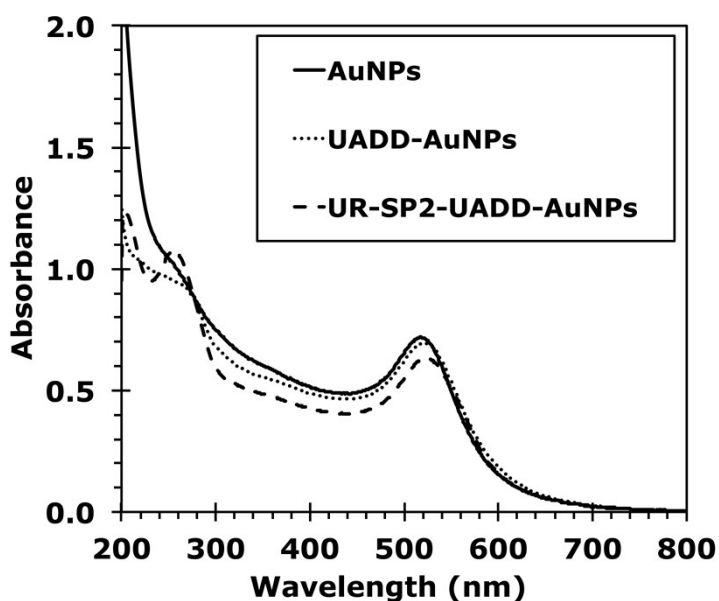


## Electronic Supplementary Information

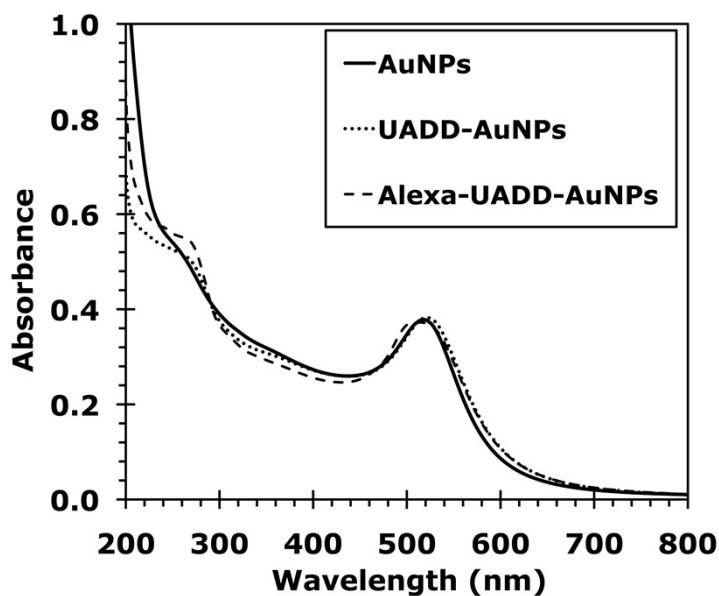
### Self-Assembled Biosensor with Universal Reporter and Dual-Quenchers for Detection of Unlabelled Nucleic Acids

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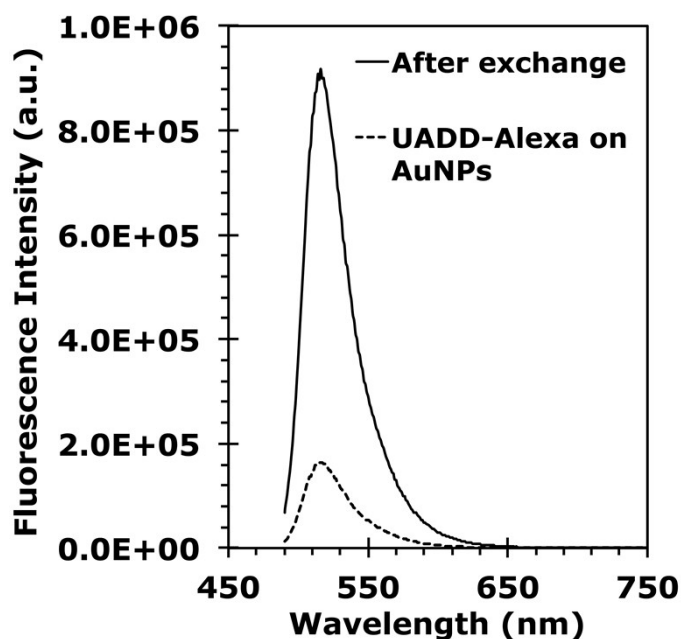
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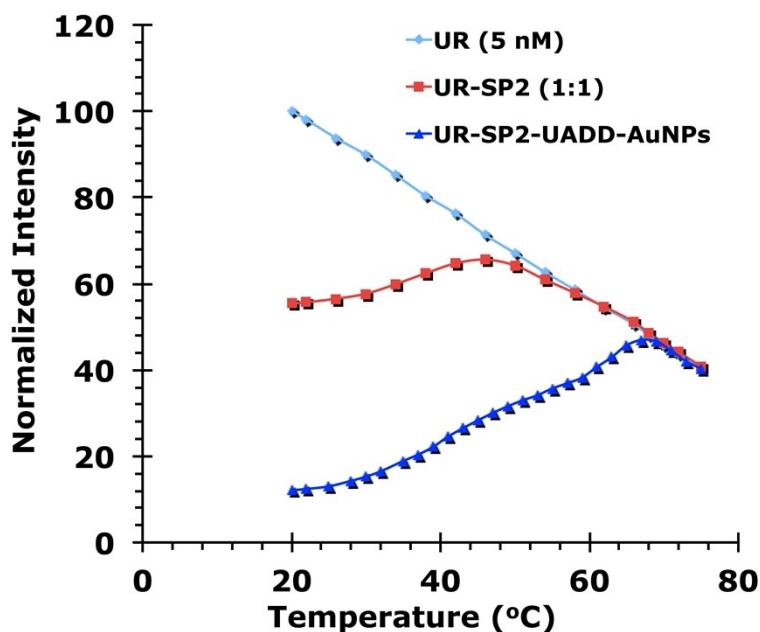
**Figure S1.** UV-vis absorption spectra of fresh AuNPs (10 nm diameter, 0.01 wt%,  $5.7 \times 10^{12}$  particles/mL  $\approx 9.5 \times 10^{-9}$  M), UADD-AuNPs, and UR-SP2-UADD-AuNPs.



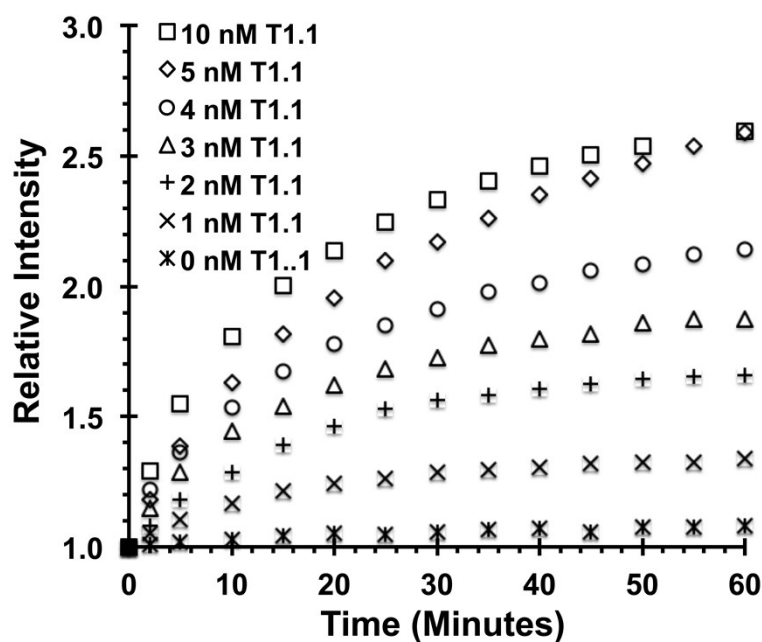
**Figure S2.** UV-vis absorption spectra of fresh AuNPs (10 nm diameter, 0.01 wt%,  $5.7 \times 10^{12}$  particles/mL  $\approx 9.5 \times 10^{-9}$  M), UADD-AuNPs and Alexa-UADD-AuNPs.



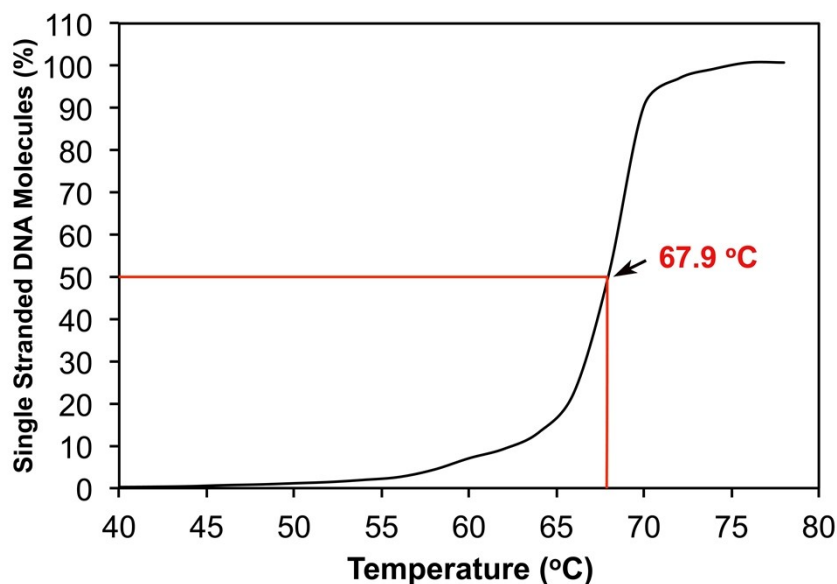
**Figure S3.** Fluorescence spectra of Alexa-UADD on AuNPs ( $\sim 0.16$  nM AuNPs) before and after replacement with excess mercaptoacetic acid (0.001 M). The fluorescence spectra were collected when samples were excited at 488 nm.



**Figure S4.** The temperature-fluorescence intensity (normalized) profiles of UR (5 nM), UR-SP2 (1:1 ratio of UR and SP2), and UR-SP2-UADD-AuNPs (5 nM of UR on AuNPs) in buffer. Fluorescence emission of samples were collected when samples were excited at 550 nm during the cooling process from 76 °C to 20 °C with a cooling rate 1 °C / 2 minutes.



**Figure S5.** The time course of relative fluorescence intensity of UR-SP1-UADD-AuNPs (UR concentration: 5nM) incubating with 0, 1, 2, 3, 4, 5, and 10 nM of T1.1 at 37 °C. Fluorescence emission of samples were collected when samples were excited at 550 nm.



**Figure S6.** Melting curve of UR (TAMRA-linker-AAA ATA ACC ACC CAC CCA CCC). The UV absorption spectra (from 300 to 200 nm) of UR (100 nM in PBS buffer pH 7.4, 137 mM NaCl and 5 mM Mg<sup>2+</sup>) were recorded every 2 °C during the heating process from 40 to 78 °C. A temperature controlled circulating water bath (model 9105, Fisher Scientific) was used for the experiment.

**Table S1.** Prices and guaranteed yields for custom synthesis of a dual-labelled MB, UR and UADD shown on [www.idtdna.com](http://www.idtdna.com)

DNA Oligo	Dual-Labelled MB 5' TAMRA-linker- <b>CCC TTA</b> <b>CAT CGT GGG TGC TTC CGT</b> <b>AAG GGT</b> -BHQ1 3'	UR 5' TAMRA-linker- <b>AAA ATA</b> <b>ACC ACC CAC CCA CCC</b> - 3'	UADD 5' C6SS- <b>CTC TCC CTC CCT</b> <b>CCC TCC C</b> - 3'
Prices for 100 nmole with 100 nmole synthetic scale	\$360 (3.2 nmol guaranteed yield)	\$167 (4.4 nmol guaranteed yield)	\$181.40 (7.1 nmol guaranteed yield)
Prices with 10 $\mu$ mole synthetic scale		\$22.7 (4.4 nmol guaranteed yield)	\$15.35 (7.1 nmol guaranteed yield)