Structural Investigation of Insulin Complexes for the Development of Drugs Against Diabetes

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Human insulin (HI) exhibits extensive molecular and crystalline polymorphism modulated by ligand binding and pH, impacting pharmaceutical formulation and pharmacokinetic properties. In this work, complementary diffraction techniques were employed to investigate insulin–ligand complexes. High angular resolution X-ray powder diffraction (XRPD) experiments at ID22[1] (ESRF) enabled rapid polymorph screening of polycrystalline samples across varying pH conditions[2]. *Pawley* analysis[3] and systematic investigation of multiple datasets facilitated phase identification and construction of phase diagrams, revealing ligand-induced polymorphic transitions and distinct space groups even under similar chemical conditions. Importantly, a monoclinic insulin polymorph $P2_1(\beta)$ [a=61.1921(7) Å, b=61.8750(5) Å, c=47.8442(7) Å, β=110.4996(9) ◦] was structurally solved from XRPD data employing a stereochemically restrained and multi-dataset Rietveld refinement[4], demonstrating the potential of XRPD for structural studies of pharmaceutical protein systems[5]. To obtain high-resolution structural information from heterogeneous and weakly diffracting samples, serial synchrotron crystallography (SSX) experiments were performed at ID23-2[6] (ESRF) using the *MeshAndCollect*[7] workflow. Data collected as small rotation wedges were grouped using *CODGAS*[8], a genetic algorithm–based approach that optimises dataset selection based on merged data quality metrics, effectively addressing non-isomorphism. The combined approach enabled structural characterisation of multiple ligand-induced insulin polymorphs, including complexes with *m*-nitrophenol, resorcinol and 4-hydroxybenzoic acid. This integrative diffraction strategy provides a robust structural framework for understanding polymorphism and supports structure-based optimisation of insulin drug formulations.

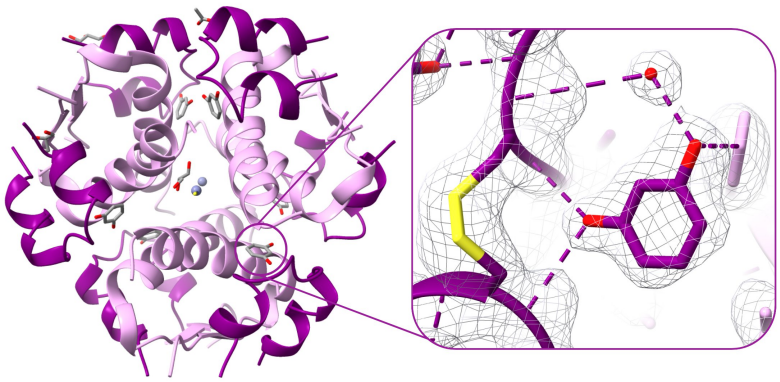


Figure 1. *Structural model of HI-resorcinol complex in a novel crystalline phase $P2_1(\gamma)$ (a=46.98 Å, b=69.09 Å, c=85.39 Å, β=105.18 °). A magnified view of one ligand-binding site is shown, with the $2F_o–F_c$ electron density map contoured at 1σ.*

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