Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

L-proline based thermoresponsive and pH-switchable nanogel as drug delivery vehicle

Yolanda Salinas, Ana M. Castilla and Marina Resmini*

Department of Chemistry and Biochemistry, SBCS, Queen Mary University of London, Mile End Road E1 4NS, London, United Kingdom.

Email: m.resmini@qmul.ac.uk

1. Supporting Tables

The amounts of monomer, cross-linker (MBA), initiator (AIBN) and solvent (DMSO) needed to prepared the 32 nanogels described in the manuscript are showed in the following Table SI-1 and Table SI-2.

Table SI-1. Chemical composition for the preparation of nanogels **N1-N25**

Nanogel n°	Feeding ratio monomers:CL	NIPAM	NPAM	NAPr	MBA CL (mmol)	AIBN initiator (mmol)	DMSO (mL)
		monomers (mmol)					
N1	**90:0:0:10**	0.884	0	0	0.098	0.011	10.36
N2	**80:0:0:20**	0.884	0	0	0.221	0.013	12.07
N3	**70:0:0:30**	0.884	0	0	0.379	0.016	14.25
N4	**60:0:0:40**	0.884	0	0	0.589	0.021	17.17
N5	**50:0:0:50**	0.884	0	0	0.884	0.026	21.26
N6	**0:90:0:10**	0	0.884	0	0.098	0.011	10.36
N7	**0:80:0:20**	0	0.884	0	0.221	0.013	12.07
N8	**0:70:0:30**	0	0.884	0	0.379	0.016	14.25
N9	**0:60:0:40**	0	0.884	0	0.589	0.021	17.17
N10	**0:50:0:50**	0	0.884	0	0.884	0.026	21.26
N11	**0:0:90:10**	0	0	0.799	0.089	0.010	10.23
N12	**0:0:80:20**	0	0	0.799	0.200	0.012	11.77
N13	**0:0:70:30**	0	0	0.799	0.342	0.015	13.75
N14	**0:0:60:40**	0	0	0.799	0.533	0.019	16.39
N15	**0:0:50:50**	0	0	0.799	0.799	0.024	20.09
N16	**40:0:50:10**	0.884	0	1.105	0.221	0.024	24.51
N17	**40:0:40:20**	0.884	0	0.884	0.442	0.026	25.09
N18	**40:0:30:30**	0.884	0	0.663	0.663	0.029	25.66
N19	**40:0:20:40**	0.884	0	0.442	0.884	0.031	26.24
N20	**40:0:10:50**	0.884	0	0.221	1.105	0.033	26.82
N21	**0:40:50:10**	0	0.884	1.105	0.221	0.024	24.51
N22	**0:40:40:20**	0	0.884	0.884	0.442	0.026	25.09
N23	**0:40:30:30**	0	0.884	0.663	0.663	0.029	25.66
N24	**0:40:20:40**	0	0.884	0.442	0.884	0.031	26.24
N25	**0:40:10:50**	0	0.884	0.221	1.105	0.033	26.82

Table SI-2. Chemical composition for the preparation of nanogels **N26-N31**

Nanogel nº	**Feeding ratio** monomers:CL	**NPAM**	**A-Pr-OH**	**NAPr**	**Aac**	**MBA** CL (mmol)	**AIBN** initiator (mmol)	**DMSO** (mL)
		monomers (mmol)						
N26	**70:20:0:0:10**	0.884	0.253	0	0	0.126	0.014	14.6
N27	**75:15:0:0:10**	0.884	0.177	0	0	0.118	0.013	14.33
N28	**80:10:0:0:10**	0.884	0.11	0	0	0.11	0.012	12.21
N29	**85:5:0:0:10**	0.884	0.052	0	0	0.104	0.012	11.23
N30	**87.5:2.5:0:0:10**	0.884	0.025	0	0	0.101	0.011	10.79
N31	**85:0:2.5:2.5:10**	0.884	0	0.026	0.026	0.104	0.014	10.74
N30D	**87.5:2.5:0:0:10**	0.884	0.025	0	0	0.101	0.011	10.79

All the nanogels chemical composition was calculated by using the following equations:

$$n_{MBA} = n_{monomer} \cdot \frac{MBA\ \%}{monomer\ \%} \quad \text{(Eq. 1)}$$

$$n_{AIBN} = (n_{monomer} + 2 \cdot n_{MBA}) \cdot 1\ \% \quad \text{(Eq. 2)}$$

$$V_{DMSO} = \left(m_{TOTAL\ monomers} \cdot \frac{100\ \%}{1\ \%} \cdot \frac{99\ \%}{100\ \%}\right) \cdot \frac{1}{\rho_{DMSO}} \quad \text{(Eq. 3)}$$

Table SI-3. Monomer, cross-linker and overall final conversions for nanogels **N1-N25**

	Feeding Composition				**Conversion**[a]				
Nanogel	**NIPAM**	**NPAM**	**NAPr**	**MBA**	**NIPAM**	**NPAM**	**NAPr**	**MBA**	**Overall**
n°	**% mol monomer**			**% mol CL**	**%**			**%**	**%**
N1	90	-	-	10	63.7	-	-	80.9	65.4
N2	80	-	-	20	67.2	-	-	91.7	72.4
N3	70	-	-	30	77.6	-	-	93.4	82.2
N4	60	-	-	40	81.1	-	-	95.7	86.7
N5	50	-	-	50	75.9	-	-	94.5	85.1
N6	-	90	-	10	-	59.3	-	80.2	61.7
N7	-	80	-	20	-	61.3	-	86.1	66.9
N8	-	70	-	30	-	64.6	-	86.9	71.4
N9	-	60	-	40	-	72.1	-	92.7	80.5
N10	-	50	-	50	-	71.7	-	92.4	82.4
N11	-	-	90	10	-	-	95.7	99.3	96.2
N12	-	-	80	20	-	-	95.4	98.8	96.2
N13	-	-	70	30	-	-	96.7	99.0	97.5
N14	-	-	60	40	-	-	96.4	98.4	97.2
N15	-	-	50	50	-	-	97.8	98.4	98.1
N16	40	-	50	10	81.6	-	94.2	94.8	89.1
N17	40	-	40	20	82.7	-	96.0	95.4	90.3
N18	40	-	30	30	81.0	-	94.1	96.8	89.4
N19	40	-	20	40	78.9	-	79.0	91.6	84.0
N20	40	-	10	50	78.4	-	94.0	95.5	88.3
N21	-	40	50	10	-	78.1	91.6	94.2	86.5
N22	-	40	40	20	-	75.8	90.5	93.3	85.3
N23	-	40	30	30	-	77.9	91.5	94.6	87.2
N24	-	40	20	40	-	79.1	93.8	95.6	88.9
N25	-	40	10	50	-	76.7	92.2	93.4	86.7

Polymerisation conditions: 48h, 70 °C, DMSO-d_6, 1% AIBN and Cm=1%. [a]Conversions were calculated from ^{1}H NMR spectra of initial and final polymerisation mixtures.

Table SI-4. Monomer, cross-linker and overall final conversions for nanogels **N26-N30**

	Feeding Composition			**Conversion**^{a}			
Nanogel	**NPAM**	**A-Pro-OH**	**MBA**	**NPAM**	**A-Pro-OH**	**MBA**	**Overall**
n°	**% mol monomer**		**% mol CL**	**%**	**%**	**%**	**%**
N26	70	20	10	67.5	87.3	85.5	73.6
N27	75	15	10	71.9	87.7	85.8	75.8
N28	80	10	10	66.3	85.5	82.0	70.0
N29	85	5	10	71.7	89.15	89.2	74.2
N30	87.5	2.5	10	62.0	79.5	85.8	65.0

Polymerisation conditions: 48h, 70 °C, DMSO-d_6, 1% AIBN and Cm=1%. ^{a}*Conversions were calculated from* ^{1}*H NMR spectra of initial and final polymerisation mixtures.*

2. Supporting Figures

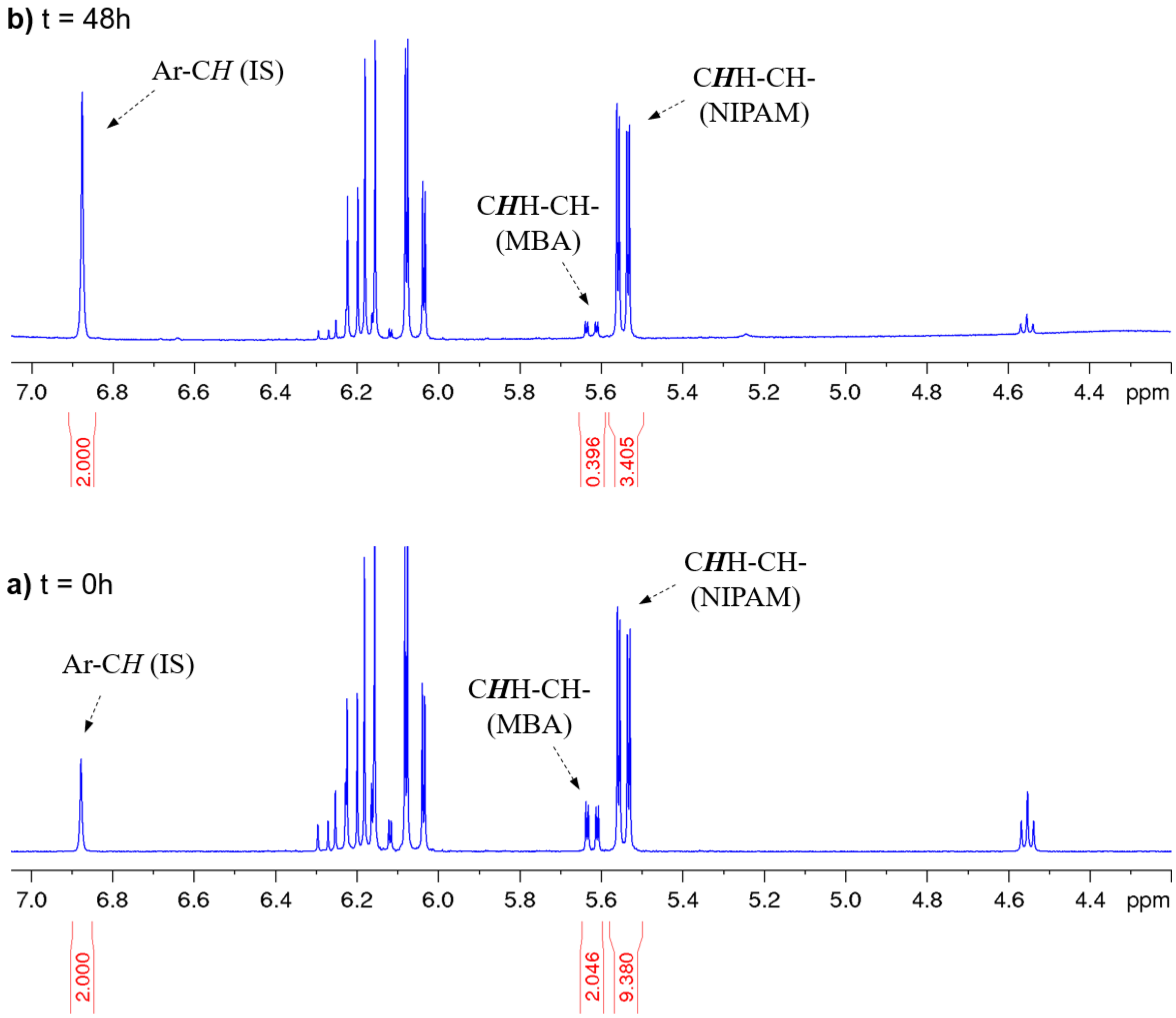

Figure SI-1. Partial ^{1}H NMR (400 MHz, 298 K, DMSO-d_6) spectra of the polymerisation mixtures for the preparation of nanogel **N1** as a representative example for all polymerisations. a) Spectrum acquired immediately after preparation of the polymerisation mixture, b) spectrum acquired after heating at 70 °C for 48 h. Peaks integrated to calculate conversions (Table SI-3 and Table SI-4) are indicated. 1,2,4,5-Tetramethylbenzene was used as internal standard (IS). Intensities have been scaled for clarity.

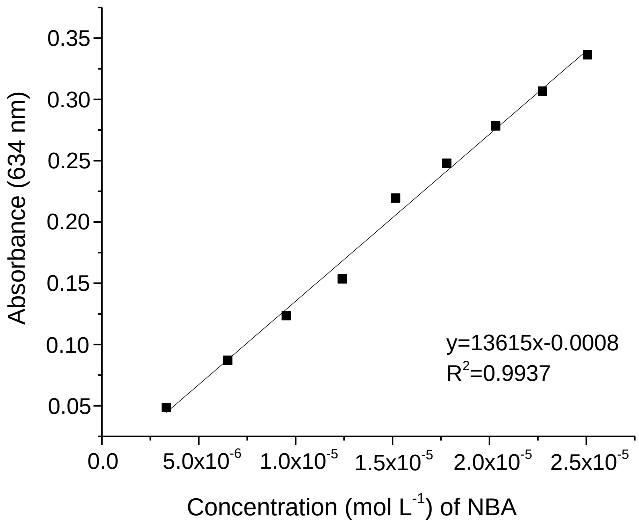

Figure SI-2. Calibration curve of NBA

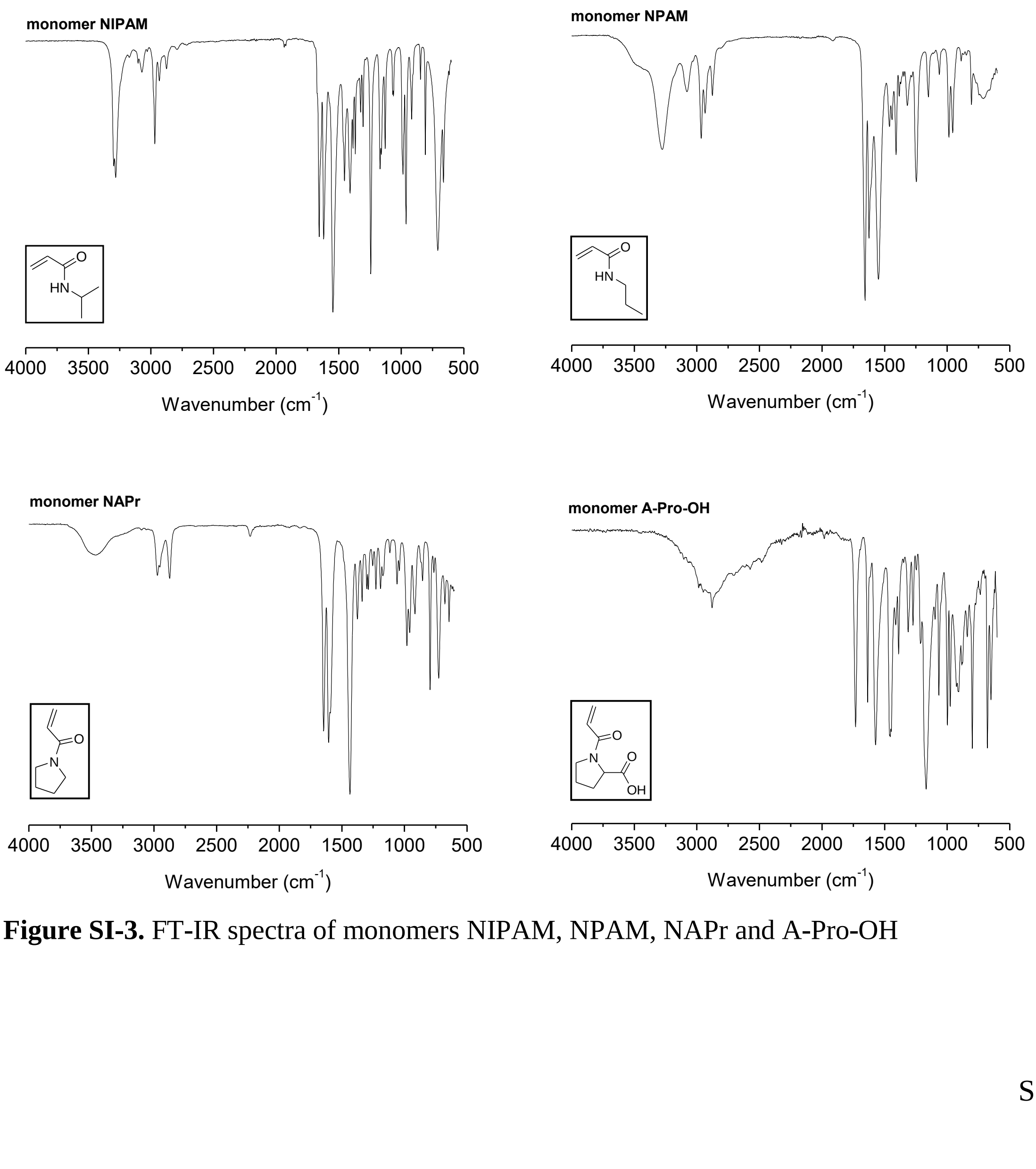

Figure SI-3. FT-IR spectra of monomers NIPAM, NPAM, NAPr and A-Pro-OH

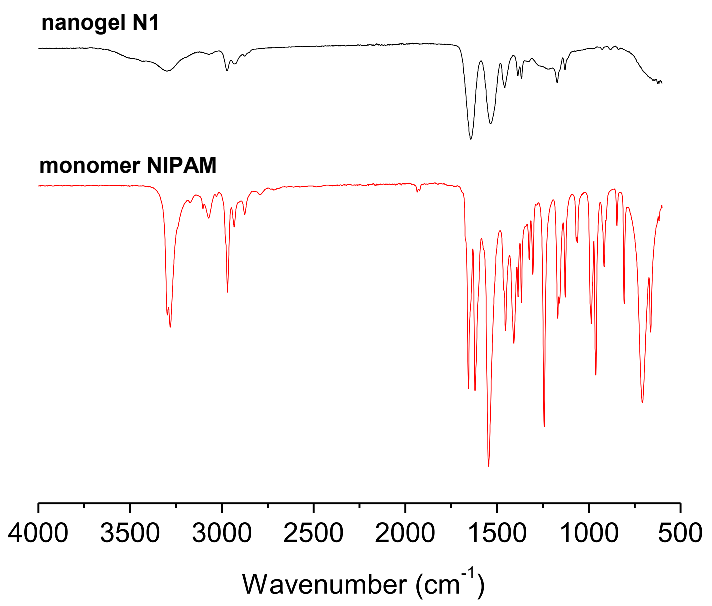

Figure SI-4. FT-IR spectra of monomer NIPAM and nanogel **N1** (NIPAM-MBA 90:10)

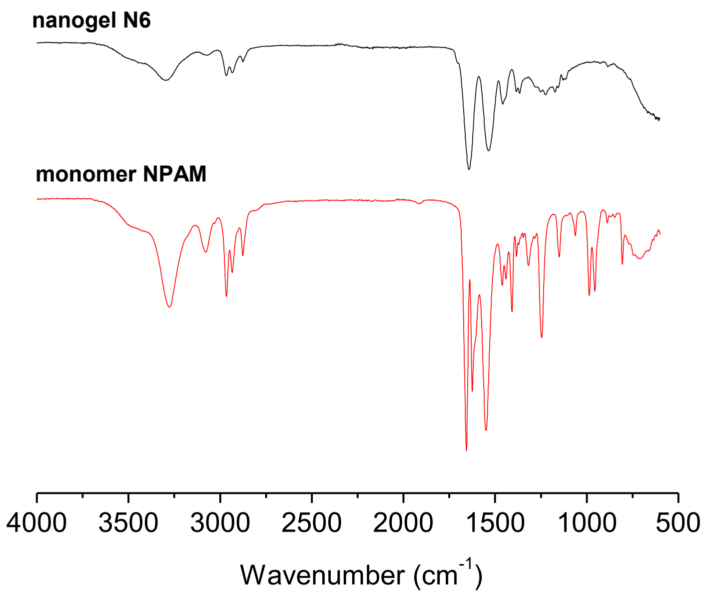

Figure SI-5. FT-IR spectra of monomer NPAM and nanogel **N6** (NPAM-MBA 90:10)

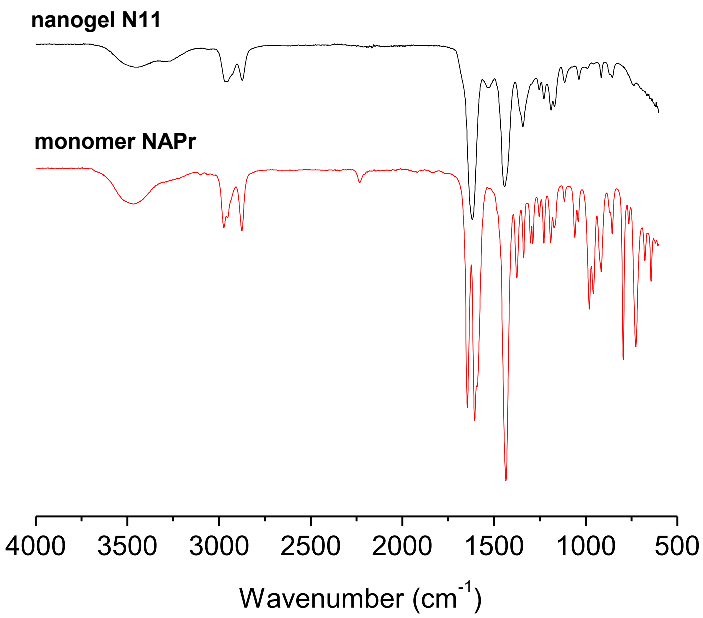

Figure SI-6. FT-IR spectra of monomer NPAr and nanogel **N11** (NPAr-MBA 90:10)

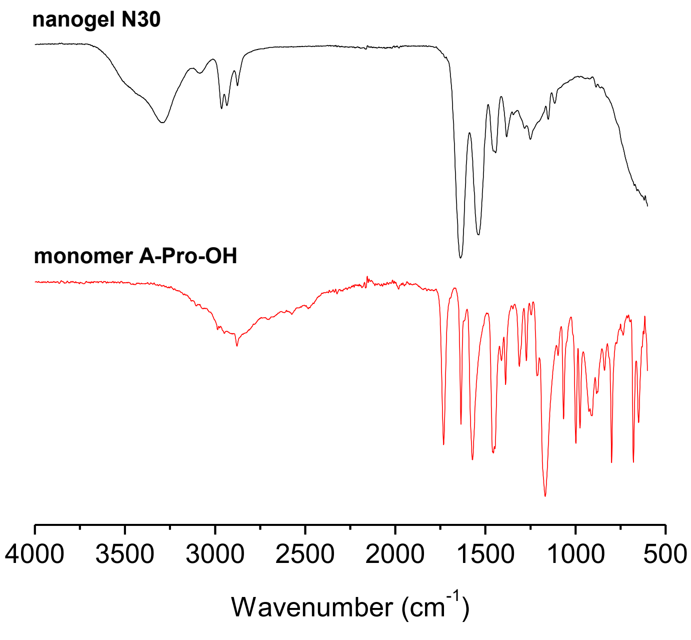

Figure SI-7. FT-IR spectra of monomer A-Pro-OH and nanogel **N30** (NPAM-A-Pro-OH-MBA 87.5:2.5:10).

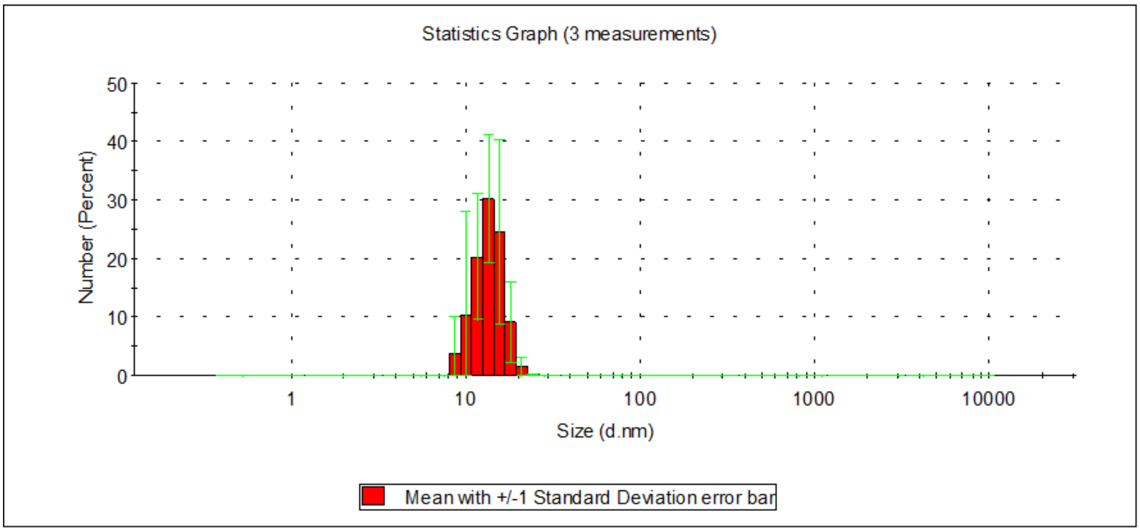

Figure SI-8. Dynamic light scattering of nanogel **N1**

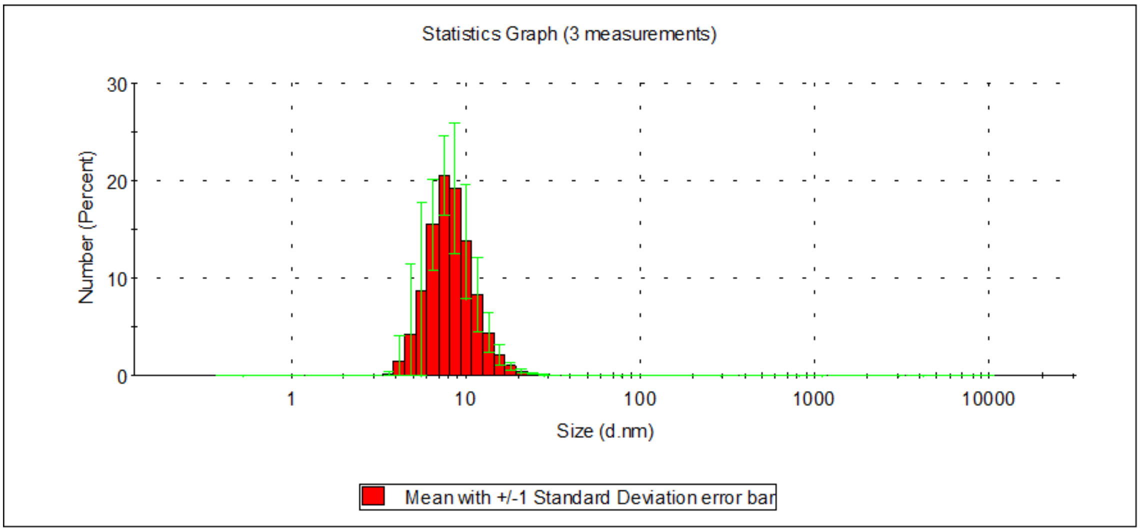

Figure SI-9. Dynamic light scattering of nanogel **N2**

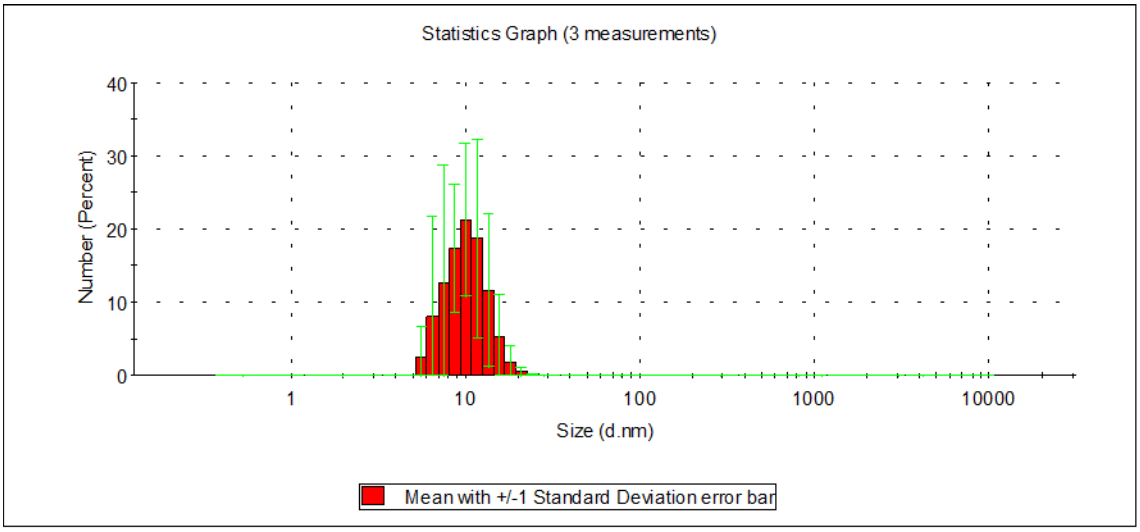

Figure SI-10. Dynamic light scattering of Nanogel **N3**

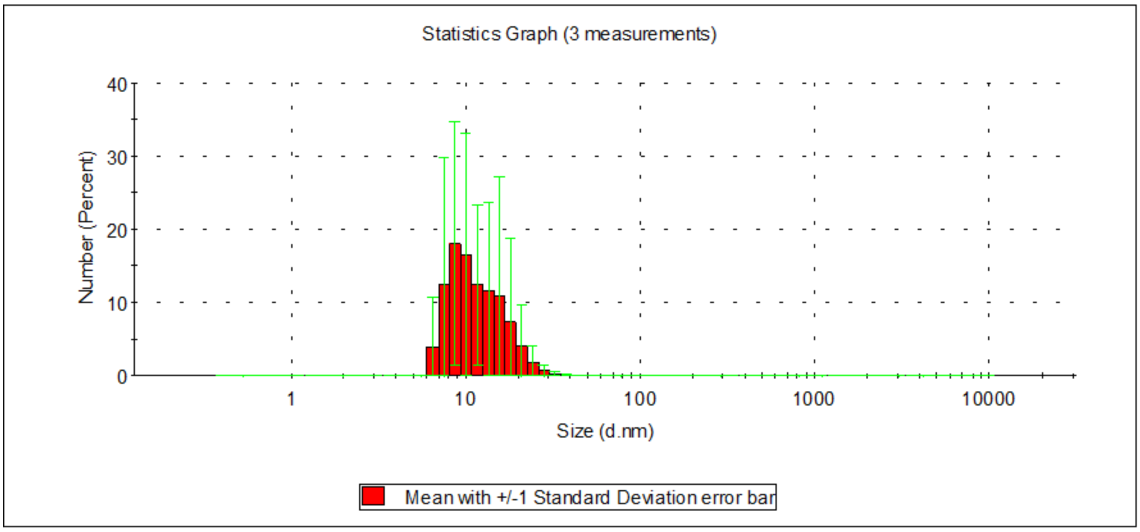

Figure SI-11. Dynamic light scattering of nanogel **N4**

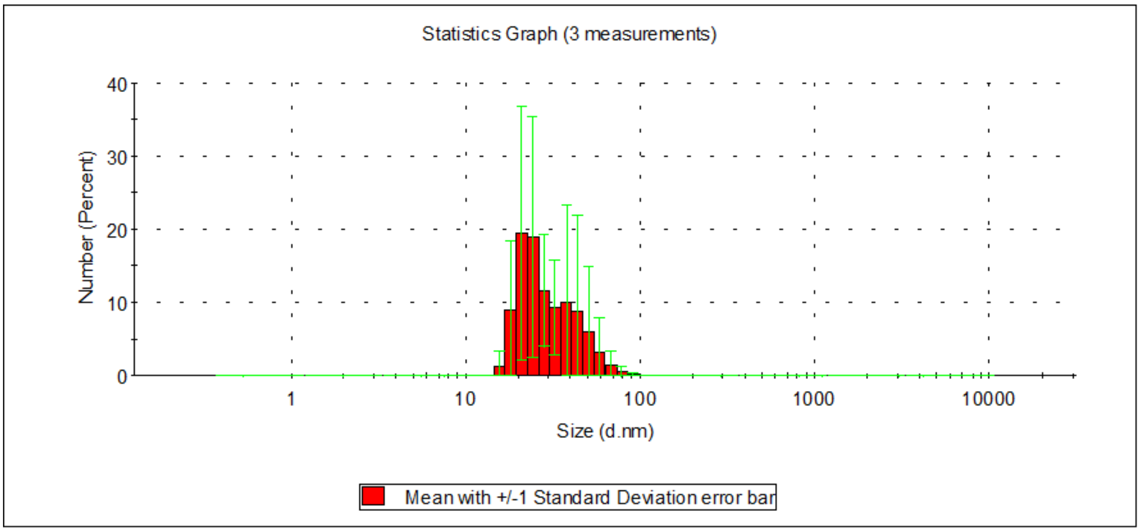

Figure SI-12. Dynamic light scattering of nanogel **N5**

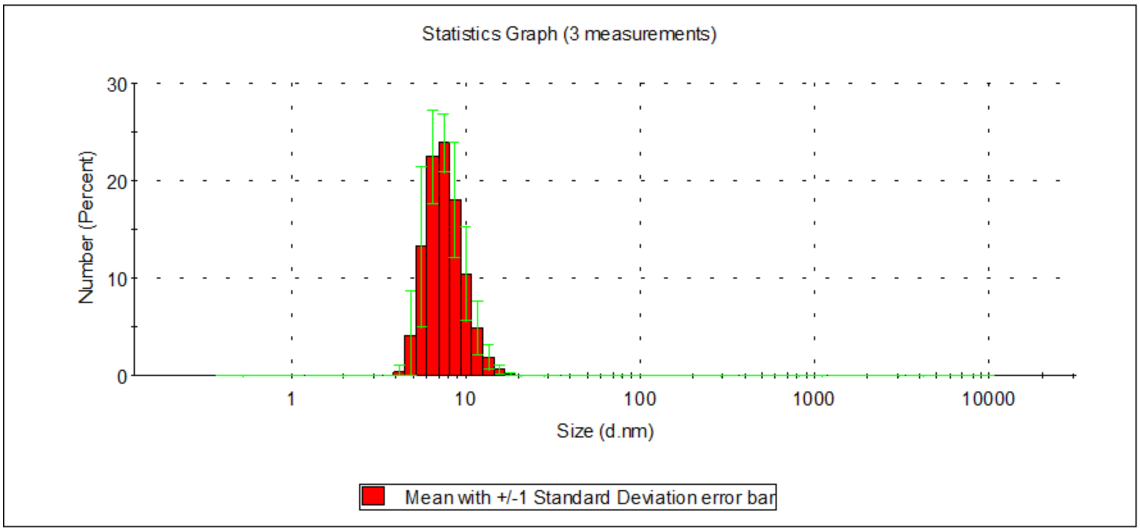

Figure SI-13. Dynamic light scattering of nanogel **N6**

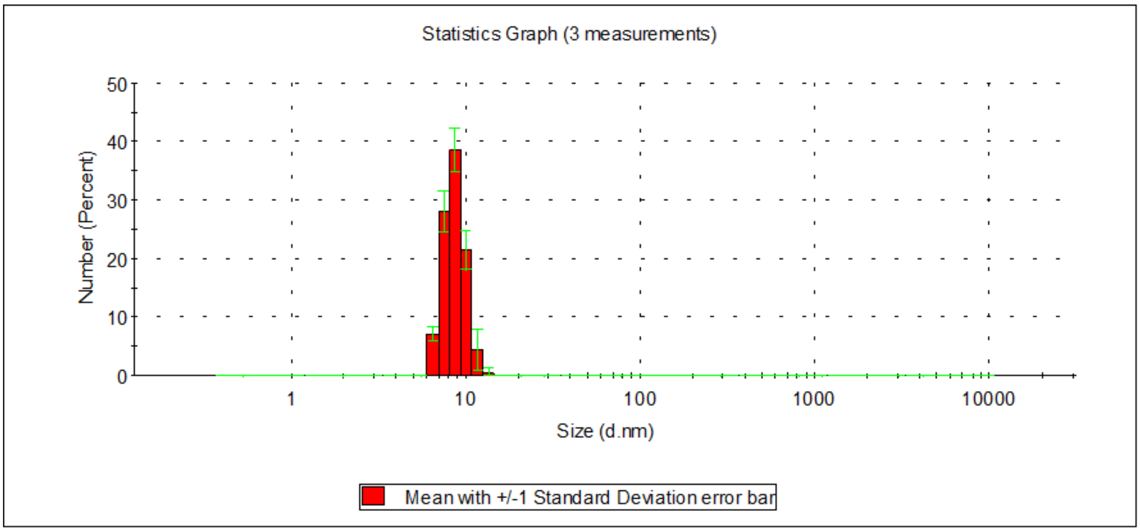

Figure SI-14. Dynamic light scattering of nanogel **N7**

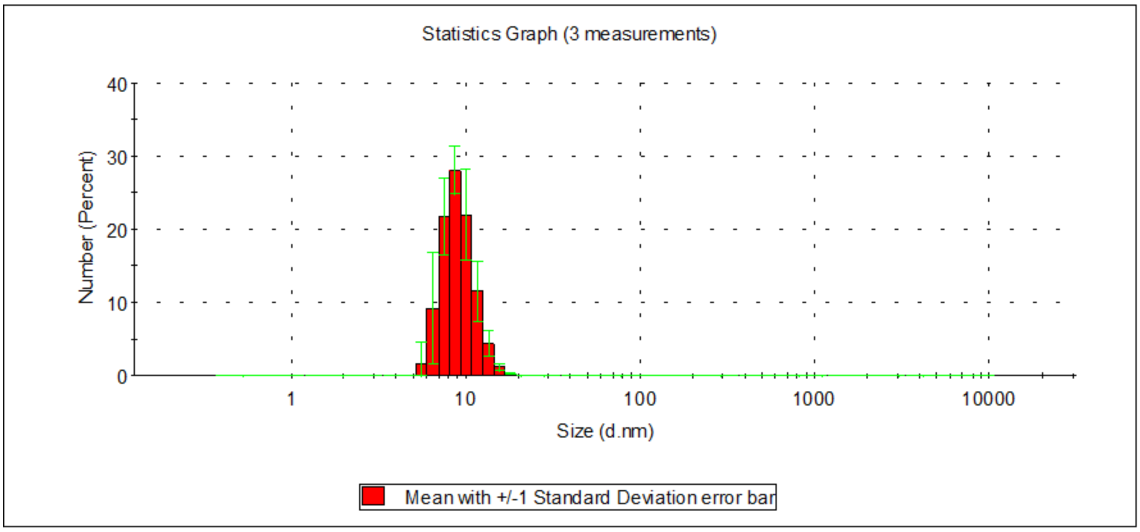

Figure SI-15. Dynamic light scattering of nanogel **N8**

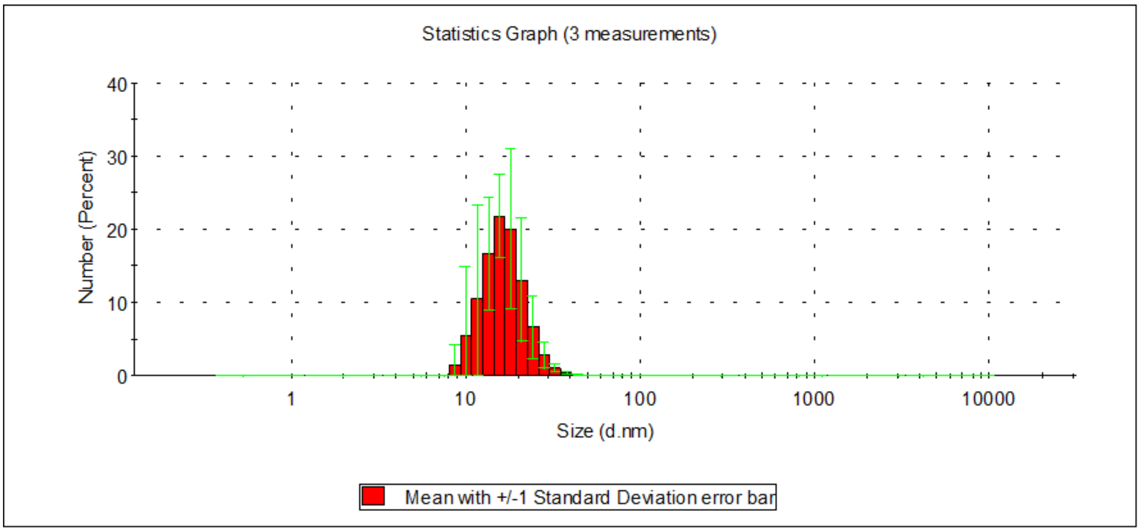

Figure SI-16. Dynamic light scattering of nanogel **N9**

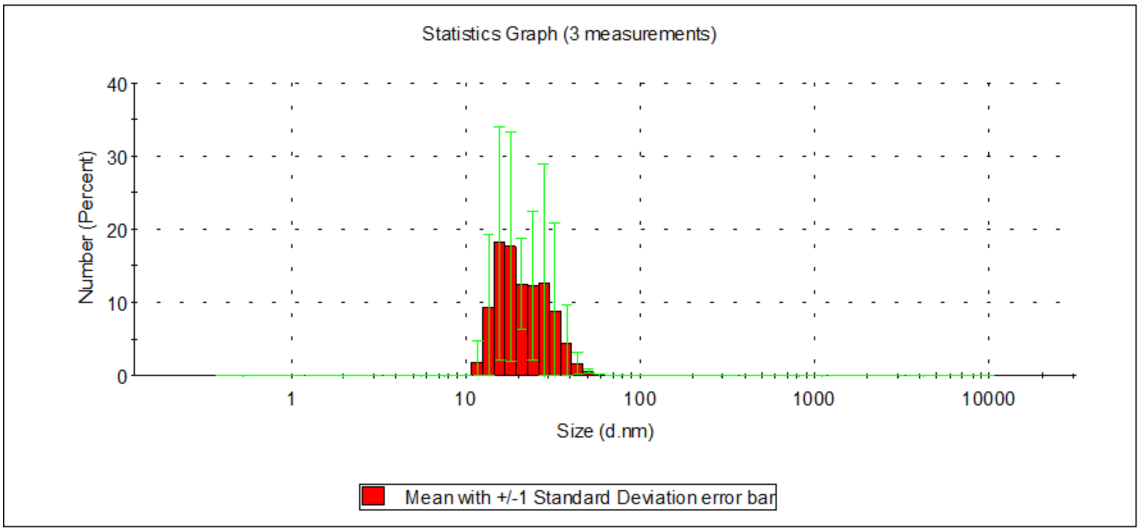

Figure SI-17. Dynamic light scattering of nanogel **N10**

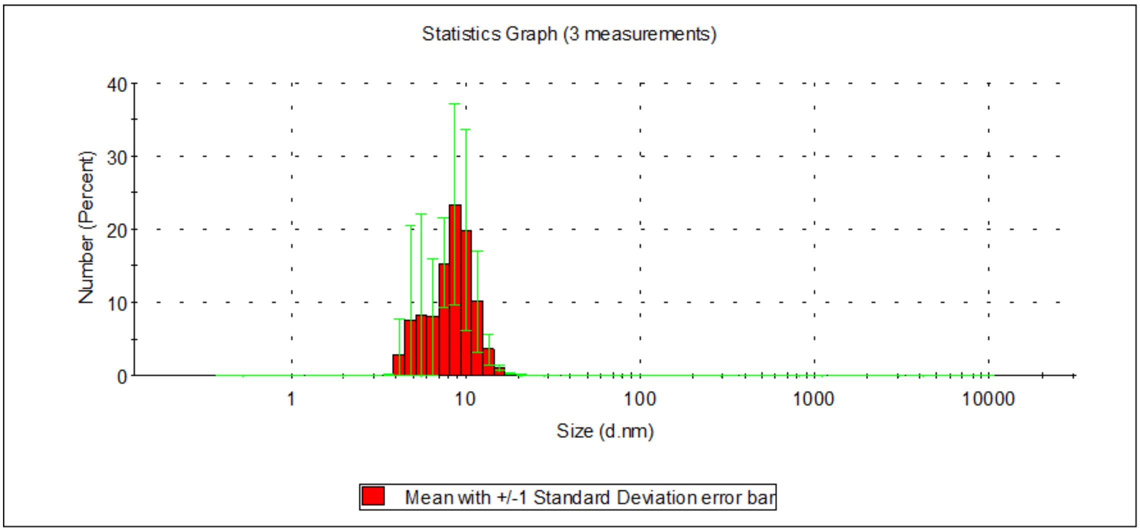

Figure SI-18. Dynamic light scattering of nanogel **N11**

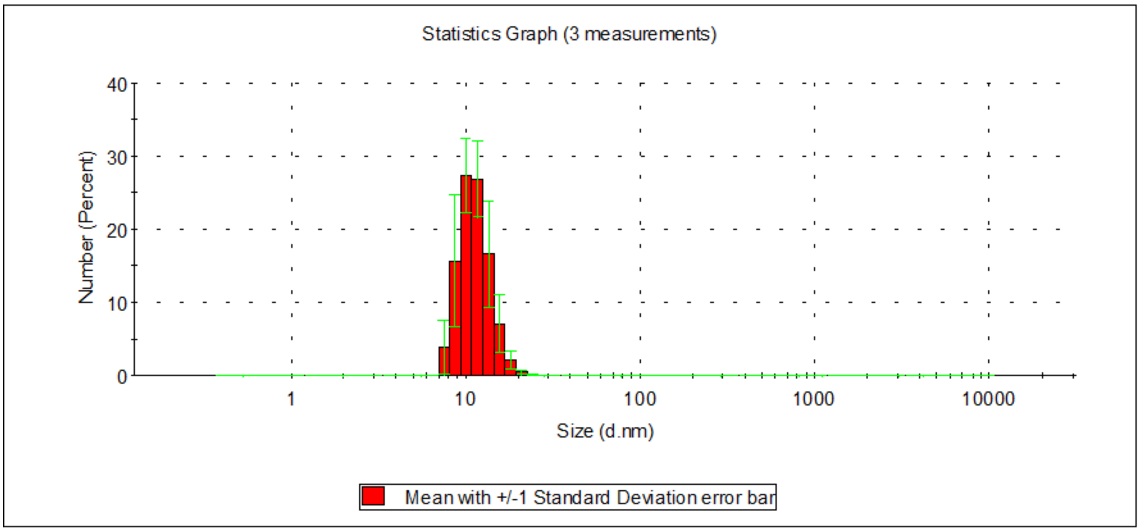

Figure SI-19. Dynamic light scattering of nanogel **N12**

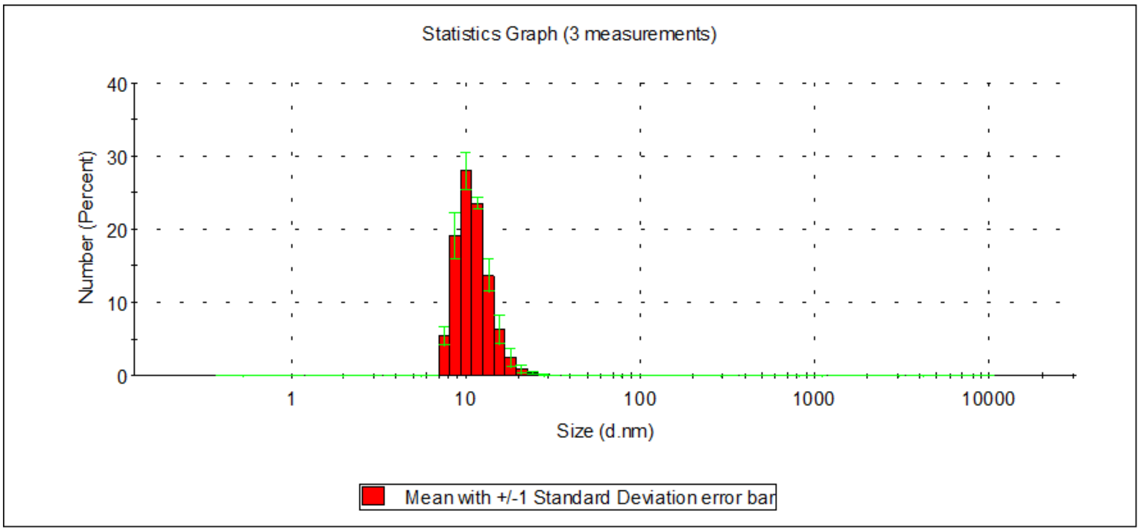

Figure SI-20. Dynamic light scattering of nanogel **N13**

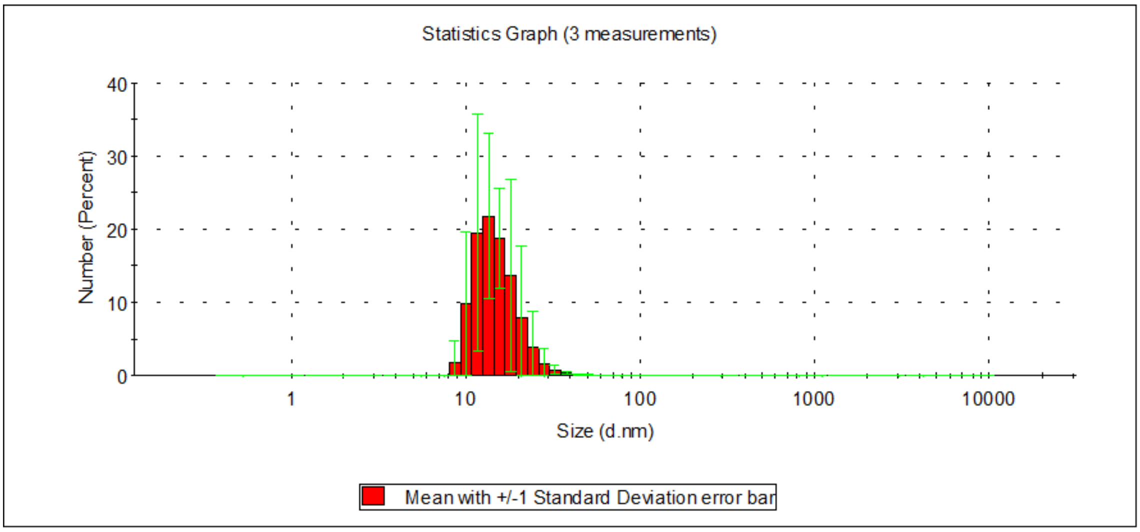

Figure SI-21. Dynamic light scattering of nanogel **N14**

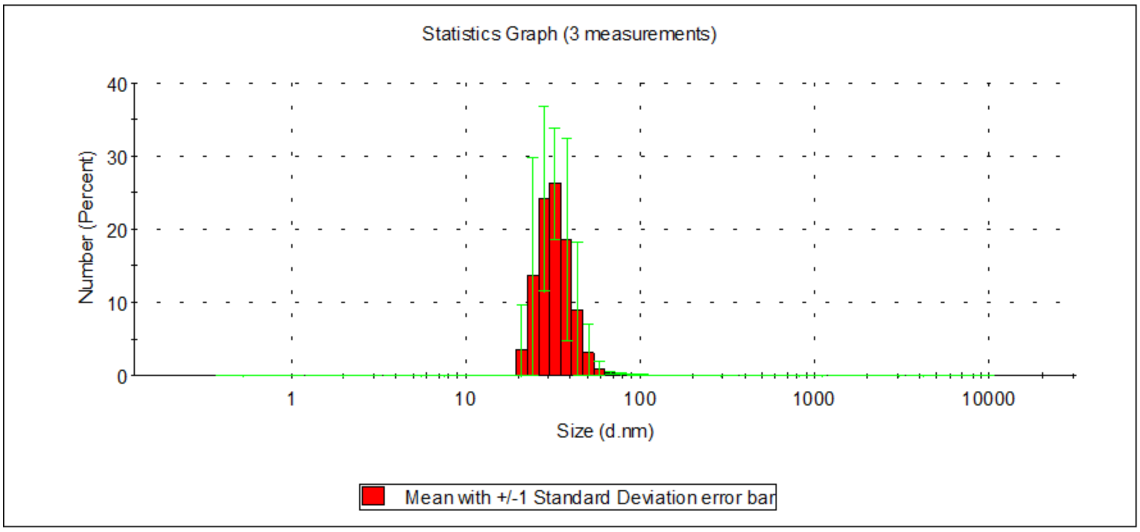

Figure SI-22. Dynamic light scattering of nanogel **N15**

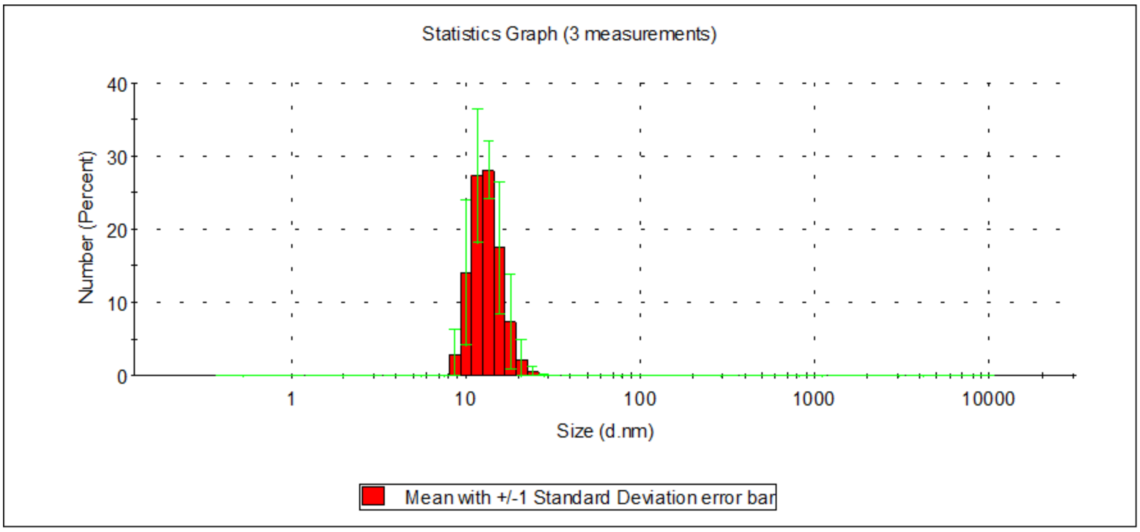

Figure SI-23. Dynamic light scattering of nanogel **N16**

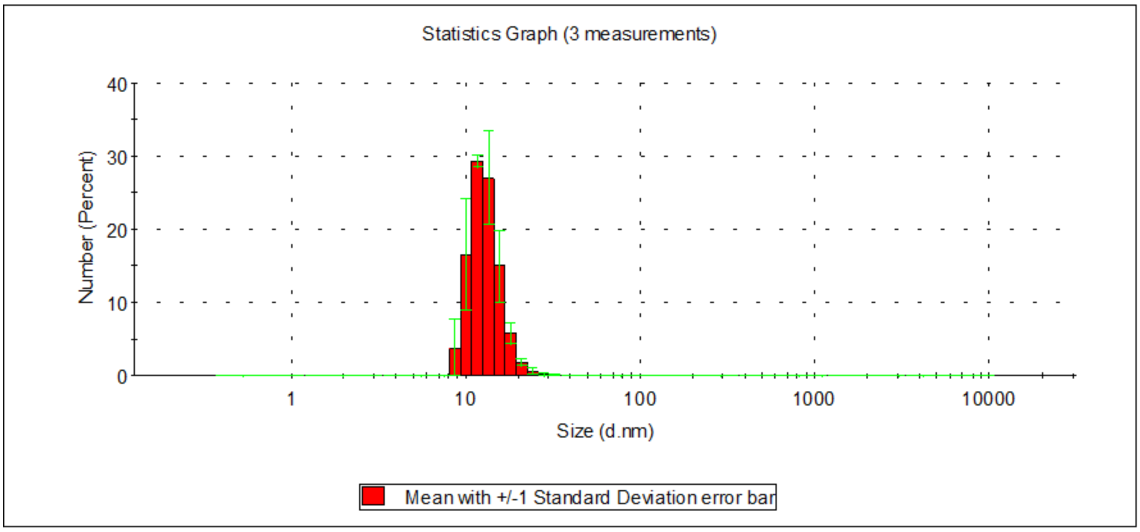

Figure SI-24. Dynamic light scattering of nanogel **N17**

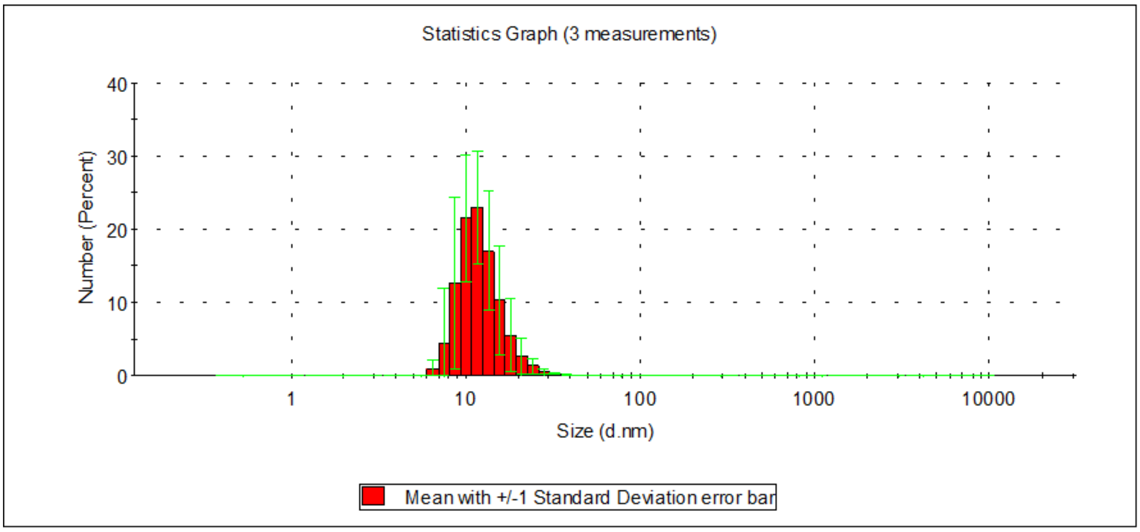

Figure SI-25. Dynamic light scattering of nanogel **N18**

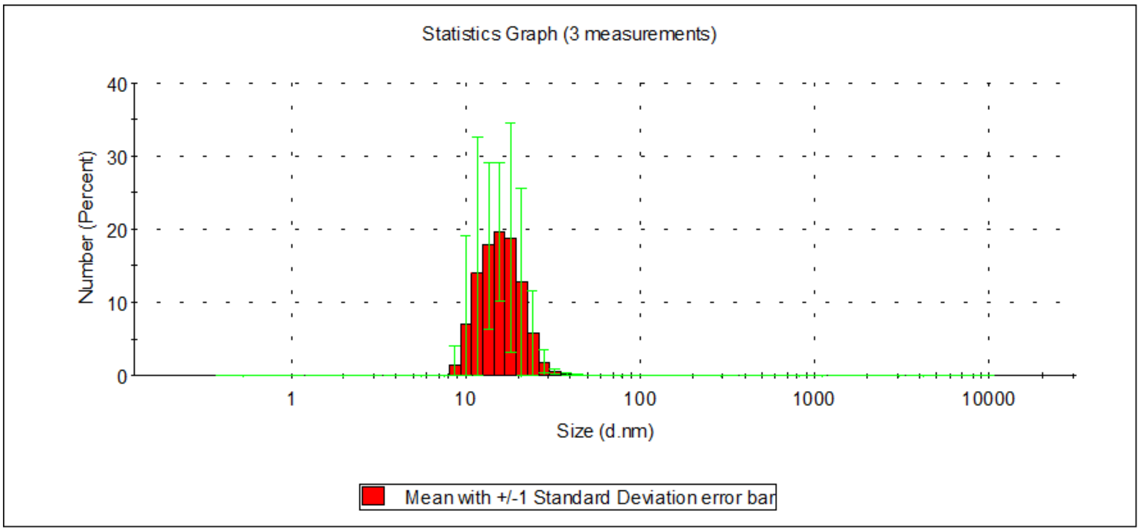

Figure SI-26. Dynamic light scattering of nanogel **N19**

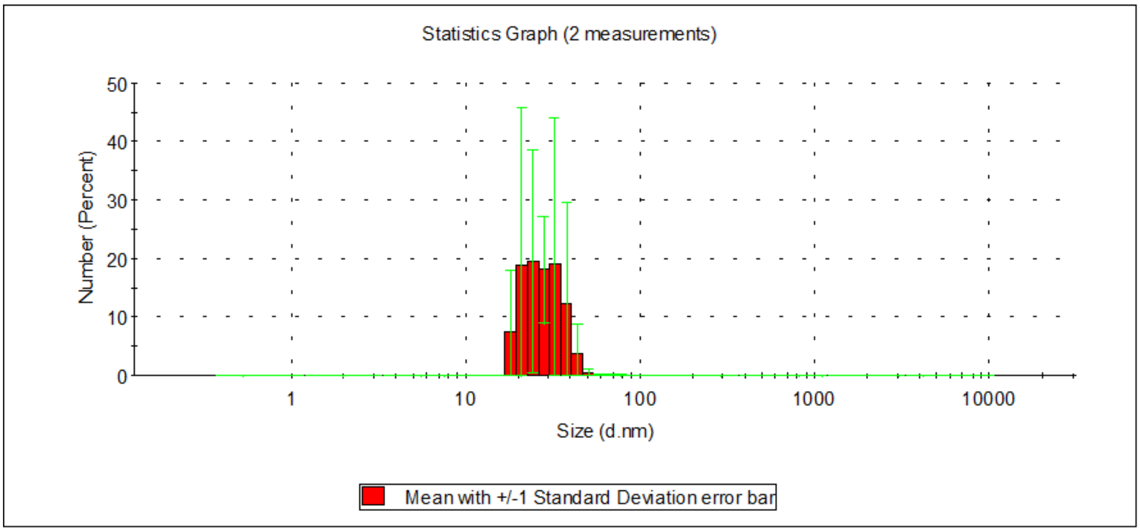

Figure SI-27. Dynamic light scattering of nanogel **N20**

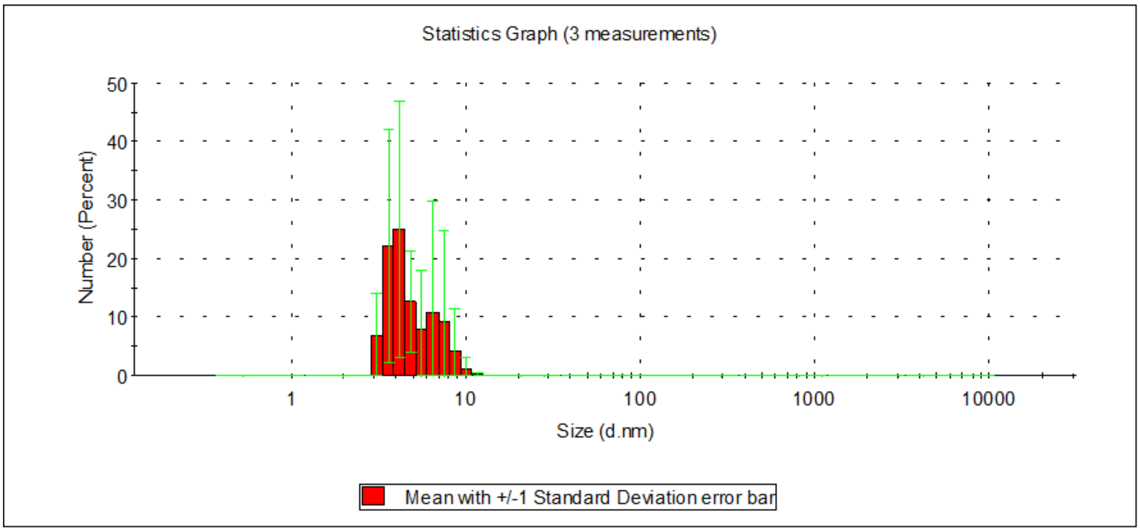

Figure SI-28. Dynamic light scattering of nanogel **N21**

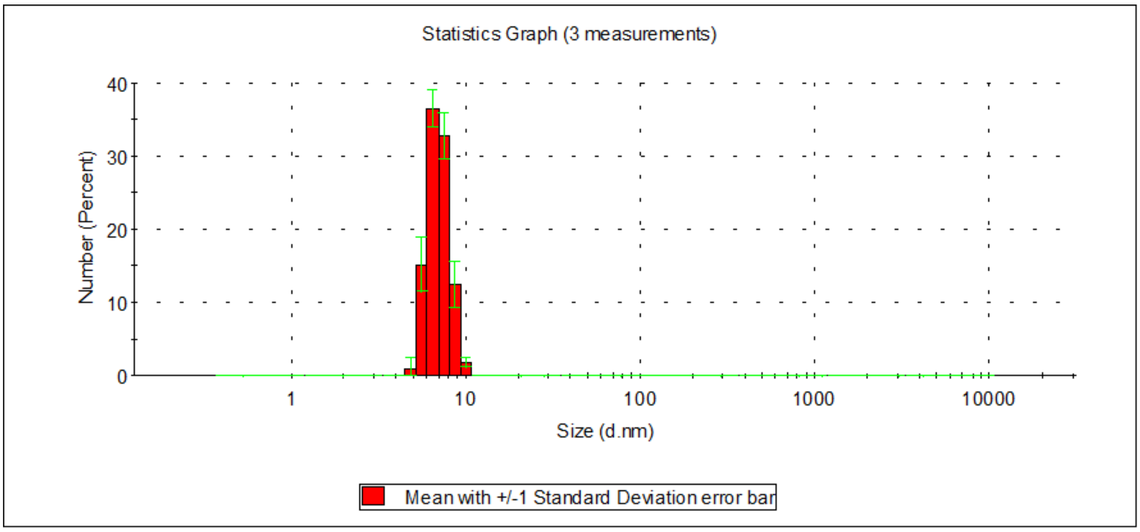

Figure SI-29. Dynamic light scattering of nanogel **N22**

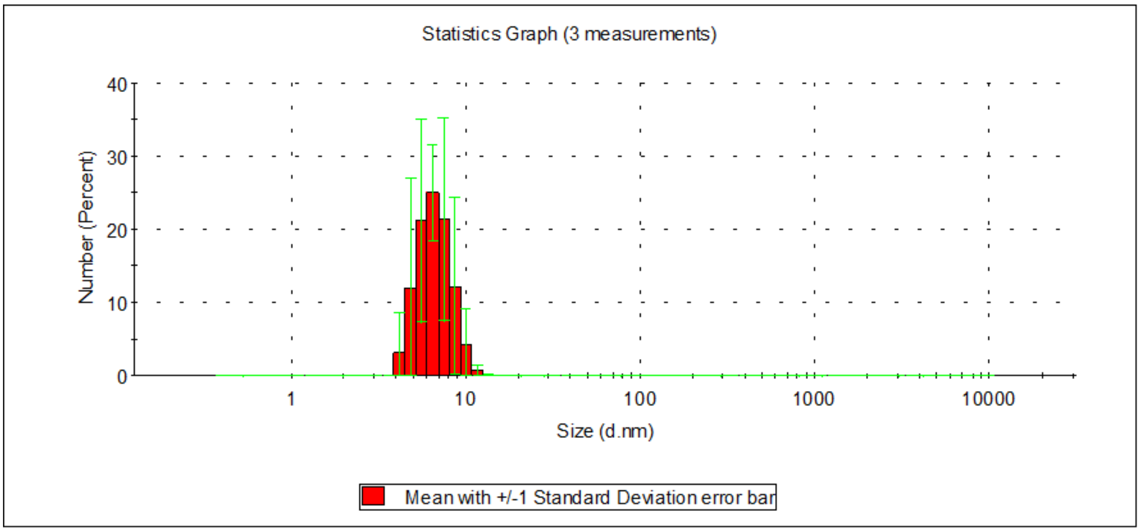

Figure SI-30. Dynamic light scattering of nanogel **N23**

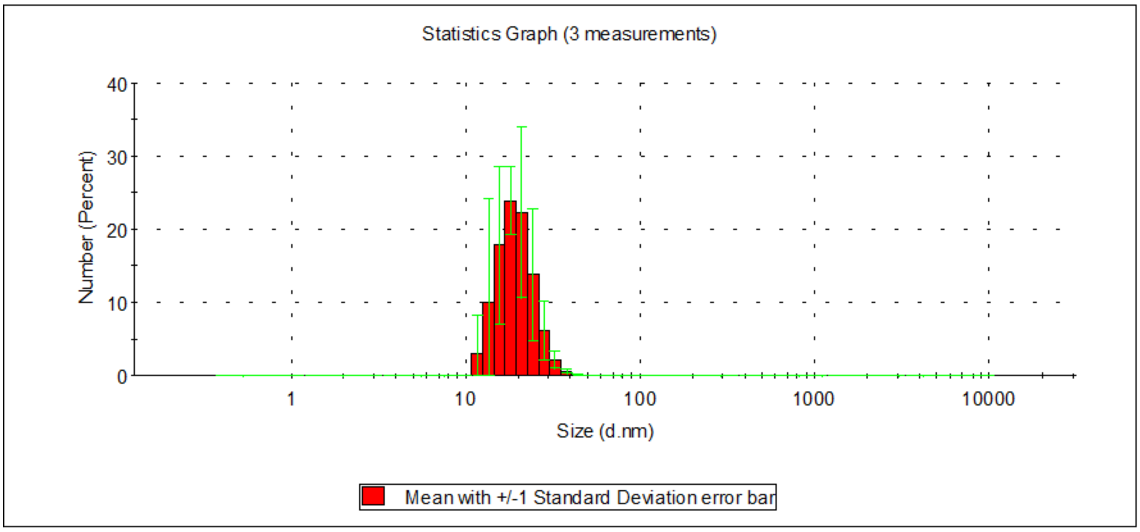

Figure SI-31. Dynamic light scattering of nanogel **N24**

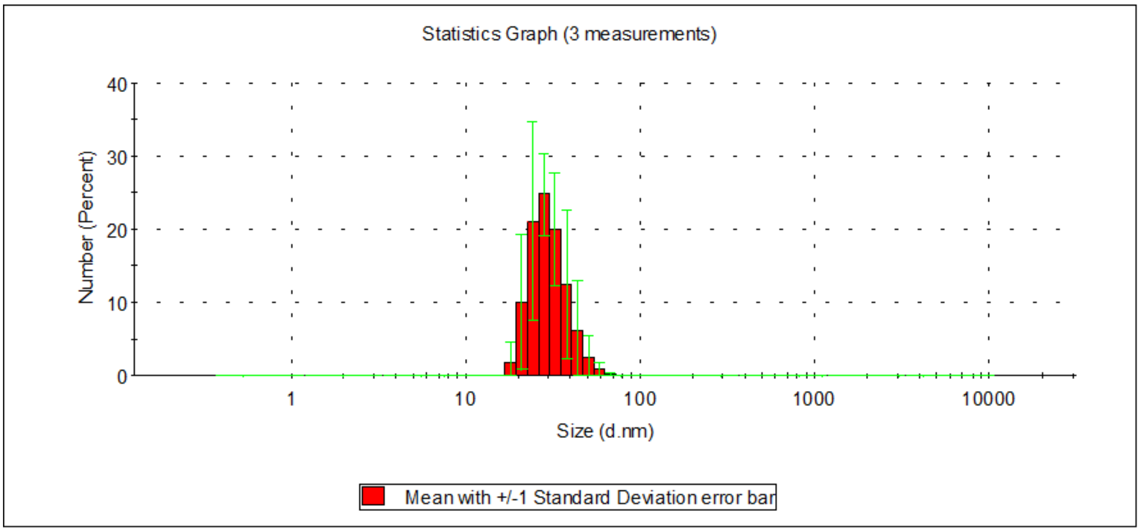

Figure SI-32. Dynamic light scattering of nanogel **N25**

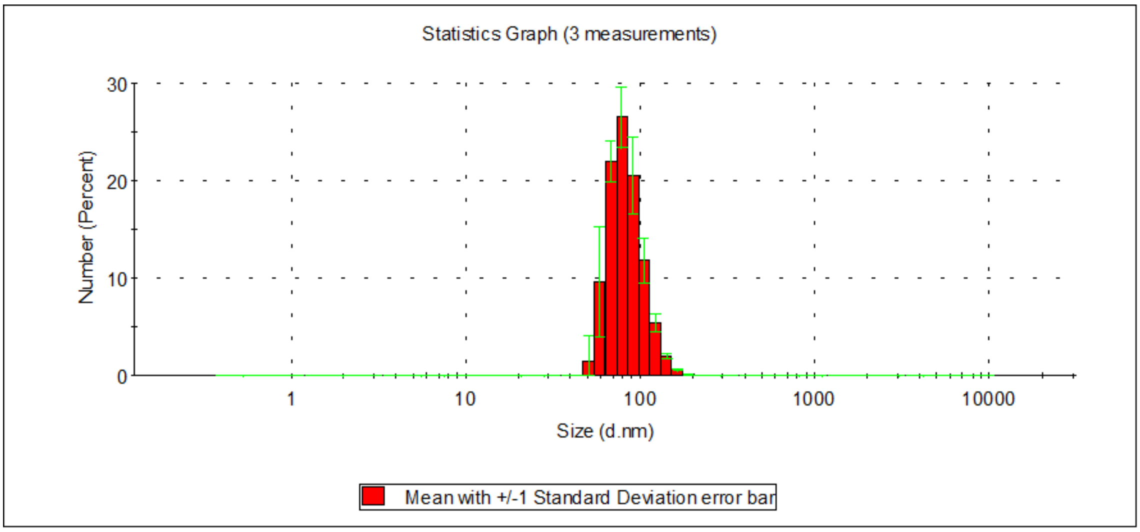

Figure SI-33. Dynamic light scattering of nanogel **N26**

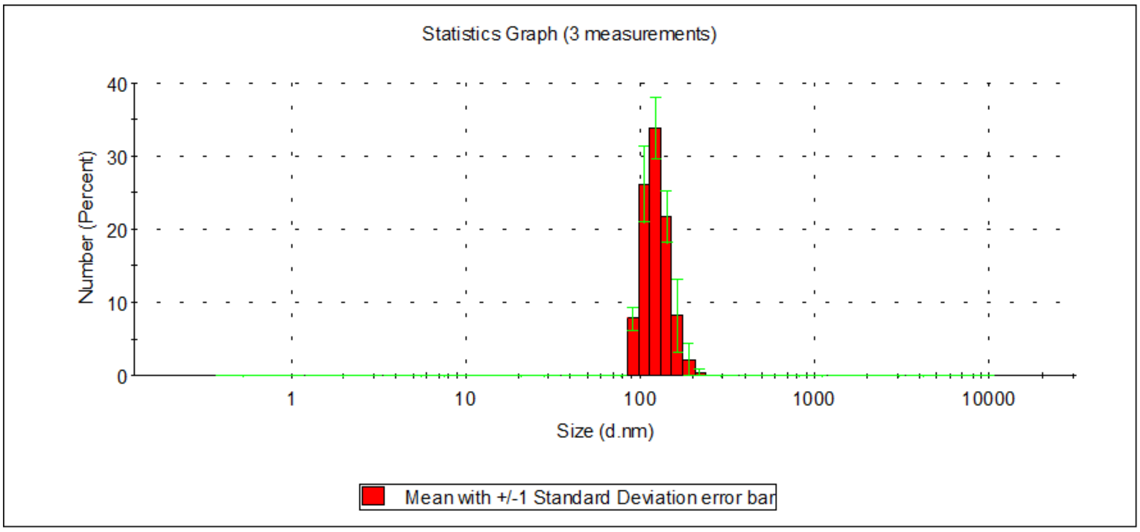

Figure SI-34. Dynamic light scattering of nanogel **N27**

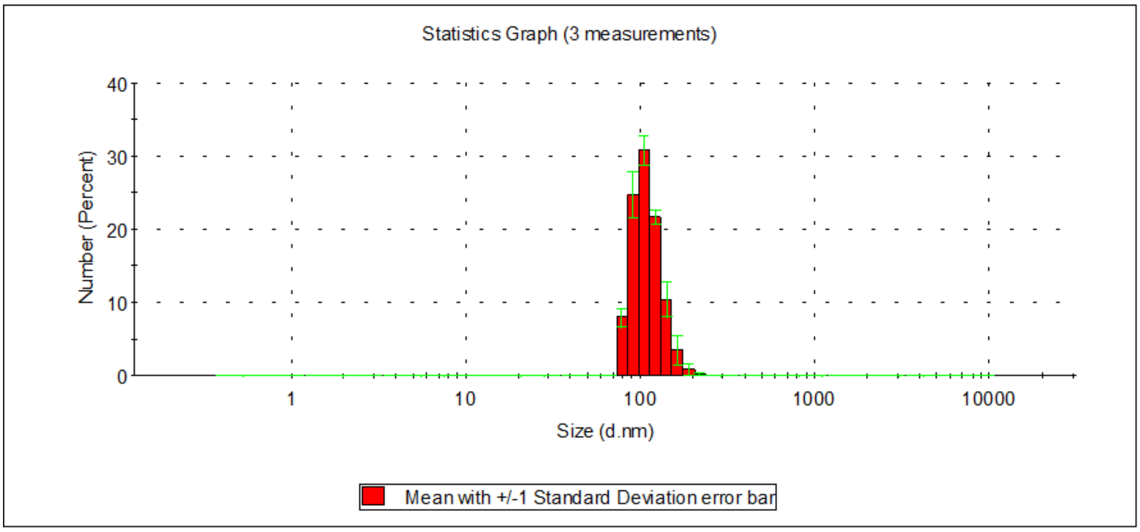

Figure SI-35. Dynamic light scattering of nanogel **N28**

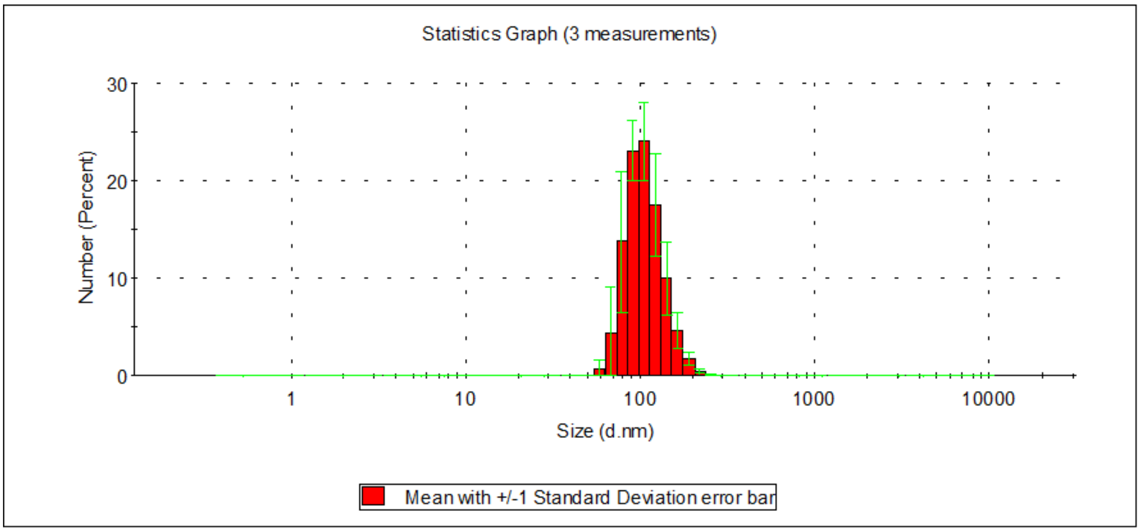

Figure SI-36. Dynamic light scattering of nanogel **N29**

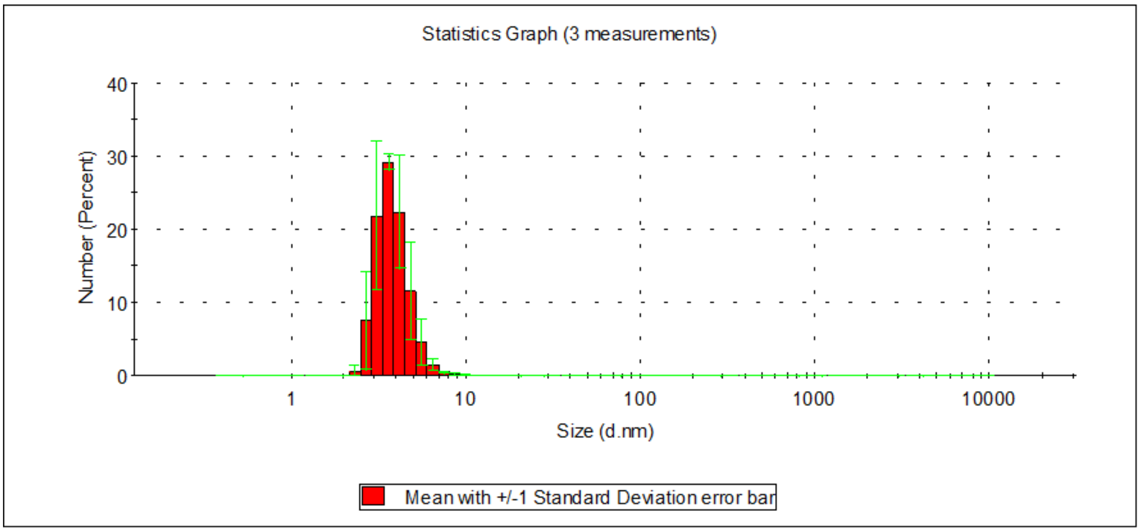

Figure SI-37. Dynamic light scattering of nanogel **N30**

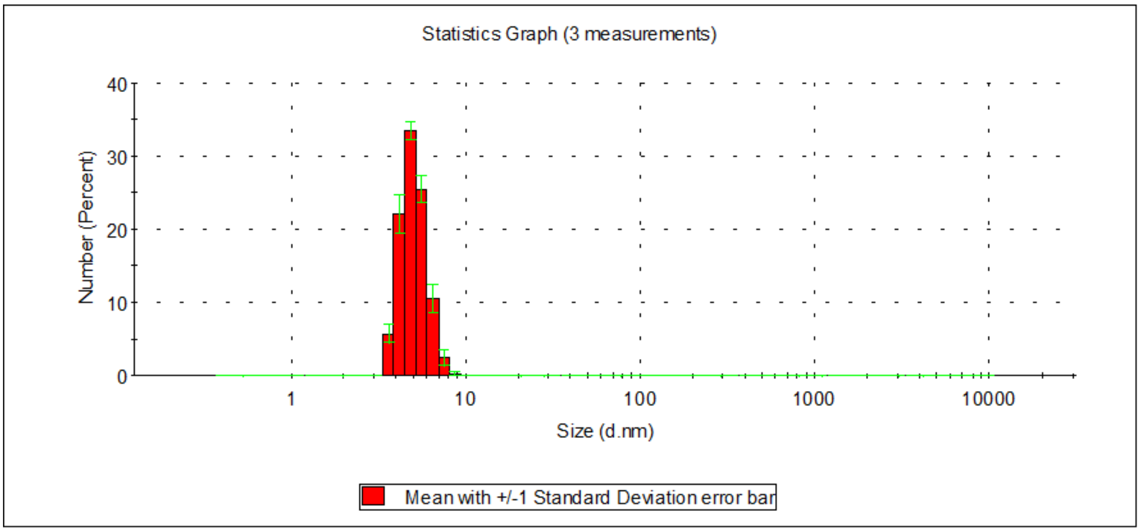

Figure SI-38. Dynamic light scattering of nanogel **N31**

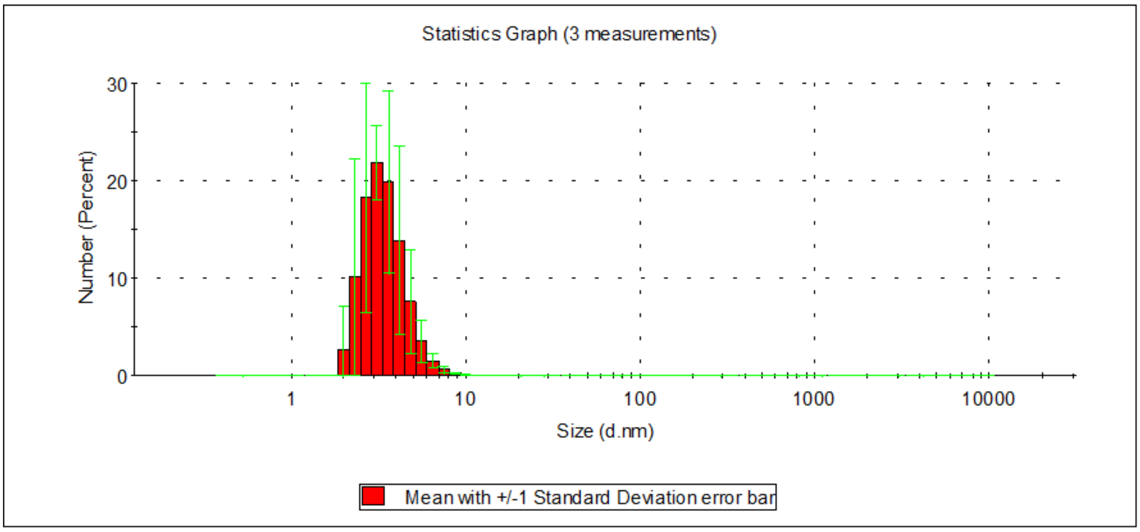

Figure SI-39. Dynamic light scattering of nanogel **N30D**

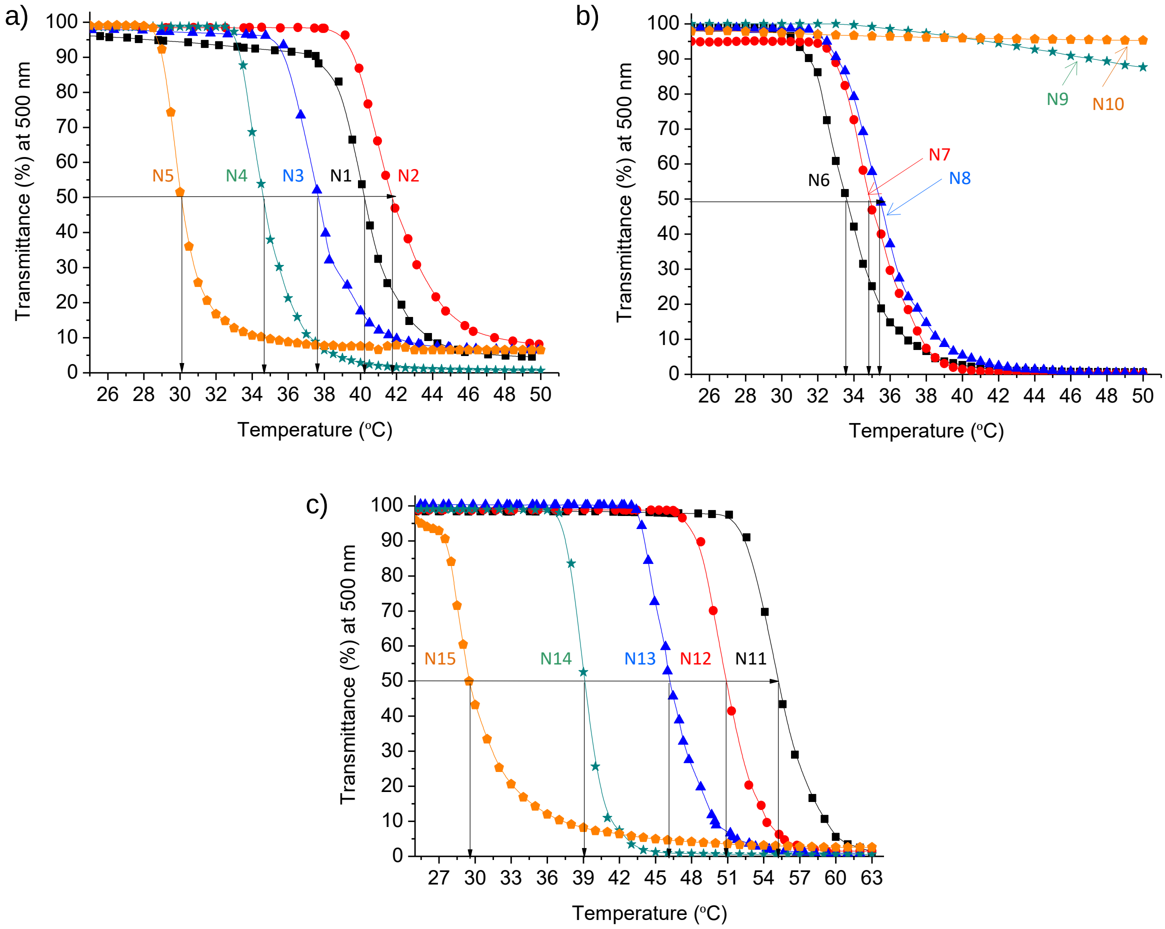

Figure SI-40 Transmittance change with increasing temperature for nanogels based on a) NIPAM (**N1-N5**), b) NPAM (**N6-N10**) and c) NAPr (**N11-N15**), cross-linked with different % mol MBA. Transmittance was measured at 500 nm at a polymer concentration of 1 mgmL^{-1} in deionised water.

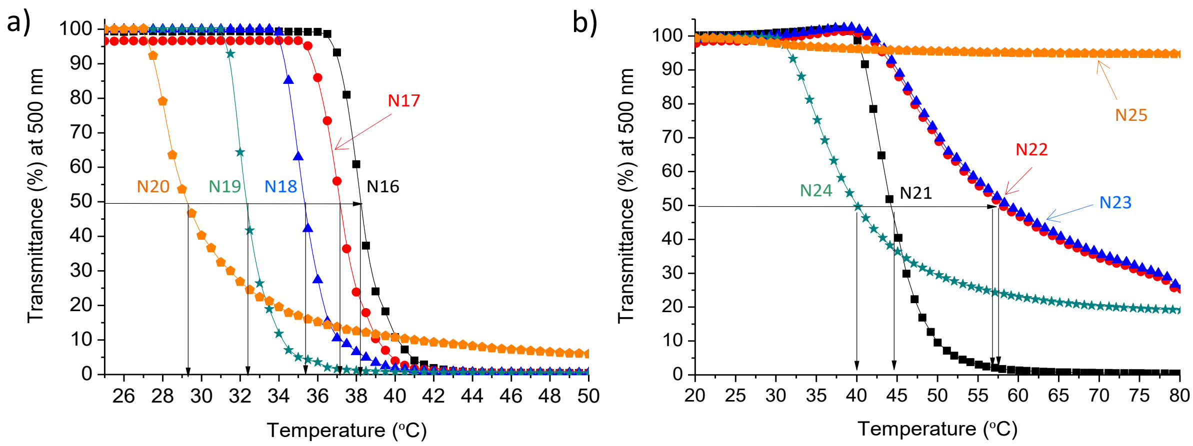

Figure SI-41. Transmittance change with increasing temperature for the nanogels based on a) NIPAM-NAPr (**N16-N20**) and b) NPAM-NAPr (**N21-N25**), cross-linked with different % mol MBA. Transmittance was measured at 500 nm at a polymer concentration of 1 mgmL^{-1} in deionised water.

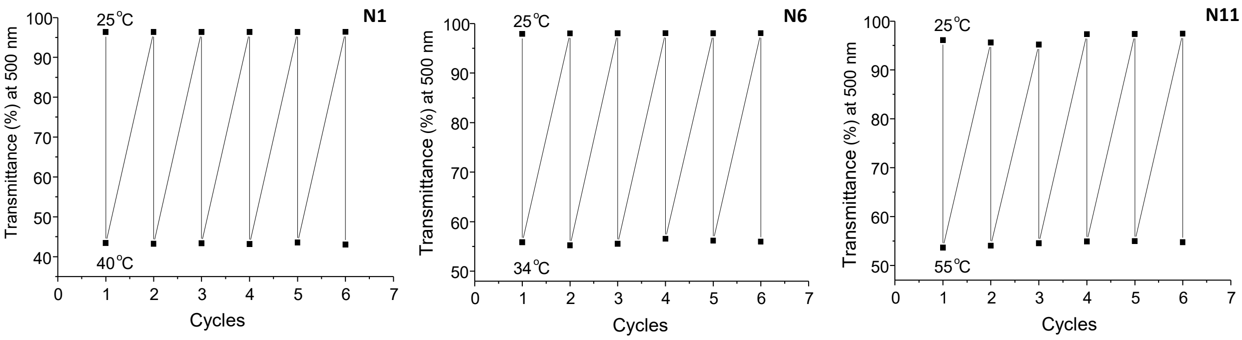

Figure SI-42. Transmittance changes at 500 nm with heating - cooling cycles for nanogels **N1**, **N6** and **N11** (conc =1 mg ml^{-1}). The temperature was cycled between 25 ºC and 40 °C, 34 °C or 55 °C for **N1**, **N6** and **N11**, respectively.

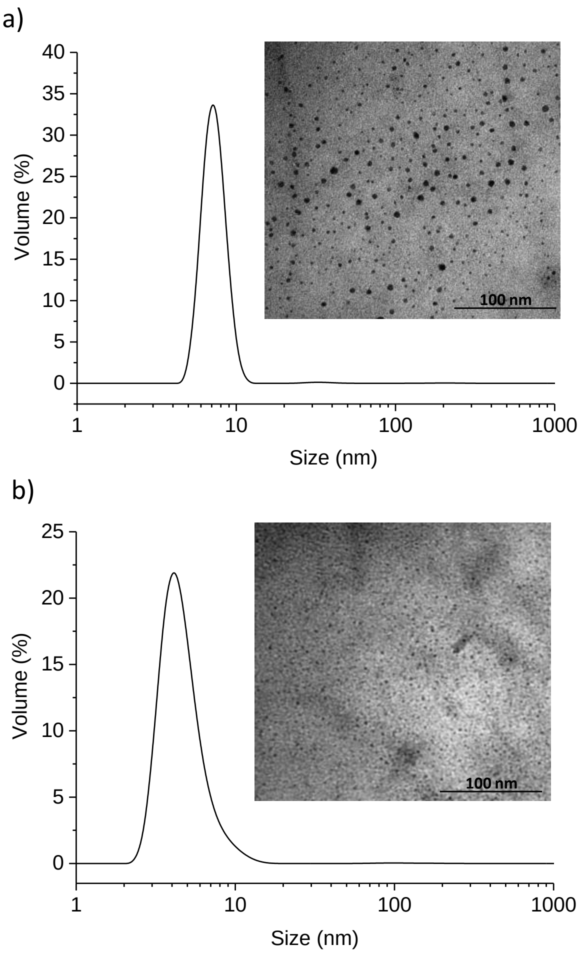

Figure SI-43. Dynamic light scattering measurements of a) nanogel **N6** (NPAM-MBA 90:10 %mol) and b) nanogel **N30** (NPAM-A-Pro-OH-MBA 87.5:2.5:10 %mol). Inset: a) TEM image of **N6**, b) TEM image of **N30**, non-stained and scale bar = 100 nm.

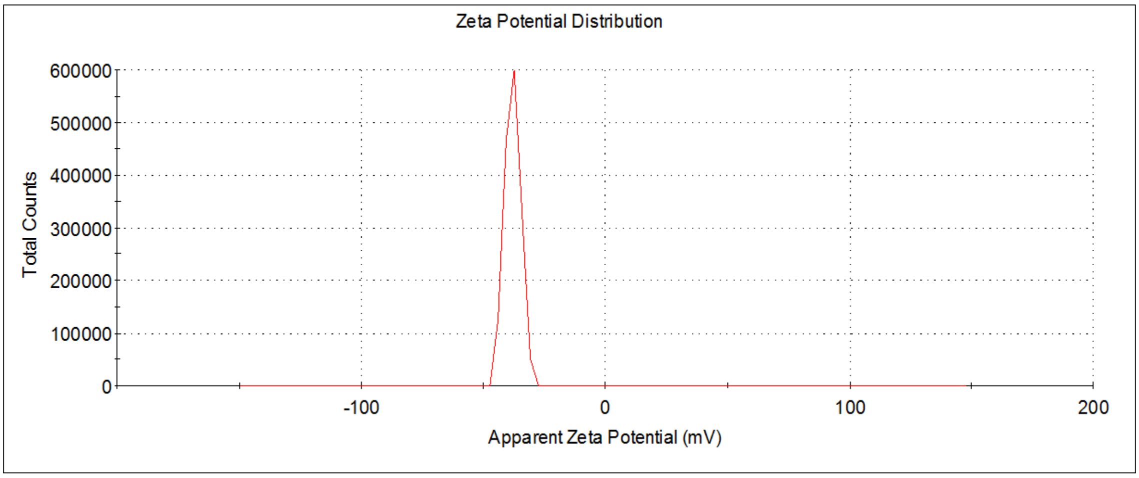

Figure SI-44. Zeta potential measurement of nanogel **N26**

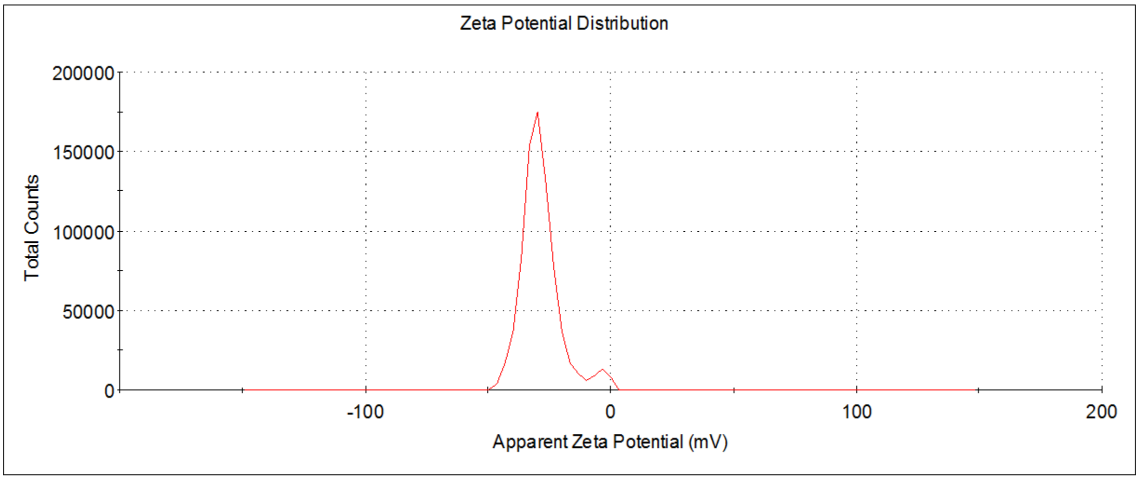

Figure SI-45. Zeta potential measurement of nanogel **N27**

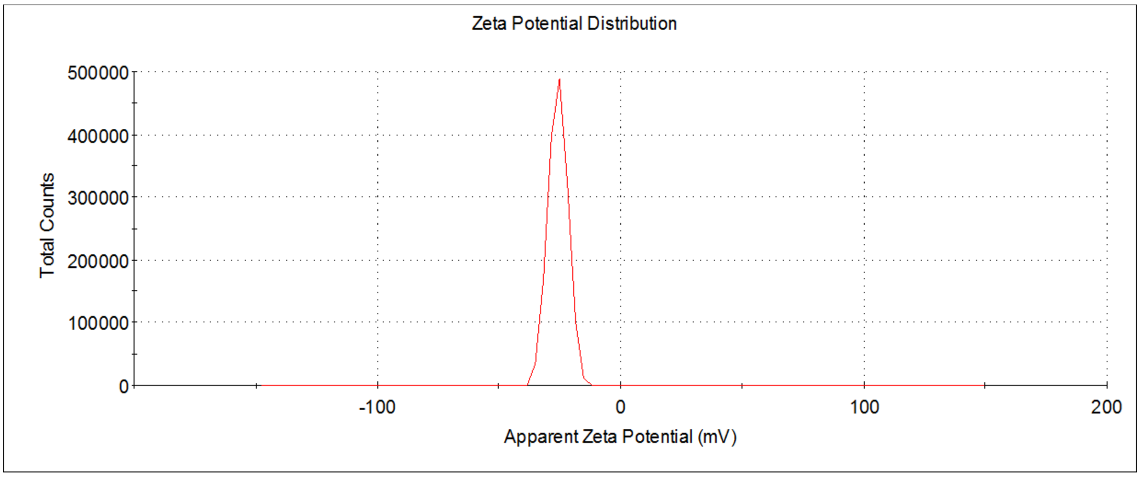

Figure SI-46. Zeta potential measurement of nanogel **N28**

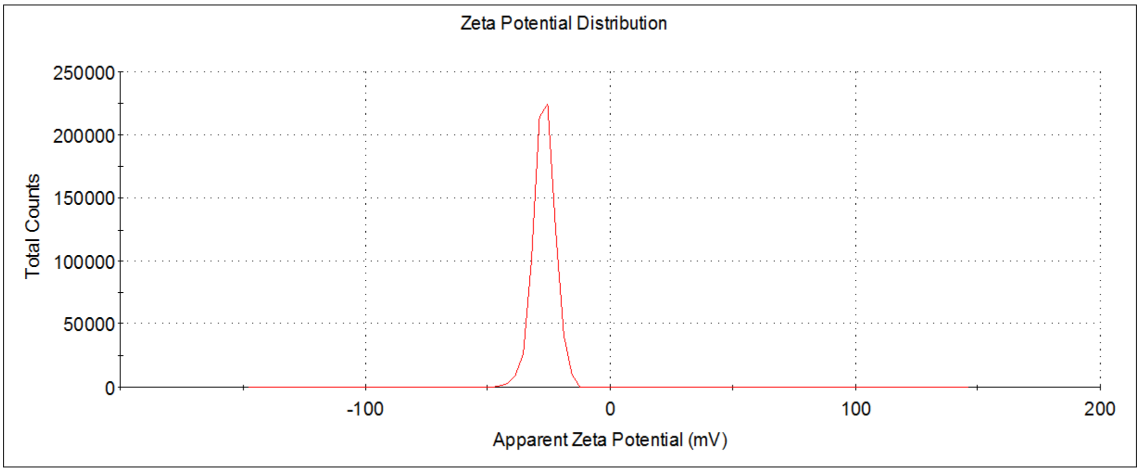

Figure SI-47. Zeta potential measurement of nanogel **N29**

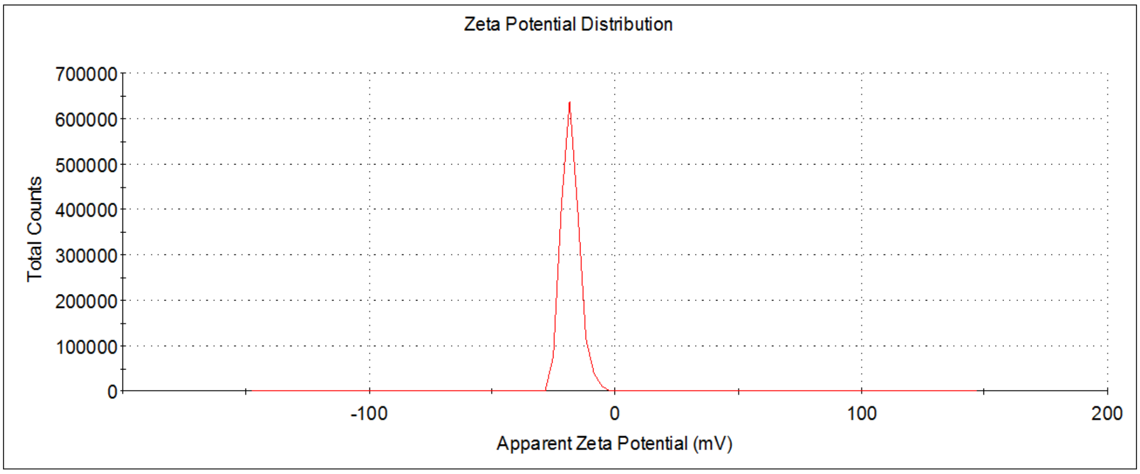

Figure SI-48. Zeta potential measurement of nanogel **N30**

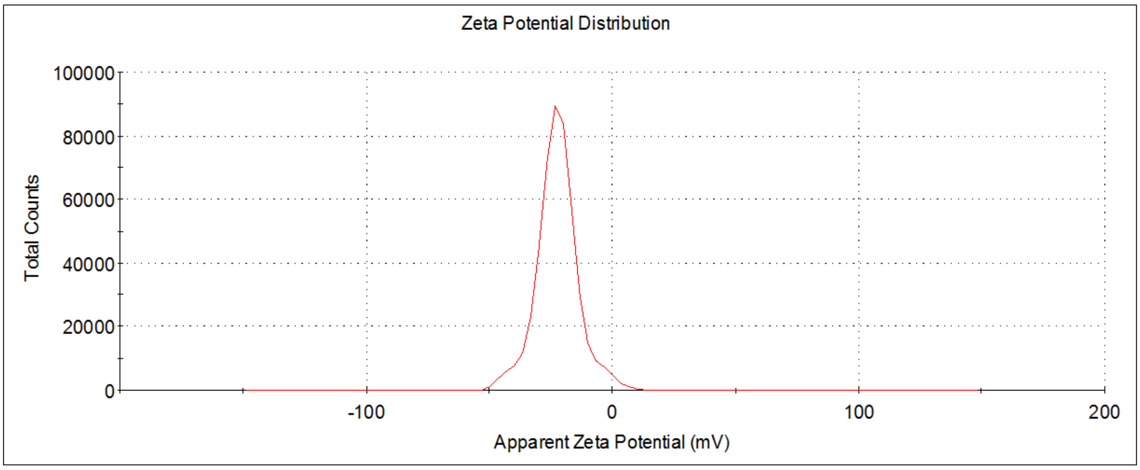

Figure SI-49. Zeta potential measurement of nanogel **N31**

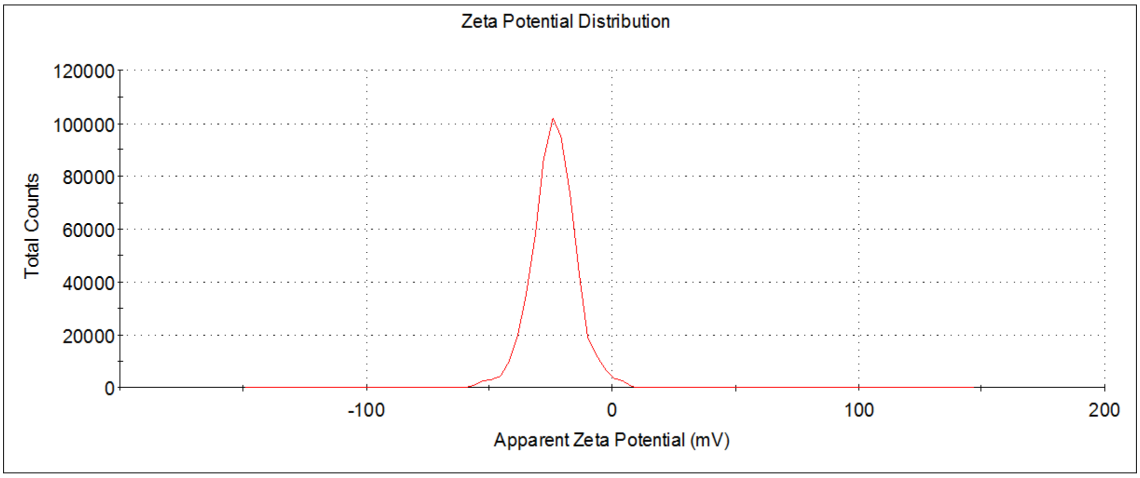

Figure SI-50. Zeta potential measurement of nanogel **N30D**

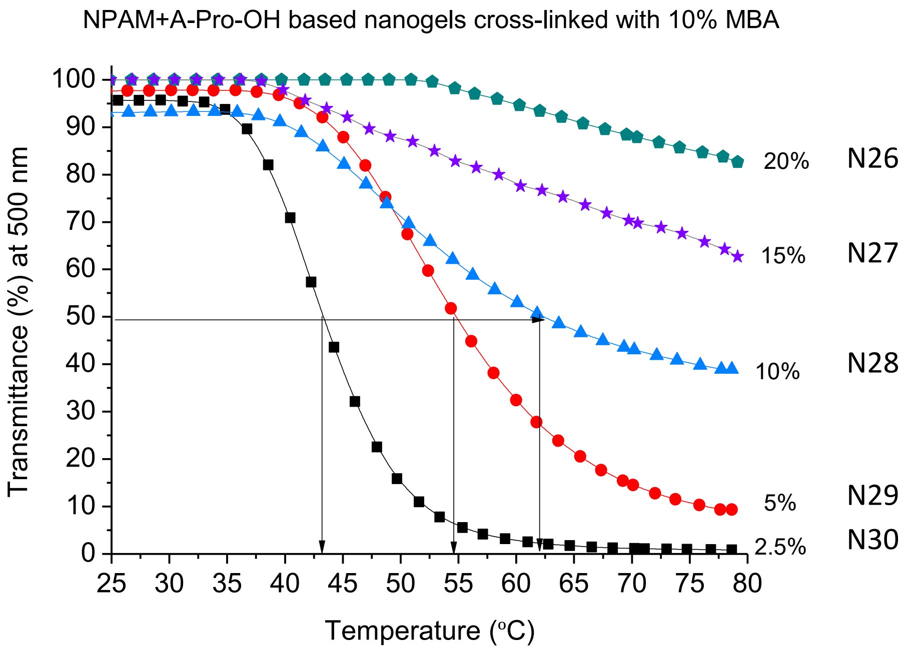

Figure SI-51. Transmittance changes with temperature for nanogels **N26-N30**, based on NPAM-10%MBA and copolymerized with A-Pro-OH from 20 to 2.5%.

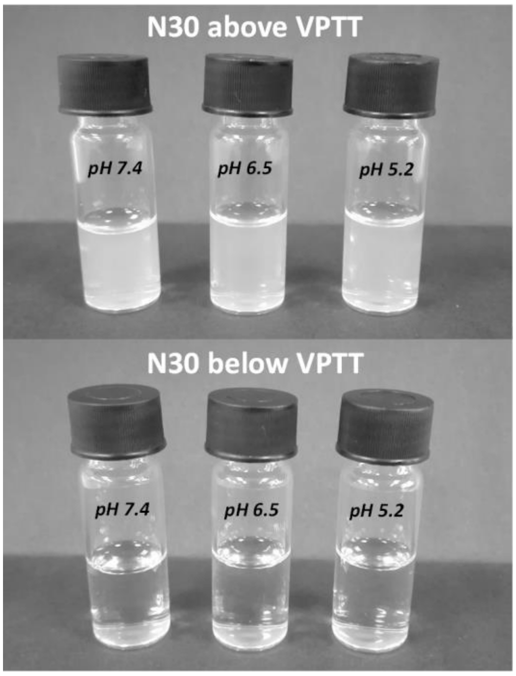

Figure SI-52. Photographs showing the visual change of nanogel **N30** in water (conc =1mgmL^{-1}) with temperature at three different pHs (7.4, 6.5 and 5.2). Bottom photograph shows the solutions at a temperature below the VPPT and the top one shows the solutions at a temperature above the VPTT.

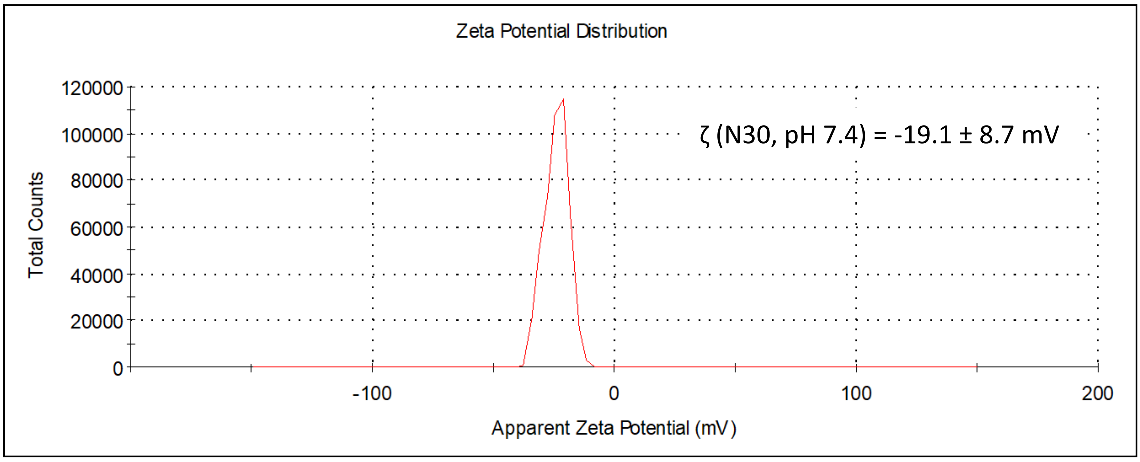

Figure SI-53. Zeta potential measurement of nanogel **N30** at pH 7.4.

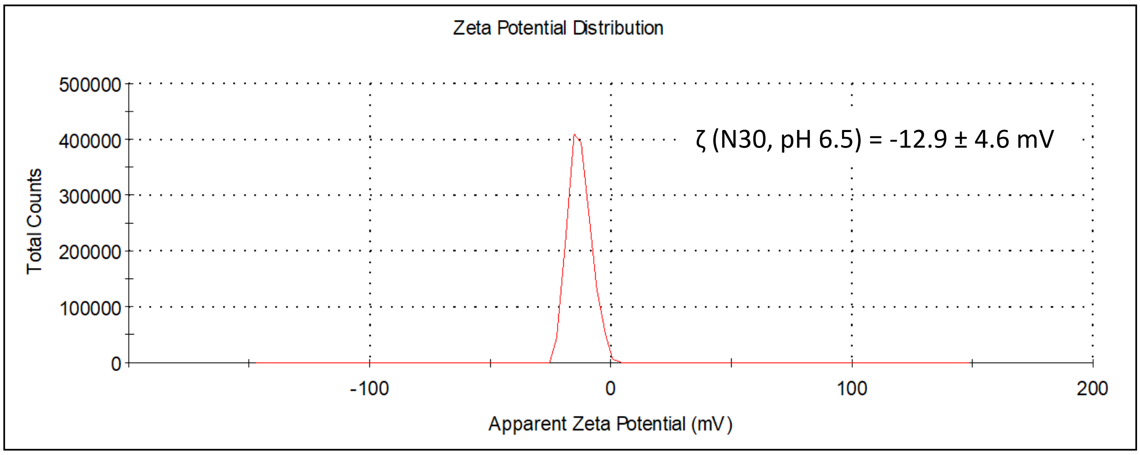

Figure SI-54. Zeta potential measurement of nanogel **N30** at pH 6.5.

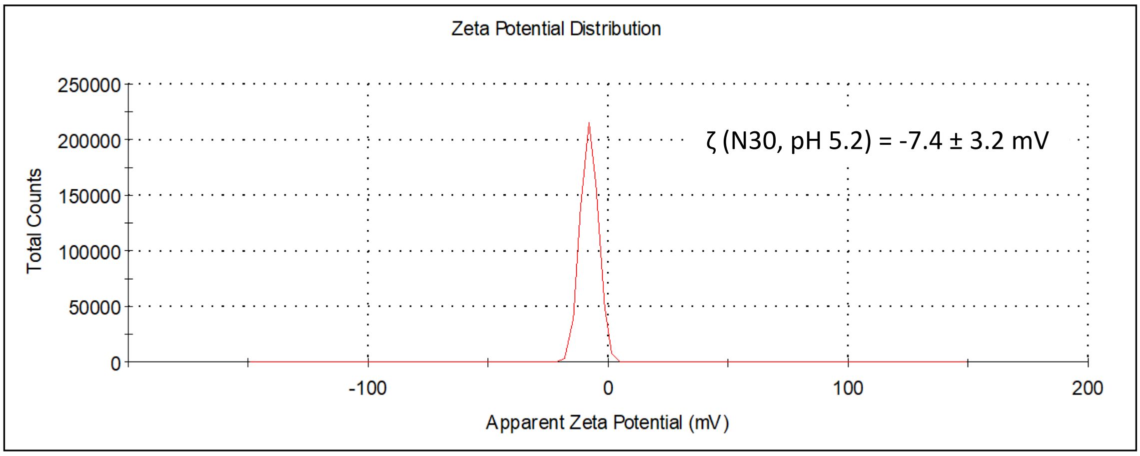

Figure SI-55. Zeta potential measurement of nanogel **N30** at pH 5.2.

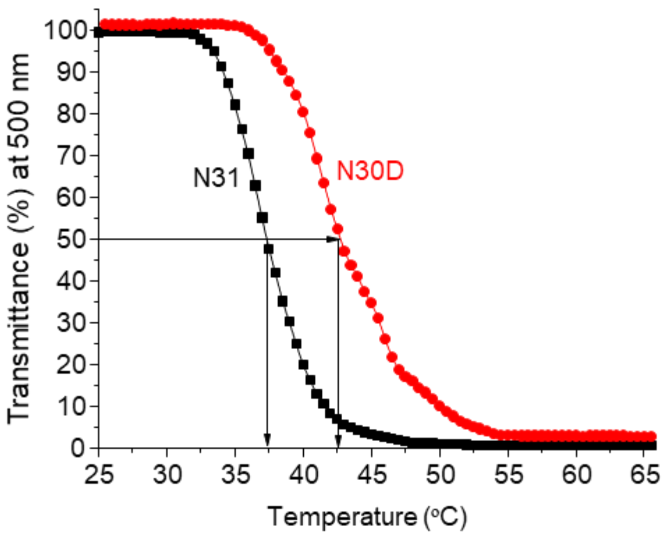

Figure SI-56. Transmittance changes at 500 nm with the temperature for nanogel **N31** (based on monomers NPAM, AAc and NAPr) and **N30D** (based on monomers NPAM, A-Pro-OH) and loaded with NBA.