Supporting Information

Materials: 3,4-Dihydroxy-L-phenyl-alanine (DOPA) (Sigma), sodium carbonate (Na_2CO_3) (UCB), sodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$) (Sigma-Aldrich), magnesium sulfate ($MgSO_4$) (BDH Prolabo), thionyl chloride ($SOCl_2$) (Sigma-Aldrich, 99 %), 4,4'-azobis(4-cyanopentanoic acid) (V-501) (Fluka, 98 %), methacryloyl chloride (Alfa Aesar, 97 %), ethyl acetate (Fischer Scientific), methylene chloride (CH_2Cl_2) (BDH Prolabo) were used without further purification. Methanol (BDH Prolabo) was dried under molecular sieves for two days before use for the synthesis of DOPA methyl ester hydrochloride. Nutrient media for antibacterial assessment: Bacto™Tryptone (ref 211705) and Bacto™Yeast Extract (ref 212750) were obtained from Becton Dickinson. Agar-agar (ref 1.016.14) was purchased from Merck. Luria-Bertani medium (LB) was prepared with 10 g Bacto™Tryptone, 5 g Bacto™ Yeast Extract, and 10 g NaCl per liter of deionized water. LB agar medium was obtained by adding 15 g agar-agar per liter of LB.

N-methacryloyl 3,4-dihydroxy-L-phenylalanine methyl ester synthesis: DOPA methyl ester hydrochloride was synthesized by reaction of $SOCl_2$ with DOPA in dried methanol as reported elsewhere[1]. The N-methacryloyl 3,4-dihydroxy-L-phenylalanine methyl ester (mDOPA) preparation occurs in water according to the synthesis described by Lee at al that we improved in the following experimental section[2]. Basically, 18.04 g (0.0473 mol) of $Na_2B_4O_7.10H_2O$ was dissolved in 540 mL of ultrapure (millQ) water and degassed with argon for 60 minutes. After that, the solution was transferred in a two-neck round-bottom flask containing 5 g (0.0237 mol) of DOPA methyl ester hydrochloride placed under argon and stirred for 15 minutes. The flask was then cooled at 0°C before adding 12.51 g (0.118 mol) of Na_2CO_3 and 11.5 mL (0.118 mol) of methacryloyl chloride, added slowly with stirring. The pH of the solution was maintained above 9 with Na_2CO_3 during the reaction. After stirring for 12 hours at room temperature, the solution was acidified to pH 2 with concentrated HCl. The mixture was extracted three times with ethyl acetate, washed with 0.1 M HCl and dried over $MgSO_4$. The solvent was removed in vacuum to yield crude orange paste. The product was further purified by elution from a silica gel column with dichloromethane/methanol (95:5) mixture (75% yield).

^{1}H NMR (250 MHz, methanol-d_4, δ (ppm)) 6.66-6.46 (m, 3H, Ph), 5.62 (d, 1H, CH_2=C-), 5.36 (d, 1H, CH_2=C-), 4.59 (q, 1H, C<u>H</u>-NH-), 3.69 (s, 3H, $COOCH_3$), 3.01-2.9 (2H, m, CH_2-Ph), 1.88 (s, 3H, CH_3-C-). ^{13}C NMR (250 MHz, methanol-d_4, δ (ppm)) 173 (1C, -O-<u>C</u>=O), 170 (1C, -NH-<u>C</u>=O), 146 (1C, Ph-OH); 145 (1C, Ph-OH), 140 (1C, -C=CH_2), 129 (1C, Ph), 121.5 (1C, Ph), 120 (1C, -C=CH_2), 117 (1C, Ph), 116 (1C, Ph), 55.5 (1C, -<u>C</u>H-NH), 53 (1C, O-<u>C</u>H_3), 37.5 (1C, <u>C</u>H_2-Ph), 19 (1C, <u>C</u>H_3-C=CH_2). No trace of the oxidized form (biquinone).

P(mDOPA) synthesis: It is important to mention that prior to polymerization, the catechol group of the mDOPA must be protected in order to avoid side reactions during the radical polymerization from its -OH groups. 2 g of mDOPA with protected catechol groups (see below for the protection step) (2.6 mmol) were placed under nitrogen in a one-necked round-bottomed flask equipped with a magnetic stirrer. At the same time, 19 mg (0.067 mmol) of V501 initiator was dissolved in 7 mL of distilled water. The pH solution was adjusted above 9 with Na_2CO_3 until complete dissolution of the white powder. The solution was then degassed by bubbling nitrogen through it for 15 minutes. Then, the aqueous solutions of the V501 initiator was transferred with a capillary under nitrogen in the glass flask containing protected mDOPA. The reactor was heated in an oil bath thermostated at 70°C during 24 hours. Then, catechol groups were deprotected by adjusting the pH around 2 with concentrated HCl. The resulting mixture was dialyzed (membrane porosity 1000 Da) against water during 48 hours, followed by lyophilization. The copolymer was recovered as a white powder with a 88 % yield.

^{1}H NMR (250 MHz, methanol-d4, δ (ppm)) 6.7 (m, 3H, Ph), 4.5 (m, 1H, C<u>H</u>-NH-), 3.6 (m, 3H, COOC<u>H</u>$_3$), 2.9 (m, 2H, C<u>H</u>$_2$-Ph(OH)$_2$), 1.6 (m, 2H, C<u>H</u>$_2$-C(CH$_3$)), 0.8 (m, 3H, CH$_2$-C(C<u>H</u>$_3$)). ^{13}C NMR (250MHz, methanol-d4, δ (ppm)) 179 (1C, -NH-<u>C</u>=O), 175 (1C, -O-<u>C</u>=O), 146 (1C, Ph-OH); 145 (1C, Ph-OH), 130 (1C, Ph), 122 (1C, Ph), 116 (2C, Ph), 55 (1C, -<u>C</u>H-NH), 55 (1C, O-<u>C</u>H$_3$), 46 (1C, C-<u>C</u>H$_2$), 38 (1C, CH$_2$-Ph(OH)$_2$), 19 (1C, <u>C</u>H$_3$-C). No trace of the oxidized form (biquinone).

Pox(mDOPA) synthesis: 20 mg of PDOPA were put in 10 ml of distilled water and NaOH solution (pH = 12.2) was slowly added in order to raise the pH above 10. This oxidation step lasts at least one night as followed by UV Vis spectroscopy.

^{1}H NMR (250 MHz, D$_2$O, δ (ppm)) 6.8 (m, 3H, Ph), 4.4 (m, 1H, C<u>H</u>-NH-), 3.6 (m, 3H, COOC<u>H</u>$_3$), 2.9 (m, 2H, -C<u>H</u>$_2$-C$_6$H$_3$O$_2$), 1.6 (m, 2H, C<u>H</u>$_2$-C(CH$_3$)), 0.8 (m, 3H, CH$_2$-C(C<u>H</u>$_3$)). ^{13}C NMR (250MHz, D$_2$O, δ (ppm)) 178 (4C, -NH-<u>C</u>=O; -O-<u>C</u>=O; Ph=O), 143 (1C, Ph), 130 (1C, Ph), 121(1C, Ph), 116 (1C, Ph), 58 (2C, -<u>C</u>H-NH; O-<u>C</u>H$_3$), 44 (1C, C-CH$_2$), 37 (1C, <u>C</u>H$_2$-Ph), 18 (1C, <u>C</u>H$_3$-C).

Protection of N-methacryloyl 3,4-dihydroxy-L-phenylalanine methyl ester: Protection was carried out by adding 160 ml of a degassed aqueous solution of $Na_2B_4O_7.10H_2O$ (0.1 M, 0.0158 mol, 2 equivalents compared to DOPA moieties) to a glass flask containing mDOPA (2.2 g, 0.00788 mol) placed under argon in a small amount of a mix water/ethanol (10 ml of water and 5 ml of ethanol). After stirring for one hour, the product is lyophilized and used without any further purification.

^{1}H NMR (250 MHz, D$_2$O, δ (ppm)) 6.6 (m, 3H, Ph), 5.61 (d, 1H, C<u>H</u>$_2$=C-), 5.45 (d, 1H, CH$_2$=C-), 4.65 (q, 1H, C<u>H</u>-NH-), 3.77 (s, 3H, COOC<u>H</u>$_3$), 3.15-2.98 (2H, m, C<u>H</u>$_2$-Ph), 1.88 (s, 3H, C<u>H</u>$_3$-C-).

Nisin purification: Nisin was purified from a nisin commercial preparation Nisaplin (Danisco, Copenhagen, Dennmark) containing 2,5% nisin, 77,5 % NaCl, 12% milk proteins, 6% carbohydrates and 2 % moisture by a simple and easily scaling up technique. First, 5 g of nisin were dialyzed against a 50 mM sodium acetate buffer, pH 4 using a 1000 Da cutoff membrane. The solution was then applied to an ion exchange HiPrep SP FF 16/10 column (GE Healthcare, England) equilibrated in buffer A (50 mM sodium acetate buffer, pH 4) and then eluted at 5 ml/min using a gradient of buffer B (50 mM sodium acetate buffer, NaCl 1M, pH 4). The major peptide fractions were pooled and prepared for layer-by-layer coating. The final yield of purified (95 % pure) peptide was approximately 75%.

Layer-by-Layer assembly: LbL deposition is conducted at room temperature. Stainless steel 304 2B surfaces were supplied by ArcelorMittal and cleaned with acetone and ethanol (scrubbing with an optical tissue) and dried under argon. Small surfaces (2 cm × 2 cm) were immediately dipped into the first copolymer solution: P(mDOPA)-co-P(DMAEMA$^+$) for 2 min. The following cycle was then used for the first fifteen bilayers: (1) Pox(mDOPA) for 2 min, (2) wash twice in water for 1 min each, (3) PAH (Mw= 15000 g/mol, Sigma-Aldrich) for 2 min, (4) wash twice in water for 1 min each. For the last five bilayers, PAH solution was replaced by nisin solution and the process keeps on running as described above with the final layer being nisin. Three control experiments were also conducted following the same cycles: one without nisin (20 bilayers with PAH), one with P(mDOPA) instead of Pox(mDOPA) and one by alternating the sodium salt of poly(acrylic acid) (PAA) (pH 5) as the polyanion and nisin as the polycation. Concentrations of all polymers were 2 g/L in deionized water and nisin concentration was 1 g/L in PBS buffer, pH 7.4. PAH solution was 0.15 M in NaCl (pH=11, adusted by NaOH).

Characterizations: ^{1}H and ^{13}C NMR spectra were recorded in deuterated solvents (CD_3OD or D_2O) with a Bruker AM spectrometer (250 MHz) at 25°C. ^{13}C CP MAS NMR spectra were recorded with 2.5-mm zirconia rotors spinning at 7 kHz on a Bruker Avance DSX 400WB spectrometer (B_0=9.04 T) working at the Larmor frequency of 100.62 MHz. Cross polarization experiments were performed with a delay time of 5 s and a contact time of 2.5 ms. UV-Vis was measured with a Hitachi spectrophotometer (U-3300). Film growth has been followed in real time using Quartz Crystal Microbalance with Dissipation technique (QCM-D). A Q-Sense E4 was used in this study. The stainless steel-coated AT-cut resonator (fundamental frequency: 5 MHz) was used as received. First distilled water was introduced in the cell and the circulation was maintained until obtaining of a stable baseline. LbL deposition was then initiated by switching the liquid exposed to the crystal from distilled water to a P(mDOPA)-co-P(DMAEMA$^+$) solution with a concentration of 2 g/L and 0.15 M NaCl (flow rate of 250 µL/min, temperature of 25.09 ± 0.02 °C). Polymer is allowed to adsorb onto the substrate for 10 minutes before being rinsed with distilled water to get a uniform positive coating on the resonator. After Pox(mDOPA), PAH and nisin were

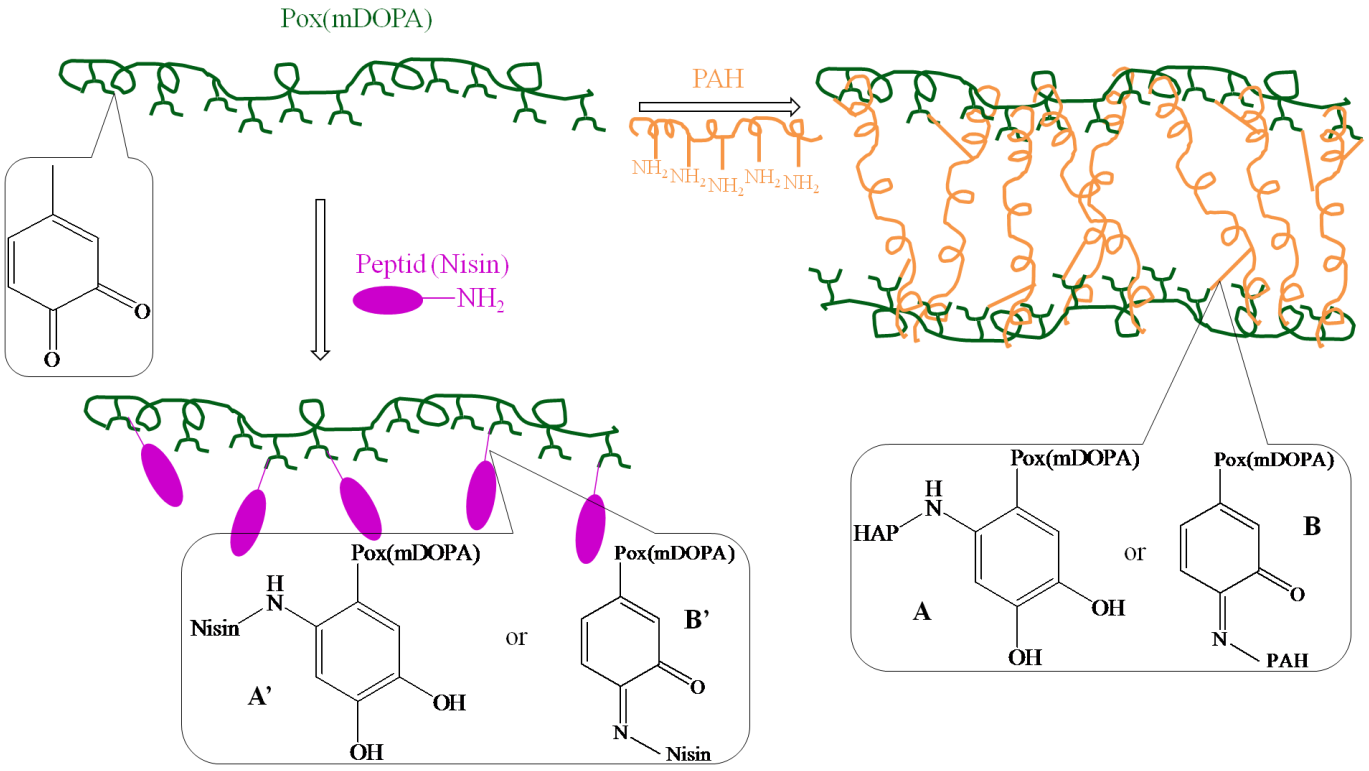
alternatively introduced (same solutions as those described here after in *Layer-by-Layer assembly* section) with distilled water rinsing in between. Field Emission Gun Scanning Electron Microscope (FEG-SEM) MEB ULTRA55 operating at 3 kV was used for sample observation after a thin layer (10 nm) of Au-Pd to increase the contrast. The XPS analyses were performed in a PHI-Quantum 2000 spectrometer. The spectrometer is characterized by a monochromatized Al kalpha primary x-ray beam and a photoelectron take-off angle of 45° against the sample normal direction. Charge effects were compensated with low energy electrons.

Antibacterial assessment: ISO 22196 (derived from JISZ2801) was used to measure the antibacterial efficacy of multilayer. A preculture of *Bacillus 168* (incubated at 37°C overnight in 3 mL Lysogeny Broth (LB)) was used to seed a fresh culture into 50 mL LB; the bacterial concentration of the test inoculum was adjusted to about to $2\text{-}4.10^8$ bacteria/mL. 2 x 2 cm SS coupons were placed in Petri dishes containing damp blotting paper; 200 µL test inoculum were pipetted onto each substrate. The inoculum was covered with a piece of polyethylene film cut from a sterile Stomacher bag (ref. B40542 Fisher Scientific) so as to ensure spreading of the inoculum onto the substrate and to avoid drop evaporation. The Petri dishes containing the inoculated coupons were incubated at 37°C for 24 hours. The SS substrates were placed, inoculated face downward, in glass jars containing 20 mL 500-fold-diluted LB and 4-mm glass beads. The jars were shaken horizontally for 10 minutes and then their content was sonicated in a water bath (50-60 KHz) for 2 minutes. Viable bacteria were counted by plating 10-fold dilutions on LB agar and incubating the plates at 37°C overnight.

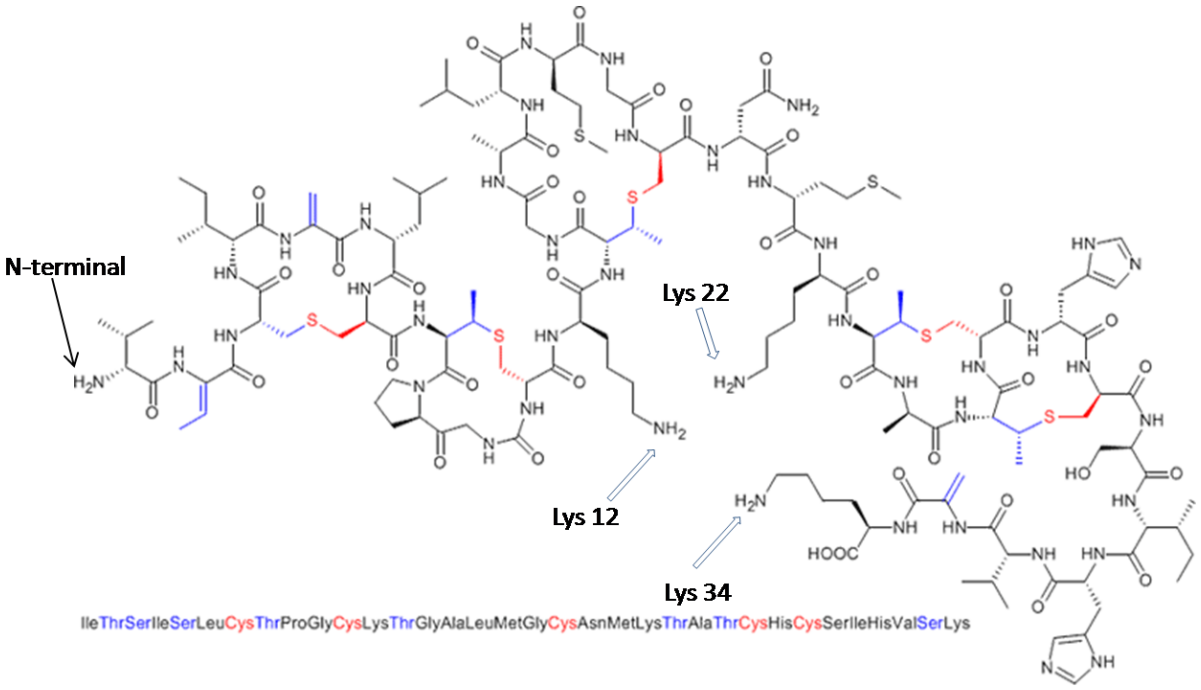
Durability assessment:

Immersion test: Stainless steel coated substrates were dipped in tap water for one night.

Mechanical test: Stainless steel coated substrates were cleaned with a sponge wetted with distilled water by 30 back and forth movements.



Scheme S1. Possible cross-linking and coupling reactions of Pox(mDOPA) with the amino-bearing molecules (PAH and Nisin): Michaël addition adducts (A, A') and Schiff bases (B, B').



Scheme S2. Primary structure of nisin; arrows indicate potential binding sites (→, α amino group; =>, lysine).

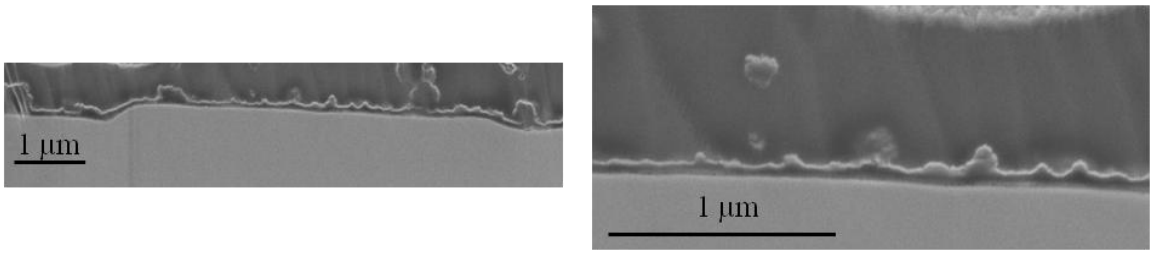


Figure S1. FEG-SEM images of cross sections of $(Pox(mDOPA)/PAH)_{15}/Nisin)_5$ multilayers on stainless steel with enlargement 8000 × (left), enlargement 20000 × (right).

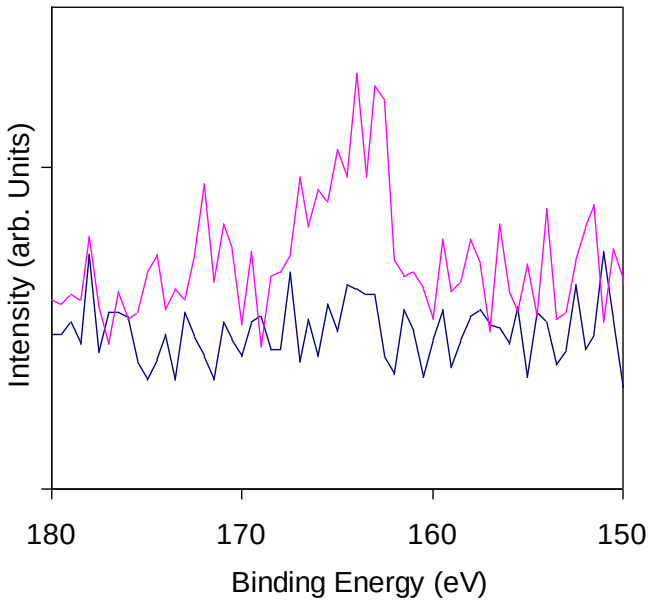


Figure S2. S_{2p} XPS spectra of Pox(mDOPA) multilayers with (pink line) and without nisin (blue line)

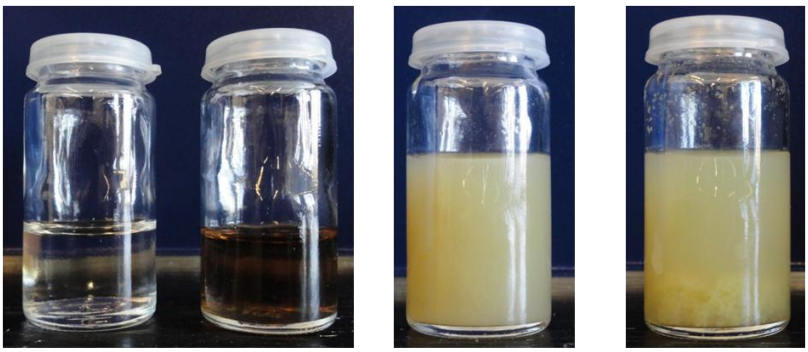


Figure S3. Images of (A) an aqueous solution of PAH (2 g/l), (B) an aqueous solution of Pox(mDOPA) (2 g/l), (C) cross-linked PAH/Pox(mDOPA) directly formed after adding A in B, (D) C after a few hours without stirring.

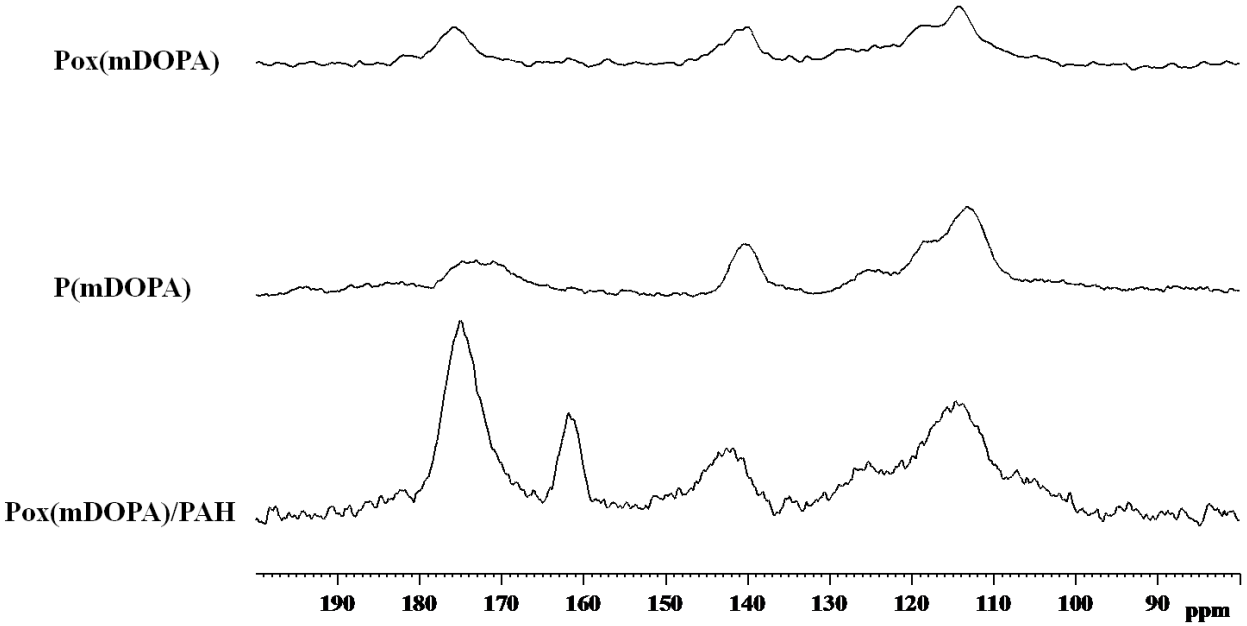


Figure S4. ^{13}C NMR spectra in the solid state of Pox(mDOPA) (upper curve), P(mDOPA) (middle curve) and Pox(mDOPA)/PAH (lower curve).

[1] R. P. Patel, S. Price, *Journal of Organic Chemistry* **1965**, *30*, 3575.
[2] B. P. Lee, K. Huang, F. N. Nunalee, K. R. Shull, P. B. Messersmith, *Journal of Biomaterials Science, Polymer Edition* **2004**, *15*, 449.