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Fast, multiplexed superresolution imaging of HER2 signaling in breast cancer with DNA-PAINT-ERS

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Abstract

Super resolution microscopy (SRM) comprises various single-molecule localization techniques that can generate images at the 20 nm scale. In recent years, SRM based on DNA point accumulation in nanoscale topology (DNA-PAINT) has become increasingly useful for biological imaging for its robust capability for multiplexing. However, the practical use of DNA-PAINT has been limited by slow imaging speed. Here, we introduce DNA-PAINT-ERS, a set of strategies that can be easily integrated into current workflows for both accelerated DNA-PAINT and improved image quality.

In DNA-PAINT, single-molecule localization events arise from reversible hybridization between a docking strand (DS) oligo immobilized on an antibody and a complementary fluorophore-conjugated imager strand (IS) oligo diffusing in solution. We found that commonly used DS-IS pairs exhibited slow binding and unbinding kinetics and proposed a set of new strategies, collectively termed E-R-S (hence the term DNA-PAINT-ERS), that significantly improves the imaging speed of DNA-PAINT. We demonstrate the general applicability of DNA-PAINT-ERS for multiplexed SRM in merely 2-5 minutes per target using previously validated oligonucleotide constructs. Additionally, we showed that DNA-PAINT-ERS significantly improved the quality of the resulting images over current DNA-PAINT.

These advances have allowed us to use DNA-PAINT-ERS for the imaging of HER2 signaling in breast cancer. HER2 is a member of the epidermal growth factor (EGF) receptor family, and HER2 gene amplification and/or protein over-expression is commonly associated with human malignancies such as breast cancer. Using multicolor SRM based on DNA-PAINT-ERS, we can now image many different targets involved in the nanoscopic organization and signaling of HER2. The imaging results start to suggest a new mechanism that could lead to persistent HER2 signaling upon HER2-targeted therapy, thus contributing to therapeutic resistance.