*Macromolecules* **2005,** *38,* 2469−2474

# Extracting Structural Information of a Heteropolymer from Force−Extension Curves

**Elena Jarkova,**[*,†] **Nam-Kyung Lee,**[†,‡,||] **and Sergei Obukhov**[†,§]

*Institut Charles Sadron, 67083 Strasbourg Cedex, France, Department of Physics, Sejong University, Seoul 143-743, South Korea, and Department of Physics, University of Florida, Gainesville, Florida 32611*

*Received October 3, 2004; Revised Manuscript Received December 21, 2004*

ABSTRACT: We present a theory for the reverse analysis on the sequence information on a single H/P two-letter random heteropolymer (RHP) from its force−extension ($f$−$z$) curves during quasi static stretching. Upon stretching of a self-assembled RHP, it undergoes several structural transitions. The typical elastic response of a heteropolymeric globule is a set of overlapping saw-tooth patterns. With consideration of the height and the position of the overlapping saw-tooth shape, we analyze the possibility of extracting the binding energies of the internal domains and the corresponding block sizes of the contributing conformations.

## I. Introduction

Within a past decade, a series of remarkable force−extension experiments was performed using atomic force microscopy (AFM). These experiments show that the elastic response of a single molecule is clearly related to the internal structure of the molecule. Force−extension profiles of single molecules such as DNA, RNA, synthetic polyelectrolytes, giant protein titin, and chromatin fibers[1−9] show characteristic saw-tooth patterns, which are interpreted as successive unfolding of internal domains. This is in agreement with theoretical studies[10−14] and computer simulations[15−17] predicting that stepwise unfolding pattern can be seen from the unfolding of pearl necklace of polyelectrolytes in a poor solvents[13,18] and protein models.[15]

In some polymer systems (in particular, biopolymers and proteins), the intrachain self-assembly produces secondary or tertiary structures and the elastic response reflects this structural hierarchy.[8] The AFM experiments show that a series of partial unfoldings of those collapsed structure occurs by applying an external force. When the elastic energy gain is comparable with increase of the potential energy, the extension increases abruptly by $\delta z$. The resulting force−extension profile is rich and reflects the domain size responding to the applied force. Information on the sequence of the linear structure reveals on the force−extension curve. In this sense it is interesting to trace back the particular sequence structure of a given chain from the measured elastic response.

In our previous study,[11,12] we minimized the free energy at the given force (which mimics a constant force measurement experiments). The obtained minimum corresponds to the ground state or to the metastable states. At several characteristic values of force, segments of linear chain in the collapsed phase unfold in the pattern of "plateaus" in the $f$−$z$ curve. However, these "plateaus" often correspond to the multiple conformational transitions going through different extensions $z$ if domains have similar binding energies. Therefore, sequence information is partly washed away under the constant force measurements.

Another experimentally common, yet theoretically more challenging, set up of AFM measurement is performed by imposing the distance and measuring the restoring force. Typically, the force−extension profile has a saw-tooth pattern. Each time an internal domain is pulled out, the contact with cantilever becomes loose resulting in a big drop of the measured force. Hence, this sequence information is more directly accessible by force−extension measurement when the distance is imposed. Then an arising question is, if it is possible to recover the information about the sequence of polymer from force−extension profiles. For this purpose, we present theoretical framework how to "read" the sequence information from the elastic response. We demonstrate the mapping of the force−extension profiles to the sequence information under the controlled displacement. We show that it is feasible to extract the composition of block sizes to some extent while the order of arrangement of those blocks still remains to be answered.

## II. General Model

We consider a polymer chain of $N$ monomers, one end of which is fixed at a reference point (i.e., $z = 0$) and the other end is brought to the distance $z$ from the reference point. The sequence consists of $n_\mathrm{h}$ of hydrophobic (h) blocks and $n_\mathrm{p}$ of hydrophilic (p) blocks in an alternating order ($n_\mathrm{h} = n_\mathrm{p}$). The size of the $i$th hydrophobic (hydrophilic) segment is $N_i^\mathrm{h}$ ($N_i^\mathrm{p}$) and the sequence of the whole chain can be represented by a series of h- and p- blocks of sizes: $\{N_i^\mathrm{p}, N_i^\mathrm{h}\}$.

We assume that hydrophobic segments $N_i^\mathrm{h}$ have a tendency to collapse into a compact globule of radius $a_i$, and these unit globules are not further stretchable. These single block globules can merge together into a larger globule leaving the connecting hydrophilic segments as loops on the surface of the large globule.

The optimal conformation of the chain is obtained from the minimum free energy under the given exten-

---

[*] Corresponding author.
[†] Institut Charles Sadron.
[‡] Sejong University.
[§] University of Florida.
[||] E-mail: lee@sejong.ac.kr.

10.1021/ma047965d CCC: $30.25

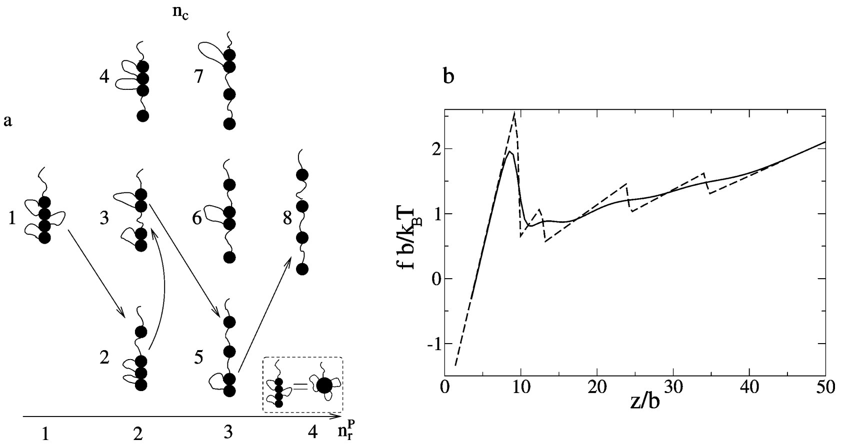

**Figure 1.** (a) Schematic picture of all possible conformations for a heteropolymer consisting of four h- and p-blocks of sequence 4p-4h-8p-6h-20p-6h-6p-6h, classified according to the number of the released p-blocks ($n_r{}^p$). (b) Force−extension curves for the same sequence. The dashed line is the force−extension curve following the minimal energy conformations according to eq 1 with $\gamma = 9k_BT/b^2$ ($\tau = 3$), and the solid-line is the statistically averaged force−extension.

sion $z$. The free energy consists of the two main contributions, the interaction energy of the collapsed h-blocks (globules) and the elastic part of the p-blocks (strings). For simplicity, here we assume that p-block strings have the elastic properties of ideal Gaussian chain (later we discuss more realistic Langevin chain model). For the chain of length $N_i^{\mathrm{p}}$ and size $z_i$, the elastic energy is $F_{\mathrm{elastic}} = z_i{}^2/N_i^{\mathrm{p}}\, k_{\mathrm{B}}T/b^2$. The elastic part of the free energy comes from the released hydrophilic segment connecting two nearest globules. Loops (hydrophilic segments whose both ends are attached to aggregated globule) do not contribute to the total elastic energy.

Initially the chain is fixed at the minimal extension $z_0$ ($z_0 \ll Nb$), all h-blocks belong to a large collapsed globule and only one end p-block is outside of this globule. As imposed distance $z$ varies, the chain adapts its conformation in order to minimize the total free energy. Each conformation can be characterized by the numbers of the released p-blocks and the position of the released p-blocks as illustrated in Figure 1a. The free energy of the conformation of which $q$th p-block is released is written as

$$\frac{E}{k_{\mathrm{B}}T} = \frac{\gamma}{k_{\mathrm{B}}T}(S_1{}^q + S_{q+1}{}^{n_{\mathrm{h}}}) + \frac{(z - 2(a_1{}^q + a_{q+1}{}^{n_{\mathrm{h}}}))^2}{(N^p{}_1 + N^p{}_q)b^2} \qquad (1)$$

where $S_k{}^m$ and $a_k{}^m$ denote the surface area and the radius of the globule consisting of $k$, $k + 1$, ..., $m$th h-blocks, respectively and $\gamma = k_{\mathrm{B}}T\tau^2/b^2$ is the surface tension with $\tau$ being reduced temperature $\tau = | T - \theta|/\theta$. Similar equations can be written for the conformations with the arbitrary number of the h-blocks. If there are $n_{\mathrm{p}}$ p-blocks, there are $n_{\mathrm{p}} - 1$ conformations of which one of the internal p-block is released. The number of conformations where $m$ out of $n_{\mathrm{p}}$ p-blocks are released is $_{n_{\mathrm{p}}}C_m = m!/(m - n_{\mathrm{p}})!n_{\mathrm{p}}!$. The total number of conformation is $\Omega = 2^{n_p-1}$. In Figure 1a, we show all $2^{4-1} = 8$ possible conformations of a heteropolymer consisting of 4 p-blocks and h-blocks. The conformations listed along the vertical lines have the same number of released p-blocks ($n^{\mathrm{p}}{}_r$) but different grouping of h-blocks.

In general, the free energy of each conformation is slightly different from each other. For any given extension $z$, there are several local energy minima with similar free energy $E_r$. These conformations contribute to the thermodynamic properties of the force−extension relation with statistical weight of $\exp(-E_r/k_{\mathrm{B}}T)$. To plot the force−extension curve, all possible conformations at given $z$ must be taken into account with this statistical weight. The statistical sum $G(z)$ of all possible conformations at the displacement $z$ is

$$G(z) = \sum_r \exp\left(-\frac{E_{\mathrm{r}}(z)}{k_{\mathrm{B}}T}\right) \qquad (2)$$

The restoring force acting on the polymer chain is

$$f = -k_{\mathrm{B}}T\frac{\partial(\ln G(z))}{\partial z} \qquad (3)$$

In Figure 1b we show a force−extension curve calculated for a randomly chosen sequence 4p-4h-8p-6h-20p-6h-6p-6h. For convenience, we choose $S_k{}^m = b^2(\sum_{i=k}^m N^h{}_i/\tau)^{2/3}$ and $a_k{}^m = b(\sum_{i=k}^m N^h{}_i/\tau)^{1/3}$.[19] The dashed line represents the force obtained from minimal energy conformation for each extension $z$. If fluctuation is negligible, the expected force−extension curve is a sharp saw-tooth pattern shown as a dashed line in Figure 1b. Each transition from one conformation to another is captured as a "drop" of a restoring force, which indicates the minimum energy conformation switches into the different conformation. The force increases with the extension until the next "drop". The force between the "drops" is proportional to $\sim z/N_{\mathrm{p}}\, k_{\mathrm{B}}T/b^2$ ($N_{\mathrm{p}}$ is a sum of free p-blocks). The longer the chain is, the easier to stretch it.

The solid line is the force−extension curve obtained from eqs 2 and 3 where all local energy minima conformations are also taken into account with proper statistical weight. The unfolding of the large globule follows the path illustrated in Figure 1a. Release of each unit globule leads to a jump. The height of each jump becomes smaller as the overall globule size becomes smaller so that surface energy difference before and after the release becomes smaller. We note that one of the transitions is between conformations with the same number of released p-blocks. (second jump from conformation 2 to the conformation 3.)

Another example of force extension curve for a different randomly chosen sequence(6 p-blocks and 6 h-blocks of different lengths) is shown in Figure 2. Here

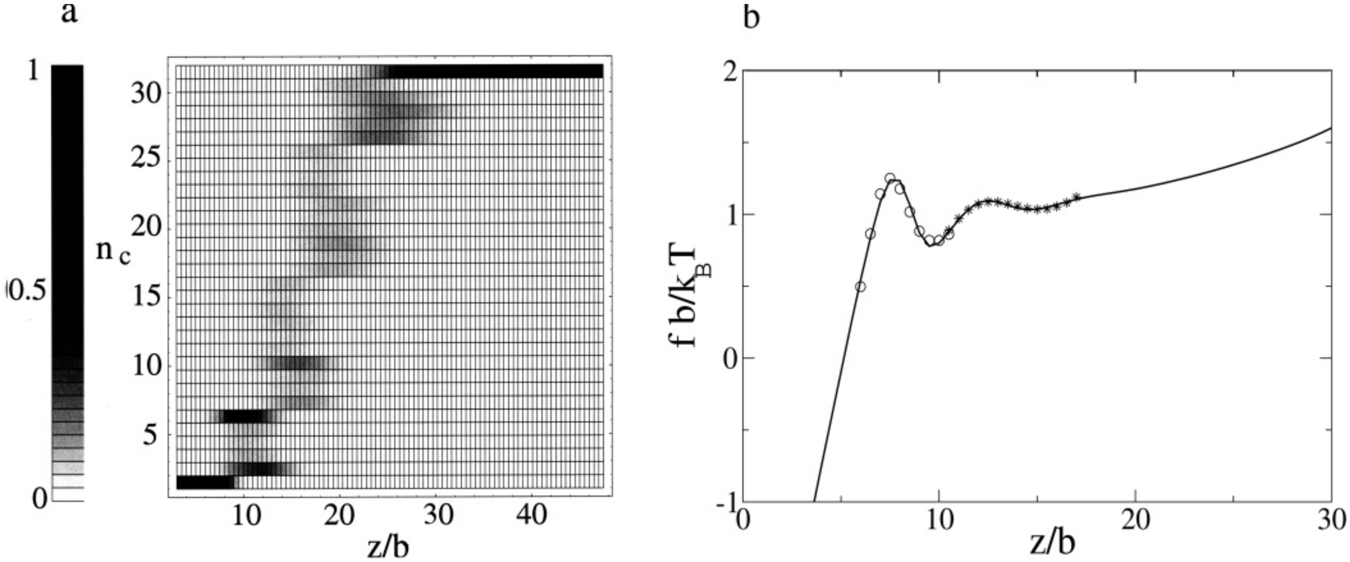

**Figure 2.** (a) Probability distribution of a sequence 3p-6h-5p-5h-3p-3h-2p-2h-3p-10h-3p-3h in the space of all possible conformations with $\gamma = 4k_BT/b^2$ ($\tau = 2$). The $x$-axis is the given extension $z$; the $y$-axis is the index of the each conformations defined in a manner similar to that illustrated in Figure 1. The gray scale bar in the left-hand side shows the probability scale. (b) Corresponding force−extension curve. The symbols (○ and +) represent the fitting results using eq 10.

the probability to be at each conformation $i$ (given by eq 2) is shown in the space of all possible $2^5$ conformations, (see Figure 2a). The dark region indicates the favorable conformations under given constraint (fixed $z$). In some range of $z$, there are several conformations with similar statistical weight. The transition from one group of conformations to another on Figure 2a near $z = 20$ does not result in any noticeable feature on (Figure 2b). There are visible only few first jumps corresponding to the conformational transitions.

Why only two or three transitions are visible on the force−extension curve? At the jump, dominant conformation shifts from one to the other. If there is a clear favorite conformation, the transition is sharp. Otherwise, if several conformations contribute with similar weights, transitions are not expected to be captured as a clear saw-tooth shape in the quasistatic measurement and the fluctuation around average force is large. Around each transition there is a region of strong fluctuations, $\delta z_n$ where difference in energy of competing conformations is smaller than $k_BT$. For the $n$th transition this region is about $\delta z_n = k_BTz_n^*/\epsilon_n$. Here $z_n^*$ is the $n$th transition point, and $\epsilon_n$ is the binding energy related to this transition. The size of fluctuation region is typically growing with $n$ because $z_n^* \propto n$. We should note that the binding energy $\epsilon_n$ cannot be much larger than $k_BT$, otherwise it is difficult to perform quasistatic experiment. It means that after several transitions $n \approx \epsilon_n/k_BT$ their fluctuation regions should overlap: $\delta z \approx z_n^* - z_{n+1}^*$ and the typical zigzag pattern of each transition starts overlapping with that of neighboring transitions. Figure 2a demonstrate such smooth $f$−$z$ curve after a few initial jumps. At large extension, when all loops are pulled out, force increases monotonically with extension.

In realistic experimental situations, one chain end is pulled with a small but finite speed. The free energy difference $\delta E^b$ between the dark and bright conformation gives typical relaxation time $\sim e^{\delta E^b/k_BT}$ for the transition between two likely conformations. Depending on the pulling speed, certain energy barrier conformations are overcome but some of them are not. Conformations separated by the large energy barrier do not contribute the $f$−$z$ when the pulling speed is faster than the chain relaxation time. Thus, the accessible conformation can be controlled by the pulling rate and this allows extracting more detailed information about the structure of polymer. We shall address this question in a future publication.

## III. Reading the Sequence Information from the *f*−*z* Curve

**Simple Model.** In the following, we show how to extract the chain sequence information from the force−extension curve. To do so, we further simplify the conformational space. As illustrated in Figure 3, we assume that globules are arranged in 1-d and interact only with neighboring globules. We denote $\epsilon_m$ as the interaction energy between $m$th and ($m + 1$)th h-block globules. The transition in conformations is related only to the releasing of a unit globule-loop pair from a larger globule. We will show that the interaction energy, $\epsilon_m$, can be extracted from the analysis of the force extension curve (see Figure 1). A more realistic assumption would be that all aggregated globules $m - 2, m - 1, m$ interact with the ($m + 1$)th globule. In this case the energy $\epsilon_m$ depends on the arrangement of globules.

In 1-d model, each conformation is completely characterized by two sets of variables: $\{\epsilon_m\}$ and $\{l_m\}$, where $\epsilon_m = \gamma[S_1^{n_h} - (S_1^m + S_{m+1}^{n_h})]$, $S_n^m$ is a surface area of globule consisting of $N_n^h, N_{n+1}^h, ..., N_m^h$ h-blocks and $l_m$ is the length of the $m$th p- block $l_m = N_m^pb$. In the absence of an external force, all h-globules are attached and aligned in one line. With the increase of the applied distance $z$, the contacts between h-globules break one after another. In the force−extension curve, these events are represented as “drops” in force. The phenomenological knowledge of $z$-coordinate of the jump (denoted as $z^*$ below) and its magnitude $\Delta f$ allows us to determine $\epsilon_m$ and $l_m$, uniquely. At the conformational transition of releasing ($m + 1$)th loop,where $z = z^*$, the energies

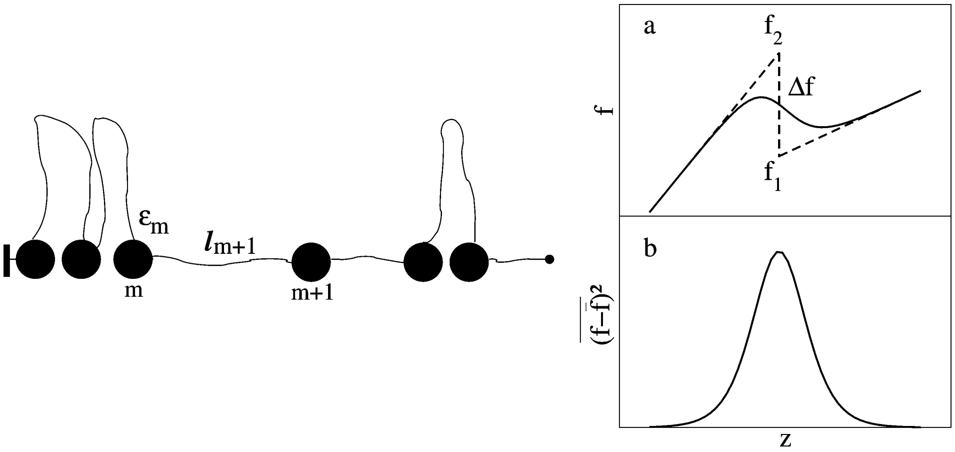

**Figure 3.** Simple model of copolymer. (a) Typical $f-z$ curve and (b) fluctuations in $f$ around a transition point.

of two conformations should be equal: $E_m = E_{m+1}$. This leads to the following relation in $k_BT$ units

$$\frac{z^2}{L_m b} = \frac{z^2}{(L_m + l_{m+1})b} + \epsilon_{m+1} \tag{4}$$

where $L_m$ is the total linear length of the chain before the transition. In this relation we assume that the p-block segments are much longer than the size of the collapsed h-blocks. Otherwise, the size of the hydrophobic globules becomes relevant as an offset of elastic energy of the chain. Then eq 4 reads

$$\frac{(z - 2{a_1}^{n_h})^2}{L_m b} = \frac{(z - 2({a_1}^{m} + {a_{m+1}}^{n_h}))^2}{(L_m + l_{m+1})b} + \epsilon_{m+1} \tag{5}$$

One can relate the height of this jump $\Delta f = f_m - f_{m+1} = \partial E_m/\partial z - \partial E_{m+1}/\partial z = [2z/L_m - 2z/(L_m + l_{m+1})](k_BT/b)$ with $\epsilon_{m+1}$:

$$\epsilon_{m+1} = \frac{\Delta f z}{2} \tag{6}$$

Similarly, the length $l_{m+1}$ can be extracted from the slope difference before and after the jump $1/f_{m+1} - 1/f_m = l_{m+1}/2z$:

$$l_{m+1} = \frac{2\Delta f z}{f_{m+1} f_m} \frac{k_BT}{b} \tag{7}$$

We notice that all inclined parts of the curve, if continued, have zero intercept. The order of releasing is determined by either the minimal interaction energy of h-globules $\epsilon_m$ among all remaining $\epsilon_k$ or the maximal length of the p-segment $l_k$. If all blocks are of similar size with small variations $\delta\epsilon_k$, $\delta l_k$ around the average values $\epsilon$ and $l$, then $L_m \approx z$, and from eq 4 we can get the condition of releasing the next segment $k$ as determined by the largest relative variation max{max($-\delta\epsilon_k/\epsilon$), max($\delta l_k/l$)}.

**Reading Thermally Averaged *f*—*z* Curves** In the vicinity of the $m$th transition point $z_m^*$ (below we simplify it as $z^*$) where the $(m + 1)$th loop is released, the difference between the energies of two states can be small and comparable to $k_BT$. Because of thermal fluctuations, the actual force—extension curve can be very noisy. If these fluctuations are properly averaged, the edge of sharp saw-tooth is rounded. (see Figure 3). We will show below, that parameters $\epsilon_m$ and $l_m$ can be extracted from rounded curve too.

In the absence of thermal fluctuations we describe the jump in a force—extension curve in Figure 3 using the well-known step-function ($\theta$-function): $f(z) = f_m(z)\theta(z^* - z) + f_{m+1}(z)\theta(z - z^*)$, where $f_m(z)$, $f_{m+1}(z)$ are force—extension curves before and after the jump ($f_m(z) = \partial E_m(z)/\partial z$). To include the rounding effect of thermal fluctuations we will replace the $\theta$-function in this equation by the thermally averaged function $\bar{\theta}$:

$$\bar{\theta}(z^* - z) = \frac{e^{-E_m(z)/k_BT}}{e^{-E_m(z)/k_BT} + e^{-E_{m+1}(z)/k_BT}} = \frac{1}{1 + e^{-(E_{m+1}(z) - E_m(z))/k_BT}} \tag{8}$$

At the transition point, where $E_{m+1}(z) - E_m(z) = 0$, the thermally averaged function is $\bar{\theta}(z^* - z) = 1/2$. In the vicinity of the transition, we can interpolate difference $E_{m+1}(z) - E_m(z)$ as $2\epsilon_{m+1}(z^* - z)/z^*$. (It follows from eq 4.) Finally we obtain

$$\bar{\theta}(z^* - z) = \frac{1}{1 + e^{-[(z^*-z)/z][2\epsilon_{m+1}/k_BT]}} \tag{9}$$

and the fitting function for a transition is

$$\overline{f(z)} = f_m(z)\bar{\theta}(z^* - z) + f_{m+1}(z_m)\bar{\theta}(z - z^*) \tag{10}$$

There are three independent variables controlling the shape of a single saw-tooth jump (see Figure 3): slopes before and after transition and the location of the transition $z^*$, where the same number of independent variables is needed for fitting of the thermally averaged curves. Each additional transition requires two additional variables for its description: $z$-coordinate of transition and the slope after the transition, which can be related to $l_m$ and $\epsilon_m$ through eqs 6 and 7.

If two or more transitions are close to each other, then it might be difficult to determine the slope of the force—extension curve in the regions between these transitions, especially with presence of noise. Here we present fitting functions for two overlapping transitions, the further generalization for multiple transitions is obvious. The combined fitting functions for two transitions can be symbolically written with the use of $\theta$ functions as: $\overline{f(z)} = f_m(z)\bar{\theta}(z_m^* - z) + f_{m+1}(z)\overline{\theta(z-z_m^*)\theta(z_{m+1}^*-z)} + f_{m+2}(z)\bar{\theta}(z - z_{m+1}^*)$.

Here the averaged product of two $\bar{\theta}$ functions represents

$$\overline{\theta(z - z_m{}^*)\theta(z_{m+1}^* - z)} = \frac{\mathrm{e}^{-E_{m+1}/k_\mathrm{B}T}}{\mathrm{e}^{-E_m/k_\mathrm{B}T} + \mathrm{e}^{-E_{m+1}/k_\mathrm{B}T} + \mathrm{e}^{-E_{m+2}/k_\mathrm{B}T}} \quad (11)$$

It should be noted that the locations of the released loops of both transitions are not necessarily next to each other along the chain. After global optimization over fitting parameters, we produce the best estimate for this circumstance. If transitions are too close to each other ($(z_m^* - z_{m+1}^*)/z < k_\mathrm{B}T/2\epsilon_m$), the fitting curve gives better estimate of the sum of energies $\epsilon_{m+1} + \epsilon_{m+2}$ and lengths $l_{m+1} + l_{m+2}$, but not estimate of these quantities by themselves.

The symbols in Figure 2b (○ and +) represent the fitting results of the function, eq 10. When the first h-block is released the unknown parameters are the transition point $z^*$ and the lengths of the released p-blocks before and after the transition: $l_1$ and $l_1 + l_2$. Notice that in the case when the total size of globules on the string before and after event, $a_m$ and $a_{m+1}$, are not small one should consider them as additional fitting parameters, so that the force has a form $f_m = 2(z - a_m)$-$k_\mathrm{B}T/L_mb$. The best fit is obtained with parameters $z^* \sim 8.3b$, $l_1 \sim 3.0b$ and $l_1 + l_2 \sim 8.0b$, $a_1 \sim 5.2b$ and $a_2 \sim 7.2b$. When the second h-block is released (+) $z^*$, $l_1 + l_2$ and $l_1 + l_2 + l_3$ are unknown. From the second event, we obtain, $z^* \sim 13.4b$, $l_1 + l_2 \sim 9.1b$ and $l_1 + l_2 + l_3 \sim 11.0b$, $a_2 \sim 6.3b$ and $a_3 \sim 10.3b$.

After all we have $l_1 = 3.0b$, $l_2 = 5.0b$ and $l_3 = 1.9b$, which are in agreement with the exact values for p-blocks 3, 5, 3 accordingly. The estimated interaction energy difference before and after event from eq 4, $\epsilon_m = (f_m(z^* - a_m) - f_{m+1}(z^* - a_{m+1}))/2$, are $\Delta\epsilon_1/k_\mathrm{B}T \sim 3.1$ and $\Delta\epsilon_2/k_\mathrm{B}T \sim 4.7$. The estimated interaction strengths are in agreement with the calculated values $\epsilon_1/k_\mathrm{B}T = \gamma/k_\mathrm{B}T((S_1{}^5) + (S_6{}^6) - (S_1{}^6)) = 4.9$ and $\epsilon_2/k_\mathrm{B}T = \gamma/k_\mathrm{B}T((S_1{}^4) + (S_5{}^5) - (S_1{}^5)) = 4.3$.

**Matching the Noise Pattern.** The above fitting was done to the thermodynamically averaged transition curve. In practice, this curve can be quite noisy especially in the transition region, because the system fluctuates between two different configurations with similar energies and time averaging could be costly. It makes sense to measure the noise directly as a function of extension $z$ and try to extract structural information from it. Calculating the average mean-square magnitude of thermal noise, we get

$$\overline{(f(z) - \overline{f(z)})^2} = \bar{\theta}(z^* - z)\cdot\bar{\theta}(z - z^*)\frac{4{\epsilon_{m+1}}^2}{z^{*2}} \quad (12)$$

This function is the product of two thermally averaged $\bar{\theta}$ function defined in eq 9 and is sharply peaked as is shown on the second inset of Figure 3b.

**Langevin Chain.** For the practical application, we consider the Langevin chain (with fixed bond length) for which the chain extension is given by the following Langevin equation.

$$z/L = \left[\coth\left(\frac{fb}{k_\mathrm{B}T}\right) - \frac{k_\mathrm{B}T}{fb}\right] \quad (13)$$

In the limit of strong stretching, this equation can be simplified to $fb/k_\mathrm{B}T \simeq 1/(1 - z/L)$ and for weak stretching limit, it reproduces the linear response behavior $fb/k_\mathrm{B}T \simeq z/L$. We can assume, that before and after transition point, the $f - z$ curve is described by strong and weak stretching behavior, respectively. Than instead of eq 7 we have

$$l_{m+1} = z\left[\frac{1}{1 - k_\mathrm{B}T/f_mb} - \frac{3}{f_{m+1}b/k_\mathrm{B}T}\right] \quad (14)$$

This reading method can be applied to the experimental curve of the protein domain unfolding where each sawtooth (jump) corresponds to the unraveling of a single domain. We do not try to fit the detail shape of the curve which often treated as wormlike-chain model. We note that the position of peaks and the depth of the jump can be directly mapped into our 1-dimension globule−string model. We may map the number of monomers in the each domain into the connecting p-block size in our model because after the unfolding of each domain, the extension increases by the length corresponding domain size. The binding energy of the each domain is now the interaction energy between two h-globules, i.e., $\epsilon_m$.

## IV. Conclusions

Using a simple model, we demonstrated that information about the sequence of heteropolymers can be to some extent extracted from the force−extension curves. In this work, we assumed that the process of pulling is so slow that the system is close to the thermodynamic equilibrium. This means that all possible conformations can contribute to the elastic response with their corresponding to thermodynamic weights. Keeping in mind that one of the potential applications of our study is long proteins, our results suggest that the recovery of the fine structure is however doubtful. Because of thermal fluctuations, the polymer is able to sample all nearby thermodynamic minima. However, the same thermal fluctuations result in smearing of the peaks in zigzag patterns, each of them corresponding to an unfolding transition. We estimated that the number of individual transitions which can be possible to detect from force−extension curve is about $\epsilon/k_\mathrm{B}T$, where $\epsilon$ is a typical binding energy involved in a transition. This ratio cannot be so large because of the quasi equilibrium condition. In this sense our work establishes the fundamental limit of force spectroscopy under the quasi equilibrium condition.

In a future publication, we will address the case of finite pulling rate where the accessible number of configurations is controlled by the pulling rate.

**Acknowledgment.** The authors are grateful to Dr. A. Johner (ICS) for his great hospitality, patience, and helpful discussions. S.O. acknowledges LEA support. N.-K.L. also acknowledges financial support from Université Louis Pasteur, Strasbourg (Professeur invité).

## References and Notes

(1) Rief, M.; Oesterhelt, F.; Heymann, B.; Gau, H. E. *Science* **1997**, *275*, 1295.
(2) Kellermayer, M.; Smith, S.; Granzier, H.; Bustamante, C. *Science* **1997**, *276*, 1112.

(3) Senden, T. J.; diMeglio, J. M.; Auroy, P. *Eur. Phys. J. B* **1998**, *3*, 211.

(4) Li, H. B.; Rief, M.; Oesterhelt, F.; Gaub, H. E. *Adv. Mater.* **1998**, *10*, 316.

(5) Courvoisier, A.; Isel, F.; François, J.; Maaloum, J. *Langmuir* **1998**, *14*, 3727.

(6) Li, H.; Oberhouser, A. F.; Fowler, S.; Clarke, J.; Fernandez, J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6527.

(7) Liphardt, J.; Onoa, B.; Smith, S.; Tinoco, I., Jr.; Bustamante, C. *Science* **2001**, *292*, 733.

(8) Gao, M.; Craig, D.; Lequin, O.; Campbell, I. D.; Vogel, V.; Schulten, K. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14784.

(9) Brower-Toland, B. D.; Smith, C. L.; Yeh, R. C.; Lis, J. T.; Peterson, C. L.; Wang, M. D. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 1960.

(10) Lee, N.-K.; Johner, A.; Vilgis, T. A. *Macromolecules* **2002**, *35*, 6043.

(11) Lee, N.-K.; Vilgis, T. A. *Europhys. Lett.* **2002**, *57*, 817.

(12) Lee, N.-K.; Vilgis, T. A.*Eur. Phys. J. B* **2002**, *28*, 451.

(13) Tamashiro, M. N.; Schiessel, H. *Macromolecules* **2000**, *33*, 5263.

(14) Geissler, P. L.; Shakhnovich, E. I. *Phys. Rev. E* **2002**, *65*, 056110. Geissler, P. L.; Shakhnovich, E. I. *Macromolecules* **2002**, *35*, 4429.

(15) Klimov, D. K.; Thirumalai, D. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6166.

(16) Paci, E.; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6521.

(17) Lu, H.; Schulten, K. *Biophys. J.* **2000**, *79*, 51.

(18) Vilgis, T. A.; Johner, A.; Joanny, J.-F. *Eur. Phys. J. E* **2000**, *2*, 289

(19) The surface area and radius of globule is not well defined if the number of consisting monomers are too few. We assume that the aggregate has a perfect spherical shape.

MA047965D