the four-noded knot has roughly tetrahedral symmetry, and it is possible to create star-shaped arrangements where the central four-noded knot coordinates to four other knots.

Received 19 March; accepted 13 May 1997.


Acknowledgements. This work was supported by the US Public Health Service, the Polish Committee of Scientific Research, the Swiss National Foundation and by Foundation Herberke, University of Lausanne. We thank R. Scharein for making available his KnotPlot program.

Correspondence should be addressed to A.S. (e-mail: Andrzej.Stasiak@laus.unil.ch).

The dynamics of partially extended single molecules of DNA

Stephen R. Quake , Hazen Babcock & Steven Chu

Physics Department, Stanford University, Stanford, California 94305, USA

The behaviour of an isolated polymer floating in a solvent forms the basis of our understanding of polymer dynamics. Classical theories describe the motion of a polymer with linear equations of motion, which yield a set of ‘normal modes’, analogous to the fundamental frequency and the harmonics of a vibrating violin string. But hydrodynamic interactions make polymer dynamics inherently nonlinear, and the linearizing approximations required for the normal-mode picture have therefore been questioned. Here we test the normal-mode theory by measuring the fluctuations of single molecules of DNA held in a partially extended state with optical tweezers. We find that the motion of the DNA can be described by linearly independent normal modes, and we have experimentally determined the eigenstates of the system. Furthermore, we show that the spectrum of relaxation times obeys a power law.

The starting point for describing the motion of a polymer is the Rouse model of beads interconnected with gaussian springs. If the interactions between different segments (beads) of the polymer \( R_p \) and \( R_m \) (\( n \) and \( m \) denote the order of the beads along the length of the polymer) are localized to nearest neighbours, each of the segments \( R_p \) satisfies a linear differential equation decoupled from the other segments. This linear system allows one to describe the dynamics in terms of a set of normal modes with mode amplitudes \( X_p \), defined as \( X_p(t) = (1/N) \sum R_p \cos(p \pi n/N) \delta_p (\delta_p = \cos(p \pi n/N) \delta_p) \) (where \( p \) is an integer denoting the \( p \)th mode and \( N \) is the total number of beads). Associated with the normal modes is a set of relaxation times, \( \tau_p \), which describe the decay in the normal-mode amplitudes \( X_p(t) \Delta X_p(0) = X_p(t) = X_p(0) e^{-\tau_p} \) (where \( q \) is the \( q \)th mode).

Hydrodynamic interactions between segments of the polymer were first included by Zimm by assuming the surrounding fluid is incompressible and obeys the Navier–Stokes equation (known as the Kirkwood approximation). The resulting coupling of each polymer segment \( R_p \) to all of the other segments \( R_m \) leads to a nonlinear set of differential equations as the coupling depends on the instantaneous configuration of the polymer. These equations can be linearized by first averaging the distance \( |R_m - R_m| \) between segments over the distribution of accessible configurations. This so-called ‘pre-averaging’ approximation linearizes the set of coupled differential equations and allows one to construct a set of normal modes similar to the Rouse modes.

It has been argued that a normal-mode description of a polymer can be only a rough approximation of the true dynamics and may not have any fundamental validity. Apart from questioning the soundness of the pre-averaging approximation, other nonlinear effects such as excluded-volume effects, forbidden crossings, and knots were not included in Zimm’s treatment. These and other nonlinear effects have led de Gennes and others to emphasize dynamical scaling laws rather than a normal-mode description. Scaling laws link relaxation rates to the spatial scale of interest, but do not require knowledge of the precise shape of the relaxation spectrum. On the other hand, the normal-mode concept requires that the relaxation spectrum be described by a set of discrete frequencies.

The analysis of the relaxation of polymers perturbed from equilibrium can yield a frequency spectrum by taking the inverse Laplace transform of the relaxation spectrum, but these methods do not provide an unambiguous decomposition into discrete frequencies. Dynamic light-scattering experiments probe relaxation rates at size scales considerably smaller than the overall coil size, but these measurements represent an average over what could be a continuous spectrum of relaxation rates. Dielectric relaxation of dipole-inverted cis-polyisoprene has been used to measure the first two normal-mode eigenfunctions, and a tentative extrapolation was used to derive the third. However, this method does not measure a relaxation mode spectrum, and does not discriminate between linear and nonlinear effects.

Figure 1 Video images of single molecules of DNA held at an extension of 12 µm. The column on the left shows a series of successive raw video images; the column on the right shows the corresponding parametrizations used in the data analysis.
optical tweezers while the images were recorded by video microscopy
the polymer is fixed19.

We have tested the supposition that polymer motion can be
described by a set of normal modes by recording the images of
partially extended 20-μm-long pieces of DNA driven by brownian
motion. The DNA is suspended in solution by attaching 0.3-μm
dyed polystyrene spheres to the ends of the molecule that serve as
‘handles’ for two optical tweezers18. The DNA, fluorescently stained
by the dye YOYO-1 (Molecular Probes, Eugene, OR) and illumi-
nated by 488-nm light, was held in a partially extended state with
two optical tweezers while the images were recorded by video microscopy
in the form

\[ R_y(t) = (1/N)^2 \sum_{k=1}^{N} R_y(t) \sin(\pi pm/N)dn. \]

The optical images were converted into parametrized descriptions
of the DNA location as described below. If the line joining
the two beads defines the x-axis, we analyse the polymer config-

uration in terms of the x-motion and the y-motion. The condition for the time correlations of the

was always degenerately small, while the amplitudes were in
agreement with our other estimations of the signal and noise. Thus, the intrinsic
noise has a systematic effect on only the \( t = 0 \) point of the correlation function.
That point was discarded before fitting the relaxation to an exponential function.

Figure 2 Comparison of the sine basis and the normal-mode basis for several
matrix elements of DNA held at 10 μm extension. The correlation function \( C_{mn}(t) \)
represents eigenbasis correlation matrix \( \langle X^m(t)X^n(0) \rangle \) for solid lines and the sine
basis correlation matrix \( \langle U^m(t)U^n(0) \rangle \) for dashed lines. We note that in the normal-
mode basis, there are no off-diagonal elements above the level of noise fluctuations.

The increasing frequency of the characteristic fluctuations for the
cross-correlation of mode 2 with successively higher modes may be seen in this
figure.

Figure 3 Relaxation curves and exponential fits for \( q = 2, 3, \) and 4 (circles,
squares and triangles, respectively) at extension 10 μm. Inset, residuals from the
fits, showing a small systematic deviation from pure exponential relaxation.
There is a measurement error in assigning the parametrization of the polymer;
this causes a systematic error in the \( t = 0 \) correlation matrix. The measured quantity is
indistinguishable from noise. If normal modes did not exist, the
measurement error should be independent of time, so \( C_{ij}(t) \) vanishes for \( t \neq 0 \).
We checked this by fitting the relaxation function to two exponentials; one
relaxation time was always degenerately small, while the amplitudes were in
agreement with our other estimations of the signal and noise. Thus, the intrinsic
noise has a systematic effect on only the \( t = 0 \) point of the correlation function.
That point was discarded before fitting the relaxation to an exponential function.

We have tested the supposition that polymer motion can be
described by a set of normal modes by recording the images of
partially extended 20-μm-long pieces of DNA driven by brownian
motion. The DNA is suspended in solution by attaching 0.3-μm
dyed polystyrene spheres to the ends of the molecule that serve as
‘handles’ for two optical tweezers18. The DNA, fluorescently stained
by the dye YOYO-1 (Molecular Probes, Eugene, OR) and illumi-
nated by 488-nm light, was held in a partially extended state with
two optical tweezers while the images were recorded by video microscopy
in the form

\[ R_y(t) = (1/N)^2 \sum_{k=1}^{N} R_y(t) \sin(\pi pm/N)dn. \]

The optical images were converted into parametrized descriptions
of the DNA location as described below. If the line joining
the two beads defines the x-axis, we analyse the polymer config-

uration in terms of the x-motion and the y-motion. The condition for the time correlations of the

was always degenerately small, while the amplitudes were in
agreement with our other estimations of the signal and noise. Thus, the intrinsic
noise has a systematic effect on only the \( t = 0 \) point of the correlation function.
That point was discarded before fitting the relaxation to an exponential function.

We have tested the supposition that polymer motion can be
described by a set of normal modes by recording the images of
partially extended 20-μm-long pieces of DNA driven by brownian
motion. The DNA is suspended in solution by attaching 0.3-μm
dyed polystyrene spheres to the ends of the molecule that serve as
‘handles’ for two optical tweezers18. The DNA, fluorescently stained
by the dye YOYO-1 (Molecular Probes, Eugene, OR) and illumi-
nated by 488-nm light, was held in a partially extended state with
two optical tweezers while the images were recorded by video microscopy
in the form

\[ R_y(t) = (1/N)^2 \sum_{k=1}^{N} R_y(t) \sin(\pi pm/N)dn. \]

The optical images were converted into parametrized descriptions
of the DNA location as described below. If the line joining
the two beads defines the x-axis, we analyse the polymer config-

uration in terms of the x-motion and the y-motion. The condition for the time correlations of the

was always degenerately small, while the amplitudes were in
agreement with our other estimations of the signal and noise. Thus, the intrinsic
noise has a systematic effect on only the \( t = 0 \) point of the correlation function.
That point was discarded before fitting the relaxation to an exponential function.

We have tested the supposition that polymer motion can be
described by a set of normal modes by recording the images of
partially extended 20-μm-long pieces of DNA driven by brownian
motion. The DNA is suspended in solution by attaching 0.3-μm
dyed polystyrene spheres to the ends of the molecule that serve as
‘handles’ for two optical tweezers18. The DNA, fluorescently stained
by the dye YOYO-1 (Molecular Probes, Eugene, OR) and illumi-
nated by 488-nm light, was held in a partially extended state with
two optical tweezers while the images were recorded by video microscopy
in the form

\[ R_y(t) = (1/N)^2 \sum_{k=1}^{N} R_y(t) \sin(\pi pm/N)dn. \]

The optical images were converted into parametrized descriptions
of the DNA location as described below. If the line joining
the two beads defines the x-axis, we analyse the polymer config-

uration in terms of the x-motion and the y-motion. The condition for the time correlations of the

was always degenerately small, while the amplitudes were in
agreement with our other estimations of the signal and noise. Thus, the intrinsic
noise has a systematic effect on only the \( t = 0 \) point of the correlation function.
That point was discarded before fitting the relaxation to an exponential function.

We have tested the supposition that polymer motion can be
described by a set of normal modes by recording the images of
partially extended 20-μm-long pieces of DNA driven by brownian
motion. The DNA is suspended in solution by attaching 0.3-μm
dyed polystyrene spheres to the ends of the molecule that serve as
‘handles’ for two optical tweezers18. The DNA, fluorescently stained
by the dye YOYO-1 (Molecular Probes, Eugene, OR) and illumi-
nated by 488-nm light, was held in a partially extended state with
two optical tweezers while the images were recorded by video microscopy
in the form

\[ R_y(t) = (1/N)^2 \sum_{k=1}^{N} R_y(t) \sin(\pi pm/N)dn. \]

The optical images were converted into parametrized descriptions
of the DNA location as described below. If the line joining
the two beads defines the x-axis, we analyse the polymer config-

uration in terms of the x-motion and the y-motion. The condition for the time correlations of the

was always degenerately small, while the amplitudes were in
agreement with our other estimations of the signal and noise. Thus, the intrinsic
noise has a systematic effect on only the \( t = 0 \) point of the correlation function.
That point was discarded before fitting the relaxation to an exponential function.

We have tested the supposition that polymer motion can be
described by a set of normal modes by recording the images of
partially extended 20-μm-long pieces of DNA driven by brownian
motion. The DNA is suspended in solution by attaching 0.3-μm
dyed polystyrene spheres to the ends of the molecule that serve as
‘handles’ for two optical tweezers18. The DNA, fluorescently stained
by the dye YOYO-1 (Molecular Probes, Eugene, OR) and illumi-
nated by 488-nm light, was held in a partially extended state with
two optical tweezers while the images were recorded by video microscopy
in the form

\[ R_y(t) = (1/N)^2 \sum_{k=1}^{N} R_y(t) \sin(\pi pm/N)dn. \]

The optical images were converted into parametrized descriptions
of the DNA location as described below. If the line joining
the two beads defines the x-axis, we analyse the polymer config-

uration in terms of the x-motion and the y-motion. The condition for the time correlations of the

was always degenerately small, while the amplitudes were in
agreement with our other estimations of the signal and noise. Thus, the intrinsic
noise has a systematic effect on only the \( t = 0 \) point of the correlation function.
That point was discarded before fitting the relaxation to an exponential function.
orthogonality of the normal modes, we show in Fig. 3 that the diagonal relaxation functions \(X_p(t)X_p(0)\) are well described by a single exponential decay as required for a linear dynamical system.

Figure 4 shows that the relaxation times \(\tau\) scale as a power law over almost two decades of time. The Zimm model for a coiled polymer predicts an exponent of \(-1.5\) for a \(\Theta\)-solvent\(^2\) and \(-1.8\) for a good solvent. DeGennes has predicted\(^3\) that a highly extended polymer will have an exponent of \(-2.0\). Caution must be used in comparing the observed power-law scaling to dynamical models that were derived for conditions where the end points are not constrained. Although it is known that our buffer conditions correspond to a good solvent for DNA\(^3\), it has been suggested that an extended polymer will behave as in an ideal solvent on moderate to long length scales such as we measured\(^2\). Also, the pre-averaged distances for a partially extended polymer are different than for a free polymer. For an unstrained chain, \(\langle R_s - R_e \rangle \sim [n - m]^{0.5}\) for an ideal solvent and \(\langle n - m \rangle^{0.8}\) for a good solvent leading to relaxation times that scale as \(\tau \sim p^{1.5}\) or \(p^{1.8}\), respectively. For partially extended polymers, at distances \(n - m > 1\), we expect \(\langle [R_s - R_e]_{pre-averaged} \rangle = [n - m]^{-1.0}\) but for \(n \approx m\), \(\langle n - m \rangle^{-1.0}\) where the exponent \(\alpha\) might be some value between 0.5 and 1.0. Clearly, a calculation of the pre-averaged hydrodynamic tensor for our geometry will have to be done before one compares these results to a particular dynamical model.

Methods

Sample preparation. \(\lambda\)-Phage DNA was attached to streptavidin-coated beads via biotinylated oligonucleotides that hybridized to the single-stranded ends of \(\lambda\) DNA. The \(\lambda\) DNA and one of the oligonucleotides were mixed in equal molar amounts in 50 mM NaCl–TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), heated to 70°C for 10 min, and allowed to anneal to room temperature for 2–3 h. The opposite oligonucleotide was mixed with streptavidin beads in about 2× excess to streptavidin binding sites, and allowed to incubate for 8 h before being washed three times by centrifugation. Equal molar amounts of biotinylated DNA, oligo-coated beads, and streptavidin beads were mixed and allowed to incubate for 12 h at room temperature in 50 mM NaCl–TE. The resulting bead–DNA–bead complexes were diluted by a factor of 10 into a staining solution contain TE, 2 mM NaCl, 100 mM YOYO, 1% β-mercaptoethanol, and 0.1% Tween-20. After 45–60 min of staining, the complexes were diluted by another factor of 10 into a high-viscosity TE buffer containing a final concentration of 71% (v/v) glycerol, 2 mM NaCl, and 0.1% Tween-20. To retard photobleaching of the dye, 0.1 mg ml\(^{-1}\) glucose oxidase, 0.04 mg ml\(^{-1}\) catalase, and 0.4% glucose were added.

Experimental procedure. The optical tweezers were made by focusing two independently controllable infrared (1.064-μm) Nd:YAG laser beams through a ×63, 1.4 numerical aperture microscope in a home-made microscope with a measured resolution of 0.25 μm. The DNA was held 10 μm below the surface of a 30-μm-deep cell made from a microscope slide and coverslip sealed with epoxy. The fluorescent images were recorded with an image-intensified CCD camera with a video rate of 30 Hz. Because the fundamental relaxation time of a polymer is proportional to viscosity, we increased the effective time resolution of the experiment by increasing the viscosity from 0.01 to 0.2 poise with the addition of 71% glycerol (w/v). We took data from 37 different molecules held at six different extensions ranging between 30% and 80% of the full length of the molecule. An average of 24 min (43,200 video frames) of data were taken at each extension.

Data analysis. Spatial noise in the images were filtered out by convolving each image with a gaussian of width 0.42 μm before finding the fluorescence maxima. Using continuity of the DNA, an algorithm tracked the fluorescent peak from one bead to the other and back again. As the DNA fluctuated in and out of the focal plane, parts of its image broadened and occasionally disappeared. The fitting program discarded those points; 98% of the data was retained, and a visual and manual check ensured that the fitting program was not giving skewed values. Although details of the DNA configuration below the 0.25 μm resolution of the microscope are not seen, we estimate that the centre of the image is determined with an uncertainty of ±1 pixel which is ≥0.14 μm. We showed that the resulting ‘pixellation’ of the images does not affect the data analysis by artificially increasing the linear pixel size by a factor of two; the results of the subsequent data analysis did not change. The beads are free to rotate in the traps; this allows the ends of the polymer a small amount of motion not greater than the radius of the bead. Also, the beads absorb some of the YOYO and fluoresce brightly; this causes blooming on the video image and prevents us from locating the precise end points of the DNA. As we infer the locations of those end points from the locations of the beads, there is a small systematic overestimation of the distance of the distance of the DNA from its equilibrium position as it fluctuates above and below the imaginary line which connects the two beads. We put that extra degree of freedom of the end points into a computer simulation of a bead–spring model with the exact hydrodynamic tensor. For exponential fits, the power-law exponent of the simulated mode structure with mobile end points was 2.5% smaller than the value for fixed end points, with a 2.5% statistical error on the fit. Thus the rotation of the beads has only a small systematic effect on the data. One can correct for this effect by multiplying the observed parameters by 1.025.

Table 1 Normal-mode eigenbasis

<table>
<thead>
<tr>
<th>Mode</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exponent</td>
<td>1.000</td>
<td>0.999</td>
<td>0.997</td>
<td>0.995</td>
<td>0.994</td>
<td>0.993</td>
<td>0.992</td>
<td>0.991</td>
</tr>
<tr>
<td>Extension (μm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.100</td>
<td>-0.027</td>
<td>-0.024</td>
<td>-0.021</td>
<td>-0.018</td>
<td>-0.015</td>
<td>-0.012</td>
<td>-0.009</td>
<td>-0.006</td>
</tr>
<tr>
<td>0.010</td>
<td>0.997</td>
<td>0.995</td>
<td>0.993</td>
<td>0.991</td>
<td>0.990</td>
<td>0.988</td>
<td>0.986</td>
<td>0.984</td>
</tr>
</tbody>
</table>

Columns show the normal modes expressed in terms of a sum of sinevectors for the 10-μm extension. The rows are the mode numbers. For example, column 8 shows that the eighth normal mode has an amplitude 0.998 of sin(8πn/N), -0.027 of sin(7πn/N) and -0.041 of sin(6πn/N).
Received 12 September 1996; accepted 15 May 1997.

Multiple episodes of aridity in southern Africa since the last interglacial period

Stephen Stokes1,* & David S. G. Thomas‡ & Richard Washington†

* School of Geography, University of Oxford, Mansfield Road, Oxford OX1 3QJ, UK
† Research Laboratory for Archaeology and the History of Art, University of Oxford, Mansfield Road, Oxford OX1 3TB, UK
‡ Sheffield Centre for International Drylands Research, Department of Geography, University of Sheffield, Sheffield S10 2TN, UK

There is generally a dearth of evidence of the nature of Quaternary climate change within desert systems, which has limited previous interpretations of past environmental change at low latitudes. The Last Glacial Maximum has previously been identified as the peak of Late Quaternary aridity, when desert systems expanded to five times their present extent1,2, and low-latitude aridity has been described for previous glaciations3. But little evidence has been derived directly for large desert basins, particularly southern Africa. Here we report new chronological (optical dating) evidence of arid episodes recorded in aeolian sediments from the Mega Kalahari sand sea. Episodic aeolian activity is recorded at the northeastern desert margin, whereas more sustained activity is evident from the southwestern desert core. Several significant arid events are apparent since the last interglacial period, with dune-building (arid) phases at ~95–115, 41–46, 20–26 and 9–16 kyr before present. Existing atmospheric general circulation model simulations and independent palaeoecological data indicate that the changes in aridity are related to changes in the northeast–southwest summer rainfall gradient, which are in turn related to sea surface temperatures in the southeastern Atlantic Ocean.

The 2.5 × 106 km2 Mega Kalahari is the world’s most continuous area of aeolian sand sea which is dominated by variably degraded and pedogenically modified, currently inactive, linear dunes4 (Fig. 1). The development and evolution of the extensive Kalahari linear dune systems has not until now been directly dated. Phases of arid and dune development have previously been inferred from gaps within subcontinental humid chronologies5,6 or by assumed mirroring of Northern Hemisphere conditions7. We have determined the timing of dune building and associated palaeoaridity along the present day northeast–southwest summer rainfall gradient by optically dating dune sediments from geographically and morphologically wide-ranging aeolian deposits. Past changes in aeolian dynamism is assumed to be related to shifting rainfall intensities along the gradient (Fig. 1).

The effect of this northeast–southwest rainfall gradient on aeolian activity provides an opportunity to determine the sensitivity of the climate system to changing boundary conditions since the last interglacial. The climatology of this gradient is an infrequently studied element of the general circulation of the subcontinent, yet one that is accurately captured in all present-day leading general circulation models8,9. It originates from the Earth’s largest cross-equatorial zonal asymmetry of tropical convection; the inter-tropical convergence zone (ITCZ) in the southwestern Indian Ocean occupies the most southerly location of any ocean at 23°S, whereas tropical convection in the eastern Atlantic Ocean is seldom found south of 5°N (ref. 10). Convection over southern Africa aligns almost meridionally over the continent. Differences in the latitude of convection in the adjacent oceans relates directly to sea surface temperatures (SSTs).

The southwestern Indian Ocean is the warmest ocean at 23°S whereas the southeastern Atlantic is the coldest (present-day January mean of 27.5 and 21.6°C, respectively). The extreme SST contrast across the subcontinent determines the southern African rainfall gradient (Fig. 1). Wetter conditions east of the Kalahari result from disturbances in the tropical easterlies associated with the Mascarene anticyclone. Westward propagation of easterly waves off the African subcontinent is blocked by cold stable air overlying the Benguela Current. Conditions west of the wave axis are therefore dry. Intense subsidence into subtropical anticyclones dominate the climatology of the austral winter, ensuring aridity. The elevated southern African land mass (2,000 m) cools through longwave emission, thereby providing an interhemispheric sink for Asian monsoon outflow. Few deserts provide a suite of controls on the rainfall regime as diverse as the Kalahari.

Although the controls on aeolian sedimentation, particularly in the case of linear dune development10, may be complex we believe that our chronological of dune construction reflects periods of regionally enhanced aridity. In the northeastern Kalahari, mean rainfall today exceeds 400 mm yr−1, and the pedogenically modified and vegetated surfaces of degraded linear dunes prohibits aeolian activity. In the southwestern Kalahari the present mean annual rainfall is 150–200 mm. Episodic, localized aeolian activity is confined to dune crests and controlled by variations in vegetation cover induced by interannual variations in rainfall and intensive grazing, but is in any case significantly restricted by present-day low...