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

Advances in optical super-resolution techniques make it possible to image biological processes below the classical diffraction limit of light. DNA Points Accumulation in Nanoscale Topography (DNA-PAINT) is a simple implementation of single-molecule localization microscopy with spatial resolution better than 5 nm demonstrated on 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.

We successfully demonstrate two different SOMAmer labeling probes for DNA-PAINT: (1) against the transmembrane protein EGFR (Figure 1) in A431 cells and (2) against GFP demonstrated in a Nup107-GFP HeLa cell line.

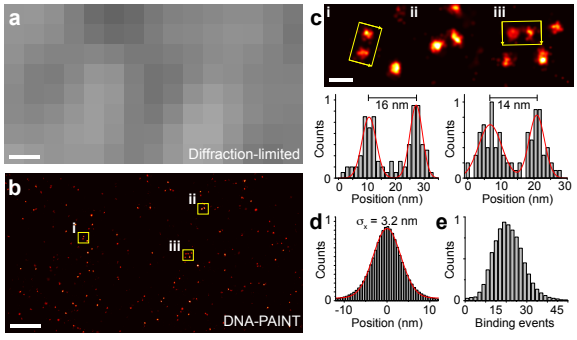

Figure 1: a) Diffraction limited image of EGFR. **b)** DNA-PAINT super-resolution image from same area as shown in a. **c)** Cross-sectional histogram analysis in i and iii respectively demonstrates high-resolution DNA-PAINT imaging of single EGFR proteins using a SOMAmer label. **d)** Fitting a Gaussian 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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