RANDOM MATRIX THEORY AND RNA FOLDING*

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I review the approach of using large N matrix field theory to fold RNA and then discuss a recent simplified model that could be solved analytically. I then outline how entropic contributions could be included starting from first principles.

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Over the last decade, RNA has transformed itself from being a relatively minor player in the central dogma of Watson and Crick to being one of the central players in molecular biology. Indeed, it has been demonstrated that in addition to its "information carrier" role in protein synthesis, some types of RNA's, known as ribozymes, have an enzymatic activity which is crucial to the functioning of the cell. As a consequence of this new prominent role of RNA, the search for the three dimensional structure of RNA has become an important problem in biology. This view was expressed forcefully by Tinoco and Bustamante [1].

With various collaborators [2–6] Henri Orland and I have developed in detail a practical program to fold RNA using large N matrix field theory. A excellent review of this program may be found in the preceding talk by Orland [7] at this conference. In this talk I focus on a simplified and soluble version of this problem [6].

Let us begin with a schematic overview of the relevant biology for the benefit of some readers. RNA is a heteropolymer constructed out of a four-letter alphabet: C,G,A, and U (for the four bases or nucleotides: cytosine, guanine, adenine, and uracil). In solution, there is an attraction between

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C and G and between A and U, with energies $\varepsilon(\mathrm{C},\mathrm{G}) \simeq -3$ kCal/mole and $\varepsilon(\mathrm{A},\mathrm{U}) \simeq -2$ kCal/mole respectively (300° K $\simeq 0.6$ kCal/mole $\simeq 1/40$ eV). There is also a weaker attraction between G and U, with $\varepsilon(\mathrm{G},\mathrm{U}) \simeq -1$ kCal/mole.

Consider an RNA sequence $\{s\} = \{s_1, s_2, \cdots, s_L\}$ (where s_i takes on one of the 4 possible values C,G,A, and U). For example, we might be given the sequence $\{s\} = \{\mathrm{CCCGUUAACCG}\cdots\}$. Given this sequence the RNA heteropolymer folds itself into a definite shape due to the attraction just mentioned between the nucleotides. The problem is to determine the configuration most favored by energetic and entropic considerations. With $L \sim 10^2$ for example the combinatorial possibilities are already staggering.

The hydrogen bond responsible for the attraction between nucleotides saturates, which merely means that once a given C is paired with a G, it cannot be paired with another G. For our purposes we could think of the nucleotides as beads on a flexible chain, with the beads to be "glued" together in pairs.

As discussed in detail in [2] and in the preceding talk by Orland [7] at this conference, the combinatorial heart of the problem is given by the following integral over L independent $N \times N$ Hermitian matrices φ_i, $i = 1, \ldots, L$:

$$Z_L(N) = \frac{1}{A_L(N)} \int \prod_{k=1}^{L} d\varphi_k \, e^{-\frac{N}{2} \sum_{ij} (V^{-1})_{ij} \mathrm{Tr}(\varphi_i \varphi_j)} \frac{1}{N} \mathrm{Tr} \prod_{l=1}^{L} (1 + \varphi_l) \quad (1)$$

with the normalization factor

$$A_L(N) = \int \prod_{k=1}^{L} d\varphi_k e^{-\frac{N}{2} \sum_{ij} (V^{-1})_{ij} \mathrm{Tr}(\varphi_i \varphi_j)} \, . \quad (2)$$

Here V_{ij} is a given, but rather complicated, L by L matrix constructed out of Boltzmann factors and so on. This Gaussian integral is easily evaluated to be

$$\begin{aligned} Z_L(N) \; = \; & 1 + \sum_{i<j} V_{ij} + \sum_{i<j<k<l} V_{ij} V_{kl} + \sum_{i<j<k<l} V_{il} V_{jk} + \ldots \\ & + \frac{1}{N^2} \left(\sum_{i<j<k<l} V_{ik} V_{jl} + \ldots \right) + \frac{1}{N^4} (\ldots) + \cdots \, . \end{aligned} \quad (3)$$

If we define $e^{-\beta\mu} \equiv \frac{1}{N^2}$ then we can write (3) as

$$Z_L(N) \; = \; 1 + \sum_{i<j} V_{ij} + \sum_{i<j<k<l} V_{ij} V_{kl} + \sum_{i<j<k<l} V_{il} V_{jk} + \ldots$$

$$+e^{-\beta\mu}\left(\sum_{i<j<k<l} V_{ik}V_{jl}+\dots\right)+e^{-2\beta\mu}\times(\text{genus 2 pseudoknots})$$
$$+e^{-3\beta\mu}\times(\text{genus 3 pseudoknots})+\dots$$
$$=\sum_{C_{ij}} e^{-\beta(E(C)+\mu g(C))}\,. \tag{4}$$

Here C denotes the $L\times L$ real symmetric contact matrix

$$C_{ij}=\begin{cases}1 & \text{if } i,j \text{ are bonded}\\ 0 & \text{otherwise}\end{cases}\,, \tag{5}$$

$E(C)$ the energy of the configuration C, $g(C)$ the genus of the configuration C, and $\sum_{C_{ij}}$ the sum over all possible contact matrices. In other words, the RNA matrix model in (1) describes the folding of an RNA molecule at zero entropy (*i.e.* very low temperature), but with a topological chemical potential $\mu\geq 0$ which effectively controls the amount of pseudoknots in the folded molecule. Each term in (4) corresponds to one particular way of folding the RNA molecule.

The expression in (1) contains information about the folding energy of an RNA molecule, but not its entropy. It was argued in [2] that for a first approximation it may suffice to suppress the entropic factor. In the Appendix we sketch how the entropic factor could be added into our formulation for a price.

In [2] the matrix integral was massaged into the form of a matrix field theory which was then studied by a steepest descent expansion in the large N limit. The resulting expression proved to be highly non-trivial, but reproduced all the known pseudoknots and more [3, 4]. In a recent paper, [6] Vernizzi, Orland, and I studied a much simplified version of this problem. We replaced the complicated matrix V by the much simpler matrix defined by $V_{ij}=v$ for $i\neq j$ and $V_{ii}=v+a$, with a a regulator so that V^{-1} exists. After a series of Hubbard–Stratanovich transformations, we can simplify (1) to

$$Z_L(N)=\frac{1}{\tilde{A}(N)}\int d\sigma\, e^{-\frac{N}{2v}\,\mathrm{Tr}\sigma^2}\,\frac{1}{N}\mathrm{Tr}(1+\sigma)^L \tag{6}$$

with $\tilde{A}(N)$ another normalization factor. The original integration over the L matrices φ_k in Eq. (1) has been reduced to an integration over a single $N\times N$ matrix σ. I may perhaps remind the reader at this point that L is the length of the RNA, while N would be related to the Mg^{++} ion concentration in biological terms. The parameter v measures the strength with which the nucleotides are "glued" to each other.

At this point, we have a standard problem in random matrix theory which could be solved in two different ways. Since this is a Gaussian problem we could, after using a series of identities, reduce the generating function for $Z_L(N)$ to a generalized Laguerre polynomial [6]. Alternatively, we could modify a method introduced by Kazakov to turn the generating function into a contour integral [8].

To understand the information contained in $Z_L(N)$ let us consider a specific example, say

$$Z_8(N) = 1 + \left(28v + 140v^2 + 140v^3 + 14v^4\right) + \frac{(70v^2 + 280v^3 + 70v^4)}{N^2} + \frac{21v^4}{N^4}. \tag{7}$$

The meaning of these numbers, 28, 140, and so on, are as follows. The power of v corresponds to the number of pairs of nucleotides that are glued together. The power of $1/N^2$ is the topological genus of the diagram as explained in [2] and [5]. Thus, for $L = 8$ there are 28 planar diagrams with one pair of nucleotides glued together, and 70 diagrams on the torus (*i.e.* genus one closed surface) with two pairs of nucleotides glued together, and so on. The total number of diagrams for each fixed genus can be obtained by putting $v = 1$. For instance, the total number of diagrams on the torus for $L = 8$ is $(70v^2+280v^3+70v^4)/v \to 1 = 420$. On the other hand, the total number of diagrams, irrespective of the genus, can be obtained by putting $N = 1$. For instance, the number of diagrams for $L = 8$ with four pairs of nucleotides glued together is $14 + 70 + 21 = 105$.

The general $1/N^2$ topological expansion of $Z_L(N)$ with $v = 1$ is:

$$Z_L(N) = \sum_{L=0}^{\infty} a_{L,g} \frac{1}{N^{2g}}\,, \tag{8}$$

where the coefficients $a_{L,g}$ give exactly the number of diagrams at fixed length L and fixed genus g. We could recursively obtain all the coefficients $a_{L,g}$. Moreover, by normalizing each $a_{L,g}$ by the total number of diagrams at fixed L, *i.e.* by $\mathcal{N} \equiv Z_L(1)$, we can obtain the distribution of the number of diagrams. Plots of the distributions of diagrams as a function of L and g are given in [6]. We note the interesting feature that for any given $L \gg 1$ most of the diagrams are not planar, and they have a genus close to a characteristic value $\langle g \rangle_L$ which increases with L: we find numerically that it scales like $\langle g \rangle_L \sim 0.23L$ at large L. Also, for each fixed L there is a maximum possible value for g, namely $g \leq L/4$. Conversely a structure can have a genus g only if it has a length at least $L \geq 4g$.

In conclusion, we have shown how to compute the number of folded structures as a function of the length and of the genus of the RNA. This model is of course very schematic and oversimplified. It shows however that

for a random RNA, the average topological character scales linearly with the length of the chain. As most wild RNA have an almost planar structure (with a genus $g \leq 2$), this implies that their sequences have been greatly designed by evolution in order to achieve this specificity.

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Appendix

We will now sketch how entropic considerations could be incorporated into our matrix theory formulation. The material in this appendix is based on work with Orland and Vernizzi [9].

It is instructive to start from the first principles of statistical mechanics. Boltzmann taught us to evaluate the sum over all configurations $Z = \sum_{\text{config}} e^{-\beta E(\text{config})}$ where $\beta = 1/T$ is the inverse temperature in units with the Boltzmann constant set to unity. In the present context we have then

$$Z = \int \cdots \int d^3x_1 \cdots d^3x_L e^{-\beta \sum_{j=1}^{L-1} K(|\vec{x}_{j+1} - \vec{x}_j|)} \times \prod_{i>j} e^{-\beta w_{hc}(|\vec{x}_i - \vec{x}_j|)} e^{-\beta E(|\vec{x}_i - \vec{x}_j|, s_i, s_j, i, j)}. \tag{9}$$

Here $K(|\vec{x}_{j+1} - \vec{x}_j|)$ denotes the energy of the backbone along the chain connecting the nucleotide at j to the next nucleotide at $j+1$, $w_{hc}(|\vec{x}_i - \vec{x}_j|)$ a hard-core potential between nucleotides at i and j, and $E(|\vec{x}_i - \vec{x}_j|, s_i, s_j, i, j)$ denotes the binding energy of a pair of nucleotides at i and j when they are bound. Note that $E(|\vec{x}_i - \vec{x}_j|, s_i, s_j, i, j)$ depends not only on the separation between the two nucleotides but also on their identities s_i and s_j and their location along the chain i and j.

We will comment on these three energies and the series of approximations that one may consider making to render the problem tractable or at least formulable as a matrix field theory.

The hard-core repulsion $w_{\text{hc}}(|\vec{x}_i - \vec{x}_j|)$ takes into account the fact that nucleotides are to first approximation hard spheres with radius b. In principle, we should also include the dependence of w_{hc} on s_i and s_j since different nucleotides have different sizes. In practice, the hard-core repulsion just means that in the Monte-Carlo integration over the spatial location of the nucleotides the integrand is to be set equal to 0 whenever $|\vec{x}_i - \vec{x}_j| < 2b$.

The energy $E(|\vec{x}_i - \vec{x}_j|, s_i, s_j, i, j)$ will in general have the form

$$E(|\vec{x}_i - \vec{x}_j|, s_i, s_j, i, j) = \theta(|i-j| > 4)\varepsilon(s_i, s_j) v(|\vec{x}_i - \vec{x}_j|, s_i, s_j)\,. \tag{10}$$

The step function $\theta(|i-j| > 4)$ takes into account the rigidity constraint, that the chain is not infinitely flexible. Only nucleotides spaced apart by

more than 4 units could bind. The 4×4 symmetric matrix $\varepsilon(s_i, s_j)$ has entries that are either 0 or 1. Thus, we could take $\varepsilon(\mathrm{C}, \mathrm{G}) = 1$, $\varepsilon(\mathrm{A}, \mathrm{U}) = 1$, $\varepsilon(\mathrm{C}, \mathrm{U}) = 0$, and so on. The attractive potential $v(|\vec{x}_i - \vec{x}_j|, s_i, s_j)$ could also depend on s_i and s_j as indicated; for instance the potential between C and G may be taken to be different from the potential between A and U. We are going to make the simplifying approximation that the most important dependence on s_i and s_j has already been taken into account by the matrix $\varepsilon(s_i, s_j)$. Evidently, we are also making the approximation that the binding between nucleotides does not depend on orientation so that v depends only on $|\vec{x}_i - \vec{x}_j|$ and not on $\vec{x}_i - \vec{x}_j$.

Since we already took out the hard-core repulsion, the attractive potential $v(r)$ goes negative for $b < r < R$, and vanishes otherwise; here R denotes the range of the potential. Two length scales b and R are involved. If the approximation $b \ll R$ is valid, then we could take $v(r)$ to be a well of depth v_0 extending from $r = 0$ to $r = R$.

Since the factor $e^{-\beta E(|\vec{x}_i - \vec{x}_j|, s_i, s_j, i, j)}$ is equal to 1 when the nucleotides are not bound, it is convenient to define U_{ij} by

$$e^{-\beta E(|\vec{x}_i - \vec{x}_j|, s_i, s_j, i, j)} = 1 + (e^{-\beta E(|\vec{x}_i - \vec{x}_j|, s_i, s_j, i, j)} - 1) \equiv 1 + U_{ij}(|\vec{x}_i - \vec{x}_j|, s_i, s_j)\,. \tag{11}$$

If the approximate form for $v(r)$ holds, then $U_{ij}(|\vec{x}_i - \vec{x}_j|, s_i, s_j) \simeq e^{\beta w} - 1$ if $|\vec{x}_i - \vec{x}_j| < R$ and $|i - j| > 4$, and $U_{ij}(|\vec{x}_i - \vec{x}_j|, s_i, s_j) \simeq 0$ otherwise. In other words, U_{ij} is non-zero when the nucleotides at i and j are close enough to be paired with each other.

Next, if w is large enough and R small enough, we could try to represent the $|\vec{x}_i - \vec{x}_j|$ dependence of $U_{ij}(|\vec{x}_i - \vec{x}_j|, s_i, s_j)$ approximately by a delta function

$$U_{ij}(|\vec{x}_i - \vec{x}_j|, s_i, s_j) = V_{ij}(s_i, s_j)\delta(|\vec{x}_i - \vec{x}_j|)\,. \tag{12}$$

In this approximation, $V_{ij}(s_i, s_j)$ depends on s_i and s_j through $\varepsilon(s_i, s_j)$: $V_{ij}(s_i, s_j) = 0$ if $\varepsilon(s_i, s_j) = 0$.

Finally, we come to $K(|\vec{x}_{j+1} - \vec{x}_j|)$, the energy of the backbone along the chain connecting the nucleotide at j to the next nucleotide at $j + 1$. This is the easiest to discuss since there is a literature on simple models of homopolymers for which the partition is given by

$$Z_{\text{simple}} = \int \cdots \int d^3x_1 \cdots d^3x_L e^{-\beta \sum_{j=1}^{L-1} K(|\vec{x}_{j+1} - \vec{x}_j|)}$$

and not the vastly more complicated (9). Various models for $K(|\vec{x}_{j+1} - \vec{x}_j|)$ could be used. For example, we could suppose the nucleotides to be connected by elastic rods of length l and spring constant k, so that $K(|\vec{x}_{j+1} - \vec{x}_j|) = \frac{1}{2}k|\vec{x}_{j+1} - \vec{x}_j|^2$. Another popular approximation is to use

rigid rods of length a and replace the Boltzmann factor $e^{-\beta K(|\vec{x}|)}$ by the probability distribution $P_\delta(\vec{x}) = \frac{1}{4\pi a^2}\delta(|\vec{x}| - a)$. Since the Gaussian is easier to handle than the delta function, a further approximation is often made replacing $P_\delta(|\vec{x}|)$ by the probability distribution $P(\vec{x}) = (\frac{3}{2\pi a^2})^{\frac{3}{2}} e^{-\frac{3}{2a^2}|\vec{x}|^2}$, where the coefficient in the width of the Gaussian is determined by demanding that the second moment $\langle|\vec{x}|^2\rangle$ is the same for the two distributions $P_\delta(\vec{x})$ and $P(\vec{x})$. (Note that the ratio of the first moment $\langle|\vec{x}|\rangle$ in P to that in P_δ is $2\sqrt{\frac{2}{3\pi}} \sim 0.92$, a number close enough to 1 for our purposes.)

We would like to generalize the matrix model in Eq. (1) to include the entropic contribution. Given the detailed discussion earlier, it is now clear that all we have to do is write, instead of (1),

$$\begin{aligned} Z_L(N) \;=\; & \int\cdots\int d^3x_1\cdots d^3x_L e^{-\beta\sum_{j=1}^{L-1} K(|\vec{x}_{j+1}-\vec{x}_j|)} \prod_{i>j} e^{-\beta w_{\rm hc}(|\vec{x}_i-\vec{x}_j|)} \\ & \times \frac{1}{A_L(N)} \int \prod_{k=1}^{L} d\varphi_k \, e^{-\frac{N}{2}\,\sum_{ij}(U^{-1})_{ij}\,{\rm Tr}(\varphi_i\varphi_j)} \frac{1}{N}{\rm Tr}\prod_{l=1}^{L}(1+\varphi_l). \end{aligned} \tag{13}$$

Note that in the integral over the φ_k's the inverse of V is now replaced by the inverse of U where $U_{ij}(|\vec{x}_i - \vec{x}_j|, s_i, s_j) = V_{ij}(s_i, s_j)\delta(|\vec{x}_i - \vec{x}_j|)$ as given in (12). The result of the integration over the φ_k's is now an expression similar to (3) but with $V_{ij}(s_i, s_j)$ replaced by $V_{ij}(s_i, s_j)\delta(|\vec{x}_i - \vec{x}_j|)$.

To write this as a matrix field theory, we need to make a further rough approximation. The radial delta function $\delta(|\vec{x}_i - \vec{x}_j|)$ is difficult to handle and so we replace it by the 3-dimensional delta function $\delta^{(3)}(\vec{x}_i - \vec{x}_j)$ through the following schematic steps

$$\delta(|\vec{x}_i - \vec{x}_j|) \sim 4\pi|\vec{x}_i - \vec{x}_j|^2\delta^{(3)}(\vec{x}_i - \vec{x}_j) \sim 4\pi R^2\delta^{(3)}(\vec{x}_i - \vec{x}_j)\,. \tag{14}$$

We then obtain (suppressing the normalization factor)

$$\begin{aligned} Z_L(N) \;=\; & \int\cdots\int d^3x_1\cdots d^3x_L e^{-\beta\sum_{j=1}^{L-1} K(|\vec{x}_{j+1}-\vec{x}_j|)} \prod_{i>j} e^{-\beta w_{hc}(|\vec{x}_i-\vec{x}_j|)} \\ & \times \int \prod_{i=1}^{L} \mathcal{D}\Phi_i(\vec{x})\, e^{-\frac{N}{2}\int d^3x\ \sum_{ij}(V^{-1})_{ij}\,{\rm Tr}(\Phi_i(\vec{x})\Phi_j(\vec{x}))} \\ & \times \left\{ \frac{1}{N}{\rm Tr}\prod_{l=1}^{L}(1+\Phi_l(\vec{x}_l)) \right\}. \end{aligned} \tag{15}$$

Note that the matrices φ_i have been promoted to matrix fields $\Phi_i(\vec{x})$.

Within this field theoretic framework we could also extend and generalize this expression further. In particular, by replacing $\mathrm{Tr}(\Phi_i(\vec{x})\Phi_j(\vec{x}))$ by $\mathrm{Tr}(\Phi_i(\vec{x})(-\nabla^2+m^2)\Phi_j(\vec{x}))$ we could have an interaction potential of finite range $\sim m^{-1}$ instead of the infinitely short ranged delta function in (14). When we carry out the Gaussian integration over the matrix fields $\Phi_i(\vec{x})$'s we obtain for every contraction a factor V_{ij} multiplied by the Yukawa function $\int \frac{d^3k}{(2\pi)^3}\frac{e^{i\vec{k}\cdot(\vec{x}_i-\vec{x}_j)}}{k^2+m^2}$.

As remarked earlier, the hard core repulsion could probably be handled only numerically, and so we suppress it here. If we also make the rigid rod approximation, then we obtain

$$Z_L(N) = \int \prod_{i=1}^{L} \mathcal{D}\Phi_i(\vec{x})\, e^{-\frac{N}{2}\int d^3x \sum_{ij}(V^{-1})_{ij}\,\mathrm{Tr}(\Phi_i(\vec{x})\Phi_j(\vec{x}))} \times \int \prod_{k=1}^{L-1} d^3x_2 e^{-\frac{3}{2a^2}|\vec{x}_{j+1}-\vec{x}_j|^2} \left\{ \frac{1}{N}\mathrm{Tr}\prod_{l=1}^{L}(1+\Phi_l(\vec{x}_l)) \right\} . \quad (16)$$

This multimatrix field theory model contains the energy rules of the RNA, the entropic contribution of the chain in 3D, some sterical constraints (the chain is not infinitely stretchable). By making a systematic expansion we could obtain the entropy factor, which in the literature is typically included in an ad hoc fashion. Note that in this field theory the action is merely Gaussian, but the observable is rather involved.

In summary, we note that the advantage of the formalism introduced in [2] and further developed here [9] is that it allows us to make a systematic expansion, in contrast to some of the more ad hoc approaches in the literature.

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