Hidden Markov Model Analysis of Motor Protein Data

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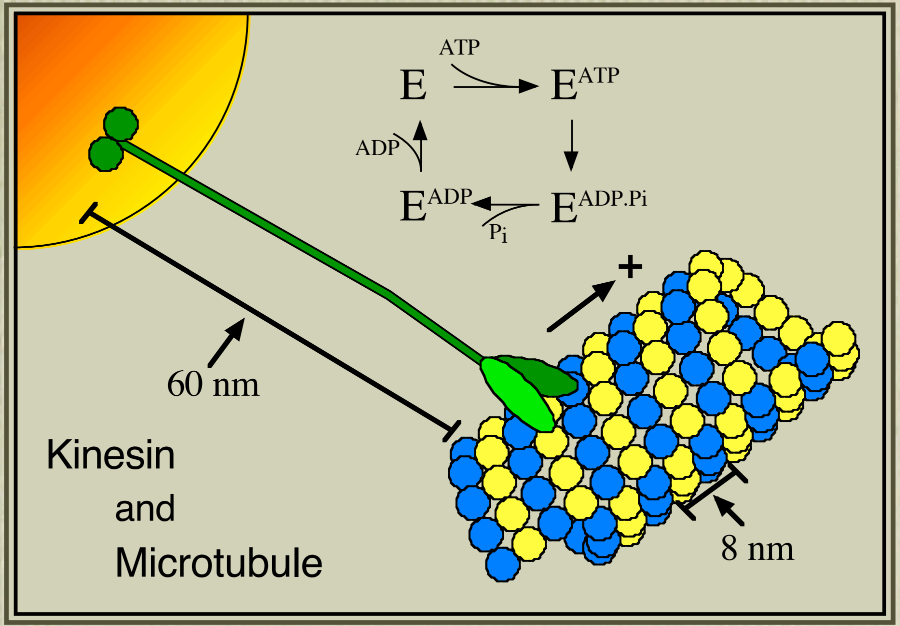
Chemical Kinetics

- Motor proteins convert chemical energy into mechanical energy.
- Mechanochemical cycle driven by chemical potential.



Properties of the cycle give insight into nature of the energy transduction.

Measuring the Kinetic Cycle

$$1_l \underset{v_2}{\overset{u_1}{\rightleftharpoons}} 2_l \underset{v_3}{\overset{u_2}{\rightleftharpoons}} \cdots \underset{v_N}{\overset{u_{N-1}}{\rightleftharpoons}} N_l \underset{v_1}{\overset{u_N}{\rightleftharpoons}} 1_{l+1}$$

- Define the cycle time τ as the time to transition from 1_l to 1_{l+1}.

- Also may consider steady-state flux

$$J = \hat{p}_j u_j - \hat{p}_{j+1} v_{j+1}$$

Then $J = \dfrac{1}{E[\tau]}$ where $E[\tau] = \langle \tau \rangle$

Kinetic Chemical Assays

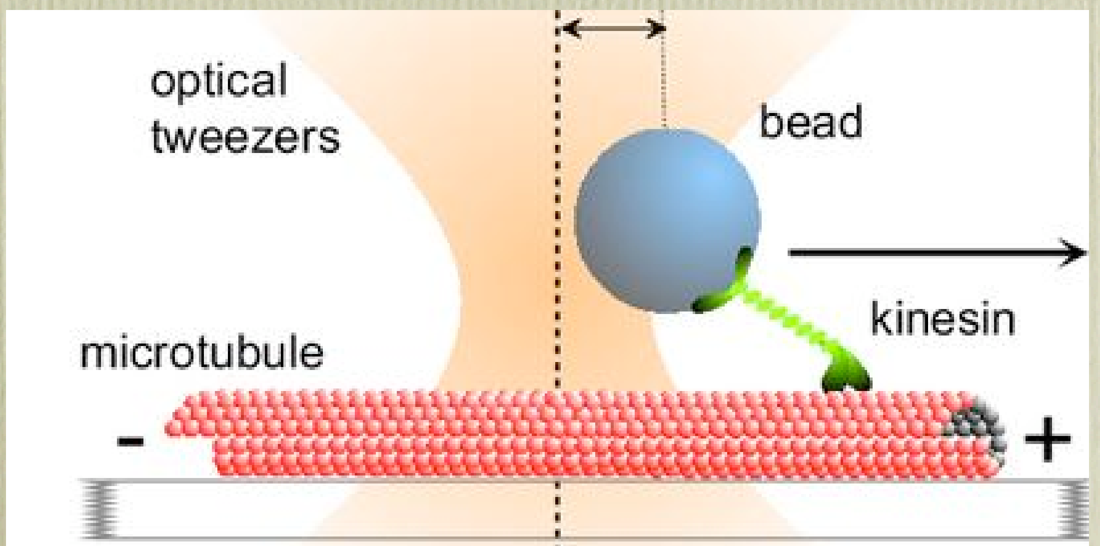
- Flux *J* relates to rate of hydrolysis

$$\frac{d}{dt}[ATP] = -J[M^*]$$

- Motor is activated by microtubule; some protein remains in solution.
- Need to identify concentration of motors that are activated M^*.

Motility Assays

- Chemical cycle coupled to motion
  - Full cycle ⇔ Step size d (tight-coupling)
  - Track individual activated motors



Courtesy: Koen Visscher

Motility Assays

- Chemical cycle coupled to motion
  - Full cycle ⇔ Step size *d* (tight-coupling)
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- Flux *J* relates to average velocity *v*

$$v = Jd$$

- Cycle time □relates to velocity *v*

$$\langle \tau \rangle = d/v$$

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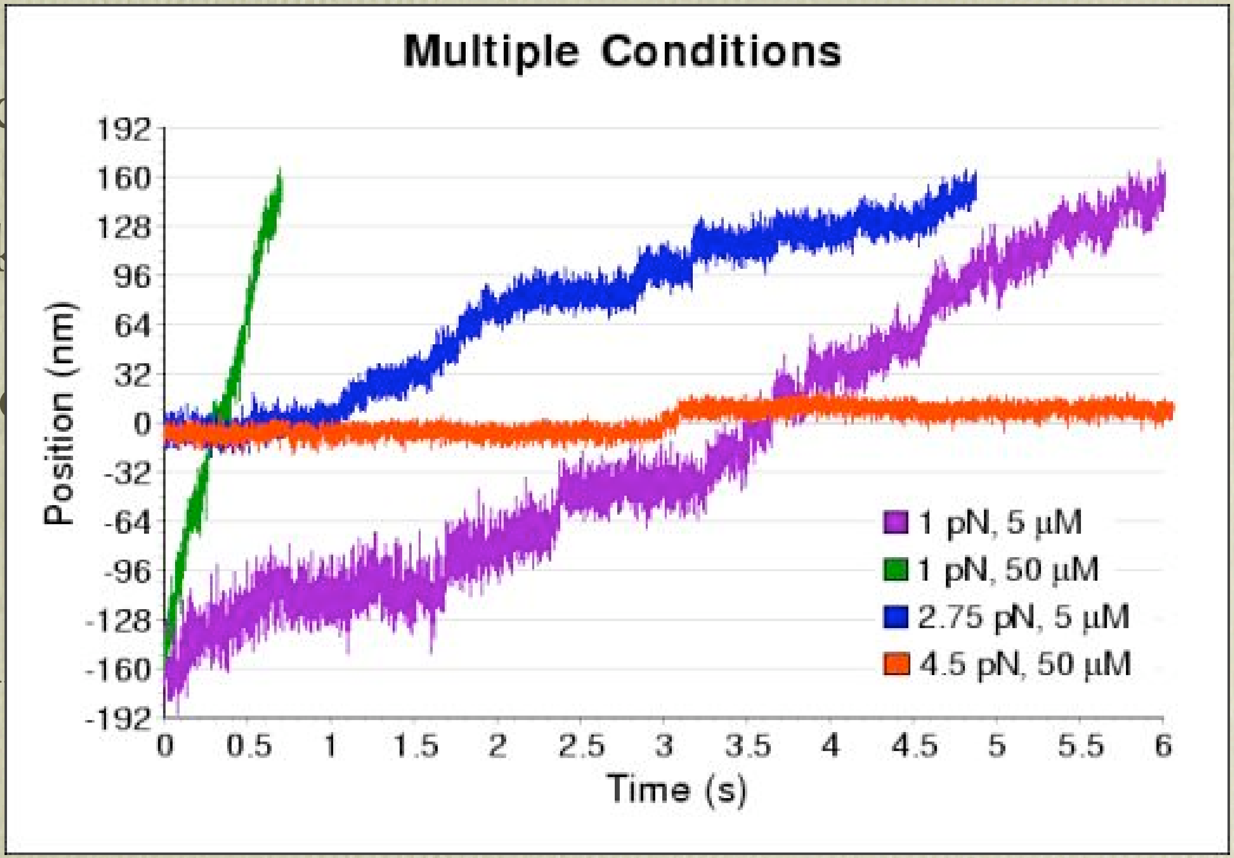

Fluctuation Analysis

- Randomness parameter (Svoboda et al. 1994, Schnitzer and Block 1995)

$$r = \lim_{t \to \infty} \frac{\mathrm{Var}[x(t)]}{d \cdot E[x(t)]} = \frac{D}{d \cdot v}$$

  - *D* is diffusion coefficient for trajectories
  - Resilient to noisy observations

- Squared coefficient of variation of cycle time

$$r = \lim_{t \to \infty} \frac{\mathrm{Var}[N(t)]}{E[N(t)]} = \frac{\sigma_\tau^2}{\langle \tau \rangle^2}$$

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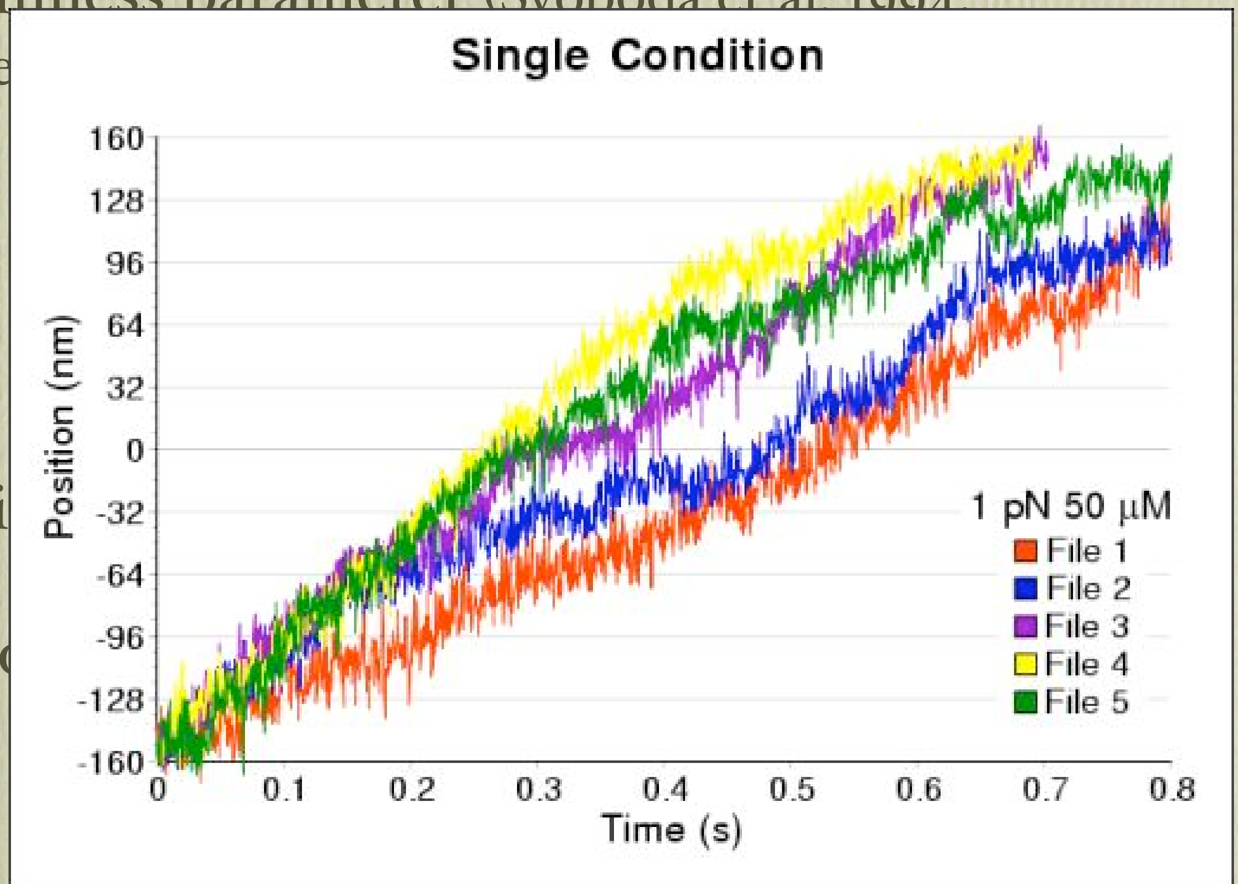

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Model Selection

- Experimentally measure average velocity and randomness
  - Load dependence
  - [ATP] dependence
- Theoretically compute mean and variance of cycle time □
- Explore parameter space on the model to see if measured response compatible with theory

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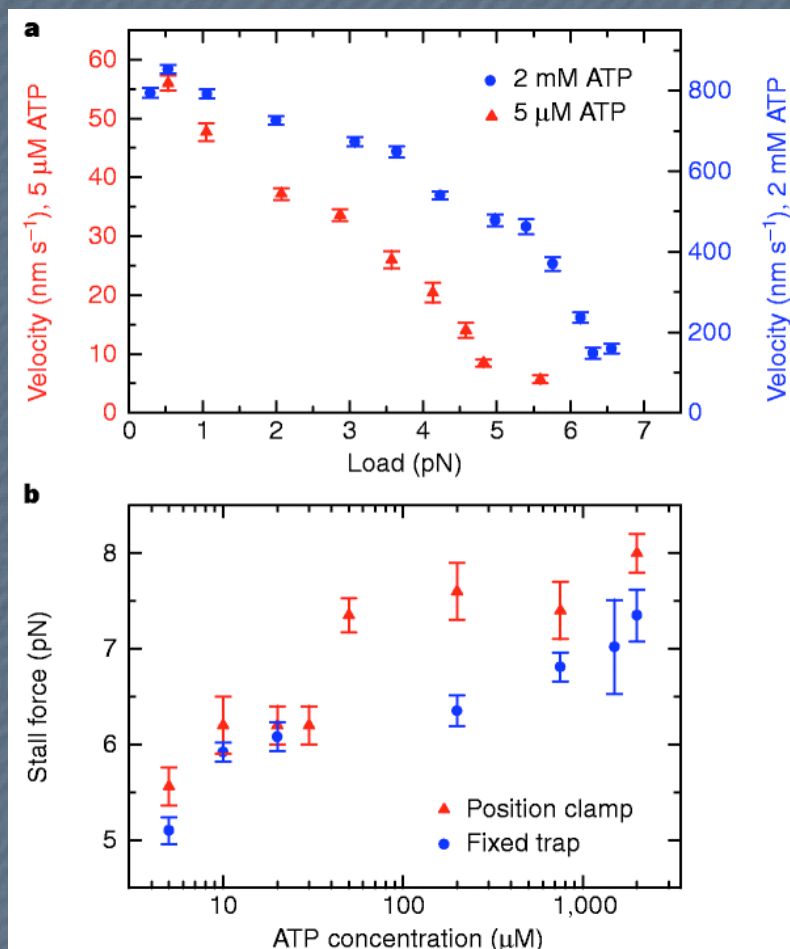

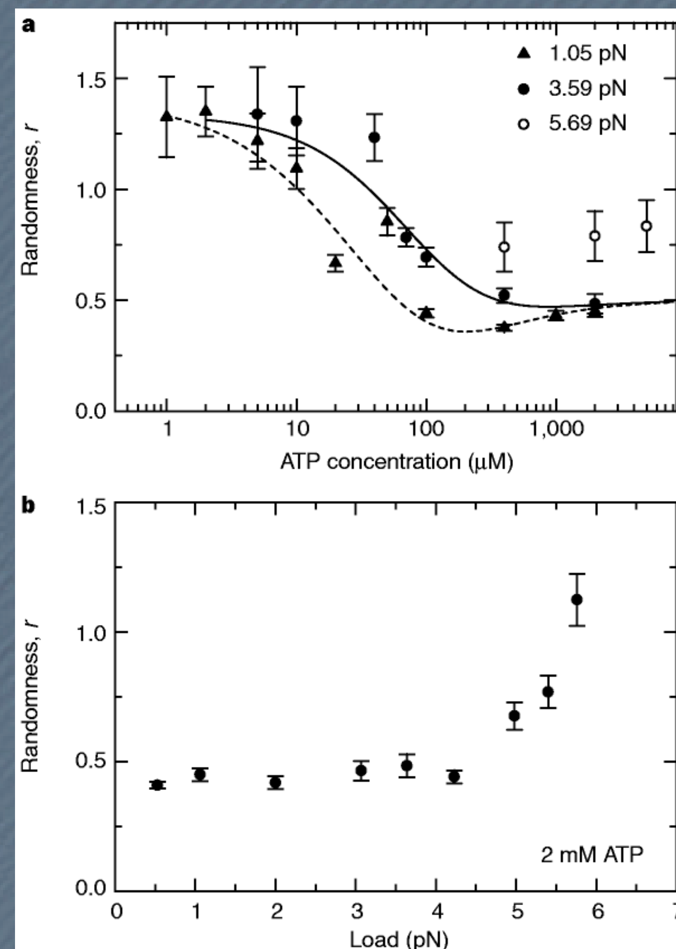

Visscher et al. 1999

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- An Idealized Experiment
  - Identify every stepping event
  - Measure dwell times (between stepping events)
  - Plot histogram of dwell times and compare to distribution of cycle time
- Why not?
  - Noise obscures identification
  - Missing events

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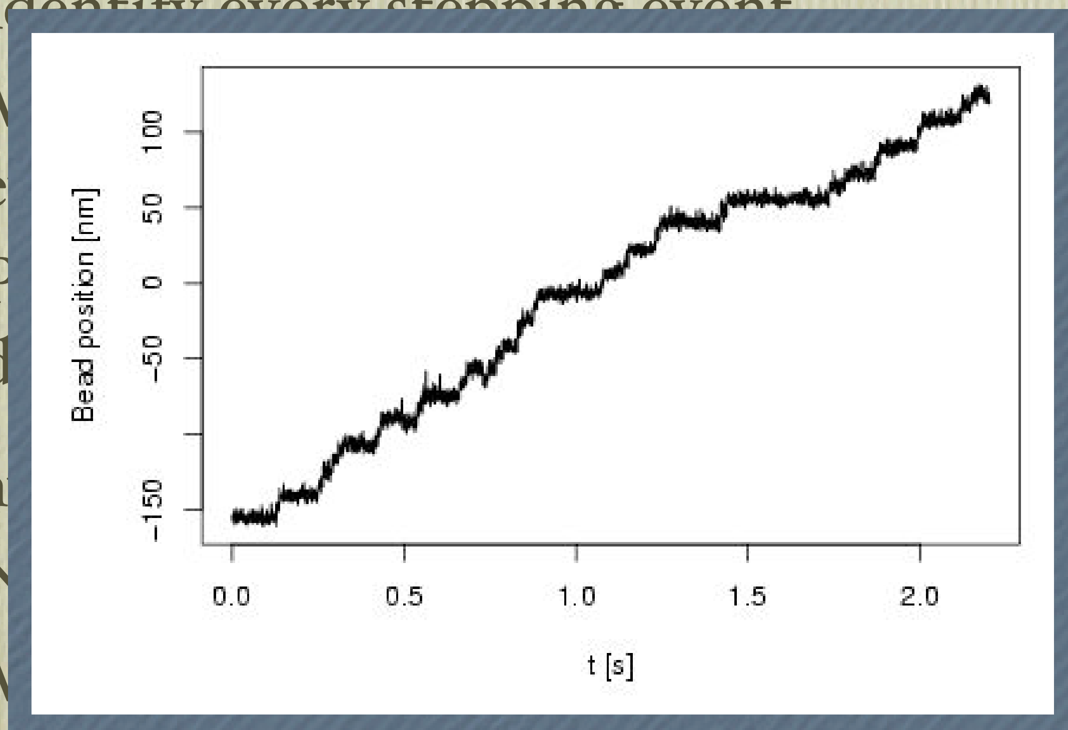

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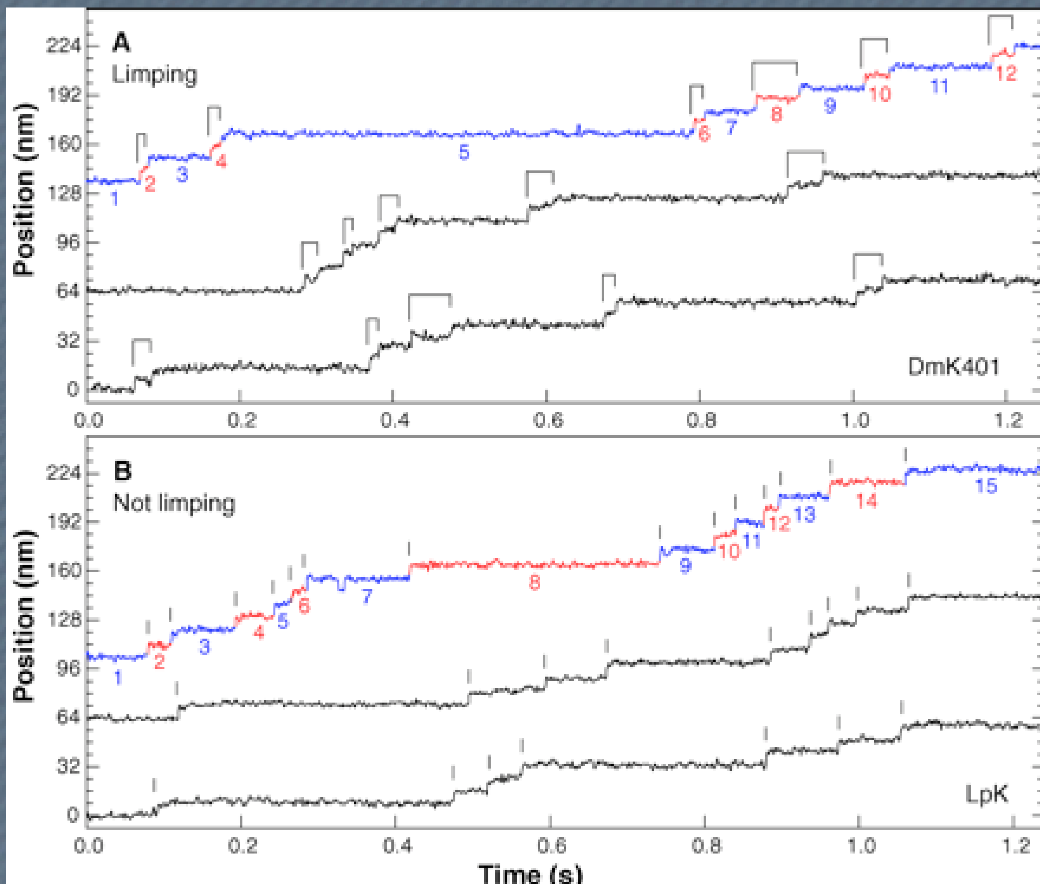

Asbury et al. 2003

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and compare to

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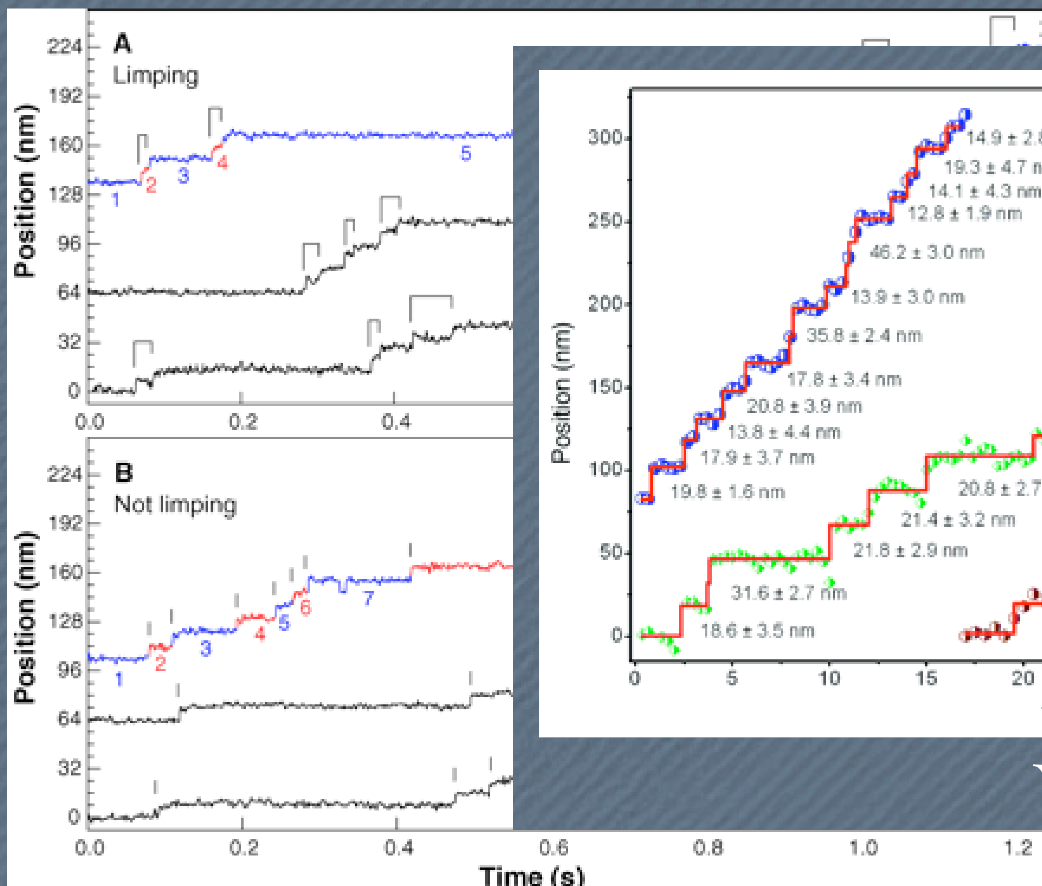

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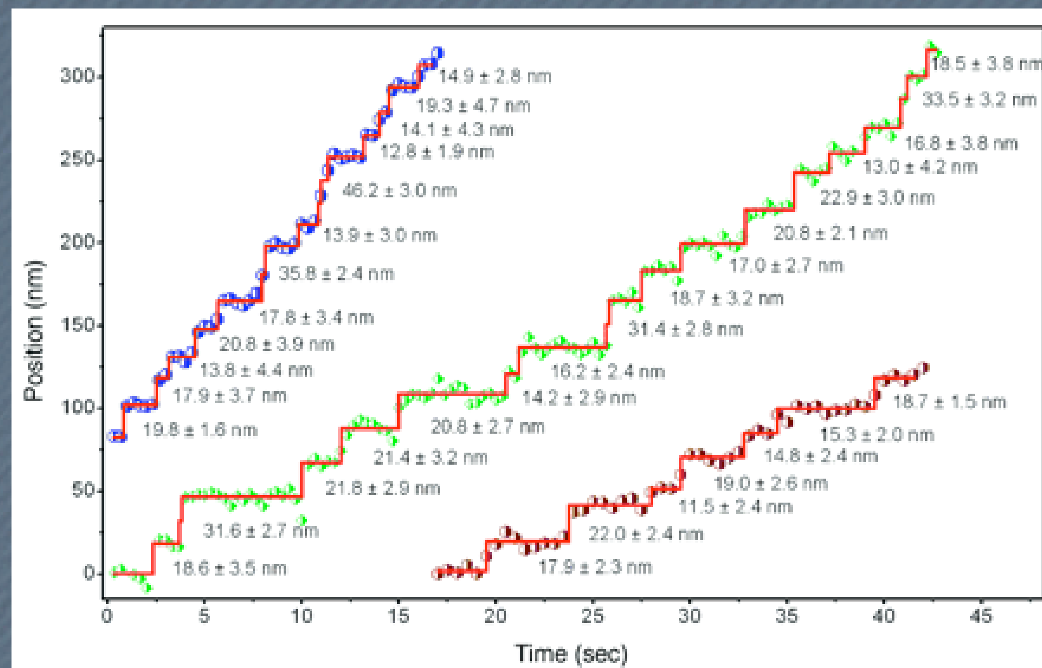

Yildiz et al. 2003

... and déjà vu

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- Similar challenges in single channel kinetics
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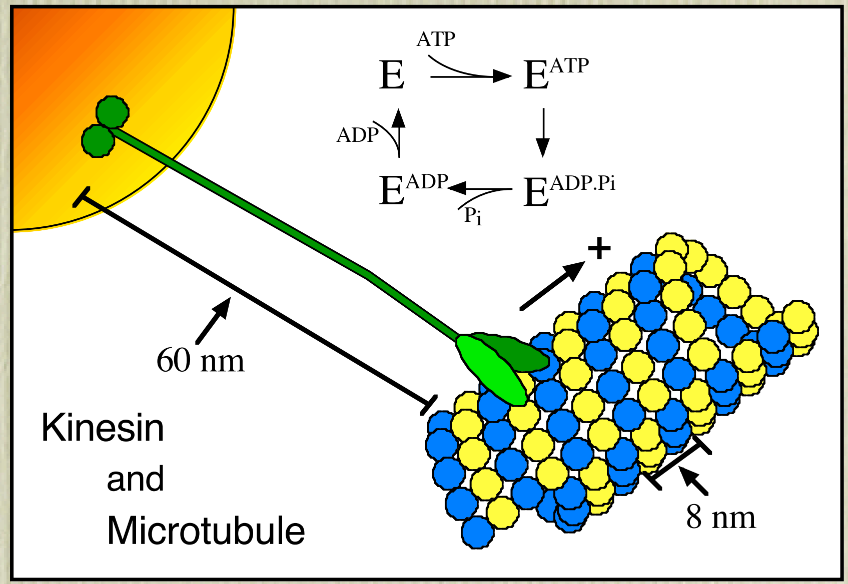
  - Current is a noisy function of channel state
- HMM for actomyosin attach/detach events (Smith et al. 2001)

Kinesin Motility

- single-protofilament
- processive
- directional
- 8 nm step, 5 pN force



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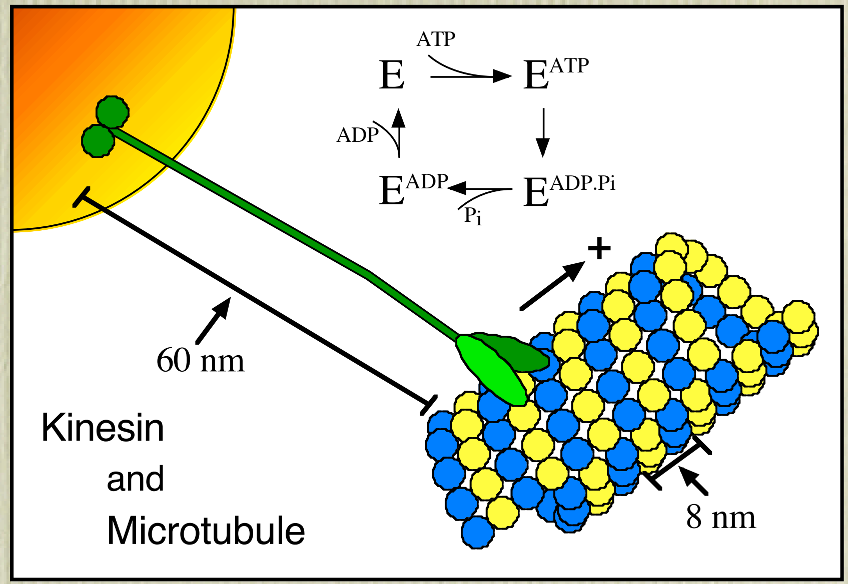


Energy Source: ATP hydrolysis

- tightly-coupled and efficient

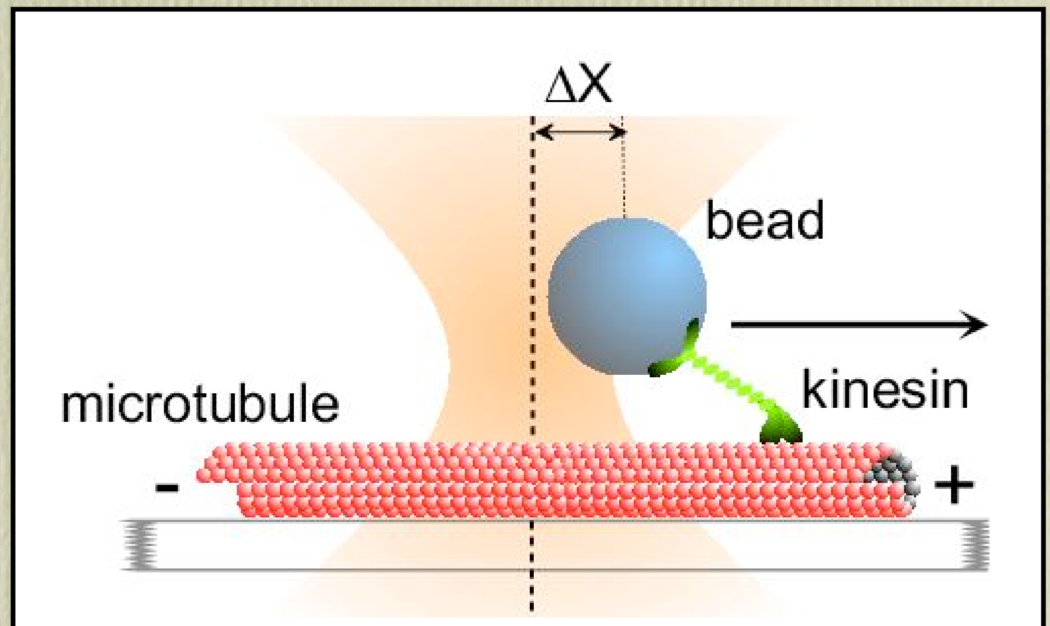
Single-Molecule Experiments

(Visscher et al., 1999)

glass bead

single protein

optical tweezers



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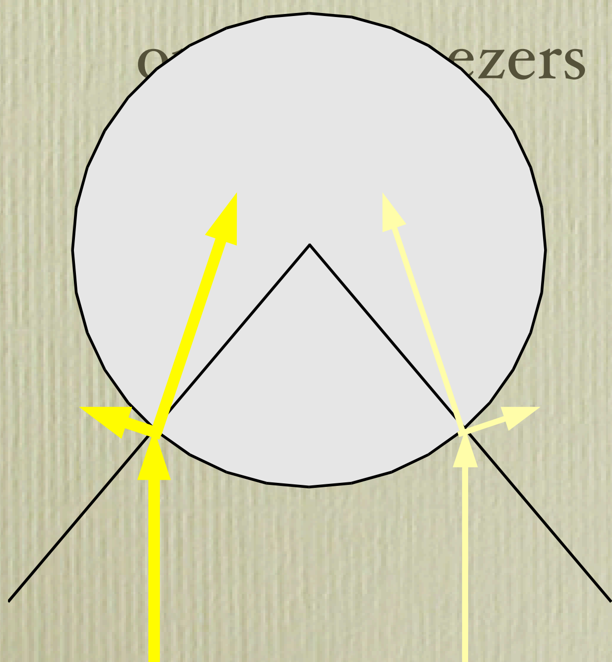

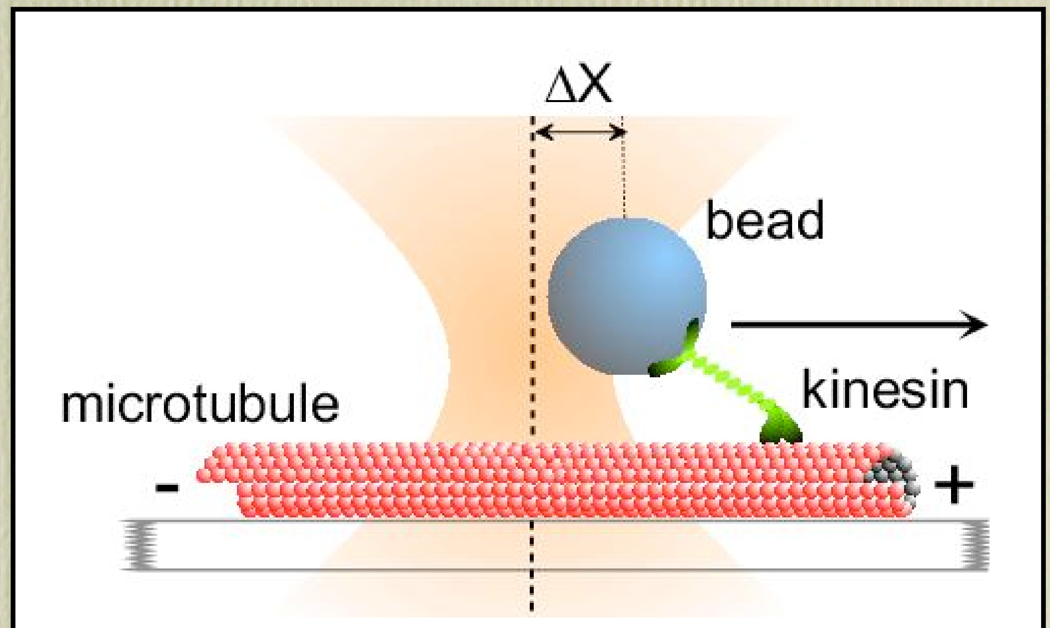

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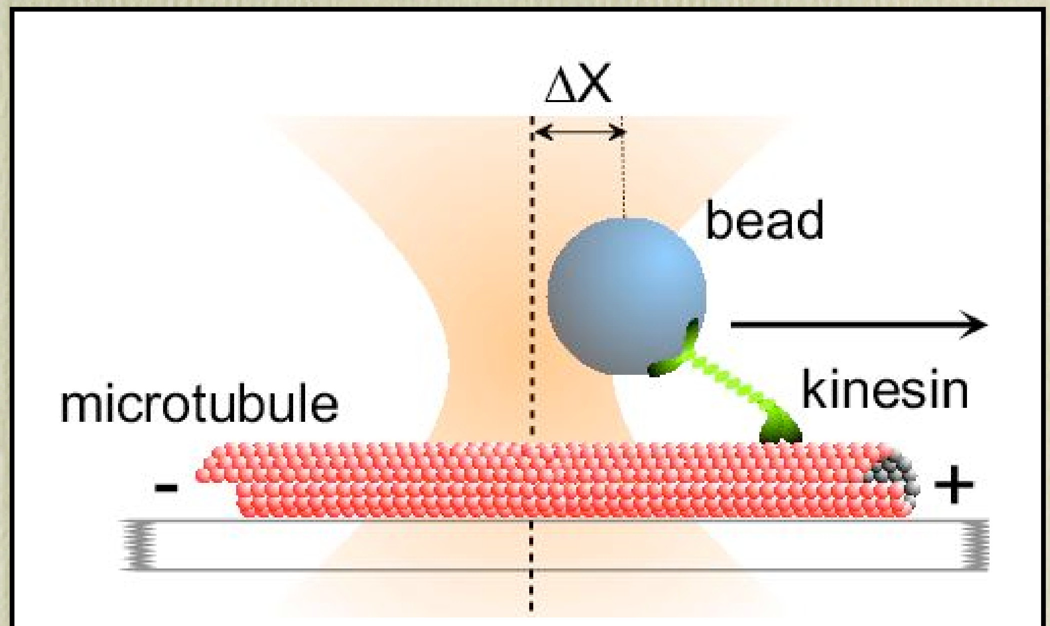

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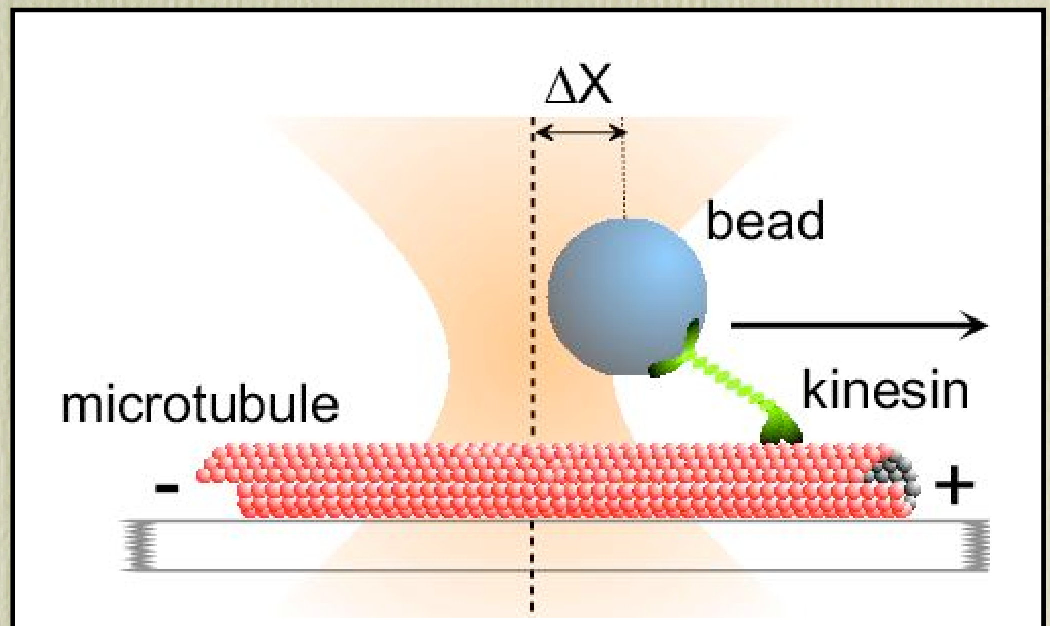

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- near center of trap, spring-like force, $F \propto \Delta X$

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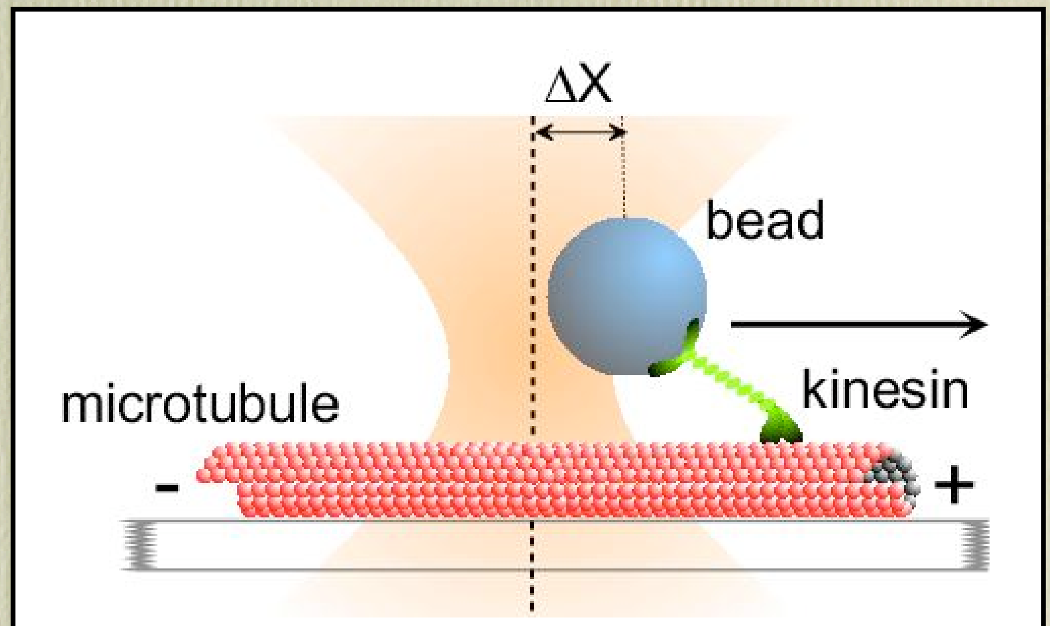

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- near center of trap, spring-like force, $F \propto \Delta X$
- optical feedback to hold ΔX constant as kinesin pulls bead forward

Hidden Markov Models

- Hidden Markov state process

$$1_k \underset{v_2}{\overset{u_1}{\rightleftharpoons}} 2_k \underset{v_3}{\overset{u_2}{\rightleftharpoons}} \cdots \underset{v_N}{\overset{u_{N-1}}{\rightleftharpoons}} N_k \underset{v_1}{\overset{u_N}{\rightleftharpoons}} 1_{k+1}$$

  - Lattice index: X_t
  - Mechanochemical state: C_t

- Observable quantity depends on hidden state
  - Bead position: Y_t
  - Thermal noise

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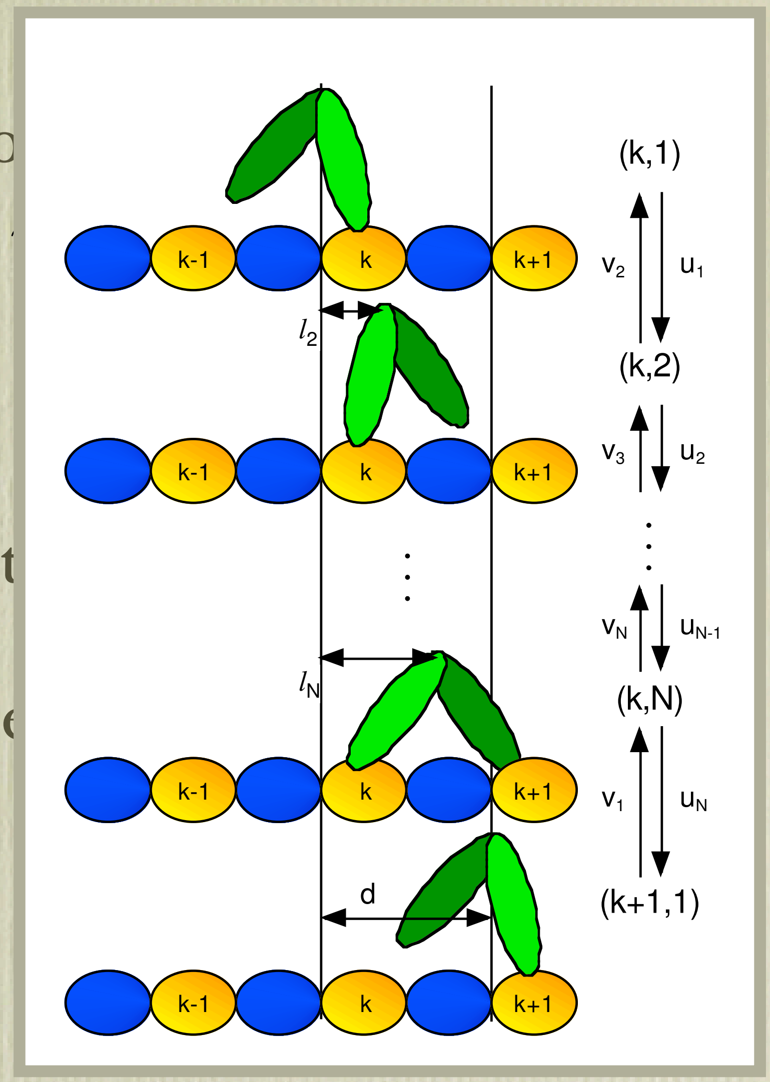

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Transition Probabilities

- Transition rates lead to transition probabilities

$$\frac{d}{dt}[p_{c_i}^{x,c}(t)] = -(u_c + v_c)p_{c_i}^{x,c}(t)$$
$$+ u_{c-1}p_{c_i}^{x,c-1}(t) + v_{c+1}p_{c_i}^{x,c+1}(t)$$

$$p_{c_i}^{x,c}(0) = \delta_{0,x}\delta_{c_i,c}$$

- Transition matrix

$$a(x_i, c_i; x, c) = p_{c_i}^{x-x_i,c}(\Delta t)$$

Bead Position Distribution

- Diffusion in quadratic potential

$$\frac{dY}{dt} = -\alpha[Y - f(X,C)] + \xi(t)$$

- Approximate the residual as AR(1) process

$$R_k = Y_k - f(X_k, C_k)$$

Bead Position Distribution

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$$\frac{dY}{dt} = -\alpha[Y - f(X, C)] + \xi(t)$$

$$f(x, c) = d(x + \epsilon_c) + \kappa$$

$$\epsilon_c = \ell_c / d$$

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$$\rho = e^{-\alpha \Delta t} \qquad \eta^2 = (1 - \rho^2)\sigma^2$$

Likelihood

- Given a kinetic model and corresponding parameterization ▯, compute the likelihood for a sequence of mechanochemical states and bead positions:

$$L(\vec{x},\vec{c},\vec{y};\theta) = \pi(c_0)\phi_0(r_0)\prod_{k=1}^{N} a(x_{k-1},c_{k-1};x_k,c_k)\phi(r_k - \rho r_{k-1})$$

- True sequence of kinetic cycle unknown:

$$L(\vec{y};\theta) = \sum_{\vec{x},\vec{c}} L(\vec{x},\vec{c},\vec{y};\theta)$$

Likelihood

- Take into account all possible transitions
  - Weighted according to observations.
  - Only likely sequences contribute significantly.
- Forward algorithm efficient (Baum et al. 1970, Rabiner 1989)

$$L(\vec{y};\theta) = (\pi \star b_0(y_0)) \cdot \prod_{k=1}^{N} (A \star B(y_{k-1}, y_k)) \cdot \vec{1}$$

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$$\boxed{\begin{array}{c} b_0(y)(x,c) = \phi_0(r) \\ r = y - \mu(x,c) \end{array}}$$

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Forward-Backward Vectors

Forward: $$\alpha_k = (\pi \star b_0(y_0)) \prod_{i=1}^{k} A \star B(y_{i-1}, y_i)$$

Backward: $$\beta_k = \prod_{i=k+1}^{N} A \star B(y_{i-1}, y_i) \cdot \vec{1}$$

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Posterior probabilities in terms of forward and backward variables:

$$P[(X_k, C_k) = (x, c) | \vec{y}] = \frac{\alpha_k(x, c)\beta_k(x, c)}{L(\vec{y}; \theta)}$$

$$L(\vec{y}; \theta) = \alpha_k \cdot \beta_k$$

HMM Calculations

- Individual terms in likelihood calculation lead to posterior probabilities for hidden variables
  - Estimated dwell times
  - Estimated transition counts (jumps)
  - Estimated statistics for regression analysis

Parameter Estimation

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    - Estimate new parameters based on statistics

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    - Experimental replicates

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  - Find the sequence of mechanochemical states with maximum likelihood

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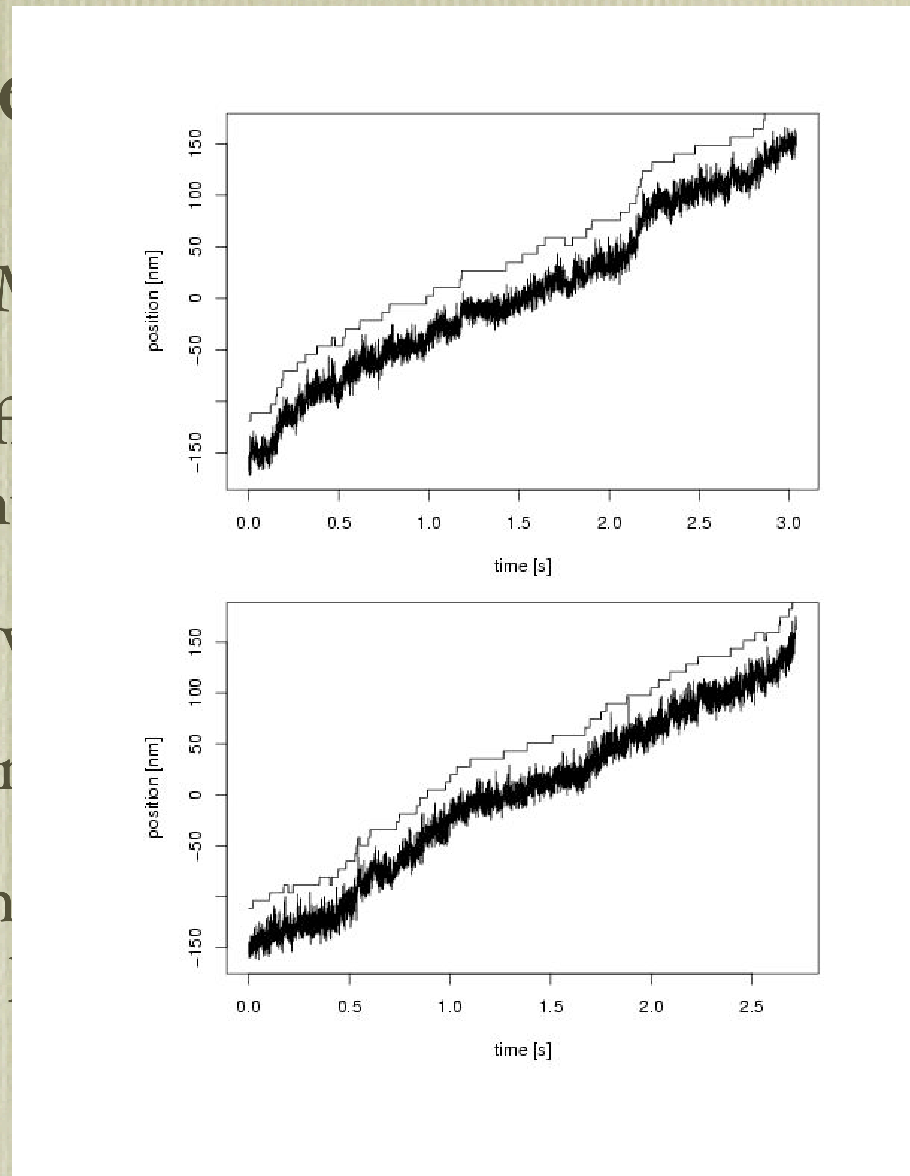
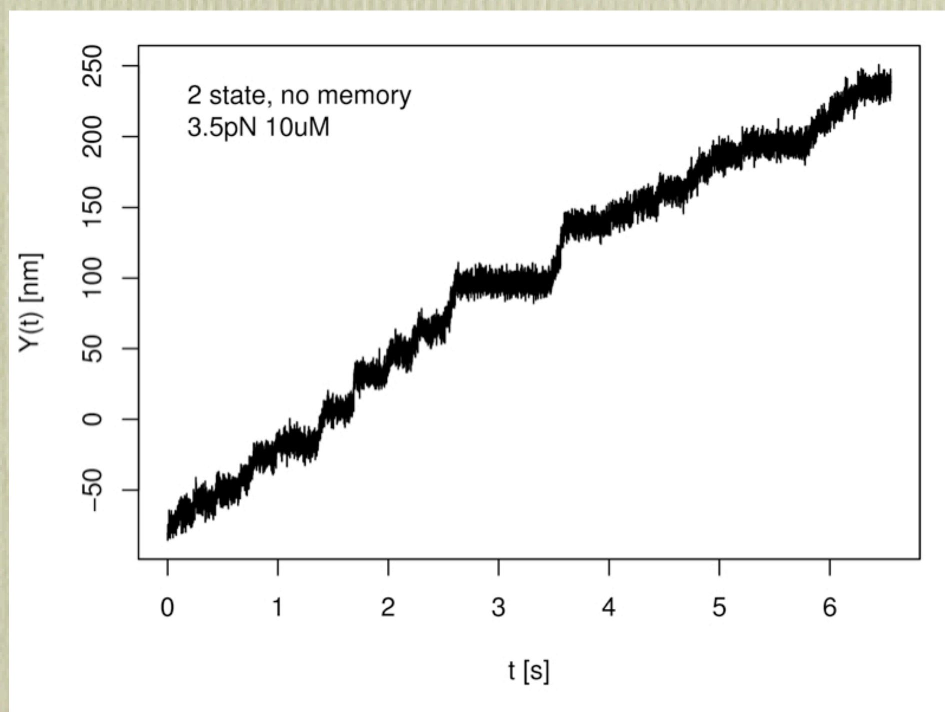


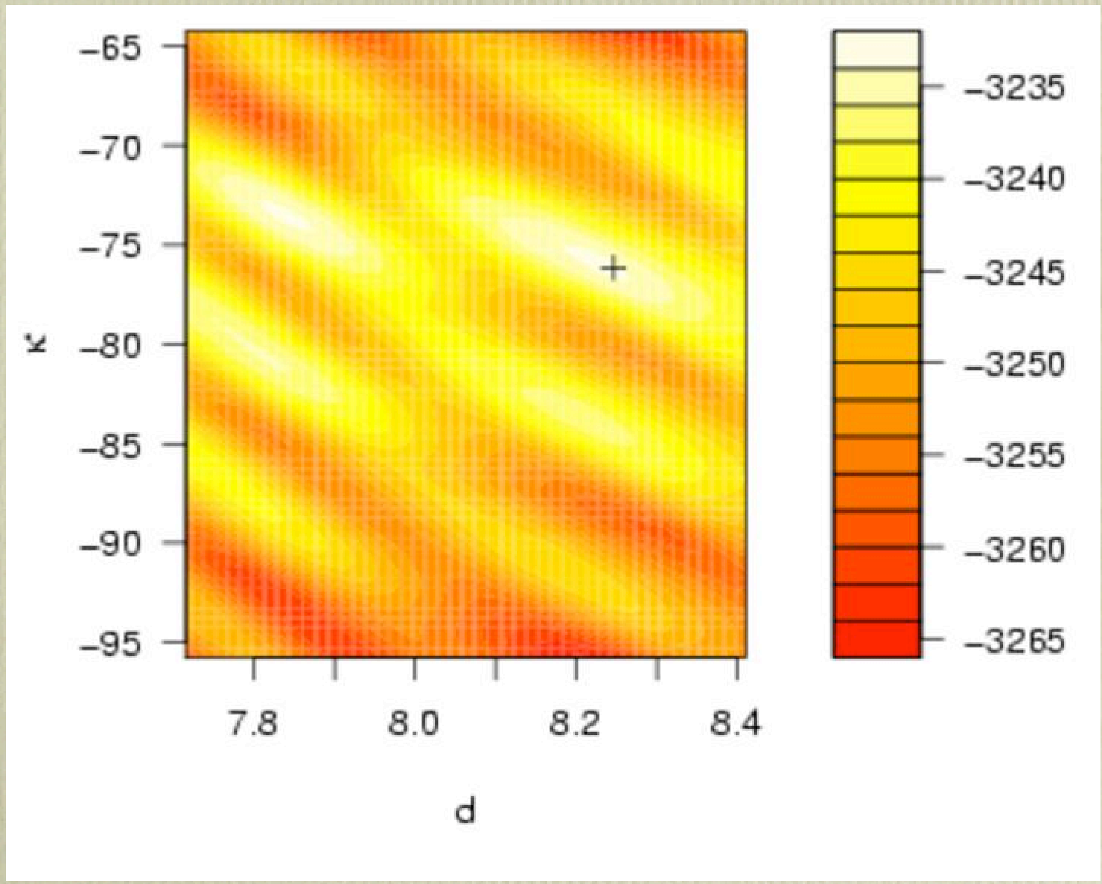
Simulation Results

- Simulations of 2-state kinetic cycle (based on model of Fisher and Kolomeisky 2001)



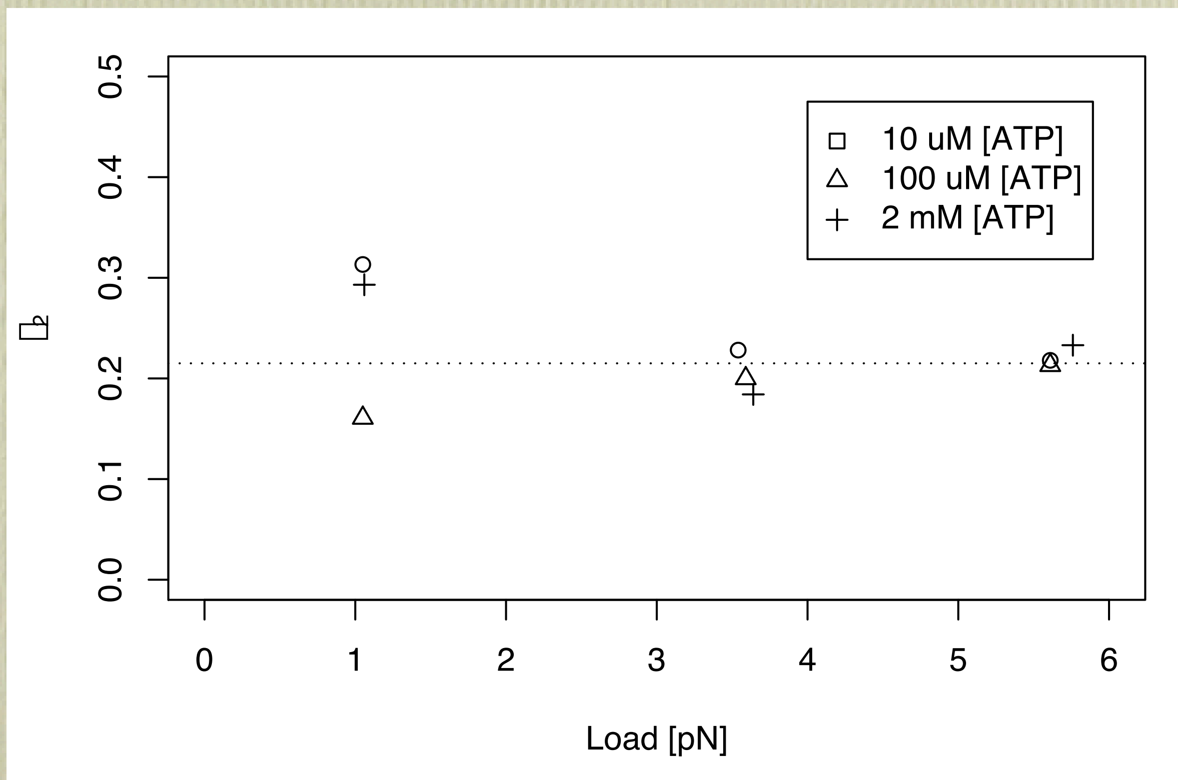
Simulation Results

- Spatial parameters (lattice spacing and offset)



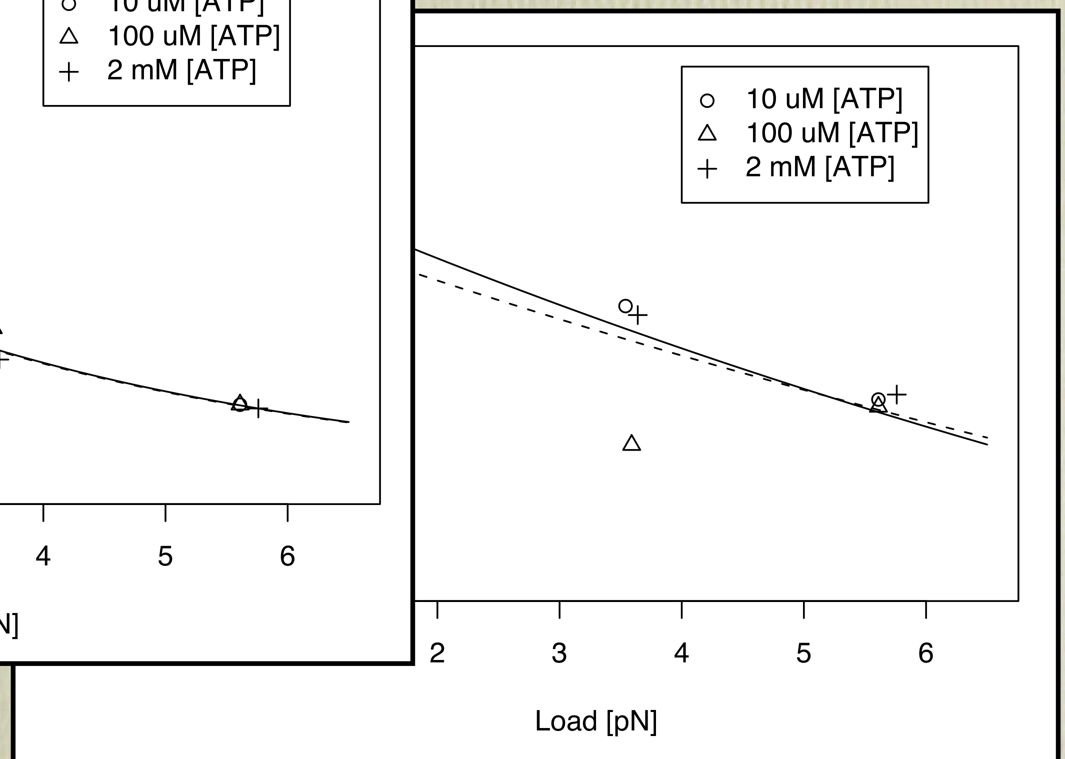
Simulation Results

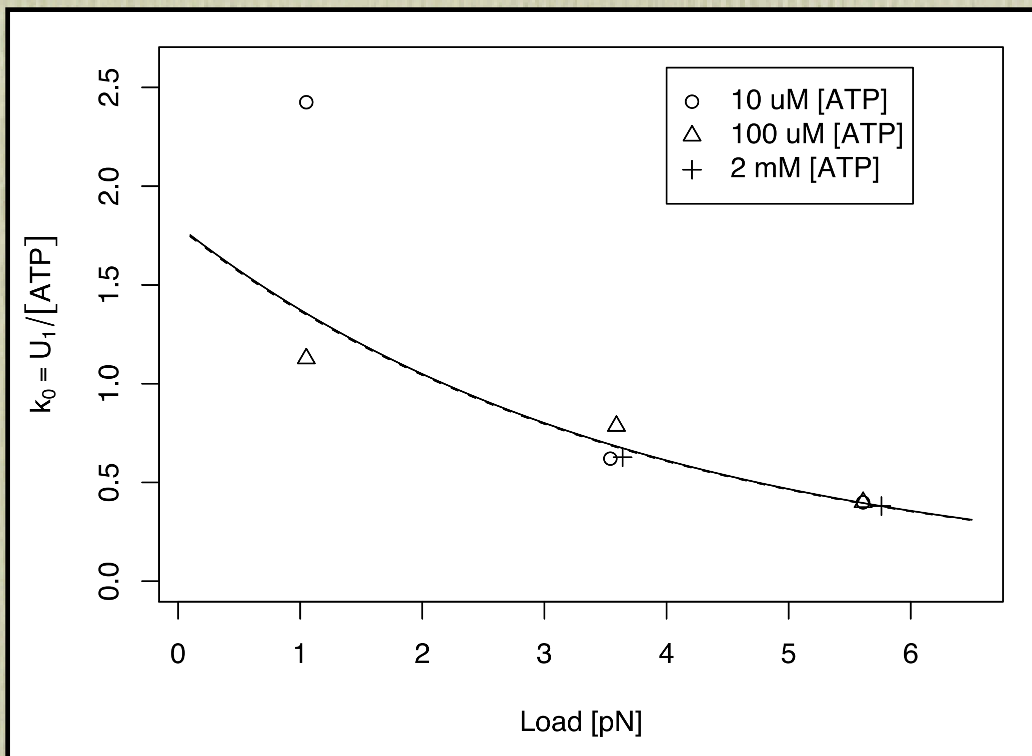
- Sub-step fractional size



Simulation Results

- Transition rates





Simulation Results

- Model selection: 1-, 2-, or 3-state model
- Log-Likelihood increase (cf. $2(\log L_1 - \log L_0) \sim \chi^2_{df}$)

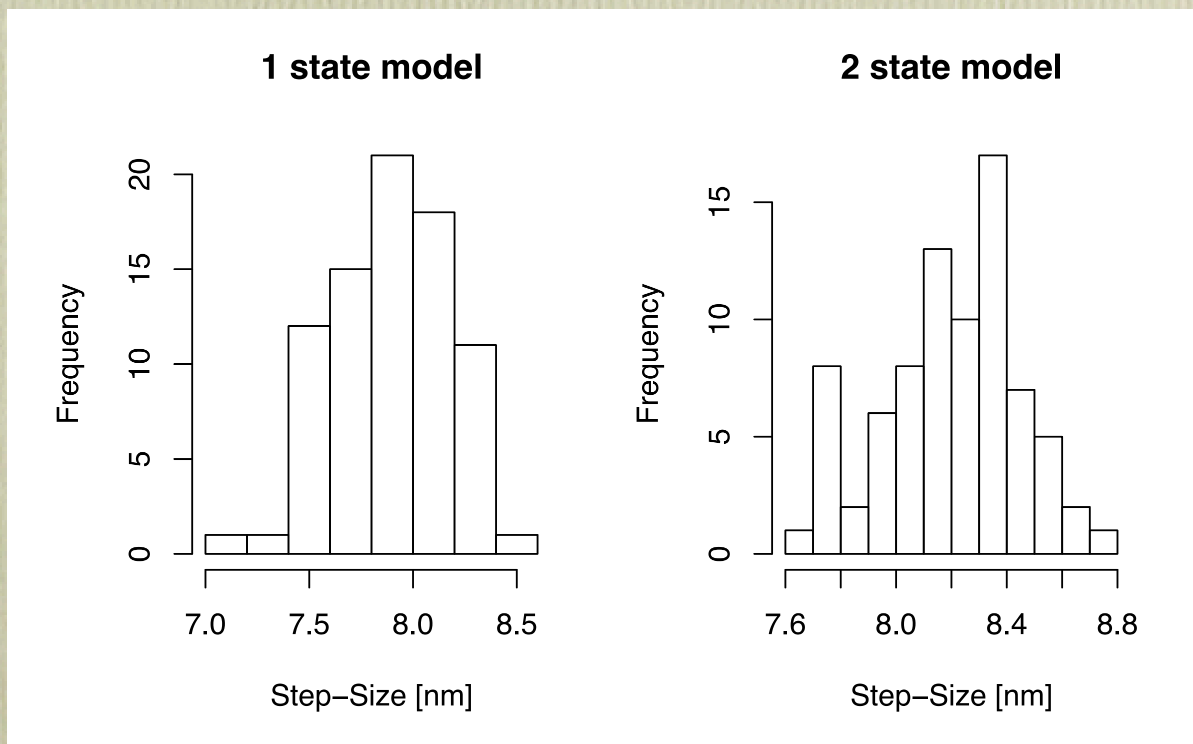
One-state to two-state: +8206.4
(P-value < 0.0001, 27 d.f.)

Two-state to three-state: +3.2
(P-value > 0.9999, 27 d.f.)

Constrain two-state model to satisfy detailed balance: -12.8
(P-value = 0.647, 29 d.f.)

Experimental Results

- Based on data of Visscher et al. 1999
- Sampling rate 2 kHz



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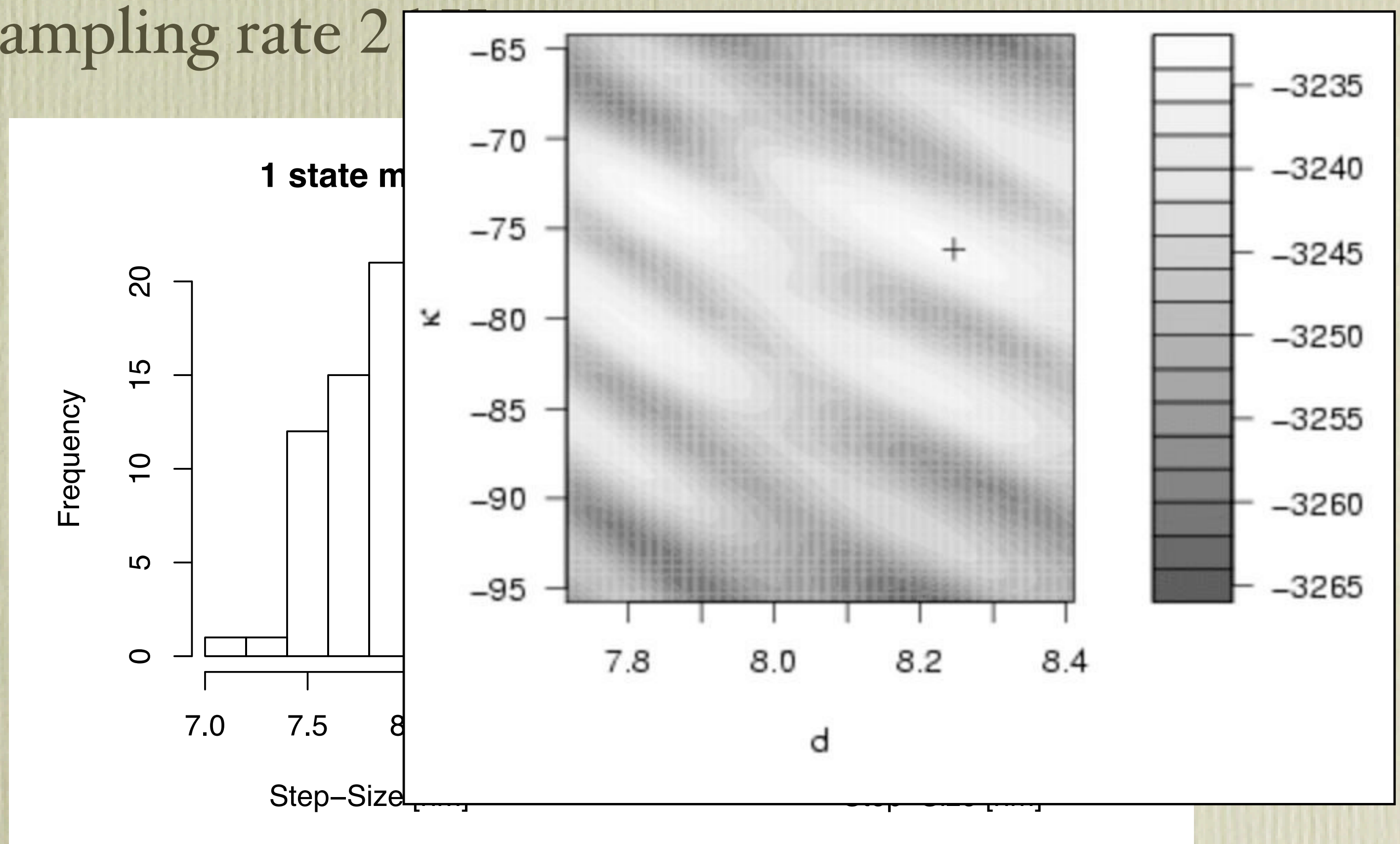

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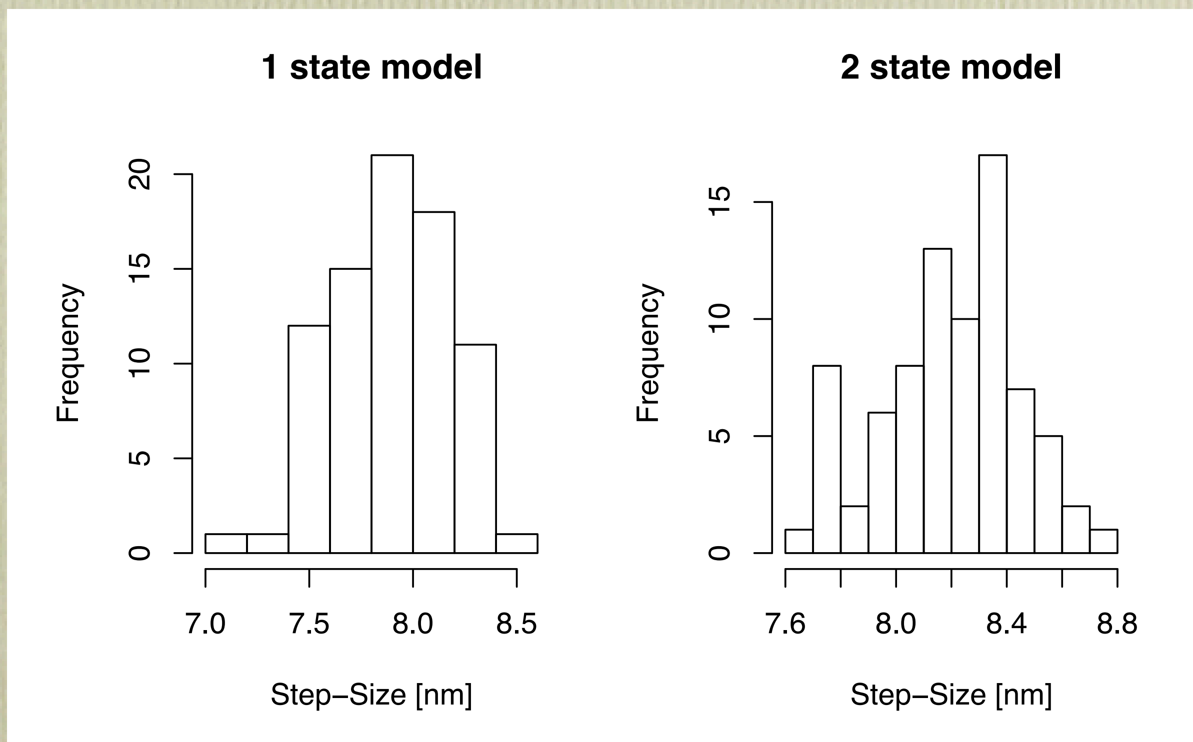
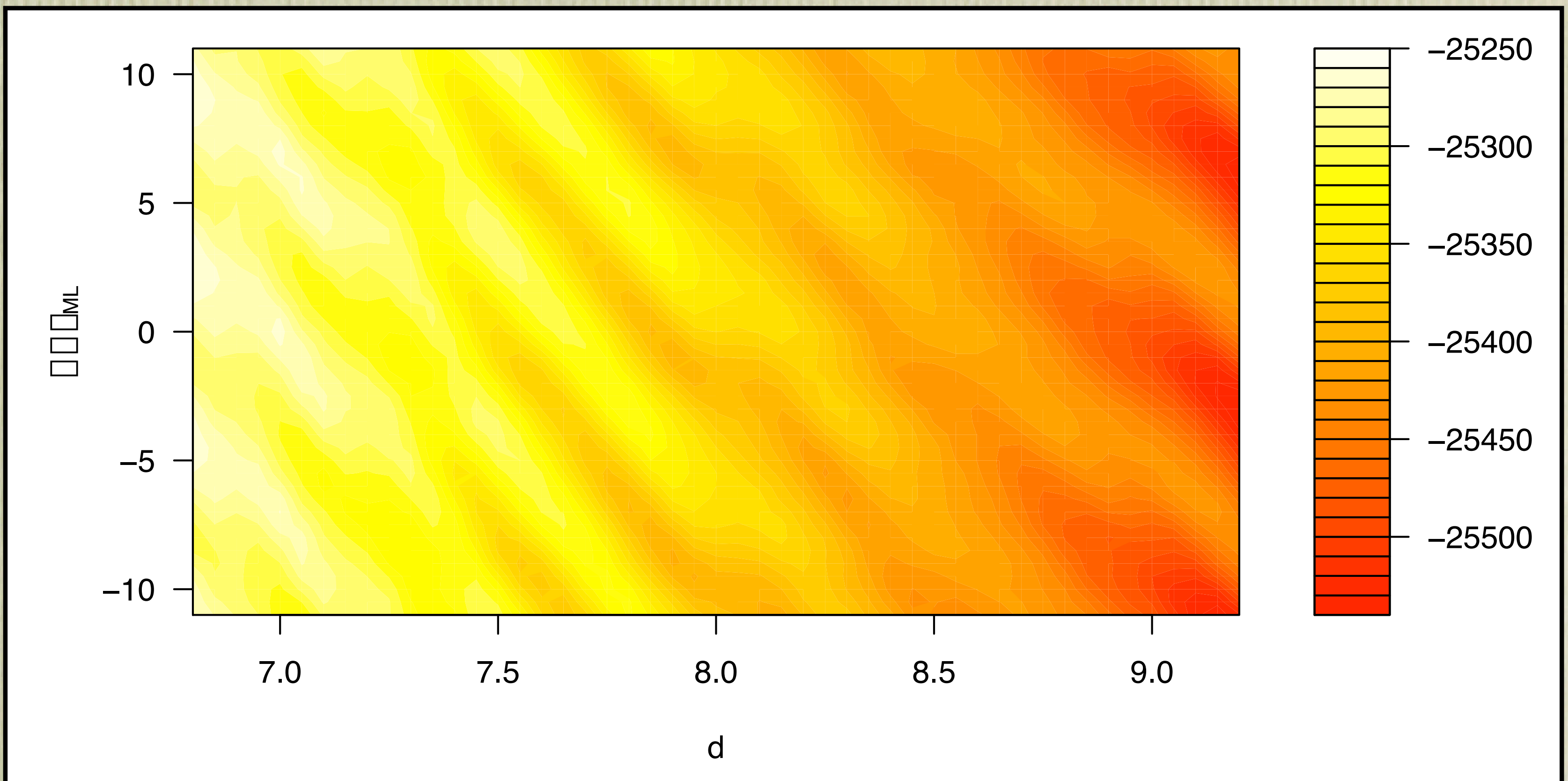
- Based on data of Visscher et al. 1999
- Sampling rate 2 kHz



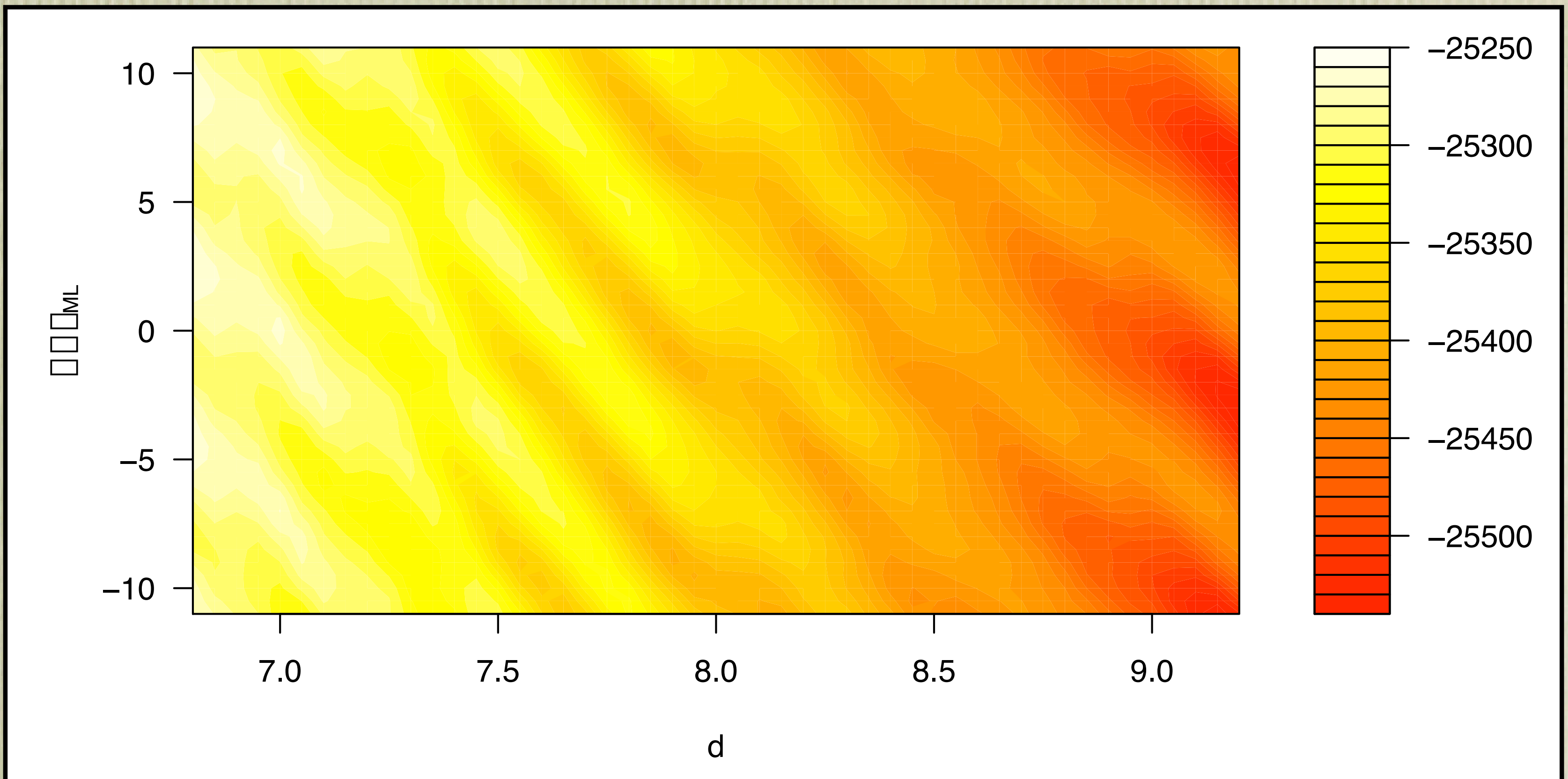
Experimental Results

- What happened?



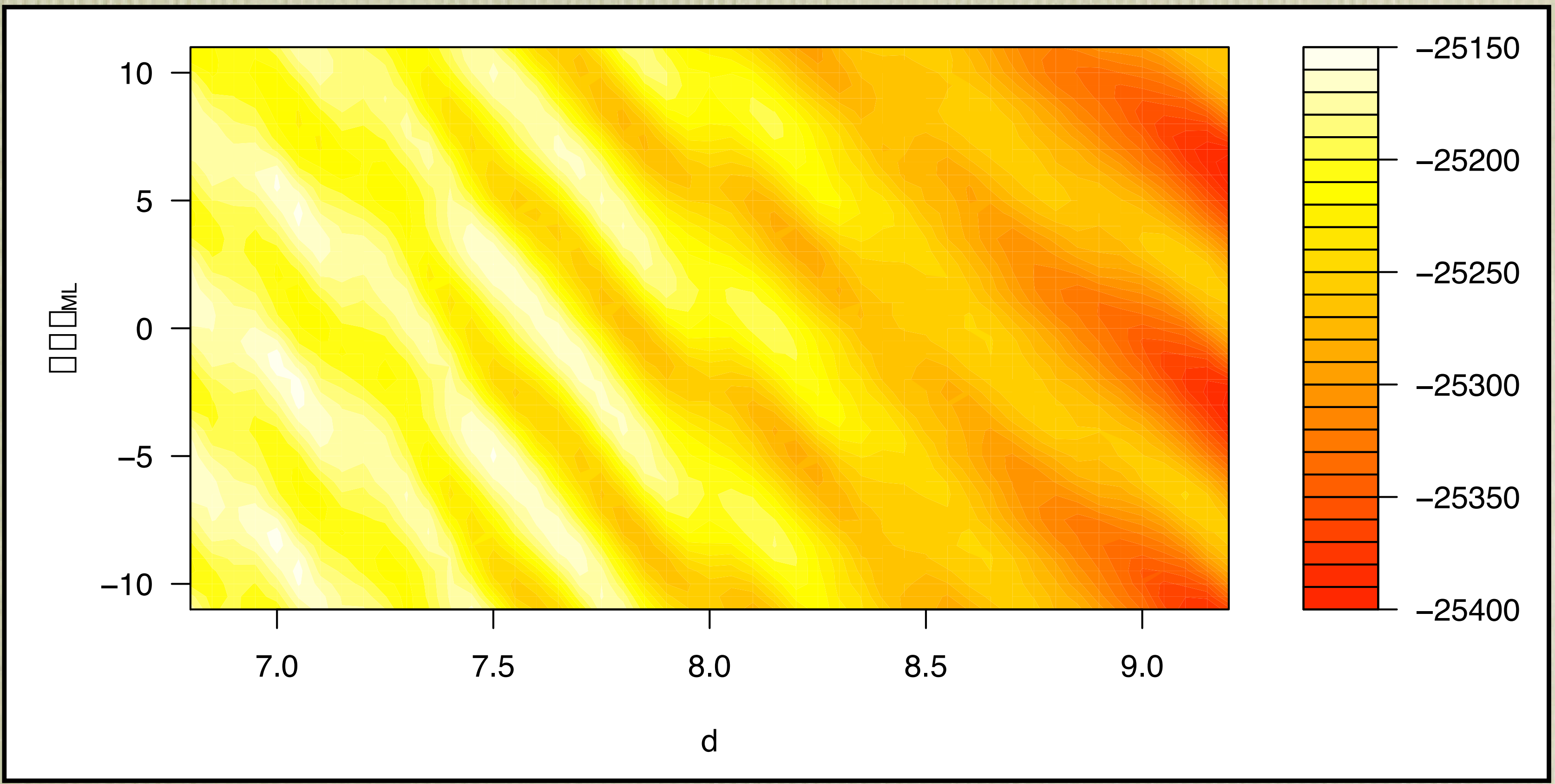
Experimental Results

- What happened?   Individual identities?



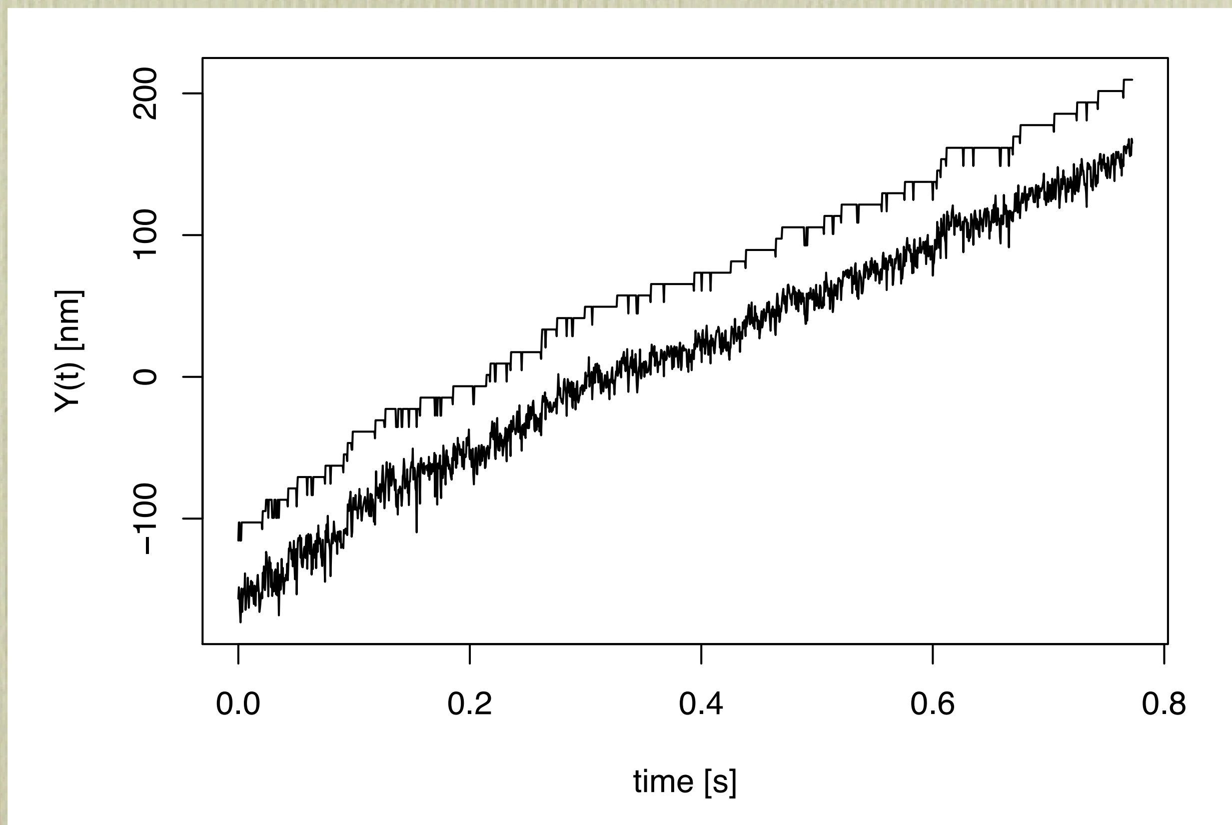
Experimental Results

- What happened?   Individual identities?



Experimental Results

- Some data sets show uncharacteristic noise



Conclusions

- Hidden Markov models incorporate explicit kinetic schemes coupled to observations
- Account for all possible unknown transitions with statistically appropriate weights
- Parameter estimation based on details of data
- Model selection using likelihood ratios

Future Work

- Optimize optimization: parameter estimation
- Account for additional experimental uncertainty
  - Backward steps or detachment/reattachment?
  - Baseline drift and variable step sizes
- Data?

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