Supporting Information

Printing “Smart” Inks of Redox Responsive Organometallic Polymers on Microelectrode Arrays for Molecular Sensing

Marco Cirelli,\textsuperscript{a,b} Jinmeng Hao,\textsuperscript{a} Teunis C. Bor,\textsuperscript{b} Joost Duvigneau,\textsuperscript{a} Niels Benson,\textsuperscript{c} Remko Akkerman,\textsuperscript{b} Mark A. Hempenius,\textsuperscript{a} G. Julius Vancso\textsuperscript{a,*}

\textsuperscript{a} Materials Science and Technology of Polymers, MESA+ Institute for Nanotechnology, University of Twente, P.O. Box 217, 7500 AE Enschede, the Netherlands.

\textsuperscript{b} Production Technology, Faculty of Engineering Technology, University of Twente, P.O. Box 217, 7500 AE Enschede, the Netherlands.

\textsuperscript{c} Institute of Technology for Nanostructures, Faculty of Engineering, University of Duisburg-Essen UDE, Bismarckstr. 81, D-47057 Duisburg, Germany.

For correspondence: g.j.vancso@utwente.nl
1 Materials

[1] Dimethyilsilaferroacenophane 1 and ([1]3-chloropropyl)methylsilaferroacenophane 2 were synthesized according to published procedures. 1 4,4'-Dithiodibutyric acid (DTDB, 95%), iodoethane (99%), 1-iodopropane (99%), hydrogen hexachloroplatinate(IV) hydrate (H₂PtCl₆·6H₂O, 99.9+%), dicyclohexano-18-crown-6 (98%), potassium iodide (KI, 99+%), sodium chloride (NaCl), sodium sulphate (Na₂SO₄, anhydrous, 99%), chloroform-d (CDCl₃, 99.8 atom % D), toluene-d₈ (99.6 atom % D) were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and used as received. For the electrochemistry experiments, sodium perchlorate (NaClO₄, ACS Reagent, >98.0%) and L-ascorbic acid (ACS Reagent, 99+%) were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and used as received. Toluene, dimethylformamide (DMF), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), ethanol (EtOH) and methanol (MeOH), all of AR grade, were obtained from Biosolve Chimie SARL (Dieuze, France) and Milli-Q grade water (Millipore Corporation) was used in all the experiments. Ultrapure nitrogen was used throughout.

2 Synthesis and characterization of disulfide-functionalized PFS 5

![Figure S1: 1H NMR spectrum of PFS 3 in toluene-d₈.](image-url)
Figure S2: $^{13}$C NMR spectrum of PFS 3 in toluene-$d_8$.

Figure S3: $^1$H NMR spectrum of PFS 4 in toluene-$d_8$. 
Figure S4: $^{13}$C NMR spectrum of PFS 4 in toluene-$d_8$.

Figure S5: $^1$H NMR spectrum of PFS 5 in toluene-$d_8$. 
Figure S6: $^{13}$C NMR spectrum of PFS 5 in toluene-d$_8$.

Figure S7 shows zoomed-in $^1$H NMR spectra of the respective PFS derivatives to confirm the quantitative conversion of the functional side groups from the chloro (CH$_2$Cl at $\delta = 3.24$ ppm), to the iodo (CH$_2$I at $\delta = 2.88$ ppm) and to the disulfide-based ester (CH$_2$SS-CH$_2$ at $\delta = 2.45$ ppm).$^2$
Figure S7: Zoom-in of the $^1$H NMR spectra of PFS 3, 4 and 5 (in toluene-$d_8$) in the region between $\delta = 0.7$ and 3.5 ppm demonstrating the complete conversion of chloropropyl- into iodopropyl side groups and the subsequent quantitative introduction of disulfide-bearing side groups.

3 Devices and instruments

The microelectrode array, MEA, biochips used in this experiment were MEA 60-200Au (Qwane Biosciences SA, Switzerland) characterized by 60 plain gold electrodes, each with 40 $\mu$m diameter, a center-to-center spacing of 200 $\mu$m, with an insulator layer of SU-8 with a thickness of 0.7 $\mu$m, and an impedance of 700–900 k$\Omega$ positioned on a transparent 10 mm x 10 mm glass chip. The MEA chip had dimensions of 49 mm x 49 mm x 1 mm in PCB base material with Au contact pads. A schematic illustration of the MEA chip design is shown in Figure S8. The Au contact pads are used for connecting the MEA to the Autolab PGSTAT 10 electrochemical workstation (Ecochemie, Utrecht, the Netherlands) through a MEA 1060-inv-BC (Multi-Channel Systems). The supporting plastic ring has an inner diameter of 26.5 mm, an outer diameter of 30 mm and a height of 6 mm.
Figure S8: Pictures and schematic representation of the MEA 60-200-Au chip used in this study. a) and b) are top and side views of the MEA chip; c) and d) show more detailed side and top views of the electrode array of the Au MEA chip.

Fourier transform infrared spectra were collected with a Bruker ALPHA single attenuated total reflection (ATR-FTIR) spectrometer equipped with an ATR single reflection crystal (Bruker Optik GmbH, Ettlingen, Germany) and a Bruker Vertex V70 equipped with an Hyperion 2000 infrared microscope (utilizing a 15x magnification objective) and a Mid-band MCT detector (liquid N₂ cooled with preamplifier, 12000–600 cm⁻¹). The spectra were collected in the range of 4000–400 cm⁻¹ with a spectral resolution of 4 cm⁻¹ employing at least 128 scans. Background spectra were recorded against air. \(^1\)H and \(^{13}\)C NMR spectra were obtained on a Bruker Avance III 400 MHz instrument at 400.1 and 100.6 MHz, respectively. \(^1\)H and \(^{13}\)C chemical shifts were based on the solvent residual signals. GPC measurements were performed using ultrastyragel columns with pore sizes of 10⁵, 10⁴, 10³ and 500 Å (Waters), equipped with a guard column of 500 Å, a 515 pump (Waters), a 1050 injector (Agilent) and a 2414 differential refractometer (Waters). All sample solutions were prepared at a concentration of 5 mg/mL and filtered through a 0.45 µm PTFE filter prior to a GPC run. The rheological properties of the inks were evaluated using a rotational UDS-200 rheometer (Anton Paar Germany GmbH, Ostfildern, Germany) equipped with parallel plates geometry and the surface tension was determined by the pendant drop method with an OCA 15 (Dataphysics Instruments, Germany). A Dimatix Piezo inkjet 2831 Printer (FUJIFILM Dimatix Inc., Santa Clara, Ca, USA) PIXDRO LP 50, featuring a DMP-2850 print head and a DMC 11610 cartridge (16 nozzles having a 23 µm
diameter, 10 pL nominal drop volume), was used to deposit the redox-active ink on the MEAs. The printer was fitted out with a horizontal CCD camera, featuring a LED light for drop jetting analysis, and with a fiducial camera that allows the alignment of the print head with respect to the sample. The nozzles were purged by jetting with pure toluene between experiments. Atomic force microscopy was performed on an MFP-3D AFM (Asylum Research, USA) in tapping mode. Images were recorded in air using commercially available Olympus silicon cantilevers (average nominal resonance frequency of 70 kHz and spring constant of 2 N/m). Surface morphology of the modified gold MEAs was investigated by high-resolution scanning electron microscopy (FESEM, JEOL, JSM-6400F, the Netherlands) at 5 and 20 keV of accelerating voltage equipped with energy dispersive X-Ray analyzer (EDX). The surface of the MEAs was examined using a laser confocal Keyence VK 9710 K microscope (LSCM, violet laser VK-9700, Keyence Corporation, Osaka, Japan) featuring a 10x magnification objective lens. To evaluate the electrochemical properties of the PFS decorated MEA biochips, an Autolab PGSTAT 10 electrochemical workstation (Ecochemie, Utrecht, the Netherlands) was used via cyclic voltammetry (CV) and chronoamperometry measurements. For the chronoamperometric experiments, the solution was mixed using a peristaltic pump (LKB Bromma, 2132 microperpex peristaltic pump).

4 Formulation and characterization of the redox stimuli-responsive PFS-based inks

Appropriate formulation of the functional ink materials will lead to a stable, well-controlled and repeatable inkjet process. Important physical properties of the inkjet inks include viscosity, density, and surface tension since they affect the droplet formation, the repeatability, and the accuracy of the inkjet deposition process. PFS 5 was dissolved in toluene at different concentrations, including 2.5, 5 and 10 wt%; corresponding solutions were named as “ink A”, “ink B” and “ink C”, respectively. The physical and chemical properties of the inks are summarized in Table S1.
| Formulation | Toluene-based inks |
|-------------|-------------------|
| Components  | Note/Function     | Ink A | Ink B | Ink C |
| Functional pigment | (wt% concentration) | Redox active | 2.5 | 5.0 | 10 |
| Solvent     | (wt% concentration) | Vehicle or carrier | 97.5 | 95.0 | 90.0 |
| Density (ρ) | (kg m⁻³ at 25 °C) | 867 | 867 | 867 |
| Surface tension (γ) | (mN m⁻¹ at 20°C) | Range 25–50 mN m⁻¹ at 20 °C | 28 | 28 | 26 |
| Viscosity (η) | (mPa·s at 20 °C, average 500–2000 s⁻¹) | Range 1–20 mPa·s at 20 °C | 1.8 ± 0.5 | 8.1 ± 0.5 | 11 ± 1 |
| Reynolds number (Re) | - | Re = vap/η | 63.6 | 14.3 | 10.4 |
| Weber number (We) | - | We = v²ap/γ | 24.5 | 24.5 | 24.5 |
| Ohnesorge number (Oh = 1/ Z) | - | Oh = We¹/₂ / Re = η / (apγ)¹/₂ | 0.08 | 0.35 | 0.48 |
| Parameter Z | Z = 1/Oh | 12 | 3 | 2 |

a: pendant drop, b: steady-shear rate sweep via rotational viscometry and c: for the evaluation of Re, We and Oh the following was considered: v is the velocity of the drop (5 m s⁻¹), a is the diameter of the nozzle, ρ is the density of the fluid, η is the viscosity and γ is the surface tension. In addition, the drop diameter was 20 µm.

The steady-shear viscosity of the inks was evaluated using steady-shear rate sweep measurements aiming to simulate the piezo inkjet printing process. The measurements were performed at 20 °C (controlled by a Peltier temperature controller) using an MP31 spindle (a plain plate with a diameter of 50 mm). Prior to performing the flow measurements, the gap (set at 500 µm) was filled with 1 mL of the ink solutions and the solution was then stabilized via a pre-shear step of 0.5 min. The rheological properties were measured by increasing the shear rate at constant logarithmic steps from 0.1 to 2400 s⁻¹, ensuring a steady flow state of the liquid. The shear stress was assessed every 3 s (measuring point duration = 1/shear rate). The experiments were repeated three times using newly prepared solutions. The viscosity was calculated as the steady-state shear stress divided by the applied shear rate. The steady-shear viscosity was average between 500–2000 s⁻¹. Surface tension values were determined by fitting the outline of pendant drops; the drop contour was fitted by the Young-Laplace equation considering the density of the ink to be the same as the density of the solvent (density of toluene is 0.87 g mL⁻¹). A needle of 0.70 mm outer
diameter was used to develop droplets of about 6 μL of volume and the average of the surface tension values and the standard deviations were calculated using at least three different measurements.

**Figure S9:** Rheology and surface tension experiments. a) Measurements of steady shear viscosity of the PFS based inks, the inset shows the shear stress curves. b) A typical photograph used in surface tension measurements via the pendant drop technique of “ink A”.

The behavior of fluids during inkjet printing can be represented by the Reynolds number (Re), the Weber number (We), and the Ohnesorge number (Oh), and be used to design a starting piezo waveform for a stable DoD inkjet printing process. Considering these physical parameters, “ink B” and “ink C” could be easily printable, while the use of “ink A”, due to the low viscosity, could result in the formation of undesired satellite drops. However, the optimization of the printing conditions must be experimentally verified and tested through the evaluation of the droplet formation as a function of the inkjet printing conditions. In practice, it turned out that “ink A” was the best compromise between printability, optimization of the formulation and quality of the redox-active coatings produced.

The droplet formation of the inks was investigated as a function of delay time. The drops were generated in the continuous inkjet, CIJ, printing mode and analyzed with the PIDRO Advanced Drop Analysis software (ADA, PixDro, Meyer Burger, the Netherlands). High-speed images of the drops were recorded and used to evaluate the speed of the drop, the drop volume, and the jet direction, as is shown in **Figure 3**.
The effect of different types of driving waveforms (e.g., single trapezoidal shaped and bipolar pulse), waveform parameters (e.g., drive voltage, dwell time, rise time, fall time, etc.) and process parameters (e.g., the temperature of the head and the backpressure of the ink reservoir) were explored in detail, eventually leading to a robust, repeatable and precise printability of the inks.

The DMC 11610 cartridge has 16 nozzles that can fire all simultaneously or in single mode. To increase the accuracy of the inkjet printing deposition in DoD mode, a single nozzle was selected during the optimization procedure and it was tested separately after the optimization of the driving waveform using all nozzles. This nozzle was used to align and calibrate the print head to the MEA substrate through fiducial alignment which consists of three reference marks with a size of 250 µm and with a minimal tolerance of 93 µm.

5 Printing and characterization of the PFS modified MEA chips

PFS 5 ink was printed on MEA chips and unpatterned gold-coated wafer samples, the latter allowing investigation of the homogeneity, step height and roughness of the printed PFS films. The MEA chips, without the ring (see Figure S8), were cleaned with ethanol and dried in a stream of N₂ before being printed with PFS-based inks. The printer was aligned with the MEA via “fiducial alignment” using three reference marks on the substrate and the pattern was designed and edited as BMP file. The three reference marks were chosen on the borders of the Au contact pads positioned at the corner of the MEA chip setting the “mark size” to 250 µm. The images were collected via the fiducial camera which was positioned on the side of the print-head close to the nozzles. Image analysis was conducted at constant focus and intensity of the coax and ring lights. The alignment was considered optimal when the score of the alignment was set at least 90 %. The PFS printed MEA biochips were rinsed with toluene, ethanol, and water, and dried in a stream of N₂. Finally, the samples were annealed at room temperature in a vacuum oven overnight. The printed patterns were rinsed with toluene, ethanol, and water, and then dried in a stream of N₂.

Following modification of the MEA with PFS 5 by inkjet printing, the quality of the printed pattern was evaluated by visual inspection through optical microscopy and laser confocal microscopy measurements as is shown in Figure S10 and Figure S11, respectively. Figure S10a shows a printed pattern on a plain gold substrate. The
The printed pattern consisted of an 8x8 array of dots (center-to-center distance was 200 µm). **Figure S10b** shows a different PFS 5 printed pattern on the MEA chip, in which the MEs of the first row were not modified demonstrating the high spatial control of the inkjet printing deposition. The precision and accuracy of the ink deposition on the MEA chip were evaluated by optical and laser confocal microscopes. The laser mode was used to adjust the filter and brightness for the capturing of a clear image, with a Z-axis scanning range set to 0.020 µm. The images were acquired with a 10x magnification objective lens and an optical zoom of 1.0x and analyzed using VK analyzer software. The surface roughness of the electrodes (mean roughness and root mean square roughness, $Ra$ and $Rq$ respectively) was measured before and after the printing process. $Ra$ and $Rq$ were evaluated on an electrode area of 1250 µm$^2$.

**Figure S10**: Typical optical microscopy image of a) an ink A printed pattern on a plain gold substrate and b) of the ink A printed pattern on a MEA chip. Printing conditions: FUJIFILM Dimatix 2831 piezo inkjet printer equipped with DMC-11610 cartridge, at room temperature, firing voltage of 50 V for 10 µs, fill time of 5 µs, rise and fall time of 1 µs and a distance of 2 mm of the print head from the surface.

3D surface images (laser confocal microscopy) and height images (in false-color view) of the clean and ink A printed MEAs show the micro-scale topography of the samples and allow one to estimate the precision and the accuracy in the deposition of the ink. The height profile and the roughness of the PFS 5 decorated ME surfaces were determined, as shown in **Figure S11**. The as-received Au-MEA chip was
characterized by an $Ra$ value of $0.8 \pm 0.2 \, \mu m$ and an $Rq$ value of $1.2 \pm 0.3 \, \mu m$, while for the “ink A” modified MEA chip the $Ra$ value was $1.3 \pm 0.3 \, \mu m$ and the $Rq$ value was $1.7 \pm 0.4 \, \mu m$. The change in the topographic properties of the samples (the increase of the roughness and the change in the height profile) point towards the successful deposition of PFS 5 on the MEAs via inkjet printing.

**Figure S11:** a) and b) are representative laser confocal microscopy images of the MEA chips, as received. c) and d) show laser confocal microscopy images of the PFS 5 modified MEAs. a) and c) are the 3-D reconstruction surface profiles; b) and d) correspond to the height images and the height profiles of an array of MEs.

**Figure S12** shows SEM images of the surfaces of PFS 5 films on the Au-ME of the MEA chip. The electrode exhibits a somewhat heterogeneous deposition of the redoxactive film. At the center and at the border of the well of the electrode there are some irregularities which one may ascribe to contamination of the MEA chip.
In order to characterize the nanoscale topology of the surfaces, the roughness and the thickness of the printed layers were measured by AFM, as shown in Figure S13. The thickness of the PFS films tethered on gold-coated surfaces was studied by scratching the polymer films with a Teflon tweezer and measuring the difference in the step height via tapping mode AFM. At least three different measurements of each sample were performed and averaged, resulting in a film thickness of 8 ± 3 nm. The topology of the polymer coatings was evaluated analyzing 3 different spots with a scan area of 1 x 1 µm². The values of the average surface roughness ($R_a$) and the root mean square average ($R_q$) were 1.2 ± 0.5 nm and 1.76 ± 0.5 nm, respectively.
Figure S13: Typical tapping mode AFM topography image a) and height profile b) of a representative dry PFS 5 film immobilized on the Au substrate over a scratch made with a Teflon tweezer.

The chemical composition of the printed MEAs was obtained by EDX-HR SEM and mapping ATR-FTIR microscopy measurements, confirming the presence of the PFS films only on the desired areas of the MEs. The morphology and the elemental mappings of the bonded coatings on the MEA biochip were evaluated by HR-SEM equipped with energy dispersive X-ray (EDX) detector at 5 and 20 keV of accelerating voltage.

Figure S14 shows the FTIR spectra in different locations of the MEA and the specific chemical groups of PFS 5 are indicated by the highlighted regions, i.e. the C-H stretch absorbance band of the Fc rings at 3087 cm$^{-1}$, and the absorbance bands ascribed to the vibration of Fc rings at 1037 and 1165 cm$^{-1}$. The FT-IR spectra of the printed
samples were collected employing at least 128 scans in the range of 4000–400 cm\(^{-1}\) with a spectral resolution of 4 cm\(^{-1}\). The background spectrum was recorded against air.

**Figure S14:** From the bottom to the top: FTIR spectrum of bulk PFS 5, of the clean AU-ME, of the insulator (SU-8) layer and of the PFS 5 printed MEs. On the left the spectrum at high energy regions (4000–2200 cm\(^{-1}\)) and on the right the FTIR spectrum in low energy regions (2200–600 cm\(^{-1}\)).

6 Classification based on the electrochemical properties of the PFS 5 printed MEAs

The PFS 5 modified MEs were classified based on the electrochemical properties of the grafted redox-active film, as shown in **Figure S15:**

a) PFS well-modified MEs are characterized by anodic and cathodic currents exceeding +5 and −5 nA, respectively, transferred charge values for the oxidation and the reduction of ferrocene sites of at least 10 nC and the presence of electrocatalytic phenomena. For example, the well-modified ME 36 is shown in **Figure S15a** and S15d.
b) PFS poorly-modified MEs are characterized by anodic and cathodic currents in the range between +5 and −5 nA, transferred charge values of 1 nC and no electrocatalytic phenomena for the oxidation of ascorbic acid. Figure S15b and S15e show the redox properties of ME 54 as an example.

c) Non-modified ME, for example ME 61, where no appreciable current signal and no electrocatalytic phenomena were detected, see Figure S15c and S15f.

**Figure S15:** Electrochemical properties of Au-MEs (representative for the MEA) modified with PFS 5, resulting in layers with different quality. a) and d) show the CVs of a well of a PFS 5 modified ME (number 36); a) shows the CV in 100 mM NaClO₄ aqueous solution [Pt(wire)-RE/CE] and d) shows the CV in presence of the analyte (ascorbic acid). b) and e) feature a PFS 5 modified ME with poor modification quality (number 54) in the absence and presence of ascorbic acid, respectively. c) and f) describe a non-modified ME (number 61) in the absence and presence of ascorbic acid, respectively. Note the different y-axis scale in a, c and d. Scan rate 50 mV s⁻¹, in 100 mM NaClO₄ and in the presence of 35 mM of ascorbic acid, Pt(wire)-RE/CE, and in the potential range between −500 and 800 mV vs. Pt.

The electrochemical stability of the redox-active PFS films was examined by successive CV cycles in aqueous electrolyte solution for two representative samples (MEs number 36 and 74), as shown in Figure S16. The oxidation and reduction current peaks were unchanged and reproducible after more than 10 cycles for both samples confirming that no PFS chains desorbed from the various ME surfaces owing to the stable S-Au bond between the redox active coating and the PFS 5 modified ME surfaces.
The electrochemical properties and the stability of the printed PFS layers were evaluated by recording CVs of the current in 100 mM aqueous NaClO₄ solution between −500 and 800 mV, using a Pt wire as a reference and counter electrode (length of 1.5 cm and 0.5 mm diameter) as a function of the number of cycles. The experiments were repeated at least 10 times in 100 mM aqueous NaClO₄ solution in the range of −500 and 800 mV with a scan rate of 50 mV s⁻¹ using the decorated PFS gold electrode as working electrode and a Pt wire as a reference and counter electrode.

The electrocatalytic response of the modified electrodes was investigated by recording CVs of modified and bare gold electrodes of the MEA in 100 mM aqueous NaClO₄ solution and in the presence of ascorbic acid with 50 mV s⁻¹ scan rate, potential range between 500 and 800 mV, and using a Pt wire as a reference and counter electrode. The chrono-amperograms were measured setting the potential at 300 mV (against Pt wire) in 2 mL of 100 mM aqueous NaClO₄ solution. Small aliquots of the ascorbic acid aqueous solution were added each 100 sec to the sensing system and mixed while recording the current response. For the current, the equilibrium value was reached in 50 sec after each addition and the average of the current was used for the calibration curve.

**Figure S16:** Typical CVs of two representative printed PFS films on different MEs number 74 and 36, respectively (a) and (b). Scan rate 50 mV s⁻¹, in 100 mM NaClO₄, Pt(wire)-RE/CE, and potential range between −500 and 800 mV vs. Pt.
7 Evaluation of the PFS-coated MEAs

The final evaluation of the PFS printed MEAs was carried out by a combination of visual inspection, chemical composition analysis, surface analysis, and electrochemical characterization of the functionalized MEAs.

Visual inspection of the printed MEA by optical microscopy confirmed a good accuracy of the inkjet printing process for deposition of “ink A”, as shown in Figure S17a. All drops were deposited on the electrodes in the designed pattern with good accuracy (few tens of microns). (We note that the deposition accuracy may be improved by a revised formulation of the redox-active ink (more viscous), working in a well-controlled environment (no airflow) and inkjet printing at a lower distance between the print-head and the substrate.) Subsequently, the chemical composition was verified. Then, the electrochemical properties of the redox-active MEs were tested as well as their performance as sensing device in absence and in presence of ascorbic acid.

As summarized in Figure S17b, there were 34 (63%) well modified, 21 (39%) poorly modified and only 4 (8%) non-modified MEs.
To further characterize the electroactive behavior of the redox-active films, we studied the scan rate dependence of the peak current as summarized in Figure S18. This included the assessment of the process to show whether it was diffusion or adsorption controlled. Figure S18a shows the effect of the scan rate on the double-wave voltammogram. As the scan rate increases, the shape of the CVs becomes more distorted and the current intensity increases as described by the Faraday and Randles-Sevcik equations. The values of the peak current as a function of the square root of the scan rate showed a linear relationship in the range of 10 - 50 mV s⁻¹, as shown in the Figure S18b and Figure S18c, which is typical for diffusion-controlled processes obeying Fick's law.⁷
Figure S18: (a) CVs of the scan rate of a typical printed PFS film immobilized on gold at different scan rates. Scan rate in the range of 10 - 50 mV s$^{-1}$, in 100 mM NaClO$_4$, Pt(wire)-RE/CE, and potential range between −500 and 800 mV vs. Pt. (b) Plot of peak currents vs. scan rate, and (c) plot of peak current vs. square root of the scan rate for these printed PFS films.

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