Karlsruhe Institute of Technology

Steinbuch Centre for Computing (SCC)
Multiscale Biomolecular Simulation (MBS)

Computational Analysis of Riboswitch Folding Under Spatial Constraints

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1. Introduction

We are interested in numerical prediction of characteristic dynamical behavior during folding and ligand binding of structured messenger RNA (mRNA). Our work utilizes molecular dynamics (MD) simulations that implement structure based models. In order to understand the functional properties, we need to answer the question whether such a process is kinetically or thermodynamically driven.

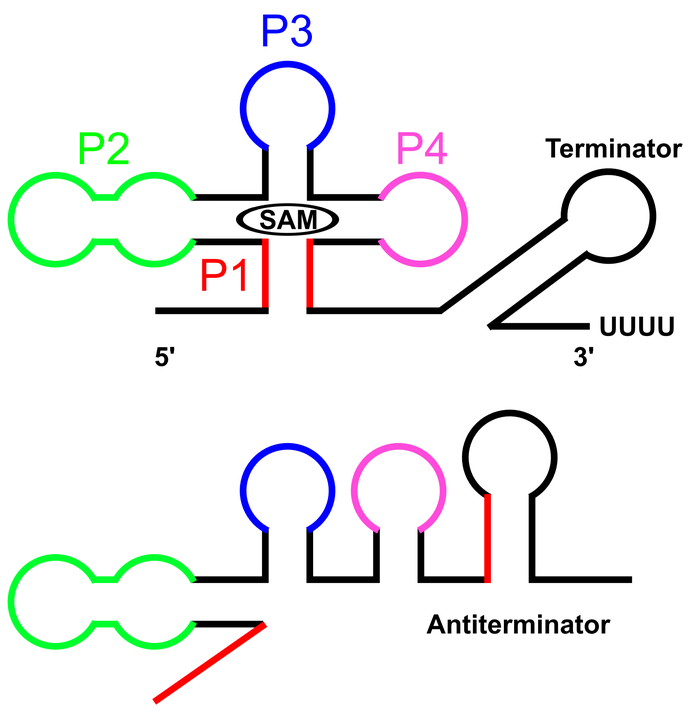
Both the role of ligand concentration and of spatial constraints in cotranscriptional folding need to be investigated in order to learn more about their impact on gene regulation.

Given the natural time scales of several hundred milliseconds for transcription, we employ a coarse-grained structure based force field to reduce computational effort.

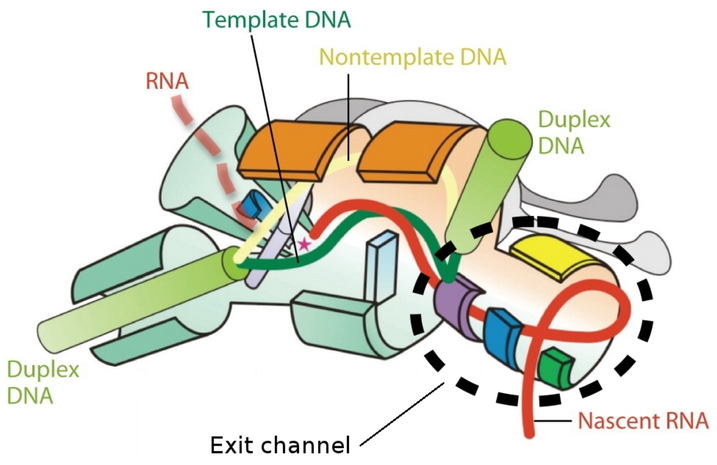
2. System of Interest

Our system of interest is a riboswitch in its natural environment during and shortly after transcription. Riboswitches are segments of the untranslated region of mRNA that regulate gene expression. During transcription, the riboswitch emerges from the RNA polymerase (RNAP) and is able to form secondary structure [1].

We study a type I S-adenosylmethionine (SAM) riboswitch (PDB ID 2GIS) which responds structurally to the presence of SAM ligands. The SAM-I riboswitch consists of four helices P1 - P4 connected by a single junction if the ligand is bound and a terminator can be formed. Without the ligand, parts of P1 form an antiterminator which prevents termination.

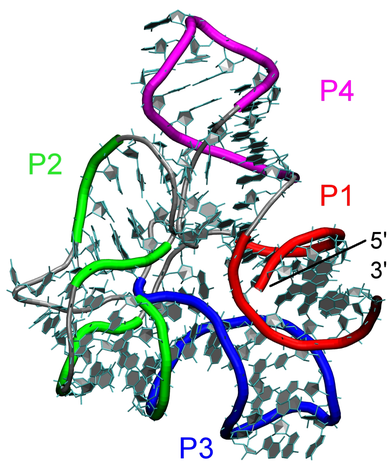


In its natural environment, the nascent riboswitch emerges from the RNAP via an exit channel. This exit channel consists of four structural elements that form a flexible claw.



[2]

3. Structure Based Model



The structure based model employs a potential that is based on the native structure $\{r_0, \theta_0, \chi_0, \phi_0, \sigma_{ij}\}$ of a biomolecular system. The potential V in an all-atom formulation reads as

$$V = \sum_{\text{bonds}} K_b(r - r_0)^2 + \sum_{\text{angles}} K_a(\theta - \theta_0)^2 + \sum_{\substack{\text{improper}\\ \text{dihedrals}}} K_i(\chi - \chi_0)^2$$
$$+ \sum_{\text{dihedrals}} K_d^{(sc)} \left[[1 - \cos(\phi - \phi_0)] + \frac{1}{2}[1 - \cos(3(\phi - \phi_0))] \right]$$
$$+ \sum_{\text{contacts}} K_c \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - 2 \cdot \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] + \sum_{\substack{\text{non-native}\\ \text{contacts}}} K_{nc} \left(\frac{\tilde{\sigma}_{ij}}{r_{ij}} \right)^{12},$$

where K_b, K_a, K_i, K_d, K_c and K_{nc} are force constants.

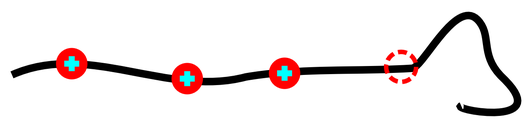
The assumption of an energy landscape based on minimally frustrated interactions decreases the computational effort drastically [3,4]. We use an all-atom force field implementation for GROMACS provided by a web-tool on the SMOG@ctbp homepage [5].

4. Polymerase Modelling

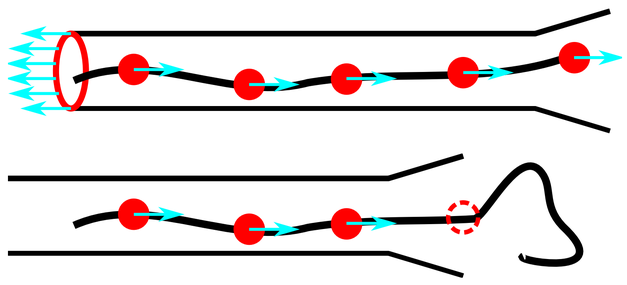
The relevant structural unit of the RNAP for our investigations is the exit channel. It is formed by four elements as a flexible claw that encompasses the channel. Due to this fact, the whole channel exhibits flexibility and can vary its width.

Based on the natural structure of the RNAP, we propose simplified scenarios to model the exit channel from which the nascent mRNA emerges.

One such scenario consists of sequential releases of several spatially restrained residues along the stretched riboswitch structure:



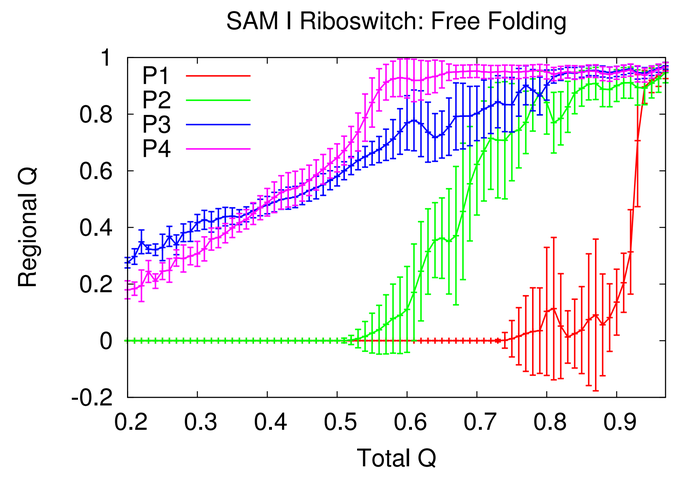
A more natural scenario is realized by pulling the stretched riboswitch out of a carbon based tube with a funnel or nozzle like exit. The residues are released sequentially when they leave the funnel:



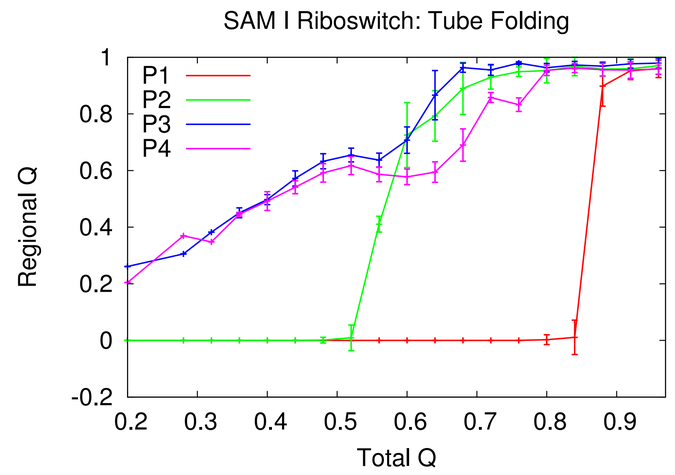
The simplified models allow us to conduct studies of geometrical and dynamical parameters such as width of the exit region, angle of the funnel, flexibility of the tube or release velocity.

5. Results and Challenges

A free folding analysis of the SAM I riboswitch represents a reference simulation. The fraction of formed native contacts Q for each helix P1 - P4 over the overall fraction of closed native contacts is plotted:



As a preliminary result, a folding analysis under spatial constraints can be compared with the free case. The stretched riboswitch is pulled out of a tube and released sequentially:



The presented approach enables the investigation of the influence of geometrical and dynamical parameters on the folding characteristics.

In future work, we will introduce a temperature scale by investigating melting curves of RNA hairpin loops and a time scale by comparing our simulations with experimental folding and ligand binding rates [6]. Therefore, it will also be necessary to implement a structure based potential for two competing structures (terminator and antiterminator).

Literature

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Acknowledgements

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