Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Optimizing locked nucleic acid modification in double-stranded biosensors for live single cell analysis

Samuel A Vilchez Mercedes[1,‖], Ian Eder [1,‖], Mona Ahmed[1], Ninghao Zhu[1] , and Pak Kin Wong[1,2]

[1]Department of Biomedical Engineering, The Pennsylvania State University, University Park, PA, USA

[2]Department of Mechanical Engineering and Department of Surgery, The Pennsylvania State University, University Park, PA, USA

[‖]These authors contributed equally to this work

Corresponding author:

Pak Kin Wong, Department of Biomedical Engineering, Department of Mechanical Engineering and Department of Surgery, The Pennsylvania State University, 516 Chemical and Biomedical Engineering Building, University Park, PA, USA.

Email: pak@engr.psu.edu

Keywords

Homogeneous biosensor, molecular beacon, 3D culture, spheroid, HeLa.

Table 1. Nucleic sequences for the dsLNA biosensor.

β-actin Probe	/5Alex647N/AG+GT+TT+TG+TC+AA+GA+AA+GG+GT
β-actin Quencher 1 (4-LNA)	GA+CA+AA+AC+CT/3IAbRQSp/
β-actin Quencher 2 (3-LNA)	GACA+AA+AC+CT/3IAbRQSp/
β-actin Quencher 3 (2S-LNA)	GACA+AAAC+CT/3IAbRQSp/
β-actin Quencher 4 (2A-LNA)	GACAAA+AC+CT/3IAbRQSp/
β-actin Quencher 5 (1-LNA)	GACAAAAC+CT/3IAbRQSp/
β-actin Quencher 6 (DNA)	GACAAAACCT/3IAbRQSp/
β-actin Target	ACCCTTTCTTGACAAAACCT
β-actin Mismatch target	ACCCTTTCTCGACAAAACCT
Random Probe	/5Alex647N/AC+GC+GA+CA+AG+CG+CA+CC+GA+TA
Random Quencher 1 (4-LNA)	CT+TG+TC+GC+GT/3IAbRQSp/
Random Quencher 2 (3-LNA)	CTTG+TC+GC+GT/3IAbRQSp/
Random Quencher 4 (2S-LNA)	CTTG+TCGC+GT/3IAbRQSp/
Random Quencher 3 (2A-LNA)	CTTGTC+GC+GT/3IAbRQSp/
Random Quencher 5 (1-LNA)	CTTGTCGC+GT/3IAbRQSp/
Random Quencher 6 (DNA)	CTTGTCGCGT/3IAbRQSp/
Random Target	TATCGGTGCGCTTGTCGCGT

Sequences are in the 5’ to 3’ direction; + indicates LNA monomers.

Table 2. Statistics for data in Figure 4B and D.

β-actin	4 LNA	3 LNA	2 LNA - A	2 LNA - S	1 LNA	DNA
4 LNA		****	****	****	**	ns
3 LNA			ns	ns	ns	****
2 LNA – A				ns	ns	****
2 LNA – S					ns	***
1 LNA						*
DNA						

Random	4 LNA	3 LNA	2 LNA - A	2 LNA - S	1 LNA	DNA
4 LNA		**	**	ns	**	ns
3 LNA			ns	ns	ns	ns
2 LNA – A				ns	ns	ns
2 LNA – S					ns	ns
1 LNA						ns
DNA						

A Brown-Forsythe and Welch ANOVA test and the Dunnet's T3 multiple comparisons test were used to evaluate differences among quenchers for a specific ratio. ns p-value > 0.05, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, and **** p-value < 0.0001.

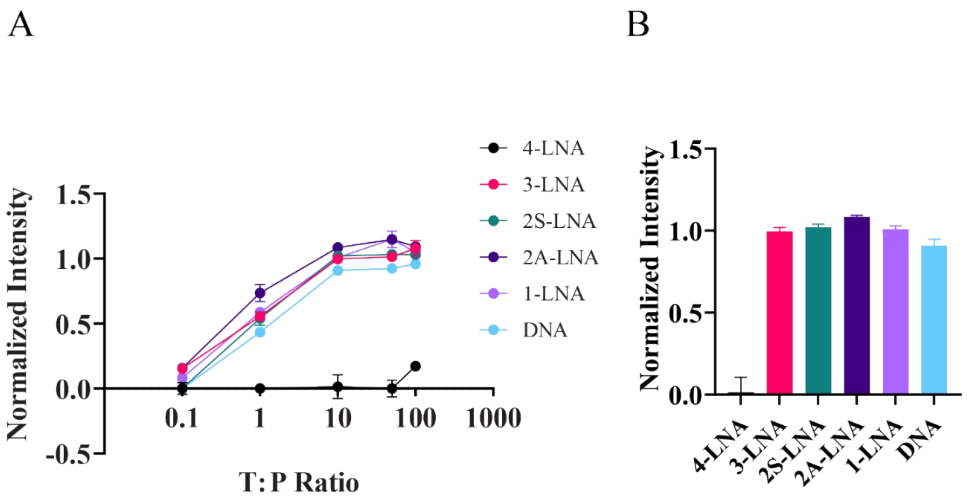

Figure S1. β-actin probe specificity against a single base mismatch target evaluated for various quencher configurations. Experimental characterization of the β-actin probe specificity against a mismatch target for the different quenchers**.** (A) Normalized intensity of the biosensor at multiple T:P ratios. (B) Bar chart of the normalized intensity of β-actin biosensor at a target-to-probe ratio of 10:1. The probe concentration is 250 nM. Error bars represent the standard deviation. A two-way ANOVA test was used to evaluate differences among quenchers and Q:P ratios. A Brown-Forsythe and Welch ANOVA test and the Dunnett's T3 multiple comparisons test were used to evaluate differences among quenchers for the T:P ratio of 10:1 (n=3).

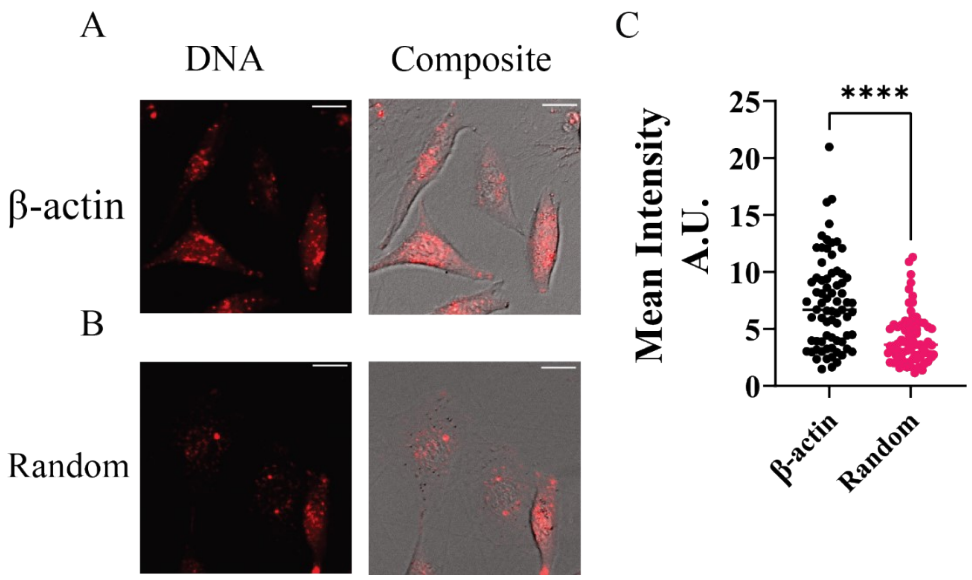

Figure S2. DNA quencher demonstrates high specificity in live cells. (A-B) Fluorescence and brightfield overlay images of HeLa cells transfected with (A) β-actin probe or (B) Random probe, respectively. Scale bars, 20 µm. **(C)** Quantification of mean fluorescence intensity per cell for both cases. The nonparametric Mann-Whitney test was used to compare across groups. For each experiment n > 100 cells per condition (****p<0.0001).

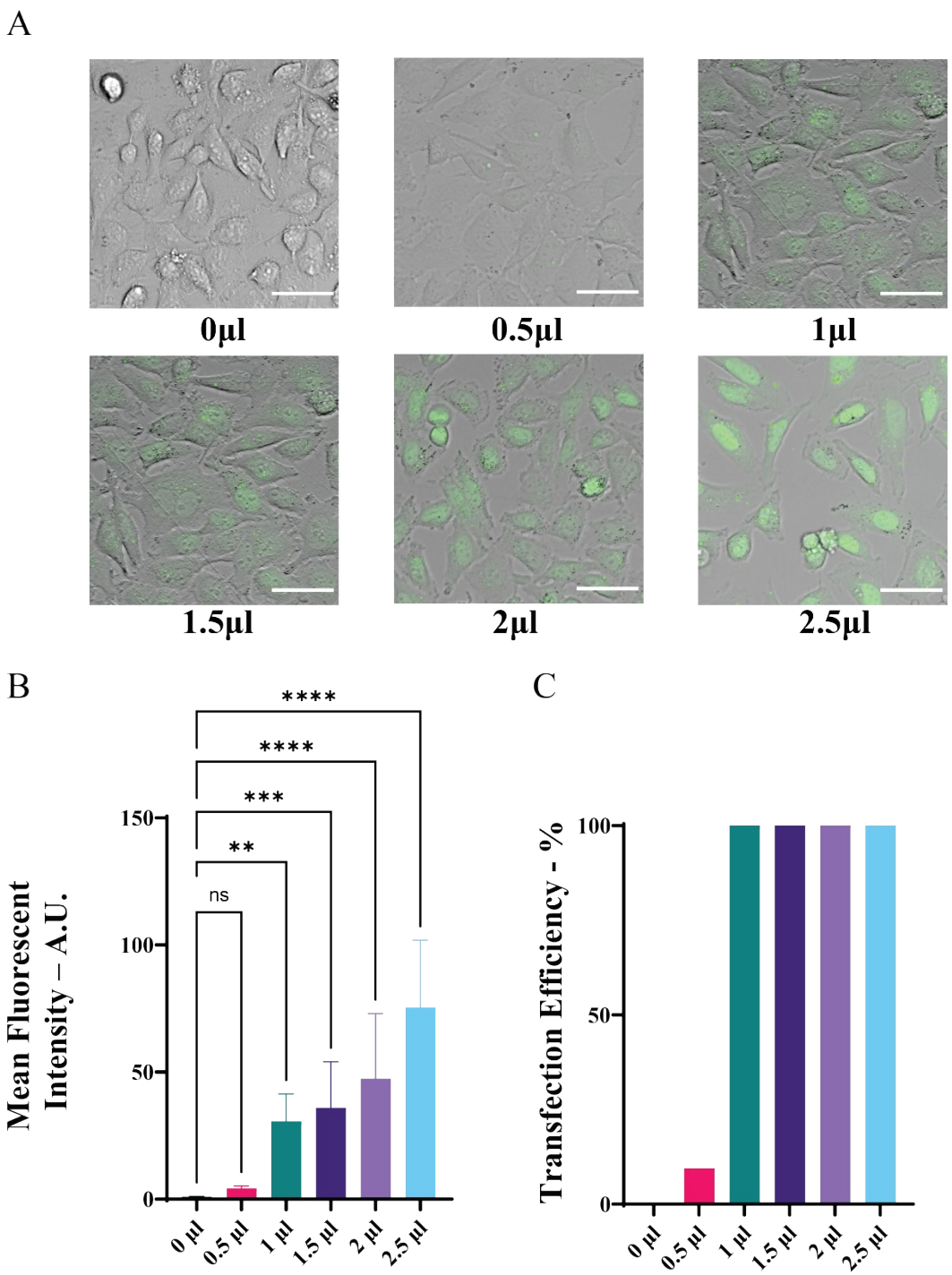

Figure S3. RNAiMAX allows for high transfection efficiency in HeLa cells. Block iT™, a non-targeting, fluorescent, double stranded RNA oligo was transfected with varying volumes of RNAiMAX in a 24 well plate according to manufacturer's protocol. Briefly, 6.5 µl of Block-iT

(corresponding to approximately 1 µg siRNA) was incubated over night with various volumes of RNAiMAX. Based on this optimization, subsequent experiments with dsLNA biosensors used 1.5µl RNAiMAX for transfection. **(A)** Representative images of HeLa cells transfected with Block iT™. Scale bars, 50 µm. **(B)** Quantification of normalized mean fluorescence intensity per cell for each condition. All values were normalized to the mean fluorescent intensity of the 0 µl RNAiMAX case. A one-way ANOVA test was used to compare between across groups. For each experiment n = 10 cells per condition (** $p < 0.01$, *** $p < 0.001$ and ****$p<0.0001$). **(C)** Percent of cells transfected with Block iT™. n > 200 cells per condition.