

Supporting Information

Amplified Detection of Protein Cancer Biomarkers Using DNzyme Functionalized Nanoprobes

Experimental

1. Chemicals

Oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. AFP, AFP monoclonal antibody, AFP polyclonal antibody were purchased from Biomade Technology Co. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), H₂O₂ and BSA were purchased from Shanghai Sangon Biotechnology Co. All other reagents were of analytical reagent grade and used without further purification. Doubly distilled water was used throughout the experiments.

2. Solutions

Washing buffer (10 mM phosphate sodium buffer solution, pH 7.5, 100 mM NaCl), stock buffer and assay buffer (10 mM phosphate sodium buffer solution, pH 7.5, 150 mM NaCl, 0.025% Tween 20 and 0.1% BSA), blocking buffer (10 mM phosphate sodium buffer solution, pH 7.5, 150 mM NaCl, 0.025% Tween 20 and 0.5% BSA).

3. GNPs Modification

The pH of citrate-stabilized GNPs (30 nm diameter) solution (about 0.5 nM) was adjusted to 9.0 by adding diluted NaOH. 10 μ L of AFP polyclonal antibody (0.1 μ g/ μ L) was added to 1 mL of GNPs solution. The mixed solution was incubated for 30 min at 10 $^{\circ}$ C under slow vortex. Subsequently, thiolated DNA

(5'-HS-(CH₂)₆-TTTTTTTTTCCCAACCCGCC) was added to the solution to a final concentration of 2.5 μM. After 16-h incubation at room temperature, the concentration of NaCl in the solution was brought to 0.1 M during 24 h by stepwise addition of 1 M NaCl/10 mM phosphate sodium buffer solution (pH 7.5). Unbound thiolated DNA was removed by repetitive centrifugation (13000 rpm, 30 min, 4 °C, supernatant decanted), followed by rinsing and resuspension in washing buffer. The centrifuging/rinsing procedure was repeated three times. The DNAzyme (5'-GGGTAGGGCGGGTTGGG) was added to the solution to a final concentration of 2.5 μM and allowed to hybridize at room temperature for 6 h. Unbound DNAzyme was removed by repetitive centrifugation (13000 rpm, 30 min, 4 °C, supernatant decanted), followed by rinsing and resuspension in washing buffer. The centrifuging/rinsing procedure was repeated three times. The final deposition was suspended in stock buffer stored at 4 °C for further use.

4. MMPs Modification

Tosylactivated MMPs (Invitrogen, 100 mg/mL) was washed twice with 0.1 M sodium borate buffer (pH 9.5). 50 μL of pre-washed MMPs (100 mg/mL) was mixed with 200 μL of 0.1 M sodium borate buffer (pH 9.5), 100 μL of 3 M (NH₄)₂SO₄ in sodium borate buffer (pH 9.5), and 100 μL of AFP monoclonal antibody solution (1.0 μg/μL). The mixed solution was incubated for 24 h at 37°C under slow vortex. Next, the MMPs solution was placed on a magnet. The supernatant was aspirated, and the MMPs were passivated by adding of blocking buffer. The passivation step proceeded for 24 hours at 37 °C under slow vortex. Then the MMPs were washed three times

with stock buffer and finally suspended in stock buffer and stored at 4 °C for further use.

5. Detection of AFP

Prior to the experiment, MMPs working solution was prepared by diluting MMPs stock solution in assay buffer to a final MMPs concentration of 0.025 mg/mL. The assay was initiated by mixing 50 μ L of assay buffer, 50 μ L of MMPs working solution and 100 μ L of AFP solution in a PCR tube. The system was incubated at 37 °C for 30 min under constant vortex to allow binding between the MMPs and the AFP. Next, the reaction tube was placed on a magnet, the supernatant aspirated, and the MMP/AFP complexes were washed twice with 200 μ L of assay buffer. 100 μ L of GNPs working solution (0.5 nM) was added to the MMP/target complexes, and the solution was incubated at 37 °C for 30 min under constant vortex. The system was washed five times with 200 μ L of assay buffer. Then 50 μ L of 25 mM HEPES-NH₄OH buffer (pH 8.0, containing 20 mM KCl, 200 mM NaCl and 1 % DMSO) was added. The system was vortexed vigorously at 65°C for 30 min to allow for full dehybridization of the DNAzyme. The complexes were again separated magnetically, and the supernatant containing the DNAzyme was collected. Then hemin was added, making sure that the final concentration of hemin was 50 nM. The system was incubated at room temperature for another 1 h, and then ABTS and H₂O₂ were added, the final concentrations of which were 2 mM. The absorption of the system was detected in the Kinetics mode of a Perkin Elmer Lambda 750 UV-Vis spectrophotometer.

6. Commercial ELISA for AFP

A commercially available ELISA assay was utilized for method comparison studies. To AFP antibody coated polystyrene 96-well plates, 50 μ L of serum sample suspension was added. The wells incubated at 37 $^{\circ}$ C for 30 min, and then rinsed 3 times with 0.1 mol/L PBS (pH 7.4) containing 0.5 mol/L NaCl and 1 mL/L Tween 20. Then 50 μ L of HRP-antibody conjugate solution was added and incubation continued for 1 h. The wells were again rinsed 3 times. Then 50 μ L of ABTS reagent solution was added and incubated at 37 $^{\circ}$ C for 10 min. The results of ELISA were measured by a spectrophotometric ELISA reader at a wavelength of 410 nm.