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# Determining RNA Flexibility by Graph Theory: Ribosomal Exit Tunnel as a Case Study

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We present an analysis of the flexibility characteristics of the ribosomal exit tunnel using concepts grounded in rigidity theory. For this, a new topological network representation of RNA structures had to be developed that allows analyzing RNA flexibility/rigidity based on constraint counting. Applied to the large ribosomal subunit, constraint counting provides new insights in atomic detail into the stability characteristics of the tunnel, which can be linked with the tunnels role in co-translational processes.

## 1 Introduction

RNA structures are highly flexible biomolecules that can undergo dramatic conformational changes required to fulfill their diverse functional roles. The determination of RNA structures, e.g., by X-ray crystallography provides us with static snapshots along these transitions, whereas the underlying dynamical processes remain largely unclear. To get a more detailed view of the dynamics of biomolecules or to illuminate experimental data, molecular dynamics simulations are very useful and widely applied. Unfortunately, the simulations are still too computationally expensive to investigate large macromolecules like the ribosomal complex on a routine basis. As a much more efficient alternative, concepts from graph theory can be used to determine flexible and rigid regions within a structure.<sup>1</sup> Thereby, a biomolecule is modeled as a topological network, where vertices (joints) represent atoms and edges (struts) represent covalent and non-covalent bond constraints (strong hydrogen bonds, salt bridges, and hydrophobic interactions) as well as angular constraints. Modeling non-covalent constraints appropriately is detrimental to the success of the analysis. Given a network representation, a fast combinatorial algorithm, the *pebble game*,<sup>2</sup> can then be applied to determine the number and spatial distribution of bond-rotational degrees of freedom in the network, which can be related to rigid regions and flexible links in between. Modeling non-covalent constraints appropriately is thereby detrimental to the success of the rigidity analysis. In this context, rigid regions are those with a well-defined equilibrium structure,<sup>3</sup> whereas biologically important diffusive motion is expected to occur at the flexible regions. At a first sight, rigidity analysis says nothing about the direction and magnitude of existing motions. However, the identification of flexible regions on a bond level gives insights into the location of possible motions or how flexibility characteristics change upon complex formation.<sup>4-6</sup>

Until recently, the approach, implemented into the FIRST software package,<sup>1</sup> has been successfully applied to the protein world, whereas a thorough validation on RNA structures has been missing. Yet, the structural stability of proteins (dominated by hydrophobic interactions) and RNA structures (dominated by hydrogen bonds and base stacking interactions) is determined by different non-covalent forces. Here, we thus aim at developing a network representation of RNA structures that allows for reliably determining flexible and rigid regions within these biomolecules. We then apply rigidity analysis to the large ribosomal subunit to gain insight into the ribosomal exit tunnel's role in co-translational processes.

## 2 Results

### 2.1 A Topological Network Representation for Analyzing Flexibility Characteristics of RNA Structures

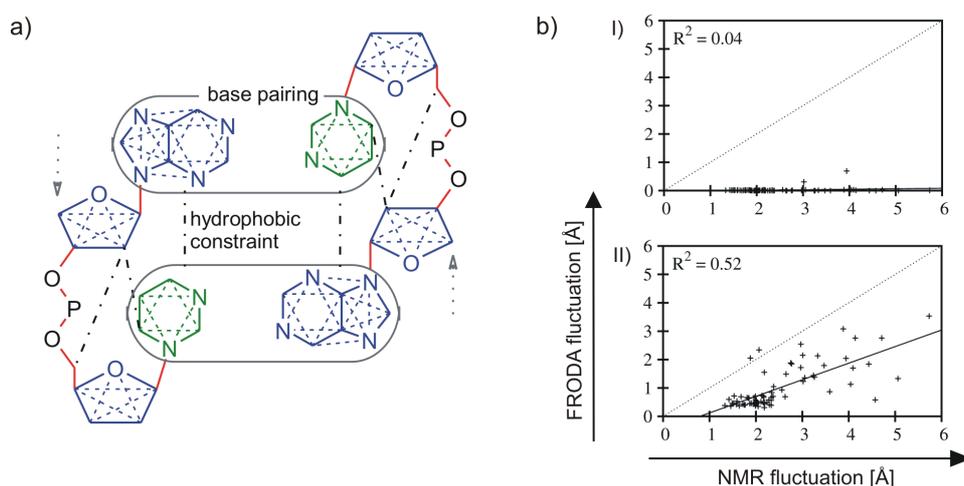


Figure 1. a) Topological network representation of a canonical A-form RNA. Constraints between nearest neighbors are indicated by straight lines, constraints between next nearest neighbors (angle constraints) by dashed lines. For reasons of clarity, angle constraints are only indicated in the sugar and base scaffolds, and hydrogen bonds between bases are omitted. Hydrophobic constraints are indicated by black dashed-dotted lines. Flexible hinges are shown in red, minimally rigid regions in green, and overconstrained regions (which contain redundant constraints) in blue. b) Atomic fluctuations predicted by FRODA simulations<sup>10</sup> vs. conformational variabilities as measured in NMR for RNA structure 1P5O. For the FRODA simulations, a topological network representation according to I) the protein-based parameterization and II) the RNA parameterization was used.

In a topological network representation a constraint is either present or not. To predict reliably the flexibility characteristics of RNA structures, parameters had to be developed for when hydrogen bonds and hydrophobic interactions are included as non-covalent constraints. These parameters were validated based on experimental mobility data of a tRNA<sup>ASP</sup> structure and all NMR-derived ensembles of RNA structures (with a chain

length  $\geq 40$ ).<sup>7</sup> We found that restricting the number of base stacking interactions between sequentially adjacent bases to one is crucial and that hydrophobic interactions in general should be considered if the distance of two hydrophobic atoms is smaller than the sum of their van der Waals radii plus a threshold of 0.15 Å. The resulting topological network representation for a canonical A-Form RNA is shown in Figure 1a. Compared to the protein parameterization,<sup>1,4</sup> the new parameters prevent the RNA network representation from being overly rigid (Figure 1b). Further details about the underlying rigidity theory and the validation on RNA and protein structures have been described elsewhere.<sup>1,5,8,9</sup>

## 2.2 Flexibility Characteristics of the Ribosomal Exit Tunnel Analyzed by Rigidity Theory

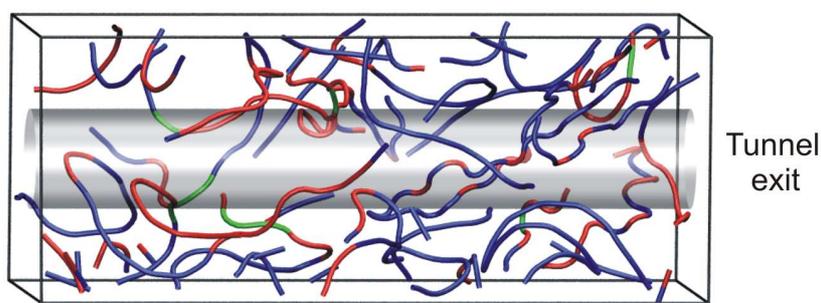


Figure 2. Color-coded representation of the flexibility characteristics of the ribosomal exit tunnel obtained by constraint counting. The coloring of the backbone atoms of the RNA part is according to the flexibility index of the P atoms and according to the  $C_{\alpha}$ -atoms in the protein part. Blue color indicates overconstrained regions and red color flexible regions.

The rigidity analysis can easily be applied to large biomolecules like the ribosomal structure, which consists of more than  $10^5$  atoms. The computational time amounts to only minutes in this case. The analysis gives new insights in atomic detail into the functional role of the ribosomal exit tunnel, e.g., during protein synthesis. Figure 2 shows a color-coded representation of the flexibility characteristics of the tunnel backbone. Blue color indicates overconstrained regions, green color isostatic (minimally rigid) regions, and flexible regions are colored in red. The approach identifies large parts of the tunnels neighboring regions to be rigid. Remarkably, this holds true for all high resolution structures of the ribosomal exit tunnel of different organism available in the PDB data base.<sup>11-13</sup> Even more striking is the finding of conserved local zones of flexible residues within the tunnel: Clusters of flexible tunnel components are located in the first half of the tunnel and around the tunnel exit. Interestingly, these regions correspond to previously identified folding zones within the tunnel.<sup>14</sup> This striking agreement between tunnel regions with low structural stability and observed folding zones implies that indeed secondary structure may be stabilized entropically there through local conformational adaptability of the ribosomal exit tunnel.

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