The increase in enzyme catalytic rates with temperature is typically modeled using Arrhenius or Eyring-Polyani relations. Substantial deviations from Arrhenius behavior may be observed at elevated temperatures where enzymes remain folded. However, structural changes that may modulate temperature-dependent rates and provide direct evidence of proposed mechanisms for non-Arrhenius behavior have rarely been described. Here, a comprehensive multi-temperature x-ray crystallography study records structural changes from -20°C – 40°C for a mesophilic enzyme in complex with inhibitors faithfully mimicking the substrate, intermediate, and product-bound states. Both substrate and catalytically relevant loop motifs evolve toward more competent conformations as temperature increases. These changes are observed even in temperature ranges where kinetics measurements show linear Arrhenius behavior. Simple analysis shows that linear temperature dependence of the underlying enthalpy of activation results in strict Arrhenius behavior with a single derived enthalpy of activation, and that even small fractional variations in either the underlying enthalpy or entropy of activation over experimental temperature ranges produce large changes in the two apparent activation parameters.

Additionally, entropy of activation temperature dependencies can manifest as significant curvature of the Arrhenius plot. Our results indicate a critical role for temperature-dependent atomic-resolution structural data in interpreting kinetics data and variations in Arrhenius plots within and between enzymatic systems.