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Supporting Information

A molecular nanocap activated by superparamagnetic heating for externally stimulated cargo release

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Methods and characterization

Nuclear magnetic resonance spectroscopy was carried out on a Bruker AV400 at room temperature in $CDCl_3$ at 400.13 MHz and 16 scans (^{1}H), or at 100.61 MHz and 512 scans (^{13}C) for 1D spectra, on a Bruker DRX500 in $CDCl_3$ at 500.33 MHz and 8 scans for 2D spectra, and on a Bruker Avance III-500 at 125.79 MHz 65272 scans for ssNMR. All FIDs were processed by zero-filling and phase correction, and liquid-state NMR FIDs are calibrated to the solvent signal. FIDs for solid state and 1D ^{13}C spectra were processed additionally by applying an exponential window function to the FID before FFT. FIDs of 2D spectra were processed additionally by applying a 0° shifted sine window function to the FID before FFT (LB=0.3 Hz and GF=0.1 Hz in the evolution (F1) domain and LB=0.3 Hz and GF=0.0 Hz in the detection (F2) domain). Peak assignments are based on multiplicity, integrals, HMBC and HMQC spectra. NMR spectra of new compounds are shown in **Figure S3-S7**.

Fourier transform infrared spectroscopy (FTIR) was carried out with a JASCO FT/IR-420 spectrometer averaging 128 scans in the range of 4000–400 cm^{-1} at a resolution of 1 cm^{-1}. KBr discs were prepared by mixing approximately 2 mg of nanoparticles with approximately 200 mg of KBr and forming the disc under pressure. The spectra shown in the main text are background corrected with a linear baseline, normalized to the symmetric Si–O–Si stretching vibration around ν = 795 cm^{-1}, and vertically offset by 0.3 units. Raw spectral data are shown in **Figure S2**.

Transmission electron microscopy (TEM) images were recorded on a Tecnai T12 Quick CryoEM and CryoET (FEI) at an accelerating voltage of 120 kV. A suspension (8 µL) of nanoparticles in ethanol (MSNs) or chloroform (SPIONs) was dropped on a 200 mesh carbon coated copper grid and the solvent was allowed to evaporate at room temperature. Energy-dispersive x-ray spectroscopy (EDX) and electron diffraction (ED) were carried out at 300 kV using a Titan 80-300 kV microscope and are shown in **Figure S10**.

Nitrogen adsorption and desorption isotherms were obtained at 77 K using an Autosorb-iQ (Quantachrome Instruments). Sample outgassing was performed for 12 hours at 493 K. Pore size distribution and pore volume were calculated by a NLDFT equilibrium model of N_2 on silica, based on the adsorption branch of the isotherms. BET surface area was calculated over the range of partial pressure between ~0.08–0.23 p/p_0. The mesopore volume was determined from NLDFT calculations for pores smaller than 6.5 nm in diameter.

Zeta-potential analysis and dynamic light scattering (DLS) were carried out on a ZetaSizer Nano (Malvern Instruments Ltd., Worcestershire, U.K.) in DI water for MSNs and in chloroform for SPION nanoparticles.

Fluorescence spectra were recorded on an Acton Spectra Pro 2300i CCD cooled below -120 °C with liquid nitrogen. For excitation, a CUBE 445-40C laser (Coherent Inc., Santa Clara, CA, USA) was used at a wavelength of 448 nm and a power of 2 mW. A 475 nm long pass filter was used to block scattered and stray light. In the experiments with conventional heating, a temperature-controlled cuvette holder (Varian Cary 1x1 Peltier) was used. Spectral calibration curves of the fluorescein emission at different temperatures and concentrations are shown in **Figure S7** and a release curve obtained at 0 °C is shown in **Figure S8**.

Superparamagnetic heating was carried out using a Magnetic Hyperthermia System manufactured by MSI Automation, Inc. The diameter and height of the five-turn copper coil that was used for the experiments was 50 mm, the oscillation frequency was 370 kHz, and the induction power was 5 kW.

Thermogravimetric analysis (TGA) was performed using a Perkin-Elmer Pyris Diamond TG/DTA under air (200 mL/min). Approximately 10-15 mg of sample was loaded into aluminum pans. The sample was held at 50 °C for ten minutes, and then the data were recorded from 50 to 550 °C at a scan rate of 5 °C/min. The plotted values are normalized to the weight at 200 °C. An empty aluminum pan was used as a reference.

Field-dependent magnetization isotherms were recorded with a MPMS-XL superconducting quantum interference device (SQUID) magnetometer (Quantum Design Inc.) at 300 K and are shown in **Figure S10**.

Experimental Section

Chemicals:

Tetraethylorthosilicate (TEOS; 99%, Aldrich), cetyltrimethylammonium bromide (CTAB; 98% , Aldrich), sodium hydroxide (99%, Fisher Scientific), maleic anhydride (99%, Aldrich), zinc chloride (anhydrous, 97%, Strem Chemicals), zinc powder (97%, Fisher Scientific), iron(III) acetylacetonate ($Fe(acac)_3$; 97%, Aldrich), manganese(II) chloride ($MnCl_2$; Merck), octylether (99%, Aldrich), triethanolamine (TEA; 98%, Aldrich), cetyltrimethylammonium chloride (CTAC; 25% in H_2O, Fluka), ammonium nitrate, absolute ethanol (EtOH; Aldrich), chloroform ($CHCl_3$; Aldrich), hexamethyldisilazane (HMDS; 99%, Aldrich), 3-aminopropyl triethoxysilane (APTES; 99%, Aldrich), furfurylamine (99%, Aldrich), 1-adamantanecarbonyl chloride (95%, Aldrich), triethylamine (99.5%, EMD), β-cyclodextrin (β-CD; 95%, TCI) and fluorescein disodium salt (90%, Aldrich) were used as received.

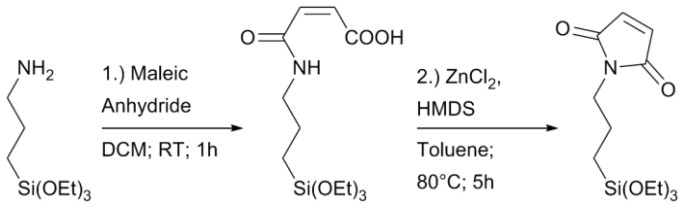
Anhydrous toluene and dichloromethane (DCM) were obtained by distillation from CaH_2 under dry nitrogen.

Zinc chloride was purified according to a literature protocol.[1] In brief, 10 g of zinc chloride and 1 g of zinc powder were refluxed in 1,4-dioxane for 1 h, the hot solution was filtered through celite to remove Zn powder, and allowed to cool to room temperature. The white crystalline solid that formed after cooling was recrystallized from 1,4-dioxane.

Oleic acid and oleylamine were distilled under reduced pressure (1 mbar and 167 °C and 1 mbar and 155°C, respectively) prior to use.

All organic reactions were carried out in dried glassware under an inert atmosphere of dry nitrogen using standard Schlenk techniques.

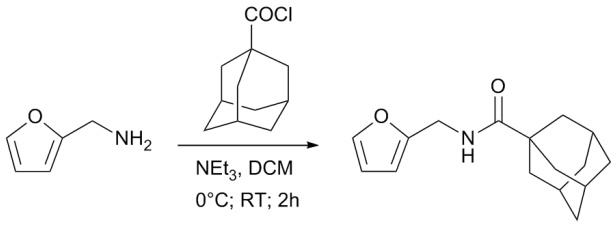
Synthesis of N-((3-Triethoxysilyl)propyl)maleimide (1):



The synthesis was carried out according to a published procedure.[2,3] In brief, 1.73 g (17.6 mmol) of maleic anhydride were stirred in 60 mL of anhydrous dichloromethane in a flame-dried 250 mL three neck round bottom flask under nitrogen for 5 minutes. Then, 4.125 mL of 3-aminopropyl triethoxysilane (17.6 mmol) in 20 mL of dry dichloromethane was added slowly under stirring, and the resulting mixture was kept at room temperature for 1 h. After that, volatiles were removed *in vacuo*, and the intermediate maleamic acid derivative was obtained as a white powder, which was directly used in the next step without further purification.

In the next step, the intermediate product was dissolved in 60 mL of dry toluene and stirred under nitrogen. Then, 2.40 g of anhydrous zinc chloride were added at once and the reaction mixture was heated to 80 °C. Next, 3.67 mL of hexamethyldisilazane (17.6 mmol) were added, and the mixture was kept at 80 °C for 5 hours. After cooling to room temperature, the solution was filtered to remove zinc chloride and the solvent was removed *in vacuo*, giving the product as a colorless oil. ^{1}H NMR (400.13 MHz; $CDCl_3$): δ = 0.53(m, 2H, SiC<u>*H*</u>$_2$), 1.16t, 9H, C<u>*H*</u>$_3$CH$_2$O), 1.64(p, 2H, SiCH$_2$C<u>*H*</u>$_2$), 3.45(t, 2H, C<u>*H*</u>$_2$N), 3.75(q, 6H, CH$_3$C<u>*H*</u>$_2$O), 6.63(s, 2H, <u>*H*</u>C=C<u>*H*</u>), ^{13}C NMR (100.61 MHz; $CDCl_3$): δ = 7.88(Si<u>*C*</u>H$_2$), 18.41(<u>*C*</u>H$_3$CH$_2$O), 22.26(SiCH$_2$<u>*C*</u>H$_2$), 40.55(<u>*C*</u>H$_2$N), 58.57(CH$_3$<u>*C*</u>H$_2$O), 134.18(H<u>*C*</u>=<u>*C*</u>H), 170.98(<u>*C*</u>=O).

Synthesis of N-(furan-2-ylmethyl)adamantane-1-carboxamide (2):



In a flame-dried 100 mL round bottom flask, a mixture of 2.0 mL of furfurylamine (22 mmol) and 3 mL of triethylamine were stirred in 45 mL of dry dichloromethane under nitrogen at 0 °C. Then, 4.4 g of 1-adamantane carbonylchloride (22 mmol) in 5 mL of dry dichloro-methane was added slowly, and the solution was allowed to warm to room temperature. After stirring for 1 h at room temperature, the solution was washed with 40 mL of an aqueous ammonium chloride solution (saturated) and 40 mL of an aqueous potassium carbonate solution (5%), the organic layer was separated, dried over $MgSO_4$, filtered, and evaporated to dryness *in vacuo*. The crude product was recrystallized from heptane/EtOAc = 1:1 (v/v) to

yield the product as off-white needles (3.15 g, 55%). ^{1}H NMR (400.13 MHz; $CDCl_3$): δ = 1.69(m, 6H, $CCH_2CHC\underline{H_2}$(Ad)), 1.84(m, 6H, $CC\underline{H_2}CHCH_2$(Ad)), 2.02(m, 3H, $CCH_2C\underline{H}CH_2$(Ad)), 4.40(d, 2H, $C\underline{H_2}NH$), 5.86(bs, 1H, N*H*), 6.18(dd, 1H, CC*H*CHCH(Fur)), 6.29(dd, 1H, CCHC*H*CH(Fur)), 7.33(dd, 1H, CCHCHC*H*(Fur)) ^{13}C NMR (100.61 MHz; $CDCl_3$): δ = 28.31($CCH_2\underline{C}HCH_2$(Ad)), 36.70($CCH_2CH\underline{C}H_2$(Ad)), 36.70($\underline{C}H_2NH$), 39.41($C\underline{C}H_2CHCH_2$(Ad)), 40.88($\underline{C}CH_2CHCH_2$(Ad)), 107.42($C\underline{C}HCHCH$(Fur)), 110.63($CCH\underline{C}HCH$(Fur)), 142.34($CCHCH\underline{C}H$(Fur)), 151.84($\underline{C}CHCHCH$(Fur)), 177.88($\underline{C}$=O).

Nanoparticle Synthesis:

1.) Sample MSN:

Unfunctionalized mesoporous silica nanoparticles were synthesized according to a published procedure.[4,5] In brief, 200 mg of CTAB and 600 µL of sodium hydroxide solution (2 M) were dissolved in 100 mL of water under stirring. The solution was heated at 80 °C for 30 minutes, followed by the addition of 1050 µL of TEOS under vigorous stirring. Stirring was continued for 2 h at 80 °C, and then the solution was allowed to cool to room temperature. The nanoparticles were collected by centrifugation (10 min at 7197 rcf), washed 2x with water (2x 90 mL), 2x with ethanol (2x 90 mL) and 2x with toluene (2x 90 mL), redispersed in 20 mL of dry toluene and directly used for further functionalization.

2.) Sample MSN-Mal:

The unfunctionalized mesoporous silica nanoparticles in 20 mL of dry toluene were stirred in a flame-dried 50 mL round bottom flask under nitrogen. Then, 40 µL of N-((3-triethoxysilyl)propyl)maleimide were added, and the resulting mixture was heated to reflux overnight. The nanoparticles were collected by centrifugation (10 min at 7197 rcf), washed 2x with toluene (2x 90 mL) and 2x with ethanol (2x 90 mL). To extract the organic template from the pores, the nanoparticles were dispersed in 90 mL of an ethanolic ammonium nitrate solution (1 mg/50 mL), refluxed for 1 h, collected by centrifugation (10 min at 7197 rcf), washed 1x with ethanol (90 mL), redispersed in 90 mL of a fresh ethanolic ammonium nitrate solution (1 mg/50 mL), refluxed again for 1 h, collected by centrifugation (10 min at 7197 rcf), washed 2x with ethanol (2x 90 mL) and stored in ethanol.

3.) Sample MSN-DA:

25 mg of MSN-Mal nanoparticles (dispersed in ethanol) were washed 2x with toluene (2x 1.5 mL), and then redispersed in 10 mL of toluene. 80 mg of N-(furan-2-ylmethyl) adamantane-1-carboxamide were added, and the resulting mixture was stirred for 3 days at 40°C. The nanoparticles were collected by centrifugation in a cooled centrifuge (5 min at 20817 rcf and 18°C), washed 2x with toluene (2x 1.5 mL), 2x with ethanol (2x 1.5 mL) and 2x with water (2x 1.5 mL).

4.) Sample MSN-CD:

For loading the model drug into the nanoparticles, 0.5 mg of sample MSN-DA were dispersed in 1 mL of an aqueous fluorescein solution (1 mM) and kept on a shaker over night at room temperature. For capping, 15 mg of β-cyclodextrin was added to the solution, and shaking was continued for 1 d at room temperature. The nanoparticles were then collected by centrifugation in a cooled centrifuge (5 min at 20817 rcf and 18 °C), washed 5x with water (5x 1.5 mL), and redispersed in 250 µL water.

5.) Superparamagnetic zinc and manganese doped iron oxide nanoparticles ($Zn_{0.4}Mn_{0.6}$)Fe_2O_4:

Zinc and manganese doped iron oxide nanoparticles were synthesized following a thermal decomposition process as previously described.[6] In brief, 0.353 g (1.00 mmol) $Fe(acac)_3$, 30.0 mg (0.220 mmol) $ZnCl_2$ and 63.3 mg (0.320 mmol) $MnCl_2$ were placed in a 50 mL three-neck round bottom flask equipped with a reflux condenser under nitrogen atmosphere. 2.00 mL oleic acid, 4.00 mL oleylamine and 2.06 mL octylether were added and the reaction mixture was heated to 300 °C (SiC bath) for 1 h. The reaction mixture was cooled to room temperature and absolute ethanol was added. The resulting nanoparticles were washed three times with a mixture of chloroform and ethanol (1:10) by centrifugation (10 min, 26892 rcf) and finally redispersed in 10 mL of chloroform.

6.) Sample SPION@MSN:

Prior to the sol-gel reaction, the SPIONs were transferred from the organic phase to the aqueous phase. 4.285 mL of a 7 mg/mL SPION dispersion in $CHCl_3$ (corresponding to 30 mg of SPIONs) were placed in a polypropylene reactor. 21.7 g H_2O and 2.41 mL of aqueous CTAC solution (25 wt%) was added, generating a second phase. The mixture was sonicated for 15 min (60% of continuous power (250 W), frequency 20 KHz) using a probe sonicator and subsequently the chloroform was evaporated at elevated temperature (70 °C) for 2 h. After a second sonication step lasting 15 min, the mixture was added to 14.3 g TEA and stirred (1000 rpm) at 60 °C. The silica source TEOS (10 times 155 µL, 692 µmol) was added

stepwise every 10 min over a total time period of 90 min at constant temperature of 60 °C. The synthesis mixture was stirred at 1000 rpm at room temperature for 12 h. After addition of ethanol (100 mL), the SPION@MSNs were separated by centrifugation (43.146 rcf for 20 min) and redispersed in ethanol. The template extraction was performed twice by heating the SPION@MSN suspension under reflux at 90 °C (oil bath) for 45 min in an ethanolic solution (100 mL) containing ammonium nitrate (2 g). The SPION@MSNs were collected by centrifugation and washed with ethanol after each extraction step. The resulting nanoparticles were stored in an ethanolic solution.

7.) Sample SPION@MSN-Mal:

20 mg of the unfunctionalized iron oxide core – mesoporous silica shell nanoparticles (SPION@MSN) were washed 2x with toluene (2x 1.5 mL), redispersed in 10 mL of dry toluene and stirred in a flame-dried 25 mL round bottom flask under nitrogen. Then, 40 µL of N-((3-triethoxysilyl)propyl)maleimide was added, and the resulting mixture was heated to reflux overnight. The nanoparticles were collected by centrifugation (5 min at 16873 rcf), washed 2x with toluene (2x 1.5 mL) and redispersed in 2.5 mL of toluene.

8.) Sample SPION@MSN-DA:

20 mg of SPION@MSN-Mal in 2.5 mL of toluene were stirred in a glass vial together with 80 mg of N-(furan-2-ylmethyl)adamantane-1-carboxamide for 3 days at 40 °C. The nanoparticles were collected by centrifugation in a cooled centrifuge (5 min at 20817 rcf and 18 °C), and washed 2x with toluene (2x 1.5 mL), 2x with ethanol (2x 1.5 mL) and 2x with water (2x 1.5 mL).

9.) Sample SPION@MSN-CD:

For loading the model drug into the nanoparticles, 1 mg of sample SPION@MSN-DA were dispersed in 1 mL of an aqueous fluorescein solution (1 mM) and kept on a shaker over night at room temperature. For capping, 15 mg of β-cyclodextrin was added to the solution, and shaking was continued for 1 d at room temperature. The nanoparticles were then collected by centrifugation in a cooled centrifuge (5 min at 20817 rcf and 18 °C), washed 5x with water (5x 1.5 mL), and redispersed in 250 µL water.

Release experiments:

For the release experiments by conventional heating (**Figure 3.a**), 0.5 mg of nanoparticles suspended in 250 µL water were added into a reservoir that was separated from an aqueous solution in a standard 1 cm fluorescence cuvette by a 14 kDa MWCO dialysis membrane (VWR). An emission scan (409.13 nm – 688.30 nm) was recorded every second, the intensity around the fluorescein emission maximum was integrated (500 nm – 550 nm) and averaged over 600 scans (corresponding to 10 minutes), and the results were plotted against time. For obtaining the spectral data of fluorescein fluorescence at different concentrations and temperatures, appropriate dilutions of fluorescein disodium salt in water were prepared and fluorescence emission data from 60 spectra were averaged. The obtained data were fitted with a linear regression model and used to calculate the amount of released fluorescein at different temperatures (see also **Figure S7**).

In the superparamagnetic heating experiments at room temperature (**Figure 3.b**), 1 mg of nanoparticles was dispersed in 250 µL water and added to a reservoir that was separated from an aqueous solution (10 mL) in a 20 mL glass vial by a 14 kDa MWCO dialysis membrane. After monitoring the release every 15 minutes for 1 hour at room temperature, the sample was exposed to an AMF for 30 minutes, followed again by monitoring the release at room temperature for 30 minutes (plotted is the mean intensity of 3 individual measurements 0 min, 15 min and 30 min after the heating cycle). In total, five such heating/monitoring cycles were performed. For measuring fluorescein emission, a 2.5 mL sample was drawn from the vial, 60 emission scans were recorded from 409.13 nm – 688.30 nm at an exposure time of 1 second, the intensity around the fluorescein emission maximum was integrated (490 nm – 550 nm), the results were averaged, and the 2.5 mL sample was added back into the vial. Here, no correction was made to the emission intensity since the effect of temperature change of the bulk solution on fluorescein fluorescence was negligible.

The procedure for the superparamagnetic heating experiment at 0 °C (**Figure S8**) was similar to the one described above. The only differences were that the sample was kept in an ice bath at 0 °C at all times, and that only three cycles with 30 minutes AMF exposure and 1 hour of monitoring time were carried out. Here, also no correction was made to the emission intensity since the temperature was always fixed at 0 °C for all measurements due to the ice bath.

Figures:

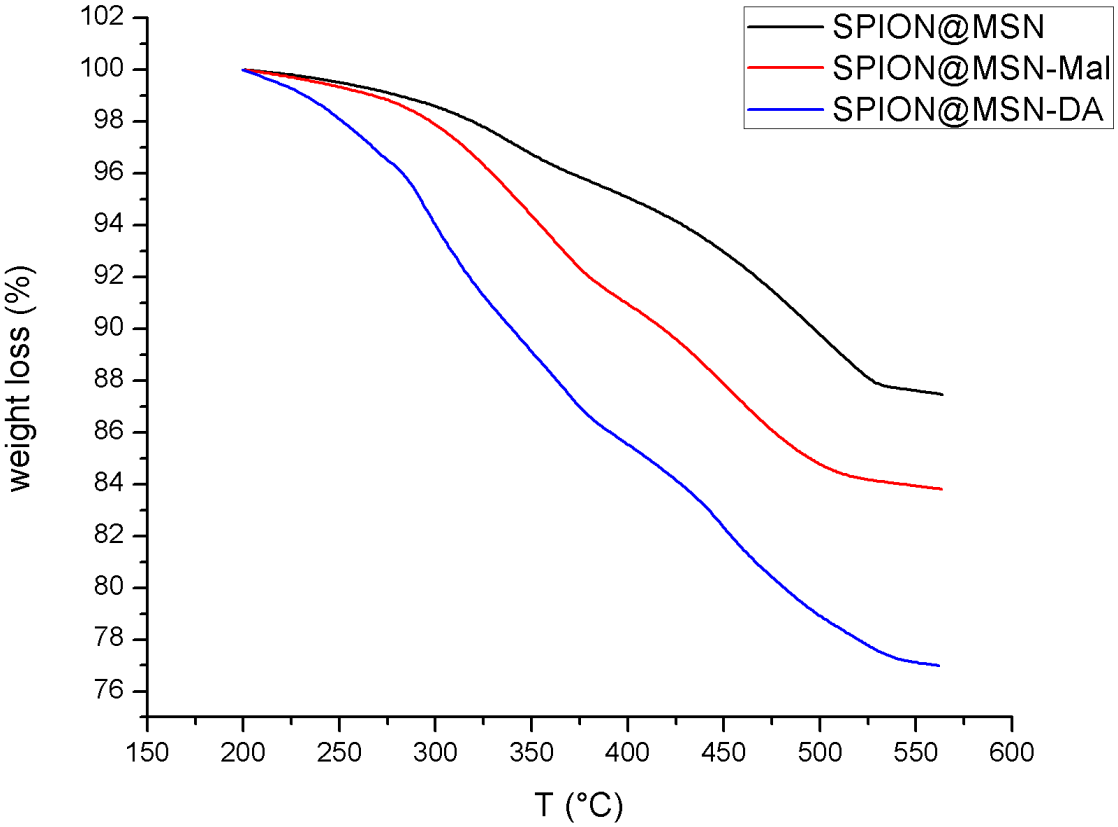


Figure S1: Thermogravimetric analysis of samples SPION@MSN (black), SPION@MSN-Mal (red) and SPION@MSN-DA (blue).

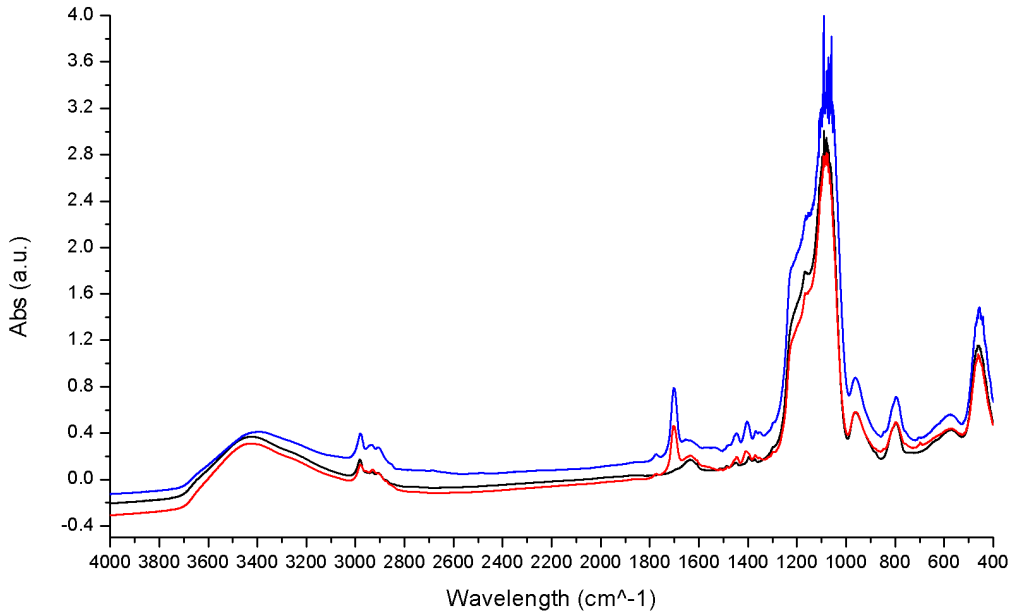


Figure S2: Raw IR spectral data for samples SPION@MSN (black), SPION@MSN-Mal (red), and SPION@MSN-DA (blue).

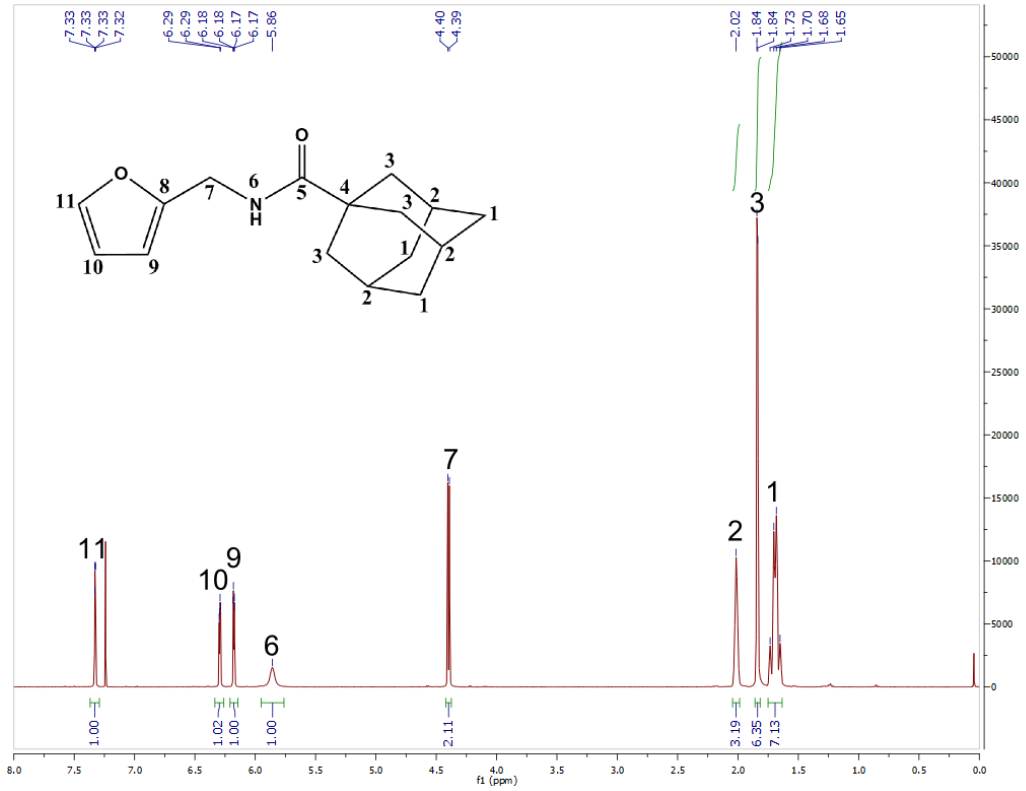


Figure S3: ^{1}H NMR ($CDCl_3$, 25 °C) of N-(furan-2-ylmethyl)adamantane-1-carboxamide.

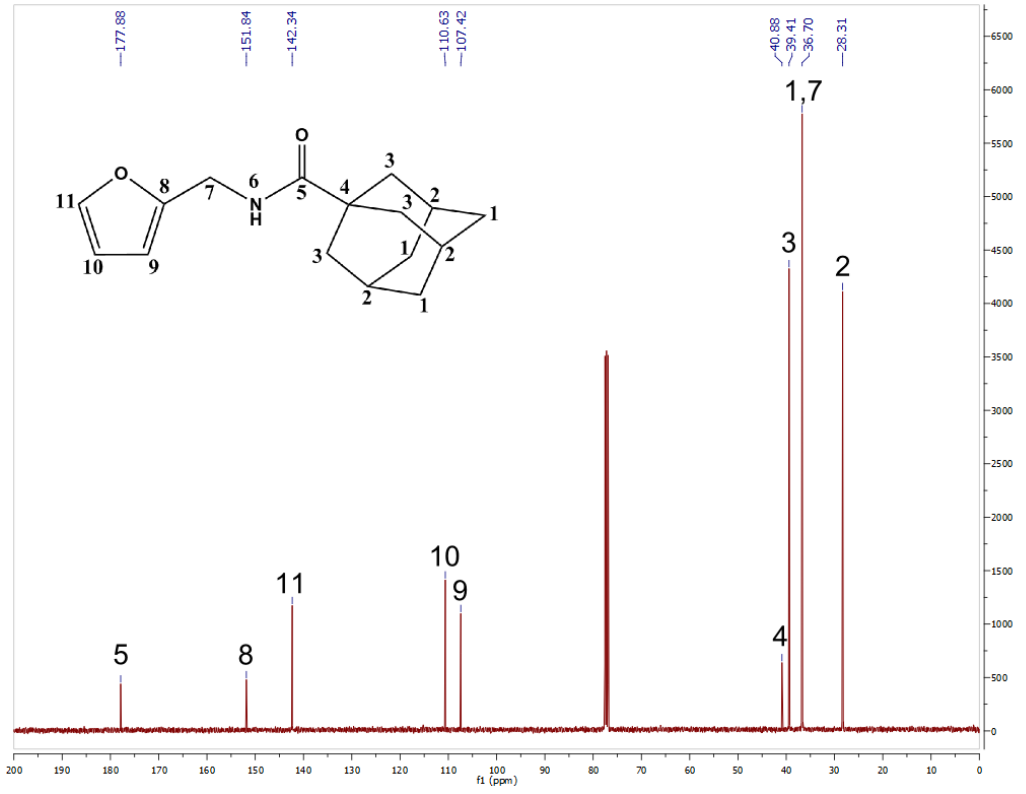


Figure S4: ^{13}C NMR ($CDCl_3$, 25 °C) of N-(furan-2-ylmethyl)adamantane-1-carboxamide.

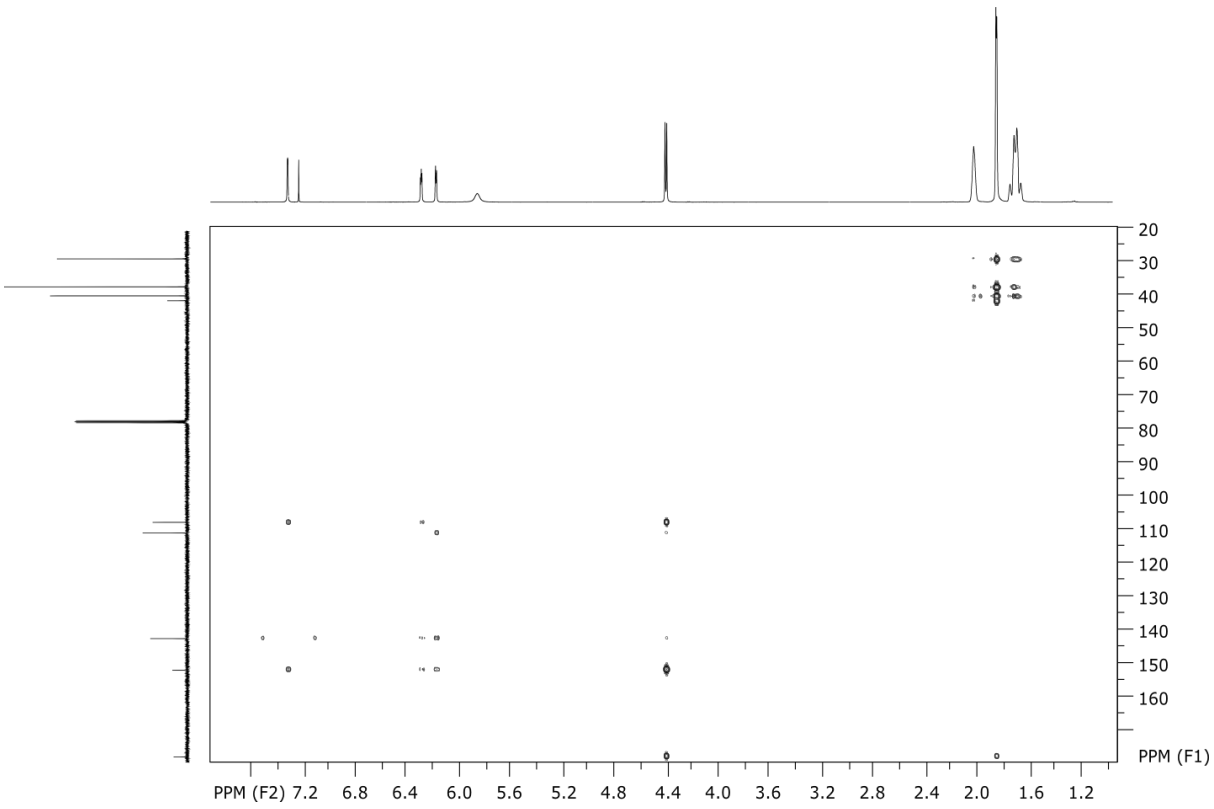


Figure S5: ^{1}H-^{13}C HMBC ($CDCl_3$, 25 °C) of N-(furan-2-ylmethyl)adamantane-1-carboxamide.

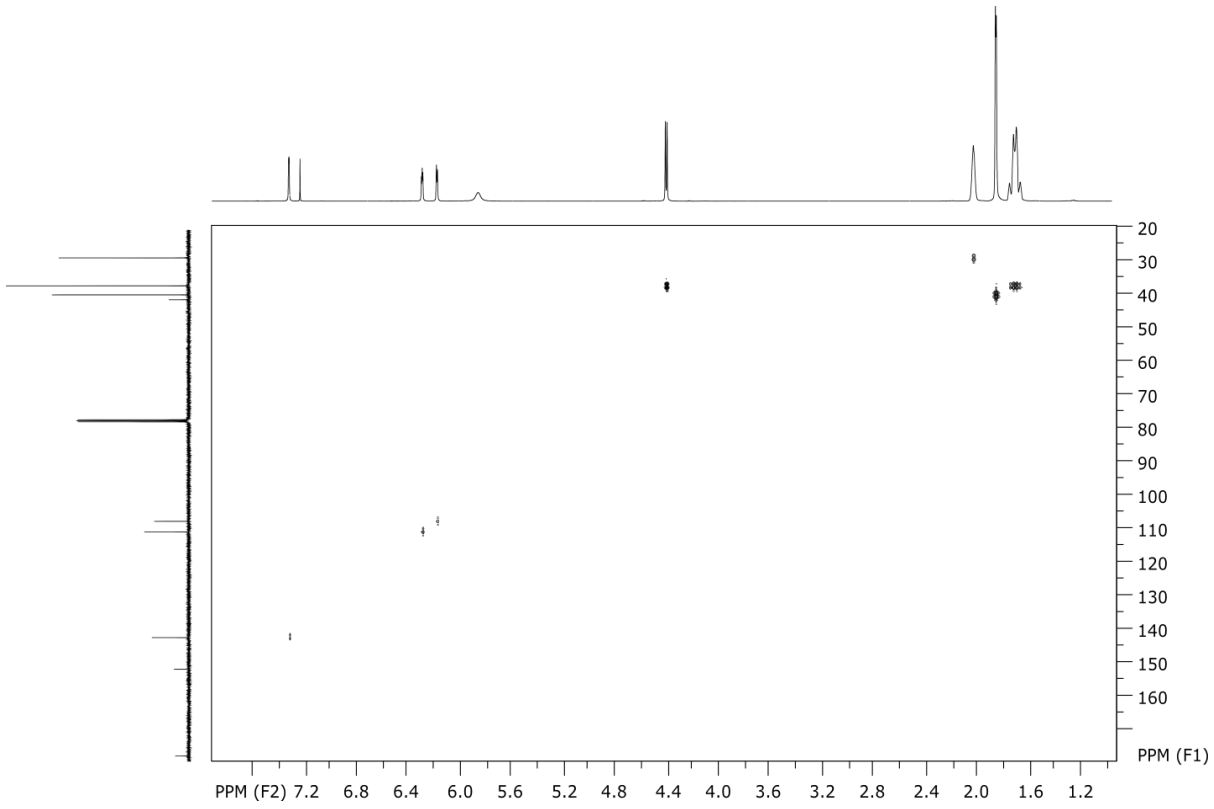


Figure S6: ^{1}H-^{13}C HMQC ($CDCl_3$, 25 °C) of N-(furan-2-ylmethyl)adamantane-1-carboxamide.

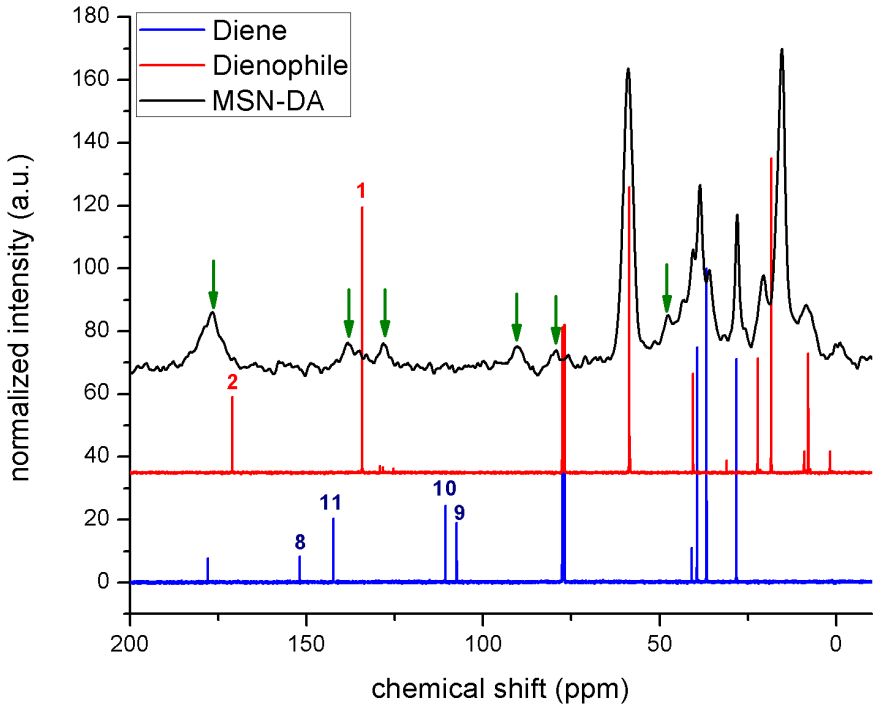
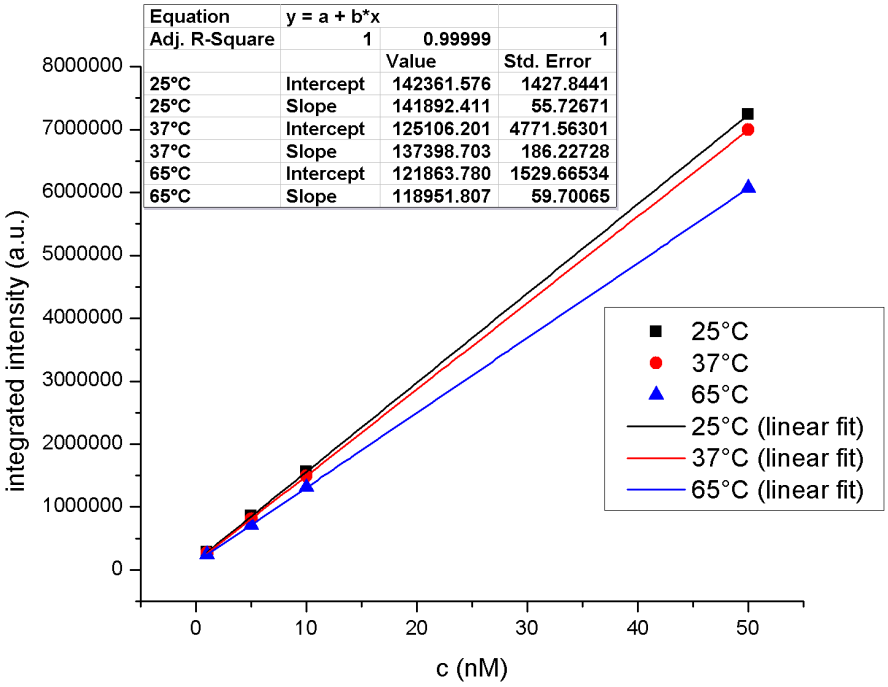


Figure S7: ^{13}C NMR data of the diene component (blue), the dienophile component (red) and the Diels-Alder cycloaddition product on the surface of the silica nanoparticles (black).



Equation	y = a + b*x		
Adj. R-Square	1	0.99999	1
		Value	Std. Error
25°C	Intercept	142361.576	1427.8441
25°C	Slope	141892.411	55.72671
37°C	Intercept	125106.201	4771.56301
37°C	Slope	137398.703	186.22728
65°C	Intercept	121863.780	1529.66534
65°C	Slope	118951.807	59.70065

Figure S8: Calibration curves for different fluorescein concentrations at 25 °C, 37 °C and 60 °C.

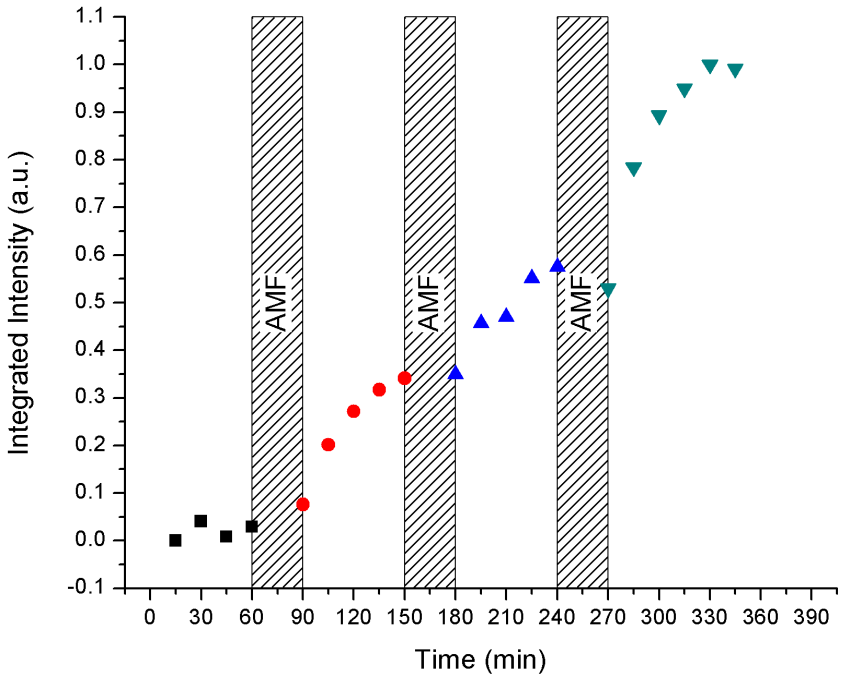


Figure S9: Release experiments with superparamagnetic heating in an ice bath at 0 °C.

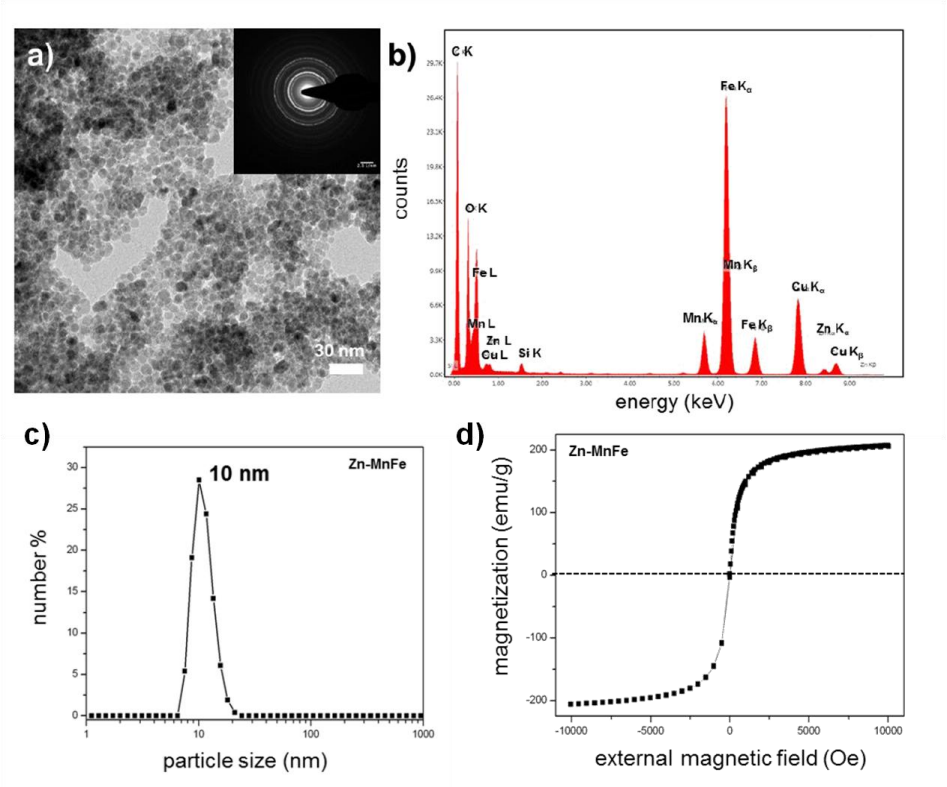


Figure S10: Further characterization of zinc and manganese-doped iron oxide nanoparticles. (a) TEM image (inset: electron diffraction pattern). (b) EDX analysis. Cu and C are from the TEM grids. (c) Dynamic light scattering in $CHCl_3$. The particle size is approximately 10 nm. (d) Field-dependent magnetization isotherms recorded at 300 K. The saturation magnetization is approximately 200 emu/g.

Table S11: Nitrogen Sorption Data

Property	SPION@MSN	SPION@MSN-DA
S_{BET} (m^2/g)	1034	859
d_{Pore} (NLDFT) (nm)	4.3	3.8
V_{tot} (NLDFT) (cc/g)	1.23	0.82
$V_{mesopores}$ (NLDFT) (cc/g)	0.80	0.59
d_{Pore} (BJH) (nm)	3.0	2.8
V_{tot} (BJH) (cc/g)	1.48	0.97
$V_{mesopores}$ (BJH) (cc/g)	1.03	0.73