Protein-Resistant Self-Assembled Monolayers on Gold with Latent Aldehyde Functions

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Received September 21, 2006. In Final Form: March 7, 2007

In the present study, oligo(ethylene glycol) (OEG)-linked alkanethiols were synthesized which carry a vicinal diol on one end of the OEG chain. After self-assembled monolayer (SAM) formation on gold, the vicinal diols were converted into aldehyde functions by exposure to aqueous NaIO₄, as previously used for SAMs with OEG chains buried in the center of the SAM [Jang et al. Nano Lett. 2003, 3, 691–694]. Mixed SAMs with latent aldehydes on 5% of the OEG termini showed high protein resistance, which greatly slowed the kinetics of protein coupling on the time scale of minutes. Small bioligands (such as biocytin hydrazide) or small heterobifunctional crosslinkers (maleimidopropionyl hydradiza, pyridyldithiopropionyl hydradiza) with hydrazide functions were efficiently bound to the aldehyde functions on the SAM, providing for specific capture of streptavidin or for fast covalent binding of proteins with free thiol or maleimide functions, respectively. In conclusion, OEG-terminated SAMs with latent aldehydes serve as protein-resistant sensor surfaces which are easily functionalized with small ligands or with heterobifunctional crosslinkers to which the bait molecule is attached in a subsequent step.

Introduction

Oligo(ethylene glycol) (OEG)-terminated alkanethiols are known to form self-assembled monolayers (SAMs) on gold which excel in high protein resistance, even in case of tri(ethylene glycol)-terminated alkanethiols.1–3 OEG-terminated SAMs are being used for suppression of nonspecific adsorption in biosensing,4–8 for passivation of the inactive areas in microarrays9,10 and nanoarrays,11,12 and as clean substrates for near-field microscopy on single molecules.8,13,14

For the use of OEG-terminated SAMs in biosensing or array formation, biomolecules need to be coupled on top of the OEG layer. For this purpose, OEG-terminated alkanethiols have been equipped with specific coupling functions, such as biotin,15–18 maleimide,5 nitrotriatoacetic acid (NTA),6,19 hydroquinone,20 acetylene,21 or suicide inhibitors,22 providing for selective, oriented, and/or triggered binding of probe molecules which then serve as capture probes in biosensors or chip assays.

As an alternative to these specific coupling functions, mixed SAMs with carboxyl functions on a fraction of the OEG chains have been used for the coupling of small ligands or of unmodified proteins via lysine residues after the COOH groups on the SAM had been activated with N-ethyl-N′-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS).6,10 The activation step (NHS ester formation) and the coupling step (amide bond formation with the capture molecule) are fast and efficient. Nevertheless, several parameters are not easily controlled: (i) The activation reagent (EDC) and the transiently formed NHS esters are very sensitive to hydrolysis,23 hampering their use in lengthy procedures such as microspotting or similar. (ii) Nonactivated or hydrolyzed carboxylates influence the adsorptive properties of the SAM.6 (iii) The kinetics of NHS ester degradation, the adsorptive effect of free carboxylates, and the kinetics of ligand coupling are strongly dependent on pH and/or the protein to be coupled, thus the coupling protocol is to be optimized for different proteins.24

SAMs with aldehyde functions are a convenient alternative to carboxylate groups for the coupling of ligands with amino groups. The activation reagent (EDC) and the transiently formed NHS esters are very sensitive to hydrolysis,23 hampering their use in lengthy procedures such as microspotting or similar. (ii) Nonactivated or hydrolyzed carboxylates influence the adsorptive properties of the SAM.6 (iii) The kinetics of NHS ester degradation, the adsorptive effect of free carboxylates, and the kinetics of ligand coupling are strongly dependent on pH and/or the protein to be coupled, thus the coupling protocol is to be optimized for different proteins.24

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10 mM NaIO₄ inside the BIAcore setup (see Figures 4, 6, and 7).

In addition, ligands with hydrazide, aminooxy groups, or N-terminal cysteines can be coupled to aldehydes via chemoselective ligation.⁹⁻¹² So far, however, the aldehyde function has yet not been used on protein-resistant SAMs. Admittedly, Jang et al.⁶⁻¹⁰ have synthesized an aldehyde-terminated SAM component containing a hexa(ethylene glycol) (EG₆) chain. However, the actual structure was HS-(CH₂)₁₁-(OCH₂CH₂)₆-O-(CH₂)₉-CHO; thus, the EG₆ region of the SAM was covered by a top layer of decanal residues which is likely to adsorb protein.⁶ The authors did not study the adsorptive properties of this SAM but used it for covalent coupling of proteins in active nanospots surrounded by inert HS-(CH₂)₁₁-(OCH₂CH₂)₆-OH.

Recently, we proposed a modular route toward aldehyde-terminated alkane thiols in which S-protected mercaptohexadecanoic acid (AcS-HDA, 1, see Figure 1) was coupled to acetonide-protected 3-amino propane-1,2-diol (APD-ac, 3, see Figure 1). In the present study, this modular route has been extended by the incorporation of an OEG chain (NH₂-EG₄-COOH, 2) to arrive at an OEG-linked alkane thiol with a terminal vicinal diol (4) that allows preparation of protein-resistant SAMs with latent aldehyde functions that can be activated with periodate (see Figure 1). A similar route was developed for ether conjugates between OEG and alkane thiol (see Figure 2), and the usefulness of both kinds of OEG-terminated SAMs with latent aldehyde functions on ~5% of the OEG chains for use in biosensing was explored.

Experimental Procedures

Thiols 5 and 8 were available from a preceding study. Thiols 4 and 7 were synthesized by standard procedures, as indicated in Figures 1 and 2 and explicitly described in the Supporting Information (see Figures 8S and 9S). Gold-coated sensor chips for the surface plasmon resonance (SPR) setup were prepared as described before. Mixed SAMs were usually prepared by incubation in acetonitrile containing 1 μM thiol 4 (MHD-EG₄-APD, rac-N-(2,3-dihydroxypropyl)-3-mercapto-22-aza-23-oxo-4,7,10,13,16,19-hexaoxa-octatriacontan-amide) and 19 μM thiol 5 (MHD-EG₈, N-(23-hydroxy-3,6,9,12,15,18,21-heptaoxatricosanyl)-16-sulfanylhexadecanoylamide; see Figure 1) or 1 μM thiol 7 (HS-C₁₂-EG₄-APD, rac-N-(2,3-dihydroxypropyl)-mercapto-4,7,10,13-tetraoxa-pentacosanamide) and 19 μM thiol 8 (HS-C₁₂-EG₈, 24-sulfanyl-3,6,9,12-tetraoxa-1-tetracosanol, see Figure 2) at room temperature in the dark for 36 h. Immunoglobulin G (IgG) from goat was derivatized either with biotin-NHS alone or with biotin-NHS in combination with SMCC (N-succinimidyl 4-(maleimidomethyl)-cyclohexane-carboxylic anhydride) or SATP (N-succinimidyl 3-(acetylthio)-propionate) or SMCC (N-succinimidyl 4-(maleimidomethyl)-cyclohexane-carboxylic anhydride) as described in the Supporting Information (see Figure 10S). Conditions were chosen which resulted in the covalent attachment of ~5 biotin residues per IgG, either alone (biotin-IgG) or in combination with ~5 protected thiol groups (biotin-IgG-SATP) or ~5 maleimide functions (biotin-IgG-SMCC).

Established procedures were used for gold cleaning, SAM formation, and rinsing, as described in the Supporting Information. The rinsed SAMs were stored under ultrapure water at 4 °C for up to 2 weeks. Before use, a chip was rinsed with water (4 °C), blown dry with nitrogen gas, inserted in a surface plasmon resonance (SPR)
In the figure legends. Stock solutions of NaIO4 and of NaCNBH3 were applied at a flow rate of 20 µL/min, and 100 µL samples of protein solution (always at 1 mg/mL protein in PBS) were injected. After two injections of commercial BSA and after rinsing with SDS, monomeric proteins, which had been gel filtered on Superdex 200 (Amersham) to remove the small amount of oligomeric protein, were applied.

The BIACore X setup used in this study has two different flow cells which are pressed onto two different spots of the functionalized gold surface so that each of these gold areas is in contact with liquid. Usually, the two flow cells (FC1 and FC2) were operated in series (see flow cell diagram in Figure 11S in the Supporting Information), with an effective delay of 0.5 s between FC1 and FC2, corresponding to a time delay of 1.5 s at a flow of 20 µL/min. For selective injection into one of the two flow cells, the liquid in the other flow cell was cut off from the flow (see Figure 11S). The SPR angle in each flow cell was measured at 1 s time intervals and plotted versus time. The trace for FC1 always showed a lower initial resonance angle than the trace for FC2. The 100 µL sample loop became part of the flow channel when the “injection” was performed by the automated system.

Measurement of nonspecific protein adsorption by SPR was performed in PBS at the same flow, applying 100 µL samples of different protein content with a concentration of 1 mg/mL, as specified in Figure 3. For the coupling of a small ligand (biocytin hydrazide, see Figure 4) or of a protein via small heterobifunctional linkers (3-(maleimido)-propionyl hydrazide, abbreviated as MPH, or 3-(2-pyridyldithio)-propionyl hydrazide, abbreviated as PDPH, see Figures 3 and 5) the vicinal diol head groups on the mixed SAM were oxidized to aldehyde functions by periodate injections in the BIACore setup (see Figures 1 and 2) and subjected to further derivatization steps, as specified in the Results section and in the figure legends. Buffers, reagent stock solutions, and the premixing of injected reagents and samples are described in the Supporting Information; the effective concentrations of the essential components are stated in the figure legends. Stock solutions of NaIO4 and of NaCNBH3 were always freshly prepared and consumed within 1 h. NaIO4 was protected from light.

**Results and Discussion**

**Syntheses of OEG-Linked Alkanethiols with Latent Aldehyde Functions.** In a preceding study,33 S-protected mercaptohexadecanoic acid (AcS-HDA, I, see Figure 1) was directly coupled to the acetonide-protected form of 3-amino-1,2-propanediol (APD-ac, 3), the protecting groups (thioester and acetonide) were removed, and a linear conjugate with a thiol and a vicinal diol terminus was obtained. The same synthetic route has now been extended, with the insertion of an OEG spacer (NH2-EG6-COOH, 2) between AcS-HDA and APD-ac (see Figure 1). The cleavage of thioester and the acetonide function gave MHD-EG6-APD (4), which nicely matched with the common OEG-terminated thiol MHD-EG8 (5)36 to give mixed SAMs with a fractional content of latent aldehyde functions that can quickly be unmasked by aqueous solutions of periodate (see Figure 1).

A similar synthetic route toward a vicinal diol-terminated SAM component with an OEG chain is shown in Figure 2. A simple S-protected OEG-terminated alkanethiol (9, Figure 2) was O-alkylated with t-butyl acrylate. The protecting groups were removed, and a dicarboxylic acid (6) was obtained to which APD-ac (3) was coupled via amide bond formation (see Figure 2). The cleavage of acetonide and disulfide gave HS-C12-EG4(APD (7), which nicely matched with the simple, OEG-terminated thiol HS-C12-EG8 (8)36 to form a mixed SAM with a fractional...
content of latent aldehyde functions (see Figure 2), in analogy to the EG₈-based SAM shown in Figure 1.

**Protein Resistance of Mixed SAMs with Latent Aldehyde Functions on ~5% of the OEG Chains.** In a previous study, different OEG-terminated SAMs had been examined for their protein resistance after 1–2 weeks of storage under water at 4 °C. Good protein resistance was observed with SAMs consisting of pure MHD-EG₅ (5) or pure HS-C₁₂-EG₄ (8), especially when the SAMs had been formed by incubation in acetonitrile containing 20 μM thiol for 36 h. In the present study, 5 mol % of the simple OEG-terminated thiols 5 and 8 were replaced by the new thiols 4 and 7, which carry a vicinal diol group on one OEG terminus, to prepare mixed SAMs with latent aldehyde functions on ~5% of the OEG chains. The nonspecific adsorption of protein was examined by SPR (see Experimental Procedures). Different protein samples were injected at 1 mg/mL protein concentration, as is visible from the transient rises in the SPR angle during the injections (see Figure 3), which are due to the higher refractory index of the protein samples in comparison to that of the running buffer (PBS).

As shown in Figure 3 for the mixed SAM of MHD-EG₅-APD (4) and MHD-EG₅ (5), the first injection of bovine serum albumin (BSA) caused a net signal increase of 22 resonance units (RU) in flow cell 1 (FC1, solid line, or 16 RU in FC2, dotted line) and the first injection of IgG after the SDS washing step gave a net rise of 30 RU in FC1 (or 32 RU in FC2). These values are slightly higher than those for SAMs of pure MHD-EG₅ (8 ± 4 RU), yet they correspond to only ~1% of maximal monolayer coverage, thus the mixed SAM in Figure 3 is to be rated as highly protein-resistant.

**Unmasking of the Latent Aldehyde Functions on the SAM and Coupling of a Small Ligand.** After examination of protein resistance, the identical chip as in Figure 3 was subjected to the derivatization steps shown in Figure 4. The vicinal diol groups on the mixed SAM of thiols 4 and 5 (see Figure 1) were converted into aldehyde functions by two injections of periodate (10 mM, in acetic buffer with pH 5.5), as seen from the transient changes in the SPR angle in Figure 4. No net change in the SPR angle was observed after periodate injection, as expected for the tiny mass change associated with the oxidation of vicinal diol functions on ~5% of the OEG chains. No net change in the SPR angle was also observed in the subsequent injections of biocytin hydrazide (in FC2 only) and ethanolamine, thus the traces in the sensogram (Figure 4) contained no information on whether biocytin hydrazide had formed hydrazone bonds with the aldehyde functions in FC2, and/or whether ethanolamine had blocked unused aldehyde functions in both flow cells by Schiff base formation. NaNBH₄ had been included in the injections of biocytin hydrazide and ethanolamine to convert the initially formed C=O double bonds into stable C–N single bonds.

After chemical derivatization, the SAM was re-examined for nonspecific protein adsorption by injection of BSA (1 mg/mL, 15 μM) as well as streptavidin (2 μM), which had been functionally blocked by a large excess of d-biotin (200 μM). As can be seen in Figure 4, neither BSA nor blocked streptavidin was adsorbed to the SAM in spite of the chemical treatments applied since the initial tests of the identical SAM shown in Figure 3.

Having demonstrated protein resistance of the functionalized SAM, the ligand function of presumably immobilized biocytin hydrazide was tested by injecting functionally active streptavidin (2 μM, not blocked with biotin). As expected, a high amount of streptavidin (~700 RU, determined ~12 min after the end of the streptavidin injection) was bound in FC2, whereas almost no streptavidin (26 RU) was bound in FC1, which had not been treated with biocytin hydrazide. The amount of streptavidin bound in FC2 corresponded to ~70% of a dense streptavidin monolayer, indicating efficient oxidation of the vicinal diols with periodate and subsequent derivatization with biocytin hydrazide in FC2. Subsequent injections of biotin-IgG, streptavidin, and again biotin-IgG led to further specific binding of these proteins in FC2, reflecting the formation of a protein multilayer with similar amounts of protein in each individual layer.

It should be noted that a fraction of specifically bound streptavidin was lost on the time scale of minutes. As explained in more detail in the Supporting Information, this must be attributed to the combination of two reasons: (i) Our aldehyde-coupled biocytin hydrazide molecules have a different molecular structure (see top of Figure 4) and obviously less affinity for streptavidin than the typical biotin-terminated SAM components used in previous studies. (ii) Our SAM has a rather low surface density (~5%) of biotin groups at which density many streptavidin molecules are known to be bound by one biotin residue only and thus with lower affinity than bivalently bound streptavidin.

The last two injections in Figure 4 were with preblocked streptavidin and with unlabeled IgG to re-examine nonspecific adsorption by the used chip surface. No binding was observed, with the slow decrease of the trace being attributed to the gradual dissociation of streptavidin from the chip. At first sight, there seems to be some nonspecific adsorption in the control cell (dotted line in Figure 4) since the injections of unblocked streptavidin, biotin-IgG, unblocked streptavidin, and again biotin-IgG led to the binding of 26, 77, 89, and 188 RU, respectively. In fact, however, these small binding responses clearly represent “undesired specific binding” in the control cell, due to an imperfection of the microfluidic system of the BIAcore X setup (as explained in context with Figure 11S in the Supporting Information). In other words, traces of biocytin hydrazide entered FC1 by diffusion while this reagent was actively pumped through FC2 only (third injection in Figure 4). Proof for truly specific binding in the control cell comes from a complete block of streptavidin binding by free d-biotin (tested twice, see Figure 4) and from a lack of binding of BSA and unlabeled IgG (Figure 4, dotted line). In conclusion, the SAM used here exhibited a perfect specificity of binding, with the apparent imperfection of the control cell being due to a technical imperfection of the SPR setup used here.

**Attempt of Direct Protein Coupling to Protein-Resistant Aldehyde-Terminated SAMs.** Due to the abundance of lysine residues in most proteins, aldehyde functions are widely used for the immobilization of proteins. In the preceding study, SAMs prepared from the amide conjugate of mercaptohexadecanoic acid and 3-amino-1,2-propanediol (analogous to thiol 4 in Figure 1, but lacking NH₂-EG₆-COOH, 2) and oxidized with periodate had shown rapid coupling of proteins by Schiff base formation. In the present study, a heptaethylene glycol spacer (2) was inserted between the fatty acid and the latent aldehyde moiety, resulting in thiol 4. Moreover, thiol 4 was diluted with

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which was applied at 5 \text{mM} and the flow rate was 10 \text{mL/min}. After reaction of the SAM with periodate, 2 \text{mM} coupling to the terminal aldehyde functions (molar ratio, see Figure 3) and subsequent attempt of direct protein binding. The second hypothesis was tested by preparing a mixed SAM of thiol. HS-C_{12}-EG_{4} (4a) and the flow rate was 10 \text{mL/min}, except for the 6.7 \text{mM} IgG injection, which was applied at 5 \text{mL/min}. 20 \text{mM NaCNBH}_{3} was included in all injections of IgG to provide for fixation of potentially formed Schiff bases. This was done by mixing 147 \text{mL} of protein solution with 3 \text{mL} of 1 \text{M NaCNBH}_{3} stock solution and then loading 140 \text{mL} thereof into the sample loop of the BIAcore setup.

![Figure 5](image)

**Figure 5.** Periodate oxidation of a mixed SAM of 4 and 5 (1/19 molar ratio, see Figure 3) and subsequent attempt of direct protein coupling to the terminal aldehyde functions (4a). Before oxidation with periodate, 2 \text{mM} IgG (0.3 mg/mL) was injected twice to test for nonspecific adsorption. After reaction of the SAM with periodate (10 \text{mM}, pH 5.5, 2 × 10 \text{min}), IgG was injected again, both at 2 \text{mM} and at 6.7 \text{mM} (1 mg/mL). All injection volumes were 100 \text{mL}, and the flow rate was 10 \text{mL/min}, except for the 6.7 \text{mM} IgG injection, which was applied at 5 \text{mL/min}. 20 \text{mM NaCNBH}_{3} was included in all injections of IgG to provide for fixation of potentially formed Schiff bases. This was done by mixing 147 \text{mL} of protein solution with 3 \text{mL} of 1 \text{M NaCNBH}_{3} stock solution and then loading 140 \text{mL} thereof into the sample loop of the BIAcore setup.

![Figure 6](image)

**Figure 6.** Periodate oxidation of a mixed SAM of 4 and 5 (1/19 molar ratio, see Figure 3) and derivatization of the terminal aldehyde function (4a) with 3-(maleimido)-propionyl hydrazide (MPH) and NaCNBH_{3} in flow cell 2 only (FC2, solid line), during which time no flow was applied in flow cell 1 (FC1, dotted line). Unused aldehyde functions in FC1 were blocked with ethanolamine. Meanwhile, the S-acetyl groups on biotin-IgG-SATP were cleaved with hydroxylamine, yielding free thiol that was coupled to the immobile maleimide functions in FC2. Subsequent injection of streptavidin served to block both unused maleimide and unused aldehyde functions. Blocked streptavidin (with a 100-fold concentration of biotin) was injected to test for nonspecific protein adsorption. Sequential injection of streptavidin, biotin-IgG, streptavidin, and again biotin-IgG showed specific capture, while subsequent injections of blocked streptavidin and unlabeled goat IgG again showed a complete absence of nonspecific adsorption. All injection volumes were 100 \text{mL}, and the flow rate was 10 \text{mL/min}, except for biocytin hydrazide and ethanolamine, which were applied at 5 \text{mL/min}, and deprotected biotin-IgG-SATP, which was applied at 2 \text{mL/min}.

The extent of IgG coupling was always below 100 RU, that is, below 4% of maximal surface coverage (see Figure 1S and Table 1S in the Supporting Information). From this, we conclude that direct coupling of protein is generally inhibited by the strong protein resistance of these OEG-terminated SAMs even if the aldehyde-terminated thiol is considerably longer than the matrix thiol.

**Fast Indirect Protein Coupling via Small Heterobifunctional Adaptor Molecules.** With the direct coupling of proteins being too slow, the aldehyde functions of the mixed SAM of thiols 4a and 5 (Figure 1) were converted into other coupling functions which afforded fast immobilization of protein. In one case, the aldehyde functions on the periodate-treated SAM were reacted with MPH (see Figure 6) to introduce covalently bound maleimide residues in FC2 (solid line), whereinupon ethanolamine was used to block unused aldehyde functions in FC1 (dotted line). The maleimide functions in FC2 provided for almost instantaneous coupling of IgG, which had been modified with both biotin and free thiol groups (biotin-IgG-SATP, see Figure 1S in the Supporting Information). Injection of cysteine served to block unused maleimide functions by thioether formation and to block unused aldehyde functions by thiazolidine ring formation.22 In the subsequent alternating injections of streptavidin (2 \text{mM}) and biotin-IgG (2 \text{mM}), progressive amounts of protein...
Subsequent injection of NaCNBH₃ and DTT served to reduce the response was caused by the DMSO content (6.7%), which came (FC1 and FC2, dotted and solid lines, respectively). The strong (molar ratio, see Figure 3) and derivatization of the terminal aldehyde functions were reacted with the hydrazide derivatives (MPH or PDPH) of two widely used heterobifunctional cross-linkers

were bound in FC2, corresponding to 24, 50, 54, and 94% of surface coverage with these proteins (see Figure 6, solid line). Blocked streptavidin (injected twice) and unlabeled IgG were bound neither in FC2 nor in FC1 (solid and dotted line), proving high protein resistance in spite of the preceding chemical steps. As in Figure 4, the minor binding of unblocked streptavidin and of biotin-IgG in the control cell (dotted line in Figure 6) must be attributed to the imperfect microfluidic system of the BIACore X setup, which allowed for cross-contamination of FC1 with FC2 only.

In the second case, the aldehyde functions of 4a were reacted with PDPH (see Figure 7) to introduce pyridyldithio groups, which were reductively cleaved with 1,4-dithiothreitol (DTT) to give free thiols on the top side of the SAM. These provided for quick coupling of maleimide-modified protein (biotin-IgG-SMCC), whereupon unused thiols on the SAM were blocked with N-ethylmaleimide. The amount of covalently bound protein (850 RU) corresponded to one-third of a dense IgG monolayer. All further results in Figure 7 were analogous to those in Figure 6, except that the progressive increase in binding capacity was more pronounced in the successive injections of streptavidin, biotin-IgG, streptavidin, and again of biotin-IgG. This progressive step increase is explained by the polyvalency of streptavidin and of multiply biotinylated IgG, which allows for “amplification” of binding in successive injections.

A comparison of Figure 6 (coupling of a thiol protein to a maleimide surface) with Figure 7 (coupling of a maleimide protein to a thiol surface) shows that the latter was less efficient. The probable explanation is a combination of the following reasons: (i) incomplete derivatization of the aldehyde functions on the surface with 20 mM PDPH within 20 min, (ii) reductive cleavage of the pyridyldithio group by the subsequently injected NaCNBH₃ (see Figure 7), and (iii) reaction of the thereby generated surface-bound thiols with adjacent unused aldehyde functions, resulting in a reduced number of surface-bound thiols available for subsequent reaction with maleimide-derivatized protein. In spite of this imperfection, the scheme shown in the top part of Figure 7 provides for easy preparation of an OEG-terminated SAM with reactive thiols on the water side of the SAM, which is otherwise a nontrivial task, due to the high affinity of thiols and disulfides to the gold support.

Comparison of EG₄-Based SAMs with EG₅-Based SAMs.

The high stability of the so generated aldehyde functions provided for convenient spontaneous coupling of the hydrazide form of a small ligand molecule (biocytin hydrazide), yielding a sensor surface which could bind streptavidin by specific recognition but did not bind blocked streptavidin or other proteins by nonspecific adsorption (Figure 4). Alternatively, the aldehyde functions were reacted with the hydrazide derivatives (MPH or PDPH) of two widely used heterobifunctional cross-linkers (BMPS or SPDP, respectively, see list of abbreviations used), affording the rapid binding of protein by thioether formation.

Conclusions

The modular strategies in Figures 1 and 2 provided for easy synthesis of alkanethiol derivatives which carry an OEG chain and a vicinal diol function on its outer end. The OEG chains in the resulting conjugates (4 and 7) allowed for mixing with simple OEG-terminated thiols (5 and 8, respectively), yielding SAMs with high protein resistance (Figure 3). The vicinal diol head groups on the SAM were converted into aldehyde functions (thiols 4a and 7a) using a dilute aqueous solution of periodate, which is nonvolatile and noncorrosive and thus compatible with any kind of device and procedure. The high reactivity and long-term stability of the so generated aldehyde functions provided for convenient spontaneous coupling of the hydrazide form of a small ligand molecule (biocytin hydrazide), yielding a sensor surface which could bind streptavidin by specific recognition but did not bind blocked streptavidin or other proteins by nonspecific adsorption (Figure 4). Alternatively, the aldehyde functions were reacted with the hydrazide derivatives (MPH or PDPH) of two widely used heterobifunctional cross-linkers (BMPS or SPDP, respectively, see list of abbreviations used), affording the rapid binding of protein by thioether formation with maleimide functions (Figures 6 and 7).

Protein resistance, that is, absence of nonspecific adhesion, was maintained throughout. The undesired side effect of high protein resistance was the exceptionally slow immobilization of protein when attempting to directly couple proteins via lysine residues to the aldehydes on the SAM. In this respect, SAMs with aldehyde functions on a minor fraction of the OEG chains cannot compete with the analogous carboxyl-terminated OEG–SAMs to which proteins can efficiently be coupled after carboxyl activation with EDC and NHS. In contrast, vicinal diol-terminated SAMs without OEG chains (or with buried OEG chains) had given fast coupling of unmodified protein after
periodate treatment. In conclusion, the performance of OEG-terminated SAMs with vicinal diol functions has been exemplified, and so have the advantages and drawbacks of the OEG chains in SAMs with latent aldehyde functions.

Acknowledgement. Consultation in NMR spectroscopy by Prof. Norbert Müller and measurement of mass spectra by Prof. Christian Klampfl are gratefully acknowledged. This work was supported by the Austrian Science Foundation (Projects P15295, N00104), and A.T. and R.T. were supported by the Deutsche Forschungsgemeinschaft (DFG, FOR495) and the Bundesministerium für Bildung und Forschung (BMBF, Programm Nanobiotechnologie).

Supporting Information Available: Syntheses of compounds 4, 5, 7, and 8. Preparation of buffers, reagent stock solutions, biotin-IgG, biotin-IgG-SATP, and biotin-IgG-SMCC. Preparation of gold-coated SPR sensor chips and of mixed SAMs on the cleaned gold surfaces. Microfluidic system of the two flow cells in the BIAcore X setup, which allows for minor cross contamination. Attempt of direct protein coupling to mixed SAMs of thiols 4a and 5 (as shown in Figure 1) or thiols 4a and 8. Preparation and functionalization of mixed SAMs (5/95) of thiols 7 and 8, in analogy to Figures 4 and 6. This material is available free of charge via the Internet at http://pubs.acs.org.

Abbreviations Used

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<th>Abbreviation</th>
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<tr>
<td>AcS-HDA</td>
<td>16-(acetylthio)-hexadecanoic acid</td>
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<td>25,25′-dithio-bis(4,7,10,13-tetraoxa-pentacosanamic acid)</td>
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<tr>
<td>HSC12-EG5</td>
<td>24-sulfanyl-3,6,9,12-tetraoxa-1-tetracosenol</td>
</tr>
<tr>
<td>HS-C12-EG5-APD</td>
<td>rac-N-(2,3-dihydroxypropyl)-25-mercaptop-4,7,10,13-tetraoxa-pentacosanamide</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin G</td>
</tr>
<tr>
<td>NHS</td>
<td>N-hydroxysuccinimide</td>
</tr>
<tr>
<td>OEG</td>
<td>oligo(ethylene glycol)</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>PDPH</td>
<td>3-(2-pyridylthio)-propionyl hydrazide</td>
</tr>
<tr>
<td>PEG</td>
<td>poly(ethylene glycol)</td>
</tr>
<tr>
<td>RU</td>
<td>resonance unit (1 RU = 0.0001° surface plasmon resonance angle shift in the BIAcore)</td>
</tr>
<tr>
<td>SAM</td>
<td>self-assembled monolayer</td>
</tr>
<tr>
<td>SATP</td>
<td>N-succinimidyl 3-(acetylthio)-propionate</td>
</tr>
<tr>
<td>SC1</td>
<td>standard cleaning solution no. 1</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SMCC</td>
<td>N-succinimidyl 4-(maleimidomethyl)-cyclohexanecarboxylate</td>
</tr>
<tr>
<td>SPDP</td>
<td>N-succinimidyl 3-(2-pyridylthio)-propionate</td>
</tr>
<tr>
<td>SPR</td>
<td>surface plasmon resonance</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
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</table>

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