Electrophoresis of composite objects:

effect of shape, topology and polymer stiffness

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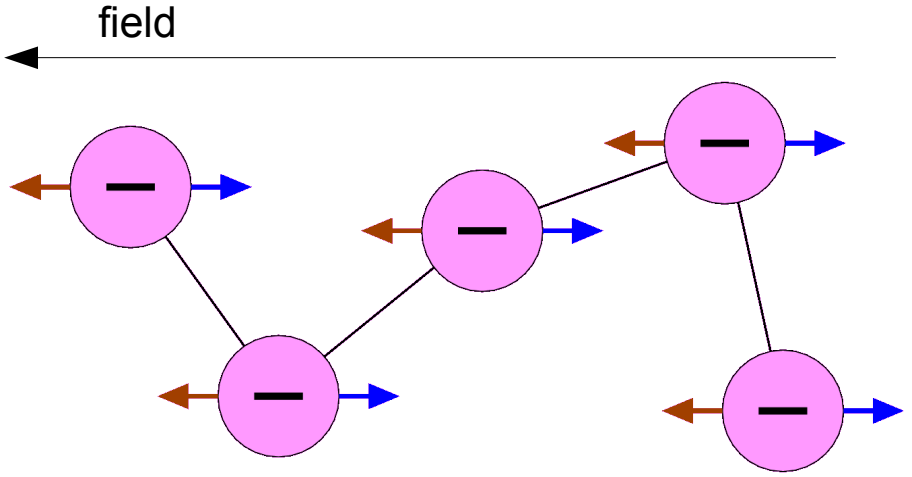
Some biomedical applications require separation of biomolecules. E.g., separation of DNA fragments by length.

Common technique: **electrophoresis**. Motion of charged objects in a fluid in an electric field.

Normally, a gel or an entangled polymer solution is used. Without it (in free solution) the electrophoretic velocity is DNA-size-independent ⇒ no separation.



Balance:

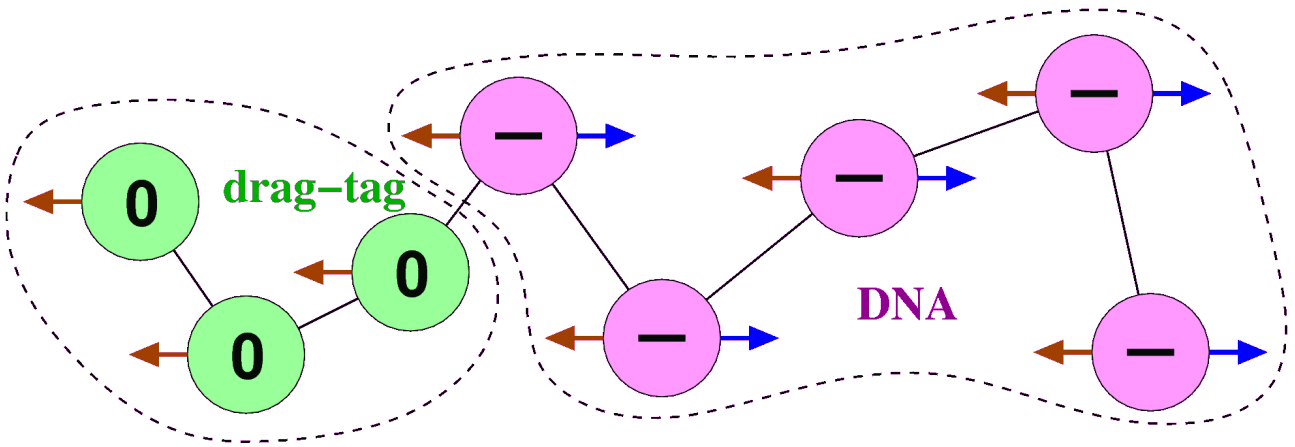
friction force electrostatic force

$$\zeta v = QE$$

$$v = \frac{Q}{\zeta} E \qquad \text{Mobility} \quad \mu \equiv \frac{v}{E} = \frac{Q}{\zeta}$$

Both Q and ζ are $\sim N_{DNA}$, so μ is independent of N_{DNA} .

Separation without a gel: attach identical neutral "drag-tags" to the DNAs.



Simplistically: Charge Q is still ~N_{DNA}. But $\zeta \sim N_{DNA} + \alpha$

$$\mu_{DNA+DT} = \frac{Q}{\zeta} \sim \frac{N_{DNA}}{N_{DNA} + \alpha}$$

Larger DNAs move faster

End-labeled free-solution electrophoresis (ELFSE)

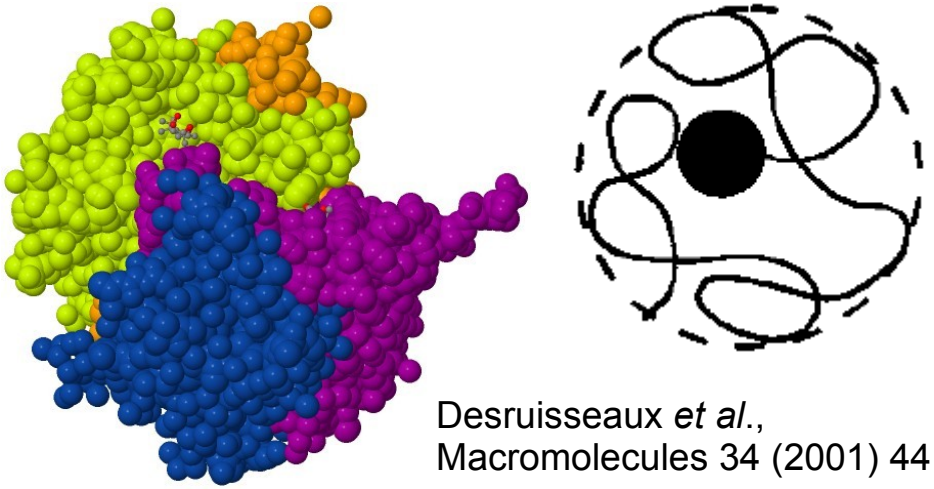
Mayer *et al.*, Anal. Chem. **66** (1994) 1777; Meagher *et al.*, Electrophoresis **26** (2005) 331

Requirement: drag-tags have to be strictly monodisperse, yet large enough.

Different kinds of drag-tags

1. Unfolded proteins (peptides) – easy to make identical ones via genetic engineering techniques [Meagher *et al.*, Anal. Chem. **80** (2008) 2842]. More flexible than even ssDNA.

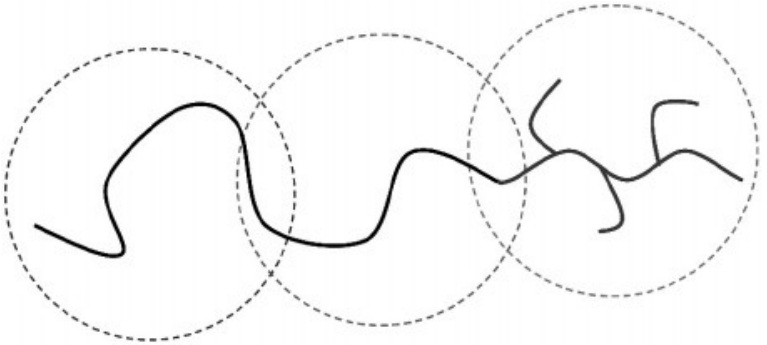
2. A globular protein (streptavidin)



Desruisseaux *et al.*,
Macromolecules 34 (2001) 44

Heller et al., J. Chromatogr. A 806 (1998) 113

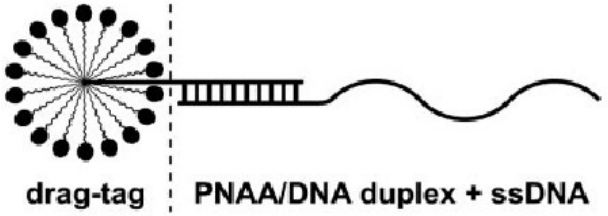
3. Branched drag-tags



S. Nedelcu and G. W. Slater,
Electrophoresis 26 (2005) 4003

Haynes et al., Bioconjugate Chem, 16 (2005) 929

4. Micelles.



Savard *et al.*, Electrophoresis 29 (2008) 2779.

In all these cases, the simplistic theory predicts the same DNA size dependence of the mobility:

$Q \sim N_{DNA}$; $\zeta \sim N_{DNA} + \alpha$

$$\mu_{DNA+DT} = \frac{Q}{\zeta} \sim \frac{N_{DNA}}{N_{DNA} + \alpha} = \frac{\mu_{DNA+DT}}{\mu_{DNA}}$$

Experimentally, measure the elution time (time to pass a certain distance): $t \sim 1/\mu$ (assuming E = const)

$$\frac{t_{DNA+DT}}{t_{DNA}} - 1 = \frac{\alpha}{N_{DNA}}$$

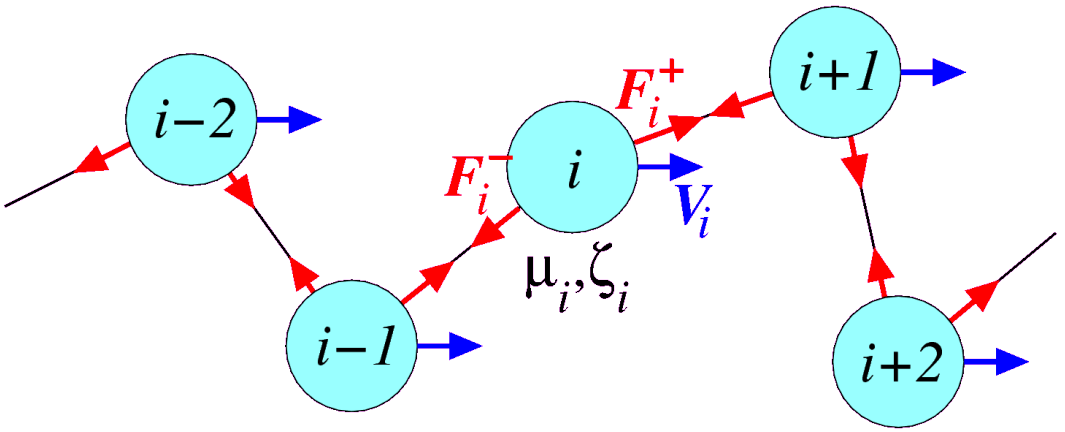
This theory neglects hydrodynamic interactions (HI) between different parts (free draining). No longer a valid assumption when the intrinsic mobilities are different – pull each other, create a long-range flow.

Nonlinearity of t_{DNA+DT}/t_{DNA} is a measure of significance of HI. There is indeed experimental evidence of such nonlinearity, even though it was not recognized until recently. See M.V. Chubynsky and G.W. Slater, *Electrophoresis*, DOI: 10.1002/elps.201300419 (2014).

Need to include HI in the calculations. Also relevant for other situations, e.g., affinity electrophoresis.

Review of the theory of electrophoresis of heterogeneous polyelectrolytes

Long *et al.*, J. Phys.: Cond. Mat. 8 (1996) 9471; J. Chem. Phys. 108 (1998) 1234



due to E-force | due to tension | due to HI

$$\boldsymbol{V}^i = \mu_i \boldsymbol{E} + \underbrace{\boldsymbol{F}^i/\zeta_i + \sum_{j\neq i} \hat{H}^{ij}\boldsymbol{F}^j}_{\sum_j \hat{H}^{ij}\boldsymbol{F}^j}$$

$$\boldsymbol{F}^i = \boldsymbol{F}_i^- + \boldsymbol{F}_i^+$$

$$\hat{H}^{ij} = \frac{1}{8\pi\eta r_{ij}}\left(\hat{I} + \frac{\boldsymbol{r}_{ij}\otimes\boldsymbol{r}_{ij}}{r_{ij}^2}\right), \quad i\neq j \qquad \text{(Oseen tensor)} \qquad \hat{H}_{ii} = \frac{1}{\zeta_i}$$

Take the average. Assume $\langle \hat{H}^{ij}\boldsymbol{F}^j\rangle = \langle \hat{H}^{ij}\rangle\langle \boldsymbol{F}^j\rangle$ (Kirkwood-Riseman approx.)

$$\langle \hat{H}^{ij}\rangle = \frac{1}{6\pi\eta r_{ij}}\hat{I} \quad \text{– scalar}$$

$$\langle \boldsymbol{V}^i\rangle = \mu_i\boldsymbol{E} + \sum_j \langle \hat{H}^{ij}\rangle\langle \boldsymbol{F}^j\rangle \quad \Rightarrow \quad \langle \boldsymbol{F}^i\rangle = \sum_j G^{ij}\left(\langle \boldsymbol{V}^j\rangle - \mu_j\boldsymbol{E}\right), \quad \text{where } G = \langle H\rangle^{-1}$$

Using $\sum_i \boldsymbol{F}^i = 0$ (no external non-electric forces) and $\langle \boldsymbol{V}^i\rangle \equiv \boldsymbol{V}$ (chain moves as a whole),

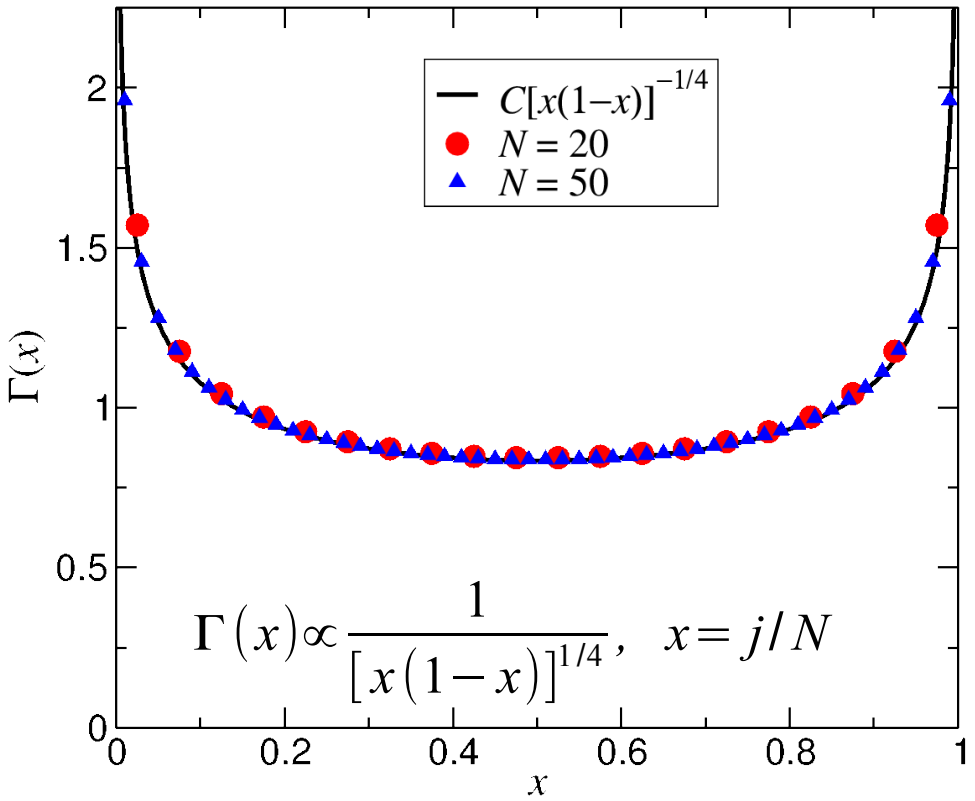
$$\mu = \frac{\boldsymbol{V}}{\boldsymbol{E}} = \frac{\sum_{i,j} G^{ij}\mu_j}{\sum_{i,j} G^{ij}} = \frac{\sum_j \Gamma_j\mu_j}{\sum_j \Gamma_j}, \quad \text{where} \quad \Gamma_j = \sum_i G^{ij}, \quad G = \langle H\rangle^{-1}$$

$$\mu = \frac{\sum_j \Gamma_j \mu_j}{\sum_j \Gamma_j}, \quad \text{where} \quad \Gamma_j = \sum_i G^{ij}, \quad G = \langle H \rangle^{-1}$$

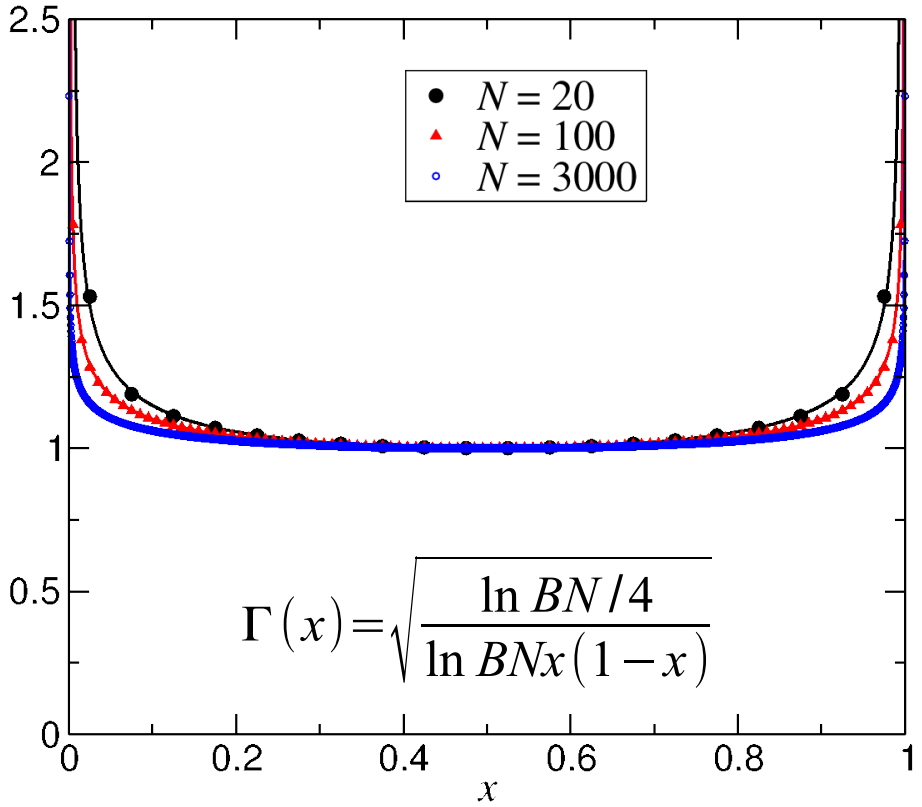
The weights depend on the geometry, but not on the mobilities or charges.

If HI are neglected and all monomers are identical, then *G* is diagonal, all weights are the same and the simple mobility formula is recovered.

Gaussian chain



Stiff rod



Zimm, JCP 24 (1956) 269

Chubynsky and Slater,
DOI: 10.1002/elps.201300419 (2014).

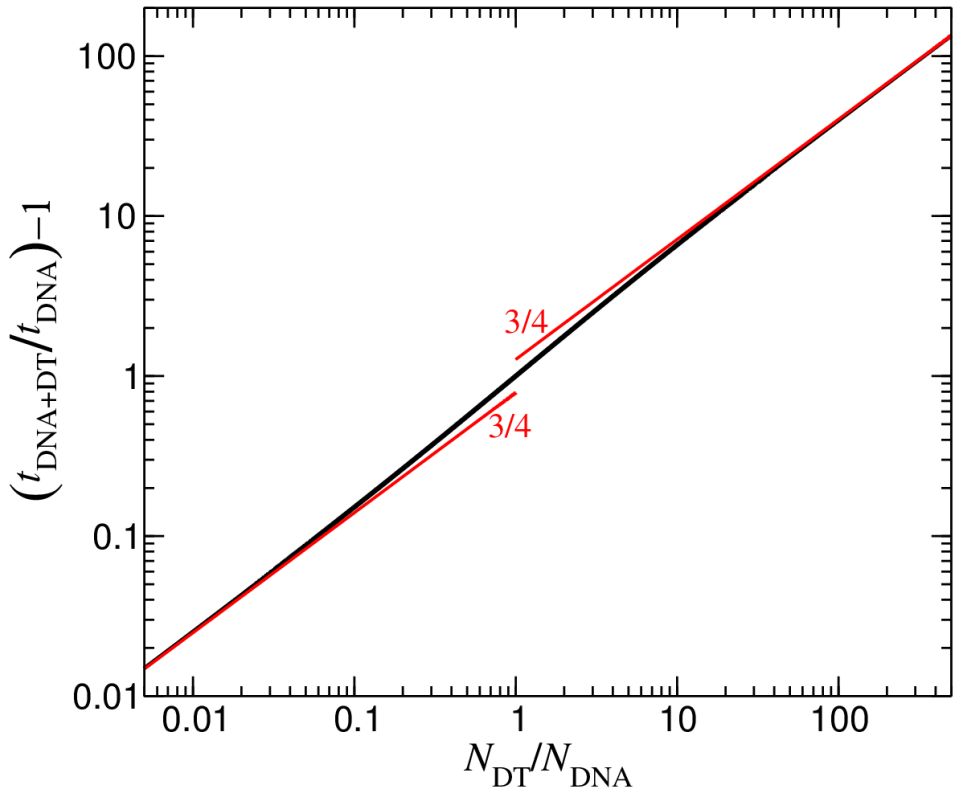
Monomers near the ends affect the mobility more than those near the middle – **end effect**.

Less significant for the rod.

Use the weights to calculate the elution times for the case when one part is neutral ($\mu = 0$) and the other is charged ($\mu = \mu_{DNA}$).

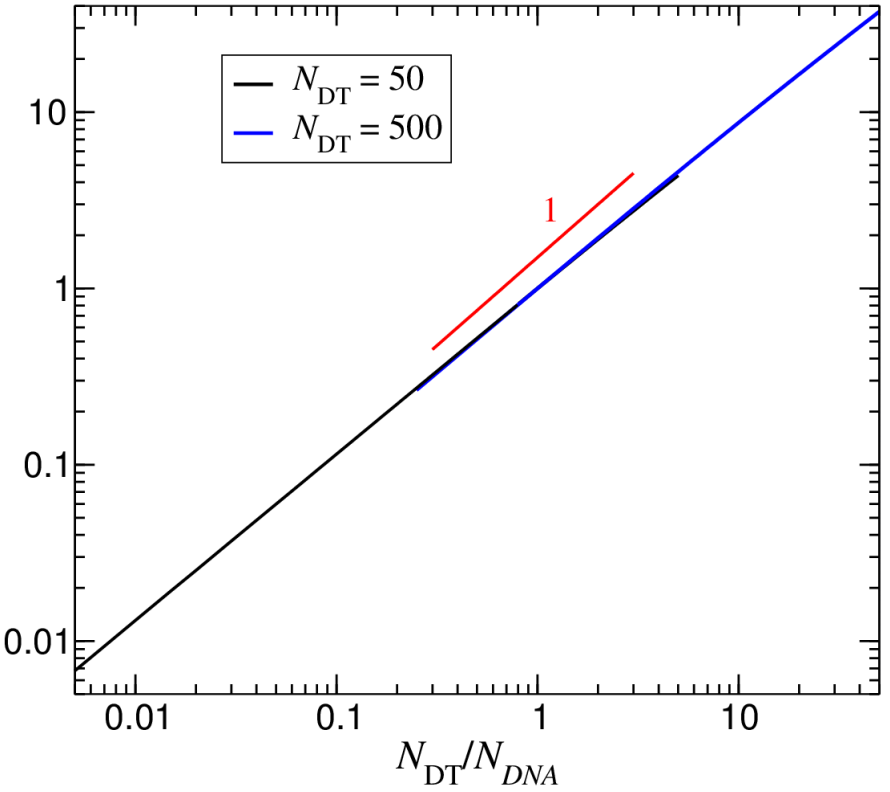
In what follows, the charged part will be referred to as “the DNA” and the neutral part as “the drag-tag”.

Gaussian chain



3/4 for both small and large N_{DNA}, with different prefactors.

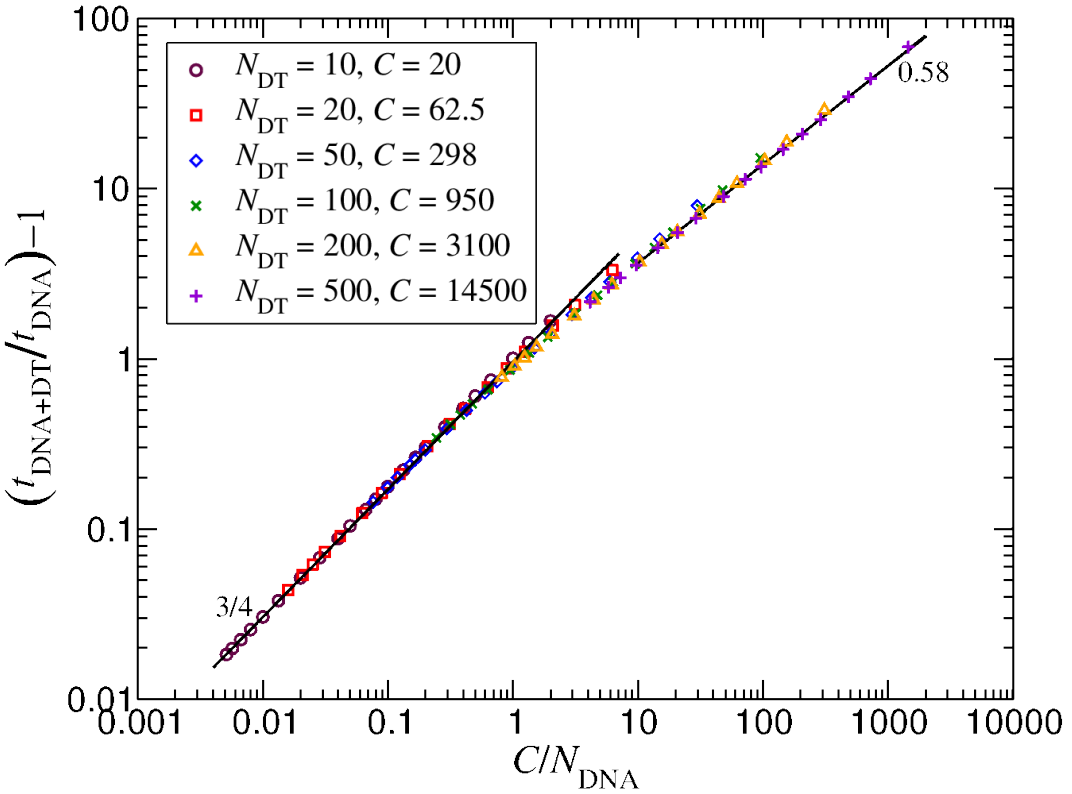
Stiff rod



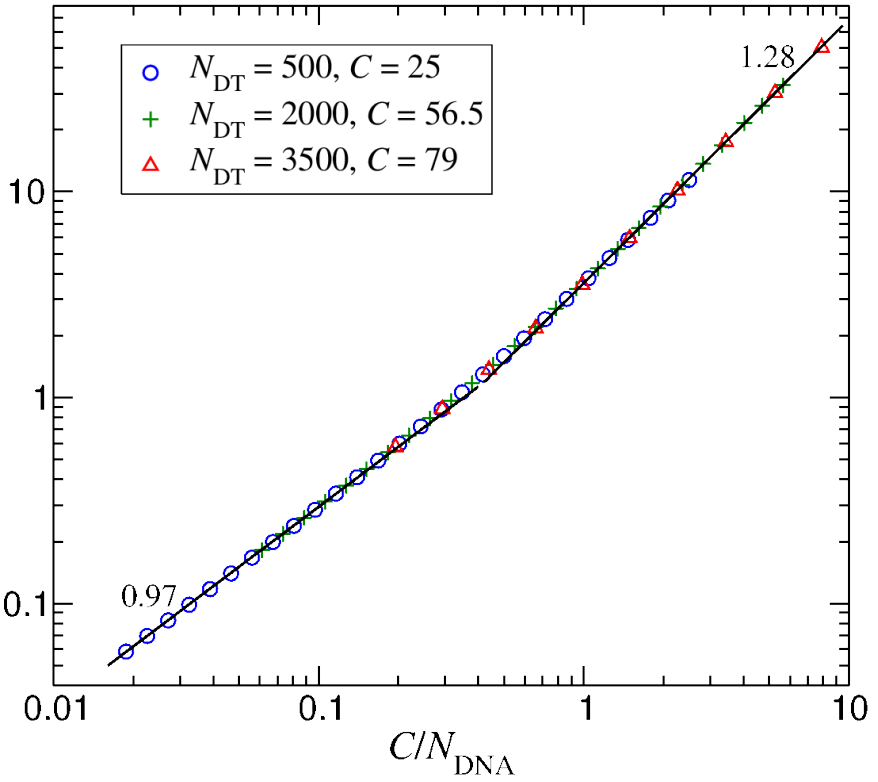
Close to linear.

Parts with different stiffness

Flexible DNA, stiff drag-tag



Stiff DNA, flexible drag-tag



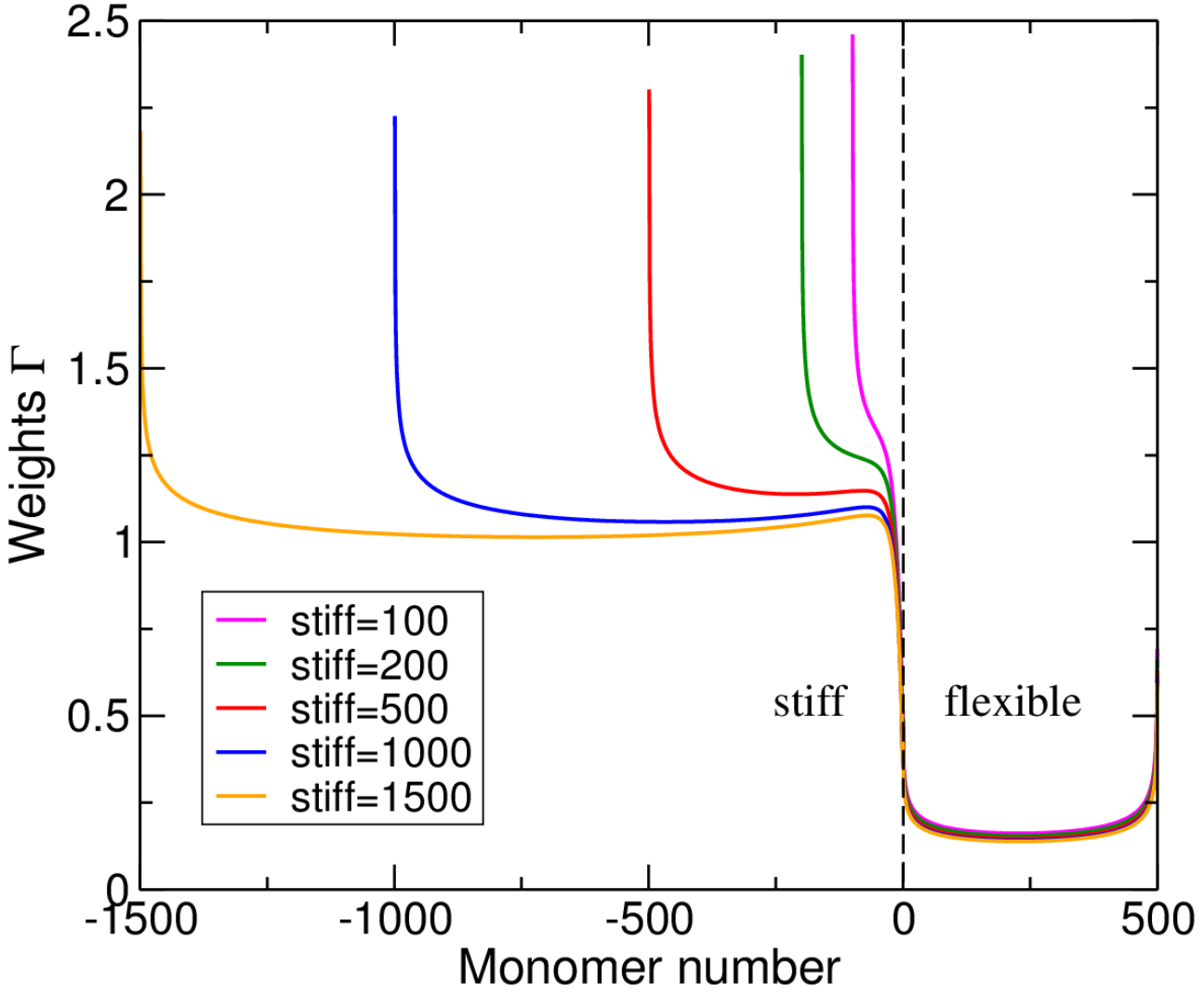
Note that if the coil and the rod are segregated (no HI between them), but the coil is not free-draining, then $\mu_{DNA+DT}=\zeta_{DNA}\mu_{DNA}/(\zeta_{DNA}+\zeta_{DT})$. Short coil, long rod.

Short DNA limit: $\mu_{DNA+DT} \propto \zeta_{DNA} \propto N_{DNA}^{1/2}$

Long DNA limit: $\dfrac{\mu_{DNA}}{\mu_{DNA+DT}} - 1 \propto \dfrac{1}{N_{DNA}} \propto \dfrac{\ln N}{N}$

Weights

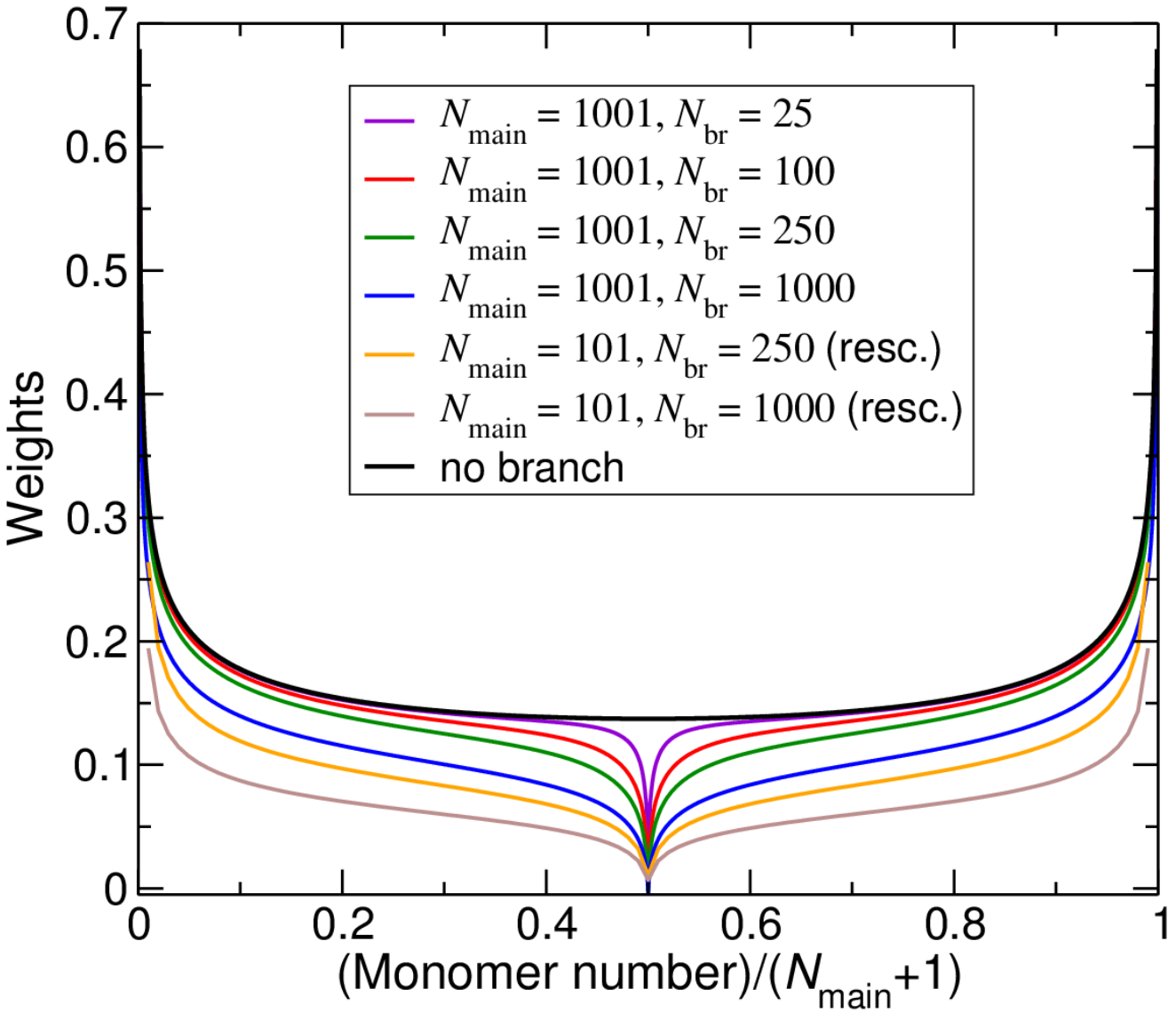
Fixed flexible segment length=500, vary stiff segment length.

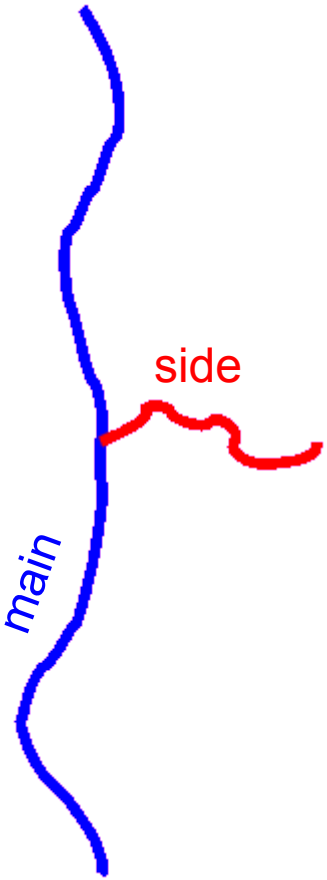


Weights of the flexible part depend very little on the length of the stiff part – little HI

Branched polymers

Main chain weights





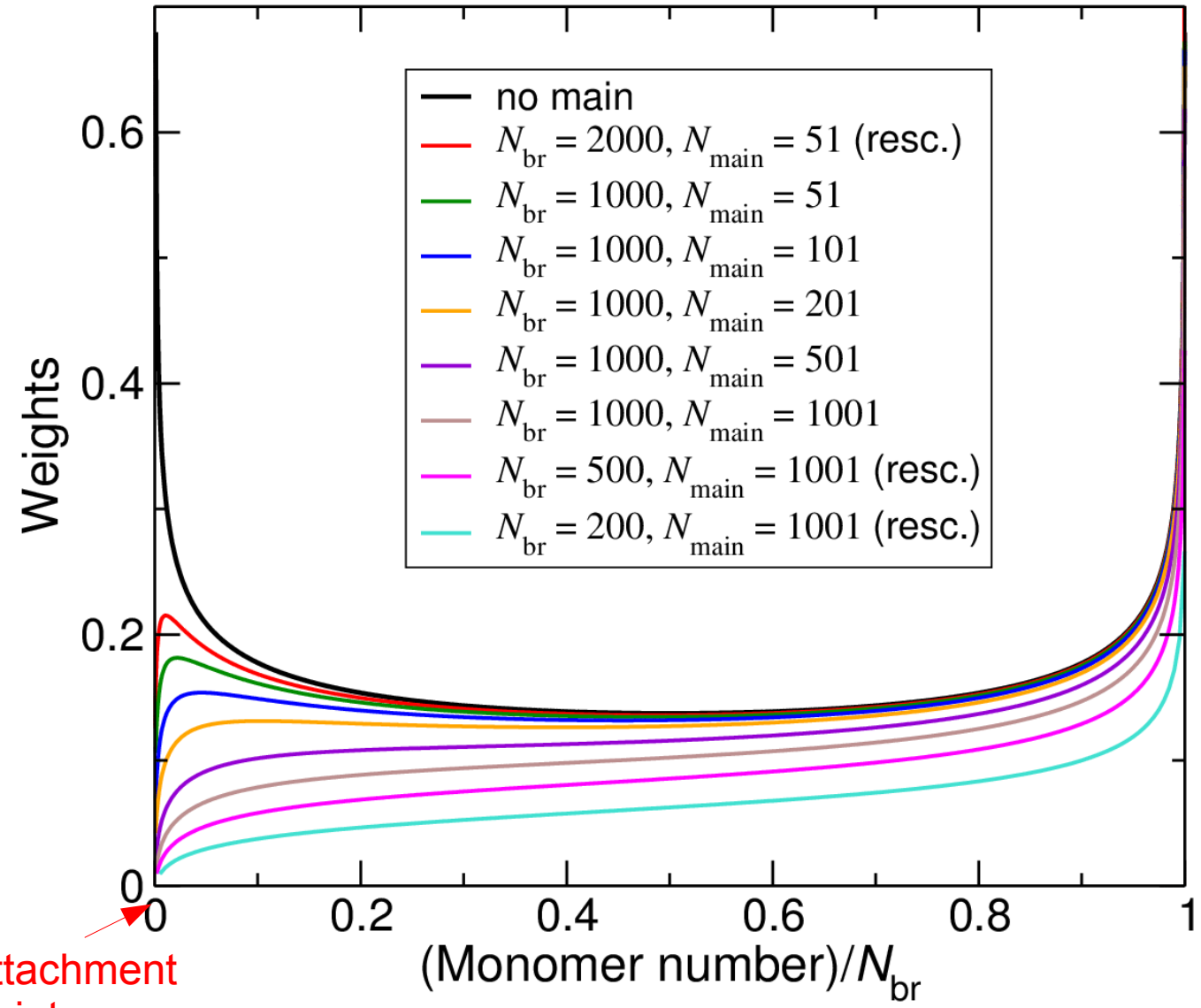
$$C \ln\left(1 + \frac{N - N_0}{w}\right)$$

Logarithmic dip near attachment point. The longer the branch the wider.

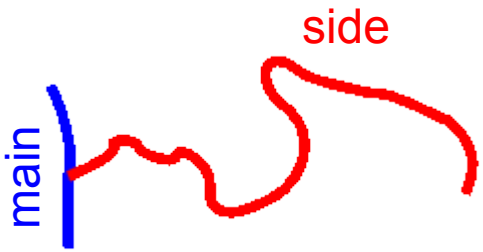
Charges near the attachment point only weakly influence the mobiity.

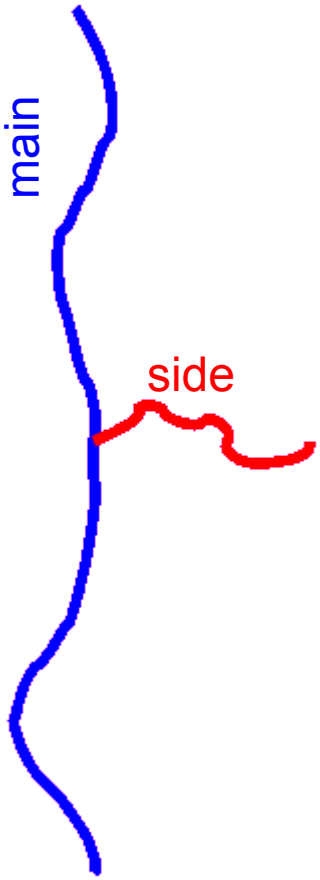
Branched polymers

Side chain weights



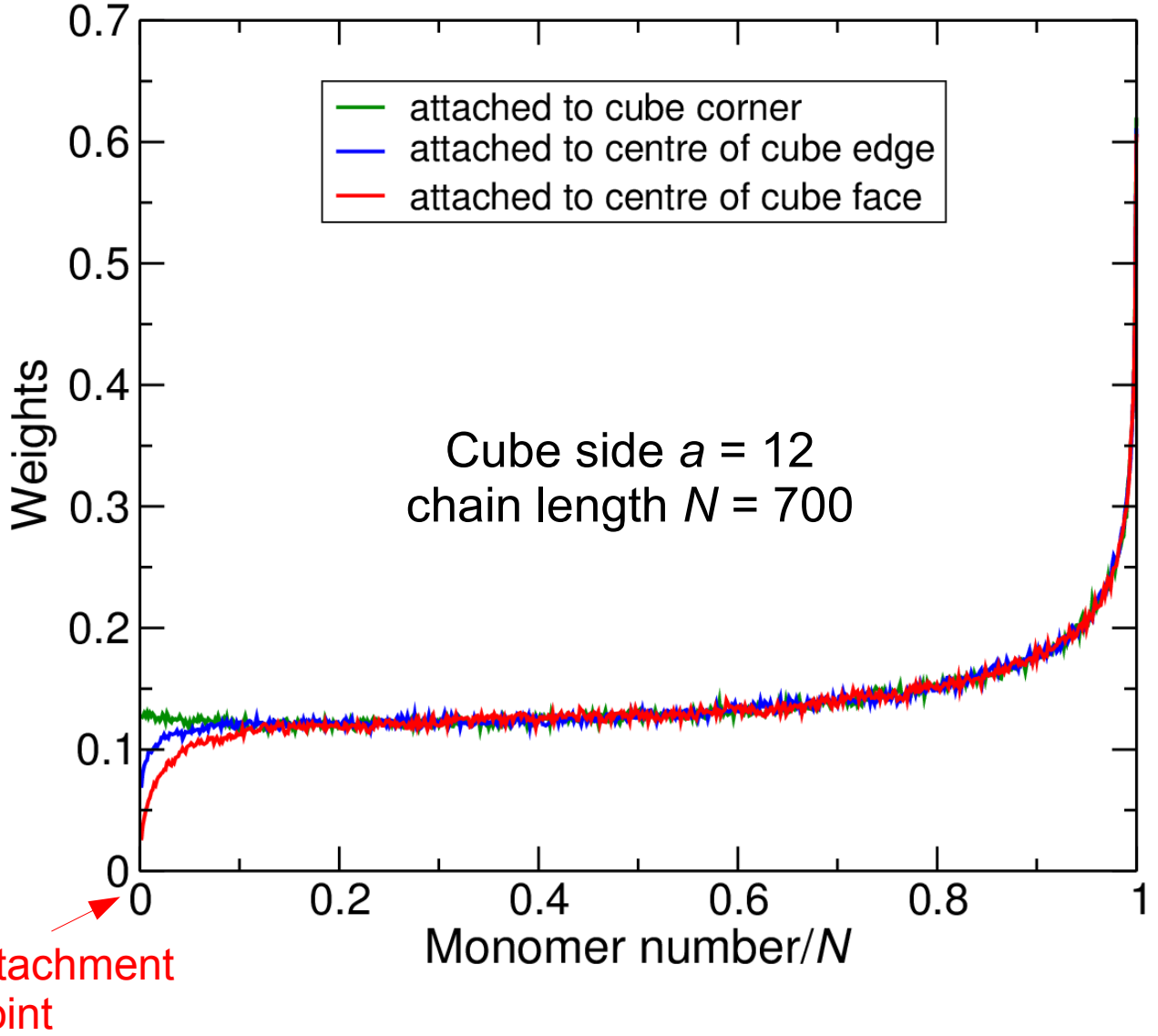
attachment point

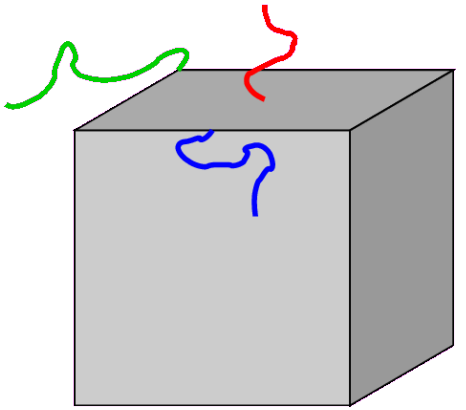




Grows then falls near attachment point when the “main” chain is short.

A flexible polymer attached to a solid object (a cube)



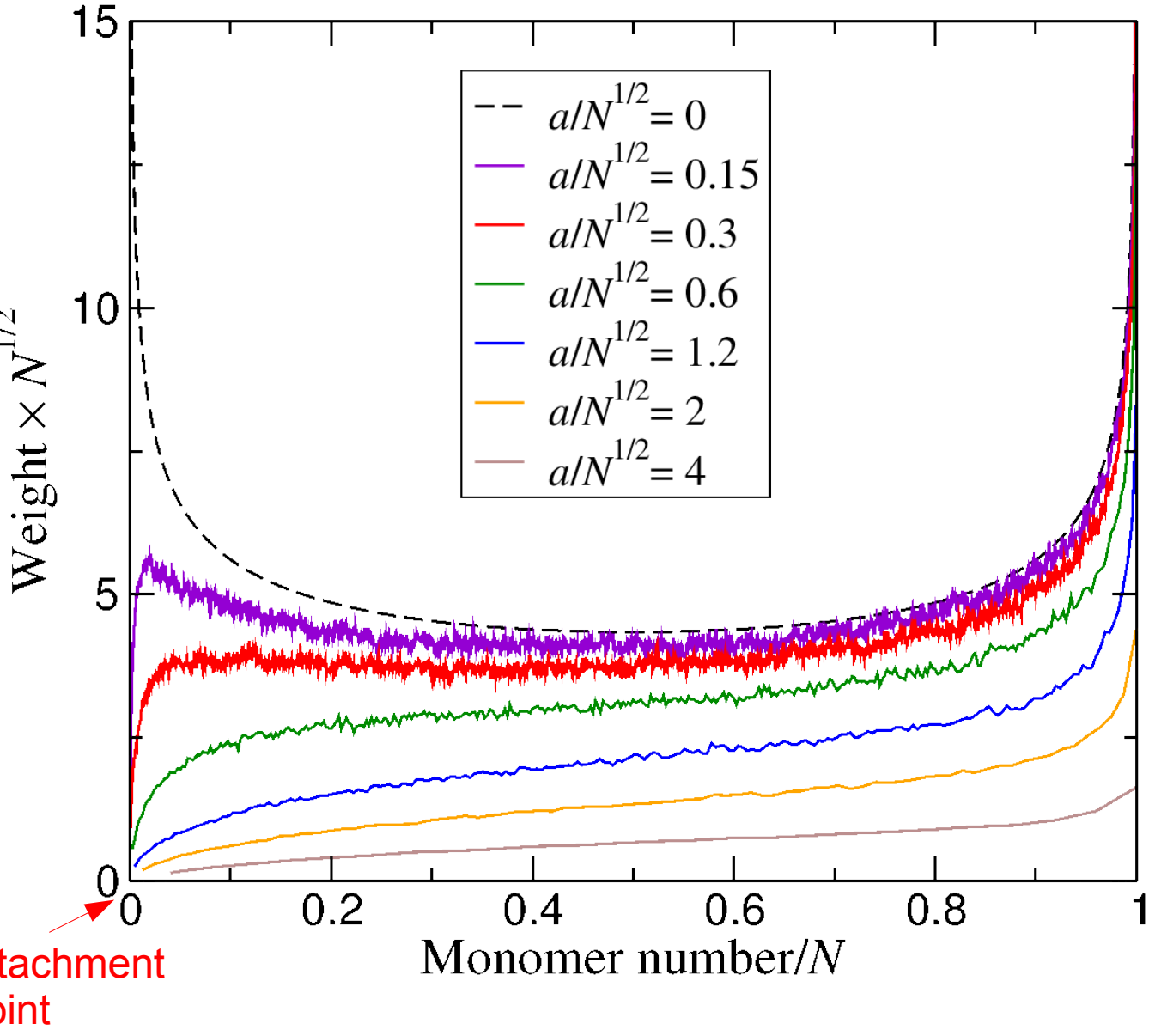


attachment point

Weights of points on the drag-tag surface close to the attachment point are also reduced.

For an arbitrary object, expect the result to depend on the curvature near the attachment point.

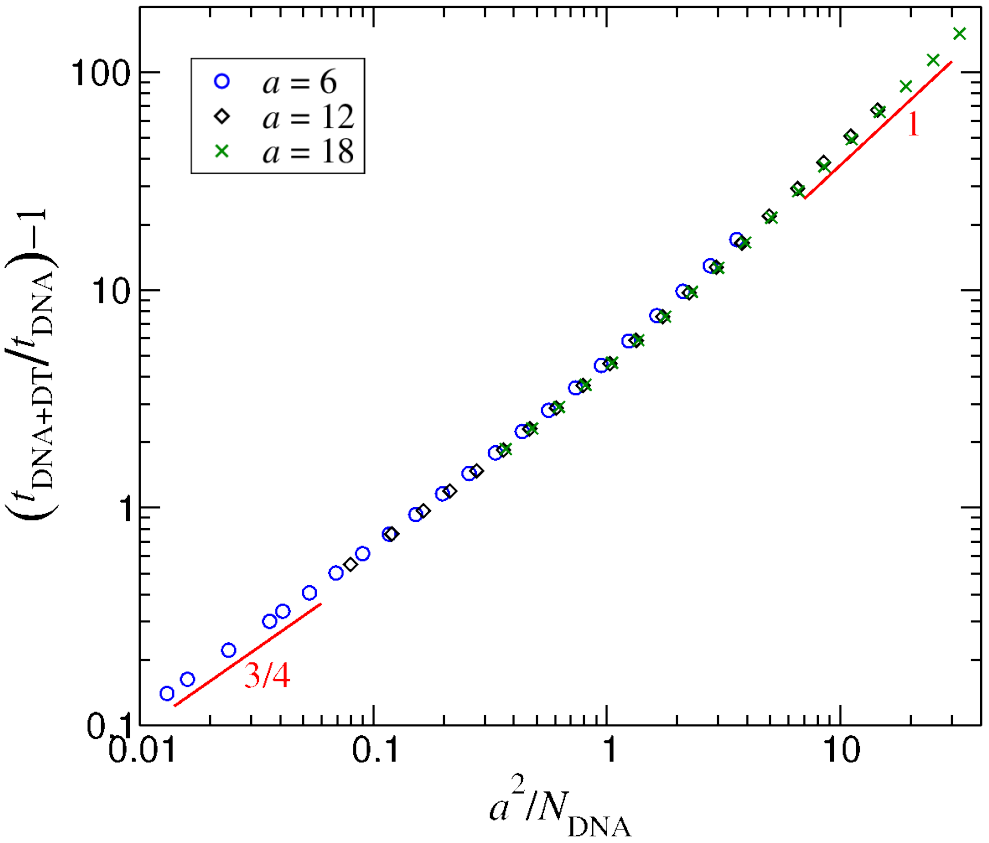
A flexible polymer attached to the center of a face of a cube. Weights.



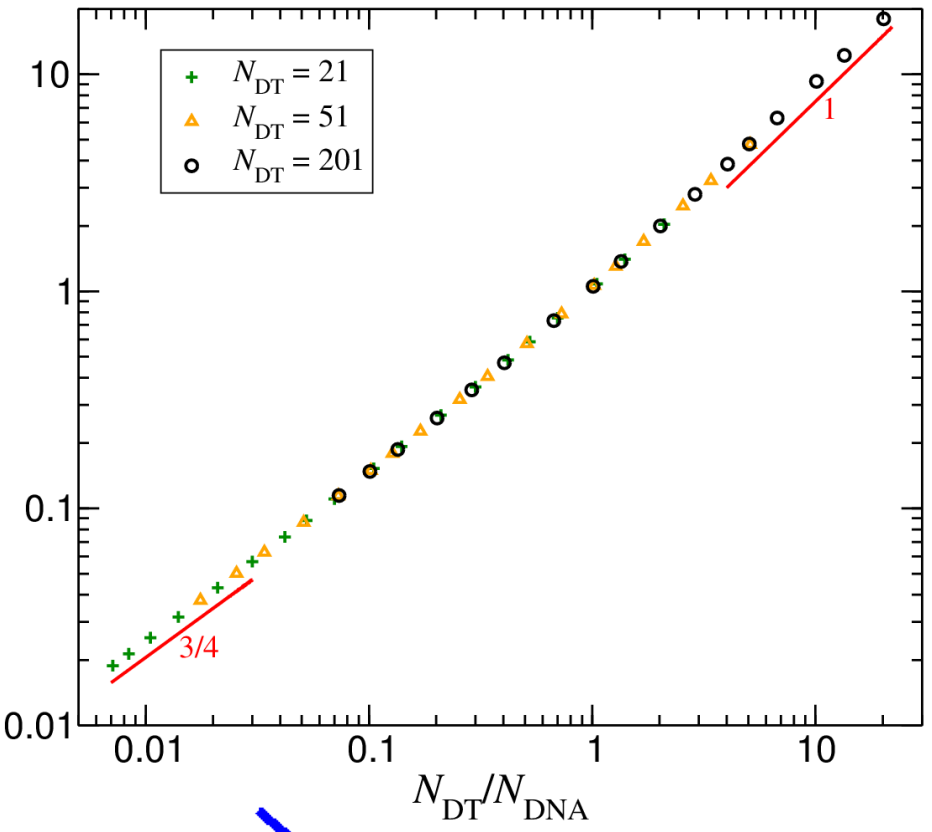
Unlike the case of attachment to a rod, significant decrease as the cube size increases.

Elution times

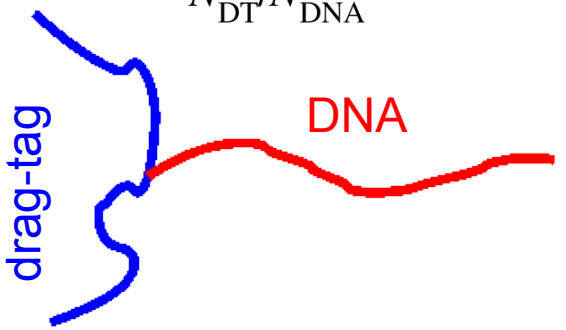
Cube drag-tag



T-shaped configuration



Same exponent as for a rod-like drag-tag for large N_{DNA}, but different for small N_{DNA}, which is a consequence of weight reduction near the attachment point.



Limiting case of a branched drag-tag

Summary and discussion

We have considered electrophoresis of various composite objects relevant to biomolecule separation. We have found that contrary to the predictions of a simple theory neglecting HI, the results depend significantly on the geometry, topology and stiffness of the objects.

Validity of the underlying Kirkwood-Riseman approximation is an issue. Hickey *et al.* [PRL 105 (2010) 148301; JCP 138 (2013) 194905] confirmed the overall shape of the weight function for the flexible chain.

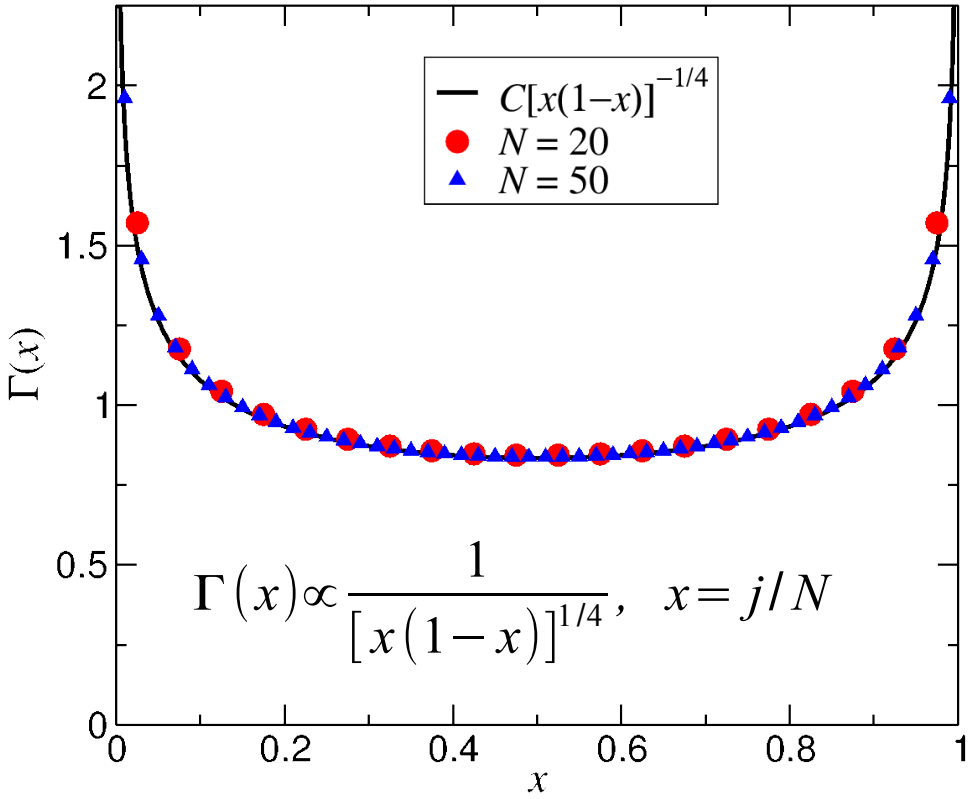
Experimental verification should in principle be rather straightforward. Most experiments so far concentrated on the regimes relevant to DNA sequencing, so the short-DNA and long-DNA limits most interesting theoretically have not been explored much.

$$\mu = \frac{\sum_j \Gamma_j \mu_j}{\sum_j \Gamma_j}, \quad \text{where} \quad \Gamma_j = \sum_i G^{ij}, \quad G = \langle H \rangle^{-1} \qquad \Sigma_j \Gamma_j \text{ is the drag coeff.}$$

The weights depend on the geometry, but not on the mobilities or charges.

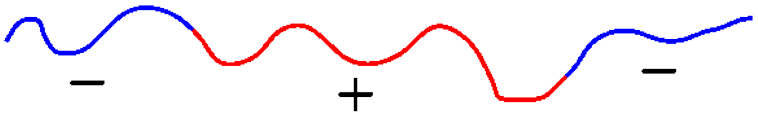
If HI are neglected and all monomers are identical, then *G* is diagonal, all weights are the same and the simple mobility formula is recovered.

Gaussian chain



Zimm (1956)
Chubynsky and Slater (2014)

Monomers near the ends of the chain affect the mobility more than those near the middle – **end effect**.



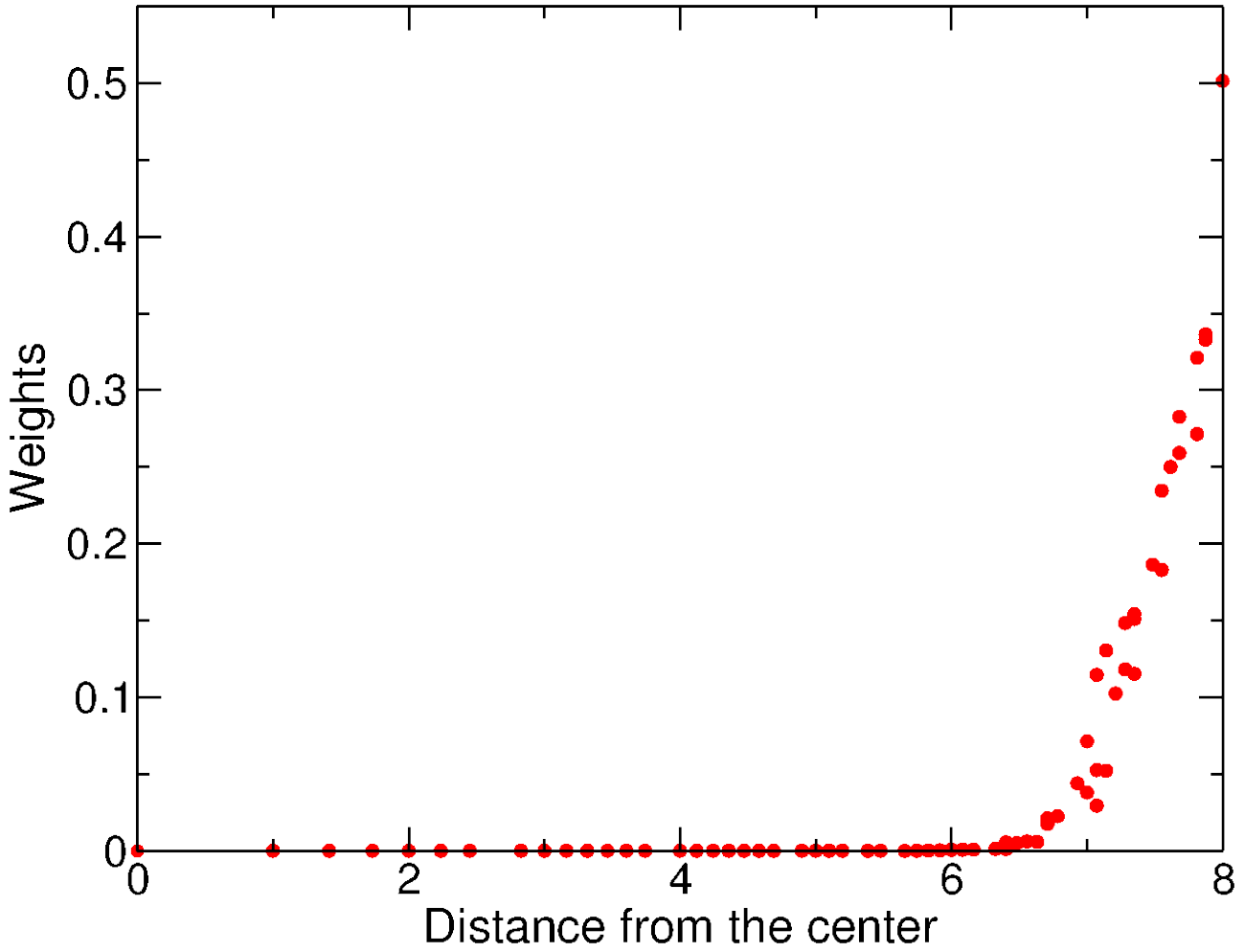
An overall neutral polymer can have nonzero mobility.

Long *et al.*, J. Chem. Phys. **108**, 1234 (1998).

Hickey et al., Phys. Rev. Lett. **105**, 148301 (2010).

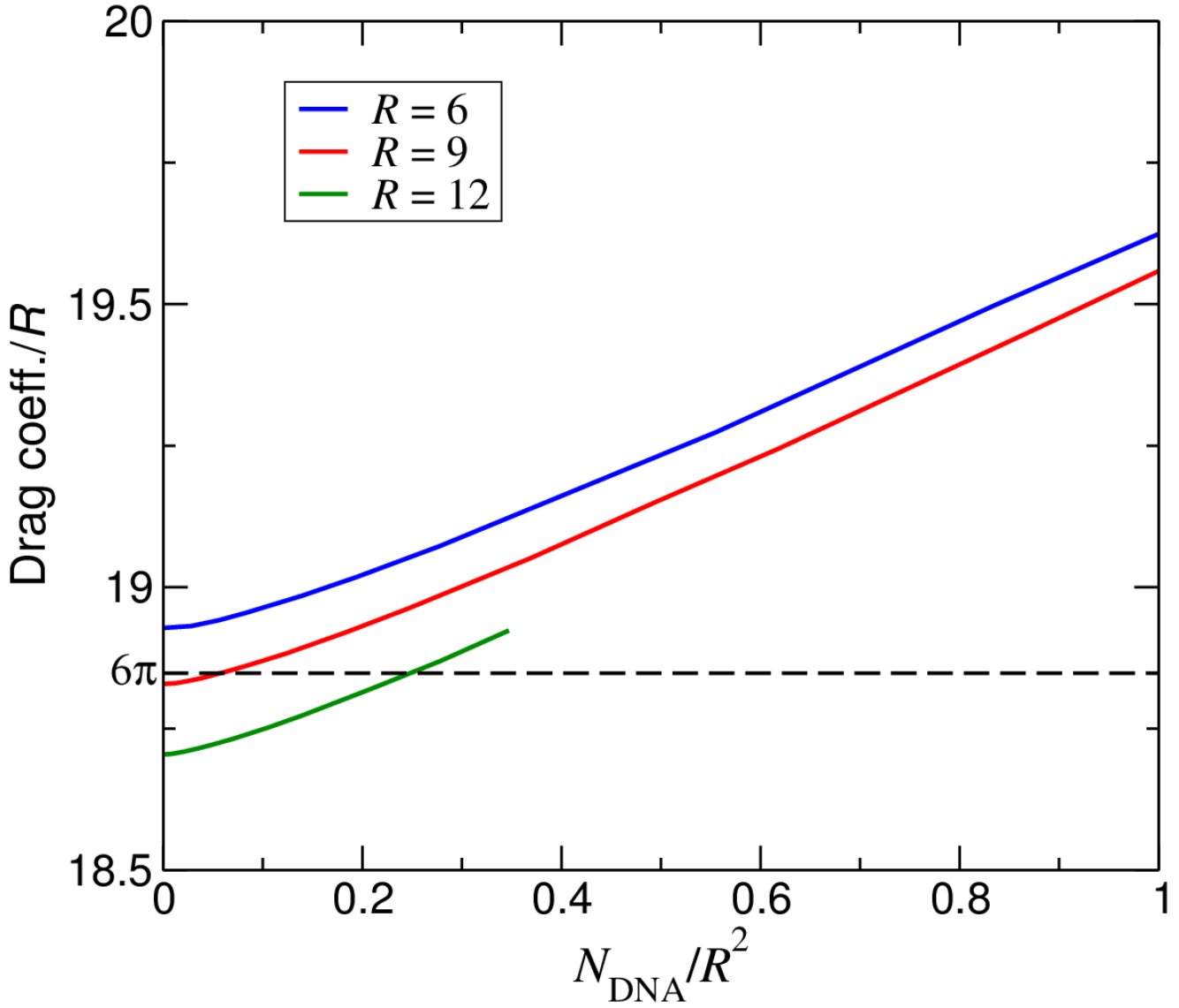
A “soft sphere” penetrable to both the solvent and the counterions:

A sphere cut out of a cubic lattice of beads:



Our result for branched polymers demonstrates that even for a 3-arm star the drop of the weights at the center is significant.

Sphere+coil – total friction (η = 1).



Grows as $N_{DNA}^{3/2}$ at small N_{DNA}. Contrast with the case of attachment to a coil:

$$\zeta \sim \sqrt{N_{DT} + N_{DNA}} \propto N_{DNA}^{1}$$