U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND
CHEMICAL BIOLOGICAL CENTER

ABERDEEN PROVING GROUND, MD 21010-5424

CCDC CBC-TR-1718

Defense Advanced Research Projects Agency Antibody Technology Program Phase II: Characterization of an Anti-BclA Antibody Produced by Illumina, Inc.

Patricia E. Buckley
Alena M. Calm
Heather Welsh
Roy Thompson
Darrel Menking
James Carney

RESEARCH AND TECHNOLOGY DIRECTORATE

Candice Warner
Melody Zacharko

EXCET, INC.
Springfield, VA 22150-2519

December 2021

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
XX-12-2021	Final Report	Sep 2012–Dec 2015

4. TITLE AND SUBTITLE
Defense Advanced Research Projects Agency Antibody Technology Program Phase II: Characterization of an Anti-BclA Antibody Produced by Illumina, Inc.

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Buckley, Patricia E.; Calm, Alena M.; Welsh, Heather; Thompson, Roy; Menking, Darrel; Carney, James (DEVCOM CBC); Warner, Candice; and Zacharko, Melody (Excet)

5d. PROJECT NUMBER
BA08DET000/1R3Z11

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, DEVCOM CBC, ATTN: FCDD-CBR-BM, APG, MD 21010-5424
Excet, Inc.; 6225 Brandon Ave., Suite 360, Springfield VA 22150-2519

8. PERFORMING ORGANIZATION REPORT NUMBER
CCDC CBC-TR-1718

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Defense Advanced Research Projects Agency; 675 N. Randolph St., Arlington, VA 22203-2114

10. SPONSOR/MONITOR'S ACRONYM(S)
DARPA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release: distribution unlimited.

13. SUPPLEMENTARY NOTES
U.S. Army Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC) was previously known as U.S. Army Edgewood Chemical Biological Center (ECBC).

14. ABSTRACT:
The Defense Advanced Research Projects Agency (DARPA; Arlington, VA) Antibody Technology Program (ATP) focused on the development of technologies that enhance the thermal stability and binding affinity of a given antibody. The U.S. Army Edgewood Chemical Biological Center (ECBC; now known as U.S. Army Combat Capabilities Development Command Chemical Biological Center; Aberdeen Proving Ground, MD) functioned as an independent testing laboratory to provide technical support on immune reagents and assist in defining the government-supplied antibody–antigen pairs. Project goals were to (1) implement standardized methods for characterizing antibodies developed at ECBC with de novo thermal and binding properties of select reagents for use by DARPA-funded investigators, and (2) use those methods to validate changes in antibody thermal stability and binding affinities achieved by DARPA investigators. The antibody chosen for this project was BA21, which detects the *Bacillus* collagen-like protein of *anthracis* (BclA). The focus was evaluation of the BA21 antibody supplied by Illumina, Inc. for affinity and stability enhancements. Results of this study include standardized parametric data on antibody properties and performance that will contribute to improvements for future detection and diagnostic platforms.

15. SUBJECT TERMS

Antibody	Biosurveillance	Enzyme-linked immunosorbent assay (ELISA)	Quality
Testing	BA21	*Bacillus* collagen-like protein of *anthracis* (BclA)	*Bacillus anthracis*

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Renu B. Rastogi
U	U	U	UU	36	**19b. TELEPHONE NUMBER** *(include area code)* (410) 436-7545

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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PREFACE

The work described in this report was authorized under project no. BA08DET000/1R3Z11. The work was started in September 2012 and completed in December 2015.

At the time this work was performed, the U.S. Army Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC; Aberdeen Proving Ground, MD) was known as the U.S. Army Edgewood Chemical Biological Center.

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This report has been approved for public release.

Acknowledgment

The authors acknowledge Ms. Kelley Betts (DEVCOM CBC) for her hard work and assistance in editing and preparing this report.

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DEFENSE ADVANCED RESEARCH PROJECTS AGENCY ANTIBODY TECHNOLOGY PROGRAM PHASE II: CHARACTERIZATION OF AN ANTI-BclA ANTIBODY PRODUCED BY ILLUMINA, INC.

1. INTRODUCTION

In an effort to more fully characterize and compare the physical and functional properties of antibody reagents in its repository, the Joint Product Management Office for Biosurveillance (Fort Detrick, MD) has instituted a quality program for the standardization of test methods. Production methods for antibodies used in detection devices have drastically changed over time. Animal models are most commonly used for producing polyclonal antibodies; however, the antibodies lack antigen-binding specificity, and the antibody affinity depends on the individual animals. Development of the monoclonal antibody (mAb) allowed for more specificity; however, the use of large numbers of animals is still required. With the advent of hybridoma cell culture production, larger quantities of high-activity antibodies were produced, and existing cell lines could be panned for higher-affinity antibodies. Recombinant methods have greatly increased researchers' ability to produce more-specific antibodies with antigen-binding fragment (Fab), single-chain variable fragment (scFv), and single-domain antibody (sdAb). Many varying recombinant production systems are being used, from basic bacteria, yeasts, and filamentous fungi to insect cell lines and mammalian cells, and including transgenic plants and animals (1).

The Defense Advanced Research Projects Agency (DARPA; Arlington, VA) Antibody Technology Program (ATP) focused on the development of technologies that enhance thermal stability and the binding affinity of a given antibody. Increased thermal stability would eliminate the need for cold storage and increase the usability of antibodies in harsh conditions, such as those experienced by troops in the field. An added benefit of increased binding affinity is the ability to develop multiplex sensors that can detect a greater number of antigens in a single immunoassay. The U.S. Army Edgewood Chemical Biological Center (ECBC; now known as the U.S. Army Combat Capabilities Development Command Chemical Biological Center; Aberdeen Proving Ground, MD) functioned as an independent testing laboratory for this program. ECBC personnel provided specific technical support on immune reagents as well as assistance in defining the government-supplied antibody–antigen pairs. Standardized methods developed at ECBC for characterizing antibodies were used for validating the changes in antibody thermal stability and binding affinity that had been achieved by DARPA investigators. Because combinatorial approaches to antibody enhancement are random and may lead to fortuitous improvements in stability or affinity, the strategies for ATP enhancement were required to be directed so they could be transferable to other antibody molecules.

The primary objectives for the Phase I ATP were to develop and demonstrate strategies that independently modulate antibody stability and affinity in a viral antibody molecule that was provided by the U.S. Government. The Phase II goal was to modify an antibody using the Phase I techniques and produce 2 g of a single protein that yielded a 100-fold increase in the affinity and a 10 °C increase in the melting temperature of a provided protein. The focus of the work highlighted in this report is the evaluation of the anti-*Bacillus anthracis* BA21 antibody supplied by the DARPA-funded investigator Illumina, Inc. (San Diego, CA) for both affinity and stability enhancements.

2. MATERIALS AND METHODS

2.1 ANTIBODY AND ANTIGEN

2.1.1 Anti-*B. anthracis* BA21 Antibody

The Critical Reagents Program collection contains large numbers of polyclonal and monoclonal anti-*B. anthracis* antibodies. For this project, the anti-*B. anthracis* BA21 antibody, which detects the *Bacillus* collagen-like protein of *anthracis* (BclA), was chosen.

2.1.2 Antibody Modification

Illumina used an unpublished method to modify the antibody BA21. The modified antibody was named AFX 1494.

2.1.3 Antigen for Testing

The BclA antigen was used for all testing. This protein is a major structural component of the spore coat protein from *B. anthracis* that has been shown to be highly immunogenic (2). We used a truncated form of the protein (tBclA) that was purified by affinity chromatography, which leads to dimerization in solution but reduces multimerization (2, 3).

2.2 TEST METHODS

For this study, the standardized parametric tests that were established during the MS2 scFv antibody DARPA ATP Phase I work were used (4).

2.2.1 Antibody Concentration Measurement

The concentrations of the antibodies were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific; Madison, WI). This instrument measures the absorbance of light at 280 nm (A_{280}) for the sample. Extinction coefficients were used in conjunction with A_{280} values to determine accurate concentrations. The concentration of each sample was determined by dividing the average A_{280} value by the extinction coefficient for each antibody. Each reading required a 2 µL sample, which was placed on the sample pedestal. The instrument was blanked using phosphate-buffered saline (PBS; Sigma-Aldrich; St. Louis, MO), and readings were taken in triplicate. As a positive control and to validate instrument operation, bovine γ-globulin (BGG; Bio-Rad; Hercules, CA) was also tested.

2.2.2 Molecular Weight and Purity Measurements

An Experion automated electrophoresis system (Bio-Rad) was used to determine the molecular weight and purity of both AFX 1494 and BA21. The microfluidic chip, in conjunction with the Experion reagents, electrophoresis station, and software, is designed to accomplish separation, staining, destaining, detection, and basic data analysis. Sample purity was determined by calculating the percent mass of the separated proteins in a sample. For Experion analysis, each antibody concentration was standardized by dilution in PBS to a final

concentration of 1 mg/mL. The control (BGG) and the antibody samples were then processed using the validated procedure specified in the *Experion Pro260 Analysis Kit Instruction Manual* (5). 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. The sample was reduced with dithiothreitol (Sigma-Aldrich) and denatured in the kit-provided sample buffer at 95 °C before it was applied to the primed chip. The instrument was operated via the Experion software. All samples were run in triplicate alongside one sample of the BGG control and the Pro260 ladder (Bio-Rad). Analysis was performed using the Experion software.

2.2.3 Protein Behavior Measurement

Dynamic light scattering (DLS) was used to evaluate how the proteins behaved in solution. Three tests were performed to determine protein uniformity in solution. Polydispersity is a measure of the size distribution of particles in solution. Protein molecules that have a polydispersion value of less than 20% are considered monodisperse. The hydrodynamic radius and molecular weight of the sample are displayed in two graphs. The correlation graph indicates the relative particle size, and the steepness of the line indicates the monodispersion level of the sample. The regularization graph (derived from the data) shows the hydrodynamic radius, percent mass, and molecular weight. The DLS software uses prediction algorithms to produce this range of values for the protein under evaluation.

For DLS analysis, five 20 µL aliquots of the antibody, along with a control (bovine serum albumin; Sigma-Aldrich), were placed into a quartz 384-well plate (Wyatt Technology; Santa Barbara, CA) and centrifuged (2 min, 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, and the plate was then placed into a temperature-controlled DynaPro plate reader (Wyatt Technology). Each well was scanned 10 times for 5 s each at 25 °C. Results were averaged, and the Wyatt Technology Dynamics software was used to measure polydispersity, hydrodynamic radius, and percent mass for each sample. The results for five wells were averaged and reported.

2.2.4 Thermal Stability Measurement

Differential scanning calorimetry (DSC) was used to obtain a quantitative melting temperature (T_m) for each antibody protein. The T_m was determined to predict results of subsequent enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) thermostability testing. A T_m above 70 °C predicts that the antibody activity after the thermal stress test will remain above 50%. A T_m below 70 °C predicts, at minimum, a 50% decrease in antibody activity after the thermal stress test. For DSC experiments, samples were diluted to 0.5 mg/mL and dialyzed overnight in PBS at pH 7.4. Samples were degassed for 5 min before analysis and injected into the sample cell of a VP-DSC calorimeter (MicroCal; North Hampton, MA). Dialysis buffer was added to the reference cell of the calorimeter, and a buffer scan was used as the baseline for all experiments. The samples were scanned in duplicate from 15 to 100 °C at a rate of 60 °C/h. The transition midpoint (measured as T_m) of the protein was determined by data analysis with Origin 7.0 software (MicroCal).

2.2.5 Thermal Stress Test

All samples were diluted to a concentration of 1 mg/mL before heat was applied to negate protective effects due to concentration. Antibodies were 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 75 °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 using ELISA.

2.2.6 ELISA Methodology

After thermal testing was complete, ELISAs were performed in triplicate using the standard capture assay technique. Each antibody sample was diluted to 20 µg/mL in PBS. A twofold serial dilution was performed across each Nunc MaxiSorp 96-well plate (Thermo Fisher Scientific). Samples 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 (MDS Analytical Technologies; Sunnyvale, CA) and then blocked with 1× milk diluent block (MDB; KPL) for 30 min at 37 °C. The plates were then washed again, and 100 µL of 0.125 µg/mL biotinylated BclA antigen was added to each well. The plates were incubated for 1 h at room temperature. Horseradish peroxidase (HRP)-labeled streptavidin (KPL) was diluted to 0.1 µg/mL in 1× MDB, 100 µL of which was added to each well and incubated at room temperature for 1 h. After the plates were washed, 100 µL of ABTS 1-component HRP substrate (KPL), at room temperature, was added to each well. After 9 min at room temperature, the optical density at the 405 nm light wavelength was determined using a Synergy H4 hybrid multi-mode microplate reader (BioTek; Winooski, VT). Data analysis was performed using Prism software, version 5.00 for Windows (GraphPad Software; San Diego, CA).

2.2.7 SPR Methodology

SPR is a method used to determine the kinetic parameters of an antibody–antigen interaction. It is a rapid means for monitoring biomolecular interactions through the excitation of surface plasmons, which results when polarized light is shined through a prism on a sensor chip with a thin metal film coating. The metal film acts as a mirror and reflects the light. When the angle of light shining through the prism is changed, the intensity of the reflected light also changes. These intensity differences can be monitored and recorded. Although the refractive index at the prism side of the chip does not change, the refractive index in the immediate vicinity of the metal surface does change when accumulated mass (bound proteins) adsorbs onto the surface. Therefore, if binding occurs, the resonance angle (SPR angle) changes, and this shift of the SPR angle provides information about the kinetics of the protein adsorption on the surface. The SPR software can then be used to accurately analyze the association and dissociation rate constants (k_a and k_d, respectively) for the antibody interactions and to calculate the overall equilibrium dissociation constants (K_D values) between antibodies and antigens.

2.2.7.1 Thermostability Testing

The Biacore T200 (Cytiva; Marlborough, MA) was used to tether 6500 response units (RUs) of BclA to one flow cell of a Biacore CM5 chip via standard amine coupling

chemistry. After thermal stress testing was performed, samples were centrifuged (5 °C, 2000×*g*, 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 antibody at 400, 350, 300, 250, 200, 150, 100, and 50 nM and then plotting the respective maximum analyte-binding capacity 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 using an 18 s injection of 0.85% phosphoric acid at a flow rate of 30 µL/min. Data were collected using concentration analysis software (Biacore T200 Evaluation software; Cytiva), and the active concentrations of heated sample were recorded. The running buffer used for this experiment was Biacore 1× HBS-EP buffer (Cytiva).

2.2.7.2 Kinetic Analysis

The Biacore T200 system and 1× HBS-EP running buffer were used to tether 102 RUs of BclA to a Biacore CM5 chip via standard amine coupling chemistry. AFX 1494 was injected across the chip surface for 120 s at a flow rate of 75 µL/min with a 900 s dissociation at 60, 20, 6.67, and 2.2 nM and 700 pM. The chip surface was regenerated using an 18 s injection of 0.85% phosphoric acid at 30 µL/min with a 60 s stabilization period. Data were analyzed using a Langmuir 1:1 fit.

3. RESULTS

3.1 ANTIBODY CONCENTRATION MEASUREMENTS

3.1.1 AFX 1494 Concentration

A_{280} values for AFX 1494 were obtained in triplicate on the NanoDrop ND-1000 spectrophotometer. The A_{280} readings are listed in Table 1.

Table 1. A_{280} Readings for AFX 1494

Replicate No.	A_{280} Value
1	10.644
2	10.641
3	10.656

An average was determined from the A_{280} results, and it was divided by the extinction coefficient of 1.77. The reported concentration was 6.02 mg/mL.

3.1.2 BA21 Concentration

A_{280} values for BA21 were obtained in triplicate on the NanoDrop ND-1000 spectrophotometer. The A_{280} readings are listed in Table 2.

Table 2. A_{280} Readings for BA21

Replicate No.	A_{280} Value
1	1.19
2	1.19
3	1.18

An average was determined from the A_{280} results (the spectrophotometer software automatically divided by the extinction coefficient). The reported concentration was 1.2 mg/mL.

3.2 MOLECULAR WEIGHT AND PURITY MEASUREMENTS

3.2.1 AFX 1494 Molecular Weight and Purity

The molecular weight of AFX 1494 was determined (Figure 1) using the Experion Pro260 analysis kit. In the figure, the thick band in the second lane corresponds to the AFX 1494 antibody chain. According to the software, the antibody was 96.4% pure, and the chain weighed 37.1 kDa.

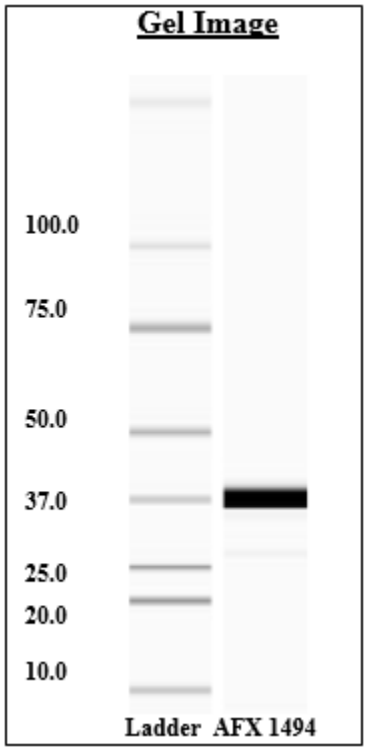


Figure 1. AFX 1494 molecular weight and purity. Digital gel of AFX 1494 antibody produced by the Experion Pro260 system. Thick band in the right lane corresponds to the AFX 1494 chain.

3.2.2 BA21 Molecular Weight and Purity

The molecular weight of BA21 was measured (Figure 2) using the Experion Pro260 analysis kit. In the figure, the thick band at the top of the second lane corresponds to the BA21 antibody heavy chain, and the lighter band corresponds to the light chain. According to the software, the antibody was 98.4% pure, the heavy chain weighed 70.0 kDa, and the light chain weighed 27.5 kDa.

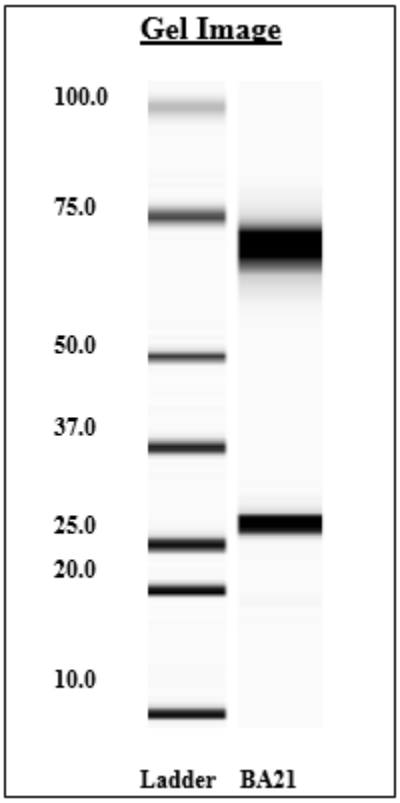


Figure 2. BA21 molecular weight and purity. Digital gel of BA21 produced by the Experion Pro260 system. Thick band at the top of the right lane corresponds to the BA21 heavy chain; thinner band at the bottom of the second lane corresponds to the light chain.

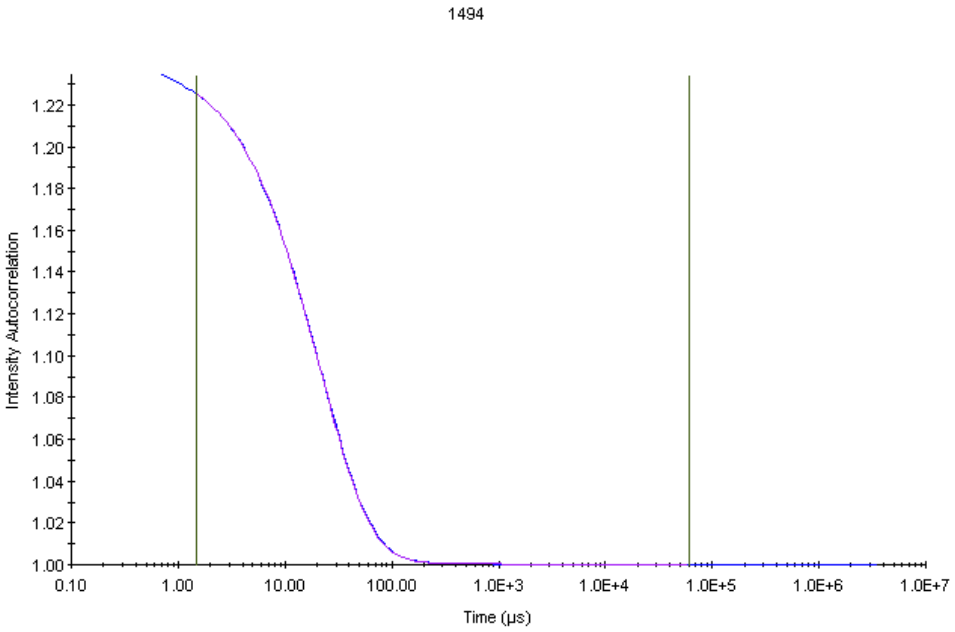
3.3 PROTEIN BEHAVIOR BY DLS

3.3.1 AFX 1494 Protein Behavior

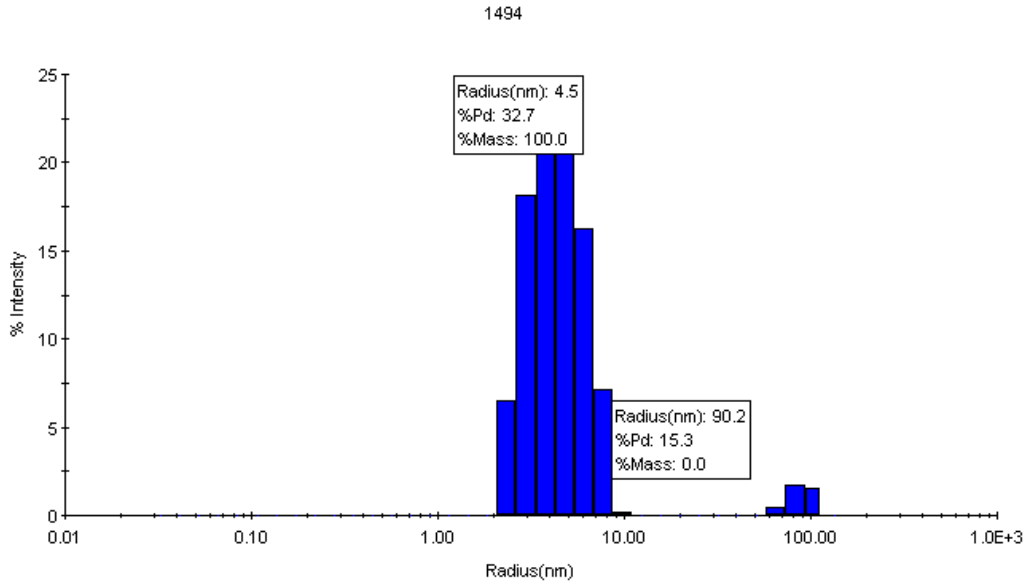
AFX 1494 was analyzed in triplicate using the DynaPro plate reader. The radius of AFX 1494 was determined to be 4.4 nm, and the polydispersity was 31.6%. Figure 3 shows representative correlation and regularization graphs for AFX 1494. The correlation graph (panel A) depicts a sigmoidal curve indicative of a valid size distribution. The regularization graph (panel B) illustrates the monodispersity of both samples. Table 3 lists the raw data produced for each replicate. Because 100% of the mass displayed favorable polydispersity and hydrodynamic radius, the sample preparations were considered to be monodisperse.

Table 3. Features of AFX 1494 in Solution

Replicate No.	Radius (nm)	Polydispersity (%)
1	4.4	31.2
2	4.2	31.3
3	4.4	30.4
4	4.5	32.7
5	4.6	32.2
Average	4.4	31.6



A. Correlation Graph for AFX 1494



B. Regularization Graph for AFX 1494

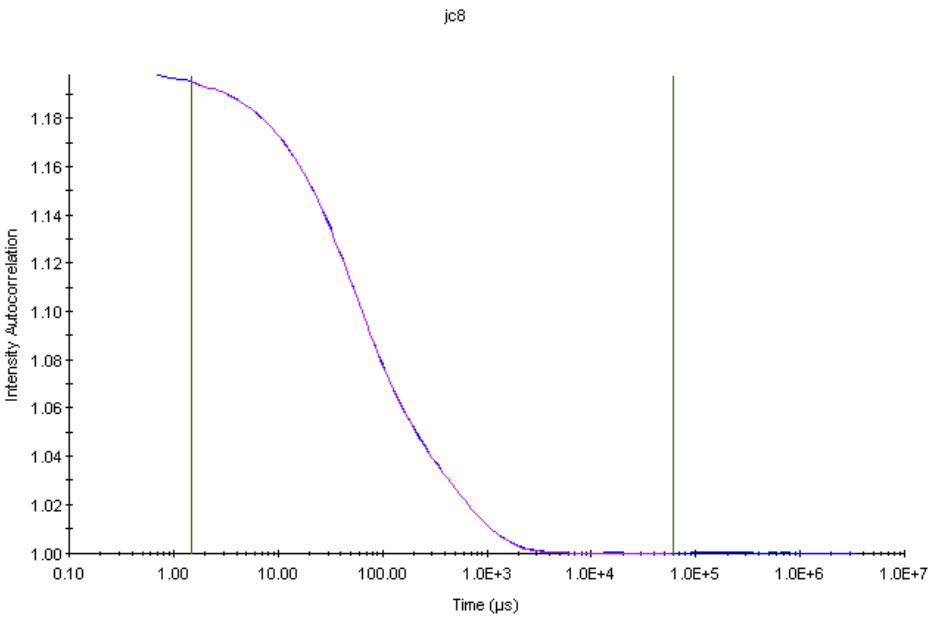
Figure 3. Radius and polydispersity (Pd) representation for AFX 1494: (A) correlation graph and (B) regularization graph.

3.3.2 BA21 Protein Behavior

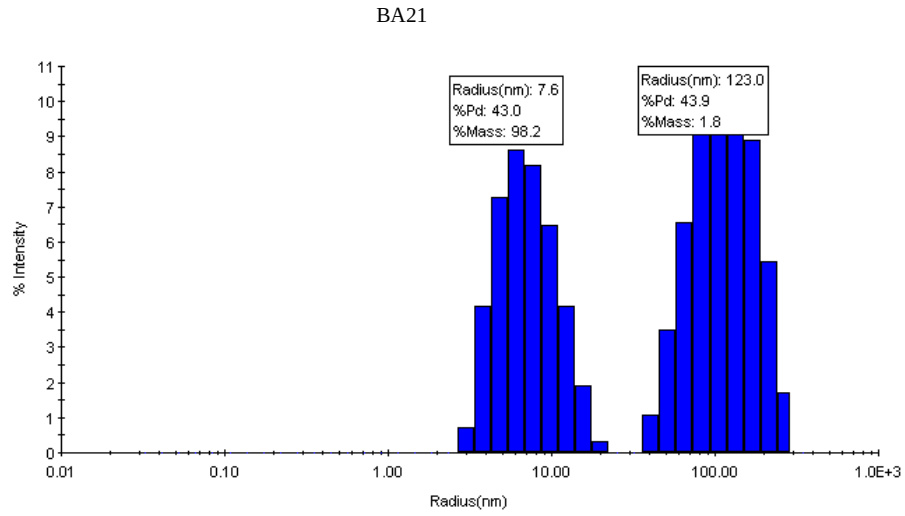
BA21 was analyzed in solution using the DynaPro plate reader. The radius of BA21 was determined to be 7.3 nm, and the polydispersity was 38.3%. Figure 4 contains representative correlation and regularization graphs for BA21. The correlation graph (panel A) depicts a sigmoidal curve indicative of a valid size distribution. The regularization graph (panel B) illustrates the polydispersity identified in the sample. Table 4 lists the raw data produced for each replicate. 98.2% of the mass displayed favorable hydrodynamic radius yet unfavorable polydispersity; thus, the sample preparation was considered to be polydisperse.

Table 4. Features of BA21 in Solution

Replicate No.	Radius (nm)	Polydispersity (%)
1	8.1	50.4
2	7.1	36.8
3	7.6	43.0
4	6.3	22.9
Average	7.3	38.3



A. Correlation Graph for BA21



B. Regularization Graph for BA21

Figure 4. Radius and polydispersity (Pd) representation for BA21: (A) correlation graph and (B) regularization graph.

3.4 DSC

3.4.1 AFX 1494 Melting Temperature

Readings for AFX 1494 were obtained in duplicate on the MicroCal VP-DSC calorimeter. The peak T_m's are presented in Table 5 and shown in Figure 5.

Table 5. AFX 1494 Melting Temperature

Replicate No.	T_m (°C)
1	71.54421
2	72.03295

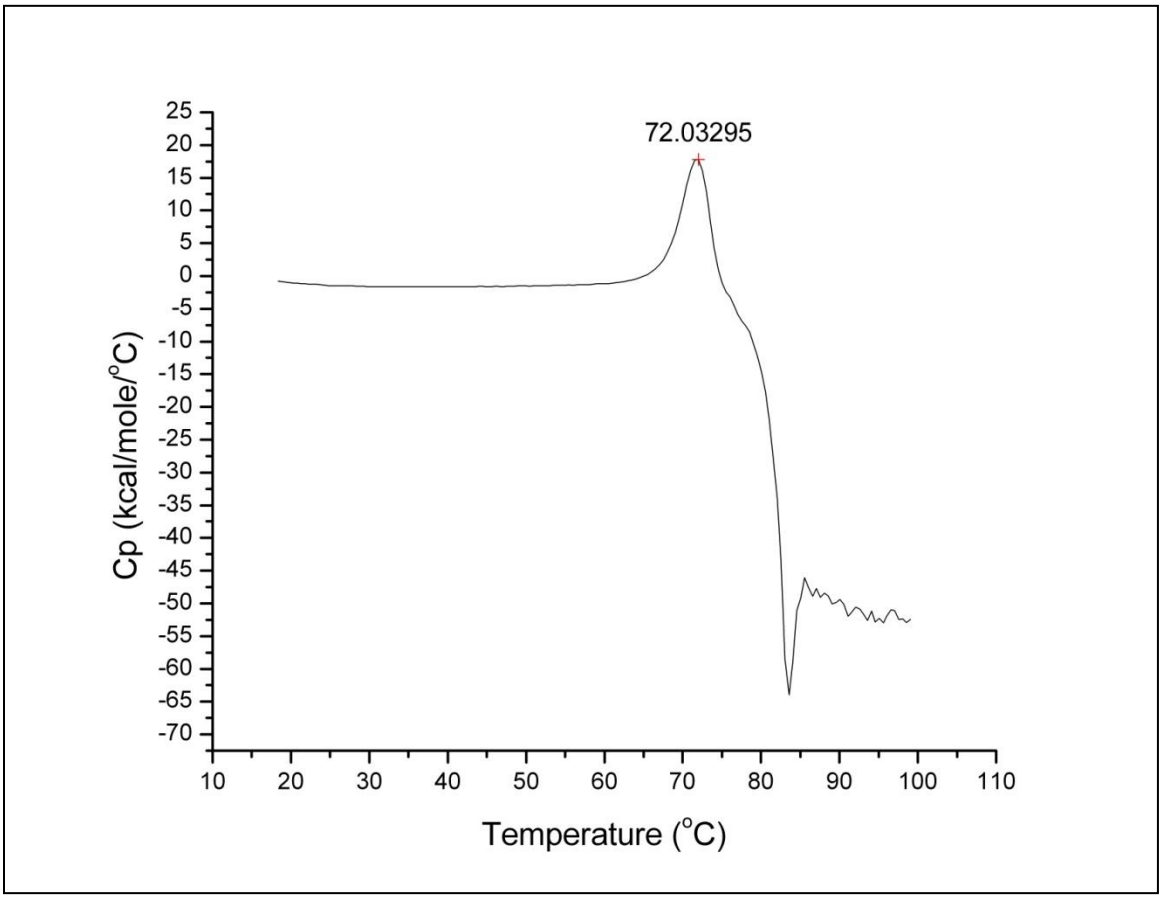


Figure 5. Transition midpoint curve for AFX 1494. The average T_m was calculated to be 72.0 °C.

3.4.2 BA21 Melting Temperature

Readings for BA21 were obtained on the MicroCal VP-DSC calorimeter. The peak T_m is shown in Figure 6.

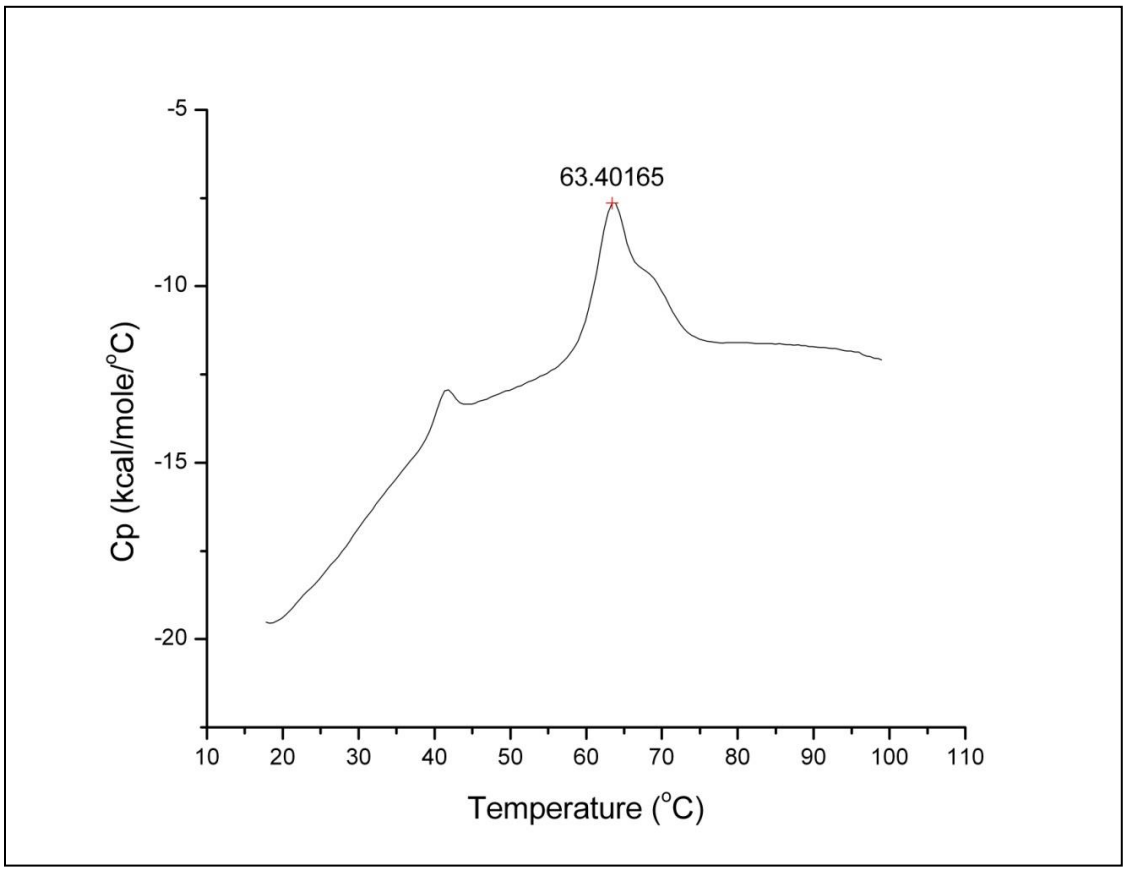


Figure 6. Transition midpoint curve for BA21. T_m was calculated to be 63.4 °C.

3.5 POST-TEMPERATURE-STRESS ELISA

3.5.1 AFX 1494 ELISA

ELISAs were used to test the functional interactions of antibodies and antigens after thermal stress at 70 ºC. Figure 7 illustrates that the antibody lost all functional capability of binding to an antigen after a 15 min exposure to 70 °C.

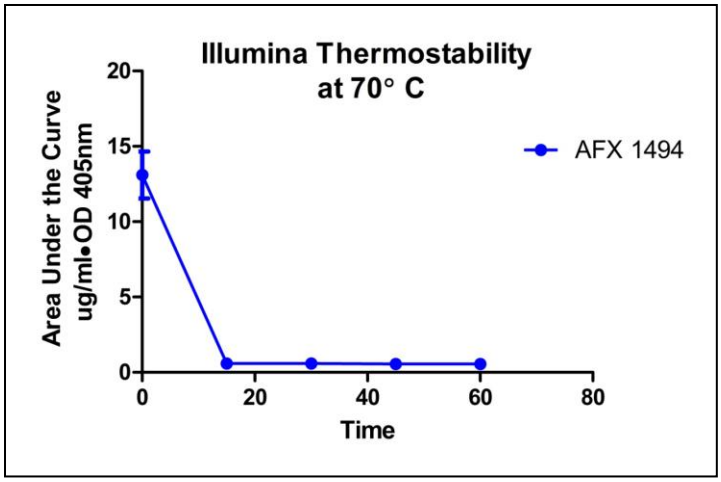


Figure 7. Thermostability of AFX 1494 ELISA. Area under the curve analysis depicts the effect of thermal stress. OD, optical density.

3.5.2 BA21 ELISA

ELISAs were used to test the functional interaction of BA21 as described in Section 3.5.1. The ELISA data (Figure 8) indicate that when the BA21 was heated to 70 °C, it ceased all activity after about 15 min.

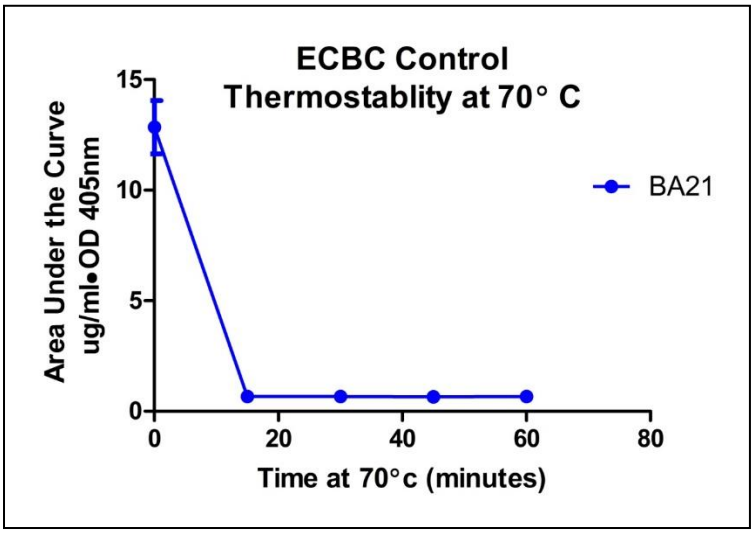


Figure 8. Thermostability of BA21 ELISA. Area under the curve analysis depicts the effect of thermal stress. OD, optical density.

3.6 SPR

3.6.1 Thermostability Testing by SPR

The functional binding between the antibodies and the antigens was also assessed by SPR after heating to 70 °C for variable time periods. Five tubes of 1 mg/mL AFX 1494 were prepared and heated to 70 °C for 15, 30, 45, and 60 min time periods and were then quenched on ice. The Biacore T200 system was used to compare the activity of each sample with a calibration curve constructed from unheated sample data (Figures 9 and 10). The AFX 1494 activity curve (Figure 9) illustrates that the antibody ceased most of its activity within 15 min and had ceased all activity after 30 min. This indicated that AFX 1494 was only slightly more resistant to heat than BA21 (Figure 10), which ceased all activity after 15 min at 70 °C.

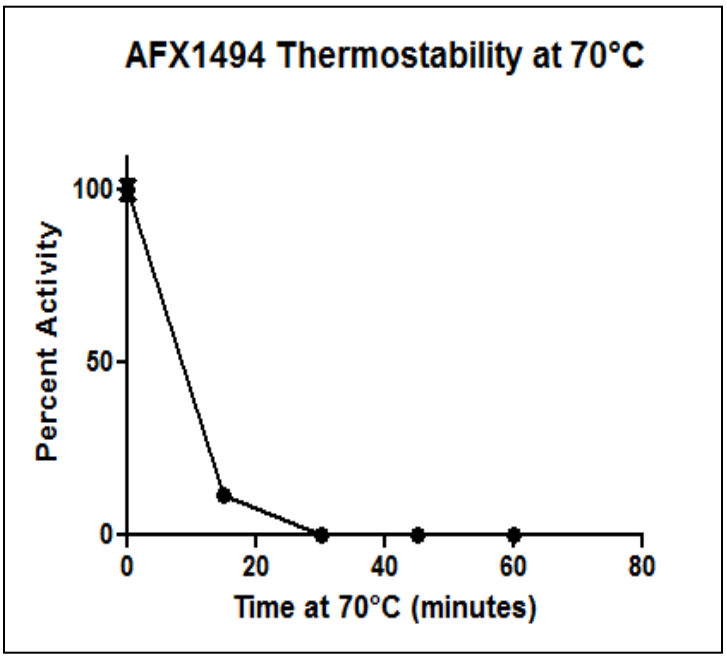


Figure 9. Thermostability of AFX 1494 as assessed using SPR. The antibody ceased most of its activity within 15 min and had ceased all activity by 30 min.

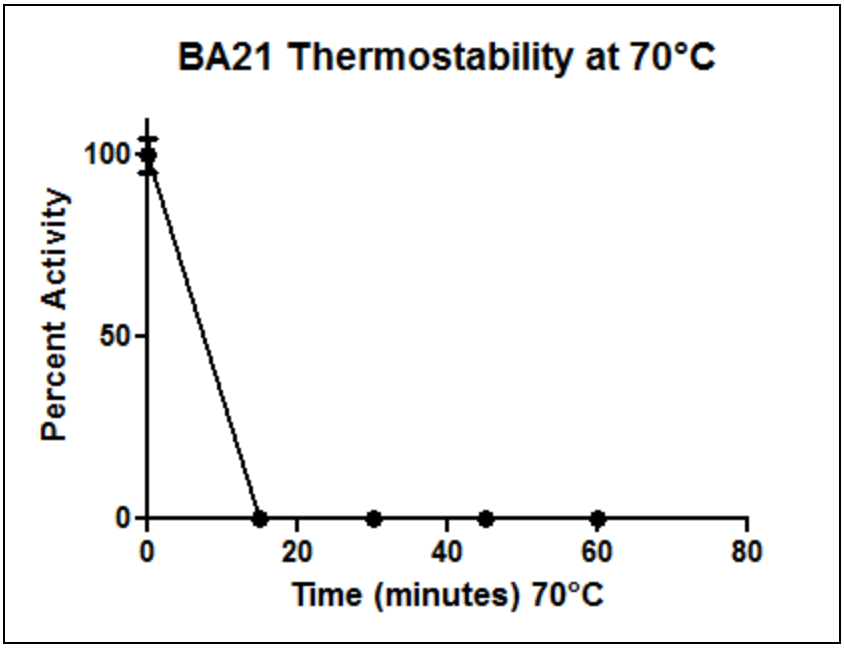


Figure 10. Thermostability of BA21 as assessed using SPR.
BA21 ceased all activity after 15 min.

3.6.2 Kinetic Analysis by SPR

Kinetic analysis of the Illumina AFX 1494 binding to the BA21 antigen was performed as a direct binding SPR experiment on the Biacore T200. Results are presented in Figure 11. Data were normalized to a blank-immobilized reference flow cell and fit to a Langmuir 1:1 model using Biacore T200 software. The K_D of AFX 1494 was determined to be 977 pM. Data from similar experiments that were performed using the original BA21 are presented in Figure 12. The K_D of BA21 was determined to be 4.99 nM; thus, Illumina provided an antibody that was well above the 100-fold improvement threshold.

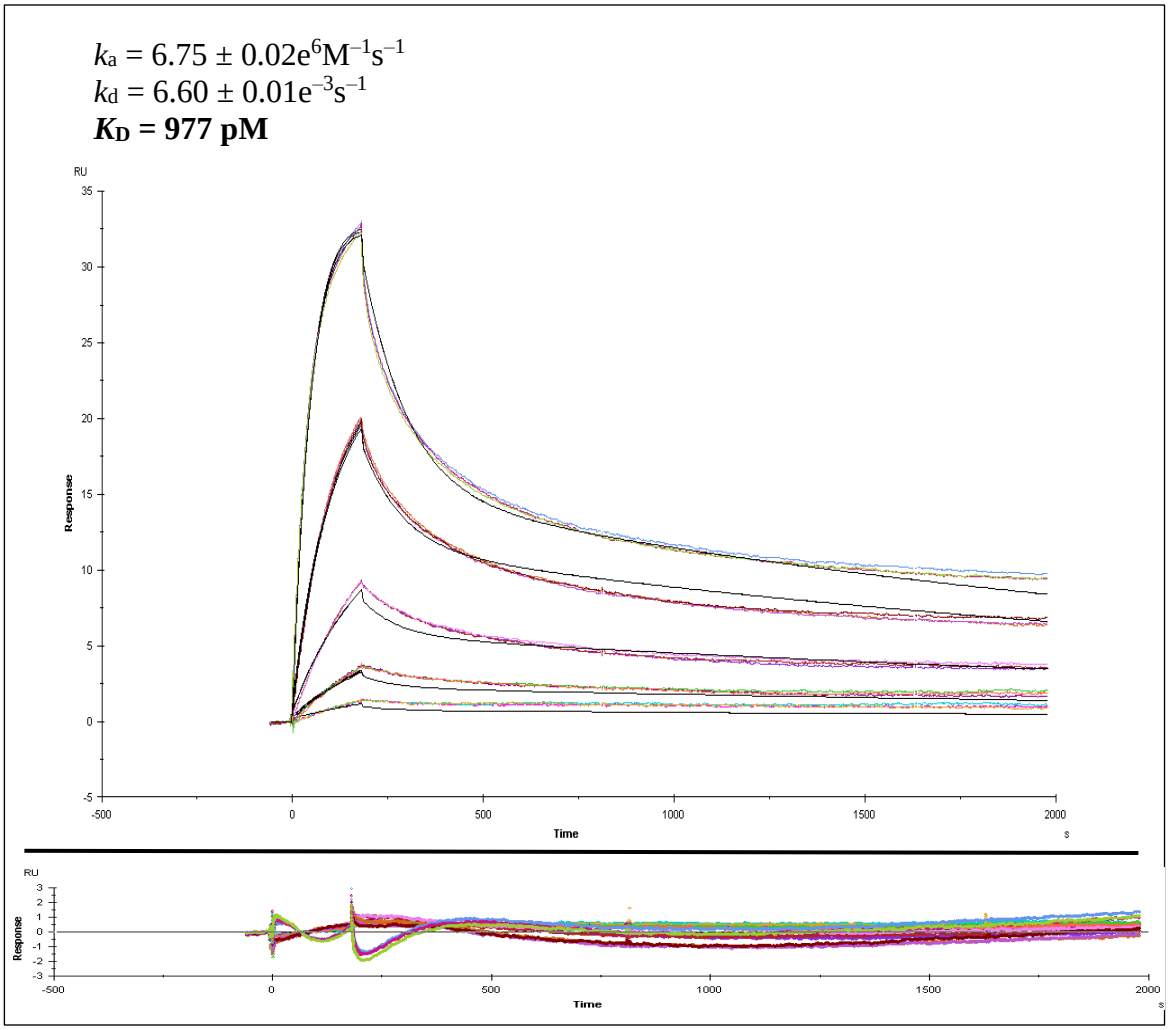


Figure 11. Kinetics of AFX 1494 as determined using a Biacore T200 system. The colors signify different test concentrations.

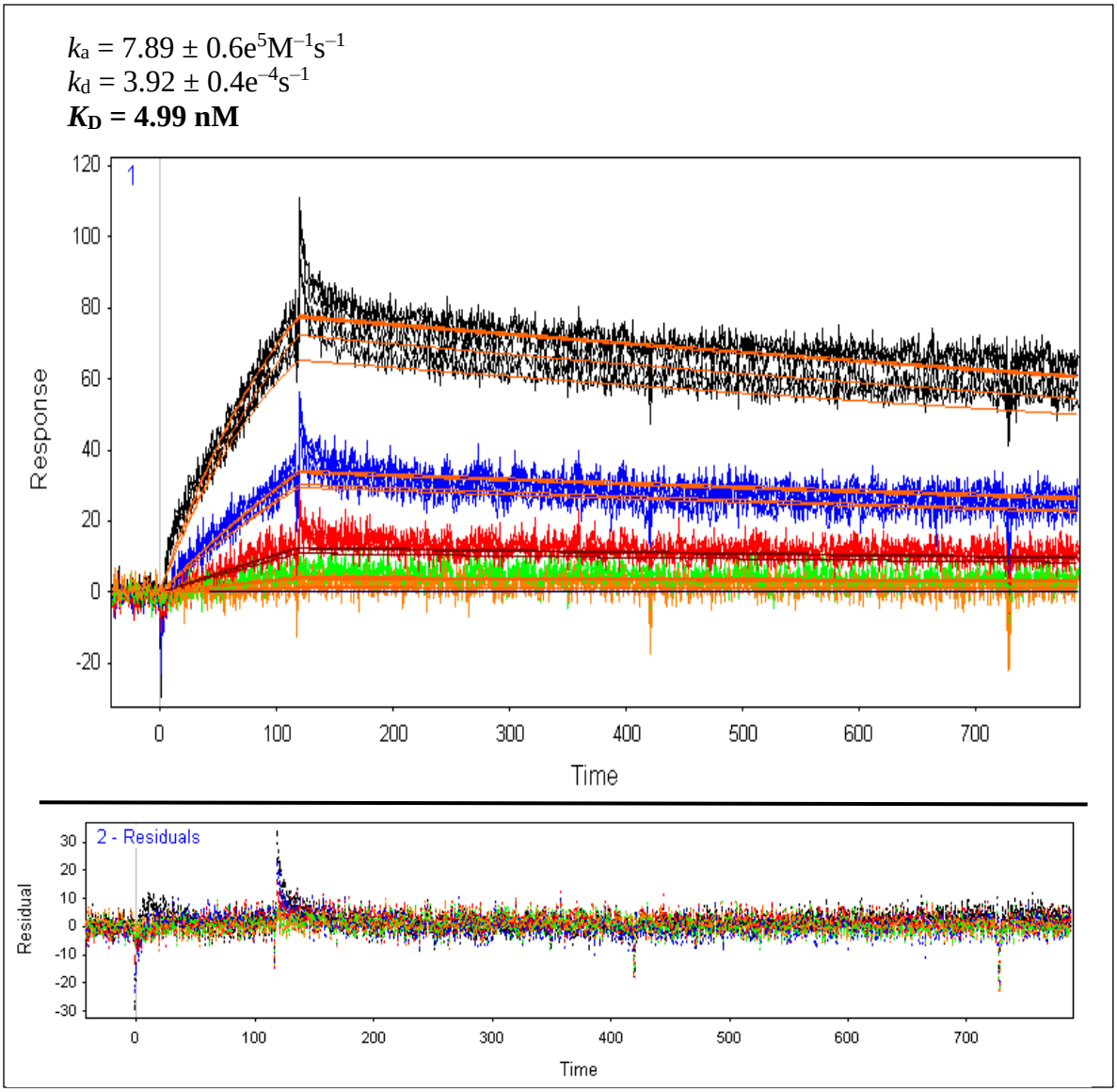


Figure 12. Kinetics of BA21 as determined using a Biacore T200 system. The colors signify different test concentrations.

4. DISCUSSION

In this study, standardized parametric tests were performed that were established during the MS2 scFv antibody DARPA ATP. The performer was able to demonstrate their molecular schemes for improving the thermal stability and affinity of an antibody for its target antigen. Images of the BA21 physical characteristics were obtained using the NanoDrop, Experion, and DLS measurement platforms, and these characteristics were compared with those of the improved antibodies submitted by Illumina. Measurements of the AFX 1494 functional characteristics were obtained using the DSC, ELISA, and SPR analytic platforms. These measurements were used to assess the effects of molecular engineering on thermal stability and affinity.

An accurate assessment of protein concentration was critically important for all of the test procedures described in this report. We applied a standard spectrophotometry technique using the NanoDrop ND-1000 system. With this, we obtained the A_{280} values of the samples. A_{280} values are influenced by the number of tryptophan and tyrosine residues in a given protein. For this reason, extinction coefficients were used in conjunction with A_{280} readings to determine accurate concentrations.

After concentrations were determined with the NanoDrop ND-1000 spectrophotometer, molecular weight and purity data were collected with the Experion automated electrophoresis system. This system uses microfluidic technology to automate electrophoresis for protein analysis. The results of Experion analysis of the Illumina AFX 1494 protein were within the acceptable purity range for use in assay development, and the molecular weight determined by the software was typical for antibodies.

DLS was used in conjunction with the Experion and NanoDrop ND-1000 systems to evaluate how the protein behaved in solution. DLS data indicated 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 AFX 1494 antibodies provided by Illumina were monomeric and monodisperse. Less than 1% of the sample mass appeared to be aggregating in solution. To mitigate the exacerbating effect of freeze–thawing on future sample aggregation and to ensure that all testing would be consistent, the AFX 1494 samples were aliquoted into single-use vials and centrifuged before use.

SPR was also used to obtain a kinetic analysis of the enhanced Illumina AFX 1494 binding to its target antigen BA21 to compare binding parameters with those for the original antibody. The K_D values show that Illumina improved the affinity measurement by 5-fold but did not meet the 100-fold improvement requested by DARPA.

5. CONCLUSION

The DARPA ATP sought to establish methods for rapidly engineering a given antibody reagent to exhibit physical and functional properties that far exceeded those of its native state. This is necessary to expand user confidence in fielding antibody-based detection and diagnostic platforms in environments and operational scenarios that degrade or interfere with the currently available reagents. By optimizing the thermal stability and binding affinity of an antibody for its biological target, the DARPA ATP sought to yield antibody reagents that can reliably function in harsh environmental conditions and increase the sensitivity of a sensor platform to detect lower levels of a threat agent.

This report documents the testing of an improved antibody produced by Illumina. The physical and functional characteristics of AFX 1494 in the ECBC testing pipeline were evaluated. The results were compared with the baseline characteristics of the original antibody's physical properties, to include concentration, molecular weight, purity, and state of aggregation in solution, as well as functional measures, such as binding affinity and thermal stability. Illumina provided a 2 g sample of AFX 1494 that produced an affinity of 977 pM. This improved the affinity measurement by fivefold but did not meet the requirement. The melting temperature of AFX 1494 was 72.0 °C, which almost met the requirement.

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ACRONYMS AND ABBREVIATIONS

A_{280}	absorbance of light at 280 nm
ATP	Antibody Technology Program
BclA	*Bacillus* collagen-like protein of *anthracis*
BGG	bovine γ-globulin
DARPA	Defense Advanced Research Projects Agency
DLS	dynamic light scattering
DSC	differential scanning calorimetry
ECBC	U.S. Army Edgewood Chemical Biological Center
ELISA	enzyme-linked immunosorbent assay
Fab	antigen-binding fragment
HRP	horseradish peroxidase
k_a	association rate constant
k_d	dissociation rate constant
K_D	equilibrium dissociation constant
mAb	monoclonal antibody
MDB	milk diluent block
PBS	phosphate-buffered saline
Pd	polydispersity
R_{Max}	maximum analyte-binding capacity of the surface
RU	response unit
scFv	single-chain variable fragment
sdAb	single-domain antibody
SPR	surface plasmon resonance
tBclA	truncated form of *Bacillus* collagen-like protein of *anthracis*
T_m	quantitative melting temperature

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