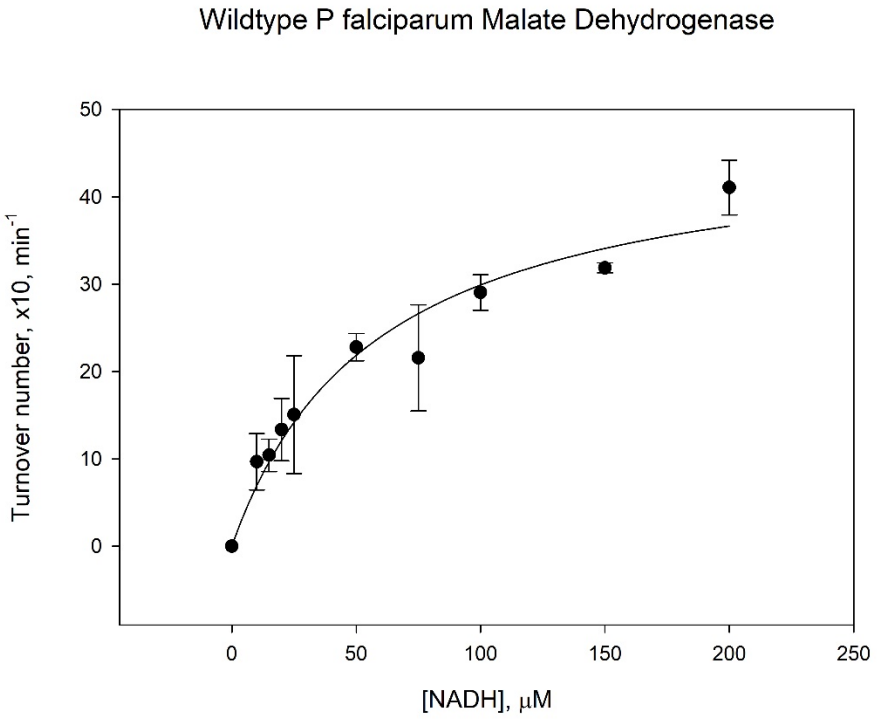
Michaelis Menten Kinetics of WildType and Individual Mutants

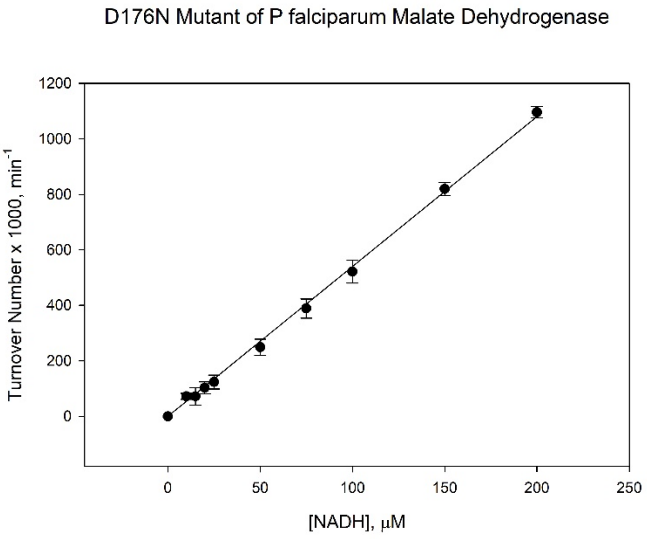
Initial Rate Kinetics experiments were performed at 0.05M Phosphate Buffer, pH 8.0 at room temperature. The rate of the reaction was determined by following absorbance at 340nm (due to the NADH) for a period of 60 seconds, the initial linear portion of the plot was used to calculate the rate (usually 30-40 seconds). Rates were calculated as apparent turnover numbers (μM product/μM enzyme added/min). All experimental data points were conducted in triplicate and averages and error bars plotted according to the Michaelis Menten Equation, Rate = Vmax[S]/(Km + [S] and the data fitted to give a value of Km +/- Standard Deviation.

Experiments with [NADH] varied were performed at a fixed Oxaloacetate concentration of 200μM.

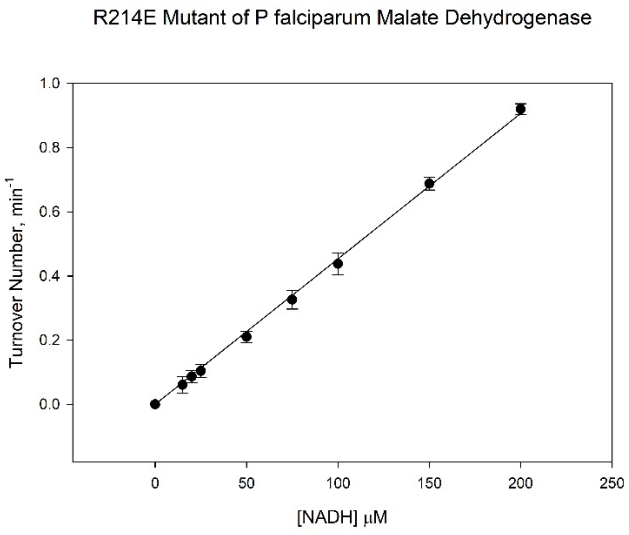
WildType:



D176N Mutant:



R214E Mutant:



In the poster this data is represented in a single graph where the rate is normalized to the apparent turnover number obtained at 200mM NADH to allow easy comparison. The error bars have also been omitted to simplify the graphic and illustrate the dramatic effect of the mutations on ther NADH Saturation versus the wildtype.