Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Cascade communication in disordered networks of enzyme-loaded microdroplets

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Table of Contents

1. Materials & Methods

1.1 Chemicals & Biologicals

All chemicals, including Span 20, Span 80, Span 85, D-(+)-glucose, xanthine, uric acid, hydrogen peroxide, Amplex red and Patent Blue V sodium salt blue dye, were purchased from Sigma-Aldrich with a p.a. purity grade. Tetradecane was purchased from TCI, sucrose from Alfa Aesar and edible red dye bought in the local convenience store. The enzymes invertase from baker's yeast (S. cerevisiae) (E.C. 3.2.1.26, Grade VII, ≥300 units/mg solid), xanthine oxidase from bovine milk (E.C. 1.17.3.2, lyophilized powder, 0.4-1.0 units/mg protein), uricase from candida sp. (E.C. 1.7.3.3, lyophilized powder, ≥2 units/mg solid), horseradish peroxidase (E.C. 1.11.1.7, lyophilized powder, ≥250 units/mg solid) and catalase (E.C. 1.11.1.6, lyophilized powder, 2,000-5,000 units/mg protein) were obtained from Sigma-Aldrich. Tetradecane from Sigma-Aldrich served as the continuous phase in all experiments. In all conducted experiments we utilized deionized water.

1.2 Droplet manufacturing

Monodisperse water droplets of various contents were produced by dispersing an aqueous phase within a continuous tetradecane phase containing surfactants (Span 20, Span 80 or Span 85) at varying concentration inside a microfluidic chip. A hydrophobically coated large “X” junction Dolomite glass chip was employed with different continuous phase/dispersed phase pressure ratios to produce droplets with a diameter of ~120 ± 5 µm for the solvent flux study and 190 ± 14 µm for the cascade reaction experiments, respectively. The flow rates were controlled by a Fluigent Flow-EZ pressure control platform.

1.3 Optical microscopy

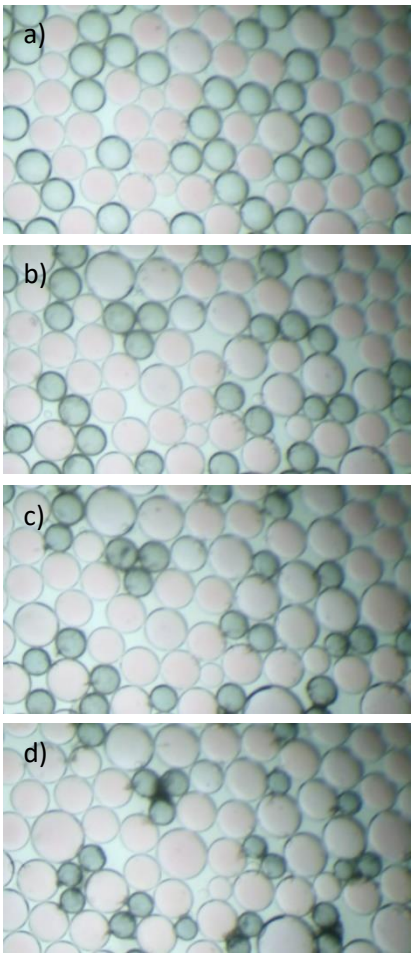
For studying the solvent exchange between aqueous droplets of different composition, droplets were freshly prior to every experiment. After preparation, droplets of different composition were carefully pre-mixed in a vial and then placed onto a Invitrogen Attofluor Cell Chamber from Thermo-Fisher Scientific. All samples were probed on a Bresser Trino or a Bresser Science IVM-401 inverted microscope. Typically, samples were recorded by acquiring an image every 10-30 sec over a course of several hours.

2. Osmotic pressure-driven directional solvent exchange

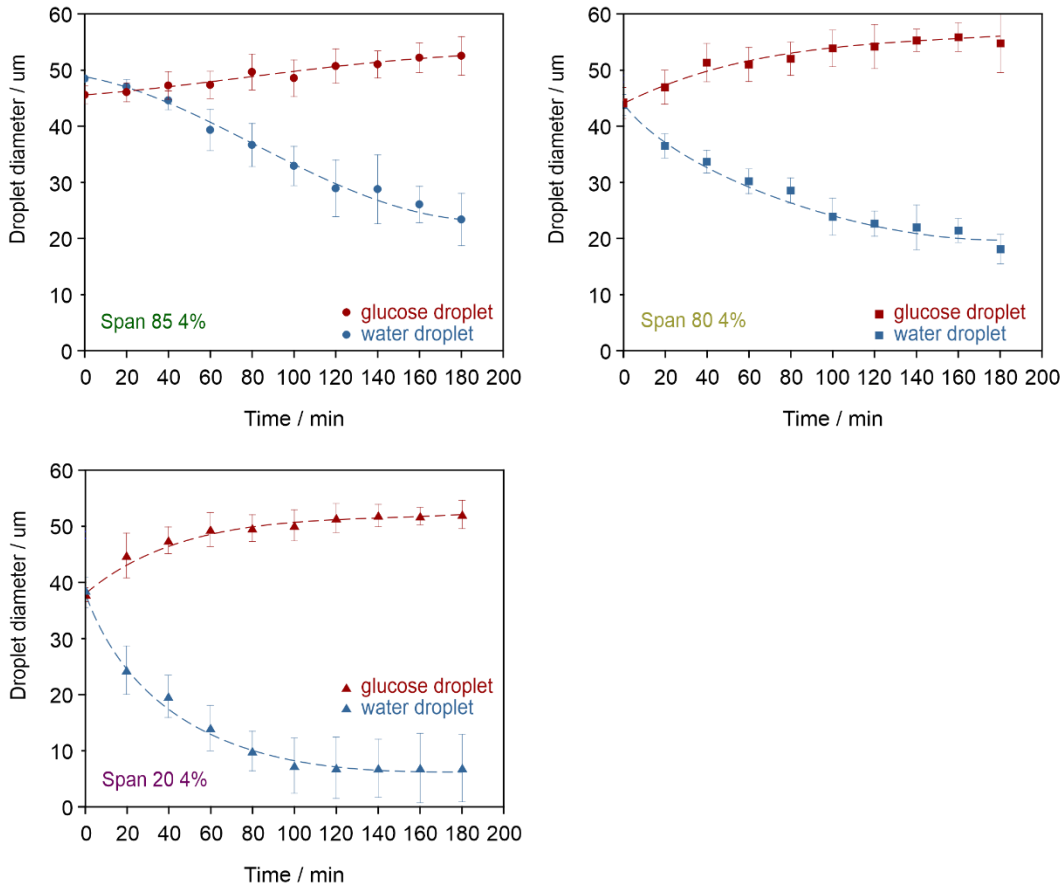
2.1 Experimental setup to monitor non-reciprocal solvent exchange between microdroplets

Droplets were monitored by optical microscopy and images were acquired every 10-30 sec. Collected time-lapse was featured either as an image sequence (example image sequence: Figure S1) or a video (example: VideoS1). Subsequently, we plotted the diameter ratio (droplet diameter of Span 80 or Span 20 4 wt.% and glucose 30 wt.% over the initial droplet diameter), or the normalized diameter ratio (diameter ratio over the initial diameter ratio) as a function of time, respectively. Due to low concentration of droplet constitutes, like enzymes or enzyme substrates (anything but osmolyte), their osmotic pressure was neglected. All experiments were performed in 1:1 populations of droplets containing different osmolytes concentrations to reveal the average droplet diameter ratio and exemplary plots are displayed in Figure S2. The smaller increase in diameter of the last droplet in comparison to a higher decrease of diameter of the first two droplets is partially a consequence of the differing influence of the volume exchange on the droplet size. In all experiments, the directional solvent flux was accompanied by molecular diffusion and solubilization of water into the continuous phase, which explained the discrepancy of the net volume uptake in these pair of droplets. As revealed by these experiments, lower osmotic pressure droplets are subject to higher rates in spontaneous secondary emulsification.

2.2 Example image sequence to investigate time-dependent solvent flux



Supporting Figure S1. Images from the time-lapse taken after a) 0 min, b) 25 min, c) 50 min and d) 100 min from the moment of mixing of transparent water droplets and edible red dye colored glucose 30 % droplets in a tetradecane containing 4% of Span 80.



Supporting Figure S2. Time-dependent evolution of the droplet diameter revealing the dependency of the osmotic pressure-driven directional solvent flux between aqueous microdroplets on the surfactant type (droplets contained 0% and 30% wt.% D-glucose).

2.3 Supporting videos

Supporting Video V1. Osmotic pressure driven non-reciprocal solvent flux between aqueous microdroplets containing different osmolyte concentrations (0 wt.% (blue) and 30 wt.% (red) D-glucose). The video further displays the anisotropic distribution of nanoemulsions formed via secondary emulsification around the droplets. Video speed: 100x real time

Supporting Video V2. Osmotic pressure driven non-reciprocal solvent flux between aqueous microdroplets containing different osmolyte concentrations (0 wt.% (blue) and 20 wt.% (red) D-glucose) and anisotropic distribution of nanoemulsions formed via secondary emulsification around the droplets. Video speed: 100x real time

3. Biochemical cascade reaction across multiple droplets

3.1 Enzymatic reaction cascade

The biochemical cascade reaction was performed across three droplets of different composition. Three samples were prepared and further emulsified using the method for the droplet preparation described above by employing dry tetradecane (dried overnight over molecular sieves and filtered through Millipore syringe filter of 0.2 μm) with 4 wt.% of Span 80. First sample contained 0.5 wt.% of sucrose, 0.3 mM of xanthine and 0.1 mg/ml of Ponceau S red dye. Second droplet was composed of 2.0 wt.% of sucrose, 0.004 U/mL of xanthine oxidase and 0.1 mg/ml of Patent Blue V sodium salt blue dye. The third droplet was prepared by emulsification of the solution containing 2.5 wt.% of glucose, 100 ppm of invertase, 100 ppm of horseradish peroxidase, 100 ppm of uricase and 0.25 mg/mL of Amplex Red. Immediately after generation, the droplets were combined in a vial and pre-mixed, followed by placement on the optical or fluorescence microscope to track the progression of the reaction via recording the droplet size variation and the fluorescence intensity, respectively.

Prior to these experiments, control reactions were performed that probed the nanoemulsion-mediated transfer of different substrate molecules between each of the two sets of droplets and the dependency of the cascade reaction on the performance of the contributing individual enzymes. Here we prepared two different types of droplets, the first set contained 0.5 wt.% of sucrose, 0.3 mM of uric acid and 0.1 mg/ml of Ponceau S red dye, while the second set was composed of 2.5 wt.% of sucrose, 50 ppm of catalase, 50 ppm of uricase and 0.25 mg/mL of Amplex Red. These two type of droplets were mixed together and successful transfer of uric acid was confirmed by the formation of Resorufin in the second droplets. This was a consequence of transport of uric acid two the second droplet where in the presence of uricase it produced hydrogen peroxide as a by-product, but also a necessary substrate for catalase to from colored product of Amplex Red,

Resorufin. This furthermore implied that we next needed to test the transfer of smaller substrates. Similarly, but we first prepared droplets by mixing 0.5 wt.% of sucrose, 2.25 w/w% of hydrogen peroxide and 0.1 mg/ml of Ponceau S red dye, while the second one possessed 2.5 wt.% of glucose, 50 ppm of catalase and 0.25 mg/mL of Amplex Red. In these experiments, over the period of one-hour, reaction did not occur due to insufficient transport of hydrogen peroxide from the first to the second type of droplets. For the purpose of confirming uric acid transfer from the second to the third droplet of the cascade, uric acid at concentration of 0.05 mg/ml was placed into the second droplet as a final product of a xanthine oxidase that was absent in this experiment, while the third droplet contained all the components as in the final cascade reaction experiment. Successful transfer of uric acid between these two droplets was also confirmed by a dye formation.

3.2 Fluorescence microscopy

Development of the fluorescent dye, resorufin, was followed on a Leica SP8 confocal microscope. We employed confocal microscopy with argon and DPSS 561 nm lasers, HC APO 10x/0.3 objective with a water drop and PMT3 571-613 nm detector. Sample was prepared and placed on a slide in a same manner as previously described in the optical microscopy section. An image was taken every 10 minutes over the course of 60 minutes.