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DARPA ANTIBODY TECHNOLOGY PROGRAM

STANDARDIZED TEST BED FOR ANTIBODY CHARACTERIZATION: CHARACTERIZATION OF AN MS2 SCFV ANTIBODY PRODUCED BY STABLEBODY TECHNOLOGIES

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The selection of antibodies for inclusion in a final assay format has primarily relied on antibody performance in enzyme-linked immunosorbent assays (ELISAs), with little regard for quantification of the full spectrum of variables affecting antibody–antigen interactions. The Joint Product Management Office for Biosurveillance (JPMO BSV) Defense Biological Product Assurance Office (formerly the Critical Reagents Program) has instituted a quality program for test method standardization to support more comprehensive characterization and comparison of the physical and functional properties of antibody reagents within its repository. Development and standardization of antibody testing provides JPMO BSV with an invaluable platform for the provision of consistent, high-quality assays and reagents for current biodetection platforms as well as the development and validation of future systems. This platform will be used to characterize and evaluate the MS2 recombinant antibody produced and supplied by the Defense Advanced Research Project Agency-funded investigator, Stablebody Technologies, LLC (Madison, WI) for affinity and stability enhancements. The study provides standardized parametric data on antibody properties and performance and contributes to the development of a decisional analysis tool for use in expanding the confidence level for selection of antibody-based reagents that will optimize field operational and performance metrics for future detection and diagnostic platforms.

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PREFACE

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DARPA ANTIBODY TECHNOLOGY PROGRAM

STANDARDIZED TEST BED FOR ANTIBODY CHARACTERIZATION: CHARACTERIZATION OF AN MS2 SCFV ANTIBODY PRODUCED BY STABLEBODY TECHNOLOGIES

1. INTRODUCTION

Current platforms for detection and diagnosis of biothreat agent exposure depend on the use of antibodies to recognize and bind to specific antigens. To date, the selection of antibodies for inclusion in a final assay format has primarily relied on an antibody's performance in an enzyme-linked immunosorbent assay (ELISA), with little regard for quantification of the full spectrum of variables affecting antibody–antigen interactions The Joint Product Management Office for Biosurveillance (JPMO BSV) Defense Biological Product Assurance Office (DBPAO; formerly the Critical Reagents Program) instituted a quality program for the standardization of test methods to more fully characterize and compare the physical and functional properties of antibody reagents in its repository. The development and standardization of antibody testing provides the JPMO BSV with an invaluable method for obtaining consistent, high-quality assays and reagents for existing biodetection platforms and also for the development and validation of future systems. This platform will be used to characterize the MS2 single-chain variable fragment (scFv) antibody produced at Stablebody Technologies (Madison, WI) for the Defense Advanced Research Projects Agency (DARPA; Arlington, VA) Antibody Technology Program (ATP).

The DARPA ATP focuses on developing technologies for enhancing the thermal stability and binding affinity of a given antibody. Functioning as an independent testing laboratory for this program, the U.S. Army Edgewood Chemical Biological Center (ECBC; Aberdeen Proving Ground, MD) has provided specific technical support on immune reagents and defined the government-supplied antibody–antigen pairs. The goal of this project was twofold: (a) select, develop, and standardize the methods for characterizing the de novo thermal and binding properties of select reagents to be used by DARPA-funded investigators; and (b) use those methods to validate the changes in antibody thermal stability and binding affinity that were achieved by the DARPA investigators. The antibody chosen for this project was the MS2 recombinant scFv produced at ECBC (*1*), which detects an MS2 coat protein (MS2CP) that forms the capsid for the MS2 bacteriophage. The focus of the work highlighted in this report is the evaluation of two MS2 antibodies supplied by the DARPA-funded investigator, Stablebody Technologies, for either affinity or stability enhancements. The results of this study provide standardized parametric data on antibody properties and performance. This information will also contribute to the development of a decisional analysis tool that will expand the confidence levels for the selection of antibody-based reagents and thereby optimize the field operational and performance metrics of future detection and diagnostic platforms.

2. MATERIALS AND METHODS

2.1 MS2 scFv and MS2CP

MS2 scFv antibody was produced from a plasmid supplied by Ellen Goldman (U.S. Naval Research Laboratory; Bethesda, MD). The plasmid was designated Gv1, and the sequence was cloned into a pET-22b(+) plasmid (EMD Millipore; Billerica, MA). The protein was produced and eluted in 20 mM sodium phosphate (pH 8.0), 0.5 M sodium chloride, and 0.5 M imidazole. Peak fractions were then collected and separated on a 16/60 Superdex 200 gel filtration column (GE Healthcare Life Sciences; Pittsburgh, PA), and fractions that corresponded with a monomeric protein were collected and flash-frozen in liquid nitrogen. These fractions were provided (along with sequence data) to Stablebody Technologies as baseline material for modification.

The MS2CP was produced from a pET-28a(+) plasmid (Novagen; Madison, WI). The MS2CP sequence was inserted with an amino acid substitution of an arginine at position 83 in a construct engineered by DNA2.0 (Menlo Park, CA). MS2CP was produced and eluted with 300 mM imidazole in pH 7.4 phosphate-buffered saline (PBS; Sigma-Aldrich Company; St. Louis, MO). Peak fractions were collected, and buffer was exchanged into PBS (pH 7.4) using a 470 mL packed volume of Sephadex G-25 fine gel chromatography media (Amersham Biosciences Corporation; Piscataway, NJ). The fractions were provided to Stablebody Technologies as antigen for the MS2 antibody.

2.2 Ultraviolet–Visible (UV–Vis) Spectrophotometry

A NanoDrop ND-1000 spectrophotometer (Thermo Scientific; Waltham, MA) was used to determine the MS2 scFv concentrations and the absorbance of light at 280 nm (A_{280}) for the samples supplied by Stablebody Technologies. The A_{280} value is influenced by the number of tryptophan and tyrosine residues in a given protein. For this reason, the extinction coefficient is used in conjunction with the A_{280} value to determine an accurate concentration. The MS2 scFv concentrations were determined by dividing the average A_{280} value by 1.77, which is the extinction coefficient for a scFv. Each reading required a 2 µL sample, which was placed on the sample pedestal. The arm of the instrument was lowered, creating a liquid column between the top of the arm and the surface of the pedestal; this was the path length through which the laser passed. The instrument was blanked using PBS, and readings were taken in triplicate. A positive control, bovine γ-globulin (BGG; Bio-Rad; Hercules, CA), was also tested to validate the instrument operation.

2.3 Electrophoresis

Molecular weight and purity data were collected with an Experion automated electrophoresis system (Bio-Rad). The Experion system employs microfluidic technology to automate electrophoresis for protein analysis. The microfluidic chip, in conjunction with the Experion reagents, electrophoresis station, and software, are designed to accomplish separation, staining, destaining, detection, and basic data analysis. The Experion Pro260 analysis kit uses engineered lower and upper internal alignment markers to provide clean baselines, accurate

molecular weight sizing, and quantitative protein analysis (*2*). The Pro260 analytical software also determines sample purity by calculating the percent mass of the separated proteins in a sample. For Experion analysis, each Stablebody Technologies MS2 scFv was standardized to a final concentration of 1 mg/mL by diluting it in PBS. The BGG control and the Stablebody Technologies samples were then processed using a validated procedure as specified in the Bio-Rad Experion Pro260 analysis kit, rev. C (*3*). Briefly, a Pro260 microfluidic chip was prepared by adding 12 µL of Pro260 gel and gel stain to the designated wells. The chip was then placed on the priming station and primed for 1 min at the medium (B) pressure setting. Priming filled the fluidic channels with gel, which was used by the instrument to form a barrier between samples during the run. The sample was reduced with dithiothrietol (Sigma-Aldrich) and denatured in the kit-provided sample buffer at 95 °C before it was applied to the primed chip. The chip was then placed in the instrument, and the lid was closed, lowering the sample needles into the wells. The instrument was operated via the Experion software; each chip took 30 min to complete. All samples were run in triplicate alongside one sample of the BGG control and the Pro260 ladder. All analysis was performed using the Experion software.

2.4 Dynamic Light Scattering (DLS)

DLS was used to paint a picture of how the proteins behaved in solution. DLS data indicates whether a protein is in solution by measuring the polydispersity, hydrodynamic radius, and molecular weight of a sample. Prediction algorithms within the software produce a range of values for the protein under evaluation. For DLS analysis, five 20 µL aliquots of the Stablebody Technologies MS2 scFvs along with the control bovine serum albumin (Sigma-Aldrich) were placed into a quartz 384-well plate (Wyatt Technology Corporation; Santa Barbara, CA) and centrifuged for 2 min at 239 ×*g* to remove trapped air bubbles from the samples. Mineral oil (Sigma-Aldrich) was applied to the top of each sample to prevent sample evaporation. The plate was placed into a DynaPro temperature-controlled plate reader (Wyatt Technology). Each well was scanned 10 times for 5 s each at 25 °C. Values were averaged to provide measurements of polydispersity, hydrodynamic radius, percent mass, and molecular weight for each sample using Dynamics software (Wyatt Technology). The results of three wells were averaged and reported.

2.5 Thermal Stress Test

Before heat was applied, all samples were diluted to 1 mg/mL to negate any protective effects that may have been due to concentration *(2*). The Stablebody Technologies thermally stabilized antibody (ANLStab 006) was diluted to 1 mg/mL in 1× PBS and divided into five tubes. One aliquot was kept on ice for the duration of the experiment and was marked time 0. The remaining four aliquots were heated to 70 °C on a calibrated heat block for 15, 30, 45, and 60 min each. After each time point, the corresponding aliquot was removed and placed in an ice bath. These samples were then tested for activity.

2.6 ELISA

ELISAs were performed in triplicate using standard techniques. After the thermal stress test, each sample was diluted to 1 µg/mL in PBS and used to coat one row each of three Nunc MaxiSorp 96-well plates (Thermo Scientific). The plates were incubated at 4 °C overnight.

In the morning, each plate was washed in 1× wash buffer (KPL; Gaithersburg, MD) using a standard wash protocol on an AquaMax 200 plate washer (Molecular Devices; Sunnyvale, CA). The plate was blocked with 1× milk diluent block (MDB; KPL) for 30 min at 37 °C. The plate was washed, and 100 µL of PBS with 0.05% Tween 20 (PBS-T; Sigma-Aldrich) was applied to each well in the plate. MS2CP was diluted in PBS-T to 2 µg/mL, and 100 µL of this solution was applied to the first well of each row. A twofold serial dilution was performed across the plate, and it was incubated for 1 h at 37 °C.

After the plate was washed, rabbit anti-MS2 (supplied by JPMO BSV DBPAO) was diluted to 5 µg/mL in 1× MDB, and 100 µL of this solution was added to each well. The plate was incubated at 37 °C for 1 h. After incubation, the plate was washed. Goat anti-rabbit IgG (H+L)-horseradish peroxidase (HRP; KPL) was diluted to 0.2 µg/mL in 1× MDB, and 100 µL of this solution was added to each well. The plate was incubated at 37 °C for 30 min. After the plate was washed, 100 µL of room-temperature 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) one-component HRP substrate (KPL) was added to each well. After 20 min at 37 °C, the optical density at the 405 nm light wavelength was determined using a Synergy H4 hybrid multimode microplate reader (BioTek Instruments; Winooski, VT). Data analysis was performed using Prism software, version 5.00 for Windows (GraphPad Software; La Jolla, CA).

2.7 Surface Plasmon Resonance (SPR) Methodology

SPR is one method used to determine the kinetic parameters of antibody–antigen interactions. It is a rapid methodology for monitoring biomolecular interactions through excitation of surface plasmons. Polarized light is shone through a prism on a sensor chip with a thin metal film coating, which reflects the light by acting as a mirror. If the angle of light shone through the prism is changed, and the intensity of the reflected light is monitored, differences in intensity can be recorded. Although the refractive index at the prism side of the sensor chip does not change, the refractive index in the immediate vicinity of the metal surface will change when accumulated mass (bound proteins) adsorbs on the surface. Therefore, if binding occurs, the resonance angle (SPR angle) changes, and this SPR angle shift provides information on the protein adsorption kinetics on the surface. The software can then provide an accurate analysis of the association (k_a) and dissociation (k_d) rate constants for the antibody interactions, as well as calculate the overall affinity constant (K_D) between antibody and antigen.

2.7.1 Thermostability Testing with SPR

Using a Biacore T200 system (GE Healthcare Life Sciences), 6500 response units (RUs) of MS2CP was tethered to one flow cell of a Biacore CM5 sensor chip using standard amine coupling chemistry. After a thermal stress test was performed, samples were centrifuged at 2000 ×g and 5 °C for 5 min. The analyte was run at 10 µL/min for 120 s. A calibration curve was created by injecting eight concentrations of the time 0 unheated Stablebody Technologies MS2 scFv samples (Stab 4B or ANLStab 006) at 400, 350, 300, 250, 200, 150, 100, and 50 nM and plotting the respective analyte-binding capacities of the surface (R_{Max}) in response units. Unheated and heated samples were then diluted 1:90 and 1:180 so that the time 0 control points would fall on the linear calibration curve. All samples were run in triplicate. The chip's surface was regenerated with an 18 s injection of 0.85% phosphoric acid at a flow rate of 30 µL/min.

Data was collected using the Biacore concentration analysis software, and the active concentration of heated sample was recorded. The running buffer used for this experiment was Biacore HBS-EP 1× buffer (GE Healthcare Life Sciences).

2.7.2 Kinetic Analysis with SPR

Using a ProteOn XPR36 SPR system (Bio-Rad) and PBS-T running buffer, 200 RU of MS2CP was tethered to a GLC sensor chip (Bio-Rad) using standard amine coupling chemistry. Stablebody Technologies MS2 scFv samples (Stab 4B or ANLStab 006) were injected across the chip's surface for 120 s at a flow rate of 100 µL/min with a 600 s dissociation at 5 nM, 1.67 nM, 560 pM, 190 pM, and 60 pM. The chip's surface was regenerated using an 18 s injection of 0.85% phosphoric acid at 100 µL/min. Data was analyzed using a Langmuir 1:1 fit.

3. RESULTS

3.1 Spectrophotometry Results

Both of the Stablebody Technologies MS2 scFvs were read in triplicate on the NanoDrop ND-1000 spectrophotometer. The A_{280} values are listed in Table 1.

Table 1. A_{280} Values for Stablebody Technologies MS2 scFvs

Replicate	**A_{280} Value**	
	Stab 4B	**ANLStab 006**
1	0.641	4.54
2	0.671	4.54
3	0.631	4.54

For each antibody, three A_{280} readings (Table 1) were averaged and divided by the extinction coefficient of 1.77. The final concentrations were determined to be 0.366 mg/mL for the affinity matured Stab 4B and 2.56 mg/mL for the thermally stabilized ANLStab 006.

3.2 Electrophoresis Results

The molecular weights of the Stablebody Technologies MS2 scFvs were determined using the Experion Pro260 analysis kit, as shown in Figure 1. The thick bands in the second and fourth lanes correspond to the MS2 scFv antibodies Stab 4B and ANLStab 006, respectively. According to the Experion software, Stab 4B was 76.1% pure and weighed 31.6 kDa, and ANLStab 006 was 93.0% pure and weighed 30.9 kDa.

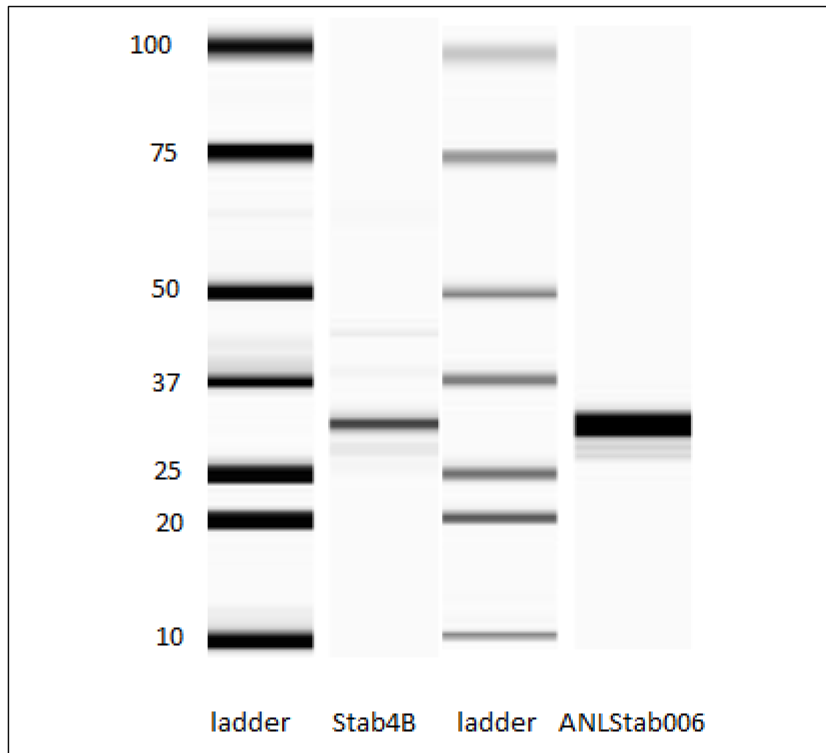


Figure 1. Molecular weight and purity. Digital gel of Stablebody MS2 scFvs produced using the Experion Pro260 analysis kit. Thick bands in the second and fourth lanes correspond to the MS2 scFv antibodies Stab 4B and ANLStab 006, respectively.

3.3 DLS Results

Both of the Stablebody Technologies MS2 scFvs were analyzed in triplicate using the DynaPro plate reader. The radius of ANLStab 006 was determined to be 2.5 nm with a polydispersity of 10.6% (Table 2), whereas the radius of Stab 4B was determined to be 9.08 nm with a polydispersity of 17.44%. Figure 2 contains representative correlation and regularization graphs for each of the MS2 scFvs. The correlation graphs (Figures 2A and 3A) depict a sigmoidal curve indicative of a valid size distribution. The regularization graphs (Figures 2B and 3B) illustrate the dispersity found in both samples. Table 2 shows the raw data produced for each replicate. Because 99.9% of the mass of ANLStab 006 displayed favorable polydispersity and hydrodynamic radius, it was considered to be monodisperse. However, the Stab 4B graphs show that ~50% of the sample in solution had a radius of ~80 nm, and the regularization graph, along with the wide range of radii and undesirable polydispersity, strongly suggests that aggregation was occurring in the solution.

Table 2. Features of MS2 ScFvs in Solution

Sample	Replicate	Radius (nm)	Polydispersity (%)
ANLStab 006	1	2.7	11.9
	2	2.2	10.3
	3	2.4	8.8
	4	2.5	10.7
	5	2.5	11.3
	Average	2.5	10.6
Stab 4B	1	13.3	24.5
	2	1.72	12.8
	3	9.38	23.1
	4	6.81	11.9
	5	14.2	14.9
	Average	9.08	17.44

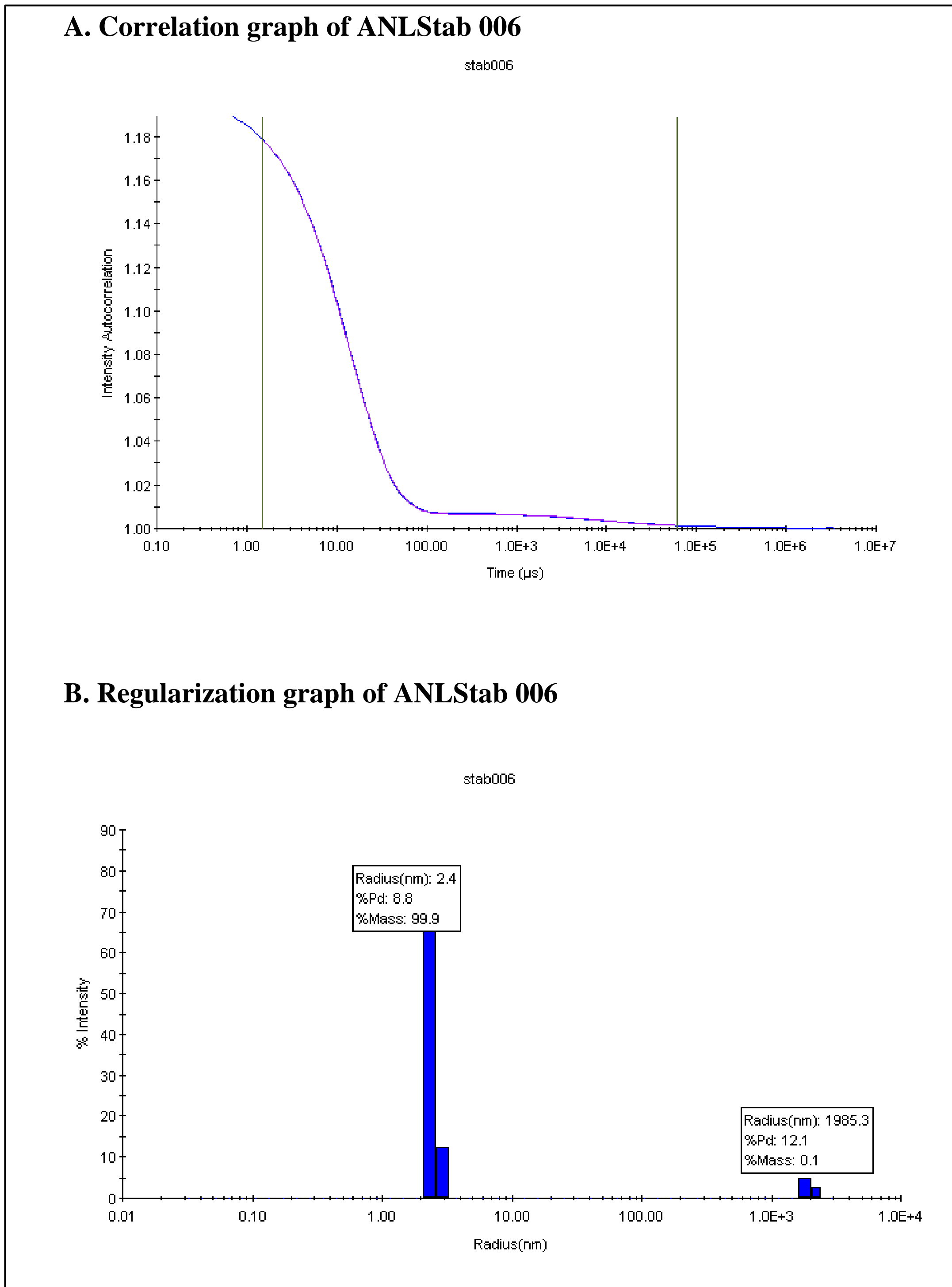


Figure 2. Radius and polydispersity representation of Stablebody thermostable scFv ANLStab 006: (A) correlation graph; and (B) regularization graph.

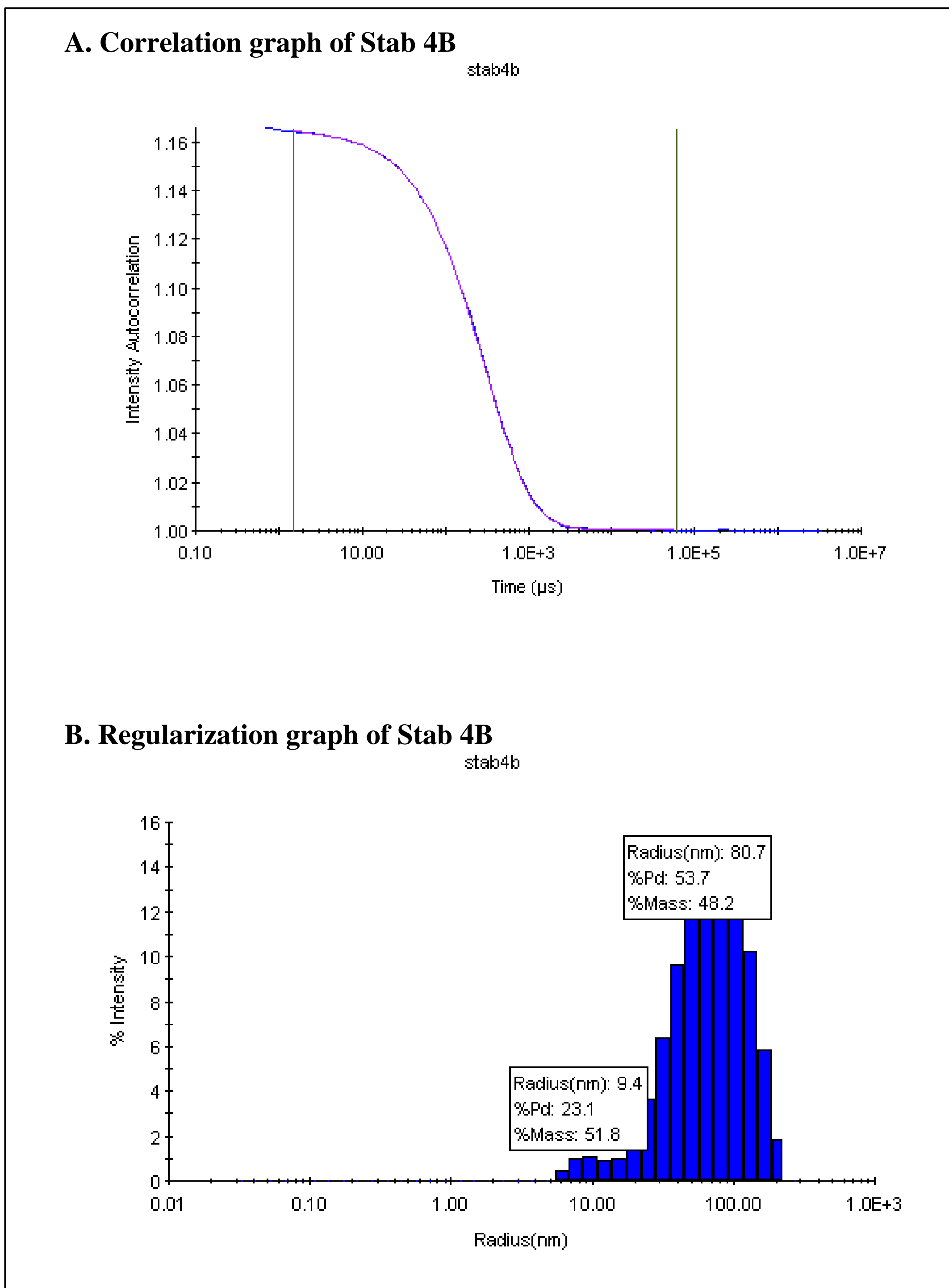


Figure 3. Radius and polydispersity representation of the Stablebody Technologies affinity-matured scFv Stab 4B, which was used for determining the radius and polydispersity of the samples: (A) correlation graph; and (B) regularization graph.

3.4 ELISA Results

ELISAs were used to test the functional interactions of antibodies and antigens after thermal stress at 70 °C. The ELISA data (Figure 4) show that when the Stablebody Technologies scFv ANLStab 006 was heated to 70 °C, it maintained all activity across all time periods of thermal stress. This was unlike the government-supplied MS2 scFv, for which less than 20% remained functionally capable of binding to antigen after a 15 min exposure to 70 °C, and a near total loss of antigen binding occurred when MS2 scFv was heated for 60 min.

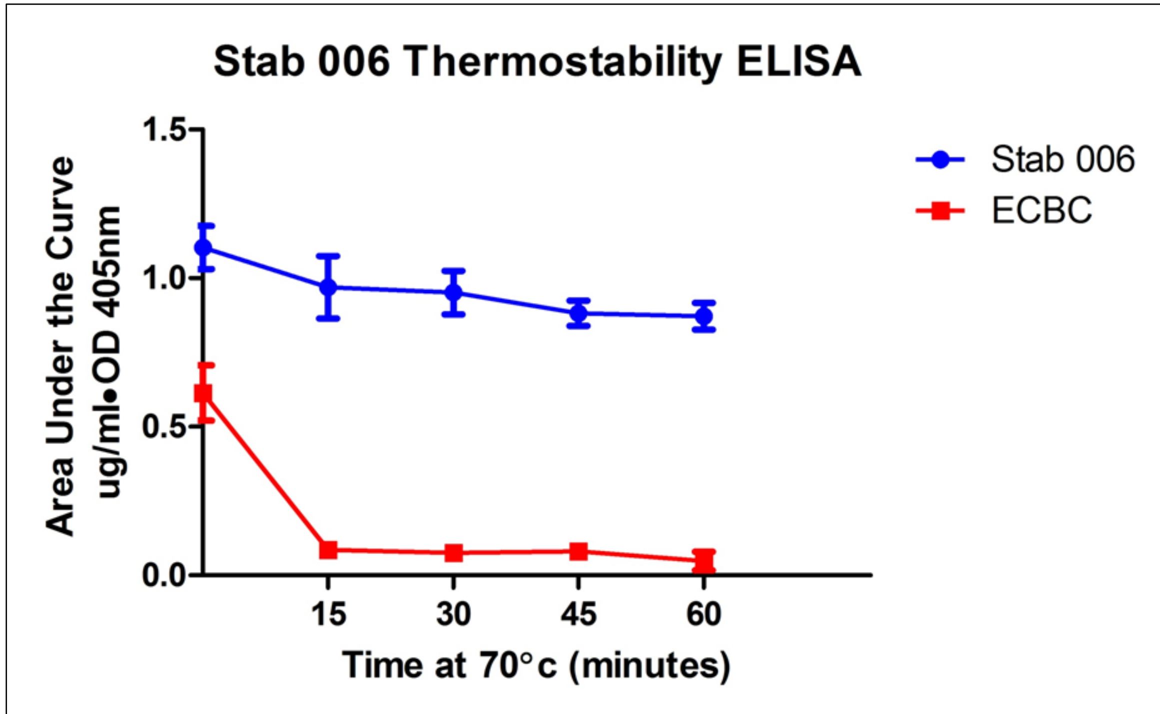


Figure 4. Thermostability of Stablebody Technologies ANLStab 006 determined with ELISA. Area under the curve analysis depicts the effect of thermal stress. ELISA was performed using ANLStab 006 as the capture antibody. MS2CP was titrated across the sample starting at 1 µg/mL. Anti-MS2 was used as the detector at 5 µg/mL.

3.5 SPR Results

3.5.1 Results of Thermostability Testing with SPR

SPR was used to assess the functional binding between the Stablebody Technologies scFv (ANLStab 006) and the antigen after the antibody–antigen complex was heated to 70 °C for variable time periods. Five tubes of 1 mg/mL ANLStab 006 were prepared and heated to 70 °C for 15, 30, 45, and 60 min time periods, followed by quenching on ice. Biacore T200 and ProteOn XPR36 systems were used to compare the activity of each sample with a calibration curve for unheated sample. The percent activity of the heated samples was plotted over time (Figure 5).

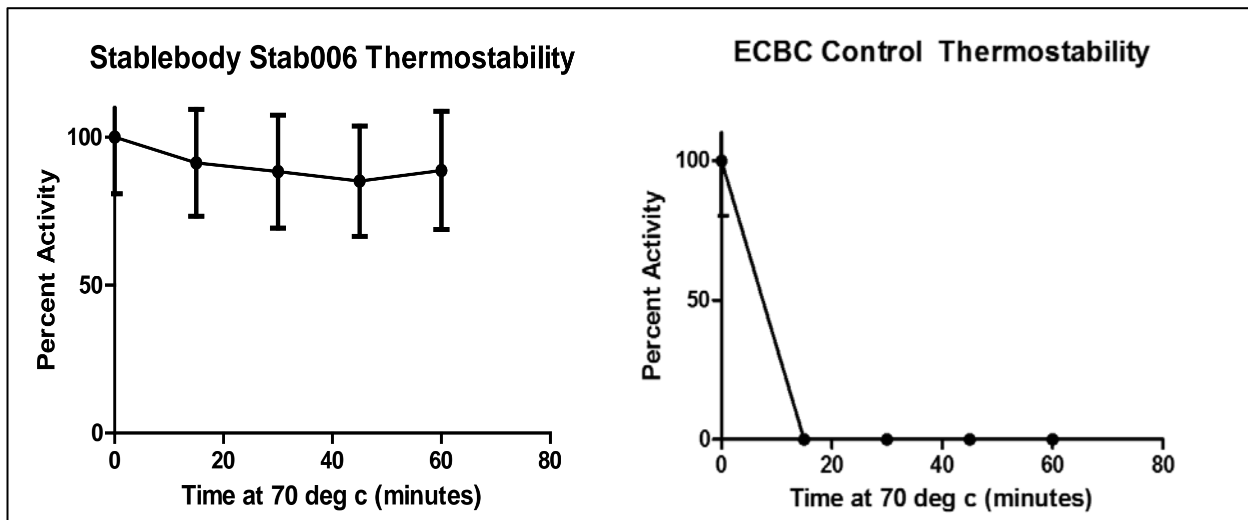
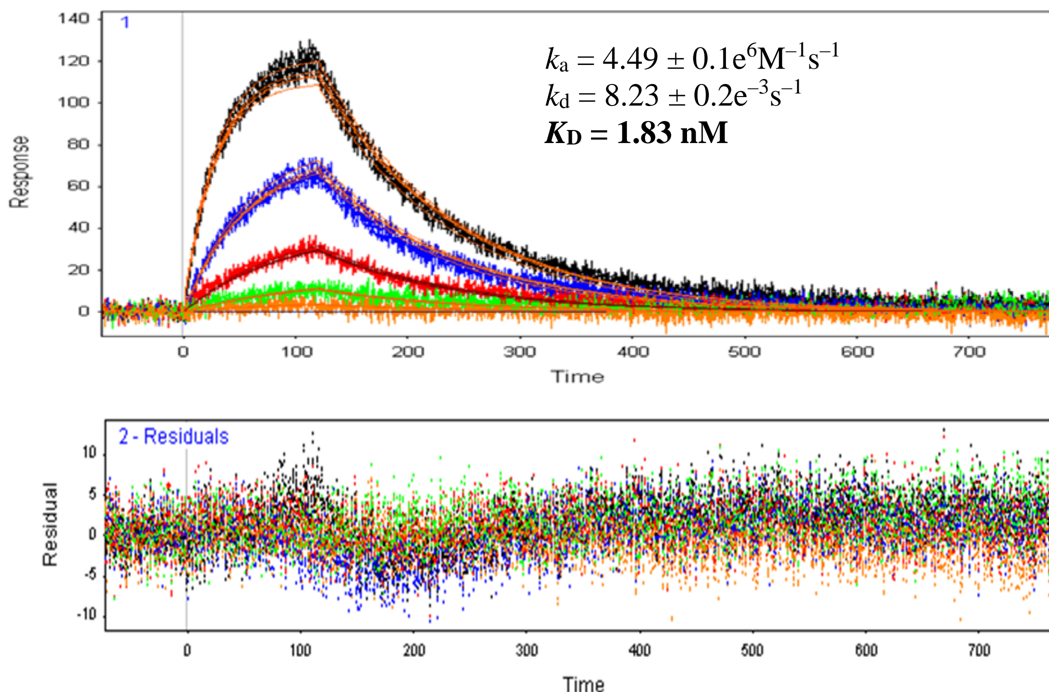


Figure 5. Thermostability of the Stablebody Technologies MS2 scFv (ANLStab 006; left) compared with the ECBC MS2 scFv by SPR (right). Results indicate that the ANLStab 006 scFv remained active over the entire 60 min, whereas the activity of the MS2 scFv dropped off completely within the first 15 min at 70 °C.

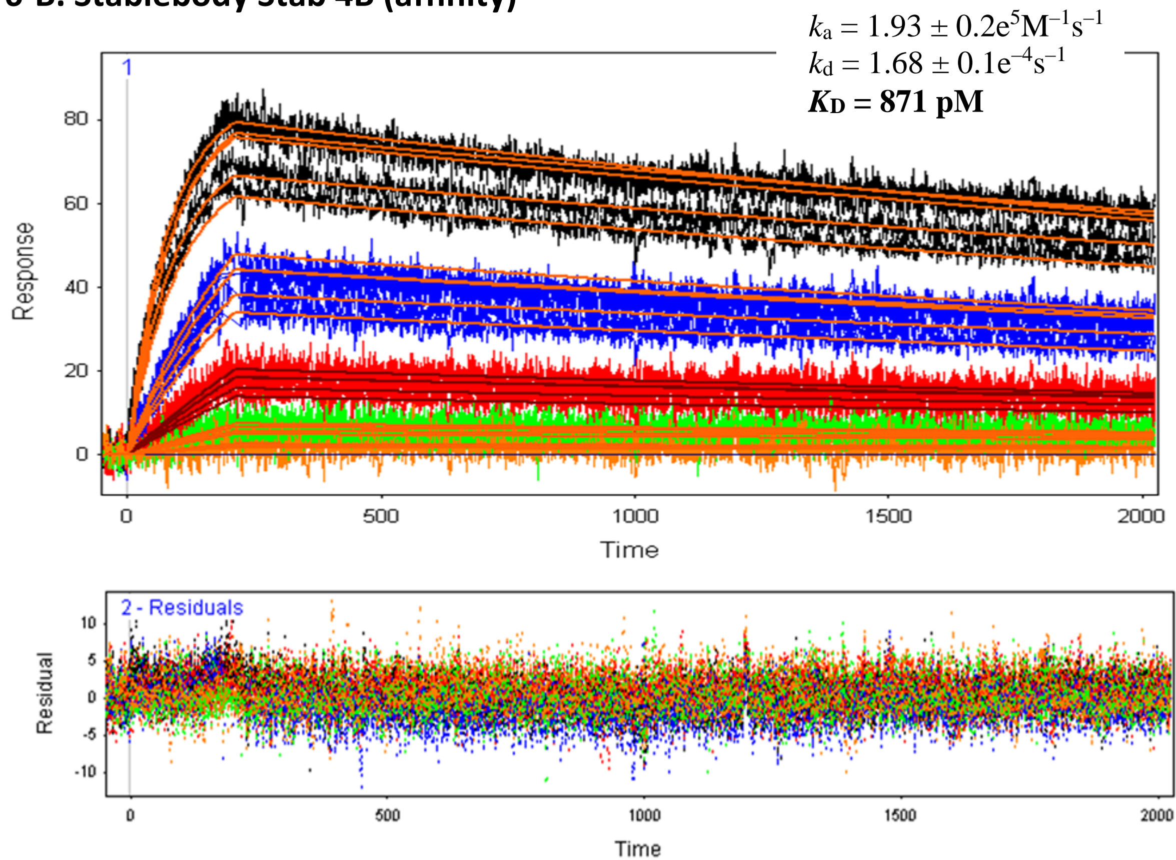
3.5.2 Results of Kinetic Analysis with SPR

Kinetic analysis of the thermostability-enhanced Stablebody Technologies MS2 scFv (ANLStab 006) binding to the MS2CP antigen was performed as a direct-binding SPR experiment on the ProteOn XPR36 system. The results are presented in Figure 6A. Data were normalized to a blank-immobilized reference flow cell and fit to a Langmuir 1:1 model using the Bio-Rad ProteOn XPR36 software. The K_D was determined to be 1.83 nM. Kinetic analysis was also performed on the affinity-enhanced Stablebody Technologies MS2 scFv (Stab 4B), and the results are presented in Figure 6B. The K_D was determined to be 871 pM. Results for similar experiments that were performed using the original ECBC MS2 scFv are presented in Figure 6C. The K_D of the original scFv was determined to be 8.66 nM; thus, Stablebody Technologies did not provide an antibody that met the 100-fold improvement threshold.

6-A. Stablebody ANLStab 006 (thermostable)



6-B. Stablebody Stab 4B (affinity)



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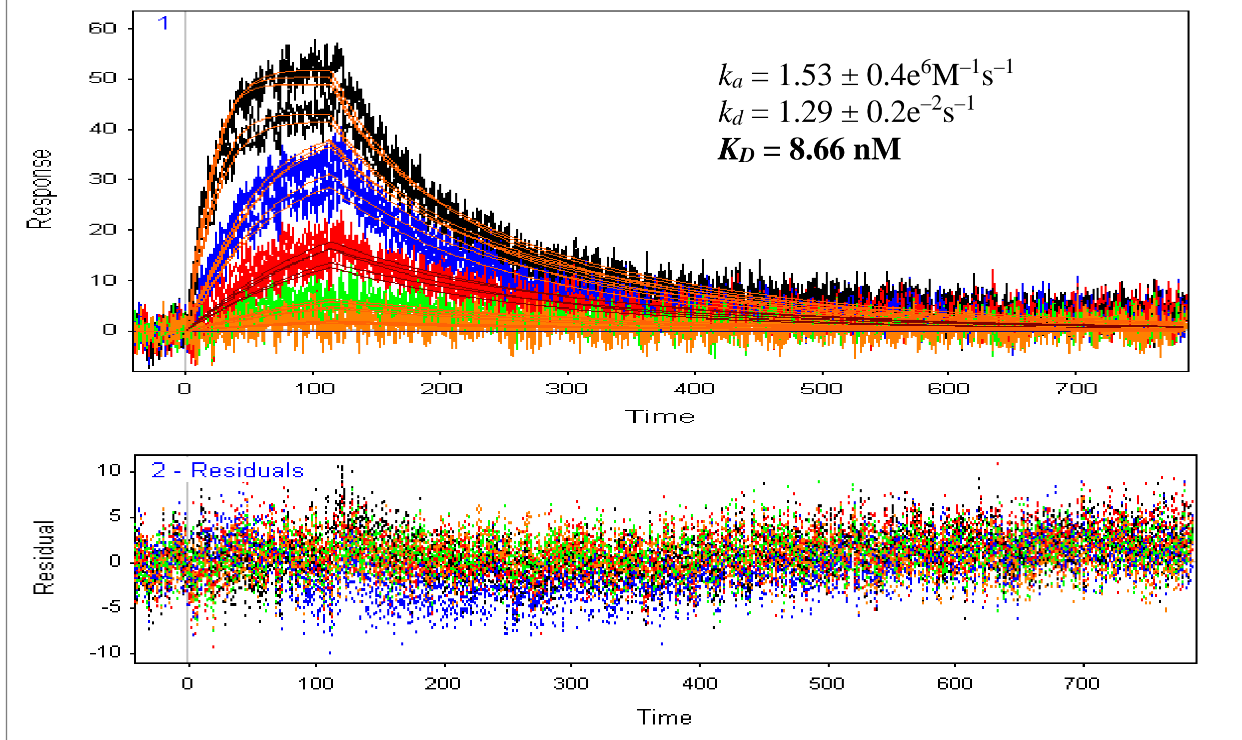

Figure 6. Comparison of the kinetic fits with residuals of the MS2 antibodies, determined using a Proteon XPR36 system. (A) Kinetics of Stablebody Technologies thermostable MS2 scFv (ANLStab 006; 1.83 nM). (B) Kinetics of Stablebody Technologies affinity-enhanced MS2 scFv (Stab 4B; 871 pM). (C) Kinetics of original MS2 scFv (8.66 nM).

4. DISCUSSION

This study established and standardized the parametric tests for performance on the MS2 scFv antibody. This antibody was selected by the DARPA ATP as the initial substrate for performers to use in demonstrating their molecular schemes for improving the thermal stability and affinity of an antibody for its target antigen. The test bed developed was used to define the physical and functional properties of the reference MS2 scFv antibody and establish the baseline for subsequent testing of the engineered antibodies submitted by the ATP performers. A snapshot of the MS2 scFv's physical characteristics was obtained using the NanoDrop, Experion, and DLS measurement platforms. Measurements of the MS2 scFv's functional characteristics, for assessing the effects of molecular engineering on thermal stability and affinity, were obtained using the differential scanning calorimetry, ELISA, and SPR analytic platforms.

An accurate assessment of protein concentration is critically important for all of the test procedures described in this report. We applied the standard technique of spectrophotometry with the NanoDrop ND-1000 system. This instrument was employed to provide the A_{280} value of the sample, which is influenced by the number of tryptophan and tyrosine residues in a given protein. For this reason, the extinction coefficient was used in conjunction with the A_{280} value to determine an accurate concentration.

After concentration was determined via spectrophotometry with the NanoDrop system, molecular weight and purity data were collected with the Experion automated electrophoresis system. This system employs microfluidic technology to automate electrophoresis for protein analysis. The results of Experion analysis of the MS2 scFv protein fell within the acceptable range of purity for use in assay development, and the molecular weight determined by the software (shown in Figure 1) was typical for an scFv.

DLS was used in conjunction with the Experion and NanoDrop systems to illustrate how the protein behaved in solution. DLS data indicate the physical state and potential aggregation of a protein in solution by measuring the polydispersity, hydrodynamic radius, and molecular weight of a sample. The DLS data established whether the MS2 scFvs provided by Stablebody Technologies were monomeric and monodisperse. Although less than 1% of the mass of the thermostablity-enhanced ANLStab 006 scFv appeared to aggregate in solution (Figure 2), the affinity-enhanced scFv, Stab 4B, appeared to aggregate considerably (Figure 3). To ensure that all testing would be consistent and to mitigate the exacerbating effect of freeze–thawing on future sample aggregation, the Stablebody Technologies MS2 scFvs were aliquoted into single-use vials and centrifuged before use.

In the next round of testing, the thermostability of the Stablebody Technologies MS2 scFvs were evaluated using ELISA and SPR systems. The results of the thermal stress test demonstrated that the Stablebody Technologies MS2 scFv ANLStab 006 remained active for over 60 min of heating at 70 °C. The ELISA and SPR data confirmed that the Stablebody Technologies scFv ANLStab 006 was able to bind the MS2CP, even after 60 min of heating, whereas the original MS2 scFv reference antibody was unable to bind the MS2CP after only 15 min of heating at 70 °C (Figures 4 and 5).

SPR was also used to obtain kinetic analyses of both the affinity-enhanced Stablebody Technologies MS2 scFv (Stab 4B) and the thermostability-enhanced ANLStab 006 binding to their target antigen MS2CP and to compare the binding parameters with the original antibodies. Kinetic data for Stab 4B MS2 scFv and ANLStab 006 binding to the MS2CP was obtained using the ProteOn XPR36 SPR platform, which yielded K_D values of 871 pM and 1.83 nM, respectively, whereas the original MS2 scFv yielded a K_D of 8.66 nM (Figure 8). The affinity K_D values show that Stablebody Technologies did not meet the 100-fold improvement requested by DARPA.

5. CONCLUSION

The DARPA ATP seeks to establish methods for rapidly engineering a given antibody reagent to exhibit physical and functional properties that far exceed those of its native state, and thereby expand user confidence in fielding antibody-based detection and diagnostic platforms in environments or operational scenarios in which currently available reagents exhibit degradation or interference. By optimizing the thermal stability and binding affinity of an antibody for its biological target, the DARPA ATP will develop antibody reagents that can reliably function in harsh environmental conditions and increase the sensitivity of sensor platforms to detect lower levels of threat agents.

This report documents the testing of an improved thermostable antibody (ANLStab 006) and an affinity-improved antibody (Stab 4B), which were both produced by Stablebody Technologies. This study evaluated the physical and functional characteristics of both scFvs in the ECBC testing pipeline. The results were compared with the baseline characteristics of the original antibody's physical properties, including concentration, molecular weight, purity, and state of aggregation in solution, as well as functional measures such as binding affinity and thermal stability. Both antibodies supplied by Stablebody Technologies exhibited enhanced thermal stability and/or affinity for binding to the MS2CP antigen.

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ACRONYMS AND ABBREVIATIONS

A_{280}	absorbance of light at 280 nm
ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ATP	Antibody Technology Program
BGG	bovine γ-globulin
DARPA	Defense Advanced Research Projects Agency
DBPAO	Defense Biological Product Assurance Office
DLS	dynamic light scattering
ECBC	U.S. Army Edgewood Chemical Biological Center
ELISA	enzyme-linked immunosorbent assay
HRP	horseradish peroxidase
IgG	immunoglobulin G
JPMO BSV	Joint Product Management Office for Biosurveillance
k_a	association rate constant
k_d	dissociation rate constant
K_D	affinity constant
MDB	milk diluent block
MS2CP	MS2 coat protein
PBS	phosphate-buffered saline
PBS-T	phosphate-buffered saline with 0.05% Tween 20
R_{Max}	maximum analyte-binding capacity of the surface
RU	response unit
scFv	single-chain fragment variable
SPR	surface plasmon resonance

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