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### ► To cite this version:

Laura Marcela Forero Ramirez, Jérôme Babin, Ariane Boudier, Caroline Gaucher, Marc Schmutz, et al.. First multi-reactive polysaccharide-based transurf to produce potentially biocompatible dextran-covered nanocapsules. Carbohydrate Polymers, 2019, 224, pp.115153. 10.1016/j.carbpol.2019.115153 . hal-02267696

HAL Id: hal-02267696

<https://hal.univ-lorraine.fr/hal-02267696>

Submitted on 20 Jul 2022

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1 **First multi-reactive polysaccharide-based transurf to produce potentially**  
2 **biocompatible dextran-covered nanocapsules**

3

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18 Additional Supporting Information will be found in the online version of this article.

19

20

21 **ABSTRACT**

22 A multi-reactive polysaccharide-based transurf (acting both as macro-Chain Transfer Agent  
23 and stabilizer) was used to confine RAFT polymerization of methyl methacrylate (MMA) at  
24 the oil/water (o/w) miniemulsion interface. Dithiobenzoate groups and hydrophobic aliphatic  
25 side chains were introduced onto dextran, conferring it both transfer agent properties and  
26 ability to stabilize direct miniemulsion of MMA in the presence of a biocompatible oil, used  
27 as co-stabilizer. Because of their amphiphilic character, transurfs were initially adsorbed at the  
28 (o/w) interface and their reactive sites mediated RAFT polymerization *via* the R-group  
29 approach. PMMA-grafted dextran glycopolymers were consequently produced at the o/w  
30 interface, thus leading to dextran coverage/PMMA shell/oily core nanocapsules (NCs) as  
31 evidenced by Cryo-TEM analyses. The influence of dextran-based transurf chemistry and oil  
32 amount on MMA RAFT polymerization control was investigated. Positive preliminary results  
33 on NCs cytotoxicity suggest the potential of these objects for biomedical applications.

34 **KEYWORDS**

35 Polysaccharide  
36 Nanocapsules  
37 Transurf  
38 Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization  
39 Miniemulsion  
40 Poly(methyl methacrylate)  
41

## 42        **1. Introduction**

43    Polymer nanocapsules (NCs) have attracted greater attention in recent years because of their  
44    unique properties and potential applications in the biomedical field, for instance as drug  
45    delivery systems (Mora-Huertas, Fessi, & Elaissari, 2010; Vrignaud, Benoit, & Saulnier,  
46    2011; Poltorak, Durand, Léonard, Six, & Nouvel, 2015, Six, & Ferji, 2019). Miniemulsion  
47    polymerization is a promising technique that allows drug encapsulation and NCs production  
48    in one-pot (Steinmacher et al., 2010). To produce oily-core NCs by this technique,  
49    nanodroplets with size ranging from 50 to 500 nm are initially obtained by dispersion of a  
50    monomer/oil mixture in an aqueous phase. Nanodroplets are protected against coalescence by  
51    the presence of stabilizer and preserved from Ostwald ripening by the addition of an osmotic  
52    pressure agent (co-stabilizer), which may be the oil constituting the inner liquid core in the  
53    final NCs (Steinmacher et al., 2010; Landfester & Mailänder, 2013; Forero Ramirez et al.,  
54    2018). In such a case, oily core and polymeric shell will be formed during polymerization by  
55    means of induced-phase separation whereby polymer migrates to the droplet/water interface  
56    due to poor solubility in the oil. However, a well-defined core/shell structure can be difficult  
57    to obtain because of the strict thermodynamic and kinetic parameters involved in polymer/oil  
58    separation. Moreover, stabilizers are usually physically adsorbed at NCs surface and can be  
59    easily removed or replaced by surface-active molecules present in physiological media, which  
60    may thus affect the colloidal stability or enhance unwanted immune responses.

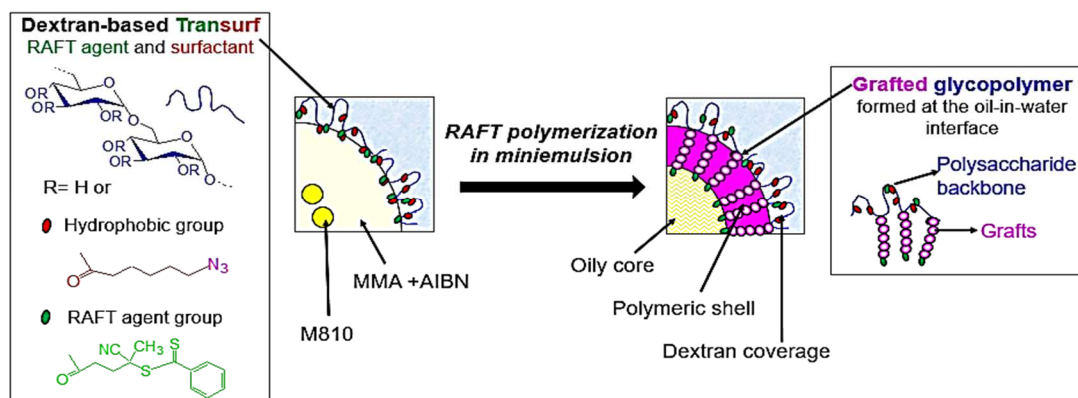
61    To overcome all the above limitations, some authors reported the combination of mono-  
62    reactive amphiphilic copolymers as miniemulsion stabilizers with Reversible-Deactivation  
63    Radical Polymerizations (RDRP) such as Atom Transfer Radical Polymerization (ATRP) or  
64    Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization to confine  
65    polymerization at the oil/water interface. While keeping their role as stabilizers, these  
66    copolymers acted either as mono-reactive inisurfs (initiators and stabilizers) (Li,

67 Matyjaszewski, Albrecht, & Möller, 2009; Li, Yoon, & Matyjaszewski, 2010; Tian et al.,  
68 2015) or transurfs (transfer agents and stabilizers) (Luo & Gu, 2007; Lu, Luo, & Li, 2010; Yu,  
69 Zhang, Zhan, & Chen, 2012). This strategy was shown to offer several advantages. Firstly,  
70 polymer chains grow directly at, or in the proximity of the interface thus allowing formation  
71 of polymeric shell at the droplet periphery. Secondly, the stabilizer provides a permanent  
72 hydrophilic covalently-linked coverage in the final NCs. Thirdly, the use of ATRP or RAFT  
73 polymerization offers the possibility of precise control of macromolecular parameters of the  
74 polymeric shell. With similar objectives, our team recently reported the first multi-reactive  
75 dextran-based inisurf used in ATRP polymerization of butyl acrylate (Wu et al., 2015) and of  
76 methyl methacrylate (MMA) (Forero Ramirez et al., 2018). In such cases, grafted dextran-  
77 based copolymers were synthesized leading to nanoparticles (NPs) with covalently-anchored  
78 hydrophilic dextran coverage. This coverage is expected to enhance NPs biocompatibility as  
79 well as to ensure their colloidal stability and stealthiness, while offering the opportunity to  
80 further functionalize their surface. Nevertheless, this ATRP process requires additional  
81 purification steps to remove remaining copper catalysts after polymerization. **We herein**  
82 **hypothesize that switching ATRP to RAFT polymerization (Scheme 1) will overcome this**  
83 **issue and will lead to the production of more suitable NCs for biomedical applications.** This  
84 new approach required the synthesis of a novel dextran derivative (transurf) carrying  
85 hydrophobic and chain transfer agent (CTA) groups and acting as stabilizer and macro-CTA  
86 during the miniemulsion process. To the best of our knowledge, it is the first time that RAFT  
87 polymerization has been performed in a miniemulsion using a multi-reactive macro-CTA and  
88 the above described dextran-based transurf is the second example thereof. In fact, one mono-  
89 reactive dextran-based macro-CTA was previously reported for vinyl acetate polymerization  
90 in emulsion, but the transurf was only formed during the polymerization process (Bernard,  
91 Save, Arathoon, & Charleux, 2008). Moreover, among all the works dealing with the use of

92 transurfs in miniemulsion polymerization, the production of oily-core NCs is scarcely related,  
93 and the preparation of PMMA-shell NCs has never been approached, which contributes to the  
94 originality of the present work.

95 **Herein, we hypothesize that using a dextran-based multi-reactive transurf can help to produce**  
96 **oily-core/PMMA shell NCs by RAFT interfacial miniemulsion polymerization** Transurfs were  
97 first designed with a precise control of hydrophobic and CTA groups numbers to provide  
98 convenient stabilizing and transfer agent properties. After model studies on MMA  
99 polymerization, RAFT polymerization of MMA in miniemulsion was carried out using such  
100 dextran-based transurfs: polymerization kinetics, molar mass control and CTA groups'  
101 efficiency were studied. Detailed characterization of NCs in terms of size distribution, amount  
102 of dextran at the surface, morphology and cytotoxicity were performed to prove the formation  
103 of oily-core NCs, usable as platform for drug delivery.

104



105

106 **Scheme 1.** Synthesis of dextran-covered oily-core NCs by MMA RAFT polymerization in  
107 miniemulsion

## 108 2. Materials and methods

### 109 2.1 Materials

110 Dextran T40 ( $\bar{M}_n = 26\ 000$  g/mol,  $\bar{D} = 1.3$ ; values determined by size exclusion  
111 chromatography coupled to a multi-angle laser light scattering detector (SEC-MALLS) in  
112 DMSO/NaNO<sub>3</sub> (0,1 M)) was purchased from Aldrich. Produced from *Leuconostoc*  
113 *mesenteroides* B 512-F, it was having very low branching (< 5% as given by the furnisher).  
114 Consequently, it could be considered as linear. MMA (99% Aldrich) was vacuum distilled  
115 from CaH<sub>2</sub>. 2,2-Azobis(isobutyronitrile) (AIBN, 99%, Aldrich) was purified by  
116 recrystallization from methanol. All those were used without further purification: N-(3-  
117 dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 99%, ABCR), 4-  
118 (dimethylamino)pyridine (DMAP, 99%, Aldrich), carbonyldiimidazole (CDI, 99%, Aldrich),  
119 2-cyanoprop-2-yl dithiobenzoate (CPDB, >97%, Aldrich), 4-cyanopentanoic acid  
120 dithiobenzoate (CPADB, >97%, Aldrich). Miglyol<sup>®</sup>810 (M810,  $d = 0.94$  g/cm<sup>3</sup>, viscosity = 28  
121 mPa.s at 20°C, water content = 0.02 wt%) was a gift from CREMER Oleo GmbH & Co.  
122 Division. M810 belonged to triacylglycerols (also called triglycerides) and was made from a  
123 mixture of saturated fatty acids (mainly caprylic acid -69.3 wt%- and capric acid -30.1 wt%),  
124 giving it a log P value of 10.2 as evaluated by group contribution methods (Ramirez, Babin,  
125 Durand, Six, & Nouvel, 2015).

### 126 2.2 Transurf synthesis

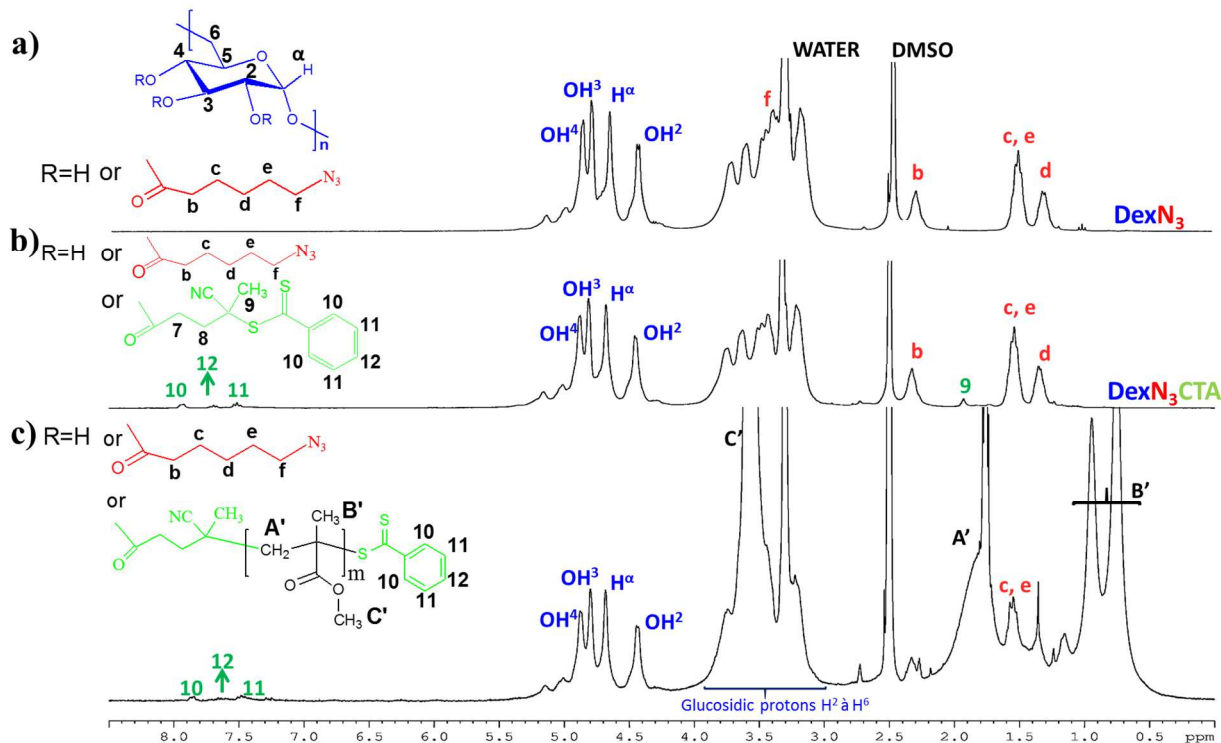
127 The transurf was synthesized in two steps. In the first step, the attachment of azide end-  
128 functionalized side chains onto dextran was performed in DMSO by reaction of 6-  
129 azidohexanoic acid activated with carbonyldiimidazole as previously described leading to  
130 DexN<sub>3</sub>- $\tau$  derivatives (Laville et al., 2013). In the second step, introduction of dithiobenzoate  
131 CTA groups was performed using classic EDC/DMAP catalyzed esterification and DexN<sub>3</sub>-  
132  $\tau$ CTA $\gamma$  transurfs were obtained (see Supporting Information).  $\tau$  and  $\gamma$  correspond to the

133 number of N<sub>3</sub>-end alkyl chains (N<sub>3</sub>) and dithiobenzoate CTA groups per 100 glucopyranosic  
 134 units, respectively, (i. e. Substitution Degrees) and were estimated by <sup>1</sup>H NMR in DMSO-*d*<sub>6</sub>  
 135 using equations 1 and 2 (Figure 1). A<sub>N<sub>3</sub></sub> corresponds to the area of 6 methylene protons of alkyl  
 136 chains (1.2 to 1.7 ppm), A<sub>R</sub> is the area of the 5 aromatic protons of dithiobenzoate groups (7.4  
 137 to 8.1 ppm) and A<sub>UG</sub> (4.3 to 5.2 ppm) corresponds globally to 4 protons per glucopyranosic  
 138 unit, whatever the modification yield. The chemical shift of protons “f” was equal to 3.3 ppm,  
 139 according the <sup>1</sup>H NMR spectrum of the 6-azidohexanoic acid in DMSO-*d*<sub>6</sub> (not shown).

$$140 \quad \tau = \frac{A_{N_3}/6}{A_{UG}/4} \times 100 \quad (1)$$

$$141 \quad \gamma = \frac{A_R/5}{A_{UG}/4} \times 100 \quad (2)$$

142



143  
 144 **Figure 1.** <sup>1</sup>H NMR spectra (DMSO-*d*<sub>6</sub>) of a) DexN<sub>3-20</sub>, b) DexN<sub>3-20</sub>CTA<sub>3.7</sub> and c) DexN<sub>3-20</sub>-g-  
 145 1.8PMMA<sub>7800</sub>.



146        2.3 RAFT polymerization of MMA in miniemulsion

147 Typical experimental conditions were as follows:  $[\text{DexN}_3\text{-}\tau\text{CTA}\gamma]_0 = 10 \text{ g/L}$ ,  $\text{DexN}_3\text{-}$   
148  $\tau\text{CTA}\gamma$  /organic phase (MMA + M810) was varied from 13 to 36 wt% and M810/MMA from  
149 10 to 50 vol.%.  $[\text{CTA groups}]_0/[\text{AIBN}]_0$  molar ratio was fixed to 3. For example, in case of  
150  $\text{DexN}_{3-20}\text{CTA}_{3.7}$  transurf and  $\text{M810/MMA} = 10 \text{ vol.}\%$  (Run 1, Table 1),  $\text{DexN}_3\text{-}$   
151  $_{20}\text{CTA}_{3.7}$ /organic phase (MMA + M810) was 13.2 wt%. 150 mg of  $\text{DexN}_{3-20}\text{CTA}_{3.7}$  were  
152 dissolved in 15 mL of MilliQ water. The organic phase was composed of 1.1 mL (10.3 mmol)  
153 of MMA, 110  $\mu\text{L}$  of M810 and 1.3 mg ( $7.9 \times 10^{-3}$  mmol) of AIBN. The aqueous phase was  
154 poured into this organic phase and the mixture was sonicated in an ice bath during 2 min  
155 (51% amplitude (power 46 W), pulsed mode) using a Vibracell 600W (Sonics & Materials,  
156 Dantbury, CT). Droplets size of the resulting miniemulsion was measured immediately after  
157 sonication. The miniemulsion was then transferred to a Schlenk tube covered with a 3-way  
158 stopcock and purged with a little nitrogen flow during 45 min at 10 °C. The reactor was then  
159 immersed in a thermostatted oil bath at 80 °C to start polymerization. To follow reaction  
160 kinetics and evolution of molar masses and particles size, the resulting suspensions were  
161 divided in three parts. The first one was kept apart for size measurements. The second one  
162 was used to determine monomer conversion (x) by a gravimetric method after vacuum drying  
163 for 24 hours. NCs were washed twice with THF then dried to obtain the grafted  
164 glycopolymers named  $\text{DexN}_3\text{-}\tau\text{-g-}n\text{PMMA}_{\bar{M}_n}$ , where n is the number of PMMA grafts  
165 (having an average molecular weight equal to  $\bar{M}_n$ ) per 100 glucopyranosic units, respectively.  
166 In order to study PMMA grafts, the dextran backbone of such glycopolymers was completely  
167 degraded under basic conditions as previously reported (Dupayage, Save, Dellacherie,  
168 Nouvel, & Six, 2008). The uncoupled PMMA grafts were analyzed by  $^1\text{H}$  NMR in  $\text{DMSO-}d_6$   
169 to verify the absence of dextran backbone. Their molar masses were then characterized by  
170 SEC-MALLS in THF. The remaining suspension was centrifuged. Recovered NCs were

171 washed twice and dispersed in deionized water for biological tests, whereas the floating was  
172 analyzed by anthrone titration (Wu et al., 2015) to estimate the quantity of DexN<sub>3</sub>- $\tau$ CTA $\gamma$   
173 remaining in aqueous solution. By difference with the initially introduced amount, one can  
174 estimate the quantity of dextran derivative adsorbed at the NC surface.

#### 175 *2.4 Characterization*

176 <sup>1</sup>H NMR and 2D DOSY <sup>1</sup>H NMR spectra were recorded on a Bruker Avance 300 apparatus  
177 (30,013 MHz, 25 °C) in DMSO-*d*<sub>6</sub>. 1024 Scans were recorded.

178 Droplet and NCs sizes were measured by using a Laser Diffraction Particle Size Analyzer  
179 (Mastersizer 2000 Malvern Instruments). Washed NCs suspension (8 wt% solid content) was  
180 diluted by a factor of 200 and NCs morphology was observed by cryo-Transmission Electron  
181 Microscopy (cryo-TEM) as reported (Forero Ramirez et al., 2018).

182

183 SEC-MALLS analyses of PMMA chains were performed in THF at 40°C. Refractive index  
184 increment (dn/dc) of 0.087 mL.g<sup>-1</sup> was used (See Supporting Information).

185 NCs cytocompatibility was evaluated on rat vascular smooth muscle cell line (A-10, ATCC\_  
186 CRL-1476™) (See Supporting Information).

187

### 188 **3. Results and discussion**

#### 189 *3.1 DexN<sub>3</sub>- $\tau$ CTA $\gamma$ transurf*

190 To the best of our knowledge, relatively few examples of polysaccharides bearing RAFT  
191 functionality have been described in the literature and only 4 examples were based on dextran.  
192 One xanthate-end functionalized dextran RAFT agent was prepared by click chemistry  
193 (Bernard et al., 2008) and three multi-reactive dextran-based macro-CTA were obtained by

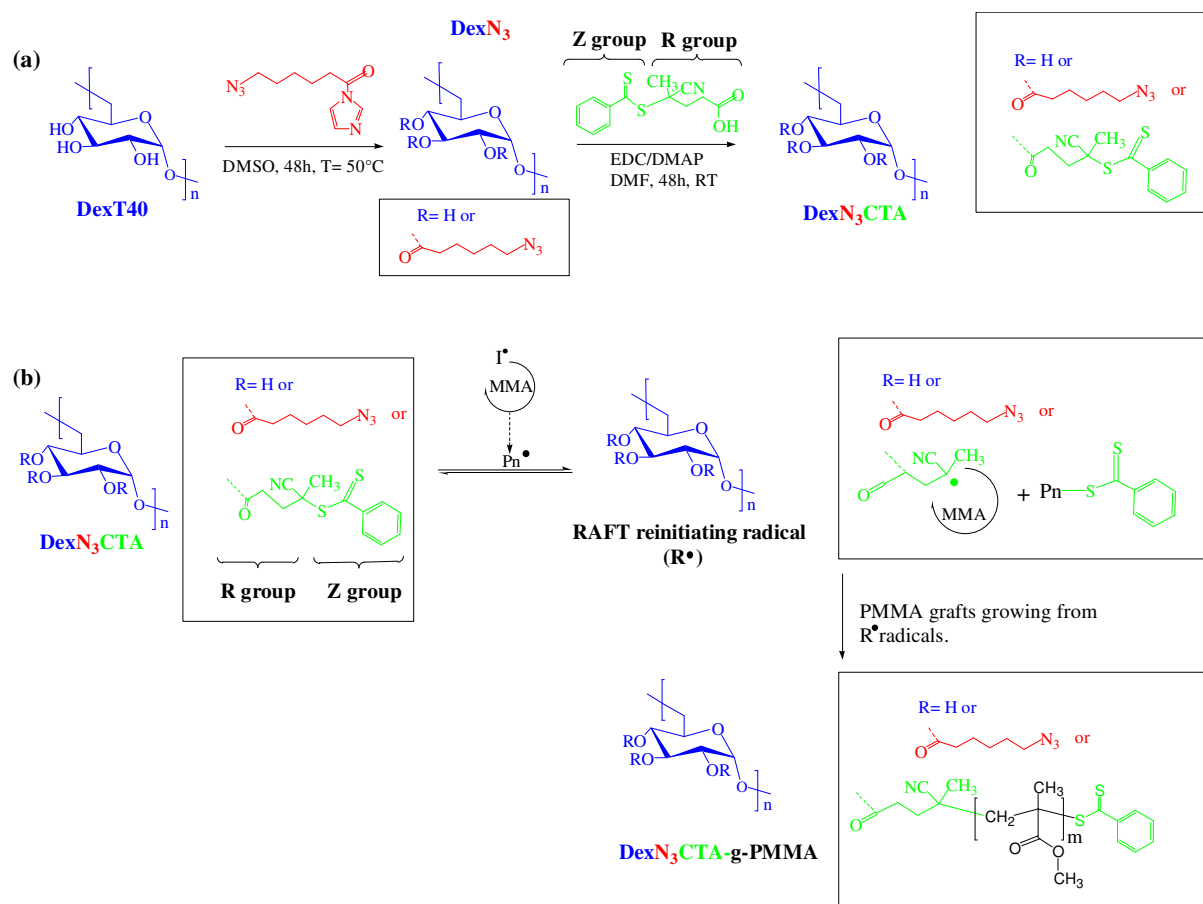
194 esterification (Duong et al., 2012; Ferji, Venturini, Cleymand, Chassenieux, & Six, 2018,  
195 Karmakar et al., 2018). Nevertheless none of them was also used as stabilizer in miniemulsion  
196 polymerization.

197 Herein, the first multi-reactive dextran-based transurf for a RAFT miniemulsion  
198 polymerization was synthesized and called  $\text{DexN}_3\text{-}\tau\text{CTA}\gamma$ , where  $\tau$  and  $\gamma$  correspond to the  
199 number of  $\text{N}_3$ -end alkyl chains ( $\text{N}_3$ ) and of dithiobenzoate CTA groups per 100  
200 glucopyranosic units, respectively (Scheme 2a). On the one hand,  $\text{N}_3$ -end alkyl chains have  
201 been chosen as hydrophobic groups due to their efficiency to hydrophobize dextran, leading to  
202 amphiphilic  $\text{DexN}_3\text{-}\tau$  dextran derivatives exhibiting surfactant properties (see Supporting  
203 Information). These derivatives have been successfully used to produce dextran-covered NPs  
204 via emulsion/evaporation (Laville et al., 2013; Poltorak et al., 2015) or nanoprecipitation  
205 (Laville et al., 2013) processes. In addition, azide functionality of such  $\text{DexN}_3\text{-}\tau$  is expected to  
206 be suitable for potential post-modification of final NPs surface by click chemistry.

207

208

209



210

211 **Scheme 2.** a) Synthesis of DexN<sub>3</sub>-τCTAγ transurfers. b) RAFT polymerization of MMA from  
 212 DexN<sub>3</sub>-τCTAγ *via* the R-group approach.

213

214 On the other hand, a dithiobenzoate group bearing cyano functionality was chosen as the  
 215 reactive side group. Indeed, molecular CTAs having similar structure (i.e. CPDB and  
 216 CPADB) showed remarkable efficiency and high degree of control over MMA RAFT  
 217 polymerizations in homogeneous (Biasutti, Davis, Lucien, & Heuts, 2005; Johnston-Hall,  
 218 Stenzel, Davis, Barner-Kowollik, & Monteiro, 2007) and heterogeneous media (Yang, Luo,  
 219 Liu, & Li, 2009; Zhou, Ni, & Yu, 2007). CTA groups were introduced (through R-group) by  
 220 reacting previous DexN<sub>3</sub>-τ with CPADB in the presence of EDC and a catalytic amount of  
 221 DMAP, resulting in DexN<sub>3</sub>-τCTAγ transurfers (Scheme 2a). No degradation of the dextran  
 222 backbone was evidenced as attested by SEC-MALLS analysis (Table S1). The integrity of  
 223 grafted CTA groups was as well confirmed by <sup>1</sup>H NMR spectroscopy as characteristic protons

224 of both Z and R moieties clearly appeared (Figure 1b), thus ensuring its reactivity as RAFT  
225 agent. Modification degree of CTA groups ( $\gamma$ ) could be tuned from 2 to 8% according to the  
226 experimental conditions. Those results were in quite good agreement with those previously  
227 obtained from native dextran (Duong et al., 2012).

228 To be used as transurfs, the designed DexN<sub>3</sub>- $\tau$ CTA $\gamma$  were expected to exhibit both surfactant  
229 and transfer agent properties. To check their surface activity, steady state surface tension  
230 measurements were performed with different series of DexN<sub>3</sub>- $\tau$  and DexN<sub>3</sub>- $\tau$ CTA $\gamma$  (Figure  
231 S1). DexN<sub>3</sub>- $\tau$  ( $9 < \tau < 27$ ) and DexN<sub>3</sub>-<sub>20</sub>CTA $\gamma$  transurfs ( $\tau=20$  and  $\gamma=3.7$  or  $7.5$ ) were able to  
232 adsorb at the air/water interface and to reduce the surface tension of aqueous solutions. In the  
233 case of transurfs, the contribution of CTA groups to the decrease of surface tension seems less  
234 than that of N<sub>3</sub>-end alkyl groups, at least in the range of CTA modification degree tested here,  
235 i. e. below 8%. In fact, similar curves have been observed in case of DexN<sub>3</sub>-<sub>24</sub> ( $\tau=24$ ), DexN<sub>3</sub>-  
236 <sub>20</sub>CTA<sub>3.7</sub> (global modification degree of  $\tau + \gamma \sim 24$ ) and DexN<sub>3</sub>-<sub>20</sub>CTA<sub>7.5</sub> ( $\tau + \gamma \sim 27$ ), whereas  
237 a bigger decrease in surface tension was observed for DexN<sub>3</sub>-<sub>27</sub> ( $\tau=27$ ). Even if CTA groups  
238 have global hydrophobic character ( $\log P \sim 3.4$  as calculated by group contribution methods,  
239 Ramirez., Babin, Durand, Six, & Nouvel, 2015 ), these results could be ascribed to the  
240 presence of thiocarbonylthio and cyano functions in CTA groups in the middle of the chain,  
241 that make them less prone to segregate towards oil phase, with respect to N<sub>3</sub>-end alkyl chains.  
242 Nevertheless, we can expect that the hydrophobic character of those CTA groups ( $\log P \sim 3.4$ )  
243 is high enough to allow direct contact of dithiobenzoate groups with the oil phase ( $\log P$   
244 ranging between 1.8 and 2.4 for initial droplet phase with M810/MMA ratio ranging from 10  
245 to 50 vol.%), thus enabling their role as transurfs.

246        *3.2 Miniemulsion polymerization using dextran-based transurf*

247    After checking both the stability of the MMA miniemulsion containing M810 (a  
248    pharmaceutically acceptable triglyceride oil (Hippalgaonkar, Majumdar, & Kansara,  
249    2010))/DexN<sub>3-τ</sub> as co-stabilizer/stabilizer system under polymerization process, and the  
250    control of the MMA RAFT polymerization in miniemulsion using model conditions  
251    (molecular CTA + no-reactive DexN<sub>3-τ</sub> stabilizer) (see Supporting Information), optimized  
252    conditions were applied to DexN<sub>3-τ</sub>CTAγ transurfs. Our main challenge was to evaluate the  
253    potential of the transurf to act both as oil/water interface stabilizer and macro-CTA to  
254    generate DexN<sub>3-τ</sub>-g-*n*PMMA<sub>M<sub>n</sub></sub> at the organic interface, in order to produce dextran-covered  
255    NCs with PMMA shell and M810 oily core. Thus, we first verified both the availability of  
256    CTA groups at the interface and their effective participation in RAFT polymerization.  
257    Secondly, we evaluated the effects of M810 content and transurf structure on the  
258    polymerization kinetics and control.

259    Table 1 summarizes all experimental recipes used in the current study, as well as the average  
260    size of initial nanodroplets and final NCs. In any case, latexes were very stable during  
261    polymerization procedure. Distribution size of the final NCs were roughly similar to the one  
262    of the initial nanodroplets, in agreement with preliminary stability assays performed with  
263    DexN<sub>3-20</sub> (see Supporting Information). Depending on experimental conditions, and especially  
264    on M810 volume ratio, final solid content varied from 8 to 13 wt% in water.

265 **Table 1.** Miniemulsion polymerization of MMA at 80°C using DexN<sub>3</sub>-τCTAγ transurf or DexN<sub>3-20</sub> stabilizer with 1.1 mL of MMA. [DexN<sub>3</sub>-  
266 τCTAγ(or DexN<sub>3-τ</sub>)] = 10 g/L and [CTA]<sub>0</sub>/[AIBN]<sub>0</sub> = 3.

Run	(DexN <sub>3</sub> -τCTAγ or DexN <sub>3-τ</sub> )/organic phase (wt%)	[AIBN] <sub>0</sub>	M810/MMA (vol.%)	Stabilizer or Transurf	DexN <sub>3</sub> -τCTAγ adsorbed (%) <sup>(a)</sup>	[MMA] <sub>0</sub> /[CTA] <sub>0</sub> (or X) <sup>(b)</sup>	Nano-objects diameter (nm) <sup>(c)</sup>		Grafts number (n) <sup>(d)</sup>	Eff <sub>CTA</sub> (%) <sup>(e)</sup>	F <sub>Homo</sub> <sup>(f)</sup>
							After degassing	Final			
0	13.2	6.7	10	DexN <sub>3-20</sub>	-	-	113 (1.30)	110 (1.10)	-	-	-
1	13.2	6.7	10	DexN <sub>3-20</sub> CTA <sub>3,7</sub>	94	393	102 (1.00)	102 (0.83)	1.8±0.3	48	20
2	11.6	5.7	25	DexN <sub>3-20</sub> CTA <sub>3,7</sub>	94	393	112 (1.49)	109 (1.30)	1.7±0.3	45	25
3	9.7	3.5	50	DexN <sub>3-20</sub> CTA <sub>3,7</sub>	92	402	117 (1.37)	119 (1.14)	1.8±0.3	48	25
4	11.6	11.9	25	DexN <sub>3-20</sub> CTA <sub>7,5</sub>	94	204	105 (1.31)	110 (1.30)	3.5±0.3	47	17

267 <sup>a</sup>Relative molar amount in NCs. Expressed as function of the initially introduced DexN<sub>3</sub>-τCTAγ.

268 <sup>b</sup>Initial molar ratio in the organic phase, estimated with the real amount of DexN<sub>3</sub>-τCTAγ at the interface evaluated in (a), and supposing that all the CTA groups were accessible  
269 for polymerization.

270 <sup>c</sup>Average nano-object diameter. Values under brackets correspond to Span (width of the distribution) calculated using  $Span = (D(0.9) - D(0.1)) / D(0.5)$ . D(0.1), D(0.5) and D(0.9)  
271 were the particle diameters at 10%, 50% and 90% of the particle volume size distribution.

272 <sup>d</sup>Average number of PMMA grafts per 100 glucopyranosic units estimated from <sup>1</sup>H NMR spectrum (Figure 1c) using  $n = [(4A_{A'B'}/5A_{UG}) \times \bar{M}_{n(SEC)DEX}/162] / \bar{X}_n$ . A<sub>A'B'</sub> and A<sub>UG</sub>  
273 are the areas under peaks (A'B') and (OH<sup>1</sup>, OH<sup>2</sup>, OH<sup>3</sup> and H<sup>α</sup>), respectively.  $\bar{M}_{n(SEC)DEX}$  is the  $\bar{M}_n$  of native dextran, 162 the molecular weight of one glucopyranosic unit and  $\bar{X}_n$  is  
274 the average polymerization degree of PMMA grafts.

275 <sup>e</sup>Average CTA groups efficiency for transurfs estimated using  $Eff_{CTA} = n/\gamma \times 100$ , where n and γ are the number of grafts and CTA groups per 100 glucopyranosic units,  
276 respectively.

277 <sup>f</sup>Weight fraction of PMMA homopolymers (non-attached to dextran) with respect to the total synthesized PMMA using  $F_{Homo} = [1 - [(m_{CTA}/M_{UG-CTA}) \times n \times \bar{M}_{n(SEC)grafts}] /$   
278  $(m_{MMA}x)] \times 100$ , with  $m_{CTA}$  the mass amount of DexN<sub>3</sub>-τCTAγ transurf at NCs surface,  $M_{UG-CTA}$  the molecular weight of one glucopyranosic unit of DexN<sub>3</sub>-τCTAγ, n the average  
279 number of PMMA grafts per 100 glucopyranosic units evaluated in (d),  $\bar{M}_{n(SEC)grafts}$  the  $\bar{M}_n$  of PMMA grafts,  $m_{MMA}$  the initial mass amount of monomer and x the monomer  
280 conversion.  $F_{Homo}$  was evaluated at two different conversions and average values are given.

281        3.2.1 *Relative amount of transurfs at nanodroplets/NCs surface.*

282    In the early stage of RAFT polymerization, DexN<sub>3</sub>-τCTAγ transurfs were located at the  
283    monomer/water interface because of their surfactant properties (Figure S1) and mediated  
284    RAFT polymerization from their multiple reactive sites. As CTA groups were attached to  
285    dextran molecules via their R moieties, re-initiating radicals (R<sup>•</sup>) were situated on the dextran  
286    backbone after the first addition-fragmentation step (Scheme 2b). Therefore, PMMA chains  
287    grew directly from the polysaccharide, via the R-group approach, to produce DexN<sub>3</sub>-τ-g-  
288    *n*PMMA <sub>$\bar{M}_n$</sub>  at the oil/water interface that will constitute the NCs surface/polymeric shell at the  
289    end.

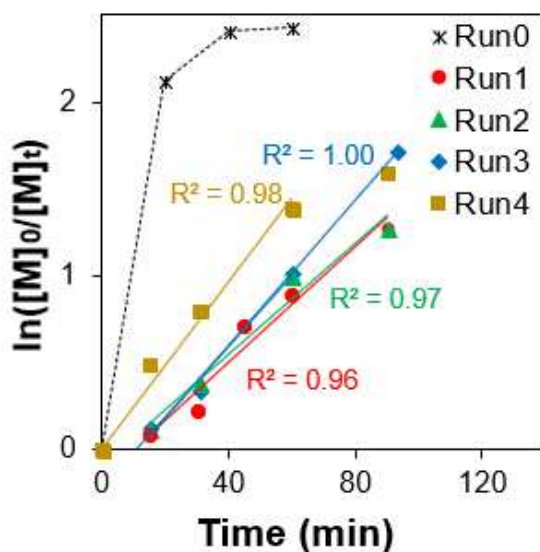
290    For Runs 1-4 (Table 1), [MMA]<sub>o</sub>/[CTA]<sub>o</sub> molar ratio (X) was estimated by taking into  
291    account the total amount of monomer in the system and the quantity of DexN<sub>3</sub>-τCTAγ  
292    adsorbed at nanodroplets surface. This last quantity was considered in first approximation to  
293    be similar to the one at the surface of the final NCs, and was indirectly determined after NCs  
294    washing by dosage of the DexN<sub>3</sub>-τCTAγ remaining in aqueous supernatant. In all cases, more  
295    than 90% of initial transurf was found to be located at the interface (Table 1). In contrast,  
296    similar analysis of PMMA latexes obtained with the model system (see Supporting  
297    Information) showed that only 60% of DexN<sub>3-20</sub> was present in final NCs. This result could be  
298    surprising as polymerization conditions and specially [DexN<sub>3</sub>-τ]/[organic phase] weight ratio  
299    were chosen to avoid the presence of stabilizer into the aqueous phase, so as to prevent  
300    micellar or homogeneous nucleation. Nevertheless, in the model system, DexN<sub>3-20</sub> desorption  
301    was more prone to occur under polymerization procedure or during particle washings as the  
302    stabilizer was only physically adsorbed onto NCs surface, whereas covalent linkage between  
303    dextran backbone and PMMA chains was expected to be formed in the case of transurfs, from  
304    the first moments of the polymerization. Transurf derivatives should thus be immobilized at



305 NCs surface *via* their CTA groups, which would limit their desorption and explain their  
306 quantitative presence in final NCs.

### 307 3.2.2 Kinetics studies.

308 Figure 2 shows the first-order kinetic plots for RAFT polymerization in miniemulsion of  
309 MMA using DexN<sub>3</sub>- $\tau$ CTA $\gamma$  transurf. A linear relationship between  $\ln([M]_0/[M]_t)$  and  
310 polymerization time was observed until at least 70% conversion, whatever the transurf or the  
311 M810 content. In addition, similar polymerization kinetics with reproducible delay period  
312 were observed when increasing M810 amount from 10 vol.% to 50 vol.% compared to MMA,  
313 while keeping  $[MMA]_0/[CTA]_0$  constant (Runs 1-3). This result was quite surprising as these  
314 experiments were carried out with the same transurf,  $[CTA]_0/[AIBN]_0$  ratio (equal to 3), and  
315 MMA volume.



316

317 **Figure 2.**  $\ln([M]_0/[M]_t)$  versus time. RAFT polymerization of MMA in miniemulsion at  
318 80°C using DexN<sub>3</sub>- $\tau$ CTA $\gamma$  transurfs (or DexN<sub>3-20</sub> stabilizer). (\*) Run 0, DexN<sub>3-20</sub>,  $[CTA]_0 = 0$ ,  
319 10 vol.% M810 (●) Run 1, DexN<sub>3-20</sub>CTA<sub>3.7</sub>, X = 393, 10 vol.% M810 (◆) Run 2, DexN<sub>3-</sub>  
320 <sub>20</sub>CTA<sub>3.7</sub>, X = 393, 25 vol.% M810 (▲) Run 3, DexN<sub>3-20</sub>CTA<sub>3.7</sub>, X = 402, 50 vol.% M810 (■)  
321 Run 4, DexN<sub>3-20</sub>CTA<sub>7.5</sub>, X = 204, 25 vol.% M810 (see Table 1).

322

323 In such conditions, AIBN initial mass remained constant while organic phase global volume  
324 increased with the amount of M810. Polymerization rate was thus expected to slow down  
325 when increasing M810/MMA volume ratio as a result of the reduction of AIBN concentration  
326 inside nanodroplets. Nevertheless, as observed in stability studies, the increase of M810  
327 content from 10 to 50% vol.% (which corresponded to initial organic phase volumes of 1.1  
328 mL and 1.65 mL, respectively) resulted only in a slight increment of initial nanodroplets  
329 average size (from 102 to 117 nm) (see Supporting Information). As a consequence, the  
330 number of nanodroplets considerably increased from one experiment to another, which led to  
331 faster polymerization kinetics and, hence, offset the effect of AIBN concentration decrease,  
332 resulting in similar kinetics.

333 As for the model system (see Supporting Information), both studied DexN<sub>3</sub>- $\tau$ CTA $\gamma$  transurfs  
334 appeared to slow down the miniemulsion polymerization of MMA. Indeed, DexN<sub>3-20</sub>CTA<sub>3.7</sub>  
335 ([AIBN]<sub>o</sub> = 5.7 mM, Run 2) and DexN<sub>3-20</sub>CTA<sub>7.5</sub> ([AIBN]<sub>o</sub> = 11.9 mM, Run 4) showed much  
336 slower kinetics when compared to a conventional radical polymerization system ([AIBN]<sub>o</sub> =  
337 6.7 mM, Run 0). This slow down effect suggested both the availability of CTA groups at the  
338 oil/water interface and their effective involvement in growth of PMMA chains. As expected,  
339 DexN<sub>3-20</sub>CTA<sub>7.5</sub> (Run 4) gave faster polymerization rate than DexN<sub>3-20</sub>CTA<sub>3.7</sub> (Run 2). Since  
340 DexN<sub>3-20</sub>CTA<sub>7.5</sub> was bearing more CTA groups, AIBN concentration was almost doubled in  
341 Run 4 with respect to Run 2 to keep constant the [CTA]<sub>o</sub>/[AIBN]<sub>o</sub> ratio. Furthermore, no  
342 delay period was observed with DexN<sub>3-20</sub>CTA<sub>7.5</sub> once again probably because of the higher  
343 initiator amount used in this experiment.

### 344 3.2.3 DexN<sub>3</sub>- $\tau$ -g-**nPMMA** $\bar{M}_n$ analysis.

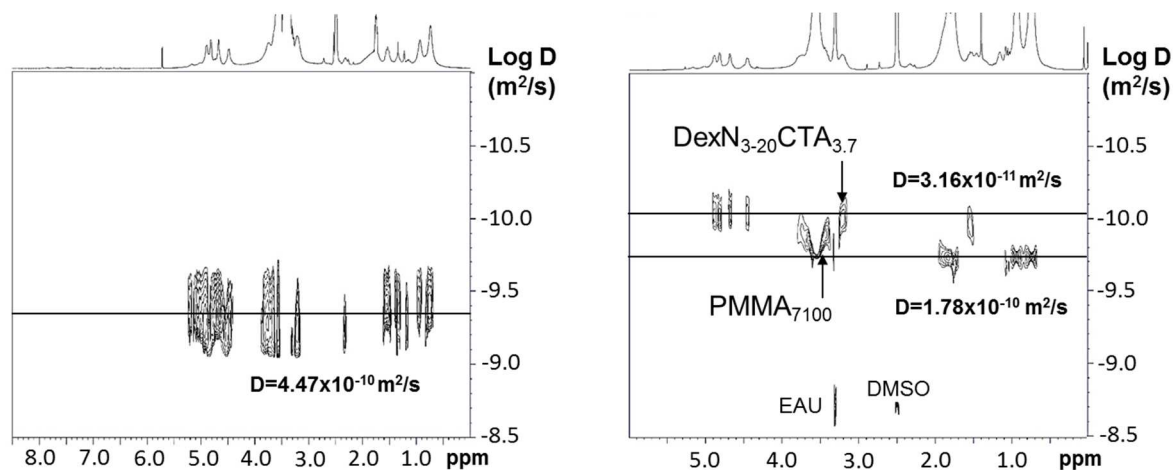
345 Attempting to confirm the covalent linkage between PMMA shell and dextran coverage of  
346 NCs, DexN<sub>3</sub>- $\tau$ -g-**nPMMA** $\bar{M}_n$  glycopolymers produced at the oil/water interface were purified

347 from M810 and from free PMMA homopolymers (not attached to dextran), by several  
348 washings of dried NCs with THF. These glycopolymers were also characterized with their  
349 weight fraction of PMMA ( $F_{PMMA}$ ) determined by equation 3, where  $M_{UG-CTA}$  is the  
350 molecular weight of one glucopyranosic unit of DexN<sub>3</sub>-τCTAγ.

$$351 \quad F_{PMMA} = \frac{n\bar{M}_n}{n\bar{M}_n + 100M_{UG-CTA}} \quad (3)$$

352 For copolymers produced in Runs 1 – 4,  $n$  was estimated from <sup>1</sup>H NMR spectra in DMSO-*d*<sub>6</sub>  
353 (Table 1). <sup>1</sup>H NMR spectrum of DexN<sub>3-20</sub>-g-1.8PMMA<sub>7800</sub> elaborated from DexN<sub>3-20</sub>CTA<sub>3.7</sub>  
354 (Run 3) is given in Figure 1c as example. Typical signals of dextran and PMMA parts are  
355 clearly observed, especially methyl (B', 0.5-1.0 ppm), methylene (A', 1.5-2.0 ppm) and O-  
356 CH<sub>3</sub> proton peaks (C', 3.6 ppm) attributed to the repeating unit of PMMA grafts, and  
357 hydroxyl and anomeric proton peaks (4.1-5.4 ppm) from the dextran backbone. Unfortunately,  
358 ester linkages between the two parts could not be proven by this method. DexN<sub>3-τ</sub>-g-  
359  $nPMMA_{\bar{M}_n}$  were therefore studied by 2D Diffusion-Ordered Spectroscopy (DOSY) <sup>1</sup>H NMR  
360 (Figure 3). This technique allowed separation of the NMR signals of different species  
361 according to their diffusion coefficient. It has previously been used to discriminate between a  
362 grafted copolymer and the corresponding homopolymer mixture (Soliman, Colombeau,  
363 Nouvel, Babin, & Six, 2016). On the one hand, the 2D DOSY <sup>1</sup>H NMR spectrum of DexN<sub>3-20</sub>-  
364 g-1.8PMMA<sub>7800</sub> was recorded in DMSO-*d*<sub>6</sub> (Figure 3a). As shown, a single self-diffusion  
365 coefficient was observed for all the protons from both dextran and PMMA parts ( $D = 4.47 \times$   
366  $10^{-10} \text{ m}^2 \text{ s}^{-1}$ ), which suggested that PMMA grew effectively from CTA groups on dextran. On  
367 the other hand, a mixture (same weight composition as for the copolymer DexN<sub>3-20</sub>-g-  
368 1.8PMMA<sub>7800</sub>) of the precursor DexN<sub>3-20</sub>CTA<sub>3.7</sub> transurf and a PMMA homopolymer having  
369 roughly the same average molecular weight of PMMA grafts was also analyzed (Figure 3b).  
370 In this last case, 2D DOSY spectrum provided two different diffusion coefficients ( $D = 3.16 \times$

371  $10^{-11} \text{ m}^2 \text{ s}^{-1}$  and  $D = 1.78 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ ) for transurf and PMMA, respectively. The presence of  
 372 these two diffusion coefficients, compared to only one (at a different value) in case of DexN<sub>3</sub>-  
 373 20-g-1.8PMMA<sub>7800</sub>, proved that PMMA grafts were really covalently linked to dextran  
 374 backbone, resulting in a DexN<sub>3</sub>- $\tau$ -g-*n*PMMA <sub>$\bar{M}_n$</sub>  glycopolymer.



375  
 376 **Figure 3.** 2D DOSY <sup>1</sup>H NMR spectra of a) DexN<sub>3</sub>-20-g-1.8PMMA<sub>7800</sub> ( $F_{\text{PMMA}} = 43\%$ ) obtained  
 377 from Run 3 (Table 1) at 12% of conversion and b) mixture of PMMA<sub>7100</sub> and DexN<sub>3</sub>-20CTA<sub>3.7</sub>  
 378 (43/57; w/w).

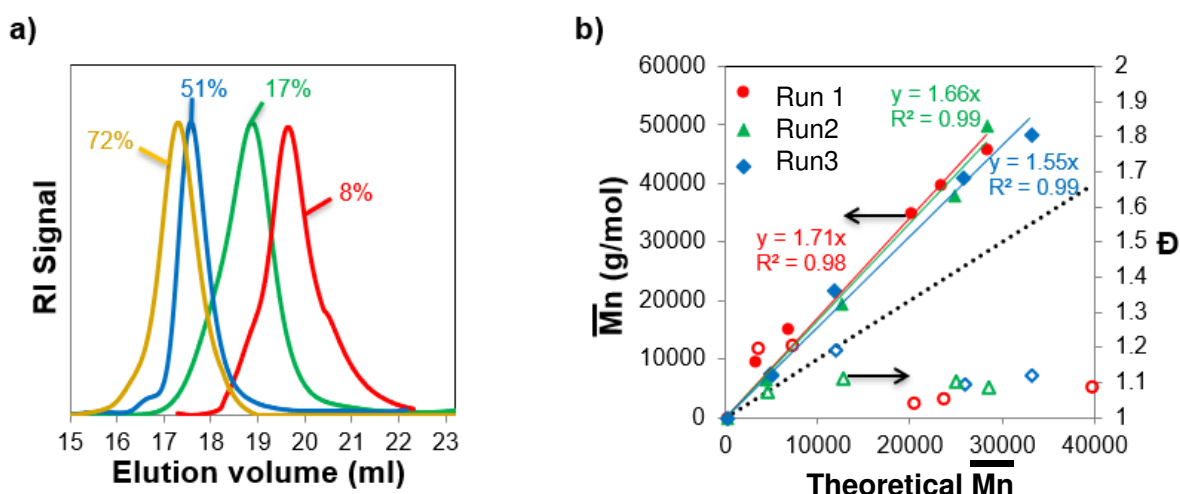
379  
 380 Finally, we wanted to characterize those DexN<sub>3</sub>- $\tau$ -g-*n*PMMA <sub>$\bar{M}_n$</sub>  glycopolymers by SEC-  
 381 MALLS. Nevertheless, it was difficult to find an appropriate solvent able to well solubilize  
 382 both the hydrophilic polysaccharide and the hydrophobic PMMA parts. Despite solubility  
 383 problems resulting in the appearance of numerous aggregate peaks on the SEC trace,  
 384 chromatograms of purified DexN<sub>3</sub>- $\tau$ -g-*n*PMMA <sub>$\bar{M}_n$</sub>  (i.e. after washing with THF- Figure S6)  
 385 clearly showed a shift of the molecular weight distribution toward higher molar masses in  
 386 comparison with the chromatogram of the precursor transurf, which further attested to the  
 387 copolymer formation. Nonetheless, accurate average molar masses were not accessible from  
 388 these SEC-data due to the above-mentioned solubility issues. Non-purified DexN<sub>3</sub>- $\tau$ -g-  
 389 *n*PMMA <sub>$\bar{M}_n$</sub>  (i.e. before THF washings) were also investigated. In comparison with  
 390 chromatograms of purified glycopolymers, an additional smaller peak evolving with

391 conversion was detected at high elution time. This peak, disappearing after purification, was  
392 found to correspond to PMMA homopolymers (non-attached to dextran) formed during  
393 polymerization, as confirmed by  $^1\text{H}$  NMR spectrum of the product extracted with THF. This  
394 observation was not a surprise. The production of a small percentage of homopolymer is  
395 inherent to the RAFT process since chains are initiated by AIBN decomposition. Thus, in  
396 addition to PMMA chains re-initiated by CTA groups and growing from the dextran backbone  
397 at the oil/water interface, there is also a small fraction of PMMA chains coming from AIBN  
398 and propagating within the organic phase. Furthermore, irreversible transfer reactions could  
399 also eventually lead to homopolymer formation. For all experiments, homopolymer fraction  
400 ( $F_{Homo}$ ) was evaluated to about 20 wt% of the total produced polymer amount (Table 1),  
401 which remains quite reasonable and below the maximum amount evaluated for a  
402  $[\text{CTA}]_0/[\text{AIBN}]_0$  ratio of 3. We can expect that this relatively low fraction would not have a  
403 significant adverse impact on the desired core-shell morphology control.

#### 404 3.2.4 Polymerization control and CTA groups efficiency.

405 In order to ascertain the degree of RAFT polymerization control, PMMA grafts were  
406 deliberately uncoupled from dextran backbone, then characterized by SEC-MALLS. Total  
407 PMMA recovery was achieved under basic conditions already proven to be harmless for  
408 PMMA grafts (Dupayage et al., 2008; Forero Ramirez et al., 2018). SEC of uncoupled  
409 PMMA chains exhibited monomodal distributions at low conversion and a clear shift toward  
410 higher molar masses with conversion (Figure 4a). For conversions above 50%, a small  
411 molecular weight shoulder was however observed suggesting a small proportion of  
412 irreversible deactivation by bimolecular termination between two PMMA grafts, or more  
413 probably between a PMMA graft and a PMMA homopolymer. When comparing  
414 polymerizations carried out with the same transurf but different M810 contents, similar SEC  
415 traces were observed at analogous conversions indicating that M810 amount has no influence

416 on RAFT polymerization control. Furthermore, molar mass distributions of PMMA  
 417 homopolymers (unlinked to dextran) overlaid pretty well with those of PMMA grafts obtained  
 418 at the same conversion (Figure S7). This showed that the exchange of thiocarbonylthio groups  
 419 between grafted and free PMMA chains was quite effective to allow the control of  
 420 macromolecular parameters of both types of chains. Overall these results indicated the  
 421 absence of noticeable transfer or termination reactions, except a small proportion of  
 422 termination by recombination of PMMA grafts (no shoulder was detected in case of  
 423 homopolymers). Bimolecular termination between a PMMA graft and a PMMA  
 424 homopolymer was more prone to occur at high conversion and with consequent amount of  
 425 M810 in the organic phase. Actually, sufficient amount of M810 in the organic phase was  
 426 expected to favor phase segregation between the oily core and the polymer shell (see 3.3), and  
 427 thus, the geographic proximity between free PMMA chains (growing into the organic phase)  
 428 and PMMA grafts (growing at the interface). Bimolecular termination between two PMMA  
 429 grafts was less probable to happen. Furthermore, such termination would have resulted in the  
 430 reticulation of the system, which was never observed in any case.



431  
 432 **Figure 4.** Evolution of a) SEC traces (Refractive Index detector) of PMMA grafts (Run 2) and  
 433 b) experimental  $\bar{M}_n$  (full symbols), Dispersity  $\mathcal{D}$  (open symbols) *versus* conversion.  
 434 Conversions are given in % on figure a). RAFT polymerization of MMA in miniemulsion at  
 435 80°C using DexN<sub>3-20</sub>CTA<sub>3.7</sub> transurf and various amounts of M810 in the organic phase (●)

436 Run 1, 10 vol.% (◆) Run 2, 25 vol.% (▲) Run 3, 50 vol.%. See Table 1 for other conditions.  
437 Theoretical  $\bar{M}_n$  (dotted lines) was evaluated using  $\bar{M}_n = 100(x)(X)$  where  $x$  and 100 are the  
438 monomer conversion and the molecular weight of a PMMA unit, respectively. Curve for  $X =$   
439 393 is given.

440  
441 The evolution of experimental  $\bar{M}_n$  of PMMA grafts with conversion for Runs 1-3 is shown as  
442 a function of theoretical  $\bar{M}_n$  in Figure 4b. In all cases, experimental  $\bar{M}_n$  evolved linearly with  
443 conversion until 80% of conversion without appearance of noticeable transfer or termination  
444 reactions. In addition,  $\bar{D}$  remained considerably below 1.2. All these results further attested of  
445 RAFT control during almost the whole polymerization. Experimental  $\bar{M}_n$  were however much  
446 higher than the theoretical ones (calculated with the adjusted average value of  $X =$   
447  $[MMA]_0/[CTA]_0=393$ ) indicating that not all CTA groups participated in the polymerization  
448 (Figure S8 for additional results of Run 4,  $X = 402$ ). The comparison between the number of  
449 grafts ( $n$ ) estimated from  $^1\text{H}$  NMR spectra and the number of CTA groups along dextran  
450 backbone ( $\gamma$ ) allowed us to evaluate an average efficiency of CTA groups ( $\text{Eff}_{\text{CTA}}$ ) of about  
451 48% (Table 1). Roughly half of the initial CTA groups of DexN<sub>3</sub>- $\tau$ CTA $\gamma$  transurfs located at  
452 the interface, effectively resulted in a PMMA grafted chain. Similar lower efficiencies have  
453 been already observed for other polysaccharides bearing reactive groups when used for  
454 controlling ATRP (Dupayage et al., 2008; Ferji et al., 2015) in homogeneous media. One  
455 possible cause seemed to be the presence of intramolecular termination reactions at the very  
456 beginning of the polymerization resulting in the loss of RAFT re-initiating radicals ( $\text{R}^*$ ). In  
457 our case, the reaction takes place in a heterogeneous environment. So, the transurf  
458 conformation at the oil/water interface is more likely the key factor limiting the availability of  
459 some CTA groups for polymerization if they are not facing to the organic phase, which was  
460 the main challenge of this work. Finally, CTA group efficiency appeared to be the same for  
461 both DexN<sub>3-20</sub>CTA<sub>3.7</sub> and DexN<sub>3-20</sub>CTA<sub>7.5</sub>, and remained unchanged whatever the  
462 M810/MMA volume ratio in agreement with our previous results for miniemulsion ATRP of

463 MMA using dextran-based inisurfs (Forero Ramirez et al., 2018). A steric hindrance effect in  
464 the vicinity of CTA groups restricting the access to C=S function does not seem to be present  
465 in the range of studied CTA modification degrees.

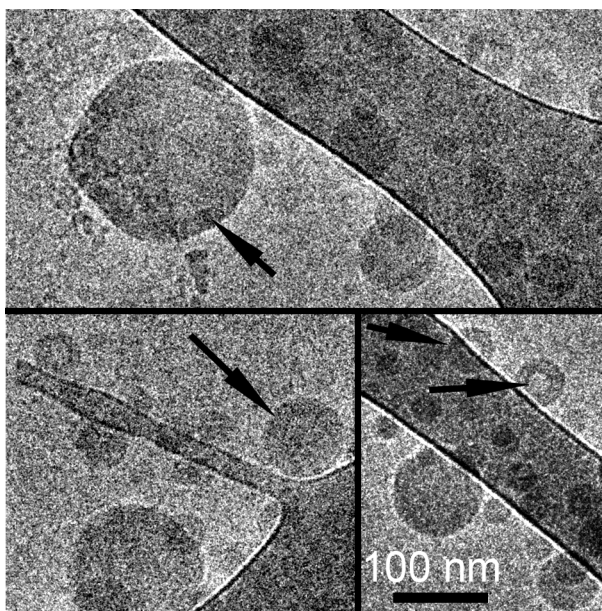
466  
467 *3.3 Nano-objects morphology*

468 Indirect confirmation of an oily-core/polymeric shell morphology of final objects was  
469 provided by MDSC analysis. The obtained thermograms (Figure S9) showed that NCs made  
470 with 25-50 vol.% of M810 (Runs 2, 3 and 4) were effectively two-phase systems having: i) a  
471 plasticized amorphous phase of PMMA/M810 (11 / 89 w / w,  $T_g = 98\text{ }^\circ\text{C}$ ) and ii) a pure M810  
472 liquid phase exhibiting one melting transition ( $T_m = -4\text{ }^\circ\text{C}$ ). On the contrary, nano-objects  
473 formulated with 10 vol.% of M810 (Run 1) displayed only a single glass transition event  
474 indicating a total miscibility of two components. These results were in quite agreement with  
475 our previous studies on PMMA/M810 blends miscibility as M810 was shown to plasticize  
476 PMMA until a weight fraction of 11 wt% (Forero Ramirez et al., 2018).

477 In order to prove NCs morphology in a direct way, final nano-objects were analyzed by  
478 Cryogenic-Transmission Electron Microscopy (cryo-TEM). Electron micrograph of NCs  
479 obtained with 25 vol.% of M810 in the organic phase (Run 2, Table 1) at 93% of conversion  
480 is shown in Figure 5. Cryo-TEM image showed the sphericity of synthesized objects as well  
481 as the average diameter determined by Laser granulometry, which varies from around 200 nm  
482 to 50 nm. A few particles are even smaller in that case the inner oily core is not clearly seen.  
483 The lack of sufficient contrast between plasticized PMMA and pure M810 phases render the  
484 visualization of the oily-core/polymeric shell structure under low dose conditions not simple.  
485 The core shell structure is best seen on small objects. On the larger objects the PMMA layer is  
486 just slightly darker than the oily core. The shell thickness is around 25 nm on all objects of  
487 run 2, Table 1. Internal liquid oil cavity was more visible for large objects after exposure to



488 the microscope electron beam since the polymeric shell was less sensitive to irradiation  
489 damage (Figure S10). These observations provided direct evidence of core-shell structure  
490 formation. Improved studies will be however needed to further characterize NCs morphology.



491  
492 **Figure 5.** Assembly of Cryo-TEM micrographs of final NCs obtained from Run 2. The black  
493 arrows point towards the shell of the particle. The scale bar is the same for each 3 images.

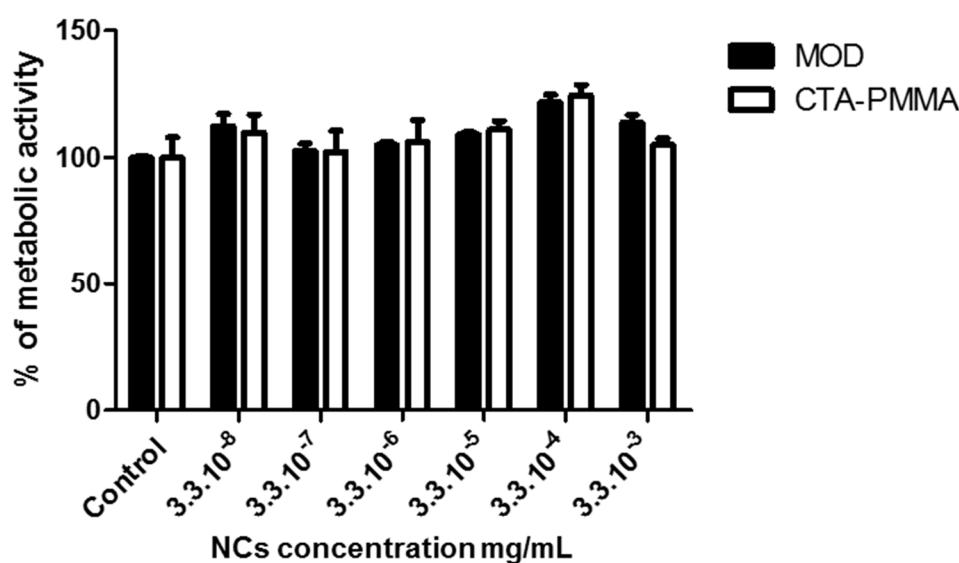
494

#### 495 *3.4 Nano-objects cytocompatibility*

496 Formulated NCs are constituted of an oily liquid biocompatible M810 core, an inner shell of  
497 PMMA (frequently used in implantable medical devices (Frazer, Byron, Osborne, & West,  
498 2005)) and an outer shell of dextran (well-known biodegradable and bioeliminable  
499 polysaccharide (Posocco et al., 2015)). In order to check the potential of these NCs for  
500 intravenous drug administration, preliminary cytocompatibility studies on vascular smooth  
501 muscle cells were performed *in vitro*. NCs prepared with the model system (see Supporting  
502 Information), called MOD, and with transurfs (called CTA-PMMA) were both analyzed.  
503 Metabolic activity of vascular cells was measured by monitoring the conversion of MTT to  
504 formazan. The reduction of MTT is catalyzed by mitochondrial dehydrogenase enzymes and

505 is therefore a measure for cell viability. After 24 hours, results expressed as percentages did  
506 not show any variation whatever the concentration or NCs type, when compared to the control  
507 condition (no NC) (Figure 6). The MOD and CTA-PMMA NCs appeared to be  
508 cytocompatible with vascular smooth muscle cells.

509



510

511 **Figure 6.** Cytocompatibility of MOD (prepared with a [MMA]<sub>o</sub>/[CPDB]<sub>o</sub> molar ratio of 700  
512 and 25 vol.% of M810 compared to MMA, see Supporting Information) or CTA-PMMA NCs  
513 (Run 2, Table 1) towards rat vascular smooth muscle cells (A-10) after 24 h incubation at  
514 37°C. 100% of metabolic activity (MTT assay) represents control cells incubated with PBS.  
515 Results expressed as mean  $\pm$  standard error of the mean, n = 3.

516

#### 517 4. Conclusions

518 As stated, we designed dextran-based transurfs able to produce oily-core/PMMA shell NCs by  
519 RAFT interfacial miniemulsion polymerization. The two major challenges of this work were  
520 to introduce CTA functionalities onto dextran in order to synthesize a multi-reactive transurf  
521 (acting both as macro-CTA and stabilizer) and to check its ability to confine RAFT  
522 miniemulsion polymerization of MMA at the oil/water interface. About a half of the CTA  
523 groups along dextran backbone were found to effectively initiate a PMMA grafted chain.

524 About 80 wt% of the total produced PMMA was effectively attached to dextran backbone. In  
525 any case, polymerization was controlled until high conversion as concluded from the analysis  
526 of uncoupled PMMA grafts deliberately removed from  $\text{DexN}_3\text{-}\tau\text{-g-}n\text{PMMA}_{\overline{M}_n}$ . Phase  
527 segregation between PMMA shell and M810 core was evidenced.

528 Overall these results highlight the value of our novel dextran-based transurf to produce  
529 dextran-covered oily-core NCs having different oil contents. Such NCs can be potentially  
530 used in drug delivery applications as indicated by preliminary *in vitro* cytotoxicity tests.  
531 Complementary biological tests, deep characterization of covalently linked dextran coverage,  
532 as well as post-functionalization of final NCs *via* the azide functions will be reported soon.  
533 The possibility of extending this method to RAFT copolymerization of MMA with a pH-  
534 responsive monomer will be also studied in the near future.

#### 535 **ACKNOWLEDGEMENTS**

536 C. Nouvel and L.M Forero Ramirez acknowledge support from ANR JCJC ANR-12-JS08-  
537 0003-01 NANOCAPDEX for funding and PhD Research Fellowship, respectively.

538 The authors express their highest gratitude to Olivier Fabre for NMR analyzes, to Marie-  
539 Christine Grassiot for help in SEC measurements and to Isabelle Fries for cytotoxicity assays.

540

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