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Supporting Information

Tailoring Substrate Hydrophilicity Using Grafted Polypeptide Nanocoatings*

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EXPERIMENTAL

Materials

H-Val-OH and H-Lys(Z)-OH were obtained from Bachem and used as received. Hyperbranched poly(ethylene imine) (PEI) (M_W ~25 kDa, 50 wt% in H_2O), triphosgene (98 %), hydrofluoric acid (48 wt% in H_2O), *n*-pentane (anhydrous, > 99 %), hydrobromic acid (33 % in acetic acid) were purchased from Aldrich and used as received. Ammonium fluoride (Fluka, 40 % in H_2O), anhydrous *N*,*N*-dimethylformamide (DMF) (Acros Organics, extra dry, > 99.8 %),

tetrahydrofuran (THF) (Honeywell, 99.9 %, HPLC grade) and sulfuric acid (H_2SO_4) (Scharlau, 99 %) were used as received. *n*-Hexane (99 %), sodium chloride (NaCl) (99 %), hydrogen peroxide (H_2O_2) (30 %), isopropanol (IPA) (99 %), *N*,*N*-dimethylformamide (DMF) (98 %) and methanol (98 %) were purchased from Chem-Supply and used as received. Anhydrous and deoxygenated THF was obtained by distillation under argon from sodium benzophenone ketyl. Deuterated dimethylsulfoxide (d_6-DMSO) was purchased from Cambridge Isotope Laboratories and was used as received. High-purity water (Milli-Q) with a resistivity greater than 18 MΩ.cm was obtained from an in-line Millipore RiOs/Origin water purification system.

Glass coverslips (No. 1, 12 mm diameter) were purchased from ProSciTech (Kirwan, Australia) and cleaned with Piranha solution (sulfuric acid: hydrogen peroxide = 7:3) – *Caution! Piranha solution is highly corrosive and extreme care should be taken during preparation and use*. The coverslips were then sonicated in isopropanol:water (1:1) solution for 20 min followed by soaking in RCA solution (water: ammonia: hydrogen peroxide = 5:1:1) for another 20 min at 60 °C. The wafers were thoroughly washed with Milli-Q after each step. Whatman 542 Filter papers (cellulose substrate, Sigma Aldrich) were cut into *ca.* 1 × 1 cm squares and used as received.

Characterization Methods

Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectroscopy was conducted using a Varian Unity 400 MHz spectrometer operating at 400 MHz and 100 MHz, respectively Deuterated dimethyl sulfoxide (d_6-DMSO) was used as the solvent and samples were prepared at a concentration of *ca.* 20 mg mL^{-1}.

Atomic force microscopy (AFM) images were acquired with a MFP-3D Asylum Research instrument. Scans were conducted in AC mode with ultrasharp SiN gold-coated cantilevers purchased from MikroMasch (Bulgaria). Image processing and surface roughness analysis were

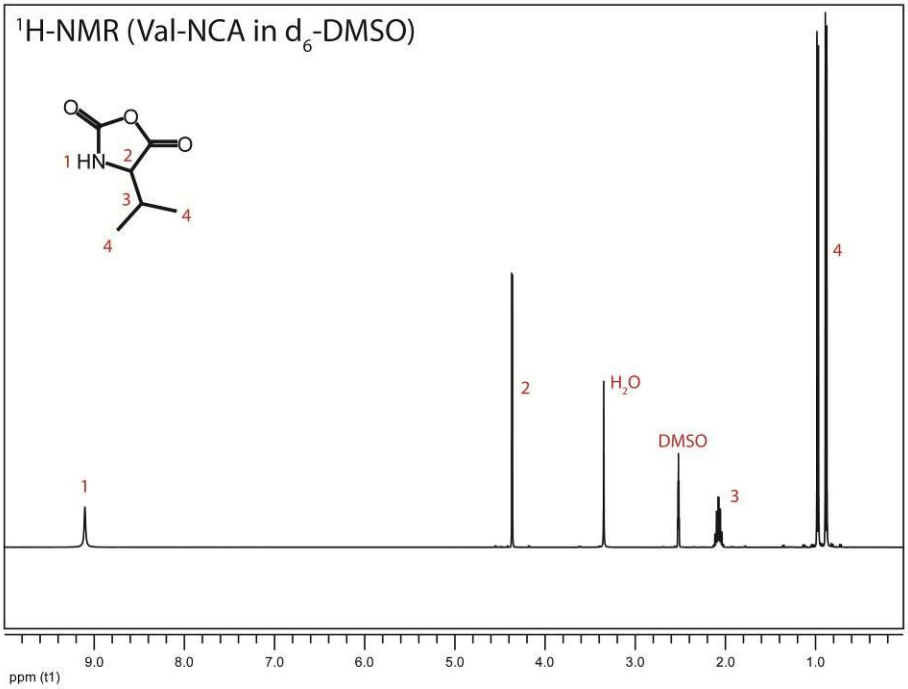
performed using the Nanoscope and IgorPro software packages. The film thicknesses were obtained by film scratching (mechanical removal) and by tracing a profile along the film and the scratched zone. The thicknesses reported represent the averaged values over analysis of 3 substrates with 3 different analysis sites per substrate.

Contact angle measurements were conducted with a Data Physics OCA-20 Tensiometer. Measurements were recorded with OCA software using sessile drop profiles. Contact angle measurements were conducted at 3 different sites per substrate and the averaged values reported represent the mean values of analysis over 3 substrates prepared independently.

Surface morphologies of unmodified and coated porous substrates (cotton and cellulose) were imaged by scanning electron microscope (SEM) using a FEI Quanta 200 ESEM FEG. Prior to imaging, samples were coated with gold using a Dynavac Mini sputter coater.

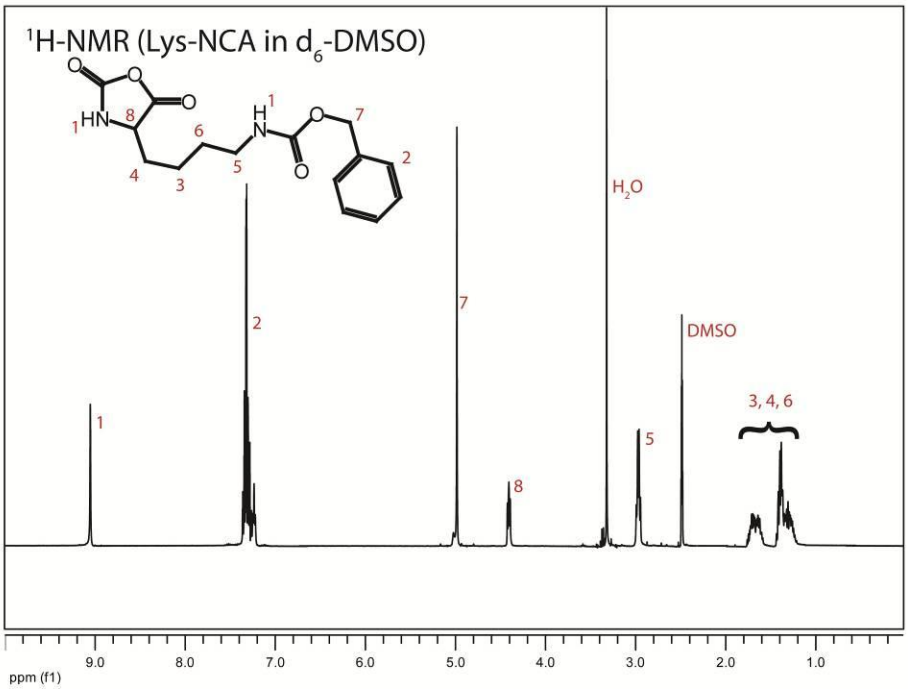
Synthesis of L-Valine NCA (Val-NCA)

H-Val-OH (1.0 g, 8.50 mmol) was suspended in anhydrous THF (20 mL) under argon before the addition of triphosgene (1.0 g, 3.40 mmol). The reaction was stirred at 60 °C until a homogenous solution was obtained (*ca.* 1 h). The reaction mixture was bubbled with argon to remove unreacted phosgene and HCl, and then concentrated *in vacuo*. The resulting residue was precipitated into anhydrous pentane (50 mL), and then the precipitate was isolated via centrifugation, washed with anhydrous pentane (3 × 50 mL) and dried *in vacuo* to afford off-white powder (0.85 g, 70%). ^{1}H NMR (400 MHz, d_6-DMSO): δ_H 0.82–0.84 (*d*, 3H, *J* = 7.0 Hz 1 C**H**$_3$), 0.91–0.93 (*d*, 3H, *J* = 7.2 Hz, 1 C**H**$_3$), 1.97–2.08 (*m*, 1H, 1 C**H**), 4.32 (*dd*, 1H, *J* = 1.2 Hz and 4.0 Hz , C**H**N), 9.06 (*br s*, 1H, 1 N**H**) ppm. ^{13}C NMR (100 MHz, d_6-DMSO): δ_C 17.0 (**C**H$_3$), 30.5 (**C**H), 56.9 (**C**HN), 63.0 (**C**H$_2$N), 152.5 (NH**C**O$_2$), 170.0 (**C**H**C**O$_2$) ppm.


1H-NMR (Val-NCA in d6-DMSO)
1 HN
2
3
4
4
1
2
H2O
DMSO
3
4
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

Synthesis of *ε*-Carboxybenzyl-L-Lysine NCA (Lys-NCA)

Triphosgene (0.40 g, 1.40 mmol) was added to a suspension of H-Lys(CBz)-OH (1.00 g, 3.56 mmol) in anhydrous THF (30 mL) under argon. After continuous stirring at 60 °C (*ca.* 1 h), the homogenous reaction mixture was cooled to room temperature, bubbled with argon to remove unreacted phosgene and HCl, and concentrated *in vacuo*. The resulting residue was then recrystallised from 2:3 anhydrous THF:hexane (100 mL) 3 times, washed with anhydrous n-pentane (20 mL) and dried *in vacuo* to afford Lys-NCA as colourless crystals (0.90 g, 82 %). ^{1}H NMR (400 MHz, d_6-DMSO): δ_H 1.22–1.45 (*m*, 4H, 2 C**H**$_2$), 1.60–1.77 (*m*, 2H, 1 C**H**$_2$), 3.01 (*q*, 2H, *J* = 6.0 Hz, C**H**$_2$N), 4.42 (*t*, 1H, *J* = 6.0 Hz, C**H**N), 5.00 (*s*, 2H, C**H**$_2$O), 7.25–7.38 (*m*, 5H, 5 Ar**H**), 9.11 (*br s*, 2H, 2 N**H**) ppm. ^{13}C NMR (100 MHz, d_6-DMSO): δ_C 21.5 (**C**H$_2$), 28.7 (**C**H$_2$), 30.6 (**C**H$_2$), 56.9 (**C**HN), 65.0 (**C**H$_2$N), 127.6 (3 Ar**C**H), 128.2 (2 Ar**C**H), 137.1 (Ar**CC**), 151.8 (NH**C**O$_2$), 156.0 (NH**C**O$_2$), 171.5 (**C**H**C**O$_2$) ppm.



Surface-initiated polymerization of amino acid NCA derivatives

Substrates were immersed in PEI solution (1 mL per substrate, 1 mg mL^{-1} in 0.5 M NaCl solution) for 30 min and washed with deionized water (3 × 10mL) to remove unbounded PEI. The amine-functionalized substrates were then washed with anhydrous THF (3 × 10 mL) and anhydrous DMF (3 × 10 mL) before being placed in a clean glass vial. The PEI-functionalized substrates were then immersed in a freshly prepared NCA solution (0.75 M, 1 mL per substrate) and allowed to react under reduced pressure. After a predetermined reaction period, the coated substrates were washed with DMF (3 × 5 mL) and then soaked in DMF (1 mL per substrate) for 15 h. The peptide-coated substrates were sonicated for 5 min to remove unbounded polymers and washed with DMF (3 × 5 mL), deionized water (3 × 5 mL) and methanol (3 × 5 mL) before drying *in vacuo*. Once dried, the poly(CBz-L-lysine)-coated substrates were immersed in HBr solution (0.5 mL per substrate) for 3 h to remove the carboxybenzyl protecting groups. The substrates were then washed with Milli-Q water (10 mL) and methanol (10 mL) before drying *in vacuo* for subsequent AFM, SEM and contact angle analysis. Poly(L-valine)-coated substrates were not treated with HBr prior to analysis.

Fabrication of Block copolypeptide nano-coatings

Substrates were functionalized with PEI by the methodology mentioned above. The PEI-functionalized substrates were then immersed in a freshly prepared Lys-NCA solution (0.75 M, 1 mL per substrate) and allowed to react under reduced pressure. After a predetermined reaction period, the coated substrates were washed with anhydrous DMF (3 × 5 mL) to remove excess unreacted Lys-NCA and then were immediately immersed in freshly prepared Val-NCA solution (0.75M, 1 mL per substrate). After a predetermined ROP time, the block copolypeptide-coated substrates were washed with DMF (3 × 5 mL) and soaked in DMF for 15 h. Subsequently, the coated substrates were sonicated for 5 minutes. The peptide-coated substrates were sonicated for 5 min to remove unbounded polymers and washed with DMF (3 × 5 mL), deionized water (3 × 5 mL) and methanol (3 × 5 mL) before drying *in vacuo*. Once dried, the poly(CBz-L-lysine-*block*-L-valine) coated substrates were immersed in HBr solution (0.5 mL per substrate) for 3 h to remove the carboxybenzyl protecting groups. The substrates were then washed with Milli-Q water (10 mL) and methanol (10 mL) before drying *in vacuo* for subsequent AFM, SEM and contact angle analysis.

SUPPORTING FIGURES

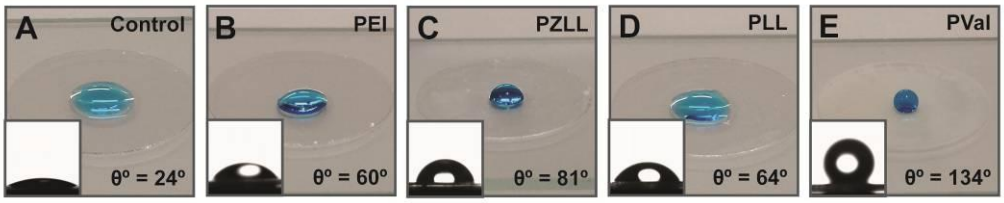


Figure S1. Digital images and water contact angles of (A) untreated glass slides, and glass slides coated with (B) PEI, (C) poly(CBz-L-Lysine) (PZLL), (D) poly(L-Lysine) (PLL), (E) poly(L-Valine) (PVal) coatings. Water droplets were dyed with methylene blue.

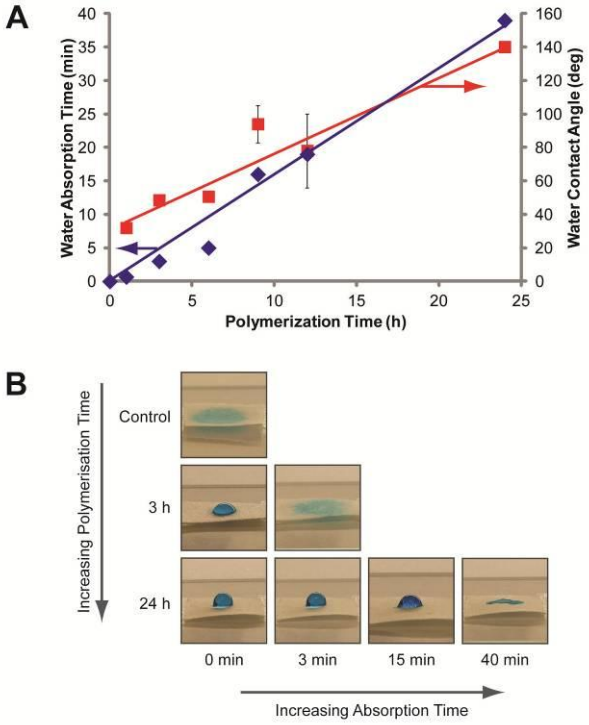


Figure S2. (A) The increase in water absorption time (blue diamond) and immediate water contact angle (red squares) with polymerization time resulting from the increased hydrophobicity

of thicker poly(L-valine) films. (B) Digital images showing the increase in water absorption time with longer L-valine NCA polymerization time.

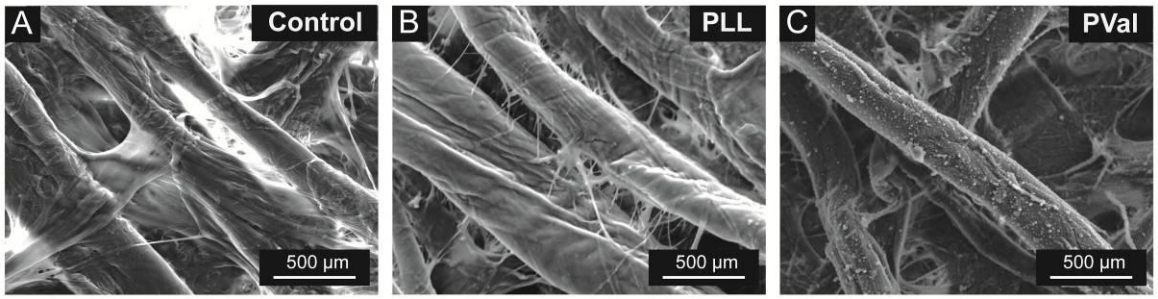


Figure S3. Scanning electron microscope (SEM) images of (A) untreated filter paper, and filter paper coated with (B) poly(L-lysine) (PLL) and (C) poly(L-valine) (PVal).