Supplementary Materials

Direct Evidence for the Polymeric Nature of Polydopamine

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1. Methods

1.1. Materials

Dopamine hydrochloride (99%, Alfa Aesar), bicine (99%, Alfa Aesar), sodium hydroxide (NaOH, pellets, Fisher Scientific), α-Cyano-4-hydroxycinnamic acid (CHCA) (Sigma Aldrich) isopropyl alcohol (99.5%, VWR Analytical), DMSO (VWR Analytical), acetone (Macron Fine Chemicals), and Simple Green (Sunshine Makers, Inc) were used as received. Mica discs were purchased from Ted Pella, Inc (Redding, CA). 1cm x 1cm 316 stainless steel (SS) samples were cut by university machine shop (Berkeley, CA), silicon wafers (SiO_2) and silicon wafers with a layer of 100 nm titanium oxide (TiO_2) were obtained from University Wafer, Inc (Boston, MA). Ultrapure (UP) water was obtained by purification of deionized water with a Barnstead Ultrapure Water Purification System (Thermo Fisher Scientific, Waltham, MA) to a resistivity of 18.2 MΩ cm.

1.2. Coating substrates with pDA

SiO_2, TiO_2, and SS substrates were first cleaned with a 3:1 mixture of water and Simple Green solution for degreasing by placing into sonication bath for 15 minutes. The substrates were then rinsed and sonicated with water followed by isopropanol and acetone, each for 15 minutes, and dried under a stream of nitrogen. Prior immersing in dopamine.HCl solution, substrates were exposed to a plasma discharge at 100 W for 5 minutes (Harrick Plasma Cleaner, Ithaca, NY, USA). Mica substrates were prepared by physically cleaving the top layer using scotch tape. AFM cantilevers were cleaned by UV-Ozone treatment (PSD Pro Series, Novascan Technologies, USA) for 6 minutes.[4] The substrates were immersed into 2 mg/ml DA.HCl in 100 mM Bicine buffer at pH 8.5 for 16 hours with constant shaking. The substrates were taken out and rinsed intensively with Milli-Q water and dried under a stream of nitrogen.

1.3. pDA NPs Synthesis

DA.HCl (2 mg/ml) was dissolved in Milli-Q water and pH was increased to 8.5 by adding NaOH. The solution was stirred at 400 rpm for 5 hours at 70 ˚C and subsequently left at ambient temperature for 3 hours without stirring. Finally, the mixture was filtered with

PVDF membrane filters with a pore size of 0.1 μm (Durapore VVPP) followed by drying at 50 ˚C under vacuum.

1.4. Scanning Electron Microscopy

Scanning electron microscopy (SEM) images were obtained using a FEI Strata 235 dual beam Focused Ion Beam (FIB). The images were taken at beam voltages of 5 and 10 kV at a working distance of ~10 mm relative to the sample surface. All samples were sputter-coated with ~20 A of Au-Pd before imaging to inhibit charging.

1.5. Single Molecule Force Spectroscopy (SMFS)

Measurements were carried out using a JPK ForceRobot 300 (JPK Instruments AG, Germany) with a tip velocity of 1000 nm.s^{-1} over a z-piezo distance of 500 nm with a dwell time of 1 second. The experiments were performed in Milli-Q water after allowing the cantilever to equilibrate in solution for at least 30 minutes. Soft silicon nitride cantilevers (MLCT from Bruker Nano Inc.) of typical spring constant of 50-60 pN.nm^{-1} were used for all experiments and calibrated using the equipartition theorem.[7] All the experiments have been repeated at least three times using different pDA-coated cantilevers and/or substrates. The force-extension traces were recorded and analyzed using data processing software from JPK and home-written procedure in IgorPro (Wavemetrics). The spikes in the force-distance curves were fitted with the Worm-like Chain (WLC) model to measure the corresponding rupture force and persistence length.

1.6. X-Ray Photoelectron Spectroscopy (XPS)

The surface chemical composition of pDA coatings on TiO_2 substrates and AFM cantilevers was measured using X-Ray photoelectron spectroscopy (XPS). XPS analyses were performed on a Perkin Elmer Phi 5600, equipped with a monochromatic Al Ka X-ray source operating at 350 W. The neutralizer was used with an emission of 1 μA to inhibit charging of the samples. All the spectra were calibrated to a C1s peak at 284.8 eV. For each sample measured, multiple survey scans were taken, on different spots, with a pass energy of 187.85 eV.

1.7. Matrix Assisted Laser Desorption/Ionization (MALDI)

MALDI spectra were recorded on an ABI Voyager DE-Pro MALDI ToF (Applied Biosystems) instrument operating in the positive reflector mode. The mass spectrometer parameters were set as recommended by the manufacturer and adjusted for optimal acquisition performance. The mass spectra data were acquired from averaging of 300 laser shots over a m/z range of 100-20000 Da. The solution of matrix CHCA (10 mg/mL) was prepared in ethanol. Sample was prepared by dissolving solid pDA powder in DMSO. Matrix solution was dropped onto the target plate and allowed to dry. A droplet of sample was pipetted on the plate and air-dried. Before analysis, matrix solution was added to the target plate and allowed to air dry.

2. Structural Models for pDA

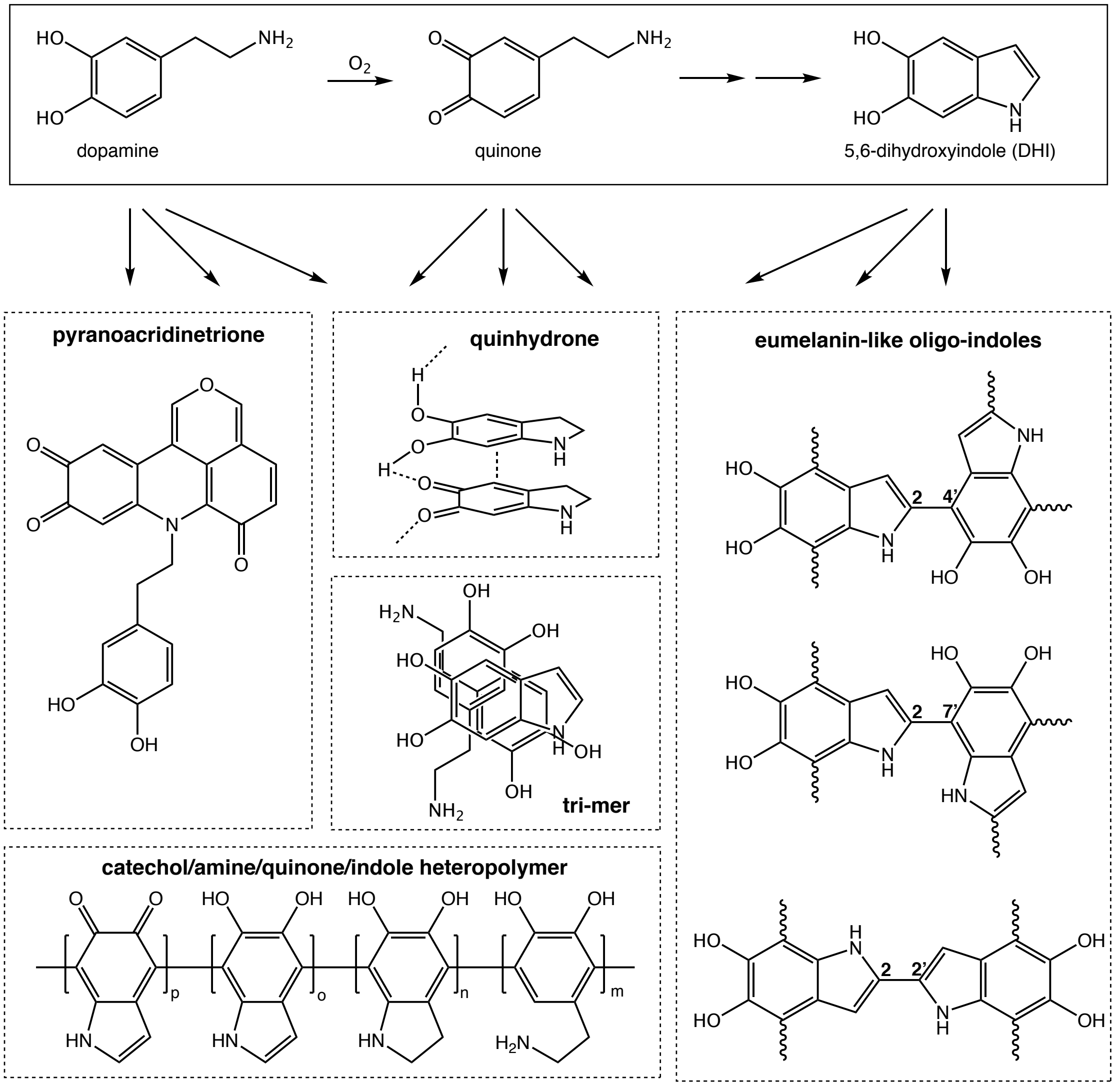

Figure S1. Current theories of polydopamine structure and formation. Auto-oxidation of dopamine leads to the formation of dopamine-quinone and 5,6-dihydroxyindole. Proposed mechanisms for polydopamine formation range from noncovalent self-assembly of subunits to form quinhydrone or trimer assemblies, and covalent coupling of subunits to yield a catecholamine/quinone/indole heteropolymer or eumelanin-like oligo-indoles. Adapted with permission from refs 1-3.[1-3] Copyright 2013 American Chemical Society, 2014 American Chemical Society, and 2012 WileyVCH.

3. Matrix Assisted Laser Desorption/Ionization (MALDI) on pDA Particles

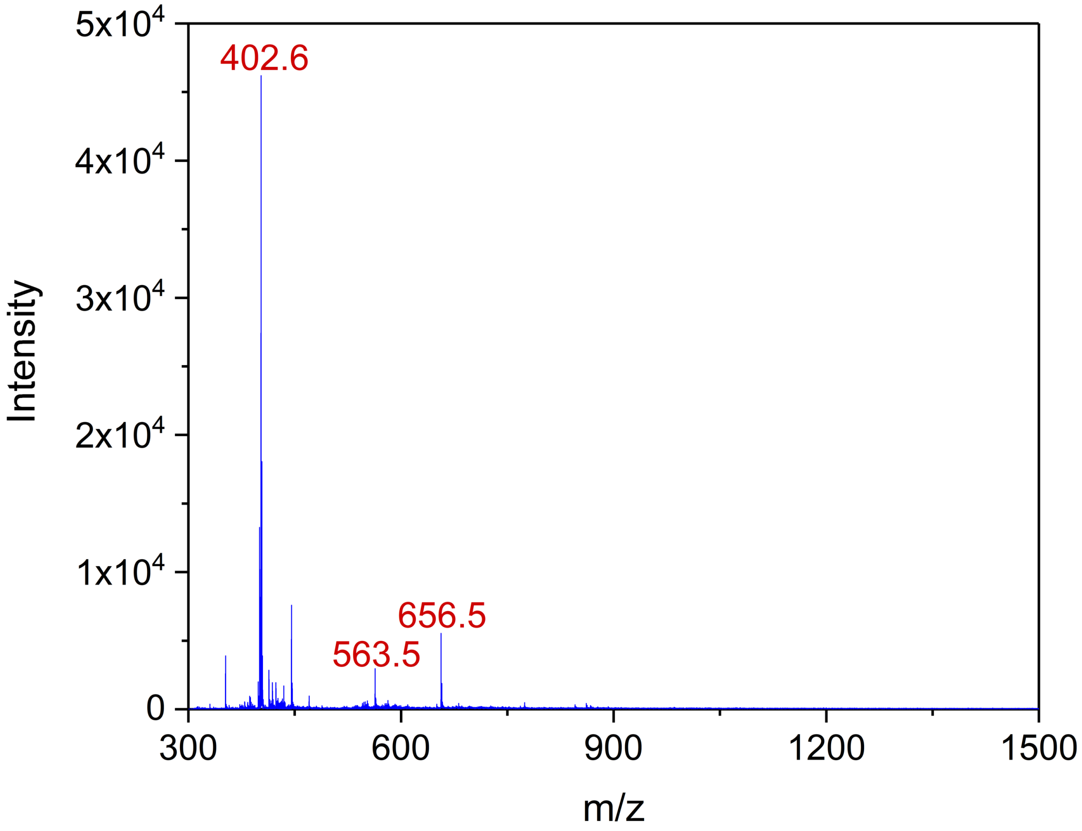

Figure S2. MALDI mass spectrum of pDA particles. Primary building block of pDA at m/z 402.6 was observed as previously reported in literature.[5,6] No major fragment was collected at m/z values of 700 or above.

4. Characterization of pDA on AFM Cantilever

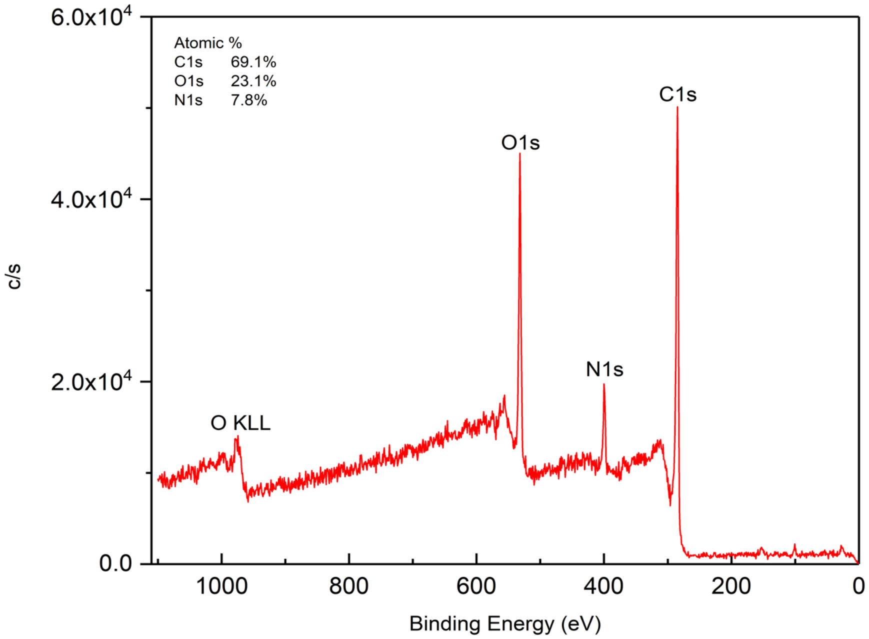

Figure S3. XPS spectrum of the pDA coated cantilever.

4. Single Molecule Force Spectroscopy (SMFS)

4.1. pDA-Coated Substrates

Force spectroscopy experiments were performed on the pDA-coated substrates to confirm that the features observed in the F-D curves are independent of the underlying substrate. In these experiments a bare AFM cantilever was approached against pDA-coated substrates and F-D traces were recorded. As shown in the Figure S3, we observed plateaus of constant force for pDA-coated mica, SS, TiO_2, and SiO_2.

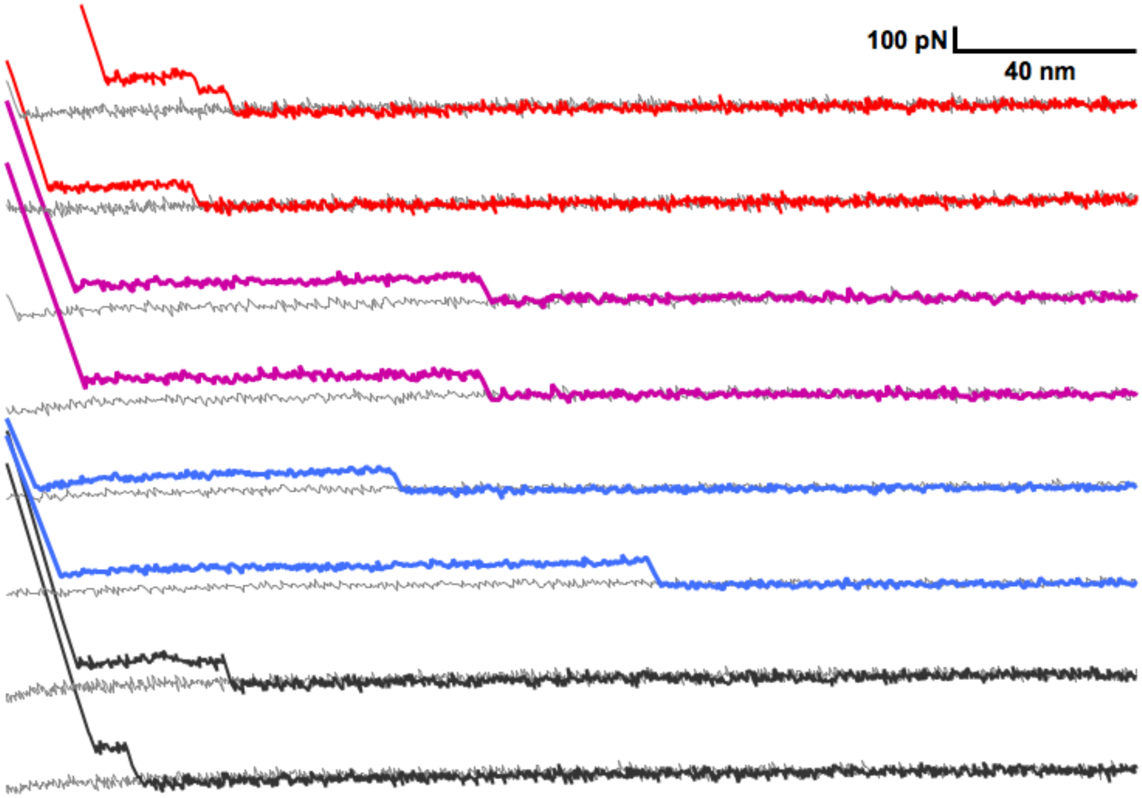

Figure S4. F-D traces for approach of a bare AFM cantilever onto pDA-coated substrates; mica (red), SS (purple), TiO_2 (blue), and SiO_2 (black).

4.2. Fitting of the Stretching Events with Polymer Chain Elasticity Model

Stretching events have been fitted with a Worm-like Chain (WLC) model, yielding a persistence length of about 0.5 nm consistent with the values reported for stretching a single polymer molecule in water. No previous studies have been done to measure persistence length for pDA chains; however, previous reports have shown values of 0.38 nm or less for PEG,[8] 0.38 nm for amyloid fibers,[9] 0.4-0.6 for ssDNA and RNA,[10-12] 0.37 nm for PS,[13] and 0.4 for polyproteins and polypeptides.[8,14]

$$F = \frac{kT}{p} * \left(\frac{1}{4\left(1 - \frac{x}{L_c}\right)^2} - \frac{1}{4} + \frac{x}{L_c} \right)$$

Where F is the applied force, p is the persistence length, k is the Boltzmann constant, L_c is the contour length, and T is the absolute temperature.[6]

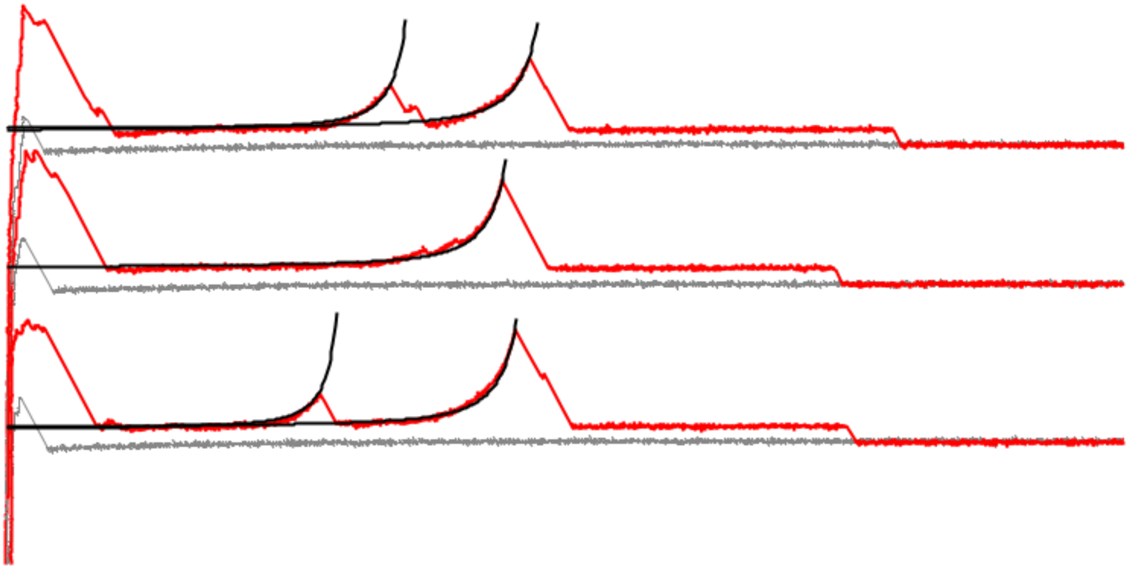

Figure S5. WLC fitting of the stretching events.

4.3. Cohesive and Intramolecular Interactions between the pDA Molecules

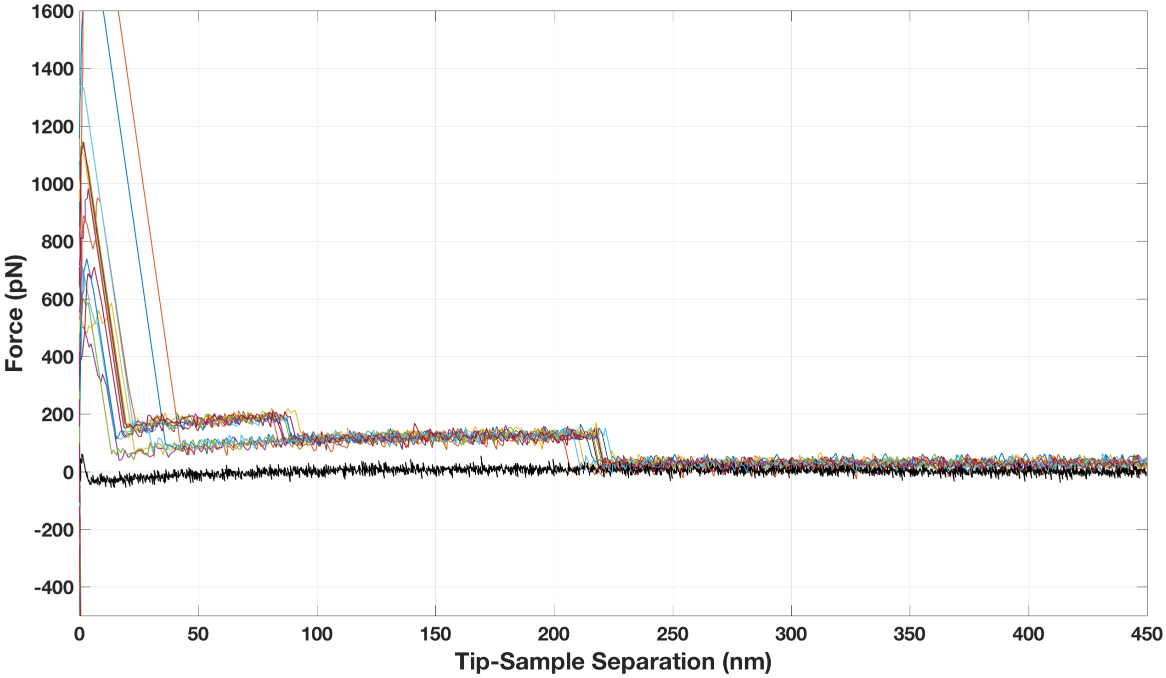

Figure S6. The superposition of the successive F-D curves obtained upon approach of a pDA coated AFM cantilever onto a pDA coated TiO_2 substrate with a piezo speed of 100 nm/s and a dwell time of 5 seconds. The presence of superimposable features indicates that the unzipped subunits can rapidly rebind to the surface prior to the subsequent mechanical cycle once given sufficient time.

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