Supporting information

Oxidant-resistant imaging and ratiometric luminescence detection by selective oxidation of silver nanodots

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Methods

Chemicals. Silver nitrate (99.9999%), sodium hypochlorite, hydrogen peroxide, potassium dichromate, potassium dioxide, Hoechst 33258, harmaline, glucose oxidase, biotin 3-sulfo-n-hydroxysuccinimide ester, iron perchlorate, *tert*-butyl hydroperoxide, tris(2-carboxyethyl)phosphine hydrochloride, sodium borohydride and polyvinyl alcohol were purchased from Sigma-Aldrich and used as received. DNA was purchased from IDT DNA. Avidin modified magnetic nanoparticles were purchased from Fisher Scientific. Hydroxyl radical and alkylperoxy radicals were generated from corresponding amount of hydrogen peroxide or *tert*-butyl hydroperoxide in the presence of 1 mM iron perchlorate, respectively.

Preparation of silver nanodots. Different silver nanodot emitters were prepared according to published data.[1-3] Brieftly, ssDNA and silver ions were mixed at a DNA base/Ag^+ ratio of 2:1. Silver nanodots were used as probes a day after chemical reduction of the mixture.

Synthesis of biotinylated GOx. 8.5 mg of GOx in 400 μL of PBS was added with 0.3 mg of biotin 3-sulfo-n-hydroxysuccinimide ester, and left at r.t. for 15 min and another 0.5 mg of biotin 3-sulfo-n-hydroxysuccinimide ester was added. 1.5 hrs later, the mixture was concentrated with 10k MWCO spin ultrafiltration membrane to 100 μL and purified on Sephadex G-100 column.

Synthesis of HAVenz and GOx conjugate. Glucose oxidase (3 mg) and sulfo-SMCC (0.5 mg) were stirred in 300 μL of PBS for 6 hrs at 4°C, and purified on sephadex G-100 column. The above product was concentrated with 10k MWCO spin ultrafiltration membrane to 100 μL and mixed with 5'-thiolated HAVenz (150 nmol) in the presence of TCEP (2 mg). The mixture was left overnight at 4°C and purified on sephadex G-100 column.

Comparison of photostability. Dyes and the blue silver nanodot were mixed with 1% PVA in the presence or absence of 5 mM of hydrogen peroxide. The above mixtures were dropped onto a glass coverslip and dried under vacuum for 15 min. The resulting films were imaged under continuous UV excitation on an Olympus IX81 inverted microscope equipped with 60× 1.35 NA objective and Andor Luca CCD camera. The excitation filter was 360-370 band pass and the emission filter BP 460-495.

HRTEM and HPLC-MS spectrometry. HRTEM images were obtained on JEM 3010 high resolution transmission electron microscope. Specific samples were analyzed with HPLC-MS system (acetonitrile/water, ESI-MS system (LCQ)).

The peak shift from the red to the blue depended on the concentration of oxidizing agents, which suggests that the remaining reducing agent (borohydride) used for AgND preparation may weaken the oxidizing capacity of oxidants. The amount of borohydride was optimized to produce maximum blue emitters with a $Ag^{+}/NaBH_4$ ratio of 6/5, slightly lower than the regular $NaBH_4$ dose.

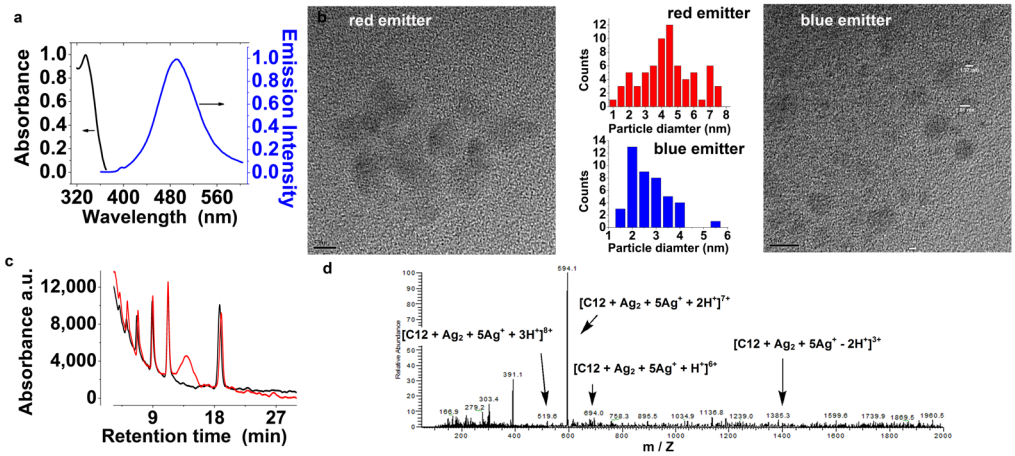

Fig. 1S Characterization of the blue silver nanodot. (a) Absorption (black) and emission (blue) spectra of the blue silver nanodot. (b) HRTEM images of the as-prepared red emitter solution (left) and the corresponding H_2O_2-oxidized blue emitter solution (right). The particle size distributions are displayed in the middle with the top for the red emitter and the bottom for the blue emitter. Scale bar: 5 nm. (c) HPLC-UV chromatograph of the blue silver nanodot (red) and a mixture of C12 and silver ions (black). (d) ESI mass spectrum of the blue silver nanodot at retention time of 14 minutes shows the possible peaks of silver-DNA complexes.

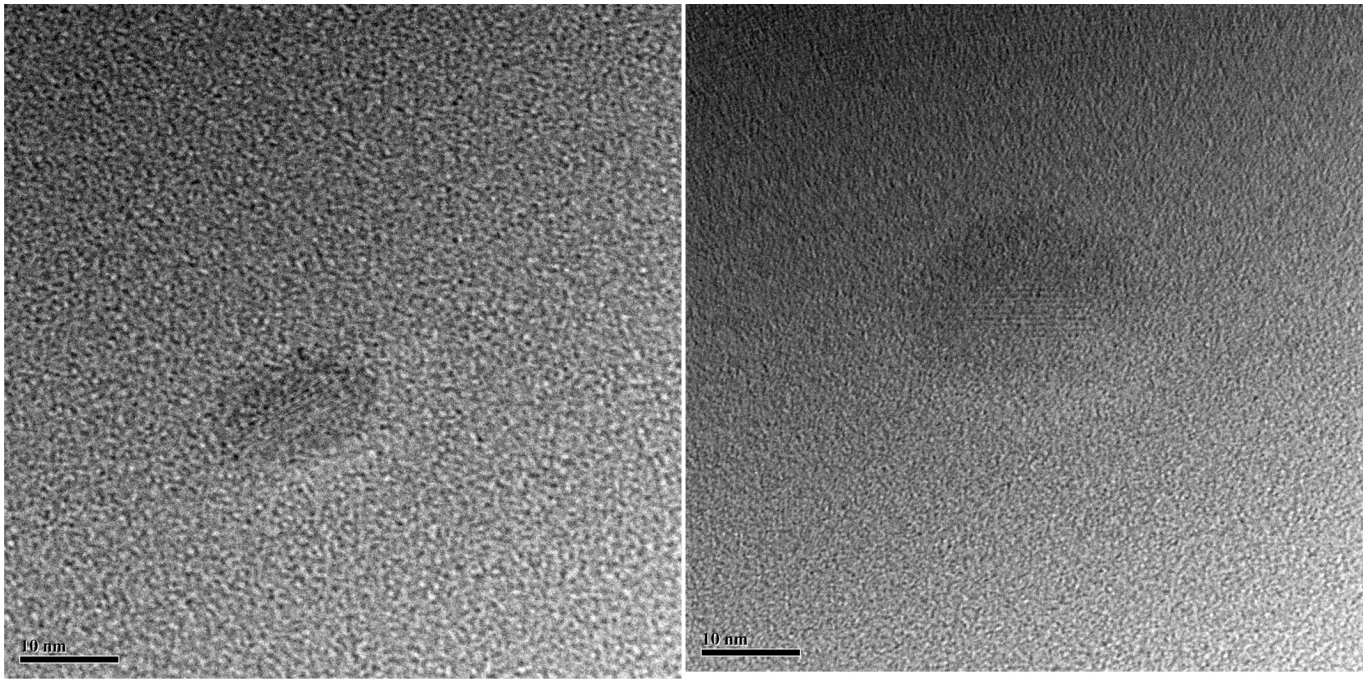

Figure 2S. HRTEM images of silver crystallites in the solution of red emitter. These large silver nanoparticles were supposed to be non-luminescent.

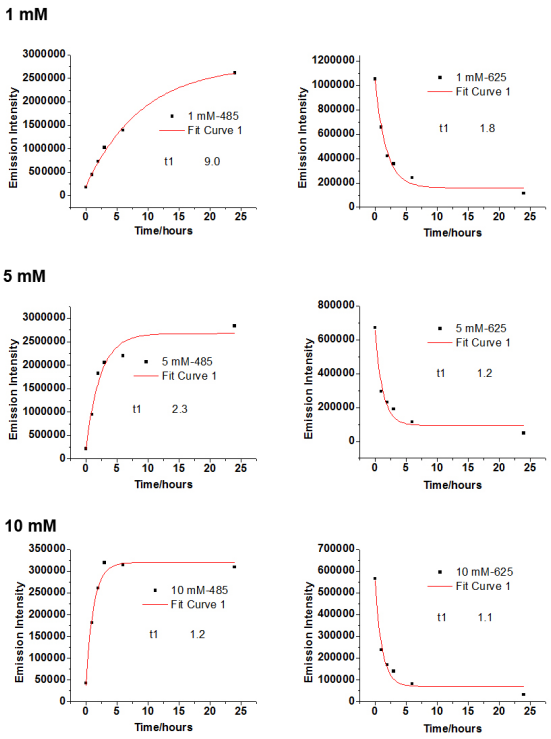

Figure 3S. The time course of C24-Ag silver nanodot emissions in the presence of 1 mM, 5 mM and 10 mM of hydrogen peroxide. The left column is the emission intensity of 485 nm, and the right is that of 625 nm (dots). The red curve is monoexponential fit of the time courses. The fitting indicates that the red emitters degraded four-fold faster than the generation of blue emitter at a hydrogen peroxide concentration of 1 mM. Higher hydrogen peroxide concentration did not bleach the red emission faster, but accelerated the generation of blue emitter significantly.

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2. Richards, C. I.; Choi, S.; Hsiang, J. C.; Antoku, Y.; Vosch, T.; Tzeng, Y. L.; Dickson, R. M. Oligonucleotide-stablized Ag nanocluster fluorophores. *Journal of the American Chemical Society* **2008,** *130* (15), 5038-5039.
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