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Supporting Information

Rational design of fluorescent barcodes for suspension array through a simple simulation strategy

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CONTENTS

Section	Page
Supporting Notes	
Supporting Note 1: Presentation of MFM	S-3
Supporting Note 2: Multiplexed detection for tumor markers	S-3~4
Supporting Figures	
Fig. S1. FMBs carboxylation reaction	S-5
Fig. S2. Microparticle size statistics of 6.59 μm QDs-encoded microbeads	S-6
Fig. S3. Fluorescence stability of FMBs	S-7
Fig. S4. Long-term stability of barcodes including G-FMBs and R-FMBs	S-8
Fig. S5. Bare MBs histograms measured by flow cytometer.	S-9
Fig. S6. The relationship between the PL peak wavelength of 516 nm (blue) and 647 nm (red) CdSe/ZnS QD-encoded.	S-10

1 Supporting Note 1: Presentation of MFM

The multicolour fluorescence model (MFM) considers the sum of the ensemble fluorescence of the detector intensities in the channels, including direct excitation, sensitization by FRET and bleed-through.[1] A general expression of the detector intensity in channel c is

$$I_{cl} = I_{cl}^{0} + \sum_{f=1}^{N} \beta_{cf} F_{f}^{e}, \quad c = 1, \dots, C, f = 1, \dots, N, l = 1, \dots, L \tag{1}$$

The ensemble fluorescence of a given fluorophore includes quenching and sensitized-fluorescence through FRET. When there is no competition between laser-induced and FRET-sensitized emission, the equation of the ensemble fluorescence can be expressed as:

$$F_{fl}^{e} = F_{fl}^{s}\left(1 - \sum_{i=f+1}^{N} E_{fi}^{em}\right) \tag{2}$$

$$F_{fl}^{s} = F_{fl}^{0} + \sum_{j=1}^{f-1} \alpha_{jf} E_{jf}^{em} F_{j}^{s} \tag{3}$$

where sensitized fluorescence F_{fl}^{s} is direct excitation by laser and FRET from donors (ie. assumes no acceptors). Hence,

$$F_{fl}^{e} = \left(F_{fl}^{0} + \sum_{j=1}^{j<f} \alpha_{jf} E_{jf}^{em} F_{jl}^{s}\right)\left(1 - \sum_{i=f+1}^{N} E_{fi}^{em}\right) \tag{4}$$

It follows from (1) and (4) that

$$I_{cl} = I_{cl}^{0} + \sum_{f=1}^{N} \beta_{cf} \left(F_{fl}^{0} + \sum_{j=1}^{j<f} \alpha_{jf} E_{jf}^{em} F_{jl}^{s}\right)\left(1 - \sum_{i=f+1}^{N} E_{fi}^{em}\right) \tag{5}$$

2 Supporting Note 2: *Multiplexed detection for tumor markers*

To verify the practicality of FMBs in multiplexed detection for tumor markers, firstly, Specific capture antibodies were immobilized onto the surface of carboxyl modified FMBs through the carbodiimide reaction for constructing the 3-plex tumor markers multiplexed assay platform. Consequently, FMBs with coding address of

G1R4, G1R3, and G3R2 were selected for three specific capture antibodies corresponding to anti-CEA antibody, anti-CA125 antibody and anti-CA199 antibody. First, 2×10^5 barcode FMBs were activated in 100 μL of activation buffer (pH = 6.2) with rotation for 20 min at room temperature, containing 0.5 mg of sulfo-NHS and 0.5 mg of EDC. And the FMBs were washed by using phosphate buffer saline (PBS, pH = 7.4) with centrifugation. Then, the appropriate amount of probe antibodies of tumor markers was added to 500 μL PBS buffer solutions of activated FMBs, and incubated at 10 ℃ for 12 h. Thereafter, unreacted antibodies in the mixture are washed away with PBS and then blocked with BSA for 30 min. Finally, the barcode FMBs were washed and stored in phosphate buffer containing 0.1% BSA at 4 ℃ for further use.

The multiplexed assay was performed in an immunoassay platform with three typical barcode FMBs to detect three tumor markers (CA125, CA199 and CEA). 2×104 FMBs (G1R4, G1R3, and G3R2) coated with probe antibody was suspended in each well of a 96-well plate containing 150 μL of buffer, and washed 3 times repeatedly. Next, 100 μL of the prepared antigen (tumor marker) solutions at different concentrations (0, 0.001 KU/L, 0.01 KU/L, 0.1 KU/L,1, 10 KU/L, 100 KU/L, and 1000 KU/L "for CA125 and CA199" as well as ng/mL "for CEA" ng/mL) were added to each well of the 96-well plate. The plate with the FMBs and antigen solutions was incubated at room temperature for 1 h in dark, and then washed three times with wash buffer. Subsequently, 100 μL of biotinylated specific antibody was added to each well, and the resulting solution was incubated at room temperature for 1 h, and then the excess secondary antibody was removed. Moreover, 100 μL S-PE solution was added to each well, and then incubated for 10 min at room temperature. Finally, barcode samples were resuspended with 200 μL of washing buffer and measured by by flow cytometry.

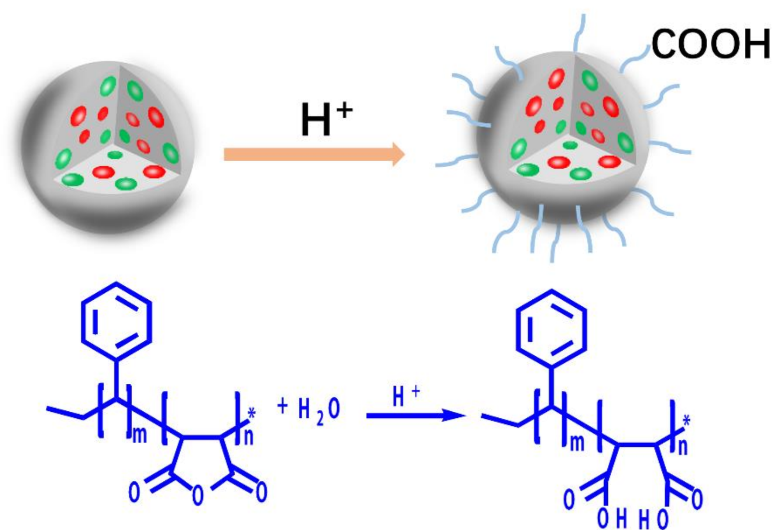

Figure S1. FMBs carboxylation reaction

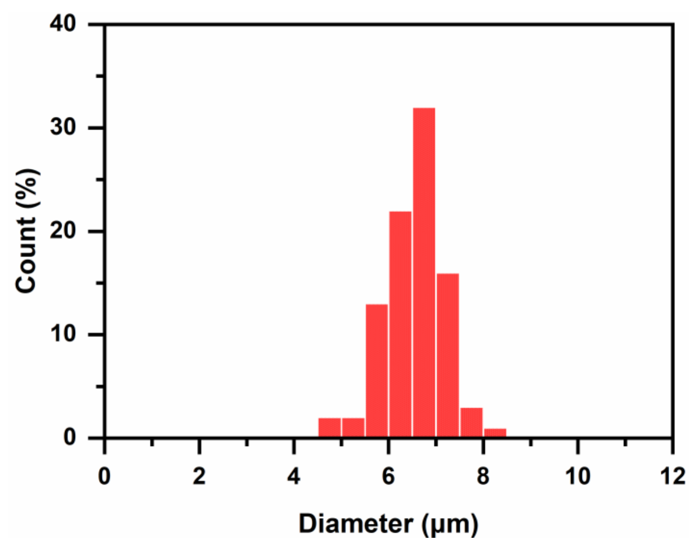

Figure S2. Microparticle size statistics of 6.59 μm QDs-encoded microbeads.

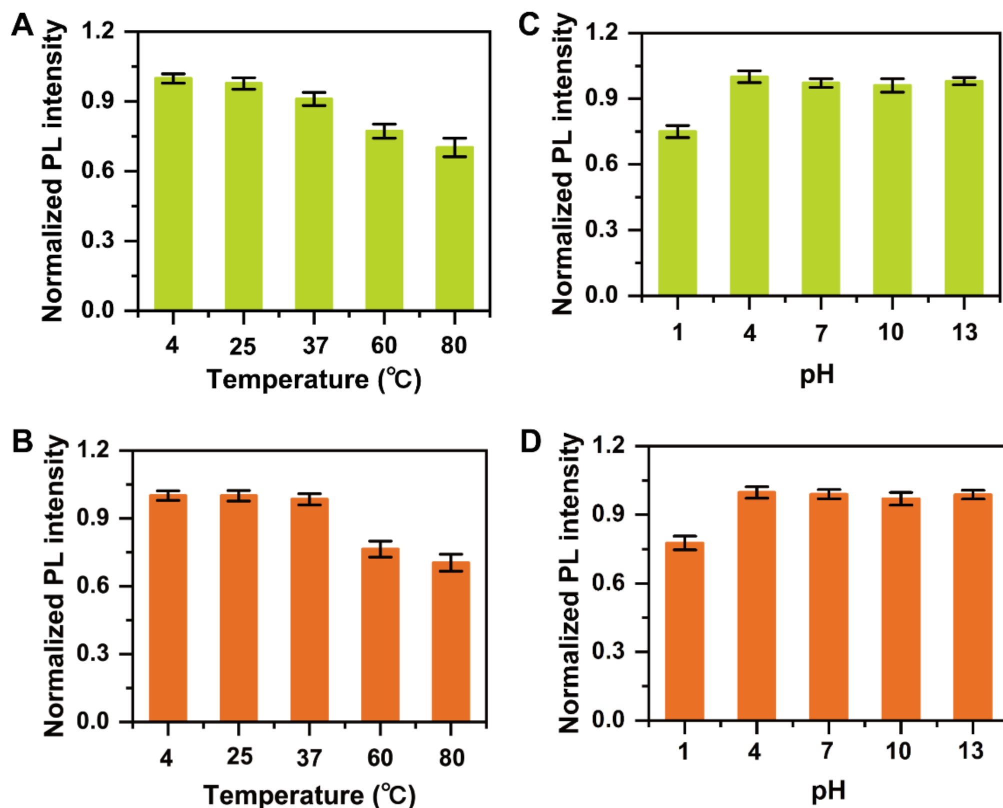

Figure S3. Fluorescence stability of FMBs. (A) and (B) Temperature dependent stability: Microparticles with G-FMBs (A) and G-FMBs (B) were suspended in PBS (pH=7.4) and stored at different temperatures including 4℃, 25℃, 37℃, 60℃, and 80℃ for 24 hours, respectively. (C) and (D) pH-dependent stability: Microparticles with G-FMBs (C) and G- FMBs (D) were suspended in PBS with different pH values (1, 4, 7, 10, and 13, respectively) and stored at room temperature for 24 hours.

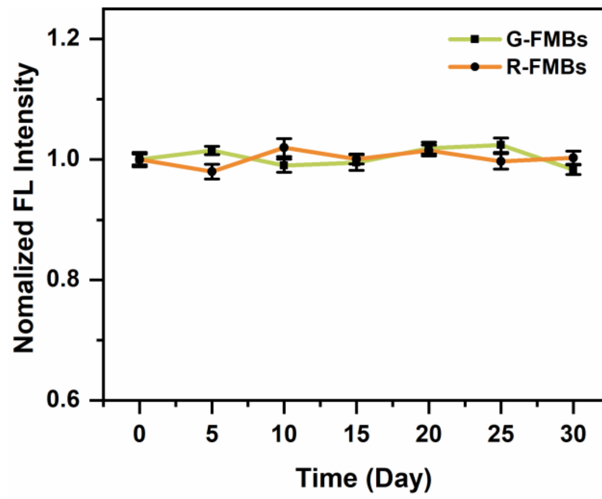

Figure S4. Long-term stability of barcodes including G-FMBs and R-FMBs. The barcodes were suspended in PBS buffer (pH=7.4) solutions at 25 ℃ and their fluorescence intensities were recorded every 5 days.

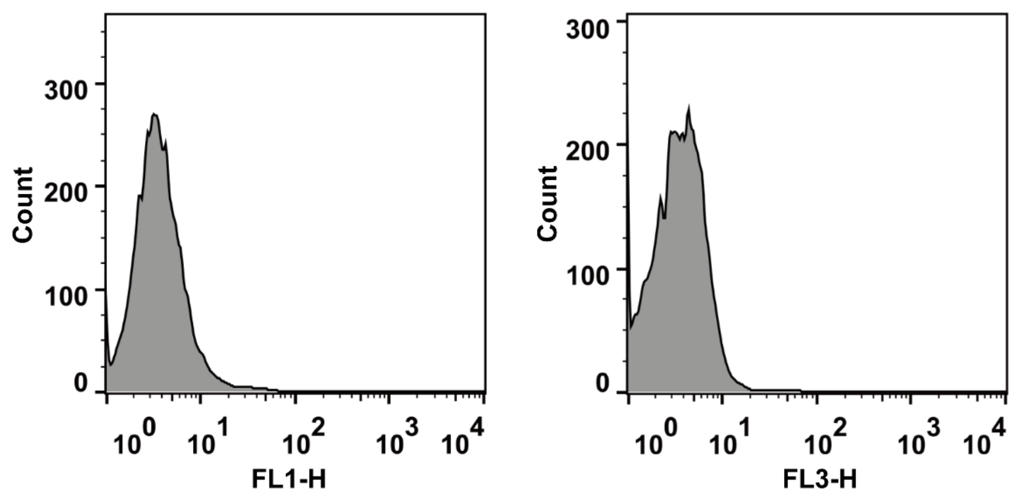

Figure S5. Bare MBs histograms measured by flow cytometer. Green detection channel (FL1-H, left) and red detection channel (FL3-H, right).

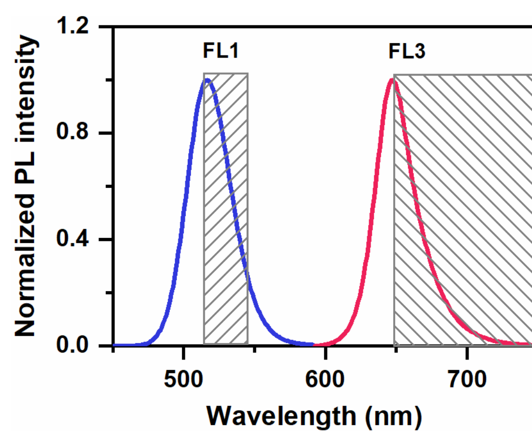

Figure S6. The relationship between the PL peak wavelength of 516 nm (blue) and 647 nm (red) CdSe/ZnS QD-encoded.

References

1 M. Dagher, M. Kleinman, A. Ng and D. Nat. Nanotechnol., 2018, **13**, 925-981.