



Modifying styrene-maleic acid co-polymer for studying lipid nanodiscs

Victoria Schmidt, James N. Sturgis

► To cite this version:

Victoria Schmidt, James N. Sturgis. Modifying styrene-maleic acid co-polymer for studying lipid nanodiscs. *Biochimica et Biophysica Acta: Biomembranes*, 2018, 1860 (3), pp.777 - 783. 10.1016/j.bbamem.2017.12.012 . hal-01769247

HAL Id: hal-01769247

<https://amu.hal.science/hal-01769247>

Submitted on 17 Apr 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Modifying styrene-maleic acid co-polymer for studying lipid nanodiscs

Victoria Schmidt and James N. Sturgis

*LISM UMR 7255, CNRS and Aix-Marseille University, 31 Chemin Joseph Aiguier,
13402 Marseille cedex 20, France*

Abstract

Recently, styrene-maleic acid copolymer lipid nanodiscs have become an increasingly popular tool for the study of membrane proteins. In the work we report here, we have developed a novel method for the efficient preparation of labeled nanodiscs, under chemically mild conditions, by modification of the hydrolyzed styrene-maleic acid copolymer. This protocol is designed to be easily accessible to biochemistry laboratories. We use this procedure to prepare various fluorescent nanodiscs labeled with different fluorophores. By studying the development of Förster resonance energy transfer, we demonstrate the rapid exchange of co-polymer between nanodiscs. This demonstration, in conjunction of previous work, indicates that the lipid nanodiscs prepared using this polymer are very dynamic structures with rapid exchange of the different components.

Keywords: SMALP, Membrane Protein, Nanodisc

1. Introduction

2 The *in vitro* study of membrane proteins can be particularly challenging
3 [1]. This challenge arises from various steps in a typical protocol, that in-
4 cludes over-expression, membrane isolation, solubilization and purification.
5 During solubilization detergents are typically used to extract the proteins
6 from their native lipid environment, and to replace the lipids with detergent
7 molecules. Finding an appropriate detergent is complex, and the delipida-
8 tion can often result in loss of activity or denaturation [2]. The difficulties
9 associated with using detergents have led to the development of several al-
10 ternative strategies for maintaining membrane proteins in solution including

11 amphipols [3], protein stabilized lipid nanodiscs [4], and polymer solubilized
12 lipid nanoparticles [5].

13 Recently the direct solubilization of membranes using the polymeric de-
14 tergent styrene maleic acid (SMA) copolymer has become increasingly pop-
15 ular as an alternative to low molecular weight detergent based strategies
16 [6][7][8]. To date, this amphipathic polymer has been shown to solubilize
17 membranes to give nanometric discs-shaped particles. These particles are
18 known as styrene maleic acid lipid particles (SMALP). When used on biolog-
19 ical membranes, these particles contain a mixture of protein, membrane lipids
20 and the SMA polymer. These nano-particles are believed to be organized as
21 a protein surrounded by native membrane lipids, the whole maintained in
22 aqueous solution thanks to a belt of the amphipathic polymer [5]. During
23 solubilization, the integral membrane proteins are thus extracted into a nan-
24 odisc along with their native lipidic environment. SMA copolymer nanodiscs
25 are compatible with conventional purification approaches, such as affinity
26 chromatography or gel filtration, allowing purification of the SMALP em-
27 bedded protein [9]. A particularly interesting point is the "almost native"
28 environment, because membrane proteins may need specific lipids to modu-
29 late their structure and exercise their function [10][11].

30 SMA have been already used to solubilize membranes from various organ-
31 isms such as Gram positive [6] or Gram negative bacteria [12], yeast [8][13],
32 insect and human cultured cells [14][8]. Solubilized proteins exhibit various
33 transmembrane segments number and are indistinctly trapped in SMALPs
34 with a average diameter around 13 nm [15]. Subsequently particles were
35 characterized by various techniques such as transmission electron microscopy
36 [6], dynamic light scattering [6], fluorescence spectroscopy [6][14][7], NMR
37 spectroscopy [16], fourier transform infra red spectroscopy [16], small angle
38 neutron scattering [16] and EPR spectroscopy [17]. SMA purified proteins
39 have also been used to resolve the structure of a bacteriorhodopsin by X-ray
40 crystallography without any conventional detergent being used [18].

41 For various protocols it could be useful to have chemically modified SMA.
42 For example, for lipid nanodisc immobilization a specific label would be use-
43 ful. Indeed, there are several articles in which labeled amphipols have been
44 used to immobilize membrane proteins, using a variety of chemistries: oligo-
45 histidine tags [19]; biotin tags [20]; and DNA oligonucleotides [21]. Labeled
46 SMA could thus be used for similar experiments while maintaining the mem-
47 brane proteins in a lipid environment. Fluorescent labeling also provides
48 interesting possibilities for studying membrane protein interactions without

49 protein modification, for example using fluorescence correlation spectroscopy
50 or energy transfer measurements.

51 A previously published method to produce labeled SMALPs is based on
52 the functionalization of styrene-maleic anhydride copolymer, and the sub-
53 sequent hydrolysis of the maleic anhydride to maleic acid under alkaline
54 conditions [22]. This protocol exploits the reactivity of anhydrides towards
55 alcohols and amines that has already been used to directly conjugate SMA
56 with fluorophores, drugs and small molecules[23][24][25]. However, the use
57 of this protocol is limited by the strong alkaline conditions and the use of
58 non-aqueous solvents not frequently found in biology laboratories.

59 Here, we propose a novel protocol for modification of SMA in aqueous
60 solution, that can be used for making labeled nanodiscs. This method is
61 based on the reaction coupling of carboxylic acids to primary amines using
62 a carbodiimide. This reaction is frequently used for protein modification
63 [26]. Unlike the protocol based on modification of the maleic anhydride
64 form, our protocol maintains chemically mild conditions compatible with
65 many biomolecules throughout the procedure. This makes the chemistry
66 easily accessible to biological laboratories, and will allow a wider range of
67 molecules to be attached to the SMA. We have used our protocol to prepare
68 fluorescent SMALPs, and used these to examine the exchange of polymer
69 between lipid-nanodiscs.

70 2. Materials and Methods

71 2.1. SMA-SH preparation

72 Hydrolyzed styrene-maleic acid copolymer (SMA) with a 3:1 styrene to
73 maleic acid ratio in NaOH solution at 25% *w/v* was provided by Polyscope
74 (Xiran SL25010 S25). All modification steps were performed at room temper-
75 ature with stirring. Typically, 1% of carboxyl groups were targeted for mod-
76 ification, based on the number of carboxylate groups present in the polymer
77 composition this is 4.47 mmol/g. To a polymer solution at a concentration
78 of 25% an appropriate amount of 1-Ethyl-3-(3-dimethylaminopropyl)carbo-
79 diimide (EDC) (Sigma, St Louis, USA) was added for the degree of label-
80 ing targeted. Thus, for modification of 1% of the carboxyl groups in 1g of
81 polymer 45 μ mole of EDC was added. To stabilize intermediates, N-hydroxy-
82 sulfo-succinimide (Sulfo-NHS) was added at a 20% molar excess (54 μ mole /
83 g polymer). Both, EDC and Sulfo-NHS were dissolved in MES 75mM pH
84 5.8 in 25% ethanol. They were then added to the polymer and incubated 20

minutes at room temperature with stirring, before a 2 fold excess (108 μ mole / g polymer) of cystamine dihydrochloride (Sigma, St Louis, USA) dissolved in 25mM MES pH 7.0 25% EtOH was added slowly with mixing. When the solution became less translucent, EtOH 96% was added drop-wise until the solution clarified. The reaction was then allowed to proceed for 12h at room temperature with stirring, and covered to avoid EtOH evaporation.

The modified polymer was then separated from reagents and low molecular weight products by dialysis for 2 hours at room temperature against 1 liter Tris 20 mM pH 8.0 with a 8000 MW cut-off membrane (Spectra/Por membrane, Spectrum Laboratories), the buffer was changed once during dialysis. SMA-SH solutions were stored at 4°C until use.

2.2. SMA-SH Labeling

Immediately prior to use the modified SMA was reduced by addition of a five fold excess (0.27 μ mole / g polymer) of DTT and dialyzed 2 hours at room temperature against 10 volumes of Tris-HCl 20 mM pH 8.0.

Reduced and dialyzed SMA-SH were labeled with Atto488- or Atto532-maleimide according to Atto-TEC recommendations [27]. Briefly, 1 mg of maleimide was dissolved in 200 μ L on DMSO, and then a 1.3 fold excess of this solution was added to the SMA-SH solution and incubated for 24 hours at 22°C in Tris-HCl 20 mM pH 8.0 NaCl 200 mM.

After the reaction, unreacted maleimides were removed with MicroBioSpin Chromatography Columns (Promega), equilibrated with Tris 20 mM pH 8.0, NaCl 200 mM, according to product recommendations. The degree of labeling was determined by decomposition of the absorption spectrum using the following formula :

$$\frac{\epsilon_{SMA} \times Abs_{Atto}}{\epsilon_{Atto} \times Abs_{SMA}} \times 100$$

Measuring SMA from absorption at 260nm and Atto fluorophores at their visible absorption maximum, 500 nm and 550 nm for Atto488 and Atto532 respectively. The extinction coefficient for SMA was ϵ_{SMA} of $365 \pm 35 \text{ M}_{carboxylate}^{-1} \text{ cm}^{-1}$, based on absorbance of SMA dilutions, and gravimetric analysis of the dried disodium salt. The reported error includes propagated contributions from the precision of the measurements (n=3) and the documented accuracy of instruments and composition. This value is in relatively good agreement with the previously reported value of $6989 \text{ M}_{polymer}^{-1} \text{ cm}^{-1}$ [28, 29], which converts using the given polymer composition to $388 \text{ M}_{carboxylate}^{-1} \text{ cm}^{-1}$. The extinction coefficients of Atto dyes were ϵ_{Atto} of $9 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ and $1.5 \times$

115 $10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for Atto488 and Atto532 respectively, based on the Atto-TEC
116 literature [27].

117 2.3. SMALP nanodisc formation and size determination

118 500 μg of *E.coli* total lipid extract (Avanti Polar Lipids) were dried under
119 nitrogen and resuspended in Tris 20 mM NaCl 200 mM with vortexing, to
120 form multi-lamellar lipid vesicles. An appropriate proportion of SMA, or
121 SMA-SH or fluorescent SMA, was added to the liposome solution mixed
122 and allowed solubilize the lipids for 10 minutes. Any unsolubilized material
123 was removed by ultracentrifugation 1 hour at $140000 \times g$. The supernatant,
124 containing nanodiscs, was collected and analyzed.

125 Dynamic light scattering (DLS) measurements were made on a Zetasizer
126 Nano-series Nano-S instrument (Malvern Instruments). Samples were briefly
127 equilibrated for 300 sec at 25°C prior to measurement. Default settings in
128 the software were used for optimizing measurement settings and duration.
129 Non-negative least squares algorithm of the correlation data was used to
130 obtain volume-based particle size distributions, assuming spherically shaped
131 particles. Volume based distributions are reported, despite the inherent as-
132 sumptions, as these are easier to understand than intensity based distribu-
133 tions. Sample homogeneity was determined using the polydispersity index
134 computed by the Malvern software. This index is a number calculated from a
135 simple 2 parameter fit to the correlation data (the cumulants analysis). The
136 polydispersity index is dimensionless and scaled such that values smaller than
137 0.05 are rarely seen other than with highly monodisperse standards. Values
138 greater than 0.7 indicate that the sample has a very broad size distribution
139 [30].

140 For electron microscopy, carbon-coated copper grids (Electron Microscopy
141 Sciences) were glow-discharged for 30 sec. A single droplet of 5 μL of sample
142 was applied to the grid and removed after 60 sec by blotting with filter paper
143 (Whatman). The grid was washed with water, and excess water was removed
144 by blotting with filter paper prior to staining with 5 μL of 2% uranyl acetate
145 for 60 sec. Excess of stain was removed by blotting with filter paper and the
146 grid was dried at room temperature. Images were recorded on a FEI Tecnai
147 200 kV electron microscope operating at a voltage of 200 kV and a defocus
148 of 2.5 μm , using a Eagle-CCD camera (FEI) at a nominal magnification of
149 50,000, yielding a pixel size of 4.4 Å.

150 *2.4. FRET measurements*

151 Förster resonance energy transfer (FRET) was measured using fluores-
 152 cence emission spectra acquired on a Olympus confocal microscope FV1000-
 153 IX81 with a 60x oil objective controlled by Fluoview FV10-ASW4.2 software.
 154 The sample was excited at 488 nm with an Argon laser, and the emission
 155 spectrum recorded as a function of time. The data was fit by adjusting the
 156 parameters A, B and τ in the equation:

$$\begin{bmatrix} F_{525} \\ F_{550} \end{bmatrix} = \begin{bmatrix} B_{525} \\ B_{550} \end{bmatrix} + \begin{bmatrix} A_{525} \\ A_{550} \end{bmatrix} \exp^{-t/\tau}$$

157 Where F are the fluorescence values at 2 wavelengths, B the endpoints and
 158 A the amplitudes of the fluorescence changes. This approach fits both fluo-
 159 rescence curves with a single rate constant.

160 **3. Results**

161 *3.1. SMA modification*

162 The protocol we have developed aims at obtaining thiol modified SMA
 163 (SMA-SH). For this we form an amide bond between a small proportion,
 164 typically 1%, of the maleic acid carboxyl groups and the amine groups of
 165 cystamine. Finally the di-thiol bridge is reduced using dithiothreitol (DTT)
 166 (figure 1).

167 In this study we have used the commercial Xiran resin with a 3:1 styrene
 168 to maleic acid ratio, however the procedure is compatible with other co-
 169 polymer compositions. EDC catalyzed cross-linking is typically performed
 170 at low pH (≈ 4.5) for improved efficiency but neutral pH conditions (up to
 171 7.2) are compatible with the reaction chemistry, albeit with lower efficiency
 172 [31]. Due to the pH dependence of the solubility of SMA 3:1 the reaction
 173 was performed at pH 6.5 [32]. The solution has a tendency to change from
 174 a transparent liquid to a slightly milky gel. For reaction efficiency it was
 175 important to avoid the formation of white granular aggregates particularly
 176 during cystamine addition. To reduce precipitate formation and aid increase
 177 reaction efficiency it proved useful to increase the solvent hydrophobicity
 178 slightly, for this we added 25% ethanol.

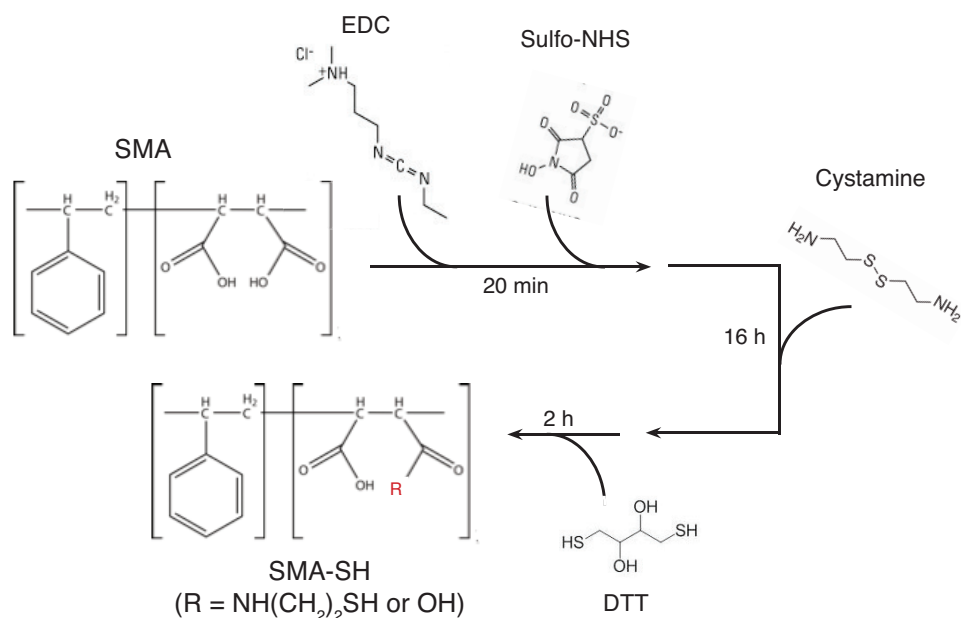


Figure 1: Reaction Scheme for the preparation of SMA-SH based on the ready-to-use Xiran SL25010 S25 from Polyscope.

3.2. Estimation of the degree of labeling

In order to estimate the degree of modification of the SMA-SH we coupled the thiol groups to fluorescent maleimides. Typically for this we used either Atto488-maleimide or Atto532-maleimide. Immediately prior to use, SMA-SH to be modified was reduced with DTT and reaction products removed by dialysis, then a 1.3 fold excess of fluorescent maleimide in dry DMSO was added. After 24 hours of reaction, unreacted fluorophores were removed using a size exclusion chromatography and labeling estimated by deconvolution of the absorption spectrum, (figure 2). For spectral deconvolution the SMA absorption maximum at 260 nm and the Atto488 absorption maximum at 500 nm, or the Atto532 absorption maximum at 550 nm, were used.

As expected, and can be readily appreciated, the modification of the SMA did not alter the UV-visible spectrum of the polymer. This is important, as we routinely estimate polymer concentration from absorption at 260 nm, using the extinction coefficient of 365 M⁻¹ cm⁻¹ we have determined. The Atto488 and Atto532 dyes, show little UV absorption, but have a strong visible absorption. Comparing the absorption of the maleimide and the labeled

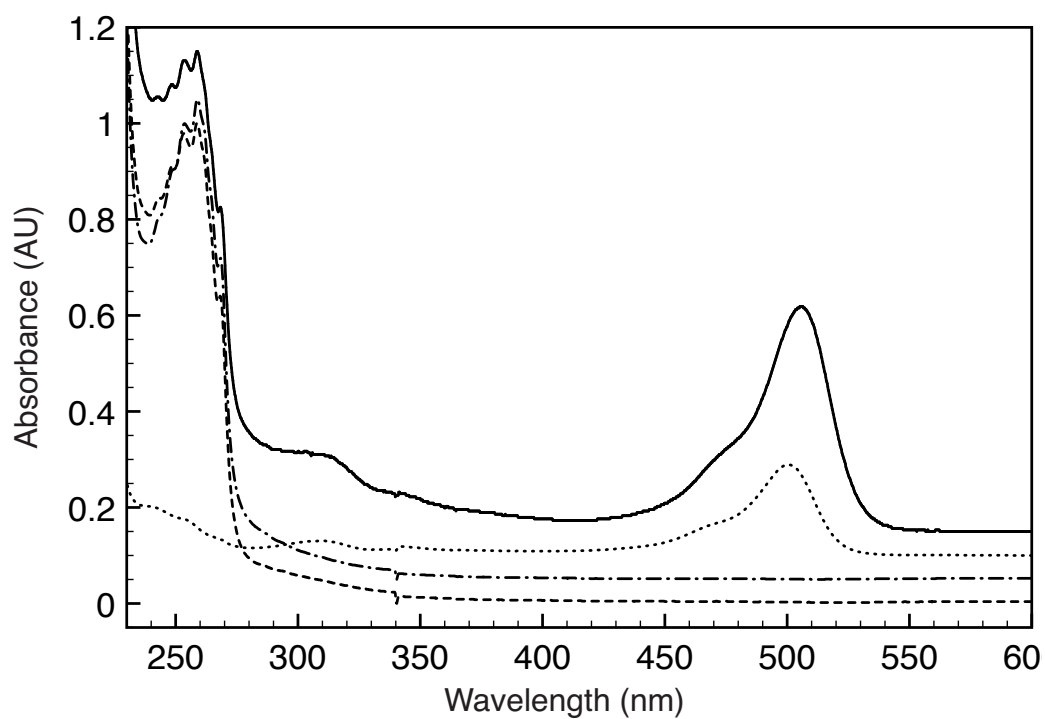


Figure 2: Absorption spectra of: SMA (dashed line); SMA-SH (dash-dotted line); Atto488 maleimide (dotted line); and Atto488 labeled SMA-SH (solid line). The various spectra are normalized and offset for improved visibility.

polymer a small red shift in the absorption maximum can be noted (from 500 to 507 nm). This small red-shift can be attributed to a change in the polarizability of the environment due to the coupling to the polymer. We have assumed for calculating degree of modification that this spectral shift is not associated with a change in the extinction coefficient.

To calculate the degree of labeling the molar ratio of dye to polymer carboxylate groups was calculated using the extinction coefficients and the fraction of maleic acid in the co-polymer, for the 3:1 copolymer we used there are 1 mole of carboxylate groups per 214.26 g of polymer. Multiple labeling reactions of the same batch of SMA-SH gave degrees of labeling between 0.16% and 0.20% for SMA-SH. At this low level of labeling the polymers have on average about 0.5 labels per chain. The final yield of labeled carboxylate groups is somewhat lower than the target, 20%, this overall yield is less than the previously reported method [22]. We believe that the EDC catalyzed derivitization is the the main reason for this low yield, longer labeling or greater excess of maleimide does not change the final yield. In particular, we suspect that this is due to working close to the solubility limit of the SMA.

3.2.1. Liposome solubilization by SMA-SH and fluorescently labelled-SMA

After performing the chemical modifications, we checked the ability of SMA-SH to solubilize liposome membranes and form SMALPs. For this multilamellar vesicles (MLV) were formed using *E.coli* total lipid extract and these were put in contact with various concentrations of polymer and the size of the resulting SMALPs was monitored using DLS (figure 3).

In agreement with previous work [33], SMALP size depends on the polymer to lipid ratio. The unmodified SMA at high polymer to lipid ratios (>3.0) gave homogeneous SMALP nanodiscs of about $7.9 \text{ nm} \pm 0.3$ (mean $\pm$ s.d.) diameter and relatively low polydispersity 0.30 ± 0.02 .

The thiol-modified polymer, SMA-SH, was slightly less efficient at solubilizing lipids, giving larger nanodiscs at all polymer lipid ratios, and with size showing a greater sensitivity to this ratio. This can be readily appreciated in figure 3A. At high polymer to lipid ratios (>3.0) nanodisc diameter was about $9.6 \pm 0.3 \text{ nm}$. Furthermore, the SMA-SH nanodiscs were slightly more heterogeneous than those formed with unmodified polymer, as can be seen in figure 3B notably with the presence of a distinct shoulder at larger sizes. This behavior can be rationalized by the slightly more hydrophobic nature of the polymer after modification. We exclude thiol oxidative cross-linking since SMA-SH was reduced immediately prior to use.

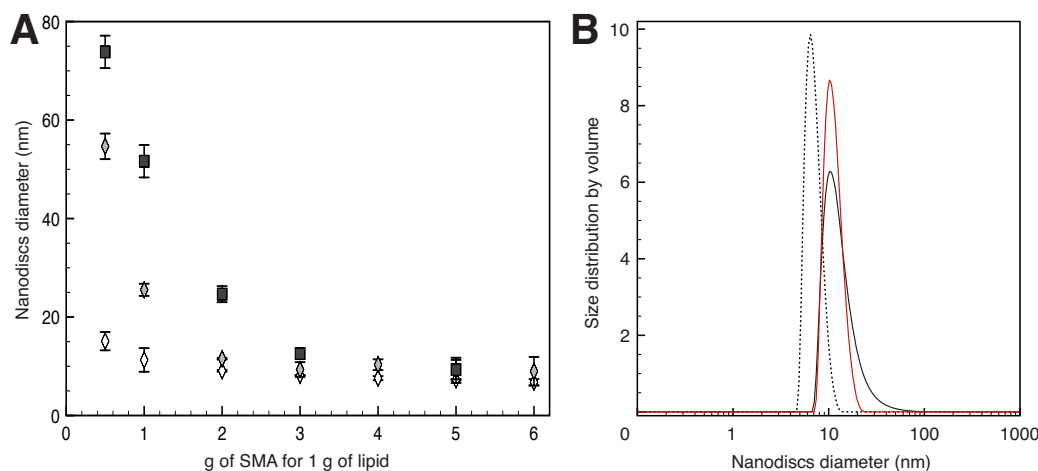


Figure 3: A. SMALP nanodisc size as a function of polymer concentration. SMALP size was determined using DLS after MLV's of *E.coli* lipids were solubilized with: SMA (open diamond); SMA-SH (gray diamond); or Atto488 labeled SMA (black square). B. DLS profiles of SMALPs formed at a polymer:lipid ratio of 3.0 *w/w* with: SMA (dashed line); SMA-SH (solid black); or Atto488 labeled SMA (red line).

233 Fluorescently labeled SMA was also able to efficiently solubilize MLV,
 234 figure 3A. However, it was slightly less efficient than the SMA-SH polymer.
 235 The nanodiscs formed at high polymer to lipid ratio appear to have a sim-
 236 ilar size to those formed with SMA-SH ($8.0 \text{ nm} \pm 0.3 \text{ nm}$ vs 9.6 ± 0.3
 237 nm), but are slightly less heterogeneous, as can be appreciated in figure 3B.
 238 These differences in solubilization may be due to slight modifications in the
 239 hydrophobic/hydrophilic balance.

240 To investigate further the heterogeneity and confirm the size difference at
 241 low polymer to lipid ratios we observed SMALPs formed at a lipid polymer
 242 ratios of 0.5 and 2.0 by transmission electron microscope (TEM), (figure 4).

243 The electron micrographs show clearly that at polymer:lipid ratio of 0.5
 244 the SMA-SH objects visible are about 50 nm in diameter and much larger
 245 than the 15 nm SMA nanodiscs, visible in figure 4C and D. Gratifyingly the
 246 sizes of objects observed in electron micrographs are in agreement with those
 247 determined by DLS. The larger objects observed at low SMA to lipid ratio,
 248 particularly in the presence of SMA-SH, figure 4B, are probably a mixture
 249 collapsed polymer saturated vesicles and membrane fragments.

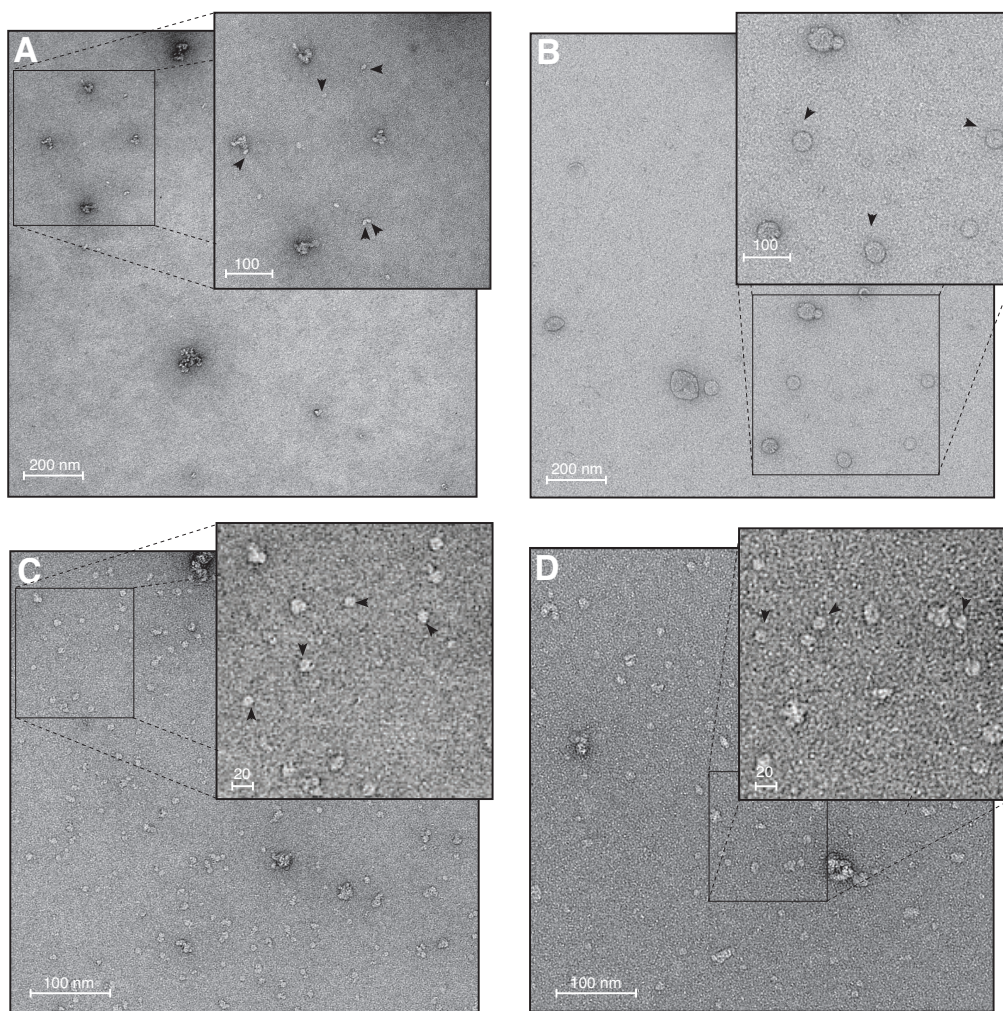


Figure 4: Negative stain transmission electron micrographs of **A)** SMA and **B)** SMA-SH nanodiscs formed at a polymer:lipid ratio of 0.5. **C)** SMA and **D)** SMA-SH nanodiscs formed at a polymer:lipid ratio of 2.0. Black arrows indicate typical individual objects in the 2 micrographs.

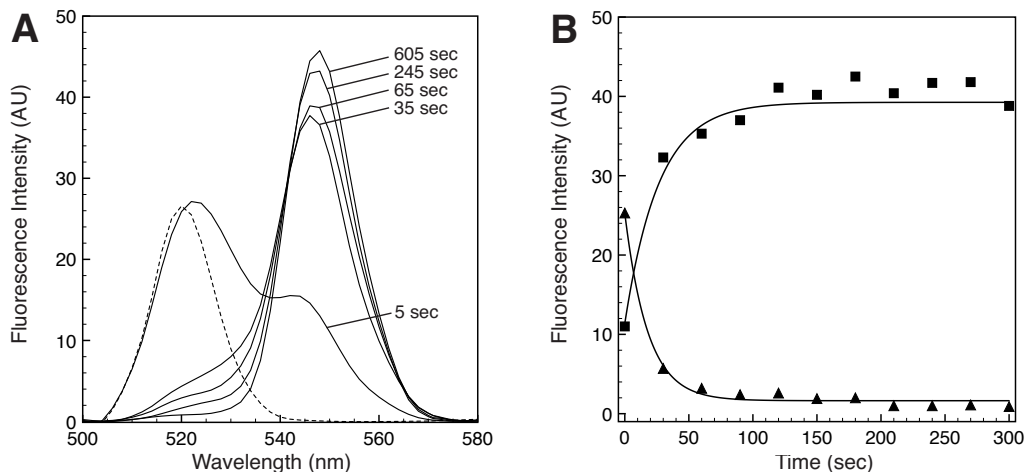


Figure 5: **A)** Emission spectra of nanodiscs labeled Atto488 (dashed black line) following mixing with and equal quantity of nanodiscs labeled with Atto532. Kinetics were recorded by measuring emission spectra every 30 seconds after sample mixing. **B)** Kinetics of FRET development followed at 525 and 550 nm. Emission at 525 nm decrease from 0 to 5 min while emission at 550nm increases. The lines show the result of a single exponential fit to the data with a time constant of 20.2 sec (see text).

250 3.3. Nanodisc dynamics

251 We have used the fluorescently labeled polymer to study the dynamics of
 252 SMALP nanodiscs. A recent study showed that lipids can exchange between
 253 nanodiscs [34]. Here we have investigated polymer exchange between nan-
 254 odiscs by mixing preformed nanodiscs labeled with different fluorophores and
 255 following the changes in Förster resonance energy transfer (FRET) between
 256 the chromophores (figure 5).

257 In figure 5A we observe the rapid decrease in donor emission, at 520
 258 nm, and the parallel increase in acceptor emission, at 550 nm, after mix-
 259 ing of the two differently colored SMALP. As the characteristic distance for
 260 transfer between these two fluorophores is 64 Å[35], and the total nanodisc
 261 concentration is low, this increase in FRET is strong evidence for exchange
 262 of polymers between nanodiscs. Development of FRET over time can be
 263 followed as seen figure 5B, and a global fit with a single exponential func-
 264 tion to fit simultaneously the changes at both 525 nm and 550 nm gives a
 265 characteristic time of 20.2 ± 0.3 sec, this is shown as the solid lines.

266 It is noteworthy, that despite the low level of modification with fluo-
 267 rophore, about 0.5 per chain, the donor fluorescence emission declines to

268 near zero. This implies that each nanodisc contains multiple polymer chains,
269 since all donors appear to be associated with acceptors after equilibration.

270 These measurements show that beyond the previously observed lipid
271 exchange between SMALPs [34] there is also polymer exchange between
272 SMALPs. These observations can explain the homogeneity of the parti-
273 cles produced during solubilization, since the rapid exchange of material,
274 lipid and polymer, will ensure that the chemical potential of the different
275 components rapidly equilibrates, resulting in homogeneous solutions.

276 4. Discussion

277 We have developed a novel protocol for the preparation of thiol labeled
278 SMA, SMA-SH, using EDC catalysed carboxylate reaction. Furthermore, we
279 have shown that this polymer can be derivatized to form fluorescent poly-
280 mers using readily available and versatile maleimide coupling chemistry. Flu-
281 orescent SMA has been previously prepared[22], so the two protocols merit
282 comparison. The previous protocol, based on anhydride chemistry, is cheap
283 and has higher yields, while the procedure we report here has the advantage
284 of using conditions compatible with most biological molecules, and reactions
285 readily available in biology laboratories. Our method thus provides a useful
286 alternative when low levels of modification are desired.

287 SMA, SMA-SH and fluorescently labeled SMA are all able to solubilize
288 membranes with the formation of SMALP nanodiscs. The chemically mod-
289 ified polymers form slightly larger nanodiscs than the unmodified polymer.
290 This is entirely consistent with the previously noted importance of the hy-
291 drophobic hydrophilic balance in modulating the energetics of membrane
292 solubilization by SMA copolymers [32].

293 Since their initial appearance as solubilizing agents [36], SMA have been
294 often been considered, or imagined, as operating like a cookie cutter, neatly
295 excising a small disc of native membrane to form stable lipid nanodiscs [6].
296 Our results, and the previous results on lipid exchange [34], clearly indicate
297 that SMALPs are much more dynamic and exchange components. Thus
298 this cookie cutter vision is no longer tenable and a more thermodynamically
299 driven, rather than kinetically driven, SMALP composition would seem ap-
300 propriate.

301 Some previous clues have suggested that polymer-bound nanodiscs might
302 be more dynamic than other system, such as nanodiscs made with membrane
303 scaffold protein [37]. For example the potassium channel KcsA can transit

304 spontaneously from SMALPs to lipid bilayer [6] and a membrane protein can
305 be transferred from SMALPs to lipid-cubic phase (LCP) in sufficient amount
306 to obtain diffracting crystals [18].

307 These observations have considerable implications for the interpretation
308 of many experiments concerning lipids and proteins in SMALPs. Previ-
309 ously, the vision of SMA driven membrane solubilization removing pieces of
310 membrane, with the composition of the resulting objects being controlled
311 by proximity in the original membrane. However, now, we must envisage
312 a dynamic system, with the composition of the resulting objects being con-
313 trolled by equilibration of chemical potential. Thus in SMALP lipidomic
314 studies some enrichment (especially in negatively charged lipids) compared
315 to protein-free SMALPs of solubilized *E.coli* native membranes has been ob-
316 served [6][7]. Such observations can no longer be considered as a result of the
317 native local environment but rather that the enrichment results from interac-
318 tions within the SMALP population. Thus, preferential presence of certain
319 lipids could result from favorable interactions with particular proteins, or
320 more indirectly due to exclusion from other SMALPs in the population. The
321 dynamic nature of SMALPs may also be useful in the pharmaceutical field
322 as many compounds have solubility problems [38][39] we can imagine the use
323 of SMALPs as transporters for hydrophobic drugs.

324 Acknowledgments

325 We would like to thank Y. Rhamani, V. Prima, and J.P. Duneau for tech-
326 nical assistance and many helpful discussions. This work was supported by
327 the CNRS and Espoir contre la Mucoviscidose.

328 Bibliography

- 329 [1] R. Grishammer, C. G. Tateu, Overexpression of integral membrane pro-
330 teins for structural studies, Quarterly Reviews of Biophysics 28 (1995)
331 315.
- 332 [2] K. Duquesne, V. Prima, J. N. Sturgis, Membrane Protein Solubilization
333 and Composition of Protein Detergent Complexes, Springer New York,
334 pp. 243–260.
- 335 [3] J. L. Popot, Amphipols, nanodiscs, and fluorinated surfactants: three
336 nonconventional approaches to studying membrane proteins in aqueous
337 solutions, Annual Review of Biochemistry 79 (2010) 737–775.

- 338 [4] I. G. Denisov, S. G. Sligar, Nanodiscs for structural and functional
339 studies of membrane proteins, *Nature Structural and Molecular Biology*
340 23 (2016) 481–486.
- 341 [5] T. J. Knowles, R. Finka, C. Smith, Y. P. Lin, T. Dafforn, M. Overduin,
342 Membrane proteins solubilized intact in lipid containing nanoparticles
343 bounded by styrene maleic acid copolymer, *Journal of the American*
344 *Chemical Society* 131 (2009) 7484–7485.
- 345 [6] J. M. Dörr, M. C. Koorengevel, M. Schäfer, A. V. Prokofyev, S. Scheide-
346 laar, E. a. W. van der Cruisen, T. R. Dafforn, M. Baldus, J. A. Killian,
347 Detergent-free isolation, characterization, and functional reconstitution
348 of a tetrameric K⁺ channel: The power of native nanodiscs, *PNAS* 111
349 (2014) 18607–18612.
- 350 [7] I. Prabudiansyah, I. Kusters, A. Caforio, A. J. Driessen, Characteriza-
351 tion of the annular lipid shell of the Sec translocon, *BBA-Biomembranes*
352 1848 (2015) 2050–2056.
- 353 [8] M. Jamshad, J. Charlton, Y. Lin, S. J. Routledge, Z. Bawa, T. J.
354 Knowles, M. Overduin, N. Dekker, T. R. Dafforn, R. M. Bill, D. R.
355 Poyner, M. Wheatley, G-protein coupled receptor solubilization and
356 purification for biophysical analysis and functional studies, in the total
357 absence of detergent, *Bioscience Reports* 35 (2015) 1–10.
- 358 [9] A. M. Seddon, P. Curnow, P. J. Booth, Membrane proteins, lipids and
359 detergents: Not just a soap opera, *BBA-Biomembranes* 1666 (2004)
360 105–117.
- 361 [10] A. Laganowsky, E. Reading, T. M. Allison, M. B. Ulmschneider, M. T.
362 Degiacomi, A. J. Baldwin, C. V. Robinson, Membrane proteins bind
363 lipids selectively to modulate their structure and function, *Nature* 510
364 (2014) 172–175.
- 365 [11] P. L. Yeagle, Non-covalent binding of membrane lipids to membrane
366 proteins, *BBA-Biomembranes* 1838 (2014) 1548 – 1559.
- 367 [12] S. Paulin, M. Jamshad, T. R. Dafforn, J. Garcia-lara, S. J. Foster, N. F.
368 Galley, D. I. Roper, Surfactant-free purification of membrane protein

369 complexes from bacteria : application to the staphylococcal penicillin-
370 binding protein complex PBP2 / PBP2a, *Nanotechnology* 25 (2014)
371 285101.

372 [13] A. R. Long, C. C. O'Brien, K. Malhotra, C. T. Schwall, A. D. Albert,
373 A. Watts, N. N. Alder, A detergent-free strategy for the reconstitu-
374 tion of active enzyme complexes from native biological membranes into
375 nanoscale discs., *BMC biotechnology* 13 (2013) 41.

376 [14] S. Gulati, M. Jamshad, T. J. Knowles, K. A. Morrison, R. Downing,
377 N. Cant, R. Collins, J. B. Koenderink, R. C. Ford, M. Overduin, I. D.
378 Kerr, T. R. Dafforn, A. J. Rothnie, Detergent free purification of ABC
379 transporters., *Biochemical Journal* 44 (2014) 1–24.

380 [15] J. M. Dörr, S. Scheidelaar, M. C. Koorengevel, J. J. Dominguez,
381 M. Schäfer, C. A. van Walree, J. A. Killian, The styrenemaleic acid
382 copolymer: a versatile tool in membrane research, *European Biophysics*
383 *Journal* 45 (2016) 3–21.

384 [16] M. Jamshad, V. Grimard, I. Idini, T. J. Knowles, M. R. Dowle,
385 N. Schofield, P. Sridhar, Y. Lin, R. Finka, M. Wheatley, O. R. T.
386 Thomas, R. E. Palmer, M. Overduin, C. Govaerts, J. M. Ruysschaert,
387 K. J. Edler, T. R. Dafforn, Structural analysis of a nanoparticle con-
388 taining a lipid bilayer used for detergent-free extraction of membrane
389 proteins, *Nano Research* 8 (2015) 774–789.

390 [17] M. Orwick-Rydmark, J. E. Lovett, A. Graziadei, L. Lindholm, M. R.
391 Hicks, A. Watts, Detergent-free incorporation of a seven-transmembrane
392 receptor protein into nanosized bilayer lipodisc particles for functional
393 and biophysical studies, *Nano Letters* 12 (2012) 4687–4692.

394 [18] J. Broecker, B. T. Eger, O. P. Ernst, Crystallogenesis of Membrane
395 Proteins Mediated by Polymer-Bounded Lipid Nanodiscs, *Structure*
396 (2017) 1–9.

397 [19] F. Giusti, P. Kessler, R. W. Hansen, E. A. Della Pia, C. Le Bon,
398 G. Mourier, J. L. Popot, K. L. Martinez, M. Zoonens, Synthesis of
399 a Polyhistidine-bearing Amphipol and its Use for Immobilizing Mem-
400 brane Proteins, *Biomacromolecules* 16 (2015) 3751–3761.

- 401 [20] D. Charvolin, J.-B. Perez, F. Rouviere, F. Giusti, P. Bazzacco, A. Ab-
 402 dine, F. Rappaport, K. L. Martinez, J.-L. Popot, The use of amphipols
 403 as universal molecular adapters to immobilize membrane proteins onto
 404 solid supports, *PNAS* 106 (2009) 405–410.
- 405 [21] C. L. Bon, E. A. D. Pia, F. Giusti, N. Lloret, M. Zoonens, K. L. Mar-
 406 tinez, J. L. Popot, Synthesis of an oligonucleotide-derivatized amphipol
 407 and its use to trap and immobilize membrane proteins, *Nucleic Acids*
 408 *Research* 42 (2014).
- 409 [22] S. Lindhoud, V. Carvalho, J. W. Pronk, M. E. Aubin-Tam, SMA-SH:
 410 Modified Styrene-Maleic Acid Copolymer for Functionalization of Lipid
 411 Nanodiscs, *Biomacromolecules* 17 (2016) 1516–1522.
- 412 [23] Y. Mu, H. Kamada, Y. Kaneda, Y. Yamamoto, H. Kodaira, S. Tsun-
 413 oda, Y. Tsutsumi, M. Maeda, K. Kawasaki, M. Nomizu, Y. Ya-
 414 mada, T. Mayumi, Bioconjugation of laminin peptide YIGSR with
 415 poly(styrene co-maleic acid) increases its antimetastatic effect on lung
 416 metastasis of B16-BL6 melanoma cells., *Biochemical and Biophysical*
 417 *Research Communications* 255 (1999) 75–9.
- 418 [24] S. Henry, M. El-Sayed, C. Pirie, pH-responsive poly (styrene-alt-
 419 maleic anhydride) alkylamide copolymers for intracellular drug delivery,
 420 *Biomacromolecules* 7 (2006) 2407–2414.
- 421 [25] Z. Li, Y. Song, Y. Yang, L. Yang, X. Huang, J. Han, S. Han, Rhodamine-
 422 deoxylactam functionalized poly[styrene-alt-(maleic acid)]s as lyso-
 423 some activatable probes for intraoperative detection of tumors, *Chemical*
 424 *Science* 3 (2012) 2941.
- 425 [26] K. L. Carraway, D. E. Koshland, Carbodiimide modification of proteins.,
 426 *Methods in Enzymology* 25 (1972) 616–623.
- 427 [27] Attotec, Recommended procedures for labeling, [http://www.](http://www.atto-tec.com/fileadmin/user_upload/Katalog_Flyer_Support/Procedures.pdf)
 428 [atto-tec.com/fileadmin/user_upload/Katalog_Flyer_Support/](http://www.atto-tec.com/fileadmin/user_upload/Katalog_Flyer_Support/Procedures.pdf)
 429 [Procedures.pdf](http://www.atto-tec.com/fileadmin/user_upload/Katalog_Flyer_Support/Procedures.pdf), 2017. Accessed: 2017-08-07.
- 430 [28] A. Grethen, A. O. Oluwole, B. Danielczak, C. Vargas, S. Keller, Thermo-
 431 dynamics of nanodisc formation mediated by styrene/maleic acid (2:1)
 432 copolymer, *Scientific Reports* 7 (2017) e11571.

- 433 [29] A. O. Oluwole, B. Danielczak, A. Meister, J. O. Babalola, C. Vargas,
434 S. Keller, Solubilization of membrane proteins into functional lipid-
435 bilayer nanodiscs using diisobutylene/maleic acid copolymer, *Ange-
436 wandte Chemie International Edition* 56 (2017) 1919–1924.
- 437 [30] Malvern, Inform White Paper Dynamic Light Scattering, Malvern Guid.
438 (2011) 1–6.
- 439 [31] Z. Grabarek, J. Gergely, Zero-Length Crosslinking Procedure with the
440 Use of Active Esters, *Analytical Biochemistry* 185 (1990) 131–135.
- 441 [32] S. Scheidelaar, M. C. Koorengevel, C. A. van Walree, J. J. Dominguez,
442 J. M. Dörr, J. A. Killian, Effect of Polymer Composition and pH on
443 Membrane Solubilization by Styrene-Maleic Acid Copolymers, *Biophys-
444 ical Journal* 111 (2016) 1974–1986.
- 445 [33] C. Vargas, R. Cuevas Arenas, E. Frotscher, S. Keller, Nanoparticle self-
446 assembly in mixtures of phospholipids with styrene/maleic acid copoly-
447 mers or fluorinated surfactants, *Nanoscale* (2015) 20685–20696.
- 448 [34] R. Cuevas Arenas, B. Danielczak, A. Martel, L. Porcar, C. Breyton,
449 C. Ebel, S. Keller, Fast collisional lipid transfer among polymer-bounded
450 nanodiscs, *Scientific Reports* (2017) 1–8.
- 451 [35] Attotec, Fluorescent labels and dyes, [https://www.atto-tec.com/
452 fileadmin/user_upload/Katalog_Flyer_Support/Catalogue_2009_
453 2010.pdf](https://www.atto-tec.com/fileadmin/user_upload/Katalog_Flyer_Support/Catalogue_2009_2010.pdf), 2017. Accessed: 2017-10-24.
- 454 [36] S. R. Tonge, B. J. Tighe, Responsive hydrophobically associating poly-
455 mers: A review of structure and properties, *Advanced Drug Delivery
456 Reviews* 53 (2001) 109–122.
- 457 [37] T. H. Bayburt, S. G. Sligar, Membrane protein assembly into Nanodiscs,
458 *FEBS Letters* 584 (2010) 1721–1727.
- 459 [38] A. M. Thayer, Finding solutions, *Chemical & Engineering News Archive*
460 88 (2010) 13–18.
- 461 [39] Y. Huang, W.-G. Dai, Fundamental aspects of solid dispersion tech-
462 nology for poorly soluble drugs, *Acta Pharmaceutica Sinica B* 4 (2014)
463 18–25.