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Biologically mediated silicification of marine cyanobacteria and implications for the Proterozoic fossil record

SUPPLEMENTAL INFORMATION

Supplemental methods

Organism collection, culturing, and enrichment

Pustular microbial mats made by coccoidal cyanobacteria and other organisms were collected from Hamelin Pool near Carbla Station in Western Australia. Small pieces of mat (~2.5 cm diameter pustules) were cut and scooped into sterile 50 mL Falcon® Centrifuge Tubes (VWR, Radnor, PA, USA, catalog # 21008-940) along with seawater. Samples were kept at room temperature during transport and were transferred to sterile culture jars containing modified hypersaline BG11 medium in the laboratory (recipe modified by Allen et al., 2009; Goh et al., 2009; Supplemental Table 3).

To enrich for the coccoidal cyanobacteria of the microbial mats and to remove other potential confounding factors from biochemical components and metabolisms of other organisms in our system, we conducted multiple transfers of cultures between agar plates and liquid medium. This selected for the coccoidal cyanobacteria with thick EPS envelopes that formed pustular mats. To do this, cyanobacteria from green pustules were first examined under a light microscope (Zeiss Axio Imager M1). Approximately 1 mL of mat material containing coccoidal cyanobacteria was selected, vortexed and streaked onto hypersaline BG11 hypersaline agar plates. After ~3 weeks of growth, individual green colonies were picked from agar plates and inoculated into liquid hypersaline BG11 hypersaline medium. The process of transferring cells between liquid culture and agar plates was repeated 5 to 6 times, until the coccoidal

cyanobacteria dominated the cultures. Enrichment cultures were grown in sterile plastic plant culture jars (BioExpress, catalog #C-3122-1, 190 mL, 68 mm x 68 mm) in hypersaline BG11 medium at room temperature. Cultures were grown in the presence of continuous light to maximize growth and the medium was replaced twice per week to maintain a pH of between 7.5 and 8.5.

Microfluidic Experimental Design

Silicification experiments were carried out in a custom-designed microfluidic flow through system modeling tidal marine environments with high silica seawater. Chips were designed in Fusion 360 (by AutoDesk, San Rafael, California) in two parts: a bottom half that had a circular central area surrounded by a separated circular channel, both of which could be used for sample containment, and a top part that covered the containment chambers and provided nozzles for attaching tubing. The cells were printed using either Dental SG or LT biocompatible resins using Form 2 stereolithographic printers (from Form Labs, Somerville, Massachusetts) at the laboratory of Systems Biophysics, Faculty of Physics at Ludwig Maximilians University of München, Germany, or by different printing factories in the United States. To simplify the coating and to avoid warping during curing, the printed cells were shipped to MIT before performing the final curing step.

Before the experiment, the sample containment areas were painted with uncured Dental SG resin and coated with carbonate sand collected in Cat Island, The Bahamas (Mariotti et al., 2014) and limestone mud (Hubercarb® Q series Q100 medium fine ground calcium carbonate). After sediment coating, the cells were cured with UV radiation for 10 minutes (in a modified MelodySusie 36W UV Nail Dryer) in a specialized fixture to avoid warping. To ensure the best fit between the top and bottom parts of the cell, both were polished manually using stearated

aluminum oxide sandpaper (#11111 from Sungold Abrasives, Port Washington, New York). Chips were sonicated in distilled water to remove non-carbonate particles and sterilized in a biological safety cabinet using ethanol and UV light for 1 hour.

For the experiments, a piece of biofilm was placed in the center of each inner chamber of the bottom part of the chip, after which the top and bottom parts were joined using ethanol sterilized acrylic adhesive transfer tape (468MP from 3M; Walsh et al., 2017). To provide more sealing pressure on the adhesive transfer tape, the cell assembly was further secured with using M3 nylon screws and nuts (Guard4U, Amazon).

During the experiment, artificial seawater medium (ASW, Supplemental Table 1), spiked with 100 ppm silica (reagent-grade sodium silicate solution ($Na_2O(SiO_2)$ x $\cdot xH_2O$, Sigma-Aldrich, St. Louis, MO), was pumped through the microfluidic chips using a Gilson Minipuls 4 channel peristaltic pump (Gilson Inc., Middleton, WI, USA). Microfluidic chips and medium bottles were upstream from the pump, and outflow containers downstream from the pump. The tubing used in the system, except for the food-grade Masterflex norprene tubing (Cole Parmer, Vernon Hills, IL, USA, Item # EW-06402-13) used inside the peristaltic pump, was clear C-Flex tubing (Cole Parmer, Vernon Hills, IL, USA, Item # EW-06422-04). The experimental medium was stored in a 1 L Corning® PYREX® media bottle (VWR, Radnor, PA, USA, catalog # 16157-282), capped with a 3D printed lid (selectively laser sintered nylon from ShapeWays, New York) that provided nozzle attachments for tubing. The medium that had passed through the system was collected in Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units (ThermoFisher Scientific, Waltham, MA, USA, catalog # 567-0020) by piercing their caps with sterile BD medical needles (VWR, Radnor, PA, USA, catalog # BD-305196).

All experiments were started by operating the peristaltic pump at a high speed (>10 mL/hr) to initiate flow through all tubes and chips and to ensure that no leaking occurred. For the actual experiment, the pumping rate was reduced to ~1 mL/hr and the cells were left sealed for up to one month. Samples were collected at time 0 and on day 1, 15, and 30. At each time point, the corresponding chip was disconnected and opened to collect samples for Scanning Electron Microscopy (SEM). The fresh biofilms were transferred to 1.5 mL Eppendorf® microtubes (Eppendorf North America, NY, USA, cat#022364111), gently centrifuged using a MicroCL 17 Microcentrifuge (ThermoFisher Scientific, NY, USA, cat#75002451) at 4,000 RPM for 10 seconds, and excess medium was removed to prevent the additional precipitation of silica during the subsequent preparation steps. Biofilm samples were subsequently fixed in 2.5% glutaraldehyde in 0.1 mM sodium cacodylate buffer with 0.1% $CaCl_2$ at pH 7.4 at 4° C overnight. The corresponding outflow medium bottle was detached, and 10 mL of the outflow medium was filtered using 10 mL syringes and 0.2 µm Pall Acrodisc® Sterile Syringe Filters with Supor® Membrane (VWR, Radnor, PA, USA, catalog # 28143-350) into sterile 15 mL Falcon® Centrifuge Tubes (VWR, Radnor, PA, USA, catalog # 21008-936) for chemical analyses. Samples of the medium were stored in the dark at room temperature. Tubing and containers were specifically selected to minimize potential sources of silica contamination.

Batch Culture Experimental Design

Batch culture experiments were conducted in sterile plastic plant culture jars (BioExpress, catalog #C-3122-1, 190 mL, 68 mm x 68 mm) at ~21° C and 1 atm with a 12 hour light/12 hour dark cycle. Four silica concentrations were tested, each in duplicate (Sup. Table 2). At time 0, ~50 microliters of biomass were inoculated into each culture jar containing 50 mL ASW medium with 15, 36, 70 or 100 ppm silica (Sup. Table 2). The batch cultures that

contained ASW with 100-ppm silica (Cultures G and H) allowed direct comparisons with continuous culture experiments. Sterile controls were included in duplicate to test whether or not silica precipitated from solutions abiotically under these conditions. Biofilm and medium samples were collected on days 0, 1, 5, and 15. At each time point, fresh biofilms were transferred into 1.5 mL Eppendorf® microtubes (Eppendorf North America, NY, USA, cat#022364111), gently spun down at 4,000 RPM for 10 seconds and medium was removed to avoid precipitation of minerals and salts from solution after the experimental time point. Cells were fixed in 2.5% glutaraldehyde in 0.1 m sodium cacodylate buffer with 0.1% $CaCl_2$ at pH 7.4 at 4° C overnight. Fixation and buffering steps were carried out in an effort to avoid cellular degradation or sample fracturing and enable sample examination by SEM/EDS. Medium was filtered using 10 mL syringes and 0.2 μm Pall Acrodisc® Sterile Syringe Filters with Supor® Membrane (VWR, Radnor, PA, USA, catalog # 28143-350) into sterile 15 mL Falcon® Centrifuge Tubes (VWR, Radnor, PA, USA, catalog # 21008-936) for chemical analyses.

Imaging, chemical and mineralogical analyses

Biofilm samples from all experiments were prepared for SEM using a standard dehydration procedure. After fixation, biofilms were washed using a 0.2 mM sodium cacodylate buffer, rinsed four times with milliQ water ≥18.2 MΩ x cm and dried using an ethanol dehydration series (50%, 80%, 90% and 100% ethanol in 10 minute steps). Samples were mounted on 12.7 mm diameter SEM stubs (Ted Pella Inc., Product #16111, Redding, CA, USA) with double-coated carbon conductive tape (Ted Pella Inc., Product #16084-7, Redding, CA, USA), coated with an 80:20 mixture of Pt:Pd on the HAR-052 Carbon Coater equipped with metal coater and imaged using a JEOL 7900F SEM at the Harvard Center for Nanoscale Systems (CNS). Images were collected at 3 keV, and Electron Dispersive X-ray spectroscopy (EDS) total

area spectra were collected at 10 keV and processed using AZtec software (Oxford Instruments, Abingdon, United Kingdom). A minimum of three regions of biofilm per sample was imaged and analyzed at magnification of 8,000x or higher.

Dissolved silica concentrations in media samples were determined using the molybdate blue spectrophotometry method (Strickland and Parsons, 1972). Water samples were diluted tenfold with milliQ water and analyzed together with a series of six standards obtained by the dilution of a silica standard solution (Sigma-Aldrich cat# 16259). The absorption values of samples and standards were measured spectroscopically at a wavelength of 810 nm using a BioTek microplate reader instrument with BioTek Gen5 Data Analysis software. All samples were measured in replicates of 5 with 1% error in absorption values and the absorption values were averaged. Characterization of precipitated material in biofilms was done using X-ray diffraction (XRD) and Fourier Transmission Infrared Spectroscopy (FT-IR). For XRD, biofilm samples were filtered by suction filtration through 0.2 μm Millipore nylon net filters (EMD Millipore, HNWP04700, Billerica, MA, USA) and left to dry overnight. Dried samples were affixed to a zero-background quartz plate using double-sided tape and analyzed on a PANalytical X'Pert PRO XRPD in the Center for Materials Science and Engineering (CMSE) at MIT from 4 to 70 degrees over 20 minutes and spectra were analyzed using HighScore Plus software. To prepare samples for FT-IR, biofilms were rinsed with milliQ water, water was removed, and samples were left to dry overnight in a 1.5 mL Eppendorf® microtube (Eppendorf North America, NY, USA, cat#022364111). Samples were analyzed on a Bruker FT-IR microscope in the Center for Nanoscale Systems (Harvard University). A minimum of six spots per sample was analyzed, and spectra were processed using Opus Spectroscopy Software.

SUPPLEMENTAL TABLE AND FIGURE CAPTIONS

Table S1. Artificial seawater medium (ASW) and stock solution recipes.

Table S2. Concentration experiment samples and silica concentrations.

Table S3. Modified hypersaline BG11 medium and stock solution recipes.

Table S4. Concentrations of silica in replicate batch culture experiments over the course of 15 days.

Figure S1: Cartoon of continuous culture experimental design with image of a microfluidic chip containing carbonate sand and green cyanobacterial biofilm. A light microscope image shows coccoidal cyanobacteria from Shark Bay used in this study with thick EPS envelopes surrounding and coating cells. These cells are analogous to fossil *Eoentophysalis.*

Figure S2: SEM image and corresponding EDS spectrum of inoculum on day 0. The cells were deflated and the EPS was shriveled due to the SEM dehydration procedure. There was no silica present in the starting material.

Figure S3: Fractured cell showing hollow interior and EDS chemical map showing silica enrichment in the EPS surrounding the cell and connecting the biofilm.

Figure S4: XRD spectrum of silicified biofilms showing a broad amorphous peak and NaCl. No other crystalline phases were present in the biofilm.

Figure S5: EDS total area spectra that correspond to SEM images in Figure 4 showing silicified cells from batch culture experiments on day 15. Spectra show high intensity Si and Mg peaks in biofilms incubated in ASW with 70 ppm silica and 100 ppm silica and low intensity Si peaks in biofilms incubated in ASW with >70 ppm silica.

Figure S6: Dissolved silica concentrations in ASW sampled from the batch cultures over two weeks. Silica concentrations remain relatively constant in samples that contained less than 70-ppm silica, but decreased in samples with 70-ppm silica or more. Initial silica concentrations for each jar were as follows: 15 ppm silica in A (and replicate B in Table S4), 36 ppm silica in C (and replicate D in Table S4), 70 ppm silica in E (and replicate F in Table S4), and 104 ppm silica in G (and replicate H in Table S4).

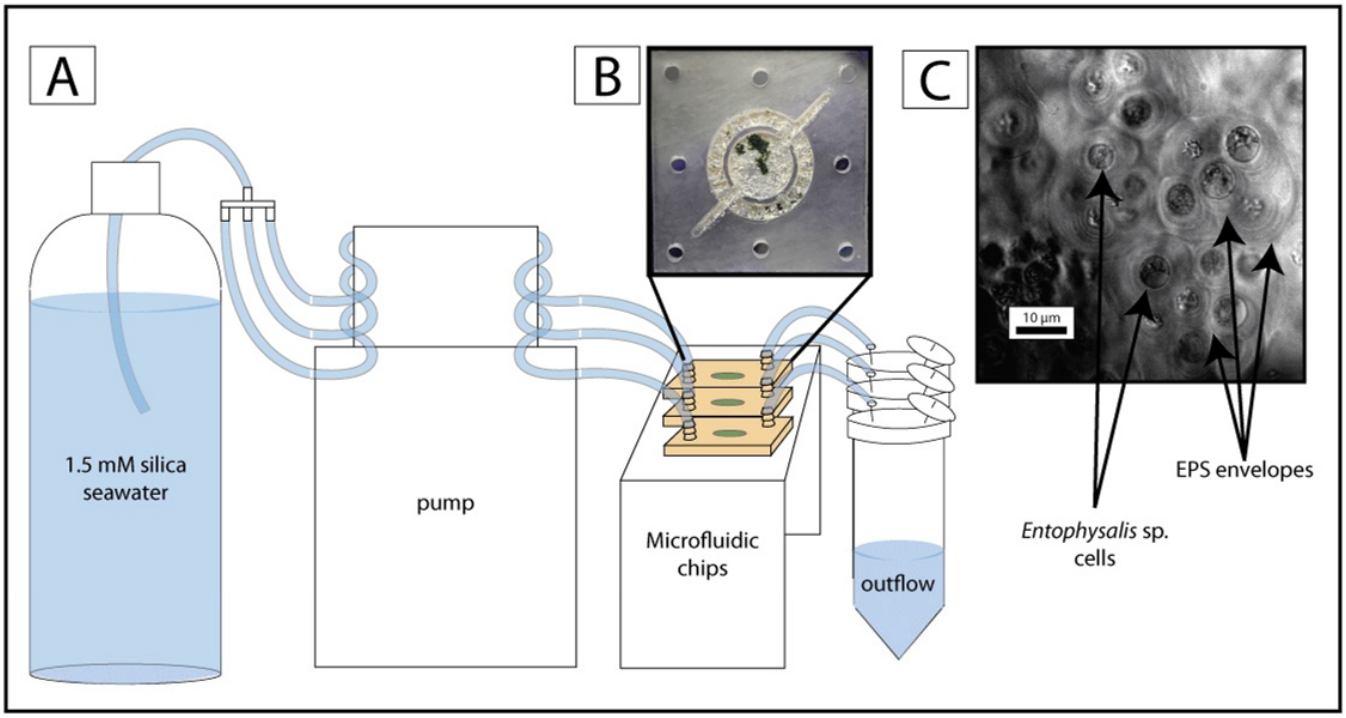
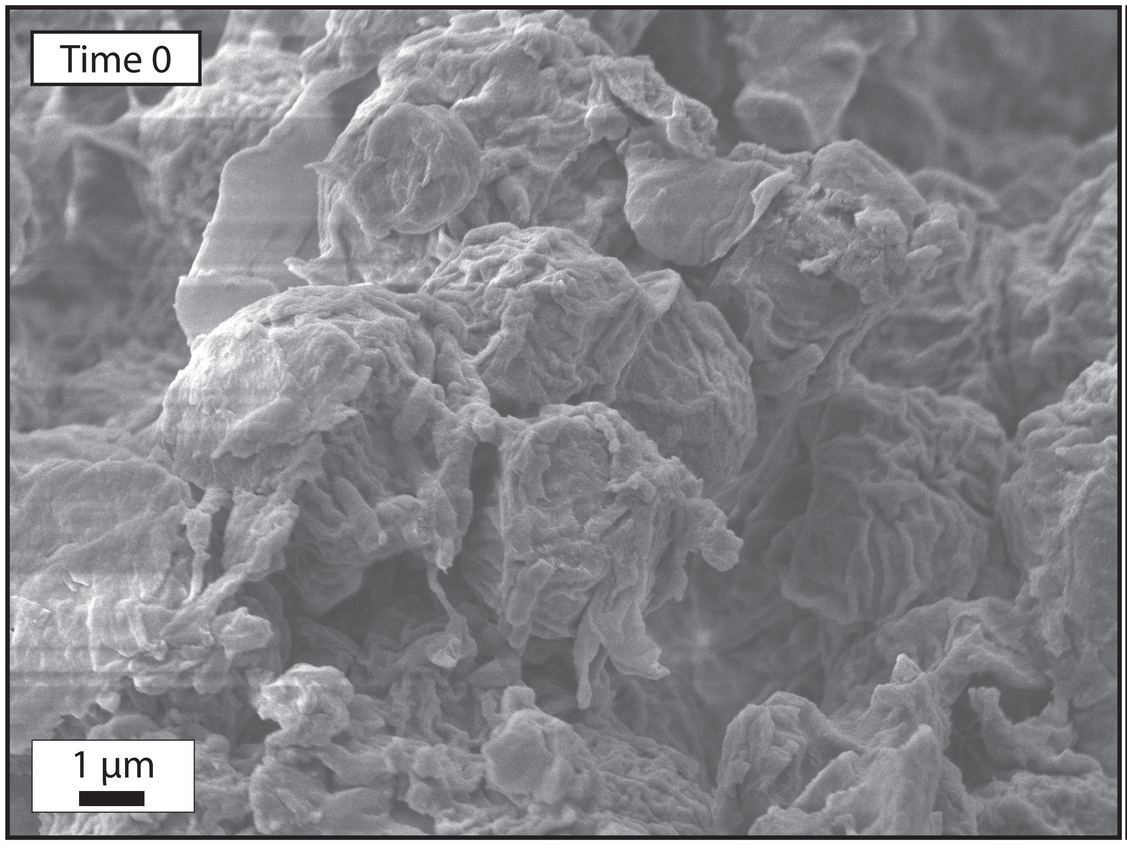

A
B
C
1.5 mM silica seawater
pump
Microfluidic chips
outflow
10 µm
EPS envelopes
Entophysalis sp. cells

Fig. S1


Time 0
1 µm

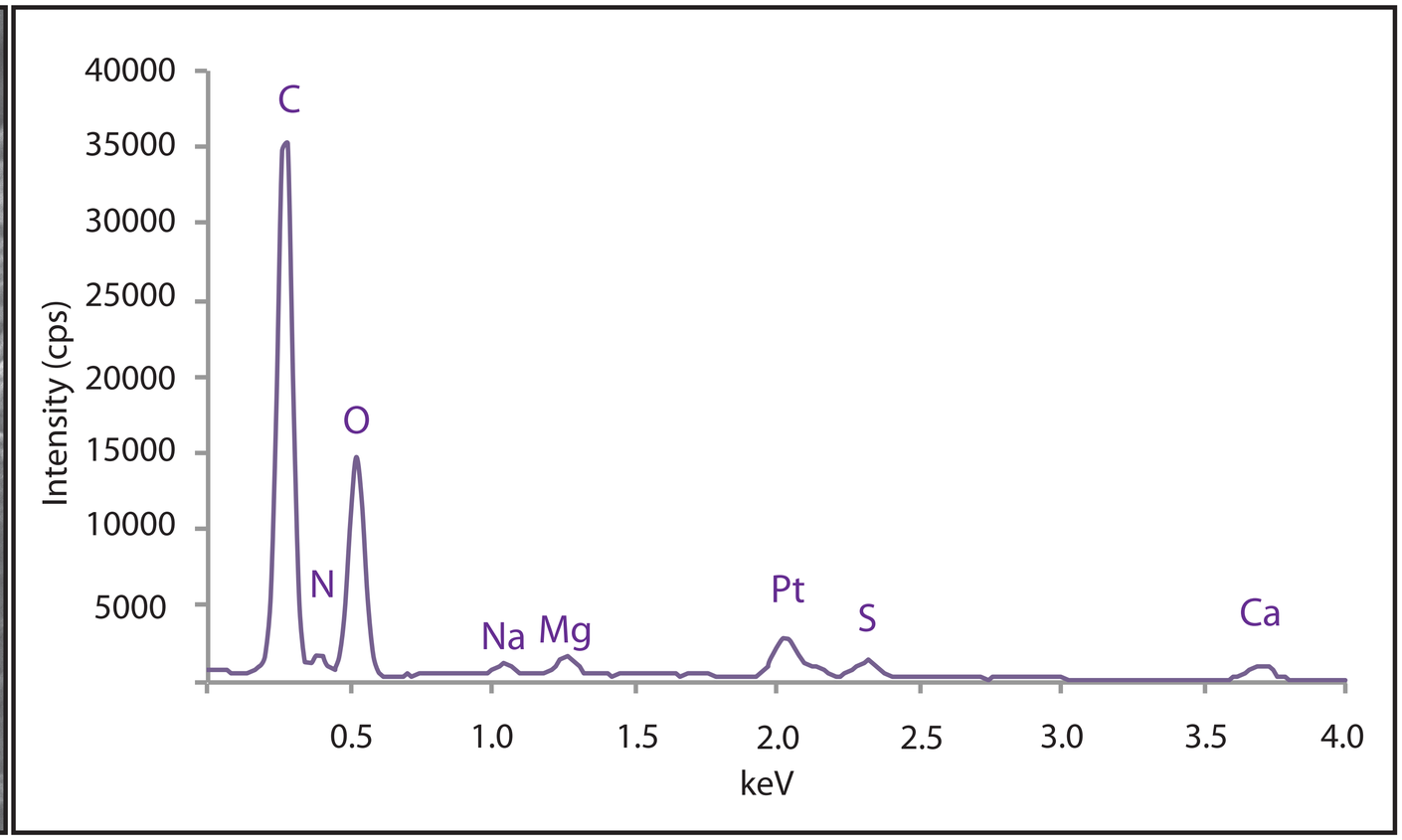

Intensity (cps)
40000
35000
30000
25000
20000
15000
10000
5000
C
O
N
Na
Mg
Pt
S
Ca
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
keV

Fig. S2

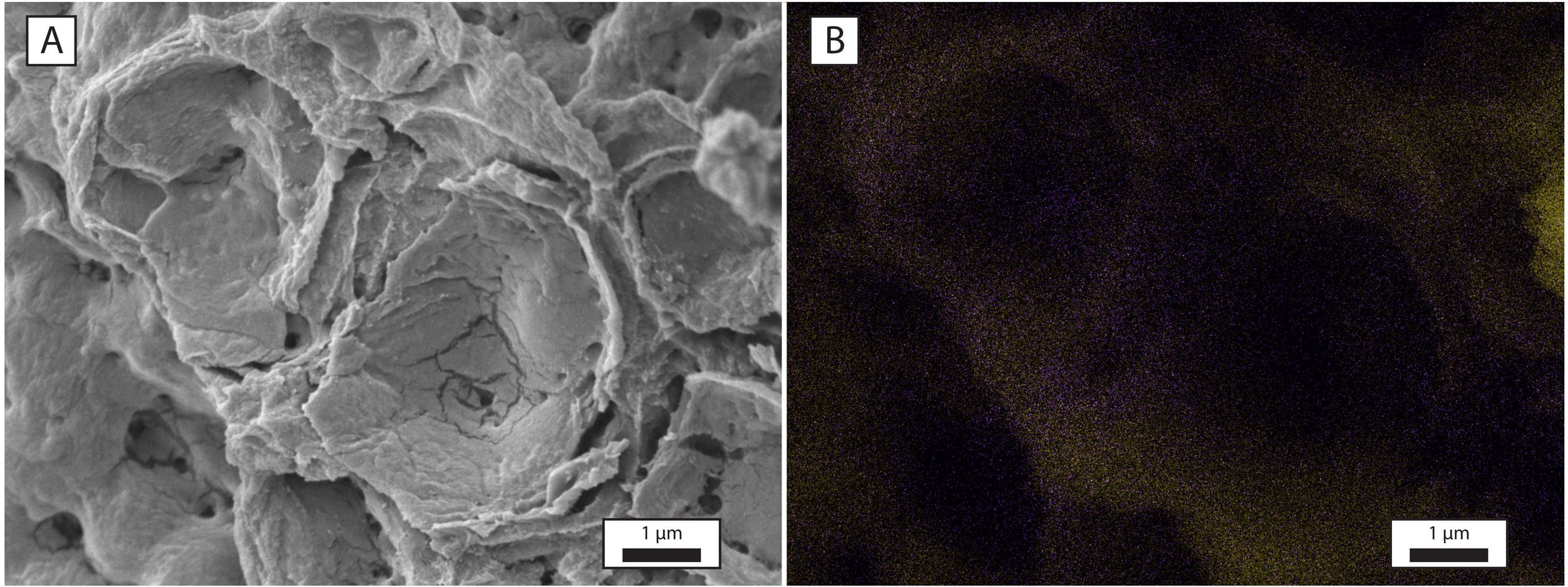


Fig. S3

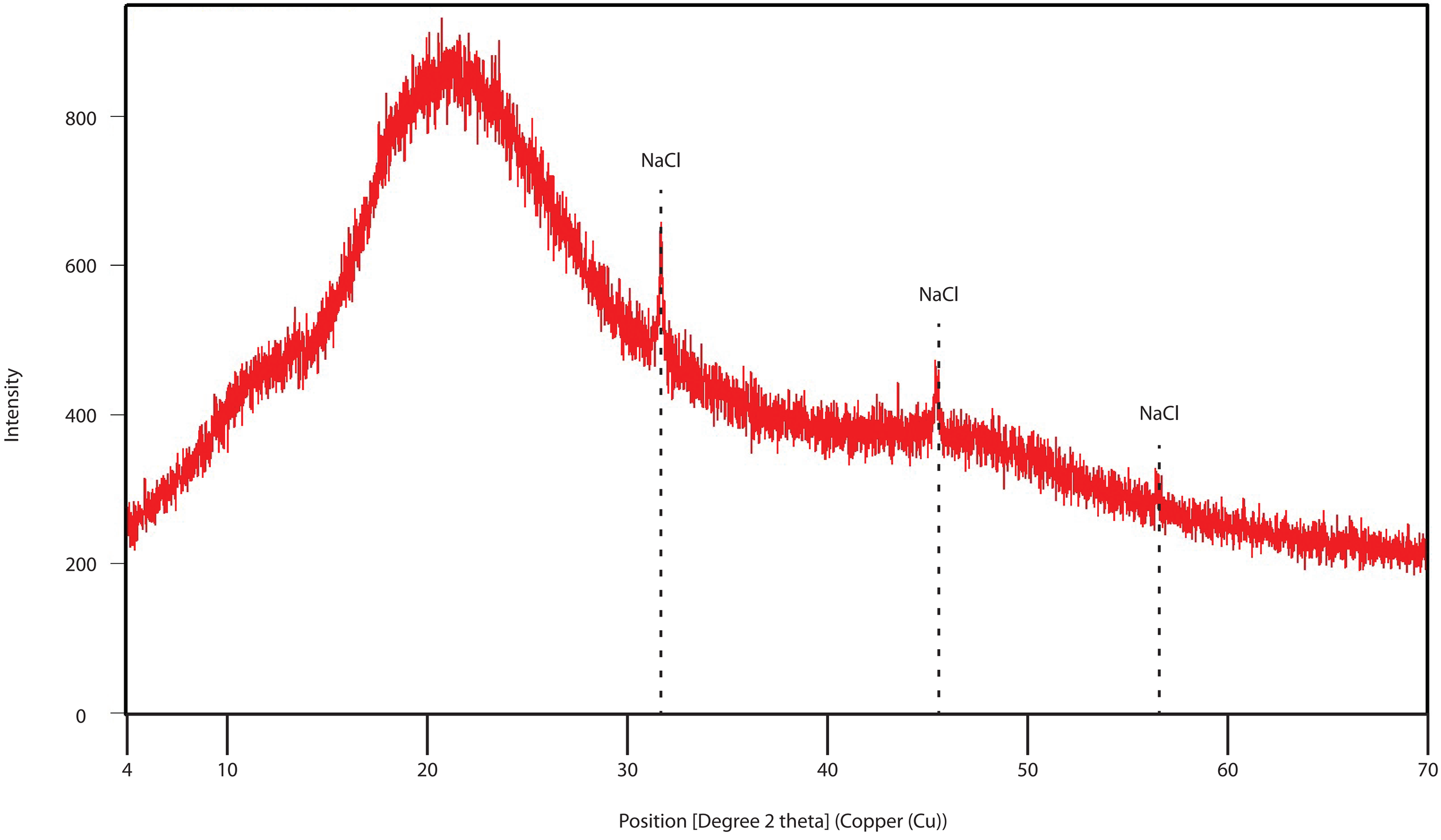

800
600
400
200
0
Intensity
NaCl
NaCl
NaCl
4
10
20
30
40
50
60
70
Position [Degree 2 theta] (Copper (Cu))

Fig. S4

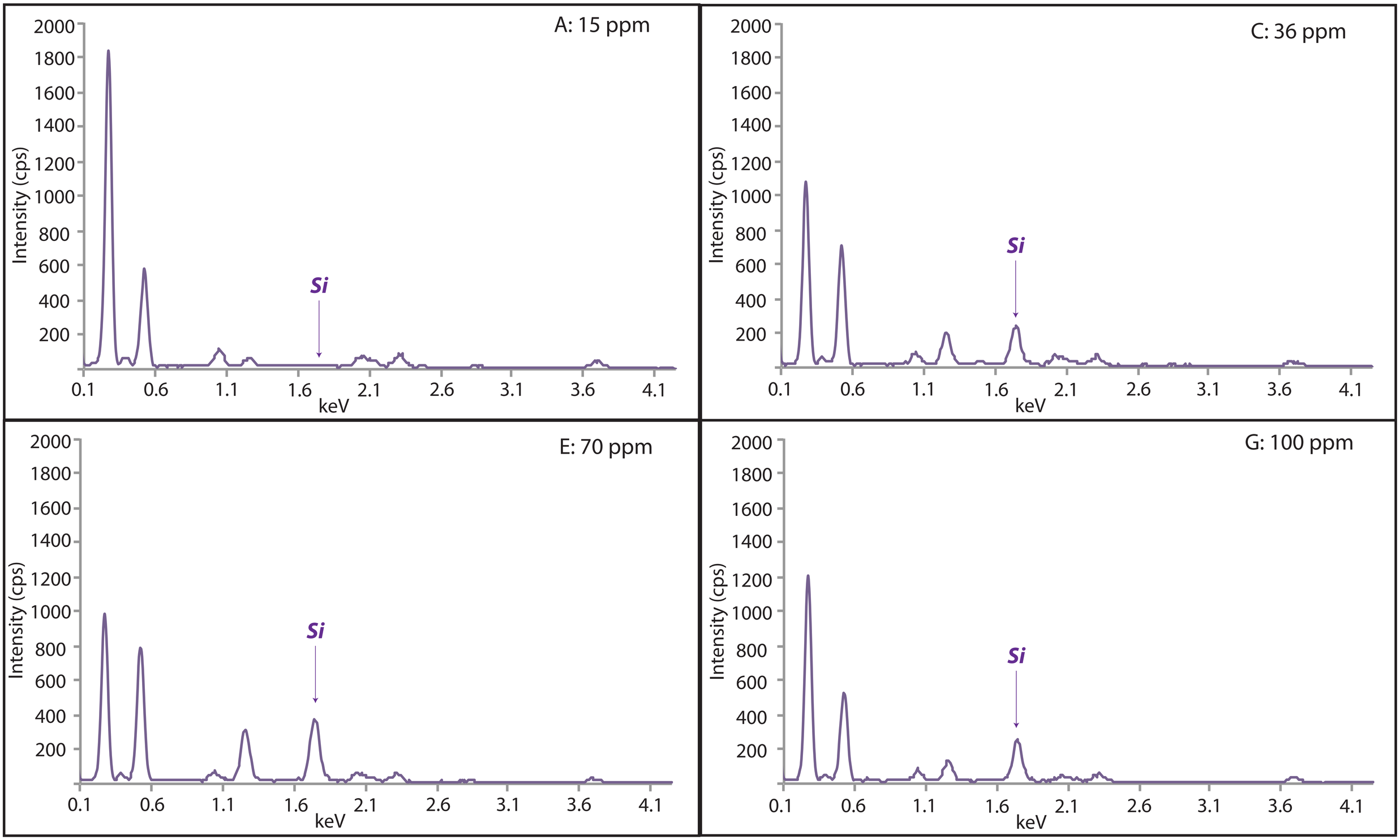

A: 15 ppm
C: 36 ppm
E: 70 ppm
G: 100 ppm
Intensity (cps)
2000
1800
1600
1400
1200
1000
800
600
400
200
Si
0.1
0.6
1.1
1.6
2.1
2.6
3.1
3.6
4.1
keV

Fig. S5

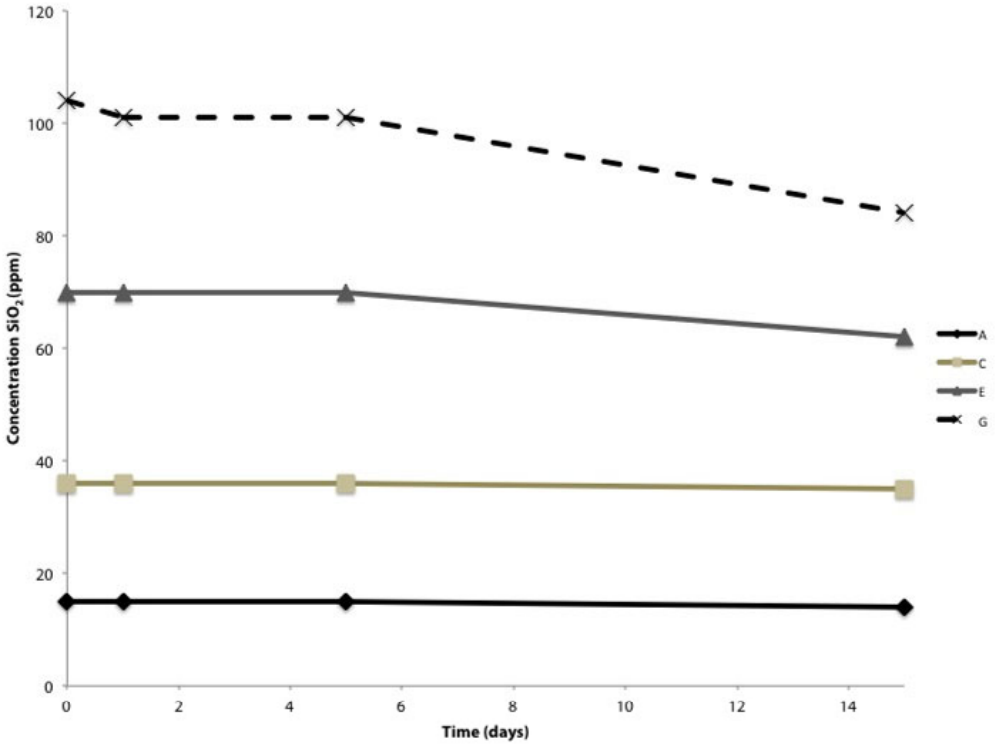

120
100
80
60
40
20
0
Concentration SiO_2 (ppm)
0
2
4
6
8
10
12
14
Time (days)
A
C
E
G

Fig. S6

Table S1

Artificial Seawater Medium (ASW)	**amount**	**unit**
Initial water	800	ml
NaCl	23	g
Na2SO4	0.71	g
KCl	1.48	g
NaHCO3	0.336	g
Nitrate sol'n	500	µl
Major "10x" sol'n	10	ml
CAST D Trace	1	ml
FeCl3	100	µl
Final water	138.4	ml
Mg/Ca solution	50	ml

Stock solutions	
Major 10X	Concentration (g/L)
Nitrilotriacetic Acid (NTA)	1
NaCl	4.8176
NaH2PO4	0.09295
KCl	0.738
Add up to 1L of dH20	

NO3 50X	Concentration (g/L)
KNO3	5.15
NaNO3	34.45
Add up to 1L of dH20	

FeCl3 10000X	Concentration (g/L)
FeCl3*6H2O	3
Add up to 1L of dH20	

Trace Metals 100X	Concentration (g/L)
MnCl2*4H2O	2.67
ZnS04*7H2O	0.5
CuSO4*5H2O	0.025
Na2MoO4	0.02128
Co(NO3)2*6H20	0.05626
H3BO3	0.5
H2SO4 (concentrated)	0.5 (mL/L)
NiCl2 (may be added from a 2000X separate solution)	0.000048

Add up to 1L of dH20	

Mg/Ca solution	Concentration (g/L)
MgCl2*6H2O	203.3
CaCl2*2H2O	29.4
Add up to 1L of dH20	

Table S2

Sample	Concentration silica (ppm)
A	15
B	15
C	36
D	36
E	70
F	70
G	100
H	100

Table S3

BG11 hypersaline medium	**amount**	**unit**
initial water	968	ml
NaCl	49.8	g
NaNO3	1.5	g
NaCO3	0.02	g
KCl	1.3867	g
MgCl2*6H2O	8.569	g
MgSO4*7H2O	6.4998	g
CaCl2*2H2O	2.042	g
Stock A	10	ml
Stock B	10	ml
Stock C	10	ml
Stock 5 (trace metals)	1	ml
Vitamins	1	ml

Stock solutions

Stock A	Concentration (g/L)
Na2MG EDTA	0.1
Ferric ammonium citrate	0.6
Citric acid*1H2O	0.6
CaCl2*2H2O	3.6
Add up to 1L of dH20	

Stock B	Concentration (g/L)
MgSO4*7H2O	7.5
Add up to 1L of dH20	

Stock C	Concentration (g/L)
K2HPO4*3H2O	4
Add up to 1L of dH20	

Stock 5	Concentration (g/L)
H3BO3	2.86
MnCl2*4H2O	1.81
ZnSO4*7H2O	0.222
CuSO4*5H2O	0.079
CoCl2*6H2O	0.05
NaMoO4*2H2O	0.391
Add up to 1L of dH20	

Table S4

Time point (days)	Culture Jar							
	A	B	C	D	E	F	G	H
0	15	15	36	36	70	70	104	104
1	15	15	36	36	70	70	101	102
5	15	14	36	36	70	68	101	102
15	14	14	34	35	62	63	84	84