Supplementary Information
for
Water-soluble dopamine-based polymers for photoacoustic imaging

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Synthesis:

3,6-dibromo-dopamine

Dopamine hydrochloride (5.0 g, 26.4 mmol) was dissolved in 150 mL of acetic acid and 150 mL of chloroform. The mixture was degassed by bubbling with argon for 30 min. Afterwards, bromine (2.8 mL, 55.4 mmol) was added dropwise to the solution and the mixture was stirred for 12 h at room temperature. The precipitated product was filtered off and washed with chloroform. The crude product was recrystallized three times from acetic acid, washed with chloroform and dried in vacuo. Yield: 11 % (white solid), ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 10.04 (s, 1H, OH), 9.56 (s, 1H, OH), 7.84 (s, 3H, NH_3), 6.81 (s, 1H, H_{ar}), 2.92 (br s, 4H, CH_2). ^{13}C-NMR (400MHz, DMSO-d_6): δ = 146.35, 143.72, 128.08, 116.11, 114.56, 113.45, 38.55, 34.42. MS (ESI): m/z (%): 311.90 (100, $[M]^+$, $C_8H_{10}Br_2NO_2^+$). Elemental analysis: $C_8H_{10}Br_3NO_2$, calcd (%): C 24.52, H 2.57, N 3.57; measd (%): C 25.51, H 2.69, N 3.47.

^{t}Boc-protected 3,6-*dibromo*-dopamine

3,6-dibromo-dopamine (1.382 g, 4.44 mmol) and di-*tert*-butyl dicarbonate (5.23 mL, 24.44 mmol) were dissolved in 20 mL DMF and degassed by bubbling with argon for 20 min. A solution of NaOH (0.444 g, 11.11 mmol) in 5 mL distilled water was degassed with argon by bubbling for 10 min and subsequently added to the reaction mixture. The mixture was stirred at room temperature for 12 hours. The precipitated solid was separated by centrifugation, dissolved in diethyl ether and dried over magnesium sulfate. The crude product was purified by column chromatography (silica gel, pentane/ethyl acetate 6.5/3.5). Yield: 45% (colorless viscous liquid). ^{1}H-NMR: (400 MHz, DMSO-d_6): δ = 7.39 (s, 1H, H_{ar}), 6.95 (m, 1H, NH_3), 3.20 (m, 2H, CH_2), 2.94 (m, 2H, CH_2), 1.48 (m, 18H, CH_3), 1.35 (s, 9H, CH_3). ^{13}C-NMR (400MHz, DMSO-d_6): δ = 155.48, 149.44, 148.85, 146.17, 142.24, 139.59, 139.37, 124.04, 123.59, 120.84, 85.53, 84.79, 84.34, 77.59, 36.91, 28.13, 27.02, 26.82. MS (ESI): m/z (%): 634.04 (100, $[M+Na^+]$, $C_{23}H_{33}Br_2NO_8Na^+$). Elemental analysis: $C_{23}H_{33}Br_2NO_8$, calcd (%): C 45.19, H 5.44, N 2.29; measd (%): C 46.52, H 5.74, N 3.01.

Polydopamine

The ^{t}Boc-protected 3,6-*dibromo*-dopamine (107 mg, 0.2 mmol) was dissolved in 20 mL anhydrous THF and was degassed by bubbling with argon for 20 min. MeMgBr (59 µL of 3M solution in diethyl ether) was added dropwise to the solution. The reaction mixture was refluxed for 3 h and then a degassed solution of Ni(dppp)Cl_2 (ca. 4.0 mg, 0.01 mmol) in 2 mL anhydrous THF was added. The mixture was refluxed for 48 h under a nitrogen blanket. The precipitated polymer was filtered, followed by washing with THF and water. Yield: 86 % (grey solid). ^{1}H-NMR: (400 MHz, DMSO-d_6): δ = 6.82 (br, 1H, NHBoc), 6.47 (br, 1H, H_{ar}), 2.99 (s, 2H, CH_2), 2.59 (s, 2H, CH_2), 1.38 (br, 9H, CH_3).

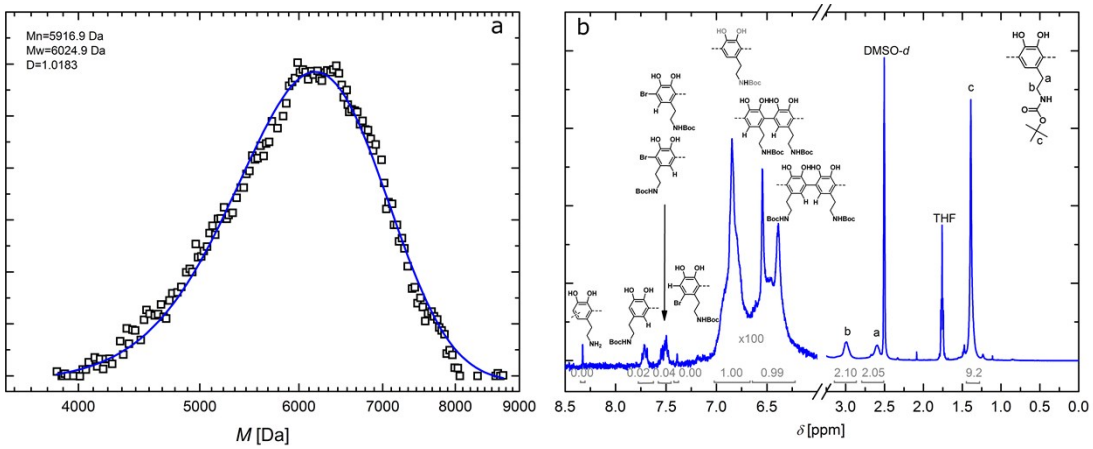

Figure S1: a) Gel permeation chromatograph of the mono-Boc protected polydopamine. Measured against polystyrene standards. b) 1H-NMR of mono-Boc protected polydopamine: the low field region (8.5 ppm – 6.0 ppm) was enhanced by a factor of 100 to clearly identify the smaller sets of peaks for endgroup analysis. The insets show the various molecular conformations possible above the corresponding peaks.

Endgroup Analysis

Per monomer unit of the polymer we obtain one H signal from the aromatic hydrogen. The signal is split into two peaks originating from *E* and *Z* conformations between 6.75 ppm and 6.25 ppm. Per polymer chain there is one bromine endgroup as well as one endgroup with two aromatic hydrogens originating from the hydrolysis of the MgBr functional group after termination. The bromine endgroups can be identified from the signal of the respective aromatic H signals between 7.60 ppm and 7.25 ppm. Per endgroup there are 25 monomer (0.99 ppm / 0.04 ppm) units resulting in an average molecular weight of 25 x 251 Da (molecular weight of the repeat unit) = 6275 Da.

Photoacoustic Imaging

To investigate the photoacoustic performance of melanin in tissue, 100 µL of aqueous solutions of melanin (containing 10 nmol, 100 nmol and 1 µmol) and saline as a control were subcutaneously injected into dead mice. After injection, B-mode ultrasound imaging was performed to localize the region of interest. Both ultrasound and photoacoustic imaging were subsequently performed using a VEVO LAZR photoacoustic imaging system equipped with a LZ250 transducer (VisualSonics, Amsterdam NL). The system delivers peak energies of 45 +/- 5 mJ, with a pulse duration of 4-6 ns at tunable wavelengths between 680 – 970 nm (2 nm step size). High resolution ultrasound images were acquired at 21 MHz.

To investigate the depth detection limit of melanin, 100 µL of 1 mM melanin solution was injected into intramuscularly into chicken breasts at different depths. For all experiments the imaging parameters were kept constant.

For the measurements in blood vessel mimicking plastic tubing phantoms, a 0.6 g/L (0.1 mM) solution of melanin was diluted 4 time by a factor of 10. For comparison a 7 g/L solution of Aurovist™ 1.9 nm gold nanoparticles was diluted 5 times and by a factor of 10. The dilutions were injected into PE plastic tubes, aligned parallel in a custom build rack and immersed in water (as background). As a control one of the tubes was filled with saline solution. The immersed tubes were then subjected to simulataneous photoacoustic and ultrasound imaging delivering photoacoustic excitation spectra and at the maximum wavelength crosssection images of the plastic tubes were recorded. To determine the *ex-vivo* detection limits the signals were compared with the control.

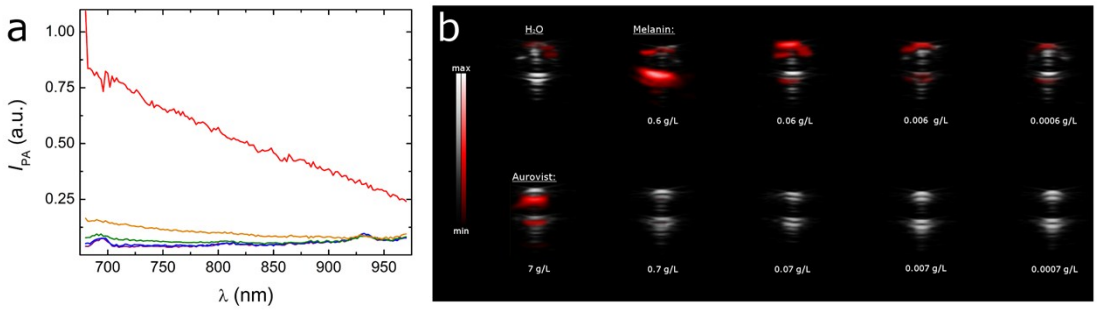

Figure S2: a) Excitation spectra for Aurovist™ gold nanoparticles with a diameter of 1.9 nm at concentration of 0.0007 g/L (purple), 0.007 g/L (blue), 0.07 g/L (green), 0.7 g/L (orange), 7 g/L (red). b) Detection limit determined in blood vessel phantoms. The crossection of the tubings are depicted where the gray scale represents the ultrasound signal overlayed with the photoacoustic signal in red. Top row: Water as a comparison and 4 concentrations of melanin solutions. The bottom row shows 5 concentrations of Aurovist™ gold nanoparticles. The detection limit for melanin is between 0.06 g/L and 0.006 g/L whereas the detection limit for the Aurovist™ particles is between 7 g/L and 0.7 g/L.

Cell toxicity

To test the toxicity of our synthetic melanin, different concentrations were tested with fibroblasts. L929 mouse fibroblast cells were cultured on fibronectin coated glass substrates that were put vertically into a 96 well plate. Three glass substrates per well were fixed in a 12 well plate, and incubated with 100 µg/mL of human fibronectin for 24 hours at 37 °C. After washing twice with phosphate buffer saline (1X PBS), 50,000 L929 cells were seeded per well for 24 hours. After cell attachment, the glass substrates were transferred to their vertical position with one glass substrate per well in the 96 well plate. The cells were then incubated for 48 hours with melanin, dissolved in PBS, at final concentrations of 10 mM, 5 mM, 1 mM, 0,1 mM, 0,01 mM, 0,001 mM, and 0 mM (n=2-3). After 48 hours, a live/dead stain was performed to determine the ratio of dead versus live cells. Images at 10X magnification were quantified. At 10 mM melanin interferes with the live/dead staining. The green fluorescence of the "live"-stain is quenched due to melanin's high absorption in the green spectrum. For the 10 mM concentration, we record the red fluorescence for "dead" cells and overlay the fluorescence image with the bright field image for analysis.

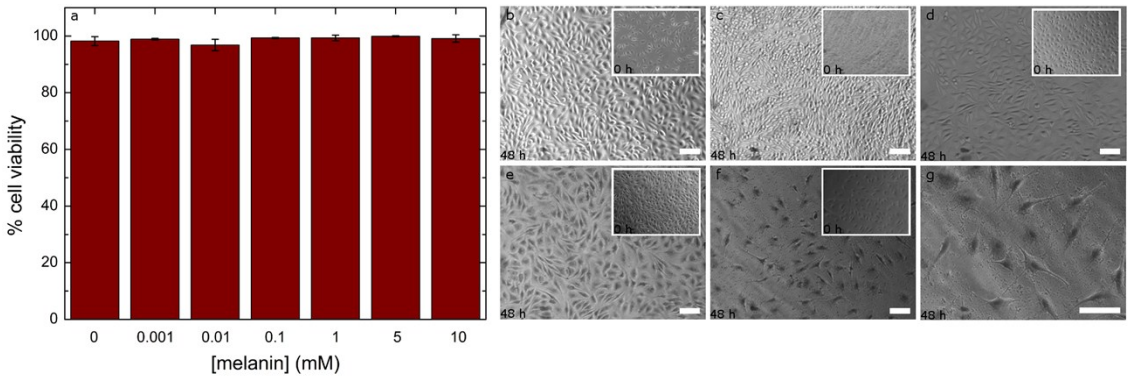

Figure S3: a) Cell viability for a variety of melanin concentrations and 0 mM as control after 48 hours of incubation. Micrographs of selected samples: b) control sample, c) 0.001 mM, d) 0.1 mM, e) 5 mM, f) 10 mM and g) close up of 10 mM sample. Insets are images of the cells before the melanin was added. Scale bars represent 100 µm.