MICRODROPLETS AS PHOTOSYNTHETIC SYSTEMS

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INTRODUCTION

One of the most fundamental biological processes is the conversion of light energy through membrane-bound protein complexes into energy-rich cofactors used to fuel metabolic reactions (adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH)). The development of microfluidic technology has permitted the generation of integrated systems that provide a platform to mimic these biological processes. Especially, microdroplets have been used as artificial cell-like compartments to manipulate and assemble constitutive elements of living systems in miniaturized and confined environments.

Thylakoids are membrane-bound compartments inside chloroplasts and cyanobacteria. They enable the light conversion of CO2 into multicarbon compounds such as glycolate. After their isolation from chloroplasts, it was hypothesized that these membranes could be used as membrane-based energy modules (TEM) to energize individual enzyme or cascade enzyme reactions.

We herein describe the use of FluoSurf-stabilized microdroplets as artificial chloroplast mimetic platforms for the encapsulation of thylakoid membranes and the subsequent light-driven conversion of CO2 into glycolate.[1]

Enzyme cascade reactions encapsulated in surfactant-stabilized microdroplets.

Light-triggered microdroplet metabolic activity

a. Encapsulation of a functional TEM in microdroplets

To demonstrate the possible regeneration of ATP and NADPH from TEM in synthetic compartments, different concentrations of TEM granules were encapsulated into 92µm (400pL) water-in-oil droplets stabilized with 3wt% FluoSurf surfactant. Thylakoid suspensions were flowed (100µL/h) in a PDMS dropmaker device treated with FluoST, and flow-focused with two streams of fluorinated oil (Novec 3000, 225µL/h). After generation, droplets were observed in a 2D-observation chamber (Figure 1) and it was calculated that about 100±20% TEM granules were encapsulated in each droplet.

Figure 1

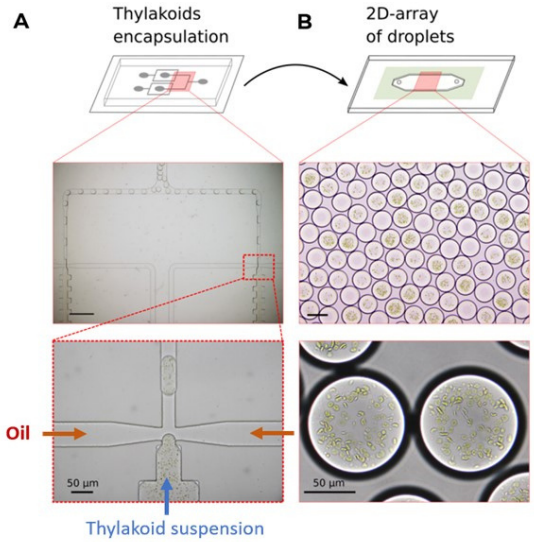

Figure 1 : *Generation and observation of water-in-oil (w/o) droplets.*
A) Encapsulation of thylakoid suspension in 92µm diameter (400pL) w/o droplets using a PDMS dropmaker device with two parallel production nozzles (30x30µm). The oil phase is 3wt% FluoSurf in Novec 3000.
B) After generation, droplets are flowed in an incubation chamber and stored as a 2D-array for light activation and time-lapse microscopy imaging.

Next, the metabolic activity of droplets was assessed by monitoring NADPH and ATP production. A white-light LED was used to irradiate the 2D-chamber in order to trigger TEM activity and subsequent photoreduction of NADP+ (Figure 2, A). The production of NADPH was monitored under microscope observation using a 365nm LED (NAPH fluorescence excitation wavelength) and was directly correlated to chlorophyll content from the TEM granules (Figure 2, B and C). A maximum photoreduction rate of 2.0 ± 0.1 NADPH µmol.min-1.µg-1 Chl at 50 µmol photons.m-2.s-1 was calculated and is comparable to rates obtained from bulk experiments. It was also demonstrated that the TEM could be switched on and off in individual droplets by performing light-dark cycles thus further proving the high level of control provided by microdroplet platforms.

Figure 2

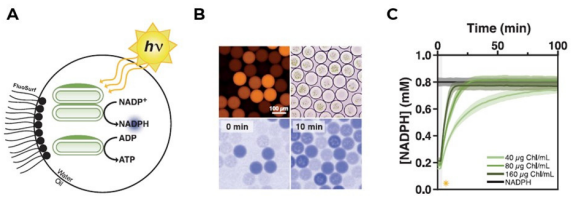

Figure 2 : *Light-driven enzymatic reaction coupled to TEM in microdroplets.*
A) Scheme of the TEM encapsulated in microdroplets. Light triggers TEM activity to produce NADPH and ATP. Hv, photon energy.
B) Microscopic photographs of droplets containing four different TEM concentrations. First row, left to right: barcoding fluorescence (550nm), bright field. Second row, left to right: NADPH fluorescence at time point 0 and NADPH fluorescence after 10min of illumination.
C) NADPH concentration versus time of microdroplets with varying TEM concentrations.

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b. Light-triggered individual enzyme reaction

The ability of microdroplets to power a single-enzyme reaction was tested by encapsulating TEM together with a glyoxylate/hydroxypyruvate reductase (Ghr) from Escherichia coli which catalyzes the NADPH-dependent reduction of glyoxylate to glycolate (Figure 3, A).

Figure 3

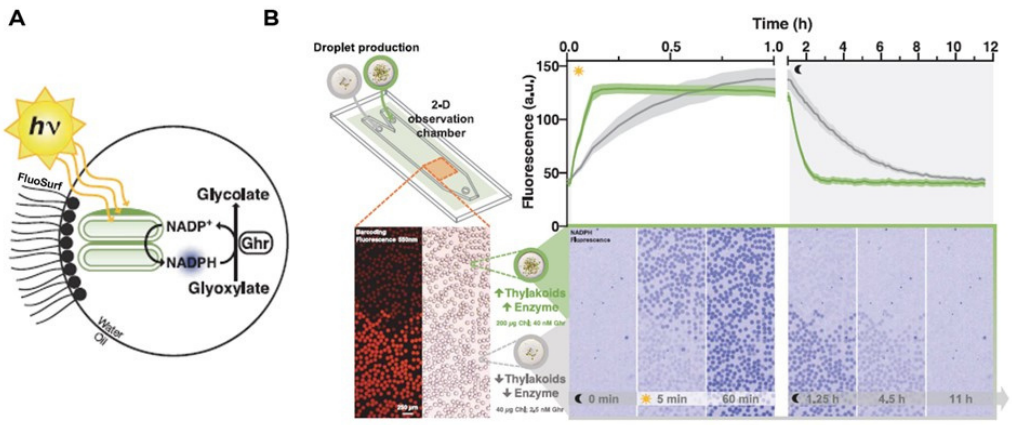

Figure 3 *: Light-driven enzymatic reaction inside microdroplets.*
A) Scheme of the encapsulated TEM-powered reduction of glyoxylate into glycolate.
B) Microscope observation of a binary emulsion of droplets with two different concentrations of TEM and Ghr, each population filling half of the observation chamber. The plot shows relative NADPH fluorescence over time under fluctuating light and dark conditions.

It was shown that distinct dynamic NADPH equilibrium states could be reached by varying the number of TEM granules as well as the concentration of substrates and Ghr in individual droplet populations. For instance, at 120µg.Chl.mL-1 reaction mixture, 8.2µg.mL-1 Ghr, and 50 µmol photons.m-2.s-1, 4.7mM glycolate could be produced (starting from 5mM glyoxylate and 0.8mM NADP+).

Time and space control of metabolic activity in droplets was further demonstrated by mixing two populations of droplets that had different TEM and Ghr concentration. These two populations each filled half of an observation chamber and thus created a spatial pattern (Figure 3, B). NADPH production and consumption rates (directly correlated with TEM and Ghr concentration respectively) could be controlled by illumination and dark cycles. As can be seen on the plots and microscope pictures, under the same illumination, fluorescence resulting from NADPH production increased faster for the population with higher TEM and enzyme concentration. Similarly, fluorescence decreased faster for that droplet population when in the dark.

Light-triggered multi-enzyme cascade reaction

After optimization of single-enzyme reactions inside microdroplets for various enzymes requiring ATP and/or NADPH, the ability of microdroplets to serve as platforms for cascade enzymatic reactions was tested. TEM was co-encapsulated with the crotonyl-coenzyme A (CoA)/ethylmalonyl-CoA/hydroxybutyryl-CoA (CETCH) cycle which is a multi-enzyme artificial pathway for the fixation of CO2 in vitro (Figure 4, A). [2] The easy tunability of microdroplet content allowed to directly quantify the behavior of different versions of the CETCH cycle in hundreds of microcompartments side by side. For each version, the dynamic equilibrium state of NADPH could be monitored. This would not have been possible with bulk experiments.

Figure 4

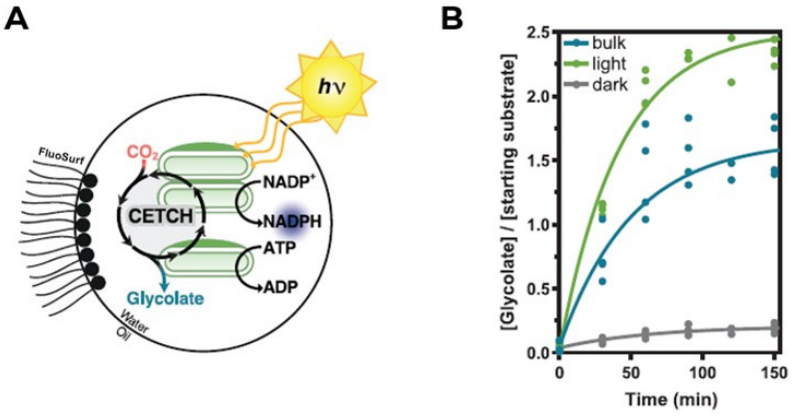

Figure 4 *: Light-driven continuous fixation of CO2 into organic acids by CETCH version 7.0 coupled to TEM in microdroplets.*
A) Scheme of the CETCH version 7.0 coupled to TEM operating inside microdroplets.
B) Glycolate formed per acceptor molecule over time in droplets and in bulk solution. Light and dark curves represent droplets kept in the light and in the dark respectively. The bulk curve shows an experiment with the same reaction mixture but on the microtube scale. The bulk solution and the droplets were simultaneously exposed to light for parallel comparison.

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Figure 4 shows the results obtained with CETCH cycle version 7.0 co-encapsulated with TEM granules in microdroplets. This version has been optimized for glycolate formation from CO2 and light. It was measured that without further optimization, this integrated system was able to produce 47 ± 5µM glycolate from CO2 over 90 minutes with a carbon-conversion efficiency of ≈3.5% for the full CETCH cycle (NADPH consumption rate of CO2 reduction divided by the measured maximum rate of NADP+ photoreduction achieved in droplets) (Figure 4, B). These values outperform other similar experiments using only single enzymes and show the potential of cell-sized compartments to be used as scaffolds for reproducing and studying cascade enzymatic reactions.

CONCLUSION

The results from this work show that microdroplets provide a solid platform for the automated assembly of metabolically active microcompartments that can be controlled and powered by external illumination and analyzed in real time. This work constitutes an example of how it is possible to interface natural and synthetic biological modules in thousands of cell-like compartments to create highly integrated biomimetic systems.

Droplet microfluidics permits high throughput generation of such microcompartments as well as parallelization of assays and multiplexing. The stability of these biomimetic droplets is crucial and ensured in this work by FluoSurf surfactant.

REFERENCES

[1] Tarryn E. Miller, Thomas Beneyton, Thomas Schwander, Christoph Diehl, Mathias Girault, Richard McLean, Tanguy Chotel, Peter Claus, Niña Socorro Cortina, Jean-Christophe Baret, Tobias J. Erb. Light-powered CO2 fixation in a chloroplast mimic with natural and synthetic parts. Science **368**, 649-654 (2020).

[2] Thomas Schwander, Lennart Schada von Borzyskowski, Simon Burgener, Niña Socorro Cortina, Tobias J. Erb. A synthetic pathway for the fixation of carbon dioxide in vitro. Science **354**, 900-904 (2016).

To learn more about surfactants and other formulation products for droplet microfluidics, please visit www.emulseo.com

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