

Supplementary Materials: Functionalizing NaGdF₄:Yb,Er Upconverting Nanoparticles with Bone-Targeting Phosphonate Ligands: Imaging and In Vivo Biodistribution

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Table S1. Gd³⁺ determination per gram of functionalized UCNPs by ICP-MS.

	g Gd³⁺ / g NP
UCNP-citrate	0.37
UCNP-EDTA	0.11
UCNP-etidronate	0.30
UCNP-alendronate	0.44
UCNP-3P	0.41

Table S2. Relaxivity r1 and r2 measurements and r1/r2 ratios for phosphonate-functionalized UCNPs obtained at 37 °C and 1.5 T.

	H₂O			MES			Human Serum		
	r1	r2	r2/r1	r1	r2	r2/r1	r1	r2	r2/r1
Gd-UCNP- etidronate	3.47±0.02	n.a	n.a	8.47±0.02	5.72±0.04	0.67	9.73±0.02	13.7±0.02	1.4
Gd-UCNP- alendronate	0.59±0.05	1.89±0.26	3.2	0.66±0.03	6.38±0.06	9.67	0.27±0.03	7.37±0.54	27.3
Gd-UCNP- 3P	0.70±0.01	4.15±0.06	5.9	0.69±0.07	1.40±0.03	2.02	0.76±0.05	1.588±0.006	2.09

Table S3. Dynamic light scattering data for UCNPs in water and MES buffer (pH 6).

d (nm) (Number (%))				
	H₂O		MES	
Gd-UCNP- etidronate	280 ± 115 (100)		46 ± 32 (100)	
Gd-UCNP- 3P	209 ± 86 (83)	1853 ± 1238 (17)	180 ± 75 (58)	1901 ± 1190 (42)
Gd-UCNP- Alendronate	2343 ± 1237 (100)		2931 ± 1213 (100)	

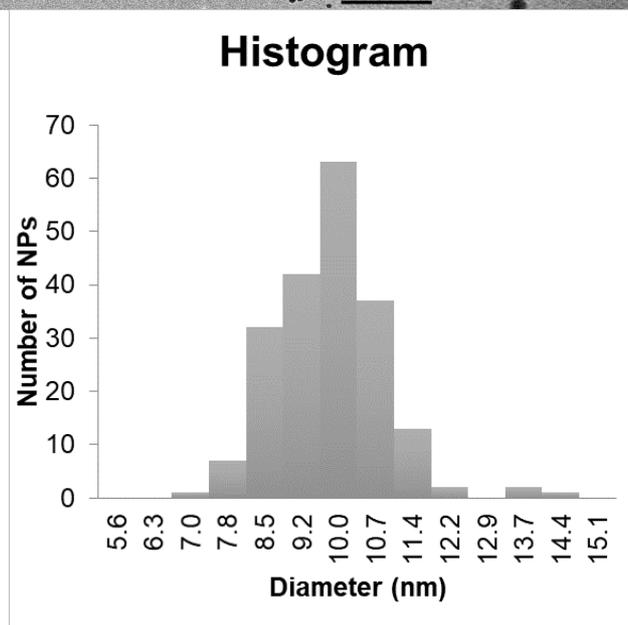
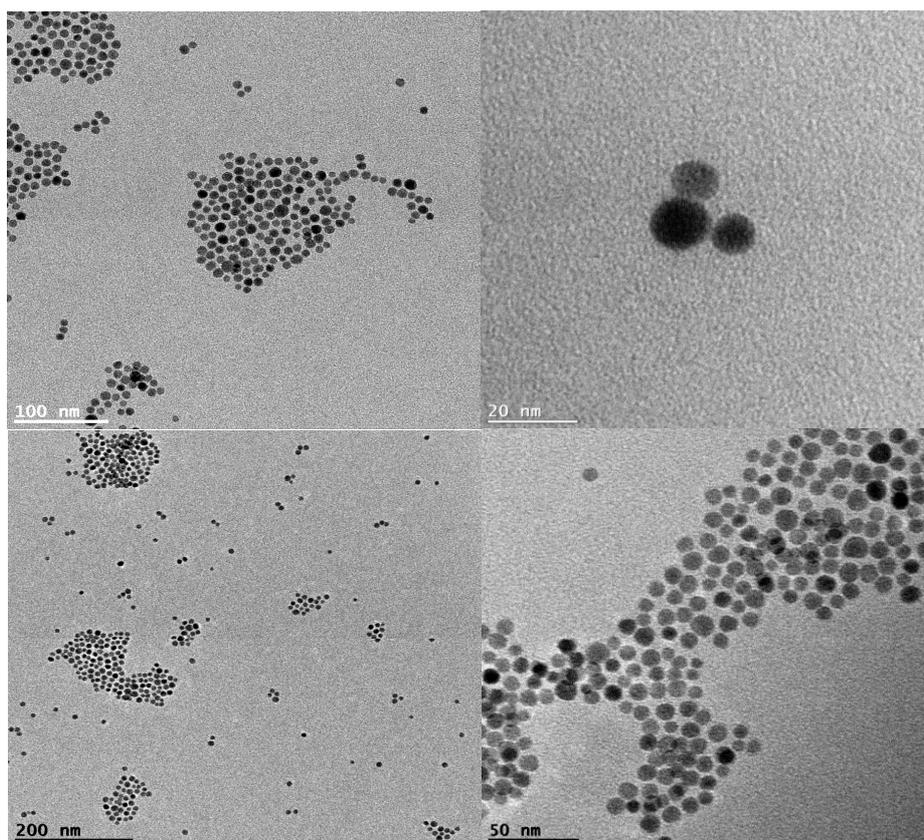


Figure S1. TEM images ($n = 150$) of NaGdF₄:Yb,Er capped with oleic acid and dissolved in THF.

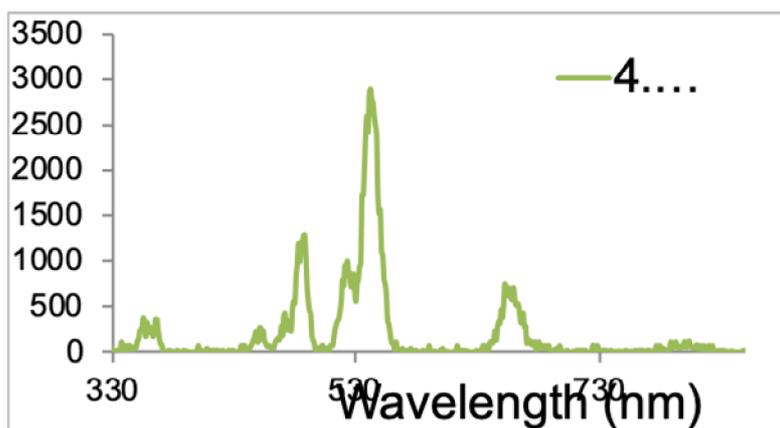
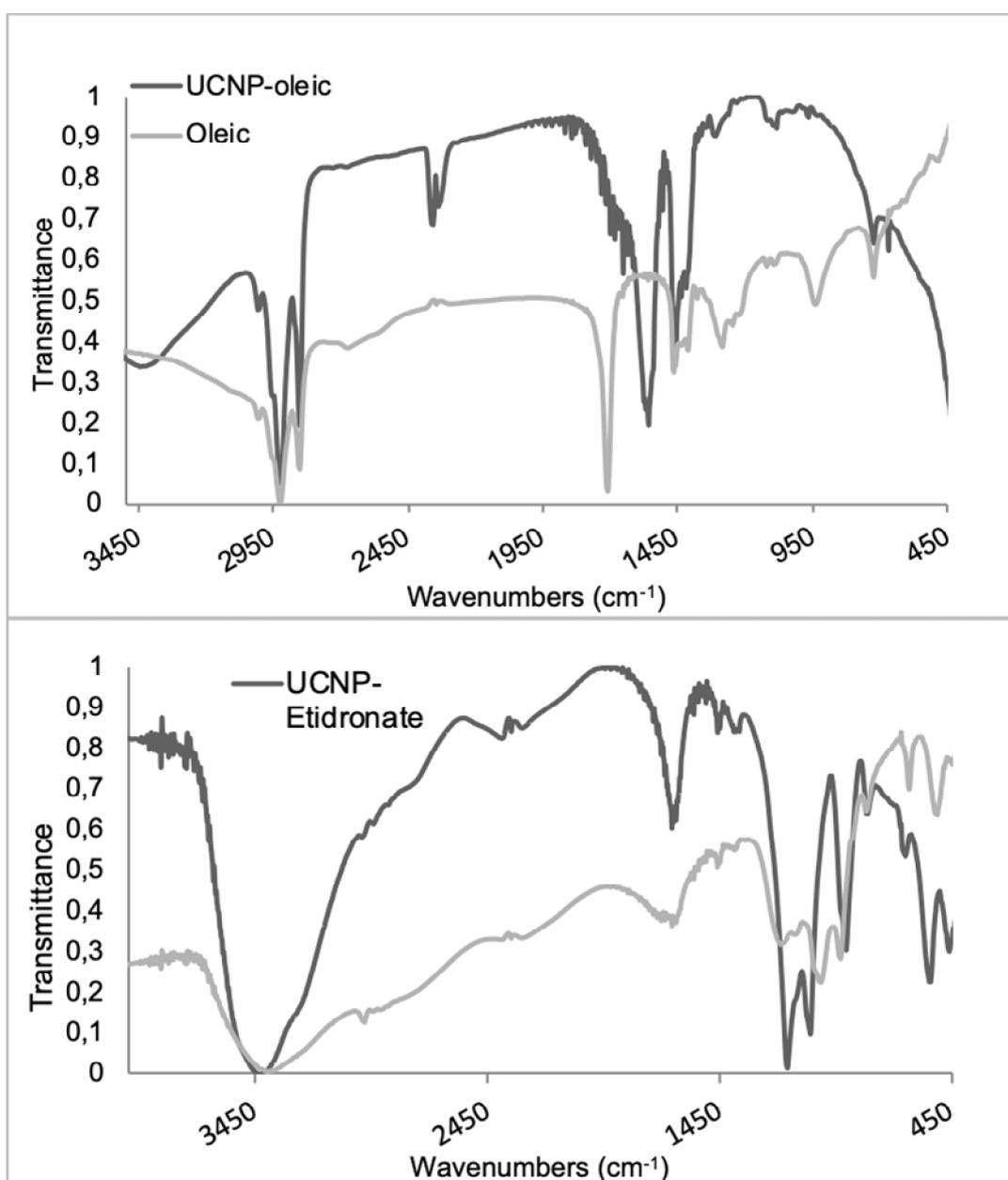


Figure S2. Emission spectra of NaYF₄ Yb:Er UCNPs capped with oleic acid irradiated at 980 nm with 4.0 W.



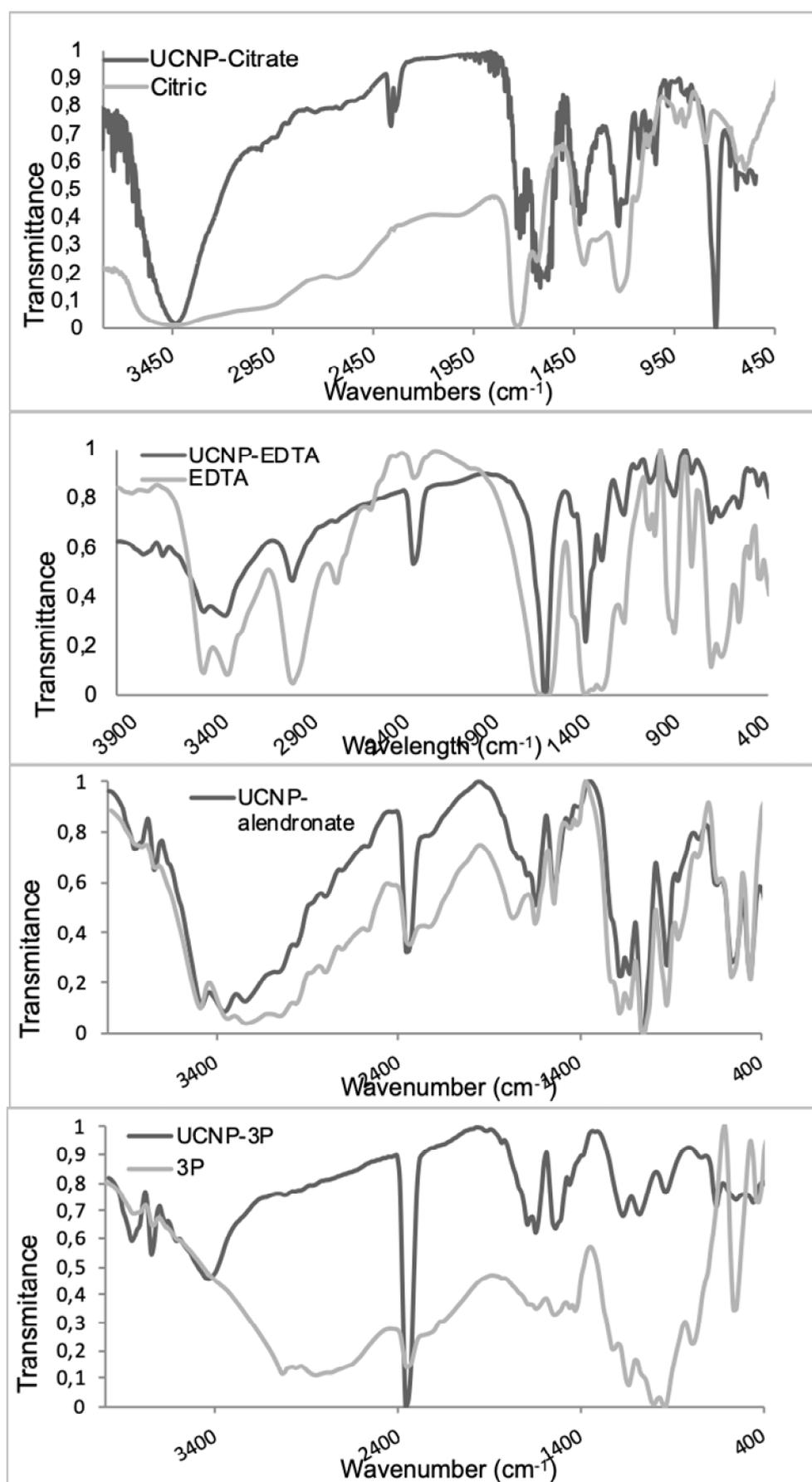


Figure S3. FT-IR spectra of NaGdF₄:Yb,Er UCNPs capped with different ligand coatings and of their corresponding free ligand.

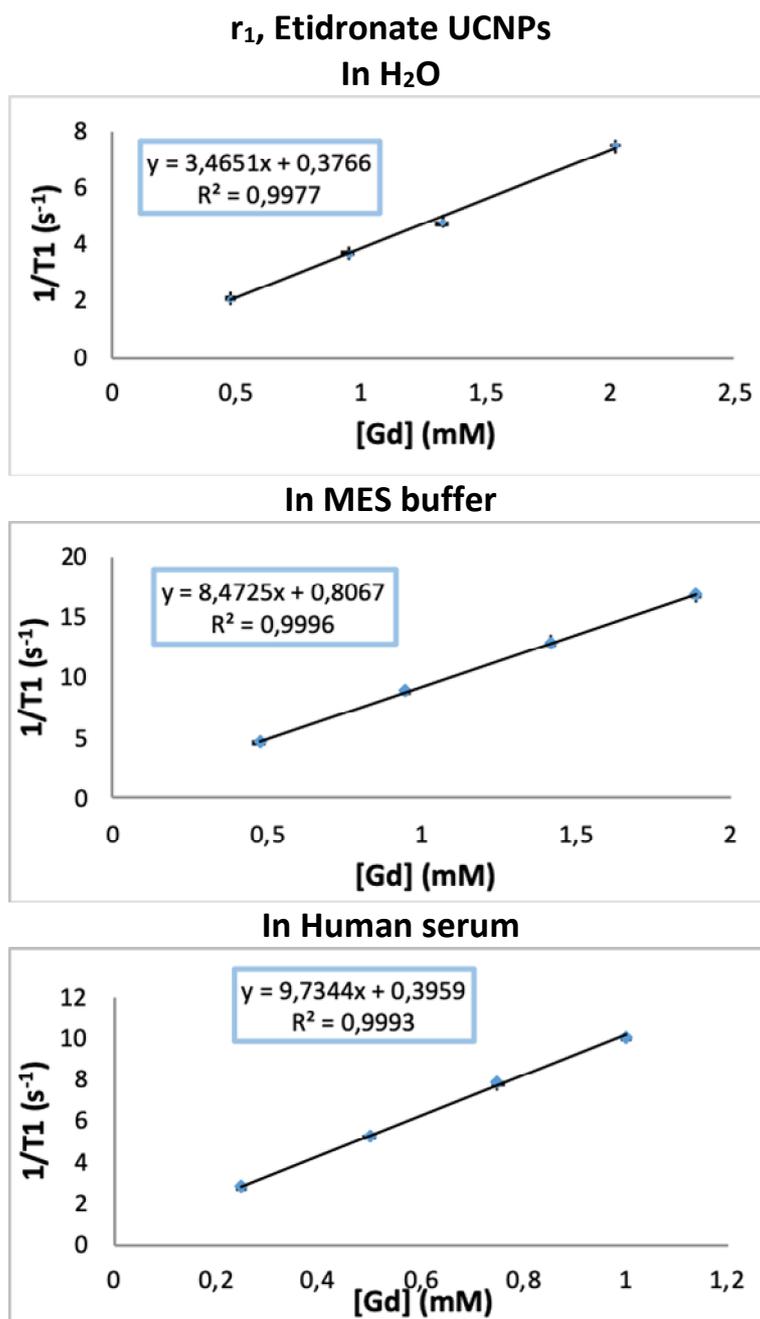


Figure S4. Relaxivity r_1 values (1.5 T, 37 °C) for etidronate UCNPs in triplicates in three different solvents (H₂O, MES buffer and human serum). Mean r_1 values are directly obtained from the slope of the linear regression generated from the plot of the measured relaxation rate versus the [Gd].

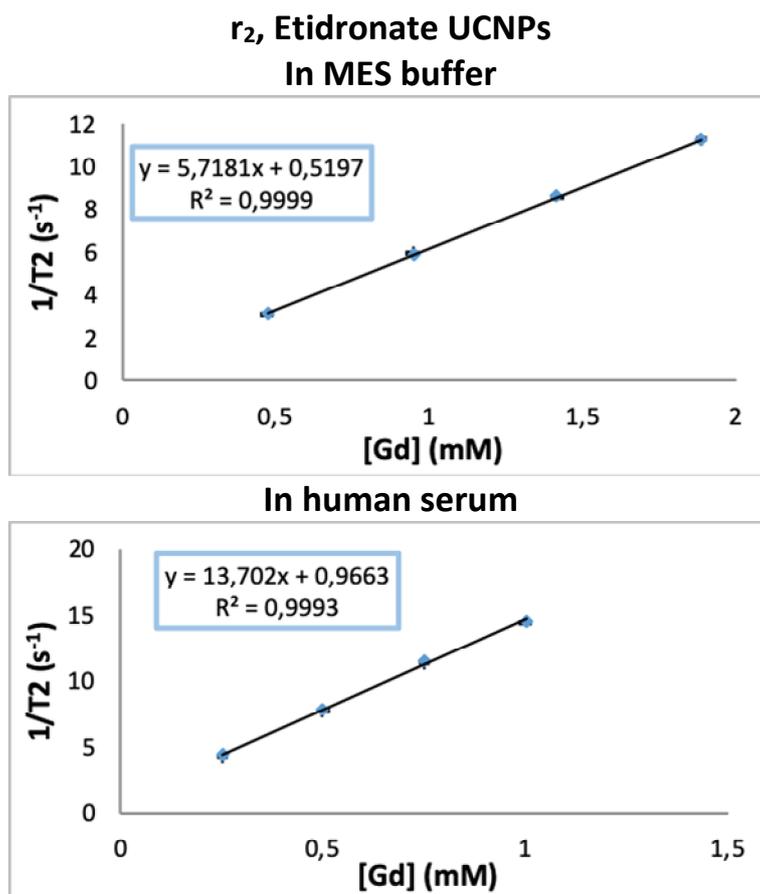


Figure S5. Relaxivity r_2 values (1.5 T, 37 °C) for etidronate UCNPs in triplicates in two different solvents (MES buffer and human serum). Mean r_2 values are directly obtained from the slope of the linear regression generated from the plot of the measured relaxation rate versus the [Gd].

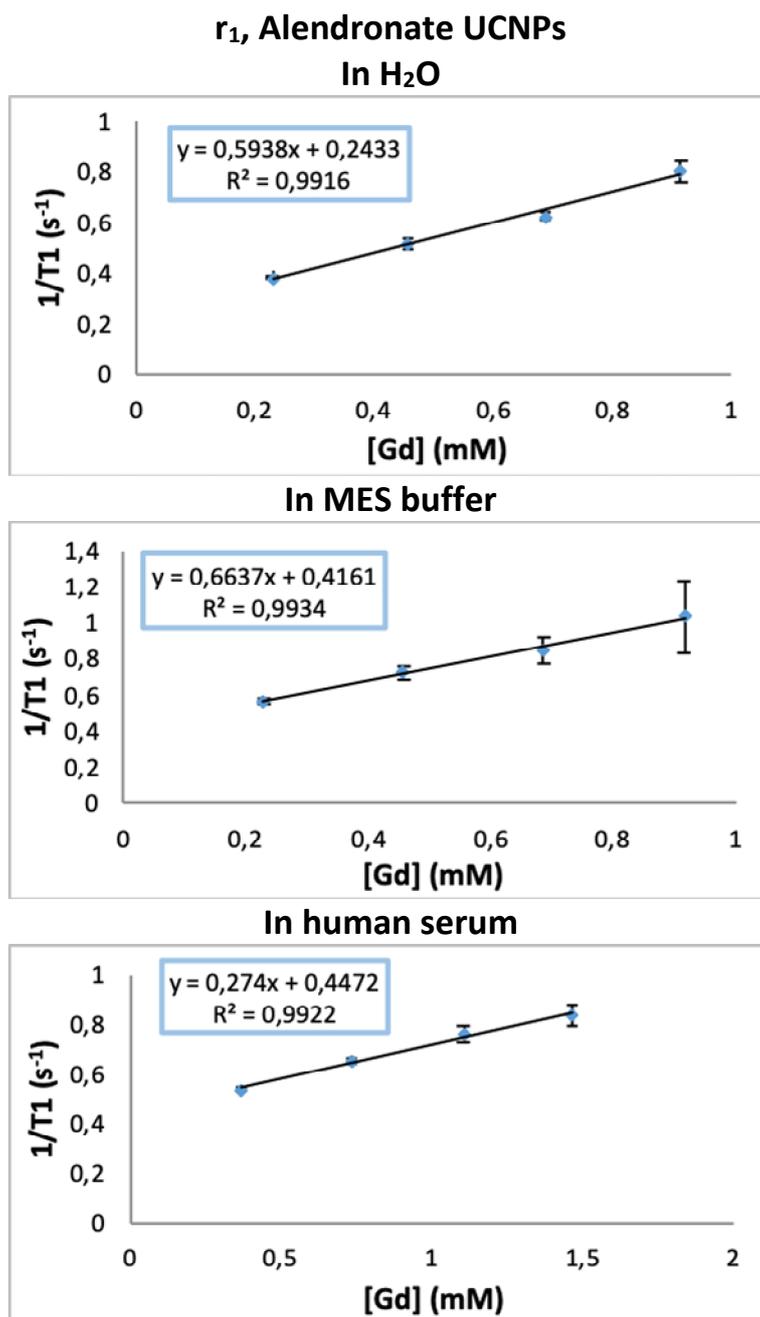


Figure S6. Relaxivity r_1 values (1.5 T, 37 °C) for alendronate UCNPs in triplicates in three different solvents (H₂O, MES buffer and human serum). Mean r_1 values are directly obtained from the slope of the linear regression generated from the plot of the measured relaxation rate versus the $[Gd]$.

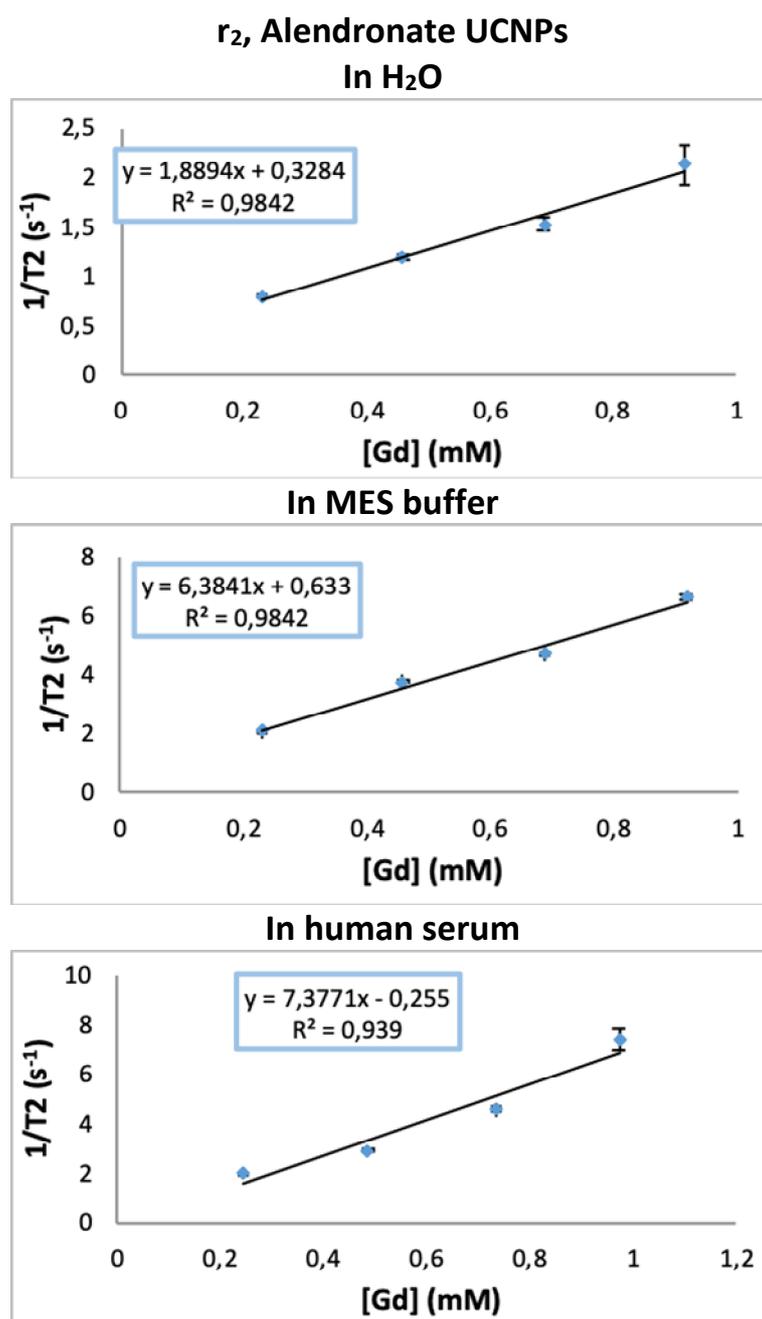


Figure S7. Relaxivity r_2 values (1.5 T, 37 °C) for alendronate UCNPs in triplicates in three different solvents (H₂O, MES buffer and human serum). Mean r_2 values are directly obtained from the slope of the linear regression generated from the plot of the measured relaxation rate versus the [Gd].

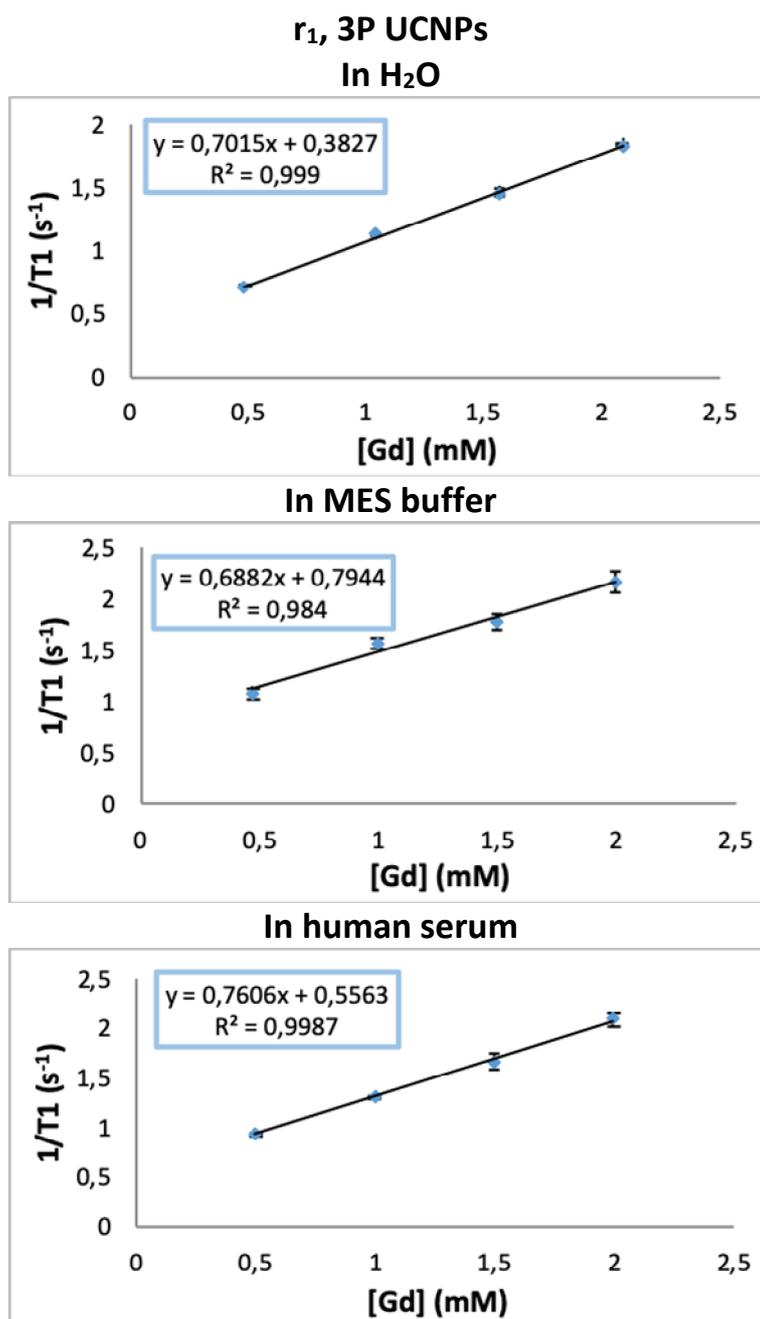


Figure S8. Relaxivity r_1 values (1.5 T, 37 °C) for 3P UCNPs in triplicates in three different solvents (H₂O, MES buffer and human serum). Mean r_1 values are directly obtained from the slope of the linear regression generated from the plot of the measured relaxation rate versus the [Gd].

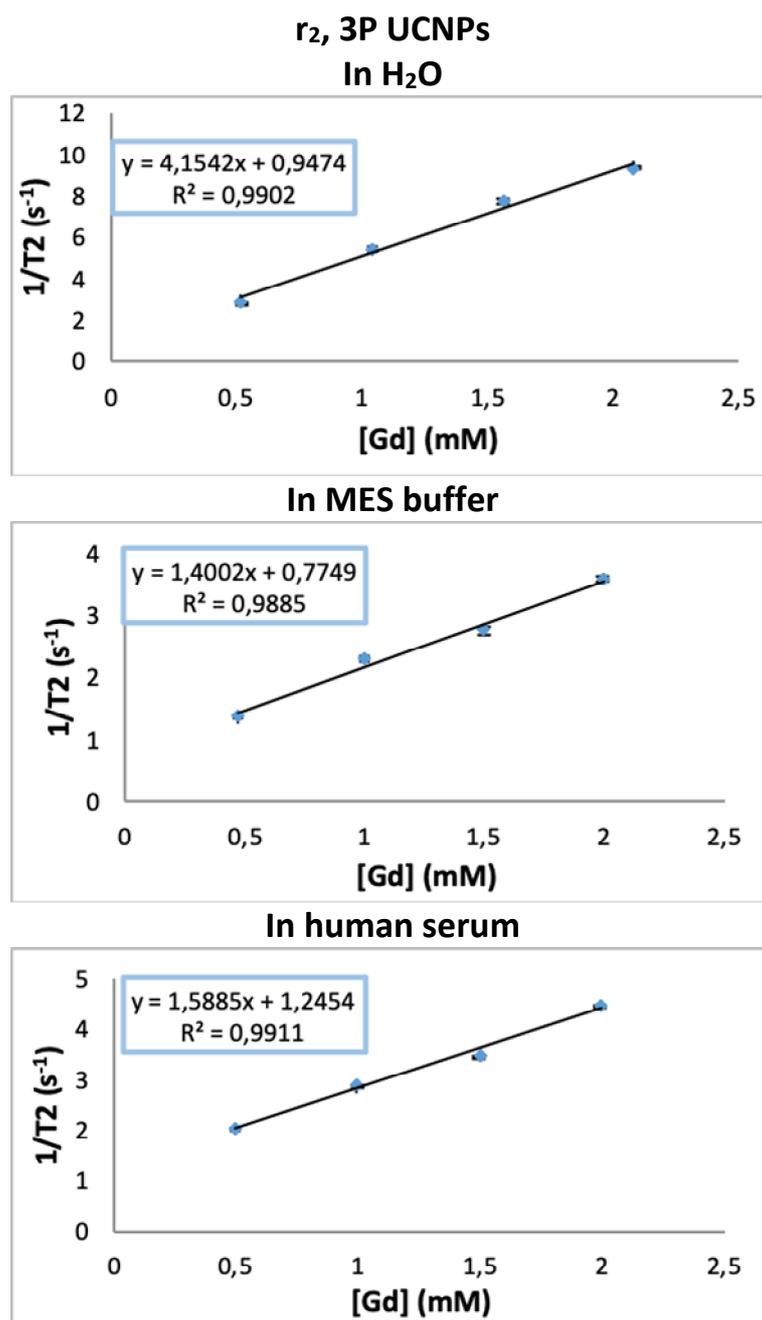


Figure S9. Relaxivity r_2 values (1.5 T, 37 °C) for 3P UCNPs in triplicates in three different solvents (H₂O, MES buffer and human serum). Mean r_2 values are directly obtained from the slope of the linear regression generated from the plot of the measured relaxation rate versus the [Gd].

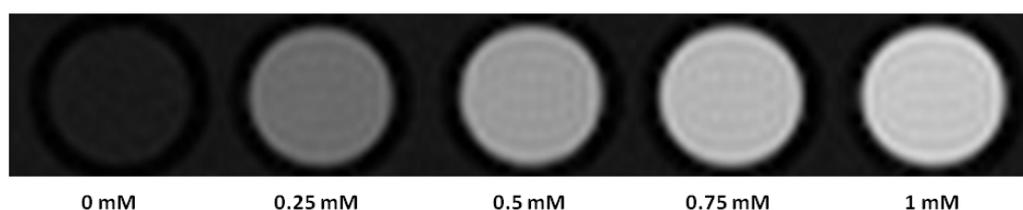


Figure S10. T1 weighted MRI image of etidronate UCNPs (7 T, 37 °C) in H₂O at different concentrations.

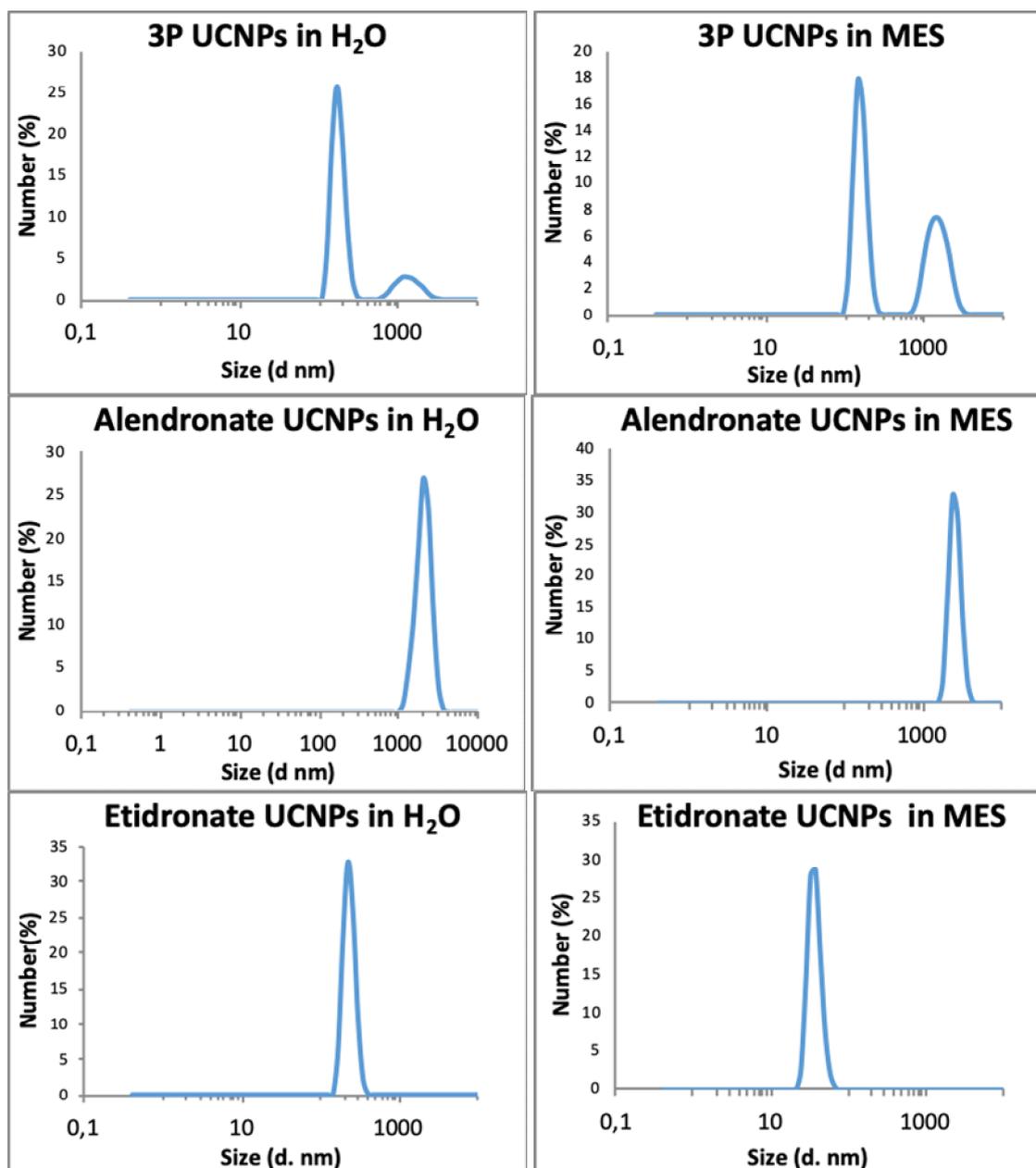


Figure S11. Dynamic Light Scattering of UCNPs coated with etidronate, alendronate, 3P and citrate measured in H₂O and MES buffer (pH 6).

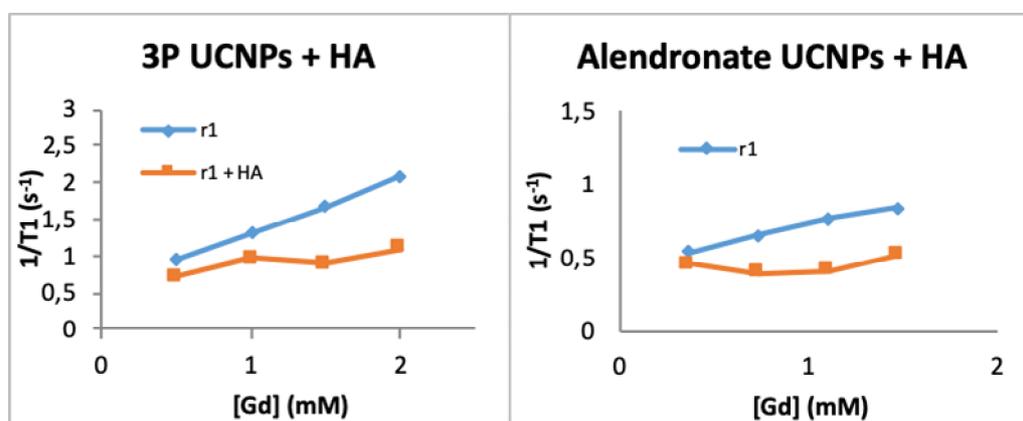


Figure S12. Relaxivity r_1 values for 3P and alendronate UCNPs in the presence and in the absence of hydroxyapatite HA (12.5 mg/ml).

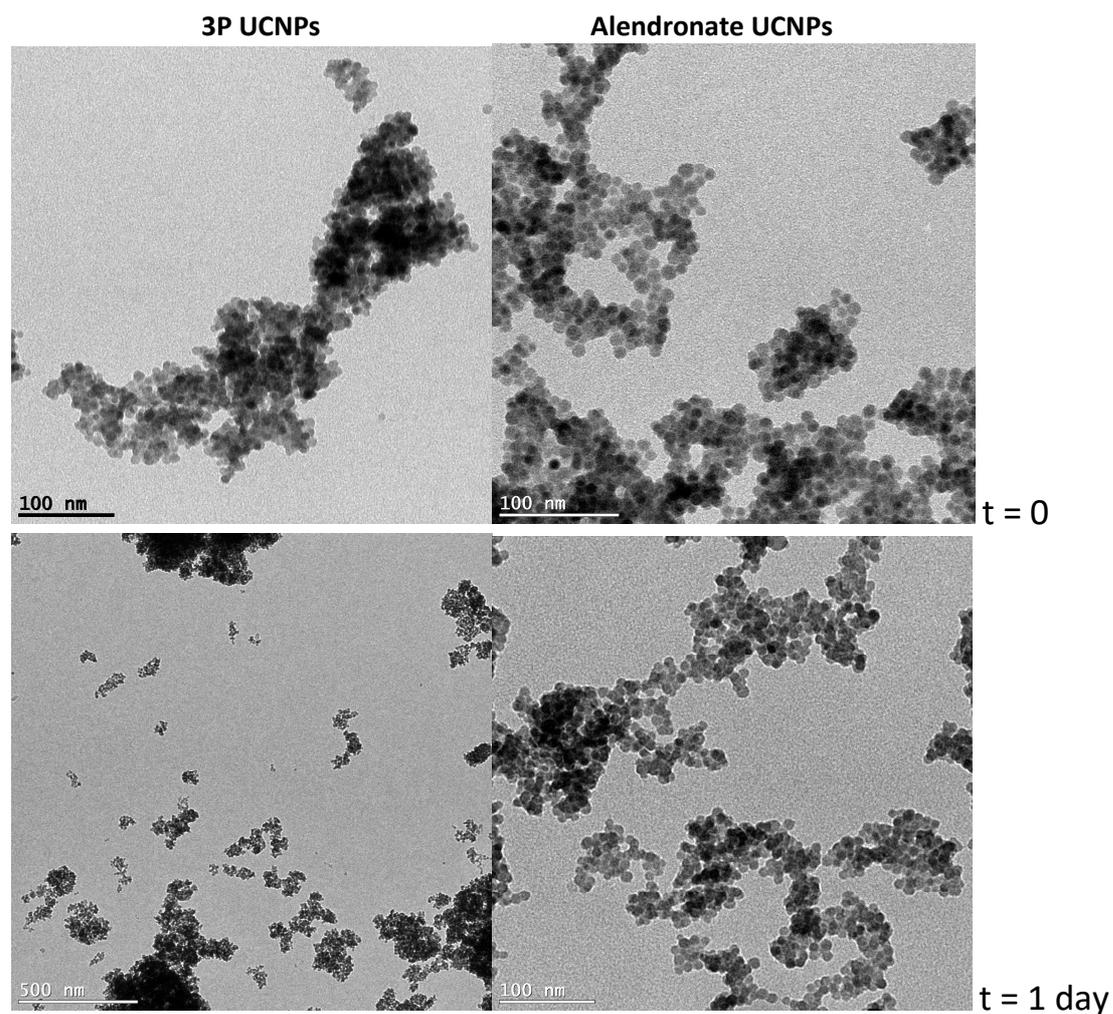


Figure S13. TEM images of 3P and alendronate UCNPs in aqueous solution measured at different time points (t = 0 and 1 day).

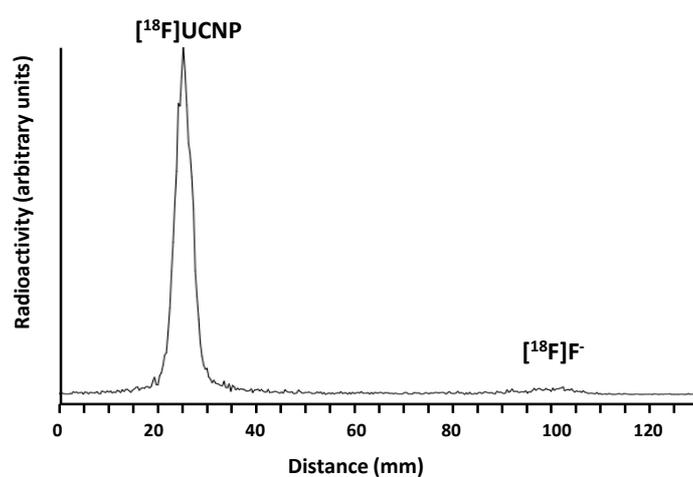


Figure S14. Diluted solution of ¹⁸F-labeled UCNPs (1:20) analysed by radio-TLC at t = 5 h of incubation in physiologic saline solution. ¹⁸F-labeled UCNPs remain at the seeding spot (d ≈ 25 mm) and the free [¹⁸F]F⁻ elutes with the solvent front (d ≈ 90–100 mm). A representative chromatogram corresponding to the analysis of 3P UCNPs is shown.

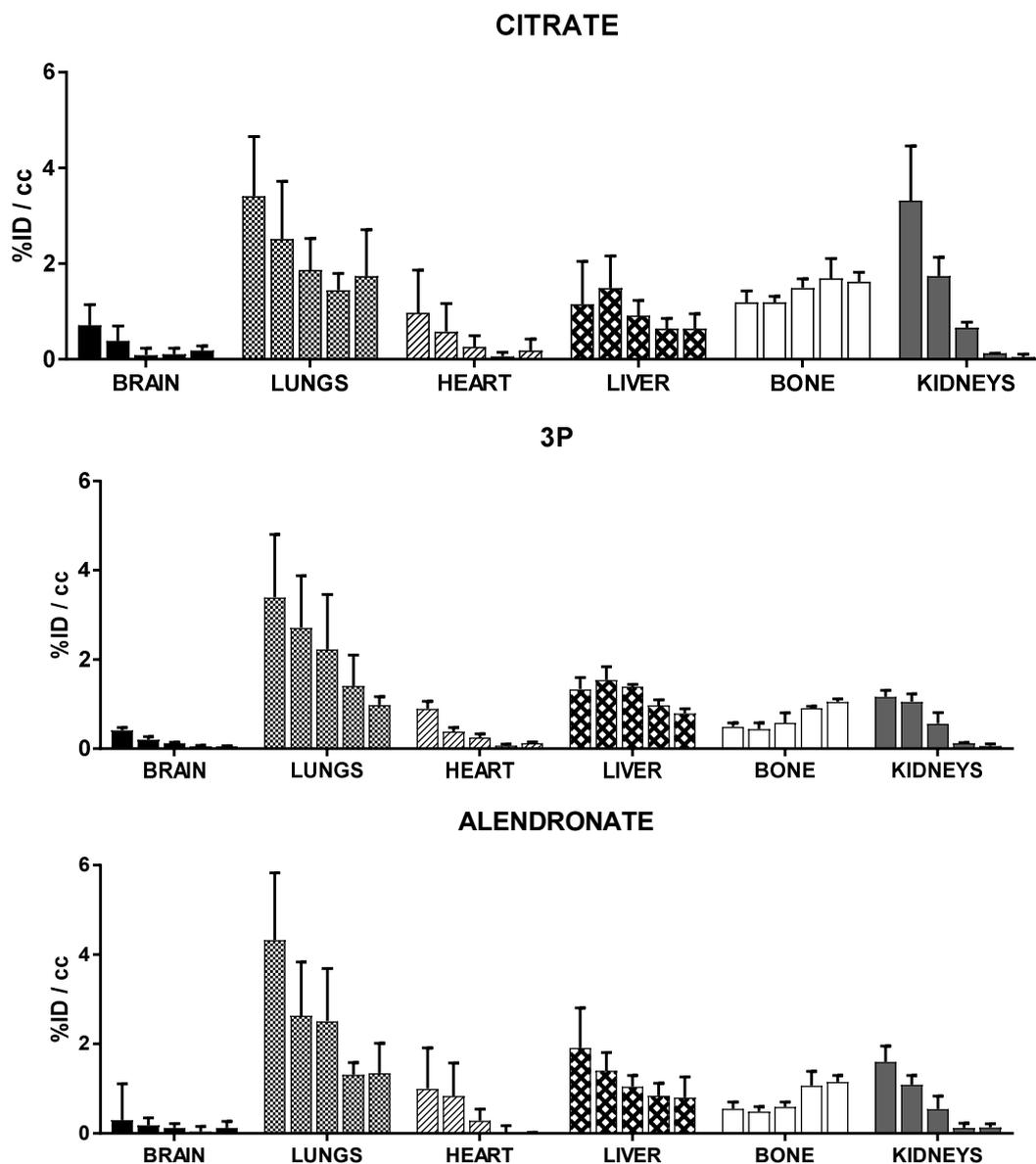


Figure S15. Concentration of radioactivity in different organs at different time points for the different labeled NPs, as determined from PET imaging. For each NP type and organ, the bars indicate, from left to right, the concentration of radioactivity in the time frames 0-2 min, 2-6 min, 6-28 min, 3h and 6 h, respectively. Results are expressed as mean±standard deviation, n=3.