

## Supporting Information

**Star-like polypeptides as simplified analogues of horseradish peroxidase (HRP) metalloenzymes**

*Antoine Tronnet<sup>a,b</sup> Pedro Salas-Ambrosio,<sup>a</sup> Rosa Roman,<sup>c</sup> Lourdes Monica Bravo-Anaya,<sup>d</sup> Marcela Ayala<sup>\*c</sup> Colin Bonduelle<sup>\*a</sup>*

<sup>a</sup> A. Tronnet, P. Salas-Ambrosio, C. Bonduelle

CNRS, LCPO (Laboratoire de Chimie des Polymères Organiques (UMR5629)), University of Bordeaux, Bordeaux INP, 16 avenue Pey Berland, F-33600 Pessac, France.

E-mail: colin.bonduelle@u-bordeaux.fr

<sup>b</sup> A. Tronnet

CNRS, LCC (Laboratoire de Chimie de Coordination (UPR8241)), University of Toulouse, 205 route de Narbonne, F-31077 Toulouse, France.

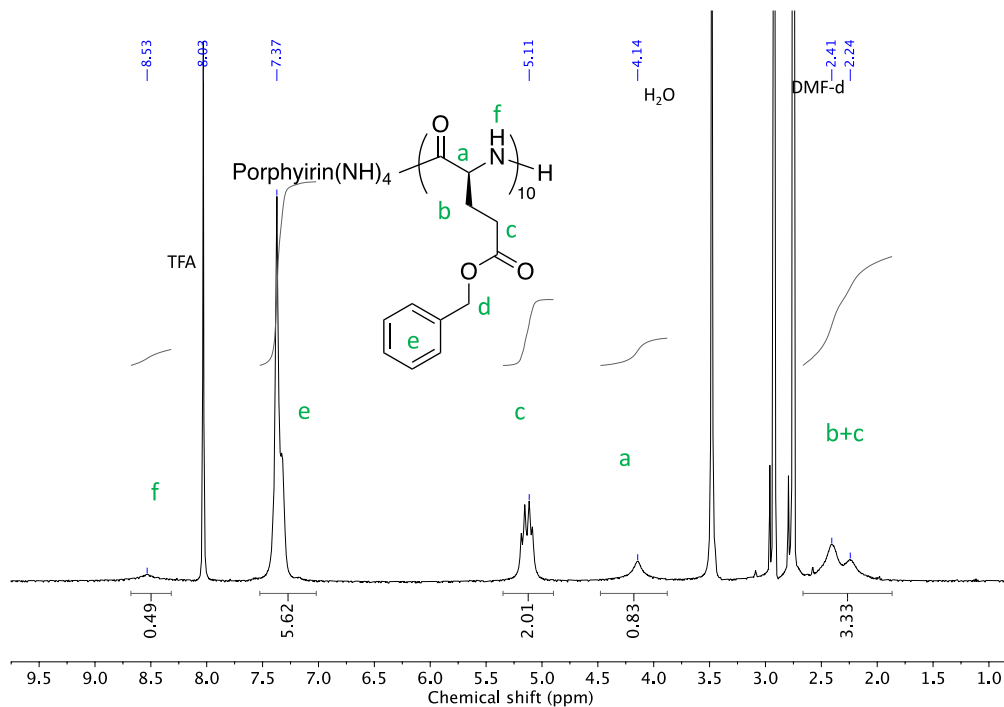
<sup>c</sup> R. Roman, M. Ayala

Departamento de Ingenieria Cellular y Biocatalisis, Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico.

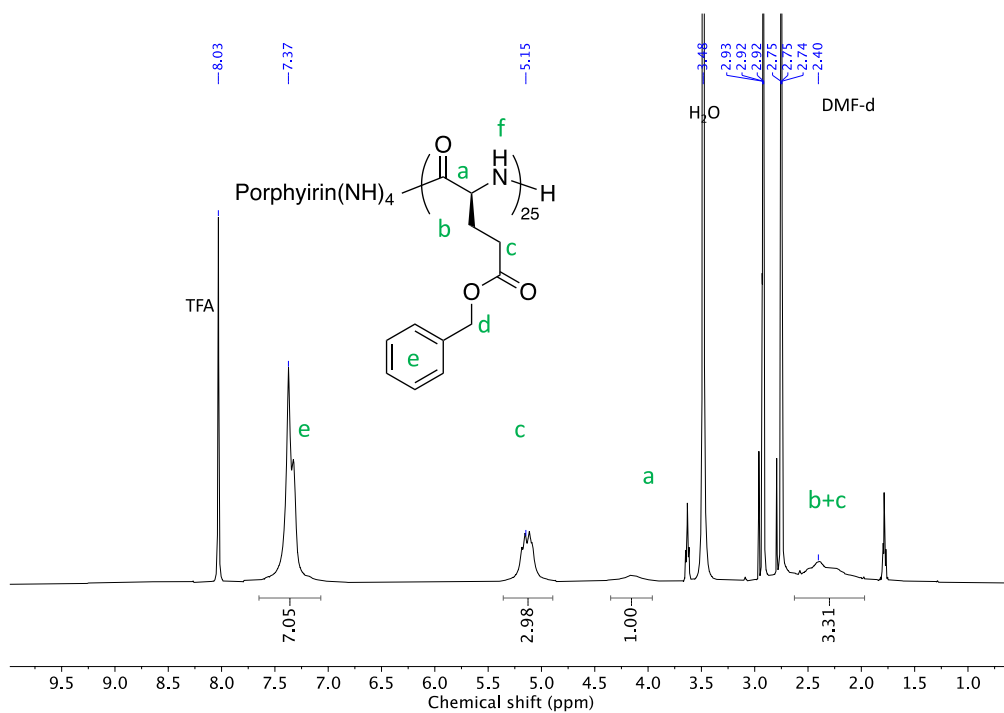
E-mail: maa@ibt.unam.mx

<sup>d</sup> L.M. Bravo-Anaya

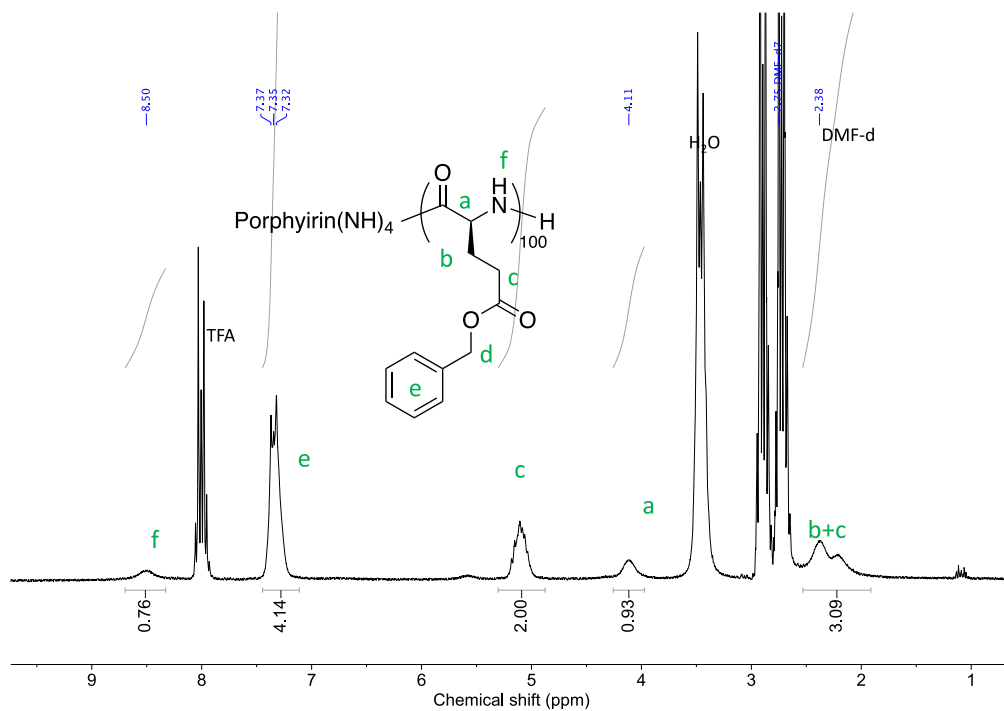
University of Rennes, Institut des Sciences Chimiques de Rennes, CNRS, UMR6226, Rennes, France.



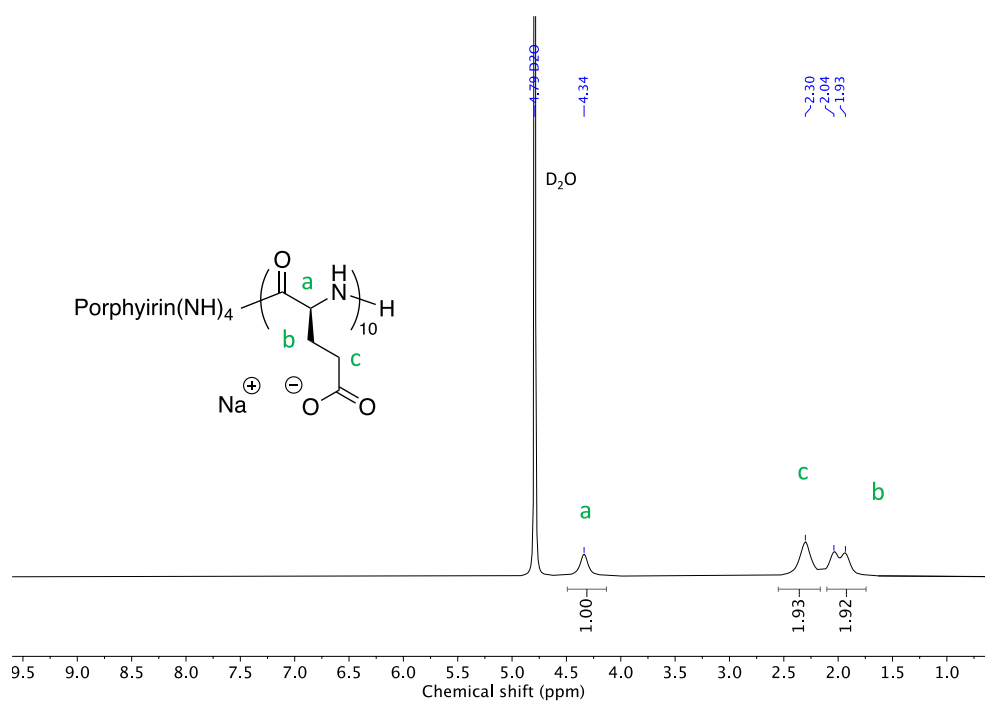
**Figure S1.**  $^1\text{H-NMR}$  of star-like PBLG initiated with porphyrin targeting a DP = 10 in DMF-d (polymer 1).



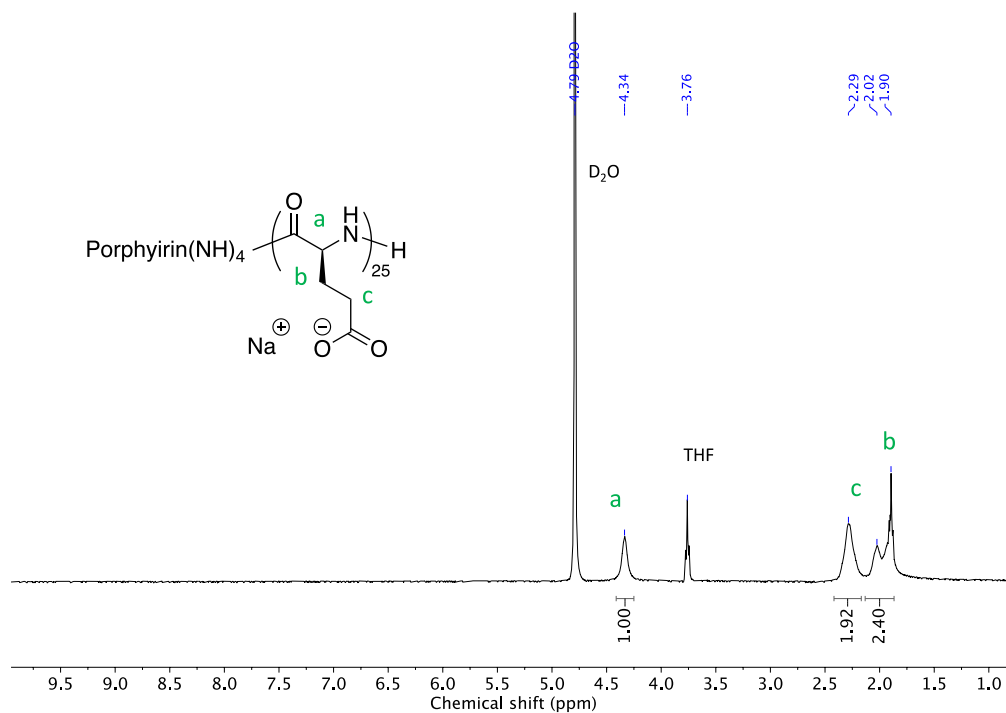
**Figure S2.**  $^1\text{H-NMR}$  of star-like PBLG initiated with porphyrin targeting a DP = 25 in DMF-d (polymer 2).



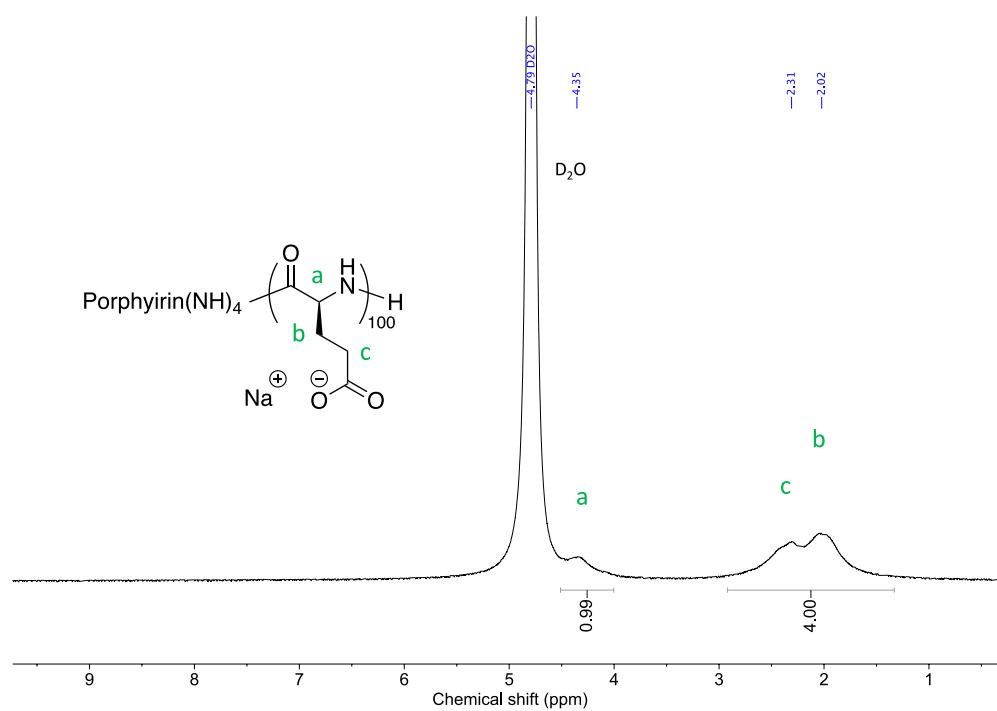
**Figure S3.**  $^1\text{H-NMR}$  of star-like PBLG initiated with porphyrin targeting a DP = 100 in DMF-d (polymer 3).



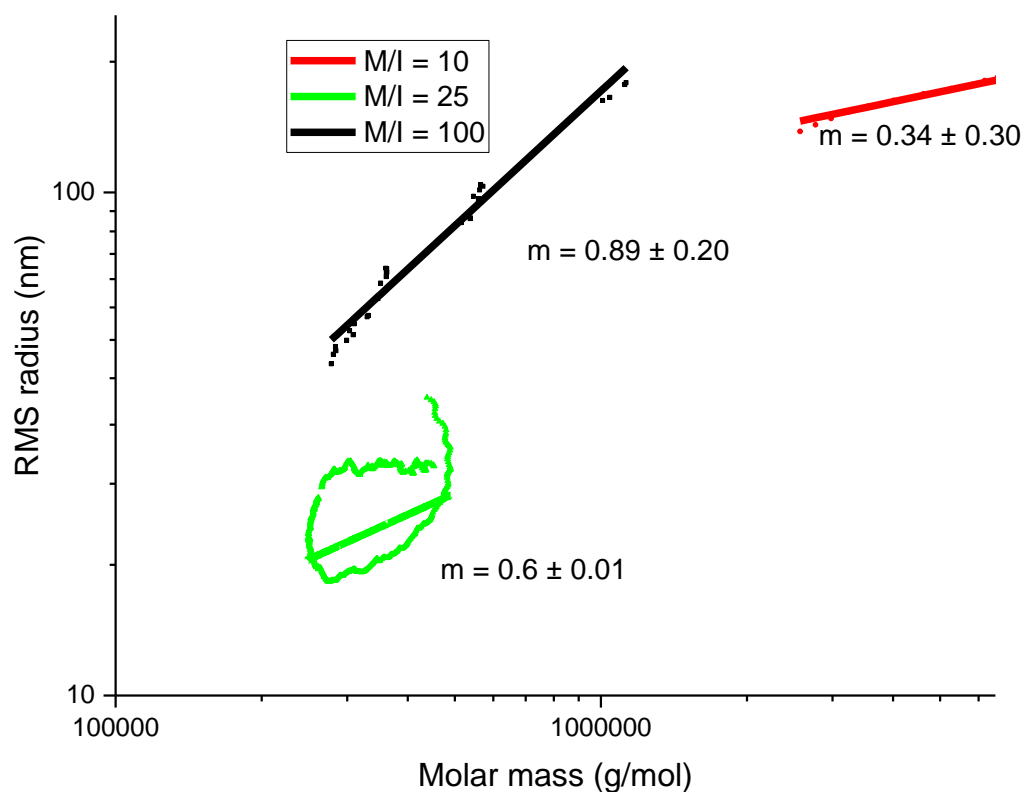
**Figure S4.**  $^1\text{H-NMR}$  of star-like PGA upon deprotection initiated with porphyrin targeting a DP = 10 in  $\text{D}_2\text{O}$  (polymer  $\mathbf{1}'$ ).



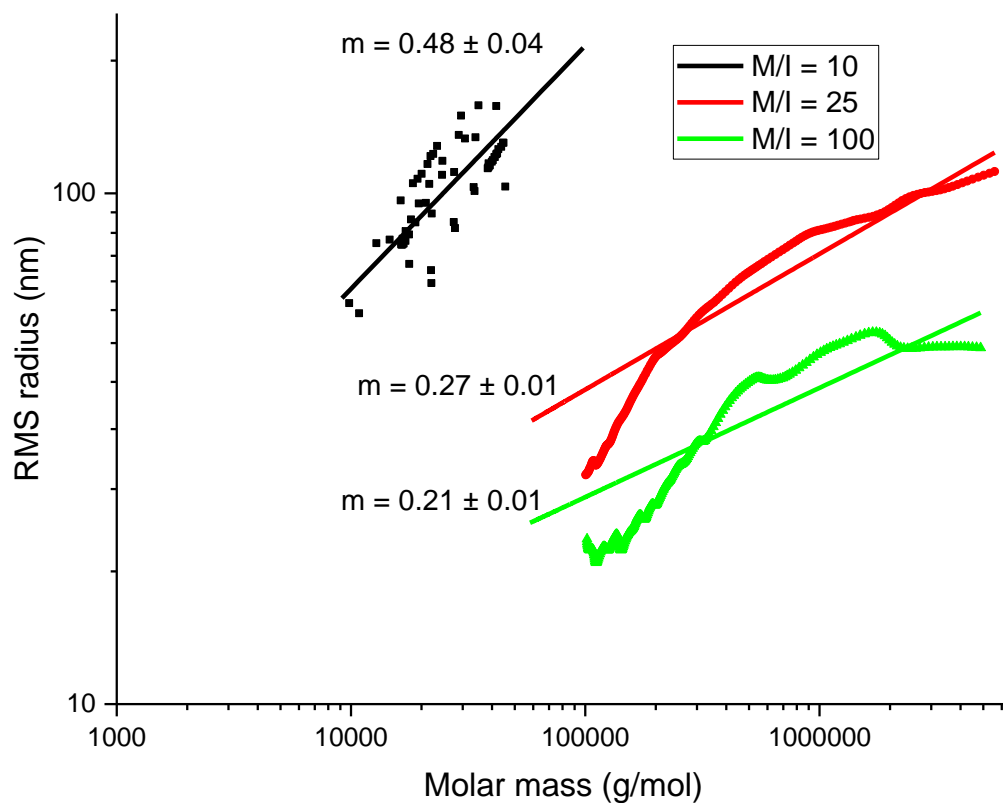
**Figure S5.**  $^1\text{H-NMR}$  of star-like PGA upon deprotection initiated with porphyrin targeting a DP = 40 in  $\text{D}_2\text{O}$  (polymer  $\mathbf{2}'$ ).



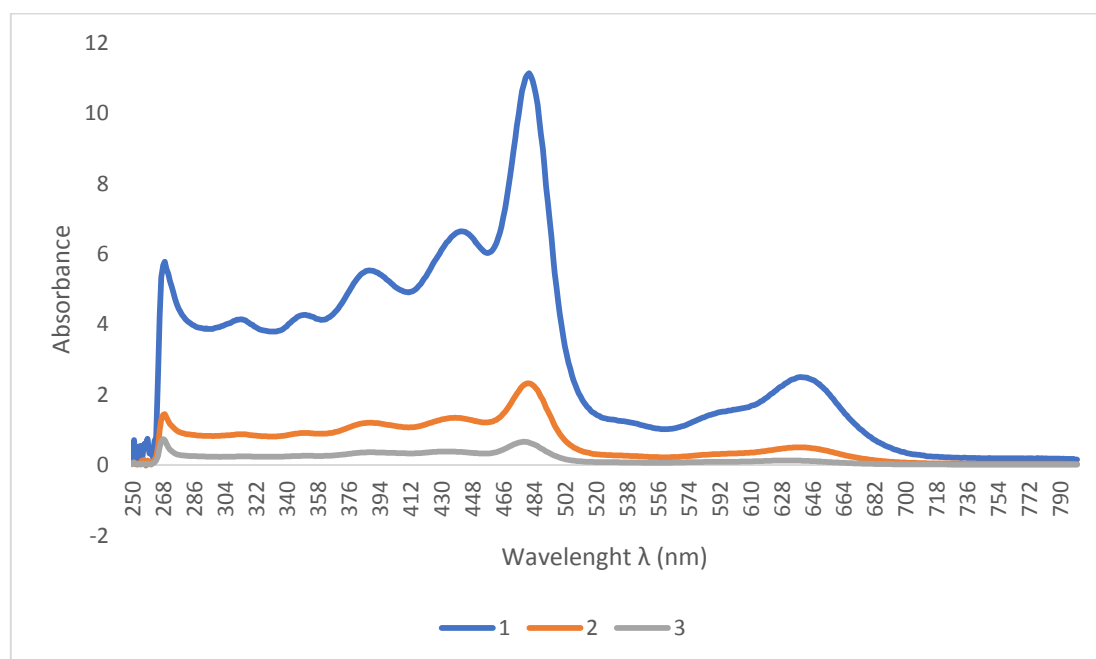
**Figure S6.**  $^1\text{H-NMR}$  of star-like PGA upon deprotection initiated with porphyrin targeting a DP = 100 in  $\text{D}_2\text{O}$  (polymer **3'**).



**Figure S7.** RMS plot versus molar of the star-like polymers (**1-3**) varying the M/I. The slope ( $m$ ) decreases due to the hydrodynamic ratio while displaying higher molar masses.



**Figure S8.** RMS plot versus molar of the star-like polymers (**1'**-**3'**) varying the M/I. The slope ( $m$ ) decreases due to the hydrodynamic radius while displaying higher molar masses.



**Figure S9.** UV-vis spectra of star-like protected conjugates 1, 2 and 3 in DMF

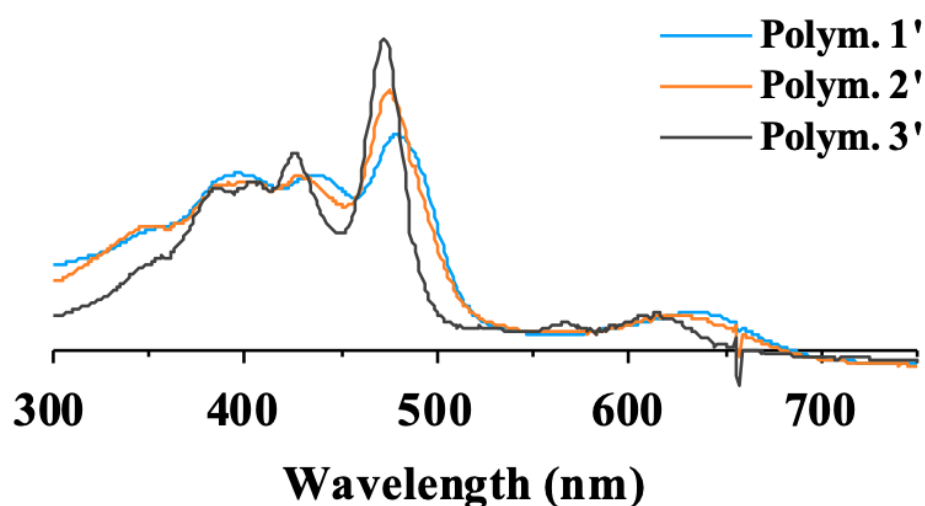
A solution of Mn-TAPP was prepared at  $1 \times 10^{-5}$  M in DMF and was analyzed by UV-vis. The molar attenuation coefficient ( $\epsilon$ ) of Mn-TAPP was calculated using the Beer-Lambert law giving  $\epsilon=90000$ . Solutions of 1 mg/mL of polymers 1, 2 or 3 in DMF were prepared and were analyzed by UV-vis (figure SX) in the same condition as Mn-TAPP. The concentration of porphyrin in the solution of polymers was calculated using  $\epsilon$  of Mn-TAPP and the Beer-Lambert law. Results for all polymers were listed in Table S1.

**Table S1. Degree of polymerization of Star-like protected conjugates determined by UV-vis before deprotection**

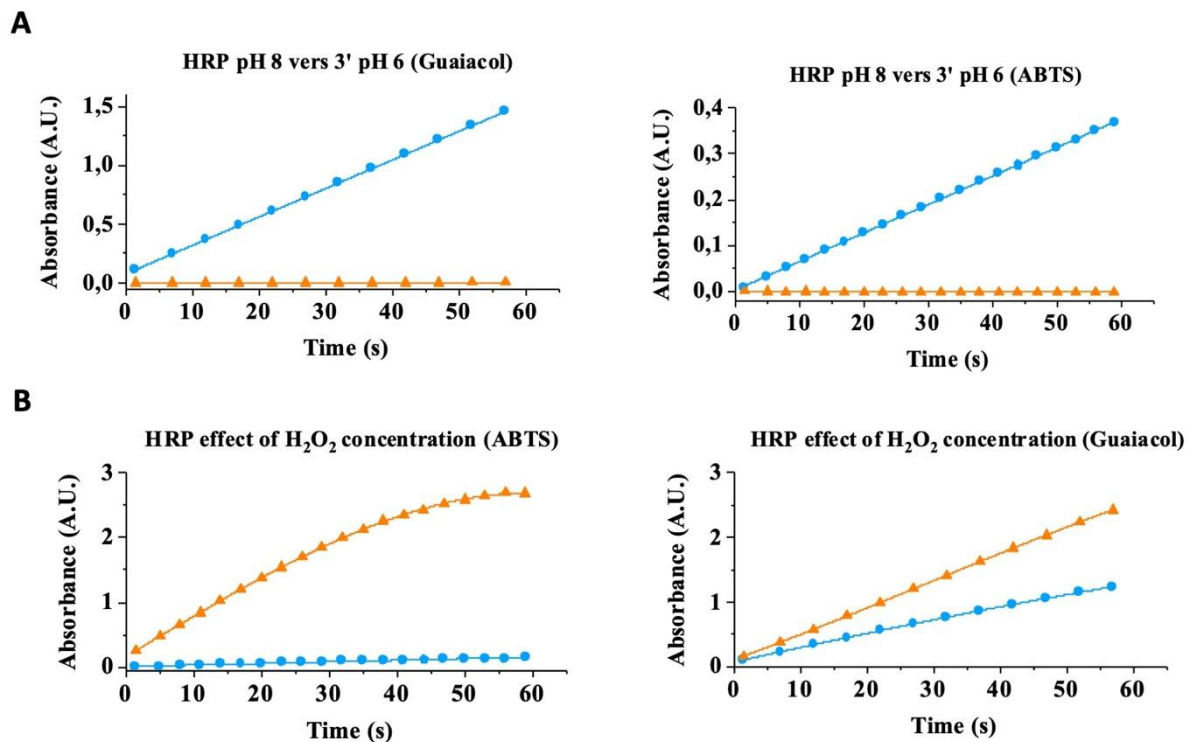
Star-like conjugate	Theoretical DP (DP / arm)	UV-vis DP (DP / arm)
1	40 (10)	34 (8.5)
2	100 (25)	172 (43)
3	400 (100)	660 (165)

**Table S2. Degree of polymerization of Star-like protected conjugates determined by SEC upon deprotection**

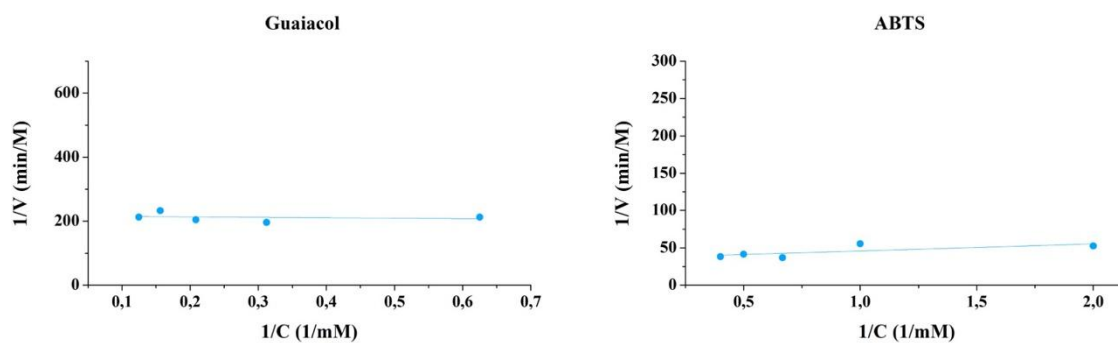
Star-like conjugate	Theoretical DP (DP / arm)	Mw (kg/mol)
1'	40 (10)	19.5
2'	100 (25)	52.7
3'	400 (100)	106



**Figure S10.** UV-vis spectra of star-like conjugates 1', 2' and 3' in imidazole buffer (Intensities were readjusted for polymers 2' and 3' to overlay signals at the same scale).

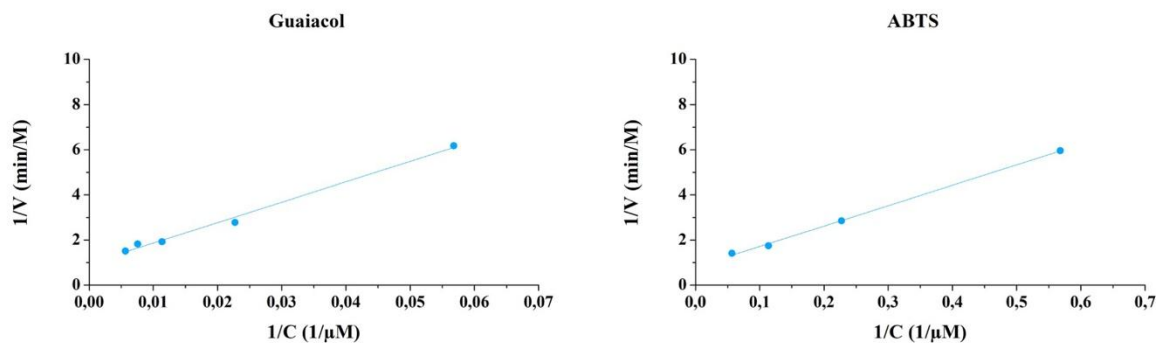


**Figure S11.** Guaiacol/ABTS oxidation by star-shaped polypeptides **3'** versus HRP: A) comparison of kinetics obtained in the worst pH conditions for HRP (pH = 8, blue) and the star-shaped polymer (pH = 6, orange); B) Effect of the concentration of H<sub>2</sub>O<sub>2</sub> on the oxidation mediated by HRP. Blue: 20 mM and orange: 1 mM.



**Figure S12.** Lineweaver-Burk plot of the H<sub>2</sub>O<sub>2</sub> mediated oxidation of various concentrations of guaiacol or ABTS using polymer **3'** (1 mM H<sub>2</sub>O<sub>2</sub>).

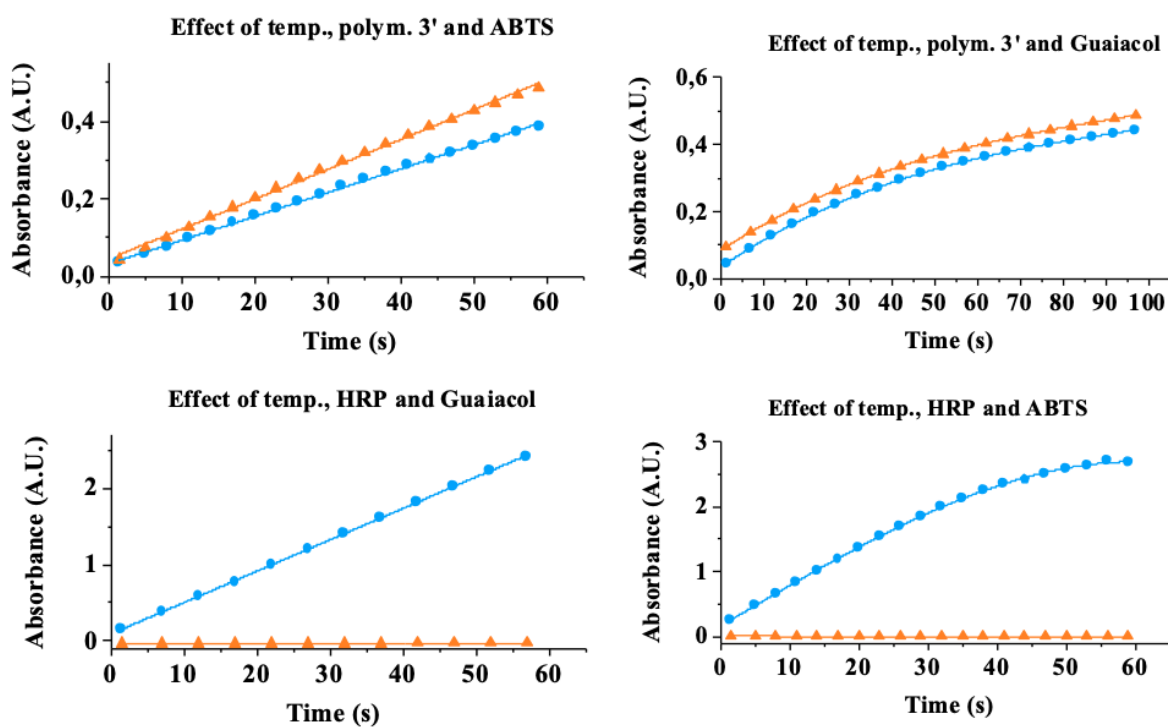




**Figure S13.** Lineweaver-Burk plot of the  $\text{H}_2\text{O}_2$  mediated oxidation of guaiacol or ABTS using HRP.

**Table S3.** Table summarizing  $K_M$  constants and  $K_M/k_{\text{cat}}$  ratios obtained with HRP (pH=6) and the polymer 3' (pH=8) using ABTS or guaiacol

Catalyst	ABTS		Guaiacol	
	$k_{\text{cat}}/K_M$	$K_M$	$k_{\text{cat}}/K_M$	$K_M$
Polymer 3'	$1155 \text{ M}^{-1} \text{ min}^{-1}$	16 mM	$714 \text{ M}^{-1} \text{ min}^{-1}$	45 mM
HRP	$57\text{E}12 \text{ M}^{-1} \text{ min}^{-1}$	11 $\mu\text{M}$	$5.7\text{E}12 \text{ M}^{-1} \text{ min}^{-1}$	100 $\mu\text{M}$



**Figure S14.** Guaiacol/ABTS oxidation by star-shaped polypeptides 3' versus HRP: effect of a pre-heating procedure (30 min.,  $80^\circ\text{C}$ ). Blue: before the treatment and orange, after the treatment.

