# Dynamics of riboswitches: molecular simulations

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Abstract:

Riboswitch RNAs play key roles in bacterial metabolism and represent a promising new class of antibiotic targets for treatment of infectious disease. While many studies of riboswitches have been performed, the exact mechanism of riboswitch operation is still not fully understood at the atomistic level of detail. Molecular dynamics simulations are useful for interpreting existing experimental data and producing predictions for new experiments. Here, a wide range of computational studies on riboswitches is reviewed. By understanding the key principles of riboswitch operation, computation may aid in the effort to design more specific antibiotics with affinities greater than those of the native ligand. Such a detailed understanding may be required to improve efficacy and reduce side effects. These studies are laying the groundwork for understanding the action mechanism of new compounds that inhibit riboswitch activity. Future directions such as magnesium effects, large-scale conformational changes, expression platforms and co-transcriptional folding are also discussed.

*Introduction:*

Riboswitches are noncoding RNAs typically found in the 5'-UTR of bacterial mRNAs and often regulate genes important for metabolism (1-3). In many cases, the presence or absence of a metabolite alters a transcriptional terminator helix, turning the gene on or off. Riboswitches have two dramatically different folds depending on the presence or absence of ligand. For many riboswitches, the presence of ligand causes a substantial collapse of the RNA into a compact aptamer (4-7). In absence of ligand, an alternative secondary structure (the expression platform) forms, destroying any chance of forming the aptamer fold. Riboswitches are promising targets for antibiotics because they (1) control important biosynthetic pathways in pathogenic bacteria, (2) have not been found in humans, and (3) are highly specific (8-11). Because riboswitches are present in multiple copies in the genome, they have the potential to be less susceptible to resistance mutations (8). With respect to therapeutic design, at least 16 human bacterial pathogens rely on riboswitch-controlled gene expression. In *B. subtilis,* the S-adenosylmethionine (SAM) riboswitches control 11 transcriptional units, including 26 genes that are critical for sulfur metabolism, amino acid metabolism and SAM biosynthesis (2; 12).

Riboswitch mechanism combines the problems of RNA folding (13-15), ligand binding and large conformational changes (16; 17). A wealth of structural studies have been performed, revealing the detailed riboswitch fold and ligand-binding interactions (18-26). Most recently, crystallographic studies have captured ligand-free states in addition to ligand-bound states (27). Many other riboswitch investigations have focused on the aptamer domain of the riboswitch (2; 13; 20; 23; 28-33). Experimental studies have examined ligand binding to riboswitch aptamer domains and associated conformational changes (13; 16; 17; 20; 22-24; 26; 30; 34-40). Fluorescence studies have shown the aptamer to undergo conformational changes (13; 30; 40; 41). The results are consistent with SAXS data and other biochemical studies (27; 30; 32). Single molecule studies have investigated the presence of intermediate states (4; 40; 42). SAXS and NMR experiments have detailed the collapse transition between extended and compact conformations (27; 43). Transcriptional termination studies of the overall effect of secondary structure change for other riboswitches have been performed with kinetic and thermodynamic experiments (12; 16; 17; 44). Very recently, several studies have examined the role of the expression platform in riboswitch function (6; 45). While experimental studies have made great progress into understanding riboswitch mechanism, computational studies play a key role in providing an atomistic interpretation and framework for riboswitch dynamics and mechanism. A wide range of computational techniques has been applied with each method giving a complementary perspective. While this short review is by no means comprehensive, an attempt is made to cover the major areas of computer simulations of riboswitch systems.

*Explicit solvent molecular dynamics simulations of riboswitch dynamics*

Many explicit solvent molecular dynamics simulations have been performed in recent years. Most studies use the AMBER or CHARMM force field and particle mesh Ewald electrostatics

(PME). Some studies use an atmosphere of excess ions in addition to water. A handful of studies have reached aggregate sampling > 1 microsecond. Simulations of the guanine riboswitch investigated the hydrogen bond network with and without ligand and guanine binding via 'pulling'(46). Here, the authors suggest pathways and compare to NMR and other experimental data. Molecular dynamics simulations of the adenine riboswitch used the CHARMM force field to examine the ligand-bound and ligand-free dynamics, suggesting mechanisms for binding and switching (47). Further studies of the adenine riboswitch using CHARMM explored the destabilization of the P1 stem and flexibility of the J2/3 junction. This same study also simulated the holo *pbuE* riboswitch in the presence of adenine (**H1**), and 2,6-diaminopurine (DAP, **H2**) (48). Studies of the SAM-II system with the AMBER force field explored pseudoknot fluctuations and ligand binding, concluding that the curvature and base-pairing of the expression platform that could affect the interactions with the ribosome(49). A second study found that unfavorable loss in entropy in SAM-II binding is greater for (*S*,*S*)- and (*R*,*S*)-SAM than SAH, which is compensated by stabilizing electrostatic interactions with the riboswitch(50). Protonation states of the glmS system concluded that the canonical form of G40 plays a structural role by stabilizing an in-line attack conformation of the cleavage site A-1(2′-OH) nucleophile (51). A second study of the glmS system suggested that GlcN6P acts as a general acid in the self-cleavage of *glmS* (52). Molecular dynamics simulations of the preQ1 riboswitch of local aptamer dynamics have been validated by 2-aminopurine fluorescence experiments(53). A very interesting study concerning the effect of urea on the preQ1 riboswitch revealed that the denaturation of RNA structures is mainly driven by the hydrogen bonds and stacking interactions of urea with the bases. This study used the CHARMM force field and had an aggregate sampling of ~5 microseconds(54). Finally, a 1.6 microsecond implicit solvent study using AMBER produced a folding energy landscape of the preQ1 system(55). In this study, an interesting comparison was performed between the *Thermoanaerobacter tengcongensis* and *Bacillus subtilis* systems.

Explicit solvent studies of the SAM-I riboswitch using AMBER with excess ions suggest that a component of the surrounding $Mg^{2+}$ cloud (outer sphere $Mg^{2+}$ ions) exhibits glass-like behavior with non-Markovian diffusion coefficients (56). Interestingly, ~80% of the $Mg^{2+}$ ions surrounding the RNA (the SAM-I riboswitch) exhibited this behavior. The aggregate sampling of this study was 20 microseconds. Replica exchange simulations of the SAM-I riboswitch binding site for wild type and diaminopurine substituted binding bases revealed relative free energies consistent with nucleotide analog interference experiments(27). A recent study of the SAM-I system has examined the role of the anti-terminator in switching. This study used AMBERbsc0 with excess ions and achieved ~17 microsecond aggregate sampling(57). We note that previous explicit solvent MD simulations have characterized the distribution of monovalent ions around RNA helices (58; 59).

*Poisson-Boltzmann calculations of riboswitch electrostatics*

Many excellent computational studies have investigated the role of the diffuse cloud of $Mg^{2+}$ ions surrounding the RNA, including explicit solvent molecular dynamics simulations and Brownian dynamics simulations and Poisson-Boltzmann studies (59-66). Poisson Boltzmann calculations use a continuum distribution of ions, via the nonlinear Poisson-Boltzmann approximation, and have been used to estimate RNA-$Mg^{2+}$ interaction free energies (67). An integrated study combining small angle X-ray scattering (SAXS), hydroxyl radical foot printing

and Poisson-Boltzmann calculations examined the cation dependence of folding for the glycine riboswitch(7). The study reported that non-specific screening facilitates folding and that ligand binding requires site-specific Mg2+ binding.

*Coarse-grained and reduced-description simulations of riboswitch dynamics*

While explicit solvent simulations have produced important insights into RNA dynamics on ~μs time scales (59; 62; 64; 68-72), coarse-grained and reduced-description models of nucleic acids are becoming more useful for long time scale dynamics (73-79). These simplified models can access long time scales (>> 1 ms) while preserving stereochemistry, and are useful in determining the geometrical features of structural basins and transition states. Three-based models of riboswitches have pioneered the simulation of force-based experiments(78). These coarse-grained models infuse extensive knowledge of RNA thermodynamics into the potential, largely based on the Turner rules of base pairing. Here, the energy landscape of the adenine riboswitch was characterized by unfolding and refolding with mechanical force using a three-bead coarse-grained self-organized polymer model and Brownian dynamics simulation. A similar study was performed for the SAM-III riboswitch(80).

A second class of models, termed "structure-based" models, has a basis in energy landscape theory and has been instrumental in forming the protein folding funnel framework (81). All-atom structure-based models were used to examine the interplay between ligand binding, folding and collapse for the SAM-I riboswitch. Here, thermodynamic and kinetic folding studies in a box of ligands predicts that ligand binding facilitates the formation of the fully collapsed form and that P1 forms last in the folding process (73). Similar folding studies in a box of ligands using all-atom structure-based models were also performed for the PreQ1 riboswitch showed that the folding pathway is cooperative and sequentially coordinated, as the folding proceeds in the 5′ → 3′ direction, suggesting a fast ligand-binding response in competition with RNA elongation (35). A second study comparing *Thermoanaerobacter tengcongensis* and *Bacillus subtilis* suggested that similar pre-folded ensembles follow distinct folding(82).

*Quantum mechanical studies of RNA ligand binding interactions.*

Quantum mechanical studies of riboswitches are a relatively new development. We note that previous important RNA simulations including quantum mechanical effects and ribozyme activity have been performed, offering complementary approaches to classical MD (83-85). Recently, quantum mechanical calculations for the optimized geometry of the hydrogen bond network for the preQ1 riboswitch ligand binding site are consistent with crystallographic studies and suggest that water may alter the ligand binding energy significantly (86). Studies of non-canonical base-phosphate interactions in the glmS riboswitch suggest that these interactions help stabilize the important regions of the RNA (87). Finally, gas phases studies of the guanine and adenine riboswitches at the B3LYP/6-31G(d,p) level indicate that stacking and $Mg^{2+}$ interactions may contribute to ligand recognition (47).

*Future Directions.*

*Collapse and folding transitions of riboswitches.* Collapse of the aptamer from the open to closed form plays a key role in riboswitch operation. As the problem of RNA riboswitch aptamer collapse is not a conventional binding or folding problem, mechanisms different from those found in proteins may be required to explain how an RNA aptamer explores its functional and folding landscape (14; 15). Core collapse has been shown experimentally in the TPP and Glycine riboswitches (4-7). Biochemical studies have shown that the SAM-I riboswitch undergoes a collapse transition from extended to compact that is strongly dependent on [$Mg^{2+}$] and [SAM] and requires cooperativity between $Mg^{2+}$ and SAM (6; 45). The results are consistent with SAXS data and other biochemical studies (27; 30; 32). Future molecular simulations of the collapse process may provide an important atomistic framework to interpret these experiments.

*Mg2+ effects on riboswitch behavior.* Almost ubiquitous among functional RNA systems is the strong dependence of RNA structure and function on magnesium concentration (88-91). For RNA, control of folding and collapse is significantly impacted by magnesium (89; 92-95). NMR studies suggest that diffusely associated metal ions play an important role in RNA structure (96) and X-ray crystallographic structures of riboswitches have been shown to contain site-specifically bound $Mg^{2+}$ ions that are positioned to be important for function (36; 37; 85; 97-99). Other work has shown that RNA structures take on compact configurations at high [$Mg^{2+}$] (36; 37; 85; 97-99). These studies suggest that $Mg^{2+}$ exerts a collective effect on the RNA causing it to collapse. Recent studies also suggest that $Mg^{2+}$ may pre-organize riboswitch ligand binding domains (13; 41; 97). While the overall effect of $Mg^{2+}$ on aptamer RNAs is known, the detailed mechanism is not (100). Rather than a single particle (*i.e.*, the ligand) determining collapse, many particles may be involved, including the ligand as well as several $Mg^{2+}$ ions. Ligand binding may be accompanied by an intricate choreography of $Mg^{2+}$ ion binding events (101). Experimental studies have shown that site-specifically bound $Mg^{2+}$ ions play important roles in RNA function. For example, nucleotide analog interference experiments show that the SAM-I riboswitch has 3 chelated $Mg^{2+}$ ions that are essential for collapse (6). While $Mg^{2+}$ effects are known to be critical for RNA function, a key riddle remains unsolved: do changes induced by $Mg^{2+}$ result from site specifically bound $Mg^{2+}$ ions or from the dense $Mg^{2+}$ cloud surrounding the RNA? Addressing this problem with molecular simulations requires (i) atomistic detail to handle site-specific ions, (ii) long time scale dynamics (>> 1 ms) to encompass the large conformational changes induced by $Mg^{2+}$, and (iii) a novel model for the chelation and dissociation of site-specific $Mg^{2+}$ ions.

*The role of the expression platform in riboswitch mechanism.* During transcription, bases in the aptamer domain and the expression platform compete with each other for the same base pairing partners (12; 44; 102). In absence of ligand, the expression platform helix dominates; at higher concentrations of ligand, the aptamer dominates. At low to medium concentrations, however, the dominant fold is not clear. This is the range of *in vivo* regulation where factors other than simple ligand:aptamer affinity alter gene regulation (12; 45). In one study, a two-piece expression platform assay targets precisely this regime, producing predictions for use in co-transcriptional studies (45). As mentioned above, a ground-breaking computational study has examined this process in the context of molecular dynamics(57).

*Kinetics and co-transcriptional folding.* A key issue in riboswitch operation is the importance of kinetics vs. thermodynamics. While studies of the FMN riboswitch suggested its function is

dictated by the kinetics of ligand binding, studies of the adenine riboswitch show it has the potential to be either kinetically or thermodynamically driven (16; 17). Cotranscriptional folding is known to play an important role in riboswitch decision making and have been investigated experimentally in recent studies(103). One computational study has examined the importance of cotranscriptional folding using a simplified model(104).

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