Research Profile

Dissecting the kinetics of self-assembly

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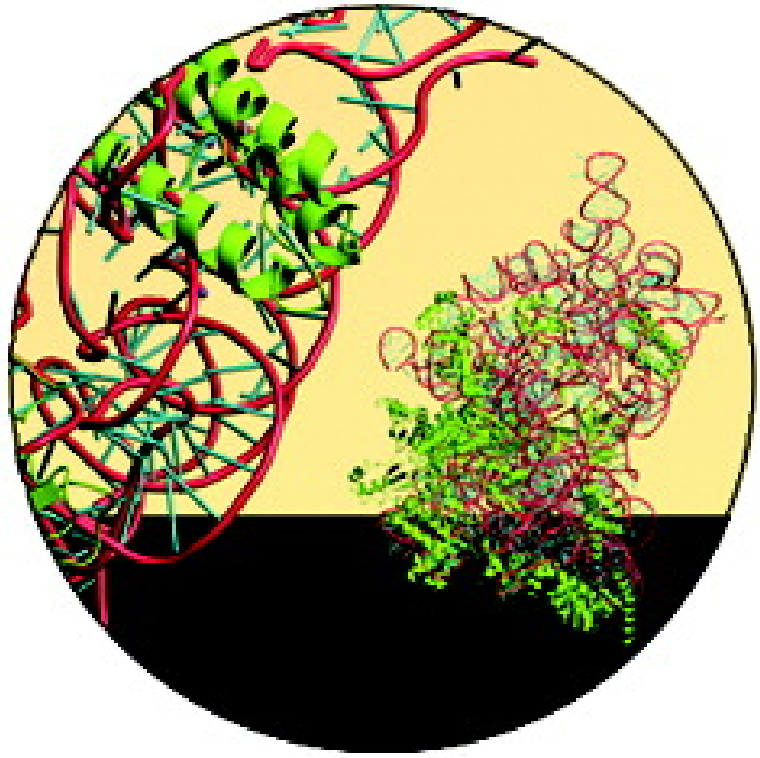

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RESEARCH PROFILE

Dissecting the kinetics of self-assembly

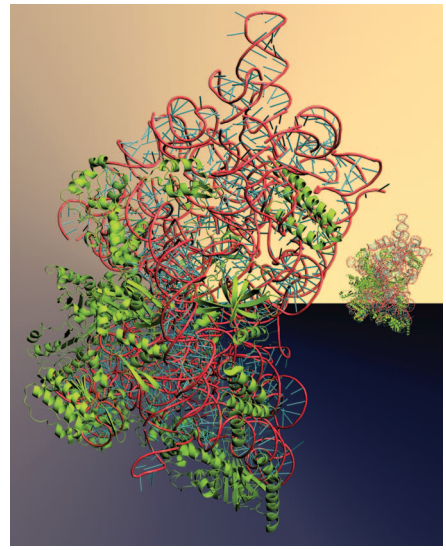
After 7 years of persistent labor, James Williamson and colleagues at Scripps Research Institute have developed an accurate, quantitative approach to kinetic studies of in vitro and in vivo ribosome assembly (*Anal. Chem.* **2008**, DOI 10.1026/ac8020505). The small (30S) ribosomal subunit is a truly remarkable device, Williamson says, because it can assemble itself in vitro from purified components without help. But Williamson had been frustrated by the inability to simultaneously study more than one or two steps in the process. The new method allows the researchers to concurrently study the binding kinetics of all 20 30S subunit proteins for the first time.

"With this analytical method in hand, we should be able to determine how temperature, magnesium concentration, protein concentration, et cetera, affect self-assembly," Williamson says. "We also hope to identify kinetic intermediates."

In the new method, the researchers first label all 20 protein building blocks of the *E. coli* 30S ribosomal subunit with ^{15}N. They then incubate an equimolar mixture of the proteins with 16S RNA (the subunit's sole RNA molecule) for varying amounts of time. The "pulse" of heavy isotope is followed by a "chase" of unlabeled versions of the same proteins. After the subunits form, the researchers dismantle them and determine what fraction of each protein in the complex is labeled with ^{15}N. The greater the fraction, the earlier a protein bound to the nascent subunit.

The proteins are identified by ESI TOFMS of tryptic digests. The peptides elute from the chromatography column in pairs because each peak contains both labeled and unlabeled versions of the protein, and the researchers take advantage of this occurrence. Even though the two peptide flavors are indistinguishable chemically, MS can separate them by m/z. "So if a peptide has 20 nitrogens, there will be a spacing of 20 daltons in the mass spectrum," Williamson explains. "That gives us a check on the identity of the peptides."

Primary MS data are used to quantify the peptides. But the group also performs a nonlinear least-squares fit to the entire profile of the $^{15}N-^{14}N$ pairs. "We use a Fourier transform convolution method that enables us to calculate the isotope distribution for both components and fit the line with the amplitude of the peak," Williamson says. "That improves precision by allowing us to integrate the entire set of features in the spectrum."



Crystal structure of the ribosome 30S subunit from *E. coli*. The binding kinetics of all 20 proteins in the assembly can now be studied simultaneously. (Structure from the Protein Data Bank: 1pnx, *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 8682–8687)

With the new method, the investigators discovered that protein S7, which initiates assembly of the 30S ribosome's 3′-domain, uniquely binds with biphasic kinetics. "So there are two populations of S7 in the sample: one that binds quickly and another that binds slowly," Williamson says. "That is unambiguous evidence for two different RNA conformations that bind this protein at different rates."

Two proteins—S2 and S21—proved to be renegades because they jumped on and off the subunit during the chase, obscuring their binding kinetics. "That was a bit of a surprise, because they are stably bound to the ribosome," Williamson says, noting that these two proteins bind to the subunit only after the other 18 are in place.

Although the 30S ribosome can self-assemble in vitro, it may not do the same within the cell. In vivo, the proteins attach to the RNA while it is still being made. Other proteins, including GTPases, helicases, and enzymes that process RNA, also assist with assembly inside a cell. By making assembly more efficient, those helpers might be especially important when bacteria have to cope with stressful conditions, such as cold, heat, lack of nutrients, or antibiotics. "But based on thermodynamic studies, it looks as if the pathway we're studying in vitro is at least related to what you see in cells," Williamson says. His group is now determining how assembly factors can benefit ribosomes in vitro and has also started to perform pulse–chase experiments with whole cells.

The new approach could be used to study the assembly of any complex particle, such as the proteasome, the spliceosome, and the degradosome, whose components can be identified and purified. But the large *E. coli* subunit and eukaryotic ribosomes head Williamson's to-do list. "This methods paper is just the tip of the iceberg," he says. "The lab is about to explode with studies of ribosome biogenesis."

—Linda Sage