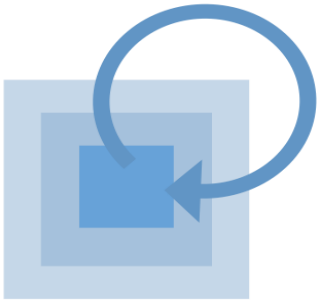

2011

Deliverable Report

D2.2 Addressing effectiveness/precision and container fusion: Demonstration of the addressing effectiveness/precision and address-directed container fusion (M24)

Matrix for Chemical IT (MATCHIT)

D2.2: Addressing effectiveness/precision and container fusion: Demonstration of the addressing effectiveness/precision and address-directed container fusion [month 24] ***Delivered in month 24***

Note: this is the third version of this deliverable. It has been expressed by Dagmar Floeck and Tracey Melvin at the second year review of the MATCHIT project on April 27, 2012 to provide a detailed deliverable report along with a value-added analysis. The previous versions of the D2.2 reports are appended at the end of this report for comprehensive clarity.

Martin Hanczyc, WP2 leader, MATCHIT

1) The primitive charge-charge addressing system

We currently have two different specific address systems in operation. In the first system (the primitive system) we use charge-charge interaction to govern specific fusion events between addressed vesicles following the simple rules of net positive charge vesicle pairs with net negative charge vesicle. Using this system we have demonstrated both **address specific assembly** and **address specific fusion**. By modulating the amount of address per vesicle we can favor assembly over fusion in a quantitative manner, see (Sunami et al 2010, Figure 3) Fig. 1 below. Controls were made to show the precision of this method, see (Sunami et al 2010, Figure 2) Fig. 2 below. Using the same chemtainer addressing system, we also demonstrated the **programmed fusion** of vesicles to produce both biochemical reactions and complete gene expression from a DNA plasmid (Caschera et al 2011). We showed how the amount of charged addresses changes the zeta potential of the vesicle chemtainers in Fig. 3 below demonstrating the effective addressing of the chemtainers. Clear proof of the fusion event producing a biochemical gene expression system is shown in (Caschera et al 2011, Figure 5, C2) Fig. 4 below.

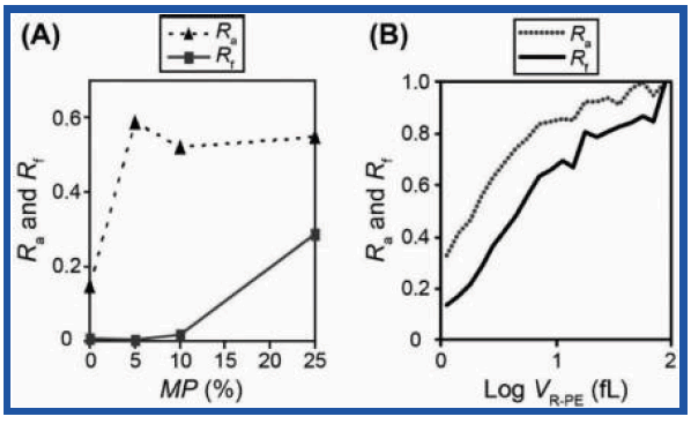

Figure 1 (Figure 3 from Sunami et al 2010). Part A shows how vesicle-vesicle association and fusion are modulated by the mole percent (MP %) of charged addresses supplied.

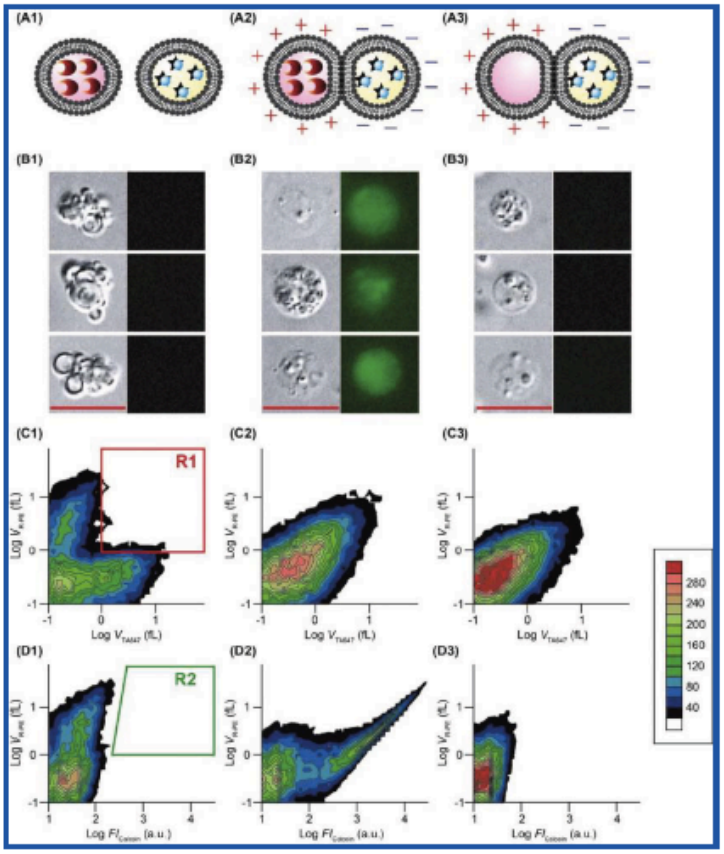

Figure 2 (Figure 2 from Sumani et al, 2010.) This shows the results of chemtainer association (C panels) and fusion (D panels) when chemainers are addressed with positive and negative charge addresses (middle column) with controls (flanking columns).

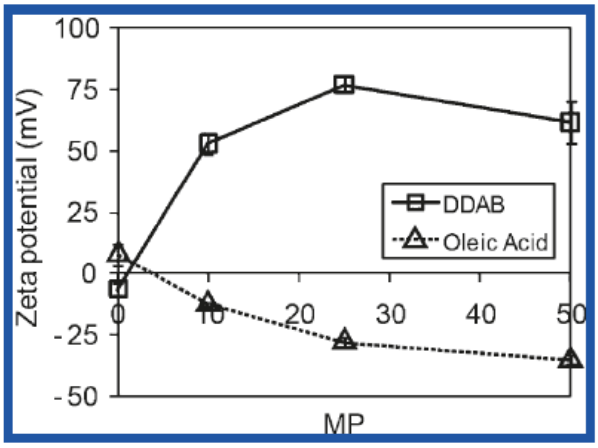

Figure 3 (Figure 2 from Caschera et al 2011) showing the zeta potential change as either anionic or cationic addresses are added to the vesicle chemtainers in varying molar percent (MP).

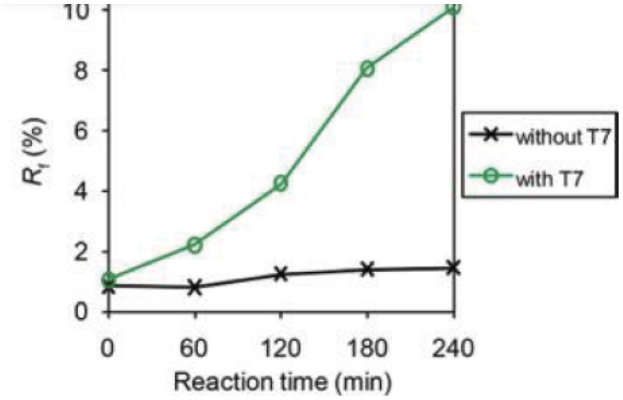

Figure 4 (Figure 5, C2 from Caschera et al, 2011) showing that properly addressed vesicle chemtainer fusion can result in the expression of a gene from a biochemical network (green line). The control (black line) is the same system but with an incomplete biochemical system that shows no gene expression.

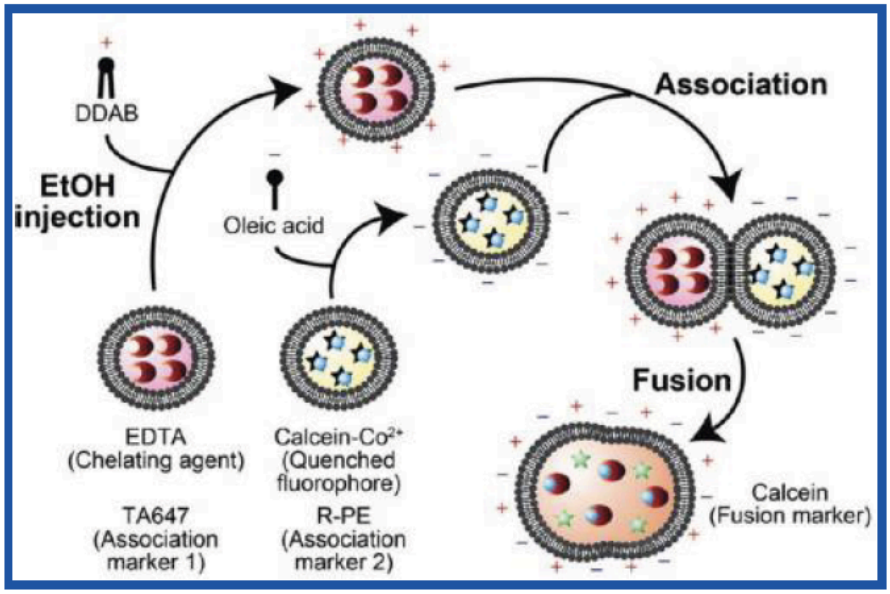

Figure 5 (Figure 1 from Sunami et al 2010.) This scheme shows how two populations of vesicle chemtainers can be addressed with charged molecules and then associate and fuse. This reflects the MATCHIT research for WP2.

This large and expensive set of experiments was mostly funded by our collaborators in Osaka Japan. The expertise of vesicle manipulation and fusion came from the MATCHIT sponsored authors. The instruments, materials as well as expertise in using the three color fluorescent system came from our long time collaborators in Oaska. The **majority of experiments** (about 90%) were executed by MATCHIT supported author Cashera under the guidance of MATCHIT sponsored Hanczyc at Osaka University. This is why Caschera either has first author position or shared first author position on both papers. Both authors Caschera and Hanczyc are in the MATCHIT consortium based at SDU. The value added therefore stems from the technical knowledge of MATCHIT and the execution of experiments in a collaborative context. The intellectual input from the MATCHIT project is directly illustrated in the first figures of both papers. Consider Sunami et al, 2010, Figure 1 (Fig. 5 above). The legend is entitled: Schematic diagram showing the induction of **vesicle fusion** by the exogenous injection of charged amphiphiles and the detection of fusion using three fluorescent markers. Compare with the wording of D2.2: Demonstration of the addressing effectiveness/precision and address-directed **container fusion**. Figure 1 from Caschera et al 2011 depicts the entire system studied in the paper. The legend reads: Schema of gene expression by **vesicle fusion**. Separate vesicle populations encapsulating DNA and T7 RNA polymerase/substrate were prepared. After **decoration** with opposite electrostatic charges by EtOH injection, the vesicles were allowed to **associate** and **fuse** to initiate gene expression. This entire

paper reflects and addresses the MATCHIT research agenda and specifically for WP2.
Without this collaborative effort between the MATCHIT supported SDU and Osaka University, there would be only a limited amount of data about this system. This set of experiments would not have been possible without MATCHIT sponsorship and contribution to the collaboration. With MATCHIT funding alone we would be able to produce a proof of principle deliverable with limited data based almost entirely on limited microscopic analysis. The instrumentation at Osaka, notably FACS, allowed for a much more comprehensive analysis and comprehension of the system. The same added value can also said for the Osaka group whom without the MATCHIT collaboration would not have the necessary expertise with chemtainers. With this synergistic collaboration we were able to produce high quality publications for wide dissemination rather than just deliverable reports.

2) The DNA-addressing system

For the second addressing system, SDUa has developed a protocol for effectively **addressing chemtainers** (precisely vesicles, oil droplets and emulsions) without disturbing the integrity or utility of the chemtainer. This is done by first forming the chemtainer using a low molar percentage of a biotinylated lipid (bPEG2000-DSPE) in the presence of POPC. A biotinylated-ssDNA address of choice is then allowed to complex with streptavidin in a separate preparation. The ssDNA address bound to streptavidin is the introduced to and incubated with the chemtainer containing the biotinylated lipid, see Hadorn and Eggenberger Hotz, 2010, Figure 1 (Fig. 6 below). After careful washing, we have shown that we can produce chemtainers with any **DNA address** of choice, see Hadorn and Eggenberger Hotz, 2010, Figure 4 (Fig. 7 below). These addresses are stable (i.e. no significant exchange between chemtainers seen) and are able to coordinate address specific hybridizaton and **assembly of chemtainer** structures.

This work on the DNA-based address system was partially funded by MATCHIT and is a follow on product of a previous EC project called PACE. Both authors Hadorn and Eggenberger Hotz are in the MATCHIT consortium working at SDU and therefore all the work was done by MATCHIT people. PACE contributed the expertise and MATCHIT contributed the conceptual direction. Materials and instruments were provided in part by MATCHIT. This set of experiments would not have been possible without MATCHIT sponsorship and contribution to the collaboration.

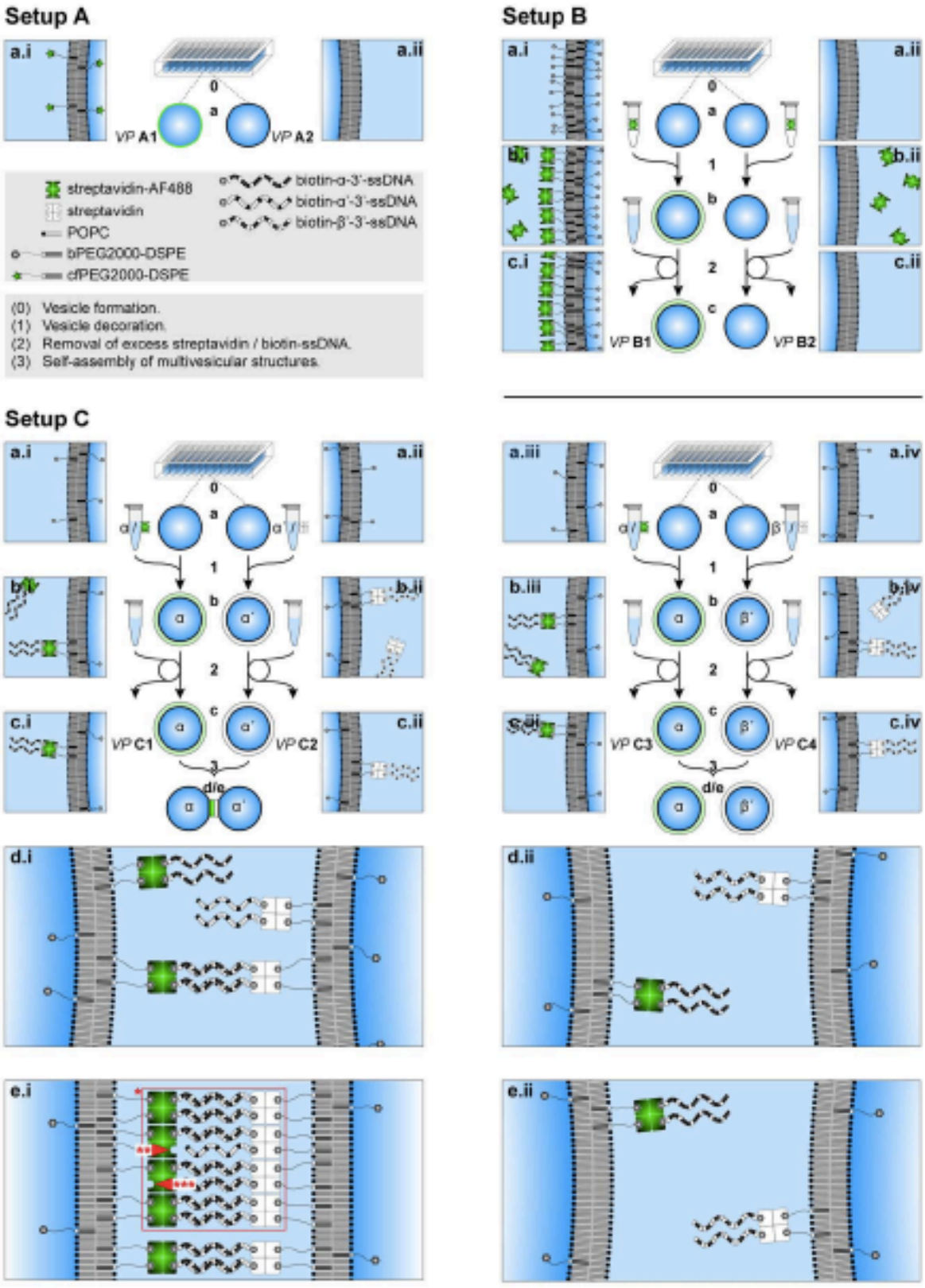

Figure 6 (Figure 1 from Hadorn and Eggenberger Hotz, 2010) detailing the ssDNA addressing of vesicle chemtainers. For details see the published work.

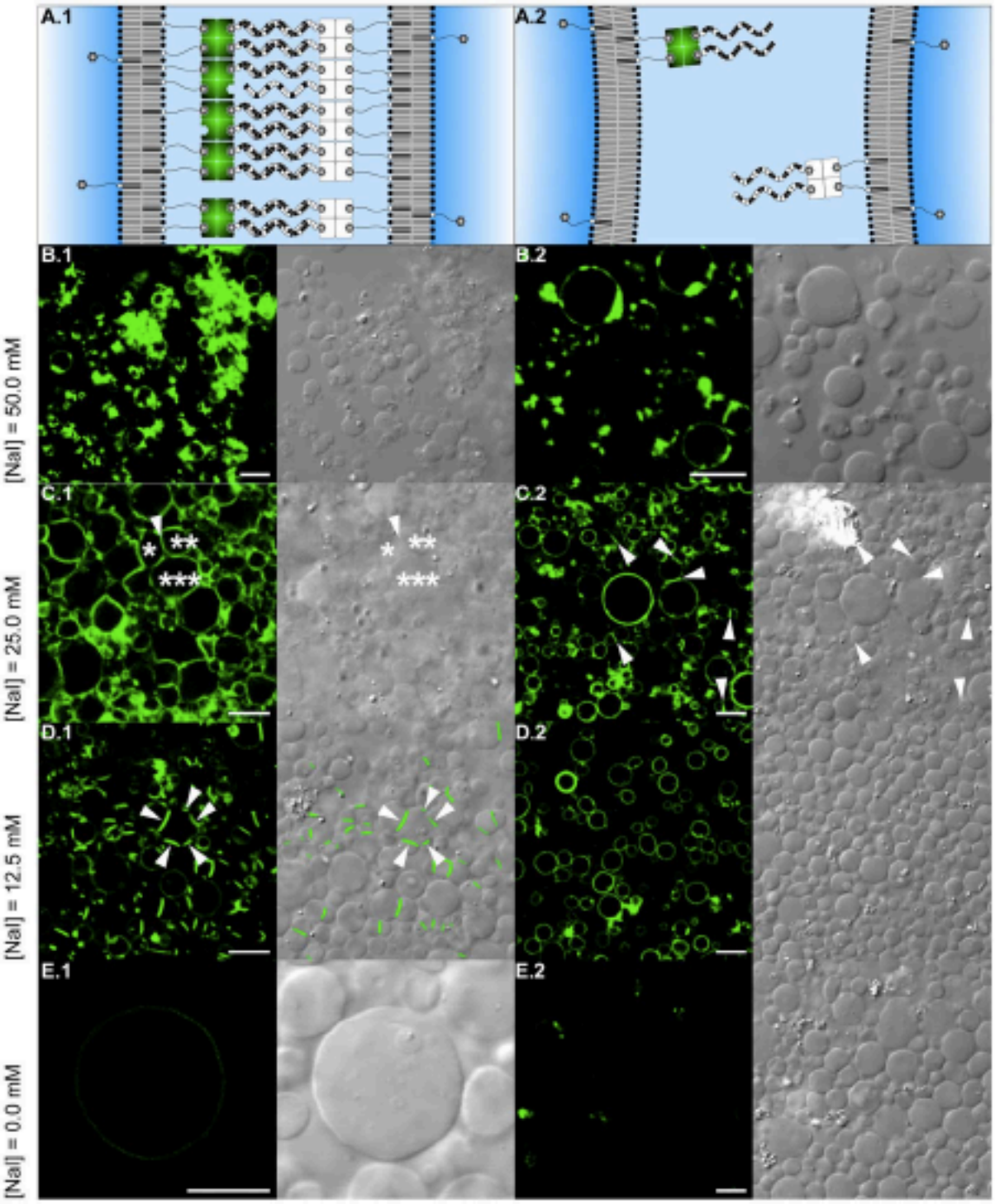

Figure 7 (Figure 4 from Hadorn and Eggenberger Hotz, 2010) showing the physical association of vesicle chemtainers based on their complementary ssDNA addresses (arrows). For details see the published work.

Summary

Overall the three peer-reviewed and published papers below directly address the following from MATCHIT WP2:

O2.1: Assembly of the DNA tag on containers
O2.4: Test intercontainer tag assembly
O2.5: Develop methods for container alterations by fusion and fission events

D2.2 Addressing effectiveness/precision and container fusion: Demonstration of the addressing effectiveness/precision and address-directed container fusion [month 24]

MS4 Tagged containers and container-container tagging [month 18]
MS5 Fusion between containers [month 24]

Peer reviewed and published papers

Hadorn M and Eggenberger Hotz P. DNA-Mediated Self-Assembly of Artificial Vesicles. *Plos One* **5**(3):e9886. (2010).

Sunami T, Caschera F, Morita Y, Toyota T, Nishimura K, Matsuura T, Suzuki H, Hanczyc MM, Yomo T. Detection of Association and Fusion of Giant Vesicles Using a Fluorescence-Activated Cell Sorter, *Langmuir* 26(19), 15098–15103. (2010).

Caschera F, Sunami T, Matsuura T, Suzuki H,. Hanczyc MM, Yomo T. Programmed Vesicle Fusion Triggers Gene Expression. *Langmuir* 2011, 27, 13082–13090. (2011).

Previous versions of this deliverable

MATCHIT deliverable report, year 2
M. Hanczyc WP2 leader

Original report:
D2.2. Addressing effectiveness/precision and container fusion

We have published three papers towards deliverable D2.2.
Addressing effectiveness and precision is the main topic of Hadorn and Eggenberger Hotz, 2010 and also in Sunami et al 2010 (see Figure 3).
Container fusion mediated by chemical addresses is the main topic in Sunami et al 2010 and Caschera et al 2011.

Revised report (2nd version) is added to answer this email:

From: "Dagmar.FLOECK@ec.europa.eu" <Dagmar.FLOECK@ec.europa.eu>
Date: April 13, 2012 5:23:41 PM GMT+02:00
To: Steen Rasmussen <steen@sdu.dk>
Subject: deliverable 2.2 and PM table

Dear Steen,

from the commission point of view the 3 papers handed in as deliverable 2.2 are not acceptable. It is not clear which part of the papers has been done by any of the consortium members - two of them don't even mention our funding. Could provide a report which extracts the work done by the consortium?

Revised report (April 13, 2012)
Hadorn M and Eggenberger Hotz P. *DNA-Mediated Self-Assembly of Artificial Vesicles.* Plos One **5**(3):e9886. (2010).

This paper addresses:
O2.1: Assembly of the DNA tag on containers
O2.4: Test intercontainer tag assembly

D2.2.2) Addressing effectiveness/precision and container fusion: Demonstration of the addressing effectiveness/precision and address-directed container fusion [month 24]

MS4 Tagged containers and container-container tagging [month 18]

This work was partially funded by MATCHIT and this was not properly acknowledged in the paper. Both authors are in the consortium working at SDU.

Sunami T, Caschera F, Morita Y, Toyota T, Nishimura K, Matsuura T, Suzuki H, Hanczyc MM, Yomo T. Detection of Association and Fusion of Giant Vesicles Using a Fluorescence-Activated Cell Sorter, Langmuir 26(19), 15098–15103. (2010).

This paper addresses:
O2.4: Test intercontainer tag assembly
O2.5: Develop methods for container alterations by fusion and fission events

D2.2.2) Addressing effectiveness/precision and container fusion : Demonstration of the addressing effectiveness/precision and address-directed container fusion [month 24]

MS4 Tagged containers and container-container tagging [month 18]
MS5 Fusion between containers [month 24]

This large and expensive set of experiments was mostly funded by our collaborators in Osaka Japan. MATCHIT supported the authors Caschera and Hanczyc as well as contributions to materials. MATCHIT was not properly acknowledged in the paper. Both authors Caschera and Hanczyc are in the consortium working at SDU.

Caschera F, Sunami T, Matsuura T, Suzuki H,. Hanczyc MM, Yomo T. Programmed Vesicle Fusion Triggers Gene Expression. Langmuir 2011, 27, 13082–13090. (2011)

This paper addresses:
O2.4: Test intercontainer tag assembly
O2.5: Develop methods for container alterations by fusion and fission events

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