Supporting Information for

Do the properties of gels constructed by interlinking triply-responsive microgels follow from those of the building blocks?

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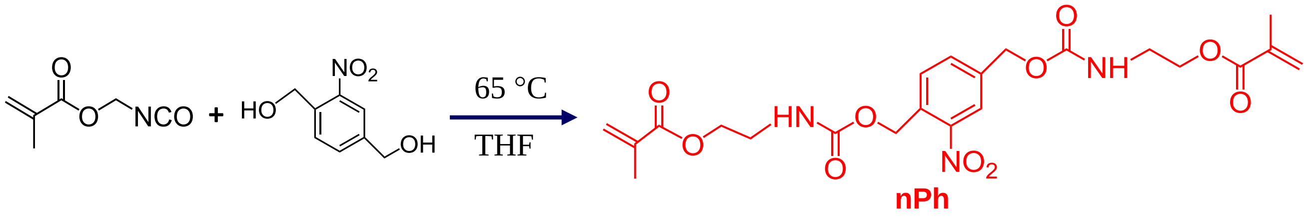
Characterisation and properties of the photocleavable crosslinker

2,2'-(2-Nitro-1,4-phenylene)bis(methylene)bis(oxy)bis(oxomethylene) bis(azane-diyl)bis(ethane-2,1-diyl) bis(2-methylacrylate) (denoted as nPh) was synthesised in one step using the method described by Klinger and Landfester[1] according to Scheme S1.



Scheme S1. Reaction scheme for the synthesis of nPh.

The successful synthesis of nPh was confirmed using ^{1}H NMR spectroscopy (see Fig. S1) and the purity was estimated at 95%. The crosslinker was UV-light photocleavable. Fig. S2 shows the changes in the UV-visible spectra that occurred upon UV irradiation. These spectra and the changes in the absorption maxima agree with those reported earlier[1].

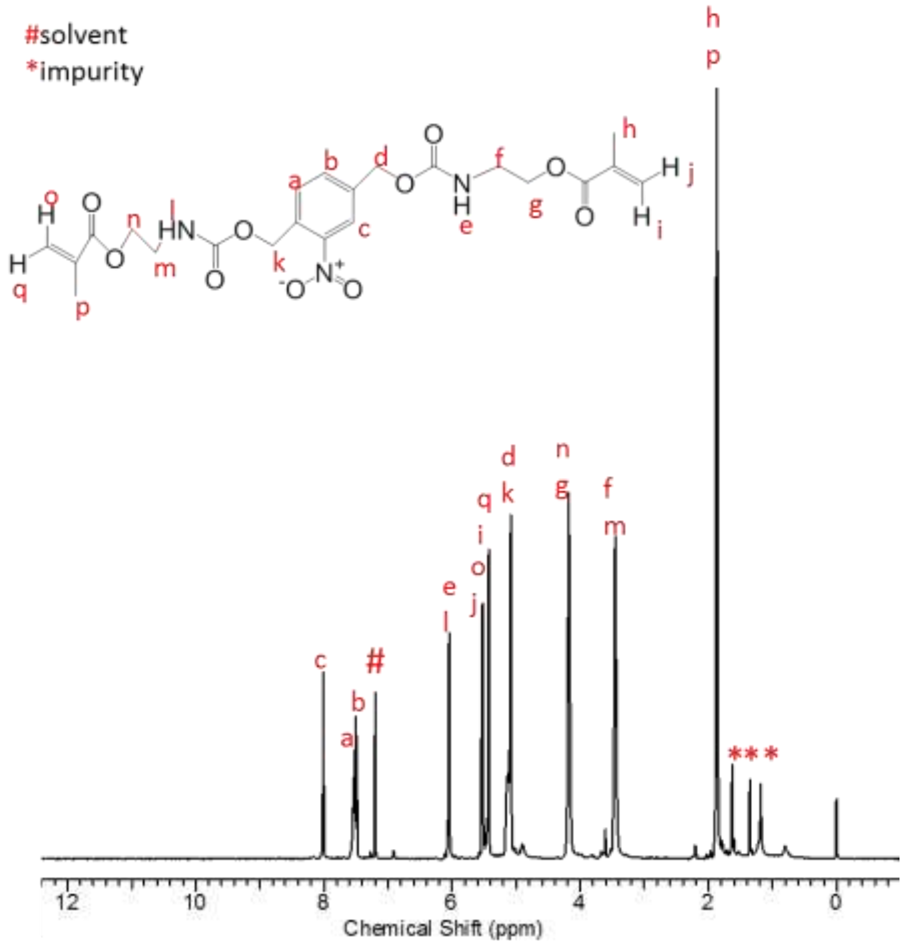


Figure S1. ^{1}H NMR spectrum and assignments for nPh.

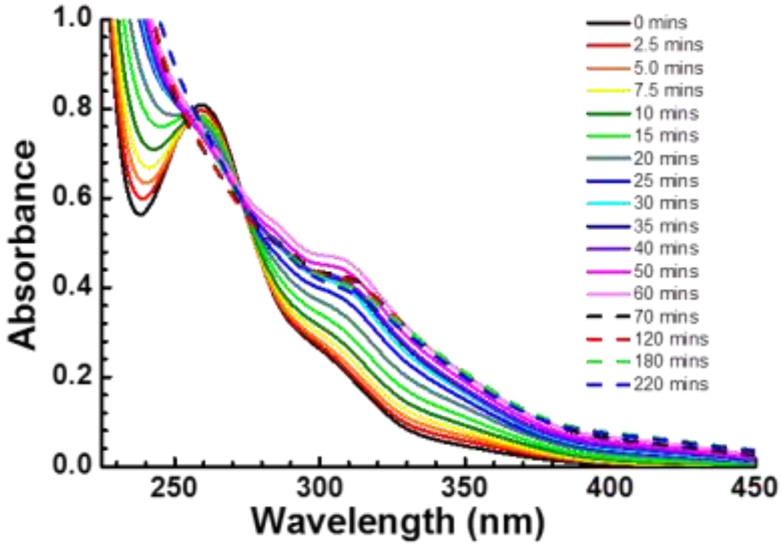


Figure S2. Time-dependent UV-vis spectra for the photocleavage of nPh in CH_3OH (0.15 mM) as a consequence of irradiation with UV light (365 nm).

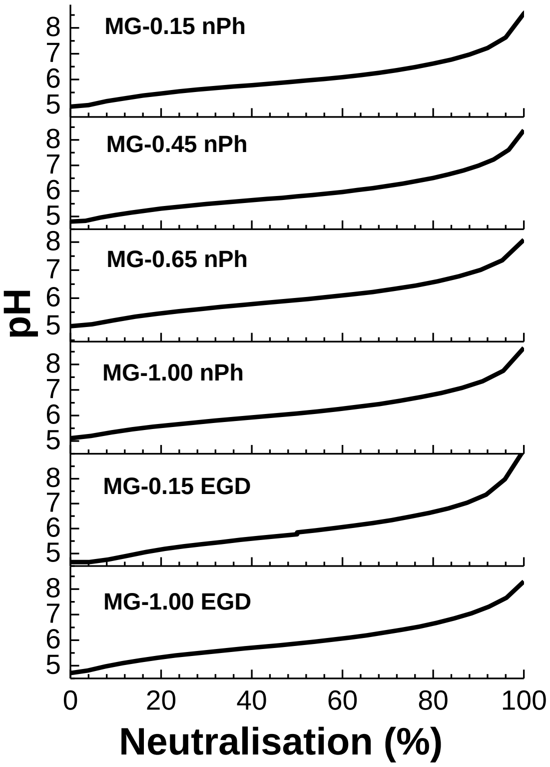


Figure S3. Potentiometric titration data for the MG dispersions. The apparent pK_a values were obtained from the pH corresponding to 50% neutralisation.

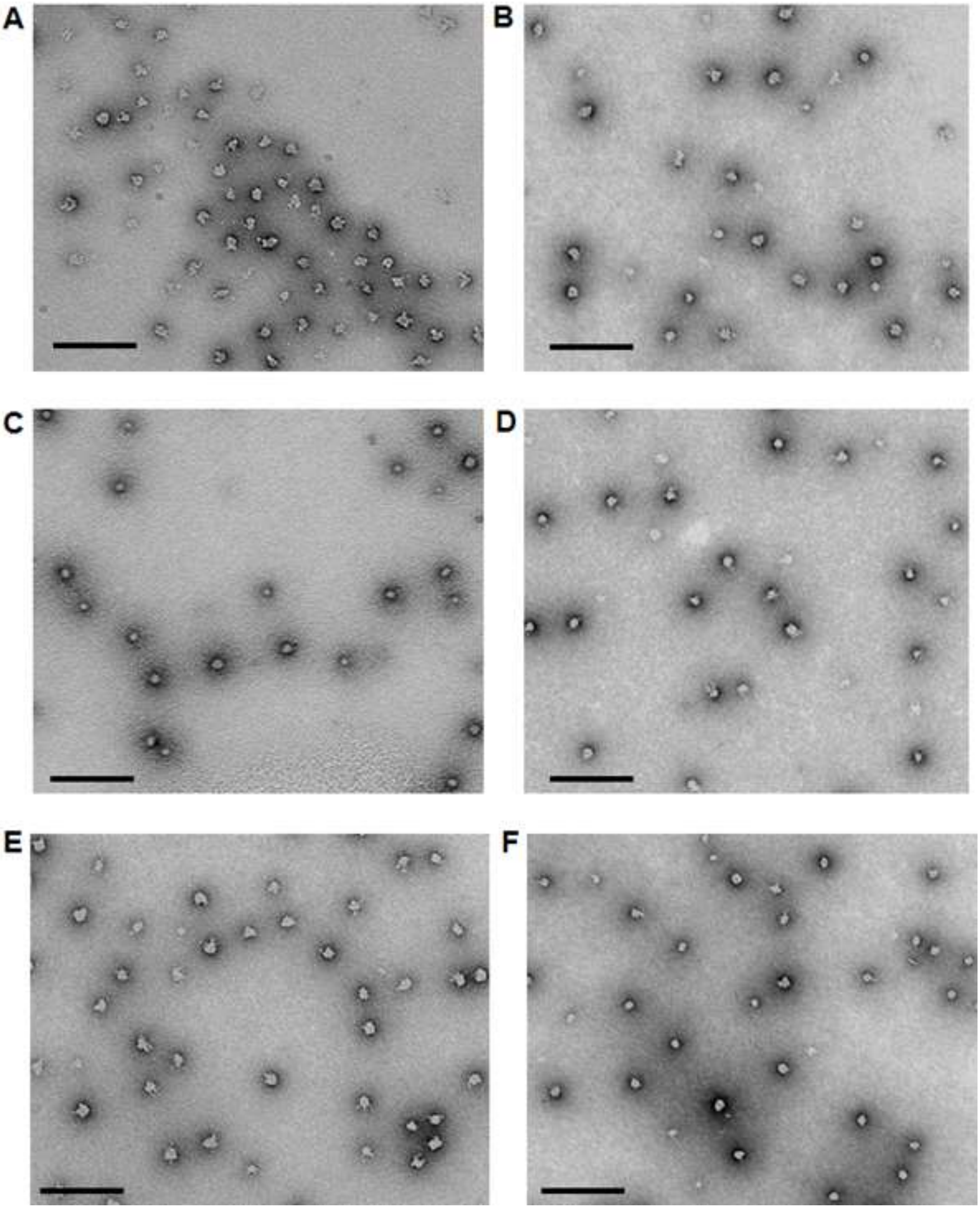


Figure S4. TEM images measured for (**A**) MG-0.15-nPh, (**B**) MG-0.45-nPh, (**C**) MG-0.65-nPh, (**D**) MG-1.00-nPh,(**E**) MG-0.15-EGD and (**F**) MG-1.00-EGD. The scale bars are 500 nm.

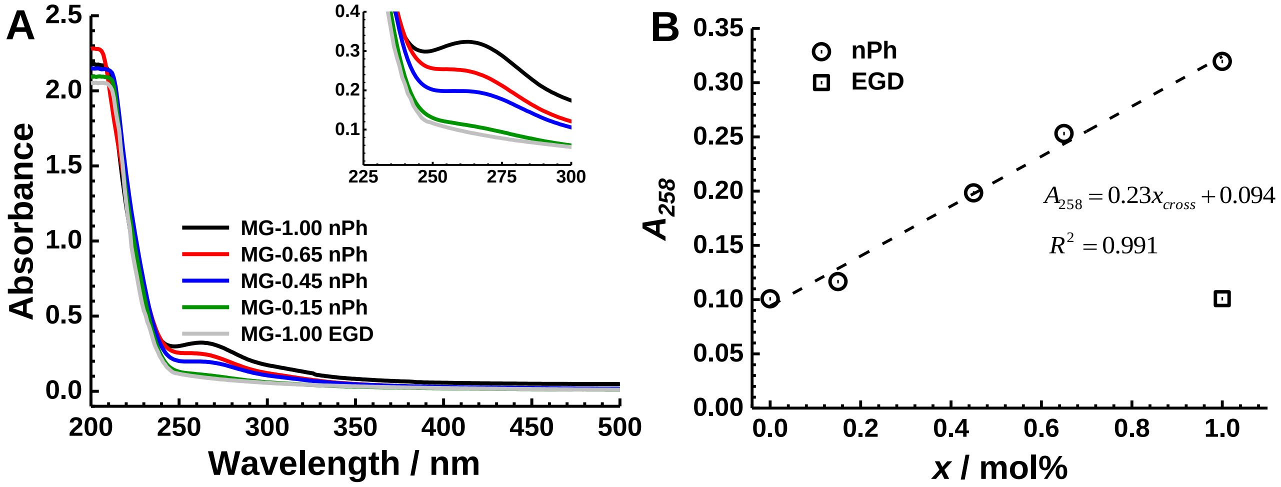


Figure S5. (**A**) UV-visible spectra for the MG-*x*-nPh dispersions measured at pH 7.4 and 25°C. The inset shows an expanded view of the maximum. (**B**) Absorbance measured at 258 nm versus mol% of crosslinker used (*x*) for MG preparation. The line of best fit obtained for the MG-*x*-nPh dispersion data is shown. Data measured for a MG-1.00-EGD dispersion are shown for comparison.

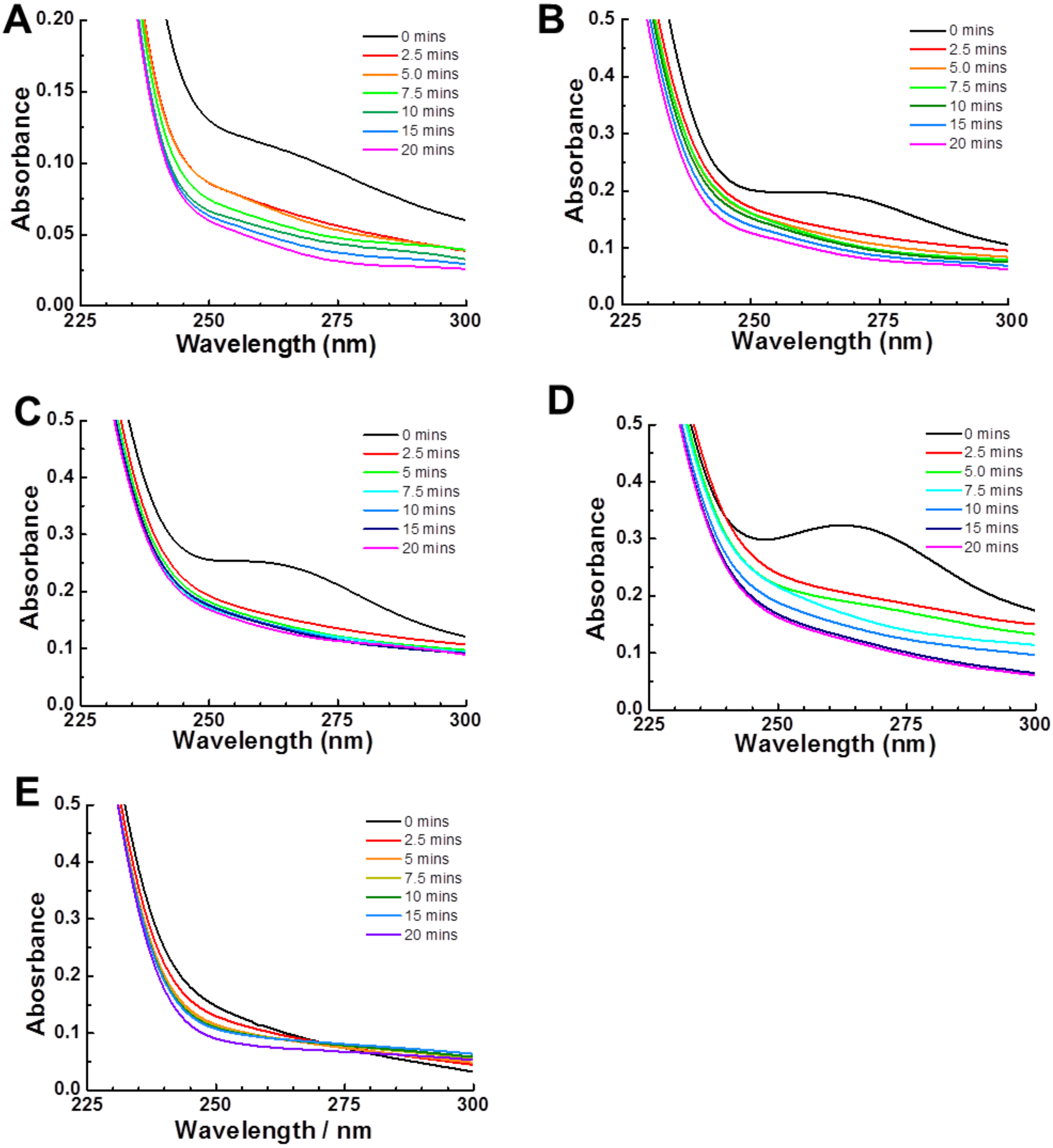


Figure S6. UV-visible spectra measured after different periods of UV irradiation for (**A**) MG-0.15-nPh, (**B**) MG-0.45-nPh, (**C**) MG-0.65-nPh, (**D**) MG-1.00-nPh and (**E**) MG-1.00-EGD. The pH and temperature were 7.4 and 25 °C.

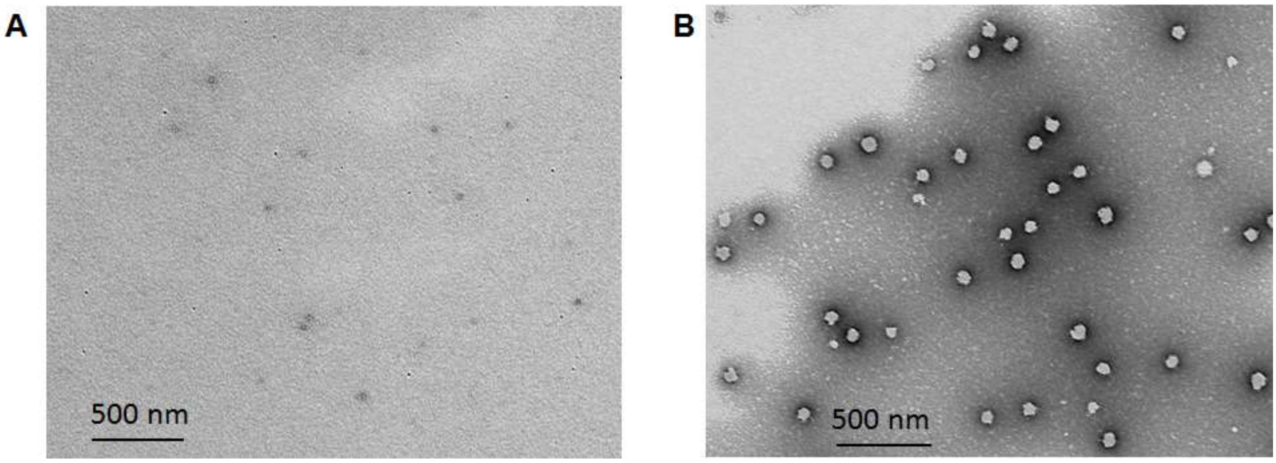


Figure S7. TEM image of (**A**) MG-1.00-nPh and (**B**) MG-1.00-EGD particles deposited after being irradiated with UV light for 20 min. Images for the same dispersions before UV-light irradiation obtained at the same magnification are shown in figures S4 (D) and (F), respectively.

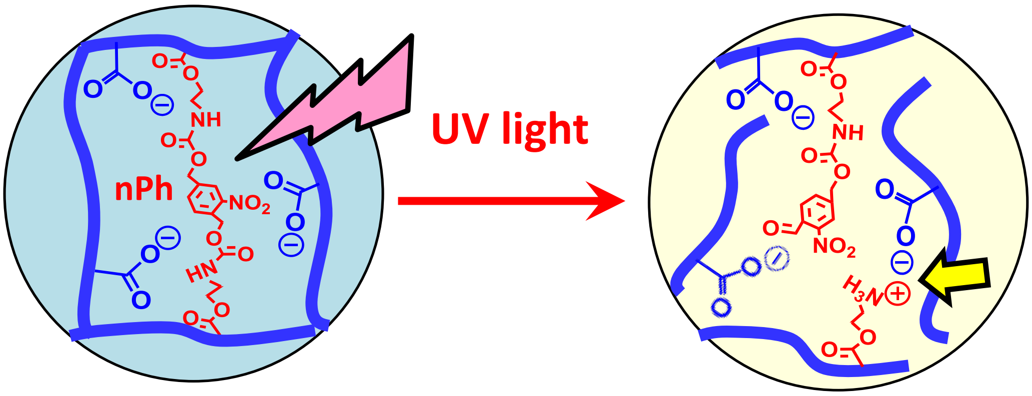


Figure S8. Depiction of the UV light-triggered cleavage of nPh crosslinker. In the presence of ionised polyacid groups ionic bond may form (yellow arrow). However, the ionic bonds are dynamic and gradually rearranged to enable MG disassembly as the permanent (covalent) crosslink concentration in the network decreased.

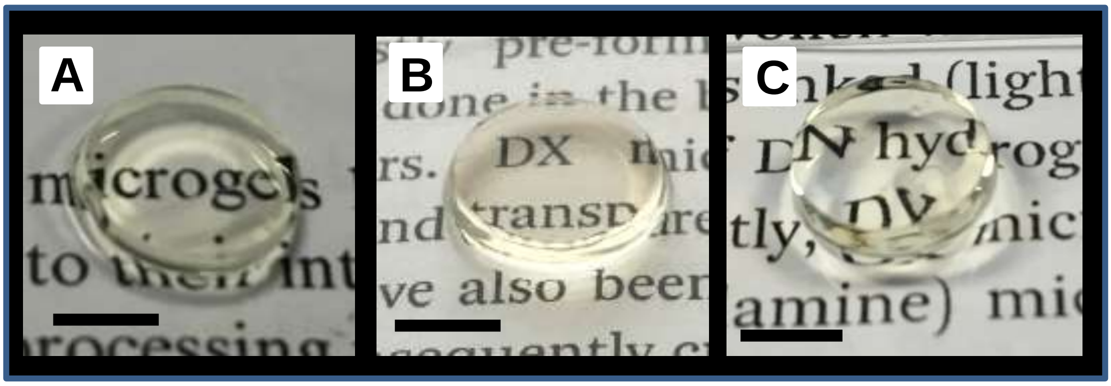


Figure S9. Digital photographs of (**A**) DX MG-0.65-nPh, (**B**) DX MG-0.15-nPh and (**C**) DX MG-0.15-EGD gels**.** The scale bars are 5 mm.

Number of elastically effective chains per glycidyl methacrylate unit within the DX MGs

The DX MGs studied here have two networks present as depicted in Scheme 1B. The MGs contain intra-MG crosslinking via the nPh or EGD crosslinking monomers. The DX MG gels also have a doubly crosslinked network formed via the glycidyl methacrylate (GMA) units. The latter plays an important role in the DX MG mechanical properties. In the following we estimate the number of elastically effective chains per GMA unit in the gel (i.e., $N_{e(GMA)}$). To calculate the latter, the value for the number density of elastically effective chains in the gel (ν_e) is first calculated from the DX MG modulus. The number of MG particles within the DX MG is then determined. These values are then used to calculate the number of elastically-effective chains per MG particle ($N_{e(MG)}$). After determining the number of GMA groups per MG particle the value for $N_{e(GMA)}$ is obtained.

From Fig. 5F it is reasonable to assume that the modulus (*E*) values for DX MG-0.15-nPh and DX MG-0.15-EGD originate primarily from the elastically effective chains from GMA groups. This assumption rests on the fact that the *E* values had reached minimum values and is supported by the very low nPh and EGD contents used (0.15 mol%). The average *E* value for these two gels is 4.0 kPa from Table S4. The polymer volume fraction in the gel (ϕ_p) was 0.12. The following equation was used from rubber elasticity theory[2] to calculate ν_e.

$$E = 3\upsilon_e kT{\phi_p}^{1/3} \qquad \text{(S1)}$$

where k and T (298 K) are the Boltzmann constant and temperature, respectively. Inserting the above values into equation (S1) and solving gives $\nu_e = 6.6 \times 10^{23}\ \text{m}^{-3}$.

The number of MG particles per m^3 of the DX MG gel (N_{MG}) can be calculated from the mass of a collapsed MG particle (m_{MG}) and the total mass of polymer in the gel per m^3 (m_{Tot}) using:

$$m_{MG} = \frac{\rho\pi d^3}{6} \qquad \text{(S2)}$$

$$N_{MG} = \frac{m_{Tot}}{m_{MG}} \qquad \text{(S3)}$$

where the values of ρ and d are, respectively, the polymer density and collapsed particle diameter. An average value of 55 nm was used for the latter from Table S3. Hence, the value for m_{MG} is 1.0 x 10^{-16} g from equation (S2). A value for m_{Tot} of 1.4 x 10^3 g per cubic metre was calculated from ϕ_p and ρ. These values gave N_{MG} = 1.4 x 10^{19} per m^3 from equation (S3). A value of $N_{e(MG)}$ = 4.7 x 10^4 was obtained by dividing ν_e by N_{MG}.

The average composition of the repeat unit for MG-0.15n-Ph and MG-0.15-EGD units is $(MEO_2MA)_{0.73}$-$(OEGMA)_{0.03}$-$(MAA)_{0.19}$-$(GMA)_{0.05}$, which neglects the minor crosslinker concentration (0.15 mol%). Hence, the average repeat unit molecular weight is 175 g/mol. The latter value enables the number of repeat units per MG particle to be calculated as $[(m_{MG}/175)N_A =]$ 3.0 x 10^5, where N_A is Avogadro’s number. Because the mole fraction of GMA is 0.05 the number of number of GMA units per MG particle is 1.5 x 10^4. Dividing the latter into the value for $N_{e(MG)}$ (above) gives $N_{e(GMA)}$ = 3.1. Hence, there were approximately three elastically effective chains per GMA group that comprised the DX network depicted in Scheme 1B.

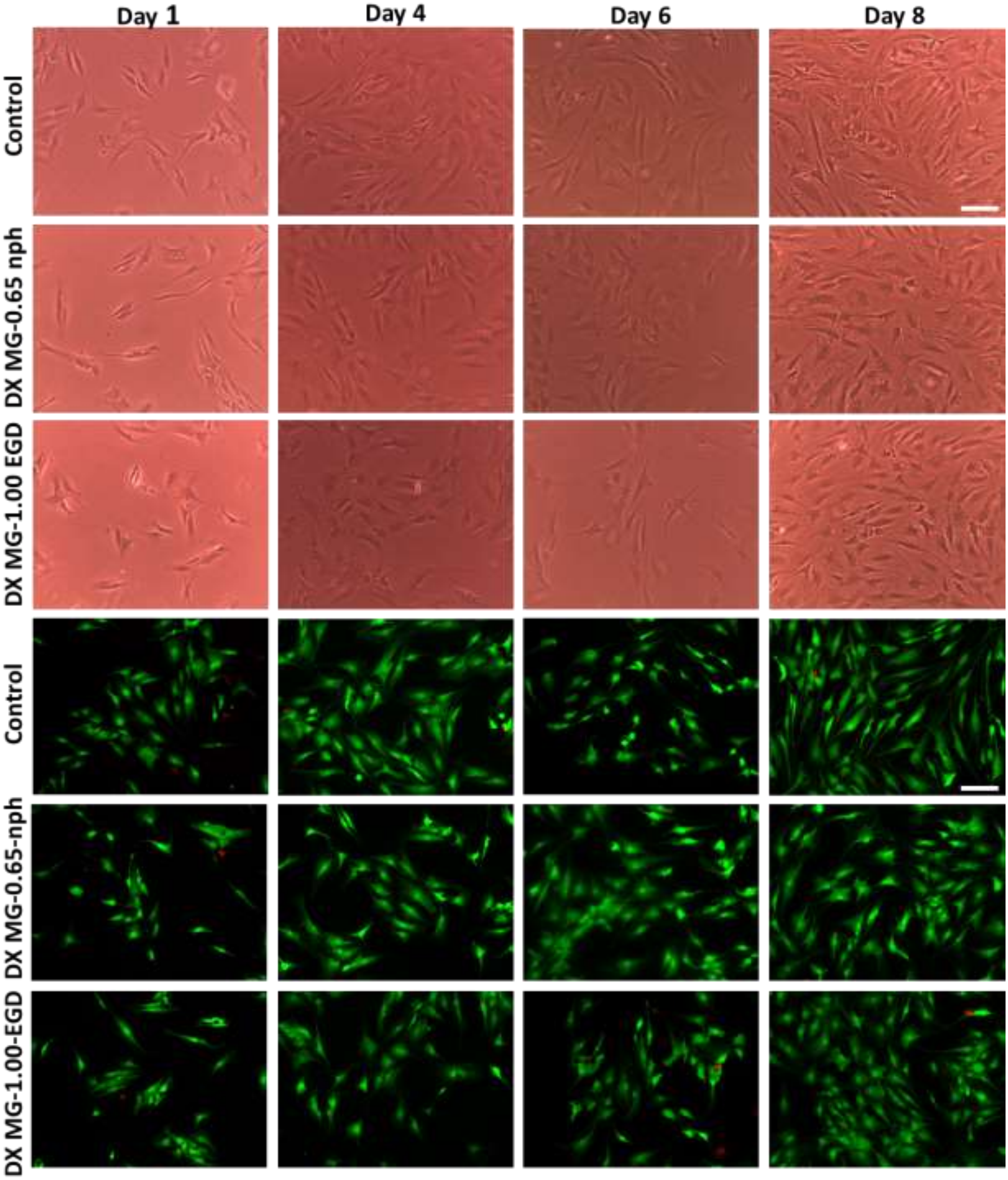
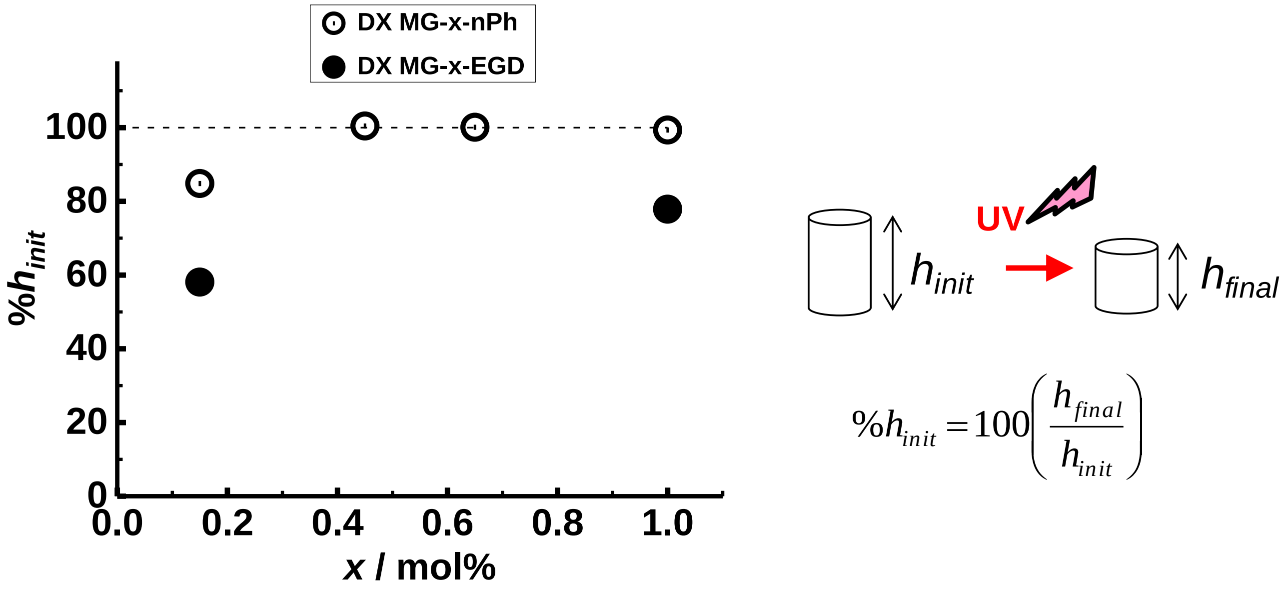


Figure S10. Cell challenge data for human nucleus pulposus (NP) cells in the presence of DX MG-0.65-nPh and DX MG-1.00-EGD. Cell morphology images are shown (top three rows). Live/Dead assay images (bottom three rows) were obtained using fluorescence microscopy. The control group used an equal volume of PBS. The scale bar applies to all images and is 100 µm.



$$\%h_{init} = 100\left(\frac{h_{final}}{h_{init}}\right)$$

Figure S11. Measured % initial gel cylinder height after UV-irradiation versus *x*.

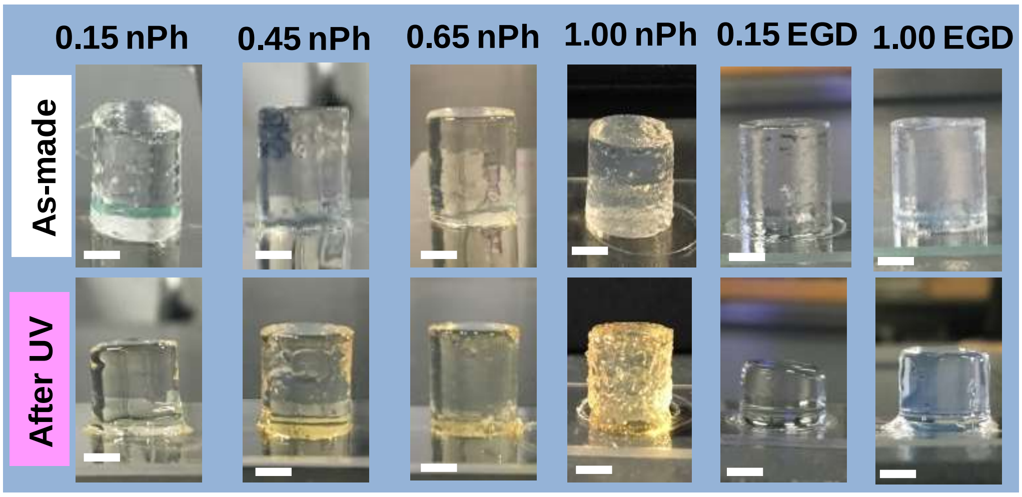


Figure S12. Photographs of DX MGs before (Top) and after (Bottom) UV-irradiation. Scale bars: 5 mm. The yellow colour after UV-irradiation is due to the photocleavage product (see Fig. S8). The appearance of DX MG-1.00-nPh after UV-irradiation is due to increased surface tackiness.

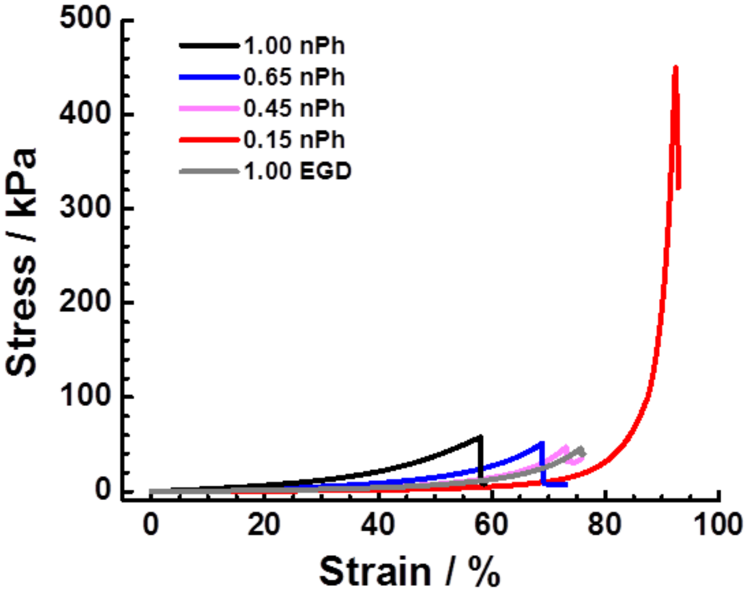


Figure S13. Compressive stress-strain data measured for various DX-MG-*x*-nPh gels and a DX MG-1.00-EGD gel after UV irradiation.

Proposed mechanism to explain effect of UV light on the properties of the DX MGs

The MG-*x*-nPh particles disassemble upon UV-light irradiation (Fig. S14A) because the ionic crosslinks that form are dynamic[3]. In contrast, the DX MG-*x*-nPh gels have a second network from the GMA-based crosslinks that prevent large-scale disassembly (see Fig. S14B). This network is proposed to have allowed significant rearrangement of the core of the MGs and stabilised the photo-generated ionic bonds. The latter cause an increase in E and decreased Q_{DXMG}. The energy of a covalent bond is 360 kJ/mol[4] (i.e., 145kT). Ionic bonds should have an energy of ~ kT [5]. Hence, the rearranged domains should contain multiple ionic bonds and provide cooperative bonding[5]. We propose that photocleavage replaced an inefficient (covalent) stress distribution network with a much more efficient (ionic) network through local structural rearrangement. It is proposed that the UV-irradiated DX MG-*x*-nPh gels are nanostructured gels with each MG containing a polyampholyte core.

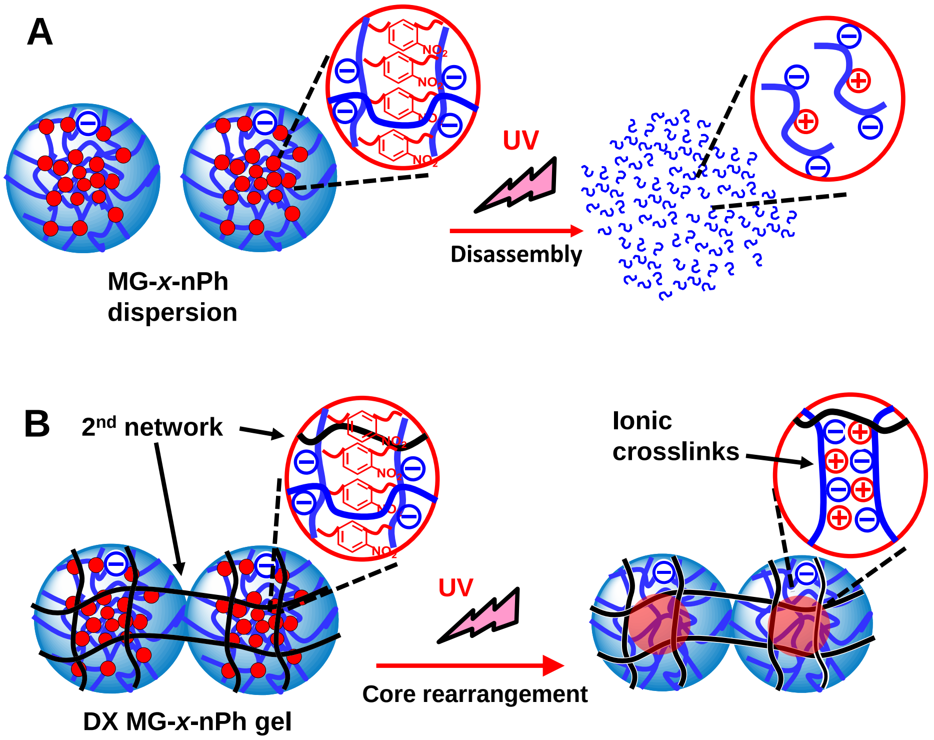


Figure S14. Depiction of proposed mechanisms to explain UV-light triggered MG-*x*-nPh disassembly (**A**) and DX MG-*x*-nPh mechanical property changes (**B**). The UV-triggered cleavage of the nPh crosslinks groups generates positive charge which forms ionic bonds with nearby -COO^- groups. For the MGs (A) the dynamic ionic bonds allow the MGs to disassemble. In the case of the DX MGs (B) the permanent linkages formed from the second GMA-based network (shown as black lines) prevent gel disassembly. Those ionic species rearrange to form multiple ionic crosslinks.

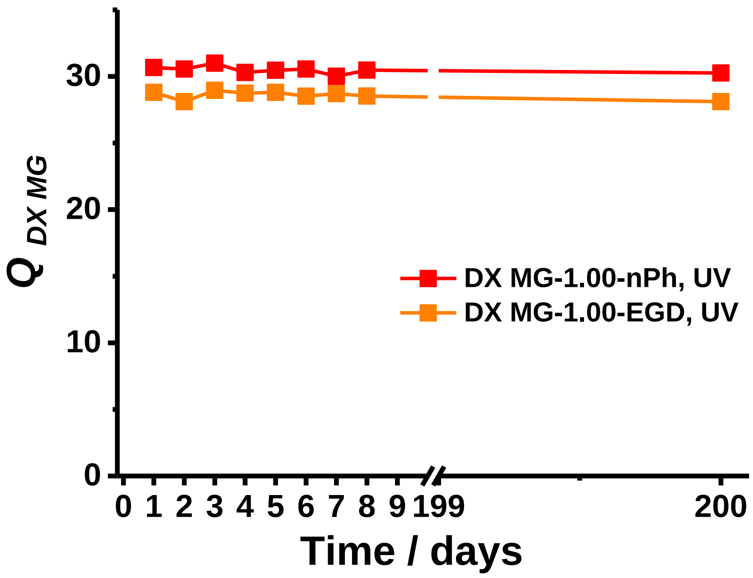


Figure S15. Variation of the volume swelling-ratio with time for DX MG-1.00-nPh and DX MG-1.00-EGD gels. The data were measured at pH 7.4 and 25°C.

Table S1 Comonomer formulations used to prepare the precursor MGs[a]

Microgels	MEO_2MA / wt.%	OEGMA / wt.%	MAA / wt.%	nPh / wt.%	EGD / wt.%
MG-0.15-nPh	80.2	8.9	10.5	0.4	-
MG-0.45-nPh	79.4	8.8	10.5	1.3	-
MG-0.65-nPh	79.0	8.8	10.4	1.8	-
MG-1.00-nPh	78.2	8.7	10.3	2.8	-
MG-0.15-EGD	80.3	8.9	10.6	-	0.20
MG-1.00-EGD	79.6	8.8	10.5	-	1.1

[a] The MGs were subsequently functionalised with GMA as described in the Experimental Details section. The values given in the table are with respect to monomer.

Table S2 Overview of the model and simulation parameters for the Monte Carlo simulations

Parameter	Value
Temperature	298.15 K
Dielectric permittivity of implicit solvent	78.3
Number of cross-links	29
Number of polymer chains	76
Number of beads per polymer chain	9
Fraction of ionisable segments per chain	9/9
Radius of cross-links	1σ
Radius of chain beads	1σ
Radius of counterions	1σ
Charge of cross-links	0e
Charge of ionisable chain beads [a]	−1e/0e
Charge of counterions [a]	+1e/0e
Length of simulation cell	750σ
Zero-force distance of bonds	3.89 $k_B T\ \sigma^{-2}$
Width of square well potential	6σ
Depth of square well potential	From 0.00 $k_B T$ to 0.18 $k_B T$ in steps of 0.02 $k_B T$

[a] Charge depends of state of ionisation (in units of the elementary charge e)

Table S3 Composition and properties of the MGs studied

Microgels	MEO_2MA [a] / mol%	OEGMA [a] / mol%	MAA [b] / mol%	nPh[a] / mol%	EGD[a]/ mol%	GMA [c] / mol%	pK_a[d]	VPTT [e] (pH, 5.4)	d_{TEM} [f] /nm	d_z /nm[g] (pH 5.4, 60°C)
MG-0.15-nPh	73.68	3.10	18.18	0.15	-	5.02	5.9	25.5	51(6)	55 (0.081)
MG-0.45-nPh	71.63	3.11	19.65	0.45	-	5.17	5.8	24.4	52(9)	54 (0.089)
MG-0.65-nPh	73.65	3.16	18.53	0.65	-	4.08	5.9	24.3	50(6)	54 (0.087)
MG-1.00-nPh	72.80	3.16	19.05	1.00	-	4.27	6.0	23.4	50(7)	60 (0.058)
MG-0.15-EGD	72.44	3.09	20.27	-	0.15	4.41	5.9	23.4	59(7)	57 (0.085)
MG-1.00-EGD	70.21	3.11	19.09	-	1.00	4.68	5.9	25.0	54(7)	65 (0.074)

[a] Nominal values based on formulation used. [b] Determined from potentiometric titration data. [c] Calculated using the difference of the MAA content before and after functionalisation. [d]Apparent pK_a value determined from potentiometric titration data. [e] Volume phase transition temperature. [f] The numbers in brackets are the standard deviation. [g] z-average diameters. The numbers in brackets are the PDI values.

Table S4 Mechanical properties of the as made DX MG gels studied

Hydrogel	Modulus / kPa	Strain at break (ε_B) / %
DX MG-0.15-nPh	4.52 ± 0.06	81.4 ± 1.7
DX MG-0.45-nPh	4.80 ± 0.44	78.5 ± 3.1
DX MG-0.65-nPh	7.46 ± 0.84	76.2 ± 2.5
DX MG-1.00-nPh	20.25 ± 2.76	63.3 ± 3.3
DX MG-0.15-EGD	3.40 ± 0.39	77.4 ± 2.3
DX MG-1.00-EGD	7.41 ± 1.43	70.1 ± 5.3

Table S5 Mechanical properties of UV-irradiated the DX MG gels

Hydrogel	Modulus / kPa	Strain at break (ε_B) / %
DX MG-0.15-nPh	2.24 ± 0.03	92.7 ± 1.9
DX MG-0.45-nPh	5.81 ± 0.15	73.1 ± 1.8
DX MG-0.65-nPh	10.61 ± 0.62	68.9 ± 1.2
DX MG-1.00-nPh,	28.04 ± 1.73	54.8 ± 3.6
DX MG-0.15-EGD	- [a]	- [a]
DX MG-1.00-EGD	4.20 ± 1.21	74.6 ± 1.6

[a] Reliable stress-strain measurements were not possible for this sample.

References

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