Supporting Information

Upconversion fluorescent and X-ray-sensitive bifunctional nanoprobes for assessing the penetrability of inorganic nanoparticles to the digestive system

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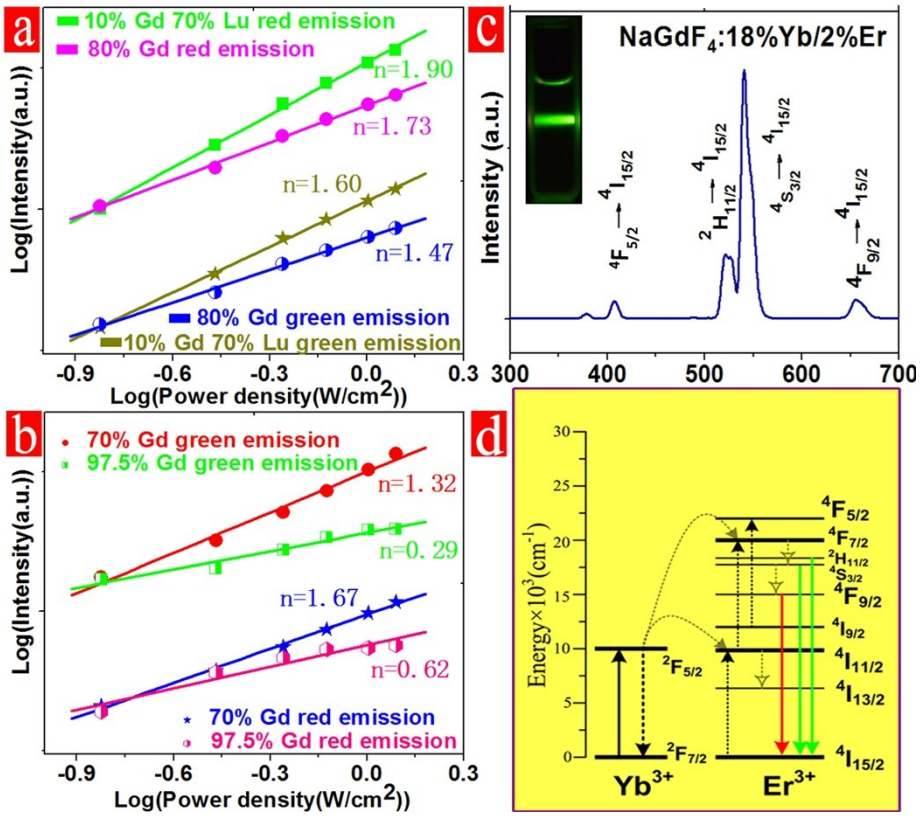

Figure S1. Comparison of the excitation power dependent upconversion emission intensity of (a) $NaGdF_4$:18%Yb^{3+}/2%Er^{3+} and $NaLuF_4$:10%Gd^{3+}/18%Yb^{3+}/2%Er^{3+} nanoparticles; (b) the $NaGdF_4$ doped with 28%Yb^{3+}/2%Er^{3+} and 2%Yb^{3+}/0.5%Er^{3+} nanoparticles. (c) UC luminescence spectrum of $NaGdF_4$:18%Yb^{3+}/2%Er^{3+}nanoparticles under different 980 nm excitation powers, the inset is corresponding luminescence photograph. (d) Schematic energy level diagram of upconversion excitation and emission processes.

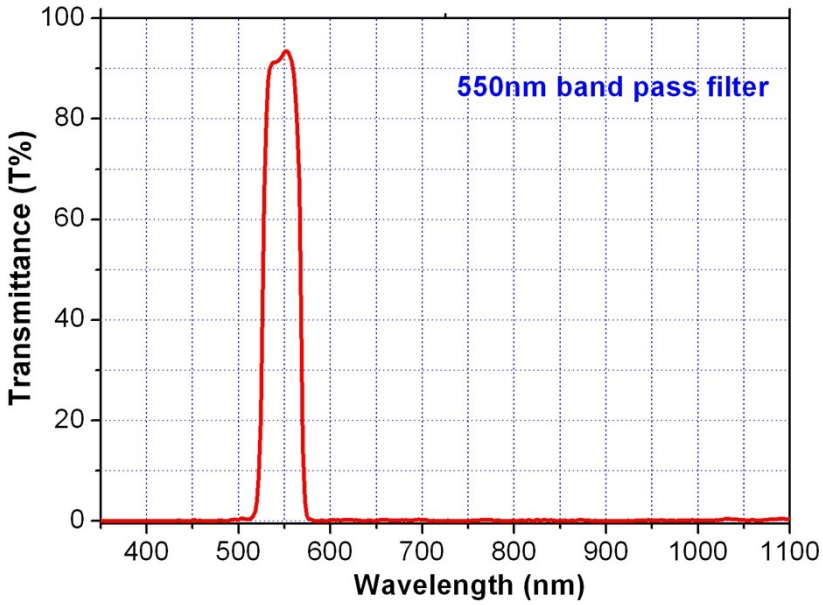

Figure S2. The transmittance spectra of the 550 nm band pass filter.

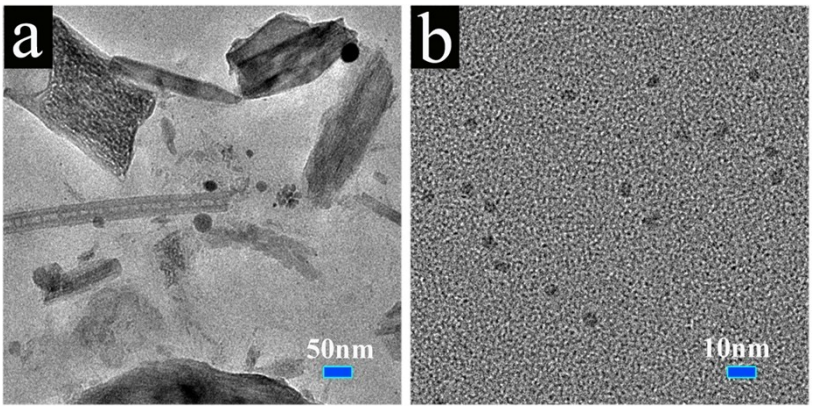

Figure S3. TEM imaging of (b) the mixture of the blood and UCNPs-1 and the blood from the mouse after oral administration of UCNPs-1 for 3 hours (a).

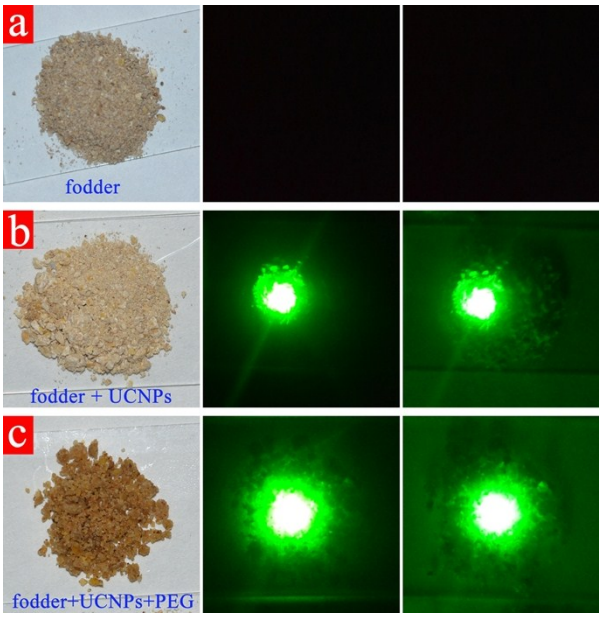

Figure S4. Upconversion luminescence photographs of the mixture excited by a 980 nm NIR laser.

(a) the pure fodder; (b) fodder and UCNPs; (c) fodder, PEG and UCNPs.

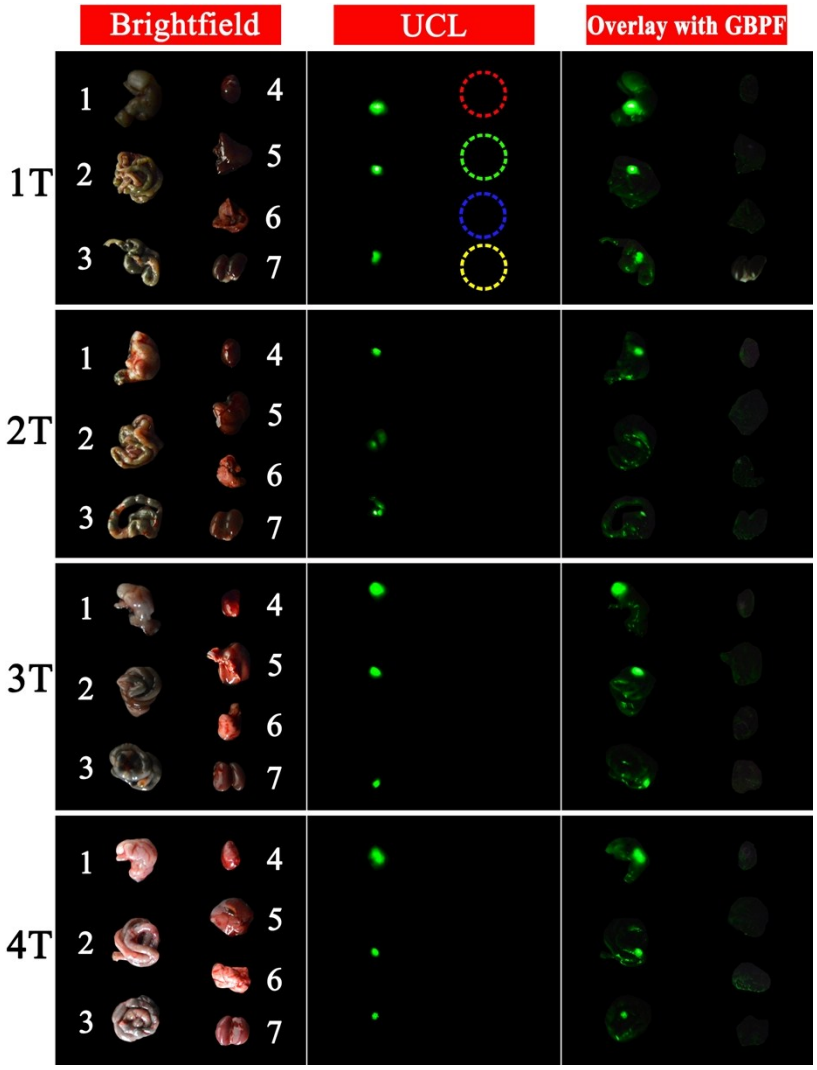

Figure S5. Middle column: Real-time ex vivo upconversion luminescence imaging of the mouse after fed with a mixture of fodder and UCNPs-2 for different times (from 1 to 4 times). The feeding period is once a day. Dose of UCNPs-2 equals to 10.0 mg/20g, that of fodder equals to 0.9 g/20g. **Left column:** conventional optical bioimaging in brightfield of organs. **Right column:** the overlay of the left and middle columns with Green Band Pass Filter (GBPF). 1: stomach; 2: small intestine; 3: large intestine; 4: heart; 5: liver; 6: lung; 7: kidney.

Supplementary Discussion

In Vivo detecation of Nile Red

Upconversion nanoparticles (UCNPs) can be excreted out almost completely from the digestion system of the mouse in several days[S1,S2]. Moreover, no UCNPs can penetrate the digestive tract into bloodstream via epidermal cells of the digestion system, and further accumulated in organs to cause damage to health of the beings according to the previous research. Therefore, the UCNPs with no residue and harmlessness show a huge application prospects in detection of chemicals in vivo quantitatively[S3].

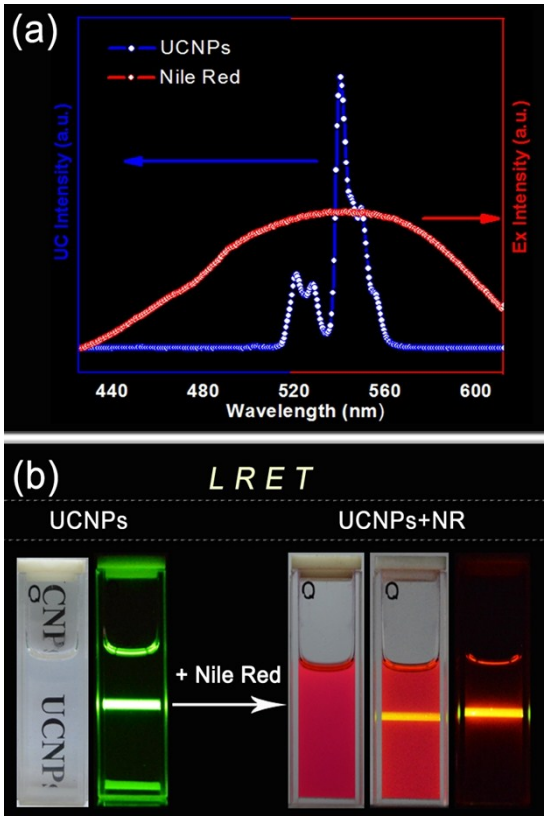

Figure S6. (**a**) The UC fluorescence spectrum of $NaGdF_4$:18%Yb^{3+},2%Er^{3+} nanocrystals (λ_{ex}=980 nm) and excitation spectrum of Nile red (λ_{em}=620 nm). (**b**) The corresponding bright-field and upconversion luminescence photos of PEG-UCNPs dispersed in cyclohexane in the absence and presence of nile red with an excitation wavelength λ= 980 nm.

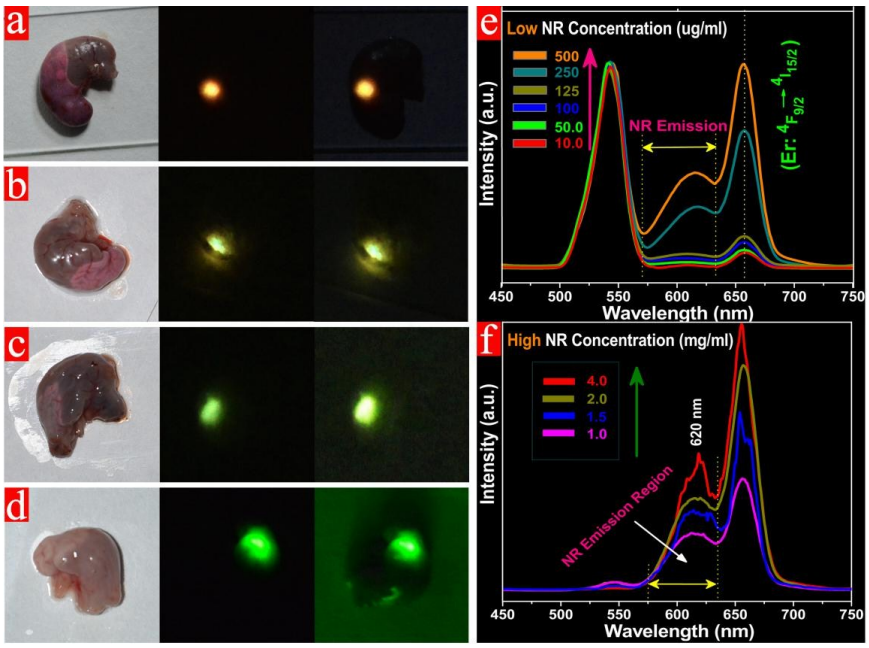

Figure S7. Middle column: *ex vivo* upconversion luminescence imaging of stomach from the mouse after fed with Nile Red for (a) 3 hours; (b) 6 hours; (c) 12 hours; (d) 24 hours using $NaGdF_4$:18%Yb^{3+},2%Er^{3+} nanocrystals as probes. Concentration of $NaGdF_4$ nanocrystals equals to 10.0 mg/ml, that of NR equals to 2.0 mg/ml. **Left column:** conventional optical bioimaging in bright-field of stomach. **Right column:** the overlay of the left and middle columns. (e,f) Evolution of the fluorescence spectra of UCNPs@NR cyclohexane solution with different concentration of Nile Red (from 10.0 to 4,000 μg/ml) under the excitation of 980 nm laser.

Here, we show that upconversion fluorescent nanoprobes is very efficient for in vivo detecting nile red (NR) in the mouse, based on the luminescent resonance energy transfer (LRET) process from UCNPs to nile red[S4]. Figure S6a showed that there is a perfect overlap bewteen the emission spectrum of $NaGdF_4$:Yb/Er and the excitation spectrum of NR, so that a UCNPs@NR detection system based on LRET can be successfully constructed by combining the UCNPs (donors) with NR (acceptor). It is clear in Figure S6b that the emission color varied from green to yellow after adding NR into UCNPs solution, this indicates the occurrence of the efficient LRET process. In

order to detect the nile red in vivo, the mice were fed with the same mixture of fodders, UCNPs, and nile red. At different incubation time point, the mouse was anesthetized, dissected, and then the stomach was obtained from the body of the mouse, imaged ex vivo by UCL imaging system. In vivo detection will be achieved truly without dissection process if fiber was placed in the body.

The fluorescence imaging of stomach with UCNPs@NR are depicted in middle column of Fig. S7a-d, where incubation time is increased from 3 to 24 hours. It is clearly observed that the stomach exhibited bright yellow color emission at the 3 h post-ingestion. Increasing the incubation time from to 6 to 24 hours discontinuously, the obvious emission color in stomach gradually varied from yellow to blue color without attenuation of comprehensive luminescence intensity. The results indicated that the nile red can be digested or excreted out in about 24 hours and can also be detected qualitatively by the emission color from the UCL images.

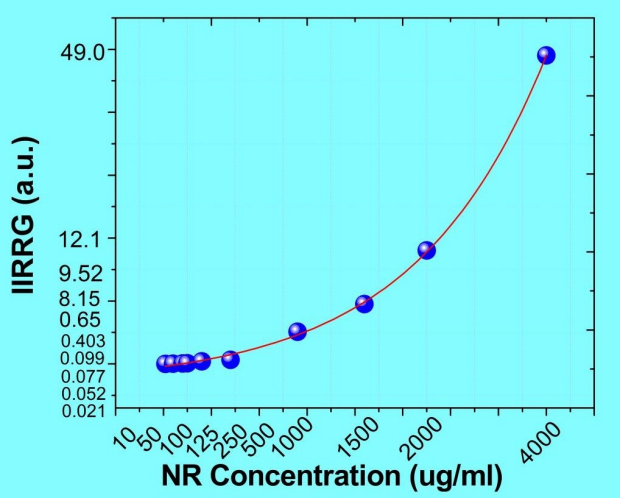

Figure S8. The curves of Integral Intensity Ratio of Red to Green (IIRRG) VS concentration of NR.

Using the corresponding UCL spectra of UCNPs@NR system, the nile red can be detected quantificationally (Figure S7e,f and Figure S8). It can be seen from Figure S7e,f that the red emission center at 620 nm increases relative to the green emission centered at 540 nm when

increasing the concentration of nile red, the corresponding integral intensity ratio of red to green emission (IIRRG) was shown in Figure S8, and that these data were fitted in a function of IIRRG vs the conventration of NR as following:

$$N_{IIRRG} = 2.84 \cdot e^{\frac{-C_{NR}}{137.3}} - 3.25$$

The concentration of nile red can be easily addressed according to IIRGB signal value, e.g., the UCNPs@NR system with 10.0 μg/ml has a IIRGB value of 0.021. The wide range of IIRGB values is beneficial to the quick and precise detection of sodium fluorescein concentration.

Supplementary References

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