JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

pubs.acs.org/JACS

Cooperative Adsorption of Lipoprotein Phospholipids, Triglycerides, and Cholesteryl Esters Are a Key Factor in Nonspecific Adsorption from Blood Plasma to Antifouling Polymer Surfaces

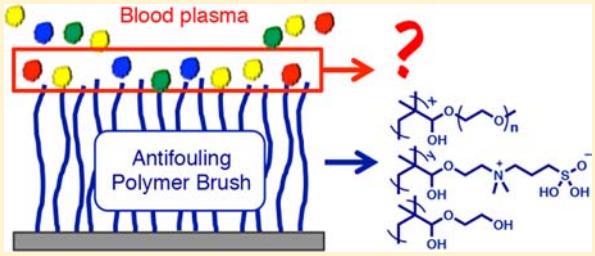
Gesine Gunkel[†] and Wilhelm T. S. Huck*[,†,‡]

[†]Melville Laboratory for Polymer Synthesis, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB21EW, United Kingdom

[‡]Institute for Molecules and Materials, Radboud University Nijmegen, Heyendaalseweg 135, 6525 Nijmegen, The Netherlands

Supporting Information

ABSTRACT: Nonspecific protein adsorption is a central challenge for the use of polymeric materials in biological media. While the quantity of adsorbed protein can be lowered, very few surfaces are protein resistant when exposed to undiluted serum or plasma. The underlying principles of this fouling and the adsorbing proteins remain to be identified. Here, we investigated adsorption from undiluted human blood plasma to three different polymer brushes. Our study showed that the polymer structure does not influence which proteins adsorb. Further, we identified 98 plasma proteins that still foul current "protein-resistant" polymer brushes. Detailed studies into the major adsorbing protein revealed the central role that lipoproteins and low density lipoprotein in particular play in fouling of plasma to polymeric biomaterials. However, although apolipoprotein B100 is found as a major fouling protein in our mass spectrometry screening, studies on individual components of lipoproteins show that it is not apoB100 but a mixture of phospholipids, triglycerides, and cholesteryl esters that plays a major role in lipoprotein adsorption.



INTRODUCTION

Fouling of surfaces in contact with tissue or blood is a major clinical problem, and there has been an enormous research effort to develop surfaces that resist the nonspecific adsorption of proteins (so-called antifouling surfaces).[1,2] Much of this effort has been guided by empirical sets of design rules obtained from model substrates such as self-assembled monolayers[1−5] and by early promising results of poly(ethylene glycol) (PEG)-based material.[4,6] In recent years, polymer brushes (surface coatings based on polymer chains tethered at one end to the surface)[7] have emerged as superior coatings that show ultralow (<5 ng cm^{-2}) fouling from single protein solutions.[8] Unfortunately, very few surfaces show equally good properties when in contact with clinically relevant complex protein mixtures such as plasma or serum, and even then, most studies deal with fetal bovine serum, which is much less fouling than human blood.[8−12] Progress in the search for better coatings is hindered by a lack of knowledge about which proteins in serum or plasma cause fouling. Therefore, the aim of the present study is to understand the interactions between plasma and antifouling polymers. This will be achieved by identifying the nonspecifically adsorbed proteins from plasma to brushes that exhibit ultralow fouling from single protein solutions. Since human blood plasma contains more than 4500 proteins,[13] identification of the proteins that adsorb is a challenging task. Besides a bioinformatical approach,[14] the combination of gel electrophoresis and liquid chromatography−tandem mass spectrometry (LC-MS/MS) has recently been used to identify proteins adsorbed to nanomaterials.[15−19] Here, we use this strategy to study fouling from plasma onto three classes of antibiofouling polymers with excellent protein resistant properties: polyether-based bottle brushes,[6,20] small side-chain zwitterionic polymers,[11,21] and hydroxylated polymers.[12,22] Further, we set out to elucidate the effect of the different polymer brush structures on plasma adsorption. It has been demonstrated in many studies that the choice of polymer can greatly reduce the amount of adsorbed protein.[8] However, it is unknown whether the composition of the adsorbed protein layer is influenced by the polymer chemistry. The polymer brushes used here, poly(oligoethylene glycol) methacrylate (POEGMA), poly(sulfobetaine methacrylate) (PSBMA), and poly(hydroxyethyl methacrylate) (PHEMA) are depicted in Figure 1.

RESULTS AND DISCUSSION

Polymer Brush Synthesis. Polymer brushes were synthesized by surface-initiated atom transfer radical polymerization (ATRP) from initiator-functionalized gold-coated surface plasmon resonance (SPR) sensor chips and silica particles. The brushes were prepared at a dry thickness between 20 and 30 nm,[23−25] which ensures complete surface coverage

Received: February 28, 2013
Published: April 12, 2013

   dx.doi.org/10.1021/ja402126t | J. Am. Chem. Soc. 2013, 135, 7047−7052

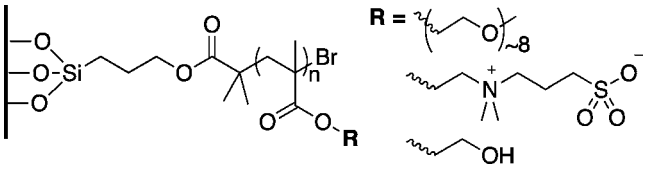


Figure 1. Chemical structures of the three polymer brushes compared: POEGMA, PSBMA, and PHEMA.

and gives optimal protein repelling properties.[22] Micrometer-sized silica particles were selected to exclude any potential effects of surface curvature on protein adsorption.[15,26] The polymer brushes were thoroughly characterized by several methods. The thickness and swelling factor in phosphate-buffered saline (PBS) buffer were measured by ellipsometry, the topography was determined by atomic force microscopy (AFM), and the wettability was probed by captive bubble contact angles (see the Supporting Information for details). Protein adsorption from undiluted and unfiltered human blood plasma was studied by SPR[27] (see Figure 2a) on flat surfaces with polymer brushes directly synthesized on SPR sensor chips.

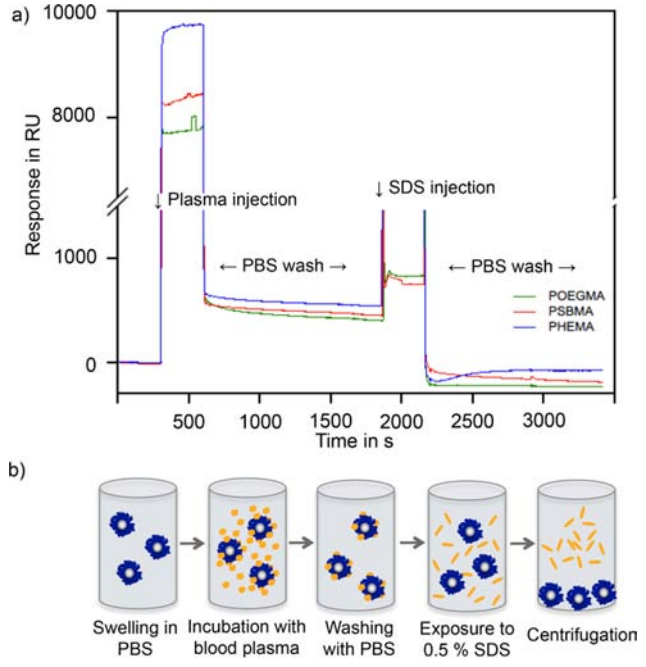


Figure 2. (a) Exemplary SPR sensorgrams showing the adsorption of plasma and complete removal of adsorbed proteins with 0.5% SDS for all three brushes. (b) Illustration of the protocol on brush-coated particles.

Protein Adsorption and Desorption. Detailed analysis of the adsorbed proteins requires complete desorption from the polymer brushes. This was achieved by exposure to 0.5% sodium dodecyl sulfate (SDS) in PBS, as can be seen in Figure 2a. After exposure to SDS, the SPR signal reaches the level of the baseline again. Further, larger sample volumes are required for analysis, which cannot be obtained by SPR. Hence, the protocol of the SPR experiment was adapted to polymer brushes on silica particles as illustrated in Figure 2b, where the amount of desorbed protein sample can be easily adjusted by the particle concentration. The amount of adsorbed protein was quantified with a bicinchoninic acid (BCA) assay after desorption from brush-coated particles. As the total surface area of the particles is known, protein adsorption in nanogram per centimeter squared can be calculated, which enables a comparison with SPR data. The results obtained for all three brushes are very similar for both methods (see Figure S7, Supporting Information), ranging 27−43 ng cm^{-2} for POEGMA, 20−49 ng cm^{-2} for PSBMA, and 38−55 ng cm^{-2} for PHEMA. These values are in line with other studies using undiluted human blood plasma.[8,11,12]

Analysis of Adsorbed Proteins. The proteins were separated and visualized by electrophoresis on a polyacrylamide gel (see Figure 3). The proteins desorbed from the three

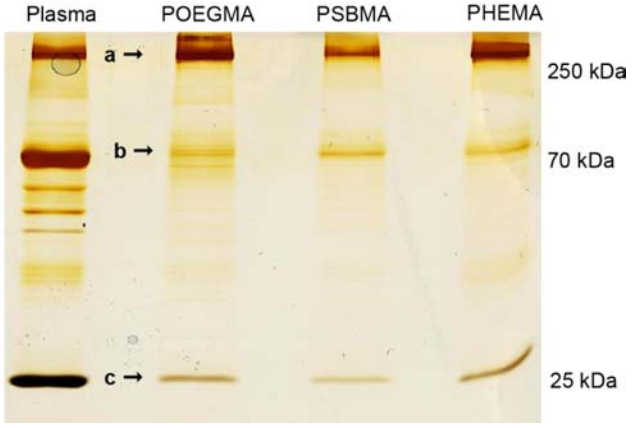


Figure 3. Silver-stained electrophoresis gel of proteins adsorbed to the polymer brushes. Human blood plasma (dilution 1/200) is shown for comparison, and major bands used for mass spectrometry are labeled.

polymer brushes were loaded on the gel together with human blood plasma for comparison. As anticipated, the gel shows that many plasma proteins are repelled by the brushes. Only a small fraction adsorbs to the polymer brushes, with the main bands identified at greater than 250 kDa, around 70 kDa, and at 25 kDa. Interestingly, the same bands are observed for all three brushes, despite their different hydroxylated, PEG-based, and zwitterionic structures. Furthermore, the absence of other bands indicates that some proteins adsorb more strongly than others. This emphasizes the need to characterize the adsorbed proteins, as knowledge of their identity will enable designing future antibiofouling coatings that also repel these proteins.

Identification of Adsorbed Proteins. In order to analyze the proteins that still adsorb to the polymer brushes further and to uncover their identify, the proteins contained in the main bands observed on the gel (labeled a−c in Figure 3) were subjected to LC-MS/MS analysis.[28,29] The results are visualized as heatmaps in Figure 4, showing the relative abundances[30] of all proteins for each polymer brush. A total of 98 different proteins were identified, which corresponds to approximately 2% of the proteins found in human blood plasma.[13] Importantly, each band observed on the gel contains two to three main proteins with high relative abundances (Figure 4, top row). These are the same for all three brushes, regardless of the zwitterionic, polyether, or hydroxylated side chains. Among the identified proteins are albumin, the most abundant protein in plasma (up to 800 μM), but also others such as S100-A8/9 proteins with natural abundances of 0.1 μM or less.[31] The results indicate that fouling is not a random process and raise the question why exactly these proteins bind. Further analysis of our results highlights the very high relative abundance of

dx.doi.org/10.1021/ja402126t | J. Am. Chem. Soc. 2013, 135, 7047−7052

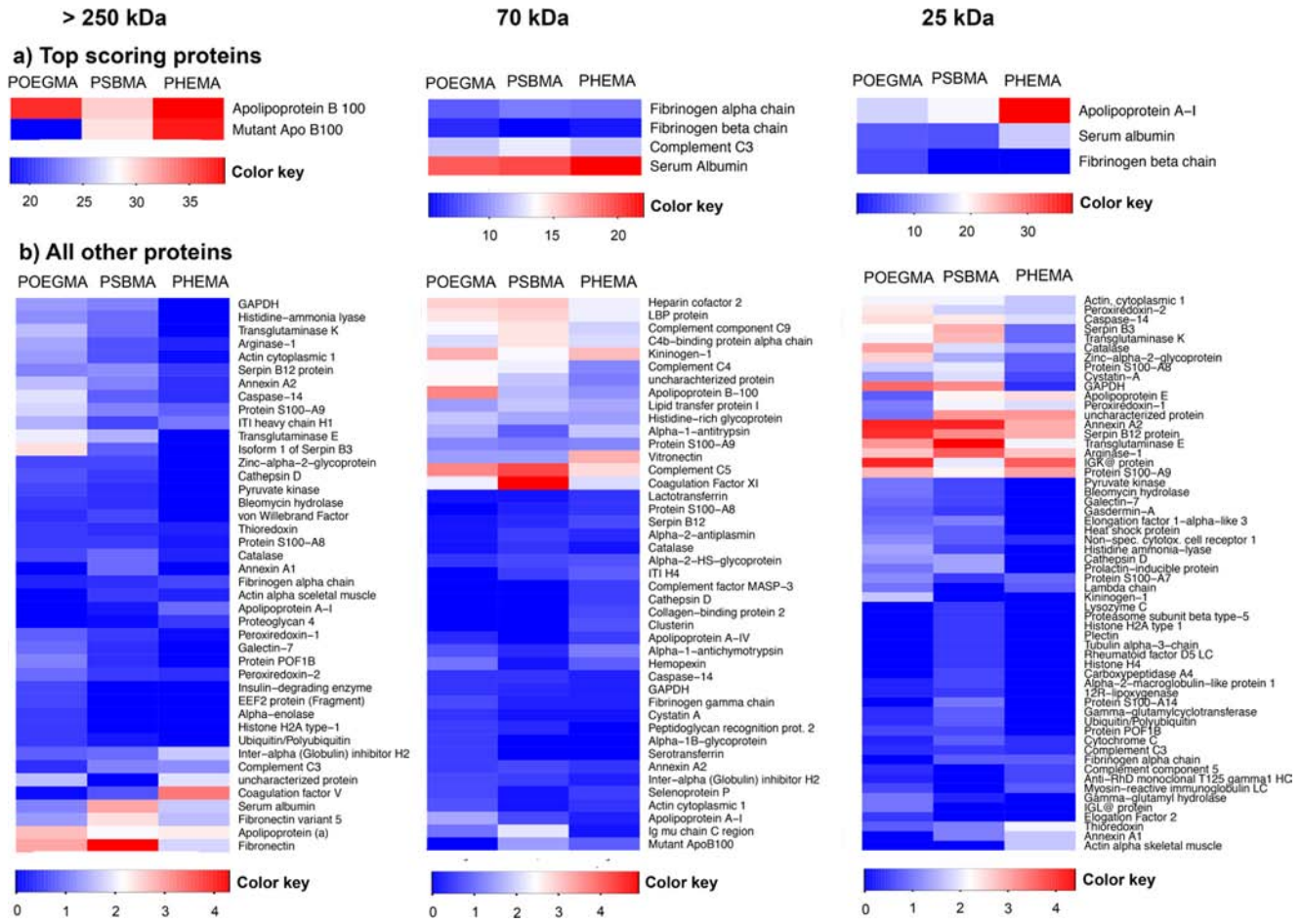


Figure 4. Heat map representing the identified proteins that adsorb to the different polymer brushes and their relative abundances. Separated in molecular weight groups as indicated above and (a) top scoring proteins with very high relative abundance and (b) all other detected proteins.

apolipoprotein B100 (apoB100) as it is the only protein exceeding 30% although it only occurs at 1−3 μM in plasma.[31]

Apolipoprotein B100 and Low Density Lipoprotein Adsorption. We studied the adsorption behavior of apoB100 to the polymer brushes by SPR in more detail. Surprisingly, no notable adsorption occurs when either polymer brush is exposed to a solution of apoB100 (Figure 5, red curve), even though it was identified as the most prominent protein by mass spectrometry. To understand this apparent contradiction, we have to note that apolipoproteins do not occur free in plasma. Instead, they are embedded in large lipoprotein particles that consist mostly of phospholipids, triglycerides, and cholesteryl esters (Figure 5, inset).[32] ApoB100 occurs in low density lipoprotein (LDL), and it would be interesting to see if the LDL particle, in contrast to apoB100 protein alone, would adsorb to our brush surfaces. The ability of LDL to adsorb to polymeric materials has been reported earlier.[33] As seen in the SPR sensorgram in Figure 5 (blue curve), LDL adsorbs very strongly and cannot be washed off the brush by PBS buffer. This indicates that not the protein alone but the lipoprotein particle is required for adsorption.

Adsorption Behavior of Other Lipoprotein Classes. We further investigated the adsorption of the two other main types of lipoproteins, high density lipoprotein (HDL) and very low density lipoprotein (VLDL) by SPR. Our results are summarized in Table 1 (for details, see Figure S9, Supporting Information) and show that both HDL and VLDL adsorb

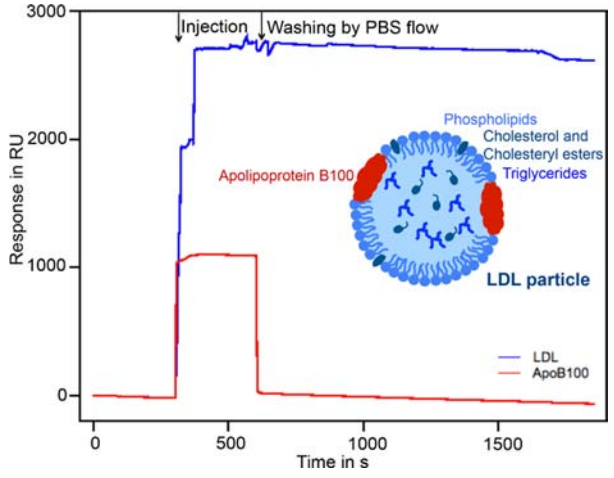


Figure 5. Representative SPR sensorgram showing the adsorption of apoB100 alone and LDL to a POEGMA brush. A schematic cross section illustrating the composition of LDL is inserted.

Table 1. Adsorption in RU of Different Lipoproteins to the Three Polymer Brushes

	VLDL	LDL	HDL
POEGMA	200−300	>2000	100−200
PSBMA	200−500	>2000	<200
PHEMA	400−900	>2000	300−400

dx.doi.org/10.1021/ja402126t | J. Am. Chem. Soc. 2013, 135, 7047−7052

significantly less to the polymer brushes compared to LDL. HDL particles show the lowest adsorption of approximately 5–10 times less than that of LDL, and also, VLDL adsorption is clearly reduced compared to LDL.

These results point to a key role for LDL in adsorption to protein-resistant brush surfaces. LDL also stands out from the lipoproteins in the body, where it accumulates in blood vessels initiating atherosclerosis.[34] It is formed from VLDL, which is accompanied by a reduction of size, softness, and surface fluidity and an increase of density caused by a change in lipid composition and protein/lipid ratio.[32,35] All of these changes can alter the interaction with polymer surfaces.

Adsorption Behavior of Lipid Micelles. Our experiments show that isolated apoB100 does not foul polymer brushes, even though it is one of the major proteins after fouling by plasma. Instead, intact lipoproteins show strong adsorption, which suggests that other components in these lipid–protein particles are involved in the adsorption of lipoproteins to polymer brushes. We investigated this hypothesis further by synthesizing phospholipid micelles, which contain the different lipid components naturally occurring in lipoproteins. Their adsorption behavior to POEGMA brushes was studied by SPR, and the results are summarized in Table 2 (for details, see Figure S10, Supporting Information). All micelles adsorbed to the POEGMA brushes[36] but in notably different amounts. The adsorption of micelles made of phospholipids only as well as those containing either cholesteryl esters or triglycerides is comparably low. However, when phospholipids, triglycerides, and cholesteryl esters are all present, the adsorption strongly increased and exceeded 300 RU. These results show that, in contrast to apoB100, the lipids do adsorb to the polymer brushes and that the lipid composition indeed affects the adsorption behavior of the micelles. The latter could also explain why lipoproteins from different classes show different degrees of adsorption (Table 1), as they also have different lipid compositions and related properties.

Table 2. Adsorption in RU of Phospholipid Micelles with Different Lipid Components to POEGMA Brushes

phospholipids	62 ± 30
phospholipids with cholesteryl ester	27 ± 14
phospholipids with triglyceride	113 ± 26
phospholipids with cholesteryl ester and triglyceride	353 ± 64

Role of LDL in Plasma Adsorption. Our studies revealed the strong adsorption behavior of LDL to protein-resistant polymer brushes; however, a list of other adsorbing proteins was also identified (see Figure 4). In order to gain more insight into the contribution of LDL to plasma fouling, we performed consecutive adsorption experiments by SPR. POEGMA brushes were first exposed to LDL, followed by injection of other strongly adsorbing single proteins. No significant protein adsorption is detected when reinjecting single protein solutions of fibrinogen or albumin (see Figure 6, blue and green curves), which is in agreement with direct exposure to POEGMA brushes (see Figure S11, Supporting Information). A reduction of the LDL concentration and an increase in single protein concentration does not alter these results (see Figure S11, Supporting Information). In addition, we studied the adsorption of undiluted blood plasma to a brush that had been exposed to LDL first. Interestingly, plasma adsorption is clearly reduced in comparison to direct contact with the brush.

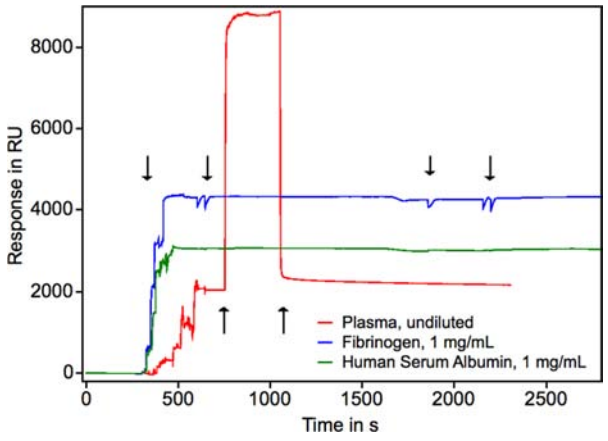


Figure 6. Consecutive injection experiments with POEGMA brushes; arrows indicate start and end points of injections. First injection of LDL (1 mg/mL) followed by rinsing with PBS and injection of fibrinogen (blue) or albumin (green) or immediate injection of plasma (red).

These findings strongly indicate that LDL does not mediate the adsorption of other proteins to the brushes.

CONCLUSIONS

In conclusion, we determined which plasma proteins foul protein-resistant surfaces by gel electrophoresis and mass spectrometry. We catalogued 98 different proteins that adsorb to the three polymer brushes under investigation, regardless of their chemical structure. This is an interesting result, as the brush structure can alter the amount of adsorbed protein[8] but not the composition of the adsorbed layer. Determination of the relative abundances of the adsorbing proteins allowed us to identify apoB100 as a major protein; it accounts for more than 30% of adsorbed protein for all three brushes. However, purified apoB100 showed no adsorption in SPR measurements, similarly to other single protein solutions.[37] Further SPR studies revealed the strong adsorption of LDL, which contains apoB100, to the brushes. The results show the key role of lipoprotein particles in plasma adsorption. LDL adsorbs very strongly, while other lipoproteins such as HDL and VLDL adsorb to a lesser extent. We additionally performed SPR measurements with phospholipid micelles containing the lipid components found in naturally occurring lipoproteins. Our data showed that only those micelles that contain a mixture of lipids adsorb in large amounts. This suggests that the lipid composition and resulting properties such as softness and flexibility[38,39] are central parameters determining adsorption, which may also explain the differences observed between lipoproteins from distinct classes. In addition, we studied the role of LDL in plasma adsorption by sequential injection experiments. The data shows clearly that LDL does not mediate the adsorption of other plasma proteins to the brushes, as other proteins do not adsorb to brushes that had been exposed to LDL first. We hypothesize that lipoprotein particles bind intact to brush-coated surfaces (supported by the SPR sensorgram shape), inhibiting protein unfolding on the surfaces and thus preventing further protein adsorption.

The results presented here provide a significant step forward toward understanding plasma fouling and ultimately the design of superior antibiofouling surface coatings. In a wider context, this will deepen the understanding of the principles of

interactions between proteins and polymer-modified surfaces, which will prove useful in many areas of biomaterials research.

EXPERIMENTAL SECTION

Polymer Brush Synthesis. ATRP initiators were synthesized as described elsewhere (ω-mercapto undecyl isobutyrate)[40] or purchased from Gelest Inc. ((3-trimethoxysilyl)propyl-2-bromo-2-methylpropionate). Known procedures for surface immobilization of the initiators were followed for gold-coated flat surfaces[37] and silica particles[41] of 2.5 μm in diameter (obtained from Bangs Laboratories). Flat surfaces were rinsed with ethanol, dried under a stream of nitrogen, and placed in a Schlenk tube for polymerization. An oxygen-free atmosphere was generated by three evacuation/backfill cycles and by bubbling the polymerization solution for at least 30 min with inert gas. Particles were washed five times by centrifugation and redispersion with toluene before gradually changing the solvent to the one used for polymerization. The suspension was degassed under vigorous stirring for at least 30 min before addition of the polymerization solution. The monomer was added to the solvent and degassed for 20 min; subsequently, ligand and copper (II) and copper (I) salts were added, and the solution was stirred and degassed for a further 10 min before injection to the substrates. Known polymerization conditions[23−25] were slightly modified to yield the desired polymer brushes as detailed in Table 3. After the polymerization flat surfaces were washed with ethanol and dried under nitrogen, particles were washed by centrifugation and redispersion cycles in the polymerization solvent.

Table 3. Polymerization Conditions for the Synthesis of POEGMA, PSBMA, and PHEMA Brushes to Yield 20−30 nm Thick Brushes

	POEGMA	PSBMA	PHEMA
monomer	5.2 mmol	17.9 mmol	22.3 mmol
CuCl	105 μmol	364 μmol	187 μmol
$CuBr_2$	10 μmol	–	18 μmol
$CuCl_2$	–	143 μmol	–
2,2′-bipy	250 μmol	896 μmol	464 μmol
solvent	0.6 mL of MeOH and 2.2 mL of water	8 mL of MeOH and 2 mL of water	2.5 mL of MeOH and 2.5 mL of water
time	1 h	3 h	45 min

Brush Characterization. Brush thickness and swelling in PBS on flat gold-coated surfaces were determined by ellipsometry onan α-SE spectroscopic ellipsometer. Captive bubble contact angles were measured as described previously[37] on a FTA1000 instrument. For both methods, the data was averaged over three separate measurements at different locations on each sample. Thermogravimetric analysis (TGA) measurements were accomplished at 10 K min^{-1} with dried samples on a Mettler Toledo TGA/DSC 1 instrument. AFM measurements were accomplished on two different samples for each polymer brush at 10 and 1 μm^2 on two separate spots on each sample. A Digital Instruments dimension 3100 AFM from Veeco Instruments Inc. was used with OMCL-AC series silicon probes from Olympus Corp. (resonance frequency ≈ 300 kHz, cantilever spring constant ≈ 42 N m^{-1}). The determined root mean square roughness was averaged over four measurements.

Incubation with Plasma, Washing, and Desorbing Bound Proteins. The brush-coated particles were transferred into PBS and washed thoroughly before all solvent was carefully removed. All experiments were performed in Protein LoBind Eppendorf tubes in a microbiological class II safety unit to reduce contamination to a minimum. A 200 μL amount of human blood plasma was added, the particles were redispersed by vortexing, and the brushes were incubated by shaking at 25 °C and 300 rpm for 15 min. Subsequently, the particles were removed by slow centrifugation, the plasma was removed by pipetting, and the particles were washed with 500 μL of PBS during five cycles of PBS addition, redispersion, centrifugation, and removal of PBS. To desorb adsorbed proteins, 100 μL of a 0.5% solution of SDS in PBS was added, and the particles were redispersed and incubated again at 25 °C and 300 rpm for 15 min. The particles were separated by rapid centrifugation, and the supernatant was taken to further analysis.

Quantification of Adsorbed Protein. Protein adsorption to flat brush-coated surfaces was determined by SPR using a BIAcore 3000 instrument. The brush-functionalized sensor chips (Ssens) were attached to sample holders, docked, and primed twice with degassed PBS buffer. The samples were equilibrated by a steady flow of PBS at 20 μL/min for at least 30 min. The brushes were exposed to undiluted human blood plasma for 5 min, followed by a flow of PBS for 20 min. Then, the brushes were exposed to 0.5% SDS in PBS solution for 5 min before a 20 min period of PBS flow. The difference before and after exposure to plasma and SDS, respectively, was determined in ΔRU, which was converted to nanogram per centimeter squared according to the following: 1 RU = 0.1 ng/cm^2. The data was baseline corrected by taking the slope of the curve when exposed to PBS before injections and extrapolating to the respective data points. Data was averaged over four SPR measurements. Protein adsorption on particles was determined with the commercially available Quanti Pro BCA assay kit in the solution of desorbed proteins. The samples were diluted 25 times with PBS, the standard protocol for 1 mL assays was followed, and the samples were measured in duplicates on a Cary 400 UV/vis instrument. The calibration curve was measured each time with freshly prepared solutions in duplicates. By taking the estimated surface area of the remainder of particles into account, the amount of adsorbed protein in nanogram per centimeter squared was calculated from this data.

Gel Electrophoresis. The samples were dissolved in loading buffer (containing 0.05% β-mercaptoethanol) and heated at 100 °C for 5 min. Human blood plasma was used as a reference in 200-fold dilution and treated similarly. After cooling to room temperature, the samples and PageRuler broad range unstained protein ladder (250−5 kDa) were loaded on precast Tris−glycine 4−20% gradient gels or 12% homogeneous gels. Gels were run at 150 V and 90 A in Tris−glycine running buffer. After completion, gels were silver stained using the Pierce Scientific silver stain kit.

Mass Spectrometry. The bands of interest were cut out of electrophoresis gels, destained, reduced with DDT, and digested with trypsin (overnight at 37 °C). The peptides were separated by reverse-phase chromatography and measured by mass spectrometry. LC-MS/MS measurements were performed on a nanoAquity UPLC (Waters Corp., Milford MA) system and an LTQ Orbitrap Velos hybrid ion trap mass spectrometer (Thermo Scientific, Waltham, MA). The obtained MS/MS files were interpreted using MASCOT software (UNIprot human database), and known contaminants were excluded. Relative abundances of the identified proteins were determined accordingly using the spectral count method,[30] by dividing the matches per identified protein by the number of overall matches. The results were averaged over two separate mass spectrometry runs of different samples, including all hits that have greater than or equal to 0.5% relative abundance for at least one brush. R software was employed to generate the heatmaps with this data.

SPR Measurements with Lipoproteins and Phospholipid Micelles. ApoB100, LDL, and HDL were reconstituted with milliQ water as indicated in the product description; VLDL was obtained in solution from Merck. Lipoproteins were diluted with PBS to give the desired concentration. LDL, VLDL, and HDL were measured at 1 mg/mL, and apoB100 was diluted to give the same molar concentration as that used with LDL. Asolectin (1 mg/mL), cholesteryl palmitate (0.5 mg/mL), and glyceryl trioleate (0.5 mg/mL), respectively, were weighed in and solubilized in PBS buffer by sonication for 20 min. The results were averaged over at least three measurements.

ASSOCIATED CONTENT

Supporting Information

Characterization of the three polymer brushes; calculations and data for plasma, lipoprotein, and phospholipid micelle

dx.doi.org/10.1021/ja402126t | J. Am. Chem. Soc. 2013, 135, 7047−7052

adsorption experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

w.huck@science.ru.nl

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Prof. C. Abell and J. Plumb for providing gel electrophoresis equipment, Dr. M. Deery, R. Feret, and J. A. Howard for mass spectrometry and Dr. J. McGregor and Z. Saracevic for TGA measurements. G.G. thanks the Daimler Benz foundation for funding.

REFERENCES

(1) Chen, H.; Yuan, L.; Song, W.; Wu, Z.; Li, D. *Prog. Polym. Sci.* **2008**, *33*, 1059−1087.
(2) Hucknall, A.; Rangarajan, S.; Chilkoti, A. *Adv. Mater.* **2009**, *21*, 2441−2446.
(3) Chapman, R. G.; Ostuni, E.; Takayama, S.; Holmlin, R. E.; Yan, L.; Whitesides, G. M. *J. Am. Chem. Soc.* **2000**, *122*, 8303−8304.
(4) Herrwerth, S.; Eck, W.; Reinhardt, S.; Grunze, M. *J. Am. Chem. Soc.* **2003**, *125*, 9359−9366.
(5) Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605−5620.
(6) Ma, H.; Hyun, J.; Stiller, P.; Chilkoti, A. *Adv. Mater.* **2004**, *16*, 338−341.
(7) Edmondson, S.; Osborne, V. L.; Huck, W. T. S. *Chem. Soc. Rev.* **2004**, *33*, 14.
(8) Blaszykowski, C.; Sheikh, S.; Thompson, M. *Chem. Soc. Rev.* **2012**, *41*, 5599−5612.
(9) Yang, W.; Xue, H.; Li, W.; Jiang, S. *Langmuir* **2009**, *25*, 11911−11916.
(10) Jiang, S.; Cao, Z. *Adv. Mater.* **2009**, *22*, 920−932.
(11) Ladd, J.; Zhang, Z.; Chen, S.; Hower, J. C.; Jiang, S. *Biomacromolecules* **2008**, *9*, 1357−1361.
(12) Rodriguez-Emmenegger, C.; Brynda, E.; Riedel, T.; Houska, M.; Šubr, V.; Alles, A. B.; Hasan, E.; Gautrot, J. E.; Huck, W. T. S. *Macromol. Rapid Commun.* **2011**, *32*, 952−957.
(13) Shen, Y.; Kim, J.; Strittmatter, E. F.; Jacobs, J. M.; Camp, D. G.; Fang, R.; Tolié, N.; Moore, R. J.; Smith, R. D. *Proteomics* **2005**, *5*, 4034−4045.
(14) White, A. D.; Nowinski, A. K.; Huang, W.; Keefe, A. J.; Sun, F.; Jiang, S. *Chem. Sci.* **2012**, *3*, 3488.
(15) Walkey, C. D.; Olsen, J. B.; Guo, H.; Emili, A.; Chan, W. C. W. *J. Am. Chem. Soc.* **2012**, *134*, 2139−2147.
(16) Walkey, C. D.; Chan, W. C. W. *Chem. Soc. Rev.* **2012**, *41*, 2780.
(17) Lai, B. F. L.; Creagh, A. L.; Janzen, J.; Haynes, C. A.; Brooks, D. E.; Kizhakkedathu, J. N. *Biomaterials* **2010**, *31*, 6710−6718.
(18) Lai, Z. W.; Yan, Y.; Caruso, F.; Nice, E. C. *ACS Nano* **2012**, *6*, 10438−10448.
(19) Riedel, T.; Riedelová-Reicheltová, Z.; Májek, P.; Rodriguez-Emmenegger, C.; Houska, M.; Dyr, J. E.; Brynda, E. *Langmuir* **2013**, *29*, 3388−3397.
(20) Gautrot, J. E.; Trappmann, B.; Oceguera-Yanez, F.; Connelly, J.; He, X.; Watt, F. M.; Huck, W. T. S. *Biomaterials* **2010**, *31*, 5030−5041.
(21) Zhang, Z.; Chen, S.; Chang, Y.; Jiang, S. *J. Phys. Chem. B* **2006**, *110*, 10799−10804.
(22) Zhao, C.; Li, L.; Wang, Q.; Yu, Q.; Zheng, J. *Langmuir* **2011**, *27*, 4906−4913.
(23) Brown, A. A.; Khan, N. S.; Steinbock, L.; Huck, W. T. S. *Eur. Polym. J.* **2005**, *41*, 1757−1765.
(24) Cheng, N.; Brown, A. A.; Azzaroni, O.; Huck, W. T. S. *Macromolecules* **2008**, *41*, 6317−6321.
(25) Rodriguez-Emmenegger, C.; Avramenko, O. A.; Brynda, E.; Skvor, J.; Alles, A. B. *Biosens. Bioelectron.* **2011**, *26*, 4545−4551.
(26) Tenzer, S.; Docter, D.; Rosfa, S.; Wlodarski, A.; Kuharev, J.; Rekik, A.; Knauer, S. K.; Bantz, C.; Nawroth, T.; Bier, C.; Sirirattanapan, J.; Mann, W.; Treuel, L.; Zellner, R.; Maskos, M.; Schild, H.; Stauber, R. H. *ACS Nano* **2011**, *5*, 7155−7167.
(27) Mrksich, M.; Sigal, G. B.; Whitesides, G. M. *Langmuir* **1995**, *11*, 4383−4385.
(28) Aebersold, R.; Mann, M. *Nature* **2003**, *422*, 198−207.
(29) Yates, J. R., III *J. Am. Chem. Soc.* **2013**, *135*, 1629−1640.
(30) Liu, H.; Sadygov, R. G.; Yates, J. R. *Anal. Chem.* **2004**, *76*, 4193−4201.
(31) Hortin, G. L.; Sviridov, D.; Anderson, N. L. *Clin. Chem.* **2008**, *54*, 1608−1616.
(32) Lodish, H.; Berk, A.; Zipurski, S. L.; Matsudaira, P.; Baltimore, D.; Darnell, J. *Molecular Cell Biology*, 5th ed.; W. H. Freeman: New York, 2004.
(33) Dong, D. E.; Andrade, J. D.; Coleman, D. L. *J. Biomed. Mater. Res.* **1987**, *21*, 683−700.
(34) Lusis, A. J. *Nature* **2000**, *407*, 233−241.
(35) Massey, J. B.; Pownall, H. J. *Biophys. J.* **1998**, *74*, 869−878.
(36) Pfeiffer, I.; Petronis, S.; Köper, I.; Kasemo, B.; Zäch, M. *J. Phys. Chem. B* **2010**, *114*, 4623−4631.
(37) Gunkel, G.; Weinhart, M.; Becherer, T.; Haag, R.; Huck, W. T. S. *Biomacromolecules* **2011**, *12*, 4169−4172.
(38) Mikl, C.; Peters, J.; Trapp, M.; Kornmueller, K.; Schneider, W. J.; Prassl, R. *J. Am. Chem. Soc.* **2011**, *133*, 13213−13215.
(39) Krilov, D.; Balarin, M.; Kosović, M.; Gamulin, O.; Brnjas-Kraljević, J. *Spectrochim. Acta, Part A* **2009**, *73*, 701−706.
(40) Jones, D. M.; Brown, A. A.; Huck, W. T. S. *Langmuir* **2002**, *18*, 1265−1269.
(41) Tan, K. Y.; Gautrot, J. E.; Huck, W. T. S. *Langmuir* **2011**, *27*, 1251−1259.