

Supporting Information: A DNA-based optical force sensor for live-cell applications

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Table 1: DNA oligos sequence

DNA oligo strands	Sequence
F	3' Thiol C3 GCGGGACTTTCGTGCGTCGC Alexa 488 5'
Q	3' Iowa blackFQ CGCGCCCGTGCGCCGAACGC C6 Thiol 5'
H	GCGAACCG GAGAGTGTTAGAGACA CGGTTCGC
C	CTCTCACAATCTCTGTCGGTTCGC

Table 2: DNA sensor crosslinking concentration

Network	R	Sensor concentration (μM)
Actin ^a	0	0
Actin ^a +DNA sensor	0.005	0.12
	0.01	0.24
	0.02	0.48
	0.1	2.4
	0.2	4.7

^a A fixed actin concentration of 23.81 μM was always used;

Table 3: Buffer compositions

Buffer types	Composition
DNA hybridization	50 mM NaCl, 10mM MgCl ₂ , 1x PBS
Actin buffer	1M KCl, 0.1M imidazole pH 7.4, 10mM ATP, 20mM MgCl ₂

Closed sensor	τ [ns] DNA hybridization buffer	τ [ns] actin buffer
F:H:Q (2:2:2)	0.62 ± 0.04 [0.25]	0.80 ± 0.08 [0.22]
	3.70 ± 0.07 [0.75]	3.58 ± 0.07 [0.78]
F:H:Q (1:2:2)	0.53 ± 0.04 [0.36]	0.59 ± 0.02 [0.25]
	3.70 ± 0.06 [0.64]	3.58 ± 0.07 [0.75]
F:H:Q (0.5:2:2)	0.53 ± 0.08 [0.38]	0.59 ± 0.04 [0.29]
	3.70 ± 0.07 [0.62]	3.58 ± 0.05 [0.71]
F:H:Q (0.25:2:2)	0.51 ± 0.08 [0.43]	0.61 ± 0.05 [0.29]
	3.70 ± 0.08 [0.57]	3.58 ± 0.05 [0.71]

Table 4: Fluorescence lifetime values (in ns) and their relative fit amplitudes

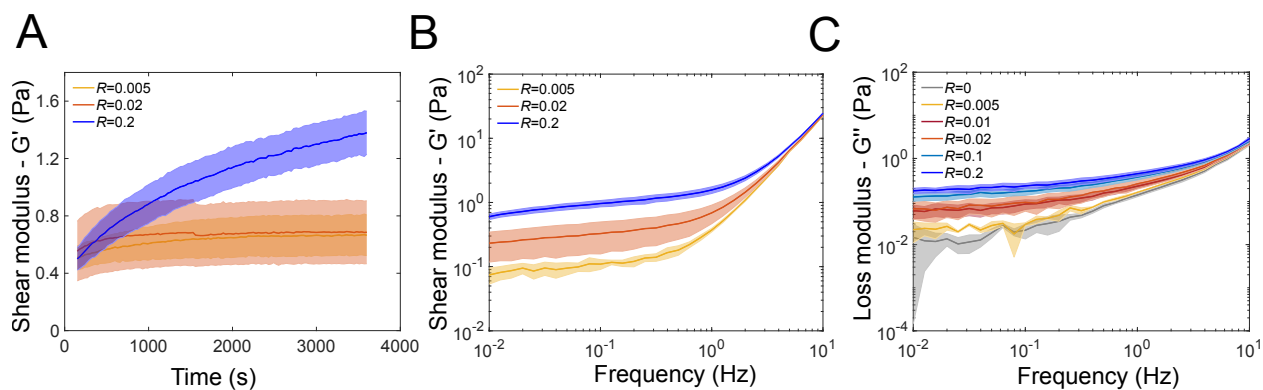


Figure 1: Gelation kinetics and frequency response of actin, actin-DNA sensors networks at different sensor concentrations. A,B,C. Gelation kinetics for sensor concentrations ($R = 0.005, 0.02, 0.2$) to show the evidence of a crosslinked network. Frequency response from these networks also do not exhibit any distinctive frequency dependent behavior. Solid lines represent mean values and the shaded area the standard error of mean.

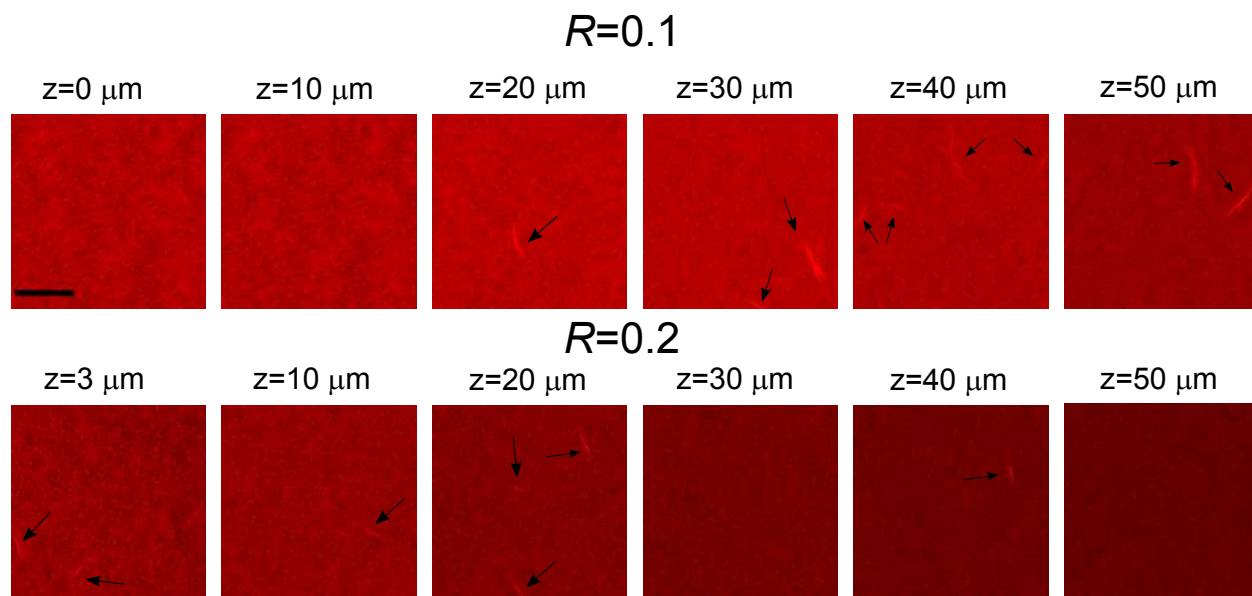


Figure 2: Microstructure of $R = 0.1$ & $R = 0.2$ actin-DNA sensor networks at varying z -heights in the network. Actin-sensor networks at high sensor concentrations ($R = 0.1, 0.2$) were imaged in a confocal z -scan which shows the existence of bundles across different z -positions. They appeared mostly in the middle of the network and were not present at the boundary surfaces of the coverslip and the microscopic slide. (Scale bar: $30 \mu\text{m}$). Actin is labeled for fluorescence with Atto 647N.

DNA Sensor Transfection

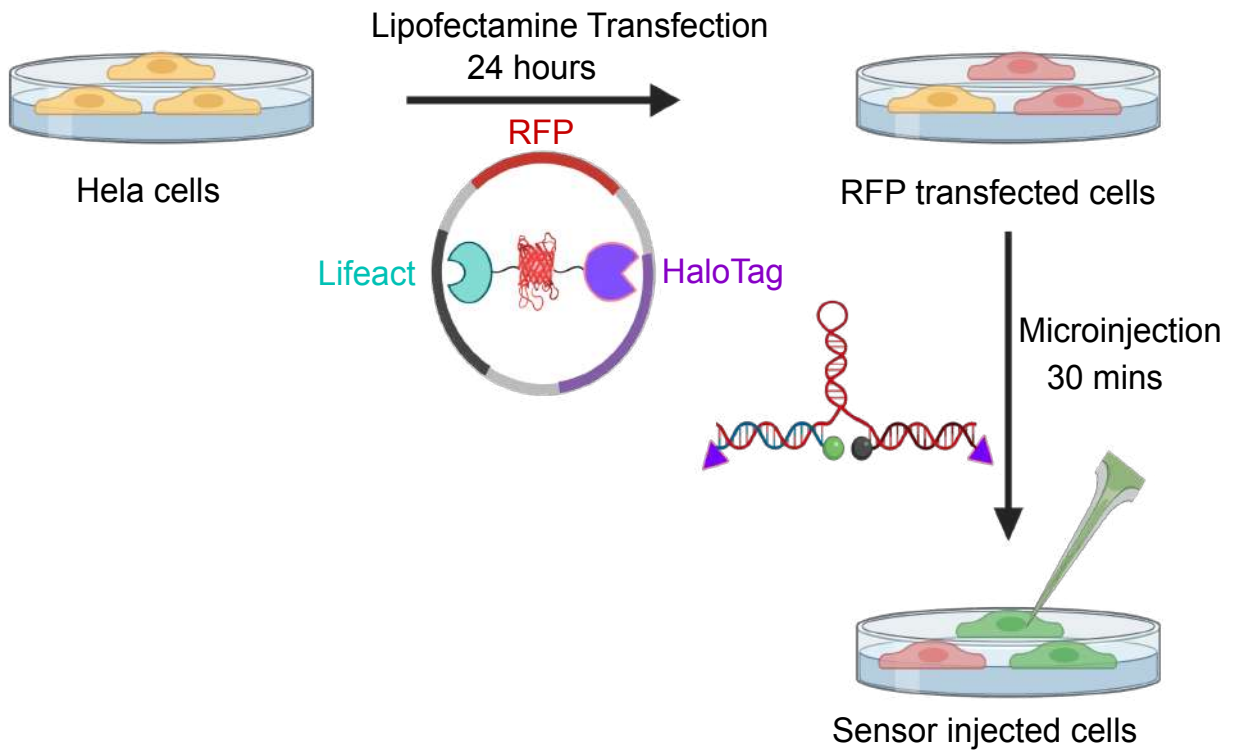


Figure 3: A schematic representation of the transfection protocol of DNA sensors. DNA sensors were transfected into HeLa cells in 2 steps. The lifeact-RFP-HaloTag® plasmid was transfected into HeLa cells with lipofectamine 3000. 24h later, a 50 nM solution of DNA sensors that were first modified, were assembled *in vitro* and microinjected near the nucleus. (Scheme is not drawn to scale.)

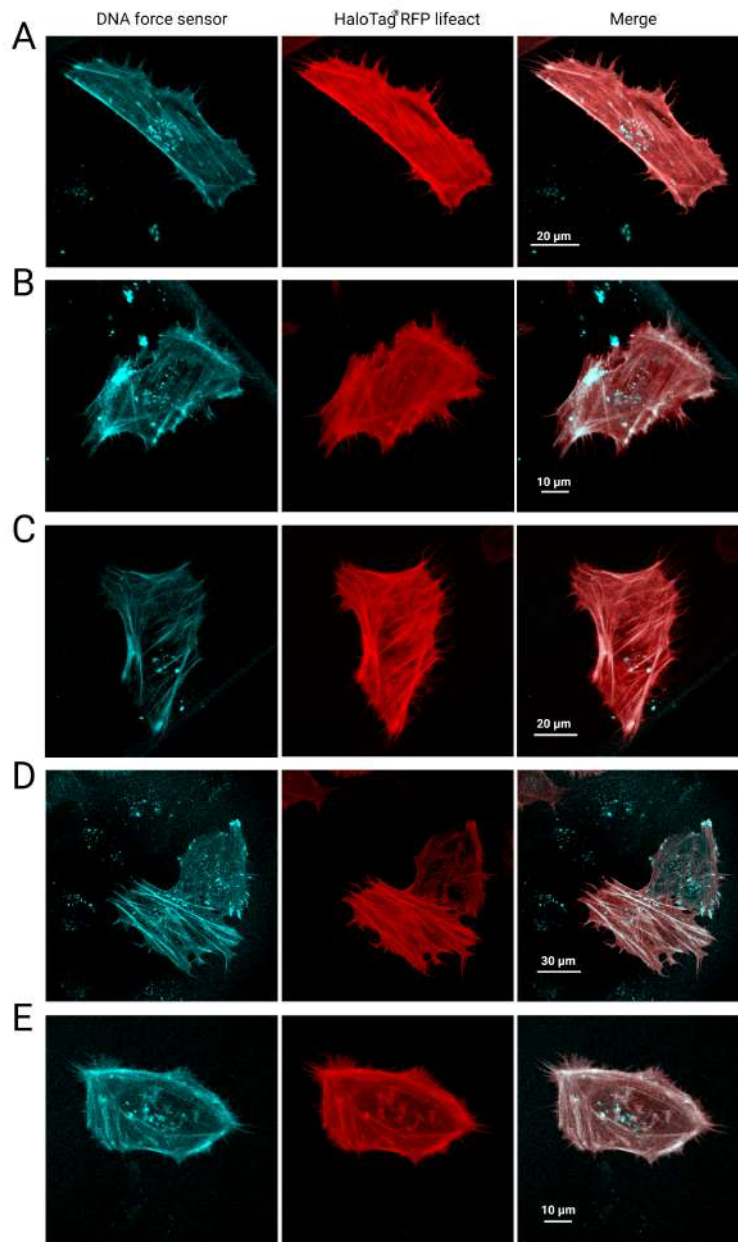


Figure 4: **A - E** HeLa cells microinjected with closed DNA sensors. Confocal micrographs of cells false-coloured for fluorescence intensity. Images corresponding to Alexa 488-tagged closed DNA sensor recorded in the DNA force sensor spectral channel (left), the same cell in RFP spectral channel (middle) and the overlay between both channels (right) confirms co-localization between DNA-sensors and actin.

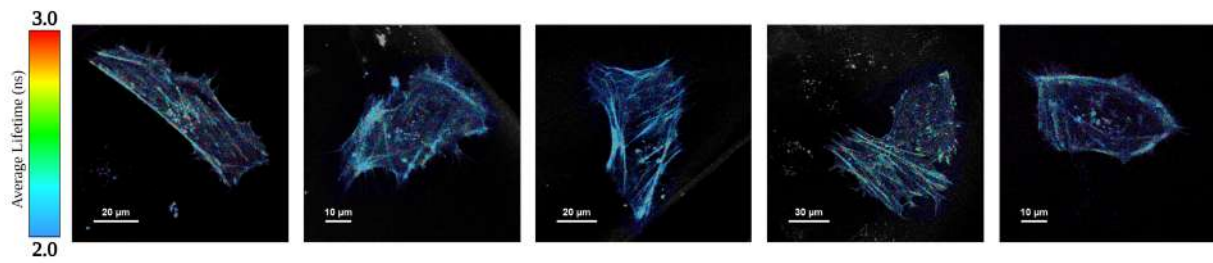


Figure 5: FLIM images of HeLa cells microinjected with DNA sensors.