Hydrothermal synthesis of novel two-dimensional α-quartz nanoplates and their applications in energy-saving, high-efficiency, microalgal biorefineries

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ABSTRACT

A facile template-free hydrothermal method was successfully developed for the controlled synthesis of ultrathin α-quartz nanoplates (NPLs) for the first time. Analyses of the α-quartz NPLs revealed the characteristic anisotropic nanostructures of highly crystalline α-quartz with an average lateral size and thickness of 1.14 ± 0.32 μm and 7.7 ± 0.6 nm, respectively. Importantly, efficient extraction of highly valuable chemicals such as astaxanthin (ATX) from the microalgal cells of Haematococcus pluvialis (H. pluvialis) was accomplished by lacerating the cell walls using the ultrathin α-quartz NPLs under mild ultrasonication. The incorporation of α-quartz NPLs enhanced the ATX extraction efficiency significantly (99%, 18.0 ± 0.6 mg ATX/g cell) when coupled with 5 min of ultrasonication. The dosage of α-quartz NPLs (800.0 mg/L) for maximum ATX extraction was reduced substantially to only 8% of the nanomaterial dose used in the extraction controls. The enhanced extraction efficiency and dosage were explained by the role of the structural anisotropy of α-quartz NPLs in multiple phase separation-extraction processes. This work provides a novel, facile, and economical route for the synthesis of uniform ultrathin nanoplates, offering a new material for the highly efficient harvesting of chemical products from various microalgal biorefinery processes.

1. Introduction

Silicon dioxide (SiO2 or silica) comprises the two most abundant elements in the Earth’s crust, silicon and oxygen. Silica is found in nature and various living organisms. Crystalline silica is an extremely common mineral that exists in at least eight different forms or polymorphs. Quartz is the most abundant polymorph because it is the most thermodynamically stable under ambient conditions. Quartz is present in rocks, soils, and sands and forms from molten magma at ~800 °C and underground hydrothermal veins at temperatures from 250 to 450 °C [1]. Quartz occurs primarily as one of two polymorphs; α-quartz or β-quartz. Both polymorphs have structures based on the helical arrangement of the tetrahedral silicate (SiO4) unit with the ridgepole of one tetrahedron connected to the keel of the next tetrahedron in a spiral conformation [2]. β-quartz exhibits hexagonal symmetry with 6- and 3-fold screw rotation axes. Structurally, α-quartz is a distorted version of β-quartz and is generally stable at ambient temperature and atmospheric pressure. In contrast, β-quartz is stable only at temperatures above ~573 °C and converts readily to α-quartz upon cooling [3].

Obtaining quartz crystals under ambient conditions is difficult because nucleating quartz normally requires harsh conditions such as high temperatures (300 ~400 °C) and pressures (15 – 100 bar) [4-6].

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Biogenic and biomimetic routes have been used to produce both amorphous and crystalline silica, including cristobalite, tridymite, and quartz [7–11]. Examples include natural biosilification processes exploited by living organisms, such as cyanobacteria [12], diatoms [13], radiolarians [14], and sponges [15] to produce complex and hierarchical silica-based nanostructured frameworks for their skeletons under mild physiological conditions [16–19]. On the other hand, the nucleation and growth processes of the natural biosilification under ambient conditions occur over geologic time. Most of the biosilification products are composed of amorphous silica.

Many synthetic techniques, such as flame, microemulsion, sol–gel, and hydrothermal methods, have been developed to produce novel silica nanostructures because of their unique properties, such as large surface area, excellent chemical, thermal, and mechanical stability [20–22]. Quartz has been produced by precipitation from saturated aqueous silica solutions under hydrothermal conditions for half a century [23,24]. One of the main characteristics of hydrothermal processes is that the temperature of the reaction solvent can be reached near its critical point by heating concurrently with autogenous pressure, making the processes amenable to grow highly crystalline nanomaterials with tunable dimensions in large quantities at relatively low costs [25–28]. There are examples of synthesizing α-quartz nanocrystals with sizes typically ranging from hundreds of micrometers to tens of nanometers. Bertone et al. first developed the synthesis of sub-μm diameter quartz nanocrystals using soluble silica precursors under hydrothermal conditions: temperature above ~250 °C and autogenic pressures within ~5 h [29]. Nevertheless, control of the nanocrystal size, shape, and crystal structure was not superb because of the extremely fast growth rates at these temperatures and the high pH required for the nucleation and rapid coarsening of nanocrystals by Ostwald ripening under basic hydrothermal conditions [29,30]. To overcome these issues, Jiang et al. used an amorphous to crystalline silica transformation to produce single-crystalline α-quartz nanoparticles with a uniform sub-100 nm diameter from Stöber silica nanoparticles and mineralizer additives, such as sodium chloride (NaCl) and sodium hydroxide (NaOH) under hydrothermal conditions of at ~200 °C and ~15 atm within ~3 days [30]. They reported that Stöber silica nanoparticles could be used as templates of uniform sizes that transform to quartz directly by devitrification and minimizing the formation of extra nuclei and aggregation at high pH and NaCl concentrations. Nevertheless, tuning the size and shape of α-quartz nanoparticles was limited by the approximately equiaxed size and shape of the Stöber nanoparticle precursors. Sochalski-Kolbus et al. prepared single-crystalline faceted α-quartz nanorods, ~100 nm × ~10 nm in size, by a microemulsion reaction under solvothermal conditions (~300 °C and < ~100 bar) within the time frame of ~14–17 h [31]. Although their techniques generated anisotropic quartz nanorods via fluoride additives that likely controlled the growth of specific facets as a template, they did not exhibit a high degree of control (±10%) of the size and uniformity of the nanorods. Recently, Buckley et al. showed that 1–5 nm diameter α-quartz nanocrystals can be initially nucleated from water-oil microemulsions under ambient conditions within ~2 days and then grown to a few tens of nm in size under hydrothermal conditions (~175 °C and ~9 bar) within ~7 days [32]. In terms of the two-dimensional (2D) architecture, 2D silica films composed of epitaxially grown ultrathin SiO2 layers were fabricated on solid substrates using various growth methods [33]. In addition, 2D mesoporous silica nanolayers and nanosheets with tunable sizes and thicknesses were also prepared in solutions using hard and soft sacrificial templates composed of graphene oxide [34,35], peptides [36,37], diblock copolymers [38], and lamellar micelles [39–40]. Their structures consisted mainly of complex amorphous silica networks with structural defects potentially available for introducing dopants or intercalating small molecular species. On the other hand, the synthesis of highly crystalline silica nanostructures with an anisotropic plate-like morphology has not been reported.

Microalgae are being exploited as the sustainable feedstock of valuable chemicals and have attracted considerable attention in the pharmaceutical, nutraceutical, aquaculture, and biofuel industries [41–46]. Microalgal biofermenters harvest bioactive proteins, carbohydrates, lipids, pigments, and other diverse metabolites produced by microalgae [47]. The processes involved multiple stages, such as cultivation, harvesting, and product extraction and recovery, of which process economics depend on the time and cost constraints for commercial-scale production [48,49]. In particular, cell disruption and intracellular product extraction are considered major technical bottlenecks that are probably due to the robustness and complexity of microagal cell walls [50]. Various mechanical methods, solely or in combination, such as bead milling, high-pressure homogenization, steam explosion, hydrodynamic cavitation, pulsed electric field, and ultrasonic and microwave agitation have been reported to be capable of disassembling structurally robust microalgal cell walls [50,51]. Mechanical methods have been favored over chemical and biological alternatives for industrial-scale biofermentation operations because of their relatively high efficiency, scalability, and cost-effectiveness, but they are energy-intensive processes. Their mainstream conditions impose thermal and chemical stresses on microalgal cells that degrade the quantity and quality of the bioactive algal products [51,52]. Moreover, their processes usually require relatively long operation times and precise temperature control. Therefore, energy-efficient, sustainable approaches for cell disruption and the extraction of highly valuable algal products under physiologically compatible, stressless conditions are required for efficient large-scale biofermentation applications. One of the promising routes towards these approaches is to apply nanotechnology to the biofermentation of microalgae. Nanomaterials have been exploited to improve the overall processes of microalgal biofermenters, including cell/biomass growth, production of intracellular bioactive compounds, and efficient cell disruption and extraction [53,54]. Nanoparticles composed of silver metal [55], nickel oxide [56,57], organic-aminoclay [58], mesoporous silica [59,60], and carbon nanodots [61] were engineered to harvest intracellular lipid compounds from various algal cells. A recent example using 3 – 5 nm diameter carbon nanodots to extract astaxanthin from lyophilized Haeematococcus pluvialis (H. pluvialis) with a robust, trilayered cell wall resulted in improved extraction efficiency (~85%), nanomaterial dosage (~10 g/L), and extraction time (~6h) [61]. Despite the notable achievements in nanomaterial applications for microalgae biofermentation, a clear picture of the enhancement mechanism has remained elusive in part because of a lack of systematic approaches to understanding the role of the nanomaterial in highly efficient cell wall disruption and extraction.

This paper describes the template-free synthesis of two-dimensional ultrathin α-quartz nanoplates (NPLs) from aqueous dissolution of silica nanoparticles within four hours under facile hydrothermal conditions. To the best of the authors’ knowledge, this is the first report on the hydrothermal synthesis of plate- or sheet-like α-quartz nanostructures. A silica precursor solution was prepared by pretreating 60 nm diameter amorphous silica nanoparticles (ASNs) with an aqueous sodium hydroxide (NaOH) solution. The precursor solution was used as a seed in the hydrothermal synthesis of α-quartz NPLs at moderate temperatures and autogenic pressures such as ~250 °C and ~2 – 3 atm. These hydrothermal methods took only a few hours rather than tens of hours to days to complete the synthesis. The α-quartz NPLs obtained had a mean lateral size (LS) and thickness (TN) of 1.14 ± 0.32 μm and 7.7 ± 0.6 nm, respectively. The as-synthesized NPLs had a highly crystalline phase of α-quartz, exhibiting excellent structural and thermal stability under basic conditions. The α-quartz NPLs were used to extract astaxanthin (ATX; a high-value red antioxidant pigment) from H. pluvialis, which is a representative commercial green microalgae with a robust, trilayered cell wall. An ATX extraction efficiency of ~99% (18.0 ± 0.6 mg ATX/g cell) was achieved from mild ultrasoundication of an α-quartz NPLs and H. pluvialis mixture for < 5 min followed by multiple phase separation–extraction processes with minimal cell damage. The α-quartz NPL dosage, i.e. the amount of α-quartz NPL required to achieve the
maximum ATX extraction, was determined to be 800.0 mg/L, which was considerably less than an excess of 10 g/L carbon nanodots necessary for disrupting the cell wall of *H. pluvialis* and extracting an equivalent amount of ATX [61]. The enhancement effects of α-quartz NPL on the recovery of ATX from *H. pluvialis* were rationalized by the characteristic anisotropic structure of α-quartz NPL that facilitated the laceration of the cell wall.

2. Experimental Section

2.1. Materials

Tetramethyl orthosilicate (TMOS, ≥99% Aldrich) and tetraethyl orthosilicate (TEOS, ≥99% Aldrich) were purchased and used as received. All other chemicals including methanol, absolute ethanol, acetone, ammonium hydroxide aqueous solution (NH₄OH, 25 wt%, Daejung), 1 N sodium hydroxide solution (NaOH, Samchun), and 1 N hydrochloric acid solution (HCl, Samchun) were of analytical ACS Regent grade and used as received.

2.2. Preparation of α-quartz NPLs

Stöber et al. reported the synthesis of ASNs via a base-catalyzed hydrolysis and condensation of TEOS [62], and a modified version of this method was used to synthesize monodisperse ASNs, which were then used as precursors for synthesizing α-quartz NPLs [63]. First, 0.225 g of 60 nm diameter ASNs and 3 mL of 1 N NaOH solution were added to 27 mL of triply deionized water and stirred vigorously at 400 rpm for one hour at 100 °C. The solution (pH ≥ 12) changed from a whitish turbid to a clear liquid after ~20 min, which was due likely to the dissolution of ASNs. After stirring for 1 h, ~1 mL of a 1 N hydrochloric acid (HCl) solution was added to neutralize the solution partially, which retained a pH of ≥ 12. The resulting clear suspension was likely composed of silicic precursor ions formed by the complete dissolution of ASNs. The solution was then transferred to a 100 mL polyether ether ketone (PEEK)-lined stainless-steel autoclave and heated at 250 °C under autogenic pressure with magnetic stirring at under 800 rpm for four hours. Once the reaction was complete, the autoclave was cooled immediately to room temperature. The solution was then filtered through a 0.2 μm nylon membrane, washed with an excess of absolute ethanol and triply deionized water, and dried in a vacuum oven at room temperature to provide ~30 mg of α-quartz NPLs as a white powder.

2.3. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray powder diffraction (XRD)

A drop of α-quartz NPL solution diluted in absolute ethanol was placed onto a silicon wafer (4Science Co, Korea) or a holy carbon Quantifoil® TEM grid (Ted Pella, Inc., USA), which had been previously undergone a hydrophilic surface treatment by glow discharge, and dried under vacuum overnight for field emission scanning electron microscopy (FESEM) or transmission electron microscopy (TEM) observations, respectively. The sizes and morphologies of the α-quartz NPLs were investigated by FESEM (Supra 40, Zeiss International, Germany) operated at an accelerating voltage ranging from 5 to 10 kV and by TEM (Hitachi H-7600, Hitachi High-tech, Germany) operated at an accelerating voltage of 80 kV. High-resolution TEM (HRTEM) and selected area electron diffraction (SAED) (TALOS F200X, FEI & Thermo Fisher Scientific Electron Microscopy Solutions, USA) analyses were performed at an accelerating voltage of 200 kV. aberration-corrected HRTEM imaging (ARM-200F, JEOL Ltd., Japan) was performed at the National Center for Inter-University Research Facilities (Seoul, Korea). The ARM-200F HRTEM microscope was equipped with an aberration corrector in the objective lens and an OneView camera (Gatan, Inc., USA). To minimize the electron-beam-induced amorphization of α-quartz NPLs, it was operated at an acceleration voltage of 60 kV with an electron dose of <~5 e/Å² to acquire each HRTEM image. The atomistic models of α-quartz NPLs were obtained using Visualization of Electronic and STructural Analysis (VESTA) software. The chemical composition of α-quartz NPLs was analyzed simultaneously using an energy-dispersive X-ray spectroscopy (EDS) detector attached to a Supra 40 FESEM (Zeiss International, Germany). X-ray powder diffraction (XRD, X’Pert-MPD, Philips Inc., USA) of α-quartz NPLs was performed using monochromatic Cu Kα radiation (λ = 0.15405 nm) operated at 1.2 kW power (40 kV, 30 mA). Custom XRD simulations were conducted using a supercell composed of layered α-quartz nanoplatelets of 1, 8.6, or 15 nm in thickness, between a vacuum slab with a 0.1 – 1 nm spacing using VESTA software [64].

2.4. Fourier transform infrared (FT-IR) and Raman spectroscopy

Fourier transformation infrared spectroscopy (FT-IR, Spectrum GX, Perkin Elmer Inc., USA) of α-quartz NPLs and ASNs was performed using KBr pellet and attenuated total reflection (ATR) techniques. Raman spectroscopy (Nanofinder 30, Tokyo Instrument Inc.) of α-quartz NPLs and ASNs was performed on a micro-Raman spectrometer system using a laser excitation wavelength of 532 nm and reduced laser power (~7 mW) to avoid heating effects under a focused laser beam at the Korea Basic Science Institute (KBIS, Jeonju Center, Korea). Each Raman peak was scanned 20 times using an 1800 G/mm grating at a spectral resolution of 0.92-cm⁻¹/pixel. The measured Raman shifts provided are the means of these measurements, and the errors are reported as ± two standard deviations of the mean.

2.5. Thermogravimetric analysis (TGA)

Thermal analyses of α-quartz NPLs and ASNs were carried out using a thermal gravimetric analysis/differential thermal analysis (TGA/DTA) analyzer (SDT Q600, TA Instrument Inc., USA). Typically a ~10 mg sample was heated in an Al₂O₃ crucible at a heating rate of 5 °C/min under an N₂ gas flow rate of 100 mL/min up to 750 °C.

2.6. ²⁹Si solid-state nuclear magnetic resonance (ssNMR) spectroscopy

²⁹Si solid-state nuclear magnetic resonance (ssNMR) spectroscopy (400 MHz AVANCE III HD, Bruker Corporation) of α-quartz NPLs and ASNs was performed using a 4 mm (outer diameter of a zirconia rotor) magic angle spinning (MAS) probe at the Korea Basic Science Institute (KBIS, Western Seoul Center, Korea). The spectra were obtained using direct excitation at 79.51 MHz with a 1.6 μs pulse width (pulse angle x/6), a 50 s recycle delay at ambient probe temperature (~25 °C), and a sample spin rate of 11 kHz. The signals from 4900 scans were accumulated. The 29Si NMR chemical shifts (δ in ppm) were referenced to an external sample of tetrakis(trimethylsilyl) silane at ~135.5 ppm with respect to tetrasmethylsilane (TMS) at 0.0 ppm.

2.7. X-ray photoelectron spectroscopy (XPS)

XPS (ESCALAB 250, Thermo Fisher Scientific Inc., USA) of α-quartz NPLs and ASNs was performed using a monochromatic X-ray excitation source of Al anode Kα radiation (1486.6 eV). The binding energies were calibrated versus the C 1 s line of carbon at 284.6 eV before the actual measurements.

2.8. Nitrogen (N₂) physisorption experiments for Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) analysis

N₂ adsorption–desorption measurements of α-quartz NPLs and ASNs were performed using an Autosorb-iQ surface area and pore size analyzer (Quantachrome Instruments, USA). Samples of α-quartz NPLs and ASNs were degassed at 300 °C for ~3 h before the measurements. The BET method was used to calculate the specific surface areas. The
pore size distributions of α-quartz NPLs and ASNs were calculated from the desorption branches of their isotherms using the BJH method.

2.9. Culture conditions of microalgae H. pluvialis cells

The H. pluvialis NIES-144 cyst cells used in this study were obtained from the National Institute for Environmental Studies (NIES), University of Tokyo, Japan. The NIES-C medium [45,49] was used for the photoautotrophic cultivation of this strain and contained the following (per liter): 0.15 g Ca(NO$_3$)$_2$, 0.10 g KNO$_3$, 0.50 g tris(hydroxymethyl)amino- nethane (Tris), 0.05 g β-glucoronaphosphate acid disodium salt pentahydrate, 0.04 g MgSO$_4$·7H$_2$O, 0.01 mg thiamine, 0.10 μg biotin, 0.10 μg vitamin B$_1$, and 3.00 mL PIV metal solution. One liter of PIV metal solution consisted of 1.0 g Na$_2$EDTA, 0.196 g FeCl$_3$·6H$_2$O, 36.0 mg MnCl$_2$·4H$_2$O, 22.0 mg ZnSO$_4$·7H$_2$O, 4.0 mg CoCl$_2$·6H$_2$O, and 2.5 mg Na$_2$MoO$_4$·2H$_2$O. After adjusting its pH to 7.5, the NIES-C medium was sterilized by filtration through a 0.2 μm polytetrafluoroethylene (PTFE) membrane filter and kept at 4 °C for two hours in the dark to allow saponification. Bead-beater assisted solvent extraction was repeated three more times until all of the red-colored cyst cells were colorless. The ATX extracts were filtered through a 0.2 μm polytetrafluoroethylene (PTFE) membrane filter and analyzed using a quantum photometer (LI-250A, LI-Cor Inc., USA) and a pH meter (HM-30R, TOADKK, Japan), respectively. Detailed of the culture conditions are reported elsewhere [45,61].

For the α-quartz NPL extraction experiments, mature H. pluvialis cyst cells (obtained by cultivation for 90 days) were harvested by centrifugation at 4,000 rpm for 10 min (Combi-S14R, Hanil Science Inc., Korea) and freeze-dried for three days (FD5512, IShhin BioBase Co., Korea). The lyophilized cyst cells were preserved in a vacuum bag at −20 °C until further use.

2.10. Determination of the total astaxanthin (ATX) content from H. pluvialis

The total ATX contents of lyophilized H. pluvialis cyst cells were determined by bead-beater assisted solvent extraction and high-performance liquid chromatography (HPLC; Agilent 1260 infinity, Hewlett Packard, USA). Briefly, 1 mL of dichloromethane/methanol (DCM/MeOH; 1:1, v/v) containing 0.025 N NaOH and 1.0 g glass beads (1.5 mm diameter, Daihan Scientific, Korea) were added to −10 μg of cyst cells in a 1.5 mL bead beating tube, and then vigorously homogenized using a FastPrep-24™ bead beater (BM Biomedicals, USA; 6 m/s for 30 s and three times). After centrifugation at 10,000 rpm for 3 min (Sorvall Legend Micro 17R, Thermo Fisher Scientific, USA), the ATX-containing solvent layer was collected in a new tube and kept at 4 °C for two hours in the dark to allow saponification. Bead-beater assisted solvent extraction was repeated three more times until all of the red-colored cyst cells were colorless. The ATX extracts were filtered through a 0.2 μm polytetrafluoroethylene (PTFE) membrane filter and analyzed by HPLC, as described previously [61]. The mean ATX content of the H. pluvialis cyst cells was determined to be 18.2 ± 0.6 mg/g cell. HPLC analysis was used to identify the other ketocarotenoid pigments without the saponification reaction. The carotenoid reference standards astaxanthin, canthaxanthin, lutein, neoxanthin, and zeaxanthin were used as supplied (Sigma Aldrich, USA).

2.11. Cell disruption and α-quartz NPL assisted ATX extraction from H. pluvialis

An algal solution of 800 mg cell/L, which was considered to be typical for H. pluvialis cell concentrations, was prepared using freeze-dried H. pluvialis and purified Milli-Q® water under ambient conditions [65]. This solution was then mixed vigorously (Vortex 3, IKA, Germany), and 5 mL aliquots were dispensed into 12 mL Pyrex-glass tubes with a Teflon-sealed screw stopper. α-Quartz NPLs (α-quartz NPLs recovery was repeated three times in duplicate. The average values were subjected to analysis, and the measurement errors were < +/- 5%.

2.13. Morphological characterisation of H. pluvialis before and after α-quartz NPLs treatment

The morphological features of H. pluvialis cyst cells, i.e., color, cell size, and cell wall thickness, were investigated using an Axio Imager.A2 optical microscope equipped with ZEISS Zen software (Carl Zeiss, Germany) [45]. Detailed surface morphologies before and after the α-quartz NPL treatment were examined by scanning electron microscopy (SEM; FEI Quanta 250 FEG, Thermo Fisher, USA). The samples for SEM...
analyses were fixed in a 1.5% paraformaldehyde-glutaraldehyde fixation solution, pasted on a glass plate, air-dried at ambient temperature, set in a sample holder, and coated with platinum at ~8 nm by sputtering for 80 s (Quorum Q150T S, UK).

2.14. Flow cytometry of H. pluvialis before and after α-quartz NPLs treatment

The cell disruption level caused by the NPL treatment was assessed using a flow cytometer (Facscanto II, Becton-Dickinson, USA) equipped with an argon-ion laser (excitation 488 nm, 20 mW power) and fluorescent phycoerythrin (PE) filter. The instrument was set up to measure the linear forward light scatter (FSC, a measure of cell size) and 90° side light scatter (SSC, a measure of cell granularity).

2.15. Zeta-potential measurements of H. pluvialis before and after α-quartz NPLs treatment

The zeta-potentials were measured in triplicate using a Zetasizer equipped with a He-Ne laser at 633 nm (Nano ZS90, Malvern Instruments, UK) at ambient temperatures.

2.16. Statistical data analysis

SPSS Statistics 17.0 software (IBM 173 Co., USA) was used for the data analysis. The experimental results are presented as the means ± standard deviations of three independent experiments. A student’s t-test was conducted using Microsoft Excel®, and P values<0.05 were considered statistically significant (*P<0.05).

3. Results and discussion

3.1. FT-IR, 29Si ssNMR, Raman, XPS, and TGA analysis of α-quartz NPLs

The FT-IR spectra of the as-synthesized α-quartz NPLs (Fig. 1A) exhibited several characteristic IR spectral bands. The IR absorption bands at 3437 and 3242 cm⁻¹ were assigned to the O–H stretch of surface silanol and adsorbed H₂O [66]. The band at ~1638 cm⁻¹ was assigned to the bending vibration of adsorbed H₂O [66]. The main IR bands at 1104, 800, and 474 cm⁻¹ were attributed to the asymmetric and symmetric stretch vibrations, and symmetric bending vibrations of the Si-O-Si bonds, respectively [67,68]. Overall, the characteristic IR bands of α-quartz NPLs were similar to those of the 60 nm diameter ASNs used as the starting materials (Fig. 1A) except for a missing band at 946 cm⁻¹, which was attributed to Si-OH bond stretch [69]. Although the

Fig. 1. (A) FT-IR, (B) 29Si ssNMR, (C) Raman, and (D) XPS spectra of α-quartz NPLs (blue) and ASNs (black). (B) 29Si ssNMR and (C) Raman spectroscopy results clearly distinguished the crystalline and amorphous phases, and results were consistent with those obtained by XRD and SAED analysis (Fig. 3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
reasons for the absence of the band at 946 cm\(^{-1}\) could not be determined, there may be fewer free hydroxyl groups on the surfaces of α-quartz NPLs than on those of the ASNs.

Fig. 1B shows the \(^{29}\)Si solid-state nuclear magnetic resonance (ssNMR) spectra of α-quartz NPLs and 60 nm diameter ASNs. The spectrum of α-quartz NPLs (blue curve) was dominated by three characteristic peaks at \(-112.29\) (including shoulders at \(-116.94\) and \(-118.75\), \(-99.96\), and \(-94.16\) ppm, which were assigned to non-hydroxy terminated Q\(_4\) ((-O)\(_4\)Si), monohydroxy terminated Q\(_3\) ((-O)\(_3\)Si(OH)), and dihydroxy terminated Q\(_2\) ((-O)\(_2\)Si(OH))\(_2\) sites, respectively. A few aspects of the spectra of α-quartz NPLs and ASNs are worth addressing. First, the spectral width of the α-quartz NPLs was much narrower than that of the 60 nm diameter ASNs (i.e., full width at half maximums (FWHMs) of α-quartz NPL peaks were < 42% of those of ASNs), indicating that variations in Si-O-Si angles from those in crystalline NPLs with ordered silica networks were smaller than those of ASNs with disordered networks (see Fig. S1A, Supplementary material). Second, when the fractions of Q\(_n\) sites (n = 2, 3, and 4) in α-quartz NPLs and ASNs were determined from deconvoluted Lorentzian-Gaussian peak areas in the \(^{29}\)Si ssNMR spectra (indicated by multi-color curves in Figs. S1B and S1C, Supplementary material, and summarized in Table S1, Supplementary material). The Q\(_2\) values for α-quartz NPLs were 0.9% for Q\(_2\), 7.6% for Q\(_3\), and 91.5% for Q\(_4\) sites, while those of ASNs were 4.6% for Q\(_2\), 37.3% for Q\(_3\), and 58.1% for Q\(_4\) sites. The 60 nm diameter ASNs would have larger Q\(_3\) and Q\(_2\) values than α-quartz NPLs because of their higher surface to volume ratios and smaller sizes. On the other hand, these values compared reasonably well with the 27.5% for Q\(_3\) and 2% for Q\(_2\) of silica nanoparticles [70], and the 35.3% for Q\(_3\) and 4.7% for Q\(_2\) of quartz nanocrystals [32] having many surfaces along, or close to, lower energy (1 0 1) planes via structural reorganizations when their sizes were reduced to the nanometer length scale. The Q\(_2\) + Q\(_3\) to Q\(_4\) ratio of α-quartz NPLs was 0.09, which was ~7.8 times smaller than the 0.72 of ASNs (Table S1, Supplementary material). By applying a simple scaling approach based on the geometric structures (Fig. S2, Supplementary material), Q\(_2\) + Q\(_3\) to Q\(_4\) ratios of α-quartz NPLs and ASNs, which were proportional to the surface to volume contribution, were estimated based on the assumption that free surface hydroxyl groups were distributed uniformly at both the Q\(_2\) and Q\(_3\) sites. Fig. S3 shows the calculated Q\(_2\) + Q\(_3\) to Q\(_4\) ratios as a function of the sizes of ASNs, α-quartz NCs, and α-quartz NPLs on this scaling approach (Supplementary material). The estimated ratios agreed with the ratios obtained from the \(^{29}\)Si ssNMR measurements provided the lateral sizes of NPLs were larger than ASN diameters by 1 – 2 orders of magnitude. This difference in the length scale was consistent with the FESEM, TEM, and atomic force microscopy (AFM) results (Fig. 2). Therefore, the lower ratio of Q\(_2\) + Q\(_3\) to Q\(_4\) sites of α-quartz NPLs was attributed to only one length scale of the anisotropic nanoplates reduced in nanometers versus the concurrence of all three nanometer scales of isotropic nanoparticles.

![Fig. 2.](image-url) Representative FESEM (A) and TEM (B) micrographs of as-synthesized α-quartz NPLs. (C) Representative AFM height images of α-quartz nanoplates and their height profiles showing layered α-quartz NPLs. (D) The corresponding distributions of the measured lateral sizes (LS) (mean 1.14 ± 0.32 μm, based on 100 counts) and thicknesses (TN) (mean 7.7 ± 0.6 nm, based on 50 counts) of α-quartz NPLs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
In addition, this decrease in ratio suggested that α-quartz NPLs had fewer free surface hydroxyl groups than ASNs, which is also consistent with the observed absence of the characteristic IR band (946 cm\(^{-1}\)) due to the surface hydroxyl groups for α-quartz NPLs.

Fig. 1C shows the Raman spectra of the as-synthesized α-quartz NPLs (blue curve) and ASNs (black curve). Bulk crystalline α-quartz has two enantiomorphs with the space group \(P\overline{3}21\) (right-handed) and \(P\overline{3}21\) (left-handed). The irreducible representation of the optical vibration for the \(D_3\) group may be expressed as \(\Gamma_{\text{vib}} = 4A_1 + 4A_2 + 8E\), where the \(A_1\) modes are Raman-active, \(A_2\) modes are IR-active, and the \(E\) modes are both Raman- and IR-active. The \(A_2\) and degenerate \(E\) modes are split by long-range Coulomb forces into longitudinal and transverse optical (LO and TO) components \([71]\). Bulk crystalline α-quartz has characteristic Raman bands at 128 (strong), 207 (strong), 265 (medium), 356 (very strong), 464 (strong), 795 (very weak), and 1085 cm\(^{-1}\) (very weak), which are due to vibrations based on the assigned symmetries of \(E(\text{LO} + \text{TO}), A_1\), \(E(\text{LO} + \text{TO}), A_2\), \(E(\text{TO}), A_1\), \(E(\text{TO}), A_1\), \(E(\text{TO}), A_1\), respectively \([71,72]\). The principal Raman modes of as-synthesized α-quartz NPLs observed at 238 and 466 cm\(^{-1}\) in Fig. 1C were attributed to the Raman-active \(A_1\) modes. The other Raman bands from the bulk α-quartz were not detected for α-quartz NPLs. Moreover, the Raman spectrum of the crystalline α-quartz NPLs (blue curve) differed appreciably from that of the ASNs (black curve). The key differences included the overall shape of the spectra, the missing \(A_1\) mode Raman band at 238 cm\(^{-1}\) (medium), and the location of the \(A_1\) mode Raman band (i.e., 466 cm\(^{-1}\) (strong) vs. 483 cm\(^{-1}\) (strong)). These bands were attributed to differences in the degree of crystallinity and structural anisotropy. Overall, Raman spectroscopy provided a better means of differentiating the crystalline and amorphous phases than IR.

Fig. 1D presents the survey XPS spectrum of the as-synthesized α-quartz NPLs. The survey XPS spectra over the full energy range confirmed the existence of common elements such as Si and O and the survey XPS spectrum of as-synthesized α-quartz NPLs resembled that of ASNs. High-resolution XPS scans of the as-synthesized α-quartz NPLs and ASNs (Fig. S4, Supplementary material) provided the following binding energies (BEs): Si 2p peaks at 103.4 and 103.2 eV and O 1 s peaks at 532.6 and 532.4 eV, respectively. Although both peaks of α-quartz NPLs were located at marginally higher BEs than those of ASNs, the BEs were reasonably consistent with the values reported for bulk quartz \([73]\), amorphous silica nanoparticles \([63,74,75]\), and mesoporous silica \([76,77]\).

TGA of the as-synthesized α-quartz NPLs and ASNs (Fig. S5, Supplementary material) showed that α-quartz NPLs had excellent thermal stability, i.e., it showed less than 4% weight loss compared to ASNs over the temperature range from 50 to 800 °C. Although the FT-IR, XPS, and TGA results indicate that the overall chemical environment of α-quartz NPLs is similar to that of ASNs, Raman and \(^{29}\)Si ssNMR spectroscopy differentiated the amorphous and crystalline forms of silica.

### 3.2. Structural analysis of α-quartz NPLs by FESEM, TEM, and AFM

Fig. 2A, 2B, and 2C present FESEM, TEM, and AFM micrographs of

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**Fig. 3.** (A) Powder XRD patterns of the as-synthesized α-quartz NPLs (black curve), bulk α-quartz (red curve), and simulated XRD patterns of layered α-quartz NPLs (blue curve). (B) Bright-field TEM image of a α-quartz NPL selected for diffraction analysis (left) and its corresponding selected area electron diffraction (SAED) pattern (right). (C) Representative HRTEM image of a α-quartz NPL. (D) Higher magnification HRTEM micrograph of single-crystalline α-quartz NPL (left) and its corresponding atomistic model projected along the \([\overline{1}1\overline{1}]\) direction showing the \([01\overline{1}]\) and \([0\overline{1}1]\) planes of α-quartz running diagonally at a dihedral angle of 85.7°. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
the as-prepared α-quartz NPLs with either a plate- or sheet-like morphology with a relatively uniform size distribution. The average lateral size (LS) and thickness (TN) of the α-quartz NPLs samples measured by FESEM, TEM, and AFM were 1.14 ± 0.32 µm and 7.7 ± 0.6 nm, respectively.

The XRD patterns of the α-quartz NPLs demonstrated their crystalline nature (Fig. 3A). Most peaks in the XRD pattern (black curve) were in accordance with that of bulk α-quartz (International Centre for Diffraction Data (ICDD) card no. 00-046-1045 and Joint Committee on Powder Diffraction Standards (JCPDS) card no. 46–1045). Other unassigned peaks were observed at low diffraction angles (0 < 2θ < 20), and a peak due to the (1 0 1) reflection showed a slight shift in its position. The simulated XRD pattern (blue curve) did not reproduce any peaks in this low 2θ range except when a vacuum slab was introduced above and below the layer of the α-quartz NPL in a supercell (Experimental Section). Although the positions of low angle peaks were not matched precisely, the best simulated XRD pattern was obtained for a 15 nm thickness of NPL with a 0.1 nm vacuum slab spacing gap. This suggests that α-quartz NPLs were likely stacked and in a powdery state presumably due to hydrophilic surface interactions.

Fig. 3B presents the selected area electron diffraction (SAED) pattern of a typical α-quartz NPL of a single crystal, as indicated by a well-ordered pattern of diffraction spots, which were assigned to the (101), (200), (101), and (01T) planes based on the d-spacings calculated from its SAED pattern (Table 1). These d-spacings matched the reflections of bulk α-quartz closely and were in good agreement with the XRD results (Fig. 3A).

The HRTEM micrographs in Fig. 3C and 3D show lattice fringes of the α-quartz NPLs consistent with an almost defect-free, single-crystalline domain with dimension up to a few µm. The interplanar distances of the lattice fringes shown in Fig. 3D were 0.334 and 0.336 nm, which were assigned to those of the (101) and (01T) planes of α-quartz, respectively. These two planes were running along diagonally with a dihedral angle of 85.7°. These findings agree well with a reported interplanar spacing of 0.334 nm and an angle of 86° for bulk α-quartz crystal, and are consistent with the lattice structure of α-quartz NPL. Fig. 3D shows the atomic arrangement projected along the [11T] zone axis [78,79].

### 3.3. Microalgae extraction experiment

The effects of the α-quartz NPLs on the extraction and recovery of ATX from H. pluvialis cysts were investigated by bright-field optical microscopy, FESEM, flow cytometry, HPLC, and using zeta-potential measurements.

Fig. 4 shows the typical cell size and cell wall thickness distributions of H. pluvialis cysts as determined by optical microscopy and image analysis. The cell diameter of H. pluvialis cysts cell ranged from 17 to 65 µm (average diameter of 31.5 ± 10.4 µm), indicating a heterogeneous cell population under typical culture conditions. Nevertheless, this distribution concurred with that reported for H. pluvialis (i.e. average diameter of 34.8 ± 5.6 µm) cultured under a red light using a bubble-column photobioreactor [80]. The average cell wall thickness of the H. pluvialis cysts cells was 2.8 ± 1.2 µm, which was comparable to that of the H. pluvialis cyst cells cultured for 2 – 3 weeks [81].

Fig. 5 presents a set of in situ optical microscopy images selected from a time sequence. The images reveal a multi-stage biorefinery process comprised of three distinct stages: (1) mature H. pluvialis cysts growth, (2) fractional disruption of cell walls induced by α-quartz NPLs under ultrasonic conditions, and (3) recovery of ATX by the solvent extraction of ATX-containing lipid droplets likely at sites lacerated by α-quartz NPLs. As shown in Fig. 5B, 5C, and 5D, mature H. pluvialis cyst cells appeared to be intact under 5 min of ultrasonication (Fig. 5B). Furthermore, even after 30 min extraction with anhydrous dichloromethane (DCM)/methanol (MeOH), the cell shapes, morphologies, and color intensities remained unchanged (Fig. 5C and 5D). In contrast, after adding α-quartz NPLs, the intracellular circular domains containing red-ATX-containing lipids appeared to be slightly smaller, and the intracellular contrast of the region between the outer perimeter of these circular domains and cell walls was altered significantly (Fig. 5B’).

Rugged granular patterns were visible within the region between the outer perimeter of these circular domains and cell walls under typical brightfield illumination or showed in differential interference contrast imaging, probably due to the light scattering effects of α-quartz NPLs. On the other hand, finer details were not observable because of the resolution limit of optical microscopes. These observations indicate that some α-quartz NPLs penetrated and accumulated within cell walls, which is in-line with the FESEM measurements shown in Fig. 6. After introducing the DCM/MeOH, red-ATX-containing lipid droplets began to emerge from the cyst cells (Fig. 5C’). In particular, after an extended extraction period of 30 min, almost all ATX was released by the α-quartz NPL treated cells (Fig. 5D’). In contrast, no ATX was retrieved from the cells not treated with α-quartz NPLs, and the cell structural integrities were preserved (Fig. 5D).

Ex situ FESEM analysis confirmed the presence of α-quartz NPLs either at or in the vicinity of the H. pluvialis cysts (Fig. 6; see Experimental Section for details of the procedure). Fig. 6C and 6D show the ATX-containing lipid residues and α-quartz NPLs embedded in the cell walls during extraction, indicating that ATX-containing lipids were likely to have been released from the lesions generated in cell walls by α-quartz NPLs.

Fig. 5 presents the ATX extraction efficiencies from the 90 day-cultured H. pluvialis cyst cells after treatments with α-quartz NPLs. The relative population P1 identified from the measurements gradually increased by ~10% after 5 min of ultrasonication in the absence of α-quartz NPL but increased by ~23% in their presence, whereas relative population P2 showed a continuous decline, indicating the extent of algal cell disruption. The forward versus side scatter profiles of P1 and P2 treated with α-quartz NPLs showed a slight shift to the left (i.e., to a smaller size) as compared to the cells exposed to ultrasonication alone, indicating a surge of forming smaller cells and cell debris from α-quartz NPLs-assisted algal cell disruption. These observations are consistent with the optical microscopy and FESEM results (Figs. 5 and 6, respectively).

Fig. 7 presents the ATX extraction efficiencies from the 90 day-cultured H. pluvialis cyst cells after treatments of α-quartz NPLs. When the cyst cells were ultrasonicated for 5 min without anisotropic α-quartz NPLs or 180 nm diameter isotropic ASNs and then extracted for 30 min with anhydrous DCM/MeOH (1:1, v/v), the ATX extraction efficiencies were 8.6 ± 0.9% and 7.0 ± 0.7%, respectively. Treatment with α-quartz NPLs improved extraction efficiency to 35.4 ± 0.5% significantly. Furthermore, the extraction efficiency of α-quartz NPLs increased gradually with increasing concentration before saturation at an α-quartz NPL concentration of > 800 mg/L (Fig. 5B, Supplementary material).

These results show that ultrasonication, followed by solvent extraction alone, did not result in effective ATX recovery. Moreover, the α-quartz

### Table 1

Summary of the diffraction analysis results and assignments for α-quartz NPLs.

<table>
<thead>
<tr>
<th>Measured diffraction SAED (1/1 nm)</th>
<th>Measured d-spacing SAED (1/nm)</th>
<th>Assignment</th>
<th>Measured diffraction XRD (2θ / intensity))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.994</td>
<td>0.334</td>
<td>α-Quartz (101)</td>
<td>25.8, 26.6(s)</td>
</tr>
<tr>
<td>2.994</td>
<td>0.334</td>
<td>α-Quartz (101)</td>
<td>25.8, 26.6(s)</td>
</tr>
<tr>
<td>3.63</td>
<td>0.423</td>
<td>α-Quartz (101)</td>
<td>20.8(m)</td>
</tr>
<tr>
<td>4.732</td>
<td>0.221</td>
<td>α-Quartz (201)</td>
<td>42.5(w)</td>
</tr>
</tbody>
</table>

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NPLs were responsible for the observed efficiency improvement. Furthermore, the structural anisotropy of ultrathin α-quartz NPLs might play a critical role in the laceration of the cell wall.

Multiple solvent extractions are always more efficient than a single extraction, particularly if the algal compound does not have an extremely high partition coefficient in the organic solvent. Fig. S9 (Supplementary material) shows ATX extraction efficiencies after three cycles of α-quartz NPL-assisted cell wall disruption followed by anhydrous DCM/MeOH (1:1, v/v) extraction. The extraction efficiency in the absence and presence of α-quartz NPLs using DCM/MeOH (1:1, v/v) increased marginally from 5.8 ± 0.1% (single extraction) to 26.0 ± 0.5% (triple extraction) and 35.4 ± 2.5% (single extraction) to 58.9 ± 9.5% (triple extraction), respectively. In addition, the extraction efficiency of single ATX extraction with α-quartz NPLs improved from of 5.8 ± 0.1% to 53.9 ± 5.8% when sufficient H2O was added to cause DCM/MeOH-H2O (1:1 v/v) aqueous-organic phase separation (see Fig. S9, Supplementary material and Fig. 7B). Surprisingly, the extraction efficiency increased dramatically from 53.9 ± 5.8% to 98.9 ± 3.1% when the phase separation-extraction process was repeated three times (Fig. 7B), indicating almost complete ATX recovery from the H. pluvialis cysts.
3.4. Discussions

3.4.1. Formation of two-dimensional α-quartz NPLs from the dissolution of ASNs under hydrothermal conditions

Several control experiments were performed to confirm the dissolution of ASNs by NaOH to assess the formation of α-quartz NPLs. First, no α-quartz NPLs were formed when the ASNs did not react with NaOH and were not partially neutralized with HCl under hydrothermal conditions. Second, almost no α-quartz NPLs formed when the ASNs were replaced with organosilicon-based precursors, such as TMOS, TEOS, and silane coupling reagent 3-aminopropyl triethoxysilane (APTES), which are typically used to synthesize and functionalize silica nanoparticles. Third, no α-quartz NPLs were produced when the NaOH to HCl molar ratio deviated from 3:1. These results suggest that an optimal amount of hydroxyl ions is needed to produce specific silicic precursor ions from ASNs. Moreover, these precursors are responsible for the nucleation and growth of α-quartz NPLs.

It is unclear why the dissolution of ASNs followed by the hydrothermal conditions leads to the formation of the anisotropic nanoplates rather than the isotropic nanoplates of α-quartz in the absence of growth modifying additives. Only two possible mechanisms could result in the preferential two-dimensional anisotropic growth of crystals considering that typical crystals grow with a very thin plate- or sheet-like morphology despite belonging to a crystal class exhibiting a natural habit that is fairly uniform in dimensions along all three principal axes. One involves the selective poisoning of particular crystal faces by ions or surfactants, for example, which force the crystal to adapt a plate-like morphology [82,83]. The other involves the specific interface where the growth actually occurs, preventing growth from occurring perpendicular to the nanoplates faces [84–87]. This system did not contain any surfactant. Hence, the second mechanism is favored, suggesting that the liquid–vapor interface potentially formed in the autoclave reactor under these hydrothermal conditions could well have a very steep temperature or concentration gradient perpendicular to the interface. Moreover, these gradients would likely cause growth to occur in the plane of the interface.

3.4.2. α-Quartz NPLs assisted microalgal extraction

The ability of microalgal cells to resist rupture by mechanical stimuli was attributed to the mechanical strength and thickness of the cell walls [88,89]. Table S3 (Supplementary material) provides a comparison of the physical and compositional characteristics of four algal cell walls and associated biotechnological applications. Note that the thickness of the *H. pluvialis* cyst cell wall ranges from 1.8 to 2.2 µm and is
Fig. 7. Astaxanthin (ATX) extraction efficiencies, expressed as percentages of the amount of ATX extracted from _H. pluvialis_ cyst cells, after α-quartz NPL treatment. (A) ATX extraction efficiencies were determined from a single 5 min ultrasonication cycle followed by extraction for 30 min by anhydrous DCM/MeOH (1:1, v/v) in the absence of neither ASNs nor α-quartz NPLs (Control, red bar), and in the presence of spherical ASNs (NP, green bar) or α-quartz NPLs (NPL, blue bar) at room temperature. (B) Comparison of (left) single and (right) multiple cycles of 5 min ultrasonication followed by 30 min DCM/MeOH/H₂O (1:1:2, v/v/v) extraction in the absence (Control, red bar) and the presence of α-quartz NPLs (NPL, blue bar). The superscripts indicate the numbers of cycles. The error bars represent the standard deviations of three independent experiments and the asterisks show the significances of differences (*, _P_ < 0.05; **, _P_ < 0.005; ***, and _P_ < 0.001). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

significantly greater than the those of _Chlamydomonas_ [90], _Chlorella_ [89], and _Nannochloropsis_ [89] (~351, ~88, and ~15 nm, respectively), which are used in widely different microalgae biorefinery purposes. Mature _H. pluvialis_ cysts develop a highly robust, three-layered rigid cell wall and synthesize various secondary ketocarotenoid pigments, such as neoxanthin, lutein, canthaxanthin, zeaxanthin, and ATX, under favorable conditions during photosynthetic growth [91]. Fig. S7 (Supplementary material) shows HPLC chromatograms of the solvent extract of α-quartz NPLs-treated _H. pluvialis_ biomass. The peaks were resolved adequately based on the information of carotenoid reference standards, suggesting that α-quartz NPL does not have any specificity for extracting certain types of carotenoid pigments. The cell walls consist of multiple layers of sporopollenin material (algaenan), nonhydrolyzable aliphatic biopolymers, proteins, cellulose, mannan, and carbohydrates [92]. The mass of the cell wall of the _H. pluvialis_ cyst was reported to be approximately 15% of the total dry cell mass after three months of cultivation [81]. Therefore, the sturdy, complex structure of the _H. pluvialis_ cyst cell wall provides excellent resistance to mechanical and chemical perturbations and constitutes the main barrier to the recovery of cell-manufactured intracellular lipids and bioactive chemicals, making the extraction of ATX quite challenging.

Understanding the mechanism of α-quartz NPLs-assisted cell wall disruption requires a consideration of the elastic modulus, i.e., the ability of the cell walls to resist and recover from deformation under the impact of mechanical forces. In one study, a large number of stiffness measurements for algal cells showed values ranging from 1 and 100 MPa, and the average values were ~1000 times softer than those of terrestrial plants [93]. The bulk elastic moduli (Young’s moduli (Eᵥ)) of living cells are directly proportional to the ∆Ρ/(∆V/V) ratios (where ∆P is the change in cell turgor pressure and ∆V/V is the fractional change in cell volume). Applying this concept to a balloon and a soccer ball, the former has a low Eᵥ and its shape changes considerably under small loads, whereas deformation of the latter requires large loads. Because the change in internal pressure (∆P) for a given change in volume (∆V/V) is significantly smaller for a balloon than a soccer ball, a balloon model would appear to reflect the elastic properties of algal cells better because their cell walls are characterized by low stiffness and high extensibility [94]. Although a determination of the absolute Eᵥ values of living cells is difficult, the force-distance curves obtained by atomic force microscopy (AFM) provided estimated Eᵥ values of the algal cell walls between 0 and 25 MPa, as determined by Hertz contact mechanics [89,94–96]. Obataya et al. conducted force measurements on human epidermal melanocytes (Eᵥ = 2–10 kPa) in a physiological medium using conventional or ultrathin nanoneedle AFM probes [97]. Their results suggested that the impact forces and indentation depths required for cell membrane penetration were much smaller when the nanoneedle used for measurements had a sharper tip (<~250 nm diameter) and a high geometric aspect ratio (~7 μm in length) than a conventional pyramidal tip with a low aspect ratio [97]. When the same logic is applied to α-quartz NPLs, their extremely narrower thickness (<~10 nm thickness) and high geometric aspect ratio (~1 μm in lateral size) will enable them to indent or penetrate cell membranes easily. Although it is unclear if their interactions with membranes under mild ultrasonic agitation provide sufficient collisional energy to rupture the membranes, ultrasonic treatment in the presence of α-quartz NPLs facilitated the efficient extraction of ATX.

These findings showing enhanced ATX recovery from _H. pluvialis_ cysts by α-quartz NPLs/ultrasonication treatment before extraction raises the question as to why this process achieves much higher ATX extraction efficiencies than the extraction processes that do not use α-quartz NPLs. Organic solvents are detrimental to microalgal cells and cause them to shrink or swell and eventually cease functioning with nonnatural conformations [98]. In _H₂O_, cells maintain their structural conformations, and solvent molecules can transit the cell membranes because of their enhanced permeability when subjected to phase separation-extraction processes [99]. These findings show that the extraction of intracellular entities is enhanced considerably when the cell walls are lacerated with α-quartz NPLs.

Two factors would be expected to boost the cell wall lesion density, i.e., α-quartz NPL concentration and biphasic separation. As the concentration increased, the extraction efficiency increased asymptotically to saturation (Fig. S8, Supplementary material). Biphasic separation, which occurred upon the addition of _H₂O_ to DCM/MeOH, resulted in an upper aqueous layer containing _H₂O_, MeOH, α-quartz NPLs, and algal cells and a lower organic DCM layer containing the extracted ATX-rich lipids exclusively (see Fig. S13, Supplementary material). Owing to the intrinsic hydrophilicity of the cell walls of the _H. pluvialis_ cysts and the hydroxyl-terminated surfaces of α-quartz NPLs, as indicated by the ²⁵Si ssNMR results, zeta-potential measurements, and the dispersion behavior of α-quartz NPLs in aqueous solution (Figs. S1, S11, and S12, Supplementary material, respectively), the interaction between the cells and NPLs was sufficient to trap them on the cell walls (see Fig. 6B).
Based on the dielectric constants ($\varepsilon$) of H$_2$O, MeOH, and DCM at 25 °C, which are 80.1, 32.7, and 8.93, respectively, the overall $\varepsilon$ of the DCM/MeOH/H$_2$O mixture increased with increasing proportion of H$_2$O. This increase, which is an $\varepsilon$ value approximately three times than that of anhydrous DCM/MeOH, is likely to reduce the attractive affinity between the cells and NPLs and enable them to separate and disperse in an aqueous layer. Redispersing and ultrasonication the algal cell-$\alpha$-quartz NPLs precipitates in an aqueous solution will allow the NPLs to increase the rate of Brownian motion promoted by the turbulence of solvent flow and circulating eddies [100]. The resulting increase in NPL dynamic motion will boost the collision rate of NPLs on the cell membranes while preventing NPLs from adhering to the cell walls. Therefore, the repeated dispersion and ultrasonication steps of phase separation-extraction processes can increase not only cell wall lesion densities but also the ATX extraction and recovery efficiencies from cells with an almost normal cellular morphology.

4. Conclusions

This paper reported a template-free, solution-phase synthesis of two-dimensional ultrathin $\alpha$-quartz NPLs, 1-2 μm in lateral size and 7-8 nm in thickness, under relatively mild hydrothermal conditions (250 °C and autogenic pressure) in the absence of any growth modifiers or additives in ~4 h. The structure and composition of the $\alpha$-quartz NPLs were characterized using a battery of analytical techniques. The as-synthesized NPLs exhibited highly crystalline lattice structures that closely matched that of $\alpha$-quartz with excellent thermal and pH stability. In addition, $\alpha$-quartz NPLs were harnessed to recover ATX from microalgal H. plувialis cells with a sturdy cell wall. ATX was secured from H. pluvialis by < 5 min facile ultrasonication followed by multiple phase separation-extraction processes with an extraction efficiency of ~99% (18.0 ± 0.6 mg ATX/g cell). The amount of anisotropic $\alpha$-quartz NPL dosing for the maximum ATX extraction was determined to be 800.0 mg/L, which was substantially lower than the routine dosages of isotropic nanomaterials for extraction controls. The enhanced extraction efficiency and dosage were attributed to the characteristic anisotropic structure of ultrathin $\alpha$-quartz NPL that expedited the formation of cell wall lesions.

The findings of the present study are meaningful in two regards. First, they provide a facile, scalable, and cost-effective means of producing novel $\alpha$-quartz nanoplates of high crystal quality and relatively narrow size distribution using template-free, mild hydrothermal reactions, potentially offering new materials for manufacturing, engineering, optoelectronic, energy storage, pharmaceutical, and biomedical applications. Second, they reveal the previously overlooked importance of an anisotropic ultrathin plate-like nanostructure that endows quartz with superior cell wall disruption activity in the microalgal extraction processes under relatively moderate conditions. Typical biofouling processes involving microalgae systems prefer mechanical over chemical or biological methods that ultimately destroy the microalgae regardless of the species or biomass conditions. Furthermore, these mechanical processes require high-energy inputs to generate high shearing forces under abrupt pressure gradients, high turbulence, and excessive hydrodynamic cavitation. Therefore, they are unsuitable for the recovery of intracellular ATX, which is quite sensitive to physical and chemical environments under harsh conditions leading to oxidation and thermal degradation [50, 51, 101]. The use of $\alpha$-quartz NPLs, ultrasonic agitation, and biphasic extraction for ATX harvesting not only provide a potential means of mitigating the risks of degradation of bioactive ATX, but offers a functional nanomaterial-based engineering approach to the efficient, low cost, large-scale extraction of other valuable algal products from sustainable algal harvesting biofouling operations. Understanding the mechanism responsible for the formation of ultrathin $\alpha$-quartz NPLs and their role in the extraction of ATX from H. pluvialis would undoubtedly represent significant progress toward the design of highly efficient microalgal biofouling processes.

5. Data availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Appendix A. Supplementary data

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References


