# The ViennaRNA Package

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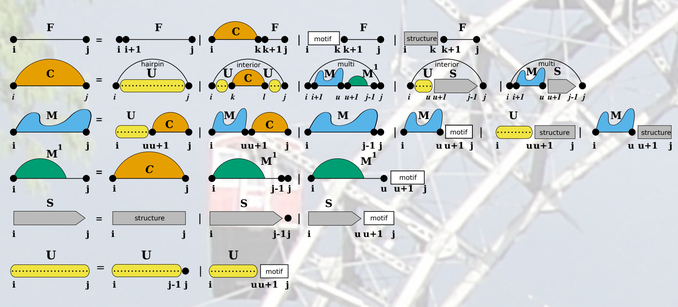
## High Quality Results

By using the **latest** available Nearest Neighbor **energy parameters** (Turner 2004) our algorithms provide best quality predictions [2]. User-defined free energy parameters can be loaded at runtime to enable adaptations of the prediction to novel measurements. The `ViennaRNA Package` already ships a set of existing parameters for RNA and DNA, derived from both, UV-melting experiments and training on a large set of reference structures.

## RNA Folding Grammar

We use an extended and **unified** recursive structure **decomposition scheme**, the RNA folding grammar. All algorithms adhere to (parts of) it to predict global or local structures for single sequences and sequence alignments [8].



## The Story of Success

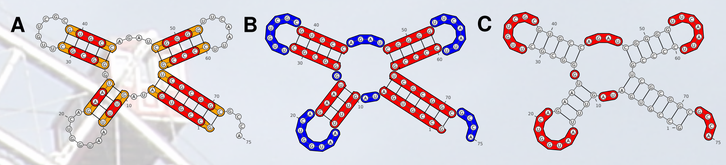
With a total of more than **6,000 citations**, the `ViennaRNA Package` is one of the most widely used tool packages for RNA secondary structure prediction in the world [1, 2, 3, 4]. It combines very good prediction accuracy paired with high performance to make everything in an RNA-bioinformaticians life easier. We constantly increase the **usability** of the command line programs and extend the underlying C-library to yield the best experience for both, **beginners** and **experts**.

## Fast folding and Comparison

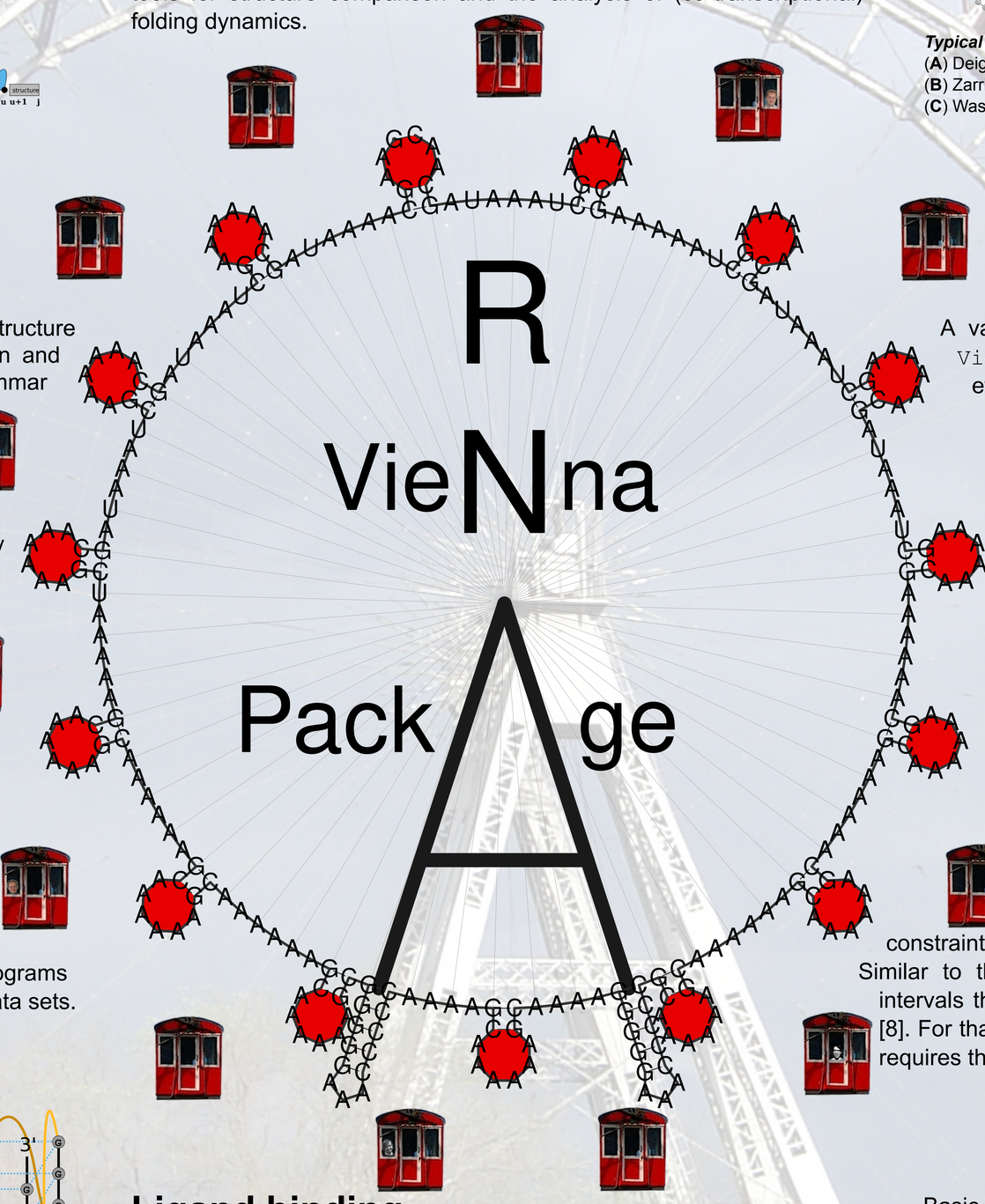
The `ViennaRNA Package` is a toolbox comprised of about 30 different instruments. It not only enables one to predict secondary structures of single sequences, intermolecular RNA-RNA interactions, and consensus structures for multiple sequence alignments, but also offers tools for structure comparison and the analysis of (co-transcriptional) folding dynamics.

## Experimental Structure Probing

Chemical and enzymatic probing of RNA structures reveals at nucleotide resolution if a nucleotide is more likely to be paired or unpaired. Especially the coupling of probing methods with high-throughput Next Generation Sequencing generates massive amounts of data suitable to guide structure prediction. For instance, most of our tools accept normalized **SHAPE reactivity data** as input which in turn is simply converted into pseudo-energy terms and added to particular conformations [6, 7].




***Typical configurations SHAPE reactivity data is applied to.***
(**A**) Deigan et al. (2009) method, stacked nucleotides
(**B**) Zarringhalam et al. (2012) method, paired and unpaired nucleotides
(**C**) Washietl et al. (2012) method, unpaired nucleotides




## Structure Constraints

We offer a generic yet systematic way to augment structure prediction. A fully **transparent access** to the derivation and energy evaluation of the implemented RNA folding grammar renders our approach most flexible [8]. Here, we distinguish two kinds of conceptually different constraints:
(i) **Hard constraints** that limit the candidate space by pruning particular derivation trees, and
(ii) **Soft constraints** acting on the evaluation level by adding "*bonus energies*".
Particular application scenarios such as guiding predictions by experimental SHAPE probing data or RNA-ligand binding are already available through high-level convenience functions.

## Complementary Software

A variety of complementary applications build upon the `ViennaRNA Package` to make use of the fast and efficient implementations of secondary structure prediction algorithms. Among them are tools for **structural sequence alignment**, such as `LocARNA`, several **RNA sequence design** approaches, e.g. `RNAblueprint`, and tools to simulate the **dynamics of the folding process** such as `Barriers/Treekin` and `DrTransformer`. Furthermore, the `ViennaRNA Package` is an essential element in many analysis pipelines for **miRNA/sRNA target detection** and numerous machine learning approaches.

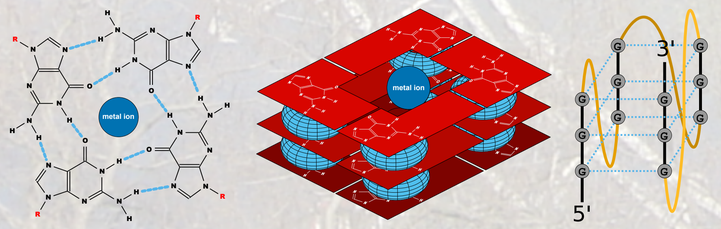
## High Speed Predictions

The `ViennaRNA Package` always was among the fastest in RNA secondary structure prediction. The latest version speeds up computations by automatically detecting **SIMD** features of the host CPU. For batch jobs (*multiple input sequences*) on **multi-core CPUs** many programs also allow for parallel processing of the individual input data sets.

## Unstructured Domains

The constraints framework alone can't treat arbitrary sequence intervals as a binding site, e.g. for **Single Strand Binding (SSB) proteins**. Such interval constraints are, however, available as additional production rules. Similar to the incorporation of G-Quadruplexes, base pair free intervals that interact with external factors are modeled explicitly [8]. For that purpose, the default implementation in `RNAfold` only requires the sequence motif and a binding free energy.

## RNA G-Quadruplexes

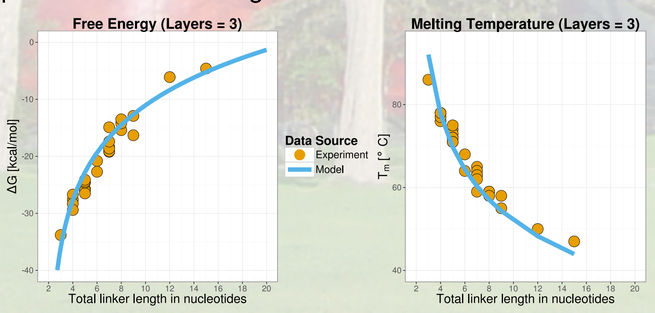



G-rich nucleic acid sequences are known to form highly stable stacks of G-quartes, also known as G-Quadruplexes. For sequence patterns that follow the canonical form


we explicitly include their potential to form a quadruplex through an extension of the RNA folding grammar [5]. Energy contributions are evaluated using a **simplified energy model**

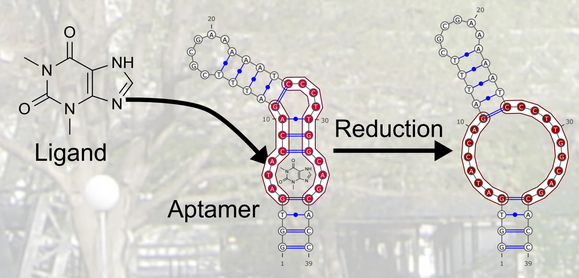
$$E(L,l,T) = a(T)(L-1) + b(T)\ln(l-2), \text{with}$$
$$a(T) = H_a + TS_a \quad \text{and} \quad b(T) = H_b + TS_b$$

that only depends on the number of stacked layers and the total length of linker sequences. Parameters have been fitted to experimental UV- melting data.




## Ligand binding

A convenience function that utilizes the soft constraints feature enables one to model **binding** of small ligands **to specific aptamer structures**. Thus, aptamers that resemble hairpin- or internal loops only require a few ingredients, (i) the binding free energy and a (ii) sequence and (iii) structure motif. Once these parameters have been suppied, the `ViennaRNA Package` will consider the bound (*Holo*) and unbound (*Apo*) conformations during MFE and partition function computations [8].




## ViennaRNA Web Services

Basic analysis and structure prediction with the most commonly used tools and most popular parameter options can be easily done **without even installing any software**. Simply submit your analysis at the ViennaRNA Web Services
**`http://rna.tbi.univie.ac.at`**
next to more than 1,500 requests by other users per day.

## Command Line Tools

The `ViennaRNA Package` includes a steadily increasing number of useful applications and scripts. While global structures can be predicted for sequences up to about 30kb, local structure prediction methods allow one to **screen entire genomes**. Most approaches are available for both, single sequences and multiple sequence alignments and the corresponding programs support common input file formats such as `FASTA`, `ClustalW`, `Stockholm 1.0`, and `MAF`.

## Programming Interface

The algorithms implemented in the `ViennaRNA Package` are available as a ***C-library*** that can be easily used by your own programs. It exclusively grants access to functionalities that might not be directly available through the command line programs. Finally, the `Perl` and `Python` wrappers allow for ***rapid prototyping*** and direct interfacing to the efficient implementations from ***analysis pipelines.***

## Acknowledgements

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