Investigating the RFAM paradox: The pseudoknot explanation
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Motivation
Given the high cost and low-throughput of experimental methods to determine the structure of RNA, computational methods based on free-energy minimization (MFE), such as RNAFold [1] are routinely used for the ab initio prediction of secondary structures of RNA. Such methods are indeed substantially successful at predicting the secondary structure, and were shown to recover about 73% of base-pairs for RNAs of length less than 700 bps [2] on established benchmarks. The RFAM database [3] groups most available RNA sequences into families sharing functional characteristics. Manually-curated multiple sequence alignments allow for the prediction of conserved structural elements through a mixture of semi-automated comparative prediction methods and experimental evidences. One of the denoted features of RFAM is the availability of a consensus secondary structure for each family, which is widely regarded as reliable, if slightly conservative. Therefore, it is expected that separate MFE predictions for family members largely overlap with their corresponding RFAM consensus.

The RFAM paradox
To confirm this intuition, we predicted MFE secondary structures for all 26,704 sequences of the 1,446 RFAM seed alignments. We systematically compared these predictions to their associated RFAM consensus secondary structure. Averaging over each family, we surprisingly observed that a large majority of families exhibited very little overlapping base-pairs between predicted structures and family consensus (Figure 1). For instance, RNAfold had absolute zero average sensitivity for around 12 % percent of the RFAM families.

The PK-oblivious algorithm explanation
As a first step towards a more comprehensive analysis of possible origins for such a discrepancy, we hypothesized the presence of complex structural features, pseudoknots (PK), as responsible for at least some of the apparent shortcomings of MFE approaches. Since PKs are typically absent from the search space of structure prediction methods, due to computational complexity reasons, the energy minimization scheme may be diverted toward structures having low-energy, yet sharing no resemblance with the RFAM consensus. Unfortunately, existing ab initio prediction
methods, including PKs, are either based on heuristics, or extremely time-consuming. Given the large scale of our experiment, this time-efficiency was especially critical. Therefore we designed a new method based on a recent contribution by one of the authors (pKiss [4]), combining an exact exploration of a restricted search space with a low time-complexity.

Pseudoknot detection
Our approach uses the pKiss software to compute the MFE conformation obtained by either excluding PKs altogether, or enforcing the presence of simple canonical or kissing hairpins PKs, the two predominant naturally-occurring PKs. For both types of PKs, the MFE differences (ΔPK and ΔKH respectively) between conformation spaces enforcing and precluding presence of pseudoknots, renormalized by the sequence length were computed (see Figure 2). If this difference in MFE is significantly large (or crosses some threshold), then the presence of PKs is suspected for the input RNA sequence. A similar approach was used for a prediction of ribosomal frameshift sites (KnotInFrame [5]).

Towards a reannotation of PK families
Turning to our initial investigation of the responsibility of PKs for RNAfold's disagreement with RFAM's consensus, we investigated the 71 families having pseudoknot annotations in RFAM; the mean structural sensitivity of the 71 families is 0.57. We found 509 RFAM families having positive ΔKH or ΔPK values in contrast to just 71 families in RFAM with pseudoknot annotations. We also manually investigated a short list of 15 of the 509 RFAM families. We found evidences of pseudoknots in the literature for at least 11 of these families. A plausible origin for the absence of such annotations may be found in the inherent conservatism of consensus approaches.

Many families remain associated with low RNAFold/RFAM overlap and low ΔKH, ΔPK values, pointing toward other explanations, such as the presence of non-canonical base-pairs (also excluded from MFE approaches) or multi-stable structure (for which the single-consensus view of RFAM may be overly restrictive). Investigating these complementary explanations should help gain insight on the shortcomings of ab initio folding methods, and help circumvent their current limitations.

References