From DNA opening to DNA sequence

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Overview

1. Introduction :

- *reminder on the DNA molecule and its opening*
- *micro-manipulation experiments*
- *unzipping of DNA/RNA at fixed force or extension*
- *modeling of the opening for a given sequence: relationship with random walks in random media*

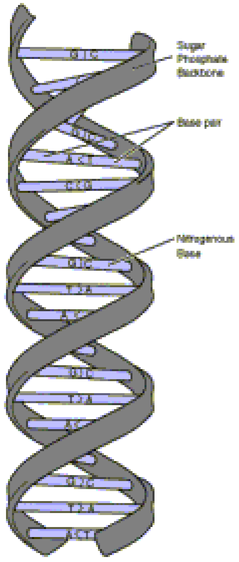
2. « Dynamical » inference

3. « Static » inference and perspectives

DNA sequence

DNA = double helix of 2 complementary strands A/T, G/C

3’ 5’

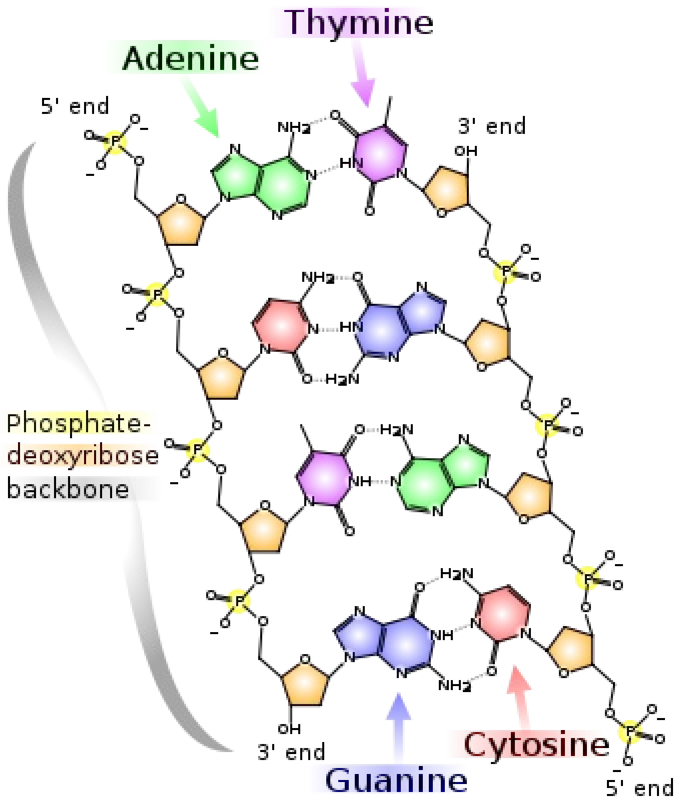


5’ 3’

5’---- ATGTGGTTAG --- 3’

3’---- TACACCAATC --- 5’

sequence = genetic information

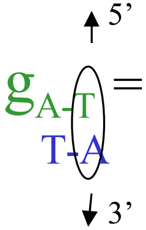


Base pairing and stacking (free) energies

Typical energy: $1\ k_BT \approx 4\ \mathrm{pN} \times \mathrm{nm}$ (at temperature T=25 °C)

H-bonds: $g_{A\text{-}T} \approx 1\ k_BT$ $g_{C\text{-}G} \approx 3\text{-}4\ k_BT$

Stacking:



$$g_{\substack{A\text{-}T\\T\text{-}A}} = 1.06\ k_BT$$

$$g_{\substack{A\text{-}T\\A\text{-}T}} = 1.78\ k_BT$$

$g(b_n,b_{n+1})$

b_{n+1}

b_n

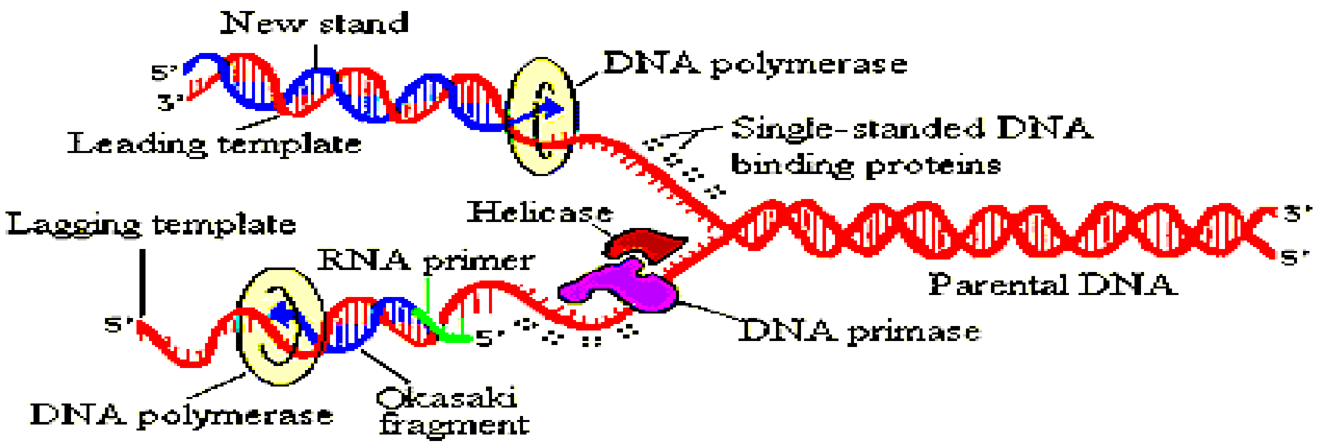
	A	T	C	G
A	1.78	1.55	2.52	2.22
T	1.06	1.78	2.28	2.54
C	2.54	2.22	3.14	3.85
G	2.28	2.52	3.90	3.14

Only 10 different values

at room temperature and standard solution conditions
(150 mM Na Cl, neutral pH)

Strand separation

In vivo opening : replication, transcription, …



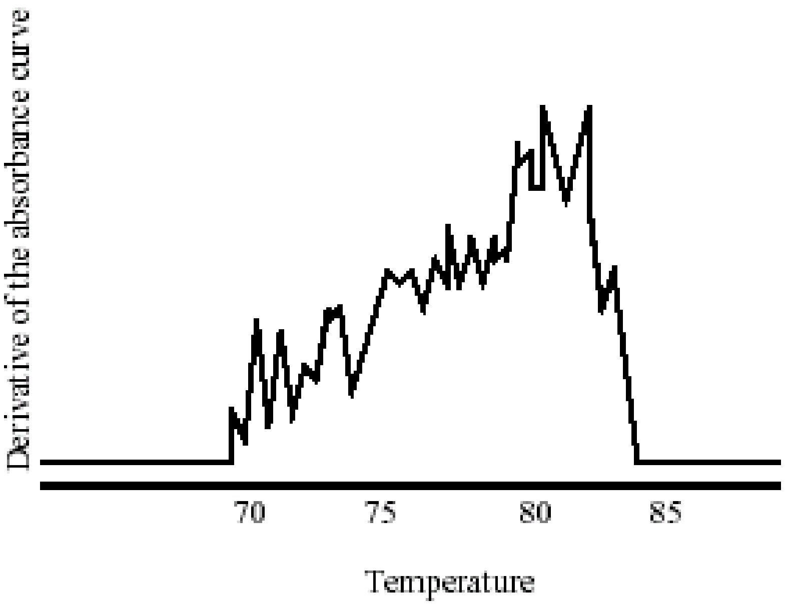
In vitro opening:

- in a bulk : thermal denaturation
- single molecule experiments

Nanopore unzipping
DNA digestion by an Exonuclease
Force Unzipping

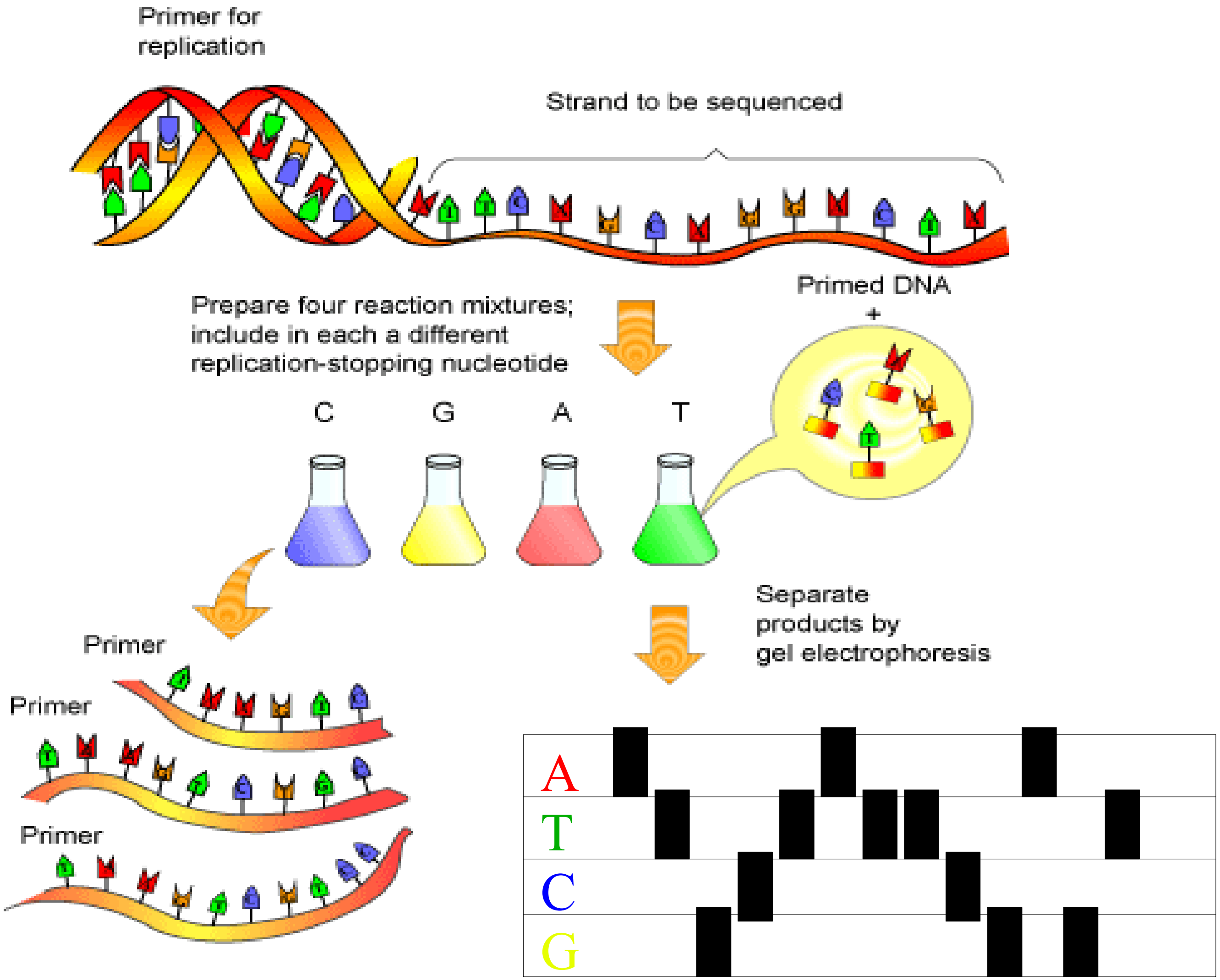
Thermal denaturation

Repeated sequences denaturate at a well defined temperature T_c (important for DNA amplification through Polymerase-Chain Reaction).
By synthetizing all 10 possible repeated sequences and measuring T_c the base pair free energies g have been evaluated.
Conversely, from T_c and the table of g, one could extract the sequence …

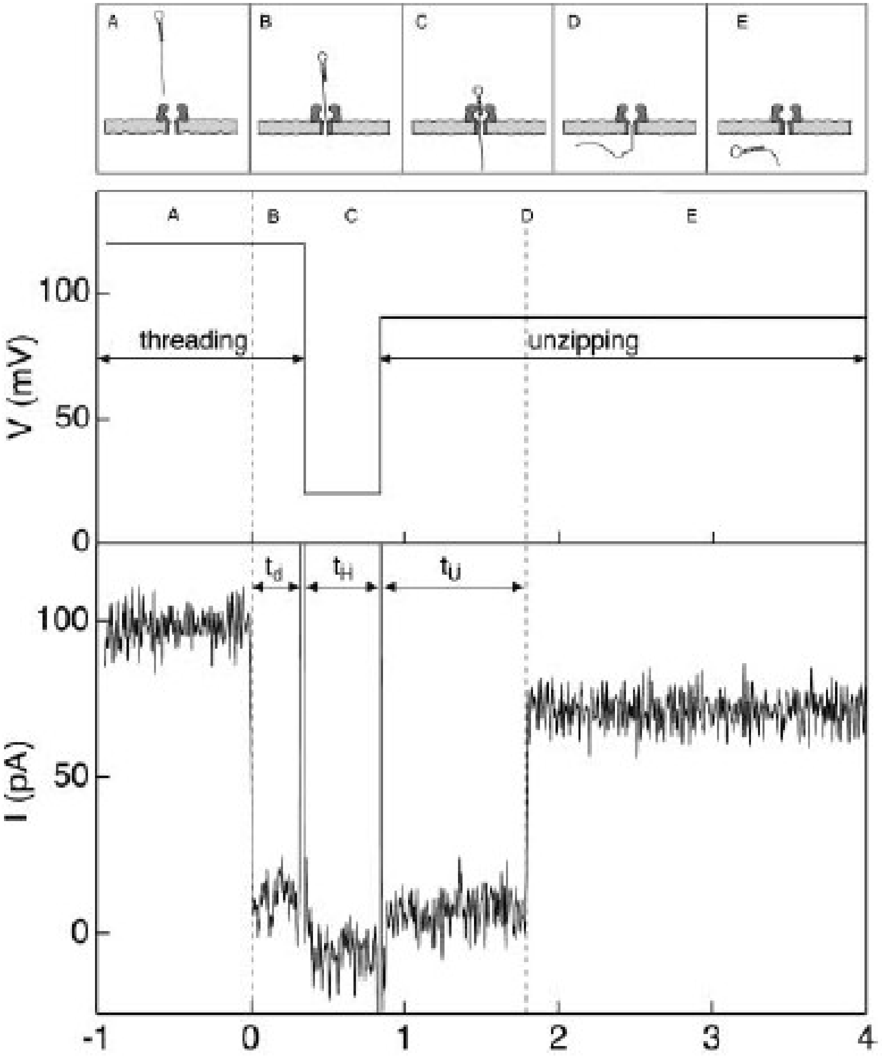


For heterogeneous sequences, AT vs GC rich region but where?

Methods for DNA sequencing



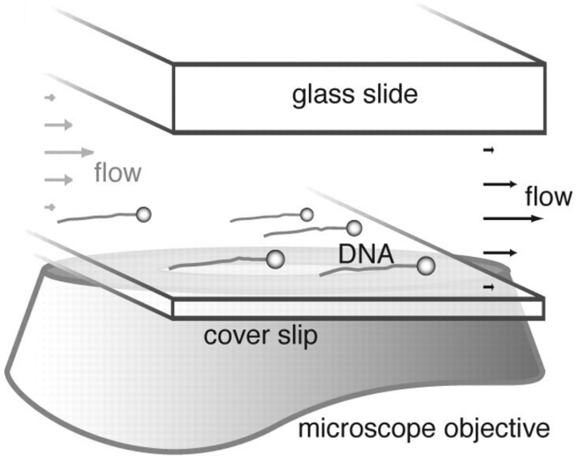
DNA translocation through a nanopore

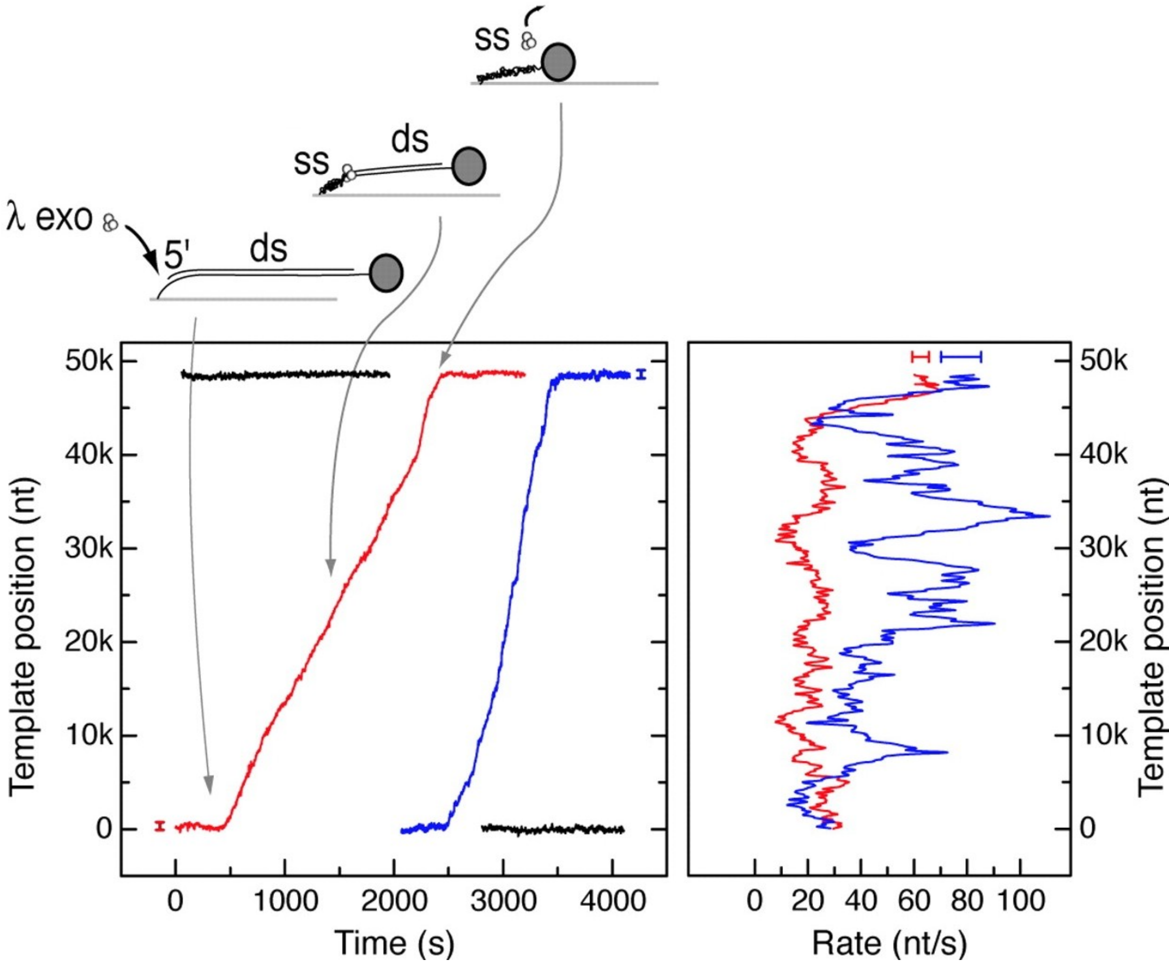


Mathé et al. (2004)

DNA digestion by the λ-exonuclease

Van Oijen et al., *Science* (2003)

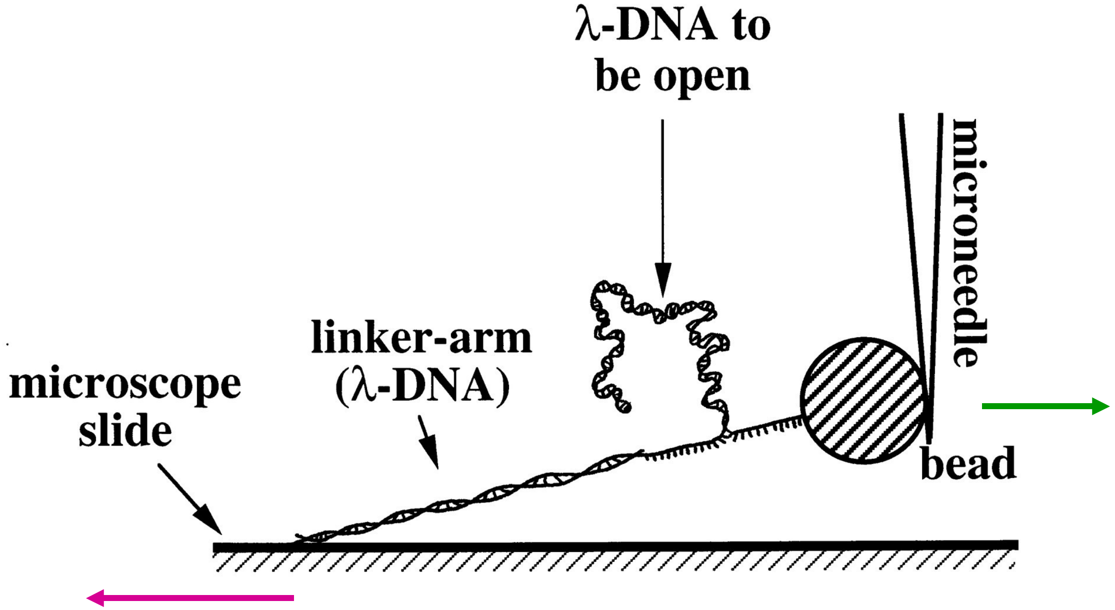




Mechanical Unzipping of DNA

Lambda-DNA : virus,
48502 base pairs

Essevaz-Roulet,
Bockelmann,
Heslot (1997)



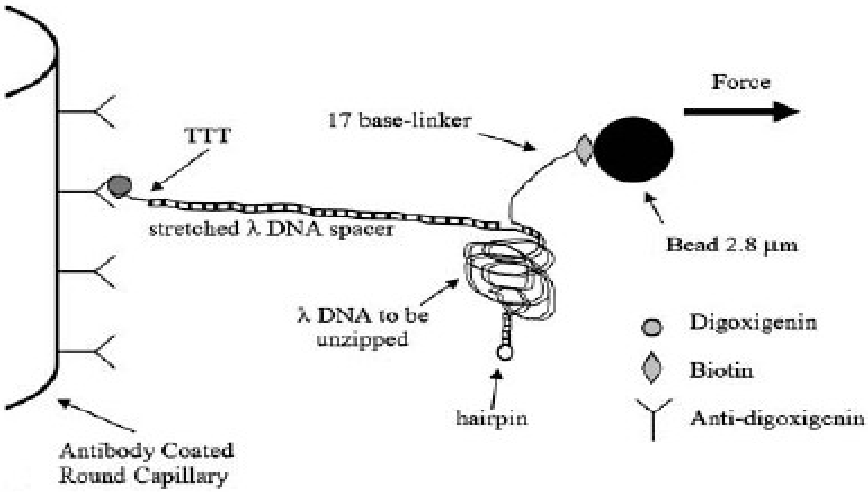
f (measured)

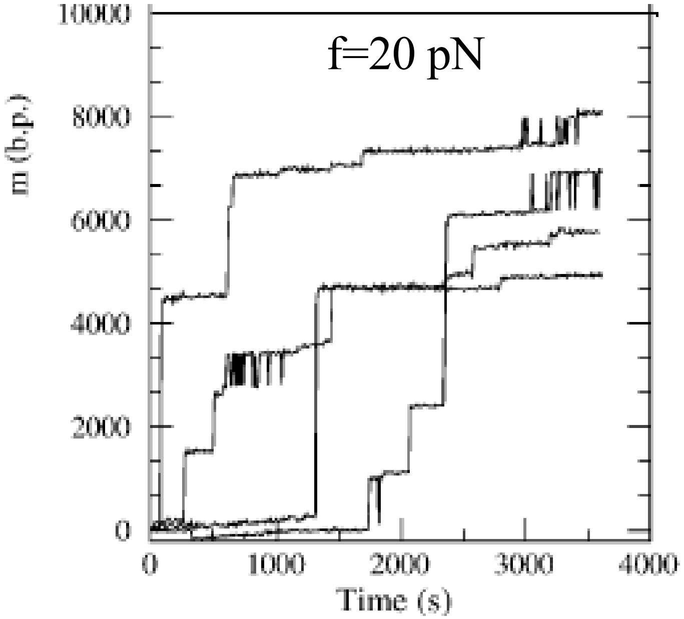
v or x (imposed)

Optical trap:
Spatial resolution ~ 1 nm
Temporal resolution ~ 1 kHz

Unzipping at constant force

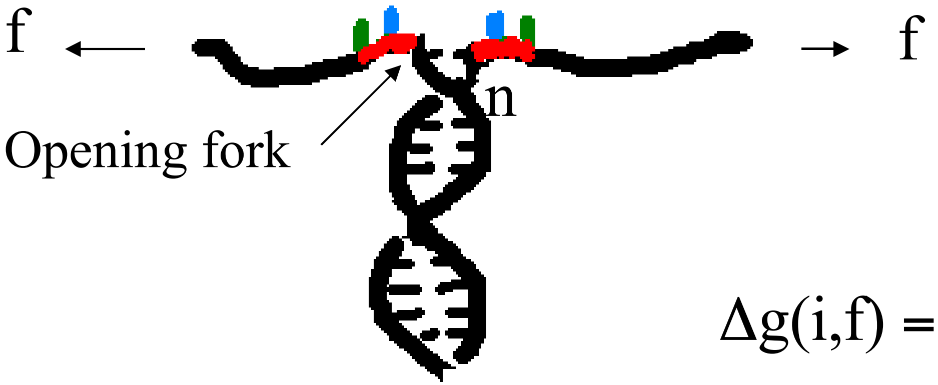
Weeks et al (2005)
Danilowitz et al. (2004)





Lionnet, Bensimon, Croquette (ENS): hairpin 200 bases

Thermodynamics of Unzipping



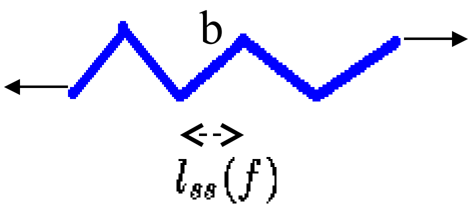
$$\Delta g(i,f) = g(b_i,b_{i+1}) - 2\ g_s(f)$$

- $g\ (b_i,b_{i+1})$ = base pair energy

- $g_s(f)$ = single strand DNA extension (ssDNA elasticity)

Elasticity of single-strand DNA (ssDNA)

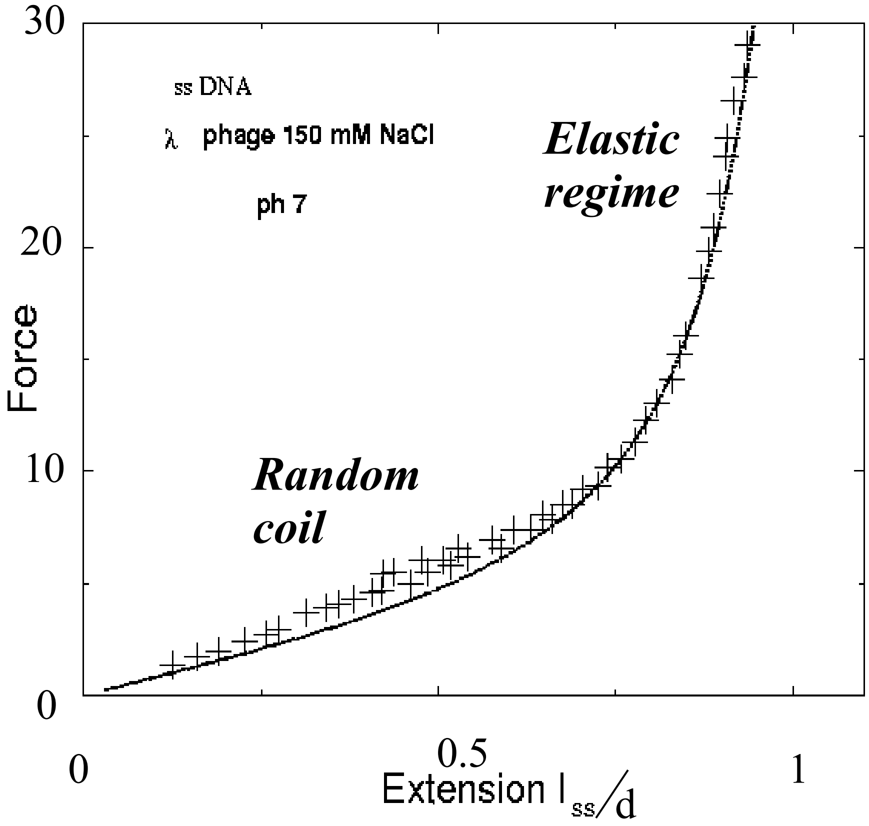
Freely-jointed chain model



$$x_{ss} = n\, l_{ss}(f)$$

$$l_{ss}(f) = d\left[Coth\left(\frac{f\,b}{k_B T}\right) - \frac{k_B T}{f\,b}\right]\left[1 + \frac{f}{\gamma_{ss}}\right]$$

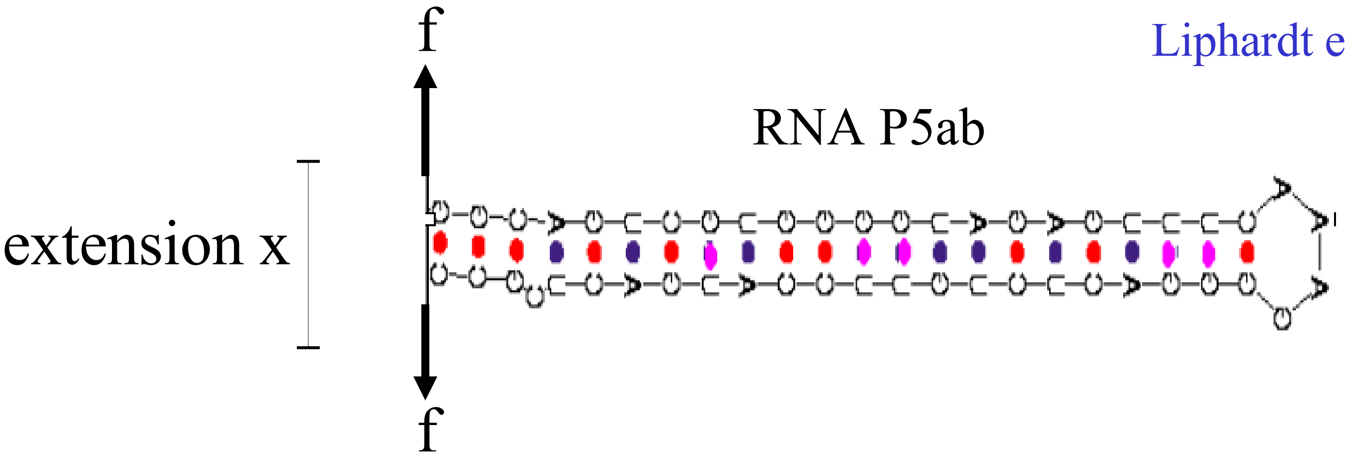
Kuhn length: b = 1.4 nm
monomer length: d = 0.56 nm
Young modulus: γ_{ss} = 800 pN

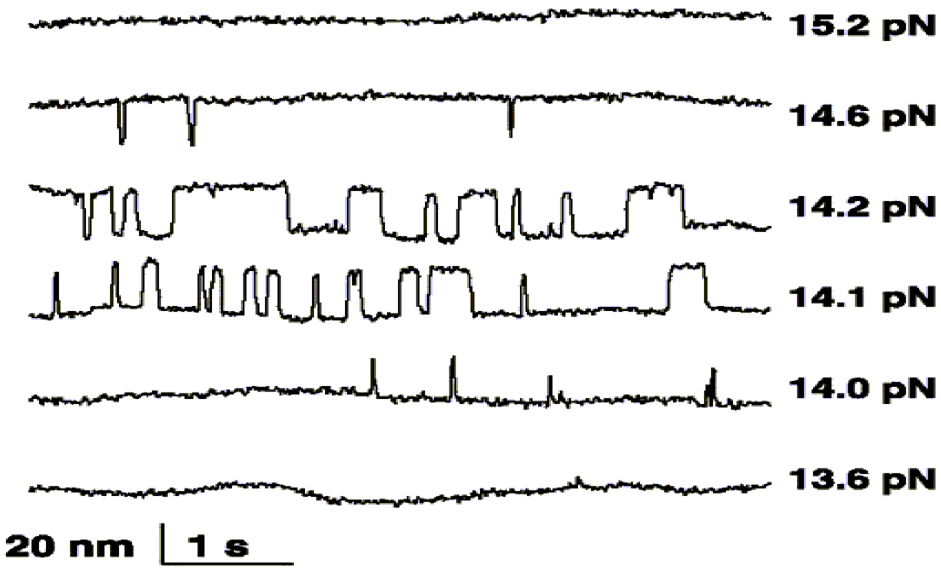


Bustamante et al. (1996)

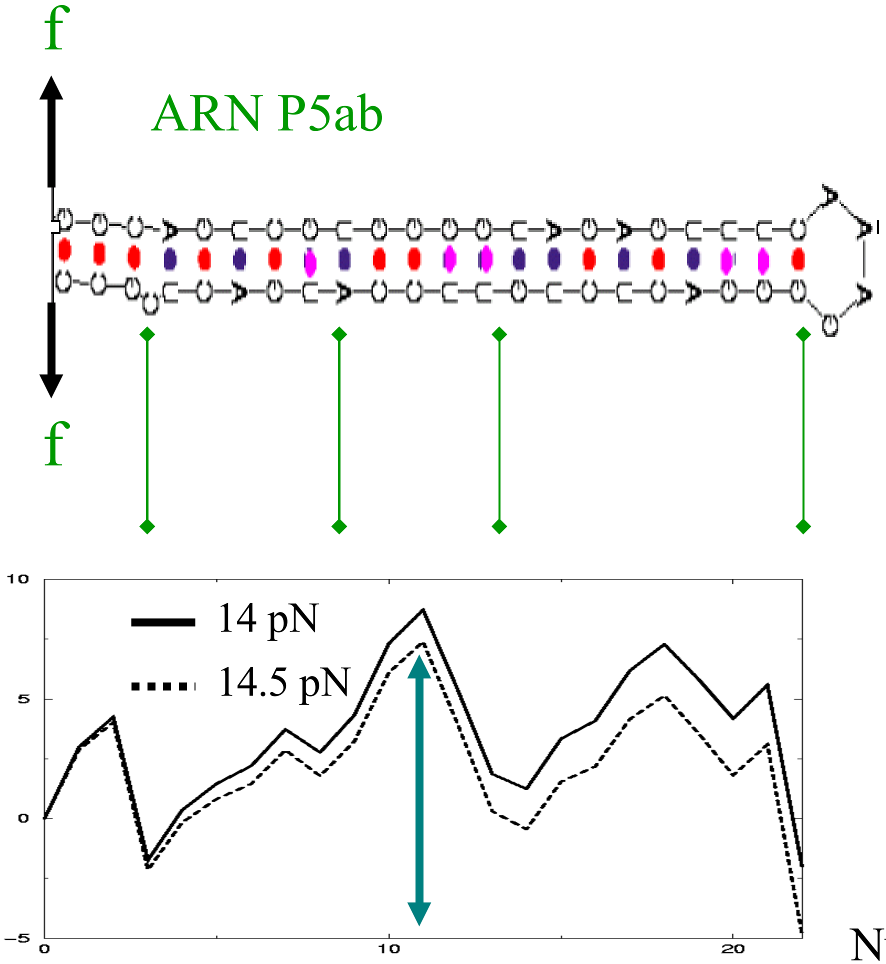
Example: opening a RNA double-helix

Liphardt et. al. (2001)





Sequence-dependent Landscape



Free energy

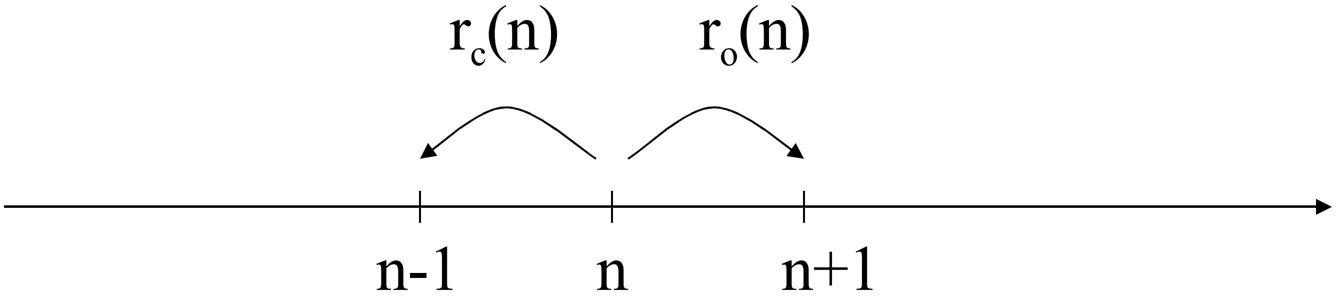
$$G(n) = \sum_{i<n} \Delta g(i,f)$$

barrier

Number n of open bases

Only two configurations can be seen in practice ...

Opening dynamics: random walk in random landscape

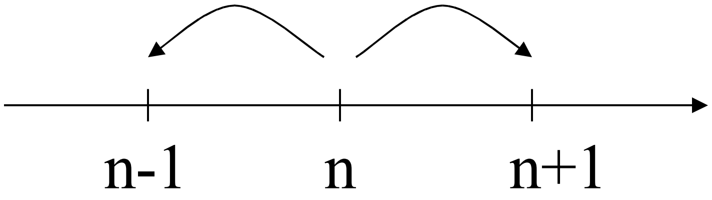


or, equivalently,

sojourn time on base n: t_n
$< t_n > \; = \; (r_c(n)+r_o(n))^{-1}$,
exponential distribution

&

$$q_n = \frac{r_c(n)}{r_c(n)+r_o(n)} \qquad 1\text{-}q_n$$



Transition matrix

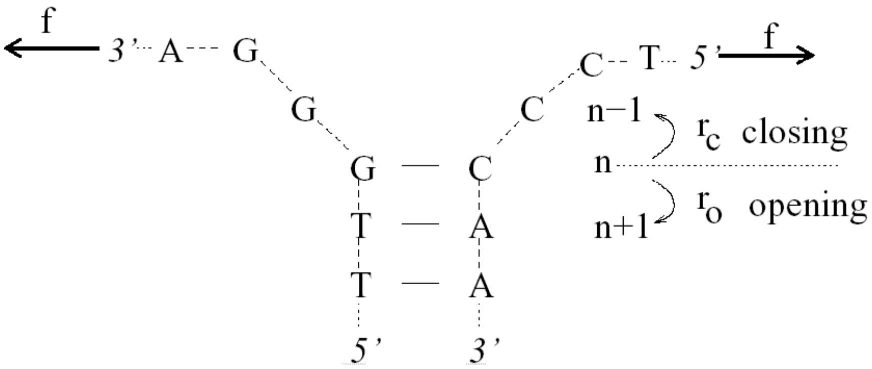
$$\text{rate matrix} = \mathbf{M} = \begin{pmatrix} -r_c(1) & r_c(2) & 0 & 0 \ldots \\ r_o(1) & -r_o(2)-r_c(2) & r_c(3) & 0 \ldots \\ 0 & r_o(2) & -r_c(3)-r_o(3) & \ldots \\ 0 & 0 & r_o(3) & \ldots \end{pmatrix}$$

$$\boxed{\frac{dP_n(t)}{dt} = \sum_{n'} M_{n,n'} P_{n'}(t)}$$

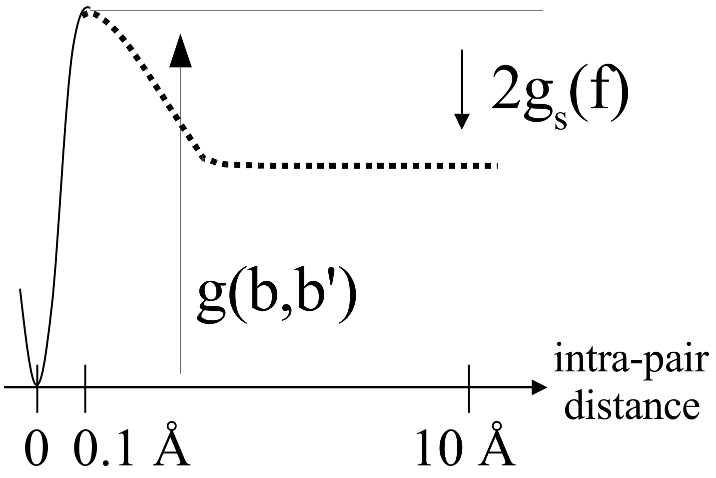
Transition matrix M?

1. equilibrium: $P_n = \exp(-G(n))/Z$
2. Rates depends on the local content of the sequence i.e. $r_o(j)$ depends on b_{j+1}, b_j …

Dynamical rates



Rates ?

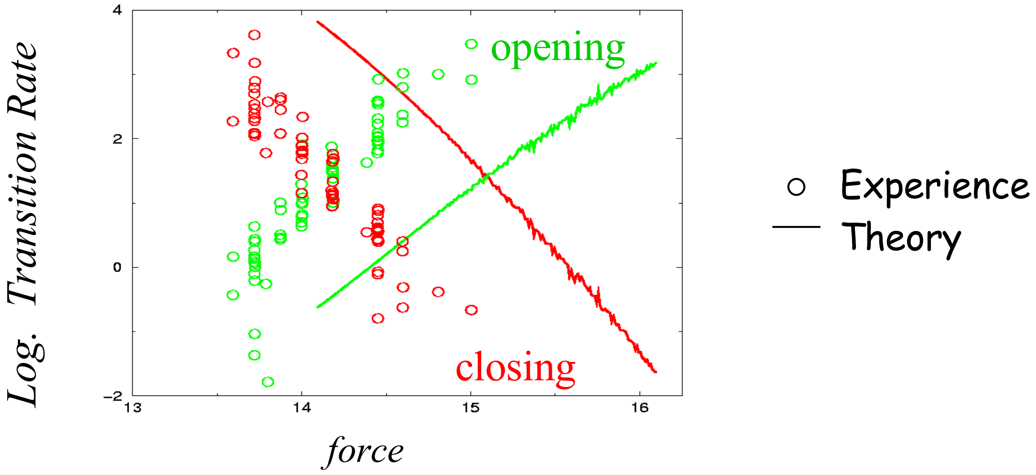


+

detailed balance

$r_o(n) = r \exp(- g(b_n,b_{n+1}))$ and $r_c(n) = r \exp(- 2g_s(f))$

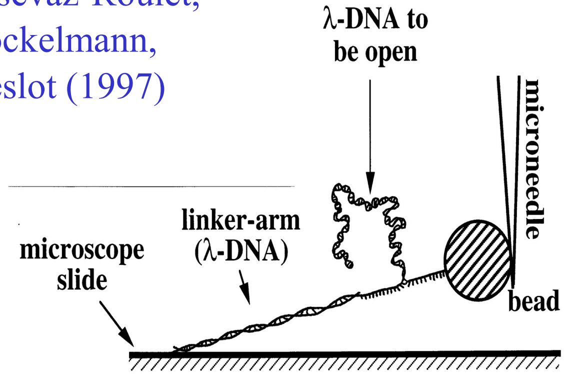
Check of the rate expressions

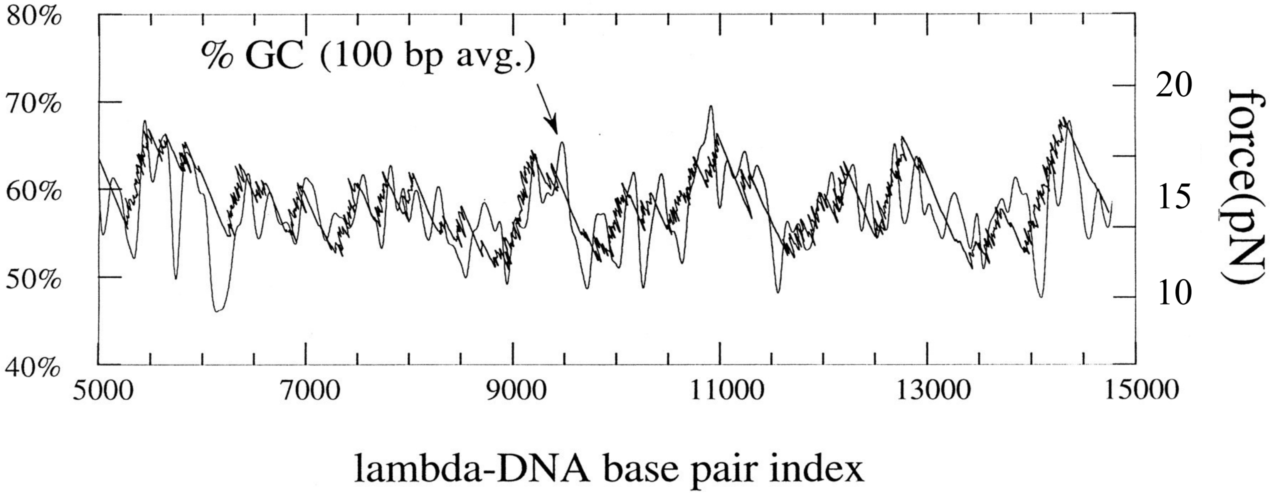


- The dependence on force seems to be ok
- Energetic parameters have to be tuned ...

Correlation between sequence and unzipping force

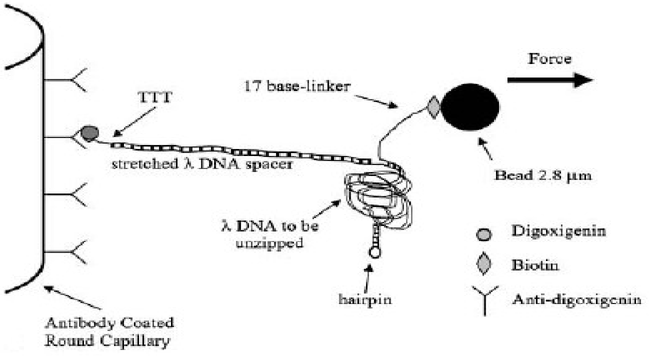
Essevaz-Roulet, Bockelmann, Heslot (1997)

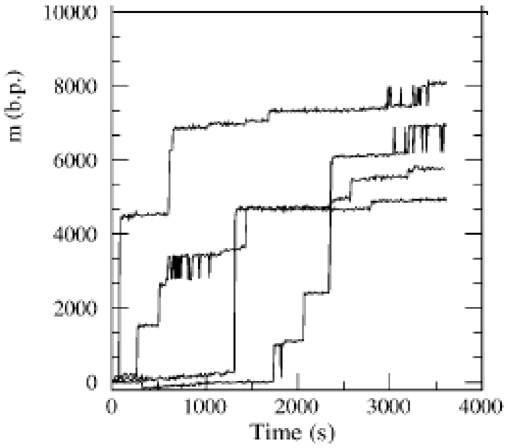


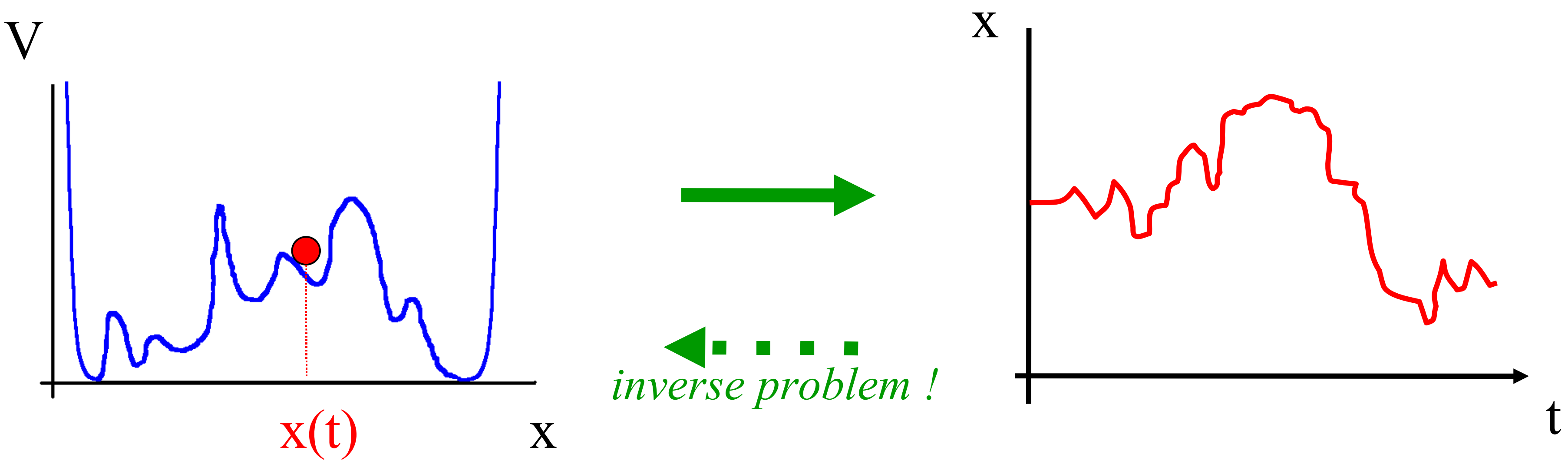


Random Walks in Random Landscape & Inverse Problem

Weeks et al (2005)
Danilowitz et al. (2004)







inverse problem !