Promoter unwinding and promoter escape by RNA polymerase: analysis by single-molecule DNA nanomanipulation

Andrei Revyakinand Richard H. Ebright
Howard Hughes Medical Institute, Rutgers University

Terence Strick
Institut Jacques Monod

background
transcription

- synthesis of an RNA copy of genetic information in DNA
- first step in gene expression
- primary regulated step in gene expression
- target of ansamycin-class antibacterial agents (e.g., rifampicin)

background
transcription

- **transcription initiation**

RNA polymerase binds to DNA and begins synthesis of an RNA molecule

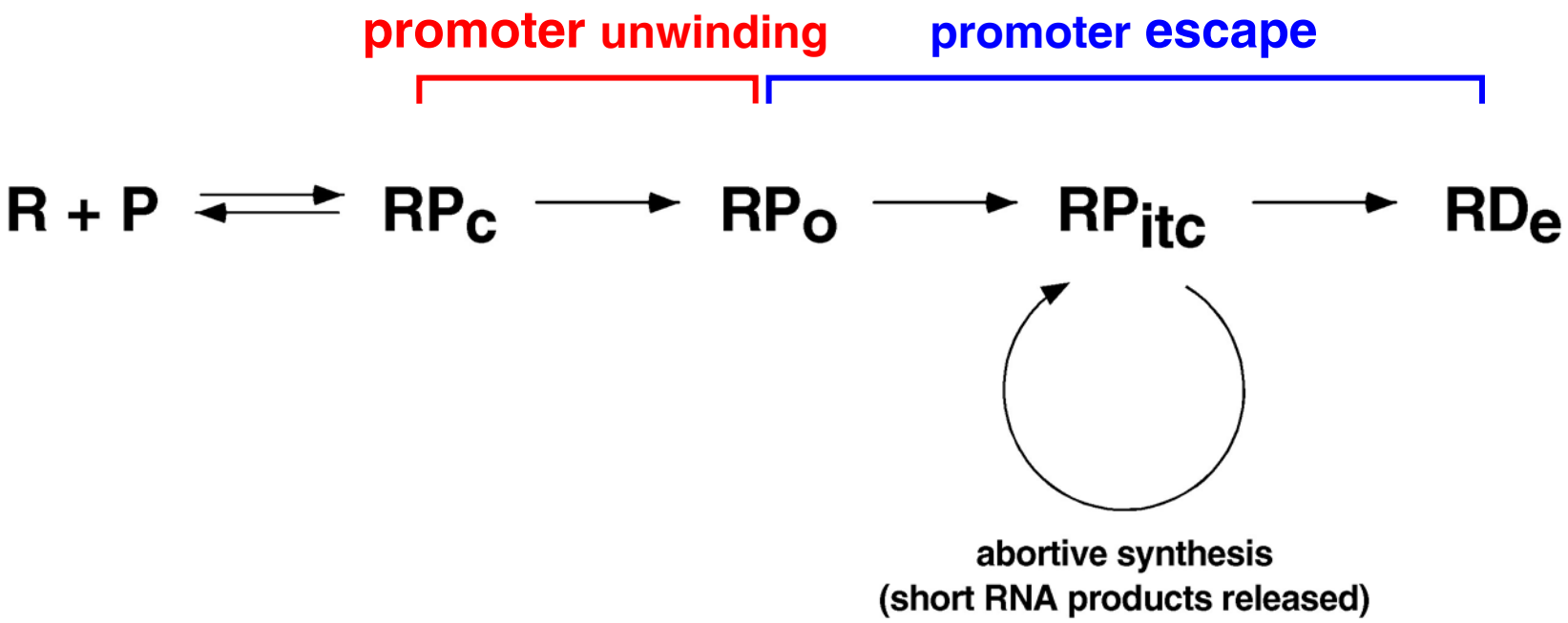
- **transcription elongation**

RNA polymerase translocates along DNA and extends the RNA molecule

- **transcription termination**

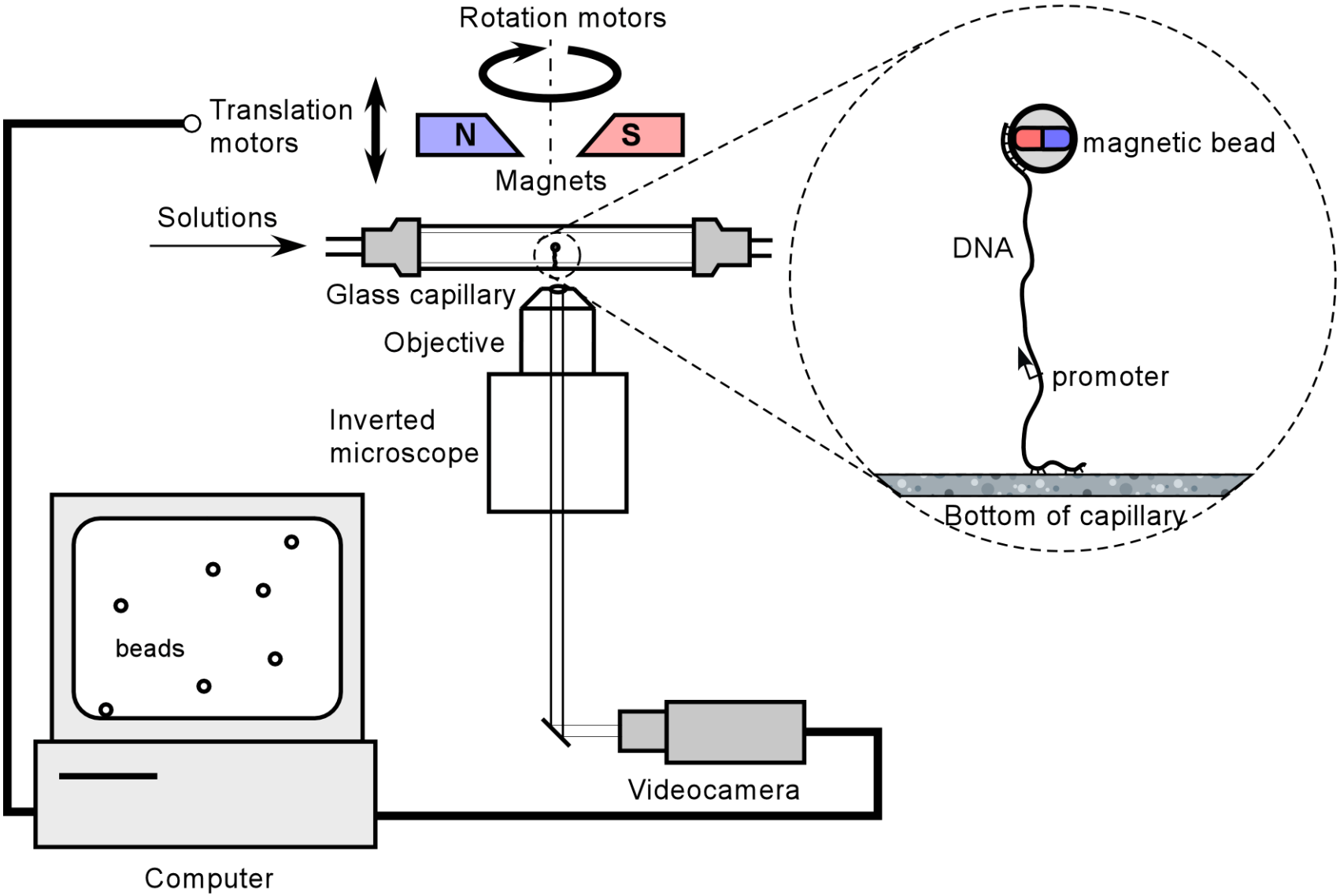
RNA polymerase dissociates from DNA and releases the RNA molecule

background
transcription initiation

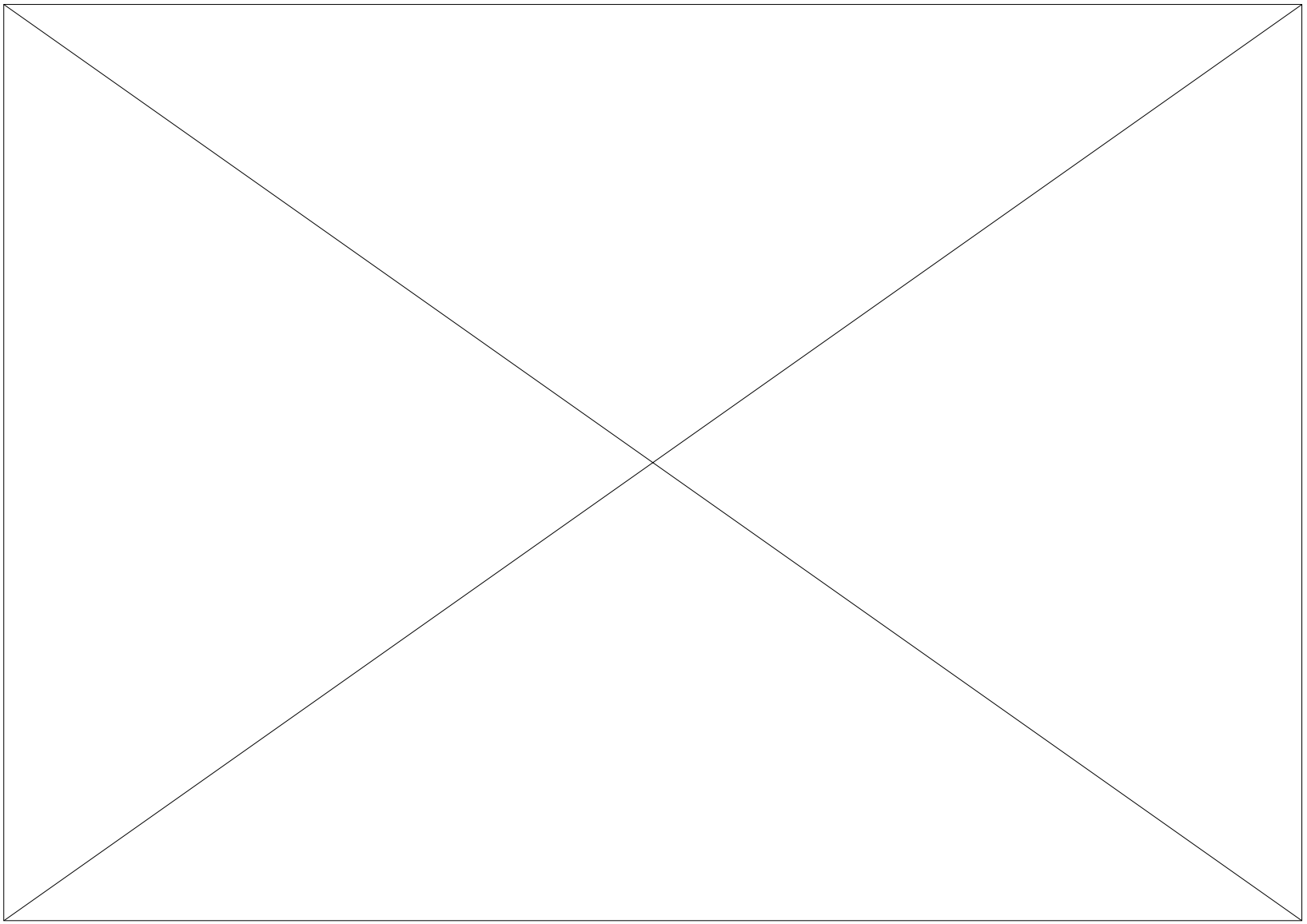


experimental approach

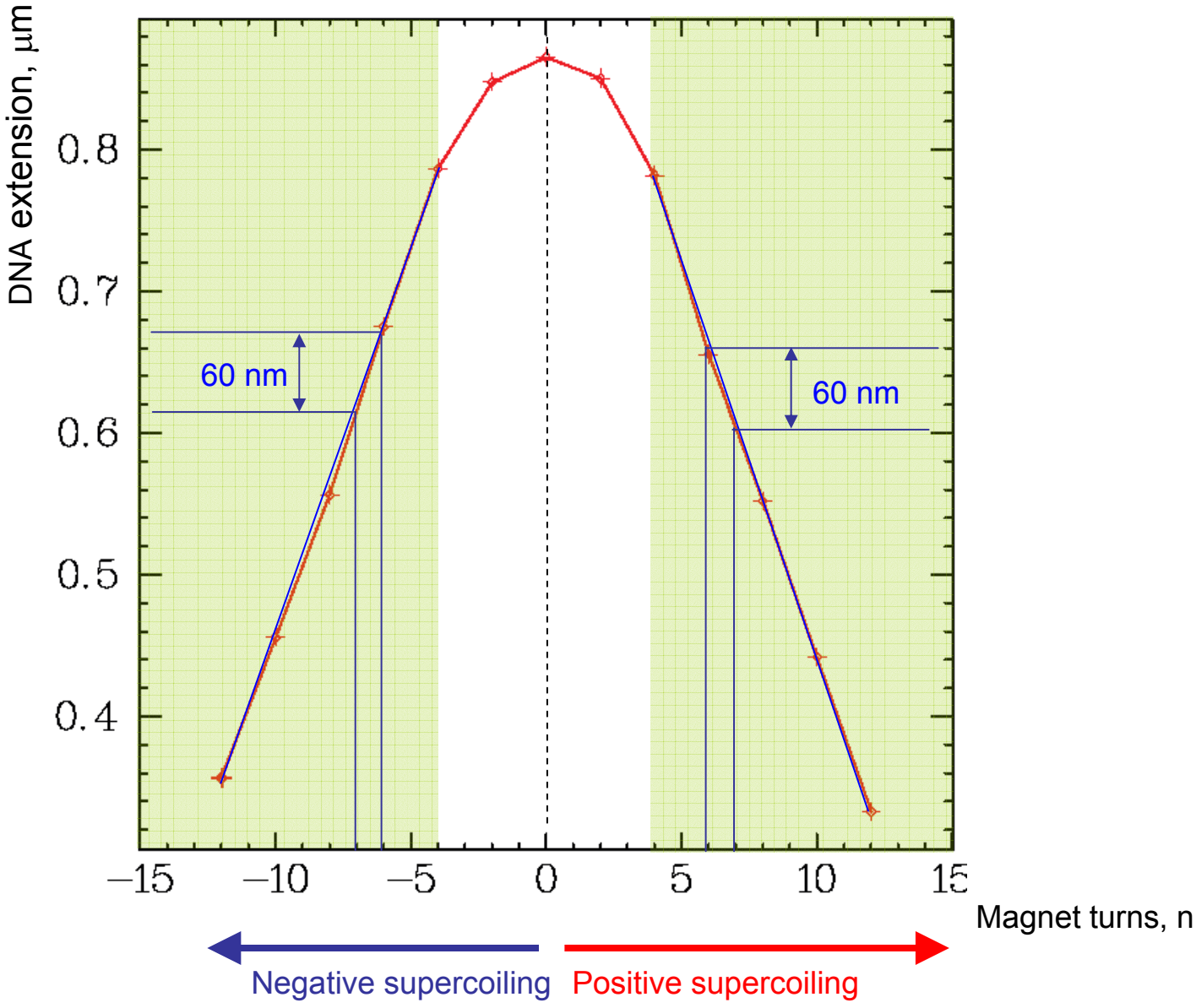
experimental approach
experimental setup (see Strick et al., 1996)



experimental approach
experimental setup (see Strick et al., 1996)

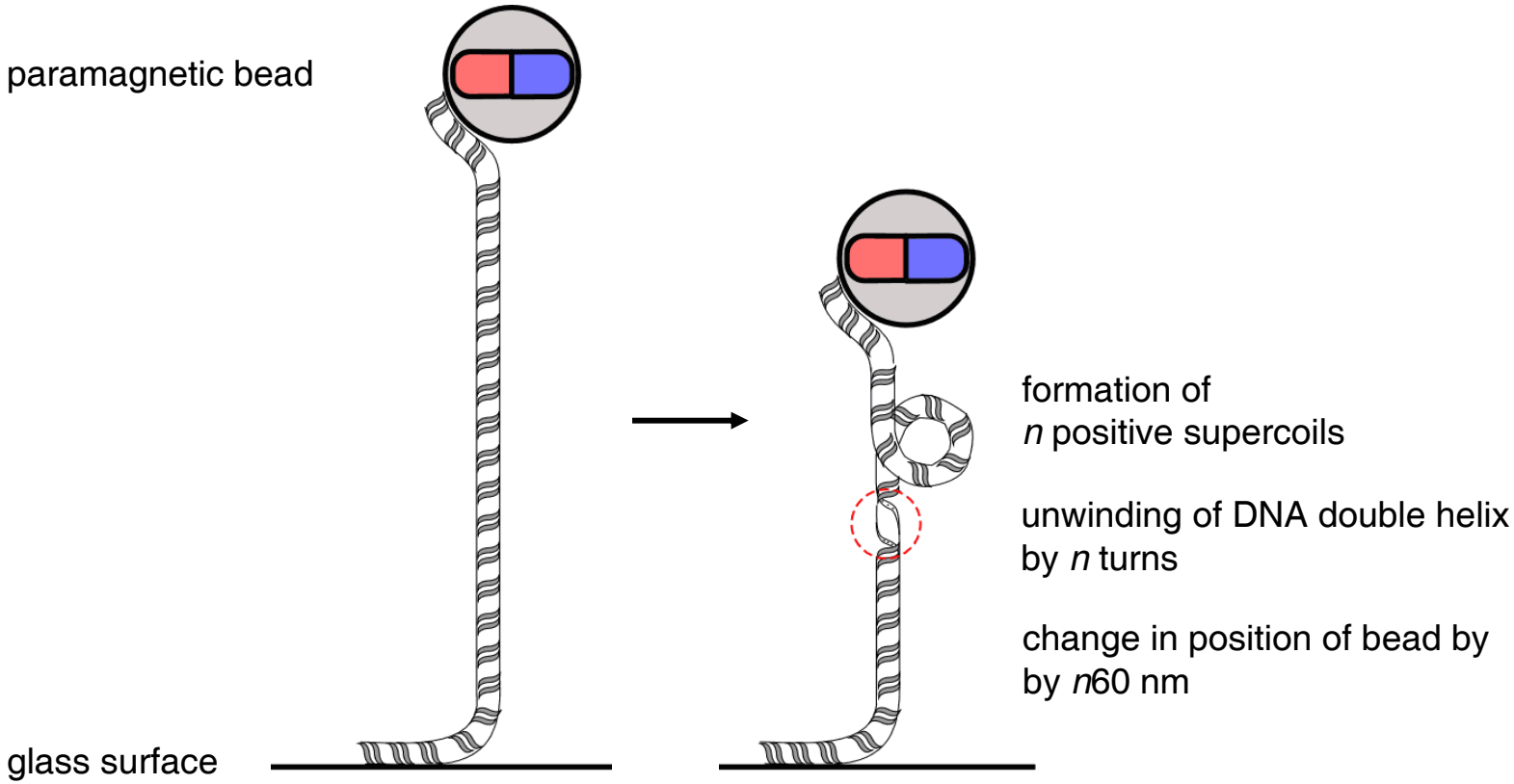


experimental approach
calibration curve

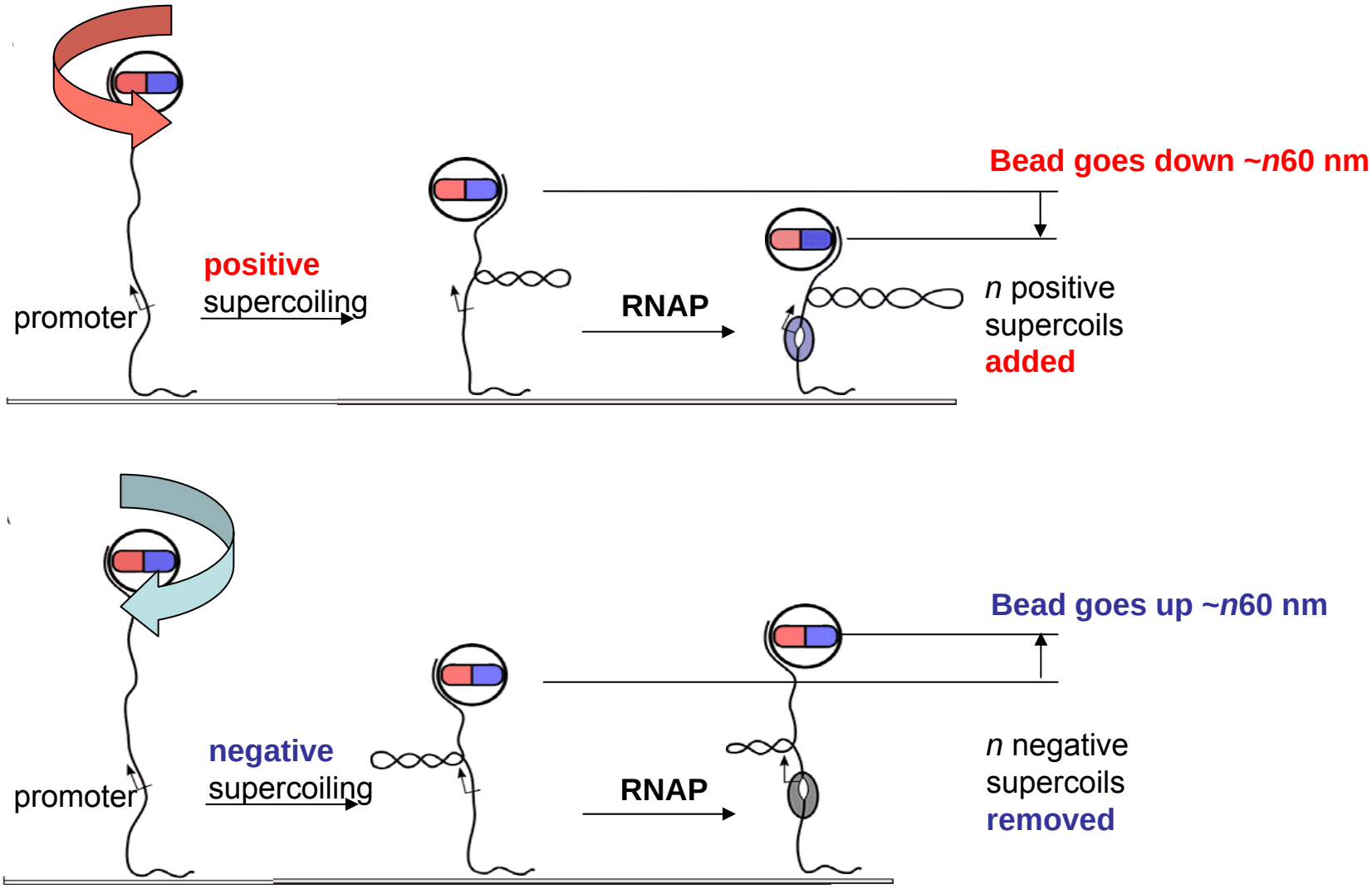


experimental approach
monitoring DNA unwinding by monitoring bead movement

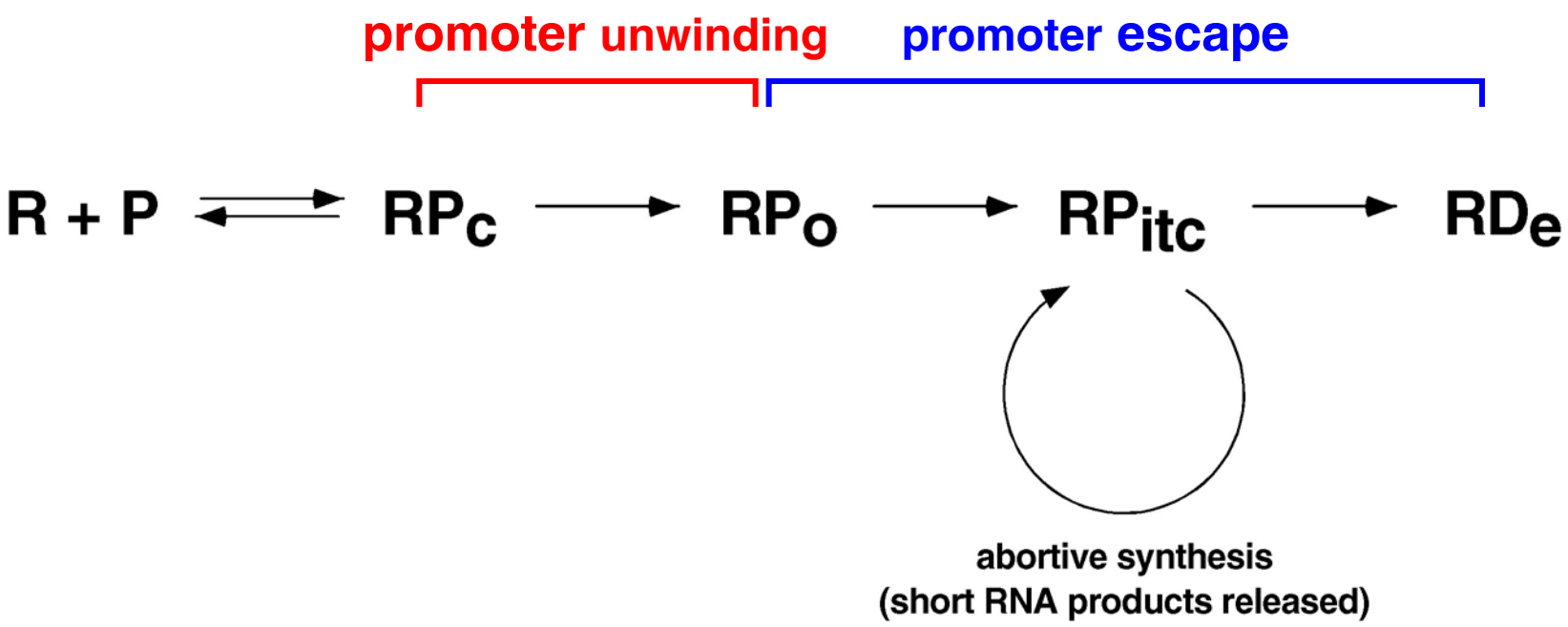
$$Lk = Tw + Wr = \text{constant}$$



experimental approach
monitoring DNA unwinding by monitoring bead movement

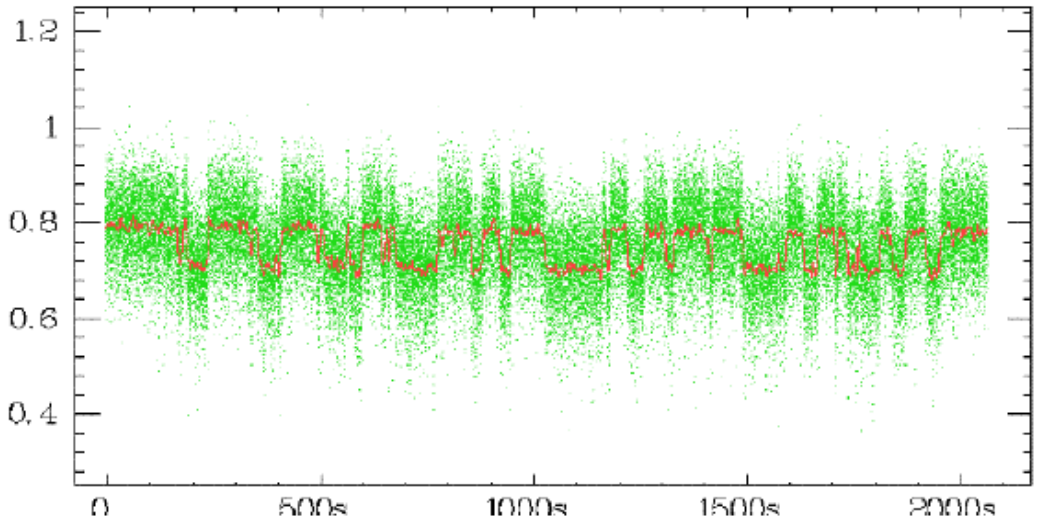


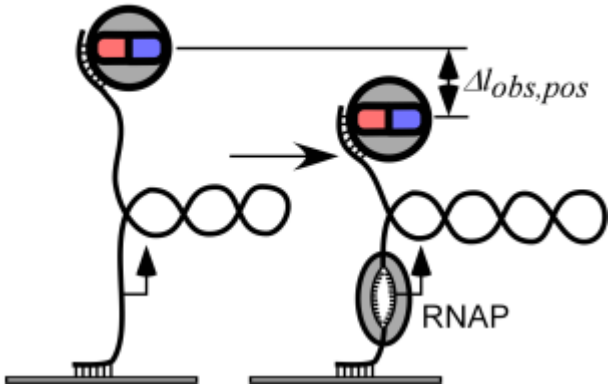
promoter unwinding


promoter unwinding
promoter escape
R + P
RPc
RPo
RPitc
RDe
abortive synthesis
(short RNA products released)

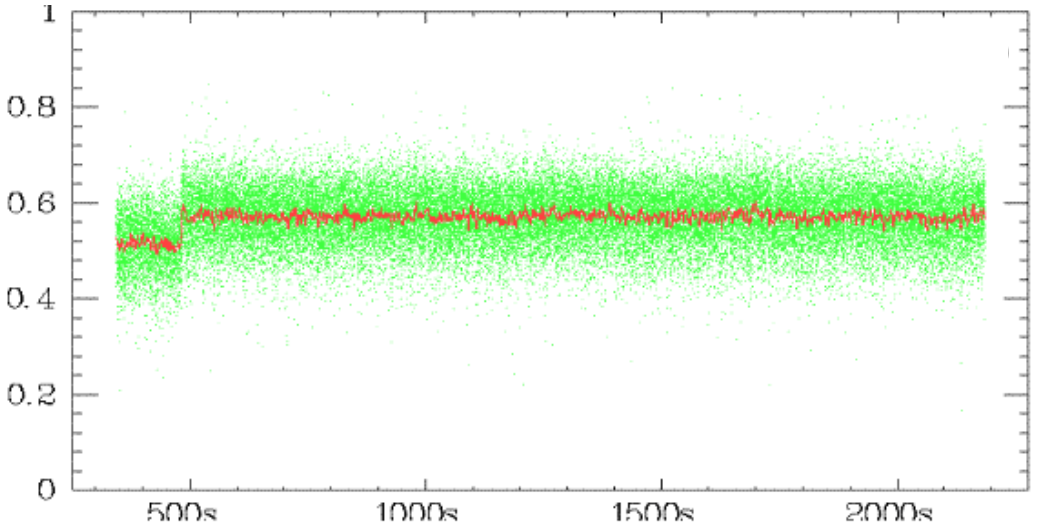
promoter unwinding detection

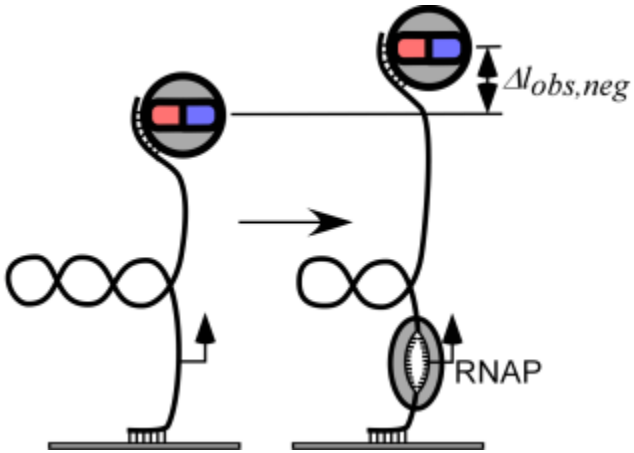
positively supercoiled DNA





negatively supercoiled DNA

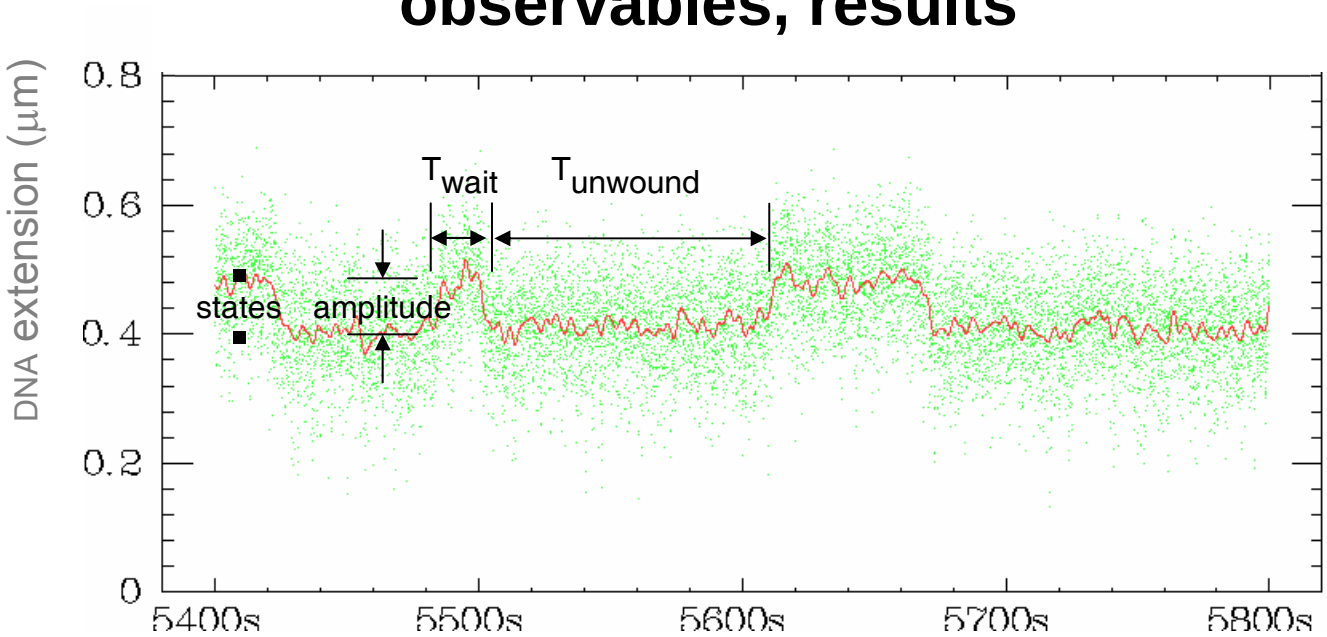




promoter unwinding control experiments

- No unwinding is observed in the absence of a promoter.
- No unwinding is observed in the absence of RNAP.
- No unwinding is observed in the absence of σ.
- No unwinding is observed at low temperatures.
- Unwinding is prevented by prior addition of heparin.
- Unwinding is not affected by subsequent addition of heparin.
- The number of unwinding events observed equals the number of promoters.
  (One unwinding event is observed on a DNA template having one promoter.
  Two unwinding events are observed on a DNA template having two promoters.
  Three unwinding events are observed on a DNA template having three promoters.)

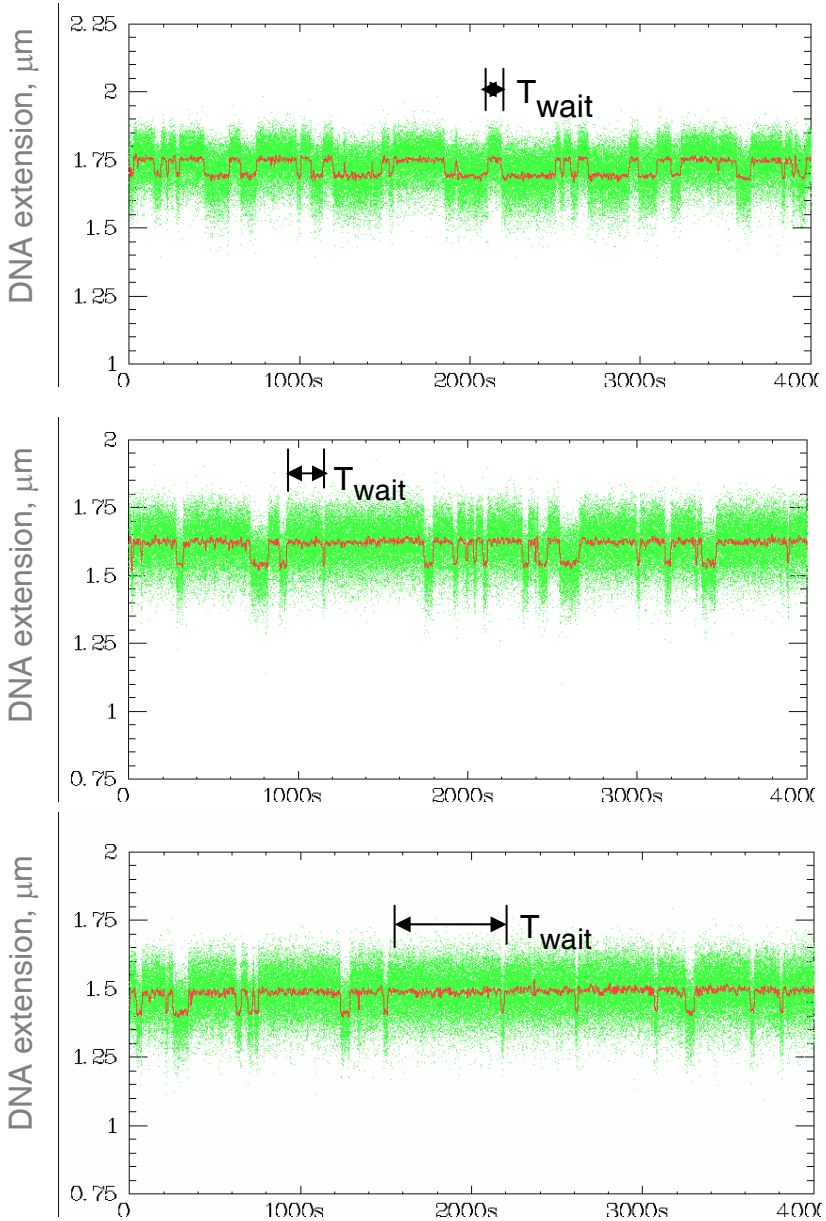
promoter unwinding observables, results



- number of states → number of intermediates: no detectable intermediates
- amplitude of change in DNA extension → extent of unwinding and compaction
  unwinding: 1.3 turn = 14 bp
  compaction: 15 nm
- time interval between events (T_{wait}) → rate of formation of unwound complex
  K_B: $1 \times 10^7\ M^{-1}$
  k_2: $1\ s^{-1}$
- lifetime of unwound complex ($T_{unwound}$) → stability of unwound complex
  k_{-2}: $0.025\ s^{-1}$

$$R + P \underset{}{\overset{K_B}{\rightleftarrows}} RP_c \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} RP_o$$

promoter unwinding: effects of supercoiling
rate of formation of the unwound complex (T_{wait})



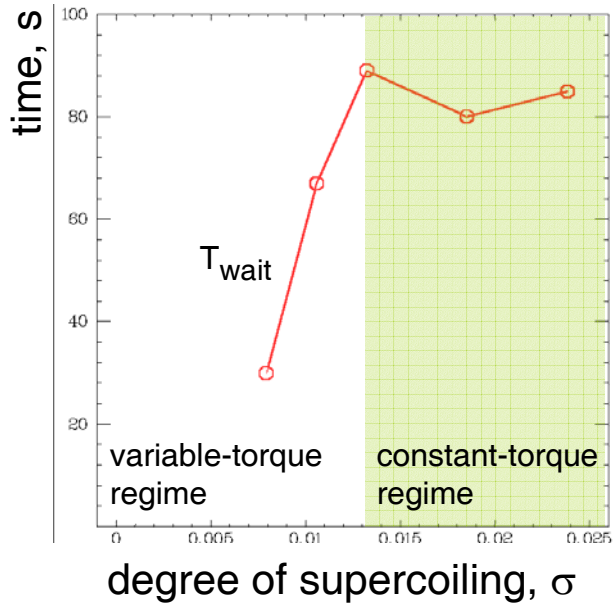
consensus promoter

positively supercoiled DNA

+5 turns

+7 turns

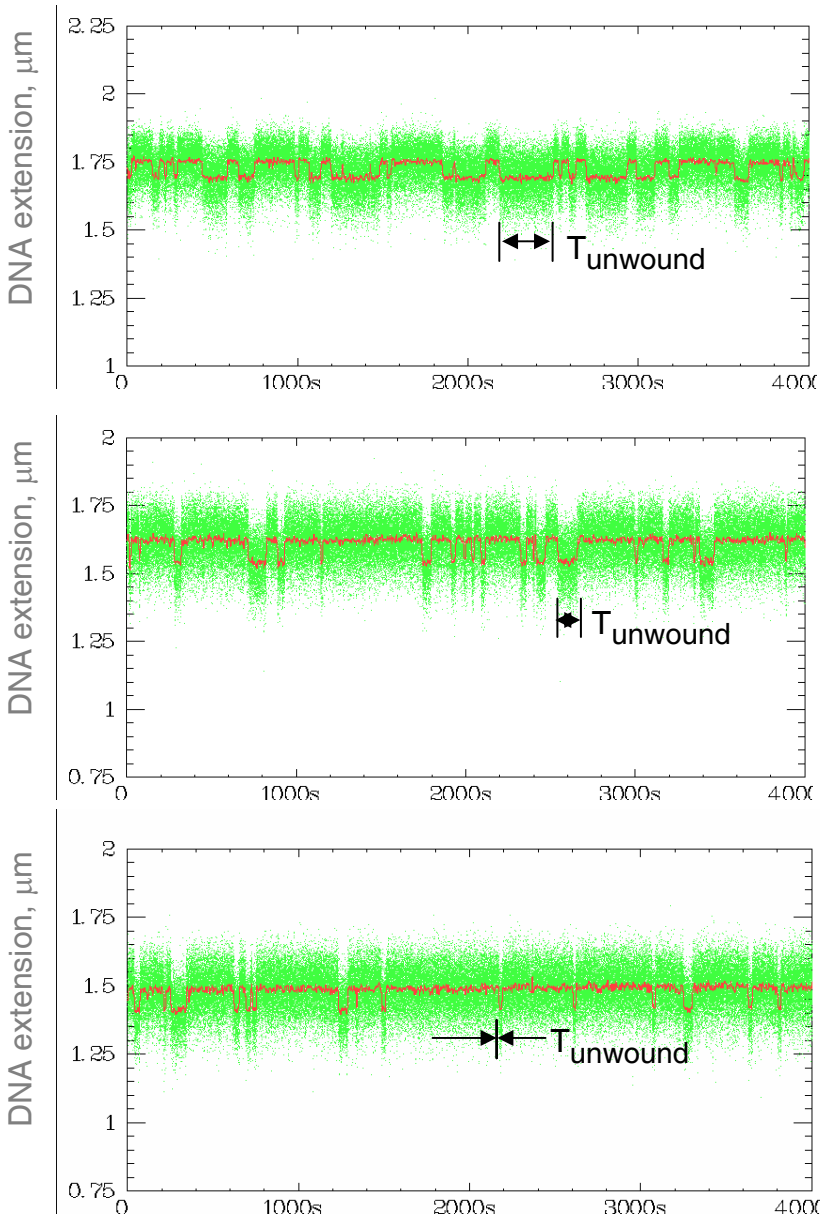
+9 turns



Positive supercoiling **decreases** the rate of formation of the unwound complex.

Effect is due to torque.

promoter unwinding: effects of supercoiling stability of the unwound complex ($T_{unwound}$)



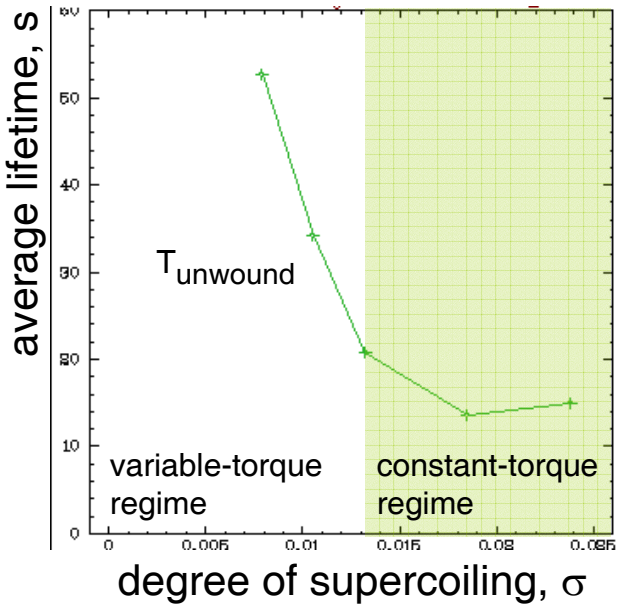
consensus promoter

positively supercoiled DNA

+5 turns

+7 turns

+9 turns



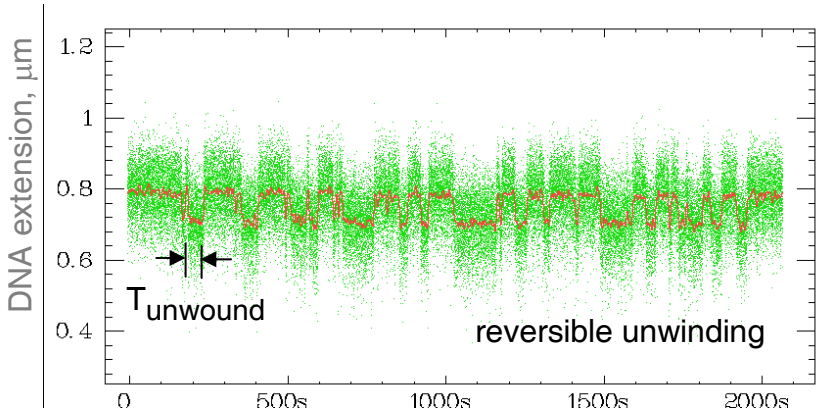
Positive supercoiling **decreases** the stability of the unwound complex.

Effect is due to torque.

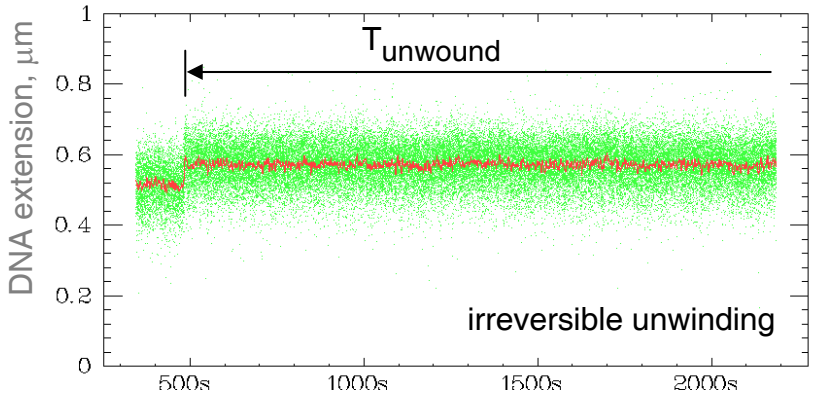
promoter unwinding: effects of promoter sequence

consensus

CTTGACACTTTATGCTTCGGCTCGTATAATGTGTGGA



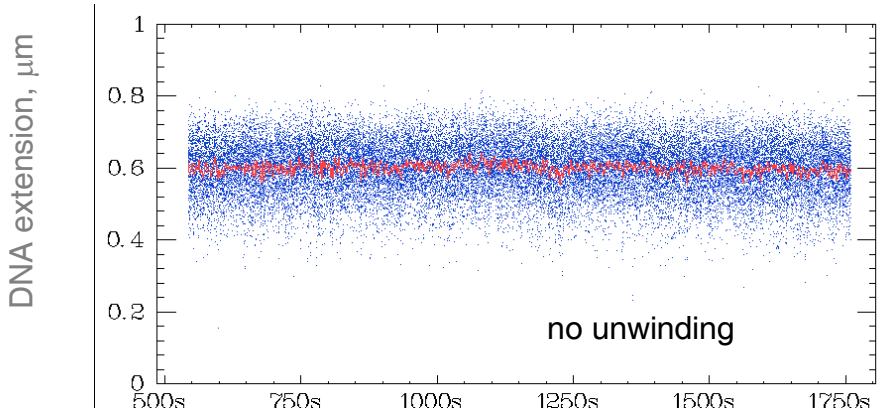
positively supercoiled DNA



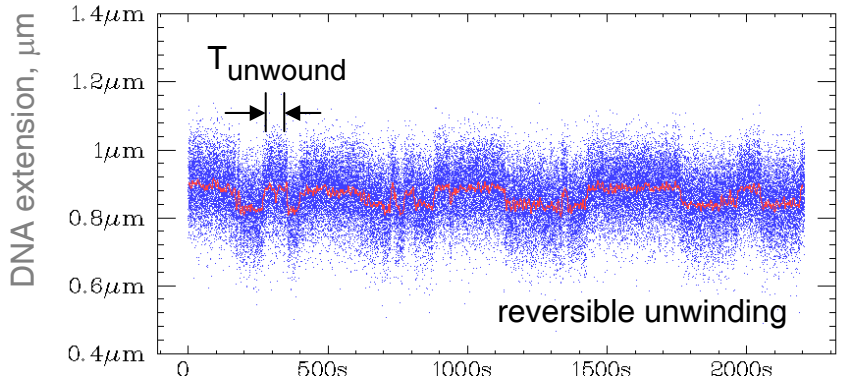
negatively supercoiled DNA

rrnB P1

CTTGTCAGGCCGGAATAACTCCCTATAATGCGCCACCA

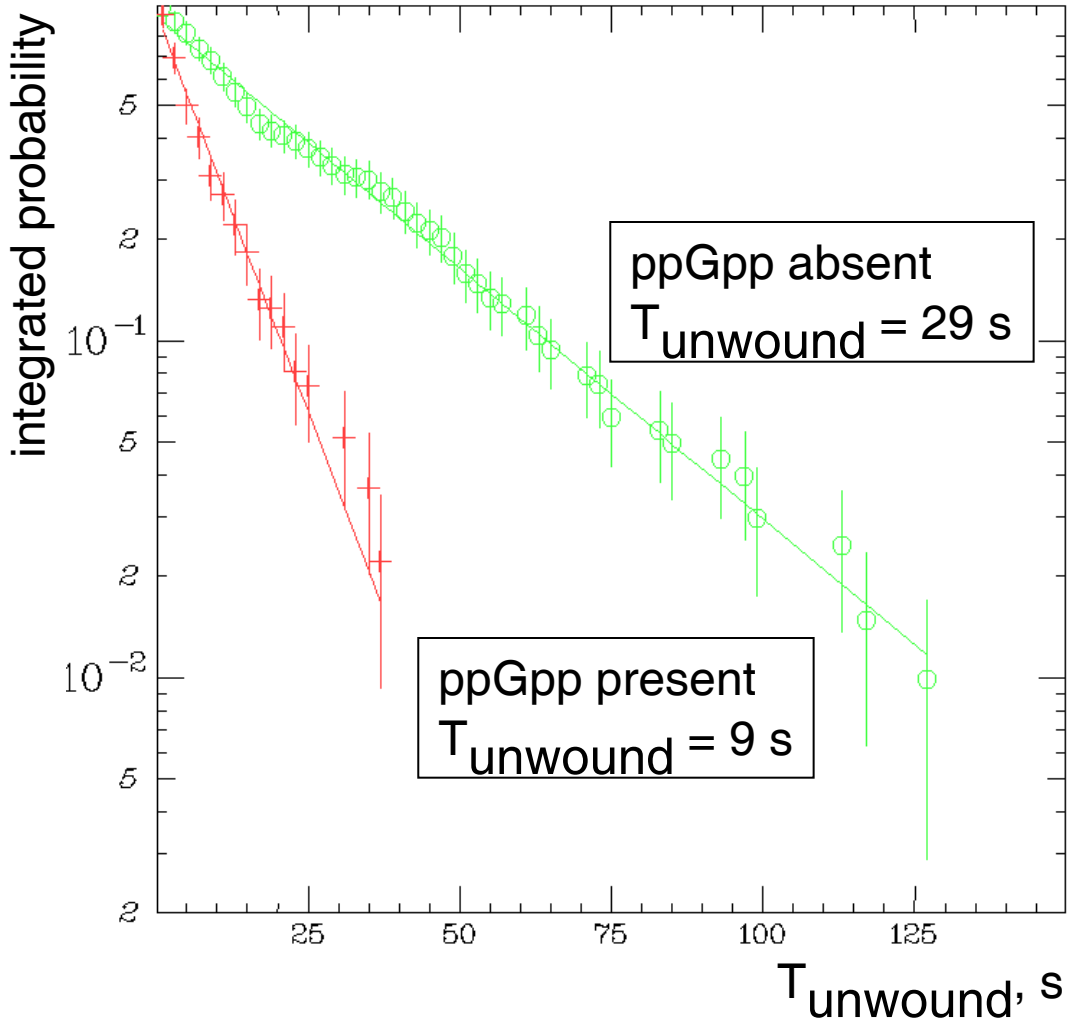


positively supercoiled DNA



negatively supercoiled DNA

promoter unwinding: effects of ppGpp

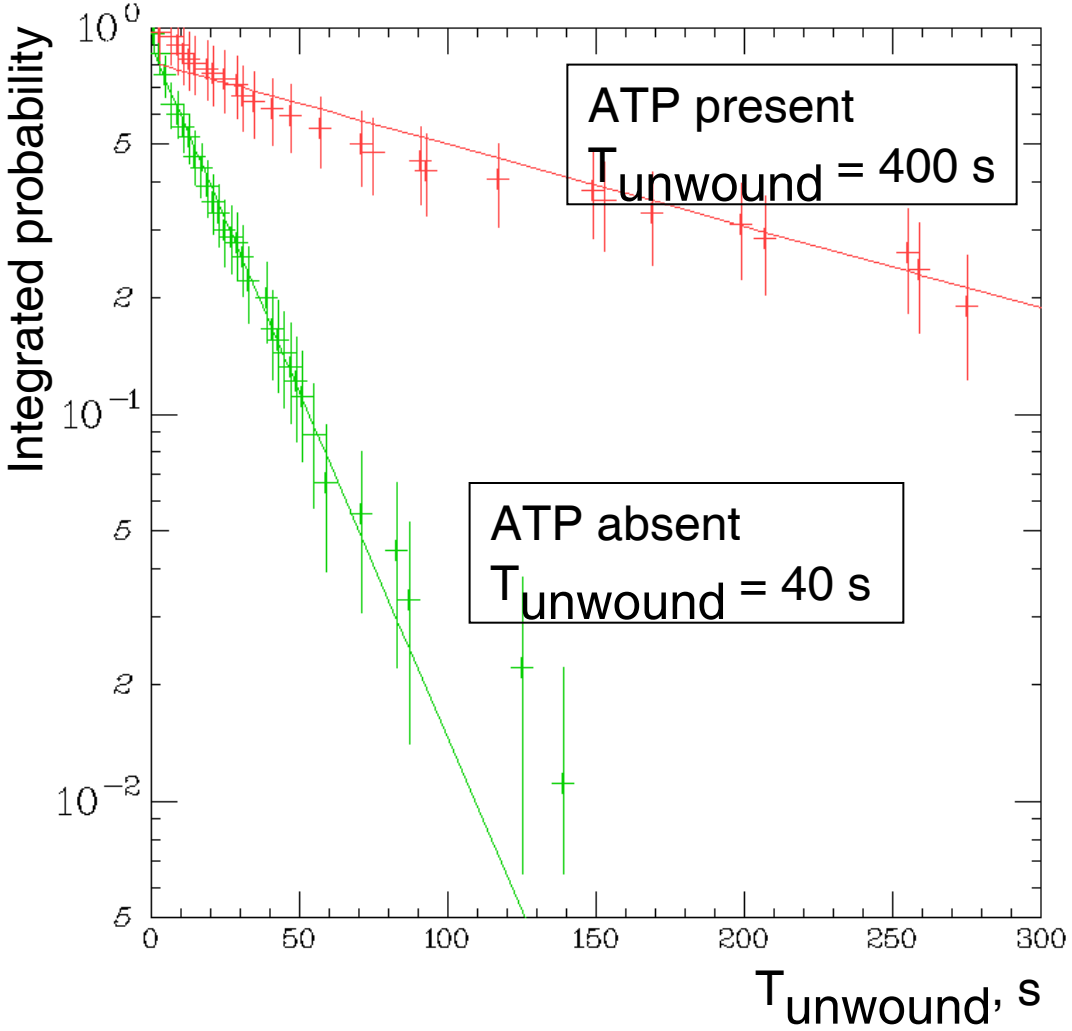


consensus

positively
supercoiled DNA

100 μM ppGpp

promoter unwinding: effects of initiating nucleotide



consensus

positively
supercoiled DNA

2 mM ATP

-10 +1

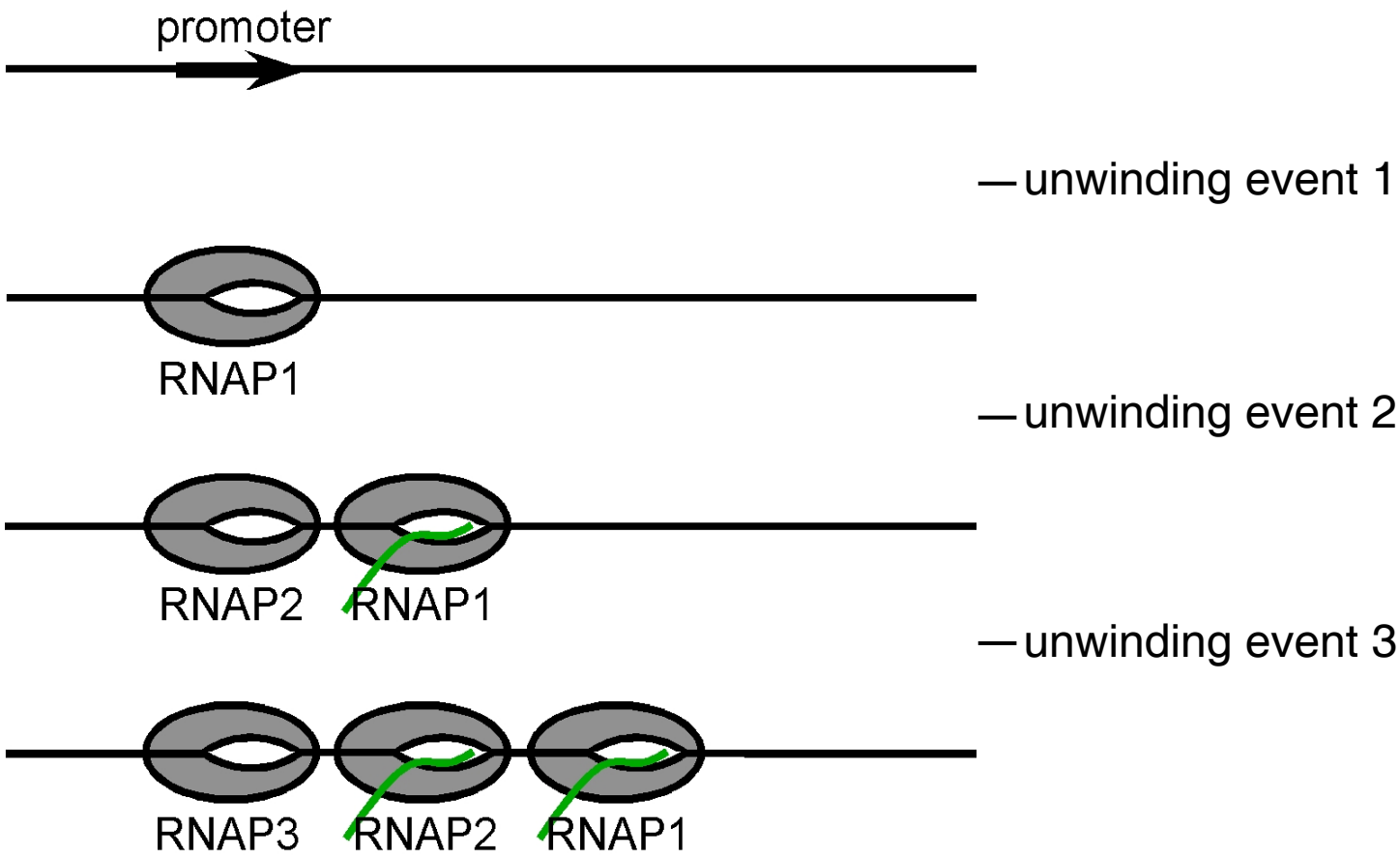
cgtataatgtgtggAatt

promoter unwinding summary

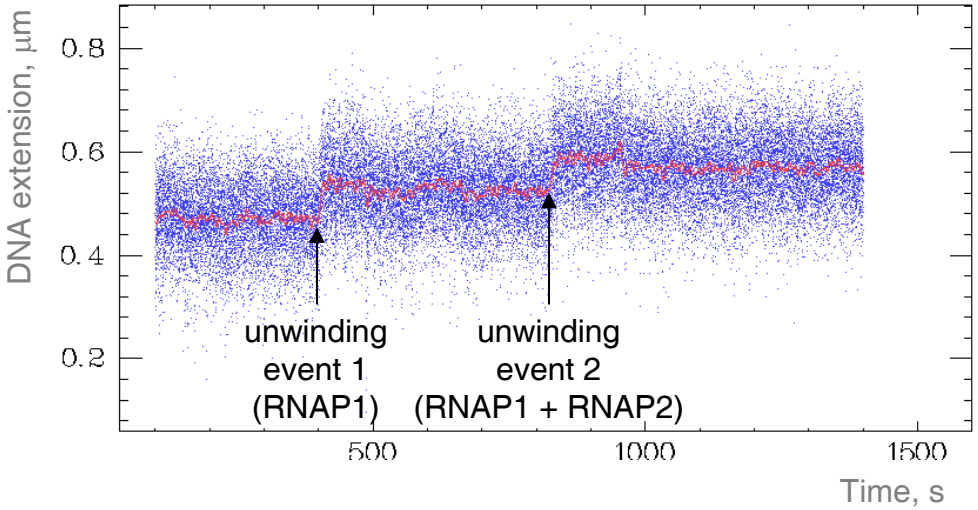
- We have developed a DNA-nanomanipulation assay for promoter unwinding
- The assay permits determination of the number of unwinding intermediates, extent of unwinding, extent of compaction, and kinetics (K_B, k_2, k_{-2})
- We have applied the assay to assess effects of supercoiling, sequence, effectors, and nucleotides
- Supercoiling influences promoter unwinding through mechanical effects (torque), not through structural effects (position or number of supercoil plectonemes)

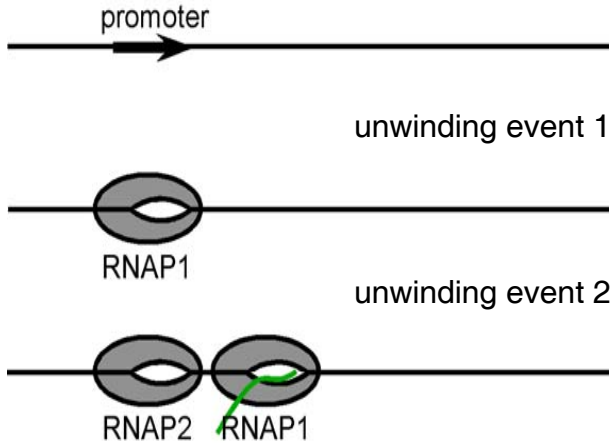
promoter escape

detection of promoter escape rationale

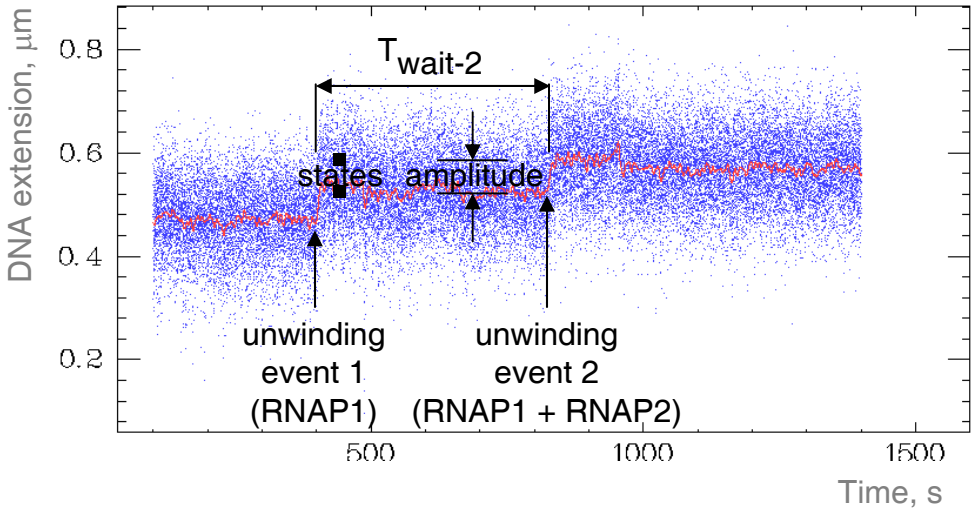


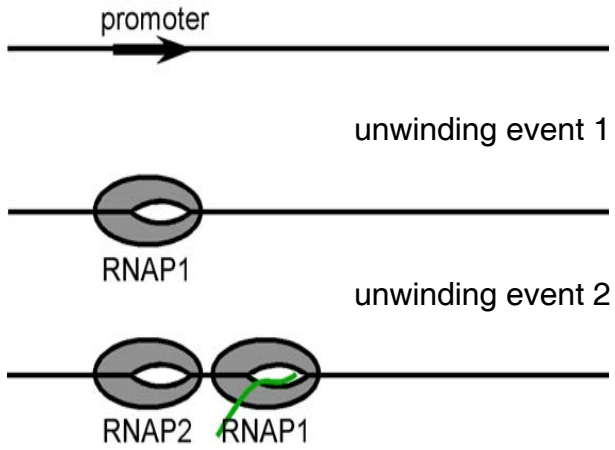
detection of promoter escape data





detection of promoter escape observables, results





- number of states in unwinding event 2 → number of unwinding intermediates in unwinding event 2
- amplitude of change in DNA extension in unwinding event 2 → extent of unwinding and compaction in elongation complex
- time interval between unwinding event 1 and unwinding event 2 (T_{wait-2}) → $T_{clear} + T_{wait}$ **k_{clear}**

$$R+P \underset{}{\overset{K_B}{\rightleftarrows}} RP_c \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} RP_o \xrightarrow{k_{clear}} RD_e$$

detection of promoter escape summary

- We have developed a DNA-nanomanipulation assay for promoter escape
- The assay permits determination of the number of unwinding intermediates in promoter clearance, extent of unwinding in elongation, extent of compaction in elongation, and kinetics (k_{clear})

objectives

objectives

applications

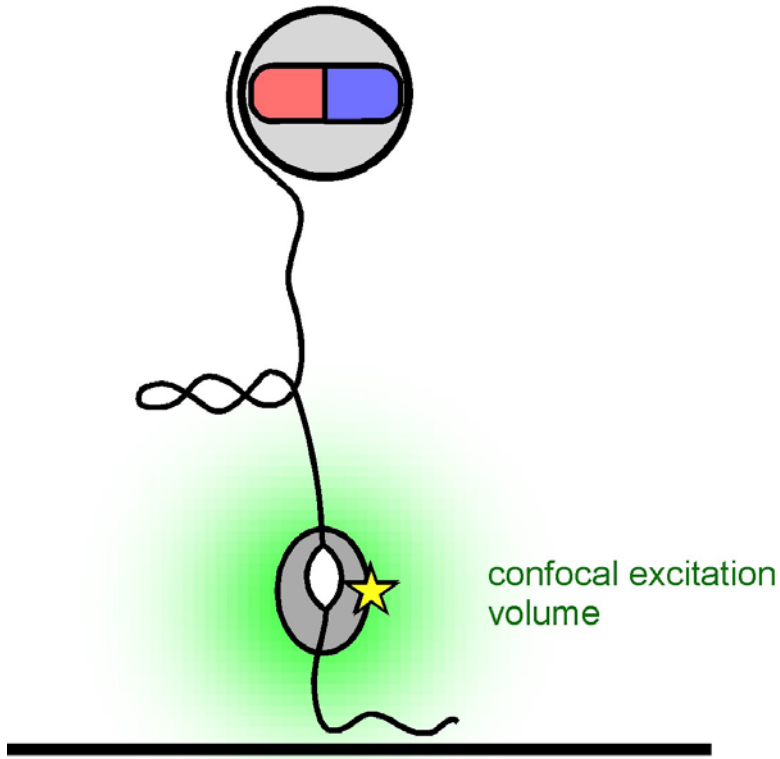
- systematic analysis of effects of activators
- systematic analysis of effects of small-molecule inhibitors
- systematic analysis of ATP-dependent, TFIIH-dependent promoter melting and promoter clearance by eukaryotic RNA polymerase II

methods development

- optimization of DNA-nanomanipulation assay for promoter escape
- development of DNA-nanomanipulation assay for elongation
- development of DNA-nanomanipulation assay for termination
- improvement of temporal resolution
- integration of DNA-nanomanipulation and single-molecule-fluorescence assays

objectives
DNA-nanomanipulation and single-molecule fluorescence

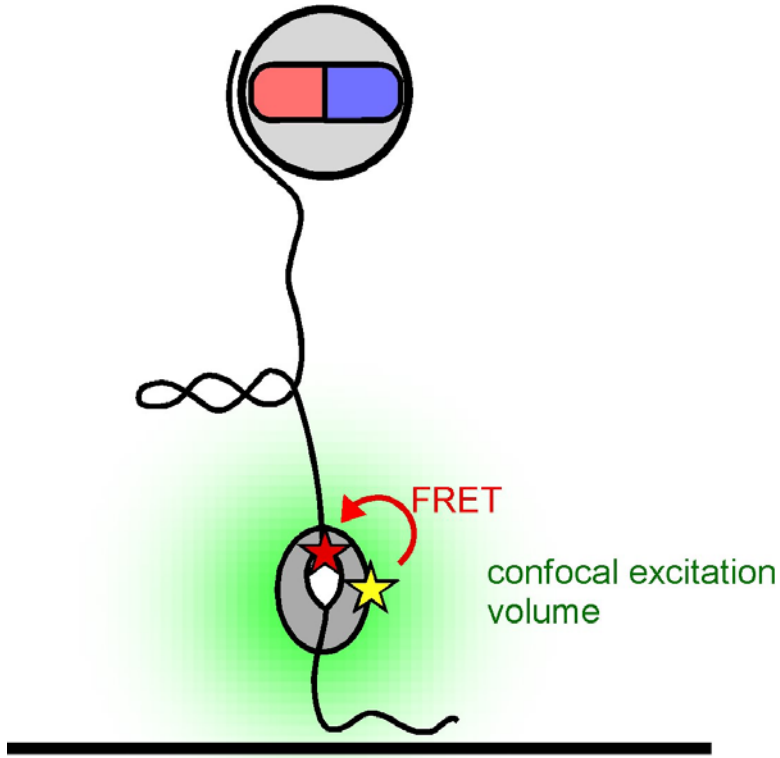
- simultaneous monitoring of DNA unwinding and RNAP binding (RNAP* imaging)



objectives
DNA-nanomanipulation and single-molecule fluorescence

- simultaneous monitoring of DNA unwinding and RNAP binding (RNAP* imaging)

- simultaneous monitoring of DNA unwinding and NTP binding (RNAP*-NTP* FRET)

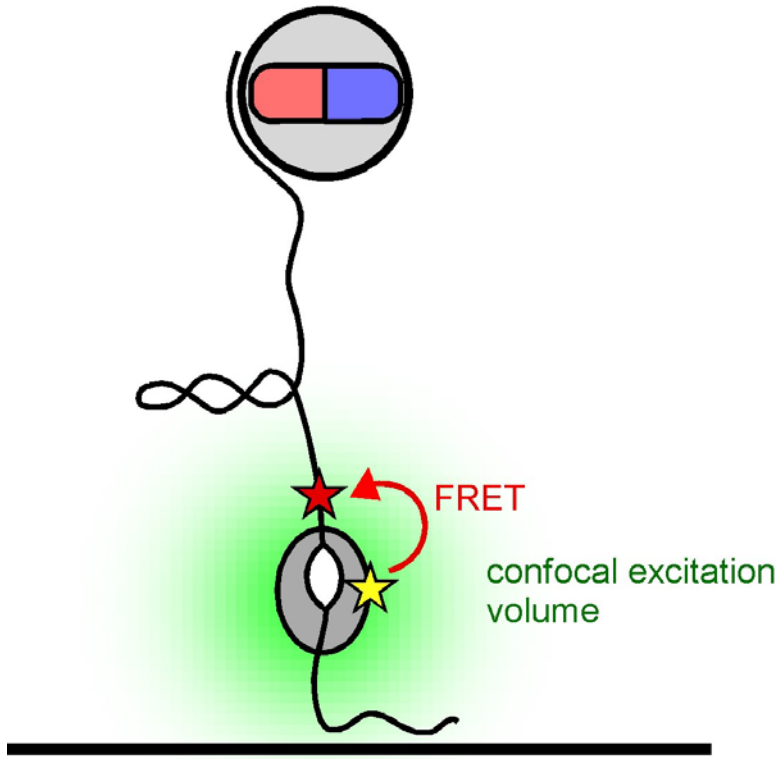


objectives
DNA-nanomanipulation and single-molecule fluorescence

- simultaneous monitoring of DNA unwinding and RNAP binding (RNAP* imaging)

- simultaneous monitoring of DNA unwinding and NTP binding (RNAP*-NTP* FRET)

- simultaneous monitoring of DNA unwinding and RNAP position (RNAP*-DNA* FRET)



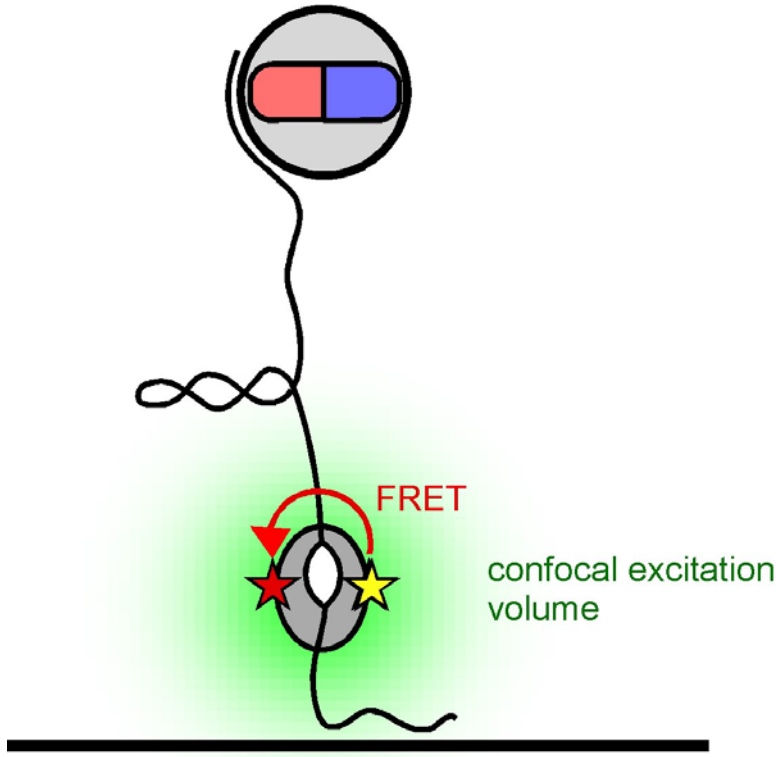
objectives
DNA-nanomanipulation and single-molecule fluorescence

- simultaneous monitoring of DNA unwinding and RNAP binding (RNAP* imaging)

- simultaneous monitoring of DNA unwinding and NTP binding (RNAP*-NTP* FRET)

- simultaneous monitoring of DNA unwinding and RNAP position (RNAP*-DNA* FRET)

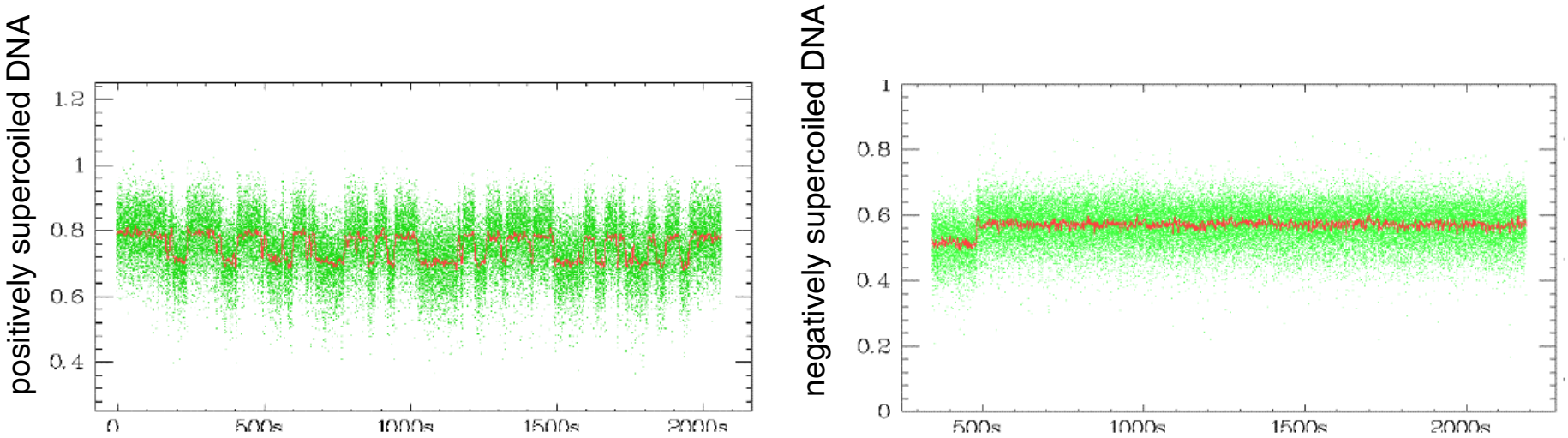
- simultaneous monitoring of DNA unwinding and RNAP conformation (RNAP*/* FRET)

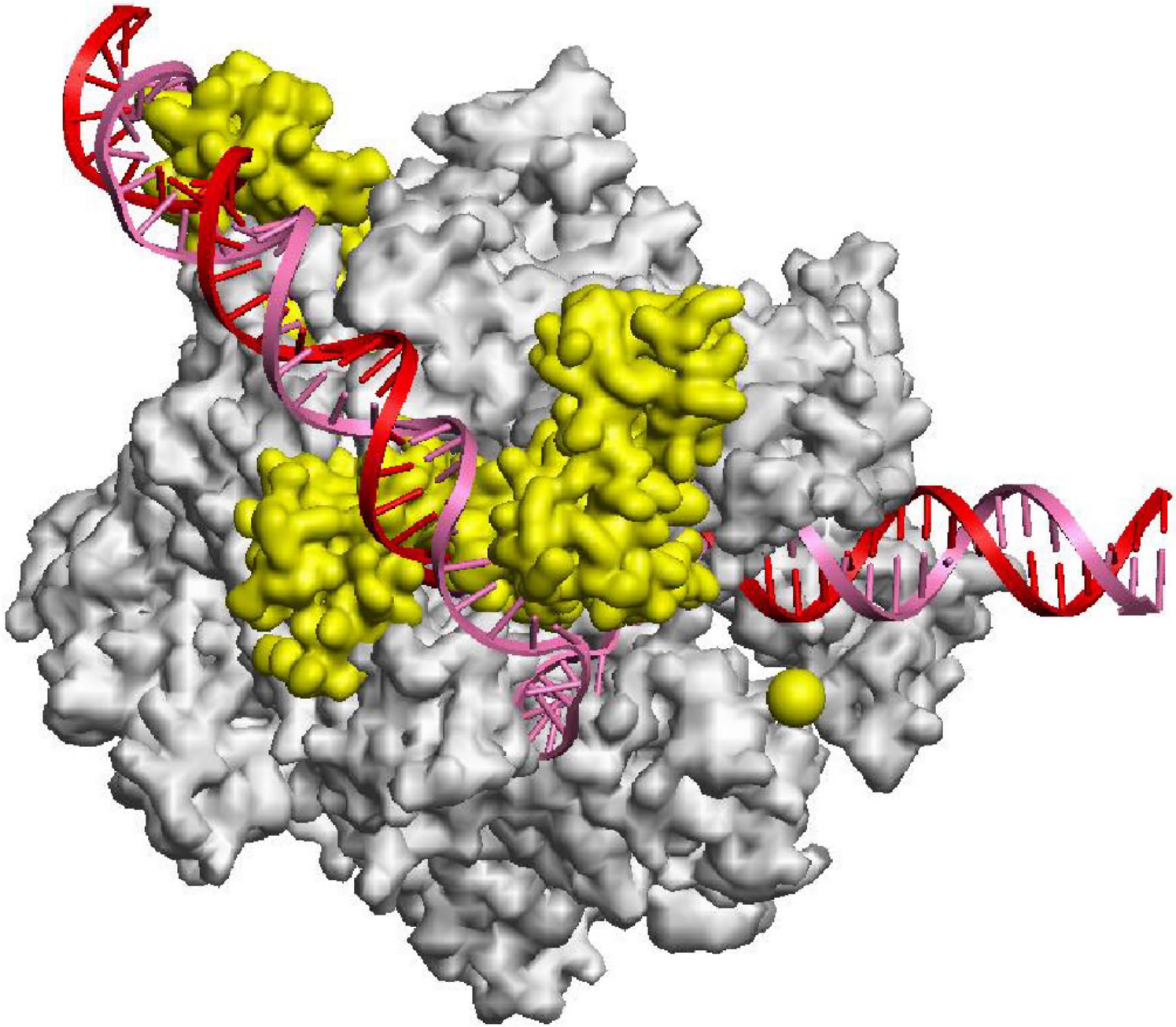


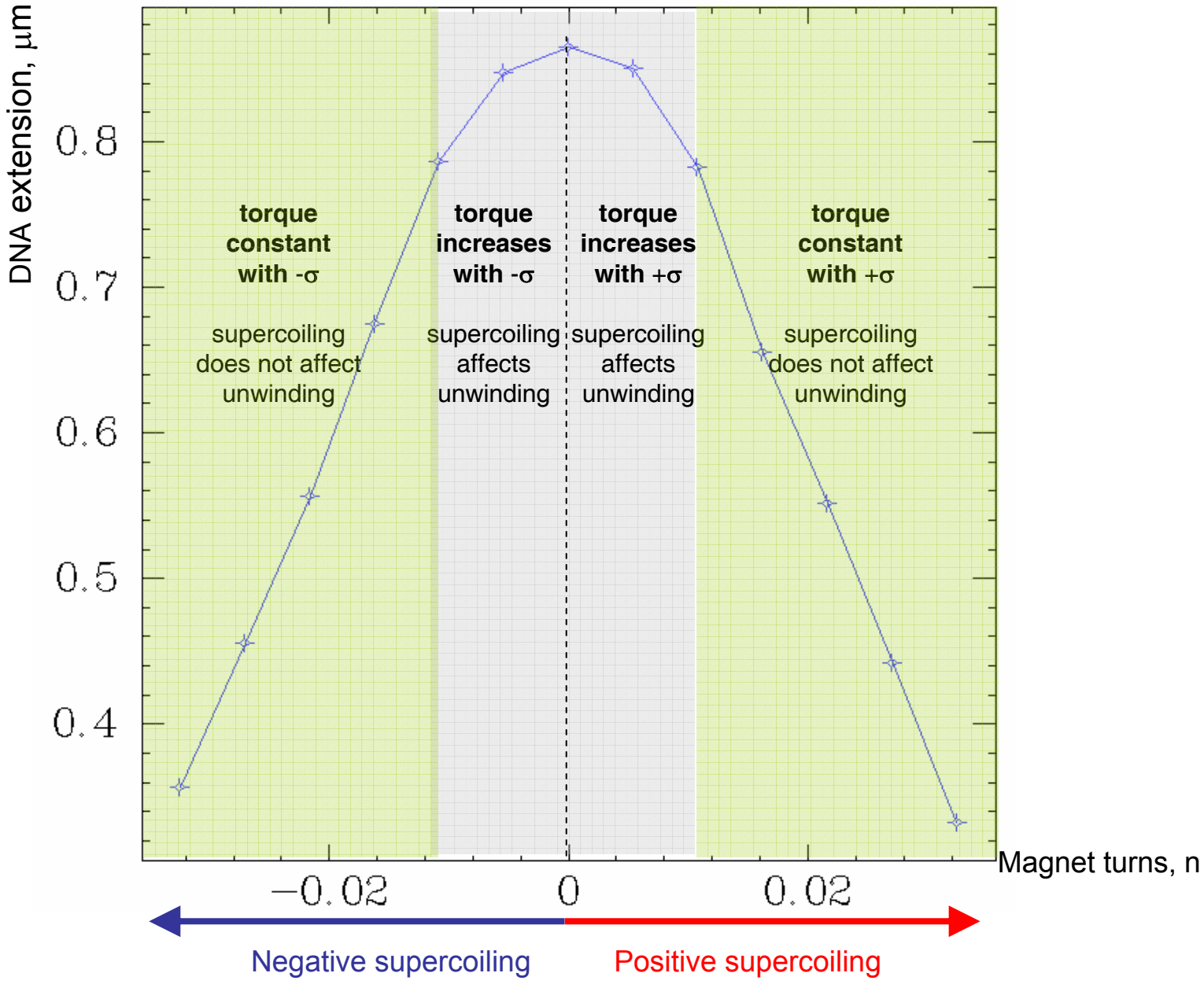
Promoter unwinding and promoter escape by RNA polymerase: analysis by single-molecule DNA nanomanipulation

Andrei Revyakin and Richard H. Ebright
Howard Hughes Medical Institute, Rutgers University

Terence Strick
Institut Jacques Monod







calibration curve: DNA extension versus number of turns