# SUPPORTING INFORMATION

# Interfacial activity of modified Dextran polysaccharide to produce enzyme-responsive oil-in-water nanoemulsions

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## **General reagents and methods**

## **Reagents**

Dextran from *Leuconostoc spp.* (DXT-40, Mn ~40 kDa), glycidyl methacrylate (GMA) (97%), dimethyl sulfoxide (DMSO) (98%), 3-mercaptopropionic acid ( $\geq$ 99%) and 2,2'- (ethylenedioxy)diethanethiol [3,6-dioxa-1,8-octane-dithiol (DODT)] (95%) were purchased from Aldrich and used as received. Phosphate-buffered saline (PBS) and dichloromethane were purchased from Scharlau and used as received. 4-(Dimethylamino)pyridine (DMAP) was purchased from Acros-Organics. Water (H<sub>2</sub>O) used in the syntheses, unless otherwise stated, was ultrapure water from a MilliQ A10 Gradient equipment (Millipore).

## Dynamic Light Scattering (DLS)

DLS analyses were conducted using a Zetasizer Nano ZS, ZEN3600 Model (Malvern Instruments Ltd). All measurements were performed in disposable sizing cuvettes at a laser wavelength of 633 nm and a scattering angle of 173°. Emulsion samples were dispersed in ultrapure water at a concentration of 2 mg oil/mL. Each measurement was repeated three times per sample at 25°C.

## Laser diffraction (LD)

LD analyses were conducted using a Mastersizer 3000 laser diffraction system (Malvern Instruments Ltd). All measurements were performed at a laser wavelength of 430 nm using 600 mL of ultrapure water. Each measurement was performed 5 times (5 seconds per run) in ultrapure water at 1500 rpm and 25°C following Mie dispersion model.

## Surface tension (ST)

Individual surface tension measurements were performed using KSV Sigma 700 Force tensiometer and "Surface Tension" program at the concentration of 5 g/L. Each sample was measured without stirring until surface tension stabilization was achieved (stabilization criteria: 4 runs with a variation below 1.5%). Description of the experimental setup: Small Vessel (diameter = 50 mm, max. volume = 70 mL), heavy phase (water) and light phase (air). Digital photography: each dextran polymer was dissolved in water at the concentration of 5 g/L (0.5wt%) and mixtures were shaken vigorously during 20 seconds and kept at rest for 10 seconds before digital photography acquisition.

### Synthesis of methacrylated dextran polymer (DXT-MA)

Dextran methacrylated polymer (DXT-MA) with different degrees of substitution (DS, percent of modified hydroxyl groups per repeating unit; DS=8-70%) were synthesized following a slightly modified published procedure.<sup>1</sup> First, dextran (DXT-40, 1g) was dissolved in 30 mL of dimethyl sulfoxide (DMSO) under a nitrogen atmosphere. To this solution, 200 mg of 4-(*N*,*N*-dimethylamino)pyridine (DMAP, 1.6 mmol) was added. Then, 1 mL of glycidyl methacrylate (GMA, 1.2 mmol) was incorporated and the mixture was stirred at room temperature. Aliquots of 5 mL were subsequently taken at different times and quenched by adding an equimolar amount of 0.1M HCl solution (0.27 mmol, 2.67 mL) to neutralize DMAP. After 4 days, the reaction mixture was quenched with 0.1M HCl and purified by dialysis against ultrapure water (MWCO 3,500 Da) at room temperature until deionized water twice per day). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) ( $\delta$  ppm) (DS=45%): 6.35-6.10 (m, 1H, methacrylic-CH), 5.92-5.72 (m, 1H, methacrylic-CH), 5.54-4.86 (2.7H, including H-1 and H-2/3 MA-substituted), 4.20-3.33 (12.4H, m, rest of hydrogen atoms of Glc), 1.98 (s, 3H, methacrylic-CH<sub>3</sub>).

Determination of the Degree of Substitution (DS): an example of this calculation with DS 45% is herein described. The most representative signals for DS calculation in the <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) spectrum are those at 6.35-6.10 ppm (m, 1H, TRANS-methacrylic-CH), 5.92-5.72 ppm (m, 1H, CIS-methacrylic-CH) and 4.20-3.33 ppm (12.4H, m, rest of hydrogen atoms of Glc). Reference signals for both molecules involved in the reaction are required. DS calculation requires the subtraction of 5H corresponding to the substituted Glc in the region of 3.3-4.2 ppm (12.4 – 5 = 7.4). This difference corresponds to 6 Hydrogen atoms of unmodified Glc. Integration of one single proton present in unmodified Glc moiety was obtained by the subsequent division against the total number of protons involved in the integral (7.4 / 6 = 1.23). DS was calculated as the ratio between the substituted Glc and the sum of both integrations (Glc + Glcsubstituted) following the formula:

Equation 1. DS calculation example (Fig. S1)

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$$DS(\%) = \frac{Glc_{substituted}}{Glc + Glc_{substituted}} x \ 100 = \frac{1}{1.23 + 1} x \ 100 = 45 \%$$



**Figure S1.** <sup>1</sup>H NMR spectrum (D<sub>2</sub>O, 500 MHz) of DXT-MA with DS=45%. For clarity reasons, only MA substitution at position 3 of glucose (Glc) has been depicted in the chemical structure insert. Protons shifted from Glc region of 3.3-4.2 ppm (H1, H1' and H3') are labelled in red and protons from MA moiety are described with letters "c" (2H, methacrylic-CH) and "a" (3H, methacrylic-CH<sub>3</sub>).

## Preparation of dextran-based single-chain polymer nanoparticles (DXT-SCPN-MA)

Following the reported procedure,<sup>2</sup> 0.413 mL of a previously prepared 0.15 M solution (2 mL, MeOH/PBS, 1:1, v/v, adjusted to pH= 9.5) of cross-linker DODT (0.06 mmol, 10.1  $\mu$ L) was added dropwise using a syringe pump (0.04 mL/h) over a 0.02 M solution of DXT-MA (DS=45%, 100 mg, 0.025 mmol of MA, 13 mL PBS, adjusted to pH= 9.5) during 8 h at room temperature and under constant stirring. After addition, the reaction was maintained under stirring at room temperature for 12 h. A 5 mL fraction was purified by dialysis against ultrapure water (MWCO 3500 Da) until deionized water conductivity values (< 1 $\mu$ S) were achieved (5 days, refreshing with 4 L of deionized water twice per day). Finally, the resulting aqueous solution was freeze-dried to obtain nanoparticles as a white solid. Yield >90%. <sup>1</sup>H NMR spectrum (**Fig. S2**, 500 MHz, D<sub>2</sub>O;  $\delta$  ppm): 6.34-6.12 (m, 1H, methacrylic-CH), 5.94-5.70 (m, 1H, methacrylic-CH), 5.55-4.85 (3.4H, including H-1 and H-2/3 MA-substituted),

4.34-3.28 (16.9H, m, rest of Glc and  $2xCH_2O$  of cross-linker), 3.06-2.53 (2.8H, m, CH(CH<sub>3</sub>)CH<sub>2</sub>S, CH<sub>2</sub>S of cross-linker), 1.98 (s, 3H, methacrylic-CH<sub>3</sub>), 1.6 (s, 3H, cross-linker-CH<sub>3</sub>). D<sub>h</sub> (DLS) = 20 ± 8 nm; PDI 0.25 (**Fig. S3**).



**Figure S2.** <sup>1</sup>H NMR spectrum (D<sub>2</sub>O, 500 MHz) of DXT-SCPN-MA. For clarity reasons, only protons from the functionalized moieties (MA and MA-Crosslinker) have been labeled.



**Figure S3.** Size distribution of intensity-average diameter for DXT-SCPN-MA with a hydrodynamic diameter of 20 nm (PDI 0.25) obtained by DLS in PBS at 10 mM, pH 7.4, and 25°C.

## Functionalization of DXT-SCPN-MA with 3-mercaptopropionic acid (DXT-SCPN-COO<sup>-</sup>)

One batch synthesis of the functionalized DXT-SCPN-COO<sup>-</sup> was achieved by slowly adding 2 mL of an aqueous solution of 3-mercapto propionic acid (61.4  $\mu$ L, 7.5 mmol, pH= 9.5) to the DXT-SCPN-MA reaction mixture previously reported.<sup>2</sup> The reaction was stirred for 24 h and the excess acid was removed by dialysis against ultrapure water (MWCO 3,500Da) until deionized water conductivity value (< 1 $\mu$ S) wase reached (5 days, refreshing with 4L of deionized water twice per day). The resulting aqueous solution was freeze-dried to obtain nanoparticles as a white solid. Yield >90%. <sup>1</sup>H NMR spectrum (Fig. S4, 500 MHz, D<sub>2</sub>O;  $\delta$  ppm): 5.55-4.85 (2.4H, including H-1 and H-2/3 MA-substituted), 4.34-3.28 (11.6H, m, rest of Glc and 2xCH<sub>2</sub>O of cross-linker), 3.11-2.5 (6.4H, m, CH(CH<sub>3</sub>)CH<sub>2</sub>S, CH<sub>2</sub>S of cross-linker and CH<sub>2</sub>S of MPA), 1.65-1.19 (3H, including MA-CH<sub>3</sub> and cross-linker-CH<sub>3</sub>). D<sub>h</sub> (DLS) = 13 ± 4 nm; PDI 0.33. Zeta-potential (pH = 7.2) = -20mV ± 5.



**Figure S4.** <sup>1</sup>H NMR spectrum (D<sub>2</sub>O, 500 MHz) of DXT-SCPN-COO<sup>-</sup>. For clarity reasons, only protons from the functionalized moieties (MPA and MA-Crosslinker) have been labeled.



**Figure S5.** Size distribution of intensity-average diameter for DXT-SCPN-COO<sup>-</sup> with a hydrodynamic diameter of 13 nm (PDI 0.33) obtained by DLS in PBS at 10 mM, pH 7.4, and 25°C.

## Functionalization of the DXT-MA (45%) with 3-mercaptopropionic acid (DXT-COO<sup>-</sup>)

An aqueous solution of 3-mercaptopropionic acid (430 µL, 5 mL H<sub>2</sub>O, pH= 9.5) was slowly added to a previously prepared solution of DXT-MA (DS=45%, 350 mg, 20 mL H<sub>2</sub>O, pH= 9.5). The reaction was maintained under constant stirring for 12 h and then purified by dialysis against ultrapure water (MWCO 3,500Da). The resulting aqueous solution was freeze-dried to obtain the resulting quenched polymer as a white solid. <sup>1</sup>H NMR (**Fig. S6**, 500 MHz, D<sub>2</sub>O)  $\delta$  ppm: 5.41-4.86 (2.5H, including H-1 and H-2/3 MPA-substituted), 4.20-3.33 (10.2H, m, rest of Glc), 3.02-2.49 (7H, m, MPA except CH<sub>3</sub>), 1.29 (s, 3H, MA-CH<sub>3</sub>). Zeta Potential (pH = 7.2) = -12 mV ± 7.



**Figure S6.** <sup>1</sup>H NMR spectrum ( $D_2O$ , 500 MHz) of DXT-COO<sup>-</sup> functionalized with mercaptopropionic acid. For clarity reasons, only protons from the MPA-functionalized moieties have been labeled.

## Production of oil-in-water (O/W) emulsions

Each dextran derivative (DXT-R) was dissolved in deionized water in an 8 mL glass vial. To this solution, the oil was added. The relative amounts of DXT-R, water and oil were modified to reach always a final amount of 2g. The emulsion was then formed by sonication (0°C, no stirring) using an UP400S (Hielscher) system at 100% of amplitude and pulse during 4 minutes (400 W) with a H3 sonotrode tip (3 mm diameter, 100 mm length).

## **Surface Tension analysis**

The surface-active properties of the four dextran derivatives, synthesised from the same batch (DXT-MA with DS=45%), were measured at the emulsion production concentration (5 g/L). For a better comparison, each solution was vigorously shaken for 20 s in order to verify the adsorption of the modified polysaccharides at the air/water interface, via the formation of foam (**Figure S7**).



**Figure S7** Surface tension values obtained for aqueous solutions of DXT-MA, DXT-COO<sup>-</sup>, DXT-SCPN-MA and DXT-SCPN-COO<sup>-</sup> at the concentration of 0.5wt% together with a representative digital picture of foam formation in the same solutions after being shacked for 20 seconds.

# Emulsion prepared in the presence of DXT-MA, DXT-COO<sup>-</sup>, DXT-SCPN-MA and DXT-SCPN-COO<sup>-</sup>

To evaluate their differences, the four dextran modified emulsifiers (DXT-MA, DXT-COO<sup>-</sup>, DXT-SCPN-MA and DXT-SCPN-COO<sup>-</sup>) synthesised from the same DXT-MA batch (DS=52%) were used at a concentration of 0.5 wt% to generate (o/w)-emulsions using 10%

wt. olive or sunflower oils. Size characterization was performed by Laser diffraction (LD) and Dynamic Light Scattering (DLS).

The effect of pH was also studied by preparing the emulsion at pH 7.4, while the one listed in Table 1 were prepared at pH 7.4 (Table S1).

Stability of the emulsion was assessed by visual inspection for any sign of demulsification (i.e. the presence of oil droplets at the surface of the aqueous phase) and DLS.

Table S1. Summary of the hydrodynamic diameter ( $D_h$ ), polydispersity (PDI), volumeaverage diameter ( $D_v$ ) and uniformity of O/W emulsions produced by sonication at 10wt% of vegetable oil (olive, O and sunflower) with an aqueous solution containing 0.5wt% of DXT, DXT-COO<sup>-</sup>, DXT-MA, DXT-SCPN-COO<sup>-</sup> or DXT-SCPN-MA at pH 7.4. All DXT derivatives proceed from the same batch of DXT-MA with DS=52%.

Emulsifier	Oil	Stability <sup>a</sup>	D <sub>h</sub> <sup>b</sup> (nm)	PDI <sup>b</sup>	D <sub>v</sub> c (d.µm )	Uniformity c	D <sub>h</sub> (nm) After 15 Days	PDI After 15 days
DXT-COO-	Olive	OK	218	0.3	1.8	1.4	217	0.25
DXT-MA	Olive	OK	385	0.50	1.3	0.57	394	0.24
DXT-SCPN- COO <sup>-</sup>	Olive	OK	237	0.26	0.9	0.7	244	0.25
DXT-SCPN- MA	Olive	OK	350	0.28	1.19	0.56	315	0.38
DXT-COO-	Sunflower	OK	227	0.3	1.6	1.3	258	0.25
DXT-MA	Sunflower	OK	342	0.25	1	0.6	345	0.3
DXT-SCPN- COO <sup>-</sup>	Sunflower	OK	271	0.24	0.8	0.67	258	0.25
DXT-SCPN- MA	Sunflower	OK	612	0.6	1.21	0.5	406	0.26

<sup>a</sup> stability after 1 week at T=4°C as judged by visual inspection and DLS; <sup>b</sup> obtained by DLS at 25°C; <sup>c</sup> obtained by LD at room temperature.

It is noteworthy that smaller droplet sizes were obtained at physiological pH in all cases.

**Table S2**. Summary of the hydrodynamic diameter ( $D_h$  in nm) obtained by DLS at 25°C of the O/W emulsions droplets produced by sonication at 10wt% of vegetable oil (olive, O, and sunflower, SF) with an aqueous solution containing 0.5wt% of DXT, DXT-COO<sup>-</sup>, DXT-MA, DXT-SCPN-COO<sup>-</sup> or DXT-SCPN-MA from Table 1.

Emulsifier	Oil	Original	1 day	1 week	2 weeks	1 month	3 months
DXT- COO <sup>-</sup>	0	300	300	310	320	340	390
DXT-MA	0	460	460	470	460	460	470
DXT- SCPN- COO <sup>-</sup>	0	230	230	240	260	290	340
DXT- SCPN- MA	0	1180	1150	1170	1170	1210	1240
DXT- COO-	SF	220	220	230	250	300	400
DXT-MA	SF	380	380	390	390	410	400
DXT- SCPN- COO-	SF	310	310	320	330	440	500
DXT- SCPN- MA	SF	1620	1620	1650	1640	1660	1650

Example of emulsion using DXT-COO<sup>-</sup> as emulsifier that demonstrate the very high polydispersity of the droplet size distribution as judged by LD, despite observing a relatively small hydrodynamic diameter by DLS (see Table 1 main manuscript) (**Fig. S8**).



**Figure S8.** Multimodal size distributions obtained for (o/w)-emulsions prepared at 10% wt. olive oil and 0.5% wt. of DXT-COO<sup>-</sup> measured by Laser diffraction (LD).

# Characterization of DXT-MA with different DS by <sup>1</sup>H NMR



Figure S9. <sup>1</sup>H NMR spectra for DXT-MA with DS = 8%.

![](_page_13_Figure_0.jpeg)

Figure S10. <sup>1</sup>H NMR spectra for DXT-MA with DS = 10%.

![](_page_14_Figure_0.jpeg)

**Figure S11.** <sup>1</sup>H NMR spectra for DXT-MA with DS = 13%.

![](_page_15_Figure_0.jpeg)

**Figure S12.** <sup>1</sup>H NMR spectra for DXT-MA with DS = 36%.

![](_page_16_Figure_0.jpeg)

Figure S13. <sup>1</sup>H NMR spectra for DXT-MA with DS = 50%.

Surface tension of water as a function of the DXT-MA concentration. The results were plotted against the logarithm of the polymer concentration in water, as shown in **Fig. S14**.

![](_page_16_Figure_3.jpeg)

**Figure S14.** Graphic representation of surface tension of aqueous polymer solutions, for pure dextran (DXT) and methacrylate functionalized dextran (DXT-MA; DS=10% and DS=36%) versus polymer concentration and a representative line at the concentration fixed for emulsions production (0.5wt%).

#### **Enzyme-responsiveness**

Experimentally, 2 mg of CALB were incubated towards 100 mg of a sunflower oil-in-water emulsion prepared at 40wt% oil and 0.5wt% of DXT-MA (DS=52%) emulsifier. An emulsion fraction of 100 mg were mixed with 100  $\mu$ L of lipase (CALB, 20 mg/mL) in an HPLC glass vial. Mixture was kept in rest 20 h at room temperature for OM analysis and 40 h for digital pictures acquisition.

The change in diameter of the droplets was monitored by LD. In addition to the optical microscope picture, the distribution of droplet diameter of the emulsions with or without CALB was determined by Laser Diffraction Figure S15.

![](_page_17_Figure_4.jpeg)

**Figure S15.** Size distribution of the droplets of sunflower oil-in-water emulsion at 40wt% using 0.5wt% DXT-MA (DS=52%) without (A) or with (B) CALB enzyme at t= 0, t= 20hrs and t=40 hrs. Note that in the absence of CALB, the emulsion remained stable with unchanged size distribution. On the other hand, in the presence of CALB, a slight shift of the droplet size distribution is observed after 20 hrs. More importantly, larger droplets could be observed after 40 hrs, indicating the destabilization of the system. It is important to point out that the process is relatively slow and more time (and surely more enzyme) would be required to achieve total demulsification.

## **References**

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