2012

Matrix for Chemical IT (MATCHIT)

Deliverable Report D4.2

Grant Agreement No. 249032

Deliverable 4.2: Integration of DNA coupling of Chemtainers

A presentation of the state of integration of DNA coupling of Chemtainers with chemical reactions that affect retagging and explore the iterative potential for the system (**M36**).

The integration presentation is comprised of three parts:

1) DNA addresses and possible operations and their iterative design.
2) Proof of concept for the ability to activate operations by bio-chemical reaction products.
3) A demonstration of the ability to apply the addresses mechanism also to MEMs in general and microfluidic MEMs in particular.

1) The first part was presented in D4.1 and MatchIT reports for years 1-3.

2) The second part is presented in MatchIT WP4 report for year 3. This demonstration includes an aptamer based mechanism, which could be integrated with the rest of MatchIT approach by designing a system in which the interaction between vA and vB' is dependent on the completion of a reaction within a (fussed) vesicle. For example, a scenario could be designed in which vA should interact (associate of fuse) with vB only after a (bio)chemical reaction had been completed inside vA (which could be the product of fusion between vC and vD). In this case, ATP (or any other small molecule) would be designed to be a byproduct of the (bio)chemical reaction. As the reaction proceeds, ATP is being accumulated in the vesicle. At a certain concentration it will be released to the medium and activate the ATP-dependent-AtoB complex which was presence *ab-initio*, but was not active. Upon activation, the AtoB molecule could change the address on vA to B, thus enabling new interaction between vesicles after the (bio)chemical reaction was completed.

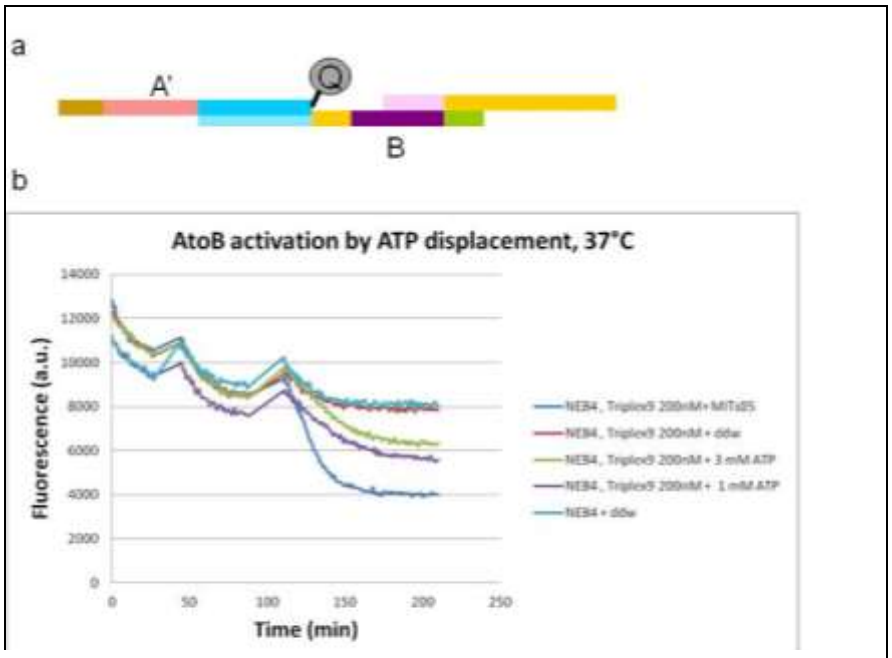

Fig. D4.2.1 ATP activation of AtoB operation. (a) design of conditional AtoB molecule. ATP aptamer blocks the molecule activity until removed by ATP binding. (b) While B' is labeled, active AtoB reduces the fluorescence by bringing the quencher close to the fluorophore. Addition of MITs05, which is complementary to the aptamer, or ATP – displaces the aptamer, thus enabling binding to B' which results in decrease in fluorescence.

An alternative approach was developed by WP3 (in collaboration with other partners), in which photochemical template-directed alteration of DNA tags was developed. This work is described in details in WP3 3rd year report and in a paper [Cape, J. L. et. al. Bioconj. Chem., 23, 2014-2019 (2012)]. In this approach the thiol-disulfide exchange between a thiomodified DNA and hexadecanethiol (C_{16}-SH), activated with DTNB (5,5'-dithiobis-(2-nitrobenzoic acid, Ellmann's reagent), was studied for the utilization as a non-enzymatic address alternation method.

3) The third part was first accomplished by RUB in years 1-2, and was perfected in year 3 by RUB and applied to direct the docking of vesicle Chemtainers to specific locations in the microfluidic system in a collaboration between WISb, SDU and RUB. These electronically directed Chemtainer synthetic experiments are described in **D5.4** as a key example of electronic chemical integration for Chemtainers. In this section we focus on the key DNA relabeling employed both on the channel wall and on the vesicles.

RUB achieved the labelling of the surface of chemical microprocessor chip with thiol-capped DNA forming stable Self Assembled Monolayers (SAM´s) capable of downstream hybridization and release of vesicles or microparticles via matching DNA. Relabeling was accomplished using adaptor DNA strands specially designed for the purpose, allowing the anchor DNA sequences on the surface to be transformed to different sequences by selective electronically assisted hybridization. Since hybridization efficiency is dependent on surface coverage, with an optimum at intermediate spacing values, the efficiency could be optimized by initially immobilizing pre-hybridized DNA in the form of duplexes (as previously reported in the literature) or hairpin triplexes (original RUB contribution) to provide a steric spacing of the immobilized strands after release of the hybridized DNA, leaving sufficient room for subsequent high efficiency hybridization.

Under electronic control, specific electrodes in the microfluidic processors were coated with immobilized DNA by this procedure, and then adaptor strands relabelled these sequences, to be suitable for either duplex or reversible triplex binding to other objects such as vesicles or beads. The experiments with vesicles proved successful with both duplexes and triplexes and are being prepared for a collaborative publication. Three lab visits for joint experimentation took place between RUB and SDU to achieve this integrated objective (2 SDU->RUB, 1 RUB->SDU), and the relabeling of WP4 played an important role in the success of these experiments.