Analysis of stability of macromolecular clusters in dilute heteropolymer solutions

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Abstract

We study the formation of clusters consisting of several chains in dilute solutions of amphiphilic heteropolymers. By means of the Gaussian variational theory we show that in a region of the phase diagram within the conventional two–phase coexistence region mesoglobules of equal size possess the lowest free energy. Monte Carlo simulation confirms that the mesoglobules are stabilised due to micro–phase separation, which introduces a preferred length scale. The very existence of such mesoscopic structures is related to a delicate balance of the energetic and entropic terms under the connectivity constraints. The issue of size monodispersity and fluctuations for mesoglobules is investigated.

I. INTRODUCTION

Conformational transitions in polymer solutions have been the subject of extensive studies for many years¹. In general, these are rather complex systems with competing interactions at different ranges with entropic contributions being equally significant. The main progress has been made in investigating the equilibrium issues of the fundamental and simplest case

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of homopolymer solution. The classical Flory–Huggins theory was further improved based on the scaling theory, the self–consistent treatment in terms of the density variables and the Lifshitz theory. There is also considerable experimental data available on the phase diagrams of model systems such as polystyrene in cyclohexane or benzene and poly-N-isopropylacrylamide (PNIPAM) in water.

However, the limit of very dilute solution appears more difficult for experimental study. There phenomena of polymer collapse and aggregation can go hand by hand, leading to a diverse range of theoretical interpretations, particularly as it may be hard to separate purely equilibrium issues from the kinetic ones (see e.g. discussion in Refs. 6,7). In recent attempts to resolve the controversy a considerable theoretical effort has been directed to understanding the collapse kinetics of a single homopolymer. The necklace mechanism and some attendant kinetic laws of the collapse transition have been obtained from the Gaussian self–consistent (GSC) method, supported in part by Monte Carlo simulations, and recently reproduced using a different analytical approach in Ref. 14.

Although block and random heteropolymers have been traditionally attracting a great deal of interest as they can exhibit ordered micro–phase separated and disordered glassy phases, their understanding is essentially limited to melts, and solutions at high concentrations (see e.g. Refs. 17,18 and references therein). It appears that the latter limitation is too restrictive for explaining some recent experimental findings. In experiments on PNIPAM copolymers with small number of ionomers in aqueous solution it has been observed that these polymers can form stable nanoparticles instead of simply aggregating on heating above the lower critical solution temperature (LCST). Such an unusual type of mesoscopic aggregates with extremely monodispersed size distribution, which we called mesoglobules, has been also reported in Ref. 21 for the homopolymer PNIPAM, in which case these structures are rather long–lived, if not truly stable.

Clearly, the appearance of such metastable structures in homopolymer solutions cannot be envisaged in the framework of conventional Flory–Huggins type theories. Thus, in Ref. 22 we have extended the GSC method to multiple chains and argued the possibility of
mesoglobules in dilute solution from thermodynamic considerations. Although the standard Flory–Huggins theory can be indeed derived from the GSC method in the thermodynamic limit in some approximation, that approximation is not reliable at low concentrations. In Ref. 23 we have also mentioned the possibility that the micro–phase separation can additionally stabilise the mesoglobules in heteropolymers, which as we have consequently learned from work Ref. 20 by Qiu et al., can take place.

In this paper, based on the success of the extended method of Ref. 24 for studying the equilibrium and kinetics of single heteropolymers chains, we would like to support that our conjecture by a direct numerical evidence. Thus, it is now possible to consider several sufficiently short heteropolymer chains of a given composition in a box of finite volume $V$ and to analyse the values of the Helmholtz free energy $\mathcal{A}$ on all its possible local minima. We note that stationary points of the GSC equations are exactly the extrema conditions for the variational free energy $\mathcal{A}$ obtained from the Gibbs–Bogoliubov principle with a quadratic trial Hamiltonian.

Traditional approach to describing phase coexistence within mean–field treatment relies on the following procedure. First, one has to obtain the dependence of the specific free energy $a = \lim \mathcal{A}/K$ (or possibly other equivalent thermodynamic potential) on the concentration $c = K/V$ (or chemical potential) in the thermodynamic limit when $V \to \infty$ and the number of particles $K$ diverges such that $c$ remains finite. Second, one has to construct the convex hull of the function $a[c]$ by applying the Maxwell construction. Within the two phase separation region, where a straight line joining the two free energy minima is drawn, the pure equilibrium states are not stable with respect to fluctuations as indicated by the wrong sign of $\partial^2 a/\partial c^2$. The theoretical argument that establishes the stability of the mixed two–phase state in this case relies heavily on thermodynamic additivity properties and that one can neglect the surface contribution of the interface between the two phases.

Polymers, however, are pretty much finite, though normally long, chains. Due to the connectivity of each chain there is no well defined interface between the high and low density phases, and, moreover, the surface entropic contribution does seem significant too. These
factors should be carefully accounted for while trying to study possible metastable states in polymer solutions. For this end, we shall consider the system of a finite number of finite length polymer chains, for which one cannot immediately apply the Maxwell construction. However, in this context the phase coexistence still means that the single–phase pure states are thermodynamically unstable. We can expect here that there are additional metastable states present at the same values of thermodynamic parameters and that strong fluctuations can bring the system from one of those states to another. Thus, the equilibrium for a finite system should be a mixed state including a large number of local free energy minima, instead of just two states corresponding to the high and low density as in the thermodynamic limit. On increasing the system size we arrive at a view of the polymer precipitate as consisting of many various sized aggregates coexisting with a few single globules. This picture certainly looks more adequate than the oversimplified mean–field inspired view of the phase coexistence between the two states of one large macroglobule and a gas of single globules.

These types of problems have been extensively studied for ternary mixtures of two immiscible liquids (water and oil) and a surfactant (e.g. diblock copolymer) (see e.g. Refs. 25–27), where metastable micelles (water in oil or oil in water surrounded by surfactant chains) are observed among many other more sophisticated favourable geometrical arrangements.

In the present work we are interested in conformational structures formed by amphiphilic (hydrophobic–hydrophilic) copolymers in fairly dilute solutions without presence of any third component. In this case, hydrophobic units would tend to escape the unfavourable contacts with the solvent, but the connectivity of each chain seriously restricts their freedom. Thus, only micro–phase separation of both types of units within the polymer globule is possible. For diblock copolymers we may expect micellar globules formed with a hydrophilic shell and hydrophobic core. At higher concentrations distinct chains may associate with each other resulting in larger micelles consisting of several chains and the repulsive shells of these can stop further aggregation. What type of structures is possible for more complicated heteropolymer sequences is not really clear as the connectivity constraints would not allow
the formation of a purely hydrophilic shell.

For heteropolymers, which possess essential heterogeneity along the chain, the situation seems even more complicated. Due to the competing hydrophobic and hydrophilic interactions the free energy profile is very rugged. Thus, here some new global minima may appear due to a specific compensation of the interactions and the entropy.

Our studies based on the Gaussian variational method here are also supported by direct Monte Carlo simulation, which allows us to visually observe the system conformations and to obtain additional insights into the problem. As kinetics after a quench to the phase separation region is very difficult to describe reliably by the Monte Carlo method, this work deals exclusively with the equilibrium and metastable states.

II. THEORETICAL MODEL AND THE GAUSSIAN VARIATIONAL METHOD

In this section we describe the model for any number of arbitrary heteropolymers in solution, and introduce the Gaussian variational method in the form derived by us in Ref. 24. There we have noted that the resulting equations are in fact covariant, i.e. their form does not depend on the structure of the connectivity matrix between monomers. In case of multiple chains, however, we should also include some kind of a box, which keeps polymers from diffusing away to infinity. This is done as in Ref. 22 by introducing a “soft” cut-off via weak “springs” connecting the centre–of–mass of each chain with the centre–of–mass of the whole system.

Let us denote by \( X^a_n \) the coordinates of the \( n \)-th monomer in the \( a \)-th chain, and to introduce multi–indices \( A = (a, n) \) and so on. Henceforth \( N \) and \( M \) will be the number of monomers in a chain and the total number of chains respectively. The effective Hamiltonian, \( H \), after exclusion of the solvent degrees of freedom is given by,

\[
H = \frac{k_B T}{2L^2} \sum_a (Y^a - \bar{Y})^2 + \frac{k_B T}{2l^2} \sum_{a,n} \left( X^a_n - X^a_{n-1} \right)^2 + \sum_{J \geq 2} \sum_{\{A\}} \sum_{i=1}^{J-1} u_{\{J\}} \prod_{i=1}^{J-1} \delta(X_{A_{i+1}} - X_{A_i}), \tag{1}
\]

where \( L \) is the box size as in Ref. 22, \( Y^a = (1/N) \sum_n X^a_n \) and \( \bar{Y} = (1/M) \sum_a Y^a \) are
the coordinates of the centre–of–mass of a chain and the total system respectively, \( l \) is the statistical segment length, and \( u^{(J)}_{\{ A \}} \) is the set of site–dependent virial coefficients.

The main idea of the Gaussian variational method is to use a generic quadratic form as the trial Hamiltonian,

\[
H_0 = \frac{1}{2} \sum_{A,A'} V_{AA'} X_A X_{A'}. \tag{2}
\]

It is possible to exclude the effective potentials \( V_{AA'} \) from the consideration and to obtain closed variational equations for the averages \( \langle X_A X_{A'} \rangle_0 \), or, equivalently, for the mean–squared distances between all pairs of monomers, whether connected or not,

\[
D_{AA'} \equiv \frac{1}{3} \left\langle (X_A - X_{A'})^2 \right\rangle. \tag{3}
\]

The trial free energy, \( A = \mathcal{E} - TS \), then is obtained according to the Gibbs–Bogoliubov variational principle,

\[
A = A_0 + \langle H - H_0 \rangle_0. \tag{4}
\]

where the prime means that the zero eigenvalue of the matrix is excluded from the determinant. The mean of trial Hamiltonian \( \langle H_0 \rangle_0 \) is a trivial constant and the mean energy term is given by

\[
\mathcal{E} = \frac{3k_B T}{2L^2} M \left( \mathcal{R}^2 - \sum_a \frac{\mathcal{R}^2_a}{M} \right) + \frac{3k_B T}{2l^2} \sum_{n,n-1} D_{nn-1}^a + \sum_{J=2,3} \sum_{\{ A \}} \hat{u}^{(J)}_{\{ A \}} (\det \Delta^{(J-1)})^{-3/2} + 3\hat{u}^{(3)}_{\{ A \}} \sum_{A \neq A'} D_{AA'}^{-3}, \tag{5}
\]

where we have included the volume interactions up to the three–body terms only, so that \( u^{(J)}_{\{ A \}} = 0 \) for \( J > 3 \), and for the discussion of the last term see Ref. 30. In Eq. (5) the total and partial radii of gyration are defined as follows,

\[
\mathcal{R}^2 = \frac{1}{2N^2 M^2} \sum_{AA'} D_{AA'}, \quad \mathcal{R}_a^2 = \frac{1}{2N^2} \sum_{nn'} D_{nn'}^{aa}. \tag{6}
\]
We shall use the following particular parametrisation for the matrix of the second virial coefficients,

\[
\bar{u}_{AA'}^{(2)} = \bar{u}^{(2)} + \Delta \sigma_A + \sigma_{A'}. \tag{7}
\]

This corresponds to the case of amphiphilic heteropolymers, for which monomers differ only in the monomer–solvent coupling constants. Then the mean second virial coefficient, \(\bar{u}^{(2)}\), is associated with the quality of the solvent and the parameter \(\Delta\) is called the degree of amphiphilicity of the chain. The set \(\{\sigma_n\}\) expresses the chemical composition, or the primary sequence of a heteropolymer chain. In our case the variables \(\sigma_A\) can take only two values: \(-1, 1\) corresponding to the hydrophobic ‘a’ and hydrophilic ‘b’ monomers respectively.

It is worthwhile to comment on the origin of this parametrisation linear in the composition variables. One usually proceeds from the effective Hamiltonian,

\[
H_{ms} = H_{solv}[R_\alpha] + H_{mon}[X_A] - \sum_{n,\alpha} I_A \delta(X_A - R_\alpha), \tag{8}
\]

which includes the terms describing the solvent degrees of freedom, \(R_\alpha\), the monomer degrees of freedom, and a ‘contact’ monomer–solvent interaction, characterised by the \(A\)-th monomer hydrophobic strengths, \(I_A\), respectively. A simple way of deriving such a term, proposed by Garel and Orland\cite{15}, would be then to explicitly use the solution incompressibility condition,

\[
\rho_{mon}(y) + \rho_{solv}(y) = \sum_A \delta(y - X_A) + \sum_\alpha \delta(y - R_\alpha) = \rho_0 = \text{const}, \tag{9}
\]

in order to integrate out the solvent degrees of freedom. This yields the partition function \(Z_{ms} = Z_{solv} Z\), where the effect of the solvent influence on the monomer degrees of freedom appears in \(Z\) only via the following term in the effective Hamiltonian,

\[
H = \sum_{A \neq A'} \left( u_2 + \frac{1}{2} (I_A + I_{A'}) \right) \delta(X_A - X_{A'}) + \ldots \tag{10}
\]

Now, by introducing \(\bar{u}_2 = u_2 + I\) and \(\sigma_A = I_A - I\), where \(I\) is the mean value of \(I_A\), we obtain exactly the linear term.

We note that the widely used quadratic term in the composition variables corresponds to the Edwards free energy functional constructed as the virial expansion, \(\sum_L \int d\mathbf{y} \left( \rho_p(\mathbf{y}) \right)^L\), in
terms of the pseudo-density, $\rho_p(y) = \sum_A \sigma_A \delta(X_A - y)$. This model was widely exploited\cite{ footnote:16}, and although it is clearly suitable for a mean-field theories, microscopically it corresponds to rather non-local monomer–solvent interactions. The model with the quadratic term in composition variables, often called the random ‘charge’ model, is appropriate for describing either true charges or non-local effective monomer–solvent interactions arising after coarse-graining of models with complex intra–molecular potentials. It is popular for the use in studying protein folding because proteins are too complicated to be described well by any model.

To be specific, we also choose to fix the third virial coefficient $u_{mm'm''}^{(3)} = 10 k_B T l^6$. As usual, we work in the system of units such that $l = 1$, and $k_B T = 1$.

III. LATTICE MODEL AND THE MONTE CARLO TECHNIQUE

For simulation we adopt the Monte Carlo technique in the lattice model of heteropolymers from Ref.\cite{ footnote:13}. Thus, apart from the connectivity and excluded volume constraints there are “weak” pair–wise interactions between lattice sites depending on the separation and sites contents, described by the Hamiltonian,

$$H = \frac{1}{2} \sum_{i \neq j} w(r_{ij}) I_{s_i s_j},$$  \hspace{1cm} (11)

where $i, j$ enumerate lattice sites; $s_i$ labels the contents of site $i$, $I_{s_i s_j}$ is a $3 \times 3$ symmetric matrix of monomer and solvent interactions, $r_{ij} = |\mathbf{r}_i - \mathbf{r}_j|$ is the separation between the two sites, and $w(r_{ij})$ is a function giving the shape of the potential.

The lattice model, similarly to Eq. (9), describes an incompressible solution — each site which is not occupied by a monomer contains a solvent molecule. The Hamiltonian (11) can be rewritten in the equivalent form,

$$H = \sum_r w(r) (I_{aa} C_{aa}(r) + I_{bb} C_{bb}(r) + I_{ss} C_{ss}(r) + I_{ab} C_{ab}(r) + I_{as} C_{as}(r) + I_{bs} C_{bs}(r)),$$  \hspace{1cm} (12)

where $C_{lm}(r)$ is the total number of $lm$-contacts at the $r$-th interaction range. Such a contact is defined as a pair of lattice sites at the distance $r$ occupied by ‘l’ and ‘m’ species.
In this model we include first nearest neighbours \( (w(r = 1) = 1) \), 2D and 3D diagonals \( (w(r = \sqrt{2}) = 1 \text{ and } w(r = \sqrt{3}) = 0.7) \), as well as the second nearest neighbours \( (w(r = 2) = 1/2) \), so that no higher interaction ranges are present \( (w(r > 2) = 0) \). Due to solution incompressibility numbers of both types of monomers and solvent molecules are fixed. This yields additional constraints on the number of contacts. Indeed, by considering contacts formed by ‘a’-monomers, we can write,

\[
2C_{aa}(r) + C_{ab}(r) + C_{as}(r) = C(r)N_a,
\]

where \( N_a \) is the total number of ‘a’ monomers on the lattice and the factor \( C(r) \) is the total number of contacts at the \( r \)-th interaction range per lattice site (in our case, \( C(1) = 6 \), \( C(\sqrt{2}) = 12 \), \( C(\sqrt{3}) = 8 \) and \( C(2) = 6 \)). Analogously, by considering ‘b’ and ‘s’ lattice sites we can write respectively,

\[
2C_{bb}(r) + C_{ab}(r) + C_{bs}(r) = C(r)N_b,
\]

\[
2C_{ss}(r) + C_{as}(r) + C_{bs}(r) = C(r)(L^3 - N_a - N_b).
\]

Using relations (13, 14, 15) one can totally exclude the monomer–solvent contacts from consideration similarly to Eqs. (8, 10) and rewrite the Hamiltonian (12) as follows,

\[
H = H_0 - k_B T \sum_r w(r) (\chi_{aa} C_{aa}(r) + \chi_{ab} C_{ab}(r) + \chi_{bb} C_{bb}(r)),
\]

\[
H_0 = (C/2)L^3 I_{ss} + C N_a (I_{as} - I_{ss}) + C N_b (I_{bs} - I_{ss}), \quad C = \sum_r w(r) C(r).
\]

Here we have introduced the so–called Flory interaction parameters,

\[
\chi_{aa} = \frac{2I_{sa} - I_{aa} - I_{ss}}{k_B T}, \quad \chi_{bb} = \frac{2I_{sb} - I_{bb} - I_{ss}}{k_B T}, \quad \chi_{ab} = \frac{I_{sa} + I_{sb} - I_{ab} - I_{ss}}{k_B T}.
\]

The first term in (16) is just trivial constant which does not depend on the system conformation and can be neglected. The combinations of interaction parameters in Eq. (18) describe the degree of corresponding monomer–monomer attraction and they are the only relevant thermodynamic parameters characterising interactions in the system for a given number of \( M \) polymer sequences of length \( N \) and lattice size \( L \).
Thus, as we have seen, the Hamiltonian (16) possesses a similar structure to the effective Hamiltonian in Eq. (1) from the Gaussian theory of the previous section. The minor distinction is that in the lattice model the connectivity and excluded volume constraints are explicitly implemented\textsuperscript{13}. The relation of each of the Flory parameters to the virial coefficients then can be worked out similarly to the derivation of the standard Flory–Huggins theory\textsuperscript{1}: $\chi^{(2)} \sim l^3 (\text{const} - 2 \chi_{im})$, $\chi^{(3)} \sim l^6$, $\ldots$, where $l$ is the lattice spacing and the $\text{const}$ depends on the particular choice of $w(r)$ weight function only. Given the latter relations, Eqs. (10) and (16, 18) differ only by replacing the Dirac delta–functions to contacts via the Kronecker symbols on the lattice. Finally, parametrisation of the second virial coefficients for amphiphilic heteropolymers Eq. (7) in the present model results in an additional relation\textsuperscript{13}, $\chi_{aa} + \chi_{bb} = 2 \chi_{ab}$.

For finding equilibrium and metastable states one is free to use a combination of various Monte Carlo moves which relax the system faster. In addition to local monomer moves\textsuperscript{13}, we argue that for a multichain system it is highly desirable to include translational moves of whole chains. This can be motivated as follows. Once a polymer has collapsed the chain mobility deteriorates significantly in the scheme with local moves only. Indeed, monomers forming a space filling core of the globule can hardly move at all, and movements of the globule shell contribute little to translations of the globule. Thus, aggregation and collapse would become oversuspended.

The situation improves dramatically by introducing translational moves representing the diffusion of chains. To be consistent, however, clumps involving several polymers should be treated by the scheme of translational moves in exactly the same manner as single chains. Thus, translational moves are applied to all clusters of chains within the interaction range with a probability inversely proportional to the number of monomers within. This ensures the Stokes law in the absence of the hydrodynamic interaction. Such a translational move results in shifting the current cluster in a random direction among 6 possible directions.
IV. HOMOPOLYMER SOLUTION

We precede the main results by considering the homopolymer solution, for which all minima of the free energy corresponding to clusters are expected to be unstable according to the standard theories.

The phase diagram of the homopolymer solution can be easily obtained from the variational equations by using the additional kinematic assumption in case of ring polymers\textsuperscript{22} that the mean squared distances between any two monomers belonging to two distinct chains are the same $D_{mm'} \equiv D$, and may be written as $D_{aa'} = D_{|m-m'|}$ for any two monomers belonging to the same chain. We have explicitly checked that in the more general formalism described here these assumptions are automatically satisfied for the thermodynamically stable states, i.e. the main minima of $A$, for solution of ring homopolymers. In Ref. \textsuperscript{22} we have concluded that the boundary of the coexistence region is well described by the Flory–Huggins theory. In fact, Eqs. (14,15,17) in the simplified treatment of Ref. \textsuperscript{22} are capable to describe only the “symmetric” phases, for which all polymer chains stay apart from each other or collapse into a single precipitate. However, for the metastable states, i.e. local free energy minima, this is not true due to the phenomenon of spontaneous symmetry breaking analogous to our discussion in Ref. \textsuperscript{24}.

Numerical analysis of the complete set of variational equations shows that there are additional states related to various local free energy minima. These minima correspond to conformations where polymers in solution form several clusters, each consisting of one or a few chains. Obviously, for a large number of chains there may be many such states. The situation is illustrated in Fig. 1 in which we present the mean squared radii of gyration of clusters formed by various number of chains in solution of $M = 4$ homopolymers. The lines are drawn as long as the corresponding minima of the free energy exist. One can see from Fig. 1 that for rather small number of chains such “asymmetric” minima exist in a certain region of the phase diagram bounded by the first point of curve $4 \times 1$ on the left and the last point of curve $1 \times 4$ on the right. Moreover, the upper bounds in $u^{(2)}$ for the existence
of such states are approximately the same.

Now let us compare the values of the free energy at minima corresponding to various possibilities to divide the system into subsets. In Tab. I we present values of the free energy for some cluster sizes, corresponding to either a symmetric division of the system into clusters of equal size, or a large precipitate plus one or two single globules. One can see from Tab. I that all asymmetric minima for the homopolymer are not stable. Note also that the system divided into a large aggregate plus a few single globules possesses the free energy value close enough to that at the global minimum, whilst the system divided into several clusters of equal size has a higher free energy. In fact, one would expect that for the homopolymer solution of a huge number of chains the minimum of the free energy should be at one of the asymmetric states consisting of one large precipitate plus a gas of single globules. Such behaviour would be consistent with the standard picture of two–phase coexistence in the thermodynamic limit. However, due to high computational expenses we have not been able to test this properly based on the variational equations as yet.

V. HETEROPOLYMER SOLUTIONS

A. Results from the variational method

First, let us overview the main results obtained by numerical analysis of the complete set of the extrema conditions of the variational free energy Eqs. (4,5). In Figs. 2 and 3 we present the equilibrium phase diagrams for solution of \( M = 4 \) heteropolymers consisting of short and long blocks respectively. These diagrams are drawn at a fixed concentration in terms of the mean second virial coefficient \( \bar{u}^{(2)} \) and the amphiphilicity \( \Delta \), which parametrise the matrix of the two–body virial coefficients in Eq. (7). For small values of the amphiphilicity \( \Delta \) the phase diagrams of heteropolymers are essentially the same as for the homopolymer. Thus, there are the low–density phase of individual globules (or coils in the repulsive regime) and the high–density macroglobule, as well as the region of their coexistence.
Let us now discuss how the situation changes with increasing $\Delta$ at a fixed low concentration. For the two heteropolymers under consideration there appears an intermediate region, in which the state corresponding to two clusters of two chains each possesses the lowest free energy. Such a minimum appears starting from some critical value of the amphiphilicity and is bound to a narrow range in the mean second virial coefficient for any fixed $\Delta$. As it is clear from Figs. 2 and 3, this region designated as the ‘Mesoglobules’ expands with increasing the amphiphilicity. The location and shape of this region turn out to be very sensitive on the heteropolymer sequence. For long blocks heteropolymers this region is narrower and appears at a weaker attraction, characterised by a smaller $|\bar{u}^{(2)}|$ compared to the case of short blocks (alternating monomers). Indeed, for the former the micro–phase separation, which stabilises the mesoglobules, proceeds easier, i.e. it requires a weaker attraction to occur. It is important to emphasise that the asymmetric clusters ‘3 + 1’ and ‘1 + 1 + 2’ always possess a higher free energy than other minima (i.e. the macroglobule ‘1 $\times$ 4’, the single globules ‘4 $\times$ 1’ or the mesoglobules ‘2 + 2’), and thus are merely metastable.

Now then, let us examine somewhat larger systems composed of $M = 12$ chains of length $N = 12$ with varying block length. Values of the free energy at various local minima, corresponding to symmetric and some asymmetric clusters, are presented in the series of Tabs. [I], [III], [IV] and [V] for different values of $\bar{u}^{(2)}$ at a fixed sufficiently high $\Delta$ for different periodic and aperiodic sequences. All considered asymmetric clusters have been found to possess a higher value of the free energy than the symmetric ones. The main conclusion from the above case of a smaller system that the mesoglobules are thermodynamically stable in some intermediate region, remains valid here as well. However, in the current case a few different mesoglobules sizes are possible, namely ‘6 $\times$ 2’, ‘4 $\times$ 3’, ‘3 $\times$ 4’ and ‘2 $\times$ 6’. We also find that at a given mean second virial coefficient, amphiphilicity, concentration and fixed sequence, only one of these is thermodynamically stable. With all other parameters fixed, the size of stable mesoglobules increases with the concentration and with $|\bar{u}^{(2)}|$. Thus, the equilibrium transition from the gas of single globules to the macroaggregate on increasing $|\bar{u}^{(2)}|$ proceeds in a few steps. Clearly, the number of various possible clusters grows exponentially with
the system size, and for a sufficiently large system it is impossible to enumerate all possible divisions. We emphasise that according to Tab. symmetric clusters have the lowest free energy not only for heteropolymers with a periodic block structure, but essentially for many aperiodic sequences as well.

B. Results from lattice Monte Carlo simulation

It is interesting to check these predictions of the Gaussian variational method by the Monte Carlo simulation on a lattice. Here we shall consider several concrete sequences of amphiphilic heteropolymers consisting of strongly hydrophobic and slightly hydrophilic units such that $\chi_{aa} = -5\chi_{bb}$. We fix the main parameters as follows: linear lattice size $L = 60$, polymer length $N = 24$ and number of chains $M = 20$. To obtain final equilibrium states we proceeded from a random coil state ($\chi_{aa} = 0.1$) and performed an instantaneous quench to $\chi_{aa} = 1$ followed by a few millions of Monte Carlo sweeps of relaxation.

Fig. 4 shows the time evolution of the mean number of macromolecular clusters $n_{cl}$ during the relaxation. The solid curve in this figure corresponds to the homopolymers consisting of only hydrophobic units, for which the final equilibrium is reached after a few hundred thousands of MC sweeps. The resulting state is a single aggregate of 20 chains, as the coexisting low density phase of single globules is virtually unobservable here due to the nearly vertical shape of the left two–phase coexistence boundary in the Flory–Huggins phase diagram. In the $n_{cl}(t)$ dependence for heteropolymers first there is a similar fast stage, which is then followed by an extremely slow further relaxation. Essentially no change in the value of $n_{cl}$ happens at very large times, which shows that the final equilibrium has indeed been reached for considered sequences. Remarkably, the number of clusters in the final state here is not equal to unity, and attempts to carry on the simulation further never changed the situation.

In Figs. and we exhibit snapshots of typical system equilibrium conformations for different heteropolymer sequences. In Fig. we have a snapshot of the initial state of
swollen coils at very weak overall monomer attraction insufficient to overcome the entropic effect. In Fig. 5b we have a snapshot of the final single aggregate in the case of diblock copolymers, which has a clear micellar structure of a hydrophobic core (black) and a shell of hydrophilic (white) subchains sticking out.

Snapshots in Figs. 6a–6d correspond to the final states of the system for different heteropolymer sequences. These correspond to a few distinct clusters each consisting of several chains, which have a large amount of hydrophilic (white) material on the outside. Strikingly, in case of sequences in Figs. 6b–6d these clusters have nearly equal size, i.e. well monodispersed particles are produced. We have already seen from the Gaussian variational theory that conformational structures corresponding to clusters of equal size (which we called symmetric there) may become most stable in some area of the phase diagram. Now, on the lattice, a direct computation shows that these mesoglobules possess a somewhat higher energy than the single macroglobule at the same values of interaction parameters. However, their entropy is, obviously, higher as well due to the gain of translational entropy, and the net result in the free energy favours the mesoglobules, which is manifested in their apparent stability. This interplay of different contributions is rather subtle, and, clearly, the micro–phase separation, which leads to a repulsive shell on the surface of the mesoglobules, does play a significant role.

Now let us examine the question about the size polydispersity of the mesoglobules in more detail. For this we have performed the above described relaxation procedure for a large ensemble consisting of $Q = 1000$ independent different initial conditions. In Figs. 7 and 8 we present the calculated histograms of the mass (i.e. number of chains in a mesoglobule) and size (i.e. squared radius of gyration of a mesoglobule) distributions in the final state for different sequences.

The most typical picture is seen for sequences s2, s3 (intermediate sized blocks) and s6 (an irregular (randomly generated) sequence). These have a single well distinguished peak in the mass and size distributions, which has a Gaussian–like shape with a fairly narrow width. This corresponds to essentially monodispersed mesoglobules, which for our particular system
size have about 10-15 percent relative dispersion in linear size. Some sequences, however, do not result in monodispersed mesoglobules. For example, sequence s1 (alternating very short blocks) has two peaks in its mass distribution: a large population of single globules \( M_{cl} = 1 \) and a smaller population of mesoglobules consisting of about 2-6 chains. A typical snapshot for this sequence in Fig. \( \text{5a} \) has two single globules and two large formations of fairly irregular shape and different size. The main reason for this is that due to a very short block length forming a core and shell structure is not possible.

The mass distribution for sequences s4 (diblock copolymer) and s5 (inverse to the irregular sequence s6, which has mostly hydrophobic ends) in addition to a mesoglobules peak possess a large population of single aggregates \( M_{cl} = 20 \). The latter circumstance is due to that the characteristic size of mesoglobules here (about 15 for s4) is quite large and comparable to the number of chains \( M = 20 \). Thus, we may expect that even for these sequences with increasing the system size (i.e. \( M \) and \( L \) so that \( c = M/L^3 = \text{const} \)) these two peaks would transform to a single mesoglobules peak as for sequences s2, s3, s6. However, to prove this reasonable conjecture by simulation would require enormous computational times. Note that, at the same time, the size distribution for s4, s5 has essentially a single fairly narrow peak even for \( M = 20 \).

Thus, we see that the size of mesoglobules varies within a few dozens percents margin due to fluctuations. This situation is analogous to that of the Gaussian variational theory, in which clusters of slightly unequal sizes have close, but somewhat higher free energy than the respective symmetric clusters. This means also that the barriers separating such slightly different minima make it hard for the system to transform from one of the metastable states to the true free energy minimum. In Monte Carlo simulation fluctuations permit to move a single chain from one cluster to another occasionally, but the average mesoglobules size does not really fluctuate. Transitions between states with different mean size of mesoglobules are strictly suppressed due to rather high activation barriers. Finally, we remark that adequate description of the nucleation process would require an introduction of collective moves, which can split clusters and form new ones, to the Monte Carlo scheme. Thus, we do not attempt
VI. CONCLUSION

In this paper we have studied the equilibrium conformational states in solutions of amphiphilic heteropolymers at relatively low concentrations. The main conclusion from our considerations is that in heteropolymers there are additional thermodynamic states obtained by association of several distinct chains. This effect is specific to heteropolymers with sufficiently strong competing interactions. In homopolymer solution clusters of several chains always possess a higher free energy than the gas of single globules or the precipitate, so that such states cannot be stable. We have introduced the term *mesoglobules* to refer to rather monodispersed (or exactly equal sized in the mean–field approximation) mesoscopic globules, i.e. globules composed of more than one and less than all chains.

The average size of the mesoglobules in heteropolymer solutions is determined by the characteristic scale of the micro–phase separation. The physical mechanism responsible for it has been discussed in Sec. \[7.13\]. Formation of mesoglobules from a single macro-aggregate at the same values of interaction parameters results in: a) a favourable gain of translational entropy, \(\Delta S \simeq n_{\text{mes}} \ln(V/n_{\text{mes}}) - \ln V\), where \(n_{\text{mes}}\) is the number of mesoglobules; b) an unfavourable gain of surface energy \(\Delta \mathcal{E} \simeq \varsigma_1(\Delta, \sigma_i) n_{\text{mes}} R_{\text{mes}}^2 - \varsigma_2(\Delta, \sigma_i) R_{\text{mac}}^2\), where the mean radii of a mesoglobule and the macroaggregate can roughly be estimated as \(R_{\text{mes}} \sim \left(\frac{NM\mu^{(3)}}{|\bar{u}^{(2)}|}\right)^{1/3}\) and \(R_{\text{mac}} \sim \left(\frac{NM\mu^{(3)}}{|\bar{u}^{(2)}|}\right)^{1/3}\) respectively. Here \(\varsigma_{1,2}(\Delta, \sigma_i)\) are some ‘effective’ surface tension coefficients which arise from a rather complicated mismatch between the amounts of hydrophobic and hydrophilic units exposed on the surface for a given sequence in the two cases. These two tendencies compete with each other, but it is more favourable to produce mesoglobules of certain size in a rather narrow domain of the phase diagram as is seen e.g. in Figs. 2,3 and Tabs. II and III. In the case of periodic copolymers with fairly long blocks it is clear that a large scale phase separation of ‘a’ and ‘b’ units would play a major role for both forming mesoglobules in dilute solution and a shell–and–
core single globule at infinite dilution[45]. Perhaps, in case of more complicated irregular sequences a sort of more refined Imry–Ma argument[22], which would take into account the above described balance of energetic and entropic terms, may provide further insight into formation of mesoglobules as a kind of localised domains appearing due to the coupling \( \sum_{a,i} \sigma_i \rho_{mon}(X_i^a) \) of the monomer density to ‘disordered’ variables \( \sigma_i \) in the Hamiltonian Eq. (II).

The size distribution of the mesoglobules is sufficiently monodisperse due to a thermodynamic preference for clusters to be of equal size. However, fluctuations can transform symmetric clusters into a slightly asymmetric ones, although the barriers separating these structures from strongly asymmetric clusters, such as macroaggregates, are very high.

We find that short blocks and certain ‘good’ irregular sequences also form mesoglobules in some narrow regions of the phase diagram. The conformation of these mesoglobules cannot have a clear micellar structure due to the connectivity constraints which make it very difficult to form a core of hydrophilic and a shell of hydrophobic units for a given sequence. Other sequences, such as some ‘bad’ aperiodic sequences and, as we have seen, in our regime also diblocks, which may be viewed as model surfactants, can only produce particles with a broad size distribution. Some of more complicated anomalous cases may nevertheless be quite interesting. So, for example, sequence s5 in Fig. 7b (but, significantly, not s6 which is obtained by ‘a’ to ‘b’ mutual replacements), which contains essentially two hydrophobic end blocks with a hydrophilic block in the middle, produces a quite polydispersed cluster distribution. Snapshots of corresponding conformations show a number of clusters interconnected by short hydrophilic bridges. These, of course, do not qualify as mesoglobules. Clearly, at higher concentrations, this localised network formation would play an increasingly important role. We hope to be able to return to the study of conformational structures produced by such tri–block and more peculiar sequences in dilute solutions at a later date.

What also seems to be essential for the existence of the mesoglobules is that there should be sufficient distinction in monomer–solvent interactions between the two types of units, one of which should be hydrophobic and another slightly hydrophilic. No mesoglobules have
been found by us for hydrophobic–neutral heteropolymers (for which \( \chi_{aa} > 0 \) and \( \chi_{bb} = 0 \)), which tend to simply aggregate similar to the homopolymer case.

Our formal observation in this work was that the mesoglobules are mean–field stable (rather than merely metastable) states in some regions of the phase diagram. However, due to fluctuations beyond mean–field they are not fully monodisperse, but possess a fairly narrow size distribution. Nevertheless, no matter how much more time elapses they do not tend to grow or aggregate and preserve their mean size and distribution. The latter conclusion is supported by our Monte Carlo simulations on extremely long times\(^{33}\) and seems to be in agreement with experimental evidence\(^{21}\). We thus believe that the observed phenomenon is generic for fairly dilute heteropolymer solutions. As for the exact regions of stability of mesoglobules and their mean size and monodispersity, these seem to be extremely sensitive on the particular heteropolymer sequence and thermodynamic parameters of the system. It also seems quite feasible that even a very weak electrostatic repulsion may play crucial role for further stabilisation of mesoglobules and that it can improve their monodispersity significantly.

Unfortunately, at the moment no theory exists that could describe the dynamic and kinetic phenomena in heteropolymer solutions at the same level of detail as we have been able to achieve here for the equilibrium and metastable states. The Gaussian self–consistent method is an optimised mean–field type theory and the account for nucleation and density fluctuations is beyond its scope. On the other hand, the Monte Carlo method is difficult to apply to kinetics as the issue of choosing a particular scheme of Monte Carlo moves is obscure. Besides, no simulation alone can completely convincingly distinguish between true thermodynamic states and very long lived metastable ones.

However, it seems that even the limited information on the depths of various local minima and barrier heights between them obtained from the Gaussian variational method should be sufficient for understanding and explaining some novel phenomena observed in recent experiments with heteropolymer solutions. For instance, it is possible that in kinetic experiments the size of mesoglobules would be dependent on the heating speed. This can happen
if the nucleation time between two mesoglobular states with different average cluster sizes is much longer than the typical measurement time involved. Thus, even though one of such mesoglobular states would have the lowest free energy at given thermodynamic parameters, the system can in principle be trapped for a rather long time in another such state which happens to be merely metastable.

It is worthwhile to emphasise that in solutions of a biopolymer, such as e.g. a protein, all chains would have exactly the same structure because they are produced by the unique rules from the same genetic code. However, synthesis normally results in a mixture of chains with somewhat varying lengths and sequences and the presence of various defects. Thus, it would be interesting to investigate the influence of weak imperfections remaining after applying physical methods such as fractionation and centrifugation on the monodispersity of the mesoglobules in solution. Technically, this requires to perform a quenched disorder averaging: first over the identical random sequences, and then also to permit randomness in the structure of each chain in solution. Replica techniques are commonly adopted for such purposes and we believe they may lead to further progress in studying the current problem and its possible variations.

Finally, we hope that mesoglobular structures may find a number of interesting industrial applications as their size distribution may be well controlled. Another potential application of these results would be in learning how to facilitate folding and suppress aggregation of proteins in vitro.

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31. These correspond to points at a fixed concentration $c$ on curves I” and I’ in Fig. 1 of Ref. 22 respectively.

32. Animation of the relaxation towards the equilibrium corresponding to mesoglobules is available at [http://darkstar.ucd.ie/animation/#2](http://darkstar.ucd.ie/animation/#2).

33. Simulations for each of sequences s1–s6 in Figs. 6 and 8 took $1.92 \cdot 10^{12}$ attempted Monte Carlo steps. The computing expenses for obtaining all Monte Carlo data in this paper were about 2 months of pure CPU time on 16 PII-400MHz processors Beowulf cluster [http://darkstar.ucd.ie/servers](http://darkstar.ucd.ie/servers).

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FIGURES

FIG. 1. Plot of the mean squared cluster size, $R_{cl}^2$, vs the second virial coefficient, $u^{(2)}$, for different cluster states. This data is obtained for open homopolymers with $N = 18$, $M = 4$ and $L = 10$. The dashed vertical line I corresponds to the transition point at which the free energies of states $4 \times 1$ (single chains) and $1 \times 4$ (aggregate) become equal.

FIG. 2. Phase diagram for solution of $M = 4$ $(ab)_6$ heteropolymers in terms of the amphiphilicity, $\Delta$, and the mean second virial coefficient, $\bar{u}^{(2)}$. The linear box size is $L = 8$. Here and below the transition curves have been determined by the condition of free energy equality on corresponding minima as in previous figure.

FIG. 3. Phase diagram for solution of $M = 4$ $(a_3b_3)_2$ heteropolymers in terms of the amphiphilicity, $\Delta$, and the mean second virial coefficient, $\bar{u}^{(2)}$. Here the linear box size is $L = 8$.

FIG. 4. Time dependence of the total number of clusters $n_{cl}$ on the approach to final equilibrium for different polymer sequences. 1 Monte Carlo sweep is defined as $NM$ attempted Monte Carlo moves. Here $L = 60$, $N = 24$, $M = 20$, $\chi_{aa} = 1$, and $\chi_{bb} = -0.2$.

FIG. 5. Snapshots of typical polymer conformations from Monte Carlo simulation: Fig. a — for the good solvent condition for sequence $(a_3b_3)_4$, and Fig. b — the single aggregate for diblock sequence $a_{12}b_{12}$. All parameters here are as in Fig. 4. Black and white circles correspond to hydrophobic and hydrophilic monomer units respectively.

FIG. 6. Snapshots of typical polymer conformations from Monte Carlo simulation for mesoglobules of different heteropolymers. Figs. a-d correspond to sequences: $(a_2b_2)_6$, $(a_3b_3)_4$, $b_3ab_2a_3a_4b_3b_2ab_3$ and $a_{12}b_{12}$ respectively (these are also called as sequences s1, s2, s6 and s4 in Figs. 7 and 8). All parameters here are as in Fig. 4. Black and white circles correspond to hydrophobic and hydrophilic monomer units respectively.
FIG. 7. Histogram of the number of chains (mass) $M_{cl}$ constituting mesoglobules for different heteropolymer sequences. These results have been obtained by analyzing data for the ensemble size (the number of initial conditions) $Q = 1000$ after equilibration time $4 \cdot 10^6$ MC sweeps (the last time point in Fig. 4). All parameters here are as in Fig. 4. Note that for some sequences there are large populations of single globules ($M_{cl} = 1$) and single aggregate ($M_{cl} = 20$).

FIG. 8. Histogram of the squared radius of gyration $R_{cl}^2$ of mesoglobules for different heteropolymer sequences. All parameters here are as in Fig. 4.
TABLE I. Values of the specific free energy, $a = A/MN$, at various minima for the system of $M = 12$ open homopolymers in a box with $L = 20$ for various values of the second virial coefficient, $u^{(2)}$. The value of the global (deepest) minimum of the free energy in each line is printed in **bold** face. The horizontal line here corresponds to the transition point, i.e. equality condition of free energies for states $12 \times 1$ (separate chains) and $1 \times 12$ (aggregate).

| $u^{(2)}$ | $12 \times 1$ | $6 \times 2$ | $4 \times 3$ | $3 \times 4$ | $2 \times 6$ | $1 \times 12$ | 1, 11 | 1, 1, 10 |
|-----------|--------------|-------------|-------------|-------------|-------------|------------|------|------------|
| $-12$     | $-1.028$     | $-0.843$    | $-0.815$    | $-0.814$    | $-0.832$    | $-0.886$   | $-0.890$ | $-0.896$   |
| $-13$     | $-1.378$     | $-1.206$    | $-1.185$    | $-1.188$    | $-1.210$    | $-1.270$   | $-1.271$ | $-1.274$   |
| $-14$     | $-1.759$     | $-1.604$    | $-1.589$    | $-1.596$    | $-1.623$    | $-1.688$   | $-1.685$ | $-1.685$   |
| $-15$     | $-2.172$     | $-2.035$    | $-2.027$    | $-2.038$    | $-2.069$    | $-2.140$   | $-2.132$ | $-2.127$   |
| $-16$     | $-2.615$     | $-2.499$    | $-2.498$    | $-2.513$    | $-2.548$    | $-2.626$   | $-2.615$ | $-2.606$   |
| $-18$     | $-3.595$     | $-3.523$    | $-3.539$    | $-3.562$    | $-3.607$    | $-3.695$   | $-3.676$ | $-3.659$   |
| $-20$     | $-4.696$     | $-4.676$    | $-4.709$    | $-4.742$    | $-4.797$    | $-4.897$   | $-4.867$ | $-4.840$   |
| $-25$     | $-7.982$     | $-8.113$    | $-8.198$    | $-8.258$    | $-8.341$    | $-8.472$   | $-8.416$ | $-8.386$   |
TABLE II. Values of the specific free energy, \( a = A/MN \), at various minima for the system of \( M = 12 \) \((ab)_6\) heteropolymers for various values of the mean second virial coefficient, \( \bar{u}^{(2)} \). Here and below the linear system size and the amphiphilicity are equal to \( L = 20 \) and \( \Delta = 30 \). The horizontal lines here and below delimit the broad region of stable mesoglobules, i.e. where the global free energy minimum is reached on states other than \( 12 \times 1 \) or \( 1 \times 12 \).  

| \( \bar{u}^{(2)} \) | \( 12 \times 1 \) | \( 6 \times 2 \) | \( 4 \times 3 \) | \( 3 \times 4 \) | \( 2 \times 6 \) | \( 1 \times 12 \) | \( 1,11 \) | \( 2,10 \) | \( 1,1,10 \) |
|----------------|-----------|-----------|-----------|-----------|-----------|----------------|----------|----------|-----------|
| -5             | -4.25     | -3.70     | -3.25     | 2.89      | -2.33     | -                | -4.17    | -3.46    | -3.56     |
| -10            | -7.08     | -6.78     | -6.42     | -6.09     | -5.57     | -4.54           | -4.88    | -5.15    | -5.20     |
| -15            | -10.31    | -10.31    | -10.05    | -9.79     | -9.32     | -8.34           | -8.63    | -8.90    | -8.90     |
| -20            | -13.96    | -14.29    | -14.16    | -13.97    | -13.58    | -12.69          | -12.91   | -13.17   | -13.12    |
| -25            | -18.04    | -18.74    | -18.75    | -18.64    | -18.35    | -17.58          | -17.72   | -17.97   | -17.85    |
| -30            | -22.61    | -23.69    | -23.86    | -23.83    | -23.65    | -23.02          | -23.07   | -23.29   | -23.12    |
| -35            | -27.73    | -29.18    | -29.51    | -29.57    | -29.50    | -29.03          | -28.99   | -29.19   | -28.94    |
| -40            | -33.87    | -35.27    | -35.75    | -35.91    | -35.95    | -35.64          | -35.51   | -35.68   | -35.37    |
| -45            | -40.41    | -42.47    | -42.65    | -42.90    | -43.04    | -42.88          | -42.67   | -42.81   | -42.45    |
| -50            | -47.64    | -50.05    | -50.65    | -50.68    | -50.83    | -50.81          | -50.52   | -50.63   | -50.21    |
| -55            | -55.57    | -58.36    | -58.69    | -59.05    | -59.34    | -59.46          | -59.08   | -59.17   | -58.69    |
TABLE III. Values of the specific free energy, $a = A/MN$, at various minima for the system of $M = 12$ $(a_2b_2)_3$ heteropolymers for various values of the mean second virial coefficient, $\bar{u}^{(2)}$.

| $\bar{u}^{(2)}$ | 12 × 1 | 6 × 2 | 4 × 3 | 3 × 4 | 2 × 6 | 1 × 12 | 1,11 | 2,10 | 1,1,10 |
|------------------|--------|-------|-------|-------|-------|--------|------|------|-------|
| −5               | −4.98  | −4.77 | −4.53 | 4.31  | −3.94 | −3.20  | −3.44| −3.63| −3.67 |
| −10              | −7.75  | −7.80 | −7.65 | −7.47 | −7.16 | −6.47  | −6.66| −6.85| −6.85 |
| −15              | −10.91 | −11.25| −11.21| −11.09| −10.85| −10.23 | −10.37| −10.55| −10.50|
| −20              | −14.47 | −15.14| −15.21| −15.17| −15.00| −14.48 | −14.55| −14.73| −14.62|
| −25              | −18.45 | −19.48| −19.69| −19.72| −19.64| −19.24 | −19.24| −19.39| −19.22|
| −30              | −22.91 | −24.30| −24.66| −24.77| −24.79| −24.42 | −24.43| −24.57| −24.34|
| −35              | −27.93 | −29.66| −30.16| −30.36| −30.48| −30.35 | −30.18| −30.29| −30.00|
| −40              | −34.02 | −36.03| −36.27| −36.55| −36.76| **36.77**| −36.52| −36.51| −36.27|
| −45              | −40.51 | −42.82| −43.27| −43.42| −43.57| **43.85**| −43.53| −43.49| −43.20|

TABLE IV. Values of the specific free energy, $a = A/MN$, at various minima for the system of $M = 12$ $(a_3b_3)_2$ heteropolymers for various values of the mean second virial coefficient, $\bar{u}^{(2)}$.

| $\bar{u}^{(2)}$ | 12 × 1 | 6 × 2 | 4 × 3 | 3 × 4 | 2 × 6 | 1 × 12 | 1,11 | 2,10 | 1,1,10 |
|------------------|--------|-------|-------|-------|-------|--------|------|------|-------|
| 0                | **3.02**| −2.81 | −2.61 | 2.45  | −2.18 | −1.65  | −1.83| −1.97| −2.01 |
| −5               | −5.38  | −5.39 | −5.27 | 5.14  | −4.91 | −4.40  | −4.55| −4.69| −4.69 |
| −10              | −8.12  | −8.37 | −8.34 | −8.26 | −8.08 | −7.63  | −7.73| −7.89| −7.83 |
| −15              | −11.23 | −11.78| −11.85| −11.83| −11.71| −11.33 | −11.38| −11.51| −11.42|
| −20              | −14.74 | −15.60| −15.80| −15.84| −15.79| −15.51 | −15.49| −15.60| −15.46|
| −25              | −18.66 | −19.87| −20.19| −20.31| −20.34| −20.16 | −20.07| −20.17| −19.97|
| −30              | −23.05 | −24.61| −25.070| −25.26| −25.39| −25.32 | −25.16| −25.24| −24.98|
| −35              | −28.00 | −29.89| −30.48| −30.75| −30.97| **31.03**| −30.78| −30.85| −30.54|
TABLE V. Values of the specific free energy, $a = A/MN$, at various minima for the system of $M = 12$ heteropolymers with the sequence $b_3a_2b_2aba$ for various values of the mean second virial coefficient, $\bar{u}^{(2)}$.

| $\bar{u}^{(2)}$ | $12 \times 1$ | $6 \times 2$ | $4 \times 3$ | $3 \times 4$ | $2 \times 6$ | $1 \times 12$ | $1, 11$ | $2, 10$ | $1, 1, 10$ |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| $-10$           | $-7.78$        | $-7.77$        | $-7.61$        | $-7.43$        | $-7.10$        | $-6.40$        | $-6.60$        | $-6.80$        | $-6.80$        |
| $-15$           | $-10.93$       | $-11.23$       | $-11.16$       | $-11.03$       | $-10.77$       | $-10.14$       | $-10.29$       | $-10.43$       | $-10.48$       |
| $-20$           | $-14.49$       | $-15.11$       | $-15.11$       | $-14.92$       | $-14.37$       | $-14.46$       | $-14.53$       | $-14.64$       | $-14.64$       |
| $-25$           | $-18.47$       | $-19.44$       | $-19.63$       | $-19.65$       | $-19.54$       | $-19.11$       | $-19.12$       | $-19.12$       | $-19.29$       |
| $-30$           | $-22.92$       | $-24.26$       | $-24.59$       | $-24.69$       | $-24.68$       | $-24.37$       | $-24.30$       | $-24.22$       | $-24.45$       |
| $-35$           | $-27.91$       | $-29.60$       | $-30.08$       | $-30.27$       | $-30.36$       | $-30.18$       | $-30.03$       | $-29.97$       | $-30.15$       |
| $-40$           | $-33.97$       | $-35.96$       | $-36.19$       | $-36.45$       | $-36.63$       | $-36.59$       | $-36.36$       | $-36.13$       | $-36.46$       |
| $-45$           | $-40.47$       | $-42.75$       | $-43.26$       | $-43.31$       | $-43.59$       | $-43.67$       | $-43.37$       | $-43.06$       | $-43.44$       |
| $-50$           | $-47.67$       | $-50.27$       | $-50.99$       | $-51.18$       | $-51.26$       | $-51.46$       | $-51.09$       | $-50.71$       | $-51.15$       |