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Repository

Cryogenic transmission electron images of *Dunaliela tertiolecta* isolates of small cellular particles

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Abstract: Cryogenic transmission electron images of small cellular particles isolated from conditioned media of microalgae *Dunaliella tertiolecta* are presented. Each image is 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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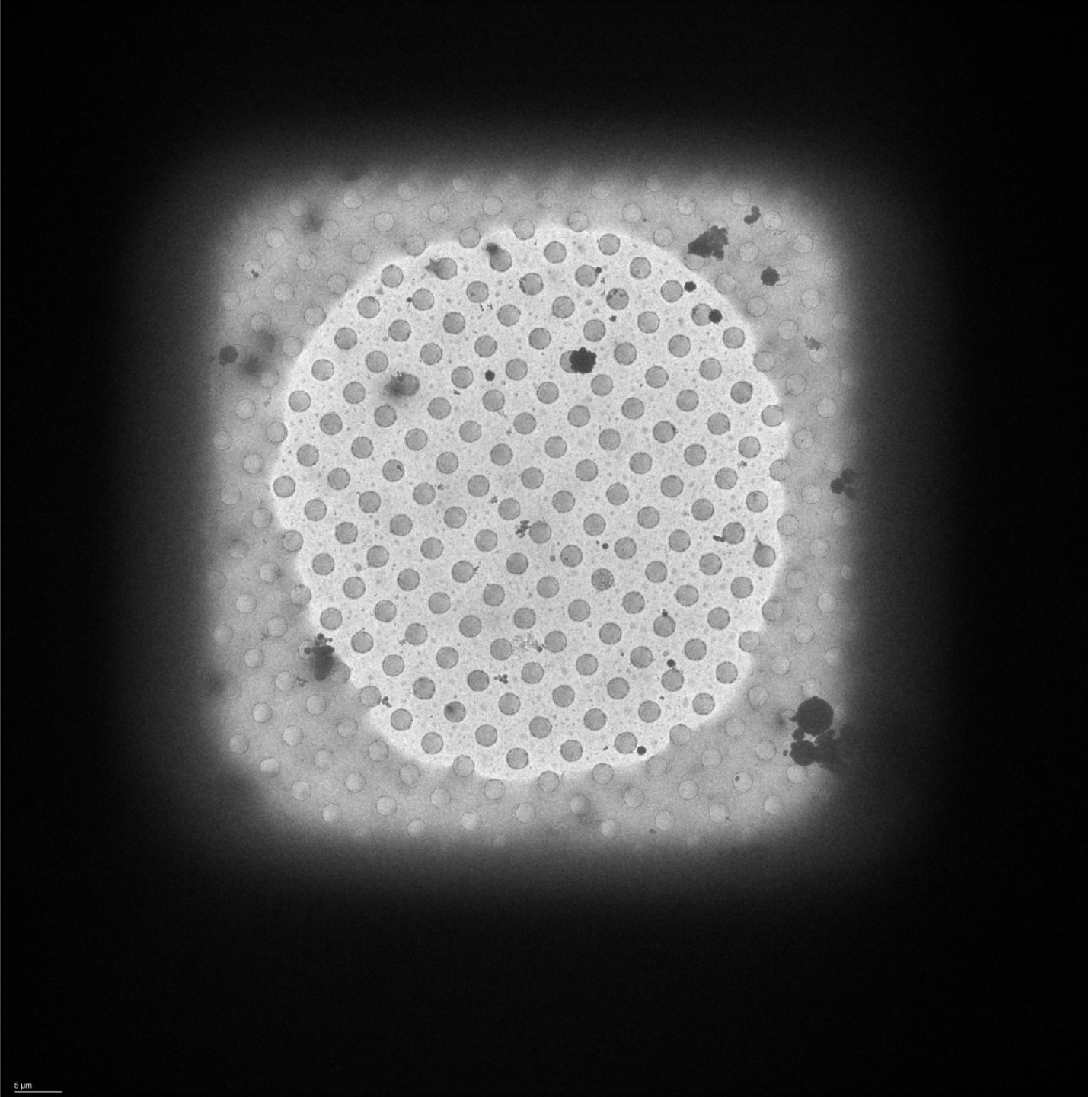

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 1.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) 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).

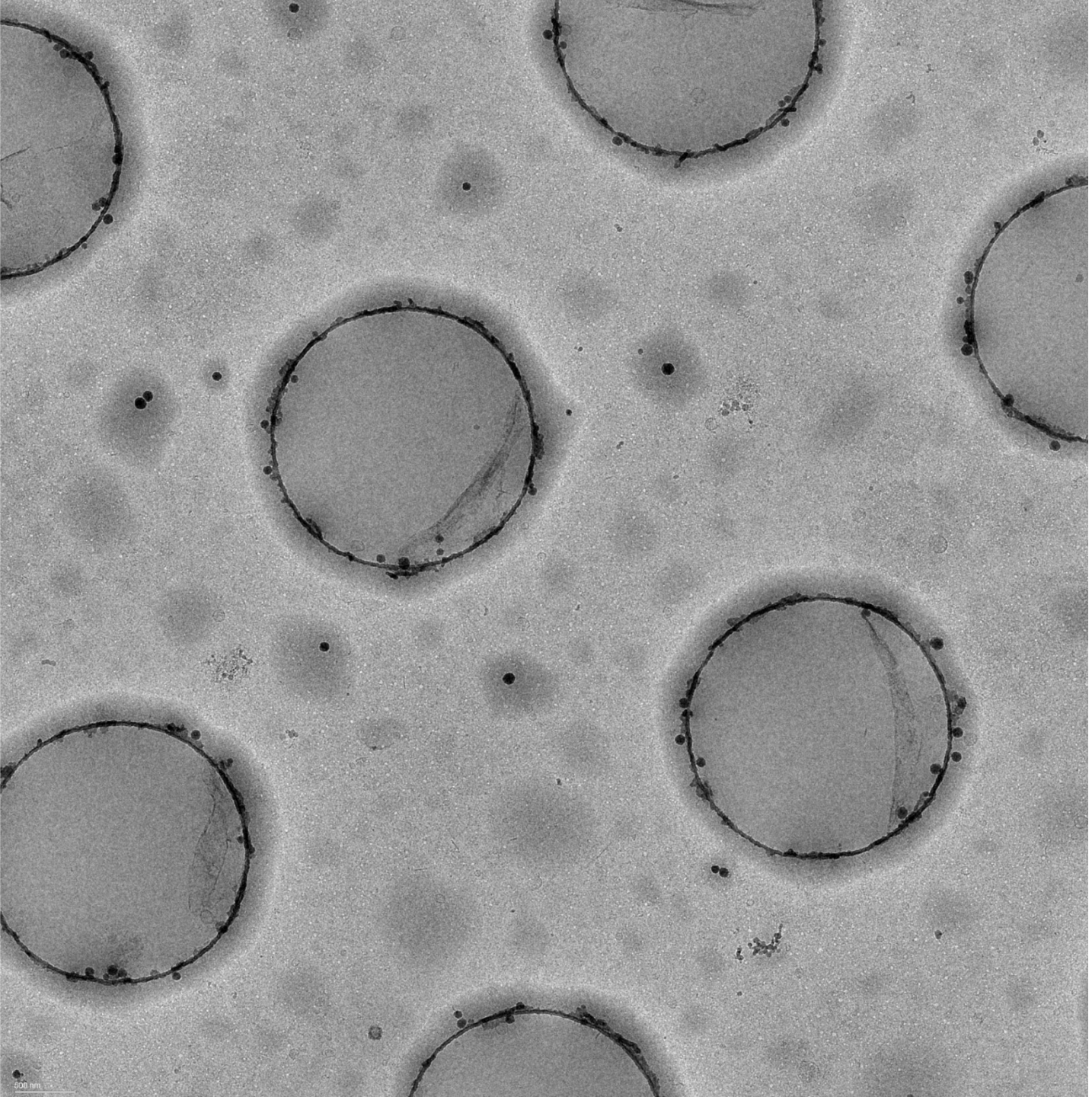

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 2.

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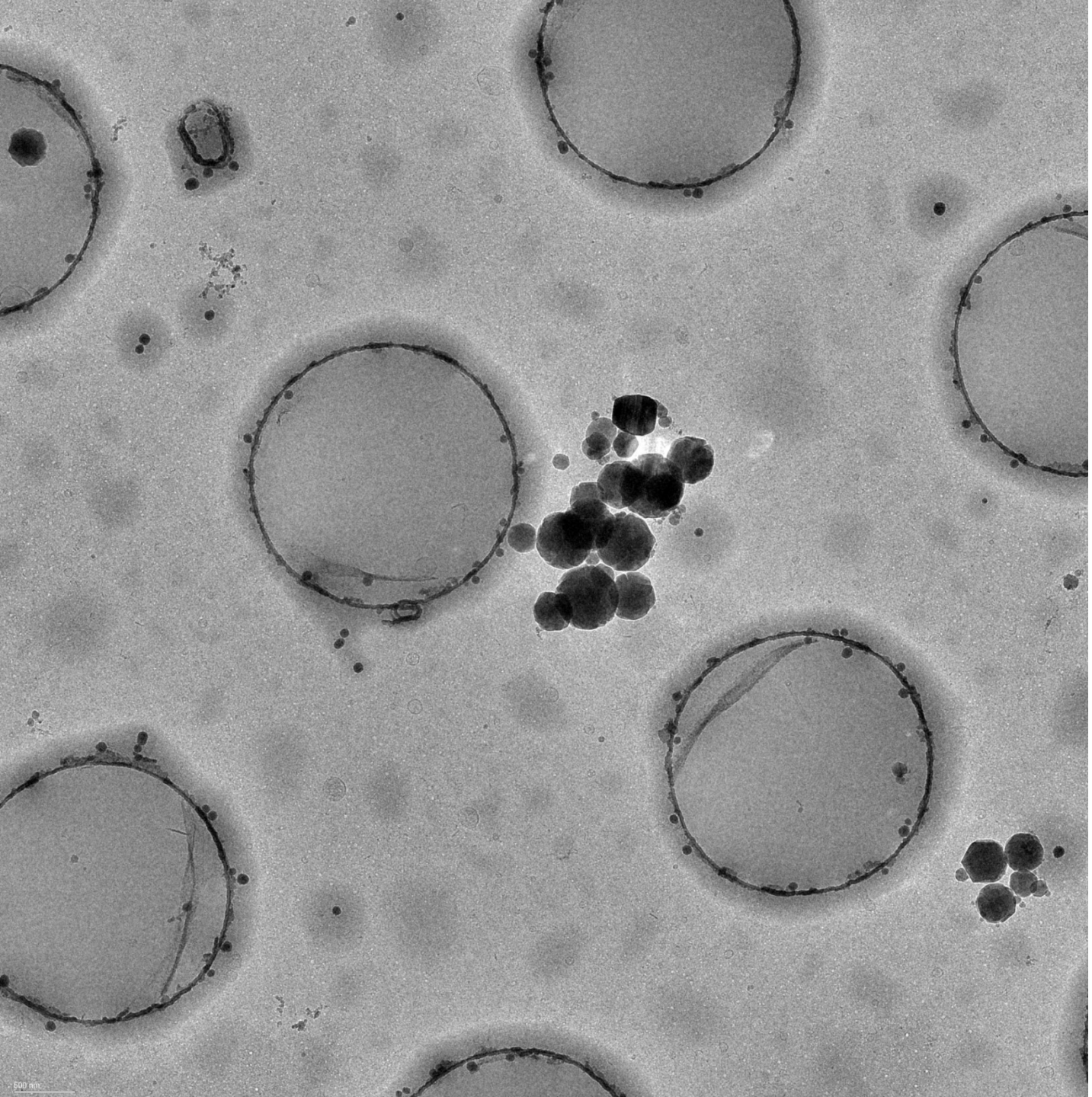

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 3.

Cultivation of the algae

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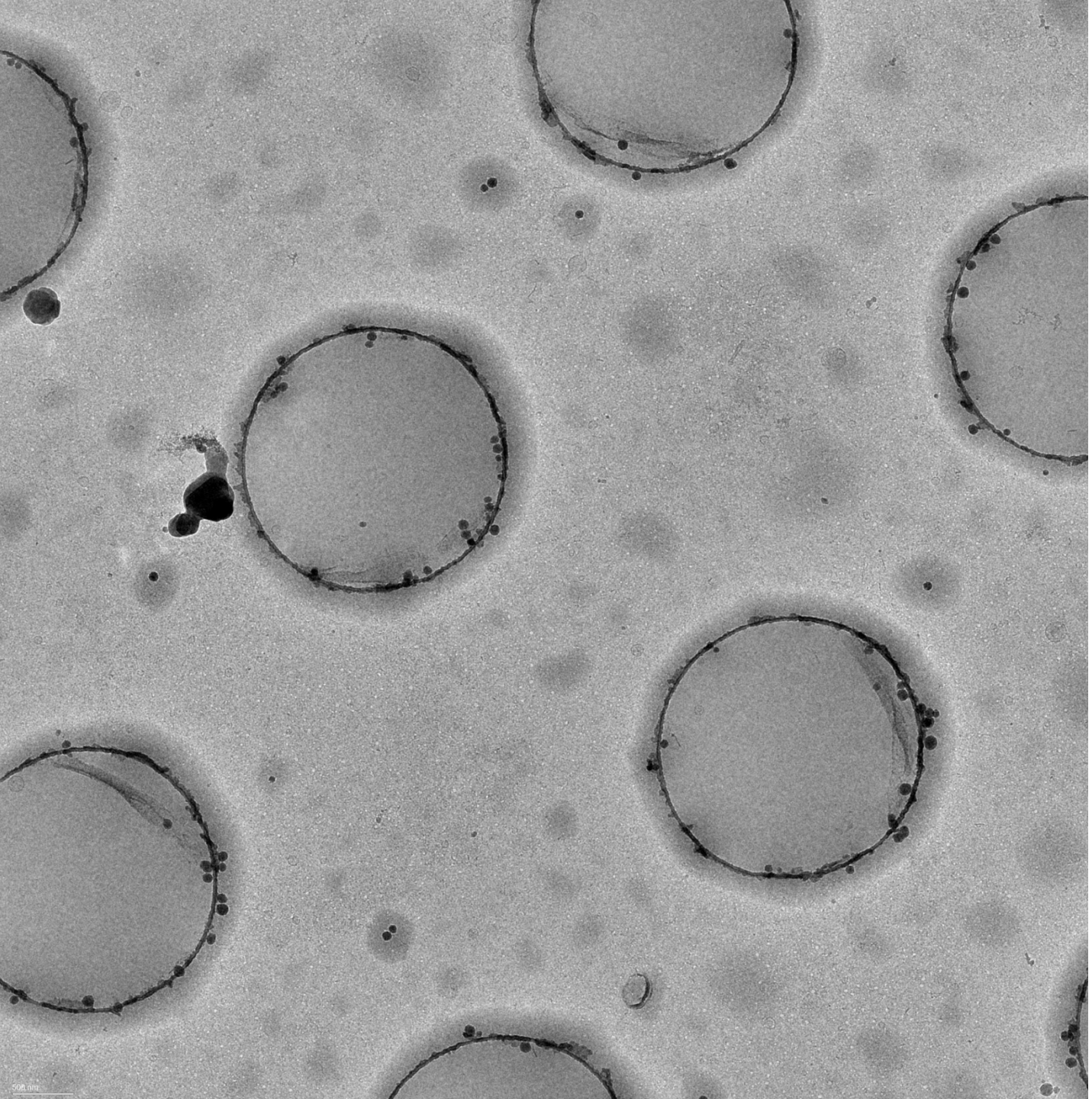

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 4.

Cultivation of the algae

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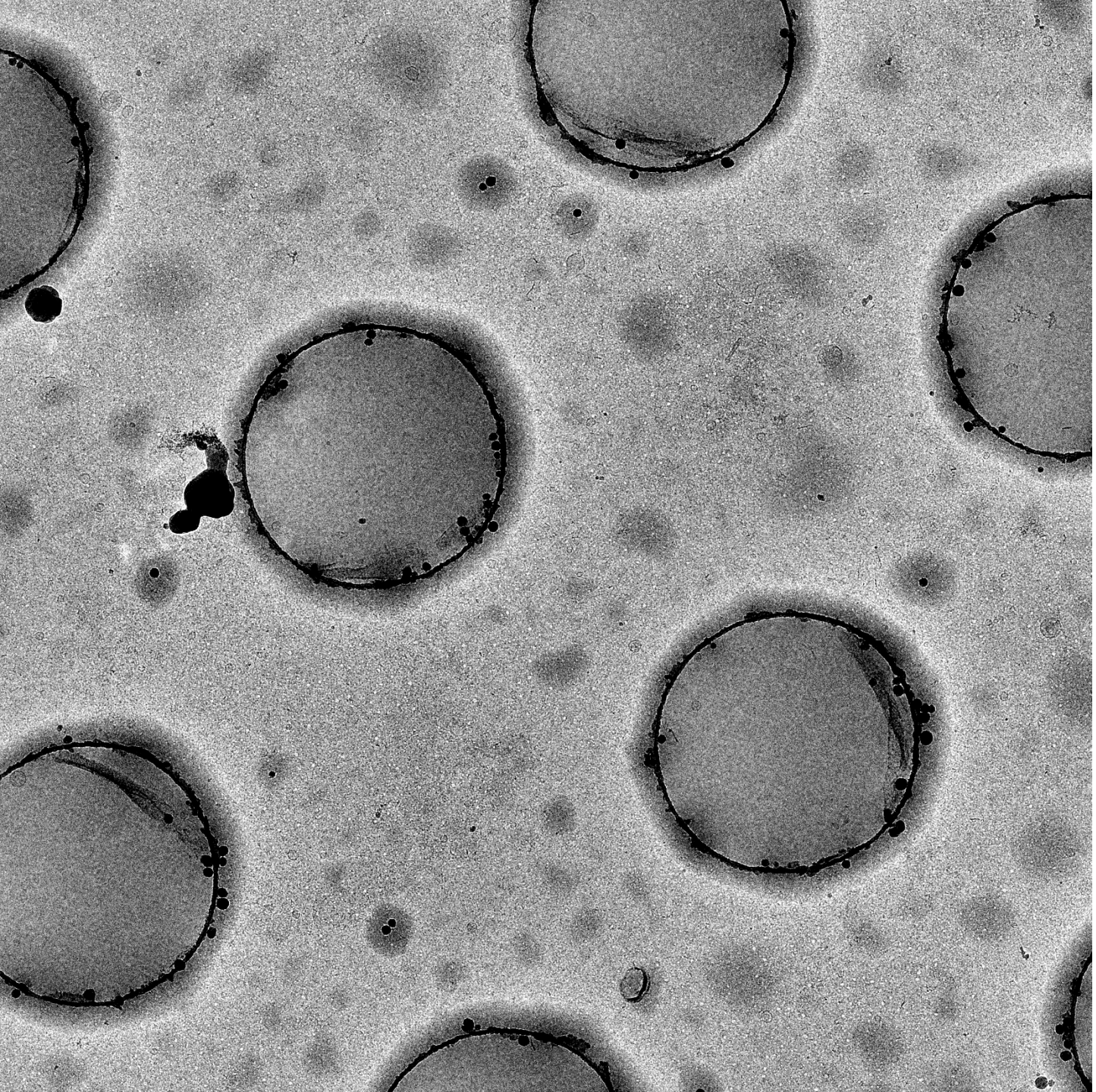

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 5.

Cultivation of the algae

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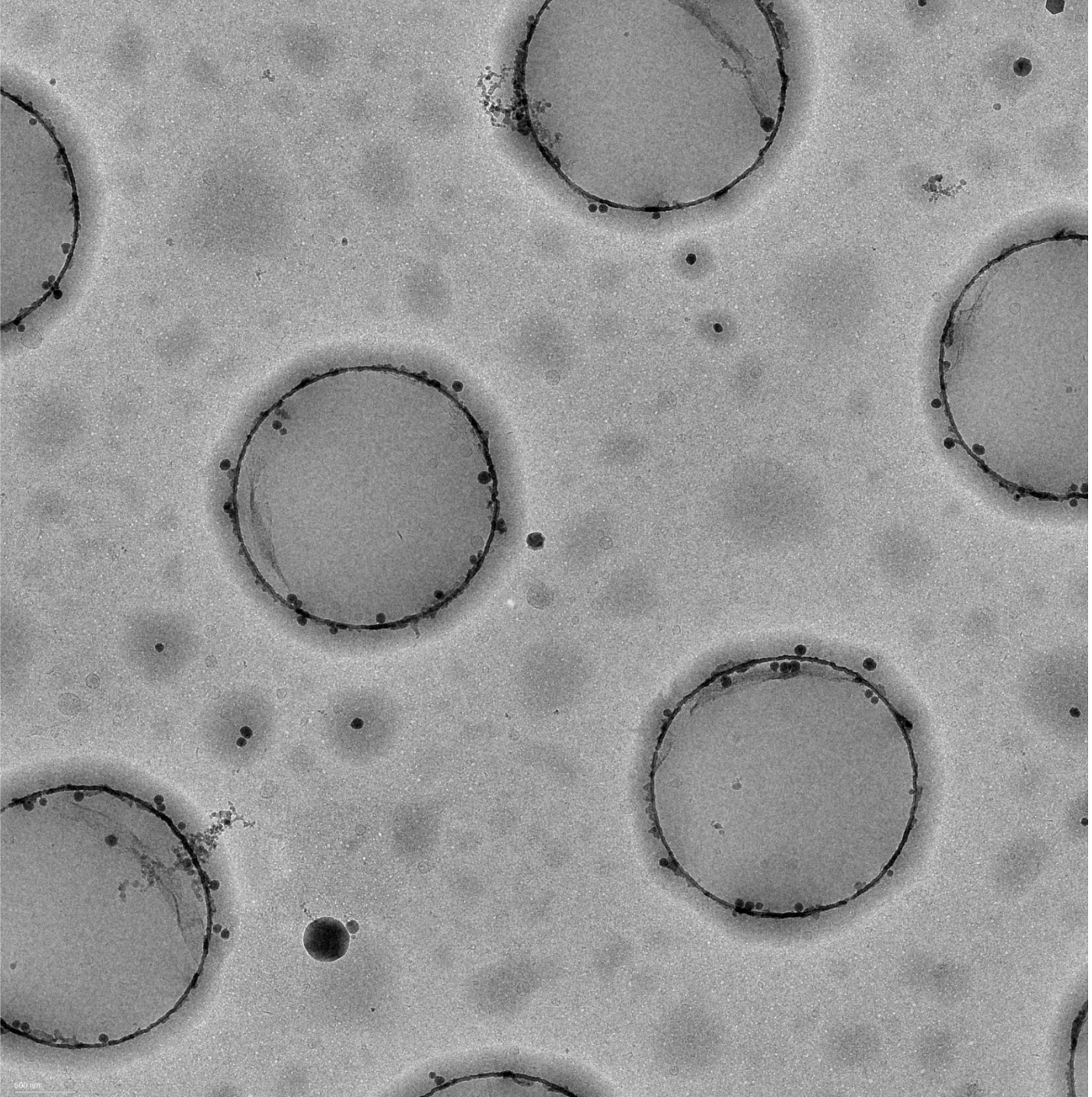

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 6.

Cultivation of the algae

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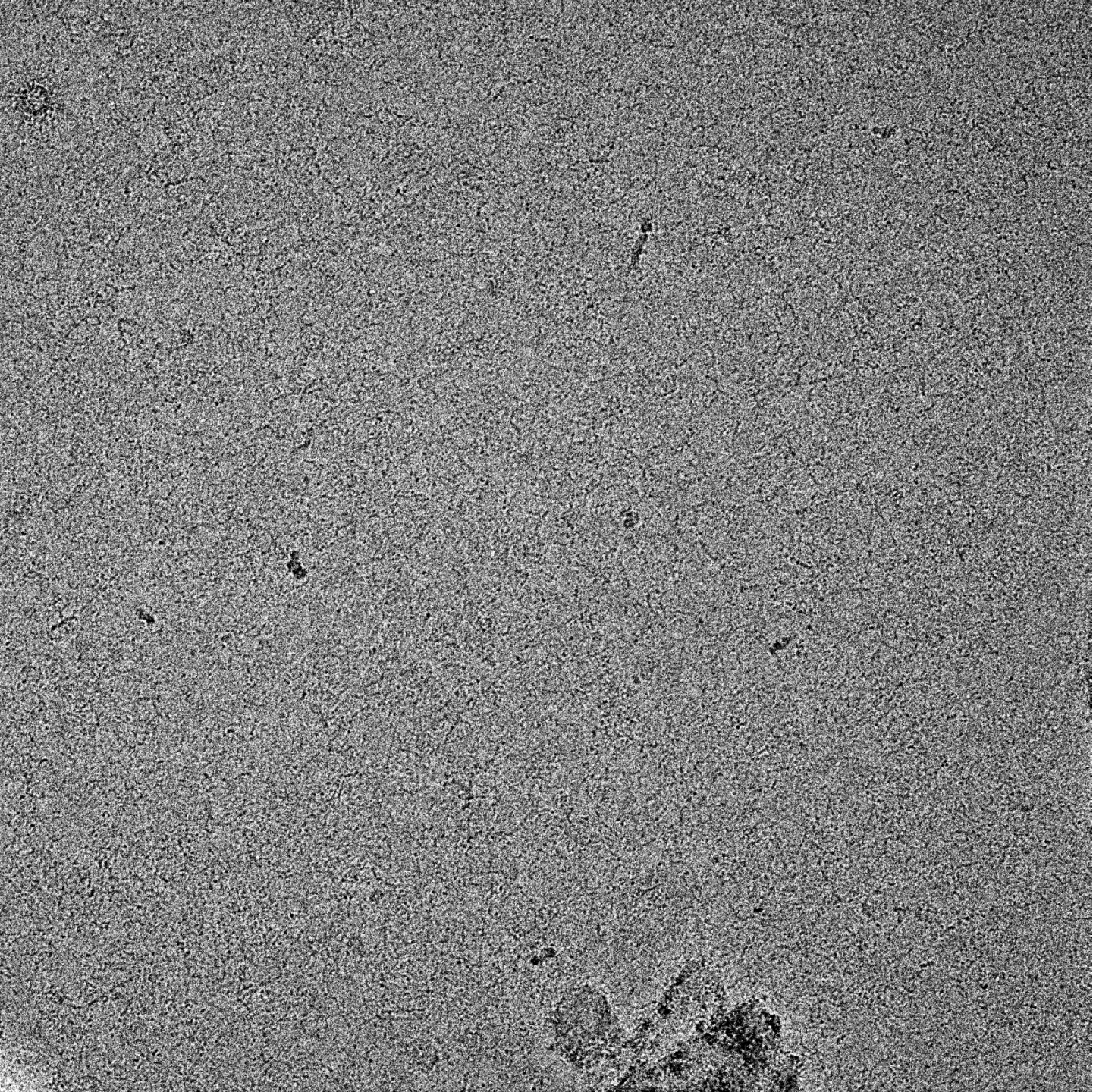

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 7.

Cultivation of the algae

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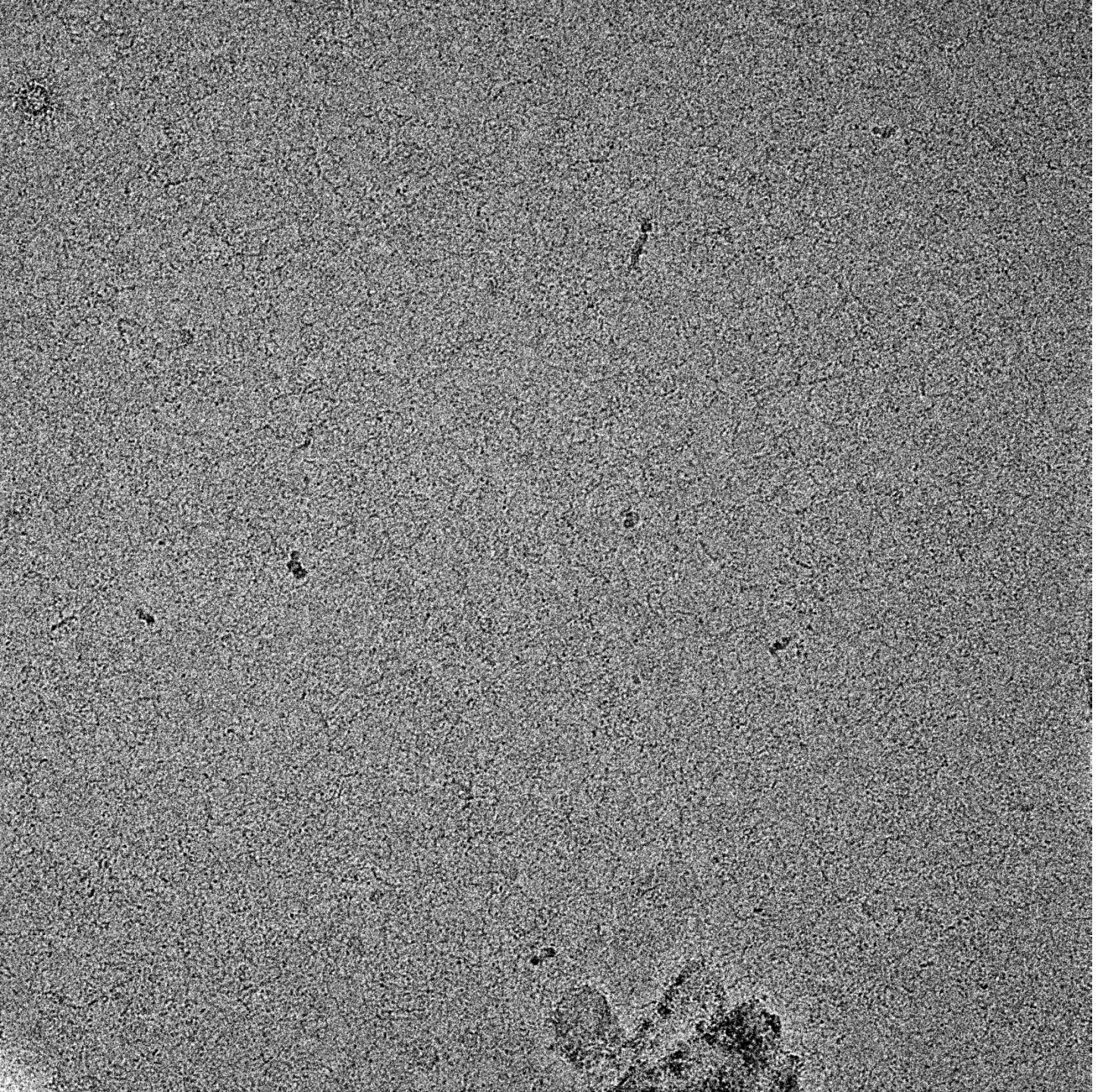

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 8.

Cultivation of the algae

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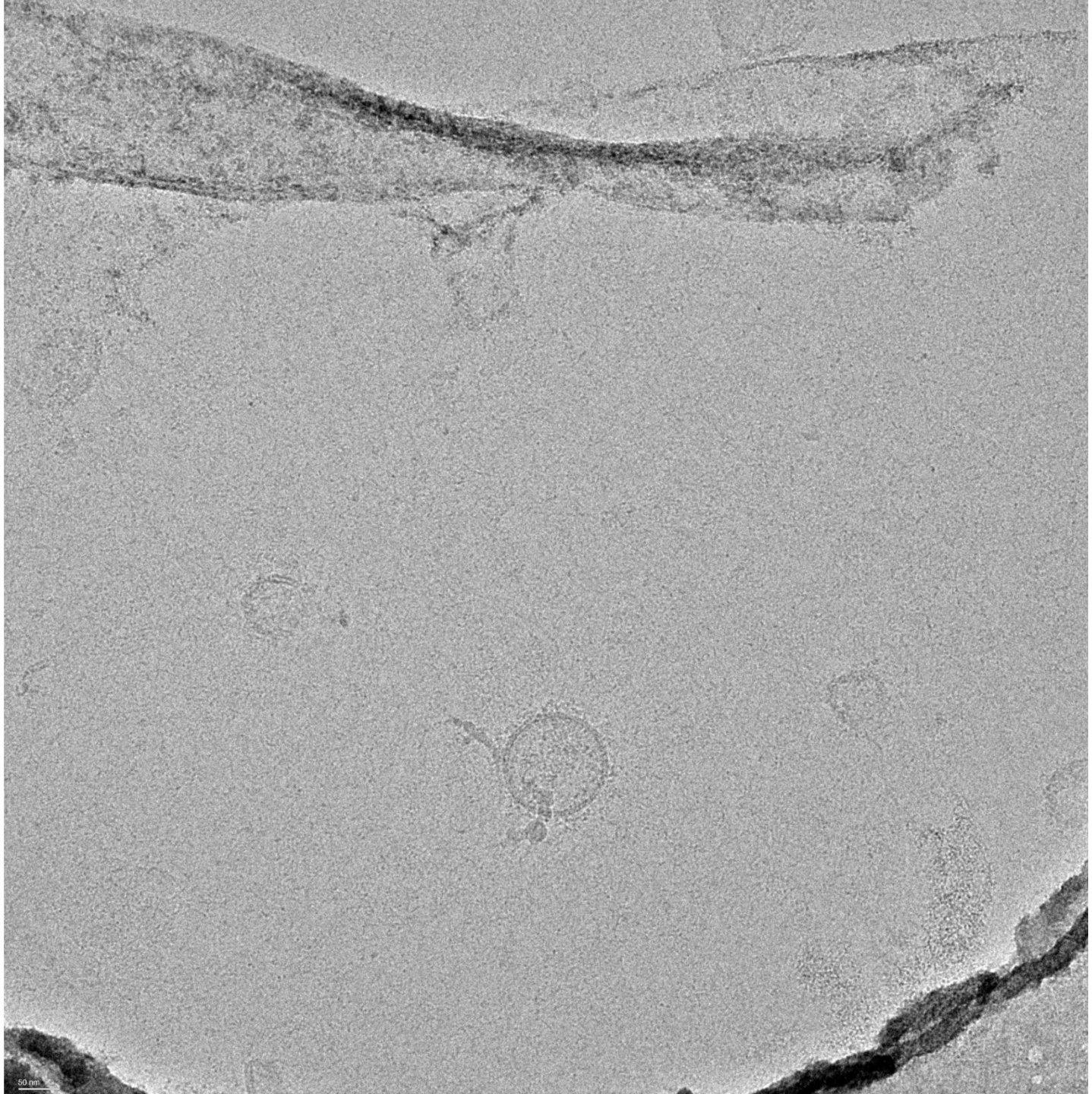

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 9.

Cultivation of the algae
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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).

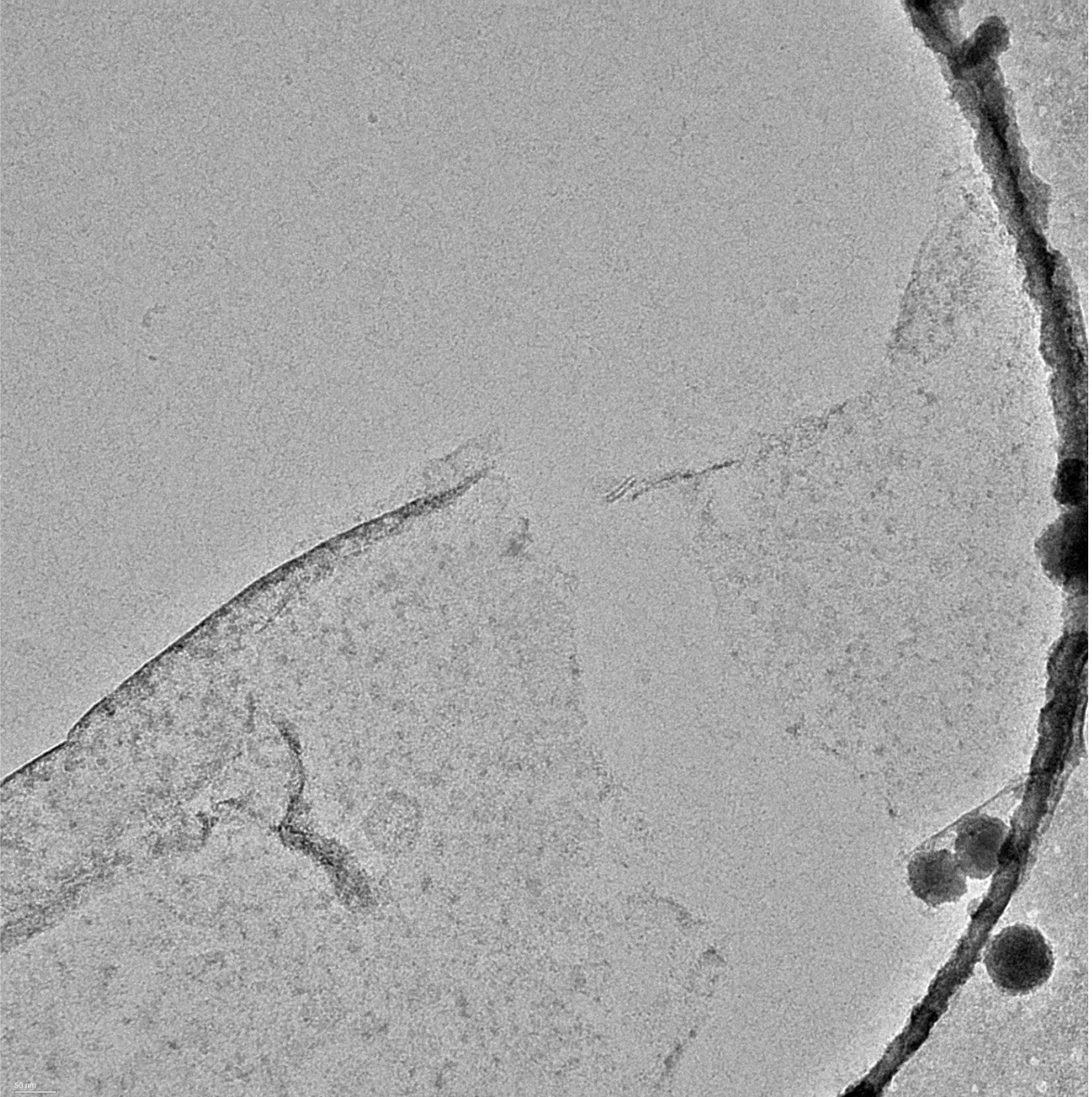

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 10.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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).

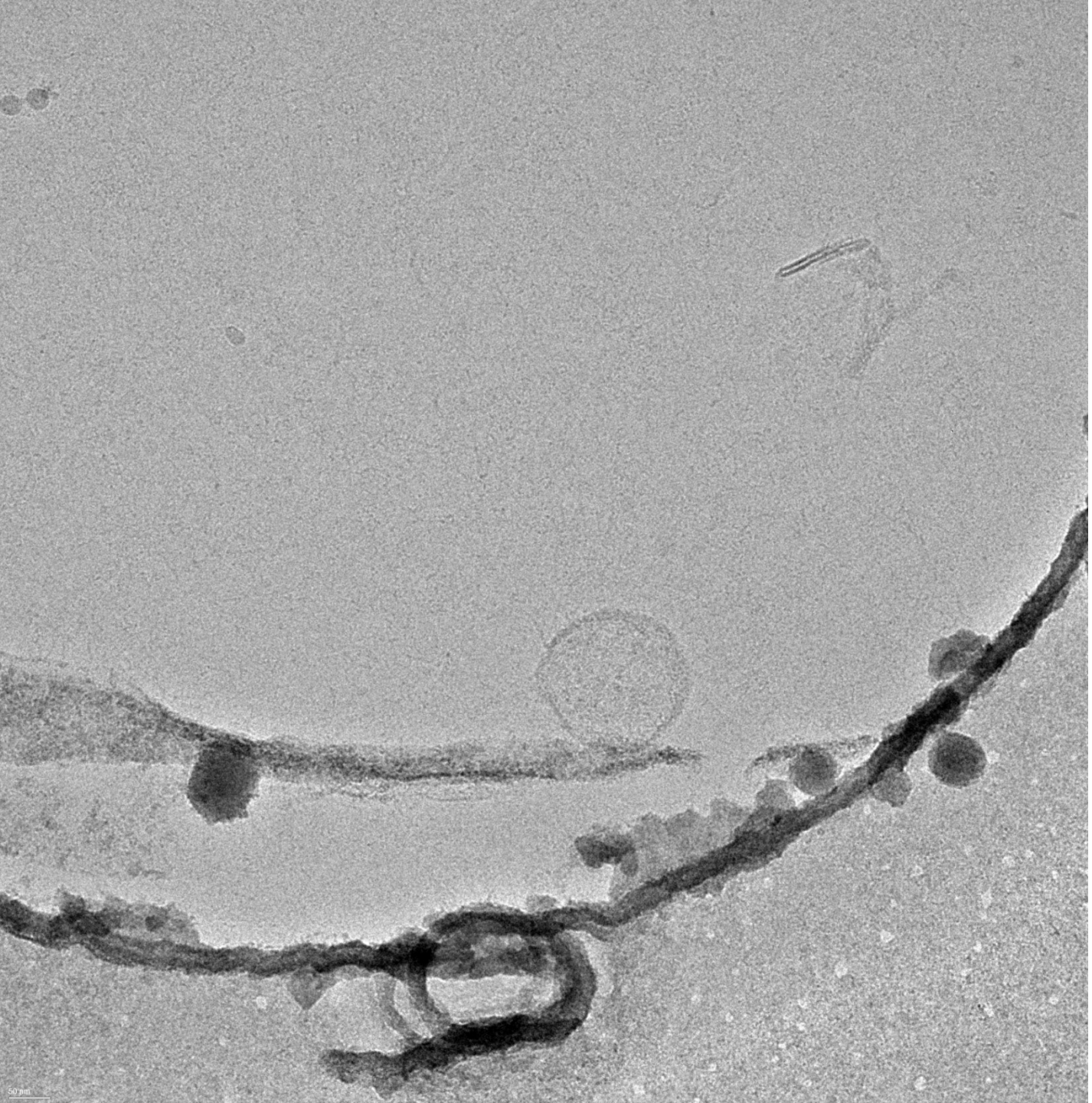

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 11.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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)

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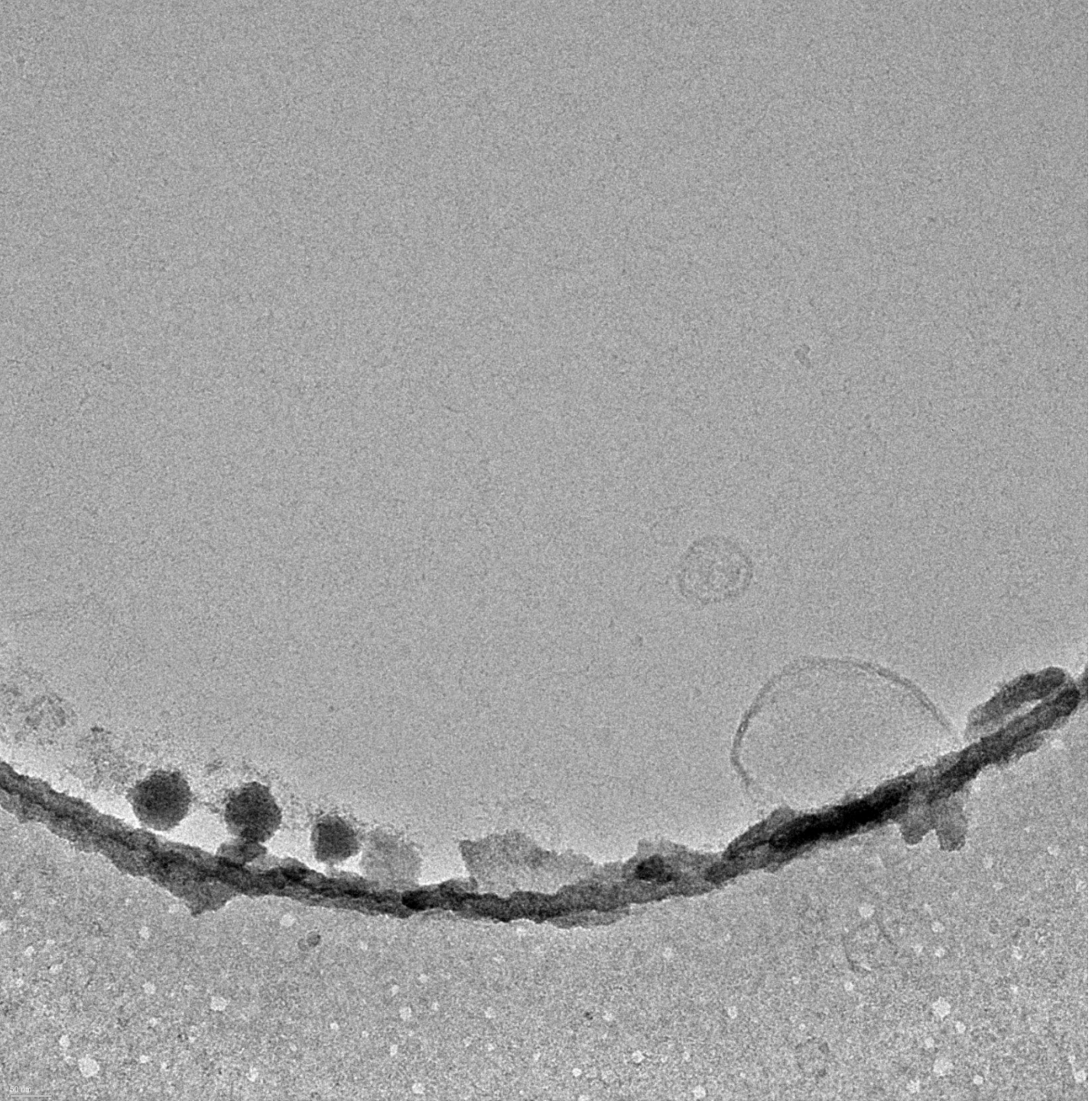

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 12.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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).

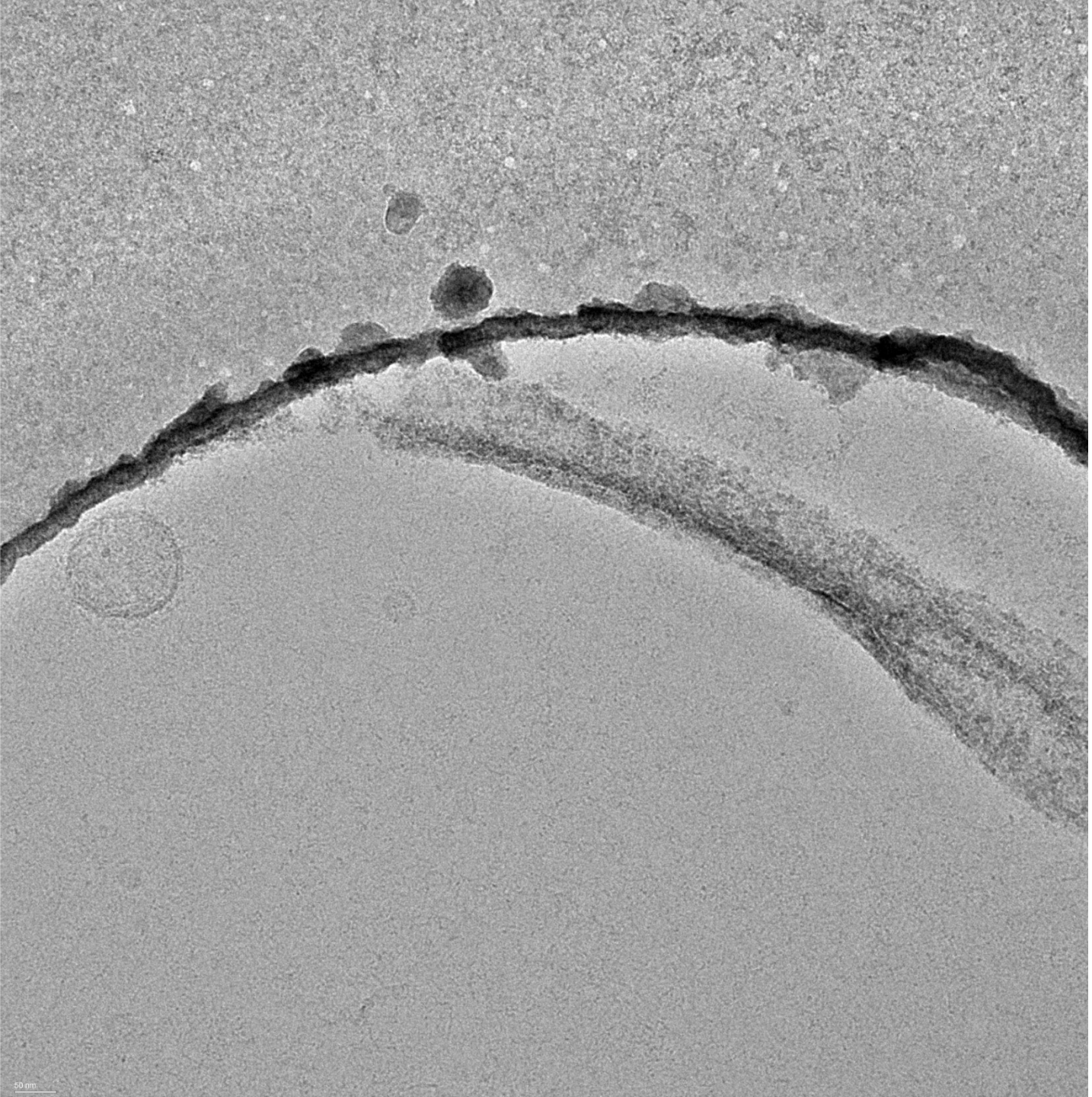

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 13.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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).

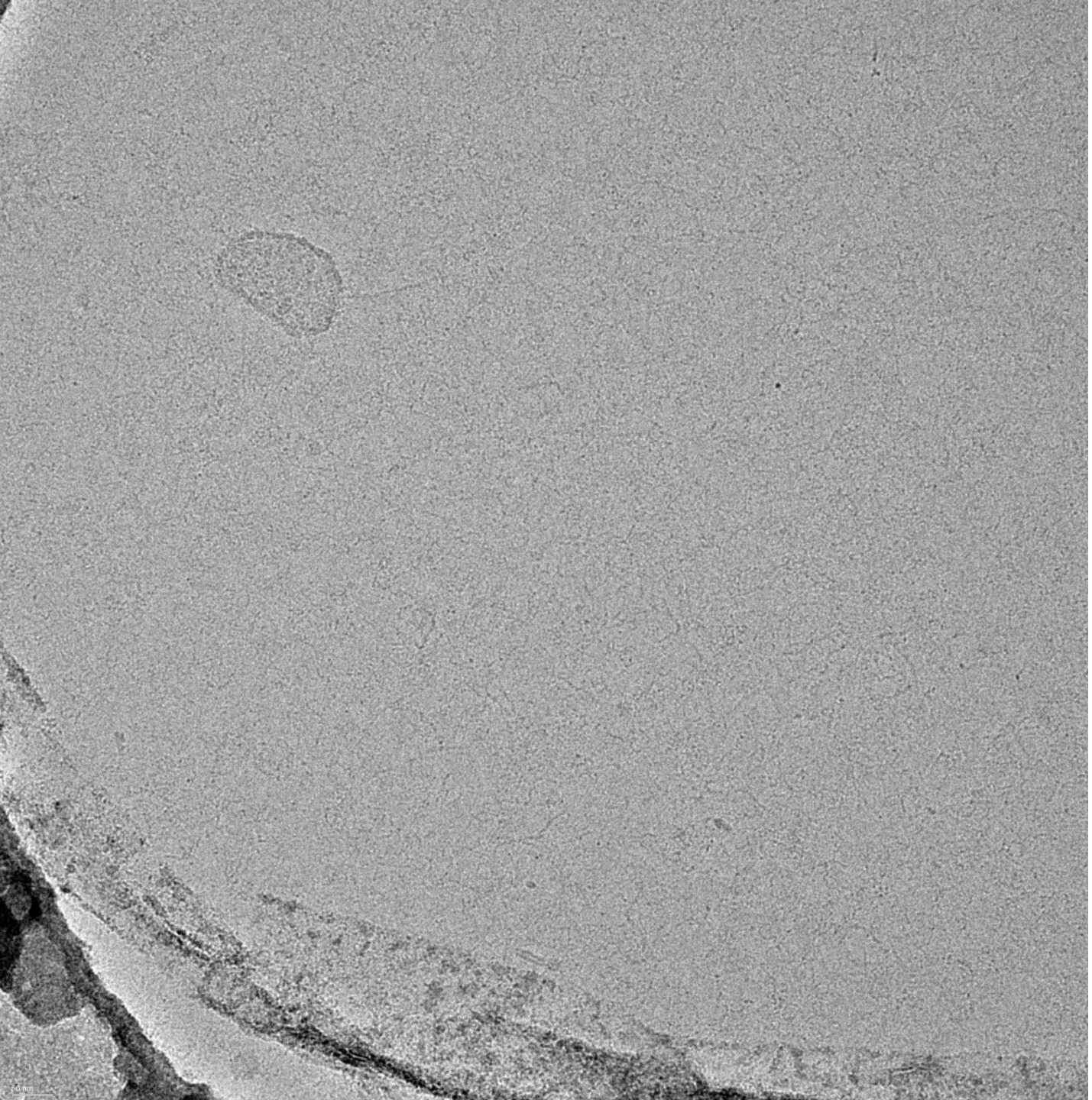

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 14.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

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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).

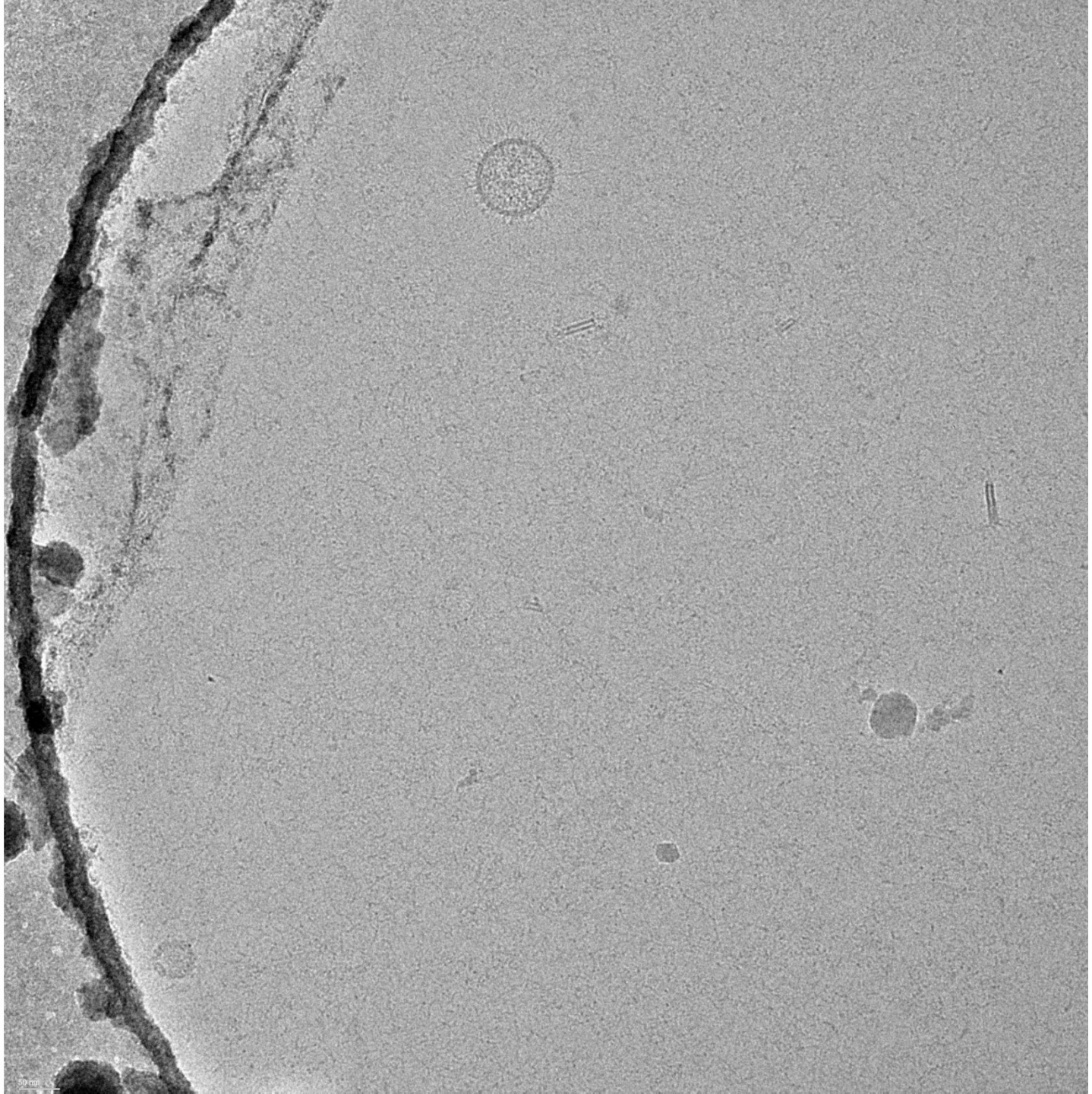

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 15.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

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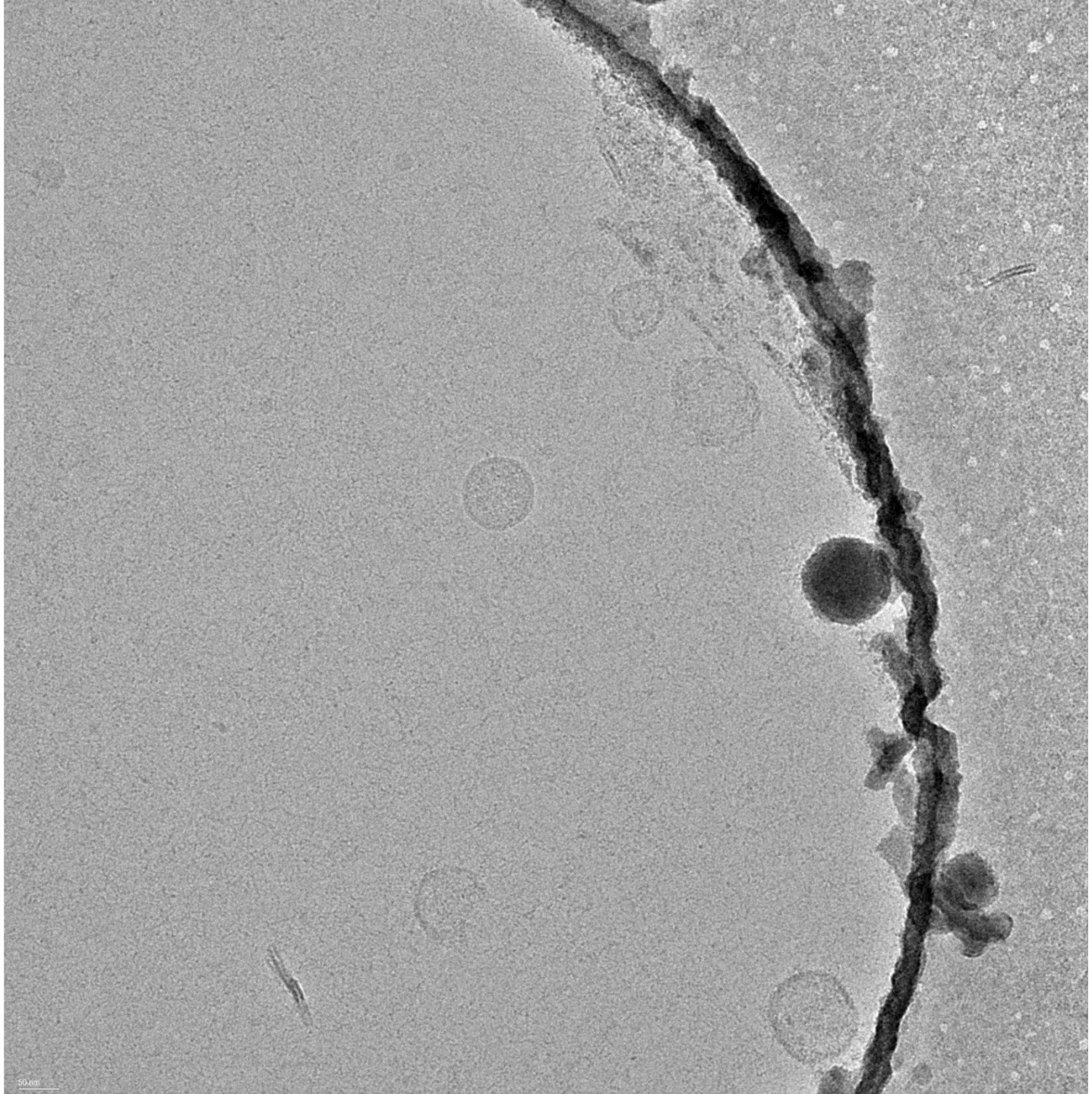

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 16.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of nanoparticles (NPs)

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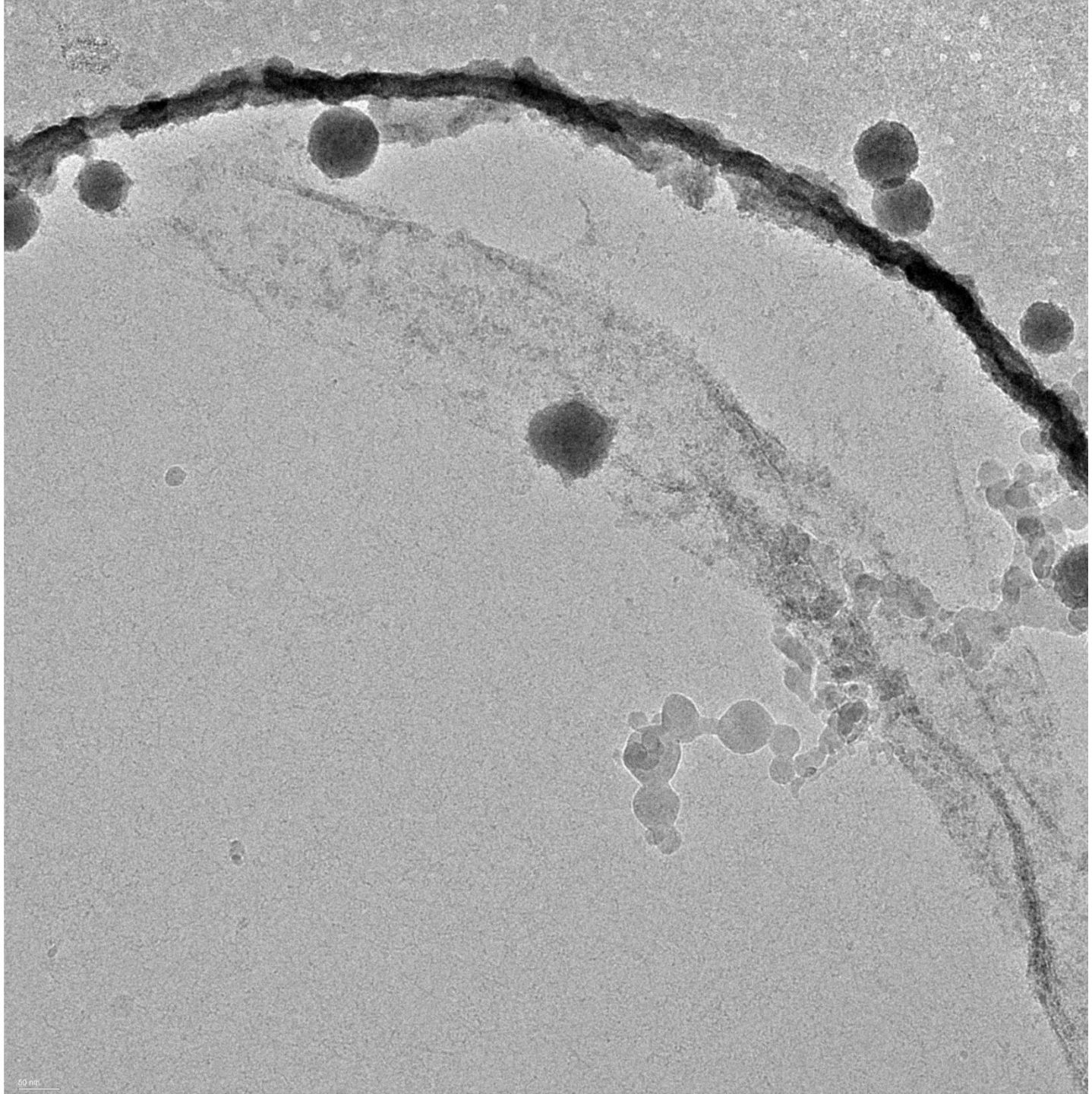

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 17.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) 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)

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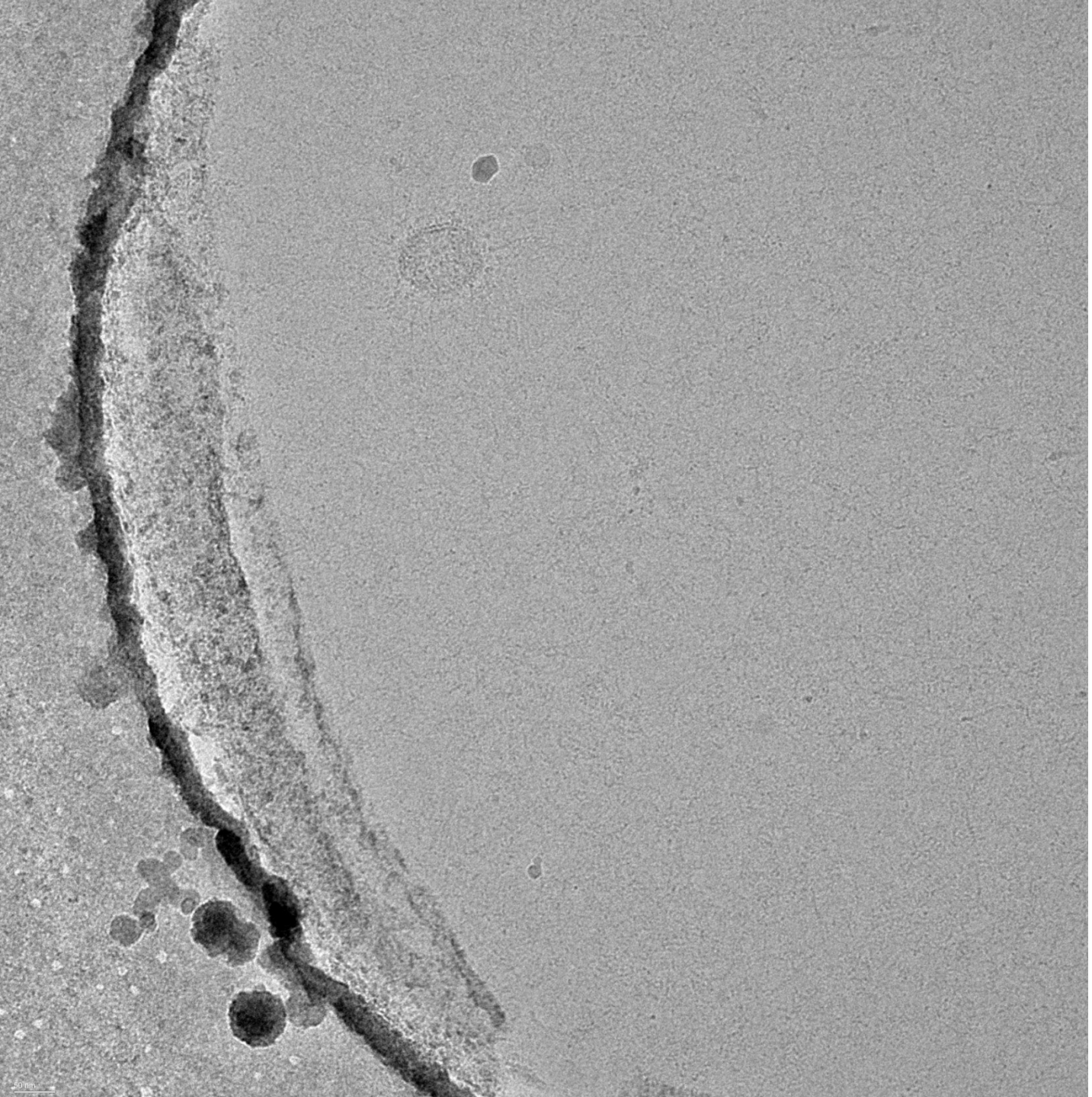

Figure *Dunnaliela tertiolecta* isolate CRYO-TEM 18.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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).

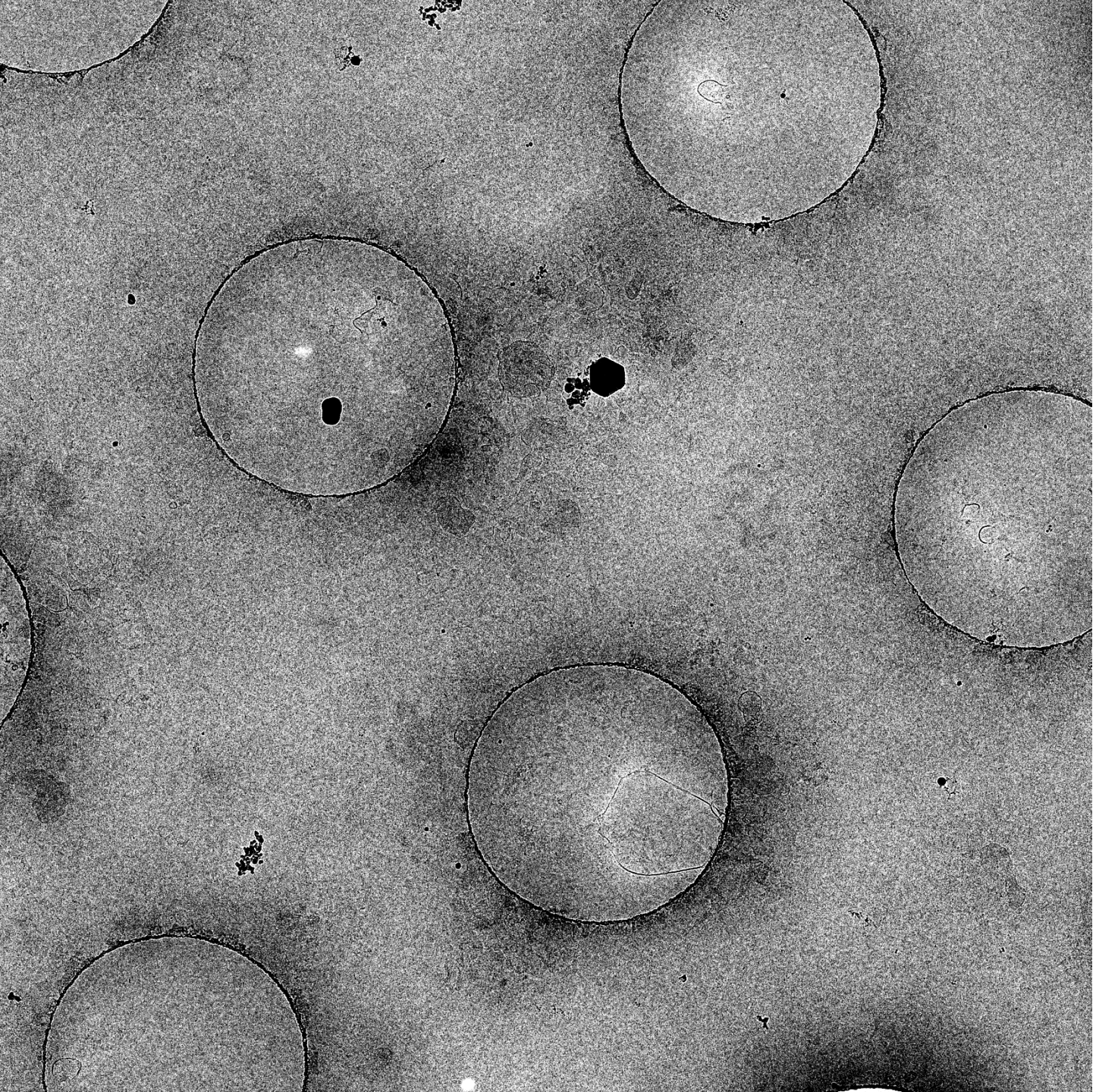

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 1.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

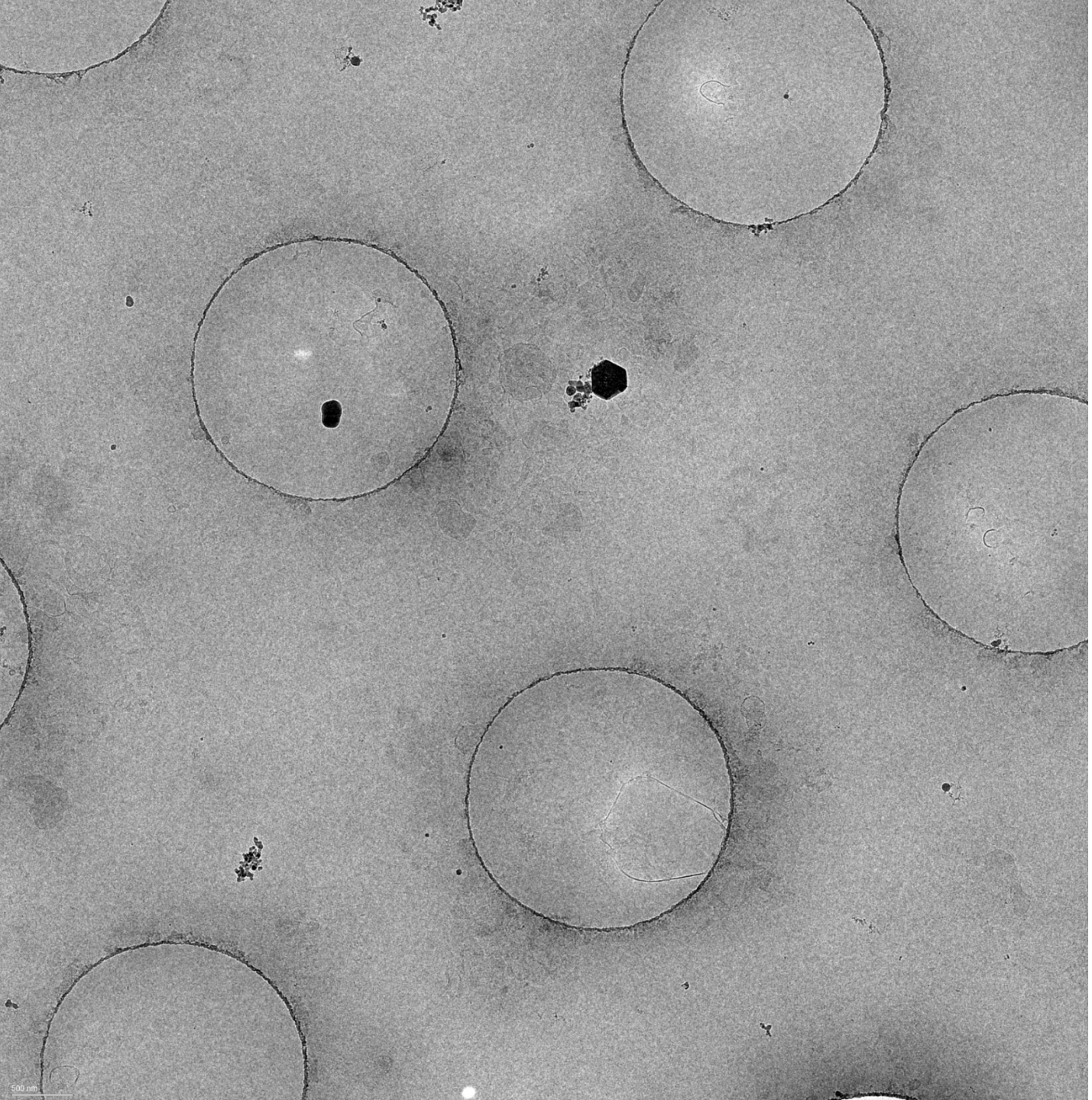

Figure *Dunaliella tertiolecta* isolate SDS CRYO-TEM 2.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

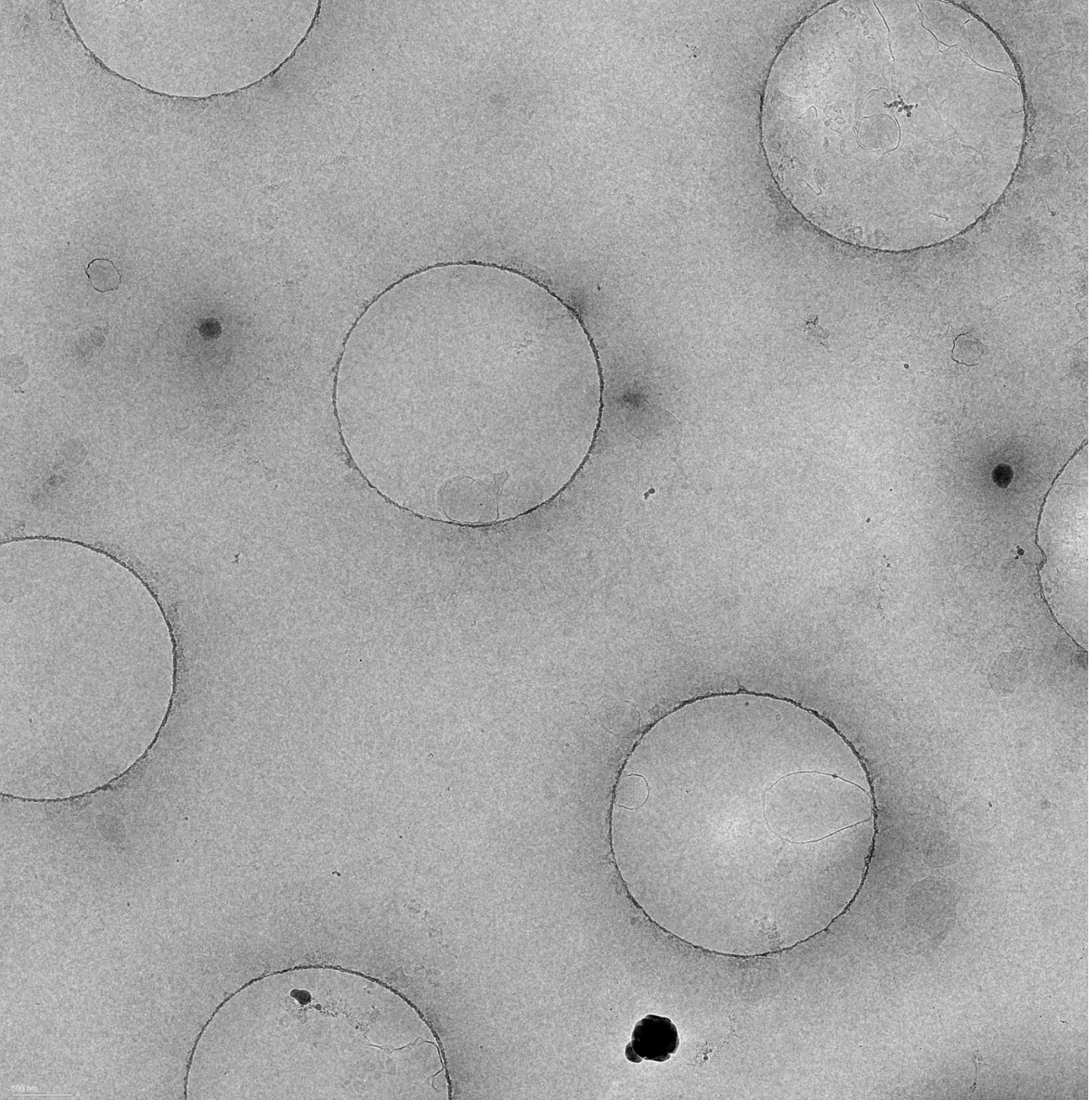

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 3.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

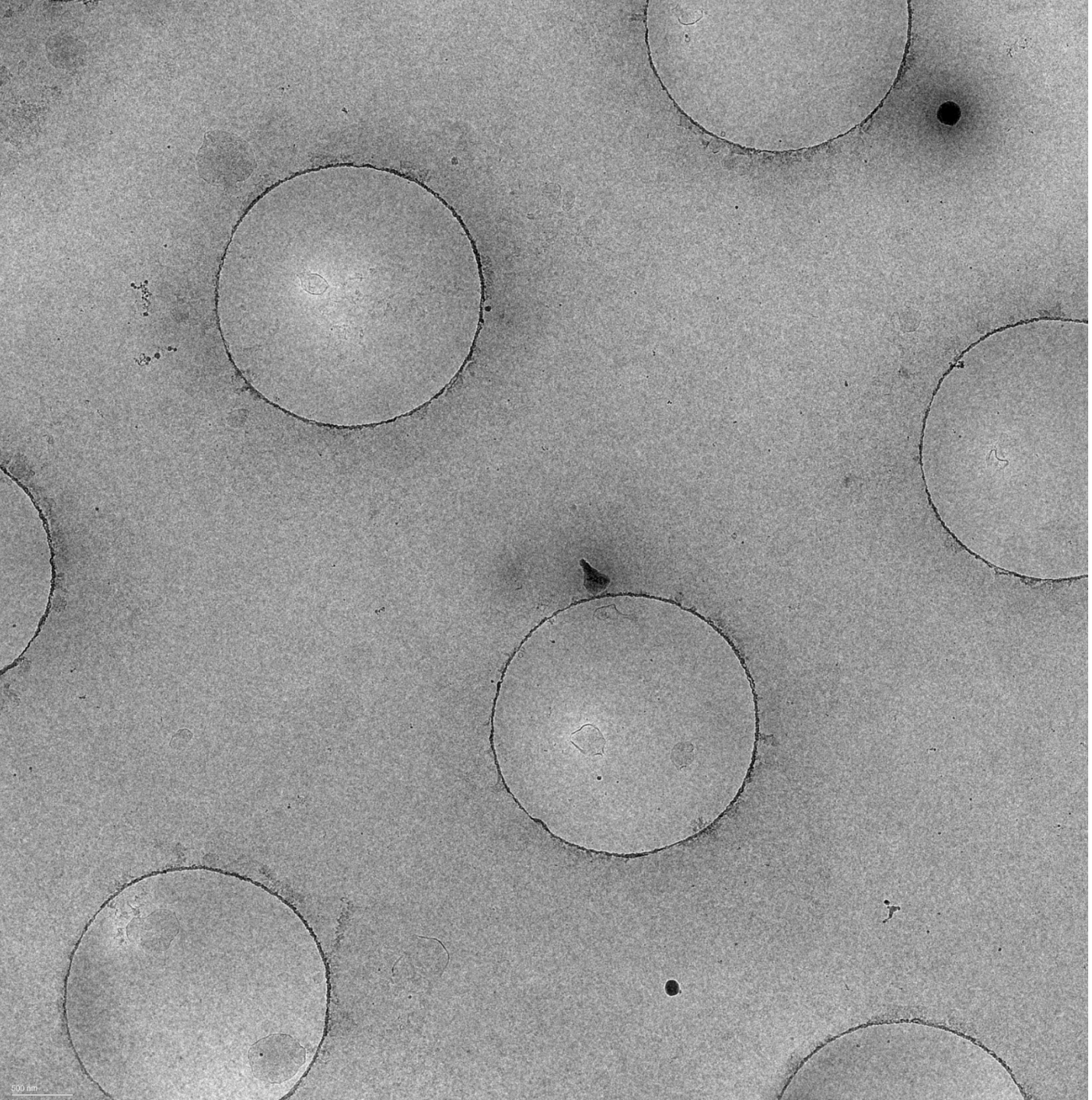

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 4.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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 $\mu mol/m^2s$) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

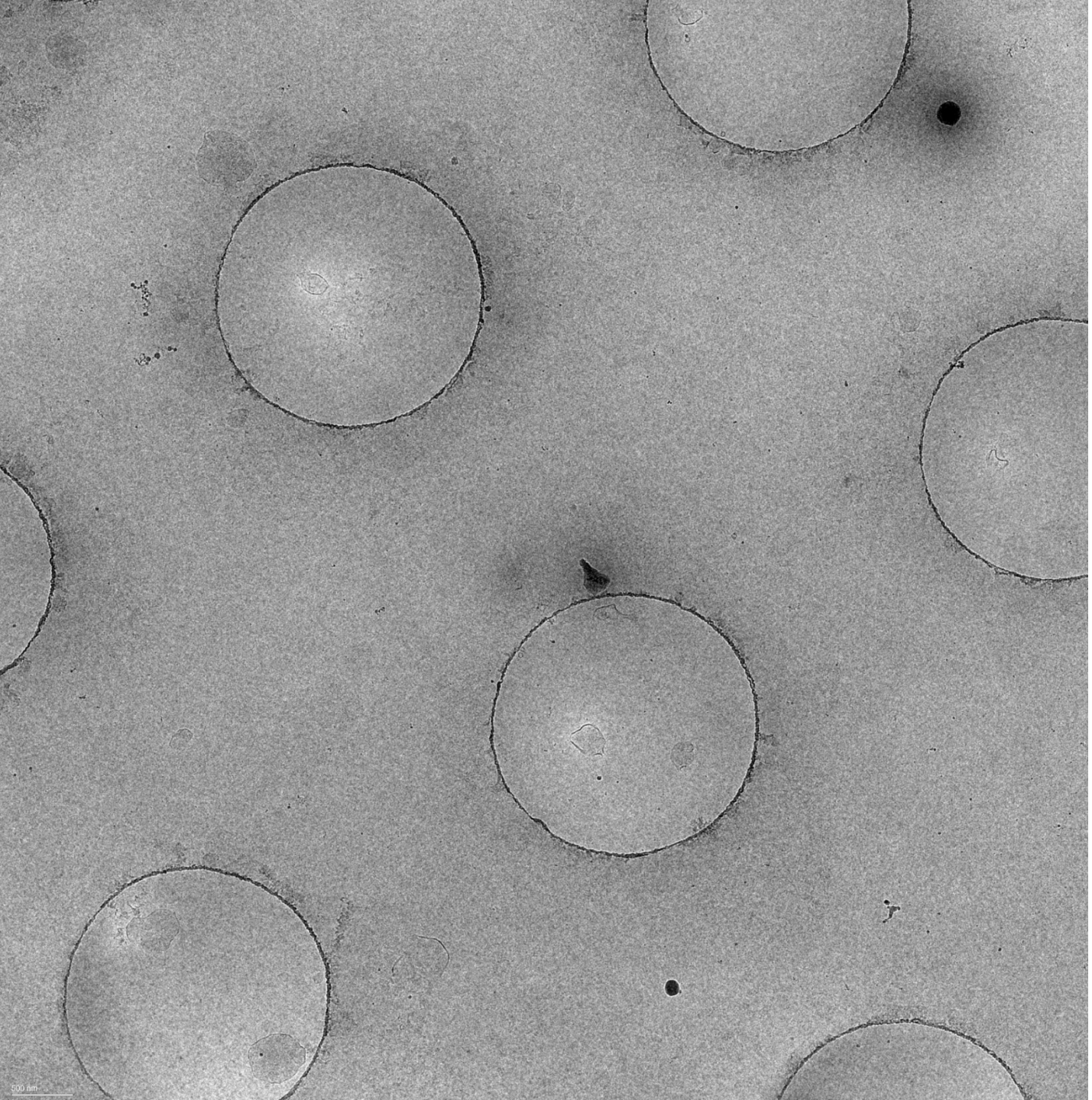

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 5.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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 $\mu mol/m^2s$) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

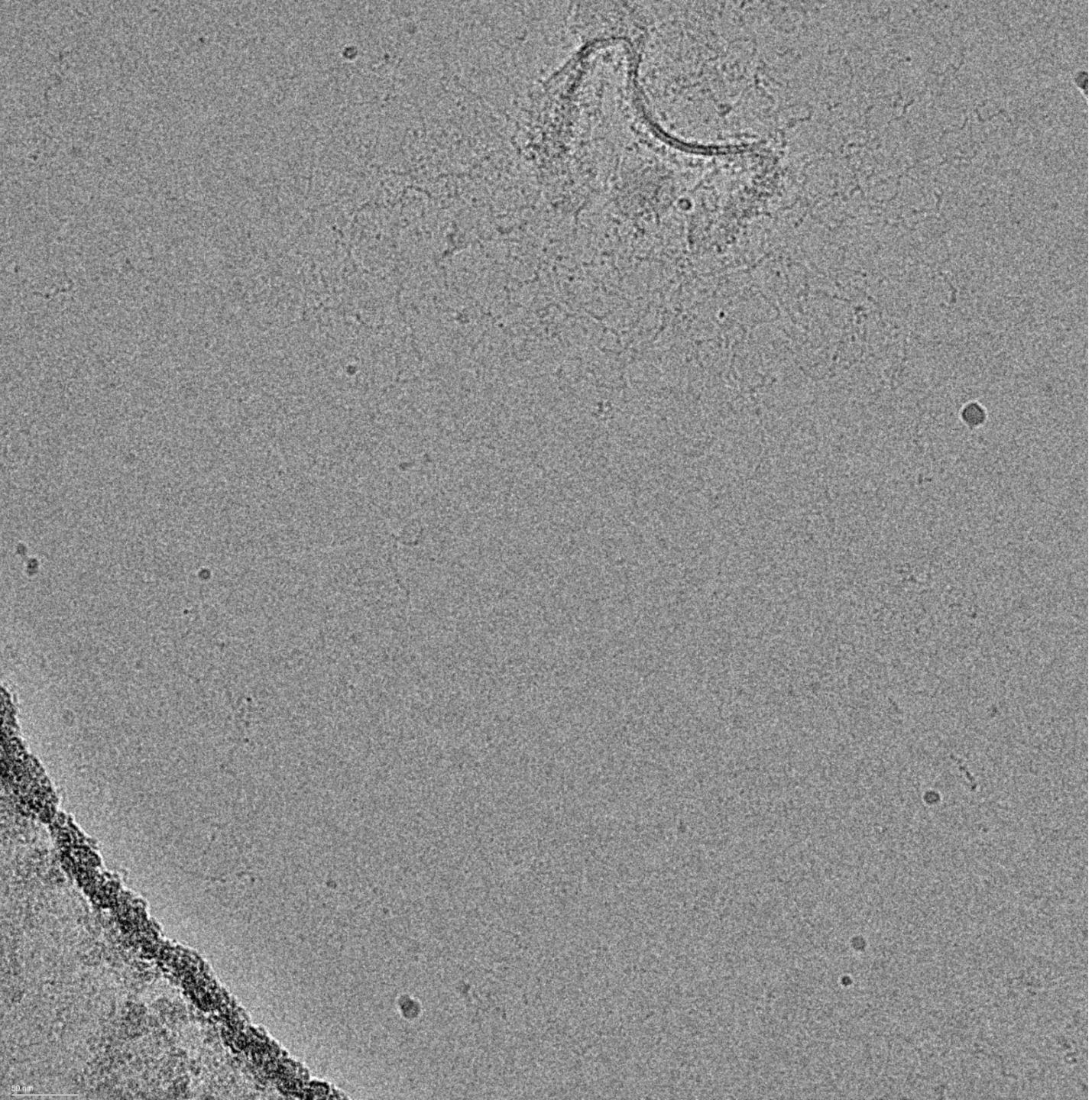

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 6.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

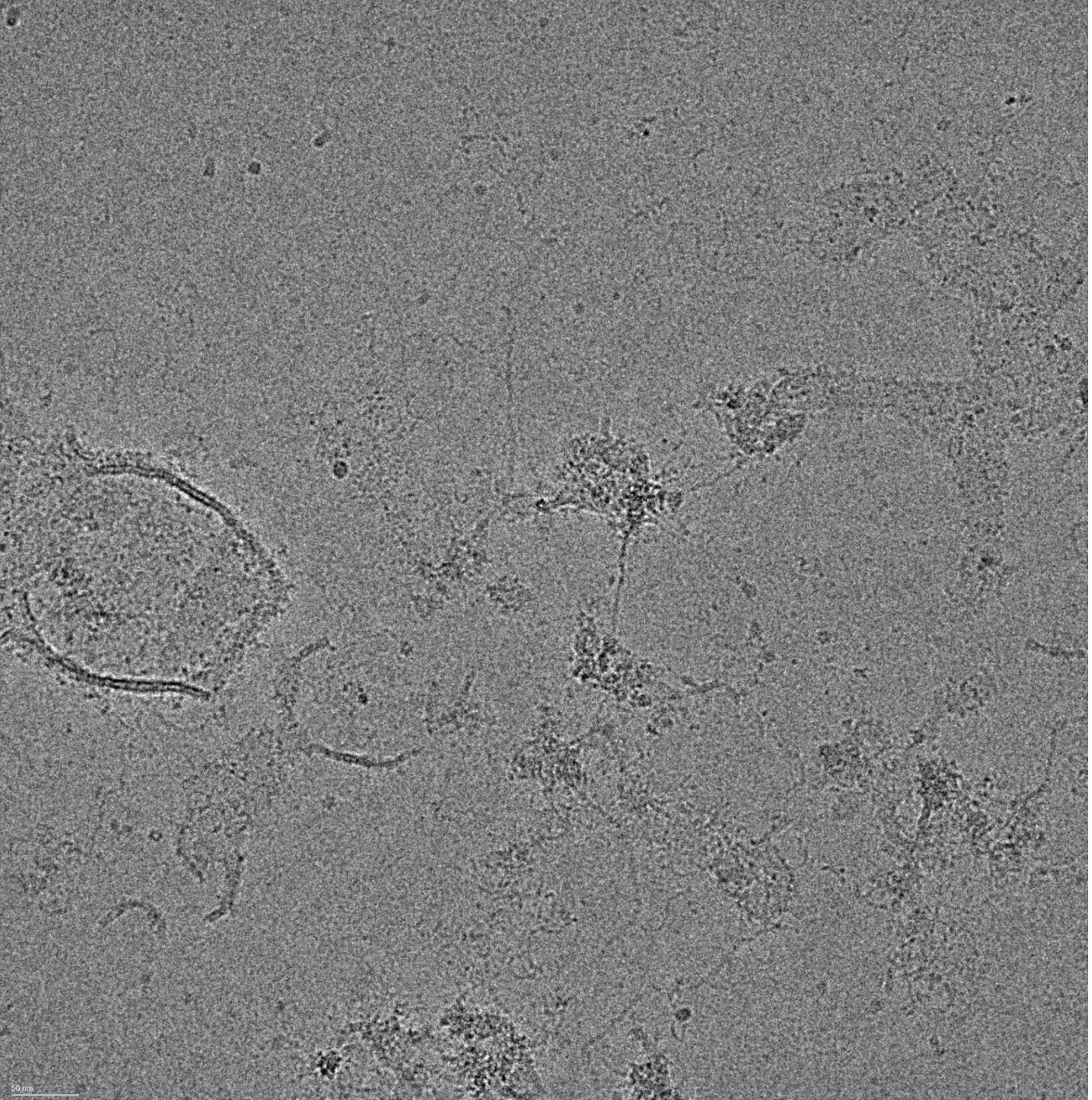

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 7.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

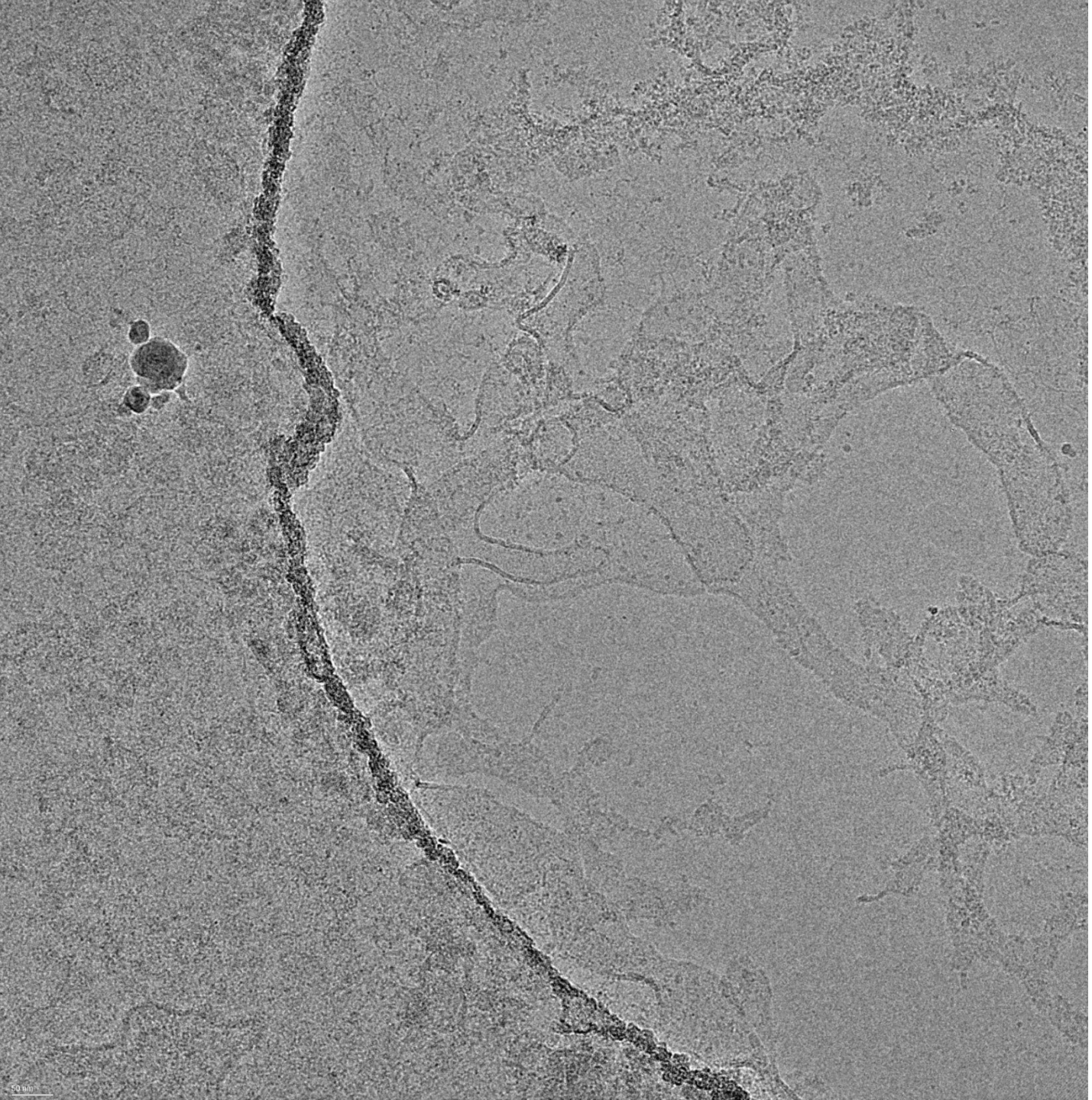

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 8.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

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Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

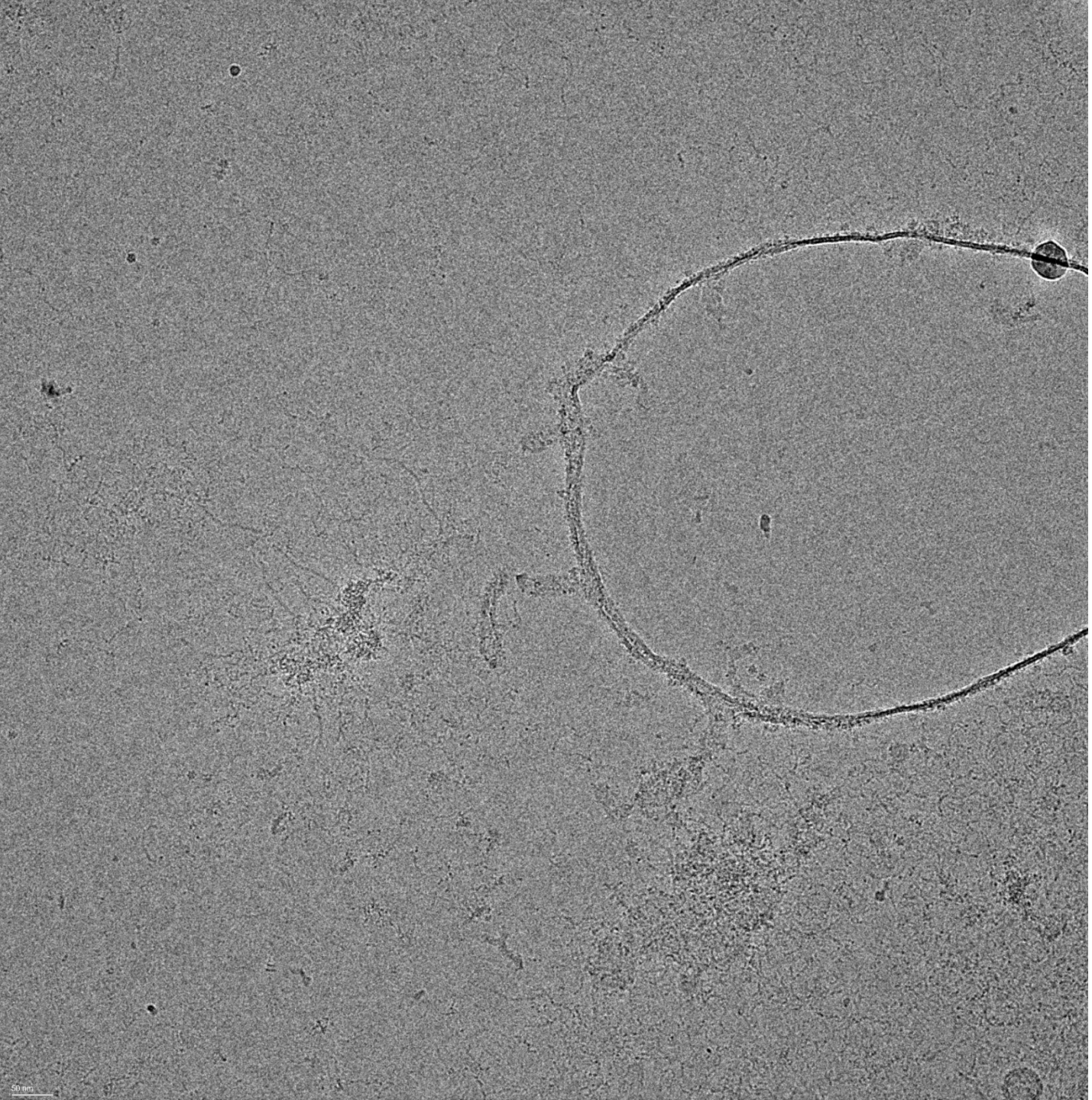

Figure *Dunaliella tertiolecta* isolate SDS CRYO-TEM 9.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

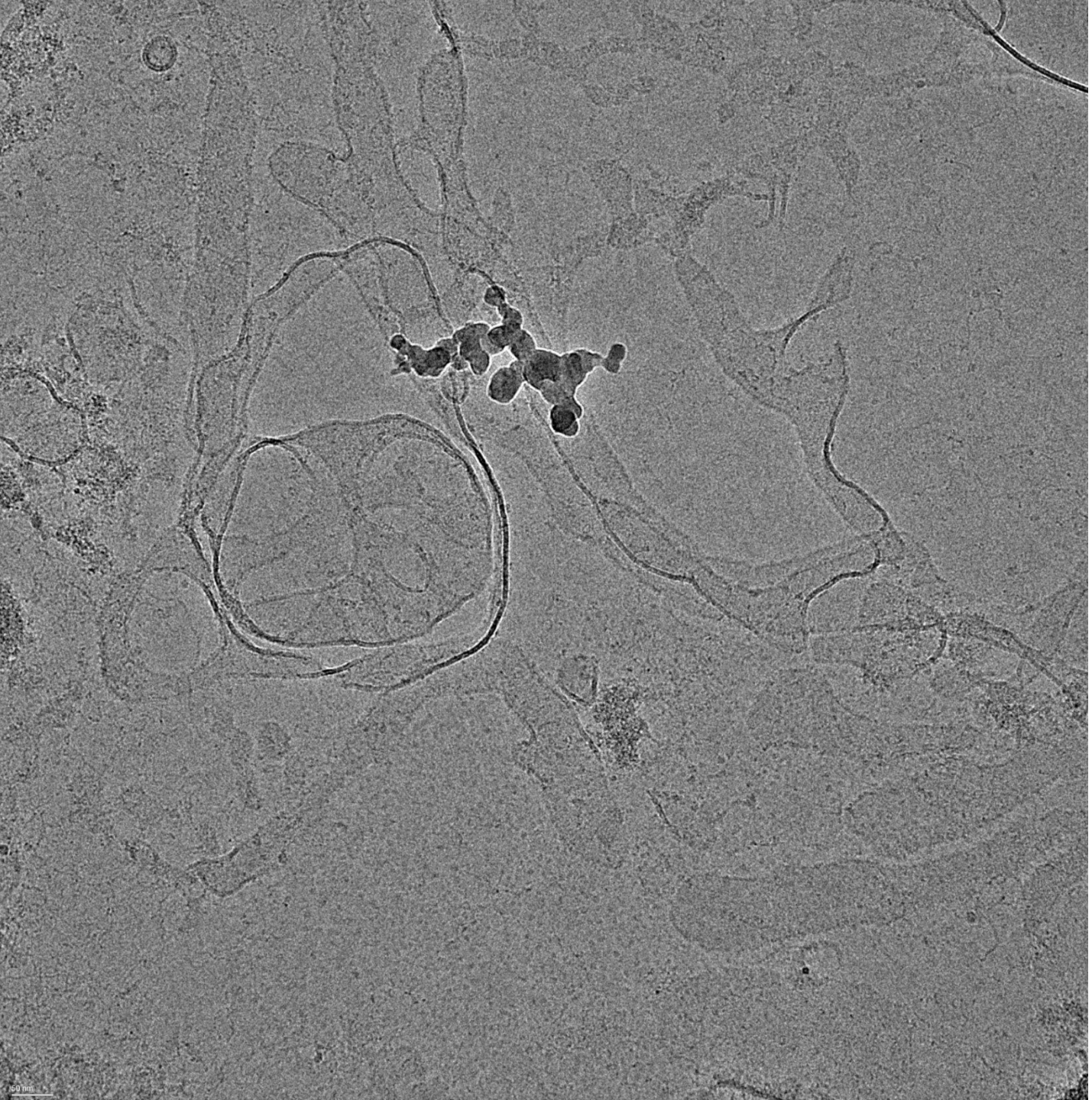

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 10.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

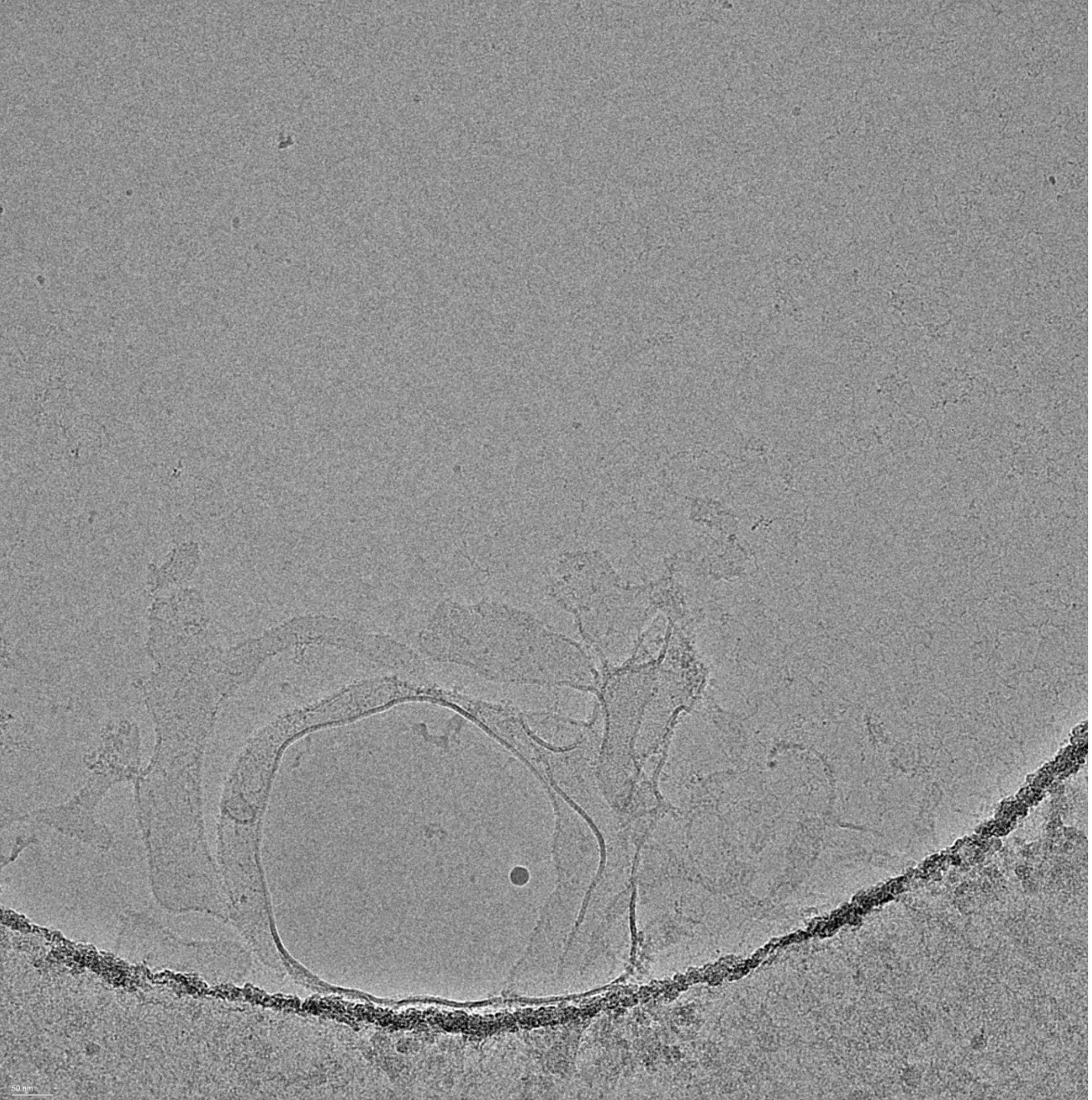

Figure *Dunaliella tertiolecta* isolate SDS CRYO-TEM 11.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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 *Dunaliela tertiolecta* isolate SDS CRYO-TEM 12.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

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Isolation of nanoparticles (NPs)

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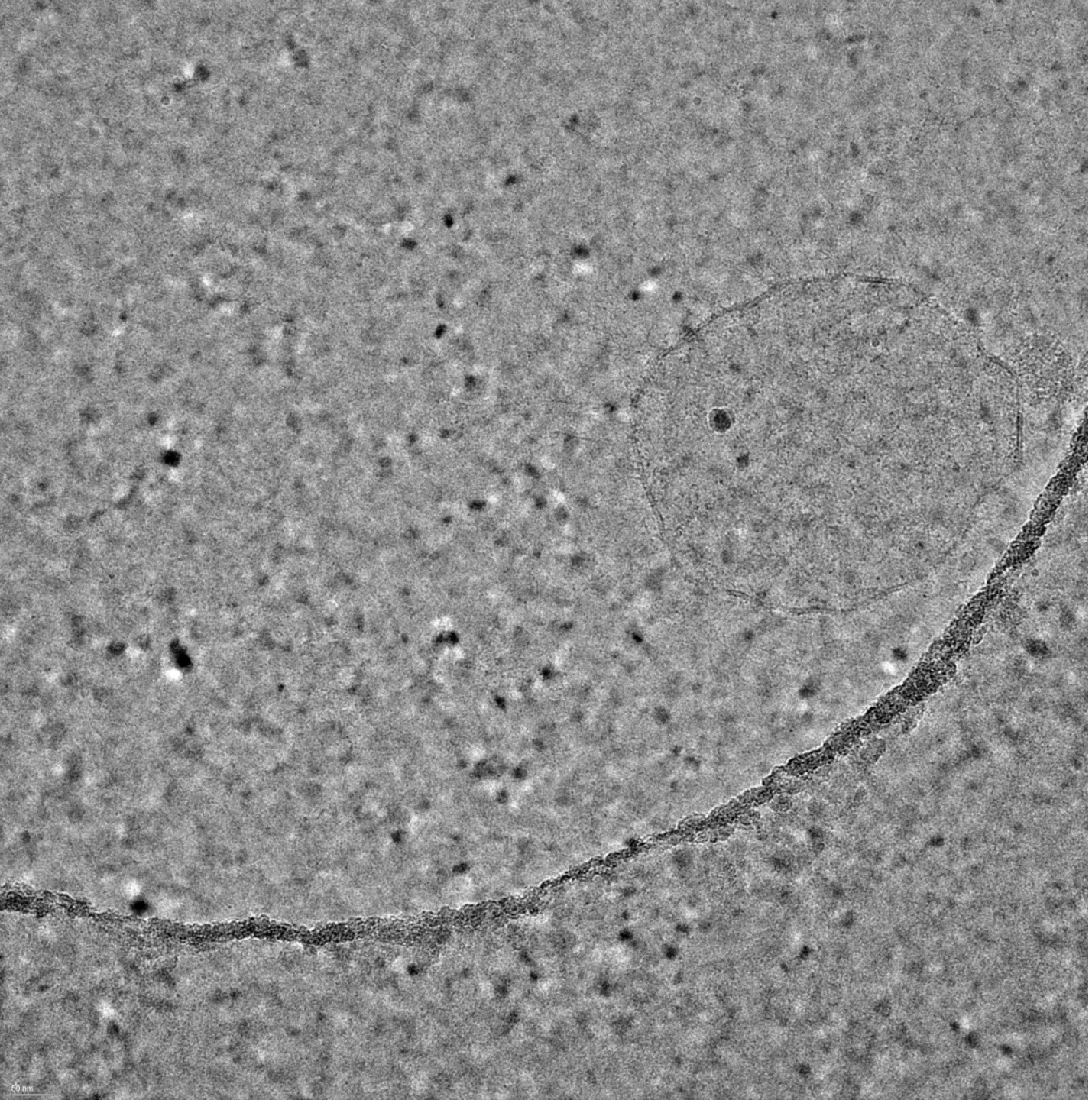

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 13.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

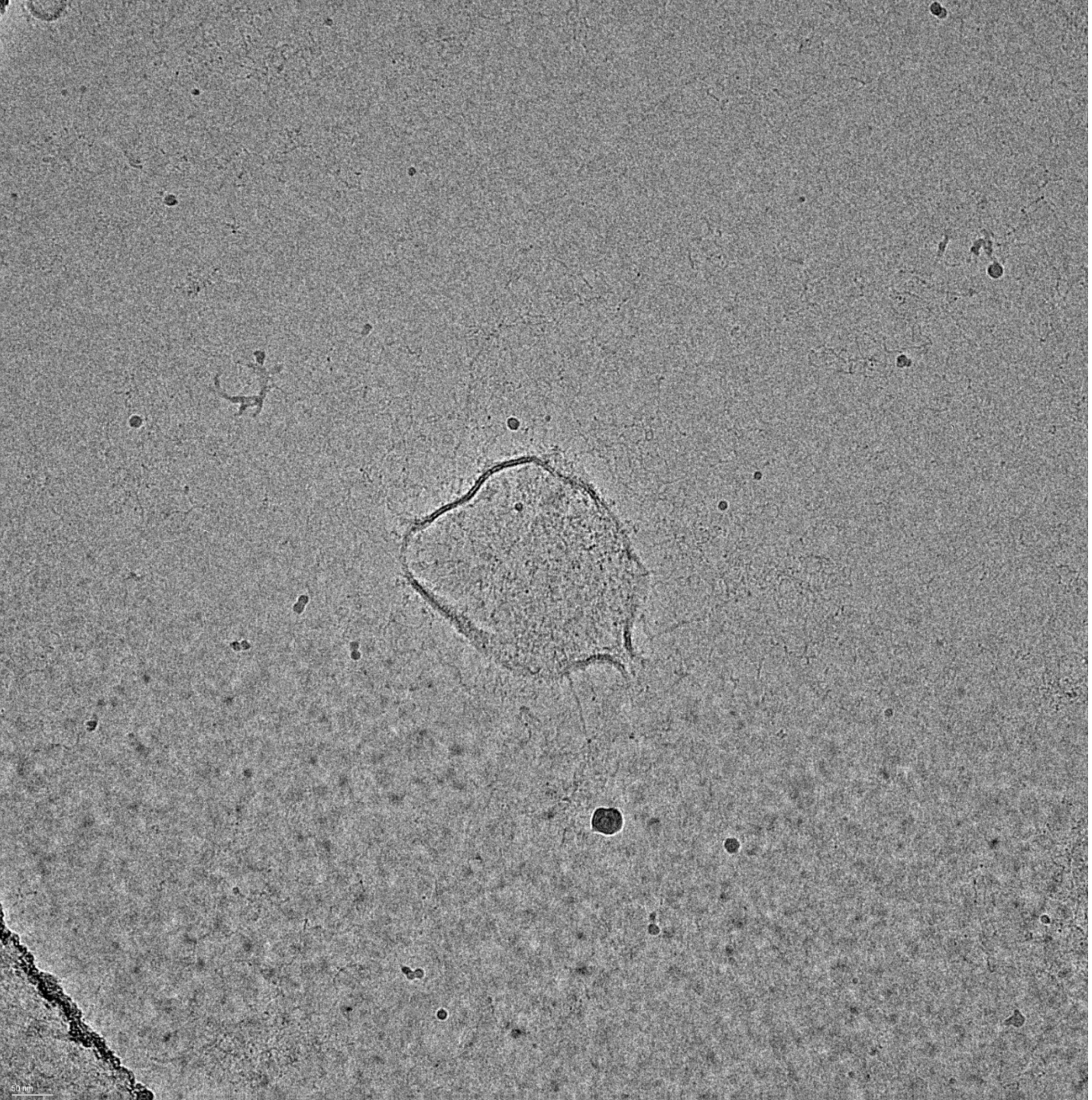

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 14.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

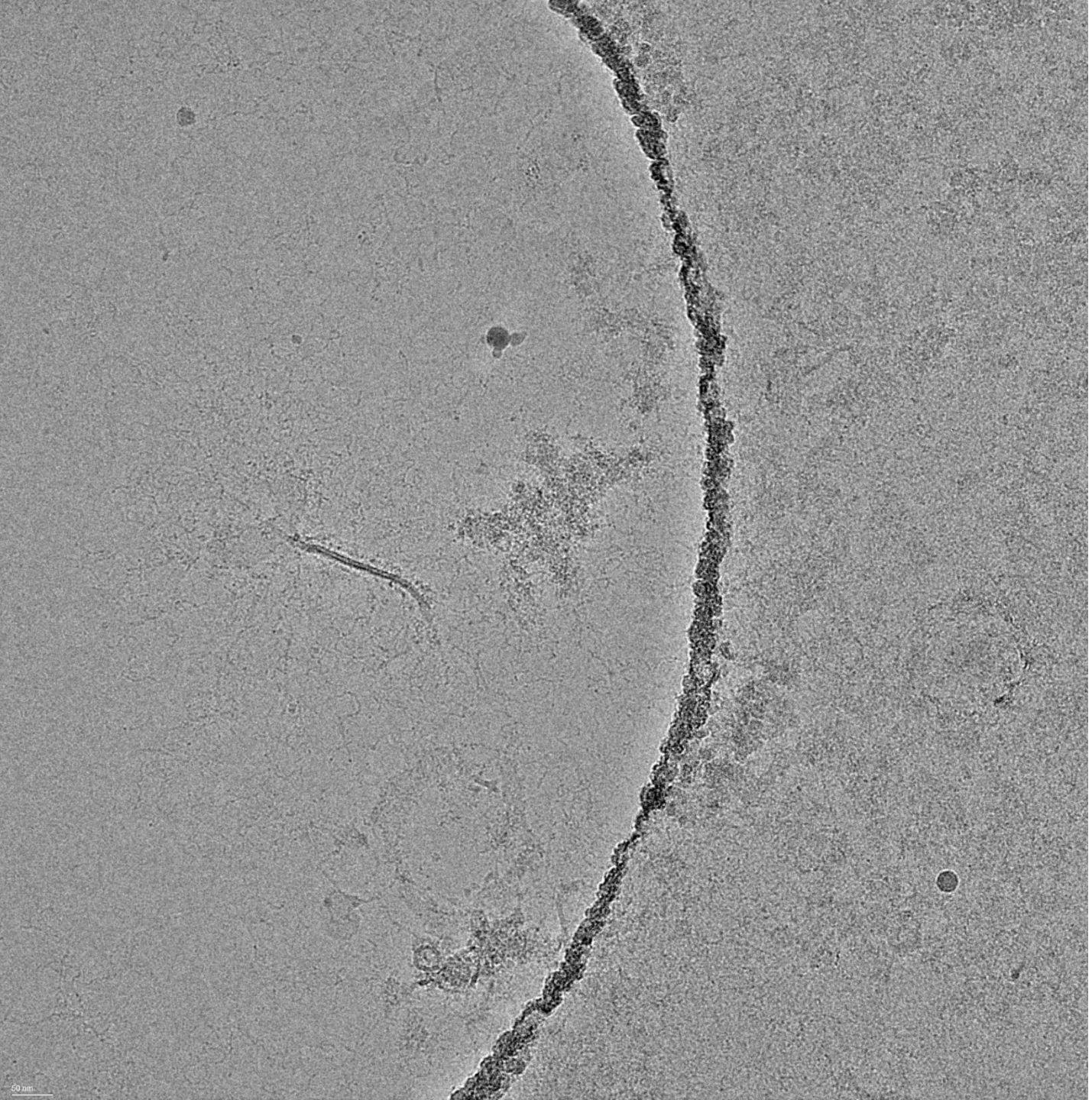

Figure *Dunaliella tertiolecta* isolate SDS CRYO-TEM 15.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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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).

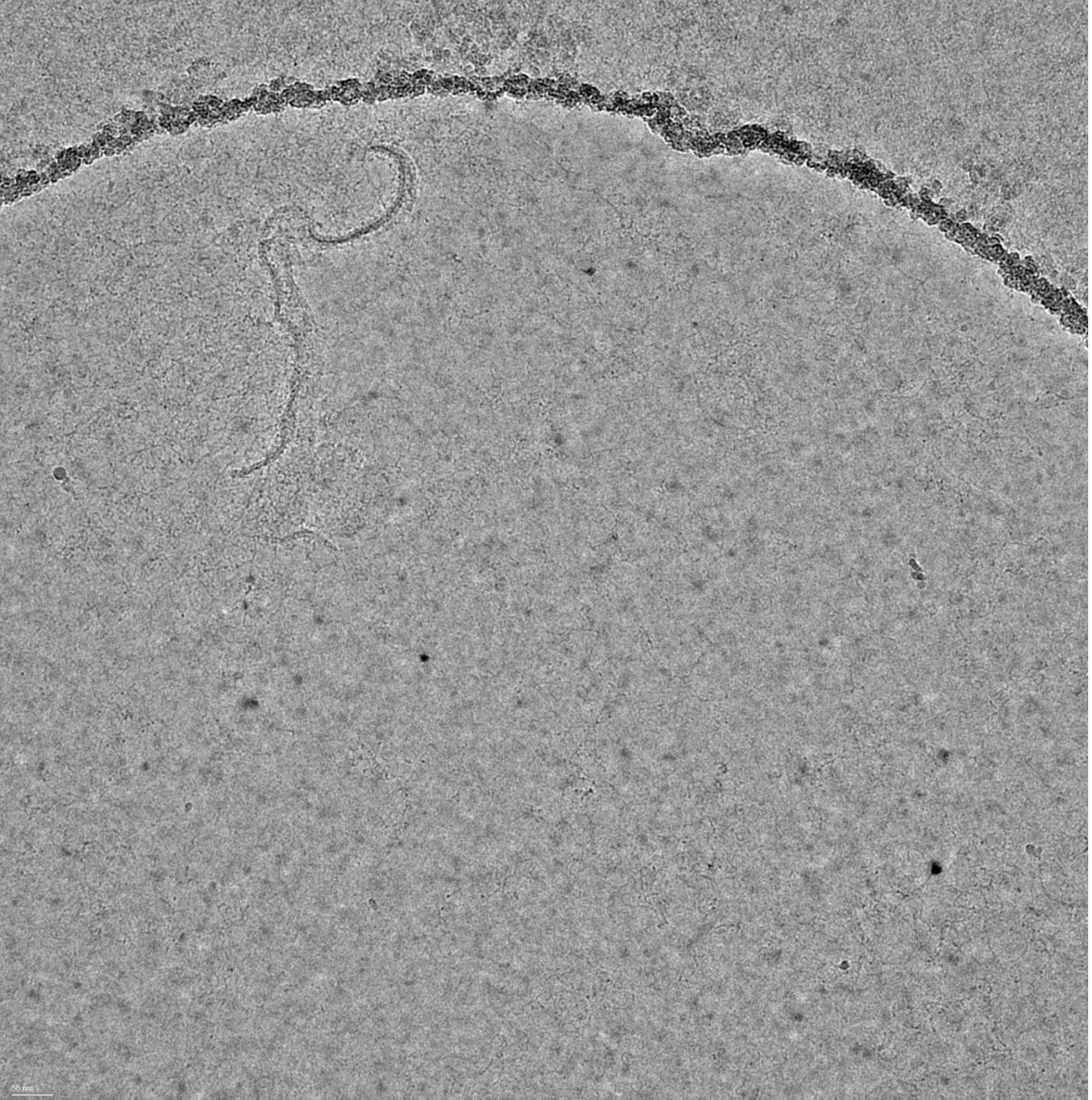

Figure *Dunaliella tertiolecta* isolate SDS CRYO-TEM 16.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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^2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

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Isolation of nanoparticles (NPs)

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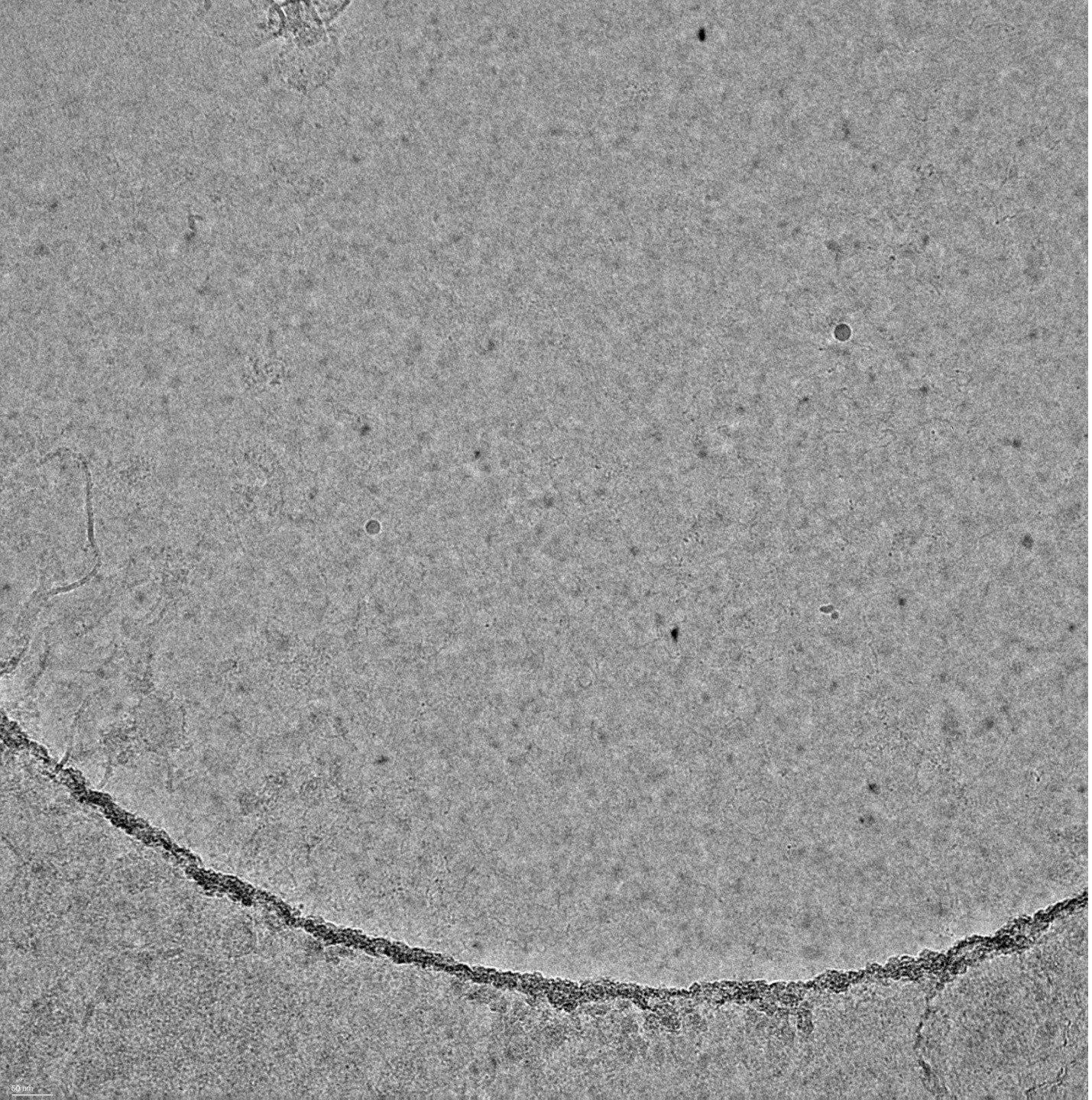

Figure *Dunaliela tertiolecta* isolate SDS CRYO-TEM 17.

Cultivation of the algae

Cultures of *D. tertiolecta* CCAP 19/22 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 $\mu mol/m^2s$) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Treatment of cells

Cells (taken in the exponential phase of growth) were centrifuged at 100g and 22°C for 10 minutes and suspended in solution of marine salt (22 g/L) to reach the concentration cca 10^6 cells/mL. The cells were treated with detergent sodium dodecylsulfate (SDS) at 1:50 proportion with respect to the volume of cell suspension for 1 hour.

Removal of cells

The cells from algal culture were centrifuged at 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); then at 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.

Isolation of nanoparticles (NPs)

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 5-mL thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 50 000 g and 4°C, for 70 min in 5-mL tubes 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).

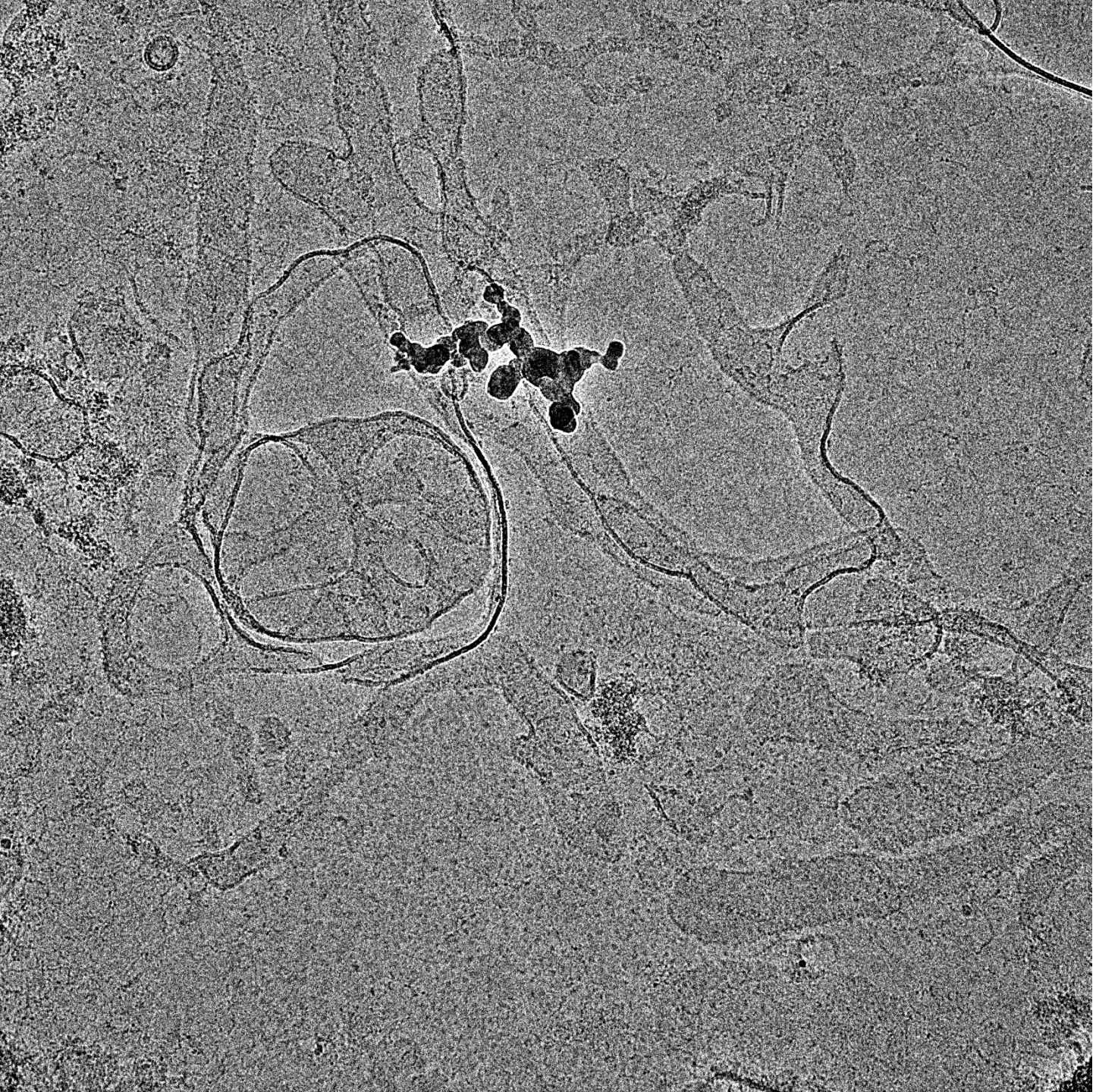

Figure *Dunaliella tertiolecta* isolate SDS CRYO-TEM 18.

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