Fluctuations in Small Systems

the case of single molecule experiments

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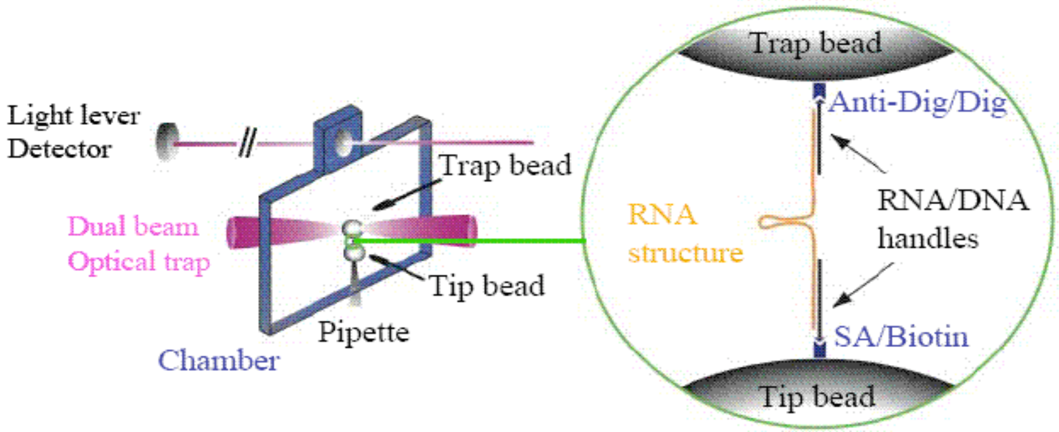
Perugia, Aug. 2nd, 2011

force × distance ≈ $k_B T$ >> quantum scale

"I do not believe a word he is saying, but I am afraid that one day I will have to learn it" (Bob Silbey)



for an introduction, **C. Bustamante** et al, Physics Today, July 2005, 43-48

Single molecule experiments
(after 1990)

- techniques to measure forces in the pN range (10^{-2}-10^{3})pN, and distances in the nm range (nanodevices);
- with high time resolution to track the trajectories of single biomolecules;
- access to new phenomena in molecular and cellular biophysics $\rightarrow$ new physical insight in non-equilibrium (n.e.) statistical physics.

The physical problem

Purely mechanical models are too naïve

- energies involved in molecular processes are a few $k_B T$ (at T=298K, 1 $k_B T$ = 4.1pN·nm = 0.6kcal/mol)

  forces ~ 10pN distances ~ 1nm

  Force scale: Brownian, 10fN; molecular motors, 10pN; folding (H-bond), 100pN; covalent bond (1eV for 1Å), 1nN

- thermal fluctuations become extremely important as they determine the efficiency of the molecular motors;

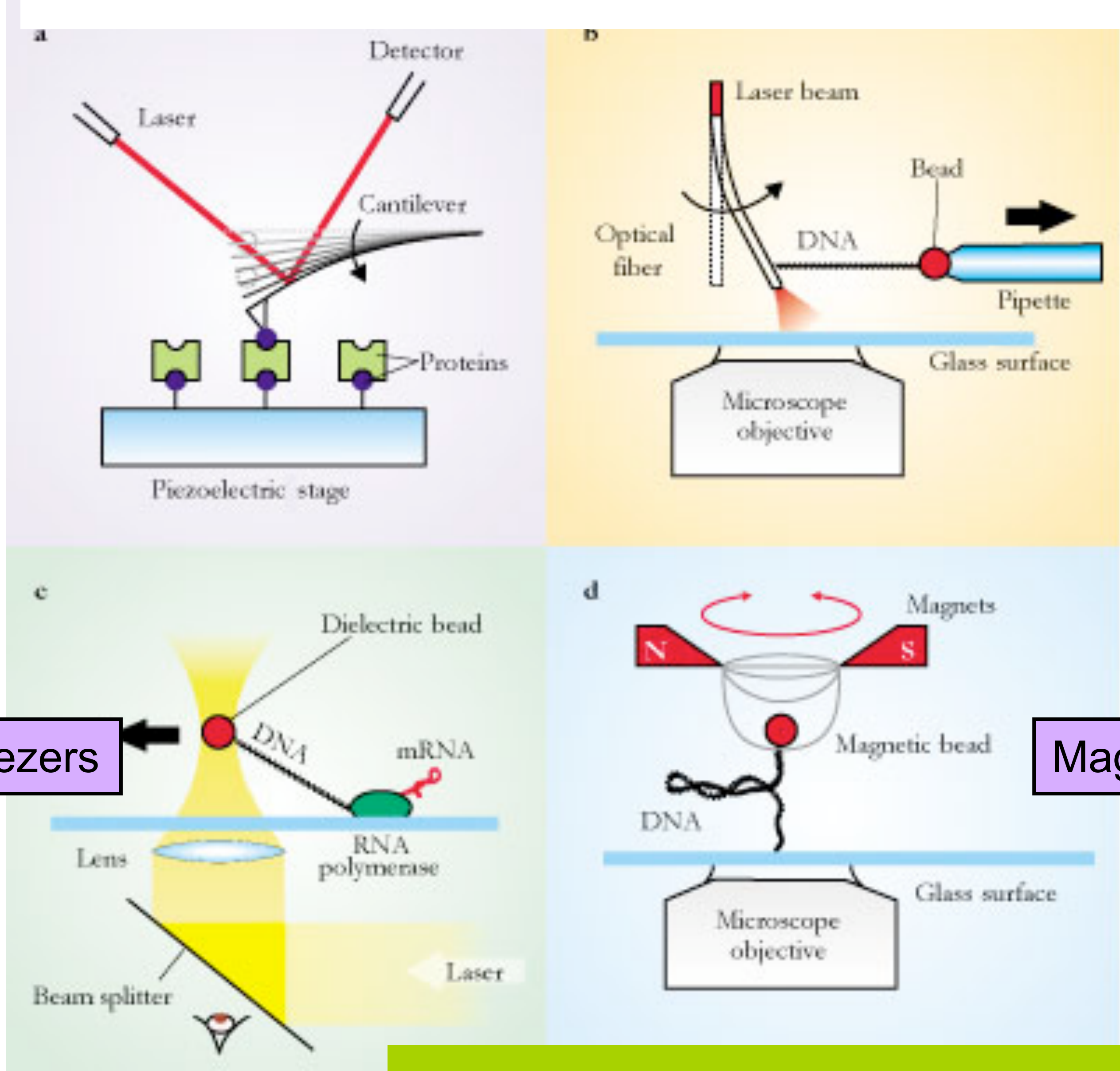
- importance of rare events and large deviations from average behavior a new thermodynamics

Mechanochemistry (after1990)

$\delta X \, \delta F \leq k_B T$

AFM

>10 pN
10^{-3}s



$\langle F \rangle = \kappa \langle X \rangle$
$\langle \delta X^2 \rangle = kT/\kappa$
$\langle \delta F^2 \rangle = \kappa\, kT$
$\langle F \rangle = kT \langle X \rangle / \langle \delta X^2 \rangle$

1pN

Optical tweezers

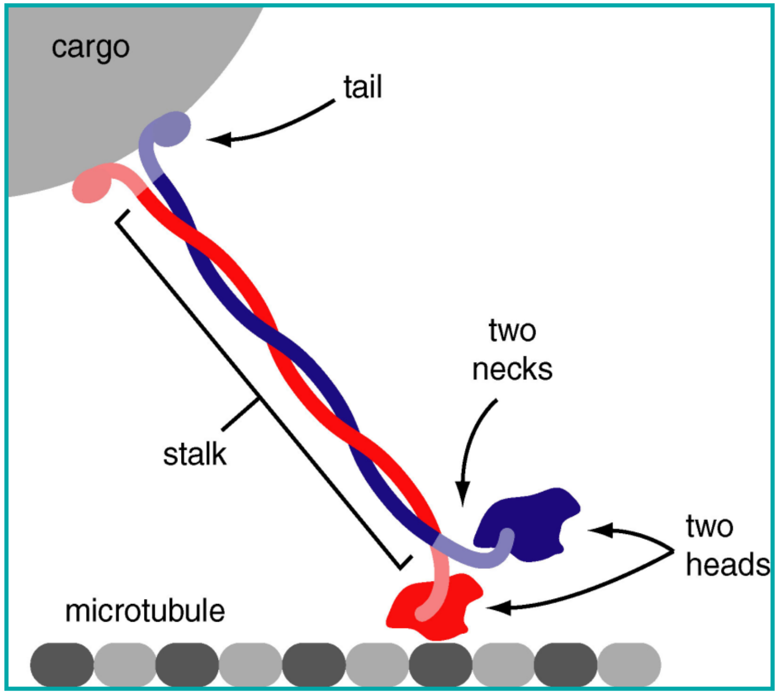
0.1-10^2pN
1nm

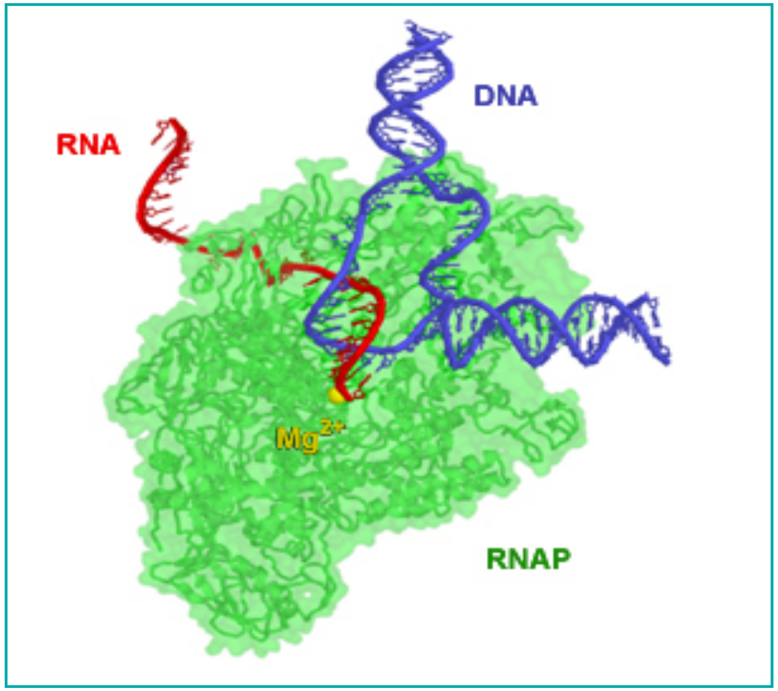
Magnetic tweezers

10^{-2}-10pN

For an introduction, **T. Strick** et al, Physics Today, Oct. 2001, 46-51

Examples





kinesin "walking" along a microtubule, fueled by ATP hydrolysis

transcription by RNA polymerase enzyme rectifies thermal noise

fuel: ATP→ADP+20 $k_B T$; high efficiency: 40-60% despite pauses, arrests, backtracking events

Small systems

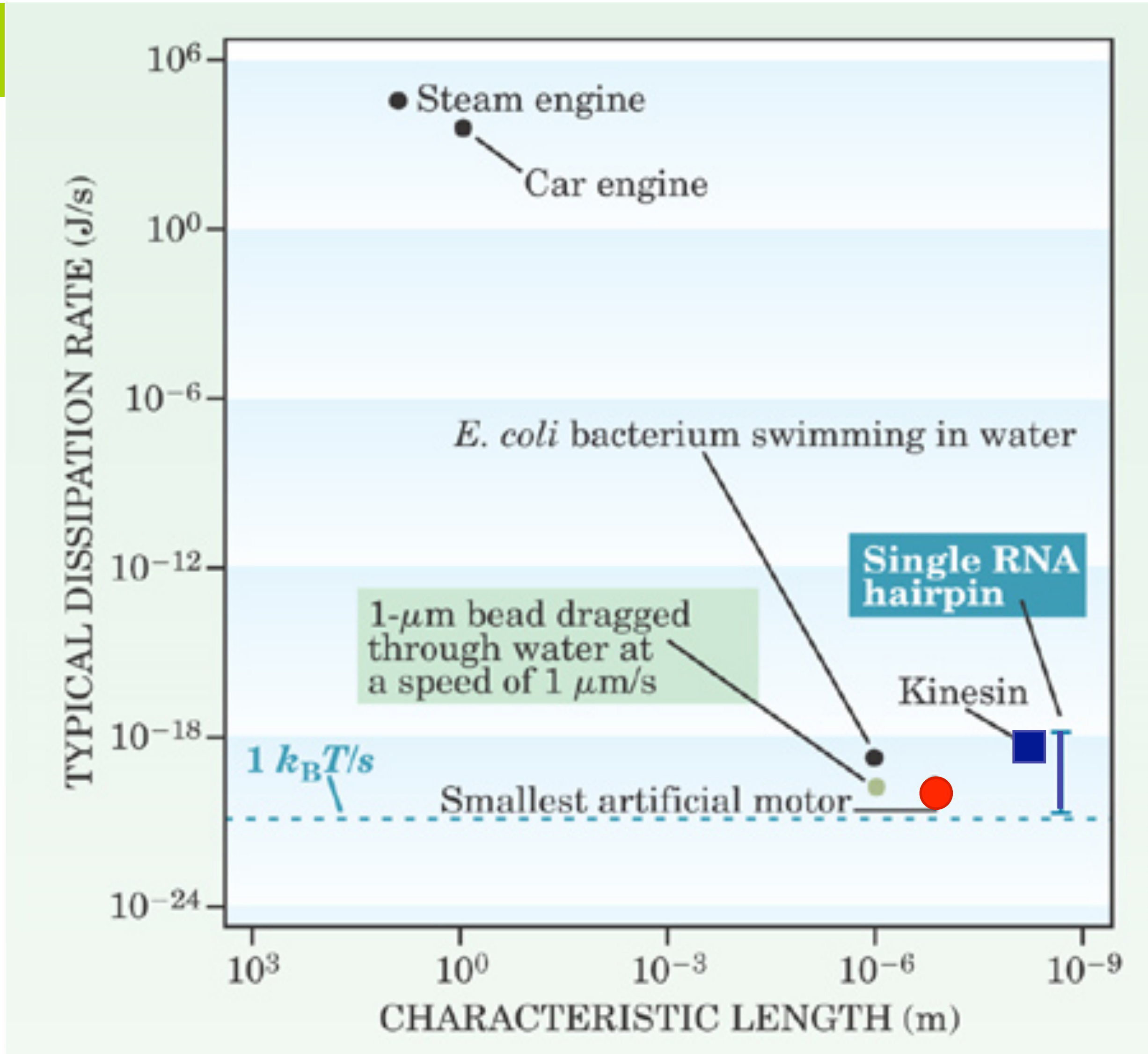
A. Mostly steady state

- net currents across (heat, electric, mass)
- dissipate energy constantly

Example: kinesin

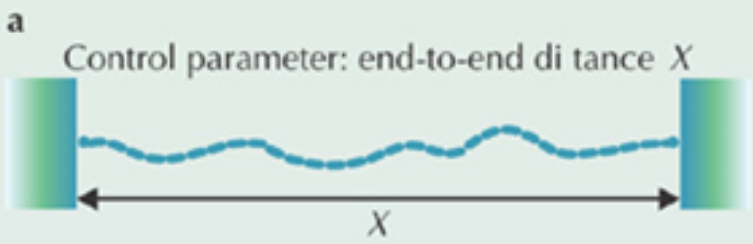
ATP $\rightarrow$ ADP + 20 k_BT
1 step: 8nm in 10-15ms
work/step: 12 k_BT (avg. load)

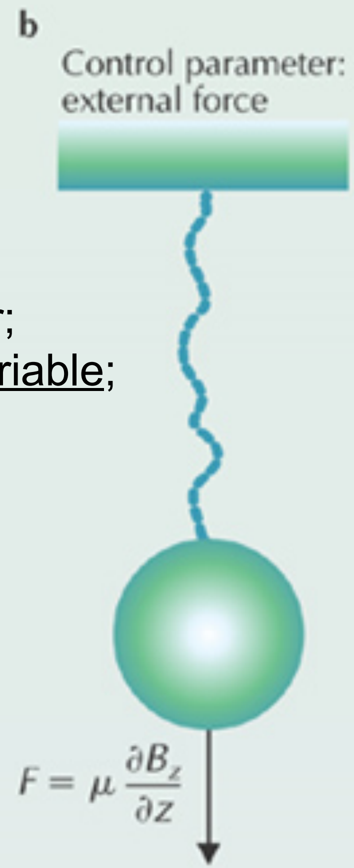
efficiency: ~60%
dissipated pwr.: 650 k_BT/s



B. Equation of state and fluctuation depend on the choice of (few) control parameters

in *small* systems
force-extension characteristics
curves not uniquely determined





a distance X: tunable control parameter;
force on one end/bead: fluctuating variable;
A(T,X) – Helmholtz f.e.

b force on free end/bead:
tunable control parameter;
chain extension X:
fluctuating variable;
G(T,F) – Gibbs f.e.

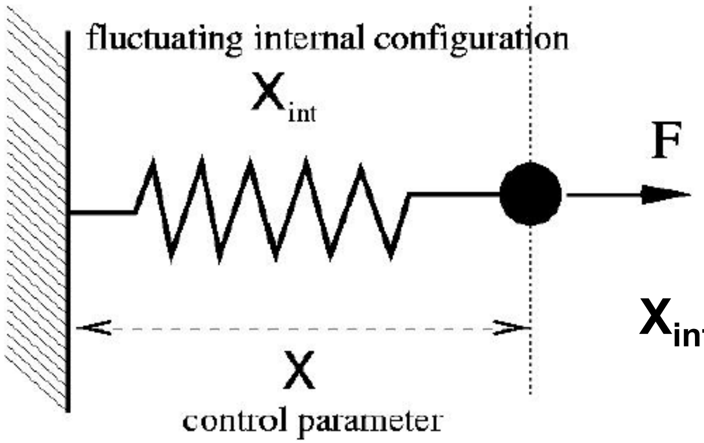
STRETCHED POLYMER

control often incomplete

C. Subjected to non-equilibrium transformations…

$$dU = \sum_i \left(\frac{\partial U}{\partial x_i}\right)_X dx_i + \left(\frac{\partial U}{\partial X}\right)_{\{x_i\}} dX = dQ + dW.$$

…according to a given protocol
$X(t)$: $X_i = 0 \rightarrow X_f$



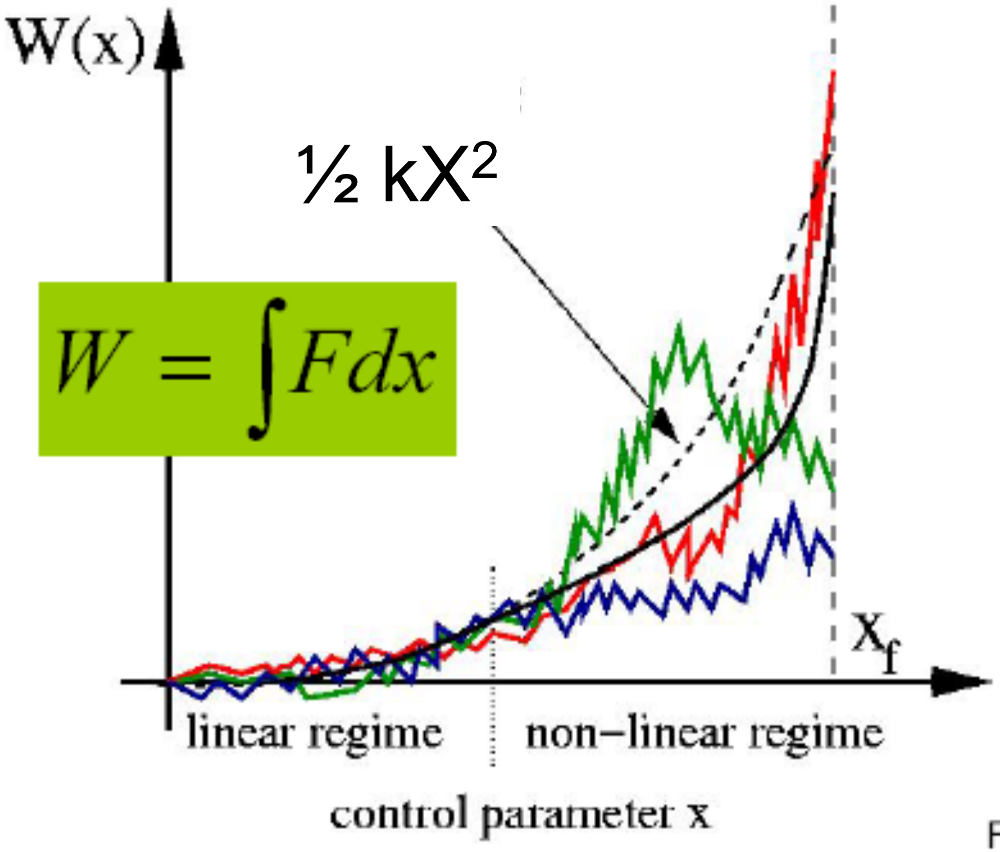
$X_{int} = \{x_i\}$

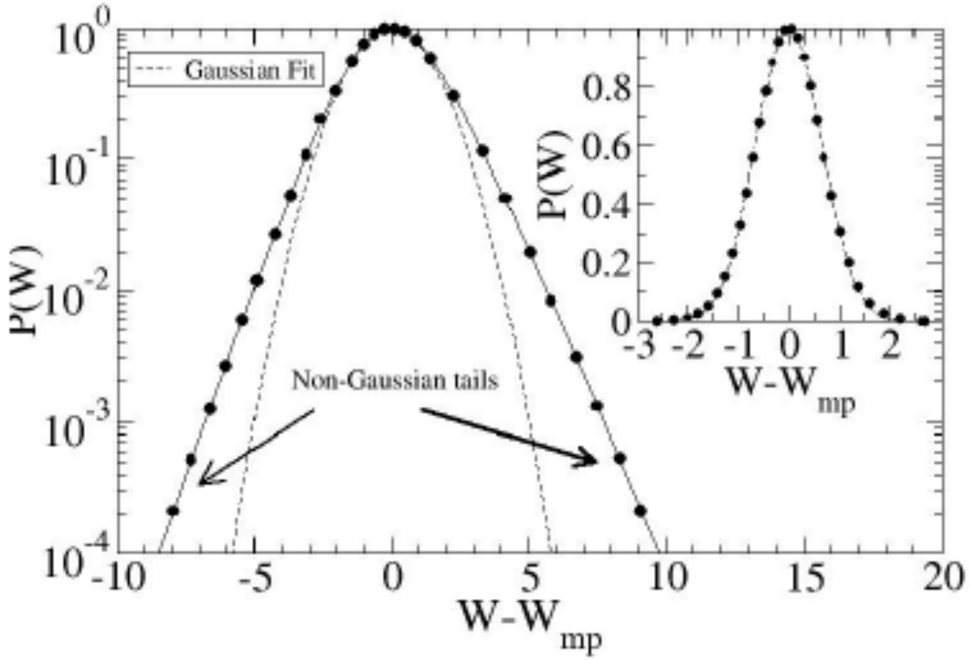
X determines the thermodynamic state of the system

Force $F=F(X_{int}, X)$ is the fluctuating variable

Q hard to measure →
[for *aperiodic* X(t)]

$$W = \int_0^{X_f} dW = \int_0^{X_f} F dX,$$





F. Ritort, J. Stat. Mech. (Theor. and Exp.) P10016 (2004)

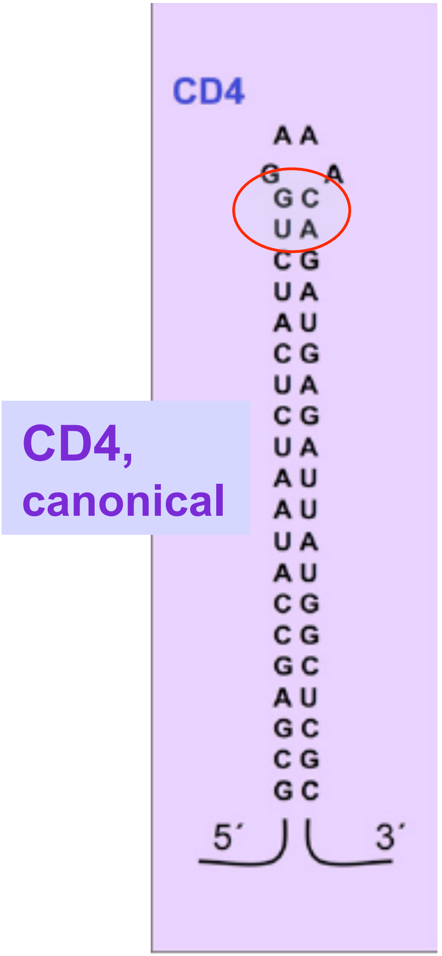
$$P(W)$$

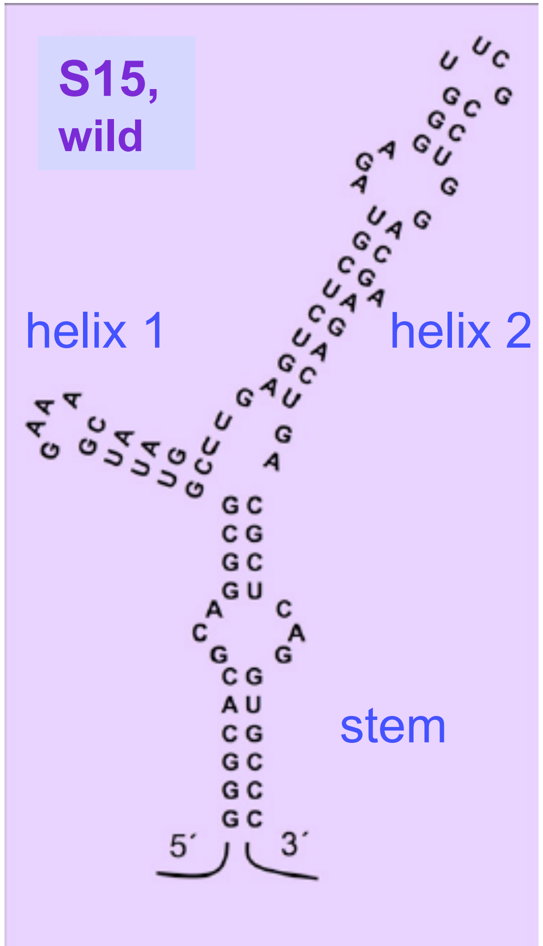
Work probability distribution

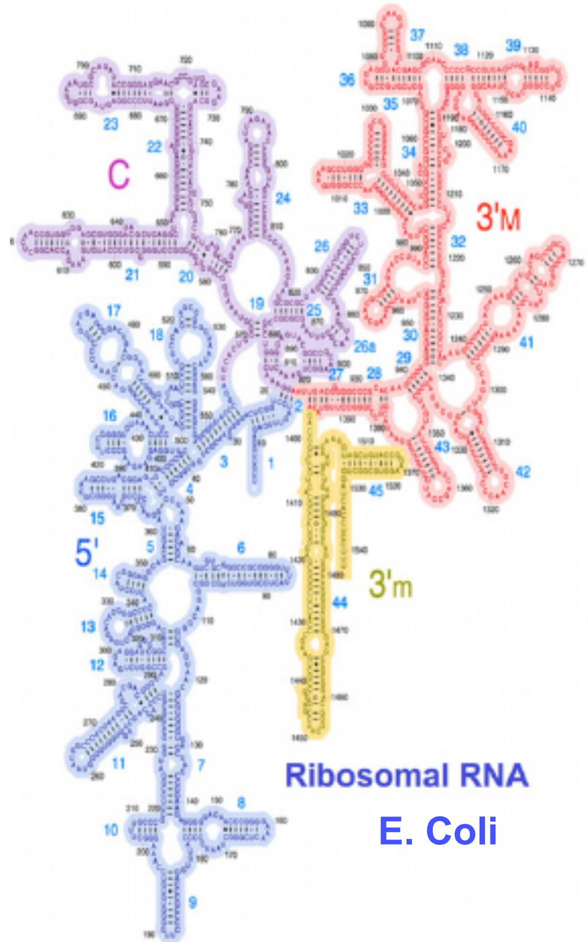
Importance of large and rare deviations respect to the average behavior:

How does RNA fold?

Single stranded RNA structures



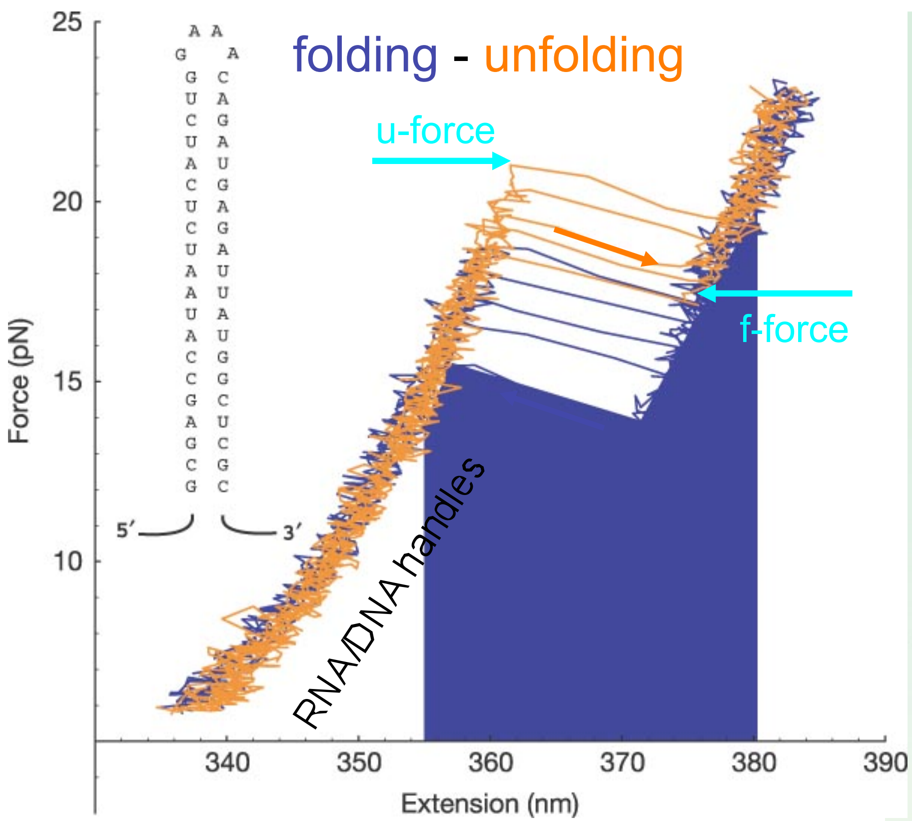


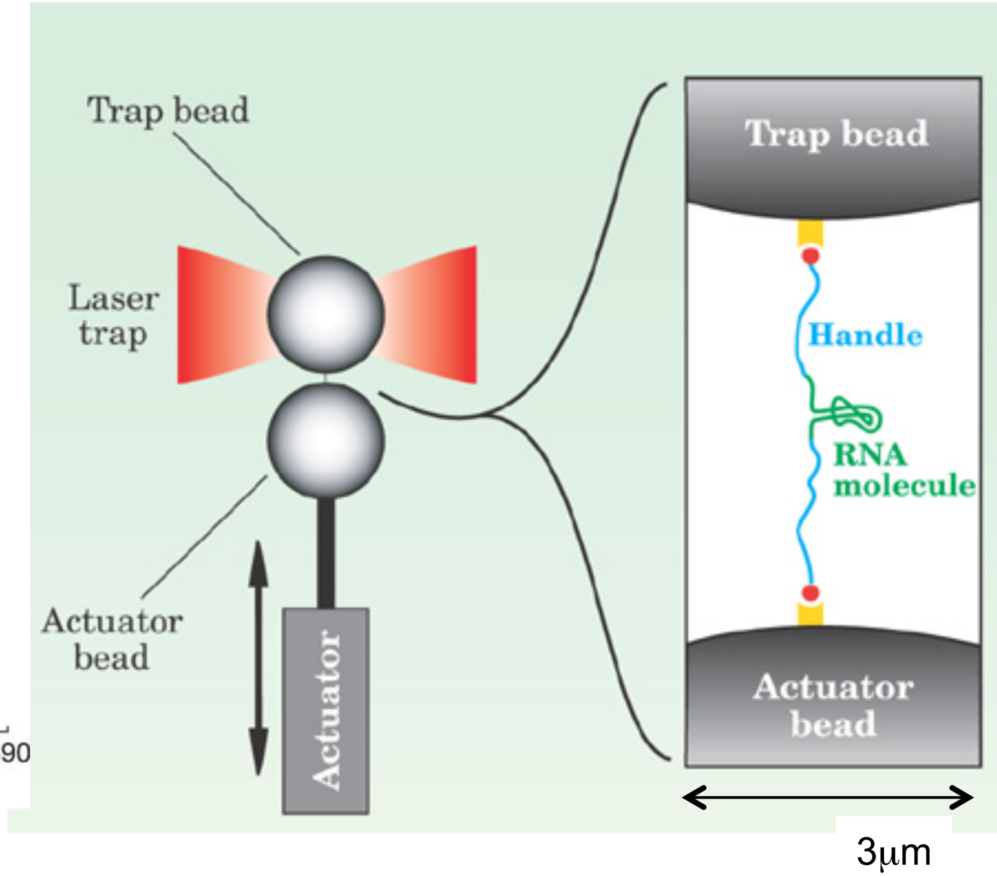


U=uracil (thymine) basepairs with Adenine and also with Guanine

J. Liphardt et al, Science (2002)

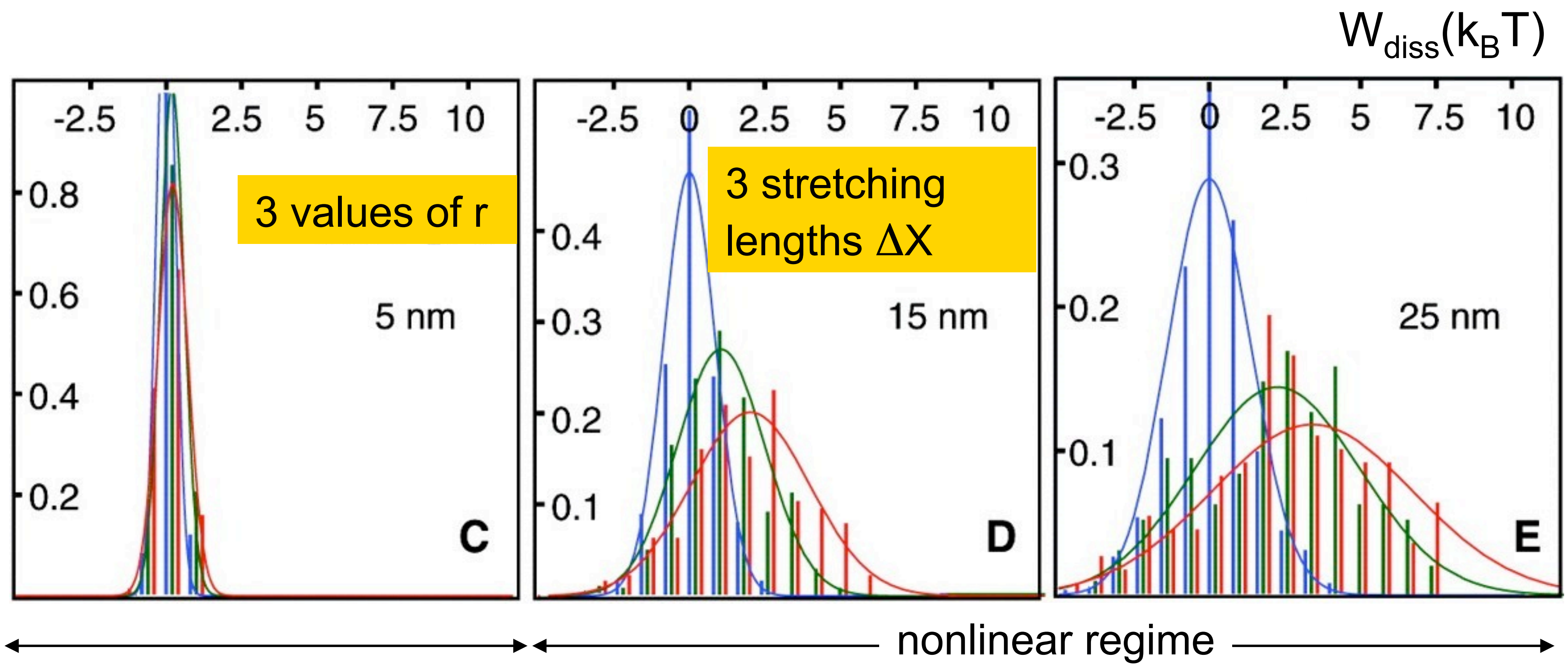
- mechanical stretching of a single RNA molecule (20nm long), at constant loading rate r (below: r = 7.5pN/s)
- irreversible folding-unfolding cycles are hysteretic → work is dissipated





• linear contributions from the *entropy loss* due to the stretching of the molecular handles and of the *elastic stretching* of single-stranded RNA

P(W) exhibits negative “fat” tails



Fluctuation Theorems

- $\Lambda_F(t)$: n.e. forward process $\boldsymbol{\lambda_F(t)}$: $\boldsymbol{\lambda_A \rightarrow \lambda_B}$; T constant;
  A: equilibrium initial state, $t = t_i$; **B***: n.e. final state, $t = t_f$

- $\Lambda_R(t)$: n.e. reverse process $\boldsymbol{\lambda_R(t')}$: $\boldsymbol{\lambda_B \rightarrow \lambda_A}$
  B: equilibrium initial state, $t' = t_i$; **A***: n.e. final state, $t' = t_f$

- $\Delta G = G_B - G_A$ free energy difference between equilibrium states A and B

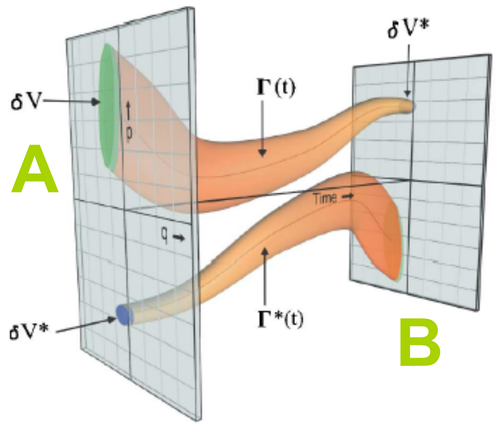
- $\Lambda_R(t)$ is time reversed with respect to $\Lambda_F(t)$, i.e. $\Lambda_R(s) = \Lambda_F(t-s)$ for $0 \leq s \leq t$, with corresponding work p.d. $P_F(W)$ and $P_R(W)$

$$\frac{P_F(W)}{P_R(-W)} = \exp\left(\frac{W - \Delta G}{k_B T}\right)$$

(Crooks, 1999)

NB: for reversible $\lambda(t)$

$$\Delta G = W_{rev}$$



applies to cyclostationary protocols, too

The Jarzynski Equality (1997)

Re-write Crooks' FT as

$$W - \Delta G = W_{dis}$$

$$P_R(-W) = P_F(W)\exp[-(W-\Delta G)/k_BT]$$
$$= P_F(W)\exp[-W_{dis}/k_BT]$$

and integrate $\left\langle \exp\left(-\frac{W_{\mathrm{dis}}}{k_BT}\right)\right\rangle = 1$ **or**

$$\exp\left(-\frac{\Delta G}{k_BT}\right) = \left\langle \exp\left(-\frac{W}{k_BT}\right)\right\rangle$$

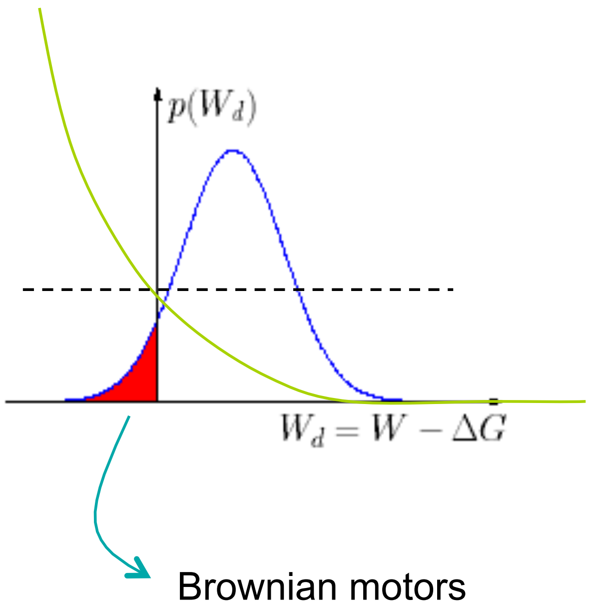
The FT physics

$$\left\langle \exp\left(-\frac{W_{\text{dis}}}{k_B T}\right)\right\rangle = 1$$

- free-energy differences can be extracted from nonequilibrium data;
- $\langle W\rangle \geq \Delta G$, or equivalently, $\langle W_{dis}\rangle \geq 0$, that is the II Law of (macroscopic) thermodynamics. Note that $\langle e^{x}\rangle \geq e^{\langle x\rangle}$ (Jensen's inequality)
- there must exist trajectories with $W_{dis} \leq 0$ (red tail) to ensure $\langle \exp(-W_{dis}/k_B T\rangle = 1$ -- transient violation of II Law due to *t*-reversal invariance (Loschmidt);
- non-Gaussian P(W): *W-cumulant generating function*

$$\Delta G =?= \langle W\rangle - \langle \sigma^2_W\rangle/2k_B T$$

beyond standard *fluctuation-dissipation theorem.*



$$\frac{\Delta G}{k_B T} = -\ln\left\langle \exp\left(-\frac{W}{k_B T}\right)\right\rangle$$

Experimental verification

- Direct method: JE

FTs work well only for small systems!

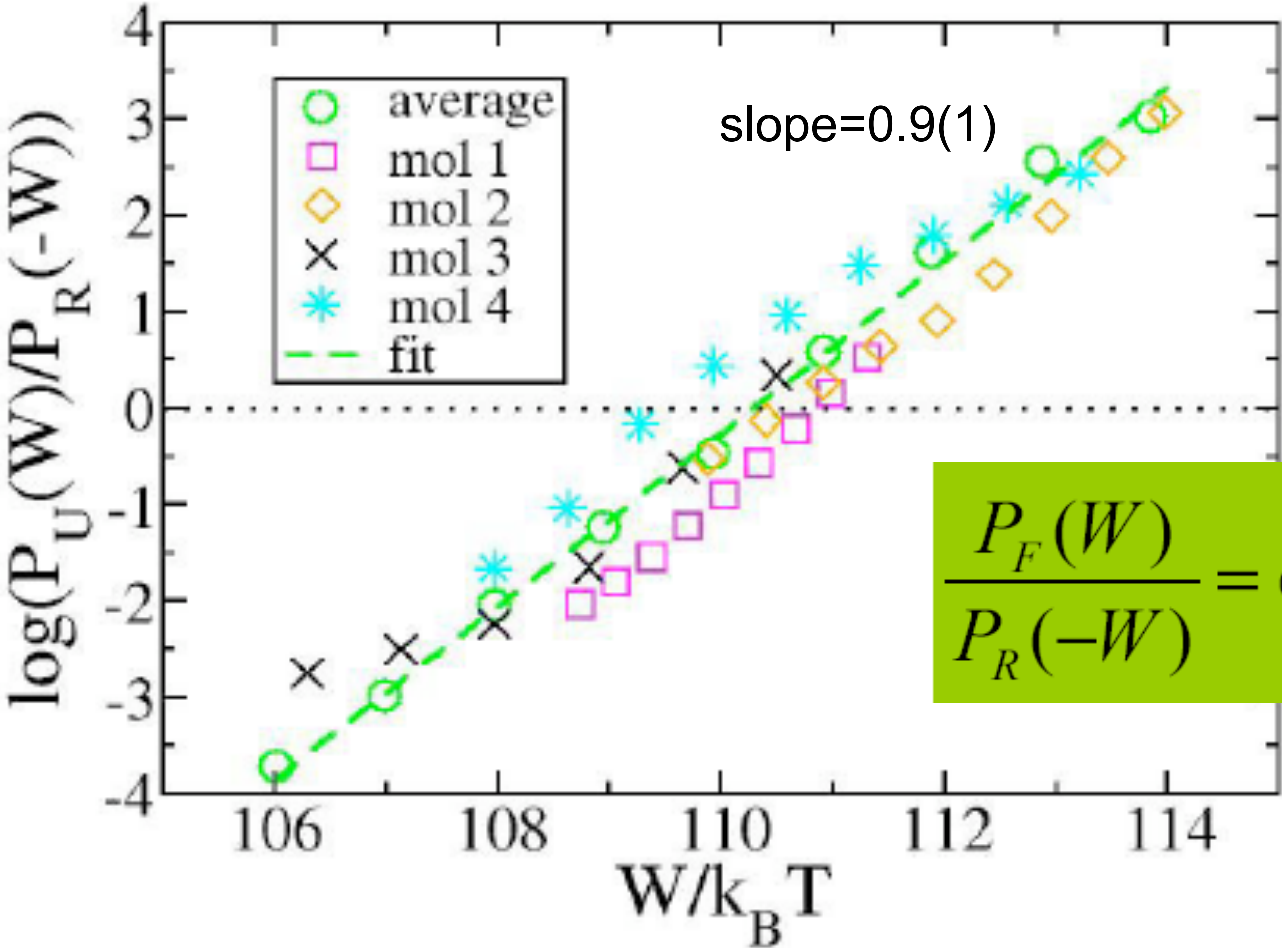
From the JE

$$\boxed{\Delta G = -k_B T \ln \left\langle \exp\left(-\frac{W}{k_B T} \right) \right\rangle_F}$$

… for a finite number of experiments this estimate is often biased

Fitted ΔG within a few $k_B T$ of best independent estimates

- Direct method: Crooks FT

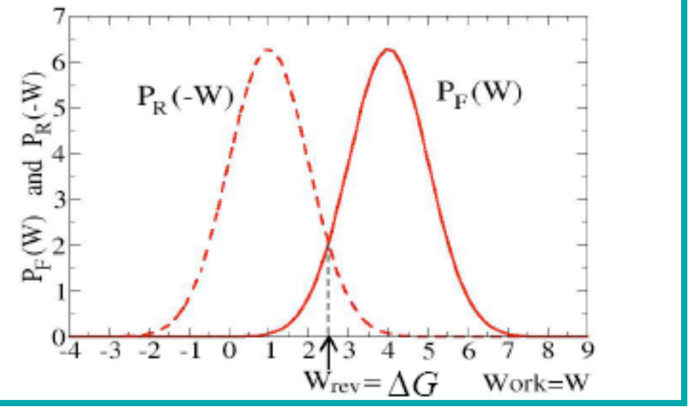


$$\frac{P_F(W)}{P_R(-W)} = \exp\left(\frac{W - \Delta G}{k_B T}\right)$$

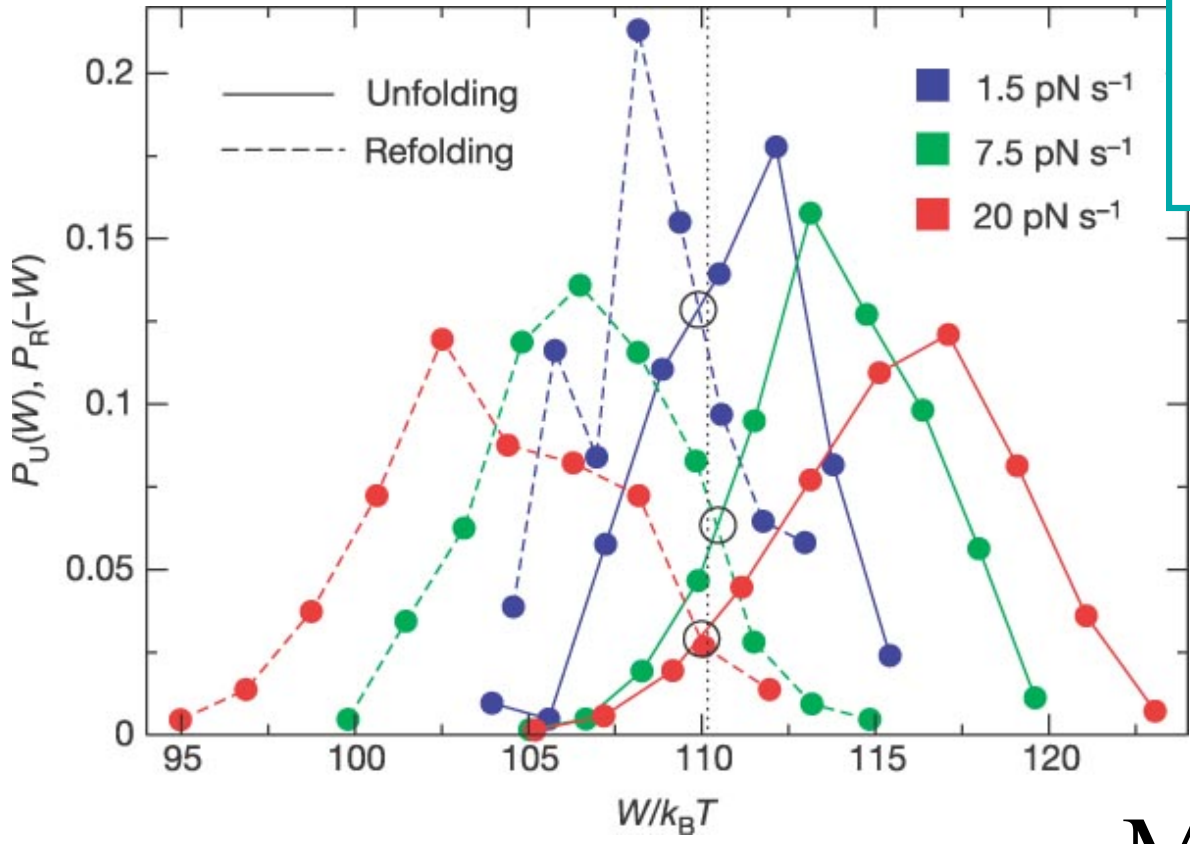
slope close to 1; x-intercept: $\Delta G^{(exp)} \approx 110\ k_BT$

- Crossing method

For $W = \Delta G \Rightarrow P_F(W) = P_R(-W)$



less systematic error



$$\text{M - fold} = 63.5 k_B T$$

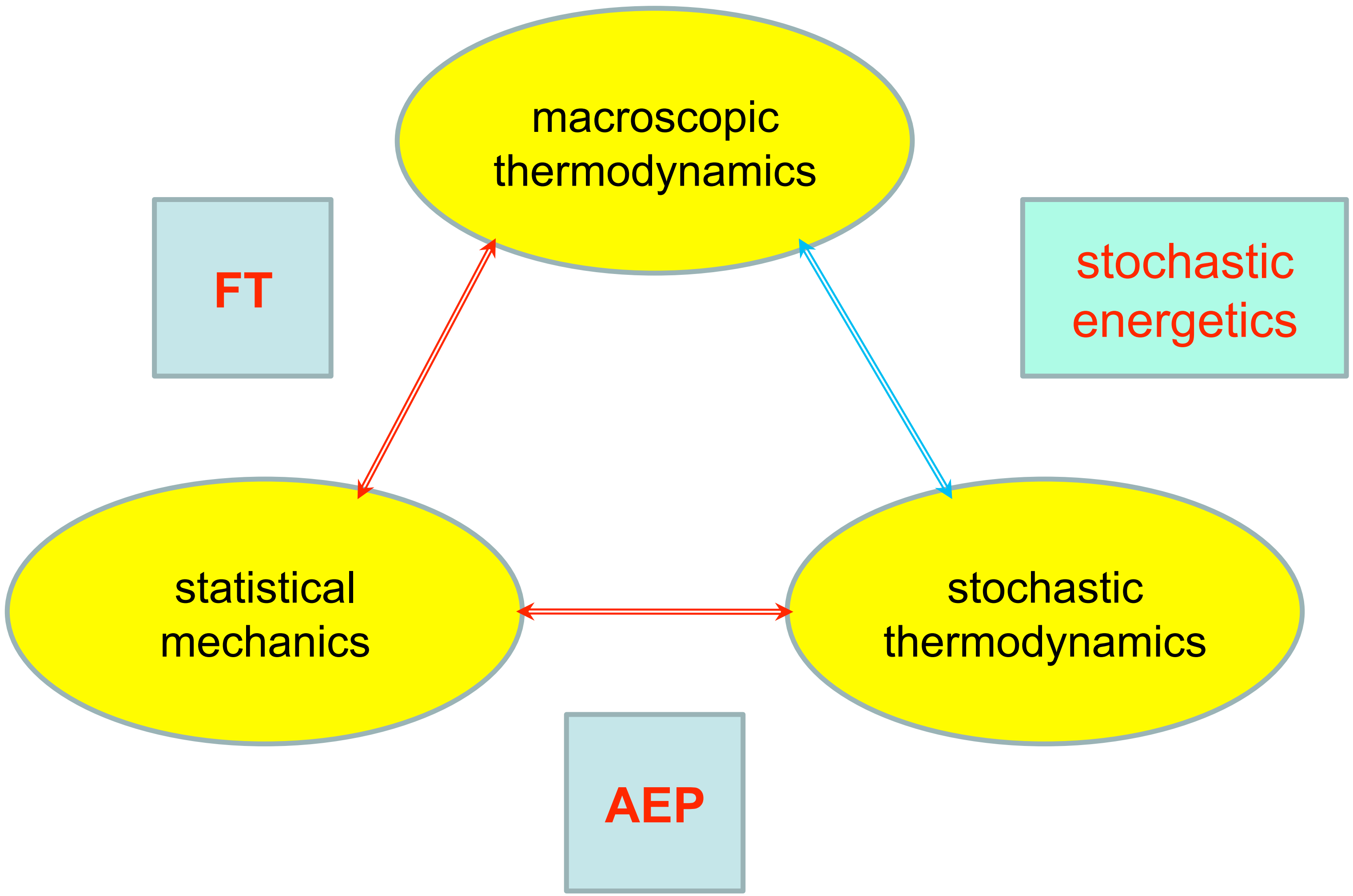
$$\Delta G^{(\exp)} = 110.3(5) k_B T \quad \Longrightarrow \quad \Delta G_0^{(\exp)} = 63 \pm 1 k_B T$$

after subtracting entropic handle stretching

difficulties

- experimental determination of small systems, control issues
- operative definition of work (inclusive vs exclusive)
- verification of FT equalities, statistics issues

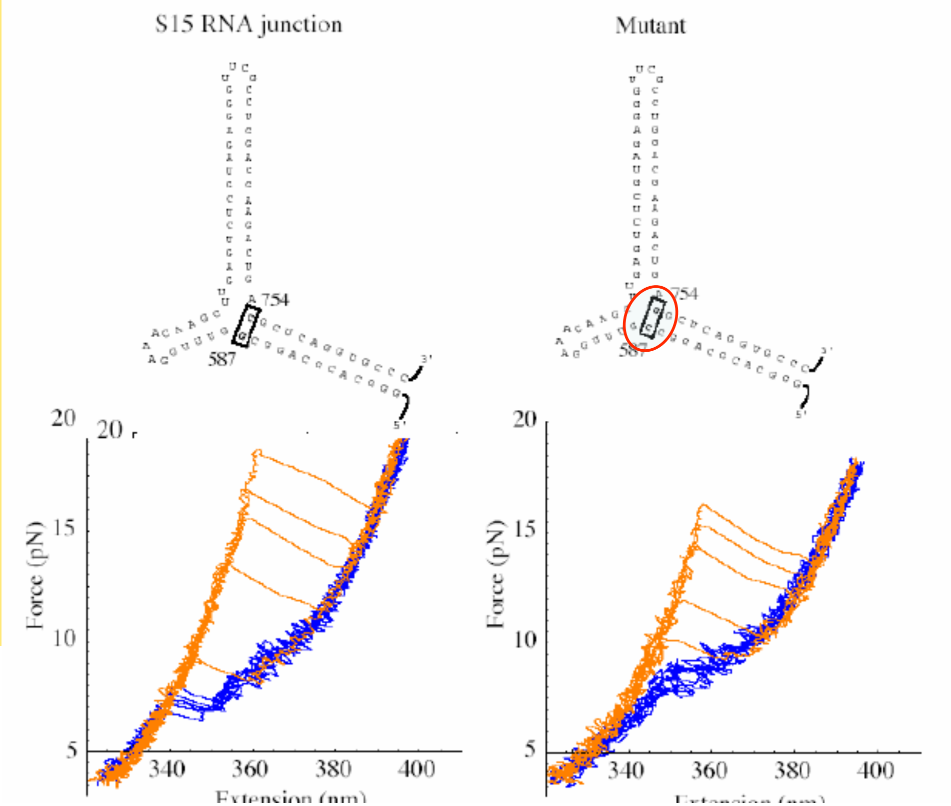
3 levels of description



<u>Conclusions</u>

- a <u>new</u> non-equilibrium thermodynamics of small systems
- more powerful tools to extract information from <u>single molecule</u> experiments
- application to <u>artificial</u> devices (possibly via *stochastic energetics*)

  Brownian Motors



References

Reviews:

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   T. Strick et al, *Physics Today, Oct. 2001, 46-51*
2. Introduction to fluctuation theorems
   C. Bustamante et al, *Physics Today, July. 2005, 43-49*
3. F. Ritort, *Séminaire Poincaré* **2**, 193 (2003),
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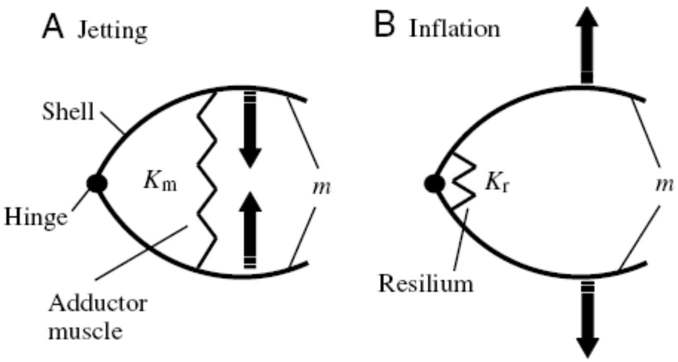
4. D. J. Evans, D. J. Searles, *Adv. Phys.* ***51****, 1529 (2002)*

Theorems:

- G. Gallavotti, E. G. D. Cohen, *Phys. Rev. Lett.* ***74****, 2694 (1995)*
- C. Jarzynski, *Phys. Rev. Lett.* ***78****, 2690 (1997)*
- G. E. Crooks, *Phys. Rev. E* ***60****, 2721 (1999)*
- U. Seifert, *Phys. Rev. E* ***95****, 040602 (2005)*

Brownian motors

from macro to micro scales



scallops, 10^{-2}m

shell flaps, jets

high Reynolds numbers

$R = av\rho/\eta \sim 100$

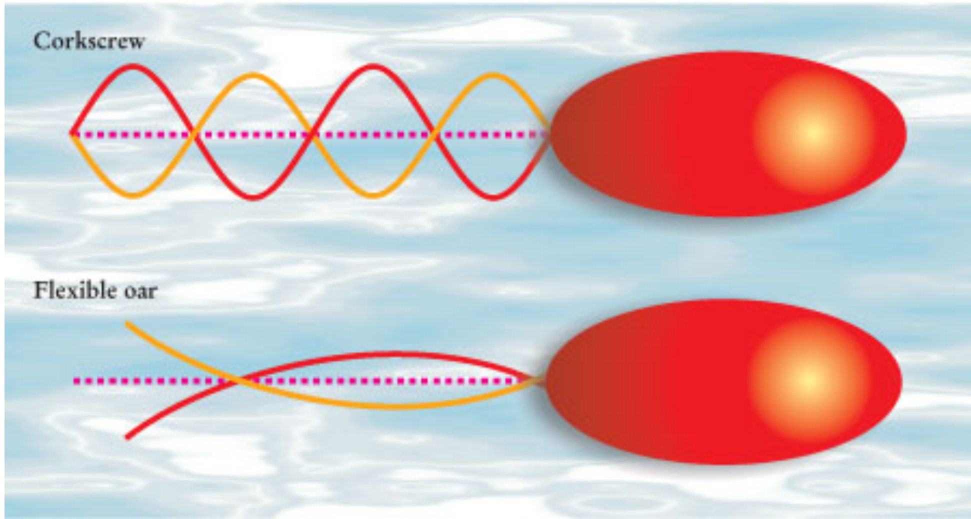
bacteria, 10^{-5}m

low Reynolds numbers $R \sim 10^{-4}$

flagellum strokes

corkscrew, $v \propto \omega$

flexible oar, $v \propto \omega^2$

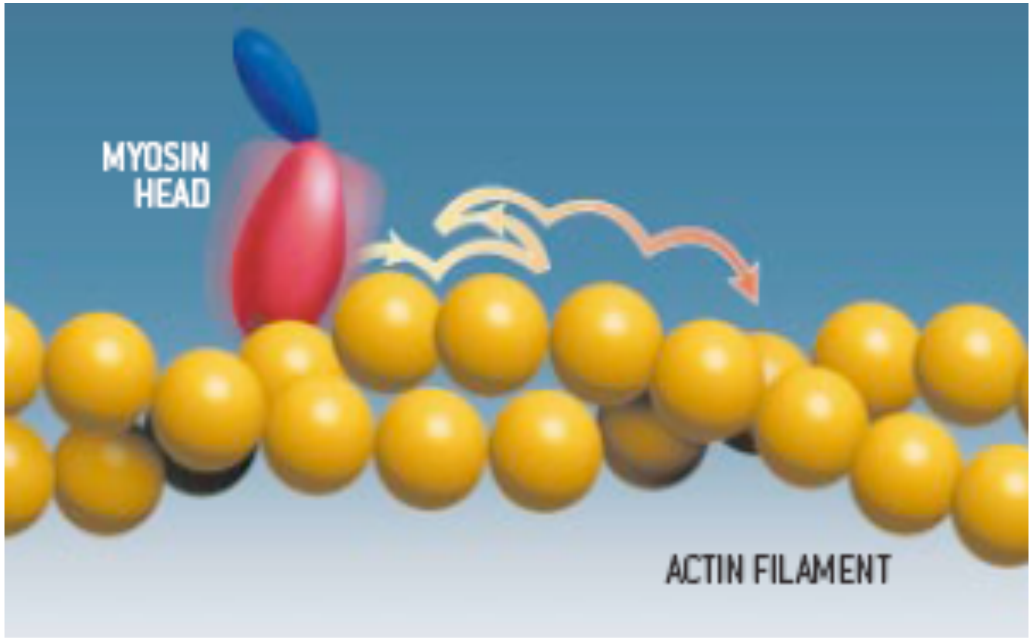


myosin, 10^{-8}m

power strokes: ATP hydrolysis, ATP$\rightarrow$ADP+20$k_B T$, efficiency ~50%; power from "fuel" comparable with power from/to environment

Brownian motion: time to diffuse a particle length is a^2/D, i.e. much shorter than the drift time a/v — $D=kT/6\pi\eta a$, v~4-5μm/s

not a deterministic engine, rather a directed random walker and still a very efficient motor!!
(Yanagida, 1999)



Proof outline:

1. <u>process protocol</u> $\lambda(t)$: $0 \rightarrow 1$ over a time t_f; fixed path in system parameter space; <u>heat bath disconnected</u> during evolution;

2. $H(z,\lambda(t)) \rightarrow H_\lambda(z)$ with $z \equiv (q,p)$;

3. $z_0 \equiv z(0) \rightarrow z \equiv z_\lambda(t)$ *deterministic* trajectory; $dW = \lambda'(t)\, \partial_\lambda H_\lambda(z) dt$
   $z_0 \in \rho_0(z_0) \equiv \mathbf{Z}_0^{-1} \exp[-\beta H_0] \rightarrow \rho(z, t) = \rho_0(z_0)$ $[\neq \rho_1(z)$ for $t=t_f]$
   (Liouville theorem);

4. $\langle \exp(-\beta W) \rangle = \int dz\, \rho(z, t_f)\, \exp[-\beta \int_0^{t_f} \lambda'(t)\, \partial_\lambda H_\lambda(z)\, dt] =$
   $= \int dz\, \rho_0(z_0)\, \exp[-\beta(H_1 - H_0)] =$
   $= \mathbf{Z}_1\, \mathbf{Z}_0^{-1} =$
   $= \exp(-\beta \Delta F)$

5. Now add <u>heat bath:</u> $H(z,z_r) = H(z) + H_{res}(z_r) + h_{int}(z,z_r)$ and assume $h_{int}(z,z_r)$ <u>small</u>

After subtracting the contribution arising from the entropy loss due to the stretching of the molecular handles, ΔG^{handle} = 23.8 kcal/mol, and of the extended single-stranded RNA, ΔG^{ssRNA} = 23.7±1 kcal/mol, we obtain ΔG_0 = 37.2 ±1 kcal/mol (at 25°C, in 100 mM Tris-HCl, pH 8.1, 1 mM EDTA), in excellent agreement with the result obtained using the Visual OMP by DNA Software, Inc. ΔG_0 = 38 kcal/mol (at 25° C, in 100 mM NaCl).

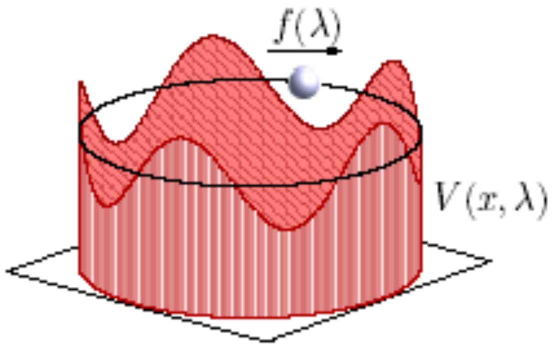
Genetic Computer Group (or Wisconsin) package

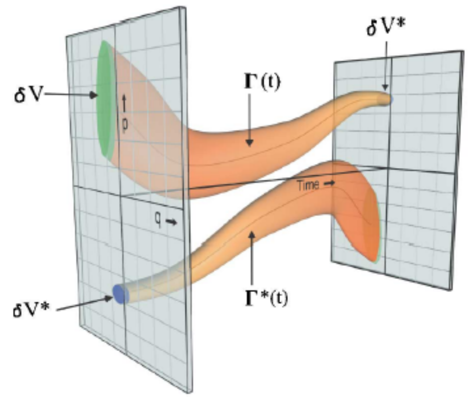
1 $k_B T$ ~ 0.6 kcal/mol at 25°C

Experimental tests: Gallavotti Cohen FT

- In <u>colloids</u>: e.g., dragging micro-particles through water (Evans, 2004)

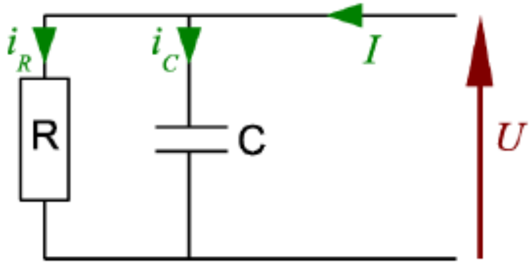
plastic bead
in an optical trap
at <u>constant v</u>

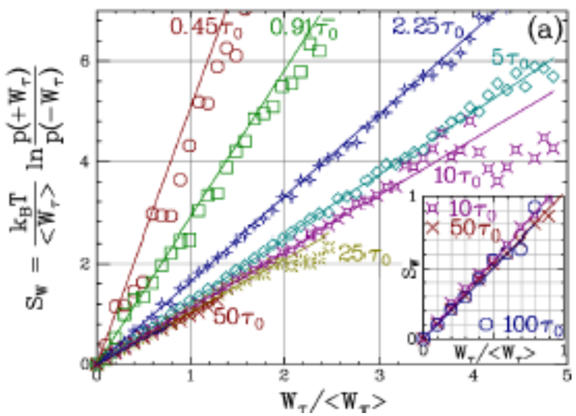




- In <u>electrical circuits</u>: more controllable dissipative system (Ciliberto, 2005)

pumped circuit:
fewer biases,
more trajectories





Fluctuation th. #1: Gallavotti-Cohen (`95)

FD for steady-state systems (SSS):

- time-reversal invariant SSS
- an external agent continuously produces heat by acting on the system; heat gets transferred to the bath. Sure, $\langle S \rangle = \langle Q \rangle / T > 0$, average total entropy increase of system+bath in a time interval t
- system entropy production (rate): $\sigma = Q/Tt$ from system → bath, trajectory dependent (fluctuates!) with t-dependent p.d. $P_t(\sigma)$

$$\lim_{t\to\infty} \frac{k_B}{t} \ln\left(\frac{P_t(\sigma)}{P_t(-\sigma)}\right) = \sigma.$$

SSS are more likely to deliver a certain amount of heat to the bath, $\sigma > 0$, than to absorb it from the bath, $\sigma < 0$.

II law of thermodynamics recovered for macro-systems: for $\sigma \to \infty$

$$P_t(\sigma)/P_t(-\sigma) \to \infty$$

heat absorption becomes insignificant!

- $\mathbf{t} \to \infty$ 'means' t >> all relaxation time scales in the system;
- molecular motors can move by rectifying thermal fluctuations (ratchets), while producing heat in average;
- Loschmidt vs Boltzmann (1876)

Q: if the microscopic law of mechanics are invariant under time-reversal, how can you rule out entropy decreasing evolutions that violate the II law?
A: time-reversed trajectories do occur, but they get vanishingly rare with system size.

Fluctuation th. #2: Jarzynski equality (`97)

- n.e. process with protocol **X(t): $X_A \to X_B$**
  X control parameter; $X(0) = X_A$ initial equilibrium state;
- [system in contact with heat bath at temperature T];
- $X(t_f) = X_B$ n.e. final state; equilibration follows for $t \to \infty$ with $X(t > t_f) = X_B$

J.E.

$$\boxed{\exp\left(-\frac{\Delta G}{k_B T}\right) = \left\langle \exp\left(-\frac{W}{k_B T}\right)\right\rangle,}$$

ΔG free-energy difference between equilibrium states $\mathbf{X_A}$, $\mathbf{X_B}$;

$\langle \dots \rangle$ average over repeated realizations of the same protocol **X(t): $X_A \to X_B$**