Supplementary Information

Magnetically-Controlled Release from Hydrogel-Supported Vesicle Assemblies

Robert J. Mart, Kwan Ping Liem and Simon J. Webb*

S.1. Materials and Instrumentation:

UV-Visible spectra were recorded on a Cary 400 Scan UV spectrophotometer and fluorescence spectra on a Perkin-Elmer LS55 fluorimeter. Images of vesicle-nanoparticle assemblies were captured using a Zeiss Axio Imager A1 fluorescence microscope fitted with a Canon Powershot G6 digital camera. Cryo-ESEM images were obtained with the help of Dr. Patrick Hill on a Philips XL30 Environmental SEM-FG with a Gatan Alto 2500 Cryotransfer system. Thermal analysis was carried out using a Perkin Elmer Diamond Differential Scanning Calorimeter. Induction heating was carried out with a water-cooled EASYHEAT 0224 induction heater (2.0 kW, 150-400 kHz) with an EASYHEAT 300P workhead, purchased from Cheltenham Induction Heating, Gloucestershire GL52 6RU, U.K. Neodymium iron boron (NdFeB) magnets, either N48 (5350 G, 23 mm diameter x 20 mm length x 6 mm diameter c/sunk hole) or N42 (4700 G, 3 mm diameter x 13 mm length), were purchased from e-magnets UK, Sheffield S2 5QT, U.K. Chemicals were used as received from Sigma-Aldrich except for **1** (2*S*)-*N*-[2-(3,4-dihydroxyphenyl)ethyl]-2-(acetamido)-3-(1*H*-imidazol-4-yl)propanamide and H_2(**2**) (8-(bis(carboxymethyl)amino)-3,6-dioxa-octyl (2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluoro-10-(pyren-1-ylmethoxy)decyloxy)acetamide), which were synthesized as detailed previously,[S1] Both **1** and H_2(**2**) gave satisfactory spectroscopic and analytical data. Magnetite nanoparticles (10 nm diameter) and [**1**-MNP] were also synthesized as detailed previously.[S1]

S.2. Preparation of [H_2(2)-TSV] vesicles at pH 7.4

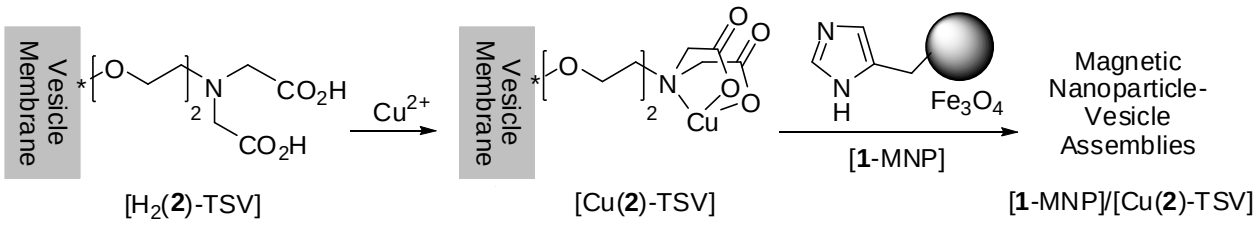
Unilamellar vesicles (800 nm diameter) with a membrane composition of 85.5% mol/mol dipalmitoyl phosphatidylcholine (DPPC), 9.5% mol/mol dimyristoyl phosphatidylcholine (DMPC) and 5% mol/mol of H_2(**2**) were prepared for vesicle-nanoparticle aggregation studies in a manner similar to previously published procedures.[S1] The required amounts of DPPC (12.55 mg, 17.1 μmol), DMPC (1.27 mg, 1.9 μmol) and H_2(**2**) (1 mL of a 1 mM solution of H_2(**2**) in chloroform) were dissolved in spectroscopic grade ethanol-free chloroform (5 mL), then the solvent removed under reduced pressure to leave a lipid thin-film on the interior wall of a round-bottomed flask. The MOPS buffer (20 mM MOPS, 100 mM NaCl, pH 7.4 at 20 ℃, 1 mL) was added to the flask and the contents vortexed to detach the thin film. This suspension of multilamellar vesicles was passed 19 times through an 800 nm polycarbonate membrane in an Avestin Liposofast extrusion apparatus to give a suspension of unilamellar vesicles (1 mL). All vesicle suspensions were freshly prepared prior to mixing. For experiments involving encapsulation, either GPC separation on Sephadex PD10 GPC columns or magnetic sedimentation was used to remove unencapsulated material:
Purification by GPC (5/6-CF encapsulation): The vesicle solution (1 mL) was diluted to 2.5 mL with additional buffer of the appropriate type and loaded onto a pre-equilibrated PD-10 Sephadex desalting column. The vesicle solution was then eluted using a further 3.5 mL of buffer.
Purification by Magnetic Sedimentation (FITC-dextran encapsulation):[S2] Vesicle suspensions were placed upon a 5 kG magnet until a compact vesicle plug had formed at the bottom of the vial and the supernatant solution was visually free of turbidity. As much of the supernatant was removed as possible without disturbing the vesicle plug (typically 60% of the initial volume), and replaced with an equal volume of the appropriate buffer solution. Briefly vortex mixing the vial regenerated the vesicle suspension. This procedure was repeated at least 6 times and until the concentration of unencapsulated material was < 0.1% of the initial concentration.

S.3. Phase separation of Cu(2) in [Cu(2)-TSV] vesicles at pH 7.4 and 20°C

Copper(II) chloride solution in MOPS buffer (20 μL, 50 mM, 1 μmol) was added to a suspension of [H_2(**2**)-TSV] vesicles (1 mL, 20 mM lipids, [H_2(**2**)] **=** 1 mM, 1 μmol) with gentle mixing to give [Cu(**2**)-

TSV] vesicles. An aliquot (4 µL) of [Cu(**2**)-TSV] vesicles in suspension at pH 7.4 (19 mM lipid, 1 mM Cu(**2**)) was added to MOPS buffer at pH 7.4 (2 mL), and the fluorescence spectrum recorded. The degree of phase separation was determined from the emission spectrum (excitation at 346 nm) by calculating the ratio of pyrene excimer emission at 480 nm to pyrene monomer emission at 379 nm. At a loading of 5% mol/mol Cu(**2**), [Cu(**2**)-TSV] vesicles exhibited an E/M ratio of 0.4 ± 0.1 at pH 7.4 and 20 ℃, indicating phase separation was comparable to that observed for our established [Cu(**2**)-DMPC/chol] vesicle composition (l_o phase, E/M ratio ~ 0.4 at pH 7.4 and 25 ℃). [S3] The observed E/M ratios decreases as the temperature was increased, falling to 0.2 ± 0.1 at 40 ℃ (pH 7.4).

S.4. General Procedure for the Preparation of [1-MNP]/[Cu(2)-TSV] Nanoparticle-Vesicle Assemblies at pH 7.4



Scheme S1: Preparation of [**1**-MNP]/[Cu(**2**)-TSV] magnetic nanoparticle/vesicle assemblies at pH 7.4.

S.4.1. Preparation: Copper(II) chloride solution in MOPS buffer (2 µL, 50 mM, 0.1 µmol) was added to a suspension of [H_2(**2**)-TSV] vesicles (1 mL, 2 mM lipids, [H_2(**2**)] = 0.1 mM, 0.1 µmol) with gentle mixing to give [Cu(**2**)-TSV] vesicles. Dry [**1**-MNP] nanoparticles (1.2 mg) were dispersed in distilled water (1 mL) by sonication to give a stock suspension of [**1**-MNP] (1.2 mg/mL). Vesicle-nanoparticle assemblies were prepared by gently mixing the suspension of [**1**-MNP] in distilled water (200 µL, 240 µg, concentration of **1** = 0.5 mM) with [Cu(**2**)-TSV] vesicles (1 mL, concentration of Cu(**2**) = 0.1 mM) at a 1:1 ratio of **1** to Cu(**2**). The mixture was then left to aggregate for 1 h, then the assemblies sedimented with a NdFeB 5 kG permanent magnet. Some of the supernatant (202 µL) was removed to return the assembly solution to its initial lipid concentration and volume (2 mM lipids, 1 mL), then the sedimented assemblies redispersed by gentle vortex mixing.

S.4.2. Characterisation: Fluorescence microscopy images of [1-MNP]/[Cu(2)-TSV] vesicle-nanoparticle assemblies: [**1**-MNP]/[Cu(**2**)-TSV] vesicle assemblies in buffer suspension (2 mM lipid concentration, Cu(**2**) = 0.1 mM) were observed on glass slides.

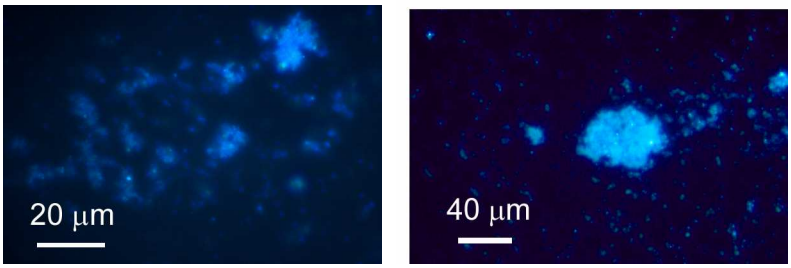


Figure S1: Representative fluorescence microscopy images of [**1**-MNP]/[Cu(**2**)-TSV] magnetic nanoparticle/vesicle assemblies at pH 7.4.

S.5. Preparation of blocks of magnetically-responsive vesicle gel containing [1-MNP]/[Cu(2)-TSV] at pH 7.4

S.5.1. Preparation: Unpatterned blocks of hydrogel were prepared by mixing a concentrated suspension of [**1**-MNP]/[Cu(**2**)-TSV] vesicles encapsulating 5/6-CF (0.5 mM 5/6-CF inside) with equal volumes of 2% sodium alginate solution. The resulting solutions were transferred into a 5

mm wide, 1 mm deep and 25 mm long trough on a microscope slide. The solution was covered by a polycarbonate membrane (as used for vesicle extrusion, 200 nm pore size) and calcium chloride solution (100 mM) placed on top of the polycarbonate membrane for ~20 minutes. The calcium chloride solution was then carefully drained and the filters removed, revealing a smooth-faced gel block.

S.5.2. Characterisation: Differential Scanning Calorimetry: Differential scanning calorimetry (DSC) showed onset of the melting phase transition (T_m) of [Cu(**2**)-TSV] to be unchanged after aggregation with [**1**-MNP] (37-38 ºC, Figure S2(d)), while immobilisation of these vesicle assemblies in the alginate matrix gave only a small rise in the onset temperature (39-40 ºC, Figure S2(e)).

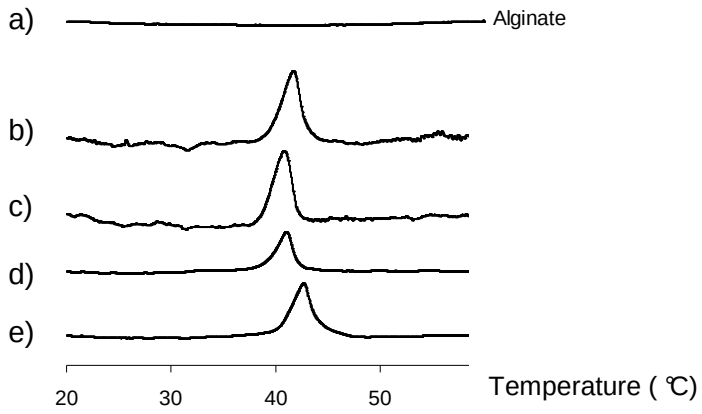


Figure S2: Differential scanning calorimetry traces for a) calcium alginate gel; b) [H_2(**2**)-TSV]; c) [Cu(**2**)-TSV]; d) Magnetic nanoparticle-vesicle assemblies [**1**-MNP]/[Cu(**2**)-TSV]; e) Magnetically sensitive vesicle gel, magnetic nanoparticle-vesicle assemblies [**1**-MNP]/[Cu(**2**)-TSV] in an calcium alginate matrix.

S.5.3. Characterisation: Cryo-environmental scanning electron microscopy (Cryo-ESEM) images of vesicle-nanoparticle assemblies in alginate gel: Cryo-ESEM samples were prepared by plunge-freezing a 25 mm^3 section of vesicle gel (2 mM lipid) on a sample stub with liquid nitrogen. Micrographs of freeze-dried vesicle gels (formed at pH 7.4) revealed the presence of spherical vesicles between 0.3 and 2 μm in diameter, both aggregated and individual, embedded within the calcium alginate matrix (Figure S3 b). In comparison, micrographs of calcium alginate gels without vesicles formed under identical conditions showed no spherical objects in the network (Figure S3 a). The alginate matrix did not appear to invade the vesicle surfaces; in some micrographs spherical cavities remained where the vesicle assemblies have been removed during freeze-fracture, revealing a dense network of fibrils that defined a smooth interface (Figure S4). Similarly, vesicle-sized depressions have been observed by Messersmith and co-workers during SEM studies of non-magnetic vesicle gels.[S4]

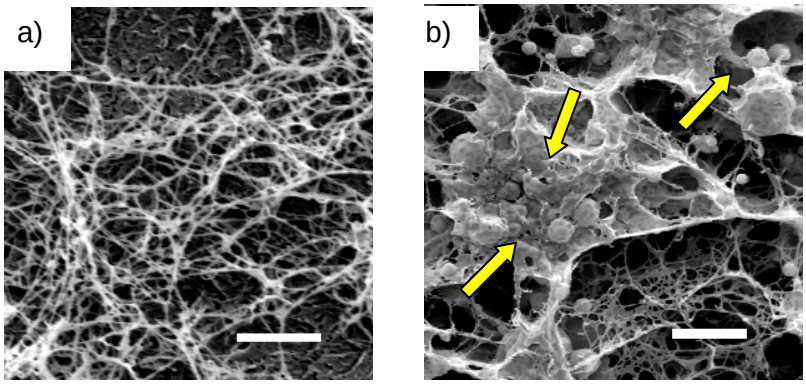


Figure S3: Cryo-ESEM images of alginate gels either a) without or b) with magnetic nanoparticle vesicle assemblies (vesicle clusters indicated with yellow arrows). Scale bar represents 1 μm.

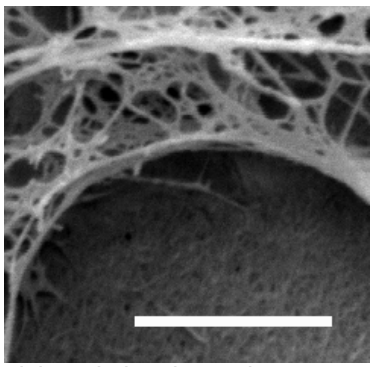


Figure S4: Cryo-ESEM images of a vesicle gel showing a dense network of fibres found around a large void that housed a vesicle assembly. Scale bar is 1 μm.

S.6. Preparation of patterned magnetically-responsive vesicle gels containing [1-MNP]/[Cu(2)-TSV] at pH 7.4

S.6.1. Preparation: Patterned vesicle gels may either be constructed by mixing 1% wt/vol sodium alginate solutions with concentrated [**1**-MNP]/[Cu(**2**)-TSV] vesicle assembly suspensions and placing them over a pattern of N42 grade 3 mm × 13 mm NdFeB rod magnets (Figure S5) or by carefully transferring by pipette aliquots (5 μL) of concentrated vesicle suspension into an alginate solution.

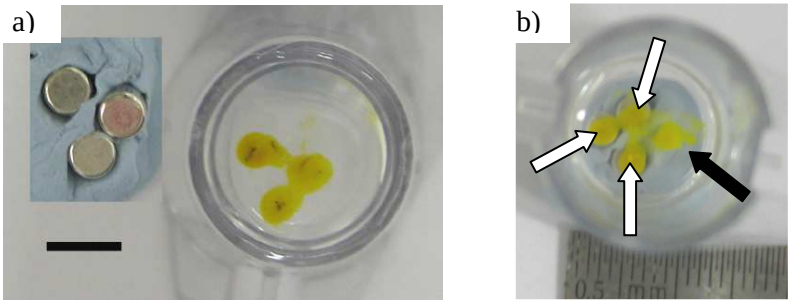


Figure S5: (a) A magnetically concentrated suspension of [**1**-MNP]/[Cu(**2**)-TSV] assemblies (~5 μL) encapsulating 5/6-CF have been spotted into a 1% alginate solution and positioned by three small patterning magnets. The gel was cured from below by calcium chloride solution (100 mM) *via* the porous base of the culture well insert. The liquid supernatant has been removed and the gel inverted. Shown top left is the array of three patterning magnets used to generate the vesicle pattern. The scale bar represents 5 mm. (b) In this control experiment, the three [**1**-MNP]/[Cu(**2**)-TSV] vesicle assembly spots to the left (white arrows) have been magnetically focused by magnets underneath the cell culture well, whilst the [**1**-MNP]/[Cu(**2**)-TSV] vesicle assemblies in the spot to the right (black arrow) are diffusing through the alginate solution because no magnetic field is present to hold them in place.

S.7. Measurement of dye retention at 20 ℃ within m agnetically-responsive vesicle gels containing [1-MNP]/[Cu(2)-TSV] at pH 7.4

S.7.1 Comparison of dye retention between magnetically-responsive vesicle gels and vesicle-free alginate: Blocks of hydrogel were prepared by mixing either a concentrated suspension of [**1**-MNP]/[Cu(**2**)-TSV] vesicles encapsulating 5/6-CF (0.5 mM 5/6-CF inside) or vesicle-free 5/6-CF solution (0.5 nM) with equal volumes of 2% wt/vol sodium alginate solution. The resulting solutions were transferred into a 5 mm wide, 1 mm deep and 25 mm long trough on a microscope slide. The solution was covered by a polycarbonate membrane (as used for vesicle extrusion, 200 nm pore size) and calcium chloride solution (100 mM) placed on top of the polycarbonate membrane for ~20 minutes. The calcium chloride solution was then carefully drained and the filters removed, revealing smooth-faced gel blocks, which were cut into 5 × 5 × 1 mm gel sections. These sections (either with or without vesicles) were placed in buffer solution (2 mL) within a cuvette, and the fluorescence of the supernatant solution monitored at 20 ºC. No

stirring was used, as stirring destroyed the gel section. Dye release from the magnetically-responsive vesicle gel sections and from gel sections that contained 5/6-CF only were normalised to the initial fluorescence and to the fluorescence obtained after overnight incubation with Triton X-100 (20 µL, 25% v/v in buffer). Only 3% of the 5/6-CF was released from vesicle gels in an hour compared to 86% for the vesicle-free gels (Figure S6).

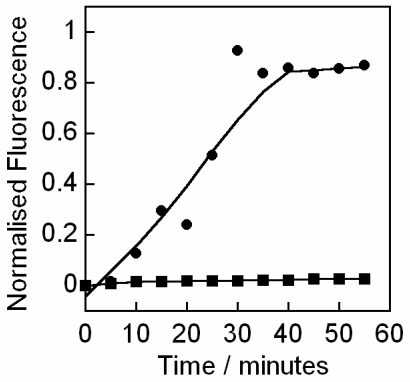


Figure S6: Comparison of 5/6-CF release from; (●) vesicle-free calcium alginate gels, (■) [**1**-MNP]/[Cu(**2**)-TSV] within magnetically-responsive vesicle gels.

S.8. Measurement of dye release at 40 ℃ from magnetically-responsive vesicle gels containing [1-MNP]/[Cu(2)-TSV] at pH 7.4

S.8.1 Measurement of 5/6-CF and FITC-dextran release from [1-MNP]/[Cu(2)-TSV] vesicle assemblies at pH 7.4: A similar procedure was followed as described for the determination of background leakage rates (S.7.1 previous). Cuvettes containing either [Cu(**2**)-TSV] encapsulating 5/6-CF (0.1 µM lipid in 2 mL buffer, 0.5 mM 5/6-CF inside), [**1**-MNP]/[Cu(**2**)-TSV] encapsulating 5/6-CF (0.1 µM lipid in 2 mL buffer, 0.5 mM 5/6-CF inside), or [**1**-MNP]/[Cu(**2**)-TSV] encapsulating FITC-dextran (0.1 µM lipid in 2 mL buffer, 20 mM FITC-dextran inside), were equilibrated in a heated sample block at 20 ºC before warming to 40 ºC. The fluorescence values were recorded, taking care to compensate for the diminished fluorescence emission of fluorescein derivatives at 40 ºC. Values were again normalised to the initial fluorescence and to the value obtained after overnight incubation with Triton X-100 (20 µL of 25% v/v in buffer).

As expected from literature precedent[S5] there was rapid and complete release of 5/6-CF from vesicles composed 85.5% mol/mol palmitoyl phosphatidylcholine (DPPC), 9.5% mol/mol mol dimyristoyl phosphatidylcholine (DMPC) and 5% mol/mol of Cu(**2**) once the triggering temperature of 40 ℃ was reached. As anticipated from the DSC data, the formation of magnetic nanoparticle-vesicle assemblies did not change this triggering temperature, and there was complete release of 5/6-CF within 30 minutes of achieving 40 ℃. Release of the large biomacromolecule 4 kDa dextran from magnetic nanoparticle-vesicle assemblies occurred at a similar rate once the triggering temperature was reached, but not all of the dextran was released, which may reflect the difficultly in transporting a large highly polar molecule across even weakened phospholipid bilayers.

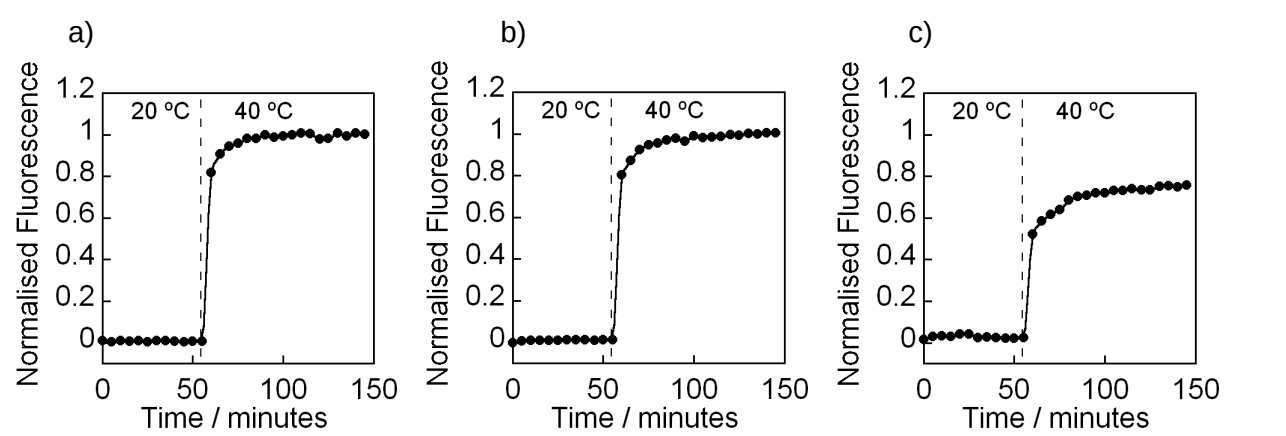


Figure S7: Thermally-triggered release of dyes from thermally-sensitive vesicles in suspension: a) 5/6-carboxyfluorescein (5/6-CF) release from [Cu(**2**)-TSV]; b) 5/6-CF release from [**1**-MNP]/[Cu(**2**)-TSV] magnetic nanoparticle vesicle assemblies. c) 4 kDa FITC-dextran release from [**1**-MNP]/[Cu(**2**)-TSV] magnetic nanoparticle-vesicle assemblies. Errors are ±0.03.

S.8.2 Measurement of 5/6-CF and FITC-dextran release from magnetically-responsive vesicle gels: A similar procedure was followed as described for the determination of background leakage rates (S.7.1 and S.8.1 previous). Cuvettes containing either a vesicle gel section with encapsulated 5/6-CF (25 mm^3 section in 2 mL buffer) or a vesicle gel section with encapsulated FITC-dextran (25 mm^3 section in 2 mL buffer) were equilibrated in a heated sample block at 20 ºC before warming to 40 ºC. The fluorescence values were recorded, taking care to compensate for the diminished fluorescence emission of fluorescein derivatives at 40 ºC. Values were again normalised to the initial fluorescence and to the value obtained after overnight incubation with Triton X-100 (20 µL of 25% v/v in buffer).

S.9. Measurement of magnetically-triggered dye release at 20 ℃ from magnetically-responsive vesicle gels containing [1-MNP]/[Cu(2)-TSV] at pH 7.4

A block of vesicle gel encapsulating 5/6-CF (created as detailed in S.5) was placed in buffer (2 mL) in a vial suspended inside the coil of the induction heater. We found it beneficial to use larger volumes of gel (125 mm^2) than in bulk heating experiments detailed previously (25 mm^2, S.8.2) because a larger proportion of the magnetic flux heated the sample, reducing the cooling needed in the work-head and extending the amount of time available for magnetic heating. The sample was inductively heated for 120 s, then an aliquot (2 µL) removed and the bulk temperature measured, before heating was resumed. The aliquots were diluted in buffer (2 mL) and the 5/6-CF fluorescence emission recorded. Maximum release of 5/6-CF from the gel was determined after incubation of the sample with Triton X-100 (20 µL, 25% in buffer) for 1 hour.

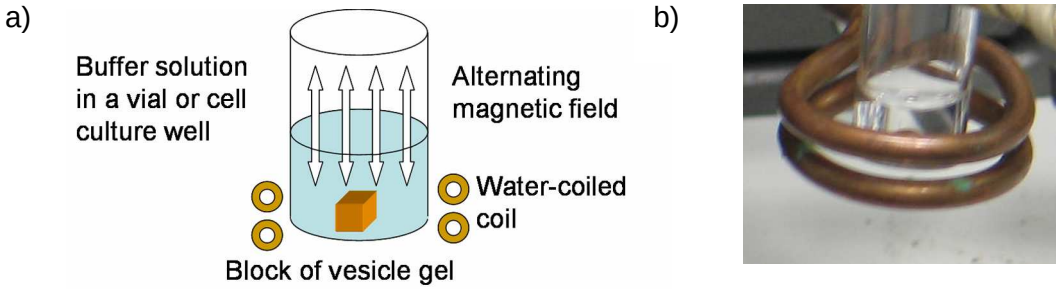


Figure S8: a) Schematic representation of the induction heating of a sample of magnetically responsive vesicle gel. b) Photograph of a vial containing a section of magnetically-responsive vesicle gel (encapsulating 5/6-CF) in MOPS buffer within the coils of the induction heater; this 2-loop coil operates at 392 kHz.

S.10. Lack of magnetically-triggered dye release at 20 ℃ from vesicle gels containing *non-crosslinked* [MNP] and [TSV] at pH 7.4

A block of vesicle gel was prepared using thermally-sensitive vesicles ([TSV]) composed of 9:1 DPPC:DMPC *without* Cu(**2**) and uncoated Fe_3O_4 magnetic nanoparticles [MNP]. A 125 mm^2 section was placed in buffer (2 mL) in a vial suspended inside the coil of the induction heater. The block of vesicle gel was exposed to an alternating magnetic field at 380 kHz, and the amount of 5/6-CF released over 20 minutes was measured.
Less than 3% of the encapsulated 5/6-CF was released after 20 minutes exposure to the AMF, demonstrating the importance of direct links (cross-links) between the magnetic nanoparticle and the thermally sensitive vesicles in magnetically-response vesicle gels containing [**1**-MNP]/[Cu(**2**)-TSV].

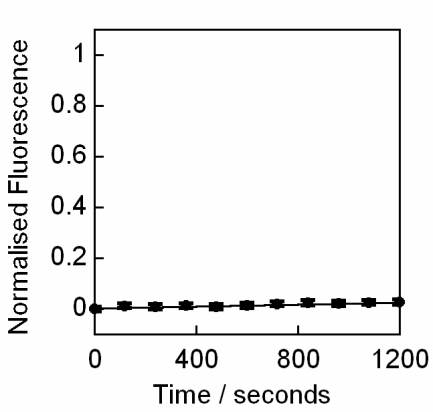


Figure S9: (●) Rate of 5/6-CF release from a 0.125 cm^3 section of vesicle gel containing non-crosslinked [MNP] and [TSV] in buffer (2 mL), after exposure to a 380 kHz alternating magnetic field (AMF). Errors are ± 0.01.

[S1] K. P. Liem, R. J. Mart and S. J. Webb, *J. Am. Chem. Soc*. 2007, **129**, 12080-12081.
[S2] R. J. Mart, K. P. Liem and S. J. Webb, *Pharm. Res.* 2008, in press
[S3] R. J. Mart, K. P. Liem, X. Wang and S. J. Webb, *J. Am. Chem. Soc.* 2006, **128**, 14462-14463.
[S4] T. J. Sanborna, P. B. Messersmith and A. E. Barron, *Biomaterials*, 2002, **23**, 2703-2710.
[S5] E. Westhaus and P. B. Messersmith, *Biomaterials*, 2001, **22**, 453-462.