Supporting Information

A Proof-of-Concept Application of Water-Soluble Ytterbium(III) Molecular Probes for *In Vivo* NIR-II Whole Body Bioimaging

Yingying Ning,[‡,a] Si Chen,[‡,b,c] Hao Chen,[b] Jing-Xiang Wang,[a] Shuqing He,[b] Yi-Wei Liu,[a] Zhen Cheng*[,b] and Jun-Long Zhang*[,a]

[a]Beijing National Laboratory for Molecular Sciences, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, P. R. China.

[b]Molecular Imaging Program at Stanford (MIPS), Bio-X Program, and Department of Radiology, Canary Center at Stanford for Cancer Early Detection, Stanford University, California 94305-5344, USA

[c]Department of Neurology, Xiangya Hospital, Central South University, Xiangya Road 88, Changsha, Hunan 410008, P. R. China

[‡]These authors contributed equally

E-mail: zhangjunlong@pku.edu.cn, zcheng@stanford.edu

Supporting figures

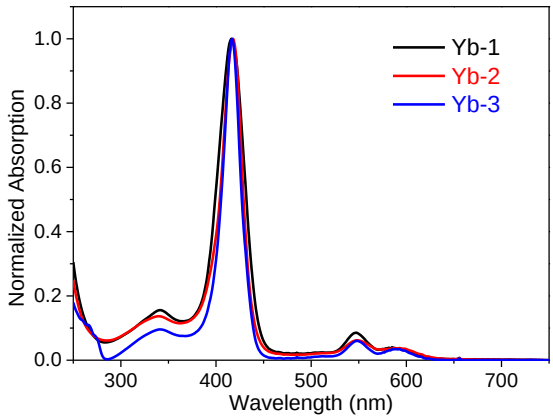

Fig. S1 Normalized absorption spectra of **Yb-1-3** in H_2O.

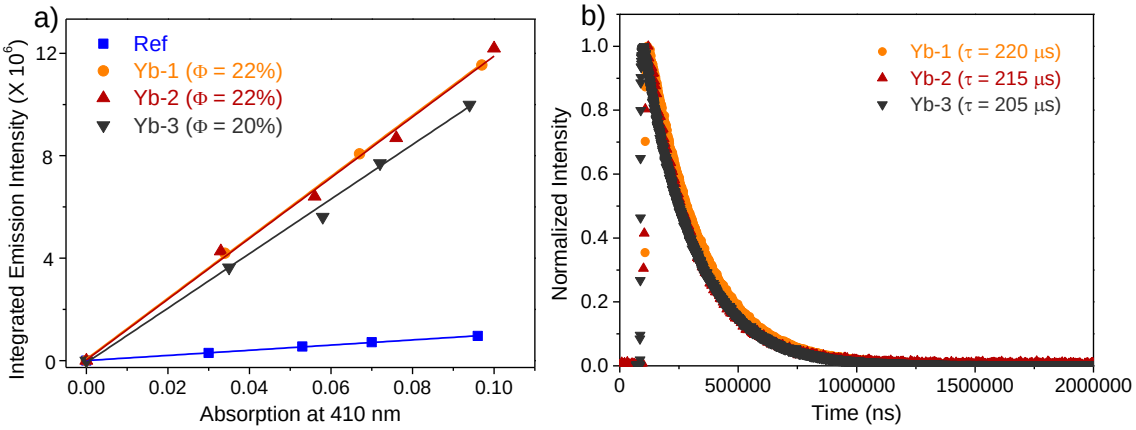

Fig. S2 Integrated emission intensity vs absorbance plots for relative quantum yield determination of **Yb-1-3** vs YbTPP(L_{OEt}) (λ_{ex} = 410 nm, CH_2Cl_2, Φ_r = 0.024) in DMSO at room temperature.

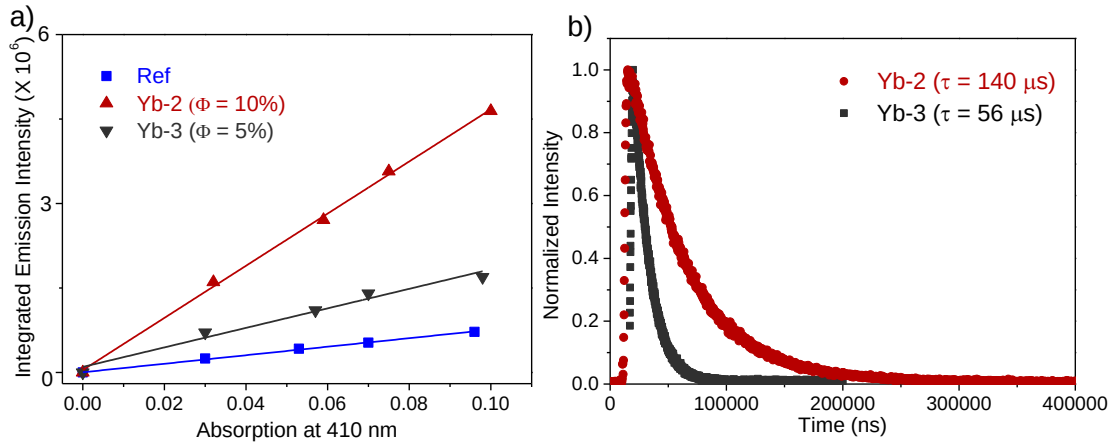

Fig. S3 Integrated emission intensity vs absorbance plots for relative quantum yield determination of **Yb-2** and **Yb-3** vs YbTPP(L_{OEt}) (λ_{ex} = 410 nm, CH_2Cl_2, Φ_r = 0.024) in water at room temperature.

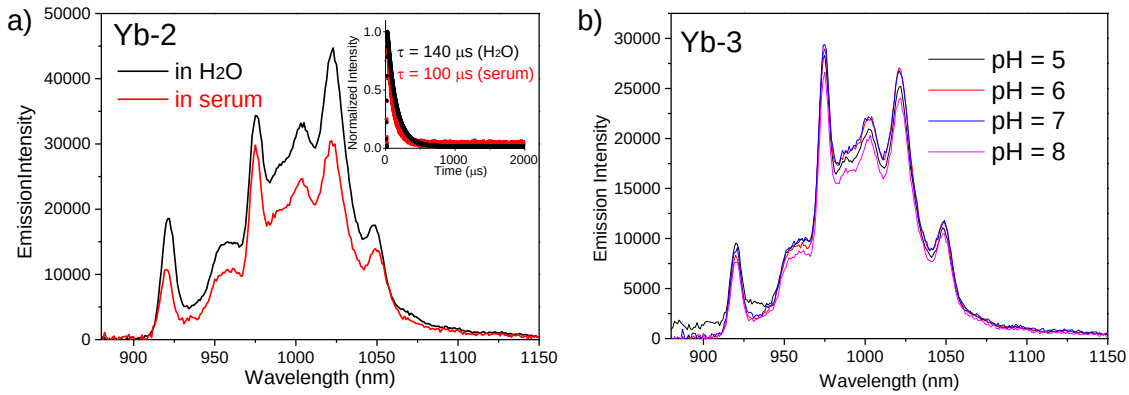

Fig. S4 a) NIR emission intensity and lifetime (inset) comparison of **Yb-2** in H_2O and serum (λ_{ex} = 410 nm, $A_{410\ nm}$ = 0.1); b) NIR emission of **Yb-3** in different pH PBS buffer (λ_{ex} = 410 nm, $A_{410\ nm}$ = 0.1).

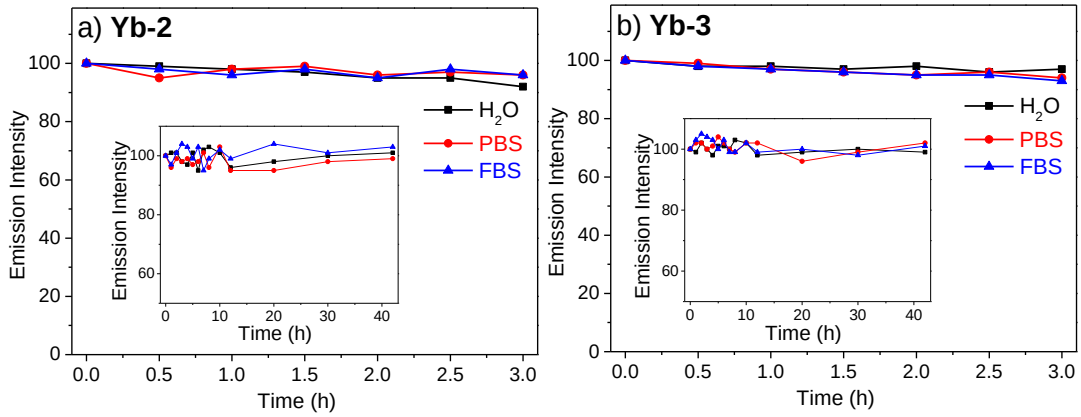

Fig. S5 Spectroscopic evaluation of a) **Yb-2** and b) **Yb-3** photostability in H_2O, phosphate buffer (PBS, pH 7.4) and fetal bovine serum (FBS) under irradiation of a 405 nm laser (0.3 Wcm^{-2}) (inset: dark stability).

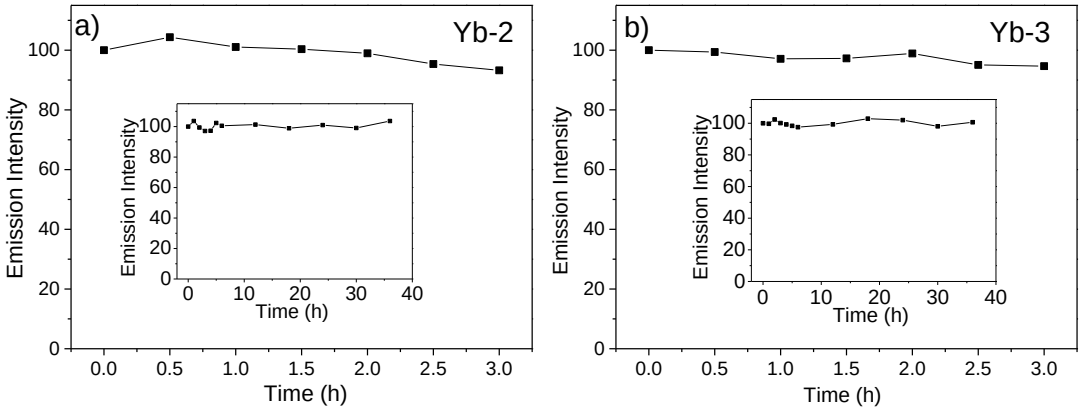

Fig. S6 Spectroscopic evaluation of a) **Yb-2** and b) **Yb-3** photostability in pH = 5 PBS buffer under irradiation of a 405 nm laser (0.3 Wcm^{-2}) (inset: dark stability).

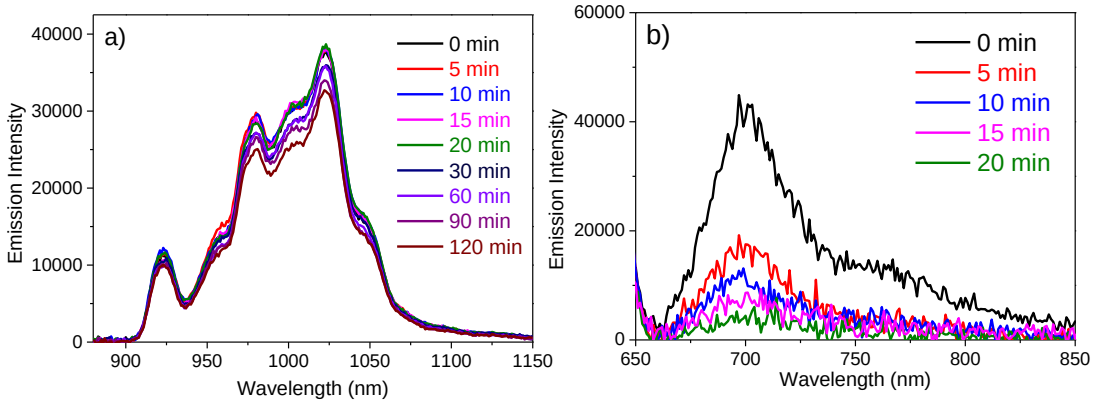

Fig. S7 Spectroscopic evaluation comparison the photostability between a) **Yb-2** and b) **Cy5.5** in water under irradiation of a 405 nm (**Yb-2**, $A_{405\ nm}$ = 0.1) or 635 nm (**Cy5.5**, $A_{635\ nm}$ = 0.1) laser (0.3 Wcm^{-2}).

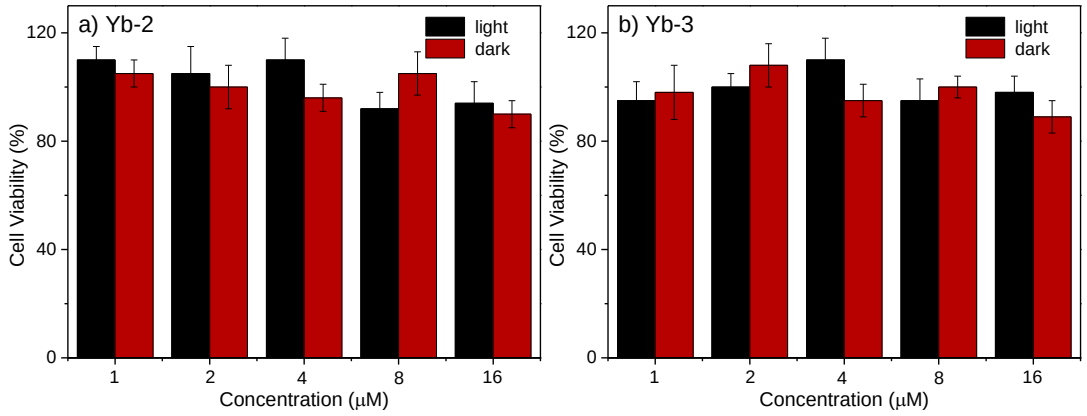

Fig. S8 Darkcytotoxicity and photocytotoxicity of a) **Yb-2** and b) **Yb-3** toward Hela cells using CCK-8 assay.

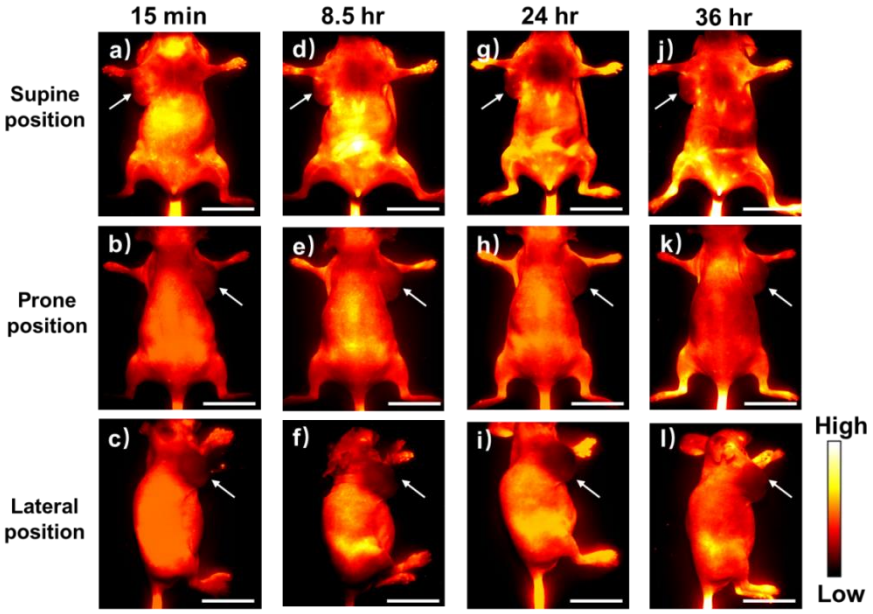

Fig. S9 *In vivo* fluorescence images of tumor captured on the 143B osteosarcoma tumor-bearing mouse (white arrow) in 15 min, 8.5 hr, 24 hr, 36 hr after **Yb-2** injection intravenously. ($\lambda_{ex,}$ 520 nm; λ_{em}, 1000 nm longpass; 3000 ms exposure; colour bar ranges from 5000 to 40000). Scale bar: 2 cm.

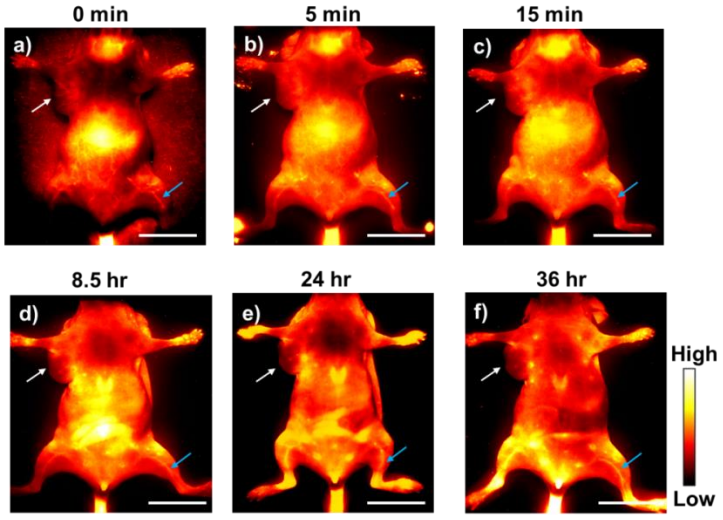

Fig. S10 *In vivo* fluorescence images of **Yb-2** obtained in corresponding time after injection through tail vein ($\lambda_{ex,}$ 520 nm; λ_{em}, 1000 nm longpass; 3000 ms exposure; colour bar ranges from 5000 to 40000). The femoral artery (blue arrow) and major artery supporting the tumor (white arrow) was observed in a), b), c), d), e), f), suggesting that the blood-circulation half-life of **Yb-2** was long. Scale bar: 2 cm.