EPFL

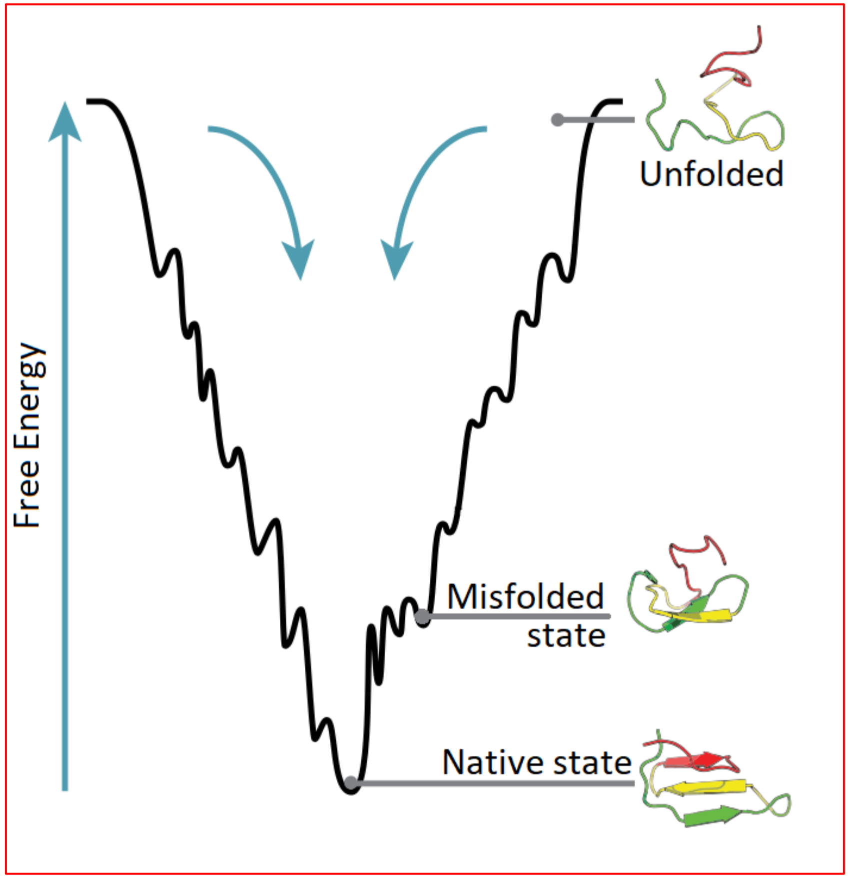
Exploration of possible structure-based models of the mechanism of action of AAA+ ATPases

Master's project defense – Antoine Maier

Supervisor: Prof. Paolo De Los Rios

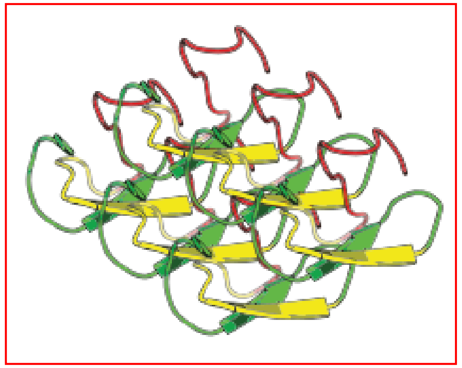
External expert: Prof. Francesco Piazza

Protein (mis)folding



By Thomas Splettstoesser (www.scistyle.com) - Own work, CC BY-SA 3.0,
https://commons.wikimedia.org/w/index.php?curid=28353539
Adapted by Antoine Maier

Aggregate



Correlated

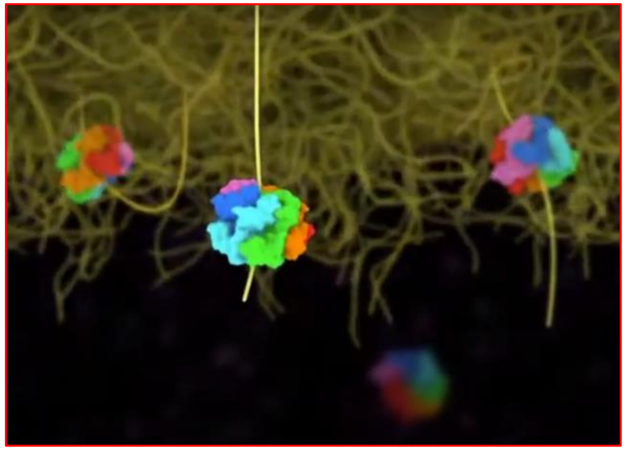
Protein misfolding diseases:

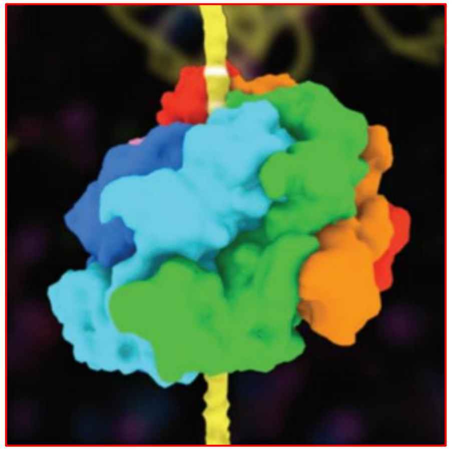
- Alzheimer's disease
- Parkinson's disease
- ...

Chaperones Hsp100

AAA+ ATPase, Hsp100 family

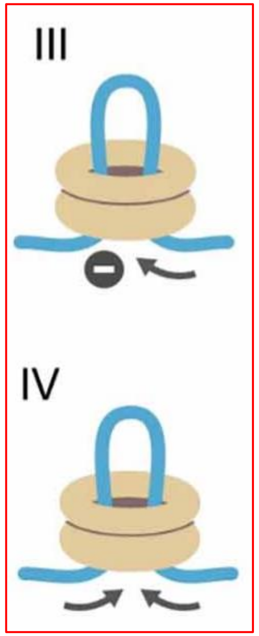
Hsp104

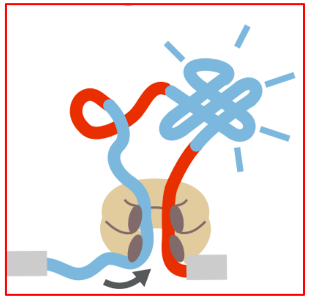




From animation related to "Ratchet-like polypeptide translocation mechanism of the AAA+ disaggregase Hsp104," Science, June 15, 2017. DOI: 10.1126/science.aan1052
URL: https://www.youtube.com/watch?v=VXupZku6lEg

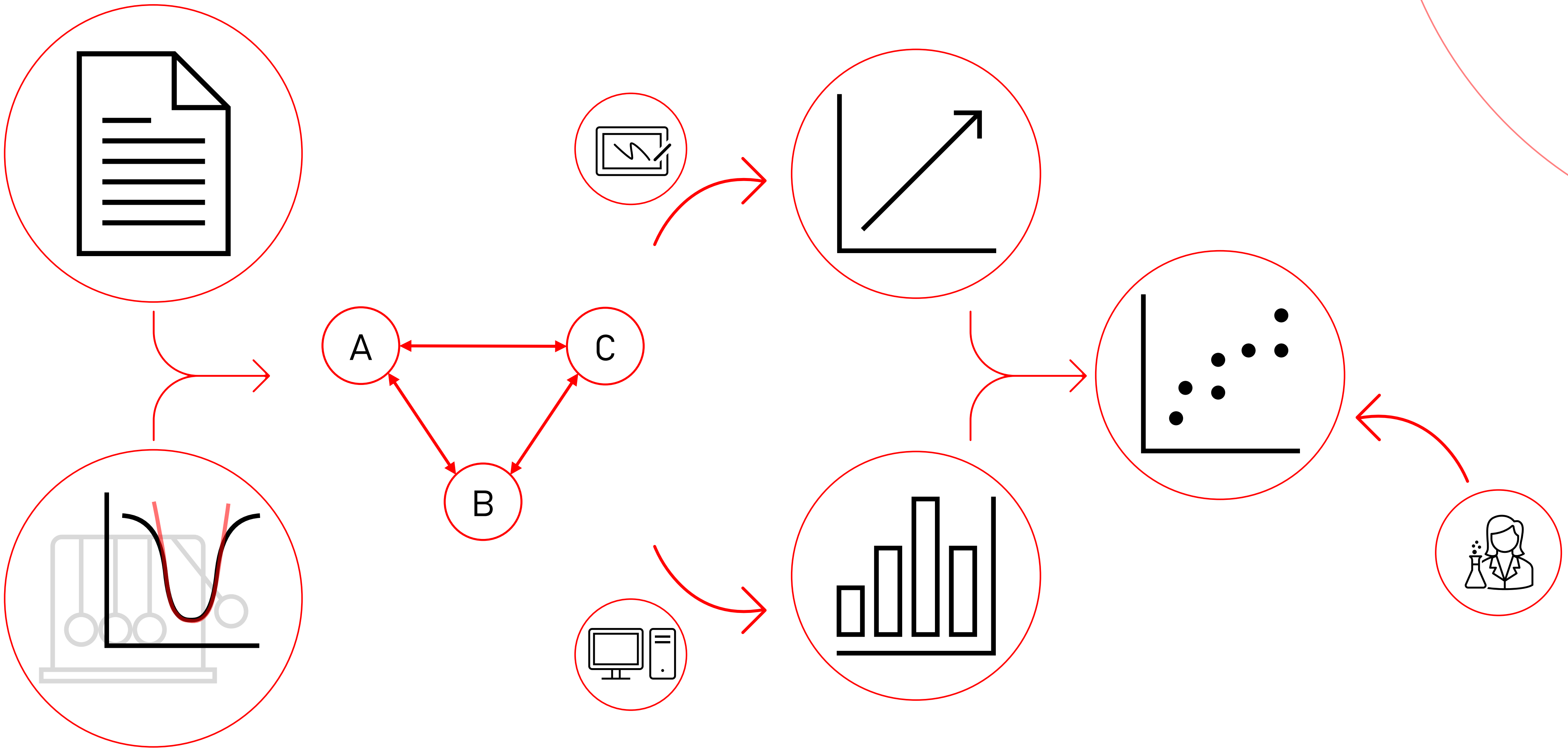
ClpB





AVELLANEDA, Mario J. et al. Processive extrusion of polypeptide loops by a Hsp100 disaggregase. Nature. 2020, vol. 578, no. 7794, pp. 317–320. DOI: 10.1038/s41586-020-1964-y.

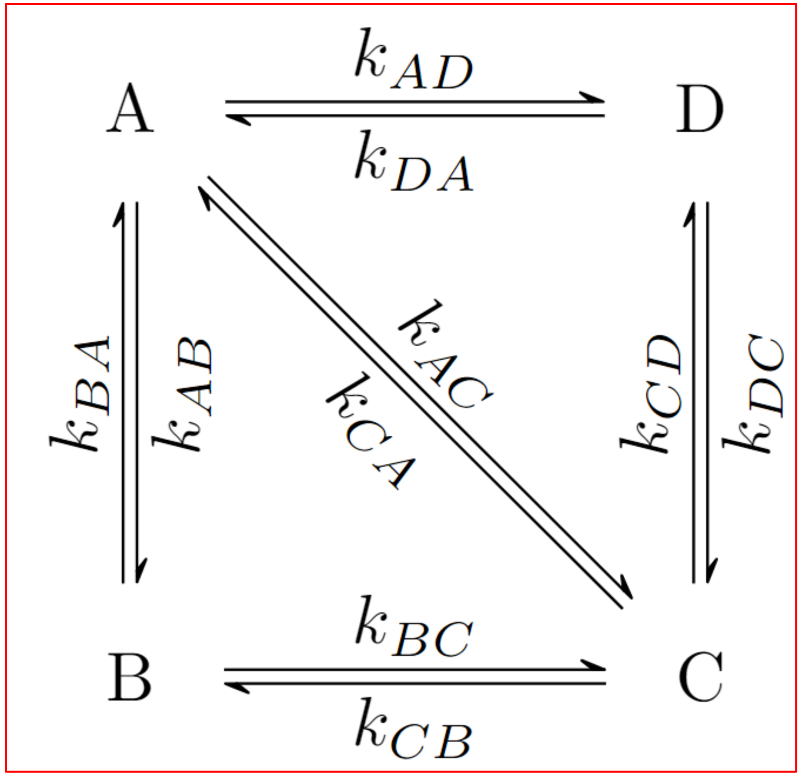
Introducing the method



Theory

Kinetic scheme

Continuous time Markov chain



$$\Longleftrightarrow$$

Master equation

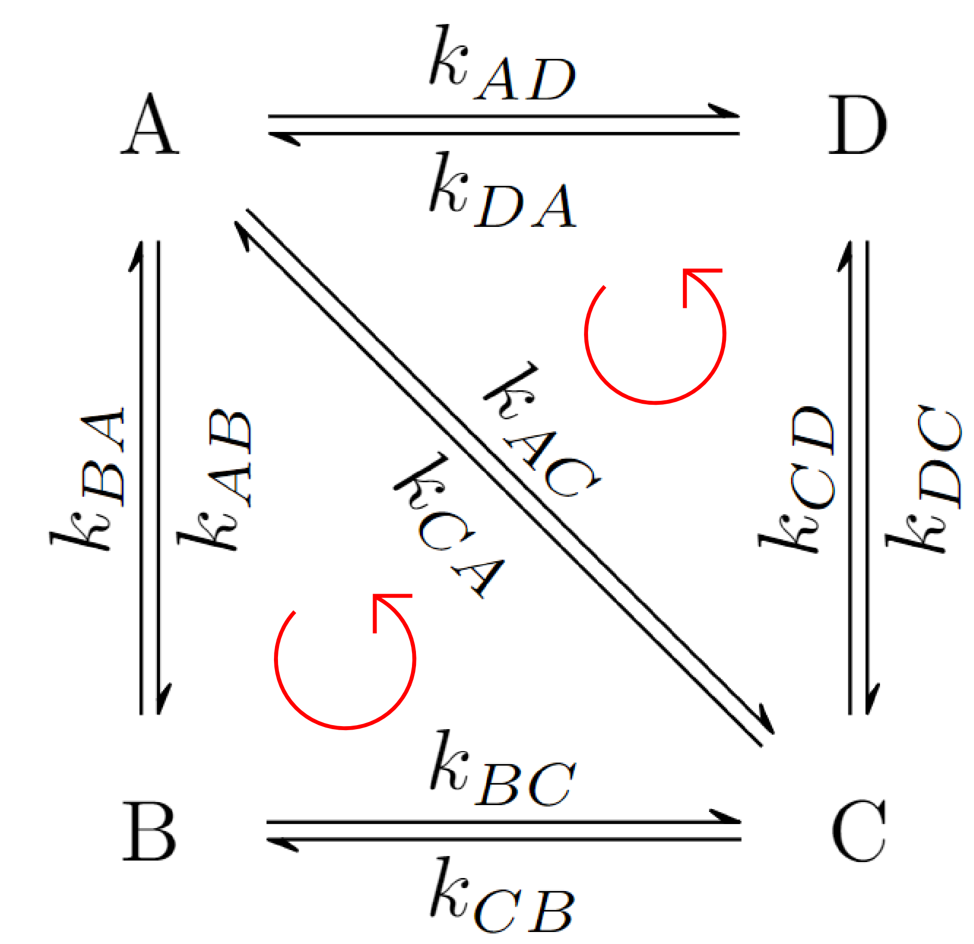
$$\dot{\mathbf{p}}(t) = \mathbf{M}\mathbf{p}(t)$$

$$M_{ij} = k_{ji} - \delta_{ij} \sum_{k=1}^{N} k_{ik}$$

Steady-state solution

$$\boxed{\mathbf{M}\mathbf{p}^* = \mathbf{0}}$$

Thermodynamic loop law



At equilibrium:

- Detailed balance

$$\left(p_i^* k_{ij}\right)\big|_{eq.} = \left(p_j^* k_{ji}\right)\big|_{eq.}$$

=> Thermodynamic loop law:

$$\left(\frac{\prod_{\circlearrowleft} k_{ij}}{\prod_{\circlearrowleft} k_{ji}}\right)\Bigg|_{eq.} = 1$$

Out of equilibrium:

$$\frac{\prod_{\circlearrowleft} k_{ij}}{\prod_{\circlearrowleft} k_{ji}} = \exp\left(\frac{\Delta\mu}{T}\right)$$

Computing statistics

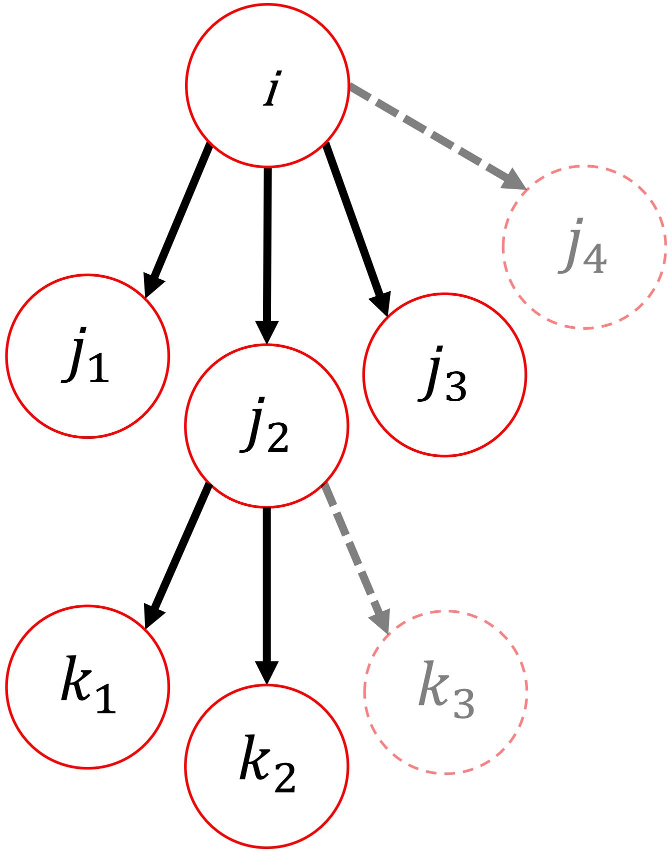
Using $w_{xy} = w_{\Delta x = y-x} = \sum_{(i \to j) \in R_{\Delta x}} p_i(t) k_{ij}$

- $\langle X(t) \rangle = t \sum_{\Delta x, R_{\Delta x}} \Delta x p_i(t) k_{ij} \; (+\langle X(0) \rangle)$
- $\mathrm{Var}\left(X(t)\right) = t \sum_{\Delta x, R_{\Delta x}} (\Delta x)^2 p_i(t) k_{ij} \; (+\mathrm{Var}\left(X(0)\right))$

$$\frac{d}{dt}\langle X^k(t) \rangle = \sum_{x,y \in \mathbb{N}} p_x(t) w_{xy} \left(y^k - x^k\right)$$

(+ characteristic function)

Gillespie algorithm

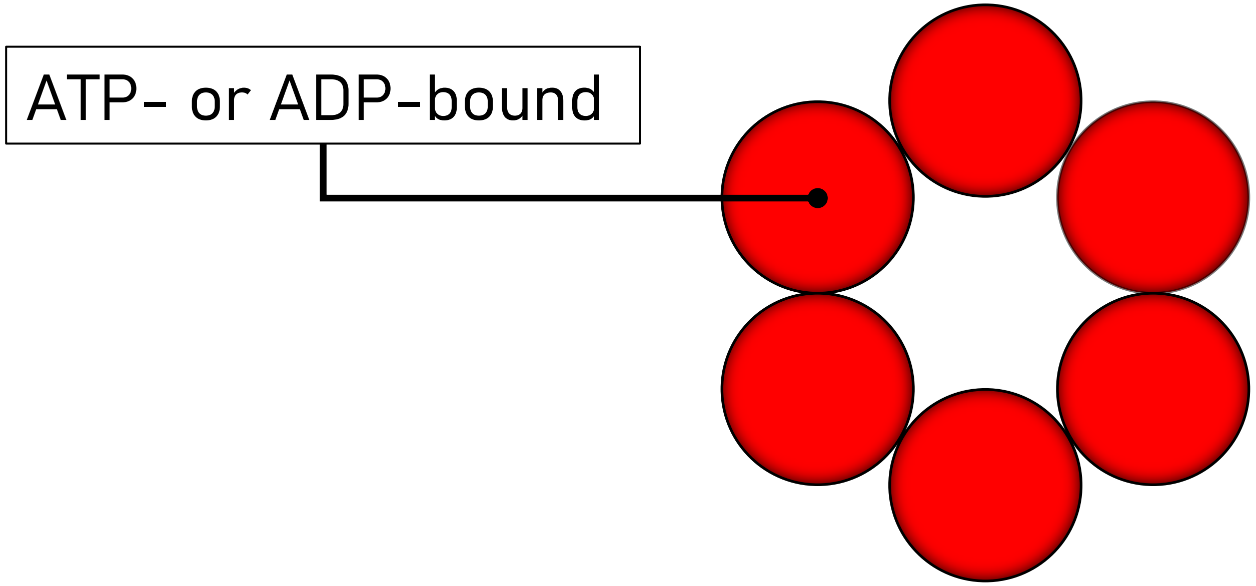


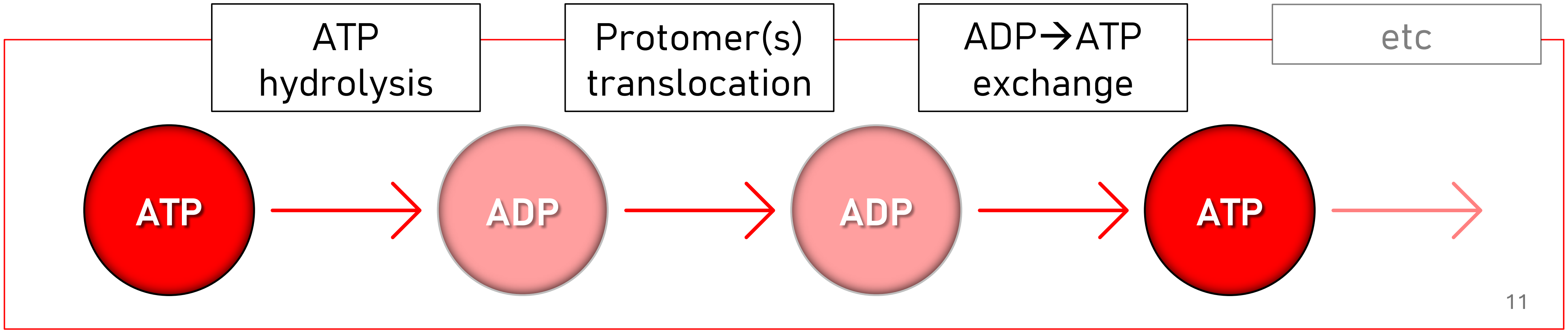
$$\tau \sim \mathrm{Exp}(k_i) \qquad k_i := \sum_j k_{ij}$$

$$j^* \sim \mathrm{WeightedSampling}\left(\frac{k_{ij^*}}{k_i}\right)$$

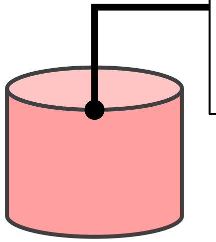
Translocation models

Coarse-grained modeling





Nucleotide exchange



- ATP [T]
- ADP [D]
- Protein [P]

$$\mathrm{PT} + \mathrm{D} \overset{{\mathrm{k}_{\mathrm{off}}}^{\mathrm{T}}}{\rightleftharpoons} \mathrm{P} + \mathrm{T} + \mathrm{D} \overset{{\mathrm{k}_{\mathrm{on}}}^{\mathrm{D}}}{\rightleftharpoons} \mathrm{PD} + \mathrm{T}$$

$$[\dot{\mathrm{P}}] = 0$$

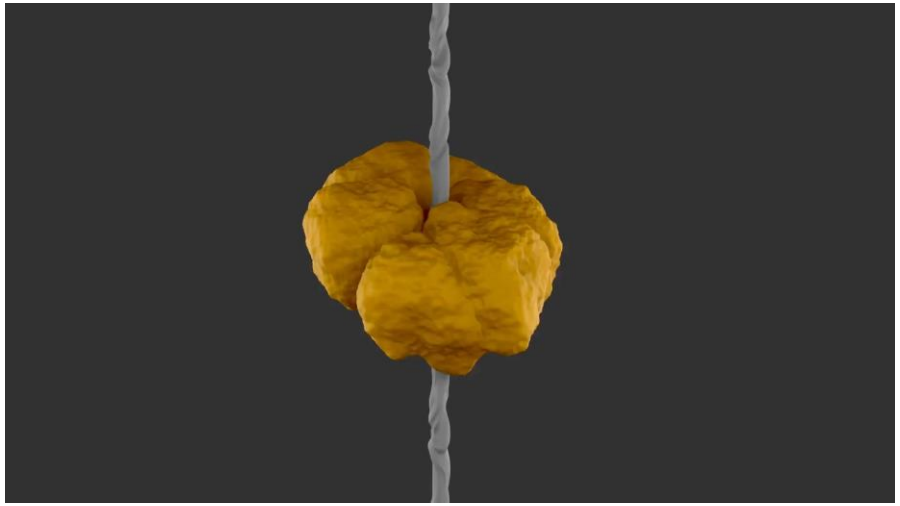
$$\frac{d[\mathrm{PT}]}{dt} = \ldots [\mathrm{P}]$$

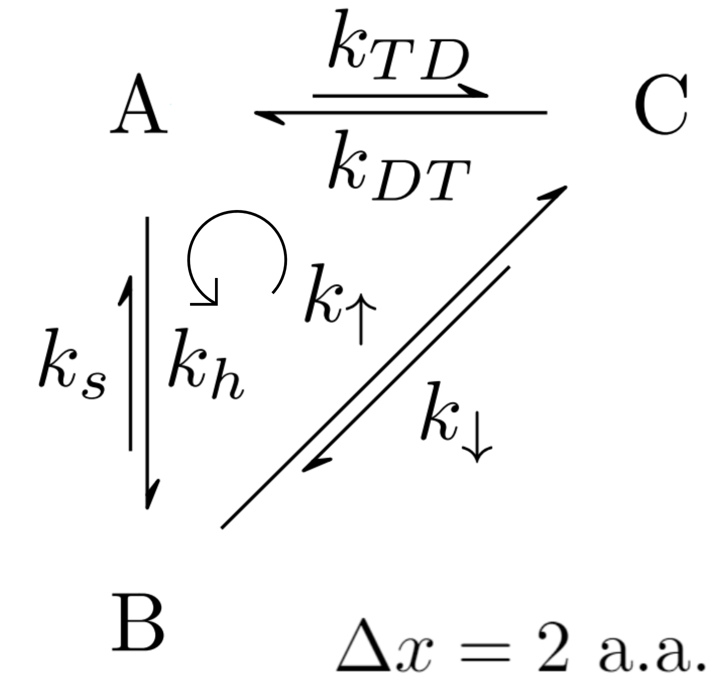
$$\frac{k_{DT}}{k_{TD}} = \left.\frac{k_{DT}}{k_{TD}}\right|_{eq.} \left(\frac{[T]}{[D]} \middle/ \left.\frac{[T]}{[D]}\right|_{eq.} \right)$$

$$\Delta\mu \propto \log \left(\frac{[T]}{[D]} \middle/ \left.\frac{[T]}{[D]}\right|_{eq.} \right)$$

Sequential Clockwise/2-Step Residue (SC/2R)

Spiraling in Control:
Structures and Mechanisms
of the Hsp104 Disaggregase

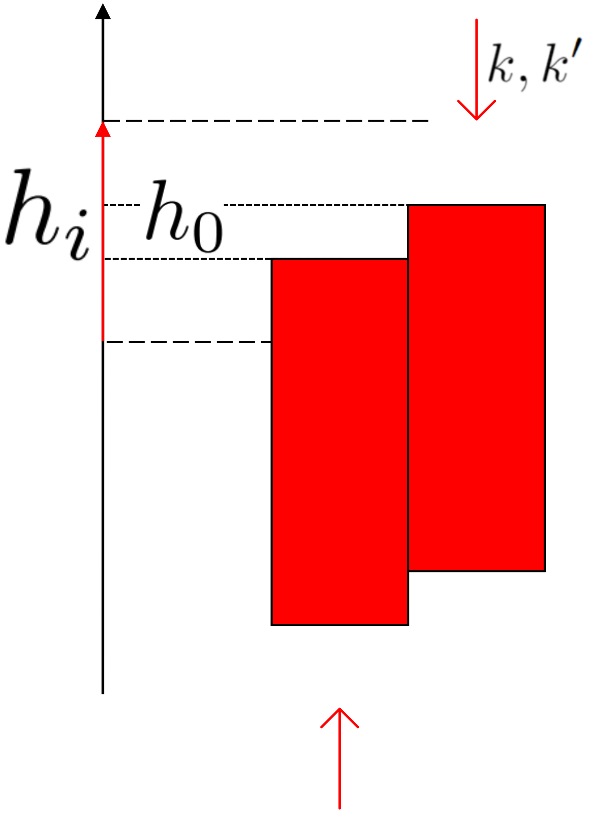




$$r_{ATP} \propto \left(1 - \frac{[T]}{[D]}\bigg|_{eq.} \bigg/ \frac{[T]}{[D]}\right) \prod_{\circlearrowleft} k$$

$$\langle v \rangle = \Delta x \cdot r_{ATP}$$

Random Protomer Concertina Locomotion (RPCL)



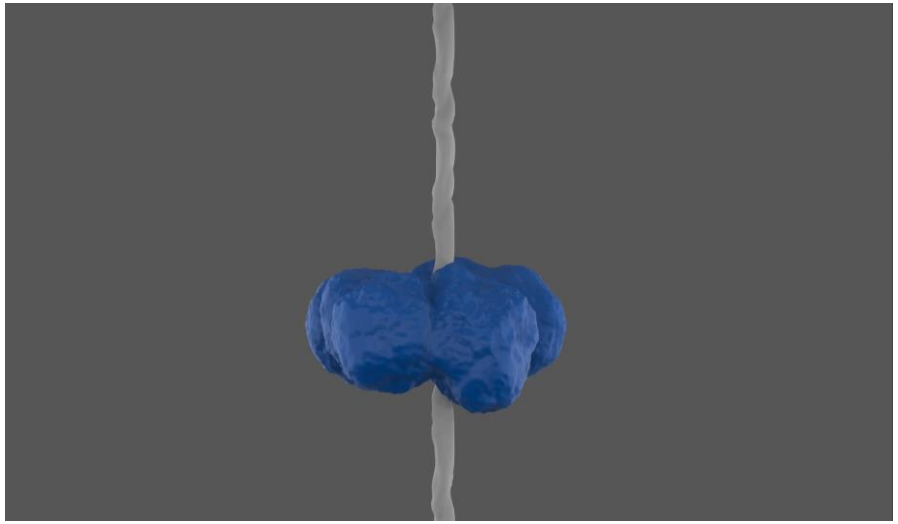
$$\mathcal{L} = \frac{1}{2}k\sum_{i=1}^{N-1}(h_i - h_0)^2 + \frac{1}{2}k'(h_N - h_0)^2 - \lambda\sum_{i=1}^{N} h_i$$

$$\Longrightarrow \begin{cases} h_{i\neq N} = h_0\frac{k-k'}{(N-1)k'+k}, \\ h_N = -(N-1)h_{i\neq N} \end{cases}$$

- *Hsp104 form[s] a closed hexameric ring.*
- *[…] the cryo-EM is at a lower resolution in this region, indicating conformational flexibility*
- *[…] we identified an additional […] conformation of Hsp104 in which all six subunits are […] in an evenly spaced helical spiral*

SHORTER, James; SOUTHWORTH, Daniel R.
Spiraling in Control: Structures and Mechanisms of the Hsp104 Disaggregase.
Cold Spring Harbor Perspectives in Biology. 2019, vol. 11, no. 8, a034033.
DOI: 10.1101/cshperspect.a034033.

Random Protomer Concertina Locomotion (RPCL)



$$\begin{tikzcd}[column sep=huge, row sep=huge]
A \arrow[r, shift left, "\bar{k}_{\downarrow ext.}"] \arrow[d, shift left, "\bar{k}_h"] & D \arrow[l, shift left, "k_{\uparrow cont.}"] \arrow[d, shift left, "k_{TD}"] \\
B \arrow[u, shift left, "k_s"] \arrow[r, shift left, "k_{\uparrow ext.}"] \arrow[ur, phantom, "\circlearrowleft"] & C \arrow[l, shift left, "k_{\downarrow cont.}"] \arrow[u, shift left, "k_{DT}"]
\end{tikzcd}$$

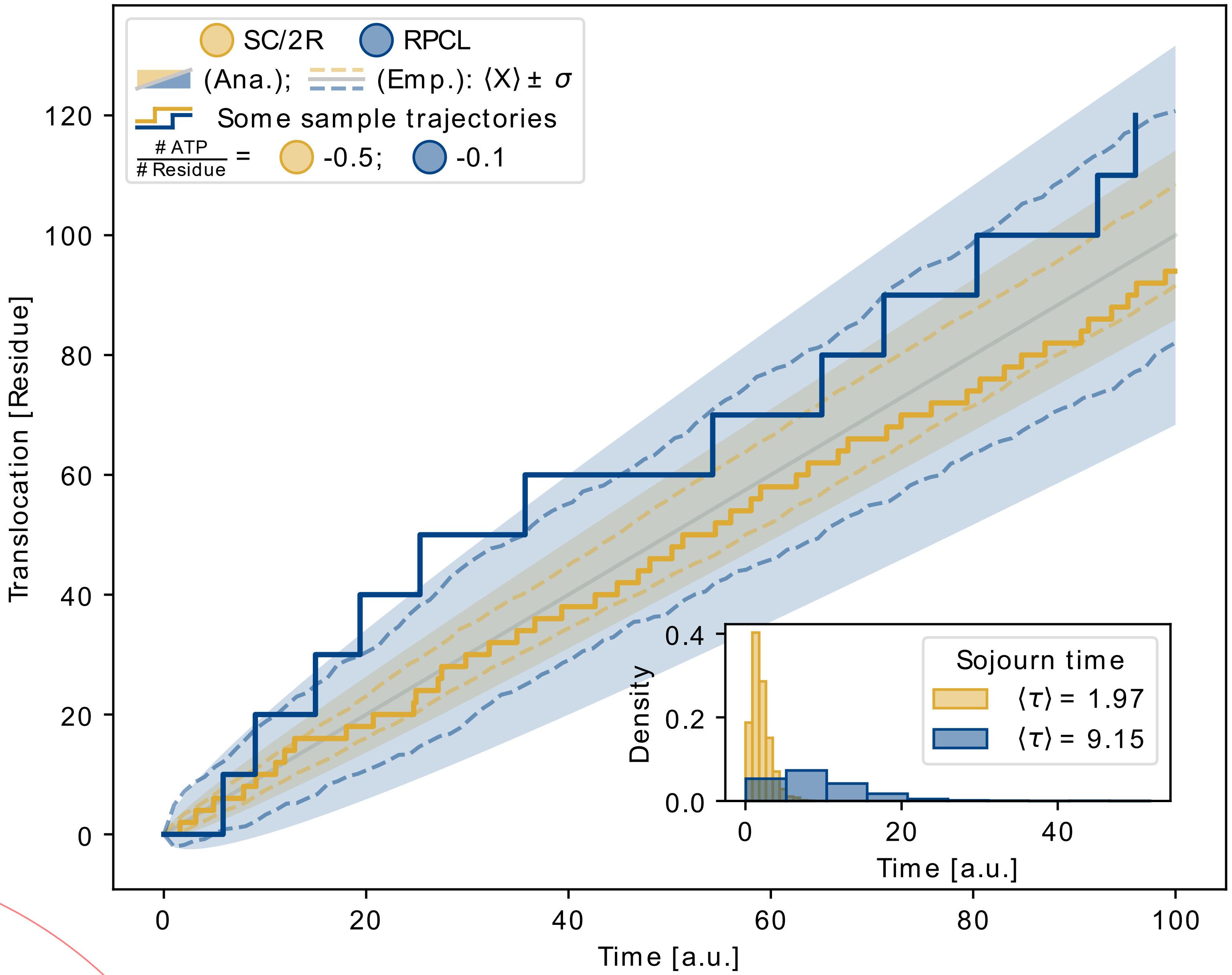
$\Delta x = 10$ a.a.

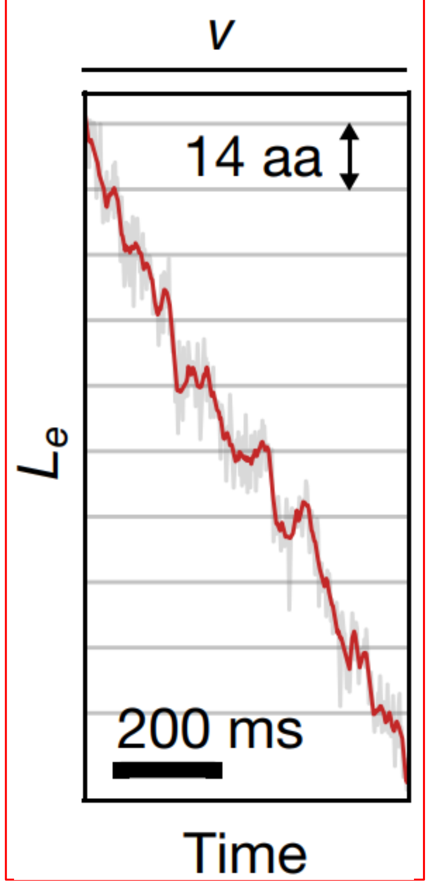
$$r_{ATP} \propto \left(1 - \left.\frac{[T]}{[D]}\right|_{eq.} \Big/ \frac{[T]}{[D]}\right) \prod_{\circlearrowleft} k$$

$$\langle v \rangle = \Delta x \cdot r_{ATP}$$

Numerical experiments

Comparing dynamics





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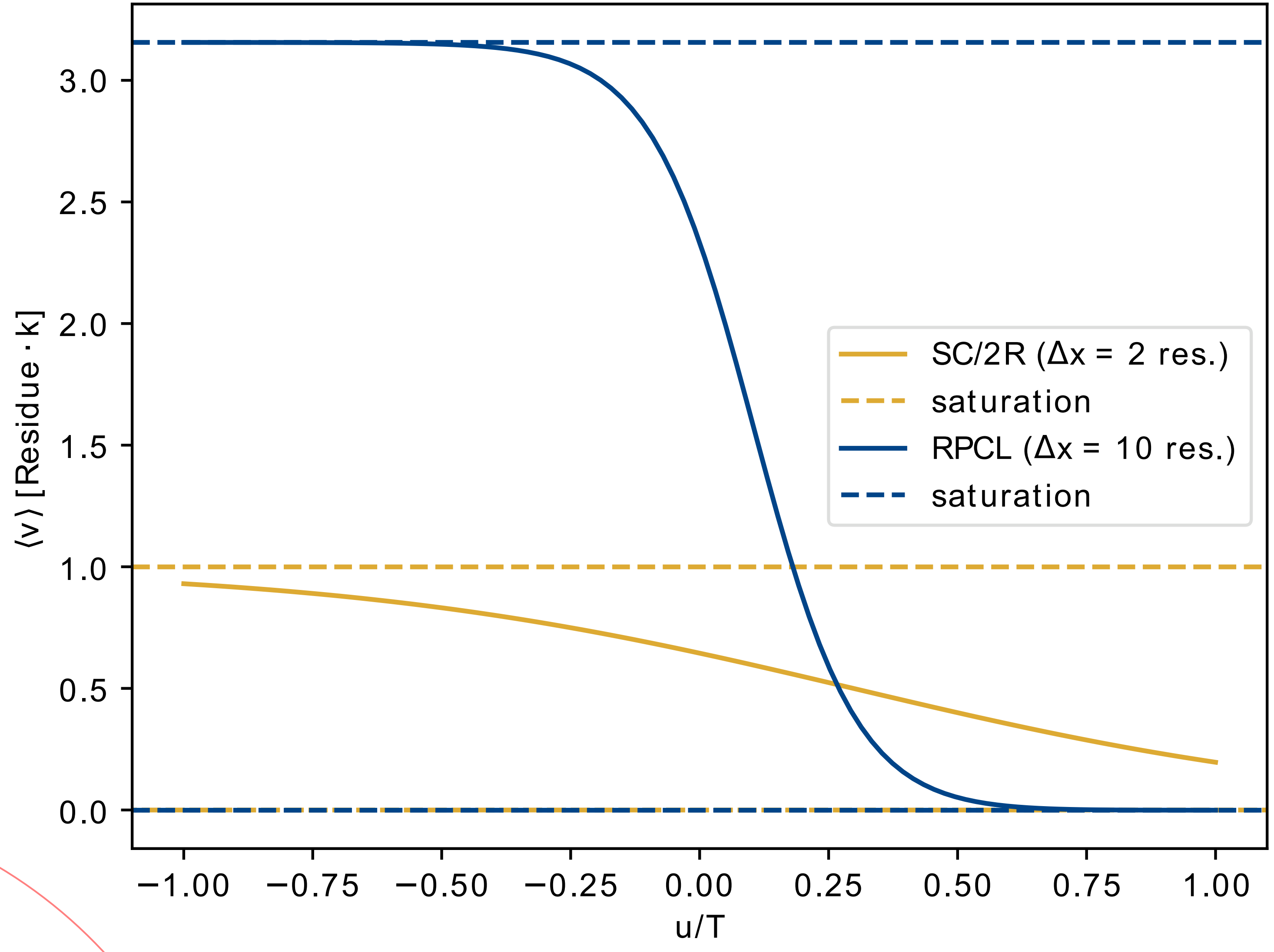
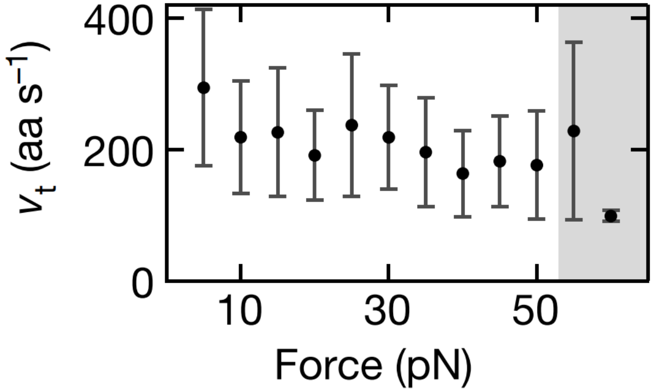
$$k \mapsto k e^{-\beta u \Delta x}$$

Pulling on the substrate





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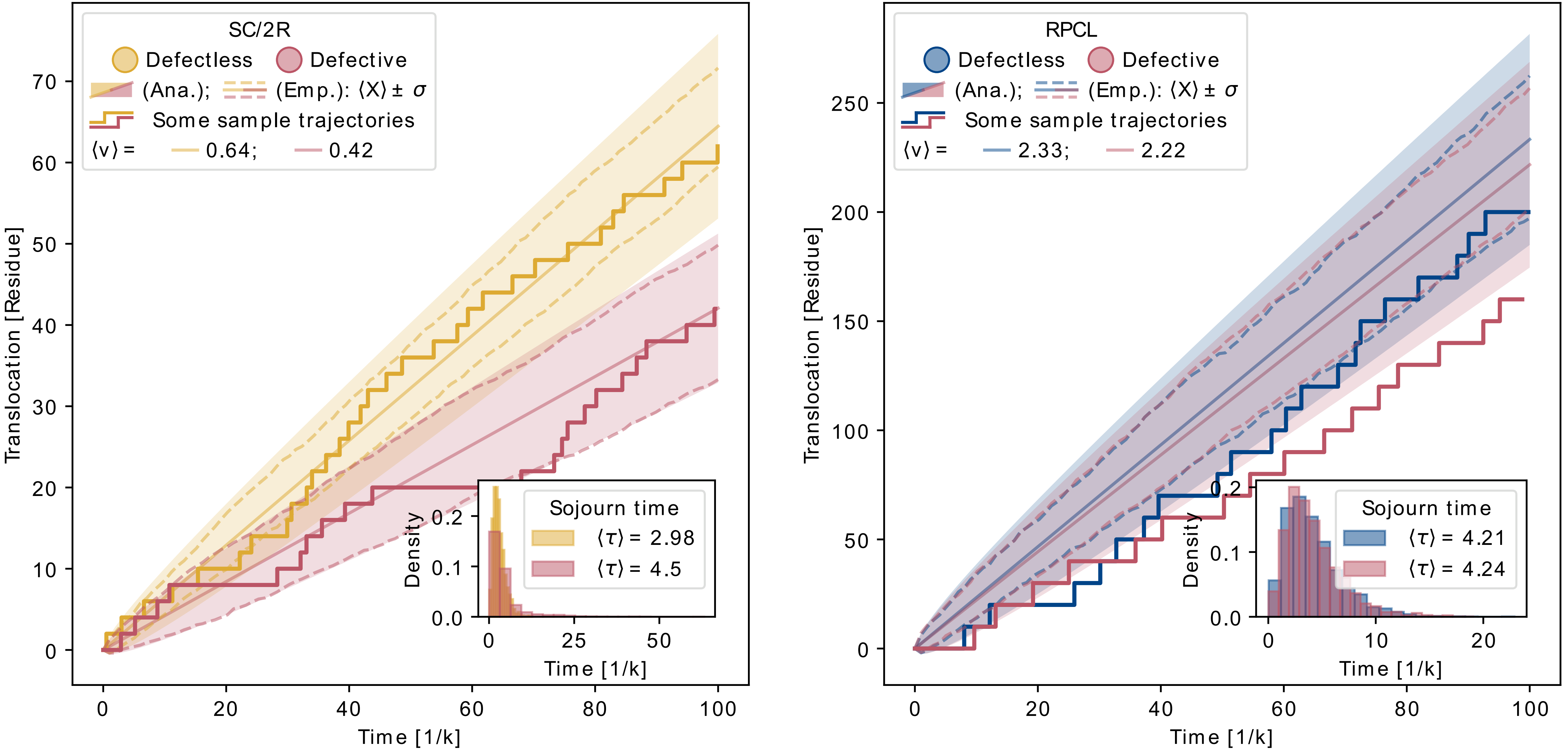


Defective protomer

[...] indicating that Hsp104 power stroke can be generated by ATP hydrolysis in a single subunit.

ESANTIS, Morgan E. et al. Operational Plasticity Enables Hsp104 to Disaggregate Diverse Amyloid and Nonamyloid Clients. Cell. 2012, vol. 151, no. 4, pp. 778– 793. DOI: 10.1016/ j.cell.2012.09.038.

Conclusion

SC/2R vs RPCL

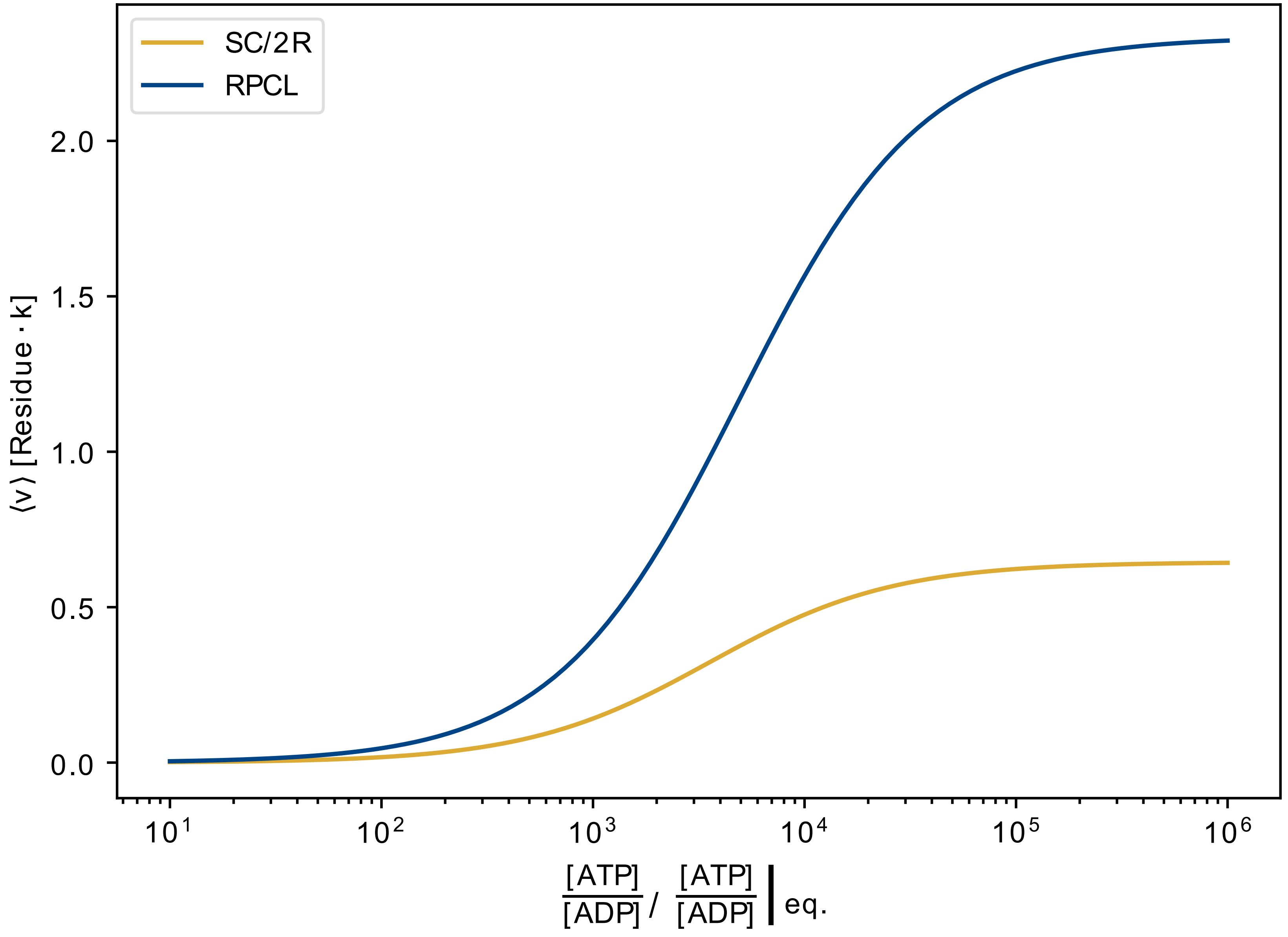
	SC/2R	**RPCL**
Compatible with cryo-EM observed contracted and extended configurations	Yes	Yes

Thank you for your attention!

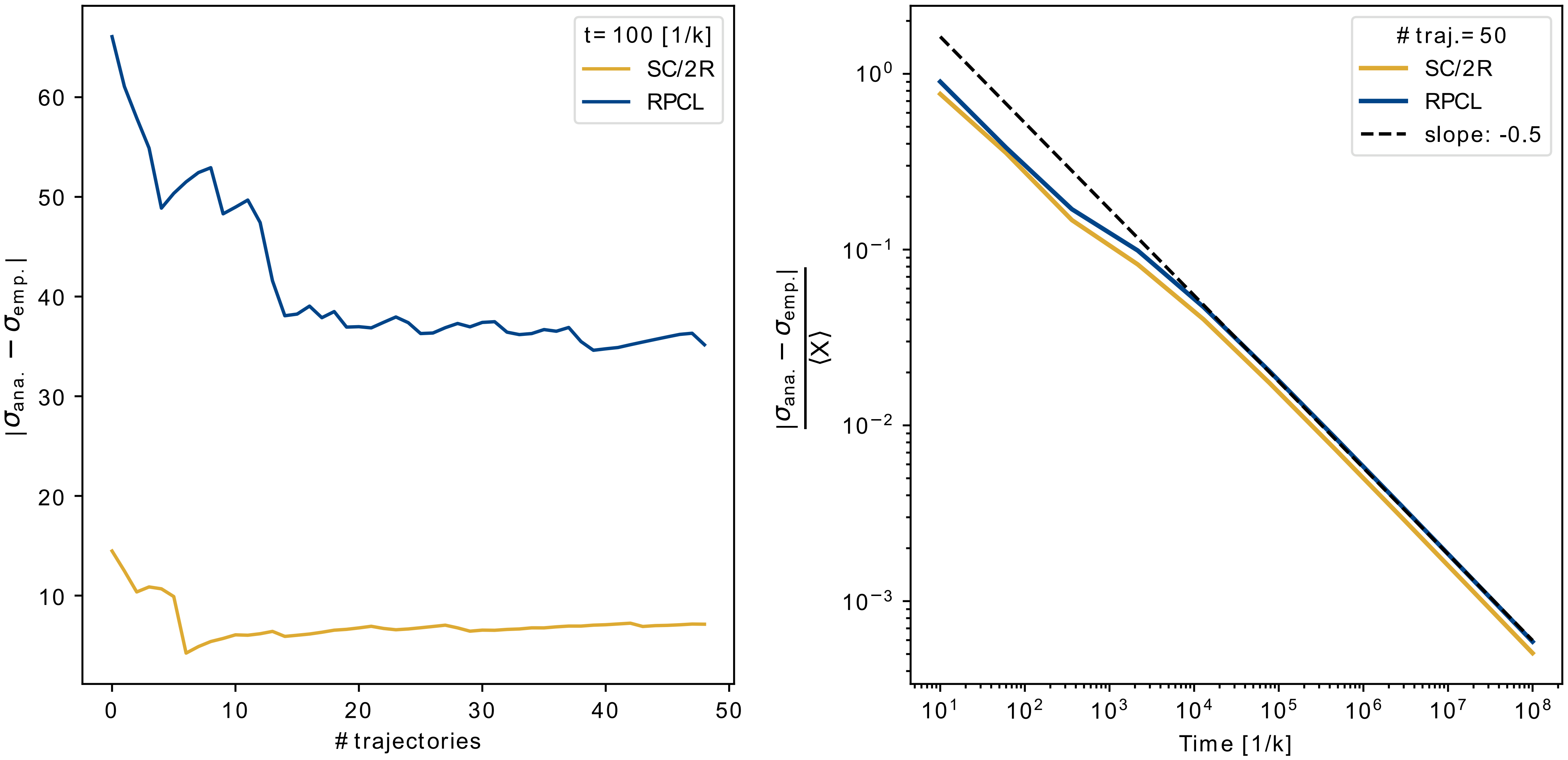
Concertina locomotion

knowerzone. Snakes can climb trees. TikTok. 2023-10-18. Available from https://www.tiktok.com/@knowerzone/video/7291299264095931681

Relation between $\langle v \rangle$ and $\frac{[\mathrm{ATP}]}{[\mathrm{ADP}]}$



Translocated length:
Error between empirical and analytical standard deviations



Saturating velocities for large forces

SC/2R

$$
\begin{tikzcd}
A \arrow[r, shift left, "k_{TD}"] \arrow[d, shift left, "k_h"] & C \arrow[l, shift left, "k_{DT}"] \arrow[dl, shift left, "k_\downarrow"] \\
B \arrow[u, shift left, "k_s"] \arrow[ur, shift left, "k_\uparrow"] &
\end{tikzcd}
$$

$$
\begin{cases}
p_A \propto k_s k_{DT} + k_\downarrow k_s + k_\uparrow k_{DT} \\
p_B \propto k_h k_\downarrow + k_{DT} k_h + k_{TD} k_\downarrow \\
p_C \propto k_\uparrow k_{TD} + k_s k_{TD} + k_h k_\uparrow
\end{cases}
$$

$$u \to \infty$$

$$\implies \begin{cases} k_\uparrow \to 0 \\ k_\downarrow \to \infty \end{cases}$$

$$\longrightarrow$$

$$
\begin{tikzcd}
A \arrow[d, shift left, "k_h"] \arrow[d, bend left=60, "k_{TD}"] \\
B \arrow[u, shift left, "k_s"]
\end{tikzcd}
$$

$$
\begin{cases}
p_A \propto k_s \\
p_B \propto k_h + k_{TD}
\end{cases}
$$

$$\langle v \rangle \propto -\Delta x k_s k_{TD}$$