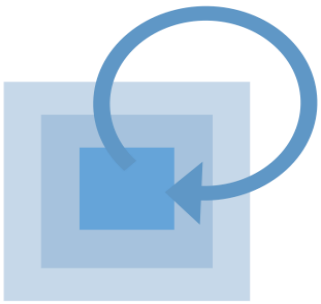

2011

Deliverable Report

D4.1 Chemtainer labelling/relabeling: Demonstration of chemtainer labelling/relabeling mechanism with DNA (M18)

Matrix for Chemical IT (MATCHIT)

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D4.1 Chemtainer labelling/relabeling: Demonstration of chemtainer labelling/relabeling mechanism with DNA (M18)

General

A paper, titled: "**DNA programming of mesoscale vesicles"** (Benny Gil, Maik Hadorn, Uri Shabi, Ehud Shapiro & Martin M Hanczyc), reporting this achievement is under preparation. The delay in submission is due to our desire to extend the scope of the publication by adding more results. Recently, we came to realize that the additional results should be published separately.

To achieve functional labeling system we have linked the computing ability of DNA to the surface of mesoscale supramolecular structures. ssDNA addresses anchored to vesicles results in the sequence specific assembly and disassembly of vesicles. We have also demonstrated programmability and control of DNA addresses through DNA-based operations. This DNA programming of supramolecular structure assembly was used to coordinate address-specific vesicle-vesicle fusion upon addition of an exogenous fusagen.

D4.1 realization part I: Chemtainer labelling

In the designed system, presented in Figure 1, each ssDNA is comprised of a contiguous 15-nt oligonucleotide stretch representing the address flanked by two regulatory regions, 5-nt long each. The address contains information to allow for selective hydrogen bonding with complementary ssDNA addresses. As shown previously, a 15-nt address is sufficient to promote specific complementary ssDNA binding and vesicle assembly. These complementary addresses may be on neighboring vesicles or part of a logical operation to be performed. We also allow for strand migration (displacement) reactions designed to perform the requisite operations on addresses. The 5-nt regulatory regions are designed to facilitate strand displacement controlled by the environmental conditions such as temperature and salinity. One regulatory region is constant for each address while the second one could vary to facilitate specific indication of the tag status. Each ssDNA also contains a linker between the address and the anchoring system consisting of biotin-streptavidin (B and Av. in Figure 1, respectively). An elongation segment (yellow segment, Fig. 1) may be used to increase the distance between the biotin-strepavidine complex and the address sequence to reduce undesired physical interference. This segment's sequence is comprised mostly of Adenines for optimal stacking.

Tag sequences

Tags were generated by using an evolutionary algorithm. Twenty sequences were tested empirically for self-dimerization and non-specific association with other addresses. Although two tags were ruled out due to self-dimerization, none of the tags were found to have non-specific interactions with other tags. Additionally, a pool of successive 20 regulatory regions was generated by the same

algorithm. The system's design ensures that non-specific interactions between regulatory regions and addresses are inconsequential.

Fig. 1

Address structure and algorithm

Figure 1: DNA addresses generation. **a)** Tag structure: each tag is a biotinylated (B, in the figure) ssDNA that encompasses two 5-nt long regulatory regions (light blue and green) and a 15-nt long specific sequence that represents an address (Red). In addition, a "spacer" sequence could be added to increase the distance between the address and the anchoring complex (biotin-avidin-biotin). B=biotin, Av.=Avidin, PL=PhosphoLipid **b)** schematic representation of the genetic address generating algorithm. Termination condition could be running time or score maximum threshold.

Empirical verification of tag sequences

Four populations of biotinylated vesicles were prepared and labeled with specific biotinylated ssDNA addresses of sequences A, A', B, and B'. The complementary vesicle populations (vA/vA' and vB/vB') were then mixed and allowed to assemble. In accordance to our previous results, only those vesicles with complementary ssDNA addresses were able to assemble (Fig. 2a,b) as a control system with non-complementary ssDNA showed no association (Fig. 2c).

D4.1 realization part II: Chemtainer relabelling

A readdressing operation that changes address A into address C (operation AtoC) was examined next. This newly generated address C is complementary to C'. When vA was mixed with vC' in the presence of the dsDNA molecule that realizes the readdressing operation AtoC significant association of vesicles could be observed (fig. 2d,e). Moreover, the association rate seemed to be higher than the association observed for vA-vA' or vC-vC'. To validate that the interaction was indeed specific, vA' was mixed with vC in the presence of the same DNA molecule (AtoC). In this experiment no association was observed (fig. f).

To conclude, we have demonstrated that simple computational operations can be applied to mesoscale supramolecular objects such as vesicles. By executing the change of address operation we can allow for the physical association of two or more vesicle populations in time and space. It is through such fine control of chemical systems that we hope to develop a system by which the information processing and the material production are integrated.

Fig. 2

Addresses' specificity

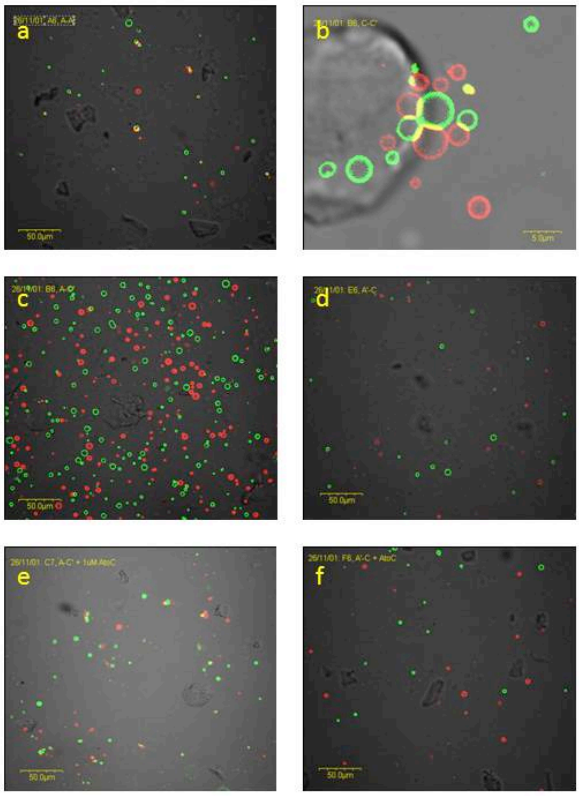

Figure 2: Specific phospholipid vesicles association. **a)** Association could be detected when vesicles labeled with address "A" (vA, green) were mixed with vesicles labeled with address vA' (red). **b)** Similarly, when vC (green) are mixed with vC' (red) significant association could be observed. **c)** Minimal or no interaction occurred when vA (green) were mixed with vC' (red). **d)** Minimal or no interaction occurred when vA' (red) were mixed with vC (green). **e)** Significant association could be observed when vA (green) are readdressed to C (with AtoC operation), in the presence of vC' (red). **f)** The reciprocal experiment (negative control) where vA' (red) are mixed with vC (green) in the presence of AtoC, show no association.