

Supplementary information :

**Electrochemically assisted localized grafting of an aptamer in a
micro channel engraved in fluorinated thermoplastic polymer**

Dyneon THV

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Materials and Methods

1. Chemicals

2,2'-dipyridyl, tetrabutylammonium tetrafluoroborate (Bu₄NBF₄), dimethylformamide (DMF), acetonitrile (ACN), ascorbic acid, hexaammineruthenium(III) chloride, copper(II) sulfate (CuSO₄) and potassium chloride (KCl) were reagent grade and purchased from Sigma-Aldrich (Isle-d'Abeau, France). Ethanol (EtOH) were HPLC-grade from SDS (Peypin, France). Acetylene-Fluor 488 was from Interchim (Montluçon, France). DNA aptamers of 75 bases with sequence: 5' ATA CCA GCT TAT TCA ATT GCA ACG TGG CGG TCA GTC AGC GGG TGG TGG GTT CGG TCC AGA TAG TAA GTG CAA TCT-3' modified with 6-carboxyfluorescein (6-FAM) at the 5' end and 5-Octadiynyl at the 3' end were purchased from Integrated DNA Technologies (Interleuvenlaan, Belgium). All these chemical products were used without further purification. Milli-Q water was used to prepare all the aqueous solutions. 4-Azidobenzediazonium was synthesized from previously reported procedure [1].

2. Materials

THV pellets (Dyneon THV 500G, 3M, USA) were first cleaned by sonication in water and ethanol for 30 min and then dried under a flow of purified argon. A few grams of the pellets were then moved onto a commercial silicone mold and melted at 210°C overnight to obtain flat sheets with a thickness of approximately 2 mm. Flat sheets obtained are then heated at

180°C on one side and pressed at an uniaxial pressure of 0.5 MPa. The sheets were then cleaned with isopropanol and cut into desired sizes for further use.

For the fabrication of microchannel plates, Dyneon THV 500G was hot embossed following a previously reported protocol [2], using an aluminum mold and a heated hydraulic press (Specac). Few grams of THV pellets were moved in a homemade PDMS mold of the same size as the aluminum mold and melted at 210°C overnight in a vacuum oven to obtain a sheet of thermoplastic. This sheet was placed in the bottom of the aluminum mold and a sheet of PDMS with few millimeters thickness is placed on the top of the plastic sheet. The mold was then closed and put inside the press. A temperature of 180 °C and a pressure of 0.5 MPa were applied for 30 minutes. After cooling the system at room temperature, the microstructured THV plate was removed from the mold and rinsed with isopropanol and water.

3. General procedure for carbonization of THV substrate surfaces

In order to control the reaction environment, the whole SECM system was placed in a glove bag (Atmosbag, Sigma-Aldrich). Dyneon THV surfaces were previously washed with EtOH and H₂O. The tip of platinum electrode (diameter $a = 25$ or $10 \mu\text{m}$) is immersed in 0.1 M KCl + 5 mM Ru(NH₃)₆Cl₃ in aqueous solution and positioned at a normalized current value equal to $i_T/i_{T,\infty} \sim 0.5$ or 0.25 which corresponds to a fixed distance from the substrate by comparing experimental and simulated approach curves. The approach curves to the Dyneon THV 500 made with Ru(NH₃)₆Cl₃ and 2,2'-dipyridyl are presented in Figure 1. As expected, the surface is seen as insulating when Ru(NH₃)₆Cl₃ mediator is used but the approach curve obtained with 2,2' dipyridyl mediator shows a slight different behavior when tip-substrate distance decreases to zero. In that case, the approach curve have been fitted by finite heterogeneous kinetics at the substrate of dimensionless rate $\lambda_{app} = k_{app} a/D$ [3] (where a is the tip radius and the D is the diffusion coefficient of the mediator) with $\lambda_{app} = 0.04$ which

gives $k_{app} = 3.2 \cdot 10^{-4} \text{ cm s}^{-1}$ (considering $D = 10^{-5} \text{ cm}^2 \text{ s}^{-1}$). This indicates that a positive feedback is observed when 2,2' dipyridyl is used and can be interpreted as a consequence of the carbonization of the substrate when the tips approaches the Dyneon.

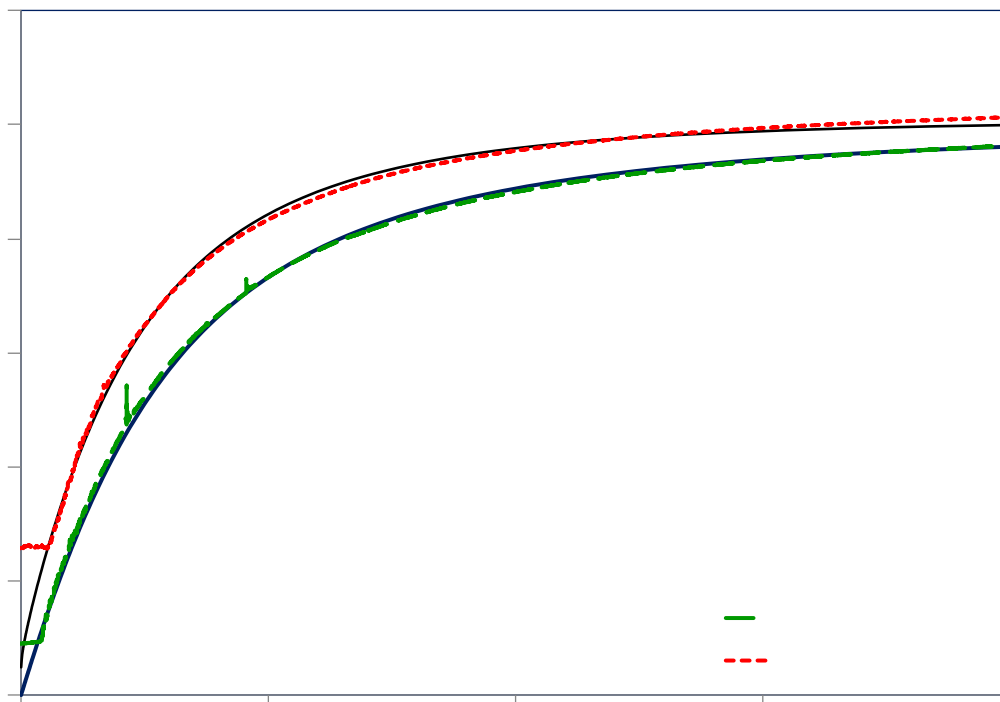
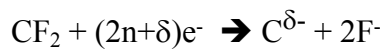


Figure 1: experimental approach curves above Dynon THV 500 with $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$ at 5 mM for a probe polarized at -0.45V vs Ag/AgCl, $RG = 10$ (RG is , diameter = $25\mu\text{m}$ and with 2,2' dipyridyl at 50 mM for a probe polarized at -2.3V vs Ag/AgCl, $RG = 9$, diameter = $25\mu\text{m}$ and $\lambda_{app} = 0.04$. Continuous lines represent theoretical curves. The RG value of the tip ($RG = a_{glass} / a$ with $a =$ tip radius and $a_{glass} =$ glass radius) was evaluated before each experiment by optical microscopy and d is the distance tip-substrate.

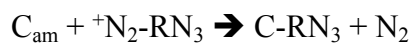
The aqueous solution was then removed and replaced by a solution of DMF containing 0.1 M Bu_4NBF_4 and 50 mM 2,2'-dipyridyl for experiment on THV plates or 100 mM 2,2' bipyridyl for experiment in THV microchannels. The system was placed under N_2 atmosphere with desiccants to obtain an environment with less than 30% humidity before starting the carbonization reaction.

4. General procedure for fluorescent dye immobilization and aptamer immobilization on N_3 -modified THV substrate surfaces through CuAAC reaction

Just after carbonization, THV substrates were immersed for 1 h in a solution of ACN containing 0,1M Bu_4NBF_4 + 5mM 4-azidobenzenediazonium for the adsorption of 4-azidobenzene groups leading to N_3 -modified THV substrate. The overall mechanism of the irreversible functionalization can be as follows:



Where CF_2 is the perfluorinated group of the polymer involved in the reaction with reduced 2,2' dipyridyl and $\text{C}^{\delta-}$ is formally reduced polymer to amorphous carbon C_{am} that allows reduction of 4-azidobenzenediazonium moieties $^+\text{N}_2\text{-RN}_3$ according:



For the AF-488 fluorescent dye immobilization by CuAAC reaction, a solution containing 100 μM AF-488, 5 mM CuSO_4 and 40 mM ascorbic acid in EtOH/ H_2O (1:1) was prepared. N_3 -modified THV substrate surfaces were immersed in the above-mentioned solution and CuAAC reaction let proceeded at room temperature for at least 18 h in dark. For the aptamer

immobilization, a solution containing 5mM CuSO₄, 40mM ascorbic acid and 1μM aptamer in EtOH/H₂O (1:1) was prepared and the same protocol was used.

The resulting modified substrate surfaces were washed with copious amounts of EtOH and Milli-Q water and then dried under a flow of purified argon.

5. Characterization of Acetylene-Fluor 488 –modified and aptamer-modified THV substrate surfaces

Fluorescence microscopy images of modified THV surfaces were recorded on an inverted fluorescence microscopic system (Axio Observer A1, Zeiss, Le Pecq, France) equipped with a 100 W metal halide lamp, 488 nm excitation filter and 525 nm emission filter (filter set 76 HE, Zeiss, France). A CCD digital camera (Pike F-145B, Allied Vision Technologies, Stadroda, Germany) was combined with HIRIS software (RD Vision, France) for detection processing.

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2. S. Begolo, G. Colas, J.-L. Viovy and L. Malaquin, *Lab. Chip*, 2012, **11**, 508.
3. C. Lefrou and R. Cornut, *Chem. Phys. Chem.*, 2010, **11**, 547.