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Preparation of ribosomes for smFRET studies: a simplified approach

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Abstract

During the past decade, single-molecule studies of the ribosome have significantly advanced our understanding of protein synthesis. The broadest application of these methods has been towards the investigation of ribosome conformational dynamics using single-molecule Förster resonance energy transfer (smFRET). The recent advances in fluorescently labeled ribosomes and translation components have resulted in success of smFRET experiments. Various methods have been employed to target fluorescent dyes to specific locations within the ribosome. Primarily, these methods have involved additional steps including subunit dissociation and/or full reconstitution, which could result in ribosomes of reduced activity and translation efficiency. In addition, substantial time and effort are required to produce limited quantities of material. To enable rapid and large-scale production of highly active, fluorescently labeled ribosomes, we have developed a procedure that combines partial reconstitution with His-tag purification. This allows for a homogeneous single-step purification of mutant ribosomes and subsequent integration of labeled proteins. Ribosomes produced with this method are shown to be as active as ribosomes purified using classical methods. While we have focused on two labeling sites in this report, the method is generalizable and can in principle be extended to any non-essential ribosomal protein.

1. Introduction

A significant component of the central dogma involves the translation of the genetic code to proteins, which function in many structural and enzymatic roles. Translation is a ubiquitous process that is shared across all domains of life. The platform upon which proteins are made is the ribosome, which is assisted by various accessory factors in an elegant and complex mechanism. At ~2.5 MDa, the bacterial ribosome is one of the largest macromolecular complexes of the cell. The ribosome, composed of two subunits (large 50S and small 30S), is assembled from an interwoven mesh of ribosomal RNA (rRNA) and a total of 52 ribosomal proteins. Given the high degree of homology and absolute conservation of specific regions within the structure,

[1] "The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors".

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