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Supplementary Information for:

Dithiol-based Modification of Poly(dopamine): Enabling Protein Resistance via Short-Chain Ethylene Oxide Oligomers

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Experimental Section:

Materials. Dopamine hydrochloride, Tris-hydrochloride, fibrinogen, phosphate buffered saline (PBS), fetal bovine serum (FBS), and octadecanethiol (ODT) were purchased from Sigma-Aldrich (St. Louis, MO). Silicon wafers were bought from Silicon Quest International (Santa Clara, CA). The synthesis of MTOEG and DTOEG was described earlier.[1,2]

^{1}H NMR for MTOEG: (270 MHz, $CDCl_3$) δ 3.70-3.5 (m, 24 H, m, ($C\mathbf{H}_2C\mathbf{H}_2O)_6OH$), 2.63 (q, J = 6 Hz, $HSC\mathbf{H}_2CH_2$), 1.88 (pentet, 2H, $CH_2C\mathbf{H}_2CH_2$), 1.84 (t, J = 7 Hz $\mathbf{H}SCH_2CH_2$).

^{1}H NMR for DTOEG: δ 3.4 – 3.7 24H, m, $[HS(CH_2)_3]_2CHO(C\mathbf{H}_2C\mathbf{H}_2O)_6H$; 3.31, 1H, pentet, J = 6 Hz, $[HS(CH_2)_3]_2C\mathbf{H}O(EO)_6H$; 2.54, 4H, dt, J = 7.2 Hz and 5.8 Hz, $[HSC\mathbf{H}_2(CH_2)_2]_2CHO(EO)_6H$; 1.5 – 1.80, 8H, m, $[HSCH_2(C\mathbf{H}_2)_2]_2CHO(EO)_6H$; 1.35, 2H, t, J = 5.8 Hz, $[\mathbf{H}S(CH_2)_3]_2CHO(EO)_6H$. MS (MALDI-ToF, no matrix) 466.99 $[M + Na]^+$, 483.11 $[M + K]^+$.

Poly(dopamine) Thin Film Formation. The coating of poly(dopamine) (PDA) thin film on Si was performed by immersing the substrates in a 10 mM Tris-HCl buffer (pH 8.5) solution containing 2mg/ml dopamine for 4 h. Samples were kept horizontally in open glass dishes under constant shaking to avoid deposition of large particles. Samples were removed from the coating solution and thoroughly rinsed with water, followed by drying in a stream of nitrogen.

Thiol Functionalization. Chemical modification of PDA-derivatized substrates was carried out by immersion in an aqueous solution of 1 mM DTOEG or MTOEG thiols for 12 h. For ODT functionalization, PDA substrates were immersed in a 1 mM ODT solution in ethanol for 12 h. Thiol monolayers were prepared on Au substrates [100 nm thick Au layer with 5 nm Ti adhesion layer on Si, (Platypus Technologies, Madison, WI)] under similar conditions as on PDA substrates.

After functionalization samples were thoroughly rinsed with deionized water followed by drying under a stream of nitrogen and used for X-ray reflectivity (XRR), *ex situ* spectroscopic ellipsometry (SE), X-ray photoelectron spectroscopy (XPS), and contact angle measurements. Thiol-functionalized PDA surfaces were extensively rinsed in the flow cell for *in situ* spectroscopic ellipsometry measurements including protein binding studies. Our initial week-long studies on stability of thiols on PDA or Au substrates indicated that DTOEG thiols were still intact for this stipulated timeframe.

X-Ray Reflectivity Measurements. XRR measurements were carried out using an X-ray reflectometer (Bruker, D8 Advance) employing Cu K_α radiation at NCNR/NIST (Gaithersburg, MD). The Cu source was operated at 40 kV and 40 mA, and the wavelength was 0.154 nm. The beam width was 10 mm and the beam height was 0.1 mm. XRR data was analyzed using the REFLPACK software package.[3] Thickness, roughness, and density of PDA and DTOEG-PDA were evaluated by getting the best-fitted parameters of reflectivity profiles for these films. The theoretical scattering length density (SLD) of PDA was calculated using the NIST SLD calculator by entering the previously published molecular formula of PDA monomeric unit; $C_8H_4NO_2$ and density 1.3 g/cm^3.[4] The XRR measurements were performed on two samples, and the reported error is the standard deviation of the mean.

Spectroscopic Ellipsometric Measurements. Multiple wavelength *ex situ* and *in situ* ellipsometric measurements were obtained on a J. A. Woollam Co., Inc. (Lincoln, NE) M2000 spectroscopic ellipsometer, as discussed earlier.[5] All measurements were performed at an incidence angle of 65º. Due to the light-absorbing property of the PDA films,[6] optical models of the system used a general oscillator in considering PDA's dielectric function and film thickness. Incorporation of an effective medium approximation (EMA) to account for the intrinsic roughness

of the PDA film had little or no effect on calculated film thicknesses. Thiol/dithiol thickness on PDA was determined by introducing a Cauchy function on the layer model with an assumed refractive index of 1.45 and average density of ~1.1 g/cm^3. Thiols interfacial mass density was calculated by multiplying the thickness with average density. The ellipsometry measurements were performed on five samples at four different spots on each sample, and the reported error bars on SE thicknesses are the standard deviation of the mean from these twenty measurements.

The adsorption of protein to the PDA or thiol-modified PDA substrates was measured using *in situ* SE in real-time in a custom built cell at room temperature.[5] After the system equilibrated, showing a stable base line, the cell was filled with either fibrinogen (1 mg/ml in PBS) or the complex biofluid FBS solution and maintained for 1 h. Protein adsorption was recorded after copious rinsing of the substrates with PBS. The adsorbed protein mass was calculated using following eq,

$$\Gamma = \frac{(n_A - n_C)d_A}{dn/dc}$$

where, Γ is adsorbed protein mass in ng/cm^2, n_A is the refractive index of protein (1.5), n_C is the refractive index of buffer (1.344), d_A is the ellipsometric thickness of adsorbed protein in nm, and dn/dc = 0.187 cm^3/g is the refractive index increment with respect to protein concentration.[7,8] The recorded mass of adsorbed protein is the average of at least five samples for all conditions, and the standard deviations calculated as ~5% of the average.

X-Ray Photoelectron Spectroscopy Analysis. The XPS analysis was obtained on a Kratos Axis Ultra DLD XPS system (Kratos Analytical, Chestnut Ridge, NY) with a monochromated Al Kα x-ray source (10 mA, 15 kV). High-resolution spectra of the C 1s, N 1s, Si 2p, O 1s, S 2p, and Au 4f regions were acquired at pass energy of 40 eV, step size 0.1 eV at three spots (spot size of 300

μm × 700 μm) for each condition, and average intensities were used for calculations. In addition, low resolution survey spectra were acquired for all spots (pass energy 160 eV, step size 0.5 eV.). Casa XPS software (v. 2.3.16 pre-rel 1.4) was used to analyze the XPS spectra. Peak energies have been calibrated to Au $4f_{7/2}$ at 84.0 eV for the ODT on Au and to the C 1s C-C peak at 284.5 eV for the PDA-based films. Thiols were quantified on PDA substrates by calculating the area under the S 2p region of functionalized samples.

Contact Angle Measurements. The static water contact angles (CA) were determined with a FTA 100 goniometer (First Ten Angstroms, Portsmouth, VA) at ambient temperature. The CA was measured immediately after the 2-3 μL drop detached from the needle tip onto the substrates. The CA values are average of at least five measurements.

Atomic Force Microscopy. All AFM images were obtained on a Dimension 3000 AFM instrument (Veeco Instruments, Santa Barabara, CA) in tapping mode. All images were acquired at a scan size of 1 μm and a scan rate of 0.3 Hz. Images were analyzed by WSxM software (Nanotec Electronica, Madrid, Spain) to determine the root-mean-squared roughness value over the entire scan area.

Thin Polydopamine Film Characterization:

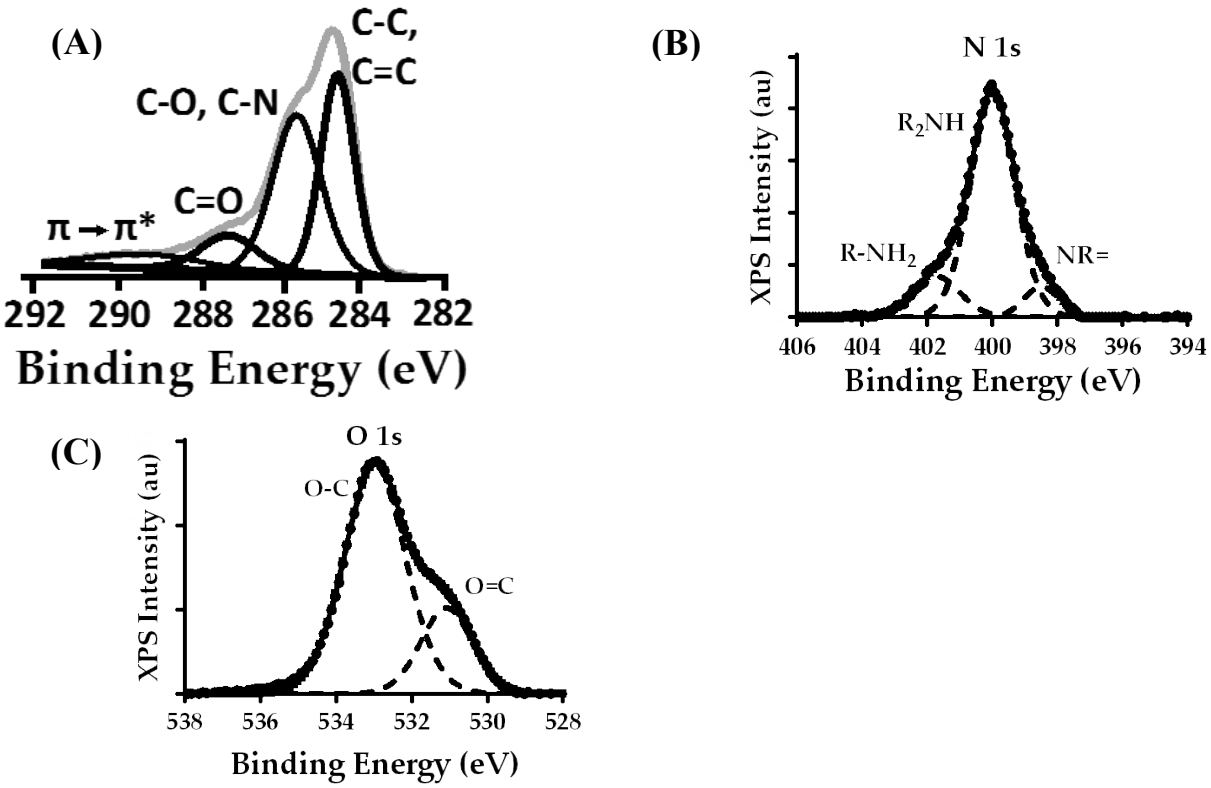

Figure S1. X-ray photoelectron spectroscopy of poly(dopamine)-modified Si substrate. (A) C 1s, **(B)** N 1s and **(C)** O 1s XPS spectra, spectra were fitted with Gaussian-Lorentzian line shapes (broken lines) to evaluate the binding energy contributions of different chemical groups

The small substrate Si peak (< 2%) in the XPS spectra indicates that the PDA films are likely continuous with thickness on the order of the XPS sampling depth (~10 nm).[9,10]

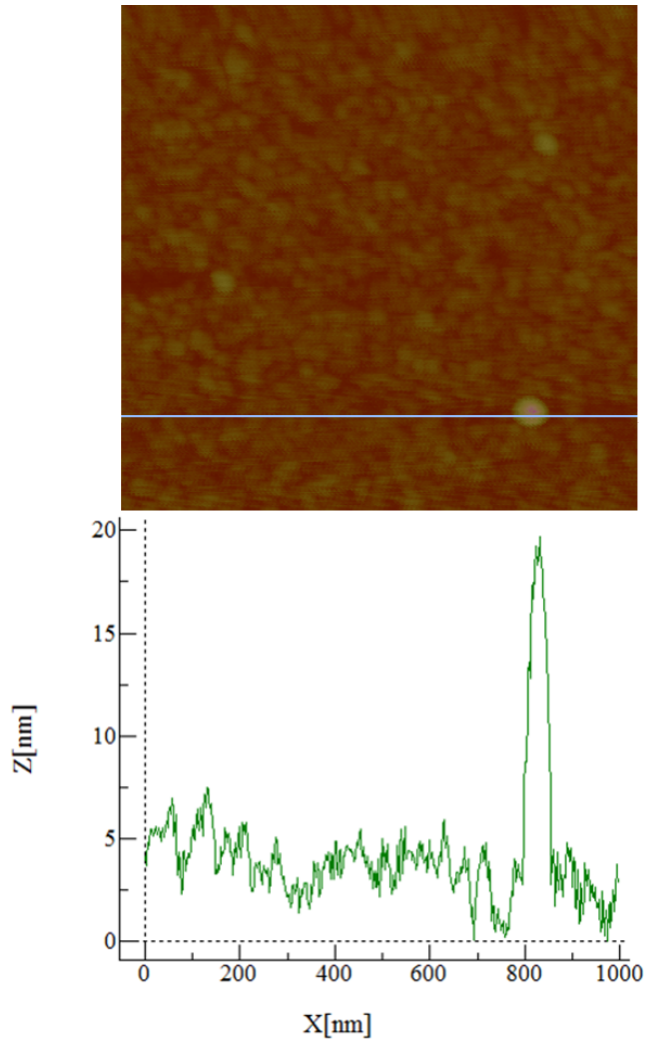

Figure S2. A representative atomic force microscope (AFM) topographical image of a PDA surface with line scan.

We also characterized the thin PDA films using SE to complement the XRR measurements. Our SE (*ex situ*) data denote PDA film thickness of (14.2 ± 2.1) nm, in good agreement with the XRR measurements. Atomic force microscopy (AFM) scans illustrate a granular PDA film with randomly distributed PDA aggregates. Additionally, AFM measurements confirm the roughness of the film indicated by XRR, and reveal an interfacial root-mean-squared roughness of (4.6 ± 2.5) nm. Previous studies also reported similar topography of PDA films[6,11,12] suggesting that this level of roughness is an intrinsic characteristic of these films.

Dithiols Oligomerization on Poly(dopamine) Surfaces:

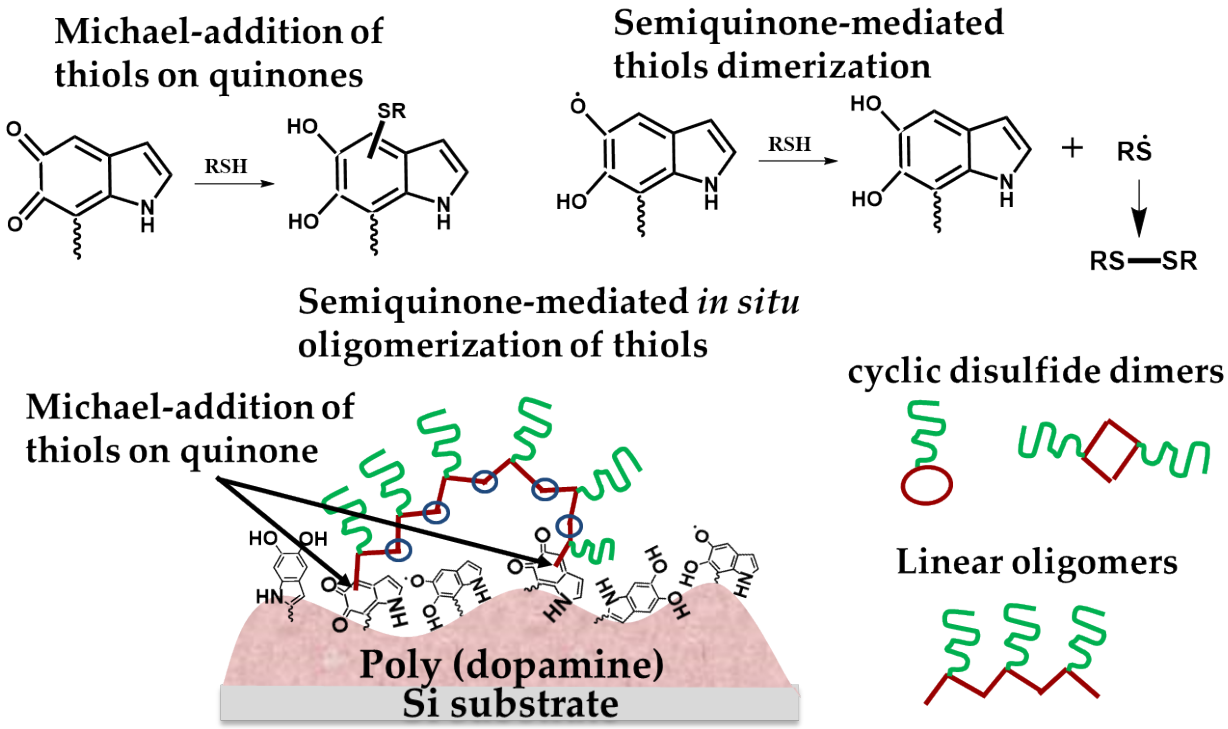

Figure S3. Schematic illustration of the proposed multilayer structure formation on a poly(dopamine) substrate. Dithiol, DTOEG, can undergo multiple reaction products by PDA interactions, ranging from Michael-addition to quinones-in-PDA to semiquinone-mediated cyclic monomer, cyclic dimer, and linear oligomeric species formation at the PDA/solution interface. Subsequently, DTOEG thiyl radicals or oligomeric species can attach to the free thiol moiety of a quinone-tethered DTOEG molecule to form a multilayer structure.

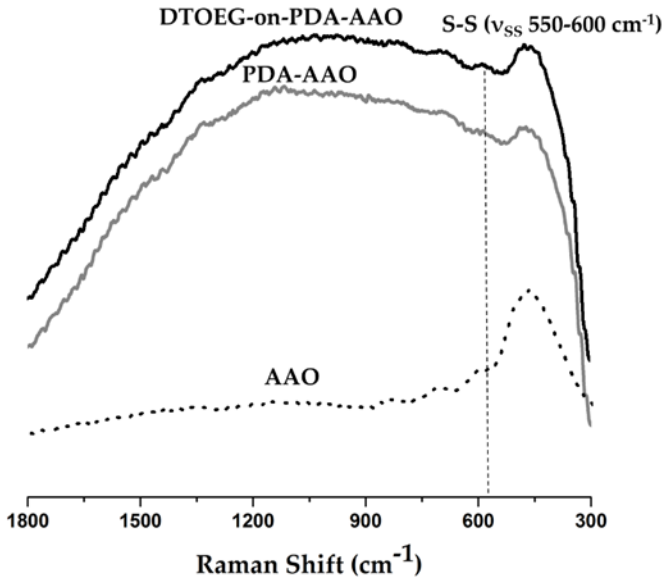

Figure S4. Raman spectra of (A) anodic alumina oxide (AAO) membrane, (B) poly(dopamine) (PDA)-functionalized AAO, and (C) modification of PDA-on-AAO by DTOEG molecules.

As illustrated in Figure S4, PDA-functionalized anodic aluminum oxide (AAO) membrane exhibits a broad peak, which is in agreement with previous reports on PDA.[13] The alumina substrate has a very weak Raman signal, except a peak (470 cm^{-1}) due to the phosphate impurities.[14] Subsequently, after the introduction of DTOEG molecules onto PDA-modified AAO substrates, a peak emerged at 550-600 cm^{-1}, which we attribute to the formation of S-S bond between DTOEG molecules as shown in the Figure S3. Previous reports also suggested similar Raman spectral peak for alkyl disulfides.[15]

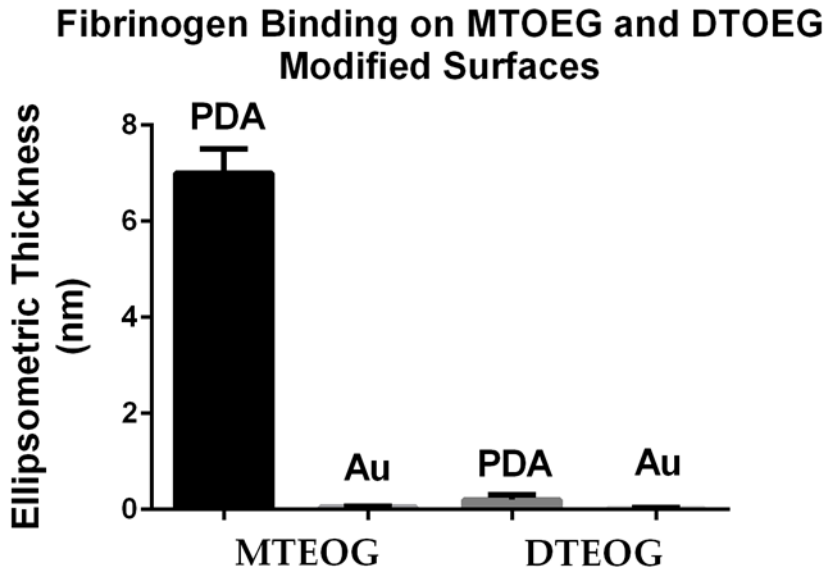

Figure S5. Ellipsometric measurements of fibrinogen binding on MTOEG and DTOEG modified Au and PDA substrates. DTEOG modified Au or PDA surfaces showed resistance to fibrinogen adsorption, contrary only MTOEG modified Au substrates exhibited resistance to fibrinogen binding.

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