

Supporting information

Amplified Fluorescence *in Situ* Hybridization by Small and Bright Dye-Loaded Polymeric Nanoparticles

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Table S1. Fluorescence quantum yields (QY) of NPs prepared with different dyes at varied loading mass ratio (*vs.* polymer).

Encapsulated dye	Loading mass ratio (%) ^a	QY (%)
R18/F5	30	52
	50	41
	70	32
Rh101-C18/F5	20	34
	30	21
	50	10
Rh101-C18/F12	20	46
	30	34
	50	18
DiD/F12	20	45
	30	42
	50	32

^aThe weight% loading of the dye with respect to the polymer.

Table S2. Sizes of bare and DNA-functionalized NPs according to TEM and DLS data.^a

Dye	Type of NPs	Size by DLS (nm)	Size by TEM (nm)
Rh101-C18/F12	bare	17.0 ± 0.4	14.1 ± 1.8
	T20-NPs	23.4 ± 1.0	16.8 ± 2.0
R18/F5	bare	17.7 ± 0.4	18.2 ± 2.7
	T20-NPs	26.9 ± 0.3	20.0 ± 2.6
DiD/F12	bare	N/A	16.3 ± 2.3
	T20-NPs	N/A	17.9 ± 2.5

^a Error bars are standard deviation of the mean ($n \geq 3$ for DLS; at least 200 NPs were analyzed for TEM).

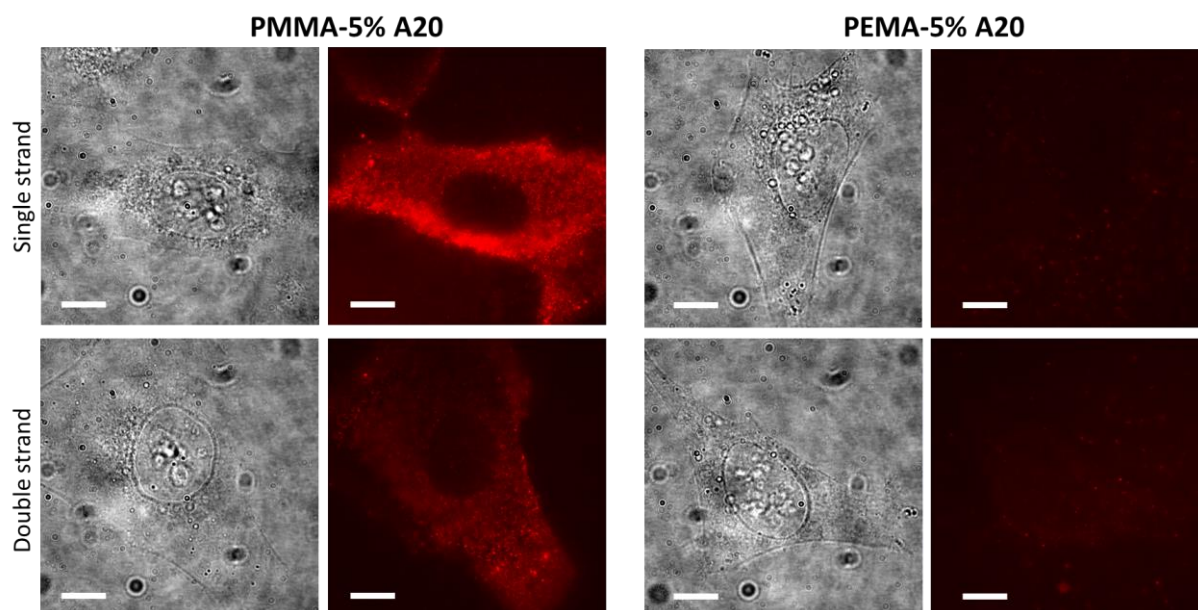


Figure S1. Effect of polymer nature and different grafted DNA sequences. Bright field (right) and fluorescence (left) images of fixed HeLa cells incubated with PMMA-based NPs and PEMA-based NPs of ~20 nm core size, functionalized with A20. Both single-stranded and double stranded (annealed with T20) DNA-NPs were tested (30 min incubation with cells). TIRF mode was used on fixed HeLa cells without washing. Scale bar: 10 μ m. PBS buffer with 50 mg/L Tween 80 was used for incubation and imaging.

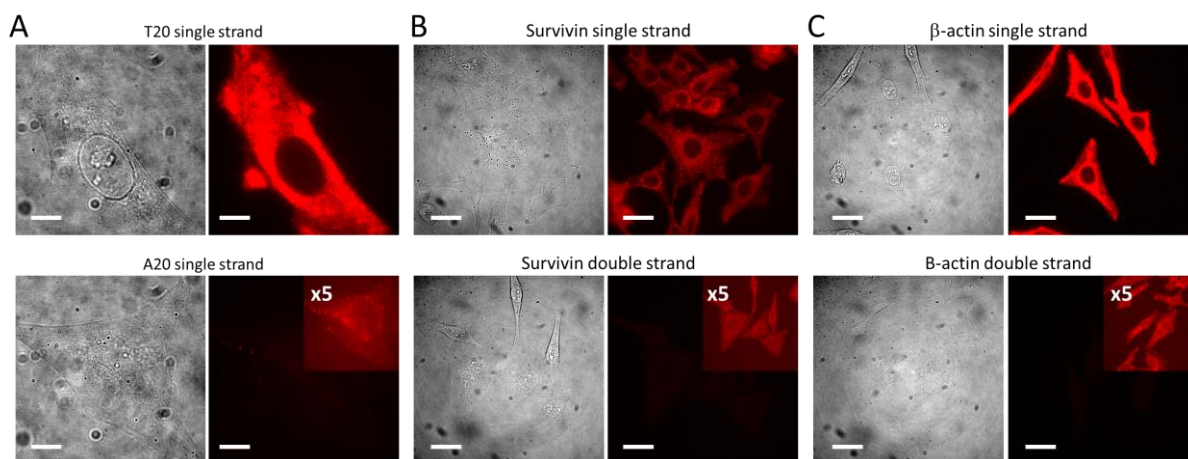


Figure S2. (A) Comparison of TIRF fluorescence images of PEMA-based NPs functionalized with T20 (upper panel) and A20 (lower panel) recorded at identical conditions (inset shows an image where signal was amplified 5-fold for visibility of the cell). Cells were incubated during 1 h with NPs, then washed two times with 0.1 % BSA / PBS. Scale bar: 10 μ m. (B,C) Epi-fluorescence microscopy of fixed HeLa cells incubated for 1 h with DNA-NPs targeting survivin (B) and β -actin (C) (the same washing protocol as in A). Images for single-stranded (upper panels) and double-stranded (annealed with complementary strands) DNA-NPs are shown. Scale bar: 50 μ m. PBS buffer with 50 mg/L Tween 80 was systematically used for incubation and imaging (A-C).

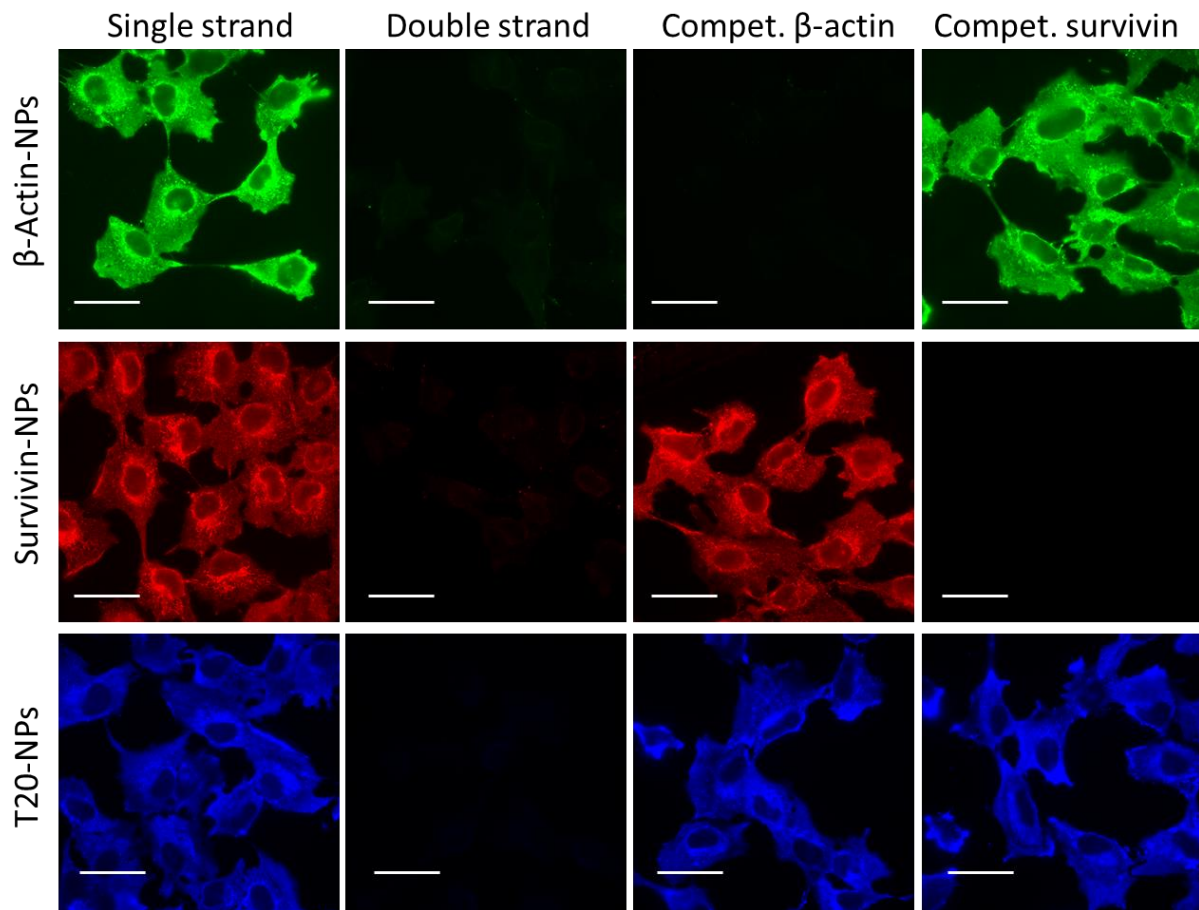


Figure S3. Validation of DNA-NPs for detection of intracellular mRNA targets in fixed U87 cells. Single stranded probes β -actin-NPs loaded with Rh110-C18/F12, survivin-NPs loaded with R18/F5 and A20-NPs loaded with DiD/F12 for β -actin, survivin and poly(A) sequences of mRNA, were compared to controls with double stranded DNA-NPs (annealed with complementary sequences) and competitor oligonucleotides (100 nM) for corresponding β -actin and survivin sequences added 1 h before addition of DNA NPs. DNA-NPs concentration expressed in encapsulated dyes was 100 nM. Scale bar: 50 μ m.

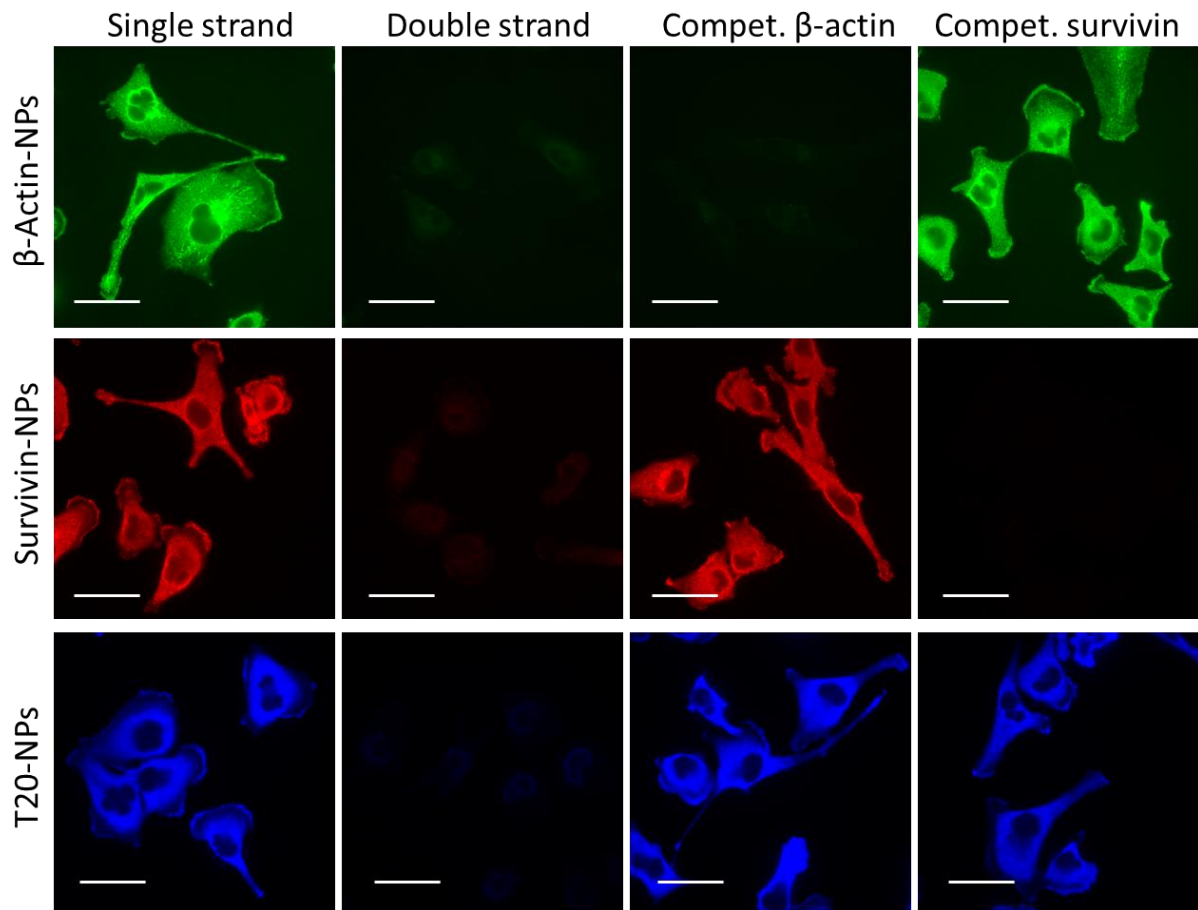


Figure S4. Validation of DNA-NPs for detection of intracellular mRNA targets in fixed MDA-MB-231 cells. Single stranded probes β -actin-NPs loaded with Rh110-C18/F12, survivin-NPs loaded with R18/F5 and A20-NPs loaded with DiD/F12 for β -actin, survivin and poly(A) sequences of mRNA, were compared to controls with double stranded DNA-NPs (annealed with complementary sequences) and competitor oligonucleotides (100 nM) for corresponding β -actin and survivin sequences added 1 h before addition of DNA NPs. DNA-NPs concentration expressed in encapsulated dyes was 100 nM. Scale bar: 50 μ m.

Table S3. Manders' colocalization coefficient M1/M2 of multiplexed detection of mRNA sequences.

Colocalization	HeLa	U87	MDA
Actin in T20	0.988	0.935	0.998
T20 in Actin	0.322	0.208	0.283
Survivin in T20	0.937	0.750	0.962
T20 in Survivin	0.275	0.208	0.437
Actin in Survivin	0.661	0.499	0.946
Survivin in Actin	0.678	0.330	0.604

Supporting videos

Video S1. Video of 3D reconstruction of survivin mRNA imaging in HeLa cells using AmpliFISH probes. HeLa cells were incubated during 1 h with DNA-NPs targeting survivin, then washed two times with 0.1 % BSA / PBS. Multiple stacks were acquired using spinning disk microscopy. 3D image reconstruction was performed using IMARIS software. Gray dots correspond to NPs identified automatically by the imaging software for quantitative analysis.

Video S2. Real-time video imaging of HeLa cells stained with actin-NPs using spinning disk fluorescence microscopy. HeLa cells were incubated during 1 h with DNA-NPs targeting β -actin mRNA, then washed two times with 0.1 % BSA / PBS. Multiple images from a single plane of HeLa cells was recorded each 100 ms using spinning disk microscopy. Scale bar is 10 μ m.