Supplementary Information

Semisynthetic Ferritin-based Nanoparticles with High Magnetic Anisotropy for Spatial Magnetic Manipulation and Inductive Heating

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Table of Content

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Supplementary Figures

DNA sequence:

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAA
CGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCAT
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Amino acid sequence:

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HVTNLRKMGAPESGLAEYLFDKHTLGDSDNES*

Figure S1 – Sequence of mEGFP–Linker–HCF.

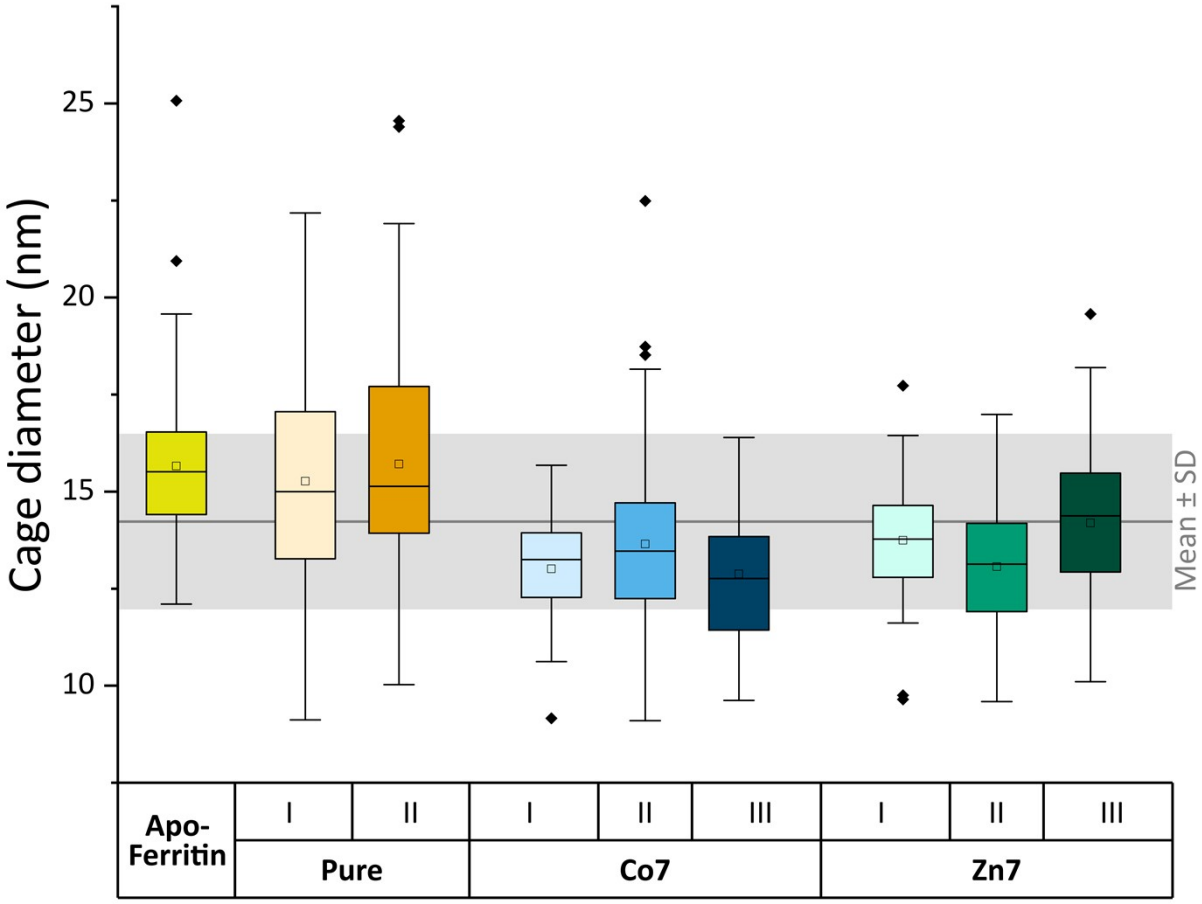

Figure S2 – Sizes of Ferritin cages.

Box plots show diameter of cages of empty Apo-Ferritin and doped Magnetoferritin of different synthesis runs (I, II, III). Mean values are shown as blank boxes, median values as horizontal lines (each $N \geq 41$; for all cages: mean ± SD = 14.2 ± 2.3 nm; for all MFt cages: mean ± SD = 13.9 ± 2.2 nm).

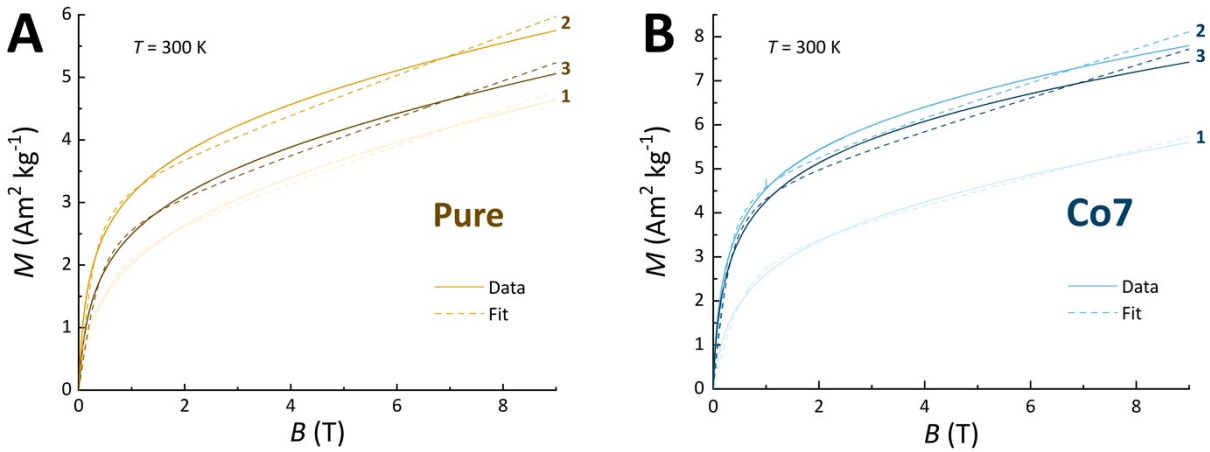

Figure S3 - Langevin Fits.

Measured magnetisation and Langevin fits of pure MFt and Co7 samples. Here, we use the Langevin function and an additional linear slope accounts for add-on contributions of salts, organics and possible antiferromagnetic contributions in the samples.

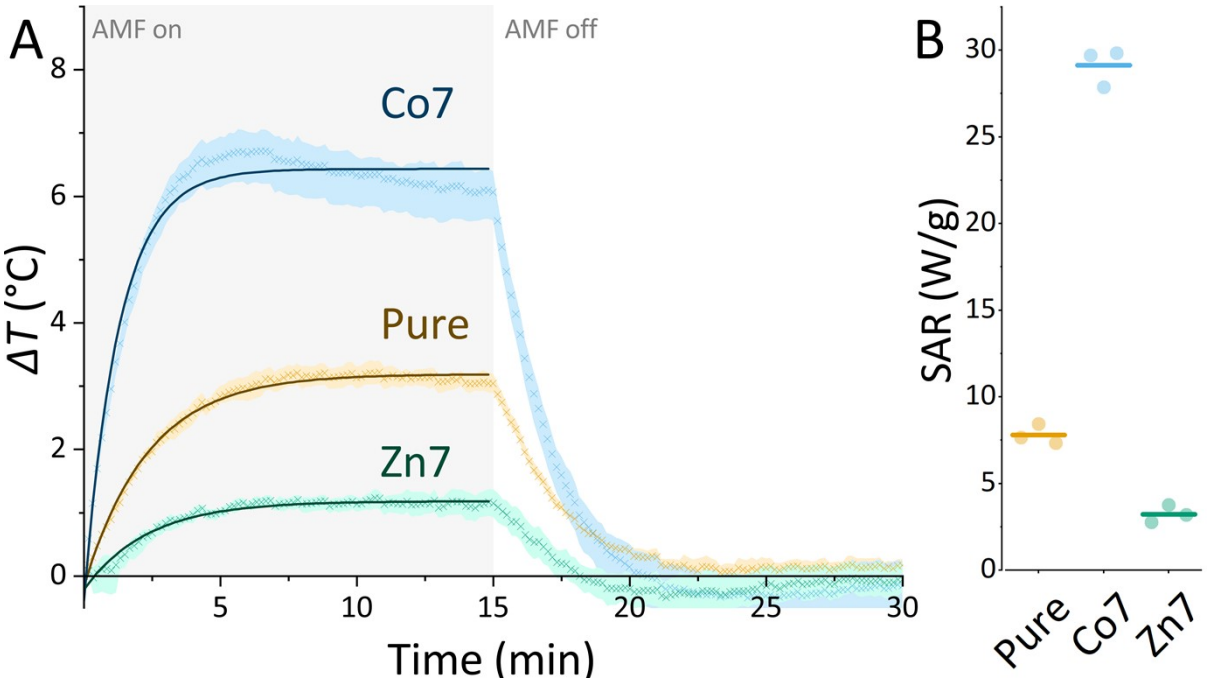

Figure S4 – Hyperthermia measurement.
(A) Temperature kinetics of Pure, Co7, and Zn7 MFt sample (each 35 µM) during exposure to the alternating magnetic field (45 mT, 104.5 kHz). Each curve is an average of three runs. The absolute temperature change $^{\Delta T}$ refers to the temperature increase above the temperature of pure buffer solution. All MFt and buffer solution samples were measured in an identical manner. (B) Specific Absorption Rate (W/g) of each run (coloured dot) and average value (coloured line).

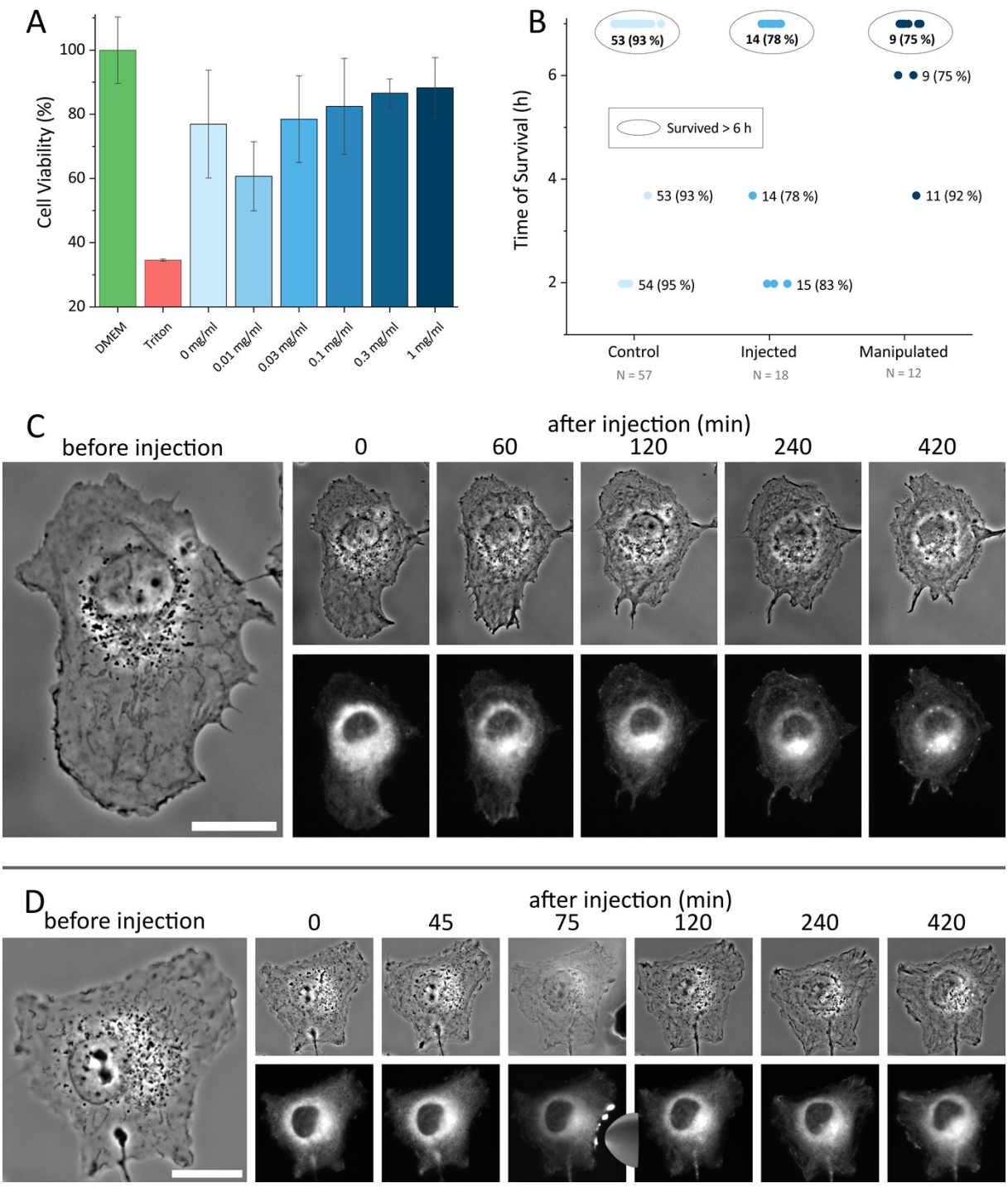

Figure S5 – Cell Viability Assays.
Biocompatibility of MFt Co7 tested in Cos7 cells. (A) CellTiter Blue assay. Cells were incubated with cell medium (DMEM) as positive control, 0.1 % Triton as negative control and several concentrations of MFt Co7 in HEPES-1 buffer (0.01 to 1 mg/ml) and with the same volume of HEPES-1 (0 mg/ml). (B) Cos7 cells were only observed ('control'), microinjected with MFt-Co7 at t = 0 h ('injected') or microinjected and magnetically manipulated for 15 min ('manipulated'). For the three conditions, 93 %, 78 %, and 75 % respectively of cells survived more than 6 h (360 min) after the beginning of the experiment/after injection (indicated by the black circle). (C) and (D) are exemplary images of Cos7 cells being (C) microinjected with MFt Co7 and observed for 420 min and (D) microinjected with MFt Co7, exposed to a magnetic stimulus after 60 min for 15 min and observed for up to 420 min. Images are taken in phase contrast (top row) and fluorescence for mEGFP (bottom row), scale bars are 20 µm.

Supplementary Tables

Table S1 – Properties of doped Magnetoferritins.
(D_H: hydrodynamic diameter, PdI: polydispersity index, both obtained from DLS measurements, ζ-Potential obtained from ELS measurements.)

Sample	Run	D_H (nm)	PdI	ζ-Potential (mV)
Pure	1	36.6 ± 1.9	0.16 ± 0.01	-5.6 ± 0.2
	2	36.8 ± 1.9	0.17 ± 0.01	-5.4 ± 0.5
	3	36.6 ± 2.2	0.16 ± 0.01	-4.4 ± 0.5
Co7	1	46.5 ± 2.5	0.16 ± 0.01	-4.7 ± 0.9
	2	45.1 ± 3.0	0.17 ± 0.01	-5.1 ± 1.6
	3	42.4 ± 3.4	0.19 ± 0.01	-4.3 ± 0.5
Zn7	1	49.9 ± 5.3	0.18 ± 0.01	-4.5 ± 0.7
	2	44.9 ± 3.1	0.17 ± 0.01	-5.1 ± 0.9
	3	35.5 ± 2.6	0.17 ± 0.01	-4.8 ± 0.8

Supplementary Videos

Video S1 – Droplet Assay with Pure MFt.
A droplet of undoped MFt was placed on a coverslip coated with PDMS. A magnetic tip consisting of a neodymium magnet with spring steel tip as described in Novoselova et al.[1] was placed at a distance of 20 µm to the edge of the droplet and left there for up to 1 h. The attraction of MFt towards the tip was recorded via the movement of mEGFP.

Video S2 – Spatial Manipulation of MFt Co7 inside a Cell.
The magnetic tip used in Video S1 is approached to a cell in which MFt Co7 was microinjected. The MFt is reversibly attracted by the magnetic field gradient emerging from the tip. The tip is placed at various positions around the cell and the particles are attracted by the tip to different sites. Particles diffuse away after tip removal within a few minutes.

Video S3 - Spatial Manipulation of MFt Co7 inside a Nucleus.
The magnetic tip used in Video S1 is approached to a cell exhibiting MFt Co7 in the cell nucleus. The MFt is reversibly attracted by the magnetic field gradient emerging from the tip. The tip is successively placed at three different positions around the cell and particles are attracted by the tip and diffuse away after tip removal within a few minutes.

1 I. P. Novoselova, A. Neusch, J. S. Brand, M. Otten, M. R. Safari, N. Bartels, M. Karg, M. Farle, U. Wiedwald and C. Monzel, *Nanomaterials*, 2021, **11**, 1–20.