# Testing of imaging buffers for Tag-PAINT

Motivation

Different single-molecule localization microscopy (SMLM) methods exist to circumvent the diffraction limit. Self-labeling protein tags e.g. HaloTag are one of a the possibilities to achieve super resolution microscopy PAINT (. point accumulation for imaging in nanoscale topography), as some ligand labeled with fluorophore are irreversible binding with modified Halo Tag. As we all know the salt concentration, pH and temperature will change proteins conformation and influence the interaction between Halo Tag and ligand. This give us a way to control the image condition by optimizing imaging buffer.

Task Description

For improving Tag PAINT with regard to localization precision and acquisition time, different imaging buffers will be tested. As model system the vimentin fused with modified Halo Tag in stable U2OS cell line will be used. The Halo Tag could be directly targeted by giving imaging buffer contains suitable ligand concentration to evaluate the effect of the labeling strategy on the quality of the super-resolved image. The binding time of single signal clusters will be analyzed as the scale of binding behavior.

Exchangeable probes

non-reactive HaloTag Ligands (nrHTLs)
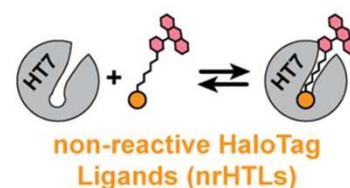
Key References

1. Frei, M.S., et al., *Engineered HaloTag variants for fluorescence lifetime multiplexing.* Nature methods, 2022. **19**(1): p. 65-70.
2. Hoelzel, C.A. and X. Zhang, *Visualizing and Manipulating Biological Processes Using HaloTag and SNAP-Tag Technologies.* Chembiochem: a European journal of chemical biology, 2020. **21**(14): p. 1935.

Work Area

Laboratory

Microscopy

Data Analysis

Programming

Time

Possible Start

Feb. 2022

Duration

4 weeks

Contact

Marius Glogger, Yunqing Li

glogger (at) chemie.uni-frankfurt.de

li (at) chemie.uni-frankfurt.de

Language

Single Molecule Biophysics