Paper Title

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1. Introduction

Studying interactions between proteins has been of utmost importance in understanding how proteins work collectively to govern cellular function.[1,2] Such collection of interactions among proteins is called a protein interaction network. Mathematically, a protein interaction network is often modeled as an edge-weighted undirected graph where each node denotes a protein and each edge represents an interaction between a pair of proteins. The weight of an edge denotes the level of confidence that this interaction truly exists.

One of the key outcomes of computational analysis of protein interaction networks is identification of signaling pathways. A signaling pathway is a series of proteins in which each protein participates in transmitting biological information by modifying its successor through an interaction. Thus, signaling pathways can be viewed as simple paths in protein interaction networks.[3]

The confidence value of an interaction between two proteins is often considered as the probability that a signal is transmitted between those two proteins. Thus, the probability that a signal moves through a pathway is the product of the confidence values of its constituting interactions. Under this model, Scott et al. conjectured that a signal tends to move through the most probable pathway.[4] They showed that such pathways yield signaling pathways, and thus help in reconstructing signaling networks. Following defines the problem of identifying the most probable pathway in a protein interaction network.

Problem Consider a protein interaction network (V, E, λ) where V denotes the set of proteins, $E = \{(u, v)|u, v \in V\}$ denotes the set of interactions, and the function $\lambda() : E \Rightarrow [0, 1]$ denotes interaction confidence for each interaction in E. Assume that we are given a set of starting proteins $S \subseteq V$ and a set of target proteins $T \subseteq V$. Given a path length denoted by a positive integer m, the problem is to find a simple path $\Phi = v_1 \rightarrow v_2 \rightarrow \ldots \rightarrow v_m$, where $\prod_{i=1}^{m-1} \lambda(v_i, v_{i+1})$ is maximum among all paths with $v_1 \in S$, $v_m \in T$ and $v_i \in V \forall i \in \{1, 2, \ldots, m\}$.

The problem above is equivalent to finding a simple path $\phi = v_1 \rightarrow v_2 \rightarrow \ldots \rightarrow v_m$, where $\sum_{i=1}^{m-1} -\log\ \lambda(v_i, v_{i+1})$ is minimum among all paths with $v_1 \in S$, $v_m \in T$ and $v_i \in V \forall i \in \{1, 2, \ldots, m\}$. The traveling-salesman problem is polynomial-time reducible to this problem;[4] therefore it is NP-hard. They developed a method using a technique devised by Alon et al.,[5] called *color-coding*. The basic idea of this method is to randomly assign each node in the graph

one of m different colors, and search for an optimal pathway in the restricted domain of colorful pathways. A pathway is colorful if and only if all of its nodes are in different color. Finding a colorful path is computationally much cheaper than finding a path without assigning colors. The drawback is that the optimal path may not be colorful in a random color assignment. If that happens, the color coding method fails to find the true optimal result. To deal with this, color coding method repeats the coloring process for several iterations. The confidence in the optimality of the result monotonically increases with each iteration until it reaches a given level of confidence that the unknown optimal pathway was among the colorful ones in at least one of these iterations. As we elaborate later in section 2, the confidence value depends solely on the pathway length m and does not capitalize on readily available information such as the network topology and color assignment. As a result, the method provides a theoretically correct but very conservative confidence value. Hence it requires many iterations in order to achieve a given confidence level, leading to an unnecessarily innefficient running time performance.

Gülsoy et al.[6] presented an enhanced color-coding technique called *k-hop coloring*. A colored network is k-hop colorable if the shortest path between all pairs of same-color nodes is more than k hops in length. This method exploits the network topology and the node colors to assign the network a maximal value k such that the network is k-hop colorable. This additional piece of information allows for higher success probability at each iteration, yielding fewer iterations than that by Scott et al. However, subnetworks with high connectivity quickly diminish the ability to k-hop color the whole network for large values of k. For example, a network containing a clique of size m cannot be colored with $(m-1)$-hop coloring using m colors.[6]

Contribution In this paper, we consider the problem of finding signaling pathways in protein interaction networks. We develop a new coloring method that overcomes the bottlenecks of exisiting coloring methods by Scott et al.[4] and Gülsoy et al.[6] Our contribution comes from a deeper understanding of the relation between network topology, random color assignment and confidence value. We assign a k value to each node individually by studying the colors of all the nodes in the network. The k value of a node v at an iteration indicates that there is no other node u that is reachable from v in k hops such that both u and v have the same color. For each node, the k value is the largest integer that satisfies the above constraint. Thus, different nodes in the network may have different k values. We also study how this reflects on the resulting success probability for each iteration. Given different k values for each node on a pathway, we show how to obtain a bound on success probability.

Based on these findings, We present a new method for detecting signaling pathways in protein interaction networks using an enhanced k-hop coloring technique. Given the parameter pathway length m, we start by randomly assigning one of m colors to each node in the graph, we then extract the optimal colorful pathway. We then calculate our new bound on success probability. We repeat this process until the cumulative success probability is at least equal to a given confidence level. Although our theoretical findings are based on assuming the knowledge of the k values assigned to the unknown global optimal pathway, we empirically demonstrate that the local optimal pathway extracted from the domain of colorful pathways yields correct

confidence values.

The coloring methods developed by Gülsoy et al.[6] and Scott et al.[4] yield special cases of our method. The first method is our method in the special case of all the nodes in the network having the same k value. The second method is our method in the special case of all the nodes in the network having k value = 0. Hence, our method is guaranteed to perform at least as good as both of them in these special cases, and better in the general case.

We provide validation experiments to test the biological significance of our results. We use *weight p-value* and *functional enrichment* as validation measures. We also compare the performance of our method against the one presented by Scott et al.[4] with respect to how fast our method reaches a given confidence level as opposed to theirs.

The rest of the paper is organized as follows. Section 2 discusses the background and related work. Section 3 explains how to obtain a tighter bound on success probability and describes our enhanced k-hop coloring method. Section 4 shows the experiments performed and their results. Section 5 is the conclusion of the paper.

2. Background

A number of methods have been developed so far to identify signaling networks from protein interaction networks. These methods differ in the way they formulate the problem. Among them, Zhao et al.[7] formulated a linear optimization problem that finds the maximum weighted subnetwork with a given size. The main difference of this approach from this paper is that it is concerned with finding signaling subnetworks rather than linear pathways. Kelley et al.[3] detected conserved signaling pathways between related organisms by performing global alignment between their protein interaction networks. They scored each pathway in terms of the probability of true homology between aligned pair of proteins, as well as the probability of true interactions between pairs of proteins along the pathway. Shlomi et al.[8] introduced QPath, a method for querying protein interaction networks for pathways using known homologous pathways as queries. They scored results based on their similarity to the query, number of insertions and deletions used, as well as the reliability of their interactions. Both Kelley et al.[3] and Shlomi et al.[8] are comparative methods. They require knowledge of multiple interaction networks. Thus, they solve a related, yet different, computational problem than the one considered in this paper.

Lu et al.[9] presented a divide-and-conquer algorithm to find signaling subnetworks in protein interaction networks. They recursively partitioned the network into two sets of vertices, enumerated substructures present in each set, and then built larger subnetworks from them. They assumed that all edges have the same weight. They scored the resulting subnetworks based on the similarity of expression profiles of their nodes to the given source and destination nodes. This method formulates a different objective. It aims to detect paths whose proteins are highest in expression similarity, and thus it does not utilize the confidence in the interactions.

Steffen et al.[10] studied detecting signaling pathways in protein interaction networks as guided by expression data. They listed all pathway candidates in a protein interaction network using exhaustive search. They scored each candidate based on how similar the expression profiles of its genes are. Bebek et al.[11] presented a method called PathFinder for finding new

signaling pathways using association rules of known ones. They started with mining association rules for known pathways, guided by the knowledge of functional annotations of their proteins. They then performed an exhaustive search for candidate pathways. From these candidates, they selected the ones having at least a certain number of the known association rules and an average interaction weight above a given threshold. The drawback of both of these methods is that the time complexity of exhaustive graph search is exponential in terms of the network size, and hence is very inefficient.

Gitter et al.[12] presented a method for discovering signaling pathways by adding edge orientation to protein interaction networks. They selected an optimal orientation of all edges in the network that maximizes the weights of all satisfied length-bound paths. They say a path is satisfied if it follows the same direction along its edges from a source node to a destination node. They proved that this problem is NP-hard. They provided two approximation algorithms for it based on available solution methods for weighted Boolean satisfiability, and a third algorithm based on probabilistic selection. As shown in their results, these methods do not scale well with increasing the number of source and destination nodes and the required path length.

The closest studies to that presented in this paper are those by Scott et al.[4] and Gülsoy et al.[6] The former detected signaling pathways in protein interaction networks using color coding. The latter developed topology-aware color coding for network alignment. We describe both methods in detail in section 1. Both methods run multiple coloring iterations. Let us denote the probability that the coloring at an iteration is successful (i.e. true optimal path is colorful) with P_s. The probability that at least one out of r iterations is successful is $1-(1-P_s)^r$. Following from this, in order to insure confidence of at least ϵ $(0 \leq \epsilon \leq 1)$, they run r iterations, such that $1-(1-P_s)^r \geq \epsilon$. Both methods calculate success probability as

$$P_s = \frac{m!}{N_c} \tag{1}$$

where N_c is the number of coloring assignments possible for the optimal pathway. They differ in the way they compute N_c. Scott et al.[4] calculated $N_c = m^m$. Gülsoy et al.[6] calculated a bound $N_c \leq (m-k)^{m-k} \prod_{i=0}^{k-1}(m-i)$ where k is the value assigned to the network such that it is k-hop colorable. Notice that in equation 1, smaller values for N_c are desirable. This is because small values for N_c increase success probability, and thus reduces the number of iterations needed to attain a given confidence level ϵ. This paper develops a novel method that computes a much smaller upper bound on N_c than both Scott et al. and Gülsoy et al., hence a better lower bound on P_s.

3. Method description

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In this section, we start by properly formulating the problem and defining common terms that we use in our methods. We then present new thoughts about pathway detection using color coding. We study the opportunity of more involving of network topology in our calculation to obtain a better success probability, and hence needing less number of iterations and improving performance. Last, we present an enhanced color-coding method for detecting pathways in

protein interaction networks.

3.1. *An overview of our method*

Consider a weighted undirected graph $G = (V, E, w)$, a path length m, a set of starting and target nodes S and T respectively, with $S, T \subseteq V$. Scott et al. has shown that it is possible to find the minimum weight path of a m nodes from S to T in G using dynamic programming.[4] As we discussed in detail in Section 2, they use color coding to provide the result faster, but with uncertainity. They limit uncertainty by computing a confidence in the result. In principle, our method follows the same steps. Algorithm 3.1 presents our method at a high level.

Algorithm 3.1 Computing the minimum weight path

Require: Input network $G = (V, E, w)$, starting and target node sets $S \subseteq V$ and $T \subseteq V$
Require: Color set $C = \{c_1, c_2, \ldots, c_m\}$
Require: Confidence cutoff ϵ
1: $P \leftarrow 0$ {Initialize overall success probability}
2: **while** $P < \epsilon$ **do**
3: Assign colors to the nodes in V randomly from the set C
4: $\Phi \leftarrow$ Find the minimum weight colorful path of length m in G
5: Store Φ in the min-heap of solutions observed so far if it is a new solution.
6: Compute the probability of success P_s for the current coloring iteration.
7: $P \leftarrow 1 - (1 - P)(1 - P_s)$ {Update the overall success probability}
8: **end while**

The algorithm works iteratively. At each iteration we randomly color the network (Step 3). We then use dynamic programming to find the minimum weight colorful path (Step 4). The dynamic programming works as follows. For any node $v \in V$, a set of colors $C' \subseteq C$ and a coloring function $c() : V \Longrightarrow C$ denoting the colors assigned to nodes in V, the minimum weight of a colorful path colored only using C', starting within S and ending at v, can be dynamically tabulated using the following recurrence:[4]

$$W(v, C') = \min_{u:c(u)\in(C'\setminus\{c(v)\})} W(u, C'\setminus\{c(v)\}) + w(u, v), |C'| > 1 \quad (2)$$

where $W(v, \{c(v)\}) = 0$ if $v \in S$ and ∞ otherwise. Once we find the best colorful path in that iteration, we store it in a min-heap (Step 5). We then compute the probability that the current iteration was successful in finding the optimal path (i.e., minimum weighted path regardless of being colorful or not) (Step 6) and update our confidence in the best result seen so far (Step 7).

As we noted earlier, Algorithm 3.1 is very similar to the method by Scott et al.[4] So, a legitimate question is what is the big challenge addressed in this paper? The answer lies in Step 6 of the algorithm where we compute the probability of success at each iteration. This step is missing in all the color coding methods to the best of our knowledge, including Scott et al.[4] among others.[5,6,8,13]

All these existing methods precompute a probability of success prior to the iterations and use the same probability value, which is $m!/m^m$, throughout the iterations (see Equation 1). As a result, they make massive worst case assumptions that has to hold regardless of which node gets which color. Our contribution is to eliminate those worst case assumptions and recompute the probability of success by carefully inspecting the colors of all the nodes. We explain how we do this in the next sections.

3.2. *Basic definitions and model*

Our key contribution is to establish the relationship among network topology, node colors and sucess probability. In this section, we build the mathematical model that will help us compute the probability of success in each iteration.

Assume that we are given a protein interaction network similar to the one described in Section 1, denoted by $G = (V, E, w)$, where $w(u, v) = -\log \lambda(u, v)$. Also assume that the colors of the nodes are already assigned in the current iteration. We denote the set of possible colors with $\{c_1, c_2, \ldots, c_m\}$ and denote the color of node $v \in V$ with $c(v)$.

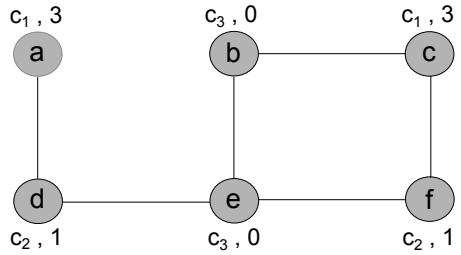

Fig. 1. A hypothetical protein interaction network with six nodes {a, b, c, d, e, f}. The network is colored using three colors {c_1, c_2, c_3}. Each node carries two labels. The label on the left denotes the color assigned to this node. The one on the right is the node's k_{max} value. For instance node d is assigned to color c_2 and its k_{max} value is 1 (i.e., there is no other node assigned to color c_2 within 1-hop of node d).

Consider any colorful path with m nodes. The number of ways to assign colors to the nodes of that path while keeping it colorful is $m!$. Notice that this is equal to the numerator in Equation 1 for probability of success. The denominator in that equation, denoted by N_c, is the total number of ways to color that path regardless of whether it yields a colorful path or not. Before we discuss how we compute N_c, we describe the following concepts.

Definition 1. (Simple path) Given a network $G = (V, E)$, a *simple path* from u to v (u, $v \in V$) is an ordering $< v_1, v_2, \ldots, v_k >$, of a subset of the vertices of G such that $v_1 = u$, $v_k = v$, $(v_i, v_{i+1}) \in E$ and no vertex v_i is repeated $\forall i$, $1 \leq i < k$.

Consider two nodes u and v in G. Let k be a positive integer. We say that v is reachable from u in k hops if there is a simple path from u to v that contains k edges.

Definition 2. (k neighborhood of a node). Let $v \in V$ be a node in G, and k be a natural number. We define the k neighborhood of node v as the set of nodes in $V \backslash \{v\}$ which are reachable from v in k hops of less. We denote this set using notation $\Psi_k(v)$.

Figure 1 shows an example of a colored network. In this example, $\Psi_1(a) = \{d\}$ because the node d is the only node that is reachable from the node a in 1 hop (or less). Similarly, $\Psi_1(f) = \{c, e\}$, $\Psi_2(a) = \{d, e\}$ and $\Psi_2(f) = \{c, e, b, d\}$. Following definition establishes the relationship between each node of the network and the rest of the network based on the colors assigned to all the nodes.

Definition 3. (k_{max} VALUE OF A NODE). Let $v \in V$ be a node in G. The k_{max} value of v, denoted with $k_{max}(v)$, is the largest valued k neighborhood of v that does not contain a node with the same color as v. Formally,

$$k_{max}(v) = \text{argmax}_k\{\forall u \in \Psi_k(v), c(u) \neq c(v)\}$$

Figure 1 shows the k_{max} values for the nodes in the network. For example, the colors of all the nodes in $\Psi_1(f) = \{c, e\}$ are different than the color of f. When we expand the neighborhood of f by one, we get $\Psi_2(f) = \{c, e, b, d\}$. In this set, $c(d) = c(f) = c_2$. Therefore $k_{max}(f) = 1$. Similarly analysis shows that, $k_{max}(a) = 3$ and $k_{max}(b) = 0$. Next definition characterizes a simple path of the network.

Definition 4. (k_{max} CONFIGURATION OF A PATH). Consider a simple path $\Phi = v_1 \rightarrow v_2 \rightarrow \ldots \rightarrow v_m$ of m nodes in G. The k_{max} configuration of Φ is the vector $[k_{max}(v_1), k_{max}(v_2), \ldots, k_{max}(v_m)]$.

As an example, in Figure 1, the k_{max} configuration of the path $\Phi = a \rightarrow d \rightarrow e \rightarrow f$ is [3, 1, 0, 1]. That for $a \rightarrow d \rightarrow e \rightarrow b$ is [3, 1, 0, 0].

3.3. *Bounding the probability of success tightly*

In this section, we focus on one coloring iteration and describe how we compute the probability of success in that iteration.

Notice that there can be many different color assignments that yield the same k_{max} configuration for the same path. Also, as we will show later, the number of possible color assignments to the nodes of a path can be different for different k_{max} configurations. Indeed, the k_{max} configuration of a path describes the constraints imposed on all the nodes of that path about how many alternative colors can be assigned to them. To understand this better, consider a hypothetical path with k_{max} configuration = [4, 4, 4, 4]. This is an extreme case in which we know that no two nodes in the path have the same color. In this case, we can assign colors in $4! = 24$ different ways. Now consider another extreme case when the k_{max} configuration = [0, 0, 0, 0]. In this case each node may or may not have the same color as its neighbor, leading to $4^4 = 256$ alternative color assignments.

Analysis of these two extreme examples above show that the the number of possible color assignments to the nodes of a path can be different for different k_{max} configurations. However, we need a systematic and efficient strategy to compute this number for any possible k_{max} configuration. To solve this problem, we first build a new undirected and unweighted graph, called the *constraint graph* from the k_{max} configuration. By utilizing the constraint graph we transform the problem of finding the number of possible colorings to the chromatic polynomial computation problem. Next, we describe how we build the constraint graph.

Assume that we are given a simple path $\Phi = v_1 \rightarrow v_2 \rightarrow \ldots \rightarrow v_m$ of m nodes along with its k_{max} configuration $[k_{max}(v_1), k_{max}(v_2), \ldots, k_{max}(v_m)]$. We build a constraint graph with m nodes $\{u_1, u_2, \ldots, u_m\}$. We denote the constraint graph as $W = (H, L)$ where H is its set of

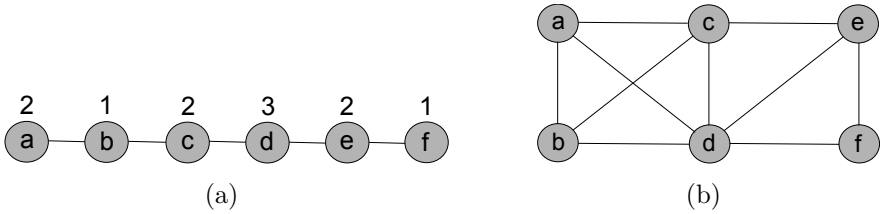

Fig. 2. (a) An example 6-node path with its max_k configuration shown above it. Each max_k value translates to the number of nodes that have to be of different color on either direction. (b) The corresponding constraint graph W: each pair of adjacent nodes have to be of different color. Finding the value of the chromatic polynomial $P(W, m)$ yields the number of coloring possibilities for the path under the given constraints.

nodes and L is its set of edges. For all pair of nodes u_i and u_j in H, we draw an undirected edge between them if the following condition holds:

$$j - i \leq \max\{k_{max}(v_i), k_{max}(v_j)\}.$$

Figure 2 shows an example of a path, its k_{max} configuration and the corresponding constraint graph.

Notice that the indices i and j in the above description show the positions of the nodes on the given path Φ. As a result, an edge between u_i and u_j in the constraint graph indicates that v_i and v_j can not be of the same color according to the underlying k_{max} configuration. Thus, each possible coloring of the given path Φ that obeys the k_{max} configuration corresponds to a chromatic coloring of the constraint graph W and vice versa.

Formally, the value of the chromatic polynomial $A(W, m)$ is equal to the number of ways of coloring W using m colors without any pair of adjacent nodes having the same color. Applying chromatic polynomials on the constraint graph of a path yields the number of possible colorings of that path. Closed form formulas of these polynomials exist in the literature for specific graph topologies, such as paths, trees, complete graphs and cycles. However, the constraint graph of a path can have any topology, and thus, these formulas will not apply for many k_{max} configurations. Therefore, we use a dynamic programming solution following edge-contraction recursive rule based on the fundamental reduction theorem.[14] To describe this, we first define two contraction operators on graph W. The first one removes one edge, (u, v) from the edge set of W. We denote this with $W - (u, v)$. The second one merges two nodes, u and v, into a single node uv. To do this, we inserting a new node uv to W that is adjacent to all the nodes which are adjacent to either u or v. We then remove the nodes u and v along with all the edges incident to them. We denote this merge operation with $W/\{u, v\}$. Using this notation, the dynamic programming works by applying the following equation.

$$A(W, m) = A(W - (u, v), m) - A(W/\{u, v\}, m) \tag{3}$$

The stopping criteria in this equation is the case when W does not contain any edge (i.e., no more constraints are remaining). In other words $W = (H, \emptyset)$. In that case, all the nodes can take any of the m colors, and thus $A(W, m) = m^{|H|}$. In Equation 3, the first term, i.e, $A(W-(u, v), m)$, formulates the number of chromatic colorings by disregarding the constraint between u and v. The second term, i.e, $A(W/\{u, v\}, m)$ corresponds to the number of colorings in which only the

constraint between u and v violates chromatic coloring of W. So, the difference the difference of these two terms tields the number of chromatic colorings of W.

Now we are ready to compute the probability of success, P_s, for a coloring instance of our method (i.e, Step 6 of Algorithm 3.1). At each iteration, we first build the constraint graph W of the best colorful path Φ found at that iteration. We compute the number of chromatic colorings of W as $A(W, m)$ as described above. We then set $N_c = A(W, m)$ and compute the probability of success using Equation 1 as $P_s = m!/N_c = m!/A(W, m)$.

3.4. *Analysis of the probability of success*

We need to answer two key questions regarding how we compute the probability of success: (i) Is it guaranteed to be better than existing methods including Scott et al.[4] and Gülsoy et al.[6] and (ii) Is it theoretically sound? In this section, we discuss these theoretically. We defer the experimental evidence to Section 4.

To answer the first question, we define a partial order between k_{max} configuration of a paths as follows: Consider two such configurations $\mathbf{x} = [x_1, x_2, \ldots, x_m]$ and $\mathbf{y} = [y_1, y_2, \ldots, y_m]$. We say that $\mathbf{x} \leq \mathbf{y}$ if and only if $\forall_i$, $x_i \leq y_i$.

Proposition 3.1. *Consider two k_{max} configurations $\mathbf{x}$ and $\mathbf{y}$ of two simple paths of m nodes. Let us denote their corresponding constraint graphs W_x and W_y respectively. If $\mathbf{x} \leq \mathbf{y}$ then $A(W_x, m) \geq A(W_y, m)$.*

We ommit detailed proof of Proposition 3.1 due to space limitation. However, briefly it follows from the observation that $\mathbf{x} \leq \mathbf{y}$ implies that W_x is topologically a subnetwork of W_y. In other words, W_x has only a subset of the constraints imposed by W_y. Thus, the chromatic polynomial $A(W_x, m)$ cannot be less than $A(W_y, m)$.

Proposition 3.1 has two important implications. First, traditional color coding method (such as Scott et al.[4]) computes $N_c = m^m$. This is the most conservative case in our model when the k_{max} configuration is $[0, \ldots, 0]$. Clearly, this will yield the worst (i.e., largest) possible value for the chromatic polynomial since $[0,\ldots,0] \leq \mathbf{y}$ for any configuration $\mathbf{y}$. Second, let t be the smallest k_{max} value among all the nodes in the network. The formulation by Gülsoy et al.[6] corresponds to k_{max} configuration is $[t, \ldots, t]$. Let $\mathbf{y}$ be the k_{max} configuration of any m-node path in the same network. We have $[t, \ldots, t] \leq \mathbf{y}$ since all the entries of $\mathbf{y}$ have value t or more. We conclude from these two implications that our method is guaranteed to produce less or same N_c value as the mentioned existing methods depending on the network topology and the color distribution. Smaller values for N_c implies larger success probability, and thus, faster convergence to the desired confidence value.

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According to this method, the value of N_c for the example path shown in Figure 2(a) is 5,760, while Scott et al.[4] and Gülsoy et al.[6] would respectively yield N_c = 46,656 and 18,750 for the same example. Such a decrease in the value of N_c leads to an increase in the value of P_s according to equation (1).

4. Experiments

In this section, we evaluate our method on real datasets. We implemented our method in Java language. We ran our experiments on Java runtime environment version 1.6 on Linux machines with 2.2-GHz dual AMD Opteron dual core processors, and 3 GBs of main memory.

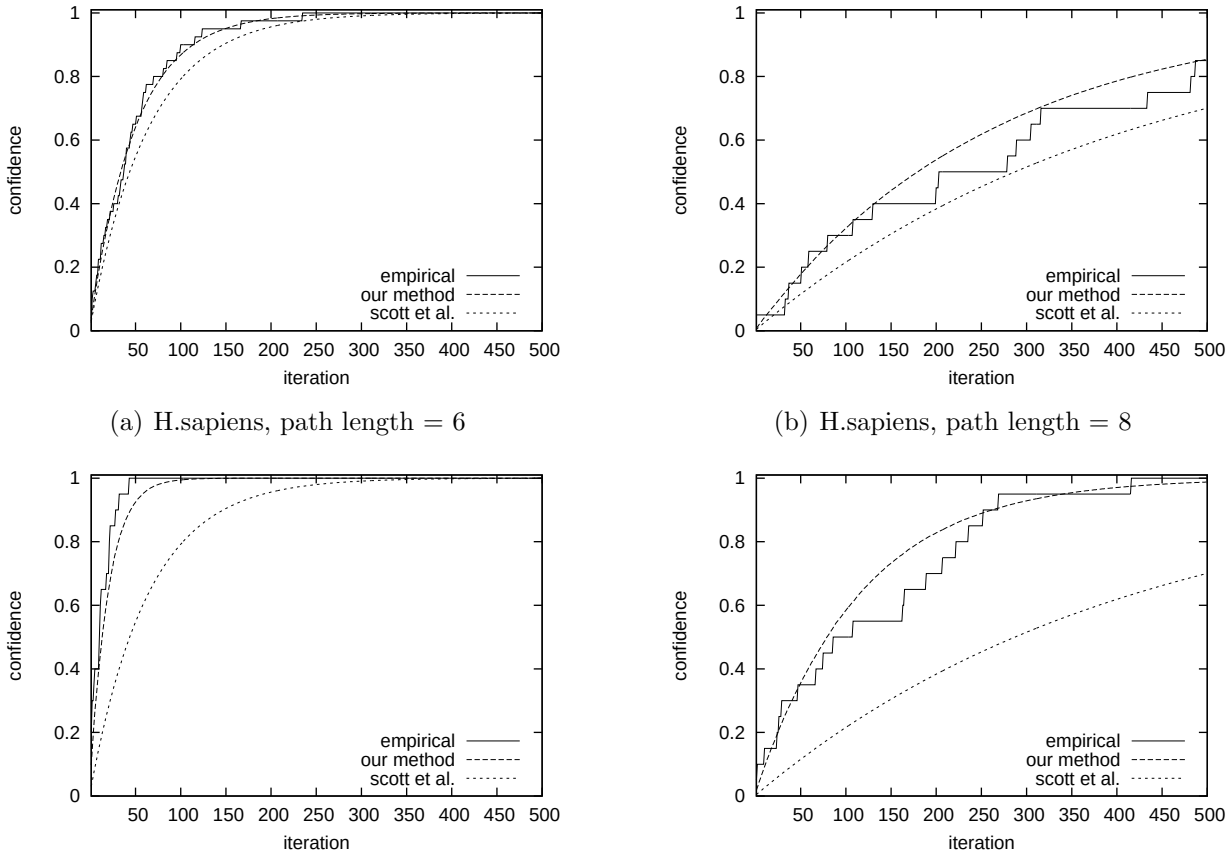
Datasets In our experiments, we used two datasets of protein interactions in Homo Sapiens and Rattus Norvegicus from MINT[15] (the Molecular INTeraction database). The first one is a large dataset of 15472 interactions among 6122 proteins. The second one is a smaller dataset of 806 interactions among 631 proteins. Each interaction is described by two interacting proteins and a reliability score between 0 and 1 that represents the level of confidence that this interaction truly exists. MINT calculates reliability scores of interactions using a heuristic formula of available evidence, including the size and type of the experiment reporting the interaction, sequence similarity of ortholog proteins and the number of publications supporting the interaction.[16]

We use the negative logarithm of MINT reliability scores as edge weights. In all experiments, we find pathways starting within the set of membrane proteins and ending within the set of transcription factors. We use the Gene Ontology database[17] to identify membrane proteins and transcription factors of each dataset. We identify membrane proteins as the ones under the terms GO:0005886 and GO:0004872, and transcription factors as the proteins under the terms GO:0000988, GO:0001071 and GO:0006351.

4.1. *Performance assessment and comparison*

We run an experiment to measure how fast the value of confidence rises with each iteration in both our method and the one presented by Scott et al.[4] We run each method for 500 iterations and measure the overall confidence after each iteration is finished. We also compare these results with the empirical confidence, which is the probability of finding the optimal pathway as empirically observed. To compute the empirical confidence, we run 500 coloring iterations and assign each iteration a value $s \in \{0, 1\}$, where $s = 1$ if the optimal pathway is found in this iteration or in a preceding one, and $s = 0$ otherwise. We repeat this experiment multiple times and take the average values of s for each iteration as the empirical confidence of this iteration. Figure 3 shows the confidence value as more iterations finish, for our method and Scott et al. and also the empirical confidence value. The figure shows a gap between Scott et al. confidence and empirical confidence, which is expected because of the conservative way they use to calculate success probability of an iteration, as discussed in section 2. This gap increases as the path length parameter increases. Our method closes this gap and produces confidence values that are closer to the empirical confidence values. The figure also shows that our method produces higher confidence values than Scott et al. for the same number of iterations.

We also measure the total time taken by our method to reach a given confidence level as opposed to that of Scott *et al.*[4] We run both methods for 500 iterations and measure, after each iteration, the confidence level reached and the overall time taken by both methods. Figure 4 shows that our method takes less time than Scott et al. to achieve the same level of



(a) H.sapiens, path length = 6

(b) H.sapiens, path length = 8

(c) R.norvegicus, path length = 6

(d) R.norvegicus, path length = 8

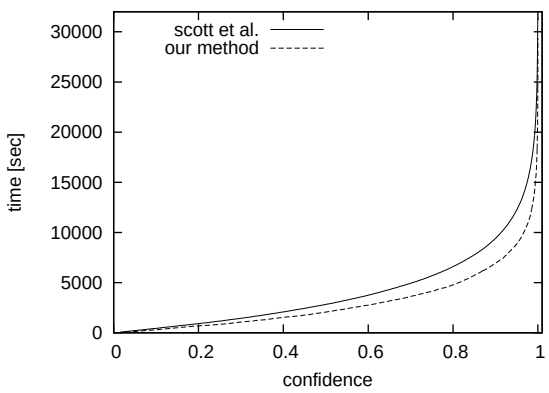
Fig. 3. Confidence level acheived after a given number of iterations, comparing our method to Scott et al. and empirical confidence. X-axis is the number of iterations finished, and Y-axis is the level of confidence in the optimality of the result, for H.sapiens and R.norvegicus at different path lengths. The empirical line denotes the observed probability that the optimal path is found at or before a given iteration, averaged over several experiments.

confidence. The figure also shows that the our performance lead increases as the path length parameter increases.

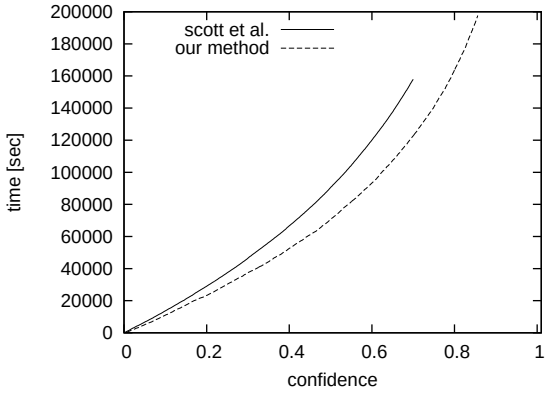
4.2. *Validation Experiments*

So far we have shown that our method outperforms existing coloring strategies in terms of the running time performance. In this section, we evaluate the biological significance of the results we can find using our method.

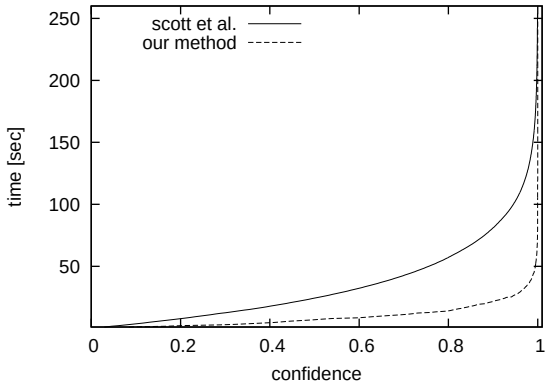
It is worth mentioning that our method returns the same results as Scott *et al.*[4] when both of them are allowed to reach a high confidence value (such as 99% confidence). The main difference is that our method scales to larger networks and longer paths. Therefore, here we will only focus on the results obtained by our method.



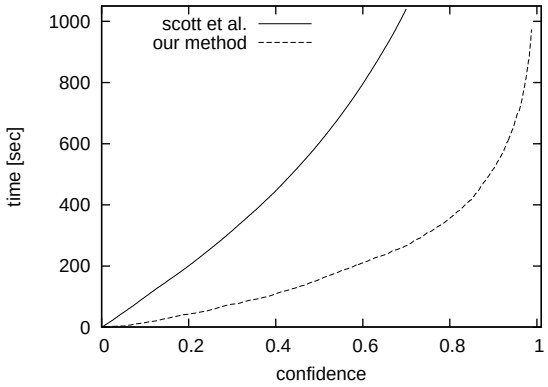
(a) H.sapiens, path length = 6



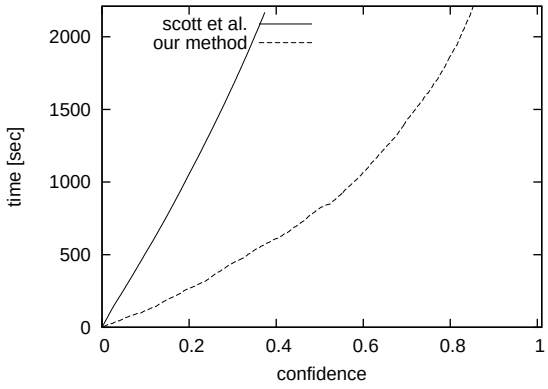
(b) H.sapiens, path length = 8



(c) R.norvegicus, path length = 6



(d) R.norvegicus, path length = 8



(e) R.norvegicus, path length = 9

Fig. 4. Total time needed to acheive a given level of confidence by our method and Scott et al. X-axis is the level of confidence in the optimality of the result, and Y-axis is the total time taken to acheive this confidence level, for H.sapiens and R.norvegicus at different path lengths. Lower values are better.

4.2.1. *Statistical significance of the results*

In this section we assess the statistical significance of the paths found of our method; how our results compare to random paths. We use Z-score to measure statistical significance. For

Table 1. Z-scores calculated for the optimal paths found by our method for H.sapiens and R.norvegicus for different path lengths. $Z = \frac{\mu-\theta}{\sigma}$ where μ is the average weight of 1000 randomly generated paths, σ is their standard deviation, and θ is the weight of the optimal path found by our method.

Dataset	Path Length								
	6			7			8		
	μ	θ	Z	μ	θ	Z	μ	θ	Z
H.sapiens	5.906	0.129	5.409	7.074	0.130	5.477	8.341	0.221	5.764
R.norvegicus	4.975	4.540	0.889	7.307	5.025	1.453	8.457	4.858	1.467

each dataset and path length, we run our method to get the path with the minimum weight θ. we then generate 1000 random simple paths that start at a membrane protein and end at a transcription factor. We compute the average weight μ of these random paths and their standard deviation σ. We then compute the Z-score as follows

$$Z = \frac{\mu - \theta}{\sigma} \tag{4}$$

Z-score indicates by how many standard deviations our optimal weight is better than the weight of an average random path, so higher values are better. Table 1 shows Z-scores for H.sapiens and R.norvegicus for path length 6, 7 and 8. Our results are always better than the random ones. The calculated Z-scores range from 0.89 to 5.76. It can also be observed that, for a given dataset, Z-score increases with increasing the path length, which is expected because increasing the size of random selection leads to less chances of the selected path being better or closer to the optimal path. From these observations observations, we can infer that our results are statistically significant.

4.2.2. *Biological significance of the results*

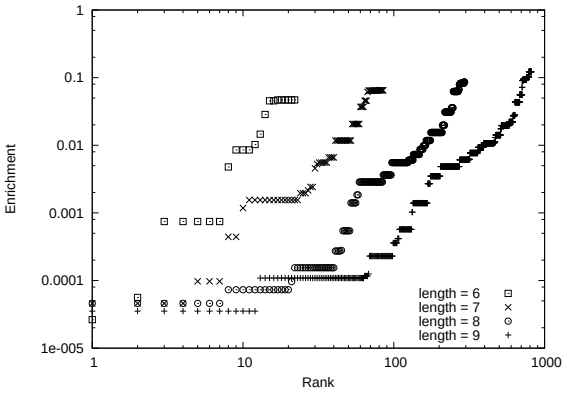


Fig. 5. Sorted values of functional enrichment of optimal and suboptimal paths found at different iterations of our method. X-axis is the rank of the path with regard to its functional enrichment in log scale, and Y-axis is the enrichment value of this path. Lower values are better.

Another important question is: how biologically significant are our results? To answer this question, we validate our results using functional enrichment. We use the Gene Ontology[17] to compute functional enrichment of paths found at different iterations of our method. Let Φ be the path being tested, T be the universal set of GO terms, m be the path length, M be the total number of proteins in the dataset, G_i be the total number of proteins annotated with the Go term t_i in the dataset, and g_i be the number of proteins annotated with t_i in Φ. We compute functional enrichment of Φ as $\min_{t_i \in T} P(X \geq g_i | M, m, G_i)$ where X is a random variable under under a hypergeometric distribution with these parameters. Lower values of are better.

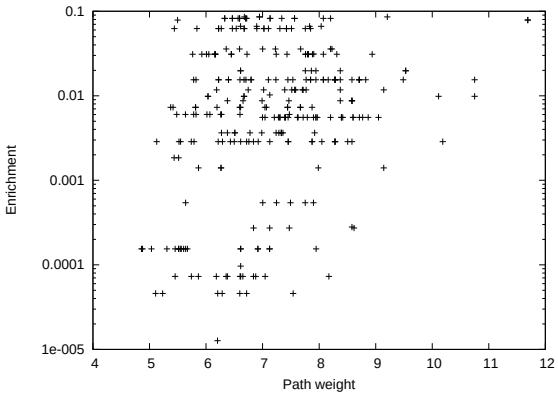
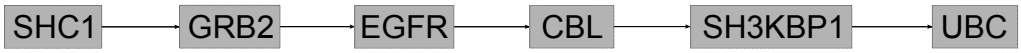
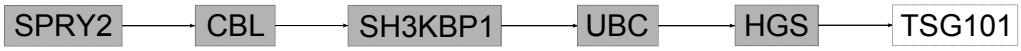


Fig. 6. Functional enrichment values of paths against their weights. X-axis is path's weight and Y-axis is its corresponding enrichment value. Lower values are better.

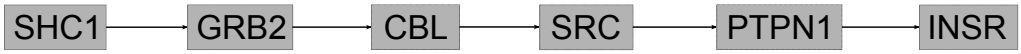
4.3. *Discussing paths found*



(a) All proteins annotated by GO:0042058 - regulation of epidermal growth factor receptor signaling pathway



(b) First five proteins annotated by GO:0042059 - negative regulation of epidermal growth factor receptor signaling pathway



(c) All proteins annotated by GO:0046875 - ephrin receptor binding

Fig. 7. Caption goes here

5. Conclusion

Here goes the conclusion.

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