Supporting information

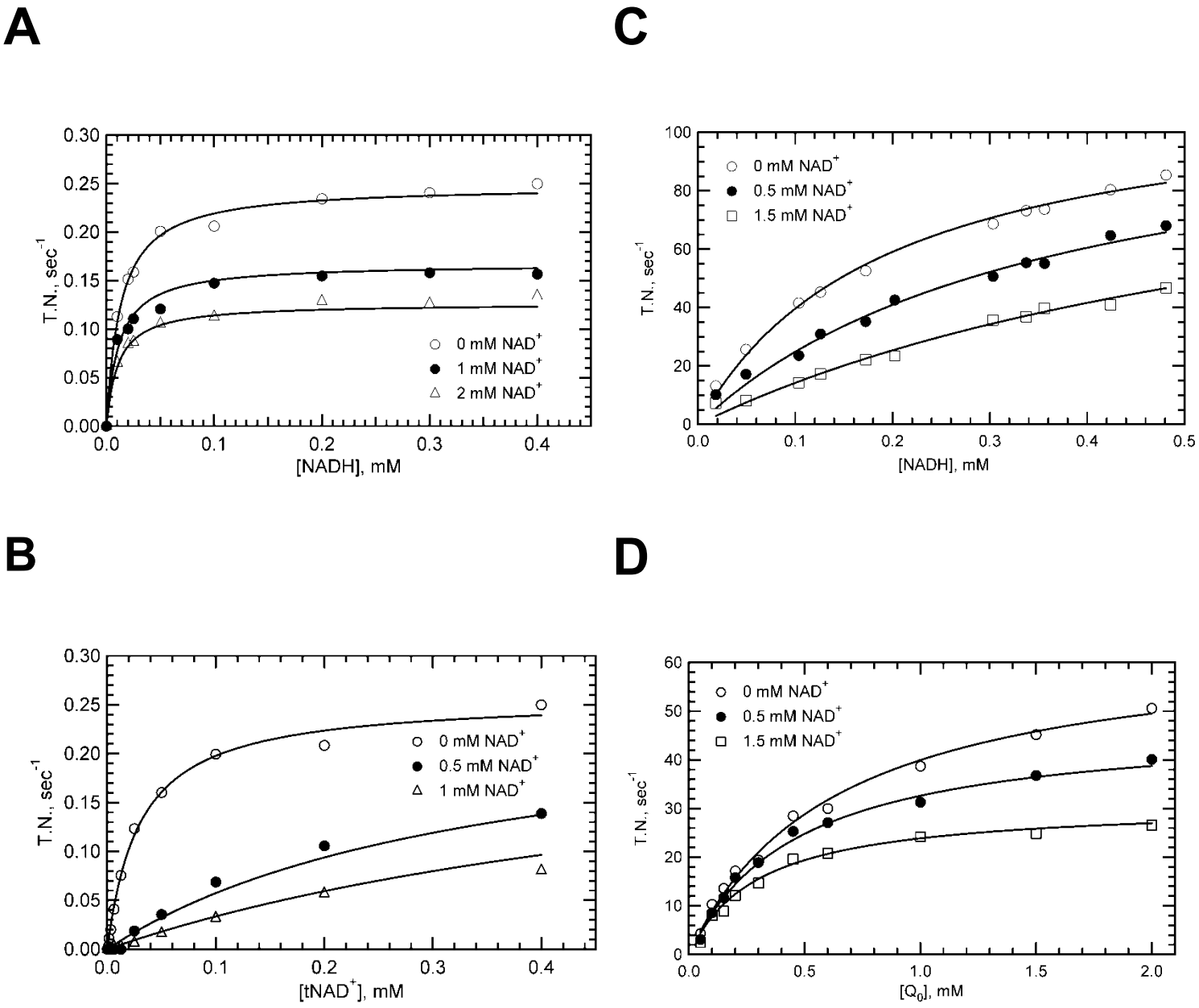

Figure S1. Kinetics of NAD^+ inhibition for NADH-$tNAD^+$ transhydrogenase and NADH-UQ_0 reductase activities as same as Figure 4 except that data were presented as s-v plots. Data were globally fit using the same parameters used in Figure 4.

Kinetic parameters for the ping-pong mechanism

Kinetic parameters for the NADH-Q reductase and transhydrogenase reactions are defined as shown below based on rate constants for microscopic steps of each reaction depicted in Scheme S1. In the NADH-Q reductase reaction, the second half-reaction (Q reduction) is much faster than the first-half reaction ($k_7 >> k_3$). Hence, K_M^{NADH} for the NADH-Q reductase reaction can be simplified to $K_{M\,(N-Q)}^{NADH} \approx \frac{k_2+k_3}{k_1}$. For the transhydrogenase reaction, $K_{M\,(Trans)}^{NADH} = \frac{k_2+k_3}{k_1} \times \frac{k_{11}}{k_8+k_{11}} = K_{M\,(N-Q)}^{NADH} \times \frac{k_{11}}{k_8+k_{11}}$. If hydride transfer from $FADH_2$ to $tNAD^+$ is rate-limiting, then $k_3 >> k_{11}$ can account for the lower K_M^{NADH} for NADH in the transhydrogenase reaction than is observed in the NADH-Q reductase reaction. These assumptions further result in $K_M^Q \approx \frac{k_3(k_6+k_7)}{k_7 k_8}$ and $K_M^{tNAD^+} \approx \frac{k_{10}+k_{11}}{k_9}$. The affinity of Q for the enzyme is most likely influenced by the off rate, k_6. We have observed a clear tendency that the K_M^Q values are much smaller if quinone has a longer isoprenoid chain, $K_M^{UQ_2} < K_M^{UQ_1} < K_M^{UQ_0}$, suggesting that the isoprenoid chain plays dominant role in substrate binding and dissociation kinetics.

Scheme S1

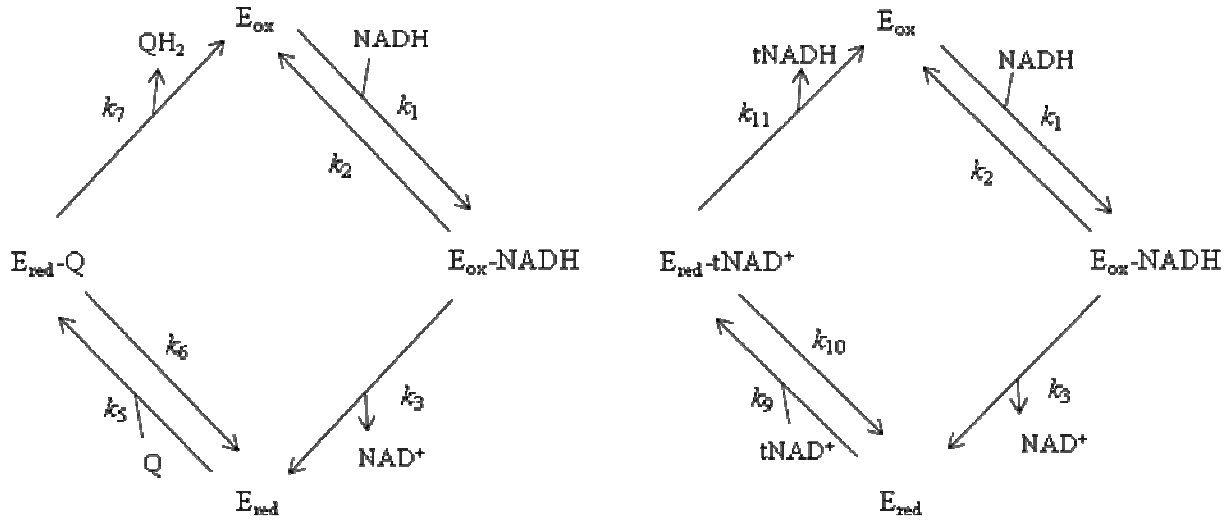

Table S1

	NADH-Q reductase reaction	NADH-tNAD$^+$ Transhydrogenase reaction
k_{cat}	$\frac{k_3 k_7}{k_3 + k_7}[E]_0$	$\frac{k_3 k_{11}}{k_3 + k_{11}}[E]_0$
K_M^{NADH}	$\frac{(k_2 + k_3)k_7}{k_1(k_3 + k_7)}$	$\frac{(k_2 + k_3)k_{11}}{k_1(k_3 + k_{11})}$
K_M^{Q}	$\frac{k_3(k_6 + k_7)}{(k_3 + k_7)k_5}$	-
$K_M^{tNAD^+}$	-	$\frac{k_3(k_{10} + k_{11})}{(k_3 + k_{11})k_9}$