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MDPI

Supplementary Materials

Dual-Signal-Encoded Barcodes with Low Background Signal for High-Sensitivity Analysis of Multiple Tumor Markers

Bo Zhang, Wan-Sheng Tang and Shou-Nian Ding *

School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, China; zhangbo@seu.edu.cn (B.Z.); wstang@seu.edu.cn (W.-S.T.)
* Correspondence: snding@seu.edu.cn; Fax: +86-25-5209-0621

Chemical and Reagents

Admium chloride hydrate ($CdCl_2·2.5H_2O$, 99.0%), sodium borohydride ($NaBH_4$, ⩾ 96.0%), tellurium powder (Te, 99.9%), tetraethyl orthosilicate (TEOS, ≥28.4%) and ammonium hydroxide ($NH_3·H_2O$, 25wt% NH_3 in water) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Poly(acrylic acid) (PAA) was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Poly(vinylpyrrolidone) PVP was supplied by Energy Chemical. 2-methoxyethanol, styrene, 2,2-azobis(isobutyronitrile) (AIBN), N-hydroxysuccinimide (NHS, 98%), 3-mercaptopropionic acid (MPA, 98%) and (3-aminopropyl) triethoxysilane (APTES, 97%) were supplied by Aladdin Industrial Corporation (Southern California, USA). N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), a 2-(N-morpholino) ethanesulfonic acid hydrate (MES) and N-Hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (Saint Louis, USA). Bovine serum albumin (BSA) was obtained from Sangon Biotech Co., Ltd (Shanghai, China). PS microbeads and silica (SiO_2) microbeads were supplied by Suzhou Nanomicro Technology Co., Ltd. (Suzhou, China). The inorganic salts ($Na_2HPO_4·12H_2O$, $NaH_2PO_4·2H_2O$, NaCl, NaOH, KCl, KH_2PO_4, HCl) for buffer preparation were brought from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

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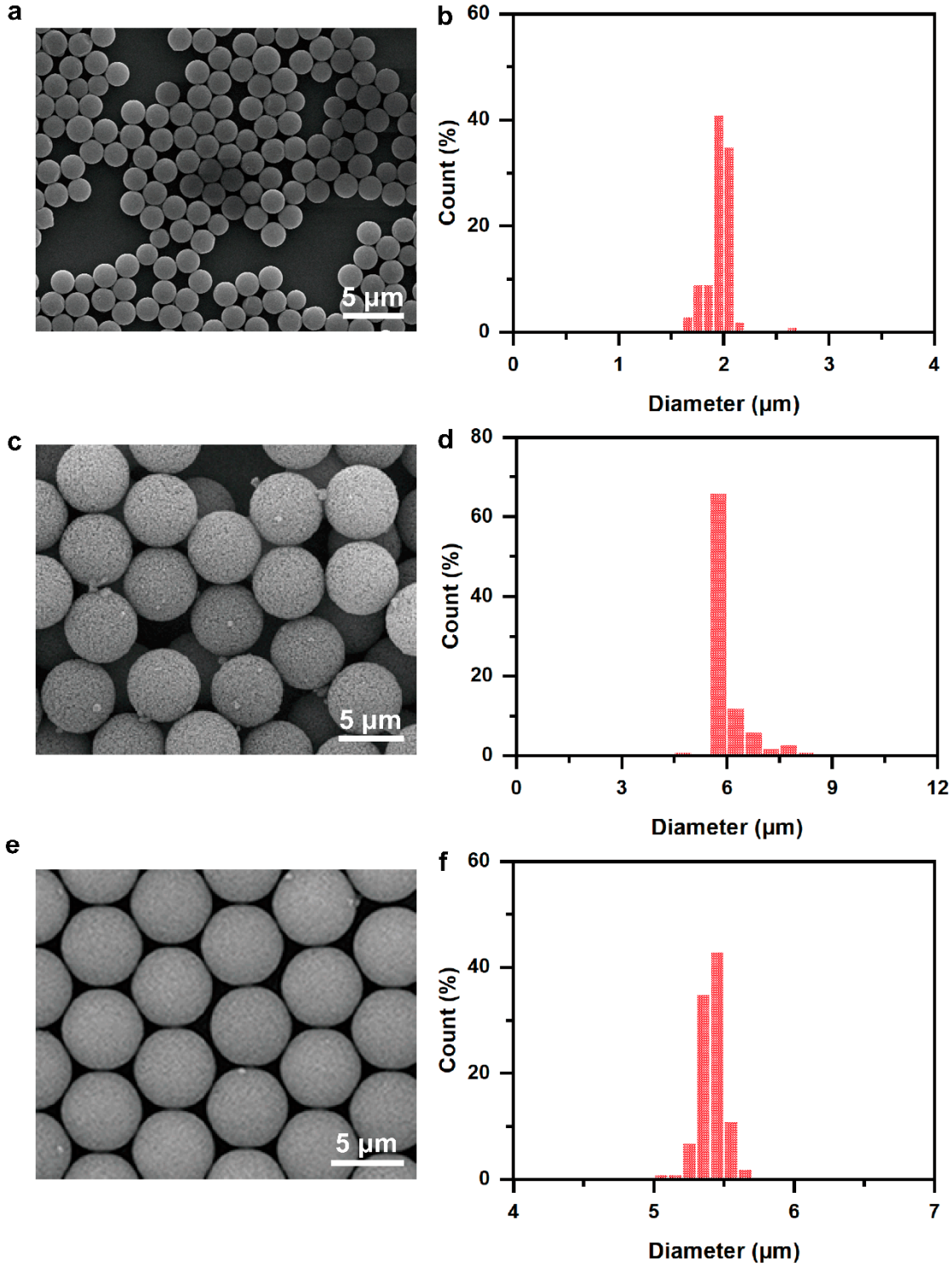

Figure S1. Representative SEM images and microparticle size statistics of MBs. (**a**) and (**b**) SEM image and microparticle size statistics of 1.96 μm PS-S, respectively. (**c**) and (**d**) SEM image and microparticle size statistics of 5.87 μm PS-L, respectively. (**e**) and (**f**) SEM image and microparticle size statistics of 5.44 μm SiO_2-L, respectively.

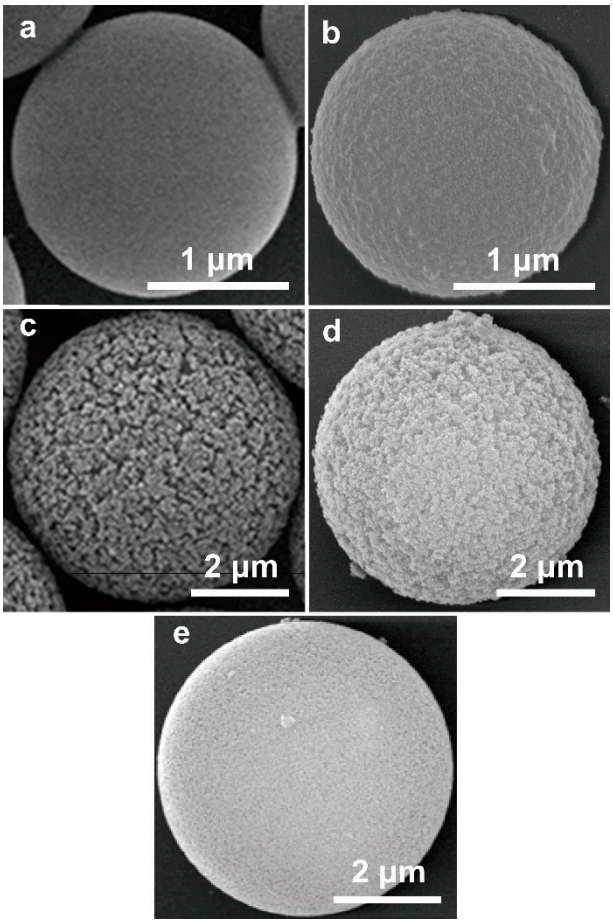

Figure S2. SEM images with high magnification of various PS and SiO_2. PS-S before (**a**) and after (**b**) coated by SiO_2, respectively. PS-L before (**c**) and after (**d**) coated by SiO_2, respectively. SiO_2-L (**e**).

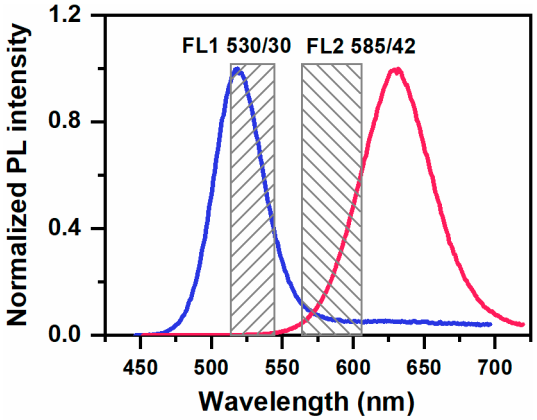

Figure S3. The relationship between the PL peak wavelength of 522 nm (blue) and 630 nm (red) CdTe.

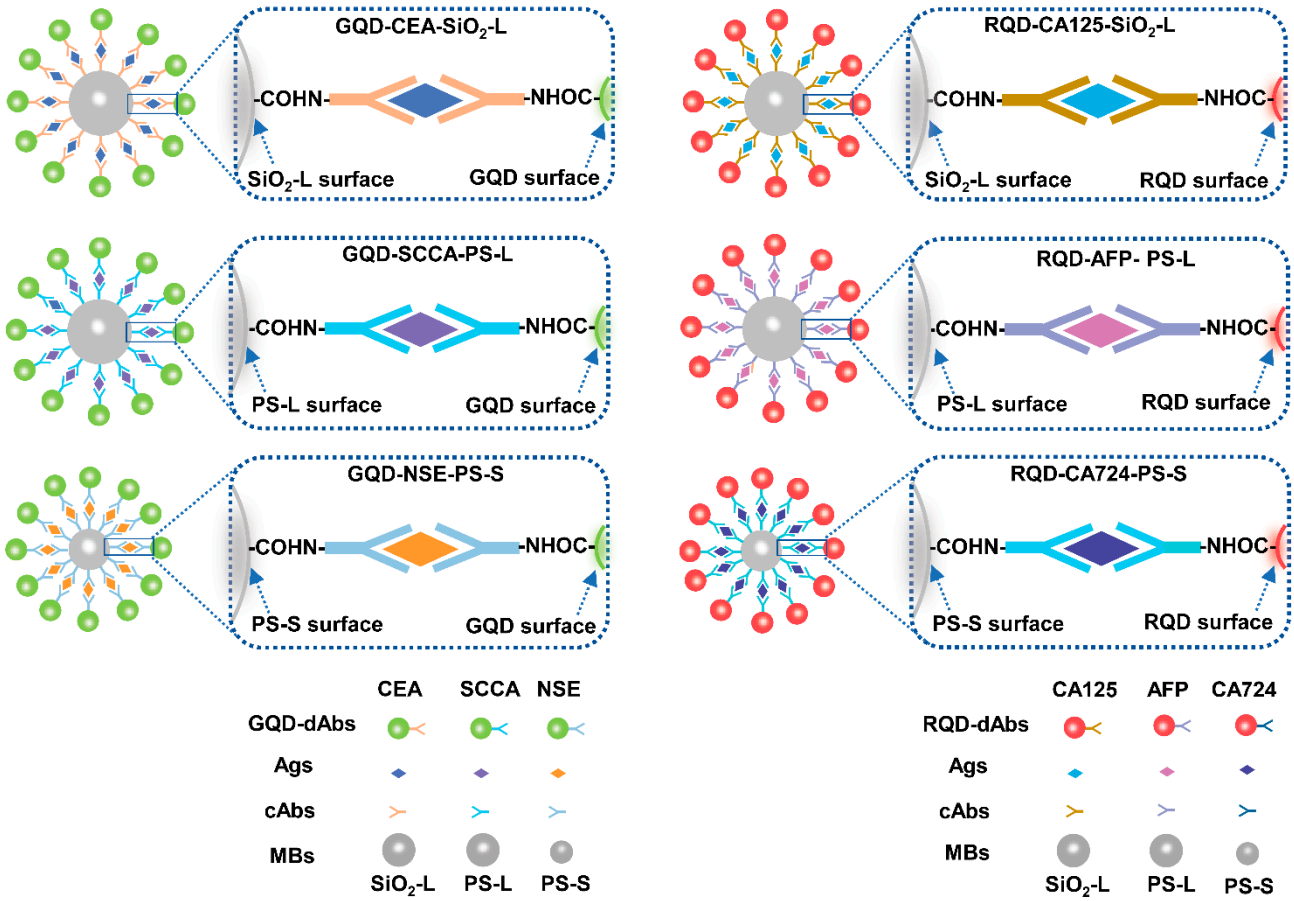

Figure S4. Schematic representation of GQDs or RQDs were coupled to the surface of MBs (SiO_2-L, PS-L and PS-S), by sandwich immune structure.

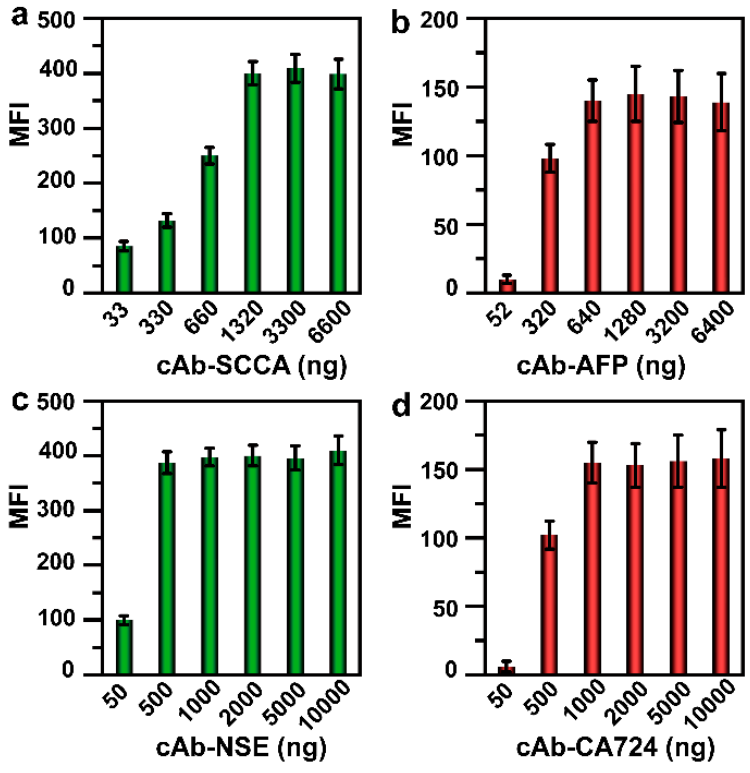

Figure S5. Optimization of added amounts of cAb. (**a**) GQD-SCCA-PS-L with 33, 330, 660, 1320, 3300, and 6600 ng of cAb-SCCA. Optimal amount of cAb-SCCA (1320 ng). (**b**) RQD-AFP-PS-L with

52, 320, 640, 1280, 3200, and 6400 ng of cAb- AFP. Optimal amount of cAb-AFP (640 ng). (**c**) GQD-NSE-PS-S with 50, 500, 1000, 2000, 5000, and 10,000 ng of cAb-NSE. Optimal amount of cAb-NSE (500 ng). (**d**) RQD-CA724-PS-S with 50, 500, 1000, 2000, 5000, and 10,000 ng of cAb-CA724. Optimal amount of cAb-CA724 (1000 ng).

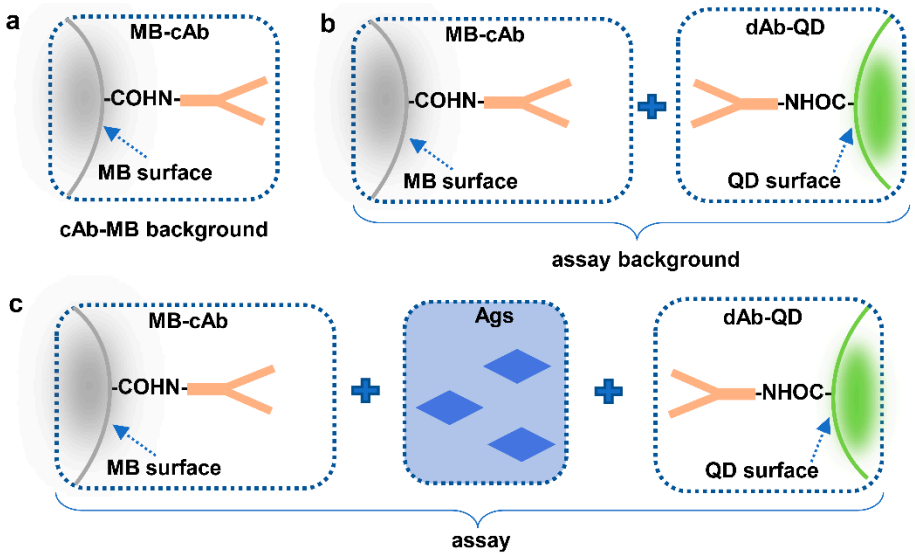

Figure S6. Schematic illustration of combinatorial experiments of 6-plex specific and non-specific binding. The cAb-MBs were incubated with different detection reagents, individual cAb-MB (**a**), cAb-MB mixture with dAb-QD (**b**), and cAb-MB mixture with Ags, followed by dAb-QD (**c**).

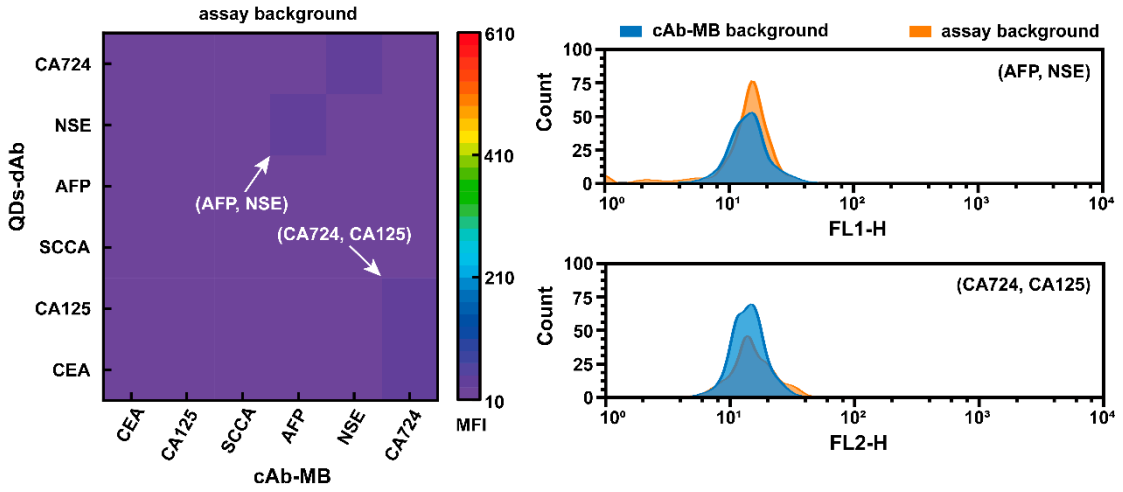

Figure S7. Combinatorial experiments of 6-plex specific and non-specific binding. cAb-MB (**column**) and QDs-dAb (**row**) incubation without Ag. Histograms of the count for (cAb, dAb) are on the right.

Table S1. Limit of detection (LOD) of six-plexed tumor markers biodetection compared to other the data of SAT.

Tumor marker	LOD	Reference
CEA	1.48 ng/mL	[1]
	0.03 ng/mL	[2]
	0.05 ng/mL	This work
CA125	0.99 IU/mL	[1]
	1.0 KU/L	[2]
	0.92 KU/L	This work
SCCA	0.39 ng/mL	[1]
	0.28 ng/mL	This work

AFP	0.17 ng/mL	[2]
	0.01 ng/mL	[2]
	0.09 ng/mL	This work
NSE	0.18 ng/mL	[1]
	0.16 ng/mL	This work
CA724	0.36 KU/L	This work

Reference

1. Wang, Y.; Chen, C.; He, J.; Cao, Y.; Fang, X.; Chi, X.; Yi, J.; Wu, J.; Guo, Q.; Masoomi, H.; et al. Precisely Encoded Barcodes through the Structure-Fluorescence Combinational Strategy: A Flexible, Robust, and Versatile Multiplexed Biodetection Platform with Ultrahigh Encoding Capacities. *Small* **2021**, *17*, 2100315. https://doi.org/10.1002/smll.202100315.
2. Leng, Y.; Wu, W.; Li, L.; Lin, K.; Sun, K. Magnetic/Fluorescent Barcodes Based on Cadmium-Free Near-Infrared-Emitting Quantum Dots for Multiplexed Detection. *Adv. Funct. Mater.* **2016**, *26*, 7581. https://doi.org/10.1002/adfm.201602900.