

Supporting Material to

Extending the range for force calibration in magnetic tweezers

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Analytical formula of the force-extension relation according to the extensible WLC model

In the following we derive an analytical solution for the force-extension relation that is defined by the extensible worm-like-chain (WLC) model (1, 2). This solution can be conveniently used to directly fit force extension data with thus to derive the contour length L_0 , the persistence length p and the stretch modulus S . Starting point is the force-extension relation from Marko and Siggia (1) that describes the entropic elasticity of inextensible DNA. The term for the relative DNA extension L/L_0 is replaced by $L/L_0 - F_{\text{DNA}}^{\text{MS}}/S$ in order to account for the additional elastic elasticity of DNA to the application of the external force $F_{\text{DNA}}^{\text{MS}}$. This provides:

$$F_{\text{DNA}}^{\text{MS}} = \frac{k_B T}{p} \left[\frac{1}{4(1 - L/L_0 + F_{\text{DNA}}^{\text{MS}}/S)^2} - \frac{1}{4} + \left(\frac{L}{L_0} - \frac{F_{\text{DNA}}^{\text{MS}}}{S} \right) \right], \quad (\text{S1})$$

where k_B is the Boltzmann constant and T the temperature. This formula can be converted to a 3rd order polynome:

$$A \cdot (F_{\text{DNA}}^{\text{MS}})^3 + B \cdot (F_{\text{DNA}}^{\text{MS}})^2 + C \cdot F_{\text{DNA}}^{\text{MS}} + D = 0, \quad (\text{S2})$$

with:

$$\begin{aligned} A &= \frac{4}{S^2} \left(\frac{p}{k_B T} + \frac{1}{S} \right) \\ B &= \frac{8}{S} \left(1 - \frac{L}{L_0} \right) \frac{p}{k_B T} + \frac{3}{S^2} \left(3 - 4 \frac{L}{L_0} \right) \\ C &= \left(1 - \frac{L}{L_0} \right) \left[4 \frac{p}{k_B T} \left(1 - \frac{L}{L_0} \right) + \frac{6}{S} \left(1 - 2 \frac{L}{L_0} \right) \right] \\ D &= -4 \left(\frac{L}{L_0} \right)^3 + 9 \left(\frac{L}{L_0} \right)^2 - 6 \left(\frac{L}{L_0} \right) \end{aligned} \quad (\text{S3})$$

According to Cardano's method equation S2 can be transformed into the reduced form:

$$z^3 + pz + q = 0, \quad (\text{S4})$$

where p and q are given by:

$$\begin{aligned} p &= \frac{C}{A} - \frac{B^2}{3A^2} \\ q &= \frac{2B^3}{27A^3} - \frac{BC}{3A^2} + \frac{D}{A} \end{aligned} \quad (\text{S5})$$

The discriminant d of Eqn. S4 is:

$$d = \left(\frac{q}{2} \right)^2 + \left(\frac{p}{3} \right)^3 \quad (\text{S6})$$

If $d > 0$, then Eqn. S2 has exactly one real solution, which is provided by:

$$F_{\text{DNA}}^{\text{MS}} = \sqrt[3]{-\frac{q}{2} + \sqrt{D}} + \sqrt[3]{-\frac{q}{2} - \sqrt{D}} - \frac{B}{3A} \quad (\text{S7})$$

For $d < 0$, Eqn. S2 has three real solutions out of which the following provides the correct result for the extensible WLC model:

$$F_{\text{DNA}}^{\text{MS}} = \sqrt{\frac{4}{3}p} \cdot \cos\left(\frac{1}{3}\arccos\left(-\frac{q}{2}\sqrt{-\frac{27}{p^3}}\right)\right) - \frac{B}{3A} \quad (\text{S8})$$

We thus obtain an analytic expression for the extensible WLC model based on the approximation by Marko and Siggia for the entropic elasticity.

The Marko-Siggia approximation is however known to deviate by up to 15% from a numeric solution for the force-extension relation of an inextensible WLC. To compensate this difference, Bouchiat et al. derived a more precise approximation by including polynomial correction terms (3):

$$F_{\text{DNA}}^{\text{B}} = \frac{k_{\text{B}}T}{p} \left[\frac{1}{4(1-L/L_0)^2} - \frac{1}{4} + \frac{L}{L_0} - 0.5164228\left(\frac{L}{L_0}\right)^2 - 2.737418\left(\frac{L}{L_0}\right)^3 \right. \\ \left. + 16.07497\left(\frac{L}{L_0}\right)^4 - 38.87607\left(\frac{L}{L_0}\right)^5 + 39.49944\left(\frac{L}{L_0}\right)^6 - 14.17718\left(\frac{L}{L_0}\right)^7 \right] \quad (\text{S9})$$

We obtain an approximation of the extensible form of this force-extension relation by replacing L/L_0 with $L/L_0 - F_{\text{DNA}}^{\text{MS}}/S$, one first solves the extensible form of the Marko-Siggia expression and then uses this force to calculate a corrected force based on the expression by Bouchiat. In case of double-stranded DNA such an analytic approximation deviates by only 0.2% from the numerical solution of the extensible form of the Bouchiat expression (Fig. S1) and can be conveniently used when fitting force extension data. The small errors with which our analytical approximation reproduces the numerical solution is due to the fact that the Marko-Siggia formula exhibits the strongest deviations from the Bouchiat formula around a relative extension of 0.5. At this extension a force of only 0.1 pN is obtained, at which the elastic stretching of DNA is negligible. At the higher forces where the elastic stretching needs to be considered (> 10 pN) both formulas are already in close agreement. This together provides the excellent agreement over the whole force range.

Correction factors for the drag coefficient of a sphere nearby a surface

The correction factors provided in the following refer to the corresponding drag coefficients of a sphere with radius R in bulk solution, i.e. far away from any surface.

$C_{\parallel}(L)$, the correction factor of the Stokes's drag coefficient for movements parallel to a nearby surface at distance L is given by Faxen's law (4):

$$C_{\parallel}(L) = \frac{1}{1 - \frac{9}{16} \left(1 + \frac{L}{R}\right)^{-1} + \frac{1}{8} \left(1 + \frac{L}{R}\right)^{-3} - \frac{45}{256} \left(1 + \frac{L}{R}\right)^{-4} - \frac{1}{16} \left(1 + \frac{L}{R}\right)^{-5}} \quad (\text{S10})$$

$C_{\perp}(L)$, the corresponding correction factor for movements perpendicular to a nearby surface, was provided by (5):

$$C_{\perp}(L) = \frac{1}{1 - \frac{9}{8} \left(1 + \frac{L}{R}\right)^{-1} + \frac{1}{2} \left(1 + \frac{L}{R}\right)^{-3} - \frac{57}{100} \left(1 + \frac{L}{R}\right)^{-4} + \frac{1}{5} \left(1 + \frac{L}{R}\right)^{-5} + \frac{7}{200} \left(1 + \frac{L}{R}\right)^{-11} - \frac{1}{25} \left(1 + \frac{L}{R}\right)^{-12}} \quad (\text{S11})$$

For $C_{\varphi}(L)$, the corresponding correction factor for rotations with the rotation axis being parallel to the surface we used (6):

$$C_{\varphi}(L) = \left[1 + \frac{5}{16} \left(1 + \frac{L}{R}\right)^{-3} \right] \quad (\text{S12})$$

Analytical formula of the PSD for coupled translational and rotational fluctuations including corrections for camera-acquisition artifacts

The analytical formula for the PSD for coupled translational and rotational fluctuations (Eqn. 15, main text) was corrected for low pass-filtering by the camera and aliasing as described for the simple harmonic model (Eqn. 5, main text). This provides the following formula:

$$S_{x,\text{coupl}}^{\text{corr}}(f) = \sum_{n=-\infty}^{\infty} \frac{4k_B T}{(2\pi)^2 (1 + C^2 \gamma_x \gamma_{\varphi} / R^2)} \left(\frac{\gamma_{\varphi} C^2}{R^2} \frac{1}{f_+^2 + |f + nf_s|^2} + \frac{1}{\gamma_x} \frac{1}{f_-^2 + |f + nf_s|^2} \right) \frac{\sin^2(\pi \tau_e |f + nf_s|)}{(\pi \tau_e |f + nf_s|)^2} \quad (\text{S13})$$

Modified equations for Brownian dynamics simulations of off-center attached beads

To establish simulations of off-center attached beads the equations describing the coordinates of the DNA attachment point and the torque on the bead (Eqn. 10) had to be modified. The translational forces (Eqn. 9) as well as the drag coefficients (Eqn. 11) remained unchanged. Essentially, the DNA attachment point is moving around the y axis of the bead on a circle with

radius $R_{\text{off}} = \sqrt{R^2 - y_{\text{off}}^2}$, where y_{off} is the off-center attachment along y (see inset in Fig. 6A). This provides lower excursions along x and z as well as a lower backdriving torque that counteracts the rotational fluctuations of the bead, R_{off} is always smaller than R . The following modified equations were applied:

$$\begin{aligned}
 x_{\text{DNA}}^{\text{off}} &= x_{\text{bead}} + R_{\text{off}} \sin \varphi_{\text{bead}} \\
 y_{\text{DNA}}^{\text{off}} &= y_{\text{bead}} + y_{\text{off}} \\
 z_{\text{DNA}}^{\text{off}} &= x_{\text{bead}} - R_{\text{off}} \cos \varphi_{\text{bead}} \\
 \Gamma_{\varphi}^{\text{off}} &= -F_{\text{DNA}} \cdot R_{\text{off}} / L (x_{\text{DNA}}^{\text{off}} \cos \varphi_{\text{bead}} + z_{\text{DNA}}^{\text{off}} \sin \varphi_{\text{bead}})
 \end{aligned}$$

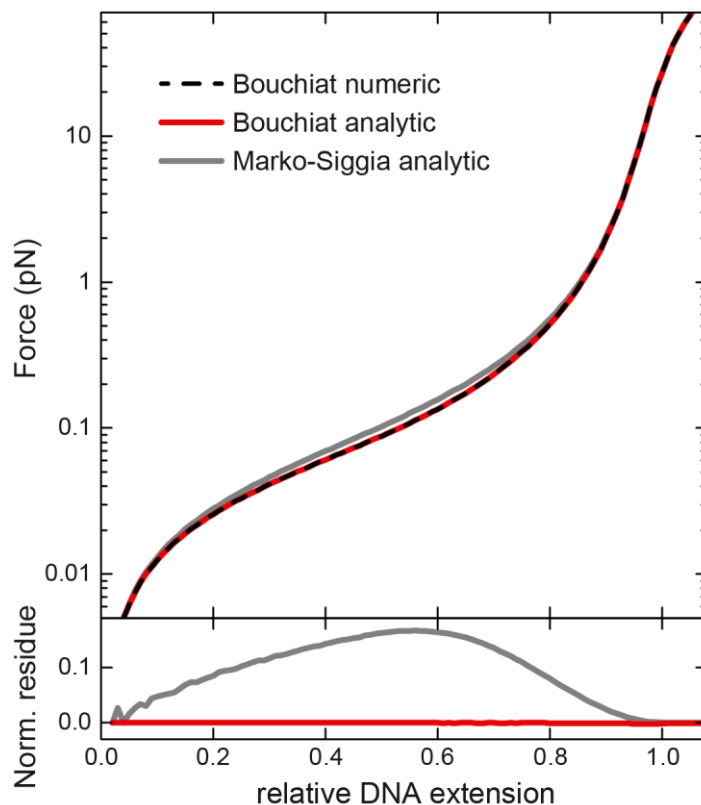


FIGURE S1 Performance of different solutions for extensible force-extension relations based on the WLC approximations according to Marko and Siggia (1) and Bouchiat et al. (3). The analytical force-extension relation based on the WLC approximation by Marko and Siggia (Eqns. S7 and S8) is shown in gray. An analytical and a numeric (black dashed line) solution for the force-extension relation that are based on the more precise WLC approximation by Bouchiat et al. (Eqn. S9) are shown as red and black dashed lines, respectively. Normalized residues of the analytical solutions with respect to the numeric solution are shown in the graph at the bottom. Despite an approximation the difference between analytical and numerical solution of the WLC approximation by Bouchiat et al. is smaller than 0.2%. Shown force extension data was calculated for a persistence length of 50 nm and a stretch modulus of 1000 pN.

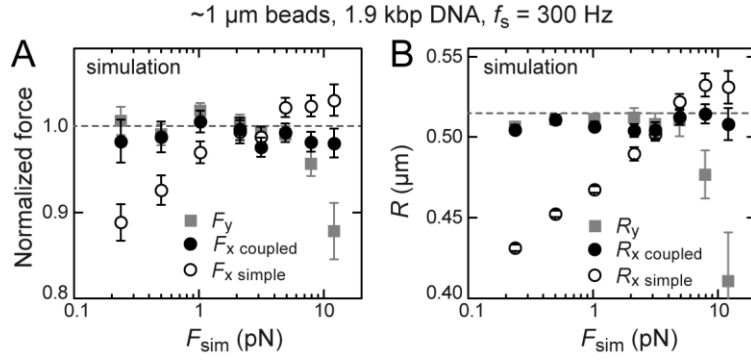


FIGURE S2 (A) Normalized forces and (B) radii obtained from PSD analysis of simulated time traces taken at 300 Hz for different external forces. The simulations used a DNA contour length of 700 nm and a bead radius of 515 nm (dashed line in B) was applied. Shown are the fit results when analyzing the PSD for the y -coordinate using a simple Lorentzian (F_y , R_y) and the PSD for the x -coordinate using a simple (F_x simple, R_x simple) as well as a double Lorentzian (F_x coupled, R_x coupled, see legend). Forces were normalized by the applied force F_{sim} . Dashed lines show the nominal values.

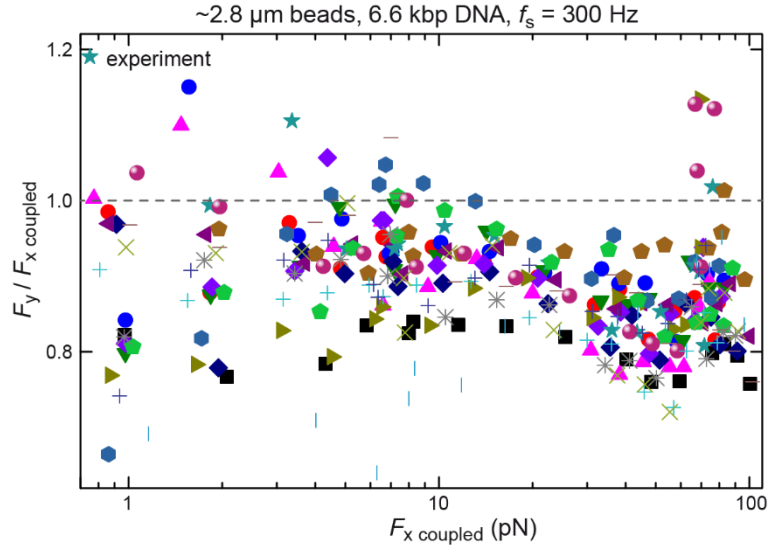


FIGURE S3 (A) Ratio $F_y / F_{x \text{ coupled}}$ between the forces obtained from the fluctuations along y and along x (double Loerentian model for the latter). Shown are the results for 21 2.8 μm beads each attached to a 6.6 kbp DNA molecule. The measurements for a single bead are represented by a unique color and a unique symbol. Overall F_y is smaller then $F_{x \text{ coupled}}$, which is most pronounced at low and high forces (see also Fig. 5, main text).

Supporting references

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