Combating Bacteria with High Performance Computing

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The objective of this work is to "map" the functional dynamics of the ribosome. This mapping information will allow for the design of novel antibiotics that target critical stages of ribosome function. Such antibiotics may combat many pathogens, including the drug-resistant bacteria that are common in hospitals. The need for atomistic resolution and long time scales (with over 109 time steps), combined with the large scale of the ribosome, puts the simulation of its dynamics at the forefront of biological computing efforts. In explicit-solvent simulations, the highly charged composition of the ribosome necessitates the frequent evaluation of long-range interactions. This highly interconnected system results in large computing loads and high inter-node communication demands.

Ribosomes are present in all cells; they serve as molecular computers that decode genetic messages and synthesize proteins. With the many functional roles of proteins, proper functioning of the ribosome is vital for cellular life. In US hospitals, approximately 50% of the antibiotics alter ribosome function. Antibiotics are small molecules that often diffuse into the ribosome and halt its function in bacteria, while remaining passive in humans. Prominent diseases that are treatable by ribosome antibiotics include Methicillin-resistant Staph infection, or MRSA (the so-called "superbugs" found in American hospitals), anthrax, and plague. The World Health Organization has also identified extensively drug-resistant (EDR) and multidrug-resistant (MDR) tuberculosis as principle challenges of national tuberculosis programs in Africa.

The exceedingly small, yet specific, interactions of antibiotics with the ribosome represent a significant challenge to molecular biologists. Some antibiotics only need to interact with about 50 atoms, out of the hundreds of thousands in the ribosome. This small set of interactions make it is extremely difficult to directly visualize antibiotic function experimentally. While single-molecule fluorescence, X-ray crystallography, and microscopy methods have revolutionized our understanding of ribosome structure and dynamics, simulations are the only means to obtain atomic resolution dynamics of ribosomal function. Simulations can also provide a structural framework for understanding experimentally measured quantities, and they can be used to predict novel motions that would not be envisioned by looking at static images.

Due to the large number of atoms in the ribosome and the long-range nature of the interactions, simulating it for biologically relevant time scales represents a formidable challenge. When simulated in full detail, with every solvent molecule explicitly represented, there are over 3 million atoms. We recently reported the most extensive sampling of the ribosome in explicit-solvent [1] (2.1 microsecond, or ~109 time steps). This is approximately 40 times more sampling than previous simulations of full ribosomes. With these simulations, we were able to provide a quantitative bridge between the thermodynamics of ribosome function and experimentally measured kinetic data. Knowing the thermodynamic properties of ribosome function will assist in the design of new generations of antibiotics, each of which can have predictable quantitative effects on the ribosome's functional dynamics.

In another recent study, we were able to demonstrate that the ribosome's resilience to antibiotics may be due to it having highly-degenerate functional dynamics [2]. That is, "back channels" are built into the ribosome and may allow it to "dodge" the deadly effects of incoming antibiotics. For that study, a simpler all-atom model was employed that allowed for the simulation of much longer effective time scales (~200 milliseconds; ~1010 time steps). With such long time scales, we calculated the statistical properties (from over 300 barrier-crossing events) of large-scale conformational rearrangements in the ribosome. These simulations predicted a specific sequence of events and they identified several new targets for antibiotic design.

With the dynamic nature of the ribosome, obtaining atomic models of transiently populated, yet functionally well-defined, configurations is a grand challenge for the field. In collaboration with the Onuchic Group at the University of California in San Diego and the Spahn Laboratory at

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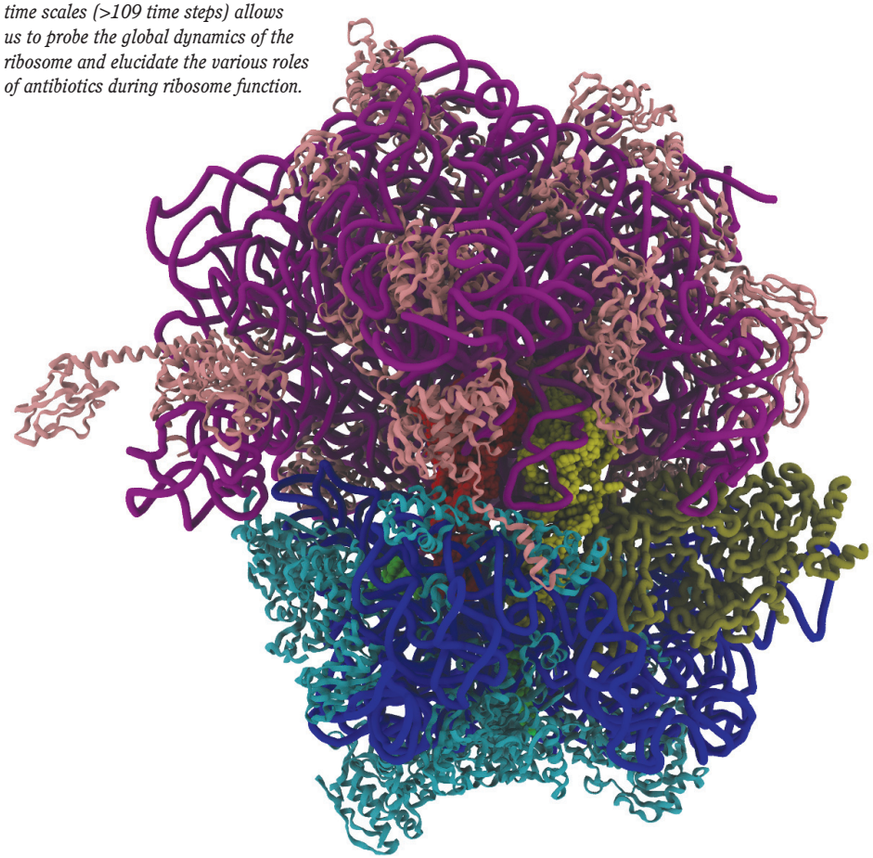
Fig. 1. Atomic structure of a 70S bacterial ribosome. The ribosome is composed of three ribonucleic acid (RNA) chains and over 50 protein chains. When simulating the ribosome with explicit-solvent and ions, simulations can exceed 3 million atoms. Evaluating long-range interactions leads to large computing loads and interconnect demands. Simulating long time scales (>109 time steps) allows us to probe the global dynamics of the ribosome and elucidate the various roles of antibiotics during ribosome function.

Charite Medical School (Berlin, Germany), we also recently developed a novel method for obtaining atomic models from cryogenic-electron microscopy data [3,4]. Due to the streamlined nature of this approach, we have already obtained atomic models for several previously unresolved functional conformations in the ribosome. Through this integrated simulation-experimental approach, we will continue to reveal the details of ribosome dynamics that occur over a vast range of length and time scales.



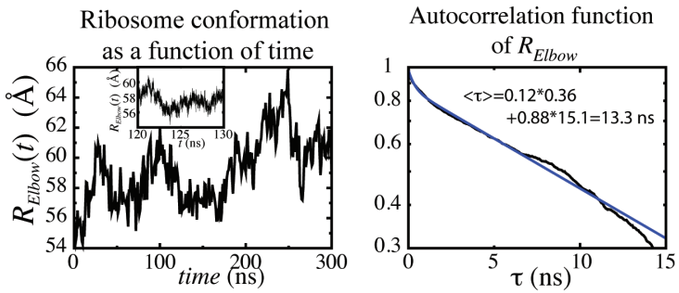


Fig. 2. Probing the dynamic properties of ribosome function (left). Movement inside the ribosome is measured by calculating the time traces for reaction coordinate (in this case RElbow). The shown trace was calculated from what is currently the longest explicit-solvent simulation of a multi-million-atom complex (301 nanoseconds). Measuring the scale of the fluctuations, and the associated decay times (right), allows one to quantify the relationship between kinetics and energetics inside this molecular machine.

[1] Whitford, P.C., et al., *J Am Chem Soc* **132**, 13170 (2010).
[2] Whitford, P.C., et al., *RNA* **16**, 1196 (2010).
[3] Ratje, A.H., et al., *Nature* **468**, 713 (2010).
[4] Whitford, P.C., et al., in preparation.

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