Supplementary Information

Spacer-enhanced chymotrypsin-activated peptide-functionalized gold nanoparticle probes: a rapid assay for the diagnosis of pancreatitis

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S1. Method of dithizone staining of islet

The islet isolation of mouse pancreas and the staining procedure were modified from Li’s protocol. Dithizone (DTZ) was used to staining isolated pancreas. Stock DTZ solution was prepared as following procedure: 25 mg DTZ was added in 0.6 mL 95% ethanol and few drops of ammonium hydroxide were added for completely dissolving. After then the solution would turn bright orange solution. DTZ solution was prepared as following: added 0.6 mL stock DTZ solution to volumetric flask and added DPBS to final 50 mL. DTZ solution was adjusted to pH 7.4 with 1N HCl.

The isolated pancreas was digested using collagenase type I (0.5 mg/mL in DPBS, 1mL) for 2 h at 37°C. The solution would be exchanged once during digestion and repoured with collagenase type I (1mL) for 2 h incubation at 37°C. Finally, the mixture processed with centrifugation (1,500 × g, 5 min). Then poured the tissue to petri-dishes and stained with prepared DTZ solution. After 2 min staining, hand-pick the isolated islets to microscope slide and observed under microscope (BX 51; Olympus, Tokyo, Japan) and digitized using an attached MagnaFireTM imaging system (Olympus, Tokyo, Japan).
Figure. S1. (A) mouse pancreas indicated by the arrow; (B) staining islets and the size is about 250 μm in diameter (scale bar in the photogram represents 120 μm); and (C) the beta-cells of islets stained by dithizone and the size is about 20 μm in diameter (scale bar in the photogram represents 20 μm).
S2. UV-spectra of different stress tests

S2.1. Method of AuNPs pH stress test

Citrate-capped AuNPs were adjusted to different pH with 1 N HCl or 1 N NaOH. Then the 100 μL of pH adjusted AuNPs were diluted with 100 μL ddH2O and the pH were confirmed as pH 4 to 11. The mixture was incubated for 30 min at room temperature. Then the total volume of 200 μL mixture was loaded in micro plate for UV-Vis absorption measurement from 400 to 700 nm wavelengths within 1nm interval.

S2.2. Method of AuNPs salt stress test

Citrate-capped AuNPs and AuNPs-peptide probe were prepared. The salt stress assays were performed as AuNP or AuNPs-peptide probe with different concentrations of NaCl (100 to 3,000 mM). The mixture was incubated for 30 min at room temperature. Then the total volume of 200 μL mixture was loaded in micro plate for UV-Vis absorption measurement from 400 to 700 nm wavelengths within 1nm interval.

S2.3  Aggregation level of AuNPs

Citrate-capped AuNPs is unstable which easy effect by ionic strength as shown in Fig. S2B. AuNPs aggregation could be observed a red shift in UV-spectra and an increase in absorption of longer wavelength at 625 nm. More specific aggregation level determination could be conducted by the parameter of A_{520} to A_{625} ratio \(^2\,^3\). The parameter of A_{520} to A_{625} ratio above 0.3 was defined as a serious aggregation level in this study. The pH of AuNPs has no effect in aggregation. For citrate-capped AuNPs, over 20 mM NaCl cause an obvious LSPR band shift and above 25 mM cause serious aggregation. For AuNPs-P2 probe without stabilizer, couldn’t bear any salt. For AuNPs-P3 probe, could bear about 50 mM NaCl.
Figure S2. (A) Absorption spectra of citrate-capped AuNPs under different pH stress; absorption spectra of (B) citrate-capped AuNPs; (C) AuNPs-P2 probe; and (D) AuNPs-P3 probe under different salt stress.
S3. Establishment of mouse model of acute pancreatitis

Acute pancreatitis (AP) could be induced by repeated cerulein injections and higher frequencies of cerulein injections will lead to rapid formation of pancreatic fibrosis. AP is initiated in the acinar cells and the proteolytic enzymes play a crucial role by inducing pancreas auto-digestion. There are several evidences from experiment models suggested that premature activation of trypsinogen represents a critical indicating event that leads to acinar cell damages, tissue destruction, and self-digestion of the organ.

The laboratory biochemical diagnosis of AP is using the plasma amylase and lipase levels which would increase over three-fold than the upper limit of the normal reference. The plasma amylase of cerulein-induced group was significantly higher than that of in the saline group, which was over 6 and 12-fold in the 8th and 10th h scarified mice, respectively (Fig. 5A). Meanwhile, the plasma lipase of cerulein-induced group was significantly higher than that of in the saline group, which was over 4 and 6-fold in the 8th and 10th h scarified mice, respectively (Fig. 5B). The cerulein-induced AP results are corresponding to the previously established model that serum amylase was about 30,000 U L$^{-1}$ at the 7th h and the reference value was about 4,000 U L$^{-1}$. Precious works showed that cerulein-induced AP model could achieve maximal pancreatic injury at the 12th h and recover spontaneously after the 24th to 48th h. The cerulein-induced AP mouse model established by Tsai et al. (2011) also showed the elevation of plasma amylase and lipase level in the 24th h and decreased in 30th h. However, the plasma amylase and lipase are decreasing to normal level in the 24th h in this study. The amylase levels with pancreatitis depended on the severity of the diseases. Van felius et al. (2003) indicated that cerulein administration induced mild pancreatitis and the
interval between injections was not frequently enough might lead to less severity \textsuperscript{10}. Therefore, the decreases of amylase and lipase levels could conclude to mild AP induced in this model.
References


