

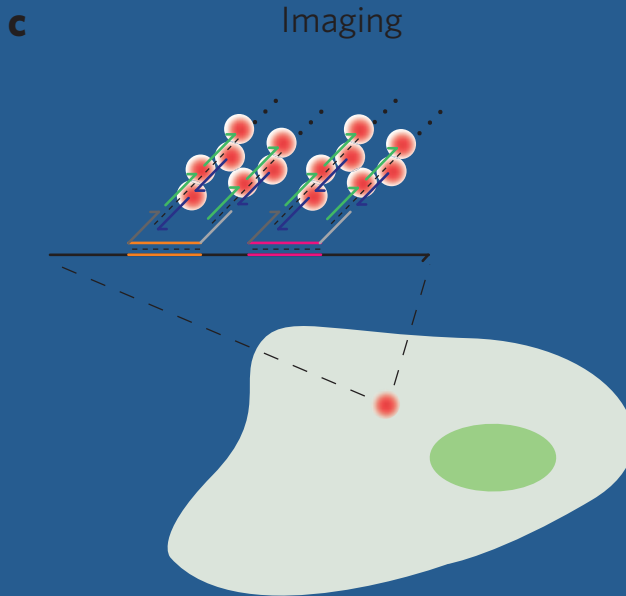
Imaging in Cells via DNA Device Technology:

Topics:

- **HCR FISH: Use HCR to Amplify Output**

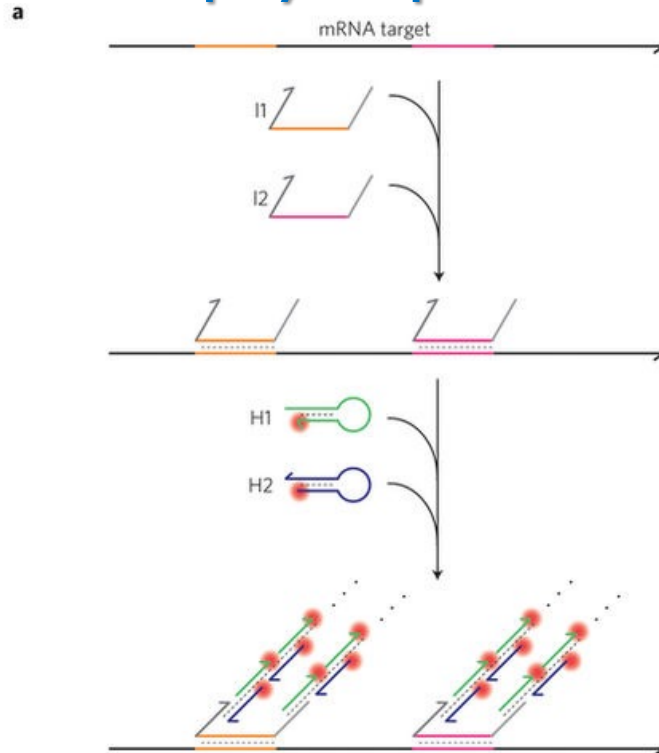
- **mRNA Imaging in Cells**
 - a. **Ratiometric bimolecular beacons (RBMBs)**
 - b. **Multiply labeled tetravalent RNA imaging probes (MTRIPs)**
 - c. **Nanoflares**

Applications of DNA nanotechnology at the interface with biology.

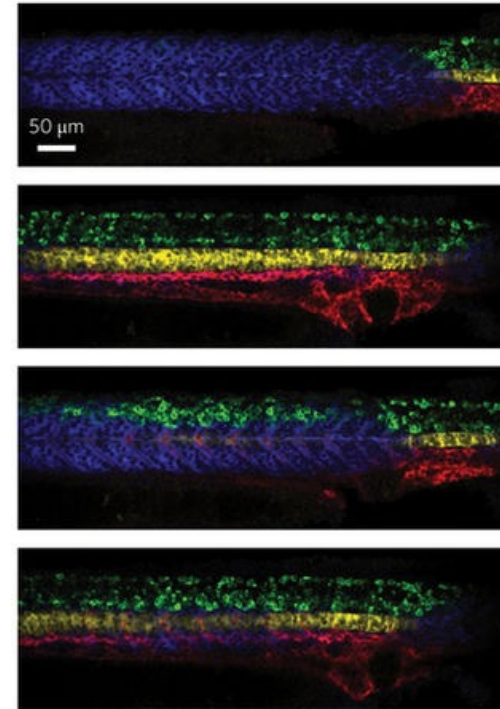


A novel class of sensitive and specific imaging probes that takes advantage of DNA-based amplification mechanisms can be programmed to sequence-specifically interact with cellular RNA. (Choi, H. M. T., Beck, V. A. & Pierce, N. A. Next-generation *in situ* hybridization chain reaction: higher gain, lower cost, greater durability. *ACS Nano* **8**, 4284–4294 (2014)).

In situ imaging of mRNA in fixed cells: HCR FISH Use HCR to Amplify Output

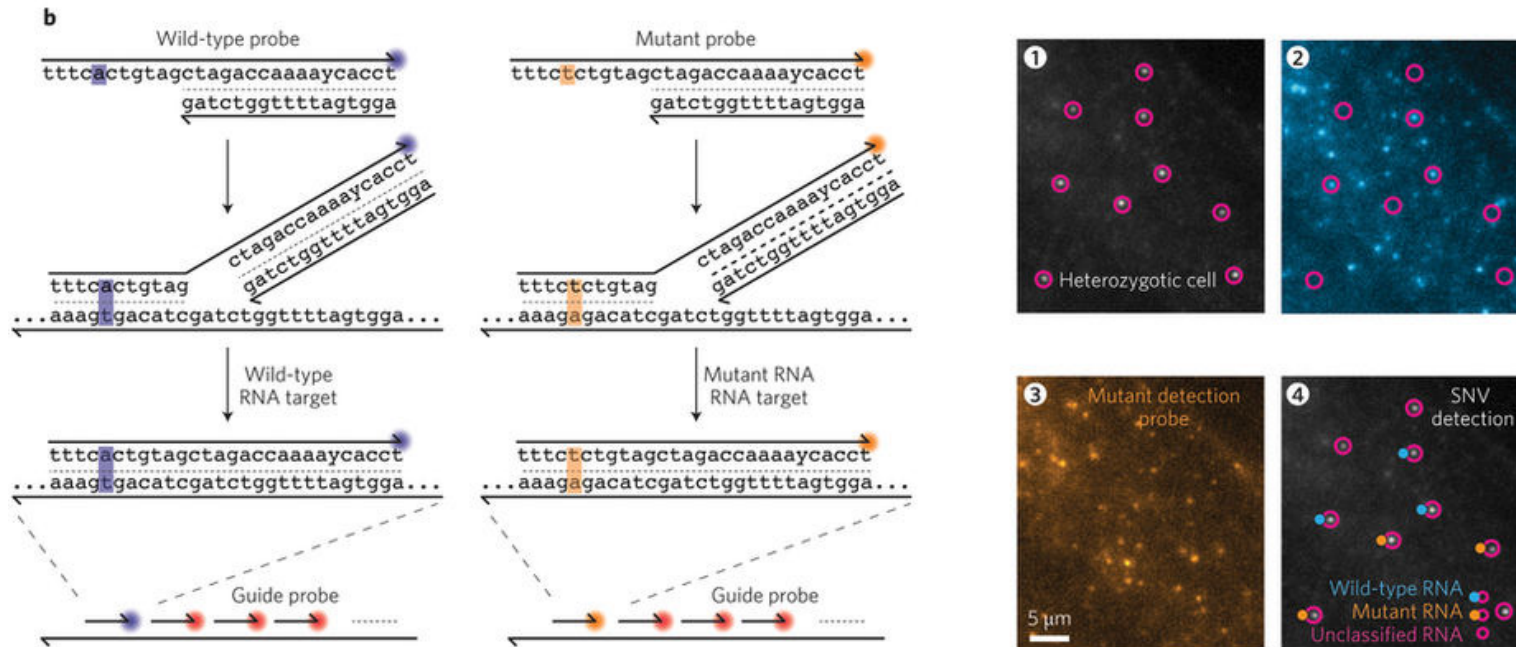


Initiator strands I1 and I2 hybridize to a target mRNA, which triggers a polymerization reaction between the two fluorescently labeled hairpin monomers H1 and H2. As a result, the target mRNA is connected to multiple fluorophores and can be visualized using fluorescence microscopy.



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: elev13; yellow: ntlα).

Detection of a Single-Nucleotide Variations Using DS



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).)

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

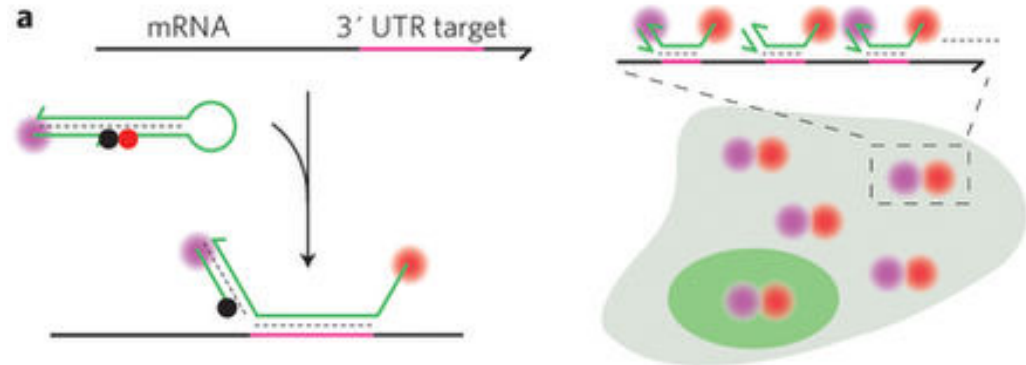
Left: Reaction mechanism. Mutant and wild-type probes compete for binding to a target mRNA. Because binding kinetics strongly depend on toehold sequence, each probe type primarily binds to the cognate mRNA. Co-localization of single-nucleotide variation detection probes with multiple mRNA-targeting guide 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 3). Image 4 shows mRNA classified as wild type or mutant. SNV, single-nucleotide variation

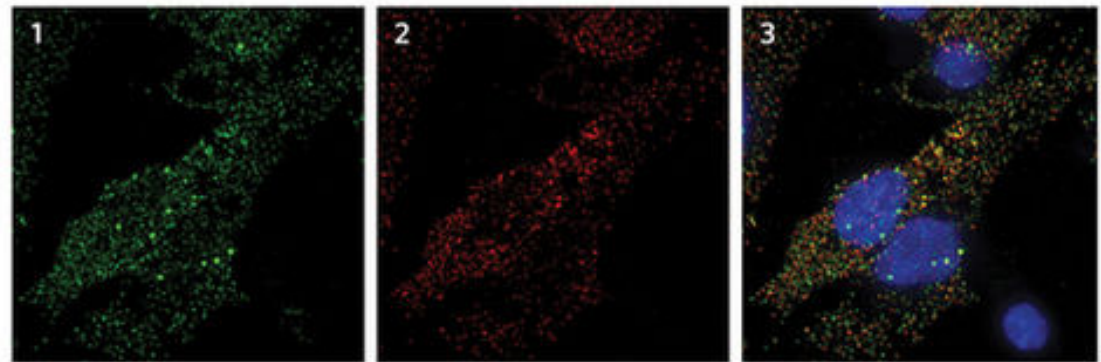
mRNA Imaging in Cells: Ratiometric bimolecular beacons (RBMBs)

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, at also includes nuclear DAPI stain (blue).



Fluorescence images of HT1080 cells using RBMB and FISH probes for the same mRNA:



Fish probes

RBMB reporter dye

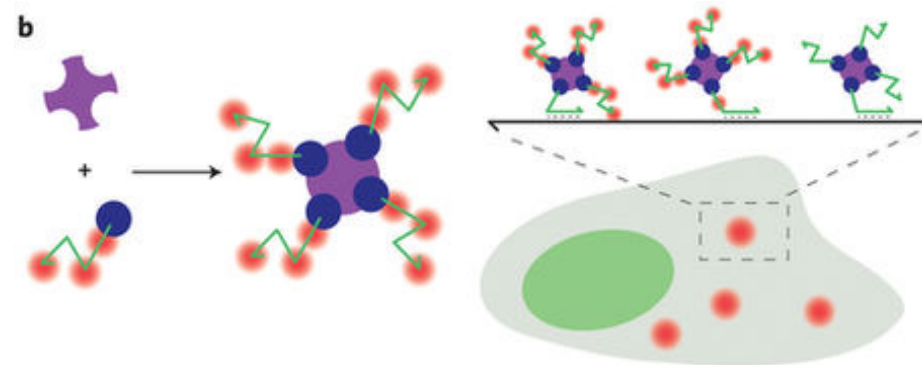
A merged image that also includes nuclear DAPI stain (blue).

Zhang, X., Song, Y., Shah, A. & Lekova, V. Quantitative assessment of bimolecular beacons as a tool for imaging single engineered RNA transcripts and measuring gene expression in living cells. *Nucleic Acids Res.* 41, e152 (2013).

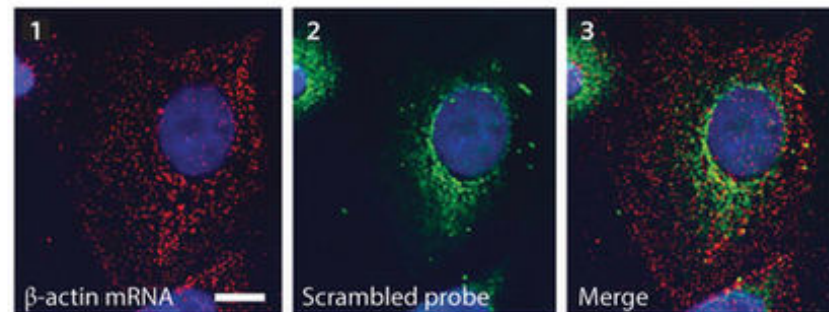
mRNA Imaging in Cells: Multiply labeled tetravalent RNA imaging probes (MTRIPs)

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..



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

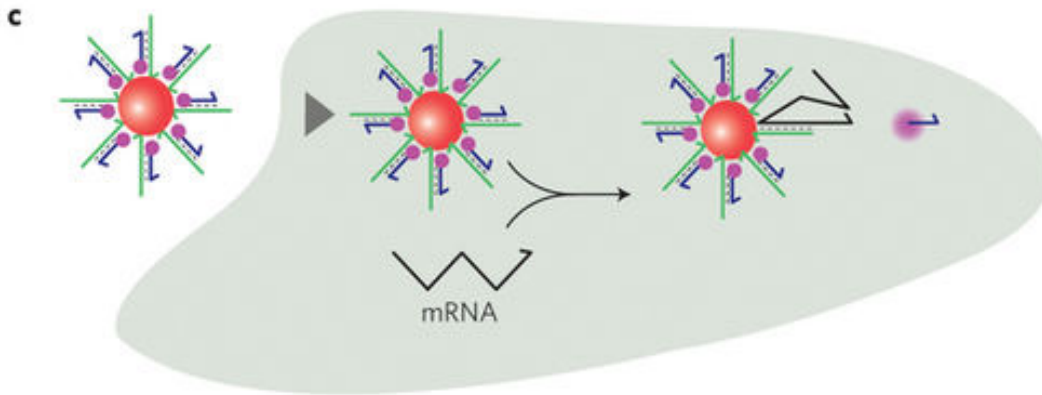


MTRIPs

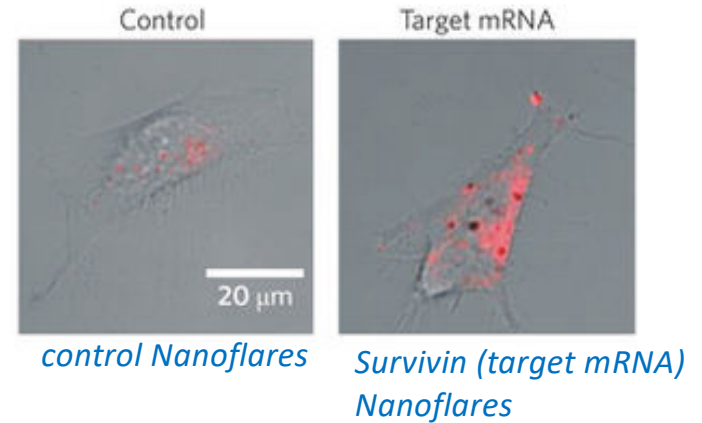
Scrambled probes

A merged image that includes nuclear DAPI stain. 6

mRNA Imaging in Cells: Nanoflares



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 'flare strand' and trigger an increase in fluorescence.



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

Rosi, N. L. et al. Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. *Science* 312, 1027–1030 (2006).

Alhasan, A. H., Patel, P. C., Choi, C. H. J. & Mirkin, C. A. Exosome encased spherical nucleic acid gold nanoparticle conjugates as potent microRNA regulation agents. *Small* 10, 186–192 (2014).

Prigodich, A. E. et al. Nanoflares for mRNA regulation and detection. *ACS Nano* 3, 2147–2152 (2009).

Halo, T. L. et al. NanoFlares for the detection, isolation, and culture of live tumor cells from human blood. *Proc. Natl Acad. Sci. USA* 111, 17104–17109 (2014).