Electronic Supplementary Material (ESI) for ChemComm.


# Supporting Information

# Reversible Speed Control of One-Stimulus-Double-Response, Temperature-Sensitive Asymmetric Micromotors

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## 1. Materials

All chemicals and enzymes were used as received unless otherwise stated. Ultrapure Milli-Q water, obtained with the help of a Labconco Water Pro PS purification system (18.2 MΩ), was used for the procedures. Poly(ethylene) tubing (0.56/1.07 mm inner/outer diameter) was purchased from ThermoFischer. Centrifugal filters, Amicon® Ultra (0.5mL, 3 kDa and 10 kDa), were purchased from Millipore. Eppendorf® centrifuge 5430 R was used for filtration. Eppendorf® ThermoMixer C was used for shaking. Fusion 100 Touch pump was purchased from KR Analytical Ltd. Asahi spectra Max-300® was used to photocure the hydrogel beads. Leica Thunder microscope was used for visualization of the microgels. SP8x AOBS-WLL confocal was used for fluorescence experiments. VAHEAT Dynamic Temperature Control from Interherence was used for temperature cycles analysis. JEOL 6330 Cryo FESEM was used for structural characterization of microgels network. Stress-controlled rheometer (Discovery HR-2, TA Instrument) was used for rheology studies. NMR characterization was carried out on a Bruker AVANCE III console with a 11.7 T UltraShield Plus magnet (500 MHz) equipped with a Bruker Prodigy cryoprobe or a JEOL ECZ-R 11.7 T magnet (500 MHz). Sylgard® 184 silicone elastomer kit was used for the fabrication of the PDMS microfluidic chip. Trichloro(1H,1H,2H,2H-perfluorooctyl)silane (97%) was purchased from Sigma-Aldrich. Dextran from leuconostoc mesenteroides (average Mn 3500-45000) was purchased from Sigma Aldrich. Poly(ethylene glycol) diacrylate (average Mn 575) was purchased from Sigma Aldrich. Poly(N-isopropylacrylamide) carboxylic acid terminated (average Mn 2 kDa, 10 kDa and 80 kDa) was purchased from AV Chemistry. Photoinitiator 2-hydroxy-4'-(2-hydroxyethoxy)-2- methylpropiophenone (98%) was purchased from Sigma-Aldrich. 1H,1H,2H,2H-Perfluoro-1- octanol (97%) was purchased from Sigma Aldrich. Cyanine 3 amine was purchased from Lumiprobe. 008-FluoroSurfactant was purchased from RAN Biotechnologies. 3M™ Novec™ 7500 Engineered Fluid was purchased from FluoroChem. Catalase form bovine liver lyophilized powder (10.000 units/mg protein) (>99%) was purchased from Sigma-Aldrich.

## 2. PDMS Microfluidic Device

Monomer and initiator (10:1 w/w) were mixed and poured onto the silicon master after which it was degassed under vacuum for at least 4 hours. The PDMS was cured at 65°C overnight, washed with isopropanol and blow dried. After oxygen plasma treatment, the PDMS was

bonded to a glass slide. The channels were coated with trichloro(1H,1H,2H,2H-perfluorooctyl)silane (2% w/w in fluorinated oil) and the device was baked at 110°C overnight.[2]

## 3. Formation of Asymmetric Hydrogel Beads

### 3.1 PEGDA:Dextran Asymmetric Microparticles Fabrication

All solutions were flushed with nitrogen for at least 0.5 hours, the fluorocarbon oil (HFE 7500) was flushed for 15 minutes, to remove dissolved oxygen. PEGDA (25% w/w and 35% w/w) and Dextran (20% w/w) were injected in the second and third inlet respectively. Irgacure® 2959 (0.4% wt final concentration) to the PEGDA solution prior to injection. The droplets were formed at the second cross-junction by the introduction of an outer phase which consisted of a fluorocarbon oil (HFE 7500) and surfactant (SS01, 2% w/w). The resulting emulsion was collected in an Eppendorf® in which Mineral oil (30 µL) was added to ensure the first particles do not break. UV curing of PEGDA was achieved by exposing the emulsion to a focused UV beam (λ = 320-500 nm, 5 min, 40% intensity). PEGDA undergoes free-radical polymerization (FRP). Because of the presence of a diacrylate functionality, FRP will happen on both sides of the polymer strand ensuring a completely crosslinked network. During cross-linking of the photopolymerisable phase, the dextran diffuses inside the PEGDA polymer network, leaving behind an opening. The emulsion was broken by adding 1H,1H,2H,2H-Perfluoro-1-octanol (300µL, 20% w/w in hexane), after which the beads were washed three times with MiliQ. Catalase (final concentration 6 mg/mL, 30000 units) was added into the PEGDA phase prior to injection. PEGDA:Dextran microgels were used as control in order to compare their behaviour with the temperature-responsive PEGDA-PNIPAm: Dextran microparticles.

Flowrates: Oil: 600 µL/h, PEGDA: 60 µL/h, Dextran: 20 µL/h

### 3.2 PEGDA-PNIPAm:Dextran Asymmetric Microparticles Fabrication

The PNIPAm is incorporated into the main phase as single stranded polymer. The average molecular weight of the PNIPAm is varied between 2 kDa, 10 kDa, and 80 kDa. All solutions and fluorocarbon oil (2% w/w surfactant SS01 in HFE 7500) were flushed with nitrogen for 15 minutes. PEGDA-PNIPAm (25% w/w, 10% w/w respectively) and dextran (20% w/w) met at the first cross-junction and emulsified at the second cross-junction by the oil. The photo-initiator is added to the main phase before injection and the particles are photocured (λ = 320-

500, 300s, LI 40%) after collection at the outlet channel in an Eppendorf® containing mineral oil (30 µL). The emulsion was broken by adding 1H,1H,2H,2H-Perfluoro-1-octanol (300 µL, 20% w/w in hexane), after which the microparticles were washed three times with Milli-Q water (7 min, 14000 rpm). Catalase was either dissolved into the PEGDA-PNIPAm phase prior to injection (final concentration 6 mg/mL, 30000 units) or cross-linked to PNIPAm before preparation of PEGDA-PNIPAm phase.

Flowrates: Oil: 600 µL/h, PEGDA-PNIPAm: 60 µL/h, Dextran: 20 µL/h.

## 4. PNIPAm Labelling with Cy3

PNIPAm-COOH 2 kDa (200 mg, 100 µmol, 1.0 eq) was dissolved in MES buffer (1 mL, pH = 4.9, 100 mM) and to the solution were added EDC (2.55 mg, 17 µmol, 0.17 eq.) and sulfo-NHS (3.6 mg, 17 µmol, 0.17 eq.). The reaction mixture was stirred for 1 hour at room temperature. The PNIPAm was purified using spin filtration (14,000 rpm for 15 min) and washed three times with PBS buffer (pH = 8.1, 100 mM). The PNIPAm was incubated with the Cyanine 3 dye (1.7 mg, 2.7 µmol, 0.03 eq.) in 10 mL PBS overnight. The dyed PNIPAm was washed with PBS over a 3 kDa spin filter until washings were colourless.

PNIPAm-COOH 10 kDa (200 mg, 20 µmol, 1.0 eq) was dissolved in MES buffer (1 mL, pH = 4.9, 100 mM) and to the solution were added EDC (0.51 mg, 3.3 µmol, 0.17 eq.) and sulfo-NHS (0.72 mg, 3.3 µmol, 0.17 eq.). The reaction mixture was stirred for 1 hour at room temperature. The PNIPAm was purified using spin filtration (14,000 rpm for 15 min) and washed three times with PBS buffer (pH = 8.1, 100 mM). The PNIPAm was incubated with the Cyanine 3 dye (1.7 mg, 2.7 µmol, 0.14 eq.) in 10 mL PBS overnight. The dyed PNIPAm was washed with PBS over a 10 kDa spin filter until washings were colourless.

PNIPAm-COOH 80 kDa (200 mg, 2.5 µmol, 1.0 eq) was dissolved in MES buffer (1 mL, pH = 4.9, 100 mM) and to the solution were added EDC (0.064 mg, 0.42 µmol, 0.17 eq.) and sulfo-NHS (0.09 mg, 0.42 µmol, 0.17 eq.). The reaction mixture was stirred for 1 hour at room temperature. The PNIPAm was purified using spin filtration (14,000 rpm for 15 min) and washed three times with PBS buffer (pH = 8.1, 100 mM). The PNIPAm was incubated with the Cyanine 3 dye (1.7 mg, 2.7 µmol, 1.08 eq.) in 10 mL PBS overnight. The dyed PNIPAm was washed with PBS over a 10 kDa spin filter until washings were colourless.

## 5. Temperature Cycles Experiments for Morphology

On a VAHEAT smart substrate (5x5 mm), SecureSeal® imaging spacers were attached to create a well. In the well, a droplet of water (20 μL) was put and the micromotors (5 μL) were added. The well was closed off be a coverslip. The smart substrate was connected to the VAHEAT heating device. Videos were recorded using Leica Thunder microscope (bright field microscopy for size analysis) and SP8x AOBS-WLL confocal (for fluorescence analysis). The micromotors sizes and fluorescence were measured over cycles: cycle 1 (t=0 at RT, t=2 at 43°C after heating up for 5 minutes), cycle 2 (t=4 at RT after cooling down for 7 min, t=6 at 43°C after heating up for 5 minutes), cycle 3 (t=8 at RT after cooling down for 7 min, t=10 at 43°C after heating up for 5 minutes) and cycle 4 (t=12 at RT after cooling down for 7 min). The temperature of 43°C was chosen because it is above the LCST of PNIPAm and it is the human heat pain threshold via skin contact.[3]

## 6. Catalase Coupling to PNIPAm

PNIPAm-COOH 2 kDa (50 mg, 25 μmol of carboxylic groups, 1.0 eq) was dissolved in 200 μL MES buffer (pH = 4.9, 100 mM) and put on ice. EDC (9.4 mg, 60 μmol, 2.4 eq) in MES buffer (50 μL, pH = 4.9, 100 mM) was added and the reaction mixture was stirred (500 rpm) for 30 minutes. Subsequently, the sulfo-NHS (12 mg, 55 μmol, 2.2 eq) in MES buffer (50 μL, pH = 4.9, 100 mM) and catalase (15 mg, 150000 units) in Milli-Q (10 μL) were added to the reaction mixture and the mixture was stirred (500 rpm) for 4 hours at room temperature. The PNIPAm was washed four times with PBS over a 3 kDa spin filter.

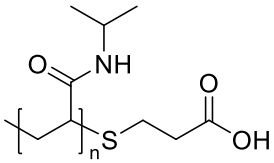
PNIPAm-COOH 10 kDa (50 mg, 5 μmol of carboxylic groups, 1.0 eq) was dissolved in 200 μL MES buffer (pH = 4.9, 100 mM) and put on ice. EDC (1.9 mg, 12 μmol, 2.4 eq) in MES buffer (50 μL, pH = 4.9, 100 mM) was added and the reaction mixture was stirred (500 rpm) for 30 minutes. Subsequently, the sulfo-NHS (2.4 mg, 11 μmol, 2.2 eq) in MES buffer (50 μL, pH = 4.9, 100 mM) and catalase (3 mg, 30000 units) in Milli-Q (10 μL) were added to the reaction mixture and the mixture was stirred (500 rpm) for 4 hours at room temperature. The PNIPAm was washed four times with PBS over a 10 kDa spin filter.

PNIPAm-COOH 80 kDa (50 mg, 0.63 μmol of carboxylic groups, 1.0 eq) was dissolved in 200 μL MES buffer (pH = 4.9, 100 mM) and put on ice. EDC (0.2 mg, 1.5 μmol, 2.4 eq) in MES buffer (50 μL, pH = 4.9, 100 mM) was added and the reaction mixture was stirred (500 rpm)

for 30 minutes. Subsequently, the sulfo-NHS (0.3 mg, 1.4 μmol, 2.2 eq) in MES buffer (50 μL, pH = 4.9, 100 mM) and catalase (0.4 mg, 4000 units) in Milli-Q (10 μL) were added to the reaction mixture and the mixture was stirred (500 rpm) for 4 hours at room temperature. The PNIPAm was washed four times with PBS over a 10 kDa spin filter.

## 7. NMR Analysis

Nuclear Magnetic resonance (NMR) characterization was carried out on a Bruker AVANCE III console with a 11.7 T UltraShield Plus magnet (500 MHz) equipped with a Bruker Prodigy cryoprobe or a JEOL ECZ-R 11.7 T magnet (500 MHz) equipped with a RoyalHFX probe, in $D_2O$. NMR spectra were recorded at 298 K unless otherwise specified. Chemical shifts are given in parts per million (ppm) with respect to water (δ 4.79 ppm) in $D_2O$ as internal standard for $^1H$ NMR. Coupling constants are reported as *J* values in Hz. Peak assignment is based on 2D gDQCOSY, $^1H^{13}C$ gHSQCED, and $^1H^{13}C$ gHMBC spectra. Diffusion spectra were measured sequentially. Side groups and end of chain signals separated from the bulk polymer $^1H$ signal are only reported when observed with clear s/n ratio and no overlap with polymer peaks, and may be (in)visible on other NMR spectrometers or with different concentrations




NMR Assignment $PNIPAm_n$:

**$^1$H NMR** (500 MHz, $D_2O$) δ 8.15 – 7.27 (m, amide), 3.83 (s, 1nH, C*H*$(CH_3)_2$), 2.72 – 2.59 (m, 2H, SC*H*$_2$), 2.59 – 2.48 (m, 2H, $SCH_2CH_2$), 2.20 − 1.79 (m, 1nH, backbone C*H*), 1.79 − 1.27 (m, 2nH, backbone C*H*$_2$), 1.08 (s, 6nH, CH(C*H*$_3$)$_2$). **$^{13}$C NMR** (125 MHz, $D_2O$) δ 178.2 (*C*OOH), 175.3 (*C*ONH), 42.6 (backbone *C*H), 41.6 (*C*H$(CH_3)_2$), 35.3 ($SCH_2CH_2$), 34.7 (backbone *C*$H_2$), 27.2 ($SCH_2CH_2$), 21.5 CH(*C*$H_3$)$_2$.

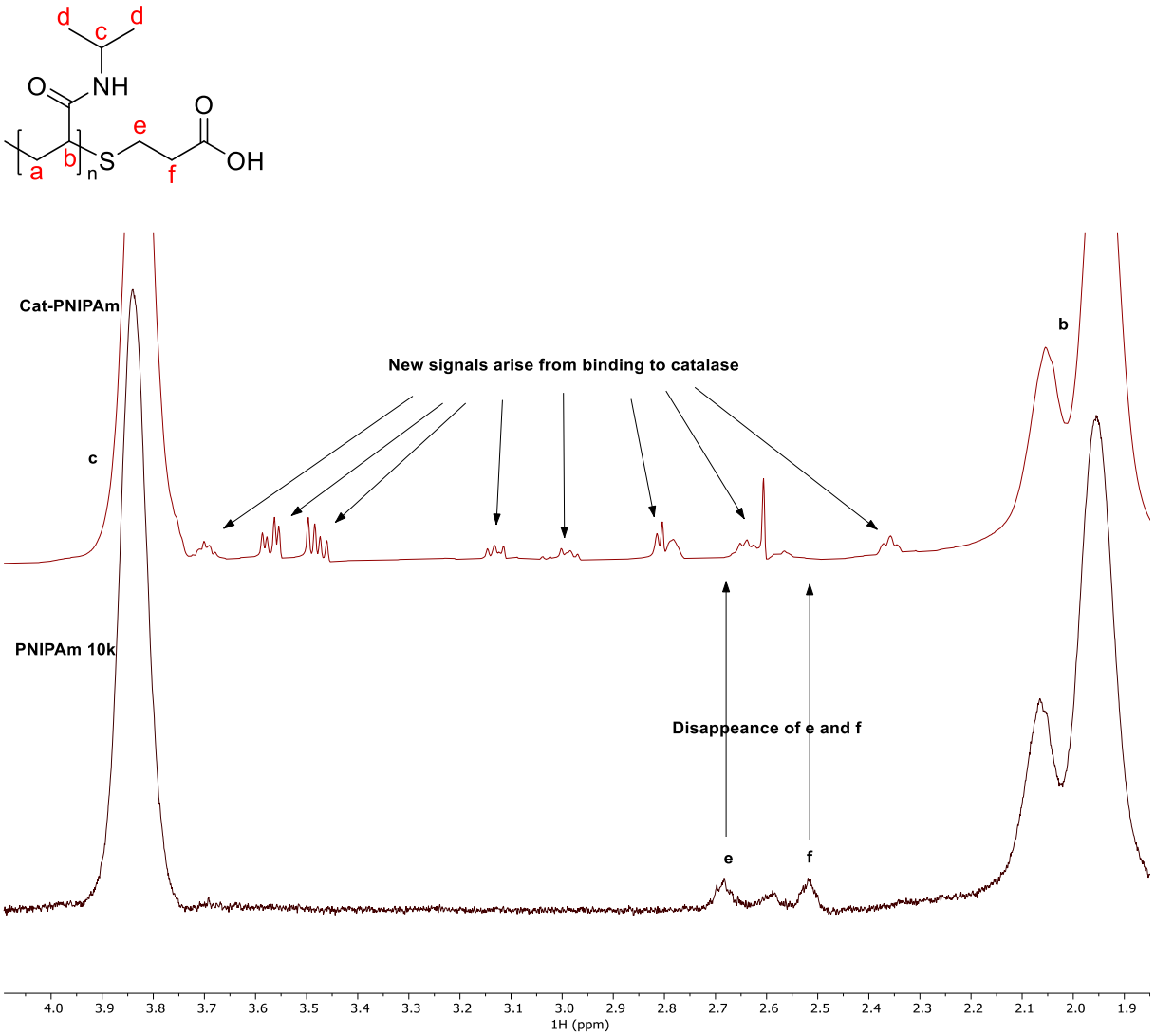



*Figure S1. $^{1}H$ NMR spectra of PNIPAm 10k (bottom) and PNIPAm 10k bound to catalase (top).*

**$^{1}H$ NMR spectra (500 MHz, 298 K) in $D_2O$**, of PNIPAm 10k (bottom) and PNIPAm 10k bound to catalase (top). PNIPAm 10k shows two distinct shifts for the functional end group ($SCH_2CH_2COOH$, e and f) between 3.7 and 2.2 ppm. The original signals of the unreacted functional end group (e and f) are no longer observed upon coupling to catalase, indicating a reaction has taken place that changed their chemical shifts. Instead, multiple extra signals are observed in this region which were not observed before. This indicates that the PNIPAm has been covalently coupled to the catalase, allowing for some parts of the catalase to more easily dissolve, thus becoming visible on liquid NMR.

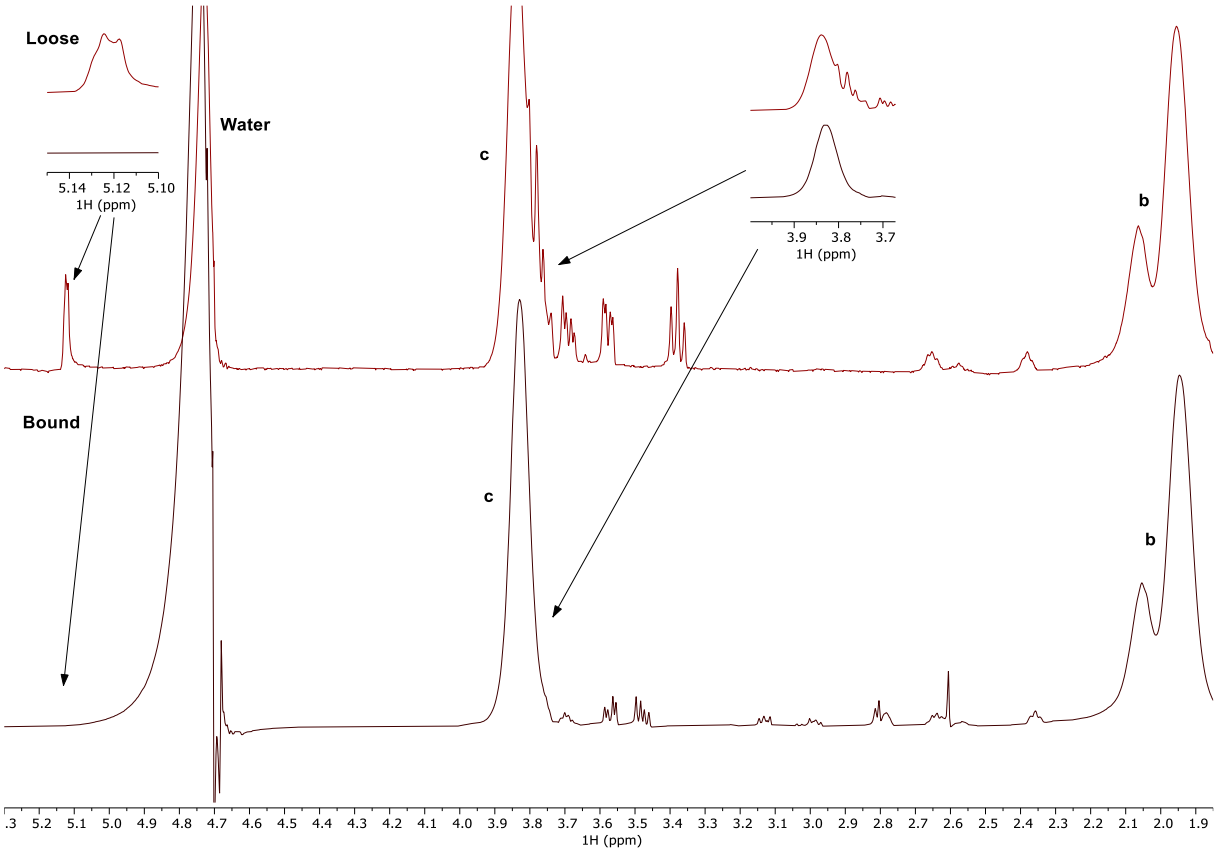



*Figure S2. $^{1}H$ NMR spectra of PNIPAm 10k and catalase loose (top) and PNIPAm 10k bound to catalase (bottom).*

**$^{1}$H NMR spectra (500 MHz, 298 K) in $D_2O$**, of PNIPAm 10k and catalase loose (top) and PNIPAm 10k bound to catalase (bottom). The loose catalase is unwashed, and still contains stabilising sugars. These signals have disappeared after attachment of PNIPAm and subsequent washing (5.12, 3.85 – 3.35 ppm). Instead, multiple signals are observed (3.6 – 2.3 ppm) which are not observed when the two components are added together without binding. This indicates that the PNIPAm has been covalently coupled to the catalase.

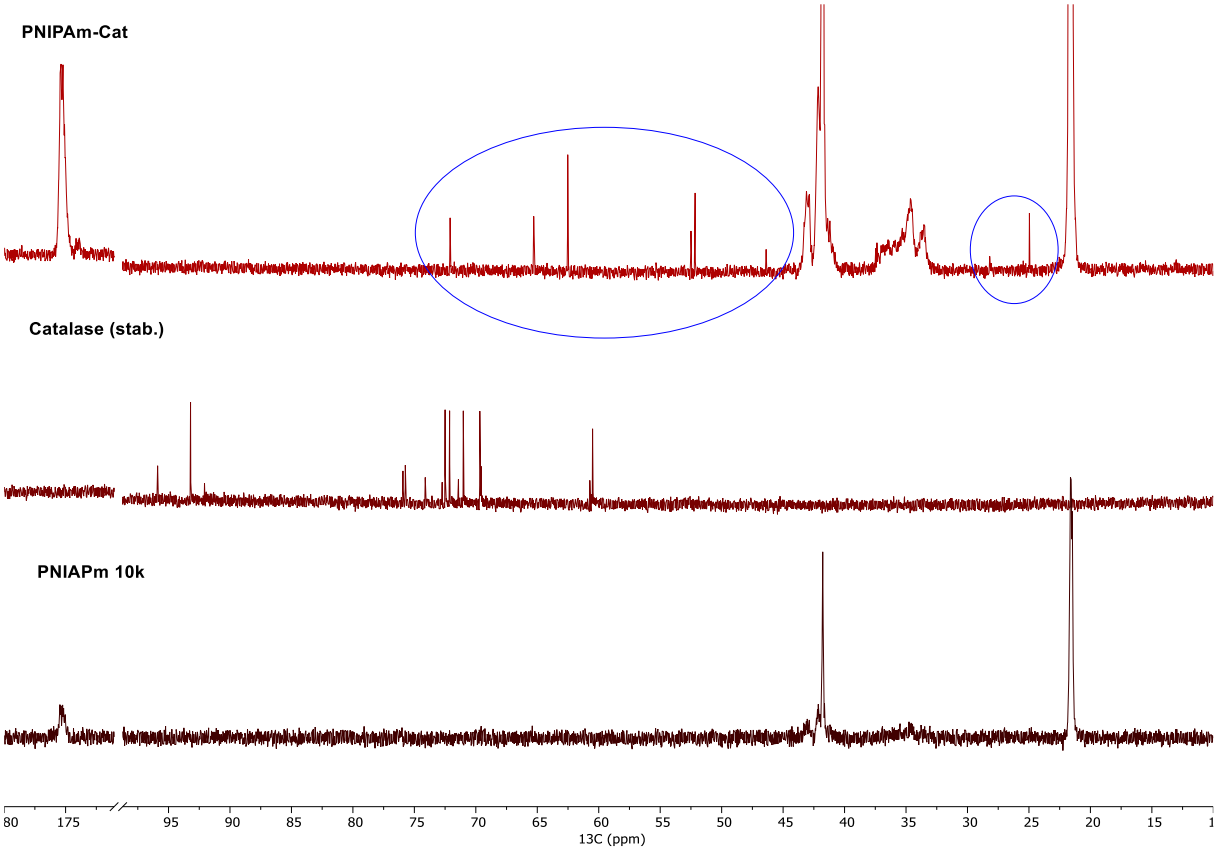



*Figure S3. $^{13}C$ NMR spectra of PNIPAm 10k and catalase bound (top), catalase (middle) and PNIPAm 10k (bottom).*

**$^{13}C$ NMR spectra (125 MHz, 298 K) in $D_2O$**, of PNIPAm 10k and catalase bound (top), catalase (middle) and PNIPAm 10k (bottom). New carbon signals in the regions of 73 – 45 ppm and 30 – 25 ppm are observed, originating from the binding between PNIPAm and catalase. Carbon signals observed in the catalase spectrum can be attributed to stabilising sugars.

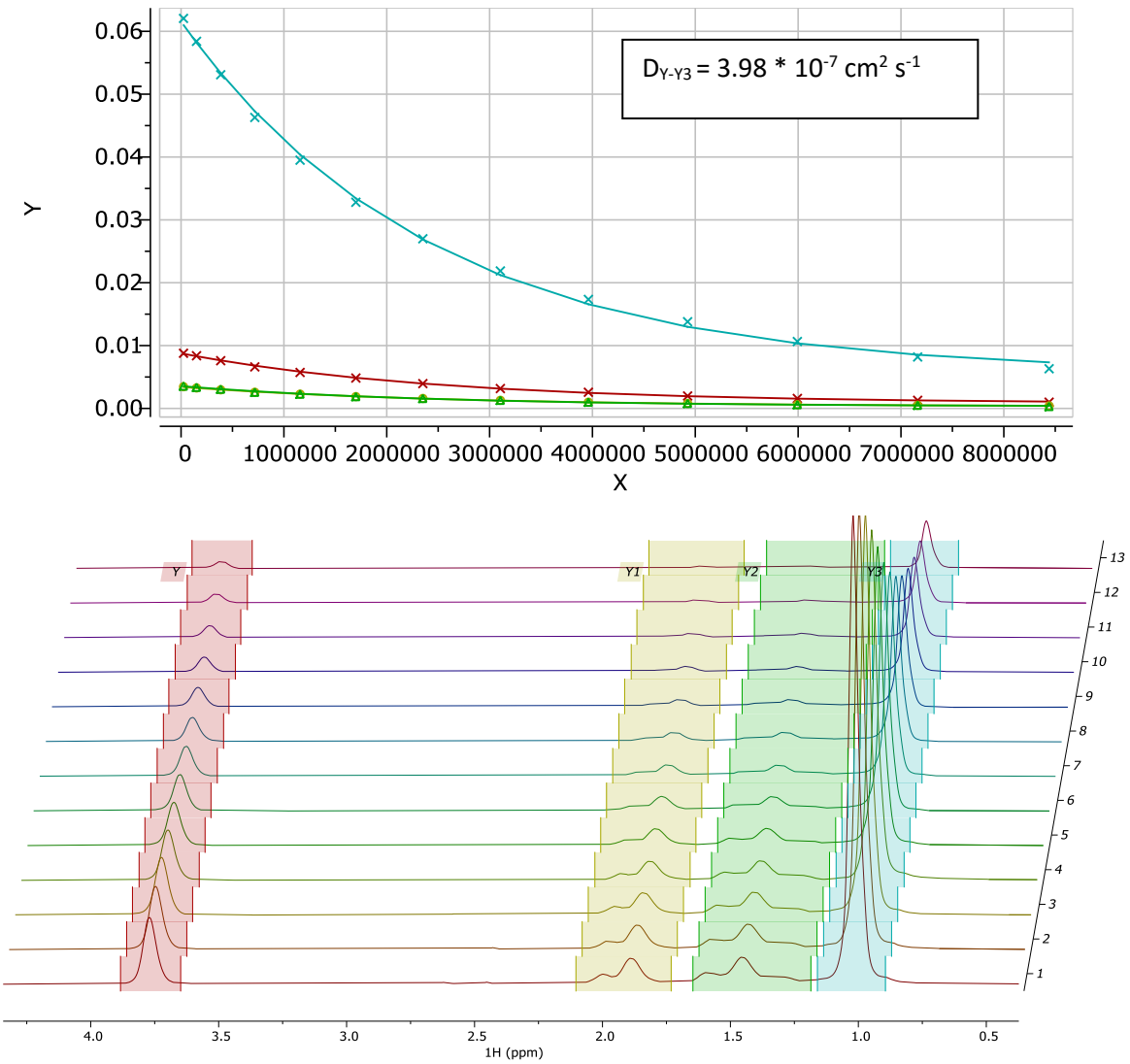



*Figure S4. $^1$H NMR diffusion spectra (500 MHz, 298 K) in $D_2O$ of $PNIPAm_{10k}$*

**$^1$H NMR diffusion spectra (500 MHz, 298 K) in $D_2O$ of $PNIPAm_{10k}$.** Gradient strengths ranged from 5% to 95%. The absolute gradient strength was not calibrated prior to use as a relative comparison was all that was desired. DY-Y3 is the average diffusion coefficient of the PNIPAm signals. End group signals were not used due to low signal to noise ratio.

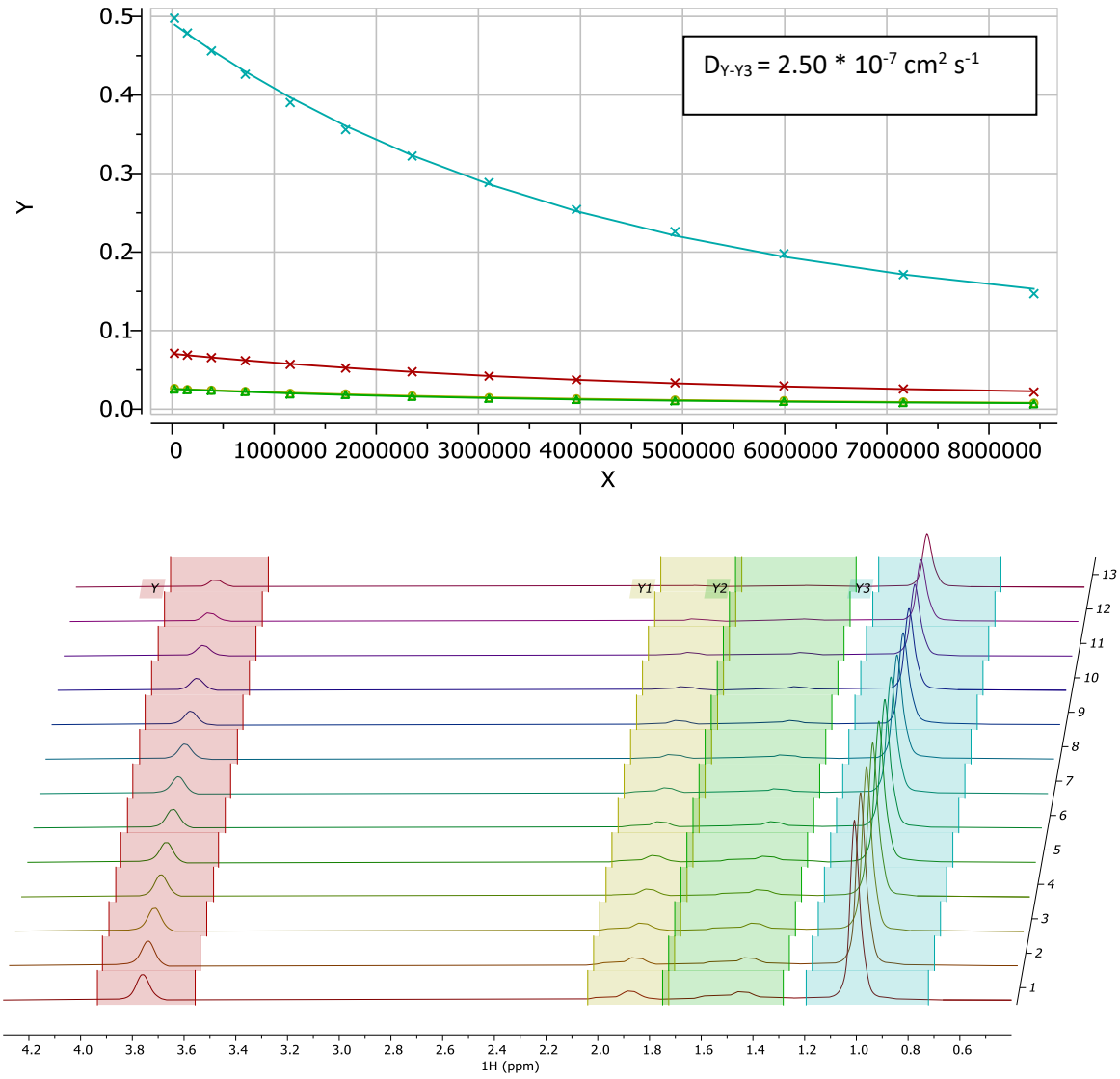



*Figure S5. $^1$H NMR diffusion spectra (500 MHz, 298 K) in $D_2O$ of $PNIPAm_{10k}$- catalase.*

**$^1$H NMR diffusion spectra (500 MHz, 298 K) in $D_2O$ of $PNIPAm_{10k}$- catalase.** Gradient strengths ranged from 5% to 95%. The absolute gradient strength was not calibrated prior to use as a relative comparison was all that was desired. DY-Y3 is the average diffusion coefficient of the PNIPAm signals. End group signals were not used due to low signal to noise ratio. The diffusion coefficient is about 40% lower compared to free PNIPAm 10k, which corresponds to a roughly six-fold increase in size according to the Stokes-Einstein equation. Catalase consists of four parts of 60 kDa, thus de increase in diffusion corresponds to the average binding of one PNIPAm polymer to every catalase part in this solution. [4]

## 8. Cryo-SEM Analysis for Structural Characterization of Microgels

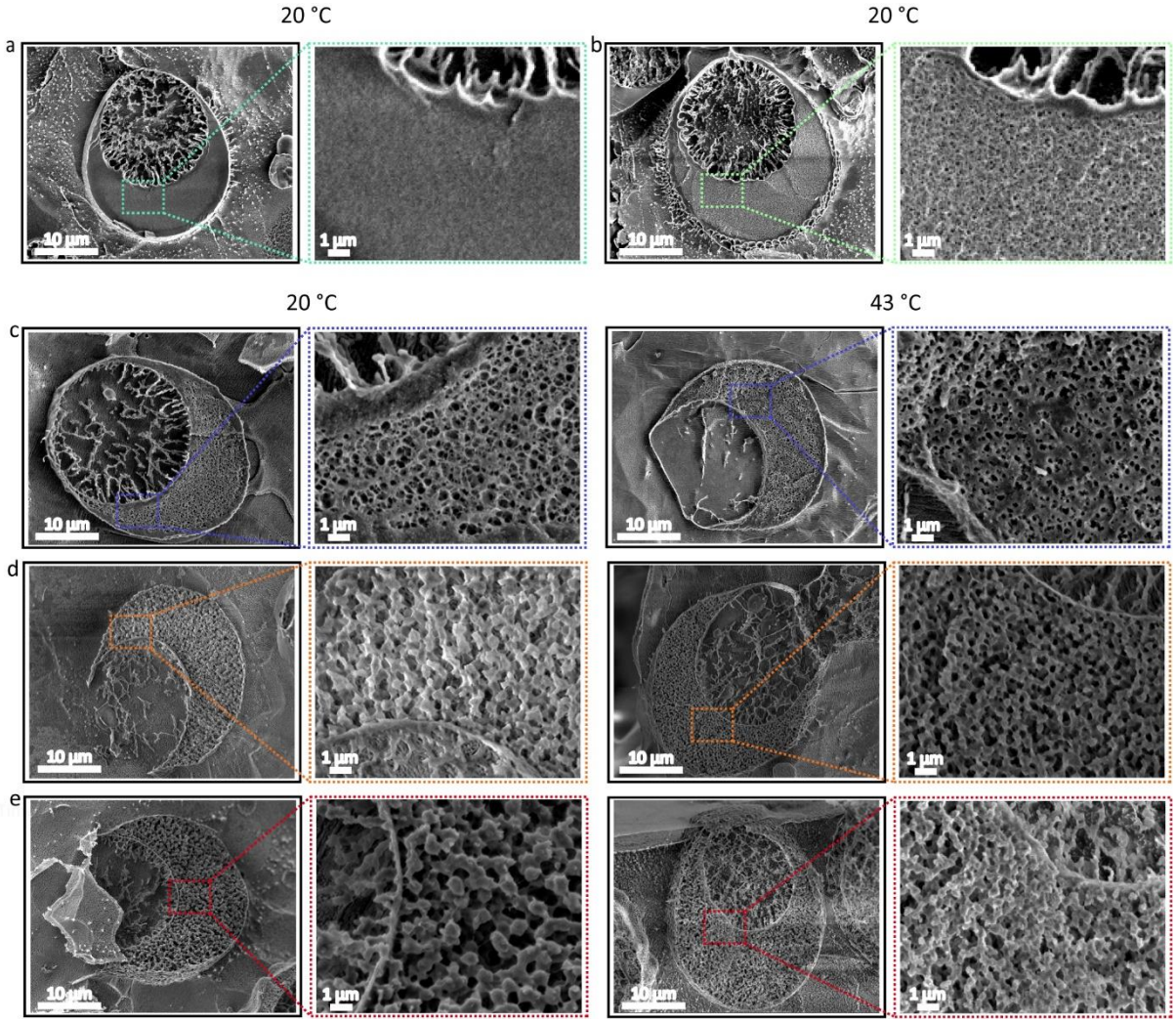



*Figure S6. Cryo-scanning electron microscopy of microgels. PEGDA:Dextran hydrogel beads at 20°C (a) PEGDA 35% w/w and (b) PEGDA 25% w/w. In the gel network at higher concentration of PEGDA pores are not visible, at concentration of PEGDA 25% w/w small pores are uniformly distributed within the structure. (c), (d) and (e) PEGDA-PNIPAm:Dextran hydrogel beads, on the left at 20°C and on the right at 43°C. MW of PNIPAm: 2 kDa (c), 10 kDa (d) and 80 kDa (e). The pores size increases by increasing the MW of PNIPAm, and decreases at 43°C as a result of deswelling due to collapse of PNIPAm and water expulsion.*

Cryo-scanning electron microscopy (cryo-SEM) was performed for structural characterization of microgels using JEOL 6330 Cryo FESEM. 100 µL of each sample was first fixed with nitrogen slush, fed under high vacuum conditions chamber, and then transferred to a cold stage in the SEM chamber for further analysis under cryogenic conditions. PEGDA:Dextran microgels (PEGDA 35% w/w and PEGDA 25% w/w) and PEGDA-PNIPAm:Dextran microgels (PEGDA 25% w/w and PNIPAm 10% w/w MW 2 kDa, 10 kDa and 80 kDa respectively) were analysed by cryo-SEM after being kept overnight at 20°C. Afterwards, PEGDA-PNIPAm:Dextran microgels (PEGDA 25% w/w and PNIPAm 10% w/w MW 2 kDa,

10 kDa and 80 kDa respectively) were kept at 43°C for 10 minutes, fixed with nitrogen slush and immediately transferred to the SEM chamber for visualization.

## 9. Rheology for Hydrogels Mechanical Properties Investigation at Different Temperature

The mechanical properties were analysed on a stress-controlled rheometer (Discovery HR-2, TA Instrument) using a steel parallel plate geometry with a plate diameter of 20 mm and a gap of 400 µm. The samples were loaded into the rheometer as thin-film hydrogels: solutions of PEGDA (25% w/w and 35% w/w) and PEGDA-PNIPAm (PNIPAm 10% w/w, 2 kDa, 10 kDa and 80 kDa) were prepared in a glass vial and treated under nitrogen flow for 15 min. Irgacure® 2959 (0.4% wt final concentration) was added and then the solutions were UV-photocured (λ = 320-500 nm, 5 min, 40% intensity) in a glass lid 20 mm in diameter. The storage modulus (G') was measured over a temperature range of 25 - 43°C.

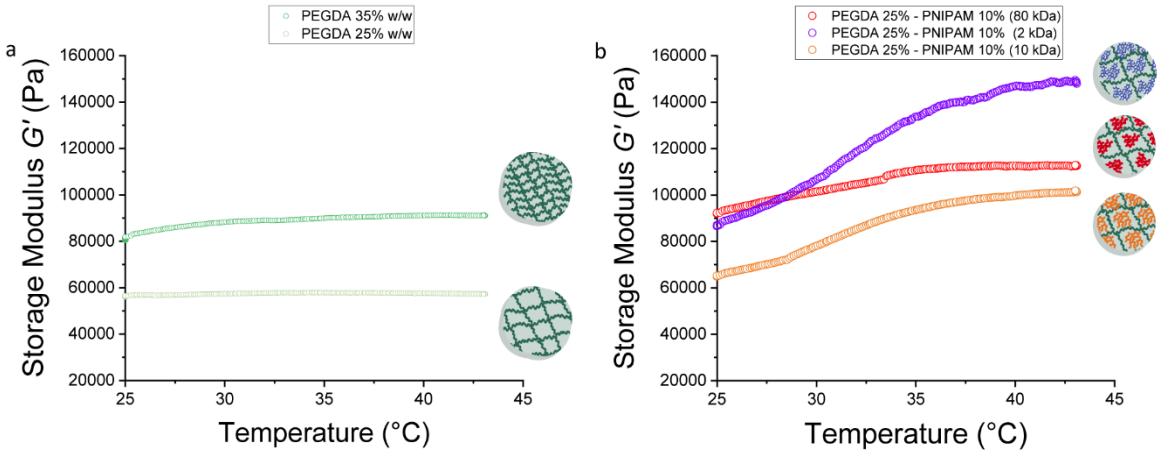



*Figure S7. Temperature sweep experiments of a range 25-43°C. (a) Storage Modulus (G') of PEGDA hydrogels: as the amount of PEGDA is increased, the water content is decreased, thus a stiffer gel is obtained. The increase of temperature does not influence the stiffness of the hydrogel. (b) Storage Modulus (G') of PEGDA-PNIPAm hydrogels for different PNIPAm MW. All the curves are of the S-shape around the LCST of PNIPAm, meaning that the single stranded PNIPAm has an influence on the overall hydrogel network and once the polymer has passed 32°C, the polymer turns hydrophobic leading to dehydration. Upon dehydration, the hydrogel network collapses, which is measured as an increase in the storage modulus of the hydrogel. The effect is bigger for lower MW of PNIPAm, leading to a stiffer hydrogel at 43°C when MW of PNIPAm is 2 kDa.*

## 10.Temperature Cycles Experiments for Autonomous Movement

Autonomous Movement Experiments at room temperature (20°C): Hydrogen peroxide solution (4% in Milli-Q) was added into a petri dish of 3 cm in diameter (3 mL). The micromotors were diluted 5x in Milli-Q (60 μL in a total volume of 300 μL) and 20 μL of the diluted solution was added to the hydrogen peroxide fuel *via* a syringe through the hole in the cover of the petri dish to minimize the drift. The micromotors were diluted 5x in Milli-Q (60 μL in a total volume of 300 μL) and 20 μL of the diluted solution was added to the hydrogen peroxide fuel *via* a syringe through the hole in the cover of the petri dish to minimize the drift.

Autonomous Movement Experiments at 43°C: Hydrogen peroxide solution (4% in Milli-Q) in falcon tubes (15 mL) was kept for 45 minutes prior experiments and for all the duration of the experiments, in a water bath at 43°C and then was added (3 mL) into a petri dish of 3 cm in diameter pre-heated at 45°C for 15 min. The sample was kept for 10 min in a thermo shaker at 43°C, then the micromotors were diluted 5x in Milli-Q (60 μL in a total volume of 300 μL) and 20 μL of the diluted solution was added to the hydrogen peroxide fuel *via* a syringe through the hole in the cover of the petri dish to minimize the drift. Milli-Q for dilution in falcon tubes (15 mL) was kept for 45 minutes prior experiments and for all the duration of the experiments, in a water bath at 43°C.

Videos were recorded using an inverted microscope equipped with a high-speed camera. Recording started upon addition of the micromotors to the fuel solution. Movies were taken of the moving micromotors for a period of 20 seconds after which they were analysed using a home build tracking software. Ten of the fastest motors were tracked and their speed was calculated as average of distances travelled by the motor for each time point tracked (0.2 sec). The speed of the micromotors was measured over three cycles at room temperature (20°C) and 43°C.

# 11.Temperature Cycles for Volume Analysis of PEGDA:Dextran microgels

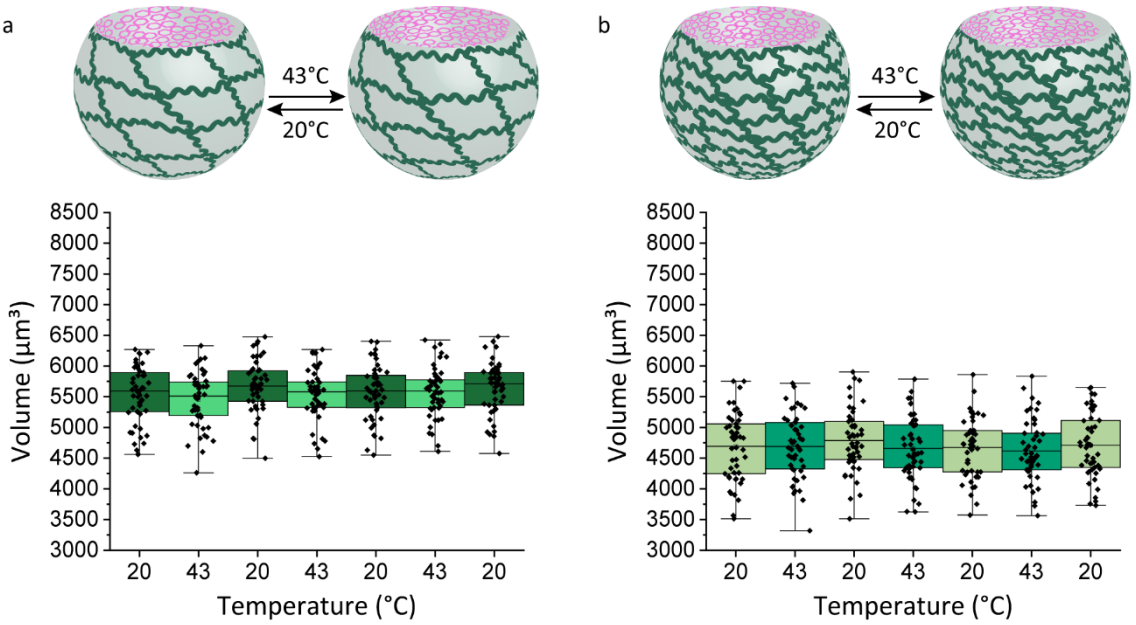



*Figure S8. Cycles of temperature at 20°C and 43°C for volume analysis of PEGDA:Dextran microgels. (a) PEGDA concentration is 25% w/w, shrinking of the volume at 43°C is 1.4%. (b) PEGDA concentration is 35% w/w, shrinking of the volume at 43°C is 1.2%. This confirms that the absence of PNIPAm brings to the generation of non thermo-responsive microgels.*

## 12. Fluorescence Experiments over Temperature Cycles of PEGDA-PNIPAm: Dextran microgels

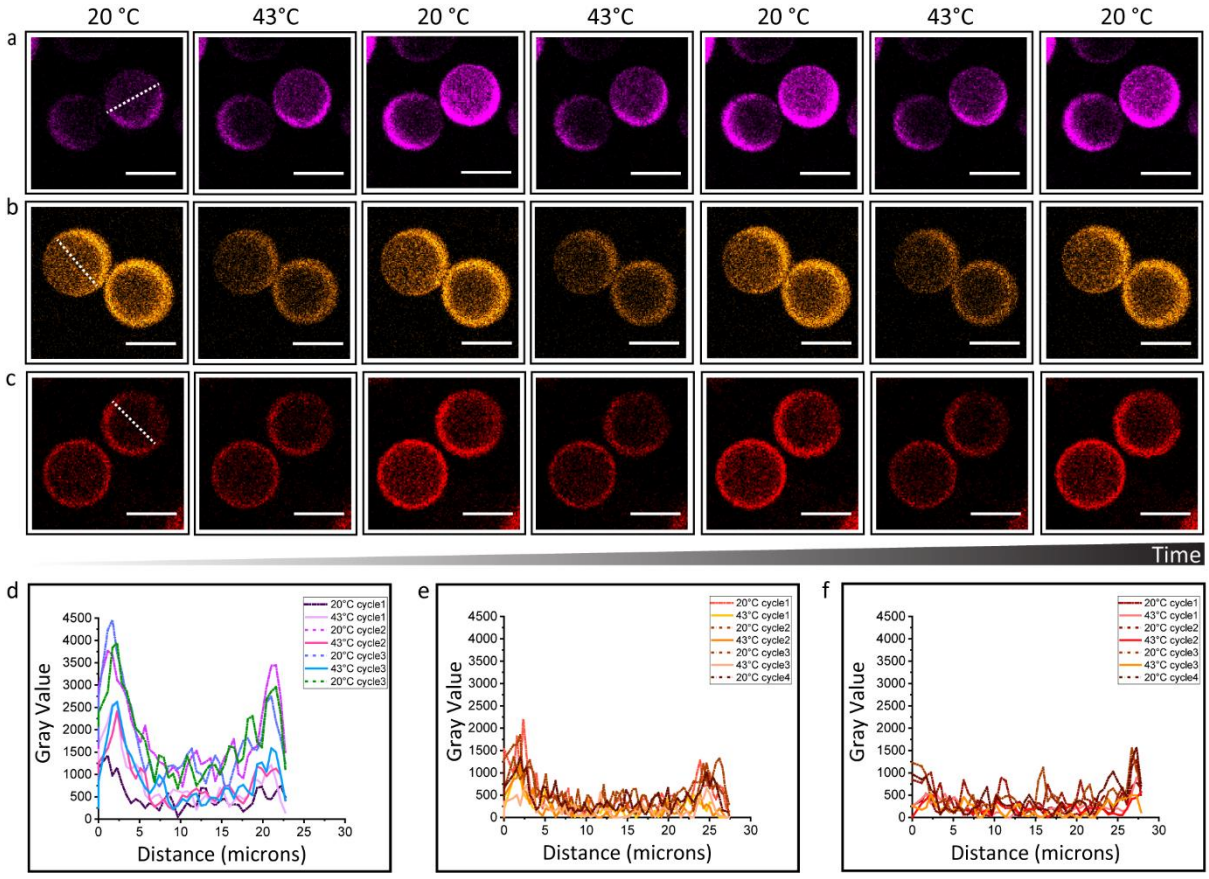



*Figure S9. Confocal images of PEGDA-PNIPAm:Dextran microgels over temperature cycles at 20°C and 43°C. Different MW of PNIPAm (a) 2 kDa, (b) 10 kDa and (c) 80 Kda labelled with Cy3 dye. The fluorescence intensity decreases at 43°C due to a change in hydrophobicity in the system (scale bar = 20 µm). Fluorescence intensity values over switches of temperature for microgels with different MW of PNIPAm. (d) 2 kDa, (e) 10 kDa, (f) 80 kDa. The dashed lines indicate where the fluorescence intensity is measured.*

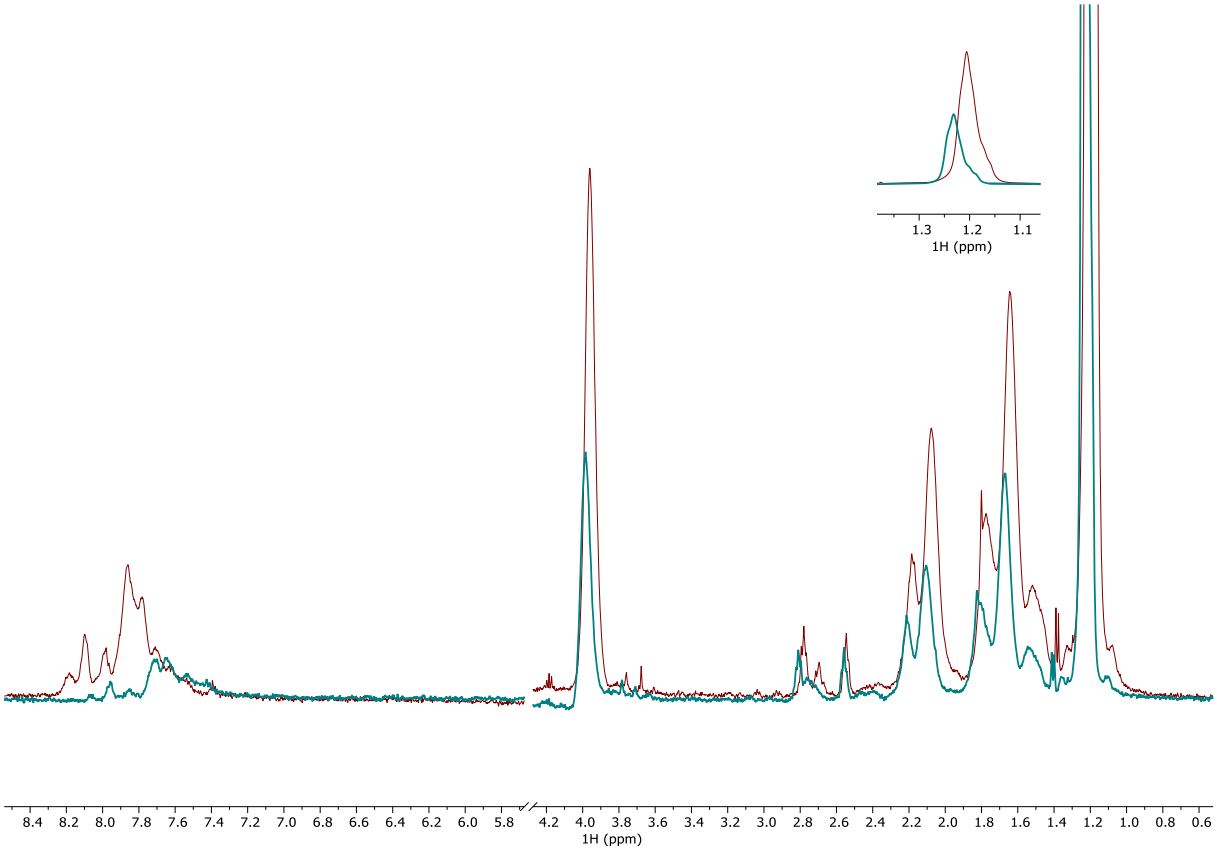



*Figure S10. $^{1}H$ NMR spectra (500 MHz) of PNIPAm-Cy3 in D2O.*

**$^{1}H$ NMR spectra (500 MHz) of PNIPAm-Cy3 in $D_2O$**, at 298 K (red) and 316 K (blue). Both spectra are measured from the same sample with equal settings (NS = 32, RG = 56). A clear decrease in intensity can be observed, which was measured to be a 49% decrease in intensity. Upon heating, conformational changes in the PNIPAm make the compound more hydrophobic, after which a part of the compound crashes out of solution and is no longer visible on liquid state NMR.

## 13. Motion Analysis over Temperature Cycles of PEGDA:Dextran micromotors

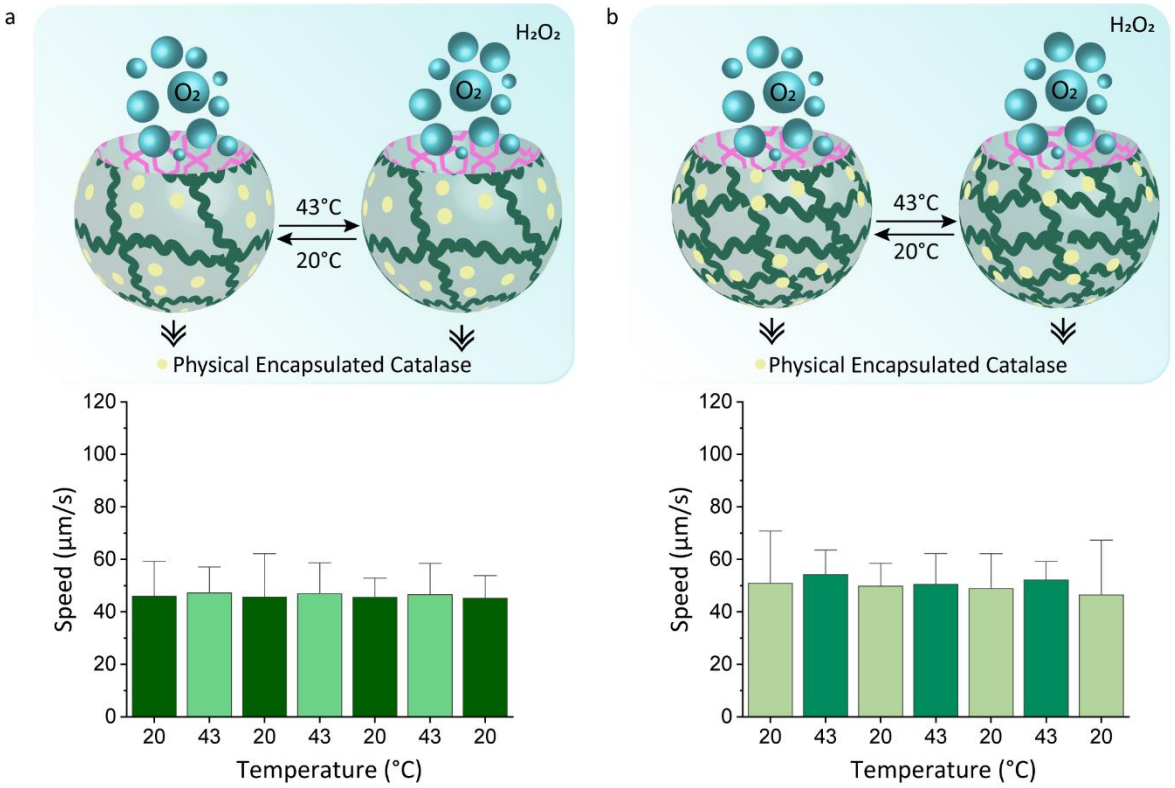
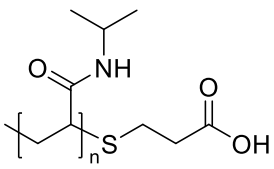


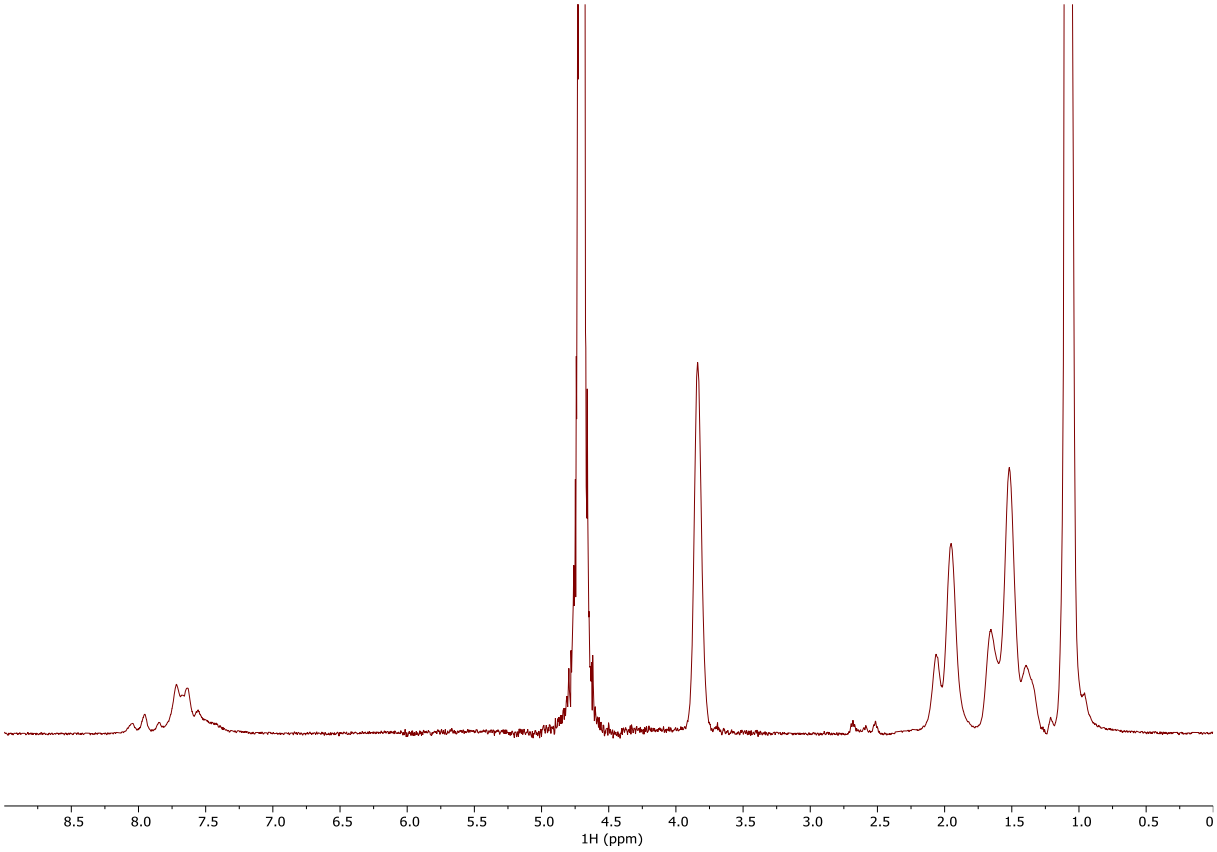

*Figure S11. Analysis of motion over cycles of temperature at 20°C and 43°C for PEGDA:Dextran micromotors. The Catalase is physically encapsulated in the microparticles (final concentration of 6 mg/mL). (a) PEGDA concentration is 25% w/w. (b) PEGDA concentration is 35% w/w. At 43°C the speeds of the motors are slightly faster, but the difference is not significant. This can be due to a higher activity of Catalase at 43°C as the enzyme is in a more optimal conformation.[1] Error bars indicate the range error.*

## NMR spectra of compounds

$^{1}$H, $^{1}$H solvent suppressed, and $^{13}$C are given.




PNIPAm-carboxylic acid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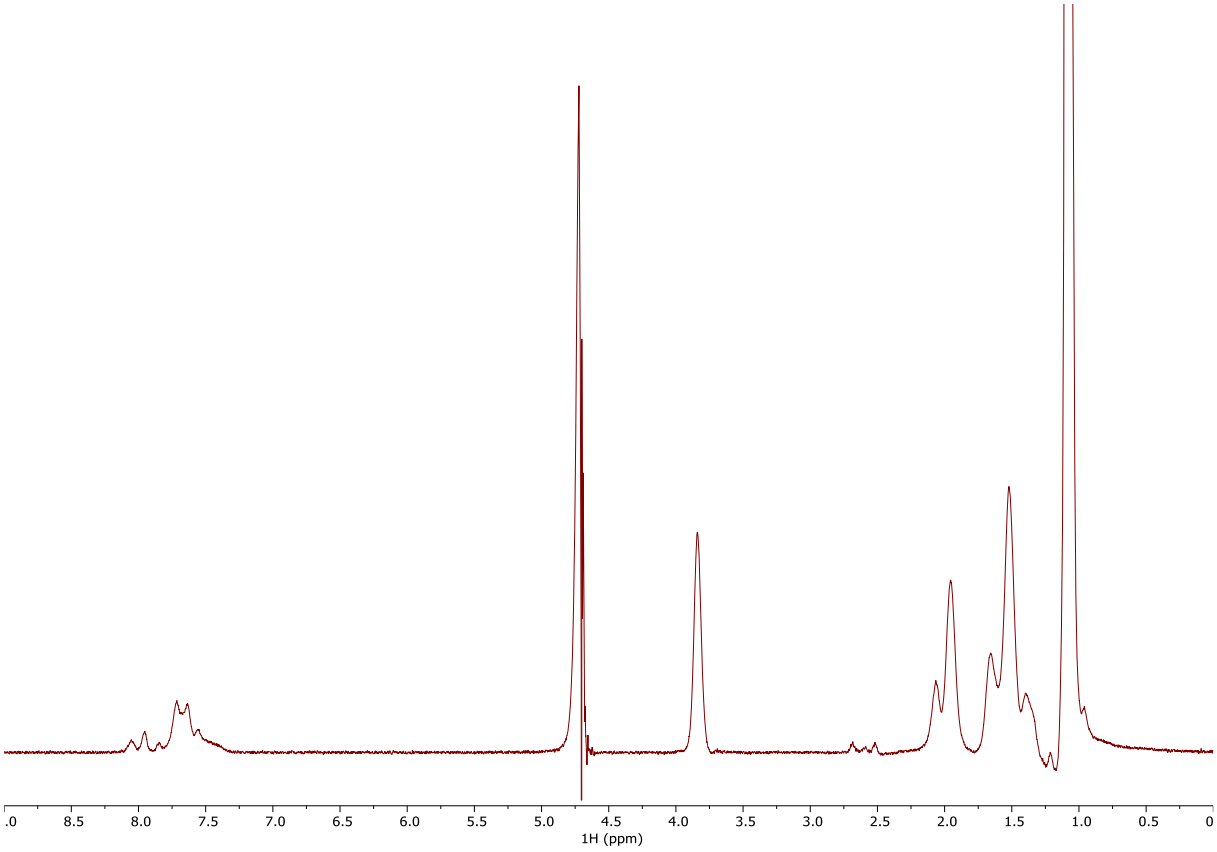
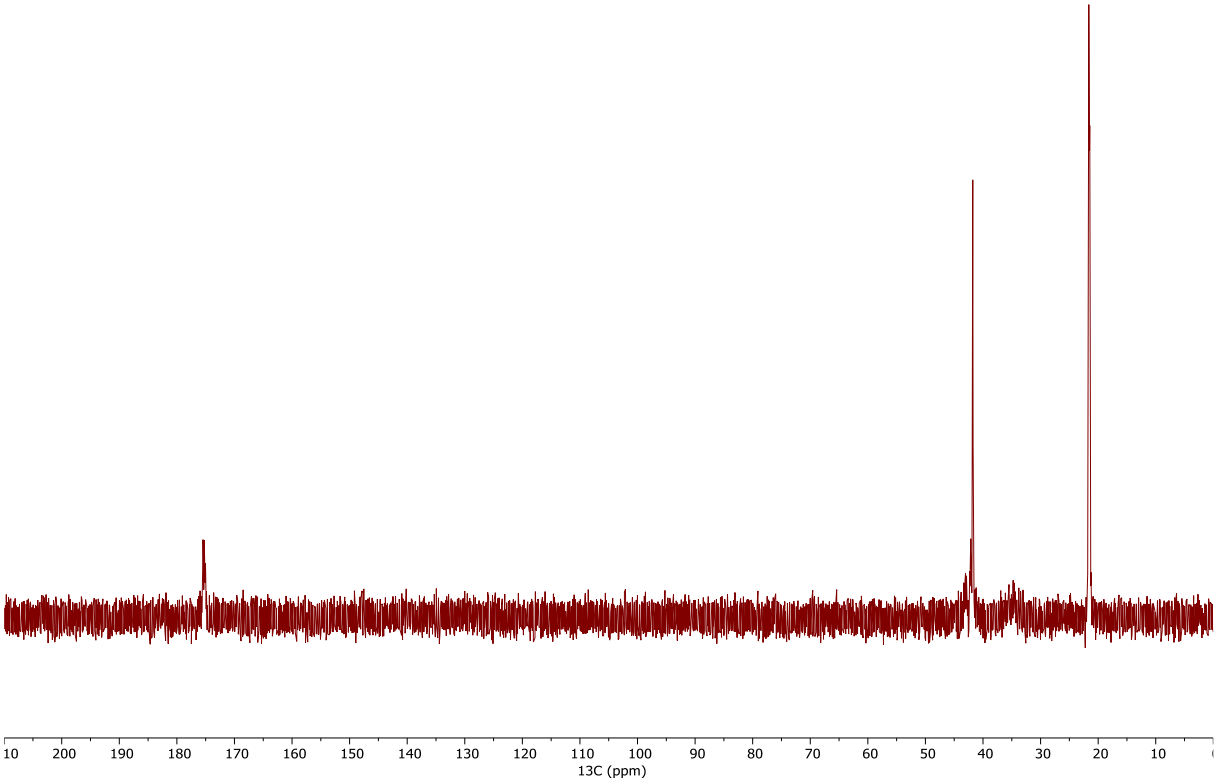
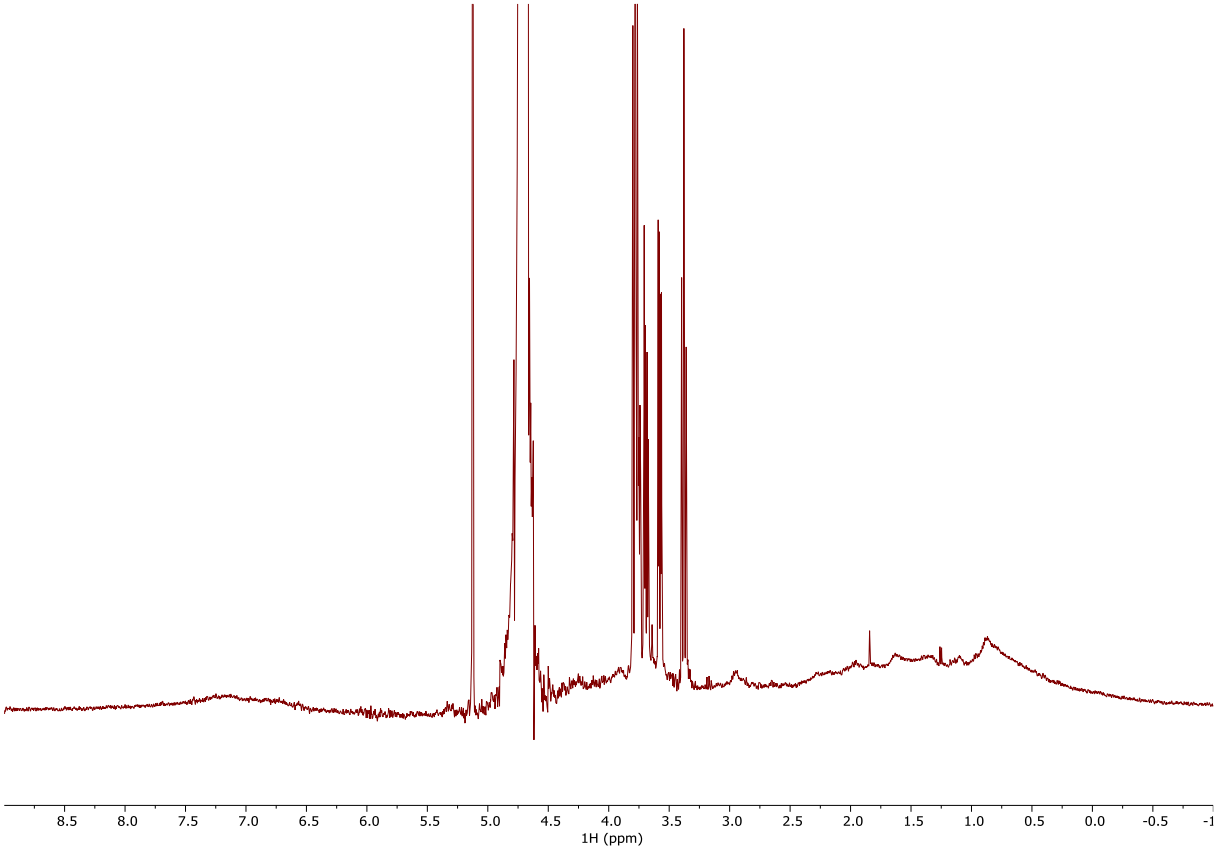
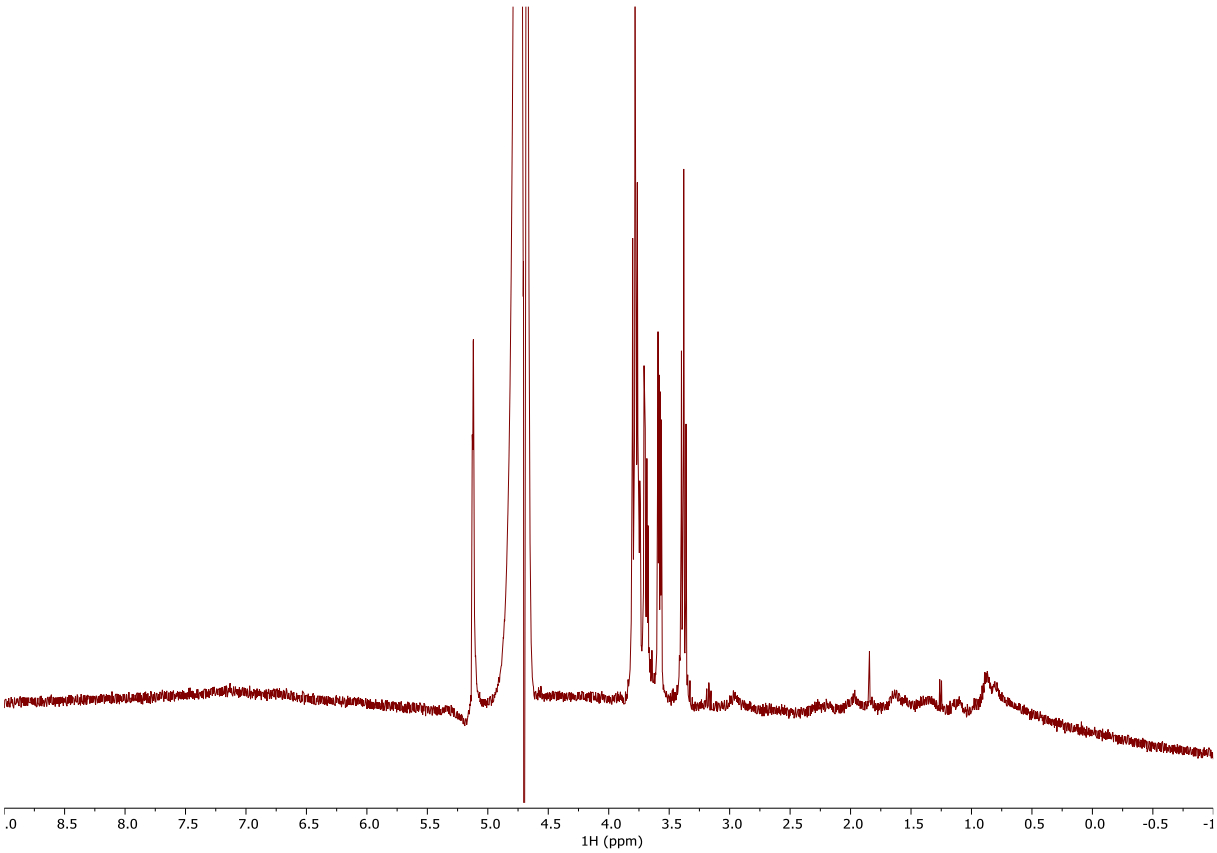
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
1H (ppm)

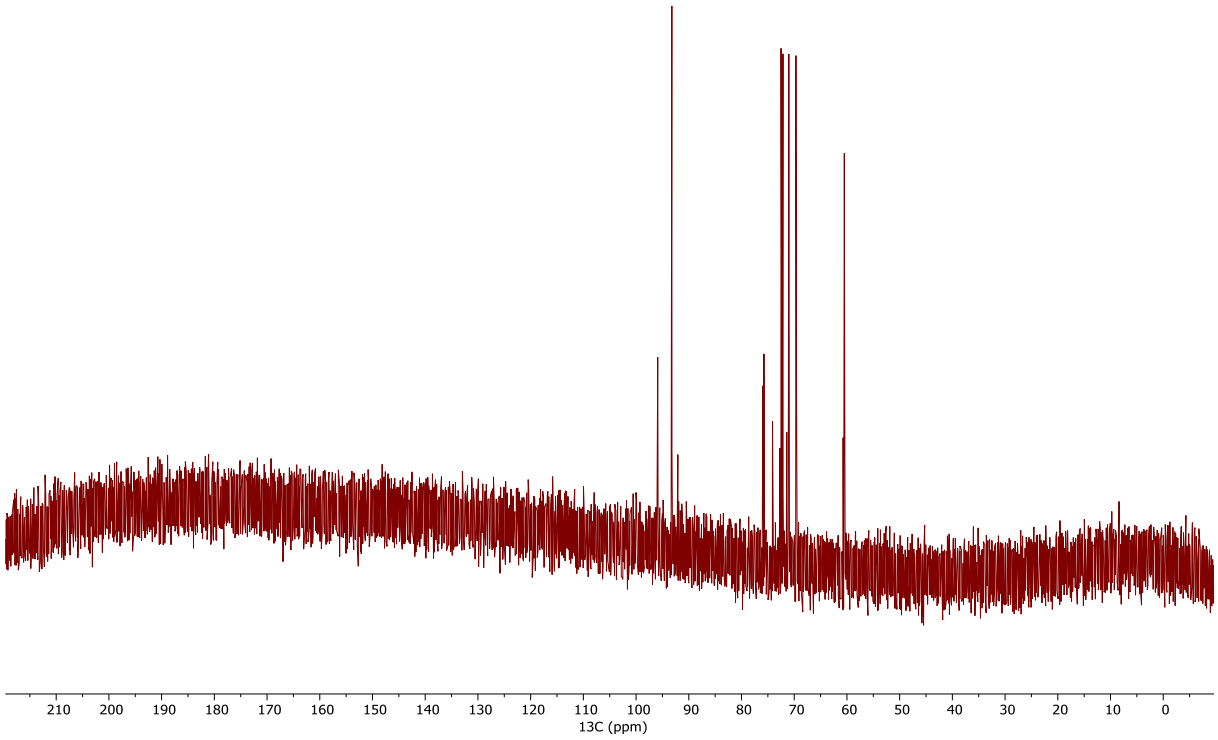

.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
1H (ppm)


10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
13C (ppm)

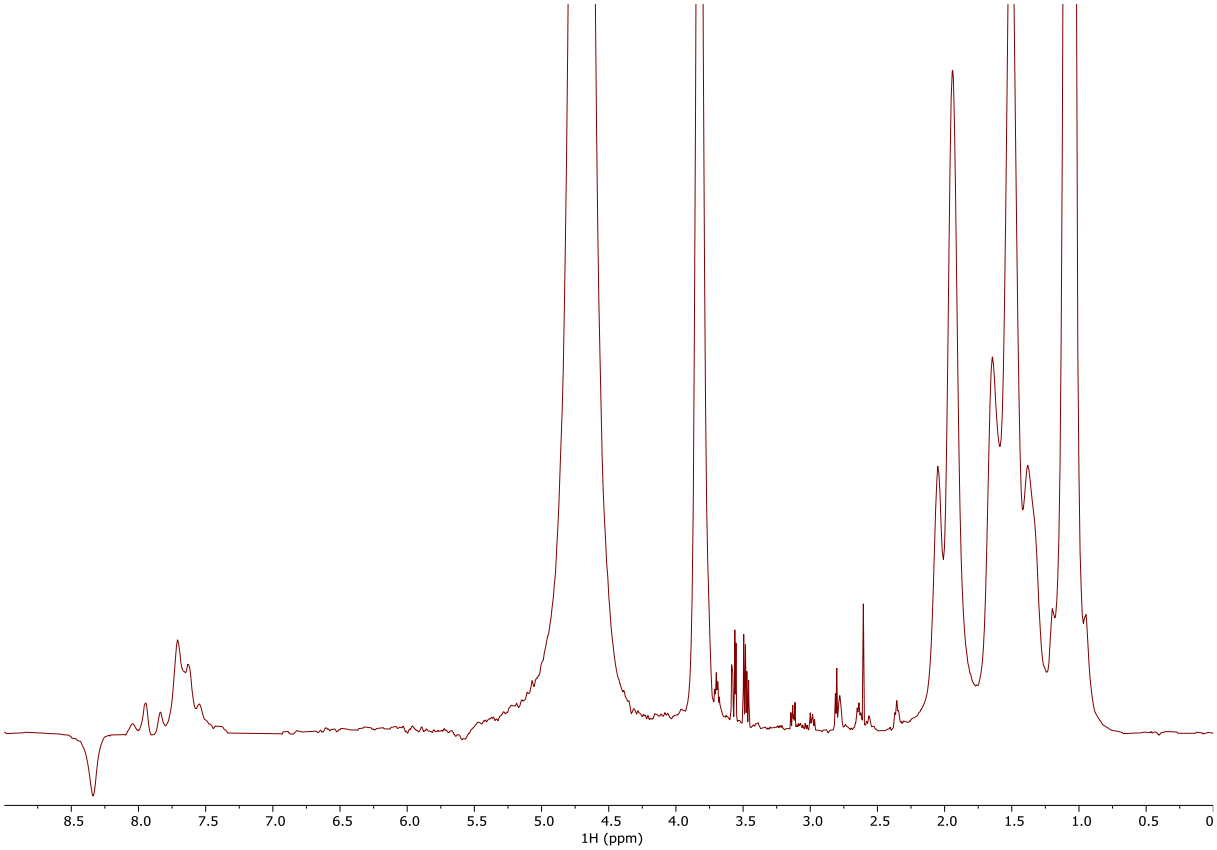
Catalase

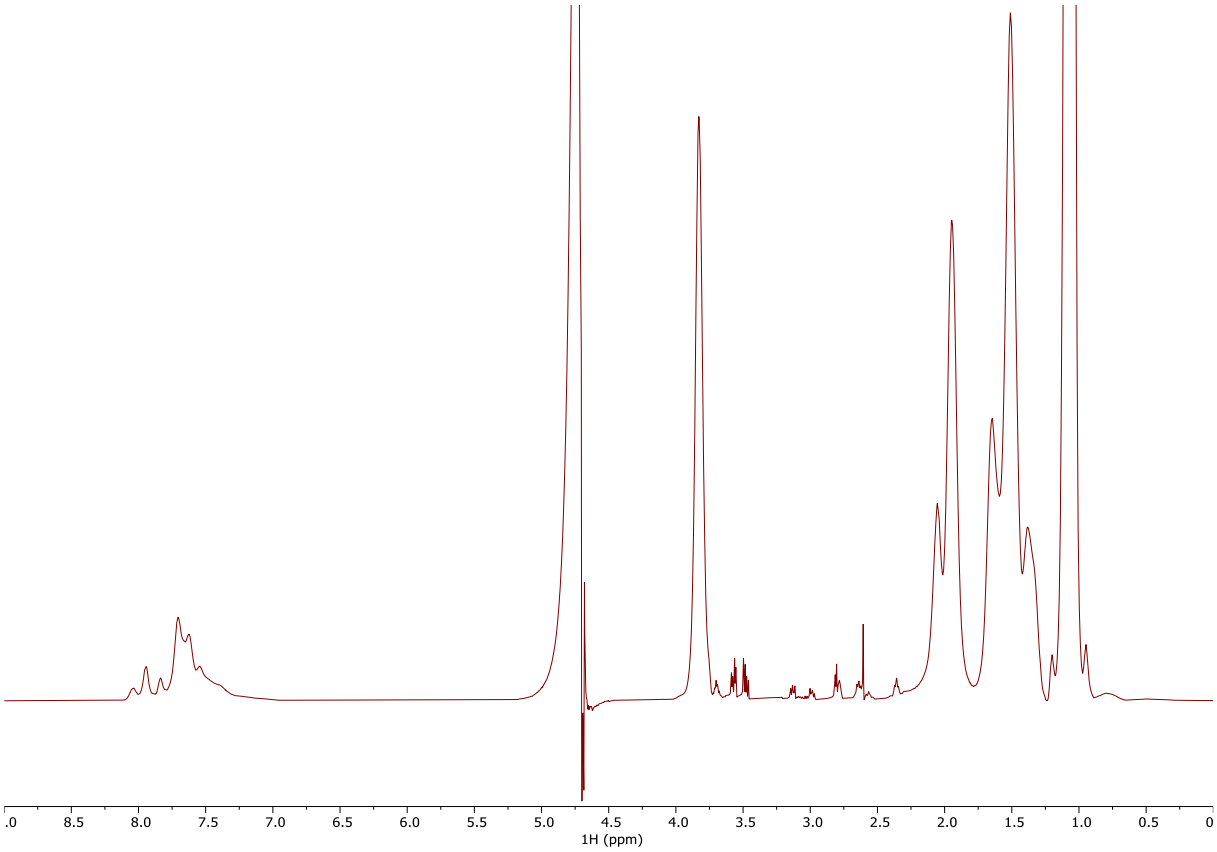

8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1
1H (ppm)

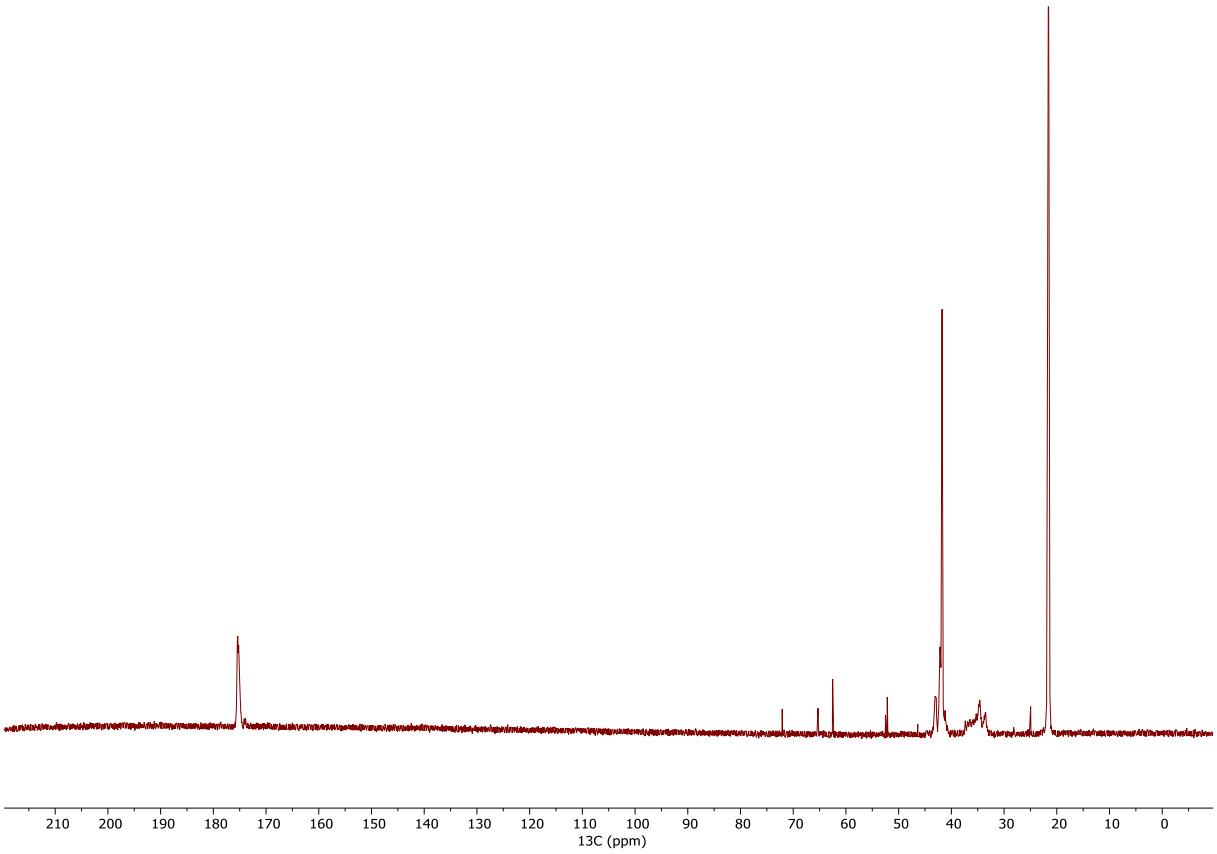

.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1
1H (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
13C (ppm)

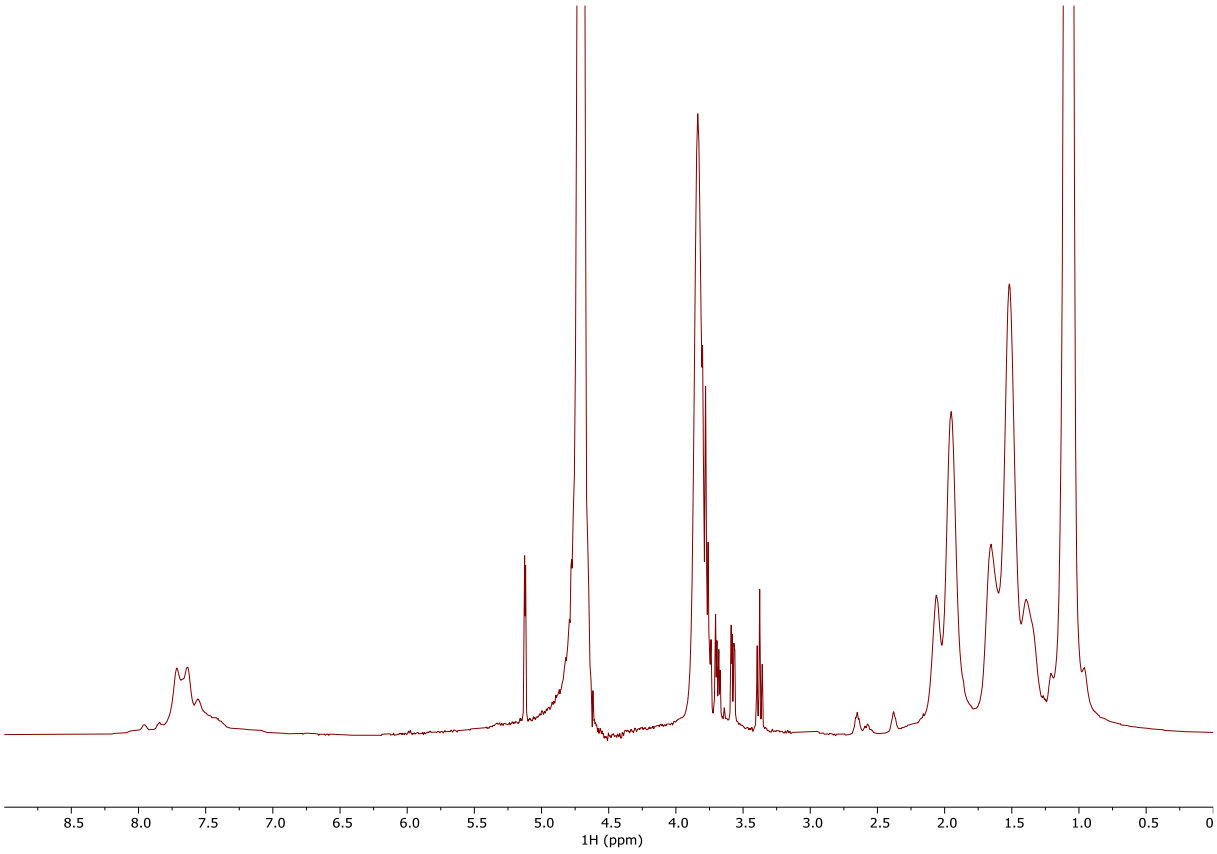
Catalase-PNIPAm

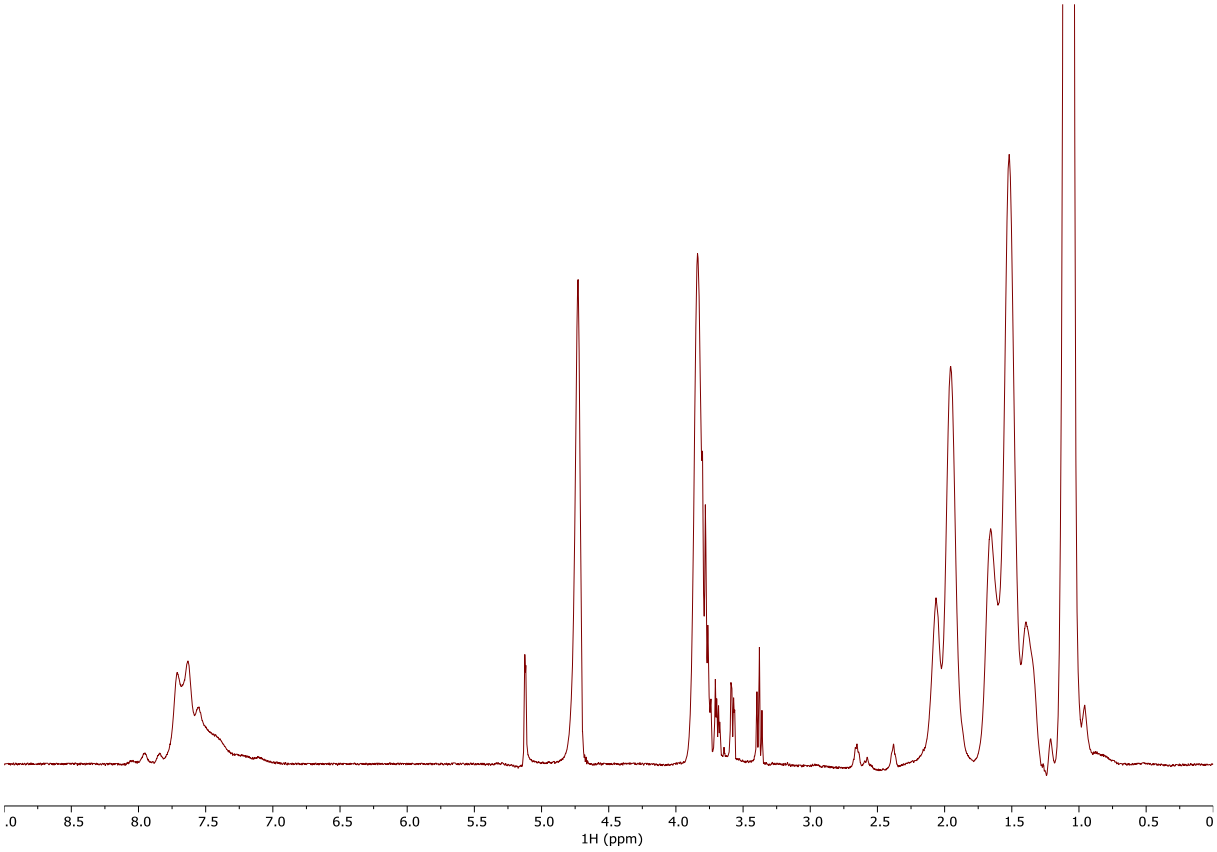


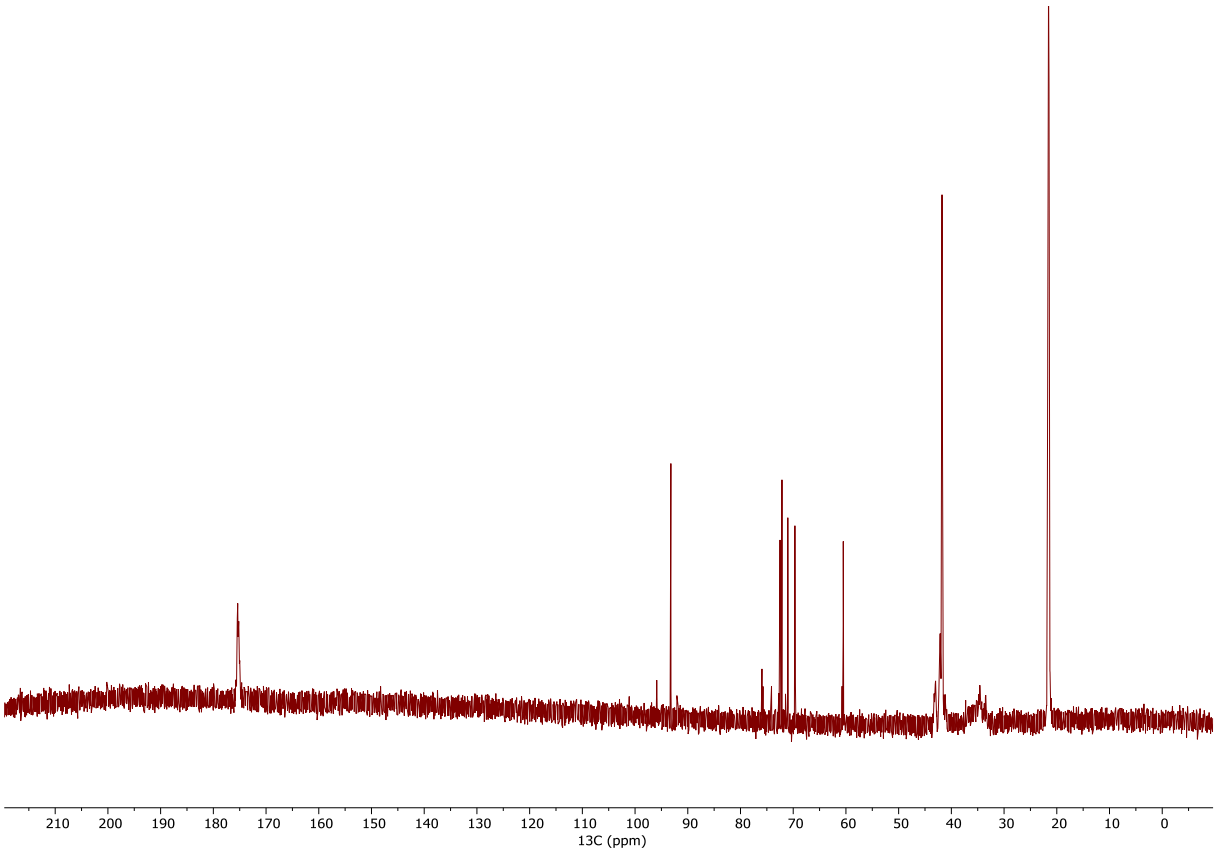




210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
13C (ppm)

Catalase and PNIPAm (loose)




.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
1H (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
13C (ppm)

# References


1. Roy M. Daniel, Michelle E. Peterson, Michael J. Danson, Nicholas C. Price, Sharon M. Kelly, Colin R. Monk, Cristina S. Weinberg, Matthew L. Oudshoorn and Charles K. Lee, *Biochemical Journal*, 2009, **425**, 353-360.
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