Small-world networks and RNA secondary structures

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Abstract

Let $S_n$ denote the network of all RNA secondary structures of length $n$, in which undirected edges exist between structures $s, t$ such that $t$ is obtained from $s$ by the addition, removal or shift of a single base pair. Using context-free grammars, generating functions and complex analysis, we show that the asymptotic average degree is $O(n)$ and that the asymptotic clustering coefficient is $O(1/n)$, from which it follows that the family $S_n, n = 1, 2, 3, \ldots$ of secondary structure networks is not small-world.

1 Introduction

Small-world networks, first introduced in \cite{18}, satisfy two properties: (1) the shortest path distance between any two nodes is “small” (e.g. six degrees of separation between any two persons), and (2) the average clustering coefficient is large (e.g. friends of a person tend to be friends of each other). Small-world networks appear to be ubiquitous in biology, sociology, and information technology; indeed, examples include the neural network of \emph{C. elegans} \cite{18}, the gene co-expression in \emph{S. cerevisiae} \cite{17}, protein folding networks \cite{16, 2}, the low energy RNA secondary structure network of \emph{E. coli} phe-tRNA \cite{19}, etc. For additional examples, see the excellent review of Albert and Barabási \cite{1}.

In this paper, we investigate asymptotic properties of degree and clustering coefficient for the ensemble of all RNA secondary structures, by using methods from algebraic combinatorics. In particular, we rigorously prove that the network of RNA secondary structures is asymptotically not small-world, although it displays strong differences from random networks.

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2 Preliminaries

In this section, we define notions of RNA secondary structure, move sets $M_{S1}, M_{S2}$, and small-world networks. An RNA secondary structure of length $n$, subsequently called length $n$ structure, is defined to be a set $s$ of ordered pairs $(i, j)$, with $1 \leq i < j \leq n$, such that:

1. There are no base triples; i.e. if $(i, j), (k, \ell) \in s$ and $\{i, j\} \cap \{k, \ell\} \neq \emptyset$, then $i = k$ and $j = \ell$.
2. There are no pseudoknots; i.e. if $(i, j), (k, \ell) \in s$, then it is not the case that $i < k < j < \ell$.
3. There are at least $\theta = 3$ unpaired bases in a hairpin loop; i.e. if $(i, j) \in s$, then $j - i > \theta = 3$.

Note that base pairs are not required to be Watson-Crick or wobble pairs, as is the case for RNA molecules, such as that depicted in Figure 1a. This definition, sometimes called homopolymer secondary structure, permits the combinatorial analysis we employ to show that RNA networks are not small-world.

Let $S_n$ denote the set of all length $n$ structures. The move sets $M_{S1}$ and $M_{S2}$, defined in [9] for RNA secondary folding kinetics, describe elementary moves that transform a structure $s$ into another structure $t$. Move set $M_{S1}$ [resp. $M_{S2}$] consists of either removing or adding [resp. removing, adding or shifting] a single base pair, provided the resulting set of base pairs constitutes a valid structure, where shift moves are depicted in Figure 2. We overload the notation $S_n$ to also denote the $M_{S1}$ network [resp. $M_{S2}$ network], whose nodes are the length $n$ structures, where an undirected edge between structures $s, t$ exists when $t$ is obtained from $s$ by a single move from $M_{S1}$ [resp. $M_{S2}$]. Figure 1b shows the $M_{S1}$ network (8 red edges) [resp. $M_{S2}$ network (8 red and 8 blue edges)] for length 7 structures, where there are 8 nodes, $M_{S1}$ degree $\frac{16}{8} = 2$ and $M_{S2}$ degree $\frac{32}{8} = 4$. See [4] and [5] for dynamic programming algorithms that compute, respectively, the $M_{S1}$ and $M_{S2}$ degree for the network of secondary structures of a given RNA sequence.

Small-world networks satisfy two conditions: (1) on average, the minimum path length between any two nodes is small, (2) neighbors of a node tend to be connected to each other. The global clustering coefficient, defined in equation (77) of [14], is given by

$$C_g(G) = \frac{3 \times \text{number of triangles}}{\text{number of connected triples}}$$

where a triangle is a set $\{x, y, z\}$ of nodes, each of which is connected by an edge, and a (connected) triple is a set $\{x, y, z\}$ of nodes, such that there is an edge from $x$ to $y$ and an edge from $x$ to $z$. Following [6], the family $\{S_n, n = 1, 2, 3, \ldots\}$ of RNA networks is small-world if the following conditions hold. (1) There is a constant $c_1 \geq 0$, such that the minimum path length between any two nodes of $S_n$ is bounded above by $c_1 \ln n$. (2) There is a constant $c_2 \geq 0$, such that the average network degree of $S_n$ is bounded above by $c_2 \ln n$. (3) The global clustering coefficient is bounded away from zero. By Theorem 2, the network size of $S_n$ is exponential in $n$. Since there are at most $\left\lfloor \frac{n-\theta}{2} \right\rfloor$ base pairs in any length $n$ structure, the minimum path length between any two structures $s$ and $t$ is at most $n - \theta$; indeed, such a path can be achieved by successively removing each base pair $(i, j) \in s$, then successively adding each base pair $(i, j) \in t$. It follows that condition (1) is satisfied for both the $M_{S1}$ and $M_{S2}$ networks of RNA structures. It is easy to see that the clustering
coefficient of the $MS_1$ network of RNA structures is zero, so in the remainder of the paper, we concentrate on conditions (2) and (3) for the $MS_2$ RNA network.

Specific properties of RNA networks critically depend on the chosen definition of neighborhood of a structure, leading to the investigation of various move sets in the RNA kinetics literature. In addition to the move sets $MS_1$ and $MS_2$ [9], which latter also models defect diffusion [15], other groups have considered more general move sets that allow helix formation and disassociations [11].

The overall method used is as follows: (1) Give a context-free grammar that generates the set of all secondary structures, possibly containing a specific motif. (2) Use Table 1 to derive and then solve a functional relation for the complex generating function $S(z)$, with the property that the $n$th Taylor coefficient of $S(z)$, denoted $[z^n]S(z)$, is equal to the number of length $n$ structures, possibly containing a specific motif. (3) Determine the dominant singularity and apply complex analysis to obtain the asymptotic value of $[z^n]S(z)$. For step (3), we use the Flajolet-Odlyzko Theorem, stated as Corollary 2, part (i) on page 224 of [7]. Before stating the theorem, we define the dominant singularity of complex function $f(z)$ to be the complex number $\rho$ having smallest absolute value (or modulus) at which $f(z)$ is not differentiable.

**Theorem 1 (Flajolet and Odlyzko)** Assume that $f(z)$ has a dominant singularity at $z = \rho > 0$, is analytic for $z \neq \rho$ satisfying $|z| \leq |\rho|$, and that

$$\lim_{z \to \rho} f(z) = K(1 - z/\rho)^\alpha.$$  

Then, as $n \to \infty$, if $\alpha \notin 0, 1, 2, \ldots$,

$$f_n = [z^n]f(z) \sim \frac{K}{\Gamma(-\alpha)} \cdot n^{-\alpha-1} \cdot \rho^{-n}$$

where $\sim$ denotes asymptotic equality and $\Gamma$ denotes the Gamma function.

The plan of the paper is now as follows. In Section 3 we show that the average $MS_2$ degree of $S_n$ is $O(n)$. In Section 4.1 [resp. 4.2] we prove that the average number of triangles [resp. triples] is $O(n)$ [resp. $O(n^2)$], which implies that the asymptotic global clustering coefficient is $O(1/n)$, hence not bounded away from zero. It follows that the family of RNA secondary structure networks is not small-world. Section 5 concludes the paper. Due to space constraints, an appendix contains supplementary Figures S1-S6, a listing of the full grammar, and short explanation of how to count all motifs necessary for an exact count of the global clustering coefficient. A Mathematica™ program to compute the asymptotic global clustering coefficient of 20.7728/n is available upon request.

### 3 Expected network degree

Due to space constraints, details for the computation of the asymptotic number of secondary structures as well as for $MS_1$ expected degree for homopolymers cannot be given here; however, these results can be found in [3], including the following.
Theorem 2 If $S(z)$ is the generating function for the number of secondary structures for a homopolymer, then

$$[z^n]S(z) \sim 0.713121 \cdot n^{-3/2} \cdot 2.28879^n$$

If $MS_1\text{degree}(n)$ denotes the $MS_1$ expected network degree for a homopolymer, then

$$MS_1\text{degree}(n) \sim 0.473475 \cdot n$$

Define the grammar $G$ to consist of the terminal symbols $\langle, \bullet, \rangle, \langle, *, \rangle$, nonterminal symbols $\widehat{S}, \widehat{T}, S, R, \theta$, with start symbol $\widehat{S}$. Shift moves are represented in the grammar by one of the three expressions: $\langle *, \rangle$, $\langle *, \langle *, \rangle$, as depicted in Figure 2. In particular, $\langle *, \rangle$ represents the right shift depicted in Figure 2a (ignoring possible intervening structure), where base pair $(x, y)$ is transformed to $(x, y')$ for $x < y' < y$; alternatively, the $\langle *, \rangle$ can represent the shift $(x, y)$ to $(x, y')$ for $x < y < y'$, as depicted in Figure 2b. The expression $\langle *, \rangle$ can represent the left shift depicted in Figure 2c, where base pair $(x, y)$ is transformed to $(x', y)$ for $x < x' < y$; alternatively, $\langle *, \rangle$ can represent the shift $(x, y)$ to $(x', y)$ for $x' < x < y$, as depicted in Figure 2d. The grammar $G$ allows us to count the number of secondary structures, that additionally contain a unique occurrence of exactly one of the three expressions: $\langle *, \rangle$, $\langle *, \rangle$. The production rules of grammar $G$ are as follows:

$$\widehat{S} \to \widehat{S} \cdot | (\widehat{S}) | S(\widehat{S}) | \widehat{S}(R) | \widehat{T}$$
$$\widehat{T} \to \ast R | S \ast R | \ast R S | S \ast R S |$$
$$\langle R \ast S \rangle \langle R \ast S \rangle | S \langle R \ast S \rangle | S \langle R \ast S \rangle |$$
$$\langle R \ast R \rangle | S \langle R \ast R \rangle$$

$S \to \bullet | S \cdot | (R) | S(R)$

$R \to \theta | R \cdot | (R) | S(R)$

$\theta \to \bullet \bullet \bullet$ (3)

The nonterminal $S$ is responsible for generating all secondary structures of length greater than or equal to 1. In contrast, the nonterminal $\widehat{S}$ is responsible for generating all well-balanced expressions of length greater than or equal to 1, that involve exactly one of the three expressions: $\langle *, \rangle$. To that end, the nonterminal $\widehat{T}$ is responsible for generating all such expressions, in which the rightmost symbol is either $\rangle$ or $\ast$, but not $\bullet$ or $\langle$. By induction on length of sequence generated, one can show that $G$ is a nonambiguous context-free grammar that generates all secondary structures having a unique occurrence of one of $\langle *, \rangle$. Since two shift moves correspond to each of the expressions $\langle *, \rangle$, $\langle *, \rangle$, it follows that the total number of $MS_2 - MS_1$ (shift-only) moves, summed over all structures for a homopolymer of length $n$ with $\theta = 3$, is equal to $2 \cdot [z^n]S(z)$. 

4
As explained in [12] and [8], it is possible to automatically transform the previous production rules into equations that relate the corresponding generating functions, where we denote generating functions of \( \hat{S}(z), \hat{T}(z), S(z), R(z) \) by the same symbols used for the corresponding nonterminals \( \hat{S}, \hat{T}, S, R \). This technique is known as DSV (Dyck, Schützenberger, Viennot) methodology in [12], or as the symbolic method in [8] – see Table 1. In this fashion, we obtain the following:

\[
\hat{S} = z\hat{S} + z^2\hat{S} + z^2 S\hat{S} + z^2 RS + \hat{T} \\
\hat{T} = 2z^3 R + 4z^3 RS + 2z^3 RS^2 + z^3 R^2 + z^3 SR^2 \\
S = z + zS + z^2 R + z^2 RS \\
R = \theta + zR + z^2 R + z^2 RS \\
\theta = z^3
\]

and by eliminating all variables except \( \hat{S} \) and \( z \), we use Mathematica to obtain the quadratic equation in \( \hat{S} \) having two solutions, for which the only solution analytic at 0 is the following:

\[
\hat{S}(z) = \hat{S} = \frac{A + B\sqrt{P}}{C}
\]  

(4)

where

\[
P = 1 - 2z - z^2 + z^3 + 3z^6 + 2z^7 + z^8 \\
A = 3 - 15z + 23z^2 - 9z^3 - z^4 - 9z^5 + \\
23z^6 - 25z^7 + 7z^8 - z^9 + 6z^{10} - \\
8z^{11} + 2z^{12} + 2z^{13} + 2z^{14} \\
B = -3 + 12z - 14z^2 + 4z^3 + 5z^5 - 10z^6 + \\
8z^7 - 2z^{10} \\
C = 2(-z^3 + 3z^4 - z^5 - z^6 - z^7 + z^8 - \\
3z^9 + z^{10} + z^{11} + z^{12})
\]

The dominant singularity \( \rho \) of \( \hat{S}(z) \) in equation (4) is the complex number having smallest absolute value (or modulus) at which \( \hat{S}(z) \) is not differentiable. For the functions in this paper, the dominant singularity will always be the (complex) root of polynomial \( P \) under the radical, having smallest modulus – since the square root function is not differentiable over the complex numbers at zero.

Letting \( \hat{F}(z) = \frac{B\sqrt{P}}{C} \) and noting that the dominant singularity \( \rho = 0.436911 \), a calculation
shows that

\[ \lim_{z \to \rho} \hat{F}(z) = \lim_{z \to \rho} \frac{B \cdot \sqrt{P'}}{C' \cdot (1 - z/\rho)} \cdot (1 - z/\rho)^{1/2} \]

\[ P' = \frac{P}{1 - z/\rho} = 1 + 0.288795z - 0.339007z^2 - 0.775919z^3 - 0.775919z^4 - 1.775919z^5 - 1.064714z^6 - 0.436911z^7 \]

\[ C' = \frac{C}{1 - z/\rho} = -2z^3 + 1.422410z^4 + 1.255605z^5 + 0.873822z^6 + 2z^8 - 1.422410z^9 - 1.255605z^{10} - 0.873822z^{11} \]

and so

\[ \lim_{z \to \rho} \hat{F}(z) = 0.684877 \cdot \lim_{z \to \rho} (1 - z/\rho)^{-1/2} \]

\[ = 0.684877 \cdot \lim_{z \to \rho} (1 - z/0.436911)^{-1/2} \]

Taking \( \alpha = -1/2 \) in the Flajolet-Odlyzko Theorem \[7\], we obtain:

\[ [z^n] \hat{F}(z) \sim \frac{0.684877}{\Gamma(1/2)} \cdot n^{-1/2} \cdot \left( \frac{1}{\rho} \right)^n \]

\[ = 0.3864 \cdot n^{-1/2} \cdot 2.28879^n \]

By Theorem \[2\] the asymptotic number of secondary structures for a homopolymer when \( \theta = 3 \) is \( 0.713121 \cdot n^{-3/2} \cdot 2.28879^n \), and noting that \( \lim_{z \to \rho} A/C = -4.36723 \), we have the following result.

**Theorem 3** The asymptotic \( MS_2 - MS_1 \) degree of \( S_n \) is

\[ \frac{2[z^n] \hat{S}(z)}{[z^n]S(z)} \sim \frac{2[z^n](A/C + B\sqrt{P'/C})(z)}{[z^n]S(z)} \sim \frac{2[z^n] F(z)}{[z^n]S(z)} \sim \frac{0.772801 \cdot n^{-1/2} \cdot 2.28879^n}{0.713121 \cdot n^{-3/2} \cdot 2.28879^n} \]

\[ = 1.083688 \cdot n \]

Adding the asymptotic values from Theorem \[2\] and Theorem \[3\] we determine the \( MS_2 \) degree.

**Corollary 4** The asymptotic \( MS_2 \) degree for the network \( S_n \) of RNA structures is \( 1.557164 \cdot n \).
Using a Taylor series expansion at zero for the functions used to determine both the $MS_1$ and $MS_2 - MS_1$ degree, we have verified that the numerical results for $S_n$ are identical with those independently computed by the dynamic programming C-implementations described in [4] and [5]. We also note that the current approach is much simpler than the program in [5], although the latter is more general, since it computes the $MS_2$ degree for any user-specified RNA sequence. Using well-known methods, these asymptotic results can be extended from homopolymers to RNA sequences with Watson-Crick and wobble base pairs by using a “stickiness model”, which stipulates the probability $p$ that any two positions can form a base pair is defined by

$$p = 2 (p_A p_U + p_G p_U + p_G p_C)$$  \hspace{1cm} (5)$$

where $p_A, p_C, p_G, p_U$ are user-specified nucleotide relative frequencies. Since we consider shifts, we need an additional stickiness parameter $q$, which specifies the probability that a shift can occur between three randomly selected positions in which one position is fixed, defined by

$$q = p_A p_U^2 + p_C p_G^2 + p_G (p_C^2 + p_U^2 + 2 p_C p_U) + p_U (p_A^2 + p_G^2 + 2 p_A p_G)$$  \hspace{1cm} (6)$$

By including stickiness parameters into our computations, we obtained values presented in Table 2, which shows asymptotic $MS_1$ and $MS_2$ degrees for a number of classes of RNA.

4 Asymptotic $MS_2$ clustering coefficient

Section 4.1 describes a grammar to count the number of triangles for $S_n$ with respect to $MS_2$ moves, while Section 4.2 describes a grammar to count two particular triples. Figure S1 provides a schematic overview of the 2 types of possible triangles and 4 types of triples. To each type of triangle or triple, there corresponds a motif, depicted as an undirected graph on 3, 4 or 5 nodes.

4.1 Counting triangles

We now define a nonambiguous context-free grammar $G$, that generates all secondary structures containing a unique triangle motif, where type A [resp. B] triangles are enumerated in Figure S2 [resp. S3], corresponding to triangle rules 1-3 [resp. 4-8] below. Grammar $G$ has the terminal symbols $(, *, )$, nonterminal symbols $S^{\triangle}, S_1, \ldots, S_8, S, R, X, \theta$, start symbol $S^{\triangle}$ and the following production rules:

$$S^{\triangle} \to S_1 | S_2 | S_3 | S_4 | S_5 | S_6 | S_7 | S_8$$
$$S \to \bullet | S \bullet | (R) | S \langle R \rangle$$
$$R \to \theta | R \bullet | (R) | S \langle R \rangle$$
$$X \to \lambda | S$$
$$\theta \to \bullet \bullet \bullet$$
where $\lambda$ denotes the empty word, and $S_1, \ldots, S_8$ are specified in the following 8 exhaustive and mutually exclusive cases. Note that $S_1, \ldots, S_3$ generate structures containing type A triangles, while $S_4, \ldots, S_8$ generate structures containing type B triangles. After each rule explanation, we give both the grammar and the corresponding DSV equations.

**Rule 1** (\* *) The following productions generate all secondary structures $s$, such that for $x < y < z$, it is the case that $s \cup \{ (x, y) \}$ and $s \cup \{ (y, z) \}$ are also secondary structures, hence form a triangle:

$$
S_1 \to S_1 \cdot \ | \ S_1 \ | \ S_1 (S_1) \ | \ S_1 (R) \ | \ X \langle \langle R * R \rangle \rangle \\
S_1 = zS_1 + z^2S_1 + z^2SS_1 + z^2RS_1 + Xz^3R^2
$$

**Rule 2** (\* *) The following productions generate all secondary structures $s$, such that for $x < y < z$, it is the case that $s \cup \{ (x, y) \}$ and $s \cup \{ (x, z) \}$ are also secondary structures, hence form a triangle:

$$
S_2 \to S_2 \cdot \ | \ S_2 \ | \ S_2 (S_2) \ | \ S_2 (R) \ | \ X \langle \langle R * \rangle \rangle X \\
S_2 = zS_2 + z^2S_2 + z^2SS_2 + z^2RS_2 + X^2z^3R
$$

**Rule 3** (\* *) The following productions generate all secondary structures $s$, such that for $x < y < z$, it is the case that $s \cup \{ (x, z) \}$ and $s \cup \{ (y, z) \}$ are also secondary structures, hence form a triangle:

$$
S_3 \to S_3 \cdot \ | \ S_3 \ | \ S_3 (S_3) \ | \ S_3 (R) \ | \ X \langle \langle R * \rangle \rangle X \\
S_3 = zS_3 + z^2S_3 + z^2SS_3 + z^2RS_3 + X^2z^3R
$$

**Rule 4** (\* *) The following productions generate all secondary structures $s$, such that for $x < y < z < w$, it is the case that $s \cup \{ (x, y) \}$, $s \cup \{ (x, z) \}$ and $s \cup \{ (x, w) \}$ are also secondary structures, hence the latter form a triangle:

$$
S_4 \to S_4 \cdot \ | \ S_4 \ | \ S_4 (S_4) \ | \ S_4 (R) \ | \ X \langle \langle R * \rangle \rangle X \rangle X \\
S_4 = zS_4 + z^2S_4 + z^2SS_4 + z^2RS_4 + X^3Rz^4
$$

**Rule 5** (\* *) For $x < y < z < w$, let $s_1 = (x, w)$, $s_2 = (y, w)$, $s_3 = (z, w)$. The following productions generate all secondary structures $s$, such that for $x < y < z$, it is the case that $s \cup \{ (x, w) \}$, $s \cup \{ (y, w) \}$ and $s \cup \{ (z, w) \}$ are also secondary structures, hence the latter form a triangle:

$$
S_5 \to S_5 \cdot \ | \ S_5 \ | \ S_5 (S_5) \ | \ S_5 (R) \ | \ X \langle \langle X \langle \langle R * \rangle \rangle \rangle X \\
S_5 = zS_5 + z^2S_5 + z^2SS_5 + z^2RS_5 + X^3z^4R
$$

8
Rule 6 \( \langle \ast \rangle \) For \( x < y < z < w \), the following productions generate all secondary structures \( s \), such that for \( x < y < z \), it is the case that \( s \cup \{(x,y)\} \), \( s \cup \{(y,z)\} \) and \( s \cup \{(y,w)\} \) are also secondary structures, hence the latter form a triangle:

\[
S_6 \rightarrow S_7 \cdot | \ ( S_6 ) \ | S \ ( S_6 ) \ | S_6 \ ( R ) \ | X \ X \ ( R \ast R ) \\
S_6 = zS_6 + z^2S_6 + z^2SS_6 + z^2RS_6 + X^2z^4R^2
\]

Rule 7 \( \langle \ast \rangle \) For \( x < y < z < w \), the following productions generate all secondary structures \( s \), such that for \( x < y < z \), it is the case that \( s \cup \{(x,z)\} \), \( s \cup \{(y,z)\} \) and \( s \cup \{(z,w)\} \) are also secondary structures, hence the latter form a triangle:

\[
S_7 \rightarrow S_7 \cdot | \ ( S_7 ) \ | S \ ( S_7 ) \ | S_7 \ ( R ) \ | X \ ( R \ast R ) \ X \\
S_7 = zS_7 + z^2S_7 + z^2SS_7 + z^2RS_7 + X^2z^4R^2
\]

Rule 8 \( \langle \ast \rangle \) bis The following productions generate all secondary structures \( s \), such that for \( x < y < z \), it is the case that \( s \cup \{(x,z)\} \), \( s \cup \{(x,y)\} \) and \( s \cup \{(y,z)\} \) are also secondary structures, hence the latter form a triangle. This grammar is identical to that in rule 1 above, with the exception that \( S_1 \) is replaced by \( S_8 \).

Let \( S^\Delta(z) \) denote the generating function for the number of structures containing a unique triangle motif, where \( triA(z) \) [resp. \( triB(z) \)] is the generating function for the collection of structures containing a unique occurrence of type A [type B] triangle, as treated in rules 1-3 [resp. rules 4-8]. We obtain the following compact form for the DSV equations for the grammar \( G \) that generates all structures containing a triangle:

\[
S^\Delta = triA + triB \\
triA = triA \cdot z + X \cdot z \cdot triA \cdot z+ \\
\quad triA \cdot z \cdot R \cdot z + X \cdot z \cdot R \cdot z \cdot R \cdot z+ \\
\quad X \cdot z \cdot R \cdot z \cdot X \cdot z + X \cdot z \cdot X \cdot z \cdot R \cdot z \\
triB = triB \cdot z + X \cdot z \cdot triB \cdot z+ \\
\quad triB \cdot z \cdot R \cdot z + X^3z^4R + X^3z^4R+ \\
\quad X^2z^4R^2 + X^2z^4R^2 + Xz^3R^2
\]

Using Mathematica, we determine the following.

\[
[z^n]S^\Delta(z) = 0.870311 \cdot 2.28879^n \cdot n^{-1/2}
\]

By Theorem 2, the asymptotic number of secondary structures is \( 0.713121 \cdot n^{-3/2} \cdot 2.28879^n \), and so we have the following result.

**Theorem 5** The asymptotic average number of triangles per structure is

\[
[z^n]S^\Delta(z) \sim 0.870331 \cdot n^{-1/2} \cdot 2.28879^n \\
[z^n]S(z) \sim 0.713121 \cdot n^{-3/2} \cdot 2.28879^n \\
\sim 1.220453 \cdot n
\]
4.2 Counting triples

In this section, we describe a grammar for two particular triples. Let $G$ be the grammar having terminal symbols $\bullet, (, , )$, nonterminal symbols $S^\dagger, S, R, X, \theta$, start symbol $S^\dagger$, and productions given in equation (7) below together with the following:

$$S \rightarrow \bullet | S \bullet | X \; (R)$$
$$R \rightarrow \theta | R \bullet | X \; (R)$$
$$X \rightarrow \lambda | S$$
$$\theta \rightarrow \bullet \bullet \bullet \bullet$$

Triple with motif [ ] [ ] or [ [ ] ]

The following grammar generates all secondary structures $s$ that have two special base pairs $(i, j)$ and $(x, y)$, designated by [ ], which are either sequential or nested, as shown in the first two panels of Figure S4. For each structure $s$, which contains a unique occurrence of the sequential motif [ ] [ ] or of the nested motif [ [ ] ], we must count four possible triples: (1) $\{ s_1, s_2, s_3 \}$, where $s_1 = s - \{(i, j), (x, y)\}$, $s_2 = s - \{(i, j)\}$, $s_3 = s - \{(x, y)\}$. (2) $\{ s_1, s_2, s_3 \}$, where $s_1 = s$, $s_2 = s - \{(i, j)\}$, $s_3 = s - \{(x, y)\}$. (3) $\{ s_1, s_2, s_3 \}$, where $s_1 = s - \{(i, j)\}$, $s_2 = s - \{(i, j), (x, y)\}$, $s_3 = s$. (4) $\{ s_1, s_2, s_3 \}$, where $s_1 = s - \{(x, y)\}$, $s_2 = s - \{(i, j)\}$, $s_3 = s$. For this reason, we multiply by 4 the asymptotic number of structures generated by the following grammar $G$, whose terminal symbols are $\bullet, (, , )$, and nonterminal symbols are $S^\dagger, S^\dagger, S, R, X, \theta$ with start symbol $S^\dagger$. Here $S^\dagger$ [resp. $S^\dagger$] generates all structures with 1 [resp. 2] distinguished base pairs ([ ] ), so $S^\dagger$ generates all structures containing a unique triple of the form [ ] [ ] or [ [ ] ].

$$S^\dagger \rightarrow S^\dagger \bullet | (S^\dagger) | S \; (S^\dagger) \; | S^\dagger \; (R) \; |$$
$$[S^\dagger] \; | S \; [S^\dagger] \; | S^\dagger \; [R] \; | S^\dagger \; (S^\dagger) \; |$$
$$S^\dagger \rightarrow S^\dagger \bullet | (S^\dagger) \; | S \; (S^\dagger) \; | S^\dagger \; (R) \; |$$
$$[R] \; | S \; [R] \; \quad (7)$$

When applying the Flajolet-Odlyzko Theorem in the current case, we have $\rho = 0.436911$ and $\alpha = -3/2$. A computation shows that

$$\lim_{z \to \rho} S^\dagger(z) = 0.0177098 \cdot (1 - z/\rho)^{-3/2}$$
$$[z^n]S^\dagger(z) \sim 0.0199834 \cdot n^{1/2} \cdot 2.28879^n$$
$$[z^n]S^\dagger(z) \sim 0.0199834 \cdot n^{1/2} \cdot 2.28879^n$$
$$[z^n]S(z) \sim 0.713121 \cdot n^{-3/2} \cdot 2.28879^n$$
$$\sim 0.0280225 \cdot n^2$$

As mentioned, the number of triples contributed in the current case is 4 times the last value. Thus the expected number of triples involving a structure containing [ ] [ ] or [ [ ] ] is $4 \cdot 0.0280224 \cdot n^2 = 0.1120896 \cdot n^2$. 

10
Theorem 6  The asymptotic average number of triples per structure, for the triples described in this section, is

\[
\frac{4[z^n]S^\dagger(z)}{[z^n]S(z)} \sim 0.11209 \cdot n^2
\]

From Theorems 5 and 6 we obtain an upper bound for the global clustering coefficient, defined in equation (1).

Theorem 7 (Bound on global clustering coefficient)

\[
C_g(G) = 3 \times \frac{\text{number of triangles}}{\text{number of connected triples}} = O \left( \frac{1}{n} \right)
\]

and hence the family \( S_n, n = 1, 2, 3, \ldots \) of RNA secondary structures is not small-world.

5 Discussion

In this paper, we have used methods from algebraic combinatorics [8] to determine the asymptotic average degree and asymptotic clustering coefficient of the \( MS_2 \) network \( S_n \) of RNA secondary structures. Since the clustering coefficient is not bounded away from zero, it follows that the family \( S_n, n = 1, 2, 3, \ldots \), of networks is not small-world. In contrast, [19] had shown by exhaustive enumeration of low energy RNA secondary structures of \( E. coli \) phetRNA, that the RNA network displays small-world properties. The seeming contradiction of our rigorous result is due to the fact that the notion of finite small-world network is not precisely defined due to absence of an exact bound for average path length and for clustering coefficient.

A related question is whether RNA secondary structure networks are scale-free, i.e. whether the tail of the degree density follows a power-law distribution. Figure 3 shows the \( MS_2 \) degree density for a 20-nt homopolymer, computed by brute-force enumeration, together with a least-squares linear fit for the tail of the log-log plot, which suggests that the distribution tail is given by a power-law with exponent \( \approx -5.6247 \). A forthcoming paper by the last author settles this question (see the arXiv preprint 1807.00215).

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| Type of nonterminal | Generating function |
|---------------------|---------------------|
| $A \to B \mid C$    | $A(z) = B(z) + C(z)$|
| $A \to BC$          | $A(z) = B(z)C(z)$   |
| $A \to t$           | $A(z) = z$          |
| $A \to \lambda$     | $A(z) = 1$          |

Table 1: Translation between context-free grammars and generating functions. Here, $G = (V, \Sigma, S, R)$ is a given context-free grammar, $A, B, C$ are any nonterminal symbols in $V$, $t$ is a terminal symbol in $\Sigma$, and $\lambda$ denotes the empty word. The generating functions for the languages $L(A), L(B), L(C)$ are respectively denoted by $A(z), B(z), C(z)$.
Table 2: The asymptotic expected degree of the network $S_{n}^{\theta,p}$ of secondary structures for move sets $\text{MS}_{1}$ and $\text{MS}_{2}$ for different values of threshold $\theta$ and base pair and triple stickiness parameters $p$ and $q$, defined in Equations (5) and (6) respectively. Five models are considered: $\text{hp}$ – homopolymer model with $p = q = 1$; $\text{wc}$ – Watson-Crick pairing model with uniform compositional frequency $p_{A} = p_{C} = p_{G} = p_{U} = \frac{1}{4}$, hence $p = \frac{1}{4}$, and $q = 0.0625$; $\text{wcw}$ – Watson-Crick and wobble pairing model with uniform compositional frequency, hence $p = \frac{3}{8}$, and $q = 0.15625$; $\text{wc tRNA}$ – Watson-Crick base pairing model based on the compositional frequencies ($p_{A} = \frac{1288}{4534}$, $p_{U} = \frac{1029}{4534}$, $p_{G} = \frac{1223}{4534}$, $p_{U} = \frac{994}{4534}$) observed in family RF00005 of 4,534 tRNAs in the Rfam 12.0 database [13], hence $p = 0.259427$, $q = 0.066394$; $\text{wcw tRNA}$ – Watson-Crick and Wobble base pairing model using compositional frequency of RF00005, so that $p = 0.377699$, $q = 0.158476$. 

| Move set  | $\theta$ | hp   | wc   | wcw  | wc tRNA | wcw tRNA |
|-----------|----------|------|------|------|---------|---------|
| $\text{MS}_{1}$ | 1        | 0.55279 | 0.42265 | 0.46158 | 0.42157 | 0.46021   |
| $\text{MS}_{1}$ | 3        | 0.47348 | 0.35130 | 0.38531 | 0.35038 | 0.38408   |
| $\text{MS}_{2} - \text{MS}_{1}$ | 1        | 1.44721 | 0.57735 | 0.84796 | 0.57985 | 0.85119   |
| $\text{MS}_{2} - \text{MS}_{1}$ | 3        | 1.08369 | 0.42908 | 0.31409 | 0.21550 | 0.63055   |
| $\text{MS}_{2}$ | 1        | 2.00000 | 1.00000 | 1.30954 | 1.00142 | 1.31140   |
| $\text{MS}_{2}$ | 3        | 1.55717 | 0.43527 | 0.32336 | 0.22162 | 0.63971   |
Figure 1: (a) Consensus secondary structure of the type III hammerhead ribozyme from Peach Latent Mosaic Viroid (PLMVd) AJ005312.1/282-335 (isolate LS35, variant ls16b), taken from Rfam [10] family RF00008. (b) Network for size 7 homopolymer with $\theta = 3$, having 8 nodes and 8 red $MS_1$ edges (base pair addition or removal), 8 blue $MS_2 - MS_1$ edges (base pair shift), hence a total of 16 $MS_2$ edges. It follows that $MS_1$ degree is $\frac{16}{8} = 2$, while $MS_2$ is $\frac{32}{8} = 4$. 
Figure 2: Illustration of possible shift moves, where each subcaption indicates the terminal symbols involved in the corresponding production rule. Solid blue [resp. dashed green] lines indicate the initial [resp. final] base pair in a shift move.

Figure 3: (a) $MS_2$-degree distribution for the 106,633 secondary structures for a 20-nt homopolymer with $\theta = 3$ (green shaded curve), with Poisson distribution of the same mean. (b) Plot of ln(density) as a function of ln(degree) for the degree distribution for $MS_2$ connectivity of the 20-nt homopolymer with $\theta = 3$, for degrees 4, \ldots, 136. The distribution tail appears to satisfy a power-law with exponent $\approx -5.6247$, i.e. $p(x) \propto x^{-5.6247}$, where $x$ is degree and $p(x)$ is the relative frequency of the number of nodes having degree $x$ (regression equation for log-log plot is $\ln(p(x)) = 14.7589 - 5.6247 \cdot x$).
A Supplementary Information

Complete grammar

In this supplement, we list the complete grammar for all triangles and connected triples, together with the corresponding functional equations. We do not provide any detailed explanation for the complete listing of all possible triples or the complete grammar, since our intent is to provide suggestive supplementary figures and a general orientation for the reader wishing to work through the Mathematica code, available upon request, for our computation of the asymptotic clustering coefficient value $20.7728/n$.

A.1 Triangles and type A triples

Figure S1 provides an overview of the classification of triangle motifs (types A and B) and non-triangle triple motifs (types A,B,C,D). In this section, we describe grammar rules for triangles and type A triples – see Figure S2 for type A triangles, Figure S3 for type B triangles, and Figure S4 for type A triples. Triangles of type A and B have been explained in the main text; type A triples are constituted by structures $s_1, s_2, s_3$, where $s_2, s_3$ are obtained from $s_1$ by a base pair addition with the property that there is a no $MS_2$ move between $s_2$ and $s_3$.

In the following production rules, nonterminal $S$ generates all non-empty secondary structures; $R$ [resp. $S2$] generates all secondary structures of length at least 3 [resp. 2]. Nonterminal $PK$ generates all structures containing a unique pseudoknot (crossing base pairs) of the form $[\{\}]$, where a second type of bracket is necessary to properly parse the expression. Nonterminal $\Delta_A$ [resp. $\Delta_B$] generates all structures containing a unique occurrence of a type A [resp. B] triangle, and nonterminal $\Lambda_A$ generates all structures containing a unique occurrence of a type A triple. Nonterminal $S^\dagger$ [resp. $S'^\dagger$] generates all secondary structures containing a unique occurrence of a motif that has exactly 1 [resp. 2] extended connected components. Thus $S^\dagger$ generates all structures having a unique occurrence of motif 1 or 2 for type A triple.
\[S \rightarrow \bullet | S \bullet | X (R)\]
\[R \rightarrow \theta | R\bullet | X (R)\]
\[S2 \rightarrow \bullet \bullet | R\]
\[\theta \rightarrow \bullet \bullet \bullet\]
\[X \rightarrow \lambda | S\]
\[\Delta_A \rightarrow \Delta_A \bullet | X (\Delta_A) | \Delta_A (R) | X (R\star\star R) | X\star R) X) | X \langle X (R\star\star R)\rangle (type A triangles)\]
\[\Delta_B \rightarrow \Delta_B \bullet | X (\Delta_B) | \Delta_B (R) | X (R\star R) X) | X \langle X (R\star)\rangle | X \langle X (R\star\star R)\rangle | X \langle R\star\star R\rangle (type B triangles)\]
\[\Delta \rightarrow \Delta_A | \Delta_B \quad (all \ triangles)\]
\[PK \rightarrow PK \bullet | X (PK) | PK (R) | X \{ S2 \} | X \{ S2 \} \} | X \{ S \} S\}
\[X \{ S2 \} S \} | X \{ S \} S\}
\[\Lambda_A \rightarrow PK \{ S\} \quad (Type A triples)\]
\[S^\dagger \rightarrow \bullet | X (S^\dagger) | S^\dagger (R) | X \{ R\]
\[S^\dagger \rightarrow S^\dagger \bullet | X (S^\dagger) | S^\dagger (R) | X \{ S\} | S^\dagger [R] | S^\dagger (S^\dagger) \quad (Note: \times 4)\]

A.2 Type B triples

Figure S5 depicts type B triples, defined to be of the form \(s_1, s_2, s_3\), where \(s_1, s_2\) are connected by a shift, as are \(s_1, s_3\), but \(s_2, s_3\) are not connected by any \(MS_2\) move. Nonterminal \(\Lambda_B\) generates all secondary structures containing a unique occurrence of one of the 12 type B triple motifs in Figure S5. Nonterminals \(R_1, \ldots, R_{12}\) generate respectively the collection of secondary structures containing a unique occurrence of motif 1, \ldots, 12. Note that \(S2\) is not the same as \(R_2\) – the former nonterminal \(S2\) simply generates all secondary structures of length 2 or greater.
A.3 Type C and D triples

Figure S6 depicts triples of type C and D, defined to be of the form are of the form $s_1, s_2, s_3$, where $s_1, s_2$ are connected by a shift, and $s_3$ is obtained from $s_1$ by a base pair addition, but $s_2, s_3$ are not connected by any $MS_2$ move.

Recall that an undirected graph is connected if there is a path between any two nodes, and that any undirected graph is either connected or can be decomposed into connected components. Note that the motif for each type B triple is connected, and that there are both connected and unconnected motifs for triples of types C and D, but that each unconnected motif has exactly two connected components. A motif is defined to contain a pseudoknot if its arc cross – this does not occur for any triangle, but it does happen in the type A triple (3), type B triples (3,4,5,12), and type C,D triples (2,4,9,10,12,13,14,15,22,23).

If there exists an edge $(x, y)$ belonging to one connected component of a motif, and another edge $(u, v)$ belonging to a different connected component of the same motif, and if $(x, y), (u, v)$ creates a pseudoknot, then both components are merged into a single extended connected component. With this definition, there are two type A motifs that have one extended connected component (3,4), and two type A motifs that have two extended connected component (1,2); all type B motifs are connected; there are 10 type C,D motifs having one extended connected component (2, 4, 9, 10, 12, 13, 14, 15, 22, 23), and 15 type C,D motifs having two extended connected components (1, 3, 5, 6, 7, 8, 11, 16, 17, 18, 19, 20, 21, 24, 25). Computations using DSV equations deriving from the grammars show that if a motif has a single extended connected component, then the asymptotic average number of triples per structure is $O(n)$, while if it has two extended connected components, then the
asymptotic average number is $O(n^2)$. Thus, the asymptotic average number of triangles per structure is $O(n)$; the asymptotic average number of triples per structure for type A triples (1,2) is $O(n^2)$ while that of type A triples (3,4) is $O(n)$. Without further computations, it immediately follows that the asymptotic clustering coefficient is $O \left( \frac{1}{n} \right)$. Nonetheless, we note more generally, that the asymptotic average number of triples per structure for any motif having two extended connected components is $O(n^2)$, while that for a motif having only one extended connected component is $O(n)$. Finally, since the $O \left( \frac{1}{n} \right)$ asymptotic clustering coefficient for the homopolymer implies an $O \left( \frac{1}{n} \right)$ asymptotic clustering coefficient for any nontrivial stickiness factors $p, q$, we will not perform computations for these cases.

We now present the corresponding grammar, where nonterminal triples generates all secondary structures containing a unique occurrence of one of the 25 type C or D triple motifs, as depicted in Figure S6. Nonterminals $\Lambda_{C_1}, \Lambda_{C_2}, \Lambda_{C_{367}}, \Lambda_{C_{4}}, \Lambda_{C_{5}}, \Lambda_{C_8}, \Lambda_{C_9}, \Lambda_{C_{10}}, \Lambda_{C_{11}}, \Lambda_{C_{12}}, \Lambda_{C_{13}}, \Lambda_{C_{14}}, \Lambda_{C_{15}}, \Lambda_{C_{\text{disc}}}, \Lambda_{C_{20}}, \Lambda_{C_{21}}, \Lambda_{C_{22}}, \Lambda_{C_{23}}$ generate respectively the collection of secondary structures containing a unique occurrence of motif 1, \ldots, 25, whereby nonterminal $\Lambda_{C_{367}}$ is the same rule for motif 3,6 and 7, and nonterminal $\Lambda_{C_{\text{disc}}}$ is the same rule for all disconnected successive motifs — i.e. the 6 type C and D triple motifs 16, 17, 18, 19, 24, 25 which have exactly 2 extended connected components of successive form [ ] [ ].
\( \Lambda_C \rightarrow \Lambda_C \bullet | X (\Lambda_C) | \Lambda_C (R) | \Lambda_C (\Lambda_C) | \Lambda_C (\Lambda_C) | \Lambda_C (\Lambda_C) \)

\( \Lambda_C \rightarrow \Lambda_C \bullet | X (\Lambda_C) | \Lambda_C (R) | \Lambda_C (\Lambda_C) | \Lambda_C (\Lambda_C) | \Lambda_C (\Lambda_C) \)

\( \Lambda_C \rightarrow \Lambda_C \bullet | X (\Lambda_C) | \Lambda_C (R) | \Lambda_C (\Lambda_C) | \Lambda_C (\Lambda_C) | \Lambda_C (\Lambda_C) \)

\( \Lambda_C \rightarrow \Lambda_C \bullet | X (\Lambda_C) | \Lambda_C (R) | \Lambda_C (\Lambda_C) | \Lambda_C (\Lambda_C) | \Lambda_C (\Lambda_C) \)

Finally, the collection of all secondary structures having a unique motif for a connected triple (both non-triangular triples of types A,B,C,D or deriving from triangles) is generated by the rule

\[ \Lambda \rightarrow \Lambda_A | \Lambda_B | \Lambda_C | \text{triangles} \quad \text{(Note: triangles must be multiplied by 3)} \]

This gives rise to the following functional equations for all connected triples, both non-triangular triples, as well as 3 triples associated with each triangle. Since rules \( R_1, \ldots, R_{12} \)
are written as \( R_1, \ldots, R_{12} \), we write \( S_2 \) in place of \( R_2 \), which latter had been defined by \( R_2 \rightarrow \bullet \cdot | R \).

\[
S^\dagger = S^\dagger \cdot z + X \cdot z \cdot S^\dagger \cdot z + S^\dagger \cdot z \cdot R \cdot z + X \cdot z \cdot S^\dagger \cdot z + S^\dagger \cdot z \cdot R \cdot z + S^\dagger \cdot z \cdot S^\dagger \cdot z
\]

\[
S^\dagger = S^\dagger \cdot z + X \cdot z \cdot S^\dagger \cdot z + S^\dagger \cdot z \cdot R \cdot z + X \cdot z \cdot R \cdot z
\]

\[
S = z + S \cdot z + X \cdot z \cdot R \cdot z
\]

\[
R = \theta + R \cdot z + X \cdot z \cdot R \cdot z
\]

\[
\theta = z \cdot z \cdot z
\]

\[
X = 1 + S
\]

\[
S_2 = z \cdot z + R
\]

\[
PK = PK \cdot z + X \cdot z \cdot PK \cdot z + PK \cdot z \cdot R \cdot z + X \cdot z^4 (S_2 + S_2^2 + 2SS_2 + S^3)
\]

\[
\Lambda_A = PK + 4 \cdot S^\dagger
\]

\[
R_1 = X^2 z^4 R^2
\]

\[
R_2 = X^3 z^4 R
\]

\[
R_3 = X z^4 R(2S_2 + S^2)
\]

\[
R_4 = X z^4 (S_2 + S_2^2 + 2SS_2 + S^3)
\]

\[
R_5 = X^3 z^4 R
\]

\[
R_6 = X^3 z^4 R
\]

\[
R_7 = X^2 z^4 R^2
\]

\[
R_8 = X^2 z^4 R^2
\]

\[
R_9 = X z^4 R^3
\]

\[
R_{10} = X^2 z^4 R^2
\]

\[
R_{11} = X^2 z^4 R^2
\]

\[
R_{12} = X z^4 (2S_2 + S^2)
\]
together with the following, where we write $\Lambda_C$ in place of $\Lambda_{CD}$ for reasons of brevity

$$\Lambda_B = R_1 + R_2 + R_3 + R_4 + R_5 + R_6 + R_7 + R_8 + R_9 + R_{10} + R_{11} + R_{12} + \Lambda_B z + X z^2 \Lambda_B + \Lambda_B z^2 R$$

$$\Lambda_C_1 = X z S \dagger z X z$$

$$\Lambda_C_2 = X z^5 (S_2 + S_2^2 + 2S S_2 + S_3^2)$$

$$\Lambda_C_{367} = X z \Delta_A z$$

$$\Lambda_C_3 = X^4 z^5 R$$

$$\Lambda_C_4 = X^4 z^5 R$$

$$\Lambda_C_5 = X z R z S \dagger z$$

$$\Lambda_C_6 = X z R z S \dagger z$$

$$\Lambda_C_7 = X^2 z^5 (2S_2 + S_2^2)$$

$$\Lambda_C_8 = X^4 z^5 R$$

$$\Lambda_C_9 = X^2 z^5 (2S_2 + S_2^2)$$

$$\Lambda_C_{10} = X^4 z^5 R$$

$$\Lambda_C_{11} = X^2 z^3 S \dagger z$$

$$\Lambda_C_{12} = X^2 z^5 R (2S_2 + S_2^2)$$

$$\Lambda_C_{13} = X^2 z^5 R (2S_2 + S_2^2)$$

$$\Lambda_C_{14} = X z^5 R (S_2 + S_2^2 + 2S S_2 + S_3^2)$$

$$\Lambda_C_{15} = X^2 z^5 (S_2 + S_2^2 + 2S S_2 + S_3^2)$$

$$\Lambda_C_{\text{disc}} = \Delta_A z R z + \Delta_A z S \dagger z + (S \dagger z R z R z + S \dagger z X z R z + S \dagger z R z X z) (1 + X^2 z^2)$$

$$\Lambda_C_{20} = X z^3 R S \dagger$$

$$\Lambda_C_{21} = X z^3 R S \dagger$$

$$\Lambda_C_{22} = X z^5 R (S_2 + S_2^2 + 2S S_2 + S_3)$$

$$\Lambda_C_{23} = X^2 z^5 R (2S_2 + S_2^2)$$

$$\Lambda = \Lambda_C \cdot z + X \cdot z \cdot \Lambda_C \cdot z + \Lambda_C \cdot z \cdot R \cdot z + 4 \cdot \Lambda_C_1 +$$

$$\Lambda_C_2 + 4 \cdot \Lambda_C_{367} + \Lambda_C_4 + 4 \cdot \Lambda_C_5 + 4 \cdot \Lambda_C_8 + \Lambda_C_9 +$$

$$\Lambda_C_{10} + 4 \cdot \Lambda_C_{11} + \Lambda_C_{12} + \Lambda_C_{13} + \Lambda_C_{14} + \Lambda_C_{15} +$$

$$\Lambda_C_{\text{disc}} + 4 \cdot \Lambda_{C_{20}} + 4 \cdot \Lambda_{C_{21}} + \Lambda_{C_{22}} + \Lambda_{C_{23}}$$

$$\Delta_A = \Delta_A \cdot z + X \cdot z \cdot \Delta_A \cdot z + \Delta_A \cdot z \cdot R \cdot z + X \cdot z \cdot R \cdot z \cdot R \cdot z +$$

$$X \cdot z \cdot R \cdot z \cdot X \cdot z + X \cdot z \cdot X \cdot z \cdot R \cdot z$$

$$\Delta_B = \Delta_B \cdot z + X \cdot z \cdot \Delta_B \cdot z + \Delta_B \cdot z \cdot R \cdot z + X^3 z^4 R + X^3 z^4 R +$$

$$X^2 z^4 R^2 + X^2 z^4 R^2 + X z^3 R^2$$

$$\Delta = \Delta_A + \Delta_B$$

$$\Lambda = \Lambda_A + 4 \cdot \Lambda_B + \Lambda_C + 3 \cdot \Delta$$
Figure S1: Complete listing of all possible moves for triangles and non-triangular connected triples with a designated first structure, up to equivalence. Consider, for instance, the triangle \( T \) (not shown) in which \( s_2 \rightarrow s_3 \) by a shift, \( s_2 \rightarrow s_1 \) by base pair removal, and \( s_1 \rightarrow s_3 \) by a base pair addition. Then \( T \) is equivalent to a triangle of type \( A \). Other instances of triangles the reader may consider are analogously equivalent to a triangle of type \( A \) or \( B \).

Figure S2: Type A triangles are constituted by structures \( s_1, s_2, s_3 \), where \( s_2, s_3 \) are obtained from \( s_1 \) by adding a base pair with the property that there is a shift move from \( s_2 \) to \( s_3 \). This type of triangle is described in rules 1,2,3 in Section 4.1. For instance, the motif for triangle rule 1 indicates that there is a base pair \((x, y)\) \( s_2 \) that can be shifted to the base pair \((y, z)\) \( s_3 \); similarly, the motif for rule 2 [resp. 3] indicates that there is a base pair \((x, y)\) \( s_2 \) [resp. \((x, z)\) \( s_2 \)] that can be shifted to the base pair \((x, z)\) \( s_3 \) [resp. \((y, z)\) \( s_3 \)].
Figure S3: Type B triangles are constituted by structures $s_1, s_2, s_3$, where $s_2, s_3$ are obtained from $s_1$ by a shift with the property that there is a shift move from $s_2$ to $s_3$. This type of triangle is described in rules 4-8 in Section 4.1. For instance, the motif in triangle rule 4 indicates that there is a base pair $(x, y) \in s_1$ can be shifted to base pair $(x, z) \in s_2$ and $(x, w) \in s_3$. The other panels have analogous meanings.
Figure S4: Type A triples are constituted by structures $s_1, s_2, s_3$, where $s_2, s_3$ are obtained from $s_1$ by a base pair addition with the property that there is no $MS_2$ move between $s_2$ and $s_3$. Type A triples are given in rules 1-3 in Section 4.2, and are represented in panels 1, 2, 3, 4 of this figure. Panel 1 [resp. 2] indicates that structures $s_2, s_3$ can be obtained from structure $s_1$ by the addition of disjoint base pairs $(u, v) (x, y)$ which are not nested, i.e. $(\ ) (\ )$, [resp. which are nested, i.e. $( ( ) )$]. Panel 3 indicates that structures $s_2, s_3$ can be obtained from structure $s_1$ by the addition of disjoint base pairs $(u, v) (x, y)$ which would form a pseudoknot if added simultaneously to $s_1$, i.e. $( [ ] )$. Panel 4, which is identical to panel 1 of Figure S2, represents a non-triangular connected triple only in the non-homopolymer case. This panel indicates that structures $s_2, s_3$ can be obtained from structure $s_1$ by the addition of non-disjoint base pairs $(u, v) (v, w)$ which share a base. To each triple motif that has 2 extended connected components (see text) there corresponds a quadrilateral, as shown in the panel with label 2-component motif, where edges are labeled by $a/r$ for base pair addition/removal. To each corner of the quadrilateral, there corresponds a unique triple – thus, panels 1 and 2 actually each represent 4 triples. It follows that the average number of triples per structure for type A(1) and type A(2) triples must be multiplied by 4. The same remark holds for type C,D triples in Figure S6 which have 2 extended connected components.
Figure S5: Type B triples are of the form \(s_1, s_2, s_3\), where \(s_1, s_2\) are connected by a shift, as are \(s_1, s_3\), but \(s_2, s_3\) are not connected by any \(MS_2\) move. Note that all motifs are connected. To each type B triple motif, there corresponds a quadrilateral, shown in panel with label 2-component motif, where edges are labeled by \(a/r\) for base pair addition/removal or by \(s\) for base pair shift. For each of these 12 motifs, there is a unique base pair that can shift to the remaining two base pairs – for instance, in motif 1, the base pairs are \((x, y), (y, z)\) and \((x, u)\), for \(x < y < z < u\), where \((x, y)\) can be shifted to each of \((y, z)\) and \((x, u)\). This uniquely defined base pair should be located in the quadrilateral diagonally opposite the corner labeled by emptyset. Since each corner of the quadrilateral corresponds to one of 4 triples associated with the motif, it follows that the average number of type B triples per structure must be multiplied by 4.
Figure S6: Type C and D triples are of the form $s_1, s_2, s_3$, where $s_1, s_2$ are connected by a shift, and $s_3$ is obtained from $s_1$ by a base pair addition, but $s_2, s_3$ are not connected by any $MS_2$ move. Triples may have either one or two extended connected components (see text); in the former case, the asymptotic expected number of triples is $O(n)$, while in the latter case the expected number of triples is $O(n^2)$, as is the case for the type A triple motifs in panels 1 and 2 of Figure S4. The 10 motifs having 1 extended connected component are: 2, 4, 9, 10, 12, 13, 14, 15, 22, 23. The 15 motifs having 2 extended connected component are: 1, 3, 5, 6, 7, 8, 11, 16, 17, 18, 19, 20, 21, 24, 25. Of the latter, the six motifs 16,17,18,19,24,25 are disconnected successive, and the nine motifs 1,3,5,6,7,8,11,20,21 are disconnected nested. To each motif, which has 2 extended connected components, there actually correspond 4 triples, as explained in the caption to Figure S4. For instance, to the motif 16, given by undirected edges $(1,2), (3,4), (3,5)$, there correspond four structures $s_1, s_2, s_3, s_4$, where $(3,4) \in s_1, (3,5) \in s_2, (1,2), (3,4) \in s_3, (1,2), (3,5) \in s_4$, with the following four triples: (1) $s_1 \rightarrow s_2, s_1 \rightarrow s_3$ (type C triple); (2) $s_2 \rightarrow s_3, s_2 \rightarrow s_1$ (type C triple); (3) $s_3 \rightarrow s_4, s_3 \rightarrow s_1$ (type D triple); (4) $s_4 \rightarrow s_3, s_4 \rightarrow s_2$ (type D triple). This is analogous to the situation summarized in the panel in Figure S4 with label 2-component motif. Note that motifs 3,6,7 have a type A triangle contained within an outer designated base pair [ ].