Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information for:

$CaCO_3$ nanoparticles as the tags for potentiometric detection of cardiac biomarker using calcium ion-selective electrode

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Experimental

S1.1. Reagents and chemicals

Monoclonal mouse anti-human myoglobin capture antibody (designated as mAb_1, 0.29 mg mL^{-1} in PBS containing 0.09% sodium azide, w/v), polyclonal rabbit anti-human myoglobin detection antibody (designated as pAb_2, application: 1 : 500 – 1 : 1000), myoglobin (Mb) standards, myc-oncogene (MYC), squamous cell carcinoma antigen (SCCA), alpha-fetoprotein (AFP), human IgG, carcinoembryonic antigen (CEA), thyroid-stimulating hormone (TSH), troponin T (TPT), troponin I (TPI), CK-MBmass (CKMB) and human myoglobin ELISA kit were purchased from Abcam (Shanghai, China). N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), thioglycolic acid, bovine serum albumin (BSA, 96-99%) and chitosan (high purity, MW 60,000 – 120,000) were acquired from Sigma-Aldrich. All other chemicals were of analytical grade and used as received. Ultrapure water obtained from a Millipore water purification system at ~18.2 $M\Omega \cdot cm^{-1}$ (Milli-Q, Millipore) was used in all runs. A pH 9.6 carbonate buffer (1.69 g Na_2CO_3 and 2.86 g $NaHCO_3$), a pH 9.8 carbonate buffer (2.33 g Na_2CO_3 and 2.35 g $NaHCO_3$), 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 in 1000-mL distilled water, respectively. The blocking buffer and washing buffer were obtained by adding 1.0 wt% BSA and 0.05% Tween 20 (v/v) in PBS, respectively.

S1.2. Preparation of BSA-functionalized $CaCO_3$ nanospheres (BSA-$CaCO_3$)

BSA-functionalized $CaCO_3$ nanoparticles (denoted as BSA-$CaCO_3$) are prepared similar to the literature [J. Zhou, J. Tang, G. Chen and D. Tang, Biosens. Bioelectron., 2014, **54**, 323-328]. Briefly, $CaCl_2$ aqueous solution (5.0 mL, 50 mM) was initially added into BSA aqueous solution (10 mL, 3.0 mg mL^{-1}) under vigorous stirring at room temperature (note: During this process, the positively charged Ca^{2+} ions were adsorbed onto the negatively charged BSA molecules because the isoelectronic point of BSA is ~4.7. Meanwhile, partial Ca^{2+} ions could be chelated onto the BSA by the residual –COOH groups of BSA molecules). Following that, 5.0 mL of 50 mM Na_2CO_3 aqueous solution was dropped into the resulting mixture under the same conditions. After reaction, the resultant suspension was aged overnight under ambient conditions. Subsequently, the suspension was separated and purified by centrifugation for 15 min at 10,000g. Finally, the obtained pellets (i.e.,

BSA-$CaCO_3$) were used for further use.

S1.3. Conjugation of BSA-$CaCO_3$ with pAb_2 antibody (pAb_2-$CaCO_3$)

Polyclonal rabbit anti-human myoglobin detection (pAb_2) were conjugated with BSA-$CaCO_3$ nanospheres (denoted as pAb_2-$CaCO_3$) through a typical carbodiimide coupling [W. Lai, J. Zhuang, J. Tang, G. Chen and D. Tang, Microchim. Acta, 2012, **178**, 357-365], schematically illustrated in Fig. S1. Initially, BSA-$CaCO_3$ nanospheres (25 mg) were dispersed into 1.0 mL of ultrapure water. Then, NHS (11 mg) and EDC (15 mg) were thrown in the resultant mixture under gentle stirring for 12 h min at 4 °C (note: The –COOH groups of BSA molecules were activated during this process). Following that, 500 µL of 1.0 mg mL^{-1} pAb_2 antibody (dispersed in 10 mM PBS, pH 7.4) was added into the mixture with gentle stirring (~150 rpm), and left overnight at 4 °C. After completion of the reaction, the conjugates were centrifuged for 10 min at 8000 g. Finally, the obtained pAb_2-$CaCO_3$ nanoconjugates were washed with pH 7.4 PBS several times, and dispersed into pH 7.4 PBS (1.0 mL, 10 mM) containing 1.0 wt % BSA and 0.1 wt % sodium azide, with a final concentration of ~25 mg mL^{-1}, and stored at 4 °C until use.

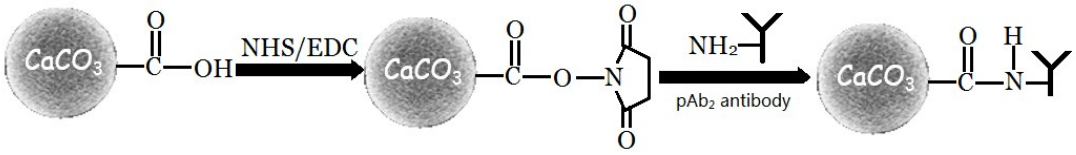

Fig. S1 Schematic diagram of BSA-$CaCO_3$ conjugation with pAb_2 antibody.

S1.4. Potentiometric measurement with Ca^{2+}ion-selective electrode

Fig. 1 represents potentiometric immunoassay protocol and measurement method. A high-binding polystyrene 96-well microplate was coated overnight at 4 ºC with mAb_1 (50 µL per well, 10 µg mL^{-1}) in 0.05 M sodium carbonate buffer (pH 9.6). The microplate was covered with adhesive plastics plate sealing film. On the following day, the microplate was washed three times with washing buffer, and then incubated with 300 µL per well of blocking buffer for 60 min at 37 °C with shaking. The microplate was then washed as before. Following that, 50 µL of myoglobin standards in pH 7.4 PBS was added into the microplate, and incubated for 60 min at 37 °C under shaking. After washing, 50 µL of pAb_2-$CaCO_3$ solution was added into the well and incubated for 60 min at 37 °C with shaking. The microplate was washed three times with washing buffer and one

time with water. Afterward, a 50-μL aliquot of 0.1 M HCl was added into each well, and reacted for 10 min (optimized) at room temperature. The resulting solution containing the released Ca^{2+} ions was transferred into 2.0 mL of 1.0 mM $NaNO_3$ solution for potentiometric measurement by using Ca^{2+} ion-selective electrode (Ca-ISE) (perfectION^{TM}, Mettler-Toledo, Switzerland). The obtained signal was registered as the sensing response to target myoglobin. According to the Nernstian equation, the reaction temperature will affect the potentiometric response of the Ca-ISE. To consider the possible application in future, however, all potentiometric measurements were carried out at room temperature (25 ± 1.0 °C).

Table S1 Comparison of the assayed results by using the developed potentiometric immunoassay and commercial human myoglobin ELISA kit for human serum samples

Sample no.	Method; concentration [ng mL^{-1}, mean ± SD, n = 3]		RSD (%)[a]
	Potentiometric immunoassay	ELISA kit	
1	23.4 ± 2.12	22.2 ± 1.97	3.72
2	0.67 ± 0.05	0.72 ± 0.06	5.09
3	12.3 ± 1.03	12.4 ± 1.01	0.57
4	34.7 ± 3.34	32.1 ± 2.98	5.51
5	68.4 ± 6.43	70.2 ± 5.53	1.84
6	19.7 ± 1.65	19.5 ± 1.42	0.72

[a]RSD was calculated according to the individual average value of two methods per sample.