

Supplementary Material

# Iron Oxide Incorporated Conjugated Polymer Nanoparticles for Simultaneous Use in Magnetic Resonance and Fluorescent Imaging of Brain Tumors

N. Arias-Ramos <sup>1</sup>, L. E. Ibarra <sup>2,4,\*</sup>, M. Serrano-Torres <sup>1</sup>, B. Yagüe <sup>1</sup>, M. D. Caverzán <sup>3,4</sup>, C. A. Chesta <sup>3,5</sup>, R. E. Palacios <sup>3,5</sup> and P. López-Larrubia <sup>1,\*</sup>

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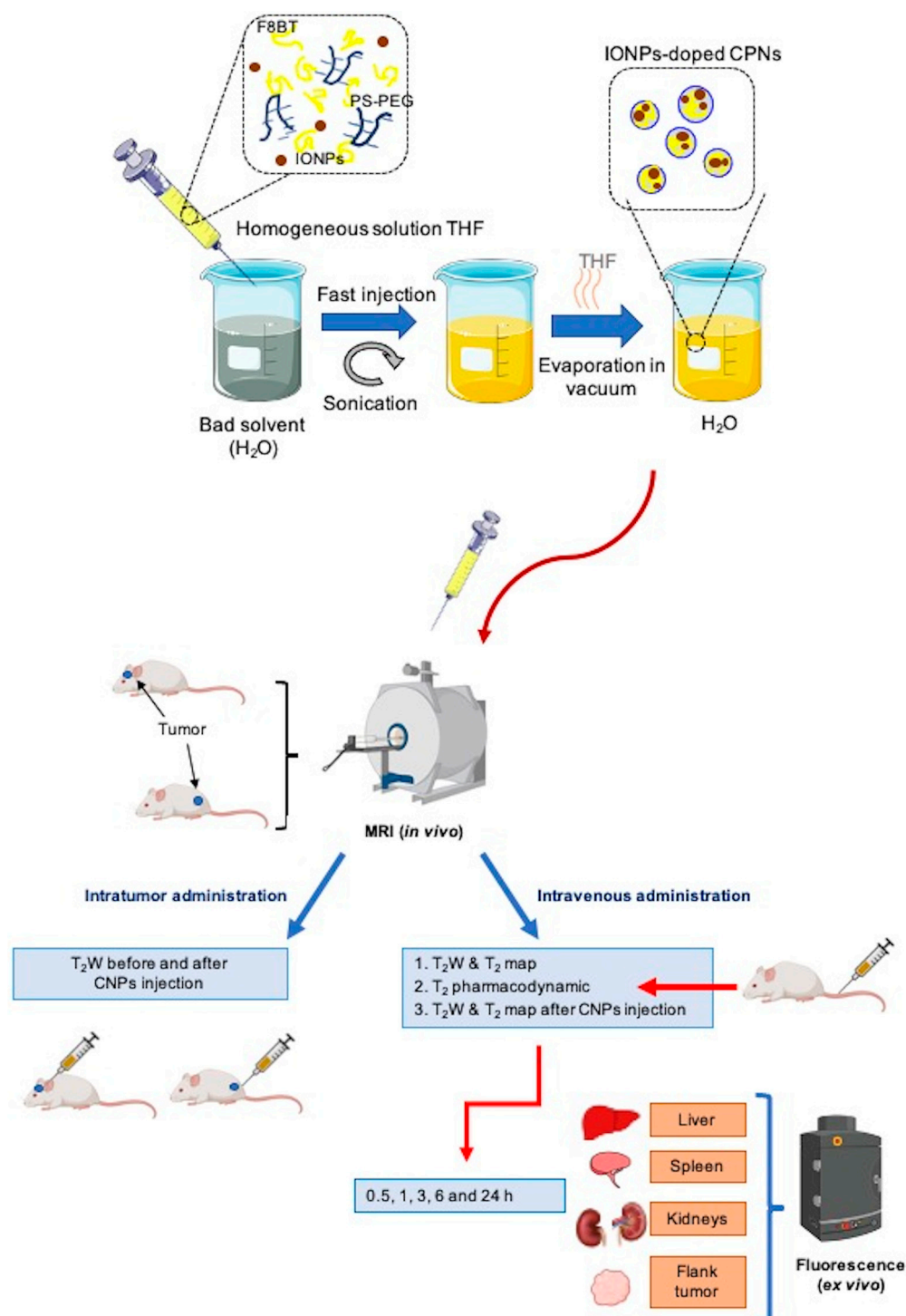
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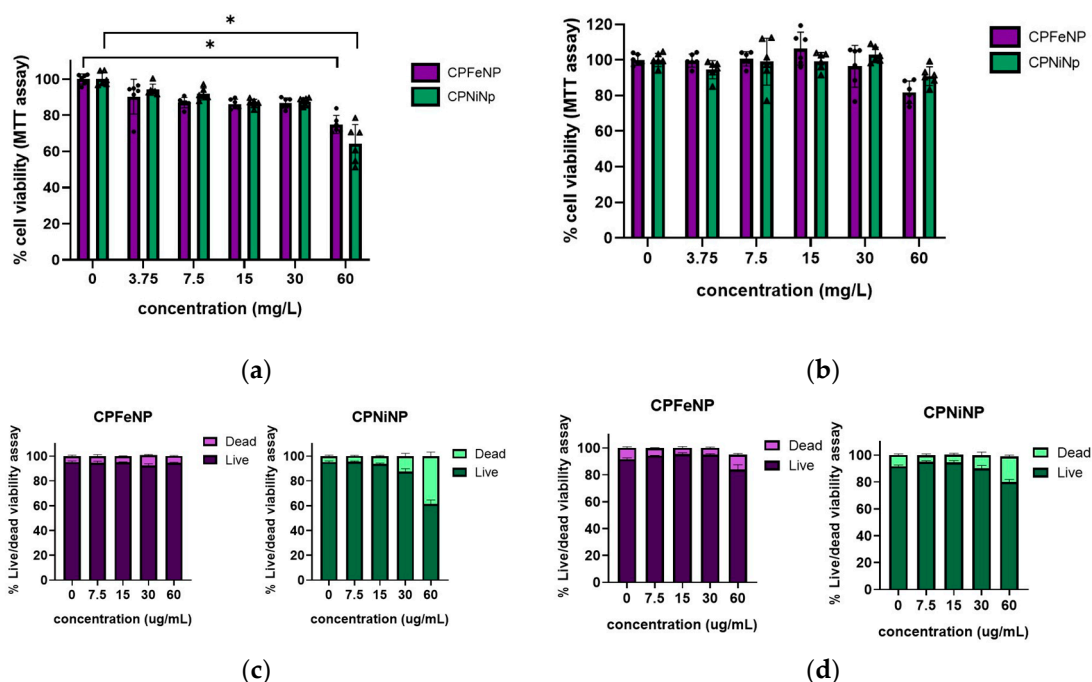
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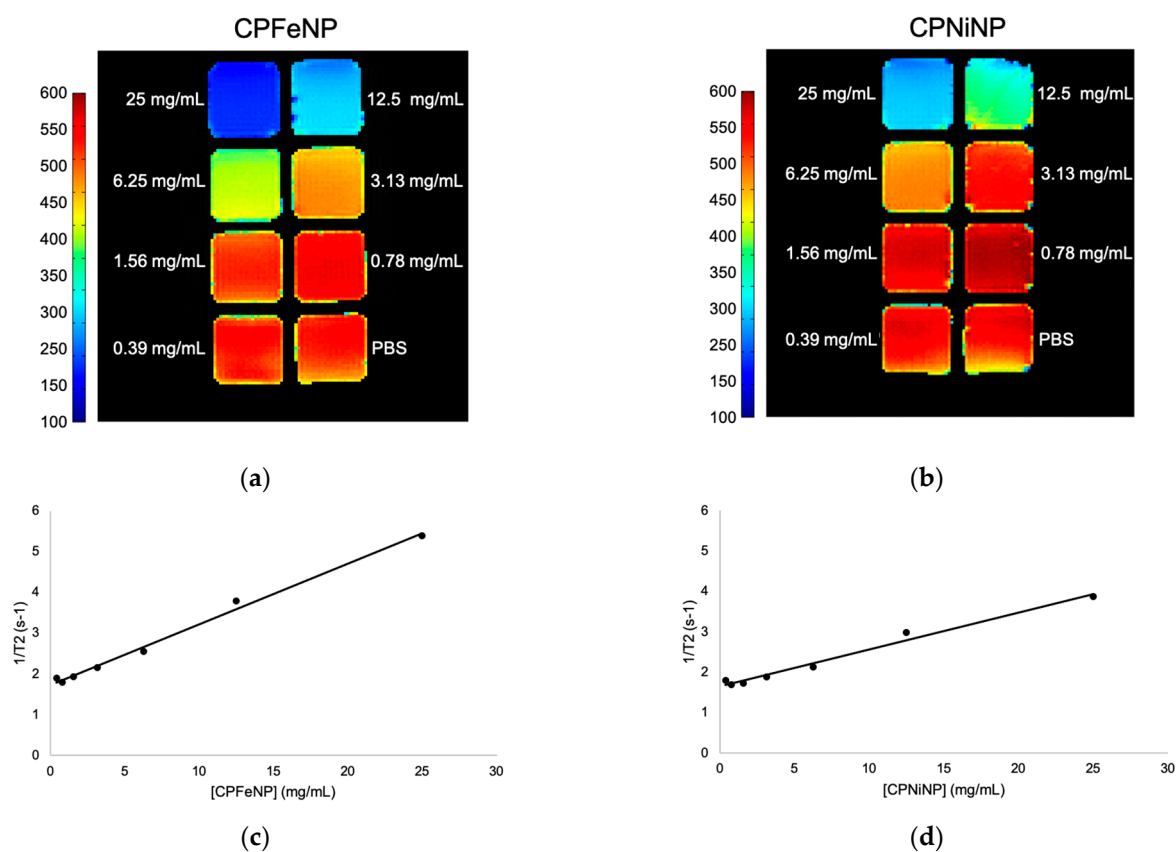
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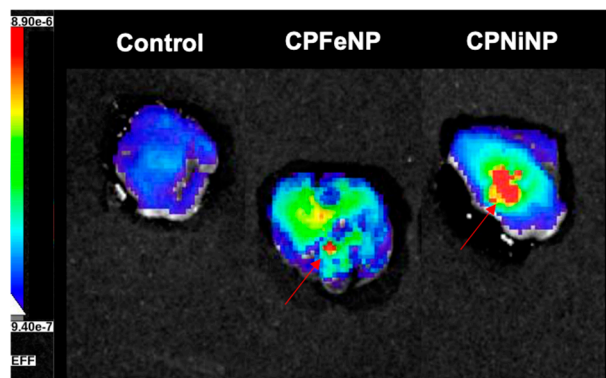
**Figure S1.** Overview of the experimental workflow followed in the preparation, characterization, in vitro and in vivo evaluation of the IONP-doped CPNs. The scheme shows the steps to synthesize and purified the IONP-doped-CPNs in the upper panels and the preclinical assessment of the nanoparticles in the lower.



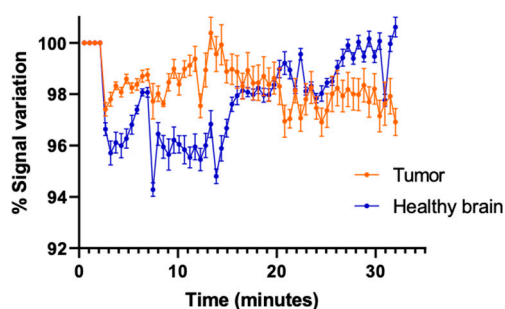
**Figure S2.** Biocompatibility evaluation of IONP-doped CPNs in GBM human cell lines. MTT viability quantification for (a) U-87 MG and (b) T98G. Cell viability quantified by flow cytometry using LIVE/DEAD cell viability assays for (c) U-87 MG and (d) T98G. (\* $p < 0.05$ ).



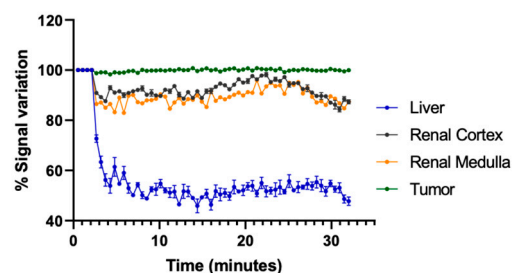
**Figure S3.** Relaxivities determination of IONP-doped CPNs. T<sub>2</sub> maps of nanoparticles at decreasing concentrations: (a) CPFeNP and (b) CPNiNP. Graphical linear regression of relaxation rates (1/T<sub>2</sub>) versus increasing nanoparticle concentration: (c) CPFeNP and (d) CPNiNP.



**Figure S4.** Fluorescence images of tumors after i.t. IONP-doped CPN administration. Images show excised tumors from a control mouse without particle administration (left), a flank tumor after CPFeNP i.t. injection (middle) and a tumor after CPNiNP i.t. injection. Red arrows point to the high fluorescence point due to IONP-doped-CPNs injection.

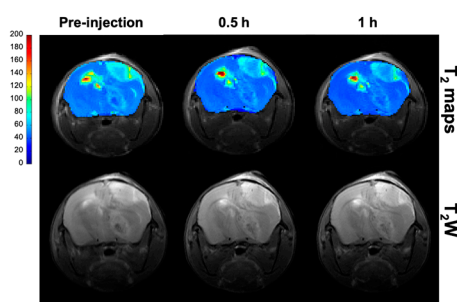


(a)

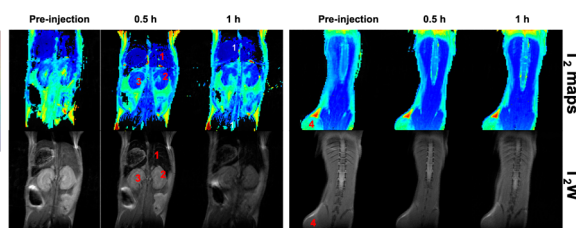


(b)

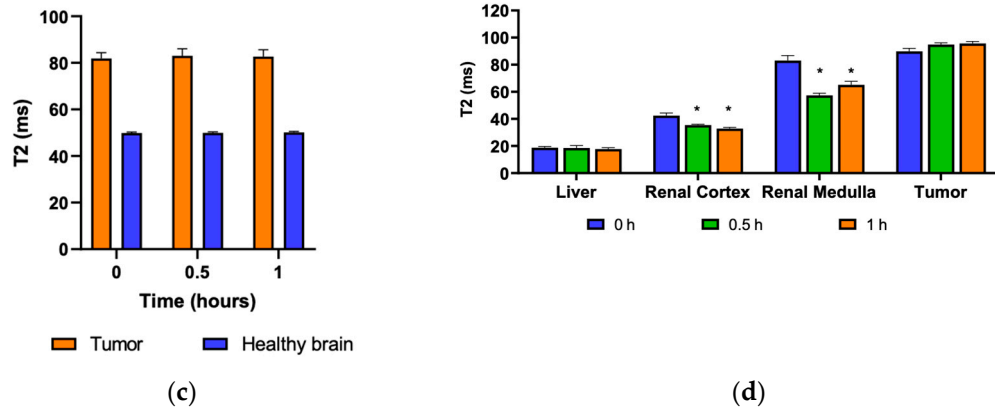
**Figure S5.** Biodistribution MRI studies of Endorem®. Graphics show the variation in MRI signal intensity (mean  $\pm$  SEM) in the different organs evaluated: tumor and contralateral healthy brain in the orthotopic glioma model (a) and liver, renal cortex, renal medulla and tumor in the heterotopic GBM model (b).



(a)



(b)



**Figure S6.** T2 maps and T2W images of GBM models injected with Endorem®. T2 color-code based maps and T2W images acquired before the i.v. administration of Endorem®, and at 0.5 and 1 h after the injection, in the of the orthotopic (a) and the heterotopic GBM model (b), numbers indicate the tissues where the ROIs were selected to do the measurements: 1, liver; 2, renal cortex; 3, renal medulla; 4, flank-tumor. Graphics show the variation in T<sub>2</sub> values (mean ± SEM) of the ROIs along the temporal evaluation in the orthotopic (c), and heterotopic tumor bearing mice (d). Statistical symbols correspond to the comparison of the data to the pre-injection value. (\* $p < 0.05$ ).