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Multiple turnovers of DNAzyme for amplified detection of ATP and reduced thiol in cell homogenate

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Materials and Apparatus

All oligonucleotides were synthesized by SangonBiotech（Shanghai）Co., Ltd.. Reduced glutathione (GSH, GB0229), cysteine (Cys, CB0132) and RPMI 1640 medium (BG028) were purchased from Shanghai Sangon BiotechCo. Ltd. (Shanghai, China). N-ethylmaleimide (NEM), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich. All solutions were prepared in Milli-Q water (resistance >18 MΩ·cm) from a Millipore system.

The fluorescence intensity was measured and recorded using F-4600 fluorometer (Hitachi, Japan). A Q-Sense E1 QCM-D instrument (Q-Sense AB, Västra Frölunda, Sweden) was used for the QCM assay. Streptavidin-modified magnetic nanoparticle (MNP, 1-2 μm), carboxyl- polystyrene microsphere (PSM, 0.05-0.1μm) and magnetic rack were obtained from BaseLine ChromTech Research Centre (Tianjin, China).

Preparation of DNA 1-Functionalized MNP

100 μL of 1.0 $\times 10^{-4}$ M biotinylated DNA 1 was reacted with 100 μL of streptavidin-modified MNP for 60 min. The excess DNA 1 was removed by magnetic separation. The resulting DNA 1-MNP was washed with 200 μL of 0.01M PBS buffer (pH 7.0) three times and then resuspended in 100 μL of PBS buffer at 4 °C for further use.

Preparation of DNA 2/3 or DNA 2/4-Functionalized PSM

In a typical experiment, 100 μL of PSM was first washed three times with 200 μL of 0.1 M imidazole buffer (pH 6.0) and then activated in 100 μL of 0.1 M imidazole buffer (pH 6.0) containing 0.2 M EDC with gentle shaking for 20 min. 50μL of 1.0 $\times 10^{-4}$ M DNA 2 and 50 μL of 1.0 $\times 10^{-5}$ M DNA 3 (or DNA 4) were added into the

activated microspheres and the resultant mixture incubated for 1 h at 37 °C with gentle mixing. The DNA 2/3 or DNA 2/4-PSMs were washed three times with 200 μL of wash buffer (7 mM Tris-HCl, pH 8.0, 0.17 M NaCl, 0.05% Tween 20) and resuspended in 100 μL of buffer (20 mM Tris-HCl, pH 8.0, 0.5 M NaCl) containing 10% BSA for 1 h to minimize nonspecific adsorption effects, and the conjugates were resuspended in 100 μL of buffer ((20 mM Tris-HCl, pH 8.0, 0.5 M NaCl) before use.

Operation Section of Amplified ATP Detection

100 μL of DNA 2/3-PSM reacted with 100 μL of DNA 1-MNPs for 60 min. After being washed with PBS buffer, the resulting MNP-PSM composites were incubated with 100 μL different concentration of ATP solution. The specific recognition process was carried out for 60 min. After magnetic separation, the supernatant containing the DNA 2/3-PSM were transferred and reacted with 100 μL of 1.0×10^{-4} M MB 5 for 120 min (Figure 1B). Finally, the signal was detected by fluorescence spectrophotometry.

Operation Section of Amplified GSH Detection

100 μL of DNA 2/4-PSMs probe reacted with streptavidin-modified MNPs for 60 min. After magnetic separation, the resulting MNPs-PSMs was washed with 200 μL of 0.01M PBS buffer (pH 7.0) three times and then reacted with 100 μL different concentration of GSH solution for 120 min. After magnetic separation, the supernatant containing the DNA2/4-PSM were transferred and reacted with 100 μL of 1.0×10^{-4} M MB 5 for 120 min. Finally, the signal was detection by fluorescence spectrophotometry.

Preparation of Cell Homogenate

K562 cells (leukemia cell) were cultured using RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 50 U/mL penicillin and 50 μg/mL streptomycin. The cell cultures were grown in a humidified atmosphere at 37°C with 95% air and 5% CO_2. The cell density was counted with a hemocytometer. A 1.0 mL of 1.2×10^6 cell/mL suspended K562 cells was centrifuged at 3500 rad min^{-1} for 5 min in culture medium, washed twice with ice-cold PBS buffer, resuspended in PBS buffer, and then stepwise diluted to different concentrations with PBS buffer, making 100 μL of K562 cell suspensions contain different amounts of cells in 0.5 mL eppendorf tube (EP tube), respectively. K562 Cell homogenates were obtained by disrupted 100 μL cell suspensions for 30 min in ultrasonic disintegrater. During sonic disruption, the temperature was maintained below 4°C with ice bath.