Supplementary Information for

Controllable Engineering and Functionalizing Nanoparticles with Specific Targeting Capability

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METHODS

Reagents. β_2-Microglobulin (B2M), ribonuclease A (RNase A), ribonuclease B (RNase B), bovine serum albumin (BSA), ovalbumin (OVA), Triton X-100, yttrium(III) chloride hexahydrate, ytterbium(III) chloride hexahydrate, thulium(III) chloride hexahydrate, oleic acid (technical grade, 90%), 1-octadecene (technical grade, 90%), oleylamine (technical grade, 70%) and iron chloride ($FeCl_3 \cdot 6H_2O$, 98%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Silver acetate (99%) and n-dodecane (99+%) were purchased from Alfa-Aesar. Sodium oleate and 2-hexyldecanoic acid (98%) were purchased from Tokyo Chemical Industry. Recombinant protein human epidermal growth factor receptor-2 (HER2) was purchased from Abcam (Shanghai, China). Human protein GPNMB, which has an additional alanine at its N-terminal, was purchased from Biorbyt (Cambridge, UK). Unmodified and modified peptides, including KIVKWDRDM, LEACTFRRP, DVWDIDNEF, RTLHRNEYG, KLTIESTPF, KRFHDVLGN, TQVCTGTDM, C13-KIVKWDRDM, AKRFHDVLGNK-C13, TQVCTGTDMK-C13 and FITC-KIVKWDRDM, were customer-ordered from Shanghai Top-Peptide Biotechnology (Shanghai, China) and their purities (HPLC) were above 98%. Aminopropyltriethoxysilane (APTES), 3-ureidopropyl-triethoxysilane (UPTES), benzyltriethoxysilane (BnTES), isobutyltriethoxysilane (IBTES) and tetraethyl orthosilicate (TEOS) were purchased from J&K Scientific (Shanghai, China). Hexane, n-Hexanol and cyclohexane were purchased from Aladdin Reagent (Shanghai, China). Ammonium hydroxide and anhydrous ethanol were purchased from Nanjing Reagent Company (Nanjing, China). Ammonium fluoride, methanol and acetonitrile (ACN) were purchased from Shanghai Macklin Biochemical (Shanghai, China). Hydrochloric acid (HCl) and acetic acid (HAc) were

purchased from Sinopharm Chemical Reagent Co. I.td. Human triple negative breast cancer cell MDA-MB-157 (over expression of GPNMB, low expression of HER2), breast cancer cell MDA-MB-361(over expression of GPNMB and HER2) and MCF-7 (low expression of GPNMB and HER2), phosphate buffer solution for cell culture (1× PBS), parenzyme cell digestion solution (containing 0.25% trypase and 0.02% EDTA), Dulbecco Modified Eagle Medium (DMEM, containing 4.5 mg/ml glucose, 80 U/ml penicillin and 0.08 mg/ml streptomycin), Roswell Park Memorial Institute 1640 medium (RPMI-1640, containing 2.0 mg/mL D-glucose, 0.3 mg/mL glutamine, 2.0 mg/mL $NaHCO_3$, 80 U/mL penicillin and 0.08 mg/mL streptomycin), dimethyl sulfoxide (DMSO) and 96-well plates were purchased from Nanjing KeyGen Biotech. (Nanjing, China). Hoechst 33342 Stain solution (ready-to-use) and 4% formaldehyde were purchased from Beijing Solarbio Science & Technology (Beijing, China). Fetal bovine serum (FBS) was purchased from Gibco (Life Technologies, Australia). Amino-modified quantum dots (QD520 and QD620) dissolved in cyclohexane were purchased from Xingzi New Material Technology Development (Shanghai, China). Culture cells for cell culture and confocal imaging were purchased from NEST Biotechnology (Wuxi, China). All other reagents used were of analytical grade or higher. Water used in all the experiments was purified by a Milli-Q Advantage A10 water purification system (Millipore, Milford, MA, USA). All chemicals were used directly without any further purification unless otherwise stated.

Instruments. Transmission electron microscopic (TEM) characterization was carried out on a JEM-2800 system (JEOL, Tokyo, Japan). Ultraviolent (UV) spectral analysis was performed with a NanoDrop 2000/2000C spectrophotometer (Thermo Fisher, MA, USA). Microplate

reader analysis was carried out on a BioTek Synergy Mx microplate reader (Winooski, VT, USA). Cell imaging was carried out on an LSM 710 laser scanning confocal microscopy (Zeiss, Oberkochen, Germany).

Optimization of imprinting conditions of B2M-specific cMIP. The specific monomer ratio and ratio between total monomer and TEOS used for the preparation of epitope-imprinted cMIPs were optimized in terms of the obtained imprinting factor (IF). Epitope-imprinted cMIPs and cNIPs (2.0 mg each) prepared at different specific molar ratios of monomers (APTES, UPTES, IBTES, and BnTES) and different total monomers/TEOS ratios were added separately to 200 μL of phosphate buffer (10 mM pH 7.4) containing 0.1 mg/mL of epitope. After incubation at room temperature for 30 min, the nanoparticles were collected by centrifugation at 4,000 rpm for 30 min and rinsed with 200 μL of phosphate buffer (10 mM, pH 7.4) three times. The nanoparticles were re-suspended and eluted in 20 μL of ACN:H_2O:HAc = 50:49:1 (v/v) at room temperature for 10 min on a rotator. Finally, the suspensions were centrifuged at 4,000 rpm for 30 min and the supernatant were collected. The amount of epitope was determined by measuring the UV absorbance of the supernatant at 214 nm. The measurement was repeated three times. For control experiments, all the procedures were the same as described above except the absence of epitope in the test samples. For optimization of conventional epitope-imprinted polymers, all the procedures were the same as described above except that cMIPs and cNIPs were replaced by MIPs and NIPs prepared under otherwise identical conditions, respectively.

Selectivity test of B2M-specific cMIP. The selectivity of B2M C-terminal epitope-imprinted cMIP at the peptide level was evaluated using the C-terminal epitopes of B2M (KIVKWDRDM), TRF (LEACTFRRP) and TfR (LEACTFRRP), and the N-terminal epitopes of alpha fetoprotein (AFP) (RTLHRNEYG) and carcinoembryonic antigen (CEA) (KLTIESTPF) as test peptides. First, each epitope standard solution (0.1 mg/mL) was separately prepared with phosphate buffer (10 mM, pH 7.4). Then equivalent corresponding cMIP and cNIP (2 mg each) were added to 200 μL of the epitope solutions in 250-μL micro-centrifugal tubes. The tubes were shaken on a rotator at room temperature for 30 min. The nanoparticles were collected by centrifugation at 4,000 rpm for 30 min and rinsed with 200 μL of phosphate buffer (10 mM, pH 7.4) three times. Second, the nanoparticles were re-suspended and eluted in 20 μL of ACN:H_2O:HAc = 50:49:1 (v/v) at room temperature for 10 min on a rotator. Finally, the nanoparticles were precipitated via centrifugation and the supernatant were collected. The amount of epitope bound by the cMIP was determined by measuring the UV absorbance of the supernatant at 214 nm. The measurement was repeated three times. For control experiments, all the procedure was the same as described above except the absence of epitope in the test samples. For the selectivity test of B2M C-terminal epitope-imprinted cMIP in the protein level, all the procedure was the same as described above except that the test peptides used were changed to the proteins B2M, RNase A, BSA, RNase B and OVA.

For the selectivity test of B2M C-terminal epitope-imprinted MIP at the peptide and protein level, all the procedures were the same as described above except that the B2M C-terminal epitope-imprinted cMIP and cNIP were replaced by B2M C-terminal epitope-imprinted MIPs and NIPs, respectively.

Measurement of adsorption isotherm. A series of standard solutions of fluorescently labeled B2M C-terminal epitope (FITC-KIVKWDRDM) of known concentrations were prepared with phosphate buffer (10 mM, pH 7.4). A volume of 200 μL of the above standard solutions was added to a 96-well plate, and the fluorescence intensity was measured by a microplate reader. Then 2 mg of the B2M C-terminal epitope-imprinted cMIP were separately added to 1 mL of the above standard solutions and shaken at room temperature for 30 min. After the nanoparticles were centrifuged, 200 μL of the supernatant was added to a 96-well plate and their fluorescence intensity was measured by the microplate reader. An adsorption isotherm was established by plotting the difference between the fluorescence intensity of the standard solution before extraction and of the supernatant after extraction using B2M C-terminal epitope-imprinted cMIP against the logarithmic concentration of FITC-KIVKWDRDM. To estimate the binding affinity of B2M C-terminal epitope-cMIP, the amount of FITC-KIVKWDRDM bound by the B2M C-terminal epitope-cMIP was plotted according to the Scatchard equation as given below:

$$\frac{Q_e}{[S]} = \frac{Q_{max}}{K_d} - \frac{Q_e}{K_d}$$

where Q_e, [S], Q_{max} and K_d are the amount of FITC-KIVKWDRDM bound by the B2M C-terminal epitope-cMIP in terms of fluorescence intensity at equilibrium, the free concentration at adsorption equilibrium, the saturated adsorption amount in terms of fluorescence and the dissociation constant, respectively. By plotting Q_e/[S] versus Q_e, K_d and Q_{max} can be calculated from the slope and intercept, respectively.

For the adsorption isotherm of B2M C-terminal epitope-imprinted MIP, all the procedure was the same as described above except that the B2M C-terminal epitope-imprinted cMIP were changed to B2M C-terminal epitope-imprinted MIP.

Preparation of GPNMB-specific QD520@cMIP. The solution S3 described in ***microemulsion formation*** procedure was used for the imprinting, while slightly different peptide sequences were used as the epitope depending on the targets to test. For selectivity test and the optimization of imprinting conditions, the decapeptide AKRFHDVLGNK, which has an additional alanine at its N-terminal as compared with the native nonapeptide epitope, was used as the epitope. This was due to the fact that the protein GPNMB used in this study remained an additional alanine as its N-terminal. However, the use of additional amino acid in the epitope will not the recognition of the prepared cMIP towards its alanine-deleted analog (KRFHDVLGNK) in selectivity test (Supplementary Fig. 13). While for the imaging of GPNMB-overexpressed cell lines, the native nonapeptide KRFHDVLGNK was used as the epitope (see Supplementary Fig. 2 for the structure). For the imprinting, the microemulsion was first stirred at 700 rpm for 10 min at 25 ºC, and 700 μL of QD520 solution (3 mg/mL in cyclohexane) was then added and stirred for 30 min. 1 mg of C13-grafted epitope was added to the above solution and continued stirring for another 30 min. After that, 1 mL of S3 was added dropwise carefully, and the mixture was allowed to stirred at 700 rpm at 25 ºC for 24 h. Subsequently, 100 μL of TEOS was added dropwise to the mixture, and the mixture was stirred at 700 rpm for another 24 h at 25 ºC. The obtained materials were released from the

microemulsion by adding acetone, followed by centrifugation at 4,000 rpm for 30 min and washed with anhydrous ethanol and water five times, respectively.

To remove the C13-grafted epitope, the obtained QD520@cMIP was dispersed into 5 mL of ACN:H_2O:HAc = 50:49:1 (v/v) and shaken for 20 min at room temperature. The above elution process was repeated three times. After removing the C13-grafted epitope template, the prepared GPNMB N-terminal epitope-imprinted QD520@cMIP was collected by centrifugation at 4,000 rpm for 30 min. The collected QD520@cMIP was washed with water and anhydrous ethanol three times each and then freeze-dried in a vacuum overnight.

For the preparation of QD520@cNIP, the process was the same except that no templates were added.

Preparation of HER2-specific QD620@cMIP. The N-terminal epitope of HER2 was used, the preparation process was the same as above except the C13-grafted GPNMB N-terminal epitope and QD520 were changed to C13-grafted HER2 N-terminal epitope and QD620, respectively.

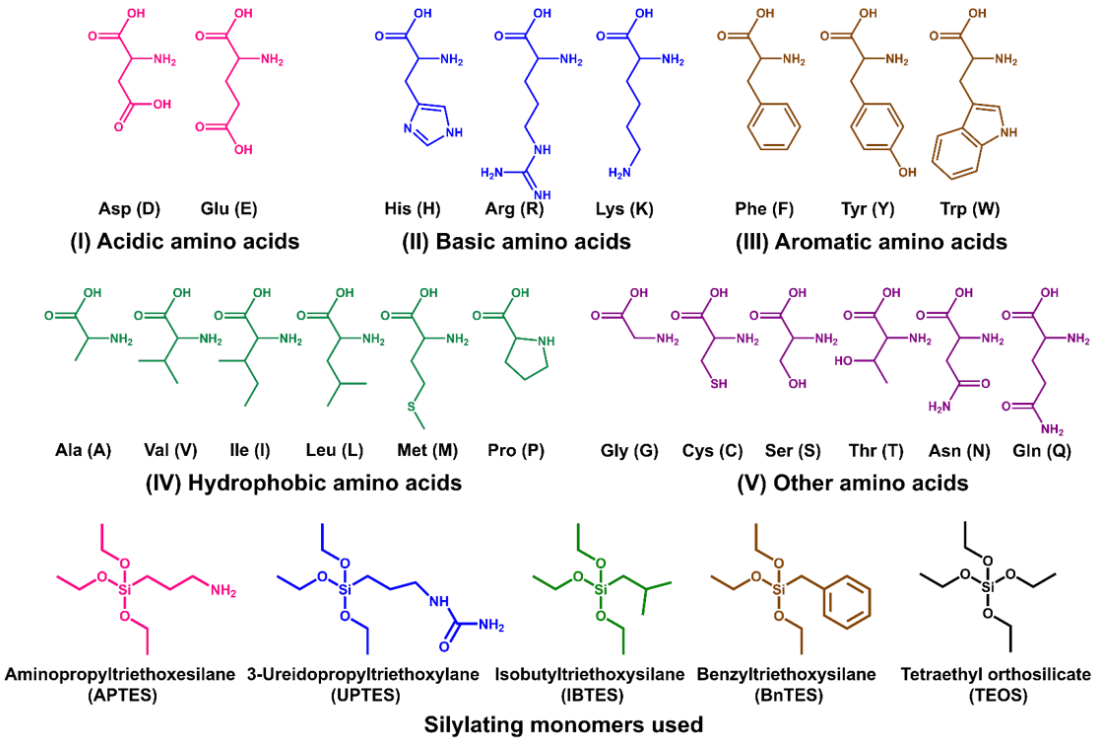
Optimization of imprinting conditions of GPNMB-specific QD520@cMIP. The specific monomer ratios and the total monomers/TEOS ratios used for the preparation of GPNMB N-terminal epitope-imprinted QD520@cMIP were optimized in terms of the obtained IF value. The optimization procedure was the same as describe above, except the materials were changed to GPNMB N-terminal epitope-imprinted QD520@cMIP.

Optimization of imprinting conditions of HER2-specific QD620@cMIP. The specific monomer ratio used for the preparation of HER2 N-terminal epitope-imprinted QD620@cMIP was optimized in terms of the obtained IF value. The optimization procedure was the same as describe above, except the materials were changed to HER2 N-terminal epitope-imprinted QD620@cMIP.

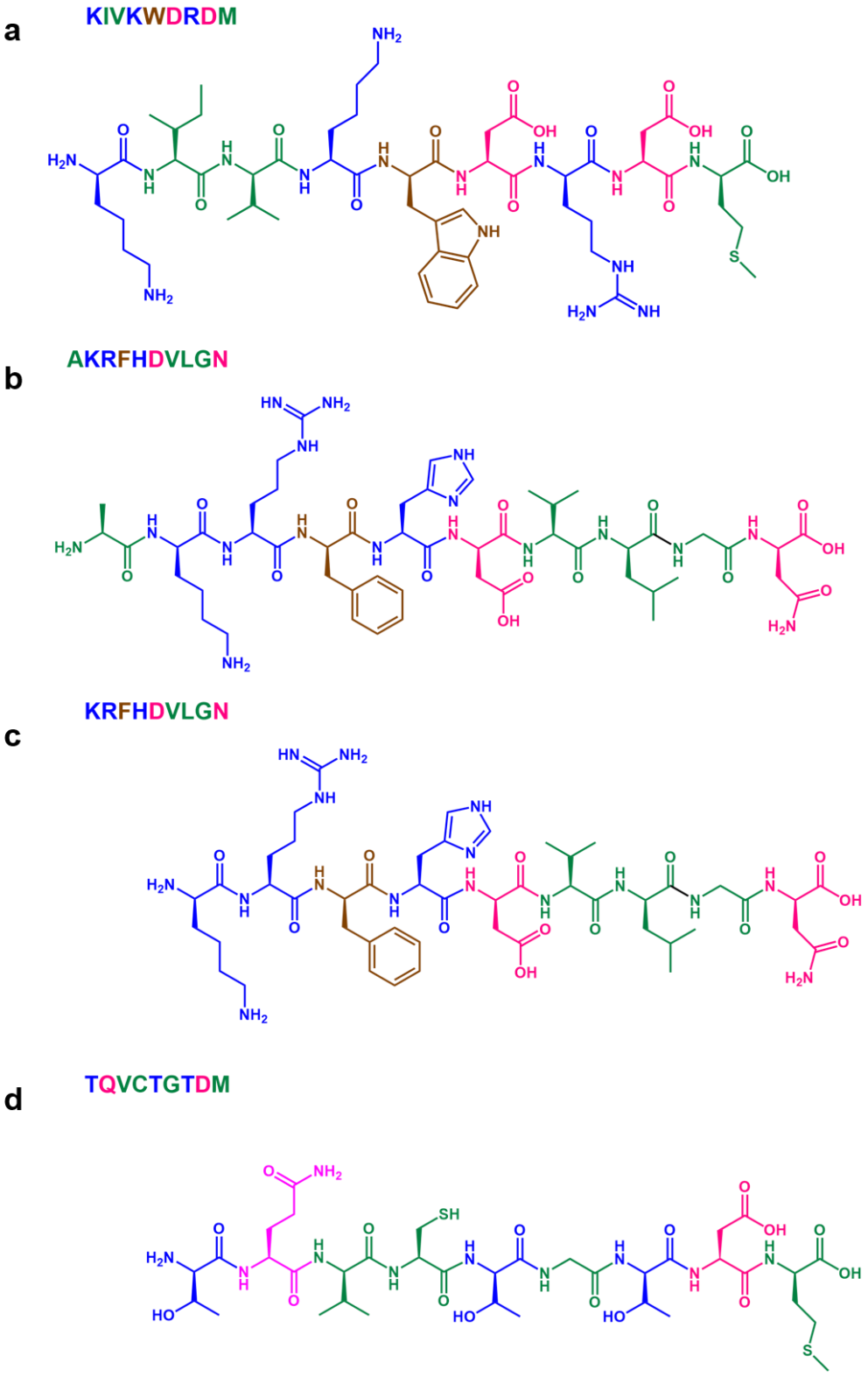
Selectivity test of GPNMB-specific QD520@cMIP and HER2-specific QD620@cMIP. The selectivity test of QD520@cMIP and QD620@cMIP at the peptide and protein levels was carried out using the same procedure described above except that the B2M C-terminal epitope-imprinted cMIP and cNIP were replaced by QD520@cMIP and QD520@cNIP or QD620@cMIP and QD620@cNIP.

Cell culture and imaging. MCF-7 cells were cultured in the RPMI-1640 medium with 10% FBS for 2-3 days (37 ºC, 5% CO_2), while MDA-MB-157 and MDA-MB-361 cells were cultured in the DMEM medium with 10% FBS for 2-3 days (37 ºC, 5% CO_2). Each cell line was cultured in four batches. The cell culture medium was removed and the cells remained on the cell culture dishes were washed with 1×PBS twice. Then the cells were fixed with 4% formaldehyde for 15 min respectively, followed by incubated with 200 μL of 200 μg/mL GPNMB N-terminal epitope-imprinted QD520@cMIP, QD520@cNIP, HER2 N-terminal epitope-imprinted QD620@cMIP and QD620@cNIP dissolved in 1× PBS for 30 min. Then 1×PBS and free nanoparticles were removed and the remaining cells were rinsed with 1×PBS three times and then stained with 100 μL of Hoechst 33342 for 10 min. After rinsed with 1×PBS

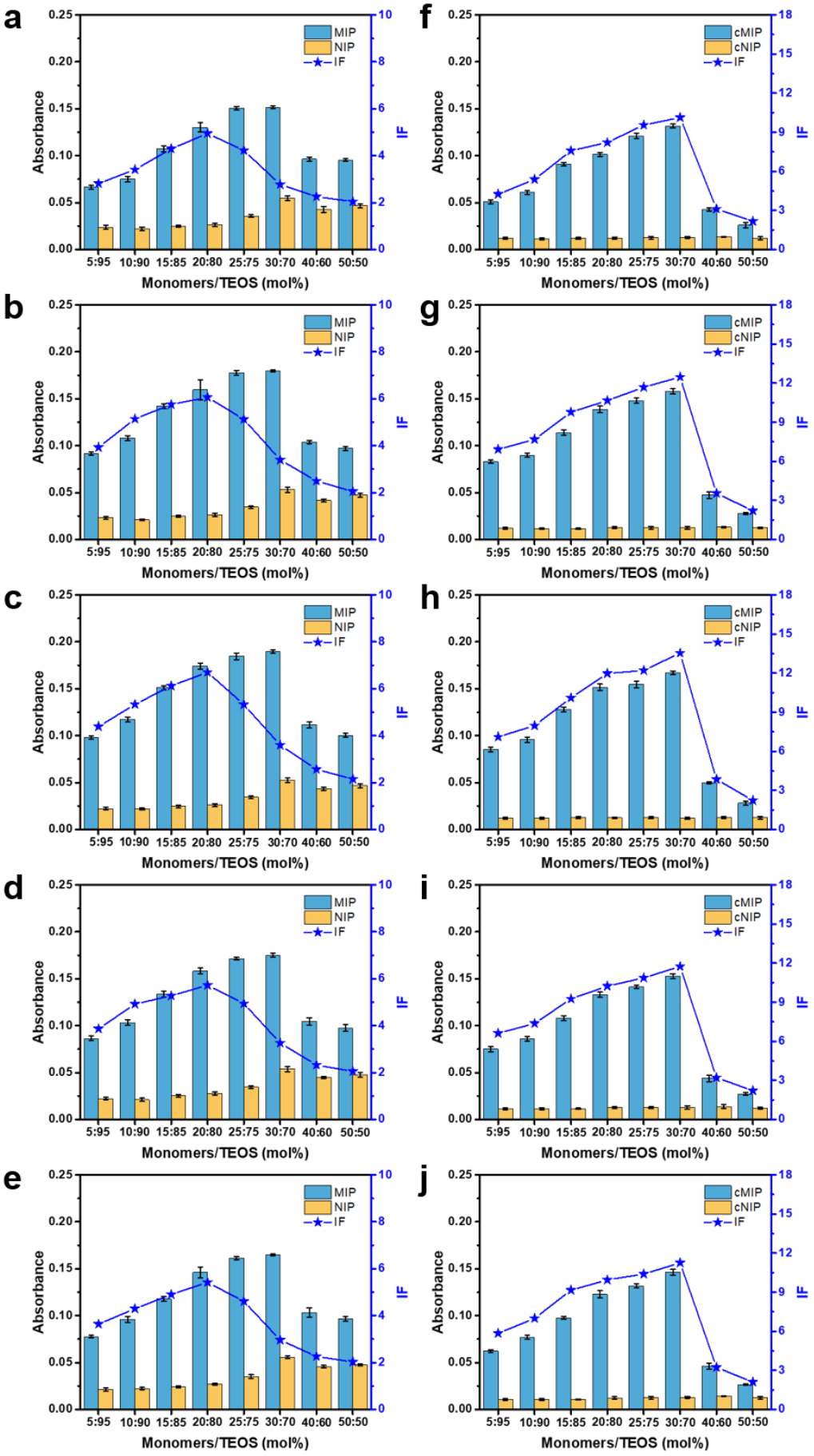
twice, the cell culture dishes were supplemented with 1 mL of 1×PBS. The obtained cells were imaged under laser scanning confocal microscopy.



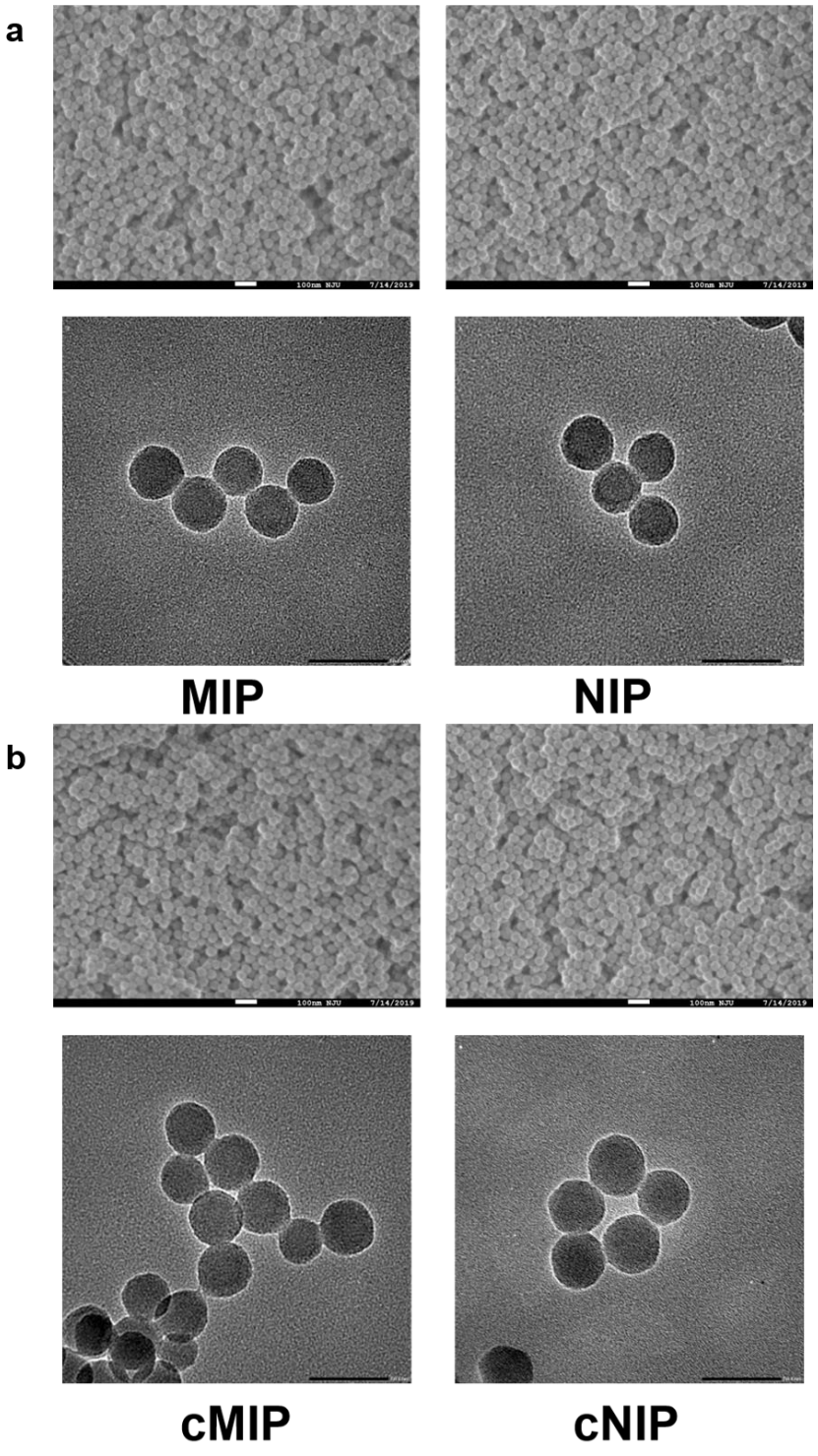
Supplementary Fig. 1 | Structures of amino acids and silylating monomers. Amino acids are classified in terms of their properties enabling interactions with silylating monomers (Amino acids and monomers shown in the same color indicate those that can interact with each other).



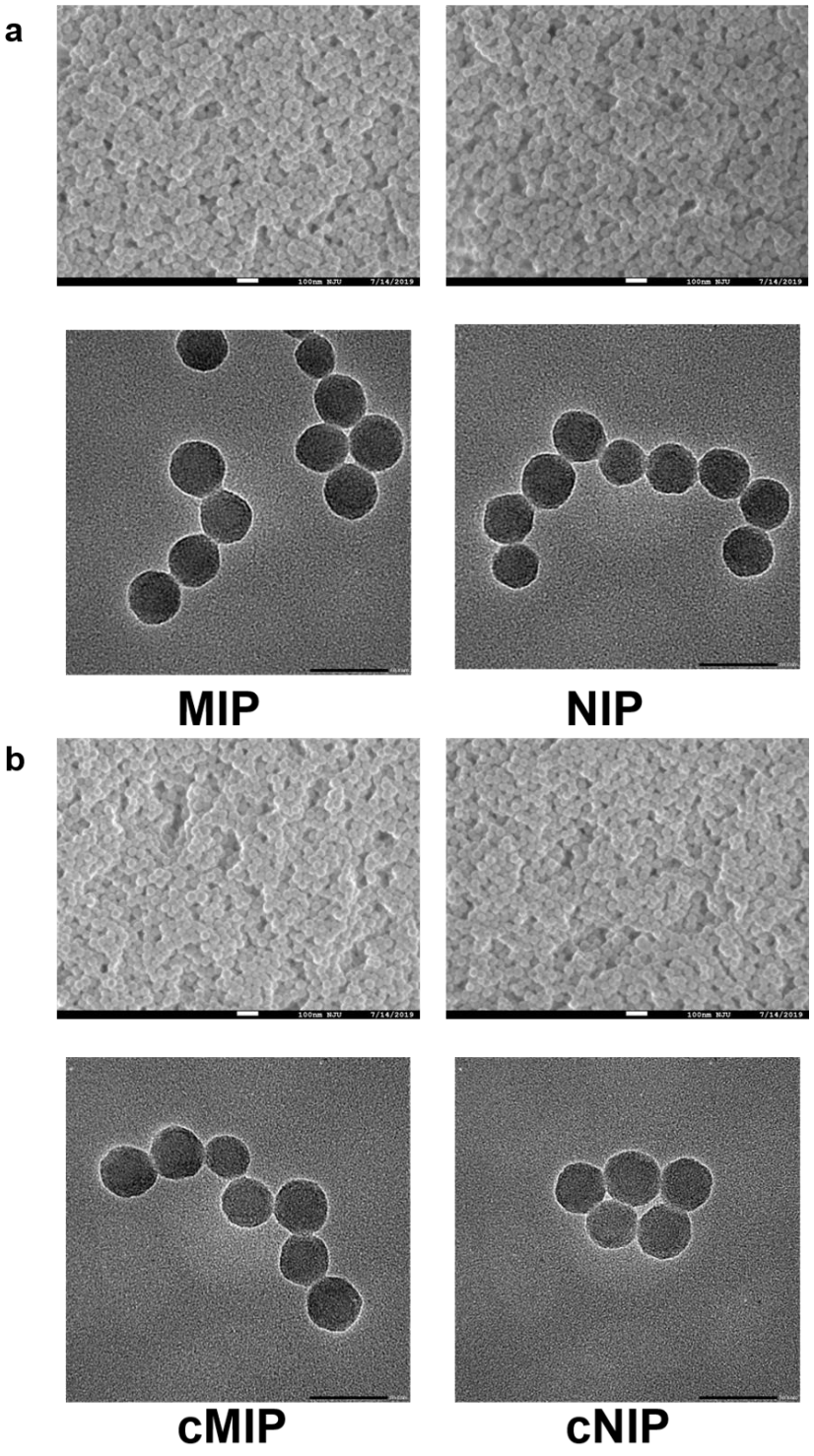
Supplementary Fig. 2 | Structures of the epitopes used in this study. a, C-terminal epitope of B2M. **b,** N-terminal epitope of GPNMB used for specificity test. **c,** N-terminal epitope of GPNMB used for cell imaging. **d,** N-terminal epitope of HER2.


a
b
c
d
e
f
g
h
i
j
MIP
NIP
IF
cMIP
cNIP
Absorbance
IF
Monomers/TEOS (mol%)
5:95
10:90
15:85
20:80
25:75
30:70
40:60
50:50

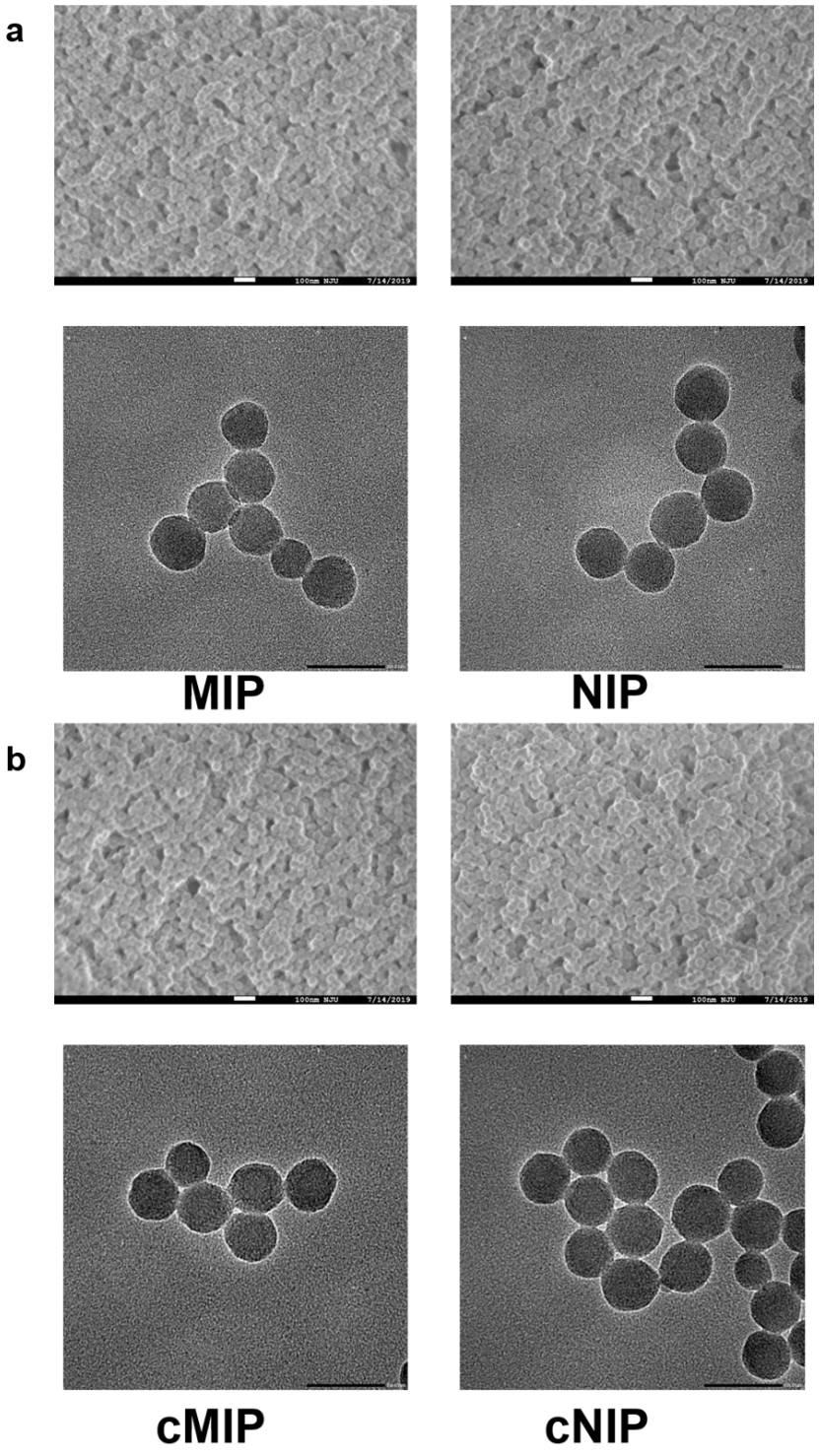
Supplementary Fig. 3 | Optimization of imprinting conditions and comparison of imprinting effect. a-e, Comparison of the absorbance of the template captured by B2M C-terminal epitope-imprinted MIPs prepared at different ratio of monomers/TEOS using the ratio of APTES/UPTES/IBTES/BnTES=20:40:40:0 (**a**), 20:30:40:10 (**b**), 20:20:50:10 (**c**), 30:20:40:10 (**d**), 10:30:50:10 (**e**). **f-j,** Comparison of the absorbance of the template captured by B2M C-terminal epitope-imprinted cMIPs prepared at different ratio of monomers/TEOS using the ratio of APTES/UPTES/IBTES/BnTES=20:40:40:0 (**f**), 20:30:40:10 (**g**), 20:20:50:10 (**h**), 30:20:40:10 (**i**), 10:30:50:10 (**j**).



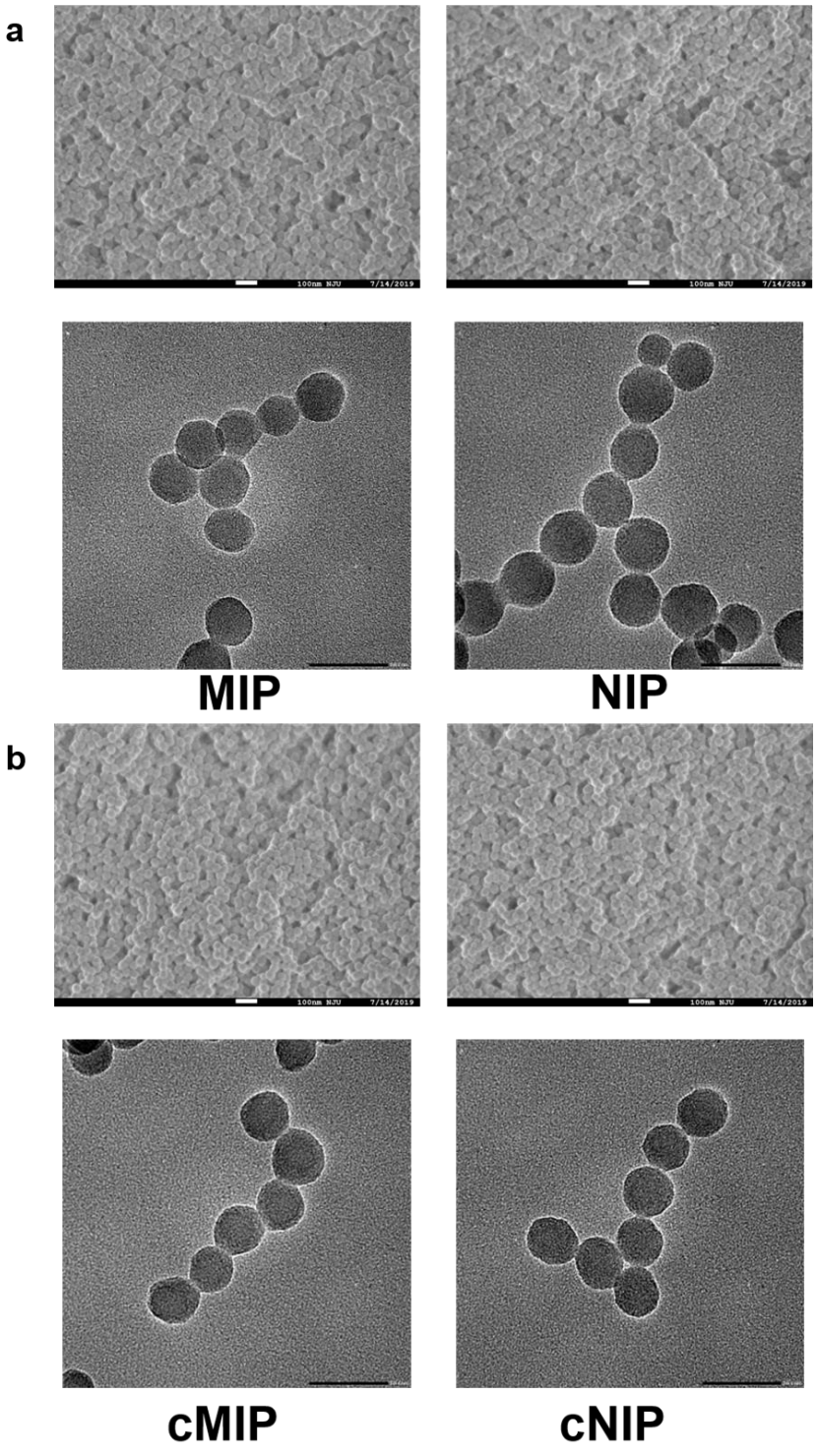
Supplementary Fig. 4 | SEM and TEM characterization of nanoparticles prepared at the total monomers/TEOS ratio of 5:95. a, MIP and NIP. **b,** cMIP and cNIP. The APTES/UPTES/IBTES/BnTES ratio was 20:20:50:10.



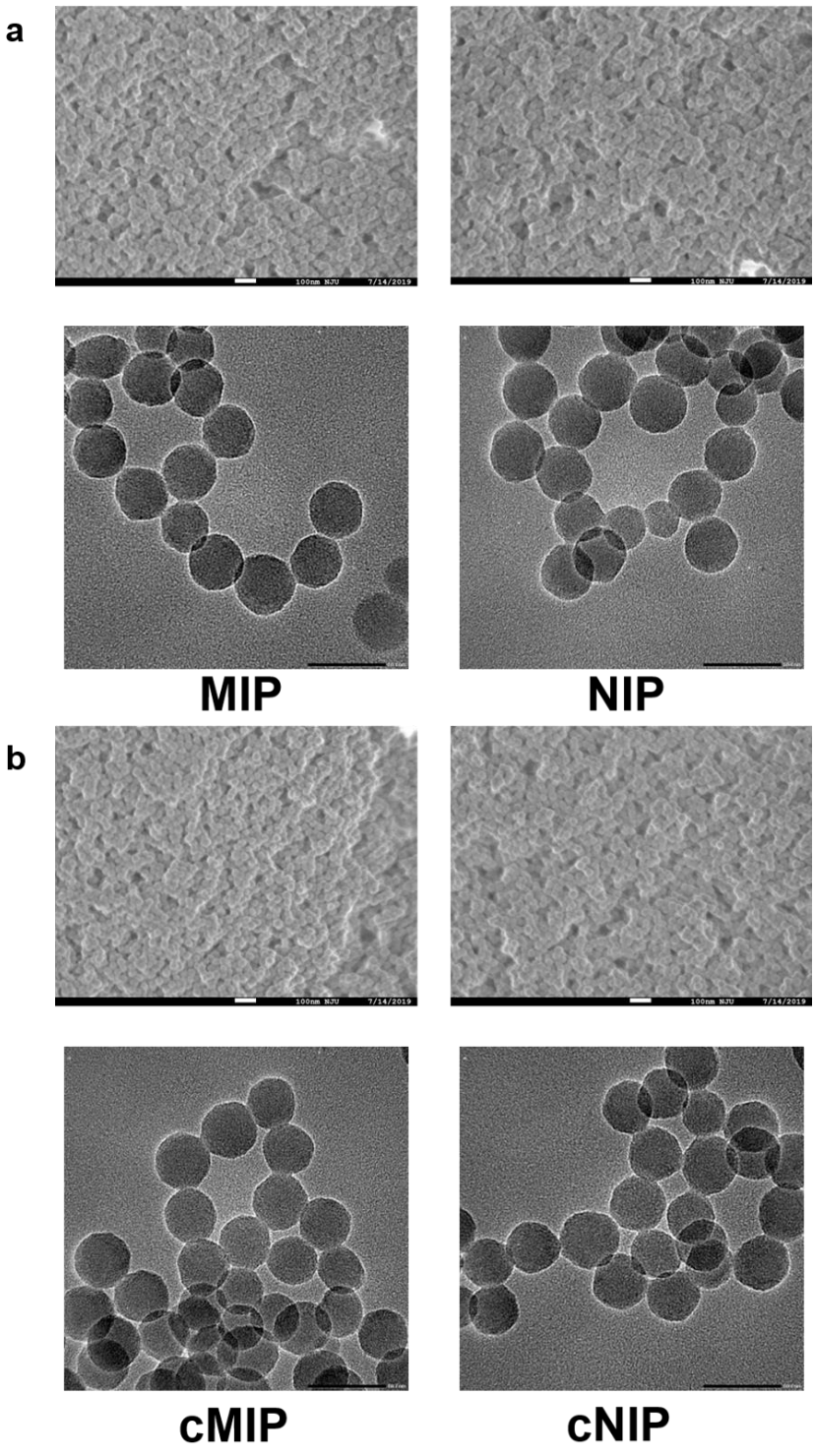
Supplementary Fig. 5 | SEM and TEM characterization of nanoparticles prepared at the ratio of total monomers/TEOS of 10:90. a, MIP and NIP. **b,** cMIP and cNIP. The APTES/UPTES/IBTES/BnTES ratio was 20:20:50:10.



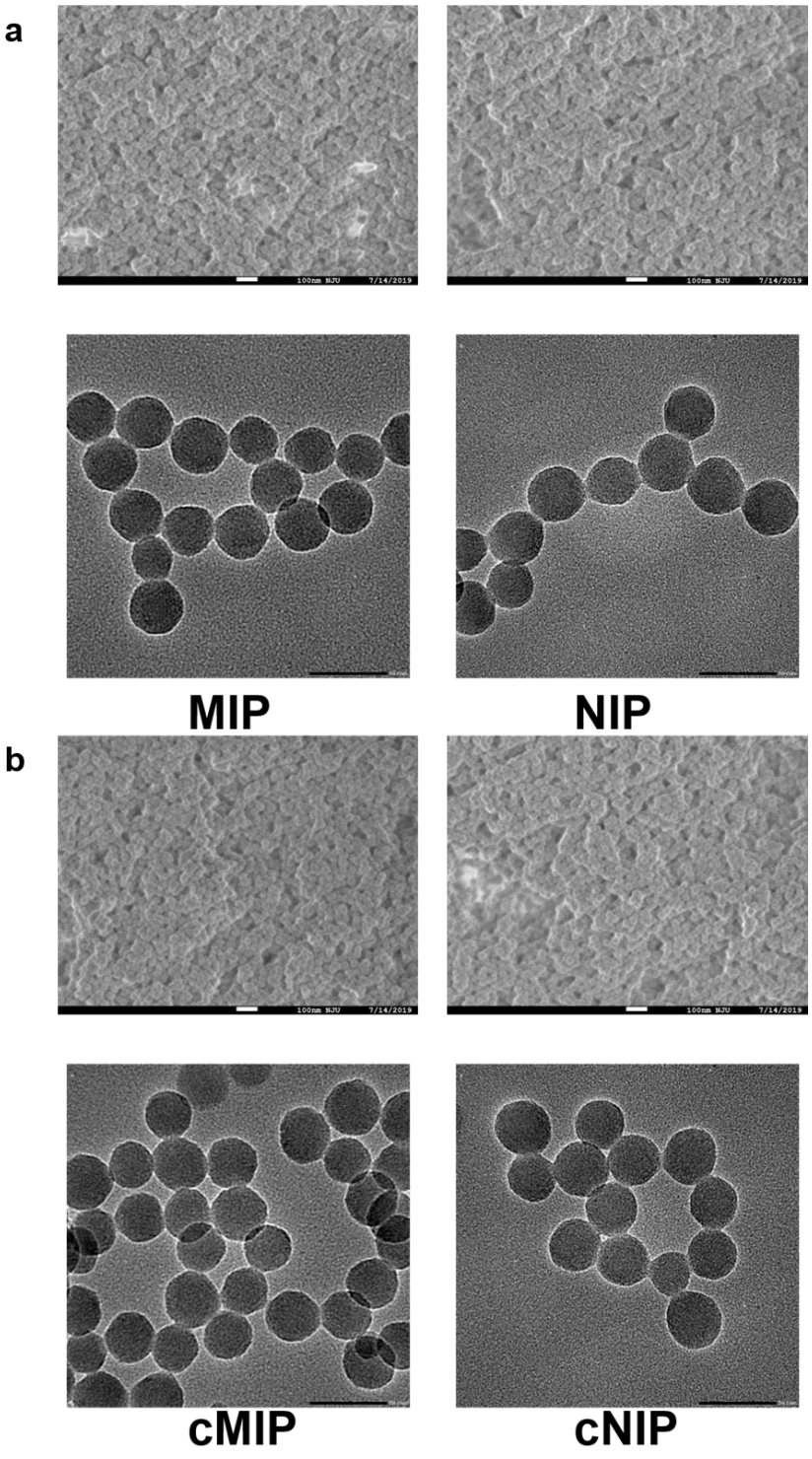
Supplementary Fig. 6 | SEM and TEM characterization of nanoparticles prepared at the monomers/TEOS ratio of 15:85. a, MIP and NIP. **b,** cMIP and cNIP. The ratio of APTES/UPTES/IBTES/BnTES was fixed at 20:20:50:10.



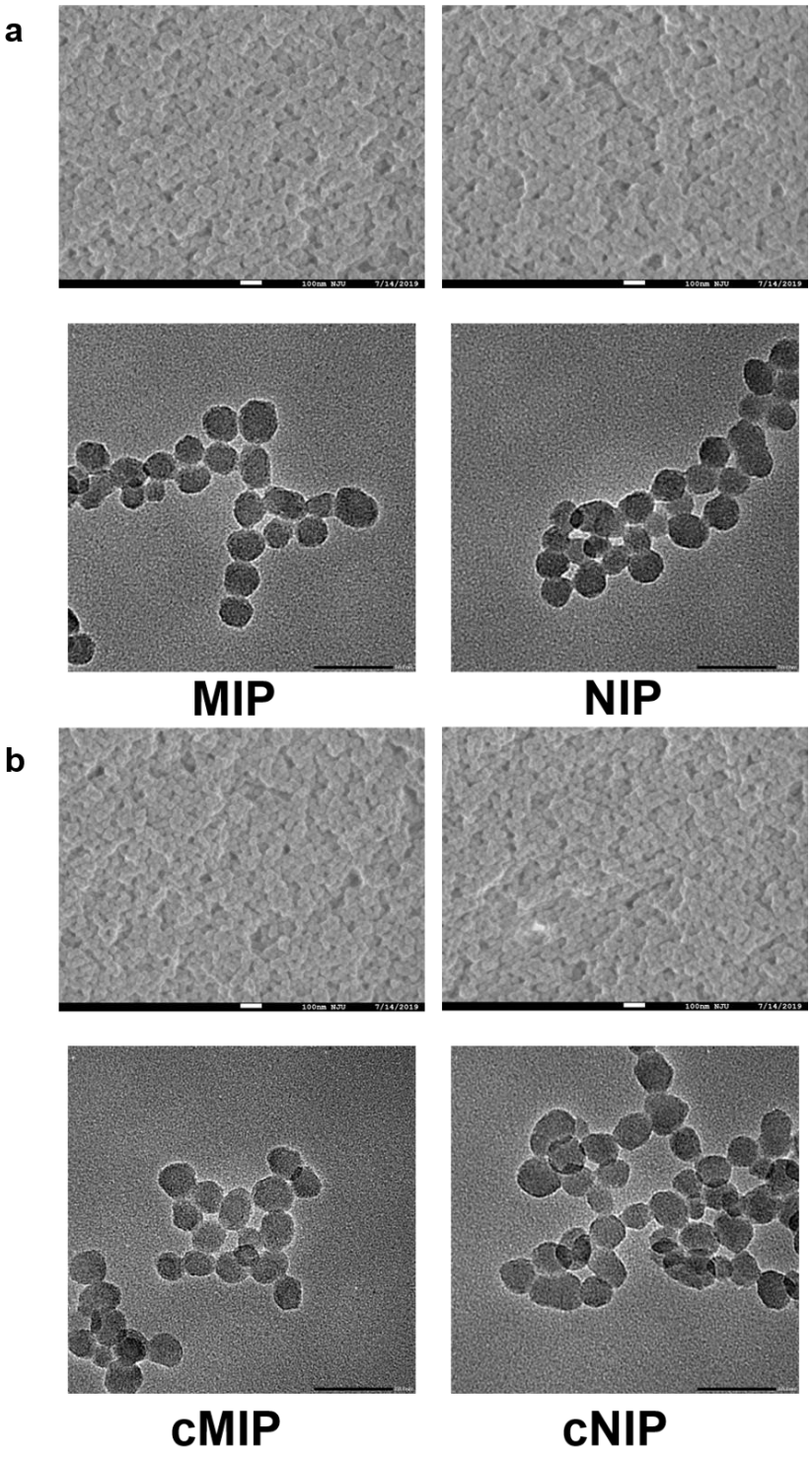
Supplementary Fig. 7 | SEM and TEM characterization of nanoparticles prepared at the total monomers/TEOS ratio of 20:80. a, MIP and NIP. **b,** cMIP and cNIP. The APTES/UPTES/IBTES/BnTES ratio was 20:20:50:10.



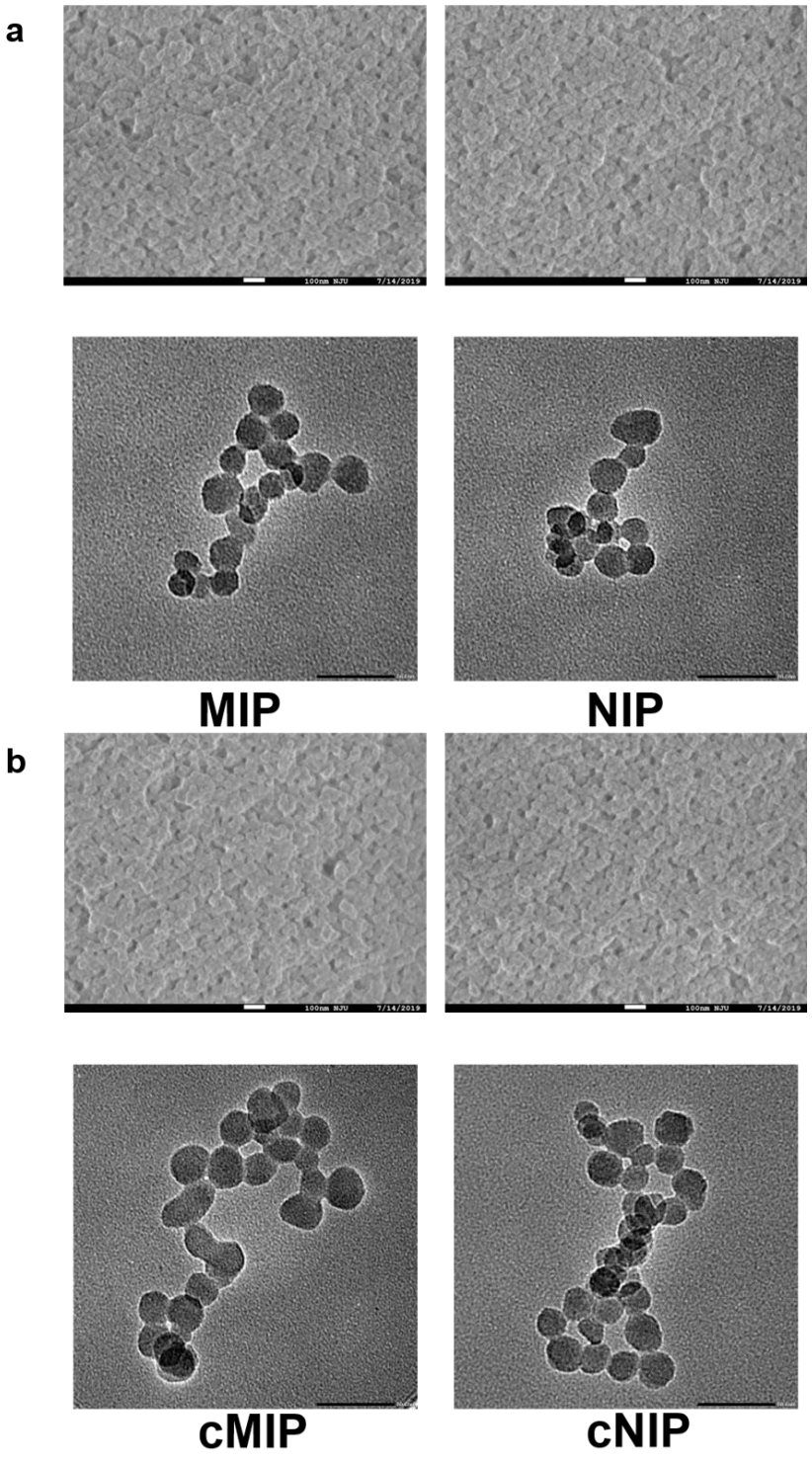
Supplementary Fig. 8 | SEM and TEM characterization of nanoparticles prepared at the total monomers/TEOS ratio of 25:75. a, MIP and NIP. **b,** cMIP and cNIP. The APTES/UPTES/IBTES/BnTES ratio was 20:20:50:10.



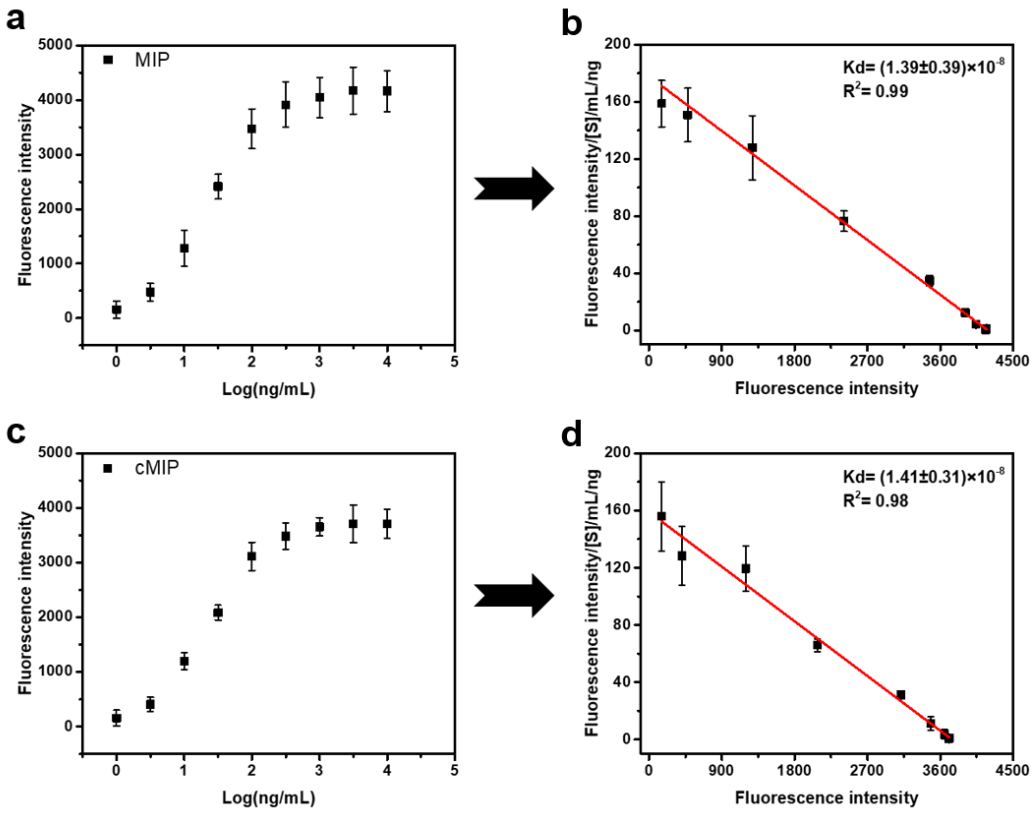
Supplementary Fig. 9 | SEM and TEM characterization of nanoparticles prepared at the total monomer/TEOS ratio of 30:70. a, MIP and NIP. **b,** cMIP and cNIP. The APTES/UPTES/IBTES/BnTES ratio was 20:20:50:10.



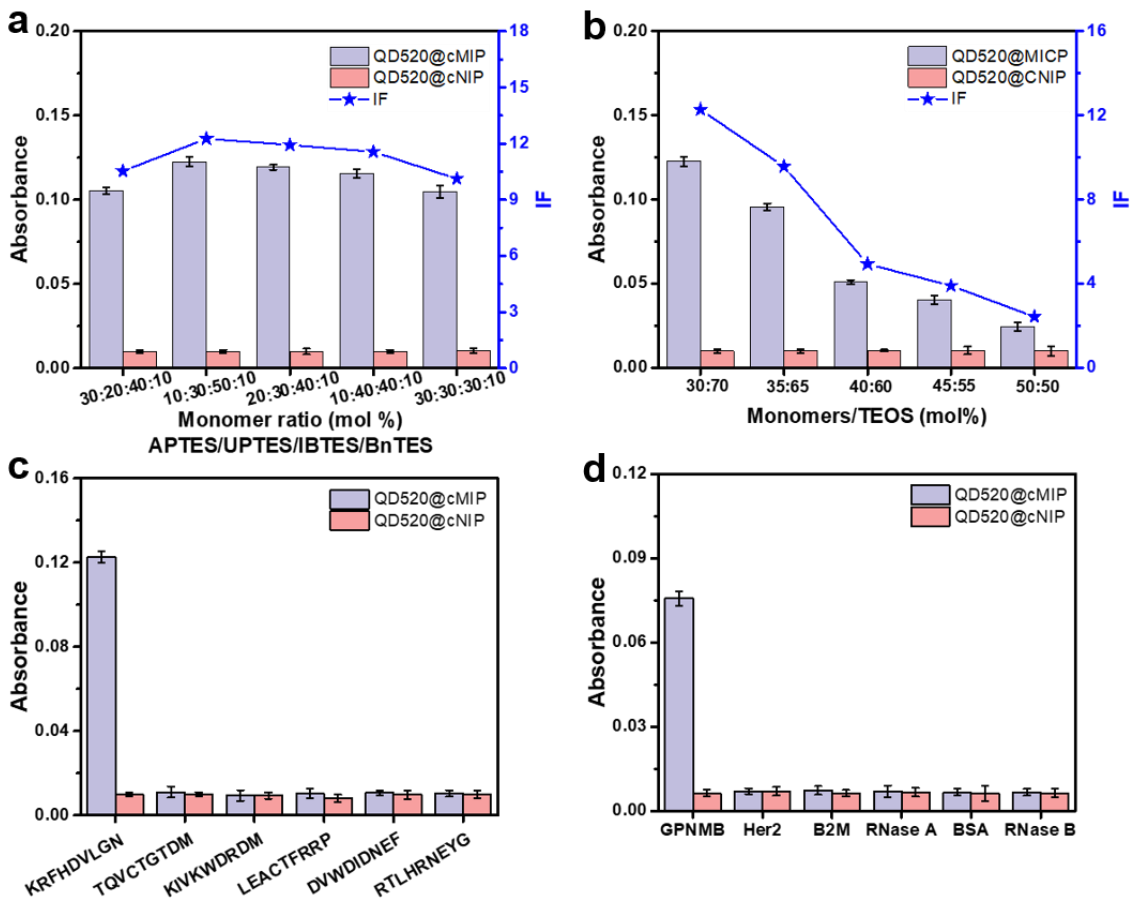
Supplementary Fig. 10 | SEM and TEM characterization of nanoparticles prepared at the total monomer/TEOS ratio of 40:60. a, MIP and NIP. **b,** cMIP and cNIP. The APTES/UPTES/IBTES/BnTES ratio was 20:20:50:10.



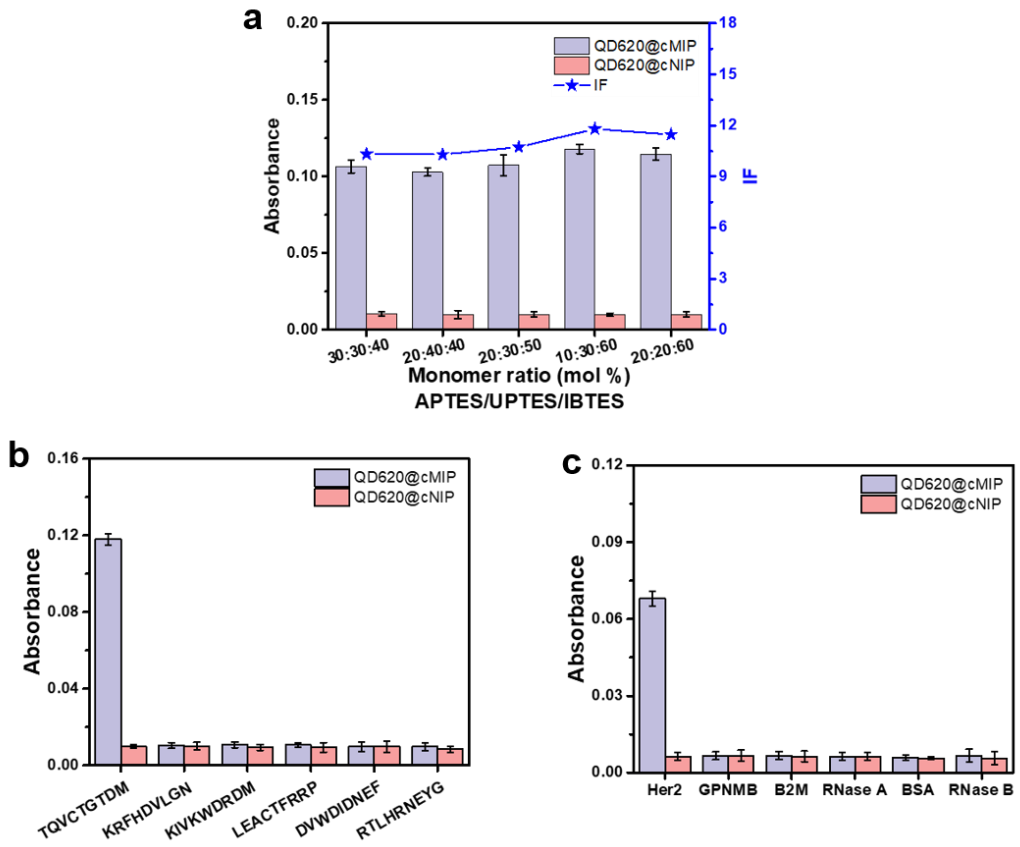
Supplementary Fig. 11 | SEM and TEM characterization of nanoparticles prepared at the total monomer/TEOS ratio of 50:50. a, MIP and NIP. **b,** cMIP and cNIP. The APTES/UPTES/IBTES/BnTES ratio was 20:20:50:10.



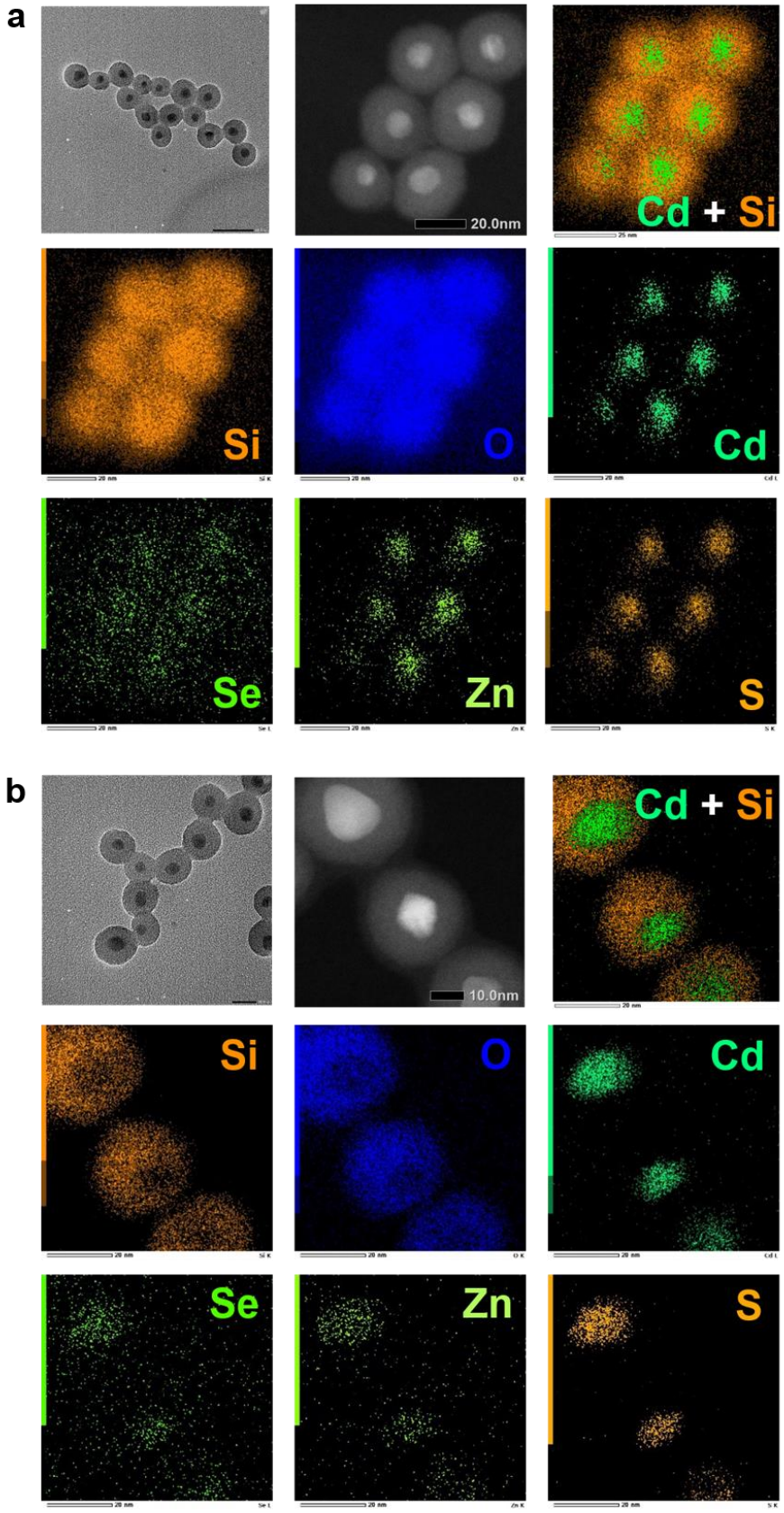
Supplementary Fig. 12 | Binding isotherms and affinity measurement. Binding isotherm (**a**) and Scatchard plot (**b**) for the binding of B2M C-terminal epitope-imprinted MIP prepared at the monomer ratio of APTES/UPTES/BnTES/IBTES = 20:20:50:10 and the ratio of monomers/TEOS = 20:80. Binding isotherm (**c**) and Scatchard plot (**d**) for the binding of B2M C-terminal epitope-imprinted cMIP prepared at the monomer ratio of APTES/UPTES/BnTES/IBTES = 20:20:50:10 and the ratio of monomers/TEOS = 30:70.



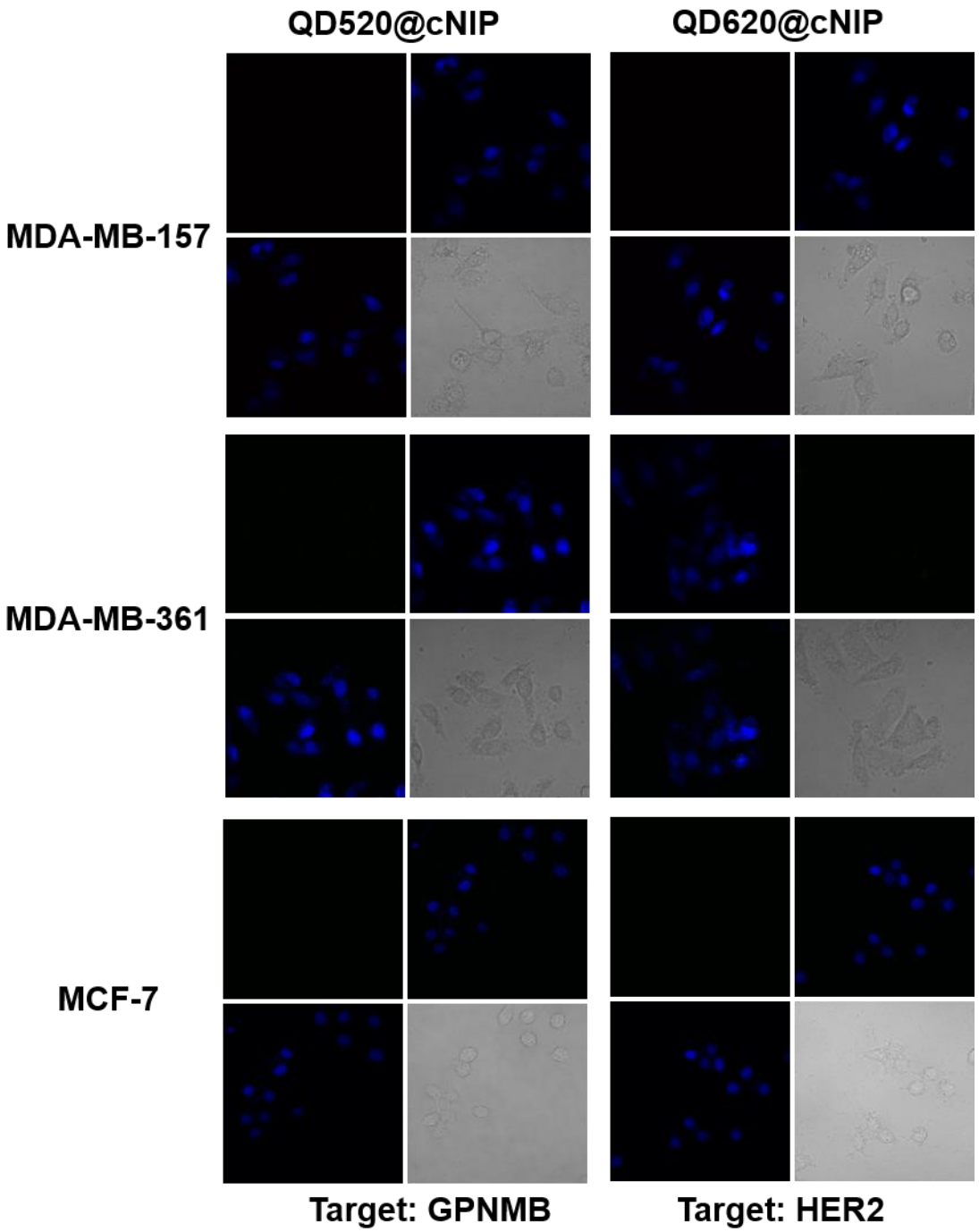
Supplementary Fig. 13 | Optimization and selectivity test of GPNMB N-terminal epitope-imprinted QD520@cMIP. Optimization of the monomer ratio of (**a)** and the total monomers/TEOS ratio (**b**), and the selectivity test of QD520@cMIP toward different peptides (**c**) and proteins (**d**).



Supplementary Fig. 14 | Optimization and selectivity test of HER2 N-terminal epitope-imprinted QD620@cMIP. Optimization of the monomer ratio of (**a**) and the selectivity test of QD620@cMIP towards different peptides (**b**) and proteins (**c**).



Supplementary Fig. 15 | TEM, STEM and EDS mapping of GPNMB-specific QD520@cMIP (a) and HER2-specific QD620@cMIP (b).



Supplementary Fig. 16 | Confocal fluorescence imaging of MDA-MB-157, MDA-MB-361 and MCF-7 cells after staining with QD520@cNIP or QD620@cNIP. Blue: nuclei stained by Hoechst 33342.