Electronic Supplementary Information

Synthesis and in vivo Evaluation of PEG-BP-BaYbF$_5$ Nanoparticles for Computed Tomography Imaging and their Toxicity

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S1. High resolution TEM images

OA-BaYbF₅

PEG(5)-BP-BaYbF₅
S2. TGA for OA-BaYbF₅ and PEG(5)-BP-BaYbF₅

![TGA graph]

Figure 1. TGA measurements performed for OA-capped and PEGylated BaYbF₅ NPs.

S3. PEG(5)-BP-BaYbF₅ PEG density calculations

Calculations of the number of PEG(5)-BP molecules bound to each NP were performed assuming the NPs are spheres of a 9.5 nm diameter resulting in a NP volume of $4.49 \times 10^{-19}$ cm$^3$, and composed of BaYbF₅. Density of BaYbF₅ was estimated to be the weighted average of YbF₃ and BaF₂ density (6.55 g/cm$^3$). Based on these assumptions, the mass of a NP BaYbF₅ core was estimated to be $2.94 \times 10^{-18}$ g.

From TGA measurements

Based on the weight losses measured we can calculate that 37% of PEG(5)-BP-BaYbF₅ are due to PEG(5)-BP. This will result in 2.96 mg of PEG(5) and 5.04 mg of BaYbF₅ for a 8.00 mg PEG(5)-BP-BaYbF₅ TGA sample, corresponding to $(2.96 \times 10^{-3}$ g/5307 g/mol) $\times$ NA $= 335.9 \times 10^{15}$ molecules of PEG and $(5.04 \times 10^{-3}$ g / $2.94 \times 10^{-18}$ g/NP) $= 1.71 \times 10^{15}$ Nanoparticles. Hence, the number of PEG molecules per nanoparticle is calculated to be 196.

Calculation of theoretical maximum density of PEG(5)-BP/NPs

The surface area of a 9.5 nm sphere is 283.4 nm$^2$. The footprint of a BP is approximately 0.85 nm$^2$ (calculated from a computational model using Chem3D, Cambridge Software). Using these values, the theoretical maximum number of BPs that can bind to a 9.5 nm sphere is 333.
Maximum number of PEG(5)-BP/NPs calculated using the amount of non-bound PEG(5)-BP after purification

Based on a scaled up version of the reaction in which 40 mg of OA-BaYBF₅ Nanoparticles were reacted with 80 mg of PEG(5)-BP.

- **Calculation of number of Yb atoms/NPs**

  The number of Yb atoms in a nanoparticle is equal to the number of BaYbF₅ units. This can be calculated as \( \text{NPs mass} \times \frac{N_a}{\text{MW BaYbF}_5} = 2.94 \times 10^{-18} \text{g} \times 6.022 \times 10^{23} \text{atoms/mol} / 405.32 \text{g/mol} \) Resulting in 4366.5 Yb atoms/NP

- **Calculation of number of BaYBF₅ NPs**

  The Yb concentration of the PEG(5)-BP-BaYBF₅ dispersion was measured to be 24.55 mM by ICP-MS, which corresponds to 9.82x 10⁻⁵ moles and 5.19 x 10¹⁹ Yb Atoms in 4 mL. Hence, 5.19 x 10¹⁹ Yb atoms/4366.5 atoms/NP = 1.35 x 10¹⁶ NPs

- **Calculation of number of PEG molecules bound to BaYBF₅ NPs**

  Using the mass of the non-bound PEG(5)-BP, obtained from the washings during purification of the pegylated nanoparticles allowed us to calculate that a maximum of 46.5 mg of PEG(5)-BP was bound to the BaYBF₅ NPs.

  Hence, using the average MW of PEG(5)-BP value of 5307 g/mol, we can calculate that this corresponds to a maximum of approximately 5.28 x 10¹⁸ PEG(5)-BP molecules.

- **Calculation of number of PEGs/NPs**

  The maximum number of PEG molecules per NPs is calculated to be \( 5.28 \times 10^{18} / 1.35 \times 10^{16} = 389.6 \text{ PEG}(5)\)-BP/NP

Since the maximum PEG(5)-BP/NPs density calculated using the recovered PEG(5)-BP was higher than the maximum theoretical density, the latter was deemed more accurate and used for comparison with value obtained from TGA measurements. Based on these value the density of PEG(5)-BP molecules on the surface of the NPs was 58.85% of the maximum theoretical density, which is consistent with the PEG moieties being arranged in a brush regime.
S4. Representative microscopy images from the *in vitro* toxicity studies.

Representative images of (**A**) untreated U937 cells, compared to those treated with (**B**) 10 mM PEG(5)-BP-BaYbF5 nanoparticles and (**C**) 0.01 mM YbCl₃. The dye cocktail used contained Mitotracker Red (red) and ImageItDead (green) dyes.