Supplementary Information

Rational Design of Functional Materials Guided by Single Particle Chemiluminescence Imaging

Weijun Kong,[a] Qi Li,[a] Wei Wang,*[b] Xiaoning Zhao,[c] Shenglong Jiang,[d] Tianhua Zheng,[a] Qun Zhang,[d] Wen Shen,[a] and Hua Cui *[a]

[a]CAS Key Laboratory of Soft Matter Chemistry, *i*ChEM (Collaborative Innovation Center of Chemistry for Energy Materials), Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, P. R. China.

[b]State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, P. R. China

[c]Beijing Institute of Metrology, Room 303, No. 10 Lishuiqiaojia, Chaoyang District. Beijing, 102200, P. R. China.

[d]Hefei National Laboratory for Physical Science at the Microscale, *i*ChEM, Synergetic Innovation Center of Quantum Information and Quantum Physics, University of Science and Technology of China, Hefei, Anhui 230026, P. R. China.

*Corresponding authors

Contents

1. Experimental section.

1.1 Reagents and materials: Fe_3O_4 seeds (~ 50 nm) were provided by Beijing Yunci Technology Co., Ltd. (China). Azobisisobutyronitrile (AIBN), styrene, polyvinyl alcohol (PVA) and methacrylic acid were purchased from Sinopharm Chemical Reagent Co. Ltd. (China). ABEI was obtained from TCI (Japan). A stock solution of ABEI (10 mM) was prepared by dissolving ABEI in NaOH solution (0.1 M) and was kept at 4 °C. TNT was purchased from Aladdin Reagent (China). The sequence of the TNT peptide aptamer (TNT-apt) is (N terminus) Trp-His-Trp-Gln-Arg-Pro-Leu-Met-Pro-Val-Ser-Ile-Lys (C terminus), which was purchased from GL Biochem Co. Ltd. (China). Sylgard 184 (including PDMS monomer and curing agent) was purchased from Dow Corning (USA). 1-(3-(Dimethylamino)propyl)-3-ethylcarbodiimidehy-drochloride (EDC), N-hydroxysuccinimide (NHS) were purchased from Aladdin Reagent (China). All other reagents were of analytical grade. Ultrapure water was prepared with a Milli-Q system (Millipore, France) and used throughout. 2-(N-Morpholino) ethanesulfonic acid (MES) (25 mM at pH 5.5) was used as activation buffer and washing buffer for EDC and NHS. Dilution buffer for TNT-apt and TNT was 0.01 M phosphate buffered saline (PBS) at pH 7.0. All the buffers were sterilized at 121 °C for 20 min in an autoclave.

1.2 Apparatus for characterization: The morphology of microbeads was characterized by SEM on a GeminiSEM-500 scanning electron microscope (Zeiss, Germany). CL spectra and FL spectra were measured on an F-7000 FL spectrometer (Hitachi, Japan). CL was measured with a centro LB960 microplate luminometer (Berthold, Germany). Porosity measurement was conducted on a Tristar II 3020M surface area and porosity analyzer (Micromeritic, USA). Confocal fluorescence microscopy images were obtained from a confocal Raman/PL system (Alpha 300R) equipped with 355 nm laser source (WITec, Germany).

2. Characterization of microbeads

2.1 Scanning electron microscopy

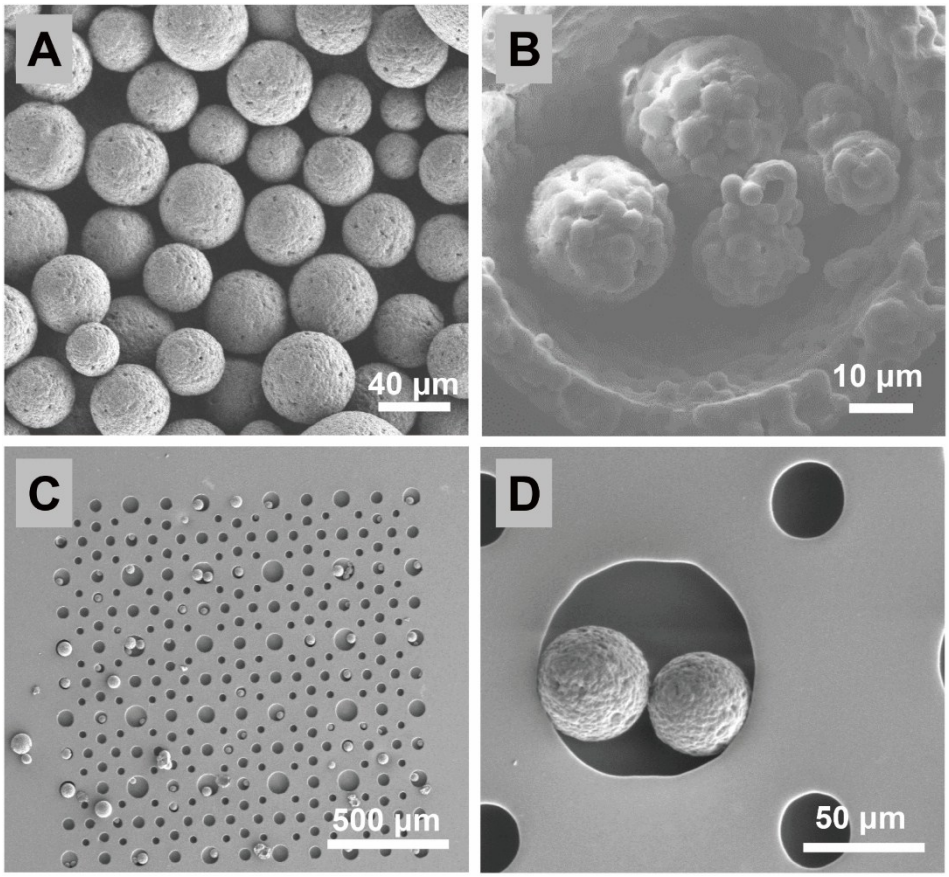

Fig. S1 SEM images of old batch of microbeads (A, B) and microbeads on polydimethylsiloxane (PDMS) array (C, D).

2.2 Fluorescence (FL) spectra and FL imaging of CL functionalized microbeads

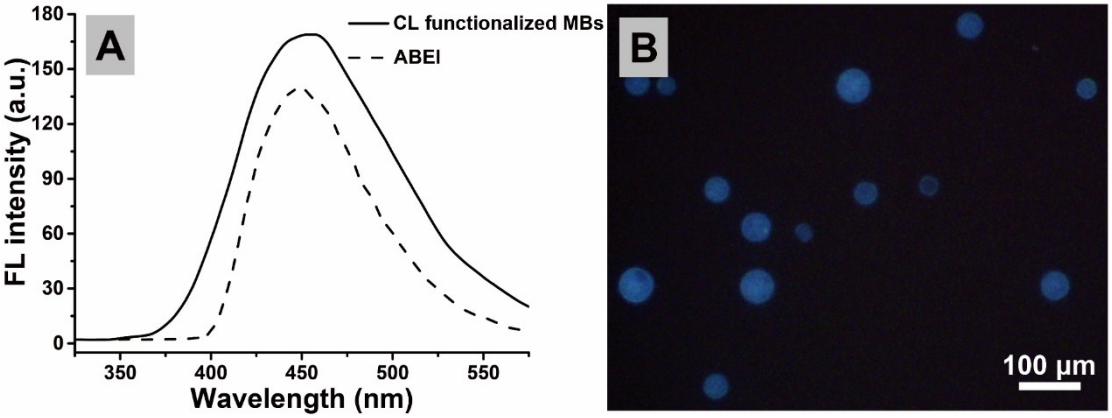

Fig. S2 (A) Fluorescence spectra of CL functionalized microbeads and ABEI. (B) Fluorescence imaging of CL functionalized single particles.

2.3 CL kinetic curves of functionalized microbeads

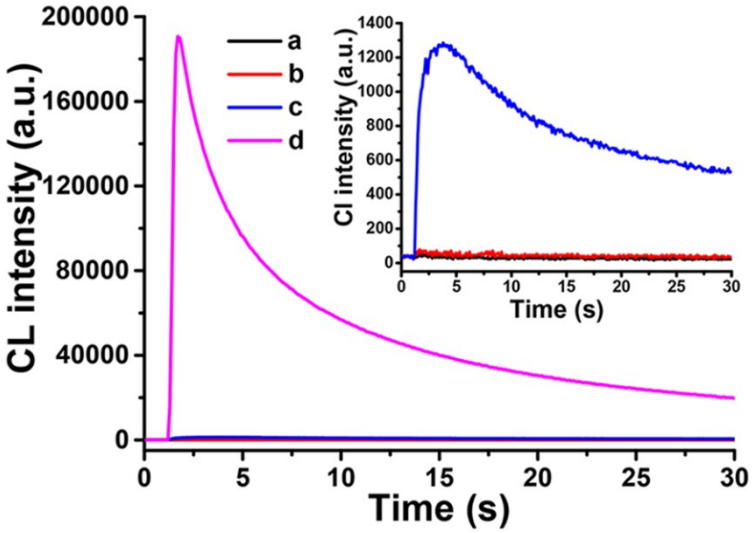

Fig. S3 CL kinetic curves of microbeads (curve a), Co^{2+} functionalized microbeads (curve b), ABEI functionalized microbeads (curve c), and Co^{2+}/ABEI co-functionalized microbeads (curve d).

3. Home-built microscope system

3.1 Photographs of home-built microscope system

CL microscopy was an upright microscope (BX53, Olympus) equipped with a 10X objective lens (numerical aperture = 0.30). CL images (exposure time is 500 ms) were captured by an iXon-Ultra-897 EMCCD (Andor, UK).

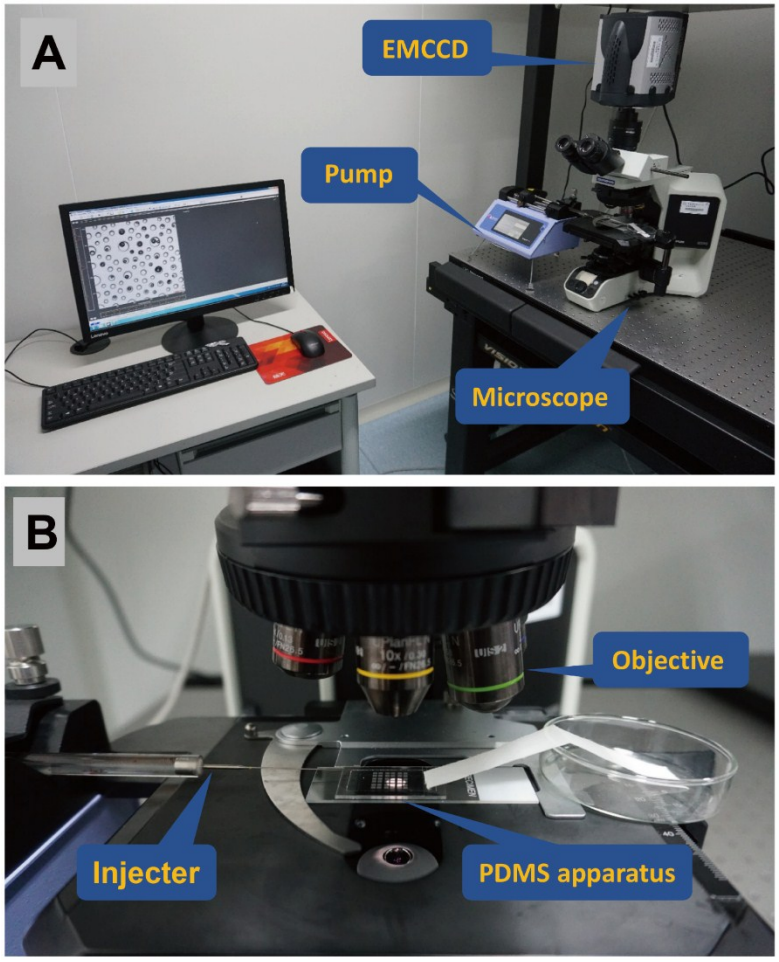

Fig. S4 Photographs of home-built microscope system.

3.2 Photographs and schematic illustration of preparation of PDMS apparatus

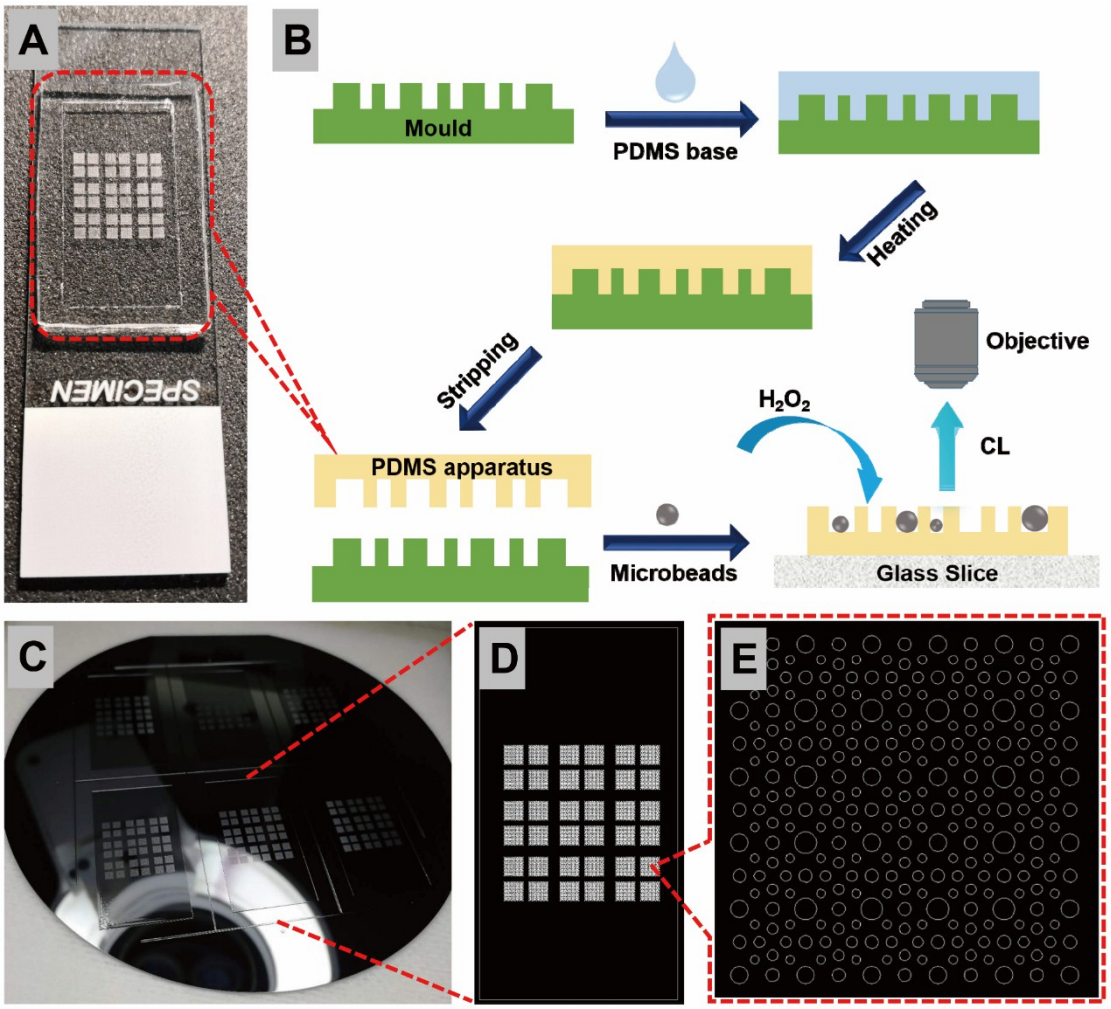

Fig. S5 (A) Photographs of home-made PDMS apparatus (with microwell array). (B) Schematic illustration of preparation of PDMS apparatus. (C) Photographs of silicon wafer mould. (D) Design sketch of PDMS apparatus. (E) Design sketch of a unit of microwell array.

4. CL histograms of old and new batch of microbeads

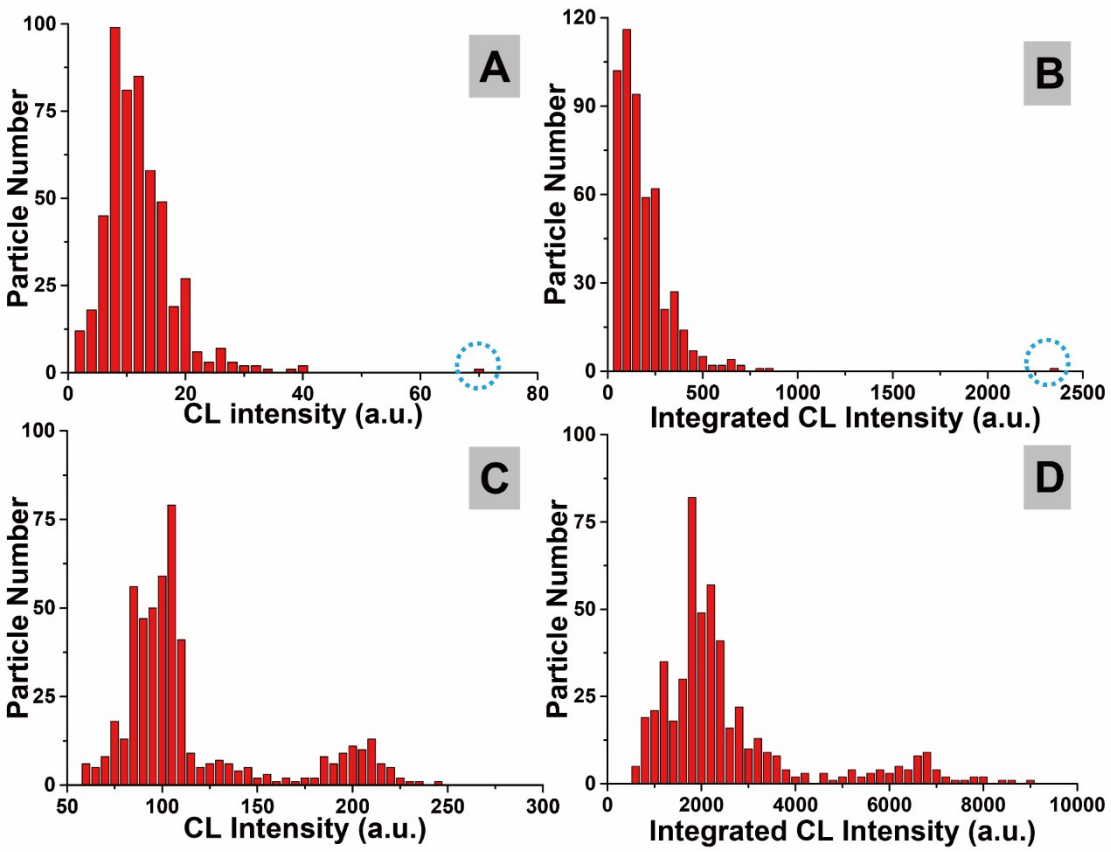

Fig. S6 CL histograms of old (A, B) and new (C, D) batch of microbeads normalized by pixel area.

5. Schematic illustration of settings on focal plane

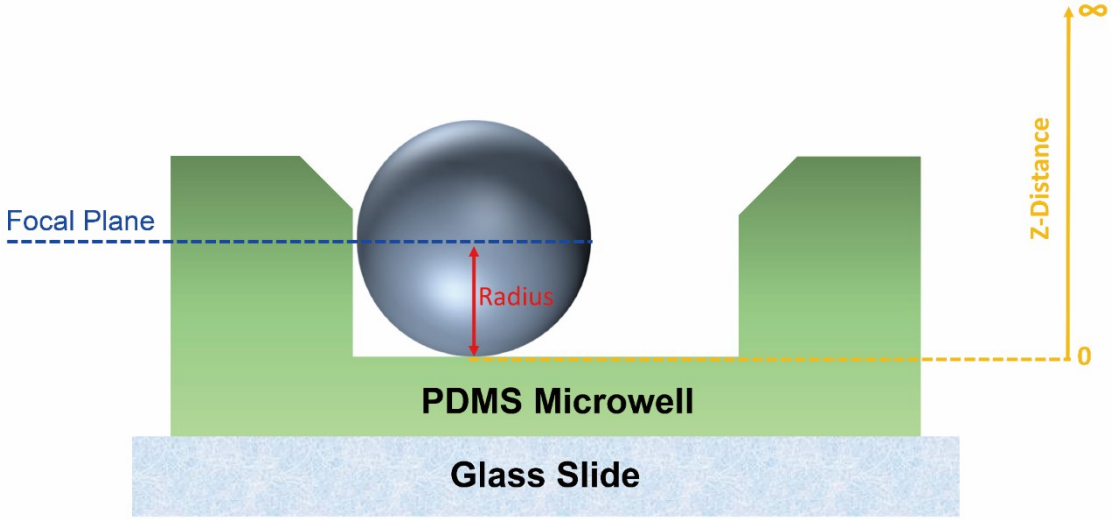

Fig. S7 Schematic illustration of settings on focal plane.

6. Correlation between CL performance and Co^{2+}/ABEI ratio

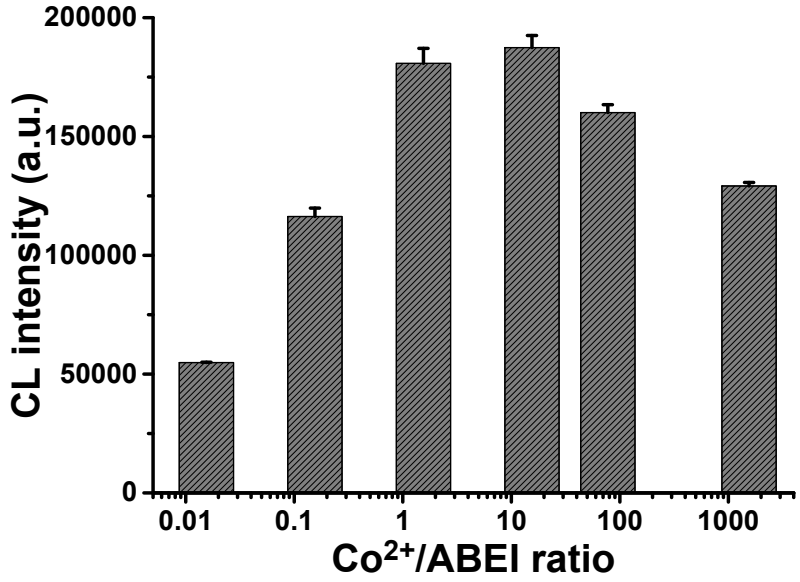

Fig. S8 Correlation between CL performance and Co^{2+}/ABEI ratio

The effect of Co^{2+}/ABEI ratio on the CL intensity was studied as shown in Fig. S8. It was found that the CL response significantly increased with the increasing of the Co^{2+}/ABEI ratio and reached the maximum value when the Co^{2+}/ABEI ratio was 15.5. Further increase in Co^{2+}/ABEI ratio caused a decrease in CL intensity. Thus, Co^{2+}/ABEI ratio with 15.5 was chosen for further experiments.

7. Detection of TNT

First, 400 μL of CL functional microbeads were adding into 4.0 mL of PBS buffer (pH 7.0) containing TNT-apt (10 μg/mL) under constant shaking. After 4-h reaction at room temperature, the suspension was washed twice and dispersed with 4.0 mL of PBS buffer for further use. For the CL detection of TNT, 1.0 mL of TNT with different concentrations (0.005-100 ng/mL) was incubate with 200 μL of the as-prepared suspension under gentle shaking at 37 °C for 30 min, followed by washing twice with PBS buffer via magnetic separation and dispersed with water. Afterward, 100 μL of H_2O_2 in 0.1 M NaOH solution (pH 13.0) was injected into the microwell of 96-well plate with 100 μL aqueous dispersion of the as-prepared hybrids after interacted with TNT, and then the CL kinetic curves of each well were recorded.

8. Descriptions of the movies

8.1 Movie S1

CL imaging of single particles of old batch of microbeads. (Reaction conditions: 0.1 M H_2O_2 in 0.1 M NaOH solution.)

8.2 Movie S2

CL imaging of single particles of optimized batch of microbeads. (Reaction conditions: 0.1 M H_2O_2 in 0.1 M NaOH solution.)