Imaging in Cells via DNA Device Technology:

- **DNA-PAINT**: A Super-Resolution Imaging Technique
- **HCR FISH**
- **mRNA Imaging in Cells**
  - a. Ratiometric bimolecular beacons (RBMBs)
  - b. Multiply labeled tetravalent RNA imaging probes (MTRIPs)
  - c. Nanoflares
DNA-PAINT: A Super-Resolution Imaging Technique

- Short, fluorescent labeled DNA imager strands are used to bind transiently to complementary docking strands attached to a target.
- The spontaneous binding and unbinding causes the fluorescence at a given point to switch between the on and off state, thus allowing individual target sites to be imaged with sub-10-nm resolution using total internal reflection microscopy.
- The reversible nature of DNA-PAINT means that it is not limited by the number of fluorophores, and sequential labelling allows the reuse of fluorescent dyes.

In situ imaging of mRNA in fixed cells: HCR FISH (Choi, H. M. T., Beck, V. A. & Pierce, N. A. Next-generation in situ hybridization chain reaction: higher gain, lower cost, greater durability. ACS Nano 8, 4284–4294 (2014).)

Left: Initiator strands I1 and I2 hybridize to a target mRNA, which triggers a polymerization reaction between the two fluorescently labeled hairpin monomers H1 and H2. As a result, the target mRNA is connected to multiple fluorophores and can be visualized using fluorescence microscopy.

Right: Confocal microscopy images at different z planes in a zebra fish embryo. HCR probes are used to identify four different mRNAs (red: Tg(k1:egfp); blue: tpm3; green: elevl3; yellow: ntlα).

(Choi, H. M. T., Beck, V. A. & Pierce, N. A. Next-generation in situ hybridization chain reaction: higher gain, lower cost, greater durability. ACS Nano 8, 4284–4294 (2014).)
Detection of a Single-Nucleotide Variation Using DS

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Detection of a single-nucleotide variation using strand displacement probes:

Left: Reaction mechanism. Mutant and wild-type probes compete for binding to a target mRNA. Because binding kinetics strongly depend on toehold sequence, each probe type primarily binds to the cognate mRNA. Co-localization of single-nucleotide variation detection probes with multiple mRNA-targeting guide probes further shows that the signal is indeed triggered to the mRNA.

Right: Fluorescence micrographs of BRAF mRNA detected using ‘guide probes’ (image 1), wild-type probes (image 2) and mutant probes (image 2). Image 4 shows mRNA classified as wild type or mutant. SNV, single-nucleotide variation.
**mRNA Imaging in Cells**

**Ratiometric bimolecular beacons (RBMBs)**


**Top:** Binding to a target mRNA separates the reporter dye (red dot) from the quencher (black dot), which results in high fluorescence. Multiple RBMBs can bind to the tandem repeat targets in the 3’UTR of a heterologous mRNA, thereby enabling visualization of a single transcript in living cells. A reference dye (pink dot) is used to control cell-to-cell variation in molecular beacon delivery.

**Bottom:** Fluorescence images of HT1080 cells using RBMB and FISH probes for the same mRNA.  
Image 1: Fish probes; Image 2: RBMB reporter dye;  
Image 3: A merged image that also includes nuclear DAPI stain (blue).
Multiply labeled tetravalent RNA imaging probes (MTRIPs)


**Top:** MTRIPs consist of multiple fluorophore labeled oligonucleotides attached to streptavidin (purple). Multiple MTRIPs can be designed to hybrid to a target mRNA, making single mRNA visible in living cells.

**Bottom:** Deconvoluted confocal microscopy images of individual beta-actin mRNA in a A549 cell.

Image 1: MTRIPs;
Image 2: Scrambled probes;
Image 3: A merged image that includes nuclear DAPI stain.
mRNA Imaging in Cells

Nanoflares:


Left: A Nanoflare contains long ‘capture strands’ and fluorophore-labeled ‘flare strands’, which are initially quenched by the gold nanoparticle. Target mRNAs can bind to ‘capture strands’, displace the ‘are strand’ and trigger an increase in fluorescence.

Right: Confocal fluorescence microscopy images of HeLa cells treated with either control Nanoflares (left) or Survivin (target mRNA) Nanoflares (right).