PREDICTIVE MODELLING AND MACHINE LEARNING-ASSISTED ENGINEERING OF *Av*PAL FOR IMPROVED THERMAL STABILITY

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Phenylalanine ammonia-lyase (PAL) is an important enzyme with biocatalytic applications in various industries as well as in the treatment of Phenylketonuria (PKU). The thermodynamic stability of PAL affects its activity, half-life, and solubility. In this study, we aimed to improve the thermodynamic stability of *Anabaena variabilis* PAL (*Av*PAL) by engineering disulfide bonds guided by three in-silico approaches: Pair Interactions Energy, Contact Maps, and Machine Learning. The predictive model developed using the Machine Learning approach was trained on structural geometry, torsion angles, the distance between sulfur atoms, and dihedral angles defining cysteine disulfides, and was used to generate plausible disulfide variants with high probability of formation. The variants were modeled using Alphafold and investigated for structural flexibility and stability of disulfide with MD simulations under constant volume and different temperatures. The designed disulfide variants were observed to not only increase the stability of *Av*PAL, but also increase its activity; amongst which variant VAL344CYS_SER271CYS (KCAT0036) showed highest activity. This study highlights the effectiveness of computational design in improving the thermodynamic stability of enzymes, which can have significant applications in industrial biocatalysis and enzyme replacement therapies.

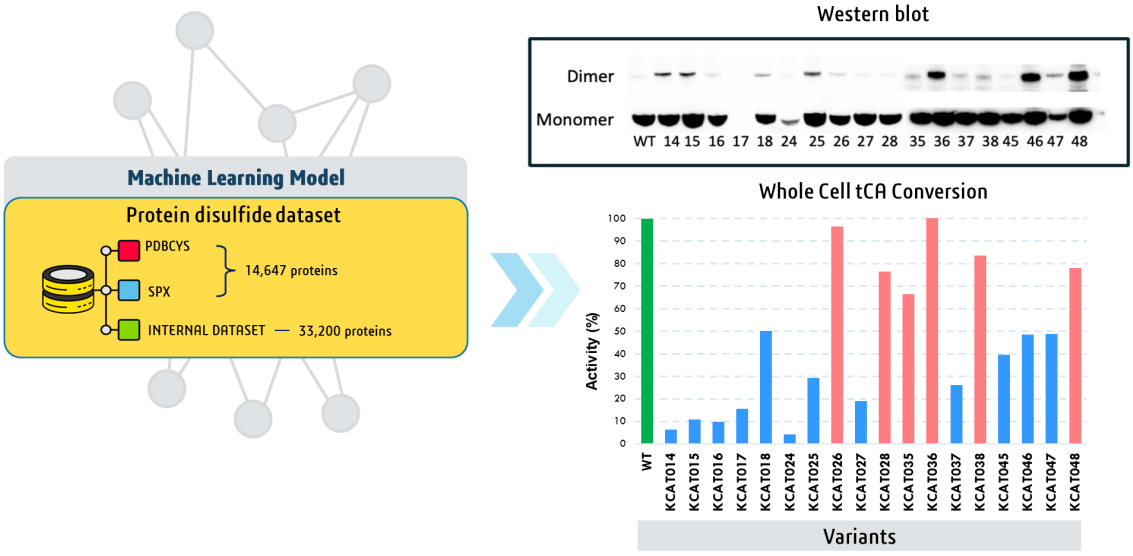

Figure 1: Machine learning-assisted computational design for improving the thermodynamic stability of AvPAL