# Supporting Information for:

# Sequence of hydrophobic and hydrophilic residues in amphiphilic polymer coatings affects surface structure and marine antifouling/fouling release properties

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## Synthesis

**PS-P(EO-co-AGE) synthesis**. PS-*b*-P(EO-*co*-AGE) with Mn(PS) = 22300, and Mn(P(EO-*co*-AGE)) = 46000 with 2.9 mol% AGE and a PDI of 1.11 was synthesized as described previously.[1] In short, alcohol-terminated polystyrene was grown anionically in cyclohexane with a *sec*-butyl lithium initiator and termination with ethylene oxide followed by isopropyl alcohol. The resulting polymer was precipitated into 50:50 methanol/isopropanol, filtered, dried under vacuum, and characterized by refractive index size-exclusion chromatrography (RI-SEC) and $^1$H NMR spectroscopy. The hydroxyl terminated PS was then used as a macroinitiator for the anionic co-polymerization of ethylene oxide and allyl glycidyl ether in THF.

**Peptoid Synthesis.** Polypeptoids were synthesized on a robotic synthesizer on 100 mg of Rink amide polystyrene resin (0.6 mmol/g, Novabiochem, San Diego, CA) using a previously described submonomer method.[2, 3] All primary amine monomers, solvents, and reagents were purchased from commercial sources and used without further purification. Amine displacement times were typically 1 hour for methoxyethylamine (1.5 M in NMP) and 2 hours for 2,2,3,3,4,4,4-heptafluorobutylamine (1.25 M in DMF) and amines coupled directly after that. Only when heptafluorobutylamine was to be coupled directly to the resin (or to one or two heptafluorobutyl groups already coupled to the resin), reaction was performed at 50 °C for 3 hours. All peptoids were thiol functionalized by reacting for 1 hr in 1 mL of 0.5 M S-tritylmercaptopropionic acid and 75 µL of DIC. Peptoid chains were cleaved from the resin by addition of 3.0 mL of cleavage cocktail (47.5 v% TFA, 47.5 v% DCM, 2.5 v% $H_2O$, 2.5 v% TIS) for 10 minutes, which was then evaporated off using a Biotage V-10 evaporator. This simultaneously resulted in deprotection of the thiol functionality (significantly longer cleavage times would result in a

cyclization reaction cleaving off the thiol and final monomer). Following cleavage, peptoids were dissolved in appropriate acetonitrile/water mixtures and lyophilized twice to obtain a fluffy white powder. All peptoids were purified by reverse-phase prep HPLC, and their purity and mass were determined by analytical reverse-phase HPLC and MALDI as previously described.[4]

**Table S1**: Thiol functionalized peptoids used in this study are shown below with their names and purity. The thiol functionality is represented by a dash, the N-(2-methoxyethyl)glycine unit is represented by a O, and the N-(heptafluorobutyl)glycine unit is represented by an X.

| Name | Sequence | Mass | Observed mass (+Na)[a] | Purity (%)[b] |
|---|---|---|---|---|
| 5merA1 | -OO**X**OO | 804.3 | 829.3 | 91 |
| 10merA2 | -OO**XX**OOOOOO | 1503.6 | 1529.0 | 92 |
| 15merA3 | -OO**XXX**OOOOOOOOO | 2202.8 | 2228.1 | 90 |
| 15merB3 | -OOOOOOO**XXX**OOOOO | 2202.8 | 2227.5 | 86 |
| 15merC3 | -OOOOOOOOOOOO**XXX** | 2202.8 | 2227.4 | 81 |
| 15merC2 | -OOOOOOOOOOOOO**XX** | 2078.9 | 2103.2 | 83 |
| 15merC1 | -OOOOOOOOOOOOOO**X** | 1954.9 | 1979.8 | 80 |
| 12merD | -OOOOOOOOOOO | 1485.8 | 1510.9 | 81 |

[a] as observed on MALDI

[b] as observed by analytical reverse-phase HPLC

**Comb-copolymer Synthesis.** Thiol-ene coupling of the thiol functionalized peptoid sequences was performed by dissolving 100 mg of polymer in a small amount of DMF (typically 550 µL per 100 mg of polymer) in a 1 dram vial. Then 2.5 equiv of thiol functionalized peptoid (with respect to alkene groups in the polymer) were added and dissolved. Finally 0.4 equiv of 2,2-dimethoxy-2-phenylacetophenone (DMPA) photoinitiator and a small stir bar were added. The vial was sealed with a screw cap fitted with a polytetrafluoroethylene (PTFE) septum, and the mixture was purged with argon for 10 min. Irradiation was carried out for 30 min with a 365 nm UV lamp. The reaction was driven to completion by an extra addition of 0.5 equiv of peptoid, 0.2 equiv of DMPA, and DMF (50 µL per 100 mg). The new mixture was purged for 10 minutes with argon before reacting 30 extra minutes under UV irradiation. After the reaction, 2.5 ml of acetonitrile was added to the reaction vessel and the mixture was suspended in 12.5 ml of Milli-Q water. These mixtures were concentrated using Amicon Ultra centrifugal filter units (30 000 cutoff Mw, Millipore) and resuspended in 15 ml of 1:6 acetonitrile/water. This procedure was repeated twice. All concentrated block copolymer solutions were lyophilized from 1:6 acetonitrile/water mixtures. The reaction was monitored by $^{1}$H NMR, shown in Figure S1.

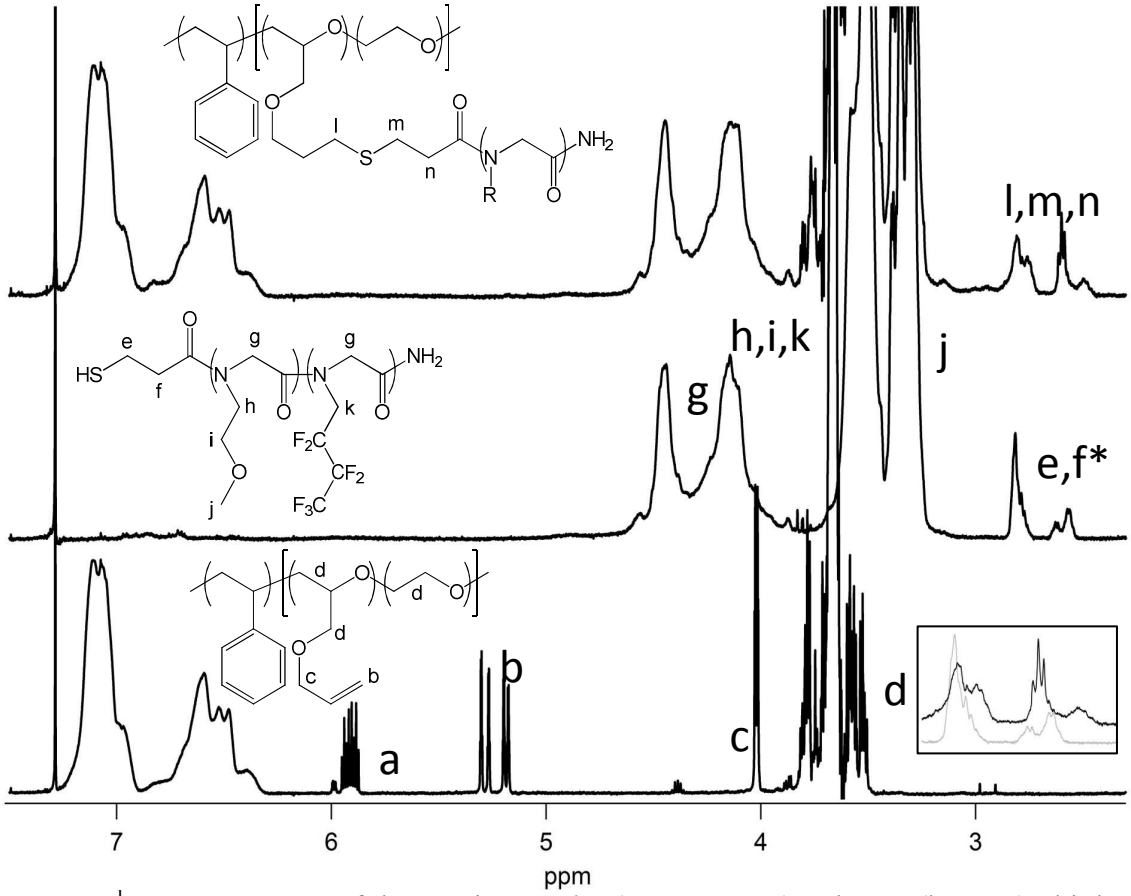


**Figure S1:** $^{1}$H NMR spectra of the starting PS-*b*-P(EO-*co*-AGE) polymer (bottom), thiol terminated peptoid (middle), and the product after thiol-ene coupling (top). The inset shows an overlay of the 3-2.5 ppm region for the peptoid product. *Four peaks are visible due to the diastereotopic protons next to the amide bond and a minor amount of dithiol impurity.

## Thin film preparation and characterization

Films with a thickness of approximately 80 nm were spin coated at 1500 rpm from 1.5% (w/v) block copolymer solutions in toluene onto plasma-cleaned silicon wafers. All films were vacuum annealed for at least 16 hours at 120 °C.

Near Edge X-ray Absorption Fine Structure (NEXAFS) experiments were conducted on the U7A NIST/Dow materials characterization end-station at the National Synchrotron Light Source at Brookhaven National Laboratory (BNL). The general NEXAFS principles and a description of the beam line at BNL have been previously reported.[1, 5-7] In short, the X-ray beam hits the sample with an angle θ to the sample surface and ejected electrons are collected at an angle ϕ from surface normal as shown in Figure S2. Because the angle between the X-ray and detector is fixed at 36°, both the surface sensitivity and the sensitivity to bond orientation can be controlled by varying θ. At a θ of 120°, ϕ is 66° and the technique is more surface sensitive. At "magic angle" of θ equal to 54.7° the technique reports chemistry irrespective of ordering or bond orientation, and provides information about the top 2nm of the film. Unless otherwise specified, all spectra were taken at the magic angle.

The PEY C1s spectra were normalized by subtracting a linear pre-edge baseline from 270 – 280 eV and setting the edge jump to unity at 320 eV.[8] The photon energy was calibrated by adjusting the peak position of the lowest π* phenyl resonance from polystyrene to 285.5 eV.[9] No further normalization was conducted for the N1s spectra. NEXAFS spectra of PS-*b*-P(EO-*co*-AGE) functionalized with peptoids are shown in Figure S3.

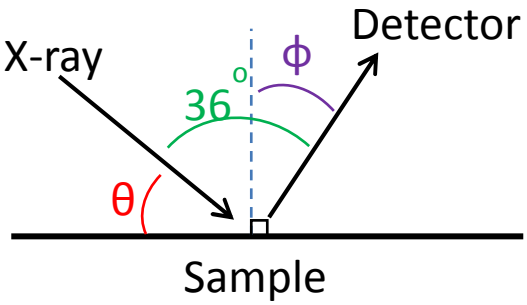


**Figure S2**: Geometry of NEXAFS set up at U7A endstation at NSLS.

Nuclear magnetic resonance ($^1$H NMR) spectra were recorded on a Bruker Biospin Avance II 500 MHz High Performance NMR Spectrometer at room temperature. Chemical shifts are reported in parts per million relative to $CHCl_3$ at 7.26 ppm for $^1$H as an internal reference. Gel permeation chromatography (GPC) was performed on an Agilent GPC system, equipped with PLgel 5µm MIXED"D columns and calibrated with narrow polystyrene standards. Using a flow rate of 1 mL/min, the mobile phase was DMF at 40°.

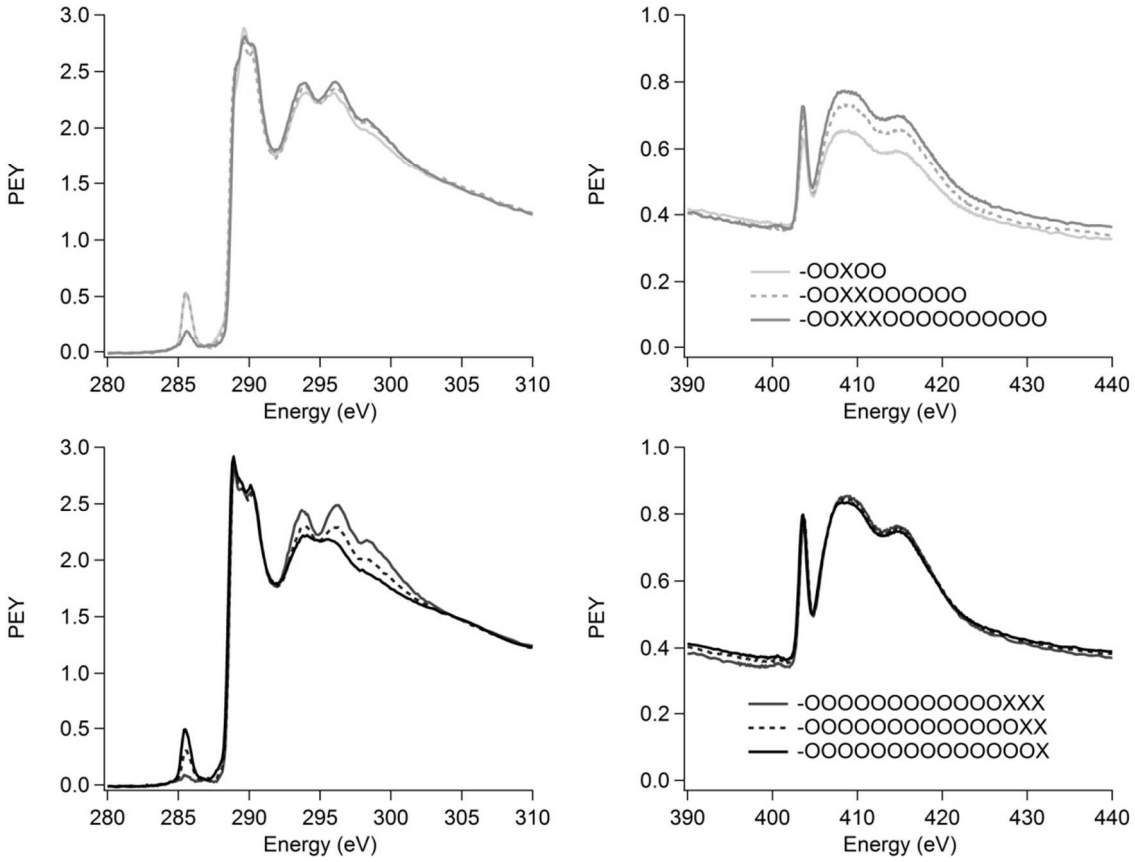


**Figure S3:** The carbon edge (left) and nitrogen edge (right) NEXAFS spectra of PS-*b*-P(EO-*co*-AGE/peptoid) copolymers show the surface chemistry of the films.

## Ulva Assays

Both antifouling and fouling-release assays were performed as previously described.[1, 10] Coatings were equilibrated in 0.22µm filtered artificial seawater (Tropic Marin®) for 24 hours before testing. Zoospores were obtained from mature plants of *U. linza*, collected at Llantwit Major in South Wales. Spores were released by the standard method. A suspension of zoospores (10 ml; $1x10^6$ spores $ml^{-1}$) was added to individual compartments of quadriperm dishes, in the dark. After 45 minutes in darkness at c. 20 $^oC$, the slides were washed by passing 10 times through a beaker of seawater to remove unsettled spores.

### Settlement of zoospores

Slides were fixed using 2.5 % glutaraldehyde in seawater. The density of zoospores attached to the surface was counted on each of 3 replicate slides using an image analysis system attached to a fluorescence microscope. Spores were visualized by autofluorescence of chlorophyll. Counts were made for 30 fields of view (each 0.15 $mm^2$) on each slide.

### Growth and Removal of Sporelings

Spores were allowed to settle on the coatings for 45 minutes and then washed as described above. The spores were cultured using supplemented seawater medium for 7 days to produce sporelings (young plants) on 6 replicate slides of each treatment. Sporeling growth medium was refreshed every 48 hours.

Sporeling biomass was determined *in situ* by measuring the fluorescence of the chlorophyll contained within the sporelings in a Tecan fluorescence plate reader. Using this method the biomass was quantified in terms of relative fluorescence units (RFU). The RFU value for each slide is the mean of 70 point fluorescence readings taken from the central portion. The sporeling growth data are expressed as the mean RFU of 6 replicate slides.

Strength of attachment of sporelings was initially assessed using a shear stress of 52 Pa in a water channel (52 Pa is the maximum achievable). There was negligible removal from any of the peptoid test surfaces and consequently the apparatus was changed for a higher powered water jet.

The relative attachment strength of sporelings on the peptoid coatings was determined by exposure to a water jet producing an impact pressure of 160 kPa (Figure S4). Biomass remaining after exposure to the water jet was determined using the fluorescence plate reader (as above). The percentage removal was calculated from readings taken before and after exposure to the shear stress.

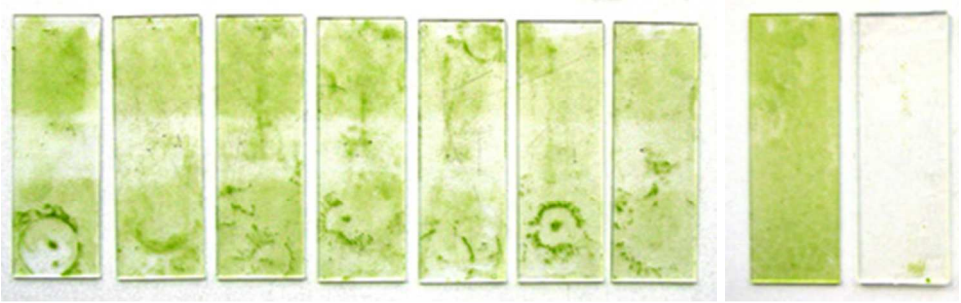

**Figure S4**: Image showing sporelings remaining on amphiphilic peptoid coatings after exposure to a shear stress of 160 kPa. From left: 15-1, 15-2, S1, S2, S3, 10, 5, glass, PDMS.

## References


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