Controlled fabrication of novel magneto-fluorescent encoded microspheres with host-guest structure for simultaneous detection of thyroxine and thyroid stimulating hormone

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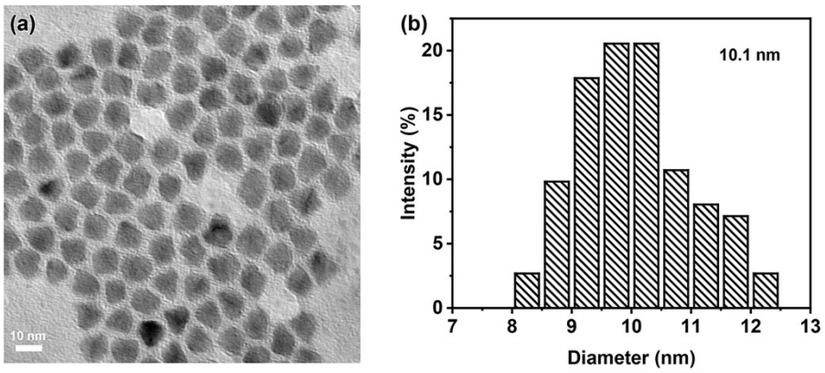

Fig. S1. TEM image (a) and size distribution (b) of QDs.

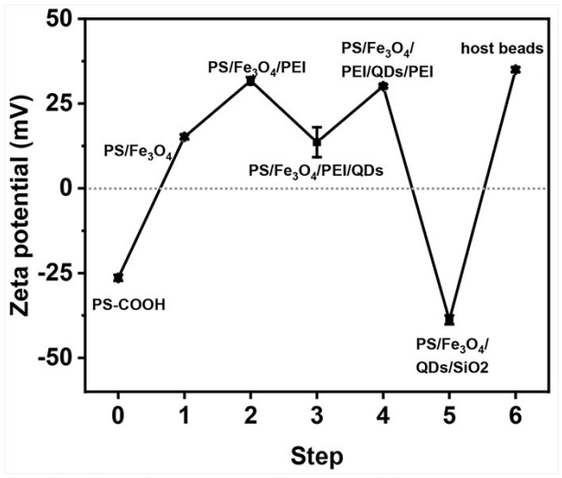

Fig. S2. Zeta potential of host beads at the corresponding assembly stages.

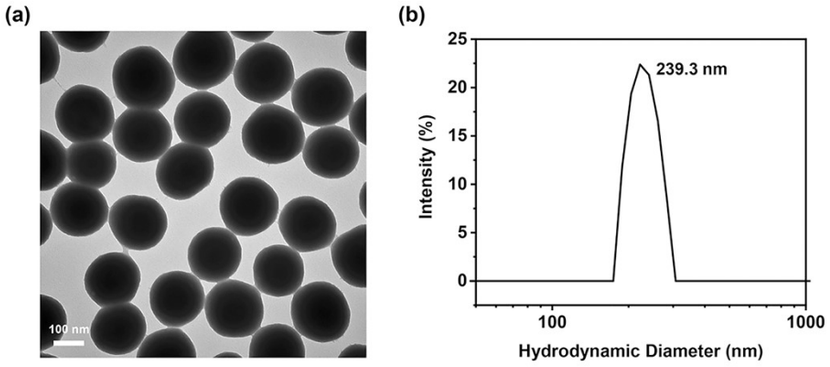

Fig. S3. TEM image (a) and hydrodynamic diameter distribution (b) of representative guest beads.

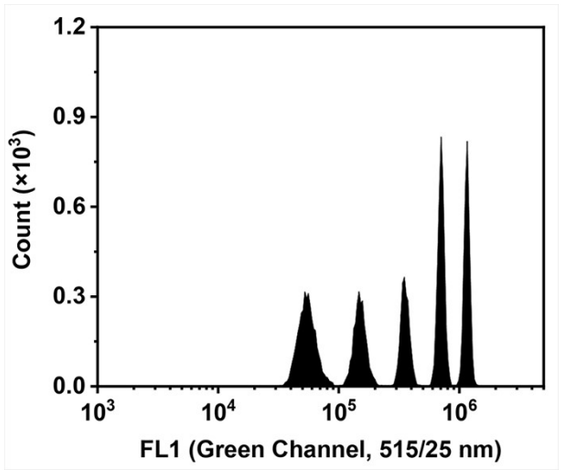

Fig. S4. Histogram plot for g1-g5 guest codes distinguished by fluorescence intensity via flow cytometry.

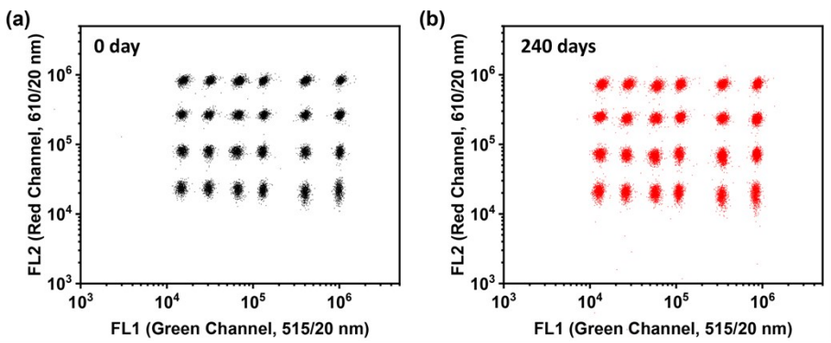

Fig. S5. Scatter plot of 24 barcodes distinguished via flow cytometry after storage in water at 2-8 ℃ for (a) 0 day and (b) 240 days.

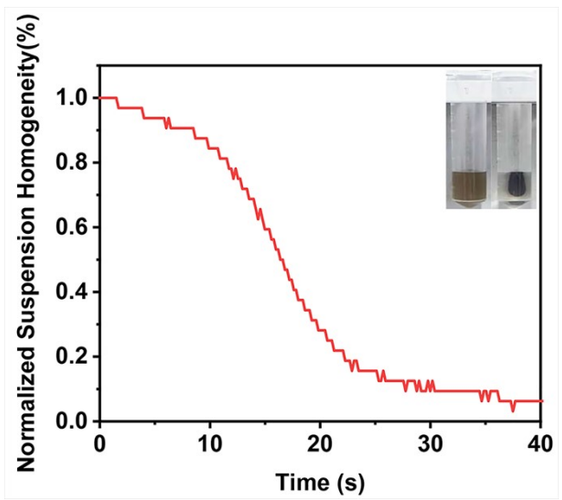

Fig. S6. Magnetic capture curve of HGBs in water on a commercial Sepmag biomagnetic separators. The inset shows the photograph of HGBs aqueous dispersion in the absence (left) and presence (right) of a magnet.

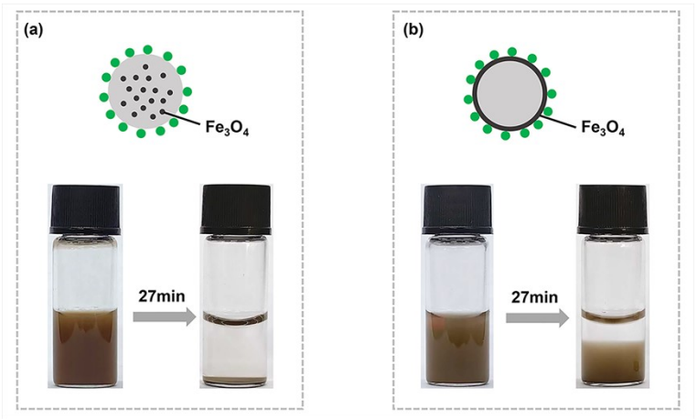

Fig. S7. The photograph of the dispersions of host-guest beads based on (a) the pitaya-like and (b) core-shell magnetic beads after standing for 27 min.

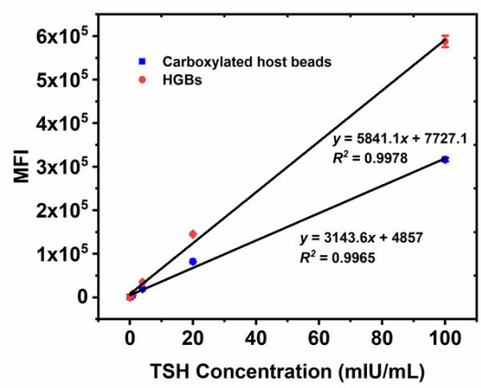

Fig. S8. The MFI of HGBs and carboxylated host beads changes with TSH concentration and the fitting curves.

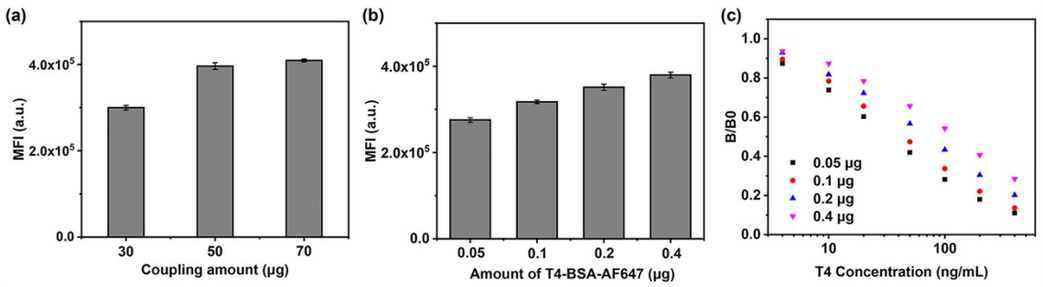

Fig. S9. (a) Relationship of the MFI with amount of T4 antibody for 1 mg HGBs. (b) Relationship of the MFI with amount of T4-BSA-AF647. (c) Relationship of B/B0 values with T4 concentration at different amount of T4-BSA-AF647.

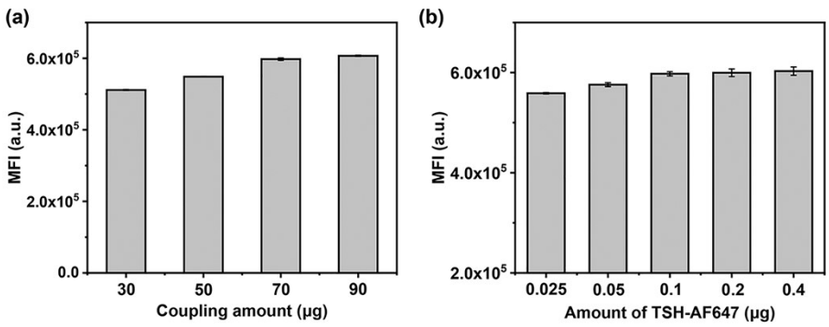

Fig. S10. (a) Relationship of the MFI with amount of TSH antibody for 1 mg HGBs. (b) Relationship of the MFI with amount of TSH-AF647.

Table S1. Encoding formula and the resulting fluorescence properties of host beads with different intensity levels.

QD Fluorescence Intensity Level	Feeding Amount of QDs Per Milligram of Host Beads （μg）	Median FL2-A	CV FL2-A (%)
Q1	1	18419	12.74%
Q2	3.3	65037	9.80%
Q3	10	224060	7.96%
Q4	30	676101	8.42%

Table S2. Amounts of reactants used in the guest beads synthesis.

Sample	Ethanol (mL)	Water (mL)	Ammonia (mL)	FITC Silane (mL)	TEOS (mL)
F0	25.3	1.6	1.4	0	2.0
F1	25.69	1.6	1.0	0.01	2.0
F2	25.675	1.6	1.0	0.025	2.0
F3	25.65	1.6	1.0	0.05	2.0
F4	25.6	1.6	1.0	0.1	2.0
F5	25.4	1.6	1.0	0.3	2.0
F6	24.7	1.6	1.0	1.0	2.0

Table S3. The resulting fluorescence properties of the as-synthesized guest beads without blending.

Guest Beads	Median FL1-A	CV FL1-A (%)
F0	1749	32.51
F1	12242	7.62
F2	31354	6.37
F3	48507	7.16
F4	109779	5.75
F5	376700	5.53
F6	1335082	5.44

Table S4. Encoding formula by blending F1 to F6 and the resulting fluorescence properties of guest codes with different intensity levels.

FITC Fluorescence Intensity Level	Recipes of Guest Beads	FL1-A by Calculation	Resulting Median FL1-A	CV FL1-A	Relative Error
G1	85.6% F1 + 14.4% F2	15000	14,995	6.82	-0.03%
G2	7.1% F1 + 92.9% F2	30000	30,334	6.26	1.11%
G3	73.1% F3 + 26.9% F4	65000	65,649	6.77	1.00%
G4	92.4% F4 + 7.6% F5	130000	132,144	5.36	1.65%
G5	97.5% F5 + 2.5% F6	400000	406,331	6.32	1.58%
G6	35.0% F5 + 65.0% F6	1000000	975,684	5.34	-2.43%

Here, relative error was used to represent the deviation between the experimental FL1 fluorescence intensity and the theoretical FL1 fluorescence intensity. The relative error is calculated using the formula (Resulting Median FL1 - FL1 by Calculation)/(FL1 by Calculation).

Table S5. The results derived by modifying and calculating Table S2 of 2014 work.

Number	Recipes of Guest Beads	FL1 by Calculation	Resulting Mean FL1	CV FL1-A (%)	Relative Error
1	100% Blank	/	832	31.3	
2	5% MF +95% Blank	2606	2620	23.0	0.54%
3	20% MF +80% Blank	7930	7311	17.8	-7.81%
4	50% MF +50% Blank	18579	16970	15.2	-8.66%
5	100% MF	/	36326	12.3	/
6	10% SF+90% Blank	58544	89157	17.4	52.29%
7	25% SF+75% Blank	145114	178424	13.4	22.95%
8	50% SF+50% Blank	289395	330482	11.7	14.20%
9	100% SF	/	577958	8.6	/

Table S6. Encoding formula by blending F0 and F6 and the resulting fluorescence properties of guest codes with different intensity levels.

FITC Fluorescence Intensity Level	Recipes Of Guest Beads	Median FL1-A	CV FL1-H
g1	96% F0 +4% F6	55,254	17.52
g2	90% F0 + 10% F6	151,234	12.14
g3	75% F0 + 25% F6	357,102	8.49
g4	50% F0 + 50% F6	709,431	6.32
g5	0% F0 + 100% F6	1335082	5.62

Table S7. Fluorescence properties of the barcodes at three distinct stages: before antibody immobilization, after antibody immobilization, and after the multiplex detection process.

	Code Address	Median FL1-A	CV FL1-A (%)	Median FL2-A	CV FL2-A (%)
Before Antibody Immobilization	Q1G2	28798	6.32	20067	12.71
	Q1G4	120492	5.68	20242	13.12
After Antibody Immobilization	Q1G2	27322	6.48	22387	14.34
	Q1G4	113126	5.45	21239	16.59
After Multiplex Detection	Q1G2	27793	7.04	21883	13.27
	Q1G4	115766	5.24	20075	16.19