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Supplementary Information

Release of proteins and enzymes from vesicular compartments by alternating magnetic fields

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S.1. Experimental details

Instrumentation: Fluorescence measurements were recorded using a Perkin Elmer LS55 fluorimeter, UV-visible spectrophotometry was performed on a V-660 spectrometer (Jasco (UK) Ltd.). Vesicles were extruded using a LiposoFast liposome (vesicle) extruder from AVESTIN Europe GmbH. Sonication was performed using a VCX130PB probe-type sonicator (Sonics & Materials Inc. 130W). Centrifugation was performed using a Heraeus Instruments Megafuge 1.0R for large tubes and a Beckman Coulter Microfuge 16 for Eppendorf tubes. Vortex mixing was performed using a Vortex Genie 2 (600-2700 rpm). Transmission electron microscopy (TEM) was performed using a Jeol 1220, 120 kV instrument with a GATAN ORIUS CCD camera or a Tecnai T20, 220 kV. Inductive heating of nanoparticles was conducted using an alternating magnetic field (AMF) generated by a water-cooled EASYHEAT 0224 induction heater (2.0 kW, 150-400 kHz, 3 cm loop diameter, 1.5 turns) with an EASYHEAT 300P workhead (392 kHz). QCM-D was performed using a Q-Sense E4 (Biolin Scientific, UK). NdFeB magnets ring/cylinder shape (5600 Gauss) were obtained from Magnet Expert Ltd.

Materials: DPPC, DOPC and biotinylated lipids were obtained from Avanti Polar Lipids Inc., (AL, USA). Avidin was obtained from Invitrogen (Thermo Fisher Scientific Inc. (USA)). Unless otherwise stated, all other reagents were obtained from Sigma-Aldrich Co. (UK). The 'Protease fluorescent detection kit' product code PF0100 was obtained from Sigma-Aldrich (UK). Polycarbonate membranes for vesicle extrusion were obtained from AVESTIN Europe GmbH. PD-10 Sephadex gel-permeation desalting columns were obtained from GE Healthcare Bio-Sciences AB (Sweden).

APTES nanoparticle coating procedure: Uncoated Fe_3O_4 MNPs (Sigma-Aldrich Co. UK, Product No. 637106) (20 mg) in a 50 mL plastic tube were suspended in MeOH (5 mL) by sonication using a probe-type sonicator until a homogeneous suspension was obtained (approximately 20 minutes). To this suspension was added (3-aminopropyl)triethoxysilane (APTES) (0.177 g, 0.187 mL, 8×10^{-4} mol) and the mixture was sonicated for a further 1 h. The sample tube was then sealed and the mixture was shaken overnight. The MNPs were sedimented by application of an NdFeB permanent magnet (5600 Gauss) to the exterior of the tube and the supernatant solution carefully decanted. The MNP mass was then resuspended in fresh MeOH (5 mL) using brief bath-type sonication, followed by sedimentation of the MNPs and removal of the supernatant. The process was then repeated at least 6 times. After the final resuspension, the suspension was transferred to a round-bottomed flask and the solvent was removed under reduced pressure using a rotary evaporator to give APTES-MNPs as a black to dark brown powder. In the acid catalysed coating procedure, two to three drops of glacial acetic acid were added to the uncoated MNP suspension immediately prior to the addition of APTES.

TNBS free amine assay: The assay described here is a modified version of the procedure developed by Edwards-Lévy *et al*. [1] Free amine content of APTES-MNP samples was determined by the reduction in absorbance at 410 nm relative to controls performed with uncoated MNPs and interpolation into a standard curve. Dry APTES-MNPs (or uncoated MNPs for controls) (5 mg) were suspended in borate buffer (10 mM sodium borate, 150 mM NaCl, pH 8, 3.2 mL) by brief sonication (~2 minutes) using a probe-type sonicator until a homogeneous suspension was obtained. To this suspension was added 2,4,6-trinitrobenzene sulfonic acid (TNBS) solution (4 mM TNBS in *N,N*-dimethylformamide (DMF), 4 mL). The sample was then incubated for 1 h in the dark at 40 °C, after which time the MNPs were magnetically sedimented by application of an NdFeB magnet and 900 µL of the supernatant was removed and transferred to a new vessel. To this 900 µL of supernatant was added valine solution (100 µL, 40 mM in borate buffer, pH readjusted to pH 8). Control samples were prepared by adding 100 µL of borate buffer without valine at this step. These samples were incubated for 1 hour at 40 °C in the dark. After this time the samples were each diluted with HCl solution (0.5 M, 11 mL). The diluted samples were then transferred to quartz UV-visible cuvettes (3 mL volume, 1 cm path length) the absorbance spectrum was measured and the absorbance at 410 nm recorded. A calibration curve was established by preparing five dilutions of the TNBS solution and using these in place of the 4 mM TNBS solution, without the addition of MNPs, which established a relation between the absorbance at 410 nm and the concentration of TNBS in the 900 µL aliquot.

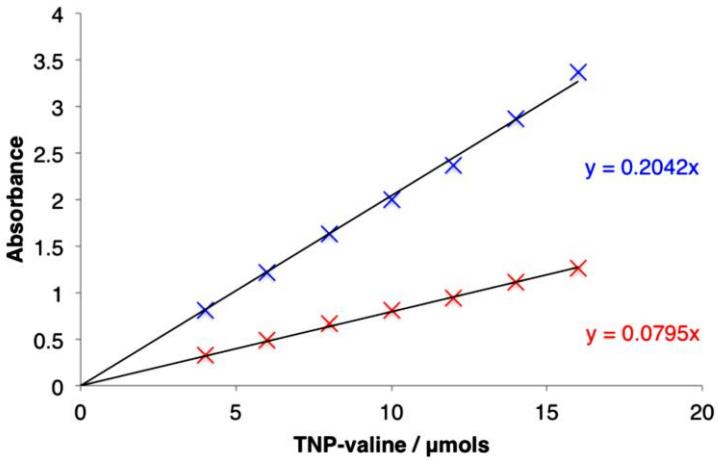

Figure S1. Calibration plot for the TNBS assay. Absorbance was measured at 410 nm (×) and 340 nm (×) for solutions of known TNBS concentration. The number of mols of TNP-valine does not refer directly to the amount in the cuvette but he amount in the assay solution (after incubation with valine solution).

Functionalisation of APTES-MNPs with 21-[D(+)-biotinylamino]-4,7,10,13,16,19-hexaoxaheneicosanoic acid 'Bt-PEG-acid': APTES-MNPs (10 mg) were resuspended in dry DMF (2 mL) using probe sonication for 10 minutes. 21-[D(+)-Biotinylamino]-4,7,10,13,16,19-hexaoxaheneicosanoic acid (5 mg, 3.45 µmol) and HBTU (1.3 mg, 3.45 µmol) were dissolved in

dry DMF (0.5 mL) with *N,N*-diisopropylethylamine (0.45 mg, 3.45 µmol), and the resulting solution was stirred for 1 h before being added to the MNP suspension. The resulting mixture was then sonicated for a further 1 h, before being sealed in a plastic centrifuge tube (15 mL) and shaken overnight. Six rounds of magnetic sedimentation and resuspension in fresh DMF were performed, followed by removal of the solvent under reduced pressure. The Bt-PEG-APTES coated MNPs were obtained as a black solid.

HABA-avidin biotin assay: A modification of the method of Lackey *et al*. was developed specifically to quantify the biotinylation of APTES-MNPs with Bt-PEG-acid (Bt-APTES-MNPs).[2] An aliquot (75 µL) of 2-(4'hydroxybenzeneazo)benzoic acid (HABA) stock solution (0.53 mM in 10 mM NaOH) was added to Dulbecco's phosphate buffered saline (PBS) (2 mL) in a quartz cuvette. Avidin (69 µL of a 10 mg mL^{-1} solution in distilled water) was then added to the cuvette, the addition was accompanied by an immediate colour change from the yellow-orange of the HABA solution to a deep red (HABA-avidin complex). The solution was then agitated to assist in mixing, transferred to a UV-visible spectrometer and the absorbance values at 500 nm and 350 nm were recorded. An aliquot of biotinylated MNP stock suspension (20 µL, 10 mg mL^{-1} in PBS) was added to the cuvette, the solution was agitated to facilitate mixing and the mixture was incubated for 20 minutes at room temperature. The cuvette was then placed directly on top of an NdFeB magnet (5600 G) for a further 20 minutes to ensure complete sedimentation of the MNPs. The sample was then returned to the spectrometer and the absorbance spectrum re- recorded, the change in absorbance at 500 nm was determined using the spectrometer software. Several aliquots (each 20 µL, 10 mg mL^{-1} in PBS) of MNPs were added sequentially to each sample of HABA-avidin in this way and the incremental change in absorbance recorded after each addition.

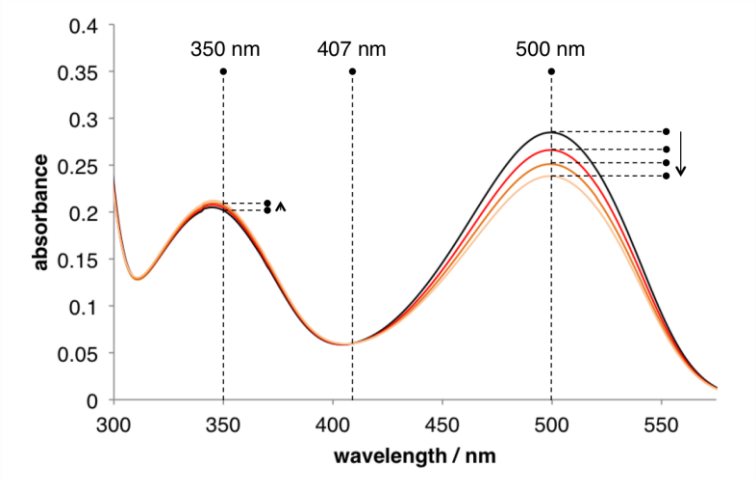

Figure S2: HABA assay plots. Absorbance spectra for the HABA assay measured after sequential additions of Bt-APTES-MNP solutions: (**–**) HABA-avidin only solution, (**–**) + 0.2 mg MNPs, (**–**) + 0.4 mg MNPs, (**–**) + 0.6 mg MNPs. An isosbestic point is observed at 407 nm, abs = 0.06

Figure S2 contains spectra for a complete HABA assay, the spectrum of the initial HABA-avidin complex solution was recorded and then spectra for sequential additions of 0.2 mg MNP in 20 µL PBS (after incubation and sedimentation). The expected decreases at 500 nm can be observed and they correlate well with the smaller, proportional increases at 350 nm. Additionally an isosbestic point is observed at 407 nm. Example calculations for the data in **Figure S2** are given in **Table S1** below.

Complex at 500 nm	**HABA + Avidin only**	**0.2 mg MNPs**	**0.4 mg MNPs**	**0.6 mg MNPs**
Absorbance	0.276571	0.259533	0.245117	0.234475
conc / M	7.8×10^{-6}	7.3×10^{-6}	6.9×10^{-6}	6.6×10^{-6}
Δ conc / M		5.08×10^{-7}	9.14×10^{-7}	1.21×10^{-6}
Δ µmols		0.001	0.002	0.0024
µmols g^{-1}		50.78	45.69	40.45
Average µmols g^{-1}				45.64
Blank subtracted				**13 ± 5 µmols g^{-1}**
HABA at 350 nm	**HABA + Avidin only**	**0.2 mg MNPs**	**0.4 mg MNPs**	**0.6 mg MNPs**
Absorbance	0.18893	0.192267	0.195454	0.206454
conc / M	9.2161×10^{-6}	9.38×10^{-6}	9.53×10^{-6}	1.01×10^{-5}
Δ conc / M		1.63×10^{-7}	3.18×10^{-7}	8.55×10^{-7}
Δ µmols		0.00033	0.00064	0.0017
µmols g^{-1}		16.28	15.91	28.49
Average µmols g^{-1}				20.23
Blank subtracted				**10 ± 7 µmols g^{-1}**

Table S1: Example calculations of the loading of biotin on MNP using HABA:avidin assay from the data presented in Figure S2.

Production of phospholipid vesicles: The desired mixture of lipids was prepared by combining quantities of lipid stock solutions in spectroscopic grade chloroform in a 5 mL round-bottomed flask such that they will afford a 20 mM (total lipid concentration) solution when resuspended in 1 mL of aqueous solution (20 µmol lipid). The chloroform was then removed under reduced pressure on a rotary evaporator to generate a thin lipid film on the walls of the flask, this was further dried on a high vacuum line for at least 1 h. The desired buffer solution (1 mL) was then added to the flask and the lipid film was suspended in the solution with vigorous vortex mixing and warming to above the T_m with the use of a heat gun until no lipid film could be observed on the walls of the flask. The

resulting suspension was passed through a polycarbonate membrane with 800 nm diameter pores using a LiposoFast extruder (AVESTIN Europe GmbH). The extruder was warmed to T>Tm using a heat gun and the suspension passed through the membrane at least 19 times (19 passes through the membrane was determined by MacDonald et al. to be the maximum number that produced a measurable improvement in the monodispersity of the vesicles).12 Care was taken that the suspension was in the initial receiving syringe at the end of the process to ensure no non-extruded material was taken forwards. The table below lists the different lipid compositions and encapsulated solutions for different vesicle experiments.

Experiment	**Lipid composition (20 µmol lipid per sample)**	**Encapsulated solution**
MNPV 5/6-CF release/ leakage	DPPC + 0.2 mol% Bt-cap-PE (4×10^{-8} mols)	0.05 M 5/6-CF in MOPS 20 mM, NaCl 100 mM, pH 7.4
MNPV protein release	DPPC + 0.2 mol% Bt-cap-PE (4×10^{-8} mols Bt-cap-PE)	10 mg mL^{-1} protein
MNPV QCM-D Bt-cap-PE	DOPC + 0.2 mol% Bt-cap-PE (4×10^{-8} mols)	HEPES 10 mM, 150 mM NaCl, $CaCl_2$ 2 mM pH 7.4
QCM-D controls (no functionalised lipid)	DOPC only	HEPES 10 mM, 150 mM NaCl, $CaCl_2$ 2 mM pH 7.4

Table S2: Lipid compositions and encapsulated solutions used for different MNPV experiments.

To improve encapsulation of proteins, a freeze-thaw procedure was added to the standard vesicle preparation. A lipid film was prepared in a 5 mL round-bottomed flask and resuspended in a solution of the desired protein (cytochrome c, trypsin, 10 mg mL^{-1} in PBS). The resulting suspension was frozen by submersion of the flask to above the level of the suspension in a bath of liquid nitrogen. Once completely frozen the flask was then gently warmed using a heat gun on a low setting, taking care to continuously move the flask to ensure even heating. Once fully thawed, the flask was returned to the liquid nitrogen bath and this process was repeated 10 times (determined to be optimum balance between improved encapsulation and freeze-thaw induced denaturation of the protein by Colletier et al. for acetylcholinesterase).[3] Once this process was complete the vesicles were then extruded as described above.

Purification of vesicles, (1) Size-exclusion 'GPC' chromatography: Unencapsulated material was removed by gel permeation chromatography (GPC), using PD-10 columns packed with Sephadex G-25 medium (size exclusion limit Mr = 7,000 Da). The standard protocol recommended by the manufacturer was employed in all cases. First the column was equilibrated with PBS (25 mL). Extruded vesicle suspension (20 mM lipid concentration, 1 mL) was diluted to 2.5 mL with

PBS and loaded onto the column, the resulting 2.5 mL of eluent from this step was discarded. Next, PBS (3.5 mL) was added to the column and the eluent collected. This suspension contained the purified vesicles, the suspension was returned to 20 mM by centrifugation at 2200 × g for 15 minutes followed by removal of the supernatant and resuspension of the vesicle mass in a total volume of 1 mL PBS. A second purification of the sample was then performed using the same procedure.

Purification of vesicles, (2) Centrifugation procedure for purification of protein-loaded vesicles: For protein-loaded vesicles, removal of unencapsulated proteins was not possible by GPC if the Mr of the protein is >7,000 Da. Therefore after the freeze-thaw cycles and extrusion of the vesicles the resulting suspension was spun in a centrifuge at 2200 × g for 15 minutes or until the vesicles had sedimented to an approximately 300 µL volume. The supernatant was then removed and replaced with an equal volume of PBS. This process was repeated as many times as necessary to reduce the concentration of unencapsulated protein to below the detection limit of the technique being used to measure its release.

Formation of MNPVs: A suspension of Bt-PEG-APTES MNPs (2 mL, 0.5 mg mL^{-1} in PBS) was prepared by sonication for ~10 minutes using a VCX130PB probe-type sonicator (Sonics & Materials Inc.) set to 50 % amplitude. Care was taken to ensure the sample has not been heated significantly by the sonication procedure, in which case the sample was allowed to cool to room temperature before continuing. Avidin (50 µL of a 10 mg mL^{-1} solution in distilled water) was added to the MNP suspension followed by brief vortex mixing and incubated for 5 minutes. Biotinylated vesicles (200 µL of a 20 mM [lipid] suspension (DPPC + 0.2 mol % Bt-cap-PE)) in PBS were then added to the avidin-MNP mixture followed by brief vortex mixing. The samples were then incubated at 37 °C for at least 40 minutes until formation of the aggregates was complete. Once the MNPVs had sedimented, the supernatant was removed and replaced with fresh PBS to give a total volume of 2 mL.

Fluorescein isothiocyanate (FITC) labelling of trypsin: Samples of lyophilised trypsin (70 mg) were dissolved in borate buffer (10 mM, pH 9.3, 5 mL). FITC (1.4 mg) was added to the solution and vortex mixing was used to aid dissolution. The sample was incubated at room temperature for 1 h in darkness, after which time the solution was purified by GPC. The labelled protein eluted as a pale green fraction that came off the column well ahead of the orange band containing unreacted FITC. The resulting solution was then lyophilised and redissolved in distilled water (as PBS salts were already present) to give a final concentration of protein of 10 mg mL^{-1}. A calibration curve was then established by sequential dilution of the protein solution and measurement of the absorbance at 495 nm (FITC absorbance maximum).

S.2. Fourier transform infrared spectroscopy (FTIR) of MNPs

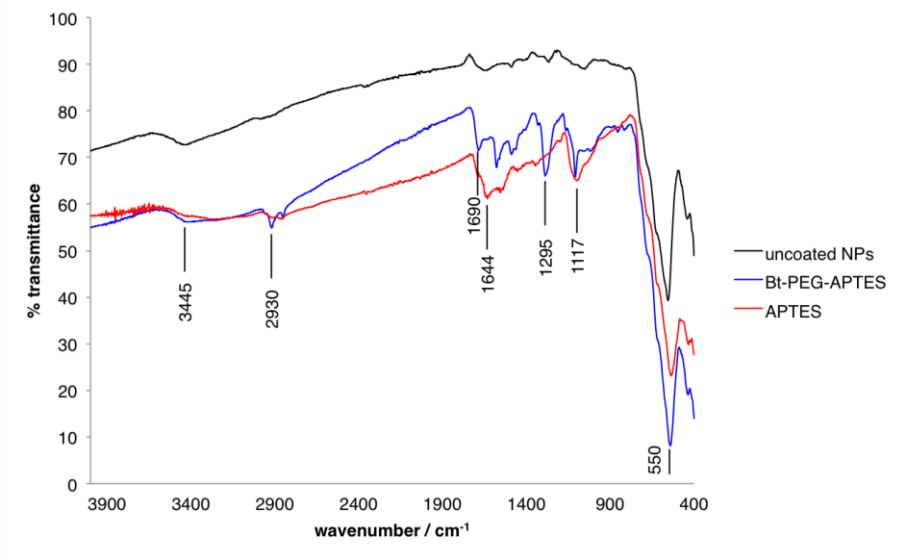

Figure S3: FTIR spectra for: (**–**) uncoated commercial MNPs, (**–**) APTES coated MNPs, (**–**) Bt-PEG- APTES coated MNPs

S.3. Quartz crystal microgravimetry with dissipation

Stock solutions: All solutions were made up in 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) buffer (10 mM, 150 mM NaCl, $CaCl_2$ 2 mM, pH 7.4). All solutions of vesicles, avidin and MNPs used in the QCM had the same mass concentration of 0.1 mg mL^{-1} in HEPES (0.1 mg mL^{-1} lipid for vesicles). Solutions were introduced to the QCM sensor *via* tubing incorporating a peristaltic pump operating at a constant flow rate of 50 µL per minute.

Experimental procedure: HEPES buffer was flowed over the sensor until a stable baseline was established and the internal temperature control was stable at 37 °C. Then a solution of vesicles (0.1 mg mL^{-1}) of the desired composition (DOPC, DOPC + 0.2 mol% Bt-cap-PE) was introduced to system. Once the characteristic 'burst effect' was observed and the frequency and dissipation had stabilised at their new levels, HEPES buffer was again flowed over the sensor. HEPES buffer was flowed over the sensor between application of each solution of vesicles, avidin or MNPs to ensure that the components only interacted on the surface of the sensor and not in solution. The flow of buffer was carried out for approximately 8 minutes, as this was the time taken for solutions introduced to the peristaltic system to reach the sensor (determined by the delay between loading and f and D response). Solutions of vesicles, proteins or MNPs were flowed over the sensor until no further change in frequency and dissipation were observed. In the case of negative controls, when it was clear that the solution had reached the sensor and elicited no frequency or dissipation

response. For details of the sequence of additions for particular experiments see the discussion of each experiment in the manuscript text.

Control experiments

Deposition of a non-biotinylated DOPC bilayer followed by addition of avidin: A DOPC bilayer was deposited on a silica sensor chip as described above (0.1 mg mL^{-1} DOPC, no biotinyl lipid, in HEPES) and a characteristic burst effect was observed (**a**, Figure S4). Avidin solution (0.1 mg mL^{-1} in HEPES) was added at point **b**, leading to a significantly reduced mass increase relative to that seen with a biotinylated bilayer.

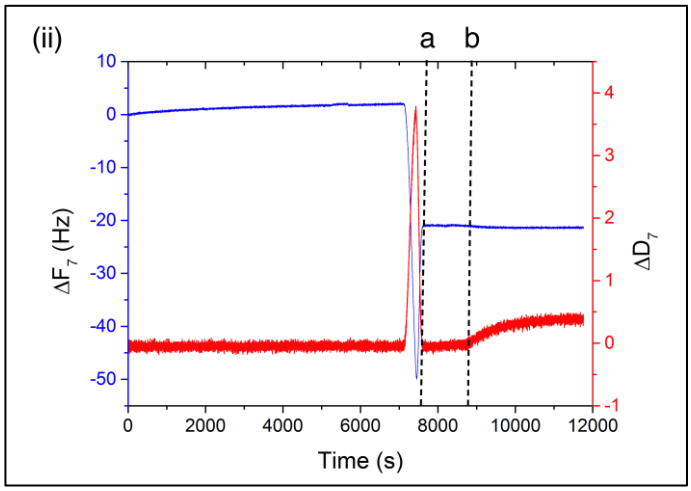

Figure S4: Plot of f and D over time **a**: deposition of DOPC (no Bt-PE), **b**: addition of avidin solution

Addition of APTES coated or uncoated MNPs to a Bt-DOPC bilayer treated with avidin, followed by addition of Bt-APTES-MNPs: A Bt-DOPC bilayer was deposited on the sensor, indicated by the burst effect seen (point **a**, Figure S5 (i) and (ii)). Avidin solution was added at point **b**, resulting in an increase in deposited mass (decrease in f) and increase in dissipation, indicative of the expected biotin-avidin binding event. At point **c**, a solution of APTES coated or uncoated MNPs (0.1 mg mL^{-1} in HEPES) was flowed over the sensor resulting in a small decrease in adsorbed mass and dissipation for uncoated MNPs, possibly due to a lack of any specific binding, resulting in an overall washing effect where the MNP suspension is removing some deposited avidin or lipids. In the latter experiement suspension of Bt-APTES-MNPs (0.1 mg mL^{-1} in HEPES) was flowed over the sensor and an increase in deposited mass and a large increase in dissipation were observed (point **d**, Figure S5 (ii)), indicating a specific binding event.

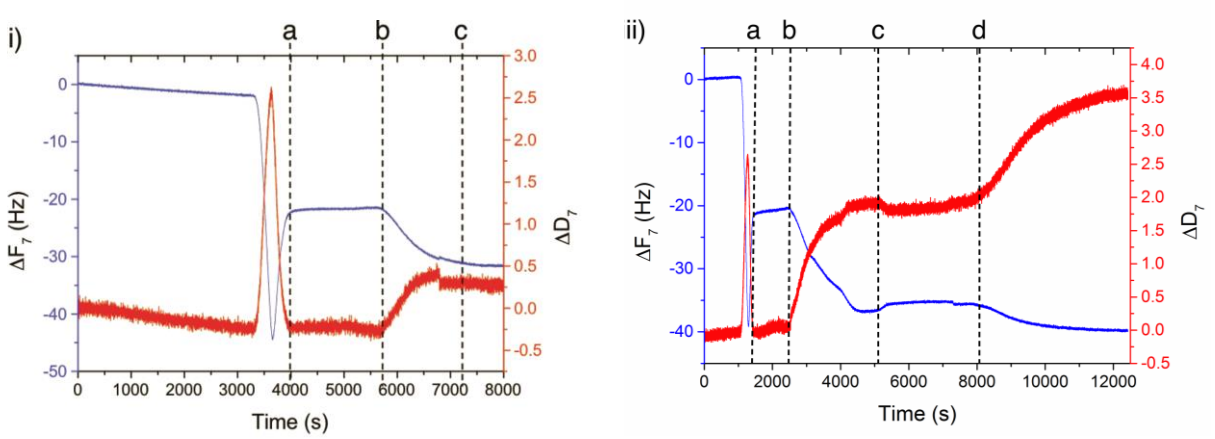

Figure S5: (i) Plot of f and D over time. **a**: Bt-DOPC vesicles 'burst effect', **b**: avidin, **c**: APTES coated MNPs. (ii) Plot of f and D over time. **a**: deposition of Bt-DOPC bilayer, **b**: addition of avidin solution, **c**: addition of uncoated MNPs, **d**: addition of Bt-APTES-MNPs

S.4. Specific Absorption Rate (SAR) of commercially available Fe_3O_4 nanoparticles

SAR values for Fe_3O_4 MNPs exposed to a 392 kHz (field strength ~42 kA m^{-1}) were determined using the calorimetric method reported by Natividad *et al.*[4] A glass vial containing 4 mL of 10 mg mL^{-1} MNP suspension in distilled water was placed in an insulating foam sleeve and cap, an alcohol thermometer was introduced into the suspension via a hole in the cap. The insulated vial was then placed inside the circular AMF coils. The temperature was recorded immediately before the AMF pulse began and at 30 s intervals during the 300 s pulse.

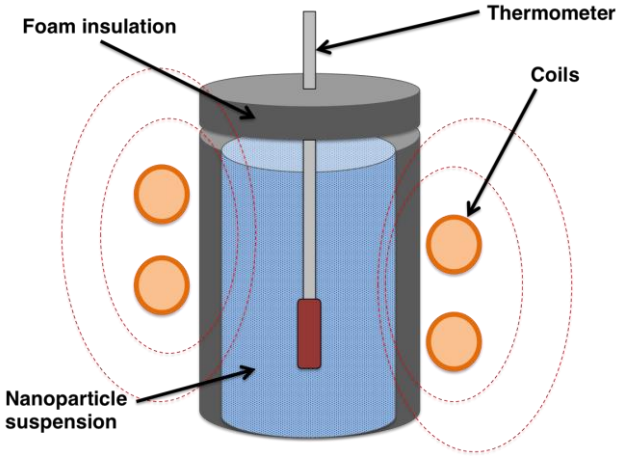

Figure S6: Experimental setup for calorimetric determination of SAR

The data was then used to determine SAR according to the relation:

$$SAR = \left(\frac{1}{m_{MNP}}\right) \times (C \times m_{water}) \times \left(\frac{\Delta T}{\Delta t}\right)$$

Equation S1: SAR is the specific absorption rate in W g^{-1}, m_{MNP} is the mass of nanoparticles in g, m_{water} is the mass of water in g, ΔT is the change in temperature in K, Δt is the time elapsed in s and C is the heat capacity of the system in J $g\ K^{-1}$.

In this experimental setup, C is approximated to the specific heat capacity of water (4.186 J $g\ K^{-1}$), and the data is corrected for eddy heating of water.

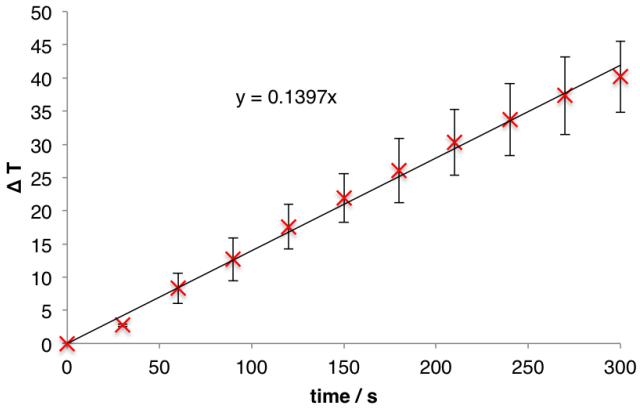

Figure S7: Average data for two SAR measurements on one batch of commercial Fe_3O_4 MNPs taken 6 months apart. The average SAR value was 225 W g^{-1} with a ΔT value of 44.2 K

S.5. Images of MNPVs and the effect of AMF on DPPC MNPVs

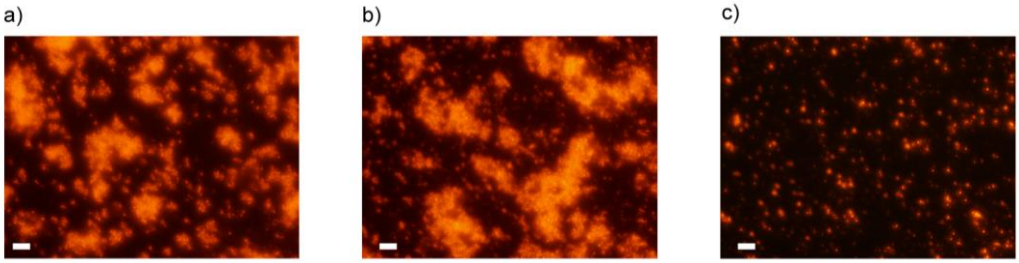

Figure S8: Fluorescence microscopy images of rhodamine-labelled MNPV samples that were either (a) incubated at 37 °C (no AMF), (b): incubated at 37 °C and exposed to an AMF pulse (392 kHz, 5 minutes) or (c): heated to 42 °C and then briefly sonicated. Scale bars are 5 μm.

MNPV samples were prepared using the method described above (DPPC + 0.2 mol% Bt-cap-PE).

MNPV samples (in 2 mL PBS, *ca*. 300 µL volume of MNPV aggregate, doped with 0.1% rhodamine-DHPE) in a 15 mL centrifuge tube were either; (i): incubated at 37 °C (no AMF), (ii): incubated at 37 °C and exposed to an AMF pulse (392 kHz, 5 minutes) or (iii): heated to 42 °C for 15 h and then briefly sonicated (~2 minutes) with a probe-type sonicator. After treatment under these conditions the MNPVs were imaged using fluorescence microscopy (Figure S8).

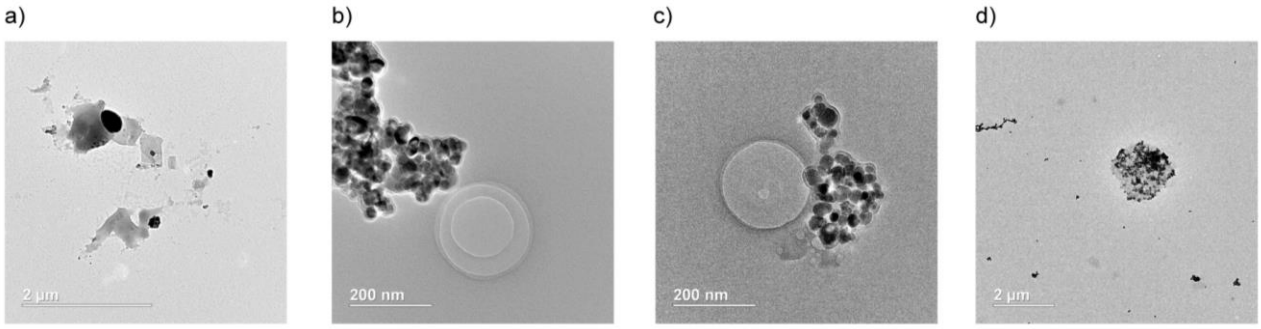

Figure S9: Transmission electron microscopy images of MNPV samples that had been (a, b) diluted with distilled water and dried onto a carbon grid. (c, d) diluted with distilled water then sonicated briefly before drying onto a carbon grid. a) disrupted DPPC vesicles attached to MNPs. b) Collapsed multilamellar vesicle linked to a cluster of MNPs. (c, d) Collapsed vesicles linked to clusters of MNPs.

Transmission electron microscopy (TEM) was undertaken using a FEI Tecnai G2 TEM (200 kV). Several drops of the samples were pipetted on holey carbon-coated copper grids (200 mesh Agar Scientific). The excess buffer was then carefully removed with a filter paper (Whatman qualitative filter paper, grade 1). Afterwards, the grids were dried in a fume hood before the TEM investigation.

Standard TEM imaging was of limited use for imaging the MNPVs. The conditions used for imaging led to the collapse of the vesicles in the MNPVs and many vesicle fragments were observed. The difference in height between the magnetic nanoparticles (ca. 50 nm) and the collapsed vesicles (two bilayers thick, ca. 4 nm) led to problems in focussing the TEM on the collapsed vesicles. A fewer images were obtained that showed the interaction of MNPs with fragments of membrane and collapsed vesicles (Figure S9).

S.6. Magnetic release from DPPC MNPVs

Release of 5/6-CF from MNPVs: MNPV samples were prepared using the method described above with 5/6-CF loaded vesicles (DPPC + 0.2 mol% Bt-cap-PE). MNPV samples (in 2 mL PBS, *ca*. 300 µL volume of MNPV aggregate) in a 15 mL centrifuge tube were either; (i): incubated at 42 °C (no AMF), (ii): incubated at 37 °C and exposed to an AMF pulse (392 kHz, 5 minutes) or (iii): incubated at 37 °C and not exposed to an AMF pulse. Aliquots of the supernatant buffer (20 µL) were taken at the desired timepoints and added to PBS (2 mL) in fluorescence cuvettes and the

fluorescence intensity measured. After the 24 hour timepoint, the samples were all heated to 42 °C for 15 hours and then briefly sonicated (~2 minutes) with a probe type sonicator. A further aliquot (20 µL) was taken and added to a fluorescence cuvette containing PBS (2 mL) and the fluorescence intensity measured. The value of the fluorescence at 517 nm (excitation at 492 nm) was used to calculate the percentage release values for the previous timepoints relative to this '100 %' value.

Release of cytochrome c from MNPVs: MNPV samples were prepared using the method described above. For cytochrome c release, at the desired timepoints the samples were sedimented by centrifugation at 2200 × g and the absorbance spectrum of the supernatant recorded (wavelength of interest: 410 nm). The supernatant was returned to the MNPVs, which were resuspended and incubated at 37 °C between timepoints. Once the timecourse had been measured, the samples were sonicated (~120 s) using a probe sonicator and incubated at 42 °C for 1 h. Percentage release values were calculated relative to the absorbance values obtained for these solutions.

Release of FITC-trypsin from MNPVs in suspension: FITC-trypsin-loaded vesicles were prepared and incorporated into MNPVs as described above. MNPV samples (in 2 mL PBS, *ca*. 300 µL volume of MNPV aggregate) were placed in a 15 mL centrifuge tube. The samples were incubated at 37 °C and were exposed to an AMF pulse (392 kHz, 5 minutes) if required. At the desired timepoints the samples were sedimented by centrifugation at 2200 × g, the PBS buffer (2 mL) transferred to a fluorescence cuvette and the fluorescein fluorescence (λ_{ex} = 492 nm; λ_{em} = 517 nm) measured. The MNPVs were resuspended and incubated at 37 °C between timepoints. These experiments were repeated using three different batches of FITC-trypsin to ensure reproducibility.

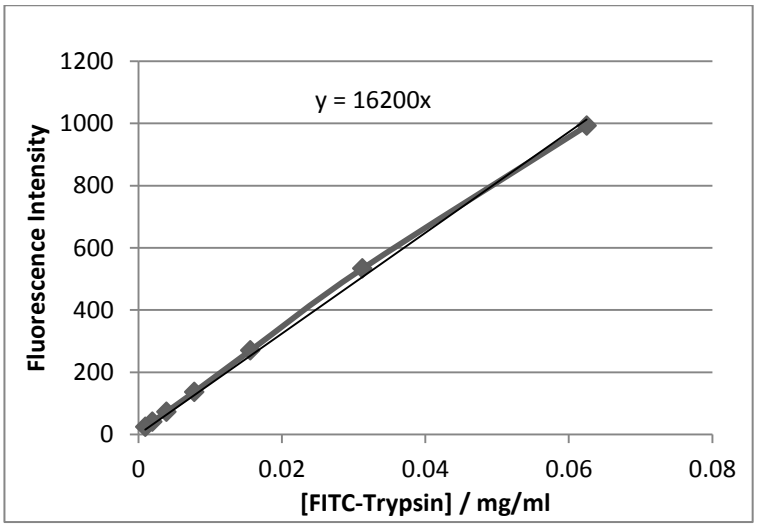

Figure S10: Fluorescence emission/FITC-trypsin calibration curve

Fluorescent protease activity assay (trypsin activity): A 'Protease fluorescent detection kit' product code PF0100 was obtained from Sigma-Aldrich (UK) and used exactly as the published instructions.[5] MNPVs containing trypsin (non-FITC labelled) were prepared in an otherwise identical fashion to those described in the FITC-trypsin release experiment. The samples were either exposed to an AMF pulse (392 kHz, 5 minutes) or not ('no AMF' control). The assay was then performed on aliquots (20 µL) of the supernatant buffer of the MNPVs at the desired timepoints.

For each sample incubation buffer (20 µL , 20 mM sodim phosphate, 150 mM NaCl, pH 7.6), FITC-casein solution (20 µL) and the solution to be assessed for protease activity (MNPV supernatant or samples of known concentration trypsin solution for the calibration plot) (10 µL) were combined in an Eppendorf tube. A blank was also prepared by combining incubation buffer (20 µL), FITC-casein solution (20 µL) and of milliQ water (10 µL). All samples were vortex mixed and incubated for 1 hour at 37 °C in darkness. After exactly 1 h, trichloroacetic acid solution (0.6 M, 150 µL) was added to each sample, vortex mixed an incubated in the dark for 0.5 h. The tubes were centrifuged at 10,000 × g for 10 minutes. The supernatant of these samples contains acid soluble FITC-casein fragments that have resulted from protease activity and these are used to determine relative activity from relative fluorescence. Aliquots (10 µL) were added to a fluorescence cuvette and diluted with assay buffer (1 mL, Tris 500 mM, pH 8.5) and the fluorescence intensity of the solution was recorded at 535 nm (excitation at 485 nm).

The fluorescence intensities were interpolated onto the calibration plot established for samples of known trypsin concentration (Figure S11).

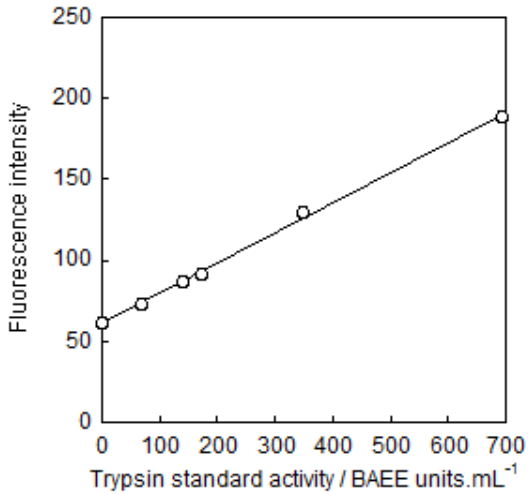

Figure S11: Calibration plot showing fluorescence intensity of supernatants containing FITC-casein fragments (em. 535 nm, ex. 485 nm) at different trypsin activities.

S.7. Control experiment: Magnetic release from DOPC MNPVs.

5/6-CF containing vesicles composed of either DPPC or DOPC were prepared and formed into aggregates (0.5 mg mL^{-1} MNP concentration) according to the standard procedure described in the experimental section. Each sample contained MNPVs prepared from 200 µL of 20 mM vesicles in 2 mL PBS. To measure release, 200 µL aliquots of the sample supernatant was taken at the desired timepoint after AMF, diluted to 2 mL in PBS and the fluorescence intensity measured. The data presented in Figure S12 below is for the 45 minutes timepoint.

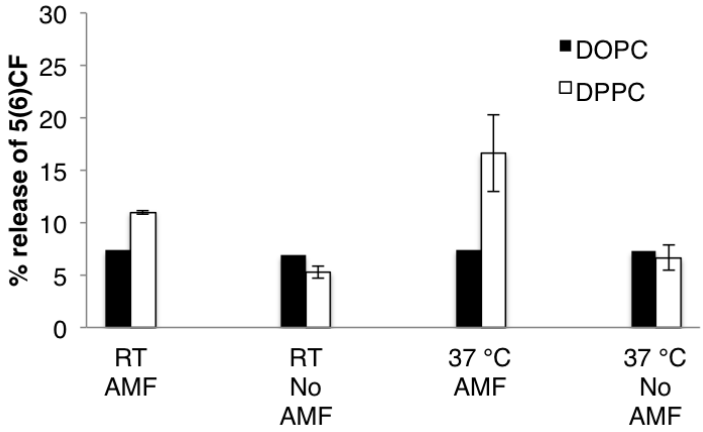

Figure S12: Percentage release of 5/6-CF from MNPVs containing DOPC or DPPC vesicles. Samples were incubated at 37 °C or RT and fluorescence intensity was measured 45 minutes after AMF or after 45 minutes of incubation (RT = room temperature).

Triggered release was observed only in the DPPC samples exposed to AMF at 37 °C, similarly treated DOPC MNPVs did not demonstrate any increased 5/6-CF release under these conditions and were comparable throughout the series of experiments. A small increase in release was observed in the DPPC vesicles exposed to AMF at room temperature, likely due to the MNPVs being near the membrane transition temperature for a shorter amount of time than those exposed to AMF at 37 °C. DPPC samples not exposed to AMF had a similar 5/6-CF concentration in the supernatant to the (non-releasing) DOPC samples, demonstrating the stability of the DPPC membranes at these temperatures.

S.8. Zeta potential measurements

A Zetasizer Nano series (Malvern Instruments) model Nano-ZS was employed with disposable capillary cell (DTS1070 and DTS1060). The Bt-APTES-MNPs were dispersed in a concentration of around 0.2 mg mL^{-1} in water by sonication (sonicator bath at 25 °C). MNPVs in PBS were not sonicated but dispersed with a 1 mL syringe to make a homogeneous suspension. Dilution was an aliquot of aggregate suspension (0.1 mL) made up to a final volume of 4 mL with distilled water. More concentrated samples of MNPVs in PBS/water gave similar results.

MNPVs: zeta potential = +4.39 ± 0.15 mV

Bt-APTES-MNPs: zeta potential = +7.98 ± 0.51 mV

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