The Freezing of Random RNA

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We study secondary structures of random RNA molecules by means of a renormalized field theory based on an expansion in the sequence disorder. We show that there is a continuous phase transition from a *molten phase* at higher temperatures to a low-temperature *glass phase*. The primary freezing occurs above the critical temperature, with local islands of stable folds forming within the molten phase. The size of these islands defines the correlation length of the transition. Our results include critical exponents at the transition and in the glass phase.

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RNA has various important functions in the cell, it forms viral genomes, and has been attributed a key role in the origin of life. RNA molecules fold into *unique* compact configurations able to perform catalytic functions, and they can act as templates for the readout of sequence information. In this sense, they are nature's compromise between DNA and proteins, which explains their likely role in early evolution as well as their ubiquity in today's molecular biology. Typical RNA folds at room temperature consist of *stems* (i.e., parts of the molecule forming a helical double strand stabilized by Watson-Crick base pairing) linked by *loops* (i.e., stretches of unpaired monomers). These conformations are governed by the energies of base pairing and backbone bending as well as by the entropy of the loops; their statistical physics is quite complicated. Yet, the problem is more tractable than protein folding since the free energy of an RNA fold can be separated energetically into that of its *secondary* and its *tertiary* structure [1, 2]. Labeling the bases consecutively along the backbone of the molecule from 1 to L_0, the secondary structure of the fold is completely defined by the Watson-Crick pairs (s,t) ($1 \leq s < t \leq L_0$) subject to the constraint that different pairs are either independent ($s < t < s' < t'$) or nested ($s < s' < t' < t$); see fig. 1. Thus, the secondary structure contains purely "topological" information about the fold, which is independent of the spatial configuration. Due to the constraint on base pairings, secondary structures can always be represented by planar diagrams as shown in fig. 1. The interactions satisfying this constraint are often the dominant part of the free energy, so the secondary structure of a fold can be determined self-consistently. There are efficient algorithms to compute the exact partition function of secondary structures for a given sequence [3, 4]. Base pairings violating the constraint (so-called *pseudoknots*) as well as additional interactions between paired bases are important for the tertiary structure of the molecule (i.e., the full spatial arrangement of stems and loops) but they generate only small-scale rearrangements of the secondary structure [1, 5]. While this separation of energies is only approximate, it can be tuned experimentally by varying salt concentrations in the solution [1]. Hence, a theory of secondary structures is an important starting point for understanding RNA conformations.

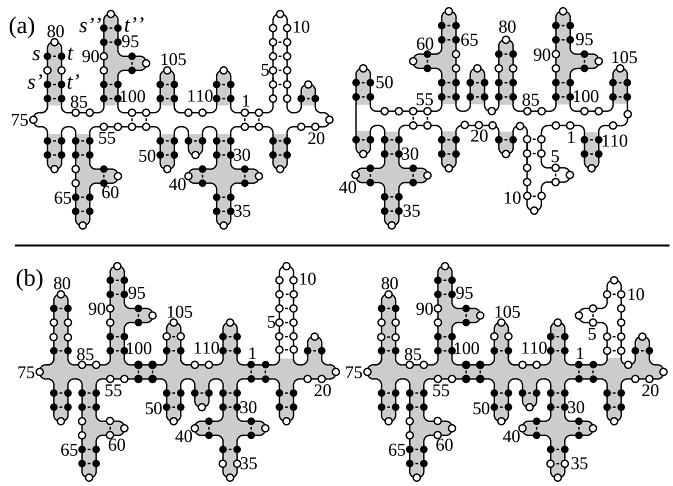

FIG. 1: Secondary structures of a random RNA molecule at distant times. Base pairings can be nested, such as (s,t) and (s',t'), or independent, such as (s,t) and (s'',t''). The pairing overlap is defined by the common base pairings between the left and right configuration (the corresponding bases are shown in black). (a) Above T_c, the molecule contains conserved subfolds on scales up to the correlation length ξ (marked by shading) and is molten on larger scales. (b) Below T_c, the molecule is locked into its minimum energy structure on all scales, up to rare fluctuations (unshaded).

The simplest class of such molecules is *homopolymers*, where all Watson-Crick pairs (s,t) contribute an equal amount f of free energy. At room temperature, where f is typically of order $k_{\rm B}T$, homopolymers have a *molten phase* of compact stem-loop folds. The fold of an individual polymer in the molten phase is not unique. It changes over time since thermal fluctuations continuously build and undo its stems. The *pairing probability* of two bases decays as a power law of their backbone distance [7], $(t-s)^{-\rho_0}$, with $\rho_0 = 3/2$. In a *heteropolymer*, the energy of a Watson-Crick pair (s,t) depends on the nucleotides at the backbone positions paired. An important class is *random* heteropolymers. In biological systems, such sequences result from evolution by *neutral* [8] mutations. For functional RNA, sequences and conformations are further modified by *selection*, but random sequences remain important as reference statistics. A well-known analytical description of this case is to approximate the pairing free energies $\eta(s,t)$ as independent Gaussian random variables given by

$$\overline{\eta(s,t)} = f, \quad \overline{\eta(s,t)\eta(s',t')} - f^2 = \sigma^2\delta(s-s')\delta(t-t'), \quad (1)$$

where f and σ are of order $k_{\rm B}T$ [9]. Free energy-estimates on the basis of this model [10, 11, 12] and numerical simulations [12, 13, 14, 15, 16, 17] indicate that RNA random heteropolymers undergo a transition at a critical temperature T_c (about room temperature) from the molten phase to a low-temperature *glass phase*. The nature of this phase is controversial [12, 13, 17], and the numerical studies may suffer from significant finite-size effects [18]. The two phases can be distinguished by disorder-induced *replica* correlations. Replicas are simply two secondary structures at distant times – i.e., drawn independently from the thermal ensemble – of the same RNA molecule, i.e., the same disorder configuration $\eta(s,t)$ as shown in fig. 1. Correlations between replicas are defined by subsequent averaging over the disorder distribution (1). The arguments of [11, 12] for the *pairing overlap* (defined as the joint probability of two bases being paired in both replicas) suggest that replicas become independent at large backbone distances in the molten phase but are essentially locked into a single conformation in the glass phase.

In this letter, we develop a systematic field theory of random RNA secondary structures. This theory has two basic fields. The *contact field* $\Phi(s,t)$ is defined to be 1 if the bases s and t are paired and 0 otherwise. The *overlap field* between two replicas α and β, defined as $\Psi_{\alpha\beta}(s,t) = \Phi_\alpha(s,t)\Phi_\beta(s,t)$, describes correlations between the replicas. By means of the *height field* $h(r) \equiv \sum_{s=1}^{r}\sum_{t=r+1}^{L_0}\Phi(s,t)$, any secondary structure can be mapped onto a random walk $h(r)$ ($r = 0,\dots,L_0$) with step size $h(r) - h(r-1) = \pm 1$ and boundary conditions $h(0) = h(L_0) = 0$. This mapping relates random RNA folds to the simpler problems of directed polymers in a disordered medium [19] and Kardar-Parisi-Zhang surface growth [20, 21]. Generalizing existing field theoretic approaches [22, 23, 24], we derive renormalization equations for the two fundamental variables of the theory, the *disorder strength* and the *backbone length*. The large-distance scaling of pairing probability and replica overlap are given by the disorder-averaged expectation values

$$\begin{aligned} \overline{\langle\Phi(s,t)\rangle} &\sim (t-s)^{-\rho_0}, \; \overline{\langle\Psi(s,t)\rangle} \sim (t-s)^{-\theta_0} \quad (T > T_c), \\ \overline{\langle\Phi(s,t)\rangle} &\sim (t-s)^{-\rho^*}, \; \overline{\langle\Psi(s,t)\rangle} \sim (t-s)^{-\theta^*} \quad (T \le T_c). \end{aligned} \quad (2)$$

Here $\rho_0 = 3/2$ and $\theta_0 = 3$ are the known exponents of the molten phase [7, 10]. At T_c, our renormalization group gives first-order values $\rho^* = \theta^* \approx 11/8$. As will become clear below, the equality $\rho^* = \theta^*$ is an exact (though not rigorous) conclusion beyond first order provided the renormalization group scenario sketched in fig. 3 is qualitatively correct, i.e., the true exponents are monotonic in p at fixed ε. This equality implies that two replicas are essentially locked into a single conformation already at the transition. Hence, the *leading* scaling is given by the minimum-energy configuration for all temperatures $T \le T_c$, i.e., the exponents $\theta^* = \rho^*$ govern the glass phase as well. The height fluctuations

$$\overline{\langle(h(r) - h(r'))^2\rangle} \sim \begin{cases} (r-r')^{2\zeta_0} & (T > T_c) \\ (r-r')^{2\zeta^*} & (T \le T_c) \end{cases} \quad (3)$$

with $\zeta^* \approx 5/8$ are linked to the contact correlations by the exact scaling relation $\zeta + \rho = 2$ in all phases, which follows from the continuum representation of the h field, $h(r) = \int_0^r {\rm d}s \int_r^{L_0} {\rm d}t\, \Phi(s,t)$ [25], and has been obtained previously in a closely related context [26]. These exponents agree well with the numerical values $\zeta_{\rm glass} = 0.65$ [12, 13] and $\rho_{\rm glass} = 1.3(4)$ [13, 27] for $T = 0$.

Our results show that the glass transition is of second order. A singular length scale

$$\xi \sim |T - T_c|^{-\nu^*}, \quad (4)$$

whose exponent $\nu^* = 1/(2-\theta^*) \approx 8/5$ is determined by hyperscaling, describes the crossover scaling above and below the critical point. The resulting freezing scenario of random RNA molecules is quite intricate. It is illustrated in fig. 1, where we show snapshots of the same molecule at two distant times for two different temperatures. Above T_c, the correlations (2), (3) scale with their critical exponents $\rho^*, \theta^*, \zeta^*$ up to backbone distances $(t-s)$ resp. $|r - r'|$ of order ξ. Hence, an RNA fold has essentially frozen "islands" of size ξ (i.e., its replicas are locked) but is molten on larger scales (its replicas become independent), see fig. 1(a). As T approaches T_c from above, the replica correlation length ξ increases according to (4), and the turnover time between conformations by thermal fluctuations grows. We call this process *primary freezing*. At criticality, there is still a power law distribution of *rare thermal fluctuations* as discussed below. Lowering the temperature below T_c, the correlation length decreases again and even these rare fluctuations are removed from larger to smaller scales; this is called *secondary freezing*, see fig. 1(b).

To derive our renormalization group, we write the secondary structure partition function of a given heteropolymer as a sum over the contact field configurations,

$$\mathcal{Z}[\eta] = \sum_{\Phi} \exp[-\beta \sum_{1\le s<t\le L_0} \eta(s,t)\Phi(s,t)], \quad (5)$$

and study the disorder-averaged free energy $\overline{\mathcal{F}} = -\beta^{-1}{\rm Tr}_\eta \log \mathcal{Z}[\eta]$ obtained from the distribution (1). In the replica formalism, this leads to a system of p interacting homopolymers, $\mathcal{Z}^{(p)} = \sum_{\Phi_1,\dots,\Phi_p} \exp(-\beta\mathcal{H}^{(p)})$, whose Hamiltonian [11, 12]

$$\mathcal{H}^{(p)} = f_0 \sum_\alpha \sum_{s<t} \Phi_\alpha(s,t) - \frac{g_0}{2} \sum_{\alpha\ne\beta} \sum_{s<t} \Psi_{\alpha\beta}(s,t) \quad (6)$$

is given in terms of the contact fields Φ_α ($1 \le \alpha \le p$) and the overlap fields $\Psi_{\alpha\beta}$ ($1 \le \alpha,\beta \le p$, $\alpha \ne \beta$) with the coupling constants $f_0 = f - \beta\sigma^2$ and $g_0 = \beta\sigma^2$. The renormalization of this theory is based on analytic continuation in the homopolymer exponent ρ_0, or equivalently, in the scaling dimension $\varepsilon := 2\rho_0 - 2$ of the coupling constant g_0 [28]. In the limit $p \to 0$, the free energy $\mathcal{F}^{(p)} = -\beta^{-1} \log \mathcal{Z}^{(p)}$ reproduces that of the random system, $\overline{\mathcal{F}} = \lim_{p\to 0} \mathcal{F}^{(p)}/p$.

The noninteracting theory ($g_0 = 0$) describes homopolymers in the molten phase and is exactly solvable in the continuum limit, i.e., for molecules of backbone length $L_0 \gg 1$. The free energy for closed rings is $\mathcal{F}_0 = p\,\rho_0 \log L_0$ [7, 10]. The correlation function of N contact fields $\Phi_\alpha(s_i, t_i)$ describes constrained configurations of the molecule with N fixed pairings (s_i, t_i) ($i = 1, \dots, N$). These pairings generate $N+1$ subrings of backbone lengths $\ell_1, \dots, \ell_N, \ell_{N+1} = L_0 - \sum_{j=1}^N \ell_j$. Since the secondary structure fluctuations in the subrings are independent, this correlation takes the factorized form

$$\langle \Phi_\alpha(s_1,t_1) \dots \Phi_\alpha(s_N,t_N)\rangle_0 = \frac{\ell_1^{-\rho_0} \dots \ell_{N+1}^{-\rho_0}}{L_0^{-\rho_0}}. \qquad (7)$$

Overlap correlations factorize further into the contributions of the single replicas upon insertion of the definition $\Psi_{\alpha\beta}(s_i,t_i) = \Phi_\alpha(s_i,t_i)\Phi_\beta(s_i,t_i)$.

In the presence of interactions, we write the free energy as a perturbation series,

$$\begin{aligned}\mathcal{F}(g_0, L_0) = \mathcal{F}_0 - \tfrac{p(p-1)}{2}\Big[g_0 \int_{0<s_1<t_1<L_0} \langle \Psi_{\alpha\beta}(s_1,t_1)\rangle_0 \\ + g_0^2 \int_{\substack{0<s_1<t_1<s_2<t_2<L_0 \\ \text{or } 0<s_1<s_2<t_2<t_1<L_0}} \Big(\langle \Psi_{\alpha\beta}(s_1,t_1)\Psi_{\alpha\beta}(s_2,t_2)\rangle_0^c \\ + 2(p-2)\langle \Psi_{\alpha\beta}(s_1,t_1)\Psi_{\alpha\gamma}(s_2,t_2)\rangle_0^c \Big)\Big] + O(g_0^3). \qquad (8)\end{aligned}$$

This series contains *connected* overlap correlations evaluated at $g_0 = 0$. The first-order term involves two, the second-order terms involve two and three pairwise different replicas, respectively; see fig. 2(a)–(c). The integration over the contact points in (8) produces a singular dependence of the free energy on g_0 as well as ultraviolet-divergent terms which are regular in g_0. Performing these integrals and expanding about the point of marginality ($\varepsilon = 0$), we obtain the leading singular part

$$\begin{aligned}\mathcal{F}(u_0, L_0) = p\Big[\log L_0 + (p-1)\frac{u_0}{\varepsilon} - \frac{(p-1)C_p u_0^2}{2\varepsilon^2} \\ + O\big(\varepsilon, u_0\varepsilon^0, u_0^2/\varepsilon, u_0^3\big)\Big] \qquad (9)\end{aligned}$$

with the dimensionless coupling constant $u_0 = g_0 L_0^{-\varepsilon}$ and $C_p = 1 - 2(p-2)$. The poles in (9) are absorbed into a renormalized coupling $g = Z_g g_0$ and a renormalized backbone length $L = Z_L L_0$, such that the free energy becomes an analytic function of the dimensionless coupling $u = gL^{-\varepsilon}$. In a minimal subtraction scheme, we extract from (9) these Z-factors to leading order,

$$Z_g = 1 - C_p \frac{u}{\varepsilon} + O(u^2), \;\; Z_L^{-1} = 1 - (p-1)\frac{u}{\varepsilon} + O(u^2). \quad (10)$$

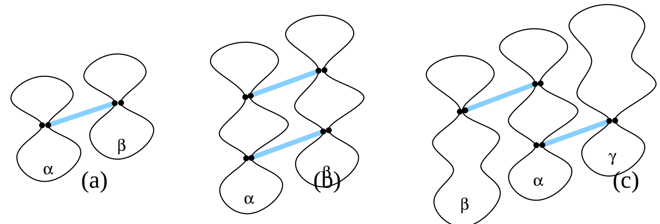

FIG. 2: Overlap correlations in the series (8). (a) Two-replica one-point function $\langle \Psi_{\alpha\beta}(s_1,t_1)\rangle_0$. (b) Two-replica two-point function $\langle \Psi_{\alpha\beta}(s_1,t_1)\Psi_{\alpha\beta}(s_2,t_2)\rangle_0$. (c) Three-replica two-point function $\langle \Psi_{\alpha\beta}(s_1,t_1)\Psi_{\alpha\gamma}(s_2,t_2)\rangle_0$.

The resulting renormalization group flow takes a simple form with respect to the renormalized scale L,

$$\tilde\beta(u) \equiv L\frac{\partial}{\partial L}u = -\varepsilon u + C_p u^2 + O(u^3), \qquad (11)$$

$$\gamma_L(u) \equiv \frac{L}{L_0}\frac{\partial}{\partial L}L_0 = 1 + (p-1)u + O(u^2). \qquad (12)$$

The beta function is defined as the flow with respect to the original scale L_0,

$$\beta(u) \equiv L_0\frac{\partial}{\partial L_0}u = \frac{\tilde\beta(u)}{\gamma_L(u)} = \frac{-\varepsilon u + C_p u^2 + O(u^3)}{1 + (p-1)u + O(u^2)}. \qquad (13)$$

It has a nontrivial fixed point $u^* = \varepsilon/C_p + O(\varepsilon^2)$ for generic p, which is ultraviolet-unstable for $\varepsilon > 0$ and marks the RNA glass transition for $\varepsilon = 1$, $p \to 0$. The ε-expansion can be analyzed at higher orders using the *operator product expansion* of the fields Φ and Ψ. Generalizing the arguments of [22, 24], we find that the theory is renormalizable in g and L (for details, see [29]). The field Φ is renormalized by a factor $Z_\Phi = Z_L^{-2} + O(u^2)$ [30]. By the scaling relation $\zeta + \rho = 2$, this implies "superdiffusive" height fluctuations with exponent $\zeta^* = \zeta_0/\gamma_L^* + O(\epsilon^2)$ for $p < 1$, where $\gamma_L^* \equiv \gamma_L(u^*)$ [30]. The renormalization of Ψ is tied to that of its conjugate coupling g. Hence, the dimensions of Φ and Ψ at the transition are two independent exponents,

$$\rho^* = \frac{\rho_0 + L\partial_L \log Z_\Phi}{\gamma_L(u^*)} = \frac{1 + \varepsilon/2 + 2(p-1)\varepsilon/C_p}{1 + (p-1)\varepsilon/C_p} + \dots,$$

$$\theta^* = 2 - \beta'(u^*) = 2 - \frac{\varepsilon}{1 + (p-1)\varepsilon/C_p} + \dots; \qquad (14)$$

the omitted terms are of order $p-2$ and ε^2. These expressions are valid within the constraints $\theta^* \geq \rho^*$, since two-replica overlap correlations decay at least as fast as single-replica ones, and $\zeta^* \geq \zeta_0$. The resulting dependence of the critical exponents on p and ϵ is shown in fig. 3. (a) For $p = 2$, we have shown that the theory is *one-loop renormalizable*, i.e., the expressions (10) to (13) and (14) for θ^* are exact [29]. This reflects the exact summability of the partition function as shown in [12] for $\varepsilon = 1$. We have generalized this solution at the transition point to arbitrary ε, giving $\zeta^* = \zeta_0$ and $\rho^* = \rho_0$ (the renormalization group results are subleading). For $\varepsilon = 1$, we thus have $\theta^* = \rho^* = 3/2$. Hence, two replicas are essentially locked into a single conformation already

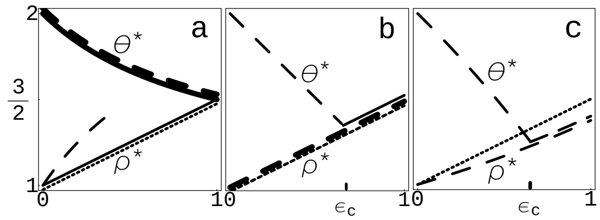

FIG. 3: The critical exponents ρ^* and θ^* as a function of ε for (a) $p = 2$, (b) $p = 1$, and (c) $p = 0$. Exact results (thick solid), renormalization group results valid to all orders (thick dashed) or to first order (thin dashed), presumably exact values (see text, thin solid), reference line $\rho_0(\varepsilon)$ (dotted).

at the transition. The borderline value $\varepsilon_c = 1$ corresponds to the upper critical dimension $d_{\rm uc} = 4$ of directed polymers [22, 31]. (b) For $p = 1$, renormalization gives $\rho^* = \rho_0$ exactly to all orders, and $\theta^* = 2 - \varepsilon + O(\varepsilon^2)$. This produces a borderline value $\varepsilon_c \approx 2/3$, beyond which $\theta^* = \rho^* = \rho_0$ exactly. (c) For $p = 0$, the first-order eq. (14) produces an even smaller value of ε_c. For $\varepsilon = 1$, we find locked configurations with $\theta^* = \rho^* = 2 - \zeta^* \approx 11/8$ as reported above. For $\varepsilon > \varepsilon_c$, the renormalization-group exponent θ^* in (14) describes a *subleading* singularity in the overlap correlations, which is related to rare critical fluctuations within the locked state [29], cf. [32] for directed polymers.

Despite its technical difficulties, our renormalization is rather intuitive since it acts directly on the fold configurations of Fig. 1. In a Wilson scheme, we would produce coarse-grained folds with varying short-distance cutoff $\ell_{\rm min}$ by integrating out subconfigurations of backbone length $\ell < \ell_{\rm min}$. This leads to a scale-dependent backbone length L and coupling constant g. For $p > 1$, the attractive replica interaction produces additional short loops, which are cut off under coarse-graining, i.e., the effective length is *shorter* than without interaction ($L \sim L_0^{1/\gamma_L^*}$ with $\gamma_L^* > 1$). For $p < 1$, however, this effect is reversed ($\gamma_L^* < 1$): L becomes *longer* and the random walk $h(r)$ becomes correlated with superdiffusive fluctuations ($\zeta^* = 1/2$, $\gamma_L^* > 1/2$). Hence, the probability of first return is shifted from small to large scales, i.e. there are more pairings between distant nucleotides ($\rho^* < \rho_0$). The locking of pairing correlations ($\theta^* = \rho^*$) at criticality means that disorder has already its maximal effect on scaling, i.e., the same exponents govern the glass phase. This prediction is remarkable in contrast to random directed polymers, where the roughening transition has no locking for $2 < d < d_{\rm uc}$ and the low-temperature physics is governed by a new strong-coupling fixed point.

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