BROWSING RNA STRUCTURES BY INTERACTIVE SONIFICATION

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ABSTRACT

This paper presents a new interactive sonification technique to browse ribonucleic acid secondary structures using a combined auditory and visual interface. Despite the existence of several optimization criteria for searching an optimal structure within the numerous possible structures of an RNA sequence, it is still necessary to manually inspect a huge number of the resulting structures in detail. We describe briefly the background of RNA structure representation and typical search scenarios. Then we discuss the audio-visual browser in detail, with a special focus on the sound design, data-to-sound mapping and interactive aspects. The sonifications we propose turn RNA structures into auditory timbre gestalts according to the shape classes they belong to. Various research-relevant phenomena become clearly audible such as transitions among shape classes and different free energies of selected folds. Both can be simultaneously assessed in an interface that allows for an integrated audio-visual perception.

1. A BRIEF REVIEW ON SONIFICATION OF NUCLEOTIDE SEQUENCES

RNA's big brother from the nucleotide family, DNA, has always been a popular target for sonification. Since its discovery, DNA has attracted lots of attention. For the general public, the iconic image of its structure, the double helix, has become widely known. But also its sequence, which is like RNA made out of 4 nucleotides, has inspired analogies with music. An early account of these cross disciplinary thoughts and works can be found in [1], [2], [3] where common issues in terms of sequence complexity are discussed.

First publications of sonifications of DNA in scientific journals followed in the 90s [4]. Meanwhile, sonification has evolved into a research field with a diversified taxonomy demonstrating its value as a scientific display [5]. A good review covering all chemistry related sonification approaches was recently published [6], many of them involving DNA.

Whilst sonifications of double stranded DNA helices address mostly aspects of the sequence, the situation for RNA is different. The single-strandedness of RNA allows for intra molecular bindings resulting in a multitude of structures. A major problem in RNA research is to handle the complexity of the structures that originate out of one sequence. Therefore the sonifications in this paper are based on the metadata representing the RNA shape, as will be explained in the sequel.

2. BACKGROUND: RNA STRUCTURE REPRESENTATION

RNA is a biologically important type of molecule that performs various functions in the living cell. It consists of a long chain of the nucleotides adenine (A), guanine (G), cytosine (C) and uracil (U). The chain molecule folds back onto itself, forming basepairs between nucleotides via hydrogen bonds. The combinatorial possibilities of paired and unpaired nucleotides in a sequence lead to a multitude of secondary structures. As a concrete example the sequence

```
GGGCCCAUAGCUCAGUGGUAGAGUGCCUCCUUUGCAAGGAGG
AUGCCCUGGGUUCGAAUCCCAGUGGGUCCA
```

leads to 9,119,914,420 possible secondary structures, four of which we have depicted in Figure 1.
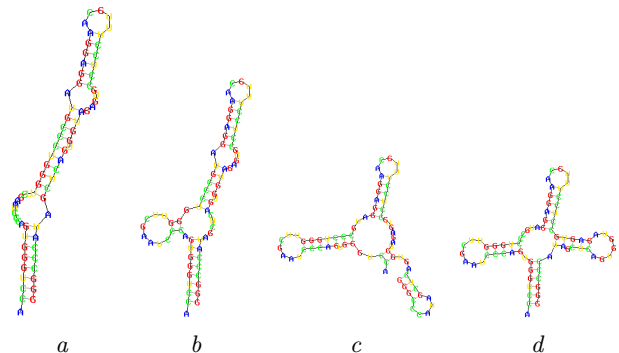

Figure 1: *Four possible secondary structures of the RNA sequence given above. The structures from left to right show possible folds from a single stem like (a) to multiple stems like a cloverleaf in (d).*

This human-readable representation of secondary structures can be translated into the machine readable dot-bracket string notation, which describes unpaired nucleotides as a dot . and pairing nucleotides as bracket pairs (). The secondary structure from Figure 1 *a* corresponds to the dot bracket string

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

Our capacity to quickly classify visually represented structures as in Figure 1 inspires the concept of abstract shapes on the level of the dot-bracket string notation. Depending on the building blocks of the molecule that are taken into account, the method currently provides five different levels of shape abstraction. In each abstraction level

information from the previous level is condensed. For the given structure the levels of shape abstraction are:

```
level 1 [_[_[_[]_]]_]_
level 2 [_[_[_[]_]]_]
level 3 [[[[]]]]
level 4 [[[]]]
level 5 []
```

We see that in level 5 structure *a* is, essentially a single stem, despite some unpaired nucleotides. With this symbolic representation at hand we may now automatically sort structures according to their shapes at different levels of abstraction.

Another important criterion for assessing the plausibility of a given structure and for comparing it with others is the free energy (FE), which reflects the thermodynamical stability of the folded molecule [7]. A low FE indicates higher stability. As a rule of thumb, the FE drops when more concatenating base pairs can be formed.

3. THE REPRESENTATION OF SHAPES IN THE INTERFACE

Although there are several optimization criteria, such as basepair maximisation [8] and free energy, and there is also the powerful method of shape abstraction [9], it is still necessary to inspect a huge part of the exponential search space in detail. The final interpretation by the biochemist is rather based on images of secondary structures, as shown in Figure 1.

The challenge for the audio-visual browsing interface consists therefore in combining all these representations. We developed a visual representation of the shapes in all five abstraction levels. Our visualization scheme is shown in Figure 2. The abstraction level drops from left (level 5) to right (level 1). The shapestring notation corresponds to the following color map: Opening brackets are encoded in red and closing brackets in yellow. Unpaired sections, which are only represented in abstraction level 1 and 2, are encoded in blue.

In Figure 2, we can also see 3 different sorting possibilities for the secondary structures. On the top, all shapes are sorted according to the size of the population of one shape class in each abstraction level. This leads to the uniform columns on the left for abstraction level 5 and 4. Column 3 shows the shape classes of this abstraction level sorted by their population size. In the central image, all shapes are sorted first according to the highest abstraction level 5, and second in ascending order of their FEs. This gives the ordered column for level 5. At the bottom all shapes are sorted in ascending order according to their FEs. Note that structures of a similar FE do not necessarily have same or similar shapes.

4. REPRESENTING SHAPES ACOUSTICALLY, MAPPING AND SOUND DESIGN

4.1. Design Requirements

In order to facilitate browsing and searching the space of secondary structures, their shapes in leves 5, 4 and 3 are also represented acoustically. For the length of the shapestring
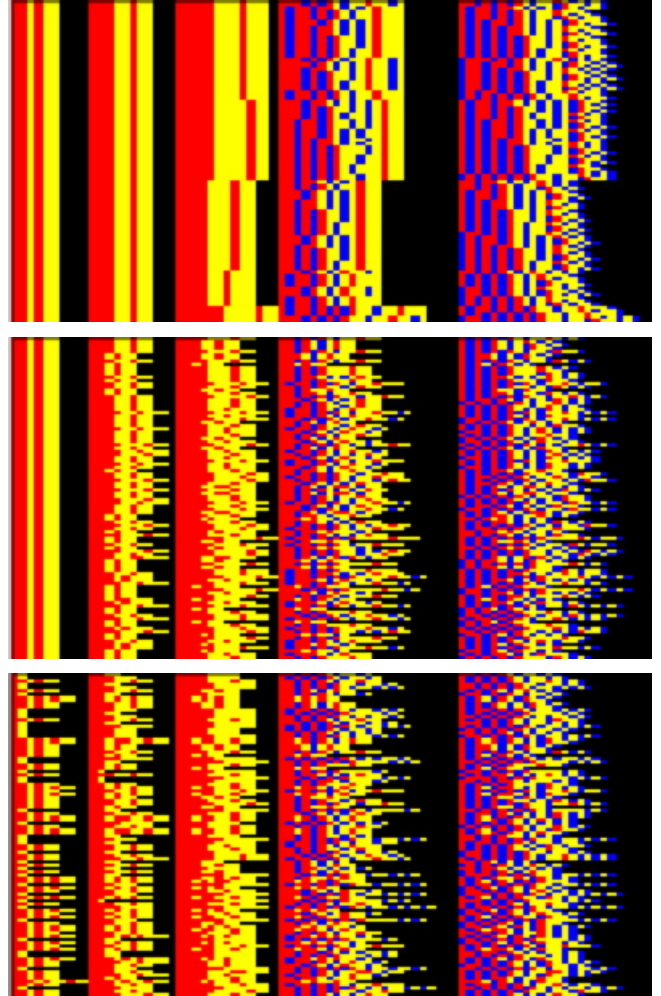

Figure 2: *A visual representation based on the shapestring notation of shapes. Depicted are approximately the first 50 structures according to a certain sorting criterion (see Section 3). Vertically you find the index of structures, the shapestring notation is encoded horizontally in colors.*

notation of the shape abstractions ($l_i, 5 > i > 3$), the following relation holds:

$$l_5 \leq l_4 \leq l_3 \qquad (1)$$

Thus, in most cases a higher abstraction level usually results in a shorter string length. If the sequence of base pairs is not interrupted by unpaired nucleotides, the highest possible abstraction can already be found at level 4 or sometimes even lower.

The shape sonification aims to fulfill the following requirements:

1. The shape sonification should be composed out of 3 individual sonic entities, representing the shape abstractions 5, 4 and 3. The parameter mapping for all abstraction levels should be the same. Their volume should be individually controllable.

2. The sound of one sonic entity should reflect the length of the string representation of a given shape abstraction, i.e the difference between [[][]] and [[]]. This helps to distinguish between different shape

classes. It further emphasizes the difference of the shape string across the various abstraction levels.

3. The information about opening and closing brackets should be acoustically distinguishable in the case were different shapes have a string representation of the same length, i. e. the difference between [[][]] and [[[]]] .

4. The sound should reflect the FE, so that for structures of the same shape further means for differentiation are available.

5. The sound should be pleasant and resemble a natural listening experience, so that user fatigue is minimized.

4.2. Parameter Mapping

The requirements mentioned above are met by mapping the shape information to timbre gestalts by mixing sounds with a certain base frequency and a series of overtones as additive synthesis with a small amount of subtractive synthesis.

The base frequency of the sound for abstraction level i is calculated according to:

$$F(l_i) = f_{min} \cdot 2^{(l_i/2)} \tag{2}$$

where $F(l_i)$ is the base frequency, f_{min} is a lower frequency limit and l corresponds to the length of the abstraction level. The limit f_{min} is set to 110 Hz [1]. By choosing this mapping requirement 2 was met.

In order to meet requirement 3, the gain of the overtones of the base frequency, $G(i)$ with $i \in \mathbb{N}^+$, is mapped from the shapestring notation s and multiplied with a decay function f_{decay}:

$$G(j) = \begin{cases} 1.00 \ f_{decay}(j) & \text{if s(j) = ''[''} \\ 0.25 \ f_{decay}(j) & \text{if s(j) = '']''} \end{cases} \tag{3}$$

with

$$f_{decay}(j) = \frac{1}{j} \tag{4}$$

Where $1 \leq j < |s|$. The decay function makes sure that the result resembles a natural sounding object. The 3 sounds for the shape abstraction levels 3 to 5 were spread out over the stereo panorama.

4.3. Implementation Details

For the implementation of the synthesis scheme, the sound synthesis language `SuperCollider` was used. In order to meet requirement 5, we used two unit generators: `DynKlang` and `DynKlank`. This combination leads to the expected natural sound. Due to its small noise component in the subtractive synthesis, a sterile timbre is avoided, which would otherwise be quickly backgrounded by the listener.

We also implemented basic psychoacoustic amplitude compensation [2]. An excerpt from the synthesis code can be found in Figure 3.

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[1] Practically, $(f_{min} + l_i).midicps$ can be used in `SuperCollider` to compute the frequencies.

[2] For further details we refer the reader to the documentation of the `AmpComp` unit generator in `SuperCollider`

```
SynthDef(\klang,
  {
  | out=0, basefreq = 100, pan = 0.0,
   lg = 0.5, volume = 0.1, mute = 0.0 |

  var klang, klank, harm, amp,
    phase, ring, noise;

  harm  = Control.names([\harm]).kr({|i| i+1}!25);
  amp   = Control.names([\amp]).kr({1/25}!25);
  phase = Control.names([\phase]).kr({1}!25);
  ring  = Control.names([\ring]).kr({4}!25);

  noise = PinkNoise.ar(0.01)  ;

  klang = DynKlang.ar(
    `[harm.lag(lg)*basefreq.lag(lg), amp.lag(lg), phase]
          ) * volume ;

  klank = DynKlank.ar(
    `[harm.lag(lg)*basefreq.lag(lg), amp.lag(lg), ring]
          , noise) * volume ;

  OffsetOut.ar(0,
      Pan2.ar( (klang + klank)
        * AmpComp.kr(basefreq.lag(lg), 30.midicps, 0.44),
        pan.lag(lg), mute.lag(lg))
        );
}).send(s);
```

Figure 3: `SuperCollider` *source code for the* `SynthDef` *using the unit generators* `DynKlang` *and* `DynKlank`.

4.4. Illustrating Example

Let us look at the spectral characteristics of the resulting sound by studying a concrete example of the secondary structure of a selected fold and its shape levels 3, 4 and 5, as depicted in Figure 4. This fold has one long stem that is interrupted by 3 loops, leading to shapes of different length. Therefore the 3 sounds show a raising base frequency. The opening and closing brackets were mapped according to eq. 3. The resulting spectrograms can be seen in Figure 5.

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


level 3 [[[[]]]]
level 4 [[[]]]
level 5 []
```
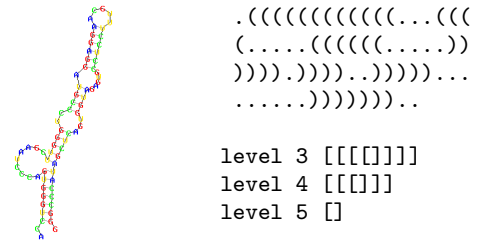
Figure 4: *Secondary structure and dot bracket notation together with the 3 highest shape abstractions of the RNA molecule from above.*

In spectrogram a of Figure 5 we can clearly identify the opening and closing bracket of the shape in level 5 as one high peak on the left and a lower one next to it on the right. The mapping from shapes to sound is also visible for shape 4 and 3 in the spectrograms b and c, respectively. The raising base frequency can also be identified as the increasing frequency bin number, where the first maximum can be found (5, 6, 7 in a, b, c, respectively). Spectrum d shows the combined spectrograms from a, b and c. The exponential decay of the overtones is less noticeable in this plot due to the logarithmic vertical dB scale.
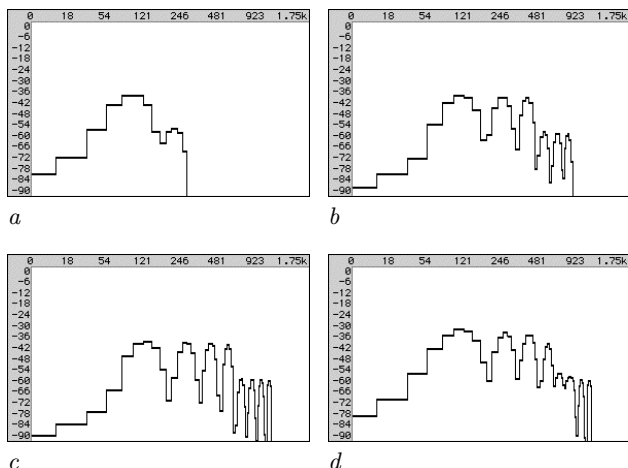
a   *b*

c   *d*

Figure 5: *Four spectrograms depicting the acoustic spectra generated by the mapping of the shape abstractions 5, 4, 3, and their sum, corresponding to a,b,c,d. The unit of the horizontal axis is Hz and of the vertical axis is sound level in dB*

5. THE SONIFICATION-ENHANCED RNA BROWSER

A typical task for an RNA researcher is to find structures with a potential biological function. The result of such a search is often not just one single optimal structure, but rather a class of structures all with the same shape and low FE for this ensemble. The different search criteria often do not coincide, therefore different searches with different search aspects return only partly overlapping collections of optimal structures as a result.

The role of sonification is therefore to support the exploratory data analysis of structures by allowing the user to rapidly compare structures based on their sonic representation. As a consequence, we set the goal for the sonification to deliver an acoustic representation that conveys the information of several abstraction levels as well as the FE for each structure. In addition, the sonification should enable the user to compare structures quickly on the basis of the shape and FE they belong to.

In Figure 6 we see our first prototype of the sonification-enhanced RNA Structure Browser. The application window contains the following elements:

Main control element: On the very left, we find a vertical slider over the visual representation of the shapes as already introduced in Figure 2. This slider allows to select the index of a certain secondary structure from a sorted order of shapes.

Zooming in for better control: Next to it the same visual representation is depicted in a slider at a higher zoom level showing the region around the selected index. This magnification allows for a more precise navigation on the indices.

Structure representation: Besides, there are images of 5 different secondary structures as shown in Figure 1. The structure in the center is the one currently selected by the slider. Next to it are its neighbors according to a certain sorting criterion. To the right of these images we find the shapestring notation of the abstract shapes.

Further control elements: The window also contains a button to toggle through the three sorting schemes introduced in Figure 2. Four horizontal sliders allow to control the gain of the abstraction levels 5 - 3 in the sonification, and to change the exponential mapping of the FE to the overall gain.

Interaction: The sonifications are played when the user browses the structures by using the sliders. They are also played when clicking onto the images of the secondary structures.
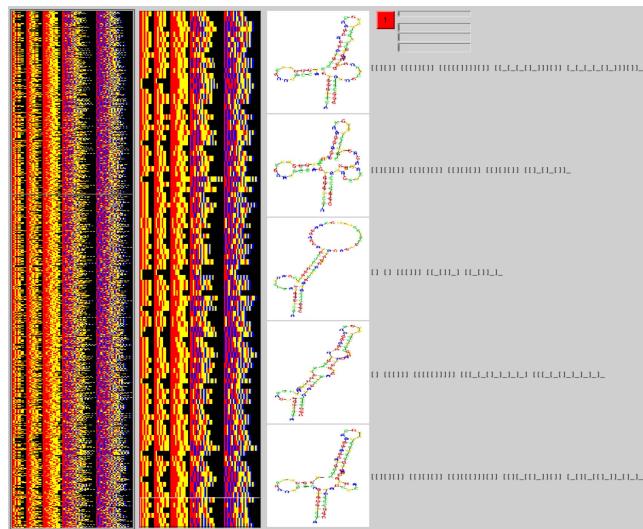

Figure 6: *A visual representation based on the shapestring notation of approximately 1000 selected structures of the RNA sequence. Horizontally, we find the index of structures, the shapestring notation is encoded vertically.*

6. THE ROLE OF INTERACTIVE SONIFICATION

6.1. Pointing and Learning

The combination of visualization, sonification and interaction has the special advantage that the user may point into an abstract representation of the sound stream. Since the sonification is played while browsing the shapes together with the image of the secondary structure representation and the shapestring notation, the meaning of the sound may be learned by interactively playing back the sound by combining two complementary visual pieces of information with one sonic representation. This is shown in example video V1, where the interplay of the browser elements is demonstrated. [3]

6.2. Complementary Information Fused by Sound

Even for the experienced reader of shapestring notations it takes a while to establish the correspondence with the secondary structure representation. This is due to the fact that the shape information, particularly at abstraction level 3 and 4, is not always easy to see in the image. The interactive sonification of the 5 secondary structures on the display

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[3]All video material can be accesses on the internet: `http://www.techfak.uni-bielefeld.de/ags/ami/publications/GJSH2010-BRS`

often reveals surprising differences or similarities. This is examplified in example video V2, where the noticeable difference in sonification originating from different groups of unpaired regions in the structure are pointed out.

6.3. Adjusting the Sonic Information

As mentioned before, the user has the possibility to adjust the gain of the sonification for each of the shape abstraction levels 3, 4 and 5. This interaction from the user adapts the sonification to task specific requirements. If the shapes are for instance sorted according to abstraction level 5, then the corresponding sonification is of less interest and the gain can be set to 0, whereas the sonification of level 4 and 3 get more importance. In example video V3 browsing interaction with different sorting criteria is demonstrated together with gain control for the abstraction level 3,4,5.

7. WHAT ARE TYPICAL TASKS, HOW DOES THIS BROWSER ASSIST?

When exploring the folding space of RNA, first of all an RNA researcher usually wants to perform exploratory data analysis to get an overview over the existing structures exhibiting a certain property. A straightforward possibility is to sort the RNA structures according to their FE, as described earlier. The relevant bioinformatic research question here was pointed out by Charles Lawrence in Benasque in 2003:

> "How much would you trust a structure with a probability of 10^{-5}, even when it is [energetically] optimal?".

The answer to this question gets clearer on inspection of the following three different scenarios:

a The simple case is a broad homogeneous ensemble of shapes with a low FE, that supports the FE prediction. It can be easily seen whether all the structures have the same shapes on level 5. Additionally, the sonification of abstraction level 4 and 3 helps to assess the homogeneity of the ensemble, while browsing over the structures with low energy.

b Broad distributions with more than one shape suggest that the different structures might act as a molecular switch [10]. The difference in shapes manifests itself here at abstraction level 5, which cannot just be easily distinguished, but it can be acoustically identified with some experience.

c A steep FE distribution within one shape class resulting in only few structures of low FE within this class makes their predicted structures less plausible.

An alternative perspective onto data exploration is to sort the structures by shape. The sonification within one shape class is consequently the same, however the mapping of FE to the overall gain is a very useful acoustic information for spotting structures of low FE within a shape class. Additionally, the transition between shape classes on the abstraction levels 4 and 3 are often not obvious on the secondary structure images at first sight, however they are clearly audible.

8. APPLICATION TO TWO RNA SEQUENCES

The RNA browser was additionally tested with two different RNA strings. Both are subject to ongoing research in the bioinformatics reseach group at CeBiTec Bielefeld University.

```
>RNA1
ATCTCATATTTTTGCAAGTGCCGGCAAATCAGGCGGCATGAGG
CGGCTTTTCAAGGCAGAGGAGGGCCAGGGTCGCCGGGG
```

```
>RNA2
CTCTTCCGTCAGTAAGCGGCGCCCCGGCTAGGGGGCGGCTTCG
TCCCGCTCTGAAGGAGAAAAACCGCGGCTCGCAAAGGG
```

The two samples were chosen to investigate if the search scenario as described in section 7 can be effectively supported through the browser interface.

The browsing interaction of the two samples above can also be found as screen captured movies on our website. The movie shows the interface as it is used to investigate the distribution of shape-classes with low FE. While browsing RNA1 you can see that one shape dominates in the region with low FE. With regards to RNA2 there are two different shapes with low energy. The interface also helps to spot structures, that do not belong to highly populated shape classes but still have a low FE.

9. DISCUSSION AND CONCLUSION

In this paper we have presented an audio-visual browser for the exploration of RNA structures. For this interface, new visual and acoustic representations of RNA shapes were developed. The resulting interactive dimension for the exploratory data analysis of RNA structures supports search tasks and brings shape information to the users immediate attention.

The browser is currently implemented in `SuperCollider`, because of the convenient sound synthesis options. For a mid-term perspective we aim at transferring it to a more portable programing language, in order to make it accessible to a wider community working in the bioinformatics field. Beyond shape and FE sonification there are further interesting data descriptors for RNA that are worth integrating in this audio-visual browser.

We also plan an evaluation of the usefulness and interaction quality of this browser. This will however most likely take the form of a qualitative study since the number of available experts who can interpret RNA shape information is limited.

10. REFERENCES

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