

# Experiments evaluating protein size distribution pattern in EPS Si<sup>+</sup> and EPS Si<sup>2</sup> using sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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**Data Type:** experimental

**Version:** 1

**Version Date:** 2019-04-10

## Project

» [Biopolymers as carrier phases for selected natural radionuclides \(of Th, Pa, Pb, Po, Be\) in diatoms and coccolithophores](#) (Biopolymers for radionuclides)

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## Abstract

Laboratory studies were conducted to examine the sorption of selected radionuclides (<sup>234</sup>Th, <sup>233</sup>Pa, <sup>210</sup>Po, <sup>210</sup>Pb, and <sup>7</sup>Be) onto inorganic (pure silica and acid-cleaned diatom frustules) and organic (diatom cells with or without silica frustules) particles in natural seawater and the role of templating biomolecules and exopolymeric substances (EPS) extracted from the same species of diatom, *Phaeodactylum tricornutum*, in the sorption process. The range of partition coefficients (*K<sub>d</sub>*, reported as log*K<sub>d</sub>*) of radionuclides between water and the different particle types was 4.78–6.69 for <sup>234</sup>Th, 5.23–6.71 for <sup>233</sup>Pa, 4.44–5.86 for <sup>210</sup>Pb, 4.47–4.92 for <sup>210</sup>Po, and 4.93–7.23 for <sup>7</sup>Be, similar to values reported for lab and field determinations. The sorption of all radionuclides was significantly enhanced in the presence of organic matter associated with particles, resulting in *K<sub>d</sub>* one to two orders of magnitude higher than for inorganic particles only, with highest values for <sup>7</sup>Be (log*K<sub>d</sub>* of 7.2). Results further indicate that EPS and frustule-embedded biomolecules in diatom cells are responsible for the sorption enhancement rather than the silica shell itself. By separating radiolabeled EPS via isoelectric focusing, we found that isoelectric points are radionuclide specific, suggesting that each radionuclide binds to specific biopolymeric functional groups, with the most efficient binding sites likely occurring in acid polysaccharides, iron hydroxides, and proteins. Further progress in evaluating the effects of diatom frustule-related biopolymers on binding, scavenging, and fractionation of radionuclides would require the application of molecular-level characterization techniques.

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## Dataset Description

Laboratory studies were conducted to examine the sorption of selected radionuclides (<sup>234</sup>Th, <sup>233</sup>Pa, <sup>210</sup>Po, <sup>210</sup>Pb, and <sup>7</sup>Be) onto inorganic (pure silica and acid-cleaned diatom frustules) and organic (diatom cells with or without silica frustules) particles in natural seawater and the role of templating biomolecules and exopolymeric substances (EPS) extracted from the same species of diatom, *Phaeodactylum tricornutum*, in the sorption

process.

The range of partition coefficients ( $K_d$ , reported as  $\log K_d$ ) of radionuclides between water and the different particle types was 4.78–6.69 for  $^{234}\text{Th}$ , 5.23–6.71 for  $^{233}\text{Pa}$ , 4.44–5.86 for  $^{210}\text{Pb}$ , 4.47–4.92 for  $^{210}\text{Po}$ , and 4.93–7.23 for  $^7\text{Be}$ , similar to values reported for lab and field determinations. The sorption of all radionuclides was

significantly enhanced in the presence of organic matter associated with particles, resulting in  $K_d$  one to two orders of magnitude higher than for inorganic particles only, with highest values for  $^7\text{Be}$  ( $\log K_d$  of 7.2). Results further indicate that EPS and frustule-embedded biomolecules in diatom cells are responsible for the sorption enhancement rather than the silica shell itself. By separating radiolabeled EPS via isoelectric focusing, we found that isoelectric points are radionuclide specific, suggesting that each radionuclide binds to specific biopolymeric functional groups, with the most efficient binding sites likely occurring in acid polysaccharides, iron hydroxides, and proteins. Further progress in evaluating the effects of diatom frustule-related biopolymers on binding, scavenging, and fractionation of radionuclides would require the application of molecular-level characterization techniques.

## Methods & Sampling

Diatom cultures, sample preparation, and EPS extraction

*P. tricornutum* (UTEX 646) was selected for culturing in autoclaved f/2 and f/2-Si media (salinity of 26) at a temperature of  $19 \pm 1^\circ\text{C}$  with a light cycling of 14 h : 10 h under a saturating irradiance of  $100 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ . In order to deplete the diatom of Si supply, cultures were transferred into f/2-Si medium over at least six generations by harvesting cells (2694 g, 30 min) and resuspending them in fresh f/2-Si medium. Sterile polycarbonate bottles were also used to prevent Si supply from glassware. The growth status of *P. tricornutum* was monitored by changes in optical density at 750 nm. Cells, frustules, and EPS were collected when *P. tricornutum* reached the stationary phase.

Laboratory cultures of *P. tricornutum* were centrifuged (2694 x g, 30 min) and filtered (0.2  $\mu\text{m}$ ) to collect the whole cells. The frustules were repeatedly treated by using a hydrogen peroxide (30%, room temperature) treatment until bubbles were no longer generated, followed by concentrated nitric acid ( $\text{HNO}_3$ ) digestion (85°C, 1 h) to remove organic matter adopted from Robinson et al. (2004).

The resulting organic carbon (C), nitrogen (N), and sulfur (S) contents of the cleaned frustules were measured using a Perkin Elmer CHNS 2400 analyzer to ensure the removal of organic materials using cysteine as a standard according to Guo and Santschi (1997).

EPS extraction was followed the procedures described in Xu et al. (2011b), which minimize cell rupture and molecular alterations and maximize extraction efficiency. EPS here is referring to those biopolymers that are attached on the diatom frustules. Hereafter, EPS Si<sup>+</sup> and EPS Si<sup>2</sup> denote the EPS extracted from diatoms cultured under Si-replete (f/2 medium) and Si-depleted (f/2-Si medium) conditions, respectively. Briefly, laboratory cultures were centrifuged (2694 x g, 30 min) and filtered (0.2  $\mu\text{m}$ ) when diatoms reached stationary phase. The diatom cells were soaked with 0.5 mol L<sup>-1</sup> sodium chloride (NaCl) solution for 10 min and followed by centrifugation at 2000 x g for 15 min to remove the medium and weakly bound organic material on the cells. The pellet from previous step was resuspended in a new 100 mL 0.5 mol L<sup>-1</sup> NaCl solution and stirred gently overnight at 4°C. The resuspended particle solution was ultracentrifuged at 12,000 x g (30 min, 4°C), and the supernatant was then filtered through a 0.2  $\mu\text{m}$  polycarbonate membrane. The filtrate was desalted and collected with a 1 kDa cutoff cross-flow ultrafiltration and diafiltration membrane and then freeze-dried for later use.

Characterization of exopolymeric substances

After partitioning EPS collected from lab cultures into aliquots for freeze-drying, subsamples were analyzed for individual components. Concentration of total carbohydrate (TCHO) concentration was determined by the TPTZ (2,4,6-tripyridyl-s-triazine) method using glucose as the standard, and uronic acids were measured by the meta-hydroxyphenyl method using glucuronic acid as the standard (Hung and Santschi 2001). Protein content was determined using a modified Lowry protein assay, using bovine serum albumin (BSA) as the standard (Pierce, Thermo Scientific). C, N, and S contents were determined as described above. Iron was measured using an atomic absorption spectrometer (Varian) after overnight digestion with 12 mol L<sup>-1</sup>  $\text{HNO}_3$  at 85°C (Von Loon 1985). To evaluate the protein size distribution pattern in EPS Si<sup>+</sup> and EPS Si<sup>2</sup>, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Sambrook et al. (1989) using standard molecular weight markers (Dual Xtra Standards, Bio-Rad). Fourier transform infrared spectroscopy (FTIR) was used to characterize samples using a Varian 3100 model interfaced with a single reflection horizontal attenuated total reflectance (ATR) accessory (PIKE Technologies).

A diamond plate was used as the internal reflection element. A freeze-dried EPS sample was mounted at the surface of the diamond. Absorbance spectra from 800 to 2000 cm<sup>-1</sup> were collected and integrated using Varian Resolution Pro 4.0 software. ATR-FTIR spectroscopy provides a noninvasive way to quickly gain information about the contents of major secondary structures of biopolymers (Xu et al. 2011b; Jiang et al. 2012). Major infrared (IR) peaks were assigned according to Xu et al. (2011b) and Jiang et al. (2012). Characteristic bands found in the IR spectra of proteins and polypeptides include the amide I (1652–1648 cm<sup>-1</sup>) and amide II (1550–1548 cm<sup>-1</sup>) band. The absorption associated with the amide I band leads to stretching vibrations of the C=O bond of the amide, and absorption associated with the amide II band leads primarily to bending vibrations of the N-H and C-N bond. The symmetric stretching peak due to deprotonated carboxyl groups is observed at 1400 cm<sup>-1</sup> along with the CH<sub>2</sub> bending mode at 1455 cm<sup>-1</sup>. In the 800–1200 cm<sup>-1</sup> regions, responses from C-O, C-O-C, P-O-P, C-O-P, and ring vibrations of the main polysaccharide functional groups are present in polysaccharide mixtures. The peaks at 1241 and 1113 cm<sup>-1</sup> correspond to P-O stretching in phosphate groups.

## Data Processing Description

BCO-DMO Processing Notes:

- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- combined the two submitted tables together on the pH column, indicating percent activity and percent composition in the parameter names.

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## Data Files

File
<b>sds_page.csv</b> (Comma Separated Values (.csv), 151 bytes) MD5:c03cd197e0c36d6b8f69372d46eefcec Primary data file for dataset ID 764688

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## Related Publications

Alvarado Quiroz, N. G., Hung, C.-C., & Santschi, P. H. (2006). Binding of thorium(IV) to carboxylate, phosphate and sulfate functional groups from marine exopolymeric substances (EPS). *Marine Chemistry*, 100(3-4), 337–353. doi:[10.1016/j.marchem.2005.10.023](https://doi.org/10.1016/j.marchem.2005.10.023)  
*Methods*

Baskaran, M., & Santschi, P. H. (1993). The role of particles and colloids in the transport of radionuclides in coastal environments of Texas. *Marine Chemistry*, 43(1-4), 95–114. doi:[10.1016/0304-4203\(93\)90218-d](https://doi.org/10.1016/0304-4203(93)90218-d)  
[https://doi.org/10.1016/0304-4203\(93\)90218-d](https://doi.org/10.1016/0304-4203(93)90218-d)  
*Methods*

Chuang, C.-Y., Santschi, P. H., Ho, Y.-F., Conte, M. H., Guo, L., Schumann, D., ... Li, Y.-H. (2013). Role of biopolymers as major carrier phases of Th, Pa, Pb, Po, and Be radionuclides in settling particles from the Atlantic Ocean. *Marine Chemistry*, 157, 131–143. doi:[10.1016/j.marchem.2013.10.002](https://doi.org/10.1016/j.marchem.2013.10.002)  
*Methods*

Chuang, C.-Y., Santschi, P. H., Jiang, Y., Ho, Y.-F., Quigg, A., Guo, L., ... Schumann, D. (2014). Important role of biomolecules from diatoms in the scavenging of particle-reactive radionuclides of thorium, protactinium, lead, polonium, and beryllium in the ocean: A case study with *Phaeodactylum tricornutum*. *Limnology and Oceanography*, 59(4), 1256–1266. doi:[10.4319/l0.2014.59.4.1256](https://doi.org/10.4319/l0.2014.59.4.1256)  
*Results*

Duan, S., & Bianchi, T. S. (2007). Particulate and dissolved amino acids in the lower Mississippi and Pearl Rivers

(USA). Marine Chemistry, 107(2), 214–229. doi:[10.1016/j.marchem.2007.07.003](https://doi.org/10.1016/j.marchem.2007.07.003)  
*Methods*

Guo, L., & Santschi, P. H. (1997). Isotopic and elemental characterization of colloidal organic matter from the Chesapeake Bay and Galveston Bay. Marine Chemistry, 59(1-2), 1–15. doi:10.1016/S0304-4203(97)00072-8  
[https://doi.org/10.1016/S0304-4203\(97\)00072-8](https://doi.org/10.1016/S0304-4203(97)00072-8)  
*Methods*

Guo, L., Santschi, P. H., Baskaran, M., & Zindler, A. (1995). Distribution of dissolved and particulate  $^{230}\text{Th}$  and  $^{232}\text{Th}$  in seawater from the Gulf of Mexico and off Cape Hatteras as measured by SIMS. Earth and Planetary Science Letters, 133(1-2), 117–128. doi:10.1016/0012-821X(95)00063-I  
[https://doi.org/10.1016/0012-821X\(95\)00063-I](https://doi.org/10.1016/0012-821X(95)00063-I)  
*Methods*

Honeyman, B. D., & Santschi, P. H. (1989). A Brownian-pumping model for oceanic trace metal scavenging: Evidence from Th isotopes. Journal of Marine Research, 47(4), 951–992. doi:[10.1357/002224089785076091](https://doi.org/10.1357/002224089785076091)  
*Methods*

Hung, C.-C., & Santschi, P. H. (2001). Spectrophotometric determination of total uronic acids in seawater using cation-exchange separation and pre-concentration by lyophilization. Analytica Chimica Acta, 427(1), 111–117. doi:10.1016/S0003-2670(00)01196-X [https://doi.org/10.1016/S0003-2670\(00\)01196-X](https://doi.org/10.1016/S0003-2670(00)01196-X)  
*Methods*

Jiang, Y., Yoshida, T., & Quigg, A. (2012). Photosynthetic performance, lipid production and biomass composition in response to nitrogen limitation in marine microalgae. Plant Physiology and Biochemistry, 54, 70–77. doi:[10.1016/j.plaphy.2012.02.012](https://doi.org/10.1016/j.plaphy.2012.02.012)  
*Methods*

Quigley, M. S., Santschi, P. H., Hung, C.-C., Guo, L., & Honeyman, B. D. (2002). Importance of acid polysaccharides for  $^{234}\text{Th}$  complexation to marine organic matter. Limnology and Oceanography, 47(2), 367–377. doi:[10.4319/lo.2002.47.2.0367](https://doi.org/10.4319/lo.2002.47.2.0367)  
*Methods*

Roberts, K. A., Xu, C., Hung, C.-C., Conte, M. H., & Santschi, P. H. (2009). Scavenging and fractionation of thorium vs. protactinium in the ocean, as determined from particle–water partitioning experiments with sediment trap material from the Gulf of Mexico and Sargasso Sea. Earth and Planetary Science Letters, 286(1-2), 131–138. doi:[10.1016/j.epsl.2009.06.029](https://doi.org/10.1016/j.epsl.2009.06.029)  
*Methods*

Robinson, R. S., Brunelle, B. G., & Sigman, D. M. (2004). Revisiting nutrient utilization in the glacial Antarctic: Evidence from a new method for diatom-bound N isotopic analysis. Paleoceanography, 19(3), n/a–n/a. doi:10.1029/2003pa000996 <https://doi.org/10.1029/2003PA000996>  
*Methods*

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). Molecular cloning: a laboratory manual (No. Ed. 2). Cold spring harbor laboratory press. <https://isbnsearch.org/isbn/0879693096>  
*Methods*

Schumann, D., Ayranov, M., Stowasser, T., Gialanella, L., Di Leva, A., Romano, M., & Schuermann, D. (2013). Radiochemical separation of  $^7\text{Be}$  from the cooling water of the neutron spallation source SINQ at PSI. Radiochimica Acta, 101(8), 509–514.  
*Methods*

Van Loon, J. C. (1985). Selected methods of trace metal analysis: biological and environmental samples. John Wiley and Sons.  
*Methods*

Walter, H. J., Rutgers van der Loeff, M. M., & Hoeltzen, H. (1997). Enhanced scavenging of  $^{231}\text{Pa}$  relative to  $^{230}\text{Th}$  in the South Atlantic south of the Polar Front: Implications for the use of the  $^{231}\text{Pa}/^{230}\text{Th}$  ratio as a paleoproductivity proxy. Earth and Planetary Science Letters, 149(1-4), 85–100. doi:10.1016/S0012-821X(97)00068-X [https://doi.org/10.1016/S0012-821X\(97\)00068-X](https://doi.org/10.1016/S0012-821X(97)00068-X)  
*Methods*

Xu, C., Zhang, S., Chuang, C., Miller, E. J., Schwehr, K. A., & Santschi, P. H. (2011). Chemical composition and relative hydrophobicity of microbial exopolymeric substances (EPS) isolated by anion exchange chromatography and their actinide-binding affinities. Marine Chemistry, 126(1-4), 27–36. doi:[10.1016/j.marchem.2011.03.004](https://doi.org/10.1016/j.marchem.2011.03.004)  
*Methods*

## Parameters

Parameter	Description	Units
Group	component group	unitless
EPS_Si_plus	exopolymeric substance extracted from diatoms cultured under Si-replete (f/2 medium) conditions	nanomole per milligram (nmol/mg)
EPS_Si_minus	exopolymeric substance extracted from diatoms cultured under Si-depleted (f/2-Si medium) conditions	nanomole per milligram (nmol/mg)

## Instruments

<b>Dataset-specific Instrument Name</b>	Pharmacia Biotech Multiphor II
<b>Generic Instrument Name</b>	Electrophoresis Chamber
<b>Dataset-specific Description</b>	For IEF (isoelectric focusing electrophoresis), a Pharmacia Biotech Multiphor II with a EPS3500 XL power supply was used.
<b>Generic Instrument Description</b>	General term for an apparatus used in clinical and research laboratories to separate charged colloidal particles (or molecules) of varying size through a medium by applying an electric field.

<b>Dataset-specific Instrument Name</b>	Canberra ultra-highpurity germanium well-type detector
<b>Generic Instrument Name</b>	Gamma Ray Spectrometer
<b>Dataset-specific Description</b>	Activity concentrations of <sup>234</sup> Th, <sup>233</sup> Pa, <sup>210</sup> Pb, <sup>210</sup> Po, and <sup>7</sup> Be were measured by gamma counting the 63.5, 312, 46.5, and 477.6 keV lines, respectively, on a Canberra ultra-highpurity germanium well-type detector.
<b>Generic Instrument Description</b>	Instruments measuring the relative levels of electromagnetic radiation of different wavelengths in the gamma-ray waveband.

<b>Dataset-specific Instrument Name</b>	Beckman Model 8100 Liquid Scintillation Counter
<b>Generic Instrument Name</b>	Liquid Scintillation Counter
<b>Dataset-specific Description</b>	The 210Po activity was analyzed by liquid scintillation counting (Beckman Model 8100 Liquid Scintillation Counter).
<b>Generic Instrument Description</b>	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used to quantify the activity of particulate emitting ( $\beta$ and $\alpha$ ) radioactive samples, it can also detect the Auger electrons emitted from 51Cr and 125I samples. Liquid scintillation counters are instruments assaying alpha and beta radiation by quantitative detection of visible light produced by the passage of rays or particles through a suitable scintillant incorporated into the sample.

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## Project Information

### **Biopolymers as carrier phases for selected natural radionuclides (of Th, Pa, Pb, Po, Be) in diatoms and coccolithophores (Biopolymers for radionuclides)**

NSF Award Abstract:

Particle-associated natural radioisotopes are transported to the ocean floor mostly via silica and carbonate ballasted particles, allowing their use as tracers for particle transport. Th(IV), Pa (IV,V), Po(IV), Pb(II) and Be(II) radionuclides are important proxies in oceanographic investigations, used for tracing particle and colloid cycling, estimating export fluxes of particulate organic carbon, tracing air-sea exchange, paleoproductivity, and/or ocean circulation in paleoceanographic studies. Even though tracer approaches are considered routine, there are cases where data interpretation or validity has become controversial, largely due to uncertainties about inorganic proxies and organic carrier molecules. Recent studies showed that cleaned diatom frustules and pure silica particles, sorb natural radionuclides to a much lower extent (by 1-2 orders of magnitude) than whole diatom cells (with or without shells). Phytoplankton that build siliceous or calcareous shells, such as the diatoms and coccolithophores, are assembled via bio-mineralization processes using biopolymers as nanoscale templates. These templates could serve as possible carriers for radionuclides and stable metals.

In this project, a research team at the Texas A & M University at Galveston hypothesizes that radionuclide sorption is controlled by selective biopolymers that are associated with biogenic opal (diatoms), CaCO<sub>3</sub> (coccolithophores) and the attached exopolymeric substances (EPS), rather than to pure mineral phase. To pursue this idea, the major objectives of their research will include separation, identification and molecular-level characterization of the individual biopolymers (e.g., polysaccharides, uronic acids, proteins, hydroquinones, hydroxamate siderophores, etc.) that are responsible for binding different radionuclides (Th, Pa, Pb, Po and Be) attached to cells or in the matrix of biogenic opal or CaCO<sub>3</sub> as well as attached EPS mixture, in laboratory grown diatom and coccolithophore cultures. Laboratory-scale radiolabeling experiments will be conducted, and different separation techniques and characterization techniques will be applied.

**Intellectual Merit :** It is expected that this study will help elucidate the molecular basis of the templated growth of diatoms and coccoliths, EPS and their role in scavenging natural radionuclides in the ocean, and help resolve debates on the oceanographic tracer applications of different natural radioisotopes (230,234Th, 231Pa, 210Po, 210Pb and 7,10Be). The proposed interdisciplinary research project will require instrumental approaches for molecular-level characterization of these radionuclides associated carrier molecules.

**Broader Impacts:** The results of this study will be relevant for understanding biologically mediated ocean scavenging of radionuclides by diatoms and coccoliths which is important for carbon cycling in the ocean, and

will contribute to improved interpretation of data obtained by field studies especially through the GEOTRACES program. This new program will enhance training programs at TAMUG for postdocs, graduate and undergraduate students. Lastly, results will be integrated in college courses and out-reach activities at Texas A&M University, including NSF-REU, Sea Camp, Elder Hostel and exhibits at the local science fair and interaction with its after-school program engaging Grade 9-12 students from groups traditionally underrepresented.

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1356453</a>

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