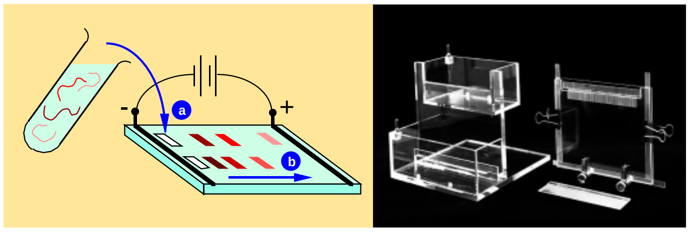
How to sort DNA molecules

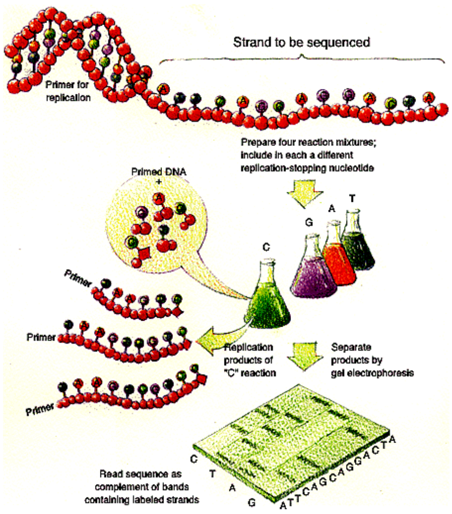
- DNA sequencing: High performance gel electrophoresis
- Separating big molecules: Pulsed-field techniques
- Microfabricated arrays
- Novel sorting devices

Gel electrophoresis

- DNA migrates at a speed that depends on its length
- mixture resolves into spatially segregated bands

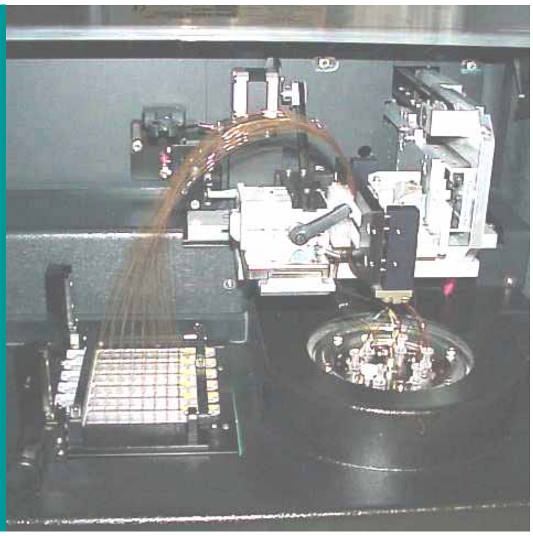


DNA sequencing

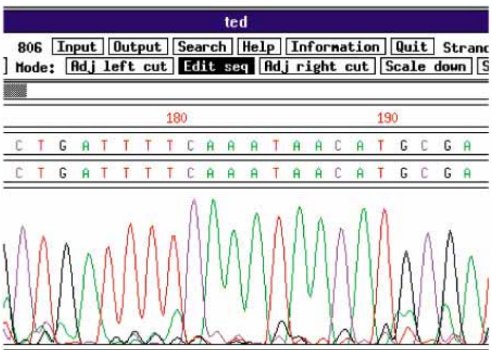


Sanger & Coulson '75

Capillary electrophoresis



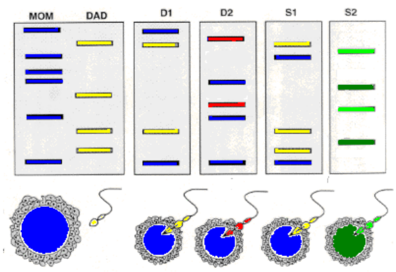
Capillary electrophoresis

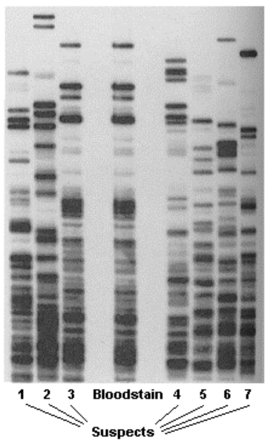


Molecular Biology of the Cell, 4th Edition.

DNA fingerprinting

Restriction fragment length polymorphism due to short tandem repeats



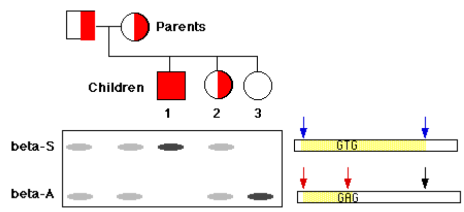


Gene typing

Sickle cell anaemia

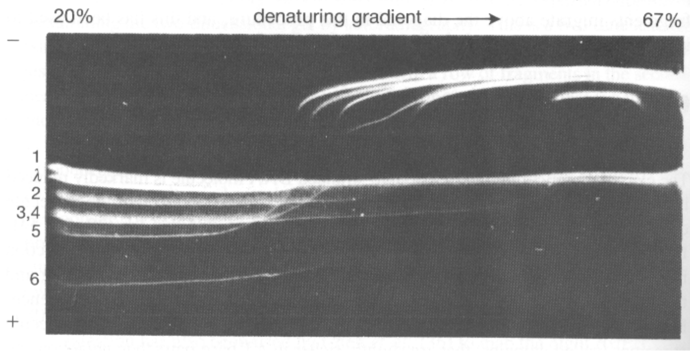
	Thr	Pro	Glu	Glu	
...A C T	C C T	G A G	G A G...		betaA chain / betaA gene
Codon #	4	5	6	7	
...A C T	C C T	G T G	G A G...		betaS gene
	Thr	Pro	Val	Glu	betaS chain

CTGAGG

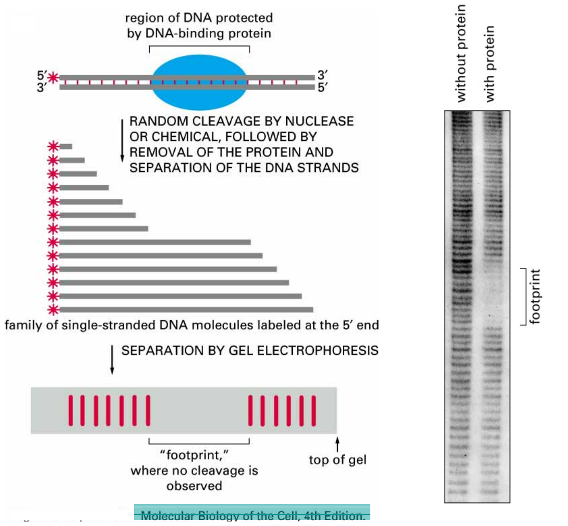


Denaturing gel electrophoresis

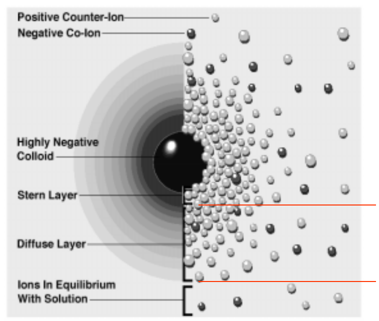
Detection of single nucleotide substitution



DNA footprinting



Charged particles in ionic solutions



Bjerrum length $\lambda_B = \frac{e^2}{4\pi\epsilon\epsilon_0 kT}$

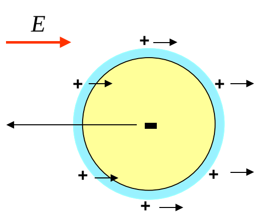
Debye length

$$\lambda_D = \left(\frac{4\pi}{\lambda_B \sum z_i^2 C_i}\right)^{1/2}$$

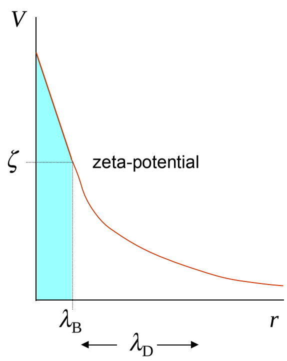
Electrophoretic mobility

Smoluchowski 1903

Mobility $\mu \equiv \frac{v}{E}$



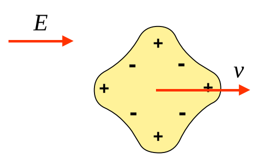
$$\mu = \frac{\epsilon\epsilon_0\zeta}{\eta}$$



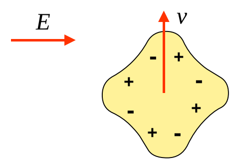
Electrophoretic mobility

Long & Ajdari '98

A neutral particle can move …

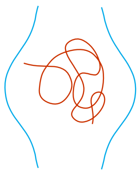


… and in an unexpected direction



Sedimentation *vs* electrophoresis

Sedimentation:
hydrodynamic interactions



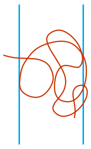
$R \sim bN^{\nu}$

random coil $\nu = \frac{1}{2}$

self-avoiding $\nu = \frac{3}{5}$

$$v = \frac{F_{tot}}{\zeta_{tot}} \sim \frac{N}{\eta R} \sim N^{1-\nu}$$

Electrophoresis:
free-draining

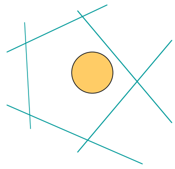


$$v = \frac{F_{tot}}{\zeta_{tot}} \sim \frac{N}{N} \sim N^0$$

Ogston sieving

Ferguson '64;Rodbard & Chrambach '70; Ogston '58

Sieving of colloidal particles in a gel



Empirical observation: $\log\left(\frac{\mu}{\mu_0}\right) = -K(R)c$

Supposition:

mobility proportional to accessible volume $\frac{\mu}{\mu_0} = f = 1 - \phi$

suspension of rods: $f = \exp\left(-\frac{\pi}{4}\frac{R^2}{a^2}\right)$, pore size $a \sim c^{-1/2}$

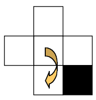
Sieving

Mercier & Slater '01

Lattice model:

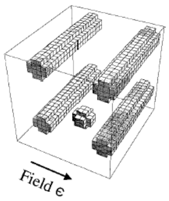
$$p_{\pm x} = p_{\pm y} = \frac{1}{6}$$

$$p_{\pm z} = \frac{1}{6}\left(1 \pm \frac{\epsilon}{2}\right)$$

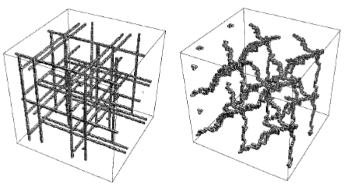


excluded volume ϕ

$$\epsilon = \frac{\Delta U}{kT}$$



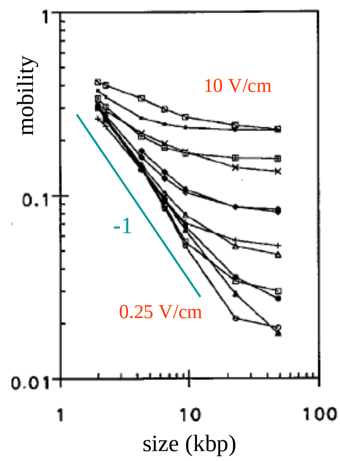
$$\frac{\mu}{\mu_0} = \frac{1}{1+\phi}$$



$$\frac{\mu}{\mu_0} = \frac{1}{1+\frac{2}{3}\phi}$$

Gel electrophoretic mobility of DNA

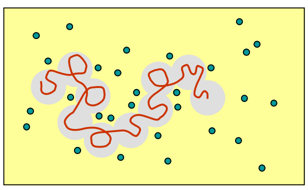
- long molecules *do* migrate
- mobility is nonlinear
- low-field mobility power-law rather than exponential ?



Reptation

de Gennes '71; Edwards '68

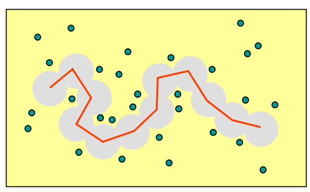
Gel fibres constrain chain, so that it can only diffuse longitudinally within a tube



Reptation

de Gennes '71; Edwards '68; Lerman & Frisch '82; Lumpkin & Zimm '82

Polymer of contour length L → primitive path of N segments $N = \frac{Lb}{a^2}$



longitudinal diffusion coeff. $D_s \sim \frac{kT}{N\zeta_a}$

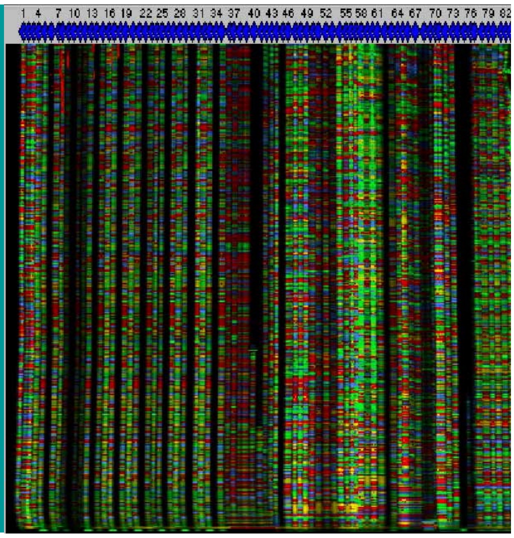
→ tube renewal time

$$\tau_{rep} \approx \frac{N^2 a^2}{D_s} \sim N^3 \tau_a$$

→ diffusion coeff. of polymer $D \sim \frac{R^2}{\tau_{rep}} \sim \frac{1}{N^2}$

fluctuation-dissipation relation → $v = \frac{F_{tot}}{\zeta_{tot}} \sim \frac{F_{tot} D}{kT} \sim \frac{1}{N}$

Sequencing electrophoresis in polyacrylamide



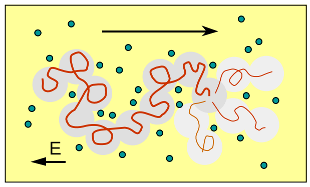
Biased reptation

Lumpkin, Dejardin & Zimm '85; Slater & Noolandi '85

Effective charge per segment q_a → mobility in free solution $\mu_0 = \frac{q_a}{\zeta_a}$

$$F_s = \sum q_a \mathbf{E}.\hat{\mathbf{s}}_\mathbf{i} = q_a E \frac{R_\parallel}{a}$$

$$\rightarrow v_s = \frac{F_s}{N\zeta_a} = \mu_0 E \frac{R_\parallel}{Na}$$



$$v = v_s \frac{R_\parallel}{Na}$$

$$\rightarrow \frac{\mu}{\mu_0} = \frac{\langle R_\parallel^2 \rangle}{N^2 a^2}$$ mobility depends on conformation

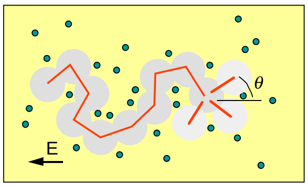
Field-induced orientation

Lumpkin, Dejardin & Zimm '85; Slater & Noolandi '85

Supposition: terminal segment is oriented by the field

$$p(\theta) \sim \exp\left(-\epsilon_a \cos\theta\right)$$

$$\epsilon_a = \frac{q_a E a}{kT}$$



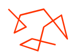
$$\langle \cos\theta \rangle = \coth\epsilon_a - \frac{1}{\epsilon_a}$$
$$\approx \frac{\epsilon_a}{3}, \qquad \epsilon_a \ll 1$$

Biased reptation model (BRM)

Lumpkin, Dejardin & Zimm '85; Slater & Noolandi '85

$$\frac{\mu}{\mu_0} = \frac{\langle R_\parallel^2 \rangle}{N^2 a^2}$$

Short chains: random coil $\langle R_\parallel^2 \rangle = \frac{Na^2}{3}$ → $\frac{\mu}{\mu_0} = \frac{1}{3N}$



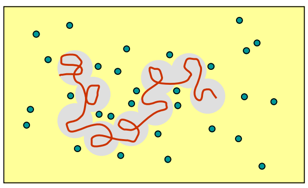
Long chains: oriented $\langle R_\parallel \rangle = \frac{Na\epsilon_a}{3}$ → $\frac{\mu}{\mu_0} \sim \epsilon_a^2$

Limit of separation: $N^* \sim \frac{1}{\epsilon_a^2}$

Tube length fluctuations

Doi '83

Breathing mode of chain in tube leads to fluctuations in the length of the primitive path



$$n_{fluc} = \left(\frac{t}{\tau_a}\right)^{1/4}, \quad t < N^2\tau_a$$
$$= N^{1/2}, \quad t > N^2\tau_a$$

$$\tau_a = \frac{\zeta_a a^2}{kT}$$

Tube orientation

Duke, Senenov & Viovy '92

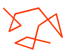
Orientation is a dynamic phenomenon

- fluctuations free a section (n segments) of the chain from the tube $\quad n = n_{fluc} = n_{drift}$
- electric field influences the orientation of this section $\quad \langle\cos\theta\rangle \approx \frac{n\epsilon_a}{3}, \quad n\epsilon_a \ll 1$
- length of section that can escape depends on drift speed $\quad n_{drift} \sim \frac{v_s}{a} t$
- drift speed depends on orientation $\quad v_s = \epsilon_a \frac{R_\parallel}{Na} \frac{a}{\tau_a}$

Biased reptation with fluctuations (BRF)

Duke, Senenov & Viovy '92

Short chains: random coil



$\langle R_{\parallel}^2 \rangle = \frac{Na^2}{3}$ → $\frac{\mu}{\mu_0} = \frac{1}{3N}$

Long chains: oriented

$\langle R_{\parallel} \rangle \sim N a \epsilon_a^{1/2}$ → $\frac{\mu}{\mu_0} \sim \epsilon_a$

Limit of separation: $N^* \sim \frac{1}{\epsilon_a}$

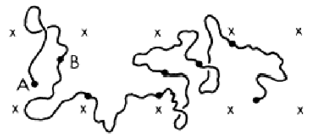
$\frac{L^*}{b} \sim \left(\frac{a}{b}\right)^{-1} \left(\frac{E}{E_0}\right)^{-1}$, $E_0 = \frac{kT}{q_b}$ $E_0 \approx 10^5 \ \mathrm{V/cm}$

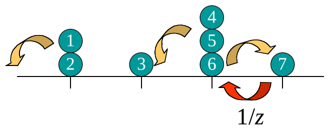
Repton model

Rubinstein '87

1d lattice model of reptation incorporating tube length fluctuations

diffusion of polymer along tube = diffusion of ordered particles on a line

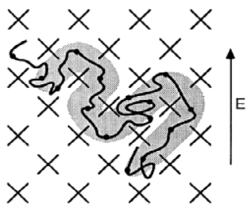


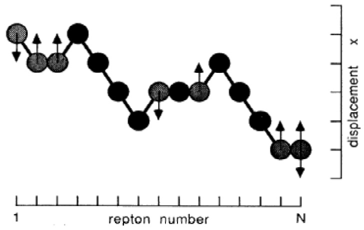


Repton model

Duke '89

3d configuration can be mapped onto a 1d lattice



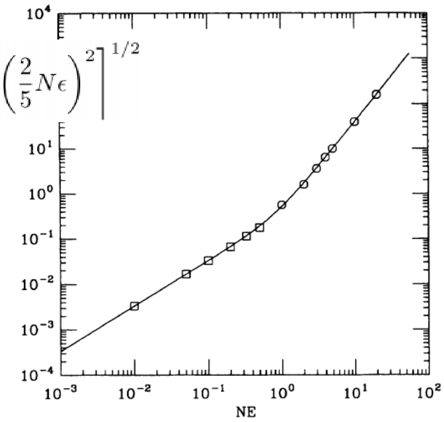


$$p_{\pm} = \frac{1}{1 + \exp(\mp\epsilon)}$$

Repton model

Barkema, Marko & Widom '94

$$N^2 v = N\epsilon \left[\left(\frac{1}{3}\right)^2 + \left(\frac{2}{5} N\epsilon\right)^2 \right]^{1/2}$$



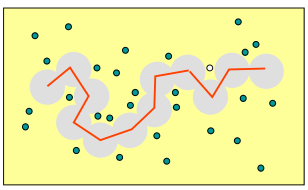
Electrophoresis in temporary gels

Duke & Viovy '91

Dynamic mechanism of orientation has a characteristic time scale $t_{eq} \sim \frac{\tau_a}{\epsilon_a^2}$

Suppose cross-links break at rate ω

- if $\omega > \frac{1}{t_{eq}}$, chain does not get oriented
- but primitive path diffuses



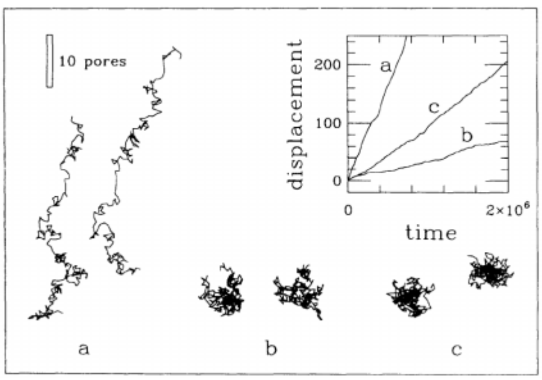
$D_{tube} \sim \frac{a^2 \omega}{N}$

$\mu_{tube} = \frac{F_{tot} D_{tube}}{kT}$

→ $\frac{\mu_{tube}}{\mu_0} \sim \omega \tau_a$

Electrophoresis in temporary gels

Duke & Viovy '91



$\omega\tau < \epsilon_a^2$ $\epsilon_a^2 < \omega\tau < \epsilon_a$ $\omega\tau > \epsilon_a$

Electrophoresis in temporary gels

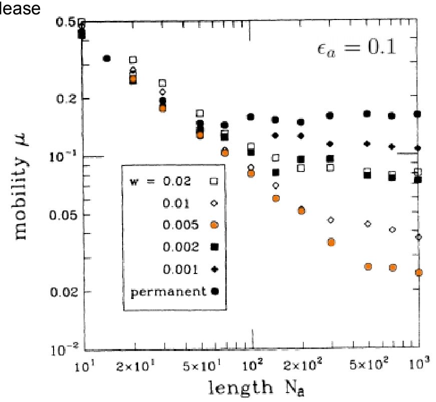
Duke & Viovy '91

- optimal rate of constraint release

$$\omega^* \tau_a \sim \epsilon_a^2$$

→ best limit of separation

$$N^* \sim \frac{1}{\epsilon_a^2}$$

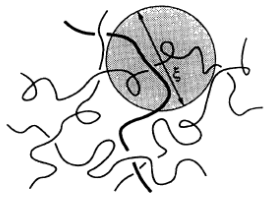


Electrophoresis in semi-dilute polymer solutions

Duke & Viovy '91

Above critical concentration $c_p^* \sim \frac{N_p}{R_p^3}$ polymers form a mesh

- mesh size $\xi \sim \left(\frac{c}{c_p^*}\right)^{-3/4} R_p$
- constraint release rate $\omega \sim \frac{1}{\tau_{rep}}$



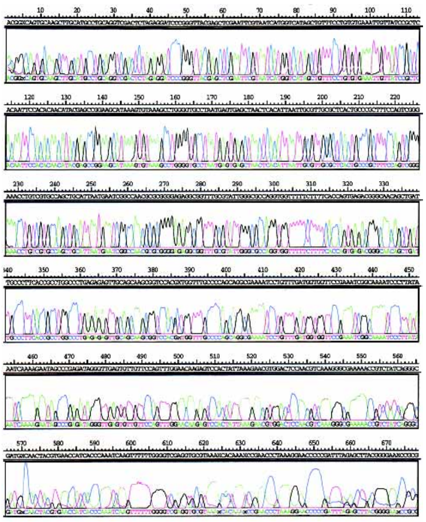
choose polymer size to fix $\omega \approx \omega^*$ → $\frac{R_p}{b} \sim \left(\frac{E}{E_0}\right)^{-2/5}$

choose concentration to fix $\xi \approx b$ → $\frac{c_p}{c_p^*} \sim \left(\frac{E}{E_0}\right)^{-3/10}$

High speed sequencing

Carrilho et al. '96
Erlich et al. '97

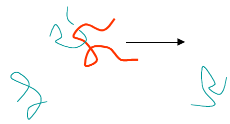
1000 bases in 80 minutes



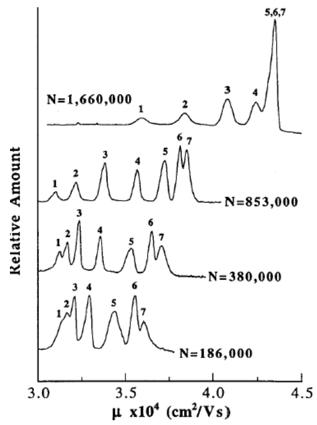
Electrophoresis in dilute solutions

Barron et al '94; Starkweather et al. '00

DNA is hindered by transient collisions with matrix polymers



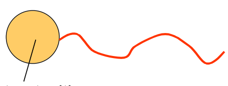
- best separation when polymer size is similar to DNA size



Medium-less sequencing

Ren et al '99

End-labelled DNA



- effective charge ~ $N_q\, q_b$
- effective drag ~ $N_\zeta\, \zeta_b$

→ $$\frac{\mu}{\mu_0} = \frac{N + N_q}{N + N_\zeta}$$