SUPPORTING INFORMATION

Glucose-doped calcium carbonate nanospheres: A new signal-transduction tag for nano-labelled immunosorbent assay (NLISA)

Yue Chen,[a,†] Bin Li,[b,†] Lilin Ge,[b,*] Fan Cai,[c,*] Dianping Tang,[d] and Yao Lin[e,*]

[a] *Pathology Department, Fujian Maternity and Child Health Hospital (Fujian Obstetrics and Gynecology Hospital), Fuzhou 350001, Fujian, China*

[b] *Jiangsu Province Engineering Research Center of Traditional Chinese Medicine health preservation, Nanjing University of Chinese Medicine, Nanjing 210000, Jiangsu, China*

[c] *College of Life Sciences, Fujian Normal University, Fuzhou 350117, Fujian, China*

[d] *Key Laboratory of Analytical Science for Food Safety and Biology (MOE & Fujian Province), Department of Chemistry, Fuzhou University, Fuzhou 350108, China*

[e] *Fujian-Macao Science and Technology Cooperation Base of Traditional Chinese Medicine-Oriented Chronic Disease Prevention and Treatment, College of Integrative Medicine, Fujian University of Traditional Chinese Medicine, Fuzhou 350122, Fujian, China*

CORRESPONDING AUTHOR INFORMATION

E-mails: gelilin@njucm.edu.cn (L. Ge), caif@fjnu.edu.cn (F. Cai), yaolin@fjtcm.edu.cn (Y. Lin).

† These authors contributed equally to this work.

EXPERIMENTAL SECTION

Materials and chemicals. Monoclonal mouse anti-human carcino embryonic antigen CEA capture antibody (mAb_1, clone number: CB30, cat# ab35657), polyclonal rabbit anti-human CEA secondary antibody (pAb_2, cat# ab131070), and human CEA ELISA kit (sensitivity: 24.68 pg mL^{-1}, range: 78.13 – 5000 pg mL^{-1}, cat# ab264604) were purchased from Abcam (Shanghai, China). All the high-binding polystyrene 96-well microtiter plates (Ref. 655061) were acquired from Greiner (Frickenhausen, Germany). Glucose, bovine serum albumin (BSA), γ-glycidoxypropyltrimethoxysilance (GPMS), and bis(2-ethylhexyl) sodium sulfosuccinate (AOT) were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). All other reagents were of analytical grade and were used without further purification, unless specified otherwise. Ultrapure water from a Milli-Q water purification system (18.2 MΩ cm^{-1}, Millipore) was used throughout this work. All the buffers including phosphate-buffered saline (PBS) solution were the product of Sigma.

A pH 9.6 coating buffer (1.59 g Na_2CO_3, 2.93 g $NaHCO_3$ and 0.2 g NaN_3) and a pH 7.4 phosphate-buffered saline (PBS, 0.01 M) (2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.24 g KH_2PO_4, 0.2 g KCl and 8.0 g NaCl) were prepared by adding the corresponding chemicals into 1000 mL distilled water, respectively. The blocking buffer and washing buffer (PBST) were obtained by adding 1.0% BSA (w/v) and 0.05% Tween 20 (v/v) in PBS, respectively.

Synthesis of glucose-doped $CaCO_3$ nanoparticles. Glucose-doped $CaCO_3$ nanoparticles (GDCaNPs) were synthesized through the reverse-micelle method similar to our previous report (D. Tang and R. Ren, *Anal. Chem.*, 2008, **80**, 8064-8070). Prior to synthesis, two solutions were prepared as follows: solution A containing $CaCl_2$ (500 μL, 10 mM) in ultrapure water, glucose (500 μL, 2.0 M) in ultrapure water and AOT in isooctane (15 mL, 0.1 M); solution B containing Na_2CO_3 (500 μL, 10 mM) in ultrapure water, glucose (500 μL, 2.0 M) in ultrapure water and AOT in isooctane (15 mL, 0.1 M). After adequate stirring, solution B was added dropwise into solution A under vigorous stirring until formation of GDCaNP colloids. Following that, absolute ethanol (3.0 mL) was injected into the mixture for stirring for another 10 min. During this process, the reverse micelle was completely breakdown to form two immiscible layers of aqueous ethanol and isooctane. The ethanol was carefully removed through a separating funnel. Finally, the as-

prepared GDCaNPs were washed 3-4 times with issoctane and centrifuged to remove residual components. The obtained GDCaNPs were collected for the subsequent use.

Conjugation of GDCaNPs with pAb_2 antibodies. Polyclonal rabbit anti-human pAb_2 secondary antibodies were modified onto the surface of GDCaNPs *via* the epoxy-amino reaction similar to our previous work (D. Tang, B. Su, J. Tang, J. Ren and G. Chen, *Anal. Chem.*, 2010, **82**, 1527-1534). Before modification, the above-prepared GDCaNPs were initially dried at 60 ^{o}C for 60 min. Then, 100 mg of GDCaNPs was thrown into 1.0 mL of 5% GPMS in dry toluene, and reacted 12 h at room temperature under continuous shaking on a shaker. Following that, the functionalized nanoparticles were thoroughly washed with toluene and ethanol through centrifugation to remove the physically adsorbed GPMS. In this case, the active epoxy group on the surface of GDCaNP could be readily reacted with the amino group of antibody. Afterward, the resulting GDCaNPs were thrown into 1.0 mL of 0.1 mg mL^{-1} pAb_2 antibody in PBS (pH 7.4, 10 mM), and reacted for 12 h at 4 ^{o}C under slight shaking. After washing with PBS (pH 7.4, 10 mM) and centrifugal separation, the residual active epoxy groups on the surface of GDCaNPs were blocked with BSA (1.0 mL, 3.0 wt%). Finally, pAb_2-functionalized GDCaNPs (designated as pAb_2-GDCaNPs) were dispersed into 5.0 mL of pH 7.4 10 mM PBS, and stored at 4 ^{o}C for the subsequent usage (note: The concentration of pAb_2-GDCaNPs was about 20 mg mL^{-1}).

Sandwiched immunoreaction and PGM measurement for CEA. Initially, a high-binding polystyrene 96-well microplate was coated overnight at 4 °C with 50 μL per well of mAb_1 at a concentration of 10 μg mL^{-1} in 0.05 M sodium carbonate buffer (pH 9.6). The microplates were covered with adhesive plastics plate sealing film to prevent evaporation. On the following day, the plates were washed three time with PBST, and then incubated with 300 μL per well of blocking buffer for 1 h at 37 °C with shaking. The plates were then washed as before. Following that, a mixture including 50 μL of CEA standards/samples with various concentrations in PBS and 50 μL of 20 mg mL^{-1} pAb_2-GDCaNPs were added into the microplates, and incubated for 20 min at room temperature under shaking. The plates were washed again, and 10 μL of 2.0 M HCl was added to each well. The plates were shaken for 2.0 min on a plate shaker at room temperature to dissolve the $CaCO_3$ and release the doped glucose molecules. Finally, a 5.0-μL aliquot of the supernatant was removed for glucose measurement using the commercialized Roche PGM. The obtained PGM signal was registered as the assaying signal relative to various-concentration target

analytes. All the determinations were made at least in duplicate. The sigmoidal curves were calculated by mathematically fitting experimental points using the Rodbard's four parameter function with Origin 6.0 software. Graphs were plotted in the form of absorbance against the logarithm of CEA concentration.

Commercial ELISA for target CEA. A commercially available ELISA assay was utilized for method comparison studies. In sandwich ELISA with standard polystyrene 96–well plates, 50 μL serum sample suspension was incubated at 37 °C for 30 min, and the wells were rinsed 3 times (3 min each) with 0.1 M PBS (pH 7.4) containing 0.5 M NaCl and 1.0 mL L^{-1} Tween 20. Then we added 50 μL conjugate solution and incubation continued for 1 h. The wells were again rinsed and 50 μL 3,3',5,5'-tetramethylbenzidine (TMB) reagent was added and incubated at 37 °C for 10 min. The enzymatic reaction was stopped by adding 50 μL of 2.0 M H_2SO_4 to each well. The results of ELISA were measured by a spectrophotometric ELISA reader at a wavelength of 450 nm.