Supporting Information

RGD-Modified Apoferritin Nanoparticles for Efficient Drug Delivery to Tumors

Zipeng Zhen,[1,2] Wei Tang,[1,2] Hongmin Chen,[1,2] Xin Lin,[3] Trever Todd,[1,2] Geoffrey Wang,[1,2] Taku Cowger,[1,2] Xiaoyuan Chen,[3] Jin Xie[1,2,*]

[1]Department of Chemistry, University of Georgia, Athens, GA 30602, USA; [2]Bio-Imaging Research Center (BIRC), University of Georgia, Athens, GA 30602; [3]National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health (NIH), Bethesda, MD 20852, USA

*To whom correspondence should be addressed.

Dr. Jin Xie: jinxie@uga.edu

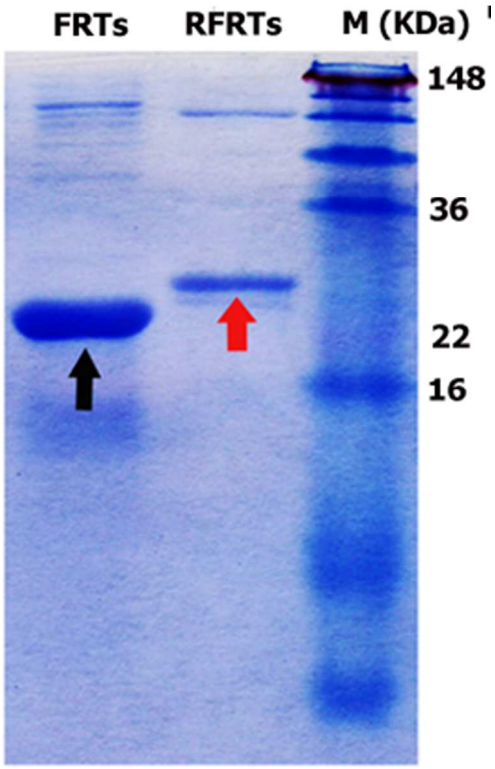

Figure S1. SDS-PAGE analyses with FRTs (the left lane) and RFRTs (the middle lane).

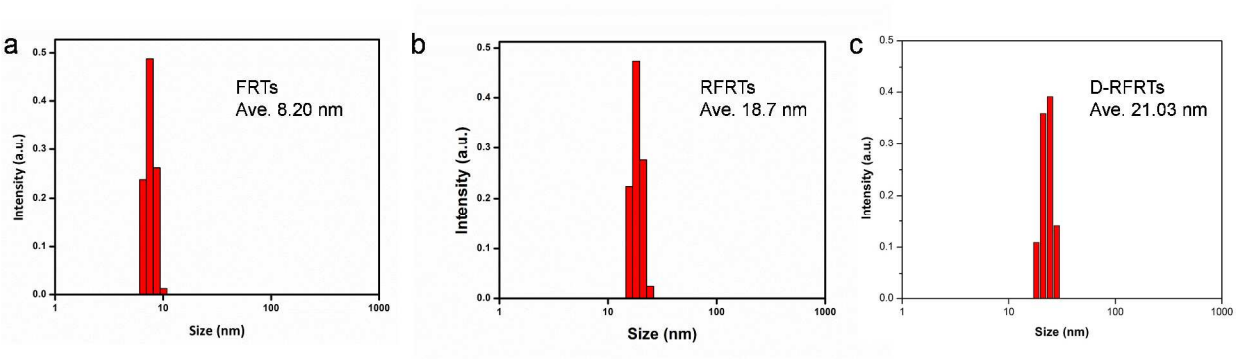

Figure S2. Dynamic light scattering analysis of a) FRTs, b) RFRTs and c) D-RFRTs. The average nanoparticle sizes are 8.2, 18.7, and 21.03 nm for FRT, RFRT, and D-RFRT, respectively.

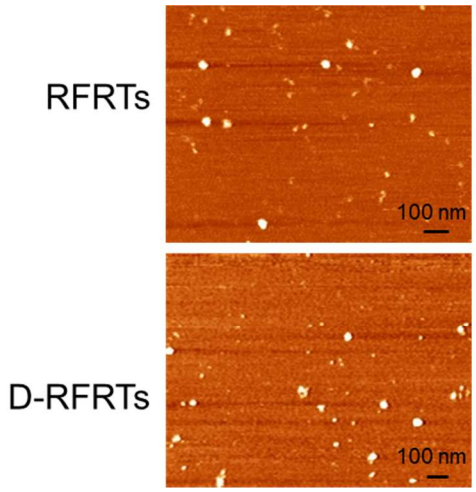

Figure S3. AFM analysis of the RFRTs and D-RFRTs. Average particle size is 18.32 ± 4.09 nm for RFRTs and 19.72 ± 2.28 nm for D-RFRTs.

	FRT or RFRT	Helper metals	Opening nanocages	Loading Rate (wt%)	Yield (%)
1	FRT	No	No	8.4	90.6
2	FRT	No	Yes	21.82	53.33
3	FRT	Mg^{2+}, 2:1	No	4.33	88.06
4	FRT	Mg^{2+}, 2:1	Yes	11.77	50.39
5	FRT	Mn^{2+}, 2:1	No	N/A*	< 15%
6	FRT	Mn^{2+}, 2:1	Yes	N/A*	< 15%
7	FRT	Zn^{2+}, 2:1	No	N/A*	<15%
8	FRT	Zn^{2+}, 2:1	Yes	N/A*	<15%
9	FRT	Fe^{3+}, 3:1	No	16.54	87
10	FRT	Fe^{3+}, 3:1	Yes	45.00	35
11	FRT	Cu^{2+}, 2:1	No	30.4	76.2
12	FRT	Cu^{2+}, 2:1	Yes	51.10	40
13	FRT	Cu^{2+}, 2:1; pre-blocking with Cu^{2+}	No	1.70	85.5
14	RFRT	No	No	14.14	78.4
15	RFRT	No	Yes	24.14	23.6
16	RFRT	Cu^{2+}; 2:1	No	73.49	33
17	RFRT	Cu^{2+}; 2:1	Yes	N/A*	< 15%
18	RFRT	Cu^{2+}, 2:1; pre-blocking with Cu^{2+}	No	8.28	56.4

Table 1. Using different conditions to load Dox into FRTs or RFRTs. Due to a relatively low RFRT production yield, we started the comparison with FRTs and then selected the best helper agent and conditions for RFRT loading. The drug loading was performed in PBS buffer (pH 7.4) with a total solution volume of 500 μl. The starting molar ratio between Dox and FRTs/RFRTs for all the conditions was 50:1. The "Help metals" column indicates the type of metal and the metal:Dox molar ratio that were used to form metal-Dox complexes. "Opening nanocages" means whether or not FRTs/RFRTs were disassembled during the drug loading. For disassembly, pH was first lowered to 2.0 and then tuned back to 7.4. "Loading rate (wt%)" means the Dox to FRTs/RFRTs weight percent in the final products. "Yield" means the weight ratio between FRTs/RFRTs in the final products and those in the initial solution. For Cu^{2+} blocking, 200× Cu^{2+} was first incubated with RFRTs. The free Cu^{2+} was removed by passing through a NAP-5 column. Incubation with Dox-Cu was performed subsequently. For all the metals, opening nanocages resulted in low loading rates. When using Zn(II) and Mn(II), we observed severe particle aggregation and because of that, very low yield (< 15%). When Mg(II) was used, we

found no significant change in drug loading. Compared with Fe(III), pre-complexation with Cu(II) led to a higher production yield and loading rate. Due to these reasons, we chose Cu(II) as the helper metal for loading Dox into RFRTs.

*Unable to compute the loading rate due to a low yield.

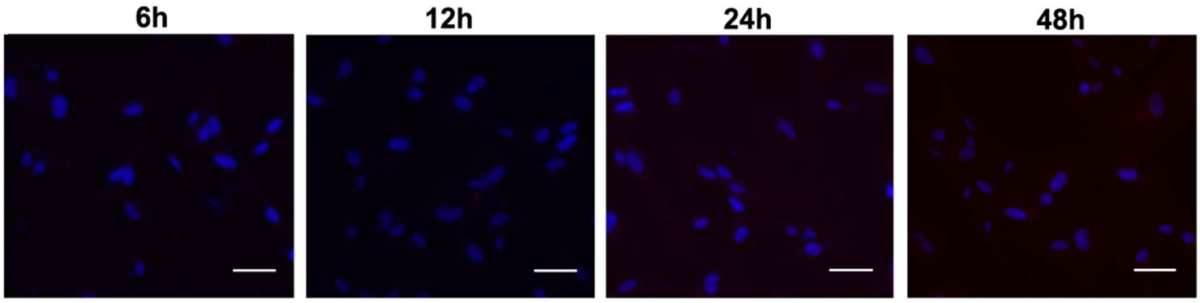

Figure S4. Co-incubating D-RFRTs with free c(RGDyK) (×20) efficiently blocked the particle internalization. Red, Dox; Blue, DAPI. Scale bars, 50 μm.

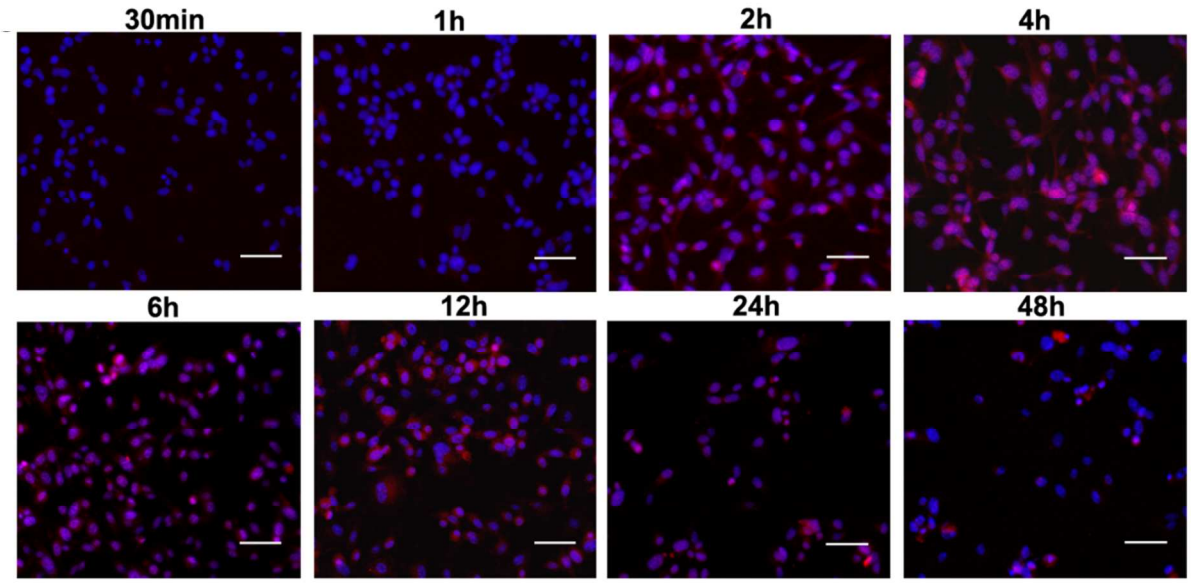

Figure S5. Internalization of D-RFRTs (12.5 μg Dox/mL) by U87MG cells at different time points. Red, Dox; blue, DAPI. Scale bars, 50 μm.

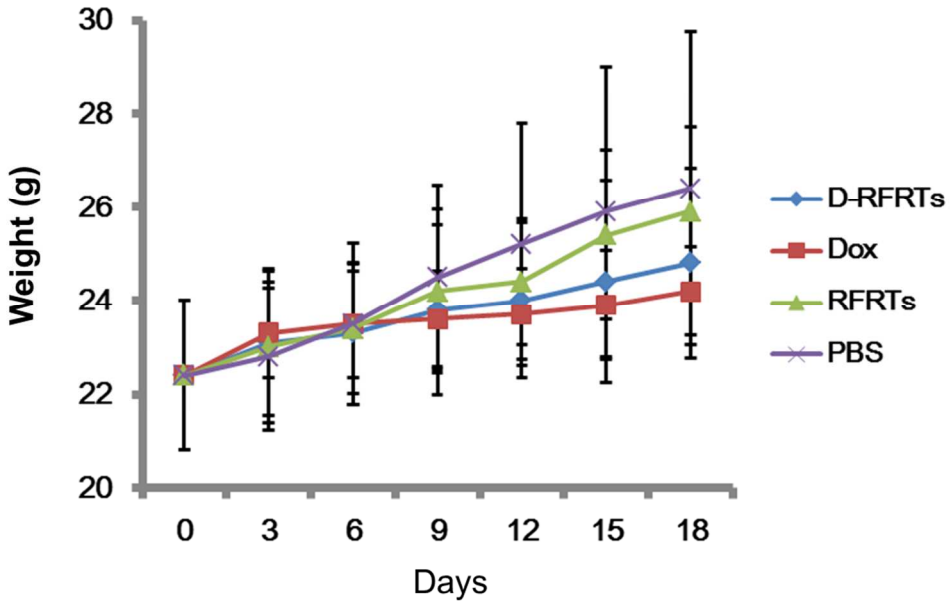

Figure S6. Body weight change for U87MG bearing mice treated with D-RFRTs, free Dox, RFRTs, and PBS. When taking into account of the tumor mass, there was no obvious weight loss for D-RFRTs treated mice.

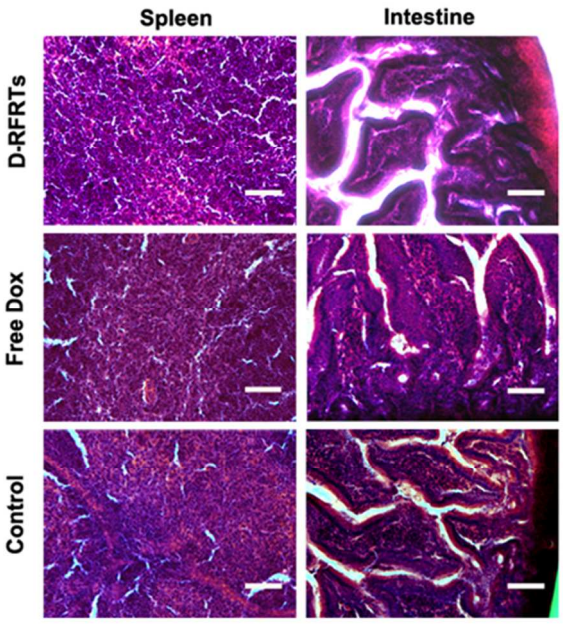

Figure S7. H&E staining on spleen and intestine samples taken from animal models treated with D-RFRTs, free dox, or PBS.