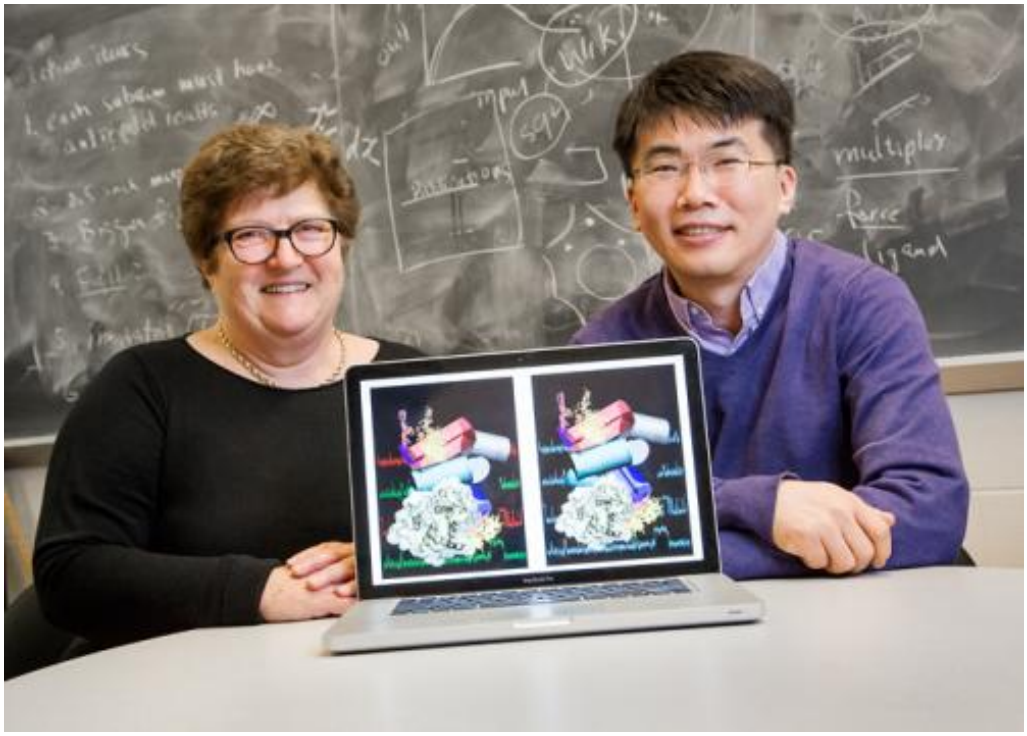
Advanced techniques yield new insights into ribosome self-assembly



University of Illinois chemistry professor Zaida Luthey-Schulten and physics professor Taekjip Ha led a study of how the ribosome assembles itself. Credit: L. Brian Stauffer

Ribosomes, the cellular machines that build proteins, are themselves made up of dozens of proteins and a few looping strands of RNA. A new study, reported in the journal *Nature*, offers new clues about how the ribosome, the master assembler of proteins, also assembles itself.

"The ribosome has more than 50 different parts – it has the complexity of a sewing machine in terms of the number of parts," said University of Illinois physics professor Taekjip Ha, who led the research with U. of I. chemistry professor Zaida Luthey-Schulten and Johns Hopkins University biophysics professor Sarah Woodson. "A sewing machine assembles other things but it cannot assemble itself if you have the parts lying around," Ha said. "The ribosome, however, can do that. It's quite amazing."

In 2000, scientists published precise atomic structures of intact ribosomes (a feat that won them a 2009 Nobel Prize in chemistry) and for decades researchers have delved into the mechanics of ribosome function. But scientists have much to learn about how the ribosome itself is built from its component parts, Luthey-Schulten said.

Solving the atomic structure was a huge step forward "that tells us what the ribosome looks like once it's assembled," she said. "But it doesn't tell you anything about how it gets there, how all these parts come together."

All ribosomes consist of two subunits, each a cluster of precisely folded proteins and RNA. The team focused on the small ribosomal subunit of the E. coli bacterium. It is made up of about 20 proteins and a ribosomal RNA (known as 16S).

The researchers labeled one of those ribosomal proteins. Known as S4, it is thought to be the first to interact with the 16S RNA during assembly. They also labeled two sites on the 16S RNA. Each label fluoresced a different color, and was designed to glow more brightly when in close proximity to another label (a technology known as FRET). These signals offered clues about how the RNA and proteins were interacting.

The team was most interested in a central region of the 16s RNA because it contains signature sequences that differentiate the three cellular "domains," or superkingdoms, of life. Previous studies suggested that this region also was key to the RNA-protein interactions that occur in the earliest stages of ribosome assembly.

Using a "computational microscope," the team compared data from their FRET experiments with an all-atom simulation of the protein and RNA interaction. Their analysis revealed that the S4 protein and the 16S ribosomal RNA were a surprisingly "dynamic duo," Ha said. The protein constrained the RNA somewhat, but still allowed it to undulate and change its conformation.

The team found that the S4 protein tends to bind to the RNA when the RNA takes on an unusual conformation – one not seen in the fully assembled ribosome. This was a surprise, since scientists generally assume that ribosomal proteins lock RNA into its final, three-dimensional shape.

"We found that the S4 and RNA complex is not static," Ha said. "It actually is dynamic and that dynamism is likely to allow binding of the next protein" in the sequence of ribosome assembly.

"Once the S4 binds, it induces other conformational changes that allow the binding sites for other proteins to appear," he said. "So the binding site for the third protein doesn't appear until after the second protein is there."

This intricate dance of molecules leading to the assembly of ribosomes occurs very fast, Luthey-Schulten said. "You can go from as few as 1,000 to 30,000 ribosomes in a bacterial cell during its cell cycle," she said. "More than 80 percent of the RNA that's in the cell is in the ribosomes."

Knowing how the ribosome is put together offers new antibiotic targets, said Ha, who is a Howard Hughes Medical Institute investigator and a co-director of the Center for the Physics of Living Cells at Illinois.

"Instead of waiting until your enemy has fully assembled its army, you want to intervene early to prevent that from happening," he said. "We know that this protein/RNA region has unique signatures in bacteria, so maybe we can target this process while keeping the human ribosome intact."

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More information: "Protein-Guided Dynamics During Early Ribosome Assembly," DOI: 10.1038/nature13039