

Single-molecule bacteriology (CRISPR-Cas)

The possibility of conducting non-invasive *in vivo* experiments with single-molecule resolution is having an increasingly profound effect on the life sciences. The Hohlbein group in the Laboratory of Biophysics recently reported on an assay in which fluorescently labelled Cas9 was used to obtain detailed information about CRISPR-Cas DNA interaction states in Gram-positive *L. lactis* (<https://doi.org/10.1101/437137>). In particular, by performing single-particle tracking, we obtained the rates by which dCas9 binds and dissociates from DNA in absence of DNA target sites. To expand our single-molecule, single-cell research, we have various exciting and highly collaborative projects for internships, BSc, and MSc theses available:

High-throughput single cell screening. Since some biological processes such as horizontal gene transfer occur very rarely, the ability to monitor many cells in parallel is very desirable. We developed microfluidic devices for quickly trapping single cells and are now looking for students to optimize experimental parameters as well as adapt software and hardware for single cell detection and segmentation. We have access to fluorescent reporter systems in *L. lactis*, *E. coli*, *P. putida*.

CRISPR-Cas target search in live bacteria. Cas9 can be employed to target specific sites on genomic or plasmid DNA in bacteria allowing, for example, to silence genes and observe the cellular response. To learn more about target finding mechanisms, we want to monitor single Cas units for as long as possible. One of the ideas is to replace the photoactivatable fluorescent proteins currently used to tag Cas9 with bright organic fluorophores. In addition to Cas9, DNA processing enzymes such as Cas12a or prokaryotic argonats promise to expand the available toolkit for genome editing or gene silencing even further and have not been analyzed at the single molecule level *in vivo*.

Single-molecule, single-cell microscopy, data acquisition and data analysis are performed at the Laboratory of Biophysics. For the developing biological assays, we closely collaborate with different groups in Wageningen (Host-Microbe Interactomics, Laboratory of Microbiology, Laboratory of Biochemistry, Systems and Synthetic Biology,...).

