

Electrochemical fouling of dopamine and recovery of carbon electrodes

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Abstract

1 A significant problem with implantable sensors is electrode fouling, which has been proposed as the
2 main reason for biosensor failures *in vivo*. Electrochemical fouling is typical for dopamine (DA) as its
3 oxidation products are very reactive and resulting polydopamine has a robust adhesion capability to
4 virtually all types of surfaces. The degree of DA fouling of different carbon electrodes with different
5 terminations was determined using cyclic voltammetry (CV) and scanning electrochemical microscopy
6 (SECM) approach curves and imaging. The rate of electron transfer kinetics at the fouled electrode
7 surface was determined from SECM approach curves allowing a comparison of insulating film
8 thickness for the different terminations. SECM imaging allowed the determination of different
9 morphologies, such as continuous layers or islands, of insulating material. We show that heterogeneous
10 modification of carbon electrodes with carboxyl-amine functionalities offers protection against
11 formation of an insulating polydopamine layer, while retaining the ability to detect DA. The benefits of
12 the heterogenous termination are proposed to be due to the electrostatic repulsion between amino-
13 functionalities and DA. Furthermore, we show that the conductivity of the surfaces as well as the
14 response towards DA was recovered close to the original performance level after cleaning the surfaces
15 for 10-20 cycles in H₂SO₄ on all materials but pyrolytic carbon (PyC). The recovery capacity of PyC
16 electrode was lower possibly due to stronger adsorption of DA on the surface.
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Introduction

Neuronal communication in the brain relies on precisely controlled dynamics of neurotransmitters, the molecules that are used for neuron-to-neuron signalling. Consequently, several diseases of the brain either are due to or associated with changes in the spatial and temporal kinetics of the neurotransmitters. One important neurotransmitter is dopamine (DA), which is involved in a variety of brain disorders such as schizophrenia and addictions as well as in Parkinson's disease, where the brain DA neurons are degenerating¹. The accurate measurement of DA would provide a better understanding of these diseases and a tool to follow up the output of the treatments. Electrochemical techniques provide a cheap method that is compatible *in vivo* for the real-time detection of DA.

A significant problem with this type of technique is electrode fouling, which has been proposed as the main reason for biosensor failure *in vivo*². The electrode fouling is the consequence of two main mechanisms, which both have to be addressed. 1) Biofouling is the passive adherence of background species, such as proteins or lipids, to the electrode surface. 2) Electrochemical fouling means the formation of an insulating film on the electrode as a sequence of the reaction used for the detection. This electrochemical fouling is typical for DA as its oxidation products are very reactive. Under proper environment (pH > 7.5, DA concentrations higher than 2 mg/mL), polydopamine formation occurs spontaneously³.

The reaction pathway leading to DA fouling is shown in Figure 1. The first step in DA oxidation into dopamine ortho-quinone (DAQ) involves a two-electron transfer accompanied with deprotonation. In the second step the DAQ undergoes intramolecular cyclization via 1,4 Michael addition which leads to the formation of leucodopaminechrome (LDAC)⁴. LDAC is further oxidized in a two-electron transfer to dopaminechrome (DAC), which may further go polymerization process and form polydopamine on the electrode. The molecular mechanism behind the formation of polydopamine is still of scientific debate, and several mechanisms have been proposed (reviewed in³). Because of the complex redox process as well as the generation of a series of intermediates during the polymerization and reaction processes, many functional groups including planar indole units, amino group, carboxylic acid group, catechol or quinone functions, and indolic/catecholic π -systems are integrated into polydopamine⁵. Consequently, polydopamine has a robust adhesion capability to virtually all types of surfaces³. This polydopamine formation results in signal attenuation and compromises the quality of the measurements, as the thick layer may prevent or hinder electron transfer. Moreover, the electrochemical oxidation of neurotransmitters is typically an inner-sphere reaction involving surface adsorption⁶, which may be blocked by the fouled layer.

This paper investigates electrochemical fouling of DA on different carbon-based electrode materials. Carbon thin films such as amorphous carbon (a-C), tetrahedral amorphous carbon (ta-C) and pyrolytic carbon are patternable and relatively cheap and easy to deposit on nearly any given surface. In comparison to metal films, a-C and ta-C films have the advantage of low fabrication temperatures. Carbon is also abundant and will therefore never become a critical material. Moreover, a-C and ta-C are completely CMOS-compatible. Both a-C and ta-C have good electrochemical properties such as a large water window and a low background signal, which makes them highly attractive for electroanalytical chemistry⁷. There are several articles reporting their qualities as sensor for neurotransmitter detection (a-C^{7,8} and ta-C^{7,9,10,11,12,13}). Due to the smoothness of these films and the inert nature of carbon, these materials are less susceptible for biological fouling than metals.

For example, diamond-like carbon resisted microbial attachment in static conditions compared to titanium, tantalum^{14,15,16} and ta-C resisted protein attachment compared to platinum¹⁷. The sensitivity of ta-C is usually not high enough for biological applications, but functionalization of the ta-C surface with different carbon allotropes, such as carbon nanotubes and nanofibers or nanodiamonds (ND), have shown great potential for the electrochemical detection of different biomolecules, such as DA, glutamate and ascorbic acid¹⁸. For example, functionalized NDs coated on ta-C decreased the detection

limit for DA by three orders of magnitude [from 10 μM to 50 nM] in comparison to ta-C¹⁹. Similarly, NDs on boron doped diamond significantly enhanced ionic currents of outer-sphere redox probe (ferrocene methanol), compared to bare BDD²⁰. Pyrolytic carbons (PyC) are a group of nanographitic thin film materials. Large densities of edge-plane sites and oxygenated functionalities on PyC make it an ideal candidate for electrochemical sensor applications²¹. Moreover, PyC enables DA detection in the physiologically relevant range (50 nm – 1 μM)^{22, 23} and PyC has dopaminergic properties, i.e. promotes neural stem cell differentiation to dopaminergic phenotype²⁴, which makes PyC a highly interesting material for DA sensors.

In a previous research on the electro-oxidation of DA on the unmodified surfaces of five different classes of carbon electrodes (glassy carbon, oxygen-terminated polycrystalline boron-doped diamond (BDD), edge plane pyrolytic graphite, basal plane pyrolytic graphite, and the basal surface of highly oriented pyrolytic graphite (HOPG)), the least susceptible material to DA surface fouling was found to be polycrystalline BDD, which also showed sluggish DA reaction kinetics, while HOPG, which gave the best voltammetric signal to DA, was very susceptible to blocking by dopaminergic products²⁵. Another study suggest that carbon nanotube fiber (CNTF) microelectrode is less susceptible for DA fouling than a traditional carbon fiber (CF) micro-electrode due to a decreased binding affinity of the insulating film to CNTF surface²⁶. Moreover, the detection of DA in complex biological environments have been investigated. The research indicates that hydrogenation of carbon electrodes has been proposed to minimize fouling during DA detection in vivo²⁷ and BDD generally exhibits the better performance in biologically fouling environments than glassy carbon²⁸.

Experimental Section

Substrate fabrication

Sample fabrication details are provided as supplementary material and the more detailed description of the a-C deposition process can be found from⁸, for ta-C and ND from¹⁹, and that of PyC from²⁹.

Amorphous carbon (a-C) films with varying oxygen or hydrogen content were deposited by closed-field unbalanced magnetron sputtering. a-C O2, a-C H2 and a-C H12 refer to oxygenated or hydrogenated a-C samples, where hydrogen or oxygen inflow was kept at 2 (a-C O2 and H2) or 12 (a-C H12) sccm during the deposition process.

The ta-C sample were prepared on p-type conductive Si substrates (0.001–0.002 Ωcm , Ultrasil, USA). First 20 nm of Ti adhesion layer was deposited by direct current magnetron sputter followed by 7 nm ta-C film deposited by pulsed filtered cathodic vacuum arc (p-FCVA).

Two types of functionalized NDs: zeta-positive ND_{andante} with amino and carboxyl functional groups and zeta-positive hydrogen terminated ND_H (Carbodeon uDiamond®, Carbodeon, Vantaa, Finland) were investigated.

For PyC coating, SU-8 resist using a BLE photoresist spinner was spin coated on silicon wafers and soft baked on a hot plate at up to 85 °C, exposed to UV light for 8 seconds (flood exposure), and post-baked on a hot plate. Pyrolysis was carried out in continuous nitrogen flow in a tube furnace at 900°C for 4 hours. The thickness of the resin layer was $13.3 \pm 0.4 \mu\text{m}$ and the thickness of the pyrolyzed layer $2.4 \pm 0.1 \mu\text{m}$.

The coated Si wafers were cut to 1 cm \times 1 cm pieces, which were then contacted to Cu slabs. For CV experiments, a circular area was defined with a Polytetrafluoroethylene tape (Irpola Oy, Finland) and for SECM measurements the samples were placed on the SECM measurement cell, where an O-ring defines a circular area.

XPS

X-ray photoelectron spectroscopy (AXIS Ultra, Kratos Analytical at Aalto Biomaterials Center) was utilized for surface chemical characterization. Elemental carbon to oxygen ratios in the surface region were determined from wide spectra, and the amount of oxygen functional groups bonded to carbon was evaluated from peak-fitted C 1s high-resolution regions. Additionally, oxygen and nitrogen high resolution regions (O 1s and N 1s) were acquired. Wide spectra were collected using 1 eV step and 80 eV pass energy and high-resolution spectra using 0.1 eV step, 20 eV pass energy.

All spectra were collected using monochromatic Al K α x-ray source at 100 W and with neutralization in the case of charging. Samples were pre-evacuated overnight in vacuum $<10^{-5}$ Pa. Analysis chamber vacuum was $<10^{-6}$ Pa during measurements. Spectra for each sample were collected from at least three locations (analysis area $\sim 400 \times 800 \mu\text{m}$, depth less than 10 nm). Filter paper (100% cellulose, Whatman) was used as in situ reference and for binding energy scale calibration (CO = 286.7 eV, C-C = 285.0)^{30, 31}. Data fitting, atomic composition ratio calculations and charge correction was done using CasaXPS software (v. 2.3.18). Peak-fitting to the C 1s high-resolution region was done using equally wide full width half maximum (FWHM) Gaussian lines for each oxygen functional group³¹.

Cyclic voltammetry

Cyclic voltammetry was carried out with a Gamry Reference 600 potentiostat in a three-electrode cell. The reference was an Ag/AgCl electrode (+0.199 V vs SHE, Radiometer Analytical) and a platinum wire was used as the counter electrode.

Surfaces were subjected to fouling in 1 mM DA in phosphate buffered saline (pH 7.4) for 10 cycles with 50 mV/s, followed by cleaning in 0.15 M H₂SO₄ for 10 cycles with 50 mV/s. After cleaning, the DA measurement was repeated. Samples were rinsed twice in dH₂O between the different electrolytes.

All the solutions were deoxygenated with argon for at least 15 minutes prior to measurements and blanketed during measurements in order to avoid DA self-polymerization.

Scanning electrochemical microscopy

Scanning electrochemical microscopy (SECM) measurements were carried out using CHI910B scanning electrochemical microscope (CH Instruments). 10 μm platinum wires sealed in glass were used as the working electrode, an Ag/AgCl electrode served as a reference electrode and a platinum wire as a counter electrode. Approach curves and SECM images were recorded using a steady state voltage of 400 mV for the working electrode and 1 mM FcMeOH in 0.15 M H₂SO₄ was used as a mediator. No potential was applied at the surface during approach curves or SECM imaging.

Substrate surfaces were the carbon electrodes under investigation. SECM approach curves and imaging were performed for untreated samples, then subsequent to cycling in DA (fouled) and subsequent to cycling in H₂SO₄ (cleaned). Fouling consisted of cycling in 1 mM DA in phosphate buffer (pH 7.4) for 10 cycles with either 50 mV/s, 400 mV/s or 1 V/s. After cycling 50 mV/s, the surfaces were subjected to cleaning in 0.15 M H₂SO₄ with 50 mV/s twice (cleaning 1: 10 cycles, cleaning 2: 10 more cycles). Samples were rinsed twice in dH₂O between the different electrolytes. All the solutions were deoxygenated with argon for at least 15 minutes prior to measurements.

SECM experimentation consists of recording approach curves where the normalized current $I = i/i_{\text{inf}}$ is plotted versus the normalized distance $L = d/a$, where i is the current at the tip electrode (radius a) localized at a distance d from the substrate, i_{inf} is the steady-state current when the tip is at an infinite

distance from the substrate. The approach rate was $2 \mu\text{m s}^{-1}$. The fittings between experimental and theoretical curves were done following C. Lefrou's approximations³² based on Bard-Mirkin's formalism, where tips parameters (a and R/G) were determined independently from approach curves of an insulator sample. A dimensionless rate constant $\kappa = k_{\text{eff}}a/D$, where k_{eff} is an apparent heterogeneous charge transfer constant between the mediator in solution and the substrate and D diffusion constant, was obtained from the theoretical approach curves. SECM imaging was recorded in feedback mode. Approach curves and imaging were performed in 1 mM FcMeOH before DA fouling, after DA fouling and after cleaning in H_2SO_4 and repeated three times in each step at different locations of the sample. The presented κ -values are average of the three measurements \pm standard deviation. H_2SO_4 was obtained from Merck or Fisher Scientific, all other chemicals from Sigma-Aldrich.

Results and Discussion

XPS

The elemental composition of the samples is presented in Table 1. The oxygen functionalities of a-C surface increased with oxygen inflow during deposition (a-C O2 sample), whereas hydrogen inflow increased the relative amount of CC bonding (a-C H2 and a-C H12 samples). $\text{ND}_{\text{andante}}$ and ND_{H} present similar surface chemistries. However, the amount of oxygen as well as COO bonding is higher on $\text{ND}_{\text{andante}}$ indicating carboxyl functionalities. XPS did not verify difference in amino functionalities between $\text{ND}_{\text{andante}}$ and ND_{H} , possibly due to the small amount (1.1–1.2%) of nitrogen in the samples. As for comparison, we have earlier functionalized ta-C surfaces with 3-Triethoxysilylpropylamine (APTES) in order to achieve amino-functionalized surface¹⁰. According to XPS analysis the amount of N (1s) on APTES functionalized surfaces was 1.2%, which is the same amount as observed here on $\text{ND}_{\text{andante}}$.

PyC consists 98.5% of carbon and only small amount of functional groups exists, which is the main difference to other carbon electrode materials investigated in this study.

Electrochemistry

The degree of fouling of different carbon electrodes with different terminations was determined using CV and SECM approach curves and imaging. DA exhibited quasi-reversible behaviour with sluggish electron transfer kinetics on all of the tested electrode materials, except on a-C H12, where the behaviour was irreversible. Based on the voltammograms of DA fouling, it is evident that polydopamine forms on all of the electrode materials. With all scanning rates (50 mV/s, 400 mV/s and 1000 mV/s), the oxidation peak current magnitude decreased significantly over consecutive scans during cycling. Figure 2 shows the voltammograms of DA fouling with 50 mV/s and the repeated DA measurement after cleaning in H_2SO_4 , and Table 2 summarizes the respective changes observed in peak separation (ΔE_p) and oxidation peak current (I_{p_o}). When cycling with 50 mV/s, the oxidation peak current decreased on average by 28% by the second cycle, while ΔE_p increased on average by 78% by 10th cycle. This indicates formation of an insulating layer on the surface, which is confirmed by the approach curves and calculated κ -values for FcMeOH (Table 3). Initially all of the materials but a-C H12 showed good electron transport properties with positive feedback ($\kappa > 2$ for FcMeOH). After the substrates had been subjected to 10 cycles in 1 mM DA using scanning speed of 50 mV/s, negative feedback ($\kappa < 1$ for FcMeOH) was recorded for all materials but $\text{ND}_{\text{andante}}$, confirming the formation of an insulating layer.

Interestingly, a positive feedback ($\kappa > 2.5$ for FcMeOH) was recorded for $\text{ND}_{\text{andante}}$ even after 10 cycles in 1 mM DA, indicating that the polydopamine layer is either nonuniform or thinner than on the other

1 tested materials. The fouling behaviour is not linked to DA detection capability as spray-coated
2 $\text{ND}_{\text{andante}}$ and ND_{H} demonstrated similar behaviour with the linear range of 500 nM–1 mM and
3 sensitivities of 0.161 and 0.172 $\text{A M}^{-1} \text{cm}^{-2}$, respectively¹⁹. It is possible that the heterogeneous surface
4 functionalities containing amino-groups protect $\text{ND}_{\text{andante}}$ surface from the formation of uniform DA
5 layer. We have previously observed that spray-coated amino functionalized nanodiamonds (ND_{amine}) on
6 ta-C did not produce oxidation or reduction peaks even at high concentrations of DA¹⁹. This could be
7 due to electrostatic repulsion between DA and protonated amine groups as, at physiological pH, DA
8 exists mostly as a cation². Heterogeneous surface functionalities containing amino-functionalities may
9 prevent DA adsorption and therefore protect surfaces from forming thick and uniform polydopamine
10 layer while at the same time the carboxyl-functionalities help to promote electron transfer and allow the
11 DA to be detected. SECM images confirm the heterogeneous surface kinetics on NDs, but it seems
12 possible that the electrochemical properties follow the topographical features of the sample to a certain
13 degree. However, after DA fouling (50 mV/s), $\text{ND}_{\text{andante}}$ represents current range from negative ($i/i_{\text{inf}} <$
14 1) to positive ($i/i_{\text{inf}} >$ 1) feedback, indicating true heterogeneity of the surface. The size of the different
15 regions of conductivity is approximately 20 μm .
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18 As mentioned, a negative feedback ($\kappa < 1$ for FcMeOH) was recorded for other tested materials
19 subsequent to DA fouling. However, some differences were observed. All of the a-C surfaces became
20 more insulating ($\kappa < 0.5$ for FcMeOH) than ta-C or PyC ($\kappa = 0.8$ FcMeOH). The chemical inertness of
21 the surface arising from the sp^3 nature of the surface may protect ta-C from fouling as severely as a-C
22 surfaces. This is in agreement with previous reports stating that the extensive sp^3 structure results in a
23 decrease in the adsorption of certain fouling agents^{33, 34}. Moreover, the initial κ -value measured in
24 FcMeOH for PyC is higher than for other materials, which may explain why the κ -value remains higher
25 even after fouling.
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28 Different a-C samples showed minor differences in their fouling behaviour. Based on the calculated κ -
29 values, a-C O2 ($\kappa = 0.22$) becomes slightly more insulating than a-C ref ($\kappa = 0.38$), which is again more
30 insulating than a-C H2 ($\kappa = 0.47$). This is possibly due to the interactions of oxygen functional groups
31 and DA. The hydrophilic functional groups may increase the adsorption of the polar dopaminechrome
32 molecules. Similar lower fouling resistance towards polar molecules on O-terminated carbon surface
33 was observed in a study of norepinephrine and theophylline electrochemistry on graphene oxide and
34 reduced graphene oxide modified glassy carbon surfaces³⁵. Note, that fouling resistance is essentially
35 depended on the fouling agent. In general, with biological fouling agents (proteins), O-termination of
36 carbon surfaces is considered to increase the fouling resistance due to the increased surface
37 hydrophilicity, which minimises the tendency of proteins to unfold and foul by hydrophobic
38 mechanisms³⁶.
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41 Subsequent to DA fouling, we examined the recovery capacity of the carbon surfaces. Cycling in H_2SO_4
42 effectively removed the insulating polydopamine layer from all the surfaces but PyC. Figure 2 shows
43 the repeated DA measurement and Table 3 the κ -values measured in FcMeOH, which both are
44 recovered close to the original level for other materials except for PyC. For PyC, the repeated DA-
45 measurement shows 50% larger ΔE_p with 12% smaller oxidation current compared to the initial DA
46 response and the κ -value measured in FcMeOH is recovered only to some degree (to 2.4 as the original
47 was 3.1). This behaviour can be caused by the adsorption of DA on PyC surfaces. The shape of the PyC
48 voltammogram with steep oxidation and reduction peaks indicates that adsorption has a role in the
49 overall kinetics (Figure 3). However, the adsorption can still be considered “weak” as there is no
50 separate peak for the adsorbed species (energy difference is relatively small between dissolved and
51 adsorbed species). Owing to adsorption, PyC also demonstrates the largest oxidation and reduction peak
52 currents of the tested materials, which is due to the fact that both soluble and adsorbed DA contribute to
53 the reaction. Thus, there are more reactants near the electrode than there would be without adsorption.
54 Indeed, on PyC the DA reaction kinetics is both diffusion and adsorption defined²⁹.
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1 Furthermore, the large drop in I_{po} by the 2nd cycle (2nd cycle 63% of the 1st on PyC, while it remains >
2 70% on other tested electrode materials) with hardly any effect on E_{po} (4 mV for PyC) indicate a
3 formation of a (probably thin) layer on the surface during the first cycle. Moreover, physisorbed or
4 chemisorbed monolayers of DA are catalytic toward DA oxidation and reduction⁶, which can explain
5 the fastest electron transfer kinetics of the tested materials ($\Delta E_p = 183$ mV). Furthermore, on PyC, the
6 shape of the return curve is significantly different from the other tested materials. The other carbon
7 electrodes demonstrate similar behaviour to ND_{andante}, with a drop in the current (at about 400 mV) prior
8 to reduction, whereas PyC demonstrates very steep reduction peak (Figure 3). On PyC, an additional
9 reduction peak appears at -315 mV on the first scan corresponding to the reduction of dopaminechrome
10 (DAC) to leucodopaminechrome (LDAC). On the subsequent scan, the corresponding oxidation of
11 leucodopaminechrome can be seen at -170 mV. Similar features can be seen on a-C ref, a-C O2 and a-C
12 H2, but not on ta-C or NDs within the tested cycling window. The adsorption of DA (and probably also
13 some subsequent reaction products) on PyC also most likely causes the observed only partial recovery
14 of the surface properties of PyC after cycling in sulfuric acid, which is also in contrast with the other
15 investigated materials. The XPS measurement showed, that PyC consist of highly homogeneous surface
16 of C-C and C-H bonding (98.5%), which may explain the DA adsorption and subsequent DA fouling on
17 PyC.
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20 The differences observed in DA fouling possibly originate from DA reaction kinetics. DA is an inner-
21 sphere redox couple that is likely to react at specific adsorption sites³⁷. These sites favor redox reactions
22 and consequently they may be exposed to a higher level of dopaminequinone, which then takes part to
23 the fouling cascade. Subsequently, these sites are the most probable to be subjected to electrochemical
24 fouling. It can be concluded that electrodes that facilitate redox reactions through enhanced adsorption
25 are more sensitive to fouling than the ones with more sluggish kinetics. The PyC surface shows very
26 sharp DA oxidation peak we have shown that the reaction kinetics both diffusion and adsorption
27 defined²⁹. Consequently, PyC surface is the most sensitive to DA fouling. ND_{andante} seems to show
28 sluggish DA kinetics (Figure 3: wider CV, lower current density, high E_p and E_{po}) but resist fouling
29 well. This resistance can be due to amino functionalities, which do not facilitate DA reactions.
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32 The fouling behavior may have been affected by the CV cycling parameters. All of the materials were
33 scanned between -350 mV and 800 mV, except a-C H12, which was scanned from -350 mV to 1.6 V.
34 PyC has the lowest DA oxidation potential (305 mV) of the tested materials, and consequently the scan
35 limit is highest with respect to the oxidation potential. By scanning the potential to higher values than
36 the oxidation peak, adsorbed species on the electrode surface undergo further chemical and
37 electrochemical reactions¹². As a result, polydopamine film structure, which cannot be as effectively
38 removed by cleaning in H₂SO₄, may be formed.
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41 Even though the DA voltammogram and the calculated κ -values for FcMeOH indicate surface recovery,
42 the SECM images (Figure 4) confirm that there are remnants of the DA (in a size range of ten μm to a
43 few dozen micrometers) still present as the surface conductivity is more heterogeneous than that of
44 pristine surface.
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46 In Figure 4, we utilized constant scale for all of the plots in order to improve comparison of the samples.
47 It is evident that the current range drops significantly after DA fouling and recovers again after cleaning
48 in H₂SO₄, except for a-C H12 which is initially insulating. The unscaled version of the Figure 4 is
49 provided as supplementary material to better visualize different features of the surfaces.
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51 With higher CV scanning rates in DA, the passivation is less significant. A clear reduction peak of
52 dopaminequinone and consequently higher I_{red}/I_{ox} ratio is observed, indicating that the intramolecular
53 cyclization does not have time to occur, instead dopaminequinone is reduced back to DA. This is in
54 agreement with a previous experiment showing that the difference in the reversibility of the dopamine
55 redox reaction is obvious at different scan rates: the I_{red}/I_{ox} relationship of DA is 0.77 for 1000 mV s⁻¹
56 and only 0.11 for 50 mV s⁻¹¹². Consequently, cycling DA at 1000 mV/s results in no changes in ΔE_p ,
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1 indicating that no continuous layer of polydopamine is formed on the surface. The approach curves
2 measured in FcMeOH support this, as no significant changes are observed in the calculated κ -values
3 (Table 3) for a substrate treated in this way, confirming the effective electron transfer. However, as the
4 oxidation current drops on average by 44% by 10th CV cycle, it is clear that DA fouling occurs on the
5 surfaces and the electroactive surface area is reduced. This is supported by the subsequent SECM image
6 (Figure 5), which show the formation of islands with decreased conductivity. Cycling DA with the
7 slower scan rate of 400 mV/s results in the increase of ΔE_p on average by 9% and decrease of the
8 oxidation current by 55% by 10th cycle. The change in ΔE_p indicates that uniform DA layer is starting
9 to form.

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11 SECM proved to be an effective tool for evaluating fouling of the carbon substrate electrode. The use of
12 DA as a mediator in SECM directly is challenging, as the ultra microelectrode utilized in SECM fouls in
13 the presence of DA as well. However, by utilizing another redox probe subsequent to substrate fouling,
14 we showed that we can “quantify” the thickness of the insulating film with SECM approach curves and
15 discriminate between continuous layers and islands of the polydopamine with SECM images. It is
16 possible that polydopamine, which forms clumps³⁸, is not strongly adhered to the substrate and the
17 clumps delaminate when taken out from liquid environment. Moreover, the melanin-like polymeric
18 molecules are of ~ 3.8 Å in size, and consequently the detection of a monolayer would be difficult with
19 microscopic methods such as scanning electron microscopy or atomic force microscopy. The
20 supplementary materials shows examples of the studied surfaces before and after DA fouling observed
21 with scanning electron microscopy and atomic force microscopy. No polydopamine layer is observed.
22 In conclusion, in-situ techniques, such as SECM, in-situ scanning tunneling microscopy or in situ
23 atomic force microscopy, may be required to reliably asses DA fouling.

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25 A high concentration of DA (1 mM) was deliberately employed to accelerate the fouling phenomenon
26 associated with DA oxidation in order to evaluate the lifetime of the electrodes. The DA concentrations
27 *in vivo* are significantly lower (5-700 nM³⁹) than what was tested here (1 mM). Therefore, similar rapid
28 fouling will not occur *in vivo*. Moreover, physiological reducing agents, such as ascorbic acid, reduce
29 dopaminequinone back to its original form before the intracyclization can occur⁴⁰. In addition to
30 judicious choice of electrode material, the scanning parameters affect severity of fouling. Here, little
31 fouling was observed with scanning speeds of 400 mV/s or 1000 mV/s. Fast scan cyclic voltammetry
32 can even further reduce electrode fouling^{41, 42} by outrunning the side reactions that lead to fouling
33 products. Alternatively, fouling may also be decreased by using high anodic–cathodic potentials
34 between voltammetric scans^{41, 42}. Moreover, biological environment can affect the electrochemical
35 fouling as different proteins can either increase or decrease the rate of electrode fouling⁴³. For example,
36 cysteine residues reduce the severity of DA electrochemical fouling⁴⁴.

37
38 Table 4 summarizes the electrochemical properties. Based on our previous research, the reaction kinetic
39 towards outer sphere probes is comparable between all the tested materials and all of them showing
40 reversible or quasi-reversible reaction kinetics, except for a-C H12, which shows sluggish reaction
41 kinetics. Reaction kinetics towards DA is significantly faster (ΔE_p 44.6 mV) on PyC than on the other
42 tested materials ($\Delta E_p > 124$ mV) and it can detect significantly lower concentrations of DA (50 mM vs 1
43 μ M) than ND or ta-C. Detection limits for the a-C samples has not been defined.

44 45 46 47 48 49 50 **Conclusions**

51 We evaluated the degree of fouling of different carbon electrodes with different terminations using CV
52 and SECM approach curves and imaging. Polydopamine was formed on all of the materials, which was
53 observed both as a drop in DA oxidation current as well as increasing ΔE_p . When cycling DA for 10
54 cycles with 50 mV/s, an insulating layer of polydopamine was formed on all of the materials except for
55 ND_{andante}, as verified by the approach curves measured in FcMeOH. We showed that heterogeneous
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1 modification of carbon electrodes with carboxyl-amine functionalities offers protection against
2 formation of an insulating polydopamine layer, while retaining the ability to detect DA. The advantages
3 of the heterogenous termination are likely due to the electrostatic repulsion between amino-
4 functionalities and DA. With higher scanning speeds (400 mV/s or 1000 mV/s) formation of an
5 insulating layers was not evident on the surfaces from CV measurements, but SECM images confirmed
6 that some fouling of the surface was still taking place. Moreover, we showed that the conductivity of the
7 surfaces as well as the response towards DA on all materials but PyC was recovered close to the original
8 performance level subsequent to cleaning the surfaces in H₂SO₄. The recovery capacity of PyC
9 electrode was inferior possibly due to stronger adsorption of DA on the surface. In conclusion,
10 polydopamine formed on all the tested materials, but the severity of fouling is affected by the surface
11 properties of the material. SECM proved to be an effective tool for evaluating electrode fouling as we
12 can “quantify” the thickness of the insulating film with SECM approach curves and discriminate
13 between continuous layers and islands of the polydopamine with SECM images.
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16 17 **Acknowledgements**

18
19 Academy of Finland (E.P. grant #274670, T.L. grants #285015 and #285526) and Orion Research
20 Foundation (E.P.) are acknowledged for funding. We acknowledge the provision of facilities and
21 technical support by Aalto University at OtaNano - Nanomicroscopy Center (Aalto-NMC) and the
22 cleanroom facilities of Micronova. Niklas Wester and Joonas J. Heikkinen from the Department of
23 Chemistry and Materials Science, School of Chemical Engineering, Aalto University are acknowledged
24 for ta-C and PyC coating, respectively. Leena-Sisko Johansson from the Department of Bioproducts and
25 Biosystems, School of Chemical Engineering, Aalto University is acknowledged for help with the XPS
26 measurements.
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30 31 **Supporting Information**

32 Information about substrate fabrication; Microscopy of the surface before and after DA fouling;
33 Unscaled figure of the fouled surfaces.
34

35 Supporting information.pdf
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38 39 **References**

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Table 1: XPS results showing the elemental composition and carbon bonding of the samples. Columns 1-3 are calculated from wide spectrum and columns 4-8 from high-resolution scan.

| | C (1s) | O (1s) | N (1s) | CC* | CC* | C-O | C=O | COO |
|-----------------------|------------|------------|-----------|------------|------------|------------|-----------|-----------|
| a-C ref | 91.1 ± 0.2 | 8.0 ± 0.1 | 0.4 ± 0.1 | 79.4 ± 0.1 | | 13.6 ± 0.1 | 4.1 ± 0.1 | 2.9 ± 0.1 |
| a-C O2 | 87.5 ± 0.4 | 11.6 ± 0.3 | 0.5 ± 0.1 | 75.9 ± 0.1 | | 15.2 ± 0.1 | 5.3 ± 0.1 | 3.5 ± 0.1 |
| a-C H2 | 91.2 ± 0.2 | 7.9 ± 0.1 | 0.3 ± 0.1 | 81.3 ± 0.2 | | 12.4 ± 0.1 | 3.7 ± 0.1 | 2.6 ± 0.1 |
| a-C H12 | 89.8 ± 0.1 | 9.7 ± 0.1 | 0.2 ± 0.1 | 84.4 ± 0.3 | | 10.6 ± 0.2 | 3.4 ± 0.1 | 1.6 ± 0.1 |
| ta-C | 89.8 ± 0.1 | 8.9 ± 0.1 | 0.8 ± 0.1 | 28.7 ± 1.3 | 57.9 ± 1.4 | 7.5 ± 0.1 | 3.7 ± 0.2 | 2.1 ± 0.1 |
| ND _H | 93.9 ± 0.4 | 4.8 ± 0.3 | 1.1 ± 0.1 | | 58.8 ± 0.2 | 37.6 ± 0.1 | 3.4 ± 0.1 | 0.2 ± 0.1 |
| ND _{andante} | 93.3 ± 0.2 | 5.4 ± 0.2 | 1.2 ± 0.1 | | 59.0 ± 0.4 | 38.5 ± 0.5 | 1.9 ± 0.2 | 0.5 ± 0.1 |
| PyC | 98.5 ± 0.1 | 1.5 ± 0.1 | 0.1 ± 0.1 | 74.4 ± 1.7 | 16.1 ± 1.8 | # | # | # |

* a-C samples were fitted with one carbon peak, whereas ta-C, NDs and PyC were fitted with two carbon peaks (first being more “graphitic” and second being more sp³-carbon)

Functional groups exists, but determination of the exact amounts is not unambiguous.

Table 2: Peak separation (ΔE_p), and oxidation peak voltage (E_p) and current (I_p) for DA during DA fouling (1st, 2nd and 10th cycle) and after the subsequent H₂SO₄ cleaning. Cycling speed 50 mV s⁻¹.

| Cycle | ΔE_p (mV) | | | | E_p (mV) | | | | I_p ($\mu A cm^{-2}$) | | | |
|-----------------------|-------------------|-----|------|--------------------------------------|------------|------|------|--------------------------------------|---------------------------|-----|------|--------------------------------------|
| | 1st | 2nd | 10th | After H ₂ SO ₄ | 1st | 2nd | 10th | After H ₂ SO ₄ | 1st | 2nd | 10th | After H ₂ SO ₄ |
| a-C ref | 215 | 270 | 496 | 267 | 308 | 357 | 505 | 331 | 249 | 180 | 139 | 243 |
| a-C O2 | 352 | 322 | 531 | 279 | 427 | 403 | 560 | 343 | 231 | 180 | 141 | 253 |
| a-C H2 | 240 | 288 | 490 | 269 | 329 | 373 | 528 | 340 | 233 | 168 | 123 | 235 |
| a-C H12 | * | * | * | * | 1390 | 1422 | # | 1400 | 173 | 140 | # | 182 |
| ta-C | 366 | 393 | 534 | 400 | 438 | 461 | 574 | 457 | 211 | 157 | 133 | 216 |
| ND _H | 374 | 438 | 603 | 375 | 367 | 424 | 574 | 408 | 253 | 185 | 150 | 274 |
| ND _{andante} | 387 | 471 | 672 | 422 | 468 | 526 | 686 | 417 | 222 | 163 | 141 | 182 |
| PyC | 183 | 190 | 285 | 276 | 305 | 309 | 367 | 376 | 347 | 219 | 157 | 307 |

*) No reduction peak, ΔE_p could not be defined

#) No peak definable at 10th cycle, at 7th cycle E_p 1546 mV and 137 $\mu A cm^{-2}$.

Table 3: κ -values for FcMeOH defined from the approach curves for pristine surface, after 1mM DA fouling with 50 mV s⁻¹ or 1 V s⁻¹ and after subsequent cleaning in 0.15 M H₂SO₄.

| κ (FcMeOH) | a-C ref | a-C O2 | a-C H2 | a-C H12 | ta-C | ND _H | ND _{andante} | PyC |
|--|-------------|-------------|-------------|-------------|-------------|-----------------|-----------------------|-----------|
| Pristine surface | 2.2 ± 0.1 | 2.29 ± 0.03 | 2.27 ± 0.03 | 0.02 ± 0.01 | 2.33 ± 0.05 | 2.17 ± 0.06 | 3.1 ± 0.3 | 3.1 ± 0.1 |
| DA 50 mV/s | 0.38 ± 0.04 | 0.22 ± 0.05 | 0.47 ± 0.05 | 0.03 ± 0.00 | 0.81 ± 0.08 | 0.30 ± 0.06 | 2.5 ± 0.1 | 0.8 ± 0.3 |
| H ₂ SO ₄ , 10 cycles | 2.0 ± 0.2 | 1.9 ± 0.2 | 2.2 ± 0.2 | 0.04 ± 0.01 | 2.2 ± 0.1 | 1.6 ± 0.9 | 2.5 ± 0.1 | 2.4 ± 0.1 |
| H ₂ SO ₄ , 20 cycles | 2.07 ± 0.01 | 2.0 ± 0.1 | 2.2 ± 0.1 | 0.05 ± 0.01 | 2.3 ± 0.08 | 2.9 ± 0.5 | 3.7 ± 0.9 | 2.7 ± 0.2 |
| Pristine surface | 2.2 ± 0.1 | 2.6 ± 0.1 | 2.6 ± 0.4 | 0.03 ± 0.00 | 2.5 ± 0.5 | 2.4 ± 0.5 | 2.9 ± 0.8 | 3.0 ± 0.2 |
| DA 1 V/s, 10 cycles | 2.2 ± 0.6 | 2.5 ± 0.1 | 2.2 ± 0.2 | 0.04 ± 0.00 | 2.5 ± 0.5 | 2.1 ± 0.2 | 2.8 ± 0.1 | 2.9 ± 0.1 |

Table 4: Advantages and disadvantages of the tested samples.

| | ΔE_p (mV) for outer sphere redox probe | ΔE_p (mV) for 100 μM DA | Lowest measured DA concentrations | DA fouling | DA recovery |
|-----------------------|--|--------------------------------------|-----------------------------------|------------|-------------|
| a-C ref | 59.8 ^{a, b} | 124 ^b | e | ++ | ++ |
| a-C O2 | 62.8 ^{a, b} | 150 ^b | e | ++ | ++ |
| a-C H2 | 58.6 | 128 ^b | e | ++ | ++ |
| a-C H12 | 789 | 1142 ^b | e | ++ | ++ |
| ta-C | 57.1 ^{a, b} | 369 ^d | 10 μM ^f | ++ | ++ |
| ND _H | 62 ^{c, d} | 192 ^d | 1 μM | ++ | ++ |
| ND _{andante} | 64 ^{c, d} | 198 ^d | 1 μM | partial | ++ |
| PyC | 62.8 ^{a, b} | 44.6 ^b | 50 nM | ++ | partial |

^aRu ^b100 mV s⁻¹ ^cFcMeOH ^d50 mV⁻¹ ^edetection limits have not been defined ^fDefined on ta-C film grown on Pt film
 ΔE_p and lowest measured DA concentrations has been previously reported: a-C and a-C O₂⁸, a-C H₂ and a-C H₁₂⁴⁵, ta-C^{11,12}, ND¹⁹ and
PyC²⁹

Figure caption

Figure 1: First steps of the formation of polydopamine.

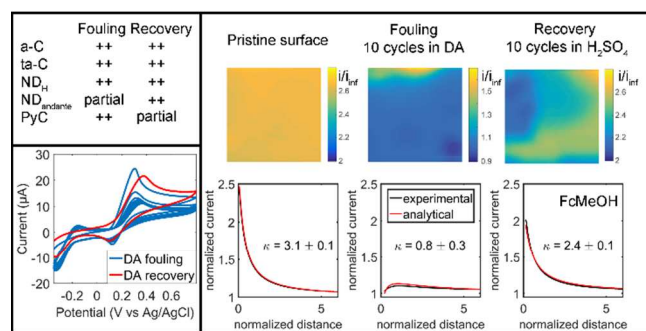
Figure 2: 1 mM DA fouling with 50 mV/s. Gray curve shows the 10 fouling cycles and dotted black curve the repeated DA measurement after 10 cleaning cycles in 0.15 M H₂SO₄.

Figure 3: 1st (gray) and 2nd (dotted black) DA fouling cycles on ND_{andante} and PyC.

Figure 4: Pristine surfaces (first column) and surfaces after cycling in 1 mM DA with 50 mV/s (second column) and after subsequent cleaning in H₂SO₄ 10 cycles (third column) or 20 cycles (fourth column). Each graph is 50 $\mu\text{m} \times 50 \mu\text{m}$ and the scale bar indicates relative current i/i_{inf} .

Figure 5: Examples of SECM images recorded in FcMeOH before and after 10 cycles of DA cycling with 1 V s⁻¹ on a-C and ta-C surfaces. Each graph is 50 $\mu\text{m} \times 50 \mu\text{m}$. Scale is kept constant for each sample.

For TOC only



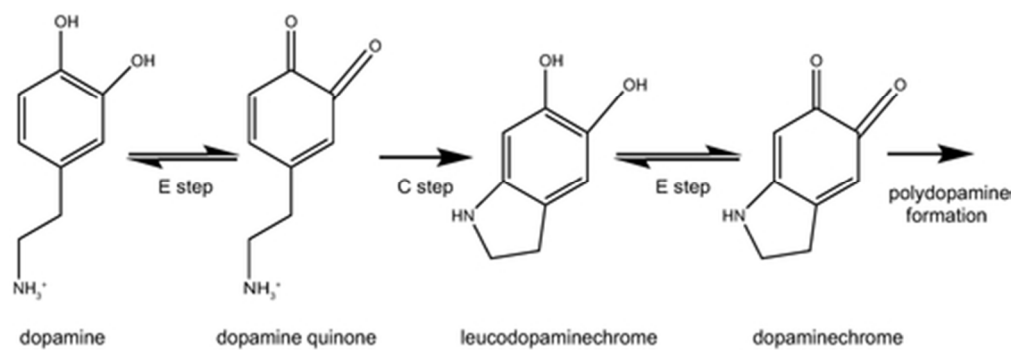


Figure 1

43x14mm (300 x 300 DPI)

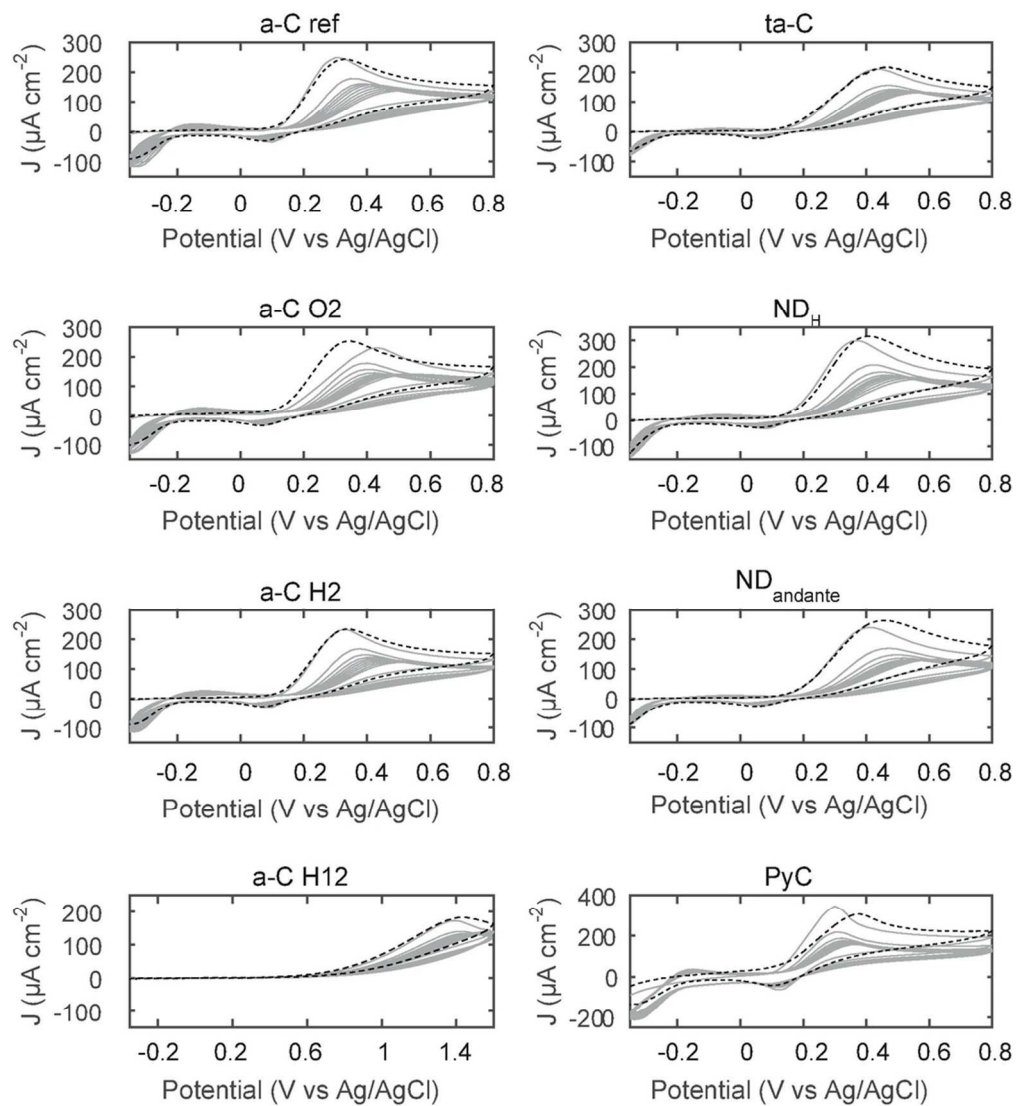


Figure 2

92x101mm (300 x 300 DPI)

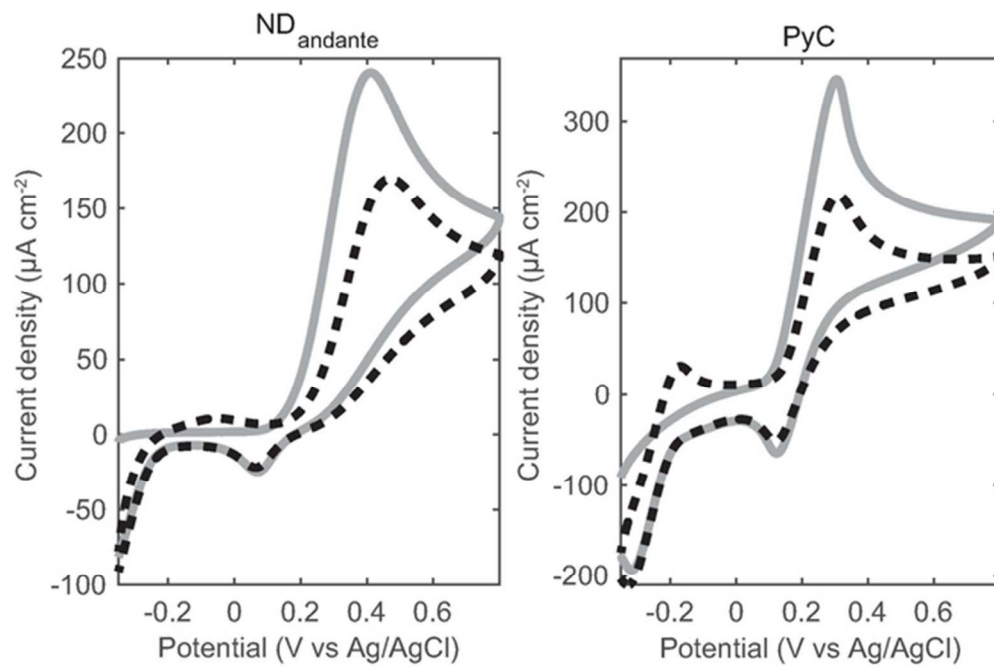


Figure 3

55x36mm (300 x 300 DPI)

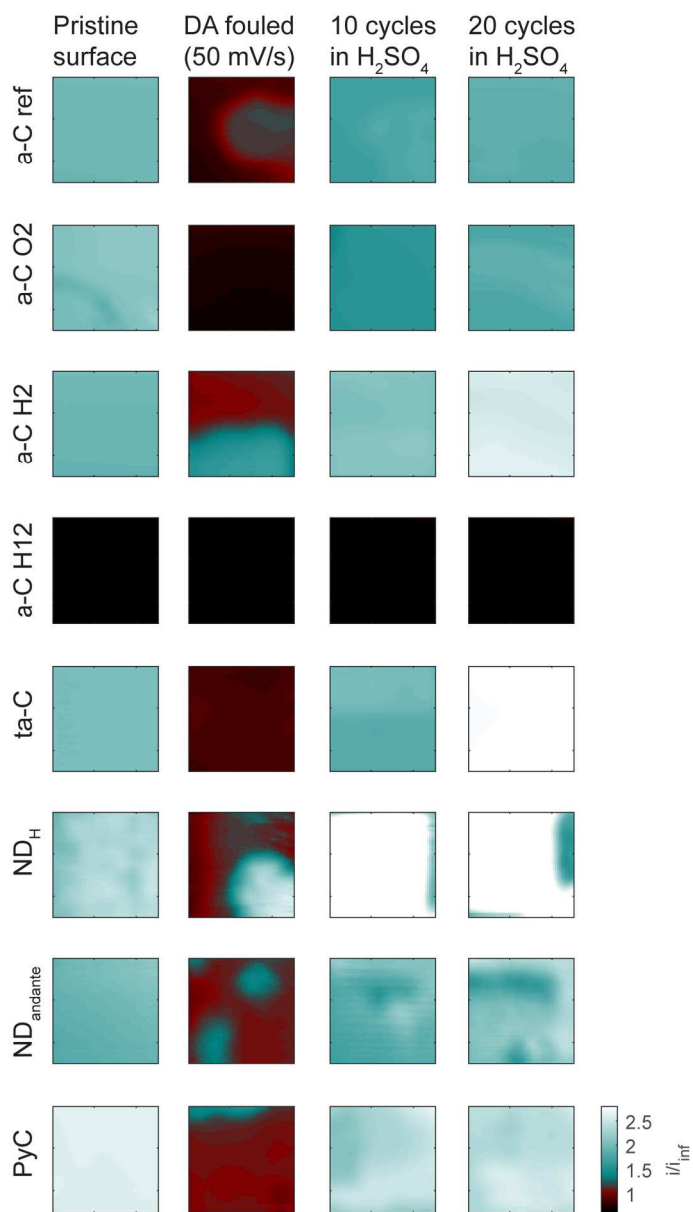


Figure 4

113x197mm (300 x 300 DPI)

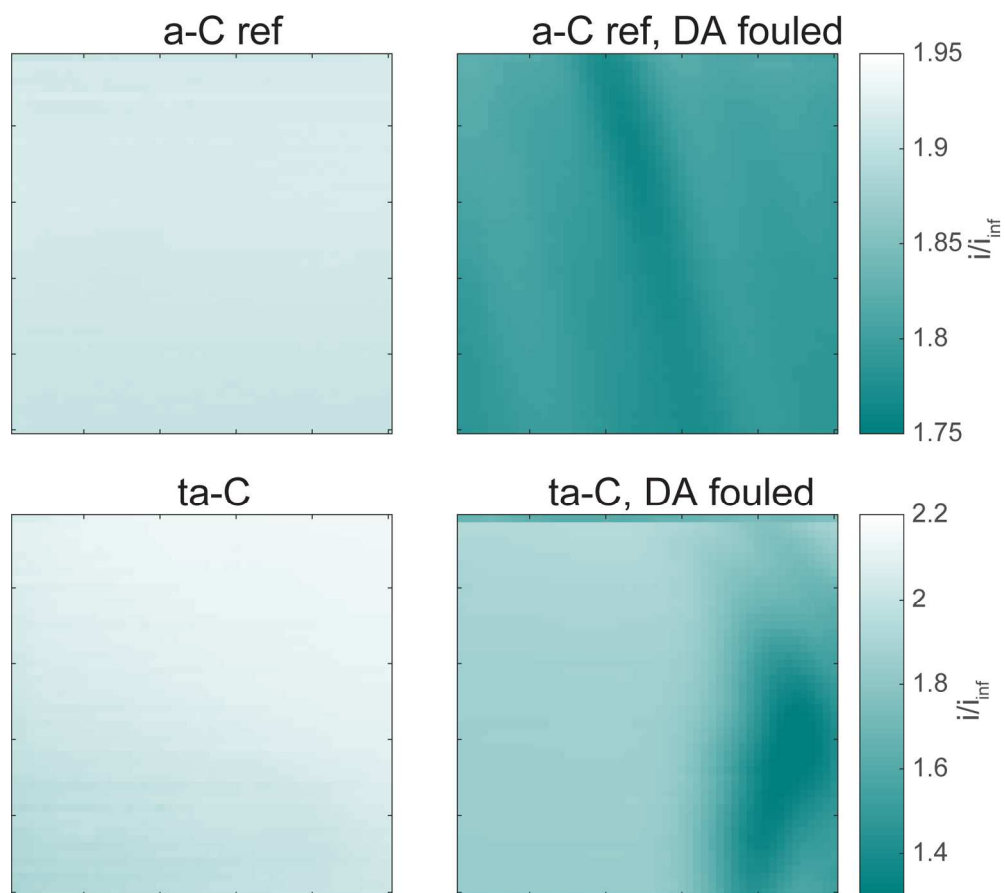


Figure 5

162x144mm (300 x 300 DPI)