On the scale-free nature of RNA secondary structure networks

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Abstract

A network is scale-free if its connectivity density function is proportional to a power-law distribution. Scale-free networks may provide an explanation for the robustness observed in certain physical and biological phenomena, since the presence of a few highly connected hub nodes and a large number of small-degree nodes may provide alternate paths between any two nodes on average—such robustness has been suggested in studies of metabolic networks, gene interaction networks and protein folding. A theoretical justification for why biological networks are often found to be scale-free may lie in the well-known fact that expanding networks in which new nodes are preferentially attached to highly connected nodes tend to be scale-free. In this paper, we provide the first efficient algorithm to compute the connectivity density function for the ensemble of all secondary structures of a user-specified length, and show both by computational and theoretical arguments that preferential attachment holds when expanding the network from length $n$ to length $n+1$ structures. Since existent power-law fitting software, such as powerlaw, cannot be used to determine a power-law fit for our exponentially large RNA connectivity data, we also implement efficient code to compute the maximum likelihood estimate for the power-law scaling factor and associated Kolmogorov-Smirnov $p$-value. Statistical goodness-of-fit tests indicate that one must reject the hypothesis that RNA connectivity data follows a power-law distribution. Nevertheless, the power-law fit is visually a good approximation for the tail of connectivity data, and provides a rationale for investigation of preferential attachment in the context of macromolecular folding.

1 Introduction

The connectivity (or degree) of a node $v$ in a network (or undirected graph) is the number of nodes (or neighbors) of $s$, connected to $s$ by an edge. A network is said to be scale-free if its connectivity function $N(k)$, which represents the number of nodes having degree $k$, satisfies the property that $N(a·k) = b·N(x)$, the unique solution of which is a power-law distribution, which by definition satisfies $N(k) \propto k^{-\alpha}$ for some scaling factor $\alpha > 1$ [22]. Scale-free networks contain a few nodes of high degree and a large number of nodes of small degree, hence may provide a reasonable model to explain the robustness often manifested in biological networks—such robustness must, of course, be present for life to exist.

Barabási and Albert [3] analyzed the emergence of scaling in random networks, and showed that two properties, previously not considered in graph theory, were responsible for the power-law scaling observed in real networks: (1) networks are not static, but grow over time, (2) during network growth, a highly connected node tends to acquire even more connections—the latter concept is known as preferential attachment. In [3], it was argued that preferential attachment of new nodes implies that the degree $N(k)$ with which a node in the network interacts with $k$ other nodes decays as a power-law, following $N(k) \propto k^{-\alpha}$, for $\alpha > 1$. This argument provides a plausible explanation for why diverse biological and physical networks appear to be scale-free. Indeed, various publications have suggested that the following biological networks are scale-free: protein-protein interaction networks [14, 24], metabolic networks [18], gene interaction networks [26], yeast co-expression networks [27], and protein folding networks [3].

How scale-free are biological networks?

The validity of a power-law fit for previously studied biological networks was first called into question in [16], where 10 published data sets of biological interaction networks were shown not to be fit by a power-law distribution, despite published claims to the contrary. Estimating an optimal power-law scaling factor by maximum likelihood and using $\chi^2$ goodness-of-fit tests, it was shown in [16] that not a
single interaction network from had a nonzero probability of being drawn from a power-law distribution; nevertheless, some of the interaction networks could be fit by a truncated power-law distribution. The data analyzed by the authors included data from protein-protein interaction networks [13], gene interaction networks determined by synthetic lethal interactions [20], metabolic interaction networks [15], etc.

In [9], 24 real-world data sets were analyzed from a variety of disciplines, each of which had been conjectured to follow a power-law distribution. Estimating an optimal power-law scaling factor by maximum likelihood and using goodness-of-fit tests based on likelihood ratios and on the Kolmogorov-Smirnov statistic for non-normal data, it was shown in [9] that some of the conjectured power-law distributions were consistent with claims in the literature, while others were not. For instance, Clauset et al. [9] found sufficient statistical evidence to reject claims of scale-free behavior for earthquake intensity and metabolic degree networks, while there was insufficient evidence to reject such claims for networks of protein interaction, Internet, and species per genus.

It is possible to come to opposite conclusions, depending on whether $\chi^2$ or Kolmogorov-Smirnov (KS) statistics are used to test the hypothesis whether a network is scale-free, i.e. follows a (possibly truncated) power-law distribution. Indeed, Khanin and Wit [16] obtained a $p$-value of $< 10^{-4}$ for $\chi^2$ goodness-of-fit for a truncated power-law distribution for the protein-protein interaction data from [14], while Clauset et al. [9] obtained a $p$-value of 0.31 for KS goodness-of-fit for a truncated power-law for the same data. This example provides the occasion for us to explain the position taken in this paper that (in our opinion) it is quite possible for a statistical test to lead to the rejection of goodness-of-fit of the power-law distribution for physical data arising from biological networks, yet the (approximate) power-law fit can possibly provide valuable insight into the nature of the data. In this manner, we sidestep the current polemic concerning the question of how wide-spread scale-free networks really are. In their preprint from Jan. 9, 2018, entitled “Scale-free networks are rare”, Broido and Clauset [6] argue that less than 45 of the 927 real-world network data sets (i.e. 4%) found in the Index of Complex Networks exhibit the “strongest level of direct evidence for scale-free structure”. In a response statement dated March 6, 2018, A.L. Barabási argued against the conclusions of Broido and Clauset – indeed, the title of Barabási’s statement sums up his position: “Love is All You Need: Clauset’s fruitless search for scale-free networks.”

Regardless of the Barabási-Clauset polemic, we stress that prior to the introduction of our novel secondary structure connectivity algorithm, only fragmentary results were possible by exhaustively enumerating all secondary structures having free energy within a certain range above the minimum free energy [28]. Indeed, using our methods, for the first time we can address the question of whether RNA secondary structure connectivity of a given homopolymer, which allows non-canonical base pairs. Though not done in this paper, this algorithm could be extended to the case of (real) RNA sequences allowing only Watson-Crick and wobble base pairs. Section 4 presents results on power-law fits of RNA connectivity data, and computational evidence that preferential attachment holds for RNA secondary structure networks. Section 5 presents concluding remarks, and the Appendix presents a mathematical proof of preferential attachment in the case of a simplified model of secondary structure.

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1It is not the first time a polemic has arisen concerning the power-law distribution – indeed, there was a heated exchange between Mandelbrot and Simon almost 60 years ago in the journal Information and Control. For details, references, and a history of the power-law distribution, see see [21].
2 Computing degree frequency

Section 2.1 presents basic definitions and notation used; Section 2.2 presents an algorithm to compute the frequency of each degree less than $K$ in the ensemble of all secondary structures with run time $O(K^2n^4)$ and memory requirements $O(Kn^3)$. Section 2.3 presents a more efficient algorithm, with run time $O(K^2n^4)$ and memory requirements $O(Kn^2)$, for the special case of a homopolymer, in which all possible non-canonical base pairs are permitted. We implemented both algorithms in Python, cross-checked for identical results, and call the resulting code RNAdensity. Since this paper is a theoretical contribution on network properties, we focus only on homopolymers and do not present the details necessary to extend the algorithm of Section 2.2 to non-homopolymer RNA, where base pairs are required to be Watson-Crick or GU wobble pairs.

2.1 Preliminaries

A secondary structure for a length $n$ homopolymer is a set $s$ of base pairs $(i, j)$, such that (1) there exist at least $\theta$ unpaired bases in every hairpin, where $\theta$ is usually taken to be 3, though sometimes 1 in the literature, (2) there do not exist base pairs $(i, j), (k, \ell) \in s$, such that $i < k < j < \ell$; i.e. a secondary structure is an outerplanar graph, where each base pair $(i, j) \in s$ satisfies $j - i > \theta$. The free energy of a homopolymer secondary structure $s$ is defined to be $-1$ times the number $|s|$ of base pairs in $s$ (Nussinov-Jacobson energy model [23]). Since entropic effects are ignored, this is not a real free energy; however it allows us to use the standard notation “MFE” for ‘minimum free energy’. Note that the MFE of a homopolymer secondary structure is an outerplanar graph, where each base pair $(i, j)$ has degree $\theta$, maxDegree$(n)$, of a secondary structure for the length $n$ homopolymer is \( \frac{n - \theta}{2} \) times the number of base pairs in

$N(k)$ is defined to be the maximum possible degree, maxDegree$(n)$, of a secondary structure for the length $n$ homopolymer is \( \frac{n - \theta}{2} \)

$N(i, j)$ denote the number of secondary structures on the interval $[i, j]$ that have exactly $k$ neighbors with respect to the $MS_1$ move set (i.e. have degree $k$). Let $N(i, j)$ denote the number of
secondary structures on interval \([i,j]\), computed by simple recursions from [25]

\[N(i,j) = \begin{cases} 
1 & \text{if } 1 \leq i \leq j \leq i + \theta \leq n \\
N(i,j-1) + N(i+1,j-1) + \sum_{r=i+1}^{j-\theta-1} N(i,r-1) \cdot N(r+1,j-1) & \text{if } 1 + \theta + 1 \leq j \leq n 
\end{cases} \quad (2)\]

or more simply

\[N(m) = \begin{cases} 
1 & \text{if } 1 \leq m \leq \theta + 1 \\
N(m-1) + N(m-2) + \sum_{r=d}^{m-3} N(m-r-2) \cdot N(r) & \text{if } \theta + 2 \leq m \leq n 
\end{cases} \quad (3)\]

Although recursion equation (2) requires \(O(n^3)\) time and \(O(n^2)\) space, it can trivially be extended to compute the number of secondary structures for an arbitrary RNA sequence \(a_1, \ldots, a_n\), where base pairs are either Watson-Crick or wobble pairs. If no such extension is necessary, then the recursion equation (3), first given in [25], requires \(O(n^2)\) time and \(O(n)\) space, hence is more efficient by a factor of \(n\). In a similar fashion, the recursion equations (5-12) and pseudocode in Section 2.2 are given in a form that allows an extension (not given here) to the general case of computing the degree density for the ensemble of secondary structures of a given RNA sequence \(a_1, \ldots, a_n\). The resulting code refalgo:degreeDensity requires \(O(n^3)\) time and \(O(n^2)\) storage, but this can be improved by a factor of \(n\).

Suppose that every hairpin loop is required to have at least \(\theta \geq 1\) unpaired positions; i.e. if \((i,j)\) is a base pair, then \(i+\theta+1 \leq j\). As in the recursions (6-12), let \(Z(i,j,k,h,v)\) denote the number of secondary structures on the interval \([i,j]\), for \(1 \leq i \leq j \leq n\) for the homopolymer model, that have exactly \(k\) neighbors, and for which there are exactly \(h\) unpaired positions (or holes) in \([i,j-\theta-1]\) and the position \(j-v\) is paired to \(r \in [i,j-v-\theta-1]\), while positions \(j-v, j-v+1, \ldots, j\) are not base-paired to any position in \([i,j]\). Additionally, define

\[Z^*(i,j,k) = \sum_{h=0}^{j-\theta-1} \sum_{v=0}^{\theta+1} Z(i,j,k,h,v) \quad (4)\]

Recalling from equation (1) that \(\text{maxDegree}(n) = \binom{n-\theta}{\theta+1}^{-1} \frac{n^2}{2}\), for any \(1 \leq i \leq j \leq n\), we clearly have that

\[N(i,j) = \sum_{k=1}^{\text{maxDegree}(j+1)} Z^*(i,j,k)\]

\[= \sum_{k=1}^{\text{maxDegree}(j+1)} \sum_{h=0}^{j-\theta-1} \sum_{v=0}^{\theta+1} Z(i,j,k,h,v)\]

The idea of our algorithm is to partition all secondary structures of the interval \([i,j]\) into those structures having exactly degree \(k\) (\(k\) MS1 neighbors, i.e. \(k\) structures that can be obtained by either adding or removing a single base pair). To support an inductive argument, in proceeding from interval \([i,j]\) to \([i,j+1]\), we need additionally to determine the number of structures having degree \(k\), which have a certain number \(h\) of positions that are visible (external to every base pair), which can be paired with the last position \(j+1\). Note that the position \(j-\theta\) can not be base-paired with \(j\) in \([i,j]\); however, \(j-\theta\) can be base-paired with \(j\) in \([i,j+1]\). Thus in addition to keeping track of the number \(h\) of holes (positions in \(i, \ldots, j-\theta-1\) that are external to all base pairs, hence can be paired with \(j\)), we introduce the variable \(v\) to keep track of the number of visible positions in \(j-\theta, \ldots, j\). This explains our need for the function \(Z(i,j,k,h,v)\) as defined in equations (5-12). We now proceed to the details, where for ease of the reader, some definitions are repeated.

Let \(\theta = 3\) denote the minimum number of unpaired positions required to be present in a hairpin loop. For a length \(n\) homopolymer, let \(1 \leq i \leq j \leq n\), \(0 \leq k \leq \binom{n-\theta}{\theta+1}\), \(0 \leq h \leq j - i - \theta\), \(0 \leq v \leq \theta + 1\). Recall that \(Z(i,j,k,h,v)\) denotes the number of secondary structures on \([i,j]\) for the homopolymer model, that have exactly \(k\) MS1 neighbors (i.e. degree \(k\)), and there are exactly \(h\) unpaired positions in \([i,j-\theta-1]\) and the position \(j-v\) is base-paired to some \(r \in [i,j-v-\theta-1]\) while positions \(j-v, j-v+1, \ldots, j\) are not base-paired to any position in \([i,j]\). The parameter \(h\) corresponds to the number of visible positions or holes \([i,j-\theta-1]\) that are external to base pairs in \([i,j]\), while the parameter \(v\) corresponds to the number of visible positions in \([j-\theta, j]\) that are external to base pairs in \([i,j]\).

Recall our notation \(Z^*(i,j,k) = \sum_v Z(i,j,k,h,v)\). We begin by initializing \(Z(i,j,k,h,v) = 0\) for all values in corresponding ranges. Letting \(N(i,j)\) denote the number of secondary structures on \([i,j]\) for
the homopolymer model, as computed by equation (2), the following recursions describe an algorithm that requires $O(K \cdot n^3)$ storage and $O(K^2 \cdot n^4)$ time to compute the probability $\text{Prob}[\deg(s) = k] = \frac{Z(1,n,k)}{N(1,n)}$ that a (uniformly chosen) random secondary structure has degree $k$ for $0 \leq k \leq K$, where $K$ is a user-defined constant bounded above by $\text{maxDegree}(n) = \frac{(n-\theta)(n-\theta-1)}{2}$.

Base Case A considers all structures on $[i,j]$, as depicted in Figure 1, that are too small to have any base pairs, hence which have degree zero.

**Base Case A:** For $j - i \leq \theta$, define

$$Z(i, j, 0, 0, j - i + 1) = 1$$

(5)

Figure 1: Structures considered in base case A.

Base Case B considers all structures on $[i,j]$, as depicted in Figure 2, that have only base pair $(i,j)$, since other potential base pairs would contain fewer than $\theta$ unpaired bases. The degree of such structures is 1, since only one base pair can be removed, and no base pairs can be added. Moreover, no position in $[i,j]$ is external to the base pair $(i,j)$, so visibility parameters $h = 0, v = 0$. The arrow in Figure 2 indicates that the sole neighbor is the empty structure, obtained by removing the base pair $(i,j)$.

**Base Case B:** For $j - i = \theta + 1$ and $(i,j)$ is a base pair, define

$$Z(i, j, 1, 0, 0) = 1$$

(6)

Figure 2: Structures considered in base case B.

Base Case C considers the converse situation, consisting of the empty structure on $[i,j]$ where $j - i > \theta + 1$, whose sole neighbor is the structure consisting of base pair $(i,j)$. The arrow is meant to indicate that the structure on the right is the only neighbor of that on the left, as depicted in Figure 3. Since the size of the empty structure on $[i,j]$ is $\theta + 2$ and every position in $[i,j]$ is visible (external to every base pair), $h = 1$ and $v = \theta + 1$. The dotted rectangle in Figure 3 indicates the $\theta + 1$ unpaired positions at the right extremity as counted by $v = \theta + 1$.

**Base Case C:** For $j - i = \theta + 1$ and $(i,j)$ not base-paired, define

$$Z(i, j, 1, 1, \theta + 1) = 1$$

(7)

Figure 3: Structures considered in base case C.

Base Case D considers the empty structure on $[i,j]$ where $j - i > \theta + 1$. The empty structure is the only structure having degree $\text{maxDegree}(i,j) = \frac{(j-i-\theta+1)(j-i-\theta)}{2}$, since $\text{maxDegree}(i,j)$ many base pairs can be added to the empty structure. In Figure 4, the dotted rectangle indicates the $\theta + 1$ rightmost unpaired positions, corresponding to visibility parameter $v = \theta + 1$, while dotted circles indicate the $h = j - i - \theta$ holes, i.e., unpaired positions that could be paired with the rightmost position $j$. 
**Base Case D:** For all \((j - i + 1) > \theta + 2\), the empty structure, as indicated by \(h + v = j - i + 1\) (so \(h = j - i - \theta\) and \(v = \theta + 1\)), has degree \(\text{maxDegree}(i, j)\) as defined by equation \(1\), where

\[
Z(i, j, \text{maxDegree}(i, j), j - i - \theta, \theta + 1) = 1
\]

\[m = j - i + 1\]

\begin{figure}[h]
\centering
\includegraphics[width=0.3\textwidth]{figure4.png}
\caption{Structures considered in base case D.}
\end{figure}

Inductive Case A considers the case where left and right extremities \(i, j\) form the base pair \((i, j)\), where \(j - i > \theta + 1\). No position in \([i, j]\) is visible (external to all base pairs), so visibility parameters \(h = 0 = v\). Recalling the definition of \(Z^* (i, j, k)\) from equation \(4\), we have the following.

**Inductive Case A:** For \(j - i > \theta + 1\) and \((i, j)\) base-paired in \([i, j]\),

\[
Z(i, j, k, 0, 0) = Z(i, j, 0, 0) + Z^*(i + 1, j - 1, k - 1)
\]

From this point on, we use the operator \(\_+\_\), so that the previous equation would be written as

\[
Z(i, j, k, 0, 0) = Z^*(i + 1, j - 1, k - 1)
\]

\begin{figure}[h]
\centering
\includegraphics[width=0.3\textwidth]{figure5.png}
\caption{Structures considered in inductive case A.}
\end{figure}

Inductive Case B considers the case where last position \(j\) base-pairs with the \(r\), where \(i < r < j - \theta\). The value \(r = i\) has already been considered in Inductive Case A, and values \(r = j - \theta + 1, \ldots, j - 1\) cannot base-pair to \(j\), since the corresponding hairpin loop would contain less than \(\theta\) unpaired positions. This situation is depicted in Figure [6], where there are \(h\) holes (positions in \([i, j - \theta - 1]\) that are external to all base pairs) and no visible positions in \([j - \theta, j]\).

**Inductive Case B:** For \(j - i > \theta + 1\) and \((r, j)\) base-paired in \([i, j]\) for some \(i < r < j - \theta\),

\[
Z(i, j, k, h, 0) = \sum_{r=i+1}^{j-\theta-1} \sum_{k_1+k_2=k-1}^{\theta+1} \sum_{w=0}^{\theta+1} Z(i, r - 1, k_1, h - w, w) \cdot Z^*(r + 1, j - 1, k_2)
\]

When implemented, this requires a check that \(h - w \geq 0\).

\begin{figure}[h]
\centering
\includegraphics[width=0.3\textwidth]{figure6.png}
\caption{Structures considered in inductive case B.}
\end{figure}

For each value \(v \in \{1, \ldots, \theta + 1\}\), inductive Case \(C(v)\) considers the case where position \(r \in [i, j - v - \theta - 1]\) forms a base pair with position \(j - v\). The value \(v = 0\) is not considered here, since it was already considered in Inductive Cases A,B. Note that a structure \(s\) of the format has \(k\) neighbors, provided the restriction of \(s\) to \([i, r - 1]\) has \(k_1\) neighbors, and the restriction of \(s\) to \([r + 1, j - 1]\) has \(k_2\) neighbors, where \(k_1 + k_2 + v h + 1 = k\). The term \(v h\) is due to the fact that since base pair \((r, j - v)\) ensures that all holes are located in \([i, r - 1]\), hence located at more than \(\theta + 1\) distance from all visible positions in \([j - v + 1, j]\),
a neighbor of \( s \) can be obtained by adding a base pair from any hole to any visible suffix position – there are \( vh \) many such possible base pairs that can be added. Finally, the last term \( +1 \) is present, since one neighbor of \( s \) can obtained by removing base pair \((r, j - v)\). This explains the summation indices and summation terms in equation (11). Figure 7 depicts a typical structure considered in case \( C(v) \).

**Inductive Case \( C(v) \), for \( v \in \{1, 2, \ldots, \theta + 1\} \):** For \( j - i > \theta + 1 \) and \((r, j - v)\) base-paired in \([i, j]\), for some \( i < r < j - v - \theta \), where \( j - v + 1, \ldots, j \) are unpaired in \([i, j]\).

\[
Z(i, j, k, h, v) + = Z^*(2, j - 1 - v, k - 1 - vh) + \sum_{r = i + 1}^{j - \theta - 1} \sum_{k_1 + k_2 = (k - 1 - vh)}^{\theta + 1} Z(i, r - 1, k_1, h - w, w) \cdot Z^*(r + 1, j - 1 - v, k_2)
\]

The first term \( Z^*(2, j - 1 - v, k - 1 - vh) \) handles the subcase where \( r = 1 \), so that \((1, j - v)\) is a base pair, while the second term handles the subcase where \( r > 1 \). Note that when implemented, this requires a test that \( h - w \geq 0 \).

![Figure 7: Structures considered in inductive case C(v).](image)

Case \( D \) considers the case where there are \( h \) holes, and positions \( j - \theta - 1, \ldots, j \) are unpaired, so that \( v = \theta + 1 \). Note that \( v = \theta + 1 \) implies only that \( j - \theta, \ldots, j \) are unpaired, so Case \( D \) includes the addition requirement that position \( j - \theta - 1 \) is unpaired. Structures \( s \) satisfying Case \( D \) can be partitioned into subcases where the restriction of \( s \) to \([i, j - \theta - 1]\) has \( h - w \) holes in \([i, (j - \theta - 1) - (\theta + 1)]\), and \( 1 \leq w \leq \theta + 1 \) visible positions in \([j - 2\theta - 1, j - \theta - 1]\). Note that \((h - w) + w = h\), accounting for the \( h \) holes in structure \( s \) in \([i, j - \theta - 1]\), and that it is essential that \( w \geq 1 \), since the case \( w = 0 \) was considered in Case \( C(\theta + 1) \).

The term \( \frac{w(w + 1)}{2} \) is due to the fact that the rightmost position \( j - \theta - 1 \) in the restriction of \( s \) to \([i, j - \theta - 1]\) can base-pair with position \( j \), but not with \( j - 1 \), etc. since this would violate the requirement of at least \( \theta \) unpaired bases in a hairpin loop. Similarly, the second rightmost position \( j - \theta - 2 \) in the restriction of \( s \) to \([i, j - \theta - 1]\) can base-pair with positions \( j \) and \( j - 1 \), but not with \( j - 2 \), etc.; as well, the third rightmost position \( j - \theta - 3 \) can base-pair with positions \( j, j - 1 \) and \( j - 2 \), but not with \( j - 3 \), etc. The number of neighbors of \( s \) produced in this fashion is thus \( \sum_{i = 1}^{w} i = \frac{w(w + 1)}{2} \). Finally, the term \((\theta + 1)(h - w)\) is due to the fact that each of the \( h - w \) holes in the restriction of \( s \) to \([i, j - \theta - 1]\) can base-pair to each of the \((\theta + 1)\) positions in \([j - \theta, j]\).

The argument just given shows the following. Let \( s \) be a structure that satisfies conditions of Case \( D \) with \( h \) holes and \( v = \theta + 1 \) visible positions, and suppose that the restriction of \( s \) to \([i, j - \theta - 1]\) has \( h - w \) holes and \( w \) visible positions. Then \( s \) has \( k \) neighbors provided that the restriction of \( s \) to \([i, j - \theta - 1]\) has \( k - \frac{w(w + 1)}{2} - (\theta + 1)(h - w) \) neighbors on interval \([i, j - \theta - 1]\). The equation (12) now follows.

**Inductive Case \( D \):** For \( j - i > \theta + 1 \) and \( j - \theta - 1, j - \theta, \ldots, j \) unpaired in \([i, j]\), and \( 1 \leq h < j - \theta - i \),

\[
Z(i, j, k, h, \theta + 1) + = \sum_{w = 1}^{\theta + 1} Z(i, j - \theta - 1, k - \frac{w(w + 1)}{2} - (\theta + 1)(h - w), h - w, w)
\]

![Figure 8: Structures considered in inductive case D.](image)

As in Case \( C(v) \), when implemented, this requires a test that \( h - w \geq 0 \).

Our implementation of the recursions (11)(12) has been cross-checked with exhaustive enumeration; moreover, we always have that \( \sum_{k} Z^*(i, j, k) = N(i, j) \), so the degree density is correctly computed.
2.3 Faster algorithm in the homopolymer case

The algorithm described in Section 2.2 requires $O(K^2n^4)$ time and $O(Kn^3)$ space, where $K$ is a user-specified degree bound $K \leq \frac{(n-\theta)n}{2}$. By minor changes, that algorithm can be modified to compute the degree density function $p(k) = \frac{Z^*(i,j,k)}{N(i,j)}$ for any given RNA sequence $a_1, \ldots, a_n$. In the case of a homopolymer, any two positions are allowed to base-pair (regardless of whether the base pair is a Watson-Crick or wobble pair), provided only that every hairpin loop contains at least $\theta$ unpaired positions.

For homopolymers, we have a faster algorithm that requires $O(\theta)$ time and $O(Kn^3)$ space. Since nucleotide identity is unimportant, instead of $Z(i,j,k,h,v)$, we describe the function $\hat{Z}(m,k,h,v)$, where $m$ corresponds to the length $j – i + 1$ of interval $[i,j]$.

$$\hat{Z}^*(m, k) = \sum_{h=0}^{m-\theta-1} \sum_{v=0}^{k}
\hat{Z}(m, k, h, v)$$

$$N(m) = \sum_{h=1}^{\theta} \hat{Z}^*(m, k)$$

We begin by initializing $\hat{Z}(m, k, h, v) = 0$ for all $1 \leq m \leq n$, $0 \leq k \leq \frac{(m-\theta)(m-\theta-1)}{2}$, $0 \leq h \leq m-2$, and $0 \leq v \leq \theta + 1$. If $h < 0$, we assume that $\hat{Z}(m, k, h, v) = 0$.

**Base Case A:** For $1 \leq m \leq \theta + 1$, define

$$\hat{Z}(m, 0, 0, m) = 1$$

**Base Case B:** For $m = \theta + 2$, define

$$\hat{Z}(m, 1, 0, 0) = 1$$

**Base Case C:** For $m = \theta + 2$, define

$$\hat{Z}(m, 1, 1, \theta + 1) = 1$$

**Base Case D:** For all $m > \theta + 2$, define

$$\hat{Z}(m, \frac{(m-\theta)(m-\theta-1)}{2}, m-\theta-1, \theta + 1) = 1$$

**Inductive Case A:** For $m > \theta + 2$ and $1 \leq k \leq \frac{(m-\theta)(m-\theta-1)}{2}$, define

$$\hat{Z}(m, k, 0, 0) = \hat{Z}^*(m-2, k-1)$$

**Inductive Case B:** For $m > \theta + 2$, $1 \leq k < \frac{(m-\theta)(m-\theta-1)}{2}$, and $0 \leq h \leq m-\theta-1$, define

$$\hat{Z}(m, k, h, 0) = \sum_{r=2}^{m-\theta-1} \sum_{k_1+k_2=k-1}^{\theta+1} \hat{Z}(r-1, k_1, h-w, w) \cdot \hat{Z}^*(m-r-1, k_2)$$

When implemented, this requires a check that $h-w \geq 0$.

**Inductive Case C:** For $v \in \{1, 2, \ldots, \theta + 1\}$ and $m > \theta + 2$, define

$$\hat{Z}(m, k, h, v) = \hat{Z}^*(m-v-2, k-1-vh)$$

$$+ \sum_{r=2}^{m-v-\theta-1} \sum_{k_1+k_2=(k-1-vh)}^{\theta+1} \hat{Z}(r-1, k_1, h-w, w) \cdot \hat{Z}^*(m-r-1, k_2)$$
Inductive Case D: For $m > \theta + 2$, $1 \leq k < \frac{(m-\theta)(m-\theta-1)}{2}$, and $1 \leq h < m - \theta - 1$,

$$
\tilde{Z}(m, k, h, \theta + 1) = \sum_{w=1}^{\theta+1} \tilde{Z}(m - \theta - 1, k - \frac{w(w+1)}{2} - (\theta + 1) \cdot (h - w), h - w, w)
$$

(20)

Note that $h$ is strictly less than $m - \theta - 1$, since the case $h = m - \theta - 1$ occurs only when additionally $v = \theta + 1$, which only arises in the empty structure. The general case for the empty structure was handled in Base Case D. When implemented, this requires a check that $h - w \geq 0$.

3 Statistical methods

Current software for probability distribution fitting of connectivity data, such as Matlab™, Mathematica™, R and powerlaw [2], appear to require an input file containing the connectivity of each node in the network. In the case of RNA secondary structures, this is only possible for very small sequence length. To analyze connectivity data computed by the algorithm of Section 2.3, we had to implement code to compute the maximum likelihood estimation for scaling factor $\alpha$ in a power-law fit, the optimal degree $k_{\text{min}}$ beyond which connectivity data is fit by a power-law, and the associated $p$-value for Kolmogorov-Smirnov goodness-of-fit, as described in [9]. We call the resulting code RNApowerlaw. This section explains those details.

Recall the definition of the zeta function

$$
\zeta(\alpha) = \sum_{n=n_0}^{\infty} n^{-\alpha}
$$

(21)

We use both the generalized zeta function (22), as well as the truncated generalized zeta function (23), defined respectively by

$$
\zeta(\alpha; n_0) = \sum_{n=n_0}^{\infty} n^{-\alpha}
$$

(22)

$$
\zeta(\alpha; n_0, n_1) = \sum_{n=n_0}^{n_1} n^{-\alpha}
$$

(23)

Given a data set $D = \{x_1, \ldots, x_n\}$ of positive integers in the range $[k_0, k_1]$, the likelihood $L(D|\alpha)$ that the data fits a truncated power-law with scaling factor $\alpha$ and range $[k_0, k_1]$ is defined by

$$
L(D|\alpha) = \prod_{i=1}^{n} \frac{x_i^{-\alpha}}{\zeta(\alpha; k_0, k_1)}
$$

(24)

Rather than sampling individual RNA secondary structures to estimate the connectivity of the secondary structure network for a given homopolymer, the algorithms from Sections 2.2 and 2.3 directly compute the exact number $N(k)$ of secondary structures having degree $k$, for all $k$ within a certain range. It follows that the likelihood $L(D|\alpha)$ that secondary structure connectivity fits a power-law with scaling factor $\alpha$ is given by

$$
L(D|\alpha, k_0, k_1) = \prod_{k=k_0}^{k_1} \left( \frac{k^{-\alpha}}{\zeta(\alpha; k_0, k_1)} \right)^{N(k)}
$$

(25)

hence the log likelihood is is given by

$$
\mathcal{L}(D|\alpha, k_0, k_1) = -\left( \log(\zeta(\alpha; k_0, k_1)) \sum_{k=k_0}^{k_1} N(k) \right) - \left( \alpha \sum_{k=k_0}^{k_1} N(k) \log(k) \right)
$$

(26)

The parameter $\hat{\alpha}$ which maximizes the log likelihood is determined by applying SciPy function minimize (with Nelder-Mead method) to the negative log likelihood, starting from initial estimate $\alpha_0$, taken from equation (3.7) of [9]

$$
\alpha_0 = 1 + n \left( \sum_{i=1}^{n} \ln \frac{x_i}{x_{\text{min}}^{1/2}} \right)^{-1}
$$

(27)
which in our notation yields

\[
\alpha_0 = 1 + \left( \sum_{k=k_0}^{k_1} N(k) \right) \cdot \left( \sum_{k=k_0}^{k_1} N(k) \cdot \log \left( \frac{k}{k_0 - 1/2} \right) \right)^{-1}
\]  

(28)

In results and tables of this paper, we often write the maximum likelihood estimate (MLE) \( \hat{\alpha} \) simply as \( \alpha \).

We compute the Kolmogorov-Smirnov (KS) \( p \)-value, following [9], as follows. Given observed relative frequency distribution \( D \) and a power-law fit \( P \) with scaling factor \( \alpha \), the KS distance is defined to be the maximum, taken over all \( k \in [k_0, k_1] \) of the absolute difference between the cumulative distribution function (CDF) for the data evaluated at \( k \), and the CDF for the power-law, evaluated at \( k \)

\[
KS(k_{\min}, k_{\max}) = \max_{k_{\min} \leq k \leq k_{\max}} |C_a(x) - C_f(x)|
\]  

(29)

where \( C_a \) and \( C_f \) are the actual and fitted cumulative density functions, respectively. The KS \( p \)-value for the fit of data \( D \) by power-law \( P \) with scaling factor \( \alpha \), is determined by (1) sampling a large number \( (N = 1000) \) of synthetic data sets \( D_i \) from a true power-law distribution with scaling factor \( \alpha \), (2) computing the KS distance between each synthetic data set \( D_i \) and its power law fit with MLE scaling factor \( \alpha \), (3) reporting the proportion of KS distances that exceed the KS distance between the original observed data set and its power-law fit with scaling factor \( \alpha \).

Following [9], \( k_{\min} \) is chosen to be that degree \( k_0 \), such that the KS distance for the optimal power-law fit is smallest. In contrast, \( k_{\max} \) is always taken to be the maximum degree in the input data. We have implemented Python code to compute \( \alpha_0, \alpha, k_{\min}, \) KS distance, \( p \)-value, etc. as described above. In Section 4, we compare results of our code with that from \texttt{powerlaw} [2] for very small homopolymers.

Though our code does not do lognormal fits, this is performed by \texttt{powerlaw}, where the density function for the lognormal distribution with parameters \( \mu, \sigma \) is defined by

\[
p(x) = \frac{\exp \left( \frac{-((\log(x) - \mu)^2)}{2\sigma^2} \right)}{x \cdot \sqrt{2\pi\sigma^2}}
\]  

(30)

In computing the \( p \)-value for power-law goodness-of-fit using Kolmogorov-Smirnov statistics, it is necessary to sample synthetic data from a (discrete) power-law distribution with scaling factor \( \alpha \), a particular type of multinomial distribution. Given an arbitrary multinomial distribution with probability \( p_i \) for each \( 1 \leq i \leq m \), it is straightforward to create \( M \) synthetic data sets, each containing \( N \) sampled values, in time \( O(mNM) \); however, since \( M = 1000 \) and \( N \) is the (exponentially large) number of all secondary structures having degrees in \( [k_{\min}, k_{\max}] \), the usual sequential method would require prohibitive run time. Instead, we implemented the much faster conditional method [19]. Our goal is to sample from a multinomial distribution given by

\[
Prob[X_1 = x_1, X_2 = x_2, \ldots, X_m] = \frac{N!}{\prod_{i=1}^{m} x_i!} \prod_{i=1}^{m} p_i^{x_i}
\]  

(31)

where \( m = k_{\max} - k_{\min} + 1 \) is the number of degrees in the synthetic data, and in the sample set of size \( N \) there are \( x_i \) many occurrences of degree \( k_{min} + i \). To do this, we sample \( X_1 \) from the binomial distribution of \( N \) coin tosses with heads probability \( p_1 \), then \( X_2 \) from the binomial distribution of \( N - x_1 \) coin tosses with heads probability \( \frac{p_2}{1-p_1} \), then \( X_3 \) from the binomial distribution of \( N - x_1 - x_2 \) coin tosses with heads probability \( \frac{p_3}{1-p_1-p_2} \), etc. where each \( x_i \) is determined with the function \texttt{binom} from Python \texttt{Scipy.stats}.

4 Results

In Section 4.1 we use the algorithms described in previous sections to compute RNA secondary structure connectivity and determine optimal power-law fits, and in Section 4.2 we show that preferential attachment holds for the network of RNA structures.
4.1 Analysis of RNA networks using RNAdensity and RNApowerlaw

The algorithm RNAdensity described in Section 2.3 was used to compute absolute and relative degree frequencies for the following cases: (1) homopolymers of length \( n = 10, 12, \ldots, 40 \) with \( \theta = 3 \) for maximum possible degree upper bound \( K = \frac{(n-\theta)(n-\theta-1)}{2} \), (2) homopolymers of length \( n = 30, 35, \ldots, 150 \) with \( \theta = 3 \), where degree upper bound \( K = 2n \) for \( n \in [30, 100] \) and \( K = n + 30 \) for \( n \in [105, 150] \), (3) homopolymers of length \( n = 30, 35, \ldots, 150 \) with \( \theta = 1 \), where degree upper bound \( K = 2n \) for \( n \in [30, 100] \) and \( K = n + 30 \) for \( n \in [105, 150] \). For small homopolymers of length at most 30, optima values for \( k_{\text{min}} \), power-law scaling factor \( \alpha \), Kolmogorov-Smirnov distance were determined using software powerlaw as well as RNApowerlaw from Section 3. Table 1 summarizes these results, which show the agreement between powerlaw and RNApowerlaw. Moreover, both both programs suggest that formal hypothesis testing should reject the null hypothesis that a power-law distribution fits connectivity data; indeed, powerlaw determines a negative log odds ratio \( R \) for the logarithm of power-law likelihood over lognormal likelihood, indicating a better fit for the lognormal distribution, and RNApowerlaw determines small \( p \)-values for Kolmogorov-Smirnov goodness-of-fit of a power-law distribution. Figure 9 shows connectivity density function for a 100-mer, with overlaid Poisson and lognormal distributions – since Erdős-Rényi random graphs have Poisson degree distribution, it follows that RNA secondary structure networks are strikingly different than random graphs. Figure 9 shows a portion of the power-law fit for degrees in \([k_{\text{min}}, k_{\text{max}}]\), where scaling factor \( \alpha \approx 7.876 \) and \( k_{\text{min}} = 83 \). Although maximum degree probability at \( k_{\text{peak}} \) is less than 0.05 for the raw data, the connectivity density for \([k_{\text{min}}, k_{\text{max}}]\) is normalized, which explains why the degree probability for \( k_{\text{min}} \) is \( \approx 0.08 \). Visual inspection suggests an excellent fit for the power-law distribution, despite a Kolmogorov-Smirnov \( p \)-value of \( \approx 0 \). This apparent contradiction highlights the point of view taken in this paper – rather than being take sides in the Barabási-Clauset polemic mentioned in the introduction, our opinion is that a power-law fit for biological data can provide valuable insight into the underlying network, even though from a technical point of view, hypothesis testing may lead to rejection of the power-law fit. The seemingly good power-law fit for RNA connectivity data indicated in Figure 9 and other figures not shown here led to the investigation of preferential attachment described in Section 4.2.

Since powerlaw requires input files of (individually observed) connectivity degrees, when creating Table 1 we could not run powerlaw for homopolymer length greater than 28, for which latter the input file contained 50,642,017 values. A potentially attractive alternative is to generate input files consisting of \( N \cdot p(k) \) many occurrences of the value \( k \), where \( N = 10^2, 10^3, \ldots, 10^7 \) denotes the total number of samples, and where relative frequency \( p(k) \) is the proportion of structures having degree \( k \). However, Table 2 shows that neither scaling factor \( \alpha \) nor \( k_{\text{min}} \) are correct with this alternative approach, even for small homopolymers of length 20, 30 and 40. This table justifies the need for our implementation of RNApowerlaw as described in Section 3. Table 3 shows maximum likelihood scaling factors \( \alpha \) and Kolmogorov-Smirnov \( p \)-values for optimal power-law fits of connectivity data for homopolymers of length from 30 to 150.

Figure 10 shows a scatter plot with regression line for the cut-off values \( x_c \), defined to be the least value such that the probability that a secondary structure for length \( n \) homopolymer has degree greater that \( x_c \) is at most 0.01. From this figure, we determined that for homopolymer length \( n > 100 \), it more than suffices to take degree upper bound \( K = n + 30 \). Figure 10 shows the connectivity degree distribution for a homopolymer of length 20, where degree \( d_g(s) \) is redefined to be the number of structures \( s \) that can be obtained from \( s \) by adding, removing, or shifting a base pair in \( s \). The so-called \( MS_2 \) move set, consisting of an addition, removal or shift of a base pair is the default move set used in RNA kinetics software kinfold [17]. Although a dynamic programming algorithm was described in [11] to compute the average \( MS_2 \) network degree, the methods of this paper do not easily generalize to \( MS_2 \) connectivity densities. Figure 11 shows a least-squares regression line for the log-log density plot for \( MS_2 \) connectivity (computed by brute-force) for a homopolymer of length 20, together with an optimal power-law fit computed by RNApowerlaw. Since there are only 106,633 secondary structures for the 20-mer with \( \theta = 3 \), we ran powerlaw on \( MS_2 \) connectivity data, which determined \( \alpha = 6.84 \), \( k_{\text{min}} = 36 \), and a log odds ratio \( R = -2.06 \) with \( p \)-value of 0.248. Since RNApowerlaw determined \( \alpha = 6.84 \), \( k_{\text{min}} = 36 \), and a Kolmogorov-Smirnov \( p \)-value of 0.219, we can not reject the null hypothesis that a power-law distribution fits the tail of \( MS_2 \) connectivity data for a 20-mer.

4.2 Preferential attachment of RNA secondary structures

In this section, we provide computational and theoretical arguments that suggest that preferential attachment holds in the homopolymer RNA secondary structure model. Before proceeding we recall basic
definitions and notation. The notion of homopolymer secondary structure was defined at the beginning of Section 2.1 throughout this section, we denote the set of all secondary structures for a length $n$ homopolymer by $S_n$. If $s \in S_n$ and $s' \in S_{n+1}$, then we say that $s'$ extends $s$, and write $s \prec s'$, if $s'$ is obtained by either (1) appending unpaired nucleotide $n+1$ to the right of $s$, so that the dot-bracket notation of $s'$ is $\bullet\bullet\ldots$, or (2) adding a base pair $(k, n+1)$ to $s$, where $k \in [1, n-\theta]$ is external to every base pair of $s$, i.e. it is not the case that $i \leq k \leq j$ for any base pair $(i, j)$ of $s$. Since the seminal papers of [25, 23], this notion of extension has been used as the basis of recursive and/or dynamic programming algorithms to count/enumerate all secondary structures and to compute minimal free energy structures.

A reasonable approach to establish preferential attachment in the context of RNA secondary structures is to show that if the degree of $s$ is greater than or equal to the degree of $t$ in the network $S_n$, then for most extensions $s'$ of $s$, and $t'$ of $t$, the degree of $s'$ is greater than or equal to the degree of $t'$ in the network $S_{n+1}$. We show that this is indeed the case for homopolymers of modest length, using brute-force, exhaustive computations in this section, and we rigorously establish this result for a relaxation $S'_n$ of the secondary structure model in Appendix A.

For fixed homopolymer length $n$, define the set $A_n$ of 4-tuples $(s, t, s', t')$ by

$$A_n = \{(s, t, s', t') : s, t \in S_n, s', t' \in S_{n+1}, s \neq t, s \prec s', t \prec t', dg(s) \geq dg(t)\}$$  \hspace{1cm} (32)

A 4-tuple $(s, t, s', t') \in A_n$ succeeds in demonstrating preferential attachment if $dg(s') \geq dg(t')$; otherwise the 4-tuple fails to demonstrate preferential attachment. Let $Succ_n$ [resp. $Fail_n$] denote the set of 4-tuples that succeed [resp. fail] to demonstrate preferential attachment, so that $A_n = Succ_n \cup Fail_n$ (when $n$ is clear, we drop the subscripts, and we ambiguously also use $Succ$ and $Fail$ to denote the sizes of these sets). Our first quantification of preferential attachment is given by the proportion $Succ/(Succ+Fail)$:

$$P(Succ_n) = \frac{|\{(s, t, s', t') \in A_n : dg(s') \geq dg(t')\}|}{|A_n|}$$  \hspace{1cm} (33)

Since secondary structures have possibly quite different degrees and numbers of extensions, a more accurate measure (in our opinion) of preferential attachment is given by $\langle p(s', t'|s, t) \rangle$, defined as follows. For distinct, fixed structures $s, t \in S_n$, define

$$p(s', t'|s, t) = P\left(\frac{dg(s') \geq dg(t')|dg(s) \geq dg(t), s \prec s', t \prec t'|dg(s) \geq dg(t)}{dg(s) \geq dg(t)}\right)$$  \hspace{1cm} (34)

$$\langle p(s', t'|s, t) \rangle = \frac{\sum_{s, t \in S_n} p(s', t'|s, t)}{|\{(s, t) : s, t \in S_n, s \neq t, dg(s) \geq dg(t)\}|}$$  \hspace{1cm} (35)

To clarify these definitions, we consider a small example. If $n = 5$, then $S_n$ consists of the two structures $\bullet\bullet\ldots\bullet\bullet\bullet$, and $\bullet\bullet\ldots\bullet\bullet\bullet$, while $S_{n+1}$ consists of the four structures $\bullet\bullet\ldots\bullet\bullet\bullet$, $\bullet\bullet\ldots\bullet\bullet\bullet$, $\bullet\bullet\ldots\bullet\bullet\bullet$, and $\bullet\bullet\ldots\bullet\bullet\bullet$. Fix $s$ to be $\bullet\bullet\ldots\bullet\bullet\bullet$, and $t$ to be $\bullet\bullet\ldots\bullet\bullet\bullet$. Since the only neighbor of $s$ is $t$, and vice-versa, it follows that $dg(s) = 1 = dg(t)$. By definition, an extension $s'$ of $s$ is obtained either by adding an unpaired nucleotide to $s$ at position $n+1$, or by adding a base pair $(k, n+1)$ to $s$, where $k$ is external to all base pairs of $s$. In the current case, the only possible extension of $s$ is produced by the former rule, thus obtaining $s' = (\bullet\bullet\ldots\bullet\bullet\bullet)$. Note that we do not consider the structure $\bullet\bullet\ldots\bullet\bullet\bullet$ to be an extension of $s$. In contrast, the structure $t = \bullet\bullet\ldots\bullet\bullet\bullet$ has three extensions: $t_1 = \bullet\bullet\ldots\bullet\bullet\bullet$, $t_2 = \bullet\bullet\ldots\bullet\bullet\bullet$, and $t_3 = \bullet\bullet\ldots\bullet\bullet\bullet$. where by definition, $t'_1 = \bullet\bullet\ldots\bullet\bullet\bullet$ is not considered to be an extension of $t$. Clearly $dg(s') = dg(t'_3) = dg(t'_2) = dg(t'_1) = 1 \neq dg(t'_1) = 3$, so $\langle p(s', t'|s, t) \rangle = 0$. If we now take $s = \bullet\bullet\ldots\bullet\bullet\bullet$, and $t = (\bullet\bullet\ldots\bullet\bullet\bullet)$, we find that

$$\frac{2}{3} = \frac{\left|\{(s', t') : dg(s') \geq dg(t') \wedge s \prec s', t \prec t', s, t \in S_{n+1}\}\right|}{\left|\{(s', t') : s \prec s', t \prec t', s, t \in S_{n+1}\}\right|}$$

so $p(s', t'|s, t) = 0.6667$. If we now take $s = \bullet\bullet\ldots\bullet\bullet\bullet$, and $t = (\bullet\bullet\ldots\bullet\bullet\bullet)$, we find that

$$\frac{3}{3} = \frac{\left|\{(s', t') : dg(s') \geq dg(t'), s \prec s', t \prec t', s, t \in S_{n+1}\}\right|}{\left|\{(s', t') : s \prec s', t \prec t', s, t \in S_{n+1}\}\right|}$$

so $p(s', t'|s, t) = 1$. The (arithmetical) average of 1 and $2/3$ is $\frac{2+3}{2+3} = 5/6 = 0.8333$, which is the value $\langle p(s', t'|s, t) \rangle$ found in the first row and last column of Table 1. In contrast to this value, averaged over all pairs $s, t \in S_n$ for which $dg(s) \geq dg(t)$, the total number of successes [resp. failures] is 5 [resp. 1], where a success [resp. failure] is defined as a 4-tuple $(s, t, s', t')$ for which $s, t \in S_n$, $s', t' \in S_{n+1}$, $s \prec s'$, $t \prec t'$, $dg(s) \geq dg(t)$ and $dg(s') \geq dg(t')$ [resp. $dg(s') < dg(t')$]. Thus we find the value $5/6 = 0.8333$
in the last column of the table. For

considered for which
dg
in 4 metazoan species analyzed (PPI networks should exhibit scale-free properties, since nature is likely to reuse and amplify fast-folding degree. On such grounds, one might argue that protein folding networks and protein-protein interaction networks are dynamic, whereby newly accrued nodes are preferentially connected to nodes already having high connectivity degree. Such a mechanism may explain preferential attachment. This, in our opinion, may provide theoretical justification for the tail of degree distributions by a power-law distribution, even though a rigorous statistical test by bootstrapping Kolmogorov-Smirnov values appears to reject this hypothesis.

## 5 Conclusion

Since the pioneering work of Zipf on the scale-free nature of natural languages [29], various groups have found scale-free networks in diverse domains ranging from communication patterns of dolphins [20], metabolic networks [15], protein-protein interaction networks [14, 24], protein folding networks [5], genetic interaction networks [26, 27] to multifractal time series [7]. These discoveries have galvanized efforts to understand biological networks from a mathematical and topological standpoint. Using mathematical analysis, Barabási and Albert [3] established that scale-free networks naturally emerge when networks are preferentially connected. This behavior is common in many biological and social networks, including protein-protein interaction (PPI) networks; i.e. such genes had a larger number of interacting partners. On similar grounds that nature should reuse and amplify successful metabolic networks, one might argue that metabolic networks should exhibit scale-free properties. However, rigorous statistical analysis has shown that metabolic networks fail a goodness-of-fit test for scale-free distribution, while PPI satisfy a goodness-of-fit test for scale-free distributions over a certain range of connectivity [19, 20].

This paper, we have introduced a novel algorithm to compute the connectivity density function for a given RNA homopolymer. Our algorithm requires \( O(K^2 n^4) \) run time and \( O(K n^3) \) storage, where \( K \) is a user-specified degree bound \( K \leq \left\lfloor \frac{(a - \theta)(a - \theta - 1)}{2} \right\rfloor \). Short of exhaustively listing secondary structures by brute-force, no such algorithm existed prior to our work. Since existing software appears unable to perform power-law fitting for exponentially large RNA connectivity data, we have implemented code to compute and statistically evaluate the maximum likelihood power-law fit for an input histogram. Perhaps this code may prove useful to other groups working with data where the underlying data set is so large that it cannot be enumerated, as is the case with connectivity of RNA secondary structure networks. Using code RNA density and RNA Powerlaw, we have computed the connectivity density function for RNA secondary structure networks for homopolymers of length up to 150. Statistical analysis shows that, almost invariably, there is no statistically significant power-law fit of connectivity density function, despite the fact the strikingly good visual fit shown in Figure 9 and other data (not shown). Nevertheless, power-law fitting provides a useful paradigm leading to the established of preferential attachment, shown in the previous section and Appendix.
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Table 1: Table comparing goodness-of-fit computations for software powerlaw [2] and RNApowerlaw for homopolymer lengths less than 30 nt. Given homopolymer length \( n \), the connectivity density is computed over all secondary structures for (all possible) degrees \( k = 1, \ldots, \frac{(n-3)(n-4)}{2} \) using the algorithm described in Section 2.3. Program powerlaw requires an input file containing the degrees of all structures (i.e. containing \( S_n \) values, where \( S_n \) is the exponentially large number of all secondary structures), while our program RNApowerlaw requires as input a list of degrees and their (absolute) frequencies. Table headers as follows: \( n \) is homopolymer length, \( S_n \) is the number of all secondary structures, \( \alpha \) is the maximum likelihood value for the scaling factor of the optimal power-law fit, as computed by powerlaw (PL) and RNApowerlaw (RNAPL), KSdist is the Kolmogorov-Smirnov (KS) distance using equation (29), \( \langle \text{KSdist} \rangle \) is the mean KS-distance obtained by replacing ‘max’ by ‘mean’ in equation (29), \( R \) is the log-odds ratio with associated \( p \)-value as computed by powerlaw, and the \( p \)-value in the last column is computed by RNApowerlaw as described in Section 3. Since powerlaw required more than 24 hours for the computation when \( n = 28 \), we did not attempt a computation for \( n = 30 \); in contrast, RNApowerlaw requires a few seconds computation time. Since the log-odds ratio \( R \) is the logarithm of the power-law likelihood divided by lognormal likelihood, a negative value \( R < 0 \) indicates that the lognormal distribution is a better fit for the tail of RNA secondary structure connectivity data. A small \( p \)-value computed by RNApowerlaw indicates that RNA connectivity data is not well-approximated by a power-law distribution. Nevertheless, we believe that the power-law paradigm provides some valuable insight, given small mean KS-distance and the fact that preferential attachment could be shown for the network of secondary structures – see Section 4.2.

| \( n \) | \( S_n \) | \( k_{\text{min}} \) | \( \alpha \) (PL) | \( \alpha \) (RNAPL) | KSdist (PL) | KSdist (RNAPL) | \( \langle \text{KSdist} \rangle \) | log odds ratio \( R \) (PL) | p-val for \( R \) (PL) | p-val (RNAPL) |
|------|-----|-----|----------|----------|---------|---------|---------|----------------|----------------|----------------|
| 10   | 65  | 3   | 3.13752  | 3.13751  | 0.00356 | 0.0576  | 0.02221 | -0.15          | 0.765          | 0.813          |
| 12   | 274 | 4   | 3.23011  | 3.23011  | 0.03650 | 0.0650  | 0.0177  | -0.81          | 0.482          | 0.746          |
| 14   | 1184| 5   | 3.38933  | 3.38935  | 0.02021 | 0.02021 | 0.00669 | -1.70          | 0.270          | 0.699          |
| 16   | 5223| 6   | 3.51289  | 3.51293  | 0.02252 | 0.02253 | 0.00633 | -6.78          | 0.029          | 0.054          |
| 18   | 23434| 9   | 3.79869  | 3.79876  | 0.02333 | 0.02333 | 0.00624 | -16.00         | 0.001          | 0.001          |
| 20   | 166633| 10  | 3.87165  | 3.87165  | 0.02116 | 0.02116 | 0.00584 | -82.12         | 0.000          | 0.000          |
| 22   | 490999| 10  | 3.85806  | 3.85809  | 0.02304 | 0.02304 | 0.00523 | -670.64        | 0.000          | 0.000          |
| 24   | 2283701| 14  | 4.16480  | 4.16477  | 0.02242 | 0.02242 | 0.00484 | -1452.24       | 0.000          | 0.000          |
| 26   | 10713941| 15  | 4.24485  | 4.24486  | 0.02298 | 0.02298 | 0.00417 | -7129.42       | 0.000          | 0.000          |
| 28   | 50642017| 16  | 4.33896  | 4.33898  | 0.02167 | 0.02168 | 0.00347 | -33020.89      | 0.000          | 0.000          |
| 30   | 24094076| —   | 4.33881  | 4.33884  | 0.02393 | 0.02393 | 0.00298 | —              | —             | 0.000          |
Table 2: Table showing that approximate [resp. exact] scaling factor $\alpha_0$ [resp. $\alpha$] and minimum degree $k_{min}$ for optimal power-law fit of homopolymer connectivity data cannot be reliably computed by using software powerlaw \cite{2} on data sampled from relative frequencies. Approximate value $\alpha_0$ is computed from equation \cite{27}, while $\alpha$ is the maximum likelihood estimate (MLE) of the optimal power-law scaling factor. Given homopolymer length $n = 20, 30, 40$, connectivity density is computed over all secondary structures for (all possible) degrees $k = 1, \ldots, \frac{(n-3)(n-4)}{2}$ using the algorithm described in Section 2.3. Since powerlaw requires input files of (individually observed) connectivity degrees, rather than a histogram of (absolute) frequencies, we generated a file consisting of $N \cdot p(k)$ many occurrences of the value $k$, where $N = 10^2, 10^3, \ldots, 10^7$ denotes the total number of samples, and where relative frequency $p(k)$ is defined by $p(k) = F(k)/\sum_{k=1}^{(n-3)(n-4)/2} F(k)$. In contrast to powerlaw, our program RNApowerlaw (RNAPL) computes exact values from connectivity degree (absolute) frequencies. When using powerlaw, it is clearly necessary to create input files of ever-increasing sizes $N$, in order to have more accurate values of $\alpha_0$, $\alpha$ and $k_{min}$. Since the number $S_n$ of RNA secondary structures is exponential in homopolymer length $n$, it rapidly becomes impossible to use powerlaw for large RNAs – for instance, table values for $n = 40$ required an overnight run of powerlaw, while our software returned the exact value within a few seconds.

| $N$ | $10^2$ | $10^3$ | $10^4$ | $10^5$ | $10^6$ | $10^7$ | RNAPL | $S_n$ |
|-----|--------|--------|--------|--------|--------|--------|-------|------|
| $\alpha_0, n = 20$ | 6.58318 | 3.66505 | 3.93389 | 3.86017 | 3.84749 | 3.84657 | 3.84648 | 1066 33 $\approx 1.1 \cdot 10^5$ |
| $k_{min}$ | 10 | 7 | 10 | 10 | 10 | 10 | 10 | |
| $\alpha_0, n = 30$ | 5.27581 | 4.42183 | 4.46307 | 4.35008 | 4.32651 | 4.32272 | 4.32213 | 240944076 $\approx 2.4 \cdot 10^8$ |
| $k_{min}$ | 12 | 13 | 16 | 16 | 16 | 16 | 16 | |
| $\alpha_0, n = 40$ | 5.15978 | 5.09714 | 5.03719 | 5.24488 | 5.16985 | 5.70916 | 5.94561 | 633180247373 $\approx 6.3 \cdot 10^{11}$ |
| $k_{min}$ | 15 | 19 | 23 | 29 | 29 | 42 | 49 | |
| $N$ | $10^2$ | $10^3$ | $10^4$ | $10^5$ | $10^6$ | $10^7$ | RNAPL | $S_n$ |
| $\alpha, n = 20$ | 6.75757 | 3.70988 | 3.96159 | 3.88570 | 3.87271 | 3.87180 | 3.87165 | 1066 33 $\approx 1.1 \cdot 10^5$ |
| $k_{min}$ | 10 | 7 | 10 | 10 | 10 | 10 | 10 | |
| $\alpha, n = 30$ | 5.33162 | 4.44651 | 4.47963 | 4.36511 | 4.34122 | 4.33739 | 4.33681 | 240944076 $\approx 2.4 \cdot 10^8$ |
| $k_{min}$ | 12 | 13 | 16 | 16 | 16 | 16 | 16 | |
| $\alpha, n = 40$ | 5.19197 | 5.11604 | 5.049419 | 5.25365 | 5.17824 | 5.65206 | 5.95033 | 633180247373 $\approx 6.3 \cdot 10^{11}$ |
| $k_{min}$ | 15 | 19 | 23 | 29 | 29 | 41 | 49 | |
Table 3: Table showing maximum likelihood scaling factors $\alpha$ with associated $p$ values for optimal power-law fits of RNA secondary structure connectivity data for homopolymers of length $n = 30$ to 150. Absolute and relative connectivity degree frequencies were computed by RNAdensity from Section 2.3, while the optimal parameters $\alpha, k_{\text{min}}$ and $p$-values were computed by RNApowerlaw from Section 3. Column headers are as follows: $n$ is sequence length, $k_{\text{max}}$ is the degree upper bound $K$ for RNAdensity, $\%$ of $S_n$ indicates the proportion of all secondary structures having degree bounded by $K = k_{\text{max}}$, $k_{\text{peak}}$ is the location of the density maximum, $k_{\text{mfe}} = \lceil \frac{n - \theta}{2} \rceil$ is the degree of the minimum free energy structure (having largest number of base pairs), $k_{\text{min}}$ is the optimal lower bound for a power-law fit, $\alpha(k_{\text{min}}, k_{\text{max}})$ is the maximum likelihood scaling factor for power-law fit, $KS(k_{\text{min}}, k_{\text{max}})$ is the Kolmogorov-Smirnov (KS) distance between connectivity data and power-law fit, $p$-val is goodness-of-fit $p$ value for Kolmogorov-Smirnov statistics, and $\langle KS \rangle$ is the average KS distance, obtained by replacing ‘max’ by ‘mean’ in equation (29). Although RNAdensity determined absolute and relative degree frequencies for homopolymers of length 130 and 150, for unexplained reasons the Scipy.optimize function minimize did not converge in the maximum likelihood computation of $\alpha$.

| n   | $k_{\text{max}}$ | $\%$ of $S_n$ | $k_{\text{peak}}$ | $k_{\text{mfe}}$ | $k_{\text{min}}$ | $\alpha(k_{\text{min}}, k_{\text{max}})$ | $KS(k_{\text{min}}, k_{\text{max}})$ | $p$-val | $\langle KS \rangle$ |
|-----|-----------------|----------------|-------------------|------------------|------------------|-------------------------------------------|-------------------------------------|---------|-------------------|
| 30  | 60              | 0.99886074     | 10                | 13               | 16               | 4.412752307                               | 0.025636172                         | 0.03149541 | 0.006849691       |
| 35  | 70              | 0.99917394     | 12                | 16               | 18               | 4.545722158                               | 0.025991642                         | 0.029727427 | 0.006009813       |
| 40  | 80              | 0.999404339    | 14                | 18               | 23               | 4.897048035                               | 0.023835647                         | 0.026543112 | 0.006547515       |
| 45  | 90              | 0.999562564    | 16                | 21               | 30               | 5.342317642                               | 0.021749037                         | 0.026034495 | 0.006104977       |
| 50  | 100             | 0.9996808      | 18                | 23               | 32               | 5.462300898                               | 0.020786348                         | 0.02382197 | 0.006154287       |
| 55  | 110             | 0.999762012    | 20                | 26               | 39               | 5.848765937                               | 0.019749956                         | 0.022546932 | 0.006518246       |
| 60  | 120             | 0.999823183    | 22                | 28               | 41               | 5.965304744                               | 0.018603143                         | 0.020882921 | 0.00652872        |
| 65  | 130             | 0.999863331    | 24                | 31               | 49               | 6.362319737                               | 0.017886705                         | 0.020202276 | 0.006522192       |
| 70  | 140             | 0.999898961    | 26                | 33               | 52               | 6.521229066                               | 0.016897879                         | 0.018714757 | 0.006036303       |
| 75  | 150             | 0.999923045    | 28                | 36               | 60               | 6.876787811                               | 0.016138551                         | 0.018129669 | 0.006015537       |
| 80  | 160             | 0.999941051    | 31                | 38               | 63               | 7.026510665                               | 0.015105392                         | 0.016718486 | 0.006597117       |
| 85  | 170             | 0.999954575    | 33                | 41               | 67               | 7.212562892                               | 0.014349852                         | 0.015688377 | 0.006328962       |
| 90  | 180             | 0.999964901    | 35                | 43               | 74               | 7.495319334                               | 0.013571271                         | 0.014903651 | 0.006193572       |
| 95  | 190             | 0.999972604    | 37                | 46               | 78               | 7.672099669                               | 0.012832491                         | 0.013074921 | 0.006298822       |
| 100 | 200             | 0.999978707    | 40                | 48               | 83               | 7.876228775                               | 0.012134176                         | 0.012832491 | 0.006276086       |
| 105 | 135             | 0.999388278    | 42                | 51               | 67               | 7.554045648                               | 0.023127812                         | 0.027684335 | 0.007516723       |
| 110 | 140             | 0.999432364    | 44                | 53               | 70               | 7.70572635                                | 0.022696603                         | 0.026966274 | 0.007443879       |
| 115 | 145             | 0.999473643    | 46                | 56               | 73               | 7.850242149                               | 0.022277404                         | 0.050607301 | 0.031881135       |
| 120 | 150             | 0.999512397    | 49                | 58               | 77               | 8.052936897                               | 0.021847326                         | 0.025869366 | 0.007113075       |
| 125 | 155             | 0.999548701    | 51                | 61               | 80               | 8.193142148                               | 0.021417147                         | 0.02250895 | 0.00670253        |
| 130 | 160             | 0.999582464    | 53                | 63               | 84               | 8.389838968                               | 0.020977798                         | 0.024783397 | 0.006763371       |
| 135 | 165             | 0.999613747    | 55                | 66               | 88               | 8.583283462                               | 0.020543854                         | 0.024364073 | 0.006753744       |
| 140 | 170             | 0.99964276     | 58                | 70               | –                | –                                         | –                                   | –        | –                 |
| 145 | 175             | 0.999690723    | 60                | 71               | 94               | 8.851385266                               | 0.019680276                         | 0.023075596 | 0.00609451        |
| 150 | 180             | 0.999694756    | 62                | 75               | –                | –                                         | –                                   | –        | –                 |
Table 4: Table showing secondary structure preferential attachment probabilities. The first two columns contain homopolymer length $n$ and $n+1$, followed by the number of secondary structures in $S_n$ and $S_{n+1}$, then the total number of 4-tuples $(s, t, s', t')$ that succeed in demonstrating [resp. fail to demonstrate] preferential attachment, denoted by Succ [resp. Fail]. The next column contains the proportion $\text{Succ}/(\text{Succ}+\text{Fail})$ of 4-tuples that demonstrate preferential attachment, defined by equation (33), while the last column contains the expected preferential attachment $\langle p(s', t'|s, t) \rangle$, defined by equation (35). This expectation is obtained by computing the arithmetical average of the conditional probabilities $p(s', t'|s, t)$, defined by $p(s', t'|s, t) = P(\text{dg}(s') \geq \text{dg}(t') | \text{dg}(s) \geq \text{dg}(t), s < s', t < t')$.

| n   | n+1 | $S_n$ | $S_{n+1}$ | Succ | Fail | Succ/(Succ+Fail) | $\langle p(s', t'|s, t) \rangle$ |
|-----|-----|------|----------|------|------|------------------|-------------------------------|
| 5   | 6   | 2    | 4        | 5    | 1    | 83.33%           | 0.8333 ± 0.1667              |
| 6   | 7   | 4    | 8        | 18   | 8    | 69.23%           | 0.7222 ± 0.4157              |
| 7   | 8   | 8    | 16       | 90   | 37   | 70.87%           | 0.7748 ± 0.3260              |
| 8   | 9   | 16   | 32       | 419  | 131  | 76.18%           | 0.8105 ± 0.2941              |
| 9   | 10  | 32   | 65       | 1,891| 575  | 76.68%           | 0.8122 ± 0.2887              |
| 10  | 11  | 65   | 133      | 7,883| 2,498| 75.94%           | 0.8125 ± 0.2891              |
| 11  | 12  | 133  | 274      | 33,069| 9,763| 77.21%           | 0.8300 ± 0.2730              |
| 12  | 13  | 274  | 568      | 142,968| 40,797| 77.80%         | 0.8322 ± 0.2709              |
| 13  | 14  | 568  | 1,184    | 621,884| 171,384| 78.40%        | 0.8366 ± 0.2646              |
| 14  | 15  | 1,184| 2,481    | 2,723,993| 723,887| 79.00%        | 0.8428 ± 0.2587              |
| 15  | 16  | 2,481| 5,223    | 12,041,929| 3,108,978| 79.48%      | 0.8478 ± 0.2556              |
| 16  | 17  | 5,223| 11,042   | 53,730,451| 13,544,005| 79.87%      | 0.8518 ± 0.2523              |
| 17  | 18  | 11,042| 23,434   | 241,738,083| 59,258,399| 80.31%      | 0.8561 ± 0.2485              |
| 18  | 19  | 23,434| 49,908   | 1,096,087,115| 261,730,198| 80.72%      | 0.8598 ± 0.2455              |
Figure 9: (a) Connectivity degree distribution for homopolymer of length 100 where $\theta = 3$, computed with the algorithm described in Section 2.3 for all degrees bounded by $K = 200$. There are $6.32 \cdot 10^{32}$ secondary structures for the 100-mer (exact number $6.319863593639685534122902079183$), and $99.9978706904\%$ of the structures have degree bounded by $K$. Using the output degree densities, the degree mean [standard deviation] is $\mu = 46.2543801196$ [resp. $\sigma = 12.2262985078$]; note that the mean computed from the algorithm in Section 2.3 is very close to the exact degree mean of $\mu = 46.2591895818$, computed over all structures using the different dynamic programming algorithm in [10]. The Poisson distribution (blue curve) with same mean $\mu$ is shown, as well as the lognormal distribution (red) with parameters $\mu_0 = 3.80467214577$ and $\sigma_0 = 0.235563374146$; i.e. $\mu_0$ [resp. $\sigma_0$] is the mean [resp. standard deviation] for logarithms of the connectivity degree – see equation (30). (b) Power-law fit of tail with scaling factor $\alpha = 7.8762287746$ and $k_{min} = 83$, determined by maximum likelihood. Kolmogorov-Smirnov (KS) distance for the fit is 0.01213 – see equation (29), while average KS distance for the alpha power-law fit 0.00400. Nevertheless, since the $p$-value 0 (to 10 decimal places), hypothesis testing would reject the null hypothesis that the power-law distribution is a good fit for the tail.

Figure 10: (a) Plot of the least cut-off value $x_c$ as a function of homopolymer length $n$, for $n = 30, 40, \ldots, 100$. Here $x_c$ is defined as the least value such that the probability that a secondary structure for length $n$ homopolymer has degree greater than $x_c$ is at most 0.01. For the least-squares fit, the regression equation is $y = 0.870714x + 38.1369$, with $p$-value of $1.65112 \cdot 10^{-15}$ for slope value, and $p$-value of $5.20963 \cdot 10^{-13}$ for the $y$-intercept. (b) $MS_2$ connectivity for the 106,633 secondary structures for a 20-nt homopolymer with $\theta = 3$ (green shaded curve), with Poisson distribution of the same mean. Connectivity values range from 4, \ldots, 136 (with many intermediate gaps before the max degree). The distribution mean [resp. standard deviation] is $\mu = 22.0531$ [resp. $\sigma = 7.333$]; these values should be contrasted with the corresponding values of $\mu' = 8.3364$ [resp. $\sigma' = 4.7690$] for $MS_1$ connectivity for the same 20-nt homopolymer (data not shown).
Figure 11: (a) 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, ..., 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 log-log plot is $\ln(p(x)) = 14.7589 - 5.6247 \cdot x$). (b) It is well-known that linear regression of the log-log plot is less reliable than using maximum likelihood when establishing whether the tail of empirical data is fit by a power-law distribution. For the $MS_2$ connectivity data of a 20-nt homopolymer, the maximum likelihood estimation (MLE) of optimal power-law scaling factor is $\alpha = 6.8257$ with $p$-value is 0.219 when $k_{\text{min}} = 36$ and $k_{\text{max}} = 136$. Since the $p$-value is not less than 0.05, we can not reject the null hypothesis that $MS_2$ connectivity is well-fit by a power-law distribution.
A Mathematical validation of preferential attachment

We now proceed to give a rigorous proof of preferential attachment for the simpler model of pseudo-secondary structure, in which pseudoknots are allowed and $\theta = 0$, so that hairpin loops are permitted that contain no unpaired nucleotides. Let $S^*_n$ denote the set of pseudo-secondary structures for a length $n$ homopolymer. By means of an example, when $n = 4$, $S^*_n$ contains the following nine structures: $\bullet \bullet \bullet \bullet$, $\bullet \bullet \bullet \circ$, $\circ \bullet \bullet \bullet$, $\bullet \circ \bullet \bullet$, $\bullet \bullet \circ \bullet$, $\circ \bullet \circ \bullet$, $\bullet \circ \circ \bullet$, $\circ \bullet \bullet \circ$, $\bullet \circ \bullet \circ$. Only the last structure contains a pseudoknot, for which a distinct type of bracket must be used. In general, if $s \in S^*_n$ contains $k$ base pairs, then $s$ can be given by the extended dot-bracket notation over alphabet $\bullet, a_1, A_1, \ldots, a_k, A_k$, where symbol $a_i$ [resp. $A_i$] occurs at position $x$ [resp. $y$] if $(x, y)$ is the $i$th base pair in the lexicographic ordering of base pairs of $s$, while $\bullet$ occurs at all remaining positions of $s$. Throughout the remainder of this section, structure will mean pseudo-secondary structure. The following lemma will be used implicitly throughout the remainder of this section when doing degree computations.

**Lemma 1.** For any structure $s \in S^*_n$, the degree of $s$ satisfies $dg(s) = |s| + \binom{n-2|s|}{2}$.

**Proof.** The first term is due to the fact that $|s|$ structural neighbors of $s$ can be obtained by removal of a base pair of $s$. By adding a base pair $(x, y)$ at any two of the $(n-2|s|)$ unpaired positions in $s$ we also obtain a neighbor of $s$. As these are the only neighbors of $s$, the lemma follows.

**Lemma 2.** Let $s, t \in S^*_n$ be two structures of length $n$. If $|s| \leq |t|$ then $dg(s) \geq dg(t)$.

**Proof.** The proof is now by induction on $|t| - |s|$. In the base case, it is obvious by the previous lemma that for any $s, t \in S^*_n$, if $|s| = |t|$ then necessarily $dg(s) = dg(t)$. Assume now that $|s| \leq |t|$ and $|t| - |s| = 1$.

It follows from the definition of binomial coefficient that

$$
\binom{n - 2|t| + 2}{2} = \binom{n - 2|t| + 1}{1} + \binom{n - 2|t|}{2} = (n - 2|t| + 1) + \binom{n - 2|t|}{2}
$$

$$
= 2n - 4|t| + 1 + \binom{n - 2|t|}{2}
$$

We now have

$$
dg(s) = |s| + \binom{n - 2|s|}{2} = (|t| - 1) + \binom{n - 2(|t| - 1)}{2}
$$

$$
= (|t| - 1) + (2n - 4|t| + 1) + \binom{n - 2|t|}{2} = 2n - 3|t| + \binom{n - 2|t|}{2}
$$

$$
dg(t) = |t| + \binom{n - 2|t|}{2}
$$

$$
dg(s) - dg(t) = 2n - 4|t|
$$

Since $t \in S^*_n$, clearly $|t| \leq \lfloor n/2 \rfloor$, so $2n - 4|t| \geq 0$, hence $dg(s) \geq dg(t)$. The proof proceeds in a similar fashion for larger values of $k = |t| - |s|$ in particular, if $|s| = |t| - k$, then a similar computation shows that

$$
dg(s) - dg(t) = (2kn - 4k|t|) + \sum_{i=1}^{2k-1} i - k
$$

$$
= (2kn - 4k|t|) + 2k(k-1) \geq 2k(k-1)
$$

The lemma now follows.

**Corollary 3.** Let $s, t \in S^*_n$ be two structures of length $n$. Suppose that $|s| < |t|$ and $k = |t| - |s| \geq 1$. Then $dg(s) > dg(t)$ holds unless $k = 1$ and $|t| = n/2$. In the latter case, $dg(s) = dg(t)$. 

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Proof. By the proof of the preceding lemma, for $k = |s| - |t|$, we have $dg(s) - dg(t) \geq 2k(k - 1)$, so that $dg(s) > dg(t)$ for any $k \geq 2$. If $k = 1$ then $dg(s) - dg(t) = 2n - 4|t|$, which is strictly greater than zero, unless $n$ is even and $|t| = n/2$. The lemma now follows. □

Lemma 4. Let $s, t \in S^*_n$ be two structures of length $n$. If $dg(s) \geq dg(t)$ then either (1) $|s| \leq |t|$, or (2) $n$ is even, $|s| = \frac{n}{2}$, $|t| = \frac{n}{2} - 1$.

Proof. We begin by a computation.

$$
dg(s) \geq dg(t) \iff |s| + \left(\frac{n - 2|s|}{2}\right) \geq |t| + \left(\frac{n - 2|t|}{2}\right)
$$

$$
\iff |s| - |t| \geq \frac{n}{2} - |t| - 1
$$

$$
\iff 2 \cdot (|s| - |t|) \geq (n^2 - 4n|t| + 4|t|^2 - n + 2t) - (n^2 - 4n|s| + 4|s|^2 - n + 2|s|)
$$

$$
\iff 2(|s| - |t|) \geq 4(|t|^2 - |s|^2) + 4n(|s| - |t|) - 2(|s| - |t|)
$$

$$
\iff 2(|s| - |t|) \geq 4(|s| - |t|)(|s| + |t|) + (|s| - |t|)(4n - 2)
$$

If $|s| > |t|$, then by dividing both sides of the last inequality by the strictly positive value $2(|s| - |t|)$, we obtain

$$
dg(s) \geq dg(t) \iff 1 \geq -2(|s| + |t|) + 2n - 1 \iff 2(|s| + |t|) \geq 2n - 2 \iff |s| + |t| \geq n - 1
$$

Now either $|s| \leq |t|$, which is one of the conclusions of the lemma, or $|s| > |t|$. In the latter case, then since $|s|, |t| \leq \frac{n}{2}$, it must be that $|s| = \lfloor n/2 \rfloor$ and $|t| = \lfloor n/2 \rfloor - 1$. If $n = 2m + 1$ is odd, then $|s| = m$, $|t| = m - 1$, so $|s| + |t| = 2m - 1 < 2m = n - 1$. It follows that $dg(s) \geq dg(t)$ and $|s| > |t|$ can only occur if $n$ is even and $|s| = \frac{n}{2}$ and $|t| = \frac{n}{2} - 1$. This completes the proof of the lemma. □

Corollary 5. Let $s, t \in S^*_n$ be two structures of length $n$. If $dg(s) > dg(t)$ then $|s| < |t|$.

Proof. Assume that $|s| \geq |t|$. Then by Lemma 3 $|s| \geq |t|$ implies that $dg(s) \leq dg(t)$, which contradicts the hypothesis of the lemma. It follows that $|s| > |t|$. □

Lemma 6. If $n \geq 2$ is an even integer, then

$$
Fail_n = \frac{n!}{(n/2)! \cdot 2^{n/2}} \cdot \frac{(n - 2)!}{(n - 2)! \cdot 2^{(n-2)/2}} \cdot \frac{n(n - 1)}{2} + \sum_{k=1}^{n/2-1} \left[ n \choose 2k \right] \cdot \left( \frac{2k!}{k! \cdot 2^k} \right) + \left[ n \choose 2 \right] \cdot \left( \frac{(2k)!}{k! \cdot 2^k} \right) - 1 \cdot 2k
$$

(36)

$$
\frac{n!}{(n/2)! \cdot 2^{n/2}} \cdot \frac{(n - 2)!}{(n - 2)! \cdot 2^{(n-2)/2}} \cdot \frac{n(n - 1)}{2}
$$

(37)

Proof. Recall that $Fail_n$ consists of all 4-tuples $(s, t, s', t')$ such that $s, t$ are distinct structures in $S^*_n$, with $dg(s) \geq dg(t)$, and that $s', t' \in S^*_n+1$ are extensions $s \prec s', t \prec t'$, but that $dg(s') < dg(t')$. If $s \prec s'$, then either $s'$ is obtained by adding an unpaired nucleotide at the end of $s$, in which case $|s'| = |s|$, or $s'$ is obtained by adding a base pair $(k, n + 1)$ to $s$, for some $k \in [1, n]$ external to every base pair of $s$, in which case $|s'| = |s| + 1$. It follows that for each 4-tuple $(s, t, s', t')$ in $Fail_n$, one of the following cases occurs.

Case 1: $|s| = |s'|$, $|t'| = |t|$, since $(s, t, s', t') \in Fail_n$, $dg(s') < dg(t')$, hence by Corollary 5 $|s'| > |t'|$. Since $|s| = |s'|$ and $|t| = |t'|$, it follows that $|s| > |t|$. Corollary 5 then implies that if $k = |s| - |t| > 1$ or $k = 1$ and $2|s| < n$, then $dg(s) < dg(t)$, a contradiction of the hypothesis that $dg(s) \geq dg(t)$. It follows that $2|s| \geq n$, and since $|s| \leq \lfloor n/2 \rfloor$ and $n$ is even, it must be that $|s| = n/2$. Now $dg(s) = |s| + \left(\frac{n-2}{2}\right)|s| = |s|$. The only manner in which $dg(s) \geq dg(t)$ is if $|t| = n/2 - 1$, in which case $dg(s) = n/2 = dg(t)$. Let $f_n(1)$ denote the number of 4-tuples in $Fail_n$ that satisfy the hypothesis of the current case. Then

$$
f_n(1) = \frac{n!}{(n/2)! \cdot 2^{n/2}} \cdot \frac{(n - 2)!}{(n - 2)! \cdot 2^{(n-2)/2}} \cdot \frac{n(n - 1)}{2}
$$

(38)

Indeed, we claim that the number of $s$ with $|s| = n/2$ for $n$ even is $\frac{n!}{(n/2)! \cdot 2^{n/2}}$, since there are $\frac{n!}{(n/2)! \cdot 2^{n/2}}$ many ways of distributing $n/2$ parentheses: $(\frac{n}{2}) \choose 2$ choices of the first parenthesis, $(\frac{n}{2} - 2) \choose 2$ choices for location of the second parenthesis, etc. However, the parentheses symbols are indistinguishable, so we then divide
by \((n/2)!\). Since \(|t| = n/2 - 1\), there are \(\binom{n}{2}\) choices for where to insert the two unpaired positions; having fixed the unpaired positions, there are \(\frac{(n-2)!}{(n/2-1)! \cdot 2^{(n/2-1)}}\) many ways of filling the remaining \(n - 2\) positions with parentheses, accounting for the fact that the parenthesis symbols are indistinguishable.

For each such \(s\), the only extension of \(s\) is \(s' = ss\); for this \(s'\), \(dg(s') = |s'| = |s| = n/2\). For each such \(t\), there are exactly three possible extensions: \(t' = \bullet \bullet t' = t \cup \{x, n + 1\}\), \(t_2 = t \cup \{y, n + 1\}\), where positions \(x, y\) are unpaired in \(t\). However, only \(t_1\) satisfies \(|t'| = |t| + 1\). Moreover, since \(t_1\) has three unpaired positions, \(dg(t_1) = |t_1'| + \binom{2}{k} = |t| + 3 = |s| + 3\), and so \(dg(s') < dg(t_1)\). It follows that \((s, t, s', t') \not\in \text{Fail}_n\), thus justifying equation (38).

**Case 2:** \(|s'| = |s|, |t'| = |t| + 1\).

Since \((s, t, s', t') \in \text{Fail}_n\), \(dg(s') < dg(t')\), hence by Corollary 5 \(|s'| > |t'|\). Since \(|s'| = |s|, and \(|t'| = |t| + 1\), it follows that \(|s| > |t| + 1\), hence \(|s| - |t| \geq 2\). Corollary 5 now implies that \(dg(s) < dg(t)\), contradicting the hypothesis that \(dg(s) \geq dg(t)\). Consequently, Case 2 contributes no 4-tuple to \(\text{Fail}_n\); however, \(\text{Succ}_n\) contains all 4-tuples \((s, t, s', t')\) that satisfy \(dg(s) \geq dg(t)\) as well as the current case assumptions \(|s'| = |s|, |t'| = |t| + 1\). In particular this includes all 4-tuples for which \(|s| < |t|, \text{ and } (s, t, s', t') \not\in \text{Fail}_n\), thus justifying equation (38).

**Case 3:** \(|s'| = |s| + 1, |t'| = |t|\).

Note first that since \(|s'| = |s| + 1\), the extension \(s'\) is obtained by adding a base pair of the form \((k, n + 1)\) to \(s\), where \(k \in [1, n]\) is external to all base pairs of \(s\). Now \(n\) is even, so it must be that \(|s| < n/2\). Since \((s, t, s', t') \in \text{Fail}_n\), \(dg(s') < dg(t')\), hence by Corollary 5 \(|s'| > |t'|\). Now \(|s'| = |s| + 1, |t'| = |t|\), so it follows that \(|s| + 1 > |t|, \text{ hence } |s| \geq |t|\). By hypothesis of the current lemma, \(dg(s) \geq dg(t)\), so by Lemma 4 either \(|s| \leq |t|, \text{ or } n\) is even and \(|s| = n/2, |t| = n/2 - 1\). However, we have already established that \(|s| < n/2\), so it must be that \(|s| \leq |t|\). It follows that \(|s| = |t|\).

Since \(s, t\) are assumed to be distinct and \(|s| = 0 = |t|\), implies that both \(s\) and \(t\) are the empty structure, we must have \(1 \leq |s| = |t|\). We have already established that \(|s| < n/2\), so if \(f_n(3)\) denotes the number of 4-tuples in \(\text{Fail}_n\) that satisfy the hypothesis of Case 3, we have

\[
f_n(3) = \binom{n}{2k} \left(\frac{(2k)!}{k! \cdot 2^k}\right) \cdot \left[\binom{n}{2k} \left(\frac{(2k)!}{k! \cdot 2^k}\right) - 1\right] \cdot 2^k
\]

Indeed, for fixed \(k\), since \(n\) is even, there are \(\binom{n}{2k} \cdot \frac{(2k)!}{k! \cdot 2^k}\) many choices of structure \(s\) having \(k = |s|\) base pairs. This holds because there are \(\binom{n}{2k}\) ways of choosing \(2k\) positions that will be occupied by \(k\) parenthesis symbols. Having selected these \(2k\) positions among positions \([1, n]\), there are \(\binom{2k}{k}\) ways of choosing where to place the first parenthesis pair, then \(\binom{2k-2}{k-1}\) ways of choosing where to place the second parenthesis pair, etc. and finally, we divide by \(k!\) since the parenthesis symbols are indistinguishable.

Since \(|t| = |s|\) and \(s \neq t\), once \(s\) is selected, there is one fewer possibilities for choice of \(t\), hence the number of choices for \(t\) is \(\binom{n}{2k} \cdot \frac{(2k)!}{k! \cdot 2^k} - 1\). For fixed \(s\) having \(k\) unpaired positions, there are \(k + 1\) possible extensions \(s < s'\), and similarly for \(t\). Enumerate the extensions of \(s\) as \(s_0, s_1', \ldots, s_{2k}\), where \(s_0 = s\bullet\), while \(s_1', \ldots, s_{2k}\) constitute the positions that are paired; similarly enumerate the extensions of \(t\) as \(t_0, t_1', \ldots, t_{2k}\). Now \(dg(s) = dg(t)\), since \(|s| = |t|\), and \(dg(s') < dg(t')\) holds if and only if \(s' \in \{s_1', \ldots, s_{2k}\}\) and \(t' = t_0\). For all such choices of \(s', t'\) we have \(|s'| = |s| + 1\) and \(|t'| = |t|\), so the case hypothesis is satisfied. This justifies equation (38).

Since \(dg(s') \geq dg(t')\) if and only if \(s' = s_0 = \bullet s\), or if \(s' \in \{s_1', \ldots, s_{2k}\}\) and \(t' \in \{t_1', \ldots, t_{2k}\}\), and for all such choices of \(s', t'\) it is not the case that \(|s'| = |s| + 1, |t'| = |t|\), it follows that there are no 4-tuples satisfying the current case hypothesis that belong to \(\text{Succ}_n\).

**Case 4:** \(|s'| = |s| + 1, |t'| = |t| + 1\).

As in previous cases, \(|s'| > |t'|\). Since \(|s'| = |s| + 1, |t'| = |t| + 1\), it follows that \(|s| > |t|\). Now \(|s| \leq |n/2|\), and \(n\) is even, so either \(2|s| = n\) or \(2|s| < n\). If \(2|s| = n\), then there are no unpaired positions in \(s\), hence the only extension of \(s\) is \(s' = ss\), where \(|s'| = |s|\). This is not possible under the hypothesis of the current case. Thus it must be that \(2|s| < n\), hence by Corollary 5 \(dg(s) < dg(t)\). But this contradicts the hypothesis that \(dg(s) \geq dg(t)\). Subsequently, Case 4 contributes no 4-tuple to \(\text{Fail}_n\).

In contrast, all 4-tuples \((s, t, s', t')\) that satisfy the hypothesis of the current case belong to \(\text{Succ}_n\); in particular, if \(0 \leq |s| < |t| < n/2\) and \(|s'| = |s| + 1, |t'| = |t| + 1\), we have \(dg(s) \geq dg(t)\) and \(dg(s') \geq dg(t')\).
In summary, we have established that

\[ Falt_n = f_n(1) + f_n(3) \]

\[ f_n(1) = \frac{n!}{(n/2)! \cdot 2^{n/2}} \cdot \frac{(n-2)!}{((n-2)/2)! \cdot 2^{(n-2)/2}} \cdot \frac{n(n-1)}{2} \]

\[ f_n(3) = \sum_{k=1}^{n/2-1} \left[ \binom{n}{2k} \cdot \frac{(2k)!}{k! \cdot 2^k} \right] \cdot \left[ \binom{n}{n/2} \cdot \left( \frac{(2k)!}{k! \cdot 2^k} - 1 \right) \cdot 2k \right] \]

This concludes the proof of the lemma. \( \square \)

**Lemma 7.** If \( n \geq 2 \) is an even integer, then

\[ \text{Succ}_n \geq \sum_{k=0}^{n/2} \sum_{\ell=k}^{n/2} \left[ \binom{n}{2k} \cdot \frac{(2k)!}{k! \cdot 2^k} \right] \cdot \left[ \binom{n}{2\ell} \cdot \frac{(2\ell)!}{\ell! \cdot 2^\ell} - 1 \right] + \]
\[ \sum_{k=0}^{n/2-2} \sum_{\ell=k+1}^{n/2-1} \left[ \binom{n}{2k} \cdot \frac{(2k)!}{k! \cdot 2^k} \right] \cdot \left[ \binom{n}{2\ell} \cdot \frac{(2\ell)!}{\ell! \cdot 2^\ell} - 1 \right] \cdot (n-2\ell) + \]
\[ \sum_{k=0}^{n/2-1} \sum_{\ell=k+1}^{n/2-1} \left[ \binom{n}{2k} \cdot \frac{(2k)!}{k! \cdot 2^k} \right] \cdot \left[ \binom{n}{2\ell} \cdot \frac{(2\ell)!}{\ell! \cdot 2^\ell} - 1 \right] \cdot (n-2k) + \]
\[ \sum_{k=0}^{n/2-1} \sum_{\ell=k+1}^{n/2-1} \left[ \binom{n}{2k} \cdot \frac{(2k)!}{k! \cdot 2^k} \right] \cdot \left[ \binom{n}{2\ell} \cdot \frac{(2\ell)!}{\ell! \cdot 2^\ell} - 1 \right] \cdot (n-2k)(n-2\ell) \]

**Proof.** Recall that \( \text{Falt}_n \) consists of all 4-tuples \((s, t, s', t')\) such that \( s, t \) are distinct structures in \( S_n \), with \( dg(s) \geq dg(t) \), and that \( s', t' \in S_{n+1} \) are extensions \( s \prec s', t \prec t' \), for which \( dg(s') \geq dg(t') \). As in the previous lemma, we consider each of the following four cases.

**CASE 1:** \(|s| = |s'|, |t'| = |t|\).

By Lemma 2, if \(|s| \leq |t|\) then \( dg(s) \geq dg(t) \); moreover, for extensions \( s' = s \bullet \) and \( t' = t \bullet \) we have \(|s'| = |s| \leq |t| = |t'|\), so \( dg(s') \geq dg(t') \). This justifies the following computation.

\[ s_n(1) = \sum_{k=0}^{n/2} \sum_{\ell=k}^{n/2} \left[ \binom{n}{2k} \cdot \frac{(2k)!}{k! \cdot 2^k} \right] \cdot \left[ \binom{n}{2\ell} \cdot \frac{(2\ell)!}{\ell! \cdot 2^\ell} - 1 \right] \]

**CASE 2:** \(|s'| = |s|, |t'| = |t| + 1\).

In the proof of the previous lemma, it was mentioned that under current case conditions, there are no 4-tuples that belong to \( \text{Falt}_n \). By Lemma 2, if \( 0 \leq |s| \leq |t| < n/2 \) we have \( dg(s) \geq dg(t) \), hence all such 4-tuples that satisfy current case conditions belong to \( \text{Succ}_n \). Noting that there are \((n-2|t|)\) extensions \( t' \) obtained by adding a base pair \((x, n+1)\) to \( t \), where \( x \) is unpaired in \( t \), we obtain \( s_n(2) \) such 4-tuples, where

\[ s_n(2) = \sum_{k=0}^{n/2-2} \sum_{\ell=k}^{n/2-1} \left[ \binom{n}{2k} \cdot \frac{(2k)!}{k! \cdot 2^k} \right] \cdot \left[ \binom{n}{2\ell} \cdot \frac{(2\ell)!}{\ell! \cdot 2^\ell} - 1 \right] \cdot (n-2\ell) \]

Here we note that the occurrence of \(-1\) in \( \left[ \binom{n}{2\ell} \cdot \frac{(2\ell)!}{\ell! \cdot 2^\ell} - 1 \right] \) is due to the requirement that \( s \not= t \).

**CASE 3:** \(|s'| = |s| + 1, |t'| = |t|\).

For any \( 0 \leq |s| < |t| < n/2 \) Corollary 3 implies that \( dg(s) > dg(t) \). As well, there are \((n-2|s|)\) many extensions \( s' \) of \( s \) obtained by adding a base pair of the form \((x, n+1)\) to \( s \), where \( x \) is unpaired in \( s \). For each such extension \( s' \) and for the extension \( t' = t \bullet \), since \(|s'| \leq |t'|\) we also have \( dg(s') \geq dg(t') \). Thus

\[ s_n(3) = \sum_{k=0}^{n/2-1} \sum_{\ell=k+1}^{n/2} \left[ \binom{n}{2k} \cdot \frac{(2k)!}{k! \cdot 2^k} \right] \cdot \left[ \binom{n}{2\ell} \cdot \frac{(2\ell)!}{\ell! \cdot 2^\ell} \right] \cdot (n-2k) \]

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Theorem 8. For each \( n \in \mathbb{N} \), \( \text{Succ}_n / (\text{Succ}_n + \text{Fail}_n) \gg 1/2 \).

Proof. We do not carry out the computation using Stirling’s factorial approximation, etc. since we believe that little is to be gained by the explicit value of this proportion; however, it suffices to note that the previous two lemmas establish that \( \text{Succ}_n \gg \text{Fail}_n \). □