SUPPORTING INFORMATION

Controlled Polymerisation and Purification of Branched Poly(lactic acid) Surfactants in Supercritical Carbon Dioxide

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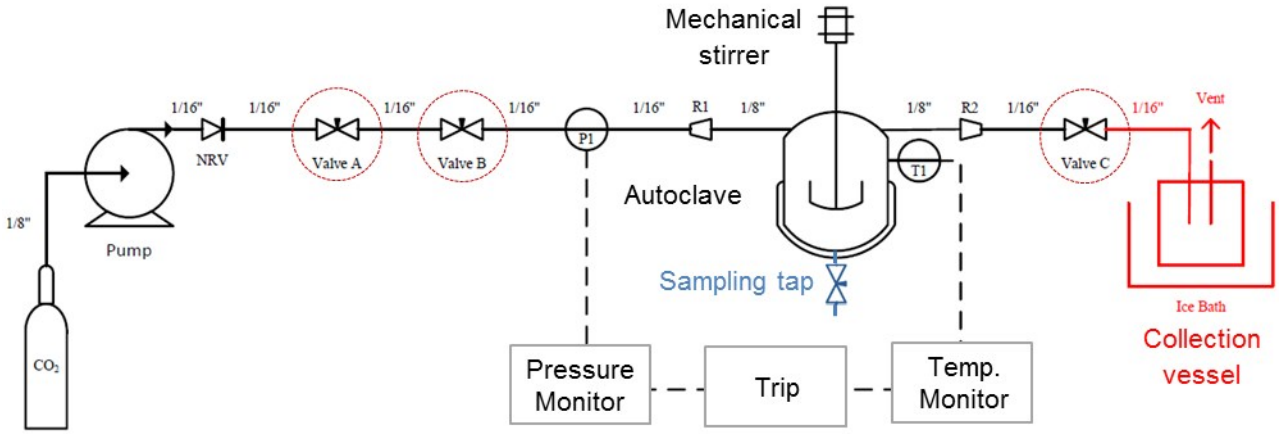

Scheme S1: Reaction schematic for the synthesis and purification of S-PLA. Valves highlighted in dashed circles are open during the purification with $scCO_2$, and recovered reagents were collected in a vial fitted with a needle valve and cooled in an ice-bath.

(**A**) $$M_n{}^{NMR} = (144\, x\, \frac{[M]_0}{[I]_0} x \frac{\%\, conversion}{100}) + 182$$ (**B**) $$M_n{}^{arm} = \frac{a' + a''}{a'} \times 72$$

Equation S1: Determination of (**A**) the molecular weight of S-PLA assuming linear growth ($M_n{}^{NMR}$) and (**B**) the molecular weight of each PLA arm ($M_n{}^{arm}$) using end-group integration values. $[M]_0$ represents the molar concentration of *D,L*-lactide and 144 the g/mol. $[I]_0$ represents the molar concentration of the co-initiator and 182 the g/mol, 72 represents the molecular weight of each lactoyl unit (*D,L*-lactide contains 2 lactoyl units), with a, a' and a'' referring to the integration of peaks from the S-PLA ^{1}H-NMR spectrum.

$$\#\ arms = \frac{PLA}{(total/6)}$$

Equation S2: Determination of the average number of hydroxyl groups corresponding to PLA end groups, giving an indication of the number of PLA arms. The total number of hydroxyl groups was divided by 6 as this is the maximum number of hydroxyls per compound.

Toxicity Assessment – Sample Preparation

Samples were prepared as 1M concentrations in DMSO, to aid addition to the broth medium. 96-well microtiter plates were used for assessment and bacteria were inoculated into the liquid growth medium (Müller Hinton Broth) in the presence of different concentrations of the compounds being assessed (serial dilutions with the highest concentration typically being 100 mmol, or 10 mmol for $Sn(Oct)_2$). Growth was assessed after incubation at 30 °C for a defined period of time (17-20 hours) and the OD was measured using an ELISA reader (Infinite M200 NanoQuant, Tecan) at λ = 405 nm after shaking the plate for 20 seconds. Controls were repeated without bacterial inoculation giving a baseline absorbance for each sample concentration. Higher absorbance than the baseline readings indicated that bacterial growth had taken place, whereas no change in absorbance indicated full inhibition of bacterial growth. The absorbance *vs*. sample concentration was plotted allowing us to determine changes in bacterial growth at varying sample quantities.. Positive and negative controls were performed in parallel.

Compounds were initially dissolved in DMSO to aid solubilisation in the growth medium. Pure DMSO at the highest concentration (100 mmol) was observed to slightly inhibit bacterial growth (by 20%). Consequently measurements made at this concentration of DMSO were disregarded when determining LC_{50} values, ensuring all growth inhibition was a result of the compound not co-solvent.

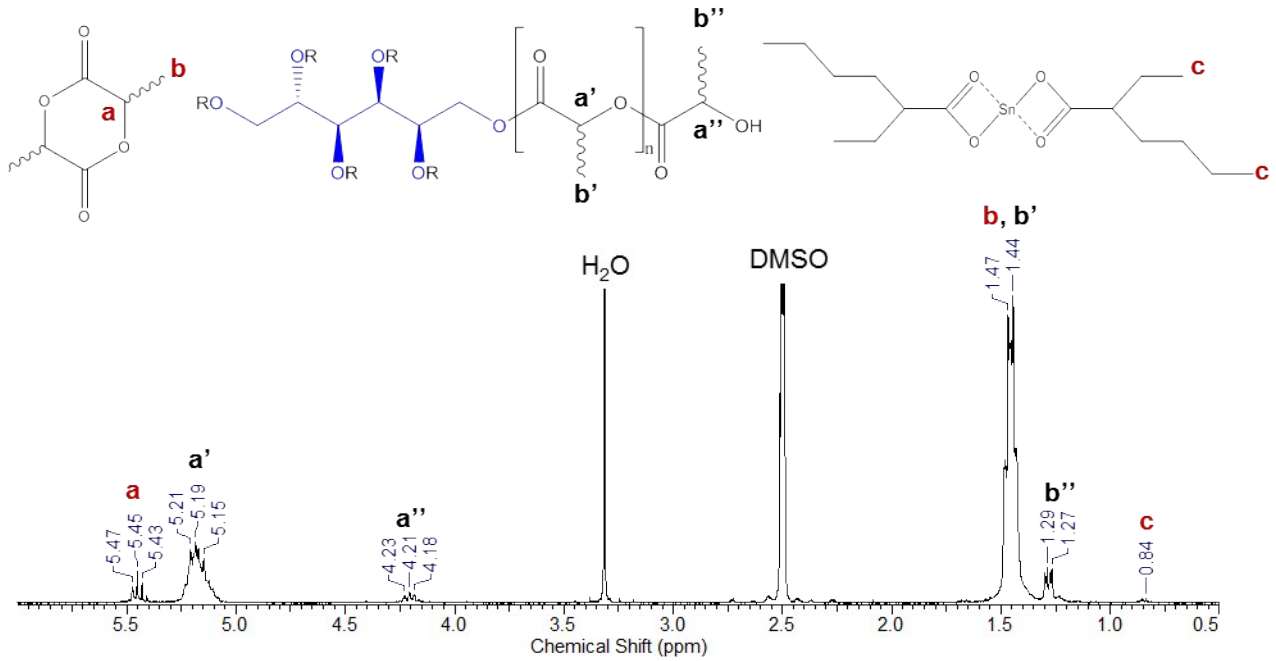

Figure S1: ^{1}H-NMR of S-PLA (6800 Da) synthesised in $scCO_2$ at 95 °C at an 85% conversion, analysed using DMSO-d_6. The conversion of the S-PLA can be determined by integration of the polymer methine peaks at **a'** and **a''** compared to the integration of all methine groups, including residual monomer identified from peaks at **a; co**

$$nv. = \frac{a' + a''}{a + a' + a''} x\ 100$$

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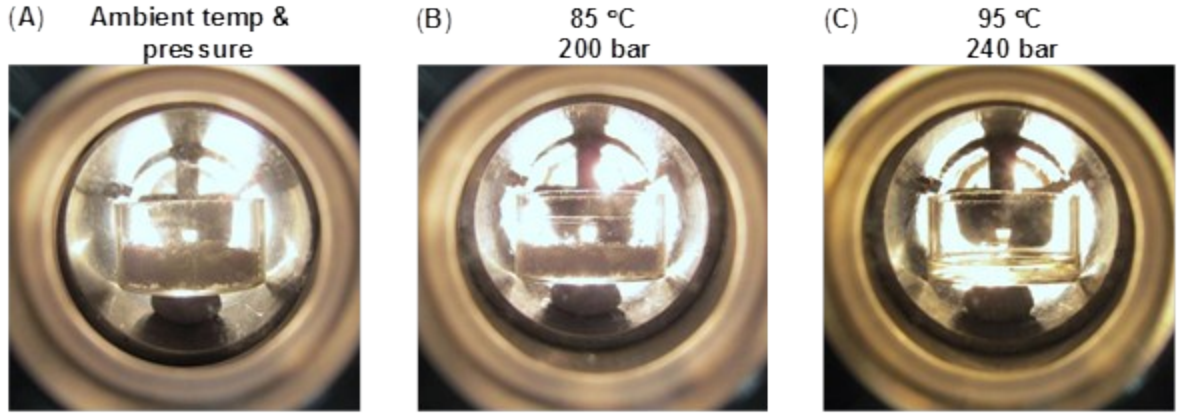
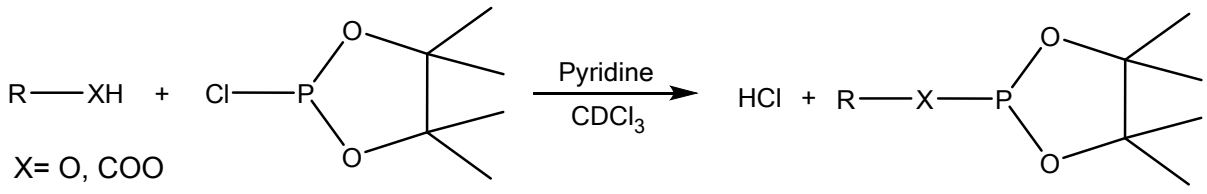


Figure S2: Transitions observed for *D,L*-lactide in the view cell at increasing temperatures & pressure; (**A**) *D,L*-lactide in the form of a white powder (room temperature and ambient pressure); (**B**) sample partially plasticised at 85 °C and 200 bar; (**C**) polymer fully plasticised at 95 °C and 240 bar.



(**I**) (**II**)

Figure S3: Phosphorylation reaction of hydroxyl group with TMDP (2-chloro-4,4,5,5 tetramethyldioxaphospholane) (**I**) and the derivatised product (**II**).

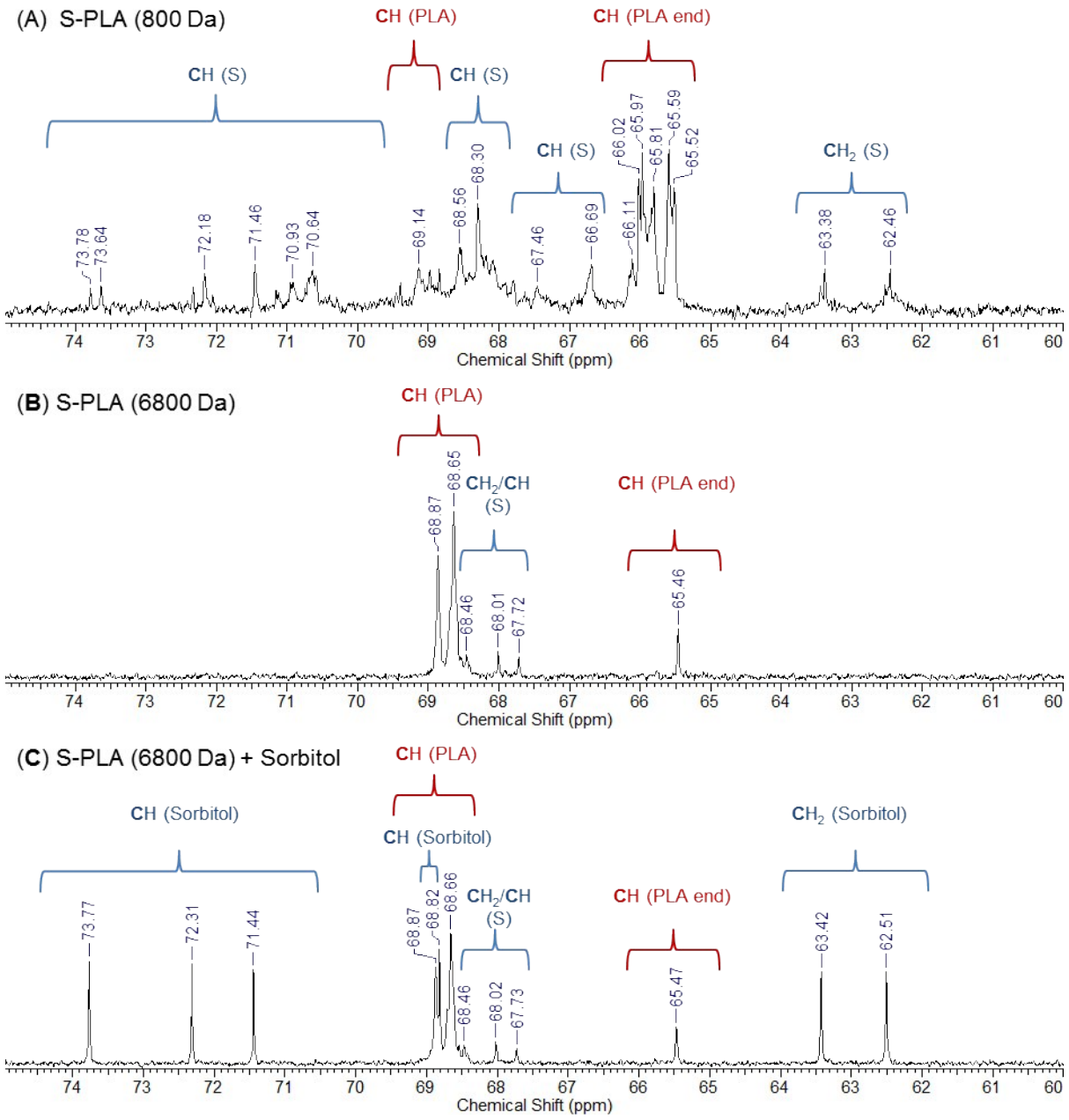


Figure S4: ^{13}C-NMR spectra of (**A**) S-PLA (800 Da), (**B**) S-PLA (6800 Da) and (**C**) S-PLA (6800 Da) spiked with sorbitol, measured in DMSO-d_6. Peaks corresponding to ^{13}C from PLA (CH) and **C**H$_2$/**C**H from *D*-sorbitol (S) have been labelled. Note the multitude of peaks identified in lower molecular weight S-PLA (**A**). These are observed as *D*-sorbitol is not fully derivatised, causing differing shifts in resonance depending on which OH groups are initiated. On average two OH groups per *D*-sorbitol molecule are derivatised, however differing peaks will be observed for compounds with one or three derivatised hydroxyls. Similar shifts to the underivatised *D*-sorbitol in spectrum (**C**) are identified alongside a narrow dispersity by GPC (Đ = 1.2). When the co-initiator is fully derivatised as seen in the spectrum obtained for S-PLA (6800 Da) (**B**) fewer peaks are observed, and these correspond well with spectra obtained by Numata *et al*.,[1] showing derivatisation of co-initiator hydroxyl groups.

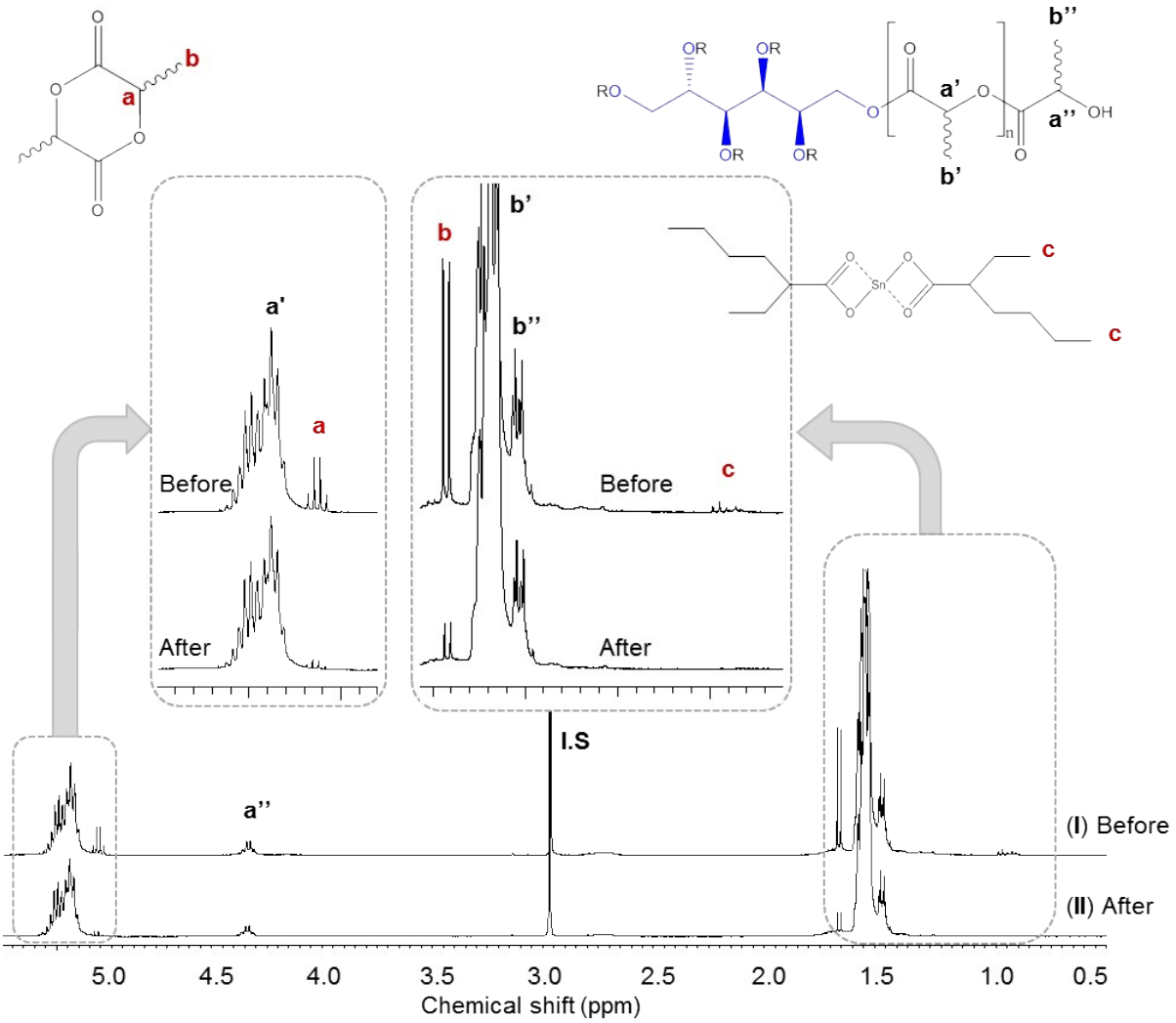


Figure S5: ^{1}H-NMR spectrum of S-PLA 6800 Da synthesised at 95 °C at 240 bar after 3 hours (**I**) before and (**II**) after purification with $scCO_2$ for 15 minutes. Dimethyl sulphone ($DMSO_2$) was used as an internal standard (I.S) (δ = 2.99). Note reduction in peaks corresponding to *D,L*-lactide (a and b) and $Sn(oct)_2$ (d) indicating that the CO_2 extraction process efficiently removes the monomer and catalyst (to below the detection limits of the technique).

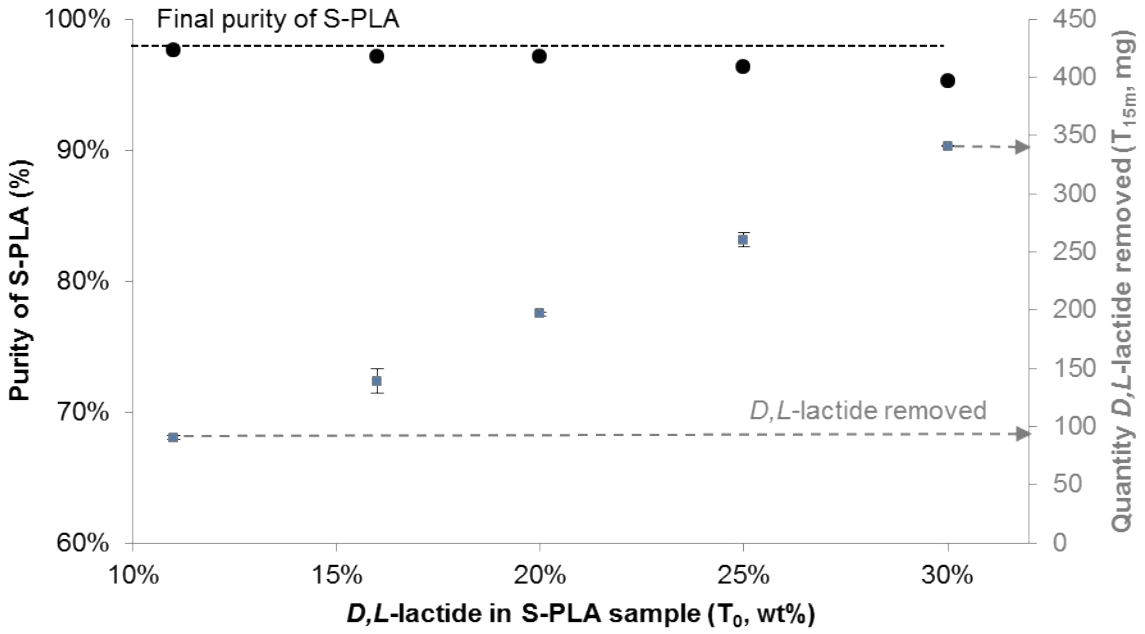


Figure S6: S-PLA deliberately spiked with increasing quantities of *D,L*-lactide and then purified using $scCO_2$ (15 min, 45 °C 240 bar). Product purity of S-PLA after processing (●) and the quantity of *D,L*-lactide removed (■) is highlighted. The black dashed line indicates 98% purity.

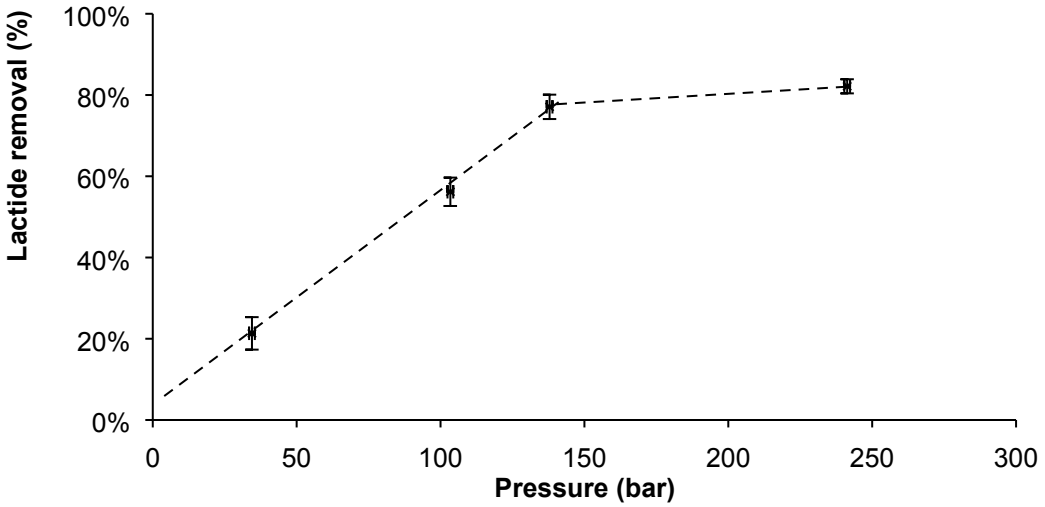


Figure S7: Purification of S-PLA 6800 Da at 45 °C when varying pressure with a purification time of 15 minutes and a constant flow rate.

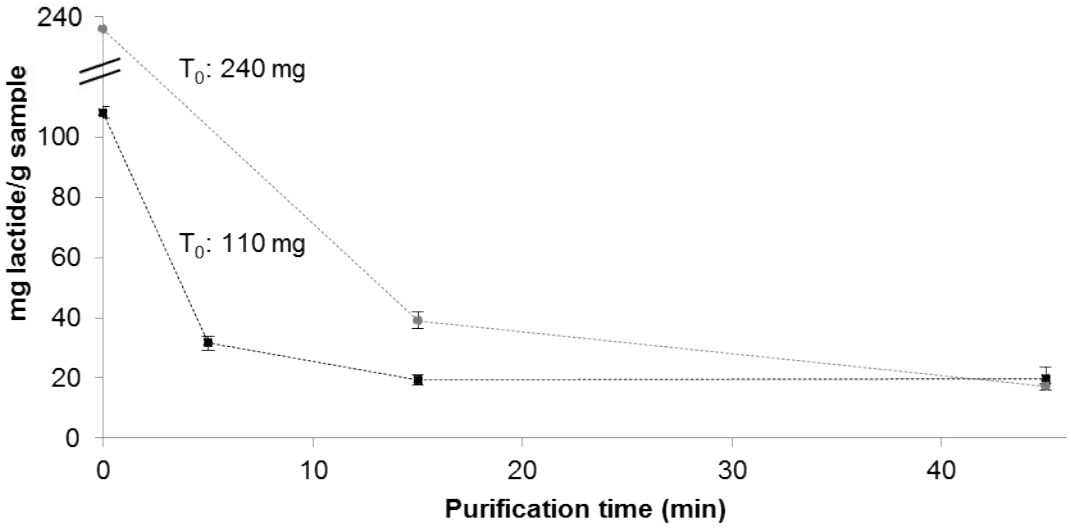


Figure S8: Purification of S-PLA 6800 Da at 45 °C when varying purification time. The starting concentrations assessed were; (■) 110 mg lactide/g sample and (●) 240 mg lactide/g sample. Note that the quantity of *D,*L-lactide removed is plateauing with time.

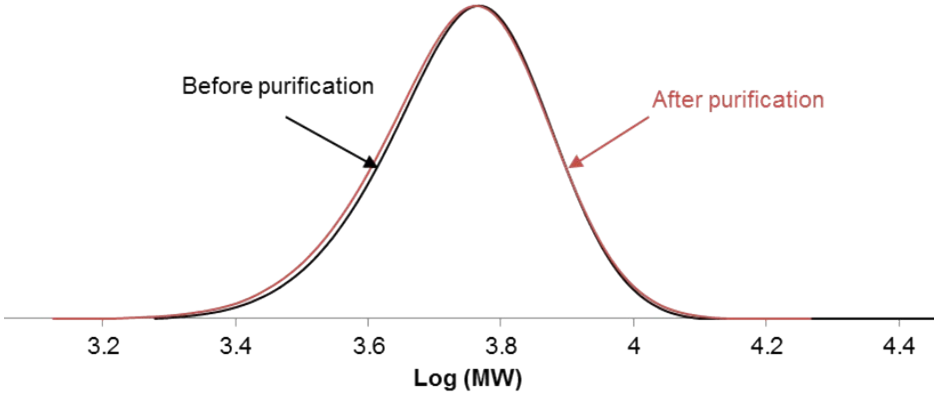


Figure S9: Overlay of GPC traces of S-PLA before (-) and after (-) purification using $scCO_2$ with a co-solvent. The response has been normalised.

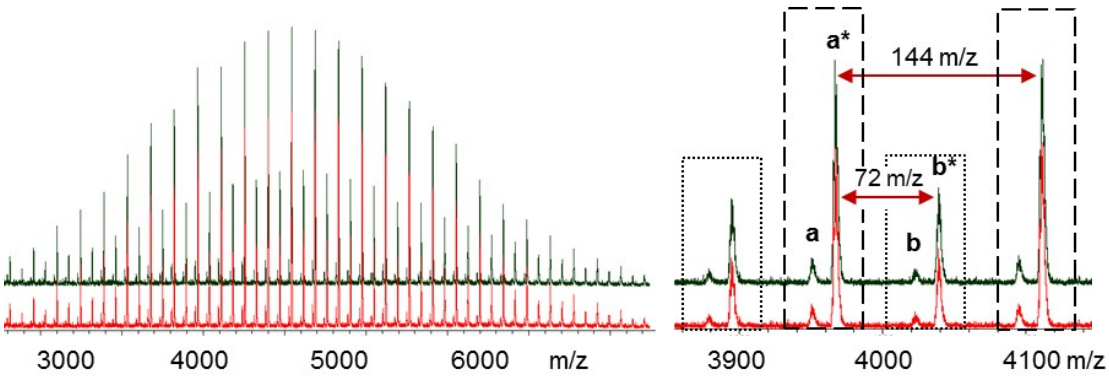


Figure S10: MALDI-ToF-MS of S-PLA (**A**) before and (**B**) after extraction using $scCO_2$ with a co-solvent. Peaks **a** and **a*** correspond to the same compound but with Na^+ and K^+ ion adducts. This is also the case for peaks labelled **b** and **b***. Note that the similarity between the two spectra strongly indicate that the use of ethanol as a co-solvent does not lead to any detectable chain transfer effects.

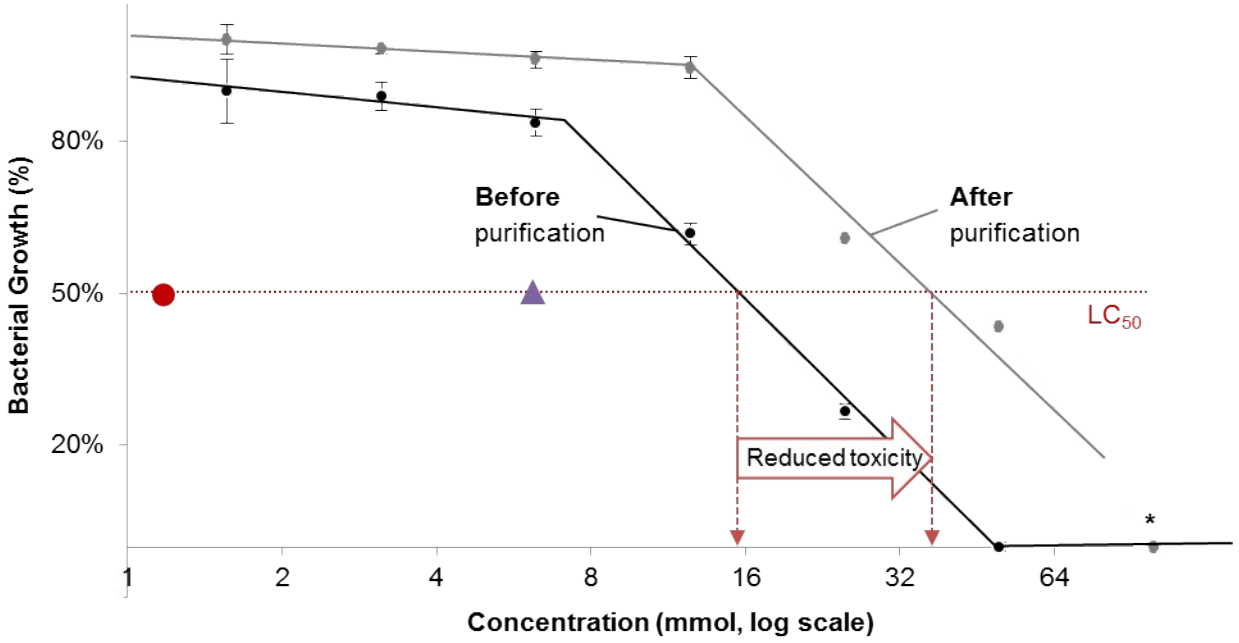


Figure S11: Bacterial growth (*E. coli)* at varying concentrations of S-PLA (1800 Da), before (—) and after (—) purification with $scCO_2$. The dashed horizontal line indicates the LC_{50} and note the shift for the purified compound. LC_{50} values for $Sn(oct)_2$ (●) and *D,L*-lactide (▲) have been included to aid interpretation. These values show that very low concentrations of $Sn(oct)_2$ (1 mmol) inhibit 50% of the microbial growth, while higher concentrations of *D,L*-lactide are needed for the equivalent inhibition (6.2 mmol). Both these concentrations are considerably lower than for S-PLA, with higher concentrations indicating reduced toxicity towards the microbe in question. * Points excluded in LC_{50} calculations.

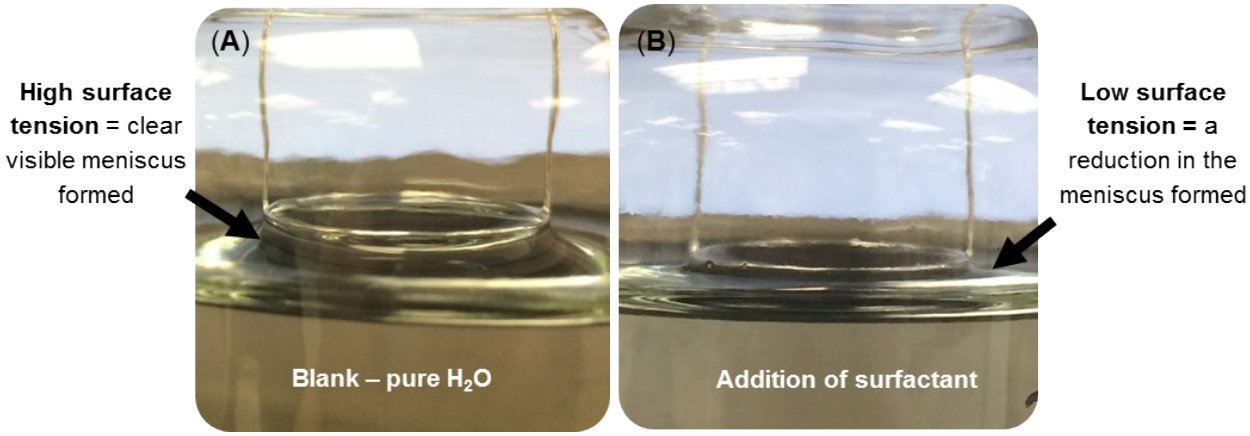


Figure S12: Images showing how the surface tension of water is measured using the du Noüy ring method (**A**) before and (**B**) after the addition of surface active agents. The platinum ring is submerged into the aqueous solution and then slowly pulled through the liquid–air interface. The surface tension (mN/m) is determined by measuring the maximum force required to lift the ring out of the aqueous phase (f_{max}).[2]

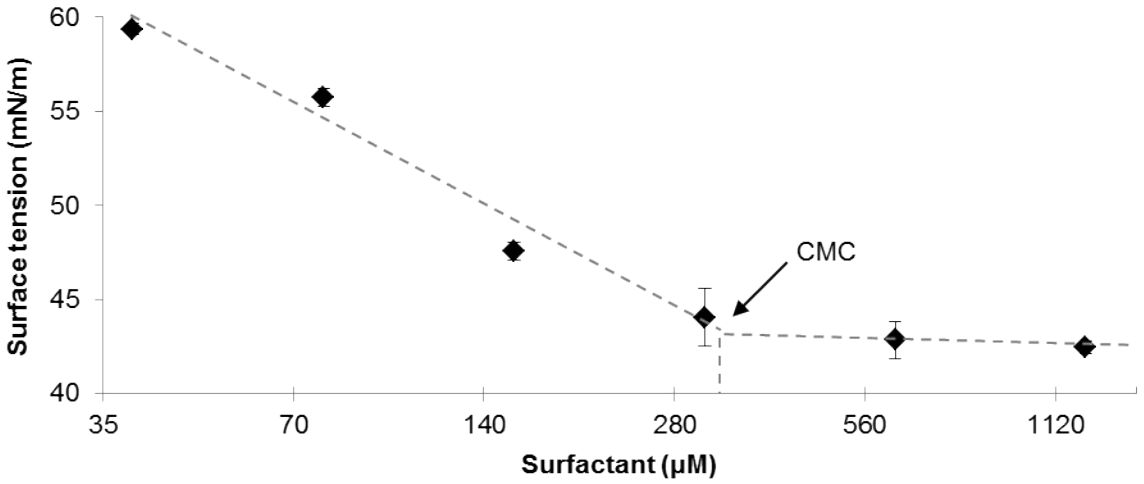


Figure S13: Surface tension measurements of S-PLA (800 Da) over a range of concentrations (plotted on a log scale). The CMC value is determined at the intersection between the two linear portions of the curve. At concentrations above the CMC value the surface tension remains almost constant, while below this level the surface tension will increase with reduced surfactant concentrations.

Compound	LC_{50} (mmol)	
	S. aureus	***E. coli***
D,L-lactide	5.0	6.2
$Sn(oct)_2$	1.1	1.0
S-PLA (1800 Da)	10.1	14.9
S-PLA (1800 Da) **Purified**	29.3	≥ 35.1*

Table S1: Summarising LC_{50} values of the S-PLA 1800 Da (before and after purification), monomer and catalyst. * Growth was not 100% inhibited at the highest concentration tested.

References

1. K. Numata, R. K. Srivastava, A. Finne-Wistrand, A.-C. Albertsson, Y. Doi and H. Abe, *Biomacromolecules*, 2007, **8**, 3115-3125.
2. H. H. Zuidema and G. W. Waters, *Industrial and Engineering Chemistry-Analytical Edition*, 1941, **13**, 312-313.