

Running NASNOX in a batch mode (updated April, 2015)

We describe here procedures for batch-mode operation of NASNOX using a script written in Matlab (The MathWorks, Inc.), which will automatically compute one set of user specified distance on multiple models. For details, see (1).

Step 1. Set-up files

The script and relevant files are compressed as the “NASOX_batch2015.zip” file, which is available for download from <http://pzqin.usc.edu/pzqhome/software>. Unzip and place the files in a folder. The folder should contain:

- (a) Matlab script, “BATCH_NASNOX.m”. The script requires Matlab version 2010b or later.
- (b) NASNOX program. An executable file compiled for the Windows operating system, and has been successfully tested with Windows XP, 7 and 8.
- (c) Nitroxide parameter files for NASNOX.
- (d) A set of example models and a readme file.

Step 2. Prepare the input files

Prepare the following input files and place them in the same folder as the Matlab script and NASNOX files:

- (a) Models of the target nucleic acid molecule:

These are a set of PDB files. Note that the NASNOX program requires a specific format, and the readers are strongly encouraged to consult the “sample.pdb” files provided. Particularly, the program identifies a specific nucleotide based on the order in which it is read, therefore, we suggest that the PDB files list the nucleotides in a consecutive fashion, with the first nucleotide set as “1”. In addition, a “TER” line is required at the end of the PDB file for it to be recognized by the NASNOX program. To check whether the input PDB files are formatted properly, one can use the web-based NASNOX_W (accessible from <http://pzqin.usc.edu/pzqhome/NASNOX/>) to execute the program on sample(s) of the input model pool.

- (b) “model_list.csv”:

This is a csv-format file that lists the names of the models described above (Figure 1). Note that the model names in this file should not contain the “.pdb” suffix.

- (c) “sites.csv”:

This is a csv-format file listing positions of the two nucleotides at which the inter-R5 distance will be computed (Figure 1). In the current version, only one pair of positions should be listed in this file. In addition, the program will model R5 onto both the R_p and S_p diastereomers at each selected nucleotide.

- (d) “parameter.csv”:

This is a csv-format file listing the search parameters for the torsion angles (Figure 1).

Step 3. Batch execution

Run the “BATCH_NASNOX.m” program in Matlab. This will execute NASNOX based on information specified above.

Step 4. Retreat output

For each entry in “model_list.csv”, corresponding “modelname_site1-site2_data.add” and “modelname_site1-site2_datalig.pdb” files are produced. These follow the same output format as those provided from the web-based NASNOX_W program (2), but with identifiers added for the respective model names and sites. In particular, for each model, the average inter-R5 distance is listed at the end of the “.add” file (see Figure 1), which can be extracted and used for model characterization as described below. For more details, one should consult reference (2) and the tutorial file provided for NASNOX_W (accessible from <http://pzqin.usc.edu/pzqhome/NASNOX/>).

Figure 1: The NASNOX program for computing expected inter-R5 distances on given nucleic acids structures. (A) Examples of input parameters. The previously reported web-interface of NASNOX_W (2) is shown to illustrate the organization of NASNOX input in the parameter files used to executed NASNOX in the batch mode. (B) An example of structure output from the “sample1_4-14_datalig.pdb file”, in which a pair of R5’s were modeled onto nucleotides 4 and 14 of the sample1.pdb input structure. The DNA is shown in red, the allowed R5 rotamers at each site are shown in blue. (C) An example of text output from the corresponding “sample1_4-14_data.add”.

(A) Input

1. Upload pdb file: sample1.pdb → “model_list.csv”

2. Nitroxide 1:
Nucleotide Position: O1P: O2P:
t1 t2 t3 → “sites.csv”

Search steps (1-12):
Fine search (0 or 1): → “parameters.csv”
Starting values:

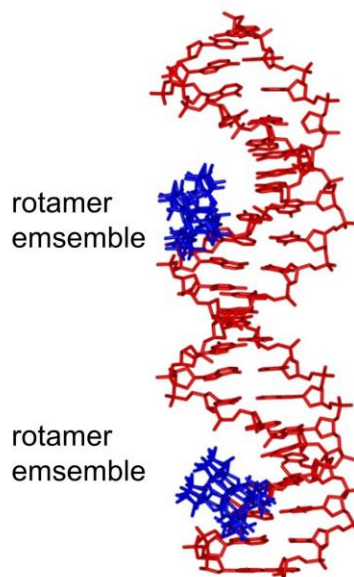
Nitroxide 2:
Nucleotide Position: O1P: O2P:
t1 t2 t3 → “sites.csv”

Search steps (1-12):
Fine search (0 or 1): → “parameters.csv”
Starting values:

3. Additional Conformer Search Criteria:
Include Hydrophobic Contacts:

4.

(B) sample1_4-14_datalig.pdb



(C) sample1_4-14_data.add

```
nitroxide - nitroxide distances (A)
nox (base) - nox (base)  N-N  O-O  mNO-mNO
1 1 ( 4) - 3 1 ( 14)  34.3  34.7  34.5
1 1 ( 4) - 3 2 ( 14)  33.6  33.1  33.3
1 1 ( 4) - 3 3 ( 14)  34.7  35.4  35.1
      ⋮
average distance = 34.2  34.5  34.4
standard deviation = 3.5  4.1  3.8  ( 256)
```

Individual inter-R5 distances

Average distance and standard deviation

References:

1. Tangprasertchai, N.S., Zhang, X., Ding, Y., Tham, K., Rohs, R., Haworth, I.S. and Qin, P.Z. (2015) An Integrated Spin-Labeling/Computational-Modeling Approach for Mapping Global Structures of Nucleic Acids. *Method Enzymol.*
2. Qin, P.Z., Haworth, I.S., Cai, Q., Kusnetzow, A.K., Grant, G.P.G., Price, E.A., Sowa, G.Z., Popova, A., Herreros, B. and He, H. (2007) Measuring nanometer distances in nucleic acids using a sequence-independent nitroxide probe. *Nat. Protocols*, **2**, 2354-2365.