

ABSTRACT

The use of antibodies in DoD field applications allows for both a rapid and simple detection approach with the advantage of high specificity and moderate sensitivity. The DARPA Antibody Technology Program opened the field to the possibility of designing thermostable monoclonal antibodies that can be used reliably in handheld devices in the field, regardless of environmental conditions. This project aimed to produce equally viable polyclonal antibodies that offer both stability and strong binding capabilities. The Sandia National Laboratory has characterized a polyclonal Ab recognition spectrum for MS2 virus-like particles (VLP's) using a protein array approach and epitope identifiers through bacteriophage complexes. They then used the protein binders and epitopes identified in the recognition spectrum to identify affinity binders via recombinational biopanning. ECBC has been assisting with the optimization of the binding affinity equation by characterizing Monoclonal Antibodies against Ricin and using different ratios of the individual antibodies in a "pseudo" polyclonal soup to see how they affect overall binding and affinity.

BACKGROUND

Sandia has shown that random peptide libraries displayed on MS2 virus-like particles (VLPs) can be subject to affinity selection against either monoclonal Abs or polyclonal sera to yield detailed information about epitopes at the amino acid level (see below). Each round of selection requires small quantities (generally < 100 ng) of Abs. Consensus sequences can be obtained in as few as two rounds of selection. However, in a case where a family of epitopes emerge from the population, multiple screening rounds may be required. The use of Next-generation DNA sequencing was applied as a means to identify consensus sequences. MS2 VLPs are structurally simple and can be synthesized entirely *in vitro* by coupled transcription and translation in water-in-oil emulsion droplets. This *in vitro* ribosomal display of random peptide libraries allows for higher complexities than are readily obtained with other display systems. And by doing so, it dramatically decreases the time required for each round of affinity selection (24 hours vs 1 week).

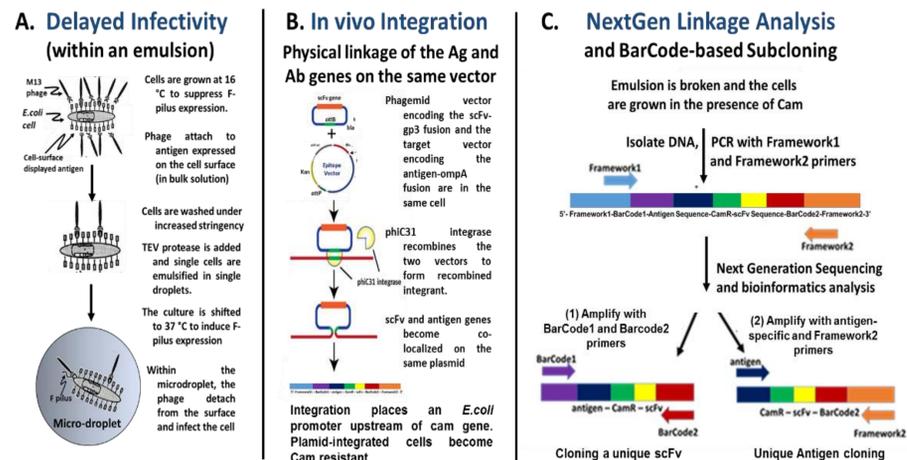


Figure 1. Recombinational Biopanning Approach. A) Antibody-antigen interactions increase specificity of phage infection and temperature is used to suppress and induce infection at the appropriate time. B) Antibiotic resistance achieved through colocalization of antibody and antigen vectors on the same plasmid and emulsion is formed C) A unique but complimentary antibody and antigen clone is created from the emulsion

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MATERIALS AND METHODS

Task 1: Characterization of a polyclonal Ab recognition spectrum using an integrated approach that included the following steps: (1) identifying proteins recognized by use of a protein array approach, and (2) identifying epitopes that primary binders recognize using phage MS2 VLP panning approach.

Task 2: The protein binders and epitopes identified in Task 1 were used to identify affinity binders using a novel approach deemed recombinational biopanning. These binders were grafted into a thermal stabilized IgG backbone and subcloned into vectors suitable for gene expression and protein production. The final clones were transitioned to the DoD for production.

Task 3: The Abs derived in Task 2 will be pooled in order to emulate the binding characteristics of the original polyclonal Ab. This will be accomplished using data from Task 1 on antigen prevalence as determined by VLP display.

Next Steps: ECBC was tasked with characterization of the individual Abs via BioLayer Interferometry (BLI). Additionally, BLI concentration analysis will be used to estimate the concentration of the individual components within the original polyclonal Ab. The goal of this task is to develop a mixture of stabilized monoclonal Abs that accurately emulate the binding characteristics of the original polyclonal Ab.

BLI Work Conducted at ECBC

- ❖ Ricin was immobilized onto the AR2G biosensor via amine coupling.
- ❖ The three chosen monoclonal antibodies were run independently in a 1:2 titration series beginning with 250 µg/ml and ending with 3.91 µg/ml.
- ❖ Nine different series of ratios comprised of the three Mab's were run in a 1:2 titration series all beginning with 250 µg/ml and ending with 3.91 µg/ml.
- ❖ Ratios: 1:1:1, 1:2:1, 1:1:2, 2:1:1, 1:3:1, 1:1:3, 3:1:1, 1:2:4, 3:1:5.

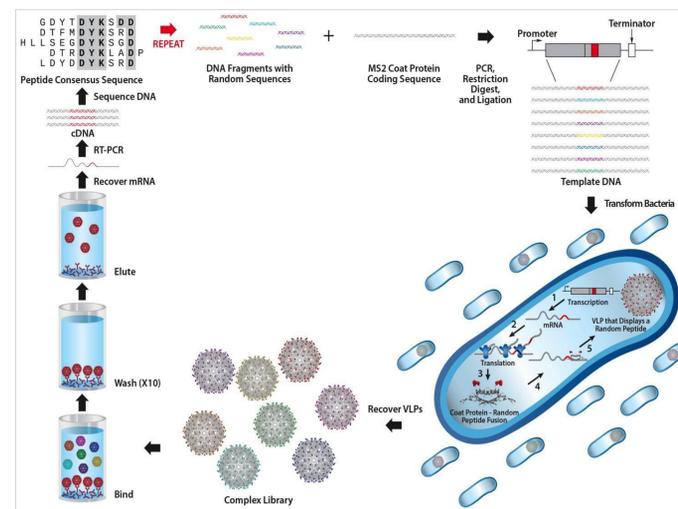


Figure 2. Library construction and affinity selection processes used to recover peptide mimics of antibody epitopes from a random sequence library displayed on MS2 VLPs.

RESULTS

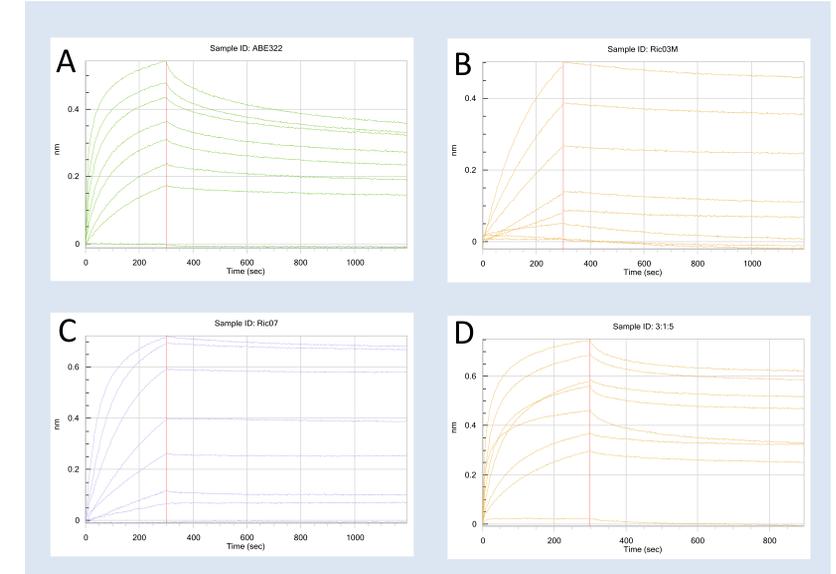


Figure 3. Sensograms of Ricin Antibodies. A) Individual Mab ABE 322 Ricin B Chain Binder B) Individual Mab RIC03M Ricin A Chain Binder C) Individual Mab RIC07 (ABE 298) Ricin A Chain Binder D) Mixture of individual Mab's (3:1:5) creating a Polyclonal "soup"

Sample	K _D	K _D Error	k _a	k _a Error	k _d	k _d Error	R _{max}	R _{max} Error	R ² Value
RIC03M	5.76E-10	8.91E-12	1.86E+05	1.51E+03	1.07E-04	1.41E-06	0.656	0.0027	0.9976
RIC07	5.20E-11	<1.0E-12	7.19E+05	1.51E+03	3.74E-05	5.97E-07	0.706	0.0003	0.9993
ABE322	9.70E-11	<1.0E-12	3.60E+06	2.83E+04	3.49E-04	2.16E-06	0.4782	0.0006	0.9759
Ratio 1	1.16E-11	<1.0E-12	1.06E+07	7.97E+04	1.22E-04	1.66E-06	0.8098	0.0009	0.9658
Ratio 2	1.18E-11	<1.0E-12	1.40E+07	1.22E+05	1.64E-04	1.88E-06	0.5013	0.0007	0.9519
Ratio 3	2.37E-11	<1.0E-12	1.08E+07	7.77E+04	2.55E-04	1.76E-06	0.2222	0.0003	0.9661
Ratio 4	1.50E-09	2.48E-11	2.13E+05	2.16E+03	3.20E-04	4.17E-06	0.366	0.0009	0.9316
Ratio 5	1.20E-09	2.58E-11	2.02E+05	2.40E+03	2.42E-04	4.36E-06	0.7494	0.0015	0.931
Ratio 6	2.22E-09	4.01E-11	1.23E+05	1.28E+03	2.73E-04	4.02E-06	0.8812	0.0014	0.9673
Ratio 7	1.81E-09	3.48E-11	1.58E+05	1.79E+03	2.85E-04	4.45E-06	0.6993	0.0013	0.9613
Ratio 8	1.59E-09	3.02E-11	1.55E+05	1.60E+03	2.46E-04	3.93E-06	0.5899	0.0013	0.966
Ratio 9	2.32E-09	4.01E-11	1.06E+05	9.11E+02	2.46E-04	3.69E-06	0.3974	0.001	0.9716

Table 1. Affinity measurements for the individual Mab's and Polyclonal "soup" ratios.

CONCLUSION

Mixing the individual Mab's into several different ratios and then running them against the Ricin target did not significantly impact the overall binding affinity or K_D of 3 of the 9 ratios. Ratios 1:1:1, 1:2:1, and 1:1:2 maintained the same level of binding affinity. The results show that mixing select monoclonal antibodies in correct proportions can mimic the multiple epitope binding properties of a polyclonal antibody.

ACKNOWLEDGEMENTS

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