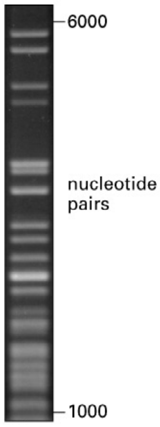
Separating double-stranded DNA

Double-stranded DNA is stiff: Kuhn length b = 100 nm

→ use agarose gel with open pores: a = 100 - 300 nm

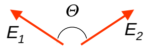
Can separate up to ~20 kb with E < 5 V/cm



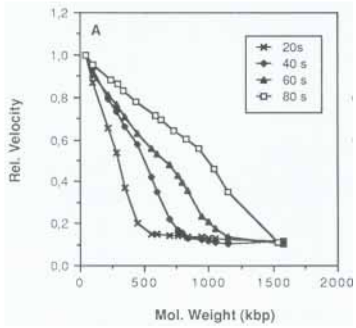
Pulsed field gel electrophoresis (PFGE)

Schwartz & Cantor '84

Apply field alternately along two different directions



- good resolution if angle Θ is obtuse
- size limit increases with pulse time T



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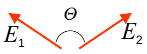
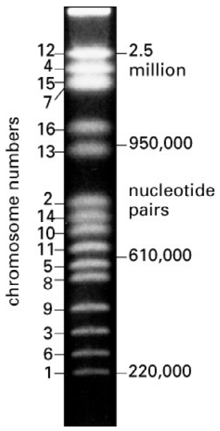


- good resolution if angle Θ is obtuse
- size limit increases with pulse time T

→ Permits separation of restriction fragments and whole chromosomes of simple organisms

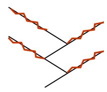


Pulsed field gel electrophoresis

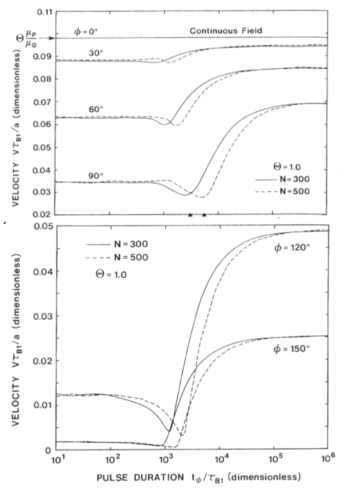
Southern et al. '87; Slater & Noolandi '89

acute

obtuse



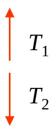
- separation due to transient dynamics



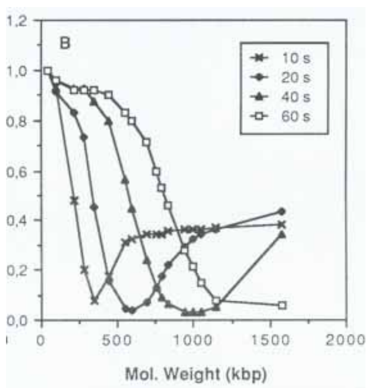
Field-inversion gel electrophoresis (FIGE)

Carle, Frank & Olson '86

Apply field in forwards and reverse directions for different periods



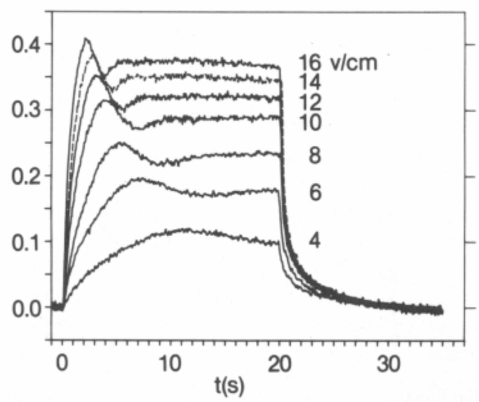
Band inversion:
large molecules travel faster than smaller ones



Transients in orientation

Holzwarth et al. '87

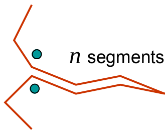
Birefringence f

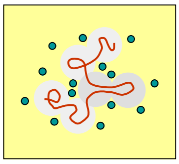


Hernias

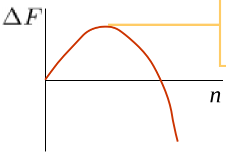
Deutsch '87

Field can pull loop of chain between adjacent gel fibres





- entropy loss $\frac{\Delta S}{k} \sim n$
- energy gain $\frac{\Delta U}{kT} \sim n^2 \epsilon_a$

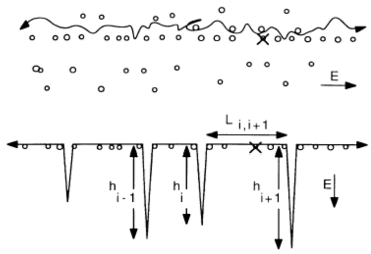


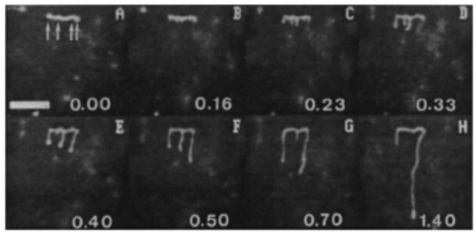
$$\frac{\Delta F^*}{kT} \sim \frac{1}{\epsilon_a}$$

→ No reptation if $\epsilon_a > 1$

Hernias

Deutsch '8; Gurrieri et al. '90





Langevin simulation

Deutsch '88

- external forces

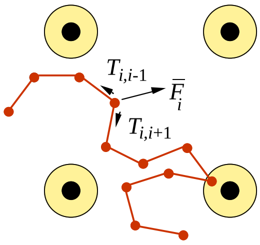
$$\bar{F}_i = \bar{F}_E + \bar{F}_{obs,i} + \bar{\xi}_i$$

- thermal noise

$$\langle \bar{\xi}_i(t).\bar{\xi}_i(t') \rangle = 2kT\zeta\,\delta(t-t')$$

- chain connectivity

$$(\bar{r}_{i+1} - \bar{r}_i).(\bar{r}_{i+1} - \bar{r}_i) = b^2 \qquad \forall i$$



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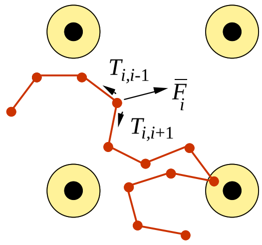
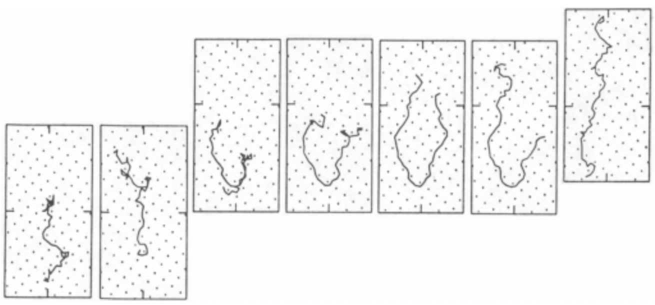


→ $$\zeta \frac{d\bar{r}_i}{dt} = \bar{F}_i + T_{i,i-1}(\bar{r}_{i-1} - \bar{r}_i) + T_{i,i+1}(\bar{r}_{i+1} - \bar{r}_i)$$

Langevin simulation
dynamics

Deutsch '88

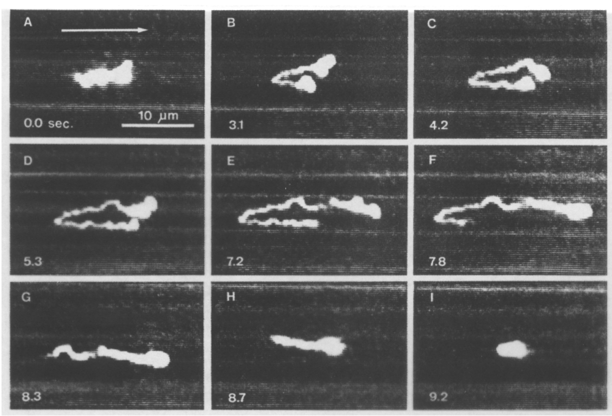
Episodic motion



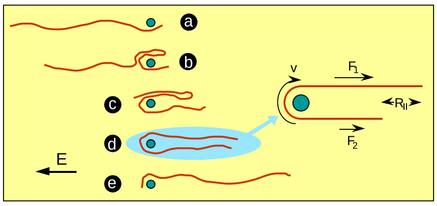
Video microscopy
dynamics

Yanagida '83; Gurrieri & Bustamante '97

T2 DNA (164 kb)



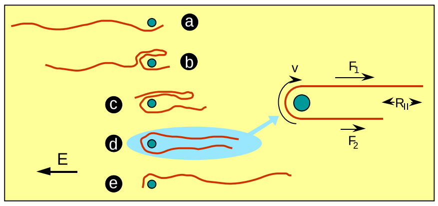
Disengagement time



$$\frac{dR_{\parallel}}{dt} = 2v = \frac{F_1 - F_2}{\zeta_{tot}} \sim \frac{R_{\parallel}}{L}$$

$$\rightarrow \quad t_{disengage} \sim \frac{L}{2\mu_0 E} \log\left(\frac{L}{R_{\parallel}(0)}\right)$$

Disengagement time



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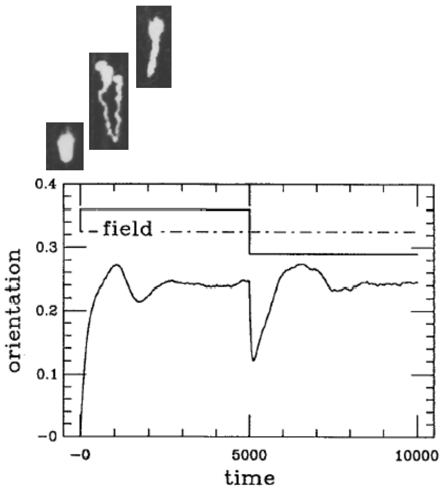
Solid friction at gel fibre ?

Burlatsky & Deutch '93; Duke & Viovy '94

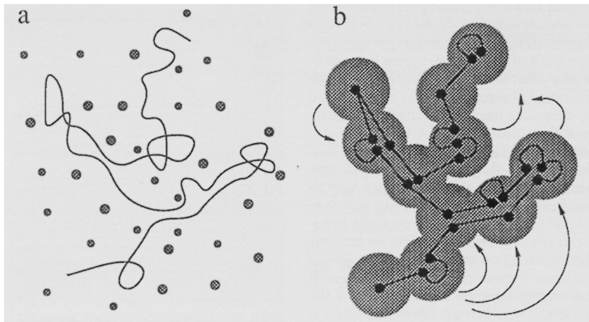
Transient orientation



Reptation in a branched tube

Duke & Viovy '92

Simulate dynamics in a branched tube



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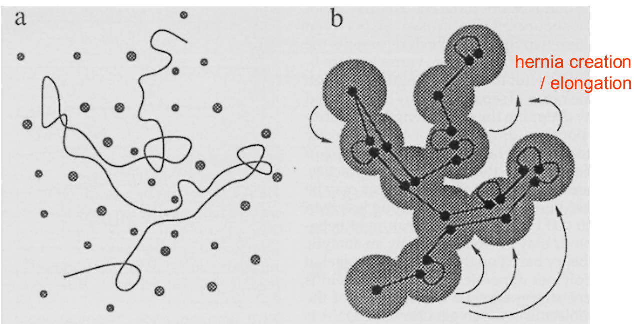

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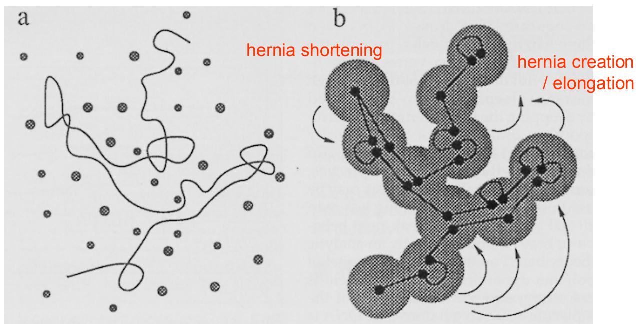

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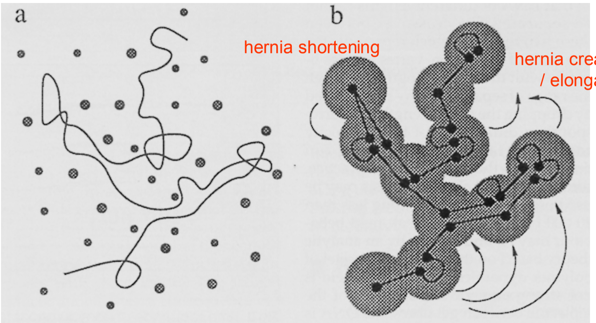
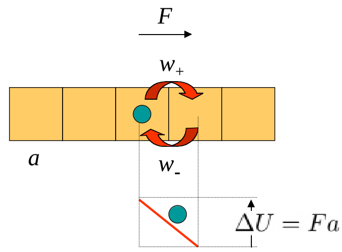


Monte Carlo dynamics on a lattice

Slater & Noolandi '87



$\Delta U = Fa$

$$\epsilon_a = \frac{Fa}{kT}$$

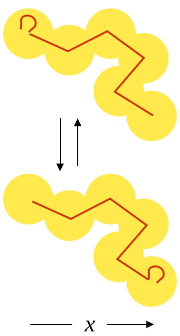
- first passage time $$\tau = \tau_a \frac{\tanh(\epsilon_a/2)}{\epsilon_a}$$
- probability of exit on either side $$p_\pm = \frac{1}{1+\exp(\mp\epsilon_a)}$$

Defect hopping dynamics

Duke & Viovy '92

Defect hop of range n ≡

sliding of string of length n through displacement of 1 pore



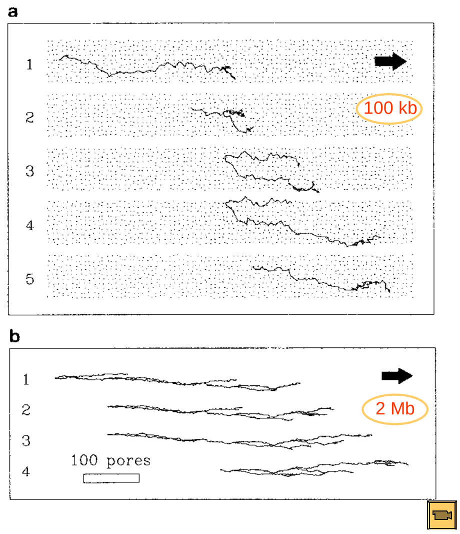
$$\tau = \tau_a n \frac{\tanh(\epsilon_a x/2a)}{\epsilon_a x/a}$$

$$p_\pm = \frac{1}{1+\exp(\mp\epsilon_a x/a)}$$

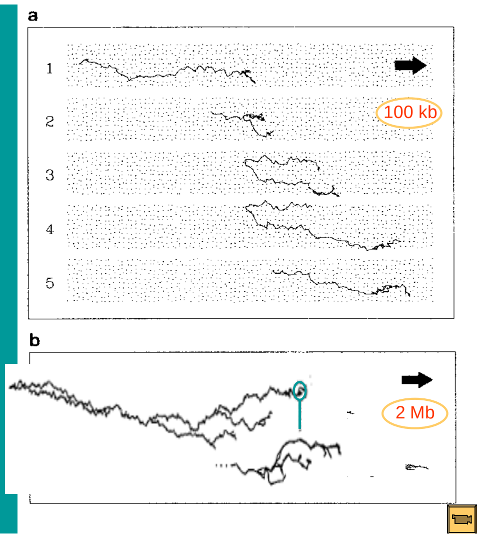
$$w_\pm = \frac{p_\pm}{\tau}$$

→ hopping rate $$w_n(x) = \frac{\epsilon_a x/a}{n\,[1-\exp(\epsilon_a x/a)]}\frac{1}{\tau_a}$$

Complex motion of large DNA molecules



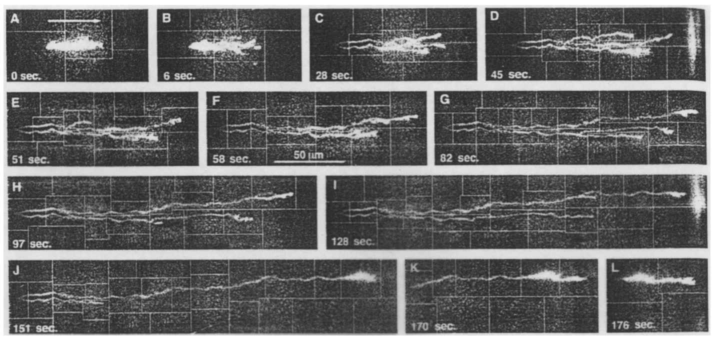
Complex motion of large DNA molecules



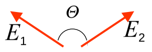
Videomicroscopy: Dynamics

Gurrieri et al. '97

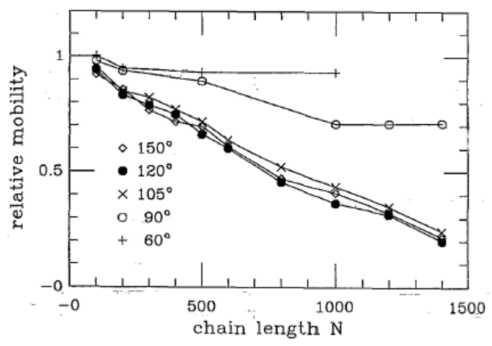
Yeast chromosome (1.6 Mb)



Pulsed-field gel electrophoresis



- good resolution if angle Θ is obtuse



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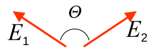
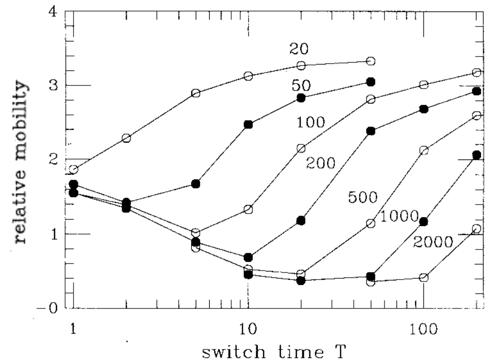
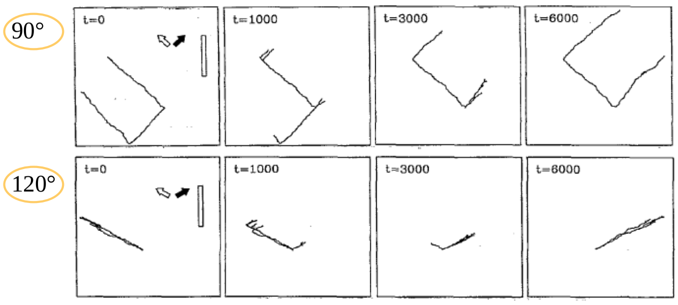


- size limit increases with pulse time T

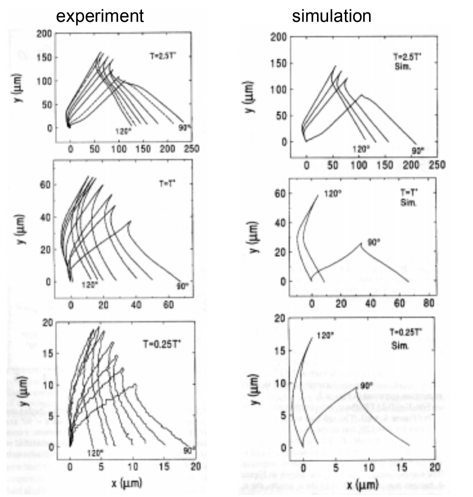


Pulsed-field gel electrophoresis: Dynamics



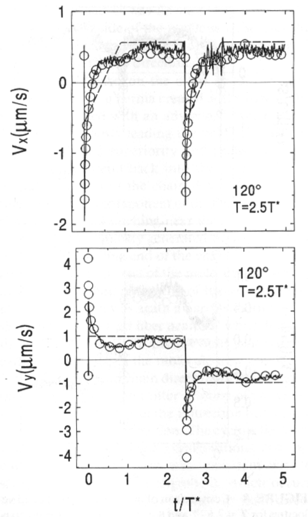
Band motion

Neitzey et al. '94



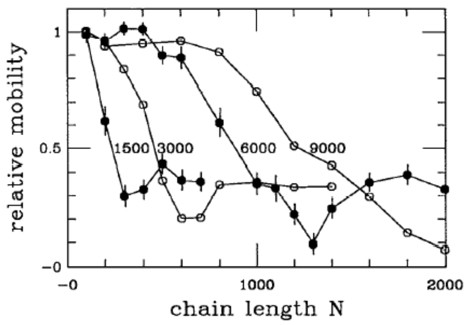
Band velocity

Neitzey et al. '94

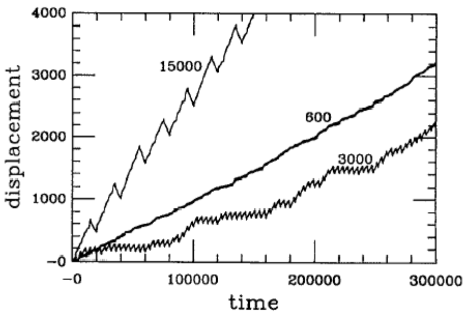


Field-inversion gel electrophoresis

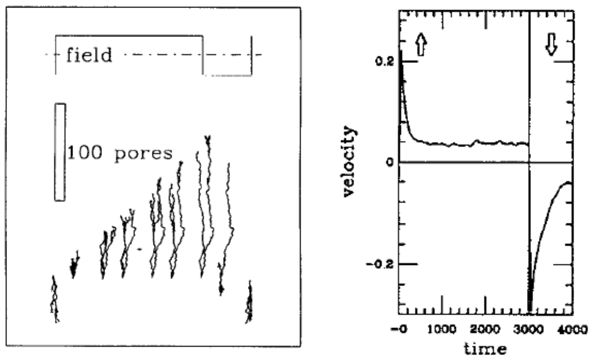
Band inversion



Field-inversion gel electrophoresis



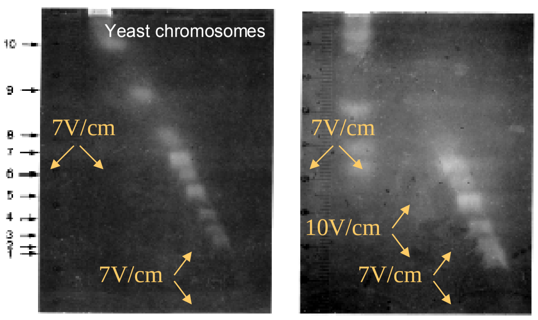
Field-inversion gel electrophoresis: Dynamics



Irreversible trapping

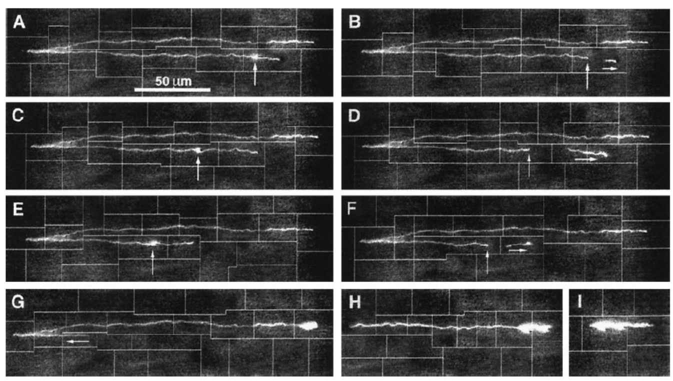
Viovy et al. '92

Large molecules get stuck at high field strengths



Reversible and irreversible trapping

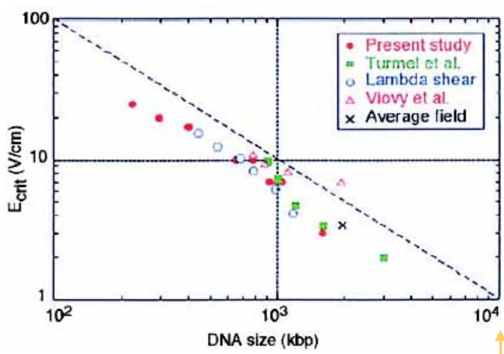
Gurrieri et al. '99



Irreversible trapping

Gurrieri et al. '99

Trapping field is inversely proportional to length



pulse time ~ 1 hour
separation time ~ 1 week

Pulsed field electrophoresis in polymer solutions

Oana et al. '97

DNA configuration is less ramified in concentrated polyacrylamide solution than in a agarose gel