Supporting Information for

A scalable synthesis of pH-responsive polyacid nanogels: Injectable gel-forming hydrogel nanoparticles with a bright future

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EXPERIMENTAL SECTION

Materials

Methacrylic acid (MAA, 99 %), methyl methacrylate (MMA, 98.5 %), , ethylene glycol dimethacrylate (EGDMA, 98 %), Ethyl acrylate (EA, 99%), 1,4-Butanediol diacrylate (BDDA, 90 %), glycidyl methacrylate, GMA, 97 %), ammonium persulfate (APS, 98 %,), sodium dodecyl sulphate (SDS, 98.5 %), N,N,N′,N′-tetramethylethylenediamine (TEMED, 99 %). 2,5-Bis(octyloxy)terephthalaldehyde (98 %), Terephthalaldehyde (99 %) α α',-dicyano-p-xylene; 1,4-phenylenediacetonitrile (98%) tetrabutylammonium hydroxide (TBAH, 1.0M in Methanol) and Tween 80 were purchased from Aldrich. Hexane was purchased from Fisher Scientific (Loughborough, UK). 2,5-Bis(octyloxy)terephthalaldehyde (98%), terephthalaldehyde (99%), α α',-dicyano-p-xylene; 1,4-phenylenediacetonitrile (98%), tetrabutylammonium hydroxide (TBAH, 1.0M in Methanol) and Tween 80 were also purchased from Aldrich. All monomers were used as received. High purity deionised water was used for all experiments.

Synthesis of vinyl-functionalized nanogels (NGs)

Four nanogels (N-x) were prepared via monomer-starved emulsion polymerisation with different monomer compositions according to Table S1. For preparation of N-1, SDS (1.2 g, 4 mmol) was dissolved in water (240 g) and the solution purged with nitrogen for 30 min. Then APS (0.20 g, 0.90 mmol) in water (2.0 ml) was added. A comonomer mixture containing MMA (42.0 g, 0.42 mol), MAA (10.0 g, 0.12 mol) and EGDMA (1.1 g, 5 mmol) was added at a feed rate of 0.30 ml / min. The product was dialysed against water for 10 days. Nanogels N-2, N-3 and N-4 were prepared using the same general conditions described above and the quantities given in Table S1.

For GMA functionalisation, the pH of the nanogel dispersion (120 g, 5 wt.%) was adjusted to 5.1 then GMA (4.5 g, 0.39 mol) was added while stirring the mixture. The dispersion was left to stir for 8 h at 40 ^{o}C. The dispersion was washed with n-hexane two times and residual solvent removed by rotary evaporation.

Synthesis of conjugated polymer nanoparticles

Both orange (OGE) and near-infrared (NIR) cyanovinylene-backboned conjugated polymer nanoparticles (CP NPs) were prepared based on the procedure established by Kim et al.[1] In situ Knoevenagal polymerisation was conducted with equimolar dialdehyde and diacetonitrile monomers in an aqueous emulsion using Tween 80 as the surfactant. The molar ratio of [dialdehyde]: [diacetonitrile]: [Tween80] was remained constant at [1.00]:[1.00]:[11.57]. The typical protocol for the synthesis of NIR CP NP synthesis was as follows. 2,5-Bis(octyloxy)terephthalaldehyde (23.22 mg, 0.059 mmol) and α α',-dicyano-p-xylene; 1,4-phenylenediacetonitrile (9.27mg, 0.059 mmol) were dissolved in Tween 80 (0.9g,

0.687 mmol) in a 25 mL round bottom flask at 65 ^{o}C. Water (15 mL) was then added at 65 ^{o}C over a period of 90 min at a constant rate of 0.17 mL/min. A clear dispersion was obtained and cooled to room temperature over a period of 30 min prior to addition of TABH (0.6 mL). The polymerisation was conducted in 14 h at room temperature in atmospheric environment under magnetic stirring.

Preparation of doubly crosslinked nanogels

To prepare DX nanogels with solid content of 12 wt.%, GMA functionalised NGs (N-xG) (1.00 g, 14.7 wt.%) was vortexed with APS solution (69 μl, 78 mM), water (50 μl) and alkaline TEMED solution (51 μl). The latter contained NaOH (4 M) with TEMED at a volume ratio of 48:2. Addition of alkaline solution to NG dispersion caused a viscosity increase of the precursor. The viscous fluid was transferred into an o-ring and sandwiched between two glass slides. The precursor was cured at 37 ºC overnight.

For preparation of DX NG/CP NP composite gels, N-1G (1.00 g, 14.7 wt.%) was mixed with 0.50 g of PD. To this, APS solution (69 μl, 78 mM) and alkaline TEMED solution (51 μl) was added and the produced precursor was left to cure at 37 ºC overnight.

Physical measurements

Potentiometric titration data were obtained in the presence of 0.05 M NaCl using a Mettler Toledo titrator. Dynamic light scattering (DLS) and zete potential data were obtained using a Malvern Zetasizer NanoZS instrument. A Hitachi U-18 00 spectrophotometer was used for UV-visible spectroscopy measurements. SEM and TEM images were obtained using Philips FEGSEM at 6 kV and Phillips CM100 at 100 kV instruments, respectively. Dynamic rheology measurements used a TA Instruments AR G2 rheometer. A 20 mm diameter plate geometry was used and the gap was set to 2500 μm. The PL spectra were obtained using an Edinburgh Instruments FLS980 photoluminescence spectrometer.

The volume swelling ratio of DX NG was studied using a gravimetric method. Samples were placed in phosphate buffer solution at different pH values (0.1 M). Each sample was weighed once a day and left in a fresh buffer. This repeated for 5 days. The volume swelling ratio (Q) for the DX NG was calculated using:

$$Q = \rho_p \left(\frac{Q_{(m)}}{\rho_s} + \frac{1}{\rho_p} \right) - \frac{\rho_p}{\rho_s} \qquad \text{(S1)}$$

where $Q_{(m)}$ is the mass swelling ratio. The ρ_s and ρ_p are the densities of the solvent and polymer, respectively. These were taken as 1.0 and 1.2 g cm^{-3}.

Cytotoxicity studies

Human nucleus pulposus (NP) cells, were cultured in Dulbecco's modified Eagle's medium

supplemented with 10% fetal bovine serum (FBS, Gibco), L-ascorbic acid 2-phosphate (10 µM) and antibiotic/antimycotic (Sigma-Aldrich, UK) at 37 °C in a humidified 5% CO_2 atmosphere. Cells were seeded at a density of 5 x 10^4 per well onto 24-well plates and cultured for 24 h. Gel samples (20 mg), sterilized with 70% ethanol and washed with phosphate buffered saline (PBS) were introduced using 0.4 µm cell-culture inserts (BD Biosciences, UK). MTT assays (Aldrich, UK) were performed after 1, 4 and 7 days and the results compared against an empty insert control. There were three points per timepoint (n = 3). Absorbance data were obtained using a BMG Labtech FLUOstar plate-reader. **[Daman: Do these details apply?]**

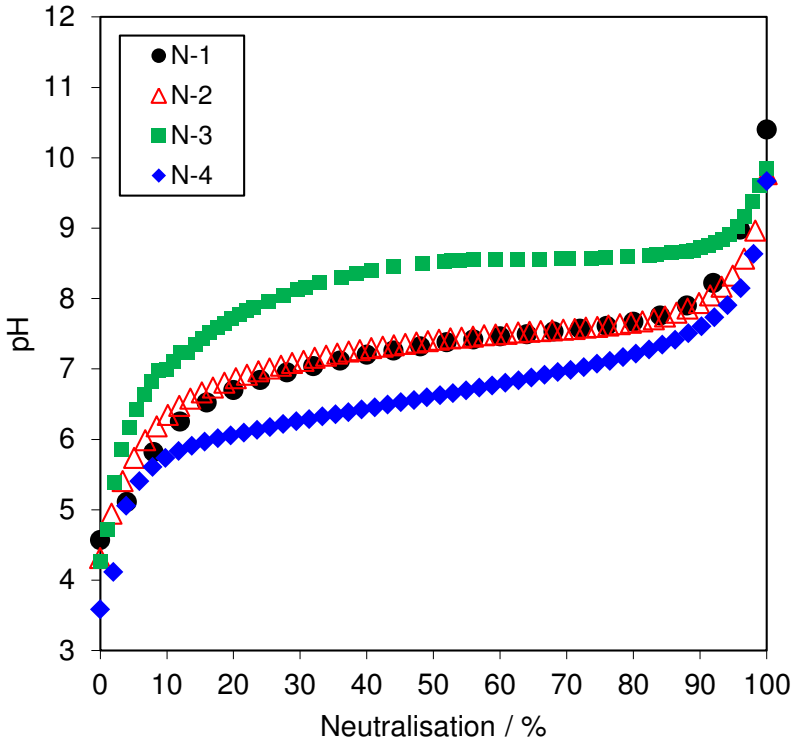

Figure S1. Potentiometric titration data for various nanogel dispersions. The apparent pK_a values were obtained from the pH corresponding to 50% neutralisation.

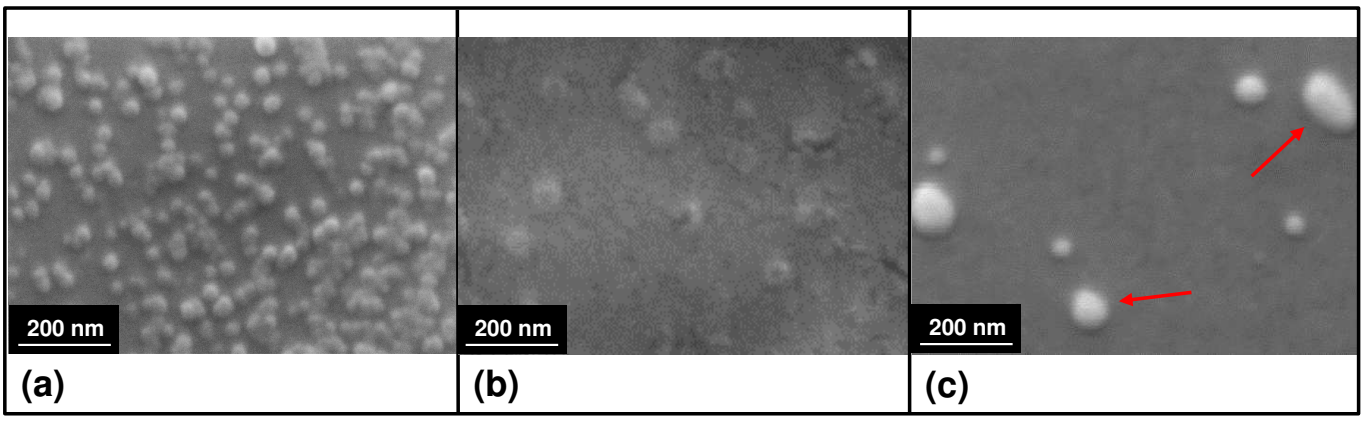

Figure S2. SEM images for (a) N-2, (b) N-3 and (c) N-4. The red arrows show particle coalescence due to sample drying.

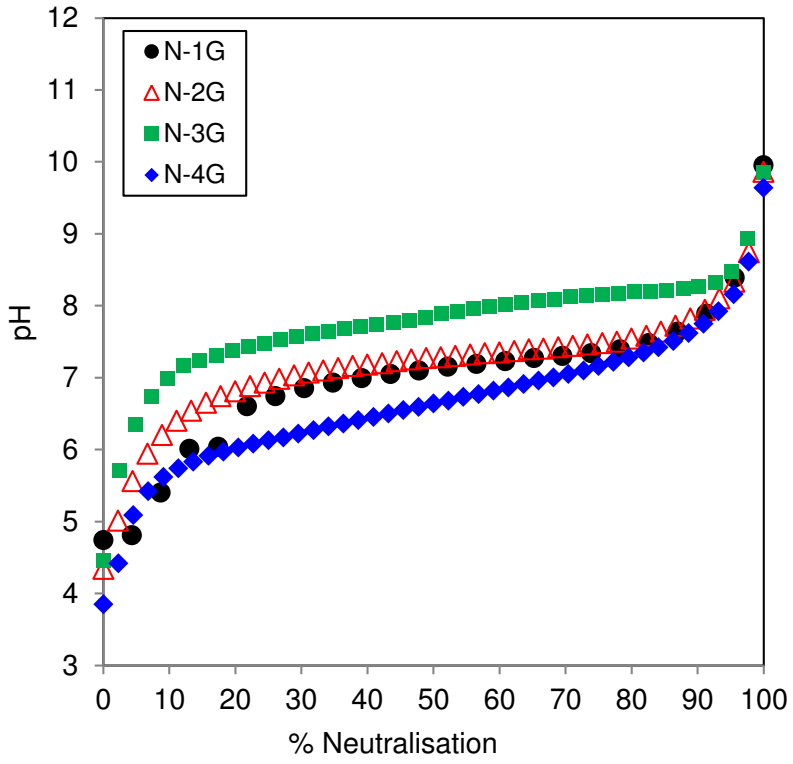

Figure S3. Potentiometric titration data for various GMA-functionalised nanogels.

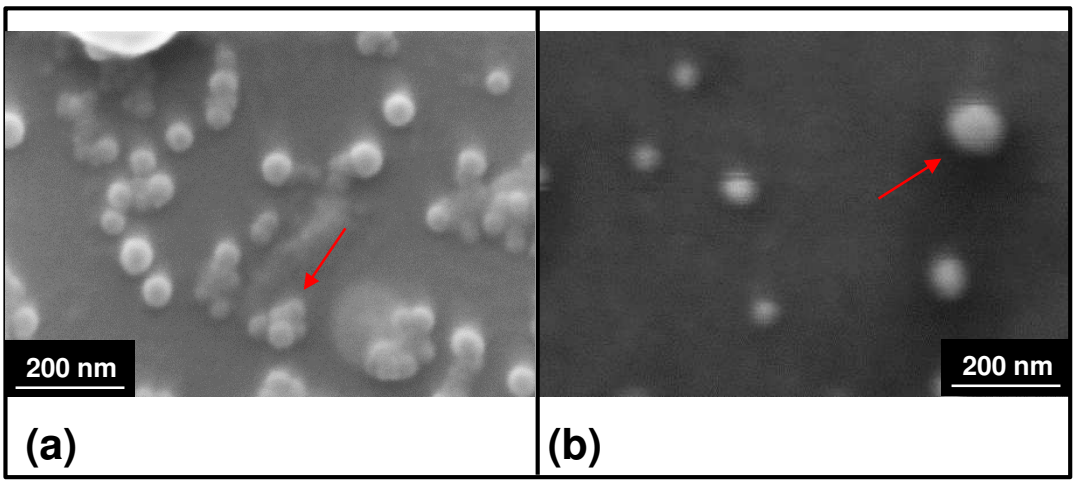

Figure S4. (a) SEM image for N-2G. (b) SEM image for N-4G. The red arrows show particle coalescence due to sample drying.

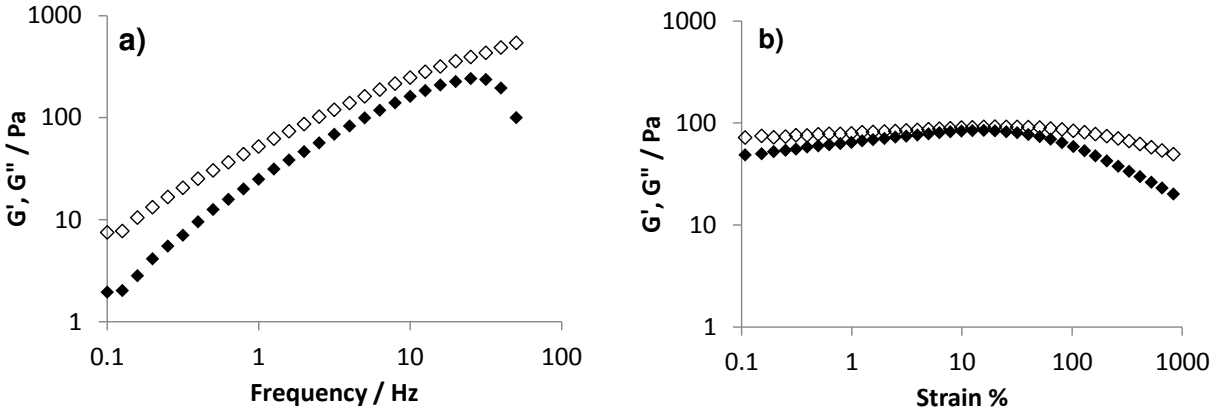

Figure S5. Rheology data for N-1G with concentration of 12 wt% and pH of 7.5. *G'* (shear modulus) and *G"* (storage modulus) are represented by the closed and open symbols, respectively.

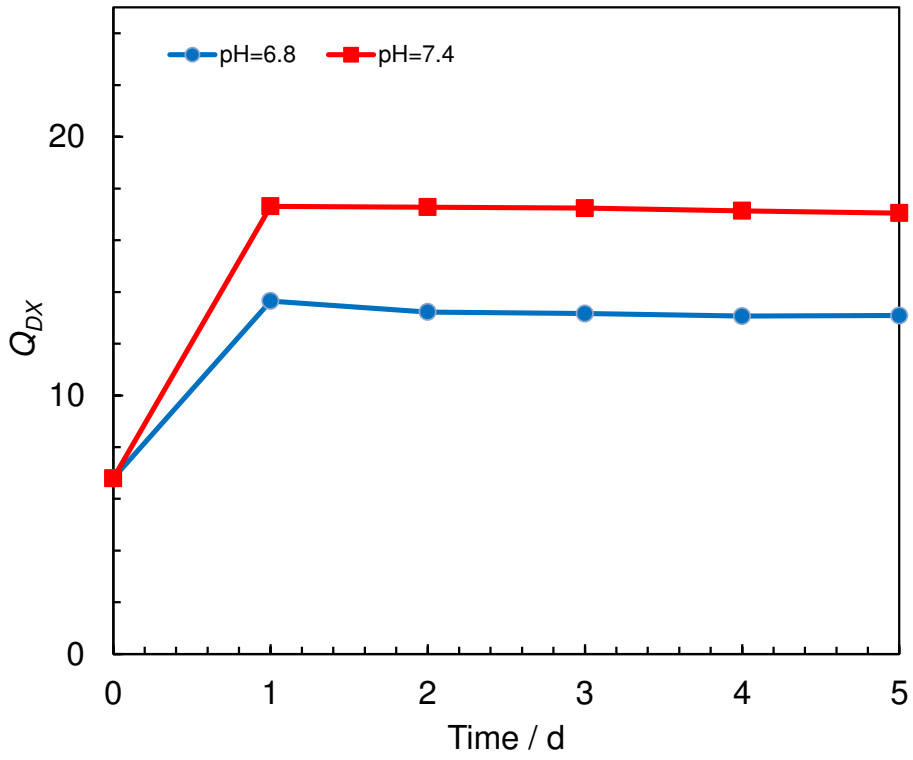

Figure S6. Variation of swelling ratio for DX N-1G as a function of time. Phosphate buffers with concentration of 0.1 M was used.

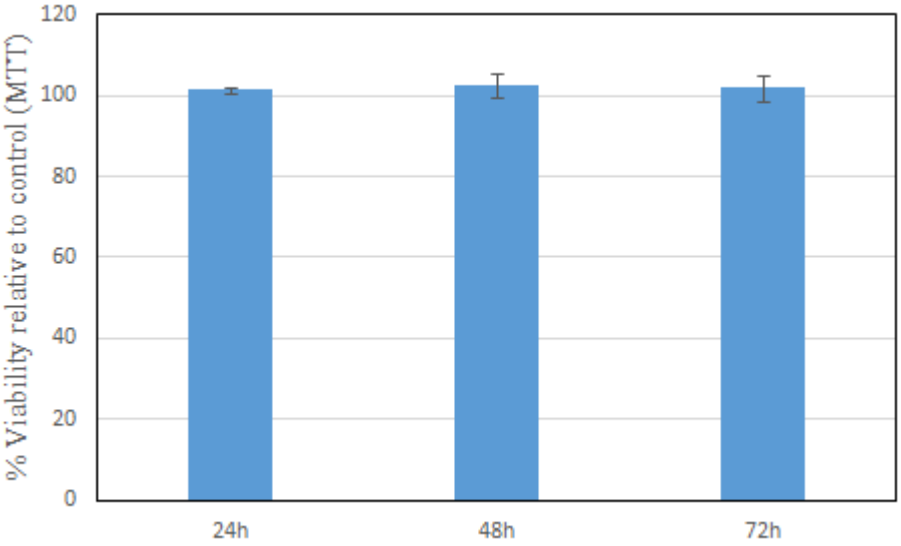

Figure S7. Relative MTT assay data for nucleus pulposus cells exposed to DX N-1G for 24, 48 and 72 h. The data are shown relative to the control sample.

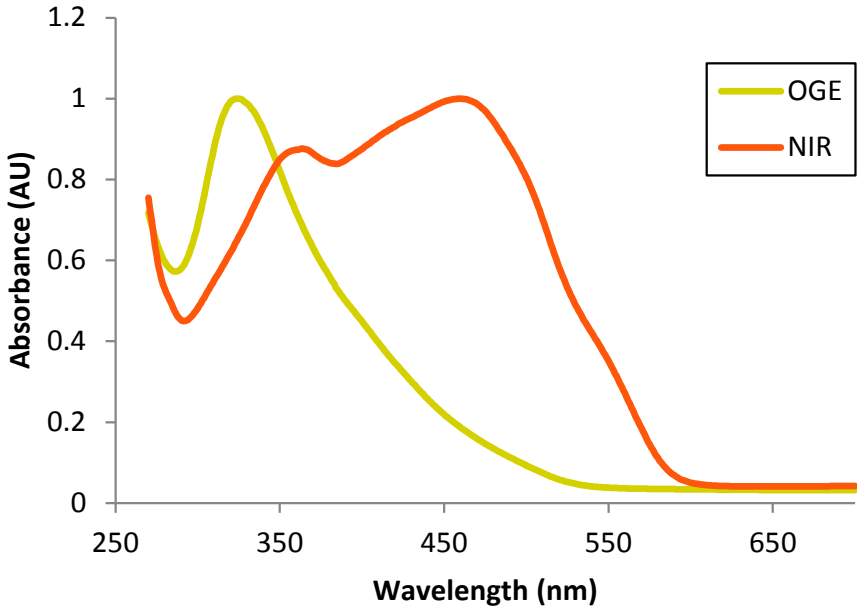

Figure S8. UV-visible spectra for OGE and NIR conjugated polymer nanoparticle dispersions (CP NPs). The structures for OGE and NIR are shown in the insets of Fig. 3a.

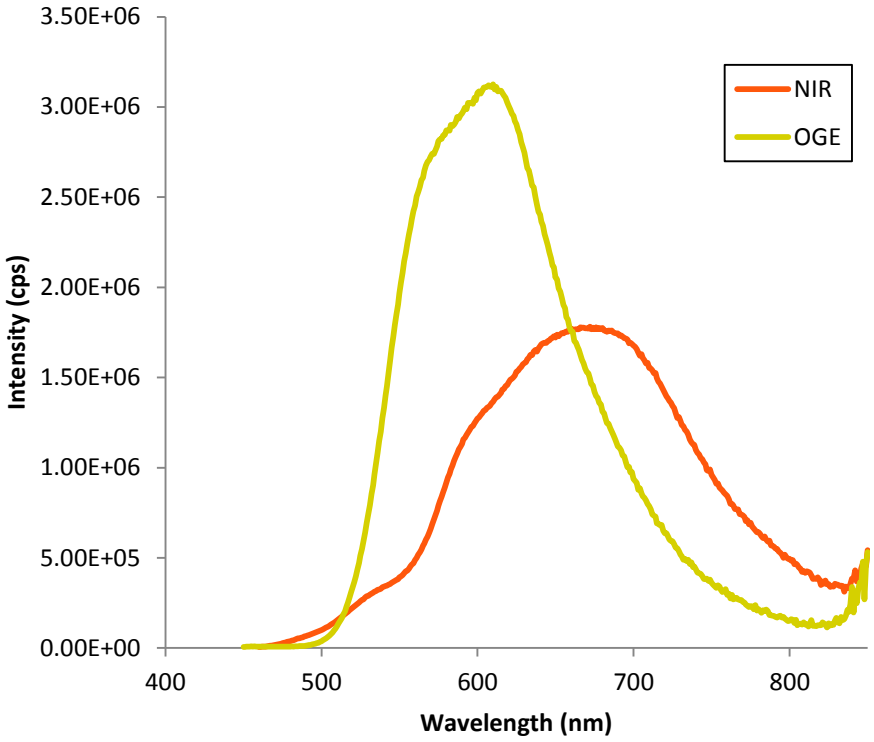

Figure S9. PL spectra for OGE and NIR CP NPs. The excitation wavelength was **xxx** nm.

[Nam: can you insert the excitation]

Table S1. Quantities of monomers used for nanogel synthesis.

Code	MMA / g	EA / g	MAA / g	EGDMA / g	BDDA / g	MAA / mol.%	Crosslinker / mol.%	TSC / wt.%[a]
N-1	42.0	-	10.00	1.10	-	24.0	1.0	12.0
N-2	35.2	-	9.05	0.439	-	29.8	0.5	15.7
N-3	44.9	-	7.39	0.528	-	20.9	0.5	18.0
N-4	-	10.7	2.55	-	0.271	24.4	1.0	5.3

[a] Nominal value based on mass of monomer used.

Table S2. Characterisation data for the nanogels

Code	MAA / mol.%[a]	GMA / mol.%[b]	pK_a[c]	d_{EM} / nm[d]	$d_{z(coll)}$ / nm[e]	$d_{z(swell)}$ / nm[f]	ζ / mV[g]
N-1	24.0	-	7.3	20 [4]	25	44	-20
N-2	29.7	-	7.4	41 [8][i]	36	58	Need
N-3	20.8	-	8.5	51 [5][i]	41	49	-
N-4	24.3	-	6.6	28 [4][i]	23	Need	-21
N-1G	21.5	2.5	7.1	17 [3]	19	53	-21
N-2G	25.3	4.4	7.3	40 [5][i]	35	105	-
N-3G	18.4	2.4	7.8	-	43	89	-
N-4G	20.2	4.1	6.6	32 [3][i]	31	54	Need

[a] Determined from titration data. [b] Calculated value using the difference of the MAA contents before and after functionalisation. [c] Apparent pK_a value determined from titration. [d] Electron microscopy includes SEM and TEM. Number-average diameters are from TEM unless otherwise stated. The number in the brackets is the standard deviation. [e] d_z values measured at pH values of ~ 6.0 which corresponds to collapsed particles. [f] Measured at pH ~ 9.0. [g] Zeta potential at pH ~ 6.0. [i] From SEM.

[MAA content for N-2 is too high and titration will need to be repeated]

Table S3. Mechanical properties for DX nanogels

Code	E / kPa[a]	σ_B / kPa	ε_B / %
DX-N-1G	Need	Need	Need
DX-N-2G	37.1	328	70.0
DX-N-3G	Need	Need	Need

[a] Compression modulus. [b] Stress-at-break. [c] Strain-at-break.

Reference

1. A. H. Milani, J. Bramhill, A. J. Freemont, and B. R. Saunders. *Soft Matter* 2015, **11**, 2586-2595.