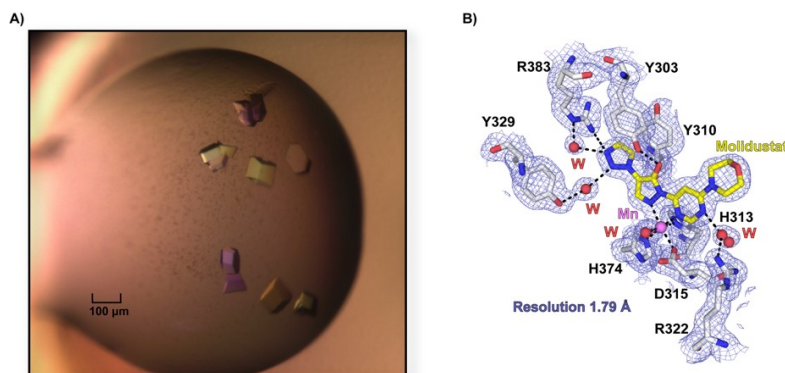


Figure 1. (A) Novel rhombohedral PHD2₁₈₁₋₄₀₇ crystals using succinate co-product. (B) PHD2₁₈₁₋₄₀₇-Molidustat complex structure (PDB: 6ZBO).

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