A TWO STATE KINETIC MODEL FOR THE UNFOLDING OF SINGLE MOLECULES BY MECHANICAL FORCE

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KITP, May 2003

Outline

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3. Transient violations of the second law
4. Pulling RNA hairpins: the experiment
5. Pulling RNA hairpins: the model
6. Fitting the experiment
7. Conclusions

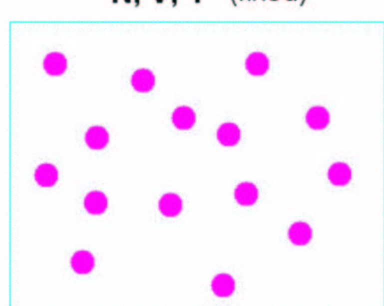
1.- INTRODUCTION

- Biological processes: E of order KT (pN nm)
- Thermal fluctuations are important
- Life processes are off-equilibrium
- Interplay of small systems and large timescales
- Importance of Non-Gaussian fluctuations as rare events

2.- A SHORT REMINDER

•Consider a gas enclosed in a vessel of fixed volume V , fixed number of particles N and in contact with a thermal bath at temperature T (so the energy E fluctuates)

N, V, T (fixed)



E fluctuate

- Imagine a transformation V to V'=V+ΔV

The entropy S(V,N,T) goes to S'(V',T,N)

Second law : $T\Delta S \geq \Delta Q$

$$\Delta W_{diss} = T\Delta S - \Delta Q \quad \text{(entropy production)}$$

In a reversible process : $\Delta W_{diss} = 0$

In the previous example:

$$\Delta E = \Delta Q + \Delta W \qquad (\Delta W = \int P dV)$$

(ΔQ: disordered; ΔW: ordered)

$$\Delta F = \Delta E - T\Delta S = \Delta Q + \Delta W - T\Delta S$$

$$\Downarrow$$

$$\Delta F = \Delta W - \Delta W_{diss} = \Delta W_{rev}$$

$$\Delta W \geq \Delta F = \Delta W_{rev}$$

OR

$$\Delta W_{diss} \geq 0$$

3.-VIOLATIONS OF THE SECOND LAW

Can the second law be violated?

YES

“Transiently” for times short or for system sizes small enough

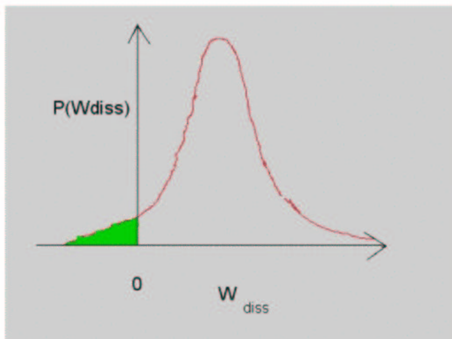
YET, THERE ARE VIOLATIONS FOR LARGE TIMES AND SMALL SIZES
SO THEY CAN BE EXPERIMENTALLY OBSERVED IN THE NANOSCALE

The fluctuation theorem measures such violations

(Cohen,Evans and Morriss 1993, Gallavotti and Cohen 1995)

A simpler form of the theorem has been introduced by Jarzynski (1997)

$$< e^{-\frac{W_{diss}}{KT}} > = 1$$

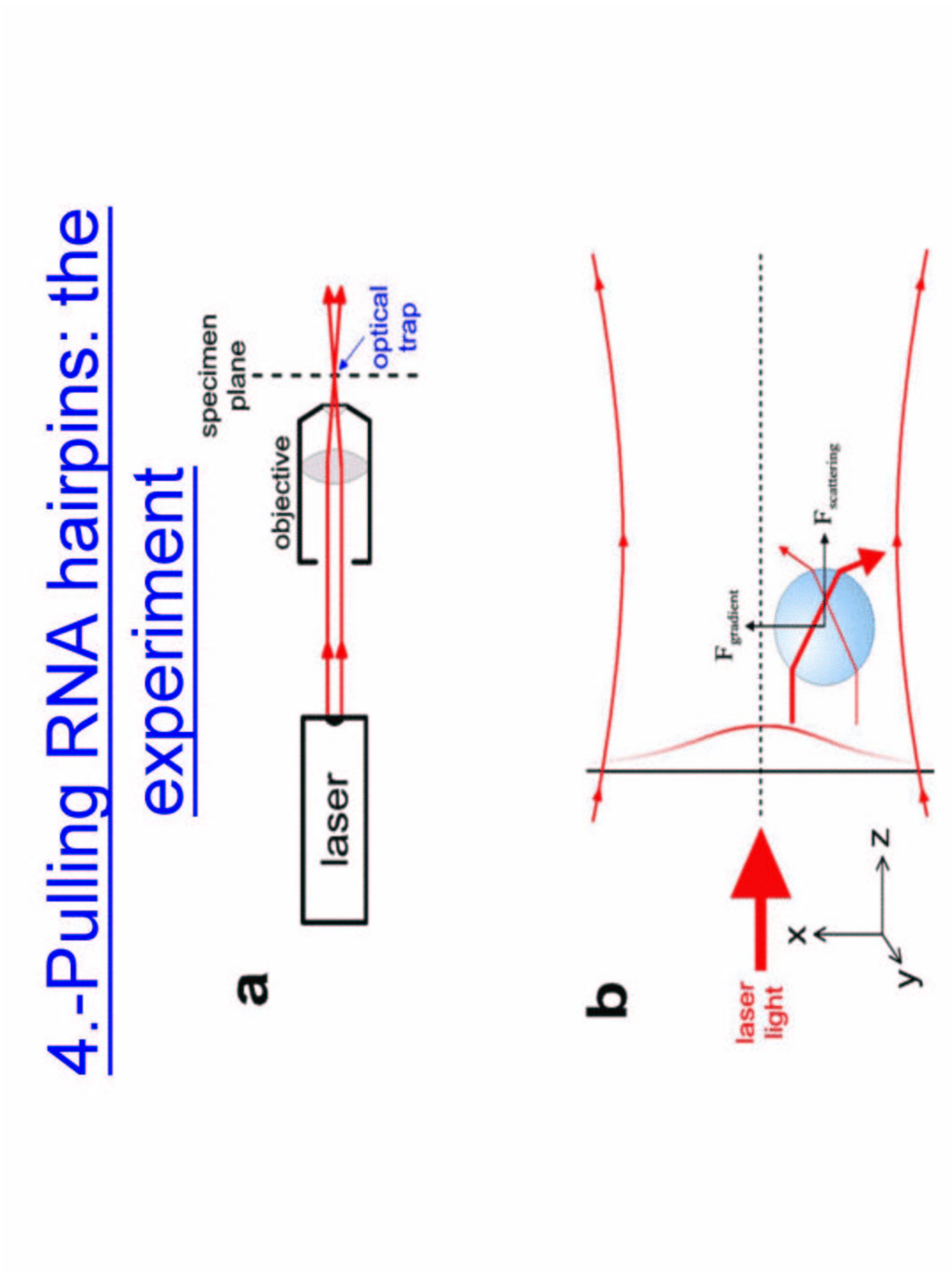


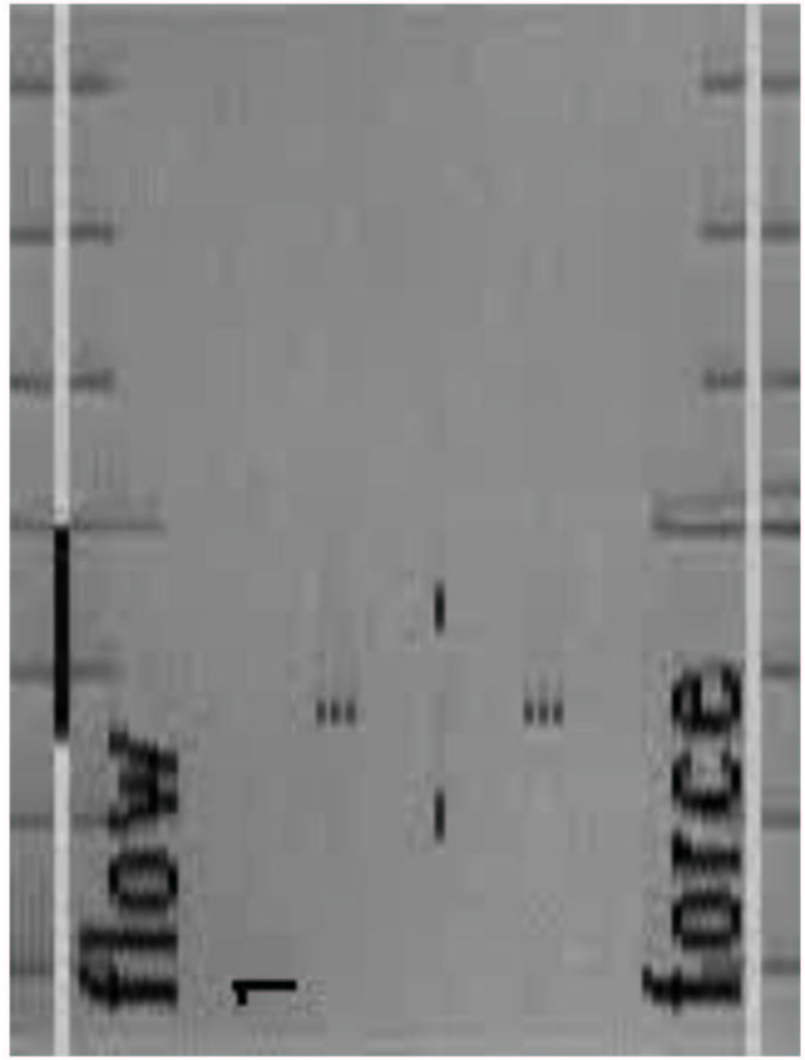
$$1 = < e^{-\frac{W_{diss}}{KT}} > = P_+ < e^{-\frac{W_{diss}}{KT}} >_+ + P_- < e^{-\frac{W_{diss}}{KT}} >_-$$

$$\text{with} \quad P_+ + P_- = 1$$

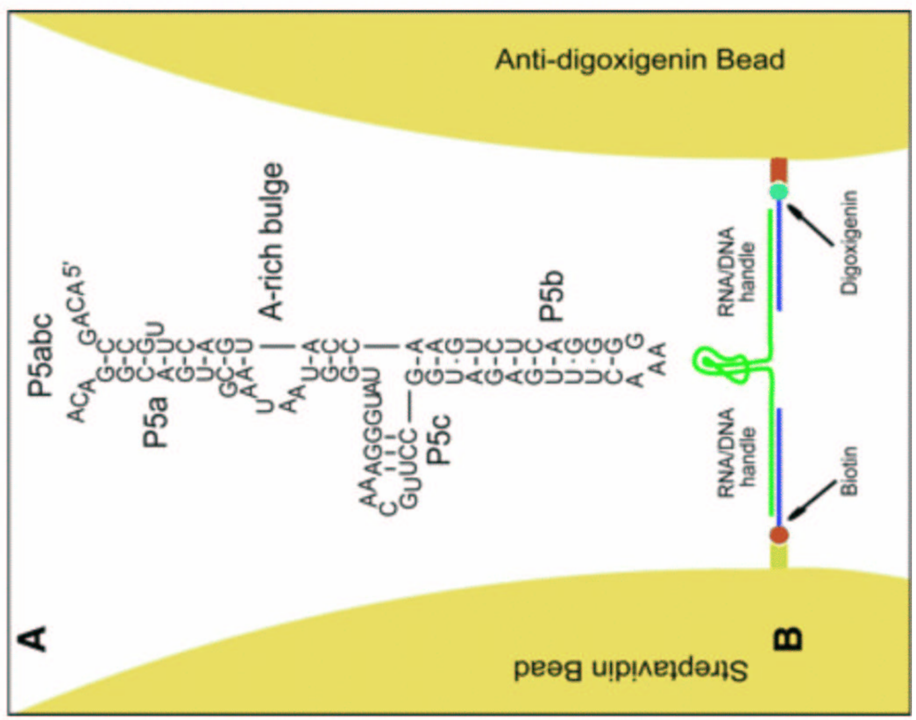
$$P_+ \approx 1 - e^{-O(V)} \quad \text{and} \quad P_- \approx e^{-O(V)}$$

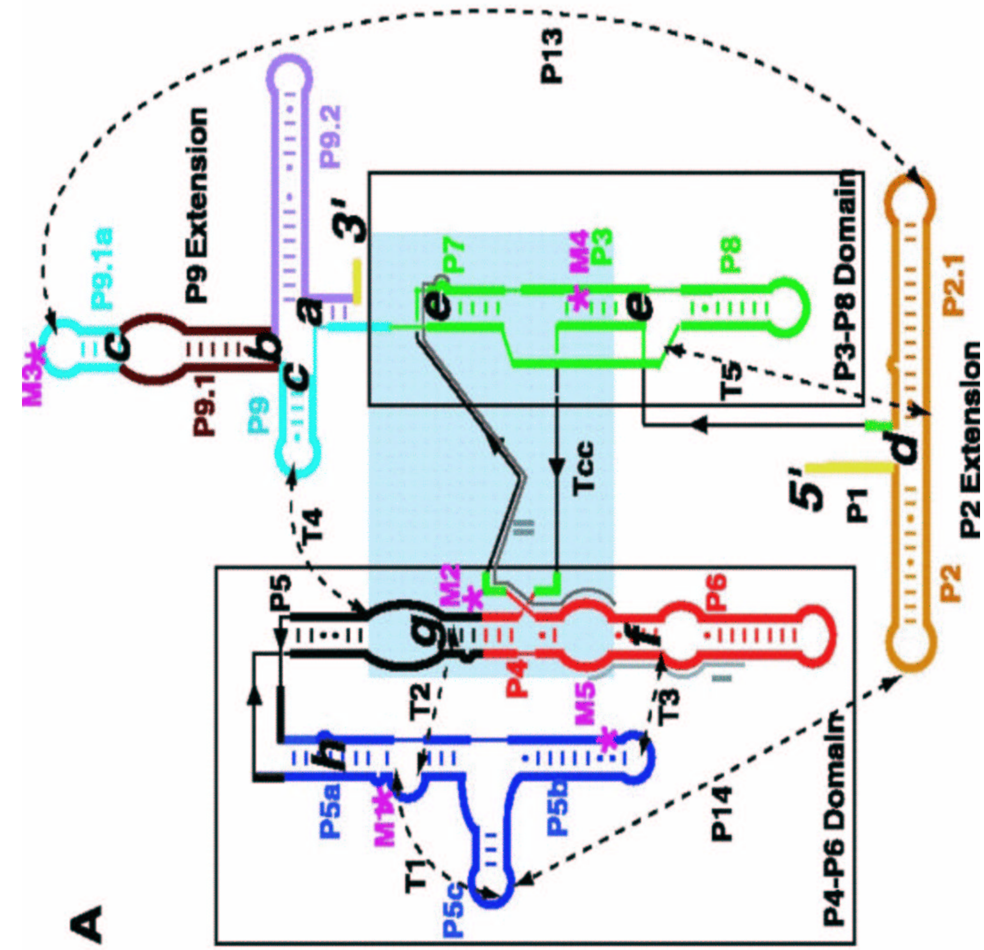
Violations are suppressed exponentially with the system size

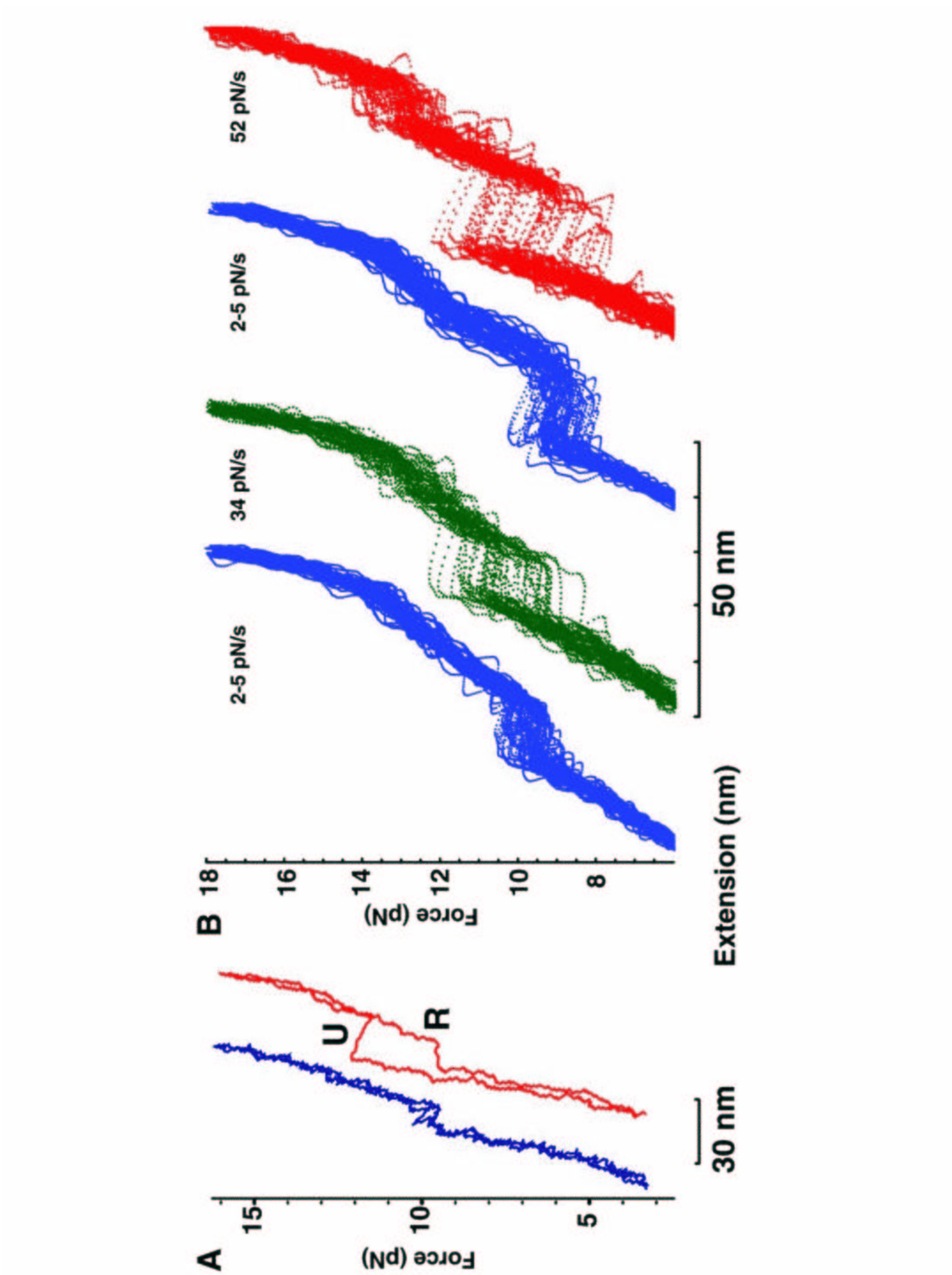

4.-Pulling RNA hairpins: the experiment
a
laser
objective
specimen plane
optical trap
b
laser light
F gradient
F scattering
x
y
z

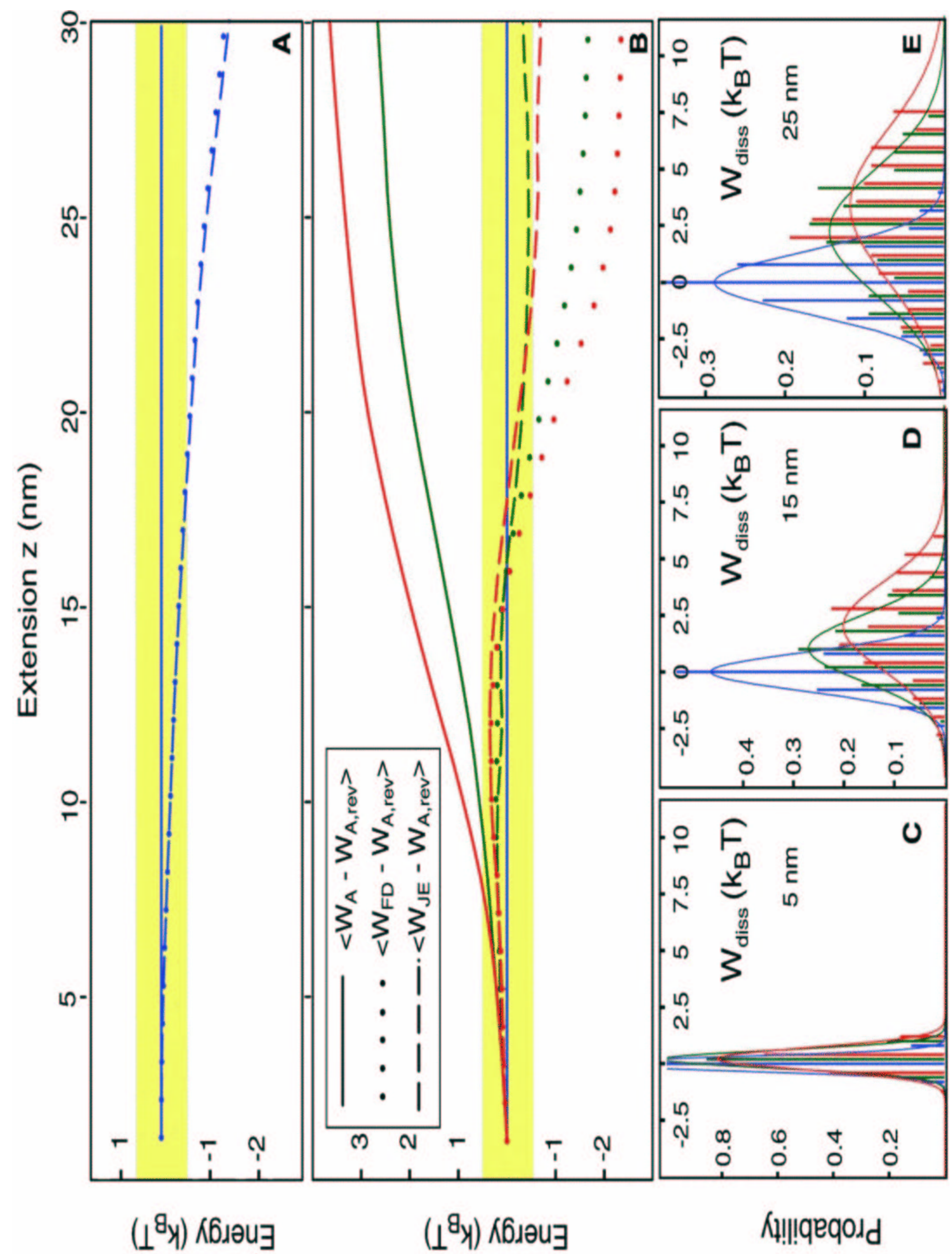

flow
force

J. Liphardt,S. Dumont,S. B. Smith, I. Tinoco and C.Bustamante,Science, 296 (2002)1832





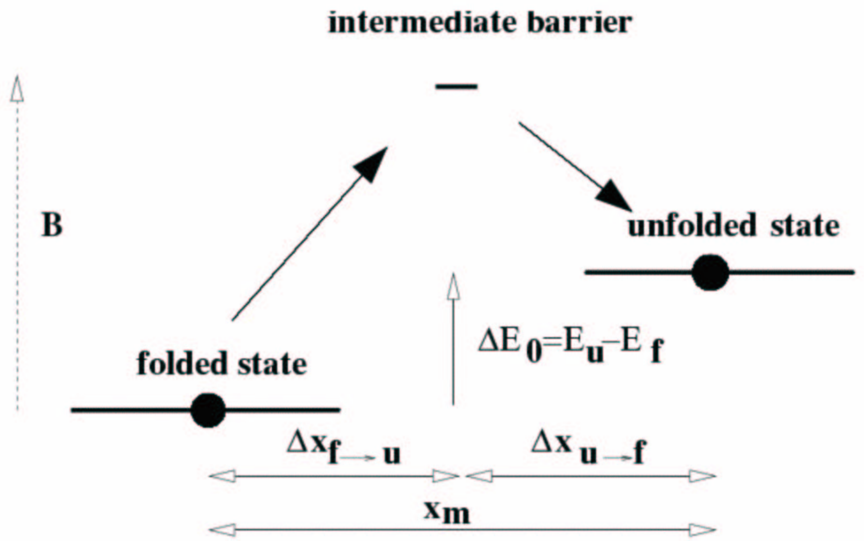

A
Force (pN)
U
R
30 nm
B
Force (pN)
2-5 pN/s
34 pN/s
2-5 pN/s
52 pN/s
50 nm
Extension (nm)


Extension z (nm)
Energy (k_BT)
A
B
<W_A - W_A,rev>
<W_FD - W_A,rev>
<W_JE - W_A,rev>
Probability
W_diss (k_BT)
5 nm
15 nm
25 nm
C
D
E

5.-Pulling RNA hairpins: the model

F. Ritort, C. Bustamante and I. Tinoco Jr, PNAS, 99 (2002) 13544

Two-level system separated by an intermediate barrier
(Experimental evidence for hopping, Liphardt et al. Science,292 (2001) 1733)



Kinetics of the barrier under force:
(G.I.Bell 1978, E.Evans and K.Richtie 1997)

$$k_{f\to u} = k_m k_0 e^{-\beta(B - F\Delta x_{f\to u})}$$

$$k_{u\to f} = k_m k_0 e^{-\beta(B - \Delta E_0 + F\Delta x_{u\to f})}$$

$k_m \;\rightarrow\;$ handle and machine contributions

$k_0 \;\rightarrow\;$ microscopic attempt frequency

$$\sigma = 0\,(\text{folded}) \qquad \sigma = 1\,(\text{unfolded})$$

$$E(F,\sigma) = E_f + (\Delta E_0 - F x_m)\sigma$$

Discretized time : $\{t_k; k = 0,1,2,\ldots,M\}$ $t_0 = 0$ $t_M = t$

Perturbation protocol : $\{F_k; k = 0,1,2,\ldots,M\}$ $F_0 = 0$ $F_M = F$

Dynamical path : $\{\sigma_k; k = 0,1,2,\ldots,M\}$ $\sigma_0 = 0$ $\sigma_M = 1$

and apply the Crooks (1998) prescription

$$W(\{\sigma_\kappa\}) = -x_m \sum_{k=0}^{M-1} (F_{k+1} - F_k)\sigma_{k+1}$$

Main purpose: Compute the P(W) over all paths

$$P(W) = \sum_{\{\sigma_k\}} \delta(W - W(\{\sigma_k\}))$$

Path integral usually very difficult to compute. We content ourselves with the first two moments:

$$\overline{W_{diss}} \quad \text{and} \quad \Delta^2 = \overline{W_{diss}^2} - (\overline{W_{diss}})^2$$

Moments of the distribution P(W)

$$\overline{W_{diss}} = -x_m \int_0^{F_m} dF' \int_0^{F'} dF'' \frac{\partial \mathrm{b}(F'')}{\partial F''} g(F',F'')$$

$$\overline{W^2{}_{diss}} - \overline{W_{diss}}^2 = 2x_m^2 \int_0^{F_m} dF' \int_0^{F'} dF'' \mathrm{d}(F'')\,(1-\mathrm{d}(F''))\, g(F',F'')$$

$$d(F) = a(F) + \int_0^{F} dF' \frac{\partial \mathrm{b}(F')}{\partial F'} g(F,F') \;;\quad \mathrm{a}(F) = \frac{k_{f\to u}(F)}{k_t(F)}$$

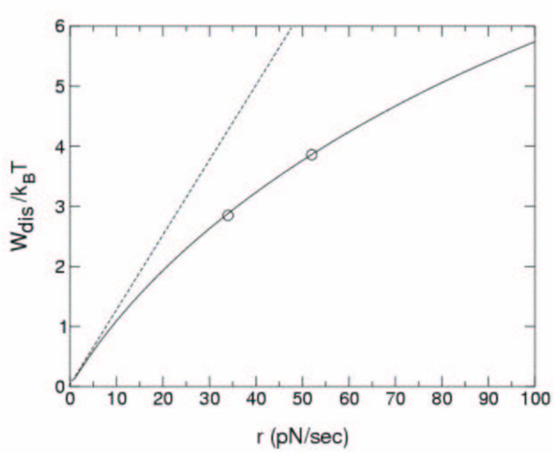
$$g(F,F') = \exp\left(-\int_0^{F'} dF'' \frac{\partial \mathrm{b}(F'')}{\partial F''}\right) \;;\qquad \mathrm{b}(F) = \frac{k_{u\to f}(F)}{k_t(F)}$$

6.-Fitting the experiment

♣ Linear response regime

$$\overline{W_{diss}} = \frac{r}{F_t k_t(F_t)} \Delta G_0 + O(r^2)$$

$$k_t(F) = k_{f\to u}(F) + k_{u\to f}(F)$$



As best fitting values to the experimental data we obtain:

$$k_t(F_t) = 20 \pm 5\,\mathrm{Hz}$$
$$x_m = 10 \pm 2\,\mathrm{nm}$$
$$\Delta x_{f\to u} = 1.6 \pm 0.4\,\mathrm{nm}$$
$$\Delta G_0 = 25\,\mathrm{k_B T}$$

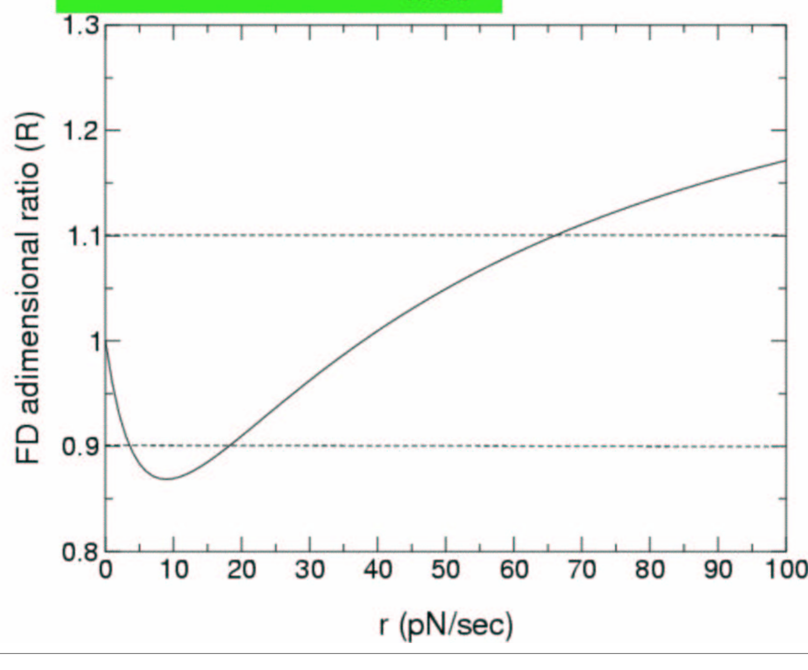
To compare with:

$$k_t(F_t)_{\exp} \cong 10 \pm 5\,\mathrm{Hz}$$
$$(x_m)_{\exp} \cong 10\,\mathrm{nm}$$
$$(\Delta x_{f\to u})_{\exp} = ?\,\mathrm{nm}$$
$$(\Delta G_0)_{\exp} \cong 25\,\mathrm{KT}$$

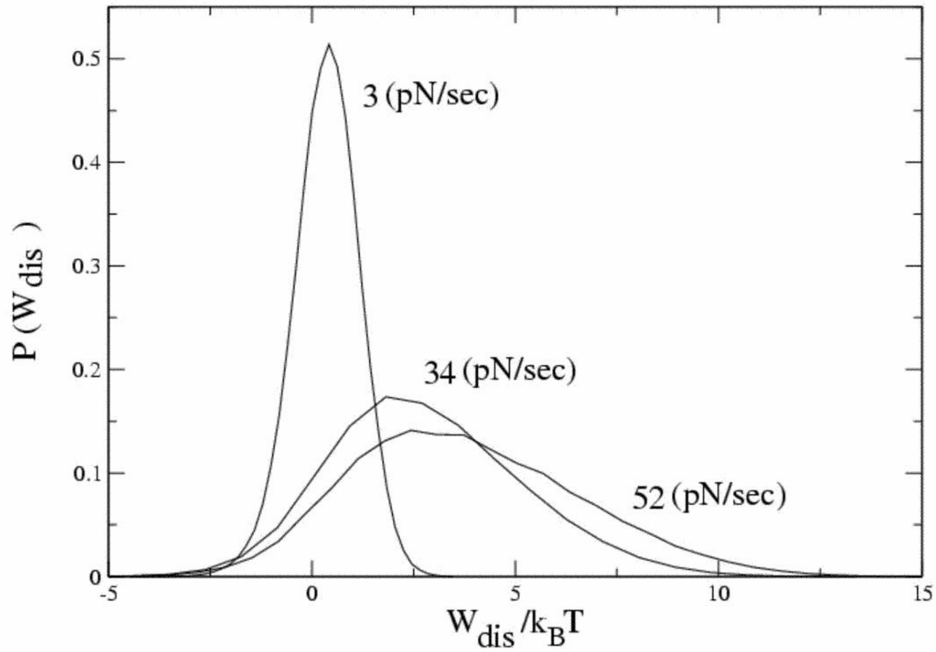
♣ Fluctuation-dissipation ratio (R)

$$R = \frac{\Delta^2}{2KTW_{diss}}$$

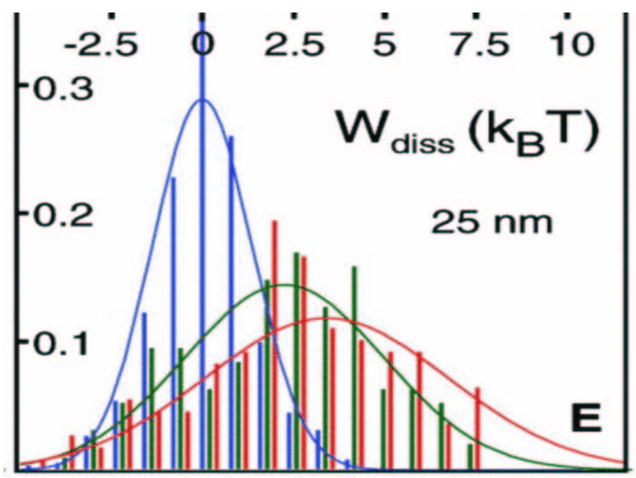
(R=1 at equilibrium)

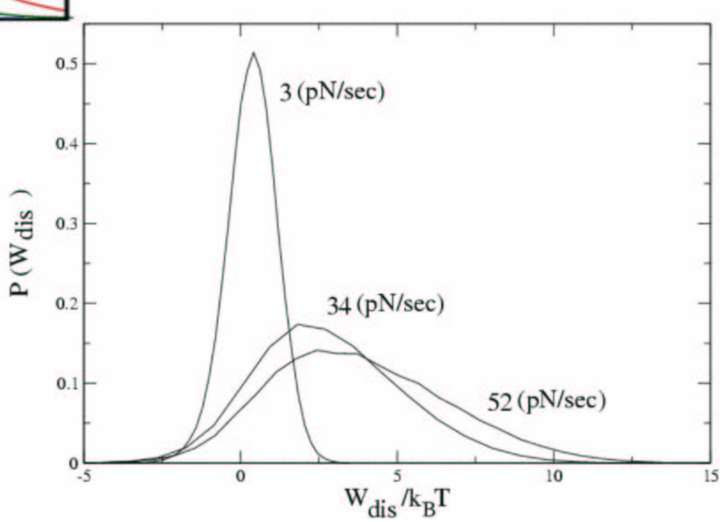


♣P(W) slightly deviates from Gaussian



Pulling rates: 3. 34, 52 (pN/sec)





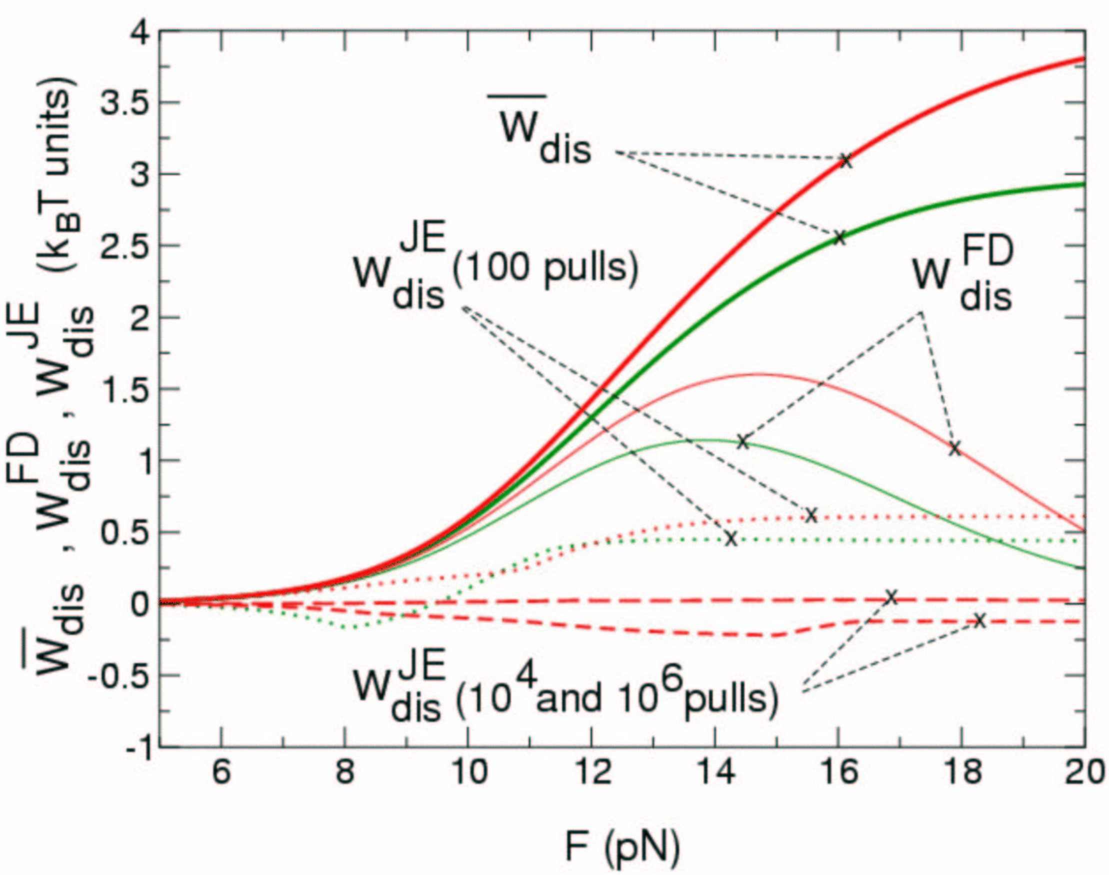
How can we recover equilibrium data from non-equilibrium ?

There are three ways to estimate the free-energy change

$$\Delta G_{diss} = \overline{W}$$

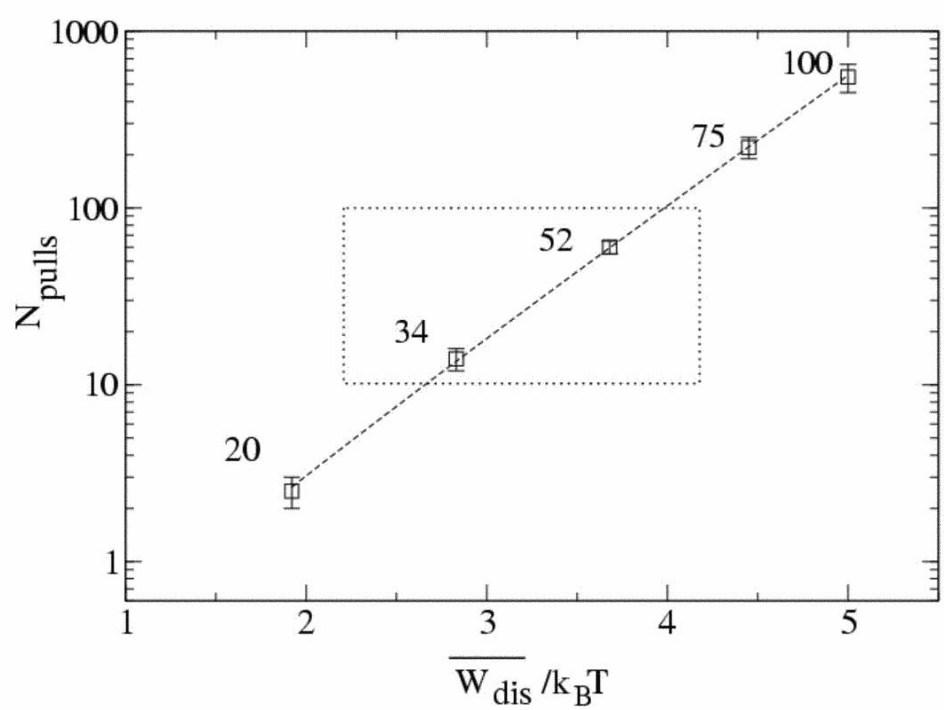
$$\Delta G_{FD} = \overline{W} - \frac{\Delta^2}{2KT}$$

$$\Delta G_{JARZYNSKI} = -KT\log(\overline{e^{-W/KT}})$$



Required number of pulls to estimate ΔG_0 within 1kT ?

$$N_{pulls} \propto e^{R_{-}\frac{\overline{W_{diss}}}{k_B T}}$$



7.- CONCLUSIONS

- KINETICS: $\overline{W_{diss}}(r)$ gives information about the kinetics of the rate limiting step in the denaturation under force. This is specially important for molecules where the unfolding cannot be done reversibly.

$$\overline{W_{diss}} = \frac{r}{F_t k_t(F_t)} \Delta G_0 + O(r^2)$$

$$R \approx 1$$

- STATICS: The required number of pulls for the Jarzynski average to estimate the free energy within 1kT grows exponentially fast with the dissipated work.

$$N_{pulls} \propto e^{R_- \frac{\overline{W_{diss}}}{k_B T}}$$

OPEN QUESTIONS

1.-Theoretical study of other more complex molecules such as multidomain proteins or RNA (kissing loops, pseudoknots, RNA junctions....)

2.- More detailed study of the Jarzynski identity: dependence of the number of pulls with the free energy estimate

3.-Ensemble of two level systems: finite-size effects (large volume limit)

Acknowledgements

- Collaborators: C. Bustamante and I.Tinoco
  (University of California in Berkeley)
- Stimulating discussions: Bustamante Lab
  - Delphine Collin
  - Jeff Gore
  - Jan Liphardt
  - Bibiana Onoa
  - Steve Smith
- Barcelona group:
  - Adan Garriga
  - Maria Mañosas
  - Sigrid Jorgensen
  - Raimon Sunyer