Structural Phases in Ca^{2+}-Triggered Alginate Assembly and Gelation: Circular Dichroism-Guided Multimodal Analysis

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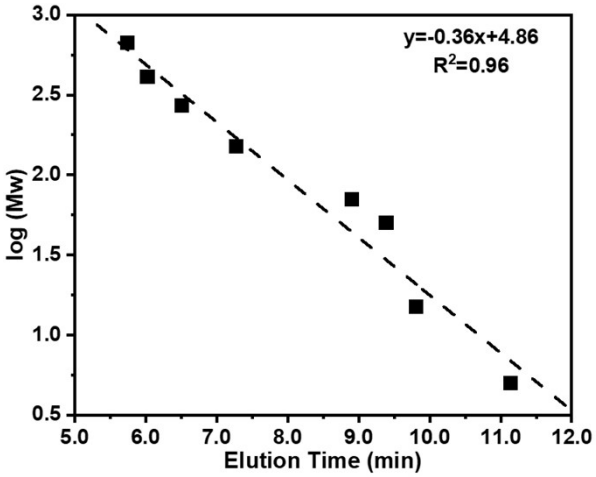
Table S1. Transition points of Ca^{2+} (mM) as a function of SA concentrations based on CD measurements.

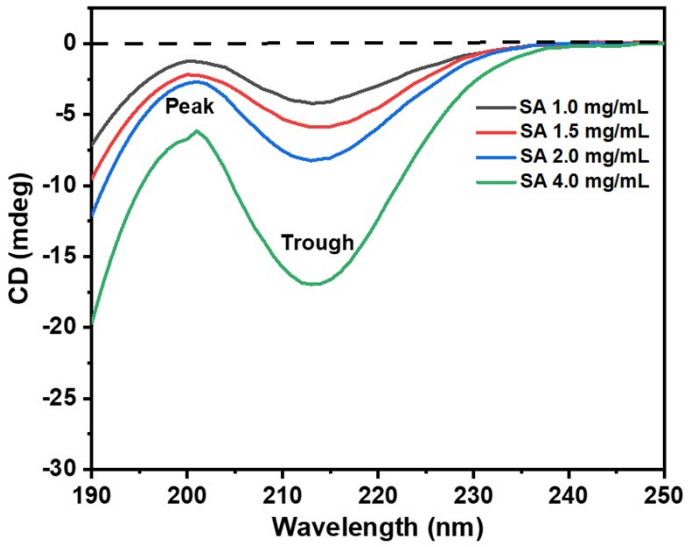
[SA] (mg/mL)	Position		Intensity		Average [Ca^{2+}] (mM)
	[Ca^{2+}] (mM)				
	Peak	Trough	Peak	Trough	
1.0	1.99	2.22	1.57	2.03	1.95 ± 0.27
1.2	1.82	2.35	1.38	1.52	1.77 ± 0.43
1.4	2.59	1.84	0.82	1.10	1.59 ± 0.79
1.6	2.40	1.88	1.86	2.25	2.10 ± 0.27
1.8	3.19	2.38	1.94	2.70	2.55 ± 0.53
2.0	3.15	2.48	1.96	2.75	2.59 ± 0.50
4.0	4.28	4.62	3.76	4.65	4.33 ± 0.41
6.0	*NC	5.30	4.69	5.21	5.07 ± 0.33

*NC: not included in the calculation



Standard	$\overline{Mw}$ (kDa)	y = log $\overline{Mw}$	x = Elution time (min)
Dex 5	5	0.699	11.14
Dex 15	15	1.176	9.81
Dex 50	50	1.699	9.39
Dex 70	70	1.845	8.91
Dex 150	150	2.176	7.28
Dex 270	270	2.431	6.51
Dex 410	410	2.613	6.03
Dex 670	670	2.826	5.75

Fig. S1. Calibration curve constructed using dextran standards at molecular weights ($\bar{M}w$) of 5, 15, 50, 70, 150, 270, 410, and 670 kDa, showing the relationship between elution time (min) and log $\bar{M}w$ as determined by HPLC.



SA concentration (mg/mL)	Trough$_{\lambda 213}$ (mdeg)	Peak$_{\lambda 200}$ (mdeg)	Peak/Trough
1.0	4.21	3.00	0.714
1.5	5.89	3.73	0.633
2.0	8.25	5.49	0.665
4.0	16.97	10.75	0.633
		Average	0.661

Fig. S2. CD spectra of SA at concentrations of 1.0, 1.5, 2.0, and 4.0 mg/mL, showing the changes in ellipticity intensity (mdeg) as a function of SA concentration.

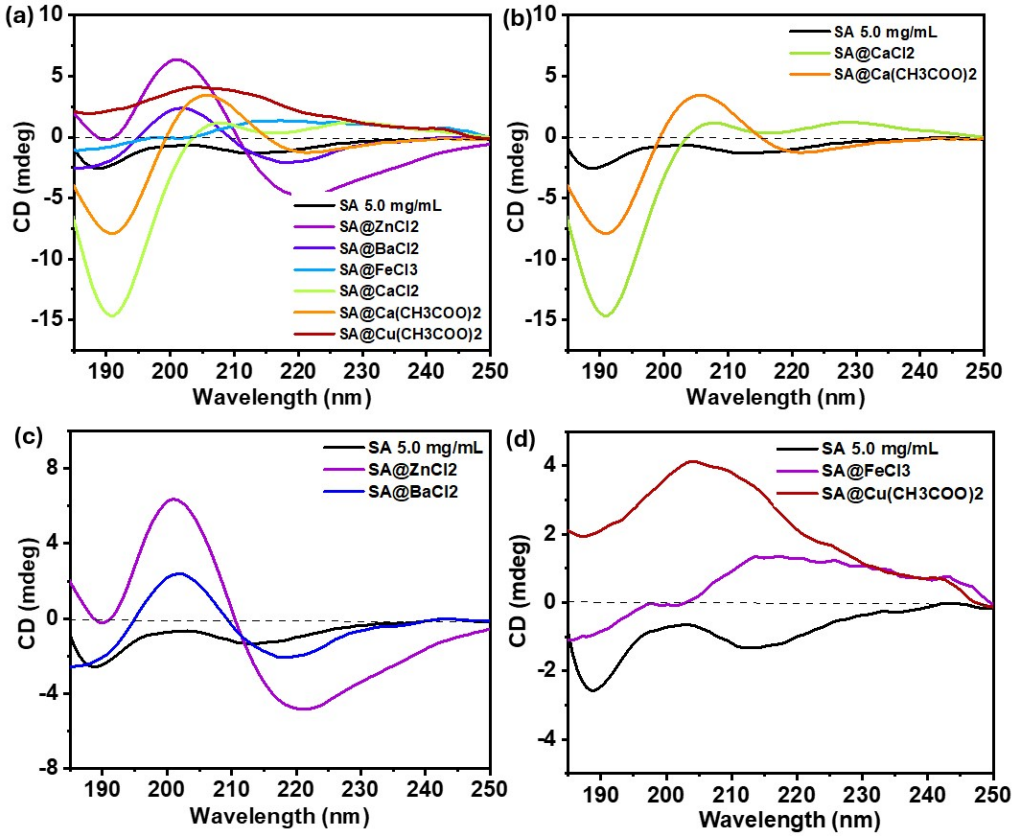


Fig. S3. CD spectra of ion-cross-linked hydrogels of SA using VUVCD at HiSOR, BL12, Japan. (a) [SA] = 5.0 mg/mL, [ion] = 50 mM; (b) SA-Ca^{2+} hydrogels; (c) SA-Ba^{2+} and SA-Zn^{2+} hydrogels; (d) SA-Cu^{2+} and SA-Fe^{3+} hydrogels.

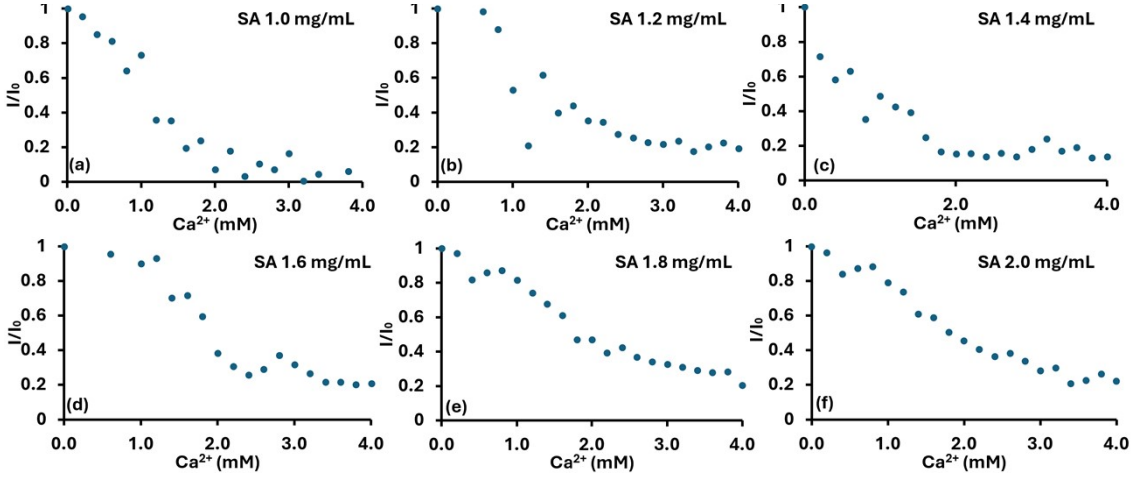


Fig. S4. Plots showing the $\frac{I}{I_0}$ ratios of the ellipticity (trough intensities) as a function of Ca^{2+} concentration (0–4.0 mM) for SA solutions in the 1.0–2.0 mg/mL range. Here, I_0 represents the ellipticity in the absence of Ca^{2+}, and I represents the ellipticity at each Ca^{2+} concentration.

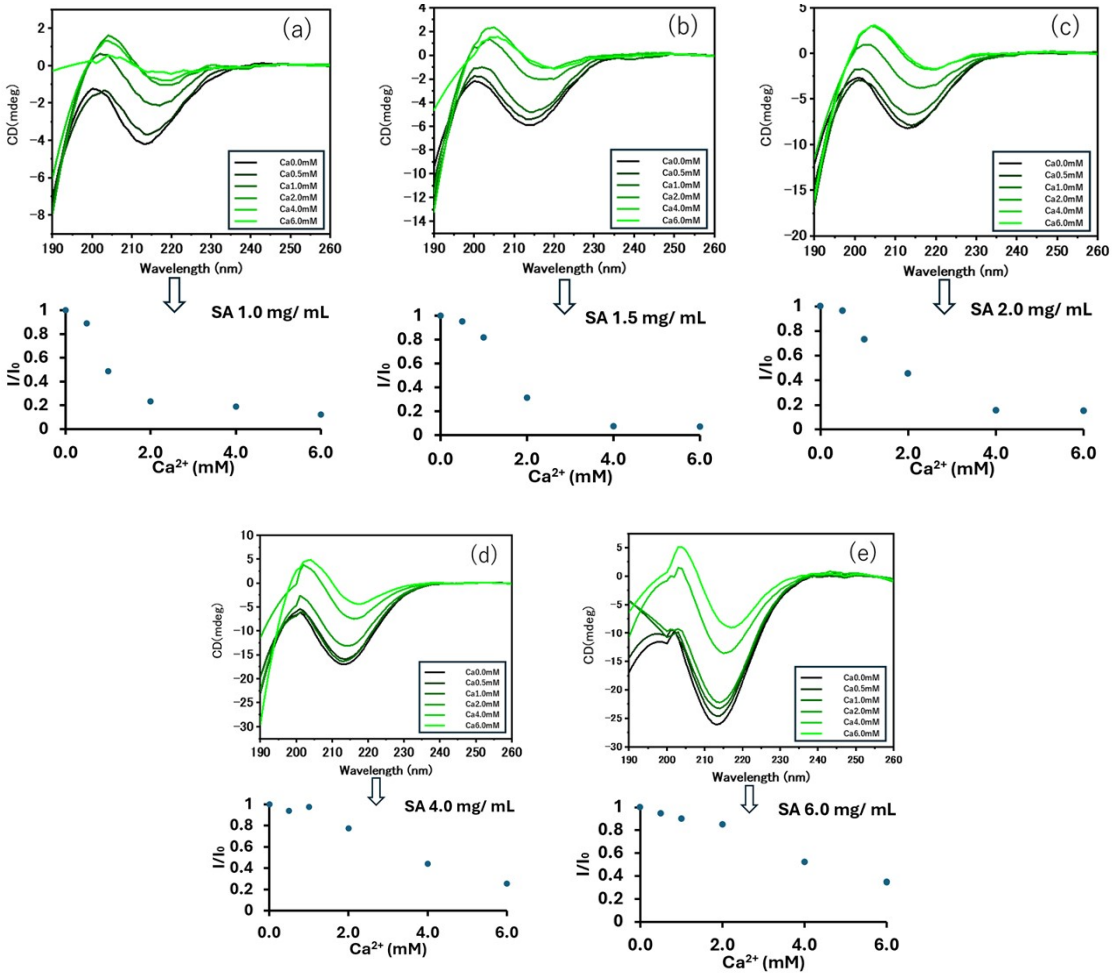


Fig. S5. CD spectra of SA-Ca^{2+} samples in the non-gel phase and the corresponding plots of the $\frac{I}{I_0}$ ratios of the ellipticity (trough intensities) as a function of Ca^{2+} concentration (0–6.0 mM), measured using a conventional CD spectropolarimeter for SA concentrations of (a) 1.0 mg/mL, (b) 1.5 mg/mL, (c) 2.0 mg/mL, (d) 4.0 mg/mL, and (e) 6.0 mg/mL. Here, I_0 represents the ellipticity in the absence of Ca^{2+}, and I represents the ellipticity at each Ca^{2+} concentration.

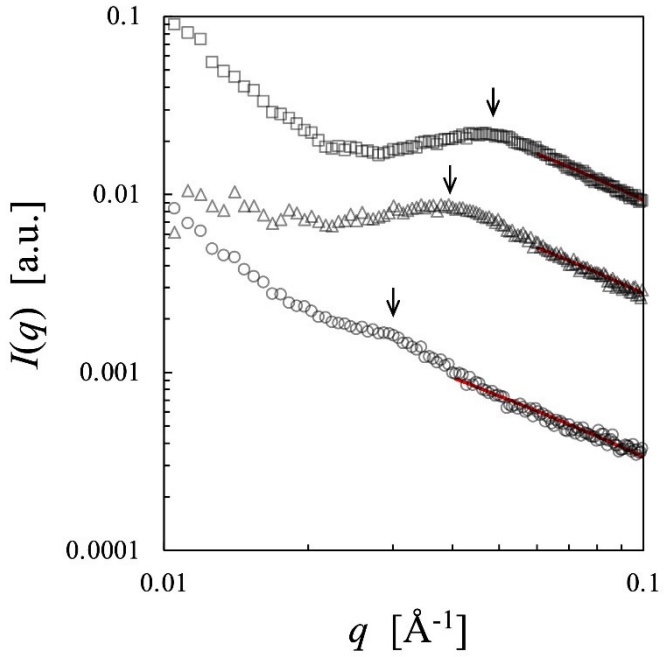
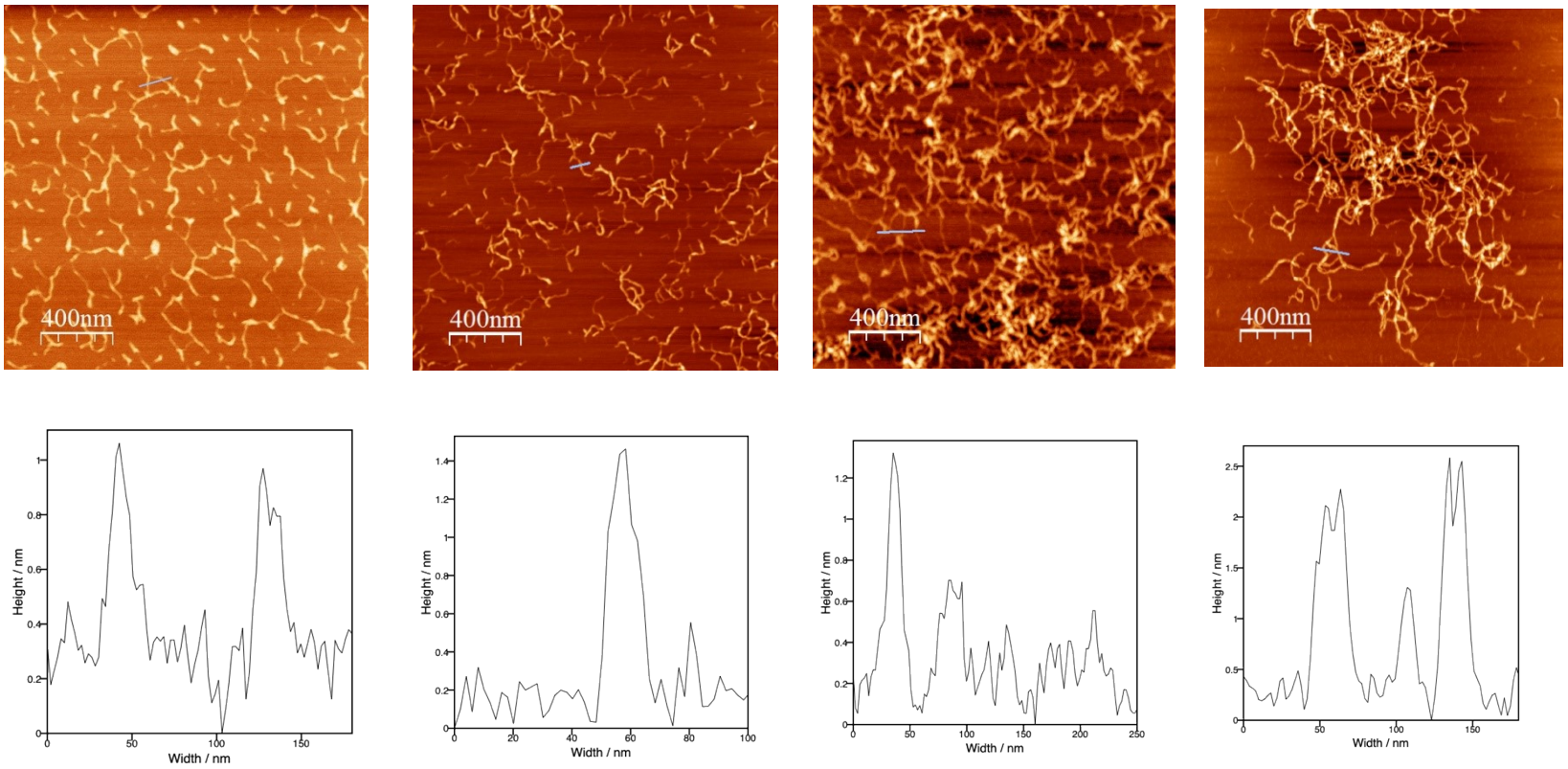
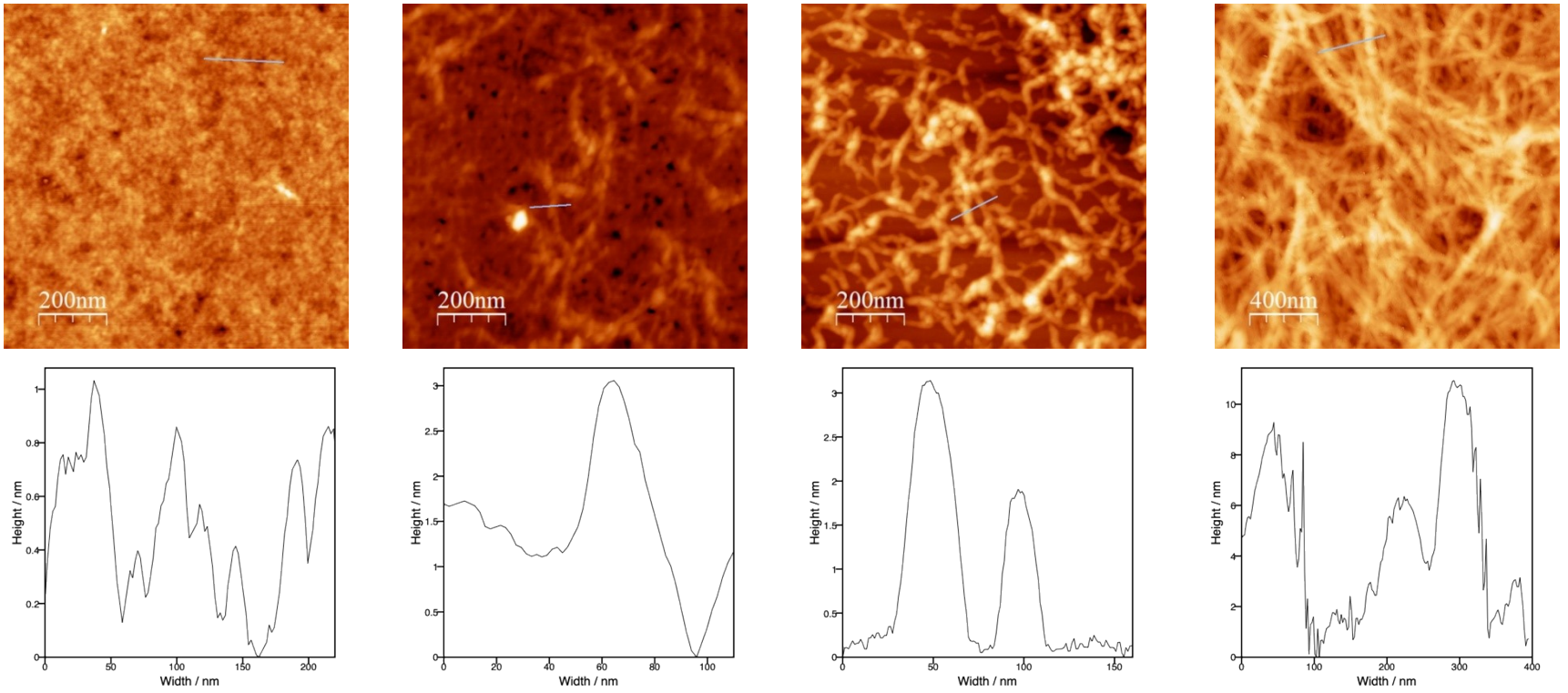


Fig. S6. Scattering profiles of SA solutions with no added salt. The concentrations of SA are 2.0 mg/mL (circles), 4.0 mg/mL (triangles), and 6.0 mg/mL (squares), respectively. The curves are vertically shifted for better visualization. The peak positions are indicated by arrows. The solid curves are calculated with **Equation 3**.



(a) SA1.0 mg/mL and Ca^{2+} 0 mM (b) SA1.0 / Ca^{2+} 1.5 mM (c) SA1.0 / Ca^{2+} 1.7 mM (d) SA1.0 / Ca^{2+} 2.5 mM



(e) SA2.0 / Ca^{2+} 0.0 mM (f) SA2.0 / Ca^{2+} 2.0 mM (g) SA2.0 / Ca^{2+} 4.0 mM (h) SA 6.0 / Ca^{2+} 50 mM

Fig. S7. Height profiles correspond to the AFM images of SA at 1.0 mg/mL and 2.0 mg/mL under varying Ca^{2+} concentrations. Panels (a, e) represent the initial state at 0 mM Ca^{2+}, (b, c, f) correspond to the nucleation phase, (d, g) show the intermediate phase, and (h) represents the gel state.