Supplementary Material

An Improved method to generate secondary nanoplastics and oligomers: Application in ecotoxicology

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Supplementary Fig. S6: Chlorophyll content expressed as percentage of variation of total chlorophylls on *C. reinhardtii* after 72 h exposure to NP/P-PBAT-NPLs & NP/P-PBAT-Olig. Asterisks indicate treatments that are significantly different (Dunnet's test, (***) $p < 0.001$.; (**) $p < 0.01$.; (*) $p < 0.05$.) from the control represented as 100 % (dot line).

Supplementary Fig. S7: Representative chlorophyll autofluorescence/bright field overlay images of confocal microscopy of *C. reinhardtii* after 72 h exposure to NP- and P-PBAT-NPLs. Image shows confocal microscopy images of the non-exposed cells (A and D); cells exposed to 0.1 and 10 mg/L of NP-PBAT-NPLs (B and C, respectively); cells exposed to 0.1 and 10 mg/L of P-PBAT-NPLs (E and F, respectively).

Supplementary Text 1: Protocol to generate, isolate and quantify nanoplastics (NPLs) and oligomers (Olig) through accelerated degradation processes: trituration (non-photooxidized (NP)-NPLs) and photooxidation followed by trituration (photooxidized (P)-NPLs).

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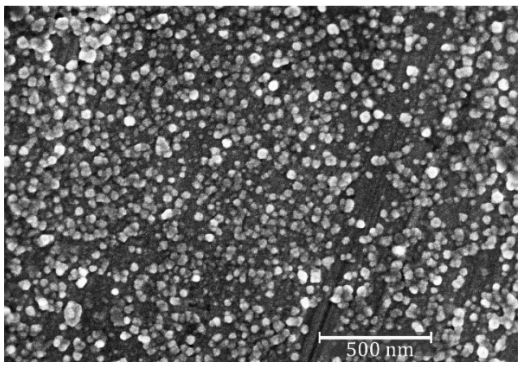
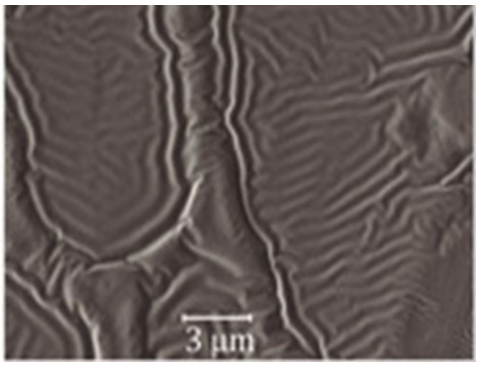


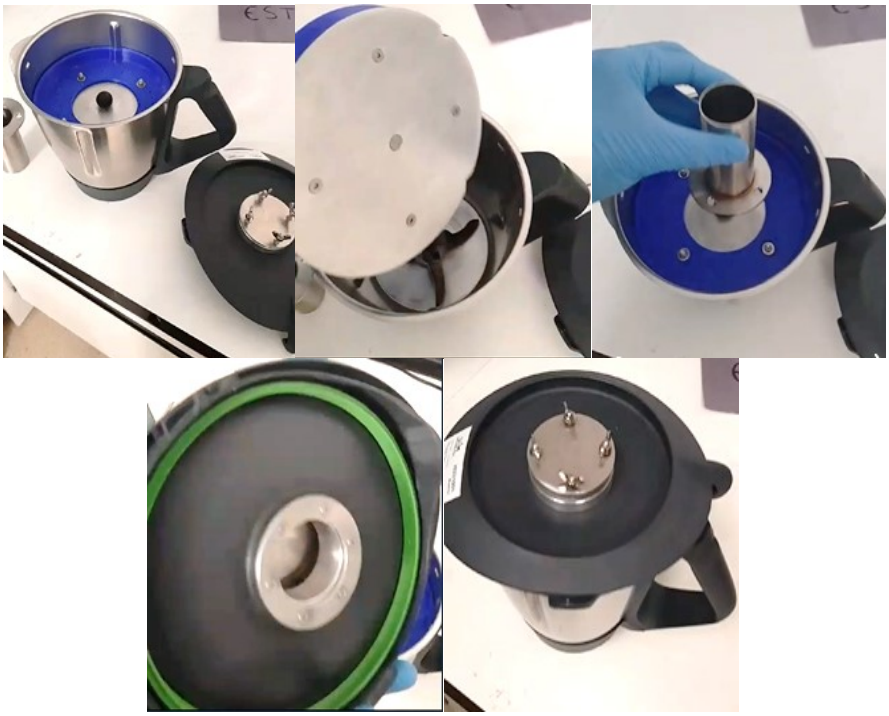
Nanoplastics



Oligomers

1 MATERIALS and EQUIPMENT

- Stainless-steel blender (Text S1 Fig. 1).



Text S1 Fig. 1: Pictures of the modified stainless-steel blender Thermomix TM31 (Vonwerk, Germany) used for the protocol.

- High power ultraviolet lamp (Text S1 Fig. 2).

Text S1 Fig. 2: 9.34 kW m^{-2} at 4 cm medium-pressure mercury lamp (Novalight TQ150) used for the protocol.

- 1mm pore metal strainer with a metal bowl or collector underneath to collect material passing through the filter.
- Liquid nitrogen.
- Magnetic stirrer and magnet.
- 50 KDa (50,000 Daltons) MWCO (Molecular Weight Cut-Off) ultrafiltration tubes.
- Ultra-pure ethanol (> 99.5%).
- 50 mL Falcon tubes.
- Tip sonicator.
- 1 µm pore filter suitable for large volumes.
- Drying oven that reaches 60°C.
- Beaker glasses
- Precision balance (capable of weighing up to 0.01 mg).
- Empty glass vials suitable for precision scales *(number the glass vial with an indelible marker before weighing and do not erase).*

Each generation process requires the use of ultrafiltration tubes, which must be CLEANED before use as follows (Section 1.1):

1.1 CLEANING THE ULTRAFILTRATION TUBES

a) Take 4 ultrafiltration tubes with a capacity of 20 ml in the upper part
b) Add 20 ml of ultra-pure water and centrifuge them for ~2 min at ~4500 rcfs.
c) Discard the filtered volume remaining in the lower part.
d) Repeat steps b) and c) 3 more times until washing the 4 tubes have been washed 4 times with ultra-pure water.

2 OBTENTION OF NON-PHOTOOXIDIZED NANOPLASTICS AND OLIGOMERS BY TRITURATION

2.1 Material trituration process

Triturate up to 300 g of microbeads in the stainless-steel blender to obtain ~210 g (~70 % of the original material) of triturated material < 1 mm:

a) Place the microbeads to be triturated in a metal or resistant plastic (polypropylene) container.
b) Fill the container with liquid nitrogen until the microbeads are covered and let it evaporate.
c) Allow the microbeads to heat up at room temperature for 1-2 minutes before triturating (if the surface of the microbeads is too cold, the blender is likely to block).
d) Add the microbeads in the blender and triturate for 1-2 min at maximum revolutions (10,000 rpm). Be careful, the material will continue to heat up during the trituration process, stop the process if the temperature exceeds 30 degrees and continue with step e).
e) Once triturated, transfer the material from the blender to the 1 mm pore strainer and shake until all the material < 1 mm passes into the collector.
f) Transfer the fragmented microbeads (> 1 mm) directly from the strainer and repeat steps a), b), c), d) and f) until an approximate amount of 30% wt of the original material remains in the strainer (this amount may vary depending on the polymer).

At this point, the small amount and small size of the fragmented microbeads make the process too inefficient).

g) Store the grinded material < 1 mm in glass bottles, avoiding exposure to light (use amber bottle, cover the bottle with aluminum, store in an opaque cabinet...).

The NPLs and oligomers generated are mixed with the triturated material < 1 mm.

2.2 Isolation, purification and quantification (dry weight) of nanoplastics and oligomers generated by trituration

Based on the application of the protocol with PBAT, we estimate that this protocol will yield between 2 and 4 mg of NPLs per 20 g of triturated material < 1mm.

This process is performed in two consecutive phases, the first aims to isolate the oligomeric fraction in ultra-pure ethanol, as the main objective, together with a small amount of the NPLs; the second phase is focused on extracting the maximum amount of NPLs using an ethanol/water mixture during the extraction. It is proposed to discard the volume retained in the lower part since it contains water, which would make the isolation of the oligomeric fraction take a considerable amount of time (the oligomers are usually most of the mass fraction, whereby enough material would be obtained only with the **Phase 1**):

a) Start transferring 80 grams of triturated material (< 1 mm) [section 2.1)] to 4 100 mL beaker glasses (20 g per beaker glass).

Phase 1:

b) Add 80 mL of ultra-pure ethanol at 30 °C to each beaker glass.

c) Sonicate each beaker glass individually for 1 min using a tip sonicator at enough power to shake the whole triturated material (typically at 60% duty cycle and 260 W using a Branson Ultrasonics™ Sonifier™ S-450A) and allow the material to settle for ~1 min.

d) Collect the maximum possible volume of ultra-pure ethanol (~320 mL) and filter through a 1 µm pore filter.

It is important not to exceed the flow rate recommended by the manufacturer, which in the case of glass fiber filters (Whatman) is 81 mL/min, to avoid the release of glass microfibers into the samples.

For greater safety, a stainless-steel filter with a 10 µm pore size can be placed between the glass fiber filter and the support (in contact with the filter) to retain the fibers that come off (most of which are larger than 10 µm) or use a nylon filter.

For avoid the filter clogging, replace the filter every 100 mL.

e) Transfer the filtered material to the upper part of 4 ultrafiltration tubes (20 mL per tube).

f) Centrifuge the ultrafiltration tubes at room temperature at ~4000 rcfs until the entire volume is ultrafiltered (~ 2-4 min).

g) The volume retained in the lower part of the tubes should be kept separately, it is the oligomeric fraction (Text S1 Fig. 3).

h) Repeat steps e), f) and g) until the entire volume (~ 320 mL) is ultrafiltered.

The last centrifugations may take longer due to the membranes clogging (usually not more than 10 minutes).

i) After ultrafiltration of the entire volume, transfer the oligomeric fraction to a beaker glass and dry in a clean oven at 60 °C until complete drying (dried oligomers have an oily appearance).

j) Resuspend the material in 2-3 mL of ultra-pure ethanol and transfer it to an empty and previously weighed small glass vial (weigh without cap) and dry it in a clean oven at 60 °C until completely dry (dried oligomers present oily appearance).

Phase 2:

k) Prepare 320 mL of 25% v/v ultra-pure ethanol in ultra-pure water (240 mL of ultra-pure water and 80 mL of ultra-pure ethanol in a glass bottle).

l) Transfer 80 mL of the solution prepared in the previous step, into 4 beaker glasses containing 20 g of triturated material (< 1 mm) that has already been extracted with ultra-pure ethanol following **Phase 1** (new triturated material can be added to increase the efficiency of NPLs extraction).

m) Cool the beaker glasses at 4 °C for 15 min to prevent the dissolution of NPLs.

n) Sonicate each beaker glass individually for 1 min using a tip sonicator at enough power to shake the whole triturated material (typically at 60% duty cycle and 260 W using a Branson Ultrasonics™ Sonifier™ S-450A) and allow the material to settle for ~1 min.

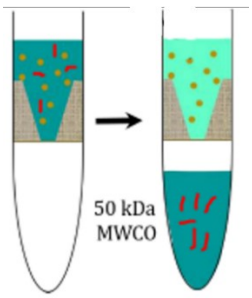
o) Collect the whole possible volume of ultra-pure ethanol at 25% in ultra-pure water (~250-300 mL) and filter through a 1 µm pore filter.

p) Store the filtered material at 4 °C for 10-15 minutes to prevent the dissolution of NPLs.

q) Transfer the filtered material to the upper part of the 4 ultrafiltration tubes used in the **Phase 1** (20 mL per tube).

r) Centrifuge the ultrafiltration tubes at room temperature at ~4000 rcfs until the entire volume is ultrafiltered (5-15 min).

s) The volume retained at the bottom of the tubes is discarded in case the triturated material used had been previously subjected to the **Phase 1** (it contains few oligomers; Text S1 Fig. 3).



Text S1 Fig. 3: Graphical image of the ultrafiltration process (yellow circles represent nanoplastics, red lines oligomers less than 50 KDa).

t) Repeat steps m), n), r), and s) until the entire volume (~ 300 mL) is ultrafiltered.

The last centrifugations may take longer due to the membranes clogging (usually not more than 10 minutes).

u) After ultrafiltering the entire volume, one more ultrafiltration should be performed using cold ultra-pure ethanol (1 h at 4 °C) to clean the fraction of NPLs that have been retained in the membranes.

v) The NPLs fraction is recovered by vigorously pipetting a volume of ~ 2 – 3 mL into the upper part of the tube, and subsequently that volume is stored in an empty and previously weighed small glass vial (weigh without cap) and dry it in a clean oven at 60 °C until completely dry.

2.3 Quantification by dry weight *(for both, Phase 1 and Phase 2)*

When both fractions are dried, the **small glass vials (without cap) containing the NPLs or the oligomers should be weighed**. From this weight, the initial weight of the empty bottle (without cap) is subtracted to obtain the mass of the material generated.

Balance accuracy were ± 0.01 mg is suggested

3 OBTENTION OF PHOTOOXIDIZED NANOPLASTICS AND OLIGOMERS BY PHOTOOXIDATION FOLLOWED BY TRITURATION

a) Place a maximum of 100 g of microbeads into the UV light lamp bottle filled with ~800 mL of ultra-pure ethanol.

b) Allow the photo-aging test to run for the days defined in the experimental design on the basis of the power applied and the photoaging scenario to be simulated.

In this study the lamp irradiates at ~ 9.34 kW m^{-2} (approximately 120 times the solar irradiance in the Iberian Peninsula) during 96 h, irradiation that corresponds to ~ 16 months of average sunlight in the Iberian Peninsula (7.7 kWh m^{-2} day^{-1}).

c) Separately collect the ultra-pure ethanol (containing NPLs, oligomers and photooxidized material > 1 µm) and the photooxidized microbeads.

d) Filter the ethanol from the previous step through 1 µm pore filters to isolate and purify the material contained in ultra-pure ethanol as follows:

a. Transfer the filtered material to the upper part of 4 ultrafiltration tubes (20 mL per tube).
b. Centrifuge the ultrafiltration tubes at room temperature at ~4000 rcfs until the entire volume is ultrafiltered (~ 2-4 min).

The last centrifugations may take longer due to the membranes clogging (usually not more than 10 minutes).

c. The volume retained in the lower part of the tubes should be kept separately, it is the oligomeric fraction (Text S1 Fig. 4).

d. After storage of the photooxidized material, one more ultrafiltration should be performed using cold ultra-pure ethanol (1 h at 4 °C) to clean the fraction of NPLs that have been retained in the membranes.
e. The NPLs fraction is recovered by vigorously pipetting a volume of ~ 2 – 3 mL into the upper part of the tube, and subsequently that volume is stored in an empty and previously weighed small glass vial (weigh without cap) and dry it in a clean oven at 60 °C until completely dry.

e) Triturate the photooxidized microbeads as described in section 2.2) and follow the **Phase 1** and **Phase 2** to obtain photooxidized nanoplastics and oligomers. Transfer these materials together with those obtained from the ultra-pure ethanol used during the photooxidation (described in section 3.d) to the same glass vials to proceed with the quantification by dry weight measurement (section 2.3).

Supplementary Table S1: pH of ultrapure water and culture medium with 50 mg/L of NP- and P-PBAT-Olig at 0 and 96 h.

Medium	Ultrapure water pH		TAP/6 pH	
	0h	96h	0h	96h
Without Oligs	5.8	5.64	6.96	7.09
NP-PBAT-Olig	5.14	5.15	6.88	7.08
P-PBAT-Olig	4.47	4.54	6.85	7.08

Supplementary Table S2: Concentrations and incubation times of the fluorochrome probes used for flow cytometry.

Fluorochrome	Acronym	Physiological parameter	Final concentration ($\mu g\ mL^{-1}$)	Incubation time (min)	Channel
Dihydrorhodamine 123	DHR123	Intracellular levels of H_2O_2	10	40	FITC
Bis-(1,3-dibutylbarbituric acid) trimethine oxonol	$DiBAC_4(3)$	Cytoplasmic membrane potential	2.5	10	FITC

Supplementary Table S3: Raw data of *C. reinhardtii* growth measured as OD_{750} after 72 h exposure to NP/P-PBAT-NPLs & NP/P-PBAT-Olig.

SAMPLE	[NPLs]	OD_{750} Raw Mean	OD_{750} Raw SD
NP-PBAT-NPLs	Control for 0.01 mg/L	1.385	0.093
	Control for 0.1 mg/L	1.090	0.049
	Control for 1 mg/L	1.090	0.049
	Control for 10 mg/L	1.090	0.049
	Control for 50 mg/L	1.385	0.093
	0.01 mg/L	1.262	0.058
	0.1 mg/L	1.036	0.050
	1 mg/L	1.019	0.076
	10 mg/L	0.981	0.016
	50 mg/L	1.248	0.101
SAMPLE	**[NPLs]**	**OD_{750} Raw Mean**	**OD_{750} Raw SD**
P-PBAT-NPLs	Control for 0.01 mg/L	1.385	0.093
	Control for 0.1 mg/L	1.385	0.093
	Control for 1 mg/L	1.385	0.093
	Control for 10 mg/L	1.385	0.093
	Control for 50 mg/L	1.385	0.093
	0.01 mg/L	1.171	0.033
	0.1 mg/L	1.238	0.050

	1 mg/L	1.275	0.074
	10 mg/L	1.268	0.062
	50 mg/L	1.263	0.142
SAMPLE	**[Olig]**	**OD_{750} Raw Mean**	**OD_{750} Raw SD**
NP-PBAT-Olig	Control for 0.01 mg/L	1.146	0.073
	Control for 0.1 mg/L	1.125	0.072
	Control for 1 mg/L	1.125	0.072
	Control for 10 mg/L	1.125	0.072
	Control for 50 mg/L	1.146	0.073
	0.01 mg/L	1.188	0.100
	0.1 mg/L	1.016	0.116
	1 mg/L	1.160	0.136
	10 mg/L	1.064	0.071
	50 mg/L	0.975	0.091
SAMPLE	**[Olig]**	**OD_{750} Raw Mean**	**OD_{750} Raw SD**
P-PBAT-Olig	Control for 0.01 mg/L	1.146	0.073
	Control for 0.1 mg/L	1.125	0.072
	Control for 1 mg/L	1.125	0.072
	Control for 10 mg/L	1.125	0.072
	Control for 50 mg/L	1.146	0.073
	0.01 mg/L	1.119	0.080
	0.1 mg/L	1.142	0.134
	1 mg/L	1.154	0.032
	10 mg/L	1.108	0.062
	50 mg/L	1.179	0.099

Supplementary Table S4: Raw data of *C. reinhardtii* chlorophyll content expressed as mg/L of total chlorophylls after 72 h exposure to NP/P-PBAT-NPLs & NP/P-PBAT-Olig.

SAMPLE	**[NPLs]**	**Σ Chlo (A + B) (mg/L) Raw Mean**	**Σ Chlo (A + B) (mg/L) Raw SD**
NP-PBAT-NPLs	Control for 0.01 mg/L	9.841	0.243
	Control for 0.1 mg/L	7.379	0.145
	Control for 1 mg/L	7.379	0.145
	Control for 10 mg/L	7.379	0.145
	Control for 50 mg/L	9.841	0.243
	0.01 mg/L	9.741	0.269
	0.1 mg/L	7.347	0.189
	1 mg/L	6.823	0.851
	10 mg/L	7.245	0.293

	50 mg/L	9.732	0.556
SAMPLE	**[NPLs]**	**Σ Chlo (A + B) (mg/L) Raw Mean**	**Σ Chlo (A + B) (mg/L) Raw SD**
P-PBAT-NPLs	Control for 0.01 mg/L	7.379	0.145
	Control for 0.1 mg/L	7.379	0.145
	Control for 1 mg/L	7.379	0.145
	Control for 10 mg/L	7.379	0.145
	Control for 50 mg/L	7.379	0.145
	0.01 mg/L	10.374	0.689
	0.1 mg/L	10.306	0.116
	1 mg/L	10.441	0.017
	10 mg/L	10.171	0.146
	50 mg/L	9.461	1.362
SAMPLE	**[Olig]**	**Σ Chlo (A + B) (mg/L) Raw Mean**	**Σ Chlo (A + B) (mg/L) Raw SD**
NP-PBAT-Olig	Control for 0.01 mg/L	8.146	0.987
	Control for 0.1 mg/L	8.336	0.788
	Control for 1 mg/L	8.336	0.788
	Control for 10 mg/L	8.336	0.788
	Control for 50 mg/L	8.146	0.987
	0.01 mg/L	7.011	1.397
	0.1 mg/L	6.782	2.288
	1 mg/L	8.687	1.266
	10 mg/L	7.525	2.729
	50 mg/L	6.430	0.650
SAMPLE	**[Olig]**	**Σ Chlo (A + B) (mg/L) Raw Mean**	**Σ Chlo (A + B) (mg/L) Raw SD**
P-PBAT-Olig	Control for 0.01 mg/L	8.146	0.987
	Control for 0.1 mg/L	8.336	0.788
	Control for 1 mg/L	8.336	0.788
	Control for 10 mg/L	8.336	0.788
	Control for 50 mg/L	8.146	0.987
	0.01 mg/L	8.335	1.749
	0.1 mg/L	8.758	1.109
	1 mg/L	8.801	1.784
	10 mg/L	8.176	2.060
	50 mg/L	5.961	0.484

Supplementary Table S5: Raw data of fluorescence intensity detected in FITC (525/40 nm) of *C. reinhardtii* cells stained with the fluorescent probe DHR123 for reactive oxygen species (ROS) indicator and after 72 h exposure to NP/P-PBAT-NPLs & NP/P-PBAT-Olig.

SAMPLE	[NPLs]	FITC Signal Raw Mean	FITC Signal Raw SD
NP-PBAT-NPLs	Control for 0.01 mg/L	26,935	2,595
	Control for 0.1 mg/L	27,819	2,482
	Control for 1 mg/L	26,093	3,366
	Control for 10 mg/L	28,709	5,572
	Control for 50 mg/L	28,898	1,068
	0.01 mg/L	34,850	4,482
	0.1 mg/L	32,914	5,397
	1 mg/L	31,900	2,771
	10 mg/L	38,858	4,565
	50 mg/L	44,520	7,955
SAMPLE	**[NPLs]**	**FITC Signal Raw Mean**	**FITC Signal Raw SD**
P-PBAT-NPLs	Control for 0.01 mg/L	21,120	684
	Control for 0.1 mg/L	22,173	2,594
	Control for 1 mg/L	20,966	863
	Control for 10 mg/L	26,824	3,117
	Control for 50 mg/L	20,602	1,461
	0.01 mg/L	24,769	1,964
	0.1 mg/L	25,531	4,387
	1 mg/L	24,989	4,862
	10 mg/L	34,040	4,871
	50 mg/L	28,291	1,447
SAMPLE	**[Olig]**	**FITC Signal Raw Mean**	**FITC Signal Raw SD**
NP-PBAT-Olig	Control for 0.01 mg/L	23,996	1,315
	Control for 0.1 mg/L	21,518	1,700
	Control for 1 mg/L	28,909	3,093
	Control for 10 mg/L	28,411	2,770
	Control for 50 mg/L	30,395	1,730
	0.01 mg/L	23,638	2,406
	0.1 mg/L	20,371	2,416
	1 mg/L	30,819	2,163
	10 mg/L	33,488	4,579
	50 mg/L	39,227	2,736
SAMPLE	**[Olig]**	**FITC Signal Raw Mean**	**FITC Signal Raw SD**
P-PBAT-Olig	Control for 0.01 mg/L	21,193	2,838
	Control for 0.1 mg/L	34,068	1,785
	Control for 1 mg/L	48,351	8,628
	Control for 10 mg/L	49,510	5,130
	Control for 50 mg/L	20,647	1,712
	0.01 mg/L	21,061	3,458
	0.1 mg/L	34,950	855
	1 mg/L	48,909	6,168
	10 mg/L	56,917	9,106
	50 mg/L	26,332	3,578

Supplementary Table S6: Raw data of fluorescence intensity detected in FITC (525/40 nm) of *C. reinhardtii* cells stained with the fluorescent probe $DiBAC_4(3)$ for membrane potential indicator and after 72 h exposure to NP/P-PBAT-NPLs & NP/P-PBAT-Olig.

SAMPLE	[NPLs]	FITC Signal Raw Mean	FITC Signal Raw SD
NP-PBAT-NPLs	Control for 0.01 mg/L	3,758	637
	Control for 0.1 mg/L	5,283	809
	Control for 1 mg/L	6,313	818
	Control for 10 mg/L	7,412	105
	Control for 50 mg/L	7,850	345
	0.01 mg/L	3,880	595
	0.1 mg/L	5,111	1,152
	1 mg/L	6,409	951
	10 mg/L	9,228	895
	50 mg/L	12,103	2,374
SAMPLE	**[NPLs]**	**FITC Signal Raw Mean**	**FITC Signal Raw SD**
P-PBAT-NPLs	Control for 0.01 mg/L	2,943	384
	Control for 0.1 mg/L	5,527	545
	Control for 1 mg/L	7,026	789
	Control for 10 mg/L	8,244	693
	Control for 50 mg/L	5,663	819
	0.01 mg/L	2,893	291
	0.1 mg/L	6,087	1,007
	1 mg/L	8,082	641
	10 mg/L	9,499	434
	50 mg/L	7,400	537
SAMPLE	**[Olig]**	**FITC Signal Raw Mean**	**FITC Signal Raw SD**
NP-PBAT-Olig	Control for 0.01 mg/L	3,181	634
	Control for 0.1 mg/L	3,105	555
	Control for 1 mg/L	2,990	262
	Control for 10 mg/L	2,762	184
	Control for 50 mg/L	2,686	54
	0.01 mg/L	3,215	417
	0.1 mg/L	3,244	531
	1 mg/L	3,300	365
	10 mg/L	3,136	284
	50 mg/L	3,418	155
SAMPLE	**[Olig]**	**FITC Signal Raw Mean**	**FITC Signal Raw SD**
P-PBAT-Olig	Control for 0.01 mg/L	2972	345
	Control for 0.1 mg/L	2890	307

	Control for 1 mg/L	2,875	200
	Control for 10 mg/L	2,690	222
	Control for 50 mg/L	2,630	99
	0.01 mg/L	3,282	233
	0.1 mg/L	3,050	500
	1 mg/L	3,242	543
	10 mg/L	3,053	454
	50 mg/L	3,210	481

Supplementary Table S7: Raw data of lipid peroxidation, measured by thiobarbituric acid reactive substances method (TBARS) and expressed as µg malondialdehyde (MDA)/mg dry weight (DW), on *C. reinhardtii* after 72 h exposure to NP/P-PBAT-NPLs & NP/P-PBAT-Olig.

SAMPLE	[NPLs]	TBARS (µg MDA/mg DW) Raw Mean	TBARS (µg MDA/mg DW) Raw SE
NP-PBAT-NPLs	Control for 0.01 mg/L	0.030	0.002
	Control for 0.1 mg/L	0.030	0.002
	Control for 1 mg/L	0.030	0.002
	Control for 10 mg/L	0.030	0.002
	Control for 50 mg/L	0.030	0.002
	0.01 mg/L	0.035	0.004
	0.1 mg/L	0.035	0.004
	1 mg/L	0.035	0.002
	10 mg/L	0.043	0.002
	50 mg/L	0.046	0.004
SAMPLE	**[NPLs]**	**TBARS (µg MDA/mg DW) Raw Mean**	**TBARS (µg MDA/mg DW) Raw SE**
P-PBAT-NPLs	Control for 0.01 mg/L	0.030	0.002
	Control for 0.1 mg/L	0.030	0.002
	Control for 1 mg/L	0.030	0.002
	Control for 10 mg/L	0.030	0.002
	Control for 50 mg/L	0.030	0.002
	0.01 mg/L	0.034	0.005
	0.1 mg/L	0.035	0.004
	1 mg/L	0.035	0.002
	10 mg/L	0.042	0.004
	50 mg/L	0.045	0.005
SAMPLE	**[Olig]**	**TBARS (µg MDA/mg DW) Raw Mean**	**TBARS (µg MDA/mg DW) Raw SE**

NP-PBAT-Olig	Control for 0.01 mg/L	0.030	0.004
	Control for 0.1 mg/L	0.030	0.004
	Control for 1 mg/L	0.030	0.004
	Control for 10 mg/L	0.030	0.004
	Control for 50 mg/L	0.030	0.004
	0.01 mg/L	0.034	0.004
	0.1 mg/L	0.034	0.003
	1 mg/L	0.034	0.002
	10 mg/L	0.035	0.004
	50 mg/L	0.038	0.006
SAMPLE	**[Olig]**	**TBARS (µg MDA/mg DW) Raw Mean**	**TBARS (µg MDA/mg DW) Raw SE**
P-PBAT-Olig	Control for 0.01 mg/L	0.030	0.004
	Control for 0.1 mg/L	0.030	0.004
	Control for 1 mg/L	0.030	0.004
	Control for 10 mg/L	0.030	0.004
	Control for 50 mg/L	0.030	0.004
	0.01 mg/L	0.034	0.002
	0.1 mg/L	0.034	0.003
	1 mg/L	0.034	0.004
	10 mg/L	0.035	0.003
	50 mg/L	0.038	0.003

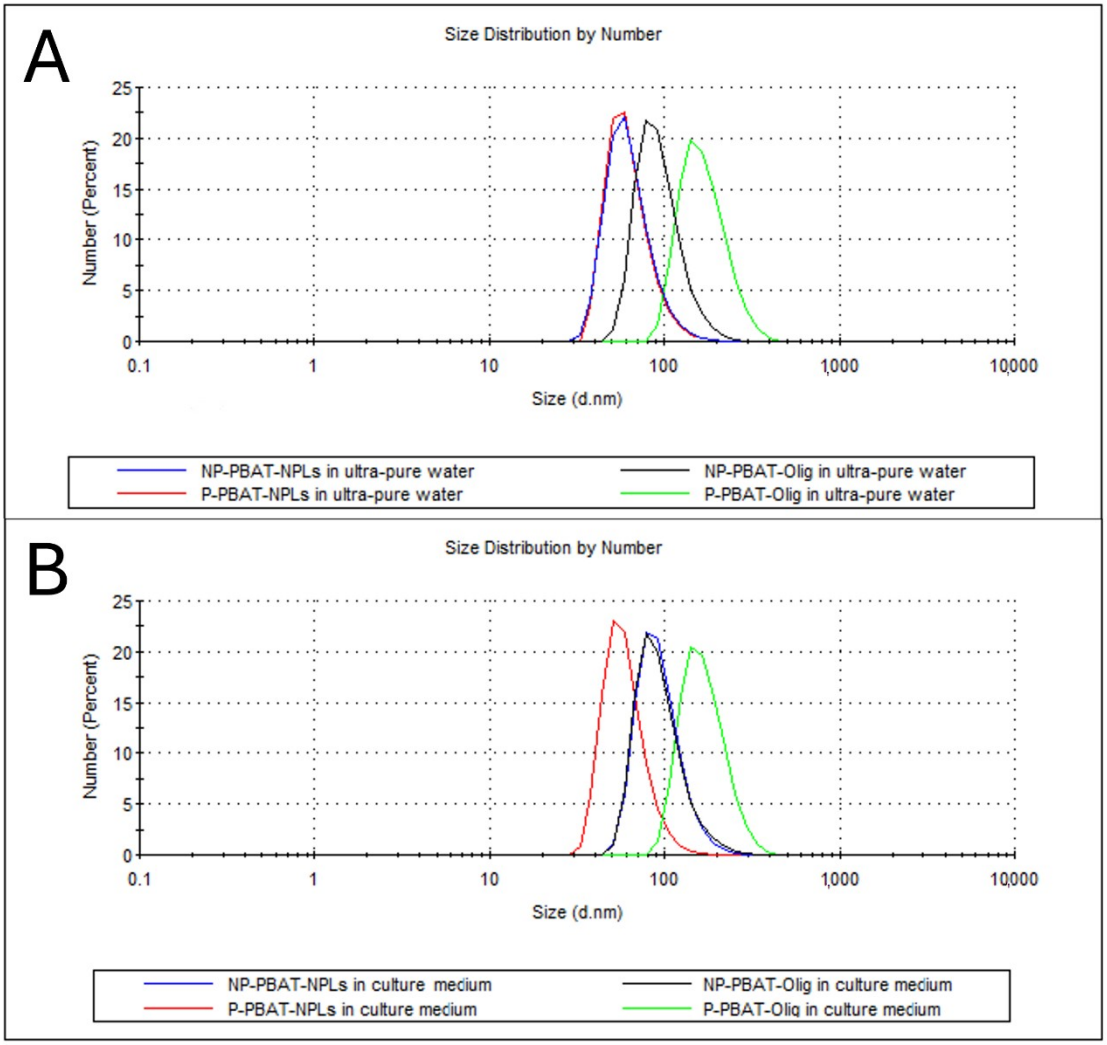
Supplementary Table S8: Raw data of oxygen evolution expressed as µmol O_2/mg Σ Chlo (A + B) · h of *C. reinhardtii* after 72 h exposure to NP/P-PBAT-NPLs & NP/P-PBAT-Olig.

SAMPLE	**[NPLs]**	**µmol O_2/mg Σ Chlo (A + B) · h Raw Mean**	**µmol O_2/mg Σ Chlo (A + B · h Raw SD**
NP-PBAT-NPLs	Control	225.4	9.5
	0.1 mg/L	201.9	15.4
	10 mg/L	187.4	2.5
SAMPLE	**[NPLs]**	**µmol O_2/mg Σ Chlo (A + B) · h Raw Mean**	**µmol O_2/mg Σ Chlo (A + B · h Raw SD**
P-PBAT-NPLs	Control	225.4	9.5
	0.1 mg/L	205.4	7.6
	10 mg/L	185.6	19.1
SAMPLE	**[NPLs]**	**µmol O_2/mg Σ Chlo (A + B) · h Raw Mean**	**µmol O_2/mg Σ Chlo (A + B · h Raw SD**
NP-PBAT-Olig	Control	232.6	19.4
	0.1 mg/L	214.3	3.7
	10 mg/L	204.9	17.5

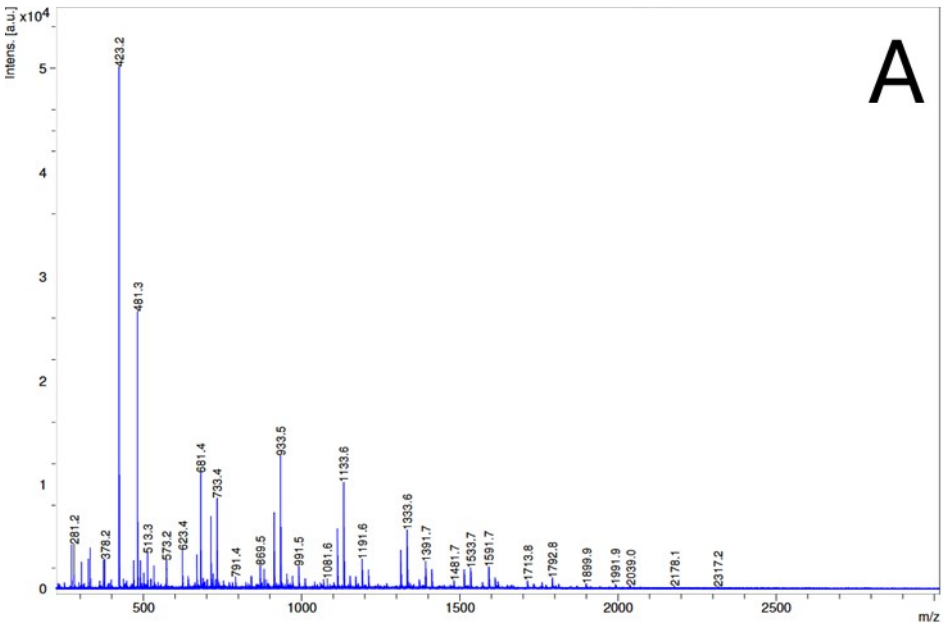
SAMPLE	[NPLs]	µmol O_2/mg Σ Chlo (A + B) · h Raw Mean	µmol O_2/mg Σ Chlo (A + B · h Raw SD
P-PBAT-Olig	Control	232.6	19.4
	0.1 mg/L	205.3	13.7
	10 mg/L	195.9	4.5

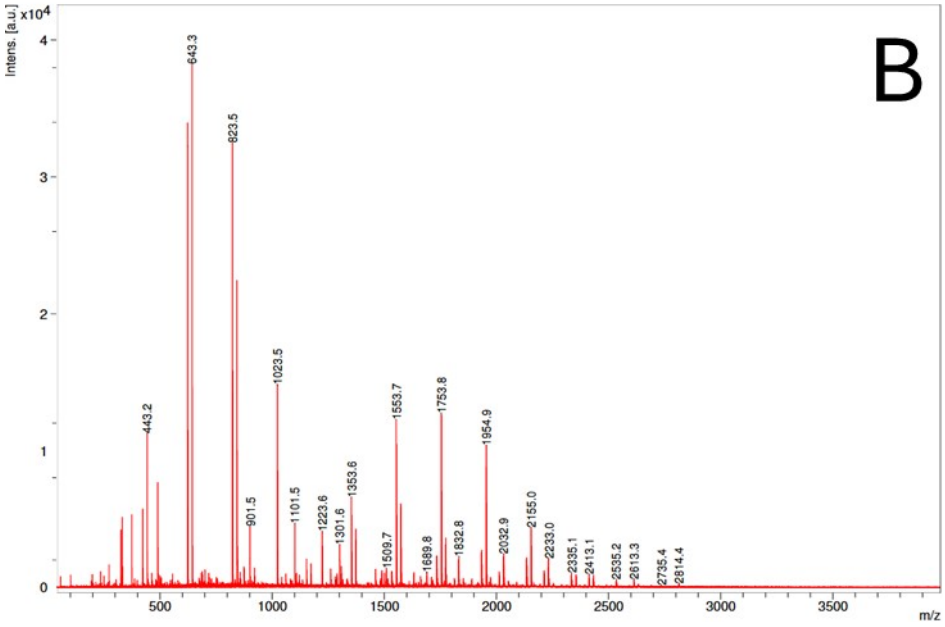
Supplementary Table S9: Raw data cell size based on flow cytometry back scatter detected with forward scatter FSC of *C. reinhardtii* cells after 72 h exposure to NP/P-PBAT-NPLs & NP/P-PBAT-Olig.

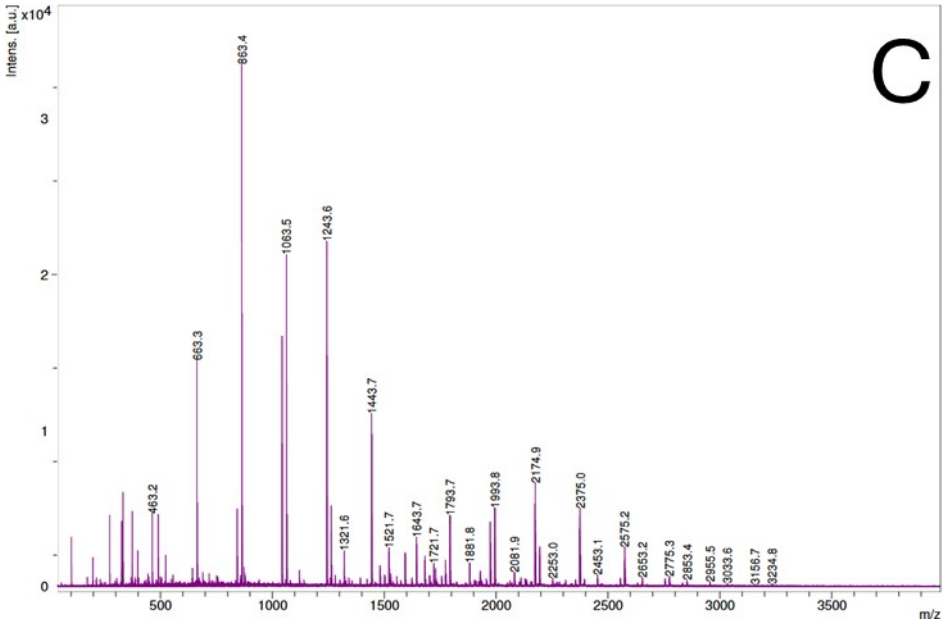
SAMPLE	[NPLs]	FSC Raw Mean	FSC Raw SD
NP-PBAT-NPLs	Control	786,653	19,144
	0.1 mg/L	789,255	17,488
	10 mg/L	834,648	20,244
SAMPLE	**[NPLs]**	**FSC Raw Mean**	**FSC Raw SD**
P-PBAT-NPLs	Control	776,479	18,606
	0.1 mg/L	783,186	8,697
	10 mg/L	822,909	23,747
SAMPLE	**[NPLs]**	**FSC Raw Mean**	**FSC Raw SD**
NP-PBAT-Olig	Control	1,004,230	139,767
	0.1 mg/L	945,057	104,617
	10 mg/L	1,000,270	101,500
SAMPLE	**[NPLs]**	**FSC Raw Mean**	**FSC Raw SD**
P-PBAT-Olig	Control	1,004,230	139,767
	0.1 mg/L	945,057	104,617
	10 mg/L	1,000,270	101,500

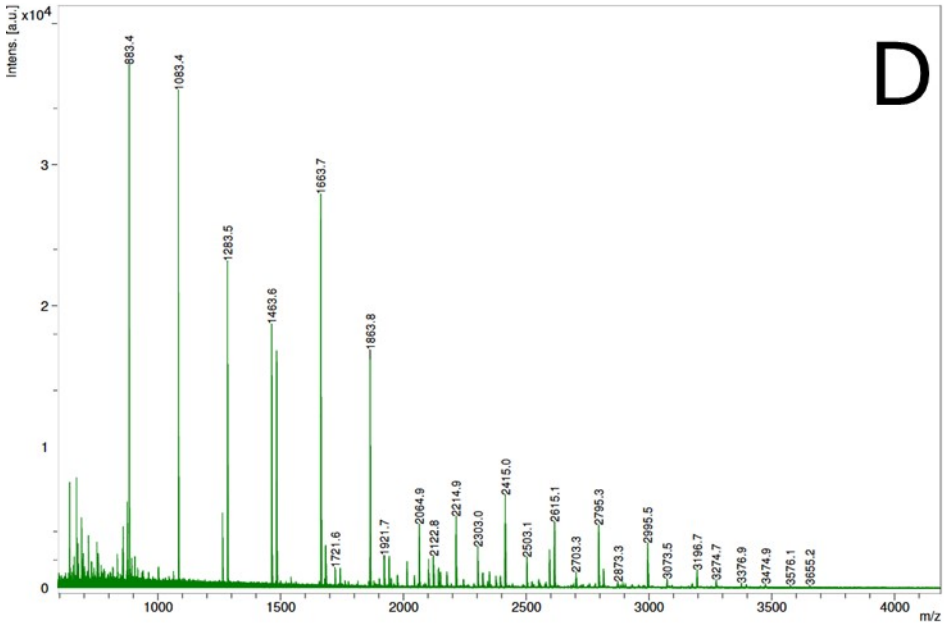


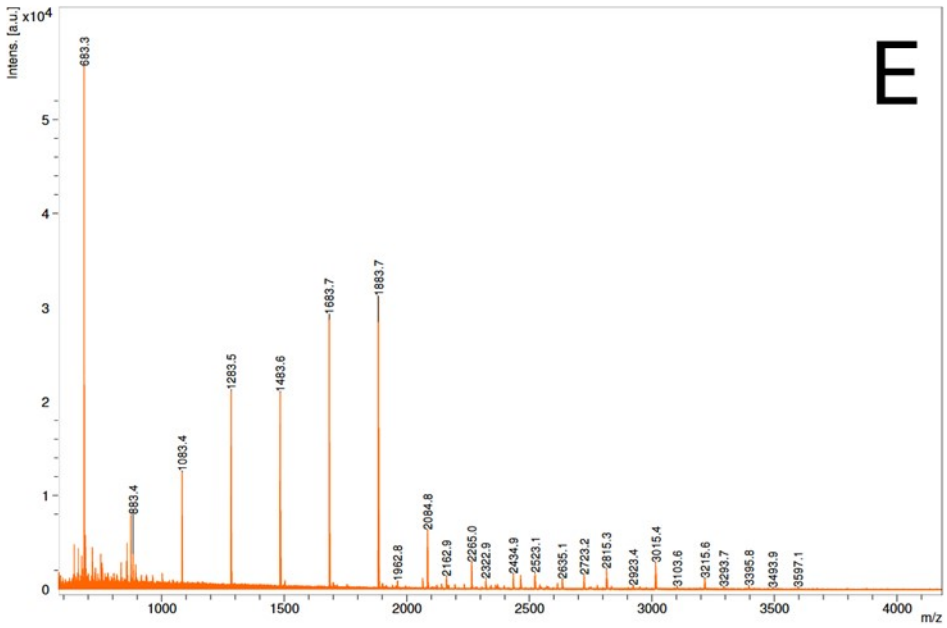
Supplementary Fig. S1: A) Hydrodynamic size distribution curves by number (measured by Dynamic Ligth Scattering) of NP- and P-PBAT-NPLs and NP- and P-PBAT-Olig in ultrapure water B) Hydrodynamic size distribution curves by number of NP- and P-PBAT-NPLs and NP- and P-PBAT-Olig in culture medium.

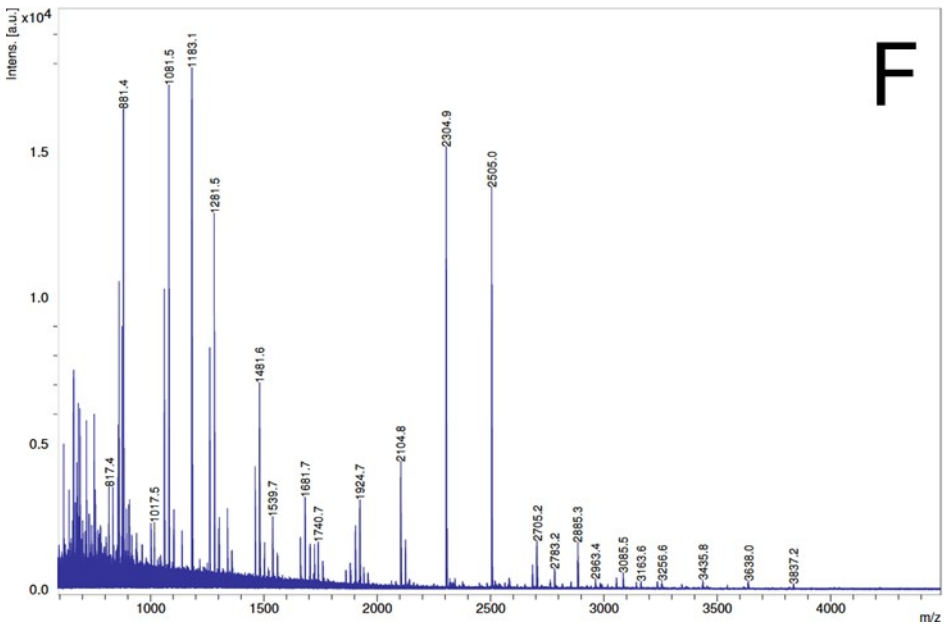

A
Intens. [a.u.]
x10^4
423.2
481.3
281.2
378.2
513.3
573.2
623.4
681.4
733.4
791.4
869.5
933.5
991.5
1081.6
1133.6
1191.6
1333.6
1391.7
1481.7
1533.7
1591.7
1713.8
1792.8
1899.9
1991.9
2039.0
2178.1
2317.2
m/z


B
Intens. [a.u.]
x10^4
643.3
823.5
443.2
901.5
1023.5
1101.5
1223.6
1301.6
1353.6
1509.7
1553.7
1689.8
1753.8
1832.8
1954.9
2032.9
2155.0
2233.0
2335.1
2413.1
2535.2
2613.3
2735.4
2814.4
m/z


C
Intens. [a.u.]
x10^4
863.4
1063.5
1243.6
663.3
463.2
1321.6
1443.7
1521.7
1643.7
1721.7
1793.7
1881.8
1993.8
2081.9
2174.9
2253.0
2375.0
2453.1
2575.2
2653.2
2775.3
2853.4
2955.5
3033.6
3156.7
3234.8
m/z


D
Intens. [a.u.]
x10^4
683.4
1083.4
1663.7
1283.5
1463.6
1863.8
1721.6
1921.7
2064.9
2122.8
2214.9
2303.0
2415.0
2503.1
2615.1
2703.3
2795.3
2873.3
2995.5
3073.5
3196.7
3274.7
3376.9
3474.9
3576.1
3655.2
m/z


E
Intens. [a.u.]
x10^4
683.3
683.4
1083.4
1283.5
1483.6
1683.7
1883.7
1962.8
2084.8
2162.9
2265.0
2322.9
2434.9
2523.1
2635.1
2723.2
2815.3
2923.4
3015.4
3103.6
3215.6
3293.7
3395.8
3493.9
3597.1
m/z


F
Intens. [a.u.]
x10^4
681.4
1081.5
1183.1
1281.5
1481.6
2304.9
2505.0
617.4
1017.5
1539.7
1681.7
1740.7
1924.7
2104.8
2705.2
2783.2
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2963.4
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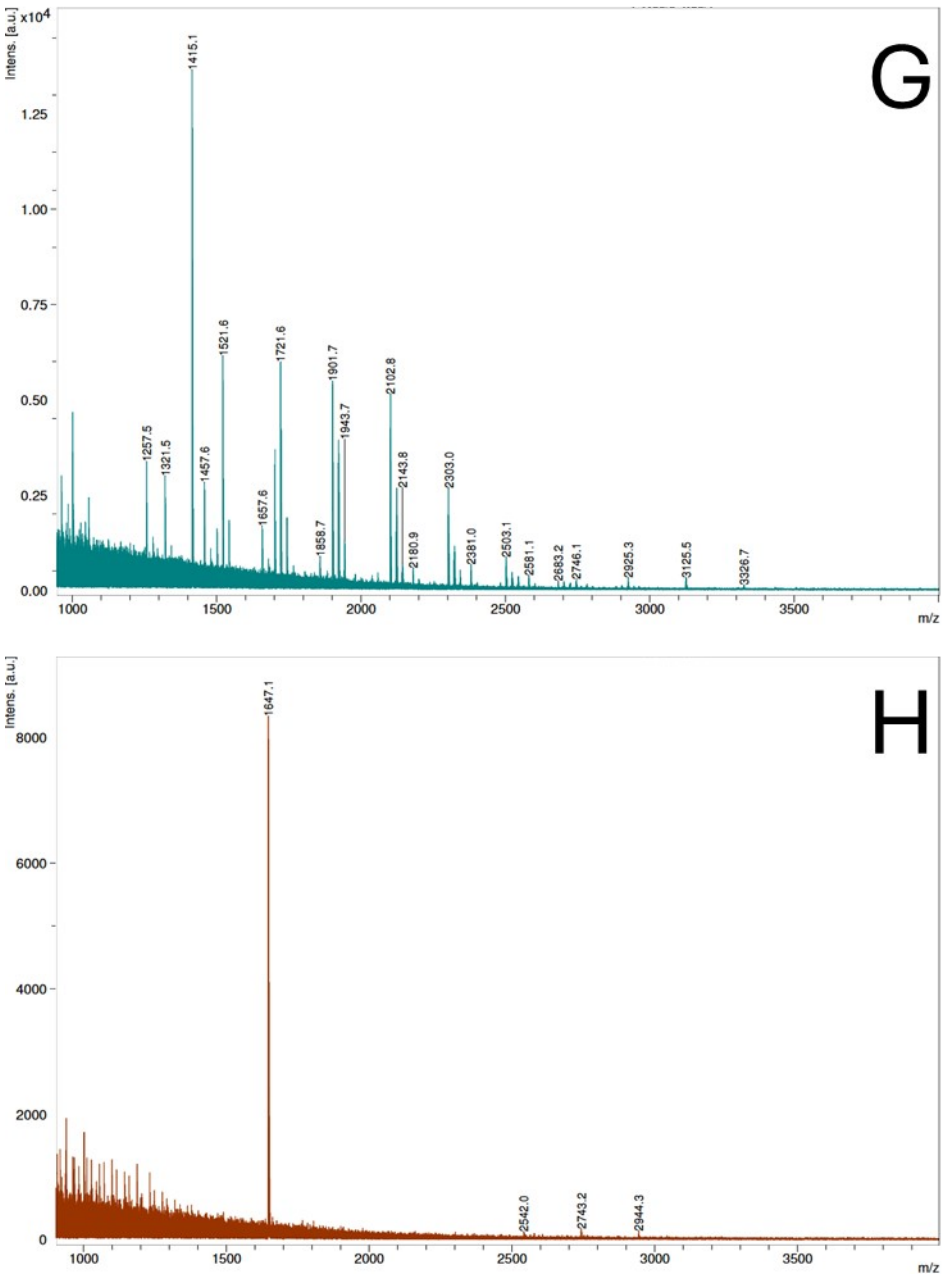
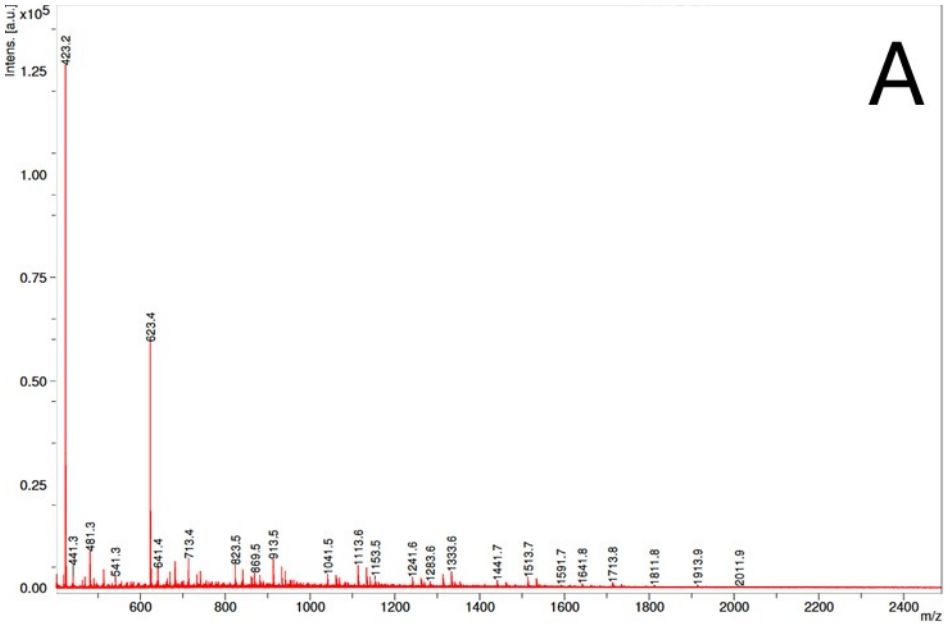
3837.2
m/z



Supplementary Fig. S2: MALDI-TOF/TOF spectrum of NP-PBAT-Olig chromatography peaks 1(A), 2(B), 3(C), 4(D), 5(E), 6(F), 7(G), and 8 (H).


A
Intens. [a.u.]
x10^5
1.25
1.00
0.75
0.50
0.25
0.00
423.2
623.4
441.3
481.3
541.3
641.4
713.4
823.5
869.5
913.5
1041.5
1113.6
1153.5
1241.6
1283.6
1333.6
1441.7
1513.7
1591.7
1641.8
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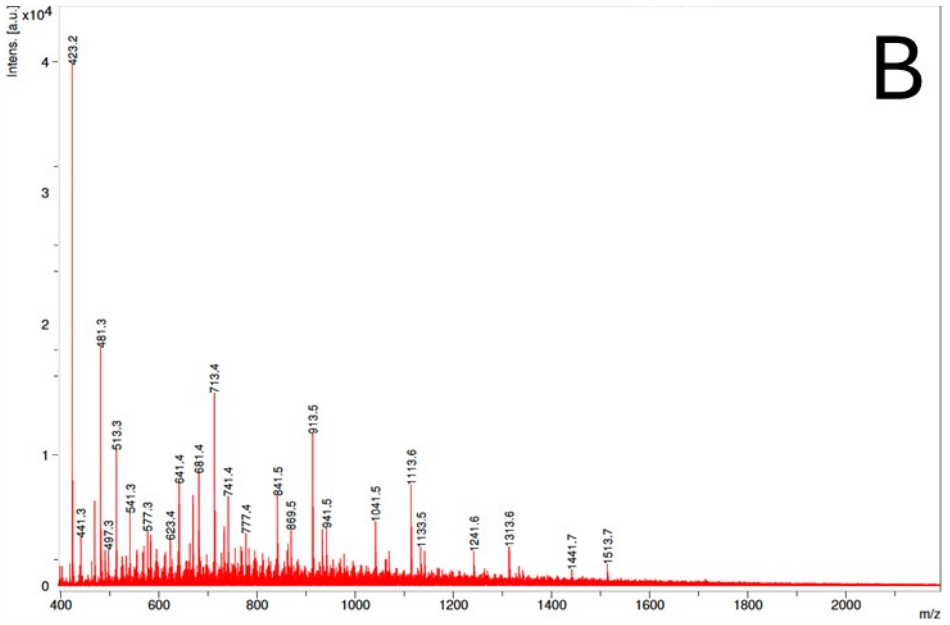
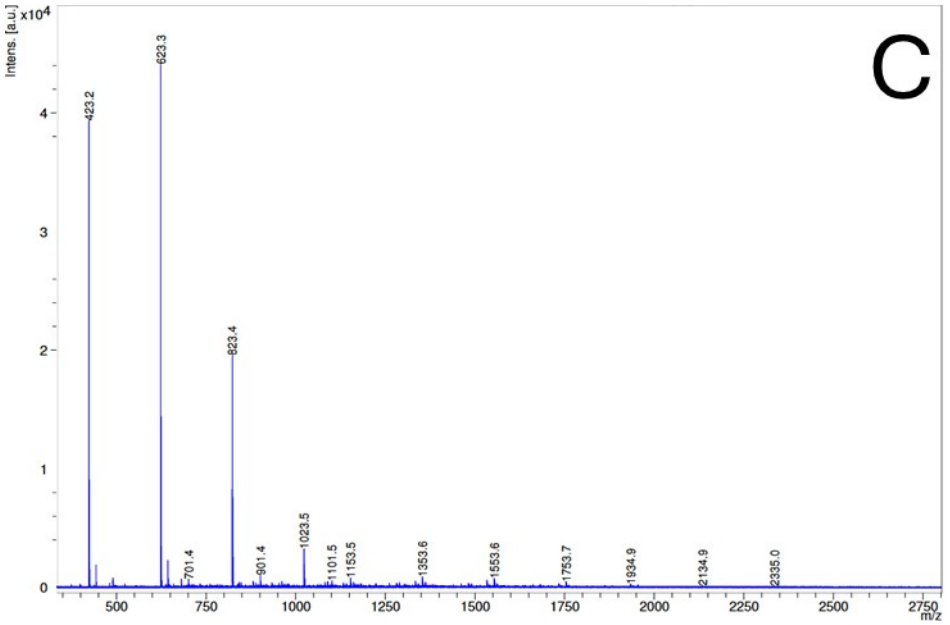
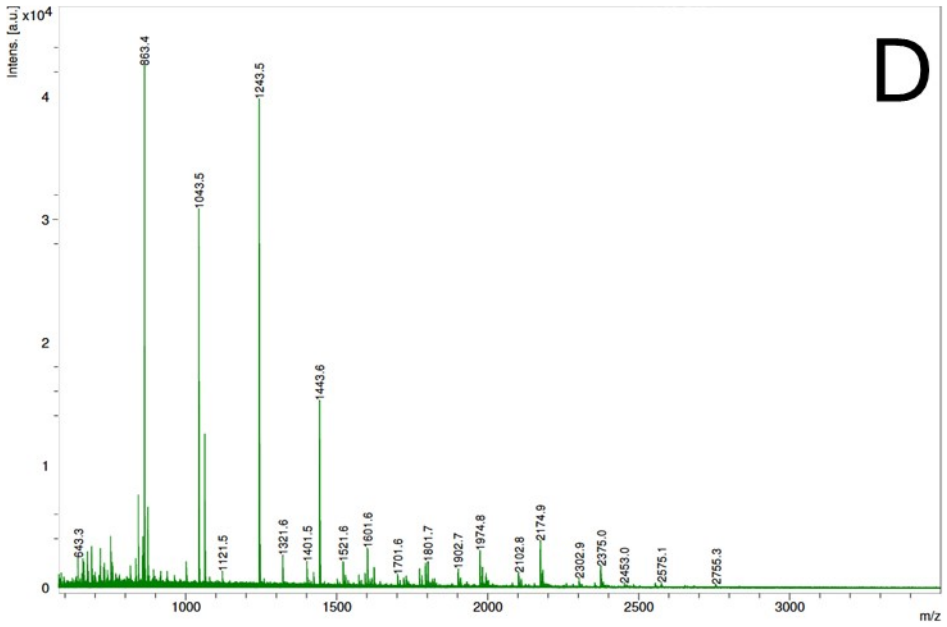
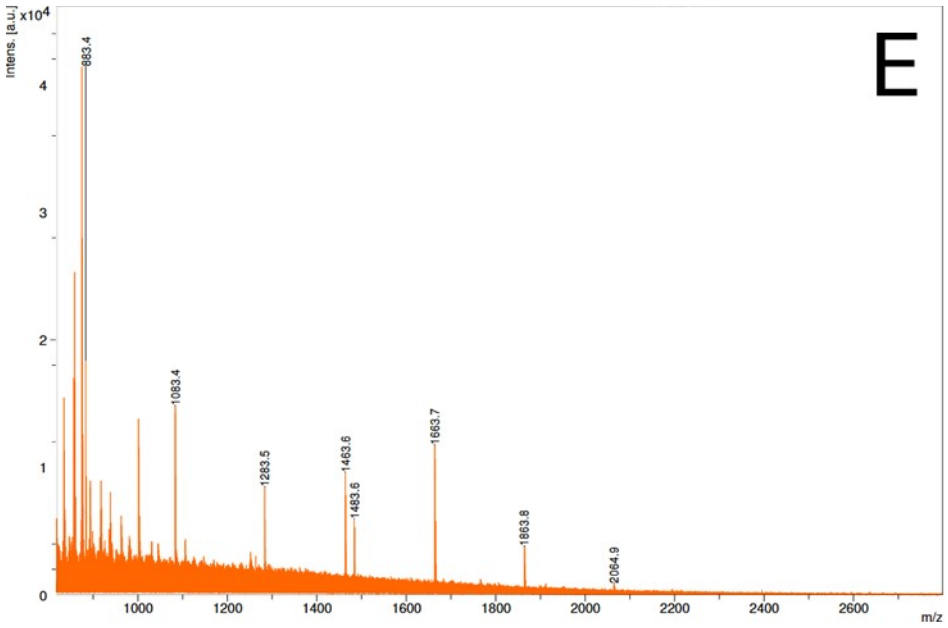
1200
1400
1600
1800
2000
2200
2400
m/z


B
Intens. [a.u.]
x10^4
4
3
2
1
0
423.2
481.3
513.3
541.3
577.3
623.4
641.4
681.4
713.4
741.4
777.4
841.5
869.5
913.5
941.5
1041.5
1113.6
1133.5
1241.6
1313.6
1441.7
1513.7
400
600
800
1000
1200
1400
1600
1800
2000
m/z


C
Intens. [a.u.]
x10^4
4
3
2
1
0
423.2
623.3
823.4
701.4
901.4
1023.5
1101.5
1153.5
1353.6
1553.6
1753.7
1934.9
2134.9
2335.0
500
750
1000
1250
1500
1750
2000
2250
2500
2750
m/z


D
Intens. [a.u.]
x10^4
663.4
1243.5
1043.5
1443.6
643.3
1121.5
1321.6
1401.5
1521.6
1601.6
1701.6
1801.7
1902.7
1974.8
2102.8
2174.9
2302.9
2375.0
2453.0
2575.1
2755.3
m/z


E
Intens. [a.u.]
x10^4
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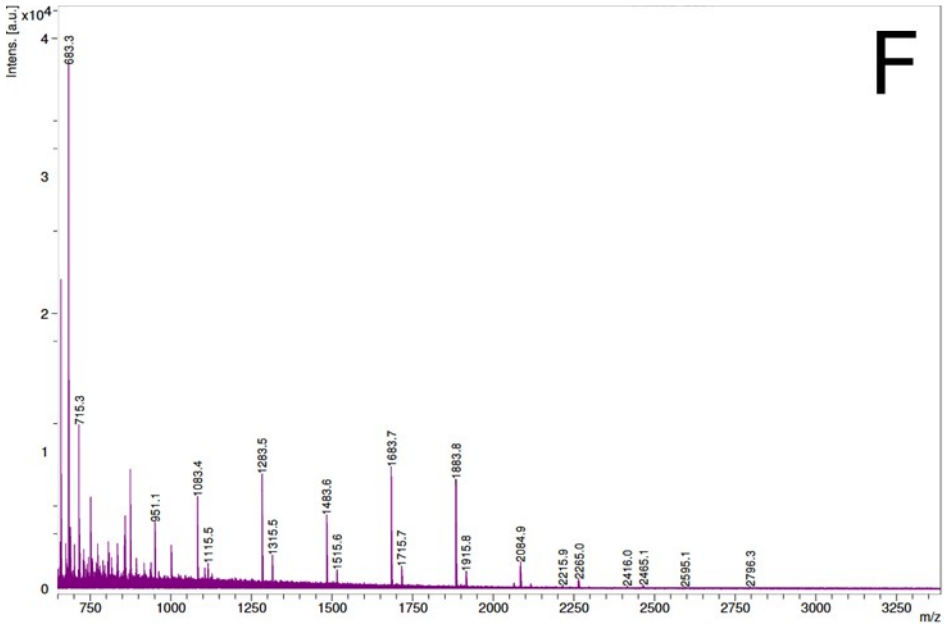
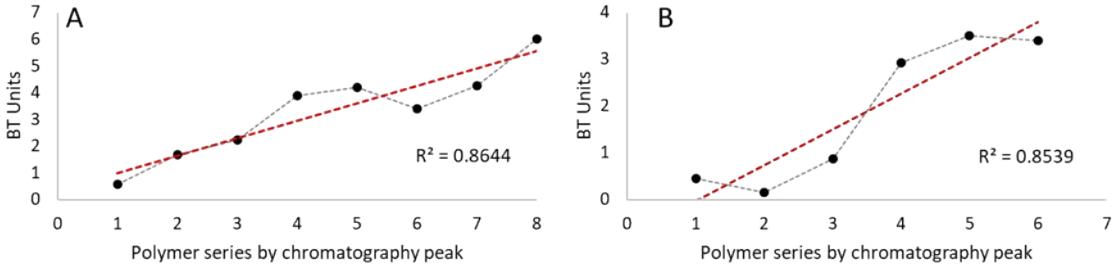
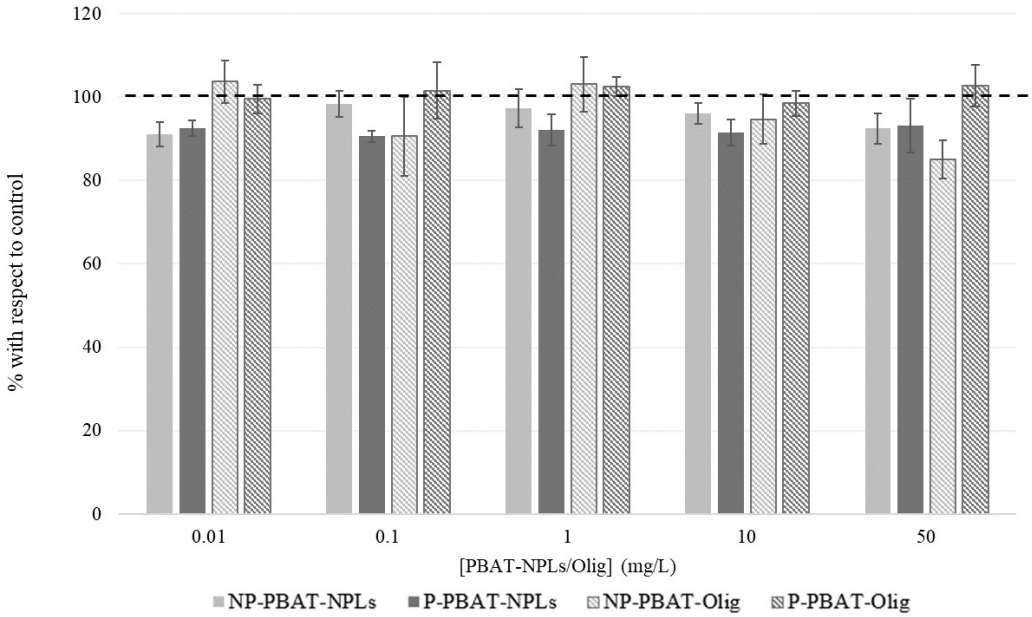
1083.4
1283.5
1463.6
1483.6
1663.7
1863.8
2064.9
m/z


F
Intens. [a.u.]
x10^4
683.3
715.3
951.1
1083.4
1115.5
1283.5
1315.5
1483.6
1515.6
1683.7
1715.7
1883.8
1915.8
2084.9
2215.9
2265.0
2416.0
2465.1
2595.1
2796.3
m/z

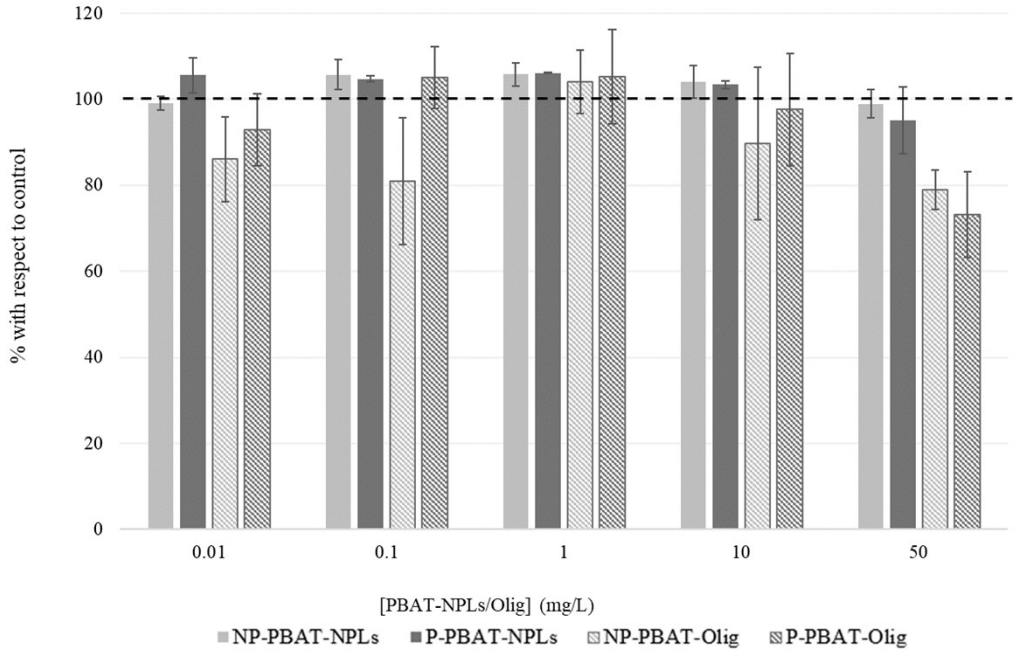
Supplementary Fig. S3: MALDI-TOF/TOF spectrum of P-PBAT-Olig chromatography peaks 1(A) 2(B), 3(C), 4(D), 5(E), and 6(F).



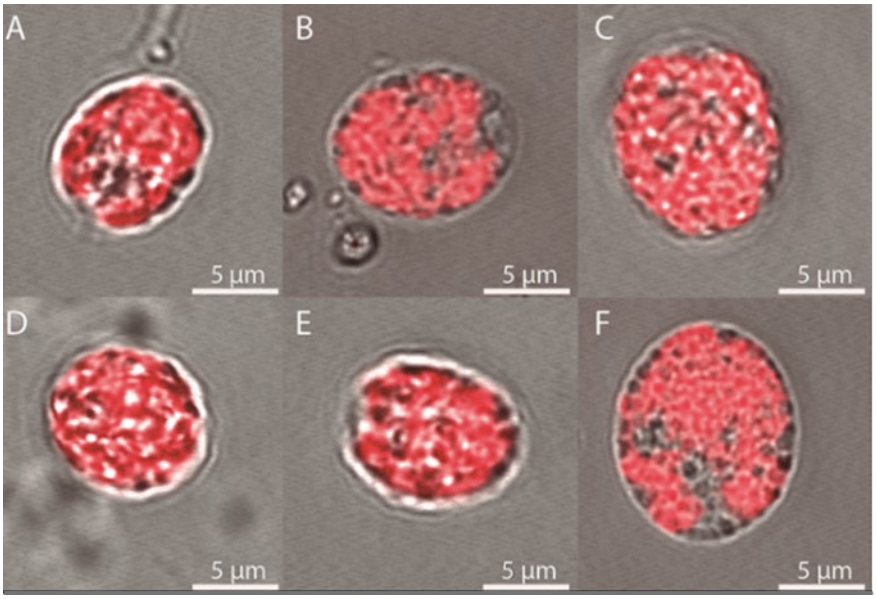
Supplementary Fig. S4: Mean value of butylene-terephthalate units per polymeric series by chromatography peak (from lower to higher hydrophobicity) of NP- (A) and P- (B) PBAT-Olig.



Supplementary Fig. S5: Growth, expressed as percentage of variation of OD_{750}, on *C. reinhardtii* after 72 h exposure to NP/P-PBAT-NPLs & NP/P-PBAT-Olig. Asterisks indicate treatments that are significantly different (Dunnet's test, (***) $p < 0.001$.; (**) $p < 0.01$.; (*) $p < 0.05$.) from the control represented as 100 % (dot line).



Supplementary Fig. S6: Chlorophyll content expressed as percentage of variation of total chlorophylls on *C. reinhardtii* after 72 h exposure to NP/P-PBAT-NPLs & NP/P-PBAT-Olig. Asterisks indicate treatments that are significantly different (Dunnet's test, (***) $p < 0.001$.; (**) $p < 0.01$.; (*) $p < 0.05$.) from the control represented as 100 % (dot line).



Supplementary Fig. S7: Representative chlorophyll autofluorescence/bright field overlay images of confocal microscopy of *C. reinhardtii* after 72 h exposure to NP- and P-PBAT-NPLs. Image shows confocal microscopy images of the non-exposed cells (A

and D); cells exposed to 0.1 and 10 mg/L of NP-PBAT-NPLs (B and C, respectively); cells exposed to 0.1 and 10 mg/L of P-PBAT-NPLs (E and F, respectively).