Nucleic Acid Beacons for Long-term Real-time Intracellular Monitoring

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Supporting Information

1. Experimental procedures

Molecular beacon synthesis. All oligonucleotides were synthesized based on solid-state phosphoramidite chemistry at a 1 μmol scale. All LNA MBs labeled by FAM were synthesized with controlled-pore glass columns with a 3'-Dabcyl molecule covalently linked to the CPG substrate. FAM phosphoramidite was used to couple to the 5' end of the sequence. The complete MB sequences were then deprotected in concentrated ammonia hydroxide at 65^{o}C overnight and further purified with reverse phase high-pressure liquid chromatography (HPLC) on a C-18 column and ion-exchange HPLC (Dionex DNAPacTM PA-100 column (40 × 250 mm, or semipreparative), 30%-70%, 45 min gradient 1 M NaCl/20 mM NaOH, pH 12). For the MBs labeled with Cyanine 3(Cy3), FAM phosphomidite was replaced by Cy3 phosphomidite, and 3'-Dabcyl CPG was replaced by 3'- Blackhole Quencher 2 (BHQ2) CPG. For the MBs labeled with AF488, off-column synthesis was required. The synthesis began with 3'-

Blackhole Quencher 1 (BHQ1) CPG and ended with 5'-amino-modifier C6 phosphomidite. After that, the sequence was purified and deprotected by 2% acetic acid to activate the amino group. Then, off-column synthesis was conducted in sodium carbonate buffer (pH=9) with Alexa Fluor 488 carboxylic acid succinimidyl ester (Invitrogen) with 10-fold higher concentrations.

Hybridization kinetics study. Hybridization experiments were obtained using 100 nM of MBs and 500 nM targets in 150 μL totally. The buffer used was 20 mM Tris-HCl (pH 7.5) containing 5 mM $MgCl_2$ and 50 mM NaCl. The fluorescence intensities were measured as a function of time.

DNase I sensitivity. To test the nuclease sensitivity of MBs, the fluorescence of a 150 ul solution containing 20 mM Tri-HCl (pH 7.5), 5 mM $MgCl_2$, 50 mM NaCl and 100 nM MBs was monitored as a function of time at room temperature. 2 units of ribonuclease-free DNase I were added and any subsequent fluorescence change was recorded.

RNase H sensitivity. To test the susceptibility of molecular beacon-target mRNA hybrids to the digestion of ribonuclease H, 100 nM of MBs were incubated with the same concentration of RNA target as indicated in the above buffer. After the hybridization reached equilibrium, 12 units of ribonuclease H were added, and the subsequent change in fluorescence was monitored as a function of time.

Protein binding study. Gel electrophoresis was performed to study the interactions between Single-Stranded DNA Binding Protein (SSB) and MBs. In the buffer containing 20 mM Tri-HCl (pH 7.5), 5 mM $MgCl_2$ and 50 mM NaCl, 5 μM MB was first incubated with the same concentration of SSB. After one hour, the solution was analyzed in a 3% agarose gel at 100V in TBE buffer for 50 minutes. The gel was then stained by Coomassie blue G 250 stain solution (Bio-Rad) for one hour and washed with de-ionized water for 30 minutes. The image of the resulting gel was achieved by scanning on a regular scanner.

2. MB design and sequences

Our design strategy consisted of two steps. First, in order to maintain the fast hybridization rate, the MB stem was consistently composed of 50% LNA in an alternating fashion. Such optimized LNA MBs would then have a fast response to excess complementary target DNA (**Supplementary Fig. 2,** online). When compared to DNA MBs, it is precisely this hybridization behavior which makes it possible to use these LNA MBs to track gene expression levels in real-time. Second, we gradually increased the LNA percentage in the loop starting from 50% until the LNA MB satisfied the biostability screening criteria (**Supplementary Fig. 1,** online).

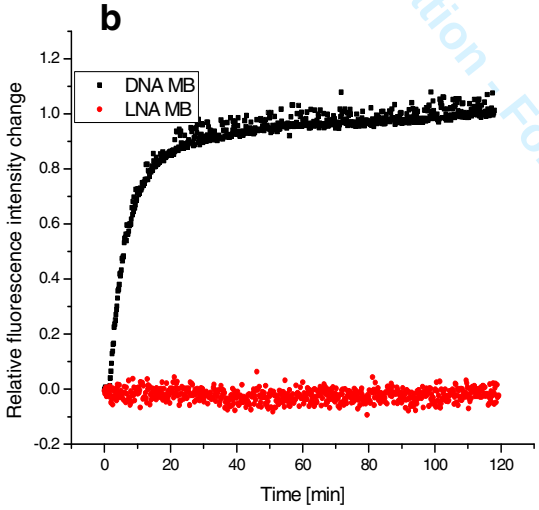
3. MBs' *in vitro* biostability tests

The following tests are the three *in vitro* biostability screening tests before the final optimized MBs were used inside the cells.

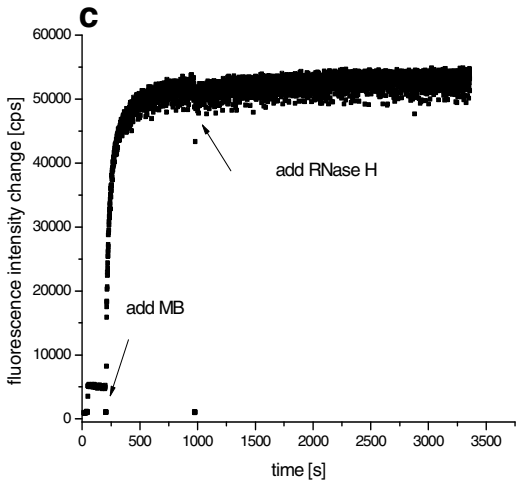
Supplementary Figure 1. Representative *in vitro* biostability experiments of the optimized LNA-MBs.

SI Fig. 1 a.

SI Fig. 1 b.



SI Fig. 1 c.



(a.) Gel electrophoresis of SSB solutions containing no MBs (lane 1); the optimized LNA-MBs (lane 2). Under the experimental conditions, SSB migrates significantly slower than the negatively charged MB and will only migrate when it is in complex with the MB. Thus, the representative gel electrophoresis result for an optimized LNA-MB showed little SSB binding.

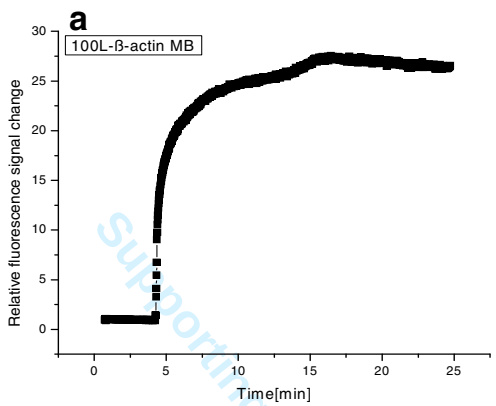
(b.) Fluorescence signal change of LNA and DNA MB upon the addition of ribonuclease-free DNase I. In the DNase I assay, the fluorescence intensity increases as the DNA MB is digested by DNase I which nonspecifically cleaves phosphodiester bounds. On the other hand, LNA MBs had no response to the addition of excess DNase I.

(c.) Fluorescence signal change of LNA-MB upon the addition of RNase H. The RNase H assay mimics false negative results that can occur when target mRNA is degraded once it is hybridized with the DNA MB. RNase H specifically cleaves RNA strands that are hybridized with DNA, thereby decreasing the target concentration and allowing the MB to reform the hairpin structure. No noticeable signal decrease was observed when RNase H was added to the duplex of RNA: LNA-MB.

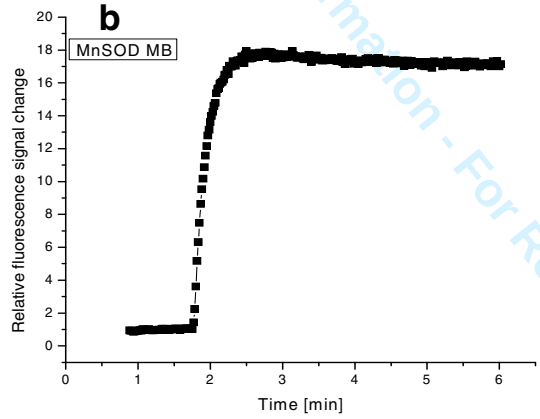
4. MBs' *in vitro* and *in vivo* hybridization tests

Supplementary Figure 2. *In vitro* hybridization kinetics of the optimized LNA-MBs.

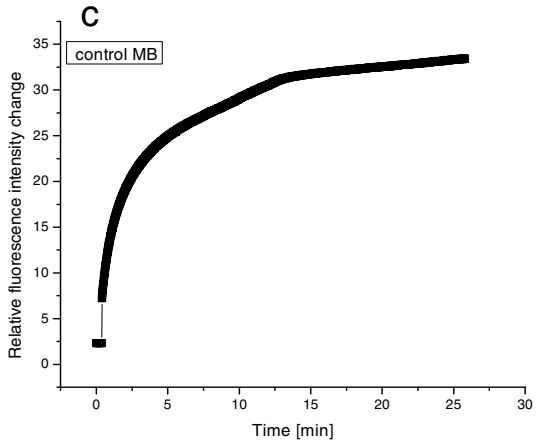
SI Fig. 2 a.



SI Fig. 2b



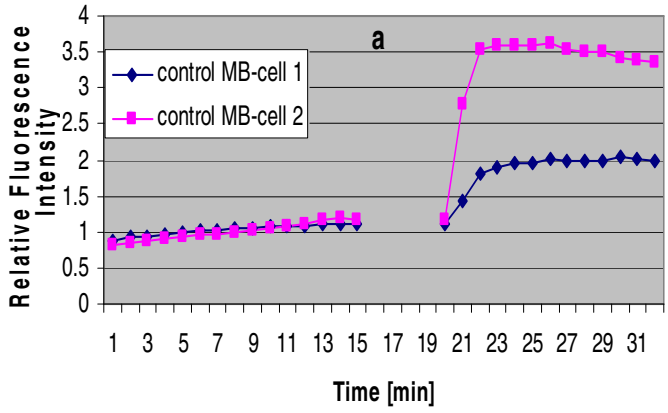
SI Fig. 2c



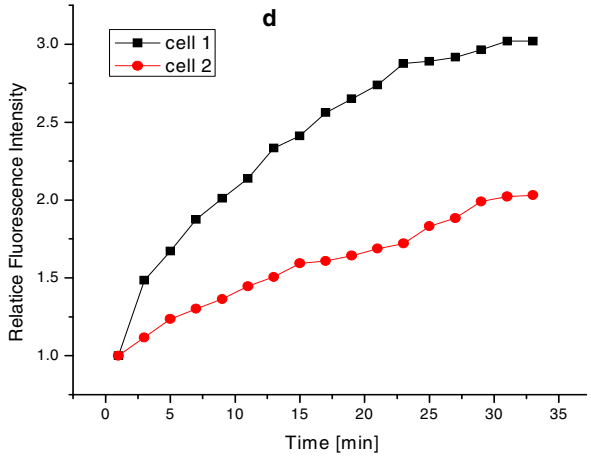
10-fold excess cDNAs (CTCTTCCAGCCTTCCTTCCT; AATCAACTGGGAGAATGT AACTG; GCGACCATAGTGATTTAGA) were added to all three optimized LNA MBs. The hybridization experiments were performed at room temperature in 20 mM Tris-HCl (pH 7.5) buffer containing 5 mM $MgCl_2$ and 50 mM NaCl.

Supplementary Figure 3. *In vivo* hybridization kinetics of the optimized control LNA-MB and ß-actin MB.

SI Fig. 3a



SI Fig. 3b



(a). The first segment represents two typical time course measurements of the fluorescence intensity of two cells after optimized LNA control MB is injected into the

cells. The second segment illustrates the hybridization kinetics of control MB with excess cDNA (GCGACCATAGTGATTTAGA) introduced into the same cells. **(b).** Time course fluorescence intensity measurements of the optimized LNA ß-actin MB with native mRNA in two living cells.