Literature Report

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BRIEF COMMUNICATION

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Left-handed DNA-PAINT for improved super-resolution imaging in the nucleus

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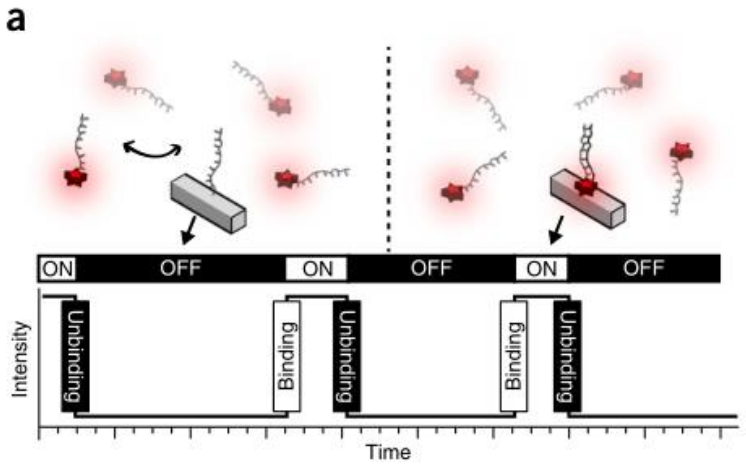
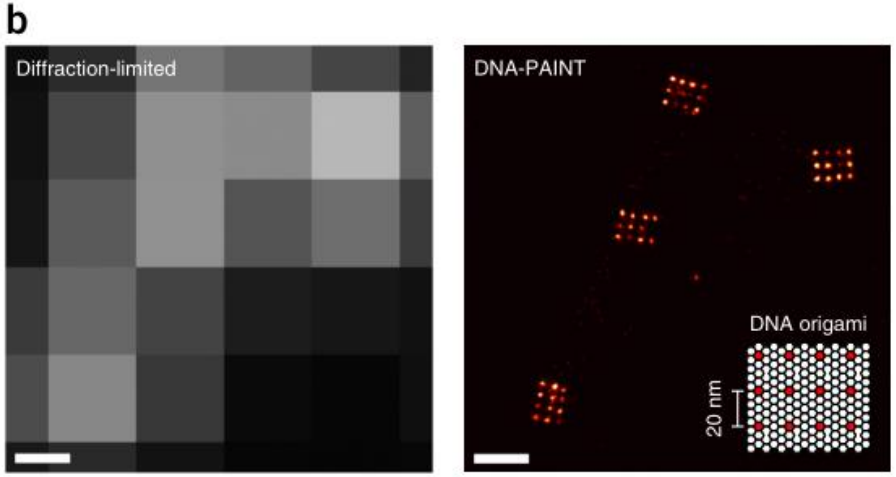
Professor for Membrane Biochemistry

- Developing superresolution microscopy as a readout of protein function
- Understanding the organization and function of cellular septin filaments.
- Understanding how the plasma membrane is affected by membrane-cytoskeleton interactions.

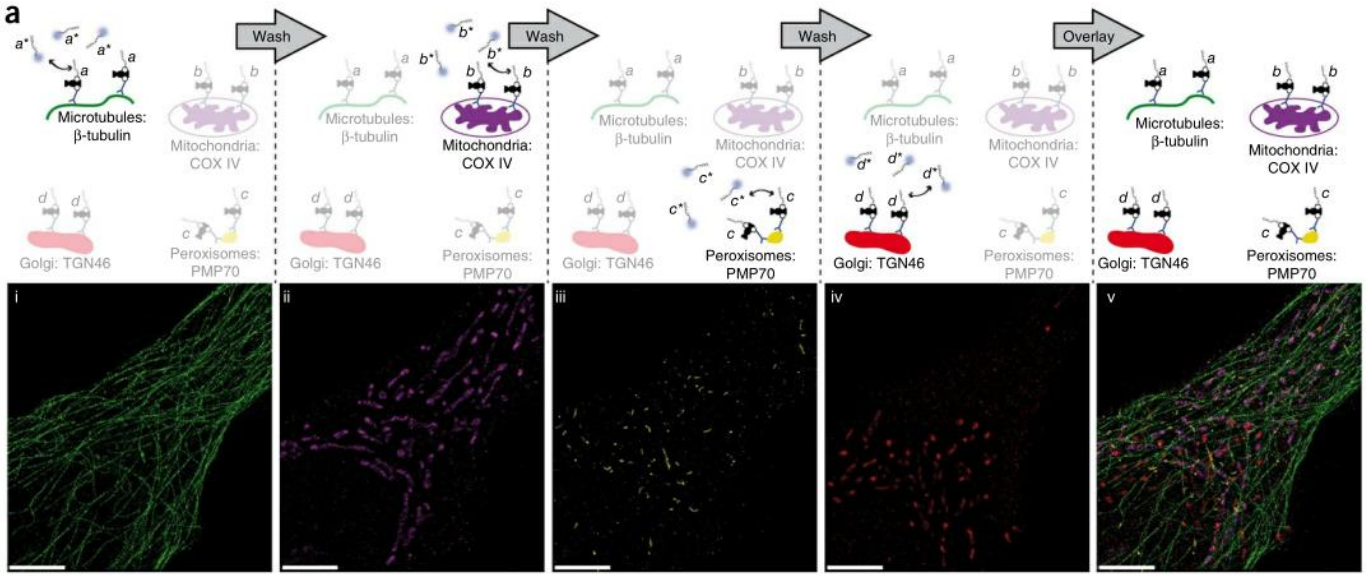
DNA-PAINT

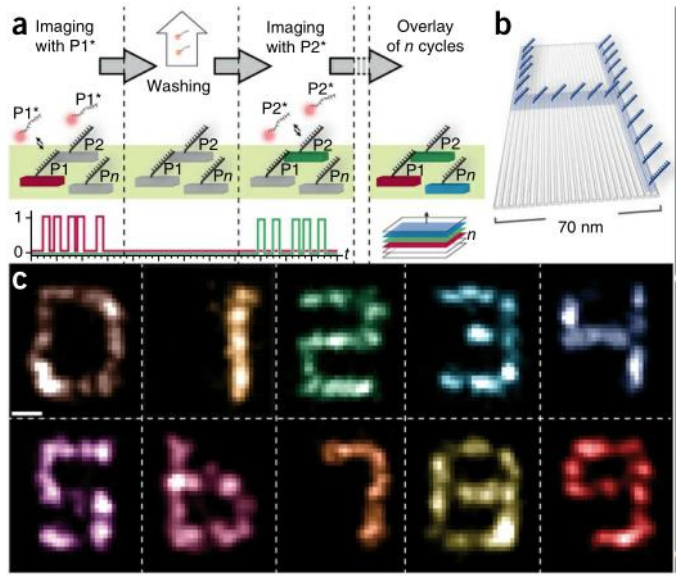




Advantage:
No photobleaching
No photoswitching of dyes necessary

Limite:
hydrophobic interactions
electrostatic coupling

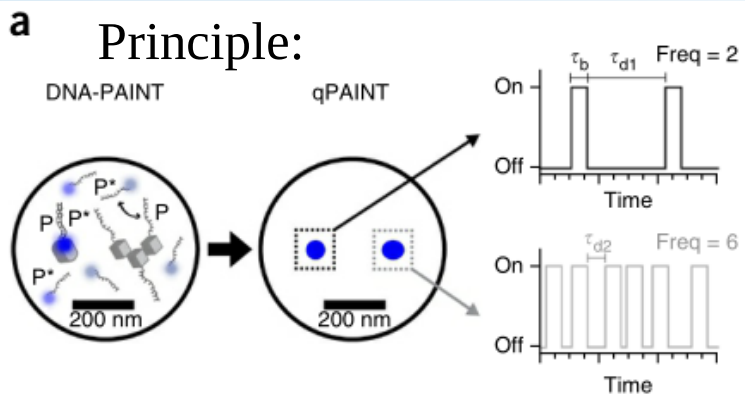


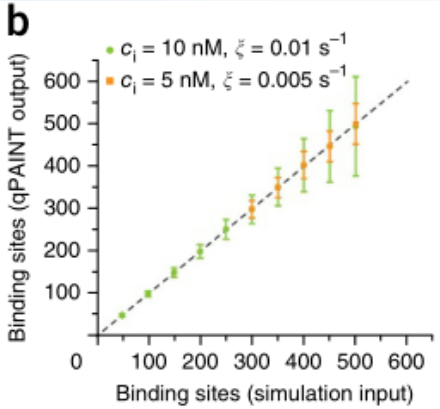


Nat methods-2014-313
Nat protoc-2017-1198

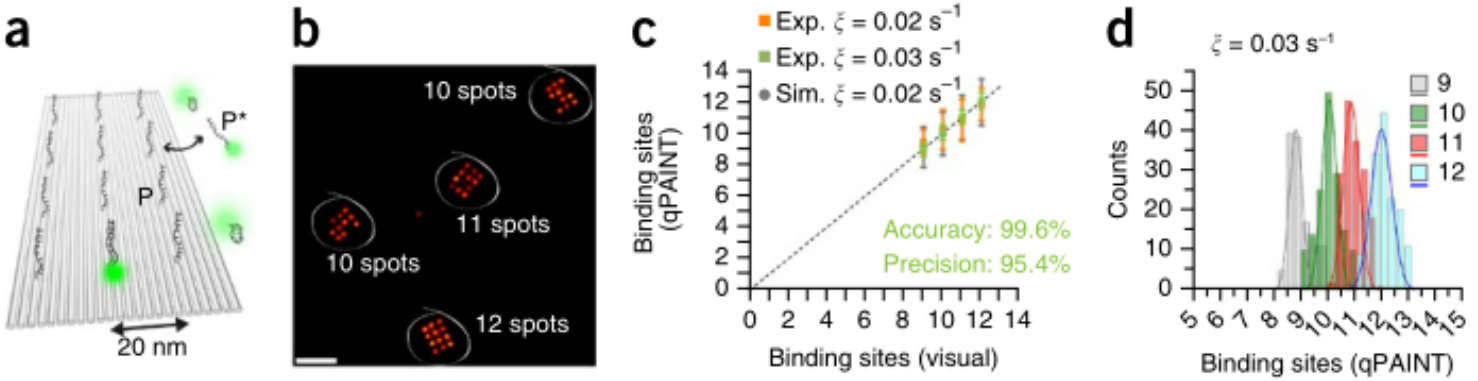
qPAINT 定量统计

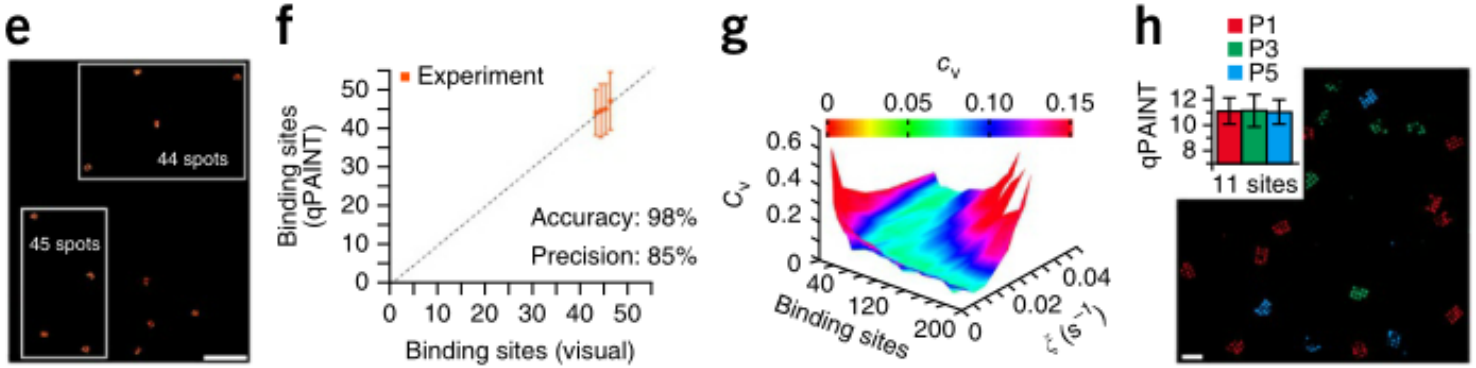
Principle:

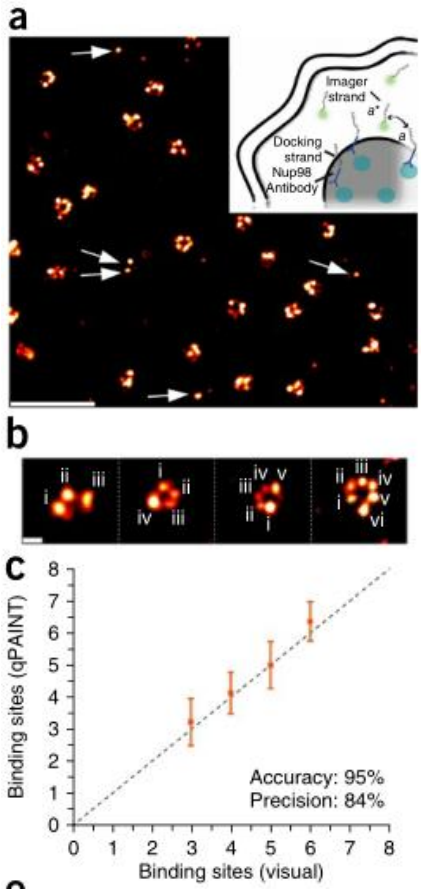




$$(k_{on} \times c_i \times \tau_{d^*})^{-1} = (\xi \times \tau_{d^*})^{-1}$$



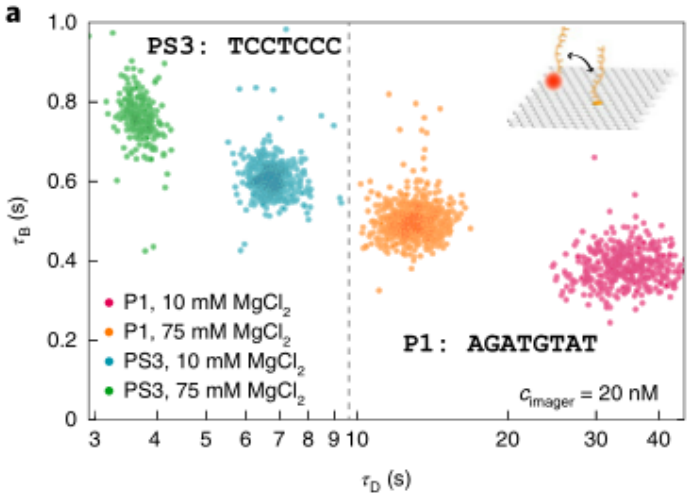


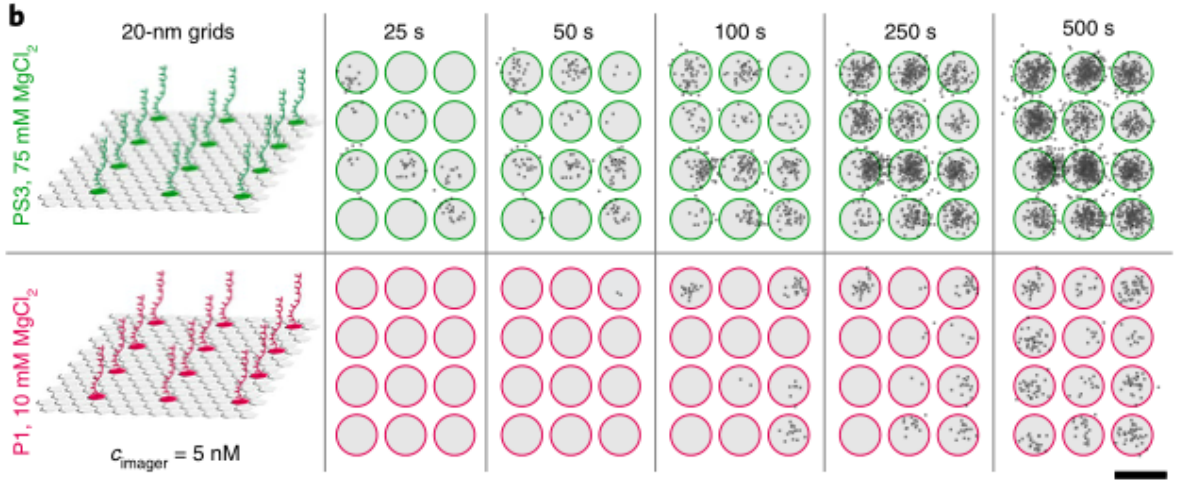


核孔复合体蛋白的定量计算

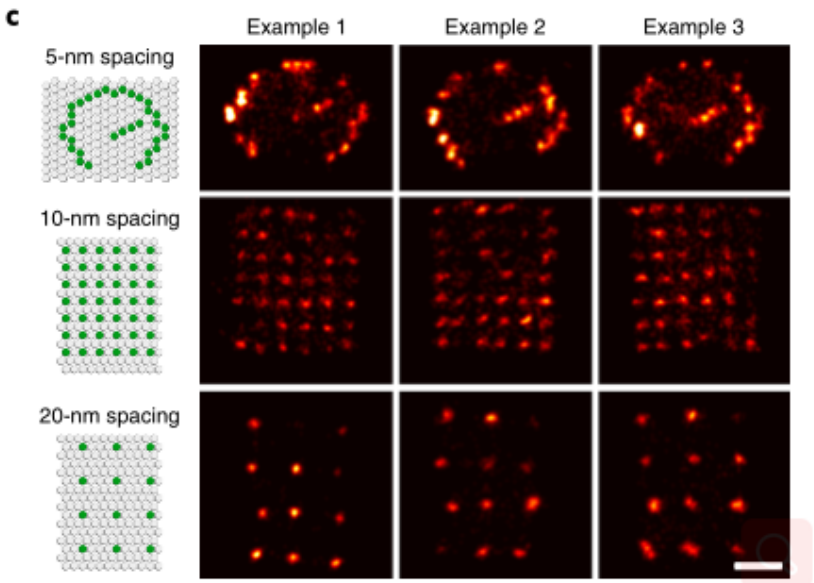
Nat methods-2016-439

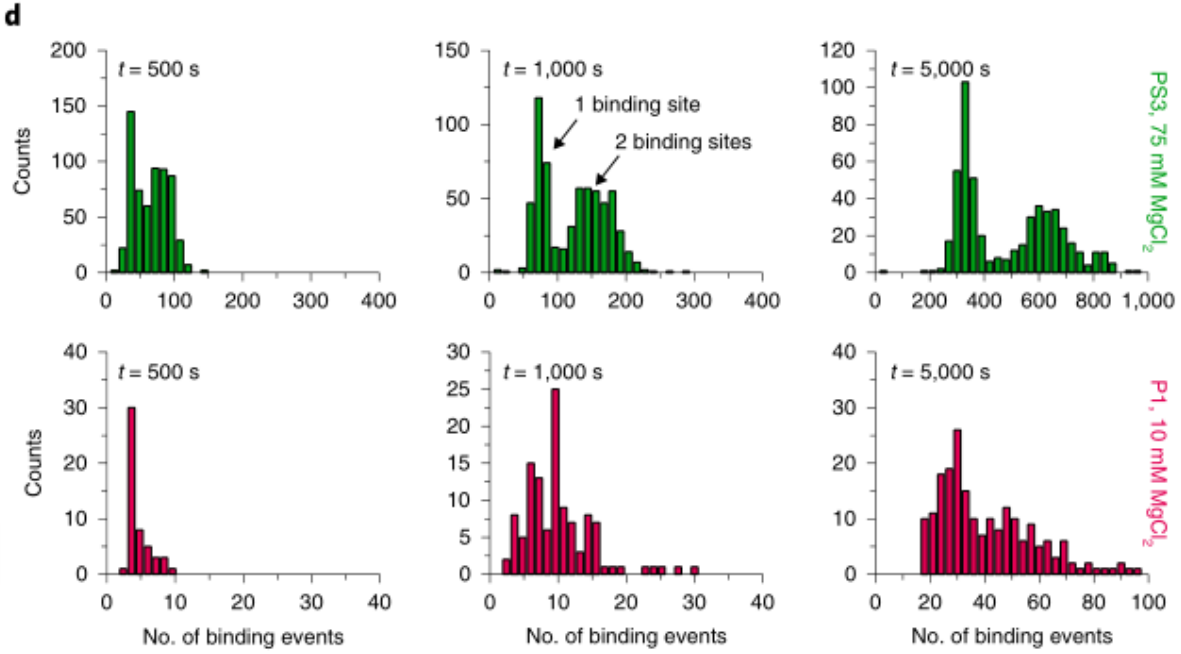
fast PAINT 提升速度

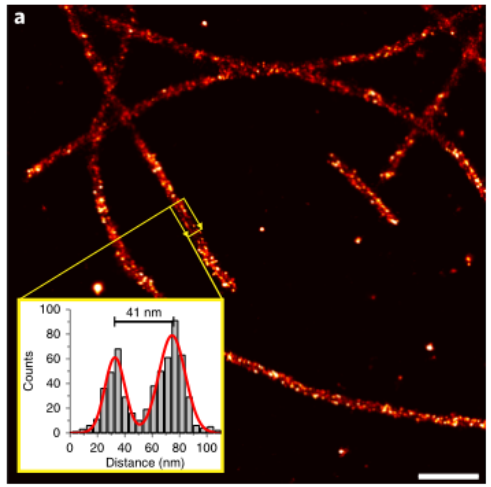




筛选条件：
核酸序列
缓冲液浓度



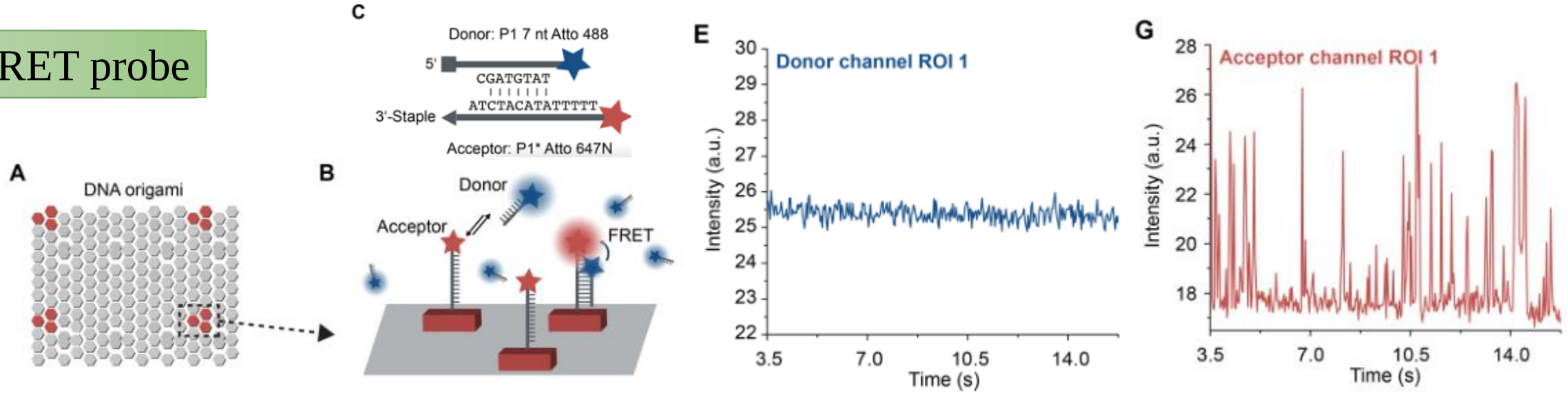




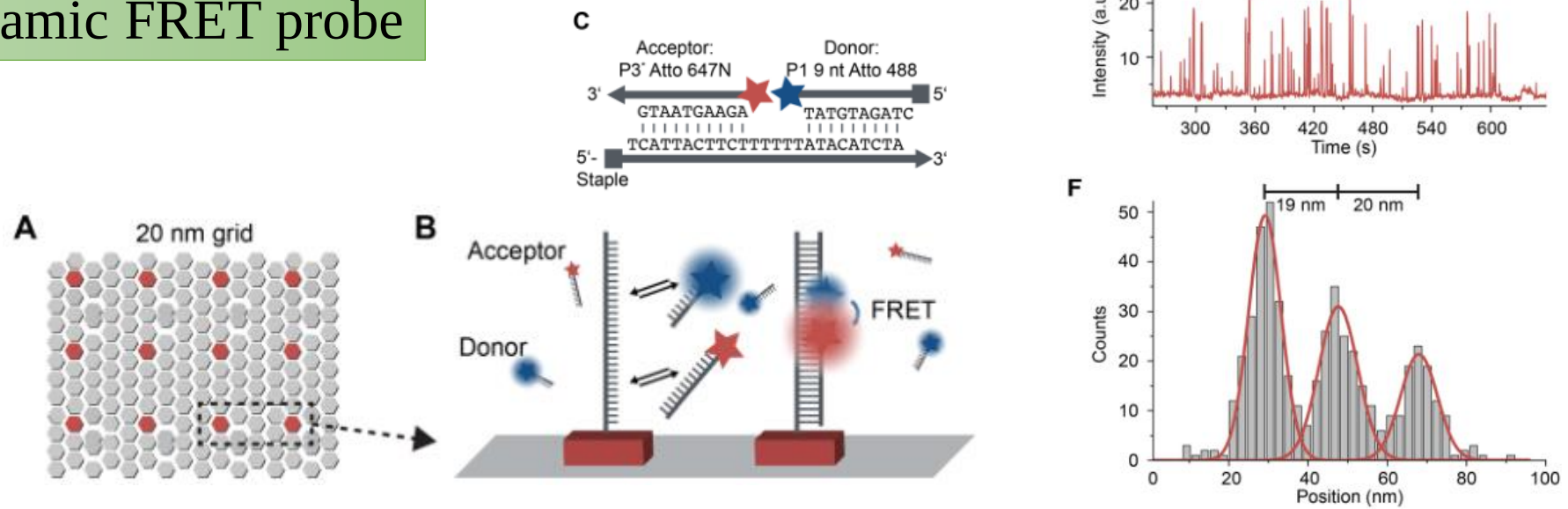
Nat Methods-2019-1101

FRET-PAINT 提升速度以及降低背景

FRET probe

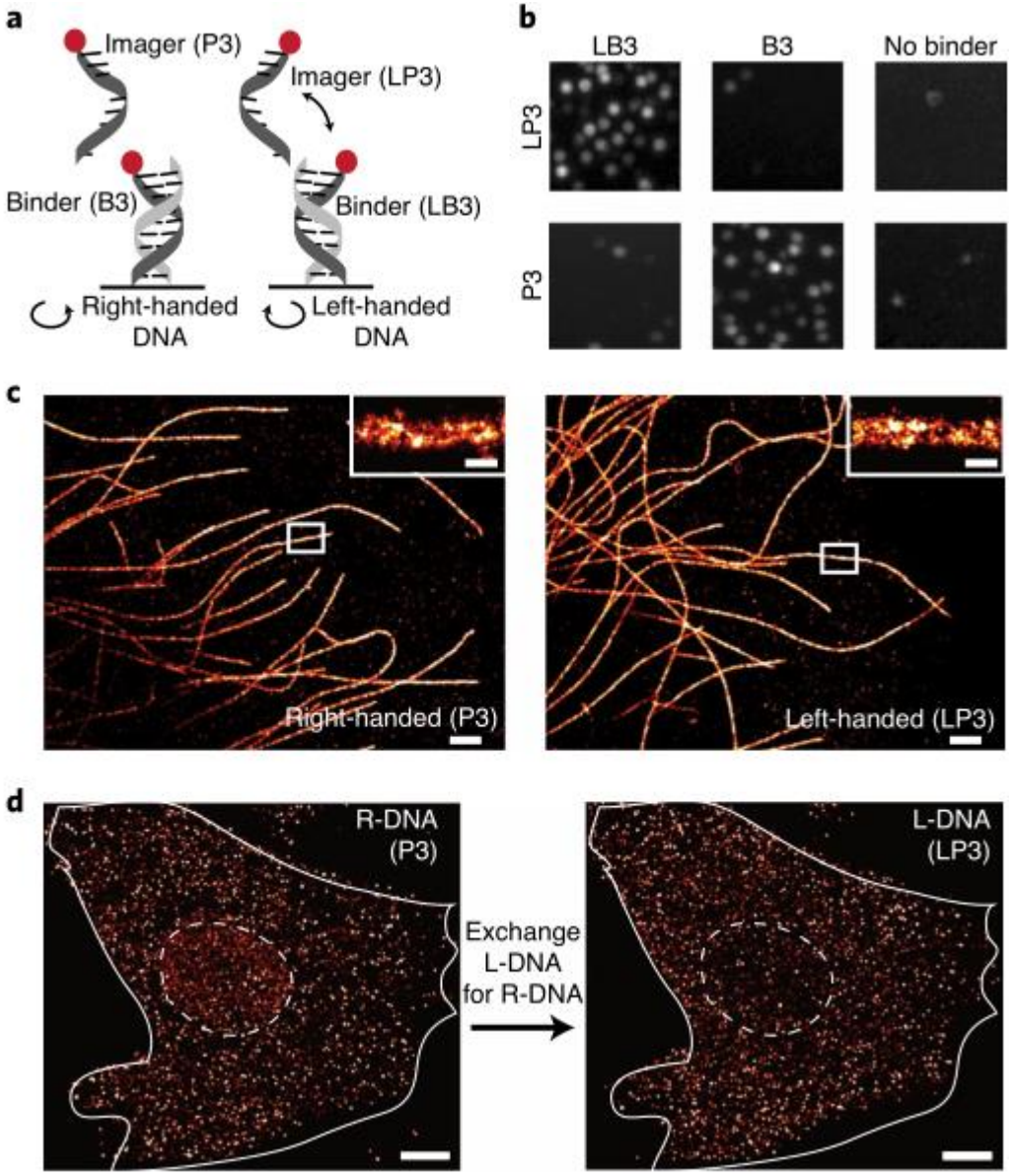


Dynamic FRET probe

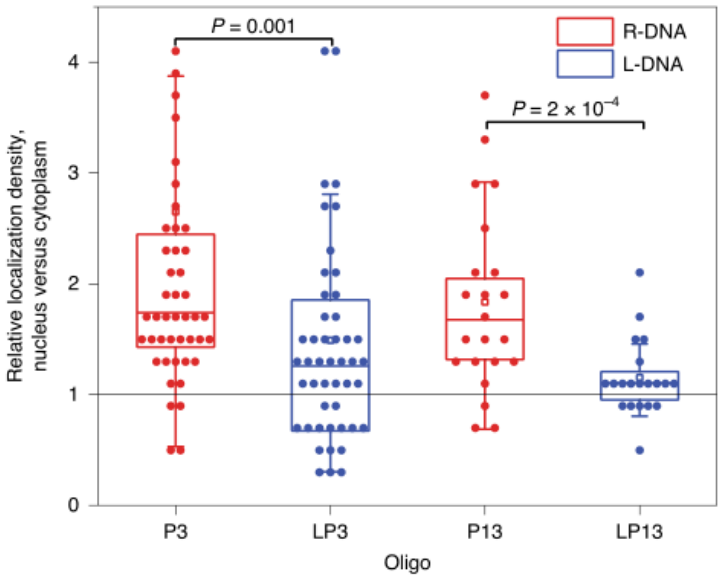


Nano Lett-2017-6428

Left-handed DNA-PAINT 设计



Oligo	Nucleotide sequence (3′–5′)
P1-Atto655	Atto655 - CTAGATGTAT
P1-Cy3B	Cy3B - CTAGATGTAT
LP1	CTAGATGTAT
B3	TTTCTTCATTA
LB3	TTTCTTCATTA
P3	GTAATGAAGA
LP3	GTAATGAAGA
LB12	TTAGTTAGAGC
P12	GCTCTAACT
LP12	GCTCTAACT
P13	CCTTCTCTA
LP13	CCTTCTCTA



对接链5’端修饰叠氮或者生物素
成像链3’ 端修饰荧光染料

Fig. 1 | Comparison of R-DNA and L-DNA oligomers for DNA-PAINT. **a**, Schematic overview of right- and left-handed DNA-PAINT. Transient hybridization events of fluorophore-labeled right- and left-handed DNA imager oligomers, with their respective binder oligomer temporally immobilizing them for single-molecule localization. **b**, Left-handed (LP3) and right-handed (P3) imager oligomers were added to surface-immobilized left-handed (LB3), right-handed (B3) binder oligos or no oligos, respectively. Right-handed imagers were detected only in the presence of right-handed binders, left-handed imagers only in the presence of left-handed binders. Single-molecule images are 5 × 5 μm². **c**, Reconstructed DNA-PAINT images generated from R- and L-DNA-PAINT experiments on immunostained microtubules in HeLa cells. Insets show representative microtubule segments. Scale bars, 1 μm (insets, 100 nm). **d**, Experimental scheme for the detection of nonspecific binding of P3 and LP3 imager strands in nuclei of HeLa cells. Fixed cells were imaged in the presence of P3 and then washed and imaged in the presence of LP3 in identical buffer and at the same concentration. Scale bars, 2 μm. **e**, Plot of relative localization density in the nucleus versus cytoplasm for different imager sequences. Sequence-identical R- and L-DNA imagers are sequentially imaged in the same cell, showing persistent enhancement of nuclear binding in the former. The horizontal line provides visual guidance for equal nuclear and cytoplasmic binding. *P* values were obtained by two-sided paired *t*-tests of 48 cells in ten independent experiments for P3 and LP3, and of 20 cells in two independent measurements for P13 and LP13. In boxplots, center is the median, boxes are interquartile range (IQR) and whiskers are 1.5 × IQR; mean values are represented by open squares.

Left-handed DNA-PAINT

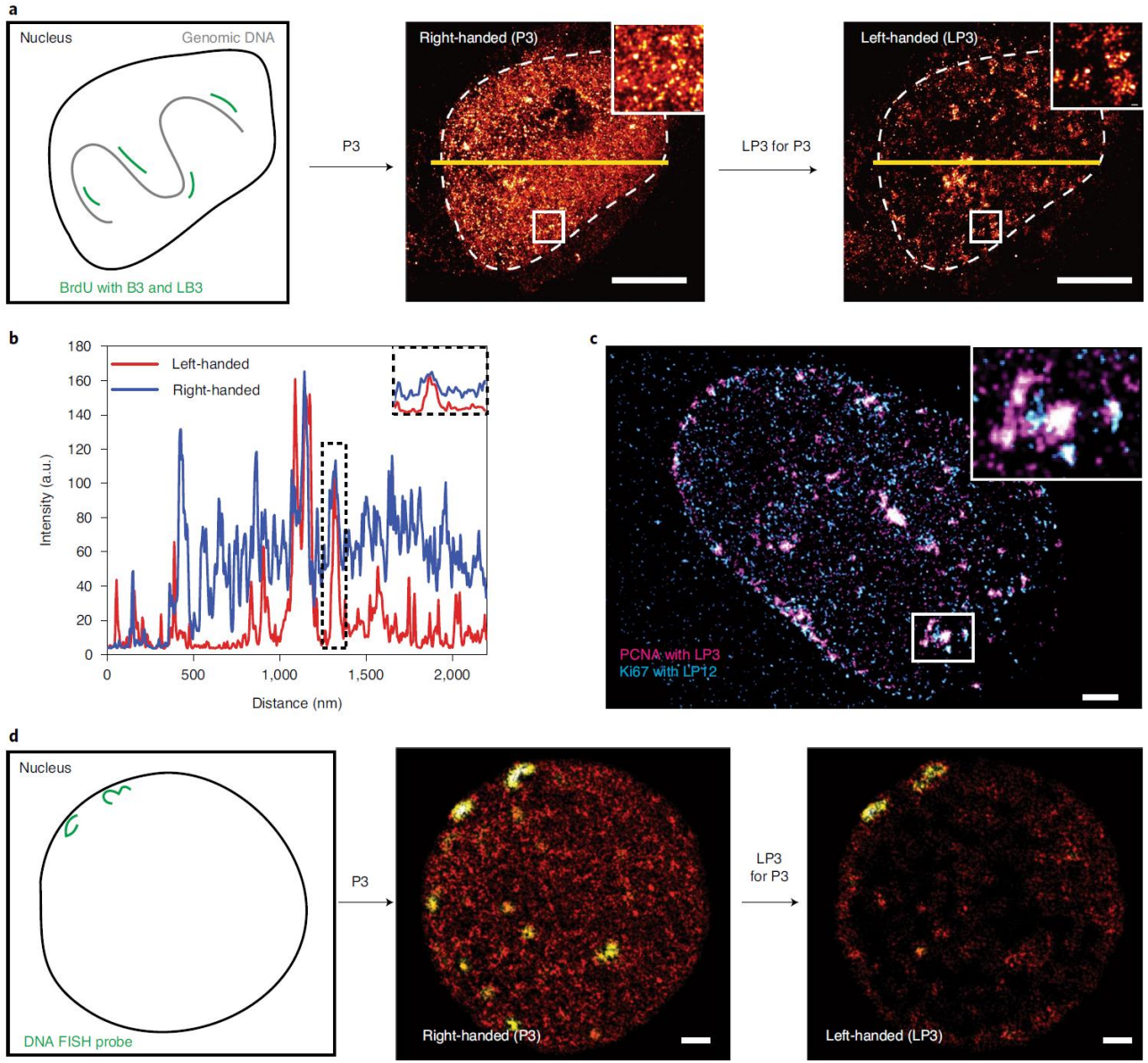


Fig. 2 | comparison of L- and r-DNA-PAINT for nuclear targets. **a**, Experimental setup for the imaging of DNA replication foci by incorporation of BrdU into chromosomes. BrdU incorporation sites were stained with a 1:1 mix of B3- and LB3-coupled antibodies and were visualized by both R- and L-DNA-PAINT. **b**, Intensity profiles of a cross-section of the BrdU-stained cell in a, colored yellow. The insets show that P3- and LP3-stained cells display the same local maxima, but the background intensity in the latter is notably reduced. **c**, L-DNA-PAINT multiplexing experiment in a HeLa cell nucleus stained with a GFP nanobody, coupled to LB3, directed against overexpressed GFP-PCNA and Ki67 labeled with an antibody linked to LB12. **d**, L-DNA-PAINT FISH experiment on HEK293T cells harboring two copies of an integrated HHV-6A genome. Integrated viral DNA was labeled with an HHV-6A-specific probe, coupled to B3 and LB3 in an equimolar ratio. Subsequent R- and L-DNA-PAINT experiments facilitated the detection of the two viral DNA loci. R-DNA-PAINT via P3 resulted in an increased amount of background localization, hampering the detection of specific FISH loci. Scale bars, 2 μm. a.u., arbitrary units.

HHV-6A：黄色部分