

Crystallization of VRK1- case study

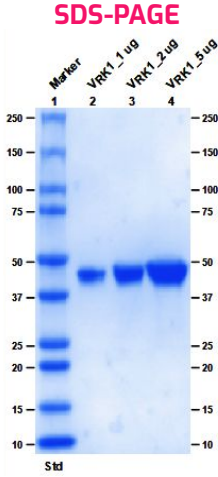
I. Protein Production & Isothermal Titration Calorimetry (ITC)

Construct

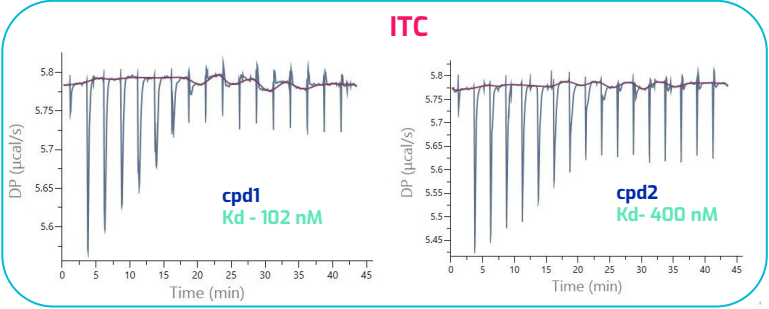
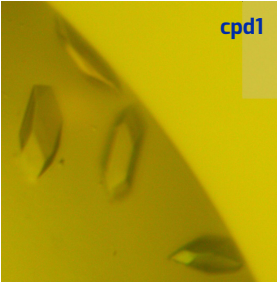
6xHis VRK1 mutant

Surface Entropy Reduction (SER) mutagenesis of flexible surface residues (Lys to Ala, Glu to Ala)

12 residues mutated to Ala (K34A, K35A, E36A, E212A, K214A, E215A, E292A, K293A, K294A, K359A, K360A, E361A)



Co-crystals



Partner

Internal case study project

Background

Inhibition of VRK1 (Vaccinia-Related Kinase 1) presents a potential synthetic lethality approach in combination with other inhibitors of DNA damage response pathways

Aim

To generate protein for co-crystallization of VRK1 with compounds to study SAR in parallel with biophysical and biochemical assays

Our Approach

Generate VRK1 mutant → optimise protein expression in *E. coli* → large scale expression → purification by IMAC/SEC → dephosphorylated with λ-phosphatase → crystallization & binding analysis by ITC

Value

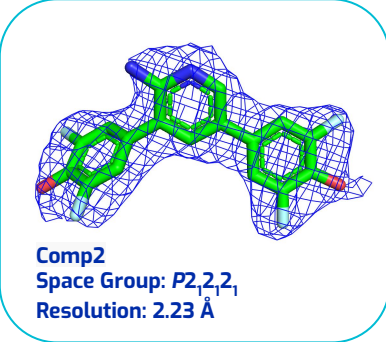
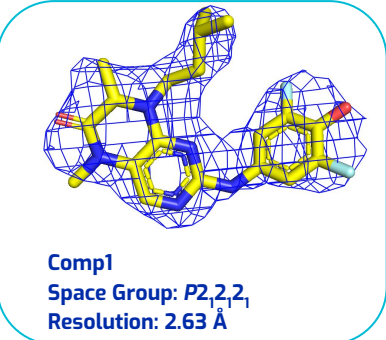
- Crystals diffract at 2.6Å (cpd1) & 2.2Å (cpd2)
- Showcasing Arctoris Gene-Protein-Structure capability



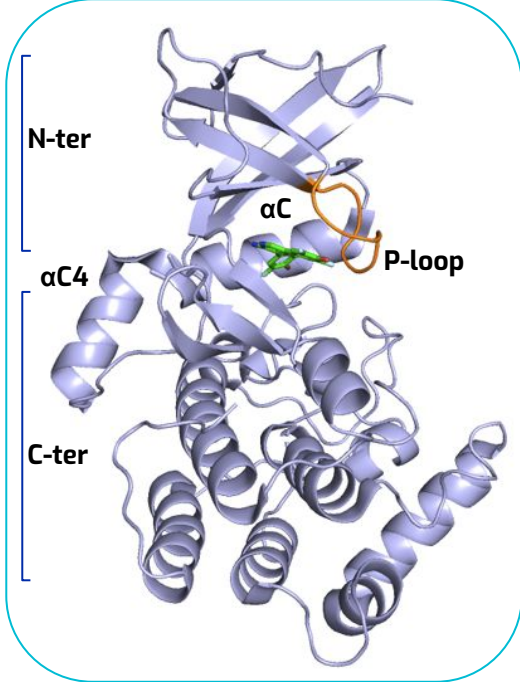
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II. Crystallization

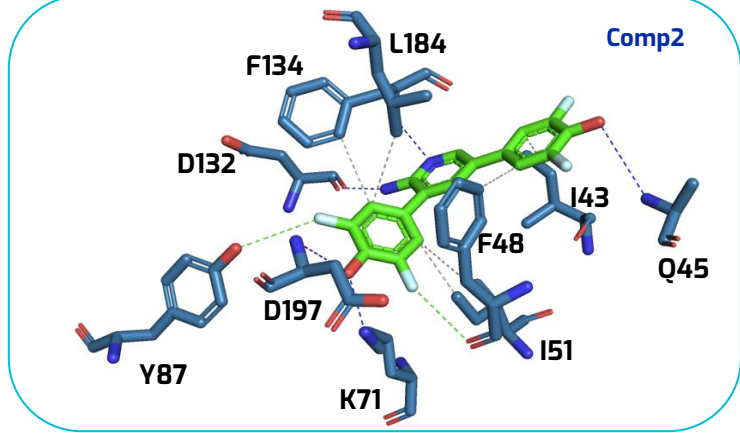
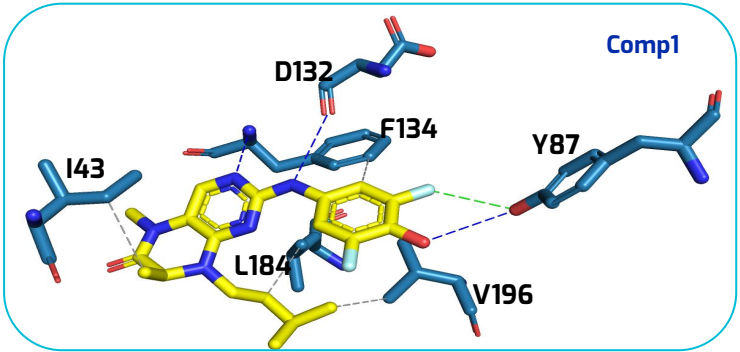
Electron density maps ($2Fo - Fc$)
contoured at 1.0σ



Overall Structure
VRK1-Comp1



VRK1-ligand interaction



Dashed line indicates a interaction between protein and ligand- green halogen bond, blue-H-Bond, Grey- Hydrophobic interaction