SimRNA (version 3.20) User Manual

1. SimRNA: Functionality

SimRNA is a tool for simulations of RNA conformational dynamics (folding, unfolding, multiple chain complex formation etc.), and its applications include RNA 3D structure prediction. SimRNA can be initiated with input files that include either the RNA sequence (or sequences) in a single line (similar to the Vienna format) or in the form of a structure written in PDB format. The PDB format should be simply the structure of the RNA with no heteroatoms or unusual names. In the current version readable residues are A, C, G and U only (i.e. no modified residues are supported as of yet).

The file outputs from SimRNA consist of the trajectory (or set of trajectories) of the RNA simulation trace (with file extension `.trafl`), a file containing bonding information (with file extension "`.bonds`"), a PDB file containing the initial structure at the beginning of the simulation (this is a circular shaped structure when only a sequence is provided) and a file indicating the initial secondary structure that was found in the starting structure (with file extension "`.ss_detected`"). All these files can be used (or reused) in various tasks of post-processing. SimRNA also generates output during the simulation specifying the total energy at a particular step of the simulation.

Additional tools that accompany this distribution are explained in the Section 6 titled **Additional Tools**.

2. SimRNA: Additional requirements

The distribution comes with a directory "`data`" that contains files with information about the energy function and important settings for the statistical potentials.

- The user **must** have the directory (or symbolic link to some other location) named 'data' inside of the working directory where SimRNA is running.
- The '`data`' directory (or link) contains the energy function files and is therefore essential to run SimRNA.
- The data stored inside these files strongly affect results the user obtains. Therefore, the user should not make changes inside these files unless the user understands the purpose and function of the files; or at least the user should make a backup copy of the data before changing it.
- If this directory is missing in the working directory where SimRNA is called or the program (for some reason) can't see it, the user will usually encounter the following error "`Error in opening file ./data/arccos`" and subsequent termination of the program.

3. SimRNA: Usage

SimRNA is fairly flexible on command line formats requirement; however, the following syntax is required to successfully run SimRNA:

```
> SimRNA -s input_file_sequence
```

or

```
> SimRNA -p input_file_PDB
```

or

```
> SimRNA -P input_file_PDB
```

Comments:

♦ Please pay attention to the format.

♦ For the “–s” option, SimRNA does not accept comment scripts; only one single line should appear in the input file, or, in the case of more than one sequence, multiple lines, each containing only one sequence, are allowed,

♦ For the “`-p`” or “`-P`” option, in the current SimRNA distribution, the input PDB file **must** contain only the RNA chains. Moreover, the residues must be complete, namely they should contain at least the atoms that are used in SimRNA representation: P, C4’, N (N1, N9), C2 and C4 (pyrimidines) or C6 (purines).

These options are explained subsequently.

`-s input_file_sequence`

This is a sequence file essentially in Vienna package format, one single line containing an *unbroken* sequence (.e.g., ″`aaaaaaccccuuuuu`″) or, if two or more independent strands are involved, these *unbroken* sequences separated by a single space on the line (e.g., ″`aaaaauuuuu aaaaauuuuu`″).

`-p input_file_PDB`

Note the *lowercase* p. Here, the input file is a structure given in PDB format.

`-P input_file_PDB`

Note the *uppercase* P. Again, the input file is a structure given in PDB format. However, the occupancy and B-factor columns of the PDB file are used to specify atom position (pinning or freezing) constraints. In such cases

- If the occupancy is equal to 0.00, then the current atom is fixed: its position is not changed during entire simulation.
- If the occupancy is between 0.00 and 1.00 (but neither 0.00 nor 1.00), then the B-factor value is treated as a radius of unrestricted movement. Hence, a B-factor of 2.00 is 2.0 Å, 4.30 would be 4.3 Å and so forth.
- If the occupancy is equal 1.00, then the atom is not restricted, while the other atoms can be.

— The PDB formatted file can also contain more than one chain. Subsequent chains are recognized only by the chain id (one character). The chains have to be in one piece; they cannot be interlaced. The PDB keyword "TER" is not mandatory.

Comments:

♦ If in the case of multichain task, initiated from a pdb file, the atom numbers have to be unique within the whole file. Because of the reduced representation, SimRNA uses "CONNECT" in the output PDB file, where "CONNECT" references the atom numbers. If the atom numbers are not unique, it is likely to result in considerable ambiguity for the display tools. Nevertheless, it doesn't affect simulation itself.

♦ If a simulation is initiated just from a sequence, the residues and atoms are numbered consecutively starting from 1. In case of multichain tasks, subsequent chains are named: A, B, C …Z, a, b, c …z.

3.1 Command line options:

`-c simulation_config_file`

The config file contains a variety of important settings and is the recommended way to start a simulation: see section 3.5 for details.

`-o output_files_basename`

If this is not specified, then the program will use pdb or sequence file name as a base name for output.

`-S secondary_strc_restraints_file`

This allows the user to specify the secondary structure restraints in bracket format. Like the sequence file input, it should consist of just a single line, or, in the case of pseudoknots, it contains consecutive dot bracket lines specifying the positions of the pseudoknots.

`-r restraints_file`

This is the file specifying restraints. (The details on the format of the file for restraints are discussed below. The format is somewhat strict, and should be followed or an error will be issued.)

`-E int_number_of_Replicas`

Request to use the replica exchange Monte Carlo method (REMC), where the program initiates a specified number of relica and perform a REMC simulation.

`-R int_number`

This allows the user to choose, generate or provide a specified random number seed for doing calculations. This is mainly useful when trying to reproduce a simulation. The user can always check the initial random seed in the SimRNA standard output, regardless of whether the random seed was specified by the user or generated by default (using the clock).

`-l`

The program generates a list of PDB files (each trajectory frame is written to a PDB file in addition to the trajectory file. This is generally not necessary and does take up a lot of space when running a long simulation, so it should not be asked for frivolously. (It is recommended that the user extract all desired PDB formatted files using SimRNA_trafl2pdb, see Section 5.)

`-n number_of_iterarions`

This specifies the number of iterations to be done in the simulation. The argument is the number of iterations in one temperature cycle of simulated annealing. (It is recommended that the user use the config file instead of this command.) This option overrides "`NUMBER_OF_ITERATIONS`" option specified in config file (see Section 3.5).

3.2 Format of sequence and structure input files

`-s input_file_sequence`

The input data is written in a single line in a basic text file with the sequence of RNA (both upper and lower cases are acceptable). For example:

```
AGACUGCUGAGAGACC
```

There should be nothing but the desired sequence or sequences contained in the file because the program will read everything in the file as though it were part of a sequence. There should be no additional spaces either.

To have more than one RNA chain as input an input, the user must separate the different chains by white spaces.
For example:

```
aagcua aaagcugggcu
```

or

```
AGACUGCUGAGAGACC
UGCUGAGAGACC
```

`-p input_file_PDB`

The user requests starting the calculation with a structure provide by the user in PDB format.

Comment:

♦ The format of the PDB file has some very strict requirements: i.e., it should be a simple structure

- no heteroatoms
- no non-standard base notations: **the readable residues are A, C, G and U only** (i.e. no modified residues are supported as of yet).
- *Every* 5′-most residue *must contain a P*! This can be added by some software tools. In a pinch, if the P of the 5′-most residue is missing *from any chain* in the PDB file, then the user can rename the O5′ atom of the 5′-most residue to P in that particular chain and the SimRNA will treat that atom as a P.

3.3 Format of secondary structure restraint files

`-S secondary_strc_restraints_file`

If the user wants to provide secondary structure restraints, the contents of the restraint file should be in the following *one-line* format

```
.((((......)))).
```

Or, when pseudoknots are involved, several lines can be provided depending on the characteristics of the pseudoknot. For example, if there is only one pseudoknot, then the following is sufficient

```
.((((......)))).
......(........)
```

If more than one pseudoknot is involved, each one should be written on a separate line. For example, the following structure would involve two pseudoknots.

```
((((.......))))...............((((.........))))).
......((((.............))))......................
..................((((..............)))).........
```

Comments:

♦ In general, it is good policy to write the dominant secondary structure on the first line and place only single hairpin loops closing the pseudoknots on subsequent lines; however, this is not strictly required.

♦ The program reads in the constraints line by line. Therefore, the following input

```
(((......)))
```

can also be written as follows

```
(..........)
.(........).
..(......)..
```

It is important emphasize that the data for secondary structure and pseudoknots must be listed on subsequent lines regardless of how the data is specified. Otherwise, SimRNA will assume that there is a second chain, and it is likely an error will occur.

Constraints can also be applied to multi chain problems. For example, in the case of two chains, restraints can be written as follows

```
aaaaccccuuuu aaaaccccuuuu
((((....(((( ))))....))))
```

The above example will form a dsRNA stem with an interior loop containing poly(C). When the sequence is written as above in the restraint file, the first line is ignored. The actual sequence must be entered using the "-s" or "-p" command-line option.

If more than two chains are involved, this can also be accommodated by the above scheme. For example, constraints on four chains could be written as follows

```
aaaaccccuuuu aaaaccccuuuu aaaaggggaaaa aaaaggggaaaa
((((....(((( ))))....)))) ............ ............
............ ....((((.... ....))))....  ............
....((((.... ............ ............ ....))))....
```

In general, there are a variety of ways that this file can be written. However, clarity is probably the most important thing to remember.

Comment: please note that SimRNA doesn't allow the user to exclude the possibility of base pairing using the secondary structure constraints file. Thus, even the dot "." does not prohibit the formation of base pairing.

3.4 Format of long-range restraint files

`-r restraints_file`

The input restraint file contains information on the type and position of the restraints between atoms.

Restraint options

A restraint can be thought of as a flexible tether that drives the selected atoms towards a certain distance by applying a penalty for distances that deviate from that range. It can also provide a reward when a desired distance is achieved. The penalty and reward are positive and negative contributions to the total energy of the simulated system, respectively.

There are two types of distance restraints: `WELL` and `SLOPE` (same as `DISTANCE` before, keyword `DISTANCE` still works for `SLOPE`). Both restraints describe the preferred distance as a range between a minimal and a maximal distance, Figure 1a and b.

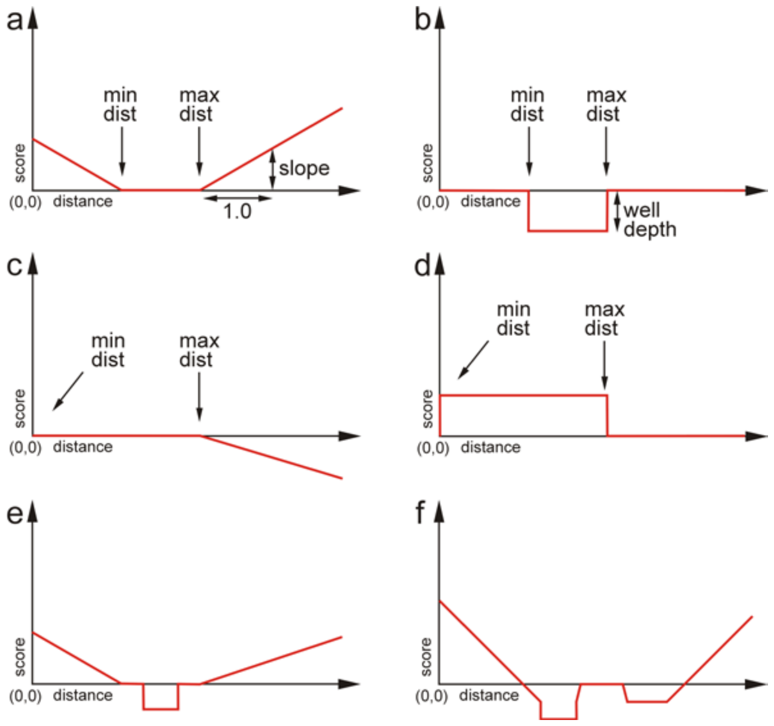


Figure 1: Examples of types of constraints. a) An elementary function of type “`SLOPE`” that depends on the following three parameters: the minimum distance, the maximum distance and the slope. b) An elementary function of type “`WELL`” that depends on three parameters: the minimum and maximum distance and the depth of the well. c) A single “`SLOPE`” function with negative weight. d) A single “`WELL`” function with negative weight. e) A combination one “`SLOPE`” function and one “`WELL`” function. f) A combination of three “`SLOPE`” and two “`WELL`” functions, both one “`WELL`” function and one “`SLOPE`” function with negative weights.

In the case of a “SLOPE”-type restraint, the two atoms are tethered towards the region by applying a linear penalty that corresponds to the degree of violation of the distance from the desired region. When the distance between the atoms becomes equal to the desired value, the value of the function reaches zero. The shape of the function resembles a “V”, with a bottom that can correspond to a single point or to a “flat” region (Figure 1a).

In case of a “WELL”-type restraint, the function is flat and equals zero for any distance outside the desired range, while the distances within the desired range correspond to the negative value of the weight (Figure 1b).

Both the “SLOPE” and “WELL” restraint functions can be “reversed” when a negative weight is applied (c.f., Figures 1c and d). For example, applying a “SLOPE”-type function with a negative weight can be used as a repelling function (Figure 1c). This function can be useful in simulations in which the user desires to study molecule stretching between terminal residues, for example. On the other hand, the “WELL”-type function can be applied with a negative weight when the user wishes to define a distance range that the atoms should avoid. When atoms are in the distance range specified by “WELL”, an additional penalty is applied (Figure 1d).

Any number of these two types of functions can be also combined in order to define complex restraints. The resulting function can adopt various shapes (Figures 1e and f). Therefore, the relative distance of two atoms under consideration can be described by a dedicated function or a linear combination of functions used as a part of the total scoring of the energy.

For both types of restraints, the user must specify the restraints in subsequent lines of the restraint file in the following format

```
ChX/i/atom_i   ChY/j/atom_j min_dist max_dist weight
```

where examples are provided in the next section. Here, `ChX` and `ChY` specify the chain index (chain name can be the same or different), `i` and `j` refer to the index of the particular residue on the respective chain, and `atom_i` and `atom_j` refer to the atom on which the constraint is applied. Restraints of type WELL are 0 except for the range between `min_dist` and `max_dist`. For the region between min/max (`min_dist` <---> `max_dist`), the value becomes -1*`weight`.

Restraints of type SLOPE are 0 within the range, `min_dist` <---> `max_dist`. Outside that range, a linear increasing positive penatly is added (the pair of atoms are attracted to each other because there is less penalty as they approach the region between `min_dist` and `max_dist`). The value for the penalty is dist_violation*`weight`.

Restraints for a given pairs of atoms can be combined (added). It requires two (or more) lines to specify subsequent contributions.

Restraint command formats

In SimRNA, distance restraints can be defined for any pair of atoms used in SimRNA representation, as well as to the central point of the nucleic acid base: P, C4′, N (N1, N9), C2, C4 (for pyrimidines) and C6 (for purines) and the midpoint MB.

The WELL restraint (a single line in the restraints file):

```
WELL      atom_1_id  atom_2_id    min_dist  max_dist  weight
```

The SLOPE restraint (a single line in the restraints file):

```
SLOPE     atom_1_id  atom_2_id    min_dist  max_dist  weight
```

or (alternative for SLOPE):

```
DISTANCE atom_1_id  atom_2_id    min_dist  max_dist  weight
```

Example line in a restraints file:

```
SLOPE   A/23/C4'  C/45/P      5.5    8.5    1.0
WELL    A/23/C4'  C/45/P      6.5    7.5    1.0
```

where

A/23/C4' means atom C4' of nucleotide 23 in chain A
C/45/P means atom P in nucleotide 45 in chain C

5.5 [Å]: minimal distance where the weight is zero (for SLOPE)
6.5 [Å]: minimal distance where the weight is −1 (for WELL)
7.5 [Å]: maximal distance where the weight is −1 (for WELL)
8.5 [Å]: maximal distance where the weight is zero (for SLOPE)
1.0 weight of this restraint weight for both SLOPE and WELL. For WELL, the value is −1 between 6.5 and 7.5 [Å], for SLOPE the value increases for distances greater than 8.5 or less than 5.5 [Å].

This is an example of a multifunctional restraint that resembles Figure 1e.

Comments:

♦ If the input file is a PDB file, then the nucleotide numbers specified in the restraints file are according to numbering in input PDB file !!!

♦ In SimRNA representation, the atom name N represents the nitrogen that binds the base to the backbone. Hence,N corresponds to N1 for pirymidines and N9 for purines! This label can also be used instead of N9 for purines and N1 for pirymidines. SimRNA also permits the user to restrain the C2 atom or and also the C6 atom for purines or the C4 atom for pirymidines.

♦ The user can also apply restraints for a middle atom of the base (pseudo atom) which is named MB !!! This is useful for restraining the bases such that they are only in contact but not specifically tied to some prescribed orientation such as Watson-Crick, or Hoogsteen edge, or whatever.

3.5 Format of the config file

`-c simulation_config file`

The argument is a file containing simulation configuration parameters. With this option, the user can configure the simulation in a more advanced way.

The configuration file is text file with one parameter specified on each line. There can be blank lines, each line must contain a command or must be empty.

Config file options

These are some of the options that can be included in a typical config file.

```
NUMBER_OF_ITERATIONS N
```

This specifies the number of unified iterations in a simulated annealing simulation. This can be overridden by −n option.

`TRA_WRITES_IN_EVERY_N_ITERATIONS N`

This specifies how many iterations should occur before the conformation is appended to the trajectory file. In general, there probably should be at least 10000 iterations of SimRNA before a write is done to generate a meaningful structural change. However, if the user desires to have a chain of quite similar structures, this value can be low.

`INIT_TEMP float`

This specifies the starting temperature of the simulation

`FINAL_STEP float`

Likewise, specifies the final temperature of simulation.

Comments:

♦ !!! The final temperature can be lower or higher that initial temperature. In the second case, the system is being gradually heated. !!!

♦ !!! The final temperature can be also equal the initial temperature. In such a case, the program maintains same temperature during entire simulation !!!

♦ The order of parameters is not important.

♦ !!! The config file lines must be in upper case only

The format of the file up to this point should be as follows

```
NUMBER_OF_ITERATIONS  1000
TRA_WRITE_IN_EVERY_N_ITERATIONS 20
INIT_TEMP          1.35
FINAL_TEMP         0.90
```

There are some additional short−range energy terms that can be set up the use in the configuration file. These options include the following:

```
BONDS_WEIGHT        1.0
ANGLES_WEIGHT       1.0
TORS_ANGLES_WEIGHT 0.0
ETA_THETA_WEIGHT   0.4
```

The BONDS_WEIGHT defines the strength of the bonds holding the structure together. A value zero would mean that there are no bonds and the atoms will all behave as though the RNA is a gas. The ANGLES_WEIGHT define the response of the flat angles to bending. In the current version of SimRNA, the TORS_ANGLES_WEIGHT is ignored, this is intended to add additional helical orientation to the RNA strand. In the place of TORS_ANGLES_WEIGHT, the option ETA_THETA_WEIGHT has been introduced and accomplishes most of the same properties.

The weight on the secondary structure (specified using the command line option "`-S secondary_structure`") can also be scaled.

```
SECOND_STRC_RESTRAINTS_WEIGHT 1.0
```

The frequencies of subsequent Monte Carlo moves can also be adjusted:

```
FRACTION_OF_NITROGEN_ATOM_MOVES 0.10
FRACTION_OF_ONE_ATOM_MOVES      0.45
FRACTION_OF_TWO_ATOMS_MOVES     0.44
FRACTION_OF_FRAGMENT_MOVES      0.01
```

Where the values listed above are default. These parameters were decided based on testing the program. The user is advised to use discretion in changing them.

For systems composed of several RNA chains additional constraint, namely limiting sphere, is activated. Chains contained within the sphere are not permitted to drift away from each other. The reason for including this range is because it reduces the search space (volume) of simulated system. When any atom drifts outside the limiting sphere, an additional positive value is added to the total energy, where the value is dist_of_violation*weight

By default radius of that sphere is calculated as: float(numberOfNucleotides) and the default weight is set to 1.0.

Those values can be specified by the user in config.dat file (i.e.):

```
LIMITING_SPHERE_RADIUS 41.5
LIMITING_SPHERE_WEIGHT 0.25
```

The user will be notified about redefinition of those variables in the SimRNA output.

4. SimRNA: Output

The main output of a simulation using SimRNA is the trajectory file (`.trafl`). At the beginning of the simulation, the program generates a PDB file (in reduced representation) of the initial structure (.pdb) and the secondary structure that was detected (.ss_detected). In the case where the user supplies only the sequence, then a structure constructed in a single circular pattern is generated, where the circle contains the full sequence or the set of sequences in consecutive order based on the input sequence file. If the user supplies a PDB formatted file, then the reduced representation of the structure is is output at the beginning of the simulation.

4.1 Trajectory format: trafl

The SimRNA output is a trajectory file "`.trafl`". If a single simulation is launched, then one trafl file is generated. If the replica exchange Monte Carlo (REMC) method is requested, the number on each trafl file corresponds to one replica. It is worth noting that, in the case of the REMC method, each trafl file corresponds to the replica, not to the temperature shelf. Hence, each trafl file maintains the conformational continuity (the current frame is always obtained from series of modifications of the previous one). However, the temperature is change according to the way that the current replica is assigned to a particular temperature shelf. The tracking of the temperature field allows the user to trace back the movement of the replica through the different temperature shelves.

The SimRNA trafl format is a simple text file. Each frame consists of two lines:
– a 5 fields header: (int) (int) (float) (float) (float)
– a line with the atomic coordinates: (a string of floats corresponding to the position of the atoms in each base.)

The format of the header is:

```
consec_write_number replica_number energy_value_plus_restraints_score
energy_value current_temperature
```

where

- `consec_write_number:` is just the order in which the file is written.
- `replica_number:` useful for tracing the replica when the files from the simulation are concatenated.
- `energy_value_plus_restraints_score:` indicates the energy including the restraints.
- `energy_value:` indicates the energy without the restraints or just the energy if no restraints are used (see Section 3.4)
- `current_temperature:` denotes current temperature, in replica exchange mode it denotes particular temperature shelf

The format of coordinates line is just:

```
x1 y1 z1 x2 y2 z2 … xN yN zN
```

The coordinates of the subsequent points corresponds to the following order of the atoms: P, C4′, N(N1 or N9 for pyrimidine or purine, respectively), B1 (C2), B2 (C4 or C6 for pyrimidine or purine, respectively). In general, the coordinate line will contain 5*numberOfNucleotides points, so 3*5*numberOfNucleotides coordinate items (in 3D space: 3 coordinates per atom, 5 atoms per residue; hence 15 coordinates per residue).

Users can write their own scripts fairly easy for trajectory processing. Reading subsequent lines: when the number of items in a line is 5 it means this is the header for next line.

Comments:

♦ The trajectories can be concatenated. An easy way is using the Unix command

```
> cat file1.trafl file2.trafl … > newfile.trafl.
```

When SimRNA is run in several instances (which is recommended), all files can be combined into a single trafl file and then can be subjected to further processing (e.g., clustering). It also is useful for outputs from simulations in replica exchange mode: especially when several instances are initiated (also recommended). The user will receive many trajectory files. The necessary requirements for trajectory files to be concatenated is that they should have the same number of atoms in coordinate lines, so basically they should originate from simulations of the same sequence. For some types of processing, an additional requirement is that the trajectories should originate from simulations under the same conditions (e.g., same energy function, similar temperature, similar restraints, or other possibilities).

♦ The trajectory may contain only one conformation. In this case it will be just a two line file.

♦ The trajectory file doesn't contain any information about the sequence: chain names, atom/residue numberings, etc Some stages of trajectory processing can be done on just trajectories, while the other stages (especially conversion to pdb) require additional information that are not in trajectory itself.
+ When displaying the files using traflView, the initial "`.bonds`" file is needed (see Section 6.4).
+ When the user desires to convert some of the trajectory frames into PDB files (Section 6.1), the missing information must be provided by the user as a PDB file (which will only be used as a template, where the actual coordinates will be provided from the given trajectory frame or frames of interest). *It is the user's responsibility is to provide the proper data.* It is recommended to use initial PDB file generated by SimRNA, at the start of the simulation, if the initial starting file was from a sequence or there is no other source of information.

♦ The 5th field of the header (the temperature field) allows for binning the conformations into specific temperature ranges or assigning them to particular temperature shelves.

5. Examples

When starting SimRNA, please make sure that there is a link to the data file in the working directory (See Section 2). This can be accessed by linking the directory where the distribution is located to the current working directory.

```
> ln -s ${path_to_SimRNA_directory}/data data
```

It is strongly recommended that the configuration file be used with the simulation

```
> cat configSA.dat
NUMBER_OF_ITERATIONS 16000000
TRA_WRITE_IN_EVERY_N_ITERATIONS 16000

INIT_TEMP  1.35
FINAL_TEMP 0.90

BONDS_WEIGHT 1.0
ANGLES_WEIGHT 1.0
TORS_ANGLES_WEIGHT 0.0
ETA_THETA_WEIGHT 0.40
```

Comment:

♦ This config file is set to a fairly long simulated annealing run where the intermittent sampling iterations per frame involve 16000 steps. (The duration of the simulation is commensurate with the total number of iterations and the number of residues.) The user will receive 1001 frames (1000+1: the first is just the starting conformation). When SimRNA is called with the option "–E `number_of_replicas`" (replica exchange), then instead of a simulated annealing run, the replica exchange protocol will be initiated. Replica swaps will be attempted with the same timing as writing into trajectory files. In this case the user will receive N trajectories (where N is the number of replicas), each file of them will contain 1001 frames.

♦ The number of structures you will obtain from a single thread is always:
`NUMBER_OF_ITERATIONS/TRA_WRITE_IN_EVERY_N_ITERATIONS + 1`

A sequence file is also required

```
> cat mytest.seq
aaaaaccccuuuuu
```

To run SimRNA, the following syntax is then recommended

```
> SimRNA –s mytest.seq –c configSA.dat –o mytest1 >& mytest1.log &
```

and the output files will be the following

```
mytest1.bonds
mytest1.trafl
mytest1-000001.pdb
mytest1-000001.ss_detected
```

Comments:

♦ The –o option is recommended if one attempts more than one calculation in the same directory, because the default output tag (if nothing is specified) is mytest.seq and in any subsequent calls to SimRNA, SimRNA will overwrite the user's preciously obtained calculations of the same name!!!

♦ Requesting the log file is recommended because it is possible to check the configurations and settings that were used to start the simulation. Since the output it to stderr, the ">&" notation should be used. The "&" at the end of the line is recommended so that the terminal is free for other activities such as checking the status of the calculations.

♦ The random seed variable for the Monte Carlo simulation is initiated from the clock. This information can be found in the log file after the simulation, in case it is desired to exactly reproduce the same calculation.

If a PDB file is available, then the following syntax can be used

```
> SimRNA –p mytest.pdb –c configSA.dat –o mytest2 >& mytest2.log &
```

with the name of the output files being the same as above for the "–s" option.

To carry out replica exchange Monte Carlo simulations (with 10 replica), the following command line is sufficient

```
> SimRNA –s mytest.seq –c configSA.dat –E 10 –o mytestE >& mytestE.log &
```

To run a simulation with a specific random seed, the following syntax is required

```
> SimRNA –s mytest.seq –c configSA.dat –R 17801 –o mytest1 >& mytest1.log &
```

Where, in this example, the random seed was set to 17801.

6. Additional Tools

Additional tools are also included with the distribution.

6.1 SimRNA_trafl2pdbs – trajectory converter

SimRNA_trafl2pdbs transforms an output from the SimRNA trajectory file into a PDB formatted file. It also makes all-atom reconstruction (optional). The SimRNA_trafl2pdbs converter requires a reference PDB structure (this can be the initial PDB structure generated at the beginning of the run), and the trajectory data (extension ".trafl") and a specification of which structure(s) is desired.

To run this application, simply type the reference PDB file, the trafl file, and the list of structures (or ranges) to be converted that is/are desired.

```
    > SimRNA_trafl2pdbs structure.pdb trajectory.trafl {list}
```

or

```
    > SimRNA_trafl2pdbs structure.pdb trajectory.trafl {list} AA
```

where `{list}` can consist of the following types of formats

`{list}`	Output
`1`	Converts the first frame of an input trajectory file (trafl) to a PDB file (input myfile.trafl: output myfile-000001.pdb).
`1 10 35`	Converts frame numbers 1, 10 and 35 (of an input trajectory file) into the PDB files with the respective indices (input myfile.trafl: output myfile-000001.pdb, myfile-000010.pdb, etc.).
`1 10 35 52:64`	Converts frame numbers 1, 10, 35, and the range of frames 52 to 64 to PDB files with the respective indices
`:`	Converts all frames of an input trajectory file. However, please see warnings in comments (below).

And the option "AA" initiates an all-atom reconstruction of the specified frames (in `{list}`)..

When AA is not used, the output PDB files will be in the reduced representation format and a file with extension `ss_detected` that indicates the RNA secondary structure that was detected. When AA is used, the output will be both the all-atom representation plus the reduced representation and `ss_detected` files.

Comments:

♦ The SimRNA_trafl2pdbs converter also requires presence of 'data' directory (or link to it), this is because it has to read in all-atom backbone conformers, as well as all-atom base representations

♦ Be forewarned that frivolous usage of the option ":" will mean that a 10000 frame trajectory file will generate 10000 independent PDB files in the directory plus 10000 ss_detected files, and if the option "AA" is used too, then 10000 files with the tag "_AA.pdb" as well. Hence **a grand total of 30000 files could suddenly overwhelm the directory**! If this is expected by the user, fine, but be sure that is what is desired.

6.2 trafl_extract_lowestE_frame.py

This is a simple python script that reads in the trajectory file, finds the lowest energy frame, and outputs it as a single frame trafl file.

Usage:

```
> trafl extract_lowestE_frame.py trajectory.trafl
```

The output (for name above) will be:

trajectory_minE.trafl

This single frame trajectory can be converted into a PDB file using the SimRNA_trafl2pdbs tool.

The user is encouraged to examine this script file if additional tools for trajectory processing are needed or desired.

Comments:

♦ Whereas obtaining the lowest energy is one way (the simplest way) of processing the trajectory files, benchmarks demonstrated that better results were generally obtained by using clustering.

♦ The current version of the tool assumes that the energy was calculated without restraints (the fourth element in the header for each frame, see Section 4.1 for the format of the trafl file). This information is read at line 30 of the script file. When the full energy (with restraints) is to be considered, this line of the script should to be changed to the third item.

6.3 Clustering

Clustering is a tool for processing trajectory file by finding and grouping similar structures together into a single group etc.. Benchmarks demonstrated that clustering provides better results in terms of structure prediction.

The input for clustering is just the trajectory.trafl file. It can be the result of concatenation of several trajectory files. Typically those trafl files originate from multi-instance runs of replica exchange methods. The output of clustering is a set of trafl files corresponding to subsequent clusters.

Usage:

```
      > clustering trajectory.trafl fraction_of_lowE_frames_to_cluster
rmsd_thrs_1 [rmsd_thrs_2] …
```

Example usage:

```
      > clustering mytest.trafl 0.01 3.5 5.0 >& mytest_clust.log
```

where:
mytest.trafl is an input trafl file,
0.01 indicates that 1% of the lowest energy frames of an input trafl will be subjected for clustering (where the remaining frames will be ignored)
3.5 is the RMSD threshold in the set for first pass of clustering
5.0 is RMSD threshold in the set for a second pass of clustering

NOTE: those two clustering passes (it can be more) are just independent clustering instances but are using the same RMSD all_vs_all matrix. The reason for that is because thecalculation of the RMSD for the all_vs_all matrix is the most computational demanding stage of the clustering procedure (it may take a lot of time).

As a result (assuming mytest.trafl input), the user will obtain:
mytest_thrs3.5A_clust01.trafl
mytest_thrs3.5A_clust02.trafl
mytest_ther3.5A_clust03.trafl
…
…
mytest_thrs5.0A_clust01.trafl
mytest_thrs5.0A_clust02.trafl
mytest_thrs5.0A_clust03.trafl
…
….

Each of the files listed above is a trajectory format file (it is not a trajectory by definition) containing elements of a given cluster. The frames of each cluster in the trafl files are sorted where the first frame corresponds for the structure closest to the center of density. Thus, conversion to a PDB file can be applied to just the first frame. For example,

```
      > SimRNA_trafl2pdbs mytest-000001.pdb mytest_thrs3.5A_clust01.trafl 1 AA
```

This command converts the first frame of the first cluster (obtained with a 3.5A RMSD threshold) to a pdb file (with all-atom reconstruction). Of course other (or all) frames of the cluster can be converted into pdbs as well.

There is one further consideration. The trafl file that is generated as a result of clustering has a different meaning in 5th fileld of the header. For a clustering output, this holds the RMSD value in relation to the center of the density (instead of current temperature)

NOTE: the current implementation of the clustering tool doesn't provide any cut-off for a number of clusters. Actually it identifies subsequent clusters until it exhausts the input data. In such cases, only the first few clusters are meaningful: the remaining clusters are usually just the sparse remains. The user should inspect the clustering output to identify the percentage of structures that are contained in the 1st, 2nd, 3rd, and so on, set of clusters.

6.4 calc_rmsd_to_1st_frame

This tool allows for calculation of the RMSD value in relation to the first frame for the entire trajectory. The input is just the trajectory (trafl) file while the output is a two column file: RMSD vs Energy. The RMSD value is calculated, while the energy value is just read from trajectory file. The calculated RMSD value is in relation to the first frame, so the RMSD in the first line will be always 0.0.

This tool is typically used for generating data for RMSD vs Energy plots, when the native structure is known. It allows for showing the projection of conformational space, rooted in the native conformation. It is worth noting that this tool allows for calculation of the RMSD for the shortest, two frame, trajectory, when each frame can be any structure. The minimum requirement is that number of atoms in each frame must be the same. The RMSD calculator assumes a 1 to 1 correspondence between the atoms. Such calculations only make sense when done for the same sequence.

The input trajectory for the tool can be any conformation; however, typically the user will want to put reference frame at the beginning. It can be done by concatenating trajectories:

```
> cat reference_frame.trafl trajectory1.trafl … > sum_trajectory_with_first_reference.trafl
```

where,

- the `reference_frame.trafl` file is one frame (one conformation) trajectory,
- it can be the native structure obtained by running SimRNA in zero interations mode (see below).

Assuming file names as above:

```
> calc_rmsd_to_1st_frame sum_trajectory_with_first_reference.trafl output_name.rmsd_e
```

The output file becomes a two column file which can be plotted in separate plotting program, like gnuplot. (see figure 3 in the main text)

6.5 TraflView

TraflView allows the user to browse the trajectory files from SimRNA directly. This program was first developed for protein structure manipulation using REFINER in the Kolinski lab[1]. The file with extension "`.bonds`" is required to view the "`.trafl`" files. This tool is still under development; nevertheless, it is a very handy tool for viewing or even monitoring the progress of a SimRNA simulation, so we decided to add it to SimRNA suite.

To browse the trajectories (run this application), simply type output SimRNA "`.trafl`" file and "`.bonds`" file.

```
traflView SimRNAoutput.trafl SimRNAoutput.bonds
```

where *`SimRNAoutput`* is output file generated by SimRNA in the given run.

TraflView can also be used to visualize a set of trajectories given in pdb format. The "`.bonds`" information is require for files with reduced representation that have bond lengths much greater than 1.5 Å. Standard PDB files serving as trajectories can be read directly without the additional information from bonds.

Comment:

♦ As a piece of browsing software,TraflView has some very specific requirements on unix libraries. If TraflView fails to run on the particular Linux system, then it is necessary to install the graphics package mesa. In general, if the package mesa is available and installable, then this can be installed using

```
> sudo aptitude install mesa
```

on a Linux operating system. It is also recommended that a relatively new version of Linux Ubuntu for Fedora Core operating system be installed to guarantee compatibility.

When the user launches TraflView (for longer trajectories it may take some time), a black graphic window will be initiated. The window can be enlarged by pulling with the mouse cursor. In the current implementation, it doesn't maintain the same horizontal/vertical proportions (that will have to be the job of the user).

The user interface is accomplished by several keys and mouse movements (keys are case sensitive):

- c (on/off): colors the structures, rainbow style, when 5' end is blue, and 3' is red
- e: (on/off): show energy plot, green line in bottom part of the window
- Esc: quitting the viewer

Selecting different frames (moving forward and backward) is accomplished by digit keys:

- 1 and 3: one step backward or forward, respectively.
- 7 and 9: ten steps backward or forward, respectively.

---

[1] Software Developed by Michal Boniecki during his PhD studies, initially dedicated for viewing Refiner and CABS trajectories.

- 4 and 6: a hundred steps backward or forward, respectively,

NOTE: TraflView only reads the digit keys. Therefore, to have this function, it is recommended that the user set the keypad such that the NumLock is switched on.

Rotating, zooming (in/out), and moving the current conformation is accomplished by using the mouse:

- Left button + mouse movement (left/right) and (up/down): rotation of a structure,
- Middle button + mouse movement (only left/right): zooming (in/out) of a structure,
- Right button + mouse movement (only left/right): moving of a structure.

It is worth noting that TraflView also outputs data that is displayed in the terminal where the application is called. In the output, there is the RMSD value to the reference frame. By default, the reference frame is first frame in the trafl file; however, it can be any frame. A new reference structure can be selected by pressing key 'r' when the desired structure is currently displayed. The current display can be also dumped into bmp file by pressing key 'S'.

7. Using SimRNA as a SS classifier, getting single frame trafl – zero interation run

SimRNA can be used as a secondary structure classifier, when input structure is a PDB file. By default, SimRNA generates a ss_detected file at the beginning of the simulation that contains secondary structure analysis of the initial structure. Hence, if the user wishes to obtain a trafl file, the secondary structure or the energy of the structure in the PDB file (i.e., if no simulation is desired), the user can run SimRNA in zero iteration mode.

This can be accomplished by:

- Setting NUMBER_OF_ITERATIONS to 0 in config file
- Using option –n 0, which overrides config setting.

When SimRNA is run in zero iteration it dumps typical output:

- The PDB file of the starting structure in reduced representation (which can be viewed by typical pdb viewers, such as rasmol, pymol …)
- A one frame trafl file, a file containing just the input structure in trafl format
- A ss_detected file, which contains the secondary structure of the initial file, according to the SimRNA classifier.

It is worth noting that both the trafl file and the PDB file contain energy value (according to the SimRNA energy function) of the input structure.

The one frame trafl file (which may contain the native conformation) can be used as a reference in calculating the E vs RMSD plot procedure (see above).

NOTE: If the user has series of PDB files, they can be converted into single frame trajectories. Those trajectories can be concatenated and processed by clustering. This procedure requires that the PDB files are in a same format and contains conformations corresponding to the same sequences.

8. Using SimRNA, repeating benchmark procedure

In our bechmarking procedure, we performed fairly long simulations using replica exchange mode, with 10 replicas. For each case of prediction, we run 8 independent runs, each of them in replica exchange mode (10 replicas). Then we clustered the results.

In order to repeat this procedure (de novo) the user should prepare the following config file:

```
> cat config.dat
      NUMBER_OF_ITERATIONS 16000000
      TRA_WRITE_IN_EVERY_N_ITERATIONS 16000

      INIT_TEMP  1.35
      FINAL_TEMP 0.90

      BONDS_WEIGHT 1.0
      ANGLES_WEIGHT 1.0
      TORS_ANGLES_WEIGHT 0.0
      ETA_THETA_WEIGHT 0.40
```

Let's assume we want to run simulations for the sequence of 2ap0. Then we must prepare the following sequence file:

```
> cat 2ap0.seq
   AGUGGCGCCGACCACUUAAAAACAACGG
```

Then just run 8 independent runs:

```
SimRNA -c config.dat -E 10 -s 2ap0.seq -o fold_2ap0_01 >& fold_2ap0_01.log
SimRNA -c config.dat -E 10 -s 2ap0.seq -o fold_2ap0_02 >& fold_2ap0_02.log
…
SimRNA -c config.dat -E 10 -s 2ap0.seq -o fold_2ap0_08 >& fold_2ap0_08.log
```

NOTE: Those runs are computationally demanding. SimRNA (replica exchange mode) is parallelized using OpenMP. The calculations specified above will be optimally executed at 8*10 = 80 CPU cores. In such a case, execution time will be several hours. It should be noted that one can obtain good predictions even after 10% of the time of the simulations above (so the config.dat file can be modified accordingly) but we chose to perform much longer runs.

The simulations above will generate files:

```
fold_2ap0_01_01.trafl
fold_2ap0_01_02.trafl
…
fold_2ap0_08_09.trafl
fold_2ap0_08_10.trafl
```

In total, there will be 80 trafl files generated, where each of them will contain 1001 frames.

Then the structures have to be concatenated:

```
> cat fold_2ap0_??_??.trafl > fold_2ap0_all.trafl
```

Then 1% of lowest energy structures will be subjected for clustering:

```
> clustering fold_2ap0_all.trafl 0.01 2.8 >& fold_2ap0_clustering.log
```

NOTE: we used 2.8 Å rmsd threshold which is 0.1*seq_length

Clustering procedure will generate files:

```
fold_2ap0_all_thrs2.80A_clust01.trafl
fold_2ap0_all_thrs2.80A_clust02.trafl
fold_2ap0_all_thrs2.80A_clust03.trafl
...
```

Let' s consider only first 3 clusters.

First frame (center of density) of each of them can be converted into pdb, with all-atom reconstruction:

```
> SimRNA_trafl2pdbs fold_2ap0_01_01-000001.pdb fold_2ap0_all_thrs2.80A_clust01.trafl 1 AA
> SimRNA_trafl2pdbs fold_2ap0_01_01-000001.pdb fold_2ap0_all_thrs2.80A_clust02.trafl 1 AA
> SimRNA_trafl2pdbs fold_2ap0_01_01-000001.pdb fold_2ap0_all_thrs2.80A_clust02.trafl 1 AA
```

In order to get E vs. RMSD plot (here native is known, so it may work as an root), 2ap0 native structure has to be converted into one frame trafl:

```
> SimRNA -c config.dat -n 0 -p 2ap0_native.pdb -o 2ap0_native >& 2ap0_native.log
```

whereupon, file `2ap0_native.trafl` will appear. Then the file should be set as a first frame of concatenated trafl file:

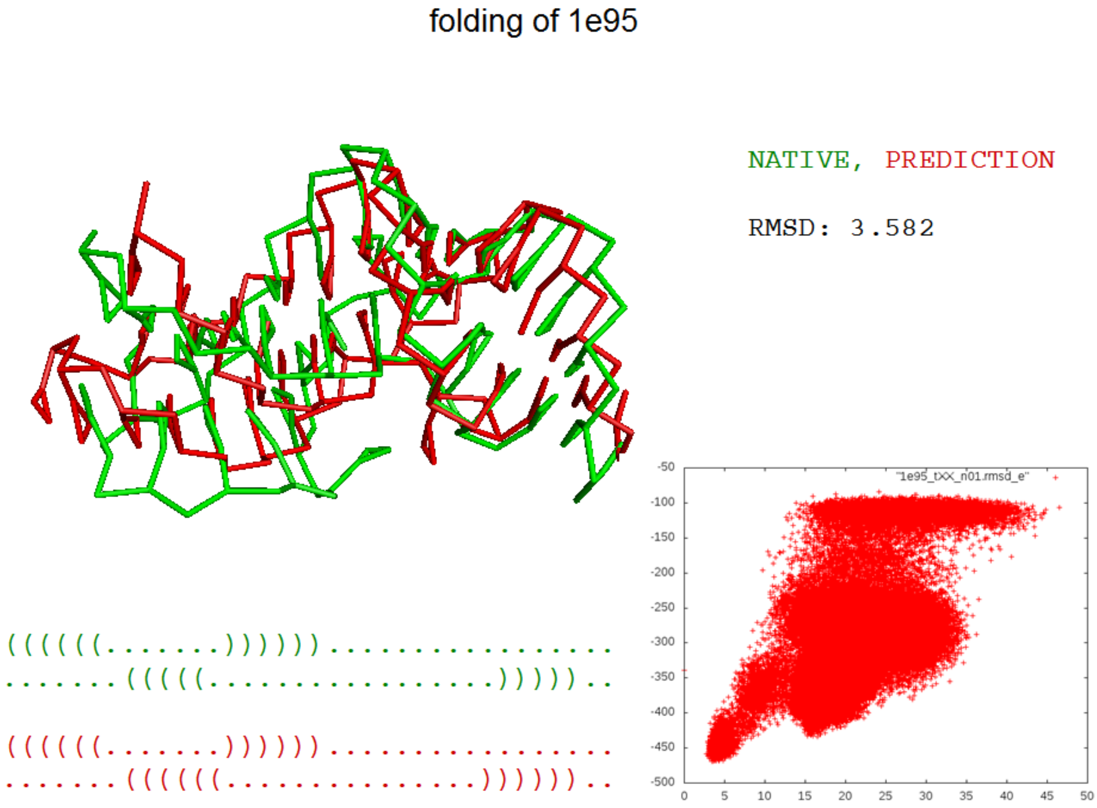
```
> cat 2ap0_native.trafl fold_2ap0_all.trafl > fold_2ap0_all_wNative.trafl
```

The resulting file, `fold_2ap0_all_wNative.trafl`, will contain the native conformation at the beginning (first index).

Then two column rmsd_e file can be generated by using:

```
> calc_rmsd_to_1st_frame fold_2ap0_all_wNative.trafl fold_2ap0_all_wNative.rmsd_e
```

The resulting file, `fold_2ap0_all_wNative.rmsd_e,` _can be plotted using standard tools like gnuplot. It will generate a plot similar to the one shown in figure 2 (see below).



```
((((((.......)))))).................
.......(((((.................)))))..

((((((.......)))))).................
.......((((((...............))))))..
```

Figure 2: Folding of 1e95. The structures are shown in reduced representation, where green is the native structure and red is the predicted structure. The secondary structure is generated from the SimRNA program, and the RSMD vs Energy plot is in the lower right corner.