

Evidence for Why Tri(ethylene oxide) Functionalized Si–C Linked Monolayers on Si(111) Have Inferior Protein Antifouling Properties Relative to the Equivalent Alkanethiol Monolayers Assembled on Gold

Till Böcking,^{A,B} Michael Gal,^B Katharina Gaus,^C and J. Justin Gooding^{A,D}

^A School of Chemistry, University of New South Wales, Sydney NSW 2052, Australia.

^B School of Physics, University of New South Wales, Sydney NSW 2052, Australia.

^C Centre for Vascular Research, School of Medical Sciences, University of New South Wales, Sydney NSW 2052, Australia.

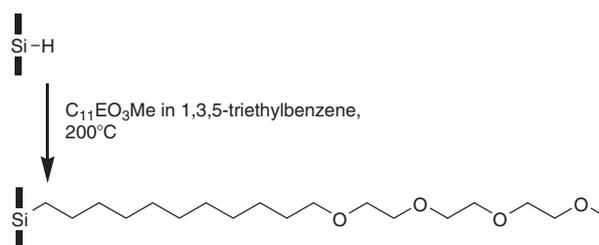
^D Corresponding author. Email: Justin.Gooding@unsw.edu.au

High quality methoxy-terminated monolayers containing a tri(ethylene oxide) moiety were formed on Si(111)–H surfaces in thermal hydrosilylation reactions. X-ray photoelectron spectroscopy, contact angle, and X-ray reflectivity measurements suggested that the suboptimal protein anti-fouling properties of these Si–C linked monolayers were due to a reduced lateral packing density of the chains resulting in a disordered layer with insufficient internal and external hydrophilicity.

Manuscript received: 16 May 2005.

Final version: 19 July 2005.

The formation of inert coatings designed to prevent bio-fouling on surfaces is crucial for improving the selectivity and sensitivity of devices for monitoring biomolecular interactions such as biosensors and microarrays. Self-assembled monolayers (SAMs) of alkanethiols on gold with hydroxyl or methoxy terminated oligo(ethylene oxide) (EO_n, *n* ≥ 3) moieties resist the non-specific adsorption of proteins.^[1,2] The mechanism of protein resistance is thought to involve the binding of interfacial water by the EO moieties^[3,4] amongst other factors including the internal and terminal hydrophilicity, and the chain packing which impacts the lateral compression in the monolayer.^[5] Recently, the formation of inert monolayers has been extended to silicon surfaces.^[6–8] Silicon substrates have advantages over gold, including smooth surfaces and compatibility with existing semiconductor nanofabrication technologies, and can be modified with extremely robust Si–C linked monolayers in thermal, catalyzed, or photochemical hydrosilylation reactions of alkenes.^[9,10] Many functional terminal groups have been incorporated on Si–C linked monolayers for immobilization of DNA,^[11–14] saccharides,^[15] and peptides,^[16] and chemical transformations and coupling chemistries have been investigated using model compounds.^[17–19] Photochemical hydrosilylation of EO_n-functionalized alkenes terminated with hydroxyl groups (*n* = 3)^[6] or methoxy groups (*n* = 3, 6, 7, 9)^[7,8] on Si(111)–H yielded monolayers with anti-fouling properties. In these studies monolayers with three EO units were less efficient in reducing the non-specific adsorption of proteins^[7] than the equivalent layers of EO₃ functionalized thiols on gold. Here the formation of monolayers formed



Scheme 1. Surface modification of Si(111)–H by thermal hydrosilylation of C₁₁EO₃Me.

by hydrosilylation of 11-(2-(2-(2-methoxyethoxy)ethoxy)undec-1-ene (C₁₁EO₃Me) using a thermal method with 1,3,5-triethylbenzene as the solvent for the reaction (Scheme 1) is reported. The chemical composition and structure of this monolayer were characterized by X-ray photoelectron spectroscopy (XPS), water contact angles, and X-ray reflectometry (XR). The purpose of this paper is to analyze and discuss the XPS, contact angle, and XR data in the context of previously observed differences in the protein resisting properties of EO₃Me terminated layers on silicon and gold.

Monolayer formation was carried out in a solution of the alkene C₁₁EO₃Me in 1,3,5-triethylbenzene at 200°C. The EO_n moieties of the functionalized alkenes are expected to trap traces of water. During the hydrosilylation reaction this trapped water reacts with the hydride-terminated silicon surface, resulting in low-quality monolayers associated with high levels of silicon dioxide. To reduce the amount of water remaining in the alkene solution, the alkene and solvent

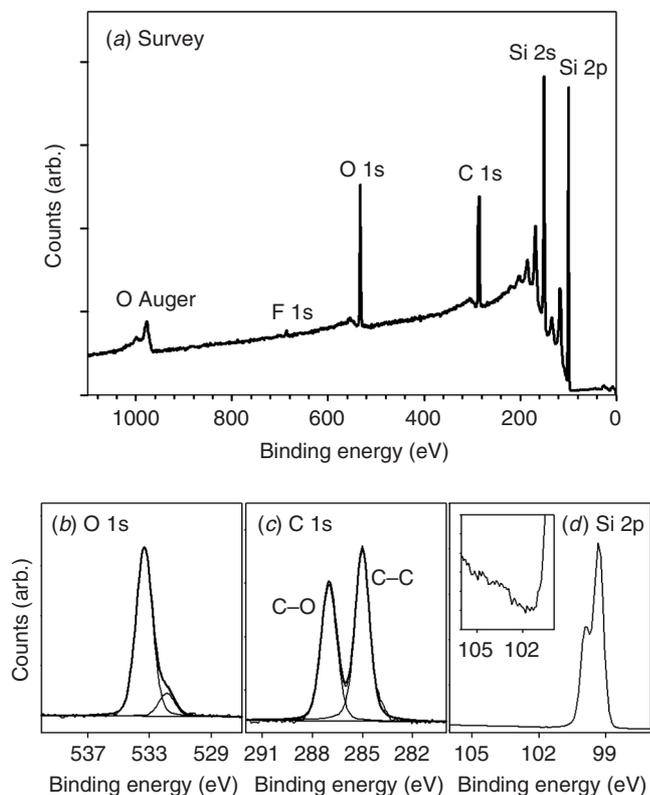


Fig. 1. (a) XPS survey spectrum, and XPS narrow scans of the (b) O 1s, (c) C 1s, and (d) Si 2p regions of Si(111) derivatized with $C_{11}EO_3Me$. The inset in (d) shows an enlargement of the Si 2p region where the peak due to oxidized silicon is expected (102–105 eV).

were stirred over sodium, redistilled under reduced pressure, and stored over molecular sieves under an argon atmosphere. Fig. 1a shows the XP survey scan of a monolayer formed by reaction of $C_{11}EO_3Me$ with Si(111)-H under conditions described above. As expected, the oxygen 1s and carbon 1s signals from the organic monolayer were detectable at 533 and 285 eV respectively, and the silicon 2s and 2p signals from the underlying substrate were present at 150 and 99 eV respectively. A small fluorine 1s signal at 686 eV indicated traces of fluoride ions remaining on the surface after etching. The high quality of the surface was evident from the silicon 2p narrow scan (Fig. 1d). Significant oxidation of the Si-H surface during or after sample preparation (as observed for low quality samples) would be detectable as a broad peak between 102 and 105 eV. Here the peak corresponding to oxidized silicon was barely detectable in the silicon 2p region. The oxygen 1s narrow scan (Fig. 1b) contained a main peak at 533.3 eV mainly due to the oxygen atoms of the organic monolayer and also a small shoulder at 531.9 eV, which was tentatively assigned to the presence of low levels of oxidized silicon.* The C 1s envelope (Fig. 1c) was fitted with two peaks. The peak at 285.0 eV was assigned to the C-C bonded carbons of the alkyl chain and the peak at 287.0 eV to the C-O bonded carbons of the triethylene oxide moiety. The area ratio of

the C-C and C-O bonded carbons was 9.4:8–9.9:8, close to the stoichiometric ratio of 10:8 for the surface-attached $C_{11}EO_3Me$ molecule.

Based on XPS data, the molecular surface coverage was estimated to be 0.38–0.40 chains per silicon surface atom.^[20] This surface coverage is consistent with that reported for the photochemically attached monolayer of $C_{11}EO_3Me$,^[7] and points to a significantly reduced packing density compared with the 0.5 chains per silicon surface atom proposed and determined experimentally for unfunctionalized Si-C linked monolayers.^[20,21] The reduced grafting density is expected to result in a some disorder in the adsorbed monolayer. Some of the oligoethoxy moieties may partially penetrate into the underlying alkyl layer,^[22] leading to mixing of the alkyl and EO_3Me chains. This picture of orientational disorder is supported by the XPS data. For an ordered bilayer structure in which the EO_3Me moieties form a separate layer on top of the alkyl chain layer, the intensity of photoelectrons originating from the alkyl layer would be attenuated by the overlying EO_3Me layer. Taking this attenuation into account, the theoretical ratio of the peak area for C-C bonded carbons to that of C-O bonded carbons in the carbon 1s narrow scan would be approximately 1:1 (see Accessory Material). This attenuation has been observed for highly ordered EO_n terminated alkanethiol SAMs on gold.^[3] For the Si-C linked monolayers described here the attenuation is not evident and instead a ratio close to the stoichiometric ether carbon-to-alkyl carbon ratio is observed, signifying disorder in these types of monolayers.

The wettability of the surface was studied using water contact angle measurements. The advancing and receding contact angles of the $C_{11}EO_3Me$ modified surface (57° , 55°) were in good agreement with those reported for the same monolayer prepared using photochemical methods (59° , 56°).^[7] However, the advancing contact angle was considerably lower than reported for the equivalent monolayer on gold and silver (63 – 65°),^[3,5] indicating greater exposure of the ether oxygen atoms to the liquid due to disorder in the chains. In contrast, the contact angles observed here were higher than those determined for Si-C linked monolayers terminated with EO_nMe ($n = 6, 9$) moieties,^[7] which indicated that in the monolayers reported here the underlying alkyl chains were insufficiently shielded by the EO_3Me chains. Finally, the low hysteresis observed here compared to thiol SAMs on gold ($\sim 10^\circ$)^[5] is attributed to the extremely low surface roughness of the Si(111) substrate (etching in 40% ammonium fluoride solution yields atomically smooth surfaces^[23]).

An indication of the packing density and structure of the monolayers can be obtained using XR. XR has been used previously for the characterization of Si-C linked monolayers.^[19,21,24] Fig. 2 shows the reflectivity curve as a function of momentum transfer (Q_z) of Si(111) derivatized with dilute $C_{11}EO_3Me$ in 1,3,5-triethylbenzene at $200^\circ C$. Structural parameters such as the thickness, electron density,

* The detection of oxygen is more sensitive than that of silicon in XPS. Thus, oxide can be detected more readily in the oxygen 1s region than in the silicon 2p region. The level of oxide detected here was considered to be insignificant. Even samples prepared from non-functionalized alkenes often reveal similar levels of surface oxidation.

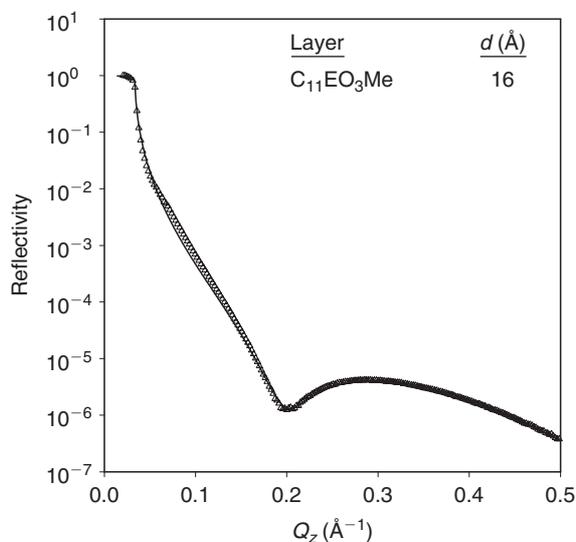


Fig. 2. XR curve of Si(111) derivatized with a monolayer formed by hydrosilylation of $C_{11}EO_3Me$. The solid line represents a model fit to the reflectivity data.

and interfacial roughness were determined by refining a structural model for the reflectivity data. A monolayer model was sufficient to achieve a satisfactory fit. The thickness of the layer was approximately 16 Å, which was around 3 Å shorter than the estimated minimum thickness of 19 Å for an ordered $C_{11}EO_3Me$ monolayer assuming fully extended alkyl chains with a tilt angle of 40–45° from surface normal, and a helical or extended conformation of the EO_3Me chains. There was ambiguity in determining the electron density of the layer because it was found to vary between 0.35 and 0.42 $e\text{Å}^{-3}$ for fits of similar quality. The range was between the electron densities of methyl-terminated Si–C linked alkyl monolayers ($\sim 0.31 e\text{Å}^{-3}$)^[21,24] and the value reported for self-assembled monolayers of low molecular weight EO_n -terminated silanes on silicon dioxide surfaces (0.44 $e\text{Å}^{-3}$).^[25] Surprisingly, a bilayer model (assuming separate electron densities for the alkyl and EO_3Me regions of the monolayer) did not result in a significantly improved fit to the XR data. The interfacial roughness at the substrate–monolayer and the monolayer–air interfaces for the monolayer in Fig. 2 was 2–3 Å, as observed previously for these Si–C linked layers.^[19,24]

The considerably smaller than expected thickness observed for the Si–C linked $C_{11}EO_3Me$ monolayer further supported the existence of a high degree of disorder leading to partial collapse. Further, the inability to obtain an improved fit using a bilayer model for the $C_{11}EO_3Me$ SAM suggested that the alkyl and EO_3Me moieties were not present as distinct separate layers.

Preliminary studies to determine the ability of the Si–C linked $C_{11}EO_3Me$ SAM prepared using thermal conditions to resist the non-specific adsorption of protein were carried out using fluorescein-labelled bovine serum albumin (F-BSA). It was found that the surface had partial anti-fouling properties, reducing the adsorption of F-BSA by 70–80% compared with adsorption on Si–C linked octadecyl monolayers.

The ability of EO_n and EO_nMe terminated monolayers to resist non-specific adsorption of proteins depends on factors including the packing density of the chains in the monolayer, which influences the conformation of the EO_n units and their ability to coordinate water.^[5] The protein-resistance of EO_3Me terminated monolayers is reduced when the packing density is too high, as observed for these types of SAMs on silver.^[26] Here it is suggested that anti-fouling properties are also reduced when the packing density falls below a critical value. The packing density of $C_{11}EO_3Me$ SAMs on silicon with an estimated 3.0–3.1 molecule nm^{-2} (based on XPS data) was considerably lower than that of the chemically equivalent but highly inert SAMs on gold (3.61 molecule nm^{-2}).^[5] This arrangement is likely to result in a collapse of the layer to the extent that partial mixing of alkyl and EO_3Me moieties occurs. As a consequence of the mixing of the monolayer components, the hydrophilicity of the interior and exterior of the SAM polyether region is reduced so to compromise the anti-fouling properties. A similar observation of protein adsorption to poorly packed EO_3Me terminated monolayers formed by electrochemical reduction of the corresponding aryl diazonium salt on glassy carbon electrodes was reported.^[27]

In conclusion, it was demonstrated that high-quality (low oxide) SAMs of $C_{11}EO_3Me$ could be formed on Si(111)–H by a thermal method. The monolayers exhibited a lower grafting density than observed for unfunctionalized Si–C linked monolayers, which may have led to partial collapse of the layer with possible partial mixing of hydrophilic and hydrophobic moieties. This orientational disorder was supported by XPS and XR data and may be responsible for the reduced hydrophilicity of the interface, and thus the suboptimal anti-fouling properties of these monolayers. Further experiments investigating the structure of these types of monolayers on silicon are required to corroborate the molecular picture suggested by the preliminary experiments presented here.

Experimental

1,3,5-triethylbenzene (97%) and octadecene (95%) were purchased from Fluka, redistilled from sodium under vacuum, and stored over molecular sieves under argon. Semiconductor grade chemicals were used for cleaning (30% H_2O_2 , 98% H_2SO_4) and etching (40% NH_4F solution) pieces of silicon wafer.

Si(111) wafer pieces (p-type, 1–10 Ωcm) were cleaned in Piranha solution (concentrated H_2SO_4 –30% H_2O_2 , 3:1, v/v) at 90°C for 20–30 min followed by rinsing with excess Milli-Q water. Hydrogen-terminated Si(111) surfaces were prepared by etching in deoxygenated 40% solution of NH_4F for 15–20 min. Monolayers were formed using thermal hydrosilylation reactions at 200°C in a deoxygenated 0.2 M solution of alkene in 1,3,5-triethylbenzene for 7 h. The sample was rinsed several times with dichloromethane and ethyl acetate and blown dry under a stream of argon.

XPS spectra were obtained by means of an EscaLab 220-IXL spectrometer with a monochromated $Al_{K\alpha}$ source (1486.6 eV), hemispherical analyzer, and multichannel detector. The spectra were accumulated at a take-off angle of 90° with a 0.79 mm^2 spot size at a pressure of less than 10^{-8} mbar.

Samples for X-ray reflectometry were packaged under argon in screw-cap glass vials for transport and storage. X-ray reflectivity curves were acquired at the Brisbane Surface Analysis Facility using

a Bruker D8 Advance diffractometer in reflectometer mode. *Parratt32* software^[29] was used to fit model parameters to measured sets of XR data with linear background correction as described previously.^[19]

Protein adsorption was studied by immersing the sample into a solution of fluorescein-labelled BSA (1 mg mL^{-1}) in phosphate-buffered saline (pH 7.4) for 1 h, followed by rinsing with Milli-Q H₂O for 1 min and drying under argon. The samples were mounted with a glycerol based mounting medium (Vectashield, Vector Labs) and covered with coverslips. Fluorescence was measured using a Fujifilm FLA 5000 fluorescence scanner. The fluorescence signal determined after adsorption of F-BSA onto an octadecyl monolayer on Si was taken as monolayer coverage with protein. The background fluorescence was determined on silicon samples derivatized with monolayers but not exposed to protein solution.

11-(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)undec-1-ene (C₁₁EO₃Me)

Triethylene glycol monomethyl ether (6.57 g, 95%, 38 mmol) was added dropwise to a cooled and stirred suspension of NaH (1.2 g, 50 mmol) in anhydrous tetrahydrofuran (80 mL) under argon followed by addition of 11-bromoundecene (9.33 g, 95%, 38 mmol). The reaction mixture was allowed to warm to room temperature and refluxed over night. After filtration by suction, the filtrate was concentrated under reduced pressure. The crude product was taken up in light petroleum and the organic phase was washed with brine, dried over Na₂SO₄, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (light petroleum:ethyl acetate 1:1, v/v) to yield the title compound as a colourless oil (8.76 g, 73%). For monolayer preparation the compound was then dried and further purified by vacuum distillation from sodium (0.01–0.02 mmHg/176°C). The distilled product was stored over molecular sieves under an Ar atmosphere. δ_{H} (300 MHz, CDCl₃) 5.67 (m, 1 H, CH₂=CH), 4.81 (m, 2 H, CH₂=CH), 3.49–3.46 (m, 12 H, (OCH₂CH₂)₃), 3.32 (t, 2 H, (CH₂)₇CH₂CH₂O), 3.24 (s, 3 H, OCH₃), 1.91 (q, 2 H, =CHCH₂), 1.44 (quint, 2 H, (CH₂)₇CH₂CH₂O), 1.16 (bsr, 12 H, =CHCH₂(CH₂)₆).

Accessory Materials

¹H NMR spectrum of C₁₁EO₃Me and calculation of attenuation factors for XPS analysis are available from the authors or, until September 2010, the *Australian Journal of Chemistry*.

Acknowledgments

This work was supported by the Australian Research Council and the Australian Institute for Nuclear Science and Engineering. The authors thank Dr Jeremy Ruggles for help with X-ray reflectometry.

References

- [1] K. L. Prime, G. M. Whitesides, *Science* **1991**, 252, 1164.
- [2] K. L. Prime, G. M. Whitesides, *J. Am. Chem. Soc.* **1993**, 115, 10714. doi:10.1021/JA00076A032
- [3] P. Harder, M. Grunze, R. Dahint, G. M. Whitesides, P. E. Laibinis, *J. Phys. Chem. B* **1998**, 102, 426. doi:10.1021/JP972635Z
- [4] E. Ostuni, R. G. Chapman, R. E. Holmlin, S. Takayama, G. M. Whitesides, *Langmuir* **2001**, 17, 5605. doi:10.1021/LA010384M
- [5] S. Herrwerth, W. Eck, S. Reinhardt, M. Grunze, *J. Am. Chem. Soc.* **2003**, 125, 9359. doi:10.1021/JA034820Y
- [6] T. L. Lasseter, B. H. Clare, N. L. Abbott, R. J. Hamers, *J. Am. Chem. Soc.* **2004**, 126, 10220. doi:10.1021/JA047642X
- [7] C. M. Yam, J. M. Lopez-Romero, J. H. Gu, C. Z. Cai, *Chem. Commun.* **2004**, 2510. doi:10.1039/B401499E
- [8] C. M. Yam, J. H. Gu, S. Li, C. Z. Cai, *J. Colloid Interface Sci.* **2005**, 285, 711. doi:10.1016/J.JCIS.2004.12.007
- [9] D. D. M. Wayner, R. A. Wolkow, *J. Chem. Soc., Perkin Trans. 2* **2002**, 23. doi:10.1039/B100704L
- [10] A. B. Sieval, R. Linke, H. Zuilhof, E. J. R. Sudholter, *Adv. Mater.* **2000**, 12, 1457. doi:10.1002/1521-4095(200010)12:19<1457::AID-ADMA1457>3.0.CO;2-#
- [11] T. Strother, R. J. Hamers, L. M. Smith, *Nucleic Acids Res.* **2000**, 28, 3535. doi:10.1093/NAR/28.18.3535
- [12] T. Strother, W. Cai, X. Zhao, R. J. Hamers, L. M. Smith, *J. Am. Chem. Soc.* **2000**, 122, 1205. doi:10.1021/JA9936161
- [13] H. B. Yin, T. Brown, J. S. Wilkinson, R. W. Eason, T. Melvin, *Nucleic Acids Res.* **2004**, 32, 118. doi:10.1093/NAR/GNH113
- [14] R. Voicu, R. Boukherroub, V. Bartzoka, T. Ward, J. T. C. Wojtyk, D. D. M. Wayner, *Langmuir* **2004**, 20, 11713. doi:10.1021/LA047886V
- [15] L. C. P. M. de Smet, G. A. Stork, G. H. F. Hurenkarnp, Q. Y. Sun, H. Topal, P. J. E. Vronen, A. B. Sieval, A. Wright, G. M. Visser, H. Zuilhof, E. J. R. Sudholter, *J. Am. Chem. Soc.* **2003**, 125, 13916. doi:10.1021/JA0374451
- [16] Y. Coffinier, C. Olivier, A. Perzyna, B. Grandidier, X. Wallart, J. O. Durand, G. Melnyk, D. Stievenard, *Langmuir* **2005**, 21, 1489. doi:10.1021/LA047781S
- [17] R. Boukherroub, D. D. M. Wayner, *J. Am. Chem. Soc.* **1999**, 121, 11513. doi:10.1021/JA992032W
- [18] A. B. Sieval, R. Linke, G. Heij, G. Meijer, H. Zuilhof, E. J. R. Sudholter, *Langmuir* **2001**, 17, 7554. doi:10.1021/LA010484S
- [19] T. Böcking, M. James, H. G. L. Coster, T. C. Chilcott, K. D. Barrow, *Langmuir* **2004**, 20, 9227. doi:10.1021/LA048474P
- [20] R. L. Cicero, M. R. Linford, C. E. D. Chidsey, *Langmuir* **2000**, 16, 5688. doi:10.1021/LA9911990
- [21] M. R. Linford, P. Fenter, P. M. Eisenberger, C. E. D. Chidsey, *J. Am. Chem. Soc.* **1995**, 117, 3145. doi:10.1021/JA00116A019
- [22] K. E. Nelson, L. Gamble, L. S. Jung, M. S. Boeckl, E. Naeemi, S. L. Golledge, T. Sasaki, D. G. Castner, C. T. Campbell, P. S. Stayton, *Langmuir* **2001**, 17, 2807. doi:10.1021/LA001111E
- [23] P. Allongue, C. Henry de Villeneuve, S. Morin, R. Boukherroub, D. D. M. Wayner, *Electrochim. Acta* **2000**, 45, 4591. doi:10.1016/S0013-4686(00)00610-1
- [24] A. B. Sieval, A. L. Demirel, J. W. M. Nissink, M. R. Linford, J. H. V. D. Maas, W. H. D. Jeu, H. Zuilhof, E. J. R. Sudholter, *Langmuir* **1998**, 14, 1759. doi:10.1021/LA971139Z
- [25] A. Papra, N. Gadegaard, N. B. Larsen, *Langmuir* **2001**, 17, 1457. doi:10.1021/LA000609D
- [26] A. J. Pertsin, M. Grunze, I. A. Garbuzova, *J. Phys. Chem. B* **1998**, 102, 4918. doi:10.1021/JP9806617
- [27] A. J. Downard, S. L. Jackson, E. S. Q. Tan, *Aust. J. Chem.* **2005**, 58, 275. doi:10.1071/CH04259
- [28] C. Braun, *Parratt32. The Reflectivity Tool 1.5.2 1999* (Hahn Meitner Institut: Berlin).