MODIFIED APTAMERS ENABLE QUANTITATIVE SUB-10 NANOMETER CELLULAR DNA-PAINT IMAGING

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KEY WORDS: labeling reagents, super-resolution, DNA-PAINT, aptamers, quantitative imaging

DNA points accumulation in nanoscale topography (DNA-PAINT) is a simple implementation of single-molecule localization microscopy that makes use of transient binding of dye-labeled DNA strands to complementary target-bound strands, thereby enabling spatial resolution better than 5 nm, as recently demonstrated with artificial DNA nanostructures [1]. However, this resolution is not easily translated to imaging cellular targets since commonly used labeling probes are relatively large (~150 kDa in case of antibodies) or only available for a limited number of targets (Nanobodies). Here, we use Slow-Off-rate Modified Aptamer (SOMAmer) reagents [2] as small (7-30 kDa), quantitative, and versatile labeling probes. These DNA aptamers contain modified bases with hydrophobic residues facilitating higher specificity and affinity against a wide range of protein targets [3]. In addition, SOMAmer reagents can be easily extended with a single fluorophore or DNA-PAINT docking site providing the capability of quantitative super-resolution microscopy on a single protein level.
To demonstrate the achievable resolution, specificity, and multiplexing capability of SOMAmers, we labeled and imaged both transmembrane and intracellular targets in fixed and live cells [4].

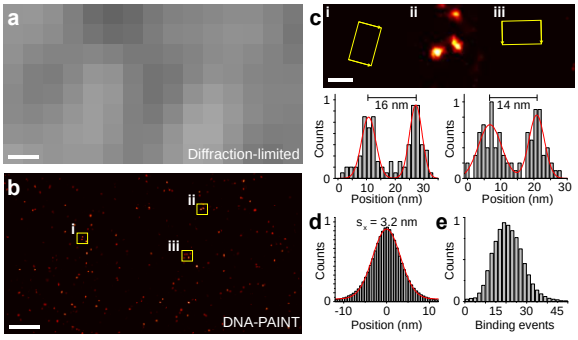

Figure 1: a) Diffraction limited image of EGFR. **b)** DNA-PAINT super-resolution image from same area as shown in a. **c)** Cross-sectional histogram analysis in i and iii respectively demonstrates high-resolution DNA-PAINT imaging of single EGFR proteins using a SOMAmer label. **d)** Fitting a Gaussian distribution to the center-of-mass aligned single-molecules localizations of ~34,000 SOMAmer labeled EGFR proteins yields a localization precision of 3.2 nm. **e)** qPAINT analysis of single EGFR proteins yields a unimodal distribution of binding events, confirming quantitative labeling of EGFR by SOMAmer reagents. Scale bars: 200 nm (a,b), 20 nm (c).

References

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