

Title: Using X-ray free electron laser to capture intermediate states

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A riboswitch is an RNA structural element generally located in the 3' untranslated region (5' UTR) of an mRNA. In the genetic regulation, ligand binding to the aptamer domain of a riboswitch triggers a signal to the downstream expression platform. A complete understanding of the structural basis for this mechanism requires the ability to study structural changes over time. Here we apply femtosecond X-ray free electron laser (XFEL) pulses to obtain structural measurements where ligand was diffused into micron/nanocrystals to trigger conformational changes within crystals. This approach is called inject-and-mix. We demonstrate this approach by determining four structures of the adenine riboswitch aptamer domain during the course of a reaction involving two apo, one ligand-bound intermediate, and the final bound states. These structures from these experiments support a reaction mechanism model with at least four states and illustrate the structural basis for signal transmission. These results all together demonstrate the potential of “mix-and-inject” time-resolved serial crystallography to study biochemically important interactions between biomacromolecules and ligands, including those involving large conformational changes.