Project acronym: **MATCHIT**

Project title: Matrix for Chemical IT

Deliverable n. 2.2.1: Report, Restricted (RE) M12
"A road-map for the chemtainer work"

Temporary only for restricted circulation

Due date of deliverable: M1

Actual submission date: March 31st 2011

Start date of project: **01.02.2010**

Duration: **3 years**

Organisation name of lead contractor for this deliverable: University of Southern Denmark, SDU

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A roadmap for the chemtainer work

Summary report evaluating our chemtainer experiences (feasibility studies) as well as provides a roadmap for the chemtainer work for the remaining part of the MATCHIT project (M12).

D2.1.a Introduction

MATrix for CHemical IT (MATCHIT) has one over-arching goal: *to develop programmable information and production chemistry by introducing an addressable chemtainer production system and interfacing it with electronic computers via MEMS technology with regulatory feedback loops.* This interface between chemistry and traditional computers will make novel use of MEMS technology and chemical addressing via DNA. Our project involves several different types of chemtainers with desired characteristics. The chemtainers should be self-assembling, self-repairing and replicable. The chemtainers will vary in terms of scale and functionality. At the nanoscale, stoichiometrically precise DNA containers will provide a programmable chemistry in which positional information can be harnessed for a range of nanoscale utilities. At the microscale containers based on DNA-labelled heterophase droplets and vesicles, will form microscopic labeled reaction vessels, which can themselves determine their next processing steps.

A key point in MATCHIT is the use of DNA addresses to coordinate the specific assembly of chemtainers in space and time. The DNA addresses will allow computation, enabling parallel chemical and internal material production programming in a new multilevel architecture. Through autonomous DNA address modification and resolution at the container-container, container-surface, and container-molecule levels, the architecture provides a concrete embedded application for information processing, computing and material production. Self-organizing container addressing allows micro- and nanoscale processing of any collection of chemicals that can be packaged in the containers. DNA-addresses can be used to bring containers together spontaneously exploiting parallel physical self-assembly, adding necessary structure and small volume control to processes normally exploited at larger scales and limited by passive diffusion.

The desired properties of chemtainers to meet the goals of MATCHIT are quite clear. The chemtainers must be:

- self-repairing
- able to self-assemble
- able to package cargo
- able to retain stability in some environmental contexts and able to release content in another contexts
- observable and controllable in the MEMS context
- compatible with DNA addressing and DNA computing
- compatible with water, oil and ionic liquids employed in the MEMS context
- compatible with micro fluidics

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- capable of material import and export under certain conditions
- able to support material replication cycles and chemical evolution

Fortunately several such chemtainers with the desired properties exist and the MATCHIT consortium possesses the expertise in developing such chemtainers towards the project goals. In section D2.1b below, each chemtainer will be presented based on the above properties.

Chemtainers will not be passive containers hosting desired chemical reactants, the chemtainers must provide an active and programmed role in chemical IT. As in real logistics, chemical containers have to be repackaged and relabeled when an assembly or manufacturing step has been carried out. Chemtainers with recursive processing provide an IT-rich generalization of self-reproducing materials with addresses. The proposed chemical matrix is a distributed system that will be able to respond to local chemical signals and mount a chemical synthetic response by activating the transport and reactivity of specific chemical containers that lie distributed throughout the matrix. While a purely autonomous response of the chemical matrix is possible, we intend to enhance the complexity, programmability and reproducibility of the matrix response making use of microfluidics with electronic actuation and optical sensing. Fluorescent and electronic monitoring of transport and reaction progress will allow feedback processing and process optimization. High-density arrays of electrodes provide a programmable transport system for both nano- and microscale containers of different kinds (both charged and uncharged). The containers in this project will be charged, containing DNA-labeled coats. We intend to also use a gel matrix both to modulate the transport of containers and confine container interchanges.

Summary of MATCHIT objectives

1. Create several types of DNA-addressable chemical micro- and nano- chemtainers in both aqueous and organic solvents.

2. Demonstrate autonomous chemical package delivery by address matching to both floating (chemtainer-chemtainer) and external (chemtainer-location) addresses.

3. Show that recursive programmable reaction processing is possible with chemtainers.

4. Use DNA computation to implement chemtainer re-addressing.

5. Construct an electronically programmable matrix for chemtainer processing with MEMS technology.

6. Develop a computer language, physical simulation, design tools and IT architecture for the artificial subcellular matrix.

We endeavor to show that this concept can be applied to make chemical processing programmable in a broad range of chemical systems, both in aqueous solution and in hydrophobic solvents. Therefore a broad range of chemtainers is necessary to show the applicability of the novel

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MATCHIT computer-chemistry interface. However this is not the only justification for using a variety of chemtainers. An important aspect of a sustainable chemical processing involves the ability to utilize the results of one chemical container process as input to another process, between and across different chemtainer platforms. Different available chemtainers with different desirable properties are necessary to meet the goals of MATCHIT. Finally different chemtainers will be developed towards the objectives of MATCHIT simply because this new frontier will challenge our knowledge about how such chemical and electronic systems can interface. By using more than one type of chemtainers we increase our chances of success for the project. Also, as chemtainer based ICT systems open a new frontier within Chembio-IT, it is important to explore the obvious possibilities and not limit this new direction *a priory*.

This report aims to justify the use of various types and size scales of chemical containers (chemtainers) in MATCHIT. Further this report will outline feasible directions for the development of chemtainer work to reach the ambitious objectives of MATCHIT as well as hopefully provide usable input to the emerging Chembio-IT community at large.

Oil droplets

Oil droplets in aqueous fluids self-assemble, are self-repairing apart from material degradation, and are able to package hydrophobic molecules and salts. Oil droplets are highly stable in many contexts but also can dissolve when surfactants (i.e. soaps) are added, providing a means for release of content. It had also been shown that droplets can release cargo such as salts and amphiphiles by diffusion. Oil droplets can be formed over several orders of magnitude in size and therefore can be tailor-made to fit an application or mode of observation. Oil droplets can also be easily labeled with hydrophobic fluorescent dyes both for tracking and for quantification.

Collaborative experiments in the first year of MATCHIT between SDUa and WISb (WP2 and WP4, respectively) have demonstrated that oil droplets (oil in water emulsions) can be successfully labeled with DNA addresses. Oil droplets with DNA addresses are currently being tested for their ability to perform DNA computing operations, see Figure 1.

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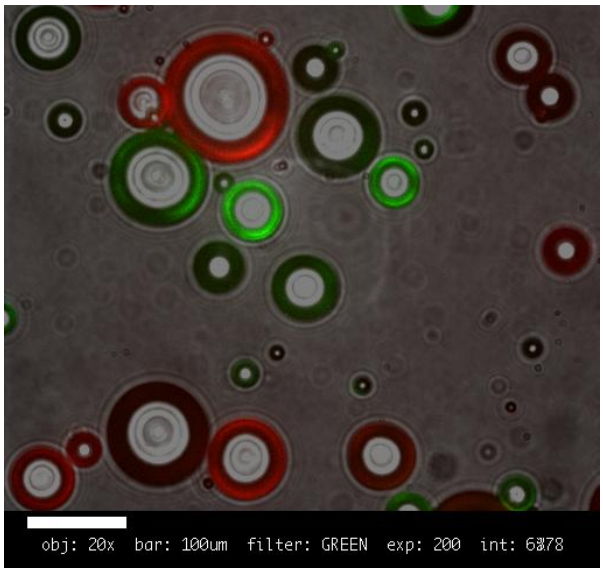

Figure 1: Oil droplets decorated with two different ssDNA addresses (with red and green fluorescence), overlayed white field and fluorescence micrographs.

Proof of concept experiments in the first year of MATCHIT at SDUa (WP2) have shown that some types of oils are compatible with various ionic liquids (IL), while other oils readily fuse with the ILs and therefore lose integrity. Conditions that support the integrity of oil droplets in the presence of ILs have been found. It has long been known that oil droplets are one of the easiest systems to make and manipulate in microfluidics.

Oil droplets are capable of material import by droplet-droplet fusion. This can be spontaneous, depending on conditions, or induced by either the droplets themselves or an external 'fusagen'. Several protocols for droplets fusion are currently working at SDUa. Export of material can be accomplished through diffusion or droplet fission. Both processes are currently working at SDUa. Within the last year SDUa (WP2) has produced an oil droplet replication cycle based on spontaneous fusion and fission events. A manuscript describing this process has been submitted for publication.

Oil droplets can be produced in MEMS, addressed with DNA tags, contain chemical components and reactions, and be monitored (i.e. by fluorescence) *in situ*. Oil droplets are fairly stable, easy and cheap to produce in great numbers, can be manipulated (e.g. motility) by external chemical and physical forces, and can grow and divide spontaneously. Therefore, oil droplets seem well suited for development and implementation in MATCHIT.

Phospholipid vesicles/liposomes

Phospholipid vesicles or liposomes can be produced by various methods and from various lipids and mixtures. Liposomes in aqueous fluids self-assemble and are self-repairing apart from material degradation. All kinds of hydrophilic substances (e.g. salts, dyes, DNA, proteins) can be easily packaged into liposomes. Hydrophobic cargo can also be integrated into the liposomal bilayer

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membrane. The complexity of the packed solutions include cell free expression systems[i], other liposomes (liposomes within liposomes) and (latex, agarose) beads. Salts are a sensitive issue. SDUb tested the influence of halogens and alkali metals on liposome formation using the W/O emulsion transfer method[ii]. For monovalent ions stability depends on character of the ion (i.e., ion size, valency, polarizability). The findings reveal a general trend in the effect of halogens and alkali metals on liposome formation. The most hydrophobic (i.e. the most chaotropic) anion affects least the vesicle formation. Interestingly, for cations the inverse series is obtained in the measurements, with the most hydrophilic (i.e. the most kosmotropic) cation affecting the liposome formation the least. This general trend exactly fits the series obtained for the influence of anions and cations on the dipole potential of PC liposomes[iii], the attraction of anions and cations to solid-supported membranes[iv], and the absorption of cations to phosphatidylserine liposomes[v]. Concerning divalent ions: Ca^{2+} affects liposome stability negatively and should not be present during liposome formation using the W/O emulsion transfer method. In addition, one has to make sure that the osmolarity of the surrounding medium is the same or higher than the medium inside the vesicles. If the surrounding medium is of lower osmolarity liposomes swell and eventually burst (burst if area expansion > 10%). On the other hand a shrinking of liposomes (if $osm_{outside} > osm_{inside}$) is not critical and results in "wobbly" liposomes that – when assembled with others – show extremely enlarged contact areas compared to assembled liposomes at $osm_{outside} = osm_{inside}$. Liposomes are not greatly affected changes in pH and protons can easily pass through the membrane. Liposomes are stable in a wide range of temperatures. Liposomes lyse when frozen, either in the presence or absence of cryopreserving media (i.e. DMSO). Liposomes become leaky at the Tm of the lipids in the membrane.

Liposomes are typically observed and analyzed by optical microscopy: bright field, dark field, phase contrast, fluorescence, confocal. Dynamic light scattering is used for size data in monodisperse polulations. . Fluorescence flow cytometry is used for both size data and fluorescence on single liposomes in a population. Therefore liposomes are easily visualized and monitored in MEMS/microfluidics using standard microscopy.

Liposomes are compatible with DNA anchoring and addressing as demonstrated by WP2 (SDUb) in the first year of MATCHIT, see reference[vi]. Biotinylated vesicles can be easily decorated with biotinylated ssDNA of every length and sequence by using streptavidin as a linking agent. The surface of a single liposome may be decorated by one or several distinct DNA tags, see Figure 2. Recent collaborative work between SDUa and WISb (WP2 and WP4) has demonstrated simple computational operations between DNA addressed liposomes.

Liposomes are compatible with microfluidics and MEMS. Initial tests regarding the compatibility of liposomes with ionic fluids are currently underway. For both the import and export of materials, membrane permeable substances (e.g. steroids) can pass through the membrane, electroporation can open temporary holes in the membrane, incorporation of pore proteins into to the membrane ensures selective permeability[i], and there exists increased membrane permeability at the phase transition temperature[vii]. Liposome-liposome fusion is used for the import of large molecules and

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assemblies[viii]. Liposome fission by extrusion or other means is used for large-scale export[ix]. Recent advances at SDUa in collaboration with Tetsuya Yomo's group at Osaka University have demonstrated the basis for a liposome based replication cycle that encapsulates chemical processes[viii].

Given that liposomes are commonly used to package chemicals, easily decorated with DNA addresses, capable of DNA computing and compatible with MEMS, they are one of the best candidate chemtainers for MATCHIT.

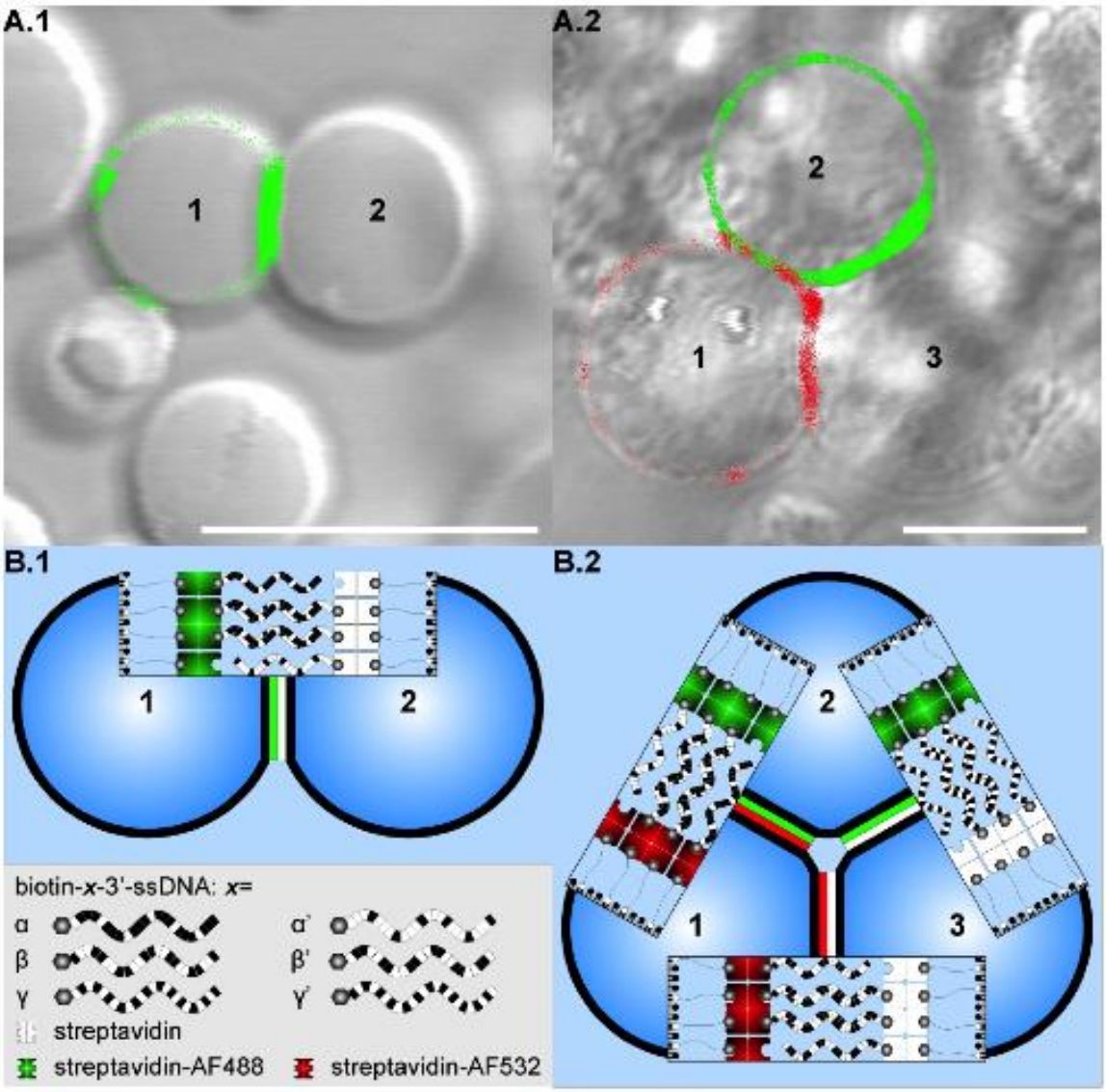

Figure 2: Programmability of the DNA-mediated liposome self-assembly process. A) Image overlays of confocal laser scanning fluorescence and differential interference contrast micrographs of merged liposome populations. (B) Schematic representation of the programmability of the DNA-mediated self-assembly process. The formation of adhesion plaques depends on the complementarity of ssDNA resulting in a sequence depend accumulation of linkers in the contact areas[vi]

Fatty acid based vesicles

Fatty acid based vesicles (FA vesicles) include vesicles made with pure fatty acids or a mix of fatty

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acids with other co-surfactants, see Figure 3. FA vesicles in aqueous fluids self-assemble and are self-repairing apart from material degradation. Many types of molecules can be packaged inside. Depending on their molecular properties, the cargo can reside in the chemtainer boundary or in the aqueous medium inside the vesicle. However, issues and limitations exist: for hydrophilic solutes, pure fatty acid vesicles are leaky even for medium sized molecules, a property that is compounded by the exposure of the vesicles to elevated temperatures[x] or high salt concentrations[xi]. Solutions, such as using co-surfactants to stabilize the vesicles exist. For hydrophobic solutes or molecules derivatized with a hydrophobic anchor (alkyl chains), the insertion into the bilayers can be easily achieved during self-assembly processes. For small amphiphile derivatized molecules, such as a single nucleobase or a ruthenium trisbipyridine, the molecules will stay very tightly bounded to the vesicles[xii].

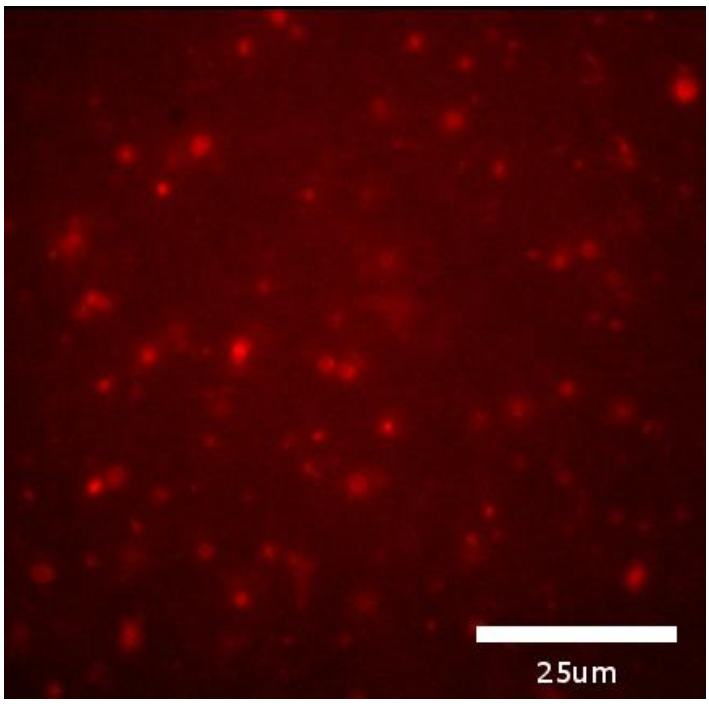

Figure 3: Micrograph showing the chemtainer, here in the form of decanoate/decanoic acid vesicles

Issues and limitations exist regarding the integrity of the vesicles: the exposure of the vesicles to elevated temperatures[xxiii] or high salt concentrations[xi] can lead to disruption or destruction of fatty acid vesicles, for example, through precipitation of fatty acid-salt complexes. Indirectly the osmolarity has been proven to have the same effects, but the degree of transmembrane osmolarity difference must be large[xivxv]. Solutions such as using co-surfactants to stabilize the vesicles exist.

pH will disrupt FA vesicles. For pure fatty acids the pH range of stability is usually 1 pH unit around the pK_a. But this can be extended using mixtures of fatty acids and cosurfactants[xvi].

A typical vesicle size distribution allows for observation and quantitation by dynamic light scattering (DLS), microscopy and fluorescence. Experiments at SDUa have shown that FA vesicles can be labeled with DNA addresses. This system will be tested for ability to perform DNA

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computing operations. Compatibility of FA vesicles with ILs is being tested. In general vesicle systems are compatible with microfluidics. However it must be noted that previously fatty acid systems have been shown to coat electrodes in MEMS and therefore interfere with the electronic control. Proper coatings and conditions need to be found to obviate this technical problem.

Materials can be exported and imported in the FA vesicle system. Small molecules can passively diffuse in and out of the vesicles. Vesicle-vesicle interactions can lead to fusion. Budding of small containers from large vesicles are postulated but not yet proven. Unlike the phospholipid liposome systems presented above, the FA vesicle systems are highly dynamic and therefore capable of being manipulated by external perturbations. Because of this property, the FA vesicle system has been shown to be capable of a replication cycle by feeding and subsequent extrusion[xvii].

At SDUa work continues on the fatty acid based containers. There are several advantages in using FA vesicles for MATCHIT: i) The production of their building blocks *in situ* can be easily achieved using one-step catalysis. ii) The structures themselves are more dynamic than phospholipids and therefore can be easily perturbed. iii) They can contain DNA tags and are (or can easily be made) compatible with the conditions required for the catalysis of DNA ligation, cleavage and other DNA alterations. The only difficulty we could face with them is the relatively high CVC compared to phospholipids. Considering the current envisioned electrodes used in the microfluidics, there will be an issue to be resolved: The accumulation of amphiphiles and perhaps medium chained ILs on the electrodes that may impair function of the MEMS device.

Reverse micelles

Reverse micelles in organic fluids self-assemble and are self-repairing apart from material degradation. Many types of hydrophilic molecules can be packaged inside. These two phase systems offer several phases in which different types of molecules can be distributed. SDUa has observed that the crowding of the aqueous phase, the smaller phase, can prevent the correct chemical reaction from occurring as water within the very small reverse-micelles starts to behave as immobilized solute[xviii]. Interestingly, apolar solutes can be dissolved into the apolar phase and still serve as "substrates" for catalytic systems present in the aqueous phase. The formation of reverse-micelles composed of fatty acids can be observed in a larger range of pH than FA vesicles (above). The pH however will affect the distribution of fatty acids between the interface and the apolar medium, thus low pH, i.e. below the pKa of the acid, could lead over time to a destabilization of the structures. Reverse-micelles are usually observed by DLS, fluorescence, and NMR.

Reverse-micelles could be dependent on the ratio of DNA size to aggregate size. During PNPase-catalysed RNA polymerization carried out in a AOT system, it was found that the RNA product (very long polymers in excess of few hundred of nucleobases) was expelled from the aqueous phase and precipitated in the apolar phase[9]. However, the water pool size can be optimized to contain short nucleic acid tags. Reverse-micelles can contain DNA but obviously cannot be decorated with it in the usual meaning of the word. However, they are very similar to water droplets in IL, thus the idea that proposed in WP5 should also apply to reverse-micelles. They could be even better: In a

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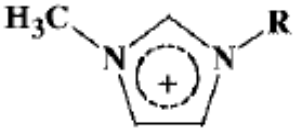
water droplet, the DNA will be floating around. In a reverse-micelle, DNA could be confined to the interface by anchoring it. The anchoring of some molecules into the interface can lead to changes in the activity of a catalytic assembly as shown by our results.



$CH_3CH_2CH_2CH_2 = [C_4mim]^+$
$CH_3CH_2CH_2CH_2CH_2CH_2 = [C_6mim]^+$
$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2 = [C_8mim]^+$

Figure 4: Formula of 1-alkyl-3-methyl imidazolium cations[xix].

Compatibility of reverse micelles with ionic liquids (IL) is being tested currently. We can make reverse-micelles using IL as apolar phase (replacing the usual organic solvent). This results in new structures that should be called water-in-IL emulsion. As literature precedents have established, reverse-micelles in IL are more stable than those in usual organic phase allowing for the formation of larger stable structures: AOT in isooctane/octanol 9:1 can have a stable size of 30 nm in diameter whereas sizes of 180 nm can be reached with the same surfactant in IL such as 1-octyl-3-methyl imidazolium bis(trifluromethyl sulfonyl) amide [C8MIM][Tf2N] with 1-hexanol present[xx]. The type of IL used seems to play a major role in the outcome of the self-assembly, both at the level of the hydrophobic chain length and the identity of the counter ion. The hydrocarbon chain on N1 can easily be varied, depending on the requirements, see Figure 4. For example, the short chain system [1-butyl-3-methyl imidazolium bis(trifluromethyl sulfonyl) amide [C4MIM][Tf2N] (shorter chains than butyl neither) will not form stable emulsion whereas the medium-chain length ones [1-octyl-3-methyl imidazolium bis(trifluromethyl sulfonyl)amide [C8MIM][Tf2N] or 1-decyl-3-methyl imidazolium bis(trifluromethyl sulfonyl) amide] [C10MIM][Tf2N] will. The role of the counter ion can be seen in the different behavior of [1-octyl-3-methyl imidazolium chloride] versus [1-octyl-3-methyl imidazolium bis(trifluromethyl sulfonyl)amide][xx], the first is miscible with water whereas the latter is not.

In general, reverse micelles are compatible with microfluidics. However, in MEMS with electronic control, the same concerns with electrode coating as noted with FA vesicles (above) is present here. Considering the reverse-micelles and medium chain length IL, how does the IL interfere with the function of the electrodes used at RUBa?

For material import and export, addition of nanodroplets[xxi], increasing the total concentration of reverse-micelles will increase the number of collision between the structures, thus the possibility of content exchange[xxii]. In addition, relatively low polarity molecules can be added to the organic phase and will equilibrate within this phase. In the process, they will come in contact with the interfaces to the water phase and can then be processed by a catalyst residing in the aqueous phase. Obviously, the knowledge accumulated in terms of solubility of compounds in the proposed IL phase that will replace the apolar phase (oil, organic solvent) of usual water-in-oil emulsion will have to be assessed on the case to case basis. Some reports already indicate that enzymatic reactions

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can be carried out more efficiently in water-in-IL emulsions[xxiii].

The main advantage of reverse micelles over vesicles are the physical properties that will govern growth and division cycles. The determining factors are the interface surface area, and the constant water volume. The ratio between interface area and volume in reverse micelles (assuming division of the internal volume by two) is theoretically described by the following equations:

$$\begin{aligned} V_1 &= 2^{-1}V_0 + 2^{-1}V_0 = V_0 \\ A_1 &= 2^{-\frac{2}{3}}A_0 + 2^{-\frac{2}{3}}A_0 \approx 1.26A_0 \end{aligned}$$

From these equations, it can be inferred that if the internal metabolic amphiphile production increases the number of amphiphiles by 26%, a "spontaneous" division of the system into containers of equal volume measuring half of the original one, would increase the surface area proportionally. Some experimental data already exist for simple surfactant production systems (hydrolysis of ester[xxiv], oxidation of alcohol into acids[xxv]).

The reverse micelle system used in MATCHIT has several advantages: i) The production of building blocks *in situ* can be easily achieved using one-step catalysis. ii) The structures themselves are more dynamic than phospholipids. iii) They can contain DNA tags and are (or can easily be made) compatible with the conditions required for the catalysis of DNA ligation, cleavage and other DNA alterations. The only difficulty we could face with them is the relatively high concentration of amphiphiles needed to stabilize the reverse-micelles could be compromised if interactions between the electrodes and the fatty acids leading to amphiphile sequestration were to occur.

Water-/Hydrogel droplets in ionic liquids

Micro and macro-scale water and hydrogel (W/H) droplets self-assemble easily in ILs and able to self-repair because of their surface tension properties. W/H droplets can be generated in a T-junction or in a flow-focusing channel.[xxvixxviixxviii] All water-soluble material can be encapsulated inside as well as hydrogel gel beads, DNA-tagged silica or polymer-beads, nanoparticles, micelles and vesicles. RUBa has noted that hydrophobic oil droplets as well as reverse micelles and vesicles lead to interaction with the hydrophobic carrier fluid, which may disrupt the W/H droplets. Specific compatibility studies need to be performed. W/H droplets are very stable. High salt concentrations are possible with no leakage of the water-soluble material into the carrier fluid (ionic liquids). They are also pH stable. It is noted that certain detergents in ILs can destabilize the W/H droplets.

W/H droplets are easily monitored by optical and fluorescence microscopy and confocal microscopy see Figure 5.

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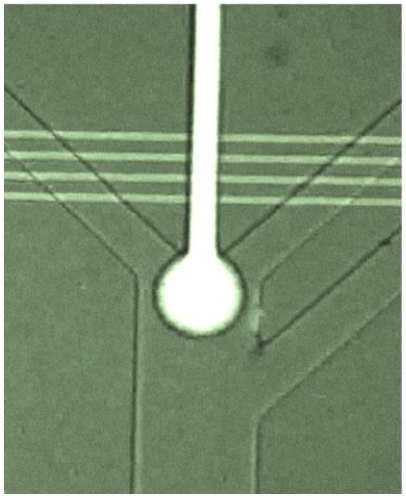

carrier fluid:

Butylmethylpyrrolidinium bis
(trifluoromethylsulfonyl)imide
[MeBuPyrr][BTA]

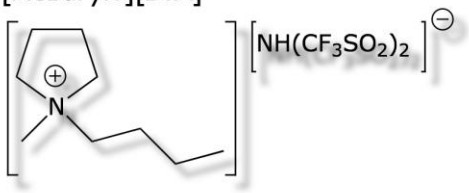

Figure 5: Droplet generation in a flow-focussing microfluidic device. Fluorescence detection of a fluorescent labeled DNA probe. Source: BioMIP.

The W/H droplet system is highly compatible with DNA oligos and DNA computing. The main idea in MATCHIT was to physically localize droplets at surface sites containing immobilized DNA complementary to DNA contained in the droplet. The surface site clearly needs to be hydrophilic to achieve this, and one strategy proposed to enhance the specific interaction was to have the DNA in the droplet attached to hydrophilic beads. Once hybridization takes place, one expects that the beads will not want to leave the droplet, so the droplet will stick to that site. An intermediate step shows the binding of DNA-labelled beads to surfaces[xxix].One way to achieve long processing times required with droplets for MATCHIT is by using the concept of trapped or parked droplets[xxx] . This can be combined with the MATCHIT- meander concept to allow content release to gel based interconnecting channels. Main steps are: 1) pattern surfaces with DNA (e.g. via thiol-groups) 2) show specific bead binding 3) use these beads in W/H droplets to show DNA addressed droplet processing.

Obviously the W/H system relies on ILs as one phase and therefore is compatible. It is also compatible with microfluidics, see Figure 6. Biomolecules can be exported and imported from aqueous to the gel phase as well as the realization of move, mix and split operations within the Chemical Microprocessor system developed by RUBa. Ongoing activities in WP5 deal with the implementation of this technology using digital droplet chains.

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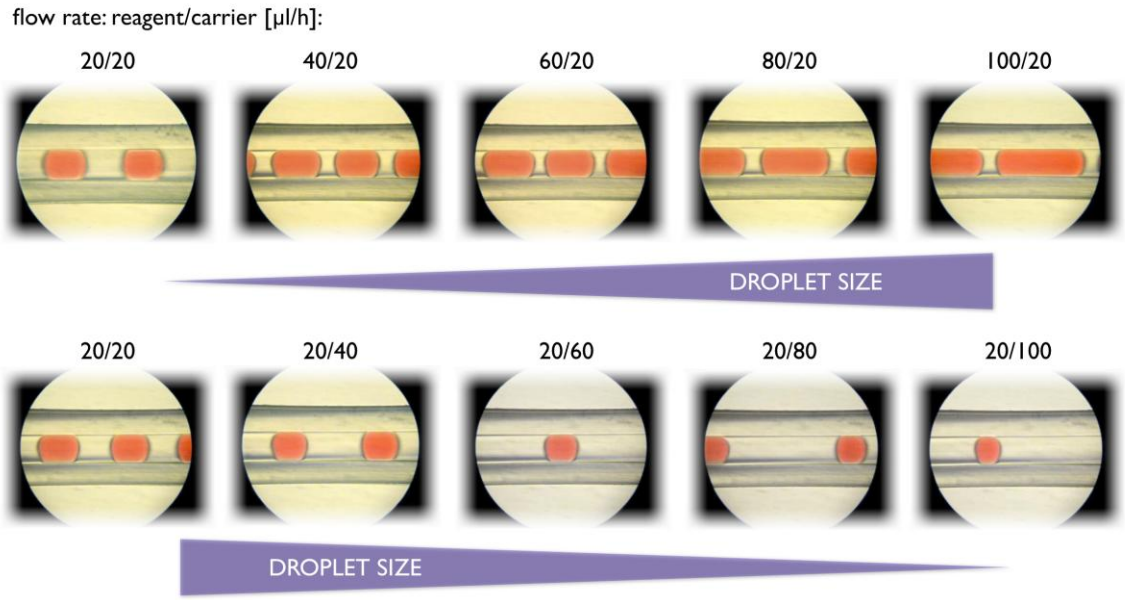

Figure 6: Droplet size modulation in a microfluidic channel by changing the relation of flow rates of the reagent droplets and the carrier solution (IL carrier fluid: Butylmethylpyrrolidinium bis(trifluoromethylsulfonyl)imide.) Source: BioMIP

A replication cycle controlled by MEMS can be implemented, WP5. Fission, fusion and sorting of generated droplets can achieved using e,g, T-junctions or obstacles installed in the channels[xxxi] as well as electrical control by methods such as dielectrophoresis and electrowetting [xxxii]. The control of droplet formation is the requirement for cycles (using the slow reaction processing tracks = hydrogel filled electro osmotic flow channels) in the proposed programmable matrix, see Figure 7.

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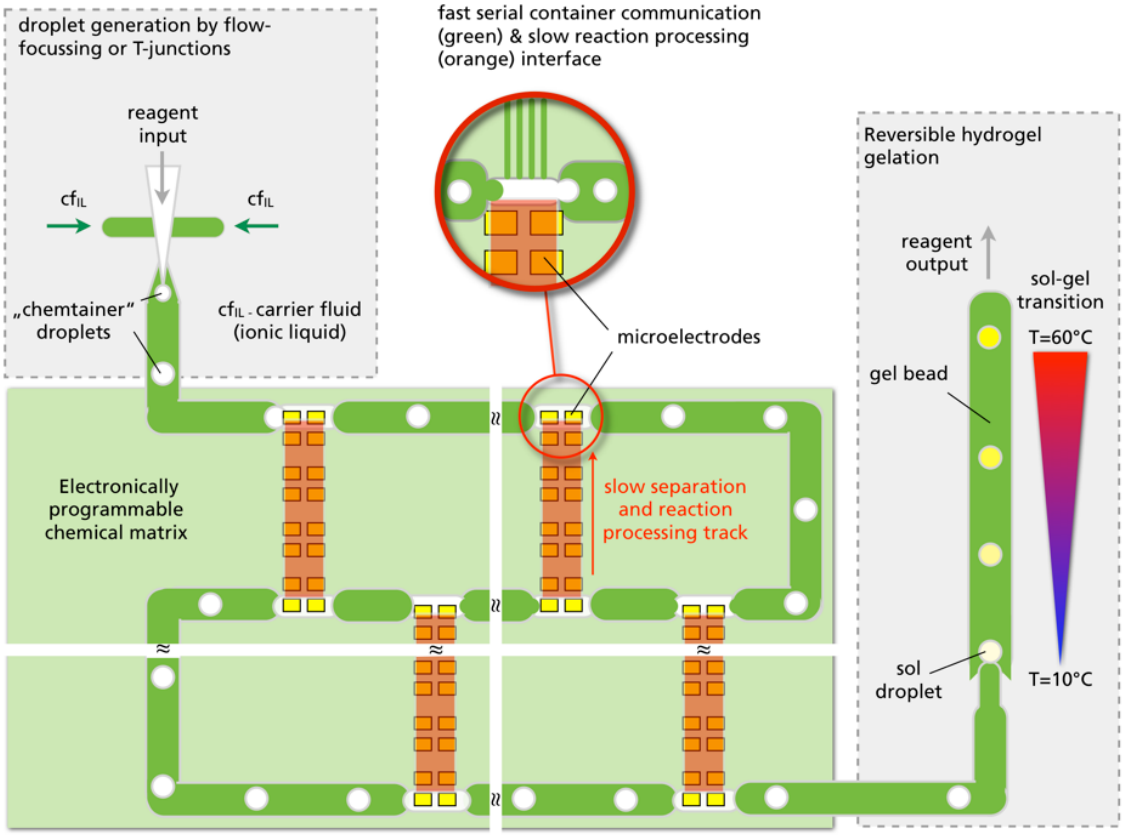

Figure 7: The MATCHIT idea implemented in MEMS.

Monodisperse W/H droplets compartmentalized in an IL phase are well suited for continuous packaging and transport of chemicals and hydrogel beads can easily immobilize with DNA tags. The formation and further processing of digital droplets is compatible with microfluidics. Considering surface-induced droplet fusion in microfluidic devices, droplet-chemtainers are suitable to initialize replication systems. Aqueous droplets, containing DNA oligomers immobilized to microbeads, when flowing through a microchannel can interact with immobilized DNA on the channel walls. The DNA-DNA hybridization process can lead to a sequence specific retention of the droplet. Gel beads labelled with DNA can also serve as an alternative container form. If we are successful in demonstrating the DNA-addressed location of these containers (e.g. using the bead-based breaking system described above) then it appears that the chemtainers present at least one of the most suitable candidates for MATCHIT chemtainers.

Electronic controlled diffusion (ECD): containers without walls

Electric fields stemming from arrays of microelectrodes can concentrate molecules in microfluidic devices using a combination of electrophoretic and potentially electroosmotic driving forces. Such ECD containers without walls can be enhanced by gelation of the entire solution and by channel networks with narrow-neck openings to further limit diffusion. The challenge is to label such containers so that their processing is DNA dependent. This is of course possible via the chemical

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microprocessor technology, provided there is a (typically optical) sensing of the presence of certain DNA sequences (e.g. fluorescent hybridization assay), see Figure 8. Then the electronics establishes the relationship between containment and the presence of certain DNA sequences.

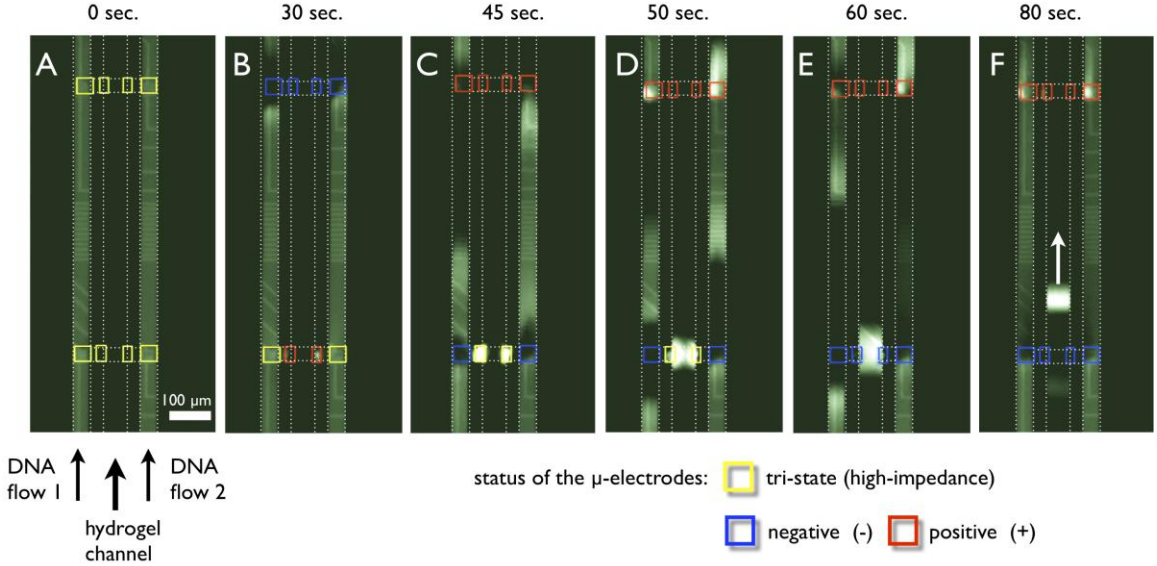

Figure 8: Electronic controlled diffusion in hydrogel channels: the fluorescence image shows a broad (60 µm) hydrogel channel supported by two continuous flow microchannels. They are connected through very thin (1-2µm) support channels. The injection from the liquid to the gelated phase starts with the concentration of the ssDNA molecules at the electrodes in the support channels by electroosmotic flow (pictures B and C) following by a subsequent formation of an molecule plug (D) inside the middle hydrogel channel. Finally the plug of material is ready for further transport or (F) as well as separation processes.

A more autonomous, DNA-based relationship can also be envisaged. In open-diffusion gels, DNA-sequence specific mobility can be achieved by immobilization of specific-sequence DNA within the gels, either covalently or by reversible hydrophobic association (as demonstrated recently in triblock copolymer gels (PEO/PPO) by RUBa in collaboration with A. Hermann, Univ. Groningen (ECCell Project). In this way, specific combinations of DNA can be processed, but the creation of transitive processing of other chemicals through a DNA tagged "open" container, would require a mechanism for DNA induced electric barriers to be created. Such sweeping effects, in which (typically long) polyelectrolyte DNA are used to sweep other lower molecular weight chemicals electrophoretically through a solution are possible, but probably difficult to control for arbitrary chemical mixtures.

The DNA dodecahedron

The DNA dodecahedron structure forms spontaneously under the right conditions, and is self-repairing according to hydrogen bonding. The dodecahedron can be used to insert objects of a definite size. We estimate the surface area of the dodecahedron as 512 nm^2 and the volume is about 950 nm^3. The size of each window is therefore about 43nm. Consequently this chemtainer is limited to a size-matched freight. This could, for example, be utilized for separation of nanoparticles.

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Due to the polyanionic nature of the DNA, the charge of an entrapped object can affect the interactions of cargo and chemtainer. Strong interactions of positive or negative charges could therefore lead to a sticking on the surface or to repulsion from the DNA structure. Affinity between the insertable object and the dodecahedron will be introduced as fluorophilicity via fluorous tags covalently bound to DNA and the packaged object.

Environmental parameters (such as salinity, pH and temperature) strongly influence the DNA base-pairing and consequently the stability of the DNA nanostructure. The insertion of photolinkers would additionally lead to a volitional photosensitivity. The DNA dodecahedron is observed and quantitated by gel electrophoresis, AFM and fluorescence- or gold-labeling. This labeling is achieved by hybridization of modified oligonucleotides to overhanging sequences. Because the DNA dodecahedron is composed of DNA and can contain overhanging sequences, it is perfectly suitable for DNA addressing and DNA computing. The dodecahedron as produced at RUBb (WP1) can easily be decorated with addresses via synthesis of trisoligonucleotides with overhanging sequences[xxxiiixxxiv], see Figure 9.

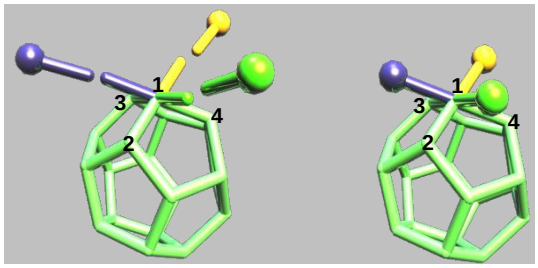

Figure 9: DNA docecahedron with overhangs on top to support several different DNA addresses.

The dodecahedron with trisoligo constructs should be as compatible with ILs as normal DNA. It is also easily compatible with microfluidics. For material import and export, the opening of the chemtainer can be achieved by strand displacement or by photocleavage. Fluorous modified macromolecules like polymers or peptides could also be inserted. The DNA dodecahedron system itself is feasible for a chemtainer replication cycle based on the chemical copying of connectivity (CCC)[xxxv] coupled to surface promoted replication and exponential amplification of DNA analogues (SPREAD).[xxxvi] Amplifying nanoobjects by spreading connectivity information is perfectly compatible with microelectrofluidic design.[xxxvii]

Overall the DNA dodecahedron is a programmable, addressable chemtainer that can easily interface with MEMS and is an appropriate chemtainer to use in MATCHIT.

Theoretical and computational chemtainers

MATCHIT has a strong theory and computation contingent (WISa, ECLT, RUBa and SDUa [WP6]). Theoretical and computational models can provide generic insights into the low level physicochemical properties of chemtainers, systemic properties at the chembio-IT level as well as

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compiler/design tool requirements at the computer science level. In this section we have selected some of the MATCHIT consortium's computational and theoretical chemtainer and MEMS chemtainer interaction models.

At the molecular level we find chemtainer self-assembly, cargo loading and release, chemtainer fission and fusion as well as information molecular dynamics such as DNA relabeling and DNA computing. At the systemic level we can study the emergent dynamics of chemtainer interactions coupled to a MEMS environment with control feed-back loops with the goal of understanding the kind of chemical reactions and the ICT-coupling that can be realized within this context. The chemtainer matrix compiler and design tools are of a different kind and will be discussed later.

The chemtainers considered by MATCHIT open at least two obvious routes of investigation: (A) coarse-grained physics based chemtainer models (SDUa and RUBa), (B) continuous mesoscale models and (C) coarse-grained systemic MATCHIT models (WISa, ECLT, SDUa and RUBa). Their applicability is determined primarily by their respective length (and time) scale. We will discuss these three routes in more detail below.

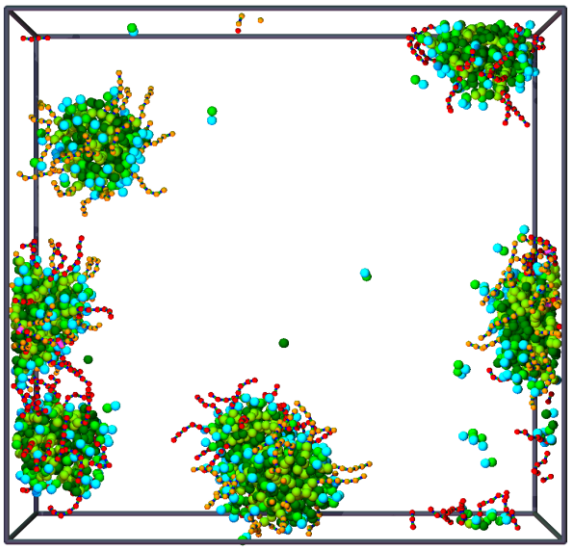

Figure 10: Dissipative Particle Dynamics simulation1 of freely floating surfactant covered oil droplets. The droplets are labelled with complimentary ssDNA labels tethered to the droplets by an anchor. At the lower left two DNA labels show magenta hybridization bonds. Periodic boundary conditions apply. Legend: surfactants (cyan-green beads), oil (dark green beads), DNA bases (red,yellow beads), and anchors (light green beads).

(A) In a coarse-grained physics based model, the solvent and all the molecules comprising the chemtainer are represented as beads and bead-spring chains, respectively. Such a model removes atomistic details in favor of a much coarser description that approximately captures the essential physical and chemical properties of the molecules such as hydrophobicity, hydrophilicity and DNA base-paring properties. When such a model is endowed with Brownian Dynamics (BD)[xxxviii] or

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Dissipative Particle Dynamics (DPD)[xxxix], we can use it to study the dynamic properties of chemtainers, see e.g. Figure 10 from SDUa. Such an approach allows simulations of systems on scales from nanometers up to micrometers to be simulated. They are thus ideally suited for simulating nano-droplets, reverse micelles, and water/hydrogel droplets in ILs, all of which are comprised of a moderate number of molecules and are dominated by short range interactions. Similarly, DNA dodecahedra can be well captured by coarse-grained particle methods.

(B) Chemtainers comprising a large number of molecules (e.g. vesicles) or where the dynamics are strongly influenced by long-range interactions (electrostatic fields) are computationally very demanding for particle-based methods. Therefore, those chemtainer types are best described in a field theoretical context. The surface energy of a vesicle is for instance best described by the Helfrich hamiltonian[xl], from which the equations of motion of the vesicle can be derived, and solved analytically or numerically. A particular challenge to field theoretical descriptions is to capture the information molecule dynamics.

(C) In the very coarse-grained systemic MATCHIT models, the entire chemtainer is represented as a single object characterised by a number of properties. Chemtainer-chemtainer interactions are described by effective emergent interactions, and the dynamics is dictated e.g. by stochastic transition rules. At this level we can study the emergent properties of the entire MATCHIT system, but we have lost the connection to the dynamics of the molecular constituents.

Some research into 2D representation of very coarse-grained systemic MATCHIT models has already been completed as part of a master thesis by ECLT, see Figure 11. In this study the simulation of a hypothetical multi chemtainer reactor is considered. The chemical processes in this reactor are controlled by the spatially heterogeneous arrangement of chemtainers and by their chemical functionalities. The assembly of the reactor is obtained by a self-assembly process of single compartments mediated by selective linkers. Stochastic simulations of the self-assembly of the reactor and an artificial polymerization reaction within the chemtainers are used to predict the potential of this concept. In this study we were able to show that in theory, complex polymerization reactions leading to branched structures can be programmed by defining the properties (specific-linkers, content and chemical functionality) of the different chemtainer types that constitute the reactor. With a predefined reactor, the production of specific types of branched polymers was increased up to 2000 times compared to random polymerization. Furthermore, we were able to show that such a reactor can self-assembly spontaneously. Even more, an increase of performance of the proposed reactors was observed in simulations of the production of polymers with increased complexity. This supports the use of several different types of chemtainer system as long they support the DNA computing platform.

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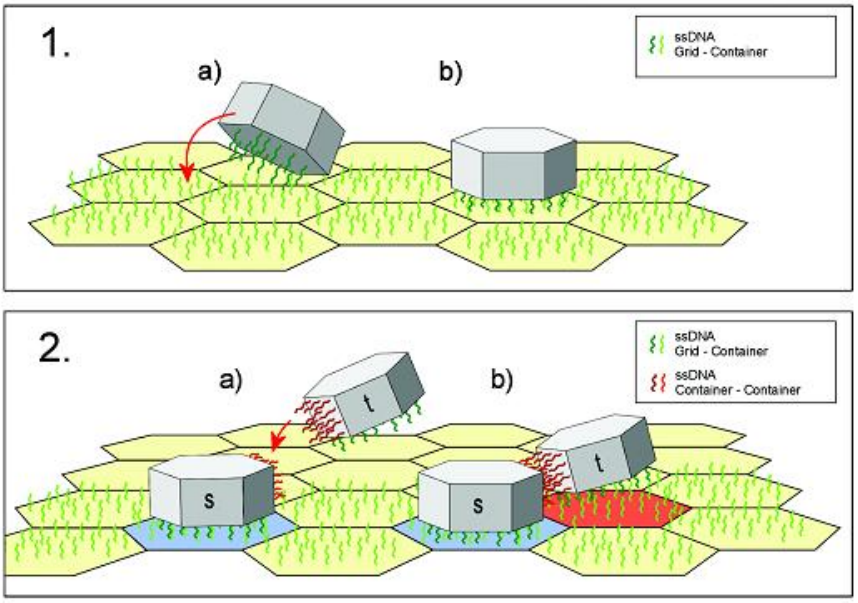

Figure 11: Self-assembly of the 2D grid of containers via specific linker molecules (ssDNA). The upper part (1) shows the association of a container to the grid, (2) illustrates the specific association between two containers (s, t) that leads to a nonrandom arrangement of the containers on the grid.

MATCHIT systems compilers to address the design and systems level programming issues as well as systemic systems level simulations are also being developed within the consortium. For details see the WP6 progress report.

D2.1c Prospective directions for chemtainer work with regard to fulfilling MATCHIT goals

The MATCHIT consortium consists of expertise on different types of chemtainers - all of which are suitable for the application to MATCHIT goals. We endeavor to show that the technology developed can be applied to make chemical processing programmable in a broad range of chemical systems, both in aqueous solution and in hydrophobic solvents. A broad range of chemtainers is necessary to show the applicability of the novel MATCHIT computer-chemistry interface. An important aspect of a sustainable chemical processing involves the ability to utilize the results of

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one chemical container process as input to another process, between and across different chemtainer platforms. Different available chemtainers with different desirable properties are necessary to meet the goals of MATCHIT. Therefore our efforts will not focus on one type of chemtainer but on three unifying aspects of MATCHIT: (i) the interface of chemtainers with ILs, (ii) functionality of chemtainer in the MEMS platform (iii) computation via DNA addresses. This clarifies immediate goals for each work package.

Table 1. Compatibility summary

	Oil Droplets	Liposomes	Fatty acid Vesicles	Reverse Micelles	W/H droplets	ECD	Dodecahedron
Compatibility with:							
Ionic liquids	Partial	Partial	Unknown	Yes	Yes	Unknown	Yes
MEMS	Partial	Partial	Partial	Unknown	Yes	Yes	Yes
DNA computing	In progress	Yes	Unknown	Unknown	Unknown	Unknown	Yes

Table 1 summarizes the main concerns for using each detailed chemtainer in the MATCHIT project. Each chemtainer satisfies most of the criteria for MATCHIT, and only those criteria where work is needed are represented in Table 1 for clarity.

For all chemtainers that consist of surfactants (oil droplets, vesicles, liposomes, reverse micelles), compatibility tests with both ILs (i) and MEMS electronics (ii) must be done immediately. In practice chemtainers such as oil droplets, liposomes, vesicles, micelles and dodecahedrons, when interfaced with the MATCHIT MEMS system, will be packaged inside of water droplets formed in the ILs. Therefore, for all of these chemtainers the compatibility tests with both MEMS and ILs have already been started with some utilizable conditions found, as reported for specific workpackages in the M12 report.

For MEMS and IL compatibility, it is clear the DNA dodecahedrons are immediately applicable to the current platform. DNA dodecahedrons are also easily compatible with DNA computing. The cargo compatibility is limited to a size matched freight yielding new opportunities to separate nanoparticles. The DNA dodecahedron can be defined as a container and a scaffold with high addressability based on sequence specific hybridization and affinity interactions such as fluorophilicity or lipophilcity.

For all other chemtainers, initial steps to interface the chemtainer with DNA computing must be executed. Since MATCHIT proposes the novel use of chemtainers for IT, the development of chemtainers to this end is still very much in progress. Initial proof of concept integration of chemtainer and DNA addressing/computation for oil droplets and liposomes will be presented at the first review meeting (M12).

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Finally, different chemtainers are being developed towards the objectives of MATCHIT simply because this new frontier will challenge our knowledge about how such chemical and electronic systems can interface. By using more than one type of chemtainers we increase our chances of success for the project.

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