SUMMARY

In the first part of the thesis, ribosomes unspecifically labeled with the fluorescent dye Atto655 were tethered onto SiO_2 beads covered with streptavidin. The ribosomes were tethered via protein L4, which was biotinylated *in vivo* and incorporated into the ribosomes.

The ribosomes tethered onto the beads were used for experiments with optical tweezers. The ribosomes synthesized the proteins GFPem and ubiquitin on a bead by adding an *in vitro* transcription-translation mix without histidine, in order to stop synthesis in a His-tag. The N-terminus of the proteins, which was biotinylated during synthesis using the suppressor tRNA technique, was barely sticking out of the ribosomal tunnel and it was accessible to bind via streptavidin to a polystyrene bead, which was trapped with the optical tweezers.

With the optical tweezers it was possible to measure forces in a pN scale. It was shown that in order to keep a steady hook-up, a force lower than 10 pN should be applied, since higher forces lead to a rupture. The next step would be to continue synthesis of the protein and determine the forces applied on the polypeptide chain during synthesis.

In the second part of the thesis, the same kind of ribosomes, unspecifically labeled with the dye Atto655, were tethered onto surfaces via the biotinylated protein L4. These ribosomes were detected on the surface due to their fluorescence using a wide field microscope and they were able to produce a fully active GFPem protein *in situ*. The protein was modified in order to remain attached to the ribosome after synthesis and it was detected due to its intrinsic fluorescence. Time resolved measurements were made and the time of appearance of fully active GFPem proteins synthesized by single ribosomes was observed.

Surface tethering of specifically labeled ribosomes on protein L24 or L29 with the dye Alexa633 (acceptor) followed. These ribosomes synthesized protein GFPem, where the dye BODIPY-TMR (donor) was incorporated during synthesis using the suppressor tRNA technique. During the experiments, the donor was excited at certain times but both donor and acceptor were detected.

Synthesis was stopped again in a His-tag, immediately after the incorporation of BODIPY-TMR. At this point, the two dyes were 111 Å apart, quite far away to

observe FRET. But by continuing synthesis, the appearance of some acceptor fluorescence was observed, exclusively due to FRET. The next step would be to determine exactly the fluorescence intensities of both dyes in a time resolved manner and calculate the distances between them. After that a first impression of how the protein folds co-translationally could be given.