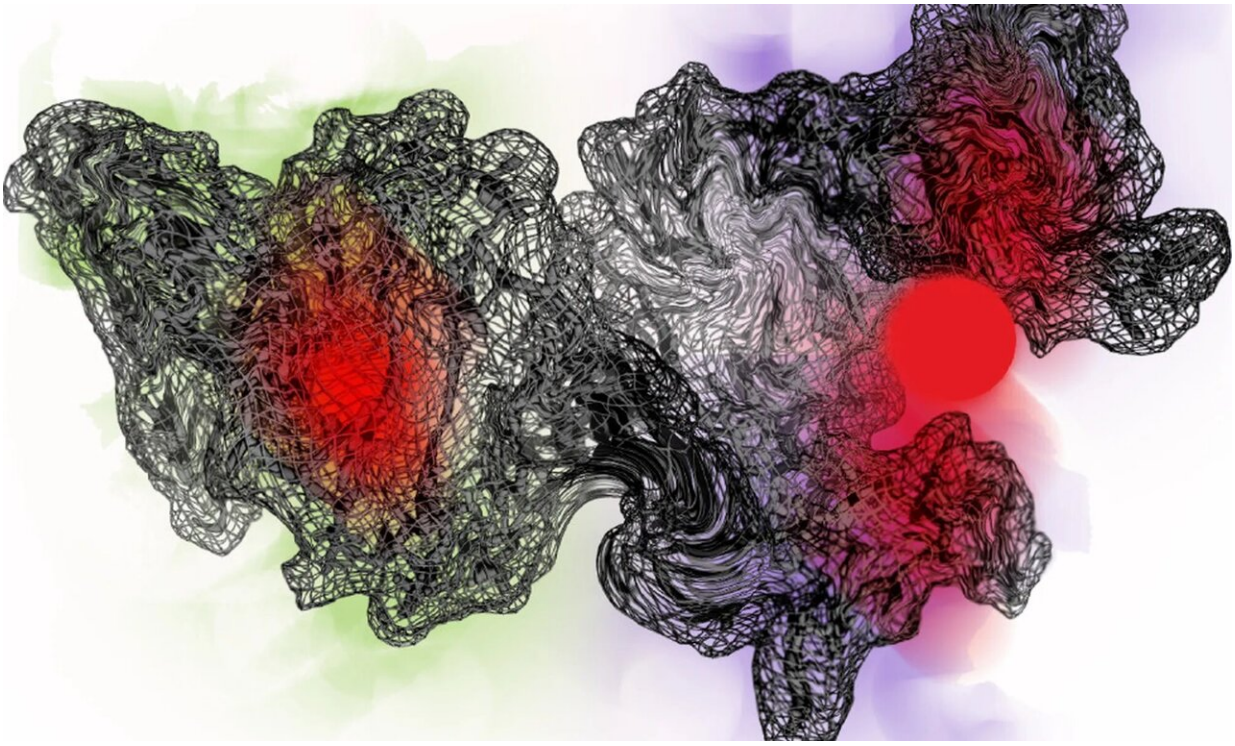
Researchers develop new method to study RNA-drug interactions

July 20 2023



Innsbruck researchers suspect that two neomycin B molecules simultaneously bind to the RNA to activate riboswitch function. Credit: University of Innsbruck

How active compounds affect RNA and thus the expression of genes is of great interest for the development of potential therapeutics. Innsbruck chemists have now used a method they recently developed to study the binding of the aminoglycoside Neomycin B to a so-called mRNA

riboswitch.

In important cellular processes, ribonucleic acids (RNA) specifically recognize certain proteins or small organic molecules as binding partners. To understand these processes and to advance the development of potential therapeutics targeting RNA, it is important to understand in detail how ligands bind to RNA.

However, one challenge in studies of RNA complexes with drugs is that RNA can offer multiple binding motifs that are difficult or impossible to resolve using conventional methods. Researchers led by Kathrin Breuker from the Department of Organic Chemistry at the University of Innsbruck have now used a method they recently developed to study the binding of the aminoglycoside Neomycin B to a so-called mRNA riboswitch. The findings are published in the *Journal of the American Chemical Society*.

Simultaneous binding

For their study, Kathrin Breuker's team used a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, which allows RNA complexes of different stoichiometries to be separated from each other in the gas phase and studied individually.

"For the RNA sequence with the highest regulatory factor for riboswitch function, we identified two different binding motifs for Neomycin B and were also able to determine their relative populations," Kathrin Breuker recounts. "By introducing RNA base mutations, we were able to specifically shift the populations of the two binding motifs and thus develop a better understanding of aminoglycoside binding to the riboswitch."

As the populations of the two binding motifs were the same for the non-

mutated sequence, the Innsbruck researchers suggest that riboswitch function is controlled by the simultaneous binding of two Neomycin B molecules.

The new experimental approach allows systematic studies of the binding specificity of RNA and, complementary to established methods for structure elucidation, will provide new insights into RNA function at the molecular level.

More information: Sarah Viola Heel et al, Native Top-Down Mass Spectrometry Uncovers Two Distinct Binding Motifs of a Functional Neomycin-Sensing Riboswitch Aptamer, *Journal of the American Chemical Society* (2023). DOI: 10.1021/jacs.3c02774