The Expected Order of Saturated RNA Secondary Structures

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Abstract

Over the last 30 years the development of RNA secondary structure prediction algorithms have been guided and inspired by corresponding combinatorial studies where the RNA molecules are modeled as certain kind of planar graphs. The other way round, new algorithmic ideas gave rise to interesting combinatorial problems asking for a deeper understanding of the structures processed. One such example is the notion *order* of a secondary structure as introduced by Waterman (as a parameter on graphs) in 1978, which reflects a structure's overall complexity: Regarding so-called *hairpin-loops* as the building blocks of a secondary structure, the order provides information on the (balanced) nesting-depth of hairpin-loops and thus on the overall complexity of the structure. In related prediction algorithms, one first searches for order 1 structures, increasing the allowed order step by step and thus considering an improved structural complexity in every iteration.

Subsequently, Zucker *et al.* and Clote introduced a more realistic combinatorial model for RNA secondary structures, the so-called *saturated secondary structures*. Compared to the traditional model of Waterman, unpaired nucleotides (vertices) which are in favorable position for a pairing do not exist, i.e. no base pair (edge) can be added without violating at least one restriction for the graphs. That way, one major shortcoming of the traditional model has been cleared. However, the resulting model gets much more challenging from a mathematical point of view. As a consequence, so far only little is known about the combinatorics of RNA saturated structures.

In this paper we show how it is possible to attack saturated structures and especially how to analyze their order. This is of special interest since in the past it has been proven to be one of the most demanding parameters to address (for the traditional model it has been an open problem for more than 20 years to find asymptotic results for the number of structures of given order and similar). We show the expected order of RNA saturated secondary structures of size n is $\log_4 n \left(1 + O\left(\frac{\log_2 n}{n}\right)\right)$, if we select the saturated secondary structure uniformly at random. Furthermore, the order of saturated secondary structures is sharply concentrated around its mean. As a consequence saturated structures and structures in the traditional model behave the same with respect to the expected order. Thus we may conclude that the traditional model has already drawn the right picture and conclusions inferred from it with respect to the order (the overall shape) of a structure remain valid even if enforcing saturation (at least in expectation).

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1. Introduction

The building blocks of RNA are four different nucleotides $\{a, c, g, u\} = \Sigma$ which are linked to each other in a linear fashion. Accordingly, the so-called primary structure of RNA, i.e. the linear sequence of building blocks, is modeled as string over Σ. In addition, non-neighboring nucleotides have a second means of binding by which certain combinations of nucleotides ($a - u$, $c - g$ and $g - u$) may form pairs, i.e. stick to each other. This gives rise to a 3D folding of the molecule which in many cases determines its biological function. Each such pair reduces the so-called free energy of the molecule and the conformation of minimal free energy is adopted in nature. Today, lab techniques to determine the primary structure of RNA are cheap and efficient while determining the 3D structure still is a time-consuming and expensive task. Accordingly one aims for algorithms to predict the structure from the sequence. However, even if building on rather simple models for the free energy, its minimization becomes an $\mathcal{N}P$-complete problem when allowing arbitrary foldings [10]. As a consequence, the set of considered structures is constrained and so-called secondary structures are considered as the first step towards understanding RNA biological function. There, only non-crossing pairings of nucleotides are allowed such that – ignoring types of nucleotides – the molecule can be represented as a planar graph [16] (see Figure 1.1) or alternatively by strings over $\{., (,)\}$ where a . represents an unpaired nucleotide and a pair of corresponding brackets represents two paired nucleotides (the left structure of Figure 1.1 is in correspondence with $((..(((......)))).))$). Even if computing a structure of minimum free energy (mfe) becomes efficient for secondary structures (algorithms with cubic time-bounds are well-known), the empiric thermodynamic data used are incomplete and erroneous such that suboptimal solutions need to be taken into consideration [11]. Computing the suboptimal structures is not difficult, however, the number of potentially interesting suboptimal conformation grows exponentially with the length of the nucleotide sequence. As one possible solution, Zucker and Sankoff suggested to restrict secondary structure folding to structures whose stacking regions (runs of consecutive brackets) extend maximally in both directions. This led to the definition of *saturated* structures for which no base pair can be added without violating the restrictions for secondary structures, see Figure 1.1. Extending the runs of consecutive brackets clears one mayor shortcoming of the traditional model, i.e. of secondary structures, which compared to native molecules tends to have ways too short stacking regions. Furthermore, in light of the asymptotic number of saturated structures determined by Clote *et al.* [4], the run time of RNA prediction algorithm should be substantially reduced if the search for suboptimal foldings is limited to saturated structures only, as observed by Bompfünewerer *et al.* for so-called canonical structures [1].

Clote initiated the combinatorial study of saturated structures [3] which gets much more challenging than that for secondary structures from a mathematical
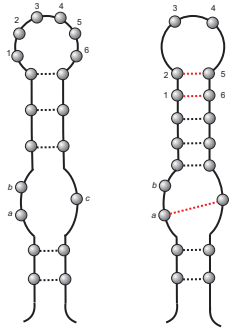
FIGURE 1.1. Secondary structure (left) and its saturated counterpart (right) where three additional links have been added (highlighted in red). The primary structure is given by the chain of vertices along the solid, pairs of nucleotides are represented by dotted edges. Note that 3 and 4 cannot be paired since both are neighbored with respect to primary structure.

point of view. He estimated the number of saturated structures by applying implicit function theory to the functional equations of its generating function $S(z)$ [4], i.e.,

$$-S(z)^3 z^4 - S(z)^2 z^2(-2 + z^2) + S(z)(-1 + z^2) + z(1 + z) = 0,$$

whereas the functional equation for secondary structures is relatively simple and given by

$$T(z) = z + zT(z) + z^2 T(z) + z^2 T(z)^2,$$

for $T(z)$ the generating function of secondary structures. Of course we observe variations of local parameters of the structures like the length and number of stacking regions or the length and number of loops (runs of symbols .). However, it is not at all obvious whether saturation has an effect on the overall shape of the structures. One parameter which allows to measure their overall shape is the so-called *order*, originally introduced by Waterman in 1978 for algorithmic purposes. A secondary structure s (saturated or not, represented in dot-bracket form) has order p if we need exactly p iterations of deleting all maximal substrings $(^k)^k$ within $\phi(s)$ in parallel to find the empty string ε. Here ϕ is the homomorphism implied by $\phi(() = (, \phi()) =)$ and $\phi(.) = \varepsilon$. Accordingly, the order provides information on the (balanced) nesting-depth of so-called hairpin-loops (substring with ϕ-image $(^n)^n$ which e.g. holds for the structures depicted in Figure 1.1) and thus on the overall complexity of the structure (it was used by algorithms to increasingly consider more and more complex foldings starting with a search space restricted to structures of order 1).

In this paper we show one way to approach the combinatorics of saturated structures and especially how to analyze their order. This – besides the motivating remarks from above – is of special interest since in the past it has been proven to be one of the most demanding parameters to address (for secondary structures it has been an open problem for more than 20 years to find asymptotic results for the number of structures of given order and similar). For that

purpose we discuss the generating function of saturated structures having order $\geq p$, denoted by $S_p(z)$, from which we extract the information of the expected order of a saturated structure of given size. We find that in expectation the order behaves the same for secondary and saturated structures such that we may conclude that the traditional model (secondary structures) has already drawn the right picture and conclusions inferred from it with respect to the order (the overall shape) of a structure remain valid even if enforcing saturation (at least in expectation).

The paper is organized as follows. We first present our main results. Afterwards we describe a streamlined analysis with details delayed till the last sections (or the appendix).

2. Main Results

Let $S(n)$ be the number of saturated RNA secondary structures of size n and $S_p(n)$ be the number of saturated RNA secondary structures of size n and having order $\geq p$, then we set ξ_n to be the random variable having probability distribution

$$\mathbb{P}(\xi_n = p) = \frac{S_p(n) - S_{p+1}(n)}{S(n)},$$

namely we select each saturated structure uniformly at random among the family of saturated RNA secondary structures of size n. Our main results are summarized as

Theorem 2.1. *The expected order of a saturated RNA secondary structure of size n is*

$$\mathbb{E}\xi_n = \log_4 n \cdot \left(1 + O\left(\frac{\log_2 n}{n}\right)\right).$$

Theorem 2.1 indicates that although the saturation of secondary structures increases the expected number of paired bases (and therefore increases the number of hairpin-loops possible) and scales down the search space, the complexity of the folding algorithm for saturated structures as given by the order stays almost the same. We may conclude that the traditional secondary structure model has already drawn the right picture and conclusions inferred from it with respect to the order of a structure (its overall shape) remain valid even if enforcing saturation (at least in expectation).

Theorem 2.2 below proves ξ_n is highly concentrated around the expected order $\mathbb{E}\xi_n$.

Theorem 2.2. *Assume we choose $0 \leq x \leq (\frac{1}{2} - \beta)\log_4 n$ for arbitrary $\beta > 0$, then we have*

$$\mathbb{P}(|\xi_n - \mathbb{E}\xi_n| \geq x) = O(2^{-x}).$$

3. Road Map of the Proof

In this section, we shall address the mayor steps and difficulties of analyzing the expected order of saturated structures by tools from analytic combinatorics [5, 6]. We start by deriving the key recursions for saturated structures of order p.

Let $S(z)$ (resp. $\mathcal{S}$) be the generating function (resp. the family) of saturated RNA structures and $R(z)$ (resp. $\mathcal{R}$) be the generating function (resp. the family) of saturated structures having the first and the last position paired, i.e., $\mathcal{R} = (\mathcal{S})$ where the parenthesis represents the paired bases and $R(z) = z^2 S(z)$. Furthermore, let $S_p(z)$ and $R_p(z)$ represent the corresponding generating function assuming order $\geq p$, $p \geq 1$. By decomposing the saturated structure into independent $\mathcal{R}$-type structures, we obtain the functional equation for $S(z)$

$$(3.1) \quad S(z) = \sum_{i=0}^{\infty} \left(1 + (i+1)(z+z^2)\right) R(z)^i - 1 = \frac{z^2 S(z)}{1 - z^2 S(z)} + \frac{z + z^2}{(1 - z^2 S(z))^2}.$$

Now, taking the order into account (omitting variable z for the ease of notation), we find the following recurrences for S_p and R_{p+1}, $p \geq 1$,

$$
\begin{aligned}
S_p &= \sum_{i \geq 1} (1 + (i+1)(z+z^2))(R^i - (R - R_p)^i) \\
(3.2) \quad &= \frac{R_p[1 + 2z^2 + 2z - 2R - 2Rz - 2Rz^2 + R^2 + (1 + z + z^2 - R)R_p]}{(R-1)^2(R - R_p - 1)^2},
\end{aligned}
$$

$$
\begin{aligned}
R_{p+1} &= R - z^2 \left[\sum_{i=1}^{\infty} (1 + (i+1)(z+z^2)) \left((R - R_p)^i + (R_p - R_{p+1})i \right) \right. \\
(3.3) \quad &\hspace{4cm} \left. \times (R - R_p)^{i-1} \right) + z + z^2 \Big] \\
(3.4) \quad &= \frac{(-R - z^2)R_p^3 + (-3R + 3R^2 + 3Rz^2 - z^2)R_p^2}{-R_p^3 + (3R - 3)R_p^2 + (6R - 3 - 3R^2 + z^2)R_p + (R-1)P_R},
\end{aligned}
$$

where $P = R^3 + (z^2 - 2)R^2 + (1 - z^2)R - z^3 - z^4$ and $P_R = \partial P / \partial R = 3R^2 + 2(z^2 - 2)R + (1 - z^2)$ and the initial conditions are $R_1 = R$ and $S_0 = S$.

Unlike for secondary structures[3], due to the non-local dependencies imposed for saturation neither the appropriate symbolic substitution nor the closed form solution of recurrence (3.2) could possibly exist, for which we have to decode the information of expected order from the recurrence itself other than attempting to solve it. Therefore, the proof for the expected order of saturated structures consists of locating the dominant singularities of $S_p(z)$ for $p \geq 0$, verifying the analytic continuation of $S_p(z)$ for some Δ-domain, which guarantees the validness of integration along Hankel contour, see Figure 3.1, and finding the singular expansion of $S_p(z)$ within the intersection of Δ-domain and a small neighborhood of the dominant singularity. Finally we apply a transfer theorem on the singular expansions of $S_p(z)$ and $S(z)$ to extract the n-th coefficient of $\sum_{p \geq 1} S_p(z)$ and $S(z)$, and conclude the expected order $\mathbb{E}\xi_n$ via

$$\mathbb{E}\xi_n = \frac{[z^n] \sum_{p \geq 1} S_p(z)}{[z^n] S(z)}.$$

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[3]For secondary structures the expected order has been analyzed by making use of well-known closed form representations of multivariate generating function for binary trees having Horton-Strahler number p. By the use of appropriate symbolic substitutions for the different variables the binary trees with Horton-Strahler number p were expanded into the secondary structures of order p and a closed form for the corresponding generating function followed [12].

The results on the deviation to the expected order follows similarly.

Before we proceed, we present the Transfer Theorem by Flajolet and Odlyzko [6]. The central point of this theorem is to use of Cauchy's formula by integrating along the Hankel contour depicted in Figure 3.1, which is guaranteed by the analytic continuation within a Δ-domain. We set

$$\Delta_{z_0}(M, \phi) = \{z \mid |z| < M, z \neq z_0, |\arg(z - z_0)| > \phi\}$$

where $M > z_0$ and $0 < \phi < \frac{\pi}{2}$. Let $U_{z_0}(r, \phi)$ be the intersection of $\Delta_{z_0}(M, \phi)$ and the neighborhood of z_0, i.e.,

$$U_{z_0}(r, \phi) = \{z \mid 0 < |z - z_0| < r, |\arg(z - z_0)| > \phi\},$$

then we have:

Theorem 3.1. (Transfer Theorem)[6] *Assume that $f(z)$ is analytic within $\Delta_1(M, \phi)$, and for $z \in U_1(r, \phi)$, $f(z)$ satisfies*

$$f(z) = O\left(\sqrt{1-z} \cdot \log_2\left(\frac{1}{1-z}\right)\right).$$

Then we have $[z^n]f(z) = O(n^{-\frac{3}{2}} \cdot \log_2 n)$.

Theorem 3.1 assumes the dominant singularity is $z = 1$. However, the case of a dominant singularity at $z = z_0 \neq 1$, can always be boiled down to the case where $z = 1$ is the dominant singularity according to

$$[z^n]f(z) = z_0^n \cdot [z^n]f\left(\frac{z}{z_0}\right).$$

In what follows we detail the steps that are needed for the singularity analysis
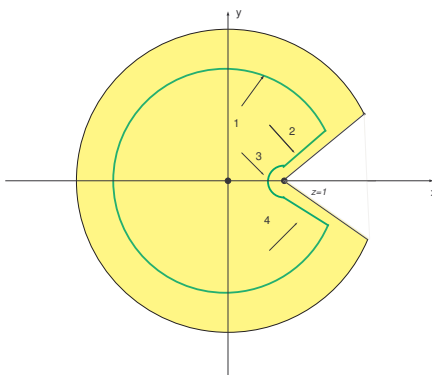

FIGURE 3.1. Δ_1-domain (yellow) and Hankel contour (green): Transfer theorem applies Cauchy's formula by integrating along the Hankel contour, colored in green. The inner incomplete circle 3, together with two rectilinear lines 2 and 4 mainly contribute to the integral. Here we assume the dominant singularity is at $z = 1$.

of $\sum_{p \geq 1} S_p(z)$.

Step 1: Locate dominant singularities: We first observe that the dominant singularity of $S(z)$ is unique since $[z^n]S(z) \neq 0$ holds for arbitrary n and

therefore $S(z)$ is aperiodic [6]. Assume z_0 is the unique dominant singularity of $S(z)$, then z_0 is also the unique dominant singularity of $S_p(z)$ for $p \geq 0$. Indeed, consider the field extension of the rational function field $Q(z)$ induced by algebraic functions $S_p(z)$, we can inductively prove that $[Q(S_p(z)) : Q(z)] = 3$ based on its tower relation $[Q(S_p(z)) : Q(z)] = [Q(S_p(z)) : Q(S_{p-1}(z))][Q(S_{p-1}(z)) : Q(z)] = [Q(S_{p-1}(z)) : Q(z)]$. In other words, $S_p(z)$ is an algebraic function of degree 3 over the field $Q(z)$. Let $S_{\leq p}(z)$ be the generating function of saturated structures having order $\leq p$, similarly we can prove $S_{\leq p}(z)$ is rational and in view of $S_p(z) = S(z) - S_{\leq p-1}(z)$, we can claim that $S_p(z)$ $(p \geq 0)$ have the same unique dominant singularity as $S(z)$. Otherwise, suppose $z = \gamma < z_0$ is the dominant singularity of $S_p(z)$ and therefore $S_p(\gamma) < S_p(z_0) < S(z_0) < \infty$, which contradicts to the fact that $S_p(\gamma) = S(\gamma) - S_{\leq p-1}(\gamma) = \infty$ since $S_{\leq p-1}(z)$ is a rational function and $z = \gamma$ must be one of the poles of $S_{\leq p-1}(z)$. Furthermore, $z = z_0$ is the unique dominant singularity of $S_p(z)$ since $[z^n]S_p(z) \neq 0$ and $S_p(z)$ is aperiodic.

Lemma 3.1. *Let z_0 be the unique dominant singularity of $S_p(z)$ $(p \geq 0)$, then $z_0 \approx 0.424687$.*

We apply the implicit function theorem on eq. (3.1) to extract the unique dominant singularity of $S(z)$, which is also the unique dominant singularity of $S_p(z)$.

Step 2: Establish the analytic continuation in some Δ_{z_0}-domain: Since $S_p(z)$ is an algebraic function of degree 3 over the rational function field $Q(z)$, $S_p(z)$ must be D-finite, which allows for analytic continuation in any Δ_{z_0}-domain containing zero [14].

Step 3: Singular expansion: We shall show the singular expansion of $S_p(z)$ within $U_{z_0}(\epsilon, \phi)$ for sufficiently small $\epsilon > 0$ and $0 < \phi < \frac{\pi}{2}$. Our strategy is to transform the fractional form of the recursion for $R_p(z)$ (eq. (3.4)) into "linear" form, based on the contributions of individual terms to the behavior of $R_p(z)$ for different p.

Case 1: $p \leq p_M = \max\left\{p : |P_R(R-1)| \leq \left|\frac{a_2}{4} \cdot R_p\right|\right\}$ for $a_2 = -3R + 3R^2 + 3Rz^2 - z^2$.

Lemma 3.2. *Assume that $z \in U_{z_0}(\epsilon, \phi)$ and $a_2 = -3R + 3R^2 + 3Rz^2 - z^2$, then*

$$
\begin{aligned}
S_{p+1}(z) &= s_p(z_0)\, 2^{-p} - \frac{1}{z_0^2} \cdot \frac{P_R(R-1)}{2a_2} \\
&\quad + O\left(\frac{p}{2^p}\left|\frac{P_R(R-1)}{a_2}\right|\right) + O\left(2^p \left|\frac{P_R(R-1)}{a_2}\right|^2\right).
\end{aligned}
$$

holds for $p \leq p_M$ and $s_p(z_0) = s + O(2^{-p})$ where $s > 0$ is constant.

Case 2: $p > p_M$. We continue analyzing the recurrence relations for $S_p(z)$ and $R_p(z)$ for $p > p_M$. Let $A'_p = -\frac{R+z^2}{a_2} \cdot R_p^3$ and $B'_p = -\frac{1}{a_2}R_p^3 + \frac{3R-3}{a_2}R_p^2 - \frac{3P_RR_p}{a_2}$. Note that $A'_p \to 0$ and $B'_p \to 0$ as $p \to \infty$ and $z \in U_{z_0}(\epsilon, \phi)$. Then we simply

have

$$(3.5) \qquad R_{p+1}(z) = \frac{R_p^2 + A_p'}{\frac{P_R(R-1)}{a_2} + 2R_p + B_p'}.$$

We observe that B_p' and A_p' converge to 0 faster than R_p as $p \to \infty$, and it only remains to determine the major contribution between R_p and $\frac{P_R(R-1)}{a_2}$ from the denominator to the behavior of R_p for different p. Here we all reduce the recursions to the function $h(x, \mu, \nu) = \frac{x^2+\mu}{1+2x+\nu}$, from which we can prove $h(x, \mu, \nu) = h(x, 0, 0) + O(\max\{|\mu|, |\nu|\})$ holds uniformly for $x \neq \frac{1}{2}$ as $\max\{|\mu|, |\nu|\} \to 0$. In order to asymptotically solve eq. (3.5), we need to avoid $R_p = \frac{1}{2}\frac{P_R(R-1)}{a_2}$, which may occur when p is sufficiently large. To this aim, we select $\lambda_1 > 0$ and $\lambda_2 > 0$ such that for $p \leq p_M + \lambda_2$, $\left|\frac{P_R(R-1)}{a_2}\right| \leq |R_p|$ and for $p \geq p_M - \lambda_1$, $|R_p| \geq 8\left|\frac{P_R(R-1)}{a_2}\right|$. Lemma 3.3 below shows the "continuity" of the phase transition around $p = p_M$.

Lemma 3.3. *Assume $z \in U_{z_0}(\epsilon, \phi)$ and $p_0 = p_M - \lambda_1$, then for arbitrary but fixed $\delta \leq \lambda_2$, we have uniformly for z and for $0 \leq k \leq \lambda_1 + \delta$ that,*

$$S_{p_0+k} = \frac{1}{z_0^2}\frac{\frac{P_R(R-1)}{a_2}}{\left(\frac{\frac{P_R(R-1)}{a_2}}{R_{p_0}}+1\right)^{2^k}-1} + O\left(\left|\frac{P_R(R-1)}{a_2}\right|^2\right),$$

where $a_2 = -3R + 3R^2 + 3Rz^2 - z^2$.

Lemma 3.4. *Assume that $z \in U_{z_0}(\epsilon, \phi)$, there exists $\kappa_0 \geq \lambda_2$ such that for $p > p_M + \kappa_0$,*

$$S_{p+1}(z) = O\left(\left|\frac{P_R(R-1)}{a_2}\right|\exp(-\ln 2 \cdot 2^p)\right).$$

Step 5: Transfer to coefficients: It only remains to translate the singular expansion of the function into an asymptotic estimate of its coefficients.

Theorem 3.2. *The expected order of a saturated secondary structures of size n is*

$$\mathbb{E}\xi_n = \log_4 n \cdot \left(1 + O\left(\frac{\log_2 n}{n}\right)\right).$$

Proof. We first analyze the expectation function $F(z) = \sum_{p \geq 1} S_p(z)$ for $z \in U_{z_0}(\epsilon, \phi)$. According to Lemma 3.2, Lemma 3.3 and Lemma 3.4, we have for

$p \geq 1$,

$$
\sum_{p \leq p_M} S_{p+1}(z) = \sum_{p \leq p_M} s_p(z_0) \, 2^{-p} - \frac{p_M}{z_0^2} \cdot \frac{P_R(R-1)}{2a_2} + O\left(\left|\frac{P_R(R-1)}{a_2}\right|\right)
$$

$$
= \sum_{p \geq 1} s_p(z_0) \, 2^{-p} - \frac{p_M}{z_0^2} \cdot \frac{P_R(R-1)}{2a_2} + O\left(\left|\frac{P_R(R-1)}{a_2}\right|\right).
$$

$$
\sum_{p > p_M} S_{p+1}(z) = \sum_{p_M < p \leq p_M + \kappa_0} S_{p+1}(z) + \sum_{p > p_M + \kappa_0} S_{p+1}(z)
$$

$$
= O\left(\left|\frac{P_R(R-1)}{a_2}\right|\right) + \sum_{p > p_M + \kappa_0} O\left(\left|\frac{P_R(R-1)}{a_2}\right| \exp(-\ln 2 \cdot 2^p)\right)
$$

$$
= O\left(\left|\frac{P_R(R-1)}{a_2}\right|\right).
$$

In combination of the cases $p \leq p_M$ and $p > p_M$, we obtain

$$
F(z) = \sum_{p \leq p_M} S_{p+1}(z) + \sum_{p > p_M} S_{p+1}(z) + S_1(z)
$$

$$
= \sum_{p \geq 0} S_{p+1}(z_0) + \left(S(z) - \frac{1}{1-z} - S_1(z_0)\right) - \frac{p_M}{z_0^2} \cdot \frac{P_R(R-1)}{2a_2}
$$

$$
+ O\left(\left|\frac{P_R(R-1)}{a_2}\right|\right).
$$

Recall that p_M is given by

$$
p_M = \max\left\{p : |P_R(R-1)| \leq \left|\frac{a_2}{4} \cdot R_p\right|\right\}
$$

and we need to find an appropriate representation for it. For $z \in U_{z_0}(\epsilon, \phi)$, $p_M \approx -\log_2\left|\frac{P_R(R-1)}{a_2}\right|$. By setting $F_0 = F(z_0)$ and $S(z) = S(z_0) - \frac{1}{z_0^2}\frac{P_R(R-1)}{2a_2} + O(z - z_0)$, we simplify $F(z)$ into

$$
F(z) = F_0 - \frac{1}{z_0^2}\frac{P_R(R-1)}{2a_2} - \frac{p_M}{z_0^2}\frac{P_R(R-1)}{2a_2} + O\left(\sqrt{1 - \frac{z}{z_0}}\right)
$$

$$
= F_0 + \frac{\log_2\left|\frac{P_R(R-1)}{a_2}\right|}{z_0^2}\frac{P_R(R-1)}{2a_2} + O\left(\sqrt{1 - \frac{z}{z_0}}\right)
$$

$$
= F_0 + \frac{1}{z_0^2}\frac{P_R(R-1)}{2a_2}\log_2\left(\frac{P_R(R-1)}{a_2}\right) + O\left(\sqrt{1 - \frac{z}{z_0}}\right)
$$

$$
= F_0 - \sqrt{\frac{P_z(z_0)}{2P_{RR}(z_0)z_0^3}}\sqrt{1 - \frac{z}{z_0}}\log_2\left(\frac{1}{1 - \frac{z}{z_0}}\right) + O\left(\sqrt{1 - \frac{z}{z_0}}\right).
$$

According to Theorem 3.1 (Transfer Theorem), for $n \geq 1$, the expected order of a saturated secondary structure is thus given by

$$
\begin{aligned}
\mathbb{E}\xi_n &= \frac{[z^n]F(z)}{[z^n]S(z)} = \frac{-n^{-\frac{3}{2}}}{\Gamma(-\frac{1}{2})} \cdot \log_2 n \cdot z_0^{-n} \cdot z_0^2 \sqrt{\frac{P_z(z_0)}{2P_{RR}(z_0)z_0^3}} \sqrt{\frac{2\pi P_{RR}(z_0)}{z_0 P_z(z_0)}} n^{\frac{3}{2}} z_0^n \\
&\quad \times \left(1 + O\left(\frac{\log_2 n}{n}\right)\right) \\
&= \log_4 n \cdot \left(1 + O\left(\frac{\log_2 n}{n}\right)\right),
\end{aligned}
$$

whence the proof is complete. $\qquad \square$

Finally we discuss the large deviation of the random variable ξ_n.

Theorem 3.3. *Assume we choose $0 \leq x \leq (\frac{1}{2} - \beta)\log_4 n$ for arbitrary $\beta > 0$, then we have*

$$
\mathbb{P}(|\xi_n - \mathbb{E}(\xi_n)| \geq x) = O(2^{-x}).
$$

Proof. For $p \leq \log_4 n$, Lemma 3.2 in combination with the Transfer Theorem implies

$$
\mathbb{P}(\xi_n \geq p) = 1 + O\left(\frac{2^p}{\sqrt{n}}\right) + O\left(\frac{p}{2^p}\right).
$$

For $p > \log_4 n$, Lemma 3.3 indicates that

$$
\mathbb{P}(\xi_n \geq p) = O\left(\exp\left(-\frac{\beta'2^p}{\sqrt{n}}\right)\right) \qquad \text{for } \beta' > 0.
$$

Consequently the theorem follows. $\qquad \square$

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