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# Single Molecule Investigations of the Stringent Response Machinery in Living Bacterial Cells

## Background
The RelA-mediated stringent response is at the heart of bacterial adaptation to starvation and stress, playing a major role in the bacterial cell cycle and virulence. RelA integrates several environmental cues and synthesizes the alarmone ppGpp, which globally reprograms transcription, translation and replication.

## Methodology
We have developed single molecule tracking techniques that for the first time make it possible to directly record the very rapid diffusion trajectories of free protein molecules throughout the bacterial cytoplasm [1]. We use Single Particle Tracking to directly validate the enzymatic mechanism of the key regulatory protein RelA in its natural environment, the cytosol of a living *Escherichia coli* cell. We have studied individual RelA enzyme molecules using a purpose-built optical setup, combining super resolution imaging and high-speed time lapse microscopy with stroboscopic laser excitation. This allows us to track individual RelA molecules in their unbound state in the cytosol of living cells, giving us a direct insight into RelA's *in vivo* catalytic cycle. As reference points for ribosome-bound and free RelA, we record trajectories of individual ribosomes, and the very fast diffusive motion of a small cytosolic protein.

## Conclusions
At the heart of RelA's catalytic cycle lies its binding to and dissociation from the ribosome. In our assay we can detect and distinguish both individual RelA molecules in complex with and unbound from the ribosome by their radically different diffusion characteristics. This provides us with a unique opportunity to validate the catalytic cycle of RelA directly in individual living bacteria [2].

[1] B.P. English; A. Sanamrad; S. Tankov; V. Hauryliuk and J. Elf, Tracking of individual freely diffusing fluorescent protein molecules in the bacterial cytoplasm, *arXiv*, 1003.2110v1 [q-bio.QM] (2010).

[2] B.P. English; A. Sanamrad; V. Hauryliuk; S. Tankov; N. Dekker and J. Elf, Single Molecule Investigations of the Stringent Response Machinery in Living Bacterial Cells, *submitted*.