

## Supporting information

### Time-domain stepwise encoding based on the stepped photon emission material

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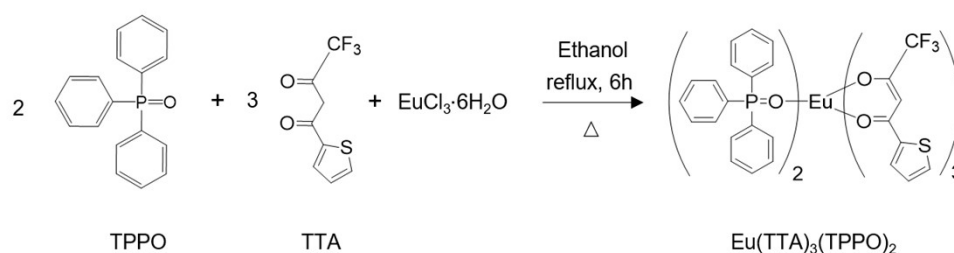
## 1. Experimental section

### 1.1. Materials and characterizations

The sensitizer PdOEP and ligands TPPO & TTA were purchased from J&K Scientific Ltd. (China). All reagents and solvents were commercially available and used without further purification. The images of nanoparticles were recorded on a transmission electron microscopy (Hitachi, HT7700 Exalens, Japan). Hydrodynamic particle size analysis was conducted on a dynamic light scattering detection system (Malvern, Nano-ZS90, England). Absorption spectra were measured with an UV-Vis spectrophotometer (Shimadzu, UV-2600, Japan). The luminescence spectra were measured on a fluorescence spectrometer (Edinburgh, FS-5, England) and a photonic multichannel analyzer (Hamamatsu, PMA-12 C14631, Japan). The particle number was recorded on a flow Nano analyzer (NanoFCM, NFCM, China). The  $\beta$ -HCG related samples and antibodies for *in vitro* whole-blood detection were provided by Shanghai Taywell Biotechnology Co., Ltd. (China).

### 1.2. Synthesis of the Eu-complex

The lanthanide emitter  $\text{Eu}(\text{TTA})_3(\text{TPPO})_2$  was synthesized according to a procedure as shown in Scheme S1. To a solution of TPPO ligand (190 mg) and TTA ligand (220 mg) in 10 mL of ethanol was added  $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$  (120 mg, in 1.25 mL water). The mixture with pH adjusted to 5~6 was stirred and heated in an oil bath at reflux for 8 hours. After cooling to the ambient temperature, the crude precipitate was washed three times with water and ethanol. The residue was dried, and ~400 mg of Eu-complex was finally obtained.

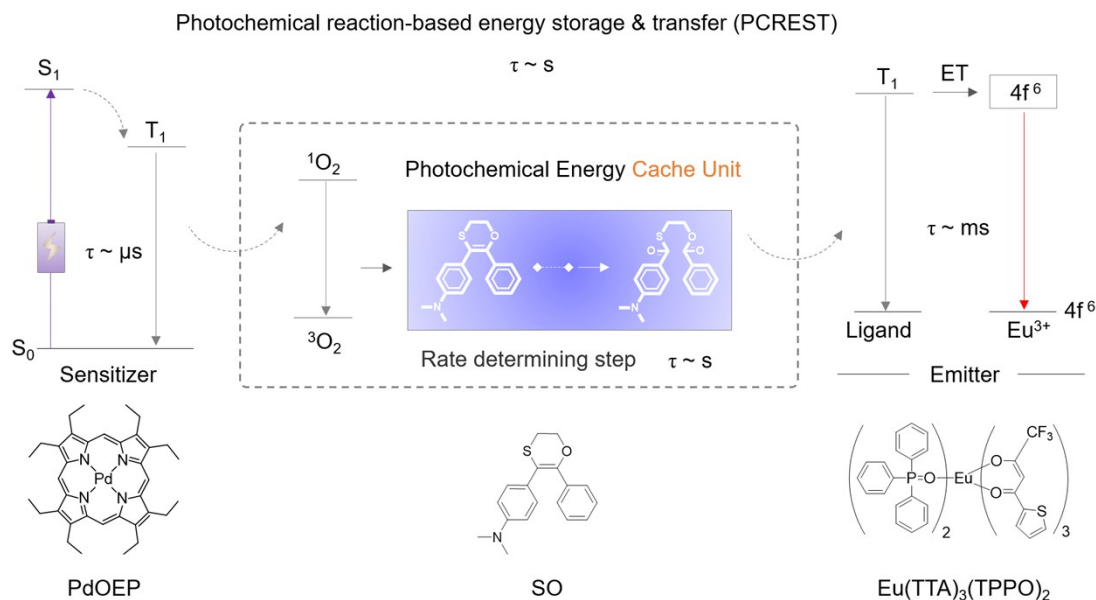


**Scheme S1** The synthesis procedure of Eu-complex.

### 1.3. Preparation of stepped photon emission nanomaterials

The nanoscale stepped photon emission materials were prepared according to a common wet-chemical procedure. In detail, the nanomaterials with Eu-complex or BODIPY as emitter were both constructed from a bottom-up synthetic method (Fig. S2a and Fig. S7a). Carboxylic crosslinked polystyrene nanoparticles were served as nanocarriers, where the carboxyls could provide hydrophilic groups and conjugation sites for promising bio-applications. The hydrophobic function molecules (sensitizer & photochemical energy cache unit & emitter, Fig. S2b and Fig. S7b) were encapsulated in the nanocarriers to achieve a homogeneous pink aqueous solution (Fig. 2a and Fig. S8a). The pink color was mainly contributed to the sensitizer PdOEP. In the wet-chemical synthetic method, tetrahydrofuran (THF) was used as the swelling agent. The mixtures were stirred in darkness for 4 h, and then the particles were washed with water and ethanol. Finally, the nanoparticles were dispersed in water for further use.

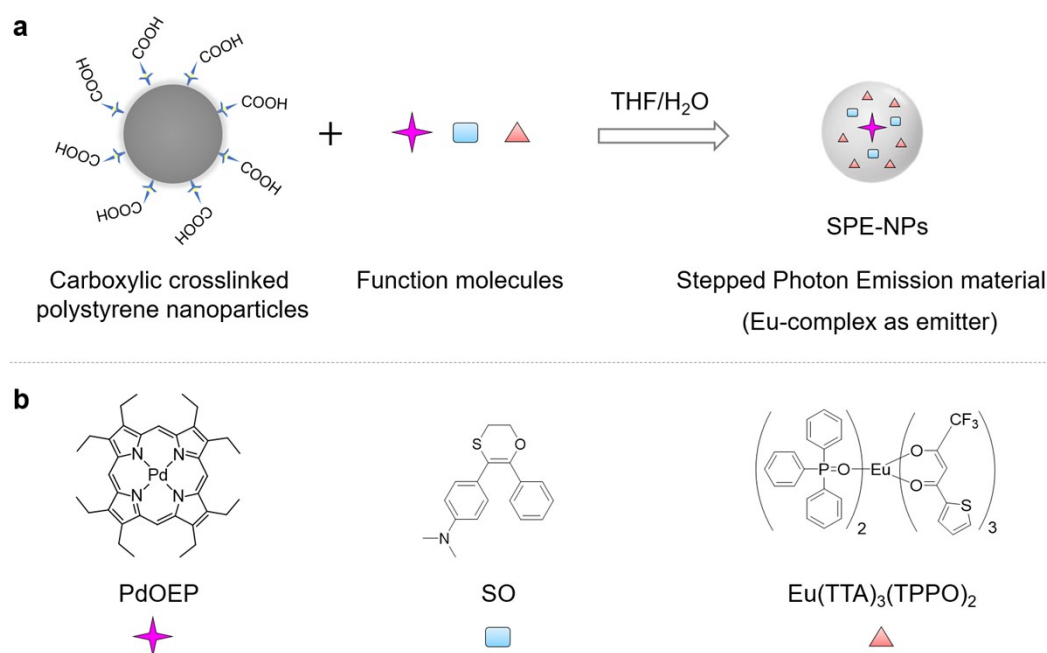
## 2. Supplementary figures



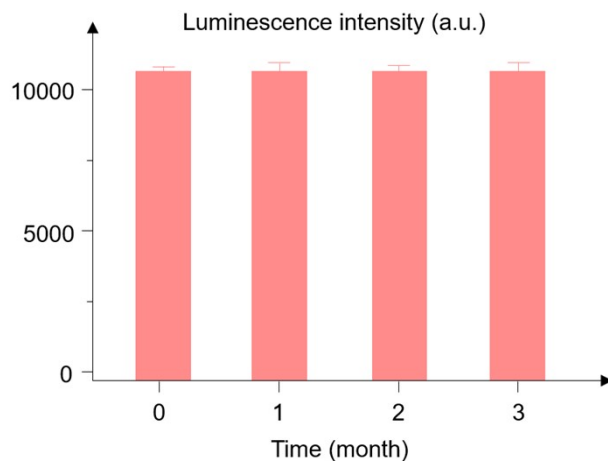
**Fig. S1** The photochemical reaction-based energy storage & transfer (PCREST) approach to achieve indirect excitation of the emitter with an ultra-long luminescence lifetime of several seconds.

Photochemical reaction-based energy storage & transfer (PCREST) leads to afterglow

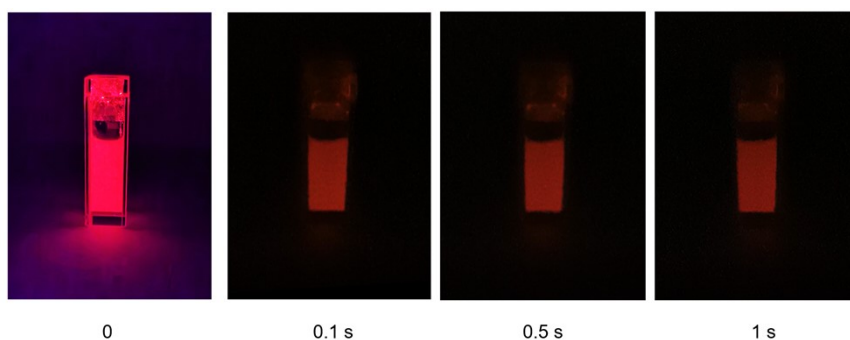
luminescence. The afterglow is correlated with a series of photochemical reactions, including the photosensitized generation of  $^1\text{O}_2$ , the cycloaddition of  $^1\text{O}_2$  to alkene and decomposition of the generated dioxetane. Four energy conversion pathways are involved in the process, namely photoexcitation and intersystem crossing to generate excited states, excitation energy transfer to generate reactive singlet oxygen, reaction-based energy cache to generate excited states, and excitation energy transfer to generate excited states for the finally output, respectively.



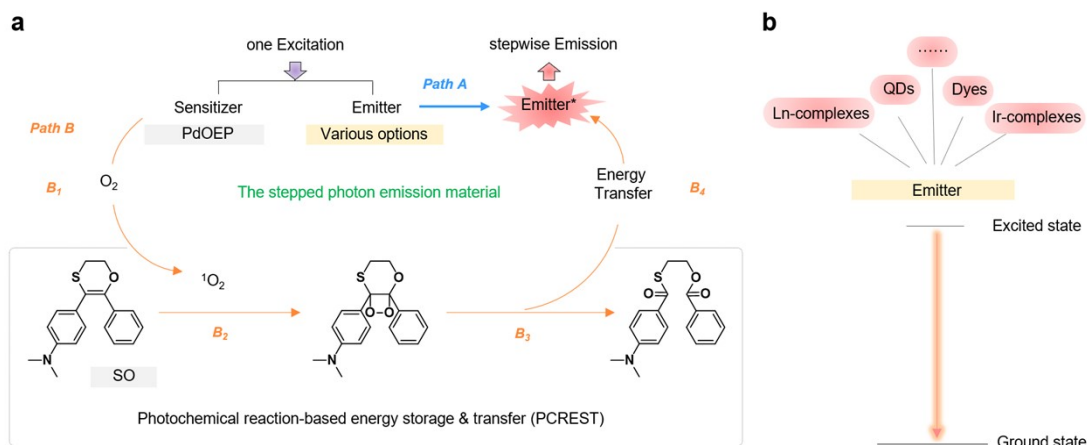
**Fig. S2** The bottom-up synthetic method to construct stepped photon emission nanoparticles (SPE-NPs) with  $\text{Eu}(\text{TTA})_3(\text{TPPO})_2$  as the emitter. This method was a wet-chemical procedure where tetrahydrofuran (THF) was used as the swelling agent.



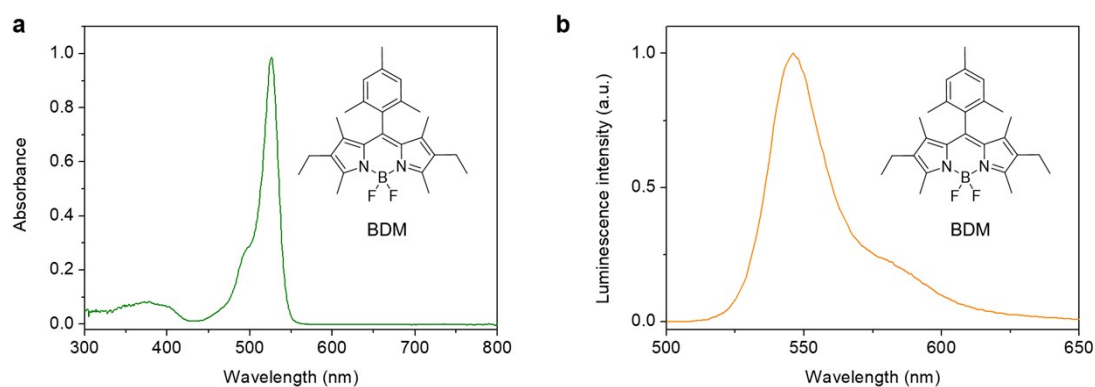
**Fig. S3** Long-term optical variations of stepped photon emission nanoparticles (SPE-NPs) in terms of the luminescence intensities. The nanoparticles were constructed with  $\text{Eu}(\text{TTA})_3(\text{TPPO})_2$  as the emitter and were dispersed in water.



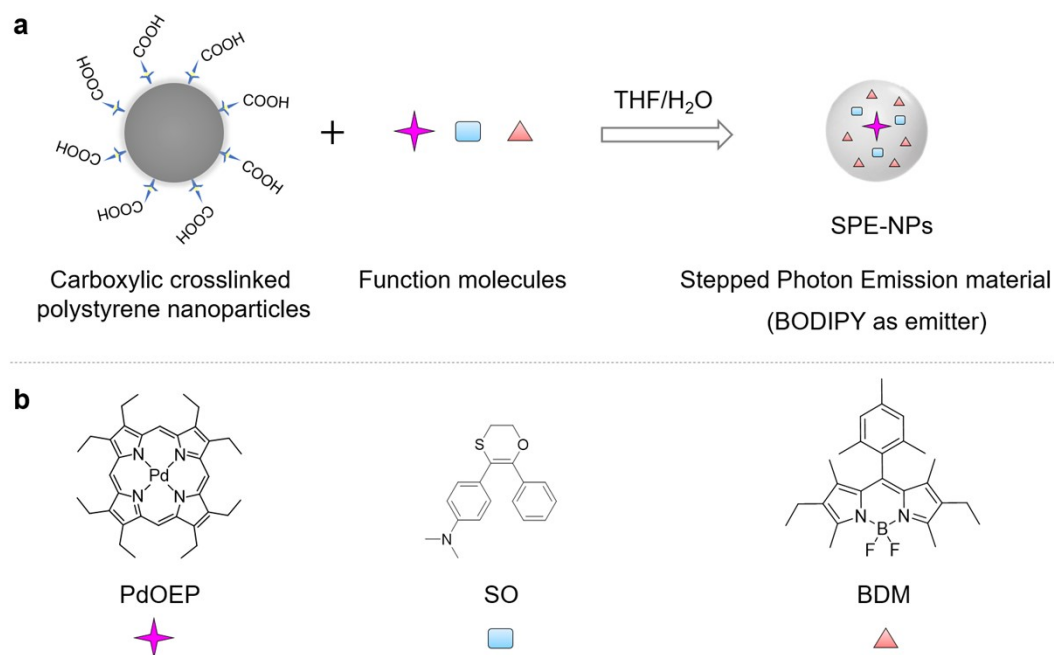
**Fig. S4** Photos of the stepped photon emission nanoparticles in water upon and after excitation of an UV LED. The emission in domain<sub>1</sub> (the first picture, 0 s, fluorescence scope) was much brighter than the emission in domain<sub>2</sub> (0.1 s-1s, afterglow scope). After a fast intensity drop upon the switching from domain<sub>1</sub> to domain<sub>2</sub>, the following intensity decay was not obvious in the time scale of sub-second (The afterglow emission could persist for tens of seconds. Therefore, intensity decay was not obvious in the very finite time scale of sub-second).



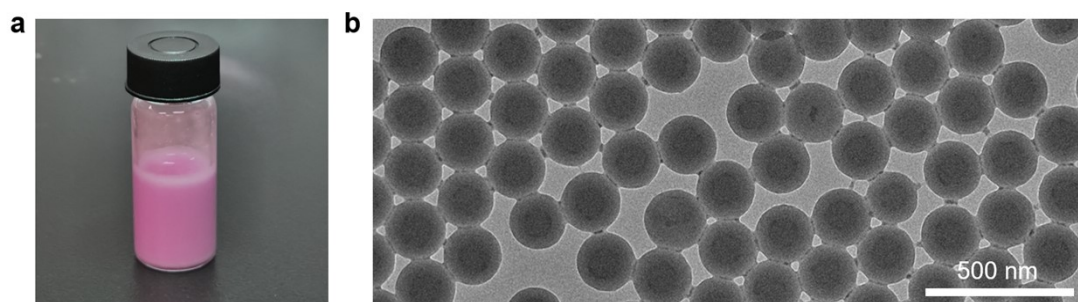
**Fig. S5** Schematic illustration of the stepped photon emission material and its stepwise emission. (a) Illustration of the pathways contributed to stepwise emission. The material was a nanoparticulate system involving three function molecules, where various options could be selected and served as the emitter, PdOEP was a sensitizer and SO was a photochemical energy cache unit. Path A was a direct excitation of the emitter, while path B was a photochemical reaction-based energy storage & transfer (PCREST) approach. (b) Illustration of the luminescent emitters suitable for constructing the stepped photon emission materials. The emitter could be selected from common luminescence materials, such as the lanthanide-complexes, Ir-complexes, QDs and organic dyes.



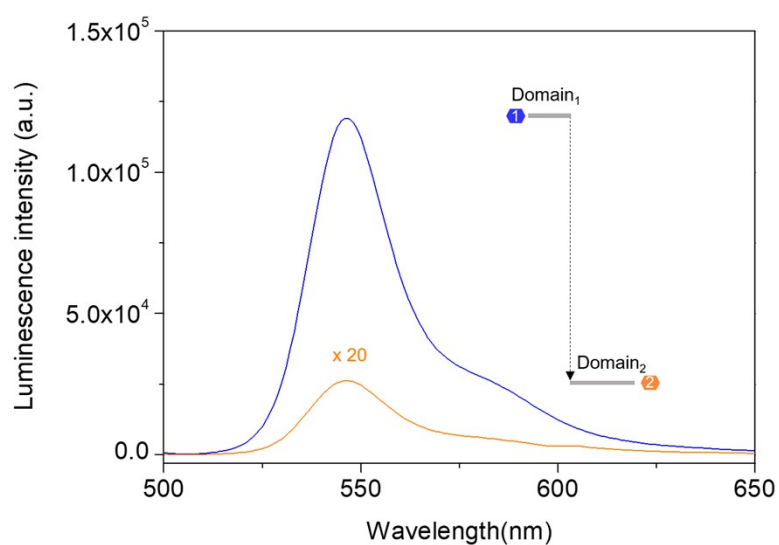
**Fig. S6** (a) The UV-vis spectrum of BDM. (b) The luminescence spectrum of BDM.



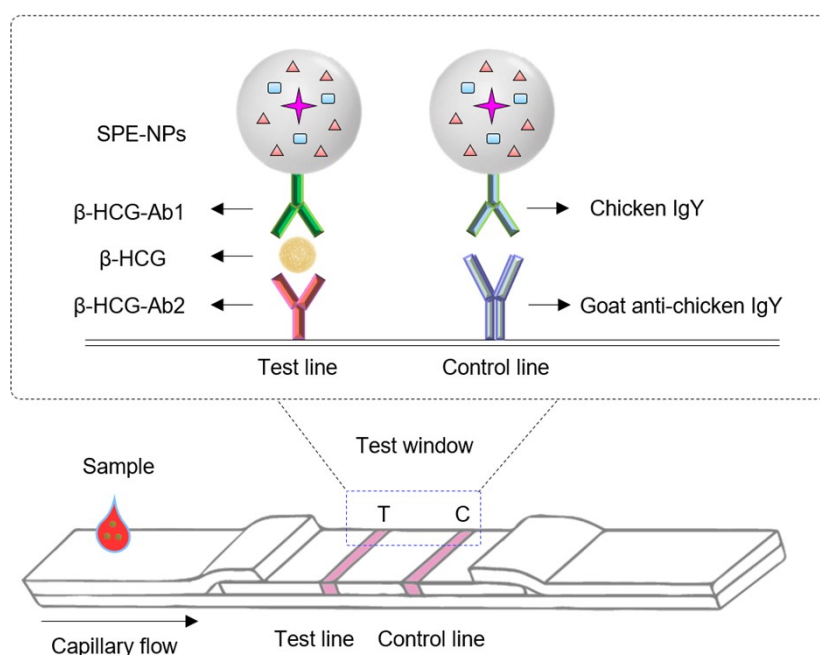
**Fig. S7** The bottom-up synthetic method to construct stepped photon emission nanoparticles (SPE-NPs) with a BODIPY dye (BDM) as the emitter.



**Fig. S8** (a) Aqueous solution of the stepped photon emission nanoparticles with BDM as the emitter. (b) Transmission electron microscope image of the nanoparticles.

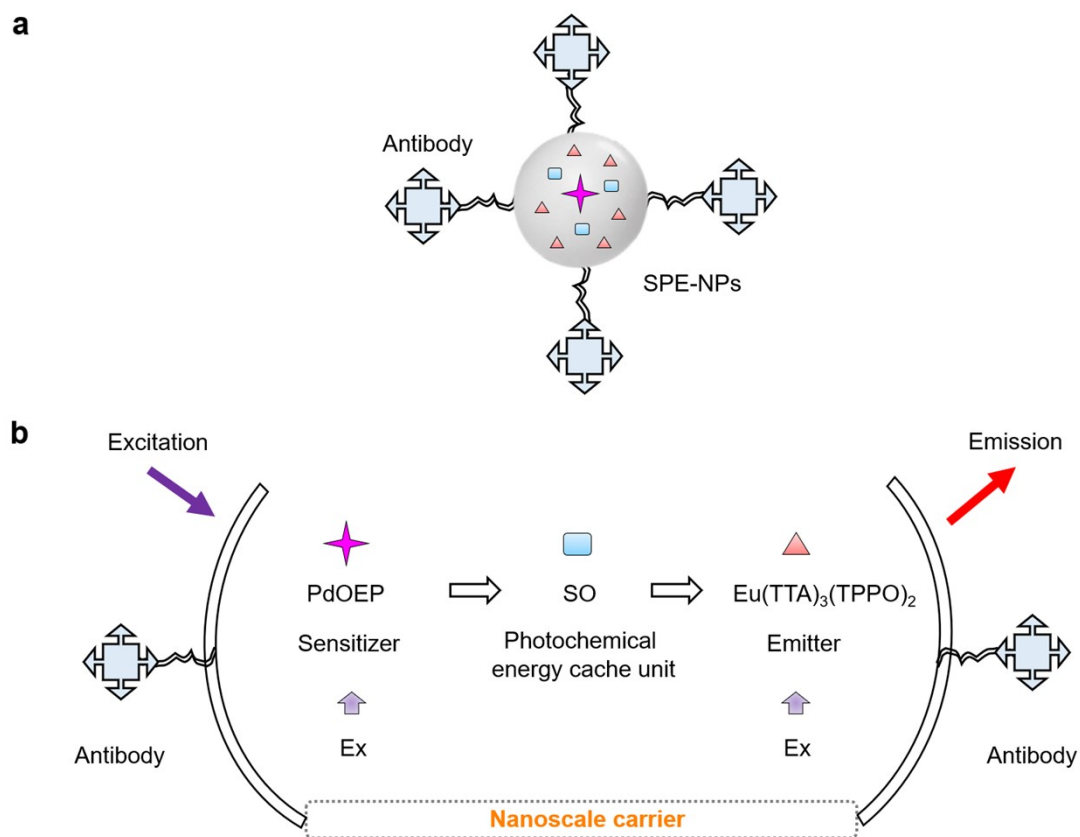


**Fig. S9** Emission spectra of the stepped photon emission nanoparticles with BDM as the emitter in different time domains. The samples in water were excited with an UV LED. The steady state was selected as one of the time domains (Domain<sub>1</sub>, blue line), and 0.1 s after the cessation of light source was selected as the other time domain (Domain<sub>2</sub>, orange line).

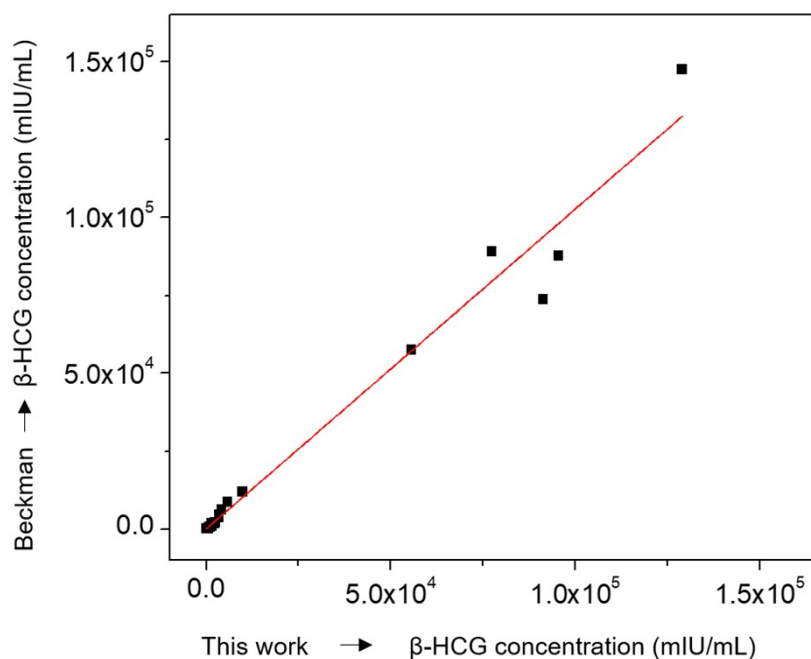


**Fig. S10** Schematic illustration of the lateral flow immunoassay for  $\beta$ -HCG detection.





**Fig. S11** Schematic illustration of the reporters based on stepped photon emission nanomaterials (SPE-NPs). For the  $\beta$ -HCG immunoassay, the antibody referred to  $\beta$ -HCG-Ab1 as illustrated in Fig. S9.



**Fig. S12** Performance of the  $\beta$ -HCG detection in a series of clinical samples. The  $\beta$ -HCG concentrations in all the samples were calibrated with chemiluminescence immunoassay method (UniCel DxI800, Beckman Coulter). And, the detection result in this work was compared with that of Beckman's method. A high correlation coefficient of 0.9885 was achieved from these two detection methods.