

Master Student Project

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Diffusion and localization of proteins involved in *E. coli* division and cell shape regulation with single molecule precision

Protein motion and the dynamics of subcellular structures in living cells are underexplored but can be researched by single cell biophysics. Most of the methods used for *in vivo* diffusion measurements such as fluorescence correlation spectroscopy (FCS) and fluorescence recovery after photobleaching (FRAP) are based on ensemble observations of labeled protein within a desired area. *In vivo* FCS and FRAP measurements of bacterial cells, e.g. *E. coli*, are also restricted in spatial precision because of their small confined volume compared to eukaryotic cells. The analyzed volume in FCS is big compared to the size of expected structures, and in FRAP typically half of the cell is bleached.

These limitations are overcome using the newly invented single molecule displacement measurement/mapping (SMdM). It is super-resolution microscopy technique combining fluorescence photo activatable localization microscopy (fPALM) with high-precision stroboscopic illumination applied in HILO (highly inclined laminated optical sheet) mode [1,2]. SMdM allows to measure displacements of single fluorophore molecules, from which the diffusion coefficients and the localization density of macromolecules can be calculated.

Some proteins involved in bacterial cell division have been shown to have membrane colocalization essential for their functioning. This membrane-protein interaction is disturbed in cells treated with CCCP – a protonophore dissipating the electrochemical proton gradient across membrane [3]. So the aim of this project is to investigate membrane-associated clustering/unclustering of selected *E. coli* proteins (FtsA, MinD and MreB) in cells treated with different protonophores and/or antibiotic inhibiting septation.

This ambitious project call for a motivated student that wants to develop and improve a new super-resolution technique and learn or master a broad experimental skill set including:

- Gene cloning and expression
- Cell cultivation and handling
- *In vivo* super-resolution microscopy (SMdM)
- Programming in Python 3

- [1] Xiang, L., Chen, K., Yan, R. et al. Single-molecule displacement mapping unveils nanoscale heterogeneities in intracellular diffusivity. *Nat Methods* 17, 524–530 (2020).
- [2] Śmigiel W., Mantovanelli L., Linnik D., et al. Protein diffusion in *Escherichia coli* cytoplasm scales with the mass of the complexes and is location dependent. *Sci Adv* 8-12, eabo5387 (2022)
- [3] Strahl H. and Hamoen L. Membrane potential is important for bacterial cell division. *PNAS* 107(27), 12281-12286 (2010)