Phase Diagram of Heteropolymers with an Imprinted Conformation

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ABSTRACT: Recently, theoretical models to employ physical interactions between monomers for the selection of appropriate heteropolymer sequences have been suggested. The Shakhnovich–Gutin model simulates the *in vivo* creation of natural proteins by molecular evolution. Our model suggests a method for the *in vitro* synthesis of heteropolymers which are "protein-like" in the sense that they have a unique 3D fold encoded in the sequence; furthermore, certain properties of this fold, such as the presence of an active site capable of specifically binding to a given target molecule, are under the control of our synthetic procedure. We show that the phase diagram of the polymer chain prepared by either method, besides random and frozen globular phases, also includes a third globular phase, which we call the target phase. The random globule is comprised of a vast number of compact conformations, and although the frozen globule is dominated by one or a few conformations, these are not under any control and generally do not possess any desirable properties. On the other hand, the target phase is dominated by the desirable conformation. Using mean field replica theory, we are able to examine a heteropolymer chain which consists of an arbitrary set of monomers with short-range interactions. We discuss crude prescriptions for the experimental realization of the target phase regime.

I. Introduction

It is well known that the equilibrium conformation of proteins is of paramount importance to its biological activity. The equilibrium conformation for a given protein is determined by the linear sequence of monomers and the interactions between them. The relationship between the heteropolymer sequence and its equilibrium conformation is still a mystery. Furthermore, while order–disorder transitions are a common theme in statistical physics, it is at first sight unclear why the equilibrium conformation should consist of one (or very few) conformations. However, there are some physical properties of this system which can immediately yield some qualitative insight. First and foremost, the nature of the polymer is fundamentally different than the behavior, for example, of its disconnected constituent monomers, due to the polymeric bonds. These connections restrict the phase space of monomer arrangements and fundamentally change the physical system by introducing frustrations: the nature of the free energy landscape for the polymer system has many local minima due to the constraints of the polymeric bonds. In other words, the polymeric bonds, in addition to a rich variation of monomer interactions (the *hetero*polymeric properties), differentiate the free energy of the conformations. This differentiation combined with the restricted phase space of monomer arrangements allows the possibility of a unique ground state. Thus, upon reducing the acting temperature on the polymer, we can induce a "freezing" transition, where the equilibrium conformation is dominated by this ground state.

With this physical principle in mind, the freezing transition was first investigated for random chains in terms of phenomenological models.[1] Using the principle of "minimal frustration", the freezing transition was shown to be similar to that of the random energy model (REM).[2] The REM transition was later derived directly from a microscopic Hamiltonian in which the interactions between each two monomers were assumed to be random independently taken from a Gaussian distribution.[3] However, this model did not explicitly include polymer sequence. The polymer sequence was later directly incorporated into the models.[4–6] In fact, a freezing transition was shown to exist for random sequences as long as the nature of the interactions were heteropolymeric.[7]

However, all of these works refer only to random sequences. It is believed that protein sequences differ in some degree from random sequences.[1,9] In the Shakhnovich–Gutin model,[8] the "design" of sequences is considered to be performed by evolution. Specifically, mutations cause changes in the heteropolymer sequence. Assuming that the fitness is related to the folding properties and therefore the polymer energy, evolution should lead to sequences which have sequences annealed to minimize the energy when in a particular target conformation $\bigstar$.

Recently, we proposed the "imprinting" model, which is a method to *in vitro* create sequences which can renature and recognize a given target molecule.[10–12] As shown in Figure 1, the general scheme is to allow the monomers to equilibrate in space prior to polymerization and then polymerize the monomers in such a way that the monomers' equilibrium positions remain unchanged. We expect that the minimization of energy of the monomer solution should lead to the minimization of the polymer energy, and therefore, the polymer would renature to the polymerization conformation, as shown in Figure 2. If a particular target molecule is placed in the monomer solution, then the monomers should also equilibrate around the target molecule and the resulting polymer should have a complementary site capable of recognizing the particular target molecule. In this sense, an imprinted heteropolymer should be able to specifically recognize the target molecule, thus acting much like an artificial antibody.

In fact, to the level of mean field, these two models are indistinguishable: in both cases, the polymer sequence resulting from the design procedure folds to a

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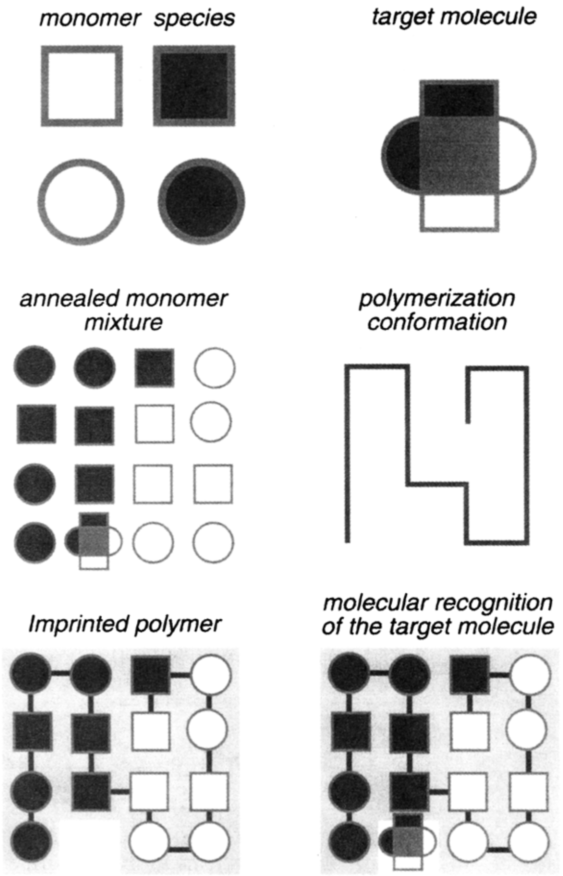

Figure 1. The imprinting process. Even though our analytic treatment is general, we schematically depict imprinting in two dimensions for monomers which interact as in the *p*-charge model (there are energetic preferences toward neighbors which have the same shape (square versus circle) and color (black versus white)). We include a target molecule which allows different interactions on each side, in this case with the four sides representing all possible monomer species. We place this target molecule in the presence of monomers prior to polymerization and allow this "monomer soup" to equilibrate, leading to an annealed monomer mixture. We model polymerization by choosing some conformation randomly and threading the monomers in the soup along the path of the polymerization conformation in order to define the sequence of the imprinted polymer. The optimization of the monomer arrangement in the monomer soup leads to an imprinted polymer which can renature to the polymerization conformation. Furthermore, the polymerization conformation includes a pocket, or "active site", allowing specific complementary interactions with respect to the target molecule. Thus, imprinted heteropolymers have the protein-like properties of renaturability and specific molecular recognition.

particular target conformation ★. In this work, we concentrate on imprinting, but we note that the general formalism derived here is applicable to the Shakhnovich–Gutin model as well.

This paper builds upon some ideas from previous works.[7,12,13] Two of them,[12,13] dealt with the analytic replica theory of designed heteropolymers but were restricted to chains of only two monomer species. Unlike these works, we make only the most basic assumptions about the nature of monomer–monomer interaction, namely that monomers interact through short-range interactions and heterogeneity comes primarily from pairwise interactions. We use a matrix B_{ij} to denote the interaction energy between monomers of *species* i and j. The same general assumptions were taken in ref 7, but for random sequences only. Unlike that work, here we address mainly the design aspect; that is, we investigate whether designed sequences will freeze into the desirable target conformation and how the nature of interactions affects the design procedure. To make this paper self-contained, we briefly repeat some steps of both ref 7 and ref 12.

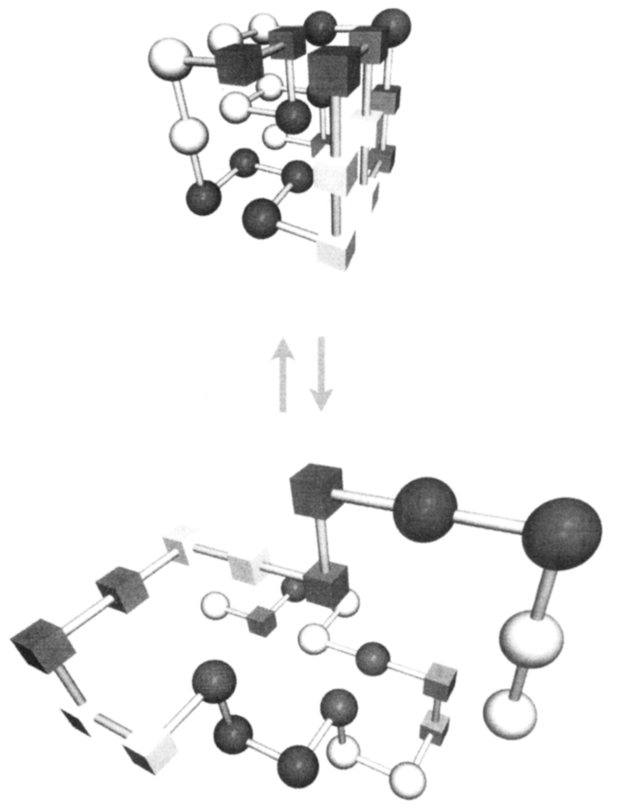

Figure 2. Renaturation of an imprinted heteropolymer. As an example, we employ *p*-charge interactions as in Figure 1, except now in three dimensions. The optimization of the spatial arrangement of the monomers prior to polymerization selects sequences which have a low energy when the polymer is arranged in the polymerization conformation. This leads to the ability of the polymer, after denaturation, to successfully fold to the polymerization conformation (renaturation).

II. Development of the Model

A. Disordered Short-Range Two-Body Interactions. In this system, there are two fixed quantities: the heteropolymer sequence and the nature of interaction between monomer species. We model both aspects explicitly. We calculate the energy of interaction between two monomers based upon the energy of interactions of the respective species of monomers and whether they are in the proximity for interaction:

$$\mathcal{H} = \sum_{i,j}^{q}\sum_{I,J}^{N} B_{ij}\delta(\mathbf{r}_I-\mathbf{r}_J)\,\delta(s_I,i)\,\delta(s_J,j) + \mathcal{H}' \tag{1}$$

where B_{ij} is the interaction energy between monomer *species* i and j, s_I is the species of monomer at position I along the chain, and $\mathbf{r}_I$ is the position of monomer I. In this paper, we will use the following notation: upper case Roman letters relate to monomer numbers along the chain, lower case Roman letters relate to monomer species, and lower case Greek letters denote replica indices. $\mathcal{H}' = C\varrho^2 + ...$ is the excluded volume virial expansion. This term is purely homopolymeric in nature. Thus, we assume that heterogeneity solely comes from pairwise interactions and all high-order interactions contribute primarily to the excluded volume effect.

Note that Hamiltonian (1) depends on both conformation (through the set of monomer coordinates $\mathbf{r}_I$) and the sequence (through s_I). We formally express that by writing $\mathscr{H} = \mathscr{H}(\mathrm{conf;seq})$.

B. Self-Averaging over the Sequences. As we take a statistical approach, we can only analyze properties of the ensemble—in this case, the ensemble of designed sequences. In each realization, the sequence is fixed, or quenched. Thus, in principle, each particular chain is characterized with the sequence-dependent free energy

$$F(\mathrm{seq}) = -T \ln Z(\mathrm{seq}) \tag{2}$$

In fact, however, free energy is believed to be a self-averaging value, which means that free energy almost does not depend on realization of the sequence, given that composition is fixed and overall length is long enough; therefore the sequence-dependent free energy is in practice almost sequence-independent and, therefore, coincides practically with the mean free energy averaged over the ensemble of sequences:

$$F(\mathrm{seq}) \simeq F^\star \equiv \langle F(\mathrm{seq})\rangle^\star_{\mathrm{seq}} = -T\langle \ln Z(\mathrm{seq})\rangle^\star_{\mathrm{seq}} \tag{3}$$

Leaving aside for a moment the difficult technical question of how to average $\ln Z$, let us discuss first the logical aspect: we have to average over the set of all possible q^N sequences, with a weighting based upon the probability of each particular sequence to appear in the ensemble of designed sequences. To specify this ensemble, recall that our polymerization scheme implies two steps: (i) prearrangement of the set of disconnected monomers in space, governed by the same monomer-to-monomer interactions involved in the Hamiltonian (1), at some polymerization temperature, T_p; (ii) formation of strong polymeric bonds between prearranged monomers along some independently chosen backbone, $\star$, so that newly prepared chain appears in the conformation $\star$. Strictly speaking, we have to consider a new ensemble of designed sequences for each preparation conformation $\star$; this is why we keep the superscript $\star$ throughout the equation (3). Doing so, we average as $\langle ...\rangle^\star_{\mathrm{seq}} = \sum_{\mathrm{seq}} ... P^\star_{\mathrm{seq}}$, and we identify probability distribution $P^\star_{\mathrm{seq}}$ with the Boltzmann weight associated with the Hamiltonian (1) at the temperature T_p. Indeed, in our polymerization procedure, each monomer is assigned with the number along the chain, thus fixing the $\{s_I\}$ variables; also, as the monomer positions are kept unchanged while polymerizing, vectors $\mathbf{r}_I$, related to conformation $\star$, are at the same time the coordinates of monomers immediately prior to polymerization. For this reason, on the mean field level the energy of the prepolymerized monomer mixture is indistinguishable from the energy of the polymer in the preparation conformation $\star$. Therefore,

$$P^\star_{\mathrm{seq}} = \exp\left[-\frac{1}{T_p}\mathscr{H}(\mathrm{conf}{=}\star;\mathrm{seq}) + \frac{1}{T_p}\sum_{I=1}^{N}\mu_{s_I}\right] \tag{4}$$

The composition of the monomer mixture prior to polymerization is maintained by equilibrium with the surrounding reservoir, where μ_s is the chemical potential of the component s. Note that we have not explicitly included any normalization for $P^\star$. However, any such normalization is just a constant factor on the partition function and is therefore irrelevant.

C. Self-Averaging over Preparation Conformation. In fact, there is a second level of frozen disorder; what is now quenched in the system is the information about preparation conformation $\star$ used to create the ensemble of sequences. At this level, we can repeat the logic of eq 3. Indeed, there are equal grounds to believe in self-averaging with respect to $\star$, just as one step before, with respect to sequences. We write therefore

$$F(\mathrm{seq}) \simeq F^\star \simeq F \equiv \langle\langle F(\mathrm{seq})\rangle^\star_{\mathrm{seq}}\rangle_\star = -T\langle \ln Z(\mathrm{seq})\rangle \tag{5}$$

where the last average over both sequence and preparation conformation $\star$ is performed as

$$\langle ...\rangle \equiv \sum_\star \sum\nolimits_{\mathrm{seq}} ... \exp\left[-\frac{1}{T_p}\mathscr{H}(\mathrm{conf}{=}\star;\mathrm{seq}) + \frac{1}{T_p}\sum_{I=1}^{N}\mu_{s_I}\right] \tag{6}$$

As to the above-mentioned question of averaging $\ln Z$, we employ the well-known replica trick, in which one solves the simpler problem of averaging Z^n with positive integer n, such that all the difficulties appear at the moment of analytic continuation to $n \to 0$:

$$F(\mathrm{seq}) \simeq F = \langle F(\mathrm{seq})\rangle = -T\langle \ln Z(\mathrm{seq})\rangle = -T \lim_{n\to 0} \frac{\langle Z^n(\mathrm{seq})\rangle - 1}{n} \tag{7}$$

As the appearance of a particular sequence is governed by the same Hamiltonian involved in monomer interactions, we can write the expression for the n-replica partition function as

$$\langle Z^n(\mathrm{seq})\rangle = \sum_{\mathrm{seq}} \mathscr{P}_{\mathrm{seq}} \sum_{C_0,C_1,...,C_n} \exp\left[-\sum_{\alpha=0}^{n}\frac{1}{T_\alpha}\mathscr{H}(C_\alpha,\mathrm{seq})\right] \tag{8}$$

where the following notations are used: $\alpha = 0, 1, ..., n$ are the numbers of replicas; $C_\alpha = C_1,...,C_n$ stand for conformations of replica number α; replica $\alpha = 0$ is attributed to the target conformation $\star$, that is, $C_0 = \star$; $T_\alpha = T$ is the acting temperature for $\alpha \neq 0$; $T_\alpha = T_p$ is the "polymerization temperature" (i.e., the selective temperature involved in the design procedure) for $\alpha = 0$; and $\mathscr{P}_{\mathrm{seq}} = \prod_{I=1}^{N} p_{s_I}$, where $p_i = \exp[\mu_i/T_p][\sum_i \exp(\mu_i/T_p)]^{-1}$ (the fraction of monomers of species i). Note, that for brevity we do not write explicitly all of the normalization factors. We will take care of all of them at the very end.

D. Manipulations with Replicas. For further notational simplification, we introduce density distributions of all species for each conformation and replica as

$$m_i^{\alpha}(\mathbf{R}) = \sum_{I=1}^{N} \delta(s_I,i)\,\delta(\mathbf{r}_I^{\alpha} - \mathbf{R}); \quad \{m_i^{\alpha}(\mathbf{R})\} \equiv \vec{m}^{(q(n+1)\infty)} \tag{9}$$

Then write in terms of those definitions

$$\langle Z^n(\mathrm{seq})\rangle_{\mathrm{seq}} = \sum_{\mathrm{seq}} \mathscr{P}_{\mathrm{seq}} \times \sum_{C_0,...,C_n} \exp\{-\langle \vec{m}|\hat{B}^{(q)} \otimes (\hat{\mathscr{T}}^{(n+1)})^{-1} \otimes \hat{I}^{(\infty)}|\vec{m}\rangle^{(q(n+1)\infty)}\} \tag{10}$$

where $\hat{\mathscr{T}} = T_\alpha \delta_{\alpha\beta}$, and $\langle|...|\rangle^{(q(n+1)\infty)}$ means scalar product in which all vectors and operators are supposed to have dimensionality as indicated ($q(n+1)\infty$ in this case). We use here the operation of direct product $\otimes$, in the following sense (identical to what was in ref 7): if there are two matrices (or operators) of different dimensionalities r and s, say $\hat{A}^{(r)}$ and $\hat{B}^{(s)}$, then $\hat{A}^{(r)}\otimes\hat{B}^{(s)}$ is the matrix of dimensionality rs obtained by mapping of the matrix $A_{uv}\hat{B}^{(s)}$ onto each matrix element (u,v) of the $\hat{A}^{(r)}$ matrix. Operator $\hat{I}^{(\infty)}$ is the identity operator with respect to real coordinate space, meaning that it has the kernel $\delta(\mathbf{R}_1-\mathbf{R}_2)$. Note that for brevity, we have not included the homopolymeric term $\mathscr{H}'$; it does not participate in any heteropolymeric effects and is therefore just a multiplicative constant insofar as the replica heteropolymeric calculations are concerned. We shall take care of this term, along with normalization constants, at the end of the calculations.

We now arrive at the formulation which is rather similar to what has been considered in ref 7, except the appearance of additional target replica 0 and matrix $\hat{\mathscr{T}}^{(n+1)}$. We repeat briefly what was done in ref 7. As monomers interact with each other, the corresponding monomer variables are coupled, which makes averaging over sequences difficult. We trade coupling of monomers for coupling of replicas by introducing fields by the Hubbard–Stratonovich transformation of the form

$$\langle Z^n(\mathrm{seq})\rangle_{\mathrm{seq}} = \sum_{C_0,\ldots,C_n} \int \mathscr{D}\{\phi\} \times \exp\left\{\frac{1}{4}\langle\vec{\phi}|(\hat{B}^{-1})^{(q)}\otimes\hat{\mathscr{T}}^{(n+1)}\otimes\hat{I}^{(\infty)}|\vec{\phi}\rangle^{(q(n+1)\infty)}\right\} \times \sum_{\mathrm{seq}} \mathscr{P}_{\mathrm{seq}} \exp\{\langle\vec{\phi}|\vec{m}\rangle^{(q(n+1)\infty)}\} \quad (11)$$

where $\{\phi_i^{\alpha}(\mathbf{R})\} = \vec{\phi}^{(q(n+1)\infty)}$ are the fields conjugated to the corresponding densities. We skip the normalization factor which comes from integration over ϕ; we will take care of this factor below.

Thus, the sum over sequences involves only uncoupled monomers in the last "source" term of the partition function above. This facilitates the summation over the sequences:

$$\exp\{\text{source term}\} = \sum_{\mathrm{seq}} \mathscr{P}_{\mathrm{seq}} \exp\{\langle\vec{\phi}|\vec{m}\rangle^{(q(n+1)\infty)}\} = \prod_{I=1}^{N} \sum_{i=1}^{q} p_i \exp\{\sum_{\alpha=0}^{n} \int \mathrm{d}\mathbf{R}\, \phi_i^{\alpha}(\mathbf{R})\, \delta(\mathbf{r}_I^{\alpha}-\mathbf{R})\} \quad (12)$$

The relevant order parameters are extracted by expansion over the powers of the fields ϕ (high-temperature expansion) up to $\mathscr{O}(\phi^2)$ (see the condition of applicability below):

$$\text{source term} = \sum_{i=1}^{q}\sum_{\alpha=0}^{n} \int \mathrm{d}\mathbf{R}\, \varrho^{\alpha}(\mathbf{R}) p_i \phi_i^{\alpha}(\mathbf{R}) + \frac{1}{2}\sum_{i,j=1}^{q} [p_i\delta_{ij} - p_i p_j] \sum_{\alpha,\beta=0}^{n} \int \mathrm{d}\mathbf{R}_1 \int \mathrm{d}\mathbf{R}_2\, \phi_i^{\alpha}(\mathbf{R}_1) \times Q_{\alpha\beta}(\mathbf{R}_1,\mathbf{R}_2)\, \phi_j^{\beta}(\mathbf{R}_2) \quad (13)$$

where we use the standard definitions[3,12,16]

$$Q_{\alpha_1,\ldots,\alpha_k}(\mathbf{R}_1,\ldots,\mathbf{R}_k) = \sum_{I=1}^{N}\prod_{\kappa=1}^{k} \delta(\mathbf{r}_I^{\alpha_\kappa}-\mathbf{R}_\kappa) \quad (14)$$

Note that $\varrho^{\alpha}(\mathbf{R})$, which in equilibrium does not depend on replica number and which within the large globule does not depend on $\mathbf{R}$ either, is the total density of the polymer chain. Following the standard interpretation, replicas are associated with the pure states of the polymer chain.[3,12,15] The k-replica order parameter $Q_{\alpha_1,\ldots,\alpha_k}$ is interpreted as the overlap between replicas $\alpha_1,\ldots,\alpha_k$. Therefore, a transition to unique structure corresponds to the equilibrium configuration where all replicas overlap, e.g., $Q_{\alpha\beta} = \varrho$.

Using the definition of the overlap order parameter, we can write the $(n + 1)$-replica partition function in a simple form:

$$\langle Z^n(\mathrm{seq})\rangle_{\mathrm{seq}} = \sum_{C_0,\ldots,C_n} \int \mathscr{D}\{\phi\} \times \exp\left\{\left\langle\vec{\phi}\left|\frac{1}{4}B_{ij}^{-1}\delta(\mathbf{R}_1-\mathbf{R}_2)\mathscr{T}_{\alpha\beta} + \frac{1}{2}Q_{\alpha\beta}(\mathbf{R}_1,\mathbf{R}_2)\Delta_{ij}\right|\vec{\phi}\right\rangle^{(q(n+1)\infty)} + \langle\vec{\varrho}\,|\vec{\phi}\rangle^{(q(n+1)\infty)}\right\} \quad (15)$$

where

$$\Delta_{ij} = p_i\delta_{ij} - p_i p_j \quad \text{and}$$

$$\vec{\varrho}^{\,(q(n+1)\infty)} \equiv \varrho_i^{\alpha}(\mathbf{R}) = p_i \sum_{I=1}^{N} \delta(\mathbf{r}_I^{\alpha}-\mathbf{R}) \quad (16)$$

Note that $\vec{\varrho}^{\,(q(n+1)\infty)} = \vec{\varrho}^{\,((n+1)\infty)}\otimes\vec{p}^{\,(q)}$. We are left with the Gaussian integral (15) for the $(n + 1)$-replica partition function, which can, of course, be evaluated. The result, however, is remarkably simplified by the argument given in refs 3, 4, and 12, which shows that the $\mathbf{R}$-dependence of $Q_{\alpha\beta}$ is of the δ-type, so that

$$Q_{\alpha\beta}(\mathbf{R}_1,\mathbf{R}_2) = \varrho q_{\alpha\beta}\delta(\mathbf{R}_1-\mathbf{R}_2) \quad (17)$$

where all the diagonal elements of the new matrix $\hat{q}^{(n+1)}$ are 1, while its off-diagonal elements are either 1 or 0. This means physically that two replicas α and β might be either uncorrelated (independent), so that $Q_{\alpha\beta} = 0$, or correlated so that one repeats the 3D fold of the other down to the microscopic length scale, so that $Q_{\alpha\beta}(\mathbf{R}_1,\mathbf{R}_2) = \varrho\delta(\mathbf{R}_1-\mathbf{R}_2)$. We do not repeat this argument here, as it is explained elsewhere (see the argument presented in ref 12 which is slightly different from the original one[3]).

Now that the $\hat{Q}$ matrix has been simplified, we perform Gaussian integration over all ϕ^{α} variables. At this moment, it is very important to take care of normalizing factors everywhere. We find that the normalization constants generated by the Hubbard–Stratonovich transformation are canceled by the Gaussian integration, yielding

$$\langle Z^n(\mathrm{seq})\rangle_{\mathrm{seq}} = \sum_{C_0,\ldots,C_n} \exp[-NE\{Q\}] \quad (18)$$

with the energy per one particle of the form

$$E = \langle\vec{\varrho}^{\,((n+1)q)}|(\hat{\mathscr{T}}^{(n+1)})^{-1}\otimes\hat{B}^{(q)}[\hat{I}^{(q(n+1))} + 2\varrho\hat{q}^{(n+1)}(\hat{\mathscr{T}}^{(n+1)})^{-1}\otimes\hat{\Delta}^{(q)}\hat{B}^{(q)}]^{-1}|\vec{\varrho}^{\,((n+1)q)}\rangle + \frac{1}{2}\ln\det[\hat{I}^{(q(n+1))} + 2\varrho\hat{q}^{(n+1)}(\hat{\mathscr{T}}^{(n+1)})^{-1}\otimes\hat{\Delta}^{(q)}\hat{B}^{(q)}] \quad (19)$$

where the rule $\det(\hat{A})\cdot\det(\hat{B}) = \det(\hat{A}\hat{B})$ has been used for simplification. The simplest way to deal with normalization is to incorporate some additive constant to the expression for energy (19) such that it yields the desirable zero level in some trivial case, for example, in the case of homopolymer, when $B_{ij} = B$ does not depend on monomer species. This is what we have done in eq 19. Indeed, for the homopolymer case eq 10 yields simply the second virial term $\varrho B[n/T + 1/T_p]$. Exactly so does the equation (19), because, as is easy to check, $\hat{\Delta}^{(q)}\hat{B}^{(q)} = 0$ in the homopolymer extreme.

Recall that the elements of the $\hat{q}$ matrix are either $q_{\alpha\beta} = 0$ or 1, which corresponds to no or complete overlap between the conformations of replicas α and β, respectively. This implies that a single-step Parisi replica symmetry-breaking (RSB) ansatz is appropriate.[17] In fact, by rearranging the matrix by row and column operations (under which the free energy is invariant), one can write the q matrix in terms of groups of overlapping replicas. Furthermore, the free energy can be calculated as a function of the nature of the grouping as well. Optimization of the free energy with respect to the nature of the grouping of replicas in fact yields the single-step Parisi RSB scheme. For the sake of simplicity, however, we omit the derivation, thus, formally employing the ansatz. Specifically, we assume that the q matrix is composed of one $(y + 1) \times (y + 1)$ "target block", i.e., a group of y replicas which overlap with the ★ conformation, and $(n - y)/x$ groups in which x replicas overlap. (See ref 12 for more details on this point.)

E. Free Energy of Replica System. With this form of $\hat{q}$ matrix in mind, we can apply matrix operations detailed in the Appendix to simplify the energy to

$$\begin{aligned} E = {} & \frac{1}{2} \ln \det\left[\hat{I} + \left(\frac{y}{T} + \frac{1}{T_p}\right) 2\varrho \hat{\Delta}\hat{B}\right] + \\ & \left(\frac{y}{T} + \frac{1}{T_p}\right)\left\langle \vec{p} \left| \varrho \hat{B}\left[\hat{I} + \left(\frac{y}{T} + \frac{1}{T_p}\right) 2\varrho\hat{\Delta}\hat{B}\right]^{-1} \right| \vec{p}\right\rangle + \\ & \frac{n-y}{2x} \ln \det\left[\hat{I} + x\frac{2\varrho}{T}\hat{\Delta}\hat{B}\right] + \\ & (n-y)\left\langle \vec{p} \left| \frac{\varrho}{T}\hat{B}\left[\hat{I} + \frac{2\varrho x}{T}\hat{\Delta}\hat{B}\right]^{-1} \right| \vec{p}\right\rangle \end{aligned} \tag{20}$$

We have summed through the replica space and now only species space remains. This is why we have dropped the labels for the dimensionality of the vectors and operators, which are now assumed to be in species space, i.e., dimensionality q.

To comment on the equation (20), which is technically the most important result of the work, we note first that it looks rather similar to what was found in ref 7 for random sequences. Recall that the expression (20) represents the energy of interaction between replicas. Replicas of different groups do not interact with each other—this is the sense of grouping. The total energy is, therefore, the sum of each independent group's contributions. We have found in ref 7 that the group of x replicas has the energy

$$\frac{1}{2} \ln \det\left[\hat{I} + \frac{2\varrho x}{T}\hat{\Delta}\hat{B}\right] + \left\langle \vec{p} \left| \frac{\varrho x}{T}\hat{B}\left[\hat{I} + \frac{2\varrho x}{T}\hat{\Delta}\hat{B}\right]^{-1} \right| \vec{p}\right\rangle \tag{21}$$

This is what eq 20 gives for $(n - y)/x$ groups with spontaneous replica symmetry breaking. As to the target group of $y + 1$ replicas, its energy is also almost of the same form, except $y/T + 1/T_p$ appears instead of x/T, because one replica has in a sense different temperature.

Now, all interesting heteropolymeric properties are seen in the nature of the phase transition of the new order parameters x and y.

We must now estimate the entropy due to the overlap of replicas. To this end, we repeat the arguments of refs 3, 4, and 12. The density of states per monomer is given by the ratio of the available volume to place additional monomers a^3, where a is the spacing between monomers, divided by the excluded volume per monomer v. For every additional replica in a group, we must consider this entropic reduction, and therefore the entropy per monomer for w replicas in a single group is $\ln(a^3/v)(w - 1)$. For $(n - y)/x$ groups of size x and one target group of size y, the entropy loss is therefore

$$\Delta S = -Ns\left[\frac{n-y}{x}(x-1) + y\right] \tag{22}$$

where $s = \ln(a^3/v)$.

We thus have the free energy

$$\begin{aligned} F = {} & \frac{1}{2} \ln \det\left[\hat{I} + \left(\frac{y}{T} + \frac{1}{T_p}\right) 2\varrho \hat{\Delta}\hat{B}\right] + \\ & \left(\frac{y}{T} + \frac{1}{T_p}\right)\left\langle \vec{p} \left| \varrho \hat{B}\left[\hat{I} + \left(\frac{y}{T} + \frac{1}{T_p}\right) 2\varrho\hat{\Delta}\hat{B}\right]^{-1} \right| \vec{p}\right\rangle + \\ & \frac{n-y}{2x} \ln \det\left[\hat{I} + x\frac{2\varrho}{T}\hat{\Delta}\hat{B}\right] + \\ & (n-y)\left\langle \vec{p} \left| \frac{\varrho}{T}\hat{B}\left[\hat{I} + \frac{2\varrho x}{T}\hat{\Delta}\hat{B}\right]^{-1} \right| \vec{p}\right\rangle + s\left[\frac{n-y}{x}(x-1) + y\right] \end{aligned} \tag{23}$$

Recall that x is the number of replicas in a nontarget group. Therefore x varies from $x = 1$, the replica symmetric case, to $x = n$, where all of the replicas are in the same (nontarget) group. Thus, we first optimize the free energy with respect to x. As x must be between 1 and n, optimization yields

$$x = \begin{cases} \xi_f T/2\varrho & \text{for } \xi_f T/2\varrho \le 1 \\ 1 & \text{for } \xi_f T/2\varrho > 1 \end{cases} \tag{24}$$

where ξ_f is the solution of the equation

$$\begin{aligned} 2s = {} & \ln \det(\hat{I} + \xi_f\hat{\Delta}\hat{B}) - \mathrm{Tr}[\xi_f\hat{\Delta}\hat{B}(\hat{I} + \xi_f\hat{\Delta}\hat{B})^{-1}] + \\ & \langle \vec{p} | \xi_f^{\,2}\hat{B}\hat{\Delta}\hat{B}(\hat{I} + \xi_f\hat{\Delta}\hat{B})^{-2} | \vec{p}\rangle \end{aligned} \tag{25}$$

We find that this result is independent of any design parameters, such as y or T_p, and is exactly the same as was found in ref 7 for random sequences. Physically, x corresponds to the number of replicas which group due to *spontaneous* symmetry breaking, not the field which draws replicas to the target replica. We interpret that $x \rightarrow n$ corresponds to maximal freezing of random sequences, while $x \rightarrow 1$ means transition between the frozen and random globular states, where frozen and random globules are characterized with a few and vast number of relevant conformations, respectively, even though they are of the same overall density.

As to the other order parameter, y, it is specifically related to the design procedure, as it represents the number of replicas in the target group (excluding replica 0); thus, y varies from 0 to n when either none or all of the replicas are in the target group. Upon performing the $n \rightarrow 0$ limit, the interpretation of y becomes somewhat obscured, as it varies from 0 to $n = 0$. It can be shown (see ref 12 for more details) that when n is arbitrarily small but still positive ($0 < n < 1$), there is no optimum of free energy (23) with respect to y within the interval $0 < y < n$. This means that the optimum is reached at the boundary of the interval, i.e., either at $y = 0$ or at $y = n$, depending simply on the slope of the F vs y dependence. Physically, this means that we predict a first-order phase transition from the state with no memory of the target state ($y = 0$) to the other state with strongly memorized target conformation ($y = n$). Therefore, the threshold between these two phases is given by the condition when the slope of the free energy in y vanishes. This is determined by

$$2s = \ln \det(\hat{I} + \xi\hat{\Delta}\hat{B}) - \mathrm{Tr}[\xi\hat{\Delta}\hat{B}(\hat{I} + \xi_p\hat{\Delta}\hat{B})^{-1}] + \langle\vec{p}|-\xi\hat{B}(\hat{I} + \xi_p\hat{\Delta}\hat{B})^{-1} + \xi_p\xi\hat{B}\hat{\Delta}\hat{B}(\hat{I} + \xi_p\hat{\Delta}\hat{B})^{-2} + \xi\hat{B}(\hat{I} + \xi\hat{\Delta}\hat{B})^{-1}|\vec{p}\rangle \quad (26)$$

where $\xi_p = 2\varrho/T_p$ and $\xi = 2x\varrho/T$.

Before passing to the discussion of the results obtained, we comment on the physical meaning of the operator $\hat{\Delta}$, which appears throughout our formulas. In brief, this operator removes the mean interactions of each species i from interaction matrix B_{ij}. Indeed, according to the definition (16), $\hat{\Delta}\hat{B} = p_i(B_{ij} - \sum_k p_k B_{ik}) = p_i(B_{ij} - \langle B_{ij}\rangle_j)$. A more detailed discussion of the $\hat{\Delta}$ operator can be found elsewhere.[7]

Also, x and y are only coupled to terms involving the $\hat{\Delta}$ operator, whereas ϱ is also coupled to $\langle\vec{p}|\hat{B}|\vec{p}\rangle$. Thus, for $B_{ij} = b_{ij} + B_0$ and $|B_0| \gg |b_{ij}|$, we can optimize the free energy with respect to density ϱ independently of optimization with respect to x and y. As for optimization with respect to ϱ, this of course includes the homopolymeric terms in $\mathscr{H}'$. Physically, density is balanced due to the competition between the attractive two-body interactions described by the average second virial coefficients and the primarily repulsive three and higher body interactions described by $\mathscr{H}'$. This is simply a homopolymeric effect. A constant density can be realized by a large ensemble of globular conformations, and the heteropolymeric effect is the selection of one conformation from this ensemble; this is described by the x and y order parameters and will be systematically described below.

III. Discussion

A. Phase Diagram. To summarize the findings of the previous section, we have shown that there are three macroscopic phases in the globule of the designed heteropolymer. One is called a "random globule", as it is comprised of a vast number of conformations; the second phase is called "frozen", as the chain freezes down to a few relevant conformations, but the choice of those conformations remains out of control of the design procedure; and the third phase is called "target", as in this phase the chain undergoes freezing to target conformation ★. These findings are organized in the form of a phase diagram (Figure 3) in the variables acting temperature T vs polymerization temperature T_p. We stress, that every vertical line on the diagram represents another physical sample of heteropolymers, which has been prepared at the given temperature T_p and is now examined at different temperatures T. So, vertical motion along the diagram means experimentally simply heating or cooling of the system, while horizontal motion means passing from one sample to another.

The lines of phase transitions are given by eqs 25 and 26. As to the transition between the random globule and frozen phase, the corresponding temperature does not depend on T_p, thus being represented by a horizontal line on the phase diagram; the transition temperature is given by $T_f = 2\varrho/\xi_f$, where ξ_f is the solution of eq 25. As to the transition between the target state and any other state, the corresponding conditions are given by eq 26. This equation should be treated independently for the $T > T_f$ and $T < T_f$ regions, as it contains x-dependence (through $\xi = 2\varrho x/T$), and $x = 1$ at $T > T_f$ and $x = T/T_f$ at $T < T_f$ (see ref 12). Combining (25) and (26), we have

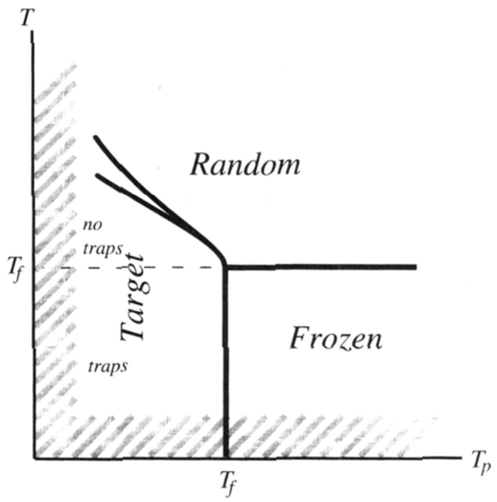

Figure 3. Phase diagram for designed heteropolymers. There are three phases: (1) *random globule*, in which a vast number of conformations (folds) are allowed for the chain in the equilibrium; (2) *frozen globule*, in which only a few conformations or even one conformation is allowed; (3) *target globule*, in which the designed conformation (★) is the only allowed one. The boundary between the target and random globule phases is given by an upper and lower line corresponding to the series expansion around the triple point eq 41 and the MJ matrix, respectively. Note that the target globule phase region of the phase diagram can be divided into two parts: the target conformation is the most stable state in both, but a few of the other random conformations may be thermodynamically either metastable, thus serving as *traps* in kinetics, or unstable *without traps*. Lines at low T and T_p represent the areas of inapplicability of the theory.

$$\langle\vec{p}|\xi\hat{B}(\hat{I} + \xi_p\hat{\Delta}\hat{B})^{-1} - \xi\hat{B}(\hat{I} + \xi\hat{\Delta}\hat{B})^{-1}|\vec{p}\rangle + \langle\vec{p}|\xi_f^2\hat{B}\hat{\Delta}\hat{B}(\hat{I} + \xi_f\hat{\Delta}\hat{B})^{-2} - \xi_p\xi\hat{B}\hat{\Delta}\hat{B}(\hat{I} + \xi_p\hat{\Delta}\hat{B})^{-2}|\vec{p}\rangle = \mathrm{Tr}\{\xi_f\hat{\Delta}\hat{B}(\hat{I} + \xi_f\hat{\Delta}\hat{B})^{-1} - \xi\hat{\Delta}\hat{B}(\hat{I} + \xi_p\hat{\Delta}\hat{B})^{-1} + \ln[(\hat{I} + \xi\hat{\Delta}\hat{B})(\hat{I} + \xi_f\hat{\Delta}\hat{B})^{-1}]\} \quad (27)$$

at $T > T_f$, where

$$\xi = 2\varrho/T; \qquad \xi_f = 2\varrho/T_f; \qquad \xi_p = 2\varrho/T_p \quad (28)$$

and

$$\langle\vec{p}|\xi_f\hat{B}(\hat{I} + \xi_p\hat{\Delta}\hat{B})^{-1} - \xi_f\hat{B}(\hat{I} + \xi_f\hat{\Delta}\hat{B})^{-1}|\vec{p}\rangle + \langle\vec{p}|\xi_f^2\hat{B}\hat{\Delta}\hat{B}(\hat{I} + \xi_f\hat{\Delta}\hat{B})^{-2} - \xi_p\xi_f\hat{B}\hat{\Delta}\hat{B}(\hat{I} + \xi_p\hat{\Delta}\hat{B})^{-2}|\vec{p}\rangle = \mathrm{Tr}\{\xi_f\hat{\Delta}\hat{B}(\hat{I} + \xi_f\hat{\Delta}\hat{B})^{-1} - \xi_f\hat{\Delta}\hat{B}(\hat{I} + \xi_p\hat{\Delta}\hat{B})^{-1}\} \quad (29)$$

at $T < T_f$.

The last equation (29) has the obvious general solution $\xi_p = \xi_f$, or $T_p = T_f$. Physically, this means that the line of transition between the frozen and target states is vertical on the phase diagram. In other words, this transition cannot be caused by acting temperature change. This is perfectly clear, because both the frozen and target states are comprised of one or a few conformations, thus having negligible entropies, and therefore their interconvertion cannot be temperature controlled.

The only part of the phase diagram remaining to be clarified is the random globule to target phase transition at $T > T_f$. We call the temperature of the transition $T_{\rm tar}$ and the transition line on the phase diagram is therefore $T_{\rm tar}(T_p)$. This is described by eq 27. In fact, the line $T_{\rm tar}(T_p)$ is the most important issue of this work, because we will speculate below that the region immediately under this line on the phase diagram (where $T_f < T < T_{\rm tar}$ and $T_p < T_f$) is the most promising for experimental realization of the target state. Thus, the elucidation of this region is very important. As eq 27 is quite cumbersome, we address some specific cases.

B. An Exactly Solvable Model: The Generalized Potts Model. The Q-Potts model of interactions assumes Q types of monomer species, with interaction energy between similar and different monomers of b and 0, respectively. On the other hand, the p-charge model, suggested in ref 5 and studied in refs 6 and 7, models the presence of p different physical short-range interactions (an abstraction of Coulomb, van der Waals, hydrophobic, etc. interactions in real chemical systems). Each monomer is depicted in this model with a set of p generalized "charges", each taking one of two possible values, say 0 or 1.

We introduce a generalized Potts model, which generalizes both the $Q-$Potts and p-charge models. In this model, each monomer has p different charges, $s^1,...,s^k,...,s^p$, and we allow the values of each charge s^k to range from 0 to $Q - 1$. Furthermore, we define the interaction between charges of monomers I and J to be of Potts form: if the value of the charges s_I^k and s_J^k are the same, then the interaction energy is b_k; otherwise it is zero. The total interaction energy between monomers I and J is given as the sum of the interaction energy of the charges of the monomers.

The Hamiltonian is therefore defined to be

$$\mathscr{H} = \sum_{I,J}^{N} \delta(\mathbf{r}_I - \mathbf{r}_J) \sum_{k=1}^{p} b_k \delta(s_I^{\,k}, s_J^{\,k}) + \mathscr{H}' \tag{30}$$

where s_I^k is the value of the charge k of monomer I. In the interaction matrix, we define each possible combination of charges as a different species. Thus, there are $q = \prod_{k=1}^{p} Q_k$ species in the interaction matrix. For species number i $(1 \le i \le q)$, the value of charge k $(0 \le k < p)$ is given by $s^k(i) = \lfloor i/(Q_k)^k \rfloor \bmod Q_k$, where $\lfloor ... \rfloor$ means truncate to the lowest integer and $a \bmod b = a - b\lfloor a/b \rfloor$.

Thus, we have an interaction matrix of the form

$$\hat{B}_{ij} = \sum_k b_k \delta\left(\left\lfloor \frac{i}{2^k} \right\rfloor \bmod Q_k, \left\lfloor \frac{j}{2^k} \right\rfloor \bmod Q_k\right) \tag{31}$$

Note that the $q-$Potts model is recovered for $p = 1$, the p-charge model is recovered for $Q_k = 2$, and the Ising model is recovered for $p = 1$ *and* $Q_k = 2$.

For simplicity, we consider the case of an even population of all monomer species, i.e., $p_i = 1/q$. In this case, the $\hat{\Delta}\hat{B}$ matrix has a $(q - p - \sum_k^p Q_k)$-degenerate eigenvalue of 0 and for each $k \in 0,1,...,p - 1$, a $(Q_k - 1)$-degenerate eigenvalue of b_k/Q_k. The energy terms of (23) involving determinants can be simplified by

$$\ln \det\left(\hat{I} + \frac{2\varrho a}{T}\hat{\Delta}\hat{B}\right) = \sum_k^p (Q_k - 1) \ln\left(1 + \frac{2b_k\varrho_a}{Q_k T}\right) \tag{32}$$

where a is some constant (either x or $y + T/T_p$). The other energy terms are drastically simplified since

$$\left\langle \vec{p} \left| \frac{\varrho}{T}\hat{B}\left(\hat{I} + \frac{2\varrho a}{T}\hat{\Delta}\hat{B}\right)^{-1} \right| \vec{p} \right\rangle = \left\langle \vec{p} \left| \frac{\varrho}{T}\hat{B} \right| \vec{p} \right\rangle \tag{33}$$

Thus, the freezing temperature is determined by

$$\sum_k^p \left[\ln\left(1 + \frac{2b_k\varrho}{T_f}\right) - \frac{2b_k\varrho/T_f}{1 + 2b_k\varrho/T_f} - \frac{2s}{p(Q_k - 1)} \right] = 0 \tag{34}$$

For the specific case $b_k = b$ and $Q_k = Q$, we have

$$T_f = -\frac{2\varrho b}{Q\Xi[2s/p(Q - 1)]} \tag{35}$$

where $\Xi(\sigma)$ is given self-consistently by

$$\Xi(\sigma):\quad \sigma = \ln(1 - \Xi) + \Xi/(1 - \Xi) \simeq \left\{ \begin{matrix} \Xi^2/2 & \text{for } \Xi \gg 1 \\ 1/(1 - \Xi) & \text{for } \Xi \to 1 \end{matrix} \right\} \tag{36}$$

The freezing temperature for the two asymptotics in flexibility are

$$T_f \simeq \left\{ \begin{matrix} -(\varrho b/Q)(p(Q-1)/s)^{1/2} & \text{for effectively flexible chain, } s/p(Q-1) \ll 1 \\ -(2\varrho b/Q)[1+(Q-1)p/2s] & \text{for effectively stiff chain, } s/p(Q-1) \gg 1 \end{matrix} \right\} \tag{37}$$

Note that the validity of these asymptotics is determined by the *effective* flexibility $\sigma = 2s/p(Q - 1)$. In particular, the regime which we call the "flexible-chain limit" (first line of (37)) is valid for even rather stiff polymers (e.g., $s > 1$) if it is has sufficient diversity of monomer species ($pQ \gg 1$). Also note that in the limits $Q_k = 2$ and $p = 1$ we recover the results previously derived for the p-charge and $Q-$Potts models.[7]

For the target transition, we have the relation

$$\sum_k^p \left[\sigma^{(k)} - \frac{\Xi_t^{(k)}}{1 - \Xi_p^{(k)}} - \ln(1 - \Xi_t^{(k)}) \right] = 0 \tag{38}$$

where $\sigma^{(k)} = 2s/p(Q_k - 1)$, $\Xi_t^{(k)} = -2b_k\varrho/Q_kT_{\rm tar}$, and $\Xi_p^{(k)} = -2b_k\varrho/Q_kT_p$. For the specific case $b_k = b$ and $Q_k = Q$, we have

$$T_p = \left\{ \begin{matrix} \dfrac{2b\varrho}{Q}\left[1 - \dfrac{2b\varrho}{QT_{\rm tar}}\left(\ln\left[\dfrac{1-2b\varrho/QT_f}{1-2b\varrho/QT_{\rm tar}}\right] + \dfrac{2b\varrho/QT_f}{1-2b\varrho/QT_f}\right)^{-1}\right]^{-1} & \text{when } T_{\rm tar} > T_f \\ T_f & \text{otherwise} \end{matrix} \right. \tag{39}$$

This equation is of the same form as was derived in ref 12 for the two-letter Potts polymer, except the value of the interaction constant B (from the two-letter Hamiltonian $\mathscr{H}_2 = B\sum_{IJ} s_I s_J \delta[\mathbf{r}_I - \mathbf{r}_J]$) is replaced by b/Q. Note that there is no p dependence other than through the freezing temperature.

C. Expansion around the Triple Point. To find the behavior for an arbitrary interaction matrix, we trade exact solvability for generality by performing an expansion in the vicinity of the $T_{\rm tar} = T_f$, $T_p = T_f$ point. We find

$$\frac{T_{\mathrm{tar}} - T_f}{T_f} \simeq \left(\frac{T_f - T_p}{T_f}\right)^{1/2} + \kappa\frac{T_f - T_p}{T_f} \qquad (40)$$

Note that the first (square root) term of this expansion is universal, as neither $\hat{B}$ nor $\vec{p}$ enters into it, other than through the value of T_f. The properties of a particular polymer, such as $\hat{B}$ and $\vec{p}$, determine the slope κ of the second (linear) term. In general form, the slope κ is given by

$$\kappa = 1 - \frac{4}{3}\xi_f\frac{\mathrm{Tr}[(\hat{\Delta}\hat{B})^3[\hat{I}+\xi_f\hat{\Delta}\hat{B}]^{-3}]+3\langle\vec{p}|\hat{B}(\hat{\Delta}\hat{B})^2[\hat{I} + \xi_f\hat{\Delta}\hat{B}]^{-4}|\vec{p}\rangle}{\mathrm{Tr}[(\hat{\Delta}\hat{B})^2[\hat{I}+\xi_f\hat{\Delta}\hat{B}]^{-2}]+2\langle\vec{p}|\hat{B}\hat{\Delta}\hat{B}[\hat{I}+\xi_f\hat{\Delta}\hat{B}]^{-3}|\vec{p}\rangle} \qquad (41)$$

This expression is rather cumbersome, but will be simplified below while considering the limiting cases of high and low flexibility.

Clearly, the expansion (40) is well applicable close to the triple point, such that $(T_f - T_p)/T_f < 1/\kappa^{1/2}$.

D. Flexible-Chain limit. As it was shown in ref 7, we can expand (25) by powers of ξ, which leads to the equation for the freezing temperature for random sequences in the form

$$2s = \sum_{k=2}^{\infty} \xi_f^k\langle B^k\rangle_m \qquad (42)$$

where "moments" of the matrix are defined as

$$\langle B^k\rangle_m = \frac{k-1}{k}\mathrm{Tr}[(-\hat{\Delta}\hat{B})^k] - (k-1)\langle\vec{p}|\hat{B}(-\hat{\Delta}\hat{B})^{k-1}|\vec{p}\rangle \qquad (43)$$

Recall that these moments do not depend on any constant (homopolymeric) contributions to the interactions: the moments are the same for B_{ij} and any $B_{ij} + B_0$. In particular, we can subtract the mean interaction defining

$$b_{ij} \equiv B_{ij} - \langle B\rangle, \qquad \langle B\rangle = \sum_{ij} p_i p_j B_{ij} \qquad (44)$$

and write definition (43) in terms of $\hat{b}$ by simply substituting $\hat{b}$ instead of $\hat{B}$.

If the chain is flexible and s is small enough, we can neglect all the terms but the first one, yielding[7]

$$T_f = \varrho\left(\frac{2}{s}\langle B^2\rangle_m\right)^{1/2}; \qquad \langle B^2\rangle_m \equiv \sum_{ij} p_i p_j b_{ij}^{\,2} \equiv \langle(B - \langle B\rangle)^2\rangle \qquad (45)$$

Note that $\langle B^2\rangle_m$ is simply the variance of the *elements* of the $\hat{B}$ matrix, irrespective of their position in the matrix, and thus T_f for flexible chains is defined mainly by the overall heterogeneity of the interaction matrix.[7]

If we apply the same expansion for the target phase transition (27), we get

$$\sum_{k=2}^{\infty}\langle B^k\rangle_m\xi_f^{\,k}\left[1 + \frac{1}{k-1}\left(\frac{\xi}{\xi_f}\right)^k - \frac{k}{k-1}\left(\frac{\xi}{\xi_f}\right)\left(\frac{\xi_p}{\xi_f}\right)^{k-1}\right] = 0 \qquad (46)$$

The simplest approximation for the flexible chain, similar to eq 45, means truncation of the series of the first nonvanishing term:

$$\xi_f^{\,2} + \xi^2 - 2\xi\xi_p = 0 \quad \text{or} \quad \frac{T_{\mathrm{tar}}}{T_f} = \frac{T_f}{T_p}\left[1 + \left(1 - \left(\frac{T_p}{T_f}\right)^2\right)^{1/2}\right] \qquad (47)$$

Note that in this approximation neither of the properties of the particular polymer, such as $\hat{B}$ and $\vec{p}$, enter into the shape of the transition line (47), except for the freezing temperature, T_f, and, therefore, except for the overall heterogeneity of the interactions.

More delicate properties of the interactions are seen to become important for not so flexible chains, i.e., in the next approximation with respect to s. In particular, already to second to lowest order, we find freezing temperature in the form

$$T_f = \varrho\left(\frac{2}{s}\langle B^2\rangle_m\right)^{1/2}\left[1 + \left(\frac{s}{2}\right)^{1/2}\frac{\langle B^3\rangle_m}{\langle B^2\rangle_m^{\,3/2}}\right] \qquad (48)$$

and, for the target transition in the vicinity of the triple point, we get (40) with the slope

$$\kappa = 1 + (2s)^{1/2}\frac{\langle B^3\rangle_m}{\langle B^2\rangle_m^{\,3/2}} \qquad (49)$$

where third moment of the interaction matrix

$$\langle B^3\rangle_m \equiv \sum_{ijk} p_i b_{ij} p_j b_{jk} p_k b_{ki} \qquad (50)$$

unlike the second one, is determined by the matrix arrangement of the elements B_{ij} and not only by their overall heterogeneity. In other words, correlations become important between interaction energies of a given monomer species to different other species.

E. Stiff-Chain Limit. For stiff chains, when s is large, the main contribution in (25) comes from divergence of the $[\hat{I} + \xi_f\hat{\Delta}\hat{B}]^{-1}$ term, which is governed by the largest eigenvalue of the $(-\hat{\Delta}\hat{B})$ operator. We call this eigenvalue and the corresponding eigenvector λ and $|\vec{\psi}\rangle$, respectively. It was shown in ref 7 that in this case the freezing transition temperature is controlled by the most attractive "mixture" of monomers (represented by $|\vec{\psi}\rangle$), where "mixing" is understood in the sense similar to quantum mechanics. This transforms (25) into

$$2s \simeq \frac{c}{(1 - \xi_f\lambda)^2} + \frac{1}{1 - \xi_f\lambda}; \qquad c = \langle\vec{p}|\vec{\psi}\rangle\langle\vec{\psi}| - \hat{B}/\lambda|\vec{p}\rangle \qquad (51)$$

where we kept the second (less divergent) term because the numerator of the first one vanishes for many particular cases with some regularities in the $\hat{B}$ matrix, such as, for example, in the Potts model, the p-charge model, and some others. In the main approximation, (51) yields $\xi_f \simeq 1/\lambda$. More accurately, we obtain

$$\xi_f \simeq \begin{cases}(1/\lambda)[1 - (c/2s)^{1/2}] & \text{for } c \neq 0\\ (1/\lambda)[1 - (1/2s)] & \text{for } c = 0\end{cases} \qquad (52)$$

A similar approach can be applied for the target transition, i.e., for eqs 27 and 41. In particular, for the vicinity of the triple point we get

$$\kappa \simeq \begin{cases}(8s/c)^{1/2} & \text{for } c \neq 0\\ 8s/3 & \text{for } c = 0\end{cases} \qquad (53)$$

In both cases, κ is rather large for stiff chains, and thus the region of the vicinity of the triple point (in the sense of applicability of the regime (40)) is small ($\sim 1/\kappa^{1/2}$). Outside this region, we can analyze the most singular terms of (27), yielding $T_{tar} \simeq T_p[(1 - \xi_p\lambda)/(1 - \xi_f\lambda)]^2$, which is almost a vertical line on the phase diagram.

F. Miyazawa–Jernigan Matrix. It is of special interest to consider the imprinting phase diagram for polymers comprised of amino acids. An interaction matrix for amino acids was derived in ref 14 based upon protein statistics. The phase diagram for imprinted sequences, numerically calculated for the Miyazawa–Jernigan (MJ) matrix, is shown in Figure 3.

It is instructive to see the significance of the particular placement of the matrix elements in the interaction matrix $\hat{B}$, which enter the higher order moments, such as $\langle B^3 \rangle_m$, and thus govern, in particular, the slope of the target transition curve near the triple point (40). To illustrate this, we also examined the curve $T_{\mathrm{tar}}(T_p)$ for an artificial interaction matrix consisting of the elements of the MJ matrix in a random (but still symmetric) arrangement. This randomized version of the MJ matrix leads to a smaller region of target phase (i.e., $T_f < T < T_{\mathrm{tar}}$ and $T_p < T_f$) above T_f.

IV. Conclusion

In general, the goal of this work was to examine the effect of a particular type of monomer species interaction on the nature of the freezing phase transformation to the target conformation and, in particular, to study whether such a transformation exists for all heteropolymers. To this end, we wrote an interaction Hamiltonian which assumed only that heterogeneity comes from two-body interactions and interactions are short range: an arbitrary matrix of monomer-species interactions was considered. We were able to calculate the heteropolymeric properties of the freezing and target transition temperatures explicitly in terms of the interaction matrix. Thus, the freezing properties of *any* heteropolymer model in which heterogeneity comes solely from binary interactions of monomers can be solved using our formalism, simply by determining the interaction matrix between species and examining the properties of this matrix.

A polymer is considered a heteropolymer if it is composed of differing monomeric species, mathematically expressed by $\hat{\Delta B} \neq \hat{0}$. All interaction matricies of this form lead to a finite freezing temperature for random and designed sequences. Thus, the particular details of the interaction matrix are vital to neither the *existence* of the freezing and target transformations nor the qualitative aspects of the phase diagram (Figure 3).

Moreover, in addition to the aspects common to all heteropolymeric interaction matricies, we can address which region of the phase diagram is the most promising from the standpoint of experimental implementation of the imprinting model. We find that the region of target phase between the freezing and target transitions ($T_f < T < T_{\mathrm{tar}}$ and $T_p < T_f$) is the optimal region. Indeed, for $T > T_{\mathrm{tar}}$, there is no unique structure. On the other hand, at $T_p > T_f$, i.e., when the sequences are almost random, the chain freezes to some state which generally has nothing in common with the target conformation. Finally, for $T < T_f$, some conformations other than target conformation ★ become thermodynamically stable as well, since the designed sequence acts much like a random sequence in a conformation other than target conformation ★. Indeed, thermodynamic stability or metastability of some conformation means that some additive (proportional to N) energy is needed to leave that state once the system is there. Therefore, the kinetic self-assembly of the target conformation, even though it is thermodynamically stable, is very problematic at $T < T_f$. These arguments are equivalent to the recently formulated criteria of reliable folding kinetics in terms of the gap in the spectrum of energies of the heteropolymer chain.[18]

Thus, the region of the phase diagram immediately below the target transition line, but above the freezing temperature, is very important because the equilibrium conformation is the designed conformation ★ and folding to ★ is fast and reliable. Moreover, we can conclude that the design of an experiment should probably include the choice of set of monomers which interact in such a way as to maximize the width of this region on the phase diagram. Also, it is vital to polymerize a dense monomer mixture; therefore, perhaps certain exotic polymerization schemes such as emulsion polymerization should be employed.

Finally, we address the applicability of our theory. First, since we have truncated the series (13) to $\mathcal{O}(\phi^2)$, we cannot describe any physical properties of the system due to phase transitions in the average value of ϕ, such as phase separation of the monomers. However, these transitions are not found in all interaction matricies; for example, they are present in ferromagnetic interaction matricies ($B_{ij} = -\delta_{ij}$)[4,19] but absent in antiferromagnetic interaction matrices ($B_{ij} = \delta_{ij}$). Second, we have assumed that all interactions are short range in space. For long-range Coulomb interactions, one must also consider the screening due to counterions and polyions. For systems with a large degree of screening (large concentration of ions), the characteristic length of interactions is short and we recover δ-function-like short-range interactions. For a small degree of screening, the interactions cannot discriminate between the placement of polyions within the characteristic length of interactions (Debye length), and thus there is no possibility for freezing to the microscopic length scale, which is the region of interest of this paper. A possible case of interest would be the freezing of a polymer which consists of monomers which interact with short-range as well as long-range interactions; however, this is currently beyond the scope of this paper. Third, although we have not explicitly included the effects due to the solution, solvent effects can be included by the appropriate redefinition of the interaction matrix.[14]

In conclusion, the fact that the specific nature of the interaction matrix is not vital to the existence of the target transition may help experimentalists in the implementation of the imprinting procedure, as one may ignore the details of the interactions chosen. It is also interesting to consider the imprinting model as a possible scheme of prebiotic evolution: monomers polymerize in a conformation capable of recognizing a given target molecule. Therefore, the diminished role of the specific form of the interaction matrix may also have helped the development of prebiotic evolution.

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Appendix A: Rotation of Replica Space

In order to simplify both terms in the energy (19), we perform the following matrix operations. Consider the structure of $\hat{\mathcal{Q}}^{(n+1)} \equiv \hat{q}^{(n+1)}(\hat{\mathcal{T}}^{(n+1)})^{-1}$. It is a block diagonal matrix. We can label these blocks: the target block of size $(y + 1) \times (y + 1)$ is called block 0 and the g blocks of size $x \times x$ are labeled from 1 to g, where $g = (n - y)/x$; we will employ the convention that capital Greek letters label replica blocks. Consider the operators $\hat{\mathcal{R}}_\Gamma^{(b)}$ which diagonalize the Γ block of $\hat{\mathcal{Q}}^{(n+1)}$; i.e., $\hat{\mathcal{R}}_\Gamma^{(b)}\hat{\mathcal{Q}}_\Gamma^{(b)}(\hat{\mathcal{R}}_\Gamma^{(b)})^{-1} \equiv \hat{\Lambda}_\Gamma^{(b)}$ is a diagonal matrix, where b is the dimensionality of the Γ block.

We then define the block matrix in replica space $\hat{\mathcal{R}}^{(n+1)}$ as the diagonal block matrix whose g diagonal blocks are $\hat{\mathcal{R}}_\Gamma^{(b)}$, $\Gamma = 0, ..., g$; finally, we extend this operator into species space by $\hat{\mathcal{R}}^{((n+1)q)} = \hat{\mathcal{R}}^{(n+1)} \otimes \hat{I}^{(q)}$. Thus, the $\hat{\mathcal{R}}^{((n+1)q)}$ operator diagonalizes the block matrix $\hat{\mathcal{Q}}^{(n+1)} \otimes \hat{A}^{(q)}$ in replica space, while rendering the species dimensions $\hat{A}^{(q)}$ unchanged. Thus,

$$(\hat{\mathcal{R}}^{((n+1)q)})^{-1}[\varrho\hat{\mathcal{Q}}^{(n+1)} \otimes \hat{\Delta}^{(q)}\hat{B}^{(q)}]\hat{\mathcal{R}}^{((n+1)q)} = \varrho\hat{\Lambda}^{(n+1)} \otimes \hat{\Delta}^{(q)}\hat{B}^{(q)} \quad \text{(A1)}$$

The eigenvalues $\Lambda_{\alpha\alpha}$ and eigenvectors of the $\hat{\mathcal{Q}}^{(n+1)}$ matrix can be calculated by elementary means.[20,21] For the $x \times x$ nontarget blocks $\hat{\mathcal{Q}}_\Gamma^{(n+1)}$, where $0 > \Gamma \geq g$, we have an $(x - 1)$-degenerate eigenvalue $\lambda = 0$ and a single nonzero eigenvalue $\lambda = x$. For the $(y + 1) \times (y + 1)$ target block $\hat{\mathcal{Q}}_0^{(n+1)}$, we have a y-degenerate eigenvalue $\lambda = 0$ and a nondegenerate eigenvalue $\lambda = y + \tau_p$, where $\tau_p = T/T_p$. Thus, for the whole $\hat{\mathcal{Q}}^{(n+1)}$ matrix we have a $[y + (n - y)(x - 1)/x]$-degenerate eigenvalue $\lambda = 0$, a $[(n - y)/x]$-degenerate nonzero eigenvalue $\lambda = x$, and a nondegenerate eigenvalue $\lambda = y + T/T_p$. Thus, we have

$$\ln \det\left[\hat{I}^{(q(n+1))} + \frac{\varrho}{T}\hat{\mathcal{Q}}^{(n+1)} \otimes \hat{\Delta}^{(q)}\hat{B}^{(q)}\right] = \ln \det\left[\hat{I}^{(q)} + (y + \tau_p)\frac{\varrho}{T}\hat{\Delta}^{(q)}\hat{B}^{(q)}\right] + \frac{n - y}{x} \ln \det\left[\hat{I}^{(q)} + x\frac{\varrho}{T}\hat{\Delta}^{(q)}\hat{B}^{(q)}\right] \quad \text{(A2)}$$

As for the second term in (19), we again use the technique of rotation in replica space in order to bring this term into block diagonal form. For each block, we have a complete set of orthonormal eigenvectors of the form

$$\hat{R}_{\alpha\beta}^{(y)} = \begin{cases} \exp[(2\pi i/z)(\alpha - 1)(\beta - 1)]z^{-1/2} & \text{for } \alpha,\beta > 0 \\ 0 & \text{for } \alpha = 0, \beta > 0 \\ \tau_p(\tau_p^{\,2} + z)^{-1/2} & \text{for } \alpha = \beta = 0 \\ (\tau_p^{\,2} + z)^{-1/2} & \text{for } \alpha > 0, \beta = 0 \end{cases} \quad \text{(A3)}$$

where $z = x$ and $0 < \alpha,\beta \leq x$ for the nontarget block, and $z = y$ and $0 \leq \alpha,\beta \leq y$ for the target block.

Using these eigenvectors, we get the second term in eq (20):

$$\left\langle \vec{p}^{(n+1)}\hat{\mathcal{R}}^{(n+1)}\right|\frac{\varrho}{T}(\hat{\mathcal{T}}^{(n+1)})^{-1} \otimes \hat{B}^{(q)}\left[\hat{I}^{((n+1)q)} + \frac{\varrho}{T}\hat{\Lambda}^{(n+1)} \otimes \hat{\Delta}^{(q)}\hat{B}^{(q)}\right]^{-1}\left|(\hat{\mathcal{R}}^{(n+1)})^{-1}\vec{p}^{(n+1)}\right\rangle^{(n+1)} = (y + \tau_p)\frac{\varrho}{T}\hat{B}^{(q)}\left[\hat{I}^{(q)} + (y + \tau_p)\frac{\varrho}{T}\hat{\Delta}^{(q)}\hat{B}^{(q)}\right]^{-1} + (n - y)\frac{\varrho}{T}\hat{B}^{(q)}\left[\hat{I}^{(q)} + \frac{\varrho x}{T}\hat{\Delta}^{(q)}\hat{B}^{(q)}\right]^{-1} \quad \text{(A4)}$$

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(20) For an $n \times n$ matrix M_n with diagonal elements $\tilde{m}$ and off-diagonal elements m, we have an $(n - 1)$-fold degenerate eigenvalue $\lambda = \tilde{m} - m$ and a nondegenerate eigenvalue of $\lambda = \tilde{m} + (n - 1)m$. Its eigenvectors are of the form $R_{\alpha\beta} = \exp[(2\pi i/n)(\alpha - 1)(\beta - 1)]n^{-1/2}$. The inverse of M_n has diagonal elements $\det M_{n-1}/\det M_n = 1/(\tilde{m} - m)$ and off-diagonal elements $-m(\tilde{m} - m)^{n-2}/\det M_n = -m/\{(\tilde{m} - m)[\tilde{m} + (n - 1)m]\}$.

(21) Consider an $n \times n$ matrix $\hat{M}$ which has one column with elements $M_{i1} = a$ and all other elements $M_{ij} = 1, j > 1$. $\hat{M}$ has an $(n - 1)$-degenerate eigenvalue $\lambda = 0$, a nondegenerate eigenvalue $\lambda = (n - 1) + a$, and eigenvectors $(-1/a, 1, 0, ..., 0), (-1/a, 0, 1, ..., 0), ..., (-1/a, 0, 0, ..., 1)$, and $(1, 1, 1, ..., 1)$.

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