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Project acronym: **ECCell**

Project title: Electronic Chemical Cell

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Thematic Priority: Theme 3 Information and Communication Technologies

**Deliverable 2.2: report, public (PU)**

# Mid-term report on programmable containment.

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Organisation name of lead contractor for this deliverable:

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# 2. Deliverable n. 2.2: Mid-term report on programmable containment.

## *2.1 Introduction*

During the second year of the ECCell project one deliverable needed to be achieved in WP2 after month 24. This deliverable D2.2 is synthetic copolymers of DNA (scpDNA) for the encapsulation of various payloads including oligodeoxynucleotides (ODNs) that are part of an electronic replication cycle. These materials should be developed by RUG and used by the partners RUBa and RUBb. These containers have been successfully produced and therewith D2.2 was successfully achieved. A detailed description of these programmable containments is given below.

Our work has been focused on the creation of programmable chemical containers. In this sense, amphiphilic DNA block copolymers (DBCs) are employed as informational molecules. On the other hand, the use of natural lipid liposomes allows constructing aqueous compartments separated from the external aqueous solution by the lipid bilayer. Liposomes are nowadays used for the solubilization of hydrophilic but also hydrophobic chemical substances. The combination of DBCs and lipidic liposomes takes advantage of both: watertight biocontainers, which can be self-assembled by DNA hybridization (figure 8).

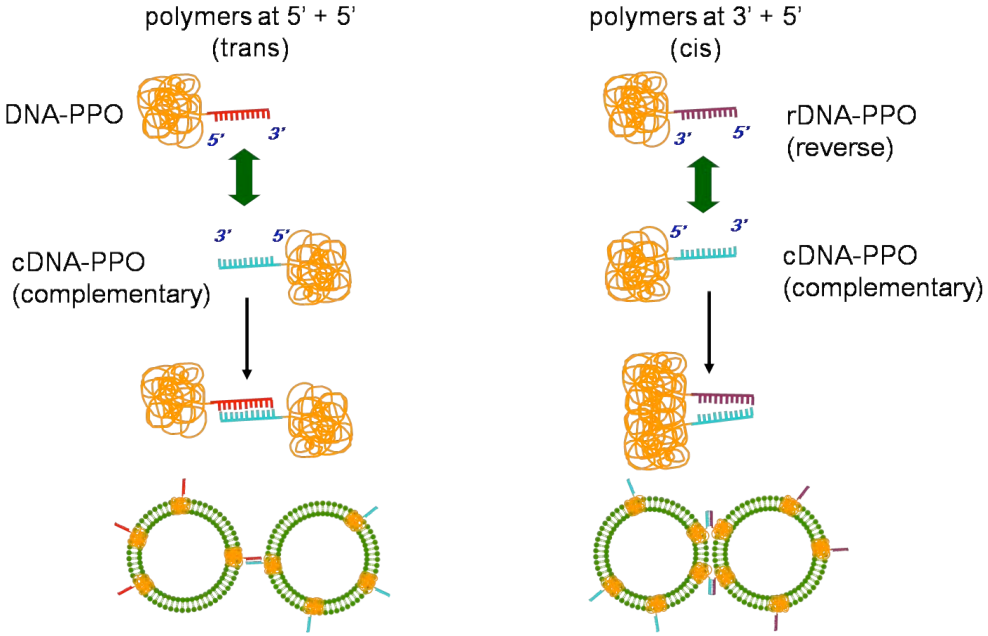


Figure 8: Two kinds of hybridization schemes between different DNA-block-poly(propylene oxide) (PPO): polymers are connected at 5'+5' ends or trans-hybridization (left) and polymers attached at 3'+5' ends or cis-hybridization (right). The latter cis-hybridization will lead to a closer proximity between the biocontainers and improved fusion between them.

## *2.2 Programmable Chemical Nano-biocontainers*

### *A) DNA Block Copolymer-lipid liposome formation.*

At RUG we have established specific techniques and methods for the creation of Large Unilamellar Vesicles (LUVs). In this sense, we have started our studies with the zwitterionic phospholipid 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhyPC), because it shows a low glass transition temperature (increasing the stability and flexibility of membranes in solution)

and a moderate fusogenic capacity between different membranes. Liposome creation methodology has been adapted and developed to the specific case of DPhyPC liposomes with DBCs incorporated in their membranes. Particularly we have used DNA-block-polypropylene oxide (DNA-b-PPO) as DBC, which is well established in our laboratory. DNA-b-PPO/DPhyPC liposomes of 200 nm diameter have been obtained efficiently by controlling parameters during the hydration (vortex and sonication times) and the extrusion process. Briefly, an appropriate amount of DPhyPC and DNA-b-PPO were dissolved in chloroform at the desired ratio. After this, the organic solution was vortexed and chloroform was removed by evaporation under a nitrogen current to yield a dry DNA-b-PPO/DPhyPC film. The resulting films were then hydrated in TRIS/NaCl buffer and alternating cycles of vigorous vortexing and sonication were used to homogenize the solutions. This combination of vortexing and sonication yields a polydisperse population of multilamellar vesicles (MLVs), which were transformed into the desired LUVs by an extrusion procedure, consisting of at least ten passes of the dispersion through polycarbonate Nucleopore membranes having 200 nm pore size.

With the aim of studying possible changes on the diameter of the DPhyPC liposomes when DNA-b-PPO is incorporated in the lipid membrane, Dynamic Light Scattering experiments have been carried out on liposome samples with and without DNA-b-PPO employing different PPO lengths (7k and 1k) and different DNA lengths (11bp and 22bp). The experiments were performed with an ALV (CGS-3) apparatus working in pseudocross-correlation mode, using a red He-Ne laser. The intensity correlation functions were obtained at a fixed temperature of 298.15 K and as a function of the scattering angle between 30º and 150º. The normalized second-order correlation functions were analyzed using CONTIN inverse Laplace algorithms. From the average relaxation times, the apparent diffusion coefficients were obtained, and using Stokes-Einstein relation, the apparent hydrodynamic radii were calculated. The results showed that the incorporation of various DNA-b-PPOs in the membranes does not affect significantly the size of liposomes compared to liposomes without DBC. There are no substantial alterations when DNA or PPO lengths are modified; the hydrodynamic diameters of 200 nm remained constant in all experiments.

***B) Stable incorporation of DBC in the lipid membrane.***

In order to create programmable chemical containers, the manipulation of which is guided by DBC hybridization, it is necessary to study the stability of incorporation of DBCs in the membrane. This parameter is important not only because a stable DBC incorporation gives rise to more selective interaction between the biocontainers, but it is also important to evaluate directly the stability of the assemblies. With the aim to study this feature, we have designed, developed and executed a Fluorescence Resonance Energy Transfer (FRET) experiment in buffer solution. 22-DNA-b-PPO/DPhyPC liposomes at ratio 1:370 have been doped with the fluorescent lipid probe N-Rhodamine-phosphatidilethanolamine (N-Rh-PE), followed by the hybridization of the DBC with complementary c22bp-DNA linked to alexa 488 (c22-alexa, c means complementary). As a result, liposomes originated, which showed FRET, as it is depicted in figure 9A. In the other figures 9B and 9C c22-DNA-PPO/DPhyPC control liposomes are presented in presence of c22-alexa (both DNAs with same sequence), and c22-DNA-PPO/DPhyPC liposomes with c22-alexa hybridized both with 22mer before the mixing, respectively. Both of them did not show FRET.

After the efficient preparation of FRET liposomes, they have been mixed with non-DNA-doped DPhyPC liposomes at different ratios 1:1, 1:10 and 1:100, as can be seen in figure 10A. Figure 10B shows the spectra of c22-alexa/22-DNA-b-PPO/N-Rh-PE//DPhyPC liposomes before and after mixing with non-fluorescent liposomes. In all the different mixing ratios no change in FRET occurred, confirming that c22-DNA-PPO is anchored stably in the membrane and does

not migrate between different liposomes. In addition, the inverse experiment was done to confirm the previous results. In this case c22-DNA-PPO/DPhyPC liposomes hybridized with c22-alexa (no FRET liposomes) were mixed with N-Rh-PE/DPhyPC liposomes, and no change in rhodamine band was observed. The stable incorporation of DBCs in the membrane was measured over time. Figure 10C shows the intensity ratio between N-Rh-PE and c22-alexa bands in FRET liposomes when they are mixed with no-FRET liposomes measured over 24 hours. As can be seen, the intensity ratio remains constant for all mixing ratios compared to the level before mixing, confirming that c22-DNA-PPO is stably incorporated in the DPhyPC liposomes for at least 24 hours. This experiment underlines the suitability of DBCs as coding molecules for liposomes.

Finally the precision of our FRET experiment, i.e., the detection limit, has been determined. Fluorescent spectra of c22-alexa/22-DNA-PPO/N-Rh-PE//DPhyPC (FRET) liposomes have been collected at different DBC:lipid ratios, and they have been compared with the fluorescence spectra of 22-DNA-PPO/N-Rh-PE//DPhyPC liposomes with c22-alexa non-hybridized (no-FRET) at the same DBC : lipid ratios. The DBC : lipid ratio at which it is not possible to discriminate between FRET and no-FRET spectra has been considered as the detection limit. This value represents 6% of DBC concentration in the stability experiment.

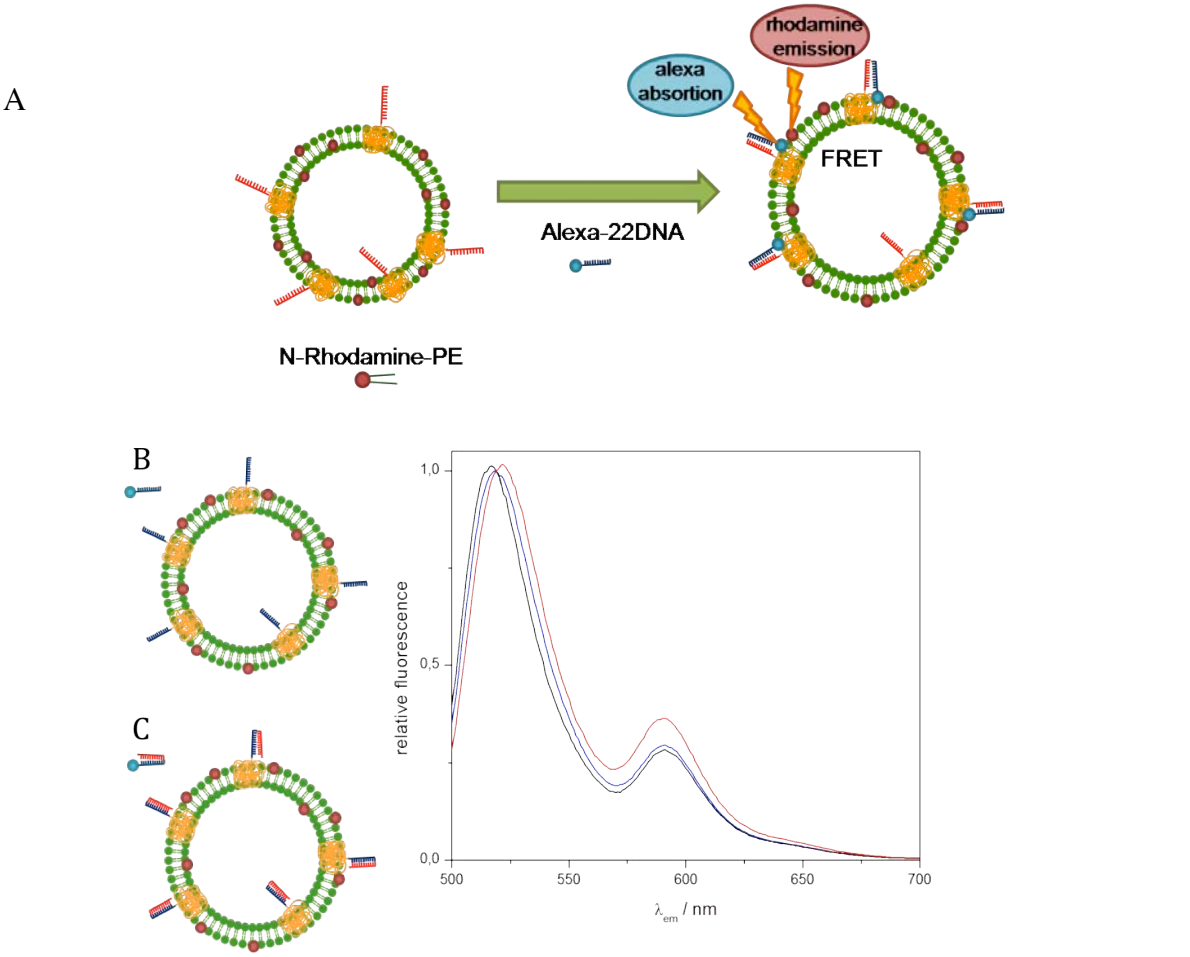


Figure 9: Scheme and spectra of designed FRET experiment. A) 22-DNA-PPO/DPhyPC liposomes doped with N-Rh-PE, which were hybridized with c22-alexa, lead to FRET liposomes (red spectrum). On the other hand, c22-DNA-PPO/N-Rh-PE/DPhyPC liposomes in the presence of c22-alexa (both DNAs with same sequence) (B and blue line), and c22-DNA-PPO/N-Rh-PE//DPhyPC liposomes with c22-alexa hybridized both with 22mer before the mixing (C and black line), respectively, do not show FRET.

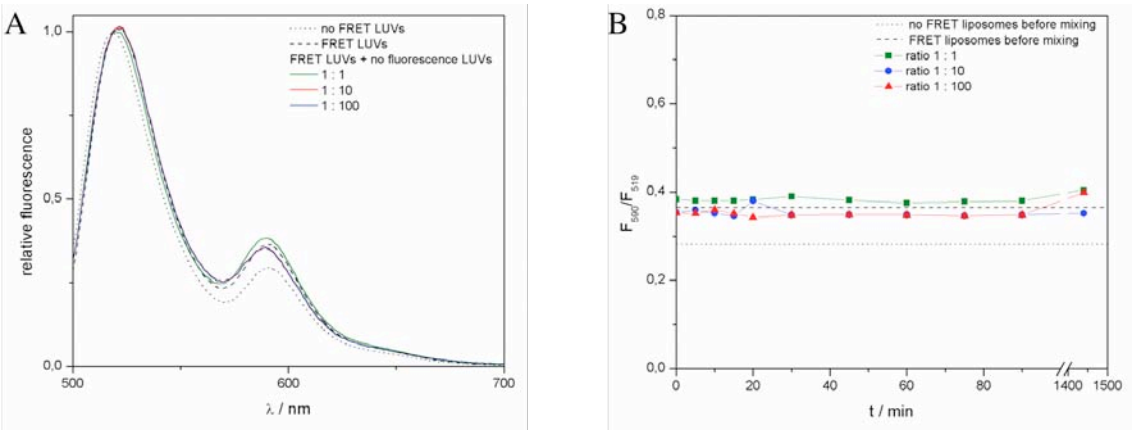


Figure 10: A) Fluorescent spectra of 22-alexa/c22-DNA-PPO/N-Rh-PE//DPhyPC liposomes before (dash line) and after (solid lines) mixing with non fluorescent DPhyPC liposomes at ratios 1:1 (green line), 1:10 (red line) and 1:100 (blue line). Dotted lines show the fluorescent spectra of c22-DNA-PPO/N-Rh-PE//DPhyPC liposomes in the presence of c22-alexa (no FRET signal). B) Values of rhodamine/alexa fluorescent intensity ratios, F590/F519, with the time.

### *C) Stability of the lipid membrane*

The effect on the DPhyPC lipid membrane stability when 11bp-DNA-PPO (1k) is inserted in the bilayer has been studied by the Black Lipid Membrane (BLM) technique. With this aim BLMs constituted of several 11bp-DNA-PPO/DPhyPC ratios have been studied. The DNA-PPO/lipid at required ratio dissolved in chloroform was dried under Ar in a glass vial, followed by resuspension in n-decane. BLM was formed by applying a small volume of DNA-PPO/lipid solution to a small hole placed between two compartments, so it separates two compartments loaded with identical saline buffer solutions, as can be seen in figure 4. Current-voltage curves were measured using a patch-CLAMP 10 to characterize the electrical properties of BLMs as a function of DNA-PPO/lipid ratio. Resistance values were monitored throughout the experiments to verify that BLM remained intact by measuring the current in response to application of 50 mV square-wave pulse. Capacitance values were determined by applying a 50 ms square-pulse of 100 mV to the BLM while recording the current response. In this sense, the membrane constituted only by DPhyPC was always stable and showed resistance when electric potential was applied. When the membrane consisted exclusively of 11bp-DNA-b-PPO it was unstable, showing low electric resistance and, finally, BLM breakage was observed. The results have shown that 1:80 is the minimum 11bp-DNA-PPO:DPhyPC ratio at which the membrane is stable and remain intact, showing properties such as ion impermeability and capacitance. An increase of DBC in the membrane gives rise to instability and subsequent breakage of the membrane.

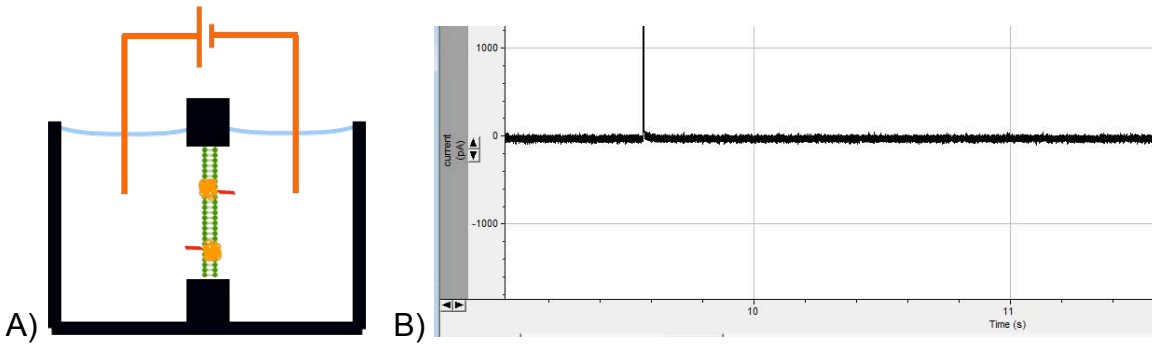


Figure 11: A) Scheme of black lipid membrane constituted by DNA block copolymer and lipids. B) Recording from stable BLM constituted by 11bp-DNA-PPO/DPhyPC at 1:780 ratio.

## *2.3 Aggregation of Giant Unilammelar vesicles (GUVs) mediated by DBCs*

The work carried out with LUVs was extended to GUVs to have containers available that can be directly visualized within microfluidic channels by optical techniques. Therefore, the GUV electroformation method was implemented at RUG and adapted to the specific case of DPhyPC GUVs with DNA-b-PPO incorporated in their membranes, as can be seen in figure 12. Briefly, an appropriate amount of DPhyPC and DNA-b-PPO were dissolved in chloroform to obtain the desired ratio and a small drop of this solution was placed on an ITO-slide. After the chloroform was removed by evaporation in an oven at 50 ºC a dry DNA-b-PPO/lipid film was obtained. This material was hydrated in buffer with a high sugar concentration. The following application of alternating current with the appropriate parameters (3 V and 5 Hz during 3 hours at 37 ºC) led to DNA-PPO/DPhyPC GUVs. With the aim of visualizing the GUVs using Confocal Laser Scanning Microscopy (CLSM), the DNA-b-PPO/DPhyPC GUVs were doped with 3,3'-dioctadecyloxacarbocyamine (DiO) fluorescent lipid probe.

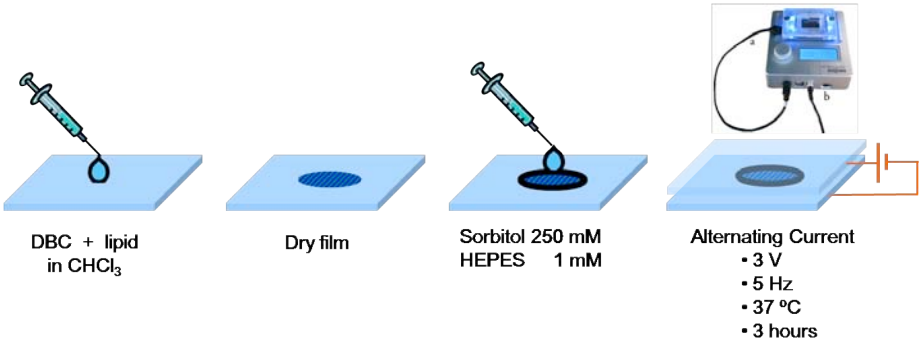


Figure 12: Scheme of the electroformation method applied for the generation of 11-DNA-PPO/DPhyPC GUVs.

Figures 13 show the micrographs obtained with 11-DNA-PPO/DPhyPC and complementary c11-DNA-PPO/DPhyPC vesicles at DBC:lipid ratio 1:$10^5$. Both figures show GUVs of various sizes (bigger than 15 micrometers) forming spherical structures. Thereby, no fusion or aggregation processes could be observed. However, when the GUVs samples were mixed containing 11-DNA-PPO/DPhyPC and c11-DNA-PPO/DPhyPC, aggregation and hemifusion processes were detected, as can be seen in figure 14. A video recording of this interaction between the DNA coded vesicles was performed, permitting to analyze the mechanism of GUV interaction. First aggregation takes place induced by the DBC hybridization and when the membranes are close together hemifusion is observed, which is accompanied by the formation of planar faces between the mebranes.

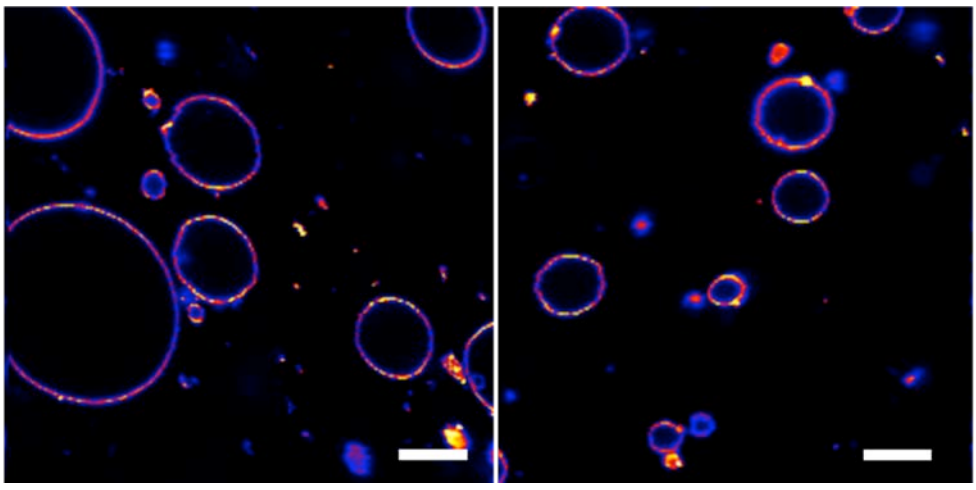

Figure 13: Micrographs of 11-DNA-PPO/DPhyPC (left) and complementary c11-DNA-PPO/DPhyPC (right) GUVs at DBC:lipid ratio 1:105. Scale bar: 25 micrometers.

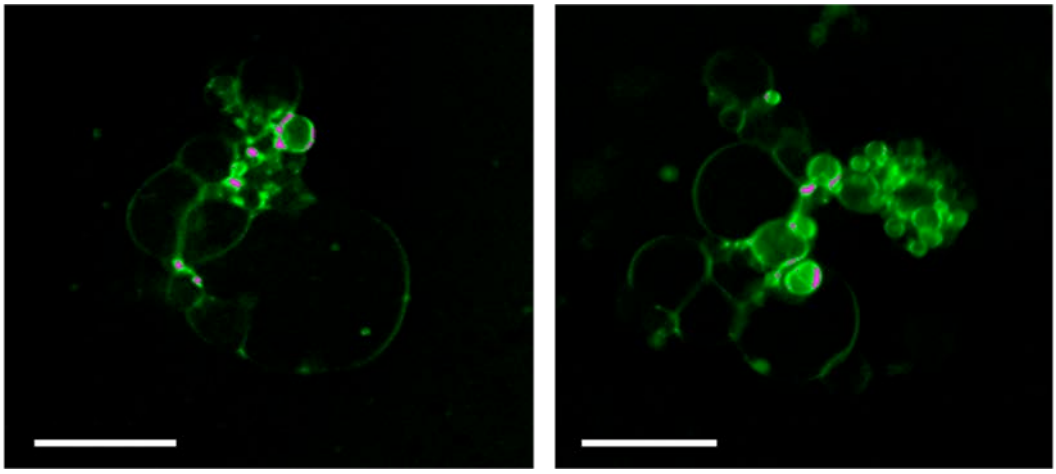

Figure 14: Micrographs of aggregation observed when 11-DNA-PPO/DPhyPC GUVs are mixed with c11-DNA-PPO/DPhyPC GUVs. Scale bar: 25 micrometers.

In summary, we have successfully incorporated DBCs that encode sequence information into the membranes of LUVs and GUVs. The stable anchoring of the informational molecules to the liposomes was undoubtedly proven by FRET experiments. Furthermore, it was demonstrated that below a certain ratio of lipid to DBC the membrane is impermeable for most ions and molecules incorporated as payloads.

Finally, vesicle aggregation can be achieved by hybridization, which clearly documents that manipulation of the containers is possible by exploiting the DNA tag. For that reason deliverable D2.3 “scpDNA for encapsulation” was successfully realized.