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Ambient Temperature Structure of Large Ribosomal Subunit from *Thermus Thermophilus*

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The introduction of ultrafast and ultrabright X-ray free electron lasers (XFELs) has allowed us to observe the structural illumination of biological macromolecules unattainable in synchrotron radiation sources at an unprecedented temporal and spatial resolution. Here we report the first near-physiological temperature structure of a large ribosomal subunit (50S) isolated from *Thermus Thermophilus*. Prokaryotic ribosomes are the target of more than half of known antibiotics and the large ribosomal subunit (50S) is targeted by blockbuster antibiotics such as macrolides and ketolides. 50S is one of the largest structures identified in an XFEL to date, with 3 MDa asymmetric units due to dimerization (1.5 MDa each) within the unit cell. A complete structure at 3.99 Å was obtained while consuming less than 100 uL of crystal sample, at a record short beam duration of 47 minutes. In the absence of radiation damage, a higher degree of solvent presence is observed, which is crucial for the stability and functionality of the ribosome. This study demonstrates the feasibility of improving the structural understanding of the unique binding sites and structural dynamics of *T. thermophilus* 50S at physiological temperatures enabling us to gain more structural insights into ribosome structure and function while enhancing our ability to develop next-generation antibiotics.

Additionally, this structure serves as the starting point for future work involving the large ribosomal subunit and time-resolved serial femtosecond crystallography (tr-SFX) experiments at XFELs.

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