The production of proteins in cells may be regulated at various stages of this process. The stage of interest to us is at the level of translation of an mRNA sequence into a sequence of protein amino acids. Some naturally found mRNA fragments change their structure upon binding of a small molecule, for example, a metabolite. Such structural change regulates the production of a protein encoded by this particular mRNA. These non-coding mRNA fragments are called riboswitches because they switch “on” and “off” translation of mRNA depending on the presence of a specific small molecule.

Riboswitches could be used to control gene expression in metabolic pathways of pathogenic bacteria. Also, there have been efforts to make riboswitches targets for antibacterial compounds.
Indeed, synthetic riboswitches were designed, incorporated into mRNA, and shown to regulate the production of proteins in cells in response to various small molecules.

A synthetic riboswitch that binds aminoglycoside antibiotics was also designed and found to have regulatory activity while incorporated into cellular mRNA. This riboswitch is a flexible 27 nucleotide long RNA fragment whose atomistic three-dimensional structure in the complex with aminoglycosides was determined by nuclear magnetic resonance spectroscopy. However, the specificity of this riboswitch towards different aminoglycosides needs to be understood to be able to design a riboswitch variant possessing better regulatory properties against only one certain aminoglycoside. Aminoglycosides, apart from their known antibacterial role, were found to bind a natural riboswitch involved in the control of bacterial resistance genes. Therefore, aminoglycosides also have the potential to regulate gene expression at the riboswitch level.

The main aim of this project is to determine the dynamics of a synthetic aminoglycoside-sensing riboswitch to understand the aminoglycoside recognition mode and the structural basis of the riboswitch activity. We will also predict the structure of aminoglycoside-free riboswitch. The resulting goal is to determine the binding affinities of aminoglycosides to this riboswitch and its variants. This will allow us to propose aminoglycoside and riboswitch modifications to improve the regulation of gene expression.

We will apply molecular modeling and high-performance computing simulations. We will extend the molecular dynamics technique to obtain realistic sampling of riboswitch conformations, positions of its atoms in space, and determine paths that an aminoglycoside has to complete to bind with the riboswitch. Simulations of the free riboswitch and in the complexes with many aminoglycosides (both with varying riboswitch sequence) will explain the reasons for the specificity and activity of the riboswitch only toward certain aminoglycosides. For example, riboswitch binds both paromomycin and neomycin but is active in cells only against neomycin even though these two aminoglycosides differ by only one small chemical group.

The collaborating partner will be the RIKEN research institute in Japan. This institute was founded in 1917 and has grown to a network of high-quality research institutes across Japan. Currently, RIKEN centers possess four supercomputers (including K-computer) listed in the Top500 list of the most powerful computer systems in the world. Prof. Sugita, our collaborator, is a Chief Scientist in RIKEN leading two groups: Theoretical Molecular Science Laboratory in Wako and Computational Biophysics Research Team at Advanced Institute for Computational Science in Kobe. Most importantly for us the collaborating groups in RIKEN (both Wako and Kobe units) will provide expertise, software, and computer time for numerous enhanced sampling simulations planned in this project. Prof. Sugita has been developing methods for enhanced and multi-dimensional sampling in molecular dynamics simulations for nearly two decades so he is the best scientist to provide the know-how for the development of this method for our system.