Magnetic tweezers to study DNA motors

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• Introduction to MT (magnetic tweezers)

• Applications:
  1. Tracking DNA motors:
     (i) Helicases
     (ii) Annealing motor
  2. Studying a multiprotein system:
     DNA replication
Single molecules force microscopy techniques

- Atomic force microscopy (AFM)
- Optical tweezers
- Magnetic tweezers
MT setup

Diagram showing an experimental setup with magnetic beads, DNA, and microscopic objective. The graph on the right depicts the relationship between magnetic field strength and force acting on the sample, with a comparison between prediction and experimental data.
MT setup
MT setup

420 nt

5'

231 bp (0.7 kbp)

50 nt
3D real time tracking

Diffraction images of a bead:
Measuring forces

The amount of brownian motion lets one measure the force:
The amount of brownian motion lets one measure the force:

\[ F_b \approx F_m \alpha = F_m \frac{x}{l} \]

\[ F_b = \left( \frac{F_m}{l} \right) x = \kappa x \]

Mean energy of a spring:

\[ E = \frac{1}{2} \kappa <x^2> \]
\[ = \frac{1}{2} \left( \frac{F_m}{l} \right) <x^2> \]
\[ = \frac{1}{2} k_B T \]

(equipartition theorem)

\[ F_m = \frac{k_B T}{<x^2>} \]
- Forces ranging from $10^{-2}$-100pN with 10% accuracy.

-(x,y,z) bead position can be determined with 1nm accuracy. Drift can be balanced by using two beads recording

-The time resolution is limited by the frequency of the camera (60-200Hz).
Multi-tracking

-multi-tracking allows performing several experiments in parallel (typically 10-100)
MT experiments

-Characterization of a biopolymers manipulating single molecules

Elasticity

Rel. DNA extension

WLC:
\[ p = 51 \pm 5 \text{ nm} \]
\[ L = 3.27 \pm 0.05 \mu\text{m} \]

Supercoiling

DNA extension (\(\mu\text{m}\))

Magnet rotations (turns)

10 kbp DNA
MT experiments

-Folding/unfolding of DNA and RNA and proteins
MT experiments

- Study of enzymes interacting with DNA: Topoisomerases
Enzymes that use the energy of ATP hydrolysis to move unidirectionally along ssDNA and unwind dsDNA.

**Gp41**
Electron microscopy image of gp41 hexamers (*Dong et al, JBC 1995*)

**RecQ**
Crystal structure of *E. coli* RecQ catalytic core (*DA Bernstein et al 2003 EMBO*)
Unwinding: passive versus active

**Passive:** helicase behaves opportunistically, relying on the fraying of the DNA fork

**Active:** direct destabilization of the DNA fork.
Detecting helicase activity
Gp41 helicase activity

unwinding

rezipping

Extension [µm]

Force=9pN
[ATP]=5mM

Gp41 Helicase +ATP

Time [s]

Extension [bp]

0 200 400 600

0 0.2 0.4 0.6
**Gp41: unwinding and ssDNA translocation**

![Graph showing the process of Gp41 unwinding and ssDNA translocation](image)

- **Force**: 9pN  
  **[ATP]**: 5mM
- **Gp41 Helicase + ATP**
- **V\text{un} = N_u / T_u**  
  ≈ 200bp/s
- **V\text{trans} = N_t / T_t**  
  ≈ 500b/s

The graph illustrates the time course of extension versus force, with two distinct phases: unwinding and rezipping.
RecQ: unwinding and ssDNA translocation
RecQ: unwinding and ssDNA translocation

\[ V_{un} = \frac{N_u}{T_u} \approx 80 \text{bp/s} \]

\[ V_{trans} = \frac{N_t}{T_t} \approx 85 \text{nt/s} \]
Force analysis

ACTIVE UNWINDING

PASSIVE UNWINDING
T. Lionnet et al., PNAS 2007

M. Manosas et al., NAR 2010
Summary

Helicases: Gp41 versus RecQ

- Gp41 shows an unwinding rate that critically depend on both force and sequence. Its behaviour is well explained by a passive model.

- RecQ unwinding behavior is almost independent on the sequence and it unwinds DNA as quick as it translocates along ssDNA: efficient active helicase.
• UvsW is one of three helicases found in T4 phage.

• It uses the energy of ATP hydrolysis to move unidirectionally along one strand of DNA to:
  1. unwind or open dsDNA molecules (helicase activity)
  2. hybridize two ssDNA segments on a dsDNA segment (annealing activity)
UvsW helicase and annealing activities

Mechanical unfolding

Unwinding assisted by force $F<15pN$

Annealing against denaturing forces $F>15pN$
Unwinding assisted by force
$F<15pN$

$T=37C$, $[UvsW]=60nM$, $[ATP]=1mM$
UvsW annealing activity

Hairpin for annealing assays:

Annealing against denaturing forces $F > 15 \text{pN}$

- $[\text{UvsW}] = 0$
  - denaturing hairpin
  - Extension [µm]: 0, 0.25, 0.5, 0.75, 1.0
  - Time [s]: 5, 10, 15, 20
- $[\text{UvsW}] = 60 \text{nM}, [\text{ATP}] = 1 \text{mM}$
  - hairpin annealing $v \sim 1500 \text{bp/s}$
  - spontaneously unfolding at 16pN
  - Extension [µm]: 0, 0.25, 0.5, 0.75, 1.0
  - Time [s]: 1, 2, 3, 4, 5
Testing DNA repair function of Uvsw

Repairing stalled fork

- uncoupled replication fork
- unwinding of leading and lagging strands and strand annealing
- "chicken foot" DNA structure
- DNA synthesis
- fork progression
- repaired replication fork
Testing DNA repair function of Uvsw

Homologous recombination for repairing ds breaks
Testing DNA repair function of Uvsw

holliday junction migration
Testing DNA repair function of UvsW

Stalled fork for testing UvsW ability to fork regression and Holliday junction migration
Testing DNA repair function of Uvsw

Fork regression and Holliday junction migration

T=37°C, [UvsW]=10nM, [ATP]=1mM

Initial DNA configuration

Fast migration down and up

F=11pN

Extension (μm)

Time (s)

600bps

Holliday junction migration

7Kbps

Holliday junction migration

Initial DNA configuration

F=8pN

Time (s)
Summary

**UvsW:**

- *Uvsw* translocates extremely fast along DNA, against denaturating forces while induces the reannealing, independently of the complementarity of DNA.

- *Uvsw* is able to generate a chicken foot structure and perform Holliday junction migration: relevant enzyme in DNA repair pathways.