

Supporting Information for

Biodegradable Lignin Nanocontainers

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Experimental Part

Materials

Lignosulfonic acid sodium salt (molecular weight Mw: 50,000) and 2,4-Toluene diisocyanate (TDI), Sulforhodamine 101 (SR101) and solvents were purchased from Sigma-Aldrich. Polyglycerol polyricinoleate (PGPR) was obtained from Danisco and used as surfactant. All chemicals were used without further purification.

The *Coprinus cinereus* laccase used within this study was purified from submerged cultures of the fungus by chromatographic methods. *C. cinereus* CBS 182.61 (CBS Fungal Biodiversity Center, Utrecht, the Netherlands) was cultivated on yeast malt glucose medium (YMG medium) consisting of 4 g yeast extract (Becton Dickinson GmbH, Heidelberg, Germany), 10 g malt extract (Fränkle & Eck, Fellbach, Germany), 10 g glucose and 18 g agar per 1 l tap H₂O. The pH was adjusted with 1 N HCl to 5.5 prior to sterilization.

Submerged cultures were grown in a 1 l flask with 500 ml of YMG (27°C, 120 r.p.m.). Several excised pieces of the fungus grown on a YMG agar plate were used as inoculum. The culture broth was separated from the mycelium by filtration after 4 days, when the glucose was depleted. An intermediate product of the laccase was obtained from 200 ml culture filtrate by ion exchange chromatography using Q-Sepharose Fast Flow (GE Healthcare, Freiburg, Germany) equilibrated

and washed after loading with 50 mM Tris-HCl pH 7.5 (buffer A). Elution was maintained with a linear gradient of buffer A supplemented with 1 M NaCl. Positive fractions (30 ml) were concentrated to 500 μ l with a Centricon Plus-70 centrifugal filter device (Millipore, Darmstadt, Germany). Final purification was achieved using a gel filtration 1 m long XK26/100 column with Superdex 75 prep grade (GE Healthcare, Freiburg, Germany) at a flow rate of 2.5 ml min⁻¹ with buffer A supplemented with 100 mM NaCl as mobile phase.

Further experiments were conducted with a crude extract of a culture supernatant of the fungal strain *Xylaria* sp. IBWF A55-2009. This strain was grown in medium Soybean-medium containing 30 g/l soybean meal (Lucas Meyer GmbH, Hamburg), Germany, 15 g/l maltose (Applichem GmbH, Darmstadt, Germany) and 5 g/l peptone (Carl Roth GmbH, Karlsruhe, Germany). Submerged cultures were incubated at 27°C and 120 rpm on an orbital shaker. The cultivation was finished after two weeks. The mycelium was separated from the supernatant by centrifugation at 10,000 g for 10 minutes. For experimental procedures within this study the supernatant stored at -20°C was used.

The laccase activity was monitored in a liquid assay in 96-well plates. 100 μ l culture fluid/protein fraction were supplemented with 100 μ l ABTS solution (2 mg ml⁻¹ ABTS in tartaric acid, pH 4.5). The mixture was immediately analysed by photometry over a period of 5 min at room temperature and a wavelength of 420 nm.

Instruments

Ultrasonication

The emulsion was treated with ultrasound by using a Branson Sonifier W-450-Digital and a 1/2" tip. For the sonication remove the screw cap from the vial, remove the magnetic stirring bar from the vial and insert the 1/2" sonication tip into the vial with the mixture. The distance between the tip and bottom of the vial has to be around 5 mm. Carry out sonication at 90% amplitude, for 180 s total sonication time applied as 20 s sonication/10 s pause. During sonication the vial has to be immersed in an ice/water bath.

Infrared (IR) spectroscopy

The chemical composition of lignin and crosslinked-lignin was studied by FTIR spectroscopy. The sample powder was obtained by freeze-drying of the capsule dispersion for overnight at -70 °C under reduced pressure. The dry samples were recorded a spectrum between 4000 and 400 cm⁻¹ was recorded using an Nicolet Nicolet iS10.

Transmission electron microscopy

Transmission electron Microscope (TEM) images were obtained on JEOL 1400. TEM samples were prepared by placing sample dilution on the 300 mesh carbon coated copper grid and dried under ambient conditions.

Scanning electron microscopy

Scanning electron microscopy (SEM) images were taken by a 1530 Gemini LEO from Zeiss with accelerating voltages between 100 V to 30 kV. For the SEM studies, the samples were diluted in cyclohexane, placed onto a 5 x 5 mm silicon wafer and dried under ambient conditions.

Dynamic light scattering (DLS)

The average capsule size and the size distribution were measured using a PSS Nicomp Particle Sizer 380 (Nicomp Particle Sizing Systems, USA) equipped with a detector at 90 °. scattering mode at 20 °C. The samples were dilute in cyclohexne or water, then sonicated for 5 minutes in sonication bath.

Synthesis

General procedure for the synthesis of cross-linked lignin nanocapsules

The lignin capsules were prepared by a polyaddition reaction performed at the miniemulsion droplet's interface. 100 mg of lignin were mixed with 20 mg of sodium chloride and dissolved in 1.3 g of demineralized water, in case of the investigation the enzymatic cleavage 1 mg of SR101 was added instead of sodium chloride. This solution was added into 7.5 g of cyclohexane containing 80 mg of PGPR at room temperature and stirred at 1000 rpm for 1 h in order to form a pre-emulsion. Then the emulsion was treated with ultrasound (for 180 s at 90% amplitude in a pulse regime (20 s sonication, 10 s pause) under ice cooling). A solution consisting of 5 g of cyclohexane, 30 mg of PGPR and 50, 100 or 150 mg of TDI was then added drop-wise (over a period of 5 min) to the earlier prepared emulsion. The reaction was carried out overnight at room temperature. Then, the nanocapsules were washed twice with cyclohexane.

Redispersion of the capsules in buffer solution

To a stirred nanocapsule dispersion in cyclohexane (1g with approximately 3% solid content) 5 g of an acetate buffer solution at pH 3 or pH 7 were added drop-wise. The mixture was stirred for 24 h with open cap in order to guarantee evaporation of the solvent, and was then sonicated in an ultrasound bath (25 kHz) for 20 min at 25 °C.

Degradation of lignin nanocapsules by C.Cinereus Laccase Lccland enzyme cocktail from mushroom extraction

To 1 ml of the aqueous nanocapsule dispersion (in buffer solution) 300 µL of a laccase solution (30 µg/ml) or an enzyme cocktail (see above) was added. The mixture was kept stirring over a period of time and samples were taken in order to determine the released dye. for 24 hours at R.T. and 50 °C.

Determination of SR101 dyes after degradation of capsules by enzyme

The lignin nanocapsules were redispersed in water (or buffer) as mentioned above and centrifuged 3000 rpm for 30 min. The supernatant was investigated by fluorescence spectroscopy in order to determine the amount of released SR101.

As control, a nanocapsule dispersion that was prepared without fluorescent dye was redispersed in water containing SR101 (the amount is equal to the amount of SR101 that was used in the encapsulation experiments) was used as a reference (blank). The fluorescence signal of the reference sample at difference concentrations of SR101 was used to set up a calibration curve (Note: SR101 has an absorption maximum at 550 nm and the emission was measured at 605 nm). The total release of SR101 from the degraded capsule by enzyme was calculated from the calibration curve.

Characterization of cross-linked-lignin nanocapsules

Table S1. Size characterization of lignin nanocapsules.

#	lignin, mmol	TDI, mmol (mg)	diameter, ¹ nm	diameter, ² nm
1	0.002	0.28(50)	162±66	311±93
2	0.002	0.56(100)	185±87	333±72
3	0.002	0.84(150)	220±81	390±90

1: diameter determined by dynamic light scattering in cyclohexane

2: diameter determined by dynamic light scattering in water

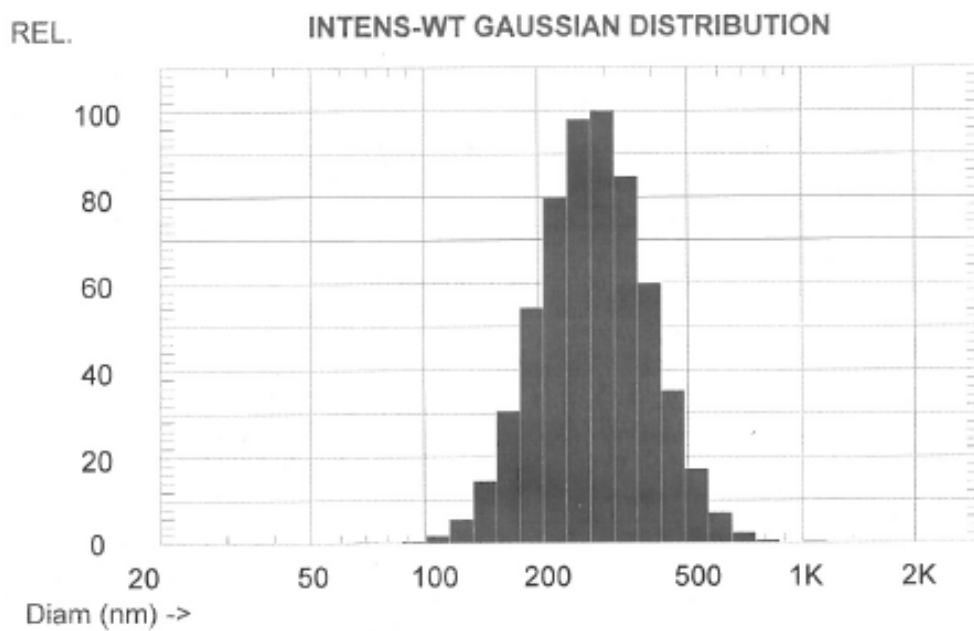
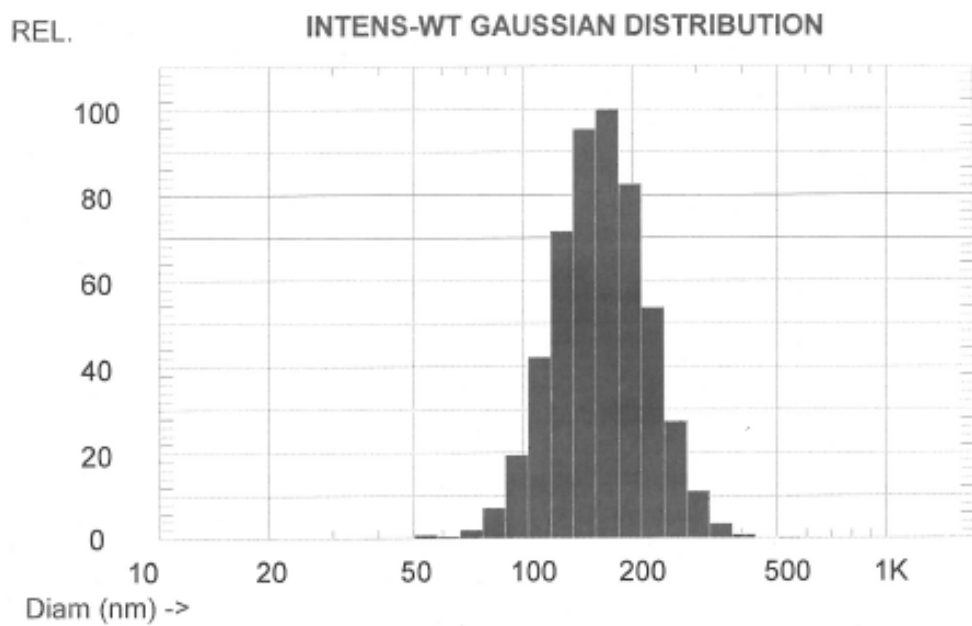


Figure S1. Size distributions of the lignin capsules of batch # 1 from DLS in cyclohexane (top) and in water (bottom).

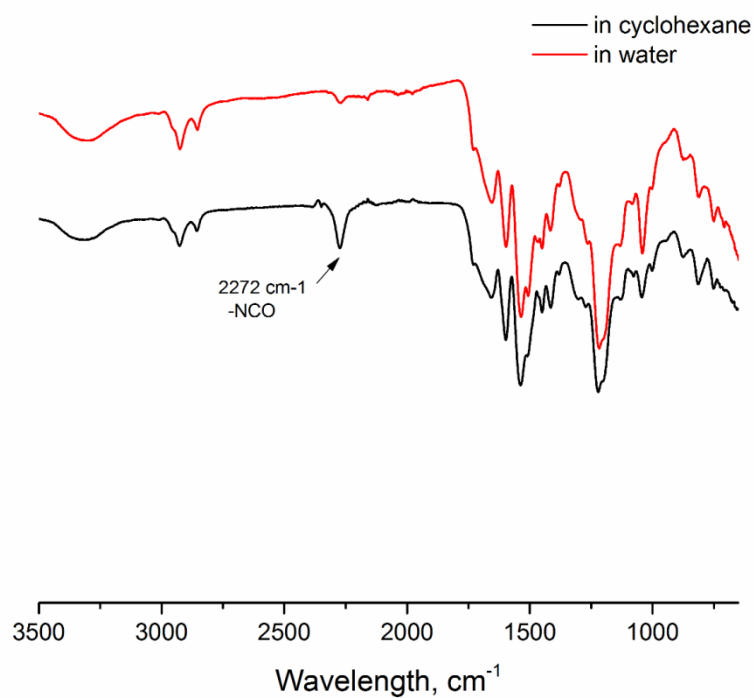


Figure S2. Comparison of the FT-IR spectra of lignin nanocapsules in cyclohexane (black line) and after redispersion in water (red line).

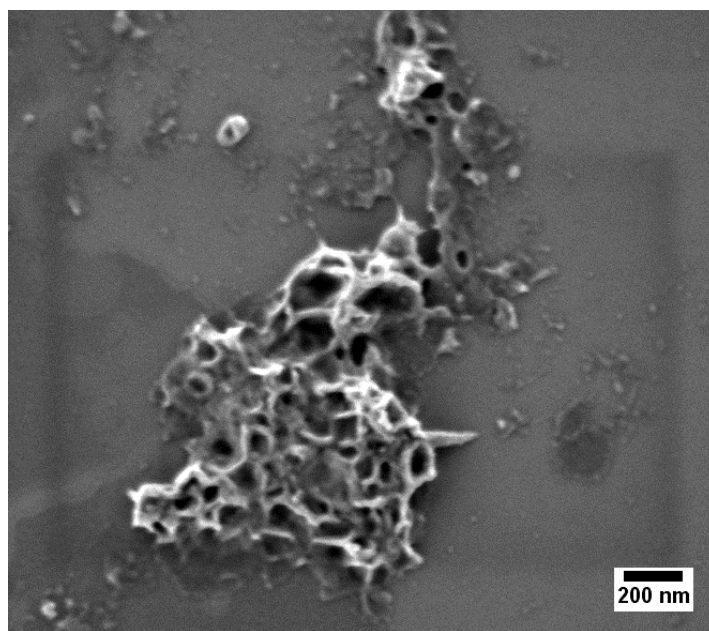


Figure S3. SEM image of lignin nanocapsules after redispersion in water.

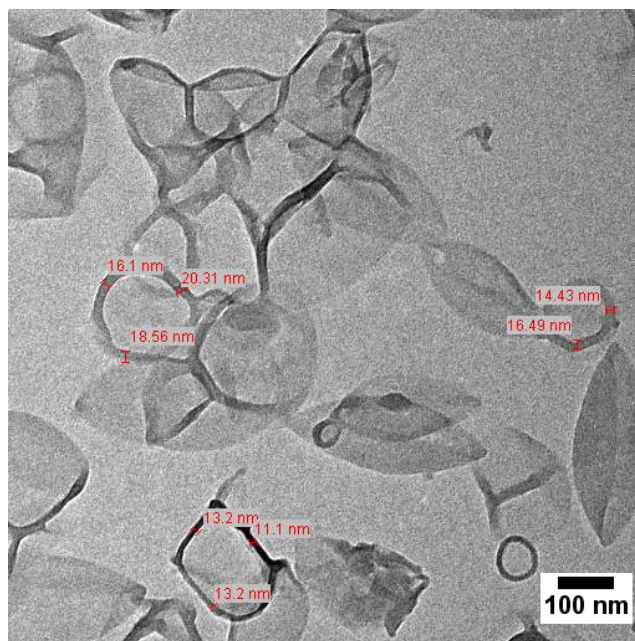


Figure S4. Estimation of the shell thickness of lignin nanocapsules from TEM.

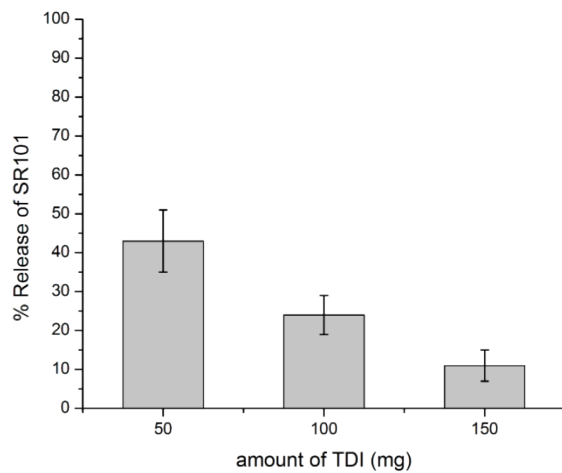


Figure S5. Release percentage of SR101 dyes from the crosslinked-lignin capsules after cleavage by enzyme cocktail at 50°C, pH 3 for 1 day.

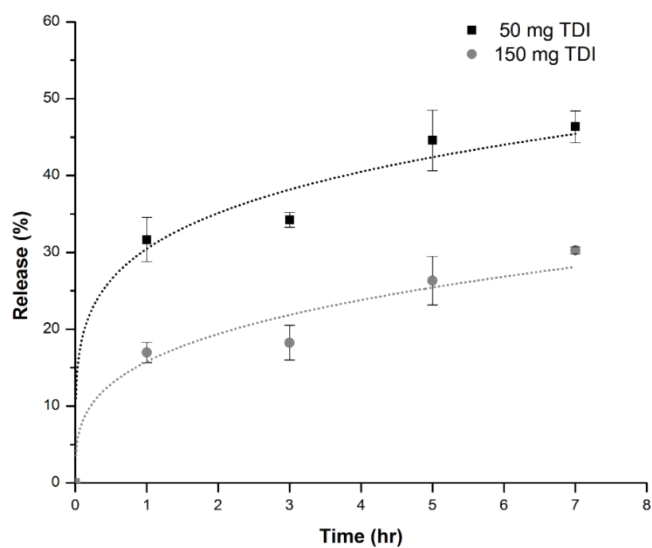


Figure S6. Release profile of SR101 dyes from the crosslinked-lignin capsules after cleavage by enzyme cocktail at 50°C, pH 3.

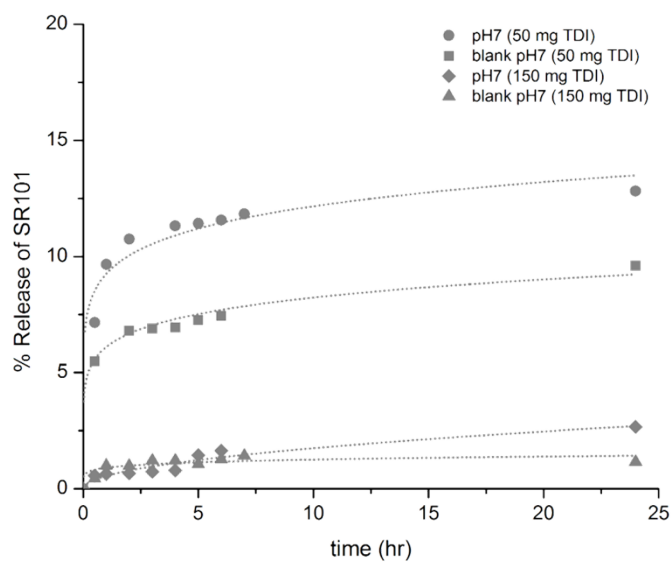


Figure S7. Release profile of the lignin nanocapsules degraded by enzyme cocktail at room temperature, pH 7.