Electronic Supplementary Information

Encoding Through Host/Guest Structure: Construction of Multiplexed Fluorescent Beads

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EXPERIMENTAL SECTION

Materials:

Fluorescein isothiocyanate isomer I (FITC, 95%) was obtained from Sangon Biotech. (3-aminopropyl)triethoxysilane (APTES, ≥98%), 2-(*N*-morpholino)ethanesulfonic acid hydrate (MES) and bovine serum albumin (BSA, ≥98%) were purchased from Sigma-Aldrich. Ammonium hydroxide (25-28%), tetraethyl orthosilicate (TEOS), *N,N*-dimethylformamide (DMF) and succinic anhydride were purchased from Sinopharm Chemical Reagent. Ethylene imine polymer (PEI, M_w=10000, 99%) was obtained from Aladdin Chemistry. *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, ≥99%) was ordered from J&K Chemical. N-hydroxysuccinimide (NHS) was purchased from Thermo Fisher Scientific. COOH-modified, magnetic beads with diameters of 5.8 μm were purchased from Bangs Laboratories. Anti-α-human chorionic gonadotropin (Anti-α-hCG) antibody was purchased from Hangzhou Clongene Biotech and goat anti-mouse IgG/APC (2mg/mL) was obtained from Beijing Biosynthesis Biotechnology. Glycine (≥99%) was product of Shanghai Shisheng Cell Biotechnology. All reagents were used without purification, and Millipore water (18.2 MΩ cm) was used in the preparation of all aqueous solutions.

Synthesis of Guest Particles through a Modified Stöber Method:[1]

To endow guest particles with fluorescent properties, a stable fluorescent conjugate (FITC-silane) was first synthesized by reacting FITC (5.25 mg, 12.8 μmol) with APTES (73 μL) in ethanol (1mL) for 12 h under stirring. Guest particles were then prepared by mixing water, ammonia, TEOS and FITC-silane in ethanol with a total volume of 30.3 mL. After reacting for 4 hours at 40°C, the product was centrifuged (10000 rpm, 10 min) and redispersed in Milli-Q water (25 mL). Guest particles with different fluorescent intensities were prepared by adjusting the amount of FITC-silane, recipe was shown in Table S1. To improve the photostability of dye molecules,[2] an additional silica layer was added using the following protocol: above-mentioned aqueous dispersion (25 mL) was mixed with ammonia (15 mL), water (85 mL), and TEOS (0.8 mL) in ethanol (124.2 mL). After reacting for 2 hours in water bath of 40°C, the product was washed with ethanol and functionalized with carboxylic acid by a two-step modification procedure.[3] Briefly, guest particles (150 mg) were dispersed in ethanol (95.0 mL) with addition of ammonia (4 mL), APTES (1mL) and stirred for 12hours. After centrifugation and washing with ethanol, the amino-modified guest particles were transferred into DMF (10 mL) with addition of succinic anhydride (28 mg) to obtain the carboxyl-modified guest particles. The final product was washed with DMF and ethanol, stored in ethanol. The whole procedure was conducted in the dark to prevent photobleaching.

Surface Modification of Host Particles:

Buffer solution (8mL) containing PEI (156.2 mg) and EDC (10.5 mg) was added to host particles (30 mg) and vertical rotated for 3 hours. The product was washed with adequate water before use. Buffer solution refers to MES aqueous solution (10 mM, pH 5.0) containing 0.05% Tween-20 (w/v).

Preparation of Host/Guest Structured Encoded Beads:

Carboxylated guest particles (8 mg) and amino-functionalized host particles (3 mg) were separately dispersed in buffer solution (300 μL). Host particles were then added to guest particles dropwise with simultaneous ultrasonic treatment and vertical rotated for 5 minutes. Buffer solution (200 μL) containing EDC (10 mg) / NHS (10 mg) was added and vertical rotated for another 3 hours. Finally, the product was washed thrice each with sodium hydroxide solution (0.1 M) and water to remove the excess guest particles. Nine distinguishable optically encoded beads were prepared by varying the types and amounts of guest particles (Table S2).

Antibody Immobilization by Covalent Coupling:

Antibody was immobilized on the encoded beads by conventional NHS/EDC process as follows: After being washed thoroughly with MES (10mM, containing 0.05%

Tween-20 (w/v), pH 5.0), encoded beads (~1.3mg) were activated in MES (400μl) for 15min with the addition of NHS (50mg/mL) and EDC (50mg/mL). After removal of supernatant, the activated beads were transformed to new tubes and washed thoroughly with MES again. Reaction buffer containing anti-α-hCG antibody (0.25mg/mL, 400μl) was then added and the reaction was allowed to proceed for 2 hours at 37°C under vertical rotation. The bead-antibody complexes were washed with blocking buffer (10mM phosphate buffer saline (PBS) containing 0.3% glycine and 0.5% BSA, pH 7.4) and rotated overnight in blocking buffer (400μl) at 4°C. Finally, the antibody-coupled beads were washed thoroughly and stored in preserving buffer (10mM PBS containing 0.1% BSA and 0.05% Tween-20, pH 7.4) at 4°C.

Procedure of Bead-based Biological Assay:

IgG-APC was first diluted to different concentrations (0, 2, 5, 10, 20, 50, 250, 500, 2000, 7000ng/mL) in PBS (10mM, pH 7.4) with a total volume of 400μL. The dilute solution was then added to tubes containing 15μg antibody-coupled beads. After 2 hours' incubation at 37°C, the products were washed thoroughly with washing buffer (10mM PBS containing 0.05% Tween-20) and stored in water for flow cytometry assay.

Characterization:

The morphology of guest particles was observed by transmission electron microscopy (TEM) using a Tecnai G2 Spirit Biotwin (FEI, USA) instrument at 120 kV. A LS 55 fluorescence spectrophotometer (PerkinElmer, USA) was used to record the spectra and intensity of guest particles and FITC. The scanning electron microscopy (SEM) images of composite particles were obtained using a Quanta 250 instrument(FEI, USA) and the laser scanning confocal microscope images were obtained by a Leica TCS SP5 (Leica, Germany) instrument. Accuri C6 (BD, USA) was used for flow cytometric analysis.

Table S1 Amounts of Reactants Used in the Guest Particles Synthesis

Sample	Ethanol [mL]	Water [mL]	Ammonia [mL]	FITC-silane [mL]	TEOS [mL]
SF	24.7	1.6	1.0	1.0	2.0
MF	25.6	1.6	1.0	0.1	2.0
Blank	25.3	1.6	1.4	0	2.0

Table S2 Amounts of Different Guest Particles Used in the Encoded Beads

Synthesis and Flow Cytometry Analysis of Nine Encoded Beads

Code Number	SF [mg]	MF [mg]	Blank [mg]	Count of Beads	Mean FL1	CV FL1 [%]
1	0	0	8	778	832	31.3
2	0	0.4	7.6	983	2620	23.0
3	0	1.6	6.4	913	7311	17.8
4	0	4	4	1109	16970	15.2
5	0	8	0	982	36326	12.3
6	0.8	0	7.2	967	89157	17.4
7	2	0	6	1090	178424	13.4
8	4	0	4	1138	330482	11.7
9	8	0	0	825	577958	8.6

Table S3 Fluorescence Stability of the Encoded Beads during 7-day Storage at 37°C (Code 5 was selected as an example)

Time [day]	Count of Beads	Mean FL1	CV FL1 [%]
1	8936	47413	13.0
2	8446	44276	13.6
3	8787	46413	12.1
4	8398	43493	13.2
5	8436	46637	13.6
6	8626	48272	12.5
7	8194	50527	12.4

Calculation of Coverage of Guest Particles on Host Particles:

Coverage of guest particles (θ) on host particle can be calculated as:

$$\theta = \frac{N}{N_{sat}} \quad (1)$$

$$N_{sat} = \frac{2\pi}{\sqrt{3}}\left(1 + \frac{R_h}{R_g}\right)^2 \quad (2)$$

where N and N_{sat} are the actual number of guest particles on the surface of host

particles and the maximum possible number by assuming a hexagonal close packing of guest particles. R_h and R_g correspond to the radius of the host and guest particles respectively.[4] In our experiment, the diameters of the guest particles and the host particles were around 190nm and 5.8μm respectively and thus N_{sat} was calculated to be 3603. The number of guest particles N was estimated by counting the particles anchored within the concentric circle with half the diameter of the composite particle drawn in the SEM image (Figure S1). Image analysis revealed that *N* was calculated to be approximately 2200, indicating a surface coverage (θ) of about 0.6.

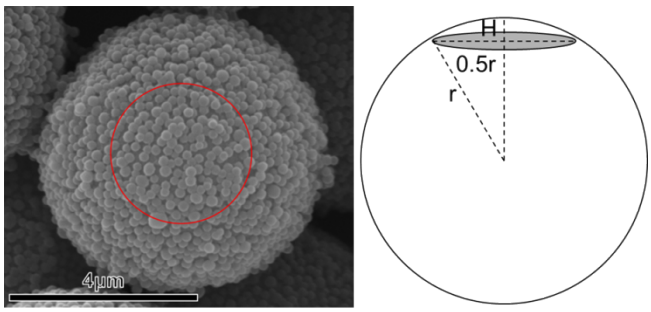

Figure S1. Schematic illustration of the estimation method used to count the actual number of guest particles *N*. Guest particles anchored within the red circle (half the diameter of the composite particle) drawn in the SEM image was counted and multiplied by a coefficient of superficial area (C≈14.928) to obtain the actual number of guest particles *N*.

Coefficient of surficial area (C) is calculated by the following equation:

$$C = \frac{S_{Sphere}}{S_{spherical\ cap}} = \frac{4\pi r^2}{2\pi r H} = \frac{2r}{H} = \frac{4}{2-\sqrt{3}} \approx 14.928$$

where r and H are radii of the composite particle and the height of the spherical cap.

Application of the Encoded Beads in Biological Assays:

To examine the applicability of the encoded beads in biosensing, a model detection system was designed. Briefly, anti-α-hCG antibody was immobilized on the beads via covalent conjugation and the bead-antibody complexes were then used to detect IgG labelled with APC. Fluorescence intensity of APC was detected on the FL4 channel (675/25nm) of flow cytometer. As shown in Figure S2, strong linear correlation was observed between the mean fluorescence intensity and the concentration of IgG-APC (0ng/mL-7000ng/mL), which demonstrated that the encoded beads synthesized herein were efficient in serving as carriers in biosensing.

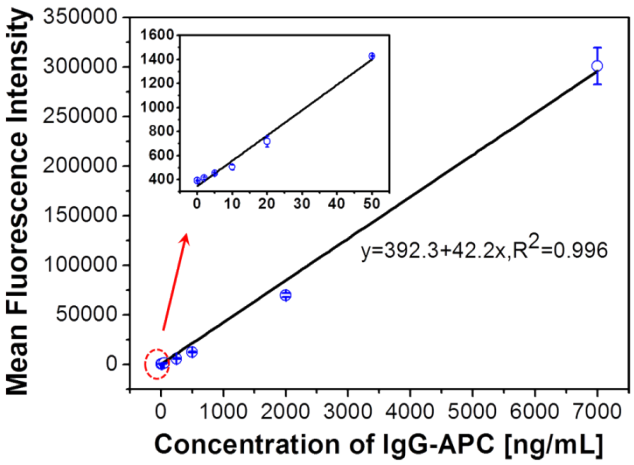

Figure S2. Relationship between the mean fluorescence intensity of FL4 channel (675/25nm) and the concentration of IgG-APC. The inset show magnified results of low IgG-APC concentration.

Magnetic Separation of Encoded Beads

As shown in Figure S3, the encoded beads were dispersed in water uniformly before magnetic separation, and the supernatant became clear soon after being subjected to the magnetic field (~250mT for 1 min), demonstrating good magnetic responsibility of the encoded beads and showed promise of automatic operation.

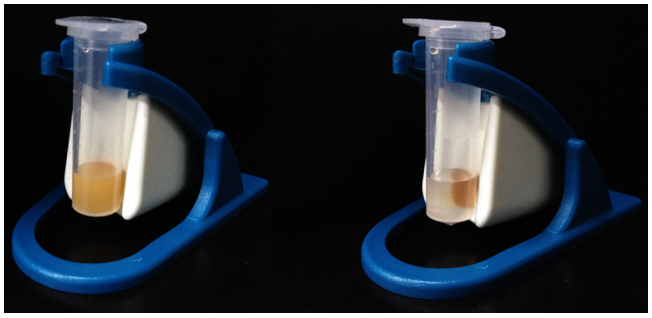

Figure S3. Photos of encoded beads before (left) and after (right) they were subjected to a magnetic field (~250mT) for 1 minute.

Reference

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4 K. Yamaguchi, M. Ito, T. Taniguchi, S. Kawaguchi and K. Nagai, *Colloid Polym. Sci.*, 2004, **282**, 366.