

# Folding of electrostatically charged beads-on-a-string as an experimental realization of a theoretical model in polymer science

Meital Reches, Phillip W. Snyder, and George M. Whitesides<sup>1</sup>

Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138

Edited by Ken A. Dill, University of California, San Francisco, CA, and approved August 31, 2009 (received for review May 21, 2009)

The “beads-on-a-string” model for folding of polymers is a cornerstone of theoretical polymer science. This communication describes a physical model of beads-on-a-string, based on the folding of flexible strings of electrostatically charged beads in two dimensions. The system comprises millimeter-scale Teflon and Nylon-6,6 (spherical or cylindrical) beads ( $\approx 6$  mm in diameter) separated by smaller ( $\approx 3$  mm) poly(methyl methacrylate) (PMMA) spherical beads, threaded on a flexible string. The smaller, uncharged beads define the distances between the larger beads, and control the flexibility of the string. During agitation of the sequence of beads on a planar, horizontal paper surface, tribocharging generates opposite electrostatic charges on the larger Nylon and Teflon beads, but leaves the smaller PMMA beads essentially uncharged; the resulting electrostatic interactions cause the string to fold. Examination and comparison of two models—one physical and one theoretical—may offer a new approach to understanding folding, collapse, and molecular recognition at an abstract level, with particular opportunity to explore the influence of the flexibility of the string and the shape of the beads on the pattern and rate of folding. The physical system is, thus, an analog computer, simulating the theoretical beads-on-a-string model in two dimensions; this system makes it possible to test hypotheses connecting “sequence” to “folding”, rapidly and conveniently, while exploring nonlinearities and other complexities omitted from the theoretical model.

self-assembly | electrets

The folding of linear polymers in solution is a subject of enormous importance in areas ranging from materials science to molecular biology. In exploring folding, theorists have developed models at every level of complexity (1–12). One of the simplest and most useful of these conceptual models is the “beads-on-a-string” model (13). This model represents each monomer of the polymer as a bead, and the backbone of the chain as a flexible string (1, 13). It has been the basis for many computational models for folding (14, 15). All theoretical models are, however, necessarily incomplete, and their failure to capture the full complexity of reality stimulates the development of more complex theory (7, 16). The strategy of the research that we describe here is opposite to that usually used in examining complex systems; rather than using complex theory to try to rationalize an even more complex reality, we have developed the simplest possible experimental system we can imagine, to match the simplest theory.

The physical system that we describe comprises beads (either spherical or cylindrical, and  $\approx 6$  mm in diameter) of two materials (Nylon-6,6 and Teflon) threaded in a defined sequence on a flexible Nylon string. Small (diameter  $\approx 3$  mm), spherical, poly(methyl methacrylate) (PMMA) beads separate the large beads, acting as spacers, and control the flexibility of the string. Agitation of the sequence of beads on a planar surface (paper) charges the large beads electrostatically; the Nylon beads charge positively, the Teflon beads negatively. The PMMA beads that determine the spacing and flexibility of the “string” remain

approximately electrically neutral (17). Electrostatic forces fold the sequence of beads.

This design provides an experimental system that makes it possible to examine the predictions of theoretical beads-on-a-string models in the simplest form we can envision: it is 2-D, the interactions among the beads are electrostatic, the shapes of the beads and properties of the string can be controlled, and the agitation of the beads is well defined. This system, although much simpler than molecular polymers in 3-D solution, and substantially different from molecules in a thermally agitated bath of solvent, still includes the inevitable nonlinearities of a real physical system. It is, thus, an analog computer designed to extend and to simulate 2-D calculations of beads-on-a-string models of polymer folding and collapse.

## Results

**Experimental Design.** We generated the beads-on-a-string by threading sequences of spherical (diameter = 6.35 mm) or cylindrical (diameter = 6.35 mm, length = 14.2 mm) Nylon and Teflon beads on a thin flexible string (see *Materials and Methods*). Beads of this size are easy to handle and machine. To increase the visual contrast between the Teflon and Nylon beads, we stained the Nylon beads with a neutral organic dye; the dye did not significantly change the charge developed on the beads (see *SI Text*).

Upon agitation on a surface (made of paper) located in the middle of the triboelectric series\* (18), Teflon and Nylon beads develop electrostatic charges of similar magnitudes and at similar rates, but with opposite electrical polarities: Teflon charges negatively and Nylon positively (19) (Fig. 1*A*). The surface on which the beads charged was planar and axially symmetrical, with a slight curvature (radius  $\approx 3$  cm) at the perimeter (Fig. 1*B* and *C*). This geometry avoided interactions of the beads with the corners of a sharply defined frame (*SI Text*).

We conducted the experiments at a relative humidity (RH) of  $\approx 15$ –30% (20) and a room temperature of  $22 \pm 2$  °C and used random lateral motion of the surface for agitation (Fig. 1*B* and Fig. S1). This motion does not simulate the impact of solvent molecules on the components of a dissolved polymer; transfer of momentum from the surface to the beads by friction is correlated across the whole surface, and the energy of impact does not follow a Boltzmann distribution. This system of agitation is,

Author contributions: M.R., P.W.S., and G.M.W. designed research; M.R. performed research; M.R. and P.W.S. analyzed data; and M.R., P.W.S., and G.M.W. wrote the paper.

The authors declare no conflict of interest.

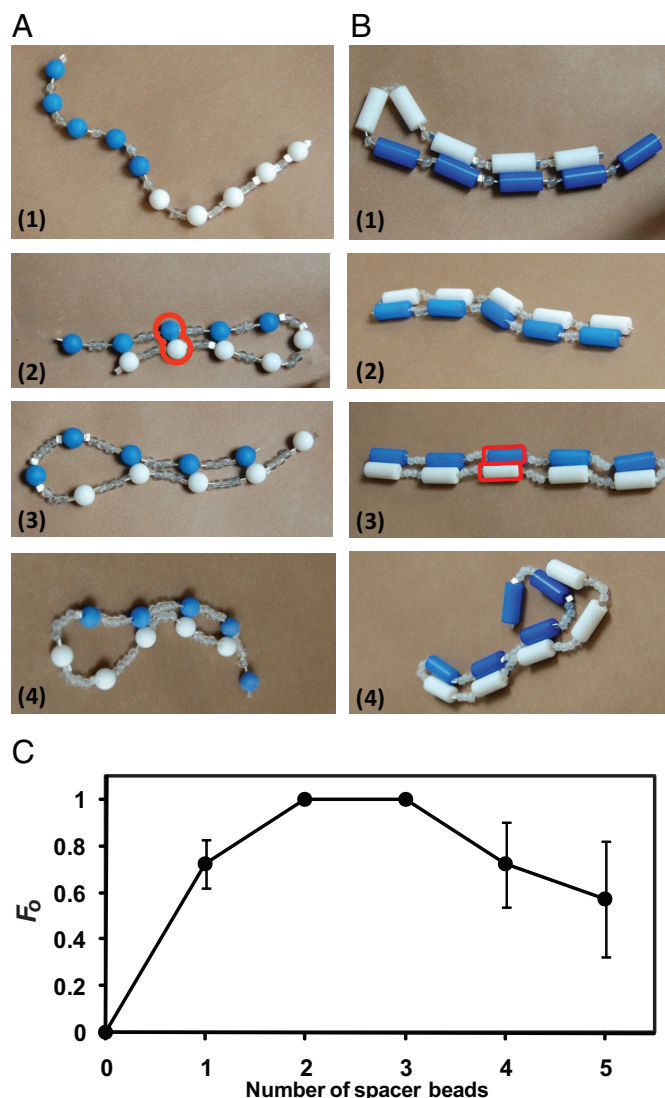
This article is a PNAS Direct Submission.

<sup>1</sup>To whom correspondence should be addressed. E-mail: gwhitesides@gmwgroup.harvard.edu.

\*The triboelectric series is an empirical list of materials that are ranked based on their tendency to acquire a positive or a negative charge upon contact with another material.

This article contains supporting information online at [www.pnas.org/cgi/content/full/090553106/DCSupplemental](http://www.pnas.org/cgi/content/full/090553106/DCSupplemental).





**Fig. 2.** Controlling the rigidity of the string with PMMA spacer beads. (A) The images are of representative conformations formed by sequences of 10 spherical beads, taken after 1 h of agitation. Color distinguishes the Nylon beads (blue) from the Teflon beads (white). The spacing between the large beads is defined by one [1], two [2], three [3], and four [4] PMMA beads. The red line outlines 1 correct pair (Nylon-Teflon). (B) The images are representative of folded conformations of sequences composed of 10 cylindrical beads. The spacing between the large beads is defined by one [1], two [2], three [3], and four [4] PMMA beads. The red line outlines 1 correct pair (Nylon-Teflon). (C) The graph plots the fraction of observed correct pairs ( $F_o = N_c/N_p$ ), where  $N_c$  is the number of observed correct pairs of beads formed when folding sequences of 10 cylindrical beads with different numbers of spacer beads, and  $N_p$  is the maximum number of possible correct pairs (i.e., five pairs for these sequences). The error bars represent one sample standard deviation calculated from eight experiments; the absence of error bars indicates that the sequences with two and three spacer beads adopted conformations with the same value of  $F_o$  in each of the eight experiments. [SI Text](#) is available for the definition of pairs.

polymers for which existing theory can predict stabilities and structures of some folded states accurately (23).

We threaded sequences composed of long (length  $\approx 14$  mm) and short (length  $\approx 7$  mm) cylindrical beads; we assumed that the net charge on the shorter cylindrical beads would be smaller than that on the longer cylinders, and the interactions between these beads would correspondingly be weaker. Our intention was that the stronger interactions between long cylindrical beads would

be analogous to those between base pairs joined by three hydrogen bonds (GC), and that the weaker interactions between shorter cylinders would model those involving two hydrogen bonds (AU). The total charge on the shorter cylinders was smaller than that on the longer ones, but the areal charge on the two lengths of beads was the same: short Nylon cylinders charged to  $+2,500 \pm 200$  pC (7 pC/mm<sup>2</sup>) and short Teflon cylinders charged to  $-2,800 \pm 300$  pC (8 pC/mm<sup>2</sup>).

Using these cylindrical beads, we constructed a palindromic sequence analogous to GGCAUAAUAGCC (24). We used one spacer bead to introduce stiffness comparable to that of RNA molecules (the persistence length of RNA molecules is  $\approx 700$  Å) (24, 25). We studied the folding of this sequence of beads by agitating it on a paper surface; it folded repeatedly, within 5 min, into the same hairpin conformation. This 2-D conformation persisted for at least 1 h under agitation; we infer that it represents the global minimum for this sequence (Fig. 3), and it corresponds to the structure predicted by theory and shown to exist in RNA by experiment (24, 26). Theoretical calculations for the inverted sequence AAUGCGGCGAUU predict that it would not spontaneously fold into a hairpin structure (i.e., the calculated free energy for formation of a hairpin from a linear sequence in solution is positive) (25). In agreement with this theoretical calculation, the analogous sequence did not fold into a single stable conformation. Instead, it sampled numerous different structures in several experiments (Fig. 3).

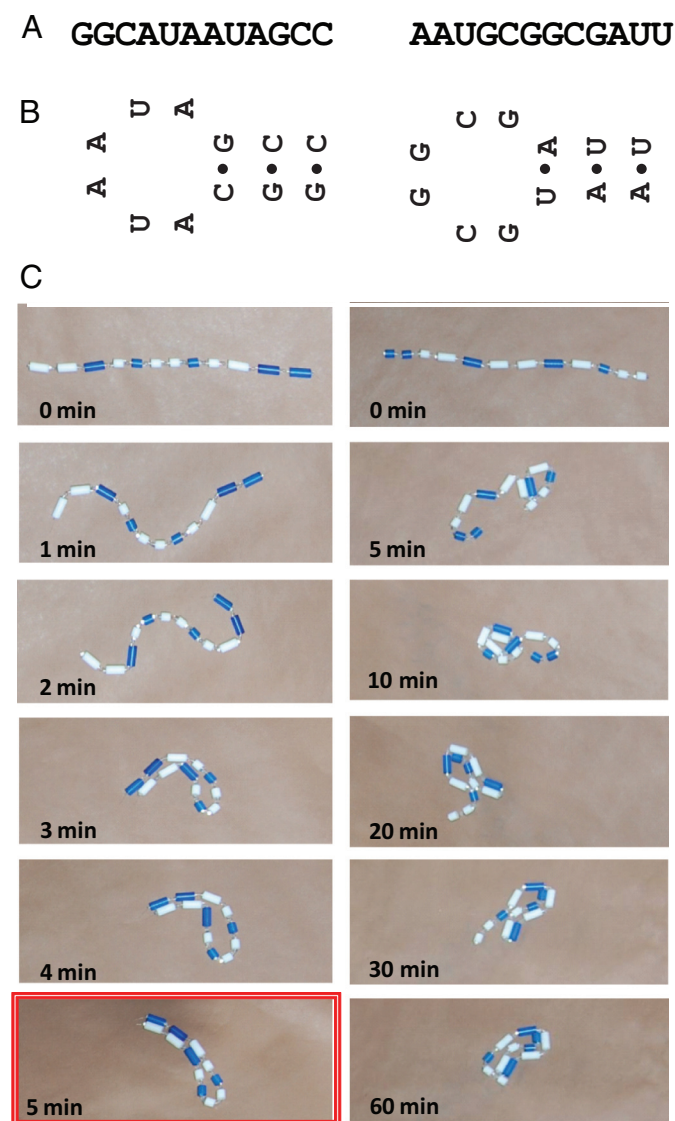
**“Polymer Chain” Models.** The still-evolving efforts of the theoretical community to resolve the Levinthal paradox—that is, to understand how a biopolymer finds its lowest-energy “native” conformation within a finite time (27)—illustrates the limits of our current understanding of folding. To model folding with length scales that correspond realistically to polymers, we constructed longer sequences of beads. Because the surface-to-volume ratio determines the physical behavior of a chain, short chains in 2-D are expected to have instructive similarities to longer chains in 3-D (28). For example, if a polymer has 200 units in 3-D, then the maximum volume of the sphere it occupies,  $V = 4\pi R^3/3 = 200$  monomeric units, has a radius of  $R = 3.62$  units. The analogous 2-D polymer with the same radius has an area of  $A = \pi R^2 \approx 40$  monomeric units.

To explore folding (or collapse) in a “long” system, we constructed a sequence composed of 40 beads with three spacer beads (chosen arbitrarily as a demonstration of principle) and agitated the sequence on the surface<sup>†</sup>. We equate folding with collapse in this system, although “collapse” is typically used to describe the nonspecific colocalization of monomers in a polymer, and “folding” is often reserved for the formation of secondary and tertiary structure stabilized by specific interactions (11, 29). The 2-D structure evolved as a function of time (i.e., the chain collapsed or folded) (Fig. 4). Fig. 5 plots the number of correct pairs (Teflon-Nylon) and incorrect pairs (Teflon-Teflon, Nylon-Nylon) as a function of time (see [SI Text](#) for the definition of a pair). After  $\approx 20$  min of agitation, the string of beads folded into a stable conformation with a low number of incorrect pairs (on average, one pair per sequence) compared with the number of correct pairs (Fig. 4 and [Fig. S3](#)). These conformations did not unfold under agitation and persisted for at least an additional 90 min. This specific sequence of beads folded into several different conformations that indicated different energetic minima (Fig. 4). A sequence of 40 Nylon beads (all positively charged) did not fold ([Fig. S4](#)).

To prove that the final conformations are the product of electrostatic interactions, we neutralized the electrostatic charge

<sup>†</sup>The longest sequence of beads that we folded had 40 beads; longer sequences could not be stretched on the surface of our apparatus.

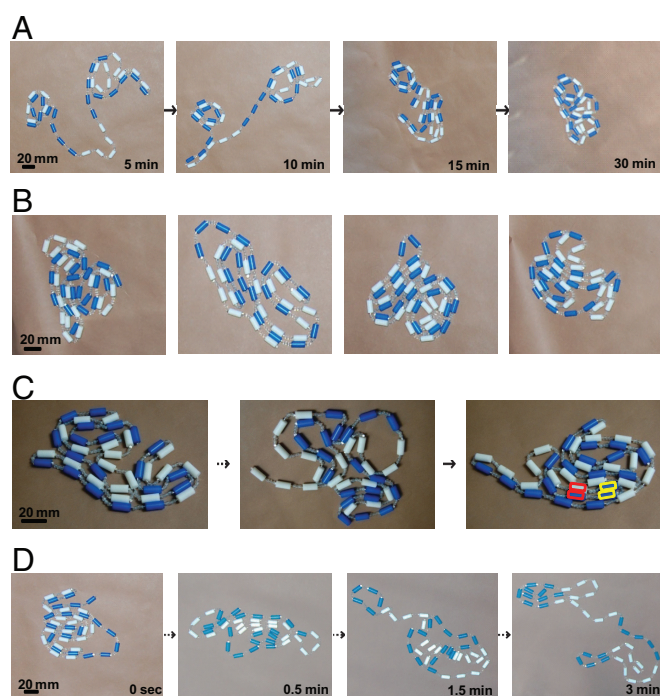




**Fig. 3.** Folding of short chain RNA. (A) The letters represent sequences of nucleosides. The two sequences are related by inversion of purines and pyrimidines. (B) Theoretical calculations predicted hairpin loop structures (with different free energies of folding) for each sequence. (C) Time lapse photographs captured the folding process for the analogous sequences composed of long ( $\approx 14$  mm) and short ( $\approx 7$  mm) cylindrical beads. The images represent the folding of the two sequences, GGCAUAAUAGCC (Left), which folded to one stable conformation (highlighted in red) in eight experiments, and AAUGCGGCGAUU (Right), the structure of which evolved continuously throughout eight independent experiments.

on the beads after 60 min of agitation using a Zerostat antistatic gun. On continuing agitation, the sequence of beads, now electrostatically neutralized, unfolded into a less compact conformation (Fig. 4C). As agitation continued the beads again charged and collapsed into a compact conformation (Fig. 4C). This cycle of charging to form an ordered structure, neutralization to break that structure, and charging to re-form a different ordered structure indicates that (i) the electrostatic charge on the beads was required for the collapse of the sequences, and (ii) the stable final conformations represent various local energetic minima of the system—since the sequence of beads folded into a unique structure in each experiment.

To establish that these final conformations can unfold completely when the electrostatic interactions are eliminated, we



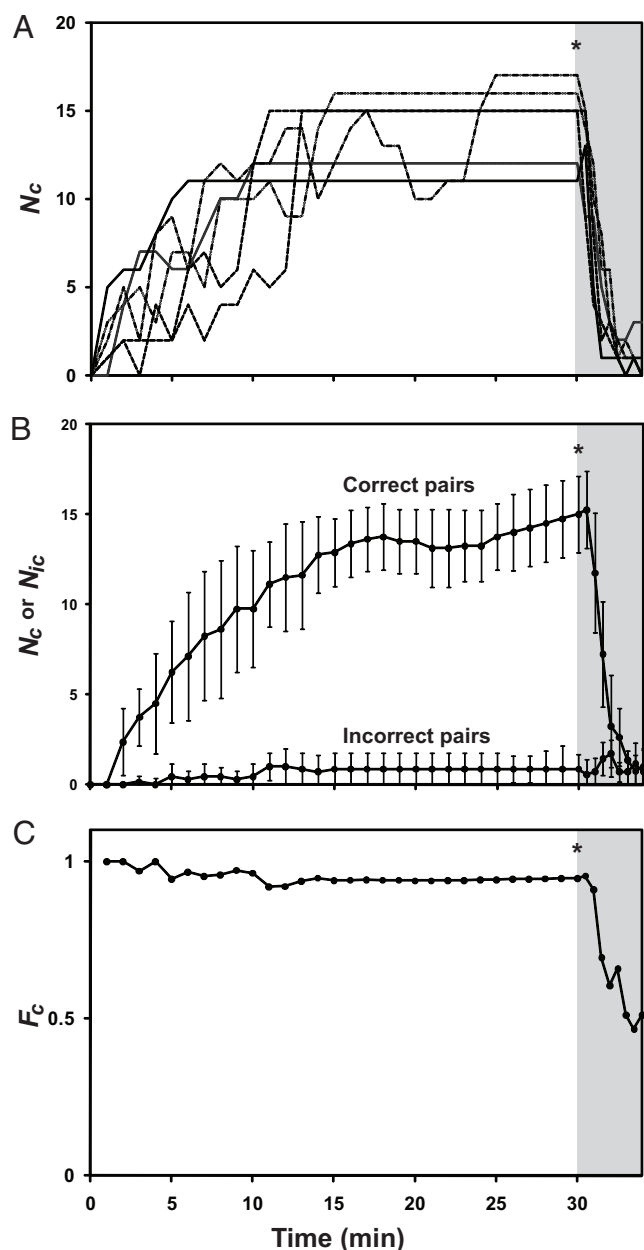
**Fig. 4.** Folding sequences of 40 cylindrical beads (with three spacer beads). (A) Time-lapse photographs capture the folding process. In this experiment, the number of correct pairs in the final conformation was 13. (B) Each image presents a different final conformation for this sequence. (C) The images show the results of one cycle of folding, unfolding, and refolding; the starting conformation had 11 correct pairs (Left). Upon discharge with a Zerostat antistatic gun, the sequence adopted a partially unfolded conformation (Center) that had two correct pairs. With continued agitation, the beads recharged and assumed a different stable conformation (Right), which also had 11 correct pairs. The red line outlines one correct pair (Nylon-Teflon), and the yellow line outlines one incorrect pair (Nylon-Nylon). (D) Time-lapse photography captures the unfolding process when agitating a stable conformation of beads in high humidity (RH  $\approx 70\%$ ). In this experiment, the number of correct pairs in the initial folded conformation was 11, and the number of incorrect pairs was 0. The number of correct pairs decreased with continued agitation in high humidity; after 3 min, the number of correct pairs was 1, and number of incorrect pairs was 1.

increased the RH in the system to  $\approx 70\%$  and agitated the surface (19). Under these conditions, the beads lost their charge (see [SI Text](#)), and the pairs of beads started to separate immediately; after  $\approx 3$  min, the number of correct pairs decreased to a value of 0 or 1, which was approximately the same as the number of incorrect pairs (Figs. 4D and 5).

## Discussion

The system emerging from this work—one comprising strings of macroscopic monomers—bridges simple theoretical and physical models of polymers. Theory and experiment provide alternative and complementary approaches to the study of polymer conformation. Many of the interactions that make the physical system complex are well-defined and (to a degree) under our control. This control provides the foundations for a system that is experimentally practical, but also retains nonlinearities of the types—although not of the specific detail—that are important in molecular systems. It allows us, for example, to examine a many-body electrostatic system, which has no analytical solution, without the use of a mean-field theory (30).

This experimental system provides examples of folding at two extremes of possible energy landscapes: (i) for short sequences (10 beads), a flat landscape with a single global minimum, in which folding is dominated by a search through conformations



**Fig. 5.** Charging (folding) and discharging (unfolding) of sequences of 40 cylindrical beads (with three spacer beads). The data derive from eight independent observations of the folding of the sequence at RH  $\approx$  20% over 30 min (white background), and the unfolding of the sequence at RH  $\approx$  70% in the subsequent 4 min (gray background). The asterisks represent the point in time at which the RH increased to  $\approx$  70%. (A) The plot shows the number of correct pairs ( $N_c$ ) formed as a function of time in the eight independent folding experiments (each represented by a line). (B) The graph plots  $N_c$  and the number of incorrect pairs ( $N_i$ ) formed when folding (and unfolding) a sequence of 40 beads. Each point is the average number of pairs formed in time in the eight independent experiments, and the error bars represent one sample standard deviation. (Discussions of the definition of pairing appear in [SI Text](#).) (C) The graph plots the evolution in time of the fraction of correct pairs [ $F_c = N_c / (N_c + N_i)$ ] formed when folding and unfolding a sequence of 40 beads.

and (ii) for longer sequences (40 beads), a more rugged landscape in which the polymer can be trapped kinetically in local minima. This system makes it possible to explore how sequences sample conformational space; we are interested in, for example, the lifetime of correct and incorrect pairs on the pathway to a

minimum, and whether longer sequences can be designed that fold to a single global minimum—in analogy to more complex problems in folding.

In considering more complex theoretical modeling, we note that the charge distribution in this system does not change as the sequence folds (i.e., the sign of charge on the beads remains the same during agitation). The sequences of beads can, therefore, be described as chains of quenched charges (i.e., the specific sequence of the chain is determined during the polymerization stage, and therefore the charge distribution is frozen) (31, 32). In this case, theories of spin glasses that deal with the nature of quenched disordered systems could be applicable (2, 32, 33).

The principles demonstrated here are a starting point for the study of more specific problems; for example, the effect of point mutation on the folding of hairpin-forming sequences in RNA and the folding of primitive “proto-proteins” using limited sets of amino acids in prebiotic chemistry (34, 35).

The physical system we describe is the simplest that we can design to simulate behaviors characteristic of polymers. Comparison of results from it with results of computations and simulations based on electrostatically interacting 2-D beads-on-a-string will allow scientists interested in collapse and folding of molecular polymers to explore the physical bases of these problems using a different perspective.

## Materials and Methods

**Materials.** Teflon and Nylon-6,6 beads with a diameter of 6.35 mm, PMMA beads with a diameter of 3.175 mm, a Nylon-6,6 string with a diameter of 0.4064 mm, and a 1.2  $\times$  1.2 m, 0.8-mm-thick aluminum sheet were purchased from McMaster Carr Supply Company. A poly(isocyanurate) board was purchased from Home Depot. Silver-coated, 2  $\times$  2-mm crimp beads were purchased from Beadalon. Holes with a diameter of 1.32 mm were drilled through the Teflon, Nylon, and PMMA beads.

**Staining the Nylon Beads.** Nylon beads were immersed in an aqueous solution of Disperse Blue 14 and placed on a hot plate at a temperature of 100  $^{\circ}$ C for 30 min. Dyed beads were washed with water and ethanol and dried with a  $N_2$  stream.

**Construction and Agitation of a Large Flat Surface.** An aluminum sheet was taped with double-sided adhesive to a poly(isocyanurate) board; this board provided a light-weight support to prevent the bending of the aluminum sheet. Next, a round aluminum frame was placed on the aluminum sheet. The sheet and frame were suspended from the ceiling by four hooks (one in each corner of the surface) which connected the surface to four chains. The chains were joined together and connected with a hook to a turnbuckle that was, in turn, connected to a Bungee cord hanging from the ceiling (Fig. 1B). We attached a pendulum to the bottom of the surface; to form the pendulum, we connected a ring to the bottom of the surface, from which we hung a 1-kg disc-shaped weight (McMaster Carr Supply Company). The overall weight of the pendulum was 1.5 kg. To agitate the surface, we connected an orbital motor to the edge of the surface via a polyurethane tie-down cord (length = 35 cm; McMaster Carr Supply Company). The orbital motor (shaking displacement  $\approx$  10 mm) was set to work at 110 rpm for sequences of spherical beads and 120 rpm for sequences of cylindrical beads. We also placed a linear motor (LinMot) below the surface to strike the pendulum. The linear motor had an amplitude of 2 cm and a frequency of 4 Hz.

To form a slight curvature (radius  $\approx$  3 cm) at the perimeter of the frame we covered the aluminum sheet with paper (Pacon Corp.) (Fig. 1C). To create a humidity-controlled environment, we covered the entire apparatus with vinyl curtains and attached them to the surface with Velcro to make a sealed enclosure; we reduced the relative humidity in the enclosure with a positive pressure of  $N_2$ . The relative humidity inside the enclosure was measured with a hygrometer (VWR). To increase the humidity, we connected a humidifier (Vicks Ultrasonic Humidifier; P&G) with a tube to the enclosed apparatus and removed the positive  $N_2$  pressure.

**Construction of a Random Motion Generator.** To construct a random motion generator, we suspended the surface supporting the beads from the ceiling of the room, and applied two mechanical inputs to the system: (i) a periodic propulsion delivered by an orbital motor attached to the surface by a flexible polyurethane cord, and (ii) an aperiodic propulsion delivered by a computer-

controlled linear actuator striking a pendulum suspended from the underside of the surface (Fig. 1B).

**Preparation of the Sequences of the Beads.** The Nylon string was loaded through the eye of a needle. Using the needle, various sequences were constructed by threading the beads onto the string. We threaded the beads in the following order: large (Teflon or Nylon) bead, crimp bead, PMMA bead(s), crimp bead, large bead, etc. The beads were fastened in position by crimping the  $2 \times 2$ -mm, silver-coated beads. The string was maintained in a horizontal position throughout the sequence-making process to ensure that it would not be deformed (stretched) under the gravitational force acting on the beads. The length of the string was the sum of the diameters of the beads comprising the sequence.

**Self-Assembly of the Sequences.** Before each experiment, the beads were rinsed with 95% ethanol and dried with a stream of  $N_2$ . An antistatic gun (Sigma) was used before each experiment to neutralize any charge accumulated on the beads.

**Measurement of Charge on the Beads.** We measured the charge on the Teflon and Nylon beads by removing them from the string, picking up each bead with poly(butylene terephthalate) (PBTP) tweezers, and dropping the bead into a Faraday cup. An electrometer (6514; Keithley), operating on the nanocoulomb scale, measured the charge inside the cup, and a homemade program in LabView recorded the charge as a function of time. The charge on the bead corresponded to the difference in charge inside the cup before and after the bead entered the cup.

**ACKNOWLEDGMENTS.** We thank Prof. Howard Stone, Prof. Eugene Shakhnovich, and Prof. Olvera de la Cruz for helpful discussions of theory; Dr. George K. Kaufman for his experimental assistance; Dr. Audrey Ellerbee for her assistance with scientific editing; and Ms. Felice Frankel for help with photography. This work was supported by Department of Energy Grant DE-FG05-00ER45852, National Science Foundation Grant CHE-0518055, Defense Advanced Research Projects Agency/Army Research Office Grant W911NF-04-1-0170, and the Xerox Corporation provided support for salaries. M.R. is supported by a postdoctoral fellowship from the European Molecular Biology Organization and Human Frontier Science Program.

1. Flory PJ (1969) in *Statistical Mechanics of Chain Molecules* (Interscience Publishers, New York, NY), pp 1–29.
2. Shakhnovich E (2006) Protein folding thermodynamics and dynamics: Where physics, chemistry, and biology meet. *Chem Rev* 106:1559–1588.
3. Alm E, Baker D (1999) Matching theory and experiment in protein folding. *Curr Opin Struct Biol* 9:189–196.
4. Dobson CM, Sali A, Karplus M (1998) Protein folding: A perspective from theory and experiment. *Angew Chem Int Ed* 37:868–893.
5. Frauenfelder H (1995) Complexity in proteins. *Nat Struct Biol* 2:821–823.
6. Sali A, Shakhnovich E, Karplus M (1994) How does a protein fold? *Nature* 369:248–251.
7. Shakhnovich EI (1997) Theoretical studies of protein-folding thermodynamics and kinetics. *Curr Opin Struct Biol* 7:29–40.
8. Wang J, Onuchic J, Wolynes P (1996) Statistics of kinetic pathways on biased rough energy landscapes with applications to protein folding. *Phys Rev Lett* 76:4861–4864.
9. Perdomo A, Truncic C, Tubert-Brohman I, Rose G, Aspuru-Guzik (2008) A construction of model hamiltonians for adiabatic quantum computation and its application to finding low-energy conformations of lattice protein models. *Phys Rev A* 78:012320.
10. Hegler JA, Weinkam P, Wolynes PG (2008) The spectrum of biomolecular states and motions. *HFSP J* 2:307–313.
11. Solis FJ, de la Cruz MO (2000) Collapse of flexible polyelectrolytes in multivalent salt solutions. *J Chem Phys* 112:2030–2035.
12. Dobrynin AV, Rubinstein M (2005) Theory of polyelectrolytes in solutions and at surfaces. *Prog Polym Sci* 30:1049–1118.
13. Lifshitz IM, Grosberg AY, Khokhlov AR (1978) Some problems of statistical physics of polymer-chains with volume interaction. *Rev Mod Phys* 50:683–713.
14. Tozzini V (2005) Coarse-grained models for proteins. *Curr Opin Struct Biol* 15:144–150.
15. Shea JE, Brooks CL (2001) From folding theories to folding proteins: A review and assessment of simulation studies of protein folding and unfolding. *Annu Rev Phys Chem* 52:499–535.
16. Go N (1983) Theoretical-studies of protein folding. *Annu Rev Biophys Bio* 12:183–210.
17. Harper WR (1998) in *Contact and Frictional Electrification* (Laplacian Press, Morgan Hill, CA), pp 1–74.
18. McCarty LS, Whitesides GM (2008) Electrostatic charging due to separation of ions at interfaces: Contact electrification of ionic electrets. *Angew Chem Int Ed* 47:2188–2207.
19. Thomas SW, Vella SJ, Kaufman GK, Whitesides GM (2008) Patterns of electrostatic charge and discharge in contact electrification. *Angew Chem Int Ed* 47:6654–6656.
20. Wiles JA, Fialkowski M, Radowski MR, Whitesides GM, Grzybowski BA (2004) Effects of surface modification and moisture on the rates of charge transfer between metals and organic materials. *J Phys Chem B* 108:20296–20302.
21. Kaufman GK, et al. (2009) Phase separation of two-dimensional Coulombic crystals of mesoscale dipolar particles from mesoscale polarizable “solvent.” *Appl Phys Lett* 94:044102.
22. Kaufman GK, et al. (2009) Phase separation of two-dimensional mesoscale Coulombic crystals from mesoscale polarizable “solvent.” *Soft Matter* 5:1188–1191.
23. Serra MJ, Axenson TJ, Turner DH (1994) A model for the stabilities of RNA hairpins based on a study of the sequence dependence of stability for hairpins of 6 nucleotides. *Biochemistry* 33:14289–14296.
24. Kebbekus P, Draper DE, Hagerman P (1995) Persistence length of RNA. *Biochemistry* 34:4354–4357.
25. Hagerman PJ (1997) Flexibility of RNA. *Annu Rev Biophys Biomol Struct* 26:139–156.
26. Vladimir N (2001) *GeneBee—Molecular Biology Server* (Belozersky Institute of Physico-Chemical Biology, Moscow, Russian Federation).
27. Levinthal C (1968) Are there pathways for protein folding? *J Chim Phys PCB* 65:44–45.
28. Lau KF, Dill KA (1989) Lattice statistical-mechanics model of the conformational and sequence-spaces of proteins. *Macromolecules* 22:3986–3997.
29. Dill KA (1999) Polymer principles and protein folding. *Protein Sci* 8:1166–1180.
30. Oppen M, Saad D (2001) in *Advanced Mean Field Methods Theory and Practice* (MIT Press, Cambridge, MA), pp 1–7.
31. Castelnovo M, Sens P, Joanny JF (2000) Charge distribution on annealed polyelectrolytes. *Eur Phys J E* 1:115–125.
32. Borukhov I, Andelman D, Orland H (1998) Random polyelectrolytes and polyampholytes in solution. *Eur Phys J B* 5:869–880.
33. Raspaud E, de la Cruz MO, Sikorav JL, Livolant F (1998) Precipitation of DNA by polyamines: A polyelectrolyte behavior. *Biophys J* 74:381–393.
34. Lazcano A, Miller SL (1996) The origin and early evolution of life: Prebiotic chemistry, the pre-RNA world, and time. *Cell* 85:793–798.
35. Joyce GF (1989) RNA evolution and the origins of life. *Nature* 338:217–224.