# Supporting Information

**Ultra-Small NIR J-aggregates of BODIPY for Potent Phototheranostics**

Kunmei Liu [a], Hongxin Liu,[a] Chaonan Li,[b] Zhigang Xie [b,]* and Min Zheng [a,]*

[a] School of Chemistry and Life Science, Advanced Institute of Materials Science, Changchun University of Technology, 2055 Yanan Street, Changchun, Jilin 130012, P. R. China

[b] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 5625 Renmin Street, Changchun, Jilin 130022, P. R. China

*Corresponding author. E-mail: zhengm@ciac.ac.cn; xiez@ciac.ac.cn

## 1. Materials

Hoechst 33258 and Calcein-AM/PI Live/Dead Viability Assay Kit were purchased from Jiangsu KeyGEN BioTECH Co., Ltd. 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) (>98%) was bought from Shanghai yuanye Bio-Technology Co., Ltd. All reagents can be used without further purification.

## 2. Characterizations

Ultraviolet-visible (UV-vis) absorption spectra were recorded on Shimadzu UV-2450 PC UV-vis spectrophotometer. Fluorescence intensity tests were obtained by using a PerkinElmer LS-55 spectrofluorophotometer. The morphology of the nanoparticles was measured by transmission electron microscopy (TEM) performed on a JEOL JEM-1011 electron microscope operating at an acceleration voltage of 100 kV. To prepare the specimen for TEM, a drop of solution was deposited onto a copper grid with a carbon coating. The specimen was air-dried and measured at room temperature. MTT assays were measured at 490 nm by a microplate reader (BioTek, EXL808). Flow cytometry analysis was performed by a flow cytometer (Beckman, USA) which collected $1\times10^4$ gated events for each sample.

## 3. The theoretical calculation

Multiwfn software was used to obtain the theoretical spectrum information as follows:

\# 1 1.4981 eV 687.61 nm f= 1.19830 Spin multiplicity= 1:

H→L 99.5%

\#   2   2.0760 eV     457.23 nm     f= 0.89210     Spin multiplicity= 1:

H-1→L 98.3%

\#   3   2.5322 eV     349.63 nm     f= 0.10010     Spin multiplicity= 1:

H-2→L 98.0%

\#   4   2.7472 eV     311.31 nm     f= 1.50910     Spin multiplicity= 1:

H→L+1 95.3%

\#   5   3.1858 eV     249.18 nm     f= 0.00510     Spin multiplicity= 1:

H-4→L 43.4%, H→L+2 38.7%, H-5→L 13.3%

\#   6   3.2514 eV     241.33 nm     f= 0.00350     Spin multiplicity= 1:

H→L+2 55.2%, H-4→L 28.2%, H-5→L 5.7%

## 4. Drug loading capability (DLC)

$$\text{DLC (Wt\%)} = \frac{M_{(mass\ of\ BODIPY\ in\ BNPs)}}{M_{(mass\ of\ BNPs)}} \times 100\%$$

## 5. Photothermal conversion efficiency

The photothermal conversion efficiency ($\eta$) was calculated according to the equation as follows according to published methods.

$$\eta = \frac{hA\Delta T_{max} - Q_S}{I(1 - 10^{A\lambda})} \tag{1}$$

where is the heat transfer coefficient, $A$ is the surface area of the container, $\Delta Tmax$ is the maximum temperature change, I is the laser power, $A\lambda$ is the absorbance at 750 nm, $Qs$ is the heat associated with the light absorbance of the solvent.

In to get the h$A$, $\theta$ defined as the ratio of $\Delta T$ to $\Delta Tmax$ was introduced :

$$\theta = \dfrac{\Delta T}{\Delta T_{max}} \qquad (2)$$

Based on the total energy balance for this system

$$\sum_i m_i c_{p,i} \dfrac{dT}{dt} = Q_{NPs} + Q_s - Q_{loss} \qquad (3)$$

where $Q_{NPs}$ is the photothermal energy input by NPs, Qs is the heat associated with the light absorbance of the solvent, $Q_{loss}$ is thermal energy lost to the surroundings. At the maximum steady-state temperature, the heat input is equal to the heat output: $Q_{NPs}+Qs=Q_{loss}=hA\Delta T_{max}$. Substituting $\theta$ into Eq.3 and rearranging:

$$\dfrac{d\theta}{dt} = \dfrac{hA}{\sum_i m_i c_{p,i}} \dfrac{Q_{NPs} + Q_s}{\left(hA\Delta T_{max} - \theta\right)} \qquad (4)$$

When the laser was shut off, the $Q_{NPs}+Qs=0$, Eq.4 changed to:

$$dt = \dfrac{\sum_i m_i c_{p,i} d\theta}{hA\,\theta} \qquad (5)$$

Integrating Eq.5 gives the expression:

$$t = \dfrac{\sum_i m_i c_{p,i}}{hA}\theta \qquad (6)$$

Then can be determined by applying the linear time data from the cooling period versus $-Ln\theta$.

## 6. Cell line culture

HeLa cells were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China. All the cells were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% heat-inactivated fetal bovine serum (FBS, GIBCO), 100 U mL$^{-1}$ penicillin and 100 μg mL$^{-1}$ streptomycin (Sigma), and the culture medium was replaced once every day. All

the cells were cultured in a humidified incubator at 37 °C with 5% $CO_2$.

7. **Cell viability assays**

The cytotoxicities of BNPs with or without laser irradiation were examined via MTT protocol. Briefly, HeLa cells harvested in a logarithmic growth phase were seeded in 96-well plates at an initial density of $2×10^3$ cells per well and incubated in DMEM for 24 h. The medium was then replaced by various concentration of BNPs. After 4 h of incubation, the cells were illuminated by 730 nm laser (0.6 W cm$^{-2}$, 5 min). The incubation was continued for 24 h. Then, 20 μL of MTT solution was added and the plates were incubated for another 4 h at 37 °C, followed by the removal of the culture media containing MTT and addition of 150 μL of DMSO to each well to dissolve the formazan crystals formed. Finally, the plates were shaken for 5 min, and the absorbance of the formazan product was measured at 490 nm using a microplate reader. The control group without laser irradiation was set up identical to that experimental group except for the illumination.

8. **Calcein-AM/PI staining tests**

HeLa cells were stained with the calcein-AM/PI to identify dead and live cells. Shortly, HeLa cells were incubated with different concentrations of BNPs (0-11.6 μM) for 4 h, and then illuminated by 730 nm laser (0.6 W cm$^{-2}$, 5 min). After additional incubation for 24 h, the medium was removed and cells were washed gently. Then HeLa cells were incubated with Calcein-AM/PI for 30 min at room temperature. Subsequently imaged by a NikonC1si laser scanning confocal microscopy.

9. **Cell apoptosis and necrosis detection assays**

HeLa cells were cultured with BNPs (11.6 µM) for 4 h, and then illuminated by 730 nm laser (0.6 W cm$^{-2}$) for 5 min. After additional incubation for 24 h, cells were washed, harvested and collected, and stained with Annexin V-FITC and PI detection kit for about 30 min. Finally, the ratio analysis of apoptosis and necrosis were determined through flow cytometer.

## 10. Cellular Uptake Measured by CLSM

HeLa cells were seeded in 6-well plates with sterile cover slips at a density of $2 \times 10^5$ cells per well the day before the experiment. After adhesion overnight, the medium was replaced with BNPs or BODIPY and diluted with fresh culture medium to a final concentration of 9.2 µM. Thereafter, cells were incubated for additional 0.5, 1, 2 and 4 h, at 37 °C, respectively, CLSM was used to examine samples.
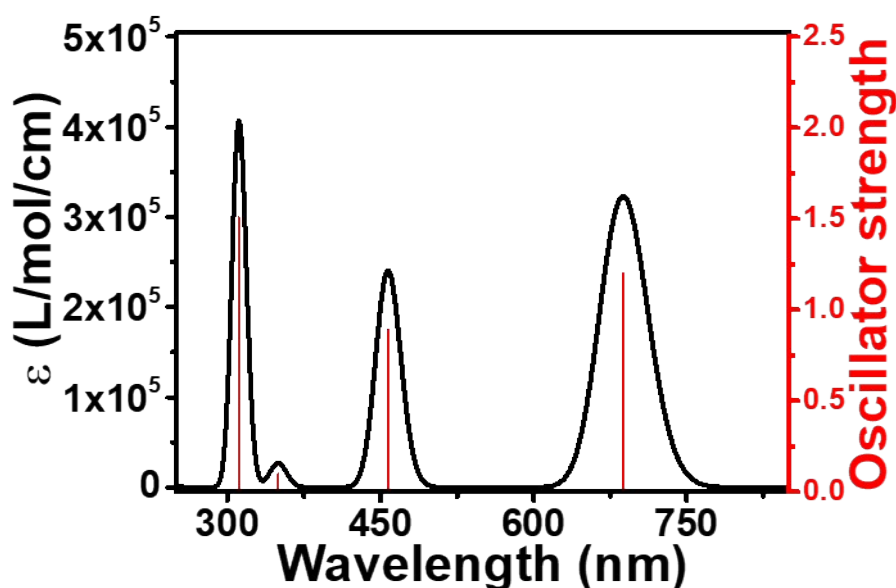


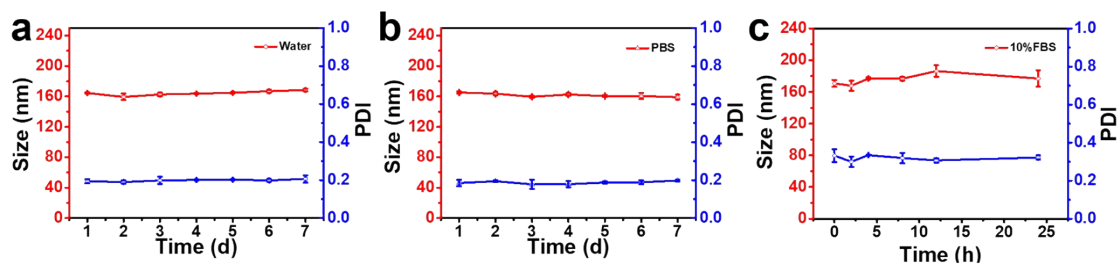**Figure S1.** Theoretical absorption spectrum of BODIPY.
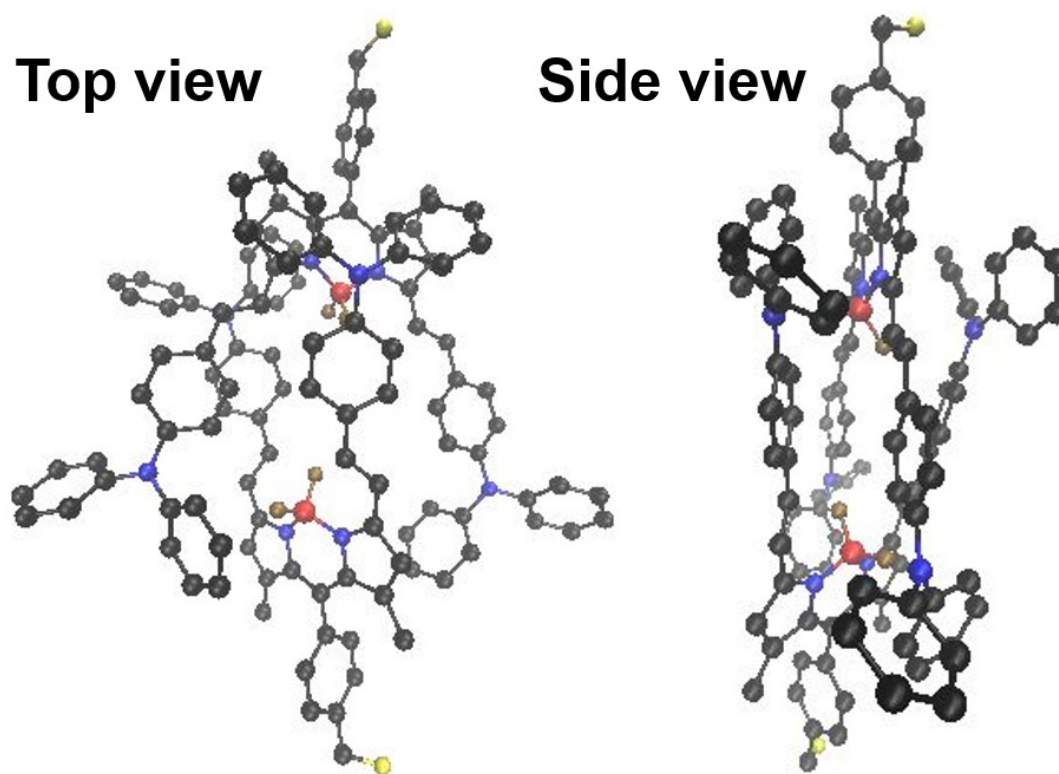
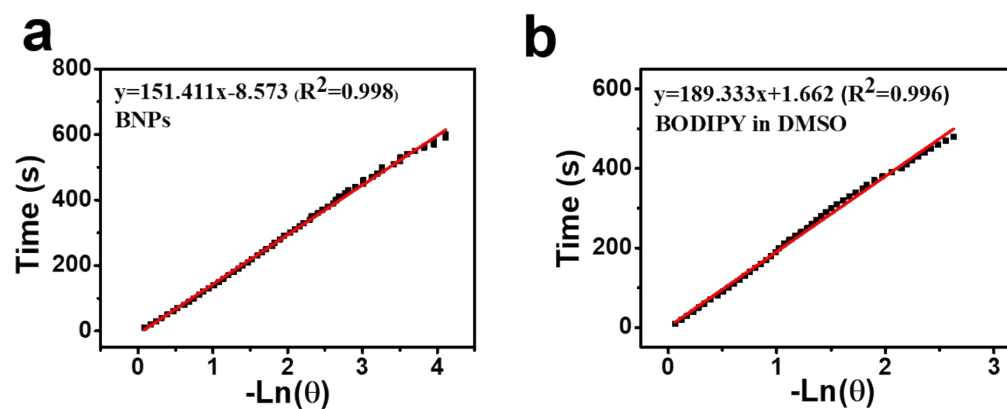**Figure S2.** Configuration diagram of J-Dimer, left: Top view; right: Side view.
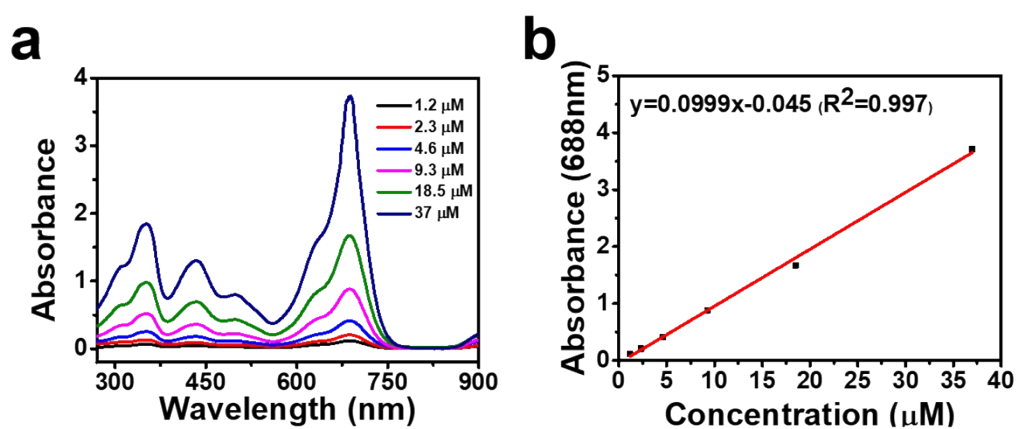


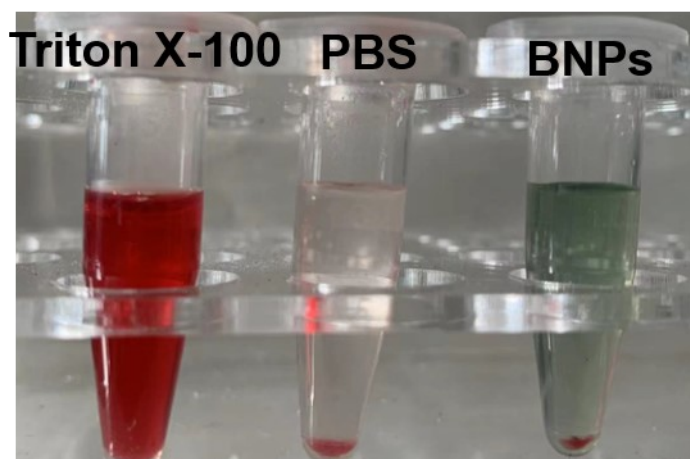**Figure S3.** (a) UV-vis absorption spectra of various concentration of BODIPY. (b)

The standard curve of BODIPY in DMF/water (v/v, 9:1).

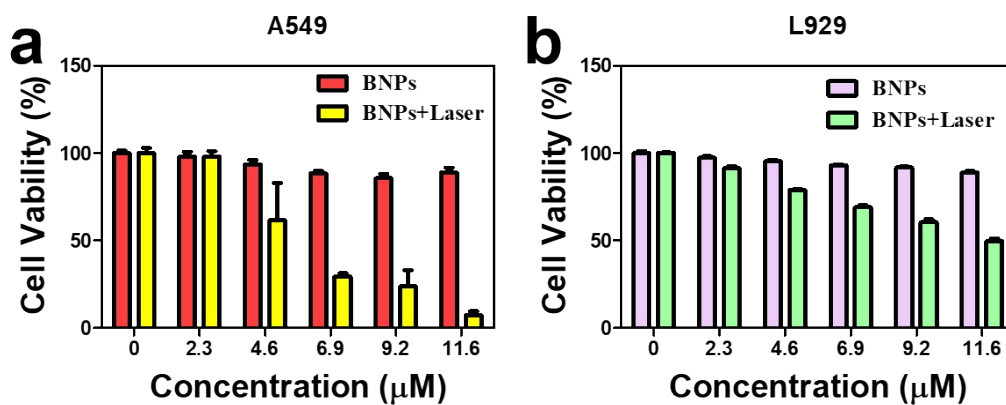**Figure S4.** The stability of BNPs in (a) water, (b) PBS, (c) 10% FBS.



**Figure S5.** Linear time data versus -Lnθ obtained from the cooling period of (a) BNPs aqueous solution and (b) BODIPY in DMSO.
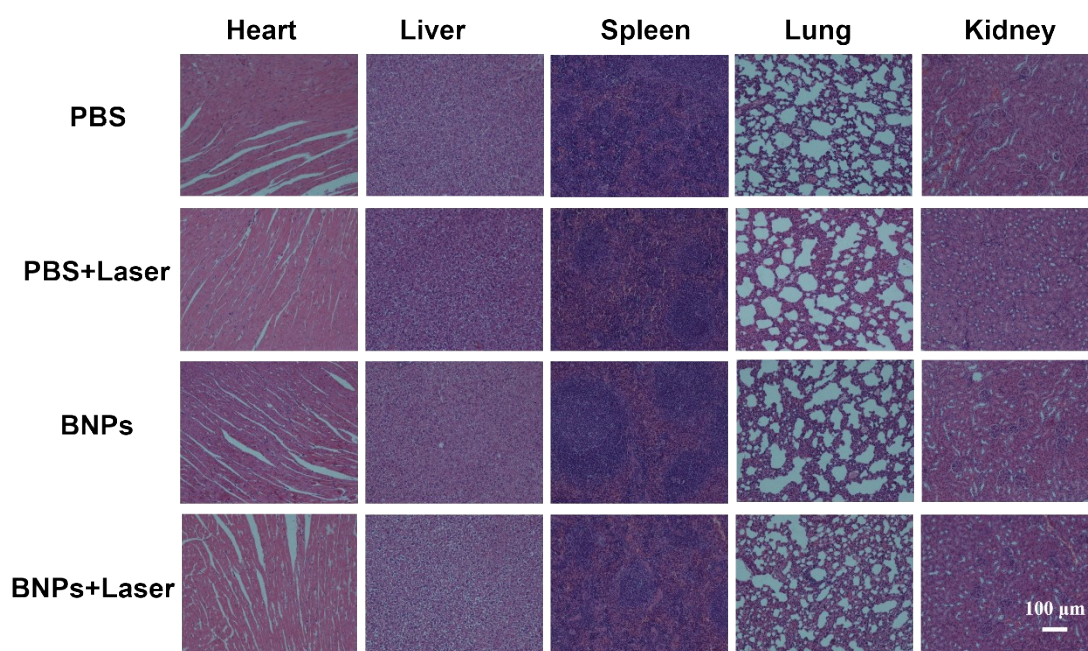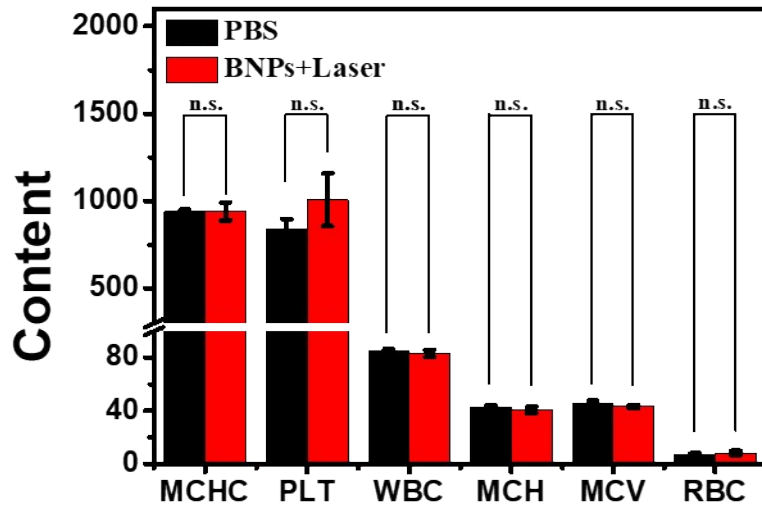


Figure S6. Hemolytic activity of BNPs.

**Figure S7.** The cytotoxic effect of BNPs on (a) A549 and (b) L929 cells.



**Figure S8.** H&E stained images of major organs and tumor slices obtained from mice after various treatments.

**Figure S9.** Routine blood biochemical indexes. Data are expressed as mean ±SD (n=4). The level of significance was set at a probability of ***p < 0.001, n.s. indicates no significant difference.