Follow reaction by appearance of NADH \( \varepsilon_{340} = 6,220 \text{ M}^{-1} \text{ cm}^{-1} \)
The ADH kinetic scheme

\[ \begin{align*}
E & \quad k_1 \quad E \cdot \text{NAD}^+ & \quad k_2 \quad E \cdot \text{NAD}^+ \cdot \text{EtOH} & \quad k_3 \quad E \cdot \text{NADH} \cdot \text{Acet.} & \quad k_4 \quad E \cdot \text{NADH} & \quad k_5 \quad E
\end{align*} \]

The Structures of NADH and NAD\(^+\)

**NADH** - nicotinamide adenine dinucleotide high-energy reduced form

**NAD\(^+\)** - oxidized form

The ADH Reaction

\[ \begin{align*}
\text{Zn} & \quad \text{ADPR} & \quad \text{H}_2\text{C} & \quad \text{H}_2\text{C} & \quad \text{Zn} & \quad \text{ADPR}
\end{align*} \]
I. Conformational Change
(induced fit)

This transition has a large interdomain conformational change of 5-6 Å, which has the effect of the tight packing of side chains, NAD⁺ and alcohol ultimately.

\[ E \xrightleftharpoons[k_{-1}]{k_1} E\cdot \text{NAD}^+ \]

II. Solvent Exclusion

In general enzyme active sites reduce solvent accessibility and thereby lower the dielectric constant.

This results in more significant ionic interactions, remember
\[ F = e_1e_2/Dr^2 \]
III. Metal Ion in Active Site

![Metal Ion in Active Site Diagram]

IV. General Acid/Base Catalysis

![General Acid/Base Catalysis Diagram]
V. Entropy Effect (increase $G$ and increased order)

A. Loss of rotational and translational entropy upon $E$ binding $S$

B. Proximity of donor and acceptor atom for hydride transfer in enzyme active site compared to what happens free in solution, more than offsets the destabilization due to increased order.

VI. Enzymes Bind Their Transition States
VII. Thermally Driven Protein Dynamics

WT

V203A