NASP: a parallel program for identifying evolutionarily conserved nucleic acid secondary structures from nucleotide sequence alignments

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ABSTRACT
Summary: Many natural nucleic acid sequences have evolutionarily conserved secondary structures with diverse biological functions. A reliable computational tool for identifying such structures would be very useful in guiding experimental analyses of their biological functions. NASP (Nucleic Acid Structure Predictor) is a program that takes into account thermodynamic stability, Boltzmann base pair probabilities, alignment uncertainty, covarying sites and evolutionary conservation to identify biologically relevant secondary structures within multiple sequence alignments. Unique to NASP is the consideration of all this information together with a recursive permutation-based approach to progressively identify and list the most probable secondary structures that are likely to have the greatest biological relevance. By focusing on identifying only evolutionarily conserved structures, NASP forgoes the prediction of complete nucleotide folds but outperforms various other secondary structure prediction methods in its ability to selectively identify actual base pairings.

Availability: Downloadable and web-based versions of NASP are freely available at http://web.cbiio.uct.ac.za/~yves/nasp_portal.php

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Supplementary information: Supplementary data are available at Bioinformatics online.

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Besides a capacity to store information within the sequences of their component nucleotides, single-stranded nucleic acids may also store information within their secondary structures. Under physiological conditions many single-stranded RNA or DNA molecules longer than approximately 20 nucleotides form metastable secondary structures, which can have important roles in genome replication and gene expression. Although a number of computational methods exist for predicting nucleic acid secondary structures from either single sequences or alignments (Bernhart et al., 2008; Hamada et al., 2009; Knudsen and Hein, 2003; Markham and Zuker, 2008), even the best of these incorrectly infer a high proportion of base pairings. Also, only a few methods provide any measures of statistical support either for their folding predictions, or for the overall presence of secondary structure (Babak et al., 2007; Simmonds et al., 2004). From the perspective of experimental biologists seeking to test the functional relevance of secondary structures, it would be very useful to have a computational tool that, with the lowest possible false positive rate, will identify sites that pair within evolutionarily conserved secondary structures.

NASP is an attempt to improve the selectivity with which individual secondary structures can be identified. It uses base pairing probabilities provided by the UNAfold nucleic acid folding program hybrid-ss (Markham and Zuker, 2008) that applies a combined partition function calculation, stochastic sampling and dynamic programming approach to compute base pairing probabilities and minimum free energy (MFE) estimates from single-stranded nucleotide sequences. The rationale behind NASP is simple: we assume that randomly shuffling nucleotides within sequences that have evolved to form stable secondary structures should influence their overall base pairing potential such that the shuffled sequences should yield higher MFE estimates than the real sequences from which they were produced. By comparing MFE estimates made with real sequences to those made with randomized versions of these sequences, NASP tests whether there is evidence that the real sequences have greater structure forming capability than can be accounted for by chance.

For each sequence, k, in an input alignment, hybrid-ss estimates the overall Gibbs free energy of an optimally folded nucleotide sequence and yields a list of Boltzmann probabilities Pk(i, j) of individual potential base pairings. NASP then computes a consensus base pairing matrix, M, whose entries satisfy

\[ M_{ij} = \exp\left(\begin{array}{c} \delta_{ij} \sum_k \frac{w_k}{w_j} \log\left(P_k(i,j)/P_k\right) \end{array}\right) \]

where: N is the number of sequences; Pk(i, j)’s are chosen to be above a user specified threshold probability Pr; wk is the mean pair-wise Hamming distance of sequence k from all others in the alignment, such that wk weights the contribution to M of more divergent sequences more heavily than those of less divergent sequences; δij is 0 if the gap frequency at either position i or j is ≥0.75 and 1 otherwise; Cij is the mutual information of columns i and j and accounts for compensatory mutations. It is given by

\[ C_{ij} = \frac{1}{\log_2(256)} \sum_{k} f_k(a,b) \log_2\left(\frac{f_k(a,b)}{f_k(a)f_k(b)}\right) \]

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Table 1. NASP compared with other RNA folding programs using sequences with known folds (best scores in bold)

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The number of sequences in the alignment.

The mean pair-wise sequence identity. M stands for medium similarity (MPI between 60% and 90%) and H for high similarity (MPI between 80% and 99%).

The number of falsely predicted base pairs.

The number of correctly predicted base pairs.

The number of correctly matched structures.

The number of sequences in the alignment.

The number of non-zero entries in M that have the highest sum. At each step:

(1) The coordinates of nucleotides within the bounds of what appear to be the largest and most evolutionarily conserved structural motifs within M are added to a list of potentially paired sites (Supplementary Figure S1).

(2) All alignment columns that are not included in this list are randomly shuffled 100 or more times with the MFEs of each sequence in each shuffled alignment being compared with those of sequences in the original alignment.

(3) The existence of additional unaccounted for structural motifs in the sequences is inferred when the MFE estimates of all sequences in the unshuffled alignment are smaller than those of at least 95% of the corresponding sequences in the shuffled alignments. The probability that there remain no unaccounted for paired nucleotides within the alignment fraction excluded from the potentially paired site list is estimated as the fraction of shuffled sequences with MFE estimates lower than those of their unshuffled counterparts.

(4) When the MFEs of the unshuffled sequences are less than those of 95% of their shuffled counterparts, the recursion continues from (1) with sites in the next most evolutionarily conserved structure being added to the paired site list.

The time complexity of NASP, which should be substantially reduced given that MFEs are computed in parallel, is O(NP^2) and the space complexity is O(L^2) where L is the length, N the number of sequences in the alignment and P the number of permutation tests. Currently our web version of NASP accepts datasets of, for example 30×3 KB sequences or 10×10 KB sequences and analyses these within 144 h (results from comparison of computational times are shown in Supplementary Table S1).

Given a sequence alignment containing evidence of evolutionarily conserved secondary structures as input, NASP outputs (i) the coordinates of potentially conserved stems and P-values indicating statistical support for additional unaccounted for secondary structures remaining in the sequences following each recursion, (ii) the consensus structure in both the Vienna bracket-dot and a concatenation file formats and (iii) the consensus base-pairing matrix, M, in both text and graphical formats.

Tests using known reference RNA structures (Table 1 for examples and Supplementary Table S1 for the complete set) indicate that the overall selectivity (the proportion of inferred base pairs that are actually in the reference structures) of NASP is considerably better than that of RNAalifold (Bernhart et al., 2008), Pfold (Knudsen and Hein, 2003), CentroidAliFold (Hamada et al., 2009) and EvoFold (Pedersen et al., 2006). The cost of NASP's low false positive rate is, however, a decreased true positive rate such that its overall accuracy (measured here using the Matthews Correlation Coefficient, MCC of Gardner and Giegerich, 2004) is slightly lower.
than that of RNAalifold (which was overall the most accurate of the programs we tested; Supplementary Table S1). Nevertheless, we must stress that the primary focus of NASP is the identification of base pairings with a false positive rate that is as low as possible: a focus that should prove particularly useful in studies aiming to evaluate the function of evolutionarily conserved (and therefore probably functional) nucleic acid secondary structures in that it should substantially reduce the time and expense needed to home in on those structures with the greatest biological relevance.

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Conflict of Interest: none declared.

REFERENCES


