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Deliverable D2.7

Replication coupled to coacervation

WP 2 – Compartmentalised autocatalytic systems

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Revision History

Author(s)	Description	Date
Lukas Herold (RUG)	Draft deliverable	18/11/2025
Sijbren Otto (RUG)	Final version	21/11/2025

Contents

Partner short names

Short name	Full name
RUG	University of Groningen
ESPCI	École Supérieure de Physique et de Chimie Industrielles de la Ville de Paris
BGU	Ben Gurion University of the Negev
PARM	Parmenides Foundation
MIC	Microfluidics Innovation Center
USAAR	Universität des Saarlandes
acceICH	Accelopment Schweiz AG
MSV	Spectrometry Vision BV
DEW	Dewpoint Therapeutics GmbH
PARA	Paradigmatic Innovations GmbH
LMU	Ludwig-Maximilians-Universität München

Abbreviations

Abbreviations	Description
D	Deliverable
EC	European Commission
EU	European Union
HEU	Horizon Europe
M	Month
MS	Milestone

Executive summary

Background

The deliverable D2.7 is part of the work package WP 2.

D2.7 describes/presents the progress made so far in coupling the replication of a self-replicator to the formation of coacervate compartments through catalysis.

Objectives

One of the objectives within D2.7 is to have a replicator catalyse the formation of a coacervate.

Methodology and implementation

Here we are using fluorescent confocal microscopy and Ultra-Performance-Liquid-Chromatography-Mass-Spectrometry (UPLC-MS) to follow the formation of coacervate.

Outcomes

We show that through adaptation of a cofactor, our replicators can catalyse the formation of coacervate.

Impact

This will help establish a compartmentalized self-replicating system, where compartmentalization is coupled to the catalytic activity of the replicator.

Next steps

We will further use this system for selection by droplet size (e.g. big coacervate droplets are more likely to survive) with the goal to achieve Darwinian evolution based on the catalytic activity of the replicator.

1 Introduction

According to Tibor Gánti's chemoton model for a minimal living system, it is essential to have self-replication functionally coupled to compartmentalization through metabolism [1, 2]. Here we achieve this by using a coacervate system that only undergoes phase separation after the formation of macrocycles through oxidation of thiols into disulfides. The replicator can catalyze this reaction through the activation of tetraphenyl porphyrin, which, upon excitation with light, generates singlet oxygen (see Figure 1) [3]. Moreover, replicator precursors cannot activate the cofactor to the same extent as fully assembled replicators. Using this difference in reactivity we are therefore able to couple replication to the formation of coacervate.

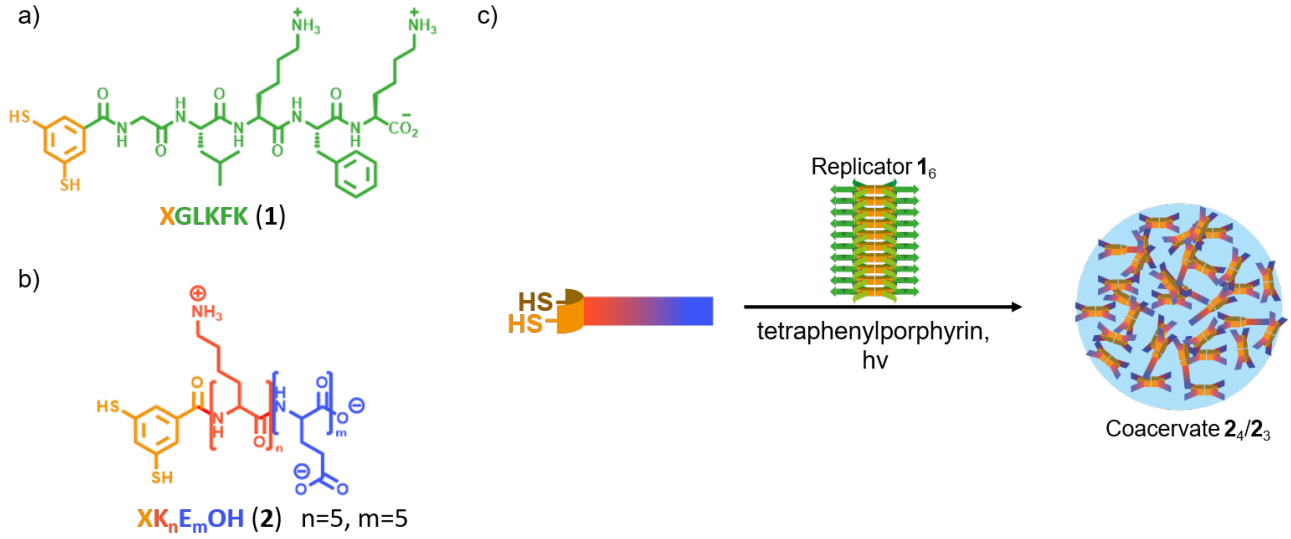

*Figure 1: a) Chemical structure of the replicator-forming building block **1**. b) Chemical structure of the coacervate-forming building block **2**. c) Reaction scheme for the replicator **1**$_6$ mediated formation of coacervate **2**$_4$/**2**$_3$.*

2 Following coacervate formation with UPLC-MS

The oxidation of monomeric building block **2** into phase-separating macrocycles **2**$_4$ and **2**$_3$ was followed by UPLC-MS. In the presence of light, replicator **1**$_6$ and tetraphenyl porphyrin, building block **2** is fully oxidized after around 10 hours and phase-separation occurs (Figure 2a). Here oxidation is fast as singlet oxygen gets produced continuously. While in the dark oxidation of **2** is considerably slower and still not completed after 22 h. (Figure 2b).

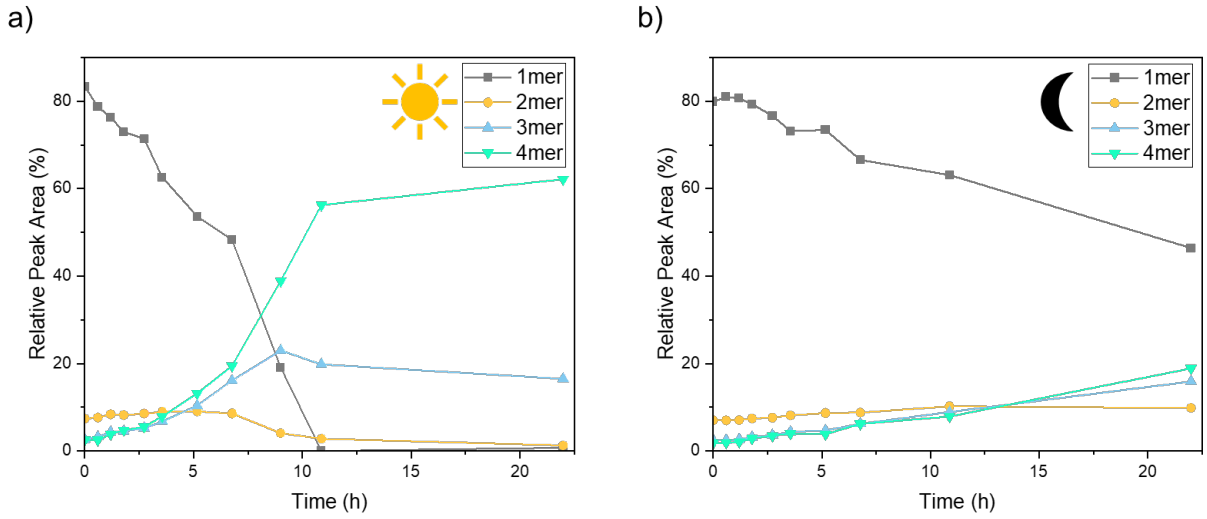

*Figure 2: a) Kinetic profile of macrocycles of **2** in presence of replicator **1**$_6$ and tetraphenylporphyrin while irradiating. b) Kinetic profile of macrocycles of **2** in presence of replicator **1**$_6$ and tetraphenylporphyrin in the dark.*

3 Following coacervate formation under the microscope

The replicator mediated formation of coacervate droplets can also be observed using confocal microscopy. Here the sample is irradiated in a well plate under the microscope. At the start of the experiment only very few small coacervates were observed, while after 60 min of irradiation, building block **2** had been oxidized to form macrocycles and consecutively form phase-separated coacervate droplets (Figure 3a). Control experiments without replicator **1**$_6$ (Figure 3b) and with only building block **2** (Figure 3c) showed little phase separation after 60 min.

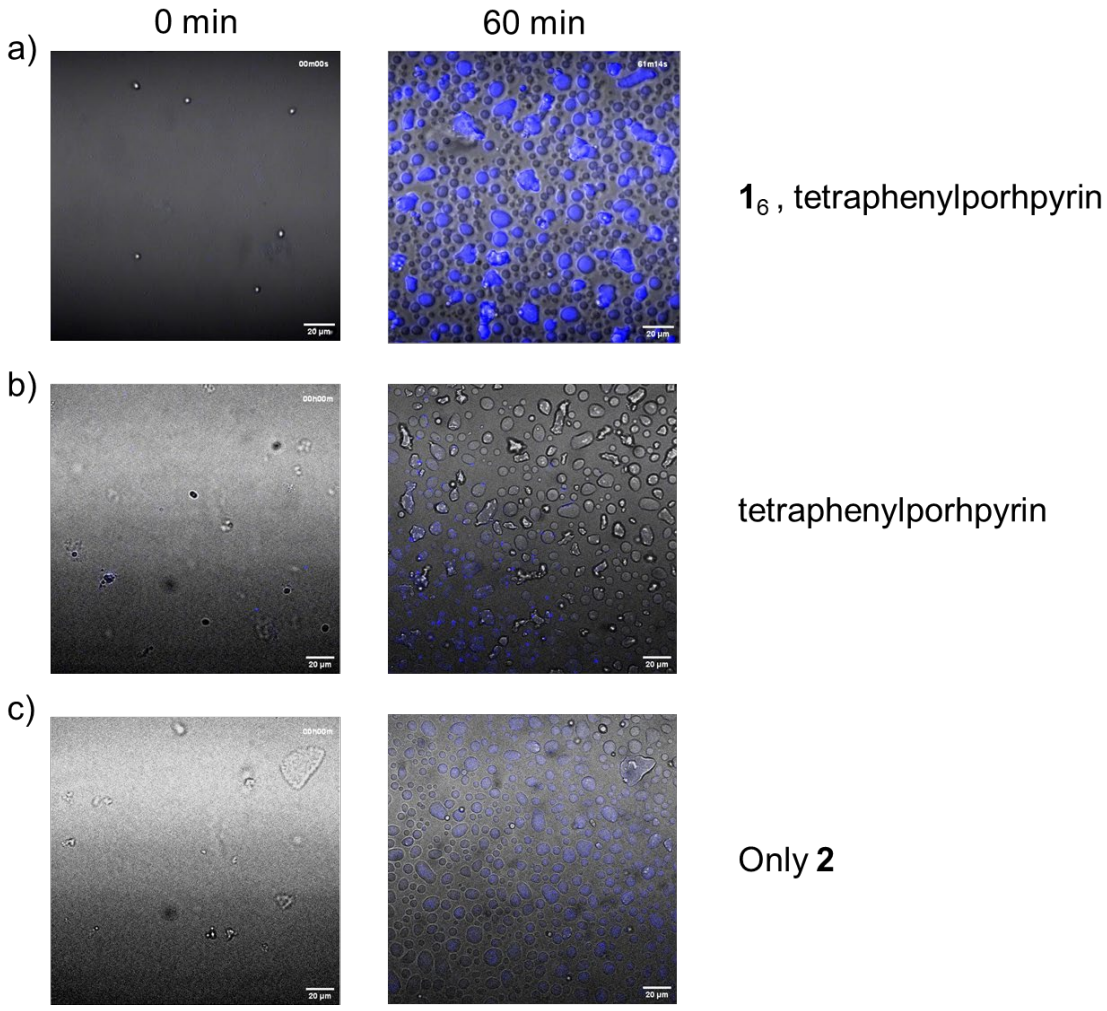

*Figure 3: Merged confocal microscopy images of the brightfield and tetraphenylporphyrin before irradiation and after 60 min of irradiation. a) sample containing replicator **1**$_6$, tetraphenylporphyrin and **2**. b) sample containing tetraphenylporphyrin and **2**. c) sample containing only **2**. Scale bar: 20 µm.*

4 Future work

Part of this work is being prepared for publication. We are currently working on understanding the interplay of replicator and coacervate by, for example, measuring water-coacervate partition coefficients (D2.8). Further work will then focus on replicator-mediated growth of the coacervate and subsequent division (D3.7). Once this is achieved, we aim to use multiple replicator building blocks with different reactivities to enable Darwinian evolution of the replicator based on coacervate growth and division dynamics (D3.8).

5 References

(1) Gánti, T. Organization of chemical reactions into dividing and metabolizing units: The chemotons. *Biosystems* **1975**, *7* (1), 15-21. DOI: https://doi.org/10.1016/0303-2647(75)90038-6.

(2) Ganti, T. *The Principles of Life*; Oxford University Press UK, 2003.

(3) Monreal Santiago, G.; Liu, K.; Browne, W. R.; Otto, S. Emergence of light-driven protometabolism on recruitment of a photocatalytic cofactor by a self-replicator. *Nature Chemistry* **2020**, *12* (7), 603-607. DOI: 10.1038/s41557-020-0494-4.