Characterization of *Skeletonema costatum* Intracellular Organic Matter and Study of Nanomechanical Properties under Different Solution Conditions

SUPPLEMENTARY DATA

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COLLOIDS AND SURFACES A: PHYSICOCHEMICAL AND ENGINEERING ASPECTS

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8 pages, 7 figures, and 1 table are included in the Supplementary Data Section.

1. Materials and Methods

1.1. Characterization of the High Molecular Weight compounds of SKC-IOM

The concentration of Carbon, Hydrogen and Nitrogen was determined using Perkin Elmer 2400 Series II CHNS/O Elemental Analyzer. This analysis was conducted in triplicate (2 mg per sample). Briefly, the sample was combusted in a pure oxygen environment to produce elemental gases (CO_2, H_2O, N_2, and SO_2), where a thermal conductivity detector measures the concentration of each species of gas. Pyrolysis-GC-MS was used to characterize the biopolymer composition of HMW SKC-IOM based on the identification of the molecules resulting from the thermal degradation of the polymeric structures. Samples were analyzed following the protocol described elsewhere [1]. Briefly, 2 mg of sample was degraded by flash pyrolysis (CDS Pyroprobe 5150) following the next temperature program: 50°C for 1 sec then 650°C for 20 sec with a ramp of 20°C/msec. The resulting compounds were subsequently separated by GC by a 0.25 µm DB-WAX column (7890A GC System, Agilent Technologies) and detected by MS (5975 inert MSD, Agilent Technologies). Samples for ^{1}H-NMR analysis were prepared by dissolving the organic compounds in 600 µl of deuterated water (D_2O). 550 µl of the solution was then transferred to 5 mm diameter NMR tubes. NMR spectra were acquired using Bruker 950 AVANAC III spectrometer equipped with 5 mm Bruker TCI CryoProbe (Bruker BioSpin, USA). The ^{1}H-NMR spectra were recorded collecting 1k scans with a 3s recycle delay time for achieving high signal to noise ratio. 3-trimethylsilylpropane sulfonic acid was used as internal chemical shift reference. Spectra was generated by excitation sculpting pulse sequence (zgesgp program, Bruker Pulse library). Free Induction Decay (FID) data was collected within a spectral width of 15243 Hz digitized into 64k data points. The FID signals were zero-filled and amplified by an exponential line-broadening factor of 0.3 Hz before Fourier transformation. Bruker Topspin 3.0 software was used for data collection and for spectral analysis.

2.   Results and Discussion

2.1. Isolation of High Molecular Weight Compounds of SKC-IOM

LC-OCD profiles showed no significant difference between the filtered culture solution and the centrifuged and filtered culture solution (Figure S1a). This result indicates that the SKC cells were not destroyed during the centrifugation process (i.e., practically undetected IOM contribution in the culture solution). The SKC cells were further re-suspended and sonicated to release all the IOM in the solution, filtered through a 0.7 μm membrane, and finally dialyzed against a 3.5 kDa membrane to isolate HMW compounds. Similarly, the HMW SKC-IOM compounds (dialysis retentate) were compared to SKC-IOM by LC-OCD. The generated profiles (Figure S1b) indicated the efficiency of the dialysis protocol to isolate the HMW fraction from IOM solutions. The proportion of HMW compounds in the IOM was 6% of the DOC. Part of the HMW SKC-IOM solution was lyophilized and the recovered material was used for further characterization, i.e., Elemental Analysis, FTIR, Pyrolysis-GC-MS, ^{13}C-NMR, and ^{1}H-NMR.

2.2. Elemental Analysis

Elemental analysis of HMW SKC-IOM was performed in triplicate. Specifically, the relative abundance (percent of mass) of C, H, N, and atomic ratios of C/N and C/H were calculated (Table S1). C/N ratio is an indicator of the presence of nitrogenous structures (e.g., proteins or similar biogenic material), while C/H ratio is an indicator of the degree of unsaturation (i.e., aromatic character). Briefly, HMW SKC-IOM showed a 51% carbon content. On the other hand, the C/H atomic ratio of HMW SKC-IOM was low (0.54). This ratio is similar in magnitude to that of freshwater biopolymers but lower than those of humic substances (i.e., 0.7 to 0.8) [2]. This result indicates that the HMW organics from SKC-IOM are characterized by a low unsaturated carbon content (i.e., low aromaticity). In addition, the C/N ratio of HMW SKC-IOM was in the order of magnitude of freshwater NOM (i.e., below 10) [3, 4].

2.3. Pyrolysis GC-MS

The different pyrolysis fragments were identified using data previously reported [5]. HMW SKC-IOM exhibited a very specific pyrochromatogram (Figure S2) with intense isocrotonic and crotonic acid peaks produced from the pyrolysis of polyhydroxybutyrates (PHBs), i.e., known as energy storage molecules synthesized by microbial cells under growth-limiting conditions [6]. Consequently, the relative abundance of the polysaccharide and protein peaks for HMW SKC-IOM was decreased. Toluene, ethyl benzene, styrene and phenols can be assigned to the pyrolysis of proteins structures; furans and several organics acids (not referenced in the chromatograms) are produced from carbohydrates. The presence of lipids is clearly demonstrated by the presence of abundant peaks of fatty acid derivatives.

2.4. ^{1}H-NMR

^{1}H-NMR was used to obtain more information on the linkage of hydrogen (Figure S3). Resonances observed between 0.5 and 1.8 ppm are assigned to alkyl proton attached to carbon removed from aromatic rings or carboxylic groups (i.e., this region have been attributed to lipids) [7]. From 1.8 to 3 ppm, the resonance corresponds to protons mostly attached to carbon positioned on α to aromatic ring and carboxylic groups, and are characteristic of proteins. The region from 3 ppm to 4.7 ppm (i.e., alcohol and ether H attached to carbon positioned on α to oxygen), and from 5 ppm to 5.5 ppm correspond to carbohydrate moieties. The resonances in the 6.4 ppm to 9 ppm region are assigned to aromatic and olefinic protons [4, 7, 8]. HMW SKC-IOM is characterized by strong resonances in the carbohydrates, proteins, and specially lipid regions (i.e., strong signal in the lipid region from 0.8 to 1.5 ppm).

Table S1. Elemental composition of HMW SKC-IOM isolate.

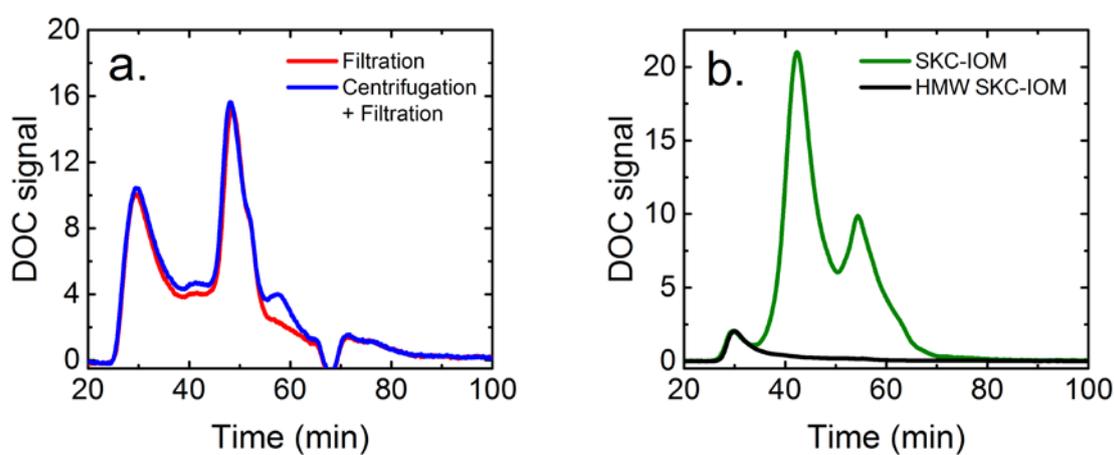|	%C	%H	%N	C/N	C/H
	(Std. dev.)	(Std. dev.)	(Std. dev.)	atomic ratio	atomic ratio
HMW SKC-IOM	51.03 (0.55)	7.84 (0.10)	6.44 (0.08)	9.24	0.54

Figure S1. LC-OCD profiles of: a) centrifuged and non-centrifuged SKC samples, and b) SKC-IOM and dialysis concentrate, i.e. HMW SKC-IOM.
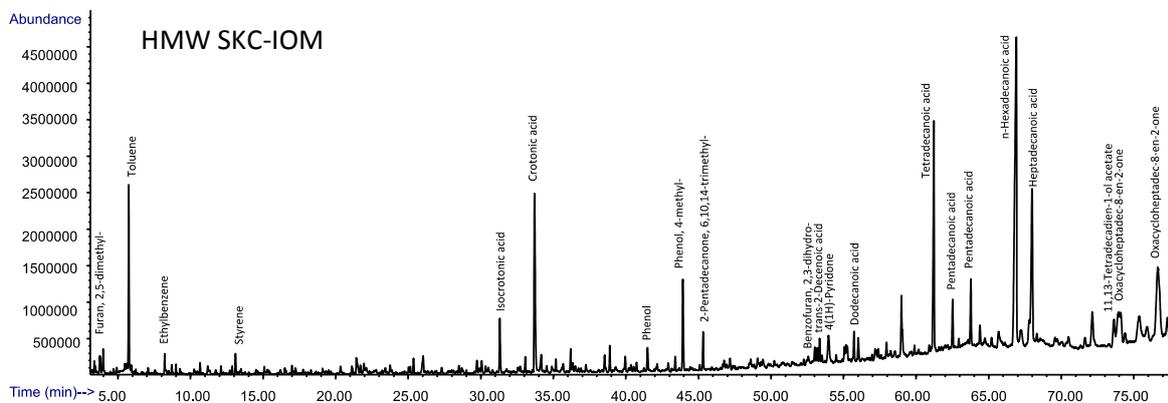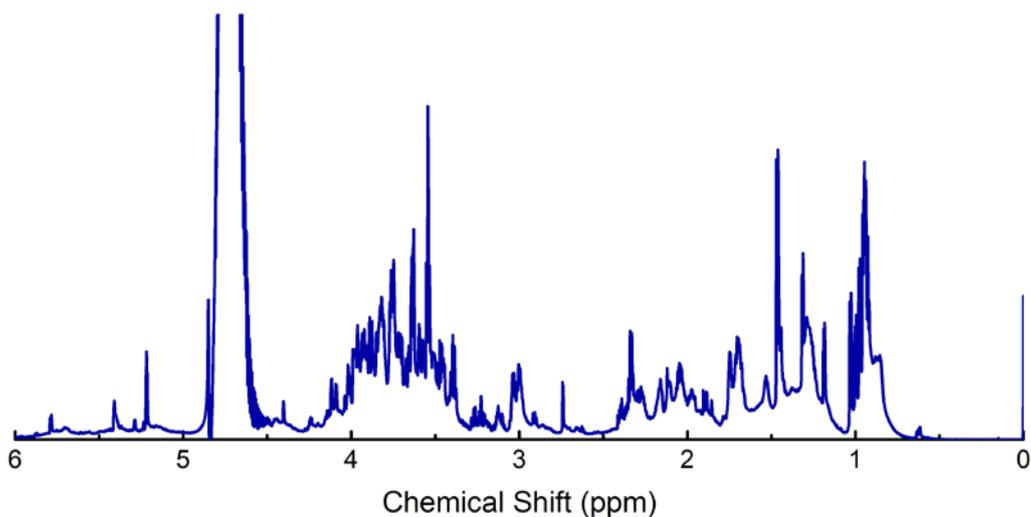

Figure S2. Pyrochromatograms of HMW SKC-IOM isolate.

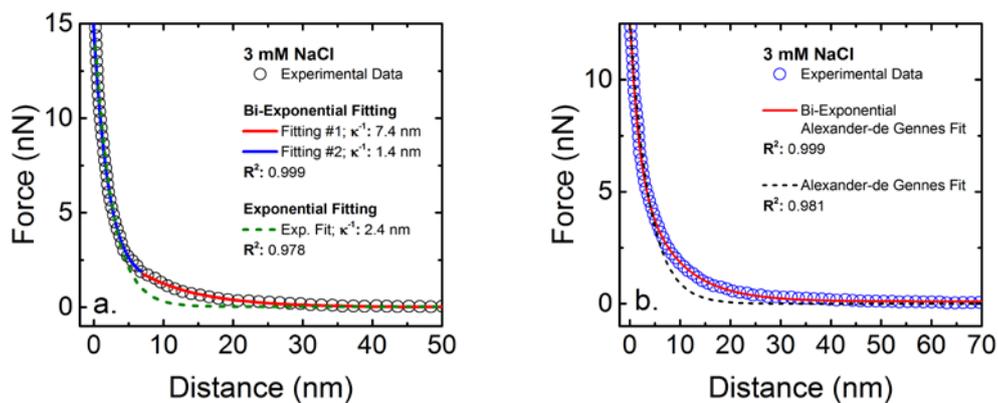**Figure S3.** ^{1}H-NMR spectra of HMW SKC-IOM isolate.

Figure S4. Force vs distance profiles at approaching regime in 3 mM NaCl. Curve fittings were conducted by: a) exponential decay (green dotted line) and bi-exponential decay (red and blue solid lines) to extract Decay Length (κ^{-1}) values; and b) Alexander-de Gennes (black dotted line) and bi-exponential Alexander-de Gennes model (red solid line).
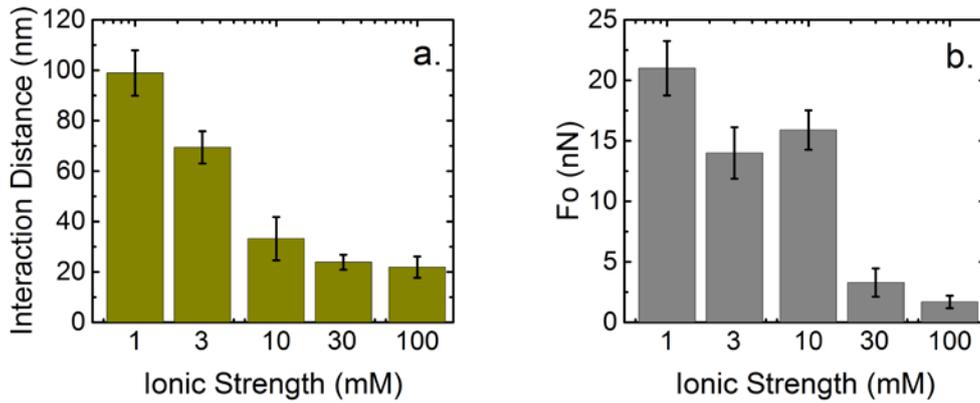
Figure S5. Change in a) interaction distance and b) F_0 during approaching regime as a function of ionic strength. Mean and variance values were determined from probability density functions.
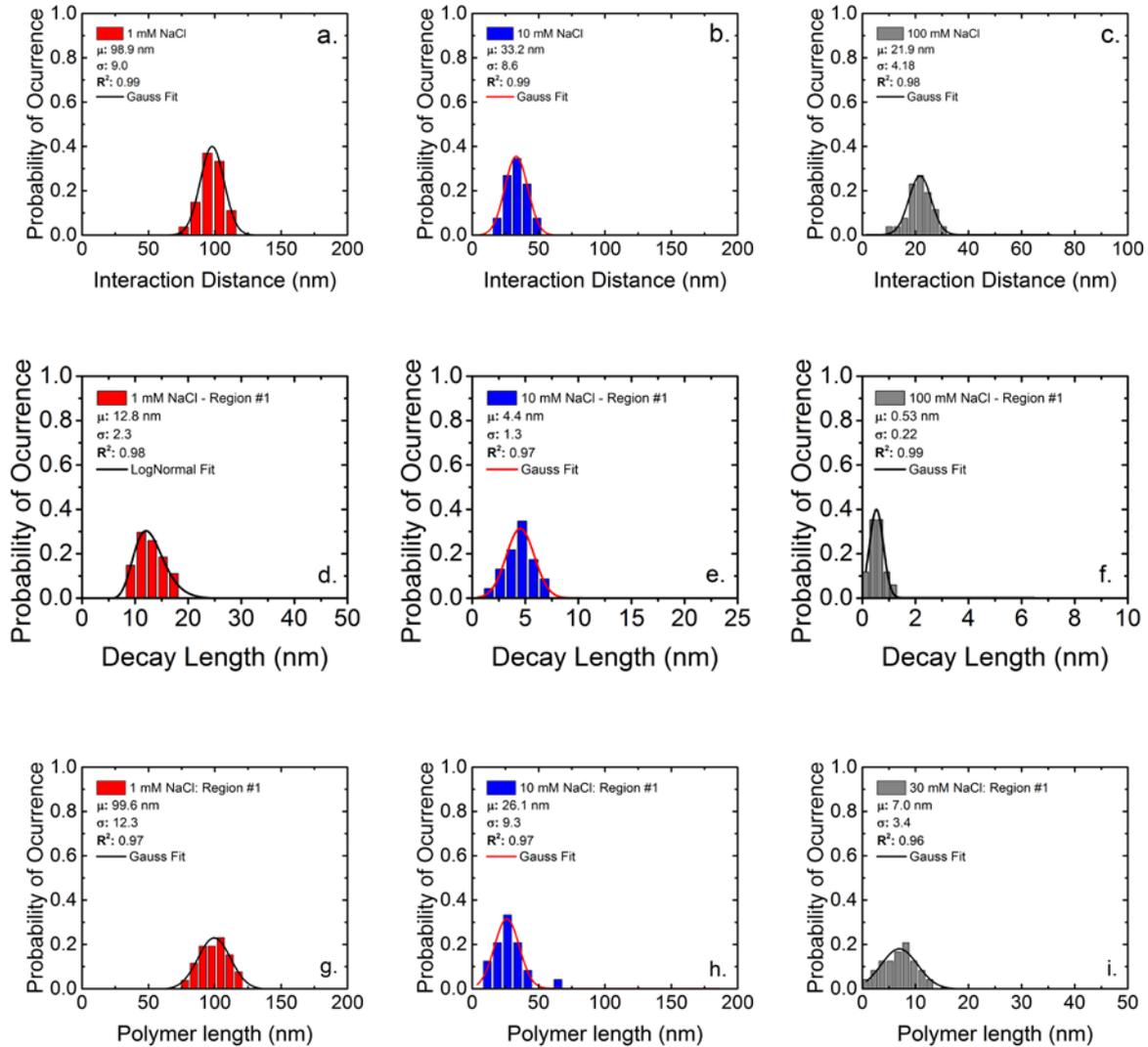

Figure S6. Probability density functions showing: a-c) interaction distance, d-f) decay length, and g-i) polymer length, as a function of ionic strength.
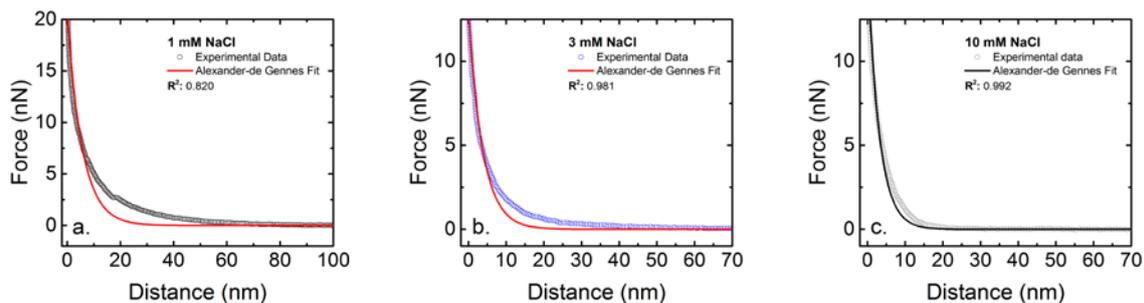
Figure S7. Representative force vs distance profiles at approaching regime in: a) 1 mM NaCl, b) 3 mM NaCl, and c) 10 mM NaCl. These curves were fitted using Alexander-de Gennes model (solid line).

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