DNA intercalation by ethidium and ruthenium complexes: A quantitative binding study using single molecule DNA stretching

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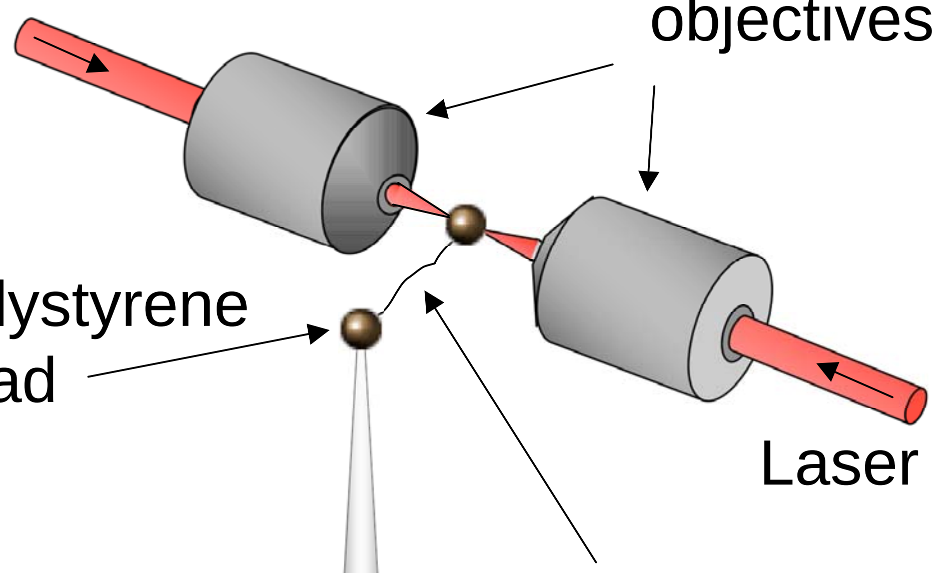
Stretching single DNA molecules

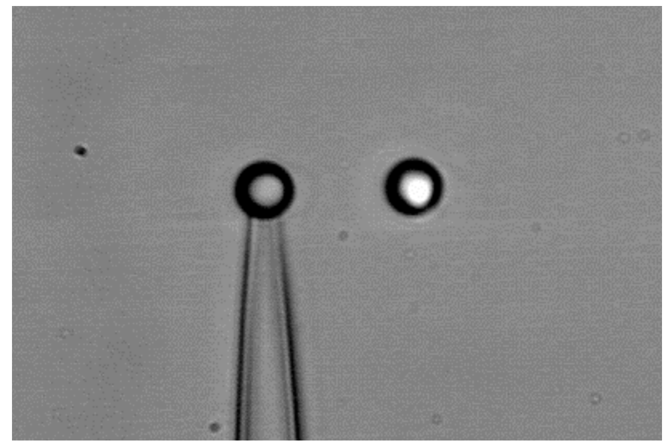
Laser beam

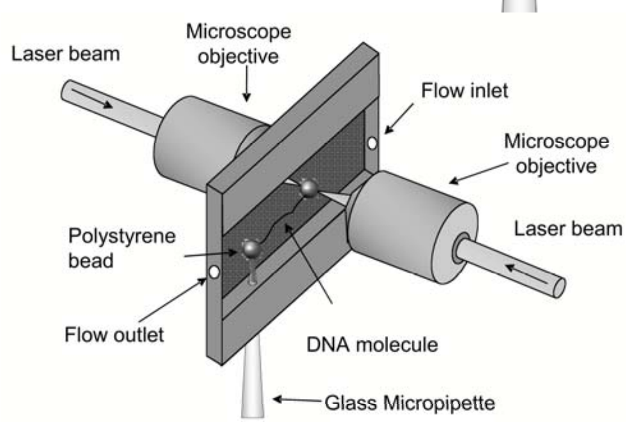
Microscope objectives



Polystyrene bead

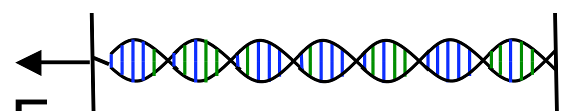
Laser beam





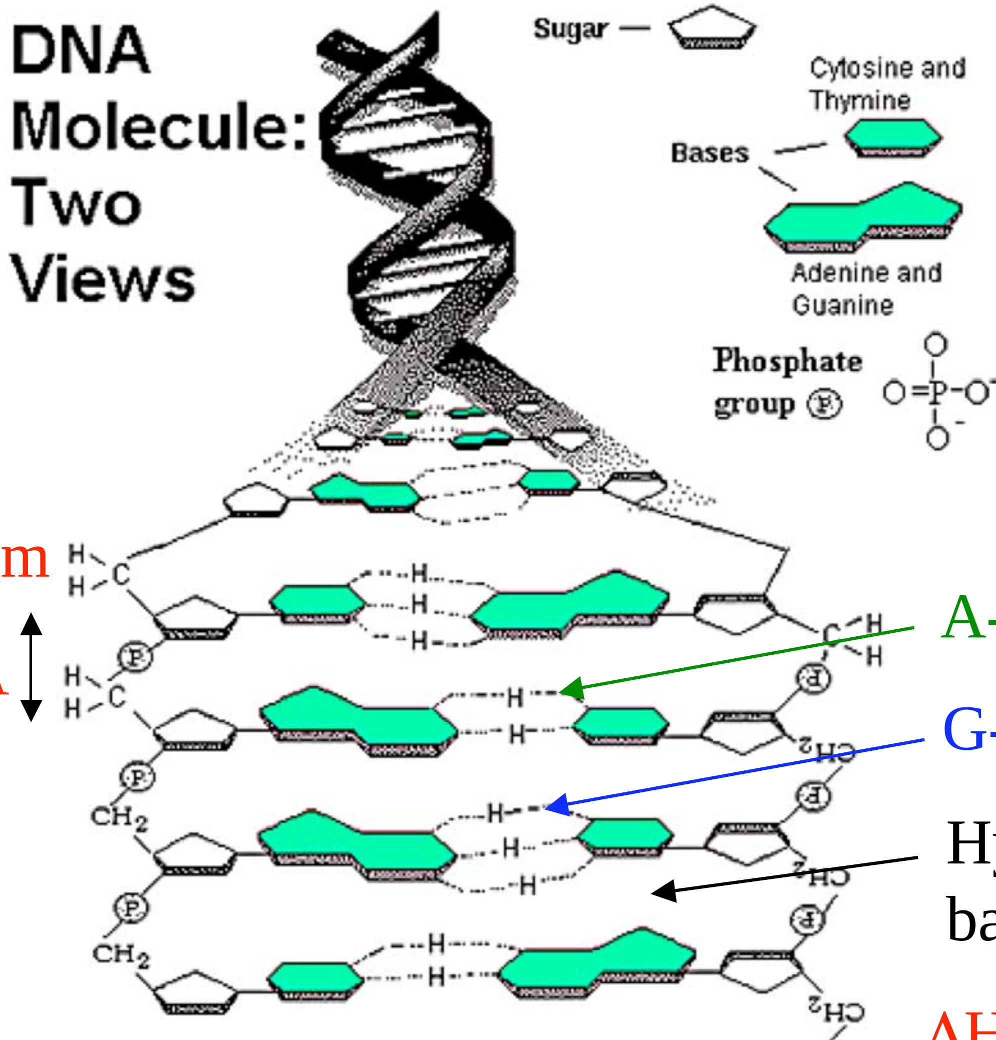
DNA molecule

Glass micropipette



Torsionally relaxed DNA

DNA structure and stability



B-form

3.4 Å

Negatively charged polymer

Cations condensed near surface in solution (Na^+)

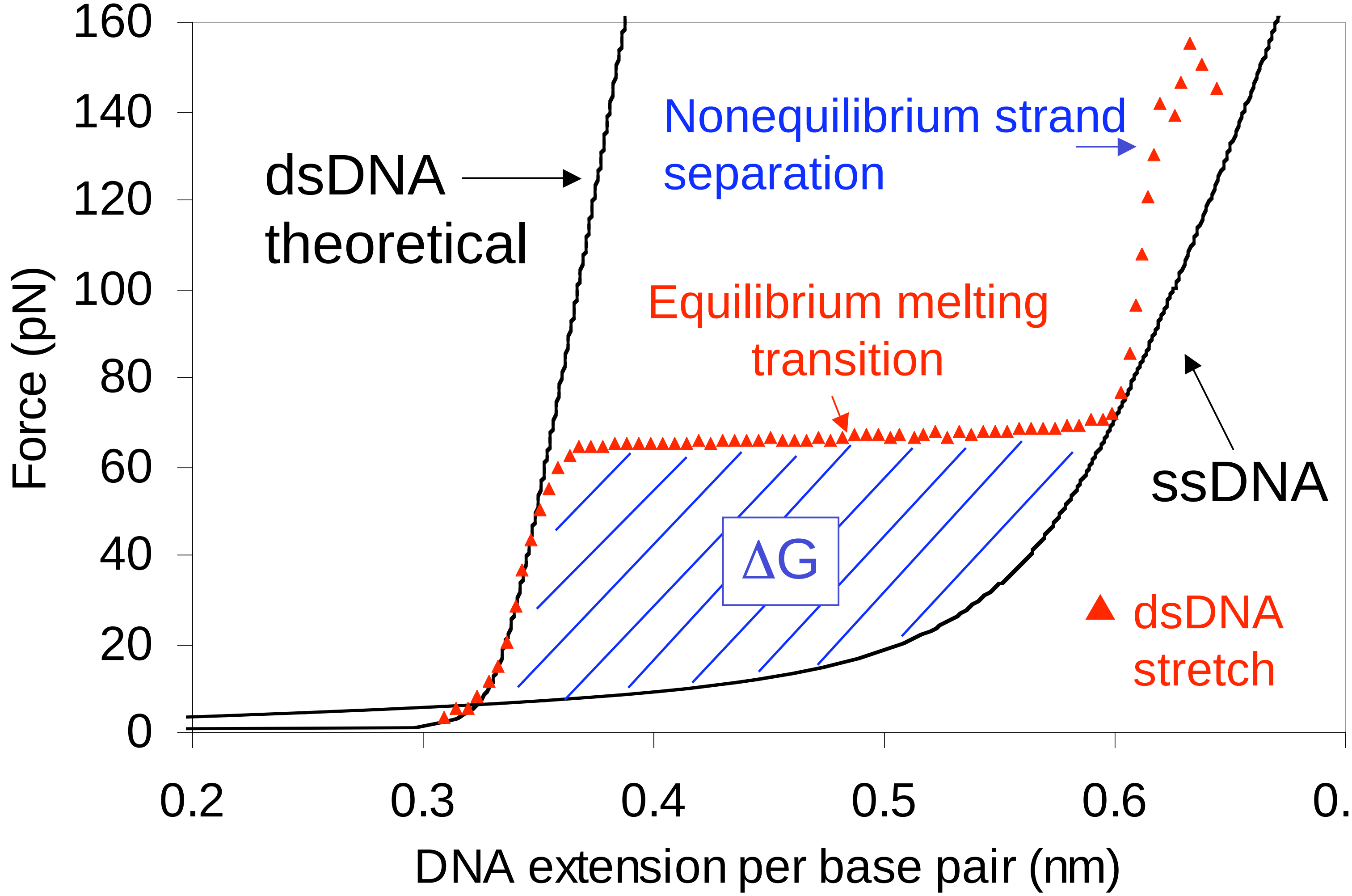
A-T base pair $\Delta H \sim -6\ k_BT$

G-C base pair $\Delta H \sim -12\ k_BT$

Hydrophobic base stacking $\Delta H \sim -6\ k_BT$

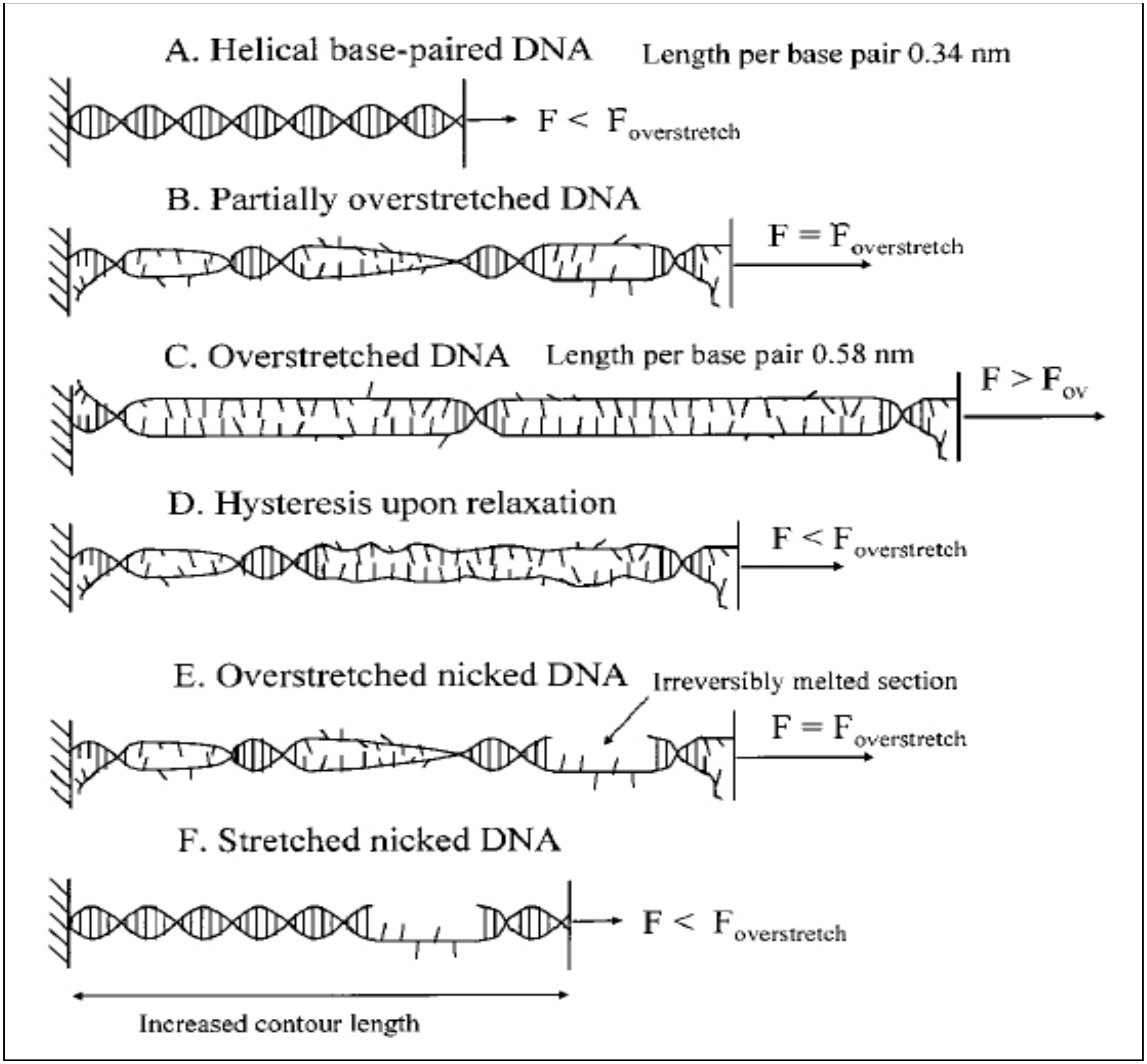
ΔH per base pair $\sim -15\ k_BT$

DNA overstretching is force-induced melting



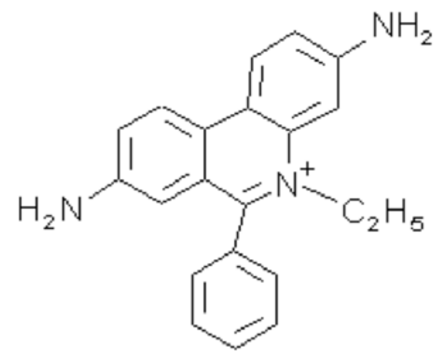
DNA melting free energy ΔG is area under transition region between dsDNA and ssDNA when transition is reversible

Force-induced melting of single DNA molecules

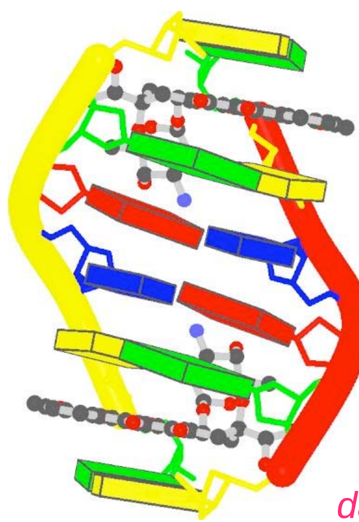


DNA Intercalation

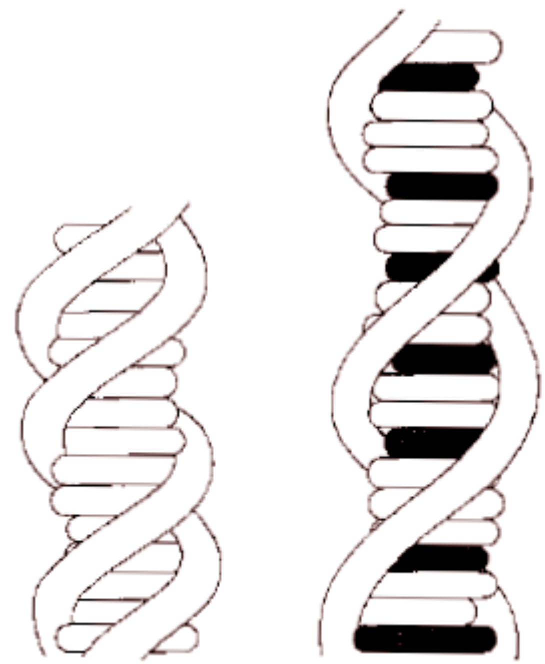
- Ethidium is paradigm for intercalative binding; studied for ~40 years
- Insertion of flat, planar molecules between base pairs of dsDNA leads to length increase by one bp
- Binding/elongation/saturation are hard to characterize.
- What limits intercalation?
- Energetics of intercalation?



Chemical structure of ethidium



Crystal structure of daunomycin- DNA complex

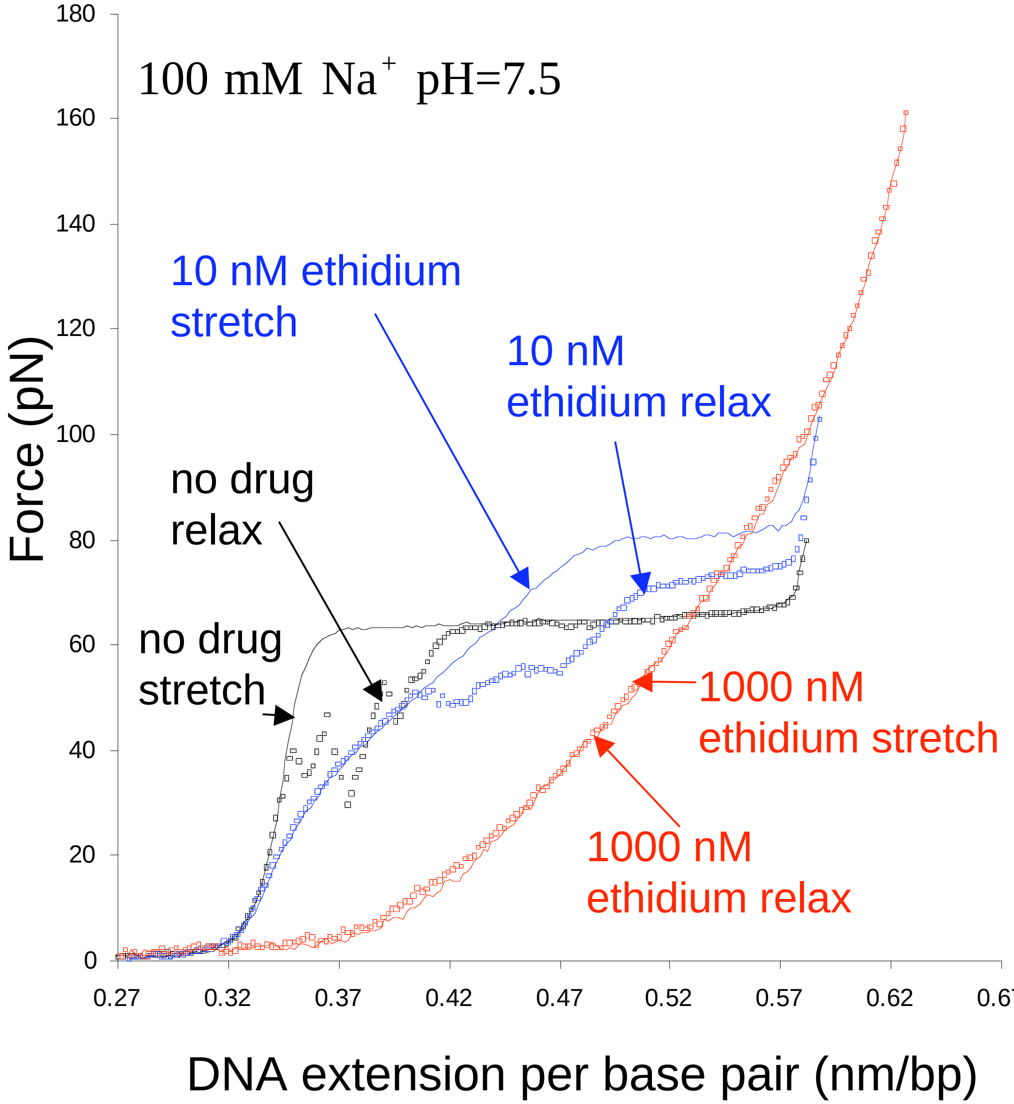


B-DNA

Intercalated DNA

DNA figures from Cantor and Schimmel

Stretch relax cycles of the DNA-ethidium complex



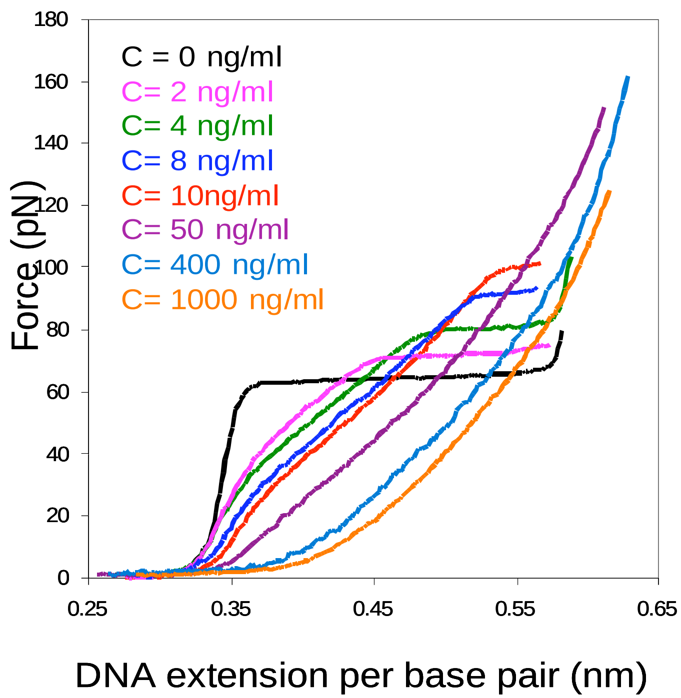
Low concentrations of Ethidium :

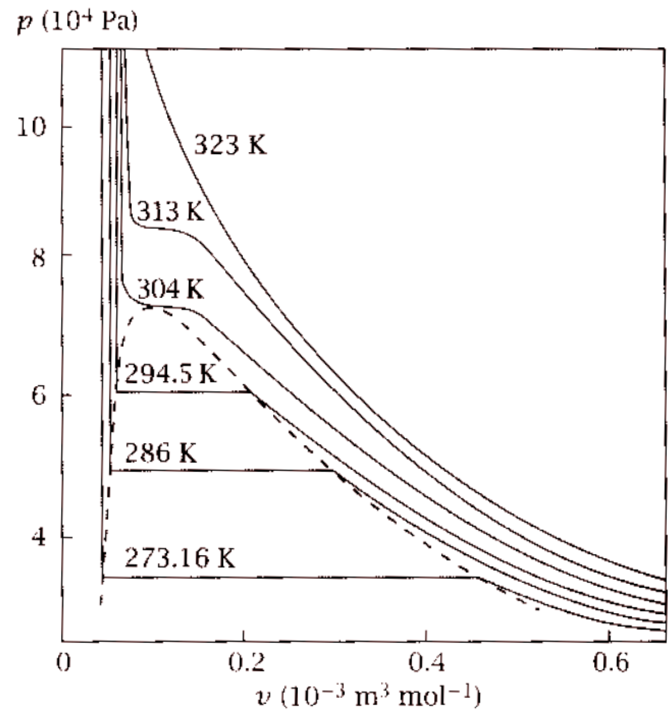
- Melting force increases
- dsDNA contour length increases
- Range of extensions of the force-induced melting transition shrinks
- Hysteresis appears upon stretching into melting plateau

High concentrations of Ethidium :

- Force-induced melting transition vanishes
- Strong increase in contour length of dsDNA
- No hysteresis

Phase diagram of single DNA molecule and critical point



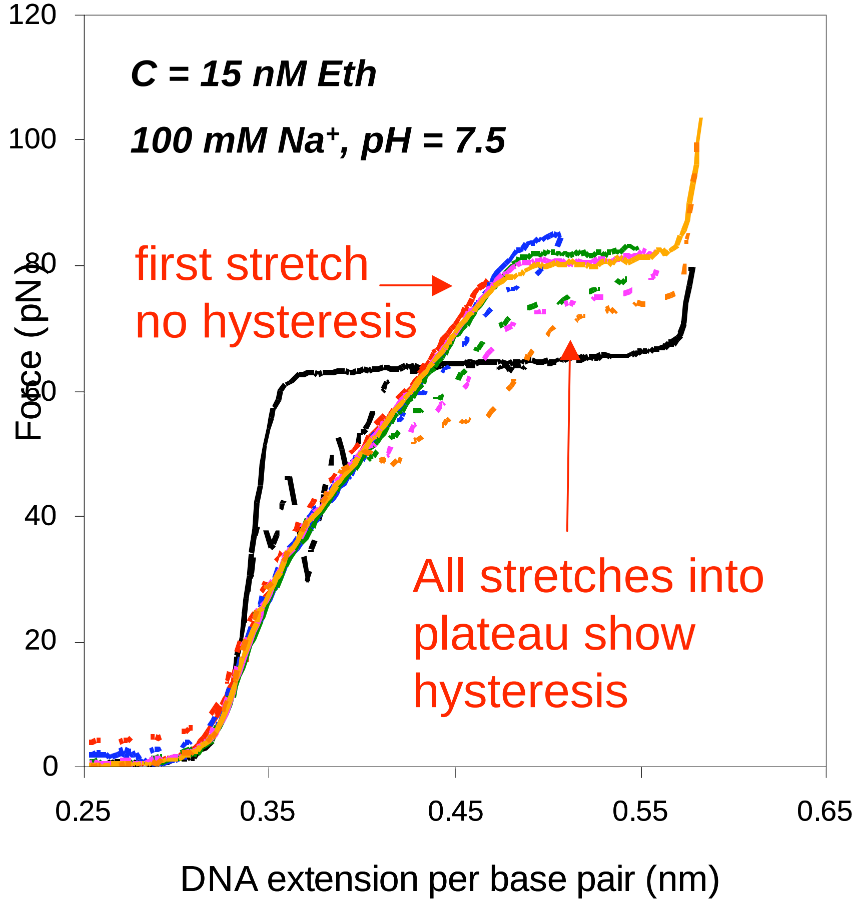


From: Dill and Bromberg, "Molecular Driving Forces"

$P \rightarrow F$ $-V \rightarrow x$ $T \rightarrow [\mathrm{EtBr}]$

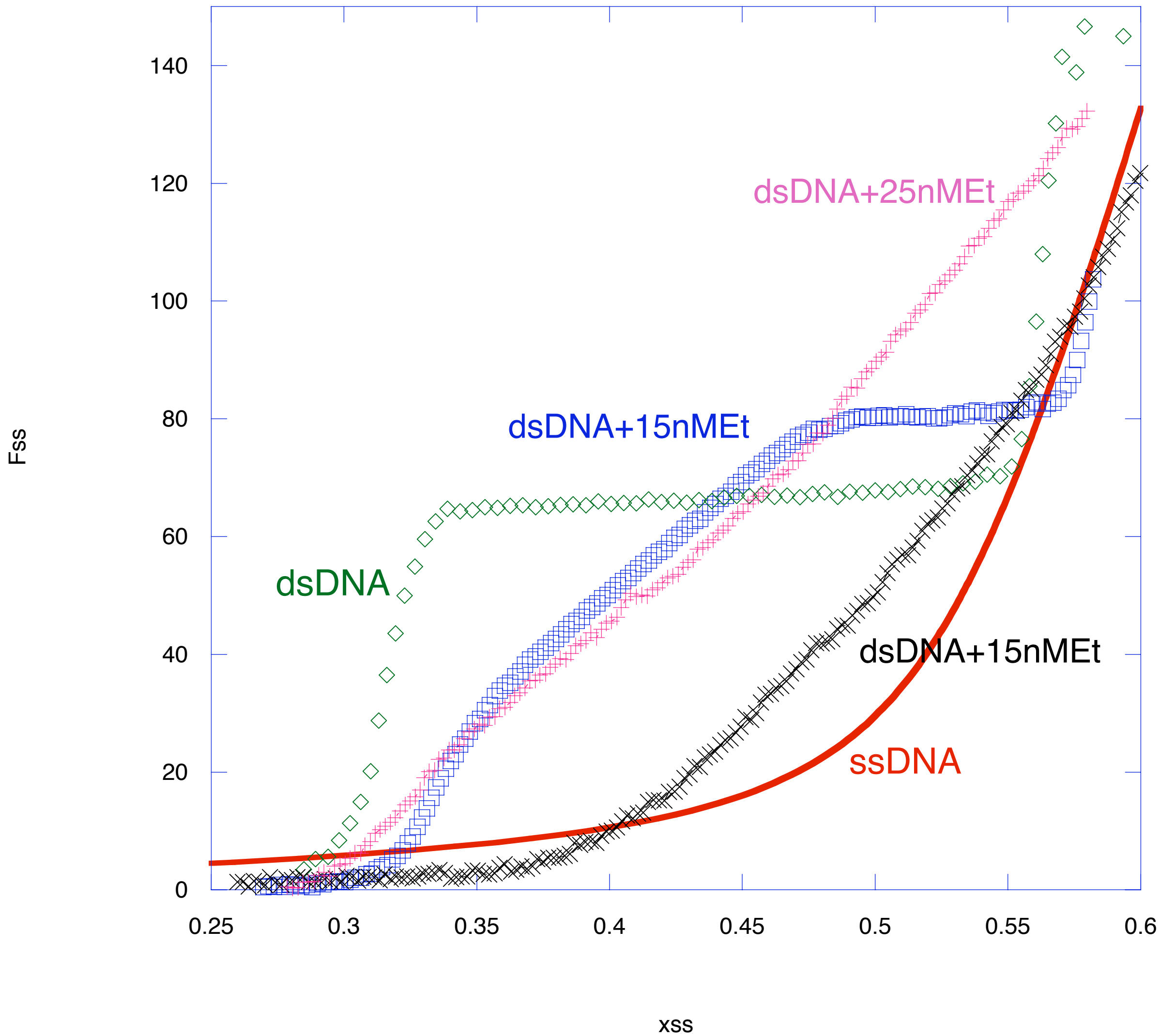
- 3D system maps to 1-D system

Hysteretic behavior confirms melting nature of force-induced transition in dsDNA



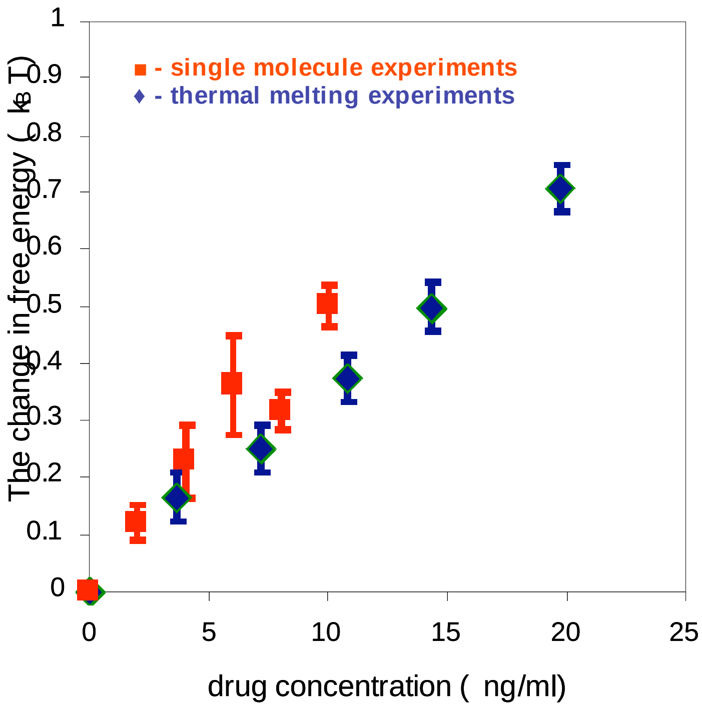
- Always hysteresis when stretching into the plateau
- No hysteresis before the transition
- Further stretching into plateau generates more hysteresis
- We never see hysteresis at high concentrations, where there is no plateau

Critical [EtBr]~25 nM is determined by condition of melting free energy per bp being equal to maximum work per bp that the force can do on dsDNA/Et to promote melting



Melting free energy increases with [Eth]; Work decreases with [Eth]-> critical point

DNA melting free energy increases upon Et or Ru intercalation



Single molecule experiments

Change in DNA melting free energy is change in work done by stretching

$$\Delta G(F, Eth) = \int_0^F df \cdot \left(x_{ss}(f) - x_{ds-Eth}(f)\right)$$

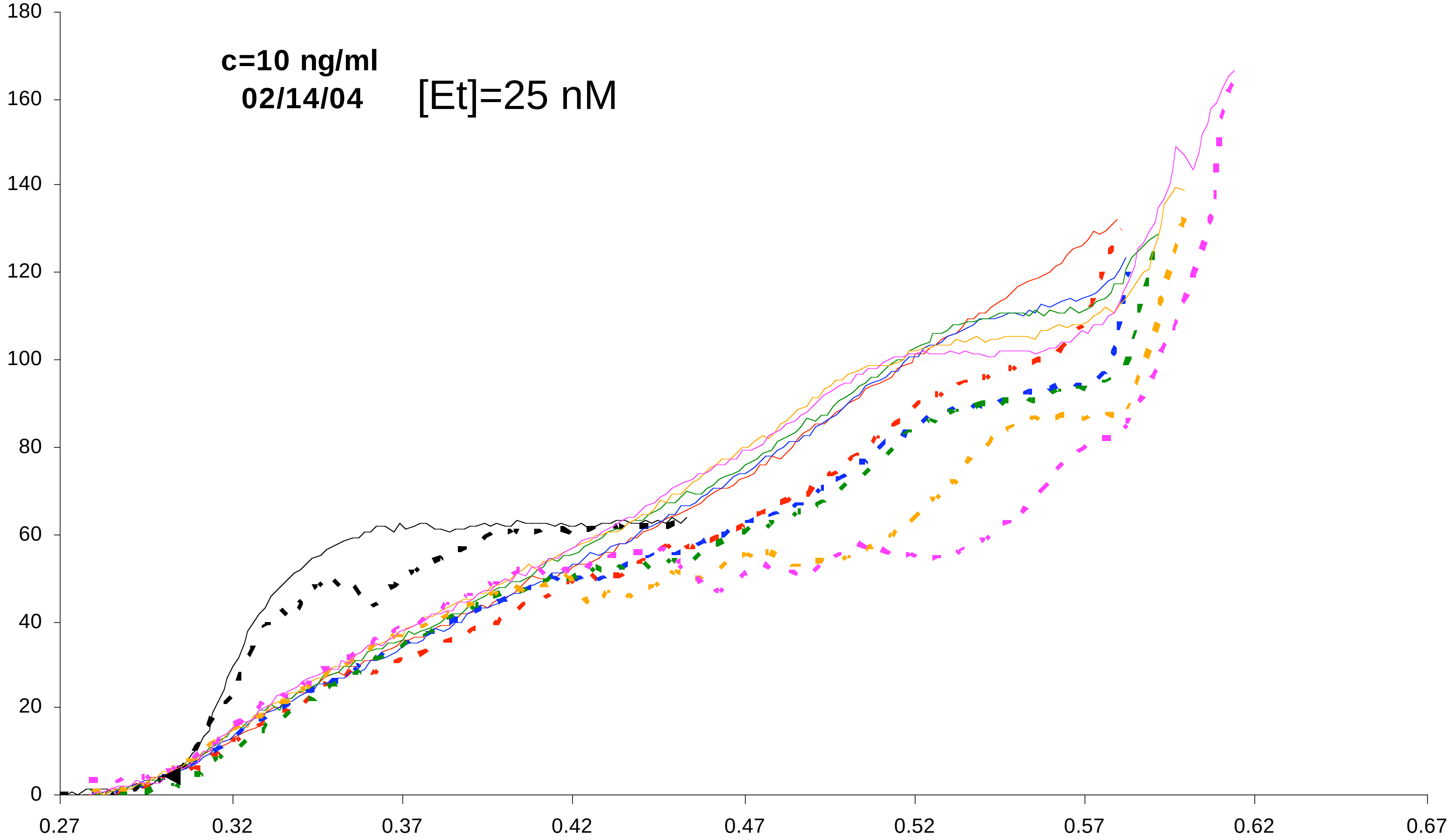
Thermal melting experiments

Change in DNA melting free energy is given by change in melting temperature

$$\Delta G^0\left(Eth\right) = \Delta S_{EtBr} \cdot \left(T_0 - T_m(Eth)\right)$$

- **DNA duplex stabilization upon ethidium binding measured by single molecule force-induced melting and conventional thermal melting are consistent with each other**
- **DNA/intercalator binding/duplex melting are in equilibrium**

Near the critical point $[Et]_{cr}$=25 nM stretching hysteresis becomes large and variable

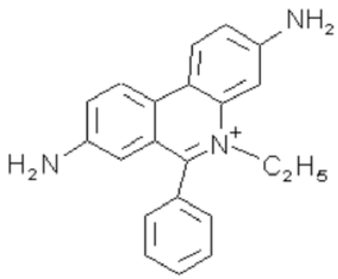


At $[Et]_{cr}$ all DNA is either dsDNA/Et or ssDNA

Transition is slow; DNA tends to be in metastable state

Force-induced melting of dsDNA-Ruthenium intercalator complex

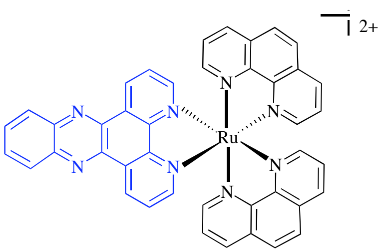
Ethidium is considered the paradigm for intercalative binding



- MW: 394.3
- Charge: +1
- Binding mode: intercalation ($K_B \sim 10^5$ M^{-1})
- Binding specificity: non-specific
- Effect on DNA: unwinding of dsDNA by 26°

Ruthenium(II) polypyridyl complexes have in common a basic motif, a Ru(II) center surrounded by three heterocyclic, aromatic ligands

- MW: 700 – 800
- Charge: +2
- Binding specificity: unknown

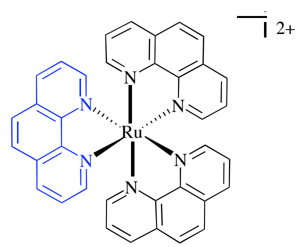


Δ-Ru(phen)$_2$(dppz)$^{2+}$

$K_B \sim 10^6$ M^{-1}

intercalative binding
(from the major groove)

positive control

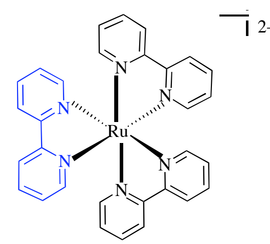


Δ-Ru(phen)$_3^{2+}$

$K_B \sim 10^4$ M^{-1}

intercalation is diminished

partial intercalation
and/or groove-binding are likely



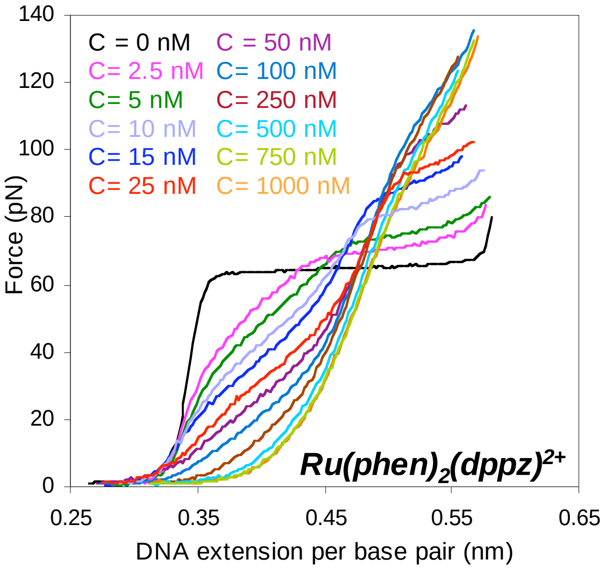
Δ-Ru(bpy)$_3^{2+}$

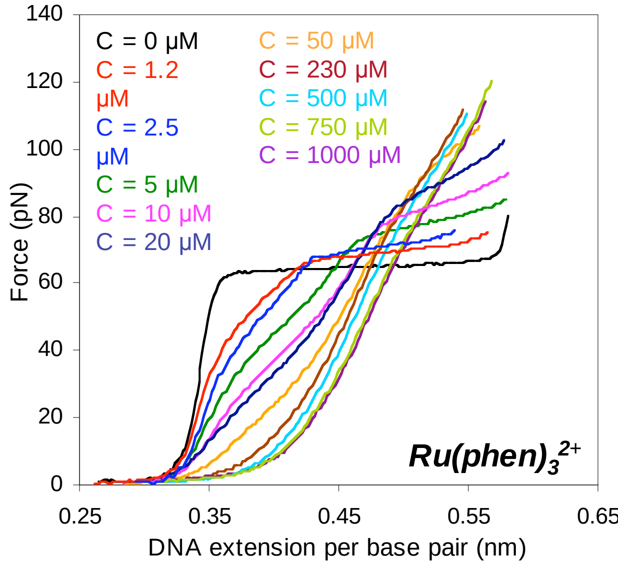
the complex does not intercalate

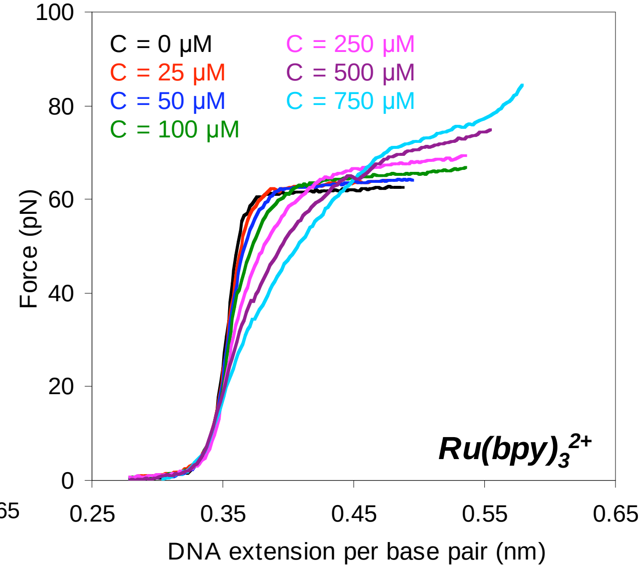
electrostatic binding

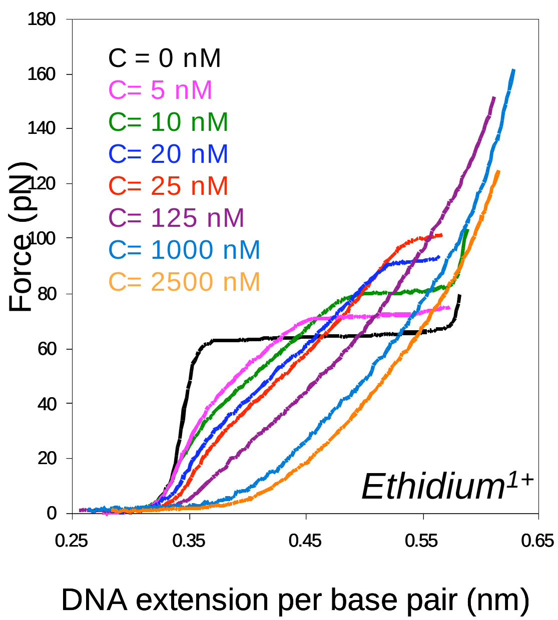
negative control

All Ru and Eth - DNA complexes have qualitatively similar force-extension behavior



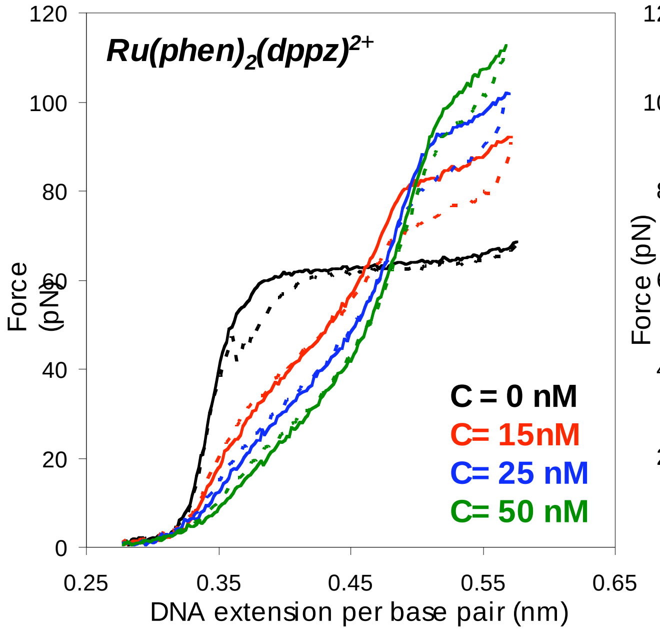


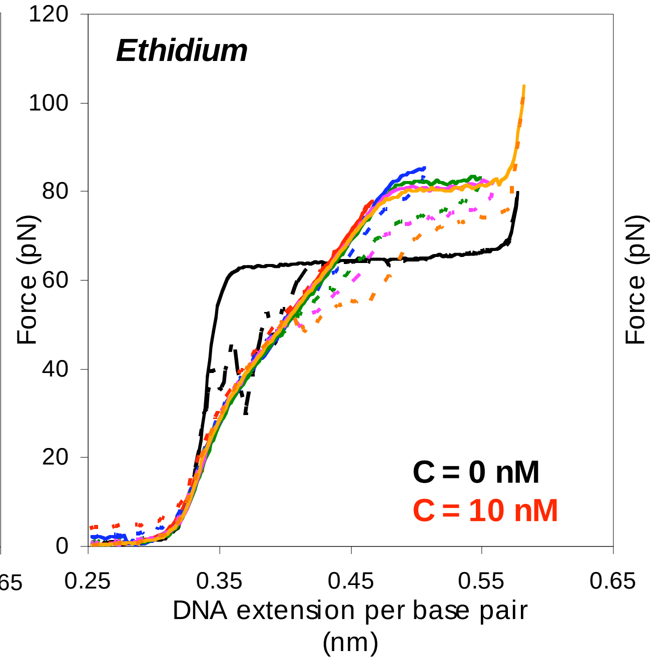


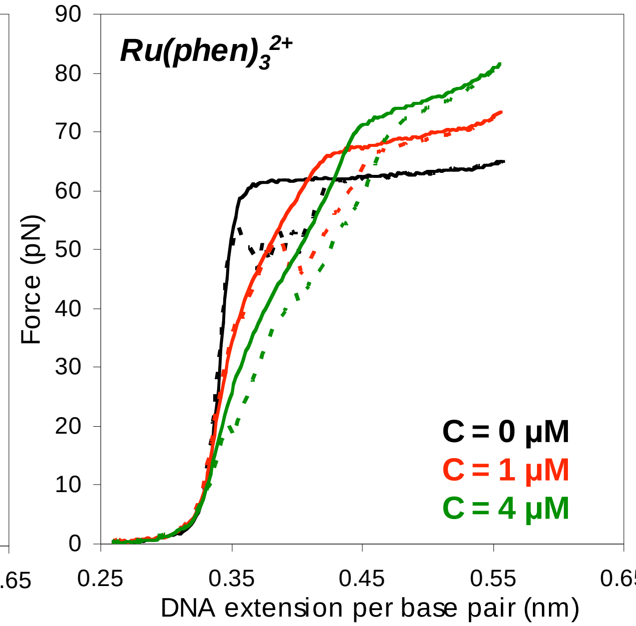


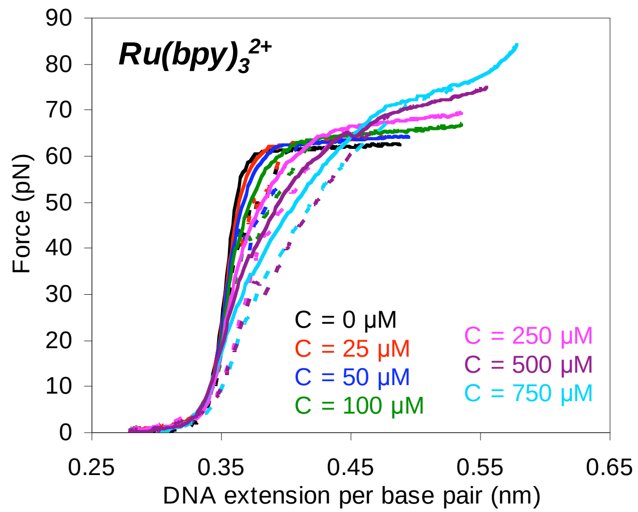
- Both $Ru(phen)_3^{2+}$ and $Ru(phen)_2(dppz)^{2+}$ intercalate to dsDNA
- $Ru(bpy)_3^{2+}$ is able to intercalate only at high forces and concentration
- $Ru(phen)_3^{2+}$ has a lower affinity for binding to DNA
- Critical point observed for phase transition at:
  - 25 nM [EtBr]
  - 100 nM [$Ru(phen)_2(dppz)^{2+}$]
  - 75 μM [$Ru(phen)_3^{2+}$]

Ru and Eth ligands are fast interaclators that stabilize dsDNA and do not bind ssDNA





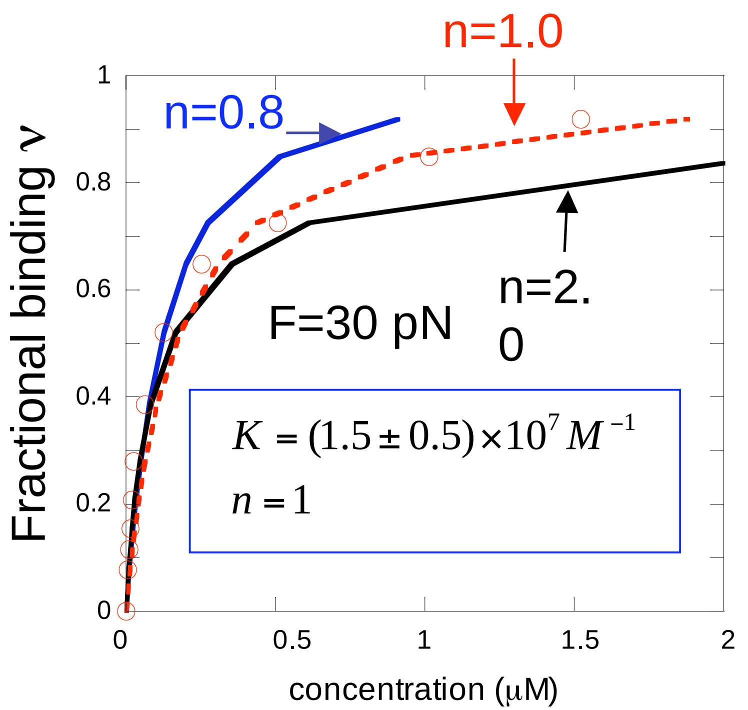




- Hysteresis observed when stretching into the plateau, never before the plateau
- Further stretching into the plateau gives more hysteresis
- Hysteresis is never observed at concentrations above the critical point

Single molecule measurement of ethidium binding constant and site size

From dsDNA/Et contour length we can estimate fractional Et binding



$$K = (1.5 \pm 0.5) \times 10^{7} M^{-1}$$
$$n = 1$$

$$\nu(F,C) = \frac{x(F,C) - x_{ds}(F)}{x_{ds}(F)}$$

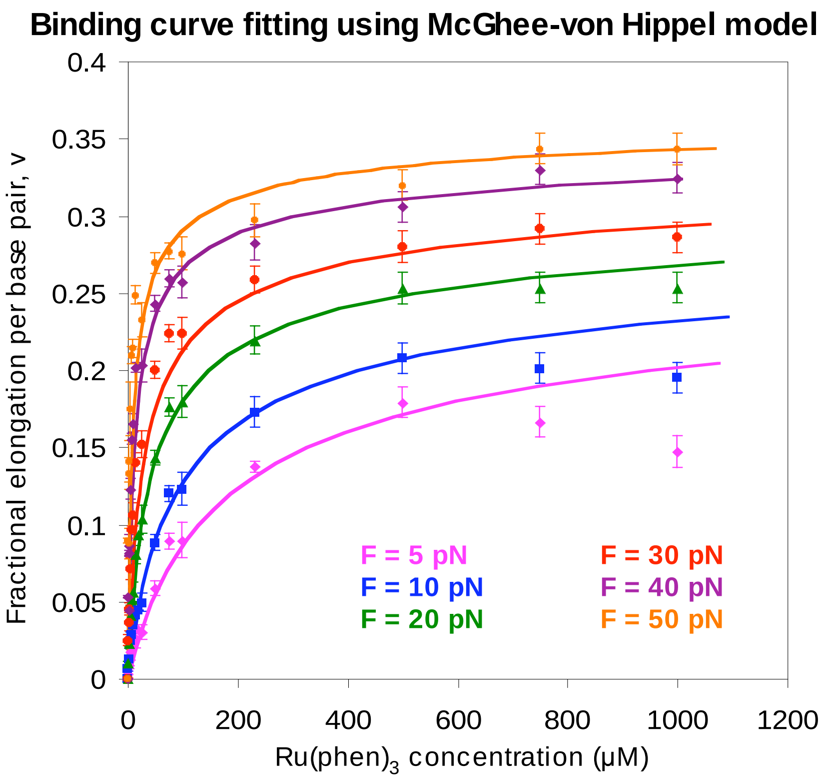
$$\nu(C) = K \cdot C \cdot \frac{(1 - n\nu)^{n}}{(1 - n\nu + \nu)^{n-1}}$$

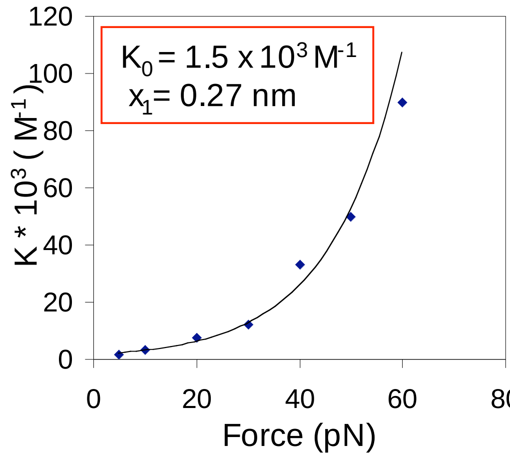
Binding curve fit using McGhee – von Hippel model

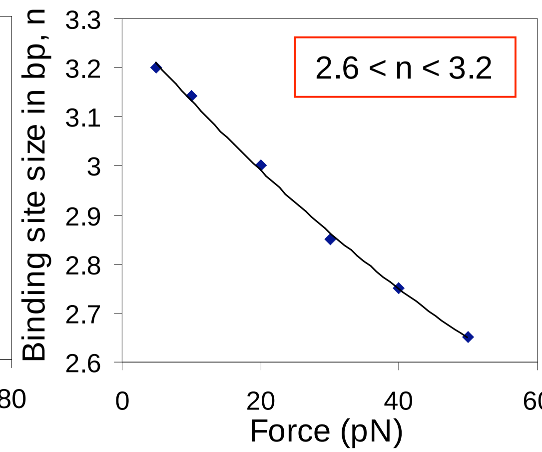
Magnitude of binding constant and binding site size depend on force!

Binding site size of n=1.0 at 30 pN shows that high force promotes ethidium intercalation between every DNA base pair

Force exponentially increases intercalator/dsDNA binding constant and promotes additional intercalation





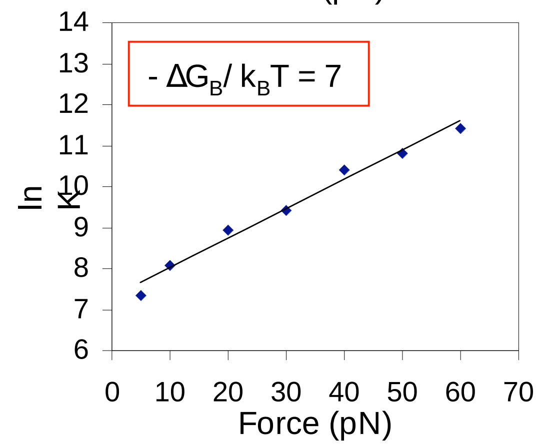


$$K(F) = K_0 \cdot e^{x_1 F / k_B T}$$

$$K_0 = \frac{1}{C_0} \cdot e^{-\Delta G_B / k_B T}$$

$$-\Delta G_B / k_B T = \ln(K_0 \cdot C_0)$$

$$C_0 \sim 1M$$

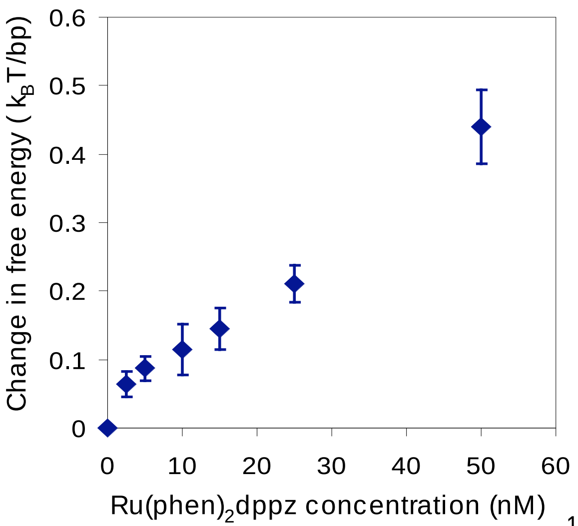


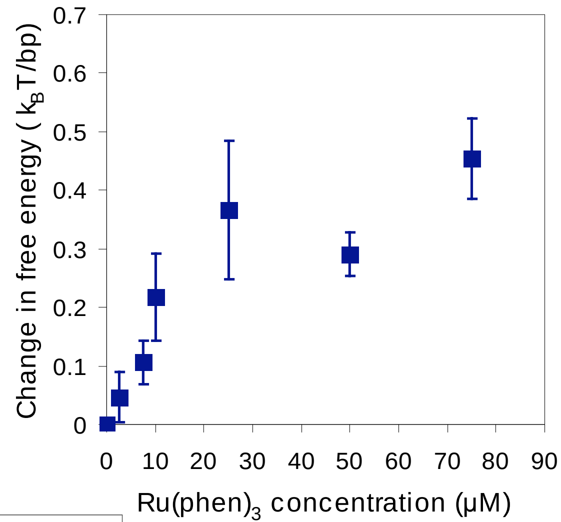
$$v(F,C) = \frac{x(F,C) - x_{ds}(F)}{x_{ds}(F)}$$

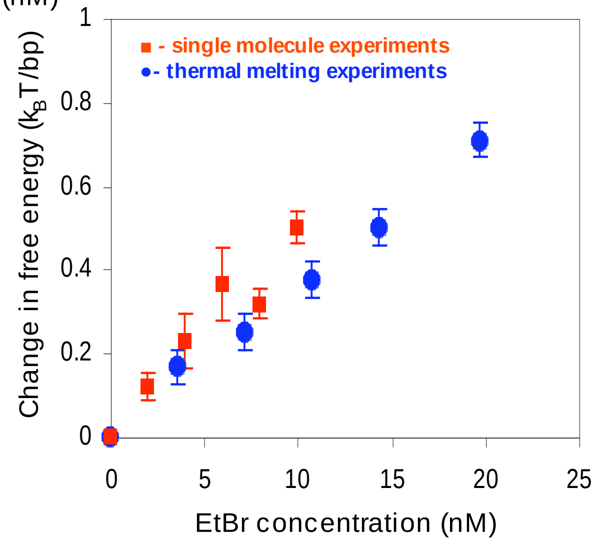
$$v(C) = K \cdot C \cdot \frac{(1 - nv)^n}{(1 - nv + v)^{n-1}}$$

v = fractional elongation/intercalation per base pair
n = binding site size in base pairs
C = drug concentration
C_0 = the maximum concentration of DNA-bound ligand
K_0 = binding constant of the ligand in the absence of applied force
x_1 = elongation upon single molecule intercalation
ΔG_B = ligand binding free energy

Ru and Eth Intercalators stabilize dsDNA







Ru intercalators stabilize dsDNA but to lesser extent then Et and at higher concentrations

Thermodynamic parameters describing DNA intercalation

	K_0, M^{-1}	$-\Delta G_B/kT$	x_1, nm	n_1-bind.site size, F=5pN	n_2-bind.site size, F=60pN
Et^{1+}	$5.0x10^5$	12	0.25	2.5	1.5
Ru $dppz^{2+}$	$1.2x10^6$	14	0.31	3.4	2.3
Ru phe^{2+}	$1.5x10^3$	7	0.27	3.2	2.6

n = binding site size in base pairs

$\mathbf{K_0}$ = binding constant of the ligand in the absence of applied force

$\mathbf{x_1}$ = elongation upon single molecule intercalation

$\mathbf{\Delta G_B}$ = ligand binding free energy