Molecular motors and mechanocchemistry

The challenge in mechanocchemistry is to connect the chemical and mechanical cycles of a motor in order to understand the transduction of chemical energy to mechanical work.

Smooth muscle myosin —► motor head

\[
\text{actin} \quad \frac{\gamma \text{ADP} \cdot \text{Pi}}{\gamma \text{ADP} \cdot \text{Pi}} \quad \rightarrow \quad \frac{\gamma \text{ADP} \cdot \text{Pi}}{\gamma \text{ADP} \cdot \text{Pi}}
\]

\[
\uparrow \quad \downarrow \quad \text{power stroke} \quad \text{product release, ADP, Pi}
\]

\[
\frac{\gamma \text{ATP}}{\gamma \text{ATP}} \quad \gamma \text{ATP} \quad \frac{\gamma \text{ATP}}{\gamma \text{ATP}} \quad \rightarrow \quad \frac{\gamma \text{ATP}}{\gamma \text{ATP}} \quad \frac{\gamma \text{ATP}}{\gamma \text{ATP}} \rightarrow
\]

\[
\text{detachment} \quad \text{recovery}
\]

- There are coupling rules between the chemical and mechanical cycle that prevent the motor from working backwards.
Landscape of a motor

- Motors are coupled to an energetically favorable reaction. Therefore, motor reactions are energetically downhill.

- Energy sources:
  \[ \text{ATP/ATP} \rightarrow \text{ADP/GDP + P;} \quad \Delta G = 25kT \]
  \[ \approx 100 \text{pN nm} \]

\[ \text{NTP} \rightarrow \text{NP + PP;} \]
\[ \text{NH}_3^{\text{high}} \rightarrow \text{NH}_3^{\text{low}} \]

\[ F = 0 \]
\[ F = F' \]
Mechanical properties of motors

- Step size: distance moved per catalytic cycle

- Stall force: The force at which the velocity of the motor reduces to zero.

  Hence, this is the maximum force that a functional motor can generate.

  Stall force of a motor may have some relevance to its biological function.

  e.g. RNAP ($F_{stall} \approx 25 \text{pN}$) has to knock off obstacles encountered while travelling on DNA.

  $\phi 29$ ($F_{stall} \approx 57 \text{pN}$) has a higher stall force presumably because of the high pressure build-up as it packages DNA inside the viral capsid.

- Mechanical efficiency: $\eta = \frac{F \cdot \Delta x}{\Delta t} = \frac{\text{output work}}{\text{input energy}}$

  - Max efficiency is achieved at stall.
  - $\eta < 100\% \rightarrow$ energy dissipated as heat.
Mechanochemistry

- Chemical reaction $\rightarrow$ force generation

- How does inhibitory or assisting force affect the output of the chemical reaction?

- Measurable outputs for a motor are quantities like velocity, stepping duration etc. Mechanochem is understood best by looking at the motor output at various chemical variables such as substrate concentrations, inhibitors etc. & simultaneously at various forces.

- A quick review of enzyme kinetics

  $E + S \xrightarrow{k_1} ES \xrightarrow{k_2} E + P$

  Under initial velocity conditions

  $v = \frac{V_{max} [S]}{[S] + K_m}$

  $K_m = \frac{k_{-1} + k_2}{k_1}$

  $v =$ motor velocity

  $V_{max} =$ maximum velocity obtainable when substrate binding is not limiting

  $K_m =$ $K_{cat}$ for a single motor enzyme

  $[S] =$ substrate concentration

  $K_m =$ $[S]$ at which $v = V_{max}$
At saturating substrate concentrations \( v = V_{\text{max}} \)

At \( S \ll \ll \text{Km} \), \( v = \frac{V_{\text{max}} S}{\text{Km}} \)

\[
\begin{align*}
\text{Initial slope} &= \frac{k_{\text{cat}}}{\text{Km}} = \text{effective 2nd order binding constant}
\end{align*}
\]

Enzyme reactions are generally more complex than the one discussed earlier.

Eg:-

\[
E+S \overset{\text{Vmax terms.}}{\rightarrow} ES_1 \overset{\text{ES2}}{\rightarrow} EP_1 \overset{\text{EP2}}{\rightarrow} E+P
\]

\( k_{\text{cat}}/\text{Km} \) terms

In general: -

\( k_{\text{cat}}/\text{Km} \) includes all steps from substrate binding to the first irreversible step

\( V_{\text{max}} \) includes all steps other than substrate binding
Force-velocity curves & mechanism

Eq: F-V curves at saturating substrate concentrations have different shapes for different enzymes.

\[ \text{in RNAP, force dependent step is not rate limiting until stall} \]

\[ \text{In } \phi 29 \text{, force dependent step is rate limiting even at low forces.} \]

There are two major classes of models to describe motor mechanism:

1. Brownian ratchet
2. Power stroke

0. Brownian ratchet

In this model, the motor can thermally visit two adjacent binding sites on its track via Brownian motion & this can be rectified by coupling it to another stochastic process which is energetically downhill such as ATP hydrolysis.
In such a mechanism:

- chemical energy favors forward motion rather than doing the mechanical work directly on the motor.
- rate of the reaction is proportional to the relative occupancies of $P^+$ and $P^-$.

$$\frac{P^+}{P^-} = K \exp \left( -\frac{F d}{k_B T} \right)$$

where $d$ is the distance between $P^-$ and $P^+$.

- The effect of force will be to push the $k_1/k_2$ equilibrium towards the "$P^-$" state. This will result in a higher $k_m$ at higher forces. In other words, more NTP will be required to reach $V_{max}$.  

experiments conducted away from small force to look at inhibition of T7 RNAP by force at various substrate concentrations

Plot of $\frac{1}{v}$ vs $\frac{1}{s}$ at various forces

Plot resembles a simple competitive inhibition plot

- $V_{max}$ is the same at all forces
- $kcat/Km$ decreases with increasing force
- $Km$ increases with increasing force

The data is fully consistent with a brownian ratchet model for T7 RNAP.
(2) Power stroke model (neurosin, $\phi 29$)

In this model, the chemical reaction is mechanically coupled to movement by some part of the molecule

For example, Chemla, Y. et al. Cell, Vol 122 (5), 683-692

Minimal motor mechanism:

- Motor
- Attachment to DNA

This model is consistent with the observation that $V_{max}$ decreases with force, where $\text{Km}$ is independent of force.

$$V_{max} = 2ae^{-Fd/kT}$$

where $d$ is the distance to the transition state and can be an indication of the size of the conformational change in the force-dependent step.
Some points to keep in mind while looking at force-vel curves

- Not all cases can be clearly identified as brownian ratchet or power strobe.

- The dependence of $V_{max}$ & $V_{max}/Km$ on force will be dictated by the details of the biochemical mechanism (i.e. first reversible step, location, two-step versus one step substrate binding mechanisms, etc). Hence interpretation of results depends on poor knowledge about the biochemical mechanism.

- Force could induce off-pathway inhibition introducing effects on $V_{max}$ & $Km$ that have nothing to do with the on-pathway mechanism of the enzyme.

Reference: