Supplementary Materials for

Dynamics of Gene Silencing in a Live Cell: Stochastic Resonance

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This file includes:

1. Experimental Section

1.1 Materials and methods:

1.1.1. Reagents: Alexa 488 tagged siRNA against human Non-muscle myosin heavy chain IIA (MYH9) and universal negative siRNA were purchased from Qiagen (Valencia, CA). The sequence of siRNA (Hs_MYH9_6) against MYH9 is as follows,

Sense Strand 5′- GGAGCAGCUCCAGGCAGAATT-3′
Anti Sense Strand 5′- UUCUGCCUGGAGCUGCUCCTG-3′. Universal negative siRNA (catalogue number 1027292) was considered as non-specific siRNA.

1.1.2. Cell Culture and Transfection: MCF-7, a human breast cancer cell line, was obtained from the American Type Culture Collection (Manassas, VA) and grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum and antibiotics (100 units/ml Penicillin and 100 μg/ml Streptomycin) in a 5% CO_2 incubator at 37°C. 2 $\times 10^3$ cells were seeded on 30 mm confocal petri dish and after 24 hour of seeding, cells were transfected with 10 pmole Alexa 488 tagged non-specific or specific siRNA using Lipofectamine^{TM} 2000 (Invitrogen, Carlsbad, CA). The transfection efficiency was > 90% as confirmed by fluorescence microscopy.

1.1.3. Real Time PCR: Total RNA from 2 X10^5 MCF-7 cells transfected with 66 pmole alexa tagged NS siRNA or specific siRNA for 0-48 h was isolated using RNeasy mini column (Qiagen). cDNA was synthesized from 1 μg of total RNA using Random hexamer and Gene amp PCR core kit (Applied Biosystems, Branchburg, NJ) at each time point (0, 6, 12, 24, 48 h). To quantify the amount of MYH9 mRNA, real time PCR was performed using SYBR green PCR master mix (Applied Biosystems). The primer pairs used to amplify the cDNA of MYH9 were as follows: forward primer, 5'-GAC CAA GGA GCG CCA GGC AG-3' ; reverse primer, 5'-TTC ATG GCA TCG GCC GTC TCA-3'; For loading control gene, GAPDH, forward primer, 5'-GAG TCA ACG GAT TTG GTC GT-3' ; reverse Primer, 5'-TTG ATT TTG GAG GGA TCT CG-3'. The real time PCR program includes an initial at 95^{0}C for 10 min and total 40 cycles at 95^{0}C for 15 sec for denaturation and at 60^{0}C for 1 min for annealing and extension. After each cycle of PCR primer melting curve and dissociation curve was analyzed to confirm that there was no nonspecific PCR product formed. Fold was calculated using an equation, fold = $2^{-\Delta\Delta Ct}$, where Ct stands for the threshold cycle number of a gene.[1] Percentage of

MYH9 mRNA present in specific siRNA treated cells was calculated considering the fold value at 0 hr as "100".

1.1.4. Electrophoresis and Immunobloting: Cell lysates of 72 h post siRNA treated MCF-7 cells were prepared by directly adding 2x Laemmli sample buffer. Proteins were separated on 8% SDS-PAGE Tris-glycine gel and transferred to PVDF membrane (Millipore Corporation, Billerica, MA, USA). The blot was blocked with 5% non-fat skim milk for 1 hour at room temperature. The upper part of the blot was incubated with antibody against NMHC II-A antibody (1: 2000, Sigma, St. Louis, USA) and lower part with antibody against Tubulin (1: 4000, Sigma) at 4^0C for overnight. The blots were then incubated with secondary antibodies against rabbit or mouse IgG for 2 hour at room temperature, and developed with SuperSignal^R West Femto luminal solution (Thermo Scientific, MA , USA). The luminescence signal was captured on Biomax MR Film (Eastman Kodak Co., Rochester, NY). Relative band intensity (NMHC IIA/Tubulin) was quantified by ImageJ (NIH, USA) software. Value was obtained by considering nonspecific siRNA treated relative intensity as 100%.

1.2 Confocal microscopy

The confocal microscope setup is described in details in our earlier publication.[2] Briefly, the setup (PicoQuant, MicroTime 200) consists of an inverted optical microscope-Olympus IX-71 with a water immersion objective (60×, 1.2 NA). The light source is picosecond diode laser (PDL 828-S "SEPIA II," PicoQuant) with excitation at 470 nm. The detectors are SPADs of MPD-PDM series.

The single molecule intensity vs. time trace was recorded using a single detector. The on-time distribution was constructed from the high fluorescence intensity spikes of single molecule time trace, in the form of a histogram, with a bin time of 1 ms with proper threshold. The values of on-time were calculated using SymPhoTime 200 software.

2. Figures and legends.

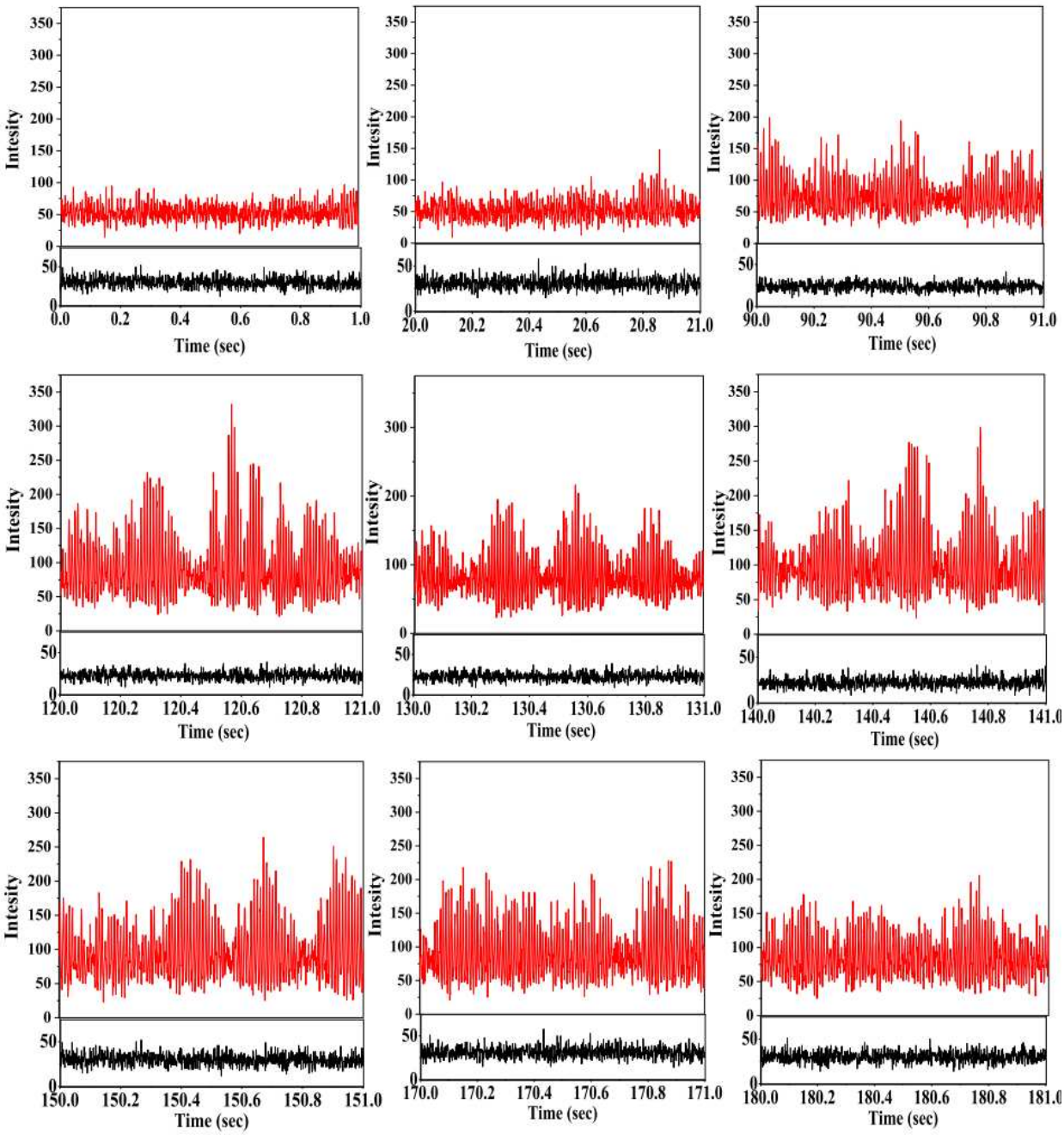

Figure S1: Comparison of single molecule fluorescence intensity vs. time trace of specific (red) and non-specific (black) siRNA inside MCF-7 cell for different time windows

3. Reference

1. Livak, K. J.; Schmittgen, T. D. *Methods* **2001**, *25*, 402–408.
2. Sen Mojumdar, S.; Chowdhury, R.; Chattoraj; S. Bhattacharyya, K. *J. Phys. Chem. B* **2012**, *116*, 12189-12198.