Supporting Information

"Hydrogels based on methylated-alginates as a platform to investigate the effect of material properties on cell activity. The role of material compliance"

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Figure 1S

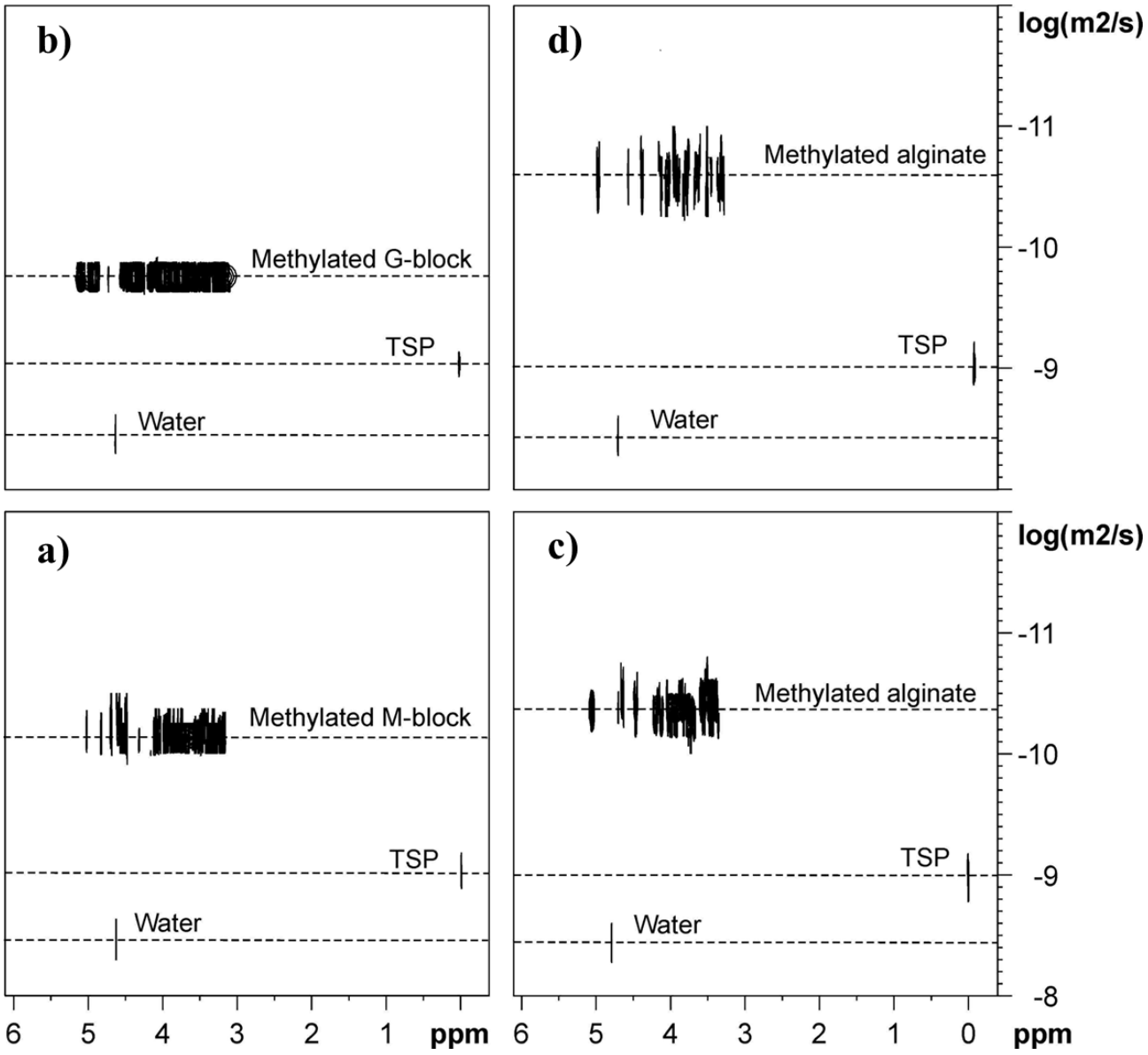

Figure 1S: Methylated alginate characterization by diffusion ordered spectroscopy (DOSY) recorded at 25°C and 800 Mhz. Dotted lines indicate the diffusion of the molecules in the same and the name of the compound is given. Panel **a**) Methylated M-blocks, **b**) methylated G-blocks, **c**) Alg_MS and **d**) Alg_MH.

Figure 2S

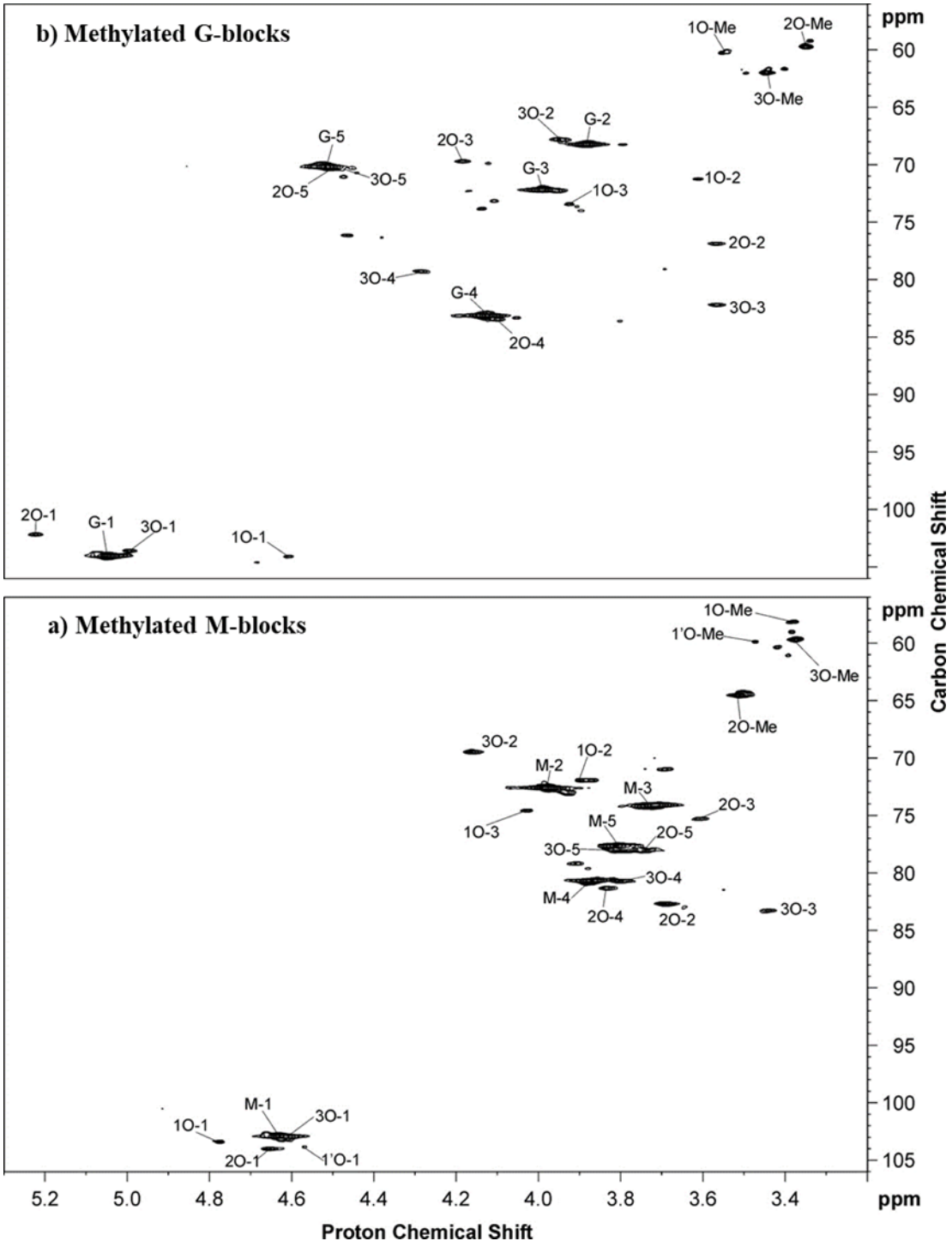

Figure 2S: ^{13}C HSQC spectra of methylated M-blocks (a) and G-blocks (b) recorded at 800 Mhz and 25°C. The resonance assignment of spectra is based on previously published assignments for alginate[1] as well as structural characterization based on 2D NMR spectra. α and β indicate the signals for the reducing end of alginate, G is guluronate, M is mannuronate, # indicate proton/carbon number in alginate sugar ring, underlined

indicate the residue giving rise to the signal, #O-# indicate 1,2,or 3-O residue on the proton/carbon number in alginate sugar ring, #O-Me indicate 2 or 3 O methyl group.

Figure 3S

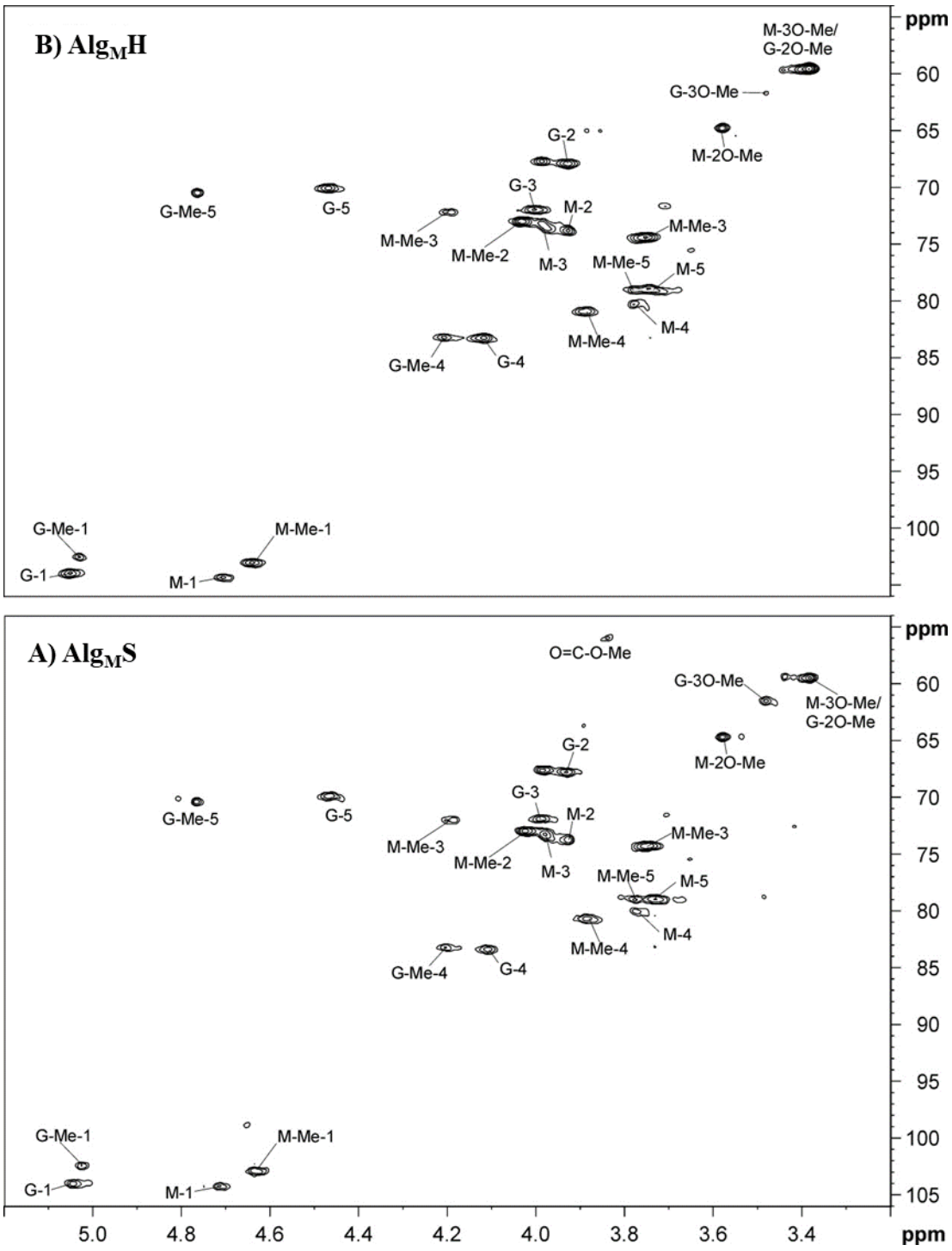

Figure 3S: ^{13}C HSQC spectra of methylated alginate products in D_2O from Alg$_M$S (a) and Alg$_M$H (b) recorded at 800 Mhz and 25°C. The resonance assignment of spectra is based on previously published

assignments for alginate[1] as well as structural characterization based on 2D NMR spectra. α and β indicate the signals for the reducing end of alginate, G is guluronate, M is mannuronate, # indicate proton/carbon number in alginate sugar ring, underlined indicate the residue giving rise to the signal, O=C-O-Me: indicate the methylated (esterified) carboxylate group in alginate, #O-Me indicate 2 or 3 O-methyl group with the proton/carbon number indicating position in the alginate sugar ring.

Figure 4S

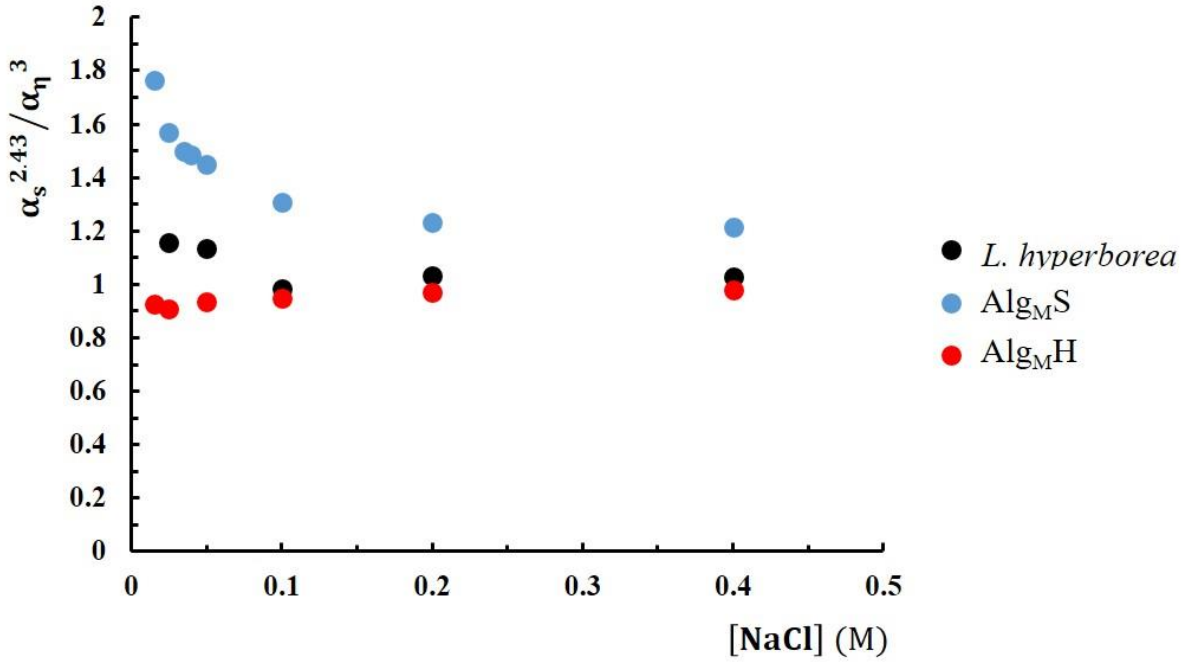

Figure 4S. Dependence of the ratio $\alpha_s^{2.43}/\alpha_\eta^3$ from the concentration of supporting salt for alginate from *L. hyperborea* (black), Alg$_M$S (blue) and Alg$_M$H (red).

Figure 5S

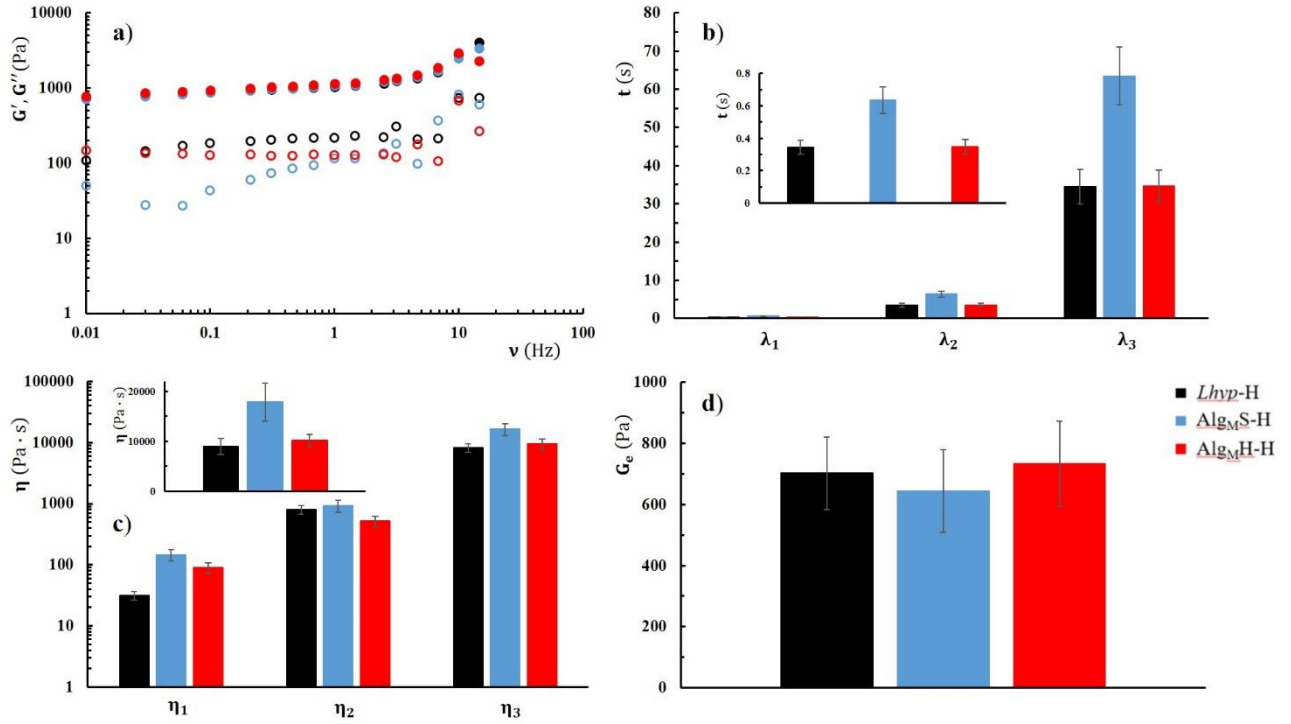

Figure 5S. **a**) Storage (G′, filled symbols) and loss (G′′, open symbols) moduli for *Lhyp*-H (black), for Alg_MS-H (blue) and for Alg_MH-H (red). **b**) Relaxation times of the three Maxwell elements (λ_1, λ_2, and λ_3) calculated from the fitting of the experimental data in a). Inset: magnification of the values of λ_1. Color code as in a). **c**) Viscosity of the three Maxwell elements (η_1, η_2, and η_3) calculated from the fitting of the experimental data in a). Inset: magnification of the values of $\eta = \eta_1 + \eta_2 + \eta_3$. Color code as in a). **d**) Additional spring constant, G_e, calculated from the fitting of the experimental data. Color code as in a).

Figure 6S

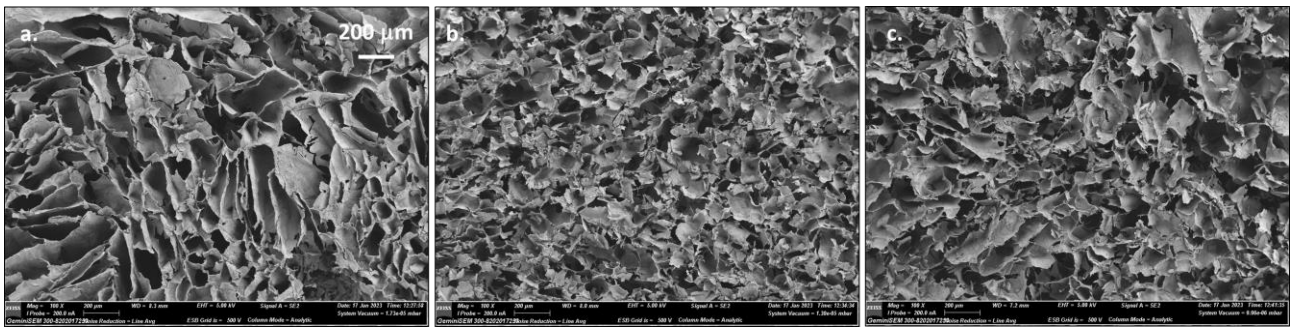

Figure 6S. SEM images of freeze-dried *Lhyp*-H (**a**), Alg_MS-H (**b**) and Alg_MH-H (**c**); (scale bar: 200 µm).

Table 1S: Chemical shift data for A) methylated M-blocks, B) methylated, C) methylated alginate (solution) and D) methylated alginate (gel) in D_2O recorded at 800 Mhz and 25°C. TSP (3-(Trimethylsilyl)propionate-2,2,3,3-d4) is used for chemical shift referance. G is guluronate, M is mannuronate, # indicate proton/carbon number in alginate sugar ring, underlined indicate the residue giving rise to the signal, O=C-O-Me: indicate the methylated (esterified) carboxylate group in alginate, #O-Me indicate 2 or 3 O-methyl group with the proton/carbon number indicating position in the alginate sugar ring.

Position	$\delta_C{}^a$ **[ppm]**	$\delta_H{}^b$ **[ppm]**	$\delta_C{}^a$ **[ppm]**	$\delta_H{}^b$ **[ppm]**	$\delta_C{}^a$ **[ppm]**	$\delta_H{}^b$ **[ppm]**	$\delta_C{}^a$ **[ppm]**	$\delta_H{}^b$ **[ppm]**	$\delta_C{}^a$ **[ppm]**	$\delta_H{}^b$ **[ppm]**
					Methylated M-blocks					
	M		**1-O-Me**		**1'-O-Me**		**2-O-Me**		**3-O-Me**	
1	4.63	103.1	4.78	103.4	4.57	99.9	4.65	103.7	4.62	103.1
2	3.99	72.6	3.90	71.9	4.78		3.69	82.4	4.16	69.5
3	3.72	74.2	4.03	74.6			3.61	75.3	3.44	83.3
4	3.77	80.4					3.84	81.3	3.80	80.7
5	3.81	77.6					3.75	78.1	3.80	77.9
6	-	176.8					-	177.2	-	177.0
1-O-Me			3.38	58.1	3.48	59.8				
2-O-Me							3.51	64.6		
3-O-Me									3.38	59.7
					Methylated G-blocks					
	G		**1-O-Me**		**-**		**2-O-Me**		**3-O-Me**	
1	5.04	104.1	4.61	104.1			5.23	102.2	4.99	103.6
2	3.88	68.3	3.61	74.2			3.57	76.9	3.95	67.8
3	3.99	72.2	3.93	73.4			4.19	69.7	3.57	82.2
4	4.12	83.1					4.10	83.4	4.29	79.3
5	4.52	70.2					4.53	69.9	4.45	70.3
6	-	178.0					-	177.4	-	177.5
1-O-Me			3.55	60.3						
2-O-Me							3.35	59.7		
3-O-Me									3.45	62.0
					Alg_MS					
	MM		**MG**		**GG**		**GM**		**O=C-O-Me**	
1	4.64	102.9	4.70	104.2	5.07	103.4	5.06	102.4		
2	4.03	72.9	3.97	73.3	3.90	68.0	3.98	67.6		
3	3.75	74.3	3.93	73.7	4.03	72.0	4.16	72.2		
4	3.88	80.9	3.77	80.2	4.14	82.6	4.23	82.7		
5	3.76	78.8	3.71	79.0	4.45	70.1	4.72	70.4		
6	-	178.4	-	177.8	-	178.4		178.6	-	173.6
	Methylation on M				**Methylation on G**					
2-O-Me	3.58	64.7	3.58	64.7	3.38	59.6	3.38	59.6		
3-O-Me	3.38	59.6	3.38	59.6	3.48	61.6	3.48	61.6		
O=C-O-Me									3.84	56.0

	Alg_MH								
	M		**M-Me**		**G**		**G-Me**		**-**
1	4.70	104.2	4.64	102.9	5.07	103.4	5.06	102.4	
2	3.97	73.3	4.03	72.9	3.90	68.0	3.98	67.6	
3	3.93	73.7	3.75	74.3	4.03	72.0	4.16	72.2	
4	3.77	80.2	3.88	80.9	4.14	82.6	4.23	82.7	
5	3.71	79.0	3.76	78.8	4.45	70.1	4.72	70.4	
6	-	177.8		178.4	-	178.4		178.6	
	Methylation on M				**Methylation on G**				
2-O-Me	3.58	64.7	3.58	64.7	3.38	59.6	3.38	59.6	
3-O-Me	3.38	59.6	3.38	59.6	3.48	61.6	3.48	61.6	

[a] ^{13}C at 200 MHz. [b] ^{1}H at 800 MHz. Chemical shifts determined from 2D experiments acquired at 25°C.

Table 2S. Intrinsic viscosity of alginate from *L. hyperborea*, Alg_MS and Alg_MG. Table reports also the results obtained for alginate from *L. hyperborea* treated as for the methylation in homogeneous (Alg_MS – No DMS) or heterogeneous (Alg_MG – No DMS) condition but without DMS.

Sample	$[\eta]_{0.1}$ (dL/g) (± s.e.)
L. hyperborea	6.13 ± 0.02
Alg_MS	3.90 ± 0.01
Alg_MS – No DMS	6.12 ± 0.01
Alg_MG	5.70 ± 0.02
Alg_MG – No DMS	5.18 ± 0.01

Table 3S. Dependence of the electrostatic persistence length (q_{el}) from the ionic strength for alginate from *L. hyperborea* used in the present study. q_{el} was calculated according to eq. 8.

NaCl (M)	q_{el} (nm)
0.015	1.91
0.025	1.20
0.035	0.88
0.04	0.77
0.05	0.62
0.1	0.32
0.2	0.16
0.4	0.08

Table 4S. Total expansion factor, α_s, and squared electrostatic expansion factor, $\alpha_{s,el}$, for the three alginate samples considered in the present study.

NaCl (M)	***Lhyp***		**Alg_MS**		**Alg_MH**	
	$\alpha_{s,el}^2$	α_s	$\alpha_{s,el}^2$	α_s	$\alpha_{s,el}^2$	α_s
0.015	1.240	1.321	1.288	1.463	1.087	1.14
0.025	1.208	1.287	1.253	1.360	1.072	1.105
0.035	1.187	1.249	1.229	1.306	1.063	1.087
0.04	1.179	1.233	1.220	1.286	1.059	1.080
0.05	1.165	1.208	1.204	1.257	1.054	1.071
0.1	1.126	1.148	1.158	1.184	1.040	1.048
0.2	1.094	1.105	1.119	1.132	1.029	1.033
0.4	1.069	1.074	1.088	1.094	1-.021	1.023

Table 5S. Composition of hydrogels from unmodified and methylated alginate samples.

Sample	**Polysaccharide**	**[$CaSO_4$]** (mM)
Lhyp-H	Alginate from *L. hyperborea*	16.5
Alg_MS-H	Alg_MS	40
Alg_MH-H	Alg_MH	25