SUPPORTING INFORMATION

Gold-Nanoparticle Coated La,Tb-encoded PS Beads and Their Application in Investigating the Performance of the Inductively Coupled Plasma of a Mass Cytometer

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Instrumentation

Inductively coupled plasma mass spectrometry (ICP-MS) measurements were carried out on an Elan 9000 instrument (Perkin-Elmer SCIEX) operating under normal Ar plasma conditions (1400 W forward plasma power, 17 L/min Ar plasma gas flow, 1.2 L/min auxiliary Ar flow, and 0.95 L/min nebulizer Ar flow). A cross flow double pass spray chamber (Perkin-Elmer SCIEX) was used in all instances. All the experiments were performed using an auto-sampler (Perkin-Elmer AS 93) modified for operation with Eppendorf 1.5 mL tubes. Sample volume was fixed at 1.5 mL. The sample uptake rate was adjusted depending on the particular experiment, typically 100 µL/min. Standards were prepared from 1000 µg/mL Perkin-Elmer pure Single-Element Standard solutions (Perkin-Elmer, Shelton, CT) by sequential dilution with high purity HNO_3. High purity HNO_3 was measured as well in each experiment to be used as blank signal. The blank signals were subtracted from the sample signals, which were then normalized to the signals of standard solutions (1 ppb).

Materials

Tetraethoxysilane (TEOS, 98 %, Aldrich), 3-(trimethoxysilyl)propyl methacrylate (MPS, 98 %, Aldrich), polyvinylpyrrolidone (PVP) (Aldrich, PVP55, M_w = 55,000), Triton-X305 (TX305, 70 % solution in water, Aldrich), 2,2'-azobis(2-methylbutyronitrile) (AMBN, Dupont USA), potassium persulfate (KPS, 99 %, Aldrich), lanthanum(III) chloride hexahydrate ($LaCl_3 \cdot 6H_2O$, 99.999 %, Aldrich), terbium(III) chloride hexahydrate ($TbCl_3 \cdot 6H_2O$, 99.999 %, Aldrich), tetraethoxysilane(TEOS, 98 %, Aldrich), 2-(diethylamino)ethyl methacrylate (DMAEMA, 99 %, Aldrich), gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9 %, Aldrich). High purity HCl and HNO_3 for mass cytometry and ICP-MS analysis were purchased from Seastar Chemical Inc. All of the reagents were used without further purification.

Synthesis

Preparation of La,Tb-encoded PS Beads by Two-stage Dispersion Polymerization. The recipe for the two-stage dispersion polymerization of St with AA, Tb and La salts in ethanol is list in **Table S1**. All of stabilizer (PVP55), co-stabilizer (TX305), initiator (AMBN), and monomer (St) and ethanol were added to a 250 mL three-necked round-bottom flask equipped with a condenser, a mechanical stirrer connected to a polytetrafluoroethylene (PTFE) half-moon stirring paddle and a gas inlet. The bottom of the hole of the stirring paddle was fixed at the same height as the surface of the solution and stirred at 100 rpm. After a homogeneous solution formed at room temperature, the solution was deoxygenated by bubbling nitrogen gas at room temperature for 30 min. Then, the flask was placed in an oil bath preheated 70 ^{o}C and a nitrogen purge was maintained during the reaction. AA, $LaCl_3$ and Tb salts were dissolved in ethanol and preheated in the 70 ^{o}C oil bath for 5 min. Then, 1 h after the polymerization reaction started, the solution was added into the reaction flask over around 1 min. The polymerization reaction was continued for 24 h. The PS beads were washed three times with ethanol by suspension and centrifugation.

Table S1. Recipe for the Preparation of La, Tb-encoded PS Beads

Materials	Two-stage reaction	
	1st stage	2nd stage[a]
Styrene (g)	6.27	
PVP(55,000) (g)	1.00	
TX-305 (g)	0.35	
AMBN (g)	0.25	
Ethanol (g)	18.97	5.01
Acrylic acid (mg)[b]		125
$LaCl_3 \cdot 6H_2O$ (mg)[c]		31
$TbCl_3 \cdot 6H_2O$ (mg)[c]		31

[a] 30 min after the reaction was initiated.

[b] 2.0 wt% of co-monomer AA based on the weight of St was added in the second stage of the reaction.

[c] 0.50 wt% of $LaCl_3 \cdot 6H_2O$ and $TbCl_3 \cdot 6H_2O$ based on the weight of St were added in the second stage of the reaction.

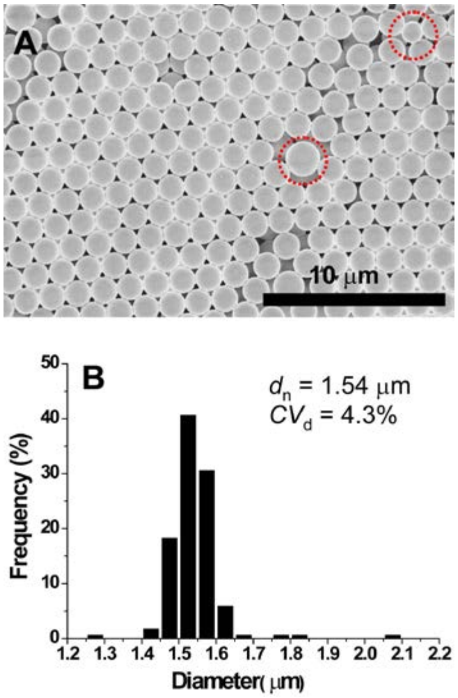

Figure S1. SEM image (A) and size distribution histogram (B) of La,Tb-encoded PS beads. In (A), the red circles identify rare examples of “outlier” particles with diameters much larger or smaller than the mean.

Preparation of PS/SiO$_2$ Beads Coated with Methacrylate Functionality. PS beads (300 mg) and ethanol (27 mL) were added into a 100 mL round-bottom flask. Then a TEOS solution (3 mL, 10 % v/v in ethanol) was added and the mixture was stirred 5 min at room temperature, followed by the addition of ammonia (0.51 mL, 28 wt% in water). After 3 h, an MPS solution (1.5 mL, 10 % v/v in ethanol) was added and the mixture was stirred for another 1 h. After the particles were washed with ethanol twice and water three times by centrifugation and resuspension. A small sample was removed to analyze for solids content gravimetrically. On this basis, we infer that 224 mg of product was formed. The remaining particle solution was used for subsequent reactions. The same strategy was used in the following experiments to determine particle yield.

Preparation of PS/SiO$_2$/PDMAEMA. PS/SiO$_2$ beads (50 mg bead dispersion in 40 mL water) was added into a 100 mL round-bottom flask. The mixture was stirred for 30 min at room temperature, followed by the addition of DMAEMA (1.0 mL) and subsequently purged with N$_2$ for 30 min. The flask was put into an oil bath preheated at 55 ^{o}C, followed by addition of the initiator KPS (1 mL, 20 mg/mL in water). After the reaction continued for 48 h, the product (42 mg) was isolated and then washed with three centrifugation-resuspension cycles.

Preparation of PS/SiO$_2$/PDMAEMA/Au. An aliquot of HAuCl$_4$ solution (160 μL, 0.5 mg/mL in water) was added into a PS/SiO$_2$/PDMAEMA solution (16.0 mg in 40 mL water). Then, the mixture was shaken in the dark at room temperature for 24 h. In a control experiment, an HAuCl$_4$ solution (160 μL, 0.5 mg/mL in water) was added into a solution of PS/SiO$_2$ (16.0 mg in 40 mL water) and shaken in the dark at room temperature for 24 h. The products were isolated and then washed with five centrifugation-resuspension cycles, yielding 14 mg of PS/SiO$_2$/PDMAEMA/Au particles and 13 mg of PS/SiO$_2$/Au particles.

Further comments on the thermal decomposition of PS/SiO$_2$ beads by TGA

The thermal decomposition of the PS beads shown in Figure 1C and D (main text) occurred in the range 262 ^{o}C to 414 ^{o}C with a peak in the DTG curve at T_d = 378 ^{o}C. These are the precursor beads for the synthesis of the silica-coated PS/SiO$_2$ beads. There was a very small residue (2.2 wt %) due to the La and Tb salts incorporated in the beads. PS beads without inorganic salts would leave a much smaller residue. The PS/SiO$_2$ beads had a slightly shifted decomposition range (271 ^{o}C to 430 ^{o}C) with T_d = 398 ^{o}C. The ceramic yield of the PS/SiO$_2$ beads at 550 ^{o}C was 3.9 wt % higher than that of the precursor PS beads. We calculated the silica content of the PS/SiO$_2$ beads on this value (see below). In the following paragraph, we offer further comments about factors that affect the behavior of PS-bead-hybrid materials in the TGA.

The weight residue at 550 °C (2.2 %) of the PS precursor beads containing La and Tb salts (main text, Figure 1C) is larger than what one might expect based on the La and Tb salt alone. Both $LaCl_3.6H_2O$ and $TbCl_3.6H_2O$ were introduced into the polymer bead synthesis at 0.5 wt % based on styrene. Some pyrolyzed organic matter must contribute to this mass. This type of degradation of polystyrene upon TGA pyrolysis has been described by Petersen et al.[1] The main message from this behavior is that one cannot use the ceramic yield obtained by TGA to quantify the lanthanide ion content of the precursor beads. We assume that this contribution to the ceramic yield remains constant for the precursor beads after they are coated with a thin shell of silica.

Li *et al.*[2] studied the thermal stability of PS beads and PS beads containing CdS quantum dots (CdS/PS) by TGA. They found that the residual mass of CdS/PS was 0.9% higher than that of PS beads themselves at around 600 ^{o}C. This difference was attributed to the component of thermally stable CdS nanoparticles. In addition, the thermal decomposition onset temperature of CdS/PS beads was higher than that of pure PS beads. They claimed that the presence of thermally stable CdS nanoparticles in the composite prevented the diffusion of the volatile component during decomposition and the chemical bonds due to the copolymerization between PS chains and the CdS nanoparticles capping agent resulted in the improvement of thermal stability in the hybrid materials of CdS/PS beads.

Previous work has shown that silica particles have excellent thermal stability, even at 800 ^{o}C.[3] Thus the additional remaining mass after pyrolysis (the 3.9% weight loss difference before and after silica coating) can be attributed to the silica layer in the sample. Based on the densities of amorphous silica (2.1 g/cm^3) and PS (0.95 g/cm^3), we can estimate a thickness of the silica layer of 5.0 nm, if we assume that each PS bead is uniformly coated. This increment to the PS bead diameter is consistent with the dark field TEM observations in Figures 2C and 2D.

Mass cytometry comparison of PS/SiO_2/PDMAEMA/Au and PS/SiO_2/Au beads

One of the design features of our synthesis was the introduction of a PDMAEMA layer to initiate the reduction of Au ions to Au^{o} and to trap the AuNPs formed. To examine the effectiveness of the PDMAEMA on the introduction of gold nanoparticles, we carried out a control experiment in which we treated PS/SiO_2 beads with $HAuCl_4$ under similar conditions as those used to prepare the PS/SiO_2/PDMAEMA/Au beads. We refer to the control sample as PS/SiO_2/Au beads. Both samples were examined by mass cytometry. In Figure S2 we present histograms of Au signal distribution of PS/SiO_2/PDMAEMA/Au and PS/SiO_2/Au beads as obtained from FlowJo software based on events gated for Tb to reflect the frequency distribution of Au signal intensities for the AuNPs on individual PS microbeads. The mean intensity of Au atoms on PS/SiO_2/PDMAEMA/Au beads is 1.3×10^3 counts compared with only 40 counts per PS/SiO_2/Au bead. The intensity of the Au signal of PS/SiO_2/PDMAEMA/Au is about 33× larger than that of PS/SiO_2/Au. These results demonstrate the importance of the PDMAEMA layer for the incorporation of AuNPs onto the surface of the silica-coated PS beads.

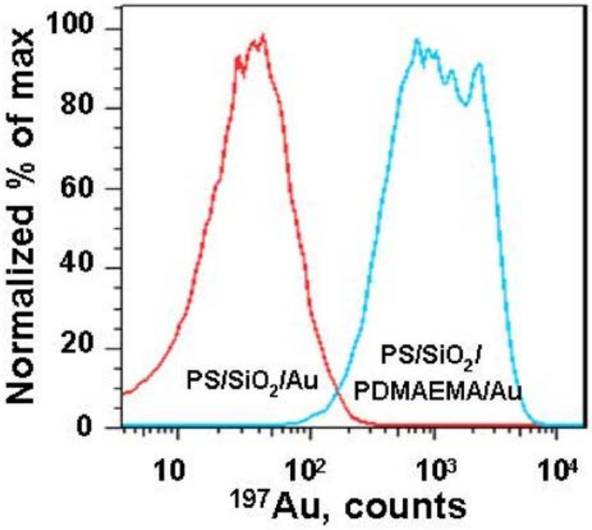

Figure S2. Distribution of the Tb-gated single bead intensities for Au for PS/SiO$_2$/PDMAEMA/Au and PS/SiO$_2$/Au beads.

Au particle release experiments

After the gold nanoparticles were incorporated onto the surface of the PS/SiO$_2$/PDMAEMA beads, these beads were designed for examining the performance of ICP-MS of mass cytometer in 1.0 % HCl aqueous solution. Our group's previous work showed that only traces of lanthanide ions encoded in the beads could leak from the beads under acidic aqueous conditions.[4] For the beads with La and Tb ions encoded in their interior and gold nanoparticles located on their surface, we need consider the stability toward the loss of gold nanoparticles in 1.0 % HCl aqueous solution. In this experiment, a sample (10 mg) of washed PS/SiO$_2$/PDMAEMA/Au particles was dispersed in 1.0 % HCl aqueous solution (20 mL). The solution was stirred continuously at room temperature. Aliquots (1 mL) were taken at different time intervals over a 2-week period (4 h, 8 h, 48 h, 120 h, 192 h and 336 h). After the beads in each sample were removed by syringe filter (PTFE, pore size = 0.2 mm), an aliquot of 100 μL of the filtrate solution was mixed with *aqua regia* solution (900 μL) and the mixture was stirred for 2 days. Then, 100 μL of the mixture was diluted with HNO$_3$ aqueous solution (3.0 %) and subsequently analyzed by ICP-MS. The content of Au in each sample taken at different intervals was used to indicate the release of gold nanoparticles from the surface of PS beads. In addition, a control experiment was carried out. An aliquot of 100 μL was taken from the PS/SiO$_2$/PDMAEMA/Au bead solution with 1.0 % HCl, followed by mixing with 900 μL *aqua regia* solution directly without filtration to remove PS beads inside. After the solution was stirred for 2 days, 100 μL of the mixture was diluted with HNO_3 aqueous solution (3.0 %), followed by analysis by ICP-MS. The content of Au in this solution was used to indicate total content of gold nanoparticles on the surface of PS beads. On the basis of these experiments, the content of lost gold ions or nanoparticles at different intervals could be calculated.

Figure S3 shows the release profile of Au gold nanoparticles from PS/SiO_2/PDMAEMA/Au beads in the 1.0 % HCl aqueous solution. The *y*-axis represents the percentage of gold released from the beads, as determined by the ratio of the Au content in the filtrate solution to that in the solution of the control experiment, assuming that all of the released gold treated with *aqua regia* could pass through the syringe filter with a pore size of 0.2 μm, but the beads with a D_n of 1.57 μm would be retained. One can see in the figure that there is almost no release of gold from the beads. Less than 1 % was lost from the surface of the beads over 336 h (14 days). This observation indicated that the gold nanoparticles located on the surface of the beads were stable in the 1.0 % HCl aqueous solution.

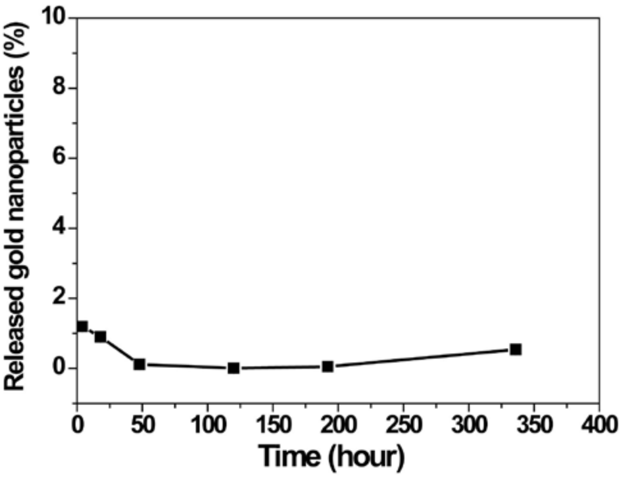

Figure S3. Release profile of gold nanoparticles from PS/SiO_2/PDMAEMA/Au beads in 1.0 %HCl aqueous solution determined by ICP-MS.

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