SUPPLEMENTARY RESULTS

This document presents a comparison of fits of competing models to experimental data obtained at pH = 7.5. Specifically, the following four models are fit to time-course data: Theorell-Chance, ping-pong, and ordered bi-bi with and without an abortive complex formed. The results presented are for data collected with pH = 7.5, T = 25 C, and I = 0.17 M. The ordered bi-bi mechanism is found to most effectively explain the experimental data. The abortive-binding model is identical to the simple ordered bi-bi model with the addition of binding of oxaloacetate competing with malate for binding to the EA state. All mechanisms are illustrated in Figure S1. Although the ordered bi-bi mechanism with the abortive complex can explain the data, the fits of this model are not substantially better than the simpler model without dead-end binding and some of this model's parameters are unidentifiable. We observed similar trends at other pH values.

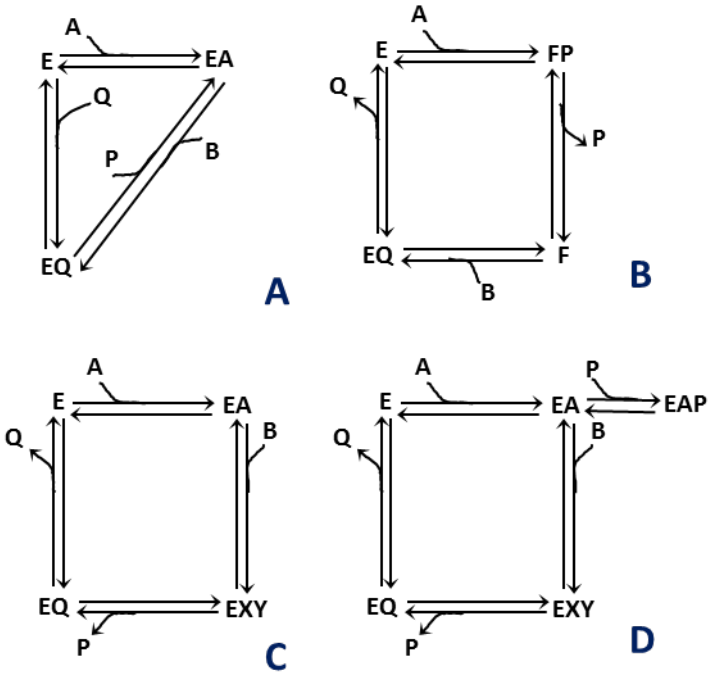

Figure S1: illustration of various mechanisms tested: A) Theorell-Chance mechanism, B) Ping-pong bi-bi mechanism, assuming NAD/NADH binds first, C) Ordered bi-bi mechanism without any abortive complex formed. D) Ordered bi-bi mechanism with EAP abortive complex formed. In all these schemes, A, B, P, Q represent NAD, MAL, OAA, and, NADH respectively.

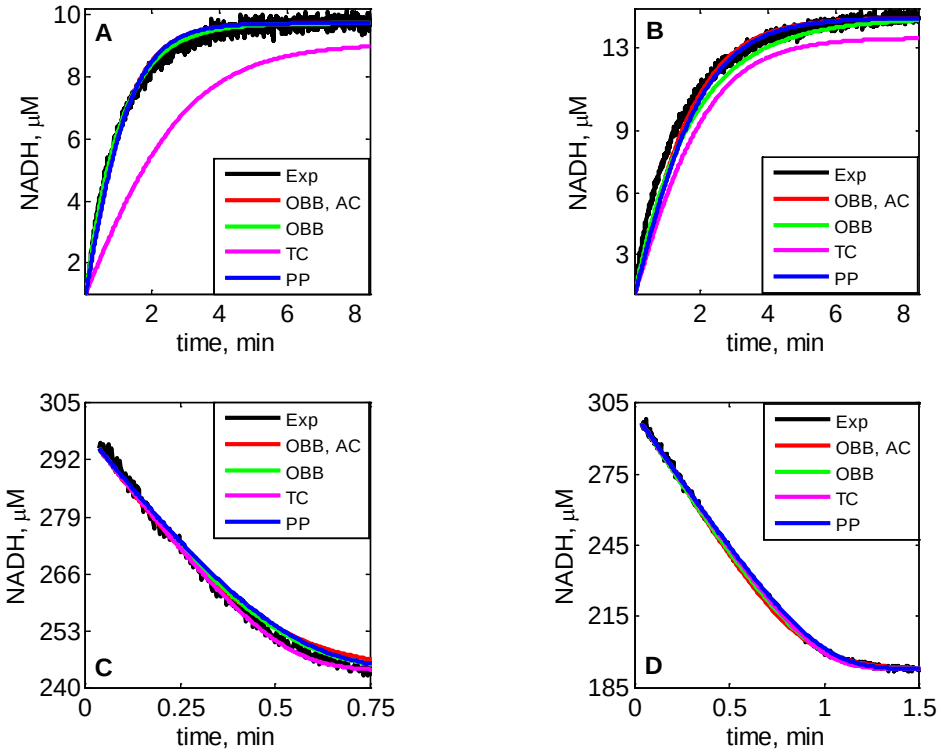

Figure S2: NADH vs time in forward and reverse direction without product inhibitor present for pH 7.5, I = 0.17 M, and T = 25 °C: A) $[NAD]_0$ = 1 mM, $[MAL]_0$ = 5 mM, B) $[NAD]_0$ = 1 mM, $[MAL]_0$ = 10 mM, C) $[NADH]_0$ = 300 µM, $[OAA]_0$ = 50 µM, D) $[NADH]_0$ = 300 µM, $[OAA]_0$ = 100 µM. In each of the plots, Exp, OBB,AC, OBB, TC and PP represent experimental data, data fits using ordered bi-bi model with EAP as abortive complex, ordered bi-bi model without abortive complex, Theorell Chance, and Ping-Pong model respectively. A simple ordered bi-bi mechanism effectively explains the data, while the addition of abortive complex formed does not improve fits significantly. Ping-pong and Theorell-Chance mechanism cannot explain the experimental data at fixed pH.

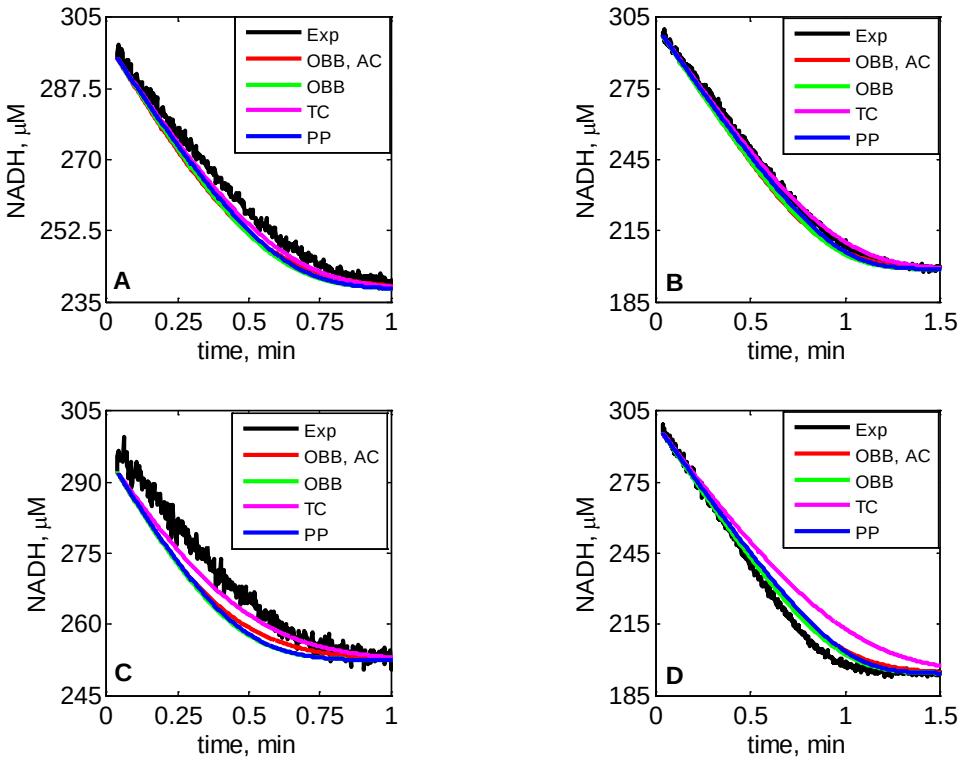

Figure S3: NADH vs time in reverse direction with NAD as product inhibitor for pH 7.5, I = 0.17 M, and T = 25 ˚C with $[NADH]_0$ = 300 μM and : A) $[OAA]_0$ = 50 μM, $[NAD]_0$ = 1 mM, B) $[OAA]_0$ = 100 μM, $[NAD]_0$ = 1 mM, C) $[OAA]_0$ = 50 μM, $[NAD]_0$ = 2 mM, D) $[OAA]_0$ = 100 μM, $[NAD]_0$ = 2 mM. In each of the plots, Exp, OBB,AC, OBB, TC and PP represent experimental data, data fits using ordered bi-bi model with EAP as abortive complex, ordered bi-bi model without abortive complex, Theorell Chance, and Ping-Pong model respectively.A simple ordered bi-bi mechanism could explain data with a give pH while assuming abortive complex formed did not improve fits significantly. Ping-pong and Theorell-Chance mechanism could not explain the experimental data at a given pH.

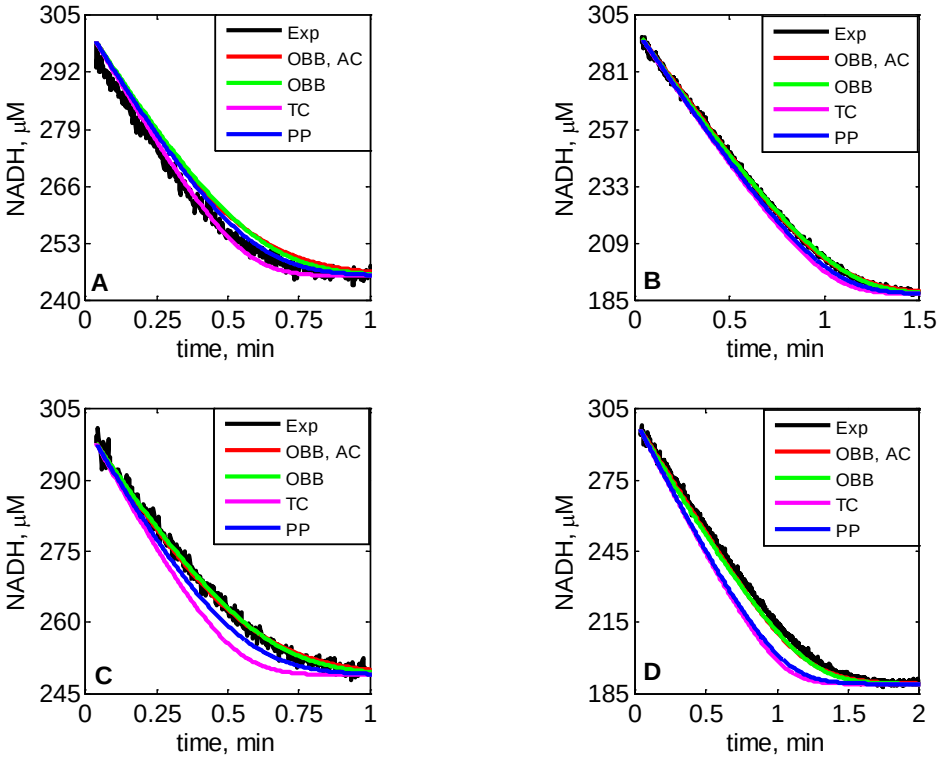

Figure S4: NADH vs time in reverse direction with MAL as product inhibitor for pH 7.5, I = 0.17 M, and T = 25 ˚C with $[NADH]_0$ = 300 µM and : A) $[OAA]_0$ = 50 µM, $[MAL]_0$ = 1 mM, B) $[OAA]_0$ = 100 µM, $[MAL]_0$ = 1 mM, C) $[OAA]_0$ = 50 µM, $[MAL]_0$ = 2 mM, D) $[OAA]_0$ = 100 µM, $[MAL]_0$ = 2 mM. In each of the plots, Exp, OBB,AC, OBB, TC and PP represent experimental data, data fits using ordered bi-bi model with EAP as abortive complex, ordered bi-bi model without abortive complex, Theorell Chance, and Ping-Pong model respectively. A simple ordered bi-bi mechanism could explain data with a give pH while assuming abortive complex formed did not improve fits significantly. Ping-pong and Theorell-Chance mechanism could not explain the experimental data at a given pH.