**Supplementary Information**

**Poly(2-ethyl-2-oxazoline)-IR780 conjugate nanoparticles for breast cancer phototherapy**

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**Synthesis and Characterization of the PEtOx-IR conjugate**

The FTIR analysis was performed to confirm the successful synthesis of the PEtOx-IR conjugate (Figure S1). The FTIR spectrum of IR780 showed peaks at 1514 and 757 cm-1 corresponding to the benzene’ ring stretch and the C-H bending, respectively (Figure S1B). On the other hand, the PEtOx-SH spectrum displayed its characteristic peak at 1628 cm-1 (amides’ C=O stretch) (Figure S1B). The FTIR spectrum of the PEtOx-IR conjugate presented the above described IR780’ and PEtOx-SH’ peaks (Figure S1B).



**Figure S1 –** Schematic representation of the PEtOx-IR conjugate synthesis (A).FTIR spectra of IR780, PEtOx-SH, and PEtOx-IR conjugate (B).

Afterwards, 1H NMR analysis was also performed to confirm the PEtOx-IR conjugate’s synthesis. The 1H NMR spectrum of IR780 showed a peak at 8.33 ppm belonging to the methine protons (C=C**H**) of the IR780’ benzene ring as well as peaks at 7.16 and 6.24 ppm corresponding to the methine protons   
(C-C**H**=C**H**-C) of the IR780’ heptamethine chain. Finally, the 1.72 ppm peak corresponding to methyl (C**H3**) protons of IR780 was also observed (Figure S2A) [1]. Furthermore, the PEtOx-SH spectrum displayed peaks at 3.48 and 2.43 ppm corresponding to the methylene protons (-N-C**H2** and -C**H2**-CH3) as well as at   
1.12 ppm belonging to the methyl protons (-CH2-C**H3**) (Figure S2B) [2, 3]. The 1H NMR spectrum of PEtOx-IR conjugate presented the above-described peaks of IR780 as well as those from PEtOx-SH (Figure S2C). Together, this data confirms the successful synthesis of the PEtOx-IR conjugate.



**Figure S2 –** 1H NMR spectra of IR780 (A), PEtOx-SH (B), and PEtOx-IR conjugate (C) dissolved in deuterated chloroform.

**Characterization of PEtOx-IR/TOS NPs**



**Figure S3 –** TEM image of PEtOx-IR/TOS NPs. Scale bar corresponds to 500 nm.

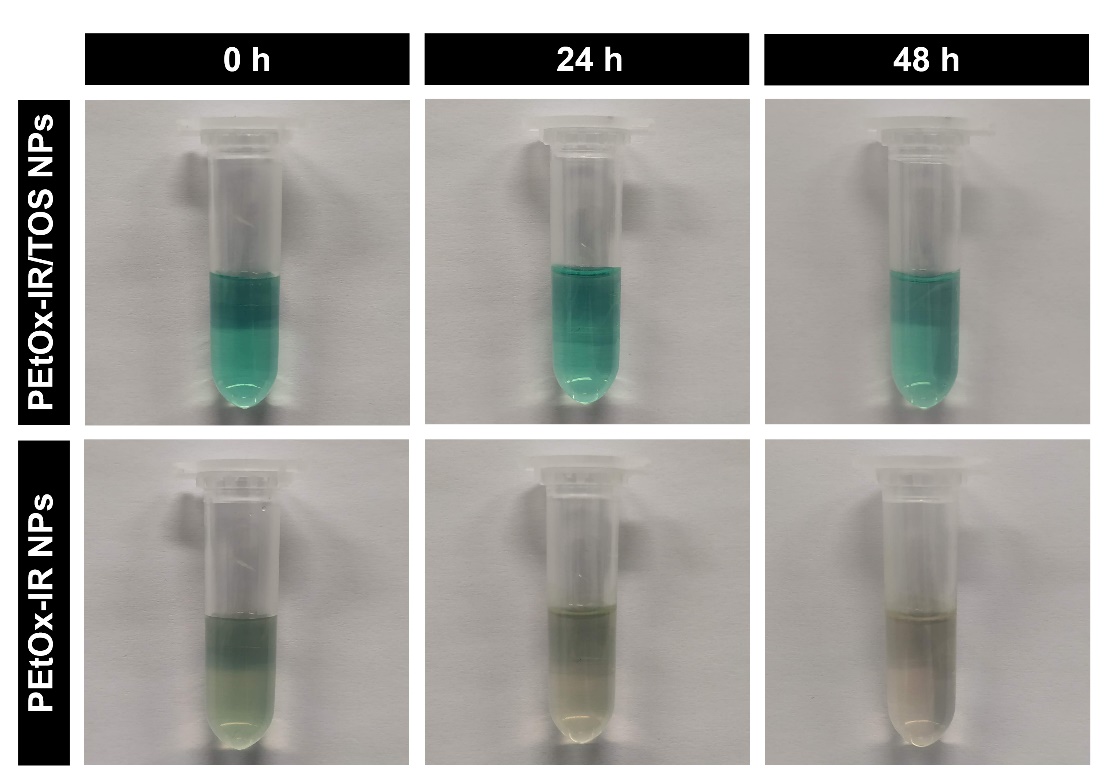
**Colloidal Stability of PEtOx-IR/TOS NPs and PEtOx-IR NPs**

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**Figure S4 –** Stability of PEtOx-IR/TOS NPs. Size variation of PEtOx-IR/TOS NPs over time when dispersed in water (A), PBS (B), and cell culture medium (C). The values were normalized using the respective initial size (t = 0 h). Each bar represents mean ± S.D. (n = 3). Vis-NIR spectra of PEtOx-IR/TOS NPs in water (D), PBS (E), and cell culture medium (F), at different time points.

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**Figure S5 –** Vis-NIR absorption spectra of PEtOx-IR NPs over a 48 h period (in water).

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**Figure S6 –** Macroscopic images of PEtOx-IR/TOS NPs and PEtOx-IR NPs, in water, over a 48 h period.

**Temperature Variation and Photothermal Stability of PEtOx-IR/TOS NPs**



**Figure S7 –** Temperature variation curves mediated by PEtOx-IR/TOS NPs (at different concentrations of PEtOx-IR conjugate equivalents) upon irradiation with NIR light (808 nm, 1.7 W cm−2) during a 5 min   
period (A). Vis-NIR spectrum of PEtOx-IR/TOS NPs (in water) during 5 minutes of NIR laser irradiation (808 nm, 1.7 W cm−2), and after 5 cycles of NIR laser exposure (B). Temperature variations induced by   
PEtOx-IR/TOS NPs (at 20 µg mL-1 of PEtOx-IR conjugate equivalents), measured at the 5th minute of NIR laser exposure (808 nm, 1.7 W cm−2, 5 min), along the different irradiation cycles (C).

**Uptake of PEtOx-IR/TOS NPs by MCF-7 cells**



**Figure S8 –** Fluorescence emission spectra of PEtOx-IR conjugate (in methanol) and PEtOx-IR/TOS NPs (in cell culture medium), using an excitation wavelength of 633 nm (A). Fluorescence intensity emitted by PEtOx-IR conjugate (in methanol) and PEtOx-IR/TOS NPs (in cell culture medium) at 800 nm, using an excitation wavelength of 780 nm (B). Uptake of PEtOx-IR/TOS NPs (2.5 µg mL-1 of PEtOx-IR conjugate equivalents) by MCF-7 cells (C). The fluorescence values were normalized using the values of cells incubated with free IR780 (2.5 µg mL-1).

**Supplementary References**

1. Yuan A, Qiu X, Tang X, Liu W, Wu J, Hu Y. Self-assembled PEG-IR-780-C13 micelle as a targeting, safe and highly-effective photothermal agent for in vivo imaging and cancer therapy*.* *Biomaterials* 51 184-193 (2015).

2. De Melo-Diogo D, Costa EC, Alves CG *et al*. POxylated graphene oxide nanomaterials for combination chemo-phototherapy of breast cancer cells*.* *Eur. J. Pharm. Biopharm.* 131 162-169 (2018).

3. Alvaradejo GG, Glassner M, Hoogenboom R, Delaittre G. Maleimide end-functionalized poly (2-oxazoline) s by the functional initiator route: synthesis and (bio) conjugation*.* *RSC Adv.* 8(17), 9471-9479 (2018).