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Coordinating Organisation

Ruhr University Bochum, BioMIP

Electronic Chemical Cell (ECCell) is an EU-sponsored project in FP7 (the 7th framework program), funded in the ICT Future Emerging Technologies by the FET-Open program. The aim of the project is to establish a novel basis for future embedded information technology by constructing the first electronically programmable chemical cell. This will lay the foundation for immersed micro- and nanoscale molecular information processing with a paradigm shift to digitally programmable chemical systems.

A central aim of ECCell is to reduce the complexity of the loop linking information processing with computer system construction that dictates the limits to adaptability of IT technology. Current computer construction involves enormous complexity associated with a global network of specialized factories. Cells are the smallest biological units that close this loop – they are capable of universal computation through genetic programming and are locally constructed. The smallest technically programmable unit that closes this loop is approached by electronic chemical cells. The objective is not to produce a computational engine able to compete with silicon on abstract problem solving. Instead, ECCell will enable novel *in situ* information processing for nano- and microscale synthetic and diagnostic process control. By so doing, it is addressing a core problem in ICT – current IT hardware is efficient but has limited adaptability – through the effective integration of programmed material construction with relevant information processing. The strong international interest in this was confirmed in 2010 by the formulation of two larger coordination initiatives COBRA[1] and SPLiT that have taken up this mission in broader contexts.

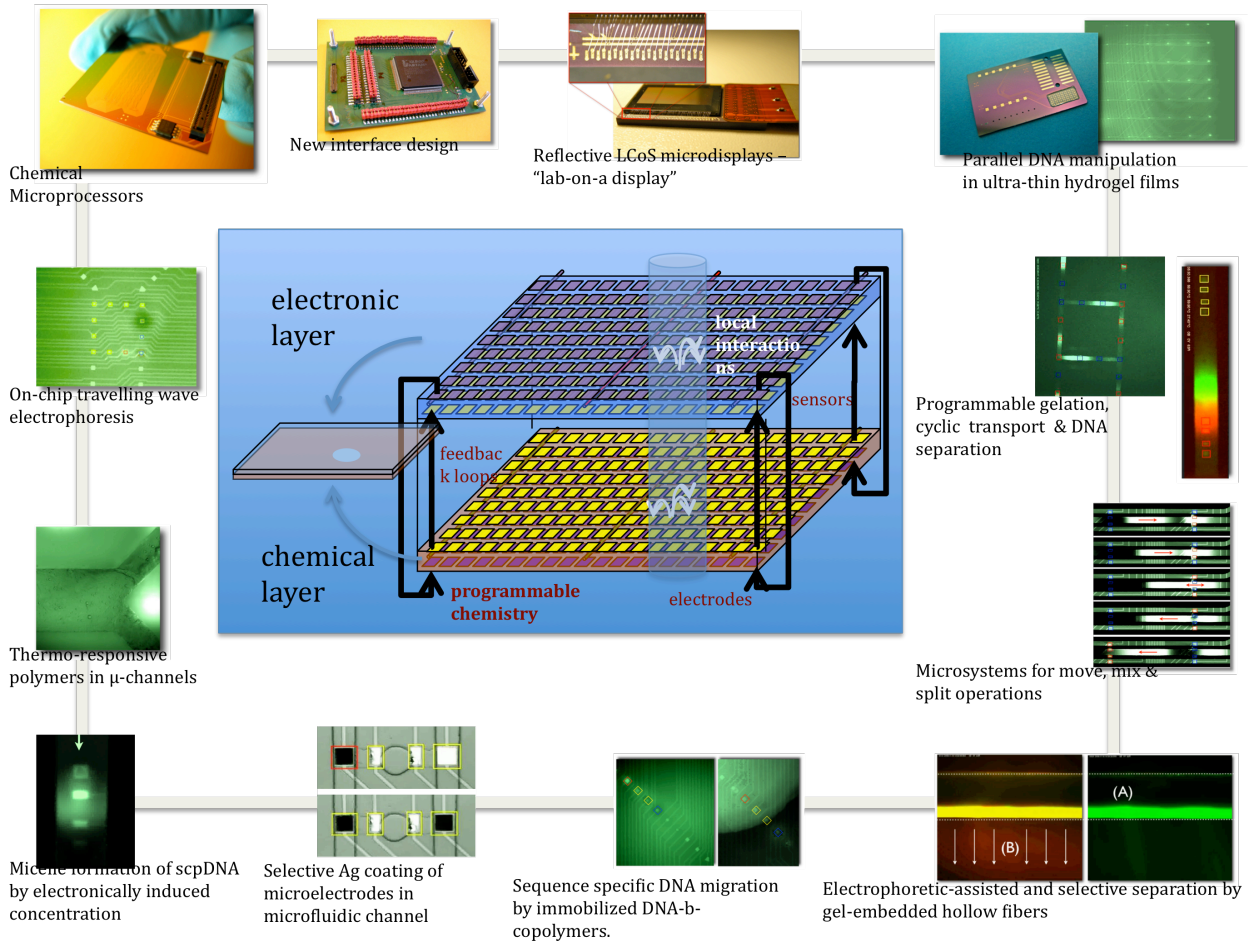

Fig. 1: Overall architecture of the material and information processing system supporting electronic chemical cells. Two layers – an electronic and a chemical layer are spatially linked to produce an electronically programmable material processing system via tight local coupling with massively parallel real-time optical sensor and electrode actuation arrays. This allows ECCells to have electronic in addition to molecular genomes. (Ruhr Universität Bochum)

The overall architecture of our physical environment system for ECCells is shown in Fig. 1. Instead of relying purely on novel chemistry, a chemical layer is tightly coupled to an electronic processing layer. It is only now becoming feasible to integrate sufficient chemistry to approach information processing via artificial cells. Most initiatives to construct an artificial cell have either been top-down, as in the Venter/Hamilton Smith path[2] to minimize the genome of existing cells, or have involved the design and integration of fully autonomous chemistry separately responsible for cell functionality, such as in the Luisi/Szostak design[3]. By contrast, in a previous FP6 project, PACE[4], we have explored the possibility of employing an electronic support system as a cofactor to bootstrap the development of an artificial cell. In ECCell, we take the next step of constructing electronic genomes, which co-regulate the chemical processes and are replicated and evolved in tight association with individual cells.

The compatibility of different chemical subsystems has proved the major barrier to progress in constructing artificial cells, even allowing very different chemical realizations of the three core functions of a cell that are: (i) the *replication* of genetic information characterizing proper functioning of the cell, (ii) the *containment* of valuable chemicals (and isolation from harmful ones) and (iii) the orchestration of a *metabolism* capable of supporting the construction and/or enrichment of all the chemical building blocks (required for the first two functions). In biological cells these three functions are attributed to nucleic acids (DNA and RNA), lipids and proteins respectively. In ECCell, we are simplifying the integration problem by focusing on a single family of informational molecules able to support all three artificial cell functionalities, one involving disulphide bonds for rapid synthesis, amphoteric groups for autonomous (isoelectric) positioning or block copolymer tails for reversible anchorage into a programmable gel matrix. The metabolism of the ECCell is regulated electronically via induced pH changes and via the selective release and uptake of small molecules via "electronic sponges". The overall scheme of a life cycle of ECCells is shown in Fig. 2.

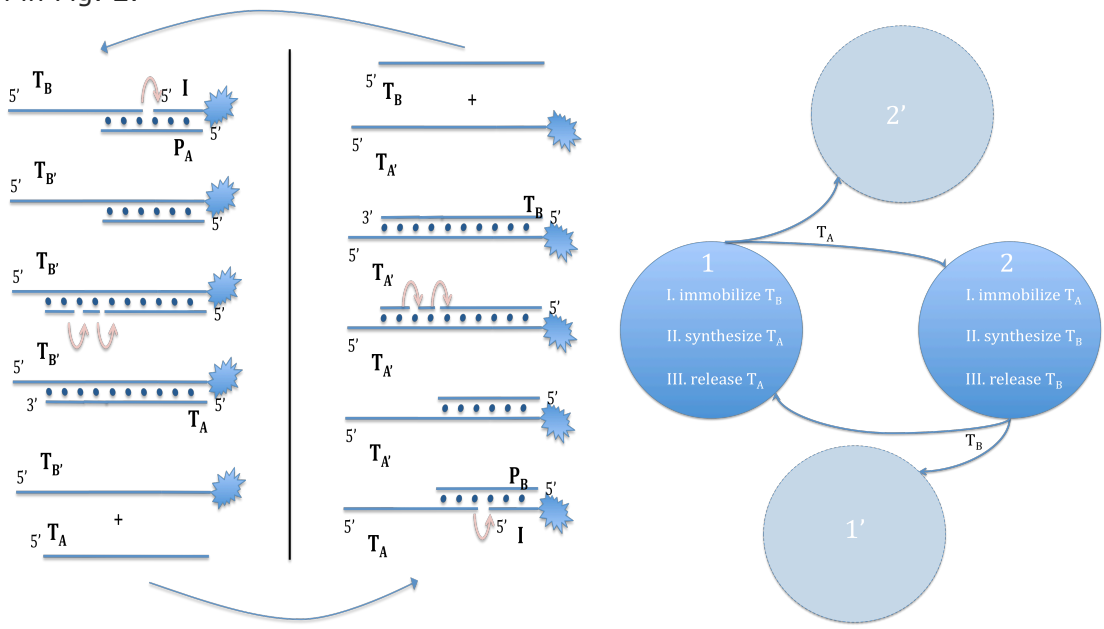

Fig 2 Overall two-phase replication scheme for ECCell (McCaskill). The overall scheme is shown on the right, with proliferation at the molecular and at the compartment level. On the left is the double strand based exponential amplification scheme of a two-compartment model. The role of the input scpDNA (labelled I) is to capture the templates from the other compartment, via an adapter primer P_X. Single or multistep ligation (e.g. using rapid disulphide bond chemistry) completes the anchorage and the template construction. An electronic pH cycle is used to program release of the newly synthesized strand in the final step. Electric fields are used to transfer the synthesized template selectively through the gel to the other compartment.

The novel informational molecules belong to the family of hybrid modified DNA, including especially scpDNA (synthetic copolymer DNA hybrids). Whereas the DNA

sequences contain genetic information about the function of the molecules in the cell cycle, the repetitive polymer conveys essentially non-evolvable (or "environmental") additional information, but imparts a significant additional functionality to the attached DNA. The primary additional functionalities being investigated in ECCell are surfactant and acid-base properties that respectively give DNA some of the characteristics of lipids and proteins and can at the same time simplify the replication process. For example, Herrmann's group is investigating the way in which block copolymers of PPO and/or PEO attached to DNA can undergo phase transitions from micelles to other structures such as gels and vesicles (similar to those found in lipids)[5]. These transitions can be induced by DNA hybridization and hence respond sensitively to sequence information. As another example, polymers of spermine attached to DNA can neutralize the DNA charge (depending on the pH) and impart the molecules with enhanced pH-sensitive hybridization[6]. Redox metabolic control of replication can be attained by developing self-replicating molecules containing modified disulphide links in the DNA backbone (von Kiedrowski's group[4]). Electronic pH control of replication can be achieved by using deprotonation of bases below pH 6 in quadruplex or triplex structures in conjunction with bis-aniline redox chemistry in gold nanoparticle enhanced electrochemistry (Willner's group). This electronic control of metabolism has now been extended to allow selective release and uptake of small molecules by "electronic sponges[7].

What does an ECCell life-cycle look like? It comprises an electronically and spatially orchestrated sequence of chemical reactions that replicates molecules, their spatial distribution and the electronic control program inside an essentially two-dimensional microfluidic array. The fixed microfluidic channel environment contains a regular network of flowing resource channels separated from electronic processing regions containing high densities (up to $10^6/cm^2$) of electrodes, by hydrodynamic barriers (see Fig. 2). scpDNA molecules are amplified, distributed in space selectively and refocused to form two daughter cells by the sequence of electrode changes defined in response to sensor signals. The sensors are provided by integrating fluorescence signals from an array of subregions, with multicolor response allowing the simultaneous monitoring of the concentration of several different labeled chemicals at video rates. Cellular containment is realized by the scpDNA synthesis being coupled to modulation of the mobility of chemicals in the electric fields induced by the electrodes (e.g. via reversible gelation or charge modulation). Simulation of the coupled reaction and transport is being performed at multiple levels of detail by Rasmussen's and McCaskill's groups. The electronic control program is attached to a particular set of chemicals to form an ECCell via a location algorithm that depends on both the previous electronic state and the current measured chemical distribution (via the sensors), and this defines the reference point for relatively addressed sensors and electrodes in the control program.

Significant progress has been made in the first two years of ECCell on the development of modules for an electronic chemical cell. High-density electrode arrays coupled to microfluidic systems with up to 2 million electrodes have been constructed. Chemical modules have been developed including a redox-sensitive ligation system using modified DNA with disulphide linkages that can be used in controlled replication protocols. scpDNA has been synthesized that can be selectively immobilized in a solution gel, and other variants that allow DNA to be used to form vesicles. The electronic regulation of DNA structural transitions, via comparatively small pH jumps (between pH5.7 and 7.2), can now readily be induced by modified microelectrodes. With natural DNA, special pH sensitive structures exist, such as the i-motif or C-quadruplex[8], which have been used by Willner's group to develop electrochemically controlled DNA processing[9]. In particular they were able to control DNAzymal processing and autocatalytic signal amplification via this structural transition and a small pH shift. Work has now commenced on triplex DNA based replication (using disulphide chemistry) that is also sensitive to pH in this range. Sequence selective gel processing of DNA has been achieved in reversible microgels using novel scpDNA variants synthesized in Hermann's group. Travelling wave and isoelectric electrophoretic manipulation of DNA has been developed for implementing the ECCell life cycle. Finally, a

novel basis for electronically controlled metabolism has been established in Willner's group through the development of "electronic sponges" for small molecule metabolites[8].

The ethics of artificial cell research has been a core concern of European researchers since the first EU project PACE in this area. That project produced a guideline document[10], that ECCell is adhering to, and that has already served as important input for the formulation of ethical guidelines for the field of Synthetic Biology. Although many issues are common to Nanotechnology and Biotechnology in general, there are a number of special issues raised by this research, in particular as the creation of novel organisms approaches. Ethical activities in 2008-9 have included coordination with Synthetic Biology in Germany[11], with the ISSP in Denmark's special initiative on Living Technology[12], with the Dutch expert meeting on Synthetic Biology[13] in addition to discussions at project workshops at the European Center of Living Technology.

ECCell Partners	**Lead Scientists**	**Country**
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http://www.dfg.de/aktuelles_presse/reden_stellungnahmen/2009/download/stellungnahme_synthetische_biologie.pdf

[12] Conference on Living Technology, org. Prof. Mark Bedau, ISSP, University of Southern Denmark, Louisiana Museum, June 9-10, 2009.
http://link.sam.sdu.dk/ISSPworkshops/index.html

[13] International Expert Meeting on Synthetic Biology, org. Prof. Patricia Ossewejer, Delft University, NL, October 3, 2009.