Nanobody Detection of Standard Fluorescent Proteins Enables Multi-Target DNA-PAINT with High Resolution and Minimal Displacement Errors

Nazar Oleksiievets [1], Shama Sograte-Idrissi [2,3,4], Sebastian Isbaner [1], Mariana Eggert-Martinez [2,3,4], Jörg Enderlein [1], Roman Tsukanov [1] and Felipe Opazo [2,3]

[1]Third Institute of Physics-Biophysics, Georg August University, 37077 Göttingen, Germany
[2] Institute of Neuro- and Sensory Physiology, University Medical Center Göttingen, 37073 Göttingen, Germany
[3]Center for Biostructural Imaging of Neurodegeneration (BIN), University of Göttingen Medical Center, 37075 Göttingen, Germany
[4]International Max Planck Research School for Molecular Biology, Göttingen, Germany
E-mail : nazar.oleksiievets@uni-goettingen.de

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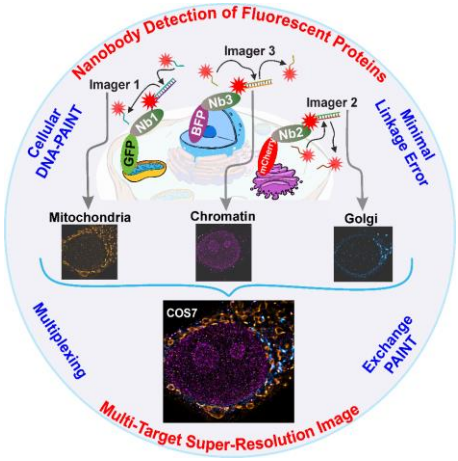

Figure 1: Graphical abstract

Abstract: DNA Point Accumulation for Imaging in Nanoscale Topography (PAINT) is a rapidly developing fluorescence super-resolution technique, which allows for reaching spatial resolutions below 10 nm. It also enables the imaging of multiple targets in the same sample [1]. However, using DNA-PAINT to observe cellular structures at such resolution remains challenging. Antibodies, which are commonly used for this purpose, lead to a displacement between the target protein and the reporting fluorophore of 20–25 nm, thus limiting the resolving power. Here, we used nanobodies [2] to minimize this linkage error to ~4 nm. We demonstrate multiplexed imaging by using three nanobodies, each able to bind to a different family of fluorescent proteins. We couple the nanobodies with single DNA strands via a straight forward and stoichiometric chemical conjugation. Additionally, we built a versatile computer-controlled microfluidic setup to enable multiplexed DNA-PAINT in an efficient manner. As a proof of principle, we labeled and imaged proteins on mitochondria, the Golgi apparatus, and chromatin. We obtained super-resolved images of the three targets with 20 nm resolution, and within only 35 minutes acquisition time [3].

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