ADDRESSING HIGH DEMANDS FOR INCREASED PRODUCTIVITY IN DOWNSTREAM OPERATIONS

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A Discussion Regarding the Productivity in Downstream Operations

At the Biotech Week Boston conference (24–28 September 2017), BioProcess International publisher Brian Caine had the opportunity to speak with Jonathan Royce, global business leader for chromatography resins at GE Healthcare. Their discussion covered current downstream challenges and the company’s next-generation chromatography resin.

Industry Challenges

Caine: What are the most critical purification challenges for biomanufacturers?

Royce: The bioindustry has seen more than a 100-fold increase in the productivity of cells. This means that downstream purification technology must continue to evolve and meet those productivity gains so that purification doesn’t become the rate-limiting step in a process.

Biomanufacturers today seek ways to use their existing equipment more effectively and even incorporate reusable raw materials more effectively. There is a lot of focus on finding ways to get efficient cleaning of chromatography resins and to ensure that they don’t get contaminated by bioburden so that end users get the value out of those resins that they expect.

Protein A and Bioburden

Caine: What role does protein A play in purification efficiency?

Royce: I always think of protein A as being the “Holy Grail of antibody purification.” Many people look for alternatives to protein A, but it’s a molecule that has developed over millions of years of evolution to protect bacterial cells against our immune systems. It has a fantastic specificity for antibodies that’s very hard to match with anything we could engineer as an alternative. It also has enabled the market to reach a state in which an antibody can go from drug discovery into manufacturing in a short time.

But at the same time, protein A is becoming a rate-limiting step in many processes because of the increasing upstream titers. The amount of mass coming onto protein A columns is increasing. That means that in many cases, the efficiency of protein A is determining the efficiency of an entire manufacturing facility.

Caine: How does protein A relate to bioburden?

Royce: Bioburden is a main concern in downstream purification today. Some regulatory agencies have asked manufacturers whether they understand sources of bioburden, what is being done to control them, and whether they are testing for it sufficiently. Protein A is challenged in that area because of two main factors. First, the protein A step is loaded with all the cell culture nutrients that cells are grown in. So the load fraction coming onto the protein A step highly promotes bacterial growth. Second, the majority of protein A resins that people use today are somewhat susceptible to damage by sodium hydroxide, which is the most common clean-in-place (CIP) agent used today.

So the highest risk factor for a bioburden outbreak occurs at a step that includes the weakest chemicals to control those outbreaks.
That means that in a risk analysis, protein A would stand out as probably one of the most susceptible downstream steps for bioburden contamination.

**Introducing MabSelect™ PrismA**

**Caine:** GE Healthcare has just introduced MabSelect PrismA chromatography resin. What makes it unique?

**Royce:** MabSelect PrismA resin comes from a big effort by GE Healthcare to address two of the main topics that we discussed. One is the throughput of downstream purification and trying to make sure that protein A is not the rate-limiting step in that process. The other is to ensure that protein A columns have a capacity that is on par with the other steps in the process.

Current cation-exchange resins have capacities for MAbs in the range of 80–100 g/L. Protein A historically lags behind that, and there’s no technical reason for that. We felt that it was important to bring protein A up into that same level in terms of capacity and productivity. At the same time, the MabSelect PrismA resin is also designed for the same CIP operations that are used in other steps in a process.

So we went back to the drawing board with the ligand. We have had the MabSelect SuRe™ ligand in our portfolio for over 10 years. It’s been a fantastic part of the story of MAb purification and the “platformability” of MAb processing, but we felt that we could do some additional work based on new technology to find ways to improve its alkaline stability. The new MabSelect PrismA resin has a stability from 0.5 to 1.0 M sodium hydroxide. That means that it can be cleaned with the same CIP solutions that customers are using for ion exchangers and hydrophobic-interaction chromatography resins. It also has capacities in the range of 80 g/L, which make it well matched in terms of capacity to the secondary and tertiary steps in a bioprocess.

**Caine:** How can MabSelect PrismA help biopharmaceutical companies produce more product with their existing platforms?

**Royce:** MabSelect PrismA is designed to be an ultrahigh-capacity protein A resin. If you have an existing facility where you have columns or systems in place, those columns and systems can now process much larger loads of antibody than what they previously could do. As upstream titers increase in your bioreactor, your downstream purification can handle those increased loads without having to make additional capital investments to buy larger systems or columns.

Using the MabSelect PrismA resin also opens a bigger window of operation for prepacked columns. Today the largest ReadyToProcess™ column that we have could harvest and purify a high-titer 2,000-L bioreactor. That’s something that we couldn’t do with any previous generation of resins in our portfolio.

As you start to increase capacity and thereby shrink the size of columns in your operations, you also get a lot of secondary effects such as smaller buffer tanks and lower consumption of buffer, which also can be important in terms of making sure that plant efficiency stays high.

**Next Steps**

**Caine:** What is next as far as helping companies reduce cost and decrease manufacturing times?

**Royce:** Going back to your first question about the diversification of the pipeline, I think there will be future opportunities for additional affinity resins that are more specific to the molecule types that companies are developing. There are more than 40 different constructs of bispecific antibodies, some of which can be purified by protein A. But some will require other types of affinity solutions. If you look even farther back in the pipeline, you can see that a lot of new types of therapies are coming, which will require totally new thoughts around production and purification. Some of those therapies are, for instance, too large to be sterile filtered. They are going to require more aseptic processing in the downstream area. Some are too large even to go into a chromatography resin. So there will be opportunities to work with membrane absorbers and other technologies that are less diffusion-limited than chromatography columns are.

I think we’re going to see continued development in the space around new technologies that enable those therapies to come to market and enable them to be manufactured efficiently and cost-effectively. 😊

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Thirty Years of Monoclonal Antibodies and Protein A

A Retrospective

Geoff Hale

In 1980 at the University of Cambridge’s department of pathology, I worked with Herman Waldmann to develop monoclonal antibodies (MAbs) as treatments for graft-versus-host disease (GVHD). That disease is associated with serious complications of stem cell transplantation when attacking T-cells can damage the lungs, liver, skin, and other organs. If we could find a specific MAb that would work with the human complement system to kill those cells, they could be selectively removed from the bone marrow. The human complement system enables the body to destroy cells targeted with antibodies.

The First Monoclonal Antibodies

From a great collection of rat monoclonal antibodies of unknown specificity (Photo 1), we selected antibodies that could kill T-cells with human complements. Because we wanted to use immunoprecipitation to purify those antibodies, we further screened selected molecules for protein A binding. The selected antibody was named Campath after the Cambridge Pathology Department.

The first MAb, Campath-IgM antibody, depleted T-cells in the blood of a patient with leukemia to <1% of starting level. Although those cells returned the next day, we could show that the treatment was fairly safe, that T-cells could be attacked, and that complement could be activated. When the same treatment was conducted with bone marrow, we found >99% of T-cells were removed from the bone marrow. When patients who needed the transplant with this bone marrow were infused, their instance of GVHD was greatly reduced (Figure 1).

However, manufacturing Campath-1 presented some problems. Although the antibody would bind to protein A, the low pH needed to elute it damaged the IgM molecule. In addition, the patient rejected some bone marrow grafts. Normally, because a recipient is immunosuppressed, T-cells from a donor get the upper hand, thereby causing GVHD (because there are a lot of T-cells). After removing those cells, the T-cells that remain in the recipient can attack the graft and cause graft rejection. Graft rejection had been seen in about 10% of patients.

In mouse models, Fc receptors on killer cells needed to be engaged to provide a way of destroying the T-cells and prevent graft rejection.
Hence, we looked for an antibody the same property in humans. The only isotype of rat antibodies that would bind with Fc receptors and engage killer cells was rat gamma 2b. Using hemagglutination, we screened a large numbers of cells to obtain a rat gamma 2b against the Campath-1 antigen.

The Campath-1G antibody was highly effective in preventing graft rejection. When tested in a patient with leukemia and previous unsuccessful treatment with Campath-1M, even small doses depleted T-cells, and the numbers stayed low for quite a long time. In this case, treatments were discontinued when the patient developed an antibody response against the rat antibody. However, Campath-1G was highly effective for preventing graft rejection in leukemia patients because it was given for only a short period at the time of the transplant. For other disease types (e.g., autoimmune diseases such as arthritis or multiple sclerosis) for which repeated doses could be needed, the antibodies would need to be humanized.

Eventually, Campath-1G was humanized, and the resulting Campath-1H progressed into the clinic from our laboratory. In a patient with end-stage lymphoma, in whom lymphoma cells had spilled out into the blood (similar to leukemia), Campath-1H depleted those cells effectively (Figure 2). When we evaluated the recovering cells (which came back after more than a month), they were healthy (Figure 3). We observed same
with the bone marrow and no signs of tumor cells, as was the case with Campath-1M and Campath-1G.

Until that point, we had conducted our work in an ordinary research laboratory. To scale up the production, we needed a better manufacturing facility with improved quality control. So the first good manufacturing practice (GMP) facility in the United Kingdom to be embedded in a university (an academic facility) was built. With increasing antibody titers, the use of hollow-fiber fermentors, and a need to work within a small budget, we had to reuse protein A resin by cleaning and sanitizing to determine whether protein A could withstand sodium hydroxide, and we investigated different brands of protein A resins. From those results, we selected protein A Sepharose™ Fast Flow resin. It could be treated with sodium hydroxide at 0.5 M (which was what we had been using for sanitizing our columns) for up to 40 cycles in pilot production scale (Figure 4).

**Increasing Number of Monoclonal Antibody Approvals**

By the 1990s, we had taken about 20 different MAbs from our research laboratory into the clinic. In 1997 and 1998, several chimeric and the first humanized antibodies were approved (Table 1). Campath-1H was approved in 2001, shortly followed by Humira™ (adalimumab). So far, the year 2017 appears to be setting a record in MAb approvals, with about eight approvals so far and perhaps more by year end (Figure 5).

Studies have shown for some time that Campath-1H could be used for treating multiple sclerosis (Figure 6). The treatment has been over a course of only five days, followed by another five-day treatment one year later. During treatment, which depletes lymphocytes and powerfully immunosuppresses patients, the instances of adverse clinical events were greatly reduced. In addition, the patients’ levels of disability seemed to improve (Figure 7). Results from a follow-up study show that clinical improvement was maintained 10 years later.

Campath antibodies recognize an epitope very close to the cell membrane (Figure 8). The proximity of that epitope to the cell membrane is one of the reasons why Campath is such a good antibody for killing cells. The antibody delivers that lytic target only to places where it is needed (close to the cell membrane). Other antibodies that are effective at killing cells almost always tend to bind closely to the cell membrane.

**Monoclonal Antibody Purification**

We developed a method for purifying Campath antibodies using the target molecule as an affinity ligand (Figure 9). This approach had the advantage of requiring only very weak acid for elution. However, this strategy was never implemented because protein A chromatography was already well established, and the lower pH

### Table 1: Some of the first approved chimeric and humanized antibodies

<table>
<thead>
<tr>
<th>INN</th>
<th>Type</th>
<th>Target</th>
<th>Class</th>
<th>Approved</th>
<th>First indication</th>
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<td>CD3</td>
<td>IgG2a</td>
<td>1986</td>
<td>Transplantation</td>
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<td>abciximab</td>
<td>chimeric</td>
<td>IIb/IIa</td>
<td>Fab</td>
<td>1994</td>
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<td>daclizumab</td>
<td>humanized</td>
<td>CD25</td>
<td>IgG1</td>
<td>1997</td>
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</tr>
<tr>
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<td>chimeric</td>
<td>CD20</td>
<td>IgG1</td>
<td>1997</td>
<td>Lymphoma</td>
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<tr>
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<td>chimeric</td>
<td>CD25</td>
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<tr>
<td>etanercept</td>
<td>Fc-fusion</td>
<td>TNF</td>
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<td>TNF</td>
<td>IgG1</td>
<td>1998</td>
<td>Crohn’s Disease</td>
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needed to elute antibodies from protein A has some advantages for virus inactivation. Several other alternatives also have been investigated, but today protein A chromatography remains useful.

A 2016 publication summarizes about 40 years of improvements made in protein A chromatography used in the therapeutic antibody industry (1). During that period, big improvements were made in the productivity of cell lines. At first, we could produce only micrograms of antibody per milliliter of culture. Today, you can get up to multimilligrams quite easily. Even bigger increases in protein A column capacity have taken place as well as the columns’ ability to withstand harsh cleaning agents such as sodium hydroxide.

Some researchers have discussed whether protein A will be displaced or remain a part of the bioindustry for many more years. Antibody therapeutics have been around now for quite a long time, and most people believe they will continue to be around for a lot longer.

One area of interest and investment is the development of biosimilar antibodies. With pipelines containing hundreds of innovator and biosimilar antibodies, I hope and believe there will be many exciting therapies to come. I see little evidence that protein A chromatography will be displaced.

With all the investments in improving this technology, immunoglobulin and protein A are a partnership: One is being used for the purification of the other to make many more powerful and effective drugs for the future.

References
1 Bolton GR, Mehta KK. The Role of More than 40 Years of Improvement in Protein A Chromatography in the Growth of the Therapeutic Antibody Industry. Biotechnol. Prog. 32(5) 2016: 1193-1202.

Formerly a visiting professor of therapeutic immunology at the University of Oxford (Oxford, UK), Geoff Hale is Managing Director at BioArchitech Ltd. in Cambridge, UK.
Reimagining Capacity for Today’s Purification of Monoclonal Antibodies

Jonathan Royce

The monoclonal antibody (MAb) market has grown over the past decade to be about half of the biomanufacturing market today. This growth should continue, driven by a strong pipeline of MAbs that are currently in phase 2 and 3 clinical trials. A synergistic evolution of MAbs and protein A resins also has taken place in the market. Figure 1 shows a graph adapted from an Amgen study of the development of protein A productivity and capacity over the past 40 years. Protein A has made MAbs highly manufacturable and easier to develop in laboratories. The purification platform approach, which has been enabled by protein A, has been a key element in this market growth.

As Figure 1 shows, productivity of protein A resins has increased >4% annually since 1978 and capacity about 6% annually. Despite that, opportunities exist for improving protein A technology. Upstream titers have improved dramatically, increasing nearly 100-fold over the past four decades. As such, protein A has become the rate-limiting step in some downstream purification suites.

In general, protein A columns are too large, which means that they either limit the use of prepacked technology, or they create a mismatch between a protein A column and downstream operations. Protein A columns also are more prone to bioburden contamination than any other step in a downstream process. Protein A resin is a reused raw material. It’s exposed to the highest load of nutrients as the entire harvest from the bioreactor is loaded onto the protein A column, but it is cleaned with weaker clean-in-place (CIP) chemicals than other downstream steps. Typically, ion-exchange and hydrophobic interaction chromatography (HIC) resins are cleaned with 1.0 M sodium hydroxide. But in a vast majority of cases today, protein A resin is cleaned with 0.1 M sodium hydroxide. At the same time, regulatory agencies are increasingly interested in sources of bioburden and the methods that manufacturers are using to limit the outbreak of bioburden in their manufacturing areas. And despite generational improvements that have led to lower cost of ownership for protein A, further cost reductions are needed.

Improving Process Productivity

Some challenges can be addressed through a simple process design. Several years ago, GE Healthcare introduced a technique called “variable residence time (VRT) loading.” VRT loading is a way to better use the capacity available in a resin while minimizing the amount of time needed during the load step. The technique is quite simple: Rather than loading a column at a constant residence time, you load a column in a step-wise manner. You start with a
short residence time, and as the resin starts to reach a saturation point, you slow down the feed pump. Then you continue to load until reaching the saturation point and then slow down again.

Figure 2 shows three different residence times for the load. The process leads to a shorter average residence time overall but maintains the capacity you would get with the longest residence time. Because the load step tends to be time-consuming during protein A chromatography, you can get significantly improved productivity. In this case, we demonstrated about a 40% productivity increase that maintains capacity and purification performance.

Another way protein A productivity can be improved is by implementing continuous capture, which is an area of focus. Continuous capture improves use of the available capacity in a resin, which allows the use of smaller equipment and in many cases, shorter process times and less manual handling. Continuous chromatography also makes it possible to manufacture smaller batches, which can be scaled-out instead of scaled-up.

Finally, fewer purification steps can be used in a process to improve overall productivity. For example, we have worked with customers to look for options for combining separate ion-exchange and HIC steps into a single multimodal step that provides the same purification performance but reduces a column in the downstream manufacturing area.

**IMPROVING RESIN PRODUCTIVITY**

Resin performance also has improved recently. To develop a next-generation version of protein A resin, we started by going back to MabSelect SuRe ligand, which uses a single domain built into a tetramer. We went back to the monomeric version and identified weak points in the protein A molecule to identify possible mutations that would improve alkaline stability. We used a high-throughput screening approach.

We also looked at the beads that we use for our protein A resins and thought a lot about the challenges the MabSelect SuRe bead was designed to address. MabSelect SuRe was developed at a time when many processes were at relatively low titers, and flow rate in the protein A step was one of the main limitations. Today most processes are at relatively high concentrations and, in fact, mass throughput is the limitation in the process, rather than volumetric throughput.

We considered opportunities to optimize a new bead for protein A chromatography, and we decided that it would be opportunistic to
cooptimize both the ligand and the bead together. We performed experiments to cooptimize pore size and ligand length. Large pores reduce steric hindrances, but at the expense of available surface area. Long ligands increase the number of binding sites but can increase steric hindrances. As a result of our experiments, we found that there was an optimal design that maximized capacity while limiting steric hindrance.

The result is a new generation of protein A resin named MabSelect PrismA. MabSelect PrismA is built on the heritage of former MabSelect products, but it is a completely new bead and new ligand designed for modern processes. It offers improved binding capacity to resolve these bottlenecks that occur in production, and exceptional alkaline stability for cleaning and sanitization operations. And it has been designed from the start with an extensive security of supply package in mind.

### Resin Performance

#### Dynamic Binding Capacity:

Figure 3 shows a comparison of MabSelect PrismA, MabSelect SuRe, and MabSelect SuRe LX resins tested at different residence times and measuring dynamic binding capacity (DBC). With MabSelect PrismA, the cooptimization of the ligand and base matrix has shown good improvement in terms of DBC at all residence times. Regardless of whether you run a continuous process in which residence times tend to be short, or you’re running a large-scale batch process in which they tend to be longer, you get a significant improvement of 25-40% improved capacity over MabSelect SuRe LX. MabSelect PrismA has a capacity that is on par with modern ion-exchange resins.

#### Flow Rate:

The new bead has been developed to meet the needs of today’s processes in terms of flow rate. Figure 4 shows data from a process-scale column packed with MabSelect PrismA. Both back pressure and flow rate were measured to determine the relationship between them. MabSelect PrismA offers very high flow rates at relatively low back pressure and does this with a bead approximately 30% smaller than MabSelect SuRe.

The combination of this higher capacity and optimized flow rate performance offers significant improvements in terms of productivity from...
existing equipment. Improved productivity means that if your plant is in a situation in which you need to make capital investments to expand, you might be able to delay those investments by making a change to MabSelect PrismA.

**Productivity:** Figure 5a shows a comparison of the mass throughput on a 45-cm column using MabSelect PrismA, MabSelect SuRe, and MabSelect SuRe LX resins. Mass throughput increases on the order of 25-50% depending on the benchmark. A similar relationship is true for volumetric throughput and the maximum-sized bioreactor that could be harvested with that column (Figure 5b). Using a 45-cm column, MabSelect PrismA could harvest a 2,000 L bioreactor with a titer of 4 g/L, whereas MabSelect SuRe LX and MabSelect SuRe resins would require larger columns for the same bioreactor.

**Reduced Cost:** The performance of MabSelect PrismA also can reduce the cost of consumables. Figure 6 shows the advantages in both resin consumption savings and buffer consumption savings compared over different resins. It shows an improvement between 20% and 33% in both resin and buffer consumption.

**Alkaline Stability and Bioburden Control:** MabSelect PrismA offers significantly improved alkaline stability over other resins. Figure 7 shows a comparison of MabSelect PrismA against MabSelect SuRe LX and MabSelect SuRe resins when cleaned with 0.5 M sodium hydroxide. MabSelect PrismA retains more than 95% of its DBC after 150 cycles, whereas MabSelect SuRe LX has lost nearly 20% of its binding capacity and MabSelect SuRe has lost nearly 40%. So when designing processes, the capacity that you measure...
at cycle zero is still available even after repeated usage as compared with MabSelect SuRe LX or MabSelect SuRe resins. Another way to think about it is that safety factors can be reduced, thereby shrinking the size of your columns. If improved cleanability or resin cleanliness is a goal, you can increase sodium hydroxide concentrations to 1.0 M. Figure 8 shows a comparison of MabSelect PrismA and MabSelect SuRe LX resins cleaned with 1.0 M sodium hydroxide. MabSelect PrismA resin retains more than 90% of its dynamic binding capacity, whereas MabSelect SuRe LX retains only about 50%. This is an important improvement in terms of performance if you want to align your CIP solutions throughout the entire downstream suite or if you want to have better control of bioburden.

Purification Performance: We have conducted a study using two of our in-house MAbs to compare the purification performance of MabSelect PrismA, MabSelect SuRe, and MabSelect SuRe LX. The load values are quite different: MabSelect PrismA has been loaded with about 50% more MAb due to its higher dynamic binding capacity.

The top of Figure 9 shows the two MAbs tested and a comparison of recovery. In all three cases, we had very similar performances. Host-cell protein levels are shown in the eluate pool at the
Evaluation of a Next-Generation Protein A Chromatography Resin for the Purification of Monoclonal Antibodies

Bryan Dransart, April Wheeler, Tony Hong, Chi Tran, Rafael Abalos, Andrew Quezada, ShiYu Wang, Brian Kluck, and Nooshafarin Sanaie

Next-generation, high-capacity, alkali-stable protein A resins have recently become available in anticipation of increased production and throughput demand for protein therapeutic processes. Efficient caustic cleaning and bioburden control regimens have allowed agarose-based, alkali-stable protein A chromatography resins to become the backbone of many commercial purification processes for over a decade and have served as the primary capture step for monoclonal antibody (MAb) purification processes. However, the relatively high raw material costs, along with limitations to binding capacity and aggressive resin stripping strategies with current resins, still represent a high contribution to overall manufacturing cost of goods. To address this, a novel higher capacity prototype protein A resin from GE Healthcare (MabSelect™ PrismA) was evaluated using a CHO-expressed MAb. Stability and performance of the resin over its usable lifetime was studied by monitoring dynamic binding capacity (DBC), protein A ligand leachate, and total protein carryover.

Protein A is a 42 kDa protein found on the cell wall of Staphylococcus aureus. Staphylococcal protein A (SpA) is composed of five IgG-binding domains (E, D, A, B, and C) folded into a triple helix bundle. All domains have affinity for, and can specifically bind to the Fc region of antibodies. It is also known that multiple domains have affinity for the antigen-binding region (Fab) of the antibody, most notably the D domain ($V_{H}$3 family). Many companies have purposely modified and mutated these domains to ensure high stability, specificity, and consistency of affinity purifications over the lifetime of the resin. These modified domains, due to their increased stability, have been produced as repeating subunits (repeating tetramer, pentamer, or hexamer) to further increase efficiency in binding capacity.

Protein A resin accounts for a large portion of the overall raw material costs to MAb processes, (Figure 1) and to a significant portion of overall operating costs (1) and cost of goods manufactured (COGM) (2). To drive down total COGM, either the raw material price must be lower or higher raw material utilization must be employed.
Advancements in Protein A Resins
Advancements in fermentation and cell culture technologies have resulted in significant upstream productivity gains, thereby creating potential bottlenecks to downstream processing. To mitigate these potential bottlenecks, many technological advances in cell harvesting and capture strategies have been developed, optimized, and implemented. Capacity bottlenecks at the protein A step have been addressed through multifaceted improvements in process conditions (e.g., dual flowrate loading), equipment strategies (e.g., continuous chromatography), and most important, the protein A ligand and bead technologies (base matrix, ligand, bead size) (Figure 2). As a result, novel protein A resins are now commercially available and claim to have increased both alkaline stability for better microbial control and capacity (>60 g MAb/L resin).

Although multiple novel alkali-stable protein A resins are now available, dynamic binding capacities can vary widely (Figure 3), and the bar to update established platforms or modify existing commercial processes may be high. To justify a change from existing protein A resins, the following baseline criteria were considered: >50% increase in DBC, caustic stability for over 150
cycles with minimal drop in DBC, high product recovery (>90%), low protein A leachate profiles, and low protein carryover (lot-to-lot). GE Healthcare has produced a new prototype (MabSelect PrismA) that has a smaller particle size ($d_{50}$) of about 60 µm, further alkaline stabilized protein A-derived ligand, and optimized bead characteristics (porosity).

**Protein A Evaluation Using MabSelect SuRe, MabSelect SuRe LX, and Prototype (MabSelect PrismA)**

**Dynamic Binding Capacity Assessment:**
Comparison DBC studies were performed on MabSelect SuRe™, MabSelect SuRe LX, and the prototype (MabSelect PrismA) at various residence times (Figure 4). At 8 minute residence time, the observed DBCs plateaued at about 50, 60, and 100 g/L for MabSelect SuRe, MabSelect SuRe LX, and the prototype (MabSelect PrismA), respectively. The prototype (MabSelect PrismA) DBC also was 50% higher in DBC at the lowest residence time of 2 minutes, whereas MabSelect SuRe and MabSelect SuRe LX appear to converge to the same value.

**Cleaning and Sanitization Efficiency:** Because of the elevated capacities observed for the prototype (MabSelect PrismA), cleaning and sanitization procedures were assessed for the potential increase in protein carryover. To assess cleaning and carryover, aged resin was generated by consecutive MAb protein load cycles at 80% DBC (4 minute residence time). A control mock elution was performed without cleaning in place (CIP) to assess total potential carryover before caustic treatment. Resin comparisons were assessed by cleaning first with mild caustic solution (25 mM sodium hydroxide), a mock elution (elution buffer only), multiple sequentially increasing caustic cleaning solutions, and finally a denaturing/reducing solution of DTT/urea to assess further requirements for sanitization procedures.

Results indicate that significant residual protein was left bound to the resin with no CIP. Mild

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**Figure 6:** (a) Normalized pool volume as a function of load factor (g/L) for MAb A and Mab B; (b) elution flowrate (residence time, min) at 80 g/L MAb A

**Figure 7:** Resin lifetime study comparison between (a) MabSelect SuRe resin (no protein load step) and (b) prototype (MabSelect PrismA) showing dynamic binding capacity (DBC) as a function of cycle number using 0.3 M and 1.0 M NaOH sanitization solution every cycle.
caustic cleaning (25 mM NaOH strip) is also insufficient to fully regenerate the resin. Prototype (MabSelect PrismA) resin was observed to have significantly more residual material as measured by total quantitative fluorescence and is most likely due to the increased loading of harvested cell culture fluid (Figure 5). Optimized cleaning and sanitization procedures may be required at these elevated loading, however, it appears that <100 µg of material was left over during 1.0 M NaOH and DTT/urea fractions combined.

**Process Performance and Resin Lifetime Studies:**

Several studies were executed to assess process performance (pool volume) on the prototype (MabSelect PrismA) and resin lifetime stability between resins. Pool volumes were evaluated for multiple MAb molecules on the prototype at protein loadings of 20, 40, 60, and 80 g/L. Results show DBC plateaus around 60 g/L with pool volumes of around 2.5–3 CV (column volume) (Figure 6a). Pool volumes also were evaluated at 80 g/L and varied elution residence times of 2, 4, 6, and 8 minutes. Experiments show consistent pool volumes of around 2.5 CV at the maximum capacity of 80 g/L (Figure 6a).

To assess the stability of protein A resin over its lifetime, cycling studies were performed comparing both MabSelect SuRe and prototype (MabSelect PrismA) resins using 0.3 M and 1.0 M NaOH sanitization solutions. Dynamic binding capacity was monitored both with and without protein load over 150 cycles to assess the effect of caustic stability and protein load independently. To eliminate the potential contribution to degradation from protein loading, cycles without protein load were performed by skipping the load step and directly washing the column after equilibration.

Cleaning and sanitization cycling (0.3 M NaOH, no protein load) on the prototype (MabSelect PrismA) resin showed no appreciable
A decrease in DBC (Figure 7b), whereas the same cycling on MabSelect SuRe showed a 17% decrease (Figure 7a). Cleaning and sanitization cycling (1.0 M NaOH, no protein load) on the prototype (MabSelect PrismA) resin showed a 28% decrease in DBC (Figure 7b), whereas the same cycling on MabSelect SuRe showed a 62% decrease (Figure 7a). Total caustic exposure (0.3 M or 1.0 M NaOH + 25mM NaOH strip) over 150 cycles was 37.5 h.

Overall resin stability was further assessed by running full protein load cycles at 80% DBC with cleaning and sanitization steps performed on every cycle. Although sanitization (0.3 M NaOH, no protein load) on the prototype (MabSelect PrismA) resin showed little decrease in DBC, the same cycling (0.3 M NaOH, with protein load) showed approximately 15% decrease. The DBC decrease suggests protein loading further degrades the prototype resin beyond caustic only cycling and a typical DBC safety factor would be required to maintain high % yield (Figure 8a). Sanitization (0.3 M NaOH) on MabSelect SuRe showed no difference between protein and no protein load and showed ~20% decrease (Figure 8a). The prototype appears to have higher caustic only stability with a total caustic exposure (0.3 M NaOH + 25mM NaOH strip) over 150 cycles of 37.5 h.

Recovery values (% yield) for the prototype (MabSelect PrismA) resin were >90% over the duration of the resin lifetime study at 67 g MAb/L resin loading (80% of DBC at 4 minute residence time) (Figure 9). Recovery values (% yield) for MabSelect SuRe resin fell <90% after 100 cycles at 36 g MAb/L resin loading (80% of DBC at 4 minute residence time). Total caustic exposure (0.3 M NaOH + 25mM NaOH strip) over 100 cycles was 25 h.

Protein carryover was monitored by collecting the elution fraction of post-sanitization (0.3 M and 1.0 M NaOH) blank runs after cycle 75 (every 25 cycles). All elution fractions were below one-thousandths of the previous elution pool concentration. Protein carryover was below the limit of quantitation (LOQ) for 1.0 M NaOH and slightly above for 0.3 M NaOH (Figure 10).

Leached protein A was monitored throughout the duration of the cycling studies performed with protein load. No significant changes were observed after cycle 25, and the relative levels of residual protein A leachate for the prototype (MabSelect PrismA) were about 4x higher than observed for the same conditions on MabSelect SuRe (Figure 11).

Continuous chromatography systems and skids are now commercially available to allow for continuous high protein loading beyond protein breakthrough. Periodic counter-current chromatography (PCC) can be operated with three or four columns attached in series with any two being loaded simultaneously. Previous findings suggest that batch protein A operations using short columns (bed height 5–10 cm) at relatively low loadings (<40 g MAb/L resin) could be a productive alternative (8–12 g/L/h) to continuous chromatography (3). However, when using three or four column PCC with the prototype (MabSelect PrismA) resin at high loading capacity (>60 g MAb/L resin) combined with reduced residence time and reduced resin volume, downstream productivity could be improved by more than twofold. Depending on the optimization parameter goal (maximum productivity or maximum capacity), productivity values can reach 26 g/L·h (L = Lresin) (Table 1).

**Summary**

Dynamic binding capacity for the prototype (MabSelect PrismA) protein A resin was observed...
to be >70 g MAb/L resin (3 minute residence
time). Cycling studies, both with and without
protein, indicate that the prototype (MabSelect
PrismA) resin was significantly more caustic stable
compared with its predecessor MabSelect SuRe
resin. Average yields were >90% over the duration
of resin lifetime studies. Total carryover was
higher for the prototype (MabSelect PrismA)
resin but well under the allowable carryover limit
of one one-thousandths of average pool
congestion from the previous cycle. The higher
cautic stability of the prototype (MabSelect
PrismA) resin allows not just more aggressive
sanitization procedures, but also more flexible
sanitization procedures — giving a wide range of
options for specific applications. Using the
prototype (MabSelect PrismA) resin in PCC
mode could improve productivity by more than
twofold.

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Protein A Capture Chromatography: Optimization Under
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Associate I; Brian Kluck is a Research Scientist I; and
Nooshafarin Sanaie is a Senior Research Scientist II, all
at Gilead Sciences.

bottom of Figure 9, which again shows
equivalent performance between the three resins.
The top of Figure 10 shows a comparison of
aggregates. The bottom of this figure shows
DNA clearance on the three resins. Purification
performance is very similar.

Figure 11 shows pool volumes and leached
protein A. Pool volumes are similar across the
three resins. For leached protein A, there are
slightly higher values of protein A for the resins
that have higher ligand densities. This is
expected, but in all cases we believe all of these
values are easily handled by the secondary and
tertiary steps in a downstream purification suite.

GE Healthcare has put extended measures
into MabSelect PrismA to ensure strong security
of supply. From the start, we’ve designed this
product to have two sources of agarose validating
into the manufacturing and two sources of the
protein A ligand. We also have taken steps to
develop in-house manufacturing for the protein
A ligand. As a second source, we have a contract
manufacturer that manufactures the ligand using
a GE-developed process.

CONCLUSION
Productivity can be improved in MAb
downstream processing, by improving both
process designs and resins. MabSelect PrismA
has been developed to address some of the
challenges we have today. It’s a new high-capacity
resin with resistance to 1.0 M sodium hydroxide
for solving both productivity and bioburden
issues. It offers significantly higher binding
capacity, which gives improved mass throughput
and productivity, especially when looking at
existing equipment and trying to get the most out
of your existing capital.

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Addressing the Risk of Bioburden and the Need for Increased Productivity in Protein A Chromatography

Jonathan Royce

Protein A has been a fantastic tool for the antibody industry. It is without doubt the most well-established purification technique in monoclonal antibody (MAb) manufacturing today due to a few key success factors. Protein A is the result of the long evolution of *Staphylococcus aureus* that developed a defense system against antibodies. Protein A exists on the cell wall of about 9% of *S. aureus* strains and immobilizes IgGs. When there is an immune response in the body, the bacterium defends itself by disabling IgG molecules, preventing them from attacking the cells. With this natural origin, it is hard to engineer an alternative with superior selectivity.

Despite protein A’s natural origins, the bioindustry has worked to make it more useful in industrial manufacturing by improving its alkaline stability. We have even played with the structure of protein A to increase the amount of binding domains. Throughout the generations of protein A resins, this engineering has made protein A a more effective tool at lower production costs.

**Challenges with Using Protein A**

Protein A is a proven technology. It is well understood by regulatory agencies, easy to move through clinical trials, and it has predictable operation. That said, there are still challenges that remain and opportunities to improve the technology. First, the protein A step is challenged by the increase of titers in bioreactors causing it to be the rate-limiting step in downstream purification. If we can increase the productivity of the protein A step, we can ultimately increase the productivity of an entire facility.

Second, protein A columns are generally too large. If you compare protein A to other types of resins, there is almost a one-to-two ratio in terms of capacity. That means that there is a limited window of operation for prepacked columns. The result is a mismatch between downstream operations and difficult choices to be made between whether you cycle a column (causing the process to take longer) or install a larger column (which involves additional maintenance and cost).

Third, protein A columns are the weakest link in downstream purification with respect to bioburden. Protein A columns are loaded with cell culture supernatant, creating a beneficial environment for bacterial growth, but the column

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Figure 1: The extreme cost of a bioburden incident

<table>
<thead>
<tr>
<th>Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial Revenue Loss</td>
<td>1 Billion USD</td>
</tr>
<tr>
<td>Risk losing trust of customers, authorities and patients</td>
<td></td>
</tr>
<tr>
<td>Raw Materials</td>
<td>3 Million USD</td>
</tr>
<tr>
<td>Resin must be discarded; estimated from a large-scale column size and resin costs</td>
<td></td>
</tr>
<tr>
<td>Failed Lots and Scrapped Batches</td>
<td>1 Million USD</td>
</tr>
<tr>
<td>2,000 L bioreactor with 5 g/L expression level; cost of MAb production 100 USD/g</td>
<td></td>
</tr>
<tr>
<td>Sanitization of Facility and Equipment</td>
<td>100,000 USD</td>
</tr>
<tr>
<td>Four weeks by five people</td>
<td></td>
</tr>
<tr>
<td>QA Investigations</td>
<td>20,000 USD</td>
</tr>
<tr>
<td>One week by three people</td>
<td></td>
</tr>
</tbody>
</table>
is subsequently cleaned with the weakest clean in place (CIP) solution in downstream purification. Today, most protein A resins are compatible with concentrations of sodium hydroxide (NaOH) about 10× lower than subsequent steps, resulting in a much lower level of assurance against bioburden contaminations.

Finally, regulatory agencies are more interested in the issue of bioburden than ever before. They are asking whether biologics producers understand sources of bioburden in their processes. They want to see evidence that controls and analytical methods are in place to identify bioburden. Even in cases where bioburden hasn’t been an issue, if you start testing for it on a regular basis and find it, you have to do something about it.

**BioBurden Concerns**
The reality of bioburden is everything from annoying to extraordinarily expensive (Figure 1). Even if a bioburden incident results only in a process deviation and investigation, most experts estimate the cost to be about 20,000 USD to investigate, perform a root cause analysis, and close the deviation. If you proceed through this escalating scale of cost, a bioburden incident could result in sanitization requirements for equipment or an entire facility, and result in failed lots of material. There are cases in which large-scale operations have had to replace the resin in a protein A column to recover from a bioburden incident. And, considering the cost, if this were to lead to an FDA warning letter or the shutdown of a facility, it could result in losses on the order of billions of dollars in reputational and commercial loss for a product on the market.

NaOH has been shown to be the most simple and effective cleaning and sanitization agent we have today (Figure 2). As the concentration of NaOH increases, it becomes increasingly effective at deactivating endotoxin and different types of both Gram-negative and Gram-positive bacteria. “Alkali-stabilized” protein A resins are still less resistant to NaOH than their counterparts in the rest of the purification suite. For example, Capto S ImpAct, an ion-exchange resin, is fully stable in 1.0 M NaOH, but MabSelect SuRe or MabSelect SuRe LX media lose binding capacity with repeated exposure to NaOH (Figure 3). This decrease in capacity occurs for essentially all protein A resins on the market today. That, of course, affects the process of economy.

**Addressing BioBurden Risk**
At GE Healthcare, we have been thinking about these issues for several years. We have taken into account customer feedback and interviews conducted between 2011 and 2014. In 2014, we started a research project to identify concepts to address these issues, and in 2015 we started developing a next-generation protein A resin designed to address productivity and bioburden.

Starting with bioburden, the main goal has been to develop new ways that further engineer protein A to increase its alkaline stability. It has been 12 years since the commercialization of MabSelect SuRe resin, and technology has advanced since then. We went back to the drawing board to look for the weak points and determine what tends to be sensitive to NaOH. We considered what we can do to change those sites and increase stability without affecting the affinity of the ligand for IgG. The key to our work was using Biacore, which is a surface plasmon
Using Biacore allowed us to evaluate ligands without having to synthesize resin. We could work at the molecular level and screen ligands using a high-throughput methodology.

We used the information gained from a series of experiments to create new libraries of ligands and make combinatorial changes in future designs. We screened more than 400 different constructs using this technique. As a result, we designed a monomer we thought was significantly more stable than what MabSelect SuRe is today.

We also looked at what would happen if we made various lengths of this new monomer, and we looked at the effect of the ligand length on various beads codeveloped at the same time. This is one of the first times we have (at least for an affinity resin) codeveloped the bead with the ligand to match the two materials to each other. If you think about how beads work, the larger the pores, the less diffusional resistance for IgG to get into pores, but there also will be less surface area.

You have an optimization exercise here in which you are trying to create as much surface area as possible and immobilize as much ligand as possible, and also bind as much IgG as possible, while also trying to prevent a steric hindrance.

A bead with very small pores has a larger surface area but also more diffusional resistance. A bead with large pores reduces this resistance but may be softer and/or have less surface area. The project team worked to optimize the ligand design and bead design so that we have a ligand design that provides high capacity as well as a bead design that is well-matched to that construction.

The Next Generation: MabSelect PrismA

The result of this work is the development of a new product we call MabSelect PrismA, the next generation of the MabSelect family. It is a new bead, and a new ligand. It is designed to solve the challenges described earlier: to resolve bottlenecks in production, and to have exceptional alkaline stability that allows for more stringent cleaning and better control of bioburden.

Alkaline Stability: As shown in Figure 4, typically when cleaning with 0.5 M NaOH, you pay a penalty in terms of loss of resin capacity. The figure shows a comparison of MabSelect PrismA, MabSelect SuRe LX, and MabSelect SuRe. You can see we have maintained more than 95% of the dynamic binding capacity (DBC) with MabSelect PrismA, even over 150 cycles with 0.5 M NaOH. If you increase the NaOH concentration to 1.0 M, you can achieve even more stringent cleaning. This might be desirable if you want to align your downstream purification suite so that all of your resins are cleaned with the same CIP solution (e.g., 1.0 M NaOH).
Figure 5 shows a comparison of MabSelect PrismA resin with MabSelect SuRe LX resin. After over 150 cycles, MabSelect PrismA loses about 10% of its DBC whereas MabSelect SuRe loses approximately 50% of its DBC. MabSelect PrismA is significantly more stable than either MabSelect SuRe or SuRe LX. This increase in NaOH stability can be used both for increased cleaning, which can reduce batch-to-batch carryover, as well as to control bioburden outbreaks on the protein A column.

Over the past few years, we have also developed a technique to recover from a spore-forming bioburden incident using an oxidizing agent called peracetic acid. This work has been completed for MabSelect SuRe (Figure 6). This recommendation will be extended to cover MabSelect PrismA as well. It can be a preventative or remedial measure to ensure that spore-forming bacteria don’t take hold on the protein A step.

Security of Supply Chain and In-House Bioburden Control: We have done considerable work around security of supply for MabSelect PrismA resin as well as work in bioburden control in our own manufacturing facilities. The agarose used to make the base matrix and the ligand itself are without doubt the two most critical raw materials, and we have validated in dual sources of both for MabSelect PrismA. For the ligand, GE is going to manufacture approximately 50% of the ligand supply, and a contract manufacturer who will use the same process will manufacture the remaining supply. This has been an additional investment on GE’s part to ensure we have full control of ligand manufacturing and to work with our contract manufacturer to make sure they have capacity to meet our ligand needs moving forward.

In our facilities, we have worked to make sure we are doing everything necessary to minimize bioburden in our products. We have a very good track record in this space, and have been operating in a scenario well within specifications for many years. In 2016 we felt it was time to make some updates to those specifications, and therefore we issued a change control notification announcing the reduction in bioburden specification from <100 cfu/mL down to <20 cfu/mL and the addition of a specification for endotoxin. Those two changes are the result of many years of in-house tests proving we have stayed well within specification. This doesn’t represent a change for end-users in the sense of what you are receiving from us because we have been well below these for many years, but it does give an added level of assurance that we will never release anything that is outside of the new specifications.

Since 2010, GE Healthcare has produced hundreds of lots of MabSelect SuRe. Our absolute, most common value for bioburden is zero. More than 94% of the lots released have no detectable bioburden. Over the same period of time, we have not measured a bioburden value higher than 5 cfu/mL. We have worked hard with our operators and manufacturing operations to help them understand the importance of hygiene on their operations and the impact it can have on our customers. We feel very proud of this result.

Summary
We recognized a couple years ago that protein A is a relatively susceptible step in downstream purification with respect to bioburden contamination. We also know that protein A in general has the potential to become rate limiting as titers increase. MabSelect PrismA is designed to address these issues by enabling the use of 1.0 M NaOH for cleaning and sanitization, offering significantly improved binding capacity.

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