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Design and synthesis of an antigenic mimic of the Ebola glycoprotein

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Abstract

An antigenic mimic of the Ebola glycoprotein was synthesized and tested for its ability to be recognized by an anti-Ebola glycoprotein antibody. Epitope-mapping procedures yielded a suitable epitope that, when presented on the surface of a nanoparticle, forms a structure that is recognized by an antibody specific for the native protein. This mimic-antibody interaction has been quantitated through ELISA and QCM-based methods and yielded an affinity ($K_d = 12 \times 10^{-6}$ M) within two orders of magnitude of the reported affinity of the native Ebola glycoprotein for the same antibody. These results suggest that the rational design approach described herein is a suitable method for the further development of protein-based antigenic mimics with potential applications in vaccine development and sensor technology.

I. INTRODUCTION

The immunological interface that exists between an antigen and an antibody remains a paradigm for biological interfaces. It occurs as a highly specific and sensitive interaction ($K_d \sim 10^{-6}$ – 10^{-12}), despite being governed by an assortment of complex, noncovalent forces (i.e., electrostatics, hydrophobics, and hydrogen bonding).^{1,2} The binding observed between an antigen and an antibody is typically caused by the antibody's recognition of a specific amino acid sequence presented in a particular conformation by the antigen.³ Recapitulation of these binding characteristics using a synthetic antigen mimic can provide a new means for studying and manipulating this interface.

The design and synthesis of immunological mimics could prove themselves useful in a number of areas, including vaccine development and sensor technology. For example, current antigen detection strategies typically use biological simulants as a form of positive control for their detection device. However, in most cases, the simulant takes the form of an irradiated or killed form of the organism they are attempting to detect (for virus or bacteria-based detection). These techniques, despite their successful adaptation, have proven to be both expensive and potentially life-threatening, thus illustrating the need for cost-effective, safe alternatives. Nanoparticle-based synthetic simulants combine the ability to mimic the antigenicity of a biological species with the nontoxicity, as compared to the viral- and bacterial-based methods, and relatively low production cost desired for such a material.^{4–6}

Successful mimicry involves the determination of the antigenic epitope, the region responsible for antibody recognition, and the presentation of that epitope in a manner similar to that of the sequence's native conformation. In this study, we describe our efforts in mimicking the antigenicity of the Ebola glycoprotein. Specifically, we have mapped an epitope region of the

glycoprotein based on the 15H10 monoclonal antibody previously shown to bind the native protein. This epitope was then presented in a fashion that allowed 15H10 to recognize and bind the complex with an affinity similar to that observed with native glycoprotein. Although our efforts were focused specifically on this one antigen, we believe this approach to be applicable to most protein-based antigens.

II. EXPERIMENTAL

A. Peptide synthesis and characterization

All of the peptides were synthesized via continuous flow fmoc solid-phase peptide synthesis methods on an Apex peptide synthesizer (Advanced Chemtech, Louisville, KY), purified by reversed-phase HPLC, and lyophilized as previously described.⁷ Identification of the peptides was confirmed by MALDI mass spectrometry.

B. Enzyme-linked immunosorbent assays (ELISAs)

ELISAs were performed in 96-well Immulon-coated plates (Thermo Electron Corp., Milford, MA). For epitope mapping, all peptides were dissolved in 50 mM carbonate buffer and added to the wells at a concentration of 6.4 μ M. These peptides were incubated for 1 h at 37 °C. Three washes were performed after this and each subsequent step using phosphate-buffered saline (PBS) containing 0.1% Tween-20. The wells were then blocked using 300 μ L of BSA solution (10%) for 2 h at 25 °C. Murine monoclonal 15H10 antibody was received as a gift via SERCEB and was isolated and purified as described previously.⁸ 15H10 antibody was added as the primary antibody at 7 μ g/mL and incubated for 1 h at 37 °C. Samples were treated with goat anti-mouse horseradish peroxidase secondary antibodies (diluted 1:5000 in PBS) and were allowed to incubate for 1 h at 37 °C. After addition of TMB substrate, the enzymatic reaction was quenched using 1 M H₂SO₄. The wells were then measured spectrophotometrically at 450 nm. Each assay was performed in triplicate. Ebola Zaire glycoprotein, received as a SERCEB gift and isolated and purified as described previously, and carbonate buffer were used as a positive and negative control, respectively.⁸

C. Monolayer-protected cluster (MPC) synthesis and characterization

Tiopronin-protected gold nanoclusters were synthesized as previously described by Templeton et al.⁹ Briefly, auric acid (2.7 mmol) was dissolved in an 85/15 solution of methanol/glacial acetic acid. *N*-(2-Mercaptopropionyl)glycine (C₅H₉NO₃S), also known as tiopronin (8.1 mmol), was added in 3-fold excess to the auric acid. Sodium borohydride (54 mmol) was then slowly added in 20-fold excess to reduce the gold. After allowing the solution to stir for 30 min, the methanol was removed under vacuum. The pH was adjusted to approximately 1.0, and the clusters were then purified by dialysis. Dialysis was performed with cellulose ester tubing with a molecular weight cut-off of 10,000. The dialysis tubing was added to 4-L of water and was allowed to stir for 3 days. The water was exchanged approximately every 12 h over the course of a 3-day period. The MPC's were then dried under ambient conditions and characterized by transmission electron microscopy (TEM), nuclear magnetic resonance spectroscopy (NMR), and thermogravimetric analysis (TGA).

D. Place exchange reaction

All place-exchange reactions were carried out as previously described by Hostetler et al.¹⁰ In a typical exchange, Au-tiopronin MPC's were dissolved in a 2 mg/mL solution of deionized water. Peptides previously dissolved in 500 μ L of deionized water were then added to the MPC solution in an approximate 7:1 tiopronin ligand-to-peptide ratio. This solution was allowed to stir at room temperature for 3 days before purification by dialysis as described above. The

extent of exchange was determined by analyzing the NMR integration of amino acid peaks from the peptide compared with integration of tiopronin peaks.

E. Determination of antigen-antibody binding by quartz crystal microbalance (QCM)

A typical QCM experiment was performed under continuous flow and focused on immobilizing the antibody while flowing across a nanocluster solution to achieve binding. Each experiment involved the use of a 5-MHz quartz crystal with gold electrodes plated on both sides. Each crystal had been previously cleaned with a solution of piranha (3:1 H₂SO₄/30% H₂O₂). After allowing the system to equilibrate in phosphate buffer for at least 1 h, the immobilization component (Protein A) was allowed to flow over the crystal until a maximal amount of binding had occurred. A 10-min phosphate buffer equilibration step was performed after this and each subsequent step. Bovine serum albumin was then added to the system for blocking purposes. Monoclonal 15H10 antibody was then added and allowed to equilibrate while binding to the Protein A followed by addition of nanocluster mimic solution for 5 min while binding was detected.

III. RESULTS AND DISCUSSION

The Ebola glycoprotein is a highly glycosylated trans-membrane protein thought to be responsible for both receptor binding and fusion of the virus with host cells.¹¹ It is the only protein expressed on the surface of the virion, and it exists in multiple forms.¹² The first form is post-translationally modified by proteolytic processing into two subunits, the N-terminal GP₁ and C-terminal GP₂, which are subsequently linked by disulfide bonds. The second form is an unedited form of GP₁; sGP, which is secreted outside the virus, and it differs from GP₁ in its last 69 a.a.^{11, 13} The version of the GP that remains with the virus forms spikes on the surface that mediate viral entry into cells.^{11, 13, 14} It is this 676-a.a. form of the glycoprotein that is the focus of this study.

The synthesis of an antigenic mimic can be thought of as a 2-fold process: epitope determination and epitope presentation. Successful mimicry of a protein's antigenicity occurs when the amino acid sequence responsible for binding the protein to an antibody is presented in a fashion similar to that found in the native structure. Oftentimes a crystal structure of the protein antigen can aid in the determination of a binding region. It is in this fashion that Gerdon et al. were able to determine an epitope of the anthrax toxin, *Bacillus anthracis*.⁶ However, in the absence of such a structure, epitope-mapping procedures can be used. Common methods that were used to explore the binding between a particular amino acid sequence and an antibody includes NMR, mass spectrometry, and surface plasmon resonance spectroscopy (SPR).^{15–17} However, our method involves ELISAs performed on potential antigenic regions.

Possible antigenic regions for the Ebola glycoprotein have previously been reported.⁸ Three principle regions were reported to have a high probability of possible antigenic activity: an N-terminal region, STDQLKSVGLNLEGSGVSTOIPSATKRWGFRSG (a.a. 59–91); a mid-peptide region, DDDAASSRITKGRISDRATRKYSIDLVPKNSPG (a.a. 318–349); and a C-terminal region, EPHDWTKNITDKINQIIHDFIDNPLPNQDNDD (a.a. 611–642). Yu et al. examined the antigenicity of these peptides.⁸ They found that antibodies raised against the C-terminal peptide reacted with the native glycoproteins from both Ebola subtypes they studied (the Zaire and Sudan-Gulu subtypes). Antibodies raised against the N-terminal and mid-region peptides failed to react with the Ebola Zaire subtype. The degree of reactivity of the C-terminal region suggested it as an epitope foundation for the design of an Ebola mimic that would effectively represent two different Ebola subtypes (Zaire and Sudan-Gulu).

In an effort to establish the specific amino acids responsible for binding 15H10, overlapping 10 a.a. peptides were determined by sequentially shifting 1 a.a. at a time to create 23 different

peptides. The ability of the antibody to recognize and bind each peptide was probed via ELISA. The signal from the assay is proportional to the degree of binding exhibited between the peptide and antibody. The results from this binding assay are shown in Fig. 1.

The highest region of binding was shown to exist near the C terminus of the sequence (a.a. 623–639), centered around one 3-a.a. sequence (F-I-D). A second fragment located near the N terminus (a.a. 614–629) showed binding (~ 0.25 – 0.27), although not as significant as the aforementioned C-terminal region. These peptides all contained a common T-D-K motif, separate from the aforementioned F-I-D pattern. The fact that binding occurred separately in two different regions suggests that the peptides contain partial epitopes and that both sequences (TDK and FID) might be responsible for complete binding of the antibody. This type of epitope is commonly referred to as a discontinuous epitope. Such epitopes are composed of discontinuous sequences of amino acids brought in a close proximity due to protein folding.³ Consequently, in an effort to construct an epitope inclusive of the major binding regions, longer peptides (16–17 a.a.) were synthesized; a 17-mer centered around the highest observed region of binding [(i) INQIIHDFIDNPLPNQD] and two 16-mers [(ii) TDKINQIIHDFIDNPL and (iii) IHDFIDNPLPNQDNDD] shifted to both sides of this potential epitope. Analysis of the expanded epitopes by ELISA showed an increased recognition and binding by the monoclonal antibody relative to their shorter counterparts (Fig. 2), highlighting the suitability of these peptides as epitopes for use in the design of an Ebola glycoprotein mimic.

For successful mimicry, the peptide epitope sequence (the primary structure of the peptide) must be presented in a manner that allows the peptide to adopt a conformation (secondary and tertiary structure) similar to the one adopted by the native protein. Because that protein structure is often difficult to predict and therefore practically impossible to purposefully mimic, a presentation method that limits the conformational freedom of the peptide epitope would go a long way towards achieving this goal. A method that provides the added dimension of multivalency would also be desirable to increase the degree of interaction between the epitope and the antibody. A few common approaches for introducing multivalency into a presentation method include the use of linear multimeric peptides, multiple-antigenic peptides (MAPs), and the use of nanoparticles.^{18–20}

Nanoparticles, in particular, have proven to be a viable presentation scaffold, capable of providing a means of recapitulating a three-dimensional structure through covalent attachment of the peptide epitope.^{4–6,21,22} Tiopronin-protected gold nanoclusters were used as a presentation scaffold for our Ebola glycoprotein mimic system. The tiopronin ligands act as stabilizing units for the nanoparticles, preventing aggregation of the particles and providing enhanced water solubility, thus allowing their integration with biosystems. Synthesis of the particles by literature methods produced a monodisperse batch of particles (3.20 ± 0.65 nm), and thermogravimetric analysis confirmed an organic composition of 20.7%.⁹ The average particle composition was calculated to be approximately $\text{Au}_{616}\text{Tiopronin}_{195}$ (MW $\sim 152,962$ Da). The monodispersity exhibited by the nanoparticles is necessary to ensure uniformity of ligand coverage on the mimic.

Peptides are functionalized onto the surface of these nanoparticles via “place-exchange reactions.”¹⁰ These well-defined reactions occur when water-soluble thiolate protected gold nanoclusters are exposed to another thiol-containing molecule, at which point the molecule exchanges with one of the tiopronin ligands.^{10,23} Each of the peptide epitopes were synthesized with an N-terminal cysteine residue to serve as the incoming thiol functionality, thus allowing an attachment point for the peptide to the nanoparticle. After place exchange, the resulting particles were characterized by NMR to determine the degree of functionalization. Place exchange involving a 5:1 tiopronin to incoming peptide ratio resulted in approximate final average compositions of $\text{Au}_{616}\text{Tiopronin}_{183}\text{Ebola-1}_{12}$ (MW $\sim 176,327$ Da),

Au₆₁₆Tiopronin₁₇₁Ebola-2₂₄ (MW ~ 197,052 Da), and Au₆₁₆Tiopronin₁₈₃Ebola-3₁₃ (MW ~ 176,660 Da) for the three separate epitopes.

Multiple analytical techniques are currently available to quantitate the interaction between an antigen and antibody, including Western blot analysis, immunofluorescence analysis, and surface plasmon resonance spectroscopy.^{24–26} This study relied upon a QCM to quantitatively determine the binding between the monoclonal antibody and our nanocluster mimic. QCM operates via an oscillating quartz crystal that measures mass adsorption due to a change in oscillation frequency. The QCM chip was prepared by adsorbing an antibody immobilization molecule, Protein A, to the gold-plated surface of the quartz crystal. After immobilization of the antibody to the Protein A, the antigenic mimic is introduced to the system (Fig. 3). QCM offers sensitivity with respect to multiple layer adsorption that is often difficult to meet with other immunological techniques, because nanogram levels of binding are detectable through multiple layers. This sensitivity, coupled with its simplicity of operation, makes QCM an advantageous technique for quantitatively measuring these types of interactions.

Initial binding studies resulted in some nonspecific binding problems for two of the potential mimics (Ebola-1 and Ebola-2), rendering them ineffective mimics. Of greater concern, however, was the complete lack of binding exhibited between 15H10 and the Ebola-3 mimic, a matter that forced the re-evaluation of the presentation method. This lack of interaction could be due to steric hindrance induced by the combination of the nano-particle and peptide conformation. The epitope of interest contains two proline residues in the middle of the sequence that probably produce a kink in the structure that renders crucial portions of the epitope (FID) inaccessible when functionalized on a nanoparticle. To account for this problem, a polyethylene glycol (PEG) linker was inserted between the peptide epitope and terminal cysteine anchor. The linker, consisting of six PEG units (PEG₆), was designed to project the peptide beyond the tiopronin cloud and away from the surface of the nanoparticle. Functionalization of the PEG-ylated epitope onto the surface of a nanoparticle introduces a new found accessibility that enhances interaction with an antibody, and it does so in a way that maintains the stabilization necessary to limit the conformational freedom of the peptide.

QCM experiments confirmed the successful recognition of the Ebola-3-PEG nanocluster complex by the monoclonal antibody (Fig. 4). The control nonfunctionalized tiopronin-protected nanoparticles did not bind to the antibody-functionalized QCM chip [Fig. 5(a)]. To ensure the specificity of the interaction, two other control experiments were performed, the first involved a different non-Ebola antibody (anti-hemagglutinin) and the Ebola-PEG-3 cluster [Fig. 5(b)], and the second involved the 15H10 anti-Ebola glycoprotein antibody and a cluster functionalized with a PEG-ylated sequence from the Respiratory Syncytial Virus F protein [Fig. 5(c)]. Both controls failed to produce any significant binding. An initial antibody concentration of 300 nM and cluster concentration of 14.4 μ M gave approximately 200 ng worth of nanocluster mimic binding. This binding confirms the success of the linker molecule in allowing accessibility of the antibody to the entire epitope structure. As a result, this type of rational approach should be applied to all future nanoparticle-based mimics in which the epitope region finds itself in close proximity to the nanoparticle core.

To act as an effective simulant, the affinity exhibited between the mimic and the antibody must be similar to that of the native protein. Determination of the equilibrium affinity constant, K_d , allows for such a comparison. Yu et al. reported a K_d value of 0.10 ± 0.01 μ M as a measure of the affinity between the 15H10 antibody and the native Ebola glycoprotein as measured by two independent techniques (QCM and SPR).⁸ The equilibrium constant for our mimic system was determined via concentration-dependent binding studies as described previously.⁴ The total mass of nanocluster bound to antibody after 5 min was determined and fit to a logarithmic curve (Fig. 6). The total mass begins to reach a constant value at the higher concentrations,

indicating the formation of a monolayer as all the antibody binding site become occupied. When fit to a Langmuir adsorption isotherm as described previously, a K_d of 20×10^{-6} M was obtained (Fig. 7).⁴ Adsorption constants can also be calculated via a kinetic method. By determining the kinetic forward (k_f) and reverse (k_r) rates obtained from time-dependent binding curves (Fig. 8), K_d can be derived. These values ($k_f = 7.3 \times 10^1 \text{ M}^{-1}\text{s}^{-1}$; $k_r = 4.0 \times 10^{-4} \text{ s}^{-1}$) produced a K_d value for the Ebola-3-PEG/15H10 monoclonal antibody system of 5.5×10^{-6} M.

The average K_d from the two methods (Langmuir isotherm and kinetic methods), as calculated from the reported native glycoprotein data, is $0.180 \mu\text{M}$.⁸ The average value for our nanocluster mimic ($12 \mu\text{M}$) falls within two orders of magnitude of this value. The lower value indicates a greater affinity of the native protein for the antibody compared with the mimic. A peptide mimic should be expected to bind with the same affinity as the native antigen. Gerdon et al. reported a K_d value for a peptide-functionalized nanoparticle-based mimic of the protective antigen of *B. anthracis* within one order of magnitude of the value obtained for the native protective antigen.⁶ Peptide mimics have also been reported, which show increased affinity compared with their native counterparts. Simmons et al. have reported on a peptide mimic of the *Plasmodium falciparum* apical membrane antigen-1 (AMA-1) that shows an increased affinity of two orders of magnitude compared with the AMA-1 antigen.²⁷ However, it should be noted that the difference observed in the case of the Ebola-3 mimic was somewhat expected because it lacked the secondary TDK motif, which showed binding in ELISA studies. This decrease in binding can probably be partially attributed to this idea that the epitope, as constructed, remains incomplete. Further investigation will center around the Ebola-2 sequence, which contained both the TDK and the FID sequences we observed as preferential for binding 15H10 and novel strategies for the presentation of a discontinuous epitope.

IV. CONCLUSIONS

A nanocluster assembly has been synthesized that was shown to be recognized and bound by the anti-Ebola glycoprotein antibody, 15H10. Control experiments show this binding to be specific for the exposed sequence when presented on a nanoparticle. QCM detection of this binding produced an average K_d of 12×10^{-6} M when calculated by two different methods. This value falls within two orders of magnitude of the reported native glycoprotein K_d (0.10×10^{-6} M). Our reported kinetic rate constants of (forward rate constant, $k_f = 7.3 \times 10^1 \text{ M}^{-1}\text{s}^{-1}$; reverse rate constant, $k_r = 4.0 \times 10^{-4} \text{ s}^{-1}$) are within a similar value. We are currently examining ways to enhance the interaction between the antibody and mimic, resulting in a higher level of binding consistent with what is reported for the native glycoprotein. One such approach involves the re-examination of how the epitope is presented on the nanoparticle surface. Methods such as bidentate attachment of the peptide and binary attachment of the desired FID and TDK sequences are being considered as possible alternatives. However, the rational design approach described here has provided a road map for future mimicry studies involving protein-based antigens.

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REFERENCES

1. Webster DM, Henry AH, Rees AR. Antibody-antigen interactions. *Curr. Opin. Struct. Biol* 1994;4:123.
2. Davies DR, Cohen GH. Interactions of protein antigens with antibodies. *Proc. Nat. Acad. Sci. U.S.A* 1996;93:7.

3. Regenmortel MHVV. The concept and operational definition of protein epitopes. *Philos. Trans. R. Soc. London, Ser. B* 1989;323:451. [PubMed: 2474169]
4. Gerdon AE, Wright DW, Cliffl DE. Quartz crystal microbalance detection of glutathione-protected nanoclusters using antibody recognition. *Anal. Chem* 2005;77:304. [PubMed: 15623309]
5. Gerdon AE, Wright DW, Cliffl DE. Hemagglutinin linear epitope presentation on monolayer-protected cluster elicits strong antibody binding. *Biomacromolecules* 2005;6:3419. [PubMed: 16283774]
6. Gerdon AE, Wright DW, Cliffl DE. Epitope mapping of the protective antigen of *B. Anthracis* by using nanoclusters presenting conformational peptide epitopes. *Angew. Chem. Int. Ed* 2006;45:594.
7. Spreitzer G, Whitting JM, Madura JD, Wright DW. Peptide-encapsulated CdS nanoclusters from a combinatorial ligand library. *Chem. Commun. (Camb.)* 2000;209
8. Yu J-S, Liao H-X, Gerdon AE, Huffman B, Searce RM, McAdams M, Alam SM, Popernack PM, Sullivan NJ, Wright D, Cliffl DE, Nabel GJ, Haynes BF. Detection of Ebola virus envelope using monoclonal and polyclonal antibodies in ELISA, surface plasmon resonance and a quartz crystal microbalance immunosensor. *J. Virol. Methods* 2006;137:219. [PubMed: 16857271]
9. Templeton AC, Chen S, Gross SM, Murray RW. Water-soluble, isolable gold clusters protected by tiopronin and coenzyme A monolayers. *Langmuir* 1999;15:66.
10. Hostetler MJ, Templeton AC, Murray RW. Dynamics of place-exchange reactions on monolayer-protected gold cluster molecules. *Langmuir* 1999;15:3782.
11. Wilson JA, Hevey M, Bakken R, Guest S, Bray M, Schmaljohn AL, Hart MK. Epitopes involved in antibody-mediated protection from Ebola virus. *Science* 2000;287:1664. [PubMed: 10698744]
12. Jasenosky LD, Kawaoka Y. Filovirus budding. *Virus Res* 2004;106:181. [PubMed: 15567496]
13. Malashkevich VN, Schneider BJ, McNally ML, Milhollen MA, Pang JX, Kim PS. Core structure of the envelope glycoprotein GP2 from Ebola virus at 1.9 Å resolution. *Proc. Nat. Acad. Sci. U.S.A* 1999;96:2662.
14. Heinz Feldmann SJ, Klenk H-D, Schnittler H-J. Ebola virus: From discovery to vaccine. *Nat. Rev. Immunol* 2003;3:677. [PubMed: 12974482]
15. Naik MT, Chang C-F, Kuo I-C, Kung CC-H, Yi F-C, Chua K-Y, Huang T-H. Roles of structure and structural dynamics in the antibody recognition of the allergen proteins: An NMR study on *blomia tropicalis* major allergen. *Structure* 2008;16:125. [PubMed: 18184590]
16. Stefanescu R, Iacob RE, Damoc EN, Marquardt A, Amstalden E, Manea M, Perdivara I, Maftai M, Paraschiv G, Przybylski M. Mass spectrometric approaches for elucidation of antigen-antibody recognition structures in molecular immunology. *Eur. J. Mass Spectrom* 2007;13:69.
17. Lanstra JMPDL, Berg CWVD, Bullido R, Almazan F, Dominguez J, Llanes D, Morgan BP. Epitope mapping of 10 monoclonal antibodies against the pig analogue of human membrane cofactor protein (MCP). *Immunology* 1999;96:663. [PubMed: 10233756]
18. Ye Y, Bloch S, Xu B, Achilefu S. Design, synthesis, and evaluation of near infrared fluorescent multimeric RGD peptides for targeting tumors. *J. Med. Chem* 2006;49:2268. [PubMed: 16570923]
19. Ziegler J, Chang RT, Wright DW. Multiple-antigenic peptides of histidine-rich protein II of *Plasmodium falciparum*: Dendrimeric biomineralization templates. *J. Am. Chem. Soc* 1999;121:2395.
20. Slocik JM, Moore JT, Wright DW. Monoclonal antibody recognition of histidine-rich peptide encapsulated nanoclusters. *Nano Lett* 2002;2:169.
21. Verma A, Rotello VM. Surface recognition of biomacromolecules using nanoparticle receptors. *Chem. Commun. (Camb.)* 2005;303
22. De M, You C-C, Srivastava S, Rotello VM. Biomimetic interactions of proteins with functionalized nanoparticles: A thermodynamic study. *J. Am. Chem. Soc* 2007;129:10747. [PubMed: 17672456]
23. Song Y, Murray RW. Dynamics and extent of ligand exchange depend on electronic charge of metal nanoparticles. *J. Am. Chem. Soc* 2002;124:7096. [PubMed: 12059234]
24. Helg A, Mueller MS, Joss A, Poltl-Frank F, Stuart F, Robinson JA, Pluschke G. Comparison of analytical methods for the evaluation of antibody responses against eptiopes of polymorphic protein antigens. *J. Immunol. Methods* 2003;276:19. [PubMed: 12738356]

25. Lyon LA, Musick MD, Natan MJ. Colloidal Au-enhanced surface plasmon resonance immunosensing. *Anal. Chem* 1998;70:5177. [PubMed: 9868916]
26. Wegner GJ, Lee HJ, Corn RM. Characterization and optimization of peptide arrays for the study of epitope-antibody in teractions using surface plasmon resonance imaging. *Anal. Chem* 2002;74:5161. [PubMed: 12403566]
27. Simmons DP, Streltsov VA, Dolezal O, Hudson PJ, Coley AM, Foley M, Proll DF, Nuttall SD. Shark IgNAR antibody mimotopes target a murine immunoglobulin through extended CDR3 loop structures. *Pro. Struct. Fund. Bioinform* 2008;71:119.

Appendix

APPENDIX: DESIGN AND SYNTHESIS OF AN IMMUNOLOGICAL MIMIC OF THE EBOLA GLYCOPROTEIN

FIG. A1. TEM image of Au-tiopronin nanoparticles.

FIG. A2. Histogram of Au-tiopronin particles.

FIG. A3. NMR of Au-tiopronin particles.

FIG. A4. Thermogravimetric analysis of Au-tiopronin particles.

FIG. A5. NMR of AuTioproninEbola-3 nanoparticles after Ebola-3 place exchange.

FIG. 1.
ELISA results for overlapping Ebola glycoprotein C-terminal peptides highlighting the specific sequences responsible for inducing antibody recognition and binding.

FIG. 2.
ELISA results of potential Ebola epitopes indicate the suitability for the use of these peptides in further mimicry studies.

FIG. 3.
Layer-by-layer assembly demonstrated by the QCM method.

FIG. 4. QCM experiment showing approximately 200 ng of Ebola-3-PEG nanocluster.

FIG. 5.

QCM control experiments: (a) Au-tiopronin particles and 15H10 antibody, (b) Ebola-PEG-3 nanocluster and anti-hemagglutinin antibody, and (c) RSV F-protein nanocluster and 15H10 antibody. All three show no significant binding.

FIG. 6. Logarithmic fit. Δm plotted versus [Ebola-3-PEG] showing immunosensor saturation at increasing concentrations.

FIG. 7. Langmuir adsorption isotherm fit. Plotting Δm versus $\Delta m/[\text{Ebola-3-PEG}]$ produces a linear plot, the slope of which allows for the calculation of $K_d = 20 \times 10^{-6}$ M.

FIG. 8.

Kinetic plot. Plotting the time constant, τ , versus [Ebola-3-PEG] allows for the determination of k_f and k_r . $k_r/k_f = K_d = 5.5 \times 10^{-6}$ M.