Advantages of Serial Femtosecond Crystallography for RNA Structure Determination

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Discovery of the important and diverse biological roles of RNA molecules is ever increasing. Similar to proteins, knowledge of the three-dimensional structures of RNAs is critical to understanding their functions. However, structure elucidation of RNAs using conventional methods has been extremely hampered by technical challenges, and is reflected in the overwhelmingly few RNA structures in the protein data bank. In addition to molecular size limitations faced by nuclear magnetic resonance (NMR), the intrinsically similar chemical signatures of nucleotides result in severe peak overlap in NMR spectra. RNAs are often difficult to crystallize, and when available, RNA crystals often exhibit high mosaicity, high solvent content, and high susceptibility to radiation damage. In addition, RNAs tend to be very dynamic, and low-temperature data collection on a single crystal may not provide the most accurate depiction of its structure. Clearly, there is an acute need for advanced methods for RNA structure determination. Serial femtosecond crystallography (SFX) using an X-ray free electron laser (XFEL) has the potential to revolutionize RNA crystallography by overcoming many of these technical challenges. Its advantages include the use of nano/micro-sized crystals, room-temperature data collection, the ability to outrun radiation damage, and a high-throughput oversampling of crystal data. We have used SFX to determine the structure of the adenine riboswitch RNA apatamer domain in the ligand-free state. For the first time, these results provide a structural basis for the ligand-induced conformational switch required for the regulation of gene expression.

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