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Project acronym: **ECCell**

Project title: Electronic Chemical Cell

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Thematic Priority: Theme 3 Information and Communication Technologies

Publishable Executive Summary

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Coordinating Organisation

Ruhr University Bochum, BioMIP

Electronic Chemical Cell (ECCell) is an EU-sponsored project in FP7, 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 tackling the construction of the first electronically programmable chemical cell. This lays the foundation for immersed micro- and nanoscale molecular information processing with a paradigm shift to digitally programmable chemical systems.

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 evolution1 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.

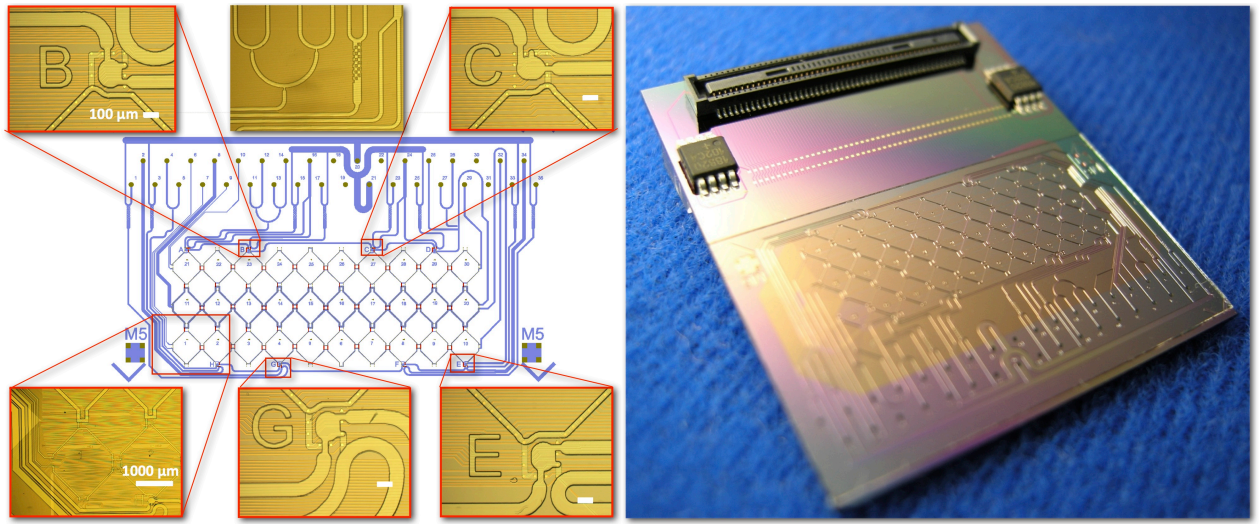


Fig. 1. One of the final integrated ECCell chemical microprocessors for exploration of electronic chemical cells. (RUB) The left image shows microscopic details of the microfluidic network environment, with the overall microchannel pattern (centre) visible inverted in the photo (right). The fluidic architecture of the chips involves three subregions: (i) resource/waste in continuous flow (ii) an array of isolated reaction sites with daisy chain fluidic IO via droplets (iii) a fractally thinned 2D communication channel network for specific information molecule exchange between reaction sites. The right 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 IO chips for electrical external connection.

The novel chemical microprocessor technology required to establish electronic chemical cells, as shown in Fig. 1, 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.

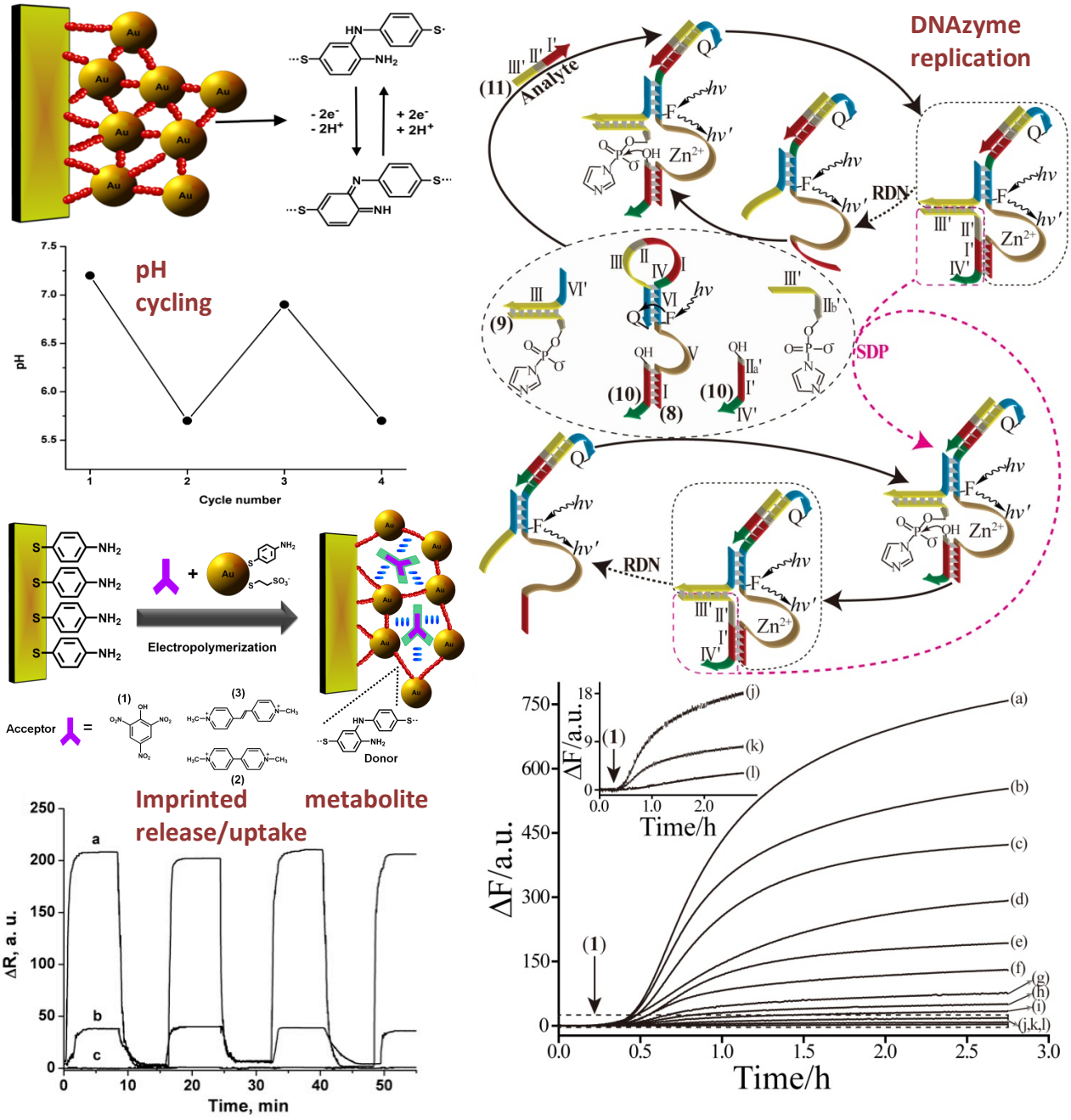
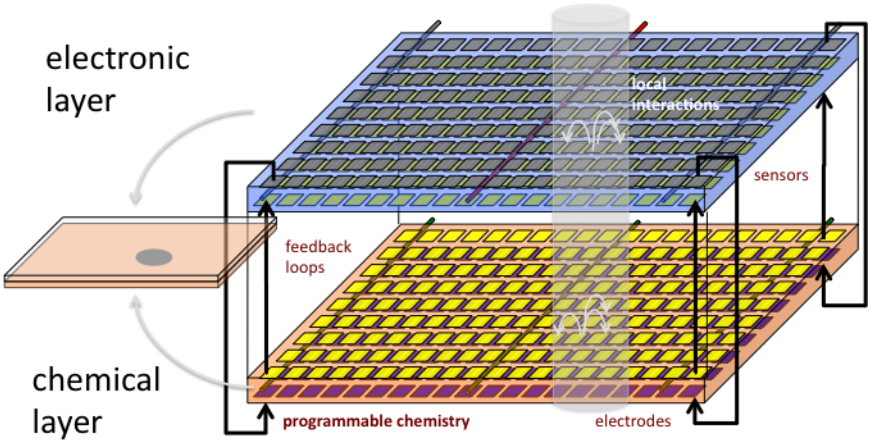


Figure 2: Electronically controlled pH cycling, imprinted metabolite release and enzyme free DNA replication (by HUJI). Willner et.al. in the ECCell project developed novel electrochemical coupling between electronic and the chemical subsystems required for cell functionality. The pH cycling (top left) operates resersibly below the electrolysis limit and in the right pH range to control DNA hybridization in triplex and quadruplex DNA structures. The programming of functionalized electrode surfaces by imprinting (bottom left) was demonstrated for a range of metabolic substrates, allowing electronic control of metabolism in the ECCell. (Right) As an alternative to triplex disulphide ligation (see Fig. 3) with high turnover, a semi-constructive isothermal DNAzyme replication scheme was established which is electrode controllable via the pH and ion release shown on the left.

Modifications of DNA chemistry have allowed it to replicate without enzymes and to act in containment and metabolic regulative capacities: thereby simplifying the chemical integration process for constructing cells. The project has developed novel rapid redox-active and pH sensitive replication chemistry based on sulfhydryl ligation (RUBb) and ion-sensitive DNAzymes (HUJI). Secondly, it has developed novel amphiphilic DNA molecules (RUG) that self-assemble into containment structures, obviating the need for lipid membranes for ECCells. Some of these assemble into vesicles, others

reversibly control molecular mobility by modulating the attachment of specific DNA to polymer gel supports. Thirdly, the project has developed electrochemical subsystems (HUJI, see fig. 2) 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 electronic chemical "hardware", the project has developed the electronic microfluidic chips (chemical microprocessors, see Fig. 1) 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 deployed effectively in the project.



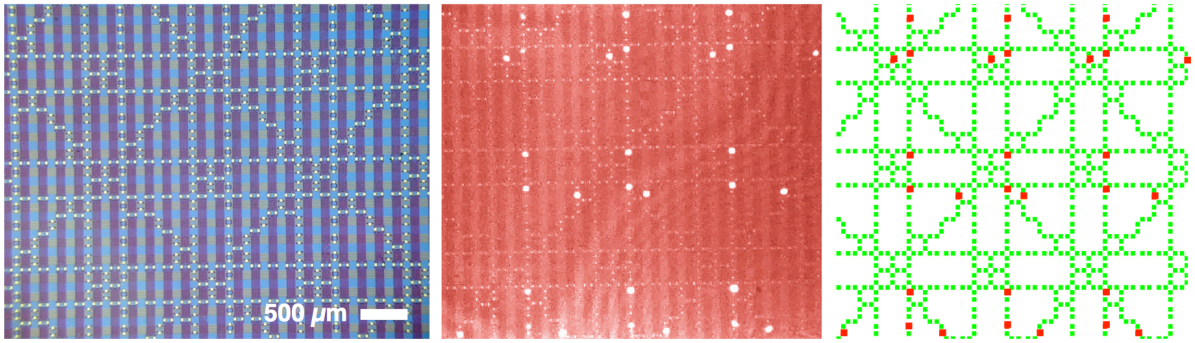


Fig.2 Architecture of electronic chemical space for ECCells. Two layers of active information processing components (chemical and electronic) are locally coupled with one another by feedback loops. Local sensors of chemical activity actuate electronic processing resulting in changes in local electrode 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. Bottom panel: electrode actuator array used in the chemical space built in the ECCell project: left: microscope image; right: pattern of electrodes (red ones active +ve); middle: chemical signals (electrochemiluminescence) providing optical feedback to the electronic processing system. In the project, we have also employed "thinned" 2D geometries (best seen at bottom right), allowing a more efficient use of independent electrodes.

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. 1). DNA 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 amphiphilic DNA 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 SDU and RUBa. 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.

The project developed novel simulation and control software. SDU has investigated multiscale particle simulations linking molecular properties with reaction-transport and self-assembly of critical subsystems and the overall cell-cycle. This work involves a novel mesoscale modelling of DNA structures, using dynamic bonds. To connect this with electronic chemical cell operation, RUBa has developed both 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. 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.

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 (RUBb) that is cycled between twin chambers at two pH levels (HUJI), with sequence specific immobilization to amphiphilic anchor DNA tags captured in a reversible gel matrix (RUG). A lasting impact of the project will also involve the completed online feedback hardware and software system (RUBa), which integrates simulation (SDU, RUBa) into the current focus of experimentation on the real devices and the chemical microprocessors themselves.

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. 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[2], that ECCell is adhering to. 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[3], with the ISSP in Denmark's special initiative on Living Technology[4], with the Dutch expert meeting on Synthetic Biology[5] in addition to discussions at project workshops at the European Center of Living Technology.

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.

References

A list of publications of the project is available online from the EU as part of the project reporting and on the project website: http://www.istpace.org/ECCell

[1] G.F.Joyce's widely accepted definition of life: "A self-sustaining chemical system capable of evolution".
[2] M. A. Bedau, E.C. Parke, U. Tangen, B. Hantsche-Tangen Ethical guidelines concerning artificial cells. (2008) Pace Final Web Report.
http://www.istpace.org/Web_Final_Report/the_pace_report/Ethics_final/PACE_ethics.pdf
[3] DFG Expert Meeting on Synthetic Biology, Berlin, February 27, 2009. Org. Dr. N. Raffler, DFG (DFG, Acatech, Leopoldina).
http://www.dfg.de/aktuelles_presse/reden_stellungnahmen/2009/download/stellungnahme_synthetische_biologie.pdf
[4] 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
[5] International Expert Meeting on Synthetic Biology, org. Prof. Patricia Ossewejer, Delft University, NL, October 3, 2009.