Supporting Information

for

In Vitro Electrochemical Characterization of Polydopamine Melanin as a Tissue Stimulating Electrode Material

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Materials and Methods

Film Preparation

Dopamine hydrochloride (98%) and sodium bicarbonate (99%) were purchased from Sigma-Aldrich (St. Louis, MO), and sodium carbonate (99%) was purchased from Fisher-Scientific (Pittsburgh, PA). Indium Tin Oxide (ITO) substrates (University Wafers, Boston, MA) were prepared by sonicating in a sequence of acetone and isopropyl alcohol (IPA) followed by rinsing in ddH_2O. Substrates were then cleaned by UV and ozone (30

mW/cm^2, 20 min; Jelight, Irvine, CA). Polydopamine melanin (PDM) films were deposited onto ITO substrates by placing cleaned ITO substrates in 2mg/ml of dopamine hydrocholoride in 50 mM carbonate/bicarbonate buffer solution (pH = 8.5) at pO_2 = 160 mmHg and temperatures of 25 ± 3 ^oC. Deposition times of 16 hr in a stock solution produce PDM films that are approximately 40 ± 5 nm in thickness. PDM films with thicknesses of 80 ± 10 nm and 120 ± 15 nm were prepared by repeatedly incubating substrates in two and three sequences of fresh stock solutions, respectively.

Morphological Characterization of Samples

Atomic force microscopy (NTegra AFM, NT-MDT, Tempe, AZ) was performed in tapping mode. Scans were 10 μm × 10 μm at 0.8 Hz using tips with a reported radius of <10 nm (Budget Sensors, Sofia, Bulgaria; k = 40 N/m). Thicknesses were determined by scoring films with a razor blade and measuring the height difference by AFM. Data were analyzed via Gwyddion AFM software (http://gwyddion.net).

Electrochemical Characterization

All electrochemical experiments were performed in a conventional three-electrode setup, with an Ag/AgCl electrode (Koslow Scientific, Englewood, NJ) as a reference electrode and with Pt foil as a counter electrode in 0.01M phosphate buffered saline (PBS) solution (Sigma-Aldrich, St. Louis, MO). All bias voltages are reported with respect to Ag/AgCl reference electrodes. No sign of delamination or morphological change was observed after the electrochemical characterization performed within the range of applied voltages in this study.

Potential Transient Measurement

Potential transients during current pulsing were measured using multistep chronopotentiometry mode in a Gamry Interface 1000 Potentiostat (Gamry Instruments, Warminster, PA). Current Pulses were charge-balanced cathodal first biphasic pair with both phases having a width of 10 ms and a built in 1ms dwell time between a cathodal and anodal phase.

Electrochemical Impedance Spectroscopy

Impedance measurements were conducted with a Gamry Interface 1000 potentiostat (Gamry Instruments, Warminster, PA) in the frequency range of f = 0.1 Hz to 100 kHz while keeping dopamine melanin films at various dc potentials from -0.6V to 0.6V versus Ag/AgCl. EIS fitting was done using Z_fit module in EC-Lab Software (Bio-Logic Science Instruments SAS, Claix, France).

UV-vis Absorption Spectrophotometry

UV-vis spectra were acquired with spectrophotometery (Shimadzu UV-2600, Kyoto, JAPAN) for PDM films deposited using previously described methods. Bare ITO substrates were used as a reference material. Before measurement, PDM films were subjected to constant dc biases using a Bio-Logic VMP3 potentiostat (Bio-Logic Science Instruments SAS, Claix, France) for 10 minutes in 0.01M PBS and were immediately rinsed with ddH_2O and dried under an N_2 stream.

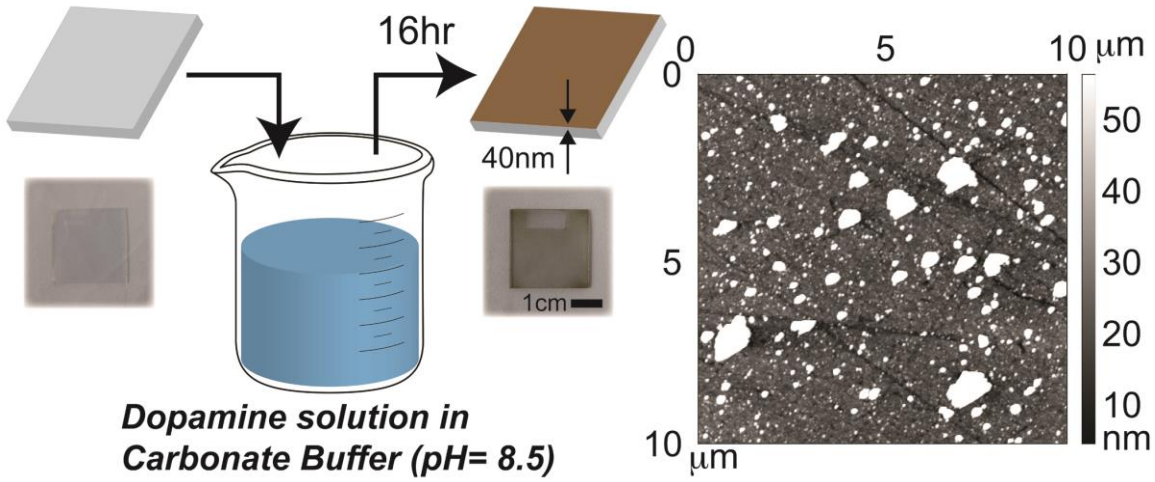

Figure S1. Schematic of polydopamine melanin (PDM) film deposition on ITO substrates. Insets are digital photos of bare ITO and PDM films deposited on ITO substrates. Representative AFM images of freshly prepared dopamine melanin films (40 nm in thickness) on ITO substrates are also shown at right.

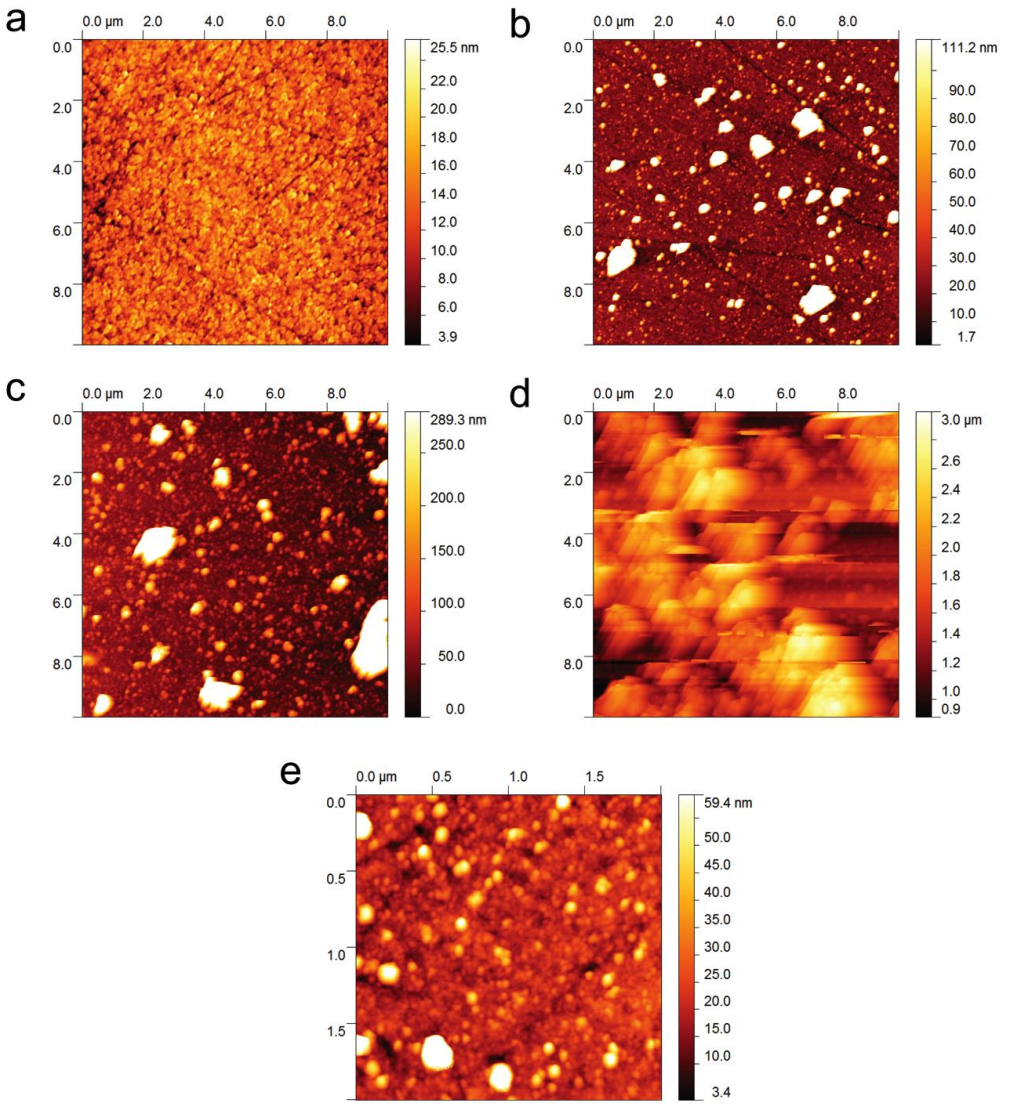

Figure S2. Atomic Force Microscopy (AFM) scans of bare ITO substrates and ITO substrates with polydopamine melanin (PDM) films. AFM scans were taken with 512 x 512 pixels: (a) 10 µm x 10 µm scan of an ITO substrate; (b) 10 µm x 10 µm scan of 40nm thick PDM film on ITO; (c) 10 µm x 10 µm scan of 80 nm thick PDM film on ITO; (d) 10 µm x 10 µm scan of 120nm thick PDM film on ITO; (e) 2 µm x 2 µm scan of 40nm thick PDM film on ITO. During initial film formation, PDM islands nucleate on the surface of the ITO as demonstrated by Klosterman et al.[1] Given the greater thickness of the films prepared here, the granule structures may be deposited in multiple layers. The fine granule structure of the 40 nm film is visible in a 2 µm x 2 µm scan. PDM films 80 nm in thickness exhibits a larger average granule size compared to PDM films 40 nm in thickness, supported by the greater RMS roughness value. Larger PDM aggregates that form in the dopamine solution also deposit on the films and are visible as the large structures with greater height than the surrounding film. These aggregates cover much of the surface in the 120 nm thick film.

Table S1. Roughness statistics for bare ITO substrates and ITO substrates with PDM films of varied thicknesses.

Electrode Material	PDM Film Thickness (nm)	R_a (nm)	RMS (nm)
ITO	n/a	1.94 ± 0.11	2.42 ± 0.13
PDM on ITO	40	41.9 ± 6.5	79.9 ± 8.7
PDM on ITO	80	98.6 ± 25.1	158 ± 33.8
PDM on ITO	120	186.7 ± 69.2	253 ± 75.5

Table S2. Film thicknesses for PDM deposited on ITO substrates as a function of time. Data presented as mean ± std. dev.

Deposition Time (hr)	Thickness (nm)
16	40.5 ± 4.8
32	81.8 ± 4.2
48	123.9 ± 7.5

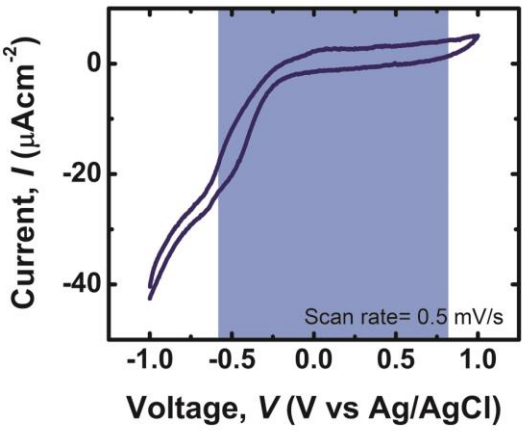

Figure S3. Cyclic voltammetry of polydopamine melanin films (40 nm thick) in 0.01PBS at 0.5 mV/s. Shaded regions correspond to the water window used in the experiment from -0.6 V to 0.8 V versus Ag/AgCl. Within the water window, the current response does not show exponential increase with respect to voltage, and thus our choice of water window is reasonable.

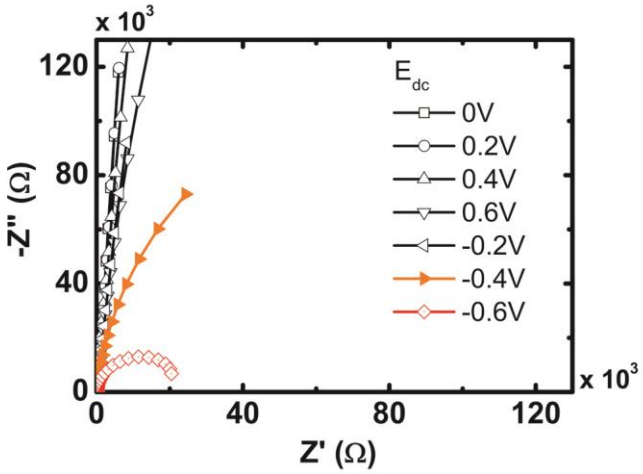

Figure S4. Nyquist plots from EIS measurements on bare ITO at various dc biases from -0.6 V to 0.6 V versus Ag/AgCl. The onset of semicircle is clear at -0.4 V and -0.6 V versus Ag/AgCl thus indicating active H^+ reduction.

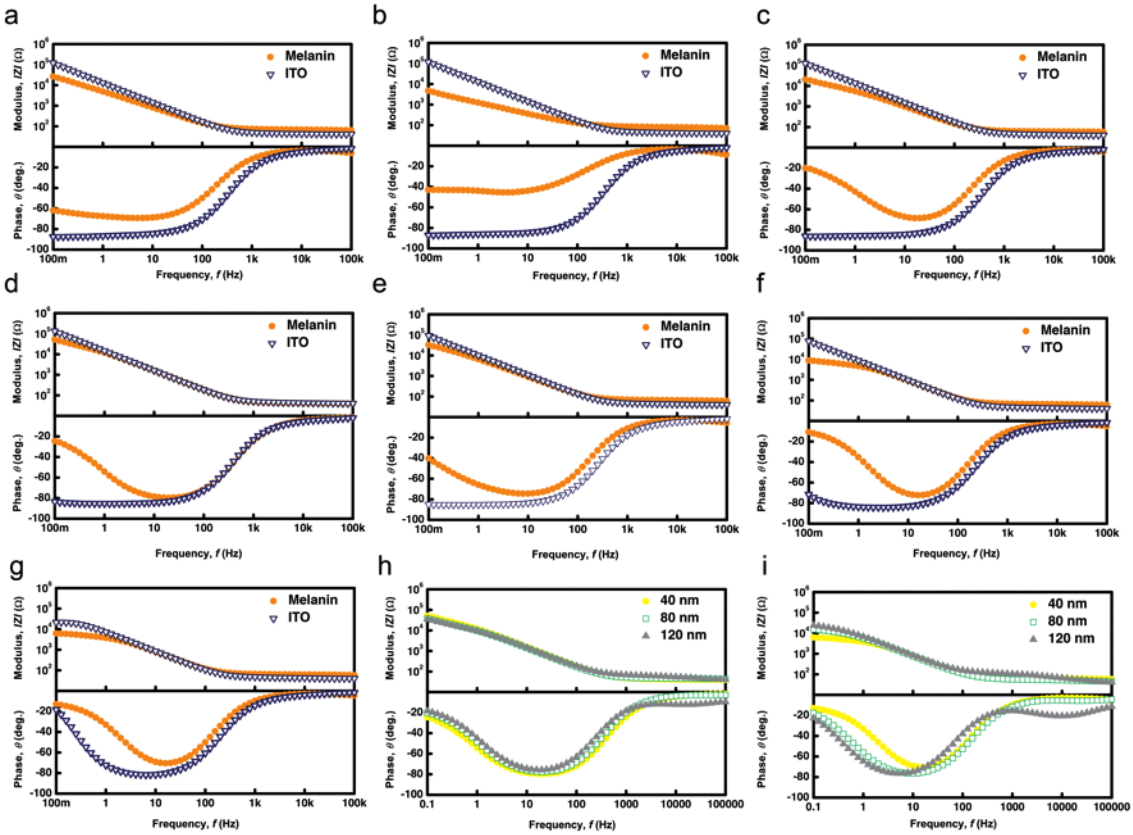

Figure S5. Representative Bode plots extracted from EIS measurements on 40nm thick PDM films deposited on bare ITO (Melanin) and bare ITO (ITO) at the following biases (vs Ag/AgCl): (a) Open Circuit Potential; (b) E_{dc} = 0.2 V; (c) E_{dc} = 0.4 V; (d) E_{dc} = 0.6 V; (e) E_{dc} = -0.2 V; (f) E_{dc} = -0.4 V; (g) E_{dc} = -0.6 V. PDM films exhibit either a comparable or lower interfacial impedance compared to bare ITO substrates throughout the bias range studied. Representative Bode plots extracted from 40 nm, 80 nm and 120 nm thick polydopamine melanin films deposited on ITO substrates at the following biases (vs Ag/AgCl): (h) E_{dc} = 0.6 V; (i) E_{dc} = -0.6 V.

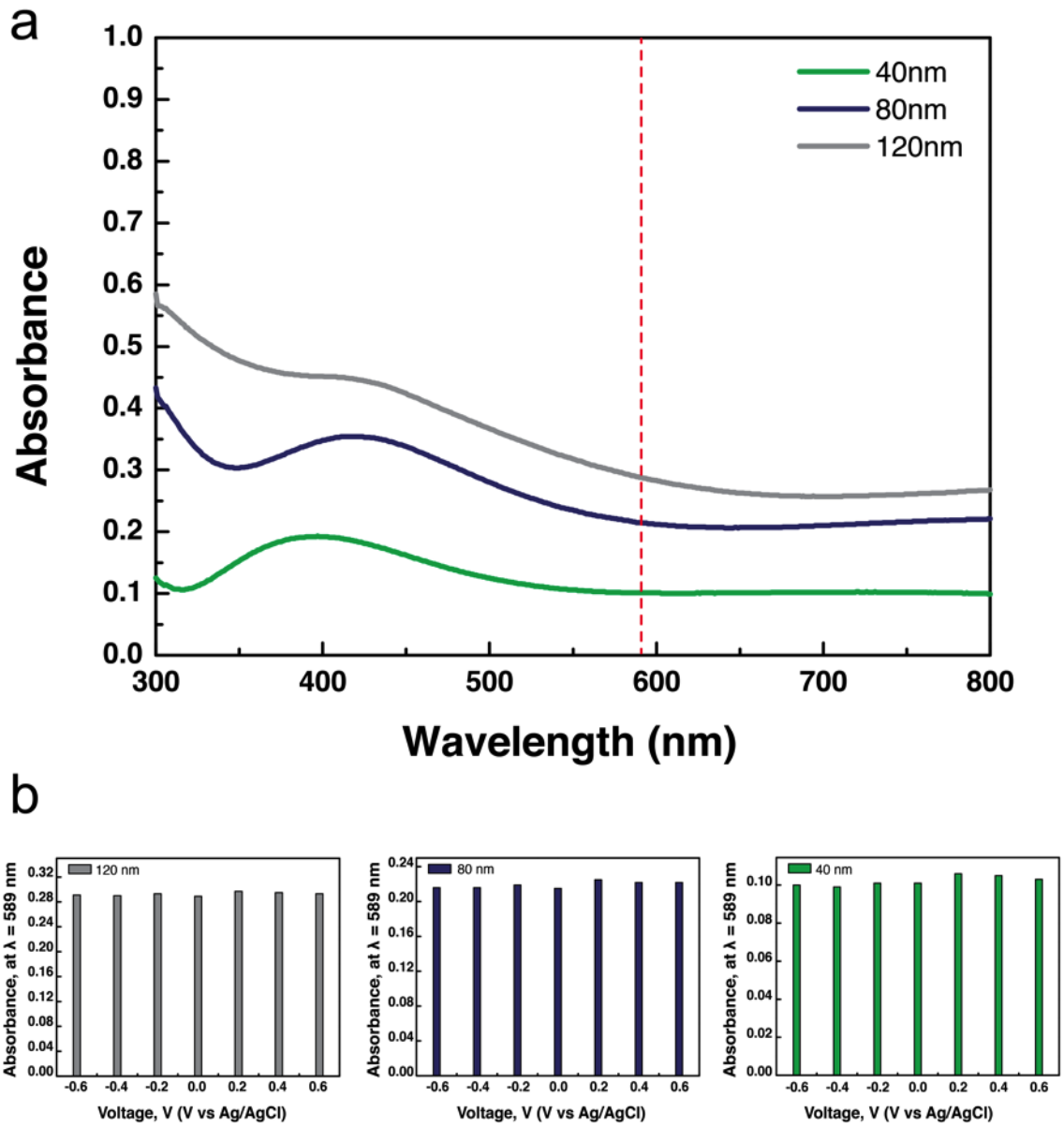

Figure S6. Thickness and voltage-dependent UV-vis absorption spectra for PDM films deposited on ITO substrates. (a) Representative UV-vis absorption spectra of PDM films with 40 nm, 80 nm, and 120 nm thicknesses at 0 V versus Ag/AgCl. While UV-vis absorption spectra of melanin films in general showed broadband absorption throughout the spectrum, broad peaks are shown for 40 nm and 80 nm films near λ_{Abs} = 400 nm and λ_{Abs} = 420 nm. These peaks could be attributed to byproducts that are co-deposited during the synthesis of PDM films through auto-oxidative polymerization of dopamine precursors.[2, 3] PDM films with thicknesses of 120 nm exhibit a subtle absorption peak at these wavelengths. (b) Summary of thickness and voltage-dependent (dc bias) absorbance at λ_{Abs} = 589 nm (red dashed line in the upper graph). The absorbance at λ_{Abs} = 589 nm (as well as other wavelengths) are largely independent of the applied dc bias.

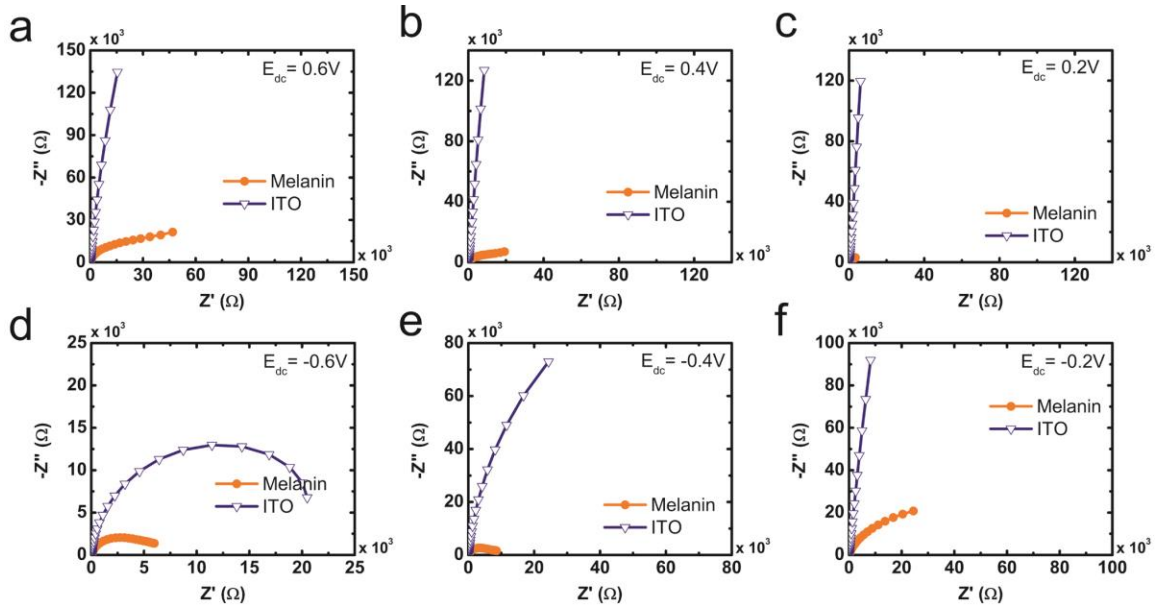

Figure S7. Representative Nyquist plots extracted from EIS measurements on 40nm thick PDM films deposited on indium tin oxide (Melanin) and bare ITO (ITO) at the following biases (vs Ag/AgCl): (a) E_{dc} = 0.6 V; (b) E_{dc} = 0.4 V; (c) E_{dc} = 0.2 V; (d) E_{dc} = -0.6 V; (e) E_{dc} = -0.4 V; (f) E_{dc} = -0.2 V.

Additional References

1. L. Klosterman, J. K. Riley and C. J. Bettinger, *Langmuir*, 2015, **31**, 3451-3458.
2. C. Lambert, J. N. Chacon, M. R. Chedekel, E. J. Land, P. A. Riley, A. Thompson and T. G. Truscott, *Biochim Biophys Acta*, 1989, **993**, 12-20.
3. F. Bernsrnann, O. Ersen, J. C. Voegel, E. Jan, N. A. Kotov and V. Ball, *Chemphyschem*, 2010, **11**, 3299-3305.