A marginal stability is required for the guanine-sensing riboswitch to function

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Riboswitches are small genetic regulatory RNA elements that control bacterial gene expression on the transcriptional or translational level upon binding of ligand molecules. The ligand binds to the riboswitch aptamer domain, inducing a conformational change in the downstream expression platform. In transcriptionally acting riboswitches, this conformational change of the expression platform leads to the formation or disintegration of an intrinsic transcription terminator loop, thus switching gene expression off or on. Since transcriptional gene regulation by riboswitches has to happen in a narrow time window during the transcription process, detailed knowledge about the unbound state of the riboswitch is crucial to understand the overall process underlying the regulation decision by the riboswitch.

While insights into the bound state of purine-sensing riboswitches on the atomic level are available from crystal structures and other experimental investigations, such atomic level details about the unbound state and its dynamics are still scarce.

We investigate the unbound state of the guanine-sensing riboswitch aptamer domain (Gsw) and a mutant using molecular dynamics (MD) simulations in explicit solvent. In all, we simulated six variants of the system with three replications each, resulting in a total simulation time of more than 10 μs. By probing the system with respect to the strength of tertiary loop-loop interactions or the presence/absence of Mg^{2+}, we gain detailed insights even at simulations times smaller than the anticipated switching time. In particular, we observe a dynamic coupling between the loop region of the Gsw and its ligand binding site located ~25 Å away. Furthermore, Mg^{2+} exerts a stabilizing effect that differently fine-tunes the structural stability of wildtype and mutant. These results suggest that the functional stability of the aptamer domain is highly sensitive to subtle changes in the structure and its environment, and that information on the ligand is transmitted to the expression platform by complex entropic control. These findings are important for understanding how Gsw functions at a molecular level.