Supporting Information

Single Coating of Zinc Ferrite Renders Magnetic Nanomotors Therapeutic and Stable against Agglomeration

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Experimental methods:

Fabrication of the zinc ferrite coated magnetic nanomotors:

Following the fabrication of nanohelices using GLancing Angle Deposition (GLAD), the substrate was subsequently tilted at a shallow angle with respect to the source, followed by the deposition of iron. The substrate contained approximately 8 X 10^8 Silica helices in a 1.5 cm × 1.5 cm wafer. These iron-coated nanomotors were used as such for zinc ferrite coating using a modified recipe. In brief, 0.4 mmol of zinc acetylacetonate and 0.8 mmol of iron acetylacetonate (Merck) were taken in a solution containing equal volumes of ethanol (HPLC grade) and decanol (SRL). This reaction mixture, with the wafer containing the Fe-SiO_2 helices immersed in it, was irradiated in a microwave synthesizer (CEM Discover SP) with total reaction and hold time of 7 minutes at 200 W, keeping the reaction temperature and pressure at 150° C and 150 psi, respectively. Annealing of the ZF-Fe-SiO_2 motors was carried out in air using a halogen lamp furnace at 300° C for 10 minutes. The ramp up and down rate for annealing was 200° C/min and 100° C/min, respectively.

Characterization of the ferrite coated nanomotors:

The morphology and surface composition of the ferrite-coated nanomotors were characterized using scanning electron microscopy and EDS (CARL ZEISS). The crystallinity of the samples was examined

using X-ray Diffractometry (XRD, Rigaku). The chemical nature of the film surface was determined by X-ray Photoelectron Spectroscopy (Kratos Analytical). An inductively coupled plasma atomic emission spectrometer (ICP-AES, iCAP 6500, Thermo) was used for the elemental analysis and concentration evaluation of zinc ferrite-coated nanomotors. The samples were prepared by overnight digestion of 150 µl of zinc ferrite-coated nanomotors solution in 2 ml of concentrated nitric acid. Subsequently, the sample was diluted with deionized water to a final volume of 25 ml, passed through 0.45 micron filter paper and used for analysis.

Magnetic actuation and stability experiments:

The details of the coil and driving electronics to generate the magnetic fields have been discussed in previous papers from our group. To determine the relative stability of the magnetic nanomotors with and without the ferrite coating, we cut out approximately equal pieces from the wafers containing the two samples: ZF-Fe-SiO_2 and Fe-SiO_2 nanomotors. These samples were sonicated, thereby releasing the nanomotors into deionized water or PBS. The samples were stored for duration greater than 6 months, after which they were shaken and re-suspended in solution. Subsequently, microfluidic chambers were prepared and the population of individual nanomotors and aggregates of nanomotors in each of the samples was estimated. The time of observation remained similar for all the samples. The aggregate area was calculated using Image J software from the sequence of images recorded for each sample.

Magnetic hyperthermia experiments:

The characterization of heat dissipation of ferrite-coated nanomotors was carried out using an induction heating system (Ambrell, Easy heat, 4.2 kW). The hyperthermia setup is detailed in supporting information (Fig S6). The ferrite coated nanomotors were sonicated out in 500 µl of 1X PBS and exposed to AC magnetic fields at 232 kHz under magnetic field amplitudes up to 28.2 kA/m. The temperature rise was measured with an alcohol thermometer. SAR calculation was carried out using the standard equation:

$$SAR\left(\frac{W}{g}\right) = \frac{C}{m}\left(\frac{dT}{dt}\right)$$

where C is the specific heat capacity of the solvent (C_{water} = 4185 J L^{-1} K^{-1}), m is the concentration (g/L of Fe) of magnetic material in solution. Note that the final values are reported as (W/g_{Fe}). The measurements were carried out in nonadiabatic conditions; thus, the slope of the curve dT/dt was measured by taking into account only the first 300 seconds of the curve.

Cell culture:

The ferrite-coated propellers were sonicated in PBS, followed by UV sterilization for 10 minutes. Hela cells (ATCC) were grown in 25 cm^2 culture flasks with Dulbecco's modified Eagle's medium (DMEM, HiMedia), supplemented by 10% Fetal Bovine Serum (Sigma Aldrich) and 1% antibiotic-antimycotic solution (Sigma Aldrich). Cells were incubated at 37^oC in CO_2 incubator (New Brunswick^{TM} Galaxy 1705) with 5% CO_2 and 90% humidity. Tissue culture polystyrene (TCPS) well plates were used as a control in the cell culture experiments.

Cell viability after magnetic hyperthermia run using ferrite-coated nanomotors:

5 x 10^4 cells were used for the heat treatment experiments after one day of incubation. After the incubation period, the ferrite-coated nanomotors (1 mg/ml and 2 mg/ml) were added and the samples were subjected to induction heating for 20 minutes at 232 kHz and 400 kA/m. Following the treatment, MTT assay was carried out, where absorbance was recorded at 570 nm using a plate reader (Tecan Infinite M 1000 PRO). In order to prevent any interference from ferrite-coated propellers during measurement; the dissolved formazan crystals were centrifuged to remove the pellet. Control samples were ferrite nanomotors with no exposure to magnetic hyperthermia run.

Biocompatibility of the ferrite-coated nanomotors:

The metabolic activity of the cells after the heat treatment and in the presence of ferrite-coated propellers was assessed by MTT assay. For the biocompatibility experiments, 5 x 10^4 cells were seeded in each well of the tissue culture plate and incubated for 24 hours. Post the incubation period, the ferrite-coated propellers in concentrations of 0.5 mg/ml, 1 mg/ml, 2mg/ml and 3mg/ml were added to the wells in 1:1 ratio of PBS and cell culture media. MTT assays were carried out after 1 day and 3 days of incubation, as described above.

Statistical Analysis:

Statistical analysis was carried out with SPSS 16.0 software (IBM, USA) using the ANOVA method. All experimental data were plotted as mean $\pm$ standard error. The differences were considered statistically significant at $p \leq 0.05$.

Cell morphology analysis:

The cellular morphology before and after hyperthermia run in the presence of ferrite-coated nanomotors was analysed using fluorescent microscopy (InCell Analyser 6000, GE Healthcare Life Sciences). About 5 x10^4 HeLa cells were incubated for 24 hours, following which ferrite-coated nanomotors of a concentration were added to the respective wells and subjected to hyperthermia run for 20 minutes. The cells were then fixed with 3.7% formaldehyde (in 1X PBS) for 20 minutes. The cells were permeabilized using .1% Triton – X 100 (in 1X PBS) and blocked using 5% FBS (in 1X PBS). ActinGreen 488 ready

probes reagent and Hoechst 33258 dyes were used to stain actin filaments and nucleus of the cells, respectively.

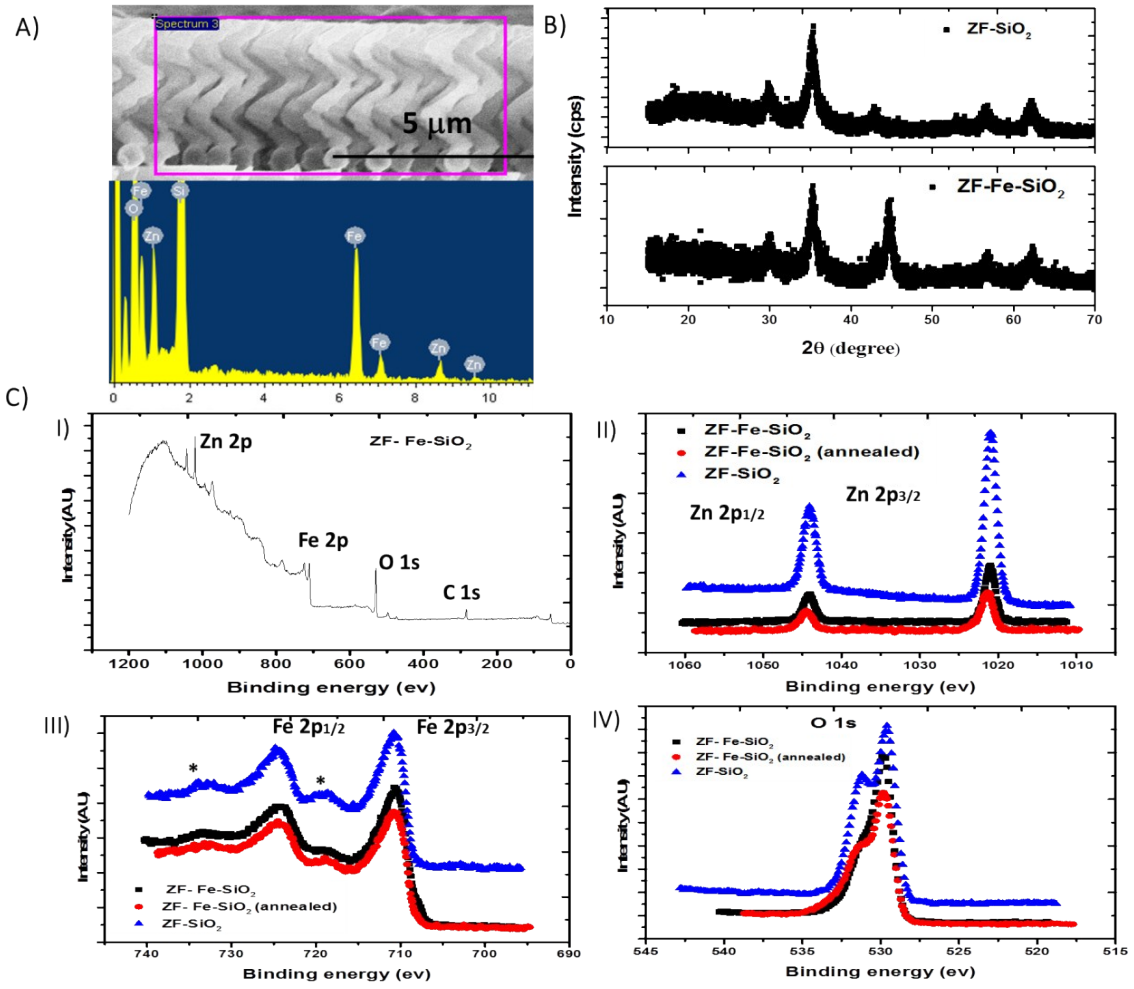

Figure S1: Characterization of the various nanomotors under study: a) EDS (Energy Dispersive X-ray Spectroscopy) b) XRD (X-ray Diffraction Spectra) and c) XPS (X-ray Photoelectron Spectroscopy).

The elemental analysis by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry) and EDS (Energy Dispersive X-Ray Spectroscopy), carried out on ZF-Fe-SiO_2 samples, showed Zn, Fe, O and Si peaks. XRD (X-ray Diffraction) studies on ZF-Fe-SiO_2 revealed peaks which could be indexed to the zinc ferrite spinel structure. The chemical states of the elements were determined using XPS (X-Ray Photoelectron Spectroscopy). The presence of the principal peak of Fe 2p3/2 at 711.2 eV and a satellite peak (denoted by *) ~8.4 eV away at 719.86 eV and another satellite peak at 733.3 eV confirmed that only Fe^{3+} was present on the film. The Zn^{2+} showed a small peak at 1022.9 eV, evidencing that Zn^{2+} ions occupy both tetrahedral A sites and octahedral B sites, leading to partially inverted spinel structure[1,2].

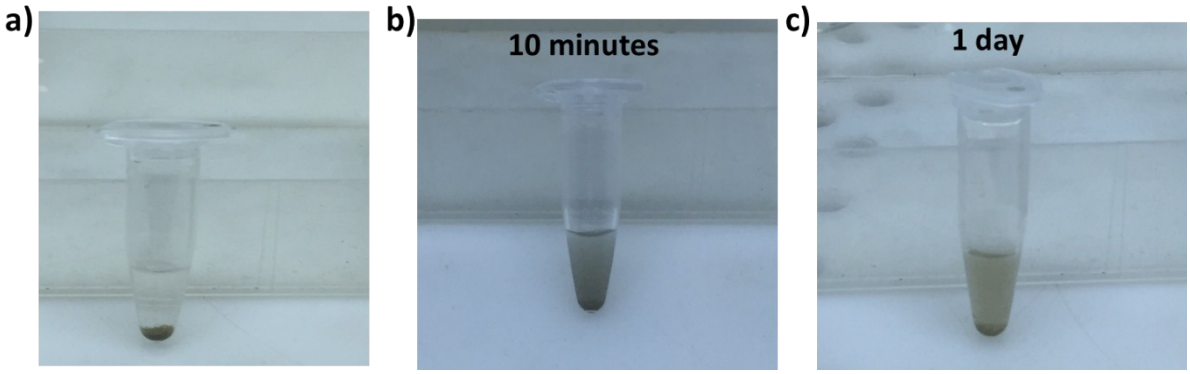

Figure S2: Suspension of Fe-SiO_2 nanomotors (a) before shaking, (b) shaking after 10 minutes and (c) shaking after 1 day of storage.

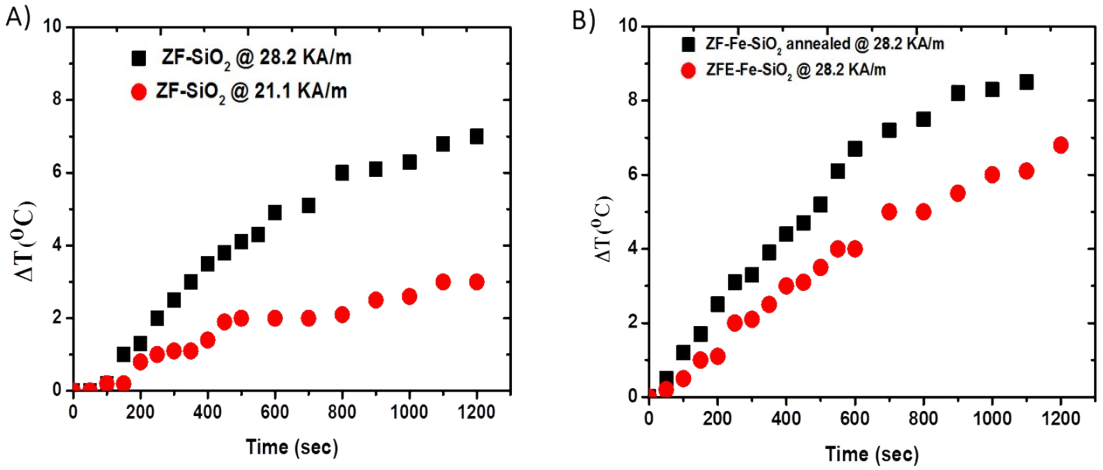

Figure S3: Effect of annealing and magnetic field strength on hyperthermia efficiency. a) Plot of temperature as a function of applied magnetic field strength for ZF-SiO_2 nanomotors in PBS. b) Plot of temperature as a function of annealing for the same applied magnetic field strength, for ZF-SiO_2 nanomotors in PBS.

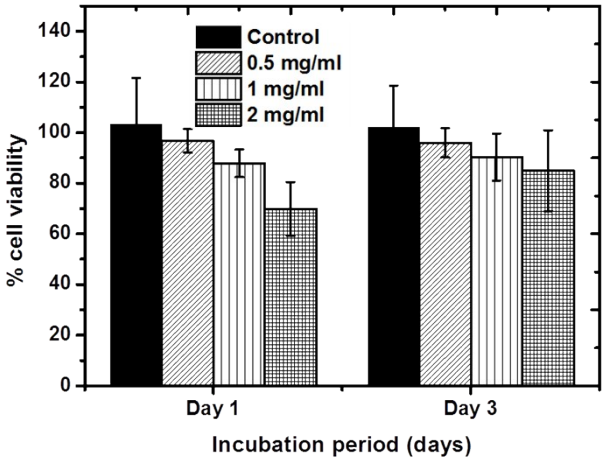

Figure S4: Biocompatibility results of different concentrations of ferrite-coated nanomotors.

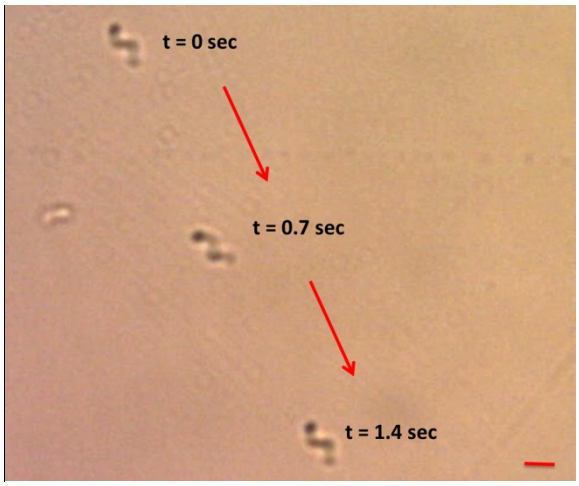

Figure S5: Microscope image showing the trajectory of ferrite-coated nanomotor moving at 6.5 body lengths/second. The movie was recorded at 102 fps. Scale bar corresponds to 2 µm.

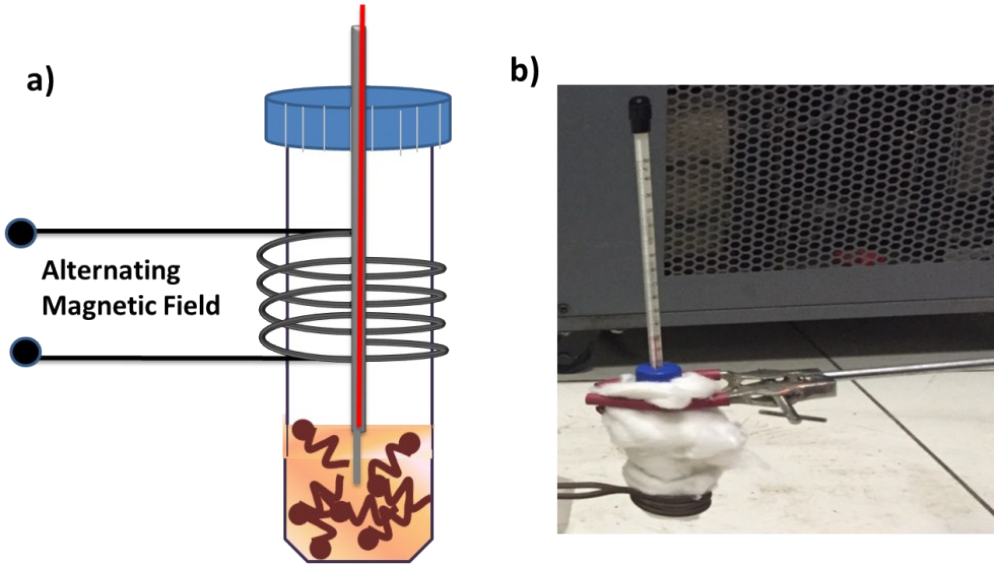

Figure S6: a) Schematic of the hyperthermia setup; b) Image showing the hyperthermia setup with the nanomotors containing solution undergoing experimentation.

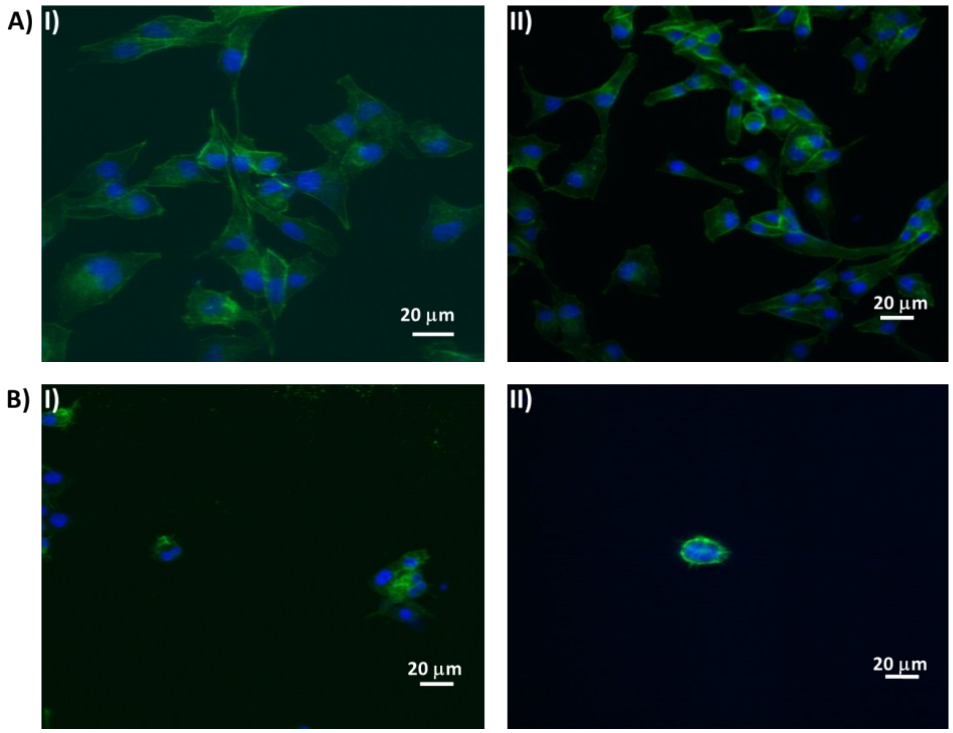

Figure S7: Representative fluorescent images of the cells with 1 mg/ml ferrite-coated nanomotors (A) before (I, II) and (B) after (I, II) the hyperthermia run.

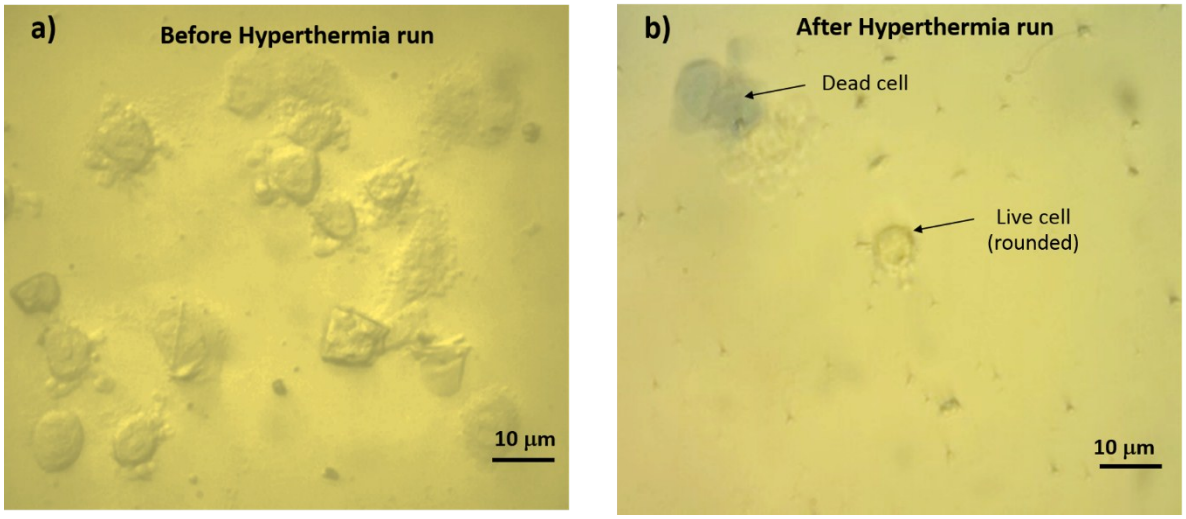

Figure S8: Targeted Hyperthermia. Representative bright field microscopic images of HeLa cells with ferrite coated nanomotors (a) before and (b) after hyperthermia run following trypan blue staining. The dead and live cells have been marked with arrows.

The hyperthermia experiments were carried out on adhered HeLa cells on coverslips in the presence of ferrite coated nanomotors and cells were stained using trypan blue to distinguish between live and dead cells. The cells were live (not stained) in the presence of ferrite coated nanomotors without hyperthermia run which implies that these nanomotors do not themselves induce cell death. As shown in Fig S8 (b), following the hyperthermia run, majority of the HeLa cells got detached from the coverslip surface, denoted by the fewer cells. A part of the remaining cells on the coverslips were dead (trypan blue stained) and the rest of the cells on the coverslip were rounded, which could get detached from the surface while still maintaining viability for long time before committing to cell death[3]. The presence of ferrite coated nanomotors near the cells is very crucial during the hyperthermia run to specifically kill cells as shown in the figure, thereby demonstrating targeted hyperthermia potential.

Movie M1: Swarm of nanomotors actuated in water at magnetic field 30 G and frequency of rotating field at 25 Hz. The movie was recorded at 65 fps.

Movie M2: Swarm of nanomotors actuated in water at magnetic field 70 G and frequency of rotating field at 30 Hz. The movie was recorded at 91 fps.

Movie M3: ZF-coated nanomotor actuated in water at magnetic field 50 G and frequency of rotating field of 50 Hz. The movie was recorded at 102 fps.

Movie M4: ZF-coated nanomotor actuated at magnetic field 60 G and frequency of rotating field of 10 Hz. The movie was recorded at 15 fps. The nanomotors were maneuvered in a dish containing adhered HeLa cells in DMEM.

References:

(1) Sai, R.; Kulkarni, S. D.; Vinoy, K. J.; Bhat, N.; Shivashankar, S. A. *J. Mater. Chem.* **2012**, *22*, 2149–2156.

(2) Sai, R.; Kulkarni, S. D.; Bhat, S. S. M.; Sundaram, N. G.; Bhat, N.; Shivashankar, S. A. *RSC Adv.* **2015**, *5*, 10267–10274.

(3) Skommer, Joanna Darzynkiewicz, Z.; Wlodkowic, D. *Cell Cycle* **2010**, *9*, 2330–2341.