



Project no. **222422**

Project acronym: **ECCell**

Project title: Programmable Artificial Cell Evolution

Instrument: STREP/FET OPEN

Thematic Priority: Theme 3 Information and Communication Technologies

# Publishable Executive Summary

**Yr 1**

Submission date: 15. 10. 2009

Start date of project: **1.09.2008**

Duration: **3 years**

Author: John S. McCaskill

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.

The basic tenant in ECCell is that the complexity of the loop linking information processing with computer system construction 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 cosntructed. 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 will address 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.

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[1] 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[2]. By contrast, in a previous FP6 project, PACE[3], 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 transcend this by 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 PACE, primarily following a design by Rasmussen *et.al.*[4], the three families of molecules were PNA (peptide nucleic acid), simple surfactants such as fatty acids and metallo-organic redox catalysts such as the Ruthenium complexes employed in solar energy research. In ECCell, we are simplifying the integration problem by

focusing on a single family of informational molecules, scpDNA, able to support all three artificial cell functionalities. In addition, we are extending the support system to include electrochemical reaction control.

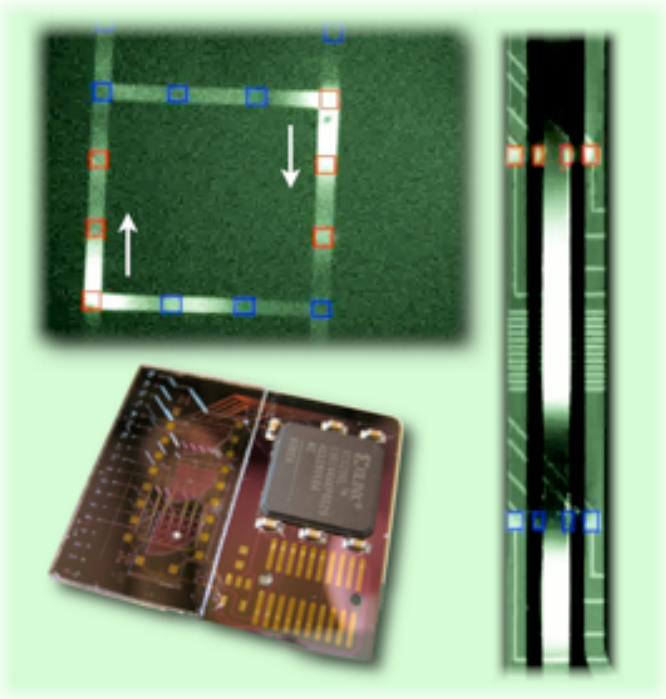

**Fig. 1 Electronically regulated DNA processing.**
*Lower left:* Chemical microprocessors developed by the Ruhr University Bochum (BioMIP group). *Upper left:* Cyclic transport and separation of DNA packets in a channel network using local electrode signals (labeled blue and red in snapshot) regulated with feedback from the imaged fluorescence signal (white) that reveals where DNA is concentrated. *Right:* two phase operation with electronic DNA processing in a central stationary channel connected to two flowing resource channels (in this case simply for DNA delivery) by hydrodynamic barriers. In contrast with standard capillary electrophoresis, these processes are locally regulated with digital voltages responding automatically to sensor signals.

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]. In addition, 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[3]).

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). 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. The project is employing a real-time dialect of UML to formalize these complex relationships [7].

Significant progress has been made in the first year of ECCell on the development of modules for an electronic chemical cell. High-density electrode arrays coupled to microfluidic systems have been constructed, to allow electronic genomes with significant complexity to be realized in the next stage of the project. 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. Perhaps most significant for the next phase of the project is the establishment of electronically regulated DNA structural transitions, via comparatively small pH jumps (between pH5.7 and 7.2), that can 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 via this structural transition and a small pH shift: see Fig. 2.

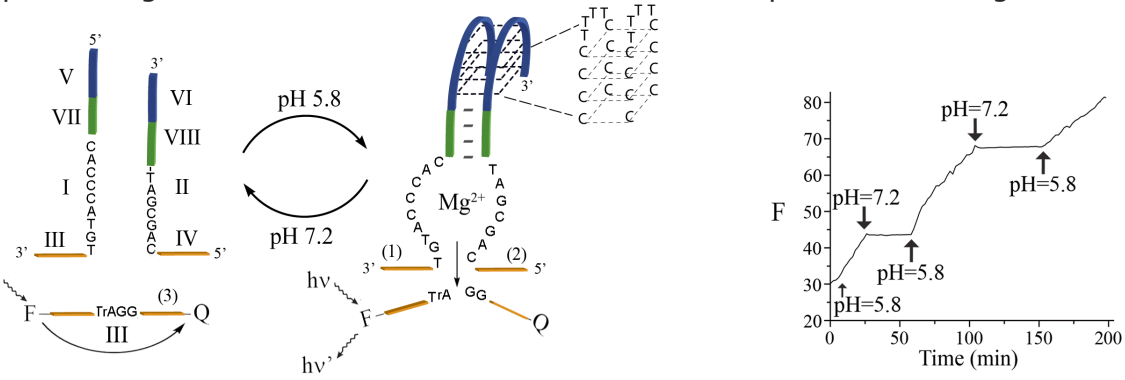


**Fig. 2 Electronic pH control of DNA cleavage and ligation.** DNAzyme subunits self-assemble at pH = 5.8 to an active C-quadruplex-bridged DNAzyme structure and separate into the inactive subunits at pH = 7.2. Reversible and switchable activation/deactivation of the DNAzyme was accomplished in the system via pH cycling[9]. This pH change could be achieved reversibly by bis-aniline-crosslinked gold nanoparticles coated on electrodes.

In the second year of the project, the separate strands of this chemical and electronic module development will be integrated and placed under local electronic control in the chemical microprocessor modules. In particular, a pH cycled replication process will be completed that involves polymer extended DNA in an essential way.

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** |
|---|---|---|
| Ruhr-Universität Bochum – BioMIP - Bioorganic Chemistry 1 | John McCaskill<br>Günter v. Kiedrowski | Germany |
| Rijksuniversiteit Groningen | Andreas Herrmann | Netherlands |
| Syddansk Universitet | Steen Rasmussen | Denmark |
| Hebrew University of Jerusalem | Itamar Willner | Israel |
| European Center of Living Technology | Kristian Lindgren | Italy |


| | |
|---|---|
| Coordinator:<br>Prof. Dr. John McCaskill<br>Ruhr-Universität Bochum<br>Organische Chemie 1, BioMIP<br>D- 44780 Bochum, Germany | Electronic Chemical Cell, ECCell<br>Web site: http://www.istpace.org/ECCell<br>Email: john.mccaskill@biomip.rub.de<br>Phone: +49-(0)234-32-27702 Fax: -14047 |


---

[1] D.G. Gibson, *et.al.* Complete Chemical Synthesis, Assembly, and Cloning of a Mycoplasma genitalium Genome. Science **319** (2008) 1215-1220.
[2] J.W. Szostak, D.P. Bartel, and P.L. Luisi Synthesizing life. Nature **409** (2001) 387-390.
[3] Programmable Artificial Cell Evolution Final Web Report. FP6-IST-FET-002035 J.S. McCaskill (coordinator) *et.al.* (2008) http://www.istpace.org
[4] S. Rasmussen, L. Chen, M. Nilsson, and S. Abe, Bridging nonliving and living matter, Artificial Life 9 (2003) 269.
[5] K. Ding, F.E. Alemdaroglu, M. Boersch, R. Berger, & A. Herrmann, Engineering the structural properties of DNA block copolymer micelles by molecular recognition. Angewandte Chemie-International Edition **46**:7 (2007) 1172-1175.
[6] R. Noir, M. Kotera, B. Pons, J. Remy, and J. Behr, Oligonucleotide–Oligospermine Conjugates (Zip Nucleic Acids): A Convenient Means of Finely Tuning Hybridization Temperatures. Journal of the American Chemical Society 130 (2008) 13500–13505.
[7] U. Tangen (2009) ECCell D5.1 http://www.istpace.org/ECCell/Research/
[8] K. Gehring, J.-L. Leroy, M. Gueron, A tetrameric DNA structure with protonated cytosine-cytosine base pairs. Nature **363 (**1993**)** 561-565.

[9] J. Elbaz, S. Shimron and I. Willner "pH-Switchable Mg2+-Dependent DNAzymes." Submitted for publication (2009).
[10] 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
[11] 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
[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.