

Lipoic Acid Glyco-Conjugates, a New Class of Agents for Controlling Nonspecific Adsorption of Blood Serum at Biointerfaces for Biosensor and Biomedical Applications

Yanyan Wang,^{†,‡} Kheireddine El-Boubbou,[§] Hovig Kouyoumdjian,[§] Bin Sun,[§] Xuefei Huang,[§] and Xiangqun Zeng^{*,†}

[†]Chemistry Department, Oakland University, Rochester, Michigan 48309, [‡]The key Laboratory of Bioactive Materials Ministry of Education, College of Life Sciences, Nankai University, Tianjin 300071, China, and [§]Chemistry Department, Michigan State University, East Lansing, Michigan 48824

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The carbohydrate-derived lipoic acid derivatives were studied as protein and cell resistant biomaterials. Six types of carbohydrates were examined for their abilities to reduce nonspecific adsorption of human serum and Hela cell using quartz crystal microbalance. Our data suggested that the structures of carbohydrates play an important role in resisting nonspecific binding. Specifically, the resistance was found to increase in the order lipoic fucose < lipoic mannose < lipoic *N*-acetyl glucosamine < lipoic glucose < lipoic sialic acid < lipoic galactose, where lipoic galactose derivative resisted most nonspecific adsorption. Furthermore, the combination of lipoic galactose and BSA was the most effective in reducing the adsorption of even undiluted human serum and the attachment of Hela cells while allowing specific binding. Several control experiments have demonstrated that the resistant-ability of mixed lipoic galactose and BSA was comparable to the best known system for decreasing nonspecific adsorption.

1. Introduction

The nonspecific adsorption from serum components is a general problem in biosensing and biomedical applications. Nonspecific adsorption of proteins to the biosensor surfaces will reduce the accuracy of the bioanalysis of blood serum samples. Biofouling is inevitable due to the accretion of contaminants over time in various biointerfaces used in the implanted medical devices, *in vivo* diagnostics, drug delivery, and polymer therapeutics.^{1,2} Human serum is the component of human blood that is composed of complex mixtures of hundreds of proteins. Serum total protein in blood is 7 g/dL including albumins (3.5–5.0 g/dL), immunoglobulin (1.0–1.5 g/dL), α -antitrypsin and regulatory proteins.^{3,4} Proteins have hydrophobic, ionic, and polar domains.⁵ In aqueous solutions, the protein adsorption onto a surface is a complex process that depends on the electrical double layer potential, the ionization tendencies of the surface, the binding properties of water, interface entropic effects, and the dynamics and hydrodynamics of the interface.⁶ The nonspecific adsorptions of human serum components on the surface of diagnostic devices or implanted materials pose significant challenges for their use and applications. Therefore, the mechanistic understanding of the protein nonspecific adsorption on surfaces and the development of biointerfaces that have the ability to resist nonspecific adsorption of protein have attracted significant

research interests.⁷ A number of blocking reagents have been studied both experimentally and theoretically. For example, biointerfaces modified with blocking reagents such as bovine serum albumin (BSA),^{8,9} poly(ethylene glycol) (PEG),^{10–12} oligo-(ethylene glycol) (OEG) based groups,^{13–15} zwitterionic materials (such as sulfobetaine methacrylate (SBMA) and carboxybetaine methacrylate (CBMA)),^{16–18} 3-mercaptopropylamino acid,¹⁹ and octenyltrimethoxysilane (OCS)²⁰ have been shown to decrease nonspecific protein adsorption. Among them, the most commonly used blocking agents are BSA and PEG containing agents. BSA is a large molecule with molecular weight of 60 KDa and is not capable of blocking small vacant sites on the surface.¹⁹ PEG is not biodegradable but is unusually effective at excluding other polymers from its presence in an aqueous environment.²¹

Though the mechanisms for protein adsorption resistance are not yet completely understood, important progress has been made in this area for the past decade. Prime et al. reported that

*Corresponding author: Telephone: 248-370-2881. Fax: 248-370-2321. E-mail: zeng@oakland.edu.

(1) Huang, R. P. *Front. Biosci.* **2003**, *8*, D559.
(2) Calvo, K. R.; Liotta, L. A.; Petricoin, E. F. *Biosci. Rep.* **2005**, *25*, 107.
(3) Zhou, M.; Lucas, D. A.; Chan, K. C.; Issaq, H. J.; Petricoin, E. F.; Liotta, L. A.; Veenstra, T. D.; Conrads, T. R. *An investigation into the human serum "interactome"*; Wiley-VCH Verlag GmbH: Weinheim, Germany, 2004; p 1289.
(4) Blankenship, J. R.; Heitman, J. *Infect. Immun.* **2005**, *73*, 5767.
(5) Milligan, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 21.
(6) Masson, J. F.; Battaglia, T. M.; Cramer, J.; Beaudoin, S.; Sierks, M.; Booksh, K. S. *Anal. Bioanal. Chem.* **2006**, *386*, 1951.
(7) Senaratne, W.; Andruzzi, L.; Ober, C. K. *Biomacromolecules* **2005**, *6*, 2427.

(8) Papavassiliou, A. G.; Bohmann, D. *Nucleic Acids Res.* **1992**, *20*, 4365.
(9) Brorson, S. H. *Micron* **1997**, *28*, 189.
(10) Elbert, D. L.; Hubbell, J. A. *Chem. Biol.* **1998**, *5*, 177.
(11) Huang, N. P.; Voros, J.; De Paul, S. M.; Textor, M.; Spencer, N. D. *Langmuir* **2002**, *18*, 220.
(12) Uchida, K.; Otsuka, H.; Kaneko, M.; Kataoka, K.; Nagasaki, Y. *Anal. Chem.* **2005**, *77*, 1075.
(13) Andruzzi, L.; Senaratne, W.; Hexemer, A.; Sheets, E. D.; Ilic, B.; Kramer, E. J.; Baird, B.; Ober, C. K. *Langmuir* **2005**, *21*, 2495.
(14) Zareie, H. M.; Boyer, C.; Bulmus, V.; Nateghi, E.; Davis, T. P. *ACS Nano* **2008**, *2*, 757.
(15) Feldman, K.; Hahner, G.; Spencer, N. D.; Harder, P.; Grunze, M. *J. Am. Chem. Soc.* **1999**, *121*, 10134.
(16) Holmlin, R. E.; Chen, X. X.; Chapman, R. G.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 2841.
(17) Ladd, J.; Zhang, Z.; Chen, S.; Hower, J. C.; Jiang, S. *Biomacromolecules* **2008**, *9*, 1357.
(18) Vaisocherova, H.; Yang, W.; Zhang, Z.; Cao, Z. Q.; Cheng, G.; Piliarik, M.; Homola, J.; Jiang, S. Y. *Anal. Chem.* **2008**, *80*, 7894.
(19) Bolduc, O. R.; Masson, J. F. *Langmuir* **2008**, *24*, 12085.
(20) Miyake, T.; Tani, T.; Kato, K.; Hosaka, T.; Kanari, Y.; Sonobe, H.; Ohdomari, I. *Chem. Phys. Lett.* **2006**, *426*, 361.
(21) Metzke, M.; Guan, Z. *Biomacromolecules* **2008**, *9*, 208.

proteins interacted more strongly with hydrophobic surfaces,²² where polar surfaces (e.g., $-\text{OH}$, and $-\text{CONH}_2$) can form hydrogen bonds with water to prevent protein adhesion more effectively than apolar surfaces (e.g., $-\text{CF}_3$, $-\text{CH}_3$, $-\text{OCH}_3$, and $-\text{CN}$).²³ Comprehensive studies of protein adhesion to solid surfaces have concluded that protein resistance is due to the formation of a tightly bound water layer at the surface²⁴ or may be related to the repulsive electrostatic interactions.¹⁵ Ostuni et al. examined a group of ca. 60 mixed self-assembled monolayers presenting a range of functional groups.²⁵ These studies suggested that groups that made surfaces inert have the following common features: the repeating unit is conformationally flexible; they contain a hydrogen bond acceptor but not a donor and are overall electrically neutral; they are water-soluble hydrophilic and self-repulsive.^{26–28} The exceptions to the above characteristics are mannitol and OEG.²⁶ Although they have many hydrogen bond donors, they are shown to be surface inert to nonspecific adsorption of proteins. One of nature's most important talents is the evolutionary development of cell surfaces that are capable of distinguishing one molecule from another as well as preventing nonspecific interactions. Carbohydrate, saccharide, or sugar, is a class of molecule identified and characterized more than 100 years ago. It is well-known that mammalian cells are covered with a dense layer of carbohydrate coating attached to lipid and proteins known as the glycocalyx. The glycocalyx passivates the cell surface while still allowing specific receptor–ligand interaction on the cell surface. Carbohydrates are small compared to BSA. They are naturally abundant with low toxicity and are synthetically available. Moreover, they exhibit vast stereochemical diversity. This difference could be explored to control the hydration layer or steric repulsion to modify their properties of protein resistance.¹⁵ Thus, we hypothesize that carbohydrates could be suitable candidates for reducing the nonspecific adsorption from serum components as well as the nonspecific cell attachment. Although Luk et al. reported that a monolayer terminated with maltose could prevent protein adsorption and mammalian 3T3 cell attachment, we envision that the carbohydrate structures could have a significant impact on resistance of nonspecific binding.²⁶ In order to study this, carbohydrates were derivatized with lipoic acid for immobilization on sensor surface. Six types of lipoic acid carbohydrate conjugates, namely lipoic mannose (LMan), lipoic galactose (LGal), lipoic glucose (LGlc), lipoic sialic acid (LSia), lipoic *N*-acetyl glucosamine (LGINAc), and lipoic fucose (LFuc) were synthesized and their properties toward protein resistance were measured using human serum samples, the most common clinical samples for bioanalysis. Resistance toward Hela cell attachment was also investigated as an example to understand the carbohydrate selectivity at cell surfaces.

We used a well-established recombinant piezoelectrode system developed in our laboratory^{29,30} with immobilized single chain antibody (scFv) that can bind with rabbit IgG to characterize the effectiveness of the lipoic carbohydrate conjugates as

blocking reagents for reducing nonspecific adsorption and improving the signal-to-noise ratio for biomedical diagnostics. The self-assembled method was chosen for surface modification, as it provided tightly controlled films.³¹ Quartz crystal microbalance (QCM) transducer offers the benefits of real-time monitoring of protein adsorption to surface coating and was used to characterize the properties of the lipoic carbohydrate biointerfaces. The immunosensor modified with various carbohydrates with or without other blocking reagents such as BSA were studied thoroughly for their effects in the resistance to nonspecific protein adsorption using the conditions most representative in most common clinical assays.

2. Experimental Section

2.1. Materials. Bovine serum albumin (BSA), human serum albumin (HSA), and rabbit IgG were purchased from Sigma, Inc. PEG–thiol was obtained from Nektar. Phosphate buffer saline pH 7.2 (PBS, Gibco) was used as received. Human blood was provided by Marian Hodge (Health Science, Oakland University). Blood samples were centrifuged at 3000g for 10 min to separate the serum, and then the serum was frozen at $-80\text{ }^\circ\text{C}$. Hela cell (ATCC) were cultured in Eagle's Minimum Essential Medium (EMEM, ATCC) plus 10% fetal bovine serum (FBS, ATCC) at $37\text{ }^\circ\text{C}$ under 5% CO_2 . Cells were removed by trypsin (ATCC) and collected. Unless otherwise indicated, all chemicals, reagents and solvents were obtained from commercial suppliers and used as supplied without further purification. All oxygen and moisture sensitive reactions were carried under nitrogen. Air sensitive solvents were transferred via syringe. Column chromatography was performed employing 230–400 mesh silica gels. Thin-layer chromatography (TLC) was performed using glass plates precoated to a depth of 0.25 mm with 230–400 mesh silica gel impregnated with a fluorescent indicator (254 nm). All compounds were visualized by the use of UV light, iodine, ninhydrin or a sulfuric acid stain (5% H_2SO_4 in methanol containing 1,3-dihydroxy-naphthalene). NMR spectra were recorded on a Varian *UnityPlus*-500 or *Inova*-600 instruments and were referenced using Me_4Si (0 ppm), residual CHCl_3 ($\delta^1\text{H}$ NMR 7.24 ppm) CDCl_3 ($\delta^{13}\text{C}$ NMR 77.0 ppm), residual CHD_2OD ($\delta^1\text{H}$ NMR 3.30, 4.78 ppm), CD_3OD ($\delta^{13}\text{C}$ NMR 49.0 ppm), and residual HDO ($\delta^1\text{H}$ NMR 4.65 ppm). Assignments of proton and carbon signals were carried out with the aid of gCOSY experiments. Electro-spray ionization mass spectra (ESI-MS) and high-resolution mass spectra (HRMS) were recorded on a Micromass Q-ToF Ultima API mass spectrometer (Waters) equipped with an orthogonal electrospray source (Z-spray) operated in positive ion mode and connected to a Waters LC 2795 Separations Module.

2.2. Synthesis of Lipoic Carbohydrate Derivatives. A solution of lipoic acid (0.50 g), Boc protected diamine 1 (1.4 g), *N*-ethyl *N,N'*-dimethylaminopropyl carbodiimide hydrochloride (EDC, 0.93 g) and triethylamine (Et_3N , 1.00 mL) in anhydrous dichloromethane CH_2Cl_2 (10 mL) was stirred under nitrogen at room temperature for 12 h. The reaction mixture was rotary evaporated and the resulting residue was purified by column chromatography eluting with 7% methanol MeOH in CH_2Cl_2 to afford the protected lipoic amide as sticky yellowish oil (0.85 g, 80%). Deprotection of the lipoic amide (0.85 g) was completed with TFA (2 mL) in CH_2Cl_2 (3 mL) after 1 h. The reaction mixture was evaporated and then purified by flash column chromatography (25% MeOH in CH_2Cl_2) to furnish lipoic amine 2 as a sticky yellowish gelatin in 84% yield (0.55 g).

To functionalize the carbohydrates, a solution of the azido propyl glycoside, succinic anhydride (1.5 equiv) and 10% Pd/C in dry THF was stirred at room temperature under H_2 . After 3 h, TLC (EtOAc –hexanes, 1:1) showed complete conversion of

(22) Prime, K. L.; Whitesides, G. M. *Science* **1991**, 252, 1164.

(23) Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, 115, 10714.

(24) Harder, P.; Grunze, M.; Dahint, R.; Whitesides, G. M.; Laibinis, P. E. *J. Phys. Chem. B* **1998**, 102, 426.

(25) Ostuni, E.; Grzybowski, B. A.; Mrksich, M.; Roberts, C. S.; Whitesides, G. M. *Langmuir* **2003**, 19(5), 1861.

(26) Luk, Y. Y.; Kato, M.; Mrksich, M. *Langmuir* **2000**, 16, 9604.

(27) Chapman, R. G.; Ostuni, E.; Takayama, S.; Holmlin, R. E.; Yan, L.; Whitesides, G. M. *J. Am. Chem. Soc.* **2000**, 122, 8303.

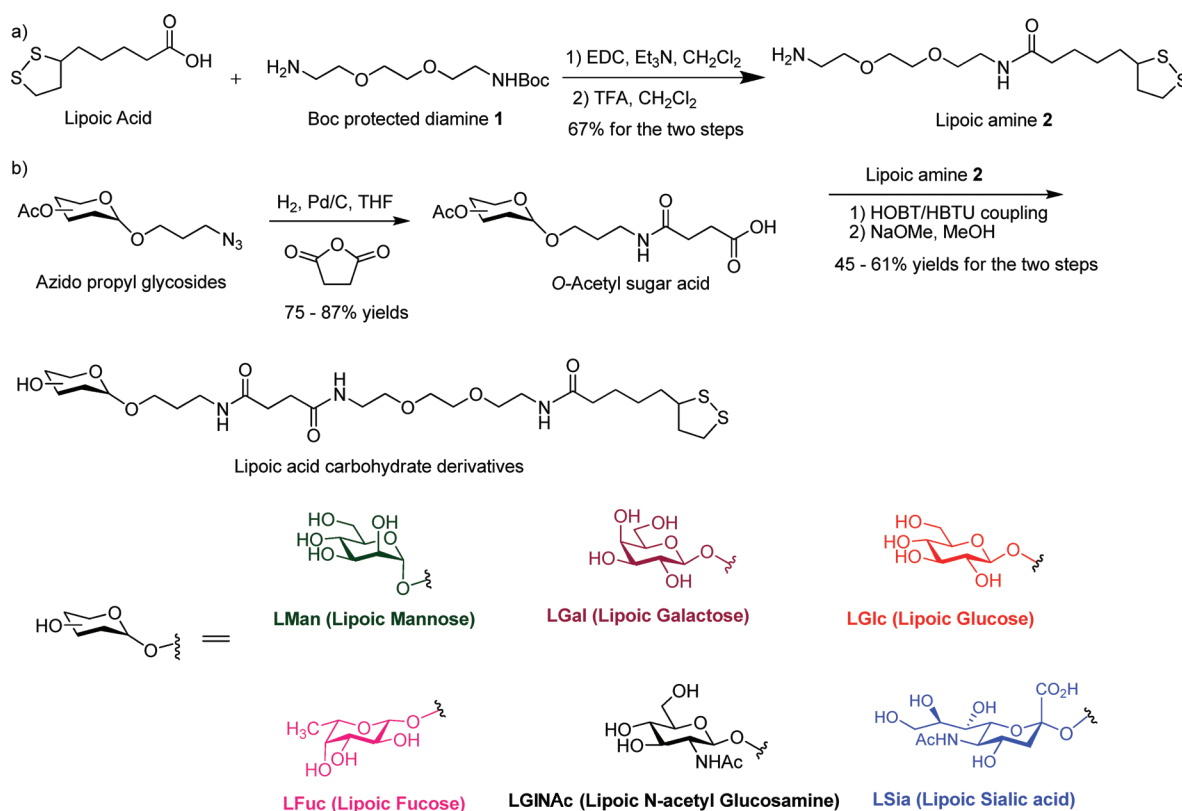
(28) Carrigan, S. D.; Tabrizian, M. *Langmuir* **2005**, 21, 12320.

(29) Shen, Z. H.; Mernaugh, R. L.; Yan, N. P.; Yu, L.; Zhang, Y.; Zeng, X. Q. *Anal. Chem.* **2005**, 77, 6834.

(30) Shen, Z. H.; Yan, H. P.; Parl, F.; Mernaugh, R.; Zeng, X. Q. *Anal. Chem.* **2005**, 79, 1283.

(31) Chaki, N. K.; Aslam, M.; Sharma, J.; Vijayamohan, K. *Proc. Indian Acad. Sci.—Chem. Sci.* **2001**, 113, 659.

Scheme 1. Synthesis of Lipoic Carbohydrate Conjugates



starting material to a major product. The reaction mixture was then filtered off through Celite and concentrated. The residue was purified by column chromatography to afford the product *O*-acetyl protected sugar acids as a gel like solid in 75–87% yield.

A solution of *O*-acetyl sugar acid, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (1 equiv), hydroxybenzotriazole (HOBT) (1 equiv), and diisopropylethylamine (DIPEA) (3.5 equiv) in anhydrous *N,N*-dimethylformamide (DMF) was stirred under nitrogen at room temperature for 5 min. Lipoic amine 2 (1.5 equiv) was then added and the reaction mixture was stirred for 3 h. The solvent was removed, and the resulting mixture was washed with water followed by brine, dried over Na_2SO_4 and rotary evaporated. The residue was purified by column chromatography eluting with 3–20% MeOH in DCM to afford the lipoic sugar acetates. Then the solution of lipoic sugar acetate (0.2 M) in MeOH (2 mL) was treated with a solution of 30% NaOMe in MeOH (0.2 mL) under nitrogen. After 1 h, the solution was neutralized with Amberlite IR-120 H^+ to pH = 6 (For lipoic sialic acid synthesis, 0.2 mL of water was added to the reaction mixture half an hour prior to neutralization). Evaporation of solvent under reduced pressure afforded the requisite final lipoic carbohydrate derivatives (LGal, LMan, LFuc, LGINAc, LGlc and LSia) in 45–61% yields for the two steps.

2.3. Electrode Modification. Nonpolished AT-cut Au quartz crystals (10-MHz, area 0.23 cm²) and Au plate electrodes (area 0.22 cm²) were used in this study. They were mounted in a custom-made Kel-F cell sealed with two Viton O-rings. The Au quartz crystal was cleaned by sequential immersion in concentrated nitric and sulfuric acid mixture, biological grade water (resistance greater than 18 MΩ, and further radiated by UV light and filtered with a 0.2 μm filter) and ethanol in series for three times to remove impurities, then dried with nitrogen. The Au plate were polished on microcloths with alumina powder and cleaned with Piranha solution ($\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ 1:3). Then one side of the Au quartz crystal or Au plate was immersed in a solution of scFv-cys (0.3 mg/mL) in PBS buffer at 4 °C overnight. After incubation, the electrode surface was rinsed with PBS buffer and biograde water

to remove the weak adsorption. Then various block reagents, such as 10 mg/mL lipoic carbohydrate, 100 mg/mL BSA, the mixture of 10 mg/mL lipoic carbohydrate and 100 mg/mL BSA, 100 mg/mL HSA or PEG thiol (1:1 v/v) was added to block the unoccupied active Au surface for several hours, respectively. After blocking, the cell was further washed by PBS buffer and biograde water to remove any unbound reagent.

2.4. QCM Measurement. The QCM cell filled with 1 mL of PBS buffer was placed in a Faraday cage at room temperature. Contents of the QCM cell were continuously stirred before, during, and after the addition of analyte, which was added to the cell in 20 μL volumes. The frequency and series damping resistance of the QCM were monitored using a network/spectrum/impedance analyzer (Agilent 4395A). The relationship between the change in resonant frequency (ΔF) resulting from a change in mass (Δm), was given by the Sauerbrey's equation, $\Delta F = -2n\Delta m F_0^2 / (\rho_q \mu_q)^{-1/2} A^{-1}$, where n is the overtone number, μ_q is the shear modulus of the quartz ($2.947 \times 10^{11} \text{ g cm}^{-1} \text{ s}^{-2}$), and ρ_q is the density of the quartz (2.648 g cm^{-3}). According to the Sauerbrey's equation, our fitted frequency change of 1 Hz corresponds to a mass increase of 1 ng for the 10 MHz quartz crystal used in this work.

2.5. Electrochemical Characterization. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were carried out with a potentiostat–galvanostat (EG&G PARC Model 2263). And a three-electrode system with a bare or modified Au plate electrode, a platinum wire counter electrode and a Ag/AgCl reference electrode (saturated KCl) was employed. All experiments were performed in Kel-F cell filled with 2 mL of 0.1 M KCl and 5 mM $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ at room temperature. CV was scanned at 50 mV/s. EIS were obtained using bias potential at open circuit potential, ac amplitude of 10 mV, and frequency from 100 kHz to 100 mHz.

3. Results and Discussion

3.1. Synthesis of Lipoic Acid Carbohydrate Derivatives. As shown in Scheme 1, the structures of the six lipoic carbohydrate

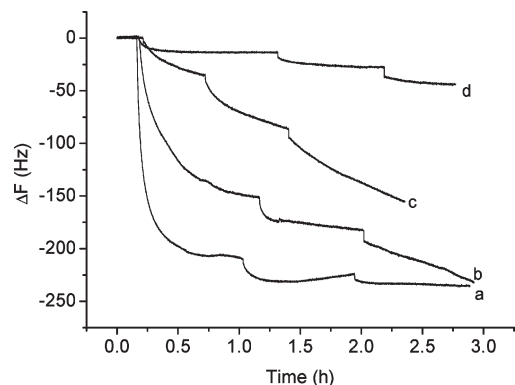


Figure 1. Human serum adsorption profiles onto bare or modified Au quartz surface. 0.2%, 0.5%, 1% human serum was added in series: (a) bare Au quartz; (b) scFv modified Au quartz; (c) scFv modified Au quartz blocking with BSA; (d) scFv modified Au quartz blocking with PEG-thiol.

derivatives consist of three components. The first component is the cyclic disulfide, which can adsorb strongly to the Au sensor surface via Au–S bond; the second is the OEG chain which can enhance aqueous solubility and form a monolayer; and the third is the carbohydrate linked through their respective reducing end.

Preparation of these compounds commenced from the commercially available lipoic acid that was coupled to the Boc-protected diamine **1**³² using a standard amide coupling condition, which was followed by removal of the Boc group promoted by trifluoroacetic acid (TFA) yielding lipoic amine **2** (Scheme 1a). The functionalization of carbohydrates with carboxylic acids was performed by reduction of the corresponding azido propyl glycosides in the presence of succinic anhydride leading to the *O*-acetyl protected sugar acids in 75–87% yields (Scheme 1b). These were then coupled to lipoic amine **2** using HOBt/HBTU with subsequent protective group removal affording the six lipoic carbohydrate derivatives.

3.2. Monitoring of Nonspecific Adsorption of Human Serum. As a blood component, human serum is a standard solution commonly used in cancer biomarker discovery or medical diagnostics. Serum protein adsorption is the first event occurring in serum-material interaction.³³ In this experiment, protein adsorptions from human serum onto various modified surfaces were tested. As 0.2%, 0.5% and 1% human serum solutions (dilution with PBS buffer) were added to the QCM cell, the serum proteins bound strongly with the bare Au quartz via van-der-Waals and electrostatic interaction, and gave rise to the frequency change (Figure 1a). These serum proteins would foul the scFv modified quartz surface and occupy the unbound site if no blocking reagents were used (Figure 1b). Serum proteins can still absorb on the surface even when the scFv surface was blocked with traditional block reagents of BSA or PEG (Figure 1c, d). As these control experiments have shown, both surfaces showed an attractive interaction with the human serum, indicating that the serum proteins established contact to these surfaces. Therefore, there is a significant need for the development of novel nonspecific adsorption barrier materials.

3.3. Blocking Effect of Lipoic Carbohydrates. To investigate the blocking effect of the lipoic acid carbohydrate conjugates, six kinds of lipoic carbohydrates, LMan, LGal, LGlc, LSia, LGINAc, and LFuc were chosen in this study, after immobilization

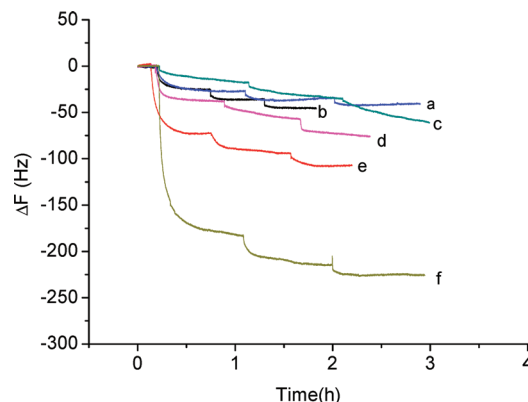


Figure 2. Blocking effect of various lipoic carbohydrates. 0.2%, 0.5%, and 1% human serum were added in series: (a) LGal, (b) LSia, (c) LGlc, (d) LGINAc, (e) LMan, and (f) LFuc.

on the bare Au quartz surface. The nonspecific adsorption resisting properties were evaluated by exposing these lipoic carbohydrate modified surfaces to 0.2%, 0.5%, 1% human serum for 1 h each. Figure 2 compares the ability of these carbohydrate modified surfaces to reduce human serum adsorption. These lipoic carbohydrates exhibited drastically different protein resistances with LGal-, LGlc-, and LSia-modified surfaces being the most effective in reducing human serum adsorption. Although the exact reasoning is not clear, the differential three-dimensional arrangements of the functional groups in these compounds may be responsible for disrupting the water lattice or hydration layer.¹⁵ From these results, we can conclude that LGlc, LGal, and LSia can be used as blocking reagents.

To select the best candidate for blocking applications, we examined LGlc, LGal, and LSia to block the scFv modified Au quartz electrodes (Figure 3A). The result showed that LGal gave the minimum serum adsorption. Interestingly, human serum adsorption increased in these experiments compared with those performed having solely carbohydrates immobilized on bare Au quartz electrode. This is presumably due to the nonspecific protein–protein interactions between the scFv and human serum proteins (Scheme 2a), which can block the recognition sites on scFv–cys resulting in much reduced IgG binding. In order to further reduce the nonspecific adsorption from serum proteins and increase the IgG binding efficiency, mixtures of 100 mg/mL BSA with 10 mg/mL LGal, LGlc, or LSia respectively (1:1 v/v) were tested. BSA has been widely used to block nonspecific interactions of various proteins.³⁴ Combining BSA with small carbohydrates would allow the flexibility and diversity of non-fouling film be made (Scheme 2b). As shown in Figure 3B, the inhibition of nonspecific adsorption of human serum was enhanced by using mixtures of selected carbohydrates and BSA, with LGal and BSA (LGal/BSA) almost totally preventing serum adsorption. Therefore, LGal/BSA was selected as the best ultralow nonspecific adsorption candidate materials and used for subsequent studies. We also examined different blocking time and found that incubating the blocking agents with the quartz surface for 3 h gave the best results (data not shown).

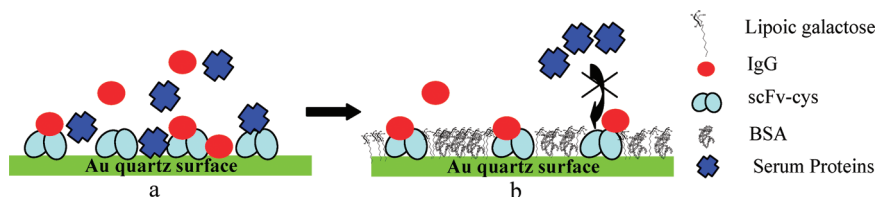
To detect antigens in a complex solution, the biosensor surface should minimize the amount of nonspecific binding from serum proteins, while maintaining a high signal due to the specific antigen interaction with the immobilized recognition element

(32) Pastorin, G.; Wu, W.; Wieckowski, S.; Briand, J.-P.; Kostarelos, K.; Prato, M.; Bianco, A. *Chem. Commun.* **2006**, *11*, 1183.

(33) Basmadjian, D.; Sefton, M. V.; Baldwin, S. A. *Biomaterials* **1997**, *18*, 1511.

(34) Jeyachandran, Y. L.; Mielczarski, J. A.; Mielczarski, E.; Rai, B. *J. Colloid Interface Sci.*, in press.

Scheme 2. Usage of Lipoic Acid Derivatized Galactose in Combination with BSA as Blocking Agents Reduces Human Serum Protein and Antibody Adsorption on the Au Quartz Surface: (a) Before Blocking, Serum Proteins and IgG Can Nonspecifically Adsorb on the Surface and (b) after Blocking, the Nonspecific Binding Will Be Reduced



such as rabbit IgG–scFv interaction exemplified in this report. We compared the efficiency of this new blocking reagent with PEG. As shown in Figure 3C, PEG reduced the nonspecific binding with the scFv-immobilized surface but led to decreased ability to detect rabbit IgG. The only blocking reagent that exhibited a reduction in protein nonspecific binding to the surface while improving the sensor response was LGal/BSA. The baseline of LGal/BSA blocked sensor surface is more stable than that blocked with PEG. This confirms the mixture of LGal/BSA can be used as a barrier to effectively block nonspecific serum adsorption.

3.4. Electrochemical Characterization of the Immobilized Layer. The electrochemical characterization of the derivatized Au surface can reveal the molecular coverage of the electrode. The bare and various modified Au plate electrodes, were characterized by CV and EIS using a 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ as the redox active couple, in 0.1 M KCl solution. As shown in Figure 4a, the redox peak currents are clearly defined in bare Au, which demonstrates that the $\text{Fe}(\text{CN})_6^{3-/4-}$ redox couple undergoes the redox process without inhibition. The peak currents reduced after the electrode was modified with scFv, indicating that the scFv was attached on the Au surface and formed an impermeable electron-transfer barrier to the $\text{Fe}(\text{CN})_6^{3-/4-}$ redox active couple. Interestingly, the peak currents increased after the electrode was blocked with LGal/BSA. This may depend on a variety of factors and the mechanism is not well understood. It is likely due to the adsorption of ferrocyanide, or the reorientation of the scFv SAM upon addition of LGal/BSA that could facilitate the electron transfer between electroactive $\text{Fe}(\text{CN})_6^{3-/4-}$ and Au electrode. Also shown in Figure 4a, after the human serum or Hela cells was added, the redox peak did not change indicating that the nonspecific adsorption of human serum and cell is inhibited by the LGal and BSA. As expected, the peak decreased when rabbit IgG was added due to the specific binding between rabbit IgG and scFv. This result also showed that the blocking reagent LGal/BSA did not interfere with the IgG binding with scFv immobilized. The same results were also obtained by impedance (Figure 4b). Figure 4 c, d gave the CV and EIS results of BSA blocked Au plate electrodes. They showed that human serum and cells could adsorb on the Au surface. This indicated that BSA can not totally inhibit the nonspecific adsorption.

3.5. Monitoring Antibody Binding in Undiluted Serum. To test the stability and the performance of LGal/BSA blocking reagents in real world complex media, the blocking properties and antibody binding activity of LGal/BSA blocked surface were evaluated by exposing the modified Au quartz surface in a QCM cell with 1 mL of 0.7%, 50%, and 100% serum solution instead of PBS buffer. To date, a few studies have been reported analyzing biomolecules in undiluted serum.^{17,18,35} All of the current reports

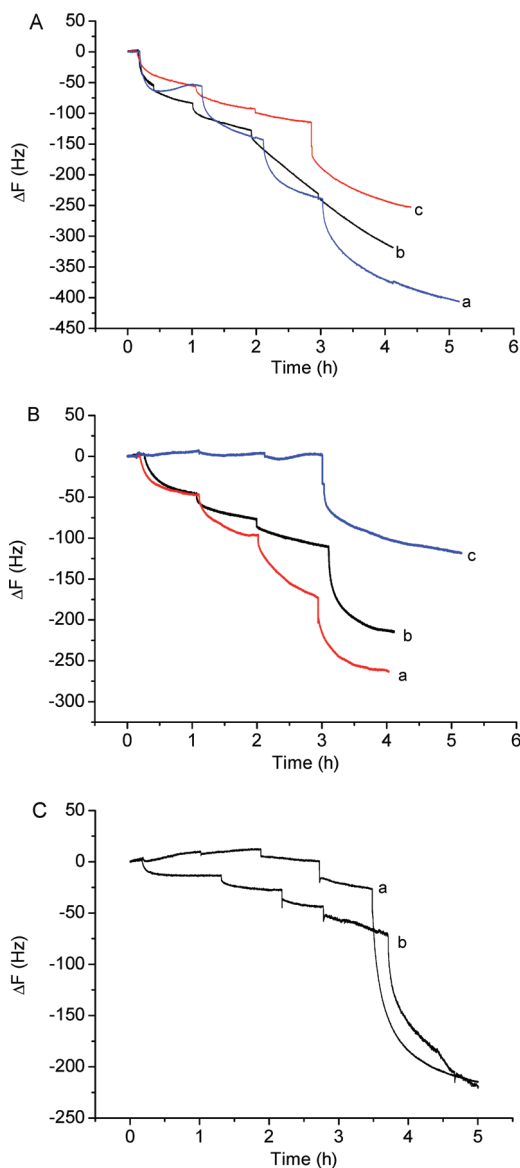


Figure 3. (A) Frequency changes of scFv–cys-immobilized Au quartz surfaces blocked using lipoic glucose (a), LSia (b) and LGal (c). (B) Frequency changes of scFv–cys-immobilized Au quartz surfaces blocked using mixture of BSA and LGal (a), LSia (b), LGal (c). (C) Comparison of the blocking effects of the mixture of LGal and BSA (a) and PEG (b). In parts A and B, 0.2%, 0.5%, and 1% human serum and 132nM IgG were added in series. In part C, 0.2%, 0.5%, 1%, and 2% human serum and 132nM rabbit IgG were added in series.

incubated with serum for no more than 30 min. Instead, in our work, we exposed the modified Au quartz electrodes to human serum over 10 h. The binding efficiency between scFv–cys and

(35) Masson, J. F.; Battaglia, T. M.; Khairallah, P.; Beaudoin, S.; Booksh, K. S. *Anal. Chem.* **2007**, *79*, 612.

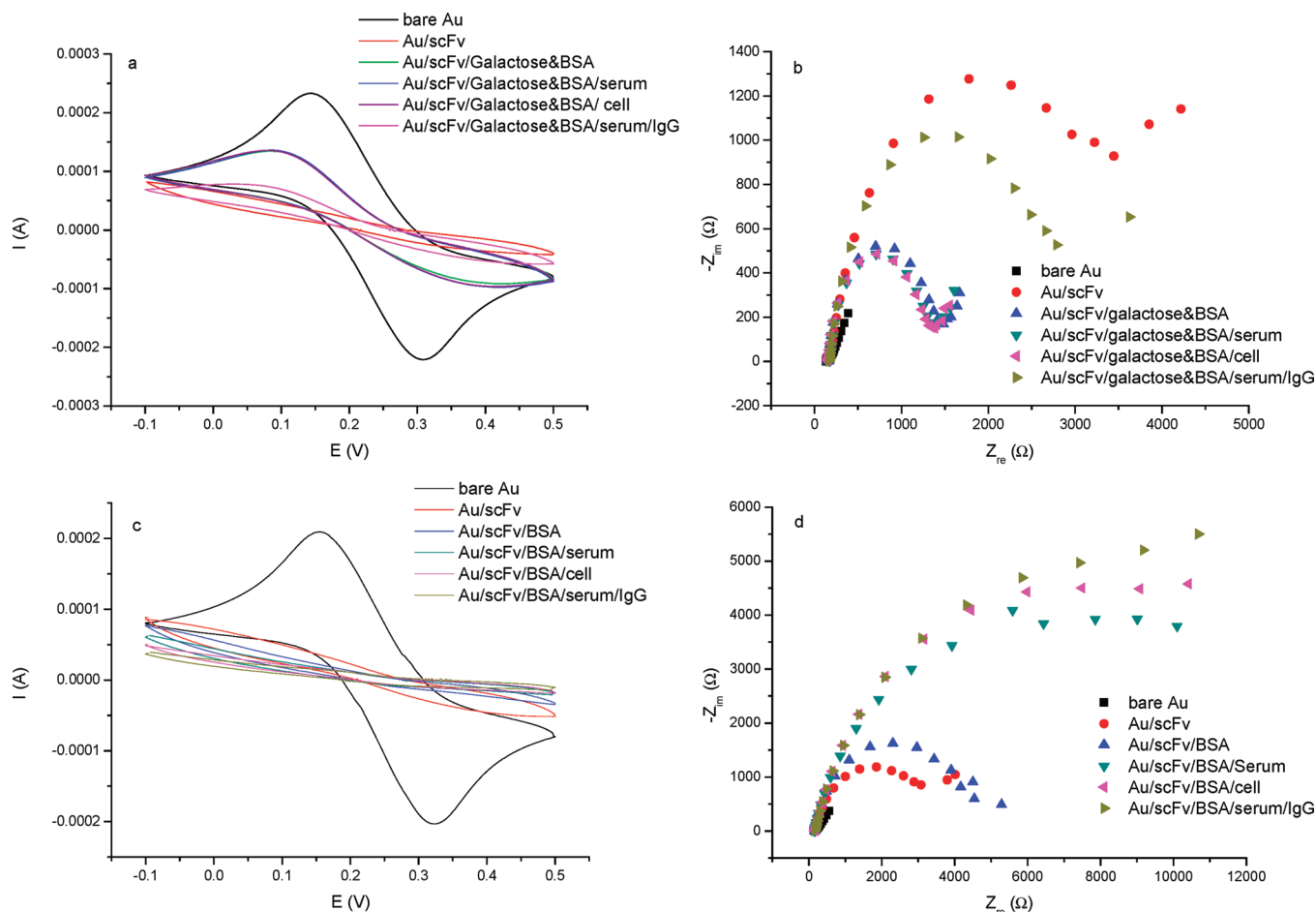


Figure 4. (a) CV and (b) complex impedance plots for bare Au, scFv, LGal/BSA–scFv, serum–LGal/BSA–scFv, cell–LGal/BSA–scFv, and IgG–serum–LGal/BSA–scFv modified Au electrode. (c) CVs and (d) complex impedance plots for bare Au, scFv, BSA–scFv, serum–BSA–scFv, cell–BSA–scFv, and IgG–serum–BSA–scFv modified Au electrode in 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}/0.1$ M KCl solution. Scan rate: 50 mV/s.

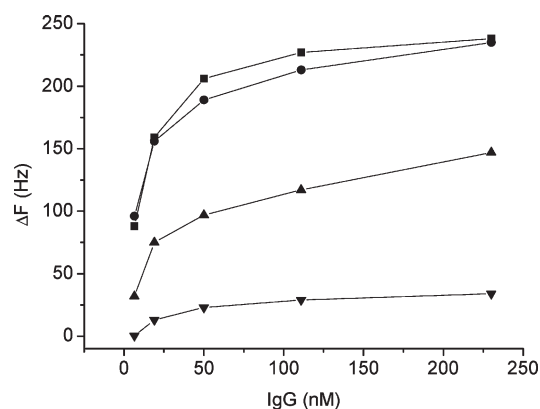


Figure 5. Frequency change vs rabbit IgG concentration on LGal/BSA or BSA blocked scFv–cys Au quartz electrode surface in human serum solutions. Concentration of rabbit IgG: 6.5, 19, 50, 111, and 230 nM. Key: (■) LGal/BSA blocked scFv–cys modified Au quartz electrode in 0.7% human serum; (●) LGal/BSA blocked scFv–cys modified Au quartz electrode in 50% human serum; (▲) LGal/BSA blocked scFv–cys modified Au quartz electrode in 100% human serum; (▼) BSA blocked scFv–cys modified Au quartz electrode in 50% human serum.

rabbit IgG were test in the serum solutions. Data are shown in Figure 5. After a series of rabbit IgG (6.5, 19, 50, 111, 230 nM) were added to the cell, frequency changes varied at the surface

with the different block reagents and serum concentrations. The rabbit IgG could hardly bind with scFv–cys and gave rise to small frequency changes at the BSA blocked Au quartz electrode. In contrast, the LGal/BSA blocked scFv–cys Au quartz electrode could bind rabbit IgG with high efficiency, even in 50% human serum. And the frequency change decreased a little when the quartz was immersed in 100% human serum. Although the rabbit IgG binding signal with scFv in the undiluted human serum was reduced, it was still much higher than the conventional BSA blocked surface in 50% human serum. This highlighted that the LGal/BSA modified surface is highly stable in serum and retains its antibody binding abilities. Thus, LGal/BSA represents a unique blocking reagent for sensing and detections in undiluted serum.

3.6. Measuring Attachment of Hela Cell. QCM technique provides a powerful means for real-time in situ analysis of cell attachment.³⁶ In this study, PEG and LGal/BSA were evaluated for their effectiveness in the cell attachment reduction. We immobilized these two blocking reagents on the Au quartz electrode, respectively. Two $\times 10^4$ Hela cells dispersed in PBS buffer were added after the baseline was stable. With the PEG surface, upon initial attachment of cells, an increase in mass was observed by QCM. This could be correlated to increased number

(36) Rodahl, M.; Hook, F.; Fredriksson, C.; Keller, C. A.; Krozer, A.; Brzezinski, P.; Voinova, M.; Kasemo, B. *Faraday Discuss* **1997**, 229.

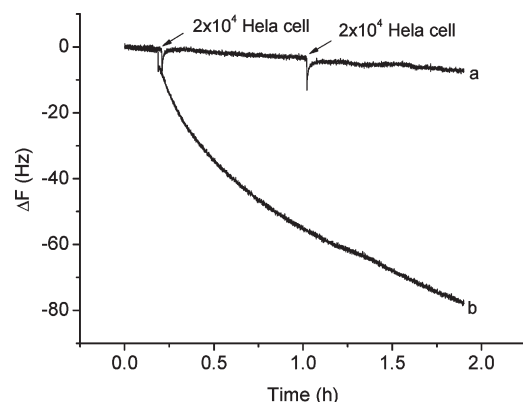


Figure 6. Real-time frequency responses to the addition of 2×10^4 HeLa cells onto (a) the mixture of LGal/BSA modified Au quartz and (b) PEG-thiol modified Au quartz.

of cells attached at the Au quartz surface (Figure 6). Hydrophobic surfaces have been shown to mediate cell attachment.³⁷ PEG is more of a hydrophilic surface but it can not completely inhibit the cell attachment. In contrast, there was little frequency change observed at LGal/BSA modified surface indicating its ability to resist cell adhesion. Thus, the LGal/BSA is not only a blocking reagent for sensor development, it can also be new materials for the development of implant and antibiofilm for medical applications.

3.7. Rigidity of the Modified Surface. Rigidity of a biofilm is important for quantitative analysis by QCM technique. The change of frequency is the sum of two contributions. The first contribution is due to the adsorption of protein to the surface. The second is due to the increase in viscosity of the solution caused by the added sticky solution and does not represent protein adsorption.³⁸ By obtaining the damping resistance through fitting the Butworth–van Dyck circuit, we can verify the validity of Sauerbrey equation (i.e., $\Delta F = -C\Delta m$) and the dissipative properties if the modified layer shows viscoelastic properties. As shown in Table 1, the damping resistance in all cases was $|\Delta R_q|/R_q (\%) < 1.5$. This proved that the attached biofilms behaved as a rigidly attached mass and the Sauerbrey equation is valid in our systems.

(37) Hou, S. Y.; Burton, E. A.; Simon, K. A.; Blodgett, D.; Luk, Y. Y.; Ren, D. C. *Appl. Environ. Microbiol.* **2007**, *73*, 4300.

(38) Martin, S. J.; Granstaff, V. E.; Frye, G. C. *Anal. Chem.* **1991**, *63*, 2272.

Table 1. Change in Damping Resistance for Experiments Shown in Figure 1, Figure 2, Figure 3 and Figure 6

$ \Delta R_q /R_q (\%)$		$ \Delta R_q /R_q (\%)$	
Figure 1, curve a	1.5	Figure 3A, curve a	0.6
Figure 1, curve b	1.2	Figure 3A, curve b	1
Figure 1, curve c	0.5	Figure 3A, curve c	0.4
Figure 1, curve d	1.0	Figure 3B, curve a	0.5
Figure 2, curve a	0.3	Figure 3B, curve b	0.8
Figure 2, curve b	0.7	Figure 3B, curve c	0.3
Figure 2, curve c	1.0	Figure 3C, curve a	1.2
Figure 2, curve d	0.6	Figure 3C, curve b	1.4
Figure 2, curve e	0.7	Figure 6, curve a	0.4
Figure 2, curve f	0.9	Figure 6, curve b	0.5

4. Conclusions

In this work, lipoic carbohydrate surfaces with different structures were studied for their abilities to reduce nonspecific protein adsorption from a human serum solution. The results of this study indicate that different stereochemical structures have different effects on protein adsorption. Specifically, the resistance was found to increase in order LFuc < LMan < LGINAc < LGlc < LSia < LGal. To our knowledge, this is the first time that the structures of carbohydrates have been shown to have a drastic effect on protein adsorption. The highest protein resistance was achieved using mixed LGal/BSA that allowed for specific binding while reducing nonspecific interactions. This mixture can also inhibit the cell attachment. Meanwhile, we compared our block reagent with those of the standard low fouling sensor surface such as those coated with BSA, PEG. The LGal/BSA represents an excellent candidate for detections in undiluted serum, with an important sensitivity improvement achieved for the immunosensor using LGal/BSA as blocking reagents. This will be particularly beneficial for real time measurements to determine kinetic rate constants and for studies of biomolecular interactions in real world complex media.

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Supporting Information Available: Text and reaction schemes showing the experimental procedures for synthesis of the lipoic carbohydrate derivatives and figures showing the NMR spectra for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.