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Hybrid giant lipid vesicles incorporating a PMMA-based copolymer

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1 STATISTICAL SUMMARY

2 Words: 6494

3 Figures/Tables: 5

4 HIGHLIGHTS

5 • Methacrylate based polymer is mixed with a lipid to produce giant vesicles

6 • Electroformation is not a suitable method to obtain such hybrid systems

7 • Phase transfer method is used to obtain hybrid giant vesicles

8 • Phase transfer method useful when the polymer has a relatively high T_g

9

1 ABSTRACT

2 Background

3 In recent years, there has been a growing interest in the formation of copolymer-lipid hybrid self-
4 assemblies, which allow combining and improving the main features of pure lipid-based and
5 copolymer-based systems known for their potential applications in the biomedical field. As the
6 most common method used to obtain giant vesicles is electroformation, most systems so far used
7 low T_g polymers for their flexibility at room temperature.

8 Methods

9 Copolymers used in the hybrid vesicles have been synthesized by a modified version of the
10 ATRP, namely the Activators ReGenerated by Electron Transfer ATRP and characterized by
11 NMR and DSC. Giant hybrid vesicles have been obtained using electroformation and droplet
12 transfer method. Confocal fluorescence microscopy was used to image the vesicles.

13 Results

14 Electroformation enabled to obtain hybrid vesicles in a narrow range of compositions (15 mol %
15 was the maximum copolymer content). This range could be extended by the use of a droplet
16 transfer method, which enabled obtaining hybrid vesicles incorporating a methacrylate-based
17 polymer in a wide range of compositions. Proof of the hybrid composition was obtained by
18 fluorescence microscopy using labelled lipids and copolymers.

19 Conclusions

20 This work describes for the first time, to the best of our knowledge, the formation of giant hybrid
21 polymer/lipid vesicles formed with such a content of a polymethylmethacrylate copolymer, the
22 glass temperature of which is above room temperature.

23 General Significance

1 This work shows that polymer structures, more complex than the ones mostly employed, can be
2 possibly included in giant hybrid vesicles by using the droplet transfer method. This will give
3 easier access to functionalized and stimuli-responsive giant vesicles and to systems exhibiting a
4 tunable permeability, these systems being relevant for biological and technological applications.

5 KEYWORDS liposomes; polymersomes; hybrid GUVs; self-assembly; surfactant; polymer;
6 phase transfer method

7 1. INTRODUCTION

8 Liposomes and polymersomes are self-assembled vesicles formed by lipids and
9 copolymers respectively and characterized by the presence of a hydrophobic double layer
10 delimitating an internal water pool. Their structure allows the encapsulation of both hydrophobic
11 and hydrophilic components with high loading efficiency, which mainly boosted the research in
12 applications related to drug delivery [1–4]. Besides, the last 10 years of research witnessed a
13 growing interest in polymersomes used as reactors [5]. For this particular application, the low
14 bilayer permeability of the high molecular weight constituents represents a drawback. Block
15 copolymers forming intrinsically permeable polymersomes [6,7], polymersomes whose
16 permeability can be modulated by a stimulus, i.e. pH [8], light [9], CO₂ [10], and self-adaptive
17 polymersomes [5] have been proposed. A further, less explored possibility is the use of hybrid
18 lipid/polymer vesicles, which could exploit the complementary properties of liposomes and
19 polymersomes, i.e. biocompatibility, biodegradability and permeability of the former and high
20 mechanical stability and chemical versatility of the latter. These hybrid vesicles have higher
21 permeability to small dyes like carboxyfluorescein or calcein with respect to polymersomes [11–
22 13] and it has been recently shown that the hybrid vesicles permeability could be increased by

1 adding phospholipase A2 that can selectively degrade lipids [14]. Examples of hybrid nanoscale
2 polymersomes have been proposed for applications in nanomedicine and seemed to improve the
3 targeting efficiency [15,16]. Interestingly, hybrid vesicles at the micron scale are valid
4 alternatives to giant liposomes as simplified cell models for a deeper understanding of
5 fundamental biological processes [17,18]. They have indeed the potential for mimicking the
6 membrane functionalities like the presence of raft-like domains [19,20], membrane asymmetry
7 [21] transport or recognition properties thanks to the insertion of proteins in the membrane [22–
8 24] and even chemical energy-driven ATP production [25] or cell compartmentalization [26]. To
9 date, the potential responsiveness of block copolymers has not yet been exploited for hybrid
10 vesicles. Indeed, only examples of temperature-responsive hybrid vesicles are reported in the
11 literature, exploiting the phase transition of dioleoylphosphatidyl choline based lipids [27].

12 One of the reasons of the lack of polymer/lipid hybrid systems is the limited choice of the
13 amphiphilic copolymers which were generally restricted to a small group based on the
14 combination of poly(ethylene oxide) or poly(oxazoline) as hydrophilic unit and either
15 poly(dimethyl siloxane) (PDMS) [28,29], poly(butadiene) (PBD) [30,31], polyisobutylene (PIB)
16 [32] and very recently butylacrilate [33] as hydrophobic moiety.

17 Different methodologies have been used to form polymer based giant unilamellar vesicles
18 (GUVs). They have been mainly inspired from those developed for lipids [34]. Electroformation
19 method is the most employed one. It is a solvent-free method where a dry amphiphilic thin film
20 is hydrated in the presence of an alternating current probably inducing fluctuations and interlayer
21 repulsions, which favours the swelling and release of giant vesicles [35]. It was applied to block-
22 copolymers for the first time in the case of poly(ethylene oxide)-*block*-poly(ethyl ethylene) [36].
23 Electroformation can be applied to a limited class of block-copolymers [33,37,38], as the choice

1 of the nature of the block copolymer is usually dictated by the hydrophobic block glass transition
2 temperature, since this should be as low as possible in order to guarantee a sufficient flexibility
3 and mobility of the polymer chains. Gentle hydration has been described even earlier than
4 electroformation for brain phospholipids [39] and is quite difficult to be applied to block-
5 copolymers as witnessed by the very limited number of publications [40–42]. Gel assisted
6 hydration using polyvinyl alcohol (PVA) [43] or agarose [44] have been proposed for lipid
7 vesicles and PVA was also used for polymer-based vesicles [45]. In this case, the presence of
8 unwanted impurities in the bilayer or lumen cannot be excluded.

9 Emulsion phase (or droplet) transfer has been developed for lipids [46], improved in
10 microfluidic devices [47,48] or special centrifugation set-ups [49–51] (continuous droplet
11 interface crossing encapsulation, i.e. cDICE) and has been rarely used for block copolymers
12 [21,52]. This method is based on a water-in-oil emulsion where water droplets are coated with
13 the amphiphilic species. These droplets are allowed to cross the interface of an oil-on-water
14 biphasic system stabilized by an amphiphilic monolayer, thus forming the vesicles. A known
15 drawback of this method is the presence of residual oil between the lipid leaflets [53]. However,
16 membrane bending rigidity analysis revealed that mineral oil doesn't affect the mechanical
17 properties of the membrane [54].

18 Because hybrid GUVs are essential tools for developing synthetic reactors or biomimetic cells
19 and are so far very limited, in this work we propose to use block copolymers based on
20 methylmethacrylate (MMA) and N,N-dimethylaminoethyl methacrylate (DMAEMA) mixed
21 with palmitoyl oleyl phosphatidyl choline (POPC) in order to obtain giant hybrid vesicles. The
22 presence of pH-sensitive DMAEMA unit could provide the access to systems with pH-
23 responsive permeability. In the literature MMA/DMAEMA based block-copolymers have been

1 mainly used to develop nano-sized polymersomes. Polymeric nano-vesicles based on butyl-
2 methacrylate (BMA) and DMAEMA were developed as reactors for the urea-urease system, for
3 example [55]. We recently showed that the copolymer poly(ethylene glycol monomethyl ether)-
4 *block*- poly(methyl methacrylate –*random*- N,N-dimethyl amino ethyl methacrylate), mPEG-
5 *block*-P(MMA-*ran*-DMAEMA), was able to form nano-vesicles in a wide range of DMAEMA
6 chemical compositions and architectures (linear and branched) [56]. Furthermore, they exhibited
7 a pH-dependent swelling characterized by a strong increase in size, up to 10 times passing from
8 pH 7.4 to pH 4.4. This swelling was attributed to electrostatic repulsions at low pH, linked to the
9 concurrent increase of protonated DMAEMA units.

10 As for giant vesicles, which were not necessarily unilamellar ones, poly(methacrylic acid)-
11 *block*-poly(methylmethacrylate-*random*-methacrylic acid), PMAA-*block*-P(MMA-*ran*-MAA)
12 could form giant vesicles in a photo-polymerization induced self-assembly process in an aqueous
13 methanol solution [57,58]. The authors investigated the pH-responsive behaviour of the vesicles
14 showing that they were disrupted in basic environment (pH 12) and they could be reversibly
15 reconstructed at neutral pH. Yoshida also characterized polymeric giant vesicles with
16 copolymers containing DMAEMA units inserted in the P(MMA-*ran*-MAA) block [59]. These
17 vesicles were unstable and had many holes on the surface. When quaternized DMAEMA was
18 used the vesicles were stable, but they had much smaller size (below 1 μm).

19 Hybrid GUVs made of poly(cholesteryl methacrylate)-*block*-poly(2-(dimethylaminoethyl
20 methacrylate) (pCMA-*block*-pDMAEMA) were characterized with confocal microscopy by W.
21 Zong et al. [16] to confirm the presence of both phospholipids and the block copolymer in the
22 same membrane, pCMA constitutes the hydrophobic block, while the pH-sensitive pDMAEMA
23 constitutes the hydrophilic block. These GUVs were electroformed and the maximum polymer

1 content was 70% w/w (corresponding to 3.6 mol % using $M_n=47.5$ kD as reported in Table 1 of
2 the cited work). Also, hybrid nano-vesicles were formed by this copolymer mixed with
3 phospholipids containing palmitoyl and oleyl as fatty acyl groups and different charged
4 headgroups (i.e. phosphatidylcholine, ethylphosphocholine and phosphatidylserine) showed a
5 prevalent cytosolic localization when incubated with mouse macrophages, indicating a good
6 potential for applications in drug delivery [16].

7 In this paper, we want to show that a block copolymer with high molecular weight and high T_g
8 can be successfully incorporated into hybrid giant vesicles. We propose to use mPEG-*block*-
9 P(MMA-*grad*-DMAEMA) in combination with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-
10 phosphocholine (POPC) to prepare vesicles by means of two formation methods, namely
11 electroformation and droplet transfer; it will be shown that the high T_g polymer diminishes the
12 efficiency of the electroformation method even at low (~ 15 mol %) polymer content, whilst the
13 droplet transfer method allows the formation of hybrid vesicles for a wide range of polymer/lipid
14 ratios. With this approach, the polymer content in the hybrid GUVs has been sensibly increased
15 with respect to that already reported in the literature (~ 4 mol %).

16 2. MATERIALS AND METHODS

17 *Materials*

18 Copper bromide (CuBr_2), 2,2'-bipyridine (bpy), tin (II) 2-ethylhexanoate ($\text{Sn}(\text{EH})_2$), 2,4-
19 dimethyl-3-ethylpyrrole, trifluoroacetic acid (TFA), dichlorodicyanoquinone (DDQ),
20 triethylamine (Et_3N), boron trifluoride etherate ($\text{BF}_3\cdot\text{Et}_2\text{O}$), butylated hydroxytoluene (BHT),
21 basic alumina, Sephadex LH-20, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, Mineral oil
22 M5904, toluene, methanol, dimethylformamide (DMF), n-hexane, poly(ethylene glycol)

1 monomethylether (mPEG, $M_n = 2000 \text{ gmol}^{-1}$, $M_w/M_n = 1.16$ and $M_n = 5000 \text{ gmol}^{-1}$, $M_w/M_n =$
2 1.02) and pyranine were purchased from Sigma-Aldrich and used without further purification.
3 18:1 Liss Rhodamine PE was used as received by Avanti Polar Lipids. Methylmethacrylate
4 (MMA) and N,N-(dimethylamino)ethylmethacrylate (DMAEMA) (Sigma-Aldrich) were purified
5 prior copolymerizations by means of a column filled with basic alumina to remove the inhibitors.
6 Dichloromethane (Sigma-Aldrich) used for BODIPY synthesis was distilled over CaCl_2 .

7 All manipulations involving air-sensitive compounds were carried out under nitrogen
8 atmosphere using Schlenk techniques.

9 *Synthesis of the fluorescent-labeled DMAEMA monomer 2,6-diethyl-1,3,5,7-tetramethyl-8-[4-*
10 *[8-(2-methacryloyloxyethyl)dimethylammoniumbromideoctyloxy]phenyl]-4,4'-difluoroboradiaza*
11 *indacene (BODIPY-DMAEMA)*

12 As a first step, the precursor 2,6-diethyl-1,3,5,7-tetramethyl-8-[4-(8-bromooctyloxy)phenyl]-4,4'-
13 difluoroboradiaza indacene was synthesized. 4-(8-bromooctyloxy)benzaldehyde was prepared as
14 previously described [60]. 720 μL of 4-(8-bromooctyloxy)benzaldehyde (2.3 mmol) and 756 μL
15 of 2,4-dimethyl-3-ethylpyrrole (5.6 mmol) were then dissolved in 50 mL of absolute CH_2Cl_2
16 under N_2 atmosphere, ten drops from a Pasteur pipette of TFA were added and the solution was
17 stirred at RT overnight or until TLC analysis showed complete consumption of the aldehyde. At
18 this time, 785 mg of DDQ (3.45 mmol) were added and stirring continued for 20 min. Then, 5 mL
19 of Et_3N and 5 mL of $\text{BF}_3 \cdot \text{OEt}_2$ were added. The mixture was stirred for 12 h and the organic layer
20 containing the crude product was subsequently washed three times with water; the organic
21 solution was dried over Na_2SO_4 , and evaporated to dryness. The raw material was purified by
22 chromatography (SiO_2 , petroleum ether - CH_2Cl_2 , 1:1) to afford 246 mg of product (yield: 18.2%)

1 in the form of orange needles. $C_{31}H_{42}BBrF_2N_2O$, MM = 587.39 $g\text{mol}^{-1}$, UV-vis (CH_2Cl_2): 528 nm
2 ($\epsilon = 92600\text{ M}^{-1}\text{ cm}^{-1}$). Quantum efficiency of fluorescence Φ_{fluo} (in CH_2Cl_2): 0.45 (544 nm) was
3 calculated according to the following equation:

$$4 \quad \Phi_{\text{fluo}}^{\text{sample}} = \Phi_{\text{fluo}}^{\text{standard}} \times (I_{\text{fluo}}^{\text{sample}} / I_{\text{fluo}}^{\text{standard}}) \times (Abs^{\text{standard}} / Abs^{\text{sample}})$$

5 where I_{fluo} is the fluorescence intensity at the specific excitation wavelength, Abs denotes the
6 absorbance at the excitation wavelength.

7 1H NMR ($CDCl_3$) δ : 1.00 (t, 6H, $2 \times CH_3$); 1.46 (s, 6H, $2 \times CH_3$); 1.45-1.55 (m, 8H, $4 \times CH_2$);
8 1.82-1.93 (m, 4H, $2 \times CH_2$); 2.32 (q, 4H, $2 \times CH_2$); 2.58 (s, 6H, $2 \times CH_3$); 3.45 (t, 2H, CH_2Br); 4.03
9 (t, 2H, CH_2O); 6.00 (s, 2H, $2 \times CH$); 7.02 (d, 2H, $2 \times CH$); 7.18 (d, 2H, $2 \times CH$).

10 To obtain the fluorescent-labeled DMAEMA monomer, 10 mg (0.045 mmol) of 2,6-diterbutyl-
11 4-methylphenol (BHT) were added to a mixture of 236 mg of 2,6-diethyl-1,3,5,7-tetramethyl-8-
12 [4-(8-bromooctyloxy)phenyl]-4,4'-difluoroboradiazindacene (0.402 mmol) in 3 mL of
13 DMAEMA (17.75 mmol), reaction scheme and chemical structures are reported in Figure S1 of
14 the supporting information. The mixture was kept at 70 $^{\circ}C$ for 24 h. During the reaction time, a
15 solid product was formed. After filtration, the product was washed 4 times with 20 mL of diethyl
16 ether affording 214 mg (71.4%) of product. $C_{39}H_{57}BBrF_2N_3O_3$, MM = 744.60 $g\text{mol}^{-1}$ (ESI-MS
17 spectrum is reported in Figure S2 of the supporting information). 1H NMR ($CDCl_3$) δ : 0.97 (t,
18 6H, $2 \times CH_3$); 1.33 (s, 6H, $2 \times CH_3$); 1.40 (m, 8H, $4 \times CH_2$); 1.80 (m, 4H, $2 \times CH_2$); 1.95 (s, 3H,
19 CH_3), 2.25-2.33 (q, 4H, $2 \times CH_2$); 2.51 (s, 6H, $2 \times CH_3$); 3.56 (s, 6H, $2 \times CH_3$); 3.61-3.65 (t, 2H,
20 CH_2); 3.97-4.02 (t, 2H, CH_2); 4.15 (t, 2H, CH_2); 4.65 (t, 2H, CH_2); 5.68 (s, 1H, CH); 6.15 (s, 1H,
21 CH); 6.96-6.99 (d, 2H, $2 \times CH$); 7.13-7.15 (d, 2H, $2 \times CH$). Details of 1H peak assignment and
22 HSQC-NMR spectra are reported in Figures S3 – S5 of the supporting information.

23 *Synthesis of mPEG-block-P(MMA-grad-DMAEMA) copolymers.*

1 Macroinitiator mPEG-Br was synthesized according to the procedure reported in literature. [61]
 2 In a typical procedure, the copolymerization of MMA/DMAEMA was carried out in a 50 mL
 3 glass flask charged, under nitrogen atmosphere, with 0.1 g of mPEG-Br macroinitiator in 5 mL
 4 of toluene. Subsequently, 100 μ L of CuBr₂ (1×10^{-3} M in DMF, 10^{-4} mmol), 100 μ L of bpy ($1 \times$
 5 10^{-2} M in DMF, 10^{-3} mmol), 100 μ L of Sn(EH)₂ (1×10^{-2} M in toluene, 10^{-3} mmol), 2-4 mL of
 6 MMA (18.8-37.6- mmol) and 1.2 – 2.5 mL of DMAEMA (7.10 – 14.8 mmol) were added (the
 7 volumes of MMA and DMAEMA were varied to change the final composition in the
 8 copolymer). The mixture was thermostated at 60 °C and magnetically stirred. The reaction was
 9 stopped after 4h by adding *n*-hexane. The copolymer was recovered by filtration, washed with
 10 cold methanol (~ 4 °C) and dried in vacuum at 40 °C. ¹H-NMR (400 MHz, CDCl₃): δ 0.87-1.03
 11 (-CH₃ main chain), 1.83-1.91 (-CH₂- main chain), 2.30 (-N(CH₃)₂), 2.58 (-OCH₂ CH₂ N(CH₃)₂),
 12 3.61 (-OCH₃), 3.66 (-OCH₂CH₂-), 4.08 (-OCH₂ CH₂ N(CH₃)₂). ¹³C-NMR(400 MHz, CDCl₃): δ
 13 16.9-19.1 (-CH₃ main chain), 44.9-45.3 (quaternary carbon in the main chain), 46.2 (-N(CH₃)₂),
 14 52.2 (-OCH₃, MMA), 54.6 (-CH₂- main chain), 57.6 (-OCH₂ CH₂ N(CH₃)₂), 63.5 (-OCH₂ CH₂
 15 N(CH₃)₂), 70.9 (-OCH₂ CH₂-), 176.3-178.2 (-C=O). Details of ¹H peak assignment and ¹³C NMR
 16 spectra are reported in Figure S6 of the supporting information.

17 Chemical composition and the molecular weight (M_n) were evaluated via ¹³C NMR. The molar
 18 fractions of the components in the final copolymers were evaluated through the equations:

$$19 \quad X_{\text{mPEG}} = \frac{I_{\text{mPEG}}}{I_{\text{mPEG}} + 2I_{\text{MMA}} + I_{\text{DMAEMA}}} \quad (1)$$

$$20 \quad X_{\text{MMA}} = \frac{2I_{\text{MMA}}}{I_{\text{mPEG}} + 2I_{\text{MMA}} + I_{\text{DMAEMA}}} \quad (2)$$

$$X_{\text{DMAEMA}} = \frac{I_{\text{DMAEMA}}}{I_{\text{mPEG}} + 2I_{\text{MMA}} + I_{\text{DMAEMA}}} \quad (3)$$

where I_{mPEG} is the integration of the signal relative to mPEG units: $-\text{OCH}_2\text{CH}_2-$, I_{MMA} is the signal corresponding to the units $-\text{OCH}_3$ of MMA and I_{DMAEMA} integrates the two carbons of the amine group of DMAEMA ($-\text{N}(\text{CH}_3)_2$). M_n was calculated from the following equation:

$$M_n = \text{DP}_{\text{mPEG}} \text{MM}(\text{EO}) + \text{DP}_{\text{MMA}} \text{MM}(\text{MMA}) + \text{DP}_{\text{DMAEMA}} \text{MM}(\text{DMAEMA}) \quad (4)$$

where:

$$\text{DP}_{\text{mPEG}} = M_{\text{nPEG}}/44$$

$$\text{DP}_{\text{MMA}} = \text{DP}_{\text{mPEG}} (2I_{\text{MMA}}/I_{\text{mPEG}})$$

$$\text{DP}_{\text{DMAEMA}} = \text{DP}_{\text{mPEG}} (I_{\text{DMAEMA}}/I_{\text{mPEG}})$$

The synthesis of the fluorescent-labelled copolymer was carried out under the same experimental conditions described above by adding 2 mL of MMA (18.8 mmol), 1.2 mL of DMAEMA (7.10 mmol), 2 mL of DMF and 5 mg of the fluorescent monomer BODIPY-DMAEMA (6.7×10^{-3} mmol) to the reaction mixture. The applied procedure of purification [62,63] was based on the use of lipophilic Sephadex LH-20 to separate molecules with different molecular weights. A mini column (Pasteur pipette) was loaded with the lipophilic stationary phase (~ 1.5 g of Sephadex previously swollen for one hour in 10 mL of methanol) and filled with methanol to elute the samples. $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 0.87-1.04 ($-\text{CH}_3$ main chain), 1.27 ppm (6 CH_2 of the alkyl chain of the BODIPY-DMAEMA), 1.83-1.91 ($-\text{CH}_2-$ main chain), 2.30 ($-\text{N}(\text{CH}_3)_2$), 2.59 ($-\text{OCH}_2 \text{CH}_2 \text{N}(\text{CH}_3)_2$), 3.61 ($-\text{OCH}_3$), 3.65 ($-\text{OCH}_2\text{CH}_2-$), 4.08 ($-\text{OCH}_2 \text{CH}_2 \text{N}(\text{CH}_3)_2$). $^{13}\text{C-NMR}$ (400 MHz, CDCl_3): δ 16.5-18.7 ($-\text{CH}_3$ main chain), 29.7 ppm (6 CH_2 of the

1 alkyl chain of the BODIPYDMAEMA), 44.6-44.9 (quaternary carbon in the main chain), 45.8 (-
 2 N(CH₃)₂), 51.8 (-OCH₃, MMA), 54.4 (-CH₂- main chain), 57.2 (-OCH₂ CH₂ N(CH₃)₂), 63.1 (-
 3 OCH₂CH₂ N(CH₃)₂), 70.6 (-OCH₂CH₂-), 176.3-178.2 (-C=O). Details of ¹H peak assignment,
 4 ¹³C NMR and HSQC-NMR spectra are reported in Figures S7 and S8 of the supporting
 5 information.

6 Chemical composition and the molecular weight (M_n) were evaluated via ¹³C NMR through
 7 the equations:

$$8 \quad X_{\text{mPEG}} = \frac{I_{\text{mPEG}}}{I_{\text{mPEG}} + 2I_{\text{MMA}} + I_{\text{DMAEMA}} + \frac{I_{\text{BODIPYDMAEMA}}}{2}} \quad (5)$$

$$9 \quad X_{\text{MMA}} = \frac{2I_{\text{MMA}}}{I_{\text{mPEG}} + 2I_{\text{MMA}} + I_{\text{DMAEMA}} + \frac{I_{\text{BODIPYDMAEMA}}}{2}} \quad (6)$$

$$10 \quad X_{\text{DMAEMA}} = \frac{I_{\text{DMAEMA}}}{I_{\text{mPEG}} + 2I_{\text{MMA}} + I_{\text{DMAEMA}} + \frac{I_{\text{BODIPYDMAEMA}}}{2}} \quad (7)$$

$$11 \quad X_{\text{BODIPYDMAEMA}} = \frac{\frac{I_{\text{BODIPYDMAEMA}}}{2}}{I_{\text{mPEG}} + 2I_{\text{MMA}} + I_{\text{DMAEMA}} + \frac{I_{\text{BODIPYDMAEMA}}}{2}} \quad (8)$$

12 where I_{mPEG} is the integration of the signal relative to mPEG units: -OCH₂CH₂-; I_{MMA} is the
 13 integration of the signal relative to MMA units: -OCH₃; I_{DMAEMA} is the integration of methyl
 14 group of the signal relative to DMAEMA units; $I_{\text{BODIPYDMAEMA}}$ are the 4 CH₂ of the fluorescent
 15 moiety (signals labeled i₁₋₄ in the NMR spectra, figures S7-S8 of the supporting info). The

1 degrees of polymerization and hence the molecular weights were calculated from the following
2 equation:

$$3 \quad M_n = DP_{mPEG} (MM_{EO}) + DP_{MMA} (MM_{MMA}) + \quad (9)$$
$$+ DP_{DMAEMA} (MM_{DMAEMA}) + DP_{BODIPYDMAEMA} (MM_{BODIPYDMAEMA})$$

4 where:

$$5 \quad DP_{mPEG} = M_{n(mPEG)} / 44$$
$$DP_{MMA} = DP_{mPEG} (2I_{MMA} / I_{mPEG})$$
$$DP_{DMAEMA} = DP_{mPEG} (I_{DMAEMA} / I_{mPEG})$$
$$DP_{BODIPYDMAEMA} = DP_{mPEG} (I_{BODIPYDMAEMA} / 2I_{mPEG})$$

6 *NMR Analysis.*

7 ¹H and ¹³C NMR spectra were recorded at 25 °C in CDCl₃ using a Bruker Avance 400 MHz
8 spectrometer (D1 = 5 s for ¹³C NMR). Samples were prepared by introducing 20 mg of
9 copolymer and 0.5 mL of CDCl₃ into a tube (5 mm outer diameter). Chemical shifts (δ) are listed
10 as parts per million: ¹H NMR spectra are referenced using the residual solvent peak at δ = 7.26
11 ppm, in ¹³C NMR spectra the residual solvent peak is at δ = 77.2 ppm.

12

13

14 *DSC.*

15 The glass transition temperatures were measured with Dynamic Scanning Calorimetry (Mettler
16 Toledo DSC1 STAR SYSTEM FRS5). About 10 mg of sample were inserted in a 100 μL
17 aluminum pan and heating/cooling cycles were registered at 20 K/min. In the first run, the

1 sample was heated from 25 °C to 165 °C, followed by cooling to -60 °C. In the other two runs,
2 the sample was heated from -60 °C to 165 °C and cooled from 165 °C to -60 °C. The T_g was
3 measured on the second heating run where the inflection point is generally more evident.

4 *Microscopy.*

5 Images were acquired on an inverted optical microscope (Eurotek Orma INV100TFL) using a
6 20× objective and then analysed with the software ImageJ. The mixed membranes were
7 characterized with a confocal microscope (Leica TCS SP2 and TIRF OLYMPUS FV1000). The
8 fluorescent polymer and the fluorescent lipid were excited respectively at 488 nm, with an Ar
9 laser, and at 561 nm, with a DPSS 561 laser and the fluorescence was collected with
10 photomultipliers tubes PMTs in the wavelength ranges 498 – 530 nm and 571 – 630 nm,
11 respectively.

12 *Preparation of Giant Vesicles.*

13 *Droplet Transfer Method.* Stock solutions of [POPC] = 3 mM (2.28 mg mL⁻¹) and [copolymer]
14 = 5 mg mL⁻¹ (0.36 mM by using the copolymer average molecular weight $M_n = 13850$ g mol⁻¹)
15 were prepared in mineral oil. The fluorescent probes, being less soluble in mineral oil, were
16 dissolved in CHCl₃ at the concentration [fluo-polymer]_{CHCl₃} = 10 mg mL⁻¹ (0.027 mM using an
17 average molecular weight $M_n = 370928$ g mol⁻¹) and [18:1 Liss Rhod PE]_{CHCl₃} = 0.77 mM (1 mg
18 mL⁻¹). Giant Unilamellar vesicles were prepared with the Droplet Transfer Method described by
19 Pautot et al. for lipid-based GUVs [46] using lipid:copolymer molar ratios 93:7, 85:15 and 60:40
20 (corresponding to lipid:copolymer weight ratios 0.42:0.58, 0.24:0.76, 0.08: 0.92 by using the
21 copolymer average molecular weight $M_n = 13850$ g mol⁻¹). An Eppendorf tube was filled with
22 500 μL of an aqueous phase containing 200 mM of glucose (O-solution), then 300 μL of a

1 hydrophobic interfacial phase containing lipids and polymers at various ratios (obtained by
2 mixing the POPC and polymer stock solution) was poured on top of the O-solution. A second
3 Eppendorf tube was used to prepare a water/oil microemulsion: 20 μL of an aqueous solution
4 containing 200 mM of sucrose (inner solution, I-solution), were mixed by pipetting with 600 μL
5 of an oil phase containing the same amount of lipids and polymers as the interfacial phase and 5
6 μL of chloroform solutions of fluorescent probes (chloroform was firstly evaporated in argon
7 stream ~~before~~ and then oil containing other amphiphiles was added). The microemulsion was
8 then poured over the interfacial phase and vesicles were formed by centrifuging the tube at 6000
9 rpm for 10 minutes at room temperature. Vesicles were collected as a pellet at the bottom of the
10 aqueous phase and gently washed with 100 μL of O-solution and finally observed at the
11 microscope. In these experimental conditions, however, pure polymeric vesicles could not be
12 formed. When POPC was not present in the initial oil solution, the precipitation of a drop of
13 coloured polymer (i.e. containing the fluorescent polymeric probe or pyranine when employed)
14 was always detected at the bottom of the Eppendorf tube. Problems might be related with
15 difference in density between the I- and O-solution that could be overcome by employing a
16 different nonpolar solvent or by changing the concentrations of the sugars.

17 *Electroformation.* GUVs were prepared on a *Vesicles prep pro* instrument produced by Nanion
18 through the electroformation method described by *Angelova et al.* [64] for lipid-based giant
19 vesicles. Stock solution of [POPC] = 1.32 mM (1 mg mL⁻¹) and [copolymer] = 1 mg mL⁻¹ (0.072
20 mM if we consider a copolymer average weight of 13850 g/mol) and POPC were prepared at 1
21 mg mL⁻¹ (both polymer and lipid) in chloroform and [copolymer] = 1 mg mL⁻¹ (0.072 mM by
22 using the copolymer average molecular weight $M_n = 13850 \text{ g mol}^{-1}$). For electroformation, they
23 were mixed in different ratios so that the total final concentration of the polymer plus POPC was

1 0.25 mg mL⁻¹ and the total deposited amount was fixed in order to avoid the formation of a thick
2 film. The POPC concentration was 0.136 mM, 0.08 mM and 0.05 mM in the samples with
3 POPC:copolymer molar ratios equal to 93:7, 85:15 and 70:30 respectively (corresponding to
4 lipid:copolymer weight ratios 0.42:0.58, 0.24: 0.76, 0.11: 0.89 by using the copolymer average
5 molecular weight $M_n = 13850 \text{ g mol}^{-1}$).

6 5 μL of the obtained chloroform solutions of the polymer and lipid at different molar ratios
7 were spread onto ITO-coated glass slide and dried under vacuum for at least 3 hours. The dry
8 film was surrounded with a 1 mm O-ring in order to delimit the electroformation chamber which
9 was subsequently filled with 250 μL of a 240 mM sucrose solution. Another ITO-coated glass
10 slide was used to close the chamber, which was further connected to an alternate voltage
11 generator. A peak-to-peak voltage of 3 V and a frequency of 10 Hz were applied at 65 °C for at
12 least 2 hours to form the GUVs. The temperature and the voltage were then slowly decreased and
13 the vesicles were collected with a pipette.

14 3. RESULTS AND DISCUSSION

15 A mandatory condition that has to be satisfied to control the formation, the stability and the
16 characteristics of mixed polymer/lipid vesicles is the similarity of the solubility parameter (δ)
17 [65,66] of the phospholipids' fatty acids chains with the hydrophobic part of the amphiphilic
18 synthetic copolymer. In this respect, the comparable values of PMMA ($\delta = 18.7 \text{ MPa}^{1/2}$ [67])
19 with that of the fatty acid chains ($\delta = 18.2 \text{ MPa}^{1/2}$ [20]), allows us to consider as good the
20 compatibility between an amphiphilic block copolymer containing PMMA and the phospholipid
21 chosen for the formation of the hybrid vesicles (POPC).

1 However, a rational design in terms of molecular weight, composition and
2 hydrophilic/hydrophobic ratio is also needed since one of the main issue for the formation of
3 hybrid vesicles also includes the similitude or the discrepancy of the size of the hydrophobic
4 segments in the copolymers and phospholipids, other than a proper glass transition temperature
5 (T_g) of the synthetic copolymer [20].

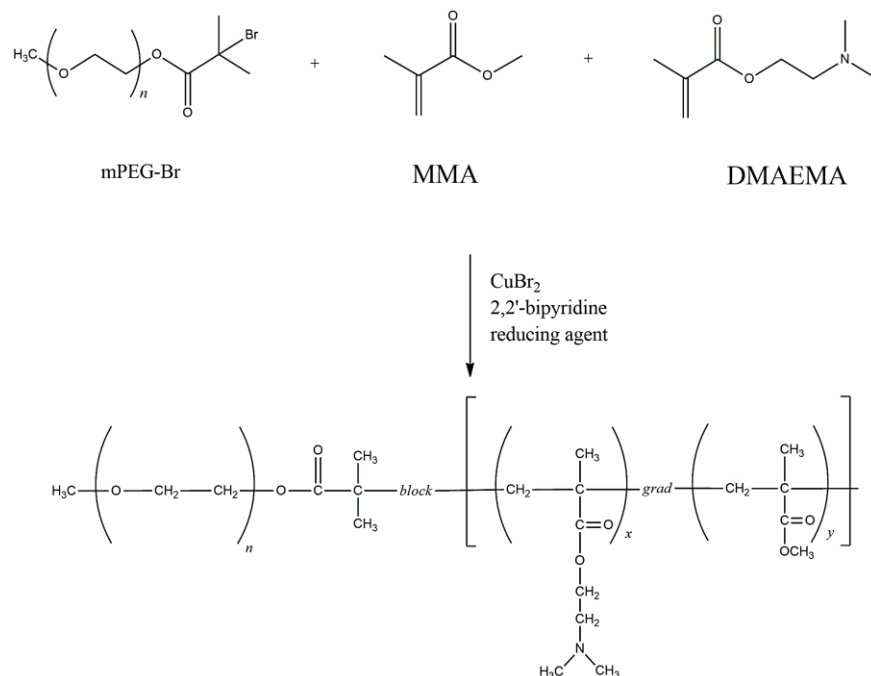
6 To this end, several MMA-based block copolymers having different properties (M_n ,
7 Hydrophilic-Lipophilic Balance HLB, etc.) were synthesized by a modified version of the
8 ATRP, namely the Activators ReGenerated by Electron Transfer ATRP (ARGET-ATRP)
9 [68,69].

10 The copolymers have a linear, *A-block*-(BC), architecture and consist of a hydrophilic block
11 (A) based on poly(ethylene glycol) monomethylether (mPEG), and a hydrophobic block (BC)
12 made of a copolymer chain containing methyl methacrylate (MMA, B) and N,N-
13 (dimethylamino)ethyl methacrylate (DMAEMA, C) as represented in Figure 1.

14 The DMAEMA monomer was introduced both to modulate the PMMA T_g , and to give
15 potential pH-sensitivity for further applications to the hybrid vesicles [56,70–72]. However,
16 though the chain based on MMA/DMAEMA has an increasing hydrophilicity with decreasing
17 pH, at pHs ranging from neutrality to basic conditions, the hydrophobicity, and consequently the
18 δ value, remains compatible with the POPC fatty acid chain.

19 Copolymers were thus synthesized using mPEG-Br ($M_n = 2000 \text{ g mol}^{-1}$ and $M_n = 5000 \text{ g mol}^{-1}$)
20 as macroinitiator in presence of CuBr_2/bpy and $\text{Sn}(\text{EH})_2$ as reducing agent in toluene at $60 \text{ }^\circ\text{C}$
21 (Figure 1). The experimental conditions, meaning relative amount of MMA and DMAEMA in

- 1 the feed, were systematically changed in order to find the most suitable microstructure for the
 2 formation of mixed vesicles with POPC.



3
 4 **Figure 1. Reaction scheme for the synthesis of mPEG-*block*-P(MMA-*grad*-DMAEMA) copolymers**

5 The large difference in the reactivity ratios found between MMA and DMAEMA
 6 suggested that the “BC” block is characterized by a *gradient-like* composition with the initial
 7 part of the chain (i.e. the one closer to the mPEG block) richer in the monomer DMAEMA, that
 8 smoothly changes towards a prevalent MMA composition [73]. This microstructure in principle
 9 could favour the formation of blended vesicles allowing the insertion of the hydrophobic moiety
 10 of the copolymer within the phospholipid carbon chains. The characteristics of all copolymers
 11 synthesized are reported in Table 1.

12 **Table 1. Chemical composition, molar masses, HLB and T_g of the copolymers synthesized**

Sample	M_n mPEG	X_{mPEG} (mol)	X_{MMA} (mol)	X_{DMAEMA} (mol)	M_n^a (kg mol^{-1})	HLB ^b	T_g^c ($^{\circ}\text{C}$)
--------	------------	----------------------------	---------------------------	------------------------------	-------------------------------------	------------------	-----------------------------------

1	2000	0.24	0.76	---	16.3	2.45	97
2	5000	0.14	0.51	0.35	62	1.61	85
3	5000	0.44	0.35	0.21	23	4.35	50
4	5000	0.50	0.27	0.23	19.4	5.15	55
5	2000	0.34	0.26	0.40	13.8	2.90	44

1 ^a Determined by ¹³C-NMR using equation (4)

2 ^b Calculated by Griffin method: $HLB = 20(M_h/M)$ where M_h is the molar mass of the hydrophilic portion (mPEG)
3 and M is the molar mass of the copolymer

4 ^c heating rate 20 K/min

5

6 Considering that the T_g of the homopolymers is ~ -65 °C for PEG, ~ 105 °C for PMMA
7 and ~ 20 °C for PDMAEMA, data reported in Table 1 show that the lower is the content of
8 MMA and the value of molecular weight (e.g. samples 3 – 5), the lower is the T_g of the
9 corresponding copolymer. Among the copolymers in Table 1, we selected the one with a molar
10 composition of 34% – 26% – 40% of mPEG – MMA – DMAEMA, respectively and $M_n = 13.8$
11 kg mol^{-1} (sample 5) to produce hybrid GUVs using the electroformation method. This copolymer
12 has the lowest T_g and average molecular weight together with a HLB (hydrophilic-lipophilic
13 balance) value similar to the one of POPC (3.98), it then appears the most suitable for the
14 preparation of hybrid vesicles. It is reasonable that the HLB value together with a low steric
15 mismatch between copolymers and POPC molecular weight should ensure a major compatibility
16 with the lipid. Besides, as it has the lowest T_g value is the best candidate for electroformation
17 where the higher the polymer chain mobility the higher the yield of vesicle formation.

18 Giant vesicles, containing different percentages of lipid and copolymer were produced by
19 using two different processes: an electroformation and a phase transfer method.

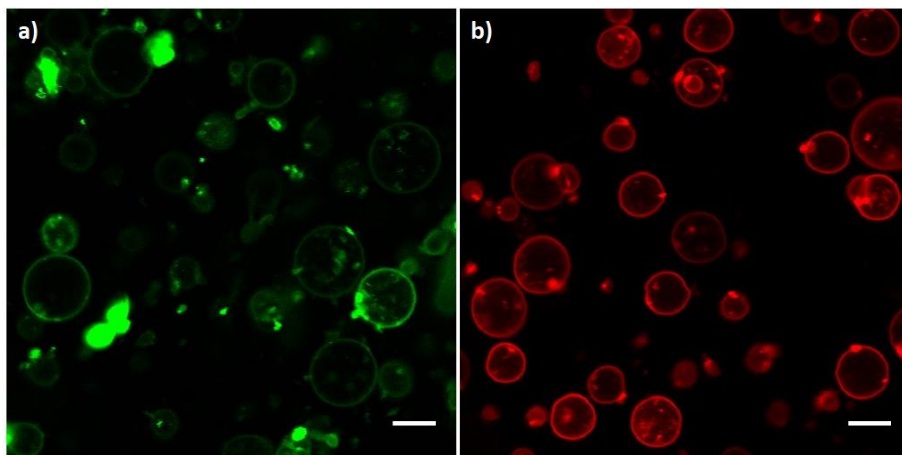
20 As copolymer 5 did not form pure GUVs by electroformation, we decided to explore
21 compositions with high molar amount of POPC. A large number of electroformed POPC giant

1 vesicles could be easily obtained within 30 minutes at room temperature, as reported in Figure
2 S9 a) of the Supporting Information. Even a small amount of copolymer (< 5 mol%) added to
3 POPC inhibited the vesicle formation at 25 °C and the temperature had to be increased till 65 °C
4 in order to be able to obtain a few vesicles after 2 hours, as shown in Figure S9 b) in the
5 Supporting Information. The maximum initial copolymer content clearly allowing vesicles
6 formation was 15 mol%, though the typical microscopy images showed few electroformed
7 aggregated vesicles (often containing solid residues of presumably precipitated polymers and/or
8 lipids), which hardly detached from the film. In the presence of higher initial percentage of
9 polymer, only scant irregular objects could be detected.

10 These results clearly show that the presence of the polymer, even at low percentage (< 15
11 mol%), with a high glass transition temperature hinders POPC vesicles electroformation. For this
12 reason we decided to use the phase transfer method, developed for giant liposomes and
13 previously employed for the synthesis of asymmetric hybrid polymer-lipid giant vesicles [21].
14 With this method, the same amount of copolymer and lipid was dispersed in both the interfacial
15 phase and in the emulsion in order to favour a symmetric composition of the two leaflets of the
16 bilayer. In order to get insight into the nature of the formed vesicles, two fluorescent probes were
17 employed to highlight the possible partitioning of the two different components, lipid and
18 copolymer, within the hybrid GUVs. A fluorescent lipid, 18:1 Liss Rhod PE, known for its
19 affinity for the liquid disordered domains in phase separated model membranes [74] and also
20 used as a marker for the lipid-rich region in hybrid vesicles [75–78], was chosen. To stain the
21 polymer-rich domains, a modified DMAEMA with a pendant group consisting of a BODIPY-
22 based fluorescent dye was synthesized and used to obtain a labeled mPEG-*block*-P(MMA-*grad*-
23 DMAEMA) copolymer (composition: mPEG = 4.4%, MMA = 51%, DMAEMA = 38%,

1 BODIPY = 6.2%, indicated as BODIPY-copolymer). The chemical structure of this BODIPY-
2 copolymer should guarantee a preferential partition of the probe in the polymer-rich region. The
3 affinity between the BODIPY-copolymer probe and the copolymer 5 has been checked by DSC
4 measurements (Figure S10 in the Supporting Information). The presence of a single T_g transition,
5 different from the ones of neat copolymers, clearly indicates that they are molecularly miscible.
6 On the other hand, blends of the BODIPY-copolymer and POPC did not show changes in phase
7 transitions (Figure S11 in the Supporting Information).

8 Because of the difficulties related to the phase transfer method in determining the final
9 composition of vesicles [79–81], the percentages of lipids and polymers reported hereon refer to
10 the initial amount of the components in the solutions prior the centrifugation phase. In a first
11 step, lipid vesicles stained with the fluorescent dye were prepared at pH ~ 7 to characterize the
12 absorption and emission spectra useful for the observation at the confocal microscope. Figure 2
13 a) and b) show confocal images, respectively, at the characteristic emission wavelengths for the
14 fluorescence of BODIPY ($498 < \lambda < 530$ nm) and for 18:1 Liss Rhod PE ($571 < \lambda < 630$ nm),
15 when blended in the double layer of POPC vesicles. Figure 2 a) clearly shows that a very low
16 amount of the BODIPY-copolymer = 0.02 mol% with respect to the total amount of amphiphiles,
17 can be homogeneously dispersed into the POPC phospholipid membranes and confirms that the
18 solubility parameter of the synthesized copolymer is close to that of pure PMMA.

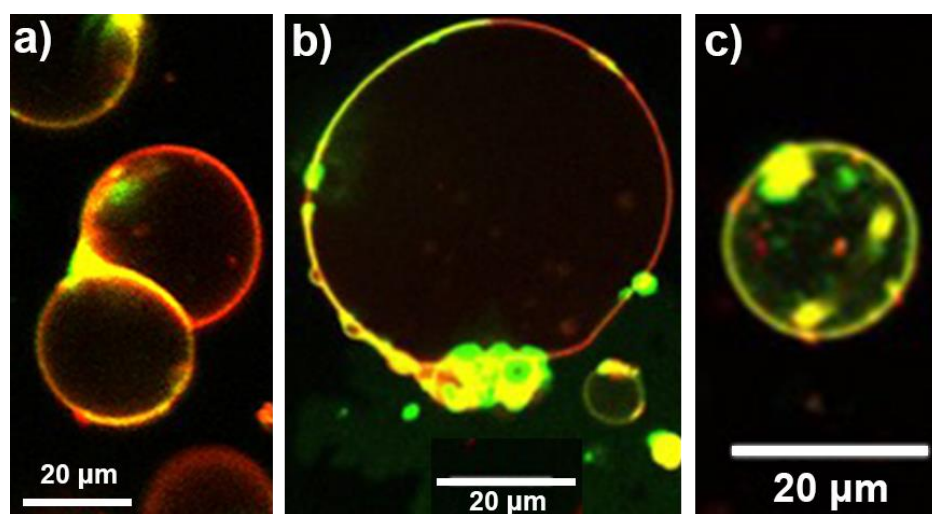


1
2 **Figure 2** POPC vesicles labelled with fluorescent probes. a) POPC and BODIPY-copolymer 99.98 %: 0.02 %;
3 b) POPC and 18:1 Liss Rhod PE 99 %: 0.8%. Small round spots in a) and b) are probably due to mineral oil
4 residues. White bar scales 10 μm .

5 Mixed vesicles were successfully prepared at $\text{pH} = 7$, where 50% of the copolymer is
6 protonated ($\text{pK}_a \sim 7$, see Figure S12 in Supporting Information), by varying the unlabeled
7 copolymer mole percentage in the range 8 – 40 % (58 – 89 wt%), higher concentration of
8 copolymer hindered the self-assembly of the amphiphiles and the formation of a pellet at the end
9 of the phase transfer procedure was not obtained. Furthermore, we could not observe the
10 formation of pure polymer GUVs.

11 Vesicles with the compositions mol % POPC – mol % copolymer 5: 92% – 8%, 85% – 15%,
12 60% – 40% labeled with 18:1 Liss Rhod PE and BODIPY-copolymer are shown in Figure 3 a) –
13 c), respectively. In each figure, the green channel represents the fluorescence of the copolymer,
14 the red channel corresponds to the fluorescence of the lipid and the yellow channel represents
15 their overlap. When the concentration of the copolymer in the vesicles was below ~ 20 mol% the
16 mixed membranes were mainly heterogeneous, with small regions where copolymer blended into
17 the lipid layers. Interestingly, the presence of the copolymer in the membranes promoted the

1 fusion of single vesicles into budded structures, as shown in Figure 3 a), or large aggregates of
2 fused vesicles. The welding surface between vesicles was indeed characterized by a large
3 concentration of copolymer, highlighted by the intense yellow colour, which probably favours
4 electrostatic interaction between the outer leaflets of the membranes, as previously observed in
5 the presence of charged fatty acids incorporated in the double layer of POPC vesicles [82,83].



6
7 **Figure 3 Mixed POPC- copolymer vesicles having molar composition: a) 92% – 8%; b) 85% – 15%; c) 60% –**
8 **40%. All systems were stained with 18:1 Liss Rhod PE (0.8%) and BODIPY-copolymer (0.02%). More**
9 **pictures are reported in the Supporting Information (Figure S13).**

10 When the percentage of copolymer increased, the length of the domains where it was present
11 seemed to increase accordingly (Figure 3 b), until a homogeneous distribution of lipids and
12 copolymers in the membrane was attained when the concentration of latter was around 40 mol%,
13 as shown in Figure 3 c). It is worth noting that mixed vesicles were obtained in presence of the
14 same amount of Liss Rhod PE (0.8%) and BODIPY-copolymer (0.02%) for all the investigated
15 compositions. At very low concentration of copolymer 5 (8%), image 3 a) shows the prevailing
16 presence of POPC as stained by Liss Rhod PE while, increasing the copolymer amount, the
17 images 3 b) and 3 c) show an increase in homogeneity of the membrane with consequent

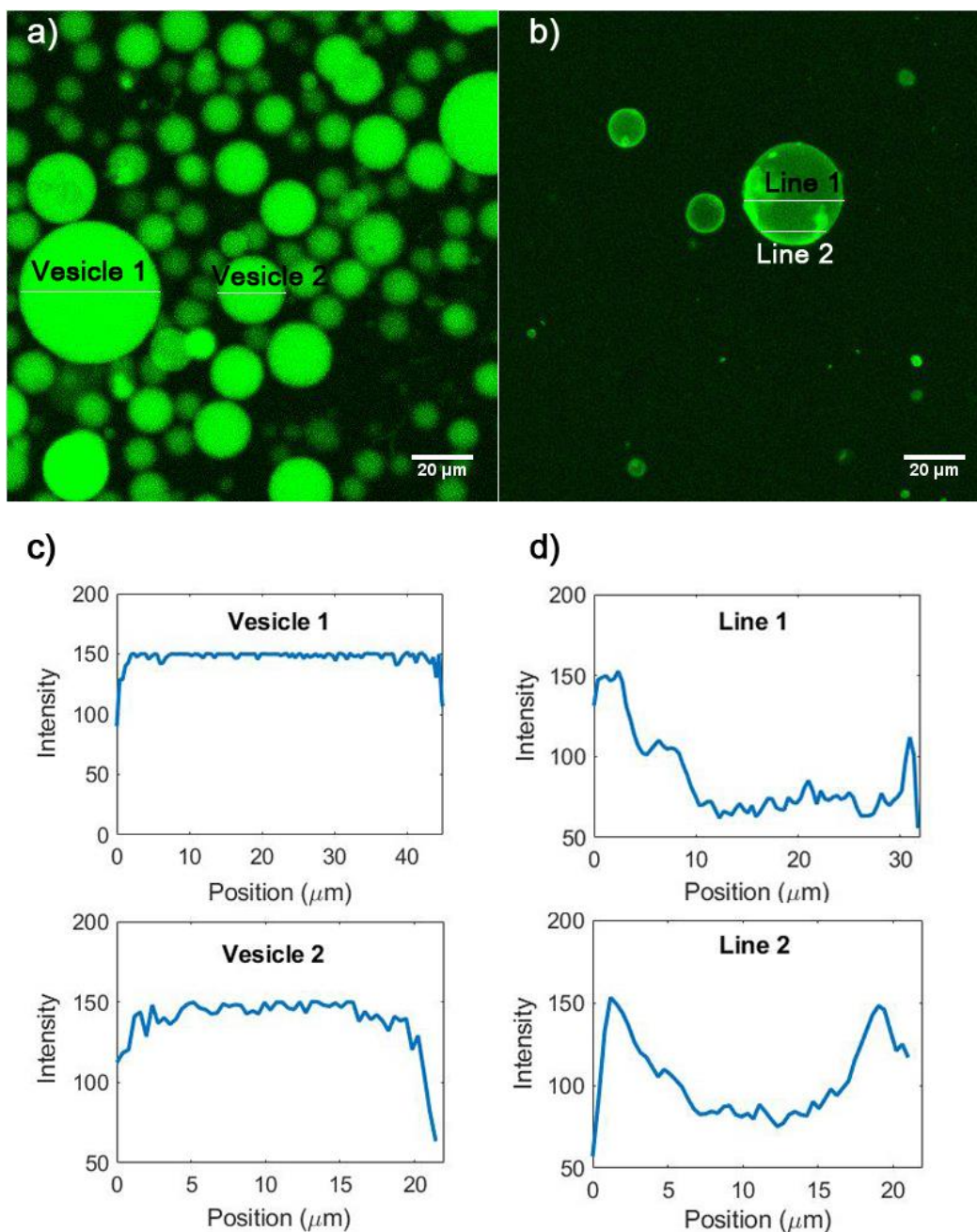
1 overlapping of both the probes. The increasing spreading of BODIPY-copolymer in the
2 membrane, despite its fixed concentration, demonstrates both the presence of the copolymer into
3 the membrane and the prevailing affinity of the fluorescent copolymer for copolymer-rich
4 regions. Although the membrane composition became more homogeneous at higher copolymer
5 percentages, the total number of stable vesicles detected in the samples drastically diminished,
6 until amphiphilic structures could not be observed at copolymer amount $> \sim 40$ mol%. The
7 concentration dependence of both the lipid/copolymer distribution in the membrane and the
8 successful formation of vesicles might depend on the complex attractive interactions taking place
9 between the two molecules, as observed for a DPPC/PIB₃₇-b-PEO₄₈ system [32].

10 It is interesting to compare our results with those previously reported in the literature
11 concerning POPC-based giant vesicles blended with PBD or PDMS. POPC is a lipid in the fluid
12 state at room temperature and in the case of PBD [13,31], homogeneous hybrid GUVs are
13 formed in a restricted range of composition (i.e. in the region of low and high mole polymer
14 content). In these homogeneous vesicles lipid molecules are dispersed in a “sea” of copolymers
15 and vice versa. This situation is the most energetically favoured. Indeed, apart from the chemical
16 affinity, also the structural compatibility has to be taken into account when dealing with
17 lipid/copolymer GUVs. The thickness of polymer bilayers varies between 5 and 50 nm, while the
18 lipid bilayer is 3-4 nm [20,84], i.e. there is a so called hydrophobic mismatch. As a consequence
19 of this hydrophobic mismatch, in phase separated systems, the copolymer has to distort in order
20 to adjust to the lipid bilayer and avoid as much as possible the contacts with water. This has a
21 high energy cost corresponding to high line tension at lipid-polymer boundary. Then, domain
22 formation is most of the time energetically disfavoured. Interestingly in the case of PDMS
23 triblock copolymers [29], hybrid GUVs were formed in all the weight concentration range and

1 were homogeneous in the polymer-rich region (till 85 wt % polymer content for 1.5k
2 copolymers, 50 wt% for 3k copolymers), while domain formation was observed at lower
3 polymer content. This phase separation was accompanied by budding and the phase-separated,
4 budded vesicles were not stable and budding degenerated in vesicle fission in the lipid rich
5 region. For molecular weight as high as 5k stable phase separated vesicles could not be observed
6 and pure lipid or pure polymer vesicles were present together with homogeneous structures.
7 Budding phenomena can evolve in fission when the line tension at boundary is higher than the
8 curvature energy associated to bending. The authors concluded that line tension at the lipid-
9 polymer boundary were too high in the case of the 5k copolymers. Besides, the authors stressed
10 that block copolymers with the same molecular weight showed even higher instability towards
11 fission than the triblock structures. As observed for PDMS based copolymers, the copolymer
12 presented here forms homogeneous GUVs at high polymer content. On the contrary domain
13 formation is observed without fission between 24 w % and 42 w % POPC despite their higher
14 hydrophobic mismatch (13.8k compared to 5k in the literature). At these compositions the
15 bending costs associated with budding and fission are still higher than the boundary line energy,
16 so phase separation is still energetically favoured to fission. We speculate that our polymer has a
17 higher T_g than PDMS based ones, and consequently a higher curvature energy.

18 In a different set of experiments, we investigated the influence of the copolymer on the
19 distribution of the water-soluble fluorescent probe pyranine (structure reported in Figure S14 in
20 the Supporting Information) that was dissolved in the I-solution. This probe is membrane
21 impermeable and in pure POPC vesicles it is firmly confined into the aqueous lumen [85,86] as
22 highlighted in Figure 4 a). However, when the copolymer was present, the pyranine tended to
23 accumulate along the membrane (Figure 4 b). The intensity profiles shown in Figure 4 c) and 4

- 1 d) highlight the different distribution of pyranine. In pure POPC vesicles the fluorescence is
- 2 constant along the line and decreases at the border, in the mixed vesicle, in contrast, two peaks
- 3 appear in proximity of the membrane where pyranine accumulates.



1
 2 **Figure 4 Vesicles made of POPC - copolymer (93% - 7% mol/mol) and pyranine 50 \square M. a) Pure POPC**
 3 **vesicles; b) Mixed vesicles; c) Intensity profile taken along a line for vesicles 1 and 2 of Panel a); d) Intensity**
 4 **profile taken along line 1 and line 2 for the vesicle of Panel b).**
 5 This behaviour could be attributed to a tendency of pyranine to accumulate in copolymer-rich
 6 domains, possibly due to the interaction of its OH moieties with the pH-sensitive units of the

1 DMAEMA group. This different behaviour observed for pyranine in the absence and in the
2 presence of the copolymer is another proof that the latter is indeed present in the POPC double
3 layer.

4 CONCLUSION

5 Hybrid lipid vesicles incorporating a copolymer with a T_g above room temperature have
6 been presented. Our study shows that electroformation cannot be used for these polymers to
7 efficiently obtain giant vesicles, probably linked to a low mobility in the electroformation
8 conditions. However, a method based on an emulsion phase transfer, so far used for lipids or
9 polymers such as PDMS or PBD has been successfully adapted. This can be preferably applied
10 to polymers compatible with lipids based on their solubility and affinity. For this, the solubility
11 parameter and the HLB value can be used to select appropriate candidates. The method presented
12 here will open up new possibilities to develop hybrid systems with a much larger chemical
13 structure variety, leading to new types of synthetic micro reactors or biomimetic compartments.
14 Possible future developments will therefore be adaptive micro-reactor with catalyst groups fixed
15 to the membrane, or pH-sensitive micro- or nano-reactors or synthetic cells with a controlled
16 permeability versus an external stimulus.

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10

1 LIST OF ABBREVIATIONS

Monomethoxypoly(ethyleneglycol)	mPEG
Poly(ethylene glycol) methyl ether 2-bromoisobutyrate	mPEG-Br
2,2'-Bipyridyl	bpy
1-palmitoyl-2-oleoyl- <i>sn</i> -glycero-3-phosphocholine	POPC
1,2-dioleoyl- <i>sn</i> -glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt)	18:1 Liss Rhod PE
Methylmetacrylate	MMA
N,N-(dimethylamino)ethyl methacrylate	DMAEMA
2,6-diethyl-1,3,5,7-tetramethyl-8-[4-[8-(2-methacryloylolethyl)dimethylammoniumbromideoctyloxy]phenyl]-4,4'-difluoroboradiaza indacene	BODIPY-DMAEMA
fluorescent mPEG- <i>block</i> -P(MMA- <i>grad</i> -DMAEMA)	BODIPY-copolymer

2

3 ASSOCIATED CONTENT

4 Supplementary information is available. It describes the characterization of the products in more
5 details and report more pictures about hybrid GUVs.

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