Free energy calculations of molecular motors: Chemomechanical Coupling in V-type ATPases

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How can motor proteins harness energy for milliseconds avoiding dissipative loss?
Biomolecules in Action: Using Titan as a “Computational Microscope” with NAMD

**Chemistry**

\[ U(\vec{R}) = \sum_{\text{bonds}} k_i (r_i - r_0)^2 + \sum_{\text{angles}} k_i (\theta_i - \theta_0)^2 + \sum_{\text{dihedrals}} k_i \left[ 1 + \cos(n_i \phi_i + \delta_i) \right] + \sum_{ij} 4k_{ij} \left[ \frac{\sigma_{ij}}{r_{ij}} \right]^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^6 + \sum_{ij} 4\tilde{q}_{ij} \frac{\sigma_{ij}}{r_{ij}} \]

**Physics**

\[ m\frac{d^2\vec{r}_i}{dt^2} = -\vec{F}_i = -\nabla U(\vec{R}) \]

**Math**

\[ \vec{r}_i(t + \Delta t) = 2\vec{r}_i(t) - \vec{r}_i(t - \Delta t) + \frac{\Delta t^2}{m} \vec{F}_i(t) \]

(repeat *one billion times* = microsecond)

**NAMD Software**

Virus

Protein Folding

ns/day

cores

128

256

512

1024

2048

4096

8192

16384

32768

Molecular Dynamics (MD) simulations

..and Supercomputers

David Hardy

Jim Phillips
Two terms are added to the MD potential

$$U_{total} = U_{MD} + U_{EM} + U_{SS}$$

An external potential derived from the EM map is defined on a grid as

$$U_{EM}(\mathbf{R}) = \sum_j w_j V_{EM}(\mathbf{r}_j)$$

$$V_{EM}(\mathbf{r}) = \begin{cases} 
\xi \left(1 - \frac{\Phi(\mathbf{r}) - \Phi_{thr}}{\Phi_{max} - \Phi_{thr}}\right) & \text{if } \Phi(\mathbf{r}) \geq \Phi_{thr}, \\
\xi & \text{if } \Phi(\mathbf{r}) < \Phi_{thr}.
\end{cases}$$

A mass-weighted force is then applied to each atom

$$\mathbf{f}_{i}^{EM} = -\nabla U_{EM}(\mathbf{R}) = -w_i \frac{\partial V_{EM}(\mathbf{r}_i)}{\partial r_i}$$


*2017 Nobel Prize in Chemistry
Hybrid Structure Determination with MD Simulations

Proteins
- Experimental Method: NMR, X-ray
- Data Types: X-ray Density
- Biological Function: Membrane Permeation

Multi-Protein Complex
- Experimental Method: cryo-EM
- Data Types: Cryo-EM Density
- Biological Function: Motor Action

Organelles
- Experimental Method: FRET
- Data Types: Cryo-EM Tomogram, Bright Field
- Biological Function: Molecular Movement

Cell
- Experimental Method: FRET
- Data Types: Bright Field
- Biological Function: Cell Division

References:

*Nat. Struct. Mol. Biol. 21, 244 (2014)*
*J. Am. Chem. Soc. 137, 8810 (2015)*
*J. Am. Chem. Soc. 138, 4843 (2016)*
*eLife 3, e03035 (2014)*
*PNAS 113, 10310 (2016)*
*eLife 5, e16105 (2016)*
*Structure 24, 2102 (2016)*
*Methods 100, 50-60 (2016)*
CONFORMATIONAL DYNAMICS

MOLECULAR DYNAMICS

ELECTRON DYNAMICS

Bottoms-up biology of entire photosynthetic cell organelle!

KINETIC MODELING

$ATP/sec$

$light intensity (W/m^2)$

$K_{ATP}(I) = \frac{1}{2}Iq \left(1 + \frac{1}{2}Iq\tau_{RC}(I)\frac{1}{n_{RC}}\right)$

eLife 5, e09541 (2016)
J. Am. Chem. Soc. 139, 293 (2017)
Science (2018) Submitted
Fokker-Plank eqn. \[
\frac{\partial P(x,t)}{\partial t} = \mu \frac{\partial}{\partial x} \left( \frac{\partial U}{\partial x} P(x,t) \right) + D \frac{\partial^2 P(x,t)}{\partial x^2}
\] (1)

Brownian dynamics eqn. \[
\frac{\partial U}{\partial x} = -6\pi \tilde{\eta} a \dot{x} + X
\] (2)
Protocol for Molecular Motor Simulations

CHEMICAL

motor model

fuel source

alchemical free-energy calculations

energy conversion efficiency

CONFORMATIONAL TRANSITION

trial pathway generation

string optimization

geometrical free-energy calculations

MECHANICAL

collective variable space definition

committer analysis

kinetics modeling

turnover rates
Anatomy of a V-type ATPase

- b = bound
- e = empty
- t = tight

V_1

V_o
Binding sites

Arai et al. Nature 2013, 493, 703
Arai et al. Nature 2013, 493, 703

Binding sites

b = bound
e = empty
t = tight
Rotatory catalysis in V-type ATPase
Protocol for Molecular Motor Simulations: Chemical Module

- Motor model
  - Conformational transition
  - Trial pathway generation
  - String optimization
- Geometrical free-energy calculations
  - Collective variable space definition
  - Committor analysis
- Alchemical free-energy calculations
- Fuel source
- Energy conversion efficiency
- Kinetics modeling
- Turnover rates
Rotatory Catalysis: Chemically charging the ATPase

ATP + ADP + Pi → ATP
Rotary Catalysis: Chemically charging the ATPase

ATP + ADP + Pi → ATP
Rotatory Catalysis: Chemically charging the ATPase

ΔG = -2 kcal/mol

ΔG = 10 kcal/mol

Alchemical free energy perturbation
Alchemical Free Energy Computations

\[
\begin{align*}
\text{ATPase} + \text{ATP}^\circ & \quad \Delta G^\circ_{\text{bind}} \quad \text{ATPase} : \text{ATP}^\circ \\
\Delta G^\text{free}_{\text{restr}} & \quad \Delta G^\text{bound}_{\text{restr}} \\
\text{ATPase} + \text{ATP}^* & \quad \Delta G^\text{alch}_{\text{restr}} \quad \text{ATPase} : \text{ATP}^* \\
\text{ATPase} + \text{nothing} & \quad \Delta G^\text{alch}_{\text{alch}} \quad \text{ATPase} : \text{nothing}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Contribution</th>
<th>Simulation time (ns)</th>
<th>Free-energy change (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP, tightly bound (t)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\Delta G^\text{bound}_{\text{alch}})</td>
<td>125</td>
<td>-443.5 ± 0.4</td>
</tr>
<tr>
<td>(\Delta G^\text{bound}_{\text{restr}})</td>
<td>7</td>
<td>14.8 ± 0.3</td>
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<tr>
<td>(\Delta G^\text{free}_{\text{restr}})</td>
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<td>-16.1 ± 0.1</td>
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<tr>
<td>(\Delta G^\text{alch}_{\text{restr}})</td>
<td>125</td>
<td>433.2 ± 0.6</td>
</tr>
<tr>
<td>(\Delta G^\circ_{\text{bind}})</td>
<td></td>
<td>-11.6 ± 0.6</td>
</tr>
</tbody>
</table>

(500 FEP Windows)
Rotatory Catalysis: Chemically charging the ATPase

ΔG = 8 kcal/mol

ΔG = 10 kcal/mol

ΔG = -2 kcal/mol

Alchemical free energy perturbation
Rotary Catalysis: Chemically charging the ATPase

8 kcal/mol

ATP

ATP
Protocol for Molecular Motor Simulations: Mechanical Module

- Motor model
  - Conformational transition
    - Trial pathway generation
      - Collective variable space definition
    - String optimization
  - Geometrical free-energy calculations
  - Energy conversion efficiency
- Fuel source
- Alchemical free-energy calculations
- Energy efficiency
- Kinetics modeling
- Turnover rates

Brownian-Ratchet

Fokker-Plank eqn.
\[
\frac{\partial P(x, t)}{\partial t} = \mu \frac{\partial}{\partial x} \left( \frac{\partial U}{\partial x} P(x, t) \right) + D \frac{\partial^2 P(x, t)}{\partial x^2}
\]
(1)

Brownian dynamics eqn.
\[
\frac{\partial U}{\partial x} = -6\pi \eta a \dot{x} + X
\]
(2)
Explicit Langevin Solvers

\[ M \dddot{X} = - \nabla U(X) - \gamma M \dot{X} + \sqrt{2\gamma k_B T M} R(t) \]

J. Chem. Phys. 2011, 134, 044104
Prog. Biophys. Mol. Biol. 2011, 107, 200
Soft Matter 2013, 9, 4319
J. Am. Chem. Soc. 2013, 135, 18458
Action of Synthetic Motors

Inchworm motion

Tetrazine

BPTz

\[
\begin{align*}
\text{Tetrazine} & \quad \text{BPTz} \\
\end{align*}
\]

\[
\begin{align*}
\text{cyanostar} & \\
\end{align*}
\]

\[
\begin{align*}
\text{Dimer Pathway} & \\
\end{align*}
\]

\[
\begin{align*}
\text{Monomer Pathway} & \\
\end{align*}
\]

\[
\begin{align*}
\text{RDS} \quad \text{RDS} \\
\end{align*}
\]

\[
\begin{align*}
\text{bis-macrocycle} & \\
\end{align*}
\]

\[
\begin{align*}
\text{cyanosolo} & \\
\end{align*}
\]

\[
\begin{align*}
\text{cyanodimer} & \\
\end{align*}
\]


(Accepted for special issue commemorating the 2016 Nobel Prize in Chemistry)
Implicit Langevin Solvers: String Simulations

- $\psi$ (degrees)
- $\phi$ (degrees)
- $\Delta G$ (kcal/mol)
- Distance (Angstrom)
- MD pathways
- Initial binding affinity
- New binding affinity
- Optimized tube
- Computational search
- Original profile
- Number of MD trajectories

$2^N N!$
Protocol for Molecular Motor Simulations: Mechanical Module

- Motor model
  - Conformational transition
    - Trial pathway generation
      - Collective variable space definition
        - Committor analysis
    - String optimization
  - Geometrical free-energy calculations
- Alchemical free-energy calculations
- Energy conversion efficiency
- Kinetics modeling
- Turnover rates

Rotary Catalysis: Chemically charging the ATPase

$8 \text{ kcal/mol}$
Rotatory Catalysis: Interface motion
Trial pathway: Anisotropic Network Model (Das & Roux) + TMD

- 392 correlated residues
- 1176 position variables
**Trial pathway**: Anisotropic Network Model (Das & Roux) + TMD

20 microsecond of cumulative simulation time with NAMD2.10

(10 x 0.35 ns cMD @ 50 Kcal/mol + 20 x 25 ps swarm)
String with Swarm of Trajectories

**Trial pathway**: MFEP of $A_3B_3$ + Flexible fitting (Singharoy & Schulten)
String with Swarm of Trajectories

**Trial pathway**: MFEP of $A_3B_3$ + Flexible fitting

- 392 correlated residues
- 1176 position variables

- 8 microsecond of cumulative simulation time with NAMD2.10

![Diagram showing conformational and stalk rotation with ATP binding and hydrolysis processes.](image)
Rotatory Catalysis: Stalk rotation

J. Am. Chem. Soc. 2017, 139, 293–310
Rotatory Catalysis: Energy Conversion

Energy conversion efficiency improves from 25% to 75%

ΔG = -2 Kcal/mol

ΔG = -6 Kcal/mol

J. Am. Chem. Soc. 2017, 139, 293–310
Rotary Catalysis and the flexible stalk

J. Am. Chem. Soc. 2017, 139, 293–310
Rotatory Catalysis in ATP synthase

Thermodynamically favorable pathway supported by crystallography:
1. ADP undocking → 2. ATP binding → 3. stalk rotation → 4. ATP hydrolysis

J. Am. Chem. Soc. 2017, 139, 293–310
Rotatory Catalysis in $V_1$-ATPase Rotor: Predicting mutations

### Computational Alanine-Scanning

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<th>chain</th>
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<th>res#</th>
<th>aa</th>
<th>DDG(complex)</th>
<th>DDG(complex,obs)</th>
<th>DG(partner)</th>
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</tbody>
</table>
1D Bias Exchange Umbrella Sampling along the MFEP defined in terms of 1176 variables. (Simulation time: 14 μs; force Constant: 2 kcal/mol ; exchange rate 29-40 %)

Generalized-WHAM

\[ w^t = \left( \sum_i T_i e^{-\beta(U_i(\xi^t) - F_i)} \right)^{-1} \]

\[ e^{-\beta F_i} = \sum_t w^t e^{-\beta U_i(\xi^t)}. \]
Rate Analysis

\[
k^{-1} = \bar{\tau}_{FP} = \sum_{i=0}^{N} \frac{\sum_{j=0}^{i} \exp\left(-\beta G(\xi_j)\right)}{D(\xi_i) \exp\left(-\beta G(\xi_i)\right)}.
\]

Szabo, Luthey-Schulten & Schulten (1980)

\[
R_{i \pm 1} = \frac{1}{\Delta t} \frac{N_{i \pm 1} + N_{i \pm 1 \pm 1}}{N_{ii} \exp\left(-\beta \left(G(\xi_{i \pm 1}) - G(\xi_{i})\right)\right) + N_{i \pm 1 \pm 1},}
\]

\[
D_{i+\frac{1}{2}} = \delta^2 \bar{\xi} \bar{\xi} R_{i+1} \exp\left(\beta \left(G(\xi_{i}) - G(\xi_{i+1})\right)\right).
\]

Ratcheting-Action in ATPase
Ratcheting-Action in ATPase
Student and Postdoctoral Positions Available

https://beckman.illinois.edu/directory/person/singharo
http://www.ks.uiuc.edu/~abhi/