Electronic Supplementary Material (ESI) for Analyst.


# Supplementary information

Aptamer-based Biosensors through the Mapping of Encoding Up-conversion Nanoparticles for Sensitive CEA Detection

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**Slides processing**: The slides and coverslips were sonicated using ultrapure water for 5-10 min, respectively, for preliminary treatment of the original impurities on the glass surface. Prepare piranha wash with concentrated sulfuric acid and hydrogen peroxide in the ratio of 7:3, soak the slides and coverslips with piranha wash for 4 hours, then remove the slides and coverslips and clean them with ultrapure water, blow dry with nitrogen, put them in a vacuum drying oven for 10 min, and remove them for use.

**Image processing:** Due to the special luminescence properties of the UCNPs and as long as the slide coverslips are kept clean in the experimental environment, theoretically, the fluorescence images of RE-UCNPs do not show strong interference bright spots similar to those caused by light scattering from environmental impurities by noble metal nanoparticles. Therefore, only the green fluorescent spots appearing on the images need to be counted. Therefore, the counting of particle images can be done with the conventional image processing software ImageJ, which requires first binarization of the image and then the setting of the grayscale threshold. The simplicity of the counting process is also an advantage of upconverting nanoparticles for counting detection. The counting results for each slide sample were averaged over 10 images. The Olympus CellSens Entry software has a resolution of 1600 × 1200 pixels, and the image size acquired after calibration is 176 × 132 $\mu m^2$, and all imaging conditions were kept consistent during the experiments for each reaction system.

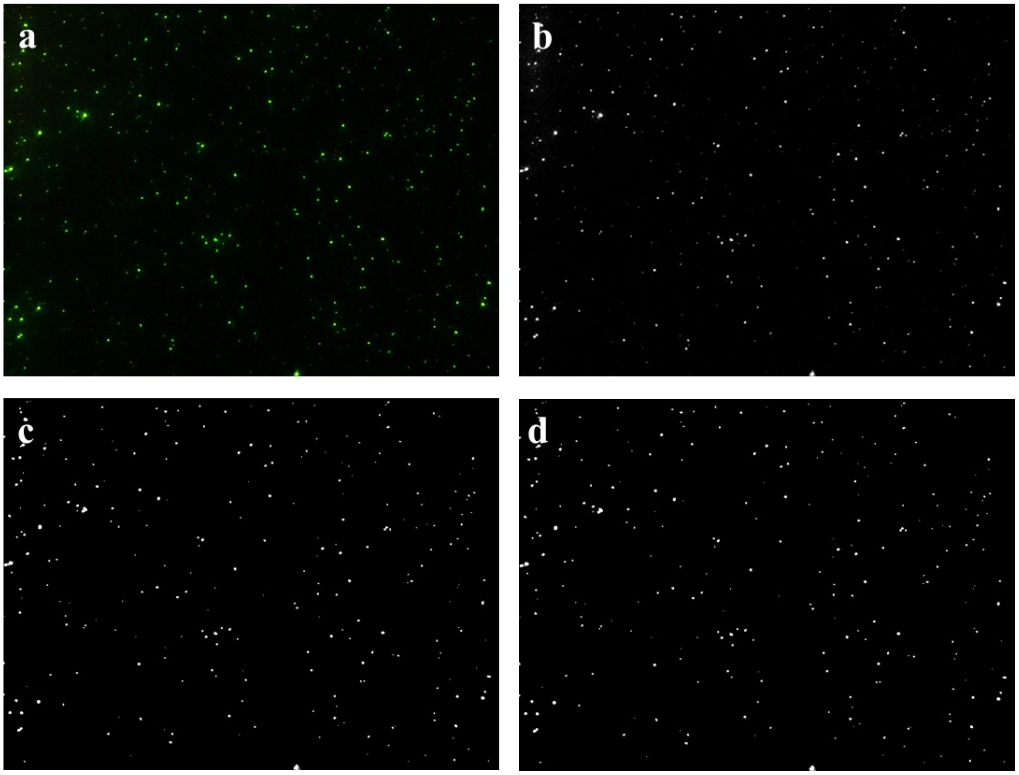


**Figure S1.** Images processing. (a) captured fluorescence images; (b) Image conversion to 8-bit format; (c) images after setting the pixel threshold; (d) images after setting the threshold for connected field area and used for counting.

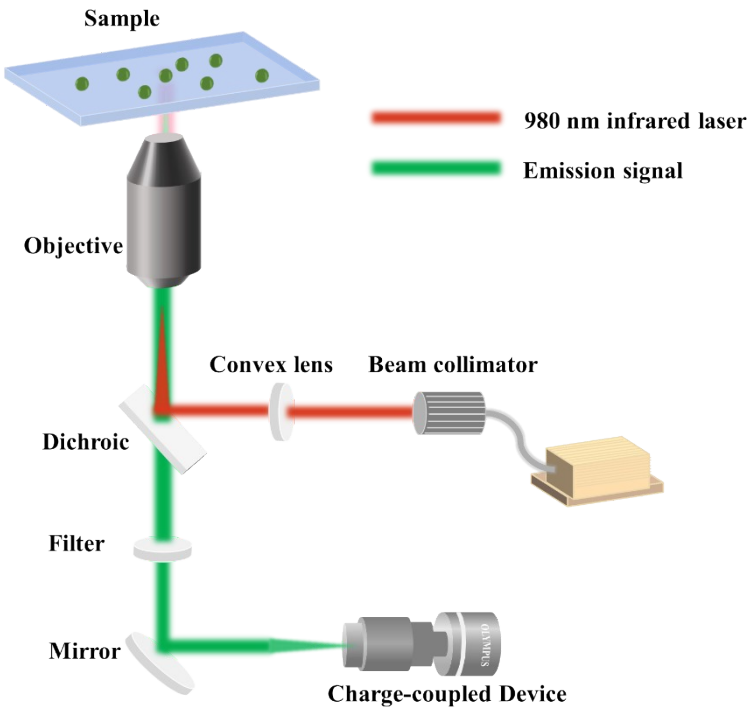


**Figure S2.** The construction diagram of the inverted fluorescence microscope we modified.

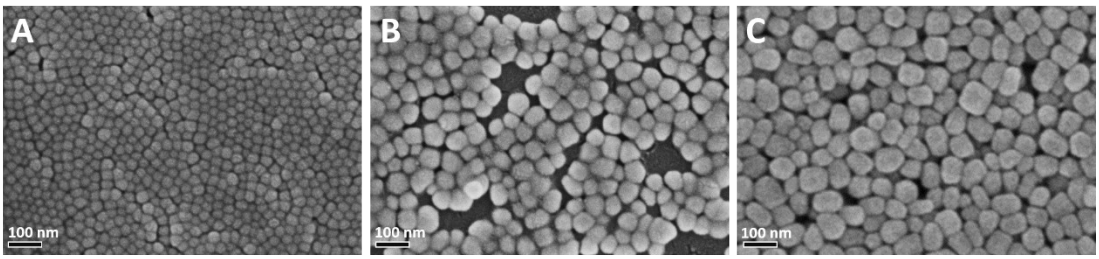


**Figure S3.** Different sizes of UCNPs under different time and temperature regulated. (A) ~30nm, heating for 1 hour under 305℃; (B) ~65nm, heating for 1.5 hours under 305℃; (C) ~105nm, heating for 1.5 hours under 310℃.

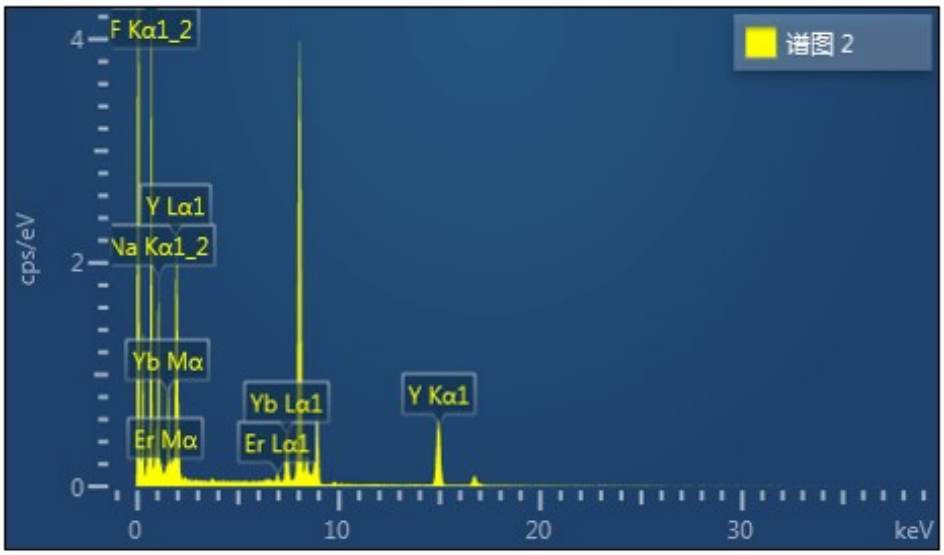


**Figure S4**. EDS Spectrogram of synthesized UCNPs.

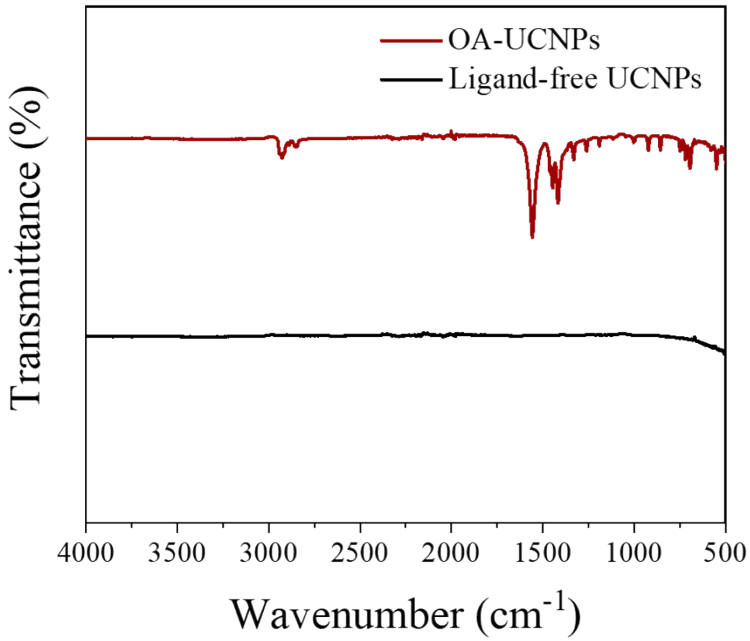


**Figure S5.** FT-IR spectra of OA-UCNPs and ligand-free UCNPs.

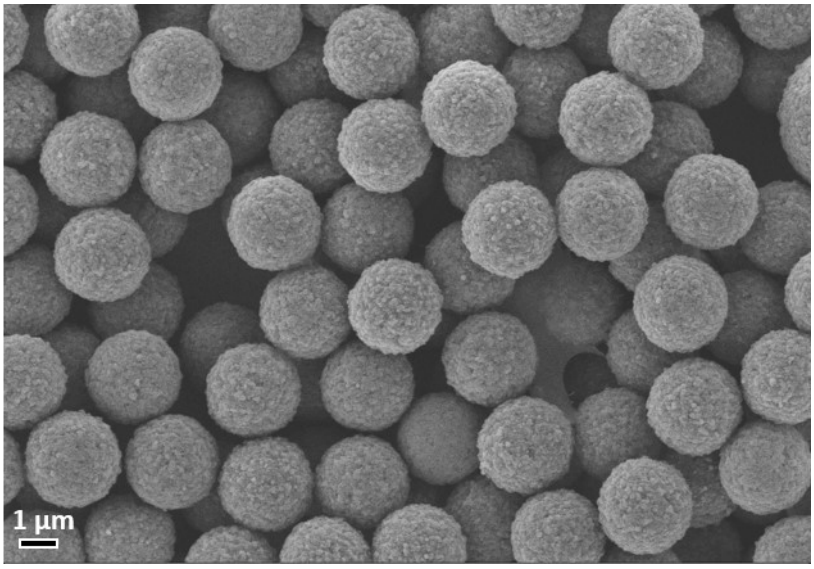


**Figure S6.** SEM images of MBs dispersed in ultrapure water.

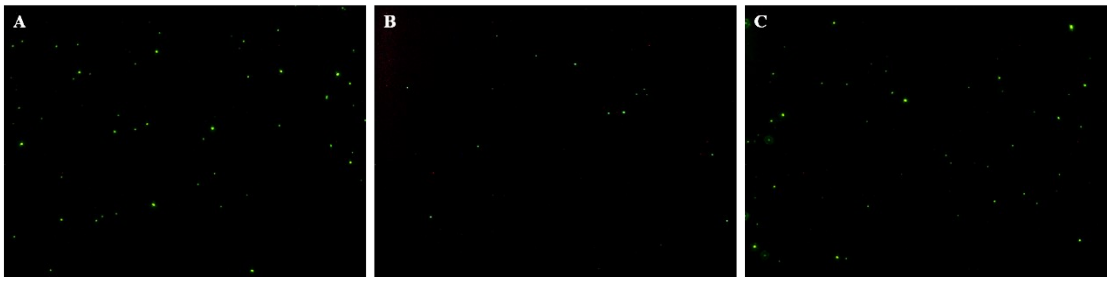


**Figure S7.** Representative upconversion microscopy images of cDNA-UCNPs on the coverslip surface.

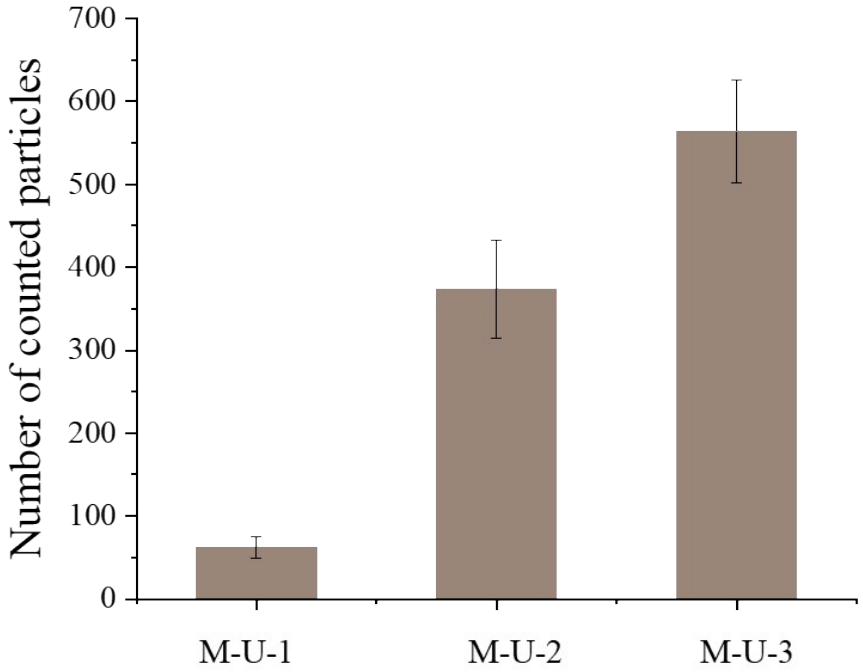


**Figure S8.** The number of counted particles of different biosensors reacted with 4 ng/mL CEA

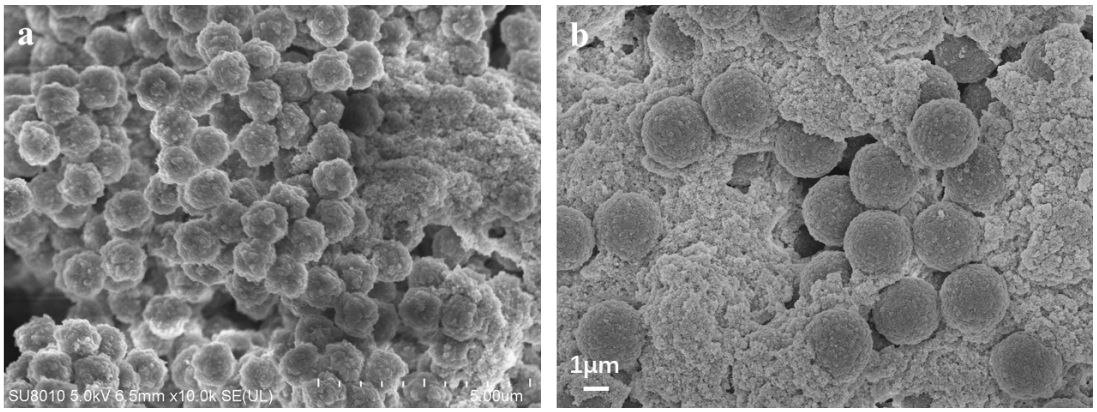


**Figure.S9** SEM images of (a) Improper control of the relationship between the amount ratio of DNA-MBs and cDNA-UCNPs for constructing biosensors; (b) Improper control of buffer salt concentration for constructing biosensors.

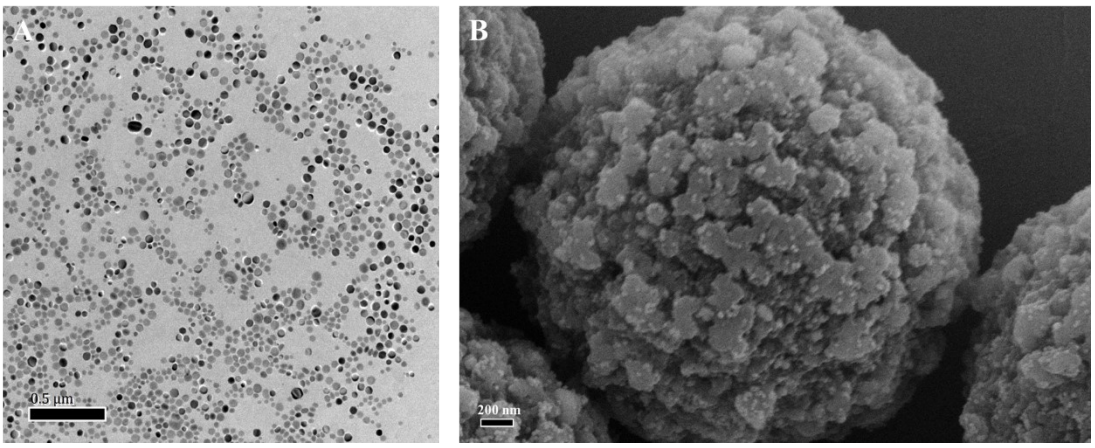


**Figure. S10** TEM images of (A) cDNA-UCNPs after stored in refrigerator at 4°C for one week; (B) MBs-DNA-cDNA-UCNPs after stored in refrigerator at 4°C for one week.

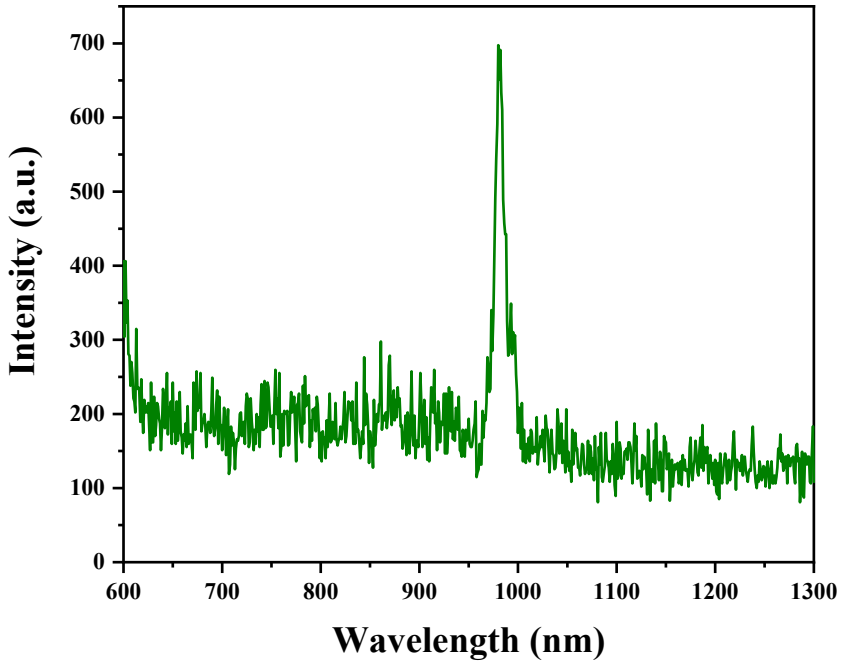


**Figure. S11** The fluorescence excitation spectra of oleic acid capped UCNPs ($NaYF_4$, Yb/Er) dispersed in cyclohexane.

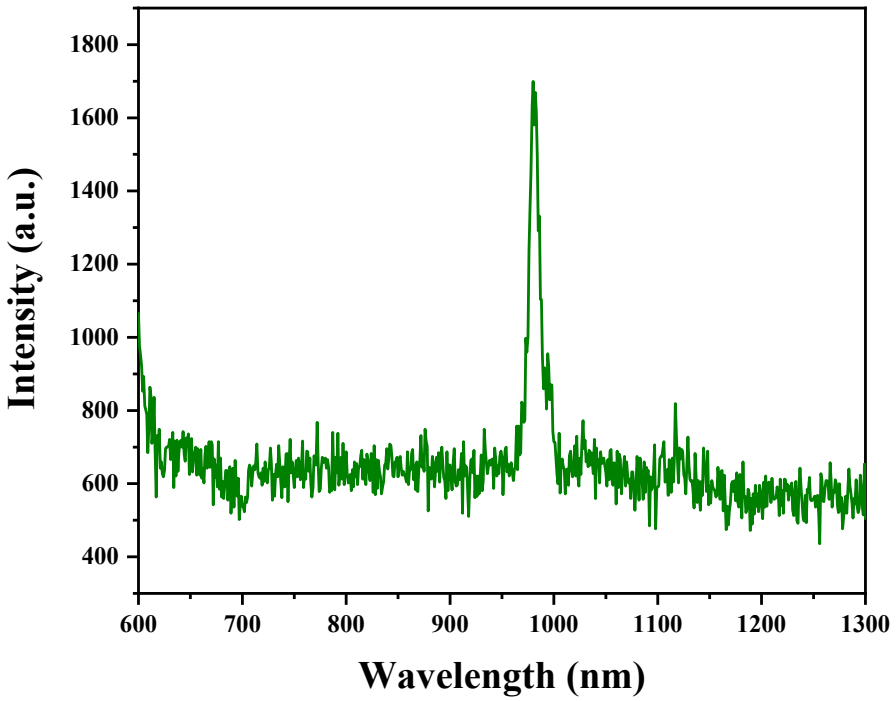


**Figure. S12** The fluorescence excitation spectra of oleic acid shedding UCNPs ($NaYF_4$, Yb/Er) dispersed in ultrapure water.

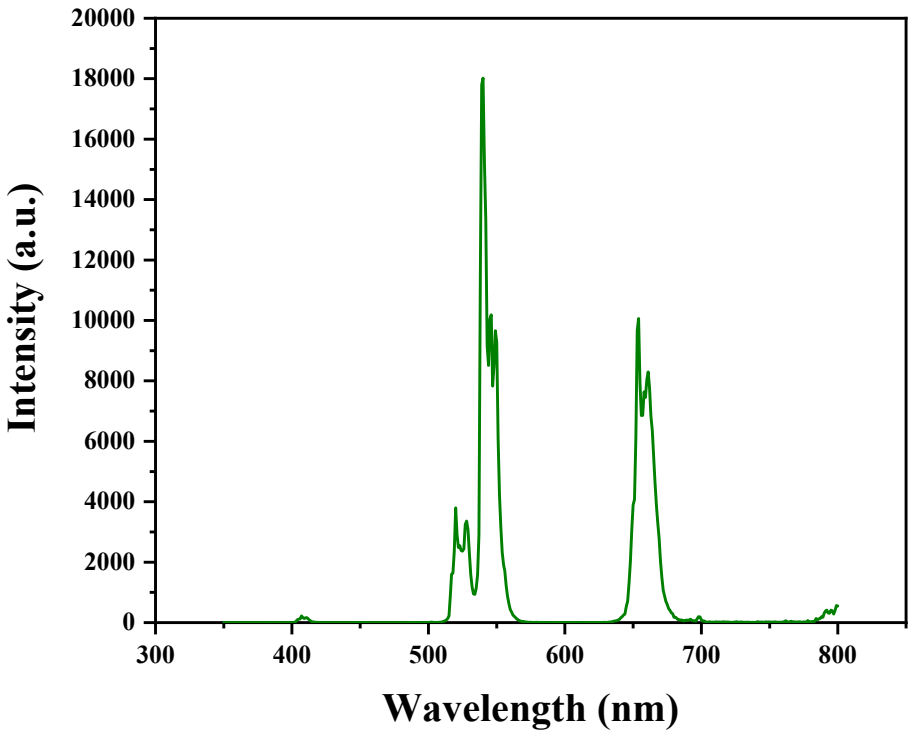


**Figure. S13** The fluorescence emission spectra of oleic acid capped UCNPs ($NaYF_4$, Yb/Er) dispersed in cyclohexane.

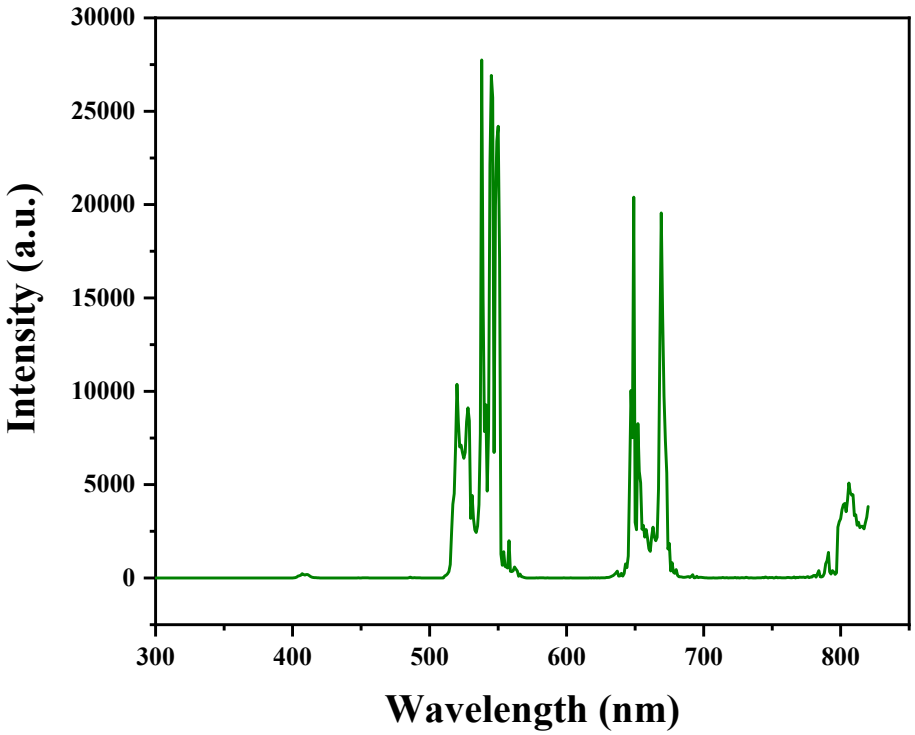


**Figure. S14** The fluorescence emission spectra of oleic acid shedding UCNPs ($NaYF_4$, Yb/Er) dispersed in ultrapure water.

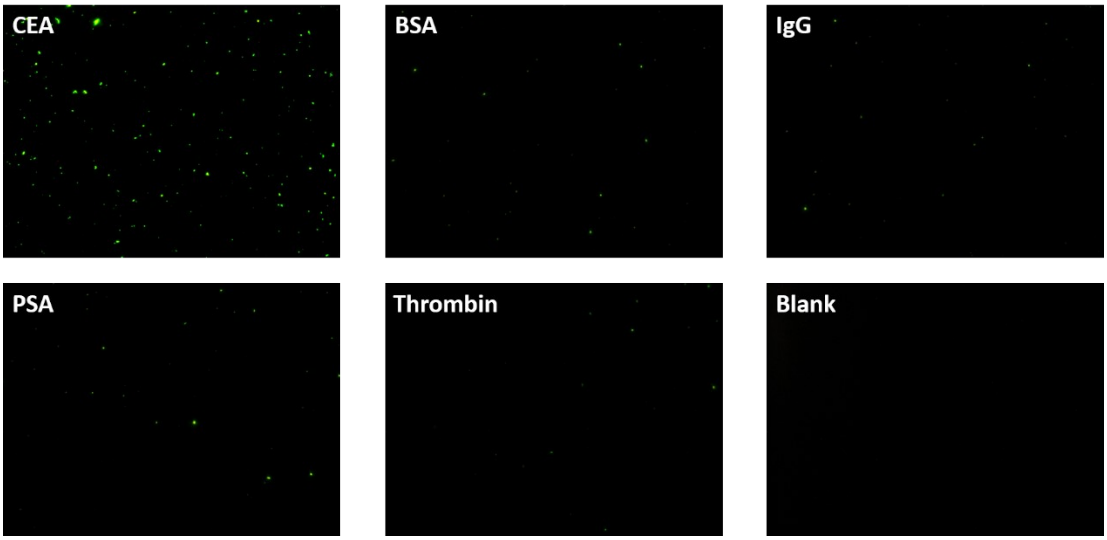


**Figure S15.** Representative upconversion microscopy images of cDNA-UCNPs on the coverslip surface in the presence of different proteins.

**Table S1** DNA sequences in this experiment

| Name | Sequences (5’-3’) |
|---|---|
| M-1 | Biotin-AAAAAAAAAAATACCAGCTTATTCAATT |
| U-1 | CCCCCCCCCCAAAAAAAAAAAAAAATTGAA |
| U-2 | CCCCCCCCCCAAAAAAAAAAAAAAATTGA |
| U-3 | CCCCCCCCCCAAAAAAAAAAAAAAATTG |
| U-4 | CCCCCCCCCCAAAAAAAAAAAAAAATT |