# Supporting Information

## Phenolic Condensation and Facilitation of Fluorescent Carbon Dot Formation: A Mechanism Study

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## Experimental Section

***Pre-treatment of basil seed*:** Whole basil seeds (Flants, Korea) were thoroughly rinsed with ethanol (Merck Millipore, Germany) before use.

***Morphological characterization of basil seed*:** Expanded polysaccharide chains were examined under a fluorescence microscope (Eclipse 80i, Nikon, Japan). The hydrocolloid structure was prepared by immersing a basil seed into deionized water for 10 minutes followed by freeze-drying it for two days. The fully dried sample was mounted onto an aluminum holder using a conductive carbon tape (Nisshin EMCO, Japan) and observed with a scanning electron microscope (S-4800, Hitachi, Japan).

***Extraction of phenolic compounds from natural basil seeds*:** For the separation of water-soluble polysaccharides from basil seed, 500 mg of basil seeds were soaked in 20 mL of distilled water, and let under stirring (~ 500 rpm) for 4 hours. The collected water extract was filtered through a cellulose acetate membranes (0.45 μm pore size, Minisart, Sartorius stedim, Germany) and directly injected (Injected volume: 5 mL) into a fast performance liquid chromatography system (UPC 900/P920, Amersham Bioscience, USA) connected to a size-exclusion column (HiLoad Superdex 75 prep grade, GE Healthcare, UK). Distilled water was used as a mobile phase buffer with a flow rate of 1 mL $min^{-1}$. A UV detector with 280 nm excitation wavelength was used to detect phenolic compounds from the extracted

polysaccharide solution. The fluorescent product showing UV absorption at 280 nm were isolated (eluent volumes of 40 mL to 60 mL) and concentrated with a rotary evaporator (HS-2001 NS, Hahnshin Scientific, Korea).

***Identification of phenolic compounds by mass analysis*:** A prepared aromatic compound (i.e., ferulic acid)-rich solution underwent high-performance liquid chromatography (HPLC) (1260 Infinity, Agilent Technologies, USA) connected to a reversed-phase column (Discovery BIO wide pore $C_{18}$, Supelco Analytical, USA) utilizing a static mode with a mobile phase of distilled water (95 vol%) and acetonitrile (5 vol%) with 0.1 vol% trifluoroacetic acid (TFA). The eluent was injected directly into a mass spectrometer (6120, Agilent Technologies, USA) for online MS analysis. The solution was released continuously through the column for 60 minutes with a flow rate of 0.5 mL $min^{-1}$.

***Carbonization of glucomannan in presence or absence of ferulic acid and carbonization of basil seed*:** The samples in presence or absence of 1 mg (for the dynamic light scattering samples: 0 mg, 1 mg, 5 mg and 10 mg, respectively.) of ferulic acid (Sigma Aldrich, USA) in 250 mg of glucomannan (Now Foods, USA) were added into 10 mL of sulfuric acid (18 M, Fisher Scientific, USA) and stirred for 20 minutes (800 rpm). For carbonization of basil seeds, 1 g of basil seeds were stirred (800 rpm) in 10 ml of sulfuric acid solution for one minute. Both carbonized solutions were diluted with 90 mL of distilled water and further neutralized by mixing with 50 mL of 10 M NaOH. Finally, salts and other chemical adducts were eliminated from the samples by dialysis (MWCO of 2000, Thermo Scientific, USA).

***Production yield and quantum yield calculation for carbonized glucomannan in presence or absence of ferulic acid*:** The amount of carbonized glucomannan solution (*i.e.*, carbon dot) prepared by the steps explained above was determined by lyophilization followed by

weighing with an extreme care. The production yield was calculated according to the following formula: (mass of carbonized glucomannan) / (initial mass of glucomannan) × 100% = production yield. To calculate the quantum yield (QY), we used the comparative method published by Williams et al.[1] In details, Coumarin 153 (Sigma Aldrich, USA, QY = 0.544[2]) dissolved in ethanol was applied as the standard compound. The equation of $\Phi_x = \Phi_{st} (S_x/S_{st}) (\eta_x/\eta_{st})^2$ was applied, in which Φ is QY, S is the slope, and η is the refractive index. The subscripts 'st' and 'x' represent the standard and the sample, respectively. The slope was obtained from the absorption (X axis) versus emission (Y axis) graph (Figure S4) measured using a UV-vis spectrometer (HP 8483, Hewlett-Packard, USA) and a spectrometer (Synergy Mx, Biotek, USA). In this study, the value of $\eta_x / \eta_{st}$ is 0.98.

***Supporting references***

[1] A. T. R. Williams, S. A. Winfield and J. N. Miller, *Analyst* 1983, **108**, 1067.

[2] K. H. Drexhage, *J. Res. Natl. Bur. Stand., Sect. A*, 1976, **80**, 421.

***Carbon dots purification using high performance liquid chromatography*:** Carbon dots were isolated from the carbonized basil seed solution (50 mL, produced by aforementioned procedures) by ethanol extraction (200 mL), which can precipitate the relatively hydrophilic polysaccharide residues. After centrifuging the solutions for 10 min at 3500 rpm, the supernatant (*i.e.*, roughly purified carbon dots) underwent rotary evaporation for ethanol removal. Then, the extracted carbon dots were further purified by HPLC (1260 Infinity, Agilent Technologies, USA) using the semi-preparative column (ZORBAX SB-C18, Agilent Technologies, USA). A deionized water with acetonitrile (ACN) mixture in 0.1% (v/v) trifluoroacetic acid (TFA) was used as a mobile phase with following intervals: (1) 95-90 % water in 3 min, (2) 90-90 % water in 8 min, (3) 90-70 % water in 38 min, (4) 70-5 % water in

42 min, (5) 5-5 % water in 47 min, (6) 5-95 % water in 52 min. Flow rate of the mobile phase was 1 mL $min^{-1}$ and the separated samples were identified by UV-vis detector at 280 nm.

***Carbonized glucomannan purification using gel permeation chromatography:*** Carbonized glucomannan solutions in presence or absence of ferulic acid addition were prepared by the sulfuric acid induced carbonization method as previously described. The solutions flowed through the column (Superose 6 Increase 5/150 GL, GE Healthcare, UK) for 60 minutes using the 1260 Infinity Quaternary LC (Agilent Technologies, USA) connected to Multi-Angle static Light Scattering (MALS) detector (DAWN HELEOS II, Wyatt Technology, USA). Phosphate buffered saline (10 mM) with sodium azide (200 pm) was used as the mobile phase with a flow rate of 0.5ml $min^{-1}$.

***$^1$H-NMR sample preparation:*** Bruker AVHD-400 spectrometer was applied to measure $^1$H NMR spectra. For the sample preparation, ferulic acid (Sigma Aldrich, USA) was added to $D_2SO_4$ solution (96-98 wt. %, Sigma Aldrich, USA) at the concentration of 0.25 mg/mL with or without glucomannan (10 mg/mL) and the total volume was adjusted to 400 μL. To the ferulic acid solution, glycine (LPS Solution, South Korea) was additionally added to 2 mg/mL concentration in $D_2SO_4$. The glycine provides quadruple peaks between 3.64 and 3.70 ppm acting as an internal quantitative standard compound. The sample containing only glucomannan (400 μL of a solution at a concentration of 8 mg/mL in $D_2SO_4$) and glycine (100 μL of a solution at a concentration of 2 mg/mL in $D_2SO_4$) was also measured to clearly distinguish the peaks of ferulic acid from the glucomannan peaks. All samples were measured after 10 minutes of vortexing and 12 hours of carbonization at ambient conditions. The chemical shift was quoted in part per million (ppm), referenced to tetramethylsilane (Sigma Aldrich, USA) observed at 0.0 ppm.

***Characterization*:** Optical and fluorescence imaging were performed with a fluorescence microscope (Eclipse 80i, Nikon, Japan) and a confocal microscope (Eclipse Ti, Nikon, Japan). Macroscale fluorescence imaging was carried out with the UV lamp (VL-215M, Viler Lourmat, France). Nanoscale images of carbonized polysaccharides were obtained with an atomic force microscope (XE-100, Park Systems, Korea) by using high-resolution tapping-mode silicon probes (Bruker, USA). UV-vis absorption spectra of each solution were measured by UV-vis spectrometry (HP 8483, Hewlett-Packard, USA) with a quartz cuvette (1 cm in length). The photoluminescence of each sample was measured by a spectrometer (Synergy Mx, Biotek, USA). Spectroscopic analyses comparing the carbon dots produced in the presence or absence of ferulic acid were performed using X-ray photoelectron spectroscopy (ESCA 2000, Thermo Scientific, USA), Fourier transform infrared spectroscopy (Nicolet iS50, Thermo Scientific, USA), and dispersive Raman spectroscopy (ARAMIS, Horiba Jobin Yvon, France) equipped with a 514.5 nm laser for the excitation energy source. To characterize the size distribution of carbon dots according to the concentration of ferulic acid, Zetasizer Nano ZS (Marven, UK) was used.

***Additional supporting figures***

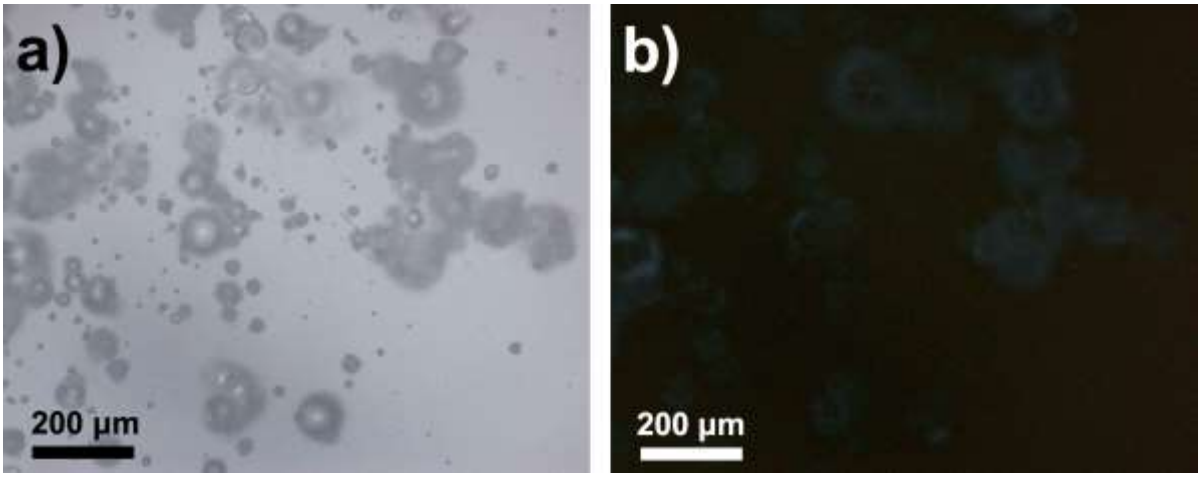


**Figure S1.** A non-fluorescent polysaccharide: dextran. a) Optical image of dextran powder and b) fluorescence image under UV excitation showing minimal fluorescence.

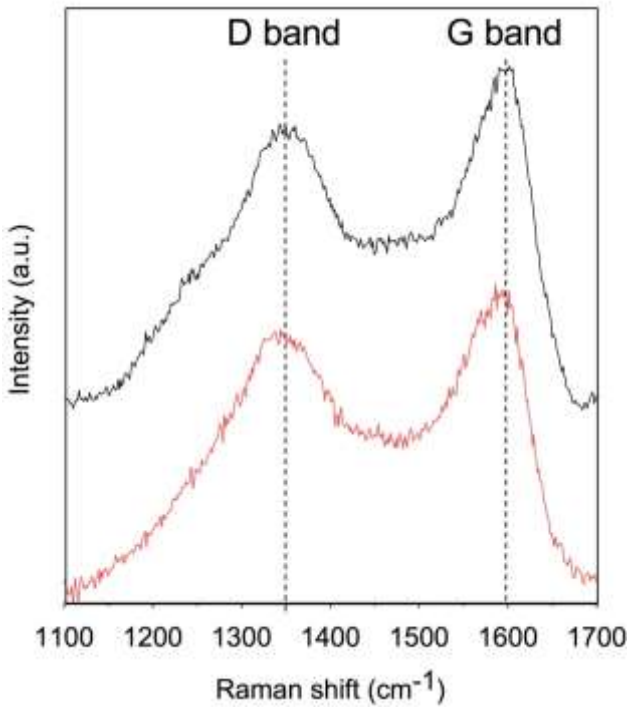


**Figure S2.** Raman spectra of the carbonized glucomannan in the presence (red) or absence (black) of ferulic acids during the carbonization process.

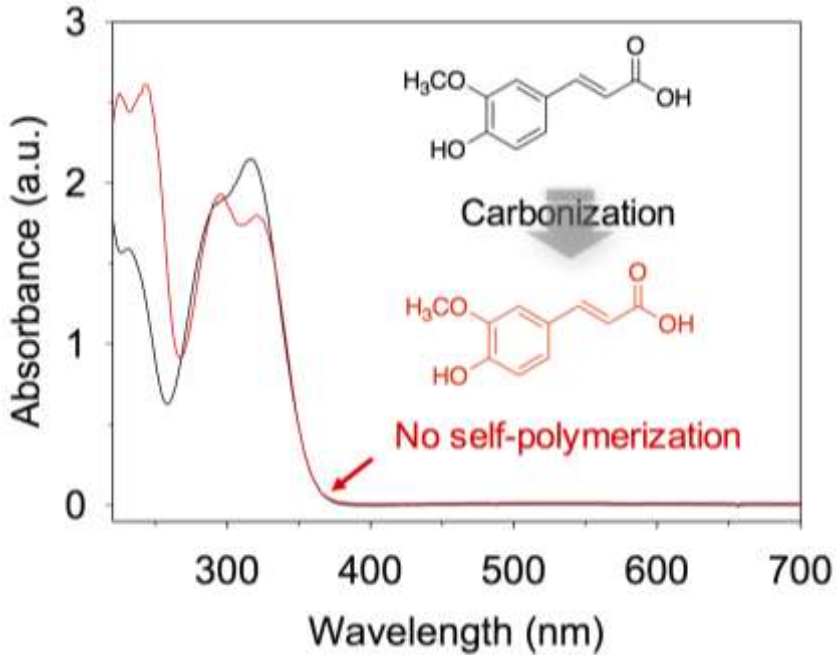


**Figure S3.** UV-Vis spectra of ferulic acid before (black) and after (red) sulfuric acid-based carbonization.

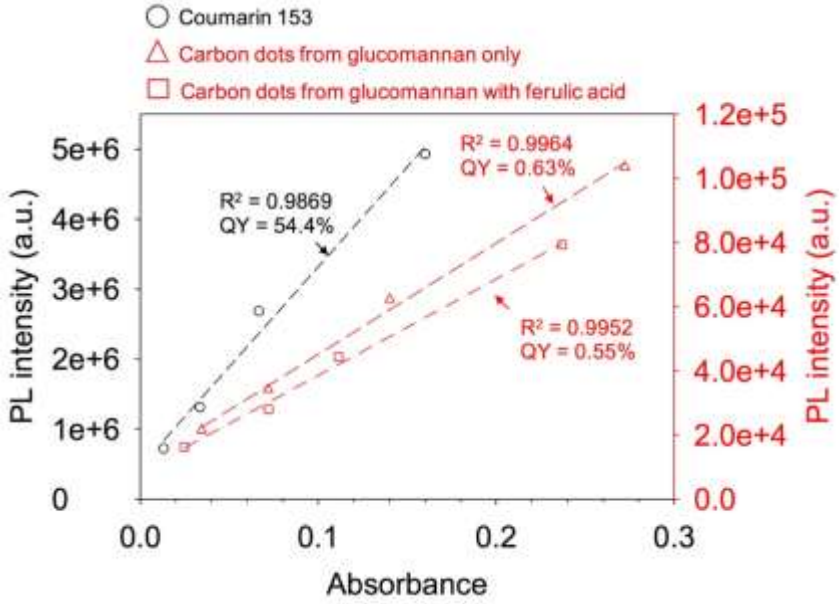


**Figure S4.** UV-vis absorbance (x-axis) versus photoluminescence (y-axis) of the carbon dots produced by glucomannan when ferulic acid is present (red, square) or absent (red, triangle). Quantum yield was calculated by applying Coumarin 153 (black, circles) as a reference.

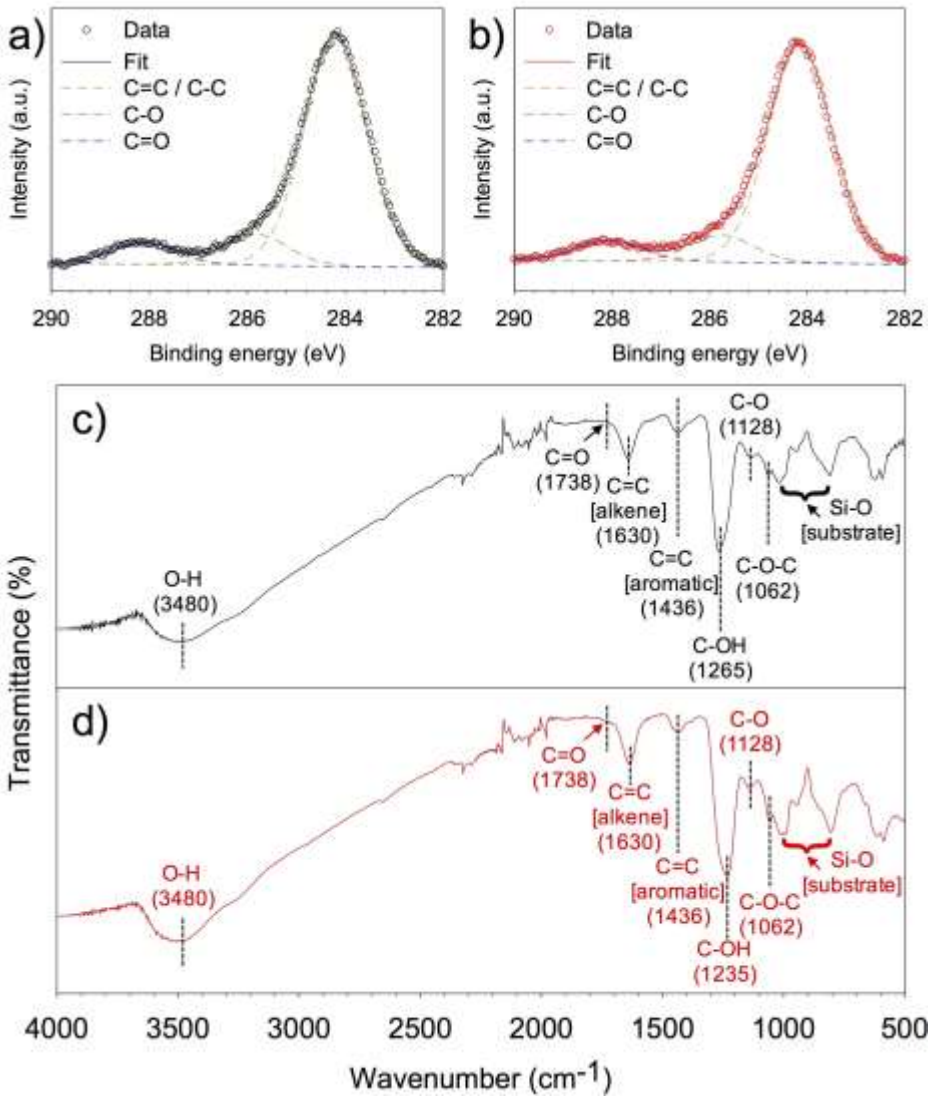


**Figure S5.** Comparisons of surface functionalities of the carbon dots produced from glucomannan carbonizations in the presence (red) or absence (black) of ferulic acids. **(**a and b) High-resolution XPS spectra of the carbon 1s and (c and d) FT-IR spectra of the carbon dots.

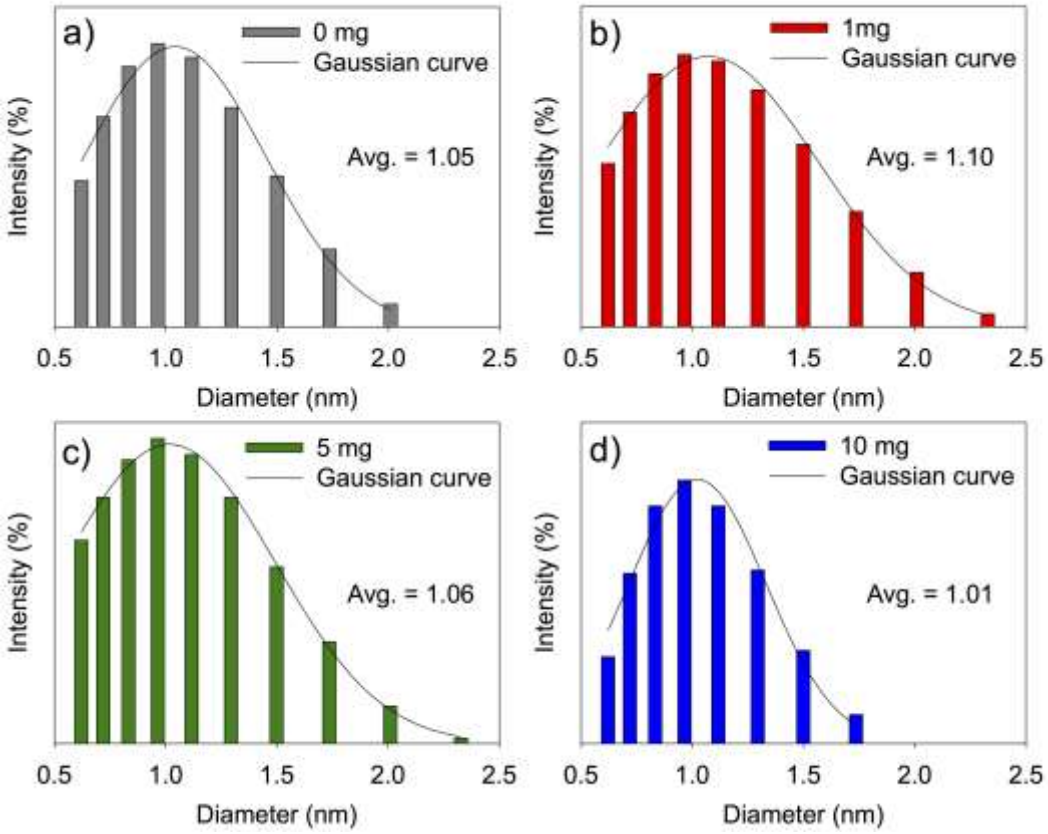


**Figure S6.** The histograms of carbon dots' diameters produced from different concentrations of ferulic acids. The indicated amount of ferulic acid was added during the sulfuric acid induced carbonization processes (total reaction volume = 10 mL) (see supporting information for the detail experimental procedures) Zero mg of ferulic acid for charcoal color, 1 mg for red, 5 mg for green and 10 mg for blue.

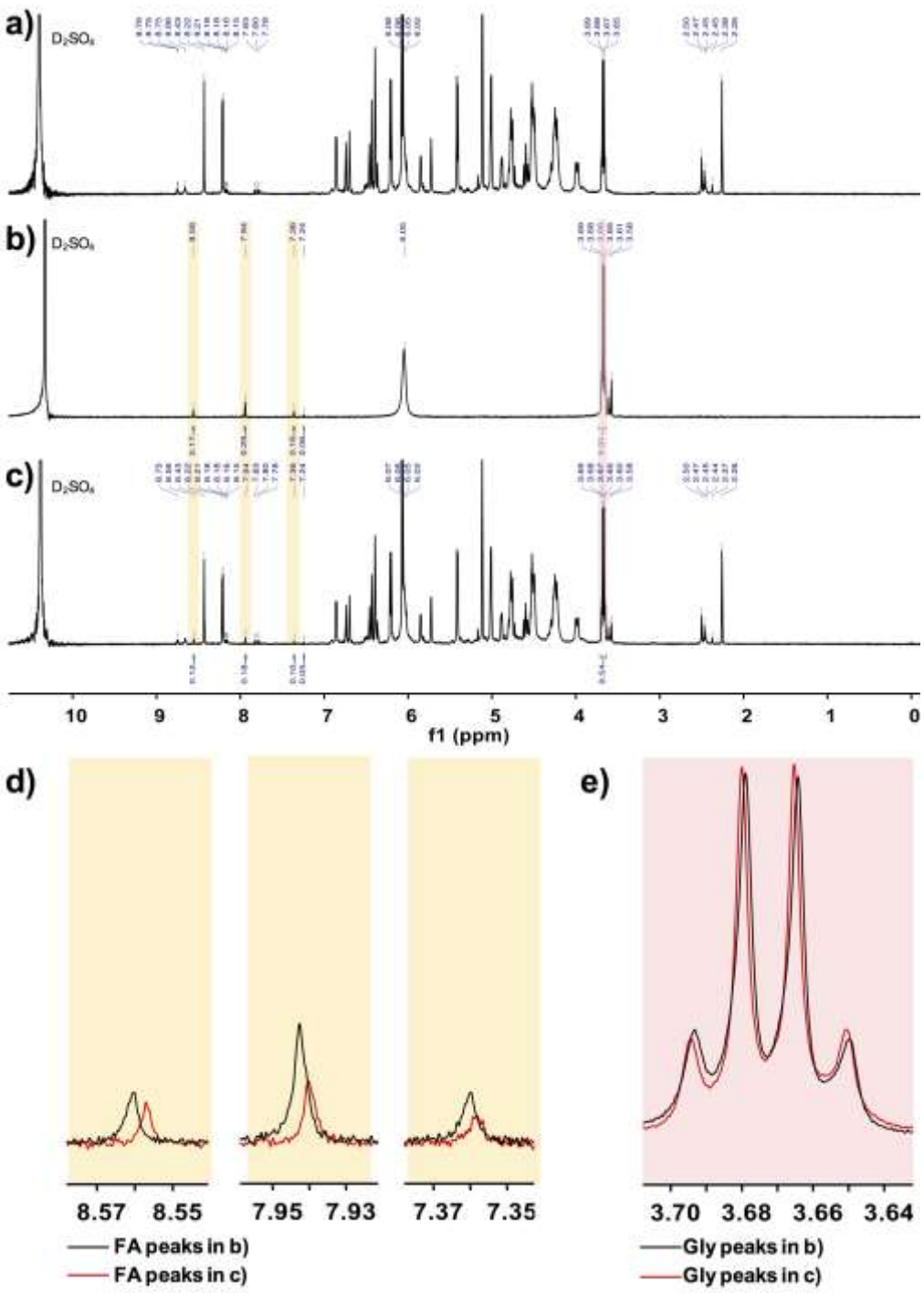


**Figure S7.** $^{1}$H-NMR results of the samples with a) glucomannan (8 mg/mL in $D_2SO_4$), b) ferulic acid (0.2 mg/mL in $D_2SO_4$) and c) glucomannan with ferulic acid (8 mg/mL and 0.2 mg/ mL, respectively in $D_2SO_4$). d) The zoom in proton peaks derived from the phenolic residue of the ferulic acid according to the presence (red) or absence (black) of glucomannan. For quantification of the integral, e) a peak from glycine present in both samples (b and c) as the internal standard.

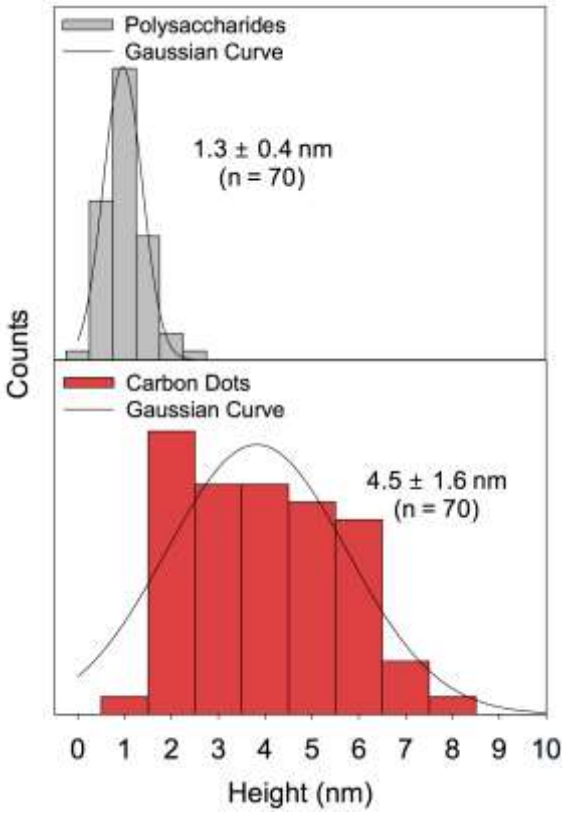


**Figure S8.** The height profiles of polysaccharide chains (black bars) and carbon dots from basil seeds (red bars) measured by atomic force microscope.