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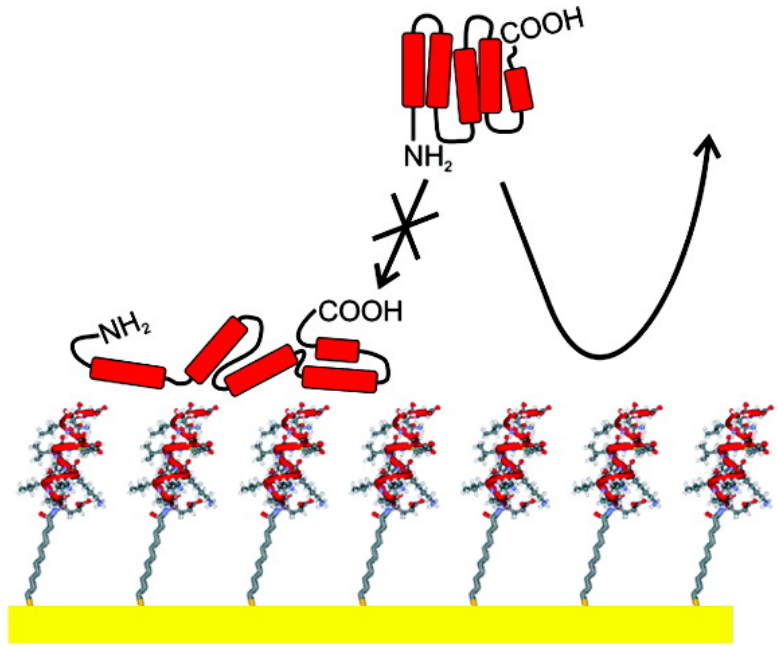
J. Am. Chem. Soc., **2008**, 130 (45), 14952-14953 • DOI: 10.1021/ja8065754 • Publication Date (Web): 18 October 2008



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Published on Web 10/18/2008

Peptide-Based SAMs that Resist the Adsorption of Proteins

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The biocompatibility of a given material is determined by the protein adlayer bound to its surface.[1,2] This adhesion layer is formed immediately after the first contact of this material (e.g., an implant) with living fluids such as blood,[3−5] and largely determines the further interaction with the surroundings. Unfortunately, an understanding of these interactions—which would be required for tailoring the biocompatibility of a material—is severely hampered by the complexity of the system; in general an adsorption of biomolecules (see Figure 1) will also lead to a structural rearrangement of the adlayer.

To reduce the complexity of this system, we propose the use of a model system employing peptide-based self-assembled monolayers, SAMs. These are rather rigid ultrathin organic layers, and the sequence of the peptides can be chosen to mimic the surface exposed by protein adlayers. The interactions of biomolecules, for example, proteins, with these biomimetic surfaces can be studied in a straightforward fashion. We go beyond previous studies on peptide SAMs made from individual peptides[6−8] by presenting a general approach suited for peptides with an arbitrary sequence employing a linker approach based on a Cu-catalyzed cycloaddition reaction (click chemistry).[9−11]

We demonstrate the high potential of such biomimetic surfaces by fabricating a proteophobic surface using a rational strategy. Although using peptides to fabricate a surface which resists the adsorption of proteins is somewhat counterintuitive, we selected a sequence of amino-acids on the basis of rules derived from previous studies on the origins of the protein resistance of organic surfaces.[12−14] The resulting peptide SAM shows a degree of resistance to unspecific adsorption of proteins which parallels that of the most proteophobic materials known to date, poly(ethylene glycol) (PEG) derivatives such as the hexamer OEG(6).[12,15,16] Unlike PEGs, however, peptides represent a "second generation" material for hydrophobic SAMs in that they have additional functionalities which can be selectively chosen or tuned for a given application.

Peptides were synthesized using a commercial microwave-supported peptide synthesizer. Starting from Wang resin that was preloaded with the first amino acid, peptides were synthesized by Fmoc solid-phase peptide synthesis (SPPS) as described before using TBTU/1-hydroxy-benzotriazole-6-sulfonamidomethyl hydrochloride (HOBt) as activators.[17] In place of a further amino acid in the last step, azido acetic acids were linked to the oligopeptide. After completion of the synthesis, peptides were cleaved from the resin, precipitated with cold ether, lyophilized, and purified by preparative HPLC when necessary. In this way, > 95% purity was ensured for all compounds. The identity of the peptides was confirmed by electrospray ionization mass spectrometry (ESI-MS)[18] (see Supporting Information). For click reactions, the peptides were incubated for 72 h with a mixture of copper iodide (CuI), *N*-ethyldiisopropylamine (DIPEA), and 11-thioacetyl-undecanoic acid

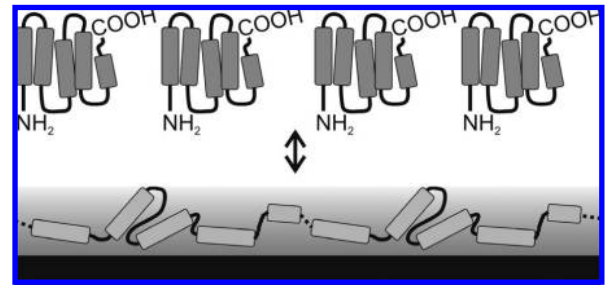

Figure 1. Schematic description of the interaction of living fluids with abiotic materials. The adsorption properties are largely determined by the protein adlayer.

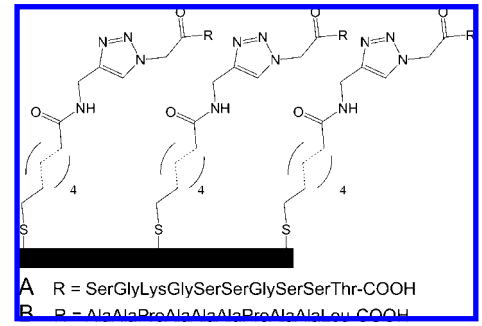

Figure 2. Schematic view of the used peptides. Peptide 1 (A) contains hydrophilic amino acids (Ser, Lys, Thr) and Gly which is not hydrophilic but important for the helical structure of the peptide. Peptide 2 (B) is a simple chain of hydrophobic side chains containing one leucin, seven alanines, and two prolines.

propargyl amid[19] in a small amount of *N,N*-dimethylformamide (DMF). Click chemistry reactions with peptides and peptide nucleic acids (PNA) oligomeres have been reported.[9,11] Concentrated stock solutions of the peptide thiols ($c \approx 36$ mM) were directly obtained. Again, ESI-MS was used on these stock solutions to verify complete conversion to the click products in all cases. The complete disappearance of characteristic alkyne bands in FTIR spectra further supported complete conversion to the desired products (see Supporting Information). For the surface coating experiments, 200 μL of the peptide stock solutions were diluted with pure ethanol to get 1 mM solutions of peptide thiol.

For this study two different peptides were synthesized. For peptide 1 (Figure 2) a sequence was chosen on the basis of the following design rules: the used amino acids should be hydrophilic, but not charged, provide hydrogen bond acceptors, and be α helix supporting, thus following design principles deduced from previous studies on the proteophobicity of organic surfaces.[12−14] To provide a reference, the sequence of peptide 2 was chosen using the opposite strategy, it almost exclusively consists of hydrophobic amino acids.

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10.1021/ja8065754 CCC: $40.75

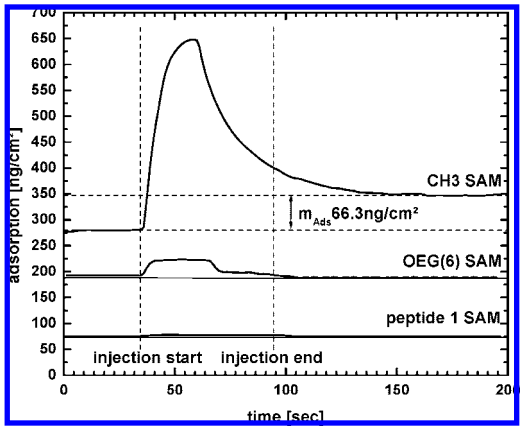

Figure 3. SPR curves of streptavidin (c = 200 nM) on differently terminated SAMs. Injection of protein runs for 1 min before starting dissociation and laundering with buffer.

Au/Si wafers and gold-coated, thin glass slides (D263, Schott) were prepared as described elsewhere[20,21] and then incubated in peptide thiol solution (c = 1 mM) for 24 h. The resulting SAMs were well-defined, densely packed as demonstrated by X-ray photoelectron spectroscopy and IR-spectroscopy (see Supporting Information). Surface plasmon resonance (SPR) measurements were carried out in a commercial system (Reichert SR7000DC). First the adsorption of streptavidin (SA) (c = 200 nM ≈ 0.01 mg/mL, Invitrogen) on the peptide SAM was monitored, then the adsorption of bovine serum albumin (BSA) (c = 2 μM ≈ 0.133 mg/mL, SIGMA) and finally the adsorption of fibronectin (c = 200 nM = 0.05 mg/mL, SIGMA).

The protocol for adsorption measurements consisted of first flowing TRIS buffer (pH 7.9, 50 mM TRIS, 5 mM $MgCl_2$) over the surface for 5 min (flow rate: 0.2 mL/min), followed by 1 min injection of protein solutions, 1 min of buffer laundering, and finally flowing TRIS buffer over the surface for a further 5 min.

Experiments with higher concentrations (c(SA) = 1 mg/mL) ensures the same protocol but with a flow rate of 0.03 mL/min and an injection time of 10 min followed by laundering with buffer for a further 10 min.

In a first step the adsorption of streptavidin on the peptide SAMs was monitored using SPR and then compared to corresponding results for a CH_3-terminated SAM (octadecane thiol). For comparison, experiments were also carried out for an OEG(6)-based thiol. Figure 3 shows the results of these measurements. The results for the CH_3-terminated SAM show the expected, high unspecific adsorption (m_{Ads} = 66.3 ng/cm^2), for the hydrophobic peptide 2 SAM the amount of adsorbed protein was only slightly smaller (m_{Ads} = 62.3 ng/cm^2, data not shown). On the OEG(6)-based SAM no adsorption of streptavidin could be observed, in agreement with earlier work.[21] The results for the peptide 1 SAM are virtually identical for those observed for the OEG(6) SAM, the amount of unspecifically adsorbed streptavidin is smaller than 0.1 ng/cm^2 (Figure 3). These results clearly demonstrate the proteophobic behavior of our peptide SAM.

To explore whether the pronounced proteophobic behavior of our peptide SAM is specific for streptavidin we have investigated two other proteins, BSA and Fibronectin. For both, BSA and Fibronectin, a small amount of unspecifically adsorbed protein was observed in contrast to the OEG-based SAM for which no measurable adsorption of protein was observed (see table 1). Also for higher concentrations of SA a small amount of unspecific adsorption can be observed (see table 2), but the high protein repelling tendency is retained.

In conclusion, we present a rational approach to produce SAMs from peptides with an arbitrary sequence. Click chemistry is particularly well suited for the production of such peptide-based SAMs since it is a chemically selective, high-yielding reaction which tolerates all functional side-chain groups in peptides. These ultrathin biomolecular layers are excellent model systems for biomimetic surfaces. We demonstrate the potential of the approach with regard to a particular important property of surfaces in contact with living fluids, that is resistance to protein adsorption. Already a first trial sequence chosen on the basis of previously established guidelines for proteophobic surfaces[12−14] yielded a surface which is comparable to the best proteophobic surfaces known to date, PEG-based SAMs.[12,15,16,22] We expect that we will be able to further improve the protein resistance of these surfaces using a combinatorial approach. Our approach is well suited to tailor and then optimize peptide sequences with regard to other properties, for example, adsorption of biomolecules and other objects such as platelets.

Table 1. Amount of Adsorption for the Used Proteins on the Differently Terminated SAMs

	m_{Ads} [ng/cm^2]		
	streptavidin	BSA	fibronectin
CH3	66.3	60	55.3
peptide 2	62.3		
OEG(6)	≤0.1	≤0.1	≤0.1
peptide 1	≤0.1	6.3	2.3

Table 2. Amount of Adsorption for Streptavidin (c = 1 mg/mL) on the Differently Terminated SAMs

	CH3	OEG(6)	peptide 1
m_{Ads} [ng/cm^2]	131.02	<0.1	4.06

Acknowledgment. We thank D. Käfer for help with the XPS analysis of the SAMs.

Supporting Information Available: ESI mass spectra of peptide 1 and peptide 2. XPS spectrum of peptide 1 and IR spectra of peptide 1 before and after SA incubation. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kidoaki, S.; Matsuda, T. *Langmuir* **1999**, *15*, 7639–7646.
(2) Kidoaki, S.; Matsuda, T. *Colloids Surf. B* **2002**, *23*, 153–163.
(3) Kurotobi, K.; Yamamoto, A.; Kikuta, A.; Hanawa, T. *J. Mater. Sci.: Mater. Med.* **2007**, *18*, 1175–1184.
(4) Hennink, W. E.; Kim, S. W.; Feijen, J. *J. Biomed. Mater. Res.* **1984**, *18*, 911–926.
(5) Sideman, S.; Mor, L.; Brandes, J. M.; Lupovitch, S. *J. Biomed. Mater. Res.* **1983**, *17*, 91–107.
(6) Gatto, E.; Stella, L.; Formaggio, F.; Toniolo, C.; Lorenzelli, L.; Venanzi, M. *J. Pept. Sci.* **2008**, *14*, 184–191.
(7) Tomoyuki, M.; Shunsaku, K. *J. Am. Chem. Soc.* **2003**, *125*, 8732–8733.
(8) Kitagawa, K.; Morita, T.; Kimura, S. *Langmuir* **2005**, *21*, 10624–10631.
(9) Köster, S. D.; Dittrich, J.; Gasser, G.; Hüsken, N.; Henao-Castañeda, I. C.; Jios, J. L.; Della-Védova, C. O.; Metzler-Nolte, N. Organometallics **2008**, in press.
(10) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem.* **2002**, *114*, 2708–2711.
(11) Gasser, G.; Hüsken, N.; Köster, S. D.; Metzler-Nolte, N. Chem. Commun. 2008, DOI:10.1039/B805369C.
(12) Herrwerth, S.; Eck, W.; Reinhardt, S.; Grunze, M. *J. Am. Chem. Soc.* **2003**, *125*, 9359–9366.
(13) Ostuni, E.; Yan, L.; Whitesides, G. M. *Colloids Surf. B* **1999**, 3–30.
(14) Chapman, R. G.; Ostuni, E.; Takayama, S.; Holmlin, R. E.; Yan, L.; Whitesides, G. M. *J. Am. Chem. Soc.* **2000**, *122*, 8303–8304.
(15) *Poly(ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications*; Harris, J. M., Ed.; Plenum Press: New York, 1992.
(16) Prime, K. L.; Whitesides, G. M. *Science* **1991**, *252*, p. 1164–1167.
(17) Kirin, S. I.; Noor, F.; Metzler-Nolte, N. *J. Chem. Educ.* **2007**, *84*, 108–111.
(18) Bakhtiar, R.; Hofstadler, S. A.; Smith, R. D. *J. Chem. Educ.* **1996**, *73*, A118−A123.
(19) Kleinert, M.; Winkler, T.; Terfort, A.; Lindhorst, T. K. *Org. Biomol. Chem.* **2008**, *6*, 2118–2132.
(20) Rajalingam, K.; Bashir, A.; Badin, M.; Schröder, F.; Hardman, N.; Strunskus, T.; Fischer, R. A.; Wöll, C. *ChemPhysChem* **2007**, *8*, 657–660.
(21) Chelmowski, R.; Prekelt, A.; Grunwald, C.; Wöll, C. *J. Phys. Chem. A* **2007**, *111*, 12295–12303.
(22) Dalsin, J. L.; Messersmith, P. B. *Mater. Res. Soc. Symp. Proc.* **2002**, *774*, 75–80.

JA8065754