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Enzymatically activating reduction-caged SERS reporters for versatile bioassays

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EXPERIMENTAL SECTION

Chemicals and Materials. Chloroauric acid, trisodium citrate dehydrate, leucomalachite green (LMG), malachite green (MG), methylene blue (MB), glucose, horseradish peroxidase (HRP), glucose oxidase (GOx, E.C.1.1.3.4, from *Aspergillus niger*), and hemoglobin (from bovine blood lyophilized powder) were obtained from Sigma and used as received. Human C-reactive protein (CRP) was obtained from Millipore. Affinity purified goat anti-human CRP was obtained from Immunology Consultants Laboratory, Inc. Leucomethylene blue (LMB) was freshly prepared by reducing MB with metal zinc. Other chemicals were of at least analytical reagent and were used as received. The stock solution of LMG was prepared by dissolving LMG into DMSO (or DMF). All aqueous solutions used in the experiments were prepared with deionized water (18.2 MΩ·cm, Millipore).

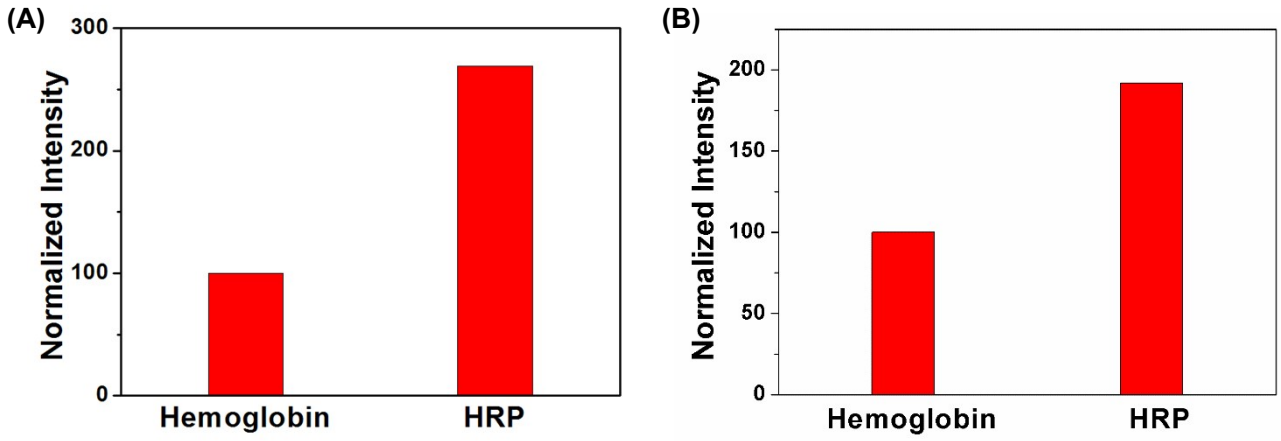
Activating Reduction Caged Raman Reporters with Enzymes for SERS measurements. For a typical test, 2 μL of 5 mM H_2O_2, 2 μL of 100 μM LMB (or Benzyol-LMB, or LMG) and 2 μL of 500 μg/mL HRP (or hemoglobin) were added into 10 μL of 50 mM pH=4.5 NaOAc buffer. After incubation, 64 μL of 50 mM pH=4.5 NaOAc buffer was further added. Then 6 μL of the above reaction solution was added into 300 μL of citrate protected AuNPs (60 nm in size) for SERS measurements. (Note: the AuNPs could be aggregated with 10 μL of the reaction solution. For LMG, high concentration of HRP would degrade MG and therefore the amount of HRP used should be optimized.) Raman spectra were obtained on an Advantage Raman spectrometer (Japan) with a 633 nm laser.

H_2O_2 and glucose bioassays with hemoglobin activated Raman reporters. For H_2O_2 detection, 2 μL of H_2O_2 with different concentrations, 2 μL of 100 μM LMG and 2 μL of 500 μg/mL hemoglobin were added into 10 μL of 50 mM pH=4.5 NaOAc buffer. After incubation, 64 μL of 50 mM pH=4.5 NaOAc buffer was further added. Then 6 μL of the above reaction solution was added into 300 μL of citrate protected AuNPs (60 nm in size) for SERS measurements.

For glucose detection, 10 μL of glucose with different concentrations in buffer (50 mM pH=4.5 NaOAc) and 2 μL of 500 μg/mL GOx in buffer (50 mM pH=4.5 NaOAc) were first mixed and incubated at 37 ºC for 1 hour. Then 2 μL of 100 μM LMG and 2 μL of 500 μg/mL hemoglobin were added. After incubation, 64 μL of 50 mM pH=4.5 NaOAc buffer was further added. Then 6 μL of the above reaction solution was added into 300 μL of citrate protected AuNPs (60 nm in size) for SERS measurements.

CRP Detection. For CRP detection, the goat anti-human CRP was first immobilized on agarose beads to form anti-CRP beads. Then, 10 μL of different concentrations of CRP was added into 20 μL of anti-CRP beads for incubation. After the incubation and washing (1% BSA in 1×PBS (3 times) and 0.1%

Tween-20 in 1×PBS (2 times)), the HRP-labelled anti-Human CRP (10 μL of 0.01 mg/mL) was added for further incubation. After the incubation and washing (1% BSA in 1×PBS (3 times) and 0.1% Tween-20 in 1×PBS (2 times)), the formed sandwich structure was re-dispersed into NaOAc buffer (pH=4.5, 50 mM). 2 μL of 100 μM LMG and 2 μL of 1 mM H_2O_2 were added to produce SERS active MG. Then 6 μL of the obtained MG solution was added into 300 μL of AuNPs (50 nm in size) for SERS measurements.



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To demonstrate the generality of the proposed activation strategy, hemoglobin was also used to activate caged Raman reporters. As shown in Figure S1, hemoglobin could activate both LMG and Benzyol LMB. Compared with HRP, the activation efficiency of hemoglobin was lower. The different activation efficiency of hemoglobin and HRP shown in Figure S1 was due to their peroxidase activities.

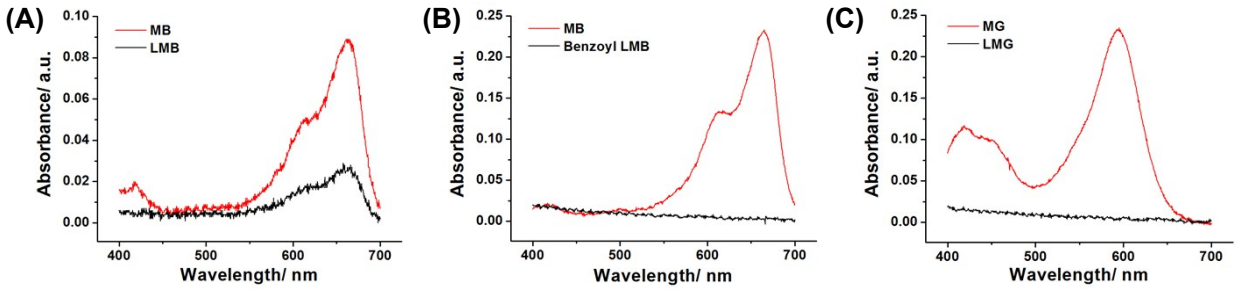
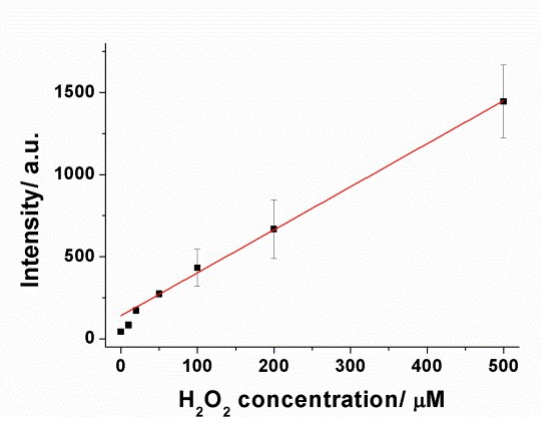


Figure S2. UV-visible absorption spectra of the caged Raman reporters before and after HRP/H_2O_2 activation.

Note: the absorption peak from 550-700 nm for LMB was due to the auto-oxidized product (i.e., MB) in the air.



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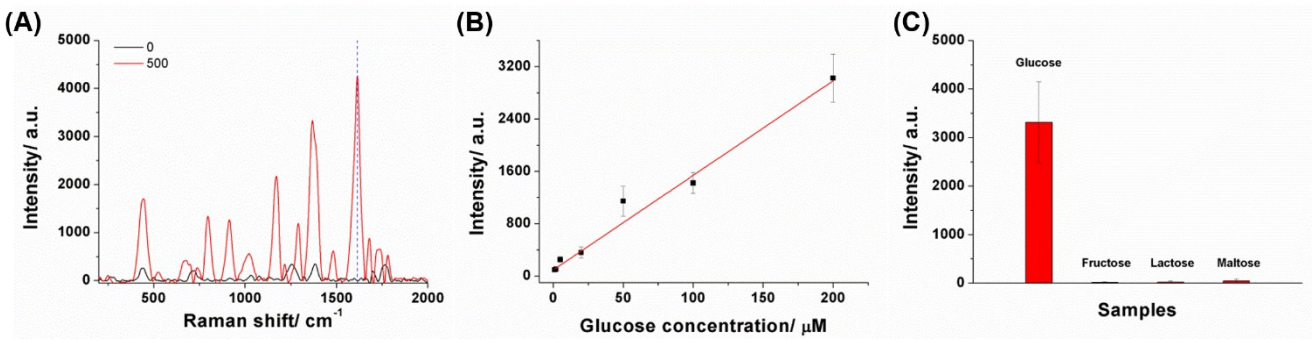

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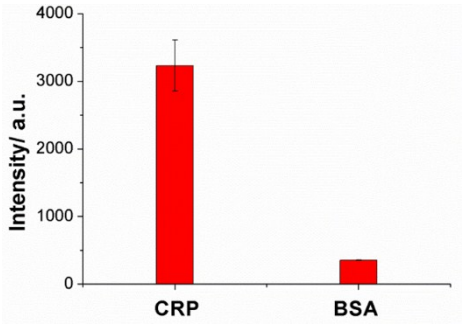

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