1.7 Cryocrystallography of native and derivatized ribosomal crystals

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Ribosomes are the universal supramolecular assemblies responsible for the translation of genetic information, encoded in mRNA, into proteins. A typical bacterial ribosome contains more than 250 000 atoms, has a molecular weight of about 2.3×10^6 and a sedimentation coefficient of 70S. It is composed of two subunits of unequal size (small: 30S, molecular weight 0.85×10^6, and large: 50S, molecular weight 1.45×10^6), which associate upon the initiation of the biosynthetic process. It contains three chains of rRNA (about 5500 nucleotides), accounting for two-thirds of its mass and 57–73 different proteins, depending on the source of the ribosome.

Of all the intracellular organelles, only ribosomes have thus far been crystallized. X-ray crystallography has recently seen major advances in the sophistication, effectiveness, efficiency, and accuracy of data collection and interpretation. Nevertheless, being ribonucleoprotein assemblies, which are notoriously flexible, unstable, and routinely prepared as conformationally mixed populations, even the best crystals of ribosomes dictate unconventional conceptual and technical approaches.

Systematic exploration of crystallization conditions combined with sophisticated seeding has led to reproducible growth of ordered three-dimensional crystals of ribosomal particles from halophilic and thermophilic bacteria, diffracting best to 2.9 Å resolution (Fig. 1). In addition, complexes of ribosomes with components of protein biosynthesis, mimicking defined functional states, as well as mutated and chemically modified ribosomal subunits have been crystallized (Table 1). In all cases, the crystalline ribosomal particles retain their integrity and biological activity for long periods despite their natural tendency to disintegrate rapidly.

In this chapter we will describe our progress in collecting and processing crystallographic data from ribosomal crystals, highlight our special problems, and discuss our strategy.

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