SUPPORTING INFORMATION

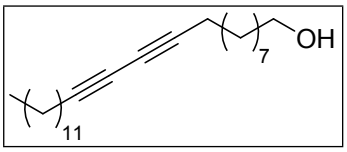
Chemical Synthesis

General

Unless otherwise specified, chemicals were purchased from Sigma-Aldrich and used without further purification. Tetrahydrofuran (THF) was distilled from sodium/benzophenone before use. Flash chromatography was carried out on Kieselgel 60 (230–240 mesh, Merck). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer at 400 and 100 MHz respectively. Chemical shifts (δ) are given in ppm relative to the NMR solvent residual peak and coupling constants (J) in Hz.

Synthesis of diacetylene polyethylene glycol (DA–PEG-OMe and DA-PEG-CO_2H) amphiphiles.

*Synthesis of pentacosa-10,12-diyn-1-ol (**DA-OH**).*



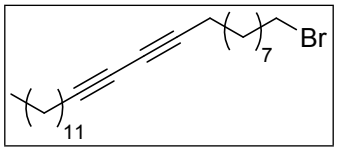
Under nitrogen atmosphere and at 0 °C (ice bath), to a solution of penatcosa-10,12-diynoic acid (1 g, 2.7 mmol, 1 equiv.) in diethyl ether (50 mL) was added lithium aluminium hydride (205 mg, 2 equiv.). After stirring for 1.5 h at room temperature, the reaction was cooled again to 0 °C and 15% sodium hydroxide (200 µL) was added, followed by water (600 µL). The resulting pink precipitate was filtered off on Celite®, the organic phase was washed with hydrochloric acid (2 ×

20 mL), and dried on magnesium sulfate. After solvent removal under vacuum, product **5** was obtained as a white solid (938 mg, yield = 96%).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.63 (t, J = 7 Hz, 2H; CH_2−OH), 2.23 (t, J = 7 Hz, 4H; CH_2−C≡), 1.60-1.45 (m, 6H; CH_2), 1.44-1.24 (m, 28H; CH_2), 0.86 ppm (t, J = 7 Hz, 3H; CH_3).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 77.5 (−C≡), 77.4 (−C≡), 65.2 (−C≡), 65.2 (−C≡), 63.1 (CH_2−OH), 32.7 (CH_2), 31.9 (CH_2), 29.6 (3 CH_2), 29.4 (CH_2), 29.3 (3 CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.3 (CH_2), 28.2 (2 CH_2), 25.7 (CH_2), 22.6 (CH_2), 19.2 (CH_2), 14.1 ppm (CH_3).

*Synthesis of 1-bromopentacosa-10,12-diyne (**DA-Br**).*

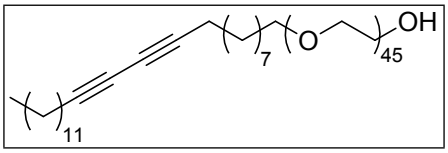


Under nitrogen atmosphere, triphenylphosphine (550 mg, 1.5 equiv.) and **DA-OH** (500 mg, 1.4 mmol, 1 equiv.) were solubilized in dichloromethane (3 mL). Tetrabromomethane (700 mg, 1.5 equiv.) was added in portions and the reaction was stirred at room temperature for 15 min. After addition of cold water (2 mL) the organic phase was separated, dried over magnesium sulfate and purified on a silica plug eluted with pure dichloromethane. Upon concentration under reduced pressure, the desired product **Da-Br** was obtained as a yellowish varnish (585 mg, yield = 100%).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.40 (t, J = 7 Hz, 2H; CH_2−Br), 2.24 (t, J = 7 Hz, 4H; CH_2−C≡), 1.85 (td, J = 7 Hz, 2H; CH_2), 1.60-1.45 (m, 6H; CH_2), 1.45-1.25 (m, 26H; CH_2), 0.88 ppm (t, J = 7 Hz, 3H; CH_3).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 77.6 (−C≡), 77.4 (−C≡), 65.3 (−C≡), 65.1 (−C≡), 34.0 (CH_2−Br), 32.8 (CH_2), 31.9 (CH_2), 29.6 (3 CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (2 CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 28.3 (CH_2), 28.2 (CH_2), 28.1 (CH_2), 22.7 (CH_2), 19.2 (CH_2), 14.1 ppm (CH_3).

*Synthesis of pentacosa-10,12-diyn-1-oxypentatetracontaethyleneglycol (**DA-PEG-OH**).*



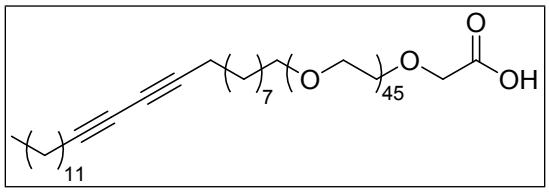
Under nitrogen atmosphere, polyethylene glycol (MW = 2 000, 1.5 g – 0.75 mmol – 1 equiv.) dissolved in anhydrous acetonitrile (15 mL) was added to a suspension of sodium hydride (36 mg, 2 equiv.) in anhydrous acetonitrile (7 mL). The mixture was heated to refluxed for 30 min and allowed to cool down to room temperature. Compound **DA-Br** (317 mg – 0.75 mmol – 1 equiv.) dissolved in tetrahydrofuran (3 mL) was slowly added and the reaction was stirred at room temperature for 96 h. After concentration under vacuum, purification by column chromatography (silica gel, dichloromethane/methanol 95:5) yielded the desired **DA-PEG-OH** product as a pale yellow solid (600 mg, yield = 40%).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.65 (m, 180H; CH_2−O), 3.42 (t, J = 7 Hz, 2H; CH_2−O), 2.22 (t, J = 7 Hz, 4H; CH_2−C≡), 1.60-1.45 (m, 6H; CH_2), 1.35-1.20 (m, 28H; CH_2), 0.88 ppm (t, J = 7 Hz, 3H; CH_3).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 77.4 (−C≡), 77.3 (−C≡), 72.4 (CH_2−O), 71.4 (CH_2−O), 70.5 (86 CH_2−O), 70.2 (CH_2−O), 69.9 (CH_2−O), 65.3 (−C≡), 65.2 (−C≡), 61.5 (CH_2−OH), 31.8 (CH_2), 29.5

(4 CH_2), 29.4 (CH_2), 29.3 (3 CH_2), 29.2 (2 CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 28.2 (2 CH_2), 25.9 (CH_2), 22.6 (CH_2), 19.1 (CH_2), 14.0 ppm (CH_3).

Synthesis of carboxylic derivative ***DA-PEG-CO$_2$H***.



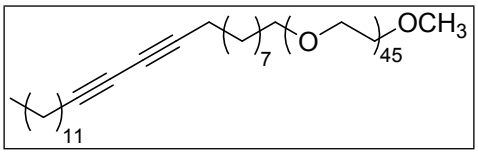
Under nitrogen atmosphere, pentacosa-10,12-diyn-1-oxypolyethyleneglycol **DA-PEG-OH** (468 mg – 0.2 mmol – 1 equiv.) dissolved in tetrahydrofuran (5 mL) was added to a suspension of sodium hydride (12 mg, 2.5 equiv.) in tetrahydrofuran (5 mL). The mixture was refluxed for 30 min and allowed to cool down to room temperature. 2-bromoacetic acid (195 mg – 1.4 mmol – 7 equiv.) dissolved in tetrahydrofuran (2 mL) was slowly added and the reaction was stirred at room temperature for 24 h. After concentration under vacuum, purification by column chromatography (silica gel, dichloromethane/methanol 95:5) yielded the desired product as a pale yellow solid (384 mg, yield = 80%).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 4.20 (s, 2H, O−C$\underline{H}_2$−CO_2H), 3.80-3.60 (m, 180H; CH_2−O), 3.51 (t, J = 7 Hz, 2H; CH_2−O), 2.24 (t, J = 7 Hz, 4H; CH_2−C≡), 1.65-1.47 (m, 6H; CH_2), 1.40-1.25 (m, 28H; CH_2), 0.88 ppm (t, J = 7 Hz, 3H; CH_3).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 169.1 (CO_2H), 77.5 (−C≡), 77.3 (−C≡), 72.4 (CH_2−O), 71.6 (CH_2−O), 70.6 (86 CH_2−O), 70.1 (CH_2−O), 70.0 (CH_2−O), 68.6 (O−$\underline{C}H_2$− CO_2H), 65.2 (−C≡), 65.1 (−C≡), 61.6 (CH_2−O), 31.6 (CH_2), 29.6 (3 CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (2 CH_2), 29.2

(CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.8 (2 CH_2), 28.7 (CH_2), 28.2 (2 CH_2), 26.2 (CH_2), 22.6 (CH_2), 19.2 (CH_2), 14.0 ppm (CH_3).

Synthesis of methyl ether derivative ***DA-PEG-OMe****.*



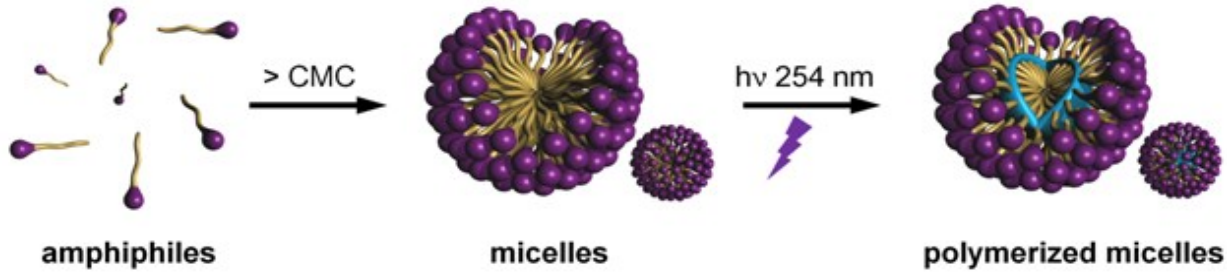
Under nitrogen atmosphere, polyethylene glycol monomethyl ether (MW = 2000, 250 mg – 0.13 mmol – 1 equiv.) dissolved in anhydrous acetonitrile (10 mL) was added to a suspension of sodium hydride (6 mg, 2 equiv.) in anhydrous acetonitrile (2 mL). The mixture was refluxed for 30 min and allowed to cool down to room temperature. Compound **DA-Br** (211 mg, 4 equiv.) dissolved in tetrahydrofuran (2 mL) was slowly added and the reaction was stirred at room temperature for 96 h. After concentration under vacuum, purification by column chromatography (silica gel, dichloromethane/methanol 95:5) yielded the desired product as pale yellow solid (265 mg, yield = 90%).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.80-3.45 (M, 180H; CH_2−O), 3.33 (s, 3H, O–CH_3), 2.19 (t, J = 7 Hz, 4H; CH_2−C≡), 1.57-1.45 (M, 6H; CH_2), 1.37-1.17 (M, 28H; CH_2), 0.83 ppm (t, J = 7 Hz, 3H; CH_3).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 77.4 (−C≡), 77.3 (−C≡), 72.5 (CH_2−O), 71.9 (CH_2−O), 70.7-70.2 (86 CH_2−O), 70.3 (CH_2−O), 70.0 (CH_2−O), 65.3 (−C≡), 65.2 (−C≡), 61.6 (CH_2−OH), 58.9 (O–CH_3), 31.8 (CH_2), 29.6 (CH_2), 29.5 (3 CH_2), 29.4 (CH_2), 29.3 (3 CH_2), 29.2 (CH_2), 29.1 (CH_2),

29.0 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.3 (2 CH_2), 26.0 (CH_2), 22.6 (CH_2), 19.1 (CH_2), 14.0 ppm (CH_3).

Micelle Assembly.



A mixture of **DA-PEG-OMe** and **DA-PEG-CO_2H** amphiphiles in a 9:1 molar ratio was solubilized in deionized water at a final 10 mg mL^{-1} concentration. The solution was sonicated with an ultrasonic probe (300 ms pulses per second, 25 W output power) for 30 min. The solution was then filtered over a 0.2 μm nylon membrane to yield conventional DA-PEG micelles. To obtain core-polymerized micelles, the DA-PEG micelle colloid was further subjected to UV irradiation (254 nm – low pressure mercury UV lamp – Heraeus) for 6 h. The volume of the solution was adjusted to the initial volume by adding deionized water, to compensate the volume lost during the photo-polymerization process. The solution was then filtered over a 0.2 μm nylon membrane to yield pDA-PEG micelle colloid.

The preparation of ^{14}C-labeled micelles was carried out in the same manner, replacing **DA-PEG-OMe** with **^{14}C-DA-PEG-OMe**. The latter was synthesized according to the procedure described above, but starting from ^{14}C-labeled pentacosa-10,12-diynoic acid (prepared according to *Org. Biomol. Chem.* **8,** 3902–3907 (2010).

Supplementary Figures

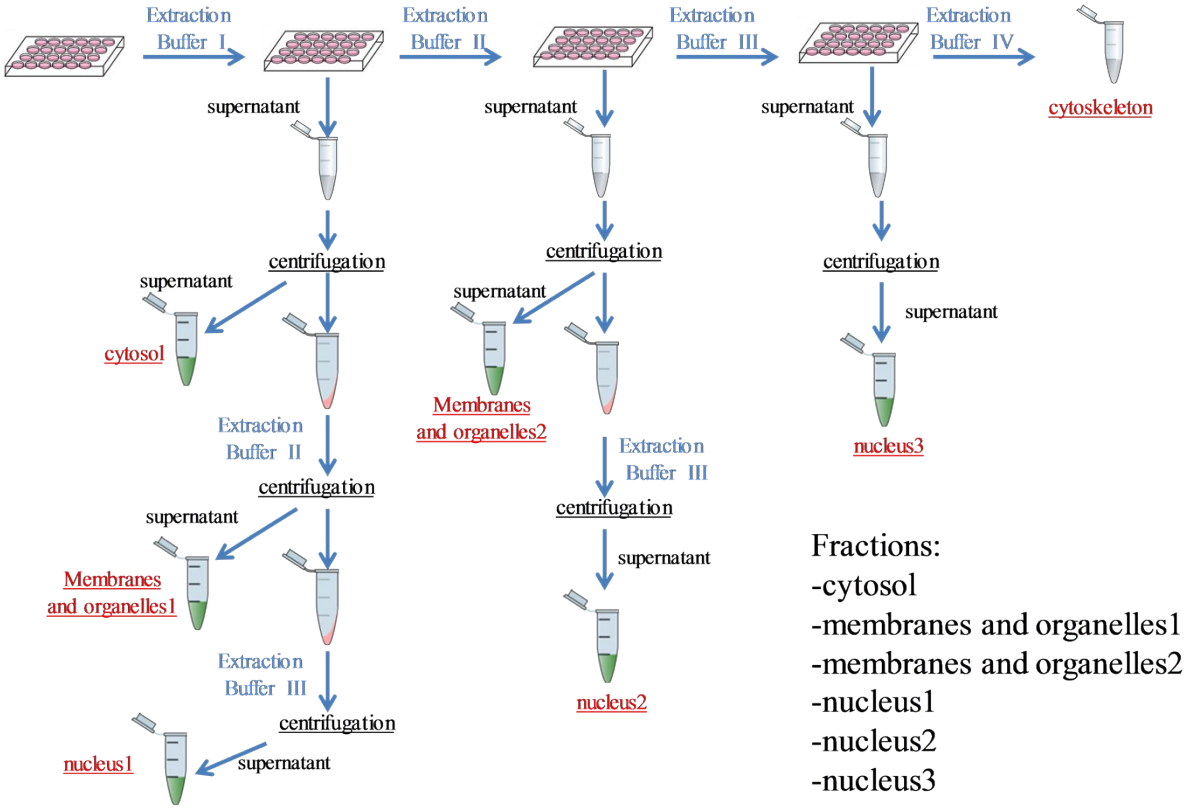


Figure S1. Initial subcellular fractionation procedure for intracellular distribution studies. The standard subcellular fractionation method provided for the ProteoExtract Subcellular Proteome Extraction Kit (Merck Millipore) was optimized to ensure a relevant separation of cytosol, membranes/organelles, nucleus and cytoskeleton in RAW 264.7 cells.

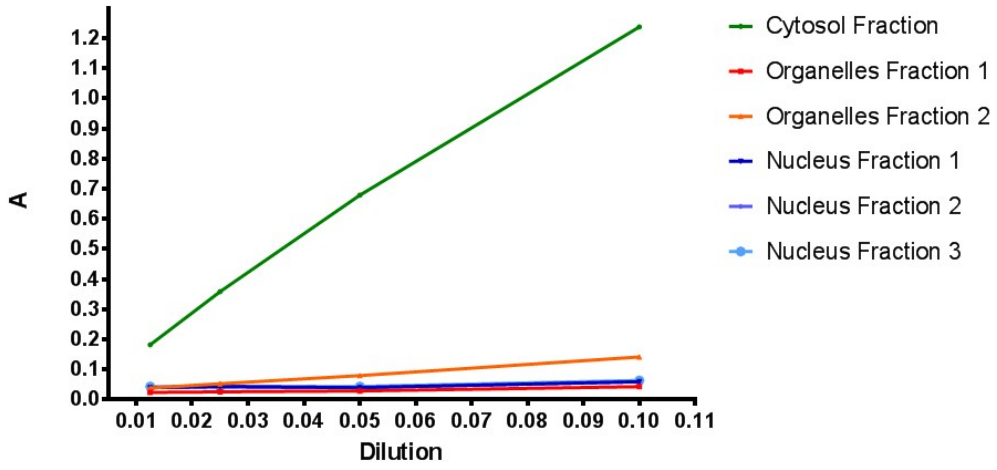


Figure S2. LDH assay on cell fractions. All fractions from the initial procedure were diluted in PBS + 1% BSA (1:10, 1:20, 1:40, 1:80) and lactate dehydrogenase (LDH) was quantified in all solutions using the CytoTox 96. Cytosolic LDH was quantified through the formation of the red formazan product in medium, measured at 492 nm. “Cytoskeleton” fraction was collected with “nucleus 3” fraction. The absorbance measured at 492 nm is proportional to the LDH content, showing that almost all the LDH is recovered in the cytosolic fraction, confirming a proper isolation of the cytosol.

Table S1. Proteins content in cell fractions. The protein content in all fractions was dosed using a Pierce BCA Protein Assay Kit (Thermoscientific), showing negligible quantities in the "Membranes and organelles 1" and "Nucleus 1" fractions, which were not selected for the optimization of the cellular fractionation procedure.

Fraction	Protein content (µg)	Proportion to total proteins
Cytosol	637	35%
Membr. and Org. 1	70	3%
Membr. and Org. 2	628	34%
Nucleus 1	9	0.5%
Nucleus 2	379	21%
Nucleus 3	100	5%

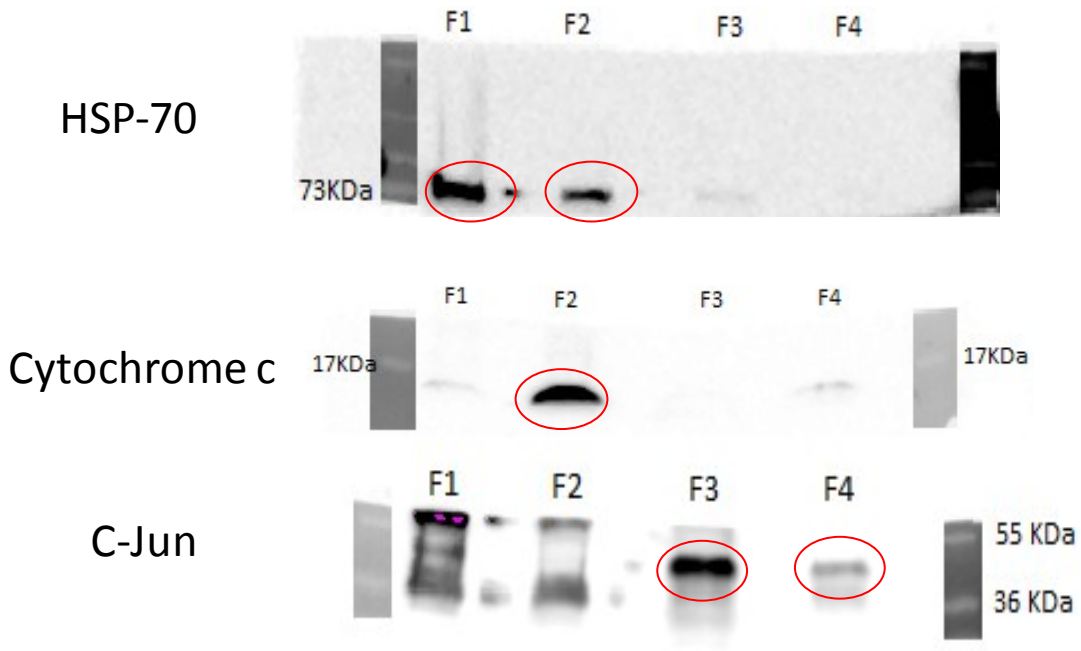


Figure S3. Identification of compartment-specific proteins in cell fractions. Three cellular proteins (HSP-70, Cytochrome c and C-Jun) were detected by Western blot on cell fractions after concentration, in the "cytosol" fraction (F1), "membranes and organelles" fraction (F2), combined "nucleus" fractions (F3) and "cytoskeleton" fraction (F4). The ubiquitous Heat shock protein HSP70 (70 kDa) was correctly detected in "cytosol" fraction (F1) and in "membranes and organelles" fraction (F2). The cytochrome complex (12 kDa) was detected only in "membranes and organelles" fraction (F2), confirming that this fraction has been well isolated. The nuclear protein c-Jun (43 kDa) was founded to be present in both the "nuclear" (F3) and the "cytoskeleton" fraction (F4), indicating that nuclear and cytoskeleton fractions were not well separated; the two fractions were therefore combined in the final fractionation protocol.

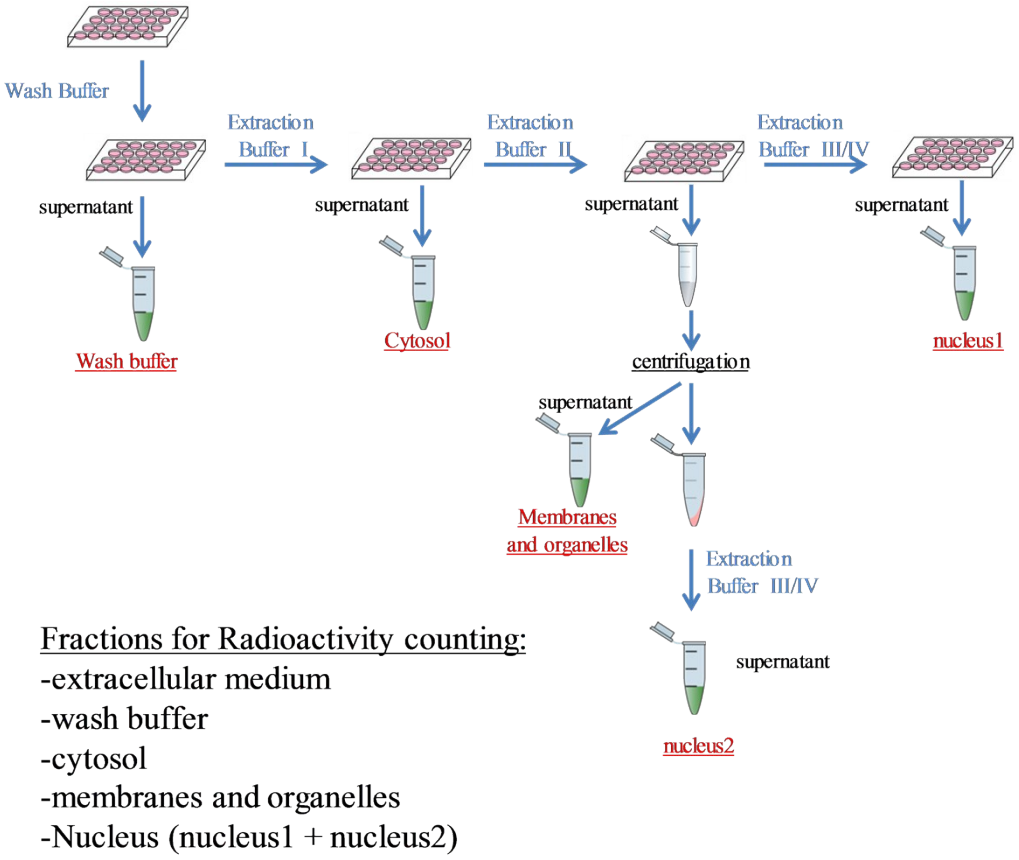


Figure S4. Final Subcellular fractionation protocol and radioactivity counting. The final subcellular fractionation protocol set up according to LDH quantification (Figure S2), protein quantification (Table S1) and identification (Figure S3). Radioactivity measurements were performed on all subcellular fractions as well as in the culture medium and in the washing buffer in order to follow the total amount of radioactivity added on cells.

Table S2. Total uptake of non-polymerized and polymerized ^{14}C-labeled micelles. The uptake (μg/million cells) by RAW cells was quantified following exposure to 0.01 mg/mL micelles at 37 °C (4–48 h incubation) or at 4 °C (4 h incubation).

	4h 4 °C	4h 37 °C	24h 37 °C	48h 37 °C
Non-polymerized micelles (μg/million cells)	0.18	0.64	1.20	1.23
Polymerized micelles (μg/million cells)	0.06	0.18	0.42	0.48

Table S3. Subcellular localization of conventional and polymerized ^{14}C-labeled micelles. Micelles (μg/million cells) were quantified in subcellular compartments following exposure of RAW cells to 0.01 mg/mL micelles at 37 °C (for 4 h and 48 h incubation).

	4h 37 °C			48h 37 °C		
	Cytosol	Membr	Nucleus	Cytosol	Membr	Nucleus
Conventional micelles (μg/million cells)	0.15	0.42	0.07	0.19	0.81	0.23
Polymerized micelles (μg/million cells)	0.03	0.12	0.03	0.11	0.30	0.08

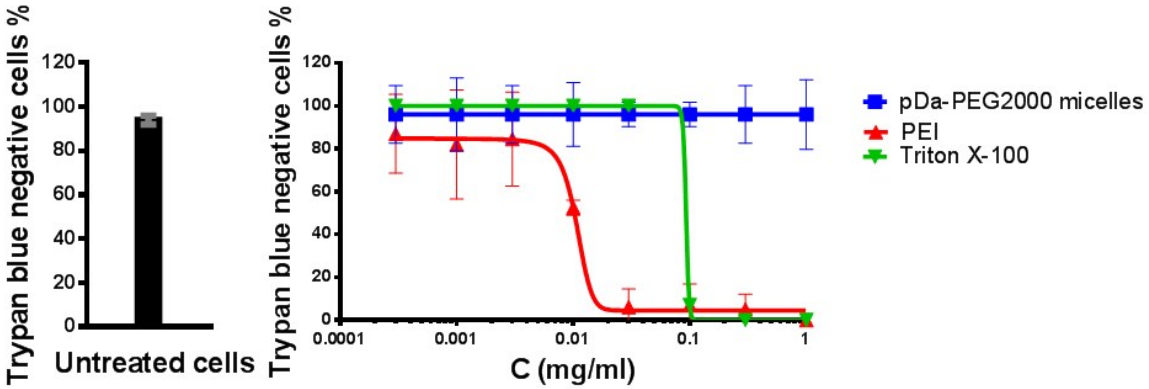


Figure S5. Evaluation of micelle impact on cell viability by the Trypan Blue test. RAW cells were treated with polymerized pDA-PEG micelles, polyethylenimine and Triton X-100 at various concentrations (0.0003-1 mg/mL) for 24 h, and further stained with Trypan Blue and observed. Results obtained with micelles were fitted by a sigmoidal curve. Data are mean values ± SD of three independent experiments.

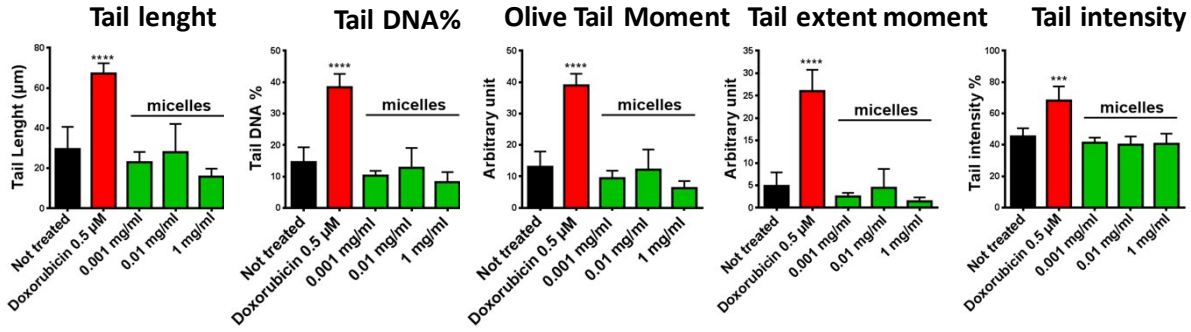


Figure S6. Comparison of genotoxicty parameters. The images of the comet assay performed on RAW cells exposed to polymerized micelles and doxorubicin (positive control) were quantified using the OpenComet plugin of the ImageJ software, and expressed by calculating different

parameters: tail length, tail DNA percentage, Olive tail moment, tail extent moment and tail intensity. Data are mean values ± SD of three independent experiments.