

Microcanonical analyses of homopolymer aggregation processes

Tao Chen, Xiangsong Lin, Yuan Liu, Teng Lu, and Haojun Liang*

Hefei National Laboratory for Physical Sciences at Microscale, Department of Polymer Science and Engineering, University of Science and Technology of China, Hefei, Anhui, 230026, People's Republic of China

(Received 14 March 2008; published 5 November 2008)

Using replica-exchange multicanonical Monte Carlo simulation, the aggregates of two homopolymers were numerically investigated through the microcanonical analysis method. The microcanonical entropy showed one convex function in the transition region, leading to a negative microcanonical specific heat. The origin of temperature backbending was the rearrangement of the segments during the process of aggregation; this aggregation process proceeded via a nucleation and growth mechanism. It was observed that the segments with a sequence number from 10 to 13 in the polymer chain have leading effects on the aggregation.

DOI: [10.1103/PhysRevE.78.056101](https://doi.org/10.1103/PhysRevE.78.056101)

PACS number(s): 82.20.Wt, 82.35.Lr, 87.15.A-, 87.15.Cc

I. INTRODUCTION

The aggregation of macromolecules, such as proteins, is of great practical importance. Protein aggregation could impose severe limitations on yields of biologically active proteins at nearly all stages of the manufacturing process [1,2]. During storage the presence of air and solid interfaces, as well as temperature fluctuations, causes the proteins to populate non-native, aggregation-prone states. Protein aggregates are suspected to be the causative agent in over 20 neurodegenerative diseases [3–6], such as Alzheimer's and Parkinson's diseases, Bovine Spongiform Encephalopathy (BSE) or mad-cow disease, and Amyotrophic Lateral Sclerosis (ALS). All these conditions appear to be related to the abnormal aggregation of proteins. Aggregated protein reduces the efficacy of formulations and leads to enhanced immunogenicity. Aggregation also limits yields of biologically active products during purification. For example, when *E. coli* is the chosen host for recombinant protein expression, the overexpressed protein often forms intracellular agglomerations termed as "inclusion bodies." The protein is not biologically active and therefore must be refolded, although the inclusion bodies are a relatively pure form of the protein. Aggregation competes with folding, often imposing severe limitations on refolding yields during the refolding step [7]. A good understanding of protein aggregation is vital to pharmaceutical and food industry processes. However, despite its importance, the physics of biomolecular aggregation is poorly understood. Many works have studied protein aggregation, but the molecular mechanisms by which the proteins aggregate are still unknown. To shed light on the general mechanisms and to better understand how proteins aggregate, the aggregation processes of polymers is worth studying.

In canonical ensemble, the state of the finite system changes discontinuously during transition when the temperature changes; that is, the process of transition could not be observed in the canonical ensemble. However, in the microcanonical ensemble where system energy E is considered as the adjustable external parameter, discontinuity does not oc-

cur during the transition, and the transitory process could be clearly observed [8,9].

Microcanonical analysis is a promising method for the investigation of a small system when it becomes cool upon absorbing energy. During the transition region, where energy E is treated as an intensive external control parameter, the microcanonical entropy $S(E) = k_B \ln n(E)$ shows a convex function of E , where $n(E)$ is the density of the states. Correspondingly, the specific heat $C_V(E) = \partial E / \partial T(E) = -(\partial S / \partial E)^2 / (\partial^2 S / \partial E^2)$ is negative, and the inverse caloric temperature $T^{-1}(E) = \partial S / \partial E$ curve exhibits an S-shaped feature. This effect has been observed in an astrophysical system [10], where energy could be added to a star or a star cluster, which subsequently cools. There is a great number of isolated finite model systems for evaporation and other melting effects [11,12]. A similar phenomenon is seen for melting atomic clusters [13,14], nuclei fragmentation [15–17], and spin models on finite lattices that experience first-order transitions in the thermodynamic limit [18,19]. Experimental evidence was found that a cluster, containing exactly 147 sodium atoms, indeed has a negative microcanonical heat capacity close to its solid-to-liquid transition [20]. In particular, this negative specific heat effect was observed by Jung-hans *et al.* in the aggregation transition of heteropolymer chains [21,22]. In our previous work, we have studied the association of hydrophobic segments in a heteropolymer, and the aforementioned negative specific heat effect was already determined [23].

In this present work, we study the aggregation of two homopolymers in detail, and investigate whether the negative specific heat effect is solely caused by the association of hydrophobic segments in polymers [21–23].

The paper is structured as follows: In Sec. II, the implementation of the method used for simulations is displayed; In Sec. III, the results of the simulation and the theory analysis are presented; and finally, the results are summarized and conclusions are drawn in Sec. IV.

II. MODEL AND SIMULATION

The conformation of two homopolymers is defined by $\{r_1, r_2, \dots, r_n\}$ and $\{r'_1, r'_2, \dots, r'_n\}$ in three-dimensional space, and a bond is used in connecting every two adjacent seg-

*Author to whom correspondence should be addressed.
hjljiang@ustc.edu.cn

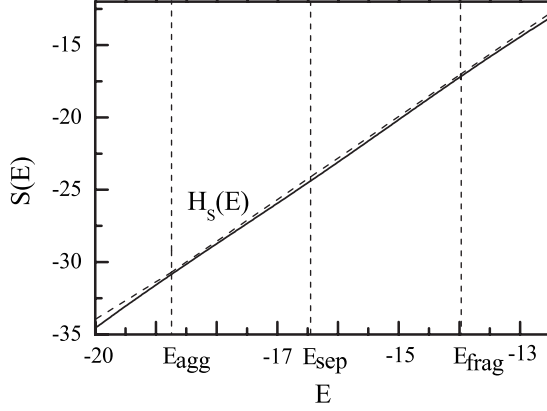


FIG. 1. Microcanonical entropy $S(E)$ is plotted as the function of energy E .

ments for each polymer. In order to keep the two polymers close to each other, they are confined into a cubic box with a length of $L=45$. We chose the off-lattice Monte Carlo model developed by Geroff *et al.* [24] and used it in our group for observing the first-order transition of homopolymer and heteropolymer chains [25]. The interaction potential between two nonbonded segments i and j in the chain for each polymer is

$$E = 4\epsilon \sum_{i=1}^{n-2} \sum_{j=i+2}^n [(1/r_{ij})^{12} - (1/r_{ij})^6], \quad (1)$$

where r_{ij} is the intersegment distance in the polymer chain, ϵ is the parameter setting energy scale, and n is the length of the polymer chain, which is 22 in the present work. Keeping the same parameter sets for the interaction of the segments of the different polymers, the model for the interaction potential between two polymers is

$$E = 4\epsilon \sum_{i=1}^n \sum_{j=1}^n [(1/r_{ij})^{12} - (1/r_{ij})^6], \quad (2)$$

where r_{ij} is the intersegment distance between two different polymers. In the present work, we have set $\epsilon=0.25$, while the bond length has been set to unity. The replica-exchange multicanonical (REMUCA) Monte Carlo simulation developed by Mitsutake *et al.* [26] was used in this study to guarantee an ergodic scan of the conformational space.

For the simulations of this system, we performed 200 multicanonical iterations in the REMUCA. A total of 3×10^{12} updates were generated. The primary result of these simulations is the density of states $n(E)$, which is determined up to a constant.

III. RESULTS AND DISCUSSION

In order to conduct the microcanonical analysis, the parameter microcanonical entropy $S(E) = \ln n(E)$ is plotted as a function of energy E in Fig. 1. The entropy curve clearly shows a convex intruder in the energy range from $E_{\text{frag}} = -13.975$ to $E_{\text{agg}} = -18.675$. The concave hull $H_S(E) = S(E_{\text{agg}}) + E/T_{\text{agg}}$, which is the tangent connecting $S(E_{\text{agg}})$

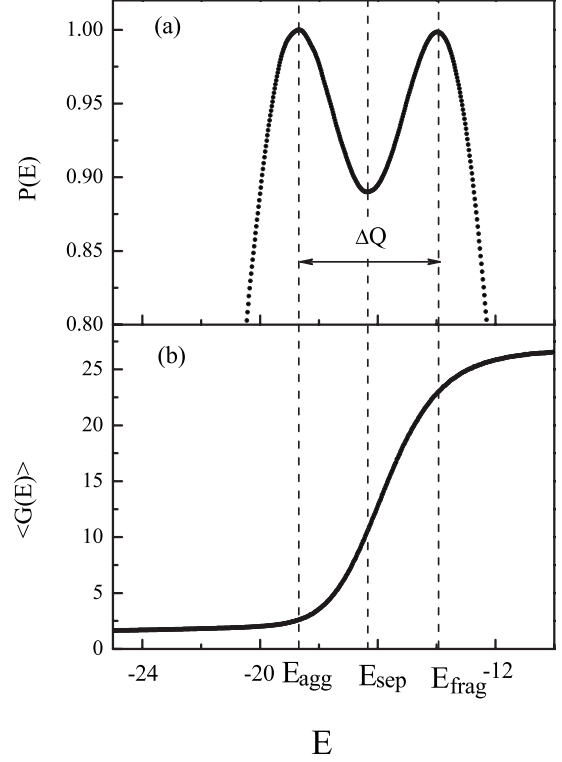


FIG. 2. Microcanonical results for the aggregation of the two homopolymers: (a) Canonical energy distribution close to the aggregation temperature $P(E)$, (b) the average distance between the center of gravity of the two homopolymers $\langle G(E) \rangle$.

and $S(E_{\text{frag}})$, is considered as the Gibbs construction. Its slope defines the inverse of the aggregation temperature $T_{\text{agg}}=0.35$. The bimodality of the canonical energy distribution $P(E) \sim n(E)\exp(-E/k_B T)$ as displayed in Fig. 2(a) indicates the coexistence of two phases at a temperatures close to T_{agg} . In Fig. 2(b), we provide a detailed analysis based on the conformations of the chain measured by the average distances between the center of gravity of the two homopolymers $\langle G(E) \rangle$. It is demonstrated that the phase corresponding to the $P(E)$ peak at $E_{\text{frag}} = -13.975$ in Fig. 2(a) represents the separation of the two homopolymers having a larger value of $\langle G(E) \rangle \sim 22.87$. Meanwhile, the phase corresponding to the $P(E)$ peak at $E_{\text{agg}} = -18.675$ represents the aggregation of the two homopolymers having a smaller value of $\langle G(E) \rangle \sim 2.6$. In Fig. 1 the interval $\Delta Q = E_{\text{frag}} - E_{\text{agg}} = T_{\text{agg}}[S(E_{\text{frag}}) - S(E_{\text{agg}})] \approx 4.7$ is the latent heat required in releasing inter-chain contacts at the aggregation temperature T_{agg} . Meanwhile, E_{sep} defines the energy at which the entropy difference $\Delta S(E) = H_S(E) - S(E)$ is at the maximum, and the corresponding maximum deviation is the interfacial entropy $\Delta S_{\text{surf}} = \Delta S(E_{\text{sep}})$.

In Fig. 3, we displayed the inverse caloric temperature $T^{-1}(E) = \partial S / \partial E$ and specific heat $C_V(E) = \partial E / \partial T(E) = -(\partial S / \partial E)^{-1} / (\partial^2 S / \partial E^2)$ as functions of energy E . Figure 3(a) shows that in both regions of $T < T_{\text{agg}} \approx 0.341$ and $T > T_{\text{agg}} \approx 0.358$ or $E < E_{\text{agg}} = -18.675$ and $E > E_{\text{frag}} = -13.975$, a bijective mapping between T and E becomes possible, indicating that temperature T is a useful control parameter. In the region of $T_{\text{agg}} \leq T \leq T_{\text{agg}}$, however, the S-shaped reciprocal ca-

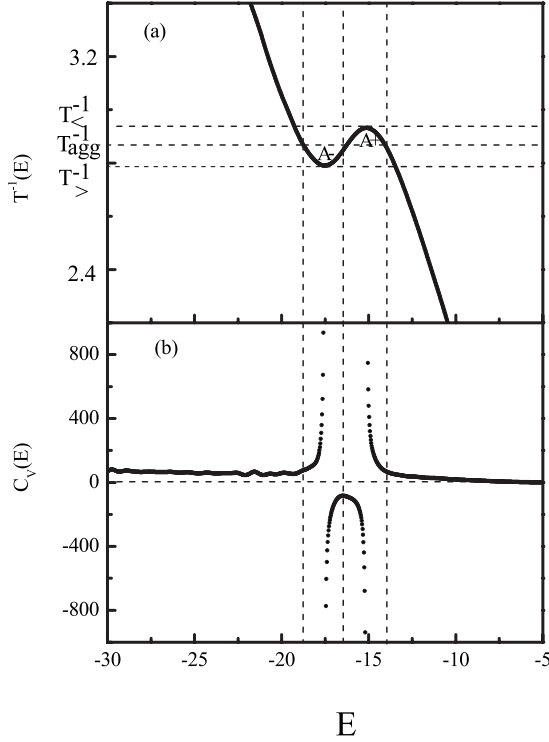


FIG. 3. Microcanonical results for the aggregation of the two homopolymers: (a) Inverse caloric temperature $T^{-1}(E)$, (b) specific heat $C_V(E)$.

loric $T^{-1}(E)$ curve shown in Fig. 3(a) exhibits a typical backbending feature, implying that the system becomes colder when the total energy is increased. Since the mapping between T and E in Fig. 3(a) is no longer in a bijective manner under such a condition, the temperature should not be considered as a proper control parameter for describing the system.

We gather from Figs. 3(a) and 2 that at $T < T_<$, the two-homopolymer system forms an aggregate. This might be due to the weak thermal energy at a low temperature, such that it could hardly overcome the cumulative interaction energy between the two polymers. Moreover, the system is within one phase, thus the energy of the system becomes extensive. On the other hand, in the case of $T > T_>$ the thermal energy is large enough to overcome the barrier of the cumulative interaction energy of the two homopolymers. As the two homopolymers cannot aggregate, the system is also within one phase, and the energy remains extensive. In the transition region $T_< \leq T \leq T_>$, however, the thermal energy of the homopolymer chains and the interaction energy between the two homopolymers are in such a delicate balance that these two switch between the two states of aggregation and fragmentation. This leads to the existence of two phases and to the total energy in the system exhibiting a nonextensive feature. Furthermore, this nonextensivity of energy leads to the negativity of the specific heat in the backbending regions as shown in Fig. 3(b), similarly indicating the coexistence of the two phases. In this region, the Maxwell line $T_{\text{agg}}^{-1} = 2.875$ divides the $T^{-1}(E)$ curve into two equal parts in areas $A_+ = T_{\text{agg}}^{-1}(E_{\text{sep}} - E_{\text{frag}}) - [S(E_{\text{sep}}) - S(E_{\text{frag}})] = 0.117$ and $A_- = T_{\text{agg}}^{-1}(E_{\text{sep}} - E_{\text{agg}}) - [S(E_{\text{sep}}) - S(E_{\text{agg}})] = 0.117$, which deter-

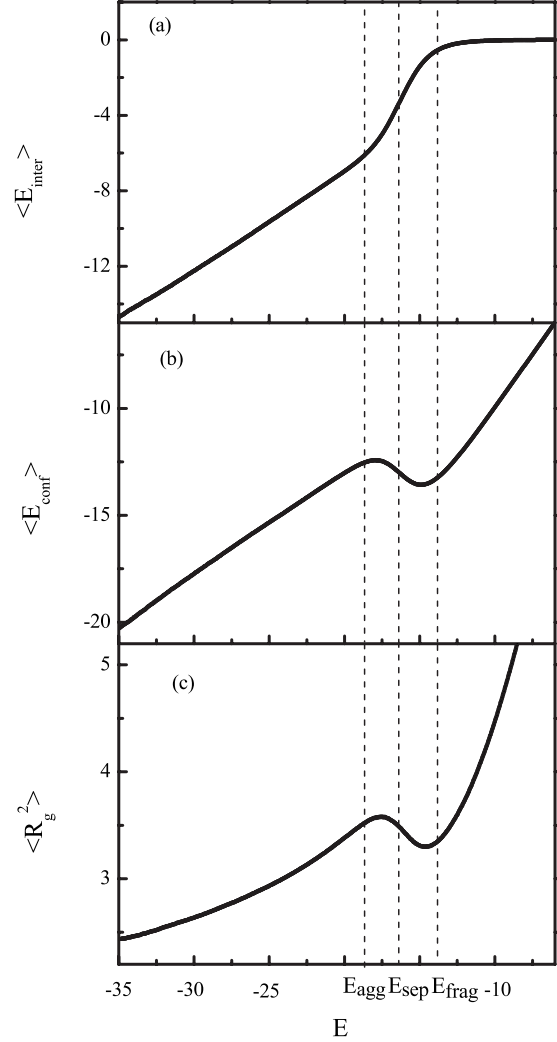


FIG. 4. Microcanonical results for the aggregation of the two homopolymers: (a) The interaction energy between the two polymers $\langle E_{\text{inter}} \rangle$, (b) conformational energy of the two homopolymers $\langle E_{\text{conf}} \rangle$, (c) the mean-square radius of gyration of one of the two homopolymers $\langle R_g^2 \rangle$.

mines the interfacial entropy $\Delta S_{\text{surf}} = A_+ = A_-$ [18] or the loss of entropy owing to the existence of an interfacial surface between the two polymers.

Pursuing the origin of this negative specific heat effect is extremely attractive. As shown in Fig. 4, when the total energy of the system is above E_{frag} , the interaction energy between the two polymers $\langle E_{\text{inter}} \rangle$, that is, the interactions between beads in different chains, remain nearly zero as shown in Fig. 4(a), indicating separation between the two polymer chains. The conformational energy $\langle E_{\text{conf}} \rangle$ of the two polymers, that is, the interactions of the beads along the same chain, and the radius of gyration $\langle R_g^2 \rangle$ of the polymer chains, decrease with energy as shown in Fig. 4(b) and Fig. 4(c), which indicates that the polymer chains collapse themselves before aggregation in the energy range $E > E_{\text{frag}}$. On the other hand, in the region of the specific heat negativity, the interaction energy between the two polymers shown in Fig. 4(a) decreases in a nonlinear manner with the reduction of the system energy, which is a signal of a system energy's

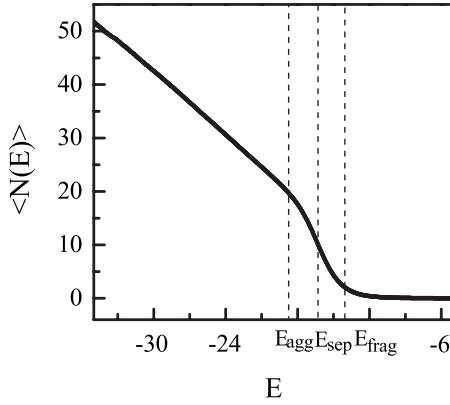


FIG. 5. The average contact number of segments between two different homopolymers $\langle N(E) \rangle$.

nonextensivity. This phenomenon occurs because the movement of the segments in the two polymer chains is constrained when the two chains aggregate, then the entropy of the system decreases. In other words, there is an interfacial surface between two aggregated polymers, and the loss of entropy is considered as interfacial entropy. Owing to the existence of an interfacial surface between the two polymers, additional energies are required to cooperatively rearrange the segments in order to reduce the surface tension. The rearrangement of the segments can be observed in Fig. 4(b) and Fig. 4(c) within the region of negative specific heat effect: The conformational energy $\langle E_{\text{conf}} \rangle$ increases from -13.57 to -12.42 with a decrease in the system energy. Accompanying this process, the mean-square radius of gyration $\langle R_g^2 \rangle$ of one of the two polymers increases rather than decreases, indicating the rearrangement of the chain. As a consequence, part of the kinetic energy is transferred into potential energy. This means that there is a decrease in temperature, or the system becomes colder as the total energy increases. This phenomenon, however, could only be observed in a small system. For a large system with two long polymer chains, the interaction between these two polymers, which could induce the constraint of a few segments, and which has less contribution to the entropy change in this large system, could rarely be observed.

By assuming a critical value ($r=1.4$) of the distance of segments between two polymers, below which the two segments are understood to be in contact, we studied the average contact number of the segments between the two polymers $\langle N(E) \rangle$ (Fig. 5). We have observed that $\langle N(E) \rangle$ increases from zero as the energy in the system decreases when the energy is above E_{frag} , which is another evidence that the two polymers are indeed separated. In the energy range from $E_{\text{frag}} = -13.975$ to $E_{\text{agg}} = -18.675$, less than 20 segments on the average are contacted. We know from the preceding sections that the system switches between the two phases of aggregation and separation in this energy range. When the total energy in the system is below E_{agg} , more than 20 contact numbers of the segments between the two polymer chains, and the two polymers are unable to separate from each other, leading to the aggregation of the two polymers. On the basis of this analysis, we could provide a comprehensive scenario for the process of aggregation of the two poly-

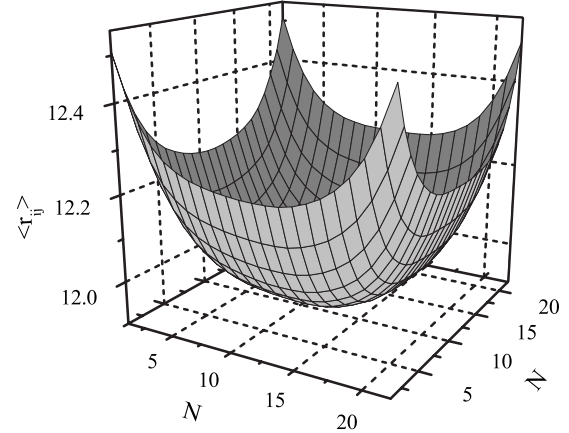


FIG. 6. The average distance of segments in two different polymers $\langle r_{ij} \rangle$ in the energy range of $E_{\text{frag}} = -13.975$ to $E_{\text{agg}} = -18.675$.

mers. That is, if the system is under higher temperatures $T > T_{>} \approx 0.358$, the system is also in a higher energy state with $E > E_{\text{frag}} = -13.975$, and the two polymers are free from each other. However, if the temperature decreases in the range $T_{<} \leq T \leq T_{>}$ ($T_{>} \approx 0.358$ and $T_{<} \approx 0.341$), the energy of the system also drops in the range of $E_{\text{frag}} = -13.975$ to $E_{\text{agg}} = -18.675$. None or less than 20 contact numbers on the average of the two polymers are bound, or the two phases exist; correspondingly, the system exhibits a backbending feature in its temperature curve and the negativity behavior for the specific heat curve. With the decrease of temperature to $T < T_{<} \approx 0.341$ (corresponding energy $E < E_{\text{agg}} = -18.675$), more than 20 contact numbers of the segments on the average are bound, and the two polymer chains aggregate as well. Therefore, the aggregation process of the two polymers has some similarities to the nucleation and the growth process. In the range of $T_{<} \leq T \leq T_{>}$, an initial nucleus is formed, with a few segments bound to each other in the two polymers. Since the initial nucleus may be unstable, it could melt as a result of thermal fluctuation; this means that the bound segments in two polymers separate from each other. Then the buildup and melting of this nucleus lead to the system switching between two phases. Once, the nucleus is made stable with more than 20 contacts of segments between two polymers on the average being bound, this nucleus becomes stable and could start growing as the temperature approaches $T_{<} \approx 0.341$; thus, the two polymers aggregate. In addition, it is clear that the backbending feature of the temperature curve and the negative behavior of the specific heat curve also occur in the process of nucleation.

The average distances between the segments of different polymer chains $\langle r_{ij} \rangle$ in the energy range from $E_{\text{frag}} = -13.975$ to $E_{\text{agg}} = -18.675$ are plotted in Fig. 6 as the sequence number of segments along the polymer chains N . The shape of the plot is similar to a funnel, implying that the connection of the middle segments between two polymer chains is relatively ready. Together with the contact number of segments in the two polymers $\langle N(E) \rangle$ in Fig. 5, the segments with a sequence number from 10 to 13 along the polymer chain are significant in inducing the aggregation. In other words, once these segments are in contact with each other, the two polymers will fully aggregate. When the cen-

tral parts of two polymer chains come in contact with each other, the number of the neighboring pairs between the two polymers is more than the other ways of contact in this short polymer chain system. Thus, the interaction energy between the two polymers is larger and easily induces the aggregation of two polymers. It is obvious that the origin of the temperature backbending is induced mostly by the contact of segments with a sequence number from 10 to 13, which are along the polymer chain. These phenomena are very similar to the origin of Alzheimer's disease, in which a few identical small fragments in large proteins show the tendency to form fibrils.

IV. CONCLUSION

We have used an off-lattice homopolymer model to demonstrate that the microcanonical analysis is very promising for the study of the process involving two homopolymer aggregates. The aggregates of two polymers have been verified as a phase separation process. Features such as the S-shaped

caloric temperatures and the negative specific heats are indicators of the existence of double phases. The loss of entropy is due to the existence of an interfacial surface between the two polymers. The system becomes colder with the total energy increase in the region of the aggregation transition. The negative specific heat effect is due to the nonextensivity of the energy, which results from the interaction between the two homopolymers in the process of aggregation. The origin of temperature backbending is mostly induced by the segment contacts in polymers with a sequence number from 10 to 13.

ACKNOWLEDGMENTS

We are grateful for the financial support provided by the Outstanding Youth Fund (Contract No. 20525416), the Programs of the National Natural Science Foundation of China (Contracts No. 50773072 and No. 20874094, and the National Basic Research Program of China (Contract No. 2005CB623800).

-
- [1] S. Frokjaer and D. E. Otzen, *Nat. Rev. Drug Discovery* **4**, 298 (2005).
 - [2] E. Y. Chi, S. Krishnan, T. W. Randolph, and J. F. Carpenter, *Pharm. Res.* **20**, 1325 (2003).
 - [3] D. Canet, M. Sunde, A. M. Last, A. Miranker, A. Spencer, C. V. Robinson, and C. M. Dobson, *Biochemistry* **38**, 6419 (1999).
 - [4] C. M. Dobson, *Trends Biochem. Sci.* **24**, 329 (1999).
 - [5] E. H. Koo, P. T. Lansbury, and J. W. Kelly, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 9989 (1999).
 - [6] S. Y. Tan and M. B. Pepys, *Histopathology* **25**, 403 (1994).
 - [7] E. D. Clark, *Curr. Opin. Biotechnol.* **12**, 202 (2001).
 - [8] D. H. E. Gross, *Microcanonical Thermodynamics* (World Scientific, Singapore, 2001).
 - [9] D. H. E. Gross and J. F. Kenney, *J. Chem. Phys.* **122**, 224111 (2005).
 - [10] W. Thirring, *Z. Phys.* **235**, 339 (1970).
 - [11] D. J. Wales and R. S. Berry, *Phys. Rev. Lett.* **73**, 2875 (1994); D. J. Wales and J. P. K. Doye, *J. Chem. Phys.* **103**, 3061 (1995).
 - [12] S. Hilbert and J. Dunkel, *Phys. Rev. E* **74**, 011120 (2006); J. Dunkel and S. Hilbert, *Physica A* **370**, 390 (2006).
 - [13] M. Bixon and J. Jortner, *J. Chem. Phys.* **91**, 1631 (1989).
 - [14] P. Labastie and R. L. Whetten, *Phys. Rev. Lett.* **65**, 1567 (1990).
 - [15] D. H. E. Gross, *Rep. Prog. Phys.* **53**, 605 (1990).
 - [16] M. Pichon, B. Tamain, R. Bougault, and O. Lopez, *Nucl. Phys. A* **749**, 93c (2005).
 - [17] O. Lopez, D. Lacroix, and E. Vient, *Phys. Rev. Lett.* **95**, 242701 (2005).
 - [18] W. Janke, *Nucl. Phys. B, Proc. Suppl.* **63A-C**, 631 (1998).
 - [19] H. Behringer and M. Pleimling, *Phys. Rev. E* **74**, 011108 (2006).
 - [20] M. Schmidt, R. Kusche, T. Hippler, J. Donges, W. Kronmüller, B. von Issendorff, and H. Haberland, *Phys. Rev. Lett.* **86**, 1191 (2001).
 - [21] C. Junghans, M. Bachmann, and W. Janke, *Phys. Rev. Lett.* **97**, 218103 (2006).
 - [22] C. Junghans, M. Bachmann, and W. Janke, *J. Chem. Phys.* **128**, 085103 (2008).
 - [23] T. Chen, X. S. Lin, Y. Liu, and H. J. Liang, *Phys. Rev. E* **76**, 046110 (2007).
 - [24] I. Gerroff, A. Milchev, K. Binder, and W. Paul, *J. Chem. Phys.* **98**, 6526 (1993).
 - [25] H. J. Liang and H. N. Chen, *J. Chem. Phys.* **113**, 4469 (2000); Y. Y. Wang, H. N. Chen, and H. J. Liang, *ibid.* **115**, 3951 (2001); B. S. Xu, L. Huang, and H. J. Liang, *ibid.* **121**, 7494 (2004).
 - [26] A. Mitsutake, Y. Sugita, and Y. Okamoto, *Biopolymers* **60**, 96 (2001); *J. Chem. Phys.* **118**, 6664 (2003); **118**, 6676 (2003).