Molecular motors and mechanochemistry

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

![Diagram of chemical and mechanical cycles]

Smooth muscle myosin

- Power stroke
- Product release
- ADP, Pi

- There are coupling rules between the chemical and mechanical cycle that prevent the motor from turning backwards.

Pat.
Landscape of a motor

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

- energy sources:

  \[ \text{ATP} \rightarrow \text{ADP} + \text{P} \quad \Delta G = 25 \text{kcal} \]

  \[ \text{NTP} \rightarrow \text{NP} + \text{PP} \]

  \[ \text{NH}_3^{\text{high}} \rightarrow \text{NH}_4^{\text{low}} \]

Diagram:

- Reaction coordinate

- \( F = 0 \)

- \( F = F' \)
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} = 25 pN) has to knock off obstacles encountered while travelling on DNA.
  
  \( \phi 29 \) (F_{stall} = 57 pN) has a higher stall force presumably because of the high pressure build-up as it packages DNA inside the viral capsid.

- Mechanical efficiency \( \eta = \frac{\text{output work}}{\text{input energy}} \)
  - Max efficiency is achieved at stall
  - \( \eta < 100\% \) \( \rightarrow \) energy dissipated as heat

pg.3.

- Work along an orthogonal axis
  \( \rightarrow \) work along an orthogonal axis
Mechanochemistry

- chemical reaction $\xrightarrow{force\ generation}$

- 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. Mechanochemistry 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

\[ v = \frac{V_{\text{max}}[S]}{[S] + K_m} \quad ; \quad K_m = \frac{k_1}{k_1 + k_2} \]

\[ v = \text{motor velocity} \]

\[ V_{\text{max}} = \text{maximum velocity obtainable when substrate}\]

\[ k_{\text{cat}} = \text{not limiting} \quad \Rightarrow \text{kcat for a single motor enzyme} \]

\[ [S] = \text{substrate concentration} \]

\[ K_m = [S] \text{ at which } v = V_{\text{max}}/2 \]
At saturating substrate concentrations \( v = V_{\text{max}} \)

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

![Graph showing enzyme kinetics](image)

**Initial slope** = \( \frac{\text{kat}}{K_m} \) = effective 2nd order binding constant

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

**Eg:-**

\[
E + S \rightleftharpoons ES_1 \rightleftharpoons ES_2 \rightarrow EP_1 \rightleftharpoons EP_2 \rightarrow E + P
\]

**kat/Km terms**

In general:

kat/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

![Diagram of F-V curves](image)

- In RNAP, force dependent step is not rate limiting until stall.

- In Ω 29, 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

**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 $P^-$

\[
\frac{P^+}{P^-} = K \exp \left(\frac{-Fd}{k_B T}\right)
\]

where $d$ is the distance btw $P^-$ & $P^+$

- The effect of force will be to push the $K_1 / K_{-1}$ 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 \( V/V_s \) vs \( 1/s \) at various forces

Plot resembles a simple
competitive inhibition plot

- \( V_{\text{max}} \) is the same at
  all forces
- \( k_\text{cat} \) or \( K_m \) decreases
  with increasing force
- \( K_m \) increases with
  increasing force

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

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

Eq $\phi 29$; Chemley, Y. et al. Cell, Vol 122 (5), 683-692

Minimal motor mechanism:

- $\ominus$ motor
- $\Delta$ attachment to DNA

\[
\begin{align*}
\text{O} & \xrightarrow{\text{I}} \text{T} \\
\text{Kcat} / \text{Km} & \xrightarrow{\text{translocation}} \\
\text{D} & \xrightarrow{\text{P}} \text{D} \\
\text{D} & \xrightarrow{\text{F}} \text{D}
\end{align*}
\]

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

\[
V_{\text{max}} = \frac{20e^{-Fd/kB}}{d}
\]

where $d$ is the distance to the transition state & 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-velocity curves:

- Not all cases can be clearly identified as Brownian ratchet or penner stroke.

- The dependence of Vmax & Vmax/km on force will be dictated by the details of the biochemical mechanism (i.e., first irreversible step location, two-step versus one-step substrate binding mechanisms etc.). Hence interpretation of results depends on prior knowledge about the biochemical mechanism.

- Force could induce off-pathway inhibition introducing effects on Vmax & kcat/km that have nothing to do with the on-pathway mechanism of the enzyme.

Reference: