Stiffness versus architecture of single helical polyisocyanopeptides

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Materials

Polyisocyanopeptides 1, 2, 3, 3 and 4^1 were prepared according to previously reported methods. The catalyst to monomer ratio of 1:10,000 was chosen to yield micrometer long polymers.

AFM. We used a commercial instrument (JPK Nanowizard 1) with software version 3.4.18. Biolever BL-AC40TS-C2 (Olympus) and the PPP-cont-50 (Nanosensors) cantilevers were used. The AFM tips were calibrated using the thermal noise method using the build in functions of the JPK software.^{4,5}

Sample preparations. Highly diluted samples were prepared by spin-coating a dilute polymer solution (3 mM) in dichloromethane on a freshly cleaved muscovite mica surface at 3000 rpm. Subsequently, these samples were rinsed with trichloroethane (TCE) to remove any non-adsorbed polymer. The resulting polymer density and morphology were then analyzed using tapping mode AFM in air (Fig. 2a). It is statistically possible that a single polymer chain is pulled more than once and hence the observed apparent L_p is biased to a lower value. However, given the extremely low concentration of polymer on the surface this is thought to be rare. By analysis of the correlation of the contour length, these events could be observed and were found to be rare (< 5 %). These events were removed from the analysis.

The interaction of the polymer molecule with the AFM tip and the surface could also change the initial n+4 conformation into the more flexible n+3 or n+2 conformations, which would explain the many flexible polymers found when probed for the first time (Fig. 2). The raw force spectroscopy data is shown in the supplementary information.

Measurement and Analysis

All force spectroscopy measurements were done in TCE. The polymers were attached to the AFM tip by non-specific adsorption.⁶ The tip was lowered with a speed of 1 μ m/s until it made contact with the sample, pressed onto the sample with a force of a few nN for several seconds and retracted from the surface with a speed of 1 μ m/s. Due to the low polymer density on the surface, a polymer was adsorbed to the AFM tip in less than 0.2 % of the approach-retract cycles. When events were seen, the majority was characterized by a single rupture. Some curves showed artifacts such as large nonspecific adhesion or multiple rupture events resulting from the simultaneous pulling of several molecules or multipoint attachment of one polymer. The few curves showing these artifacts or no event at all were filtered using a custom Matlab program and JPK data processing software version 3.4.18. Some of the curves that showed multiple rupture events were fitted for each individual force-distance curve. It should be noted that the eWLC fit showed deviations at forces above 200 pN indicating that enthalpic contributions (*e.g.* bond angle deformations in the polymer backbone) start to play a role at these high forces. Therefore, the force-distance curves were fitted only up to 200 pN.

The data were fitted using the modified Marko-Siggia WLC model (Ref 3, main text):

$$F = \left(\frac{k_{\rm B}T}{L_{\rm p}}\right) \left[\frac{1}{4(1 - x/L_0 + F/K_0)^2} - \frac{1}{4} + \frac{x}{L_0} - \frac{F}{K_0}\right]$$

Fitting parameters: L_p : persistence length, L_0 : contour length and K_0 : elastic modulus. In each iteration in the fitting procedure F(x) was calculated self-consistently:

$$F_0(x) = 200 \text{pN}; F_n(x) = 0.9F_{n-1}(x) + 0.1 \left(\frac{k_B T}{L_p}\right) \left[\frac{1}{4(1-x/L_0+F_{n-1}(x)/K_0)^2} - \frac{1}{4} + \frac{x}{L_0} - \frac{F_{n-1}(x)}{K_0}\right] \text{until} \langle F_n - F_{n-1} \rangle < 10^{-30} \text{N}$$



Fig. 1. a, Force-distance curve showing desorption like features. b, Force-distance curve with multiple ruptures. c, Force-distance curve (blue) with multiple ruptures. The last rupture could still be fitted to the eWLC model (red).

A few remarks should be made about the differences between the L_p 's for the same polymer obtained with different techniques and the potential influence that SMFS has on the results. The average L_p of polyisocyanodipeptide **1** obtained with SMFS is 17 nm, which is much shorter than the previously measured 76 nm for the same polymer.⁷ The large difference between the results obtained with different methods suggests that the apparent L_p is influenced by the measurement technique used. This can also be seen during the repeated stretching of the same polymer chain where the L_p was found to decrease. Possibly, picking up the polymer could be similarly invasive since the AFM tip is pressed with a high force (up to 3 nN) on the polymer which could potentially cause defects in the polymer. The surface could also influence the polymer upon desorption from the surface as the polymer is locally bend, perhaps disrupting hydrogen bonds and locally inducing a conformational change. Furthermore, this kind of invasive treatment differs from the environment in which we previously measured polyisocyanodipeptide **1** or the light scattering as used by Okoshi et al.⁷

In order to catch a polymer which can be stretched repeatedly, a force trigger of 200 pN at a minimum distance of 500 nm was applied. After a polymer is caught, the distance the AFM tip travels is locked. No pause was held during either direction reversal.

The following procedure was used to correct the background of the force-distance curves which did not fully extend to the sample surface. The first part of the retract curve, and the last part of the approach curve were set to zero force by subtracting the linear fit to this part. Subsequently, the complete curve was fitted to the eWLC model. The L_p obtained with this fit was used to add an offset equal to the Gaussian chain approximation at low extensions:

$$F(x) = \frac{k_B T}{L_p} \frac{3x}{2L_c}$$

with k_B Boltzmann's constant, T the absolute temperature, x the end to end distance of the polymer, L_p the persistence length and L_c the contour length. The curve was again fitted to the eWLC model and the difference between the old and the new offset was again added. These steps were repeated till the L_p remained constant.

Dispersity index (of L_p) is defined analogues to the Polydispersity Index (PDI) and is a measure of the heterogeneity:

$$DI(L_p) = \frac{n \sum L_{p(i)}^2}{\left(\sum L_{p(i)}\right)^2}$$

Where n is the total amount of measured polymers.

In Fig. 2a is illustrated that when a high force of more than 200 pN is applied to the polymer, large deviations of the eWLC are observed (Fig. 2a). In Fig. 2b it can be seen that fitting to 200 pN gives a reasonable. The deviations of the eWLC model at forces higher than 200 pN are probably due to the fact that friction is not incorporated in this model (Fig. 2). But since no model exists that includes friction, we used the eWLC model to extract the L_p from the force-distance curves.



Fig. 2 Deviation from the eWLC model. a, Part of the force extension curve where a high force is applied to polyisocyanodipeptide 1. The retract trace (blue rectangles) and the approach trace (red dots) do not completely overlap. The continuous red and blue lines show the eWLC fits to the complete approach and retract curve, respectively, showing a clear deviation from the experimental data when the complete curve is fitted. The inset shows the full approach and retract cycle. b, The same force-distance curves as in a, now fitted up to only 200 pN, giving a better fit.

Molecular modeling

The polymer model was stretched by applying a harmonic potential between the terminal atoms of the polymer chain. A very high force constant of 10.000 kcal/mol.Å² was used to fix the stretching distance at the equilibrium distance of the harmonic potential. The equilibrium distance was then increased with steps of 0.1 nm and after each step a geometric optimization at 0 K was performed to relax the polymer. The molecular mechanics energy minimizations were performed with a conjugate gradient algorithm until a convergence criterion of 0.001 kcal/mol Å was reached. The long-range interaction cutoff distance was set to 14 Å with a spline width of 3 Å. The charges were assigned from the PCFF force field. The resulting structure was then subjected to the Molecular Dynamics simulation at room temperature and afterwards again geometrically optimized at 0 K. The Molecular Dynamics simulations were performed on an isolated chain of 60 units in the NVT ensemble (*i.e.*, particle number, volume, and temperature are kept constant). The Nose thermal bath coupling scheme was used to maintain a constant temperature of 298 K with a coupling constant of 0.5. A Verlet velocity algorithm was used to integrate the equations of motion with 1 fs time steps.

Speed dependence



Here we present the data measured to distinguish between friction and non-equilibrium stretching (Fig. 3).

Fig. 3 Speed dependence of the hysteresis. a-d, Stretching and relaxation cycles measured at increasing speeds of a, 0.3, b, 1, c, 5 and d, 10 μ m/s. Note that d clearly shows an oscillation after the AFM piëzo direction is reversed due to the abruptness of this change. This artifact limits the maximum speed achievable. e, Hundreds of cycles were measured for each pulling speed and fitted to a Gaussian distribution. Here the distribution measured at a speed of 5 μ m/s is shown. The small shoulder at 0.12 aJ is probably the result of the polymers sometimes slightly adsorbing to the surface which increases the dissipated energy. f, The resulting mean value and standard deviation of the Gaussian fit are plotted versus the pulling speed. The error bars give the interval which accounts for 68 % of the set.

Raw data and histograms

Here we present the corresponding raw force-distance curves and fits, contour length distribution histogram, persistence length distribution histogram, rupture force distribution histogram and specific stiffness distribution histogram of polyisocyanodipeptide 1:



Fig. 4 Molecular structure of polyisocyanide 1.



Fig. 5 Raw force-distance curves (green - blue) and eWLC fits (red) of polyisocyanodipeptide 1.



Fig. 6 Contour length distribution histogram of polyisocyanodipeptide 1.



Fig. 7 Persistence length distribution histogram of polyisocyanodipeptide 1



Fig. 8 Rupture force distribution histogram of polyisocyanodipeptide 1.



Fig. 9 Specific stiffness distribution histogram of polyisocyanodipeptide 1.



Fig. 10 Initial contour length versus initial persistence length of polyisocyanodipeptide 1.



Fig. 11 Rupture force versus persistence length of polyisocyanodipeptide 1.

Here we present the corresponding raw force-distance curves and fits, contour length distribution histogram, persistence length distribution histogram, rupture force distribution histogram and specific stiffness distribution histogram of perylenepolyisocyanodipeptide 2:



Fig. 12 Molecular structure of polyisocyanide 2.



Fig. 13 Raw force-distance curves (green - blue) and eWLC fits (red) of perylenepolyisocyanodipeptide 2.



Fig. 14 Contour length distribution histogram of perylenepolyisocyanodipeptide 2.



Fig. 15 Persistence length distribution histogram of perylenepolyisocyanodipeptide 2.



Fig. 16 Rupture force distribution histogram of perylenepolyisocyanodipeptide 2.



Fig. 17 Specific stiffness distribution histogram of perylenepolyisocyanodipeptide 2.



Fig. 18 Initial contour length versus initial persistence length of perylenepolyisocyanodipeptide 2.



Fig. 19 Rupture force versus persistence length of perylenepolyisocyanodipeptide 2.

Here we present the corresponding raw force-distance curves and fits, contour length distribution histogram, persistence length distribution histogram, rupture force distribution histogram and specific stiffness distribution histogram of polyisocyanotripeptide **3**.



Fig. 20 Molecular structure of polyisocyanide 3.



Fig. 21 Raw force-distance curves (green - blue) and eWLC fits (red) of polyisocyanotripeptide 3.



Fig. 22 Contour length distribution histogram of polyisocyanotripeptide 3.



Fig. 23 Persistence length distribution histogram of polyisocyanotripeptide 3.



Fig. 24 Rupture force distribution histogram of polyisocyanotripeptide 3.



Fig. 25 Specific stiffness distribution histogram of polyisocyanotripeptide 3.



Fig. 26 Initial contour length versus initial persistence length of polyisocyanotripeptide 3.



Fig. 27 Rupture force versus persistence length of polyisocyanotripeptide 3.



Fig. 28 Raw force-distance curves (green-blue) and WLC fits of the low-force part (<25pN) of the curves of polyisocyanotripeptide 3.



Fig. 29 Persistence length distribution histogram of polyisocyanotripeptide 3, based on WLC fits of the low force part of force-distance curves.

Here we present the corresponding raw force-distance curves and fits, contour length distribution histogram, persistence length distribution histogram, rupture force distribution histogram and specific stiffness distribution histogram of polyisocyanodipeptide **4**.



Fig. 30 Molecular structure of polyisocyanide 4.



Fig. 31 Raw force-distance curves (green - blue) and eWLC fits (red) of polyisocyanodipeptide 4.



Fig. 32 Contour length distribution histogram of polyisocyanodipeptide 4.



Fig. 33 Persistence length distribution histogram of polyisocyanodipeptide 4.



Fig. 34 Rupture force distribution histogram of polyisocyanodipeptide 4.



Fig. 35 Specific stiffness distribution histogram of polyisocyanodipeptide 4. A few curves with a negative specific stiffness were discarded.



Fig. 36 Initial contour length versus initial persistence length of polyisocyanodipeptide 4.



Fig. 37 Rupture force versus persistence length of polyisocyanodipeptide 4.



Fig. 38 Raw force-distance curves (green-blue) and WLC fits of the low-force part (<25pN) of the curves of polyisocyanotripeptide 4.



Fig. 39 Persistence length distribution histogram of polyisocyanotripeptide 3, based on WLC fits of the low force part of force-distance curves.

In the following we present the corresponding raw force-distance curves and fits, contour length distribution histogram, persistence length distribution histogram, rupture force distribution histogram and specific stiffness distribution histogram of polyisocyanodipeptide 1 + TFA.



Fig. 40 Raw force-distance curves (green - blue) and eWLC fits (red) of polyisocyanodipeptide 1 + TFA.



Fig. 41 Contour length distribution histogram of polyisocyanodipeptide 1 + TFA.



Fig. 42 Persistence length distribution histogram of polyisocyanodipeptide 1 + TFA.



Fig. 43 Rupture force distribution histogram of polyisocyanodipeptide 1 + TFA.



Fig. 44 Specific stiffness distribution histogram of polyisocyanodipeptide 1 + TFA.

In the following we present the corresponding raw force-distance curves and fits, contour length distribution histogram, persistence length distribution histogram, rupture force distribution histogram and specific stiffness distribution histogram of perylenepolyisocyanodipeptide 2 + TFA.



Fig. 45 Raw force-distance curves (green - blue) and eWLC fits (red) of perylenepolyisocyanodipeptide 2 + TFA.



Fig. 46 Contour length distribution histogram of perylenepolyisocyanodipeptide 2 + TFA.



Fig. 47 Persistence length distribution histogram of perylenepolyisocyanodipeptide 2 + TFA.



Fig. 48 Rupture force distribution histogram of perylenepolyisocyanodipeptide 2 + TFA.



Fig. 49 Specific stiffness distribution histogram of perylenepolyisocyanodipeptide 2 + TFA.

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