PROJECT FINAL REPORT

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Executive Summary

The aim of the project was to establish a novel basis for future adaptive embedded information technology at the molecular level by constructing the first electronically programmable chemical cells (ECCells). The novel chemical microprocessor technology required to do this has also provided a programmable real-time interface to control other complex chemical information systems. Chemical cells are microscopically contained synthetic chemical systems in which the reactions occurring are directed by informational molecules and self-sustaining as in living cells. They should combine the three core

architectural features of living cells: a hereditary information system, a containment system and a metabolic system that produces necessary energized components. Electronic chemical cells interface a microscopic self-regulating electronic subsystem with each microscopic chemical system via microelectrode arrays, with the impact that digital electronic information, in addition to genetic molecular information, can control them.

Extensions of DNA chemistry have allowed its function without enzymes and in containment and metabolic capacities: thereby simplifying the chemical integration process for constructing cells. The project has developed novel redox-active and pH sensitive replication chemistry based on sulfhydryl ligation and on ion-sensitive DNAzymes. Secondly, it has developed novel DNA-block copolymer hybrid molecules that self-assemble into containment structures, obviating the need for lipid membranes for ECCells. Some of these have surfactant properties like lipids, others reversibly control molecular mobility by modulating the attachment of specific DNA to polymer gel supports. Thirdly, the project has developed electrochemical subsystems that allow the reversible uptake and release of H+, metal cations as well as small metabolites and has shown how electrical control of processes involving DNA can thereby be achieved. As "hardware" the project has developed the electronic microfluidic chips and their chemical, optical and electronic interfaces and the integrated workstation platform. Each of the three chemical subsystems has separately been integrated into the microscopic electronic chemical cell processing system, and tested there using fluorescence imaging to feedback information to the electronic system. Three levels of electrode array integration were investigated to support increasingly fine grained ECCells: single layer electrode arrays, dual layer electrode arrays and active matrix electrode arrays based on LCOS displays, with the former two levels reaching fruition in the project.

The project also developed novel simulation and control software. The ECCell project has investigated multiscale particle simulations linking molecular properties with reaction-transport and self-assembly of critical subsystems and the overall cell-cycle, it has developed an efficient general purpose simulator for the integrated nonlinear electrochemical transport equations and it has integrated a particle tracer simulator with the experimental autonomous control system software, to allow an interpretation of online imaging data streams arising during the operation of electronic chemical cells. Finally, a general purpose local feedback control system has been implemented that will in future allow the complete integration of all chemical subsystems into electronic chemical cells. Ultimately, the ability of the chemical systems to synthesize information-rich components will also allow the electronic subsystems themselves to be repaired and copied, enabling the true integration of electronics production and their deployment as embedded systems. The ethical and social implications of this have also been investigated as part of a systematic policy of responsible engagement.

An international team from Germany (2), Israel, The Netherlands, Denmark, and Italy has pioneered this development, publishing widely in peer reviewed journals and contributing to a series of ongoing projects and coordination actions for the chem/bio ICT area. The work has been covered in press reports and is the subject of summer schools and ongoing dissemination. It was cooperatively and efficiently managed by the Ruhr University Bochum.

ECCell project context and objectives

The aim of the project has been to establish a novel basis for future adaptive embedded information technology at the molecular level by constructing the first electronically programmable chemical cells (ECCell). These ECCells will function through the interplay of chemical microprocessors and information molecule chemistry. Chemical microprocessors act as coprocessors coupled to chemical information systems through a digital electronically programmable microelectrode MEMS[1] interface, taking advantage of integrated electronics and microfluidics. Information processing in molecular systems is not in direct competition with silicon technology, but the long-term goal is to integrate information processing with self-contained molecular construction of

information processing materials and components. ECCells will provide a paradigmatic proof of principle that such technology is possible and already useful in nano- and microscale embedded system applications. The novel chemical microprocessor technology required to do this will also provide a programmable real-time interface to control other complex chemical information systems. This is naturally a high-risk, embryonic research project, but based on solid interdisciplinary research and aimed at a breakthrough which will lay the foundation of a new embedded IT for immersed micro- and nanoscale molecular information processing, with a paradigm shift to digitally programmable chemical systems.

We first clarify the conceptual ICT objectives briefly, before we turn to the more technical system objectives. A more detailed analysis of the overall ICT vision and applications can be found in the Deliverable 6.2. The core vision is the value of programmable molecular synthesis "hardware" for molecular scale adaptive ICT. As in natural cellular processing, building the computer needs to be part of the computation for ongoing adaptability. Moreover, as the information content of the world of artefacts that surround us increases – whether in health, energy, food, the environment or education, entertainment or security – the need for local information-instructed synthesis of smart materials from raw materials will increase. In addition to powerful centralized abstract information processors, the dominant mode of ICT activity will shift to ubiquitous smart devices interacting with materials (and organisms like us), and these need to be constructed and deployed intelligently without filling the environment with otherwise useless construction and delivery machinery.

Natural cells achieve local complex synthesis, although the protein-based construction machinery provides strong constraints on chemical compatibility. The main consideration that distinguishes ECCell from other molecular computing conceptions is that of the value of a microscopic closure of the construction-information processing loop. This is not feasible without collective self-organization: the collective interactions between molecules suffice to create positioning constraints and functional material properties for their operation as material information processing devices. Von Neumann's self-reproducing automata and Drexler's nanotech assemblers, in contrast with Turing's morphogenesis, failed to appreciate the importance of collective self-assembly in bridging the information gap to self-construction. Building detailed programmable molecular nanoscaffolds is now possible with DNA self-assembly, and partners in this project have contributed to this development, but not the assembly of prefabricated molecules but the synthesis of informational molecules is the main focus of ECCell.

Finally, non-biological autonomous chemical self-organization is currently limited in information content, and to bootstrap this technology we need a powerful and direct interface to electronic information processing. ECCell will build a prototype electronic-chemical hybrid material and information-processing computer, which will guide the way to this future ICT technology. Electronic genomes associated with each cell will be interpreted as microelectrode control algorithms, providing a major boost to the functional information content of the cells. The project will demonstrate the power of this technology by establishing the first technical artificial cells – electronic chemical cells.

The overall conceptual ICT objectives are thus to:

I. Demonstrate that electronic chemical cells can form a nucleus for a paradigm shift to reflexive embedded adaptive information processing modules for smart material processing
II. Establish how chemical self-organization can be integrated with digital signal processing without building biological cells to create powerful information processing devices for nanoscale synthesis
III. Establish an effective programming language linking combinatorial feedback control to chemical self-organization adaptively to produce desired functionality reproducibly in complex material systems

Cell like molecular information processing requires programmed self-assembly, selective transport (separation) and reactions of informational molecules. We maintain that all three can be programmed by digital electric fields and hence interfaced directly with existing computer technology. In the past, the complexities of different chemistries and replication of basic cell functions have posed a major obstacle to a breakthrough molecular processing technology via artificial cells. We shall employ novel families of synthetic copolymers linked covalently to a novel form of reversibly ligatable DNA (scpDNA) that simultaneously support the three basic cell functionalities: self-

replication, self-containment and self-sustenance, to overcome this roadblock. The scpDNA will be assembled from subsequences using chemically modified reversible backbone linkages based on disulphide or other redox-active and hence electrically programmable bonds. This assembly process can be directed as in template chemistry using the information in already assembled entities, enabling self-replication and ultimately also component sequence evolution.

Whereas DNA alone supports specific-sequence self-assembly, scpDNA also supports collective physical aggregate phases (and transitions between them) that are operationally more powerful for directing chemical synthesis. Thus these molecules simplify the task of chemical integration, allowing templated replication, self-assembling containers and self-sustaining coupling with an energy source under electric field control. The novel replicable hybrid molecules can be equipped with redox active chemical links, enabling catalytic electrochemical control, and with tunably hydrophobic regions, as in triblock copolymers, allowing them to self-assemble into chemical containers such as micelles, vesicles and hydrogels. A single family of multi-functional replicable molecules thus replaces the complex biotriad (lipids, proteins and nucleic acids), with membranes complemented or replaced by gel boundaries. The proposal is directed at novel self-organizing IT technology, not at the origin of life, so that considerations of natural synthesis routes, or the historical route to autonomous boot strapping of complexity, are not relevant.

Microfluidic-electronic devices allow multi-electrode structures at the same scale as cells, so that individual control of emergent spatial structures is achievable. Our electronic chemical cells will exploit electronic chips as coprocessors and memories for genetic information. Local molecular concentrations can be changed by digital voltages (1-5 V) that induce directed transport (electrophoresis and electroosmosis), guiding molecular self-assembly and inducing phase transitions and hence controlling the mobility, separation and transport of molecules. scpDNA will autonomously contribute to selective containment of different polyelectrolytes by supporting reversible hydrogel structures inside MEMS channels. Secondly, electrochemical control (via electrodes) of key redox reactions for chemical synthesis will provide a direct microscopic local control of the energy flow and reactions in the electronic cell. Redox reactions provide a universal currency for regulating chemistry coupled to an external energy source: hence this opens the door to synthetic programmability. Thirdly, electronic control of replication and self-reproduction will be achieved by combining these effects.

While it would be possible to use electric fields both as actuators and sensors, and this is the ultimate intention, the parallelization of direct electronic sensing is still limited because of signal amplification sensitivity and cross talk, so that this proposal utilizes rapid fluorescence imaging of chemical reactions (with sensitivity down to single molecule levels) for sensory feedback to the electronic system. Meanwhile, this optical sensory system gives us reliable diagnostic tools and supports a range of subsidiary applications to analytical biotechnology. ECCells depend on external energy for their operation, in the form of chemical building blocks and electrical power, but they must self-organize to be able to make use of this energy for their construction and operation. In principle, ECCells could eventually be used to close the design loop and evolve intelligent solar energy cells that adapt their properties to local environmental, but this is outside the scope of the current project.

Redox processes coupled electrochemically to microelectrode operation allow a rather general chemical energy currency to be employed which can be coupled to drive a huge range of potential reactions. Similarly, the complex ion gradients created by electric fields provide a universal motor for directing chemical reactions : both simple pH gradients but also charged informational molecule gradients play a role here. Incidentally, natural cells employ both proton gradients and redox chemistry to couple many processes to their energy sources. The ECCell electronic activation thus can support adaptive changes in the synthetic chemical reactions being performed and is thus worthy of the title of a metabolism.

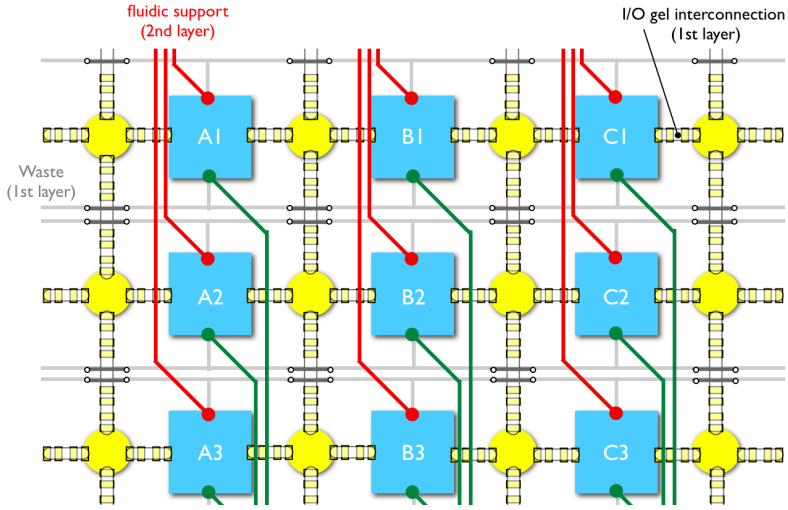


Figure 1 ECCell MEMS architecture. This architecture shows the proposed cell site

modules (blue) that may have significant internal structure to support electronic cell proliferation and proc-essing. The ECCell modules (blue) are connected to a pressure driven resource and waste system (analogously to Vcc and Gnd in electrical circuits). Here there are two separate resource mixes (shown in red and green) that flow by to the waste collection (grey). The ECCell modules are pressure decoupled from these flows via hydrodynamic barriers that may also involve gels. Internal electrically directed communication of specific molecules between modules occurs via electrophoretic transport of charged informational molecules along the yellow channels.

Apart from building the systems, a core objective of the ECCell project is to develop an effective programming methodology for this type of coupled synthetic information processing and material construction system. While extensions to real time UML can provide a language for modelling such systems, in which physical and chemical simulation and theoretical models can be inserted, programming them effectively is another matter. Chemical microprocessors cannot simply execute electrode instruction sequences, because the chemical systems may not respond as expected, and immediate corrective action is necessary to ensure successful operation. Thus the basic objective is to explore chemical system programming by means of an instruction level optimal process control approach using local feedback loops (based on real time fluorescence imaging sensory data). Instruction level feedback means that for each induced combinatorial transformation step of the chemical system, a quality measure of effective completion is defined and electric actuation regulated by feedback towards this goal.

Implementing such feedback processes effectively requires a reasonable model of system behaviour, and this will be generated in ECCell by integrating scientific simulation and modelling into the description of modules. Doing this effectively, despite problems with multiscale, requires scientific expertise and the consortium is fortunate to have two groups with leading competency in the field of scientific simulation. Conventional MEMS simulation tools will be extended with novel particle based approaches and systems chemistry approaches incorporating the influence of electric field gradients and complex boundary conditions. These extensions are necessary to allow electrically induced collective phase-transitions and electrochemical processes to be included in the basic processing steps. The strong adaptivity of the system resides in part in the deployment of these combinatorial feedback processes at a low level in the basic architecture of the system. More fundamentally, the local association of electrode control instructions and programs with individual ECCells (and their precursors) allows this electronic information to be inherited in conjunction with cellular reproduction. These electronic genomes provide another major innovation of this project. This approach will also allow a seamless evolution and optimisation of the system, since the basic instruction sets are continuously modifiable via the completion objectives. Failure to reach an instruction objective is registered and taken into account in the conditional steps of downstream processing. The algorithms defining transformation sequences are also potentially different for each cell and hence evolvable. The memory required to harbour this information is electronic. The ECCell development system will also support complex interactions between cells, constructing an electronically driven communication network between cells. This network, necessary for controlled proliferation, will also allow early application of the technology to complex molecular programming.

In summary, the technical objectives of ECCell were:

I. To deliver a fully functional simple *electronic chemical cell*. Fully functional means that the ECCell will be "alive", capable of evolution[2] and able to process molecular information.
II. To develop functional modules for programmable chemical templating/replication, containment/separation, and activation/reaction control and *evolution*.
III. To develop the *reconfigurable chemical microprocessor* technology to the point where they can effectively interface programmable chemistry with electronic microprocessors.
IV. To demonstrate the effective integration of physico-chemical models confirmed by scientific simulation in the programmable control structures of these hybrid electronic-chemical systems.
V. To develop an evolvable programming system taking advantage of the adaptive self-organizing chemical information subsystems.
VI. To demonstrate the broad range of ICT applications of ECCells and the chemical microprocessor technology and information chemistry used to generate them.

Reaching these objectives, in part or in full, will both validate online programmability of chemical systems as achievable, opening a broad arena of future ICT applications, and provide valuable insight for future conceptions of ICT as intelligent semi-autonomous soft matter.

Science and technology results of the ECCell project

Introduction

The project began with the formulation of a new architecture for hybrid electronic-chemical processing as shown in Fig. 2. This differs from now conventional lab-on-a-chip architectures in that the electronic and chemical layers play symmetric and complementary roles with a common topology of interactions within each layer. As in the spatially resolved chemical system, there is a spatially resolved electronic system. Although it is currently embedded in an external computer chip and connected by microfabricated wires and optical sensing to the chemical layer, in future parallel direct contact realizations will be possible. The ECCell project is an advance exploration of the capabilities that such fine-grained-hybrid coupled systems bring to information processing with chemical systems.

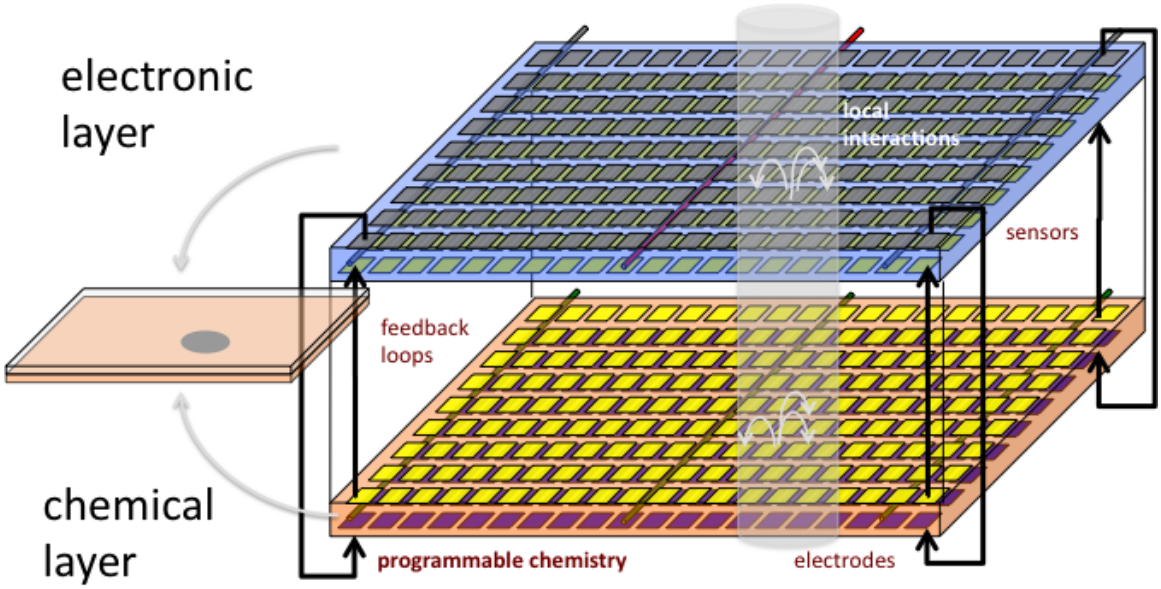


Fig.2 Architecture of electronic chemical space for ECCells. Two layers of active information processing components (chemical and electronic) are locally coupled with one another by feedback loops. Local sensors of chemical activity actuate electronic processing resulting in changes in local electrode activation patterns and these initiate new chemical activity. In the project, the sensors of chemical activity were implemented indirectly, but in real time, mediated by fluorescent light originating from labelled molecules, via microscopic imaging and a CCD camera. In future, such systems will employ direct local electronic sensing (e.g. via chemFETs). While the current architecture is essentially two dimensional, as dictated by the planar optical sensing employed, true three dimensional extensions of this architecture will be achievable with direct electronic sensing. In the project, we have also employed "thinned" 2D geometries, allowing a more efficient use of electrodes.

The realization of this architecture involved the design and fabrication of a series of special electronic-microfluidic chips that we call chemical microprocessors and the completion of an integrated experimental platform that links the system to the optical feedback system, an external computer, chemical reservoirs and physical ambient environment control (e.g. temperature). This physical support system is depicted further in section 4 below. To illustrate more concretely the technical development in the project we include a representation of the functioning interplay between the chemical and electronic layers in the real devices in Fig. 3. The image involves the intermediate integration density of the three tackled in the project, and shows how electrode signals produced by the electronic layer result in chemical reactions that give spatially resolved local optical signals that are relayed back to the local electronics using a CCD camera. It also shows the type of "fractal" thinning used in the project to optimize the tradeoff between the number of electrodes in a neighbourhood and the distance to the nearest non-independently controlled electrode.

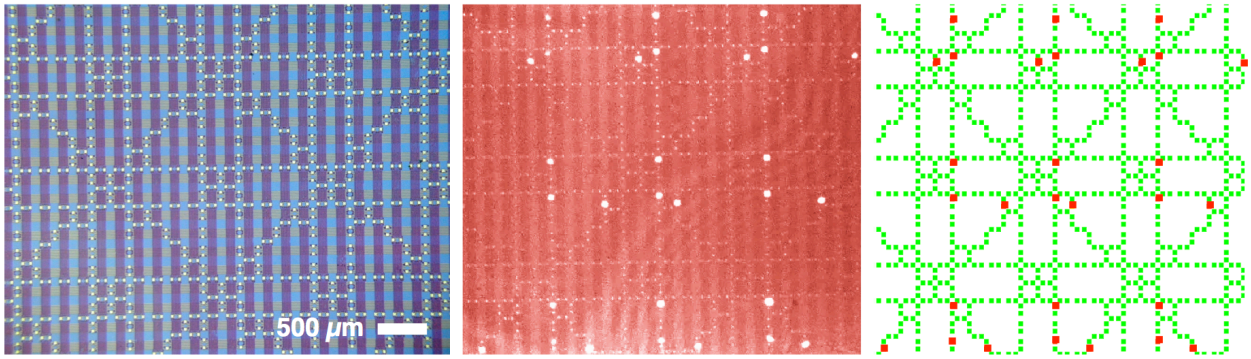


Figure 3: Experimental actuation and sensing network realized in the project in a thinned ("fractal") electrode space. The direct optical monitoring of the double layer multilayer electrode array network using electrochemiluminescence ECL is shown in the middle panel. Left: Light microscopic image of a small portion of the microelectrode array, connected by two-layer wiring. Middle: Fluorescence image of an electrochemically active test chemical layer covering the electrode array (0.1 M phosphate buffer electrolyte solution (pH 6.9) 5 mM $Ru(bpy)_3^{2+}$, 25 mM TPA and 100 µM benzyl viologen. (ΔE_{elec}=1.8V, λ_{max} = 610 nm)). Right: Snapshot of an electronically programmed sequence of electrode activation, showing activated electrodes in red. Note the perfect transfer of this activation pattern to chemical changes in the solution, and their optical detection, closing the feedback loop as input to the electronic layer for further computed changes in electrode activation.

For the majority of the project, the chemical layer was also thinned from a fully connected 2D film using microfluidic channels. Actual implementations of the abstract scheme of Fig. 2 evolved during the course of the project to address experimental artefacts, robust performance and new ideas to the regular structured arrays with local structures as shown in Fig. 4.

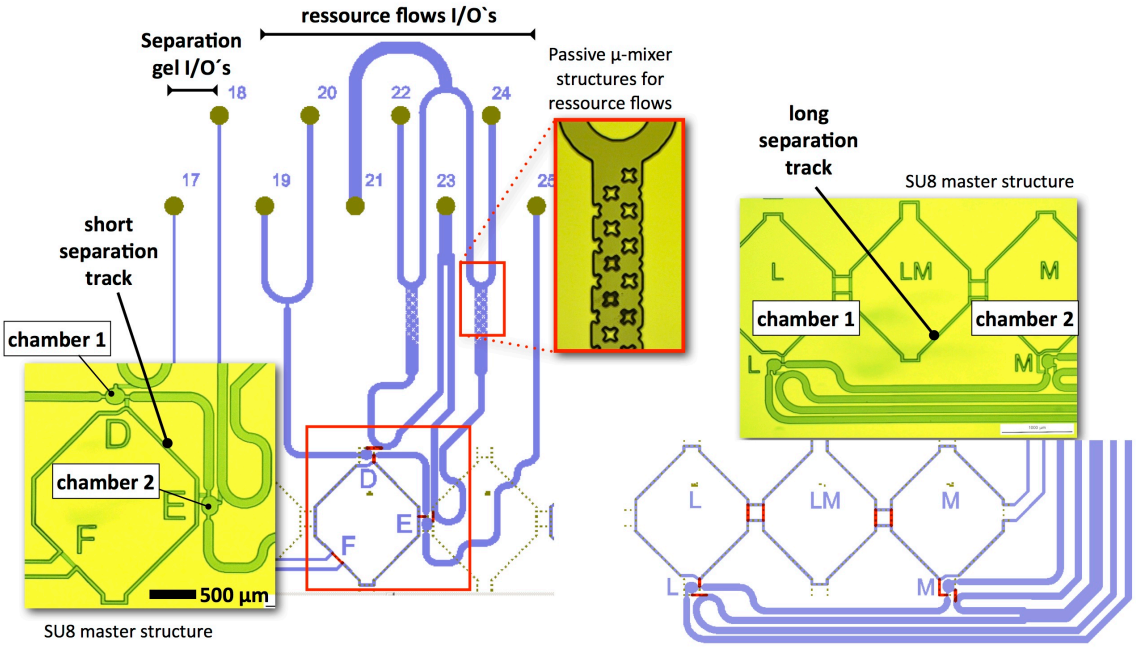


Figure 4: Two microfluidic twin chamber designs for electronically regulated isothermal amplification in ECCell. The basic architecture involves three types of chemical channels: (i) continuously flowing resource channels supplying nutrients and removing waste (21-24) (ii) mostly static segmented (two-phase) chain of chemical reaction chambers that can be stepped through the system for diagnostic purposes (19,20,25) (iii) static gelled chemical separation and communication channels (17,18). In the full architecture, the communication network connects pairs of such chambers to other pairs (see section 6) for proliferation and cooperation. The complexities of the supply channel and geometry are dictated here in part by the desire to perform analytical chemical work to diagnose the performance of the ECCell operation: the segmented structure in (ii) allows the contents of individual local chemical reactions to be extracted from the device for analysis.

Several integrative schemes were proposed for the life cycle of an ECCell, and prompted work on component chemistries, electrochemical interfaces and microfluidic support by the consortium. The first is a novel electronically clocked two-phase replication scheme that makes use of the mobility control provided by amphiphilic DNA in gels (containment) and the electrochemical pH cycling (metabolism). A variant of this is the scheme shown in Fig. 5. The second alternative is an isoelectric replication scheme based on autonomous amphoteric DNA positioning. These two schemes span the

spectrum between fully clocked and fully autonomous schemes making use of dynamic local or static spatial control. The consortium focussed on the former scheme, after initial work with candidate chemicals such as ZNA for the latter scheme proved problematic for reasons of solubility. It also developed novel DNA replication chemistry based on disulphides and DNAzymes (see Section 1), which are both compatible with the former scheme. An interesting alternative membrane-based compartmentation system based on DNA-amphiphiles developed in Section 2 was deemed less distinctive for ECCell and not integrated in further developments.

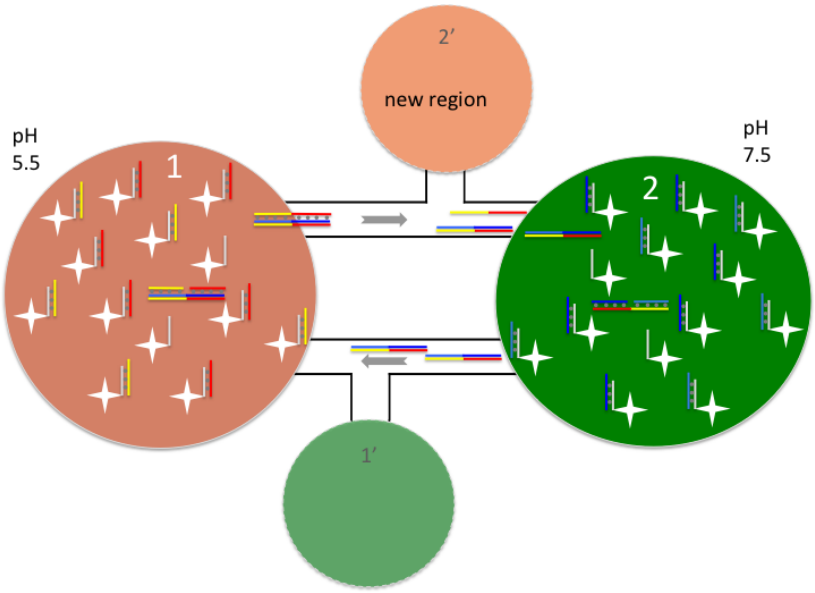


Figure 5: One architecture for overall life cycle for ECCell. Modified DNA molecules with disulphide ligation chemistry (see Section 1) are shuttled electronically between two reaction chambers (1 and 2) embedded in the ECCell two-layer architecture (cf Fig. 2,3,4). Amphiphilic DNA (See Section 2, shown here as DNA strand attached to a hydrophobic tail as a white star) is used as a reversible anchor to partially immobilize DNA in solution to a reversible gel matrix. The scheme utilizes triple helix formation at acidic pH 5.5 in chamber 1 to avoid the double stranded information end-point produced in chamber 2 at neutral pH 7.5. pH cycling in a single chamber, or maintenance of a steady pH difference, is possible via the electro-chemical activation developed in Section 3. Electric fields are used to transfer the synthesized template selectively through the gel to the other compartment as in Section 4 and 6. The required electronic feedback control system and simulation system is described in Section 5. The overall scheme has proliferation at the molecular and at the compartment level, through spin-off to new compartments 1' and 2' shown.

The chemical microprocessors used in the project are exemplified by the one shown in fig. 6, that was fabricated by the project (RUBa) in a variety of designs both for project development and final applications.

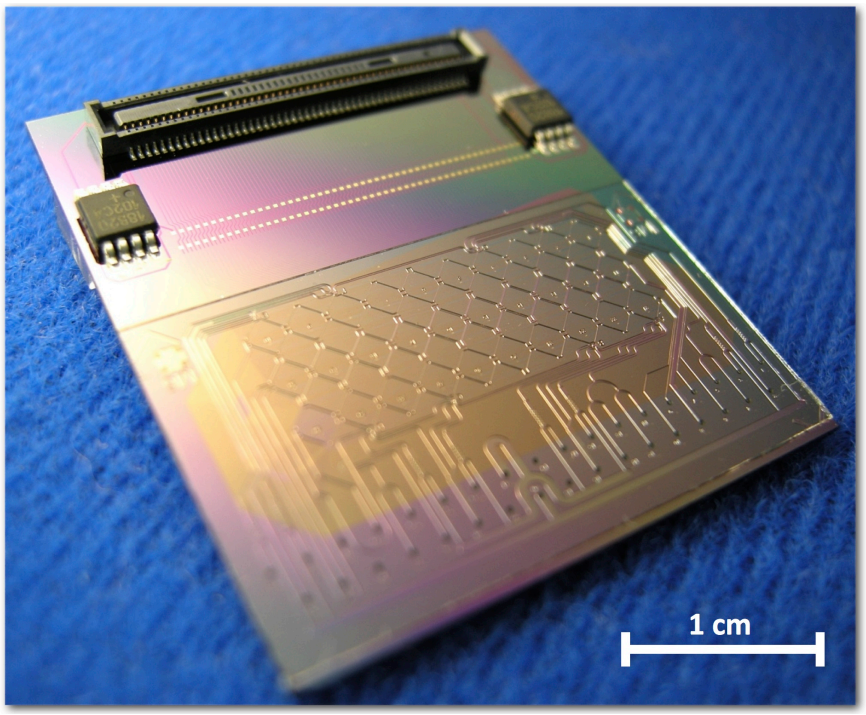


Fig. 6. Final integrated ECCell device for DNA processing and exploration of electronic chemical cells. The image shows the silicon chip base with the lower 2/3 covered with PDMS microfluidic structures. The chemical IO is from the rear, entering through the two rows of dark holes at the base. Complex fluidic IO channeling connects these to the regular ECCell matrix in the centre of the chip. The upper part of the chip contains ID, temperature sensor and electrical IO chips.

Significant novel chemical synthesis work was required and achieved to approach the goals of the project. These are described in the Sections 1, 2 and 3 that roughly follow the three types of subsystem necessary for a cell: replication, containment and metabolic regulation. In addition to the development of electronic and microfluidic hardware, culminating in the chemical microprocessors described in Section 4, a significant development in software was required both to support the system development through simulation and to implement the online control and feedback system that couples the electronic and chemical layers, as outlined in Section 5. A uniform sensor/actuator architecture was developed and employed in the project. Finally the applications of the technology developed are discussed briefly in Section 6, with reference to a separate public deliverable addressing this topic.

Was the project successful? In terms of advancing the state of knowledge and technology major progress was achieved and research is in a very different, more cohesive situation to that before the project started. Compatible chemical subsystems were developed that can interact with electronics in an elect5ronic chemical cell. Important insights were gained and new concepts tested for extending ICT into the realm of systems capable of chemical construction. In terms of the single overarching goal of completing an electronic chemical cell, the project reached the point of having all pieces functional in a single coherent framework but despite a cost-neutral extension and considerable efforts, fell short of demonstrating the ambitious final integration of the ECCell within the lifetime of the project. The major reasons for this were the amount of detailed research, cross-checking and redesign needed to establish the novel subsystems in the project, whether chemical, electronic, microfluidic or in software. The other major tasks II-V were all achieved and like the achieved concretization of an ECCell design in Task I will have an ongoing impact on integrating ICT research in this area.

Dissemination and management are covered in detail in separate reports and summarized in the conclusions. The European Center for Living Technology, inaugurated by and during the PACE project that preceded ECCell, was the seat of most project workshops and has played an active role in the dissemination and coordination of results in the area supported by the project. Any activity in artificial cell research should involve an enquiry into ethical and social implications of the technology, and we are happy to report that such flanking measures were carried out. In addition to the usual channels, dissemination activities also involved the formulation of major integrative coordination actions such as SPLiT (flagship contender) and COBRA (now running) based on the insights of the ECCell and related projects. In the conclusions we also outline briefly the direction of future research that has been applied for to further exploit the exciting developments of this project and to move the technology to a more completely autonomous integration.

Table 1. Work packages in ECCell and main contributors

WP#	Section	Theme	Organization	Leader	2nd Contributor
WP1	1	Programmable Replication	RUBb	von Kiedrowski	HUJI Willner
WP2	2	Programmable Containment	RUG	Herrmann	SDU Rasmussen
WP3	3	Electrochemical Activation	HUJI	Willner	
WP4	4	ECCell MEMS Integration	RUBa	Wagler/McCaskill	
WP5	5	ECCell Simulation and Programming	SDU	Rasmussen	RUBa Tangen/McCaskill
WP6	6 & D6.2	Functionality and ICT Applications	RUBa	McCaskill	SDU Rasmussen
WP7	Concl.	Dissemination and Workshops	ECLT	Lindgren	RUBa McCaskill
WP8	Concl.	Management	RUBa	McCaskill	

1 Programmable replication (RUBb)

The chemical requirements for a programmable spatially resolved replication-system in the ECCell are:

1. Fast templated ligation reaction with limited background- and side-reactions including a pH-independent online detection method.
2. Compatibility with an electrochemically induced pH-switch from pH 5.8 to pH 7.2 and v.v. (WP3).
3. The sequence design must be chosen in correspondence with the amphiphilic DNA, designed for mobility control of containment (WP2).

1.1 Rapid disulphide linked DNA ligation and replication. (RUBb)

The main objective was to develop a programmable chemical ligation of DNA involving a programmable pH-controlled (via WP3) copy release that is compatible with the containment chemistry (developed in WP2). This chemistry relies on the spatially resolved electronic regulation environment (WP4) not only to achieve programmable control but also to solve the chemical kinetic problem of product inhibition. Initially the chemistry was developed and analysed for DNA duplex-based template replication, and later extended to a triplex-based scheme (generalizing the original proposal of Nicoloau) to allow pH cycling of templating in a spatially resolved system.

Disulfide chemistry creates a new window of opportunity for DNA replication without enzymes, in that it is a fast reaction with low background that is stable towards wide changes in temperature and pH and so can potentially be extended to include cycling without degradation of active building blocks. Further advantages of the disulfide ligation chemistry are reversibility and its structural similarity to the native phosphodiester bond.

We demonstrated the compatibility of the DNA block copolymer synthesis with thiol oligonucleotide chemistry by synthesizing PPO-thiol-oligonucleotides and utilizing scp-DNA in chemical ligation experiments as a template. Nevertheless, for the integration of chemical replication and its control in the ECCell, the immobilization process in the microfluidic system via scp-DNA and the replication process were separated in different strands for reasons of higher flexibility in sequence design. The interaction of immobilization and replication can thereby be achieved via DNA helper-strands, binding the ligation part to the polymer-DNA on hybridization.

1.2 pH cycling of triplex-based DNA templating. (RUBb, RUBa)

For the established disulfide-DNA ligation system, based on Watson-Crick base pairing and a CG-ligation motif, the pH-variation is insufficient for an effective product-template-separation. On the other hand, the triplex formation of DNA in a parallel motif requires a pH value of 6 for an effective base pairing, due to the protonation of cytosine. The triplex-strand release is achieved at pH 7 and therefore a triplex replication can be induced and controlled via hybridization events under pH-switch in the given range.

Our aim was to use the pH sensitivity of triplex-DNA, in conjunction with disulphide chemistry, to develop a programmable replication-system which is implementable in the ECCell. The system is based on the self replication of a palindromic DNA seqeunce, previously described by Li and Nicolaou.3 In their system the ligation sites are phosphordiester bonds formed next to thymine and adenine nucleobases. The replication of the DNA duplex herein proceeds (slowly on the timescale of days) via two ligation steps, one on the Hoogsteen- and one on the Watson-Crick-side of the symmetrical triplex-DNA. Both ligation steps could be separately performed under varying pH conditions but the slow timescale precludes both using many cycles and spatially resolved regulation in the ECCell.

Since previous replication experiments with disulfide chemistry were based on a CG-ligation motif, new nucleoside modifications had to be synthesized to provide a TT-ligation for the Hoogsteen-side and an AA-ligation for the Watson-Crick-side of the system. We focussed on the syntheses of the Hoogsteen-ligation, since a Watson-Crick-disulfide ligation was already shown in previous experiments with the CG-motif. A significant variety of reactivity was not to be expected here by the introduction of thiol-adenosine and the synthetic effort for the full system appeared too high for the full replication circle. Nevertheless a full replication- or amplification-circle is accessible in principle

without Watson-Crick ligation by a spatially resolved and pH-controlled amplification process in the microfluidic environment (Figure 1).

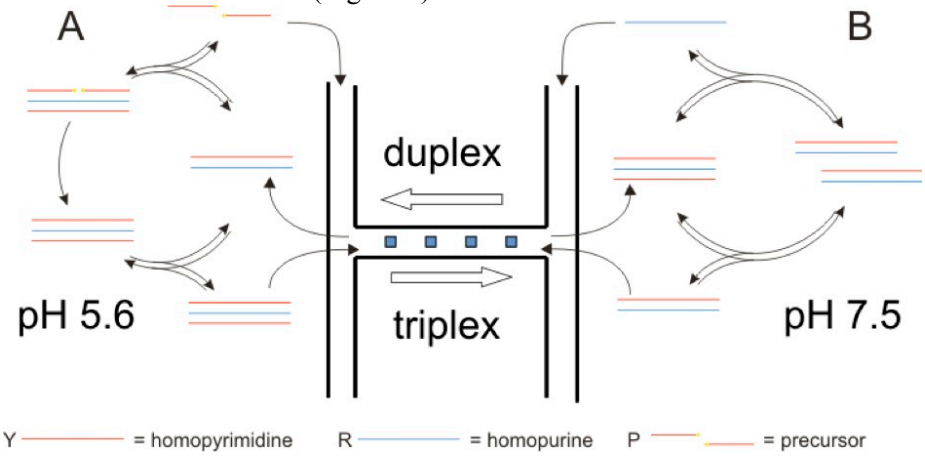


Figure 1.1: Programmable Chemical Replication in the ECCell; triplex ligation and duplex amplification result in self replication of double stranded DNA.

A full exponential replication circle is achieved when triplex DNA, synthesized by chemical disulfide ligation at the Hoogsteen-side of duplex DNA at pH 5-6 in position A, is transferred to position B, where pH 7.5 and the homopurine strand R is given. Hereby, one triplex strand gives two duplex strands which can, after transfer to position A, both serve as templates for triplex ligation. The sequence design was performed according to the Nicolaou-System.[3]

Table 1.1: Sequence pool for triplex-amplification in the ECCell.

A-strand	5´ TTT TGC GGA TTC
B-strand	5´ GAG AAA AAA GAG GAA GGG GAA TCC GCA AAA
C-strand	5´ CTC TTT TTT CTC CTT CCC GAA TCC GCA AAA
R-strand	5´ GGG AAG GAG AAA AAA GAG GAA GGG
Y-strand	5´ CCC TTC CTC TTT TTT CTC CTT CCC

The sequence pool was extended for the immobilisation process with an amphiphilic lipid-DNA (A-strand) strand and two counterstrands (B- and C-strand), binding the homopurine- or the homopyrimidine-strand, respectively (Table1). The retention mechanism (Figure 2, exemplary for the homopyrimidine binding) further contains a toe-hold of 6 base pairs for a controlled release via strand displacement.

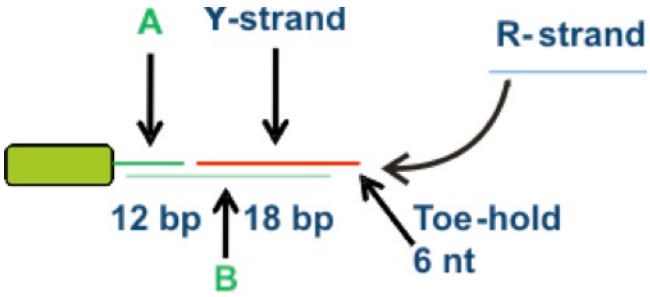
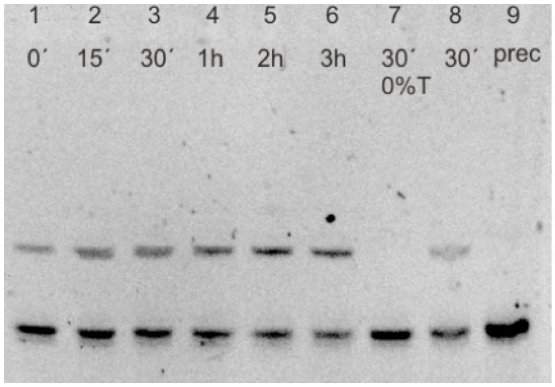
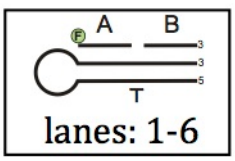


Figure 1.2: Retention mechanism on amphiphilic lipid-DNA with helper-strand B.

Both DNA-strands of the replication system can herewith be localized in the pluronic matrix of the ECCell and released as a duplex- or triplex-strand for cycling events (see also WP2, WP4).

The system was synthesized during the project, building on earlier work[4]. In order to simplify the analysis of the system, a loop was added to connect two of the triplex strands in the characterization experiments. Gel analysis revealed a fast and clean template disulphide ligation reaction (Figure 1.3).





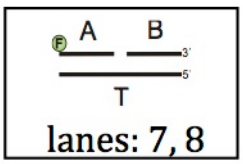


Figure 1.3: Triplex-ligation; reaction conditions: pH 5.3, 50mM NaCl, 1μM A + T, 2μM B ; preannealing of A + T, 90°C (5min)=> 15min=> 20°C.

The system was synthesized during the project, building on earlier

work[4]. In order to simplify the analysis of the system, a loop was added to connect two of the triplex strands in the characterization experiments. Gel analysis revealed a fast and clean template disulphide ligation reaction (Figure 3). From the start (lane 1) a continuous product formation was observed for 3h (lanes 1-6) without any side products detectable. Furthermore, the control experiment without template addition showed no product band (lane 7), which indicates that the product formation undergoes the templated reaction path, only. The control experiment with the Watson-Crick template showed less product formation (lane 8) at pH 5.3 than for the Hoogsteen template. Furthermore, even after 3h reaction time no untemplated product formation was observed and no side products are detected. This indicates that the triplex ligation exclusively follows the templated reaction pathway without any significant background reaction. We also performed a pH-switch from pH 5.3 to pH 7 and observed that the template effect of the looped template is drastically reduced at pH 7 and only very little product formation becomes monitorable after 16h. The variation of template concentration also showed the expected effect.

These results on the triplex ligation prove the programmability of the chemical ligation and the pH-controllability of the copy release. We have shown that the pH-switch from pH 5.3 to pH 7 effectively prohibits the product formation because the triplex-hybridisation gets prevented. Consequently, the pH-switch enables the copy release and simultaneously stops the templated ligation at the Hoogsteen-side.

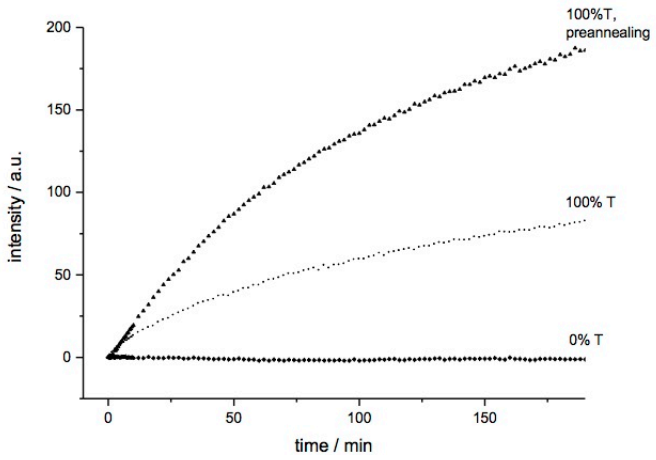


Figure 1.4: Online monitoring of the triplex ligation; pH 6, 50mM NaCl, 1µM A, 2µM B; preannealing of A + T, 90°C (5min) => 15min => 20°C. A FRET quencher system involving Alexa dye and a dabcyl quencher was used, with ligation causing concomitant release of the qunecher.

With the online measurements (Fig. 1.4) we could verify the absence of a background reaction as well as the influence of a preannealing on the ligation rate. The relatively weak Hoogsteen-basepairing and the low salt concentrations appear to retard the formation of the precursor complex. This can be compensated by the preannealing.

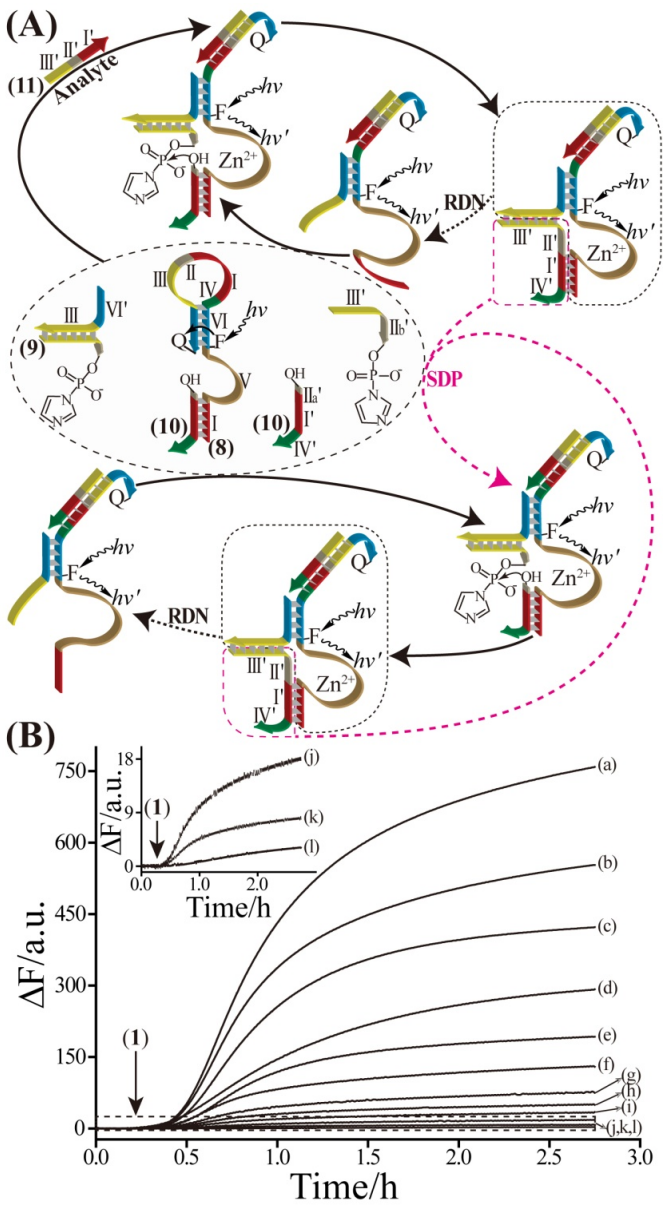
We have demonstrated that the templated disulfide ligation of DNA is highly efficient and fast as a ligation chemistry, without significant side reactions. Untemplated background reactions were efficiently suppressed (Watson-Crick-ligation) or not observed (Hoogsteen-ligation). We developed two means of control for a programmable replication in the ECCell, temperature- and pH-switch. In the case of the triplex-ligation we are able to switch the ligation by pH variation from pH 5.3 to pH 7 and to induce a programmable copy release with this mechanism. The combination of this programmable ligation with the induced immobilization, transport and pH-switch in the ECCell leads to a programmable replication system.

1.3 Autocatalytic isothermal reactions involving nucleic acids. (HUJI)

Different approaches to develop isothermal autocatalytic transformations that involve nucleic acids were developed. The following systems were developed and characterized.

1. Systems that result in the autonomous formation of catalytic nucleic acid nanostructures were developed. In these systems, predesigned hairpin structures were designed, and in the presence of an activator nucleic acid, the cross-opening of the hairpin structures was activated to yield catalytic nucleic acid nanowires structures. These systems were implemented for the ultrasensitive detection of DNA (BRCA1 oncogene).

Figure 1.5: Autocatalytic signal amplification via replication A) Schematic autonomous replication of an analyte reporter unit by the Zn^{2+}-dependent ligation DNAzyme upon sensing the analyte and the autonomous synthesis of the DNAzyme nanostructures. Throughout the paper, domains X and X′ in the respective analyte and DNAzyme or substrates subunits represent complementary base pair regions. (B) Time-dependent fluorescence changes upon analyzing different concentrations of the analyte (**11**): (a) 4.0 x 10^{-7} M, (b) 2.0 x 10^{-7} M, (c) 1.0 x 10^{-7} M, (d) 4.0 x 10^{-8} M, (e) 2.0 x 10^{-8} M, (f) 1.0 x 10^{-8} M, (g) 4.0 x 10^{-9} M, (h) 2.0 x 10^{-9} M, (i) 1.0 x 10^{-9} M, (j) 1.0 x 10^{-10} M, (k) 1.0 x 10^{-11} M, (l) 0 M. Arrow indicates the time of addition of the analyte. Inset: Enlargement of curves (j)-(l).



2.The autonomous activation of isothermal ligation/replication machineries was demonstrated with DNAzyme machineries and using strand-displacement or pH as triggering signal. This approach is highlighted in Figure 1.5. The hairpin structure (**8**) includes in domains (IV), (V) and (I) the Zn^{2+}-dependent ligation sequence, and in domains (II) and (III) of the single-stranded loop the recognition sequence for the activator (**11**). The two subunits, the imidazole-modified nucleic acid (**9**), and (**10**), act as the substrate for ligation. In the presence of the activator (**11**), hairpin (**8**) opens, resulting in the hybridization of the subunits (**9**) and (**10**) to the DNAzyme and their ligation. Since the ligated product is complementary to the loop region of (**8**), strand displacement of the ligated product is energetically favored, leading to the generation of additional ligation DNAzyme units. The major accomplishments of this autonomous biocatalytic ligation cycle are: (i) The ligated replication product acted as reporter unit of the activator. It included a copy of the activator sequence. (ii) The strand-displacement principle provided a mechanism for separation of the ligated product and the regeneration of the ligation DNAzyme. (iii) The ligated product provided an input for the further formation of the ligation DNAzyme units. (iv) The entire system is an isothermal replication system. (v) The paradigm was extended by the pH-induced separation of the ligated product. (vi) The replication of the reporter units represents a route to enhance the sequence of the activator, and thus provides a means to amplify the detection of a gene (activator sequence). By the appropriate labeling of the hairpin (**8**) with a fluorophore/quencher pair, the autonomous ligation and opening of hairpin (**8**) was followed by the fluorescence of the fluorophore units.

It should be noted that this replication scheme, in contrast with the disulphide chemistry system shown above and with Zhang and Winfree's purely conformational replicator[5], is a semisynthetic scheme. Longer DNA sequences are built up during the replication, but the fuel for the process involves relatively complex primed structures. This a novel intermediate position in the landscape of replication mechanisms and an important by-product of the project. The integration of DNAzyme based replication into the electronic chemical cell would be facilitated by the ability to electronically regulate pH or other ion concentrations and to perform molecular separation, but this must be the subject of future work.

2 Programmable containment (RUG)

The major goal of the RUG group, led by A. Herrmann, was to design and synthesize materials that can be employed to form programmable containments for an electronically programmable chemical cell. During the course of the project several strategies were envisaged to generate such structures from informational molecules like DNA extended with amphiphilic properties (see Fig. 2.1), as in DNA block copolymers (DBCs). In particular the reversible associated of amphiphilic DNA with amphiphilic gels has proved an effective informational modulation of genetic transport. On the other hand, the use of natural lipid liposomes allows the construction of aqueous compartments separated from the external aqueous solution by the lipid bilayer. Liposomes are commonly used for the solubilization of hydrophilic but also hydrophobic chemical substances. The combination of DBCs and lipidic liposomes takes advantage of both properties: watertight biocontainers, which can undergo self-assembly by DNA hybridization.

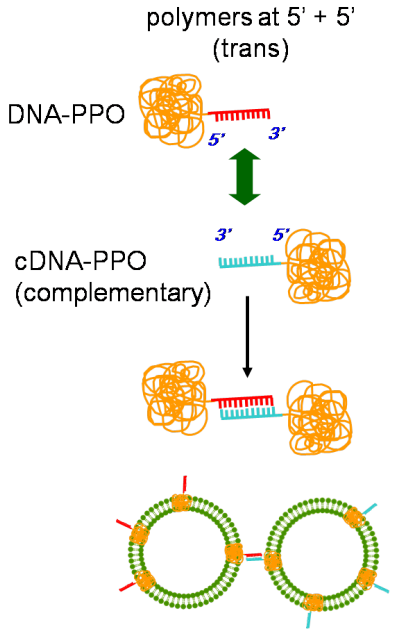


Figure 2.1: Schematic of DNA-directed hybridization of vesicle compartments. The vesicles are equipped with different DNA block copolymers in the membrane.

In ECCell, the interaction of amphiphilic DNA block copolymers (elsewhere referred to as scpDNA) with reversible block copolymer gels was the main development integrated into the electronic chemical cell architecture (cf. WP 1 and 4) and used to test ICT applications (WP 6). This comparatively simple functionality, allowed us to make the transport of DNA sequence specific, allowing the design of novel coupled transport and replication processes controlled by electronics. Sequence selectivity was achieved without having to synthesize special purpose covalent separation materials (as in previous work on DNA computing in Adleman's lab, for example6). This work was also made possible by the developments in this work package, and is reported next, however the main activity of RUG here focussed on more complete programmable containments, involving membranes.

2.1 Programmable sequence dependent mobility control (RUG)

The first approach pursued involved using trisoligonucleotides (with three arms) to investigate the formation of gels formed not by amphiphilic interactions but by DNA hybridization.

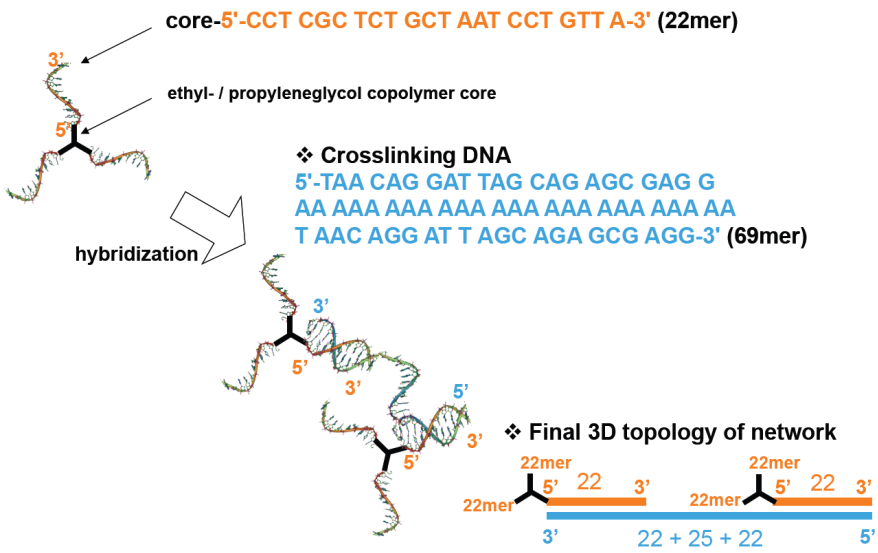


Figure 2.2: DNA three-arm star-polymers for the generation of DNA networks within microfluidic devices.

However since the microfluidic investigation (Section 4) of potential gelation revealed that cross-linked DNA networks were not achieved by hybridization of these trisoligonucleotides, probably

owing to the low concentrations achievable, we developed a new strategy for the reversible retention of DNA within another reversible hydrogel matrix. Polymeric DNA nanoparticles were produced that are retained (on the basis of size) compared with pristine DNA sequences. Three different materials were fabricated for that purpose.

a) DNA block copolymer micelles stabilized by a semi-interpenetrating network in the core

Since we had synthesized DNA-b-PPO but could not realize concentration and micellization in the microfluidic channels with it, we generated micelles from this material that were stabilized by a semi-interpenetrating polymer network in the hydrophobic core outside the microfluidic devices. To further increase the molecular interactions between the DNA-b-PPO particles and the Pluronics gel matrix, for better retention, the micelles were themselves blended with Pluronics F127 before the cross-linking reaction within the core. The stabilization reaction was carried out by internalization of pentaerythrytol tetraacrylate (PETA) in the micelles and UV induced cross-linking. Figure 2.3 gives a schematic representation of the architecture of the blended DNA block copolymer aggregates.

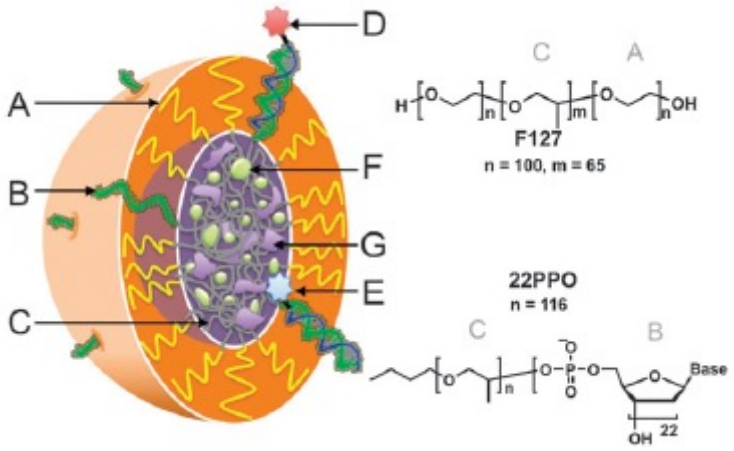


Figure 2.3: Schematic of the mixed micelle architecture and chemical structures of the polymeric components. (A) PEO block of Pluronic. (B) DNA block of DBC. (C) PPO blocks of Pluronic or DBC. (D) and (E) Probes at 5'- and 3'-ends of the complementary DNA, respectively. (F) Hydrophobic compound loaded into the hydrophobic core. (G) Cross-linked nanodomains of PETA in the core.

The stabilization of the micelles by forming a semi-interpenetrating network in the core that contained Pluronics F127 and DNA-b-PPO in a ratio of 5:1 was assessed by incorporation of pyrene and cooling below the critical micelle temperature (CMT). The fluorescence spectroscopic analysis revealed that core cross-linked micelles retained the pyrene in the core while non cross-linked blended micelles released the payload and disassembled into their individual components. The ability of the aggregates to undergo Watson-Crick base pairing with complementary DNA was successfully proven with FRET experiments employing fluorescently labelled Pluronics F127 and labelled ODNs, with ODN-labelled gold nanoparticles and TEM analysis and by AFM and dynamic light scattering (DLS)[7].

b) DNA block copolymer micelles containing more hydrophobic polymer cores.

Another method to stabilize micelles is the utilization of a more hydrophobic polymer than PPO such as polyisoprene (PI). PI exhibits a very low glass transition temperature, which allows easy fabrication of micelles by just dissolving the amphiphilic block copolymer in aqueous solution. Moreover, these materials contain double bonds that could be utilized for further stabilization of the particles by introduction of covalent bonds between the PI chains within the micelle core. Several PIs of varying molecular weight with a terminal hydroxyl group were prepared by anionic polymerization. Subsequently, the semitelechelic polymers were converted into the corresponding phosphoramidites (Figure 2.4 A) and coupled to ODNs present on the solid support to form DNA-b-PI in excellent yields (Figure 2.4 B and C).

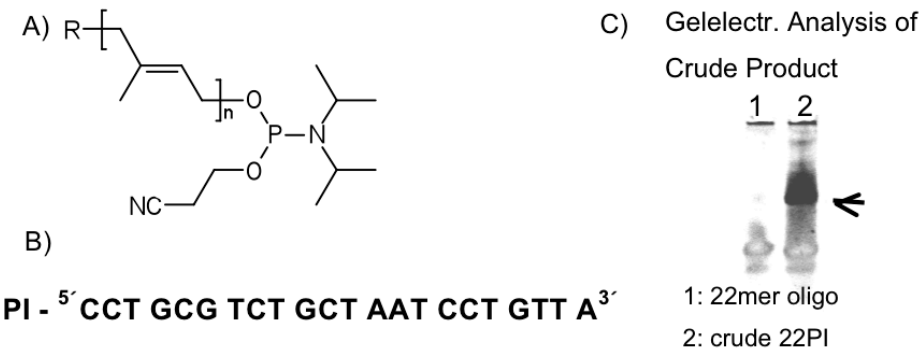


Figure 2.4: Hydrophobic core stabilization. A) PI-phosphoramidite, B) Sequence composition of DNA-b-PI and C) Gel-electrophoretic analysis of the crude product of

DNA-b-PI demonstrating the high efficiency of the coupling reaction on the solid support.

After micellization, the corresponding aggregates were analysed by AFM and DLS, revealing particles with a diameter of 23 ± 3 nm. Once again, complementary DNA hybridization experiments with these self-assembled structures proved successful.

c) DNA nanoparticles stabilized by hydrophobic chains attached to the nucleobase

As a third alternative to produce DNA nanoparticles that can retain DNA in a reversible Pluronics gel matrix, DBC aggregates were produced that are functionalized with hydrophobic chains at the nucleobases: a dodec-1-yne chain at the 5-position of the uracil base, which allowed precise and simple tuning of the hydrophobic properties through solid-phase DNA synthesis. Figure 2.5 gives an overview of the materials employed, including the phosphoramidite building block, the incorporation into ODNs and three different architectures with their corresponding hybridization products.

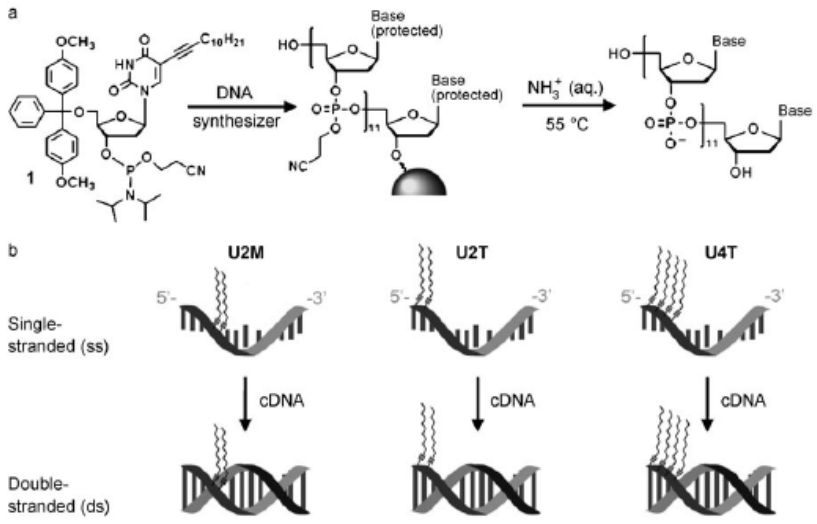
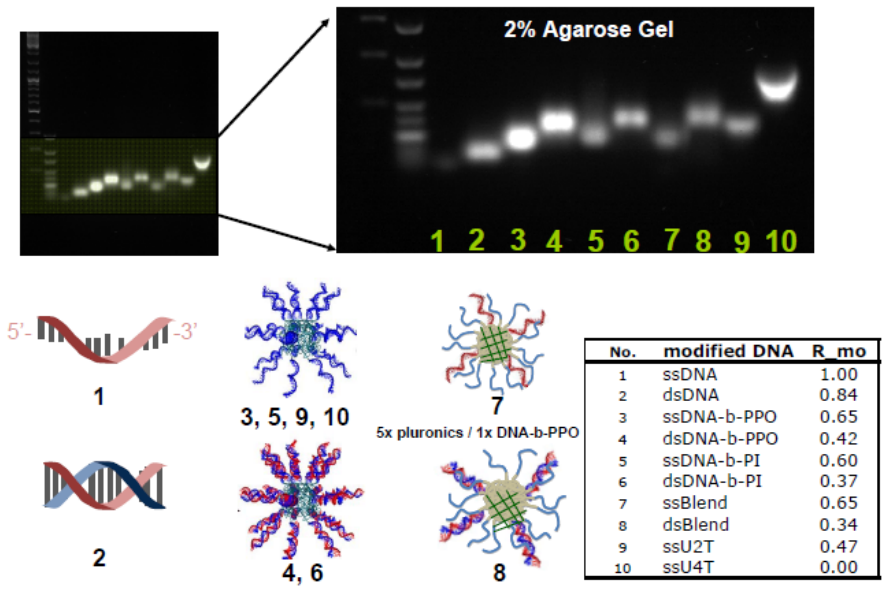


Figure 2.5: Synthetic scheme and representation of lipid–DNAs. a) The precursor, 5-(dodec-1-ynyl)uracil deoxyribophosphoramidite (left) was used in conventional solid-phase DNA synthesis (center), and deprotection yielded the lipid–DNA (right). b) Schematic representation of the ss and ds lipid–DNA amphiphiles (U2M, U2T, and U4T) investigated.

The micelles formed from these modified DNA sequences were characterized by atomic force microscopy, dynamic light scattering, and polyacrylamide gel electrophoresis8. These experiments revealed the role of the quantity and location of the hydrophobic units in determining the morphology and stability of the micelles. The effects of hybridization on the physical characteristics of the DNA micelles were also studied; these results showed potential for the sequence-specific noncovalent functionalization of the self-assembled aggregates and their utilization in the microfluidic devices.

Testing the ability of DNA aggregates to retain DNA in reversible gels (RUG, RUBa)

We tested the ability of the DNA aggregates to retain DNA sequence-specifically in conventional gels that can be disaggregated after electrophoresis, i.e. agarose and Pluronics gels. In Figure 2.6 the electrophoretic analyses of the mobility of DNA aggregates in an agarose gel are summarized. These results clearly show that all the DNA polymer and DNA alkyl modified materials exhibit significantly less electrophoretic mobility than non-modified DNA. All hydridized samples, as expected, migrate less than their corresponding double stranded counterparts. Finally, the 12mer ODN functionalized with four hydrophobic chains (U4T) exhibited an extremely low electrophoretic mobility compared to the other aggregates. Very similar results were obtained in experiments where Pluronics acting as a gel matrix (data not shown). In conclusion, all the modified DNA materials should be very well suited to retain DNA sequence specifically and reversibly in gel matrices in microfluidic devices. All the materials were handed over to RUBa to be tested in the micron-sized channels (see WP 6 and 4).



No.	modified DNA	R_mo
1	ssDNA	1.00
2	dsDNA	0.84
3	ssDNA-b-PPO	0.65
4	dsDNA-b-PPO	0.42
5	ssDNA-b-PI	0.60
6	dsDNA-b-PI	0.37
7	ssBlend	0.65
8	dsBlend	0.34
9	ssU2T	0.47
10	ssU4T	0.00

Figure 2.6: Electrophoretic analysis of DNA aggregates vs. non-modified DNA in agarose. The relative mobilities in the table were calculated in respect to ssU4T and ssDNA as slowest and fastest moving species, respectively.

2.2 Programmable Chemical Nano-biocontainers (RUG)

DNA Block Copolymer-lipid liposome formation.

RUG commenced 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 at RUG was adapted and developed to the specific case of DPhyPC liposomes with DBCs incorporated in their membranes. As DBC, we used DNA-b-PPO, as above. DNA-b-PPO/DPhyPC liposomes of 200 nm diameter were obtained efficiently by controlling parameters during the hydration (vortexing and sonication) and the extrusion process. The hydrodynamic results showed that the incorporation of DNA-b-PPOs (with various DNA or PPO lengths) in the membranes does not significantly affect the size of liposomes compared to liposomes without DBC.

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. Fluorescence resonance transfer experiments were employed to this end (Fig. 2.7).

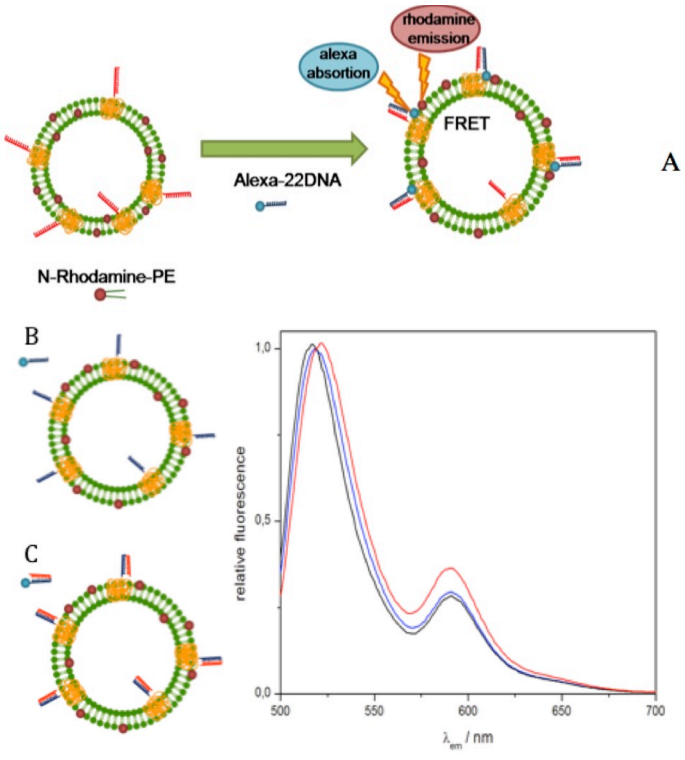


Figure 2.7: Scheme and spectra of a FRET experiment to test the incorporation of DBC's into membranes. A) 22-DNA-PPO/DPhyPC liposomes doped with N-Rh-PE, which were hybridized with c22-alexa, lead to FRET liposomes (red spectrum). On the other hand, c22-DNA-PPO/N-Rh-PE/DPhyPC liposomes in the presence 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.

After the efficient preparation of FRET liposomes, they were mixed with non-DNA-doped liposomes at different ratios 1:1, 1:10 and 1:100, figure 2.5A. Figure 2.5B shows the spectra of liposomes before and after mixing with non-fluorescent liposomes. 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 under-lines the suitability of DBCs as coding molecules for liposomes. In addition a low (6%) detection limit in the FRET experiments was ascertained.

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. 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, see Deliverable 2.4. To visualize the GUVs using Confocal Laser Scanning Microscopy (CLSM), the GUVs were doped with 3,3'-dioctadecyloxacarb-ocyamine (DiO) fluorescent lipid probe.

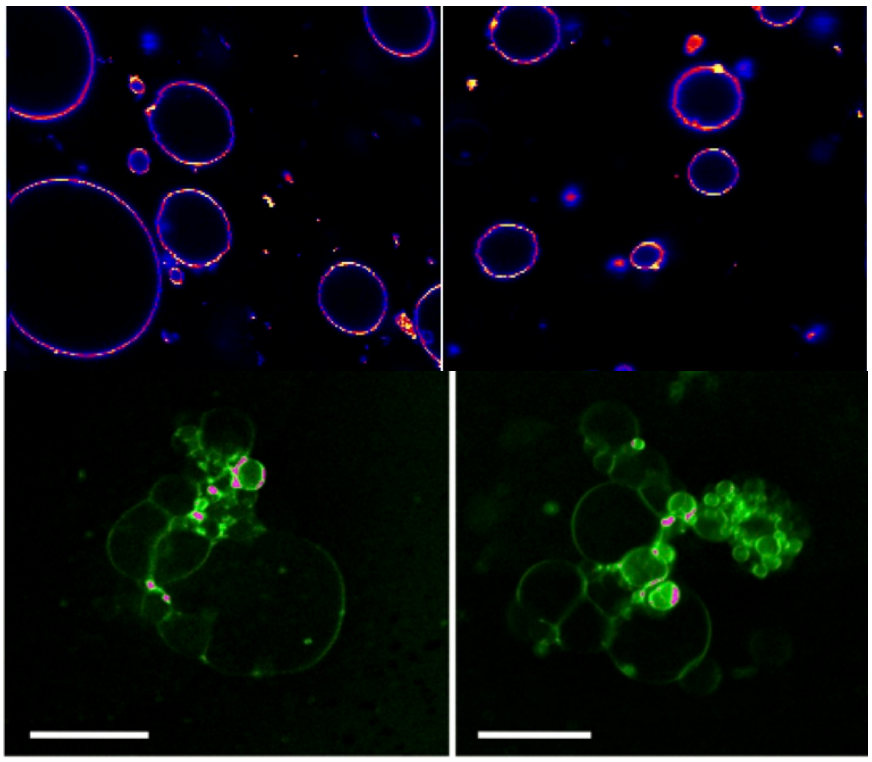
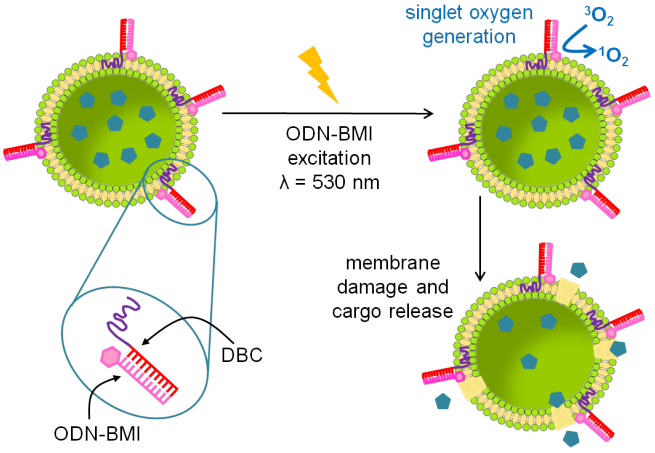


Figure 2.8: Matching DNA induced aggregation of tagged vesicular containers.

Top. Separated populations of DBC labelled vesicles. 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.

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

When the GUVs, containing separately 11-DNA-PPO/DPhyPC and complementary c11-DNA-PPO/DPhyPC, were mixed, sequence-directed aggregation and planarization of the membrane were detected as can be seen in bottom panels of figure 2.8 (for schematic see figure 2.1). Video recording of this interaction between the DNA coded vesicles permitted us to analyse the mechanism of GUV interaction. First aggregation takes place induced by the DBC hybridization and when the membranes are close together more hybridization events occur in a zipper-like fashion, which is accompanied by the formation of planar faces between the membranes.



Sequence specific release of cargo from DBC tagged vesicles

Figure 2.9: Schematic representation of sequence-specific cargo release from DBC-lipid vesicles induced by light irradiation. DBC-lipid vesicles are hybridized with an ODN-photosensitizer based on BODIPY monoiodine (ODN-BMI). The irradiation of ODN-BMI generates singlet oxygen, which is able to damage the bilayer and release the cargo.

After vesicle aggregation through hybridization was successfully realized, the next step was to achieve the sequence-specific release of encapsulated molecules out of these containers as a second important functionality of such programmable containments. For that purpose, the protruding oligonucleotides (ODN) on the vesicle surface were functionalized with ODN-photosensitizer conjugates by Watson-Crick base pairing and after light irradiation that induced the formation of singlet oxygen selective cargo release was achieved depending on the DNA code of the vesicles (Figure 2.9).

For the cargo release from the DNA tagged vesicles several parameters were investigated to control the liberation of encapsulated molecules. The first one was the amount of DNA block copolymers (DBC) and hybridized photo-sensitizer molecules on the vesicle surface. Figure 2.10 shows the proportion of calcein release from irradiated liposomes over time for several different DBC/liposome ratios. Figure 2.10 also shows the release profiles of two controls, which cannot hybridize to the surface.

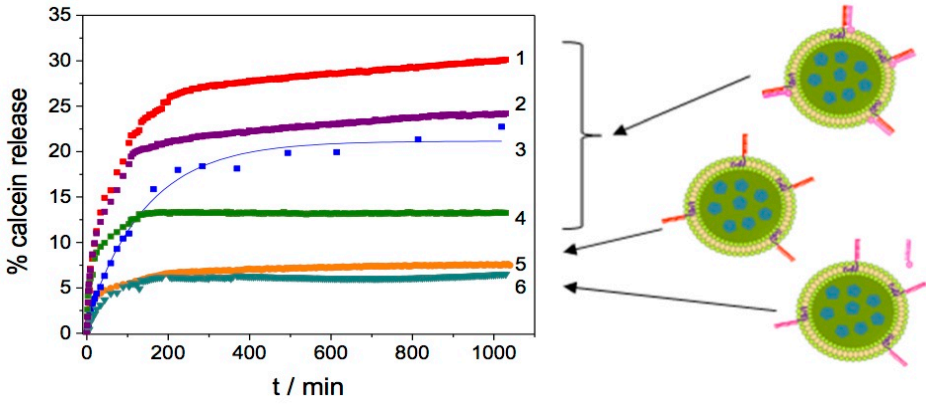


Figure 2.10: Kinetics of cargo release (%) from DBC-coated vesicles induced by $^1O_2$ formation: r22-b-PPO/DPhyPC liposomes hybridized with c22-BMI at different lipid/DBC ratios (curves 1 to 4: 1048, 2316, 5321 and 9271); r22-b-PPO/DPhyPC liposomes in the absence of c22-BMI (curve 5); c22-b-PPO/DPhyPC liposomes in the presence of c22-BMI (curve 6).

In summary, we have successfully and stably incorporated DBCs that encode sequence information into the membranes of conventional liposomes, LUVs and GUVs. Vesicle aggregation was achieved by hybridization of complementary sequences in the membrane, which clearly documents that manipulation of the containers is possible by exploiting the DNA tag. Finally, sequence specific cargo release was successfully demonstrated that can be controlled by several parameters. In conclusion, vesicles encoded with DNA are becoming a valuable technology platform for manipulation and handling of minute volumes.

3 Electrochemical interface and control of ECCells (HUJI)

The activities (led by Itamar Willner, HUJI) were directed towards electrochemically controllable processes relevant to the construction and operation of electronic chemical cells. This involved the interplay between electrode voltages and chemical processes influencing (i) nucleic acid replication, (ii) containment of chemicals and (iii) metabolism and regulation. In the course of these investigations novel isothermal amplification systems for DNA were also developed, making an important contribution to Section 1 on replication.

The main research topics involved:

1) The modification of electrodes with redox-active groups for the reversible switching of pH changes by electrochemically induced redox process and their application to drive nucleic acids dehybridization and pH-switchable chemical transformations.

2) The design of electrochemically switchable containments that enable controlled uptake and release of components:

- To electropolymerize thermo-sensitive polymers on surfaces that undergo thermal and thermal/electrochemical solid-gel transitions.
- To develop electrochemically triggered “sponges” for the uptake and release of pre-designed molecular substrates, and to control surface hydrophilicity.

3) Additional research was directed to assist the activities of other WPs in the project:

- The synthesis of functionalized Au nanoparticles for electrochemical immobilization in microfluidic channels for electrochemical control of pH changes.

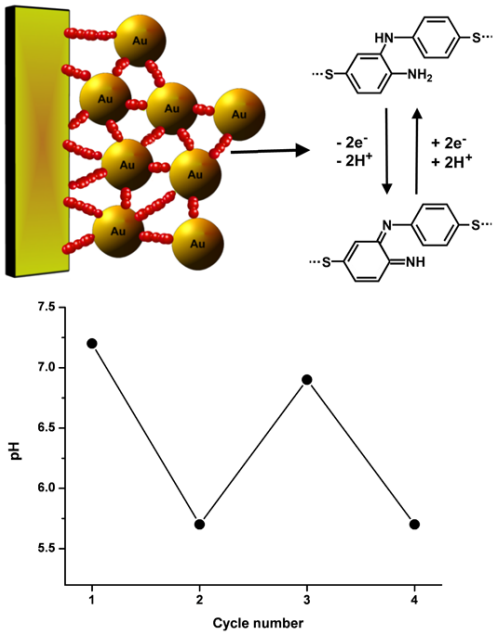
- Introduction of new paradigms for enzyme-free replication, ligation and activation of catalytic assemblies that include nucleic acids. (see WP 1)

The following highlights only the most important developments originating from the project.

3.1 A nanoparticle-modified electrode for the reversible electrochemical switching of pH, and its application for pH-switchable dehybridization and DNAzyme catalysis.

A modified electrode that stimulates electrochemically driven pH changes was constructed, and the electrode was implemented to control dehybridization of duplex DNAs through the formation of i-motif structures. The separation of the duplex DNAs was used to control and switch DNAzyme activities.

An Au-nanoparticle-modified electrode for controlled reversible pH-changes.



A modified electrode that stimulates reversible pH changes of an aqueous solution was developed. The electrode involves the modification of a base electrode with bis-aniline-crosslinked Au nanoparticles (NPs). The reversible oxidation and reduction of the bis-aniline bridges resulted in the release or uptake of protons to (or from) the aqueous solution, reversibly. We were able to demonstrate reversible pH changes between pH = 5.7 and pH = 7.2. To reach these 100-fold changes of H+ in the solution, the following parameters were adapted for fabricating the electrode, Figure 3.1: (1)A base Au electrode was modified with a high surface-area Pt-sponge coating. (2) The Pt-functionalized Au electrode was modified with a bis-aniline-crosslinked Au NPs composite by applying variable numbers of electropolymerization cycles (optimum number was 60 electropolymerization cycles).

Figure 3.1: Electrochemical pH Switching without electrolysis.

pH-Switchable DNAzyme reactions.

Two different model systems of duplex oligonucleotide structures separable by pH changes were developed, Figure 3.2.

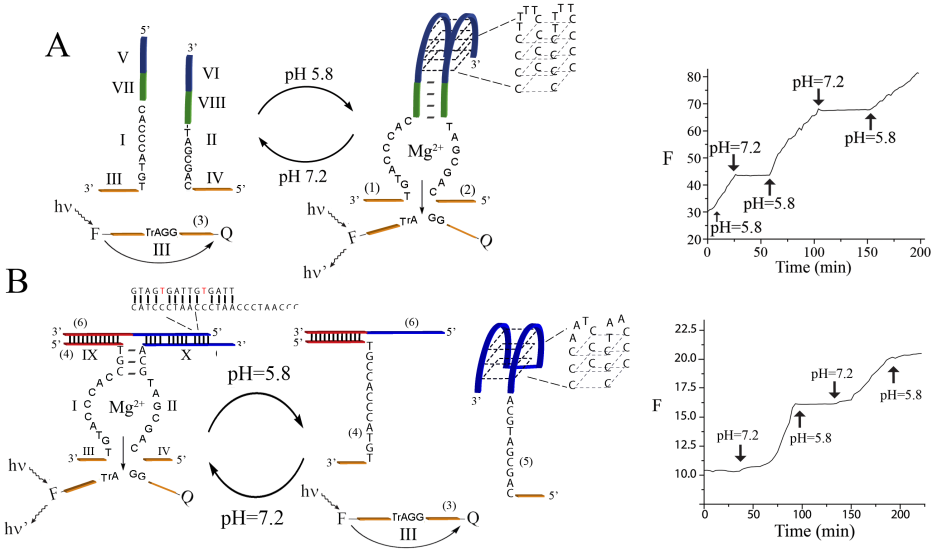


Figure 3.2: pH switchable DNAzyme reactions making use of the C quadraplex.

One configuration involved the use of the Mg2+-dependent DNAzyme subunits that self-assemble at pH = 5.8 to an active C-quadruplex-bridged DNAzyme structure and separate into the inactive subunits at pH = 7.2, Figure 3.2(A). Reversible and switchable activation/deactivation of the DNAzyme was accomplished in the system. The second

pH-triggered separation of oligonucleotide duplexes involved the use of an oligonucleotide-bridged active DNAzyme structure at pH = 7.2 that is separated at pH = 5.8, while generating a C-quadruplex structure, Figure 3.2(B). Reversible activation/deactivation of the DNAzyme structure by switching of the pH was demonstrated. A fluorescence method was developed to probe the pH-triggered functions of the DNAzyme.

3.2 Development of electro-switchable containments for the controlled uptake/release of substrates and for controlling the wettability of surfaces.

The efforts in this sub-project involved the electropolymerization of thin polymer films or Au-nanoparticle composites on conductive surfaces as functional matrices for the electro-switchable uptake/release of substrates and for the control of surface properties.

One system involved the electropolymerization of poly-isopropyl-acrylamide, p-NIPAM on electrode surfaces and the incorporation of different metal ions (Ag+, Hg2+, Cu2+) into the polymer films. These polymers acted as thermo-sensitive polymers that undergo reversible solid-to-gel transitions thereby controlling the electronic and uptake/release of molecular substrates. The systems revealed the following important functions:

- The solid-to-gel transition temperatures were controlled by the metal ions incorporated into the polymer matrices.
- The electronic properties of the polymer could be electro-switched by the electrochemical transformation of the "in-polymer" metal ions into metal nanoclusters, and by the thermal solid-gel transitions of the polymer in the presence of the metal nanoclusters.

The p-NIPAM matrix that included Ag0 nanoclusters revealed electro-switchable catalytic activities upon transition between the solid and gel states of the polymer.

The electropolymerization of thioaniline-functionalized Au nanoparticles (NPs) on Pt-black roughened surfaces in the presence of the electron acceptor substrates: picric acid (1), N,N'-dimethyl-4,4'-bipyridinium, (2), MV2+, or bis-N-methylpyridinium ethylene, (3), BPE2+, led to three-dimensional imprinted Au matrices for the respective substrates, Figure 3.3.

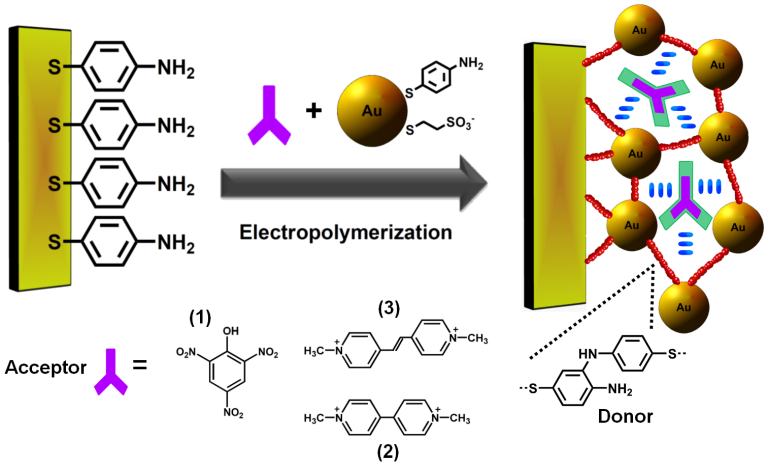


Figure 3.3: Schematic presentation of the electropolymerization of the bis-aniline-crosslinked Au NP composite and the imprinting of the π-acceptor molecules picric acid (**1**), N,N'-dimethyl-4,4'-bipyridinium (**2**), and N,N'-dimethyl bipyridinium-4,4'-ethylene dichloride (**3**).

The imprinted sites revealed high specificity towards the association of the imprinted substrates (**1**), (**2**), or (**3**).

The electrochemical uptake/release of the substrates to, and from, the imprinted sites were electro-switched through the cyclic reduction or oxidation of the bis-aniline bridging units. While the bis-aniline bridges act as π-donors that bind the π-acceptor substrates through donor-acceptor interactions, the oxidation of the bridging units to the quinoid acceptor state results in the release of the acceptor substrates from the matrix. By the cyclic reduction and oxidation of the bridging units, the selective uptake and release of the imprinted substrates were demonstrated, respectively.

The uptake and release of the imprinted substrates were followed by surface plasmon resonance spectroscopy (SPR), and by analyzing the quenching of external fluorophores (meso-tetramethyl pyridinium porphyrin for (**1**) or Zn(II)-meso-tetraphenylsulfonatoporphyrin for (**2**)), upon the uptake or release of the imprinted substrates. Figures 3.4 and 3.5 exemplify the uptake/release of (**1**) and (**2**), respectively.

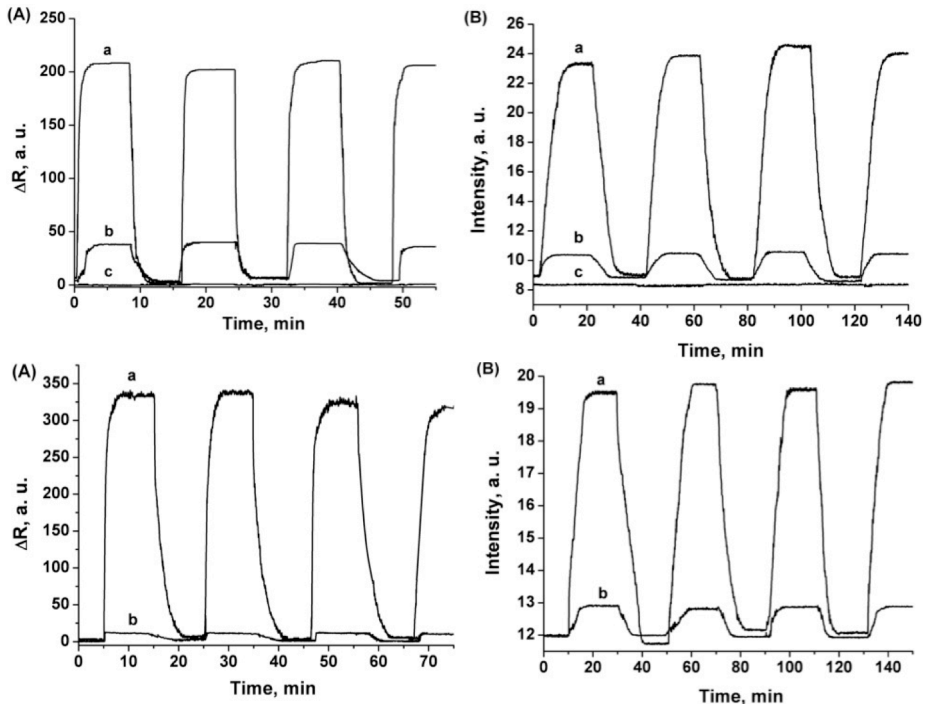


Figure 3.4: Electronic uptake and release cycles of (1, upper) and (2, lower) A) Reflectance changes, and (B) Fluorescence intensity changes following the cyclic electro-stimulated uptake and release by: (a) The (1)-imprinted, and (b) The non-imprinted bis-aniline-crosslinked Au NP composites. Curves (c) correspond to the cyclic application of the reduction and oxidation potentials on thioaniline monolayer-modified Au surfaces.

The significance of the imprinting process to yield effective electrochemically driven sponges for the uptake/release of the substrates was demonstrated by comparing the performance of the imprinted Au NPs composites to non-imprinted composites. The significance of the imprinting process to yield selective uptake/release sponges was demonstrated by analyzing the uptake/release of the substrate to its imprinted substrate and by probing the affinity of the template to the non-imprinted substrate (for example, we demonstrated that (**1**) exhibits high affinity for binding to the (**1**)-imprinted composite, while (**2**) revealed a low affinity for the (**1**)-imprinted matrix. These results were reversed for the (**2**)-imprinted Au NPs composite).

Controlling the hydrophilic/hydrophobic properties of microfluidic channels is a general method to block or trigger the transfer of fluids through the device. We have implemented the imprinted bis-aniline-crosslinked Au NPs composites as electroactive matrices for controlling the wettability of the surface, Figure 3.5.

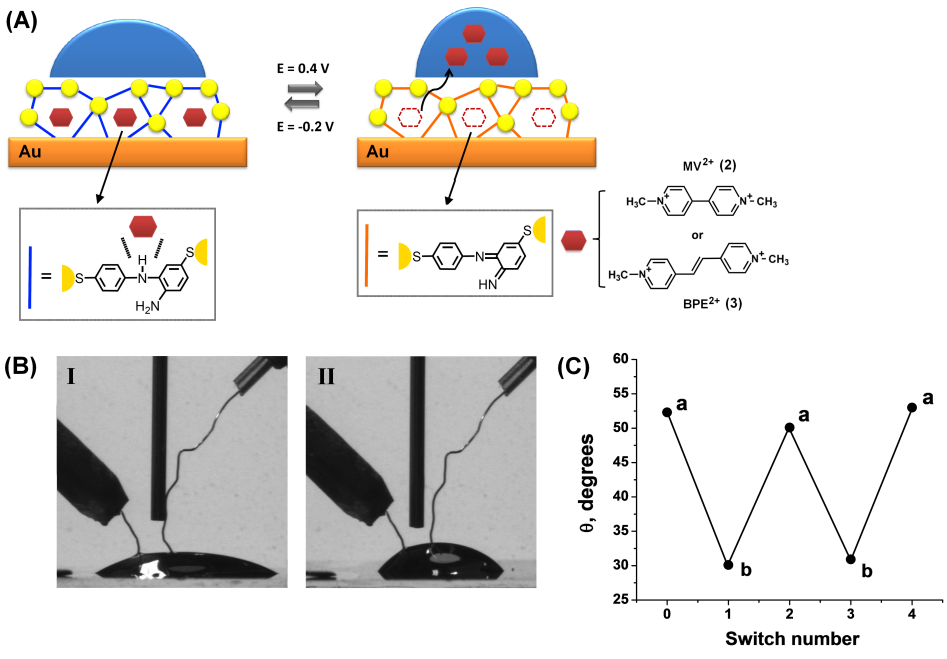


Figure 3.5: (A) Schematic presentation of **e**lectro-stimulated wettability changes by the uptake and release of π-acceptor molecules using the imprinted bis-aniline-crosslinked Au NPs composite. (B) Images showing changes in the contact angle for a droplet containing MV^{2+}, on the MV^{2+}-imprinted bis-aniline-crosslinked Au NPs electrode, upon the application of: (I) A reductive potential pulse, and (II) An oxidative potential pulse. (C) Cyclic electrical switching of the contact angle of the droplet in (B) by the application of: (a) an oxidative potential pulse, and (b) a reductive potential pulse.

The uptake of the imprinted substrates (**2**) or (**3**) by the imprinted bis-aniline-crosslinked Au NPs composites resulted in hydrophilic surfaces, whereas the electrochemical oxidation of the bridging units to the quinoid state released (**2**) or (**3**) and yielded surface of lower hydrophilicity, Figure 3.5(A). The hydrophilic/hydrophobic properties of the surfaces were probed by contact-angle measurements. Cyclic control of the wettability of the surface was demonstrated by the reversible oxidation and reduction of the bridging units, Figures 3.5(B) and (C). The imprinted template controlled the selectivity for binding the acceptor units (**1**) or (**2**).

These results pave a way to generate different imprinted Au NPs composites in the different channels of the microfluidic devices that enable a programmed delivery of chemical components by electrical stimuli. In conjunction with the parallel work on DNAzyme based replication (see Section 1), the results provide a powerful set of tools for future electronic chemical cells. Further work remains to ensure the integrability of these new concepts and techniques into closed channel networks, although apart from the generic problems of reference electrodes and electrode cleaning, which generally makes use of mechanical procedures such as polishing (in additional to chemical and electrochemical ones) to ensure reproducibility, no principle barriers to use are foreseen. Certainly the capabilities of controlled delivery and uptake of small molecules, regulated by the electronic system, will provide an exciting future metabolism control system for electronic chemical cells.

4 ECCell MEMS Integration (RUBa)

The construction of the two layer ECCell architecture depicted in Fig. 2 comprised a whole range of technical problems and challenges that needed to be addressed in ECCell. Not only the specific integration of the three functionalities addressed in Section 1-3, but a variety of overarching technical domains involving:

(i) high density electrode array design and fabrication (with interconnect);
(ii) electrochemical surface modification of electrodes and verification with CV, including decoupling from FPGA perturbations
(iii) development of a robust medium density parallel fluidic interconnect;
(iv) electronic interconnect and adapter modules, including FPGA programming;
(v) optical sensor system integration, including fully programmable high-speed real-time confocal fluorescence microscope with cylindrical optics scanning system;
(vi) development of an electrochemiluminscence test system;
(vii) microfluidics design and fabrication in PDMS prototyping;
(viii) droplet based compartmentation and IO for analysis and separation;
(ix) development of a reversible in-channel temperature control and gelation system for separation and selective transport of DNA;
(x) chip ID, documentation, and use and defect reporting system;
(xi) integration of all programmable elements in a common computer control system.

All of these aspects were dealt with during the project by the BioMIP team at RUB. We do not deal with these issues in detail in this summary report, but make space to highlight a few of the achievements. Some successive intermediate developments are shown in Fig. 4.1.

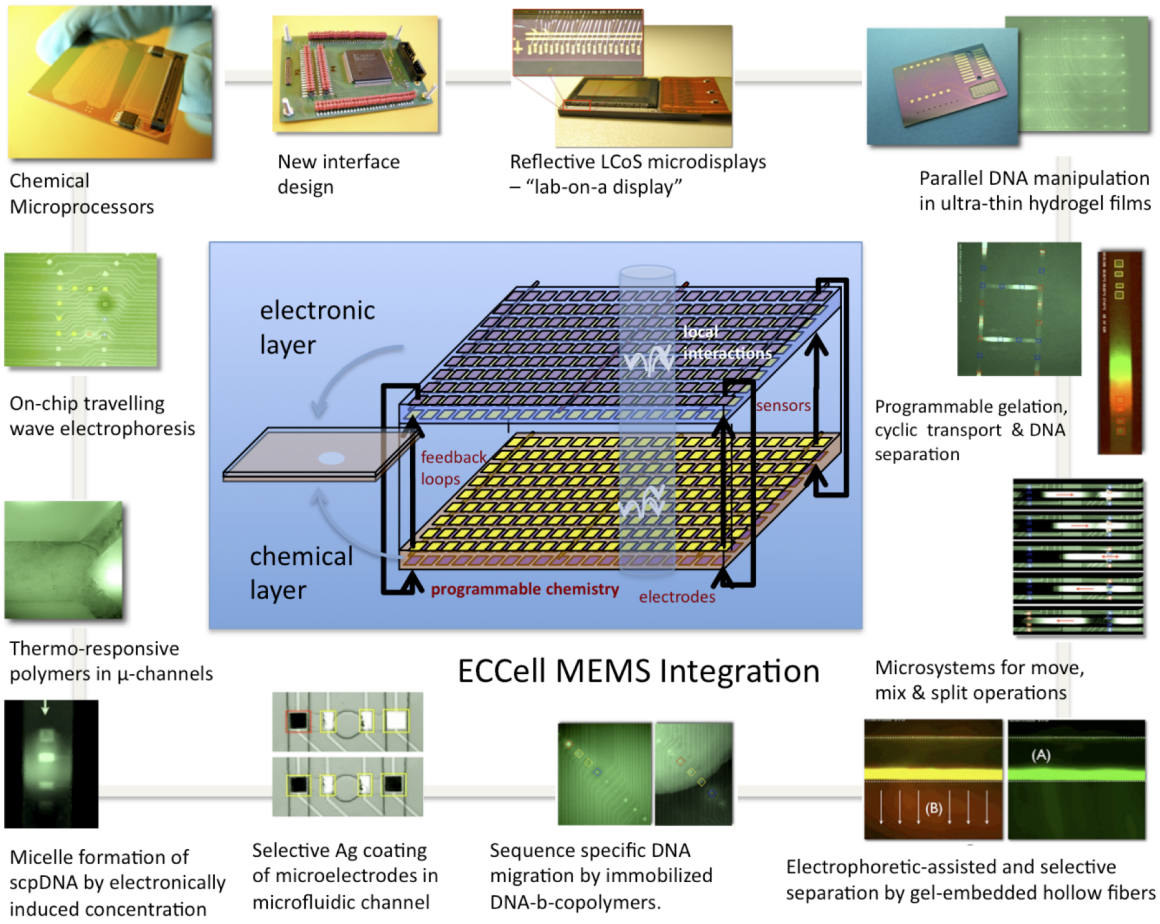


Figure 4.1: Overview of early progress in MEMS integration of ECCells. The figure shows the overall architecture and its implementation with chemical microprocessors. The images on the border illustrate separate MEMS solutions for aspects of the three components of the integrated ECCell: the replication system, the containment system and the metabolic system.

4.1 Electronic structures (RUBa)

Since integrated electronics with active transistor components, at the scale of cms needed for electrode arrays in the ECCell project, proved prohibitive in terms of cost, attention initially focussed on passive electrode arrays driven by an external reconfigurable electronics chips such as FPGAs. These structures can be produced with a limited number of supra-micrometer masks at whole wafer scale at reasonable costs. The most economical approach with a single layer routing design and embedded electrodes accompanied the work in most of the project. Through careful design optimization, sufficient (of the order of 100) independently controlled electrodes could be achieved in a periodic structure, at the cost of smaller electrodes (20 µm) in the one layer design, to test functionality and emulate the properties of the electronic chemical local feedback. A series of fabrication strategies were explored to attain reliable multilayer structuring of gold electrode interconnect on our chemical microprocessor chips, with the final approach, developed by RUB together with iX-factory in Dortmund, resulting in a robust cross-over wiring of the chemical microprocessor chips.

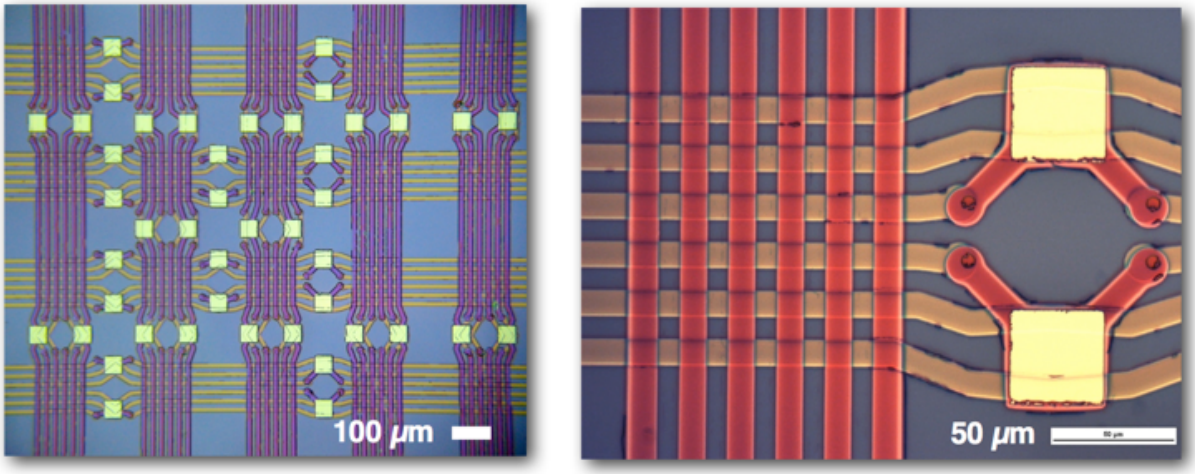


Figure 4.2: The light microscopic images showing the successful processed double layer multilayer electrode array network. The close-up (right) shows the exposed gold electrodes (gold) and two insulated (beneath SiO_2) layers of gold interconnect (red and brown shades in right picture).

The technological transition to the two-layer electronic chip design (figure 4.2) leads to an increased integration density to more than 5.000 electrodes for the same chip space, which allows a more effective platform for future ECCells and other applications. Because connections are possible underneath the electrodes, the electrodes can also be made twice as large as in the single layer designs, which allows larger surface and diffusion-limited currents to be sustained. The production of these robust chemical microprocessors will be a lasting dividend of the project that can be exploited in a wide range of follow-on activities.

A second transition to over a million electrodes, using a prefabricated active electronics chip designed for LCOS displays (Aurora) was also investigated, see also Fig. 4.1. The 8x8µm Al coated electrodes used in the device are closely spaced (1µm gap) but electrically insulated and used to drive a liquid crystal layer. However, Faradaic reactions are necessary to maintain the DC electrophoretic drive of molecules in the chemical microprocessor chips, and Al electrodes are too reactive with water to be useful. We removed the insulating layer with a selective plasma etcher, checking the residual electrical integrity of the device. We then coated the device with a monolayer film to (partially) inhibit Al electrode reactivity. Although electrode patterned processing of DNA could be demonstrated, the artefacts associated with Al electrode reactivity were too large to allow extensive use in the project. Production development costs associated with modified noble metallization (Pt, Au) for this commercial product would require large investments and significant development time. Software was developed to control the voltages and duty cycle of the LCOS display device to allow asymmetric driving potentials and variable effective voltages, so that the time response of the system would prove adequate for real time control. In summary, in contrast with dielectrophoretic manipulators for larger particles, the use of LCOS displays for electrophoretic manipulation of molecules currently suffers from the lack of inertness of the electrodes despite its eminent high integration density appeal.

4.2 Microfluidic structures (RUBa)

The microfluidic architecture started with a clear conception of the ECCell chemical medium as a two phase system, with continuously flowing resources separated by hydrodynamic barriers from (but connected via diffusion with) a stationary (reversibly gelled) array where the chemical reactions and information processing chemistry (including electronic separations) takes place. The architectural design tasks were primarily to allow resources (and wastes) access to as many points of the ECCell medium as possible, without disrupting the ability to renew the gelled reaction medium and extract products for analysis. Although multilevel fluidic channels would allow a more flexible topology of individual access of resources to local sites in a 2D array, the higher fan-out/in associated with individual branched access brings additional technical integration challenges and were avoided in this project. Instead, the simpler and originally proposed scheme of flow-by resources (like a river serving a neighboring billabong lake) was pursued. However, it proved beneficial to divide the active information processing part of the array into two subsystems: chemical reaction chambers and gelled communication network.

Thus, compared with the originally proposed design in fig. 4.3, the communication network was connected more extensively, to allow packet bypass and parallel processing with a limited number of electrode control lines, and the microreaction chambers (round in fig. 4.3) themselves became a connected subnetwork as shown in the implementation of fig. 4.4, separated from the resource and communication networks by hydrodynamic barriers, and allowing separate filling. The direct short-circuit connection between these reaction chambers (such as A and B in fig. 4.4) via their filling channel line is either long enough to be isolated, or separated by ionic liquid in a two phase droplet chain filling of the reactor network. The latter allows reactor contents to exported from the chip without longitudinal mixing along the reaction chamber IO. The communication channels in fig 4.4 are aligned with chains of electrodes to allow local feedback controlled electronic transport and separations.

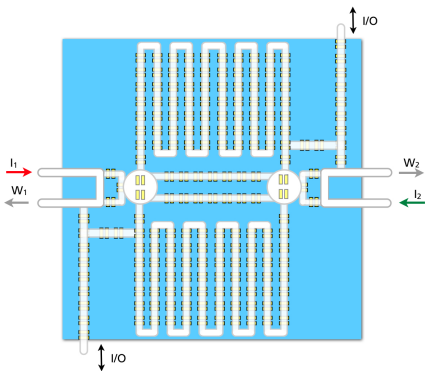


Figure 3.3 Original schematic of an ECCell hardware module. The scheme shows a twin chamber molecular amplification module operating via pH modulation cycles and a feedback separation line for periodic molecular separation. Furthermore, it contains microfluidic input channels (I_1, I_2) and output channels (W_1 and W_2) connected to the external pressure driven fluidic system.

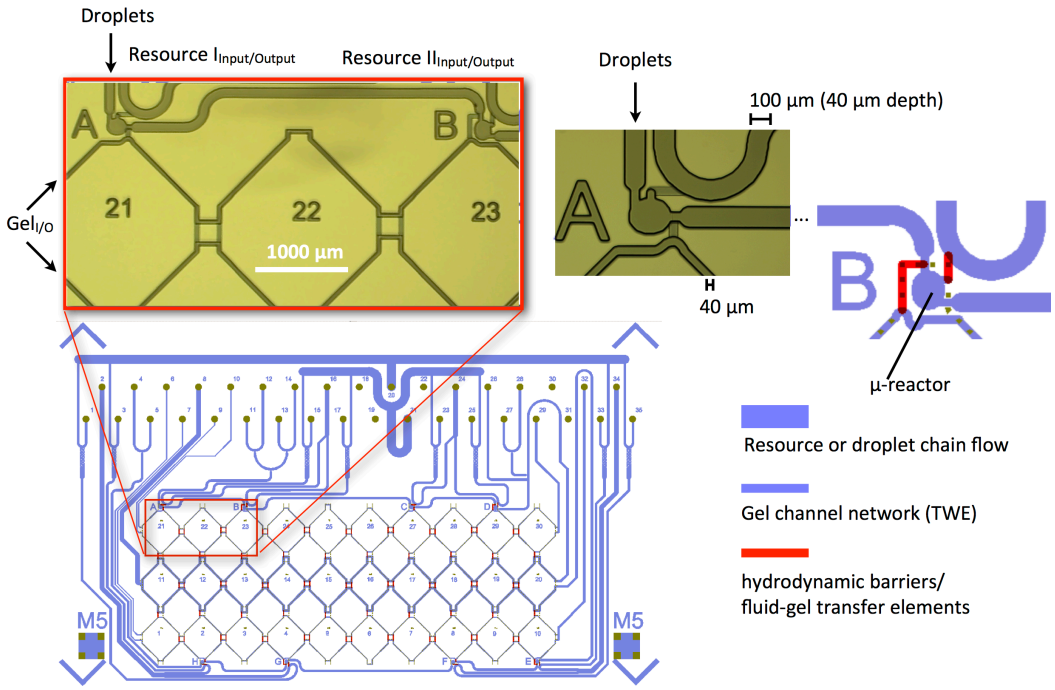


Figure 4.4 Final mask design for the microfluidic integration to do a complete cycle of droplet reaction, extraction to gel, product separation in gel as well as the content transport between the two chambers. The light microscopic images show the master structures for micro-moulding process in PDMS. The twin reactors A and B together allow the functioning a single ECCell as described in 4.6.

4.3 Autonomous selective transport of DNA with feedback-coupled electrodes (RUBa)

The first major roles of electrodes in the project was to provide programmable containment and molecular selective mobility, to allow membrane-less "cells" to function with the equivalent of cellular compartmentation. More prosaically, the integration of chemical reactions involves product cleanup steps that generically involve molecular separations, and so electronically integrated separations become a fundamental operation in chemical processing. The obvious approach to this problem is to scale down the voltage and distance between electrodes, keeping the field strength constant, to allow local control of the separation process. Difficulties in this procedure involve the necessary Faradaic reactions at the proximate electrodes and their cumulative impact on the solution constitution and transport properties. At low carrier salt concentrations fields are largest, but the impact of electrochemical reactions on the solution are fastest and largest. The use of a zwitterionic buffer like histidine is well established to help deal with these problems, and maintain constant pH, and this technique was also employed in our research. In addition we optimized and employed a reversible gel system involving various length triblock copolymers (Pluronic) for viscosity and separation properties.

Conventional travelling wave electrode control sequences suffer from lack of integrated sensing for optimal separation. Because electrodes vary in the rates of chemical reactions at their surface, and because of the hysteresis induced by heterogeneous solution concentrations, it is essential to provide feedback on the response of the molecular system to the electric fields into the control process to ensure reliable separation. This example of fine-grained systematic local feedback control was fully developed and tested in the project (including the major software development reported in Section 5). An example of successful autonomous separation of two DNA sequences locally on the timescale of a minute (compatible with typical replication times) is shown in Fig 4.5.

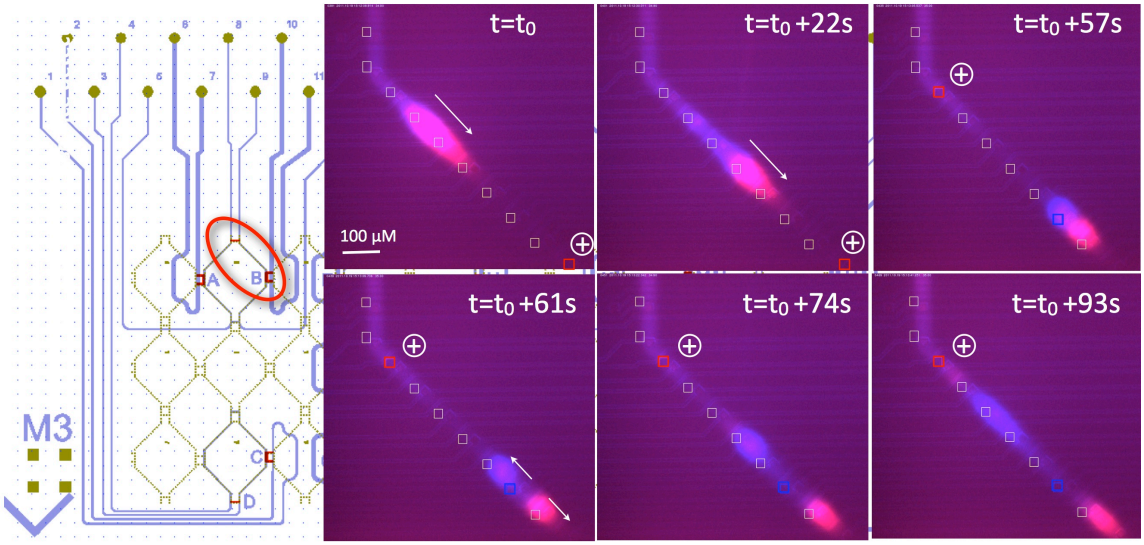


Figure 4.5: Selective transfer of DNA between sites under autonomous feedback electronic control. Two different oligonucleotides (labelled red and blue) in a mixture could be autonomously separated and one transferred to a second reaction site by the coupled two-layer electronic-chemical sensor-actuator system developed in ECCell. Channel 9 is filled with 30% Pluronic (F87 : F127; 2:1) in His-buffer 50 mM pH 7.2 + 5 mM Na_2HPO_4 Oligo20 (8nt) Alexa647 10^{-7} M + Oligo1 (30nt) OregonGreen 10^{-7} M. Channels 10, 11, 5 and 8 are filled with H_2O. Pluronic melting temperature T_m = 22°C.

4.4 Electrochemical reversible local pH and DNA hybridization cycling (RUBa)

Ideally, the surface modified electrodes developed by Willner's group at HUJI (see Section 3) would provide a local cycling of pH (between 5.7 and 7.2 or more because of the larger surface volume ratio) that is relatively independent of solution chemistry. Attempts to extend this procedure to inaccessible microelectrodes in microchannels at RUB, however, have not yet proved completely successful, although other electrochemical reactions were successfully miniaturized to the gold microarray, with cyclic voltammetry indicating that although all the electrochemical steps were functional, insufficient active surface area was achieved probably owing to the incumbent inflexibility to adjust the crystalline properties of the gold surface of the microelectrodes after or during the complex fabrication process of the devices. Nonetheless, a functional electrochemical system was

ported to the chemical microprocessor context and various other electrochemical reactions, including ruthenium bipy complexes (also used in artificial cell metabolism by SDU and Los Alamos), PANI polymerization and silver deposition, were measured using cyclic voltammetry (CV). The scaling of CV sensitivity down to the chemical microprocessor microelectrodes was also analysed.

In order to complete the pH cycling actuation, another system, involving quinhydrone chemistry, in the bulk solution was established, using SNARF fluorescence and ratio imaging to calibrate the pH changes. The former had originally been proposed for free flow electrophoresis experiments[9]. In fig. 4.6 we show the systematic inversion of pH (color codes as in litmus paper but for the pH range 5.5-8) achieved without gas formation using small groups of electrodes and the hydroquinone (HQ) and p-benzoquinone (BQ) system. In fig. 4.7, the effective switching of local pH at a single electrode is demonstrated. The color code used here is different to improve the contrast, but the fluorescent signals are as in fig. 4.6. In Section 4.6, this switchable pH will be used to switch triplex DNA hybridization.

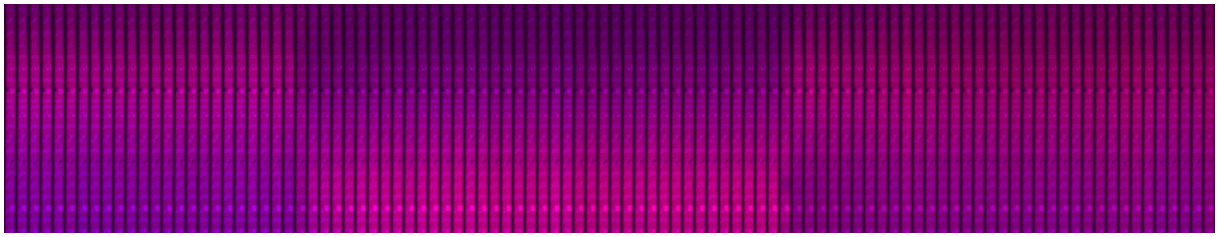


Figure 4.6 Electronic regulation of pH using hydroquinone sytem. A stack representing emission wavelength shift of SNARF-4F dye induced by pH changes taking place on electrodes in a single microchannel. Fluorescence passed by 560-600 nm filter was colored as red, whereas 660-700 nm filter as blue to reflect litmus coloring of pH responsible for these bands. Among nine electrodes in the channel three at the bottom and three at the top of the channel were active, whereas three in the middle were neutral. There are five series of images with reverse palette indicating opposite configuration of active electrodes. Each series was recorded with 488 nm laser excitation over 6 min and images were acquired at exposure time 1 s using the BioPro software (Section 5). The external potential applied was 1.8 V without any alternating duty cycle. Red color is concerned with production of protons at positive electrodes, whereas blue - with production of hydroxy-ions at negative electrodes. pH changes were induced via corresponding reduction of quinone and oxidation of hydroquinone at the electrodes. Concentrations: 30 mM BQ, 30 mM HQ, Sörensen buffer 8 mM.

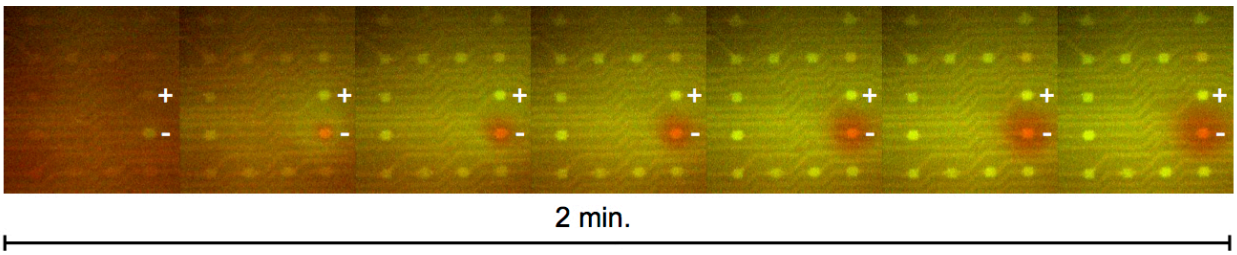


Figure 4.7 pH change at a single microelectrode. Quinhydrone redox-couple detected by pH-dependent SNARF fluorescence and ratio imaging. Fluorescence image series: red and green colours indicate 660-700 nm and 560-600 nm emission filters, respectively. Two electrodes on the right hand side of the electrode array were on. Corresponding to high pH produced at negative electrode, we observed the SNARF wavelength shift towards 700 nm.

4.5 Sequence specific reversible transport modulation (RUBa, RUG)

The sequence-specific retention of DNA in reversibly programmable gel matrices was developed in Section 2, and was integrated as a basic functionality into the chemical microprocessor system for ECCell. Its potential application in the ECCell life cycle is discussed in the next subsection and its application in more general DNA processing in "smart gels" is presented in Section 6.1. In two decisive experiments, the sequence specific modulation of transport properties was demonstrated as shown in fig. 4.8.

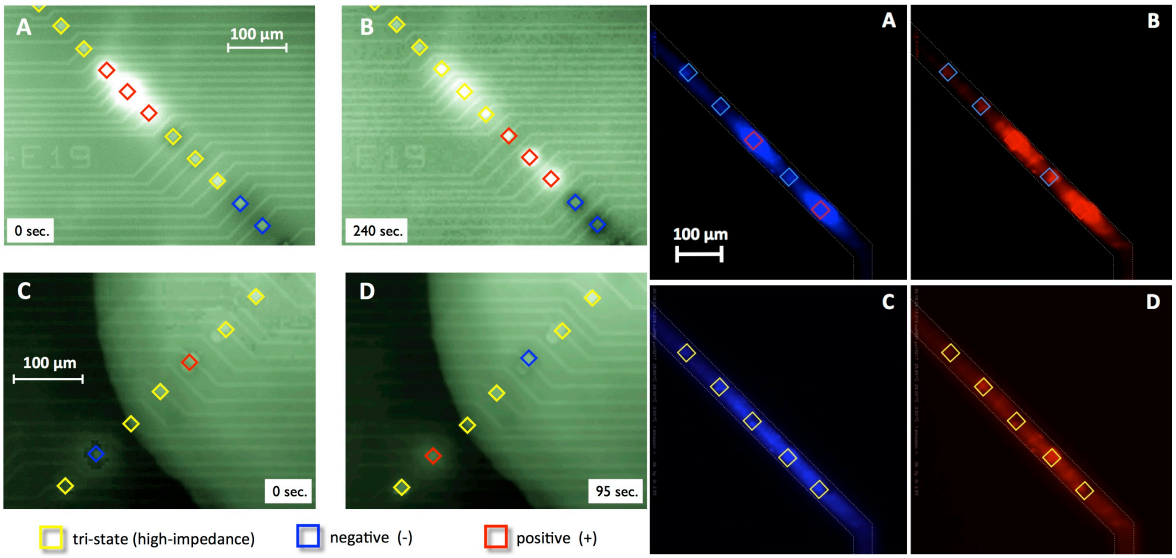


Figure 4.8: Sequence specific mobility of DNA in amphiphilic-DNA (scpDNA) loaded gels. Top left: Non matching DNA in gel with amphiphilic DNA anchor. The images **A** and **B** (from a movie, supplementary material) show that negative charged electrodes repel the non-complementary strands and positive charged electrodes lead to a concentration of the non-matching species from the surrounding region. **Bottom left**: two different gels, one (light region) with matching amphiphilic DNA, the other (dark) without. The images **C** and **D** show only marginal mobility of complementary strands in the light region when driven by the electrodes. **Right**: mobility difference between matching (blue) and non-matching (red) DNA in microfluidic channels filled with a Pluronics gel containing amphiphilic DNA anchors as developed in WP2 (Section 2). Concentration by the electrodes is slower and less complete for the matching DNA, consistent with the significantly lower mobility measured in gel shift assays. **A,B** electrodes on (see color coded legend of voltages) **C,D** electrodes in high Z state (off). Material: embedded amphiphilic DNA 12mer ssU4T : 25% Pluronic F127 = 1 : 100, $2x10^{-6}$M Atto488 5'-GAA TCC GCA AAA-3' (cDNA 12-mer), $2*10^{-6}$ M Alexa488 5'-TTC GAT GAT GCG C-3' (non matching DNA) Experimental conditions: 30°C, 25% w/v Pluronic F127. Excitation wavelength: 488 nm.

4.6 Steps towards a DNA triplex-based life-cycle and other achievements (RUBa, RUBb)

Other achievements of WP 4 in the project include the design and extensive deployment of an integrated capillary electrophoresis setup, compatible with our control/monitoring environment, the design of a ZNA based autonomous amplification system, solutions for all the problem domains listed at the beginning of this section (resulting in several publications) and the work presented in the introduction. We complete this section with a brief analysis of the microfluidic experimentation towards the DNA triplex-based life cycle.

Firstly, two triplex systems (one with a hairpin connector and one without) and adapter and toehold DNA strands to allow the attachment of the system to amphiphilic DNA anchors (as in Section 2 and 4.5) were designed and tested. In particular, the thermodynamics and kinetics of hybridization to form double stranded and triplex complexes was analysed as a function of temperature and pH. Similar results are known in the literature for other DNA sequences. Capillary electrophoresis was then used to analyse coupled migration and hybridization using multiple colour fluorescent labels on the DNA strands. A consistent picture confirming triplex formation at low pH (5.5-6) and dissociation at higher pH (over 7) emerged, including the known but tolerable delays in association kinetics for the triplexes (these can be accelerated electronically by migration).

Fig. 4 of the introduction shows two alternative versions of the local two chamber microfluidic systems for electronically regulated replication in ECCell. These are employed in a two stage electronically synchronized cycle as outlined in fig. 5, another variant of which is described in Section 1.2. This type of cycle was pretested extensively in a custom capillary electrophoresis setup using macroscopic chambers and high voltages. The design and testing of the toehold DNA resource release system (coupled to the amphilic DNA anchors of Section 2 and 4.5) and the mobility assays for the triplex hybridization system was completed by RUBa. This work will be published elsewhere. The operation of the entire cyclic amplification system has not yet been achieved under electronic control in the chemical microprocessor.

5 ECCell Simulation and Programming (SDU, RUBa)

Three main tiers of simulation and programming are: (i) a mesoscale simulation facility based on a novel extension of DPD (dissipative particle dynamics) with dynamic bonds, which provides tabular data describing more accurately properties of DNA-strands subjected to electric fields (ii) an ionic and electrochemical based calculation of dynamic field and concentration effects based on nonlinear partial differential equations (extending the Poisson Nernst Planck equations to an efficient pseudo 1-dimensional analysis including finite ion-size effects) which finally is integrated into (iii) the simulation package of the software (ng_biopro) controlling real experiments. The following table explains some of the phenomena and methods used to work on these scales.

Scale	Technique/Software	Physical phenomena	Goals
Molecular	Dissipative Particle dynamics simulations using a customized version of Large-scale Atomic / Molecular Massively Parallel Simulator (LAMMPS) that incorporates electric fields and pH fields from the microscopic scale.	Molecular architecture, hydrodynamics, self-assembly. DNA template replication. DNA electrophoresis, isoelectric focussing.	Understanding the fundamental physics of self-assembly, isoelectric focusing, DNA hybrid-izations. Predicting electric mobility and diffusivities.
Ionic reaction-transport	Symbolic algebra driven automatic numerical code creation via Mathematica to perform analytic and numerical analysis of electrochemical reaction-transport theory.	Dynamics of Faradaic reactions, the electric double layer at the electrode interface, DNA and solution ion transport and surface electrochemistry.	Predicting the electric and chemical potentials, electric field and ionic concentrations, including pH in the bulk.
Systemic	Custom written systemic simulation/control program making use of simplified mobility and diffusivity data obtained from mesoscopic scale.	ECCell systemic response to spatially and temporally varying potentials incorporating all emergent properties at the scales above.	Achieving transparent ECCell simulation and control.

Table 6.1: Overview of scales. Three levels of scale are involved in the ECCell software development.

In addition to this simulation-centred software development, a major achievement in the project was the development of a realtime control system for the ECCell laboratory environment, including the chemical microprocessor and coupled microfluidic control and fluorescence microscope feedback system. A complete experimental control system was developed by RUB (building on earliear work in the PACE project of FP6).

5.1 Dissipative particle simulation of life-cycle components (SDU)

Classical DPD must be extended with angle dependent bonding to model the details of DNA hybridization. The coarse-grained modelling of DNA and its template-directed replication is done via a Dynamic Bonding approach. We have chosen to model recognition/hybridisation using bonded interactions rather than non-bonded interactions. The latter method has the advantage that it is much simpler to implement, but requires quite detailed attention to create complex directional interactions that ensure strong recognition and stable double strands while avoiding unspecific aggregation.[10] Dynamic bonding requires that we continually check for hybridisation bonds to create and break, based on the distance between complimentary beads. However, unspecific aggregation can be solved simply by restricting each bead to have maximally one hybridisation bond at a time. Furthermore, we can extend the dynamic bonding to include dynamic angular and dihedral interactions to stabilise the desired double stranded conformation. The introduction of a hybridisation bond is a binary event, hence the "chemical" structure of a complex of strands is always well defined.
Using this approach, the life cycle of various versions of the ECCell can be simulated, incorporating the interplay between reactions and transport with specific reference to structural features associated with the complex hydrogel milieu and the interplay of hybridization kinetics with transport and drift in the applied electric fields.

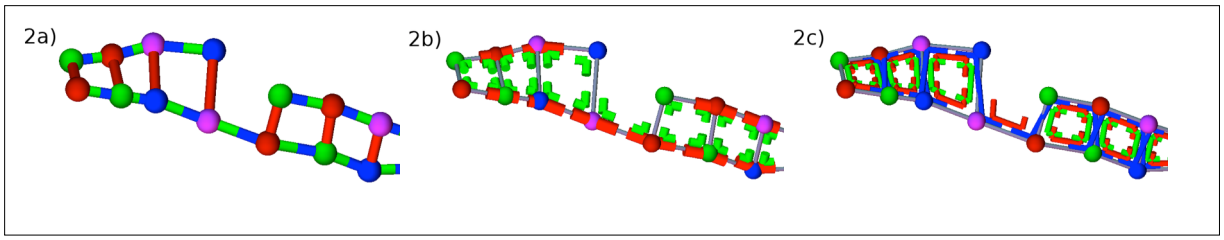


Figure 5.1 Angular bonding extension to DPD. The figure shows the same configuration visualising bond (left), angular (middle), and dihedral interactions (right). The middle visualisation shows the angular interactions as red and green cylinders around the bead that forms the angle with its two bonds. The right visualisation shows all the dihedral interactions. These are visualised as three connected cylinders slightly offset from the three bonds across which the dihedral interaction is defined. The red angular interactions stabilise the single strand backbone stiffness, while the green angular interactions ensure that hybridization bonds are orthogonal to the strand backbone.

The DPD-based simulations allow the complex molecular interactions and self-assembly processes associated with reversible immobilization and transport-mediated ECCell life-cycle to be simulated, on timescales that are inaccessible to molecular dynamics. Of course, the loss in detail is significant. Fig. 5.2 shows some examples from the double stranded life cycle proposed earlier in the project.

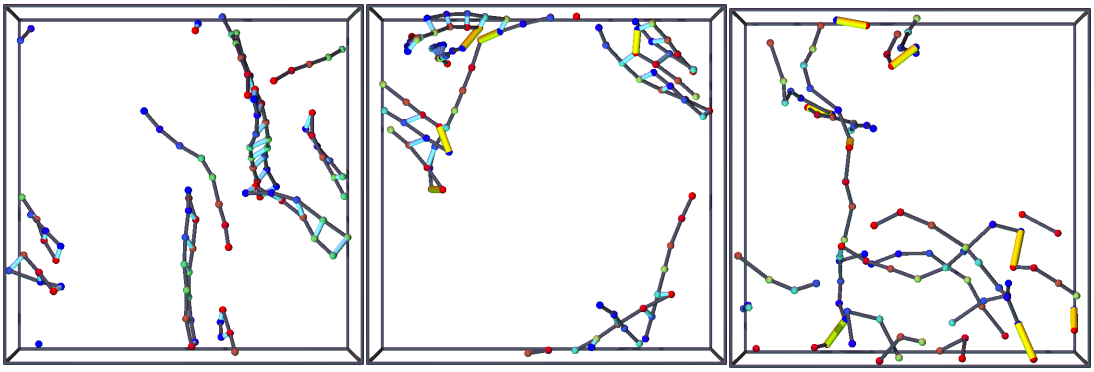


Figure 5.2 Snapshots from part of the ECCell lifecycle simulated by dynamic bonding DPD. The figure shows hybridization (left), ligation (middle), and dissociation (right) of DNA.

5.2 Electrochemical simulation of reaction-transport for ECCell (RUBa)

Electronic chemical cells depend on the interplay between local electrode switching and chemical reactions. Understanding the complex and nonlinear physical and chemical phenomena resulting from this interaction is still a subject of intensive research in electrokinetics and electrochemistry[11]. The reaction-extended and modified Poisson Nernst Planck (PNP) equations, which are coupled time and space dependent partial differential equations, provide a useful nonlinear point of departure, extending reaction diffusion (pattern forming) systems with collective electrostatic effects and the interaction with the external fields induced by electrodes. The digital voltages employed (1.2-3.3 V) are large (100X) compared with the thermal voltages of 25 mV and so in equilibrium the potential must be computed from the nonlinear modified Poisson Boltzmann equation, where the Bikerman modification is for the excluded volume resulting from finite ion size effects[12]. These arise even in dilute solution when ions "condense" near a charged surface, resulting in an excess chemical potential μ that can be employed in the full extended PNP equations for concentrations c_i and electric potential $\boldsymbol{\phi}$, which, assuming constant dielectric constant $\varepsilon = \varepsilon_0 \varepsilon_r$ and negligible cross-effects, take the form[13,14]:

$$\frac{\partial c_i}{\partial t} = \nabla D_i(\nabla c_i + \frac{z_i e \nabla \phi + \nabla \mu}{k_B T} c_i) \qquad \nabla^2 \phi = -\frac{F}{\varepsilon_0 \varepsilon_r} \sum_i z_i c_i \qquad \mu_{ex} = -k_B T \ln(1 - \sum_i a_i^3 c_i)$$

where F is Faraday's constant, a_i^3 is the bulk volume fraction, D_i the diffusion coefficient and z_i e the charge of ion i. The boundary conditions at the electrodes involve the reaction flux of redox active species which undergo Faradaic reactions at the surface, which can be expressed by the (nonlinear) Butler-Volmer formula with the Frumkin correction[15]. The direct numerical solution of these equations in 3D is complicated by the sharp boundary layers (double layer) and nonlinearities, and prohibits their use in online applications and for combinatorial explorative time dependent fields resulting from

complex electrode voltage switching in microfluidic systems. A procedure employing matched asymptotic expansions to couple different space scales e.g. [16], is difficult to extend from parallel plate capacitor geometries to 3D, and would also not meet the above requirements.
Earlier work in the project concentrated on independent migration of DNA in an electric field resulting from a potential computed by the solution to Laplace's equation, which is approximately valid in the limit where ion concentration gradients are small[15]. The boundary conditions for Laplace equations are of Robin type if reactions are taken into account and depend on the time development of surface charge. The initial plan was to generalize the iterative self-consistent (matched asymptotic expansions based) procedure proposed by Suh and Kang[17] but this is also computationally intensive in 3D and involves some questionable approximations for non-planar geometries. Instead, we built on a one-dimensional solution of the full modified PNP equations, generalizing work on modelling ion channels in membranes with complex geometries[18,19], which complement the work on (potential free) reaction diffusion equations[20], that field driven ionic migration in the PNP equations can be effectively described as one-dimensional if the varying area of the equipotential surfaces along the one-dimensional coordinate is taken into account – it effects both the ion and potential flux and the volumes of successive 1D slices:

$$\frac{\partial c_i}{\partial t} = D \ln A(x) \left(\frac{\partial}{\partial x} c_i + \frac{z_i e \frac{\partial}{\partial x}(\phi+\mu)}{k_B T} c_i\right) + D \frac{\partial}{\partial x}\left(\frac{\partial}{\partial x} c_i + \frac{z_i e \frac{\partial}{\partial x}(\phi+\mu)}{k_B T} c_i\right) \qquad \frac{\partial^2 \phi}{\partial x} + \frac{\partial \phi}{\partial x}\frac{\partial}{\partial x} \ln A(x) = -\frac{F}{\varepsilon_0 \varepsilon_r} \sum_i z_i c_i$$

We found that a transformation to appropriate area weighted coordinates (found by solving an ancillary differential equation) further simplifies these equations by removing the logarithm terms and leads to a robust numerical procedure for electrodes in microfluidic channel geometries. We employ a once off solution to Laplace's equation for electrodes in closed channels to derive this area function. Calculations for pairs of banded electrodes using analytic conformal solutions to the Laplace equation revealed that the equipotential surfaces in microfluidic channels could be well approximated as perpendicular to superellipses, with similar results expected for square electrodes and superellipsoids. This observation was used to map back the computed one-dimensional results to three dimensions for the simulation and interpretation of experimental observations. In particular, if one of the species is H+, this formalism may be used to describe electronically induced migration of DNA interacting with electronically induced pH effects. The coupled time and one-space-dimensional system of equations for $c_i(x), \phi(x), \mu(x)$ is solved using a semi implicit Crank Nicholson scheme on an expanding space grid (with more points near the electrodes). The nonlinear update rule for each new time step is solved using a rapidly converging iterative Newton Rhapson procedure employing the solution of a linear Jacobian matrix equation at each iteration as proposed for example in[21].

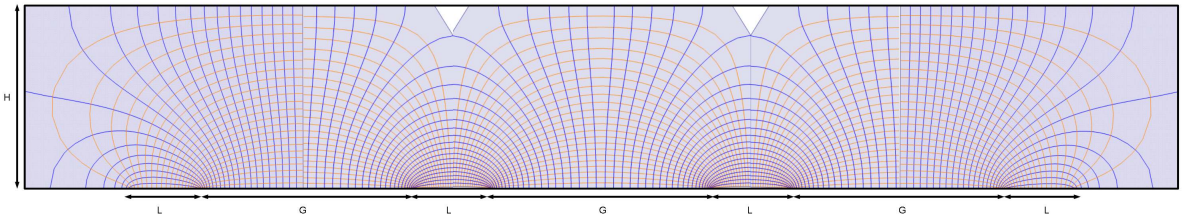


Figure 5.3 Spliced conformal solution of potential (blue) for banded electrodes of length L with gaps G in a channel of finite height H according to the Laplace equation. The dielectric constants of PDMS and SiO_2 are low enough to allow a zero gradient boundary condition at the channel walls. The potential contours are used to convert the one-dimensional solution of the fully non-linear finite salt solution with Faradaic reactions to the potential as a two dimensional function of channel height and distance along the channel. Three dimensional solutions have been found using the NEBEM method numerically[22,23], and both can be approximated using simple analytical functions in the form of superellipses (not shown).

5.3 Integrated experimental workstation software (RUBa)

An extensive integrated software environment for programming electronic chemical cells has been developed at RUBa. The overall goal for this software is to facilitate and operate the electronic chemical microprocessor chips with a hundred or more electrodes and many different variants of fluidic designs. To illustrate the complexity of the software: about 200,000 lines of code and several man-years of development are behind it, providing a general framework for experimentation and documentation in both manual and automated modes. The developments in ECCell involved feedback

algorithms, additional device interfaces and functionalities, including an integrated simulator to accompany real-time experiments. The software runs on Linux and MacOS-X.

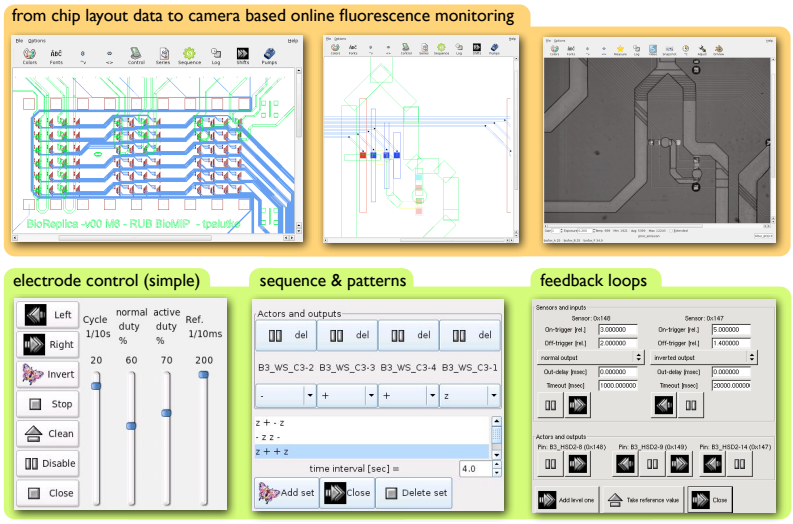


Fig. 5.4 Control and simulation software interface (ng_biopro). The image shows several of the many control and monitoring windows of the software. Design information of electronics and microfluidics is integrated with current optical microscopic feedback to allow user-mediated or autonomous local feedback algorithms to couple chemistry and electronics.

The control-software (ng_biopro) has been extended with an electrokinetic based particle swarm simulator package. All geometries are taken from the real design data used to produce the actual devices. This simulation package is fully integrated into the ng_biopro-software and usable in parallel with ongoing real experiments. The simulation itself is mapped via a camera-view into the user-interface and it thus it represents an additional device in the normal experimental procedure. An extensive user manual is available with the software.

5.4 Feedback control autonomy and software development (RUBa)

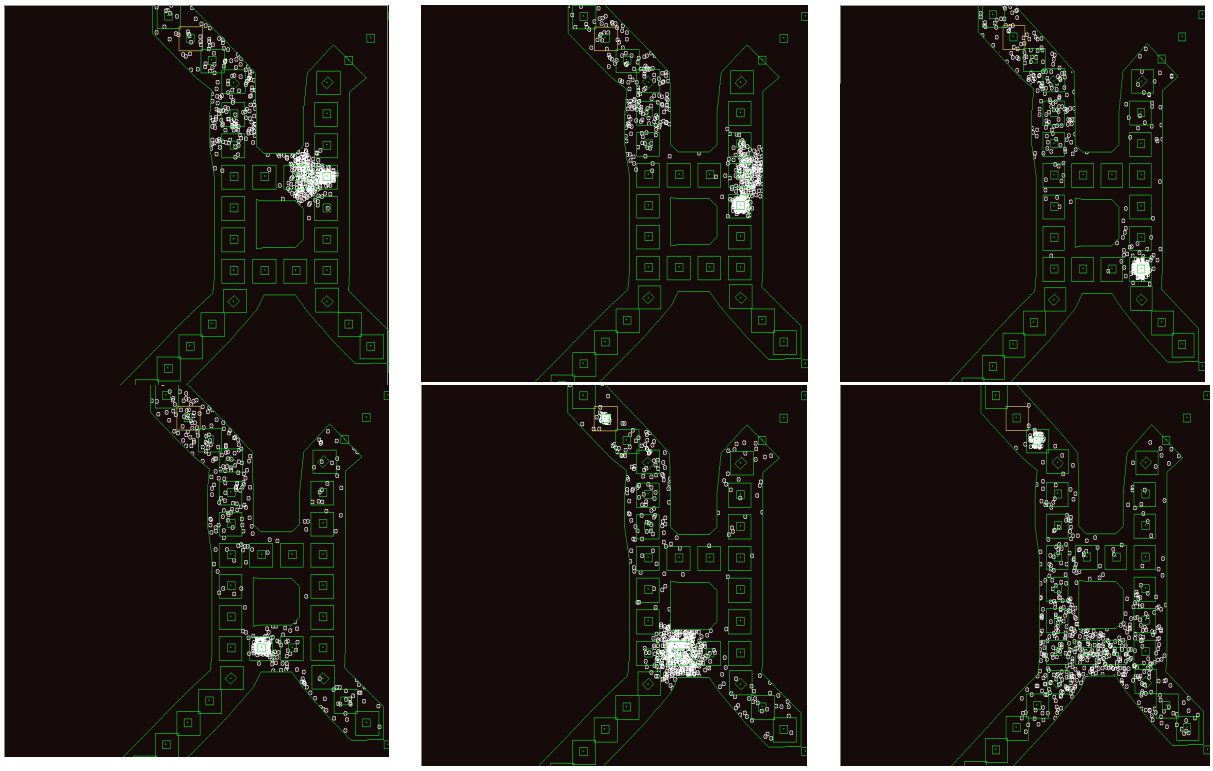
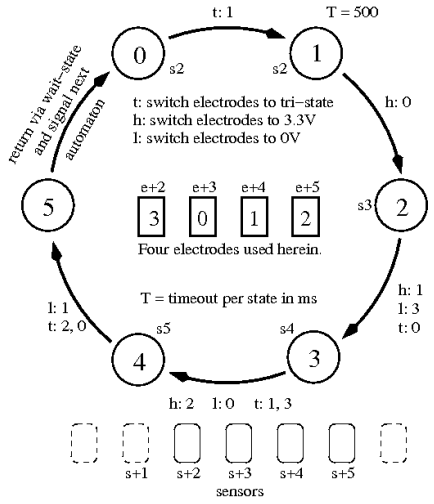


Figure 5.5: The particle cloud movement under feedback control after the second state-machine (extract_middle, see scheme 2) has been invoked. The purpose of this state-machine is to pull the material around the circle. When reaching the left channel again all electrodes are switched of and the particle diffuse freely.

Currently, integrated separation using electrophoresis relies on non-local electrodes with high voltages or on a form of Travelling Wave Electrophoresis (TWE). Usually TWE only works for larger particles, since it is based on blocking electrodes, supporting only dielectrophoresis, and requires

analogue control potentials, working with a repeat pattern involving a very small number (*e.g.* 4) of individually controlled electrodes. The target is to get maximally uniform fields to prevent particle dispersion and optimize the separation capability. With reactive electrodes as employed in ECCell, electrode switching timing depends on variations in electrode surface reaction kinetics (and coating variations) and other factors with extensive potential switching history dependence. The only robust way to achieve desired goals under these circumstances is to employ feedback controllers, rather than apply a predetermined time switching pattern, and for this, individually controllable digital electrodes are required.Instead of using subtle phase relationships of the moving particles and the sinusoidal potentials, our idea was to use a forward and backward tracking of the particles. The time constants used for forward tracking are chosen such that the desired particles are optimally drawn in the right direction and the undesired particles are optimally drawn backward. In a fundamental test case of such a problem, we constructed a system of autonomous controllers to first collect material from an input channel (top left in fig. 5.5) and then pass it around a loop. Two independent feedback-controllers are in action. The first feedback-controller is moving the cloud from the top left channel down into the loop. The second feedback-controller, is activated by the first feedback-controller after reaching the first junction. The first feedback-controller is stopped after handing over the activity to the second feedback-controller. In this case the handing-over is unconditional.

After the second feedback-controller is finished it restarts the first feedback-controller, which is launched from the beginning. Interestingly, in this second round all missing particles are collected from the first round and without changing thresholds and parameters the feedback-controller is robust enough to continue working even with hundreds of particles disturbing the reference system of the controllers. Finally, in fig. 5.6 we show the structure of a similar controller used to regulate the separative transport of DNA in the real experiment (shown in fig. 4.3).




```
control_group $1 0 $3
control_group $1 1 $4
control_group $1 2 $5
control_group $1 3 $2
control_sensor $1 $3 $4 $5
control_reference $1 $2 $3 $4 $5
set_sensor sen_$2 high_thr 1.70
set_sensor sen_$3 high_thr 1.70
set_sensor sen_$4 high_thr 1.70
set_sensor sen_$5 high_thr 1.70
nr_states $1 6 # 0->1 event=1, 1->0 event=0
# name state sensor event prio. tiout nstate
nrhigh {list} nrlow {list} nr-tri {list}
add_state $1 0 sen_$2 1 5 0 1 0 0 1 1
add_state $1 1 sen_$2 1 5 500 2 1 0 0 0
add_state $1 2 sen_$3 1 5 0 3 1 1 1 3 1 0
add_state $1 3 sen_$4 1 5 0 4 1 2 1 0 2 1 3
add_state $1 4 sen_$5 1 5 0 5 0 1 1 2 2 0
add_state $1 5 $6 1 5 0 9999 0 0 0
```

Figure 5.6: Left: State-machine diagram to transport DNA oligos. Sensors are coincident with electrodes with the only different the eight times larger area when compared in the camera image. This state-machine has six states and at the end is signalling the following state machine to commence operation. Separation is then realized via a second state-machine which declaration can be seen in Scheme 4.3. The resulting video can be seen in the supplementary material as ng_biopro_vid_20111019_1505xxx_0_elec.mpg. See also Figure 4.7 as an overview of the whole experiments with all chemical details used within. Right: The six-state state-machine used to transport the molecules along the chain.

5.5 The Electronic Cell and Electronic Genomes (RUBa)

For us to be able to consider the electronic control structure as "belonging" to the electronic cell, it is necessary to have a definition of cell ownership. The simplest structure of this type is a space associated one: co-localization of chemicals with the region of application of electronic control (electrodes and sensors) control creates the connection between the chemicals and the control program. While other exotic modes of connection (e.g. pattern based) are conceivable, we stay with this most obvious choice of topo-association. In principle this can be electronically or chemically determined or both. The chemical determination requires a mechanism of deducing a spatial location from a spatial sensor pattern. If there is only one cell in the system, then a sufficient algorithm would find the centre of intensity from the sensor array (when fluorescence intensity is proportional to concentration) and then establish a region (e.g. linear, circular or rectangular depending on microfluidic structures) of a fixed size about this centre. A more elaborate algorithm would also compute the range of the cell region. An electronic determination may associate the boundary of a cell with a particular pattern of electrode states (a field barrier preventing boundary crossing of key components analogously to a lipid membrane preventing material exchange). When more than one association between chemicals and electronic regulators must be established, this is relatively straightforward in the cases where cell sizes are fixed or the boundaries are clearly recognizable. It is clear that the definition of the boundary codetermines the behaviour of the system, so that simple physical principles are likely to prove the most apt for later evolution.

The model hierarchy that we have employed already contains (through inheritance) a location in each of its elements. A topo-association as described above comes into force when for example these locations are treated as relative to a centre of mass of replicating molecules. In a particularly simple case, imagine a molecular amplification process combined with an electrophoretic separation, product isolation and split control program. The control program will walk such a set of amplified molecules via travelling wave electrophoresis along a certain path, with its sensors and electrodes virtually staying within a fixed distance of the centre of mass of the set of molecules during movement, and the pulse-forming regulators virtually moving together with the majority of molecules. Of course, this process can be codetermined by the chemical properties (migration and reaction rates determining location and quantities) so that for example the initiation time, transport rates and splitting events are codetermined by the chemistry (e.g. splitting via reaction –diffusion non-linearity's in an electric field). Thus these processes can be achieved with a suitable control of electrodes or happen autonomously as a side effect of the regulator algorithms.

Having the controllers moving with the centre of masses of molecule collections, it is straightforward to consider also the parameters of these controllers moving together with the molecules virtually. If now, after each separation- or splitting-event, these parameters are slightly changed by random perturbations, an evolving population is already instantiated and evolution sets in to optimize the parameters. Parameter sets that are bad for replication yield diminishing sets of molecules, and this causes the deletion of the moving controllers and their associated parameters. This reflects death in the evolving system. The electronic genome concept thus involves a second type of genetic information associated with the cell: i.e. that involved in specifying the parameters and/or structure of the regulatory control program.

In summary, we have shown the role of both multi-level simulation and integrated feedback in fostering the development of ECCell component functionality, for all of the processes in the life cycle. The system is now fully automated and ripe to tackle the final unachieved task of integrating and optimizing all these component functionalities into a fully functional ECCell. This must remain the subject of future research.

6 Functionalization and ICT Applications (RUBa & all partners)

6.1 DNA processor as an application of ECCell technology (RUBa)

The full DNA Processor couples up to 8 separate ECCells for DNA processing reactions via DNA exchange and should allow separate regulated reactions and programmable selective DNA transfer via flexible gel separation tracks between reactors. It follows the same architectural principles of ECCell, as described in Section 4.2, with three separate subnetworks of channels: resource, reactor and communication. Enzymatic and non-enzymatic amplification reactions can be performed in the reaction chambers, and the products extracted and selectively delivered to other microreactors. The selective transport of special sequences, as required for one implementation of the ECCell life-cycle, has more general utilization in allowing specific processing of the products of DNA reactions and thereby the interconnection of different DNA processes. In order to test this general functionality, a test system with a programmable variety of functions, depending on the DNA sequences, and involving DNA amplification was sought. The choice of test system was alleviated by the recent progress in programmable DNA kinetic systems by the group of Montagne.

An enzymatic test system based on the Strand Displacement Reaction (in contrast with the DNAzymic reactions in Section 1.3) was employed because of its robust qualities[24]. This system was reoptimized in the RUB lab in another project (FP7 CADMAD #265505) and used as a test system in ECCell. Depending on the sets of DNA sequences employed, the system behaves as an exponential isothermal amplification system (that can be used to test DNA injection and electronically regulated replication) or as an oscillator. Both functionalities, found by Montagne et.al. have been confirmed in the RUB lab. Actually, this system is a non-evolvable (pinned) system that is different in the respect from the earlier oscillatory predator prey system of the McCaskill lab[25]. The DNA processor is capable of combining the parallel electronically regulated operation of these reactions with programmed transfer of DNA between processes to make online decisions of coupling subsystems, employing the local length based separations (Section 4.3) or sequence specific smart gels (developed in 2.1 and 4.5) to deliver only specific DNA sequences from one process to another. Details will be published elsewhere.

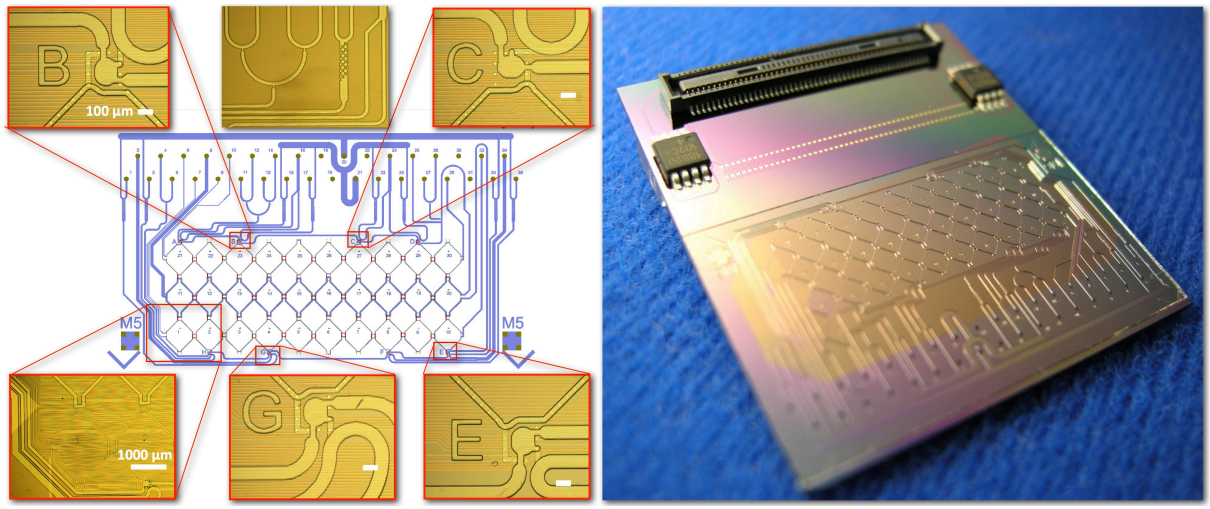


Figure 6.1 The final design for a multipurpose DNA processor arising as an application of the development of the ECCell technology. The chemical reaction chambers B,C,G,E (left image in the fluidic structure blow-ups) are adjusted slightly to match the pattern of independent electrodes available at the different locations, so as to ensure optimally independent operation of DNA sample extraction and dosing at the different sites. This DNA processor can simultaneously process 8 chemical reactions with programmed product cleanup and interchange between the processes.

6.2 Survey of ICT applications of ECCell (RUBa & all partners)

Electronic chemical cells provide an integration of chemical and electronic information processing, so that chemical systems can operate with enhanced local information control and the electronic systems can benefit from runtime chemical construction processes. Whereas the integration of chemical construction and information processing is complete in living cells, current chemical experimentation separates the control process, through macroscopic external systems such as pipetting

robots or pumped microfluidic systems, from the systems themselves. Electronic chemical cells target a modular cellular functionality, which involves the local control of ongoing chemical and electronic reconstruction processes and an ability to replicate both the chemical and electronic information. Current technology limits electronic reconstruction to reconfiguration, but both the electrochemical interfaces and the chemical systems can be fully reconstructed, allowing such systems to evolve increasing material and functional complexity once established.

The published survey (Deliverable 6.2) covers several distinct aspects of the ICT applications of electronic chemical cells:

(i) Applications to DNA information processing
(ii) Applications to molecular electronics and hybrid devices
(iii) Applications to Sustainable Personal Living Technology
(iv) Applications towards autonomous electronic chemical micro-reagents.

It does not deal with other non-ICT-centred applications for chemistry, biotechnology and biomedicine, although the programmable chemical processing capability of electronic cells suggest broad domains of applications to these areas. In particular, the ability to program the production of microscopic quantities of fresh chemicals on demand may have broad applications in chemical synthesis, and the ability to construct and deploy custom chemical analyses may have broad application in analytical chemistry and diagnostics.

Conclusions

The ECCell project has established a new domain of integrated electronic-chemical ICT, by researching and implementing tightly coupled twin-layer electronic and chemical systems with key examples from the domain of autocatalytic chemistry relevant to the construction of an electronic chemical cell. The project was a pioneering, decidedly non-incremental step into unknown territory. While the project was not in its lifetime able to deliver a fully functional electronic chemical cell, an architecture for this cell and concrete implementations of its component functionalities have been achieved, and we are confident that this objective can be reached in the near future. The remainder of the six main objectives of the project were all achieved. The joint architecture tested involves amphiphilic and disulphide triplex DNA that is cycled between twin chambers at two pH levels, with sequence specific immobilization to amphiphilic anchor DNA tags captured in a reversible gel matrix. In the course of this work, novel synthetic chemistry involving rapid disulphide DNA ligation, novel polymer chemistry involving amphiphilic DNA labelled compartments, novel analytical and electrochemistry involving electronically regulated ionic uptake and release systems and replication-based sensing were developed. A lasting impact of the project will also involve the completed online feedback hardware and software system, which integrates simulation into the current focus of experimentation on the real devices and the chemical microprocessors themselves.

About 50 publications in peer reviewed journals, as well as numerous conference and guest seminars have been held, and the project results have been the subject of press and media coverage including TV documentation. The project has given rise to extensive foreground that will be exploited in future projects and coordination actions, and in fact has helped to coordinate and focus the area of chem/bio ICT in Europe. An additional embedding in a larger framework of sustainable personal living technology (SPLiT) with connections to programmable fabrication technology and internet communicable chemical experimentation will carry the project results forward in future years.

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PI: principal investigator; TA technical assistant; St student.

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4.2. *Use and dissemination of foreground*

Section A. Dissemination Measures

This section lists the dissemination measures and includes two tables

- Table A1: List of all scientific (peer reviewed) publications relating to the foreground of the project.
- Table A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

During the 3 years of the project, a total of **57 peer reviewed publications** were published. It should be noted that some of these publications have been co-sponsored by other projects, (i.e. MATCHIT, CADMAD), partner universities or national funds, such as the Danish Science Foundation.

The **dissemination table** lists in **total 210 activities** in Europe and abroad, 170 of which were participations in conferences, workshops and meetings with more than 125 poster and oral presentations. The target groups included not only members from science and higher research worldwide, but in many instances civil society and policy makers, bearing witness of a wide dissemination activity during the lifetime of the project.

The content of the above tables are made available on the project website (http://www.istpace.org/ECCell) and in the EU public domain to demonstrating the added-value and positive impact of the project on the European Union.

Table A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS

	Title	Main author	Title of the periodical or the series	Number	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available)	Open access provided ?
1a	Electronically programmable membranes for improved biomolecule handling in micro-compartments on-chip	Chemnitz, S.,Tangen, U., Wagler,P., Maeke,T., McCaskill, J. S.	Chemical Engineering Journal	135S	Elsevier	Amsterdam	2008	276-279	http://dx.doi.org/10.1016/j.cej.2007.07.061	No
	Evolutionary microfluidic complementation towards artificial cells	McCaskill, J.S.	Protocells: Bridging nonliving and living matter		MIT Press	Cambridge, USA	2008	253-295		No
	A roadmap to protocells	Rasmussen, S., M.A. Bedau, M.A., McCaskill, J. S., Packard N H	Protocells: bridging nonliving and living matter		MIT Press	Cambridge, USA	2008	71-101		No
	Social and ethical checkpoints for bottom-up synthetic biology, or protocells	Bedau, M., Tangen, U., Hantsche-Tangen,	Syst. Synth. Biol.	3	Springer	N.Y., Heidelberg	2009	65-75	10.1007/s11693-009-9039-2	No

Spatially resolved simulation of membrane reactions and dynamics: Multipolar reaction DPD	Füchslin, R., Maeke, T., McCaskill, J.S.	European Physical Journal E (EPJE)	29	Springer	N.Y., Heidelberg	2009	413-448	10.1140/epje/i2009-10482-x	No
Coarse-Graining and Scaling in Dissipative Particle Dynamics	Füchslin, R.M., Fellermann, H., Eriksson,A., Ziock, H.-J.	Journal of Chemical Physics	130	ACS Publications	Washington, USA	2009	214102-1 - 214102-8	10.1063/1.3143976	No
Living technology: Exploiting life's principles in technology	Bedau, M., McCaskill, J.S., Packard, N., Rasmussen, S.	Artificial Life	16	MIT Press	Cambridge, USA	2010	89-97	10.1162/artl.2009.16.1.16103	No
Living technology: 5 Questions, Contribution John McCaskill	McCaskill, J.S.	Living technology: 5 Questions	Book	Automatic Press/VIP	Denmark	2010	111-121	http://www.vince-inc.com/automatic.html	No
Enzyme-like replication de novo in a micro-controller	Tangen, U	Artificial Life	16 (4)	MIT Press	Cambridge, USA	2010	311-328	DOI:10.1162/artl_a_00012	No
The emergence of replication in a digital evolution system using a secondary structure approach	Tangen, U	Alife 12		MIT Press	Cambridge, USA	2010	168-175		No
Biological and Chemical Information Technologies	Amos, M., Dittrich, P., McCaskill, J.S., Rasmussen, S.	Procedia Computer Science	7	Elsevier	online journal	2011	56–60.	http://www.sciencedirect.com/science/journal/18770509	No

	Field programmable chemistry: Integrated chemical and electronic processing of informational molecules towards electronic chemical cells	Wagler, P., Tangen, U., Maeke,T., McCaskill, J. S.	Biosystems	in press	Elsevier	Amsterdam	2012		http://dx.doi.org/10.1016/j.biosystems.2012.01.005	No
1b	Self-Assembly of a DNA Dodecahedron from 20 Trisoligonucleotides with C3h Linkers	Zimmermann, J., Cebulla, M., Mönninghoff, S., von Kiedrowski, G.	Angew. Chem.-Int. Edit.	47(19)	Wiley VCH	Weinheim	2008	3626-3630	DOI: 10.1002/anie.200702682	No
	Systems chemistry on ribozyme self-construction: evidence for anabolic autocatalysis in a recombination network	Hayden, E. J., von Kiedrowski, G., Lehman, N.	Angew. Chem.-Int. Edit.,	47(44)	Wiley VCH	Weinheim	2008	8424-8428	DOI: 10.1002/ange.200802177	No
	Self-Replication and Autocatalysis, in Protocells: Bridging Nonliving and Living Matter	Patzke, V., von Kiedrowski, G.	Protocells: Bridging Nonliving and Living Matter		MIT press	Cambridge, USA	2008	200-316	ISBN-10: 0262182688	No
	Welcome Home, Systems Chemists!	von Kiedrowski, G., Otto, S., Herdewijn, P.	Journal of Systems Chemistry 2010,	0,042361111	BioMed Central Ltd.	London	2010	1:1.	doi:10.1186/1759-2208-1-1	Yes
	Synthesis of information-carrying polymers of mixed sequences from double stranded short deoxynucleotides	Taran, O, Thoennessen, O, Achilles, K, von Kiedrowski, G	Journal of Systems Chemistry	0,047916667	BioMed Central Ltd.	London	2010	1:9.	doi:10.1186/1759-2208-1-1	Yes

	Unravelling a fulvene based Replicator: Experiment and Theory in Interplay	Dieckmann, A, Beniken, S, Lorenz, C, Doltsinis, NL, von Kiedrowski, G	Journal of Systems Chemistry	0,048 61111 1	BioMed Central Ltd.	London	2010	1:10.	doi:10.1186/1 759-2208-1-10	Yes
	Elucidating the Origin of Diastereoselectivity in a Self-Replicating System: Selfishness versus Altruism.	Dieckmann, A., Beniken, S., Lorenz, C. D., Doltsinis, N. L., von Kiedrowski, G.	Chemistry - A European Journal,:.	17			2011	468–480	DOI: 10.1002 /chem.201002 325	No
	Improved Large-Scale Liquid-Phase Synthesis and High-Temperature NMR Characterization of Short (F-)PNAs	Plöger,T. A., von Kiedrowski, G.	Helvetica Chimica Acta	94(11)	Helvetica	Zürich	2011	1952–1980	DOI: 10.1002 /hlca.2011002 43	No
	A Self-Replicating Peptide Nucleic Acid	Plöger,T. A., von Kiedrowski, G.	(arXiv e-print)		Cornell University	Ithaca, NY	2011	36	arXiv:1112.49 52v1 [q-bio.MN]	Yes
2	Non Covalent Monolayer-Piercing Anchoring of Lipophilic Nucleic Acids: Preparation, Characterization, and Sensing Applications	Patolsky, F. , Herrmann, A. et.al.	J. Am. Chem. Soc.	134	American Chemical Society	Washington, USA	2012	280-292		No
	Nucleic Acid Amphiphiles: Synthesis and Self-Assembled Nanostructures	Kwak M., Herrmann A.	Chem. Soc. Rev.	40	Royal Society ofChem-istry		2011	5745-5755		No
	DNA Block Copolymer Doing It All: From Selection to Self-Assembly of Semiconducting Carbon Nanotubes	Loi, M., Herrmann, A. et al.	Angew. Chem. Int. Ed.	50	Wiley VCH		2011	3206-3210		No

	Tunable Hydrophobicity in DNA micelles: design, synthesis, and characterization of a new family of DNA amphiphiles	Anaya, M, Kwak, M, Musser, AJ, Müllen, K, Herrmann, A	Chem. Eur. J.	16	Wiley VCH		2010	12852-12859	http://dx.doi.org/10.1002/chem.201001816%22 %5Ct %22_blank	No
	DNA-functionalised blend micelles: mix and fix polymeric hybrid nanostructures	Kwak, M, Musser, AJ, Lee, J, Herrmann, A	Chem. Commun.	46	Royal Society of Chemistry		2010	4935-4937	http://www.rsc.org/Publishing/Journals/CC/article.asp?doi=c0cc00855a%22 %5Ct %22_blank	No
	Virus-like Particles Templated by DNA Micelles: A General Method for Loading Virus Nanocarriers	Kwak, M, Minten, IJ, Anaya, D-M, Musser, A J, Brasch, M, Nolte, RJM, Müllen, K, Cornelissen, JJLM, Herrmann, A	J. Am. Chem. Soc.	132	American Chemical Society	Washington, USA	2010	7834-7835	http://www.rsc.org/Publishing/Journals/CC/article.asp?doi=c0cc00855a%22 %5Ct %22_blank	No
	Nucleic Acid/Organic Polymer Hybrid Materials: Synthesis, Superstructures and Applications	Kwak, M, Herrmann, A	Angew. Chem. Int. Ed.	46	Wiley VCH		2010	8574-8587	http://dx.doi.org/10.1002/anie.200906820%22 %5Ct %22_blank	No
3	Coarse Graining and Scaling in Dissipative Particle Dynamics	Füchslin, R., et.al.	Journal of Chemical physics	Vol. 130 no. 21	American Institute of Physics	Washington, USA	2009	214102	http://jcp.aip.org/resource/1/jcpsa6/v130/i21/p214102_s1?isAuthorized=no	No

Living Technology; Exploiting life´s principles in technology	Rasmussen, S., et.al.	Artificial Life	Vol. 16, no. 1	MIT Press Journals	Cambridge, USA	2009	89-97	http://www.mitpressjournals.org/doi/abs/10.1162/artl.2009.16.1.16103	Yes
Nucleobase Mediated Photocatalytic Vesicle Formation from Ester Precursor	DeClue, et al.	Journal of American Society	Vol. 131 (3)	ACS Publications	USA	2009	931-933	http://pubs.acs.org/doi/abs/10.1021/ja808200n	Yes
Life after the Synthetic Cell	Rasmussen, S.	Nature/Opinion	Vol. 465 (7297)	Nature	USA	2010	422	http://www.nature.com/nature/journal/v465/n7297/full/465422a.html	Yes
Chemical Basis for Minimal Cognition	Hanczyc, M. et al.	Artificial Life	Vol. 16 (3)	MIT Press Journals	Cambridge, USA	2010	233-244	http://www.mitpressjournals.org/doi/abs/10.1162/artl_a_00002	No
Stress Relaxation in Entangled Polymer Melts	Svaneborg, C. et al.	Physical Review Letters	Vol. 105	American Physical Society	USA	2010		http://prl.aps.org/abstract/PRL/v105/i6/e068301	Yes

Wet artificial life: the creation of artificial living systems	Fellermann, H.	H. Meyer-Ortmanns and S. Turner (eds.) Principles of Evolution.		SpringerLink		2010	261-280	http://www.springerlink.com/content/gu3r773g78j76820/	Yes
Living Technology: 5 questions	Bedau M. et al.	Book		Automatic Press/VIP	Denmark	2010		http://www.vince-inc.com/automatic.html	No
Proceedings of the 12th International Conference on Synthesis and Simulation of Artificial Life	Fellermann, H. et al.	Proceedings of the 12th International Conference on Synthesis and Simulation of Artificial Life		MIT Press	Cambridge, USA	2010		http://mitpress.mit.edu/catalog/item/default.asp?ttype=2&tid=12433	Yes

Designing a Protocell: Attempt at a Systemic Design Linking Informatin, Metabolism and Container	Maurer, S.E. et al	Proceedings of the 12th International Conference on Synthesis and Simulation of Artificial Life		MIT Press	Cambridge, USA	2010		http://mitpress.mit.edu/catalog/item/default.asp?ttype=2&tid=12433	Yes
On the Interplay of Kinetics, Thermodynamics and Information in Simple Replicating Systems	Corominas-Murtra, B. et al.	Proceedings of the 12th International Conference on Synthesis and Simulation of Artificial Life		MIT Press	Cambridge, USA	2010		http://mitpress.mit.edu/catalog/item/default.asp?ttype=2&tid=12433	Yes
Interactions Between catalyst and amphiphile structures and their implications for a protocell model	Maurer, S.E. et al.	ChemPhysChem	12 (4)	Wiley-VCH Verlag GmbH & Co. KGaA	Weinheim, Germany	2011	828-835	http://onlinelibrary.wiley.com/doi/10.1002/cphc.201000843/abstract	No

A Review of one Approach to Bottom-Up Assembly of Minimal Life	Rasmussen, S. et al.	Proceedings of the Eleventh European Conference on the Synthesis of Simulation of Living Systems, ECAL 2011		MIT Press	Cambridge, USA	2011		http://mitpress.mit.edu/catalog/item/default.asp?ttype=2&tid=12760&mode=toc	Yes
On the Growth Rate of Non-Enzymatic Molecular Replicators	Rasmussen, S., Fellermann, H.	Entropy	13 (10)			2011	1882-1903		Yes
The Ten Grand Challenges of Synthetic Life	Porcar, M. et al.	Systems and Synthetic Biology	5(1-2)	SpringerLink	N.Y., Heidelberg	2011	41153	http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3159694/	Yes
Primitive Membrane Formation, Characteristics and roles in Emergent Properties of a Protocell.	Mauer, S.E., Monnard, P.-A.	Entropy	13	MPDI Edition	Basel, Switzerland		466-484	http://www.mdpi.com/1099-4300/13/2/466/	Yes

	Assembling Living Materials and Engineering Life-Like Technologies	Rasmussen, S. et al.	Proceedings of the 13th Annual Conference Companion on Genetic and Evolutionary Computation (GECCO 11)		ACM	New York	2011		http://dl.acm.org/citation.cfm?id=2001579	Yes
	Lipid protocells	Albertsen, A.N., Monnard, P.-A.	Encyclopedia of Biophysics		Springer Science	N.Y., Heidelberg	2012			No
4	Electrochemically Stimulated pH Changes: A Route to Control Chemical Reactivity	Willner, I.	J. Am. Chem. Soc.	132	ACS Publications	Washington, USA	2010	2029-2036		No
	pH-Triggered Switchable Mg2+-Dependent DNAzymes	Willner, I.	Chem. Commun	46	Royal Society of Chemistry		2010	1209-1211		No
	Amplified Analysis of DNA by the Autonomous Assembly of Polymers Consisting of DNAzyme Wires	Willner, I.	J. Am. Chem. Soc.	133	ACS Publications	Washington, USA	2011	17149-17151		No
	Enzyme-Free Amplified Detection of DNA by an Autonomous Ligation and Replication DNAzyme Machinery	Willner, I.	Submitted							No

Electrified Au Nanoparticle Sponges with Controlled Hydrophilic/Hydrophobic Properties	Willner, I.	ACS Nano	5	ACS Publications	Washington, USA	2011	299-306		No
Electrified Selective "Sponges" Made of Au Nanoparticles	Willner, I.	J. Am. Chem. Soc.	132	ACS Publications	Washington, USA	2010	9373-9382		No
Amplified Detection of DNA through the Enzyme-Free Autonomous Assembly of Hemin/G-Quadruplex DNAzyme Nanowires	Willner, I.	Anal. Chem.	84	ACS Publications	Washington, USA	2012	1042-1048		No
Photochemical Switching of the Phase-Transition Temperatures of Poly(N-Isopropyl-acrylamide)/Pt Nanoparticles Thermosensitive Polymer Composites Associated with Electrodes: Functional Electrodes for Switchable Electrocatalysis	Willner, I.	Chem. Eur. J	17	Wiley-VCH		2011	11237-11242		No
Switchable Motion of DNA on Solid Supports	Willner, I.	Angew. Chem. Int. Ed.	48	Wiley-VCH		2009	133-137		No
Thermo-Switchable Charge-Transport and Electrocatalysis Using Metal-Ion Modified pNIPAM-Functionalized Electrodes	Willner, I.	Adv. Funct. Mater.	19	Wiley-VCH		2009	2474-2480		No
Amplified Detection of DNA through the Autocatalytic and Catabolic Mediated DNAzyme-Process	Willner, I.	Angew. Chem. Int. Ed.	50	Wiley-VCH		2011	295-299		No

TABLE A2: LIST OF DISSEMINATION ACTIVITIES

No.	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed	Year
1	Articles published in popular press	J. S. McCaskill	"Die Gottes-Maschine. Ist das Leben ganz neu zu erfinden?"	08.08.2009	Germany	Civil Society			2009
2	Articles published in popular press	J. S. McCaskill	'Konkurrenz für Gott'	January, 2010	Germany	Civil Society			2010
3	Articles published in popular press	S. Rasmussen	Aspekter af liv, information, virkelighed og fysik	2011	Kvant (2)	Civil Society	2200	Denmark	2011
4	Articles published in popular press	C. Svaneborg et al.	Fra syntetisk liv til levende teknologi	Sep.11	Aktuel Naturvidenskab	Civil Society & Scientific Community	8600	Denmark	2011
5	Colloquium	G. von Kiedrowski	Autocatalysis, Self-Replication and the Origin of Life. - Kolloquium der Israelian Chemical Society, Ben Gurion University of the Negev	23.05.2010	Tel Aviv, Israel	Scientific Community (higher education, Research)	40		2010
1	Colloquium	I. Willner	Colloquium Series of the Zernike Institute for Advanced Materials, University of Groningen	01.12.2011	Groningen, The Netherlands	Scientific Community		Mostly European	2011
2	Colloquium	J. S. McCaskill	Frankfurter Sonderkolloquium der Dechema 'Künstliches Leben'. Presentation' Leben zwischen Elektronik und Chemie'	27.01.2011	Frankfurt, Germany	Civil Society		Germany	2011
3	Conference	J. S. McCaskill	'Chembiogenenesis', COST CMO703 Systems Chemistry WG 05	10.-11.10.2008	Maratea, Italy	Scientific Community	50	Europe	2008
4	Conference	J. S. McCaskill	EU-ICT 2008 Conference	25.11.2008	Lyon, France	Scientific Community		Europe	2008

5	Conference	S. Rasmussen; M. Hanczyc; H. Fellermann; W. H. Jørgensen; A.N. Albertsen & L.L. Laursen	Public Opening of the Initiative for Science, Society and Policy	9.06. 2009	Louisiana Museum of Modern Art, Humlebæk, Denmark	Policy Makers	150	Europe	2009
6	Conference	S. Rasmussen	2nd Workshop on Living Technology	9.-11.06.2009	Humlebæk, Denmark	Scientific Community	100	Europe	2009
7	Conference	S. Rasmussen; M. Dörr; S. Maurer & P.-A. Monnard.	Emergence in Chemical Systems 2.0	June, 2009	University of Alaska, Anchorage	Scientific Community	300	Worldwide	2009
8	Conference	S. Rasmussen & P.-A. Monnard	BioControl Workshops,	24.-28.08.2009	Odense, Denmark.	Scientific Community	80	Denmark	2009
9	Conference	S. Rasmussen & P.-A. Monnard	Chembiogenesis 2009. Systems Chemistry, COST Action CMO703 Meeting	24.-26.10.2009	Lake Balaton, Hungary	Scientific Community	80	Europe	2009
10	Conference	J. S. McCaskill	FET Conference 'Science beyond Fiction' 2009	21.04.2009	Prag, Czech Rep.	Scientific Community		Internation	2009
11	Conference	Herrmann A.	Advances in Synthetic Biology	05.02.2010	London, Uk	Scientific Community	50	International	2010
12	Conference	I. Willner	The 3rd International NanoBio Conference	24.-27.08.2010	Zurich, Switzerland	Scientific Community		Worldwide	2010
13	Conference	I. Willner	XiangShan Science Conference on Multilevel Molecular Assemblies: Structure, Dynamics and Functions	27.-29.10.2010	Beijing, China	Scientific Community		Worldwide	2010
14	Conference	M.M. Hanczyc	Unconventional Computing and Architecture.	26.-27.02.2010.	Building Centre, London, UK	Scientific Community	150	Europe	2010

15	Conference	S. Rasmussen	Conference on “Scientific Social Responsibility”.	17.06.2010	Dansk Erhvervs Akademi (DEA), Copenhagen	Policy Makers	50	Denmark	2010
16	Conference	S. Rasmussen; P.-A. Monnard; M.M. Hanczyc; H. Fellermann; M. Dörr; C. Svaneborg, S. Maurer; A.N. Albertsen; W.H. Jørgensen; P.L. Pedersen & L.L. Laursen. (Organizers)	The 12th International Conference on the Synthesis and Simulation of Living Systems (AlifeXII)	19.-23.08.2010	University of Southern Denmark, Odense, Denmark	Scientific Community	300	Worldwide	2010
17	Conference	P.A. Monnard	Designing a Protocell: Attempt at a Systemic Design linking Information, Metabolism and Container	19.-23.08.2011	12th international conference on Artificial Life, Odense, Denmark	Scientific Community		Worldwide	2010
18	Conference	S. Rasmussen	ICT 2010 Congress	Sep.10	Brussels, Belgium	Scientific Community/Policy Makers/Industry	600	Worldwide	2010
19	Conference	Herrmann A.	ACS Meeting	24.03.2010	San Francisco USA	Scientific Community	50	International	2010

20	Conference	S. Rasmussen	Bottom-Up Assembly of Minimal Life	Aug.11	The 70th Harden Conference on Synthetic Biology: Design and Engineering through Understandin g	Scientific Community	200	Mostly European	2011
21	Conference	S. Rasmussen	FET 11. The European Future Technologies Conference and Exhibition. Science beyond Fiction	4.-6.05.2011	Budapest, Hungary	Scientific Community		Europe	2011
22	Conference	S. Rasmussen	Novozymes Technology Conference 2011	17.-18.05.2011	Copenhagen, Denmark	Industry / Scientific Community		Europe	2011
23	Conference	S. Rasmussen; M.M. Hanczyc; C. Svaneborg; M.C. Wamberg & Eva Bönzli.	The 6th Danish Conference on Biotechnology and Molecular Biology: Synthetic Biology and Cell Factories. May 2011.	26.05.2011	Vejle, Denmark	Scientific Community & Industry		Denmark	2011
24	Conference	S. Rasmussen	Renaissance Weekend	1.-4.07.2011	Jackson Hole, Wyoming, USA	Civil Society		North America	2011
25	Conference	S. Rasmussen	The 11th European Conference for Artificial Life (ECAL 11)	8.-14.08.2011	Paris, France	Scientific Community		Worldwide	2011
26	Conference	S. Rasmussen	The 70th Harden Conference on Synthetic Biology: Design and Engineering Through Understanding	22.-26.08.2011	Keele University, UK	Scientific Community		Worldwide	2011
27	Conference	S. Rasmussen	New Thinking about Global Challenges	10.-11.10.2011	Berlin, Germany	Scientific Community		Worldwide	2011

28	Conference	P.-A. Monnard, S.E. Maurer & S. Rasmussen.	ESF-COST High-level Research Conference on Systems Chemistry III.	23.-28.10.2011	Crete, Greece	Scientific Community		Mostly Europe	2011
29	Conference	P.-A. Monnard, S.E. Maurer & S. Rasmussen.	COST CMO703 Systems Chemistry WG03.	28.-30.10.2011	Crete, Greece	Scientific Community		Mostly Europe	2011
30	Conference	P.-A. Monnard, M.M. Hanczyc, M.C. Wamberg, E. Bönzli, M. Hadorn, W.H. Jørgensen & S.E. Maurer.	COST CMO703 Systems Chemistry WG03.	8.-10.12.2011	Odense, Denmark.	Scientific Community		Mostly Europe	2011
31	Conference	Herrmann A.	Makromolekulares Colloquium	25.02.2011	Freiburg, Germany	Scientific Community	1200	Germany, Austria, Switzerland	2011
32	Conference	Herrmann A.	ACS Meeting	28.03.2011	Anaheim USA	Scientific Community	50	International	2011
33	Conference	U. Tangen	FET 11. The European Future Technologies Conference and Exhibition. Science beyond Fiction, Panel session BioChemIT	4.-7.05.2011	Budapest, Hungary	Scientific Community		Europe	2011
34	Conference	J. S. McCaskill	'Chembiogenenesis', COST CMO703 Systems Chemistry WG 05	28.-29.10.2011	Heraklion, Greece	Scientific Community	50	Europe	2011
35	Exhibition	J. S. McCaskill	Contributions to the project 'Expedition Future 2020' of the Max-Planck Society	2009	Science Train of the Max-Planck-Society	Civil Society		Germany	2009

36	Exhibition	J. S. McCaskill	Exhibits on second creation (or "Manmade evolution")	2009	Deutsches Museum München,Zentrum Neue Technologien	Civil Society		Germany	2009
37	Interview	J. S. McCaskill	Chemie pflanzt sich fort - Forscher aus Bochum erwecken tote Moleküle zum Leben	13.01.2010	Germany, www.dradio.de/streaming/dlf.m3u	Civil Society			2010
38	Meeting	I. Willner	Swiss Chemical Society Spring Meeting 2009, on "Nanomaterials by Chemical Design"	11.02.2009	Neuchatel, Switzerland	Scientific Community		Worldwide	2009
39	Meeting	I. Willner	CSCB (Centre for Synthesis & Chemical Biology) Meeting 2010 on Recent Advances in Synthesis and Chemical Biology IX	10.12.2010	Dublin, Ireland	Scientific Community		Worldwide	2010
40	Meeting	J. S. McCaskill	FET Flagship Information Day	30.09.2010	Brussels, Belgium	Scientific Community		Europe	2010
41	Meeting	J. S. McCaskill	FET Information Day	12.10.2011	Brussels, Belgium	Scientific Community		Europe	2011
42	Meeting	J. S. McCaskill	FET Workshop 'Living Technologies, Artificial Systems, Embodied Evolution'	10.11.2011	Brussels, Belgium	Scientific Community		Europe	2011
43	Other	S. Rasmussen	Initiativ for Science, Society and Policy. In: Viljen til Visdom: En bog om dannelse og uddannelse	2009	Forlaget Slagmark, Denmark	Civil Society and Policy Makers		Denmark	2009
44	Other	H. Fellermann	Wet artificial life: the creation of artificial living systems	2010	H. Meyer-Ortmanns and S. Turner (eds.) Principles of Evolution. Springer	Scientific Community		World Wide	2010

45	Other	H. Fellermann et al.	Proceedings of the Twelfth International Conference on the Synthesis and Simulation of Living Systems	2010	MIT Press 2010, ISBN 0-262-29075-8	Scientific Community		Worldwide	2010
46	Other	S. Rasmussen	Synthetic Biology: A Discussion Paper	May, 2011	The Danish Board of Technology	Policy Makers		Denmark	2011
47	Poster	A.N. Albertsen	Attempt at a Systemic Design of a Protocell: Linking Information, Metabolism and Container	Aug.11	Challenges in Chemical Biology, Manchester, UK	Scientific Community	200	Mostly European	2011
48	Poster	U. Tangen	MPI Winterseminar on 'Biophysical Chemistry', Poster presentation 'Towards an integrated feedback control of chemical cell autonomy'	16.-20.01.2011	Klosters, Switzerland	Scientific Community	80	Internation	2011
49	Poster	A. Minero	JCS Ruhr 2011, Universität Duisburg-Essen, Poster: Monitoring and fast analysis of triplex formation'	22.09.2011	Essen, Germany	Students, Scientific Community	70	national	2011
50	Poster	A. Minero	Dechema Summer school 'Biotransformations 2011'. Poster: Murine DNA methyltransferase Dnmt3a: probing of DNA-enzyme interactions with benzo[a]pyrene-containing DNA by fluorescence	22.-26.08.2011	Bad Herrenalb, Germany	Young Scientific Community	110	Europe	2011
51	Presentation	J. S. McCaskill	'Phase-modulatin chemical reactions towards artificial cells',invited lecture given at ESF-COST High-level Research Conference on Systems Chemistry	4.-9.10.2008	Maratea, Italy	Scientific Community	100	Internation	2008

52	Presentation	J. S. McCaskill	Seminar of the dept. of theoretical chemistry, Presentation 'Physical self-assembly and chemical reactions towards artificial cells: A tool for systems chemistry'	05.11.2008	Bochum, Germany	Scientific Community	20	Germany	2008
53	Presentation	G. von Kiedrowski	Systems Chemistry, Chemical Self-Replication and Multicomponent Assembly. - Plenarvortrag auf “Systems Chemistry: Center Opening Symposium and COST CM0703 WG1 Kickoff“	28.-29.04.2009	Groningen, The Netherlands	Scientific Community (higher education, Research)	40		2009
54	Presentation	G. von Kiedrowski	Replicators and Assemblers. - ESF-COST High-Level Research Conference “Systems Chemistry II - Evolution and Systems”,	18.-23.10. 2009	Lake Balaton, Hungary	Scientific Community (higher education, Research)	100		2009
55	Presentation	G. von Kiedrowski	Replication versus Metabolic Autocatalysis in Asymmetric Mannich- Reactions. - “ChemBioGenesis 2009”,	23.-27.10.2009	Lake Balaton, Hungary	Scientific Community (higher education, Research)	60		2009
56	Presentation	G. von Kiedrowski	Systems Chemistry: From chemical self-replication to trisoligo-based nanoconstruction. - Plenary lecture 5th German Chemoinformatics Conference, ,	8.-10.11.2009	Goslar, Germany	Scientific Community (higher education, Research)	80		2009
57	Presentation	J. S. McCaskill	Ist Leben machbar? Möglichkeiten und Perspektiven einer synthetischen Biologie	13.-14.11.2009	Tagung der Ev. Akad. Rheinland, Bonn, Germany	Civil Society & Scientific Community	80		2009

58	Presentation	G. von Kiedrowski	Chemical Self-Replication and Multicomponent Assembly	12.01.2009	Dortmund, Germany	Scientific Community (higher education, Research)	40		2009
59	Presentation	G. von Kiedrowski	Chemical Self-Replication and Multicomponent Assembly	26.01.2009	München, Germany	Scientific Community (higher education, Research)	50		2009
60	Presentation	G. von Kiedrowski	Systems Chemistry and the Origin of Life. - 1384. Sitzung der Chemischen Gesellschaft zu Heidelberg	07.04.2009	Heidelberg, Germany	Scientific Community (higher education, Research)	40		2009
61	Presentation	G. von Kiedrowski	Systemchemie und der Ursprung des Lebens. -Nordrhein-Westfälische Akademie der Wissenschaften	24.04.2009	Düsseldorf, Germany	Scientific Community (higher education, Research)	60		2009
62	Presentation	G. von Kiedrowski	Systems Chemistry, Chemical Self-Replication and Multicomponent Assembly. - Symposium “Physics of DNA Assembly and Applications”, École de Physique, Les Houches,	3.-8.05.2009	Les Houches, France	Scientific Community (higher education, Research)	25		2009
63	Presentation	G. von Kiedrowski	Spontaneous Emergence of Optical Acitivity in Organic SystemsChemistry - Rare or Frequent? - Bürgenstock-Konferenz,	17.-20.05.2009	Brunnen, Zwitzerland	Scientific Community (higher education, Research)	60		2009

64	Presentation	G. von Kiedrowski	Systems Chemistry: Chemical Self-Replication and Multicomponent Assembly. GDC-Kolloquium - Universität Konstanz.	04.06.2009	Konstanz, Germany	Scientific Community (higher education, Research)	30		2009
65	Presentation	G. von Kiedrowski	Systems Chemistry: From chemical self-replication to trisoligo-based nanoconstruction. - CENS Seminar, LMU München.	23.07.2009	München, Germany	Scientific Community (higher education, Research)	35		2009
66	Presentation	G. von Kiedrowski	Systems Chemistry CM0703: Spontaneous Emergence of Optical Activity in Organic Reactions. -COST DC-Meeting, La Sapienza University of Rome	18.09.2009	Rom, Italy	Scientific Community (higher education, Research)	40		2009
67	Presentation	G. von Kiedrowski	Replicator Systems Chemistry. Regio-Symposium on Organic and Bioorganic Chemistry,	23.09.2009	Rheinfelden, Germany	Scientific Community (higher education, Research)	30		2009
68	Presentation	G. von Kiedrowski	Systems Chemistry: artificial replication, organo-autocatalysis and multicomponent assembly. - GDCh Kolloquium, Ortsverband Kiel.	26.11.2009	Kiel, Germany	Scientific Community (higher education, Research)	35		2009
69	Presentation	S. Rasmussen	Creating Artificial Life.	5.02.2009	Nørre Gymnasium, Copenhagen, Denmark	Civil Society	75	Denmark	2009

70	Presentation	S. Rasmussen	Kunstigt liv og livets oprindelse	18.03.2009	Folkeuniversitetet, University of Southern Denmark	Civil Society	40	Denmark	2009
71	Presentation	H. Fellermann	Introduction to Molecular and Coarse-grained Modeling	20.03.2009	University of Southern Denmark,	Scientific Community	35	Denmark	2009
72	Presentation	S. Rasmussen	Public Opening of the ISSP meeting at the Louisiana Museum of Modern Art as well as moderate the open public discussion after the presentation	11.06.2009	Louisiana Museum of Modern Art, Denmark	Policy Makers	150	Europe	2009
73	Presentation	S. Rasmussen	Assembly of Minimal Living Systems	23.06.2009	Emergence in Chemical Systems 2.0, Anchorage, USA, University of Alaska, Anchorage,	Scientific Community	200	Worldwide	2009
74	Presentation	P.-A. Monnard	Non-enzymatic Polymerization of RNA in water-ice	23.06.2009	Emergence in Chemical Systems 2.0, University of Alaska, Anchorage	Scientific Community	200	Worldwide	2009
75	Presentation	S. Rasmussen	Assembly of a Minimal Protocell	2.07.2009	Argonne National Laboratory, USA	Scientific Community	50	Worldwide	2009

76	Presentation	H. Fellermann	Artificial Life: on the Construction of Artificial Living Systems	3.-4.07.2009	Jacobs University, Bremen Germany	Scientific Community	40	Germany	2009
77	Presentation	H. Fellermann	Physically Embedded Minimal Self-Replicating Systems: Studies by Simulation. Fellermann, H. Germany,	4.08.2009	University of Osnabruck, Germany	Scientific Community	40	Germany	2009
78	Presentation	S. Rasmussen	Artificial Life	24.08.2009	BioControl Workshops, Denmark	Scientific Community	80	Denmark	2009
79	Presentation	S. Rasmussen	Self-organized Systems: About parts and pieces	25.08.2009	BioControl Workshops, Odense, Denmark	Scientific Community	80	Denmark	2009
80	Presentation	S. Rasmussen	Scientific Responsibility	8.09.2009	Syddansk Universitet, Det Naturvidensk abelige Fakultet, Odense, Denmark	Scientific Community	50	Denmark	2009
81	Presentation	P.-A. Monnard	Genetic Material Synthesis in Structures Environments	14.09.2009	European Systems & Synthetic Biology 2009, Evry, France	Scientific Community	30	Worldwide	2009

82	Presentation	S. Rasmussen	Coupling of Minimal Protocellular Information, Metabolic and Container System: Status Report	25.10.2009	Chembiogenesis 2009, Systems Chemistry, COST, Lake Balaton, Hungary,	Scientific Community	80	Europe	2009
83	Presentation	P.-A. Monnard	Coupling of a minimal protocellular information, metabolic and container system: status report	23.-27.10.2009	ESF-COST Action Meeting, Lake Balaton, Hungary		30	Worldwide	2009
84	Presentation	S. Rasmussen	Naturvidenskab, samfund og politik - nye perspektiver i gymnasiets undervisning i naturvidenskab	5.11.2009	Science Day 2009, Denmark, Det Naturvidenskabelige Fakultet, SDU, Odense, Denmark	Scientific Community		Denmark	2009
85	Presentation	S. Rasmussen	What is the idea Behind The Initiative for Science, Society and Policy (ISSP)?	13.11.2009	Scientific stakeholder meeting for the Initiative for ISSP, Copenhagen Business School, Denmark	Policy Makers	50	Denmark	2009
86	Presentation	S. Rasmussen	Coupling of Minimal Protocellular Information, Metabolic and Container System	16.11.2009	Workshop on Streamlined and Synthetic Genomes, Valencia, Spain	Scientific Community	35	Europe	2009

87	Presentation	S. Rasmussen	Protocell Activities within FLinT	7.12.2009	Los Alamos National Laboratory (LANL). USA	Scientific Community	40	North America	2009
88	Presentation	H. Fellermann	Progress on the Creation of a Minimal Protocell	3.12.2009	Parc de Recerca Biomedicà Barcelona, Barcelona, Spain	Scientific Community	25	Europe	2009
89	Presentation	S. Rasmussen	Protolife: Assembly of Minimal Self-replicating Nano-machines	15.12.2009	University of Kiel, Germany	Scientific Community	30	Germany	2009
90	Presentation	U. Tangen	Emergence of replication using catalytic micro-controllers	19.01.2009	Klosters, Switzerland	Scientific Community (higher education, Research)			2009
91	Presentation	J. S. McCaskill	Seminar given at FIAS, Univ. Frankfurt	15.05.2009	Frankfurt, Germany	Scientific Community	20	Germany	2009
92	Presentation	J. S. McCaskill	Seminar given at FIAS, Univ. Frankfurt	18.06.2009	Frankfurt, Germany	Scientific Community		Germany	2009
93	Presentation	G. von Kiedrowski	Nanosystems Chemistry: Procedural Replication and Programmable Assembly. - Symposium “Recent Topics in Systems Chemistry: Molecular Replication and Computation”,	24.-26.05.2010	Tel Aviv, Israel	Scientific Community (higher education, Research)	40		2010
94	Presentation	G. von Kiedrowski	Systems Chemistry: Autocatalysis, Replication, Assembly. - Gordon Research Conference “Oscillations and Dynamic Instabilities in Chemical Systems”,	4.-9.07.2010	Lucca, Italy	Scientific Community (higher education, Research)	80		2010

95	Presentation	G. von Kiedrowski	Systems Chemistry: Replicators and Assemblers. -Symposium “From Molecular Structure to Systems Biology”, Societá Chimica Italiana, S	9.-11.09.2010	an Vito di Cadore, Italy	Scientific Community (higher education, Research)	50		2010
96	Presentation	U. Tangen	ALifeXII, The Emergence of Replication in a Digital Evolution System using a Secondary Structure Approach	23.08.2010	Odense, Denmark	Scientific Community		Internation	2010
97	Presentation	J. S. McCaskill	Keynote lecture at ALife XII, SDU, 'From electronic evolution to electronic chemical cells'	19.-24.08.2010	Odense, Denmark	Scientific Community		Internation	2010
98	Presentation	J. S. McCaskill	FET ICT Conference 'Digitally Driven', Session on Bio-chemistry based IT, Presentation on ECCell 'Chembio-IT in a nutshell'	30.09.2010	Brussels, Belgium	Scientific Community/Policy Makers/Industry	600	Internation	2010
99	Presentation	G. von Kiedrowski	Systems Chemistry. -“Konstanz Symposium Chemical Biology”, Steigenberger Inselhotel, Konstanz,	16.-18.06.2010	Konstanz, Germany	Scientific Community (higher education, Research)	30		2010
100	Presentation	G. von Kiedrowski	Nucleic Acid Systems Chemistry: Non enzymatic copying, template-free polymerization, and programmable multimodularity.	29.08.-03.09.2010	Lyon, France	Scientific Community (higher education, Research)	40		2010
101	Presentation	S. Rasmussen (Invited speaker)	Conference on Genomes and the Synthesis of Life: Technological, Philosophical and Cultural Aspects.	October 2010	Copenhagen, Denmark	Scientific Community	100	Europe	2010

102	Presentation	J. S. McCaskill	FET Coordination Action Meeting, Presentation	26.-27.10.2010	Brussels, Belgium	Scientific Community		Europe	2010
103	Presentation	P.-A. Monnard	Linking a catalytic reaction to the replication of primitive membranes: A step towards protocell integration	10.-15.01.2010	Gordon Research Conference on Origins of Life, Galveston, Texas, USA	Scientific Community	120	Worldwide	2010
104	Presentation	S. Rasmussen	Living Technology	22.01.2010	FET Flagship Workshop, Brussels, Belgium	Scientific Community	80	Europe	2010
105	Presentation	S. Rasmussen	Living Technology Focus Area.	25.03.2010	ISSP Workshop on Living Technology, Odense, Denmark	Policy Makers	50	Denmark	2010
106	Presentation	S. Rasmussen	Citizens in Science	22.05.2010	European Environmental Agency (EEA) biodiversity workshop “Citizens in Science”, Copenhagen, Denmark	Policy Makers & Civil Society	100	Denmark	2010

107	Presentation	S. Rasmussen	Origins of Life	04.06.2010	Annual Meeting, Department of Molecular Biology, University of Århus, Denmark	Scientific Community	90	Denmark	2010
108	Presentation	S. Rasmussen	What is Life?	04.06.2010	Annual meeting in the Danish Chemical Society, University of Southern Denmark	Scientific Community	150	Denmark	2010
109	Presentation	P.A. Monnard	Self-assembled amphiphile structures: Could there be more to expect than simple compartments	9.06.2010	Deutsche Chemische Gesellschaft Seminar Serie at Friedrich Schiller Universitat Jena, Germany	Scientific Community	40	Germany	2010
110	Presentation	S. Rasmussen	Living Technology	18.06.2010	FET Flagship Workshop, Berlin, Germany	Scientific Community	70	Europe	2010
111	Presentation	S. Rasmussen.	Living Technology.	21.06.2010	FET Flagship Workshop, Venice, Italy	Scientific Community	25	Europe	2010

112	Presentation	S. Rasmussen	Bottom-up Assembly of Life	28.06.2010	Los Alamos National Laboratory Synthetic Biology Workshop 2010	Scientific Community	45	North America	2010
113	Presentation	P.A. Monnard	Nucleobase-mediated, photocatalytic production of amphiphiles to promote the self-assembly of a simple self-replicating protocell	18.-25.07.2010	COSPAR Scientific Assembly, Bremen Germany	Scientific Community	650	Worldwide	2010
114	Presentation	S. E. Maurer	Designing a Protocell: Attempt at a Systemic Design linking Information, Metabolism and Container	20.08.2010	12th international conference on Artificial Life, Odense, Denmark	Scientific Community	300	Worldwide	2010
115	Presentation	S. E. Maurer	Why the FLinT Protocell is Unique	21.08.2010	12th international conference on Artificial Life, Odense, Denmark	Scientific Community	300	Worldwide	2010
116	Presentation	S. Rasmussen.	Synthesizing and Assembling Minimal Self-replicating Molecular Machines	27.08.2010	Synthetic Biology International Workshop, Copenhagen, Denmark	Scientific Community	200	Europe	2010

117	Presentation	S. Rasmussen	Minimal Living Systems and Chemical IT	9.09.2010	Public lecture at the Weizmann Institute of Science, Israel	Scientific Community	30	Israeli	2010
118	Presentation	S. Rasmussen	Minimal Life	29.10.2010	Conference on Genomes and the Synthesis of Life: Technological, Philosophical and Cultural Aspects. Copenhagen, Denmark	Scientific Community	150	Europe	2010
119	Presentation	C. Svaneborg	Kunstigt liv	29.10.2010	Brobygningsaktivitet at University of Southern Denmark	Civil Society	100	Denmark	2010
120	Presentation	S. Rasmussen	Assembling Minimal Life.	10.11.2010	Bioteknologisk studenterforening, DTU, Denmark	Civil Society	45	Denmark	2010
121	Presentation	S. Rasmussen.	Livets anden oprindelse.	11.11.2010	Dansk Naturhistorisk forening, København, Denmark	Civil Society	60	Denmark	2010

122	Presentation	P.A. Monnard	What is FLiNT?	13.11.2010	Meeting of the Alumni of the Department of Physics and Chemistry at the University of Southern Denmark	Civil Society	50	Denmark	2010
123	Presentation	S. Rasmussen.	Assembly of Minimal Living Systems from Bottum-up.	13.12.2010	Life, Evolution and Complexity - Academic Seminar of Sophia Iberia, Madrid, Spain	Scientific Community	75	Europe	2010
124	Presentation	J. S. McCaskill	Presentation at the FET Flagship Consultation 2010	9.-10.07.2010	Brussels, Belgium	Scientific Community		Europe	2010
125	Presentation	J. S. McCaskill	Electronic & chemical co-evolution	21.09.2010	Public lecture at Weizmann Institute, Rehovot, Israel	Scientific Community			2010
126	Presentation	U. Tangen	MPI Winterseminar on 'Biophysical Chemistry, molecular biology and cybernetics of cell functions'. Presentation 'Emergence of replication using catalytic micro-controllers'	16.-21.01.2010	Klosters, Switzerland	Scientific Community	80	Internation	2010
127	Presentation	S. Rasmussen (Invited speaker)	Synthetic Biology International Workshop	August, 2010	Copenhagen, Denmark	Scientific Community	100	Europe	2010
128	Presentation	S. Rasmussen (Invited speaker)	Life, Evolution and Complexity - Academic Seminar of Sophia-Iberia	December, 2010	Madrid, Spain	Scientific Community	50	Europe	2010

129	Presentation	G. von Kiedrowski	Systems Chemistry: Some Ideas about Molecular Replication (outside the Standard Model). - Gordon Research Conference “Nucleosides, Nucleotides, Oligonucleotides”,	3.-8.07.2011	Newport, RL, USA	Scientific Community (higher education, Research)	80		2011
130	Presentation	J. S. McCaskill	FET 11. The European Future Technologies Conference and Exhibition. Science beyond Fiction, Presentation at Panel session BioChemIT 'Biological and Chemical Information Technologies'	4.-7.05.2011	Budapest, Hungary	Scientific Community		Europe	2011
131	Presentation	J. S. McCaskill	The 11th European Conference for Artificial Life (ECAL 11), presentation at satellite workshop session held on BioChemIT 'Spatially interfaces Chem-IT: Towards electronic chemical cells'	8.-12.08.2011	Paris, France	Scientific Community	200	Internation	2011
132	Presentation	U. Tangen	The 11th European Conference for Artificial Life (ECAL 11), presentation at satellite workshop session held on BioChemIT 'On the external programming of a self-referential evolving micro-controller system"	8.-12.08.2011	Paris, France	Scientific Community	200	Internation	2011
133	Presentation	J. S. McCaskill	'Electronic Systems Chemistry', lecture given at ESF-COST High-level Research Conference on Systems Chemistry III	23.-27.10.2011	Heraklion, Greece	Scientific Community	100	Internation	2011
134	Presentation	G. von Kiedrowski	Systems Chemistry of Self-Replication and Self-Assembly	08.07.2011	Göttingen, Germany	Scientific Community (higher	40		2011

						education, Research)			
135	Presentation	S. Rasmussen	Fra syntesebiologi til levende teknologi.	6.01.2011	Synthetic Biology Workshop, The Danish Board of Technology, Copenhagen, Denmark	Policy Makers	50	Denmark	2011
136	Presentation	S. Rasmussen	NASA´s nye fund og muligheden for liv i rummet.	25.01.2011	Vin & Videnskab på Geologisk Museum, Copenhagen, Denmark	Civil Society	50	Denmark	2011
137	Presentation	P.A. Monnard	Can emergent properties be realized in chemical complex systems?	8.03.2011	Department of Logic and Phil. Of Science, University of the Basque Country, San Sebastian, Spain	Scientific Community	40	Spain	2011

138	Presentation	P.A. Monnard	Self-assembly, self-replication and reproduction: the three processes that shape a protocell	9.03.2011	Biophysical Unit, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), March 9, Bilbao, Spain	Scientific Community	50	Spain	2011
139	Presentation	M. Hanczyc	The Path Towards Living Systems.	18.05.2011	TED Salon	Scientific Community		Worldwide	2011
140	Presentation	P.A. Monnard	Research on the origins of life: How to handle this problem? Complex systems	20.05.2011	Origins of Life - Brainstormin g Workshop, CERN, Geneva, Switzerland	Scientific Community	15	Worldwide	2011
141	Presentation	C. Svaneborg	DNA Dymanics and Self-Assembly of Soft-Condensed Matter	26.05.2011	Conference on Synthetic Biology and Cell Factories , Vejle, Denmark	Scientific Community & Industry	50	Denmark	2011
142	Presentation	S. Rasmussen	Bottom-Up Assembly of Minimal Life	26.05.2011	Conference on Synthetic Biology and Cell Factories , Vejle, Denmark	Scientific Community & Industry	50	Denmark	2011
143	Presentation	S. Rasmussen		02.07.2011	Renaissance Weekend, Jackson Hole, Wyoming,	Civil Society	200	North America	2011

					USA				
144	Presentation	S. Rasmussen	Life Science 2.0.	3.07.2011	Renaissance Weekend, Jackson Hole, Wyoming, USA	Civil Society	200	North America	2011
145	Presentation	S. Rasmussen	Artificial Life: Creating Life from Scratch from Non-living Materials.	03.07.2011	Renaissance Weekend, Jackson Hole, Wyoming, USA	Civil Society	200	North America	2011
146	Presentation	P.A. Monnard	Attempt at a Systemic Design of a Protocell: Connecting information, Metabolism and Container	3.-8.07.2011	ISSOL conference, Montpellier, France	Scientific Community	500	Worldwide	2011
147	Presentation	S. Rasmussen	Bottom Up Assembly of Minimal Life.	8.07.2011	Santa Fe Institute, New Mexico, USA	Scientific Community	25	North America	2011
148	Presentation	S. Rasmussen	Assembling Living Materials and Life-like Technologies. S. Rasmussen @. July 11, 2011	11.07.2011	Los Alamos National Laboratories, New Mexico, USA	Scientific Community	30	North America	2011

149	Presentation	S. Rasmussen	Bottom Up Assembly of Minimal Life	14.07.2011	The 13th Annual Conference Companion on Genetic and Evolutionary Computation (GECCO 11). Dublin, Ireland	Scientific Community		World Wide	2011
150	Presentation	S. Rasmussen	Assembling Minimal Life from Bottom Up. S. Rasmussen @ August 25, 2011 (Keynote)	25.08.2011	The 70th Harden Conference on Synthetic Biology: Design and Engineering Through Understandin g. Keele University, UK	Scientific Community		World Wide	2011
151	Presentation	H. Fellermann	Thermodynamics and Kinetics of Protocells: Towards a quantitative Theory of Early Life	Sep.11	European Conference on Complex Systems, ECCS 11, Austria	Scientific Community	700	Mostly European	2011

152	Presentation	S. Rasmussen	Science Sharing, Open Source, Bio Hacking and the Emerging Science-engineering-design-art Interface in Synthetic Biology.	2.09.2011	Workshop on Engineering Life - Philosophical and Societal Perspectives on Synthetic Biology in Copenhagen, Denmark	Policy Makers	50	Denmark	2011
153	Presentation	C. Svaneborg	DNA Dymanics and Self-Assembly of Soft-Condensed Matter	27.09.2011	SimBioMa Conference, Konstanz, Germany	Scientific Community	200	Mostly European	2011
154	Presentation	P.A. Monnard	Non-Enzymatic biopolymerization reactions supported by heterogeneous media.	10.-11.10.2011	Second International Neurodegenerative Diseases & Genomics Symposium, Roma, Italy	Scientific Community	40	Worldwide	2011
155	Presentation	P.A. Monnard	Heterogeneous self-assembled media for biopolymerization	23.10. 2011	ESF-COST Conference on Systems Chemistry III, Heraklion, Greece	Scientific Community	120	Worldwide	2011
156	Presentation	P.A. Monnard	Exploring single chain amphiphile self-assembly and their possible roles in light transduction	27.-30.10.2011	COST Action CM0703, CHEMBIOGENESIS 2011, Heraklion, Greece	Scientific Community	50	Europe	2011

157	Presentation	S. Rasmussen	Challenges for Assembling Minimal Living Physicochemical Systems.	27.10.2011	ESF-COST High-level Research Conference on Systems Chemistry III. Crete, Greece	Scientific Community		Europe	2011
158	Presentation	H. Fellermann	A calculus for integrated information processing and material production.	4.12.2011	Genetic Group Seminar, The Weizmann Institute, Israel	Scientific Community	20	Israeli	2011
159	Presentation	P.A. Monnard	Single chain amphiphile membranes and their potential to sustain chemical reactions	9.-10.12.2011	COST Action CM0703 Working Group 3, Odense, Denmark	Scientific Community	25-30	Europe	2011
160	Presentation	H. Fellermann	On the growth rate of minimal molecular replicators.	9.12.2011	COST Action CMO703 - Systems Chemistry, 2nd meeting of the Workgroup 3, Odense, Denmark	Scientific Community	25	Europe	2011
161	Presentation	J. S. McCaskill	MPI Winterseminar on 'Biophysical Chemistry'. Presentation 'From electronic chemical cells to living technology'	16.-20.01.2011	Klosters, Switzerland	Scientific Community	80	Internation	2011
162	Presentation	J. S. McCaskill	Workshop on 'Autonomous electronic-chemical hybrid systems', ECLT Venice	15.-16.11.2011	Venice, Italy	Scientific Community	25	Internation	2011

163	Presentation	G. von Kiedrowski	Systems Chemistry: Replicators and Assemblers. - GDCh Kolloquium, Ortsverband Tübingen.		Tübingen, Germany	Scientific Community (higher education, Research)	30		
164	Presentation	J. S. McCaskill	Seminar at Univer. Of Southern Denmark, FLINT, Presentation 'Towards electronic chemical cells'	10.11.2009	Odense, Denmark	Scientific Community	25	Denmark	
165	Public Lecture	J. S. McCaskill	Public lecture at Weizmann Institute, Israel, 'Electronic & chemical co-evolution'	21.09.2010	Rehovot, Israel	Scientific Community	30	Israel	2010
166	Publication	S. Rasmussen et al.	Synthetic Biology: Challenges and Debate	June 2011	The Danish Board of Technology, Denmark	Policy Makers		Denmark	2011
167	Symposium	I. Willner	The 4th Nucleic Acid Chemical Biology (NACB) Symposium	24.-25.06.2009	Odense, Denmark	Scientific Community		Worldwide	2009
168	Symposium	I. Willner	The 16th Roche PCR Symposium	05.11.2009	Basel, Switzerland	Scientific Community		Worldwide	2009
169	Symposium	J. S. McCaskill	Formal Opening Symposium of the Centre of Systems Chemistry	28.-29.4.2009	Groningen, The Netherlands	Scientific Community	200	Europe	2009
170	Symposium	I. Willner	Hangzhou Symposium on Supramolecular Systems and Biomaterials	31.10.-1.11.2010	Hangzhou, China	Scientific Community		Worldwide	2010
171	Video (DVD)	J. S. McCaskill	'Future and Emerging Information Technologies'	November, 2008	EU	Civil Society and Policy Makers		Europe	2008
172	Web	C. Svaneborg	Programmable DNA self-assembly of three-dimensionally defined nano-objects.	2011	Internet publication	Scientific Community		World Wide	2011

173	Web	C. Svaneborg	Simulation of non-enzymatic DNA replication chemistry	2011	Internet publication	Scientific Community		World Wide	2011
174	Web	C. Svaneborg	DPD simulation of coarsening dynamics of oil-surfactant-water mixtures (excess oil)	2011	Internet publication	Scientific Community		World Wide	2011
175	Web	C. Svaneborg	DPD simulation of coarsening dynamics of oil-surfactant-water (surfactant)	2011	Internet publication	Scientific Community		World Wide	2011
176	Web	C. Svaneborg	Computer simulation of the thermal motion of poly-AT DNA under standard conditinos	2011	Internet publication	Scientific Community		World Wide	2011
177	Web	C. Svaneborg	Dynamic Bonding Framework and an Application Modelling DNA	Accepted 2012	Computer Physics CommunicationOpen Access Software submitted to Computer Physics Communications Program Library	Scientific Community		World Wide	2012
178	Workhop	J. S. McCaskill	MPI Winterseminar on 'Biophysical Chemistry, molecular biology and cybernetics of cell functions'	16.-21.01.2010	Klosters, Switzerland	Scientific Community	80	Internation	2010
179	Workhop	A. Minero, A. Sharma	MPI Winterseminar on 'Biophysical Chemistry'	16.-20.01.2011	Klosters, Switzerland	Scientific Community	80	Internation	2011

180	Workshop	I. Willner	International Workshop on "DNA-Based Nanotechnology: Construction, Mechanics, and Electronics"	11.-15.05.2009	Dresden, Germany	Scientific Community		Worldwide	2009
181	Workshop	H. Fellermann	WE-Heraeus-Summerschool "Steps in Evolution"	04.07.2009	Jacobs University, Bremen, Germany	Scientific Community	40	Germany	2009
182	Workshop	S. Rasmussen	Science Day 2009	5.11.2009	University of Southern Denmark, Odense, Denmark	Civil Society	75	Denmark	2009
183	Workshop	S. Rasmussen	Scientific Stakeholder Meeting: Initiative for Science, Society and Policy.	13.11.2009	University of Southern Denmark, Odense, Denmark	Policy Makers & Scientific Society	50	Denmark	2009
184	Workshop	S. Rasmussen	Workshop on Streamlined and Synthetic Genomes	16.-18.11.2009	Valencia, Spain	Scientific Community	40	Europe	2009
185	Workshop	S. Rasmussen	Workshop on joint US-SDU program development efforts within the areas of protocells and living technology	7.-11.12.2009	Los Alamos National Laboratory, Los Alamos, New Mexico, USA	Scientific Community	25	Worldwide	2009
186	Workshop	S. Rasmussen	University of Southern Denmark (SDU) - Carl Albrechts Universität Kiel (CAU) Workshop	15.12.2009	Sønderborg, Denmark	Scientific Community	15	Denmark & Germany	2009

187	Workshop	G. von Kiedrowski	Systems Chemistry: Artificial replication and multicomponent assembly. “Synthetic Bio(techno)logy”, Frankfurt, Dechema-Haus,	9.- 10.11. 2009	Frankfurt, Germany	Scientific Community (higher education, Research)	40		2009
188	Workshop	P. Wagler	FET Workshop 'Chem-IT', ICT Proposers' Day 2009	21.- 22.1.2009	Budapest, Hungary	Scientific Community		Europe	2009
189	Workshop	J. S. McCaskill	DFG workshop on Synthetic Biology, Presentation 'Protocells'	26.02.2009	Berlin, Germany	Scientific Community	20	Europe	2009
190	Workshop	U. Tangen	Expert Meeting on Synthetic Biology	03.10.2009	Delft, The Netherlands	Scientific Community		Europe	2009
191	Workshop	J. S. McCaskill	FET Expert Consultation Workshop on Atomic & molecular scale devices and systems, Presentation on Chem IT	22.- 23.10.2009	Brussels, Belgium	Scientific Community		Europe	2009
192	Workshop	S. Rasmussen	FET Flagship Initiatives Workshop	January, 2010	Brussels, Belgium	Scientific Community	100	Europe	2010
193	Workshop	S. Rasmussen	ISSP Workshop on Living Technology	March, 2010	University of Southern Denmark, Odense, Denmark	Scientific Community	100	Europe	2010
194	Workshop	S. Rasmussen	European Environmental Agency (EEA) biodiversity workshop “Citiziens in Science	May, 2010	Copenhagen, Denmark	Scientific Community	80	Europe	2010
195	Workshop	S. Rasmussen	Annual Meeting at Department of Molecular Biology, Origins of Life	June, 2010	Århus, Denmark	Scientific Community	75	Europe	2010
196	Workshop	S. Rasmussen	FET Flagship Workshop	18.06.2010	Berlin, Germany	Scientific Community	80	Europe	2010

197	Workshop	S. Rasmussen	FET Flagship Workshop.	20.-21.06.2010	Venice, Italy	Scientific Community	25	Europe	2010
198	Workshop	S. Rasmussen	Los Alamos National Laboratory Synthetic Biology Workshop 2010..	28.-29.06.2010	Los Alamos National Laboratory, USA	Scientific Community	45	North America	2010
199	Workshop	S. Rasmussen	ICT for Governance and Policy Modelling Constituence Building Workshop.	13.07.2010.	Brussels, Belgium	Policy Makers		Europe	2010
200	Workshop	S. Rasmussen	FET Flagship Workshop “Science & Policy Forum”	July, 2010	Brussels, Belgium	Policy Makers / Scientific Community		Europe	2010
201	Workshop	S. Rasmussen; M. Hanczyc	Workshop in Living Technology- Putting People in the Present.	24.-25.08.2010.	University of Southern Denmark, Odense, Denmark	Policy Makers / Scientific Community	50	Europe	2010
202	Workshop	S. Rasmussen	Workshop for FET 2011 Science Conference.	17.08.2010	Brussels, Belgium	Scientific Community	40	Europe	2010
203	Workshop	J. S. McCaskill	ISSP Workshop on Living Technology	March, 2010	University of Southern Denmark, Odense, Denmark	Scientific Community	100	Europe	2010
204	Workshop	J. S. McCaskill	FET Flagship Workshop 'Living Technology'	09.-10.06.2010	Brussels, Belgium	Scientific Community		Europe	2010
205	Workshop	S. Rasmussen	Synthetic Biology Workshop.	6.01.2011	The Danish Board of Technology, Copenhagen, Denmark	Scientific Community		Denmark	2011

206	Workshop	S. Rasmussen	Workshop on Engineering Life - Philosophical and Societal Perspectives on Synthetic Biology	2.09.2011	Copenhagen, Denmark	Policy Makers		Denmark	2011
207	Workshop	S. Rasmussen	When Science Meets the Headlines	05.10.2011	Copenhagen, Denmark	Policy Makers		Denmark	2011
208	Workshop	P.A. Monnard	COST Action CM0703 Working Group 3, (Organizer)	9.-10.12.2011	University of Southern Denmark, Odense, Denmark	Scientific Community	25-30	Denmark, Germany, Italy, France, Switzerland	2011
209	Workshop	G. von Kiedrowski	Overview of Origin of Life Problem (Part 2: Systems Chemistry). - "Origin of Life - Brainstorming Workshop",	20.-22.05.2011	Cern, Zwitzerland	Scientific Community (higher education, Research)	20		2011
210	Workshop	J. S. McCaskill	New Principles of unbound embodied evolution (EVOBODY)	24.-25.03.2011	La Palma, Spain	Scientific Community	21	EU	2011

Section B Exploitable foreground

This section specifies the exploitable foreground and provides the plans for exploitation. All these data can be public or confidential; the report must clearly mark non-publishable (confidential) parts that will be treated as such by the Commission. Information under Section B that is not marked as confidential will be made available in the public domain thus demonstrating the added-value and positive impact of the project on the European Union.

No applications for patents, trademarks, registered designs, etc. were made during the project lifetime.

Table B2: Exploitable foreground

Type of Exploitable Foreground[xxvi]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[xxvii]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
1 General advancement of knowledge	*Method for product extraction and separation in reversible hydrogel as well as content transport between two microreaction chambers*	*Yes*		*Microfluidic Device*	*M 72, 1.1*	*2012-2014*	*Proprietary knowledge and know-how*	*RUB-BioMIP*
2 General advancement of knowledge	*Intelligent controller board Bio@Fox for complex machines*	*Yes*		*Electronic Hardware*	*M 72, 1.1*	*2012-2014*	*Proprietary knowledge and know-how*	*RUB-BioMIP*
3 General advancement of knowledge	*On-chip concentration, dosing and preparation of genetic molecules, and move, mix and split operations in reversible gels in μ-channels*	*Yes*		*Molecular manipulation technique*	*M 72, 1.1*	*2012-2014*	*Proprietary knowledge and know-how*	*RUB-BioMIP*
4 General advancement of knowledge	*Electronically programmable chemical matrix (DNA processor)*	*Yes*		*Hybrid microfluidic device*	*M 72, 1.1*	*2012-2014*	*Proprietary knowledge and know-how*	*RUB-BioMIP*
5 General advancement of knowledge	*Experimental platform in ECCell*	*Yes*		*Programmable microfluidic control environment*	*M 72, 1.1*	*Current*	*Proprietary knowledge and know-how*	*RUB-BioMIP*
6 General advancement of knowledge	*ng_BioPro Software Package*	*Yes*		*Software*	*M 72, 1.1*	*Current*	*Software copyright*	*RUB-BioMIP*
7 General advancement	*Platform for the selective and specific sensing of*	*Yes*		*Analytical tool for*	*M 72, 1.1*	*2012-2014*	*Proprietary knowledge and*	*HUJI*

Type of Exploitable Foreground[xxvi]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[xxvii]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
of knowledge	*any DNA analyte*			*medical research (ie breast cancer, genetic disorders)*			*know-how*	
8 General advancement of knowledge	*Platform for nucleic acid detection by an electrical read out*	*Yes*		*Detection technology for analytes*	*M 72, 1.1*	*2012-2014*	*Proprietary knowledge and know-how*	*RUG*

[xxvi] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[xxvii] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

Description of exploitable foreground

1. Method for product extraction and separation in reversible hydrogel as well as content transport between two microreaction chambers

The method developed enables the transfer of different (bio-)chemical substances as well as products of microreactions in programmable microfluidic devices. The procedure based on a modulation between separative and non-separative transport in microchannels using feedback controlled Travelling-Wave-Electrophoresis (TWE). Application for such sophisticated microfluidic technology may be found quite generally in biochemical and molecular biological microfluidic applications. Potential sectors are:

1. Lab-on-a-chip
2. General microfluidic operations
3. Combinatorial and synthetic biology
4. Analytical Chemistry

Further experiments are required to verify the reproducibility of the separation capability of microelectrodes.

2. Intelligent controller board Bio@Fox for complex machines

The developed intelligent controller board *Bio@Fox* is a universal computer to measure and control processes in real time on programmable microfluidic chips. A custom designed adapter PCB, allows individual connection of electrodes (via jumpers) either to the micro controller board or to an electrochemical control system. It contains an FPGA (Xilinx xc3s500e) producing programmable digital control sequences for the microelectrodes (96 I/O´s; 1.2 - 3.3 Volt) as well as an alternative switchable connection to low-noise and continuously variable voltage electrochemical interfaces to perform e.g. cyclic voltammetry or impedance spectroscopy on-chip.

Application fields for this sophisticated process engineering technology are manifold, however a potential sector is the chemical process engineering.

3. On-chip concentration, dosing and preparation of genetic molecules, and move, mix and split operations in reversible gels in microchannels

Transport and concentration of molecules microfluidic channels

Two different physical effects are contributing to this molecular manipulation technique: electrophoresis (positive electrode, 1.2 - 3.3V) attracts the negatively charged DNA and probably electroosmotic-flow (EOF) which transport fluids in microchannels with low channel cross sections (1-2 µm). The two field effects, EOF and electrophoresis, help each other in this case.

Electronically programmable artificial membranes

In the simplest case, electronically programmable membranes do have one connection channel between two reservoirs, which is controlled by switch on or off electrical potentials. This allows the transport of chemicals from one reservoir to another. On-line control via fluorescence detection yields closed loop regulation in real-time is also available.

Programmable gelation and DNA-separation in a microfluidic environment

We developed a procedure to enable a programmable gelation in Lab-on-a-Chip devices using reversible, thermal-responsive hydrogels following by on-chip separation of biomolecules (DNA).

Programmable injection, extraction, mix and split operations

We evaluated the ability to process DNA from two flowing, dilute aqueous solution domains (which could be used as inputs and outputs) in an intervening gelled domain under electronic control. The transfer of informational molecules from the liquid to the gel phase is a prerequisite for renewable processing in a continuous flow setup. Possible applications for this sophisticated Lab-on-a-Chip/microfluidic operations are to use it for the controlled dosing of catalytic/initiator chemicals in micro-reactors as well as analytical issues, for instance. Potential application sectors are:

1. Lab-on-a-chip/Microfluidics
2. Combinatorial and synthetic biology
3. Nanobiotech

4. Electronically programmable chemical matrix (DNA processor)

The electronically programmable chemical matrix (DNA Processor) is an experimental microfluidic platform with integrated gold electrodes and a micromoulded fluidic layer, which uses electroosmotic flow and electrophoresis in order to 'live'control flows in biochemical processes. This involves both the real time detection and data analysis of the biomolecular system and the active control of biomolecules in a hybrid system. The DNA Processor couples up to 8 separate ECCells for DNA processing reactions via DNA exchange and should allow separate regulated reactions and programmable selective DNA transfer via flexible gel separation tracks between reactors.

The new technology can provide a generic programmable platform for complex molecular processing tasks in Field Programmable Chemistry, including the grand challenge of constructing the first electronic chemical cells. Further experiments are required to investigate new application in the field of DNA-Processing. Furthermore, potential sectors of application for this sophisticated Lab-on-a-Chip/microfluidic technology are also:

1. Lab-on-a-chip
2. Combinatorial and synthetic biology

5. Experimental platform in ECCell

This is a programmable hybrid electronic-microfluidic control system that can "see" the microscopic states of a chemical system and control it in real time, allowing a smooth transfer functionality and information between the chemical and electronic systems. Potential sectors of application for this sophisticated Lab-on-a-Chip/microfluidic technology are found in the areas of

1. Lab-on-a-chip
2. Combinatorial and synthetic biology
3. Nanobiotech
4. Fluorescence microscopy

6. ng_BioPro software package

The goal of this software is to facilitate and operate electronic Biochips (e.g. the electronically programmable chemical matrix) with thousands of electrodes and many different variants of fluidic designs, since higher-level control like feedback-loops in particular require a tight control of actors and sensors. Potential sectors of application for this sophisticated software technology are in the areas of:

1. Lab-on-a-chip
2. Combinatorial chemistry
3. Nanobiotech
4. Fluorescence Microscopy
5. Diagnostics

7. Platform for the selective and specific sensing of any DNA analyte

The different amplified DNA detection schemes developed within the ECCell project provide a generic sensing platform for the selective and specific sensing of any DNA analyte. Within these efforts, HUJI optimized the different sensing platforms and added to the system an exchangeable hairpin structure that can be implemented to sense any desired target. They have implemented the different sensing platforms to analyze the breast cancer biomarker gene BRCA1 and to detect the DNA mutants causing the Tay-Sachs genetic disorder and Cystic Fibrosis genetic disorder. Potential sectors of application are found in the areas of medical research and diagnostics.

8. Platform for nucleic acid detection by an electrical read out

In the course of the ECCell project RUG generated DNA sequences that are modified with lipid tails. These materials are the basis for a novel DNA surface anchoring process that was successfully applied for the selective sensing of DNAs by Si-NanoWire Field-Effect Transistor device arrays, and their regeneration through heat treatment and re-incubation was demonstrated. This represents a generic and cost-effective platform for nucleic acid detection by an electrical read out. Moreover, this technology may be readily applied for the non-covalent immobilization of other biomolecular species, such as proteins and antibodies for the detection of other analytes. Potential sectors of application are found in the areas of medical research and diagnostics.

4.3. *Report on societal implications*

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

This report has been collated from the input of the individual partner groups.

A General Information *(completed automatically when **Grant Agreement number** is entered.*

Grant Agreement Number:	**222422**
Title of Project:	**Electronic Chemical Cell (ECCell)**
Name and Title of Coordinator:	**Prof. John McCaskill, Ruhr-Universität Bochum**

B Ethics

1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***No***
2. Please indicate whether your project involved any of the following issues (tick box) :	***NO YES***
RESEARCH ON HUMANS	
• Did the project involve children?	X
• Did the project involve patients?	X
• Did the project involve persons not able to give consent?	X
• Did the project involve adult healthy volunteers?	X
• Did the project involve Human genetic material?	X
• Did the project involve Human biological samples?	X
• Did the project involve Human data collection?	X
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	X
• Did the project involve Human Foetal Tissue / Cells?	X
• Did the project involve Human Embryonic Stem Cells (hESCs)?	X
• Did the project on human Embryonic Stem Cells involve cells in culture?	X
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	X
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	X
• Did the project involve tracking the location or observation of people?	X
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	X
• Were those animals transgenic small laboratory animals?	X
• Were those animals transgenic farm animals?	X

• Were those animals cloned farm animals?	X
• Were those animals non-human primates?	X
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	**X**
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	**X**
DUAL USE	
• Research having direct military use	No
• Research having the potential for terrorist abuse	No

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	0	3
Work package leaders	0	7
Experienced researchers (i.e. PhD holders)	4	15
PhD Students	6	9
Other	5	3

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**3**
Of which, indicate the number of men:	3

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?

○ Yes
⊙ No

6. Which of the following actions did you carry out and how effective were they?

	Not at all effective				Very effective
❑ Design and implement an equal opportunity policy	○	○	○	○	○
❑ Set targets to achieve a gender balance in the workforce	○	○	○	○	○
❑ Organise conferences and workshops on gender	○	○	○	○	○
❑ Actions to improve work-life balance	○	○	○	○	○

○ Other:

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

⊙ No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

⊙ Yes- please specify **PhD Course and undergraduate students. SDU engagement of high school students as well as hosted introduction presentations to first year university students (at no cost to ECCell)**

○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

⊙ Yes- please specify: **Public project website, DVD (EU_FET)**

○ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

⊙ Main discipline[xxviii]:**1.1 Computer sciences, 1.3 Chemistry, 2.2 Electrical engineering, 2.3. Other engineering sciences**

⊙ Associated discipline:**1.2 Physics, 1.5 Biological sciences, 3.1. Basic medicine**

⊙ Associated discipline: **Nanoscience**

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14*) SDU involvement was at no Cost to ECCell budget

⊙ Yes
○ No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

○ No

○ Yes- in determining what research should be performed

○ Yes - in implementing the research ⊙ Yes, in communicating /disseminating / using the results of the project	
11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	⊙ Yes ○ No
12. Did you engage with government / public bodies or policy makers (including international organisations)	
○ No ⊙ Yes- in framing the research agenda ○ Yes - in implementing the research agenda ⊙ Yes, in communicating /disseminating / using the results of the project	
13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers? ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible) ⊙ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible) ○ No	
13b If Yes, in which fields? Energy; Environment; Information Society; Public Health; Research and Innovation	

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
Education, Training, Youth		Foreign and Security Policy		Space	
Employment and Social Affairs		Fraud		Taxation	
		Humanitarian aid		Transport	

13c If Yes, at which level?

- ⊙ Local / regional levels
- ⊙ National level
- ⊙ European level
- ⊙ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**57**
To how many of these is open access[xxix] provided?	**15**
How many of these are published in open access journals?	**3**
How many of these are published in open repositories?	**1**
To how many of these is open access not provided?	**42**
Please check all applicable reasons for not providing open access:	
☒ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[xxx]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**0**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

☒	Increase in employment, or	☒	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
❑	Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs, incl. students, approx. Difficult to estimate / not possible to quantify:	*Indicate figure:* ***45***

	❑

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes ⊙ No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes ⊙ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

☒	Press Release	☒	Coverage in specialist press
❑	Media briefing	☒	Coverage in general (non-specialist) press
❑	TV coverage / report	☒	Coverage in national press
☒	Radio coverage / report	☒	Coverage in international press
☒	Brochures /posters / flyers	☒	Website for the general public / internet
☒	DVD /Film /Multimedia	☒	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

❑	Language of the coordinator	☒	English
❑	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as

geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

[xxviii] Insert number from list below (Frascati Manual).

[xxix] Open Access is defined as free of charge access for anyone via Internet.

[xxx] For instance: classification for security project.