Glass transition in secondary structures formed by random RNA sequences

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(Dated: February 1, 2008)

Formation of RNA secondary structures is an example of the sequence-structure problem omnipresent in biopolymers. A theoretical question of recent interest is whether a random RNA sequence undergoes a finite temperature glass transition. We answer this question affirmatively by first establishing the perturbative stability of the high temperature phase via a two replica calculation. Subsequently, we show that this phase cannot persist down to zero temperature by considering energetic contributions due to rare regions of complementary subsequences.

PACS numbers: 87.15.Aa, 05.40.-a, 87.15.Cc, 64.60.Fr

Introduction: RNA is an important biopolymer critical to all living systems. Like in DNA, there are four types of nucleotides (or bases) A, C, G, and U which, when polymerized can form double helical structures consisting of stacks of stable Watson-Crick (A–U or G–C) pairs. However unlike a long polymer of DNA, which is often accompanied by a complementary strand and forms otherwise featureless double helical structures, a polymer of RNA is usually single-stranded. It bends onto itself and forms elaborate spatial structures in order for bases located on different parts of the backbone to pair with each other. The structures encoded by the primary sequences often have important biological functions, much like how the primary sequence of a protein encodes its structure.

Understanding the encoding of structure from the primary sequence has been an outstanding problem of theoretical biophysics. Most work in the past decade has been focused on the problem of protein folding, which is very difficult analytically and numerically. Here, we study the problem of RNA folding, specifically the formation of RNA secondary structures which is more amenable to analytical and numerical approaches due to a separation of energy scales. Efficient algorithms together with carefully measured free energy parameters describing the formation of various microscopic structures (e.g., stacks, loops, hairpins, etc.) allow the exact calculation of the ensemble of secondary structures formed by a given RNA molecule of up to a few thousand bases.

In this work, we are not concerned with the structure formed by a specific sequence. Instead, we will study the statistics of secondary structures formed by the ensemble of long random RNA sequences. It has been debated recently whether such an ensemble undergoes a finite temperature glass transition. However, the numerical results these studies are based on are not clear enough to allow unambiguous interpretation. In this letter, we provide analytical evidence supporting the existence of a finite temperature glass transition by studying some toy models of RNA folding. We characterize the behavior of RNA in the absence of disorder and show with the help of a two-replica calculation that disorder is perturbatively irrelevant. We then show that the assumption that the high-temperature phase exists at all temperatures leads to a contradiction below some finite temperature, thereby necessitating a finite temperature phase transition.

Model: A secondary structure $S$ of a polymer of RNA is the set of pairings formed between all of its monomers (or bases), with each base allowed in at most one pairing. We denote the pairing between the $i^{th}$ and $j^{th}$ monomer by $(i, j)$, with $1 \leq i < j \leq N$. Each such structure can be represented by a diagram like the one shown in Fig. 1(a).

In addition, it is common to exclude “pseudo-knots” like the one shown in Fig. 1(b) from the definition of secondary structures, so that any two base pairs $(i, j)$ and $(k, l)$ are either independent, i.e., $i < j < k < l$, or nested, i.e., $i < k < l < j$. This is permissible since long pseudo-knots are kinetically difficult to form and even the short ones occur infrequently due to energetic reasons. Experimentally, it is possible to “turn off” the pseudo-knots and other complicated tertiary contacts and study exclusively the class of secondary structures defined above.

In order to calculate Boltzmann factors for an ensemble of secondary structures, we need to assign an energy $E[S]$ to each structure $S$. For the purpose of secondary structure prediction, it is essential to model the energy as accurately as possible. However, for our interest in the universal statistical properties of long, random RNA sequences far below the denaturation temperature, it suffices to consider simplified models along the line of those used in earlier studies.

We associate an interaction energy $\varepsilon_{i,j}$ with every pairing $(i, j)$ and assign $E[S] = \sum_{(i,j) \in S} \varepsilon_{i,j}$ as the total en-

FIG. 1: Secondary structures of an RNA molecule: The solid and dashed lines represent the backbone and the base pairs respectively. (a) shows a valid secondary structure while (b) contains a pseudo-knot as indicated by the arrow.
ergy of the structure $S$. To retain the spirit of Watson-Crick pairing, we choose random sequences $b_1 \ldots b_N$ of the four bases $A$, $U$, $C$, and $G$ and then assign

$$
\varepsilon_{i,j} = \begin{cases} 
-\mu_m \text{ (}b_i,b_j\text{)} & \text{is a Watson-Crick base pair} \\
\varepsilon_{mm} & \text{otherwise}
\end{cases}
$$

(1)

with $\mu_m, \varepsilon_{mm} > 0$. Here, $\mu_m$ sets the energy scale. The value of $\varepsilon_{mm}$ is not essential as long as it is repulsive, since there is always the option to not bind at all (with energy “0”) rather than to bind with a repulsive energy.

For analytical work, it is convenient to take the $\varepsilon_{i,j}$ to be independent Gaussian random variables specified by the mean $\overline{\varepsilon}$ and variance

$$
(\varepsilon_{i,j} - \overline{\varepsilon})(\varepsilon_{k,l} - \overline{\varepsilon}) = D \delta_{i,k} \delta_{j,l}.
$$

(2)

Throughout the text, we will use the overline to denote averages over the ensemble of random pairing energies. Here, the parameter $D$ provides a measure of the strength of the randomness. It is an approximation to the model \([\text{1}], \text{2}\) in two respects: First, it replaces the discrete distribution of energies by a Gaussian distribution. Moreover, it neglects the correlations between $\varepsilon_{i,j}$ and $\varepsilon_{i,k}$ induced by the shared base $b_i$. We do not anticipate universal quantities to depend on the subtle differences in the statistics of the $\varepsilon_{i,j}$’s. This will be tested numerically by comparing the scaling behavior produced by the two models.

Given the energy of each secondary structure, we can study the partition function $Z(N) = \sum_{S \in \mathcal{S}(N)} e^{-\beta E[S]}$ where $\mathcal{S}(N)$ comprises all valid secondary structures of a molecule of length $N$. This function can be conveniently computed in terms of the restricted partition function $Z_{i,j}$ for the substrand $b_i \ldots b_j$. For the simple energy model $E[S]$ described above, $Z_{i,j}$ can be split up according to the possible pairings of position $j$ to yield the exact recursion relation \([\text{1}] \text{[1]} \text{[2]}\)

$$
Z_{i,j} = Z_{i,j-1} + \sum_{k=i}^{j-1} Z_{i,k-1} \cdot e^{-\beta \varepsilon_{k,j}} \cdot Z_{k+1,j-1}.
$$

(3)

This equation can then be iterated to compute the full partition function $Z(N) = Z_{1,N}$ for arbitrary interaction energies $\varepsilon_{i,j}$’s in $O(N^2)$ time \([\text{1}] \text{[1]} \text{[2]}\). It also forms the basis of analytical approaches to the problem.

The molten phase: If sequence disorder does not play an important role, we may describe the RNA molecule by replacing all the binding energies $\varepsilon_{i,j}$ by some effective value $\varepsilon_0$. As we will see later, this is an adequate description of random RNA at high enough temperatures (but below denaturation). Below we briefly review the properties of RNA in this high temperature “molten phase”.

Since the $Z_{i,j}$’s become translationally invariant in the absence of disorder, it is straightforward to solve Eq. \([3]\) for the molten phase partition function $Z_0(N)$ using the $z$-transform. For large $N$, it has the form \([\text{1}] \text{[1]} \text{[2]}\)

$$
Z_0(N) \approx N^{-\theta_0} \exp[-\beta N f_0(q)]
$$

(4)

where $q \equiv e^{-\beta \varepsilon_0}$ is the only parameter, $\theta_0 = 3/2$ is a universal exponent, and $f_0(q) = -k_B T \ln(1+2\sqrt{q})$ is the free energy per length.

One useful observable characterizing the state of the RNA is the free energy cost $\Delta F(N)$ of pinching together one end (say $i = 1$) and the mid-point ($i = N/2$) of the polymer relative to the unperturbed state. The pinch effectively separates the polymer into two pieces of length $N/2$. In the molten phase, we simply have

$$
\Delta F = -k_B T \ln[Z_0(N)/Z_0^2(N/2)] \approx \frac{1}{2} k_B T \ln N.
$$

(5)

It reflects the loss of configurational entropy in the allowed secondary structures due to the pinch.

Numerics: Before we delve into the analysis, we first present some numerical evidence for a phase transition between the high- and low-temperature behavior for the ensemble of random RNA. To this end, we generate many configurations of interaction energies $\varepsilon_{i,j}$’s according to both models \([\text{1}] \text{[1]} \text{[2]}\), with $\mu_m = \varepsilon_{mm} = 1$ and $\varepsilon = 1/2$, $D = 3/4$ respectively. Then for a wide range of temperatures we calculate the pinching free energy, which is given by $\Delta F = -k_B T \ln[e^{-\beta \varepsilon_{1,N/2}} Z_{2N/2-1} Z_{2N/2+1} / Z_{1,N}]$ in terms of the quantity $Z_{i,j}$ in \([3]\) for sequences without translational invariance. The result is then averaged over 1000 to 10000 disorder configurations and illustrated in Fig. \([2]\) for two representative temperatures.

At high temperature ($k_B T = 2$), the pinch energy follows precisely the molten phase behavior expected according to Eq. \([5]\) up to an additive constant. Thus, at high temperatures the molten phase description is applicable even if the interaction energies $\varepsilon_{i,j}$ are not uniform. Moreover, we find no difference between the two models of disorder at $k_B T = 2$. At low temperature ($k_B T = 0.025$), the picture is different. The length dependence of $\Delta F$ is still logarithmic. However, the prefactors differ between the two disorder choices and they are both far larger than the prefactor 0.0375 expected if the molten phase result is extrapolated to this temperature. This suggests that there is a distinct low temperature phase; this finding is reinforced by similar changes detected in other observables \([13]\).

High temperature behavior: Now we will establish the stability of the molten phase against weak disorder by a perturbative analysis. Assuming that the specific choice of disorder does not matter at weak disorder (or high temperature) as supported by the numerical results above, we will use the uncorrelated Gaussian disorder characterized by Eq. \([3]\) for the purpose of this analysis. The behavior of the system at weak disorder is determined by the lowest order terms in the perturbative expansion of the ensemble averaged free energy in the disorder strength $D$. This term is given by the two-replica partition function $Z(N)$ of two RNA molecules sharing the same disorder. With the uncorrelated Gaussian energies
to a logarithmic behavior (dotted lines) give the ensemble average can be explicitly performed, 

\[ Z^2(N) = \sum_{\{S_1, S_2 \in S(N)\}} q^{|S_1|} q^{|S_2|} q^{S_1 \cap S_2} \]  

(6)

where \( q \equiv \exp( -\beta \varepsilon + \frac{1}{2} \beta^2 D ) \) and \( \tilde{q} \equiv \exp( \beta^2 D ) \) are the two relevant “Boltzmann factors”, and \(|S_i|\) and \(|S_1 \cap S_2|\) are the number of bases contained in structure \(S_i\) or common to \(S_1\) and \(S_2\) respectively. This effective partition function has a simple physical interpretation: It describes two RNA molecules subject to a homogeneous attractive with effective interaction energy \(\varepsilon_0 \equiv -\beta^{-1} \ln q = -\frac{1}{2} \beta D\) between any two bases of the same molecule. In addition, there is an inter-replica attraction characterized by the factor \(\tilde{q}\) for each bond shared between the two replicas. The inter-replica attraction is induced by the same disorder shared by the replicas. It can potentially force the replicas to “lock” together, i.e., to become correlated.

It turns out that this two-replica problem can be solved exactly [3]. The key idea leading to the solution is that the sum over the pairs of secondary structures in Eq. (6) can be reordered by first summing over the bonds which are common to the two structures, noting that the possible configurations of the common bonds are themselves the set \(S(N)\) of valid secondary structures. Within a given configuration of common bonds, all possibilities to place non-common bonds in the two individual structures can then be summed over, leading to an effective single RNA problem. However, the necessary algebra is quite involved [3]: here we just quote the results. The solution has the form \( Z^2(N) \sim N^{-\theta} \exp( -\beta N (\tilde{q}^2 - 1) ) \), with two different expressions for \(\theta\) and \(\tilde{q}\) depending on whether \(\bar{q}\) is above or below a critical value \(\bar{q}_c = 1 + 1/|q^2 \sum_{N=1}^{\infty} Ng^2(N)|\), where \(g(N) = Z_0(N)/(1+2\sqrt{q})^{-N-1} \sim N^{-3/2}\) for large \(N\).

For \(\bar{q} < \bar{q}_c\), we have \(\theta = 2\theta_0 = 3\). In addition, \(f(q, \bar{q})\) is basically a modified version of \(2f_0(q)\). In this regime, the two-replica partition function \(Z^2(N)\) is essentially a product of two single-replica partition functions \(Z_0(N)\). Since there is no coupling between the two replicas, we conclude that the effect of disorder is irrelevant in this regime. For \(\bar{q} > \bar{q}_c\), we have \(\theta = 3/2\) and \(f\) given by a complicated function of \(Z_0(N)\) [3]. Here, the two-replica partition function has the same asymptotic form as that of the single-replica system in [3]. This implies that the disorder coupling locks the two replicas together.

Of course, as already explained above, only the weak-disorder limit (i.e., \(\bar{q} \gg 1\)) of the two-replica problem can be applied to the full random RNA problem. While \(\bar{q}_c\) itself depends on the disorder strength \(\beta^2 D\), it converges in the weak disorder limit (\(\beta^2 D \ll 1\)) towards a constant \(\bar{q}_c(\beta^2 D = 0) > 1\). Therefore, for \(\beta^2 D \ll 1\) we always have \(\bar{q} < \bar{q}_c\). The molten phase is perturbatively stable upon the introduction of disorder, making it the appropriate description of random RNA at high temperatures.

**Low temperature behavior:** Next we determine whether the molten phase persists for all temperatures down to \(T = 0^+\). In the following, we will assume that long random RNA is in the molten phase for all temperatures, i.e., that the partition function for any substrand of large length is given by Eq. (3), with some effective value of \(\bar{q}\).

Then, we will show that this assumption leads to a contradiction below some temperature \(T^* > 0\). This contradiction implies that the molten phase description breaks down at some finite \(T_c \geq T^*\). To be specific, we will consider the sequence disorder model [3] in this analysis.

We will again focus on the pinching free energy \(\Delta F\). Under the assumption that the random sequences are described by the molten phase, it is given by Eq. (3) for large \(N\) and all \(T\) independently of the effective value of \(\bar{q}\). On the other hand, we can study this pinching free energy for each given sequence of bases drawn from the ensemble of random sequences. For each such sequence, we can look for a continuous segment of length \(\ell\) in a sequence of length \(N\) in which the two complementary segments are paired in the molten phase. Thus, for \(\ell < N\) Watson-Crick pairs \((b_i, b_j)(b_{i+1}, b_{j-1})\ldots(b_{i+\ell-1}, b_{j-\ell+1})\) where the bases \(b_i\ldots b_i \ell-1\) are within the first half of the molecule and the bases \(b_{j-\ell+1}\ldots b_j\) are in the second half. For random sequences, the probability of finding such complementary segments decreases exponentially with the length \(\ell\) in a given region, with the largest \(\ell\) in a sequence of length \(N\) being typically \(\ln N/\ln 2\) [3].

Now we calculate the pinching free energy \(\Delta F = F_{\text{pinched}} - F_{\text{unpinched}}\) by evaluating the two terms separately. The partition function corresponding to the unpinched free energy contains at least all the configurations in which the two complementary segments \(b_i \ldots b_i \ell-1\) and \(b_{j-\ell+1}\ldots b_j\) are completely paired. Thus,

\[ F_{\text{unpinched}} \leq F_{\text{paired}} \]  

(7)

where \(F_{\text{paired}}\) is the free energy of the ensemble of structures in which the two complementary segments are...
paired. The latter is the sum of the energy of the paired segments and those of the two remaining substrands of lengths $L_1 = j - i - 2\ell + 1$ and $L_2 = N + i - j - 1$, i.e.,

$$F_{\text{paired}} = -\ell u_m + (N - 2\ell) f_0 + \frac{3}{2} k_B T \left[ \ln(L_1) + \ln(L_2) \right].$$  \hspace{1cm} (8)

The free energy $F_{\text{pinched}}$ is, by the assumption of the molten phase, the interaction energy of the pinched base pair $(b_1, b_{N/2})$ plus the free energy of the two substrands $b_2 \ldots b_{N/2 - 1}$ and $b_{N/2 + 1} \ldots b_N$. According to Eq. (5), this is $F_{\text{pinched}} = f_0 N + 2 \times \frac{3}{2} k_B T \ln N$ up to terms independent of $N$. Combining this with Eqs. (3) and (8), and noting that $\ell$ is typically of the order $\ln N/\ln 2$ and $L_1, L_2$ are typically proportional to $N$, we finally obtain

$$\Delta F \geq [u_m + 2f_0] \ln N/\ln 2$$

for very large $N$. This is only consistent with Eq. (8) if

$$\frac{3}{2} k_B T \geq [u_m + 2f_0]/\ln 2.$$  \hspace{1cm} (9)

Since $f_0$ is a free energy per length, the right hand side of this equation is a decreasing function of temperature. Moreover, $f_0(T = 0)$ is the average free energy per length of the minimum energy secondary structures $L\ni N$ dependent of $T$. Moreover, if the sequence disorder leads to frustration, there is always a finite fraction of bases which cannot be incorporated into Watson-Crick base pairs. However, if the sequence disorder leads to frustration, there is always a finite fraction of bases which cannot be incorporated into Watson-Crick base pairs and thus $f_0(T = 0) > -u_m/2$. Therefore, the right hand side of (9) is a decreasing function of $T$ starting at some positive value at $T = 0$. It follows that there is some unique temperature $T^*$ below which the consistency condition (9) breaks down, implying the inconsistency of the molten phase assumption in this regime. From this we conclude that there must be a phase transition away from the molten phase at some critical temperature $T_c > T^* > 0$. Numerically, we find that $f_0(T = 0) \approx -0.46$ for $u_m = 1$, yielding $k_B T^* \approx 0.75$. This is consistent with the numerically observed change between the low- and high-temperature behaviors at $k_B T_c \approx 0.25$. Improved estimates of $T_c$ can be made by relaxing the condition of perfect complementarity of the two segments; this as well as a detailed characterization of the low temperature phase will be discussed elsewhere.

**Conclusions:** We studied the behavior of random RNA sequences in the regime below the denaturation transition. A two-replica calculation shows that disorder is perturbatively irrelevant, i.e., an RNA molecule with weak sequence disorder is in the molten phase where many secondary structures with comparable total energy coexist. By further considering the rare regions of strong sequence complementarity, we show that the molten phase cannot exist down to arbitrarily low temperatures, and thereby establish the existence of a distinct low temperature phase below some critical temperature $T_c > 0$. Our analysis follows the approach used in Ref. [14] to show the relevance of disorder in the denaturation of double-stranded DNA. It will be interesting to see whether a renormalization group theory along the line of Ref. [15] may be constructed to elucidate the low-temperature phase of the random RNA problem.

**Acknowledgments:** The authors benefited from discussions with U. Gerland, D. Moroz, and especially L.-H. Tang regarding the role of rare regions. This work is supported by the NSF through grants no. DMR-9971456 and DBI-9970199.

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