Elucidation of first ambient-temperature 50S Ribosomal subunit & Selenium-SAD de-novo phasing using an XFEL

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An emerging form of X-ray crystallography is Serial femtosecond X-ray Crystallography (SFX) which utilizes micrometer-sized crystals of uniform dimensions which differs from the traditional large-sized crystals employed in traditional Synchrotron crystallography. SFX requires an X-ray Free Electron Laser (XFEL) which allows for the imaging of biological macromolecules at near-physiological temperatures compared to the cryo-temperatures incorporated in traditional Synchrotron crystallography.

The ribosome is a biological macromolecule responsible for decoding the genetic code and translating it into proteins. It has long been the target of antibiotics because life cannot exist without functional proteins. Many antibiotic mechanisms which target the ribosome have been illuminated through high-resolution structures utilizing X-ray crystallography. However, Antibiotic resistance has been a growing concern in the scientific field as bacteria adapt and reject commonly prescribed antibiotics. This necessitates the study of novel antibiotics at ambient temperatures to understand how they interact with the dynamic systems of biological macromolecules.

This investigation utilized SFX to obtain the first ambient-temperature structure of the large ribosomal subunit (50S) of *Thermus thermophillus* in record time consuming only microliters of sample. Macrolides and ketolides are a class of antibiotics which target the peptidyl-transferase center of the 50S subunit, specifically binding to the P-site thus inhibiting the peptide bond formation for proteins. This structure opens the door to study novel antibiotics on the 50S subunit at ambient temperatures to resemble the *in vivo* antibiotic mechanism.

Majority of structures solved at an XFEL utilize external information through techniques such as Molecular Replacement to phase the structure. In this investigation, Single Anomalous Dispersion (SAD) was applied to phase the structure of selenobiotinyl-streptavidin using the Selenium heavy-atom K-edge (Se-SAD). This experiment demonstrates the efficacy of applying SAD to SFX experiments and illustrates that *de novo* phasing is possible at XFELs. This experiment opens the door to further this finding an employ common techniques such as Multiple wavelength anomalous Dispersion (MAD) or utilize the K-edge of other atoms to accomplish *de novo* phasing.