Crystallography of ribosomes

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Electron density maps of the small ribosomal subunit from *Thermus thermophilus* (T30S) and the large subunit from *Haloarcula marismortui* (H50S) were constructed. These maps show recognizable morphologies as well as internal features similar to those observed by cryogenic electron microscopy. They contain elongated dense regions that span the particles in various directions and are traceable as RNA chains as well as globular patches of lower density, readily distinguishable from the above.

The small ribosomal subunits exhibit the lowest level of stability and the highest level of flexibility among the ribosomal particles. Multiconformational states were suggested to account for the inconsistencies in locations of selected components revealed by surface probing or by monitoring the ribosomal activity. Thus, the early T30S crystals yielded satisfactory data only to 10–12 Å resolution. However, treatment with a W cluster improved the crystal quality dramatically and high-quality diffraction data to 3.2 Å were collected at the Advanced Photon Source beamline 19-ID.

The current 3.6 Å electron density map of the small subunit shows the traditional division of this subunit into three main parts: a head, a neck, and a bulky lower body. A large part of this map has been interpreted as 800 RNA nucleotides by visual fittings of known motifs of RNA chains as well as by the placement of all structures of ribosomal proteins that were determined individually by x-ray crystallography or NMR. The features identified revealed diverse modes of inter-component recognition.

To facilitate unbiased map interpretation, markers inserted in predetermined sites are being exploited. These markers are composed of heavy-atom compounds attached either directly to the T30S particle or through carriers that bind to the ribosomal particles with high affinity. Candidates are antibiotics, complementary DNA (cDNA) oligomers, charged tRNA molecules, and factors participating in the translation process. In this way, various exposed RNA strands were targeted, the position of the 3' end of the 16S RNA was identified, and two surface cysteines belonging to proteins S11 and S13 were located.

The packing diagram of the crystals of H50S provides possible reasoning for the odd combination of the properties of these crystals: high resolution (2.7 Å) accompanied by problematic diffraction. The high resolution may result from the extensive interparticle interactions that are concentrated in parts of the unit cell. In contrast, there is only a small interparticle contact area along the c axis (564 Å), which is surrounded by an extremely large solvent region. The small number of inter-particle interactions may cause the poor isomorphism, the unit cell polymorphism, the unfavorable crystal habit (thickness of a few microns), and the multilayer structure.

Based on the packing rearrangement performed within the crystals of the small subunits, controlled conformational engineering, carried out either before crystallization or within the crystals, led to improved diffraction. Employing similar strategies to large ribosomal subunits form *T. thermophilus*, diffraction to 3.4 Å was observed from crystals that previously led to 9–10 Å resolution.