Electronic Supplementary Material (ESI) for Nanoscale.

Real-Time Label-Free Quantitative Fluorescence Microscopy-Based Detection of ATP Using a Tunable Fluorescent Nano-Aptasensor Platform

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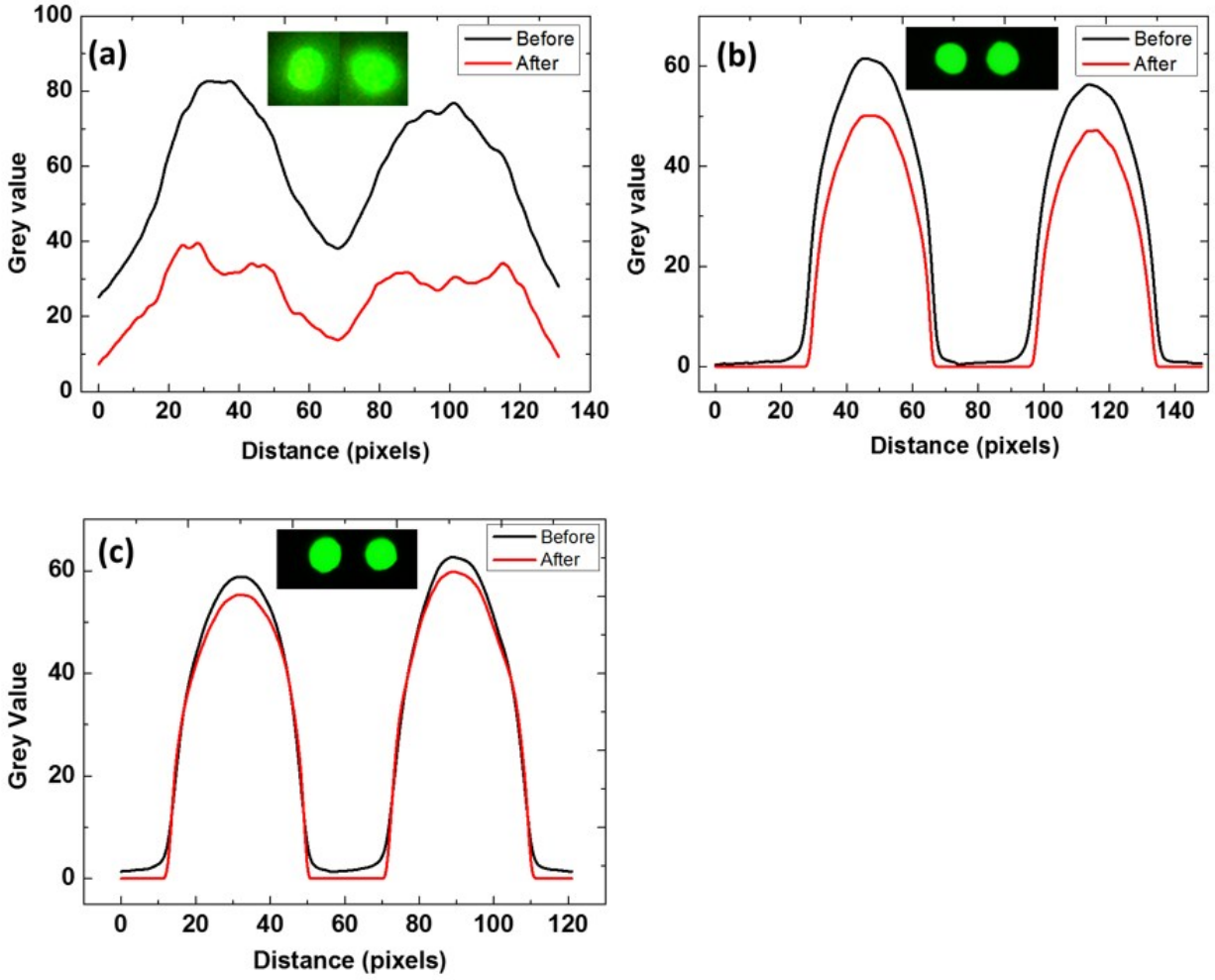
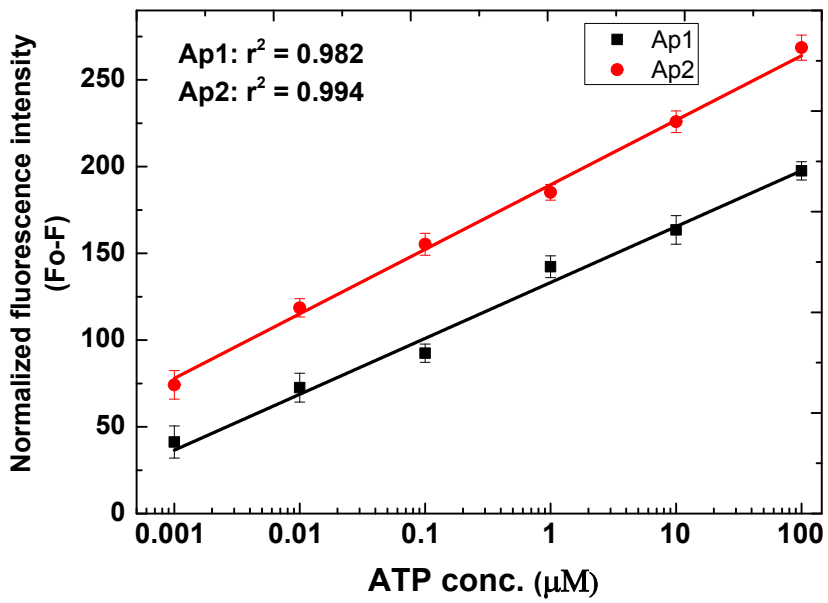
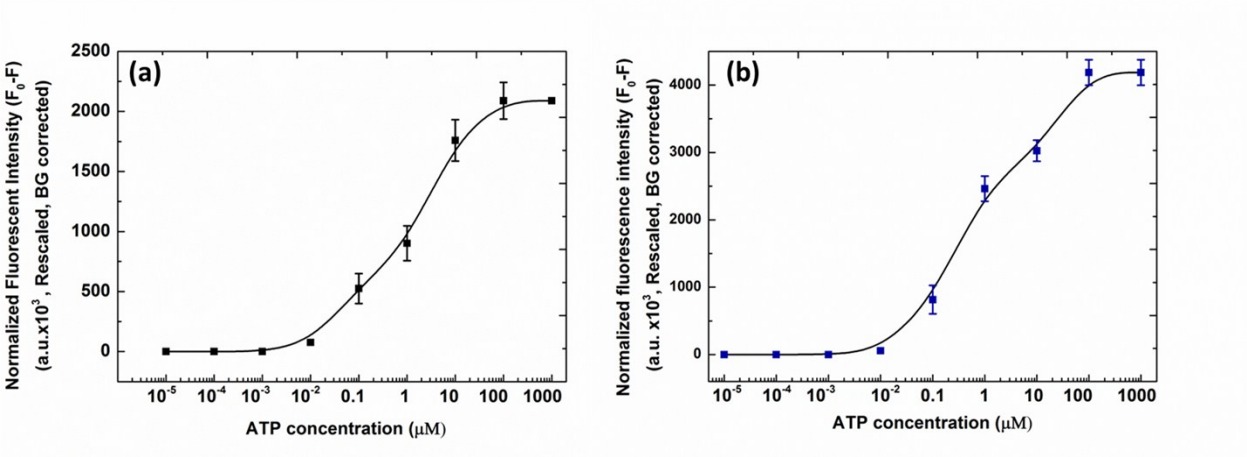


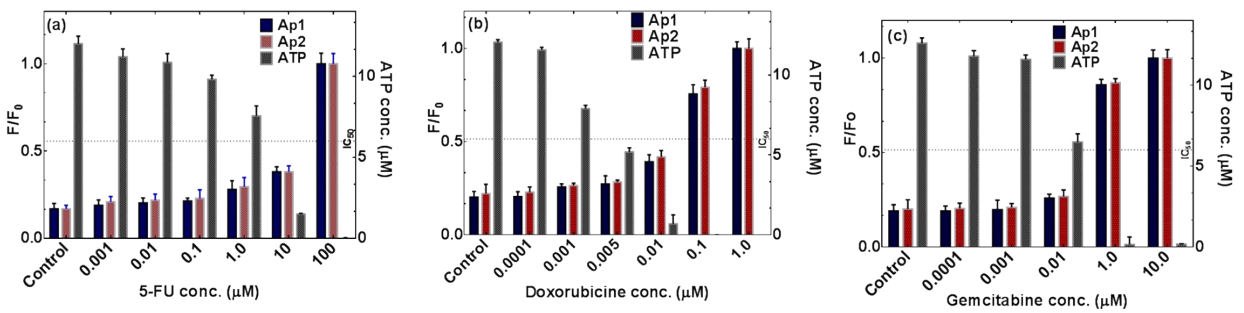
Supporting Figure S1. Effects of background subtraction from aptasensing fluorescence signals on glass, Gr and rGO substrates on signal-to-noise ratio. The fluorescent images as insets represent a row of fluorescence spots before background subtraction and the graph plots grey value vs position along a line through the spots. The graphs demonstrate the underlying effects of background subtraction on removal of noise in aptasensing on (a) glass substrates without Gr or rGO, (b) Gr and (c) rGO aptasensors. Black- and red-colored lines represent the fluorescence intensity before and after background subtraction, respectively. A lower background noise and, in turn, higher signal-to-noise ratio can be easily seen for the Gr and rGO aptasensors compared to aptasensing on the glass without Gr or rGO.



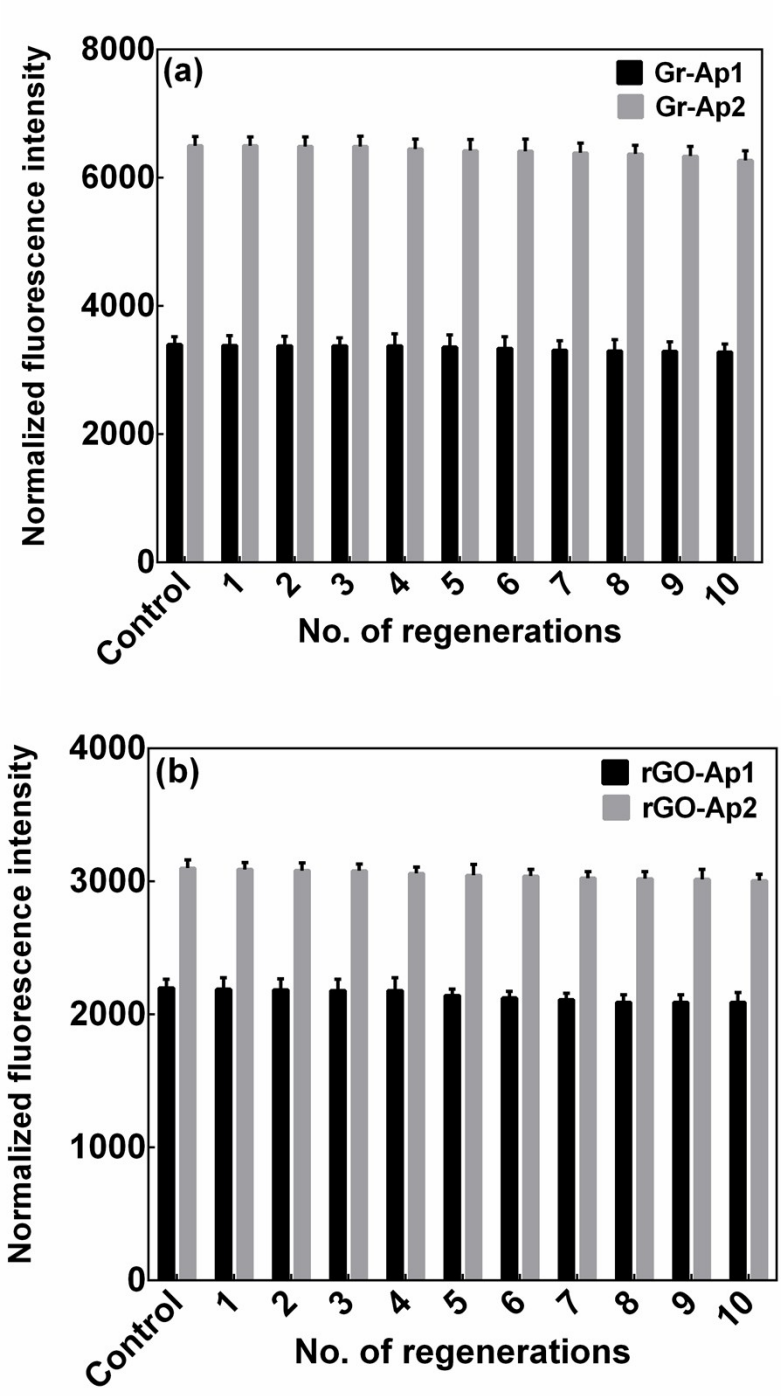
Supporting Figure S2. Spectrometry-based ATP quantification. Fluorescence spectra of solutions containing Ap1 and Ap2 complexes at various ATP concentrations. Aptamer solutions (1 nM of Ap1 or Ap2) were incubated with ATP at concentrations ranging from 0.001 to 100 μM. The normalized fluorescence intensity was calculated for each concentration. All data were expressed as mean ± SEM.



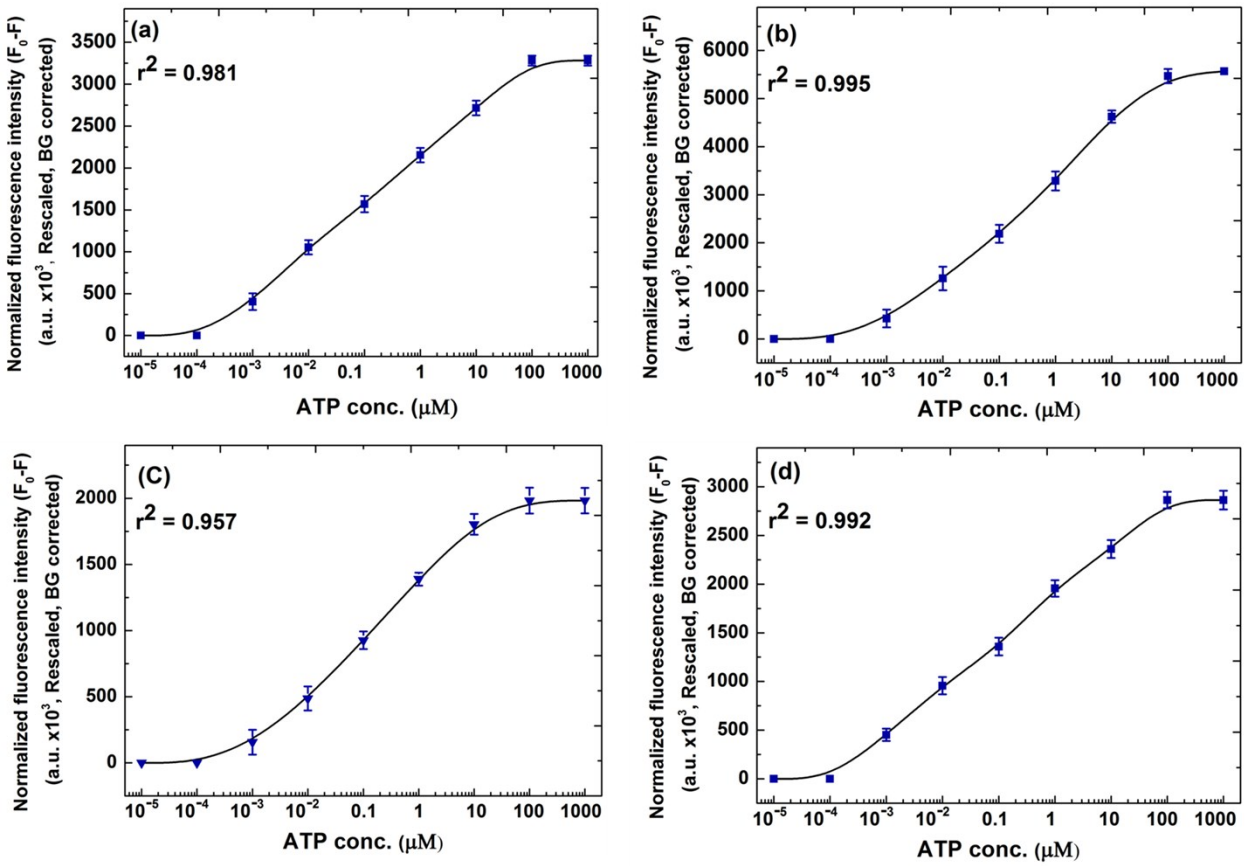
Supporting Figure S3. ATP quantification on glass substrate immobilized with SGI-intercalated aptamers of different lengths. The normalized changes in fluorescence intensity ($F-F_o$) at various concentrations of ATP were quantified on (a) a glass-Ap1 aptasensor and (b) a glass-Ap2 aptasensor.



Supporting Figure S4. Spectrometry-based ATP quantification after treatment of HeLa cells with cytotoxic drugs. The relative fluorescence intensity, F/F_o, is shown along with the corresponding ATP concentration after exposure of cells to different drugs. ATP was quantified by either Ap1 or Ap2 after exposure of cells to (a) 5-fluorouracil, (b) doxorubicin, or (c) gemcitabine. The dotted line represents the IC_{50} range; all data are presented as ±SEM.



Supporting Figure S5. Fluorescence signal recovery after regeneration of ATP aptasensors. The Gr (a) and rGO (b) aptasensors immobilized with either Ap1 or Ap2 were regenerated and the normalized fluorescence intensity was measured after each regeneration. All data are presented as ±SEM.



Supporting Figure S6. ATP quantification on Gr and rGO surfaces after regeneration of 10 times. A similar trend was observed from the regenerated (a) Gr-Ap1, (b) Gr-Ap2, (c) rGO-Ap1, and (d) rGO-Ap2 aptasensors as compared to that of the new aptasensor (before regeneration). All data were expressed as mean ± SEM.