ACS Macro Letters

Letter

pubs.acs.org/macroletters

Sequence of Hydrophobic and Hydrophilic Residues in Amphiphilic Polymer Coatings Affects Surface Structure and Marine Antifouling/Fouling Release Properties

Wendy van Zoelen,[†,○] Hilda G. Buss,[†,○] Nathan C. Ellebracht,[†] Nathaniel A. Lynd,[‡] Daniel A. Fischer,[∥] John Finlay,[⊥] Sophie Hill,[⊥] Maureen E. Callow,[⊥] James A. Callow,[⊥] Edward J. Kramer,[#,▽] Ronald N. Zuckermann,[§] and Rachel A. Segalman*[,†,§]

[†]Department of Chemical and Biomolecular Engineering, University of California, Berkeley, California 94720, United States

[‡]Joint Center for Artificial Photosynthesis and [§]The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States

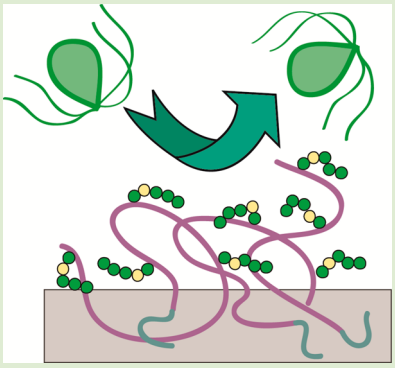
[∥]Materials Science and Engineering Laboratory, National Institute for Standards and Technology, Gaithersburg, Maryland 20899, United States

[⊥]School of Biosciences, University of Birmingham, West Midlands B15 2TT, U.K.

[#]Department of Materials and [▽]Department of Chemical Engineering, University of California, Santa Barbara, California 93106, United States

Supporting Information

ABSTRACT: Amphiphilic polymers, specifically combinations of hydrophilic and hydrophobic residues, have been shown to be effective as antifouling materials against the algae *Ulva linza* and *Navicula* diatoms. Here we use the inherent sequence specificity of polypeptoids made by solid-phase synthesis to show that the sequence of hydrophilic (methoxy) and hydrophobic (fluorinated) moieties affects both antifouling and fouling release of *U. linza*. The platform used to test these sequences was a polystyrene-*b*-poly(ethylene oxide-*co*-allyl glycidyl ether) (PS-*b*-P(EO-*co*-AGE)) scaffold, where the polypeptoids are attached to the scaffold using thiol−ene click chemistry. The fluorinated moiety is very surface active and directs the surface composition of the polymer thin film. The position and number of fluorinated groups in the polypeptoid are shown to affect both the surface composition and antifouling properties of the film. Specifically, the position of the fluorinated units in the peptoid chain changes the surface chemistry and the antifouling behavior, while the number of fluorinated residues affects the fouling-release properties.



In nature, the sequence specificity of polypeptides mediates the complex interactions between organisms and their environment. Small variations in sequence, size, and composition can have large effects on the structure of a protein or on binding affinity. In this study we use sequence-specific polypeptoids,[1] a class of oligomers similar to polypeptides, to study these effects on the interaction between the fouling alga *Ulva linza* and the surfaces they colonize. There are a large number of studies investigating amphiphilic coatings for antifouling, but synthetic challenges have made it difficult to understand how polymer architecture and the combination of the hydrophilic and hydrophobic groups affect the settlement and adhesion of fouling organisms. Most groups have investigated randomly combined hydrophobic and hydrophilic oligomers[2−4] or diblock chains with hydrophilic and hydrophobic blocks.[5−8] Additionally, molecular patterning of two distinct polymer chemistries on the length scale of micrometers[9] and tens of nanometers[10] has been shown to affect the behavior of zoospores of *U. linza*.

In this study we use peptoids containing only the two functional groups shown in Figure 1a, a hydrophilic *N*-(2-methoxyethyl)glycine unit, and a hydrophobic *N*-(heptafluorobutyl)glycine unit. These functional groups were chosen to match the chemistry of amphiphilic polymer films shown by other groups to be antifouling[11,12] and allow us to explore sequence space to determine design rules for amphiphilic antifouling polymers. The polypeptoids were presented on the surface of a thin film using a polystyrene-*b*-poly(ethylene oxide-*co*-allyl glycidyl ether) (PS-*b*-P(EO-*co*-AGE))[13,14] scaffold, where the peptoids are attached to the polymer via thiol−ene click chemistry[12] as shown in Figure 1b.

A previous study of PS-*b*-peptoid model block copolymer systems indicated that fluorinated peptoid monomers drive surface segregation of a predominantly hydrophilic peptoid

Received: February 11, 2014
Accepted: March 21, 2014
Published: March 28, 2014

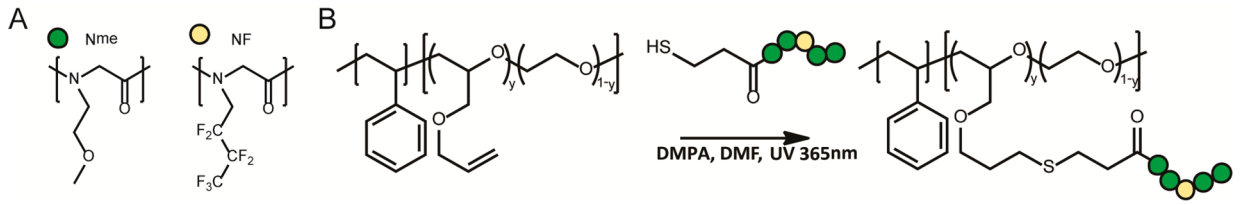


Figure 1. (a) Peptoid monomers used in this study, symbol, and abbreviation used to present them. (b) Thiol−ene click chemistry is used to functionalize PS-P(EO-co-AGE) with thiol-terminated peptoids to make a comb-shaped polymer. This functionalized polymer will be called AGE-(peptoid name).

block.[15] The carbon edge near edge X-ray absorption fine structure (NEXAFS) spectrum shown in Figure 2 shows that

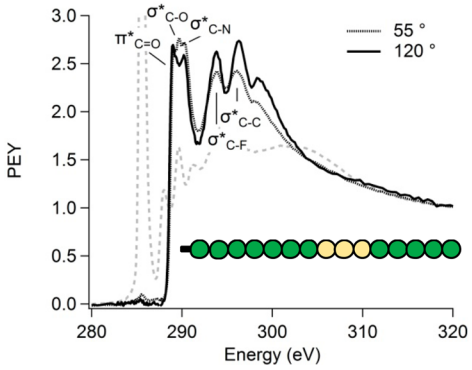


Figure 2. Carbon K-edge NEXAFS spectra of AGE-S2 (sequence on right) at two different θ. The dashed line represents a polystyrene surface spectrum, visible for the parent AGE copolymer or when only a hydrophilic thiol 12mer is coupled to the AGE copolymer.

the same is true for the PS-*b*-P(EO-*co*-AGE) copolymer system used here (23k PS, 46k PEO, 3 mol % AGE). In Figure 2 it is apparent that in the case of the fluorine-deficient peptoid, AGE-12merD shown in Table 1, PS segregates at the top 2 nm[16] of

Table 1. Polypeptoid Sequences Used in This Study, Where Yellow (Light) Is *N*-(Heptafluorobutyl)glycine, Green (Dark) Is *N*-(2-Methoxyethyl)glycine, and the Dash Is the Thiol End of the Peptoid[a]

Code	Sequence	PEY at 408.5 eV	PEY at 293.8 eV
5		0.65	2.30
10		0.73	2.34
S3		0.77	2.40
S2		0.80	2.42
S1		0.85	2.43
15 2		0.84	2.29
15 1		0.83	2.22
12mer		N/A	N/A

[a]The PEY at 408.5 and 293.8 eV is indicative of the amount of peptoid and fluorine at the surface, respectively.

the surface as characterized in Figure 2 by the sharp C 1s → $\pi^*_{C=C}$ transition at 285.5 eV, and there is no evidence of the C 1s → $\pi^*_{C=O}$ or the C 1s → σ^*_{C-O} transition (at 288.6 and 289.5 eV) that is characteristic of polypeptoids and PEO, respectively. In this case, the PS has a lower surface free energy than the peptoid or the PEO and therefore forms a wetting layer at the surface of the film. However, fluorocarbon bonds have an even lower surface free energy and will segregate to the surface on top of the PS. When a fluorocarbon group is attached to the polypeptoid and PEO domains of the polymer, it provides a sufficient driving force to drag the polypeptoid and the PEO to the surface as well, effectively pushing the PS deeper into the film. This can be seen in the case of the fluorinated peptoid AGE-S2 in Figure 2 where no C 1s → $\pi^*_{C=C}$ transition is observed. Instead, typical PEO and peptoid π and σ transitions can be observed as well as the σ^*_{C-F} transition at 293 eV. Moreover, Figure 2 shows that closer to the surface (θ = 120° is more surface sensitive than θ = 55° as discussed in Figure S2, Supporting Information) the $\pi^*_{C=O}$, σ^*_{C-N}, and σ^*_{C-F} transitions are more pronounced, indicating that peptoid chains are concentrated at the surface since the fluorocarbon is attached directly to the peptoid.

A systematic study of the surface segregation behavior of the complete fluorinated peptoid series listed in Table 1 gives further insight into our control of surface composition with the modular PS-*b*-P(EO-*co*-AGE/peptoid) copolymers. There is a strong dependence of surface chemistry on the position of the fluorinated moiety in the peptoid, despite the identical overall chemical composition of the peptoid and therefore the overall polymer. The fluorinated groups were placed at the beginning of the peptoid (closest to the PEO backbone), in the middle of the peptoid, or at the end of the peptoid (farthest from the PEO backbone). All of these peptoids were 15 repeat units long, containing three *N*-(heptafluorobutyl)glycine residues and 12 *N*-(2-methoxyethyl)glycine residues. They are compositionally identical with a molar mass of 2228 Da as observed by matrix-assisted laser desorption ionization (MALDI). The carbon edge NEXAFS spectra shown in Figure 3a show that the σ^*_{C-F} peak from the peptoid and the $\pi^*_{C=C}$ peak from the PS are similar for all three sequences. For all three the surface is covered by the surface-segregating fluorocarbon as indicated by a large σ^*_{C-F} peak, and PS is effectively covered by PEO and peptoid as indicated by the very small $\pi^*_{C=C}$ peak. However, there is a clear difference between the different sequences in the amount of PEO as compared to peptoid as seen in the σ^*_{C-N} and σ^*_{C-O} peaks. Because these peaks are close together and the peptoid is the only source of nitrogen in the system, it is informative to use the nitrogen edge NEXAFS spectrum shown in Figure 3b as an indicator of peptoid content at the surface (partial electron yield at this energy is shown in Table 1). As the fluorinated units were moved from the outer edge of the peptoid toward the PEO backbone, the amount of peptoid at the surfaces decreased as seen in Figure 3b, and the amount of PEO increased because when the fluorine is at the end of the peptoid it dragged only peptoid toward the surfaces, covering the surface with fluorinated peptoid chain ends. However, when the fluorine is close to the PEO backbone, both the PEO and

dx.doi.org/10.1021/mz500090n | ACS Macro Lett. 2014, 3, 364−368

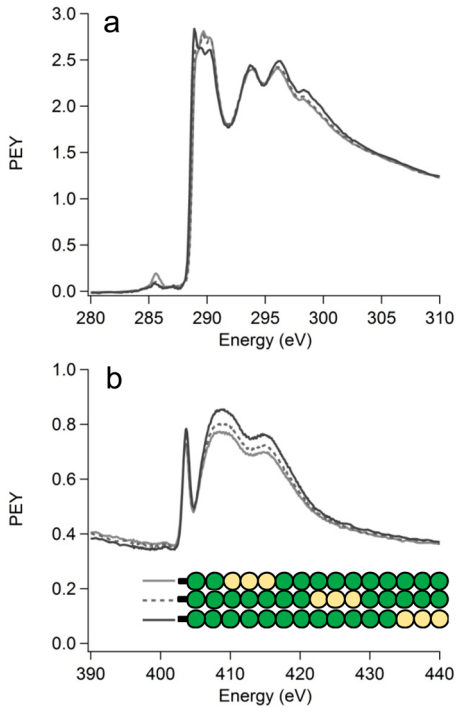


Figure 3. NEXAFS spectra of AGE-(peptoid) copolymers show the surface chemistry of the films. (a) The carbon K-edge spectra show that the surface is dominated by peptoid, PEO, and fluorocarbon units and that there is negligible PS at the surface. They also show that the overall composition of the three surfaces is very similar. (b) The nitrogen K-edge spectra show that there is slightly more peptoid at the surface when the fluorine is at the end of the peptoid.

the peptoid are dragged to the surface, and the surface is covered with fluorinated peptoid loops.

As one might expect, the length of the peptoids also has an effect on the thin-film surface composition. In this case, peptoids were five, ten, and fifteen repeat units long and contained one, two, and three fluorinated residues, respectively. The nitrogen NEXAFS spectra in Figure S3 (Supporting Information) and PEY in Table 1 show that there is less peptoid at the surface of the films with shorter peptoids due to a lower volume fraction of peptoid in the film. From the carbon edge we see that there is a small increase in σ^*_{C-F} peak height with increasing fluorinated residue content. However, there is an increase in the size of the $\pi^*_{C=C}$ peak, indicating an increase in PS content with decreasing peptoid size. This indicates that the five unit and ten unit long peptoids are not able to completely cover the PS and that to achieve a PS-free surface a higher peptoid volume fraction or larger groups are needed.

Finally, Figure S3 (Supporting Information) shows that the fraction of fluorinated groups can be used to control the surface segregation. Decreasing the number of heptafluorobutyl groups does not change the amount of peptoid at the surface as seen in the nitrogen edge NEXAFS spectra, indicating that one group is sufficient to drag all geometrically accessible peptoids to the surface. As expected, we observe an increased σ^*_{C-F} signal for higher fluorine content polymers, but we also see a corresponding decrease in PS because the heptafluorobutyl groups are larger than the methoxyethyl groups and are more effective at covering the underlying PS.

These surfaces were used to study the effect of sequence and surface chemistry on the settlement (attachment) of zoospores and the adhesion strength of sporelings (young plants) of *U. linza*, a widely studied fouling alga. In this system, polystyrene imparts stability to the film, but it is known to be attractive to spore settlement and is thus undesirable at the surface.[10] In this case, the heptafluorobutyl groups are used not only to direct the surface chemistry but also as the hydrophobic residue of our amphiphilic coating.

Assays with zoospores are shown in Figure 4 and demonstrated that while all the surfaces were antifouling with

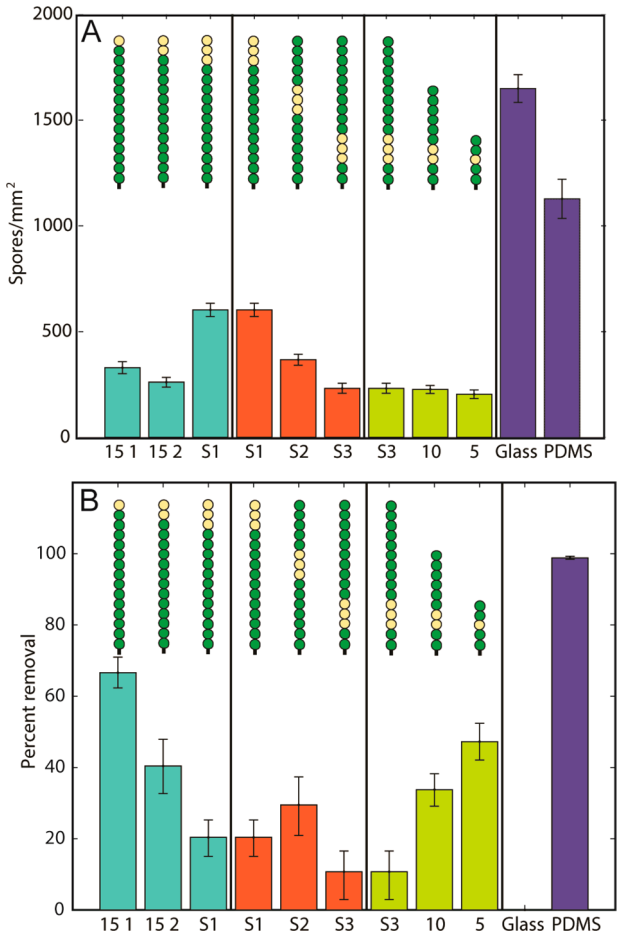


Figure 4. Antifouling and fouling-release assays on peptoid surfaces. (a) Density of attached spores on peptoid surfaces after 45 min settlement. All surfaces performed well as antifouling materials compared to the glass and PDMS standards. The position of the fluorine in the peptoid affects spore settlement, while peptoid length and fluorine number do not have a marked effect. Each bar is the mean from 90 counts on three replicate slides. Bars show 95% confidence limits. (b) Percent removal of sporelings (young plants) from the surfaces after exposure to an impact pressure of 160 kPa, generated by a calibrated water jet. The fluorine content of the peptoid and peptoid length affect fouling release, whereas fluorine position has no effect. As expected, there is high removal of sporelings from the PDMS (fouling-release) standard and no removal from the glass standard. Each bar shows the mean percentage removal of sporeling biomass from six replicate slides. Bars show standard error of the mean.

respect to the glass and PDMS standard the sequence in the peptoid affects both settlement behavior as well as the release properties of sporelings, the young plants that develop from settled spores. Notably, the position of the fluorinated residues in the peptoid has a large effect on the spore settlement density, with the most settlement on the peptoid with the fluorines on the end of the peptoid. This may be due to the decreased

dx.doi.org/10.1021/mz500090n | *ACS Macro Lett.* 2014, 3, 364−368

amount of PEO at the surface, meaning that PEO is better at antifouling than the peptoid itself. While methoxyethyl peptoids have not been previously tested against *Ulva*, they have been shown to be resistant to protein adsorption and cell adhesion, similarly to PEO.[17] Alternatively, the geometry of the polymers at the surface may play a role. When the fluorine is at the end of the peptoid farthest from the PEO backbone, the surface is populated by fluorinated chain ends, whereas when the fluorine moves toward the PEO backbone, the surface is populated by fluorinated polymer loops. The chain ends will have more available conformations and a lower surface energy than the corresponding loops and may have caused the difference in settlement density. The position does not markedly affect the fouling-release properties of the surface.

Peptoid length does not markedly affect antifouling as seen in Figure 4. However, both length and number of fluorinated residues have a large effect on fouling release, where peptoids with fewer heptafluorobutyl groups have better fouling release (i.e., higher % removal of sporelings). While this is unusual as increased fouling release is often associated with higher fluorocarbon content,[18] a similar trend was observed by Dimitriou et al. at very low fluorocarbon content.[12] It is interesting to note that the dominant factor in the peptoids of different length is not the size of the peptoid, but the number of fluorinated residues. In this series, the heptafluorobutyl volume fraction in the peptoid was kept the same, meaning that they contained different numbers of fluorine groups. Both the spore settlement density and the release of sporelings from these peptoids reflected the results obtained for the peptoids with a different number of heptafluorobutyl groups.

We have found that peptoid sequence in our PS-*b*-P(EO-*co*-AGE/peptoid) thin films has a profound effect on both surface structure and marine antifouling properties. Using only two peptoid units, a hydrophilic *N*-(2-methoxyethyl)glycine unit and a hydrophobic *N*-(heptafluorobutyl)glycine unit, we are able to explore the effect of sequence on the properties of interest. For example, polymer thin films with identical chemical composition but different fluorinated group position have different surface composition because the fluorinated group will drag whatever portion of the polymer it is closest to the surface. These films also have different antifouling and fouling-release properties, though it is unclear whether these differences are due to the difference in surface chemical composition, surface structure, or a combination of the two. Studies of sequence-specific peptoid films must be expanded to identify the most important aspects of the sequence to obtain good antifouling properties. Additionally, the highly controllable system presented in this work is a unique opportunity to study the effect of grouping and sequence of other antifouling chemistries such as zwitterions.[19,20] These insights into the relationship between architecture and sequence can be used to design the next generation of amphiphilic antifouling/fouling-release coatings.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic details and additional NEXAFS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: segalman@berkeley.edu.

Author Contributions

○These authors (W.v.Z. and H.G.B.) contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Office of Naval Research (ONR) in the form of a Presidential Early Career Award (PECASE) in Science and Engineering for R.A.S. and via awards N00014-08-1-0010 (J.A.C. and M.E.C.) and N000141110325 (E.J.K.). W.v.Z. gratefully acknowledges The Netherlands Organization for Scientific Research (NWO) for a Rubicon fellowship. H.G.B. acknowledges the National Science Foundation for a graduate fellowship. Polypeptoid synthesis and characterization were performed at the Molecular Foundry, a Lawrence Berkeley National Laboratory user Facility supported by the Office of Science, Office of Basic Energy Sciences, U.S. Department of Energy, under Contract DE-AC02-05CH11231. Use of the National Synchrotron Light Source, Brookhaven National Laboratory, was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract DE-AC02-98CH10.

■ REFERENCES

(1) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646−10647.
(2) Gudipati, C. S.; Finlay, J. A.; Callow, J. A.; Callow, M. E.; Wooley, K. L. *Langmuir* **2005**, *21*, 3044−3053.
(3) Sundaram, H. S.; Cho, Y. J.; Dimitriou, M. D.; Weinman, C. J.; Finlay, J. A.; Cone, G.; Callow, M. E.; Callow, J. A.; Kramer, E. J.; Ober, C. K. *Biofouling* **2011**, *27*, 589−601.
(4) Callow, J. A.; Callow, M. E.; Ista, L. K.; Lopez, G.; Chaudhury, M. K. *J. R. Soc. Interface* **2005**, *2*, 319−325.
(5) Cho, Y. J.; Sundaram, H. S.; Weinman, C. J.; Paik, M. Y.; Dimitriou, M. D.; Finlay, J. A.; Callow, M. E.; Callow, J. A.; Kramer, E. J.; Ober, C. K. *Macromolecules* **2011**, *44*, 4783−4792.
(6) Weinman, C. J.; Finlay, J. A.; Park, D.; Paik, M. Y.; Krishnan, S.; Sundaram, H. S.; Dimitriou, M.; Sohn, K. E.; Callow, M. E.; Callow, J. A.; Handlin, D. L.; Willis, C. L.; Kramer, E. J.; Ober, C. K. *Langmuir* **2009**, *25*, 12266−12274.
(7) Martinelli, E.; Suffredini, M.; Galli, G.; Glisenti, A.; Pettitt, M. E.; Callow, M. E.; Callow, J. A.; Williams, D.; Lyall, G. *Biofouling* **2011**, *27*, 529−541.
(8) Krishnan, S.; Weinman, C. J.; Ober, C. K. *J. Mater. Chem.* **2008**, *18*, 3405−3413.
(9) Finlay, J. A.; Krishnan, S.; Callow, M. E.; Callow, J. A.; Dong, R.; Asgill, N.; Wong, K.; Kramer, E. J.; Ober, C. K. *Langmuir* **2008**, *24*, 503−510.
(10) Grozea, C. M.; Gunari, N.; Finlay, J. A.; Grozea, D.; Callow, M. E.; Callow, J. A.; Lu, Z. H.; Walker, G. C. *Biomacromolecules* **2009**, *10*, 1004−1012.
(11) Krishnan, S.; Ayothi, R.; Hexemer, A.; Finlay, J. A.; Sohn, K. E.; Perry, R.; Ober, C. K.; Kramer, E. J.; Callow, M. E.; Callow, J. A.; Fischer, D. A. *Langmuir* **2006**, *22*, 5075−5086.
(12) Dimitriou, M. D.; Zhou, Z. L.; Yoo, H. S.; Killops, K. L.; Finlay, J. A.; Cone, G.; Sundaram, H. S.; Lynd, N. A.; Barteau, K. P.; Campos, L. M.; Fischer, D. A.; Callow, M. E.; Callow, J. A.; Ober, C. K.; Hawker, C. J.; Kramer, E. J. *Langmuir* **2011**, *27*, 13762−13772.
(13) Lee, B. F.; Kade, M. J.; Chute, J. A.; Gupta, N.; Campos, L. M.; Fredrickson, G. H.; Kramer, E. J.; Lynd, N. A.; Hawker, C. J. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 4498−4504.
(14) Lee, B. F.; Wolffs, M.; Delaney, K. T.; Sprafke, J. K.; Leibfarth, F. A.; Hawker, C. J.; Lynd, N. A. *Macromolecules* **2012**, *45*, 3722−3731.

(15) van Zoelen, W.; Zuckermann, R. N.; Segalman, R. A. *Macromolecules* **2012**, *45*, 7072−7082.
(16) Sohn, K. E.; Dimitriou, M. D.; Genzer, J.; Fischer, D. A.; Hawker, C. J.; Kramer, E. J. *Langmuir* **2009**, *25*, 6341−6348.
(17) Statz, A. R.; Meagher, R. J.; Barron, A. E.; Messersmith, P. B. *J. Am. Chem. Soc.* **2005**, *127*, 7972−7973.
(18) Imbesi, P. M.; Finlay, J. A.; Aldred, N.; Eller, M. J.; Felder, S. E.; Pollack, K. A.; Lonnecker, A. T.; Raymond, J. E.; Mackay, M. E.; Schweikert, E. A.; Clare, A. S.; Callow, J. A.; Callow, M. E.; Wooley, K. L. *Polym. Chem.* **2012**, *3*, 3121−3131.
(19) Zhang, Z.; Finlay, J. A.; Wang, L. F.; Gao, Y.; Callow, J. A.; Callow, M. E.; Jiang, S. Y. *Langmuir* **2009**, *25*, 13516−13521.
(20) Aldred, N.; Li, G. Z.; Gao, Y.; Clare, A. S.; Jiang, S. Y. *Biofouling* **2010**, *26*, 673−683.