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Andrew Gross, Raoudha Haddad, Christophe Travelet, Eric Reynaud, Pierre Audebert, et al.. Redox-Active Carbohydrate-Coated Nanoparticles: Self-Assembly of a Cyclodextrin–Polystyrene Glycopolymer with Tetrazine–Naphthalimide. *Langmuir*, 2016, 32 (45), pp.11939 - 11945. 10.1021/acs.langmuir.6b03512 . hal-01644738

**HAL Id: hal-01644738**

**<https://hal.science/hal-01644738v1>**

Submitted on 19 Nov 2020

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## 1 Redox-Active Carbohydrate-Coated Nanoparticles: Self-Assembly of 2 a Cyclodextrin–Polystyrene Glycopolymer with Tetrazine– 3 Naphthalimide

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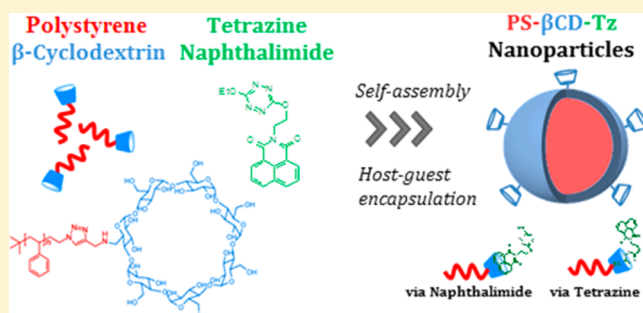
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9  Supporting Information

10 **ABSTRACT:** The controlled self-assembly of precise and  
11 well-defined photochemically and electrochemically active  
12 carbohydrate-coated nanoparticles offers the exciting prospect  
13 of biocompatible catalysts for energy storage/conversion and  
14 biolabeling applications. Here an aqueous nanoparticle system  
15 has been developed with a versatile outer layer for host–guest  
16 molecule encapsulation via  $\beta$ -cyclodextrin inclusion complexes.  
17 A  $\beta$ -cyclodextrin-modified polystyrene polymer was first  
18 obtained by copper nanopowder click chemistry. The  
19 glycopolymer enables self-assembly and controlled encapsula-  
20 tion of tetrazine-naphthalimide, as a model redox-active agent,  
21 into nanoparticles via nanoprecipitation. Cyclodextrin host–  
22 guest interactions permit encapsulation and internanoparticle cross-linking for the formation of fluorescent compounds and  
23 clustered self-assemblies with chemically reversible electroactivity in aqueous solution. Light scattering experiments revealed  
24 stable particles with hydrodynamic diameters of 138 and 654 nm for nanoparticles prepared with tetrazine, of which 95% of the  
25 nanoparticles represent the smaller objects by number. Dynamic light scattering revealed differences as a function of preparation  
26 method in terms of size, 3-month stability, polydispersity, radius of gyration, and shape factor. Individual self-assemblies were  
27 visualized by atomic force microscopy and fluorescence microscopy and monitored in real-time by nanoparticle tracking analysis.  
28 UV–vis and fluorescence spectra provided insight into the optical properties and critical evidence for host–guest encapsulation  
29 as evidenced by solvachromatism and enhanced tetrazine uptake. Cyclic voltammetry was used to investigate the electrochemical  
30 properties and provided further support for encapsulation and an estimate of the tetrazine loading capacity in tandem with light  
31 scattering data.



### 32 ■ INTRODUCTION

33 The development of biocompatible nanoassembled particles  
34 with controlled chemical and physical properties has attracted  
35 significant attention for applications such as imaging,  
36 biosensing, drug delivery, and biocatalysis.<sup>1,2</sup> Nanoparticles  
37 such as micelles capable of carrying and delivering redox  
38 molecules in a selective manner have shown great benefits  
39 including enhanced bioavailability,<sup>3</sup> stimuli-responsiveness,<sup>4</sup>  
40 and reduced toxicity.<sup>5</sup> However, polymer synthesis and particle  
41 assembly methods are often complicated and time-consuming.<sup>6</sup>  
42 Furthermore, challenges remain for in vivo and in vitro  
43 applications including solubility and stability issues.

44 The preparation of amphiphilic glycopolymers provides a  
45 route to biocompatible and bioinspired nanoparticles but  
46 remains relatively undeveloped because of their tricky  
47 preparation with many synthesis steps.<sup>7–9</sup> Several groups  
48 have now reported on the preparation and self-assembly of  
49 block copolymers containing polysaccharides and oligosacchar-

ides (maltoheptaose,<sup>10</sup> dextrans,<sup>9,11</sup> and amylose<sup>12</sup> with  
synthetic polymers such as polystyrenes,<sup>9–13</sup> poly( $\epsilon$ -  
51 caprolactone)s,<sup>14</sup> poly(ethylene glycol)s<sup>15</sup> and poly(methyl  
52 methacrylate)s<sup>16</sup>). Amphiphilic block copolymer formation is  
53 generally achieved using combinations of controlled living  
54 radical polymerizations, transition-metal-mediated coupling  
55 chemistry, and tedious purification procedures.<sup>7</sup> Many methods  
56 report the self-assembly of amphiphilic block copolymers into  
57 nanoparticles by direct dissolution into a selective solvent for  
58 one of the block moieties.<sup>9,11,17,18</sup> However, with this approach  
59 it is difficult to avoid random aggregation and control particle  
60 size at the nanoscale. Clever manipulation strategies involving  
61 indirect dissolution methods such as nanoprecipitation facilitate  
62 the preparation of well-defined micellar nanoparticles with 63

Received: September 25, 2016

Revised: October 20, 2016

Published: October 25, 2016

64 narrow polydispersities from water or mixed solvent–water  
65 copolymer solutions.<sup>13,16,19</sup> Nanoprecipitation, first reported by  
66 Fessi and co-workers, involves the dissolution of both blocks  
67 followed by precipitation and selective solvent removal.<sup>20,21</sup> A  
68 further advantage is that redox molecules or drugs can be  
69 dissolved in copolymer solution prior to precipitation. In this  
70 case, hydrophobic species can be trapped as cargo inside the  
71 dense hydrophobic core of nanoassembled particles.<sup>10,22</sup>

72 A more sophisticated approach to encapsulation is to  
73 incorporate chemical handles capable not only of trapping  
74 but also of orienting and stimuli-controlled releasing of redox  
75 molecules in aqueous solution.<sup>23</sup> Methods capable of molecule  
76 encapsulation in the outer layer may further benefit from  
77 improved redox molecule bioavailability and accessibility.  
78 Cyclodextrins are one class of functional molecules capable of  
79 selectively binding a vast array of compounds via host–guest  
80 interactions.<sup>24–27</sup> Their low solubility in water leads to  
81 spontaneous aggregation, but functionalized cyclodextrins  
82 with carefully designed polymer blocks have been used to  
83 prepare nanoassembled particles.<sup>7,9,13</sup>

84 In this article, we describe a new straightforward synthesis  
85 route for the synthesis of a  $\beta$ -cyclodextrin-modified polystyrene  
86 polymer (PS-CD) and its use in the self-assembly of redox-  
87 active nanoparticles in water via encapsulation of a model  
88 hydrophobic molecule, tetrazine-naphthalimide (Tz). This  
89 molecule was recently developed by us and is of interest for  
90 future applications in fluorescence sensing and labeling.<sup>28,29</sup>

91 The PS-CD glycopolymer was synthesized according to a  
92 simple procedure that uses a copper nanopowder catalyst rather  
93 than copper in solution to covalently couple functionalized  $\beta$ -  
94 cyclodextrin and polystyrene blocks via click chemistry with a  
95 high yield of 90%. (See the [Supporting Information](#) for  
96 synthesis and characterization details.) We previously used CuI  
97 to couple  $\beta$ -cyclodextrin and PS groups, but the Cu in solution  
98 was difficult to remove so it was not possible to synthesize a  
99 large amount of clean glycopolymer.<sup>13</sup> In contrast, the Cu  
100 nanopowder employed here is very easy to remove via classical  
101 filtration and CupriSorb resin, facilitating preparation and  
102 purification of the modified polymer on a larger scale.

## 103 ■ EXPERIMENTAL SECTION

104 **General Materials and Apparatus.** The  $\omega$ -hydroxy-polystyrene  
105 ( $M_n = 3800 \text{ g mol}^{-1}$ ) was purchased from Polymer Source. Tetrazine-  
106 naphthalimide was synthesized according to our previously reported  
107 work.<sup>29</sup> Propargylamine, sodium hydroxide (NaOH), tetrahydrofuran  
108 (THF), *N,N*-dimethylformamide (DMF), sodium azide ( $\text{NaN}_3$ ), and  
109 diatomaceous earth were purchased from Sigma-Aldrich.  $\beta$ -Cyclo-  
110 dextrin and Cu/CuO nanopowder were purchased from Alfa Aesar.  
111 Tosyl chloride (TsCl) was obtained from Fluka, and pyridine was  
112 purchased at SDS. Methanol (MeOH) and dichloromethane  
113 ( $\text{CH}_2\text{Cl}_2$ ) were purchased from Biosolve. Milli-Q water was obtained  
114 by water purification to a resistivity of 18.2 M $\Omega$  cm using a Millipore  
115 Ultrapure system. Deuterated solvents were purchased from Euriso-  
116 top, the Cuprisorb resin was purchased from Seachem, and the  
117 Si(100) wafers were purchased from Siltronix. All reagents and  
118 solvents used for the synthesis of tetrazine-naphthalimide were  
119 purchased from Sigma-Aldrich or Carlo-Erba.

120 <sup>1</sup>H NMR spectra were recorded on a Bruker Avance 400 MHz  
121 spectrometer with a frequency of 400.13 MHz and calibrated with the  
122 deuterated solvent signal.<sup>30</sup> The analysis characteristics were  $z\theta = 90^\circ$ ,  
123  $D1 = 1 \text{ s}$ , and  $NS = 128$  scans. Matrix-assisted laser desorption  
124 ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was  
125 performed using an Applied Biosystems Voyager-DE STR-H equipped  
126 with a nitrogen laser (337 nm and 3 ns pulse width).

UV–vis absorption spectra were recorded in quartz cuvettes using a 127  
PerkinElmer UV-Lambda 650 spectrophotometer, and fluorescence 128  
spectra were recorded using a PerkinElmer LS50. 129

**Synthesis of  $\beta$ -Cyclodextrin-Modified Polystyrene Polymer.** 130  
The amphiphilic glycopolymer was obtained by click chemistry. The 131  
first step was to functionalize each block: the hydrophobic block,  $\omega$ - 132  
hydroxyl-terminated polystyrene, was functionalized to form  $\omega$ -azido- 133  
terminated polystyrene via the  $\omega$ -tosyl terminated polystyrene 134  
intermediate with an overall yield of 75% according to the method 135  
of Fallais and co-workers.<sup>31</sup> The hydrophilic block, native  $\beta$ - 136  
cyclodextrin, was monofunctionalized into mono-6-alkyne- $\beta$ -cyclo- 137  
dextrin via the mono-6-tosyl- $\beta$ -cyclodextrin intermediate with an 138  
overall yield of 7% according to the procedure developed by 139  
Moutard.<sup>32,33</sup> Both blocks with their complementary chemical 140  
functions (alkyne and azido) were subsequently linked together via a 141  
click reaction in heterogeneous media catalyzed by Cu nano- 142  
powder.<sup>34,35</sup> to obtain the  $\beta$ -cyclodextrin-modified polystyrene polymer 143  
( $M_n = 4900 \text{ g mol}^{-1}$ , PDI = 1.01) (Scheme S1). To perform the click 144  
reaction, a degassed solution containing the azido-functionalized 145  
polystyrene (1 equiv, 8.1 g,  $1.8 \times 10^{-3} \text{ mol}$ ) and the alkynyl- 146  
functionalized cyclodextrin (1.2 equiv, 2.7 g,  $2.2 \times 10^{-3} \text{ mol}$ ) in DMF 147  
was first prepared. An excess of Cu nanopowder (1.8 equiv, 260 mg, 148  
 $3.2 \times 10^{-3} \text{ mol}$ ) was added to the solution and the mixture stirred 149  
under Ar at 65 °C for several hours until the azide <sup>1</sup>H NMR signal 150  
disappeared. At the end of the reaction, the crude heterogeneous 151  
mixture was filtered off under diatomaceous earth, and the obtained 152  
filtrate was stirred several hours in the presence of the Cuprisorb resin. 153  
The mixture was finally precipitated in cold MeOH to give a white 154  
powder with a yield of 90% (mass = 8 g). The modified polymer 155  
structure was confirmed using NMR and MALDI analysis (Figure S1). 156

**Preparation of PS-CD<sub>NP</sub> and PS-CD-Tz<sub>NP</sub> Nanoparticles via** 157  
**Nanoprecipitation.** Polymer solutions were prepared by dissolving 158  
either 5 mg of PS-CD or 5 mg of PS-CD together with 1 mg of 159  
tetrazine in 5 g of a THF/H<sub>2</sub>O solution (80:20 v/v %, unless 160  
otherwise stated). For nanoprecipitation by method A, 1 g of the PS- 161  
CD solution was slowly added dropwise to 40 g of Milli-Q water under 162  
stirring at 500 rpm at room temperature. For nanoprecipitation by 163  
method B, 40 g of water was slowly added to 1 g of the PS-CD 164  
solution under stirring at 500 rpm at room temperature. The solutions 165  
were subsequently stirred at 500 rpm at room temperature for 1.5 h 166  
and then preconcentrated to 4 g by evaporation under reduced 167  
pressure at 50 °C using a rotary evaporator. The nanoparticle 168  
suspensions were stored at room temperature in the dark. 169

**Static Light Scattering (SLS)/Dynamic Light Scattering** 170  
**(DLS).** Light scattering measurements were performed using an 171  
ALV/CGS-8FS/N069 goniometer with an ALV/LSE-5004 multiple- $\tau$  172  
digital corrector with a 125 ns initial sampling time and a 35 mW red 173  
HeNe linearly polarized laser operating at  $\lambda = 632.8 \text{ nm}$ . Nanoparticle 174  
suspensions were loaded into 10-mm-diameter quartz cells thermo- 175  
stated at  $25 \pm 0.1 \text{ }^\circ\text{C}$ . Data were collected using the digital ALV 176  
correlator control software at observation angles relative to the 177  
transmitted beam of 90/150° (scattering vector moduli  $1.87 \times 10^{-2}/$  178  
 $2.56 \times 10^{-2} \text{ nm}^{-1}$ ) for a counting time of typically 180 s. In DLS 179  
measurements, relaxation time distributions were determined using a 180  
Contin analysis of the autocorrelation functions. Polydispersity values 181  
were calculated from the cumulant analysis of the DLS autocorrela- 182  
tions ( $g^{(2)} - 1$ ). 183

Diffusion coefficients were obtained from eq 1 where  $D$  is the 184  
diffusion coefficient,  $\tau$  is the relaxation time, and  $q$  is the modulus of 185  
the scattering vector: 186

$$D = \frac{1}{\tau q^2} \quad (1) \quad 187$$

Hydrodynamic diameters,  $D_h$ , were calculated using the Stokes– 188  
Einstein equation (eq 2), where  $K_B$  is the Boltzmann constant,  $T$  is the 189  
temperature,  $\eta$  is the medium viscosity, and  $D$  is the diffusion 190  
coefficient: 191

$$D_h = \frac{K_B T}{3\pi\eta D} \quad (2) \quad 192$$

193 The percentage of small nanoparticles was calculated using eq 3,  
 194 assuming that the nanoparticles behave like hard spheres in water and  
 195 have the same density, where  $N_{\text{small}}$  refers to the number of small  
 196 nanoparticles (fast relaxation mode),  $\tau_{\text{small}}$  is the relaxation time  
 197 corresponding to the small nanoparticles, and  $S_{\text{small}}$  is the surface area  
 198 under the peak related to the small nanoparticles:<sup>36</sup>

$$\frac{N_{\text{small}}}{N_{\text{big}}} = \left( \frac{\tau_{\text{big}}}{\tau_{\text{small}}} \right)^3 \left( \frac{S_{\text{small}}}{S_{\text{big}}} \right) \quad (3)$$

200 For SLS measurements, scattering intensities were corrected by the  
 201 background solvent signal. The diameter of gyration,  $D_g$ , was  
 202 determined from eq 4, where  $n$  is the number of repetitive units  
 203 and  $b$  is the Kuhn length:

$$D_g = 2 \left( \frac{nb^2}{6} \right)^{0.5} \quad (4)$$

205 **Electrochemistry.** Electrochemical measurements were performed  
 206 at room temperature using an Eco Chemie Autolab PGSTAT 100  
 207 potentiostat running GPES 4.9 software. A conventional three-  
 208 electrode cell setup was used for all electrochemical experiments  
 209 comprising a glassy carbon working electrode (GC, 3 mm diameter), a  
 210 saturated calomel reference electrode (SCE), and a Pt wire counter  
 211 electrode. The concentration of tetrazine-naphthalimide was estimated  
 212 according to the Randles–Sevcik equation (eq 5) where  $I_p$  is the peak  
 213 current,  $n$  is the number of electrons transferred in the redox event,  $F$   
 214 is the Faraday constant,  $C$  is the concentration of electroactive species,  
 215  $D$  is the diffusion coefficient (here estimated from dynamic light  
 216 scattering),  $A$  is the geometric electrode area,  $\nu$  is the scan rate,  $R$  is the  
 217 gas constant, and  $T$  is room temperature:

$$I_p = nFAC \left( \frac{nF\nu D}{RT} \right)^{0.5} \quad (5)$$

219 **Nanoparticle Tracking Analysis (NTA).** NTA was carried out  
 220 using a Nanosight LM10HS optical microscope setup equipped with a  
 221 blue-purple laser ( $\lambda_{\text{ex}} = 405 \text{ nm}$ ), a camera, and a chamber mounted  
 222 on a modified microscope stage (Nanosight, Amesbury, U.K.). The  
 223 original suspensions of nanoparticles were diluted with Milli-Q water  
 224 and introduced into the chamber with a syringe. Video clips of the  
 225 nanoparticles subjected to their natural Brownian motion were  
 226 captured over 60 s at 25.0 °C and analyzed using analytical software  
 227 version 2.1, giving access to the number-weighted size distributions.  
 228 (See the Supporting Information for 10 s video clips.)

229 **Atomic Force Microscopy (AFM).** Twenty microliters of a  
 230 nanoparticle suspension was dropped onto a Si(100) substrate, and  
 231 the sample was left to dry at room temperature overnight in a  
 232 desiccator. AFM measurements were performed using a Dimension  
 233 Icon (Bruker, Santa Barbara, CA) with SCANASIST-Air (Bruker,  
 234 Santa Barbara, CA) probes in peak-force mode with a scan rate of 1  
 235 Hz. The data was processed using Gwyddion 2.41 microscope  
 236 software.

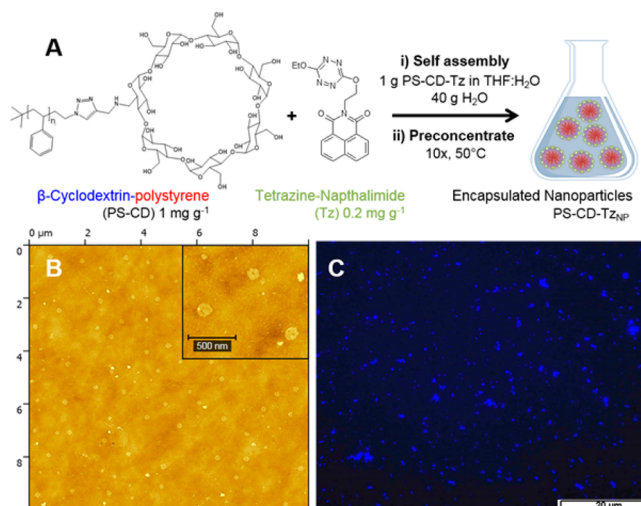
237 **Fluorescence Microscopy.** Twenty microliters of a nanoparticle  
 238 suspension was dropped onto a Si(100) substrate, and the sample was  
 239 left to dry at room temperature overnight in a desiccator. Fluorescence  
 240 microscopy images were recorded using an Olympus BX61 micro-  
 241 scope coupled with an Olympus DP30BW camera and CellP imaging  
 242 software. A DAPI (4',6-diamidino-2-phenylindole) filter set was used:  
 243 DAPI with  $\lambda_{\text{ex}} = 340\text{--}380 \text{ nm}$  and  $\lambda_{\text{em}} = 450\text{--}490 \text{ nm}$ . Images were  
 244 recorded with an exposure time of 500 ms.

## 245 ■ RESULTS AND DISCUSSION

246 For the preparation of nanoparticles in aqueous solution via  
 247 nanoprecipitation, we focused our initial studies on optimizing  
 248 the solvent system for both blocks of the PS-CD glycopolymer.  
 249 Tetrahydrofuran was chosen as the organic solvent because of  
 250 its good solubility toward polystyrenes and Tz, water  
 251 miscibility, and good evaporation rate. Static light scattering

experiments were recorded for a series of PS-CD solutions 252  
 prepared in THF with water content of between 0 and 50% 253  
 (Figure S2). Very low scattering intensities were observed in 254  
 the presence of 5 to 20% water, consistent with desirable 255  
 polymer chain dissolution and few aggregates in a mixed 256  
 solvent. In the presence of  $\geq 20\%$  water, a significant increase in 257  
 scattering intensity was observed because of the unwanted 258  
 formation of self-assemblies.  $\beta$ -Cyclodextrin-modified polymer 259  
 solutions were therefore investigated in 10 and 20% water 260  
 solutions. However, further observations proved that suspen- 261  
 sions based on 10% water solutions were more unstable, with 262  
 microscopic particles visible after just a few days of storage, and 263  
 thus the 80:20 THF/water ratio was adopted for further 264  
 investigations. 265

We explored the controlled self-assembly of PS-CD (1 mg 266  
 $\text{g}^{-1}$ ) in the presence and absence of Tz (0.2 mg  $\text{g}^{-1}$ ) in THF/ 267  
 $\text{H}_2\text{O}$  via nanoprecipitation in a large excess of water (Figure 268  
 1A). For the PS-CD with Tz solution, the PS-CD/Tz mole-to- 269



**Figure 1.** (A) Synthesis route for the preparation of encapsulated nanoparticles (PS-CD-Tz<sub>NP</sub>). (B, C) Images of PS-CD-Tz<sub>NP</sub> particles prepared by method A on silicon recorded by (B) AFM in peak-force mode at 1 Hz and (C) fluorescence microscopy with a DAPI filter and a 500 ms exposure time.

mole ratio used was 1:3. Herein, suspensions obtained from PS- 270  
 CD and PS-CD with Tz will be referred to as PS-CD<sub>NP</sub> and PS- 271  
 CD-Tz<sub>NP</sub>, respectively. Two methods of nanoprecipitation were 272  
 explored: method A, where 1 g of PS-CD polymer solution was 273  
 slowly added to 40 g of water (solvent-to-water method), and 274  
 method B, where 40 g of water was slowly added to 1 g of PS- 275  
 CD polymer solution (water-to-solvent method). For both 276  
 methods A and B, the same precipitation and evaporation 277  
 procedures were performed (Experimental Section) to obtain 278  
 aqueous PS-CD<sub>NP</sub> and PS-CD-Tz<sub>NP</sub> suspensions. 279

The ability of the  $\beta$ -cyclodextrin-modified polystyrene 280  
 solutions to spontaneously assemble into nanoparticles was 281  
 initially confirmed by atomic force microscopy and fluorescence 282  
 microscopy after solution drop-casting and drying on a silicon 283  
 wafer (Figure 1B,C). AFM imaging of the PS-CD-Tz<sub>NP</sub> sample 284  
 prepared by method A revealed the presence of individual 285  
 nanoparticles with an average particle diameter of  $167 \pm 30 \text{ nm}$  286  
 ( $n = 89$  particles). Importantly, the brief AFM analysis proved 287  
 the successful formation of nanoparticle self-assemblies using 288  
 our nanoprecipitation protocol. Figure 1C shows a fluorescence 289

290 microscopy image of the PS-CD-Tz<sub>NP</sub> sample recorded with a  
 291 DAPI filter at  $\lambda_{\text{ex}} = 340\text{--}380$  nm and  $\lambda_{\text{em}} = 450\text{--}490$  nm.  
 292 Bright fluorescence over a long lifetime using a DAPI filter was  
 293 observed from the particles on the surface. In contrast,  
 294 insignificant fluorescence was observed for PS-CD<sub>NP</sub> (data  
 295 not shown), consistent with the successful encapsulation of the  
 296 small organic tetrazine-containing bifluorophore in PS-CD-  
 297 Tz<sub>NP</sub> particles.<sup>28</sup>

298 Dynamic light scattering experiments were performed to  
 299 examine the suspensions in more detail. Initial DLS was  
 300 performed on PS-CD<sub>NP</sub> (Figure 2A) and PS-CD-Tz<sub>NP</sub> (Figure  
 301 2B) prepared by methods A and B to optimize the  
 302 nanoprecipitation protocol for the preparation of well-defined  
 303 nanoassemblies.

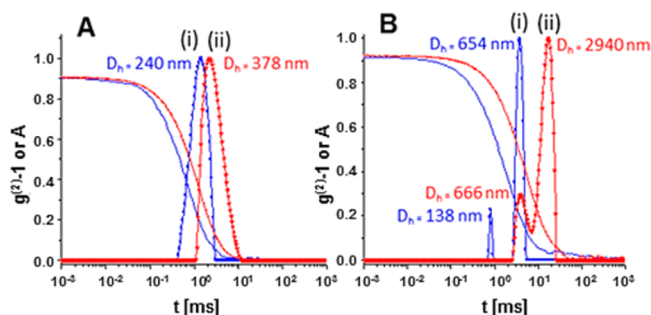


Figure 2. DLS autocorrelation ( $g^{(2)} - 1$ ) measured at  $90^\circ$  and relaxation time distribution of aqueous suspensions of (A) PS-CD<sub>NP</sub> prepared by (i) method A and (ii) method B and (B) PS-CD-Tz<sub>NP</sub> prepared by (i) method A and (ii) method B.

304 Figure 2A shows the DLS autocorrelation function ( $g^{(2)} - 1$ )  
 305 and relaxation time distribution for the PS-CD<sub>NP</sub> suspensions.  
 306 For PS-CD<sub>NP</sub> prepared by methods A (Figure 2Ai) and B  
 307 (Figure 2Aii), unimodal distributions of relaxation times were  
 308 observed corresponding to mean hydrodynamic diameters of  
 309  $D_h = 240$  and  $378$  nm with polydispersities of  $\mathcal{D} = 1.34$  and  
 310  $1.45$ , respectively. The particles prepared by method A are  
 311 therefore smaller and less polydisperse than those prepared by  
 312 method B. The PS-CD<sub>NP</sub> particles prepared by method A are  
 313 comparable in size to previously reported compound micelles  
 314 with  $D_h \approx 160$  nm prepared by the self-assembly of  
 315 polystyrene-containing diblock polymers.<sup>10,15</sup> Figure 2B  
 316 shows the DLS autocorrelation function ( $g^{(2)} - 1$ ) and  
 317 relaxation time distribution for the PS-CD-Tz<sub>NP</sub> suspensions  
 318 prepared by methods A (Figure 2Bi) and B (Figure 2Bii). The  
 319 relaxation time distribution plots, reflecting mass-weighted  
 320 distributions, show the appearance of two particle sizes for PS-  
 321 CD-Tz<sub>NP</sub>. The PS-CD-Tz<sub>NP</sub> suspension obtained by method B  
 322 exhibits greater polydispersity ( $\mathcal{D} = 1.43$ ) and contains  
 323 microscale particles ( $D_h = 2940$  nm). In contrast, smaller  
 324 nanoparticles with a lower polydispersity ( $\mathcal{D} = 1.32$ ) are  
 325 obtained by method A, and thus this method of nano-  
 326 precipitation was adopted for further analysis.

327 The smaller particle size of  $D_h = 138$  nm observed for PS-  
 328 CD-Tz<sub>NP</sub> prepared by method A is attributed to small self-  
 329 assemblies that have decreased in size compared to the PS-  
 330 CD<sub>NP</sub> nanoparticles prepared by the same method as a result of  
 331 the encapsulation of Tz molecules and the shrinking of the  
 332 more hydrophobic core. A change in self-assembly behavior of  
 333 the PS-CD polymer in the presence of Tz was expected because  
 334 the cone-shaped hydrophobic cavities of the  $\beta$ -cyclodextrin  
 335 groups drive the 1:1 inclusion of guest Tz molecules via 1,8-

naphthalimide (equatorial inclusion) and 1,2,4,5-tetrazine 336  
 groups.<sup>29,37</sup> A decrease in size with encapsulation has previously 337  
 been reported for self-assembled nanoparticles with encapsu- 338  
 lated hydrophobic molecules.<sup>15</sup> The larger nanoparticles of PS- 339  
 CD-Tz<sub>NP</sub> with  $D_h = 654$  nm, observed only after self-assembly 340  
 in the presence of Tz, are attributed to nanoparticle clusters. 341  
 The formation of clusters is attributed to the cross-linking of 342  
 individual particles via host-guest interactions between Tz 343  
 molecules and host  $\beta$ -cyclodextrin groups located at the 344  
 periphery of the particles. (Both tetrazine and naphthalimide 345  
 functional groups can function as guests.) Particle aggregation 346  
 due to nonspecific interactions<sup>3</sup> must be considered and likely 347  
 contributes to the self-assembly of the larger particle 348  
 distribution. Regardless of the nature of formation of the larger 349  
 particles, their presence in terms of number is very small 350  
 compared to the smaller particles. On the basis of 12 DLS 351  
 measurements at  $90^\circ$ , the estimated number of smaller objects 352  
 is 95.1% ( $N_{\text{small}} \approx 19.1N_{\text{big}}$ ), according to eq 3. 353

To complete the nanoparticle sizing experiments, nano- 354  
 particle tracking analysis was performed. Nanoparticles were 355  
 illuminated with a 405 nm blue laser and tracked individually 356  
 (Figure 3, Figure S3 and corresponding video clips in the 357 358

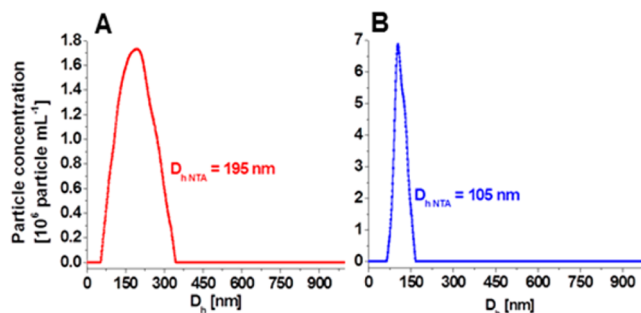
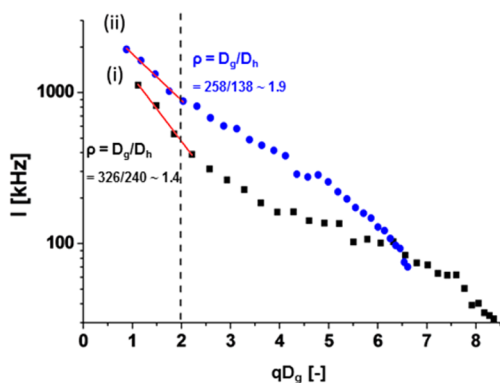


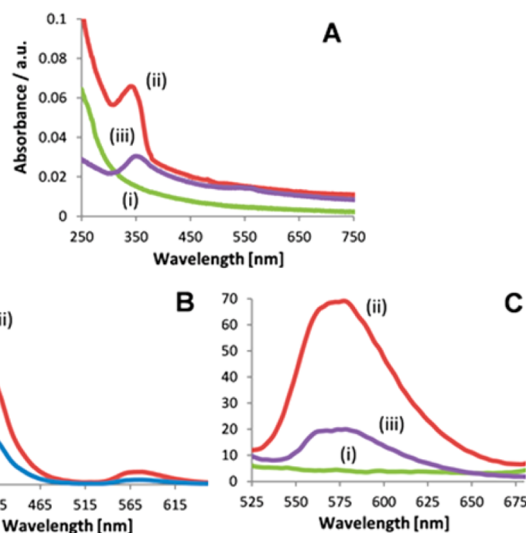
Figure 3. Hydrodynamic number-weighted size distributions by NTA of (A) PS-CD<sub>NP</sub> and (B) PS-CD-Tz<sub>NP</sub> suspensions prepared by method A.

Supporting Information). Nanoparticles that undergo Brownian 358  
 motion were taken into account, and average  $D_{h,\text{NTA}}$  values of 359  
 195 nm for PS-CD<sub>NP</sub> and 105 nm for PS-CD-Tz<sub>NP</sub> were 360  
 acquired. These values are smaller than those obtained by DLS 361  
 because of the fact that number-weighted distributions are 362  
 obtained. Nevertheless, the sizes obtained by NTA are 363  
 complementary, with the corresponding particle sizes obtained 364  
 by DLS ( $D_h = 240$  and  $138$  nm). The absence of the larger 365  
 cluster distribution for PS-CD-Tz<sub>NP</sub> by NTA is not at all 366  
 surprising given that (i) NTA gives access to number-weighted 367  
 distributions of which only  $\sim 4.9\%$  are the clusters and (ii) only 368  
 a few nanoparticles are imaged, as can be seen in Figure S3 and 369  
 the video clips. 370

Further analysis of the DLS and SLS scattering data was 371  
 performed to shed light on the shape of the nanoparticles. First, 372  
 the diameter of gyration,  $D_g$ , was determined from SLS angular 373  
 dependence experiments via Guinier plots (Figure 4). Values of 374 375  
 $D_g = 326$  and  $258$  nm were obtained from the slope of the 376  
 linear fit in the low  $qD_g$  region for PS-CD<sub>NP</sub> and PS-CD-Tz<sub>NP</sub>,  
 respectively. Next, the shape factor,  $\rho$ , was obtained from the 377  
 ratio  $\rho = D_g/D_h$ , as proposed by Burchard.<sup>38</sup> The  $\rho$  values of 1.4 378  
 for PS-CD<sub>NP</sub> and 1.9 for PS-CD-Tz<sub>NP</sub> fall between those of 379  
 vesicles ( $\rho = 1$ ) and rigid rods ( $\rho \geq 2.0$ ), and thus PS-CD<sub>NP</sub> 380  
 and PS-CD-Tz<sub>NP</sub> particles can be ascribed to particles whose 381



**Figure 4.** SLS Guinier plots of the scattering intensity of (i) PS-CD<sub>NP</sub> and (ii) PS-CD-Tz<sub>NP</sub> suspensions prepared by method A.



**Figure 5.** (A) UV–vis absorption spectra for (i) PS-CD<sub>NP</sub>, (ii) PS-CD-Tz<sub>NP</sub>, and (iii) H<sub>2</sub>O-Tz. (B, C) Fluorescence emission spectra recorded at  $\lambda_{\text{ex}} = 353$  nm for (B) PS-CD-Tz<sub>NP</sub> and (C) (i) PS-CD<sub>NP</sub>, (ii) PS-CD-Tz<sub>NP</sub>, and (iii) H<sub>2</sub>O-Tz at 525 to 680 nm.

382 overall shape is spherical (1.4) and polydisperse spheres (1.9),  
383 respectively.<sup>38</sup>

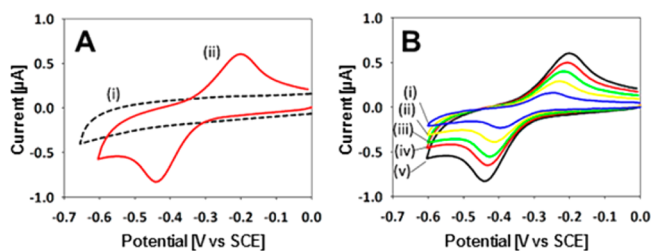
384 An important issue largely overlooked in previous studies on  
385 the self-assembly of saccharide-based polymers is their stability.  
386 In this study, we tested the long-term stability of PS-CD<sub>NP</sub> and  
387 PS-CD-Tz<sub>NP</sub> suspensions by monitoring the particle size  
388 change after storage in the dark at ambient temperature for 3  
389 months. The DLS data (Figure S4) reveal relatively small  
390 increases in hydrodynamic radii for PS-CD-Tz<sub>NP</sub> compared to  
391 markedly larger increases in radii for PS-CD<sub>NP</sub> particles. The  
392 apparent low stability of the nonencapsulated PS-CD<sub>NP</sub>  
393 particles is attributed to nonspecific hydrophobic interactions  
394 of  $\beta$ -cyclodextrin groups at the periphery of the nanoparticles,  
395 which give larger aggregations over time. The higher stability of  
396 PS-CD-Tz<sub>NP</sub> reflects the saturation of these  $\beta$ -cyclodextrin  
397 groups, which in turn suppresses particle aggregation over time.  
398 Crucially, the stability of PS-CD-Tz<sub>NP</sub> suggests little evidence of  
399 the presence of Tz molecules in solution that would likely  
400 increase particle aggregation.

401 UV–vis and fluorescence spectra were recorded to probe the  
402 ability of the PS-CD polymer to encapsulate tetrazine-  
403 naphthalimide during self-assembly. Three solutions were  
404 investigated: as-prepared PS-CD<sub>NP</sub>, PS-CD-Tz<sub>NP</sub> (Tz = 0.06  
405 mg mL<sup>-1</sup>), and a saturated tetrazine-naphthalimide in water  
406 solution (H<sub>2</sub>O-Tz) prepared by addition of 0.3 mg Tz in 3 mL  
407 (Tz < 0.1 mg mL<sup>-1</sup>). The absorption spectrum for PS-CD<sub>NP</sub> in  
408 the absence of Tz shows weak background absorption due to  
409 the polymer but no well-defined absorption peaks (Figure 5Ai).  
410 In contrast, both PS-CD-Tz<sub>NP</sub> (Figure 5Aii) and H<sub>2</sub>O-Tz  
411 (Figure 5Aiii) solutions exhibit a strong absorption band at  
412 340–380 nm, ascribed to both the imide group and the  $\pi$ - $\pi^*$   
413 band transition of the tetrazine group. Significantly larger  
414 absorption is observed for PS-CD-Tz<sub>NP</sub> compared to the H<sub>2</sub>O-  
415 Tz, consistent with improved solubilization of the Tz via host-  
416 guest encapsulation. The small hypsochromic shift (blue shift)  
417 from  $\lambda_{\text{max}} = 353$  nm for H<sub>2</sub>O-Tz to 341 nm for PS-CD-Tz<sub>NP</sub> is  
418 consistent with the Tz molecules shifting from a polar to a  
419 nonpolar environment. The shift therefore provides further  
420 evidence of the inclusion of Tz in the hydrophobic  $\beta$ -  
421 cyclodextrin cavities. Such small blue shifts in UV–vis spectra  
422 due to host–guest complexation have previously been reported  
423 for 4-amino-1,8-naphthalimides with  $\beta$ -cyclodextrin mole-  
424 cules.<sup>37</sup> Another remarkable feature of the fluorescence  
425 emission of the encapsulated molecules is that both the imide  
426 antenna and the tetrazine acceptor emit together (producing  
427 almost white light), proving that the energy transfer is partially

428 blocked as a consequence of the encapsulation process. This is  
429 in sharp contrast to the situation encountered when the  
430 molecules are dissolved in an organic solvent, where the energy  
431 transfer occurs with a ca. 90% efficiency and only tetrazine  
432 fluorescence is observed.<sup>28</sup>

433 Fluorescence spectra of PS-CD-Tz<sub>NP</sub> nanoparticles were  
434 initially recorded at different emission wavelengths. Strong  
435 fluorescence was observed only upon excitation in the UV  
436 region at  $\lambda_{\text{ex}} = 353$  nm (Figure 5B) and  $\lambda_{\text{ex}} = 340$  nm (data not  
437 shown) and not at higher wavelengths (for example,  $\lambda_{\text{ex}} = 518$   
438 and 544 nm, data not shown). The classical strong fluorescence  
439 of 1,8-naphthalimide was observed between 370 and 420 nm  
440 along with a weak fluorescence band at 550–600 nm due to the  
441 tetrazine moiety. Further evidence for the improved uptake of  
442 fluorescent Tz molecules via molecular encapsulation was  
443 obtained by comparison of the fluorescence spectra recorded at  
444  $\lambda_{\text{ex}} = 340$  nm for PS-CD<sub>NP</sub>, PS-CD-Tz<sub>NP</sub>, and H<sub>2</sub>O-Tz solutions  
445 (Figure 5C). No fluorescence was observed for nanoparticles  
446 free of Tz (Figure 5Ci), as expected. A clear enhancement in  
447 fluorescence was, however, observed at  $\lambda_{\text{em}} = 570$  nm for PS-  
448 CD-Tz<sub>NP</sub> (Figure 5Cii) compared to the Tz-saturated H<sub>2</sub>O-Tz  
449 solution, reflecting the improved solubility of tetrazine-  
450 naphthalimide in the nanoparticle solution compared to water.

451 Cyclic voltammograms (CVs) of the solutions were also  
452 performed to test the electroactivity of the nanoparticles. CVs  
453 were recorded at glassy carbon electrodes without supporting  
454 electrolyte to avoid aggregation due to increased ionic strength.  
455 In the absence of Tz, a typical background response of a GC  
456 electrode in aqueous solution was observed (Figure 6Ai). For  
457 PS-CD-Tz<sub>NP</sub> nanoparticles, a single well-defined and chemically  
458 reversible redox couple at  $E_{1/2} = -0.32$  V vs SCE was observed  
459 (Figure 6Aii). This system is attributed to a two-electron, two-  
460 proton reduction of 1,4-tetrazine to its protonated form, 1,4-  
461 dihydrotetrazine.<sup>29,39</sup> This behavior is analogous to the two-  
462 electron reduction of quinones to hydroquinones in aqueous  
463 solution. It should be noted that the CV recorded in H<sub>2</sub>O-Tz  
464 solution (Figure 6Si) shows an almost identical  $E_{1/2}$  but with an  
465 extremely weak peak intensity, reflecting the trace presence of



**Figure 6.** CVs recorded at glassy carbon on (A) (i) PS-CD<sub>NP</sub> and (ii) PS-CD-Tz<sub>NP</sub> at 100 mV s<sup>-1</sup> and (B) PS-CD-Tz<sub>NP</sub> at (i) 20, (ii) 40, (iii) 60, (iv) 80, and (v) 100 mV s<sup>-1</sup>.

466 Tz for the Tz-saturated H<sub>2</sub>O solution. However, the peak-to-  
 467 peak separation for H<sub>2</sub>O-Tz ( $\Delta E_p = 149$  mV) is smaller than  
 468 that recorded for PS-CD-Tz<sub>NP</sub>, suggesting slower apparent  
 469 electron-transfer kinetics for the encapsulated nanoparticles.  
 470 The latter may be assigned to a phenomenon of electron  
 471 hopping between Tz molecules encapsulated within cyclo-  
 472 dextrin groups located at the periphery and inside the poorly  
 473 conducting polystyrene core. Thus, the tetrazine-containing  
 474 nanoparticles are redox-active and electrochemically accessible,  
 475 which has important implications for future catalytic and  
 476 sensing applications.

477 Finally, CVs of the PS-CD-Tz<sub>NP</sub> solution were recorded at  
 478 scan rates in the range of 20 to 100 mV s<sup>-1</sup>. A linear  
 479 dependence between the peak current and the square root of  
 480 the scan rate, indicative of diffusion-controlled behavior (Figure  
 481 S6), was observed. Assuming diffusion-controlled behavior, we  
 482 applied the Randles–Sevcik equation (eq 5) to estimate the  
 483 average concentration of electroactive Tz molecules encapsu-  
 484 lated in the nanoparticles, thus providing an idea of possible  
 485 drug or redox molecule loading capacity. From the oxidative  
 486 peak current shown in Figure 6A and using the diffusion  
 487 coefficient obtained for the small particles of  $3.57 \times 10^{-8}$  cm<sup>2</sup>  
 488 s<sup>-1</sup> via DLS analysis using eq 1, the estimated Tz concentration  
 489 for PS-CD-Tz<sub>NP</sub> is  $1.5 \times 10^{-4}$  mol L<sup>-1</sup>.

## 490 ■ CONCLUSIONS

491 A new bioinspired  $\beta$ -cyclodextrin-modified polystyrene poly-  
 492 mer has been prepared using a heterogeneous copper  
 493 nanocatalyst that facilitates glycopolymer purification and  
 494 preparation on a larger scale. Self-assembly via a “solvent-to-  
 495 water” nanoprecipitation method, in the presence of tetrazine-  
 496 naphthalimide, forms stable redox-active nanoassembled  
 497 particles and clusters with mean hydrodynamic diameters of  
 498 138 and 654 nm, respectively. The nanoparticles are considered  
 499 to be spheres constituting  $\beta$ -cyclodextrin and polystyrene  
 500 blocks, with  $\beta$ -cyclodextrin groups present in the outer layer.  
 501 Increased UV absorption, fluorescence, and electrochemical  
 502 signals confirm both the presence and improved uptake of  
 503 tetrazine-naphthalimide molecules in nanoparticles compared  
 504 to water. Solvchromatic shifts and a slight decrease in  
 505 electrochemical reversibility for the redox-active molecules in  
 506 the nanoparticles are consistent with their encapsulation in the  
 507 nanoparticles and their surface via Tz inclusion in  $\beta$ -  
 508 cyclodextrin groups. Furthermore, the fluorescence emission  
 509 reveals that both the imide antenna and tetrazine acceptor emit  
 510 together, proving that the energy transfer between the  
 511 chromophores is partially deactivated. As a consequence, the  
 512 fluorescence emission is less brilliant than that observed for the  
 513 tetrazine-naphthalimide dyad in organic solvent. The formation  
 514 of a small number of large clusters, observed here only by light

scattering experiments, is possible via a novel type of host–  
 guest interparticle cross-linking where tetrazine-naphthalimide  
 molecules act as a nanoparticle cross-linker. The potential use  
 of the new glycopolymer for host–guest encapsulation of  
 electrocatalysts for the construction of enzymatic biofuel cells  
 with improved performance and biocompatibility is currently  
 underway.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the  
 ACS Publications website at DOI: 10.1021/acs.lang-  
 muir.6b03512.

Glycopolymer synthesis details and characterization data  
 by DLS, NTA, and voltammetry (PDF)  
 Nanoparticle tracking analysis of PS-CD and PS-CD-  
 TzNP suspensions prepared by method A (ZIP)

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This work was supported by LABEX ARCANÉ (ANR-11-  
 LABX-0003-01) and platform Chimie NanoBio ICMG FR  
 2607 (PCN-ICMG). We gratefully acknowledge the assistance  
 of Hugues Bonnet with AFM imaging.

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