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*Repository*

# Cryogenic transmission electron images of *Phaeodactylum tricornutum* isolates of small cellular particles

Bedina Zavec Apolonija[1], Božič Darja[2,3], Iglič Aleš[3,4], Jeran Marko[2,3], Kisovec Matic[1], Kralj-Iglič Veronika[2,*], Romolo Anna[2]
[1]National Institute of Chemistry, University of Ljubljana, [2]University of Ljubljana, Faculty of Health Sciences, Laboratory of Clinical Biophysics,[3]University of Ljubljana, Faculty of Electrical Engineering, Laboratory of Physics, [4]University of Ljubljana, Faculty of Medicine, Laboratory of Clinical Biophysics, Ljubljana, Slovenia

* Corresponding author: Veronika Kralj-Iglič, veronika.kralj-iglic@zf.uni-lj.si

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**Abstract**: Cryogenic transmission electron images of small cellular particles isolated from conditioned media of microalgae *Phaeodactylum tricornutun* are presented. Each image is identified by the name and number that constitutes the title of the Figure, and supplemented by description of the preparation of the sample and the data on the imaging technique and equipment.

The data curators of the repository are Veronika Kralj-Iglič and Anna Romolo. More data on experiments with microalgae small cellular particles can be found in (Adamo et al., 2021), (Picciotto et al., 2021) and (Božič et al., 2022).

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**Keywords**: Extracellular vesicles, Extracellular particles, Nanoalgosomes, Exosomes

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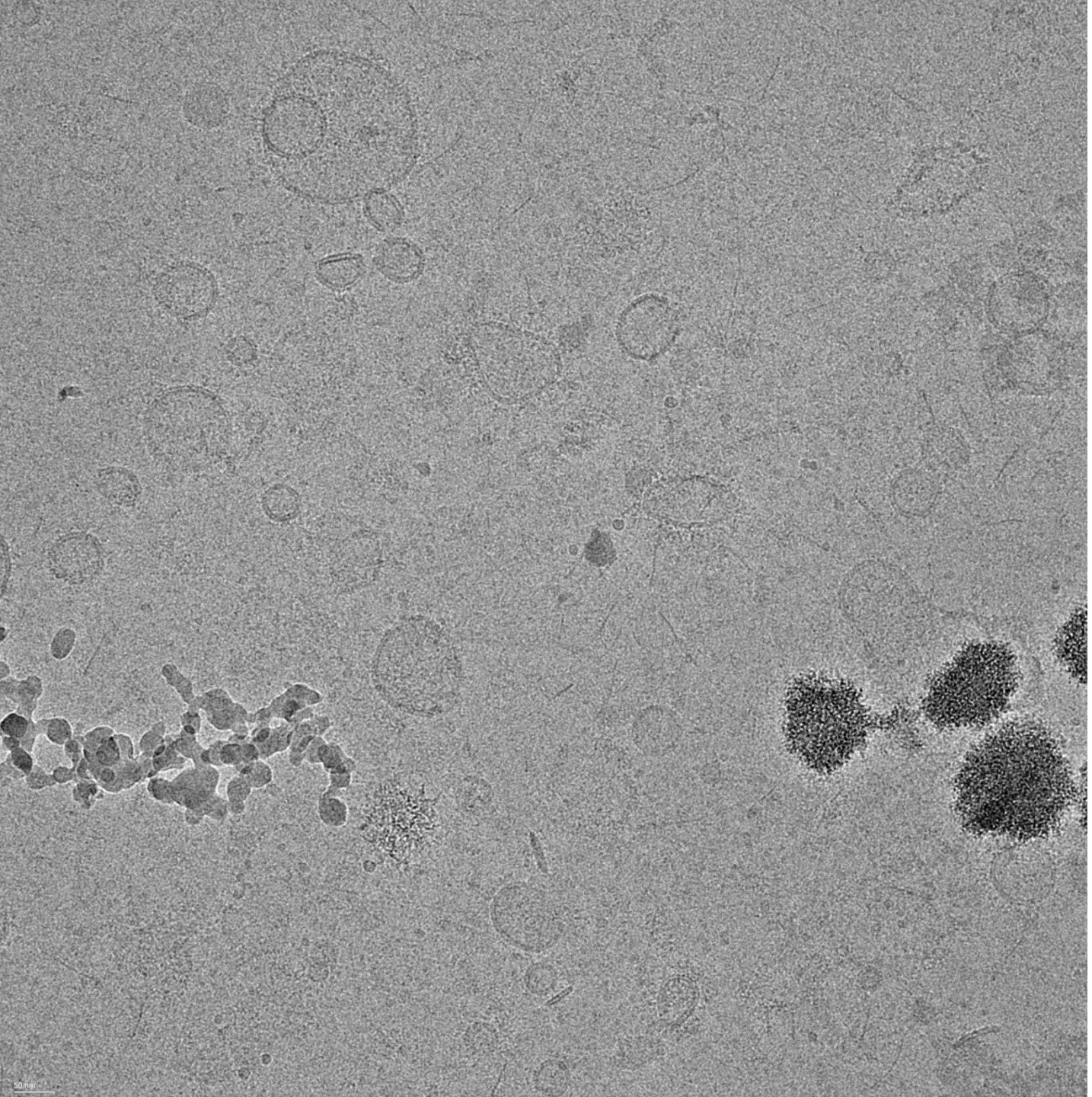


**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 26.**

***Cultivation of the algae***
Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
***Isolation of nanoparticles (NPs)***
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
***Cryogenic Electron Microscopy (Cryo-TEM)***
Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).
From https://zenodo.org/record/6908895. Image 16
DOI 10.5281/zenodo.6908895.

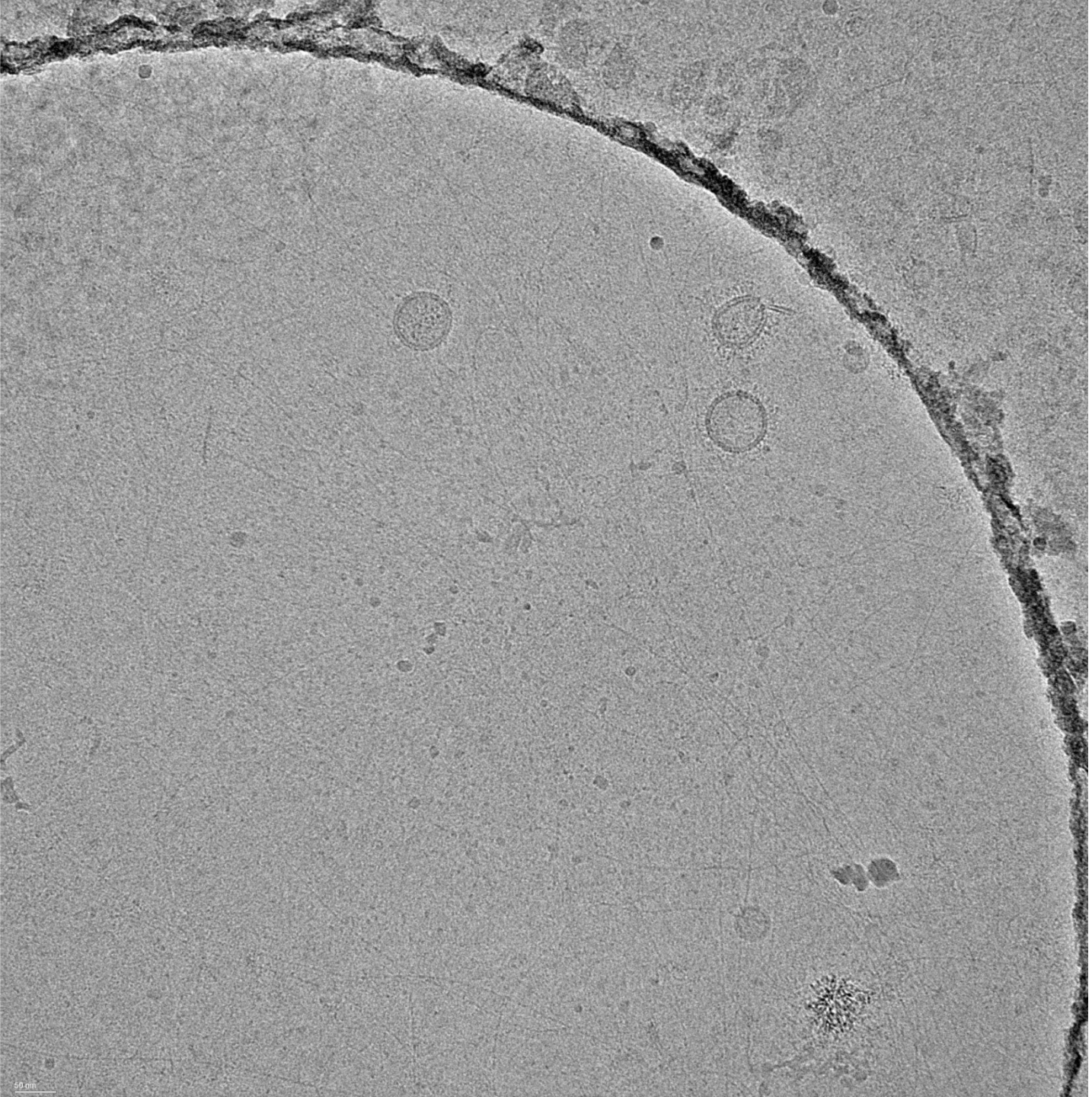


**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 27.**

***Cultivation of the algae***
Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
***Isolation of nanoparticles (NPs)***
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
***Cryogenic Electron Microscopy (Cryo-TEM)***
Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).
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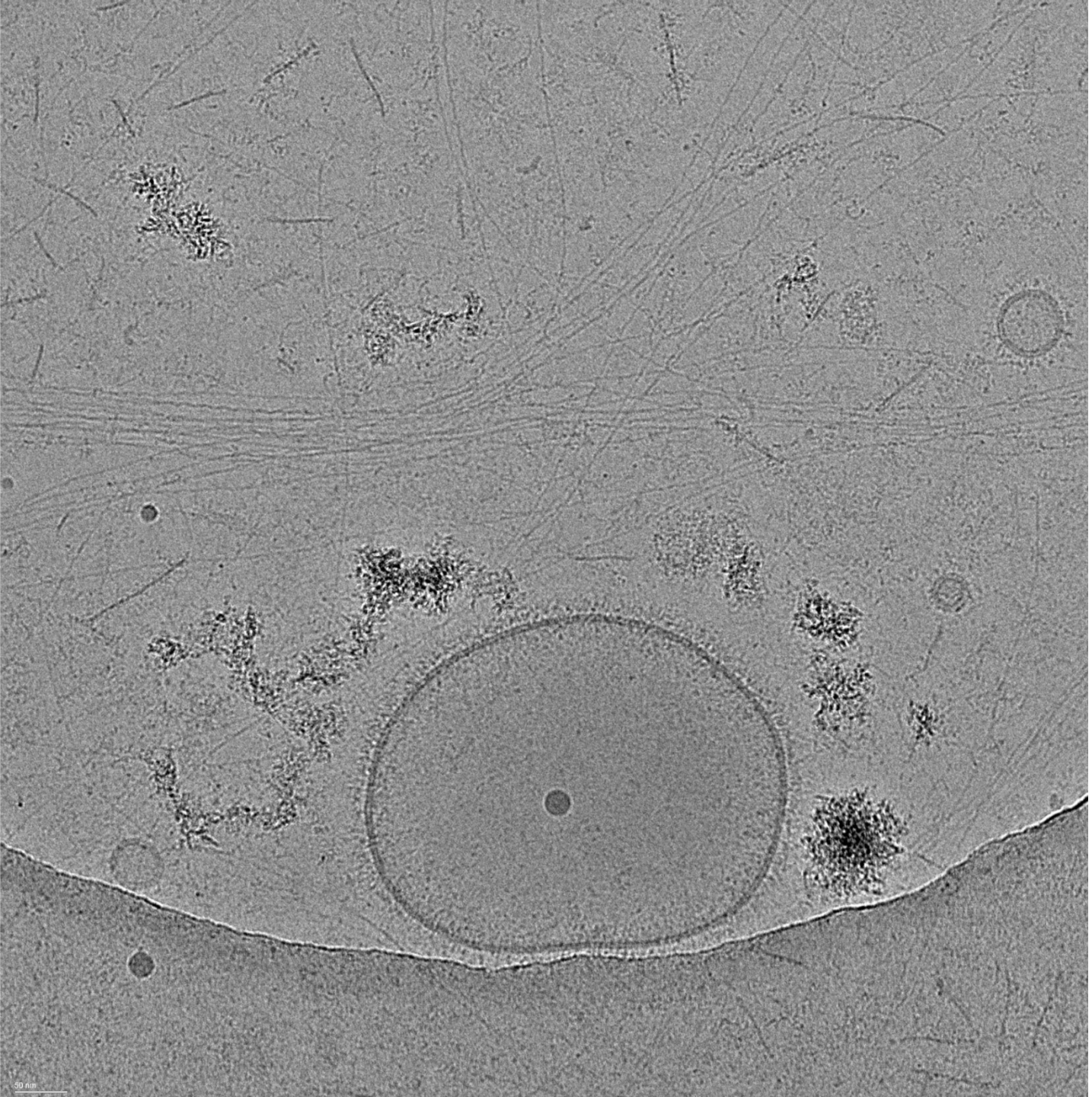


**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 28.**

***Cultivation of the algae***
Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
***Isolation of nanoparticles (NPs)***
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
***Cryogenic Electron Microscopy (Cryo-TEM)***
Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).
From Božič et al., 2022, https://doi.org/10.12688/openreseurope.14896.1.

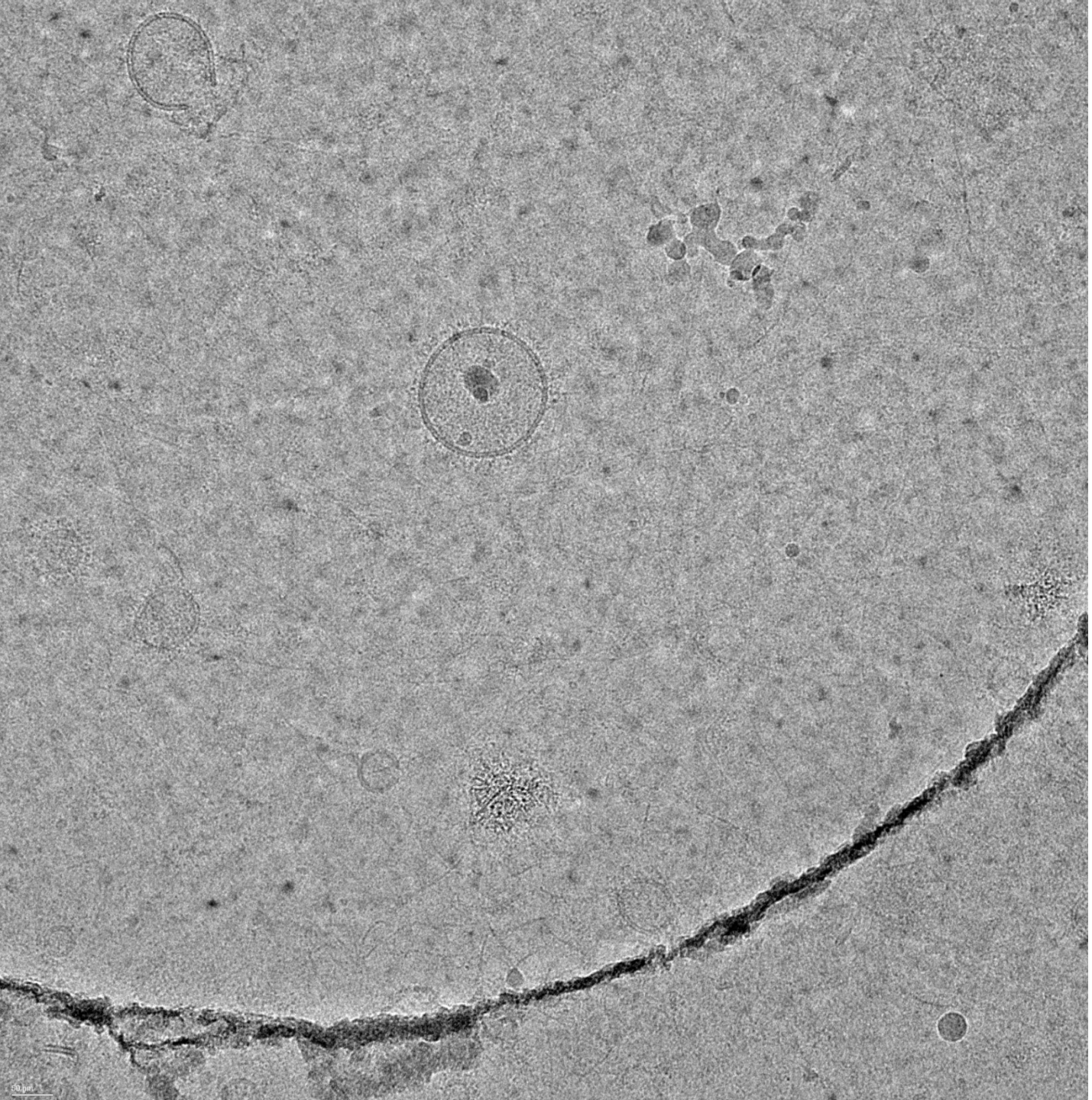

**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 29.**

***Cultivation of the algae***
Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.
***Isolation of nanoparticles (NPs)***
NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.
***Cryogenic Electron Microscopy (Cryo-TEM)***
Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).
From https://zenodo.org/record/6908895. Image 18
DOI 10.5281/zenodo.6908895.

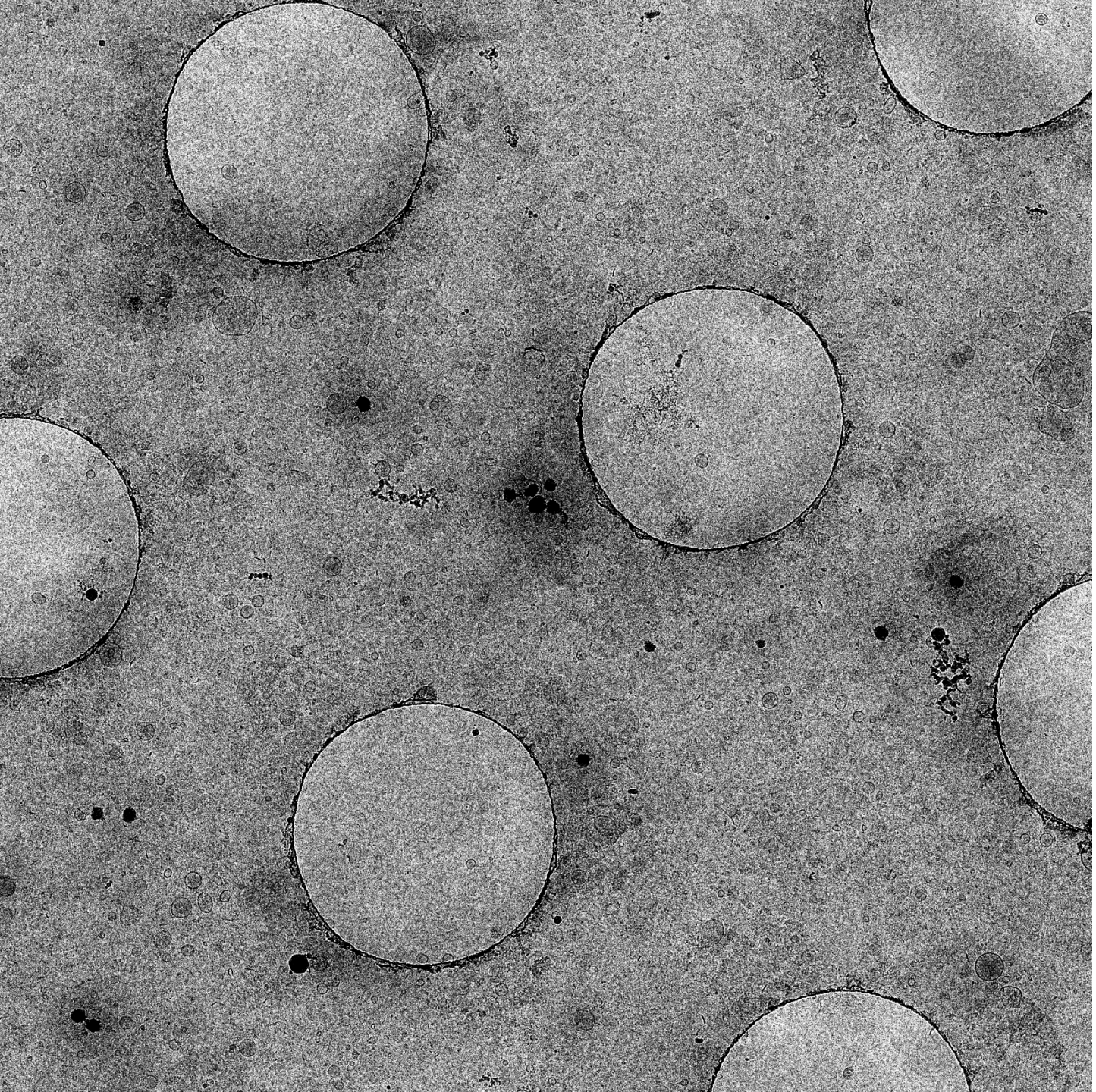

**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 30.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

***Isolation of nanoparticles (NPs)***

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

***Cryogenic Electron Microscopy (Cryo-TEM)***

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

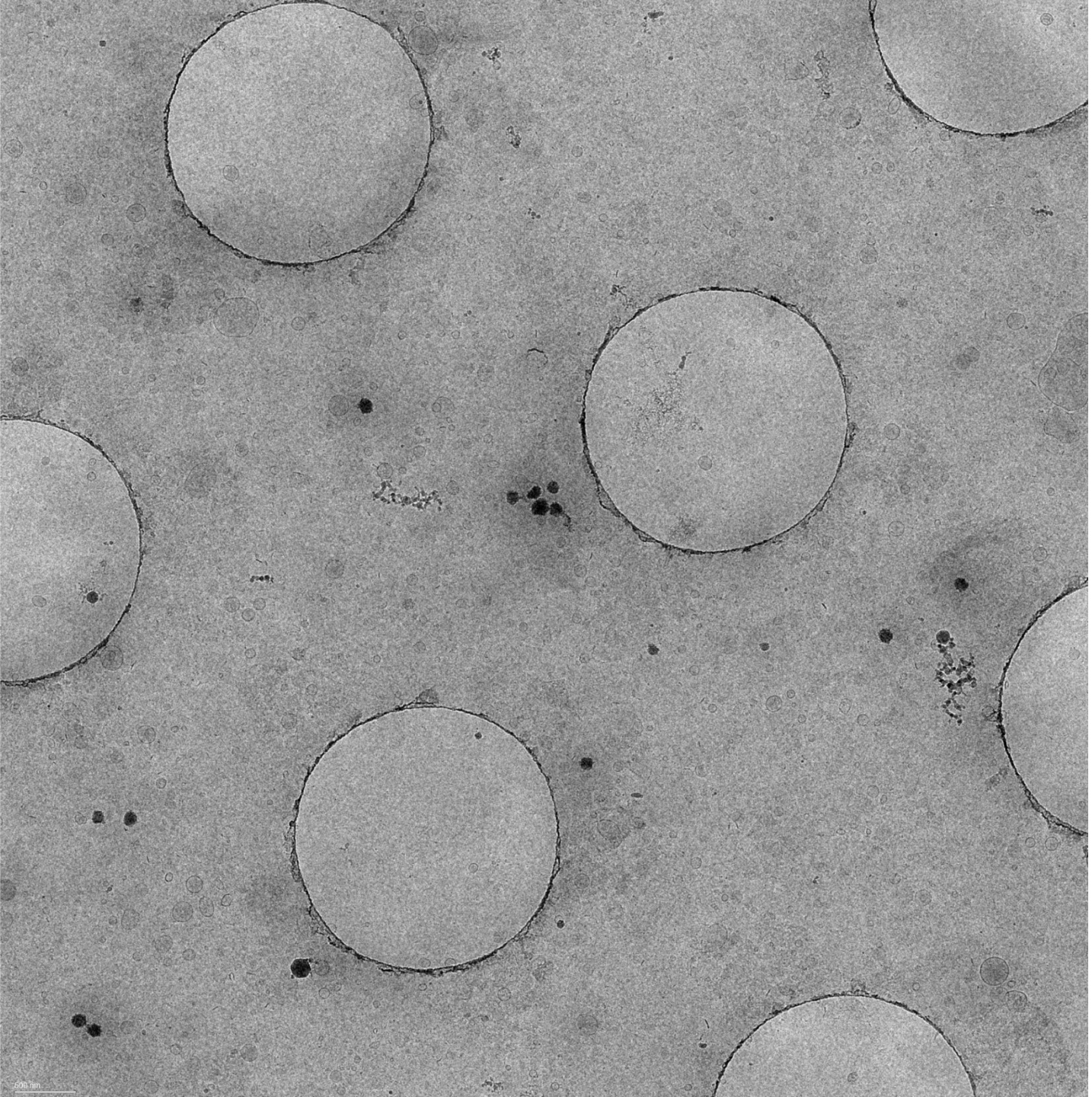

**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 31.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

***Isolation of nanoparticles (NPs)***

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

***Cryogenic Electron Microscopy (Cryo-TEM)***

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

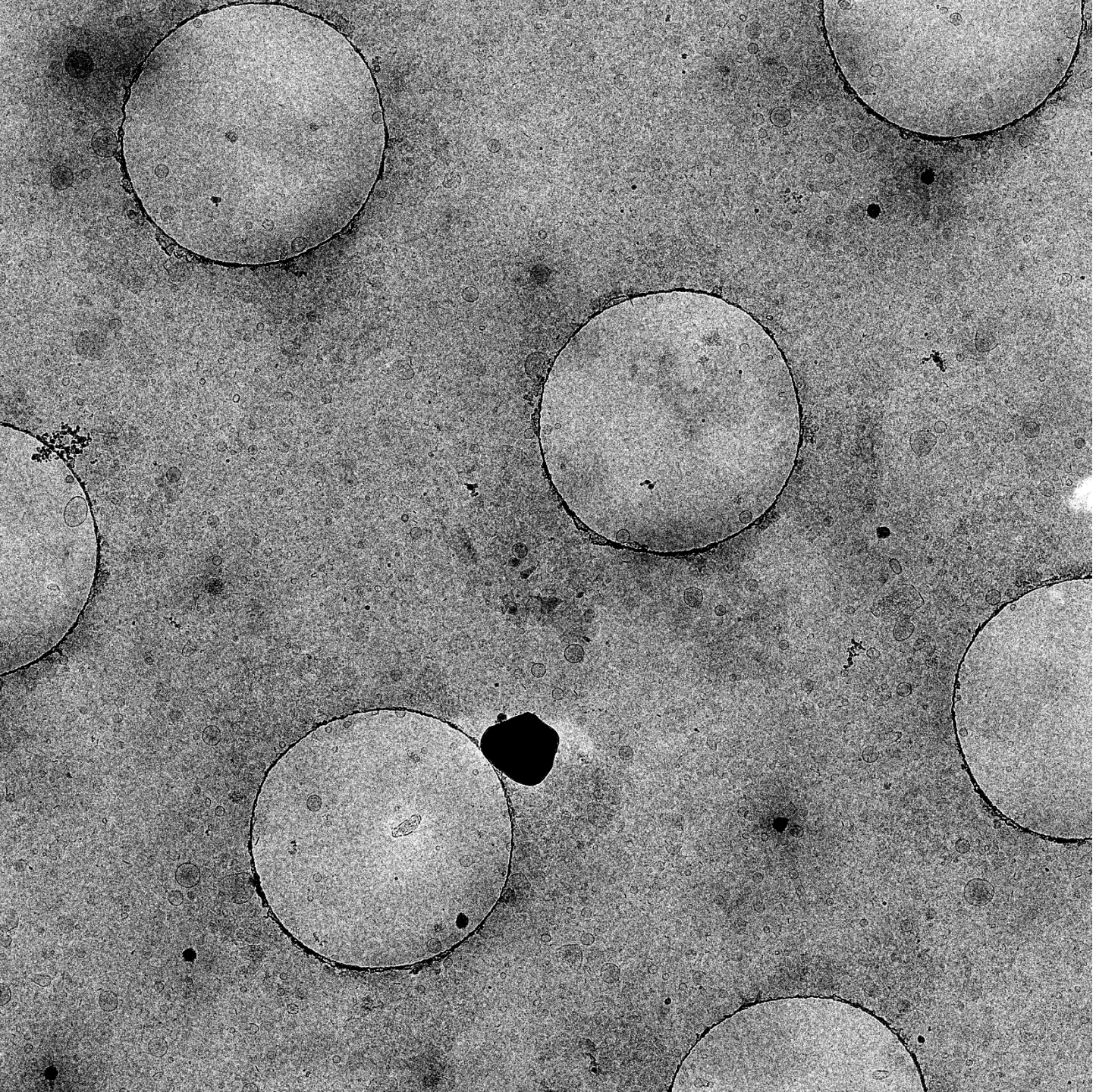

**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 32.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

***Isolation of nanoparticles (NPs)***

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***Cryogenic Electron Microscopy (Cryo-TEM)***

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

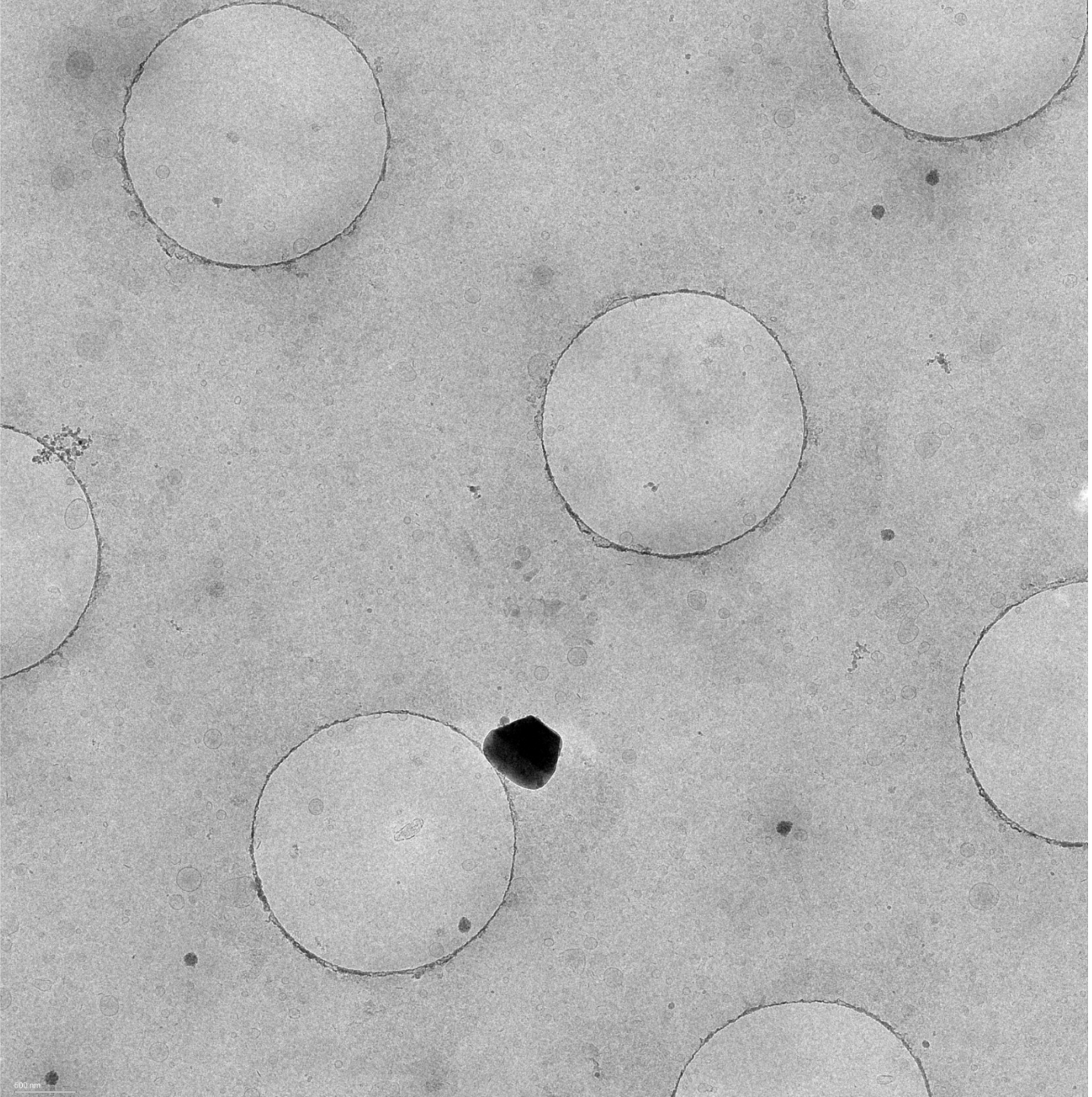


**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 33.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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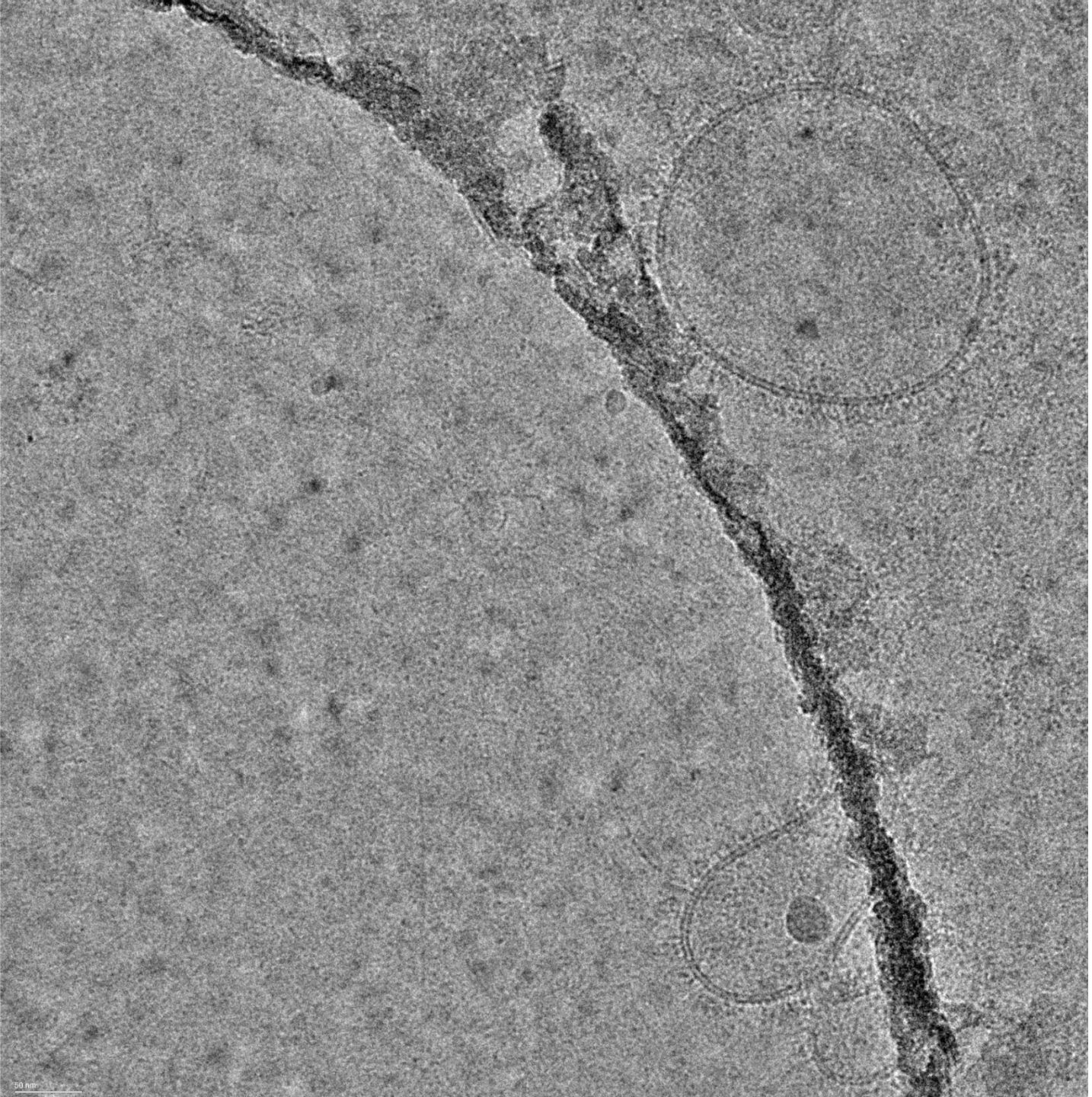

**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 34.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

***Isolation of nanoparticles (NPs)***

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

***Cryogenic Electron Microscopy (Cryo-TEM)***

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

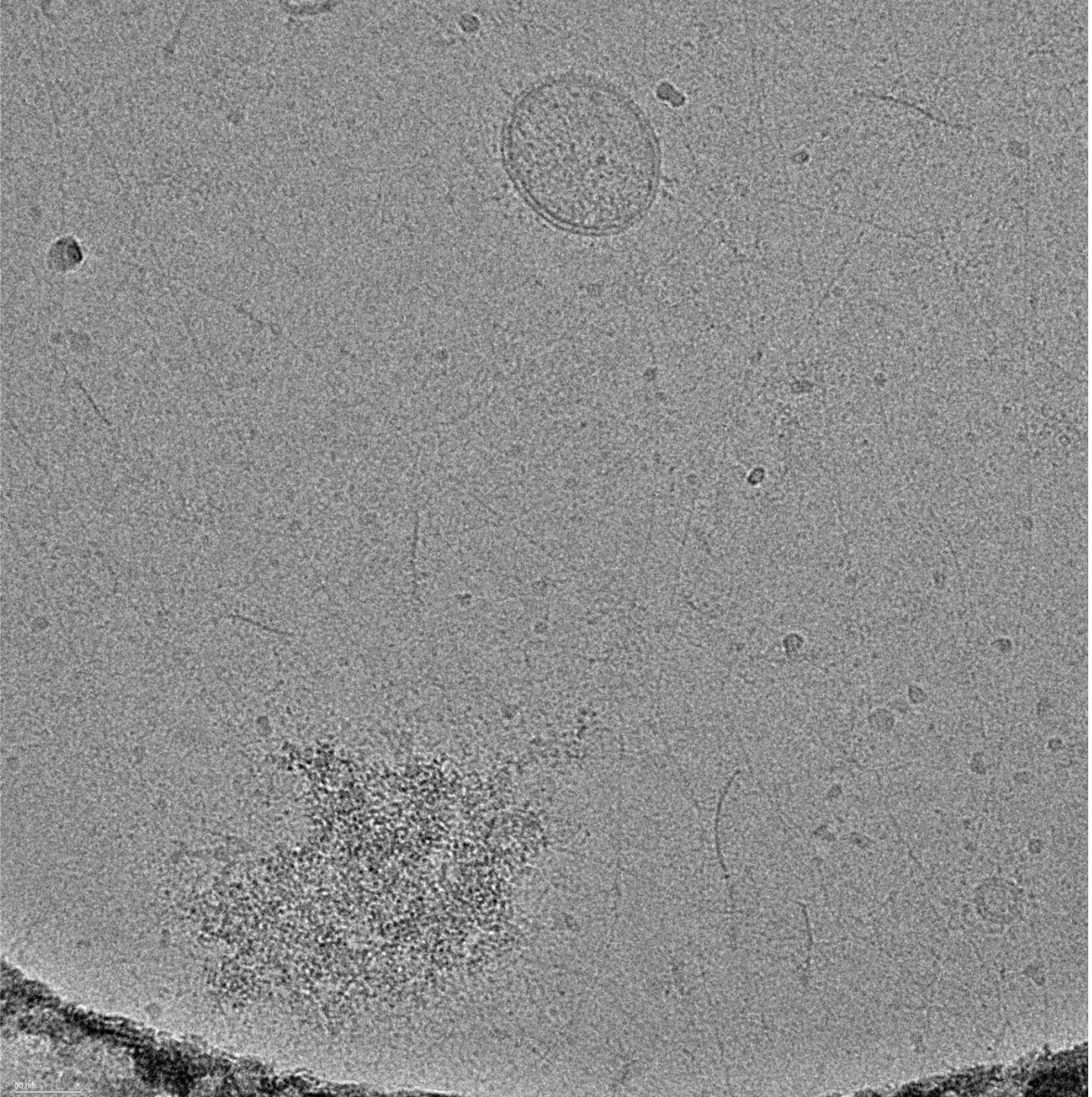

**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 35.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

***Isolation of nanoparticles (NPs)***

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

***Cryogenic Electron Microscopy (Cryo-TEM)***

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

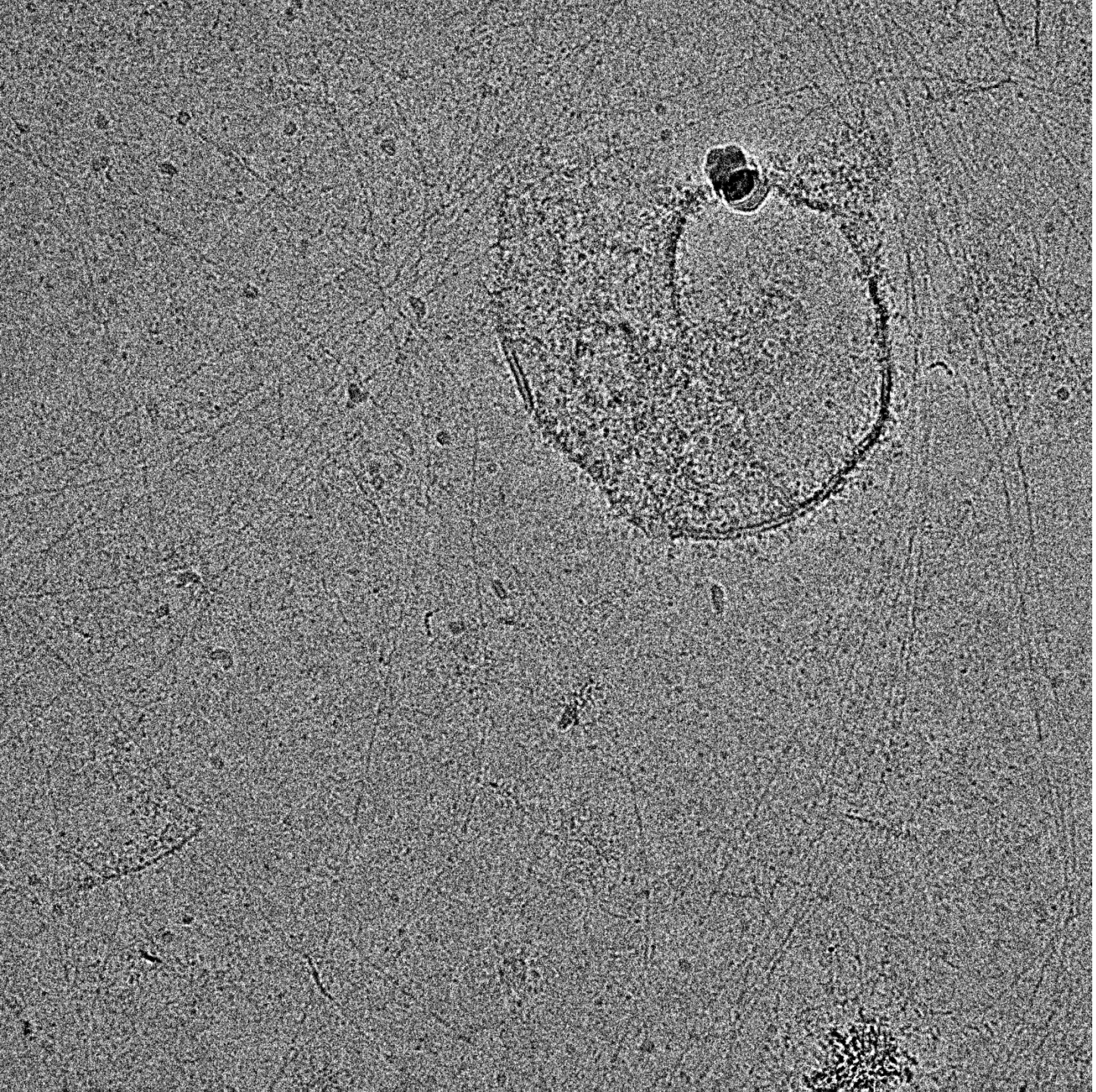

**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 36.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

***Isolation of nanoparticles (NPs)***

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

***Cryogenic Electron Microscopy (Cryo-TEM)***

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

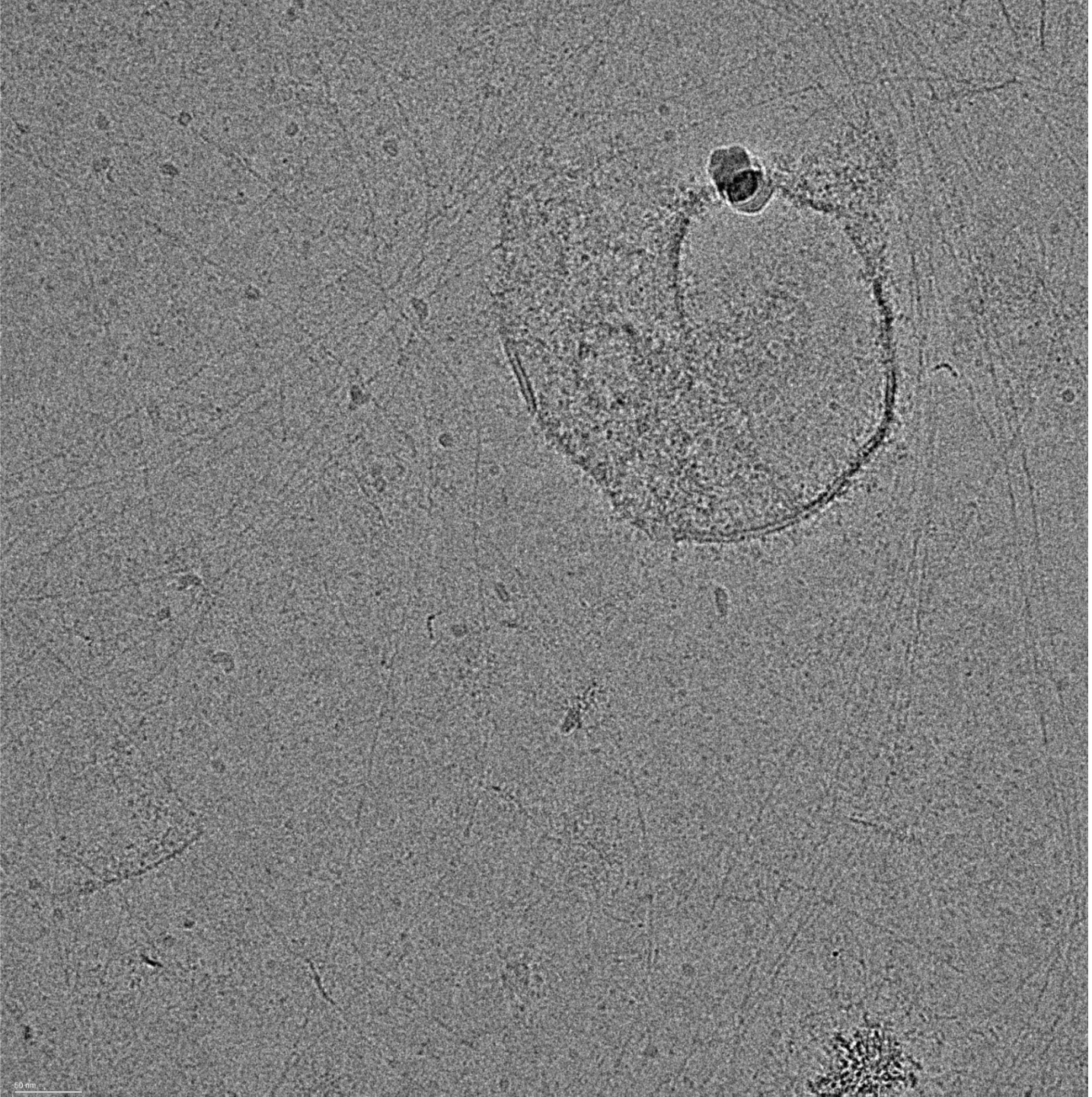


**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 37.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

***Isolation of nanoparticles (NPs)***

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

***Cryogenic Electron Microscopy (Cryo-TEM)***

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

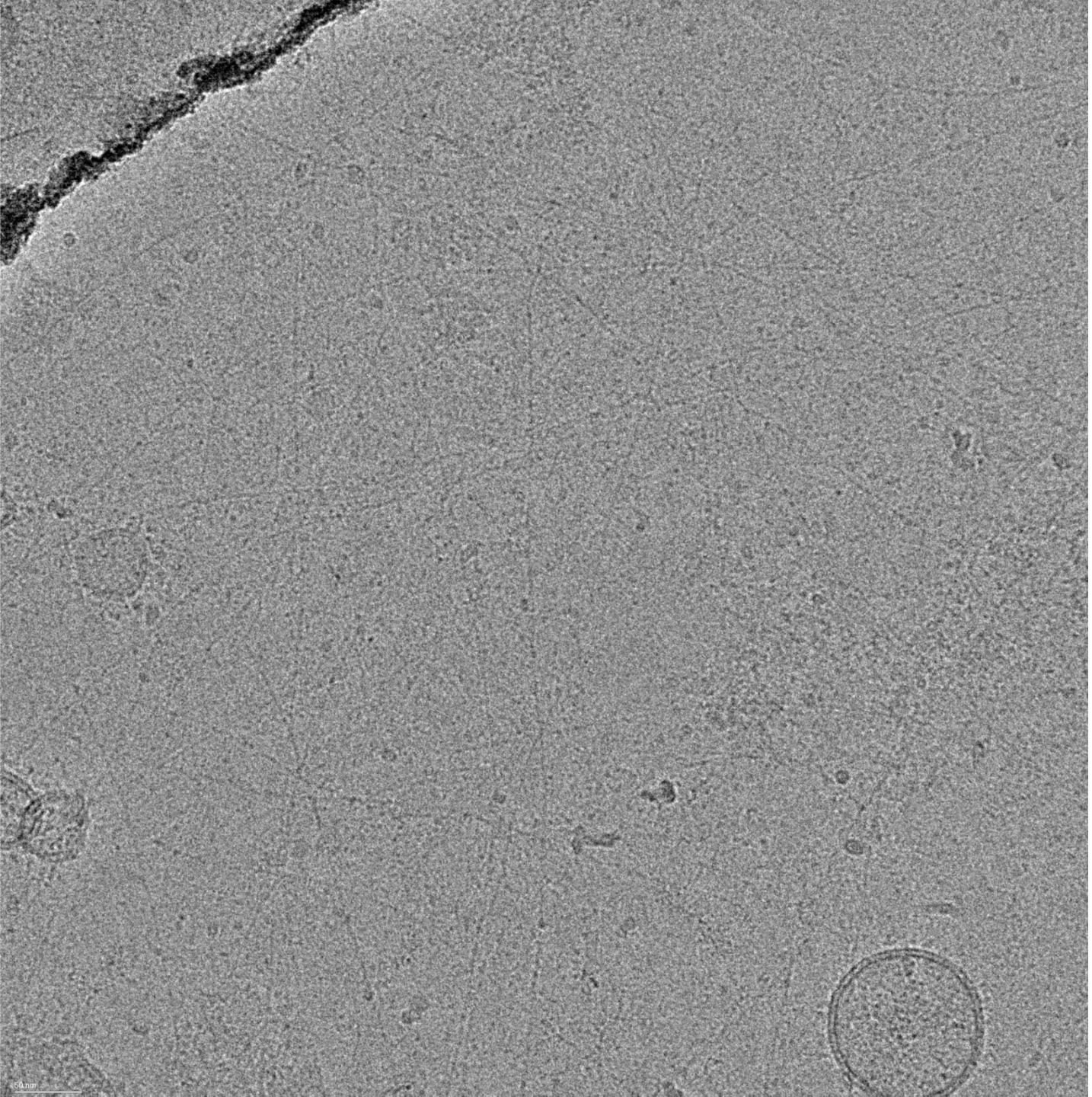

**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 38.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

***Isolation of nanoparticles (NPs)***

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***Cryogenic Electron Microscopy (Cryo-TEM)***

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

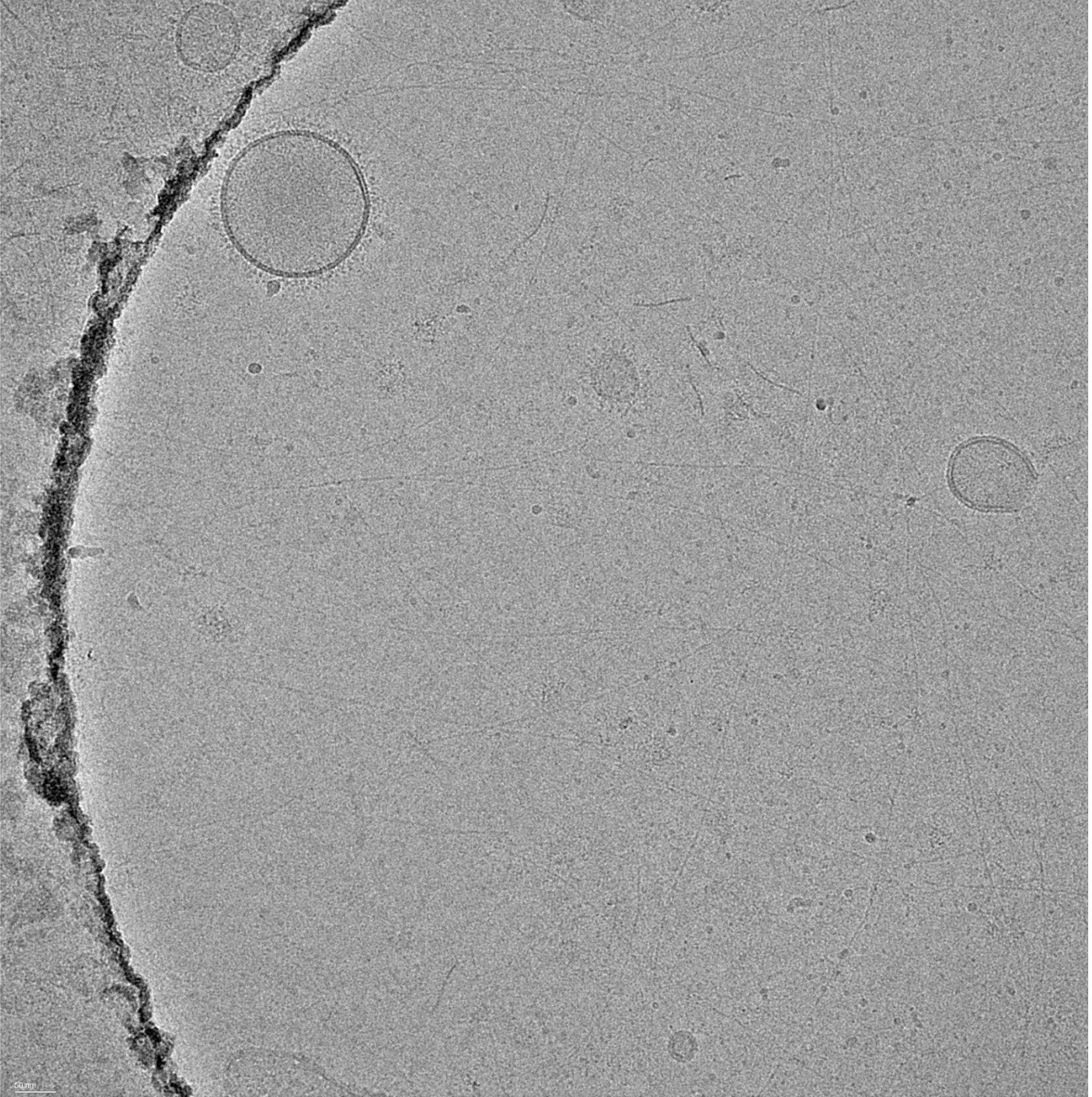

**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 39.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

***Isolation of nanoparticles (NPs)***

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

***Cryogenic Electron Microscopy (Cryo-TEM)***

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

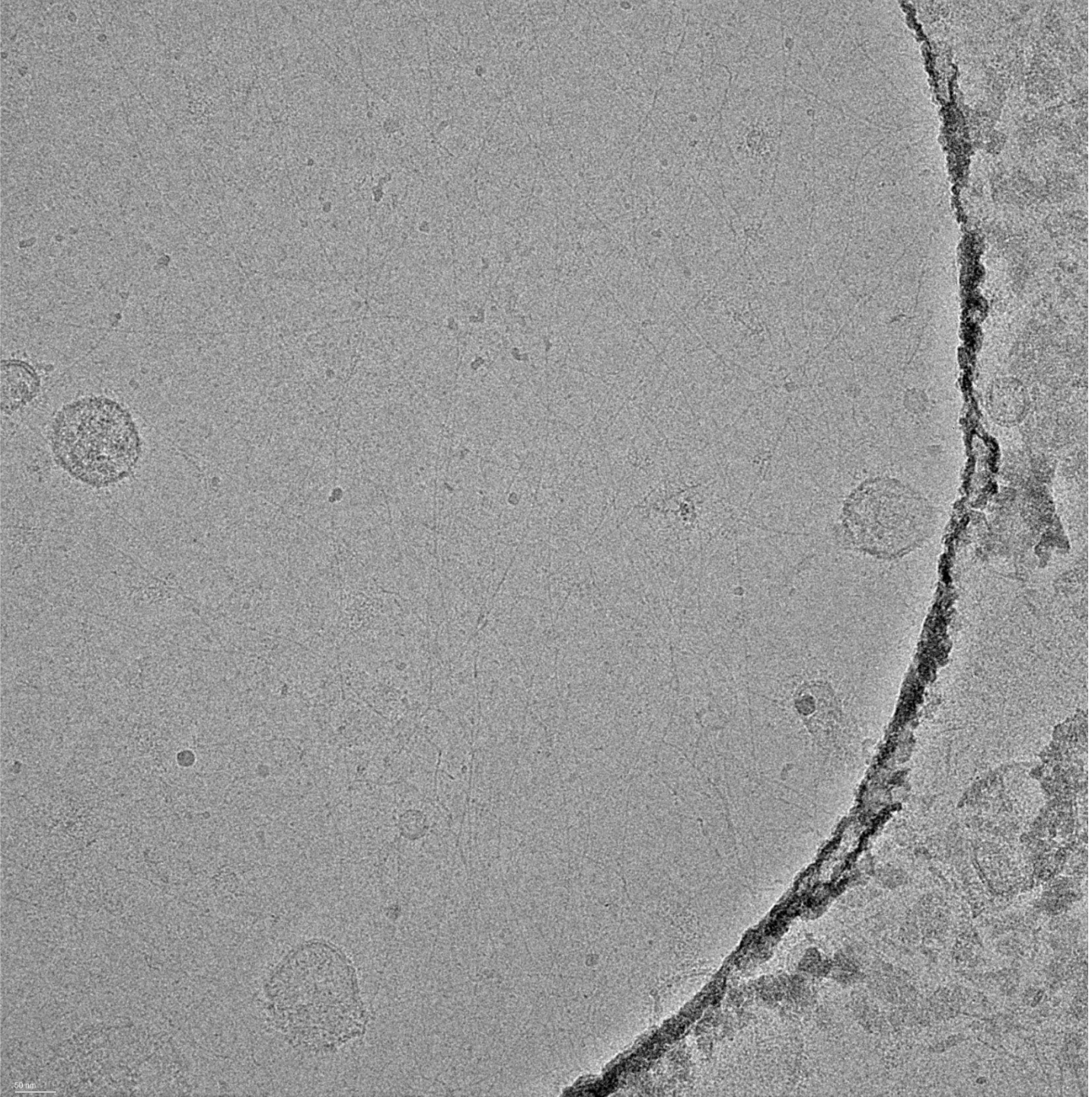


**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 40.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

***Isolation of nanoparticles (NPs)***

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

***Cryogenic Electron Microscopy (Cryo-TEM)***

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

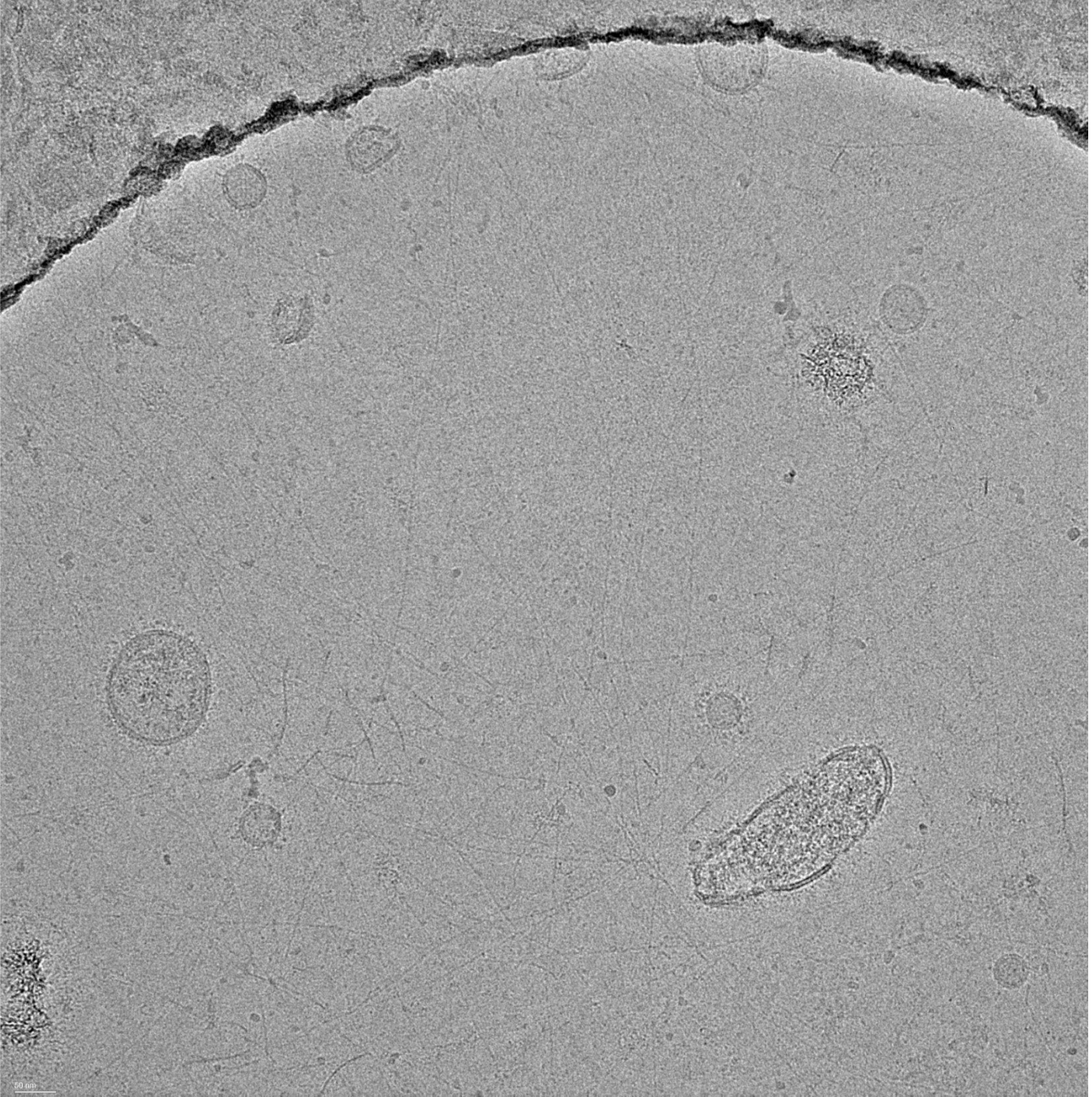


**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 41.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

***Isolation of nanoparticles (NPs)***

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

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Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

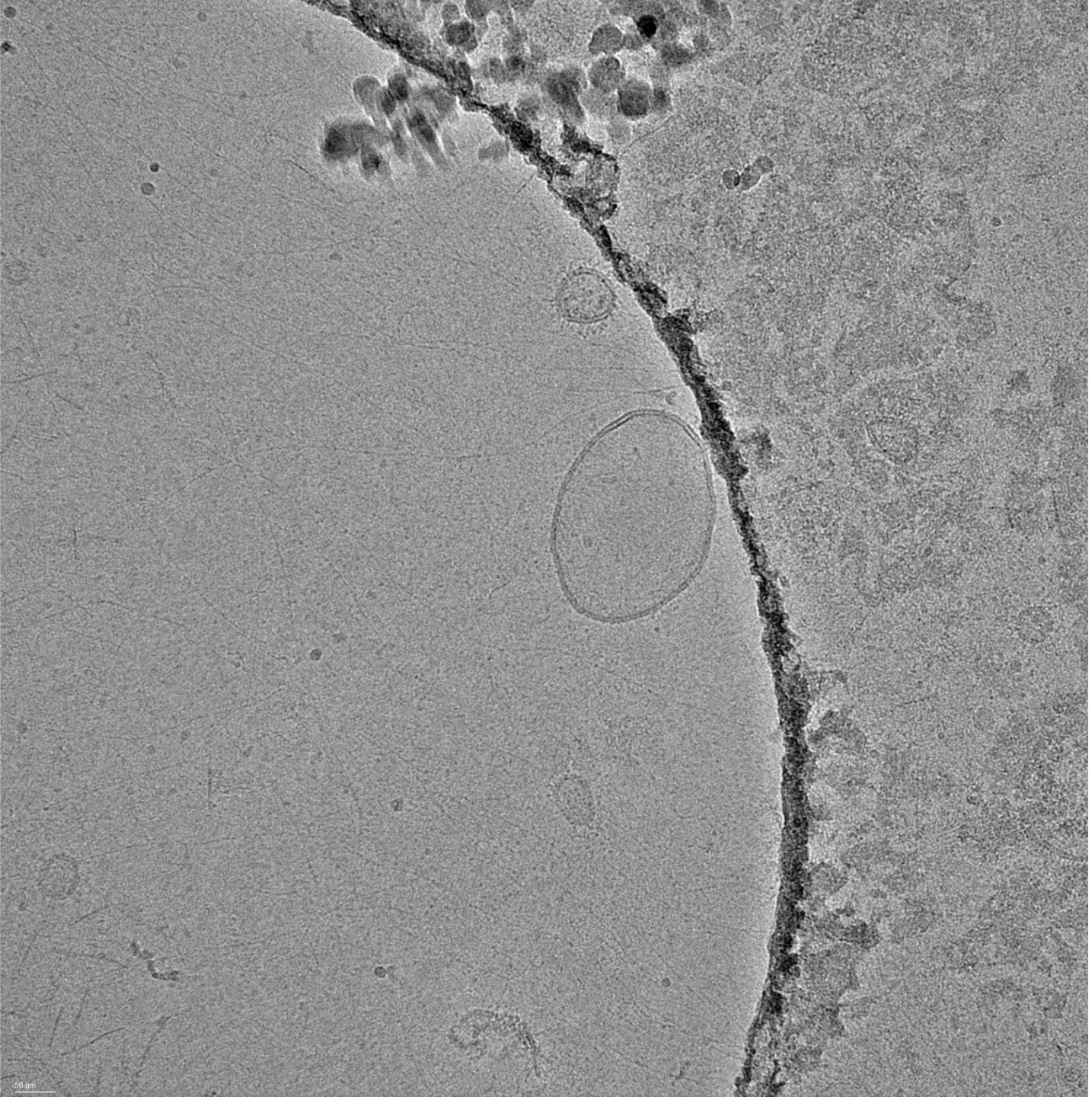


**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 42.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

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***Cryogenic Electron Microscopy (Cryo-TEM)***

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

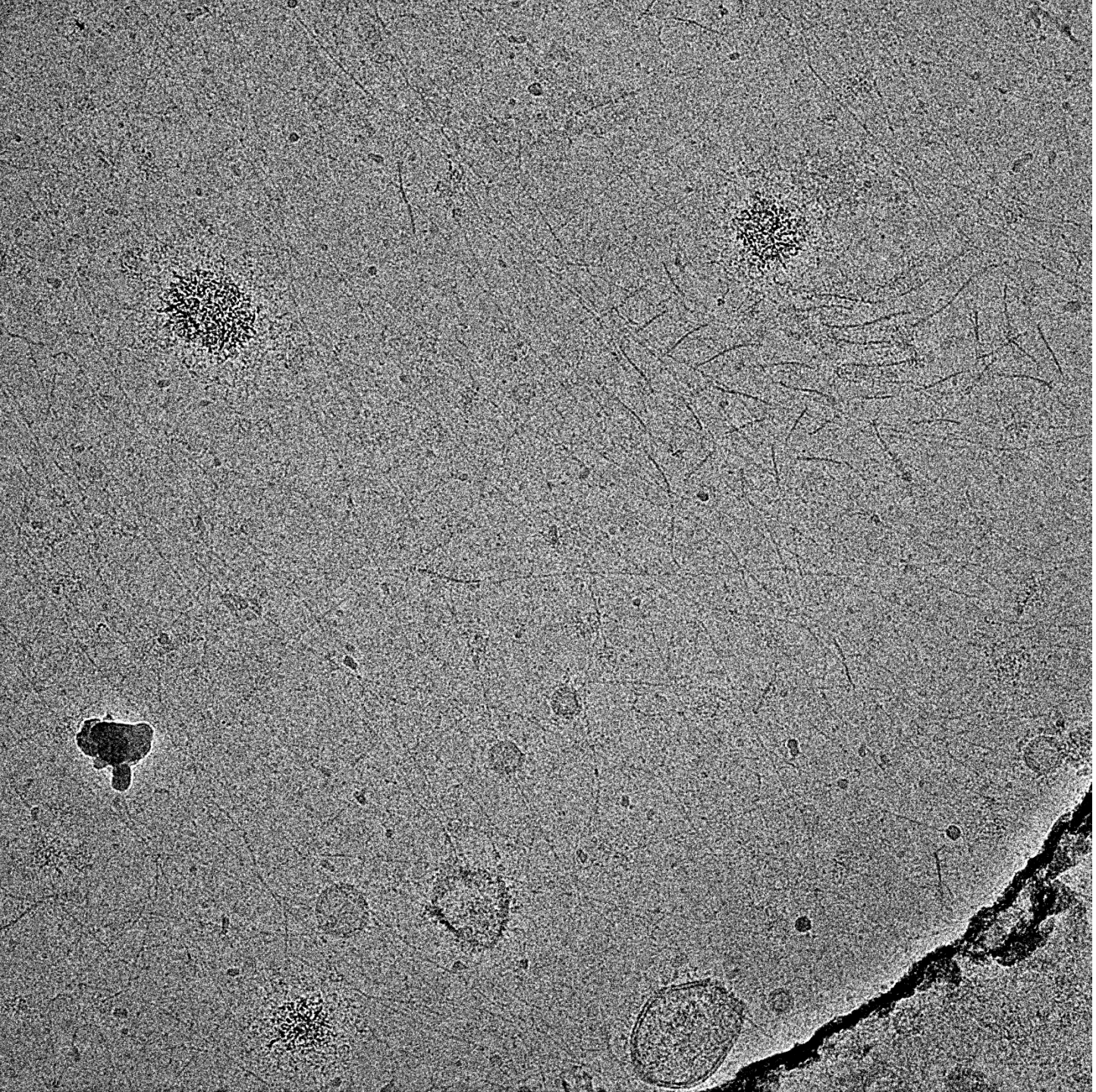

**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 43.**

***Cultivation of the algae***

Cultures of *P. tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) were grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)[17]. Cultures were grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/$m^2$s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

***Isolation of nanoparticles (NPs)***

NPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

***Cryogenic Electron Microscopy (Cryo-TEM)***

Samples of NPs isolates were prepared for Cryo-TEM using Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA, US). Quantifoil® R 2/2 (or 1.2/1.3), 200 mesh holey carbon grids (Quantifoil Micro Tools GmbH, Großlöbichau, Germany) were glow discharged for 60 s at 20 mA and positive polarity in the air atmosphere (GloQube® Plus, Quorum, Laughton, UK). Conditions were set at 4 °C, 95% relative humidity, blot time: 5 s, and blot force: 4. 2 μL of the sample with nanoparticles in suspension was applied to the grid, blotted, and vitrified in liquid ethane. Samples were visualized under cryogenic conditions using a 200 kV microscope Glacios with Falcon 3EC detector (Thermo Fisher Scientific, Waltham, MA, US).

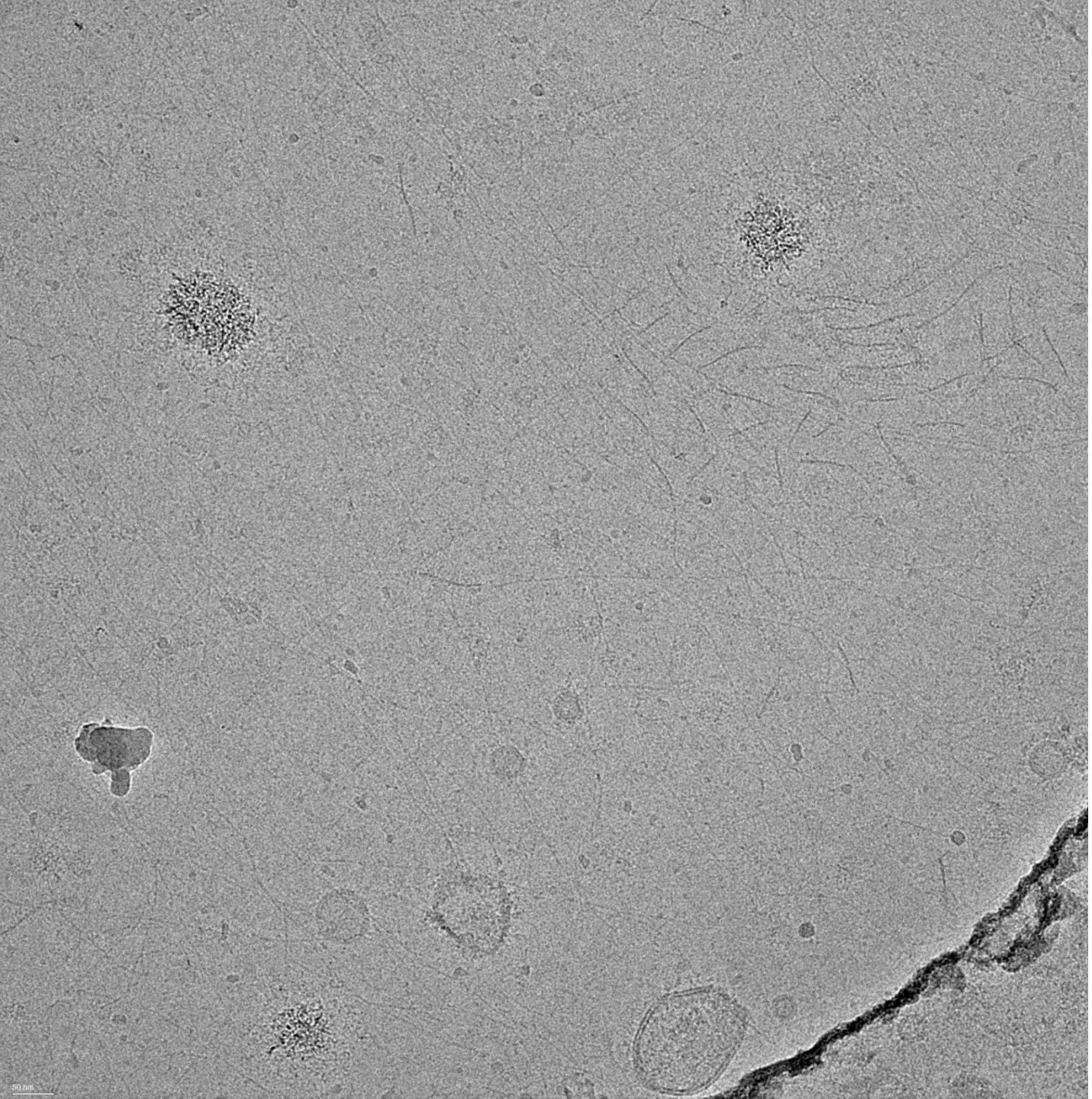


**Figure *Phaeodactylum tricornutum* isolate CRYO-TEM 44.**

***Cultivation of the algae***

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