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On-Demand Electrical Switching of Antibody−Antigen Binding on Surfaces

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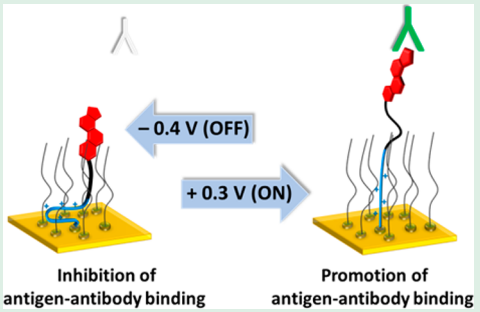
S *Supporting Information*

ABSTRACT: The development of stimuli-responsive interfaces between synthetic materials and biological systems is providing the unprecedented ability to modulate biomolecular interactions for a diverse range of biotechnological and biomedical applications. Antibody−antigen binding interactions are at the heart of many biosensing platforms, but no attempts have been made yet to control antibody−antigen binding in an on-demand fashion. Herein, a molecular surface was designed and developed that utilizes an electric potential to drive a conformational change in surface bound peptide moiety, to give on-demand control over antigen−antibody interactions on sensor chips. The molecularly engineered surfaces allow for propagation of conformational changes from the molecular switching unit to a distal progesterone antigen, resulting in promotion (ON state) or inhibition (OFF state) of progesterone antibody binding. The approach presented here can be generally applicable to other antigen−antibody systems and meets the technological needs for in situ long-term assessment of biological processes and disease monitoring on-demand.



KEYWORDS: *switchable surfaces, antibody−antigen binding, self-assembled monolayers, surface plasmon resonance, on-demand binding*

1. INTRODUCTION

Antibody−antigen binding interactions are at the heart of many biosensing platforms.[1−3] The incorporation of an antigen or antibody on surfaces and detection of their binding partner in solution has been developed into numerous clinical, nonclinical, and research applications. In particular, the quantitative detection of antibodies is critical in the monitoring of its production in cell culture, many bioanalytical assays and surveillance of a broad range of diseases, including autoimmune diseases, infectious diseases, and allergies.[4,5] Furthermore, detection of antidrug antibodies, which can be elicited *in vivo* to a therapeutic antigen, forms the core of the evaluation of drug immunogenicity.[6] While a wide variety of sensitive antibody detection strategies have been developed,[7−9] a limitation in the inability to exert on-demand control over the sensing of target antibodies still exists. On-demand specific capture of antibodies on surfaces provides the opportunity for detection only when required, leading to the development of sophisticated multianalyte sensors. Such sensors could be applied, for instance, in the near real-time, long-term monitoring of biological processes in cell culture systems or in diagnostic devices for disease detection and surveillance on-demand.[10,11]

Surfaces with temporal control attributes are the focus of many recent efforts, where materials exhibit the ability to modulate their properties in response to diverse external stimuli, such as electric, optical, thermal, and chemical.[12−14] Surfaces have been created that are able to control bacterial adhesion via modulation of nonspecific interactions.[15,16] Furthermore, different stimuli have been employed to control

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protein[17,18] and cell[19−21] adhesion on surfaces. For instance, we have developed a new class of electrically responsive surfaces based on the response of a charged molecular switching unit on the structure of a mixed self-assembled monolayer (SAM) to control the binding activity of a surface-tethered biotin to neutravidin in solution[17,22,23] and surface-tethered arginylglycylaspartic acid (RGD) to macrophage cells.[21] These studies were based on the switching units being located in close proximity to the binding site and the binding sites being highly flexible. However, it has not yet been shown that electrically responsive surfaces can be developed to control antigen−antibody interactions, in which the presence of structurally rigid binding sites and the requirement for a linker segment to promote high affinity binding represent key features to consider in the rational design of high-performing and efficient switchable sensors.

Herein, we report on a new molecular design that harnesses our surface-based conformational switching mechanism[22,23] to introduce, for the first time, a sensing platform that allows for efficient and specific capture of antibodies on surfaces only when desired. The switchable surface can be manipulated to promote (ON state) or inhibit (OFF state) antibody binding. The on-demand sensing of antibodies is based on an electrical stimulus that offers several attractive features to be integrated with sensing platforms since it (i) provides fast response times, (ii) allows for easy creation of multiple individually addressable switchable regions on the same surface, and (iii) uses low applied voltage and electrical field strengths that are biocompatible.[24] Herein, the smart surfaces are integrated with the surface plasmon resonance sensing platform, allowing label-free and real-time detection. Progesterone was selected as a low molecular weight model antigen. Progesterone is characterized by a rigid core chemical structure, which is also present in many steroid hormones and many drug antigens where the steroid skeleton is combined with structural elements possessing appropriate biological activities.[25,26] An IgG antiprogesterone antibody, progesterone-3 antimouse monoclonal antibody (anti-Pg mAb), was selected and employed as the target for on-demand sensing.

The stimuli-responsive surface is created on a gold sensor chip and comprises a mixed SAM with two components (Figure 1): (i) an oligopeptide covalently linked to the distal progesterone moiety (progesterone-C7-4KC) and (ii) a hexaethylene glycol-terminated thiol (C11EG6). The oligopeptide contains (i) a terminal cysteine for attachment to the gold surface, (ii) four lysine residues (protonated and therefore a tetracation at physiological pH) as the electrically responsive switching unit, (iii) an alkyl linker that separates the switching unit (lysine moieties) from the distal progesterone while also presenting the antigen above the surface for optimal antibody recognition, and (iv) the rigid progesterone antigen binding unit. The C11EG6 moieties on the mixed SAM have a triple function, namely (i) preventing nonspecific binding such that only specific binding can take place between the surface-tethered antigen and antibody, (ii) assisting in spacing out the oligopeptides to enable conformational changes to occur in the lysine moieties, and (iii) promoting accessibility of the antibody toward the surface-tethered progesterone such that binding can be maximized in the ON state. Since the surface-tethered protonated lysine residues can adopt two distinct conformations, namely *linear* and *folded*, under a positive and negative potential, respectively,[22,23] we reasoned that such conformational modulation could be potentially applied to provide precise temporal control over the activity and steric accessibility of the progesterone antigen to the antibody.

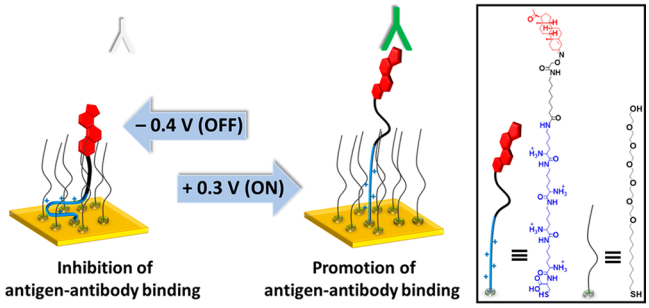

Figure 1. Schematic of the dynamic progesterone-C7-4KC oligopeptide SAM harnessed for temporal control of antibody binding. The electrically responsive SAM conceals the rigid progesterone unit under a negative potential (−0.4 V) (folded conformation), inhibiting antibody binding, via folding of the peptide residues pulling the progesterone into the surface of the SAM. Conversely, a positive potential (+0.3 V) promotes antigen activity and consequently allows high antibody binding capacity, via unfolding of the peptide residues to the linear conformation, releasing the progesterone from the surface of the SAM. (inset) Chemical structures and cartoons of the progesterone-C7-4KC oligopeptide (left) and oligo(ethylene glycol) thiol (C11EG6) (right) used for mixed SAM formation.

2. RESULTS AND DISCUSSION

2.1. Mixed SAM Characterization. A pure C11EG6 SAM, a pure progesterone-C7-4KC SAM, and a mixed SAM with mole fractions in solution of 0.98 for C11EG6 and 0.02 for progesterone-C7-4KC were prepared and analyzed by contact angle and ellipsometry (Table 1). These mole fractions were selected based on our previous studies[17,23] with a peptide system where different ratios of peptide and spacer were investigated in order to maximize the switching efficiency. Contact angle data are in line with expectations, with the progesterone surface being the most hydrophobic, and becoming more hydrophilic in the presence of C11EG6 in the mixed SAM. By comparing the thickness obtained for pure progesterone-C7-4KC (4.4 nm) with the theoretical length of the oligopeptide as calculated by MD simulations (5.4 nm), one can postulate that the peptide molecules in the monolayer are not arranged in a fully upright conformation. It is important to note though that the contact angle hysteresis (advancing contact angle minus receding contact angle) is low (ranging from 2° to 5°), providing an indication of a reasonable degree of molecular ordering in the SAMs. The pure C11EG6 SAM

Table 1. Advancing and Receding Water Contact Angle and Ellipsometric Thickness for the Pure Progesterone-C7-4KC SAM, the progesterone-C7-4KC:C11EG6 mixed SAM, and the pure C11EG6 SAM on Gold Surfaces[a]

	contact angle (deg)		thickness (nm)	
SAM	adv	rec	measured	theor
progesterone-C7−4KC	70 ± 3	67 ± 4	4.4 ± 0.2	5.4
progesterone-C7-4KC:C11EG6	43 ± 2	38 ± 4	3.1 ± 0.4	3.2
C11EG6	41 ± 2	39 ± 3	2.4 ± 0.1	2.9

[a]The values are the average of three measurements per sample, prepared in triplicate, with the errors reported as standard deviation. The average theoretical molecular lengths were obtained from molecular dynamics (MD) simulations.

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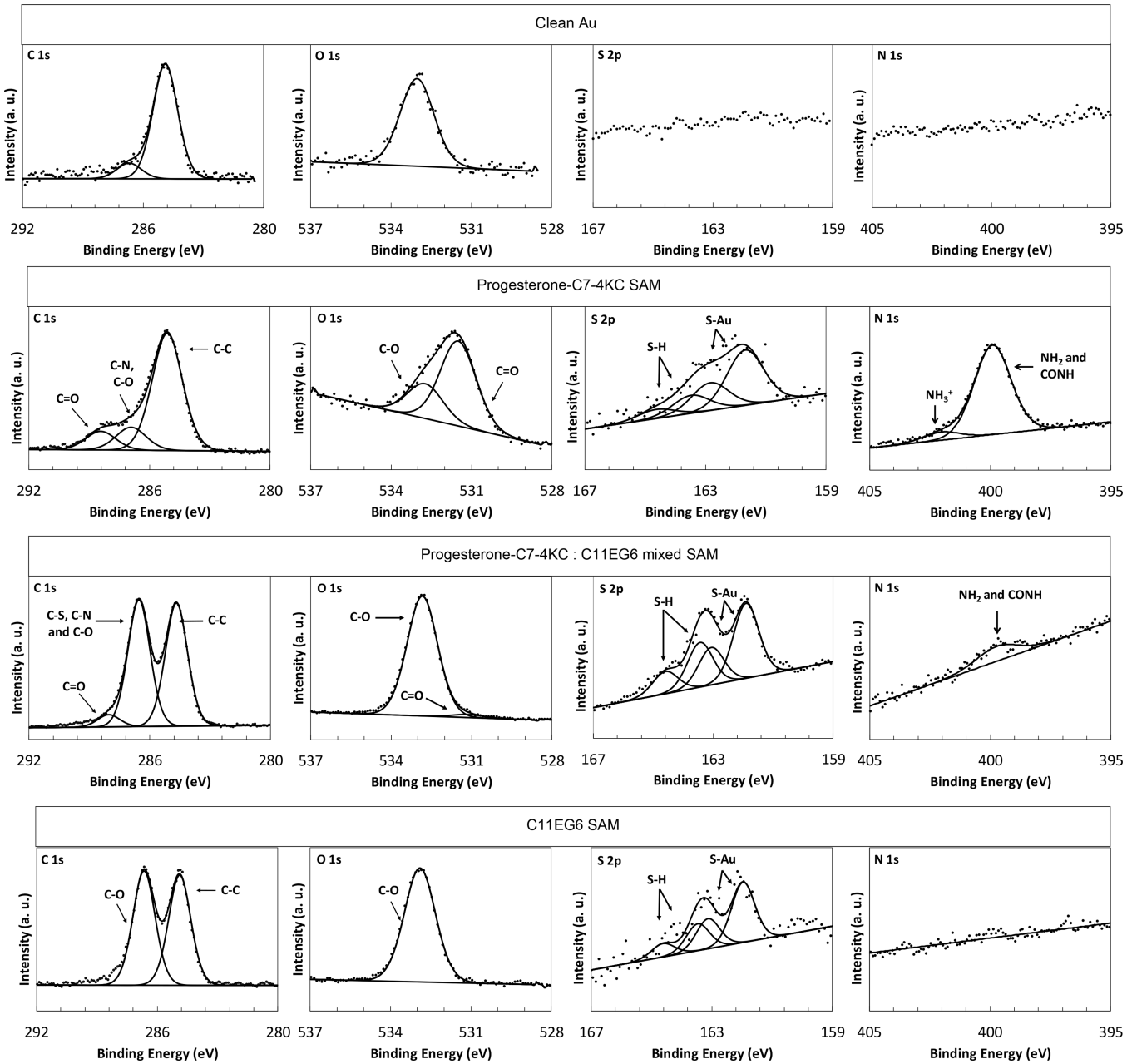

Figure 2. XPS spectra of the C 1s, O 1s, S 2p, and N 1s regions for clean gold, progesterone-C7-4KC SAM, progesterone-C7-4KC:C11EG6 mixed SAM, and C11EG6 SAM.

exhibits contact angle and thickness values lower than those obtained for pure progesterone-C7-4KC and similar to those reported in the literature.[27] The values of contact angle and thickness for the progesterone-C7-4KC:C11EG6 mixed SAM are in between those of pure progesterone-C7-4KC and C11EG6.

In order to confirm the chemical composition of the binary SAM, XPS analysis was performed on clean gold, progesterone-C7-4KC SAM, progesterone-C7-4KC:C11EG6 mixed SAM, and C11EG6 SAM (Figure 2). XPS confirmed the presence of carbonyl carbon (C═O) from the peptide (progesterone-C7-4KC), and ether carbon (C−O) from the spacer (C11EG6) through the fitting of the C 1s and O 1s XPS spectra.[28] The S 2p spectra consisted of two doublet peaks, with the doublet peak at lower binding energy indicating that the sulfur was chemisorbed on the gold surface.[29,30] A second doublet peak was observed at higher binding energy, which can be attributed to the S−H bonds, indicating presence of some unbound sulfur.[30] No sulfur peaks above 166 eV were observed, indicating that no oxidized sulfur was present on the surface. Furthermore, N 1s peaks centered at 399.7 eV, which are characteristic of amino (NH_2) and amide (CONH) moieties,[31] were observed. XPS analysis of clean gold showed the absence of N or S species, only revealing C 1s and O 1s signals due to the typical presence of adventitious carbon contamination[32] (Figure 2). Thus, the observed N 1s and S 2p signals on the mixed SAM can be fully ascribed to the presence of the progesterone-C7-4KC and C11EG6 molecules.

From the integration of the elemental peak areas, relative atomic percentages for each element were calculated and are shown in Tables 2−4, as well as the relative component percentage for each element. These values are in accordance

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Table 2. Relative Atomic Percentages and Relative Component Percentages for the Progesterone-C7-4KC:C11EG6 Mixed SAM Calculated from XPS[a]

	relative atomic percentages			relative component percentages	
	measured	theor	components	measured	theor
O	21.2 ± 1.3	21.7	O−C	97.7 ± 1.0	95.8
			O═C	2.3 ± 1.0	4.2
C			C−C	48.2 ± 1.7	45.3
	75.7 ± 1.1	74.2	C−S, C−N, and C−O	47.3 ± 2.1	53.6
			C═O	4.5 ± 0.3	1.1
S	2.3 ± 0.2	3.1	S−Au	63.2 ± 0.7	
			S−H	36.8 ± 0.7	
N	0.9 ± 0.1	1.1	-		

[a]The values are the average of two measurements per sample (prepared in triplicate), with the errors reported as standard deviation.

Table 3. Relative Atomic Percentages and Relative Component Percentages for the Progesterone-C7-4KC SAM Calculated from XPS[a]

	relative atomic percentages			relative component percentages	
	measured	theor	components	measured	theor
O	13.0 ± 0.6	12.3	O−C	23.5 ± 3.6	20.0
			O═C	76.5 ± 3.6	80.0
C			C−C	73.3 ± 0.2	66.1
	66.9 ± 0.6	72.8	C−N and C−O	14.1 ± 0.2	20.3
			C═O	12.5 ± 0.2	13.6
S	3.0 ± 0.2	1.2	S−Au	78.6 ± 3.9	
			S−H	21.4 ± 3.9	
N	17.1 ± 0.4	13.6			

[a]The values are the average of two measurements per sample (prepared in triplicate), with the errors reported as standard deviation.

Table 4. Relative Atomic Percentages and Relative Component Percentages for the C11EG6 SAM Calculated from XPS[a]

	relative atomic percentages			relative component percentages	
	measured	theor	components	measured	theor
O	26.5 ± 1.2	22.6	O−C	100	100
			O═C	0	0
C			C−C	48.8 ± 3.4	43.5
	71.7 ± 1.1	74.2	C−O	51.2 ± 3.4	56.5
			C═O	0	0
S	1.7 ± 0.1	3.2	S−Au	67.4 ± 8.1	
			S−H	32.6 ± 8.1	
N	0	0			

[a]The values are the average of two measurements per sample (prepared in triplicate), with the errors reported as standard deviation.

with the theoretical ones, based on the stoichiometry of the molecules, providing further evidence of the formation of the progesterone-C7-4KC:C11EG6 mixed SAM. Small deviations of the calculated atomic percentages from the theoretical values are mainly attributed to the level of accuracy of the relative sensitivity factors used for quantification.[33]

From integrating the area of the S 2p and N 1s peaks and taking into consideration that the progesterone-C7-4KC oligopeptide contains 11 N atoms and 1 S atom and the C11EG6 presents no N atoms and only 1 S atom only, an average mole fraction in the mixed SAM of 0.03 ± 0.01 for progesterone-C7-4KC and 0.97 ± 0.01 for C11EG6 was obtained (see ESI for more details on the calculations). These results illustrate comparable surface fractions, within the error, to the solution fractions used to prepare the progesterone-C7-4KC:C11EG6 mixed SAM.

2.2. Switching Properties. Following the characterization of the mixed SAM, attention was turned to the investigation of its antibody binding capability and ability to switch progesterone antigen activity on-demand. The antibody binding to the switchable SAMs was investigated using electrochemical SPR (eSPR), which allowed monitoring of surface binding while an electrical potential was applied to the surface using a three-electrode electrochemical cell and a potentiostat. In this system, the gold surfaces act as the working electrode, a Pt wire as the counter electrode, and an Ag/AgCl electrode as the reference electrode. Monitoring of surface binding was initiated by establishing a baseline using phosphate buffer saline (PBS) solution while (i) no electrical potential (open circuit (OC) conditions), (ii) negative (−0.4 V), or (iii) positive (+0.3 V) potential was being applied. Subsequently, the antibody for progesterone (anti-Pg Mab) diluted in degassed PBS (conc. 250 nM) was flowed over the surface, after which the surface was rinsed with a continuous flow of PBS to remove any unbound antibody.

The recorded sensorgrams for the progesterone-C7-4KC:C11EG6 mixed SAM at different electrical potential conditions (OC (no applied potential), −0.4, and +0.3 V) are illustrated in Figure 3. Table 5 summarizes the final binding capacity (BC) at the three electrically different states, as well as the switching efficiency (SE) for the mixed SAM. The BC is defined as the difference in the SPR response units between the beginning of the antibody injection and the end of washing

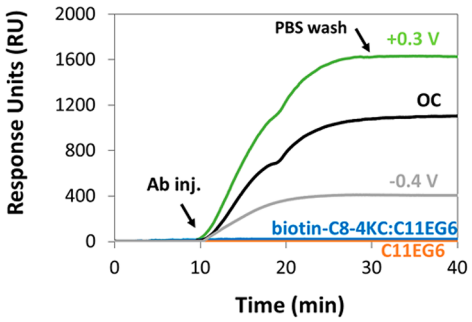

Figure 3. SPR sensorgrams for the binding of anti-Pg mAb to the progesterone-C7-4KC:C11EG6 mixed SAM, in PBS, under OC (no applied potential), ON (+0.3 V) and OFF (−0.4 V) conditions; control sensorgrams for biotin-C8-4KC:C11EG6 mixed SAM and C11EG6 SAM under OC (no applied potential).

Table 5. Binding Capacity under OFF (−0.4 V), OC (no applied potential), and ON (+0.3 V) Conditions, Expressed in Response Units (RU), and Switching Efficiency of the Progesterone-C7-4KC:C11EG6 Mixed SAM[a]

binding capacity, BC (RU)			
−0.4 V	OC	+0.3 V	switching efficiency, SE (%)
478 ± 39	1195 ± 39	1744 ± 90	73 ± 3

[a]The values are the average of three samples, with the errors reported as standard deviation.

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with PBS. The SE was calculated as the percentage difference between the binding capacity when a positive potential was applied (BC_{ON}) and the binding capacity when a negative potential was applied (BC_{OFF}), divided by BC_{ON}:

$$SE = \frac{BC_{ON} - BC_{OFF}}{BC_{ON}} \times 100 \quad (1)$$

The progesterone-C7-4KC:C11EG6 mixed SAM shows high antibody binding with immobilization capacity of at least 1.2 ng/mm^2 in OC conditions and 1.7 ng/mm^2 for an applied potential of +0.3 V (1 000 RU = 1 ng/mm^2).[17] The very high binding affinity (7.5×10^{11} M^{-1}) of the antibody for the progesterone antigen has promoted such high binding and no dissociation of the captured antibodies upon rinsing for all different electrical potential conditions (OC, −0.4, and +0.3 V). As for a nonswitching sensing system, parameters, such as the antibody affinity, the amount of the antigen ligands immobilized on the sensor surface and the sensing platform, will impact on the detection sensitivity that can be achieved.

The antibody interaction with the surface-tethered progesterone is specific, since negligible binding was observed for the control monolayer surfaces, namely a pure C11EG6 SAM and a biotin-C8−4KC:C11EG6 mixed SAM where the progesterone was replaced by a biotin moiety. The biotin ligand, which is well-known for its strong and rapid interaction with avidin protein, did not bind to the progesterone antibody. Additional control experiments were performed and will be discussed later in the manuscript (section 2.3).

While good accessibility occurs in OC conditions, the presence of +0.3 V induces further increase in binding capacity that can be explained by the *linear* peptide conformation of the oligolysines under a positive electrical potential.[22,23] The results suggest that in a fully extended state, the progesterone moieties are largely free from steric interactions from the surface and, thus, are entirely exposed for antibody binding. A switching efficiency above 70% was achieved for this system. The switching efficiency is highly dependent on the ability of concealing the progesterone moiety under a negative potential and herein there is a substantial reduction on the binding capacity, from ∼1700 to ∼500 RU. Based on our previous studies,[22,23] we interpret this binding activity inhibition as being the result of the progesterone being in close proximity to the ethylene glycol matrix, hindering molecular recognition. It is of significance to highlight that despite the presence of a heptyl linker between the oligolysine switching unit and the antigen binding site, the electrically triggered conformational changes in the oligolysine are able to distort the progesterone into a conformation that lacks affinity for the antibody. In addition, the findings revealed that the conformational rigidity of the progesterone does not affect the ability of the oligolysines to induce conformational changes that either promote or inhibit progesterone−antibody interactions. Notably, the findings suggest that the oligolysine conformational changes upon application of an electrical potential can be propagated over a span of at least 2.6 nm (i.e., length of the heptyl linker-progesterone units).

Based on previous potential and time-dependent electrochemical stability experiments conducted on peptide-based SAMs,[17] eSPR experiments were run using a maximum negative electrical potential of −0.4 V and a maximum positive potential of +0.3 V in order to prevent any undesirable loss of monolayer stability. However, in order to understand the effect of the electrical potential on the switching efficiency, SPR experiments were conducted using lower negative (−0.2 V) and positive potentials (+0.2 V). As illustrated in Table 6, the switching efficiencies, as determined using eq 1, were shown to decrease if such potentials are used as ON and/or OFF conditions. Thus, higher switching efficiencies are achieved by using high electrical potentials within the range of electrochemical stability of the SAM. Thus, it is likely that at high positive or negative potentials there is a stronger repulsion or attraction between the positively charged oligolysine SAMs and the positively or negatively polarized surface, respectively.

Table 6. Switching Efficiencies of the Progesterone-C7-4KC:C11EG6 Mixed SAM when Different Combinations of Positive or Negative Potentials Are Used[a]

ON potential (V)	OFF potential (V)	switching efficiency, SE (%)
+0.2	−0.2	4 ± 1
+0.2	−0.4	46 ± 8
+0.3	−0.2	38 ± 7

[a]The values are the average of three samples, with the errors reported as standard deviation.

2.3. Control Mixed SAMs. In order to demonstrate that electrical potential control over antibody binding occurs due to conformational changes of the oligopeptide that lead to the concealment or exposure of the progesterone antigen, two different type of control SAMs were prepared and analyzed. The first control SAM comprises a 5KC:C11EG6 mixed SAM, in which no progesterone is present, to address the question whether antibody binding occurs due to the presence of an electrical potential that attracts or repels the anti-Pg mAb. The 5KC:C11EG6 mixed SAM was prepared at a mole fraction in solution of 0.98 for C11EG6 and characterized by contact angle and ellipsometry as shown in Table 7. As expected, the contact angle data shows the presence of a hydrophilic surface, with similar thickness to that of the progesterone-C7-4KC:C11EG6 mixed SAM.

Table 7. Advancing Water Contact Angle and Ellipsometric Thickness Values for the Control 5KC:C11EG6 Mixed SAM on a Gold Surface[a]

		thickness (nm)	
SAM	contact angle (deg)	measured	theor
5KC	74 ± 5	3.3 ± 0.4	4.3
5KC:C11EG6	44 ± 1	3.0 ± 0.1	
C11EG6	41 ± 2	2.4 ± 0.1	2.9

[a]The values are the average of three measurements per sample, prepared in triplicate, with the errors reported as standard deviation.

XPS analysis was performed (Figure 4) to confirm the chemical composition of the formed mixed SAM. From the integration of the elemental peak areas, relative atomic percentages for each element were calculated and are shown in Table 8, as well as the relative component percentage for each element. These values are in accordance with the theoretical ones, based on the stoichiometry of the molecules, providing further evidence of the formation of the 5KC:C11EG6 mixed SAM. From integrating the area of the S 2p and N 1s peaks and taking into consideration that the 5KC oligopeptide contains 11 N atoms and 1 S atom and the C11EG6 presents no N atoms and only 1 S atom only, an

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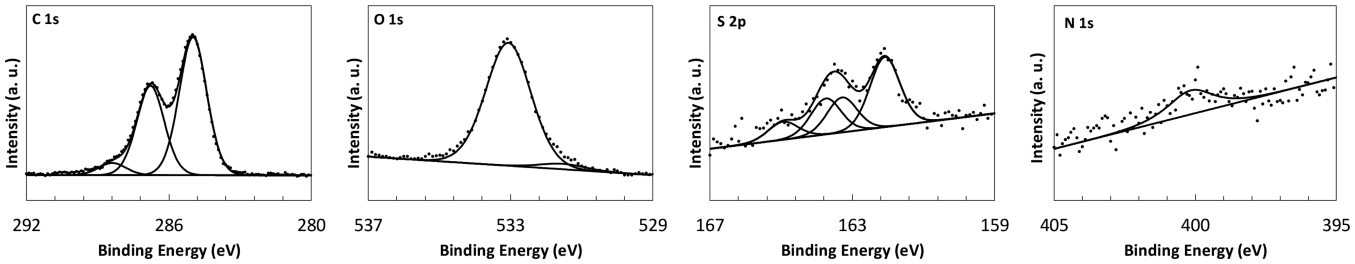

Figure 4. XPS spectra of the C 1s, O 1s, S 2p, and N 1s regions for the 5KC:C11EG6 mixed SAM.

Table 8. Relative Atomic Percentages and Relative Component Percentages for the 5KC: C11EG6 Mixed SAM Calculated from XPS[a]

	relative atomic percentages			relative component percentages	
	measured	theor	components	measured	theor
O	16.5 ± 4.2	22.2	O−C	92.2 ± 1.2	97.8
			O═C	7.8 ± 1.2	2.2
C			C−C	59.8 ± 5.4	39.4
	78.4 ± 4.3	73.7	C−S, C−N, and C−O	34.9 ± 5.5	59.9
			C═O	5.3 ± 0.1	0.7
S	3.9 ± 0.3	3.2	S−Au	66.9 ± 2.4	
			S−H	33.1 ± 2.4	
N	1.2 ± 0.1	1.0			

[a]The values are the average of two measurements per sample (prepared in triplicate), with the errors reported as standard deviation.

average mole fraction in the mixed SAM of 0.03 ± 0.01 for 5KC and 0.97 ± 0.01 for C11EG6 was obtained.

The recorded sensorgrams for the 5KC:C11EG6 mixed SAM at different electrical potential conditions (OC, −0.4, and +0.3 V) are illustrated in Figure 5. The sensorgrams for the progesterone-C7-4KC:C11EG6 mixed SAM at different electrical potential conditions (OC, −0.4, and +0.3 V), previously shown in Figure 3, were added to this figure for comparison purposes. In contrast to different binding capabilities of the antibody for progesterone-C7-4KC:C11EG6 mixed SAM at different electrical potentials, the amount of antibody that adsorbed nonspecifically to the 5KC:C11EG6 mixed SAM under OC, −0.4, and +0.3 V was very limited. These results demonstrate that the electrical potential has minimal direct effect on attracting or repelling the antibody to or from the surface.

Another control mixed SAM was designed, prepared and characterized to demonstrate that modulation of antibody binding was due to the conformational changes of the progesterone oligopeptide on the surface induced by an electrical potential. The control mixed SAM was prepared with a nonswitchable progesterone thiol (progesterone-C11-SH) and a tri(ethylene glycol) thiol (EG3SH). The progesterone-C11-SH compound was synthesized by EDC/HOBt coupling of progesterone 3-(O-carboxymethyl)oxime and 11-amino-1-undecanethiol hydrochloride and isolated after column chromatography purification (see SI for details on the progesterone-C11-SH synthesis and characterization). EG3SH was used instead of EG6OH due to the chain length of the progesterone-C11-SH, which is shorter than the progesterone-C7-4KC. The use of EG6OH in this case could potentially inhibit the access of the antibody to the progesterone molecule. The progesterone-C11-SH:EG3SH mixed SAM was prepared at a mole fraction in solution of 0.98 for EG3SH and characterized by contact angle and ellipsometry as shown in Table 9. The advancing contact angle value obtained for the mixed SAM was between the contact angle values obtained for the pure SAMs of each component.

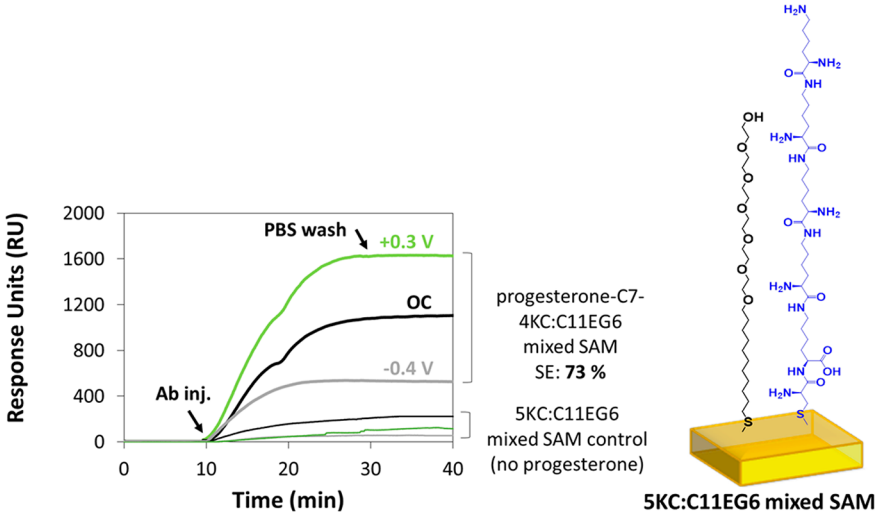

Figure 5. SPR sensorgrams for the binding of anti-Pg mAb to the control 5KC:C11EG6 mixed SAM (no progesterone), in PBS, under OC (no applied potential), ON (+0.3 V), and OFF (−0.4 V) conditions; sensorgrams for the binding of anti-Pg mAb to the progesterone-C7-4KC:C11EG6 mixed SAM are also included, for comparison purposes. (right) Chemical structures of the components used for the formation of the 5KC:C11EG6 mixed SAM.

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Table 9. Advancing Water Contact Angle and Ellipsometric Thickness Values for the Control Progesterone-C11-SH:EG3SH Mixed SAM on Gold Surfaces[a]

SAM	contact angle (deg)	thickness (nm)	
		measured	theor
progesterone-C11-SH	73 ± 2	2.2 ± 0.1	2.8
progesterone-C11-SH:EG3SH	37 ± 1	1.9 ± 0.4	
EG3SH	32 ± 2	0.7 ± 0.1	1.4

[a]The values are the average of three measurements per sample, prepared in triplicate, with the errors reported as standard deviation.

Table 10. Relative Atomic Percentages and Relative Component Percentages for the Progesterone-C11-SH:EG3SH Mixed SAM Calculated from XPS[a]

	relative atomic percentages			relative component percentages	
	measured	theor.	components	measured	theor.
O	20.1 ± 0.9	23.7	O−C	92.9 ± 1.3	93.2
			O═C	7.1 ± 1.3	6.8
C			C−C	39.9 ± 2.4	30.7
	60.0 ± 0.5	68.5	C−S, C−N, and C−O	52.6 ± 1.9	67.0
			C═O	7.5 ± 0.8	2.4
S	15.7 ± 0.9	6.2	S−Au	90.5 ± 1.8	
			S−H	9.5 ± 1.8	
N	4.2 ± 0.4	1.6			

[a]The values are the average of two measurements per sample (prepared in triplicate), with the errors reported as standard deviation.

The experimental thickness value obtained for the progesterone-C11-SH:EG3SH mixed SAM is also situated between the theoretical molecular length of both molecules, constituting another indication of the presence of a binary monolayer.

As for the previous SAMs, XPS analysis was performed (Figure 6) to confirm the chemical composition of the formed mixed SAM. Table 10 shows the relative atomic percentages and relative component percentages for the progesterone-C11-SH:EG3SH mixed SAM. From integrating the area of the S 2p and N 1s peaks and taking into consideration that the progesterone-C11-SH contains 2 atoms of N and 1 atom of S and the EG3SH presents no N atoms and only 1 S atom only, an average mole fraction in the mixed SAM of 0.13 ± 0.02 for progesterone-C11-SH and 0.87 ± 0.02 for EG3SH was obtained.

The recorded sensorgrams for the progesterone-C11-SH:EG3SH mixed SAM at different electrical potential conditions (OC, −0.4, and +0.3 V) are illustrated in Figure 7. While the progesterone antibody binds to this surface, the effect when different electrical potential conditions is applied is limited, with a calculated switching efficiency of 18 ± 5%. The differences in binding observed for the different applied potentials might arise from a small effect that the OC, −0.4, and +0.3 V might have on attracting or repelling the antibody to or from the surface, thus, to a small extent, promoting or inhibiting antibody binding. However, the switching efficiency of 18 ± 5% for the control is significantly lower than that obtained for the switchable progesterone-C7-4KC:C11EG6 mixed SAM, which exhibits a switching efficiency of 73 ± 3%. This result is another indication that the switching is due mainly to the oligolysine conformational changes. It is relevant to note that the higher binding capacity for the progesterone-C11-SH:EG3SH mixed SAM when compared with the progesterone-C7-4KC:C11EG6 mixed SAM is due to the higher density of progesterone ligands on the former, as shown from the XPS results. The mole fraction of progesterone-C11-SH in the mixed SAM is 0.13, whereas the progesterone-C7-4KC fraction on the progesterone-C7-4KC:C11EG6 mixed SAM is 0.03, despite starting from a similar molar fraction in solution for the creation of both surfaces.

These control experiments provide valuable evidence that antibody binding is controlled by the exposure of the progesterone moiety, which in turn is controlled by the oligolysine conformational changes induced by the application of an electrical potential.

2.4. MD Simulations. To confirm our interpretation of the switching mechanism for the progesterone-C7-4KC:C11EG6 mixed SAM, molecular dynamics (MD) simulations were carried out. The consistent-valence force field (cvff) was chosen as it was previously validated for another surface system comprising a similar conformational switching mechanism.[23] As shown in Scheme 1, the progesterone-C7-4KC:C11EG6 mixed SAM was modeled using slab models with two-dimensional rhombic periodic boundary conditions. The PBS solution was treated as a simplified model to water molecules and chloride ions. A mole fraction of 0.94 C11EG6 on the surface was chosen. The theoretical SAM thicknesses obtained from MD simulations agree well with the experimental measurements (Table 1), which validates the packing density of our model. The detailed model parameters are listed in Table S1. The electrical potentials used in the experiment were modeled with electric fields oriented vertically upward (E_{+z} = +6 V/nm) or downward (E_{-z} = −8 V/nm) from the gold surface. The oligolysines adopted a fully extended conformation and the progesterone head pointed upward and was entirely exposed under E_{+z} (Figure 8a), corresponding to the ON state (positive potential) with high binding capacity. In contrast, when E_{-z} was applied to mimic the OFF condition (negative potential), the oligolysines collapsed to a folded conformation, pulling the progesterone head partially into the C11EG6 SAM and concealing it from the subphase (Figure

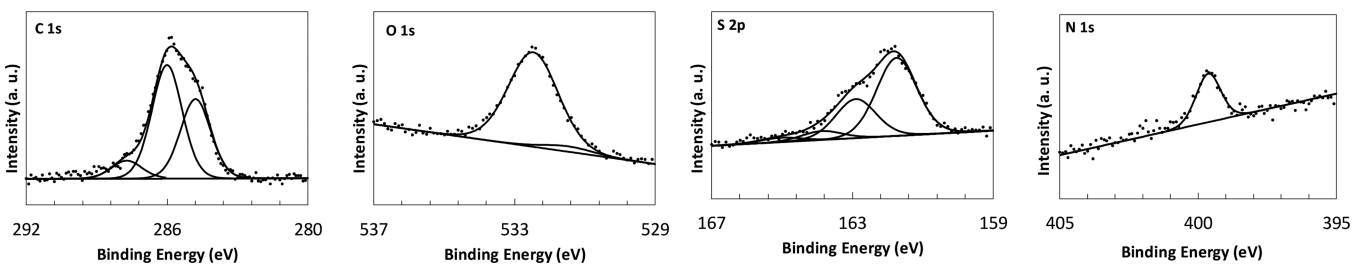

Figure 6. XPS spectra of the C 1s, O 1s, S 2p, and N 1s regions for the progesterone-C11-SH:EG3SH mixed SAM.

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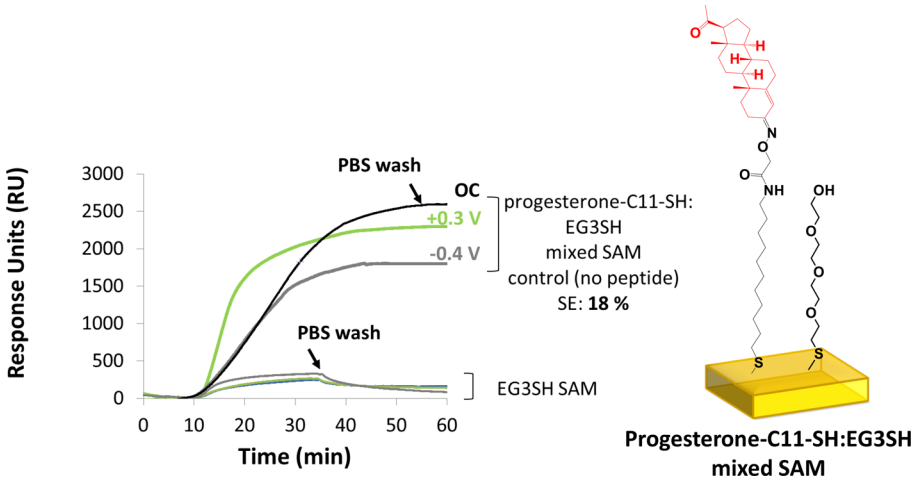
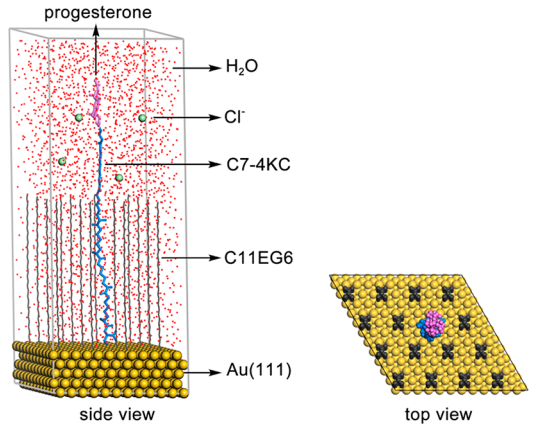


Figure 7. SPR sensorgrams for the binding of anti-Pg mAb to the control progesterone-C11-SH:EG3SH mixed SAM (no peptide) and to the EG3SH SAM, in PBS, under OC, ON (+0.3 V) and OFF (−0.4 V) conditions. (right) Chemical structures of the components used for the formation of the progesterone-C11-SH:EG3SH mixed SAM.

Scheme 1. Models Used in the MD Simulations[a]



[a]The progesterone moiety, alkyl-lysine-cysteine groups (C7-4KC), and C11EG6 are coloured in purple, blue, and grey, respectively. The red dots and green and yellow balls represent water molecules, chloride ions, and gold atoms, respectively.

8b) and, hence, resulting in decreased binding capacity. In the OC condition, the progesterone-C7-4KC chain was more flexible and adopted multiple conformations that were partially or totally buried by the C11EG6 chains (Figure 8c), displaying medium binding affinity. In comparison to the former biotin-4KC:tri(ethylene glycol)-terminated thiol SAM,[23] the inclusion of an alkyl linker leads to a more flexible OC state while the presence of a rigid binding site (i.e., progesterone) introduces further requirements related with its concealment by the EG matrix for controlling bioactivity.

3. CONCLUSION

In summary, we have developed a new surface molecular design with electrically responsive properties for on-demand antibody−antigen recognition on sensor chips. While we chose to implement the strategy using the progesterone antigen-progesterone antibody system, this approach is a versatile platform for on-demand biosensing of a broad range of low molecular weight antigens and their complementary antibodies. Fundamental surface structure−activity studies were conducted and design rules to guide the construction of such on-demand sensor chips have been established. The results demonstrate that mixed SAMs comprising an antigen end-functionalized switchable component and a C11EG6 spacer are able to prevent nonspecific binding and control antigen activity with high switching efficiency (above 70%). In such a system, the switching performance is intimately linked with the capability to perturb the presentation of the antigen targeting moiety. The molecular architecture chosen for the antigen end-functionalized switchable component allows for such modulation to occur, in which we demonstrate for the first time that the conformational changes of a four lysine molecular switch can be propagated to a distal rigid antigen site and regulate its

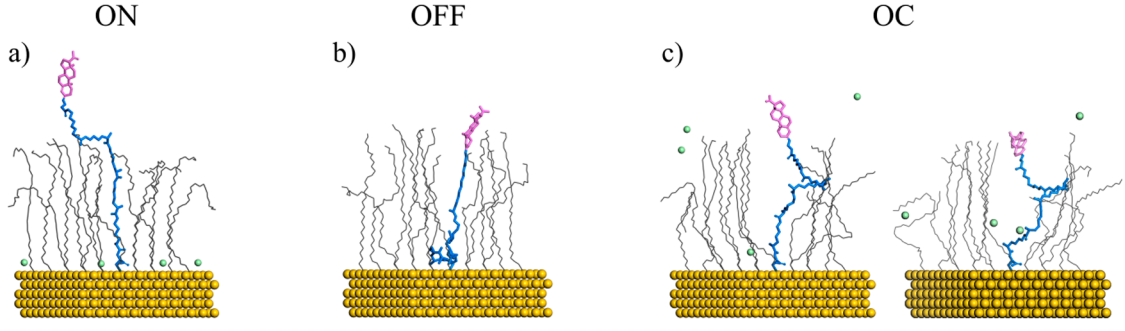


Figure 8. MD simulation snapshots showing the progesterone-C7-4KC:C11EG6 mixed SAM conformations of (a) ON, (b) OFF, and (c) OC states.

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activity. The design principles and strategies for achieving high switching efficiencies described herein will be able to be adapted, in future studies, to other highly relevant antigen−antibody systems. Electrical switching is easy to be applied and regulated, making it an excellent remote trigger to biosensing applications. To date antibody−antigen interactions have always been evaluated in static conditions, and this work paves the way toward the development of on-demand antibody biosensors for a wide range of medical, biotechnological, and pharmaceutical applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsabm.8b00201.

Experimental details of progesterone-C11-SH synthesis, SAM preparation and characterization, and MD simulation details (PDF)

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Notes

The authors declare no competing financial interest.

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