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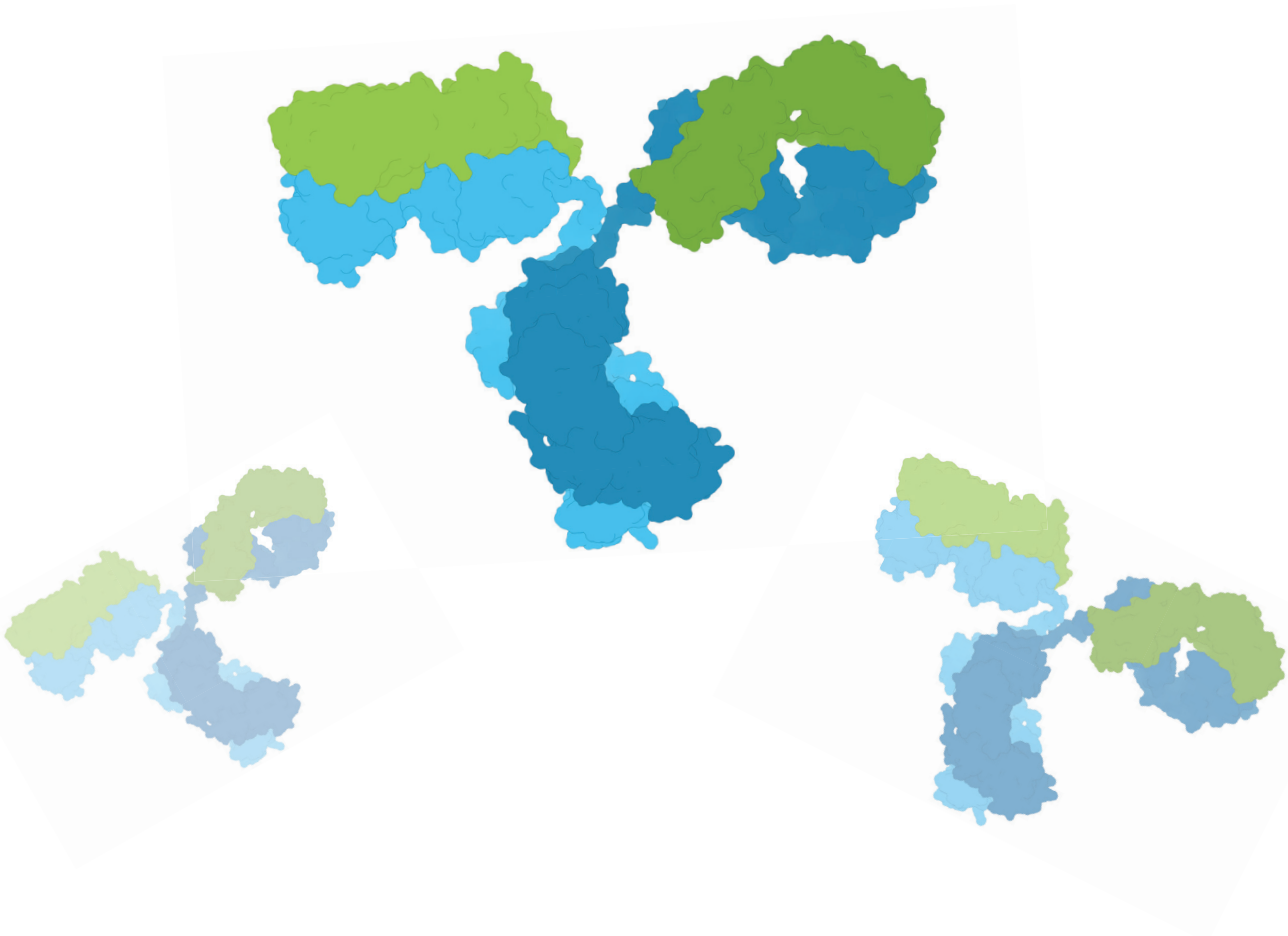
Recombinant Antibody Discovery and Development

From Antigen Design to Characterization with Aviva Systems Biology



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Recent analyses indicate as much as 50% of commercial antibody products do not work as intended. To address the outsized effect of poor antibodies on the reproducibility crisis, researchers are pushing for a transition to recombinant antibody production systems.

This whitepaper discusses our newly-launched recombinant antibody manufacturing platform, while presenting in-house data from the development of anti-NFκB antibodies, from antigen design to production.

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Recombinant Antibody Discovery and Development

From Antigen Design to Characterization

INTRODUCTION

Antibodies are some of the most essential and widely used biotechnologies. Following basic immunology research describing immunization and antibody production, antibody-based detection techniques quickly became a foundational pillar of life science research.[1] Antibodies form a critical basis for the specific detection of analytes in a variety of biological assay formats, including Western Blotting (WB), Enzyme-linked immunosorbent assays (ELISA), Immunoprecipitation (IP), Chromatin Immunoprecipitation (ChIP), Immunohistochemistry (IHC), Immunocytochemistry (ICC), Immunofluorescence (IF) Flow cytometry, and Fluorescence-Assisted Cell Sorting (FACS).[2,3,4,5,6] Researchers apply these assays everywhere, from basic research, drug development, clinical diagnostics, and pharmaceutical manufacturing quality control.

Responding to the demand for target-specific antibodies, a large cohort of antibody discovery and manufacturing companies sprung up to serve researchers. The antibody biotechnology industry has continued to evolve and mature, alongside impressive growth in the number of targets covered by commercial antibodies, including ~96% of the human proteome.[7]

Polyclonal Antibodies

The longest-standing antibody products are polyclonal preparations, which collect serum post-immunization to extract antibodies from multiple B-cell lineages. Polyclonal antibodies can produce strong assay signals, in part because their antibodies may bind distinct epitopes on the same protein. Though polyclonal antibodies benefit from *in vivo* affinity and quick development timelines, their uncharacterized and heterogeneous antibody composition can complicate analyses and lead to non-specific and off-target binding. The dependence on repeated animal immunizations can also create batch-to-batch variation associated with varied individual immune responses.[8]

Hybridoma Monoclonal Antibodies

Beyond polyclonals, monoclonal antibodies produced from hybridomas, which apply the research and methods that led to the 1984 Nobel Prize in Medicine,[9] have also become commonplace. Since monoclonal antibodies derive from single B cell clones, these antibodies are homogenous and can be readily characterized, though they often require longer timelines. However, monoclonal antibody-producing hybridoma cell lines can also display low yields, inconsistent performance, and quality changes associated with long-term continuous production, due to the accumulation of mutations and chromosomal instability.[10]

Recombinant Antibodies

Together, polyclonal and monoclonal preparations have made up the bulk of historical and current antibody products used in countless studies and assays. More recently, antibody providers have begun switching to recombinant antibody production, where antibody-coding DNA sequences are cloned and expressed in cell culture.[11,12] Largely, this push is driven by a need for greater reproducibility and more reliable biomanufacturing.

Figure 1: Aviva Systems Biology recombinant antibody manufacturing process

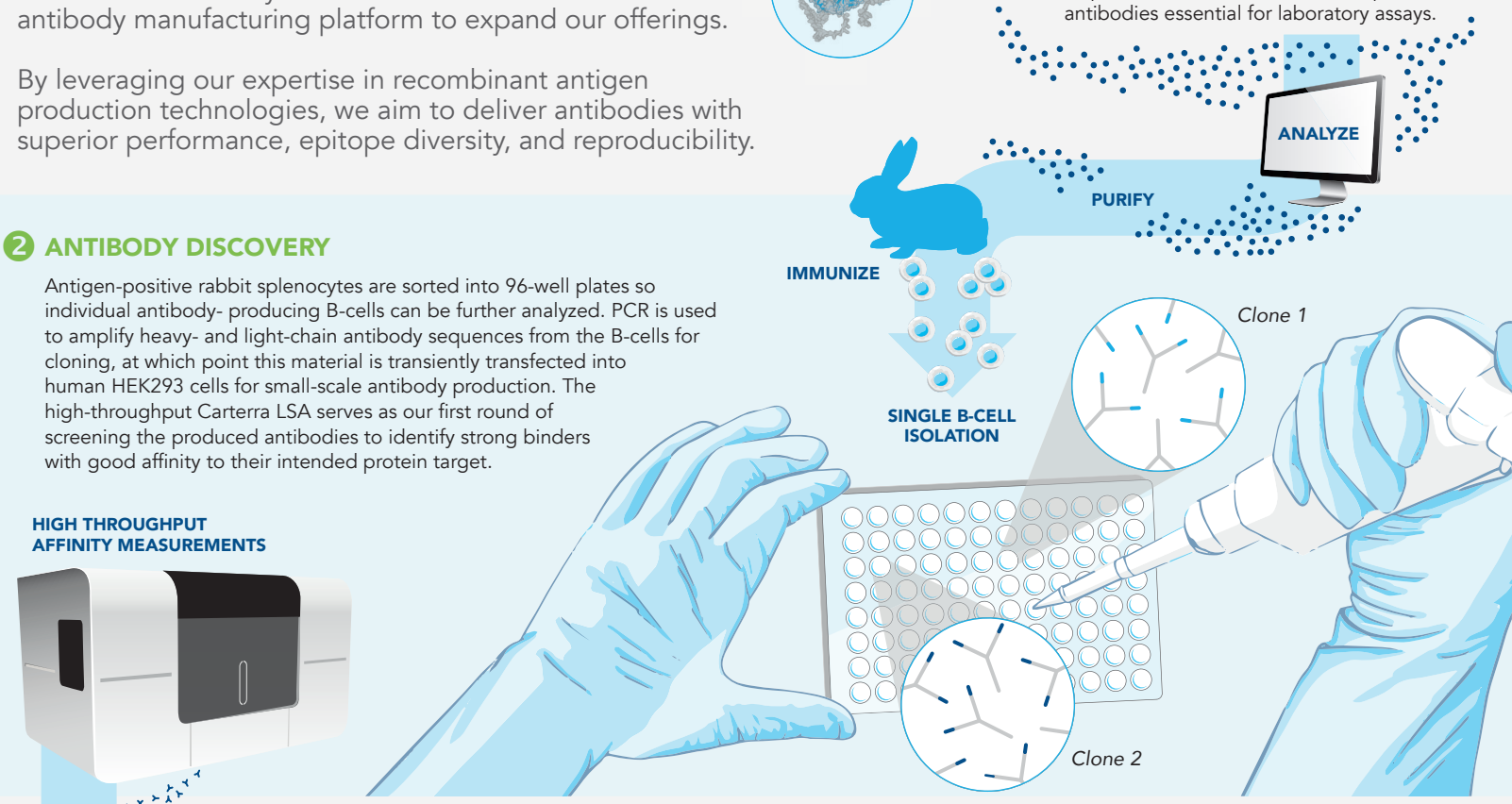
From Antigen Design to Characterization

Aviva Systems Biology has been a trusted provider of off-the-shelf and custom antibody products for decades, and we have recently launched a new recombinant antibody manufacturing platform to expand our offerings.

By leveraging our expertise in recombinant antigen production technologies, we aim to deliver antibodies with superior performance, epitope diversity, and reproducibility.

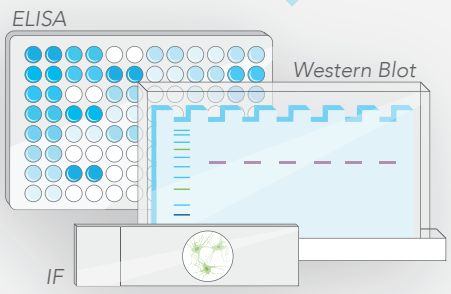
1 ANTIGEN DESIGN

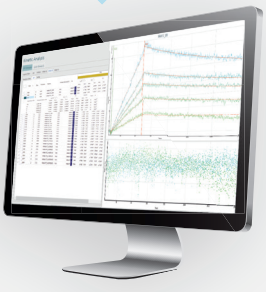
- For a single target, we start by designing a variety of antigenic protein fragments, evaluated on their key structural and biophysical characteristics.
- This approach ensures a breadth of immune responses, generating a diverse repertoire of antibodies essential for laboratory assays.

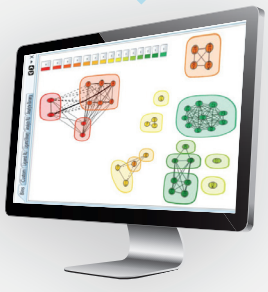


2 ANTIBODY DISCOVERY

Antigen-positive rabbit splenocytes are sorted into 96-well plates so individual antibody- producing B-cells can be further analyzed. PCR is used to amplify heavy- and light-chain antibody sequences from the B-cells for cloning, at which point this material is transiently transfected into human HEK293 cells for small-scale antibody production. The high-throughput Carterra LSA serves as our first round of screening the produced antibodies to identify strong binders with good affinity to their intended protein target.







3 APPLICATION TESTING

To demonstrate antibody performance and reproducibility, antibody clones are characterized via Western Blot, ICC, SPR, and ELISA.

4 KINETIC PROFILING

The performance of antibodies can be evaluated by their kinetic properties, characterized by rates of binding and dissociation (k_{on}, k_{off}), and the affinity of the antibody for the antigen, K_D.

5 BINNING & MAPPING

To develop high-precision ELISAs, we identify robust antibody pairs by testing pair-wise combinations in sandwich assays. The results allow us to assign epitope bins and build community plots.

THE CENTRAL ROLE OF ANTIBODIES IN THE REPRODUCIBILITY CRISIS

Despite the central importance of antibodies to the life science sector, it's clear that a significant portion of commercial antibody products do not work as intended. Recent studies have suggested that as much as ~50% of antibody products[13,14] either struggle to bind their target or showcase significant off-target binding, with other reports suggesting an even more pessimistic view.[15]

To emphasize the cost of inadequate antibody performance, it has been estimated that the US alone wastes between 350 Million[16] and 1.7 Billion[17] USD annually on poor-performing antibody products, separate from the cost of time lost, other reagents, and instrument use. As the antibody market continues to grow, so too will the expected waste. More broadly, the cost of irreproducible pre-clinical research in the US alone has been estimated to be well over 20 billion USD,[18,19] in addition to its negative impact on the sustainability of the scientific enterprise.[20]

To address this far-reaching and costly challenge, many antibody researchers and non-profit scientific organizations have called for standardizing analysis workflows and a transition to recombinant antibody production systems.[7,13,16,17,18,21,22,23,24]

Rigorous standardized antibody validation and data registration allow for more accurate and consistent characterization of antibody specificity ahead of their use in assay formats. Furthermore, transparent data sharing allows users to clearly understand the potential performance limitations.

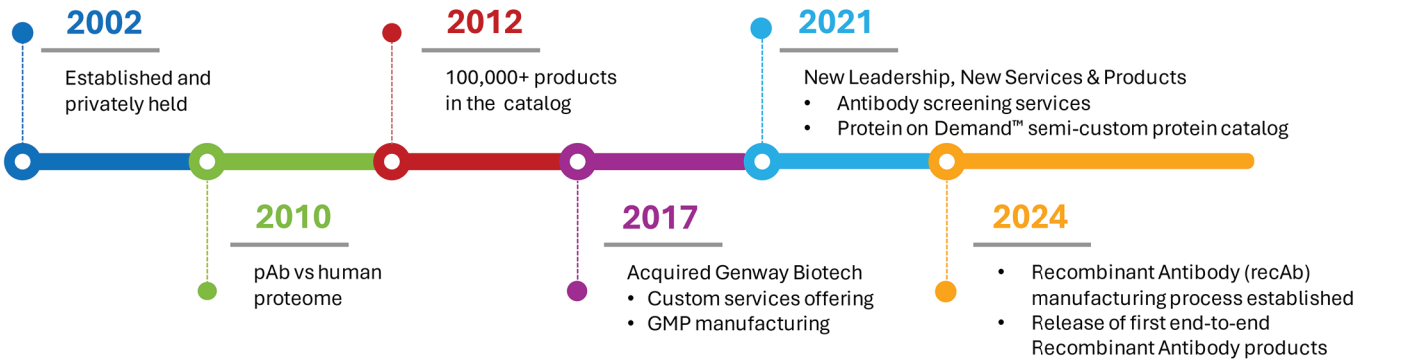
Even with better characterization and data sharing, the inherent limitations of polyclonal and hybridoma-based monoclonal antibodies remain. Thus, shifting the sector towards recombinant production systems is perhaps most important to the long-term improvement of antibody performance, due to their great renewable potential and strict genetic characterization.

To highlight this, Ayoubi et al. assessed the performance of 614 commercial antibodies (258 polyclonal, 165 monoclonal, and 191 recombinant antibodies), including those from Aviva Systems Biology. The authors found that a higher percentage of recombinant antibodies performed better in all formats (WB, IP, IF) compared to polyclonal and monoclonal antibodies (**Table 1**).

Table 1: Percent of evaluated commercial antibodies that correctly bind intended target[13]

Assay Format	Western Blot (WB)	Immunoprecipitation (IP)	Cellular Immunoflouresence (IF)
Recombinant	67%	54%	48%
Polyclonal	27%	39%	22%
Monoclonal	41%	32%	31%

Figure 2: Aviva System's Biology Through the Years



MAKING ANTIBODY ASSAYS MORE RELIABLE AND REPRODUCIBLE

Aviva Systems Biology has spent decades as a leading provider of off-the-shelf and custom antibody products for research, diagnostic, and quality control purposes (**Figure 2**). Though Aviva Systems Biology has long maintained a careful emphasis on antibody characterization and quality control in our products, our extensive portfolio has historically been made up of polyclonal antibodies.

Recognizing the significant need of the life science and biomedical communities, Aviva Systems Biology launched a recombinant antibody platform and intends to expand our offerings in that direction going forward. Combining our expertise in antigen design, immunization, and antibody validation with recombinant production technologies, we can provide researchers with greater performance, target diversity, and reproducibility to benefit their research and assay applications.

The remainder of this whitepaper describes Aviva Systems Biology's recombinant antibody platform, providing an overview of our processes for discovering and manufacturing high-performance recombinant antibody products: from antigen design to characterization and validation. More specifically, this piece will explore data from the development of recombinant antibodies that bind NFκB, a key transcription factor at the center of several cellular pathways.[25] More specifically, this program targeted NFκB1, an important regulator of NFκB activity *in vivo* that plays a critical role in inflammation, aging, and cancer.[26]

ANTIGEN DEVELOPMENT

After selecting a molecular target, the first step toward a high-performing antibody reagent is antigen development. For *in vivo* immunization, targets must be both sufficiently specific and immunogenic such that the immune system responds appropriately to the antigen and initiates affinity maturation towards its target. Done correctly, B cells will produce antibodies that are more likely to bind to a target with high affinity and specificity. Yet, predicting antigenicity remains quite challenging. Though antigenicity assessments can help researchers make predictions about candidate antigen success, it is difficult to account for the inherent complexity of immunization and B-cell maturation, resulting in varied responses due to inaccurate or incomplete models.

Even with good predictive tools, balancing those two parameters is no easy feat, especially when researchers must also factor in the ability to express and solubilize the target in sufficient amounts and dissolve them into solution at high enough concentrations. Together, these variables often make the use of full-length protein antigens less practical, since cloning, expressing, and solubilizing larger genes is often more difficult.[27] Full-length proteins may also have hydrophobic or intrinsically disordered regions that complicate structural presentation in immunization formats. As a result, immune reactions might produce antibodies against unnatural epitope conformations of the antigen that don't bind the native protein consistently.

For these reasons, many antibody developers opt to use peptide antigens, given their relative simplicity compared to full-length proteins and protein fragments.[28] Peptides are relatively simple to produce and solubilize (depending on the amino acid sequence) and can provide some ability to target highly specific protein sequences. However, the simplicity of peptide antigens also comes with some drawbacks (Table 2).

Though peptides do provide some ability to target highly specific sequences, they lack the ability to act as conformational antigens. Similarly, a protein's 3D structure can obscure unique peptide sequences such that they are not presented linearly in its native conformation. Unless the linear epitope is exposed, antibodies raised against peptides may fail to bind the target in an assay-specific manner, resulting in cross-assay performance issues.

To circumvent these reproducibility challenges, Aviva Systems Biology developed a proprietary method for antigen design that operates from larger protein fragments. Larger protein fragments are easier to express and purify compared to full-length proteins, while also providing more quantifiable, reproducible antigen selection akin to peptide options (Table 2). Importantly, provided that the fragment maintains its native structure in the full-length protein, we can also generate conformational antigens for improved affinity and specificity of the antibody, while also reducing non-

Table 2: Advantages and disadvantages of different antigen types.

Antigen Type	Pros	Cons
Full-Length Protein	• Conformational antigens if folded successfully • Some full-length protein has market value	• Human proteome has high intrinsic disorder • Larger genes have higher chance for cloning errors • Expression and analysis of large proteins is difficult • Classic approach often not reproducible
Peptide	• Cheaper and faster to produce • Only requires protein sequence (not structure) • Useful when protein has high sequence similarity to others • Effective for detection of post-translational modifications	• Only recognize linear epitopes • Less likely to be effective for recognizing protein when in native state, thereby often limiting applications to western blotting.
Protein Fragment	• Smaller, easier to express and purify • Ease of cloning and validation • Quantifiable, reproducible selection • AlphaFold-mediated secondary, tertiary structure • Conformational antigens if folded successfully	• Potential loss of tertiary structure • Stability issues can arise

specific binding. In addition, larger protein fragment antigens make studying molecular interactions by surface plasmon resonance (SPR) easier and more precise. SPR is an analytical method used to measure binding affinities, and SPR detection improves when the ligand and analyte are closer in size.[29]

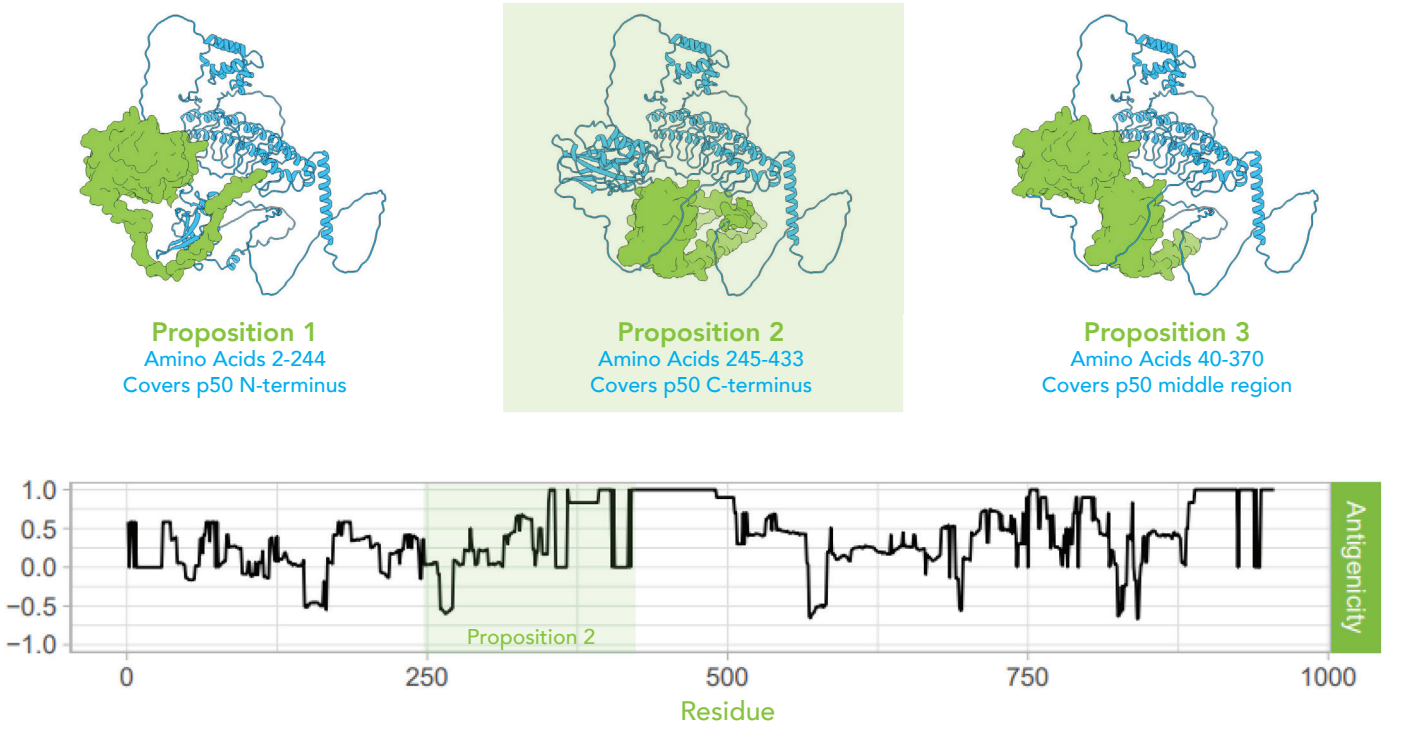
To improve the reliability and throughput, Aviva Systems Biology's R&D team developed an AI-guided antigen design method, which allows for rapid, quantifiable identification and scoring of potential antigens (which we call propositions, **Figure 3**) from target proteins. Though the model takes traditional immunogenicity and solubility indicators into consideration, we further fortified its output with our own internal data. Using 20+ years of performance and sales data from ~24,000 antibody products, our R&D team extracted valuable information about what makes an effective and expressible antigen and incorporated those learnings into the model.

The antigen design process also incorporates structural information to ensure that fragments recapitulate their native structure. However, structural data is still unavailable for many relevant protein targets as well as for specific protein fragments. To work around this, Aviva Systems Biology applies AlphaFold[30] to generate fragment structures. In doing so, we can better understand the fragment's possible structure, evaluate its solubility, and predict stability.

Through the inclusion of AlphaFold-generated fragment structural information into our proprietary antigenicity and expression algorithm, Aviva can reliably generate protein fragment propositions with a greater likelihood of success as antigens.

Examining NFκB, Aviva was able to generate and explore a number of propositions using our antigenicity model (**Figure 3**). Based on the outcome of our analysis

Figure 3: Example fragment antigen propositions and antigenicity score from NFκB. Positions are highlighted in green.



(Figure 4A), which factors details like rolling averages for amino acid enrichment, predicted disorder, and solvent accessibility, NFκB1 Prop. 2 (the p50 subunit) was carried forward to expression and purification. NFκB1 p50 is a key subunit of the NFκB complex produced via proteolytic processing of the full NFκB1 gene product (p105). Typical rabbit immunizations require at least 10 - 100 µg in approximately 500 µL to provide high enough antigen concentration for effective immunization.[31] As predicted, Prop. 2 expression produced sufficient antigen for immunization (Figure 4B).

Leveraging decades of experience and data collection with modern AI technologies, Aviva Systems Biology generated a model that makes antigen design faster and more effective. Using this AI-guided approach, Aviva Systems Biology improved our rate of antigen development from single antigen propositions to up to 60 antigen propositions per month. This allows our team to generate numerous antigens from the same protein for parallel immunization, offering increased success rates and potential for greater epitope diversity.

Figure 4A: Detailed antigenicity evaluation of NFκB Proposition 2

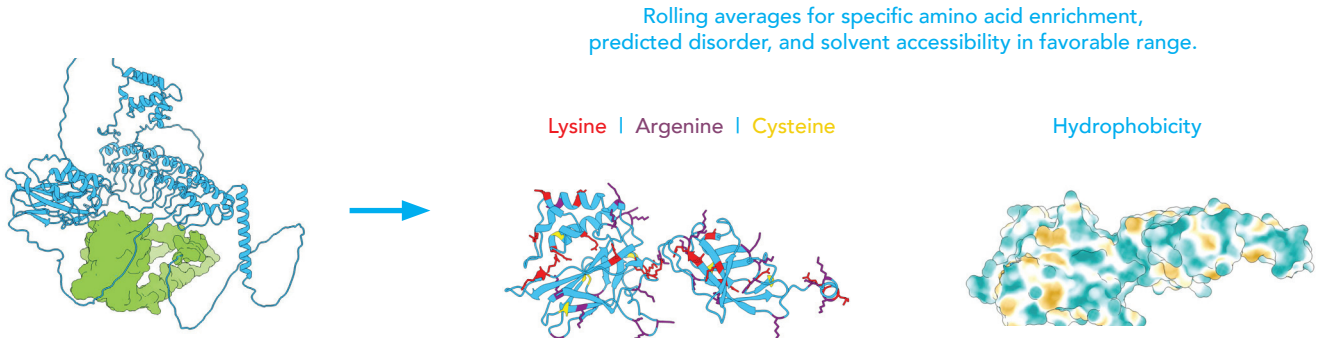
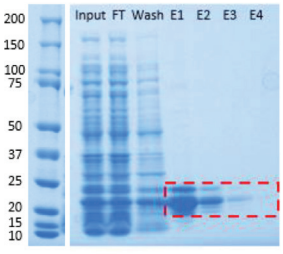


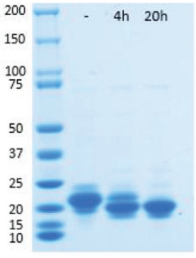
Figure 4B: Expression data and PAGE gels of NFκB Proposition 2

NFκB1 p50	Proposition 2
Range (aa)	245 - 433
Fusion	6xHis-tag
MW (kDa)	23.15 (20.48 untagged)
pI	8.19
ε	17,670
Yield	56 mg @ 2mg/mL

Purification
- Flowthrough (FT)
- Elution fractions (E1-E4)



Tag Removal
- TEV protease
- Final Antigen



IMMUNIZATION & AFFINITY MATURATION

After settling on antigen candidates, antibodies must undergo an affinity maturation process, either *in vivo* in specific organisms or *in vitro* using methods like display technologies.

Though *in vitro* methods like phage display are becoming increasingly prevalent,[32] animal immunization remains the primary mechanism for generating antibody candidates. The advantages of *in vivo* affinity maturation are a major contributing factor.[33] Natural immune evolution continually optimized this complex process over hundreds of millions of years, creating incredible paratope diversity and high affinity antibodies.[33] Immunization also allows researchers to access valuable information about an antibody's lineage and paratope patterning.[34] Though animal immunization can be time-consuming (~2-3 months) compared to *in vitro* technologies, phage display technologies typically require additional expense, due to library acquisition, infrastructure, and expertise.

For these reasons, Aviva Systems Biology utilizes rabbit immunization (**Box 1**) to elicit a strong immune response and generate B-cells expressing target-specific antibodies.[33] By combining animal immunization and single B-cell cloning, Aviva Systems Biology harnesses nature's preeminent affinity maturation capabilities with the reproducibility and renewability of recombinant heavy and light chain gene expression.

Our current recombinant antibody development pipeline has hundreds of antigen targets in rabbits, and we have witnessed strong immune responses in nearly everyone. Additionally, serum extracted from rabbits immunized for our recombinant antibody pipeline demonstrates potent serum titers against their target.

For NFκB antibody development, strong immune responses were detected in a number of rabbits. Two rabbits immunized with the NFκB p50 antigen proposition displayed detectable titers at serum dilutions below 1:500,000, indicating significant potency and highly sensitive detection of the antigen. The best-performing rabbit was carried forward for B-cell isolation, antibody cloning, and characterization.

Box 1: Why rabbits?

Rabbits have become a model of choice for animal immunization over rodent systems, due to their more complex B-cell maturation process,[35,36] genetic diversity,[37] stronger immune responses,[38,39] and greater evolutionary distance to humans and rodents.[37] Together, these features allow rabbits to generate antibodies with greater diversity and a wider range of epitope targets that are non-immunogenic in other systems, like mice or rats. It also means rabbits can produce antibodies against mouse and rat antigens.

The unique structure of rabbit immunoglobulins also provides greater antibody stability,[36,38] while their larger bodies allow for greater immune cell collection yields. There is also only one IgG isotype in rabbits, which means rabbit antibodies are uniform in structure.

Finally, the third complementarity-determining region (CDR3) of the light chain is longer in rabbits compared to humans and mice,[40] which may explain why in some studies rabbit antibodies demonstrate higher affinities than mouse antibodies against the same target.[41]

B-CELL ISOLATION AND GENE CLONING

To identify cells expressing antigen-binding IgG antibodies, our researchers use 6-color cell sorting instrumentation to screen rabbit blood cells. Starting from hundreds of millions of cells, the first color is used to gate living cells and exclude dead ones, resulting in approximately ~10 million cells. The second gate uses another color to gate singlet cells from clumps, trimming the cell count by a few million. The next two colors are used to screen for IgG-producing immune cells, selecting CD4-, CD8-, and IgM- cells, which further reduces the candidate cell count.

With the remaining millions of IgG producing cells, the final two colors are used to stain membrane-bound B-cell receptors (BCRs) with antigen bait to identify cells producing antigen-specific antibodies. Antigens are

biotinylated and stained with two anti-biotin binding antibodies, each with a distinct fluorophore label. This double-staining confirmation helps reduce false positives associated with non-specific binding and sticky antigens. Given that only ~0.05% of IgG-positive cells recognize the target antigen, this results in approximately ~1 to 3 thousand double positive antigen-binding cells. These cells are directly sorted into well plates containing lysis buffer for RT-PCR amplification of single-cell RNA, followed by further amplification of heavy and light chain variable genes.

Candidates progress if the clonal pair of heavy and light genes are detected. Clonal pairs are transfected and transiently expressed in cells to produce antibody candidates at small scales in supernatants. This allows Aviva Systems Biology to move to deeper candidate profiling much more rapidly, since it eliminates the generation and transfection of thousands of plasmids containing antibody candidates. After detailed characterization (discussed below), researchers cull the candidate pool to best options and then clone heavy and light chain genes into microbial chassis for long term recombinant expression.

Collectively, this approach provides rapid and high-throughput B-cell screening, allowing the identification of a multitude of antibody candidates, including rare binders.[10] But before finalizing the best candidates from the library pool, we explore their activities in both kinetic profiling and application testing.

ANTIBODY CHARACTERIZATION & PERFORMANCE

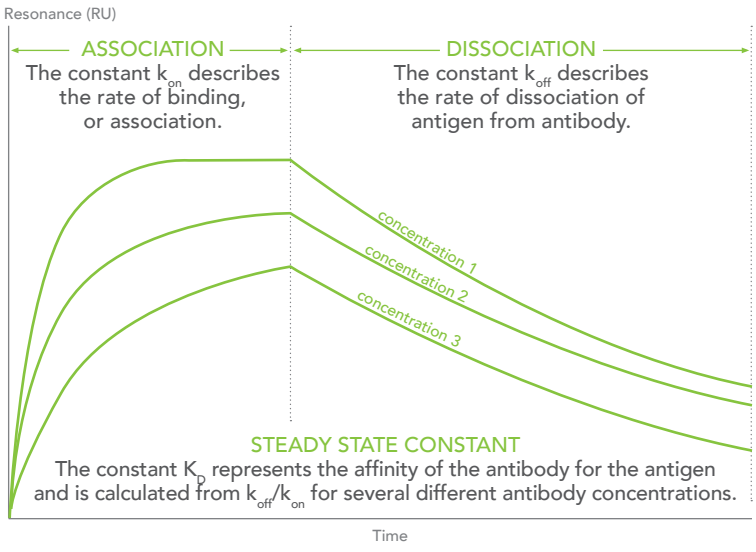
Detailed, individualized antibody validation helps establish greater reproducibility in commercial antibodies.[21] For this reason, Aviva Systems Biology has long implemented detailed characterization and quality control methods to ensure the robust use and reliability of our antibody products. As part of this, we have maintained a partnership and collaboration with Antibody Characterization through Open Science (YCharOs), an open science company with the mission of characterizing commercially available reagent antibodies for every human protein. Aviva Systems Biology has also contributed to validation method research with these external collaborators, including recent work published in eLife from Ayoubi et al.[13]

High-throughput Kinetics Profiling

To take our analysis deeper, we execute kinetic profiling of all candidates using our high-throughput SPR workflow.[42] To our knowledge, virtually no other antibody companies do this as routinely and extensively as Aviva.

After immobilizing antibody candidates, antigen analytes are run over the entire array of antibodies, allowing kinetic screening of up to 1,152 mAbs and 384 clones in parallel to generate affinity curves and perform true analysis. Using software tools, these curves can be rapidly fitted and analyzed to determine K_D and k_{on}/k_{off} rates. For context on SPR data analysis, **Figure 5** provides an overview of SPR curves to help explain curve fitting and constant extraction.

Figure 5: Understanding and Analyzing SPR Binding Curves



Sensorgrams represent the kinetic responses of the analyte A (e.g., antigen), at different concentrations, binding to the ligand L attached to the surface (e.g., antibody) and dissociating when the flow switches back to the buffer.

When the binding phase is fast and dissociation is slow, the K_D parameter is very low (in the picomolar range) and indicates tight binding between antibody and antigen. Whereas, when the dissociation curve is steep, the off-rate is fast, indicating the antigen and antibody are weakly bound.

In this manner, antibodies generated from NFκB immunizations were analyzed. Following analysis, a number of viable antibody candidates were identified. Table 3 shows a collection of four representative high-affinity antibodies analyzed in this study, each with low nanomolar K_D values (1.8 to 24 nM).

Even in this four-member collection (anti-NFkB clones 8M5-I, 8M5-5, 8M5-L, and 8M5-M), different kinetic profiles were found. Clone 8M5-I has slightly slower on- and off-rates compared to clone 8M5-L, resulting in these clones having similar affinities (22 and 24 nM, respectively. Though clone 8M5-5 has the highest overall affinity (1.8nM), clone 8M5-M has a very gradual off-rate that is slower than clone 8M5-5.

Kinetic diversity in antibody selection provides researchers with an additional parameter they can tune for their experiments. Choosing the highest affinity (lowest K_D) antibody is not necessarily the most desirable option. For optimal efficacy, a higher K_D is sometimes preferred. For example, antibodies intended to function as agonists of specific receptors can deliver greater activity at lower affinity.[43,44] Through our recombinant platform, Aviva can produce and characterize a range of antibodies with varying K_D values and on/off rates, allowing fit-for-purpose selection.

Application Testing

Application testing demonstrates antibody performance in common assays, such that users understand their

Table 3: Kinetic profile of select anti-NFκB antibody clones

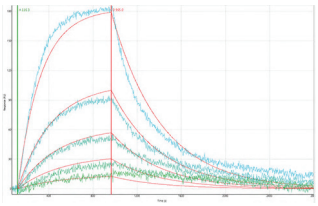
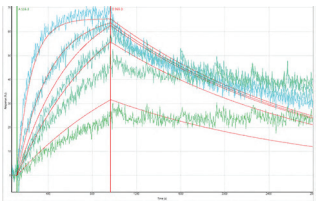
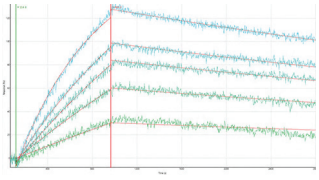
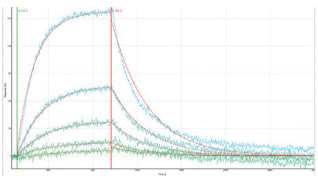
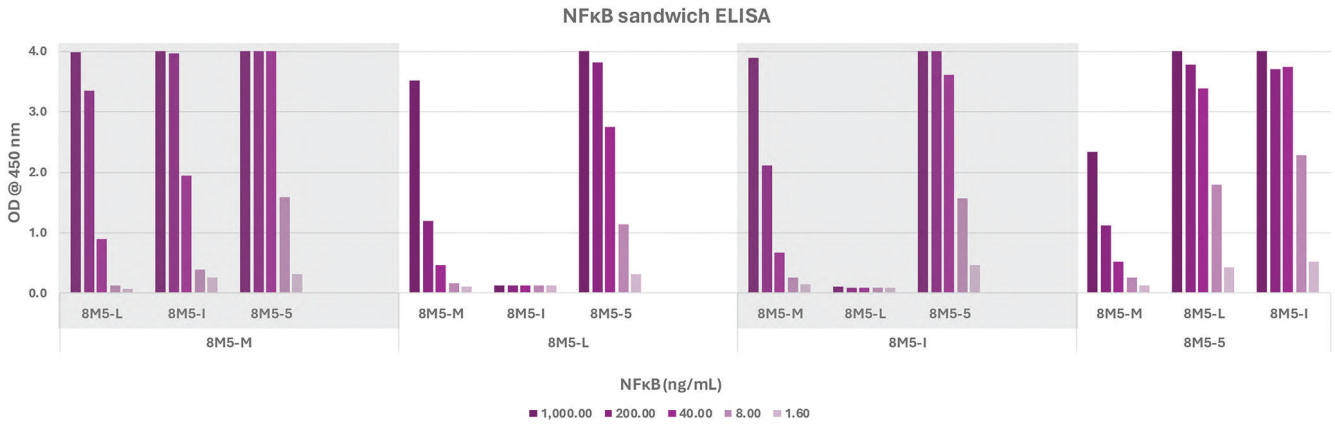
Antibody Clone ID	K_{on} ($M^{-1}s^{-1}$)	K_{off} (s^{-1})	K_D (nM)	SPR Sensorgram
8M5-I	1.0e5	2.2e-3	22	
8M5-5	3.0e5	5.3e-4	1.8	
8M5-M	5.2e4	1.3e-4	2.4	
8M5-L	1.3e5	3.1e-3	24	

Table 4: Summary of application testing results

Clone ID	Western	ICC	sELISA compatibility	KD	Epitope
8M5-I	-	N/A	8M5-5, 8M5-M	22nM	Shared with 85M-L
8M5-5	+	-	All	1.8nM	Distinct
8M5-M	+	cytoplasmic	All	2.4nM	Distinct
8M5-L	-	nuclear	8M5-5, 8M5-M	24nM	Shared with 85M-I

Figure 6: Sandwich ELISA Binding Data at Various Antibody Concentrations



behavior and reproducibility in preferred formats. For each candidate, researchers at Aviva Systems Biology test performance in multiple assay formats using independent antibodies and genetic validation (where reagents are available). To this end, each selected NFκB clone was evaluated by SPR, Western Blot, ICC, and ELISA assays (Summarized in **Table 4**).

All four clones bound NFκB in ELISAs (**Figure 6**) across a variety of NFκB concentrations. Though 8M5-I and 8M5-L did not bind in Western blots, both 8M5-5 and 8M5-M bound specifically to NFκB p50 across six different cell lines (**Figure 7**, band at ~50 kDa), with some additional faint bands in some cases. This likely indicates that 8M5-5 and 8M5-M can bind both denatured and native protein, whereas 8M5-I and 8M5-L can recognize native protein, but not the denatured form.

Figure 7:
Representative Western Blots for Two NFκB Clones

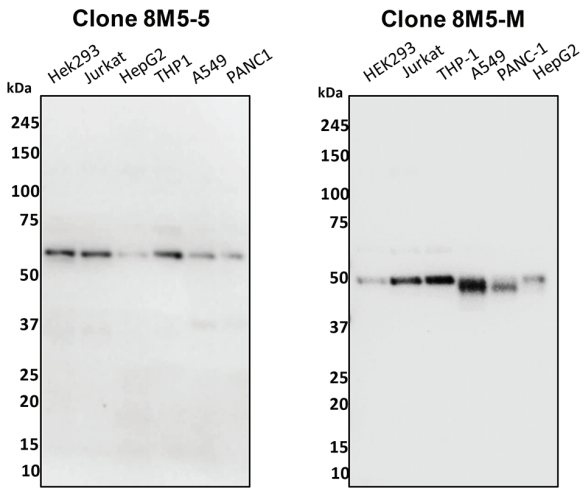


Figure 8: Immunocytochemical Staining of PANC-1 cells with NFκB Clones 8M5-M and 8M5-L

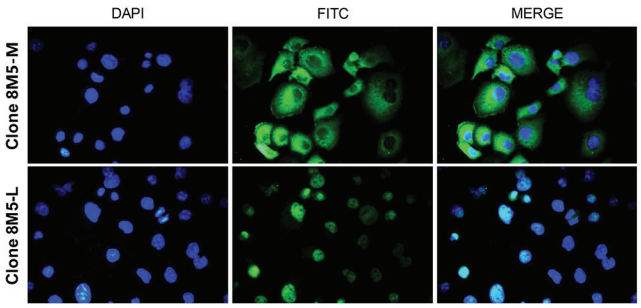
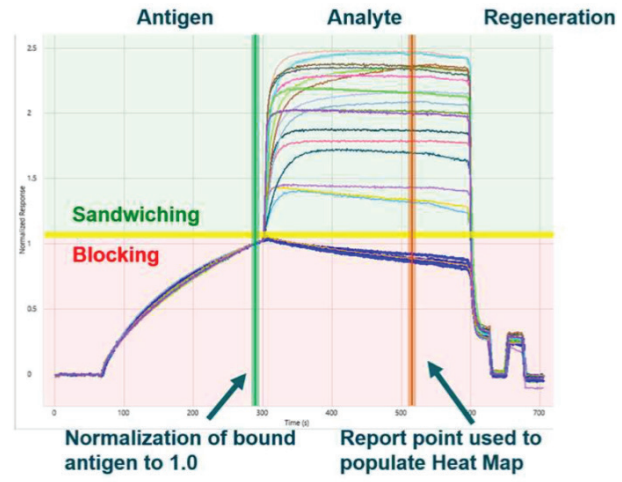


Figure 9:
Epitope Binning Sandwich Assay Visualization

- Per ligand cut-off adjustment is used to determine the Sandwichers in green and competitors in red.
- The first vertical bar (green) is the antigen bound normalization tool which sets all sensorgrams to 1.0, the second vertical bar (Orange) is used to calculate the sandwich value for the heatmap and apply the cut-off.
- The blue curves are the buffer control cycles where running buffer is injected instead of analyte antibody. These values are used to gauge baseline for sandwiching calls

Blue – buffers; Green – Sandwichers; Red - competitors



For ICC, 8M5-M and 8M5-L each were effective for staining NFκB (Figure 8). Interestingly, 8M5-M stained cytoplasmic NFκB p50, whereas 8M5-L stained NFκB p50 in the nucleus. NFκB p50 is found in both the cytoplasm and nucleus, depending on whether it is activated. This may indicate that the 8M5-L is recognizing NFκB p50 in an active NFκB complex bound to genomic DNA, whereas 8M5-M may be staining inactive p50 prior to nuclear localization. Though more studies are needed to confirm that these two different epitopes distinguish between active and inactive p50, these results may point to the potential of using these clones to closely track NFκB activation. In both cases, negative controls lacking primary antibodies exhibited no signal (data not shown).

Epitope Binning

The functionality and application of a specific antibody is often linked to its epitope. For example, two antibodies with distinct epitopes on the same protein can be used as sandwich assay partners or to create super-clonal mixes that increase assay sensitivity through multiple simultaneous binding events. As another, it's common for the intellectual property of commercial antibodies to be linked to the epitope they recognize. Thus, it is crucial to understand epitope recognition, and epitope mapping assessments act as a surrogate for the functional diversity of antibody library pools.

To evaluate and bin epitopes, Aviva Systems Biology performs sandwich assays using our SPR analysis workflow (Figure 9). First, an individual antibody is bound to the surface. Antigens are then flowed over them, followed by delivery of a second antibody to the original bound antigen-antibody complex. If additional signal is detected, this indicates that the two antibody epitopes are mutually exclusive. If the signal is lost or stagnant, then the antibodies are competitive. Using a round-robin of sandwich assays of all candidates generated, Aviva Systems Biology then bins epitopes to visualize diversity. Our high-throughput automated SPR workflow allows us to bin up 96 candidates simultaneously, compared to traditional approaches where only a few clones can be explored at time.

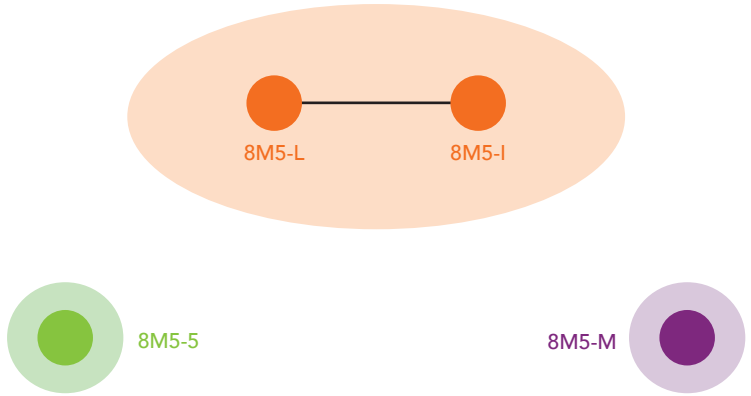
Putting this to practice for the selection of anti-NFκB antibodies, sandwich assays revealed competitive binding between one antibody pair: 8M5-I and 8M5-L (Figure 10A, red squares). In all other combinations, an additive signal

Figure 10A: Epitope Binning

Note: Values are normalized to an individual antibody surface and are not globally normalized. Thus, numerical comparisons can only be made horizontally across the heatmap and not vertically.

	Bins					
Bins	Clone ID	8M5-M	8M5-5	8M5-I	8M5-L	Buffer
	8M5-M		3.17	1.67	1.57	
	8M5-5	2.09		0.64	0.60	
	8M5-I	1.52	1.46		0.01	
	8M5-L	1.44	1.50	0.08		

Figure 10B: Mapping of Select Anti-NFκB Antibody Clones by SPR



(**Figure 10A**, green squares) was detected. Collectively, these results indicate that this selection of four antibodies includes three bins, indicative of three distinct epitopes (**Figure 10B**), in agreement with sandwich ELISA data (**Figure 6**). Though a select example, mapping these antibodies helps show the epitope diversity outcomes possible through the antigen design and immunization workflows in our recombinant antibody pipeline.

SCALING FOR THE FUTURE OF REPRODUCIBILITY

Shifting to recombinant antibodies represents a vital step for securing better reproducibility without sacrificing performance. By cloning and expressing recombinant DNA, at-scale antibody manufacturing provides greater performance consistency.

Through our recombinant antibody platform, Aviva provides application-specific validation of key assay formats, detailed kinetic characterization, and epitope evaluations to source multiple viable antibodies. In turn, customers can select the reagent most applicable to their use case.

Though this piece explored our recombinant antibody R&D process using NFκB as a target, it's worth noting that our team is actively working to expand our recombinant portfolio and accepts custom recombinant antibody discovery and assay development projects. As it stands, there are more than 500 clinically relevant biomarkers and disease targets in the pipeline. In addition, our teams are working to further automate single B-cell cloning and scale biomanufacturing to bring greater speed and reliable supply to our partners.

Though Aviva will continue to offer polyclonal antibody products, our team is eager to advance our recombinant portfolio to support future research, diagnostic, and quality control assays. We believe that the future of biotechnology will need to rely on increasingly reproducible high-performance antibodies, and we are excited to play a key role in that future.

Clones 8M5-I, 8M5-5, 8M5-5, and 8M5-L were developed using Active Motif's AbFlex® antibody technology.

Aviva Systems Biology:
Comprehensive portfolio of products and services

- 24,000 manufactured polyclonal antibodies
- 20,000 ELISA kits
- 58,000 off the shelf proteins
- 339,000 semi-custom Protein on Demand™ recombinant proteins
- Recombinant Antibody development
- Custom ELISA development
- High throughput antibody characterization
- Large scale recombinant protein expression
- GMP, ISO13485 manufacturing

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