Chapter 2
Antifouling Surfaces of Self-assembled Thin Layer

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Abstract Advances in new technologies such as biosensors, biomedical implants rely greatly on the performance of devices. In this chapter, strategies for preventing fouling of proteins, bacteria, and marine fouling organisms by using self-assembled thin layers are reviewed. One of the commonly used methods for inhibiting the adhesion of proteins, bacteria, and marine organisms is the modification of the surfaces with poly(ethylene glycol) (PEG) monolayers or PEG-based alternatives, others such as oligo(ethylene glycol), zwitterionic molecules, enzymes, and functional polymers have also been used for antifouling materials with much less environmental impact than traditional biocides. Protein-resistant coatings may also resist bacterial attachment and the subsequent biofilm formation. The emergence of environmental issues has necessitated the development of nontoxic and biocompatible antifouling surfaces under marine environments. Although considerable progress has been made in the design of antifouling coatings, challenges still remain, including comprehensive understanding of the underlying adhesion mechanisms, seeking for more environmentally friendly and effective, and even "universal" nonfouling materials in the future.

2.1 Introduction

Materials with anti-biofouling properties, i.e., materials that resist the nonspecific adsorption of proteins, bacteria, or other biological species, are of great interest for a variety of biomedical and biotechnological applications ranging from medical implants to contact lenses, drug delivery, biosensors, as well as marine applications such as coatings of ship hulls [1–5]. Fouling issues will lead to energy dissipation and device failure, resulting in massive economic and environmental costs [6–10]. Biofouling is the contamination of unwanted biological matters on a surface, with two categories called microfouling (leading to biofilms) and

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macrofouling. Examples of microfouling organisms include bacteria and algae, whereas macrofouling organisms include larger barnacles and mussels [11–13]. Protein adsorption is a nonspecific event that is related to the solvent–protein interactions on the surfaces, or as a result of complex interactions between proteins and surfaces including van der Waal's interactions, electrostatic interactions, hydrogen bonding, and hydrophobic interactions [14–18], as well as the surface topography [19]. Protein fouling on biological implants reduces the efficiency of the devices and may also result in harmful side-effects, such as infections of catheters, prosthetic devices, and immunological assays [20, 21]. Moreover, protein adsorption on the surfaces of biological implants would provide a conditioning layer for microbial colonization and the subsequent biofilm formation. Bacterial biofilms are ubiquitous and are the major cause of chronic infections in humans and persistent biofouling in industry [22]. The attachment of bacteria to a surface leads to subsequent colonization and results in the formation of robust, surface-associated communities known as biofilms that exist in natural and anthropogenic environments. Additionally, any defects in the surface chemistry can serve as nucleation sites for bacterial attachment, and the treatment of the adherent biofilm is difficult and costly. Meanwhile, strategies for biofilm prevention based on surface chemistry treatments or surface microstructure have been found to be effective [23]. Biofouling associated with marine environments which initiates the accumulation of bacterial biofilm and is followed by the attachment of larger marine organisms is a worldwide problem, in particular for the naval industry. Aquatic fouling on ships and underwater structures causes the deterioration of the surfaces, increased ship hull drag, corrosion, and fuel consumption.

The past decade has witnessed significant advances in the development of antifouling coatings. Most approaches for preventing biofouling caused by proteins, microbes, and marine organisms involve developing coatings that resist the adhesion of biofoulants or degrade them [24]. Clearly, it is extremely desirable to prevent rather than cure biofilm formation. Traditional techniques involve the designing of coatings that release biocidal agents, including antibiotics, quaternary ammonium salts, and silver into the surrounding aqueous environment [25]. However, the emergence of toxic and environmental issues has necessitated the development of biocompatible nonfouling strategies. Therefore, other techniques based on the use of polymers, enzymes, and photoactive agents are being investigated. Furthermore, natural antifouling surfaces also provide an inspiration for developing novel antifouling coatings, which can be realized by the combination of the structure or topography with specialized surface chemistry to control the adhesion process. For example, low surface-energy models through biomimetic nonfouling "lotus leaf" effect [24, 26], or by reducing van der Waals dispersion force inspired by geckos [27]. A commonly employed method is focused on the use of chemicals with functional groups as a means to inhibit adhesion, such as hydrophilic polymeric materials, forming highly hydrated layers to inhibit adhesion [3, 28–32]. In this chapter, we mainly highlight antifouling surfaces based on self-assembled monolayers (SAMs) and layer-by-layer (LBL) assembly techniques.

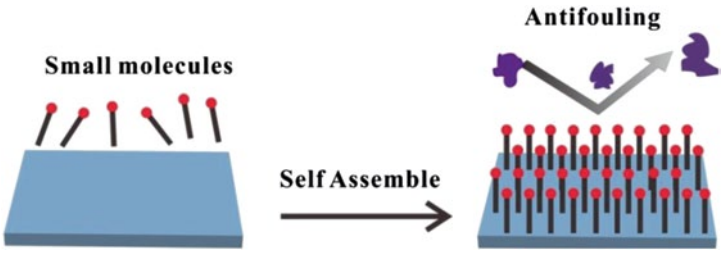

Fig. 2.1 Schematic formation of antifouling coatings of self-assembled monolayers (SAMs)

2.2 Self-Assembled Monolayers (SAMs) Coatings that Resist Protein Fouling

2.2.1 PEG-based SAMs

Considerable progress has been made in the design of antifouling coatings, surfaces with micro/nanotopography, as well as coatings of different functionality have been studied for a surface to be resistant to proteins. Several materials that exhibit a significant reduction in nonspecific adsorption of proteins, one of the most commonly used SAMs strategies for imparting adhesion resistance involves the functionalization of surfaces with polyethylene glycol (PEG) or oligo(ethylene glycol) (OEG) and other PEG-based coatings [28, 33–35]. At the same time, zwitterionic SAMs, and other hydrophilic materials, tetraglyme, dextran, mannitol, polyamines, peptidomimetic polymers, peptide-based SAMs and synthetic polymers are also studied [20, 36–41].

In the case of SAMs, active groups are needed (e.g., thiols or silane) in order to anchor them to a substrate (Fig. 2.1). Surfaces coated with PEG or OEG are generally serving as the preferred materials for preventing bio-adhesion. The PEG-based coatings with antifouling properties have been widely described in the literature [1, 42]. A thin layer of PEG was found to exhibit excellent resistance to proteins in the biological media [19, 43]. However, the kinetics and thermodynamic origins of the protein resistance of PEG remain a matter of debate [20]. PEG coatings of long chain can significantly reduce protein adsorption. The PEG coatings can be prepared through the physical/chemical adsorption and covalently grafted techniques [19, 20, 28, 44]. In design of these nonfouling surfaces, three characteristics of the PEG or PEG-based chains should be preserved: (i) a hydrophilic repeating unit; (ii) a unit that is well solvated and can form hydrogen bond with water; (iii) an oligomer that is of conformational freedom in water.

It is generally believed that water plays an important role in surface resistance to protein adsorption [45, 46]. Such antifouling behavior is mainly due to the steric repulsion between hydrated neutral PEG chains and proteins [47]. A hydration layer forms on hydrophilic or neutral PEG via hydrogen bonds, and zwitterions form a hydration layer via electrostatic interactions [41, 48]. The protein

resistance of PEG can be attributed to the "steric repulsion" between hydrated neutral PEG chains and proteins, which is an entropic effect caused by the unfavorable change in free energy associated with the terminal hydrophilicity of head groups and the high conformational freedom [25, 49]. Furthermore, as cell-adhesion mechanisms are generally protein-mediated, PEG-modified surfaces exhibit effective depression of both protein adsorption and cell attachment, and protein-resistant coatings may also resist bacterial attachment and the subsequent biofilm formation, rendering PEG-coated materials to be extensively used as antifouling coatings [50, 51].

Since the early 1980s, PEG has been widely used for proteins and cells resistant coatings in aqueous solution [52]. SAMs of alkanethiolates on gold is a classical model surface to study the relationships between the structure of a substrate and the adsorption of protein. Pioneer work has been systematically taken out to elucidate underlying mechanisms of the protein resistance, monolayers with different chain lengths, and alkyl termination, as well as the packing density and chemical composition have been investigated. Whitesides and coworkers [19] reported a study of the adsorption of four proteins (fibrinogen, lysozyme, pyruvate kinase, and RNAse A) to self-assembled monolayers derived from thiols of the structure $HS(CH_2)_{10}R$ which differed in both structure and chain length, where R is CH_3, CH_2OH, or oligo(ethylene oxide). Results indicated that SAMs with high concentrations of chains effectively resisted the adsorption of proteins, and coatings of longer chains are more effective in resisting the adsorption than those of shorter chains. The efficiency of the protein resistance increased with the length of the OEG chains. Also, little difference of protein adsorption between a CH_3 group and a OH group of the oligo(ethylene oxide) end group was observed. Meanwhile, oligo(ethylene oxide) monolayers with longer chains were found to decrease the protein adsorption at lower mole fractions [53, 54]. The protein adsorption is also related to the wettability of the SAMs; the protein resistance ability increases with the hydrophilicity for a given hydrophilic surface, but the wettability only serves as a general predictor of protein resistant.

Oligo(ethylene oxide) SAMs repelling protein adsorption is mainly due to the repulsive hydration forces between the water layer around the OEG chains and the protein [55, 56]. Prime and Grunze suggested that protein resistance surfaces be coated with OEG self-assembled monolayers with different chain lengths and end alkyl groups [57, 58]. Both internal and terminal hydrophilicity is vital to the protein resistance. Monolayers with a hydrophobic interior, such as those containing oligo(propylene glycol), have little effect on protein resistance, only those that have hydrophilic interior segments suppress the protein adsorption. Vanderah et al. reported that a methoxy-terminated hexa(ethylene glycol) SAMs on gold which have a significant component of well-ordered helical conformations exhibit better inhibition of protein adsorption [59]. Protein resistance by oligo(ethylene oxide) (OEO)-modified surfaces was described as a result of changes in free energy due to oligomer–oligomer interactions. In the case of a fully covered surface, there is no free energy perturbation for resisting the protein adsorption due to little conformational changes [60]. SAMs of lower packing densities with more

chain flexibility can be explained by the 'steric' model [61]. Also, the penetration of water molecules in the interior of the SAMs of lower packing densities is necessary for protein resistance due to the water osmotic effects [62]. Thus, SAMs are protein resistant only when several key factors are under consideration, such as the hydrophilicity of the termination and the internal units, the lateral packing density, and any of the related factors that will affect the overall protein resistance. Grunze group used Monte Carlo technique to simulate the behavior of water near the surface of an oligo(ethylene glycol)-terminated alkanethiol self-assembled monolayer; it was shown that water molecules could penetrate into the near-surface region of the helical SAM and resulted in conformational disordering of the SAM on the gold substrate. Chains which favor all-*trans* SAMs conformation, are much more resistant to the penetration of water molecules and have a noticeably lower surface density of hydrogen bonds with water molecules, suggesting that the interaction between the chains and water molecules plays a vital role in determining protein resistance [63]. Recently, Jiang et al. reported a hybrid PEG chains and polyhedral oligosilsesquioxane (POSS) on a gold surface through thio-ene photo-click reaction that exhibited excellent protein resistance and long-term stability. The amount of protein adsorption decreased with the increasing of the lengths and the number of PEG chains [64]. Gooding and coworkers reported a stepwise construction method to build antifouling surface with excellent protein repellence [65]. Acetylene-terminated surfaces were functionalized via a copper-catalyzed azide−alkyne cycloaddition reaction to produce an amine-terminated layer, and subsequently the amine-terminated layer was further conjugated with PEG to produce an antifouling surface. Minute amounts of fouling of lysozyme and no fouling of HSA were observed. The surface is completely antifouling to larger proteins while smaller biological species are not completely repelled because the smaller biological species may be able to penetrate into the PEG layer and hence less effectively.

Various techniques for fabricating antifouling surfaces have been developed by anchoring PEG on different substrates. New biomimetic strategies for modification of biomaterial surfaces with PEG were developed [66–71]. Messersmith and coworkers [68, 69] used X-ray photoelectron spectroscopy (XPS), spectroscopic ellipsometry, and optical waveguide light mode spectroscopy (OWLS) to examine the surface adsorption and protein resistance behavior of bio-inspired polymers consisting of poly(ethylene glycol) conjugated to peptide mimics of mussel adhesive proteins (Fig. 2.2). 3,4-Dihydroxyphenylalanine (DOPA) containing peptides conjugated to PEG (mPEG-DOPA) adsorbed onto Au and Ti surfaces, rendering these surfaces resistant to cell attachment for a long period. By using DOPA and PEG, surface densities are higher than other existing PEG immobilization strategies, thus afford a strong correlation between PEG surface density and protein resistance [68]. They also reported an entirely new class of synthetic antifouling macromolecules that mimic polypeptides. These polymers are specifically designed for surfaces with robust, and long-term resistance to fouling in the biological environment [39]. Gademann and coworkers developed a novel biomimetic strategy based on the cyanobacterial iron chelator anachelin for

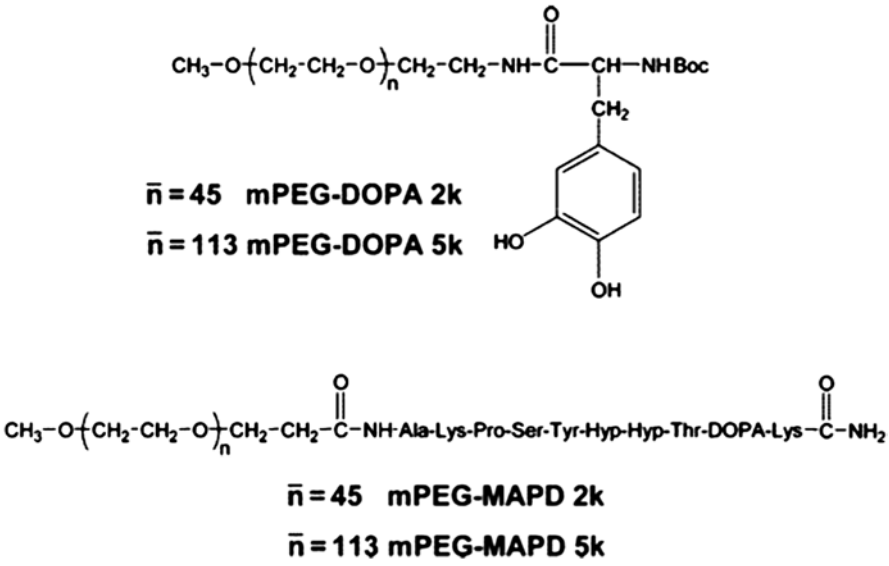

Fig. 2.2 Biomimetic PEG conjugates used for surface modification. PEG polyethylene glycol. (Reprinted with permission from Ref. [69] Copyright 2003, American Chemical Society)

the formation of a stable, protein-resistant adlayer [72]. Stagnaro and colleagues have reported monomethoxy poly(ethylene glycol) (mPEG) with two different molecular weights that were grafted on polyethylene to elucidate the adsorption mechanisms of plasma protein by quartz crystal microbalance with dissipation (QCM-D) [73]. Results revealed that the preadsorbed bovine serum albumin (BSA) on the surfaces of PE-g-mPEG films reduced the subsequent adsorption of fibrinogen. The surface coverage but not the chain length of PEG seemed to have a predominant effect on the nonspecific protein adsorption of BSA because the dense PEG chains could release more trapped water molecules to resist BSA. The preadsorbed fibrinogen could be gradually displaced by high-concentration BSA, but the adsorption and displacement of fibrinogen depended on the surface hydrophilicity of biomaterials. Recently, Hunt and colleagues covalently bound PEG coatings of varying thickness to investigate the effect of PEG chain length on minimizing nonspecifc protein (lysozyme and fibrinogen) adsorption onto the biosensor surface. PEG SAMs significantly reduced the nonspecific protein adsorption to the surface [74]. Surprisingly, PEG coating of short chain is more effective in reducing both lysozyme and fibrinogen adsorption on the surface of the microsphere in comparison to the long-chain PEG coating, which is somewhat atypical for PEG functionalized systems where longer PEG chains are preferred. Perhaps, PEG of short chains can form denser coating and the high surface density is more significant than long chain length in preventing the adsorption [19, 55, 75].

The physical adsorption or covalent attachment of PEG chains (the "*grafting to*" approach) usually cannot reduce protein adsorption below a certain limit because of the steric effect so as to the low surface density of PEG chains on the surface, which can be attributed to steric issues that limit the density of the attached polymer chains [33, 76]. Many studies have reported that antifouling surfaces decorated with PEG and its derivatives cannot endure long-term stability due to the degradation or detachment of SAMs. Meanwhile, PEG has a number of inherent limitations, including thermal instability, non-degradability, susceptible to oxidation, especially under physiological conditions, and is difficult to functionalize. For example, PEG SAMs would decompose in the presence of oxygen and transition metal ions which were found in most biochemically relevant solutions [37, 77]. It was also shown that the grafted PEG would lose the protein repulsive ability above 35 °C [78], or lose the nonfouling property after a certain period. These limitations of PEG and its derivatives have prompted the search of alternative protein-resistant materials other than PEG [32, 79]. Common strategies for enhancing the coating's stability include choosing a more stable SAMs or polymer brushes anchoring to the substrate, or using polymers with a more stable chemical structure and network [20, 50].

2.2.2 Other SAMs Decorated Surfaces

Whitesides and coworkers also reported SAMs of alkanethiolates on gold bearing tri(propylene sulfoxide) groups to prevent the nonspecific adsorption of protein and the subsequent attachment of cells. They used surface plasmon resonance (SPR) spectroscopy to measure the adsorption of the proteins RNAse A and fibrinogen. The results indicated that SAMs presenting tri(propylene sulfoxide) groups are more hydrophilic than those presenting hexa(ethylene glycol) groups, and the functional groups dimethyl sulfoxides are more biocompatible than ethylene glycol [80]. In their following work, they investigated proteins resistance effect of self-assembled monolayers of functional groups by using SPR spectroscopy. They have identified four functional groups that show proteins resistance when presented on mixed SAMs (Fig. 2.3) [81]. The absence of hydrogen bond donor groups improved the inertness of the substrate in protein adsorption. Surfaces that are coated with compounds with NCH_3 and OCH_3 groups are more effective in protein resistant than those that expose their more polar analogues with NH and OH groups. They also prepared mixed SAMs presenting different functional groups using a synthetic protocol based on the reaction of organic amines with a SAM terminated by interchain carboxylic anhydride groups. Surfaces that presented derivatives of oligo(sarcosine), *N*-acetylpiperazine, and permethylated sorbitol groups were particularly effective in resisting proteins adsorption. These functional groups that resist the adsorption of proteins have the following properties: they are hydrophilic; they have hydrogen bond accepting functional groups instead of the donating groups, and no net charge. And the most protein-resistant surfaces are hydrophilic [77, 81].

When the hydrogen bond donors were screened by using SAMs of $(EG)_n$ and other ether derivatives with different functional groups, the resistance ability of

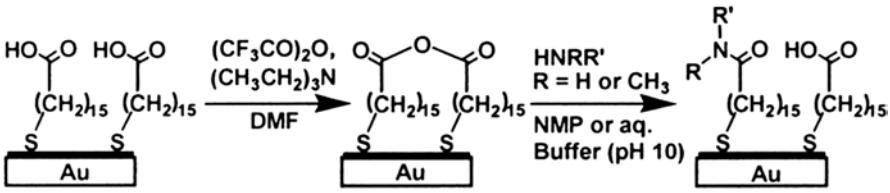

Fig. 2.3 Schematic illustration of the synthesis of mixed SAMs that presents a 1:1 mixture of –CONRR' and CO_2H/CO_2^- groups using the anhydride method. SAMs self-assembled monolayers. (Reprinted with permission from Ref. [81]. Copyright 2000, American Chemical Society)

these surfaces increased [77, 82, 83], EG or amino groups exhibit better protein resistance due to the positive charge. For example, the replacement of hydrogen-bond donor hydroxyl groups with methyl groups, or increasing the number of terminal –CN groups will improve the inertness of the surface significantly. The mannitol-terminated SAMs were reported as a highly protein-preventing surface [37]. Surface plasmon resonance spectroscopy showed that the mannitol-terminated SAMs prevented the adsorption of several proteins and were indistinguishable from a SAM presenting tri(ethylene glycol)groups. These works afford to evaluate the hypotheses relating to molecular structures and biological properties of surfaces. Experiment results of chemicals and the structures of surfaces that resist the adsorption of proteins offer a guide to design antifouling surface/interface.

Zwitterionic SAMs Coatings with zwitterionic groups can bind water molecules via electrostatically induced hydration, and the zwitterionic chains are electrically overall neutral and highly resistant to nonspecific protein adsorption, bacterial adhesion, and biofilm formation [32]. These inert surfaces based on zwitterionic groups are probably more stable to oxidation over those based on EG layers. Jiang and coworkers reported the strong resistance of zwitterionic phosphorylcholine (PC) SAMs to protein adsorption [41]. They used both experimental and molecular simulation techniques to examine key factors of their antifouling properties. PC head groups having similar packing densities to membrane lipids favor an antiparallel orientation for the dipole minimization. Strong hydration capacity via electrostatic interactions and a balanced charge that minimize dipole are two key factors for their nonfouling behavior, and rendering the zwitterions excellent candidates for nonfouling materials. Nonspecific protein-resistant mixed SAMs were also extended to various counter-charged terminal groups of different valence and protonation/deprotonation [84]. It is demonstrated that excellent nonfouling surfaces can be readily constructed from mixed positively- and negatively charged components of equal valence in a wide range of thiol solution compositions. Results showed that a single compact layer of charged groups of balanced charge with a crystalline structure can resist nonspecific protein adsorption, but conformational flexibility is not required for protein resistance of a surface, the hydration layer formed on the mixed SAMs plays a dominant role in surface resistance to nonspecific protein adsorption [85, 86]. Holmlin et al. reported that mixed SAMs of a 1:1 mixture of thiols terminated in a negatively charged group and in a positively charged group had very low protein adsorption [86].

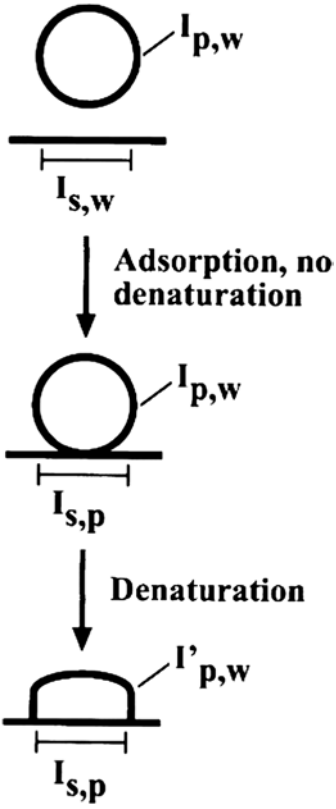

Fig. 2.4 Schematic illustration of the interfaces that are involved in the process of protein adsorption onto surfaces. Legend for the labels: I = interface, p = protein, w = water, s = solid. Upon adsorption, the protein can undergo conformational changes that cause a change in its interaction with water ($I'_{p,w}$). (Reprinted with permission from Ref. [77]. Copyright 2000, American Chemical Society)

Gooding and colleagues [87] reported that the zwitterionic phenyl layers can perform as low impedance anti-biofouling coating to the electrode surface with greater long-term stability; they coated a zwitterionic phenyl phosphorylcholine (PPC) diazonium salt, or a mixture (1:1) of zwitterionic sulfophenyl aryl diazonium salt and trimethylammonium phenyl aryl diazonium salt (mix-GC) on the electrode. The phenyl-based zwitterionic coatings are comparable to the OEG SAMs at resisting the nonspecific adsorption of bovine serum albumin and cytochrome c. And the PPC-GC and mix-GC layers are even better than OEG-SAM-Au at resisting the adsorption of negatively charged protein BSA.

Peptide-Based and Peptoid-Based Protein-Resistant Surfaces Unlike PEGs, peptides represent a "second generation" biodegradable material for antifouling SAMs; they have additional functionalities which can be selectively chosen or post-modified and can be used in the application of protein-resistant surfaces, which benefit from protease resistance property of the backbone, precise control of molecular weight, and side chain composition versatility [39, 40, 88]. The concepts for the design of the side chain and backbone for effective antifouling surfaces adhere to general principles (Fig. 2.4) [77]. Wöll and coworkers proposed a model system by employing peptide-based ultrathin SAMs with an arbitrary sequence to study the

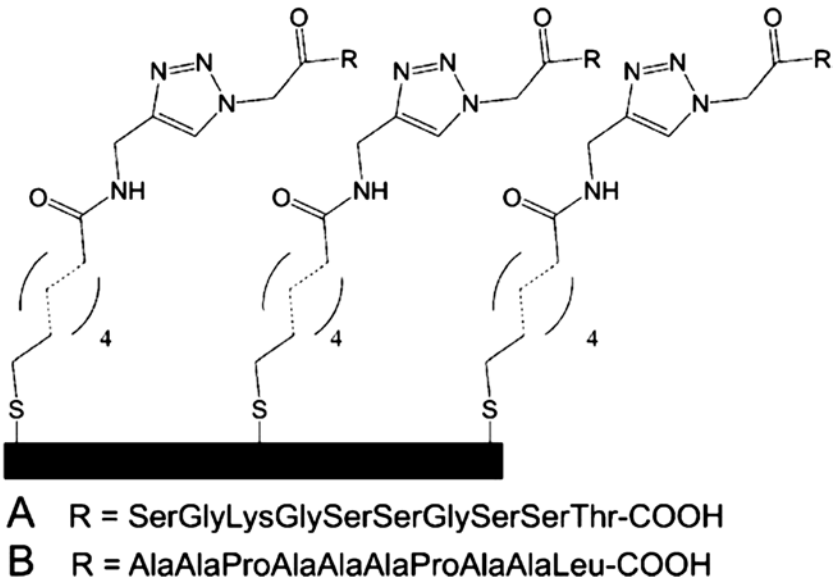

Fig. 2.5 Schematic view of the used peptides. Peptide 1 (**a**) contains hydrophilic amino acids (Ser, Lys, Thr) and Gly which is not hydrophilic but important for the helical structure of the peptide. Peptide 2 (**b**) is a simple chain of hydrophobic side chains containing one leucin, seven alanines, and two prolines. (Reprinted with permission from Ref. [40]. Copyright 2008, American Chemical Society)

interactions of proteins with these biomimetic surfaces (Fig. 2.5). Although using peptides to fabricate a surface which resists the adsorption of proteins is somewhat counterintuitive, the resulting peptide SAMs show resistance to nonspecific adsorption of proteins including streptavidin, bovine serum albumin (BSA), and fibronectin, which is comparable to the PEG-based SAMs [40]. Chen et al. reported the ultra-low fouling natural peptides composing of negatively and positively charged residues, such as glutamic acid, aspartic acid, and lysine, in the form of either alternating or randomly mixed charge. The natural high resistance to nonspecific protein adsorption is comparable to that of PEG-based materials [89].

Messersmith and colleagues reported a new class of synthetic antifouling macromolecules that mimics polypeptides attached to biomaterial surfaces for long-term resistance to fouling in the biological environment [39]. Peptidomimetic polymers consist of a short functional peptide domain containing alternate DOPA and lysine residues to adhere to surfaces, and have a N-substituted glycine (peptoid) oligomer of variable length which has a protein-like backbone with side chain derivatization on the amide nitrogen; the methoxyethyl side chains provide fouling resistance to surfaces and resembles the repeat unit of PEG, resulting in excellent protein resistance.

Glycerol and Carbohydrate Derivatives Functionalized Nonfouling Surfaces Haag and colleagues attached a novel biocompatible SAM of dendritic polyglycerols

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