Supporting Information for: Primary, Secondary, Tertiary and Quaternary Structure Levels in Linear Polysaccharides - from Random Coil, to Single Helix to Supramolecular Assembly

Michael Diener,[†] Jozef Adamcik,[†] Antoni Sánchez-Ferrer,[†] Florian Jädig,[†] Larissa Schefer,[†] and Raffaele Mezzenga[*,†,‡]

[†]*Department of Health Sciences and Technology, ETH Zrich, 8092 Zrich, Switzerland*

[‡]*Department of Materials, ETH Zrich, 8093 Zrich*

E-mail: raffaele.mezzenga@hest.ehtz.ch

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Supporting Material

Gellan gum (Phytagel^{TM}, lot no. SLBX8796) was purchased from Sigma Aldrich.

Supporting Techniques

Fourier-Transform Infrared Spectroscopy (FTIR)

Solid-FTIR-attenauated total reflectance experiment were conducted on Varian 640 FTIR Spectrometer, equipped with an MKII golden gate single attenuated total reflectance system, at normal atmosphere and room temperature by collecting 64 scans from 4000 to 600 cm^{-1} and with a resolution of 4 cm^{-1}. Background measurements were done before each sample and subtracted automatically.

Optical rotatory dispersion (ORD)

Dilution series ranging from 0.0005 to 0.25 wt-% were prepared and measured to determine the polymer concentration after purification by linear regression of the ORD values at 550 nm. Values were obtained from 600 - 400 nm at 100 nm/s, with a data pitch of 0.2 nm, with standard sensitivity, a digital integration time of 1 s, a bandwidth of 5 nm and averaging two accumulations on a J-815-150S CD spectrometer with an ORDE-402/15 accessory (Jasco Inc.).

Flame-Atomic Absorption Spectroscopy (Flame-AAS)

An air/acetylene flame in a 240FS AA fast sequential atomic absorption spectrometer (Agilent technologies) was used at 589.0 nm for Na^+, 766.5 nm for K^+ and 422.7 nm for Ca^{2+} to determine the ionic compositions of the carrageenan samples. KNO_3 was used as matrix modifier for the determination of Na^+ and Ca^{2+} whereas $CsNO_3$ was used for K^+.

Supporting Results

Table S1. Ionic composition of various κ-, ι- and λ-carrageenan solutions as determined by Flame-Atomic Absorption Spectroscopy.

	Na^+ [wt-%]	K^+ [wt-%]	Ca^{2+} [wt-%]
Commercial κ-carrageenan	0.63	2.16	1.03
Commercial ι-carrageenan	1.30	1.10	3.34
Commercial λ-carrageenan	3.90	0.91	0.21
Purified κ-carrageenan	$<$ detection	6.49	$<$ detection
Purified ι-carrageenan	$<$ detection	8.96	$<$ detection

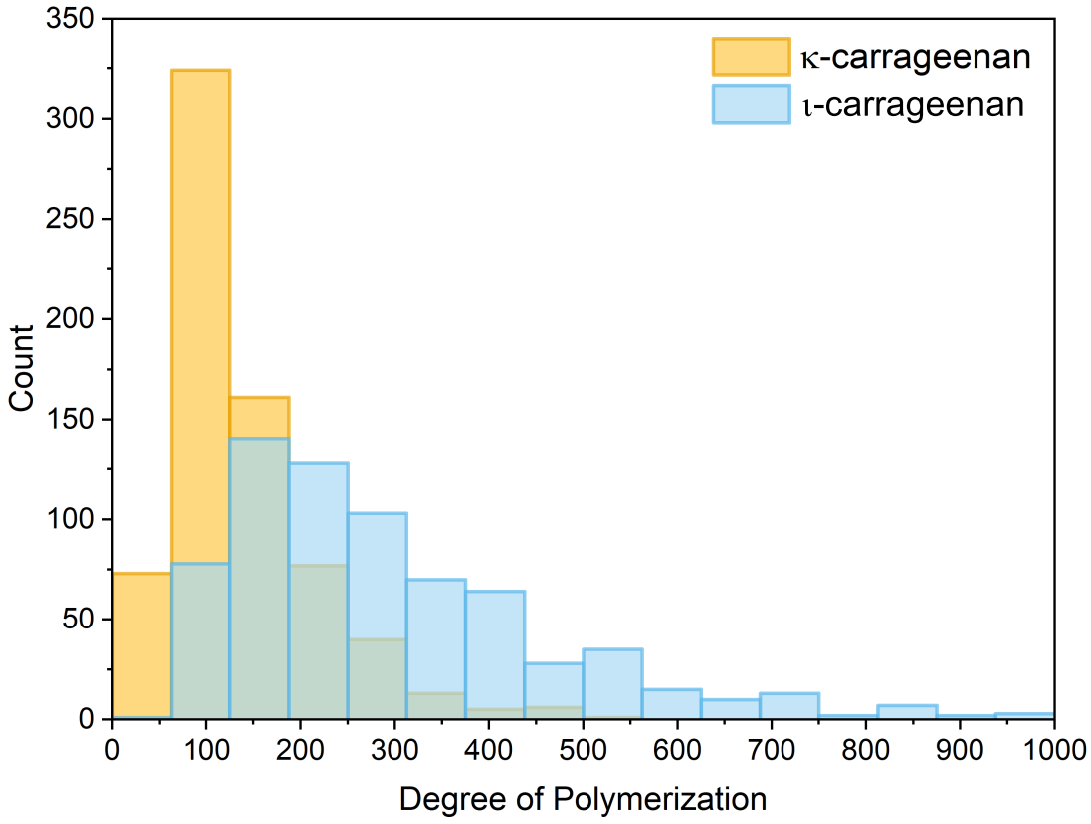

Figure S1. Degree of polymerization histograms of 700 traced polymer chains of κ- and ι-carrageenan used to calculate the weight average molecular weight.

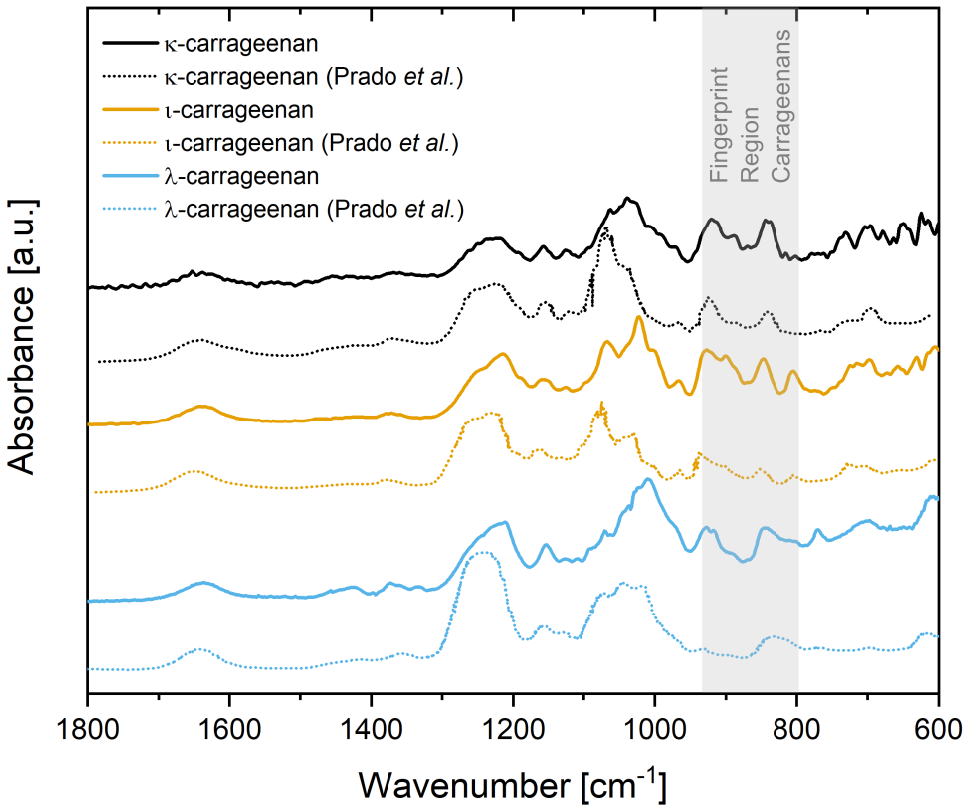

Figure S2. Solid state ATR-FTIR spectra of commercial κ-, ι- and λ-carrageenans (solid lines) with corresponding curves from literature (dotted lines).[1]

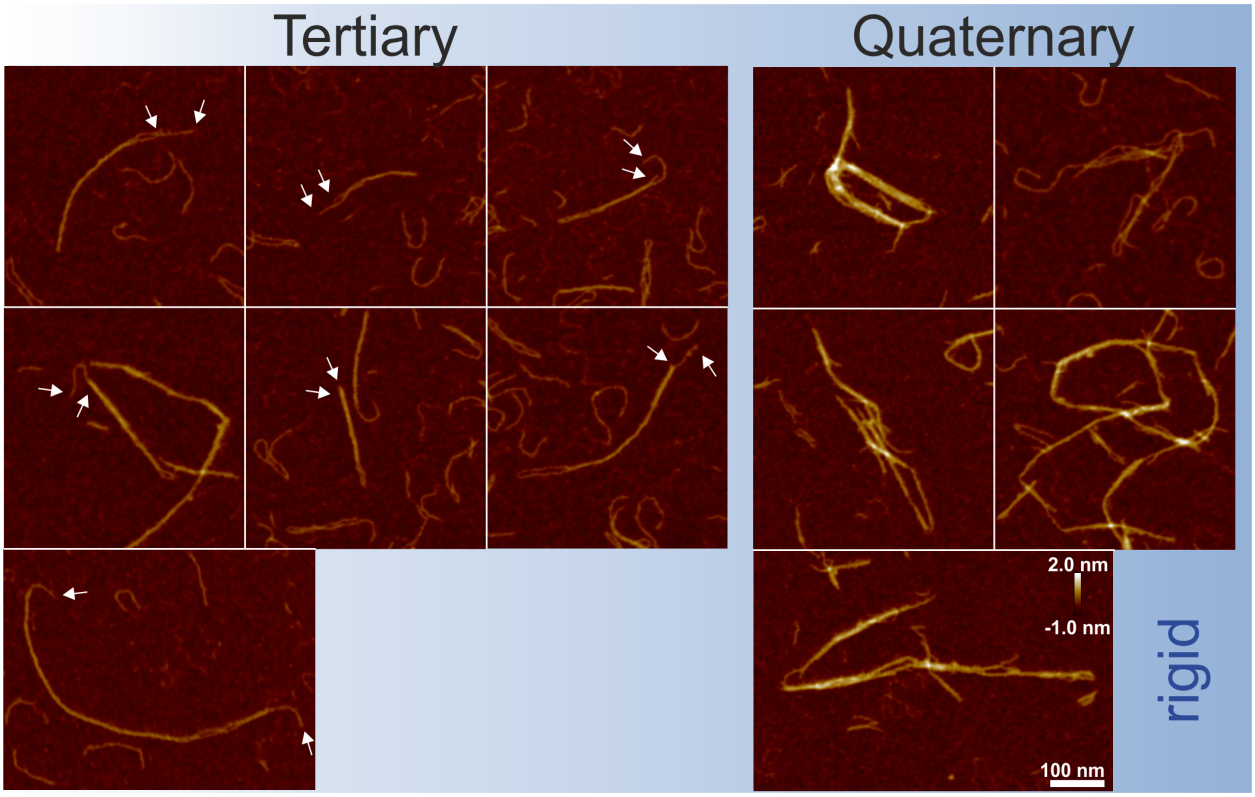

Figure S3. AFM height images of rigid tertiary and quaternary structures. White arrows indicate endings of the polymer chain. Scale bar and height applies to all images.

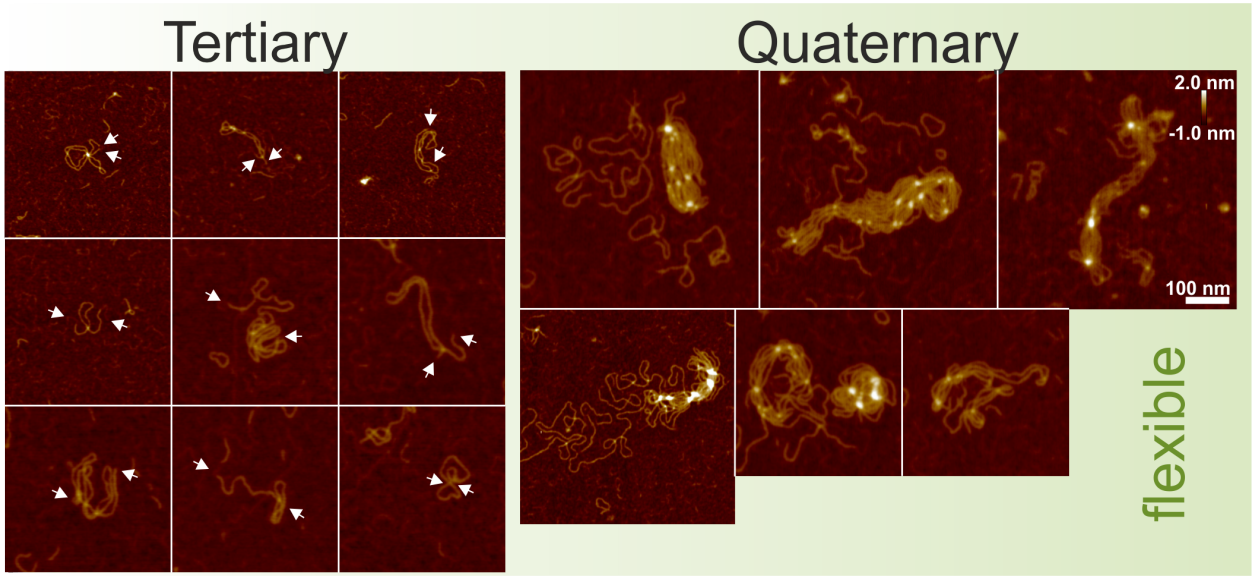

Figure S4. AFM height images of flexible tertiary and quaternary structures. White arrows indicate endings of the polymer chain. Scale bar and height applies to all images.

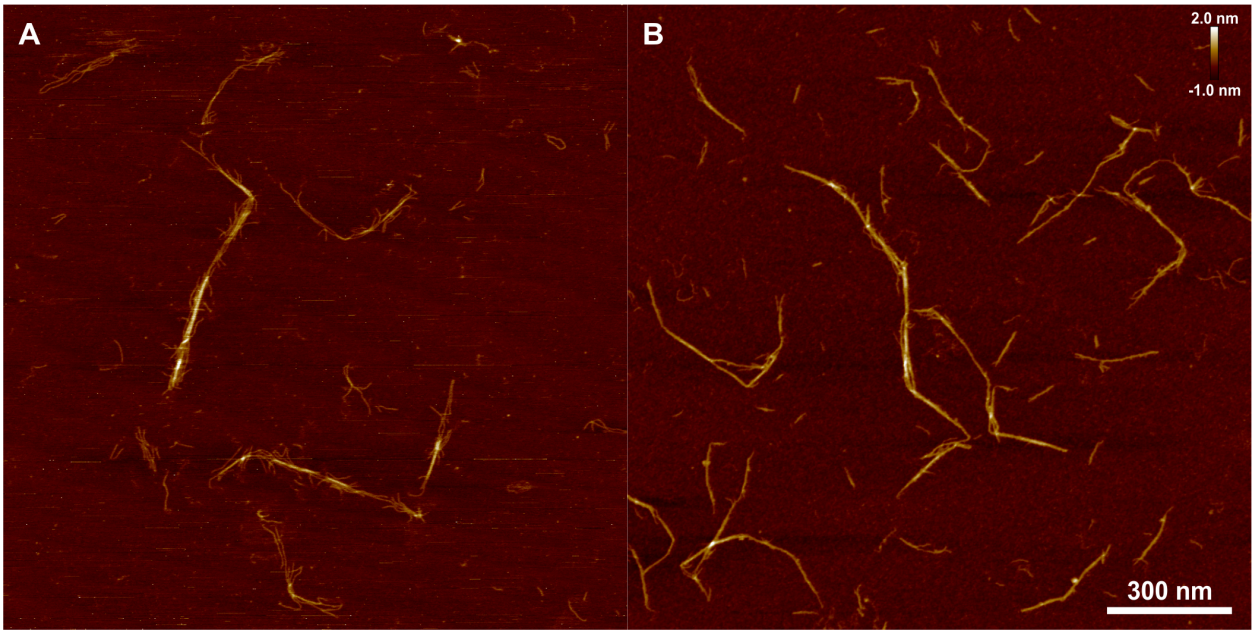

Figure S5. Representative AFM height images for purified κ-carrageenan in the K-form at 0.0005 wt-% imaged in (A) liquid and (B) dry state. Scale bars apply to both images.

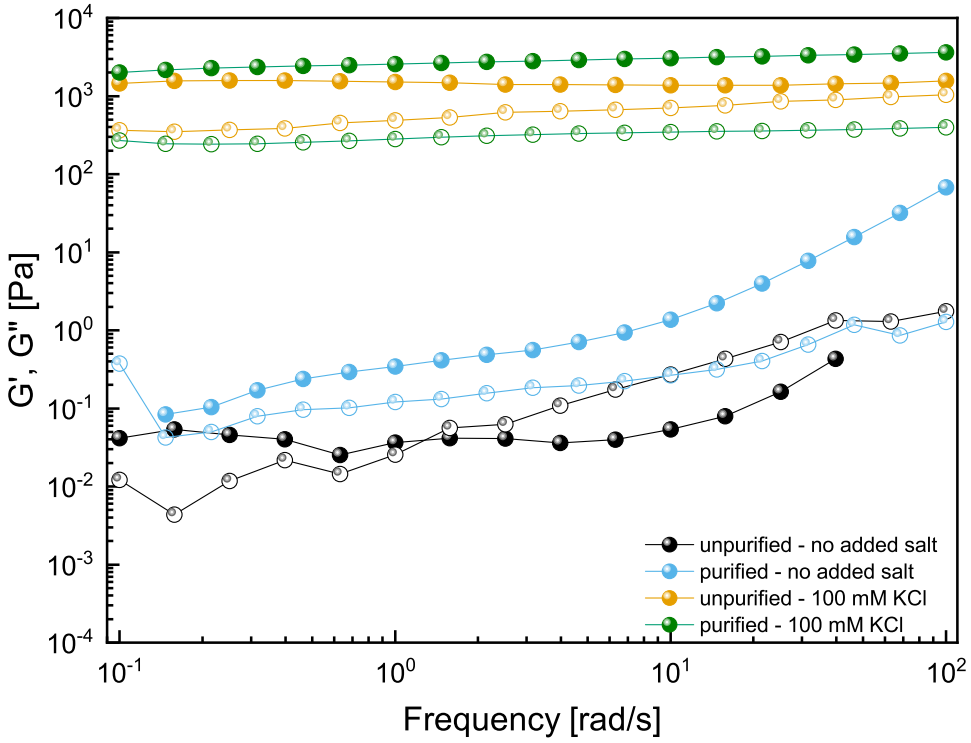

Figure S6. Storage G′ (full) and loss G″ (empty) moduli at 1% strain as a function of angular frequency for 0.5 wt-% solutions of commercial κ-carrageenan - as well as purifed κ-carrageenan in the potassium form without any salt added and at 100 mM KCl.

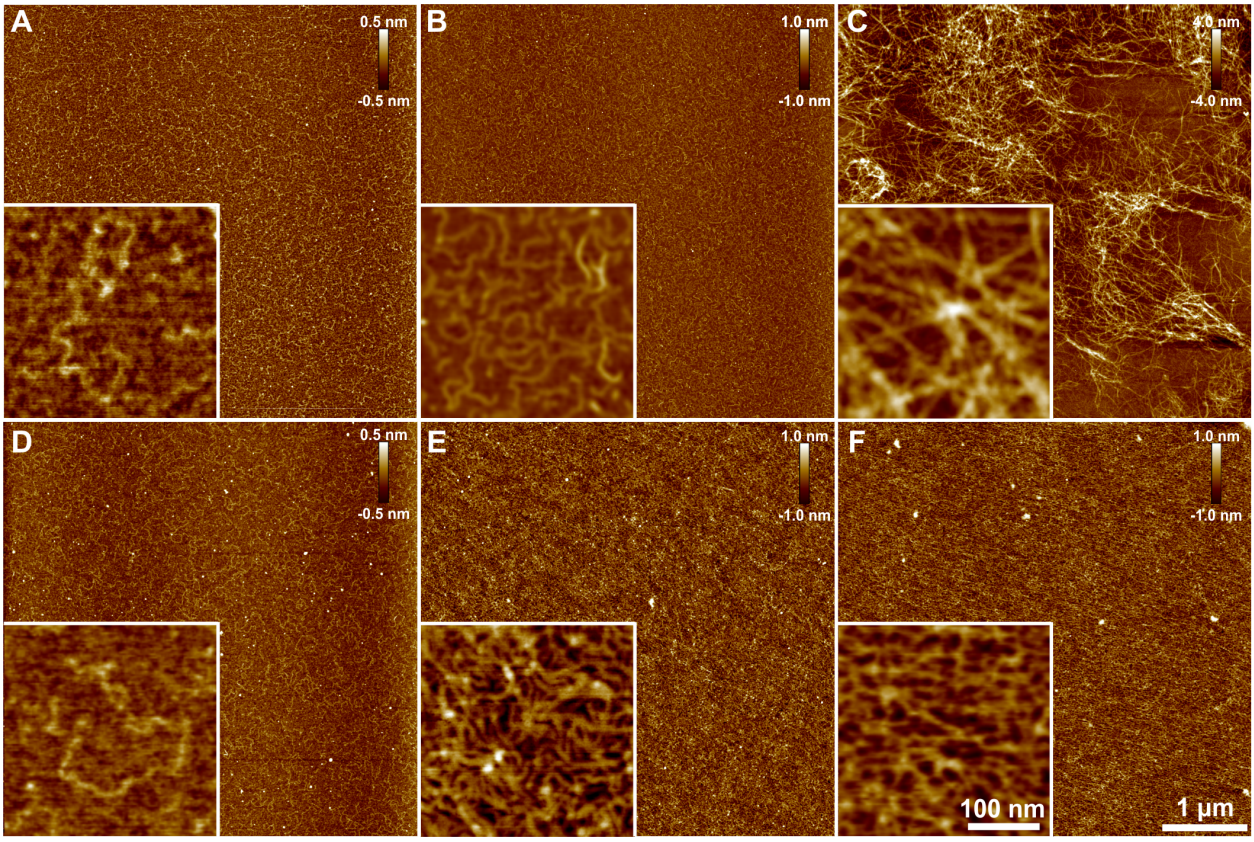

Figure S7. Representative AFM height images for purified (A-C) κ-carrageenan in the K-form (A) at 0.0005 wt-% and (B) at 0.5 wt-% and (C) at 0.5 wt-% with 200 mM KCl added. (D-F) ι-carrageenan (D) at 0.0005 wt-% and (E) at 0.5 wt-% and (F) at 0.5 wt-% with 500 mM KCl added. Insets show enlarged regions of interest. Scale bars apply to all images of same category.

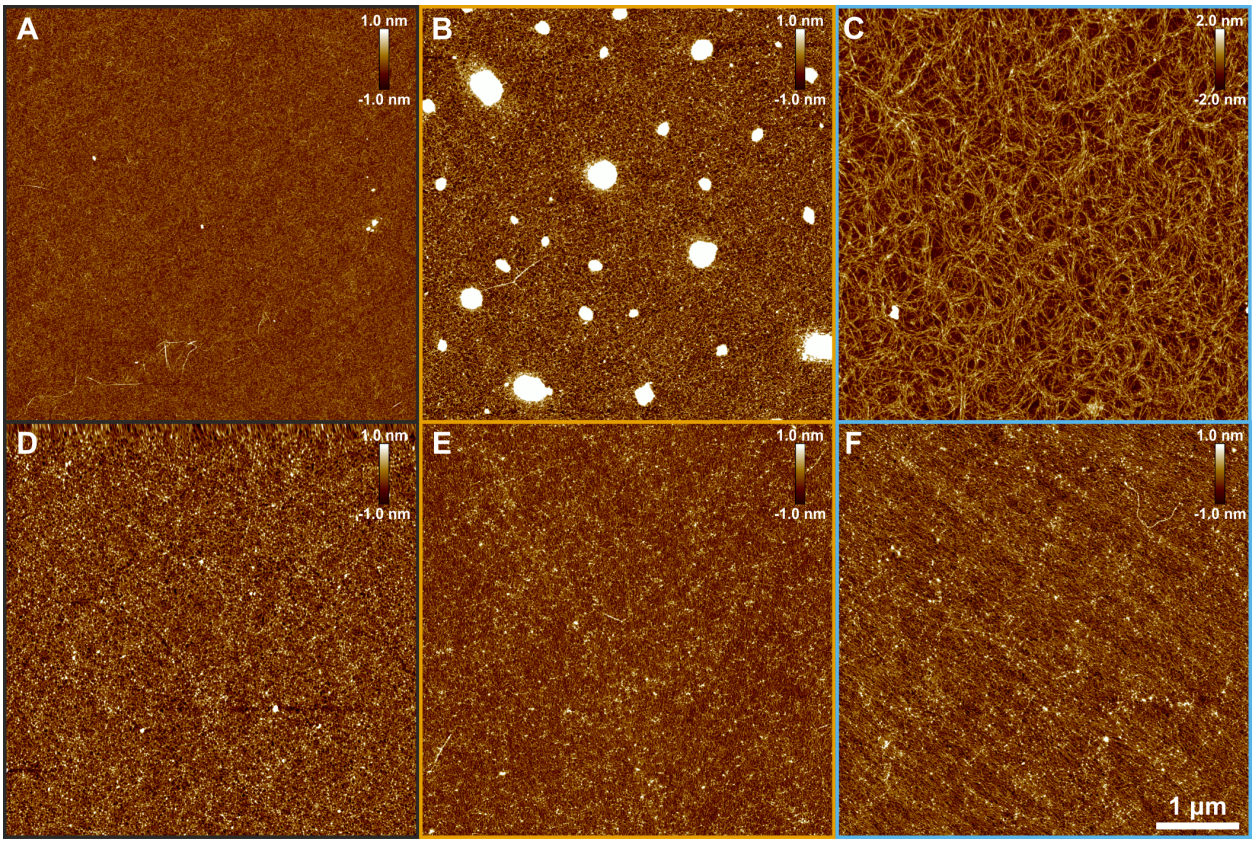

Figure S8. Representative AFM height images of 0.5 wt-% (A-C) κ- and (D-F) ι-carrageenan. (A, D) without any salt addition, (B, E) with 100 mM NaCl and (C, F) 100 mM KCl. Scale bar applies to all images.

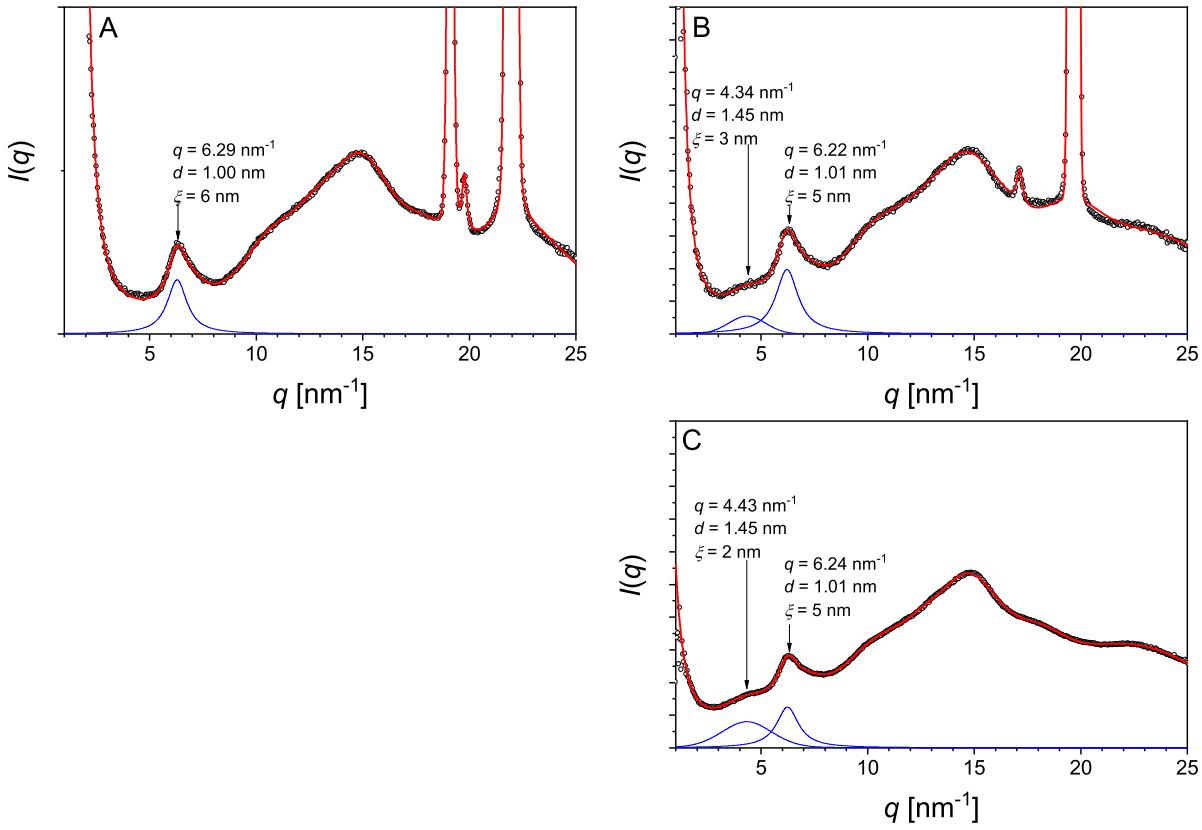

Figure S9. WAXS intensity profiles with the corresponding deconvoluted peaks for primary and secondary structure of lyophilized κ-carrageenan at (A) 0.5 wt-% and 100 mM NaCl, (B) 0.5 wt-% and 100 mM KCl and (C) 10 wt-% and 100 mM KCl added. Sharp peaks correspond to salt crystals.

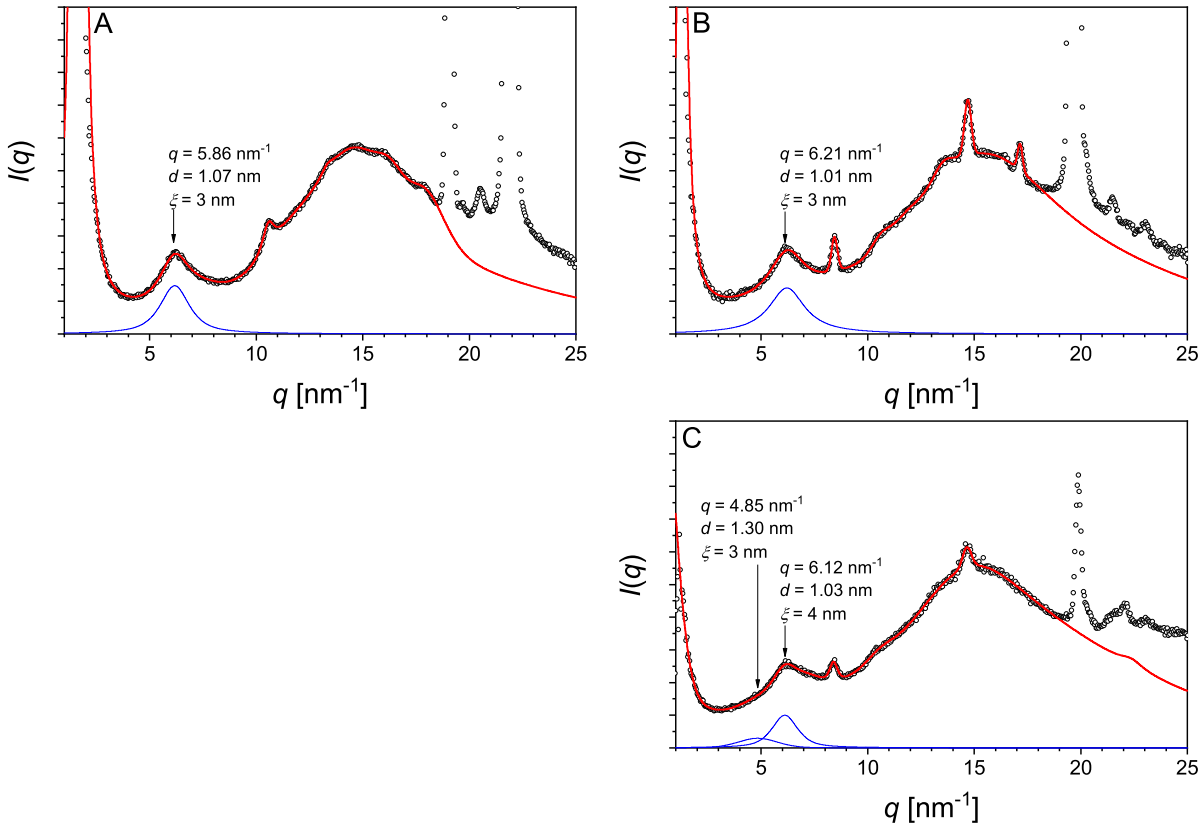

Figure S10. WAXS intensity profiles with the corresponding deconvoluted peaks for primary and secondary structure of lyophilized ι-carrageenan at (A) 0.5 wt-% and 100 mM NaCl, (B) 0.5 wt-% and 100 mM KCl and (C) 10 wt-% and 100 mM KCl added. Sharp peaks correspond to salt crystals.

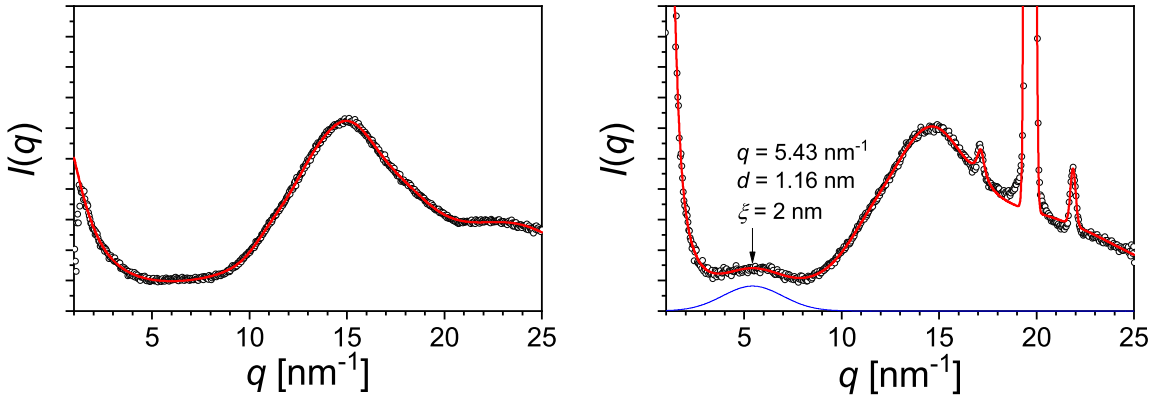

Figure S11. WAXS intensity profiles with the corresponding deconvoluted peaks for primary and secondary structure of lyophilized λ-carrageenan (A) at 10 wt-% without salt addition and (B) 0.5 wt-% and 100 mM KCl added. Sharp peaks correspond to salt crystals.

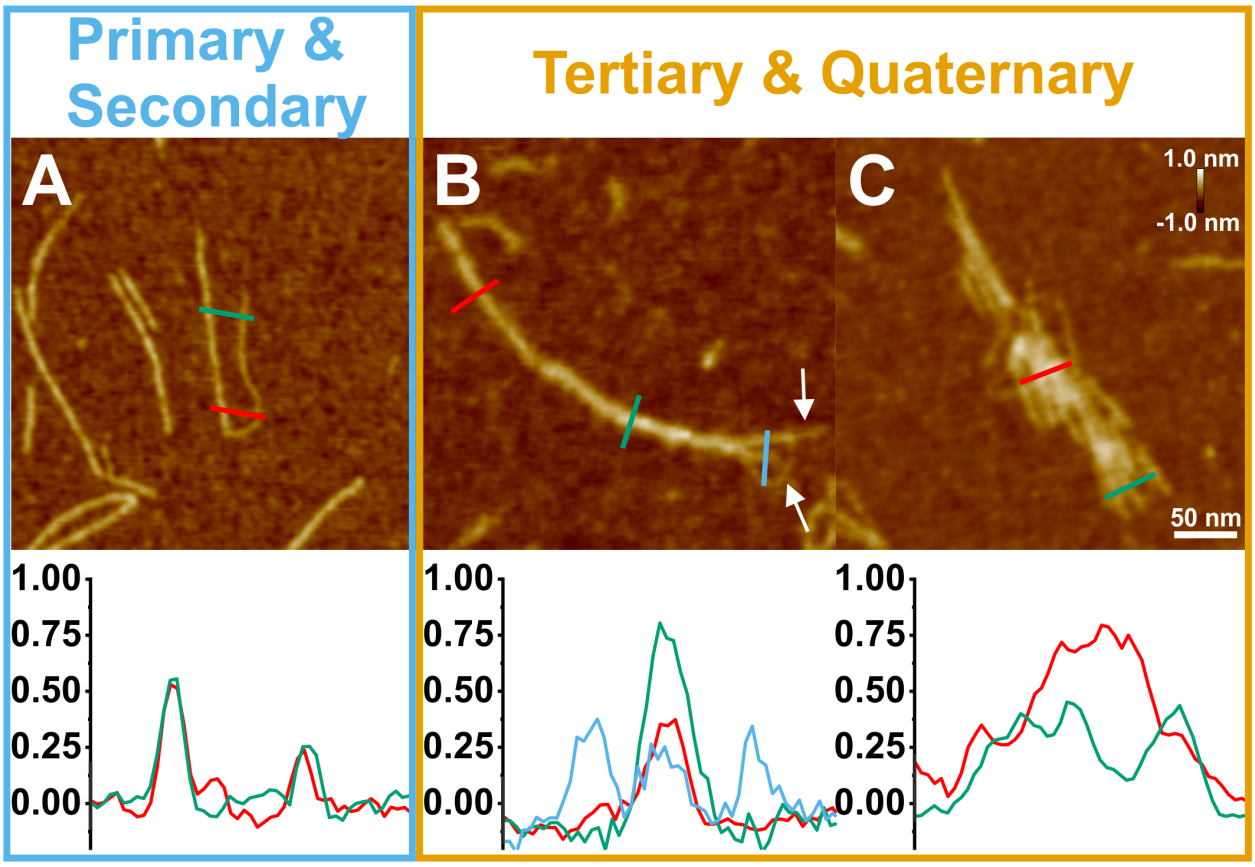

Figure S12. Representative AFM height images with corresponding height profiles of selected cross sections in nm for Gellan gum at 1 μg/mL. (A) Intramolecular formation of a helix defined as secondary structure as well as (B) further intrachain supercoiling events and (C) multiple chains laterally aggregating upon the addition of 100 mM NaCl or KCl. White arrows point to a hairpin loop indicating tertiary structure as well as the loose end of the second involved polysaccharide chain.

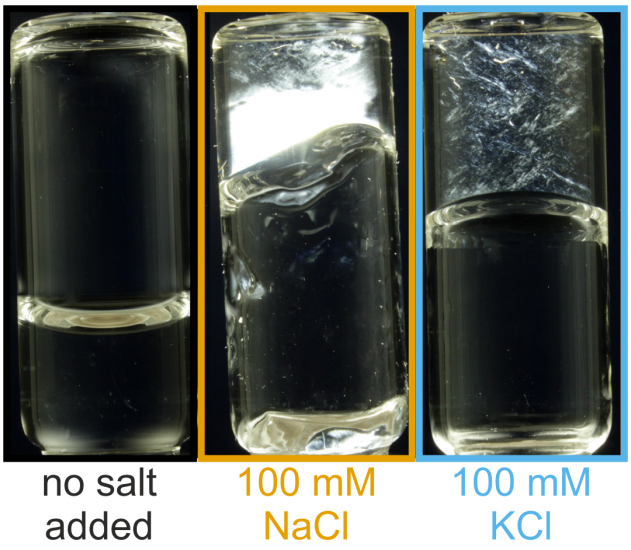

Figure S13. Gellan gum solutions at 0.5 wt-% with different added chloride salts placed between cross-polarizers in order to show the birefringence and turned upside down to visualize the solid-like behaviour upon gelation.

References

(1) Prado-Fernández, J.; Rodríguez-Vázquez, J.; Tojo, E.; Andrade, J. Quantitation of κ-, ι- and λ-Carrageenans by Mid-Infrared Spectroscopy and PLS Regression. *Analytica Chimica Acta* **2003**, *480*, 23–37.