Infrared laser triggered release of bioactive compounds from single hard shell microcapsules

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MATERIALS AND METHODS

Reagents

Polyvinyl alcohol (PVA; MW: 13,000-23,000 g/mol, 87-89 % hydrolyzed) and all other reagents were obtained in the highest purity from Sigma-Aldrich GmbH (Germany). Witepsol W31, a hard fat consisting of a mixture of tri-, di- and monoglycerides with a melting temperature of 35-37 °C, was obtained from CREMER OLEO GmbH & Co. KG (Germany).

Protein preparation

eYFP with an N-terminal 6×His-Tag was expressed in the *Escherichia Coli* strain TG1. The cells were grown in TB-media at 37 °C until the OD_{600} reached 0.8. The temperature was then decreased to 30 °C and protein expression was induced by the addition of 250 µM Isopropyl β-D-1-thiogalactopyranoside (IPTG) overnight. The cells were harvested and disrupted using an ultrasonic homogenizer (BANDELIN electronic GmbH & Co. KG, Germany). Cell debris was pelleted at 17,000 rpm and 4 °C. The supernatant was applied to a column packed with Ni-NTA resin (GE healthcare GmbH, Germany). The column was washed with buffer containing 50 mM potassium phosphate (KPi), 150 mM sodium chloride (NaCl), 10 mM Imidazole pH 7.4 until the OD_{280} reached a minimum baseline. The protein was eluted from the column using a buffer containing 500 mM Imidazole. The fractions containing protein were concentrated using a Vivaspin 20 ultrafiltration unit (Sartorius AG, Germany). Protein purity was verified by SDS-PAGE. The concentration was determined by the protein absorbance at 280 nm (ε_{280} (eYFP) = 21,800 M^{-1} cm^{-1}) in buffer containing 20 mM KPi, 6 M guanidinium hydrochloride, pH 6.5 according to the method described by Gill and von Hippel[1].

Microcapsule preparation

5 g of Witepsol W31 were melted at a temperature of 40 °C. To the molten sample 0.26 g of a Dulbecco's Phosphate Buffered Saline (DPBS) buffered solution containing 100, 150 or 200 µM of eYFP respectively were added. Additionally the buffer contained different concentrations (10 %, 22.5 % or 45 % w/w) of glucose or trehalose. The mixture was stirred using a magnetic stirrer at constant heating for 2 minutes to form a water in oil (Witepsol) emulsion. 10 % PVA solution at 40 °C was added to the mixture while stirring. The mass ratio between the fat and the PVA solution was 1:6. This led to the formation of a W/O/W emulsion. After stirring the sample for another 5 minutes, 80 g of cold water (approximately 4 °C) were added to decrease the temperature of the emulsion below the melting temperature of the hard fat thereby solidifying it. To avoid drop coalescence in a not-mixed environment the water was added while stirring. The formed microcapsules were separated by filtration using a Sartorius quantitative grade 393 filter paper (Sartorius AG, Germany).

Fluorescence imaging and temperature controlled release

We have built an experiment consisting of a combination of high speed imaging using a Zeiss Axio Observer Z1 inverted microscope equipped with a Colibri 2 LED light source (Carl Zeiss AG, Germany) and laser heating. Therefor we coupled an

infrared laser (m2k laser GmbH, Germany) with a wavelength of 2,200 ± 20 nm and a power stability of ± 1 % into the upright beam path of the inverted microscope (Scheme 1)[2]. The infrared laser is coupled via a multimode optical fiber into the microscope setup. The output power is adjusted by changing the diode current using a custom-written software (LabView, National Instruments Germany GmbH, Germany). The laser is focused into a microfluidic sample chamber with a channel height of 400 µm (ibidi µ-Slides VI, ibidi GmbH, Germany). The sample chamber was filled with DPBS buffer containing the microcapsules. Individual microcapsules within the microfluidic chamber were selected and imaged by fluorescence microscopy. Single capsules were heated by focused infrared laser radiation using specific output laser waveforms to achieve instantaneous heating and subsequent thermostatization[2]. The laser heats specifically the liquid phase, the water solvating the microcapsules and the encapsulated water by excitation of hydroxyl group vibrations (absorption coefficient $\alpha = 20$ cm^{-1} at 2,200 nm)[3]. The infrared laser is focused on the sample to achieve heating of single particles only, however the beam diameter is larger than the capsule to ensure homogeneous heating of the sample (typically 480 µm FWHM (full width at half maximum)). A heating profile with 25 individual steps each with a temperature increase of 1 °C each was used to heat up the samples starting at room temperature. The heating profile of each individual heating step was adjusted to allow for rapid heating and for temperature equilibration. For each individual 1 °C heating step, the tailored waveform consists of a short heating phase at maximal laser power (270 mW) to rapidly increase the temperature within 50 ms. Temperature equilibration of the sample at the elevated temperature, is then achieved by exponentially decreasing the laser power with time. The heating waveform was adjusted using repetitive jumps and measurements of the induced temperature. The temperature profile of the laser was determined by imaging the temperature-dependent fluorescence quantum yield of Rhodamine B (excitation: 555 nm, emission: 575 - 680 nm)[4, 5]. For calibration, the Rhodamine B fluorescence decrease was recorded in a temperature controlled petri dish (Delta T5, Bioptechs Inc., USA). Typical heating profiles are shown in Figure S1.

Image processing and data analysis

Image processing and data analysis was done using Zeiss Axiovision and Zenpro, OriginPro 9 g (OriginLab, USA) and ImageJ [6]. The regions of interest (ROI) were analyzed by measuring the mean gray values using ImageJ. The resulting values were used to calculate the normalized fluorescence intensities.

Particle size determination

The volumetric particle size distribution (PSD) was measured using a Malvern Mastersizer 2000 (Malvern Instruments, UK). This method is based on laser diffraction. The particles were dispersed in water. In order to get a homogeneous suspension the stirring speed was set at 2,500 rpm. The d_{50} value represents the diameter of the sample where 50 % of all particles are finer than the determined value. The span is a measure for the polydispersity of the sample.

SUPPLEMENTAL RESULTS

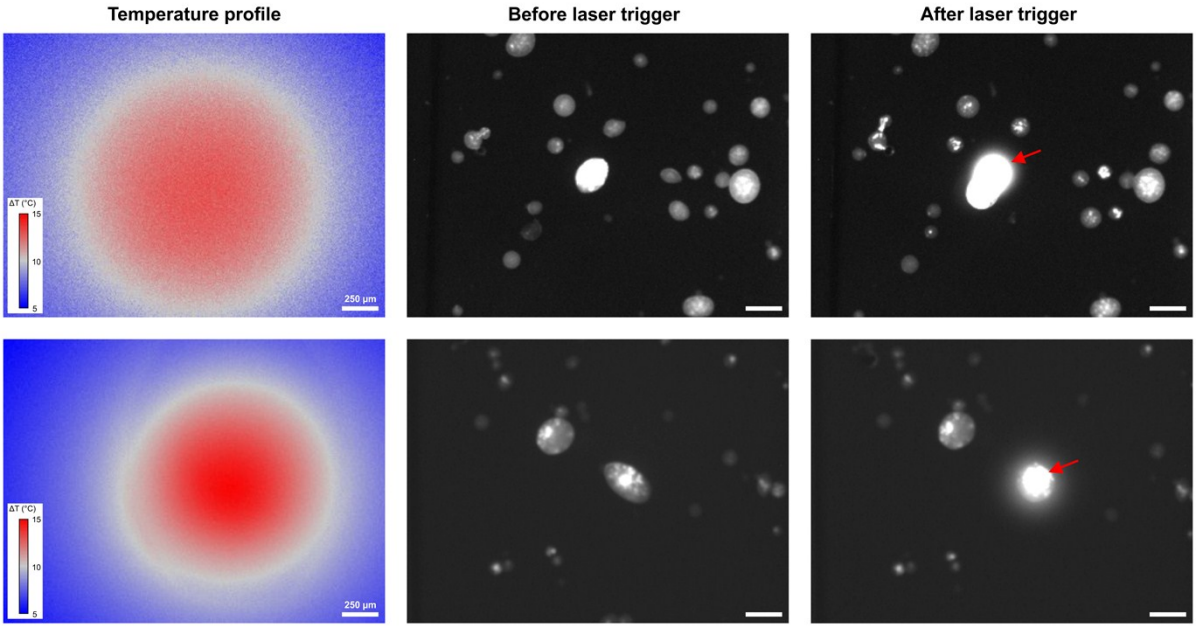

Figure S1. Temperature triggered release of eYFP from preselected single microcapsules. Two experiments (top row and bottom row) are shown with different heating profiles. The heating ramp corresponds to the one shown in Figure 3 A, the laser induced temperature profiles (measured by Rhodamine B) are shown on the left. The artificial color codes for the temperature increase ΔT measured from room temperature (25°C). The red color corresponds to a temperature increase above the melting temperature of pure Witepsol W31. Fluorescent images of the microcapsules at 25 times magnification are shown before (middle column) and after microcapsule release (right column). The red arrows highlight the release site of eYFP.

Video S1. Melting of a mononuclear capsule with a single temperature increase.

Video S2. Melting and release characteristics of a mononuclear capsule.

SUPPLEMENTAL REFERENCES

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