Using Bifunctional Polymers Presenting Vancomycin and Fluorescein Groups To Direct Anti-Fluorescein Antibodies to Self-Assembled Monolayers Presenting d-Alanine-d-Alanine Groups

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Abstract: This paper describes the synthesis of bifunctional polyacrylamides containing pendant vancomycin (Van) and fluorescein groups, and the use of these polymers to direct antibodies to fluorescein to self-assembled monolayers (SAMs) presenting d-alanine-d-alanine (dADA) groups. These polymers bind biospecifically to these SAMs via interactions between the dADA and Van groups and serve as a molecular bridge between the anti-fluorescein antibodies and the SAM. The binding events were characterized using surface plasmon resonance spectroscopy and fluorescence microscopy. The paper demonstrates that polyvalent, biospecific, noncovalent interactions between a polymer and a surface can be used to tailor the properties of the surface in molecular recognition. It also represents a first step toward the design of polymers that direct arbitrarily chosen antibodies to the surfaces of cells.

Introduction

Polyvalent interactions—interactions that involve the simultaneous binding of multiple ligands on one entity to multiple receptors on another—are ubiquitous in biology. We and others have synthesized polymers that present multiple copies of suitable ligands and that bind polyvalently to the surfaces of pathogens. If polymers presenting multiple copies of a ligand directed toward a specific target—pathogen or cell (a cell endogenous to the organism, a tumor cell, a virally infected cell, etc.)—were to incorporate a pendant antigen that recruited antibodies to the target of the polymer bound to it, then it might be possible to direct or modulate the interaction of the target with the immune system. Polymers possessing both multiple target-binding sites and ligands that interacted with elements of the immune system would be loosely functionally analogous, but structurally dissimilar, to antibodies.

Bertozzi and Bednarski have synthesized a biotinylated C-glycoside of mannone. This conjugate was allowed to bind to avidin, and the resulting aggregate was then exposed to anti-avidin antibodies. The resulting complex bound to cells of Escherichia coli expressing type 1 pili (these pili contain mannone-specific receptors) via the mannosides present and displayed the antibody portion of the complex; this region could be recognized by components of the immune system. Li et al. have synthesized polymers presenting mannone and Gal(1→3)Gal, and have demonstrated the ability of these polymers to bind human anti-Gal antibodies and to prevent the agglutination of yeast by E. coli (expressing type 1 pilus mannose-binding sites on their surface). They did not, however, demonstrate the ability of these polymers to target anti-Gal antibodies to E. coli.

As a first step toward the development of a general strategy for targeting antibodies to the surfaces of cells and to other biological surfaces, we have synthesized bifunctional polymers presenting both vancomycin (Van) and an antigen (fluorescein) that is recognized specifically by an antibody (anti-fluorescein IgG, IgG3). Vancomycin is a broad-spectrum glycopeptide antibiotic that inhibits the growth of Gram-positive bacterial cells by inhibiting the synthesis of the cell wall. It functions

(12) Antibodies are at least bivalent and have domains (for example, the Fc region) that mediate the clearance of target pathogens or cells by macrophages.

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by binding to terminal DADA residues in the precursors of the cell wall of Gram-positive bacteria. 16–18 Fluorescein is a low molecular weight antigen representative of many that might be used to recruit antibodies. This paper describes a test of the ability of bifunctional polymers presenting vancomycin and fluorescein groups to direct anti-fluorescein antibodies to surfaces in a model system based on self-assembled monolayers (SAMs) presenting d-alanine-d-alanine (dAdA) groups (Scheme 1). SAMs of alkanethiols on gold allow molecular control over the properties of solid–liquid interfaces and allow the interaction between the polymer and dAdA to be investigated. 19 SAMs of alkanethiols on gold are also compatible with measurements of binding by surface plasmon resonance spectroscopy (SPR). 19–23 We used SPR to study both the binding of the polymer 24–26 to the SAM and the subsequent binding of antibodies to the surface comprising the SAM and a film of adsorbed polymer. Finally, we confirmed that the polymer bound to live bacteria at a concentration and a time scale consistent with observations of binding to the model surface.

Results and Discussion

Synthesis of Polymers. Poly(N-acryloyloxy succinimide) (PNAS) 27 was available from a previous study. Polyacrylamide (pA) presenting 1 mol % 28 fluorescein groups (pA-F) was synthesized by the reaction of PNAS with 5-((5-aminopentyl)-thioureidyl) fluorescein (fluorescein cadaverine) in anhydrous N,N-dimethylformamide (DMF) in the presence of triethylamine, followed by a quenching of the reaction mixture with aqueous ammonium hydroxide. Polyacrylamide presenting 5 mol % vancomycin and 1 mol % fluorescein groups (pA-V-F) was synthesized as shown in Scheme 2. Although the vancomycin derivative 1 used for the synthesis of pA-V-F has three potentially reactive amines, the reaction with the hindered secondary or primary amine does not compete significantly with the reaction with the primary amine from the diaminobutane linker. 29

Preparation of Surfaces. Mixed SAMs presenting dAdA groups and tri(ethylene glycol) groups (which resist the non-specific adsorption of biomolecules) were generated as described by Lahiri et al. 30 A mixed SAM was generated from ethanolic solutions of a mixture of HS(CH 2 ) 11 (OCH 2 CH 2 ) 3 OH (1.8 mM) and HS(CH 2 ) 11 (OCH 2 CH 2 ) 3 OCH 2 COOH (0.2 mM), 31 Reaction of N-α-acetyl-L-lysine-d-alanine-d-alanine (N-α-Ac-KDADA) with the carboxylic acid groups of the SAM (activated as N-hydroxysuccinimidyl esters) generated SAMs presenting dAdA groups. To confirm the coupling of N-α-Ac-KDADA to the SAM, we used polarized infrared external reflectance spectroscopy (PIERS). PIERS provides evidence for the presence


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Schema 1. Illustration of the Use of Bifunctional Polymers Presenting Vancomycin and Fluorescein Groups to Direct Anti-fluorescein Antibodies to a Surface

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* The model surface used in this study consisted of SAMs presenting dAdA groups in a background of tri(ethylene glycol) groups. Passing a solution of the bifunctional polymer over the SAM resulted in the adsorption of the polymer on the surface. The adsorbed polymer film presented fluorescein groups that enabled the binding of IgG but not of other antibodies, to the surface. The SAM, the antibody, and the pendant vancomycin and fluorescein groups are drawn approximately to scale. The scheme shows only a part of a polymer chain.
ence of specific functional groups on the surface of the SAM. After the reaction of N-α-R-Ac-KADA with the SAM, we observed the appearance of bands at 1550 and 1660 cm⁻¹, corresponding to the NH bending modes and the C=O stretch of the amide bonds.³⁰ We also estimated the extent of reaction of DADA groups with the activated esters of the SAM crudely by using ellipsometry. We observed an increase in the thickness of the SAM of 2 ± 1 Å after the reaction with N-α-R-Ac-KADA. We infer that the yield for the coupling of N-α-R-Ac-KADA to the SAM was in the range 50–100%, a range similar to that reported by Lahiri et al.³⁰ for the coupling of a benzenesulfonamide ligand to a mixed SAM having the same composition.

Characterization of the Binding of Polymers to SAMs Presenting DADA Groups. The binding of the polymers pA, pA-F, and pA-V-F to SAMs presenting DADA was characterized using SPR.²⁰–²³,³² The BIAcore 1000 instrument used in this study reports changes in the resonance angle in resonance units (RU). For most proteins, a change in the resonance angle of 1 RU corresponds to a change of ~0.1 ng/cm² in the quantity of protein adsorbed to the surface.

Figure 1 shows SPR sensorgrams for the interaction of the polymers (pA, pA-F, pA-V-F) with SAMs presenting DADA groups (γ(dAda) = 0.1). Each of these binding experiments had three stages: (a) buffer (phosphate-buffered saline solution, PBS) was passed over the sensing surface for 10 min. (b) A solution of the polymer in PBS was passed over the sensing surface for 30 min—a period of time that was sufficient for the amount of polymer adsorbed to approach a constant value. (c) A solution of PBS was passed over the sensing surface for 15 min. Figure 1A demonstrates that neither pA nor pA-F bind to SAMs presenting DADA. We also observed that pA-V-F does not bind to single-component SAMs presenting tri(ethylene glycol) groups (sensorgram not shown). These controls indicate that this polymer does not bind to the SAM nonspecifically when either pendant vancomycin or surface-displayed DADA is absent.

Figure 1A also demonstrates that pA-V-F binds to SAMs presenting DADA. The polymer desorbed from the surface very slowly in PBS buffer (k_off ≈ 2 × 10⁻⁶ s⁻¹) or in 10 mg/mL (35 mM) sodium dodecyl sulfate (SDS) (k_off ≈ 3 × 10⁻⁶ s⁻¹) (Figure 1B). The concentration of SDS is well above the critical micelle concentration (cmc = 8 mM)³³ and is a concentration typically used to dissociate proteins from a sensor chip. The rate of dissociation of the polymer increased by a factor of ~50 (to k_off) in the presence of the soluble ligand (0.5 mM N-α,N-diAc-LKADA). These sensorgrams indicate that pA-V-F forms a tightly bound film on the surface of SAMs presenting DADA groups, presumably through polyvalent interactions between the multiple vancomycin groups on the polymer and multiple DADA groups on the SAM. The increase in the rate of dissociation of the polymer in the presence of soluble ligand relative to the rate of dissociation in PBS suggests that vancomycin groups on the polymer rebind to the surface during the dissociation in the absence of the soluble DADA.³⁴

We also measured the thickness of the layer of adsorbed pA-V-F by using ellipsometry. The measured thickness (65 Å) lies within the range reported for monolayers of adsorbed poly-


of anti-fluorescein IgG (IgGF) to adsorbed pA-V-F are shown in Figure 3. The rate of dissociation of 
IgGF to adsorbed pA-V-F due to divalency is a factor of $14 \text{nM}$. The inset shows sensorgrams for the binding of 
IgGF to the adsorbed pA-V-F coated with $8 \mu M$, $K_d^D = 14 \text{nM}$ for the interaction between IgGF and the adsorbed pA-V-F were close to the values of the dissociation constants ($K_a^D = 8 \mu M$, $K_d^D = 2 \text{nM}$) for the binding of IgGF to SAMs presenting fluorescein groups ($\chi_{fluorescein} = 0.1$) and corresponds to a monolayer of IgGF.

The presence of an adsorption plateau in the SPR sensorgram for the adsorption of pA-V-F (Figure 1A) is also consistent with the adsorption of a monolayer of pA-V-F.

**Characterization of the Binding of Antibodies to pA-V-F Adsorbed onto SAMs Presenting tADA Groups.** Figure 1 indicates that pA-V-F adsorbs strongly to the surface of SAMs presenting tADA groups. The pendant fluorescein groups of the adsorbed pA-V-F should then be available for binding to anti-fluorescein antibodies. SPR sensorgrams for the binding of anti-fluorescein IgG (IgGF) to adsorbed pA-V-F are shown in the inset to Figure 2. We observed that this antibody binds to the adsorbed pA-V-F, and the binding of the antibody can be inhibited by using soluble fluorescein. Anti-dinitrophenyl IgE (IgEDNP) did not bind to the adsorbed pA-V-F (Figure 2). These results indicate that the binding of the antibody is a result of the biospecific interaction between IgGF and the fluorescein groups displayed on the exposed region of the adsorbed polymer.

We analyzed the data (Figure 2) for the inhibition of the binding of IgGF to the adsorbed pA-V-F in the presence of soluble fluorescein to obtain equilibrium dissociation constants for the monovalent ($K_a^D$) and divalent ($K_d^D$) binding of IgGF to the fluorescein groups displayed on the surface. To simplify the analysis, we assumed that the equilibrium constant for the monovalent binding of IgGF to the fluorescein groups displayed on the surface ($K_a^D$) has the same magnitude as the equilibrium constant for the monovalent interaction between IgGF and soluble fluorescein ($K_a^D$). We also assumed that there is no cooperativity between the binding sites of IgGF for the binding to soluble fluorescein. Analysis of the data, subject to these assumptions, yielded the line illustrated in Figure 2. The values of the dissociation constants ($K_a^D = 8 \mu M$, $K_d^D = 14 \text{nM}$) for the interaction between IgGF and the adsorbed pA-V-F were close to the values of the dissociation constants ($K_a^D = 8 \mu M$, $K_d^D = 2 \text{nM}$) for the binding of IgGF to SAMs presenting fluorescein groups ($\chi_{fluorescein} = 0.1$; data not shown). The enhancement in the affinity of IgGF for adsorbed pA-V-F due to divalency is a factor of $\sim 570$. This enhancement in affinity lies in the range (100–1000) usually observed for the interaction of divalent molecules with surface-bound ligands.

Sensorgrams for the dissociation of bound IgGF from the adsorbed pA-V-F are shown in Figure 3. The rate of dissociation of IgGF ($k_{off} = 1.1 \times 10^{-5} \text{s}^{-1}$ in PBS) increased in the presence of soluble ligand ($k_{off} = 3.6 \times 10^{-4} \text{s}^{-1}$) in $10 \mu M$ fluorescein in PBS; $k_{off} = 3.9 \times 10^{-3} \text{s}^{-1}$ in 1 mM fluorescein in PBS). The increase in the rate of dissociation in the presence of soluble

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(37) Klein, J. J. Colloid Interface Sci. 1986, 111, 305–313.
(39) Shiratori and Rubner have reported that the thickness of a single layer of adsorbed poly(acrylic acid) or poly(allylamine hydrochloride) can range between 5 and 80 Å depending on the conditions under which deposition is carried out.
fluorescein suggests that the antibody rebinds to ligands on the surface during the dissociation.

From the data shown in Figures 1–3, we conclude that pA-V-F forms a molecular bridge between IgGF and SAMs presenting DAda groups as a result of two independent, high-affinity interactions—the polyvalent interaction between pA-V-F and DAda groups of the SAM and the divalent interaction between IgGF and the adsorbed pA-V-F.

**Immunofluorescence.** The binding of antibodies to the bifunctional polymer was also visualized by immunofluorescent staining after laminar flow patterning. A poly(dimethylsiloxane) (PDMS) stamp having 300-μm channels molded in its surface (Figure 4A) was placed in conformal contact with a self-assembled monolayer presenting DAda groups (DAda = 0.1). A solution of pA-V-F (10 μM vancomycin, 2 μM fluorescein) was added to inlets 1 and 2 and was allowed to flow through the channel (Figure 4B). The channel was rinsed with PBS buffer. PBS buffer was then added to inlet 1, a solution of IgGF was added to inlet 2, and these solutions were allowed to flow through the channel. Finally, a solution of tetramethylrhodamine isothiocyanate (TRITC)-labeled Fc-specific, antimouse, secondary antibody (IgGFcAM) was added to both inlets with PBS buffer. PBS buffer was then added to inlet 1, a solution of IgGFcAM was added to inlet 2, and these solutions were allowed to flow through the channel. The PDMS stamp was removed and the pattern of fluorescence characterized by fluorescence microscopy. The fluorescence micrograph (green fluorescence detection) shown in Figure 4B illustrates that pA-V-F adsorbed uniformly in the channel. The fluorescence micrograph (red fluorescence mode) illustrates that IgGFcAM has adsorbed to the surface only in the region where IgGF was allowed to flow.

**Binding of pA-V-F to Enterococcus faecalis.** The SAM model provided a reproducible, controlled system for experimentation with biologically relevant polymer–surface interactions that would be difficult to systematically study otherwise. We wanted to confirm the relevance of the SAM model to biological surfaces; specifically, we wanted to establish whether a living bacterium presented sufficient numbers of accessible 1,kDAda moieties in the cell wall to allow polyvalent interaction with a vancomycin-presenting polymer. We determined the ability of the bifunctional polymer to bind to the Gram-positive bacteria *E. faecalis* (Figure 5A). By using the fluorescein attached to pA-V-F as a fluorescent marker instead of an antigen, we directly observed binding of the polymer to the target bacteria.4,49 The bacteria were exposed to a red-fluorescent, intercalating dye (SYTO 63) and to pA-V-F in PBS. After several washings with PBS, the presence of the fluorescent polymer on the bacteria was determined by fluorescence microscopy. Comparison of the red fluorescence due to the nucleic acid stain (Figure 5B) with the green fluorescence due to fluorescein (Figure 5C), which indicates the presence of pA-V-F, shows that the large majority of bacteria have detectable amounts of bifunctional polymer bound to them. Some bacteria either do not have polymer bound to them or have substantially less polymer bound than the majority of the bacteria. Exposure of *E. faecalis* to the control polymer, pA-F, yielded nonfluorescent bacteria. When the Gram-negative bacterium *E. coli* was exposed to pA-V-F, no fluorescence was observed (data not shown). These experiments indicate that the bifunctional polymer binds specifically to Gram-positive bacteria through interactions with the vancomycin moiety.

Although the surface of a cell wall is much more complex than the surface of the model SAMs, the bifunctional polymer bound to the bacteria at densities similar to those found in the experiments using SAMs and SPR, and with similar rates. Furthermore, the polymer remained bound to the bacterial surface through multiple rounds of washing; this adherence

indicates that the strong binding of the polymer to a polyvalent substrate that was observed in the model system was retained in the bacterial system.

Conclusions

We have synthesized bifunctional polymers (pA-V-F) that bind biospecifically to SAMs presenting hAbA groups and serve as a molecular bridge between the SAM and an antibody (IgG) that does not otherwise bind to the surface. Experiments confirm that, under conditions similar to those used in the model study, the bifunctional polymer binds to the surface of \textit{E. faecalis}. Polymers with pendant antigens able to recruit antibodies to the surfaces of Gram-positive bacteria (or other cells) might influence the interaction of these cells with the immune system. Of note was the extent and the stability of the interaction of the IgG targeted to an antigen displayed by the polymer. The antigen, fluorescein, was present on average every 100 polymer units yet a surface on which this polymer was bound was able to recruit the same amount of antibody with nearly the same affinity as a surface presenting a high density (10%) of fluorescein molecules. Perhaps due to the flexibility of the polymer as well the large size and flexibility of the IgG molecules it may not be necessary to display a dense array of antigens at a surface in order to achieve a high degree of stable binding by antibodies. Further work has indicated that the bifunctional polymer has the ability to recruit anti-fluorescein antibodies to the bacterial surface (Metallo and Whitesides, unpublished results).

More generally, our work demonstrates that biospecific, polyvalent interactions can be used to engineer molecular recognition at a surface. The use of polyvalent interactions to cause the adsorption of polyelectrolytes on charged surfaces is well-established.\textsuperscript{50--52} Since most biological surfaces carry a net charge, it is difficult to modify specific surfaces selectively by adsorption of polyelectrolytes. The change in the types of molecular recognition that occur at a surface, based on the polyvalent strategy outlined in this paper, is selective, stable, and can be carried out under mild conditions.

Modification using multifunctional polymers might enable us to change the chemistry of various kinds of surfaces, including those of mammalian cells.\textsuperscript{53} While our preliminary efforts have been focused on designing polymers that adsorb on bacteria, it should also be possible to design multifunctional polymers that direct antibodies toward other types of cells.

Experimental Section

Materials. All materials and reagents were used as received. N-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-3-ethylcarboxyhemodiode hydrochloride (EDC), O-benzotriazole-1-yl-N,N',N'',N'''-tetramethyluroniumhexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBT), diaminobutane, and \textit{N},\textit{N}-disopropylethylamine (DIEA) were purchased from Aldrich (Milwaukee, WI), Vancomycin (V-2002), \textit{N},\textit{N}-Ac-KnA	extit{b} (A-6950), IgG (F-5636), IgG\textit{F}cAM (T-7657), and IgE DNP (D-8406) were purchased from Sigma (St. Louis, MO). Fluorescein cadaverine was purchased from Molecular Probes (Eugene, OR). Anhydrous DMF and dimethyl sulfoxide (DMSO) were purchased from EM Science (Gibbstown, NJ) and were used without further purification. Silicon wafers were purchased from Silicon Sense (Nashua, NH).

\textbf{Synthesis of the Vancomycin Conjugate 1.} The synthesis of 1 was carried out according to the method of Sundaram et al.\textsuperscript{29} Diaminobutane (5.3 mg, 60 \textmu mol) was dissolved in 0.5 mL of DMSO and added to a solution of vancomycin (50 mg, 34 \textmu mol) in 0.5 mL of DMF. The combined solution was cooled to 4 °C, and HBTU (19.2 mg, 50 \textmu mol) and HOBT (6.6 mg, 49 \textmu mol) were added in 0.2 mL of DMF. DIEA (10 \textmu L, 57 \textmu mol) was added, and the reaction mixture was allowed to warm to room temperature and was stirred overnight. The precipitate obtained by adding methane chloride to the reaction mixture was filtered, washed, and purified by RP-HPLC on a C18 column. The \textsuperscript{1}H NMR showed resonances attributable to vancomycin as well as the amide and methylenes expected for the linker. The electrospray MS showed an \textit{M} + \textit{Na} ion at \textit{m} / \textit{z} = 1540 (expected \textit{M} + \textit{Na}: 1540).

\textbf{Synthesis of the Polymers.} PNAS was available from a previous study.\textsuperscript{27} PNAS (50 mg, 296 \textmu mol in monomer units) was dissolved in 1.5 mL of anhydrous DMF. Triethylamine (100 \textmu L, 720 \textmu mol) and a solution of fluorescein cadaverine (3.8 mg, 5.8 \textmu mol) in anhydrous DMF were added. The reaction was stirred overnight in the dark. A part (75%) of the reaction mixture was removed and quenched with aqueous ammonium hydroxide (30%) to give pA-F. To the remainder (74 \textmu mol in monomer units) of the reaction mixture was added 5.6 mg (3.7 \textmu mol) of the vancomycin conjugate 1 in anhydrous DMF. The reaction mixture was stirred overnight in the dark, and then 200 \textmu L of aqueous ammonium hydroxide (30%) was added to give pA-V-F.

\textsuperscript{50} Decher, G. Science 1997, 277, 1232--1237.
\textsuperscript{51} Hammond, P. T. \textit{Curr. Opin. Colloid Interface Sci.} 1999, 4, 430--442.
\textsuperscript{52} Yoo, D.; Shiratori, S. S.; Rubner, M. F. \textit{Macromolecules} 1998, 31, 4309--4318.
water again and then stored frozen at −20 °C. While there is no indication (from NMR of pA-V-F) that reaction between the nitrogen of the hindered N-terminus or of the vancomysine moiety of 1 and NPM occurs, a low level of multipoint attachment to the polymer backbone that would result from reaction at these additional sites cannot be definitely excluded.

**Characterization of Polymers.** Poly(β-acryloyloxyxysuccinimide) was hydrolyzed by a reaction with aqueous sodium hydroxide (0.1 N). The polymer was dialyzed against deionized water, followed by dephosphorylation, and the molecular weight distribution was characterized by GPC: $M_w = 96,500$; $M_n = 65,000$; PDI = 1.48; degree of polymerization $\approx 900$. The fractional coupling of fluorescein and vancomycin on the polymers was determined by $^1$H NMR and absorption spectroscopy. Characteristic aromatic peaks of fluorescein were integrated and compared with the integrated signal from the protons on the polycrylamide backbone to determine the percent substitution with fluorescein. The NMR signals from vancomycin on pA-V-F were too broad to provide reliable integrated values, so the percent substitution was determined based on the vancomycin-to-fluorescein ratio. Due to the method of synthesis, the mole percent substitution of fluorescein on the pA-V-F polymer was the same as on the pA-F polymer. The mole percent vancomycin on pA-V-F was determined using its absorbance at 280 nm, where both vancomycin and fluorescein absorb, and 450 nm, where only fluorescein absorbs. The molar absorbivity at 280 and 450 nm was determined from Beer’s law plots of vancomycin ($\epsilon_{280} = 6030 \text{ M}^{-1} \text{ cm}^{-1}$; $\epsilon_{450} = 0 \text{ M}^{-1} \text{ cm}^{-1}$) and fluorescein cadaverine ($\epsilon_{280} = 20,800 \text{ M}^{-1} \text{ cm}^{-1}$; $\epsilon_{450} = 20,600 \text{ M}^{-1} \text{ cm}^{-1}$). From these values and the absorbance of pA-V-F at the two wavelengths, the concentration of vancomycin and fluorescein was determined. The ratio of their concentrations, in conjunction with the mole percent substitution of fluorescein on the polymer, yielded the mole percent vancomycin. The polymers used in this work (pA, pA-V-F, pA-F) are soluble in water and PBS.

**Preparation of Gold Substrates Presenting Mixed SAMs.** Gold substrates were prepared by evaporating titanium (1.5 nm, as an adhesion layer) and then gold (100 nm) onto silicon wafers. We commercial ellipsometer (AutoEL II, Rudolph, Inc.). Substrates for ellipsometry were prepared by evaporating titanium (1.5 nm, as an adhesion layer) and then gold (100 nm) onto silicon wafers. We determined the optical constants for each substrate. The substrates were then immersed in ethanolic solutions of a mixture of HS(CH$_2$)$_{11}$(OCH$_2$CH$_2$)$_3$OH (1.8 mM) and HS(CH$_2$)$_3$OCH$_2$:COOH (0.2 mM) overnight. The coupling of N-α-Ac-KDADA to the mixed SAMs was carried out as described above. The SAMs presenting KDADA were immersed in a solution of pA-V-F (10 μM vancomycin, 2 μM fluorescein) in PBS for 6 h. The substrate was rinsed with PBS and then with distilled water and dried under a stream of nitrogen. Ellipsometric thicknesses were determined (at each step) using the previously determined optical constants.

**Polarized Infrared External Reflectance Spectroscopy.** PIES spectra were measured using a DigiLab Fourier transform infrared spectrometer (BioRad) equipped with a liquid N$_2$-cooled mercury–cadmium–telluride detector. The p-polarized light was incident at 80° relative to the surface normal of the substrate. Substrates were prepared in a manner identical to those used for ellipsometric measurements.

**Bacterial Fluorescence.** Bacteria (E. faecalis, ATCC 49332) were grown overnight in brain–heart infusion broth at 37 °C, with shaking. The bacteria were harvested by centrifugation and washed by resuspension in PBS and centrifugation. The bacteria were then resuspended to an OD$_{600}$ = 1 in PBS containing 5 μM SYTO 63 (Molecular Probes) and pA-V-F (15 μM in vancomycin, 3 μM in fluorescein). After 30 min at room temperature, 50 μL of the bacterial suspension was transferred to an ELF spin filter (0.2-μm pore size, Molecular Probes) and centrifuged to remove the polymer- and dye-containing solution. The bacteria were washed by being resuspended in 200 μL of PBS and centrifuged. Samples were washed three times. After the final washing, the bacteria were resuspended in a one-to-one mixture of PBS and SlowFade antifade reagent (Molecular Probes). Control samples using SYTO 63 and pA-F, or only SYTO 63 were prepared using the same procedure. Samples containing the Gram-negative bacteria E. coli were prepared in the same manner as the E. faecalis samples. Bacterial samples were imaged using a Leica confocal microscope equipped with an Ar–Kr laser.

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