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Deliverable 6.1: report, public (PU)

Demonstration of sequence-specific DNA processing with programmable ECCell gels.

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Organisation name of lead contractor for this deliverable:

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9. Deliverable n. 6.1: Demonstration of sequence-specific DNA processing with programmable ECCell gels.

9.1 Introduction

During the course of the ECCell project, the consortium leader RUBa, who is dealing with microfluidic devices as the electronic scaffold for the electrochemical cell, made some important findings. The triblock copolymer Pluronics, which has the molecular structure poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (PEO-b-PPO-b-PEO), was employed as a gel matrix within microfluidic channels to separate ODNs of various lengths. The striking feature of this material to other gel matrices is that it can be reversibly dissolved within the microfluidic device by electronically induced heating to a sol state. In this way, ODN separation can be carried out in the gel state and subsequently different ODNs can be directed to different positions within the microfluidic channels after dissolution of the gel by travelling wave electrophoresis. This is a significant step forward to realize an electronically controlled cell because it allows performing anabolic reactions and separation of the resulting products. However, since the genetic material of the cell is oligo- and polynucleotides one obstacle is remaining, i.e., the separation of equally long, single stranded (ss) DNA sequences that usually exhibit the same electrophoretic mobilities.

In the following, three different materials are described, with which this goal was successfully realized. It is well known that ssDNA sequences can be specifically retained when the complement is covalently connected to the gel matrix. However, the resulting gels (mostly polyacrylamide) are not reversible. We decided to generate soft matter DNA nanoparticle systems that due to their size and molecular interactions are retained in the reversible Pluronics matrix.

This allows the development of sequence-specific programmable gel processing as an application of the technology being developed in ECCell. We commence this report on the deliverable with section son the synthesis and characterization of the new materials and then with microchannel experiments (via CGE and chemical microprocessors) on their use to program sequence-specific separations of DNA.

9.2 DNA block copolymer micelles stabilized by formation of a semi-interpenetrating network in the core

DNA-b-PPO micelles were combined with Pluronics to form blended micelles. In this case a strong molecular interaction between the DNA particles and the Pluronics gel matrix should be achieved. For stabilization of the particles a polymernetwork was generated in the core of the micelles. The stabilization reaction was carried out by internalization of pentaerythrytol tetraacrylate (PETA) in the micelles and UV induced cross-linking. Figure 32 gives a schematic representation of the architecture of the blended DNA block copolymer aggregates.

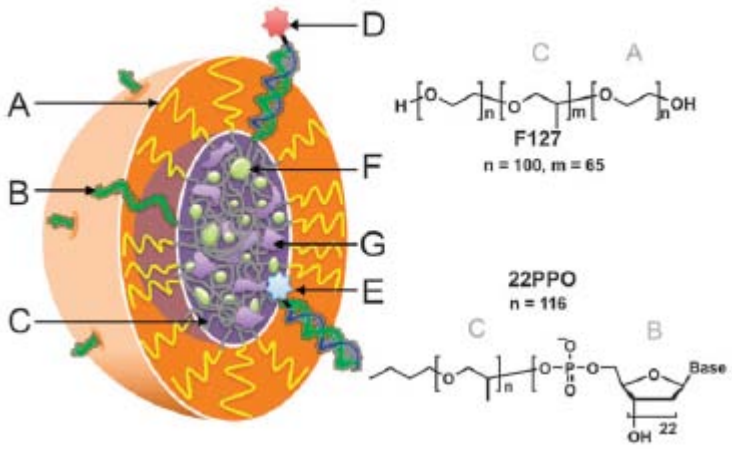


Figure 32: Schematic of the mixed micelle architecture and chemical structures of the polymeric components. (A) PEO block of Pluronic. (B) DNA block of DBC. (C) PPO blocks of Pluronic or DBC. (D) and (E) Probes at 5'- and 3'-ends of the complementary DNA, respectively. (F) Hydrophobic compound loaded 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. In a next step it needed to be demonstrated that the cross-linked blended micelles are still able to hybridize with complementary DNA. The ability of the aggregates to undergo Watson-Crick base pairing was successfully proven with FRET experiments employing fluorophore labelled Pluronics F127 and labelled ODNs. Additional proof for the ability of the blended micelles to hybridize was achieved with ODN-labelled gold nanoparticles and subsequent TEM analysis. Moreover, the particles were analyzed by AFM and dynamic light scattering (DLS) (see A. Herrmann et al, Chem Commun. 2010, 46, 4935 for further information).

9.3 DNA block copolymer micelles containing more hydrophobic polymer cores.

Several PIs (Mw = 950, 2810, 4710, and 7630 g/mol) with a terminal hydroxyl group were prepared by anionic polymerization. Subsequently, the semitelechelic polymers were converted into the corresponding phosphoramidites (Figure 33A) and coupled to ODNs present on the solid support to form DNA-b-PI in excellent yields (Figure 33B and 33C).

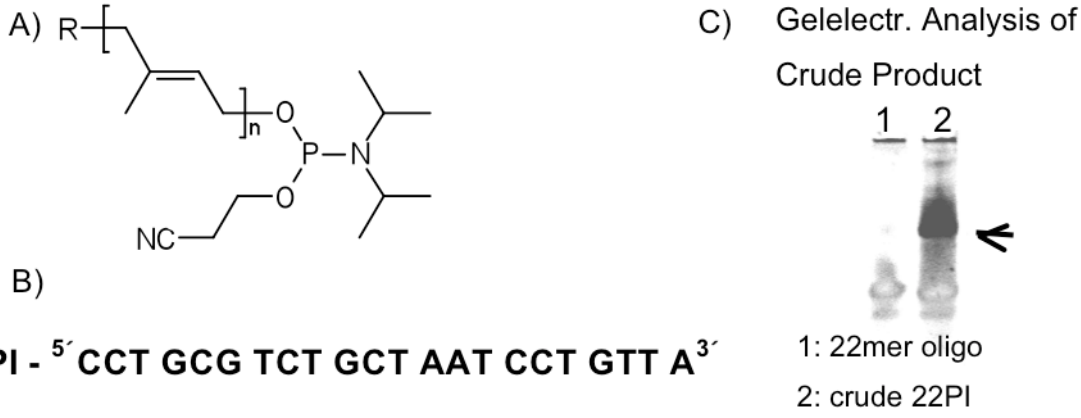


Figure 33: A) PI-phosphoramidite, B) Sequence composition of DNA-b-PI and C) Gel-electrophoretic analysis of the crude product of DNA-b-PI demonstrating the high efficiency of the coupling reaction on the solid support.

After micellisation the corresponding aggregates were analyzed by AFM and DLS revealing particles with a diameter of 23 + 3 nm. Again hybridization experiments with these self-assembled structures proved to be successful.

9.4 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 aggregates were produced that are composed of ODNs which are functionalized with hydrophobic chains at the nucleobases. For that purpose hydrophobicity was imparted by the introduction of 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 34 gives an overview of the employed materials including the phosphoramidite building block, the incorporation into ODNs and three different architectures with their corresponding hybridization products.

The micelles formed from these modified DNA sequences were characterized by atomic force microscopy, dynamic light scattering, and polyacrylamide gel electrophoresis (see A. Herrmann et al, Chem. Eur. J. 2010, DOI: 10.1002/chem.201001816.) 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.

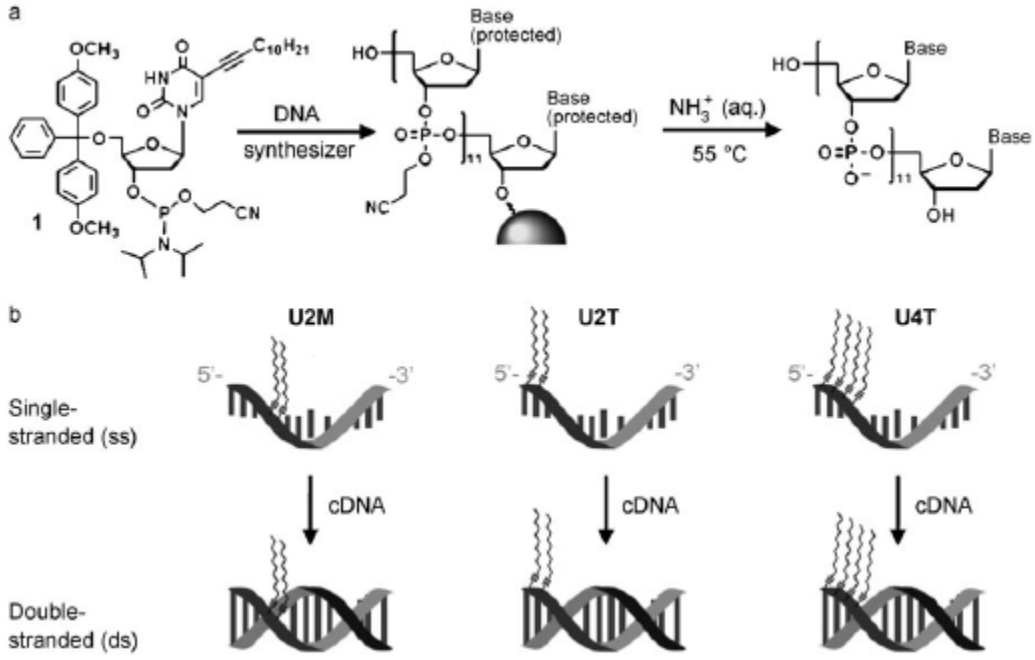
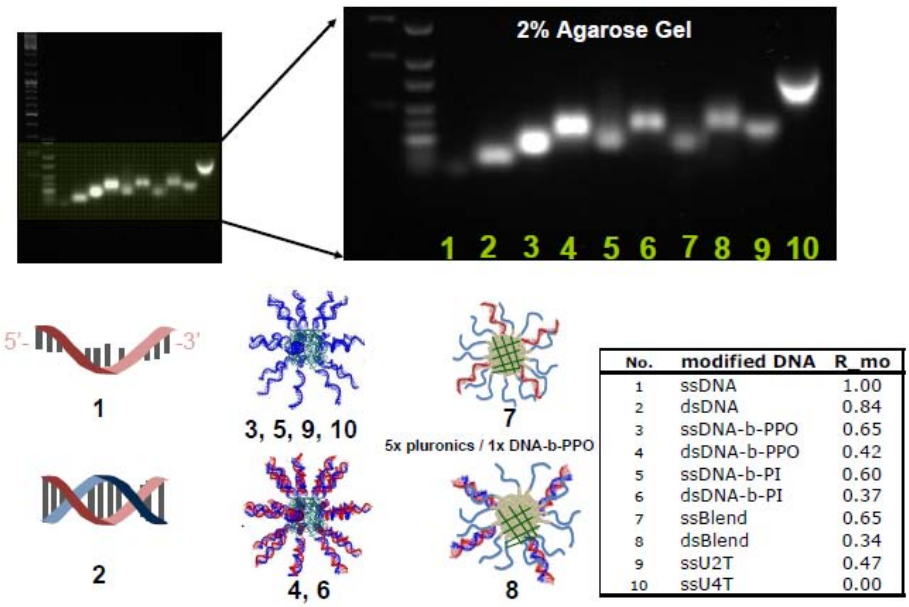


Figure 34: Synthetic scheme and representation of lipid–DNAs. a) The precursor, 5-(dodec-1-ynyl)uracil deoxyribophosphoramidite (left) was used in conventional solid-phase DNA synthesis (center), and deprotection yielded the lipid–DNA (right). b) Schematic representation of the ss and ds lipid–DNA amphiphiles (U2M, U2T, and U4T) investigated.

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

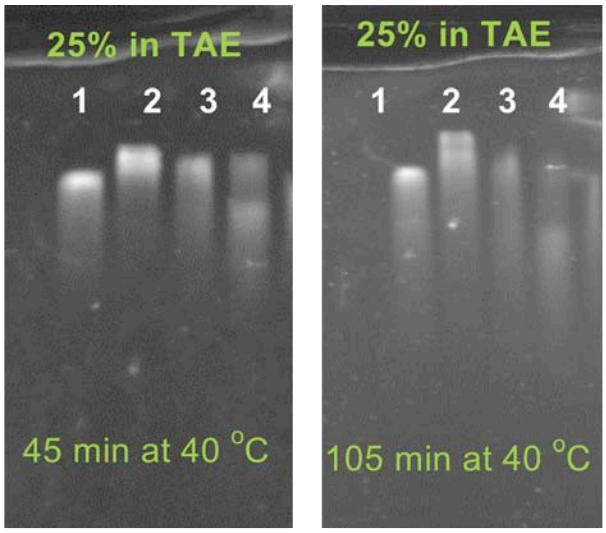
After preparation we tested the ability of the DNA aggregates to sequence specifically retain DNA in conventional gels that can be disaggregated after electrophoresis, i.e. agarose and Pluronics gels.



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 35: 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.

In Figure 35 the electrophoretic analysis 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 significant 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 acted as a gel matrix. Figure 27 shows the migration of polymeric DNA aggregates in the Pluronics gel. Pristine DNA-b-PPO micelles (lane 2) and DNA-b-PI micelles (lane 3) migrate significantly slower than the ds DNA control. Blended micelles (lane 4) seem to disaggregate upon longer running times.



Lane 1: 22 mer dsDNA

Lane2: 22 mer dsDNA-b-PPO micelle

Lane3: 22 mer dsDNA-b-PI micelle

Lane4: 22 mer ds blend micelle

Figure 36: Gel electrophoretic analysis of DNA polymer aggregates in a Pluronics matrix.

9.6 Demonstration of programming containment via sequence specific DNA retention with ECCell gels in CGE (RUBa)

As the separation matrix we used the same hydrogel material (Pluronic® F127) as successfully tested in the chemical microprocessor chip in the first project year. The advantage in terms of microfluidic integration is, that these kinds of hydrogel monomers are reversible thermo-responsive gels. At low temperatures, the dissolved materials acts as a free flowing solution while at elevated temperatures self assembled micelles form a quasi-lattice with a face-centered cubic structure leading to an increase in viscosity and hence to the formation of a gel. Furthermore, Pluronic® F127 is known to be a good separation medium for electrophoresis.

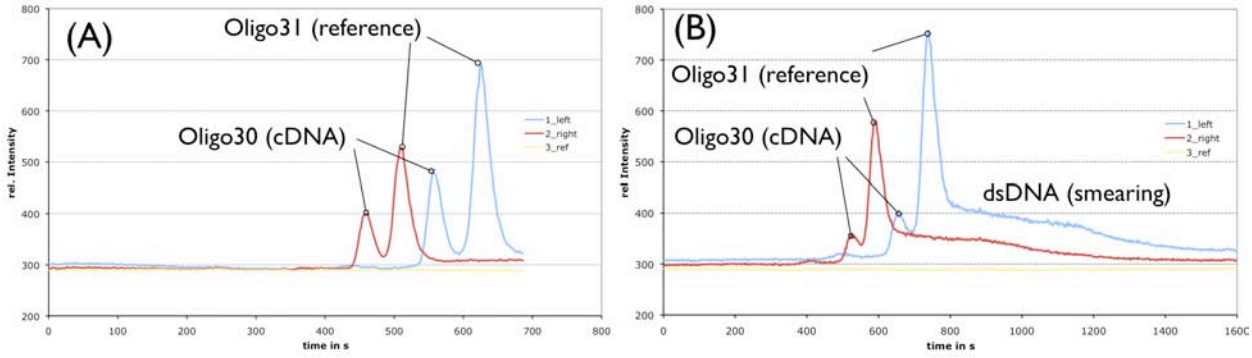


Figure 37: (A) Linear electrophoretic DNA-transport of a reference Oligo31 3'-GGAGCGAGACGATTAGGACAAT-5'Alexa488 (22-mer) and Oligo30 Atto488-5'-GAA TCC GCA AAA-3' (cDNA) in a capillary as well as (B) a hybridised solution of ssU4T + cDNA (dsDNA-b-copolymer). CGE conditions: T = 30°C, pH 7.5, TBE running buffer, 600 V (56 μA), injection 3 sec., C = 2*10-6 M.

The CGE pre-experiments shown in figure 37 were designed to give us information about (A) the mobility of the cDNA in comparison with a reference ssDNA (22mer) and (B) the duplex of scp-DNA and the complementary cDNA in Pluronic F127. The hydridized duplexes migrate less than their corresponding single stranded counterparts, and the 22mer reference ssDNA, and show a diffuse peak signal. To find out the best conditions for integration in the microfluidic environment of the various modified DNA oligomers, especially the one functionalized with four alkyl groups (ssU4T **10** as synthesized by the group of RUG, see also WP2 & 4) capillary gel electrophoresis (CGE) analysis was performed for the modified oligonucleotides.

To test the sequence specific retention the cDNA (Oligo30) as well as a non matching sequence (Oligo 03) was injected in a hydrogel mixture containing Pluronic F127 and the 12mer DNA functionalized with four hydrophobic chains (ssU4T **10**) as shown in figure 38. The result is, that the non-complementary strand moves as a single strand through the gel whereas the complementary oligonucleotide forms the duplex with the scp-DNA in combination with a significant reduced electrophoretic mobility.

A movie regarding sequence specific DNA migration by immobilized DNA-b-copolymers in CGE-capillaries (CGE_scpDNA_retention.mov) as a part of the Devireable 4.2 is shown in the download section for reviewers at the ECCell homepage.

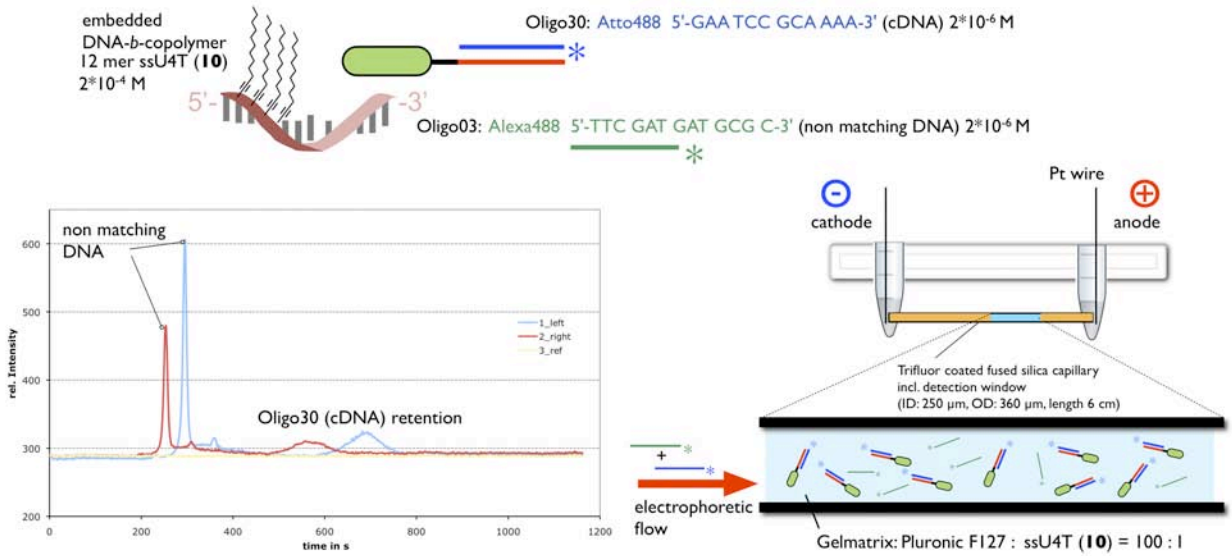


Figure 38: Sequence specific DNA retention of Oligo30 Atto488-5'-GAA TCC GCA AAA-3' (cDNA) in a CGE experiments in comparison to non-matching DNA. CGE conditions: T = 30°C, pH 7.5, TBE running buffer, 600 V (88 μA), injection 3 sec., C = 2*10^{-6} M.

9.7 µ-fluidic integration experiments of sequence specific DNA retention in ultra-thin hydrogel films using the chemical microprocessor

Besides the investigation of the mobility using CGE we also tested the system in ultra-thin hydrogel films on-chip. The preparation procedure is shown in figure 39. The chip was treated with tiny droplets of a cold mixture (5°C) of Pluronic F 127 and 12mer scpDNA (ssU4T **10**) (mixing ratio = 100:1) as well as the same mixture containing non-matching DNA (2*10^{-6} M Alexa488-5'-TTC GAT GAT GCG C-3'). The samples were heated up to 30°C to trigger gelation embedded in a pure Pluronic F127 hydrogel matrix. Finally the samples were covered by a thin glass slide.

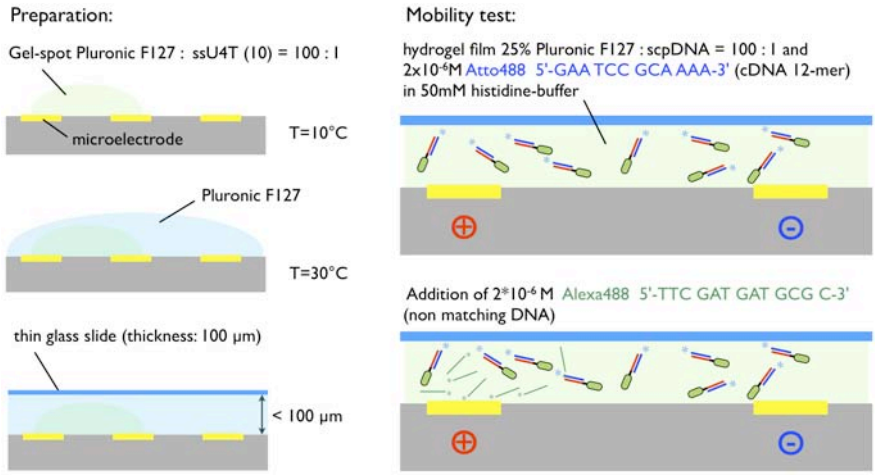


Figure 39: Left: Illustrated procedure to produce ultra-thin hydrogel films. Right: Schematic presentation of sequence specific DNA retention in ultra-thin hydrogel films.

Regarding the results of the CGE, the non-complementary DNA should not interact with the scp-DNA and therefore they were concentrated if the electrodes are activated (positive or negative voltage) and transported using the electric fields inside the hydrogel matrix as shown in figure 40.

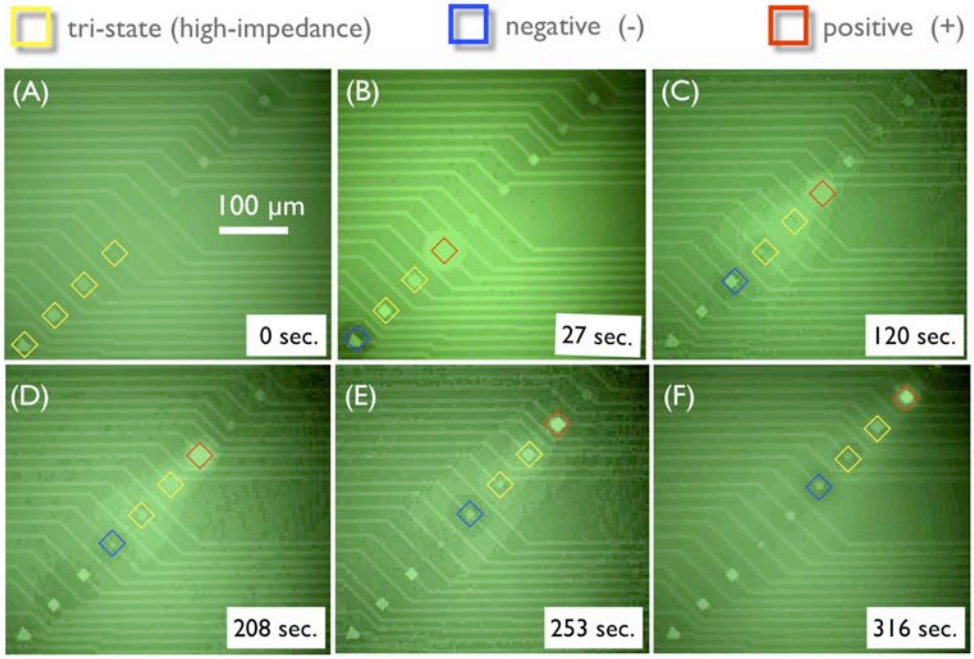


Figure 40: The movie series show that negative charged electrodes repel the non-complementary strands. Positive charged electrodes lead to a concentration of the non-matching species. Experimental conditions: 30°C, 25% w/v Pluronic F 127. Excitation wavelength: 488 nm.

The control experiment performed in an embedded scpDNA/Pluronic spot without the non-complementary strands shows no interactions with the microelectrodes except for a low concentration of non-hybridised scpDNA species outside of the gel-spot, which diffused inside the pure Pluronic F127 matrix (figure 41).

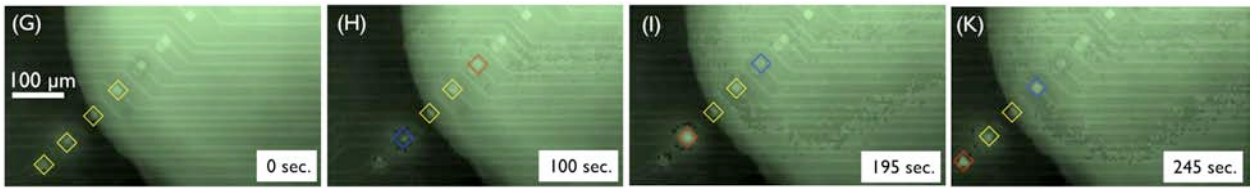


Figure 41: The movie series show or minor interactions with the microelectrodes respectively. Experimental conditions: 30°C, 25% w/v Pluronic F 127. Excitation wavelength: 488 nm.