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# Half Antibody Fragments Improve Biosensor Sensitivity without Loss of Selectivity

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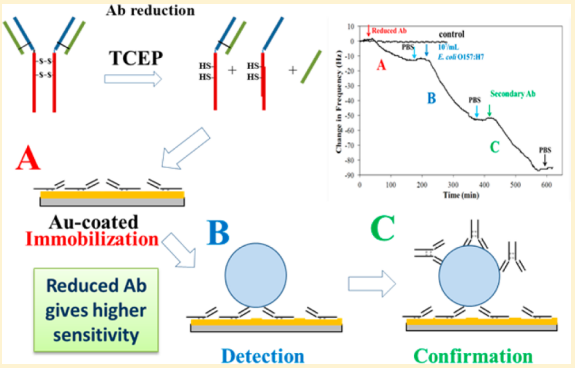
**ABSTRACT:** We show for the first time that half antibody fragments obtained by reduction via tris(2-carboxyethyl)phosphine (TCEP) gave a larger response with shear-mode resonating mass sensors than physisorbed whole antibody or antibody immobilized via Protein G. The reduced antibody is shown to preserve the antigen-binding region and was determined via the antigen binding response. Reduction exposed the native thiol group in the antibody that readily chemisorbed onto the gold-coated sensor surfaces with the right orientation for antigen binding. Comparing responses obtained on a quartz crystal microbalance for the detection of pathogenic *Escherichia coli* O157:H7 using TCEP-reduced antibody with native antibody showed that the proposed method enhances device sensitivity. Examining the half antibody fragments for detection of the pathogen in the presence of the nonpathogenic wild strain showed that the antibody fragments retained their specific antigen binding capability without loss of selectivity.




Antibodies, because of their selectivity, are commonly used as recognition molecules in biosensing. For use in biosensors, they are to be immobilized on the sensor surfaces. Traditionally, reactive surface functional groups are generated on the sensor using wet-chemistry methods such as silanization on silica that are then reacted with complementary functional group(s) on antibodies for immobilization.[1] For sensors with a gold surface, self-assembled monolayers (SAM) of thiolated molecules containing a reactive functional group are used.[1] Physisorption of proteins such as protein A and protein G are commonly used as an initial layer for antibody immobilization via the $F_c$ region for achieving the right orientation.[2] We hypothesize that the use of the intermediate layer of protein G for immobilization reduces device sensitivity as the added effective mass sensed may be reduced.

Antibodies are made of two heavy chains and two light chains of amino acids held together by disulfide bridges between the cysteine residues.[3] Reduction of these disulfide bridges will cleave an antibody into two equal halves, and each of these halves will have active thiol groups that can directly chemisorb on Au ⟨111⟩ sites. Immobilization of reduced antibody can help with proper orientation of the antigen-binding $F_{ab}$ regions because the thiol groups are at the far end and away from the $F_{ab}$ region. In this work we wish to examine if half antibody immobilization can simplify not only the preparation method but also lead to the right orientation of the antibody resulting in improved device sensitivity. We test this hypothesis by comparing the responses obtained for the detection of pathogenic *Escherichia coli* O157:H7 using Protein G for the right orientation of the antibody with the responses obtained by direct physisorption of whole antibody molecules as well as the proposed reduced antibody. An established biosensing platform, quartz crystal microbalance (QCM), was used to measure the extent of immobilization and detection and binding responses.

Enzymes such as pepsin and papain are used to digest antibody (Ab) for various preparations (Figure 1).[1] Pepsin digests the $F_c$ region of the antibody to give a divalent Ab structure, whereas the papain digest gives individual antigen-

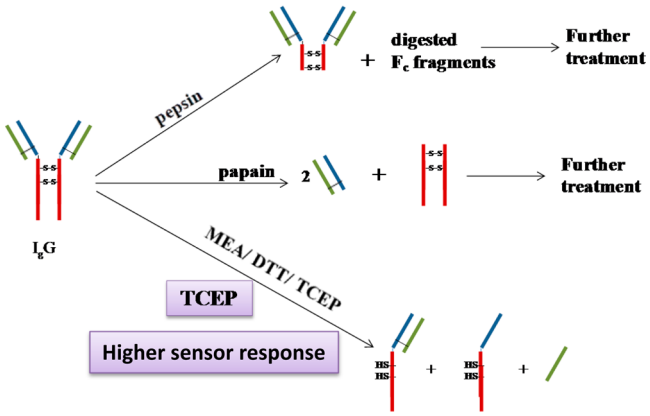



**Figure 1.** Schematic of the typical reactions used to cleave antibodies. Enzymes such as pepsin and papain cleave the antibodies around the disulfide bridges, and these fragments can then be reacted for immobilization. MEA, DTT, and TCEP reduce the disulfide bridges in the antibody both in the $F_c$ and $F_{ab}$ regions.

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binding $F_{ab}$ fragments. The fragments produced by enzyme digestion need further processing steps for oriented immobilization on biosensor surfaces. Chemicals commonly used for reducing antibodies are 2-mercaptoethanol (MEA) and dithiothreitol (DTT).[4] DTT is a stronger reducing agent than MEA. Both MEA and DTT contain thiol groups that would compete with the reduced antibodies for gold ⟨111⟩ sites on the sensor surface. Therefore, the reducing agent is to be removed prior to immobilization on the sensor gold surface. Such a separation step adds one more variability to the protocol, leave alone the loss of the reduced Ab in handling and trace reducing agent contamination.

The use of MEA as a reducing agent for biosensing application has been investigated.[5] Since MEA contains a thiol group, the unreacted MEA is removed from the cleaved antibody fragments using gel columns. Inefficiency of the separation step can lead to MEA being present in the reduced antibody sample that will compete for gold sites and also continue reduction of the disulfide group in the $F_{ab}$ region of the antibody molecule. Some of the Ab fragments are lost during the separation step which might also explain why the studies did not find any improvement in sensitivity. In the second study, gold nanoparticles were used to increase the surface area for immobilization of half Ab fragments which led to enhanced sensitivity and faster response rates.

An alternate to the sulfur containing reducing agents, trialkylphosphine such as tris(2-carboxyethyl)phosphine (TCEP) can be used for reduction of disulfides.[6] To the authors' knowledge, the use of TCEP for cleaving the antibody for use as recognition molecules on a biosensor platform has not been investigated. A schematic of the reaction between the TCEP and the disulfide bonds in the antibody is shown in Figure 1. TCEP is a more stable and a stronger reducing agent than DTT. Since TCEP is devoid of sulfur-containing groups, it does not compete with the reduced antibody for Au ⟨111⟩ sites, and therefore a separation step is not essential, making it a more convenient reagent for gold-coated biosensor applications.[7]

## EXPERIMENTAL SECTION

**Reagents and Equipment.** All solutions were prepared using deionized (18 MΩ) water. Bond-Breaker TCEP solution was purchased from Thermo Scientific. Polyclonal goat antibody against *Escherichia coli* O157:H7 as well as the heat-killed positive *Escherichia coli* O157:H7, and rabbit antigoat IgG antibody were purchased from Kirkegaard and Perry Laboratories, Inc. Bovine serum albumin (BSA), phosphate-buffered saline (PBS), sinapinic acid, and acetonitrile were purchased from Sigma-Aldrich. The heat-killed *E. coli* O157:H7 is a nontoxic reagent and was handled as a nontoxic chemical. Used samples were mixed with household Chlorox to a final concentration of 10% and disposed of via the lab drain.

Detection experiments were performed using a 5 MHz AT-cut quartz crystal and a Teflon flow cell (Stanford Research Systems). The resonance frequency was measured using an impedance analyzer (HP 4192A) which swept the frequency in the vicinity of 5 MHz at high resolution, and the resulting peak in the phase angle value was used to determine the resonance frequency. The impedance analyzer was managed by a PC using a custom designed LabView program.

**Reduction of Antibodies.** TCEP solutions for antibody reduction were prepared in 10 mM PBS at 5 mM concentration. A volume of 10 μL of TCEP was added to 1 mL of 10 μg/mL antibody in PBS and mixed well and allowed to react at room temperature for 60 min before the sample was directly injected into the flow loop.

Various mole ratios of TCEP:Ab were tested for two different lengths of time, and the cleaved products were measured using MALDI-MS to determine the extent of reaction. The reaction mixture of a mole ratio of 100:1 TCEP−Ab for 1 h gave the desired results and was used in all subsequent experiments. Further optimization of this step was carried out in this study.

**MALDI Mass Spectrometry.** MALDI mass spectrometry (MALDI-MS) analysis was carried out using a linear detector on a Bruker Daltonics autoflex III equipped with a smartbeam. Spectra obtained were externally calibrated using bovine serum albumin. For MALDI experiments, rabbit antigoat antibody was used as a substitute for the anti-*E. coli* O157:H7. Protein samples at 1 mg/mL in DI water were typically deposited (1 μL) on a dried film of 1 μL of aqueous solution of sinapinic acid containing 50% acetonitrile on a stainless steel stage. All spectra were obtained under identical instrument settings.

**QCM Measurements.** The quartz crystal was cleaned by exposing the gold surface to piranha solution (3:1 concentrated sulfuric acid to hydrogen peroxide) for 30 s followed by rinsing with copious amounts of DI water. The crystal was then exposed to oxygen plasma for 2 min which ensured a clean gold surface. The crystal was then rinsed with copious amounts of DI water and dried in a nitrogen stream before installation in the Teflon flow-cell. The reagent reservoirs were connected to the flow-cell via a 5-port manifold and a syringe pump downstream from the flow-cell pumped at a constant flow-rate of 5 μL/min.

Once the crystal resonance frequency reached a stable value, 1 mL of reagent was injected via the 5-port manifold for contacting with the crystal surface. The flow loop was flushed thoroughly with PBS before introduction of the next reagent to the crystal. As the reagents became immobilized on the crystal surface, a decrease in resonance frequency was observed and recorded.

**Controls.** Prior to conducting a detection experiment, two types of control experiments were performed. In the first, QCM not immobilized with antibody was exposed to a sample containing the target antigen, *E. coli* O157:H7. In the second, an antibody-immobilized QCM was exposed to nontarget cells (*E. coli* JM101) at a concentration of $10^7$/mL. Neither of the two control experiments gave a decrease in resonance frequency that was statistically significant. The maximum deviation obtained was <2 Hz. In presenting the responses in the Results and Discussion, we have labeled the two control responses as control.

## RESULTS AND DISCUSSION

**MALDI-MS Analysis.** In Figure 2 we compare the antibody that was treated with TCEP for reducing the disulfide bridges with the control, untreated antibody. The spectrum for native Ab exhibits the peaks for the monovalent (a), divalent (b), and trivalent (c) antibody molecular ions. The molecular weight of the antibody monomer is ∼143.6 kDa. The intensities of the monovalent and the divalent molecular ions are almost identical for the native Ab sample. On the other hand, the spectrum for TCEP-reduced Ab exhibited almost double intensity for the divalent molecular ion peak (b). The peak widths for the divalent, trivalent, and quatravalent ions should be $^1/_2$, $^1/_3$, and $^1/_4$ of the peak width for the monovalent molecular ion.[8]


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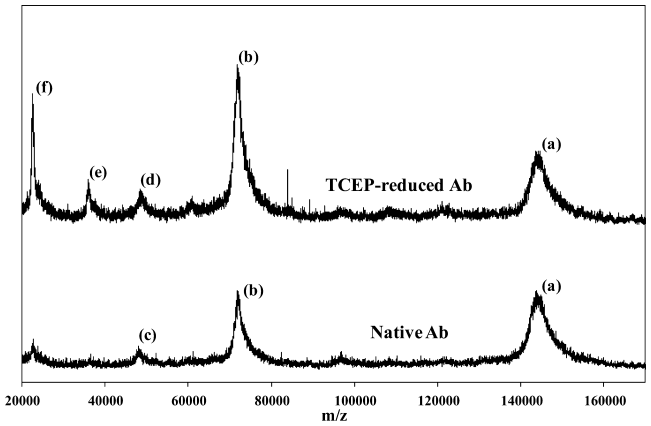


**Figure 2.** Typical MALDI-MS of native and TCEP-reduced $I_g$G. The native Ab exhibits 3 peaks at ~143.6 kDa (a), ~71.8 kDa (b), and ~47.9 kDa (c), which correspond to the monovalent, divalent, and trivalent molecular ions of whole antibody, respectively. The upper spectrum of TCEP-reduced Ab exhibits the peaks a and b in addition to three other peaks ~48.5 kDa (d), ~36 kDa (e), and ~22.6 kDa (f). Peak e can correspond to a quatravalent ion of the whole antibody or a divalent ion of half antibody molecules. The presence of the peak e along with the almost double intensity of peak b corresponding to the divalent whole antibody or monovalent half antibody molecules for TCEP reduced Ab leads to the conclusion that TCEP-reduction cleaves the antibody molecules into halves. The presence of peaks d and f suggests that TCEP also reduces the disulfides holding the heavy and light chains together.

This can sometimes be difficult to see for polyclonal antibodies but is discernible in Figure 2. We expect TCEP to cleave the disulfides holding the two halves of the antibody molecules. A monovalent molecular ion of half the antibody molecule will have the same mass as the divalent ion of the whole antibody molecule, and thus we expect to observe overlap in the spectrum. For the spectrum of TCEP-reduced Ab, the intensity of the peak at ~71.8 kDa increased which can be attributed to the presence of half antibody molecules. The TCEP-reduced Ab spectrum also exhibited increased intensity for the peak at ~35.9 kDa (e) which can correspond to quatravalent ion of the whole antibody or divalent ion for half antibody. Since the native Ab spectrum does not show a peak at ~35.9 kDa, the inference is that the increase in intensity of peak e was due to the presence of the half antibody fragments.

Since TCEP does not selectively target disulfide bridges, we expected the disulfide bridge holding the light and heavy chains in the $F_{ab}$ regions to be also reduced. As compared to the native Ab spectrum, we see increased intensity of peaks at ~48.5 kDa (d) and ~22.6 kDa (f) in the TCEP-reduced Ab spectrum. The heavy chains and light chains have molecular weights of ~50 kDa and ~25 kDa, respectively.[9] The increased intensities of peaks d and f in the TCEP-reduced Ab spectrum suggests that some of the disulfide bridges between the heavy and light chains were also reduced and were observed via MALDI-MS. It is reasonable to conclude that 1 h of reaction of 100:1 mole ratio of TCEP−antibody reduced the disulfides in the $F_c$ region more than the ones in the $F_{ab}$ region and gave half antibody fragments that can be immobilized on gold surfaces.

**TCEP-Reduced Antibodies As Recognition Molecules.** A clean QCM crystal was installed in the flow-cell with PBS as the circulating buffer. The crystal resonance frequency was measured and recorded as it came to a stable value which took ~7 min. The reduced antibody solution was introduced into the flow loop for immobilization on the clean gold surface. As the antibody fragments chemisorbed on the gold surface, the mass of the oscillating crystal increased and the resonance frequency decreased in value. As shown in Figure 2, immobilization of 10 $\mu$g/mL TCEP-reduced antibody lead to a resonance frequency decrease of 13 ± 3 Hz ($n$ = 5). Following this immobilization, the flow-cell was flushed thoroughly with PBS to remove any loosely bound antibody fragments and to prepare the flow loop for the next reagent. The PBS flush was followed by the introduction of 1 mL of $10^7$/mL *E. coli* O157:H7 antigen in the flow loop. As the pathogen became bound to the chemisorbed antibody, the resonance frequency decreased further by 41 ± 4 Hz ($n$ = 5) as shown in Figure 3. Following the binding of the pathogenic

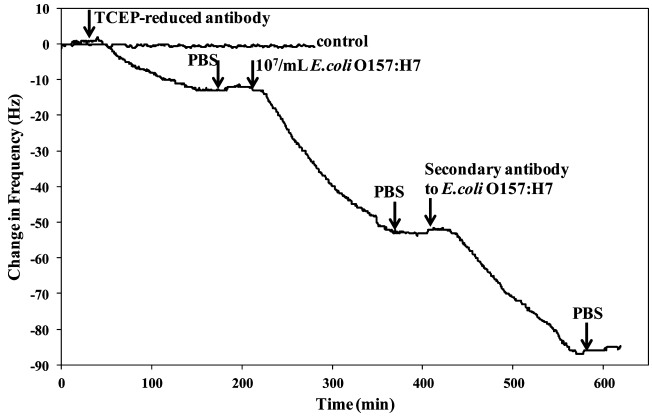


**Figure 3.** TCEP-reduced Ab works well for biosensing. Change in frequency vs time plot of a typical experiment is shown with time points for introduction of each reagent to the QCM crystal; 10 $\mu$g/mL of TCEP-reduced Ab led to a response of 13 Hz; $10^7$/mL of *E. coli* O157:H7, when introduced to the sensor after a PBS rinse, bound to the TCEP-reduced Ab immobilized on the QCM and gave a shift of 40 Hz. A confirmation step of antigen binding using whole antibodies subsequently gave a frequency shift of 30 Hz.

cells to the sensor-bound antibody fragments, 1 mL of anti-*E. coli* O157:H7, at 10 $\mu$g/mL, was introduced to the flow loop which bound to the sensor-bound target cells and induced a further resonance frequency decrease of 30 ± 3 Hz ($n$ = 5). The secondary antibody step confirms that the antibody fragments formed by TCEP-reduction retain the activity in the target-sensing $F_{ab}$ region. As a negative control, 1 mL of whole antibody (anti-*E. coli* O157:H7 at 10 $\mu$g/mL) was sent right after immobilization of the TCEP-reduced fragments without an antigen-binding step in between. No sensor response was observed in the control experiments.

After showing that the TCEP-reduced antibody fragments retain their antigen binding property, the next steps were to examine if the proposed method enhances biosensor sensitivity and if the antibody fragments retain selectivity.

**TCEP-Reduced Antibodies Improve Sensitivity.** Establishing that TCEP-reduced antibody can be used for biosensing lead us to examine how the performance of the cleaved antibody compares to some commonly used protocols for antibody immobilization. For this study, we chose two methods to contrast against the half antibody method: (1) direct physisorption of whole antibody on the gold crystal surface and (2) the method of chemisorption of protein G followed by the whole antibody. In the latter method, the $F_c$ region of the antibody binds to the surface-bound protein G and correctly


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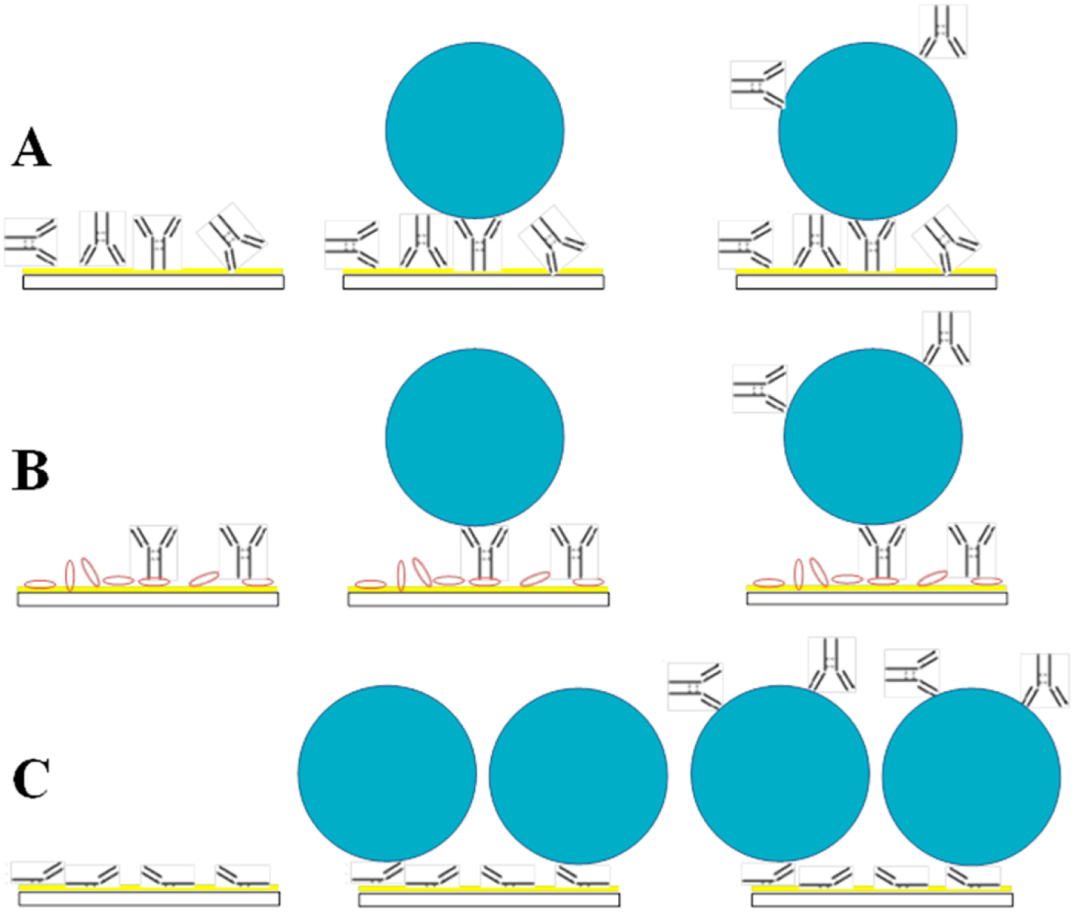


**Figure 4.** A schematic representation of the three methods investigated. The left most picture shows immobilization, followed by antigen binding and then sandwich binding for the three schemes. Panel A shows the direct physisorption of whole antibody on the gold-coated sensor surface. Panel B shows protein G mediated oriented immobilization of whole antibodies on the sensor surface. Panel C depicts oriented immobilization of TCEP-reduced half antibody molecules on gold surfaces.

orients the antigen binding $F_{ab}$ region. For the antigen, we chose *E. coli* O157:H7, a common food-borne pathogen in pure samples as well as samples mixed with a wild strain, *E. coli* JM 101. A schematic of the three immobilization methods investigated in this study followed by target binding and the second antibody confirmation step is illustrated in Figure 4.

Various antigen concentrations, $10^5$–$10^6$–$10^7$/mL, were used to compare the observed binding responses using the three immobilization methods. A summary of the resonance frequency response curves on the 5 MHz QCM crystal to $10^7$/mL concentration of *E. coli* O157:H7 is shown in Figure 5. For the same target concentration, the response for directly physisorbed whole antibody molecules as the sensing layer was 17 ± 2 Hz ($n$ = 3) and was lower than that observed with protein G as an intermediate layer, 29 ± 3 Hz ($n$ = 4). TCEP-reduced antibody fragments immobilized on gold manifested the largest frequency decrease of 39 ± 5 Hz ($n$ = 5) for the same antibody concentrations. Given that the noise level was comparable, the antibody fragments were approximately twice as sensitive as the native antibody.

We believe that direct physisorption of antibody on gold leads to random orientation of antibodies. This random orientation causes a steric hindrance of the target binding $F_{ab}$ region, thereby limiting the number of binding interactions with the antigen resulting in a smaller sensor response. Use of protein G as an intermediate layer leads to oriented

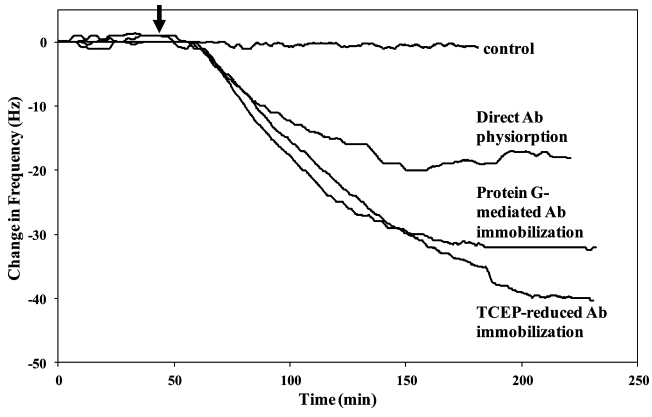


**Figure 5.** Comparison of sensor response to 1 mL of $10^7$/mL *E. coli* O157:H7 samples obtained with the three methods. The control experiment consisted of the cell sample exposed to sensor without antibody did not cause any change in the frequency. When direct physisorption of antibody was used for immobilization, the response was 17 Hz as compared to 29 Hz when protein G was used to oriented immobilization of antibody and 39 Hz when TCEP-reduced Ab was used for sensing.

immobilization of antibody molecules, but the extent of immobilization is dependent on the amount of protein G bound to and its orientation on the gold surface. Since only a fraction of the initial concentration of protein G introduced to


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the sensor surface binds, we believe that the total amount of oriented antibody immobilized on the sensor surface is lower. TCEP-reduced antibody, on the other hand, contains thiol groups that lead to oriented chemisorption of the half antibody fragments on the sensor surface that caused a larger shift in resonance frequency for binding to the same initial concentration of the target cells.

The above trend observed in sensor responses held true for all concentrations of the target cells investigated. A summary of the changes in frequency observed for the three concentrations for the three methods of antibody immobilization is shown in Figure 6. A linear regression fit of the data in Figure 4 lead to $y$

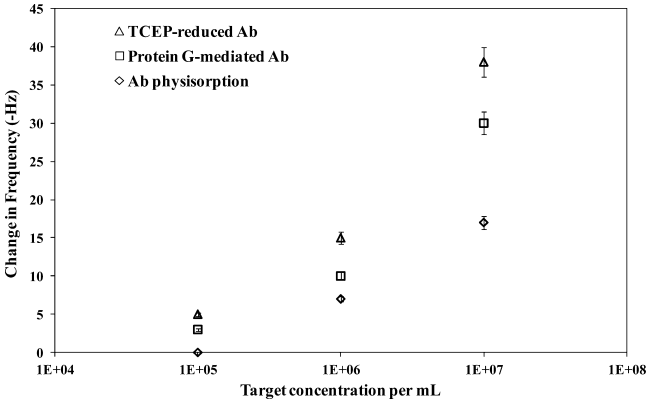


**Figure 6.** Comparison of responses to three different concentrations, $10^5$–$10^6$–$10^7$/mL of *E. coli* O157:H7. This comparison establishes that TCEP-reduction of antibody improves sensor response. A linear regression for the three methods of immobilization gave $R^2$ = 0.95, 0.97, and 0.90 for the TCEP-reduced method, Protein G mediated antibody, and direct antibody physisorption, respectively.

$= 3 \times 10^{-6}x + 8.17$ ($R^2 = 0.95$), $y = 3 \times 10^{-6}x + 5$ ($R^2 = 0.97$), and $y = 1 \times 10^{-6}x + 2.56$ ($R^2 = 0.90$), respectively, for TCEP-reduced antibodies, Protein G mediated antibody immobilization, and direct physisorption of whole antibodies. The slope for TCEP-reduced antibodies and Protein G mediated immobilization of antibody is comparable and is higher than that for direct antibody physisorption method. We can therefore conclude that TCEP-reduction of antibody does not interfere with the antigen binding property of the halved antibody fragments.

**Selectivity of TCEP-Reduced Antibodies.** The ability of antibody molecules to bind only to the specific targets has led to their widespread use for biosensing. To ensure that the proposed method of generating two half antibody fragments via TCEP-reduction is useful, it is important to show that their specificity is not compromised. We examine specificity by introducing spiked samples of *E. coli* O157:H7 with *E. coli* JM 101. To examine selectivity we used three ratios of the pathogenic target cells to nonpathogenic cells, 1:0, 1:1, and 1:10, where the concentration of the target cells was kept constant at $10^7$ cells/mL. Figure 7A compares the sensor responses for the three ratios of target to nontarget cells when TCEP-reduced antibody was used. For the pure sample of target *E. coli* O157:H7 cells, the observed frequency decrease was 38 ± 2 Hz ($n$ = 5). As the concentration of the nontarget cells increased to the ratios of 1:1 and 1:10, the frequency response decreased. For 1:1, the observed response was 25 ± 1 Hz ($n$ = 5) which was higher than that observed for 1:10 ratio of target to nontarget cells, 20 ± 1 Hz ($n$ = 5). The control

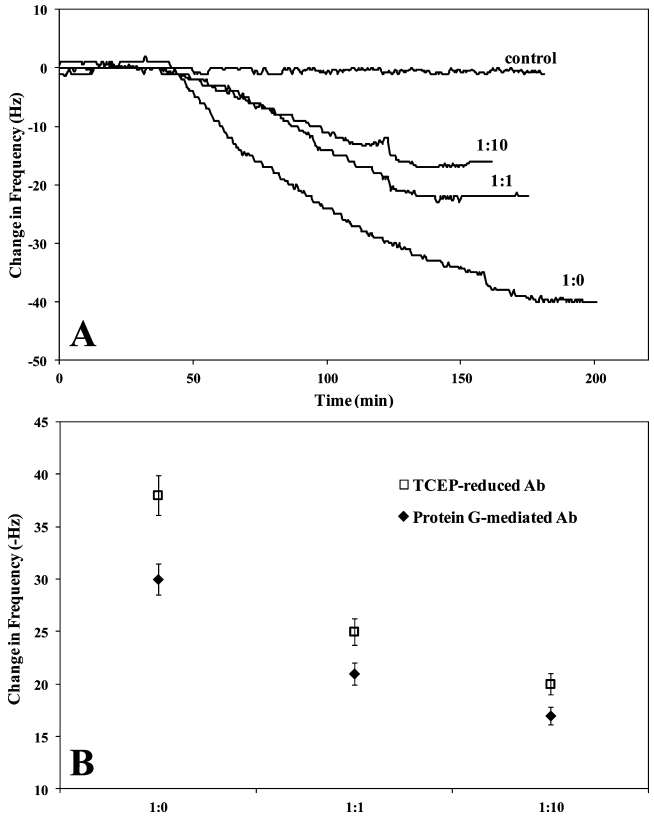


**Figure 7.** Selectivity of TCEP-reduced antibody. (Panel A) QCM responses to detection of *E. coli* O157:H7 mixed with nontarget *E. coli* JM101 at ratios of 1:0, 1:1, and 1:10 with the concentration *E. coli* O157:H7 kept constant at $10^7$cells/mL is shown. The response labeled as a control represents a response to 0:1, meaning the sample contained only JM101 at $10^7$ cells/mL. The second control (not shown) consisted of a bare sensor that was exposed to $10^7$ cells/mL *E. coli* O157:H7 that also gave a very small response (<2 Hz). (Panel B) Comparison of selectivity responses: Protein G mediated oriented immobilization vs TCEP-reduced immobilization. For protein G, mediated immobilization response reduction was 30% and 44% at 1:1 and 1:10, respectively, as compared to a 1:0 response. For TCEP-reduced antibody, the reductions were comparable, 35% and 48%, respectively.

response shown in Figure 7A is for nontarget *E. coli* JM101 ($10^7$/mL; 1 mL) which did not manifest any significant change in sensor resonance frequency.

We compared the sensor responses observed for TCEP-reduced antibody fragments against those observed for protein G mediated immobilized antibody. A summary chart for this comparison is shown in Figure 7B. For Protein G mediated antibody immobilization, the response to the ratio; 1:1, for target to nontarget cells was ∼30% lower compared to the pure sample with only target cells (1:0). The response was ∼44% lower in the presence of 10 times higher concentration of nontarget cells (1:10). For the TCEP-reduced antibody, the response was ∼35% lower for 1:1 and ∼48% lower when this ratio was 1:10, when compared to the pure sample response. The decrease in sensor response magnitude can be attributed to the interfering presence of nontarget cells that limit the binding interactions between the antigenic sites and the immobilized antibody. The control experiments response to 0:1, meaning the sample contained only JM101, gave no response as shown in Figure 6. Second control (not shown in figure) consisting of the bare sensor that was exposed to $10^7$ cells/mL *E. coli*


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O157:H7 also gave a response that was <2 Hz. We can, thus, conclude that the TCEP-reduction does not compromise the selectivity of antibody. It should be noted that the compromise in selectivity might be a function of the affinity of the antibody to its specific target. A poorly binding antibody might exhibit a larger loss in selectivity.

The TCEP-reduced antibody was shown to generate a larger sensor response for the same concentration of antigen as compared protein G mediated antibody immobilization without loss of selectivity in the presence of nontarget cells. The next step should be to examine the kinetics of antibody immobilization via the three methods to show the usefulness of the proposed method. In the following section we characterize the kinetics of the selectivity experiments in which the TCEP-reduced antibody fragments were used.

**Effect of Immobilization Method on Kinetics.** The observed rate constant of antigen binding has been shown to follow Langmuir kinetics.[5b,10] Antibody binding to the sensor by the three methods employed in this study can also be similarly modeled. Since the bulk concentration of the antibody introduced to the flow loop and that of *E. coli* O157:H7 is known initially, the rate analysis was limited to the initial time period. The observed rate constants were determined by analyzing the experimental data as per the following relationship,

$$\ln\left(\frac{(-\Delta f_\infty) - (-\Delta f)}{(-\Delta f_\infty)}\right) = -k_{obs}t \quad (1)$$

where $(-\Delta f)$ and $(-\Delta f_\infty)$ are the changes in frequency at time $t$ and at steady state, respectively.

For the antibody directly physisorbing on the sensor surface, fitting the experimental data gave an observed rate constant of 0.0094 $min^{-1}$ ($R^2$ = 0.91) while the rate constants obtained similarly for protein G mediated immobilization and TCEP-reduced antibody immobilization were 0.0135 $min^{-1}$ ($R^2$ = 0.92) and 0.0174 $min^{-1}$ ($R^2$ = 0.96), respectively.

The native thiol groups on TCEP-reduced antibody fragments directly and strongly bind to gold surfaces unlike physisorption of whole antibody. For the other antibody immobilization method, the amount and rate of immobilization strongly depends on the amount of protein G present on the sensor surface. We find the kinetics of TCEP-reduced antibody to be faster than the other methods investigated in this study.

Similar analysis of the sensor response for the pure sample of target *E. coli* O157:H7 binding to the sensor-bound TCEP-reduced antibody fragments gave a value of 0.0164 $min^{-1}$ ($R^2$ = 0.99). In the presence of nontarget cells (1:1 and 1:10), the observed rate constants were 0.0174 $min^{-1}$ ($R^2$ = 0.97) and 0.0166 $min^{-1}$ ($R^2$ = 0.92), respectively. In the presence of competing nontarget cells, only the magnitude of sensor response decreased whereas the kinetics of binding were comparable.

## CONCLUSION

Disulfide bridges between the light and heavy chains of antibodies were shown to be reduced by TCEP. The thiol groups on the half antibody fragments thus obtained cause direct, oriented immobilization on gold surfaces as measured by QCM. This method of antibody immobilization gave approximately twice the sensor response, for the same concentration of antigen, when compared to the conventional protein G mediated immobilization method. The property of these fragments to bind to target antigens is retained without loss of selectivity.

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### Notes

The authors declare no competing financial interest.

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