Chapter 18

An Introductory Guide to Aligning Networks Using SANA, the Simulated Annealing Network Aligner

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Abstract

Sequence alignment has had an enormous impact on our understanding of biology, evolution, and disease. The alignment of biological *networks* holds similar promise. Biological networks generally model interactions between biomolecules such as proteins, genes, metabolites, or mRNAs. There is strong evidence that the network topology—the "structure" of the network—is correlated with the functions performed, so that network topology can be used to help predict or understand function. However, unlike sequence comparison and alignment—which is an essentially solved problem—network comparison and alignment is an NP-complete problem for which heuristic algorithms must be used.

Here we introduce SANA, the *Simulated Annealing Network Aligner*. SANA is one of many algorithms proposed for the arena of biological network alignment. In the context of global network alignment, SANA stands out for its speed, memory efficiency, ease-of-use, and flexibility in the arena of producing alignments between two or more networks. SANA produces better alignments in minutes on a laptop than most other algorithms can produce in hours or days of CPU time on large server-class machines. We walk the user through how to use SANA for several types of biomolecular networks.

Key words Network alignment, Biological networks, Simulated annealing

1 Introduction

A biological network consists of a set of nodes representing entities, with edges connecting entities that are related in some way. They come in many varieties, such as protein–protein interaction (PPI) networks [1, 2], gene regulatory networks [3, 4], gene-μRNA networks [5–9], metabolic networks [10], brain connectomes [11], and many others [12]. It is believed that the *structure* of the networks, in the form of the network topology, is related to the function of the entities [3, 13, 14]. The *alignment* of such networks aims to use connectivity between nodes—the *topology* of the network—to aid extraction of information about the nodes and their function. Network alignments can be used to build taxonomic trees and find highly conserved pathways across distant species

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[15]; and by extension finding such topological similarities may aid in transferring functional knowledge from better-understood species to less well-understood ones, much like how sequence alignment has been doing so for sequence for decades. Networks are even starting to have an influence on individual human health [16].

Network alignment is a fundamentally difficult problem: it is a generalization of the NP-complete subgraph isomorphism problem [17, 18]; and adding to the difficulty is that current data sets are very noisy [19]. Therefore, modern alignment algorithms try to approximate solutions using heuristic approaches.

There are several sub-classes of network alignment. *Global Network Alignment* (GNA) is the task of attempting to completely align entire networks to each other; GNA applied to just two networks is called *pairwise* GNA [15, 20–25], while aligning more than two whole networks is called *multiple* GNA. In contrast, *Local Network Alignment* (LNA) attempts to find similarity in the local wiring patterns among small groups of nodes, either in the same network or across many networks. In all of these cases, alignments can map nodes 1-to-1 or many-to-many; the latter is more biologically realistic since, for example, one gene in yeast may have multiple homologs in mammals. However, the 1-to-1 assumption makes programming simpler and so the majority of aligners take the 1-to-1 mapping as a simplifying assumption. A more recent version of network alignment looks into modeling dynamic networks (see, for example, [26]). An excellent comprehensive survey of all these types of alignments is provided by Faisal et al. [27]. SANA was originally a 1-to-1 pairwise global network alignment algorithm, although we here also introduce a prototype multiple network alignment version.

1.1 User/System Requirements

Source code to SANA is available on GitHub at
http://github.com/waynebhayes/SANA, and is best cloned from GitHub on the Unix command line using

`git clone` http://github.com/waynebhayes/SANA

SANA is written in C++ and runs best on the Unix command line. It has been tested with gcc 4.8, 4.9, 5.2, and 5.4, and runs on Unix, Linux, Mac OS/X, and under the Windows-based Unix emulator Cygwin (http://cygwin.com), 32-bit or 64-bit. SANA has a rudimentary Web interface at http://sana.ics.uci.edu, and a rudimentary SANA app is available in the Cytoscape app store. SANA expects its input networks to be in a two-column ASCII format we call *edge list format*: each line is one edge, specified by listing the two nodes at each end of the edge in arbitrary order (unless `-bipartite` is specified, see below). Duplicate edges and self-loops are not allowed. We also supply a program called `createEdgeList` that can convert the following types of formats into SANA's edgeList format: XML, GML, LEDA, .gw, CSV, LGF.

1.2 Alignment Measures and Objective Functions

An *alignment measure* is any quantity designed to evaluate the quality of a network alignment. Alignment measures can be classified along many axes.

1.2.1 Objective vs. Non-objective Measures

The first axis is the distinction between *objectives* and what we call *post-hoc* measures. While both can be evaluated on any given alignment, any measure used to *guide* an alignment *as it is being created* is called an *objective function*; any measure not used to guide the alignment is generally applied after-the-fact as an independent measure of quality. A good alignment algorithm should be able to use virtually any measure as an objective, and also evaluate the alignment after-the-fact using any other measures which were not used as objectives.

1.2.2 Graph Topology vs. Biological Measures

Another axis along which measures can be classified is *topological* vs *biological* measures.

A Topological Measure quantifies a network alignment based solely on graph-theoretic grounds. Several such measures exist: *EC* [15], *ICS* [25], and S^3 [21] quantify the number of edges in one network that are mapped to edges in the other network(s); they are all described in more detail below. Other topological measures use graphlets to quantify local structure [20, 28–30], while still others use graph measures such as spectral analysis [25] and degree similarity-based measures such as Importance [23].

Biological Measures: In contrast, biological measures are usually used to compare the nodes from different networks that have been paired together by the alignment. For genes or proteins, a common measure is the sequence similarity or BLAST score between the aligned nodes [31]; sequence similarity is also frequently combined with topology to produce a hybrid objective function (see, for example, [20–22, 32], among many others). Another biology-based measure is the *functional* similarity between pairs of aligned proteins as expressed by Gene Ontology (GO) terms [33]. While many authors quantify the functional similarity exposed by an alignment using the mean value of various pairwise GO similarity measures across the alignment, such mean-of-pairwise-scores assume each pair of aligned proteins is independent of all others, which is not true in an alignment since every pair is implicitly related to every other pair via the alignment itself. This problem is alleviated by the NetGO score as implemented in SANA [34], which is a global rather than local scoring mechanism (see below for the meaning of local vs. global measures).

1.2.3 Local vs. Global Measures

The final axis along which network alignment measures can be classified is what we refer as *local* vs. *global* measures.

A Local Measure is one that involves evaluating node pairs that are aligned to each other, and has no explicit dependence on the alignment edges and thus has no explicit dependence on network topology. Examples of local measures include sequence similarity and pairwise GO term similarity as described above; some local measures such as graphlet similarity [15, 20, 21] and Importance [23] include topology indirectly by pre-computing all-by-all pairwise local topological similarities between all pairs of nodes in one network and all pairs of nodes in the other.

Global Measures are ones that implicitly or explicitly can be computed only on the entire alignment and have nothing to do with pairwise node similarities. The most common global measures are *EC*, *ICS*, and S^3, described in more detail below.

1.3 Major Topological Measures

1.3.1 A Useful Analogy for Topological Measures

In order to more easily understand and discuss topological measures, we introduce an analogy between pairwise network alignment and the old board game of *Battleship*. A Battleship game consists of many holes in a board, and some pegs that are placed into the holes. In our analogy, assume G_1 is a "smaller" network with n_1 nodes and m_1 edges, and G_2 is a "larger" network with n_2 nodes and m_2 edges, and we assume that $n_1 \leq n_2$—that is, G_1 is the smaller network in terms of number of nodes. We will furthermore depict G_1 as blue and G_2 as red. Consider Fig. 1: this board has $n_2 = 6$ red holes with red edges painted between two holes if there is an edge between the two corresponding nodes in G_2. The smaller network G_1 is represented by $n_1 = 4$ blue pegs; edges between the pegs are represented by blue "laser beams" between the corresponding pegs (because laser beams don't get tangled as pegs are moved from hole to hole). Any placement of the n_1 pegs

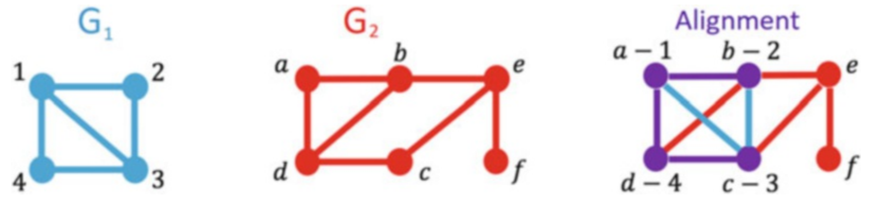


Fig. 1 A simple example of a network alignment. The smaller network G_1 (far left) has its pegs, numbered 1–4, and edges ("laser beams") depicted in blue; the larger network G_2 (middle) has its holes and painted edges depicted in red. One possible alignment (in this case the "visually obvious" one) is depicted at the far right. Here, aligned nodes and edges are depicted as purple; unaligned laser beams from G_1 are still blue, and unaligned holes and edges from G_2 are still red. As stated in the text, in an alignment figure like the one on the right, the number of edges in G_1 is always $m_1 =$ (purple + blue edges), and the number of edges in G_2 is always $m_2 =$ (purple + red edges). Thus, from the figure, it can be easily seen that $EC = 3/5$, and $S^3 = 3/6$ (where 6 is the total number of edges visible across all colors on the subgraph induced by the alignment); also $ICS = 3/4$, since there are 4 edges induced in G_2 by the alignment (i.e., by purple nodes). The purple network is called the *Common Subgraph*, and it can consist of several connected components. In this case, there is only one *Common Connected Subgraph* consisting of 4 nodes and 3 edges

into the n_2 holes represents an alignment between G_1 and G_2; for now we assume that each peg is placed into exactly one hole, so that there are exactly $n_2 - n_1$ empty holes. Furthermore, since mixing red and blue creates purple, we depict the alignment (far right of Fig. 1) in purple: a blue peg in a red hole is purple, and a blue edge lying on top of a red one is also depicted as purple.

1.3.2 Edge-Based Measures: EC, ICS, S^3

We can now define some edge-based topological measures based on this analogy. The fraction of laser beams that lie on top of painted edges is called the *EC* (Variously called Edge Coverage, Edge Correspondence, or Edge Correctness by various authors) [15]. The numerator of *EC* is the number of (purple) edges that are aligned between the two networks, call it *AE* (an integer), while the denominator is m_1; note that since at most m_1 edges can be aligned, the value $EC = AE/m_1$ is always less than or equal to 1. The authors of MAGNA [21] noted that *EC* is asymmetric: in particular, if $n_1 = n_2$, then we can "turn the board upside down," swapping the roles of pegs and holes. In that case, the *EC* changes because G_1 and G_2 are swapped: in particular, the numerator is always the number of aligned edges *AE*, but the denominator switches from m_1 to m_2.

The authors of MAGNA fixed the asymmetry of *EC* by introducing the *Symmetric Substructure Score* or S^3. Consider the rightmost section of Fig. 1, which depicts a proposed alignment. In our analogy, if we "look down" on the alignment from above, we can see four different types of edges. They are: (1) *AE* aligned (purple) edges; (2) UE_1 unaligned (blue) edges from G_1; (3) UE_{2in} unaligned (red) edges in G_2 induced between purple nodes; and (4) UE_{2out} unaligned (red) edges outside the alignment (i.e., not induced between purple nodes). Note that the following equations always hold: $m_1 = AE + UE_1$ and $m_2 = AE + UE_{2in} + UE_{2out}$. Whereas $EC = AE/m_1$, S^3 is defined as $AE/(AE + UE_1 + UE_{2in})$, and is thus symmetric with respect to the interchange of G_1 and G_2. Another way of saying this is that both *EC* and S^3 are rewarded for purple edges in the numerator, but *EC*'s denominator is penalized only for blue edges in its denominator, whereas S^3 is penalized in its denominator for both blue and red edges induced by the alignment.

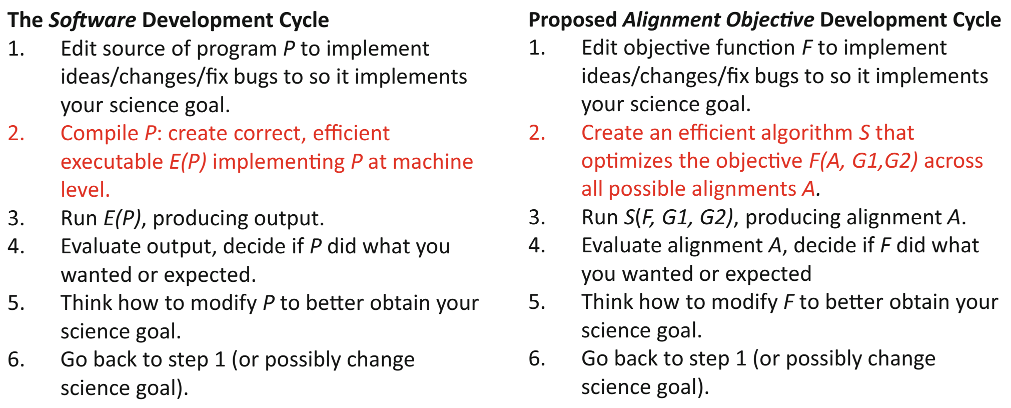
Another measure called ICS *Induced Conserved Substructure* [25] measures *AE* divided by the number of painted edges that exist only between holes that have pegs in them. ICS has the significant disadvantage that it can be maximized by finding a network alignment that *minimizes* the number of edges between filled holes [21, 22, 35], which can hardly be said to be a good alignment. Consider again Fig. 1. The reason ICS is a bad measure is because we could make it equal to 2/2, i.e. 1, by moving node 2 to align with *e* and 3 to align with *f*; then there would be 2 purple edges (*a*-1 to *d*-4, and *e*-2 to *f*-3) and no red edges *induced by the* *alignment* on G_2, even though there would be 3 blue edges (1–2, 4–3, and 1–3) unaligned from G_1. Thus there exists an alignment with $ICS = 1$ even though it only exposes 2 edges of common topology, which is less common topology discovered by maximizing either EC or S^3. This demonstrates the general principle that *choosing the right objective function is* ***crucial*** *to getting good alignments.*

1.3.3 Graphlet-Based Measures

Graphlets [28, 36] are small, connected, induced subgraphs on a larger graph. They have myriad uses, such as quantifying global topological structure [28, 30]. Enumerating graphlets in a large graph is an *NP*-hard problem and much work has gone into heuristics to make their enumeration more efficient. SANA uses ORCA [37] to exhaustively enumerate graphlets in a network. By computing an *orbit degree vector* [29], one can create a local measure that compares the orbit degree vectors of two nodes (one from each network); that local measure can then be used as an objective to guide the alignment. GRAAL [15] was the first to use orbit degree vectors. (In the GRAAL paper, we used the term "graphlet degree vector" but it's more correctly called an "orbit degree vector" because it's a vector of orbit counts, not graphlet counts.) SANA uses the exact same mechanism; however, as networks grow larger, the exhaustive enumeration of its graphlets is becoming very expensive. For example, ORCA takes more than 24 h to compute the orbit degree vectors when aligning the 2018 BioGRID [38] networks of *H. sapiens* and *S. cerevisiae*. Instead, we intend to move SANA towards statistical sampling of graphlets which can be accomplished *far* faster and produce results with low frequency error and high confidence (see, for example, [39–41]).

1.3.4 Which Topological Score to Use?

We believe that one of the major outstanding questions in network alignment is the design of good topological objective functions. While most measures that currently exist have been shown to correlate with interesting biological information, none have been shown to be substantially better than any other in terms of recovering relevant biology. For example, while S^3 is symmetric and can thus be considered a more aesthetically pleasing measure from a mathematical standpoint, it's by no means clear that it actually produces better correlations with biology than EC. And while graphlets have been shown to correlate with biological information [13, 15, 20], it is not clear that we know the best way to *use* them to recover the greatest amount of relevant biological information (cf. Subheading 3.1, especially Table 4). In general, the design of good topological objective functions is a wide-open area of research that deserves to be explored. SANA, with its speed and accuracy, is an ideal playground for exploring objective functions.



	The *Software* Development Cycle		Proposed *Alignment Objective* Development Cycle
1.	Edit source of program *P* to implement ideas/changes/fix bugs to so it implements your science goal.	1.	Edit objective function *F* to implement ideas/changes/fix bugs to so it implements your science goal.
2.	Compile *P*: create correct, efficient executable *E(P)* implementing *P* at machine level.	2.	Create an efficient algorithm *S* that optimizes the objective *F(A, G1,G2)* across all possible alignments *A*.
3.	Run *E(P)*, producing output.	3.	Run *S*(*F, G1, G2*), producing alignment *A*.
4.	Evaluate output, decide if *P* did what you wanted or expected.	4.	Evaluate alignment *A*, decide if *F* did what you wanted or expected
5.	Think how to modify *P* to better obtain your science goal.	5.	Think how to modify *F* to better obtain your science goal.
6.	Go back to step 1 (or possibly change science goal).	6.	Go back to step 1 (or possibly change science goal).

Fig. 2 Comparison of the standard software development cycle (left) and proposed cycle for developing new objective functions for alignment (right). Red highlights the step that should be entirely automated and requiring no effort on the user's part

To explain what we mean by experimenting with objective functions, consider Fig. 2. There are three orthogonal components to network alignment: (1) a (possibly vague) scientific or informational goal *G*; (2) an objective function *M* created by the user that attempts to formally encode *G*; and (3) an alignment algorithm *S* that builds an alignment trying to optimize *M*. In sequence alignment, the three orthogonal components are clearly delimited: the substitution/indel cost matrix encodes the goal the user wants, and tools like BLAST [31] quickly find (near-)optimal solutions. Practitioners can *use* BLAST without having to understand the details of how it works. It is a trusted tool, like a C++ compiler is to a developer, or a linear solver to a scientist solving a linear system; practitioners iterate the familiar edit-compile-debug loop, gaining knowledge from the feedback process until they are satisfied that they have achieved their goal. Unfortunately, this edit-compile-debug loop is virtually impossible in the network alignment arena, due to (a) the lack of an algorithm fast enough to perform effective edit-compile-debug loops, (b) the lack of a generally accepted "gold standard" of network alignment, and (c) the lack of a clear separation of the *goal*, its formalized *objective*, and the *alignment tool*. SANA fixes the first two; the third is a matter of scientific culture in the network alignment community that we hope to influence by spreading the use of SANA in conjunction with the process depicted in Fig. 2.

1.3.5 Using Sequence Similarity as an Objective: A Necessary but Hopefully Temporary Evil

It may help here to (re-)state the obvious: the whole point of *network* alignment is to align networks based upon their network topology. This is a desirable goal because there is a strong belief that the topology of a network is somehow related to its function. For example, we believe that humans and chimpanzees are very close relatives, taxonomically speaking. If there is a particular protein h_0 in humans that performs a certain function by interacting with seven other proteins $h_1, h_2, \ldots, h_7$, then it is quite likely that there is a very similar protein c_0 in chimpanzees that also interacts with (close to) seven proteins $c_1, c_2, \ldots, c_7$ to perform virtually the same function. Another way of saying this is that the network topology of the protein–protein interaction networks of human and chimp is likely to be very similar in the vicinity of h_0 and c_0, respectively. As such, a natural network alignment between human and chimp should contain the ordered pairs $(h_0, c_0), (h_1, c_1), \ldots, (h_7, c_7)$. If the network of interactions around h_0 and c_0 are in fact similar, then any network alignment algorithm worth its mettle, optimizing an objective that highlights such network similarities, should include the above pairs with high likelihood.

The problem, at least in the research area of protein–protein interaction (PPI) networks, is that the data on current PPI networks is *extremely* incomplete in terms of enumerating the edges in the PPI networks. For example, as of 2018, the most complete PPI network is that of *S. cerevisiae*, and it may be only about 50% complete; the human PPI network is probably less than 10% complete [42]; other species are even far less complete. For instance, we'd expect most mammals to have about the same number of interactions in their PPI networks, and yet the 2018 BioGRID Human network has almost 300,000 interactions, but mouse and rat have only 38,000 and 5000 interactions listed, respectively. If Human is only 10% complete and currently contains 300,000 interactions, then we may expect the complete interactome to have over one million interactions. By this measure, mouse and rat are at most a few percent, and well less than 1% complete, respectively. Here's the crux: if we are missing 90% or more of the edges in most mammal PPI networks, *no network alignment algorithm based solely upon network topology has any hope of providing good alignments.* This is the state of affairs in PPI network alignment.

Thus, it is no surprise that virtually every network alignment algorithm currently in existence must rely on using sequence similarity information to help give network alignments that show decent functional similarity. However, *if network alignment is of any worth whatsoever, the use of sequence similarity should be viewed only as a* **temporary** *crutch—a necessary evil—until such time as the interactions in PPI networks are more completely enumerated.*

On the other hand, since protein function is defined by the shape of the folded protein, and disrupting the function of a protein can be lethal, the folded structure of a protein tends to be better conserved than its sequence [43]. This in turn suggests that the network of interactions may also be better conserved than sequence. If this is the case, then network alignment may ultimately be at least as useful as sequence alignment in terms of learning about protein function. Alas, we must wait until PPI networks are far more complete than they are today to test this hypothesis.

1.4 Search Algorithms

Given two networks with $n_1 \leq n_2$ nodes, respectively, the number of possible 1-to-1 pairwise global network alignments between them is exactly $\frac{n_2!}{(n_2-n_1)!}$. This is an *enormous* number; for example, if the two networks each have thousands of nodes (not uncommon for protein–protein interaction networks), the number of possible alignments can easily exceed $10^{100,\,000}$. This is an enormous search space, *far* larger, for example, than the number of elementary particles in the known universe—which according to Wikipedia is a paltry 10^{100}.

The task of a network alignment algorithm is to search through this enormous space of possible alignments, looking for ones that score well according to one or more of the measures described in Subheading 1.2. Since network alignment is an NP-complete problem (for those who are inclined to graph theory, the proof is trivial: finding a network alignment with an EC of exactly 1 is equivalent to solving the subgraph isomorphism problem), all such algorithms must use heuristics to navigate this enormous search space. Search methods abound; several good review papers exist [11, 44–46]; for an extensive comparison specifically showing that SANA outperforms about a dozen of the best existing algorithms, *see* ref. 22. SANA is virtually unique in that it was designed from the start to be able to optimize *any* objective function, including the objective functions introduced by other researchers; a preliminary report shows that SANA outperforms over a dozen other algorithms at optimizing their own objective functions [47].

1.5 Requirements of a Good Alignment Algorithm

We believe that, in order to be of general use, a network alignment algorithm must satisfy the following properties:

Speed, If So Desired: SANA can produce better alignments in minutes that most other aligners can in hours. This is useful for many reasons: to perform test alignments; to experiment with objective functions; to perform multiple alignments of the same pair of networks in order to see which parts of the alignment, if any, come out the same each time (more on this later).

High Quality of Results, If So Desired: SANA's primary user-tunable parameter is the amount of time the user wishes to wait. While SANA can produce better alignments in 1 min on a laptop than many existing algorithms can do given hours of CPU, users can also tell SANA to spend any amount of time improving the alignment, such as 5 min, 3 h, or a week. SANA generally produces better scoring alignments with longer runtimes, although we generally see a point of diminishing returns beyond a few hours.

It Should be Simple to Use: By this we mean that if there are any algorithmic parameters that crucially control the quality of the result, those parameters should be tuned automatically without user input—in other words, the user should not need be an expert on the algorithm in order to understand how to use it. The primary parameter controlling the anneal is how long to spend annealing. By default SANA spends a minute or two automatically finding near-optimal starting and ending temperature extremes, before annealing for the amount of time specified by the user. (Another algorithm called SailMCS [48] also uses simulated annealing but fails to automatically determine good temperature extremes, and so SANA produces alignments that are far superior to those of SailMCS [47].)

Providing Confidence Estimates on the Quality of the Alignment: For example, if some set of pegs P_1 always end up in the same holes every time SANA is run and another set of pegs P_2 end up in different holes each time SANA is run, this suggests the set P_1 is *confidently* aligned, whereas we should be suspicious about the alignment of pegs in P_2. Few algorithms are capable of this sort of confidence testing of the alignment; SANA, on the other hand, is so fast that it is easy to look for such *core* alignments [49]—cf. Subheading 3.1.

Flexible with Objective Functions: SANA has over a dozen pre-programmed objective functions that users can experiment with. In addition, users can supply SANA with externally computed similarity matrices, either node-to-node or edge-to-edge. Finally, we have tried to make the code base of SANA clear so that anybody familiar with C++ can program new objective functions easily.

Able to Handle Nodes That Have ASCII Names Rather Than Only Allowing Integers as Node Identifiers: To a programmer, creating a mapping between ASCII names and integers is easy. To non-programmers this is not so easy, and many aligners have the inexcusable fault of insisting that nodes are named by sequential integers. SANA does this internally but allows users to use whatever names they want to identify nodes.

Available to Plug in to Existing Popular Tools such as Cytoscape: SANA is available in the Cytoscape App store.

Able to Handle Multiple Input Graph Formats: Currently, SANA only natively accepts networks in edge list format and LEDA.gw format. The former is a line-by-line list of edges (two nodes from the same network listed on one line), while the latter is a rather deprecated format used by an old version of LEDA [50]. However, we do provide a converter called `createEdgeList` that outputs our edge list format given any of the following input formats: GML, XML, graphML, LEDA, CSV, and LGF.

Able to Perform Multi-objective Optimization, If So Desired: SANA has the ability to create a Pareto front across all measures the user chooses to optimize. So far as we are aware, the only other network alignment tool that does this is OptNetAlign [51]. Essentially, a Pareto front consists of a family of alignments that explore the trade-off between all the objective functions being optimized.

In general, we can only increase the value of one objective at the expense of one or more others. The Pareto front approximates the "frontier" of this trade-off; we use the method outlined in [52].

1.6 The Value of Randomness: Core Alignments

SANA shares one important aspect with a few other aligners including MAGNA [21, 35] and OptNetAlign [51]: it is a randomized search algorithm. Like these other algorithms, SANA starts with a random alignment and then starts to move pegs around between holes; each time it tries to swap or move pegs around, it asks if the objective function has gotten better or not. As time progresses, the alignment gets better according to the objective function. If the objective function is an easy one to optimize, SANA will quickly find the optimal or near-optimal alignment [22, 47]; in harder cases, it will simply find better-and-better solutions as it is given more time.

The fact that SANA intentionally injects randomness has some surprising positive aspects. In particular, if there exist highly similar regions between the two networks G_1 and G_2, SANA is likely to find them and align them identically every time, despite starting with a different random alignment each time. If there are other parts of the networks that are dissimilar and there is no obvious way to align them correctly, those regions are likely to get aligned differently each time SANA is run. Given two regions R_1 in G_1 and R_2 in G_2, the more topologically similar R_1 is to R_2, the more likely it is that SANA will align them the same way every time it is run, independent of the randomness. Since SANA is extremely fast, and since it has this random aspect, it is relatively painless to run SANA many times on the same pair of networks and look for pairs of nodes that are aligned together frequently. We use the term *core alignment* to refer to pairs of nodes that are stable across many runs of SANA; the more frequently a pair of nodes is aligned together, the more confident we are that they truly *belong* together according to the objective function being optimized. So for example, if we run SANA 10 times on the same 2 networks and produce output files out0.align, out1.align, out2.align, ..., out9.align, then we can trivially measure the core frequencies on the Unix command line as follows:

```
$ sort out?.align | uniq -c | sort -nr
```

The first `sort` puts identical lines from all 10 files side-by-side; the `uniq -c` counts how many unique lines are side-by-side (thus measuring core frequency), and the final `sort -nr` then sorts the aligned pairs of nodes by frequency, most frequent pairs of nodes first—that is, the most confident parts of the alignment are listed first. Note that the output of the above command line is a list of pairs preceded by their frequency. Note in particular that, even though SANA is a 1-to-1 aligner *per run*, with multiple runs we can produce non-1-to-1 mappings between the two networks, along with a confidence level for each particular pair. We are also working on functionality to produce core alignments in one run of SANA; that functionality may exist by the time this article goes to press and accessible via the command-line option "`-cores`".

1.7 Limitations of SANA

Currently, SANA aligns only two networks at a time. Each time, it produces a 1-to-1 mapping between the nodes of the smaller network to the nodes of the larger one (i.e., an arrangement of pegs into holes). So technically, SANA is a global, pairwise, 1-to-1 alignment algorithm—the simplest type of global alignment algorithm. However, as we described above, SANA produces *good* alignments so quickly that it can be run many times on the same pair of networks in the same time it takes to run most other algorithms just once; by running SANA many times we effectively produce not only a non-1-to-1 mapping, but also a *confidence estimate* of each pair of nodes we output. So far as we are aware, no other algorithm produces such confidence estimates.

Furthermore, even though SANA technically aligns only 2 networks at a time, in **Note 1** we describe a prototype version of multi-SANA that uses pairwise alignments to construct a multiple network alignment.

Thus, although SANA is technically only a 1-to-1 pairwise network aligner, it can effectively produce both many-to-many alignments (with confidences) and multiple alignments.

2 Examples of Usage

2.1 Getting Started with SANA

Table 1 contains a sequence of Unix Shell commands that will download the repo from GitHub, compile SANA, and perform your first test of SANA to ensure everything works.

The most basic run of SANA requires the user only to specify which two networks to align; in Table 1 it is the 2018 BioGRID renditions of *Rattus norvegicus* (the common sewer rat, aka lab rat), and the single-celled yeast *Schizosaccharomyces pombe.* SANA defaults to using S^3 as the objective function, and 5 min as the amount of time to perform simulated annealing. Total runtime is about 6–7 min including the initial computation of the temperature schedule, which we now describe.

Simulated annealing only works well if the temperature schedule is chosen carefully. We must start with a temperature high enough that moves are essentially random, so that even bad moves are frequently accepted (this keeps us out of local minima); and then end with a temperature low enough that only good moves are accepted (to hone in on the best local maximum once we've found its general vicinity). Empirically, we are controlling the *probability of accepting a bad move* or `pBad`; it must start close to 1, and end close to zero. Unfortunately there's no analytical method to compute these extremes, so the first 1–2 min of SANA are spent

Table 1
Getting started with SANA on the Unix command line

```
# Lines like this are comments. The Unix/Bash prompt is the dollar sign.
# First use "git" to clone the repo:
$ git clone http://github.com/waynebhayes/SANA
Cloning into 'SANA'... #git output deleted
$ cd SANA; make   # now wait a few minutes...
# Run SANA for the first time on the 2018 BioGRID networks of rat and S. pombe:
$ ./sana -fg1 networks/RNorvegicus18.el -fg2 networks/SPombe18.el
# wait while SANA computes a temperature schedule and then performs the alignment...
$ cat sana.out # look at the output file; first line is an internal
# representation of the alignment and can be ignored.
$ head -3 sana.align # left column is a BioGRID node name from rat, right from S.pombe.
361207  2542195
316265  2541287
499382  2539901
$
```

We first clone the repo from GitHub, then "make" SANA, then run it on the two smallest BioGRID 2018 networks: *R. norvegicus* and *S. pombe*. We then look at the output file `sana.out`, which contains scores and other useful information, as well as the actual alignment file `sana.align`. SANA has *many* command-line options; type "`../sana -h | less`" to see a long list of them

estimating the initial temperature $t_{initial}$, the final temperature t_{final} that gives a pBad starting near 1 and ending near zero, along with the t_{decay}, the temperature decay rate that gets us from one to the other in the allotted time (5 min by default).

Next you will see the statement, `Start execution of SANA_s3` which says SANA is finally starting the anneal, optimizing `s3`. After that, you'll see updates every few seconds as SANA progresses. These updates show the update number, the elapsed time so far, the current score, some statistical theoretical values that don't concern us here, and the sampled pBad, which should start above 0.98 and end somewhere below about 1e−6.

Once SANA is finished running, there are exactly two output files (whose names can be changed with the "-o" option): `sana.out` contains as its first (long) line an internal representation of the alignment, followed by some human-readable statistics; an example is in Table 2. The second file, called `sana.align`, contains the actual alignment in two-column format: on each line, the left column contains a node ("peg") from G_1 and the right column is the aligned node ("hole") from G_2.

The default objective function is S^3; changing the objective function is easy on the command line. For example to have SANA optimize a 50-50 combination of *EC* and S^3, type

```
./sana -ec 0.5 -s3 0.5 -fg1 ...
```

To turn off S^3 entirely and perform an *EC*-only alignment, do

```
./sana -s3 0 -ec 1 -fg1 ...
```

To perform an alignment that optimizes 90% Importance as defined by HubAlign [23] 5% graphlets as used by GRAAL [15], 5% EC, and no S^3, do

Table 2
The `sana.out` file (whose name can be changed using the `-o` command-line option) contains information about the input networks (nodes, edges, connected components) and an analysis of the alignment (various measures applied to the entire alignment and also applied to the common connected subgraphs)

```
2018-06-15 15:21:57

G1: yeast
n    = 2390
m    = 16127
#connectedComponents = 158
Largest connectedComponents (nodes, edges) = (1994, 15819) (10, 32) (6, 11)

G2: human
n    = 9141
m    = 41456
#connectedComponents = 94
Largest connectedComponents (nodes, edges) = (8934, 41341) (5, 4) (4, 3)

Method: SANA_s3
Temperature schedule:
T_initial: 0.000316228
T_decay: 6.61993
Optimize:
weight s3: 1
Requested Execution time: 5 minutes

Actual execution time = 300.976 seconds

Random Seed: 514154230

Scores:
ec: 0.397966
mec: 1
ses: 35381
ics: 0.831563
s3: 0.368279
lccs: 0.248768
sec: 0.222913

Common subgraph:
n    = 2390
m    = 6418
#connectedComponents = 395
Largest connectedComponents (nodes, edges) = (1059, 4805) (53, 263) (48, 69)

Common connected subgraphs:
Graph  n     m      alig-edges  indu-edges  EC        ICS       S3
G1     2390  16127  6418        7718        0.397966  0.831563  0.368279
CCS_0  1059  4805   4805        5790        1.000000  0.829879  0.829879
CCS_1  53    263    263         268         1.000000  0.981343  0.981343
CCS_2  48    69     69          73          1.000000  0.945205  0.945205
CCS_3  34    68     68          70          1.000000  0.971429  0.971429
CCS_4  33    50     50          52          1.000000  0.961538  0.961538
```

Table 3
Measures accepted by SANA on the command line

Name	Description
`s3`	Symmetric Substructure Score [21]
`ec`	Edge Coverage/Correspondence/Correctness [15]
`ics`	Induced Conserved Structure [25]
`graphlet`	Orbit Degree Vector (ODV) Similarity [15, 29]
`graphletlgraal`	LGRAAL-normalization of ODV sim [20]
`go`	Mean ResnikMax GO similarity [62, 63]
`netgo`	Network-alignment-based GO similarity [34]
`wec`	Weighted EC [24]
`esim`	External file defining node-pair similarities
`sequence`	BLANT-based sequence similarities [31]
`lccs`	Largest Common Connected Subgraph [15]
`nc`	Node Correctness (if known, defines the exact alignment)
`spc`	Shortest Path Conservation [22]
`edgeCount`	Degree difference
`edgeDensity`	Relative degree difference
`importance`	HubAlign's Importance [23]
`nodeDensity`	Local node density
`ewec`	External edge-based similarity matrix, e.g., edge-graphlet similarity [56]
`sequence`	BLAST bit scores based on protein sequence similarity [31]

Note that "Name" means "command-line option," so for example to give `ec` a weight of 0.5, use "`-ec 0.5`" on the SANA command line. As more objectives become available, they can be listed by running "./sana -help".

`./sana -s3 0 -importance 0.9 -graphlets 0.05 -ec 0.05 ...` Note that one does not need to manually ensure that all the weights specified on the command line add to 1; if they do not, SANA will simply re-normalize them all so that they add to 1.

Similarly, the are many other objective functions defined by SANA; currently implemented ones are listed in Table 3.

Finally, as mentioned previously, SANA is capable of approximating the Pareto Frontier with a family of solutions called the *Pareto Front*. This mode is invoked using `-mode pareto`. See the output of `./sana --help` for more details on usage.

2.2 Direct Comparison with Other Aligners

As a part of our first publication on SANA [22], we wanted to automate the process of directly comparing to many other existing aligners. Thus, the external source code of over a dozen existing aligners was directly incorporated into SANA so that they can be called from the SANA command line. This was done to ensure consistent calling conventions to these other aligners during our comparisons. These other methods can be called from the SANA command line using the `-method` argument. In the SANA repo, these other aligners are in the directory `wrappedAlgorithms`; see the online SANA documentation for more details. (If you are an author of one of these aligners and notice that SANA is not using your algorithm optimally, feel free to contact us with any corrections.) The other aligners currently incorporated into SANA are LGRAAL [20], MAGNA++ [35], HubAlign [23], WAVE [24], NETAL [53], MIGRAAL [32], GHOST [25], PISWAP [54], OptNetAlign [51], SPINAL [55], GREAT [56], NATALIE 2.0 [57], GEDEVO [58], CytoGEDEVO [59], BEAMS [60], HGRAAL [49], PINALOG [61].

3 An Example of Objective Function Experimentation

As shown in Fig. 2, SANA can be used to experiment with objective functions; we believe that such experimentation is one of the most important but apparently under-appreciated aspects of the science of network alignment. Here, we describe one such experiment with a very well-defined scientific goal.

3.1 Gene–microRNA Networks

Consider a set of gene–microRNA (mRNA) networks [9], one network for each species. These networks are bipartite, meaning that genes interact with microRNAs, but neither genes nor microRNAs interact with their own type. Thus, when aligning two gene–mRNA networks, we wish to align genes from one network to genes in the other, and mRNAs in one to mRNAs in the other, but we should never align a gene to an mRNA or vice versa. In essence, the nodes have two *types*, and we must provide a type-specific network alignment.

At first, SANA did not have the functionality to provide a typed-node alignment. (It does now, using the `-bipartite` argument, in which case we assume that the first column in the edge list is one type, and the second column is the other type. Only two types are supported at the moment, but more may become available; run "./sana -help" for more info). The question was: how do the various topological objective functions compare in their ability to *automatically* align types correctly, given that typing is not *enforced* by the alignment algorithm?

Referring to Fig. 2, the scientific goal is clear: **maximize the fraction of nodes that are aligned to like-type nodes in the other network**. The question is now, *which topological objective function best achieves this scientific goal?*

We received 535 networks directly from one of the authors of [9]. We chose 1000 pairs of networks at random out of the $\binom{535}{2} = 142,845$ possible pairs of networks. For each pair of networks, we tested the following objective functions for their ability to correctly align nodes of like type to each other when this was not enforced: *EC*, S^3, Importance [23], GRAAL-type graphlet orbit signatures [15, 29], and LGRAAL-type graphlet orbit signatures [20]. To further test the dependence on runtime, we ran SANA on all the above objectives for all 1000 networks for runtimes of 1 and 4 min. Finally, to look at the frequency of core alignments, we performed each of the above pairs 5 times each. The results are in Table 4.

Table 4
Table of results when testing various objective functions (leftmost column) for their ability to correctly align genes-to-genes, and mRNAs-to-mRNAs, when aligning a pair of gene–mRNA networks [9]

Objective	Pairs	2*Gene	mix	2*RNA	coreFreq (GG)> 1	coreFreq (MG)> 1	coreFreq (MM)> 1
1 minute runs							
ec	30424880	29953074	198792	273014	1268806	570	3169
s3	30424880	29986047	284470	154363	1093307	2947	688
Importance	30241594	25434876	4658345	148373	651969	114137	1386
graphlet-GRAAL	30424880	24109670	6176510	138700	1902554	449738	17331
graphlet-LGRAAL	30424880	23056815	7305611	62454	1718519	584735	7086
4 minute runs							
ec	30424880	30055465	97811	271604	1245103	208	5908
s3	30424880	29953309	283313	188258	1092319	3779	1508
Importance	30292547	25473995	4669942	148610	652830	114815	1621
graphlet-GRAAL	30424880	24104880	6180836	139164	2208583	502806	25308
graphlet-LGRAAL	30424880	23051615	7310109	63156	2090416	692752	10504

Objectives tested were *EC* [15], S^3 [21], Importance [23], graphlet [15, 29], and graphlet-LGRAAL [20]. The columns are as follows. **pairs**: total number of pairs of nodes aligned in all 1000 network pairs that were run 5 times each. **2*Gene**: number of pairs in which a gene was correctly aligned to another gene. **mix**: number of pairs in which a gene in one network was aligned to an mRNA in the other. **2*RNA**: number of pairs in which an mRNA was aligned to another mRNA. **coreFreq(XY)**$>$ 1: the number of aligned pairs that had a core frequency greater than 1 (indicating the objective function strongly prefers to align this pair of nodes together) for type-pairs GG, MG, and MM

One column of great interest is the "mix" column, which counts the number of times, out of the approximately 30 million pairs of aligned nodes, in which a gene from one network was aligned to an mRNA in the other network—which is the kind of mis-typed node alignment we are trying to avoid. The rows are sorted best-to-worst by this measure, in each of the 1-min and 4-min sub-tables. As we can see, the *EC* objective scores best at avoiding this kind of mis-typed alignment. In the 1-min runs, *EC* aligns unlike typed node-pairs in only 0.65% of cases; S^3 is a close second, mis-typing just under 1% of the aligned pairs of nodes. In contrast, HubAlign's Importance measure [23] is almost 20 times worse in terms of incorrectly aligning nodes of different types, doing so in about 15% of aligned pairs of nodes, while both graphlet measures fare the worst, aligning unlike-type nodes in over 20% of cases.

Even more interesting is the 4 min runs, in which *EC* cuts its mis-typed node alignment in half, down to about 0.3% of aligned pairs, while all other measures fail to improve their "mix" column with the longer runtime.

Recall that if SANA aligns the same pair of nodes together in more than one run, we say that pair is in the *core* alignment, because the objective function is unlikely to align two nodes together more than once by chance. Another column of great interest is thus the **coreFreq(MG)**>1 column, which tells us how frequently the objective function seems to *strongly* prefer mis-aligning a pair of nodes of different types. Again we see that the *EC* measure is by far the best measure by this criterion: in the 1 min runs, only 570 mis-typed pairs appear out of 30 million (about 2 per 100,000 pairs), while the 4 min runs cut that "error rate" in half, suggesting that longer runs will do a better job of correctly aligning types. Meanwhile, S^3 does $10\times$ worse at 1 min and gets *more* bad in the 4 min runs, while importance and both graphlet measures misalign orders of magnitude more typed pairs, presenting a strong preference for misaligning nodes in about 1–2% of pairs.

We conclude that the *EC* measure is, *by far*, the best available objective function for this particular purpose among those we tested. For the moment we do not hypothesize why this is the case, but empirically the result seems iron-clad. While we agree that the S^3 measure is mathematically more aesthetically pleasing and would seem to be a better measure intuitively, for this particular purpose *EC* seems to work better. The author finds the poor performance of graphlet-based measures particularly surprising, since the author is a strong believer that graphlets are a useful tool for network analysis (see, for example, [41])—and graphlets have certainly demonstrated their value in other contexts [13, 30]. However, these results suggest that perhaps orbit degree signatures as they are currently defined [15, 20, 29] may not be the best way to leverage graphlet-based information in the context of global pairwise network alignment.

4 Conclusion

We have described the use of SANA [22], the *Simulated Annealing Network Aligner*, in the context of the pairwise 1-to-1 global alignment of biological networks. SANA provides many advantages over the many other aligners currently available: as a search algorithm, it is lightning fast, producing well-scoring alignments in minutes rather than hours; it provides a large array of objective functions users may wish to experiment with, as well as the facility to add more objectives in the future; it does not require the user to know much about the internal workings of the aligner in order to use it; and it is well on the way toward being fully integrated into popular network analysis tools such as Cytoscape.

We have introduced the concept of *objective function experimentation* (cf. Fig. 2 and Subheading 3.1), which we believe is at the core of future developments in network alignment. SANA's speed and effectiveness makes it the ideal aligner to implement the process depicted in Fig. 2.

5 Note

1. A prototype of a multiple-network-alignment version of SANA is available in the SANA GitHub repo. Simply re-compile SANA with the `-DMULTI_PAIRWISE` option on the command line (see the `Makefile`), and consult the Bourne shell script `multi-pairwise.sh`; running it without any arguments provides a short help message.

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