Simplifying Progress

Thoughtful Process Development for Antibodies With Sartobind® Rapid A

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Introduction

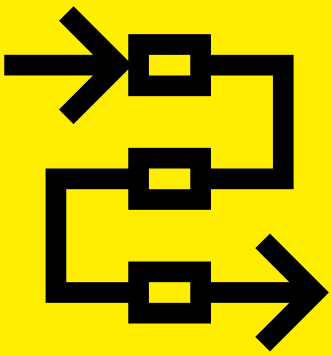
With global supply chain shortages, personnel shortages, and increased pressure to bring antibody therapeutics to the market on accelerated timelines, process development (PD) must ensure that scale-up can proceed rapidly with large quantities being produced, while simultaneously minimizing suite time. In this article, we will discuss a concept that addresses these factors referred to as 'Thoughtful PD,' and how membrane chromatography using Sartobind® Rapid A for capture of antibodies can naturally address such considerations when implementing such a strategy.

What is Thoughtful PD?

Thoughtful PD considers many factors, and how these considerations will impact the groups that will receive the developed process downstream of PD. Groups impacted subsequent to PD are: Tech Transfer, MS&T, Pilot and Scaled-Up Manufacturing.
For chromatography, some examples of factors to consider making thoughtful PD are:

- **Suite space required for the final scaled-up process**
- **Capital expenses for both PD and manufacturing spaces**
- **Personnel training skill level to execute an affinity chromatography unit operation**
- **Set-up, staging, and breakdown activities required outside operating the chromatographic step**
- **Chromatographic media supply chain**
- **Scalability**
- **Regulatory guidance**

Failure to align these factors at the PD stage could mean that the process is rejected by subsequent groups that receive the process and must be sent back to PD to be reoptimized to some degree. This would be considered a wasteful activity, and negatively impact overall project timelines. Thoughtful PD considers all, or at minimum, the vast majority of the aforementioned factors, allowing the process to move forward to the next stage efficiently. Inherently, membrane chromatography addresses most of the factors required by 'Thoughtful PD'. Sartobind® Rapid A is a scalable Protein A affinity membrane for the capture of Fc containing antibodies, that provides the perfect opportunity for 'Thoughtful PD' for capture of antibodies from cell culture.



Critical Benchmarks

When switching to a more productive method of capture for antibodies, one of the primary considerations, is to ensure that the critical quality attributes (CQAs) that the purification unit operation impacts will achieve the quality target product profile (QTPP) for drug substance match or improve upon the legacy unit operation. It is essential that the selectivity of a unit operation can remove critical product and process related impurities efficiently, while meeting economic targets such as dynamic binding capacity (DBC) given at a certain residence time (rT). It is also essential that the unit operation takes regulatory factors into account, such as the minimization of leached protein A (LPrA). Technologies to be implemented must meet or exceed that of legacy industry standards. Traditionally, the antibody capture step is accomplished using Protein A chromatography media in packed columns. Membrane chromatography using Sartobind® Rapid A has demonstrated equivalent or better performance to legacy Protein A chromatography media, while providing substantial gains in process economics and productivity.

Dynamic Binding Capacity and Residence Time

In addition to buffer selectivity conditions such as buffering system, buffer strength, conductivity, pH, and solubility additives, DBC is typically a trade-off between rT and the pressure that a chromatographic system, including the chromatographic media, permits. With traditional chromatography media, depending on load viscosity and mass, loading rT is indirectly proportional to pressure, and the amount of antibody bound per volume of PrA chromatography media. The convective mass transport of Sartobind® Rapid A means that less pressure is generated than in chromatography media-based systems where diffusive mass transfer is a limiting factor. With pressure minimized in this relationship, the trade-off between rT, antibody load mass (viscosity), pressure, and DBC is now simplified from an engineering perspective (Figure 1). This provides a much larger design space for manufacturing to operate in during tech transfer. Faster rT and maximized DBC means a smaller footprint, less suite time, and lower CoGS for consumables leading to higher productivity.

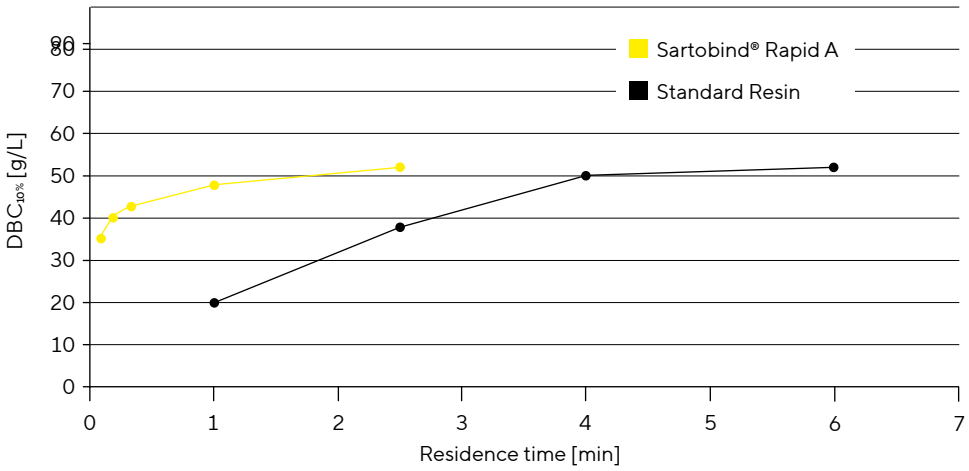


Figure 1: *Comparison of DBC at 10% Breakthrough Between Sartobind® Rapid A and Standard Resin[1].*

Yield

The main driver of process intensification in upstream is to increase the amount of antibody that is produced with the existing equipment. One of the challenges in downstream is to minimize product loss during the different purification and filtration steps. Therefore, the protein A media should be able to provide high yields. Sartobind® Rapid A was shown to result in comparable yields to industry accepted protein A resins (see Figure 2). With this, it is ensured that the overall yield in downstream is not impacted.

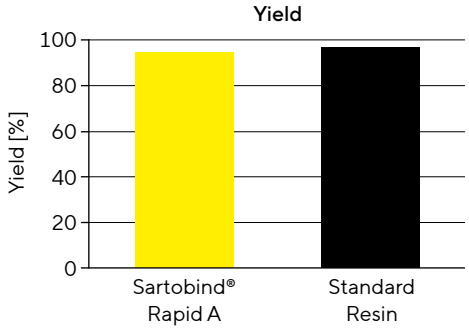


Figure 2: *Comparison of Yield Between Sartobind® Rapid A and Standard Resin[1].*

Contaminant Removal

For the production of antibodies, regulatory agencies require process and product related impurities, such as host cell proteins (HCP), DNA, virus, and aggregates | clipped forms, to be reduced below a certain level to ensure the safety of patients when the therapeutic is administered at a prescribed dosage. Although subsequent chromatographic steps employing orthogonal modes (AEX, HIC, CEX, MM) are used to perform final polishing of such impurities, the bulk of these impurities are removed in the flow through of a bind and elute antibody capture step. Sartobind® Rapid A effectively removes the bulk of all the main impurities generated by mammalian expression systems. Membrane chromatography achieves this despite the fact that unit operation processing time is typically an order of magnitude faster than legacy chromatography media-based antibody capture unit operations.

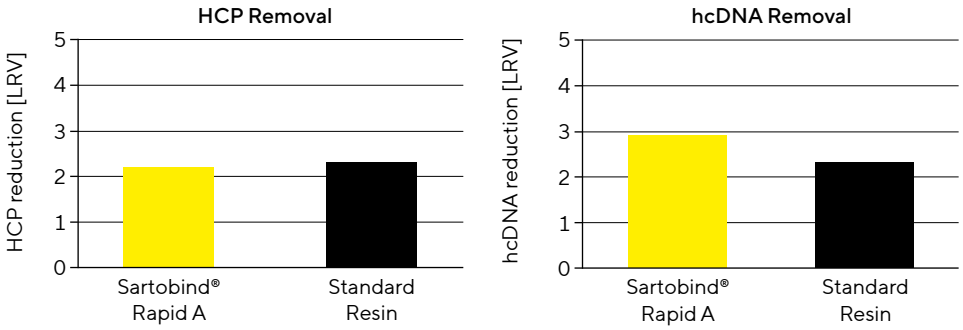


Figure 3 : *Comparison of HCP and hcDNA Removal Between Sartobind® Rapid A and Standard Resin[1].*

Elution pH

Aggregation of antibodies can occur at lower pH. This creates an additional impurity that places burden on the following chromatographic unit operations. This increases the chromatographic media amount required for subsequent polishing chromatography processing steps and potentially, yield loss. The objective is to minimize aggregation as much as possible with an increased elution pH and reduce exposure time to low pH to minimize on-column aggregation. Both legacy resins and Sartobind® Rapid A utilized descending pH for elution of the antibody from the chromatographic media. Considering residence times are typically a magnitude of order faster with membrane chromatography, therefore time for on-column aggregation due to low pH elution is minimized. Where a higher pH elution is not possible from a recovery perspective or pH-sensitive antibodies, elution pools for Sartobind® Rapid A may be directed into a neutralization buffer containing additives that prevent aggregation such as arginine, similar to traditional Protein A chromatography media.

Leached Protein A

Over time, PrA from chromatography media and membranes can leach into the product pools. Although cleared downstream in the process by additional purification steps, LPrA is a regulatory concern since protein A is derived from human pathogen, Staphylococcus aureus. When used within the specifications deemed appropriate for the chromatography media or membrane, both chromatography media and membranes leach negligible Protein A ligand. The amount of Protein A ligand that is leached from Sartobind® Rapid A is lower compared to legacy Protein A chromatography media.

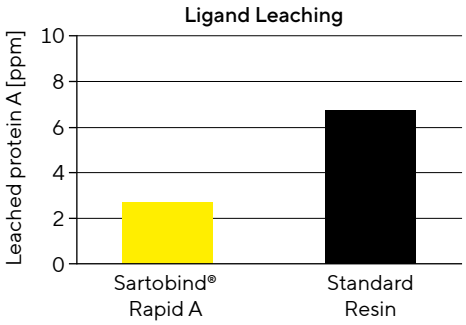


Figure 4 : *Comparison of Leached Protein A from Sartobind® Rapid A and Standard Resin[1].*

Chemical Compatibility

Modern Protein A chromatography media are alkaline tolerant for CIP cycles up to >1M NaOH. Studies show that up to 0.5M NaOH is sufficient for adequate cleaning-in-place (CIP) of Protein A chromatography media: Sartobind® Rapid A is no different. Sartobind® Rapid A is compatible with 0.5 M NaOH for bioburden reduction, and 0.2 M NaOH has been shown to be appropriate for intra-batch cycling. Additionally, Sartobind® Rapid A is compatible with most other Protein A affinity chromatography buffers and additives such as acetate, citrate, glycine, arginine, glutamine, high salt, and detergents.

Using High Throughput DOE and Column-Based Screening for Process Selectivity Conditions

After discussing key considerations in implementing Thoughtful PD to an antibody capture purification unit operation at the bench scale, with the needs of future groups responsible for technical transfer scale-up in mind, here are some tools and considerations for the process development group itself .

Sartobind® Rapid A is offered in convenient screening formats such as 96 well-plate formats for performing buffer selectivity and static binding DOE, as well as nano devices for optimizing engineering parameters such as DBC given residence times, flow rates and elution pools. This allows for a robust design space that can be optimized in PD for the seamless, robust technical transfer by MS&T to pilot and large-scale manufacturing.

Additionally, lifetime studies are a necessary, but very time-consuming, part of PD. It is essential to validate how the equipment and processes will respond over the time that they are used. One benefit of using membranes is that they are only used for one production batch, so the lifetimes are very short. This means that lifetime studies can be performed much faster because cycles take 10 – 15 minutes, compared to 2 – 4 hours with resin-based systems. It is also not necessary to study the effects of multiple cleaning rounds, or the effect of long-term storage.

Membrane chromatography using Sartobind® Rapid A, coupled with a robust design space that can be developed with scaled down tools, is the definition of 'Thoughtful PD' and creates synergy between PD and manufacturing groups.

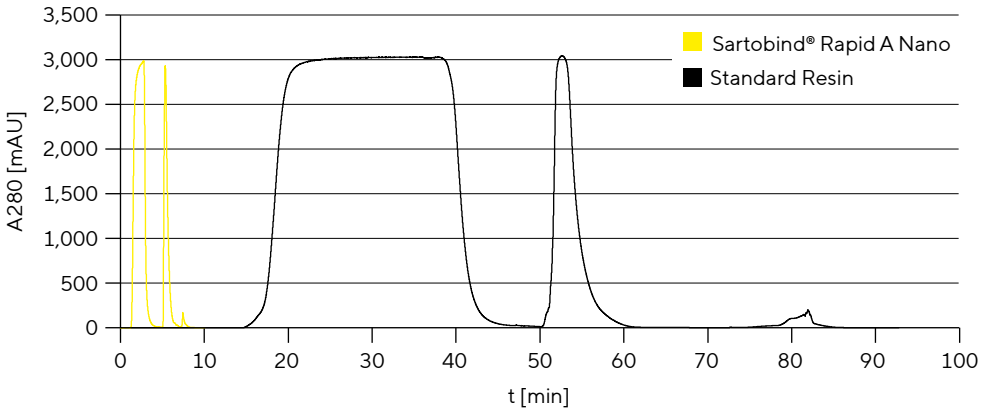


Figure 5: *Comparison of Cycle Times of Sartobind® Rapid A and a Standard Resin.*

The chromogram in Figure 5 illustrates the process PD could transfer to manufacturing with Sartobind® Rapid A. Note that equilibration, loading, elution and stripping in Sartobind® Rapid A occurs even before the loading of the legacy chromatography media. The synergy that this created between PD and manufacturing makes membrane chromatography a very easy technology to consider adopting in manufacturing from PD.

Process Productivity: Adopting a Holistic Approach

Sartobind® Rapid A provides several additional process improvements. Firstly, the cycle speed is dramatically decreased from several hours using legacy chromatographic media to 10 – 15 minutes with membranes (Figure 5). Secondly, the membrane is provided in ready-to-use devices and are discarded after one batch, which eliminates the overall column handling activities in the facility. This reduces the overall time needed to complete a batch and increases the productivity of the plant. Thirdly, the need for column qualification and CIP is eliminated when using membrane chromatography. Sartobind® Rapid A allows for a chromatographic capture process with less equipment, and therefore a smaller overall plant footprint. A production plant can, for example, eliminate cold storage and other suite space associated with column storage, packing and qualification. Additionally, consumables and capital expenses such as the need for large quantities of resin and respective large diameter column, are also minimized. With these items considered, labor associated with set-up and break down are also substantially decreased with a membrane chromatography unit operation.

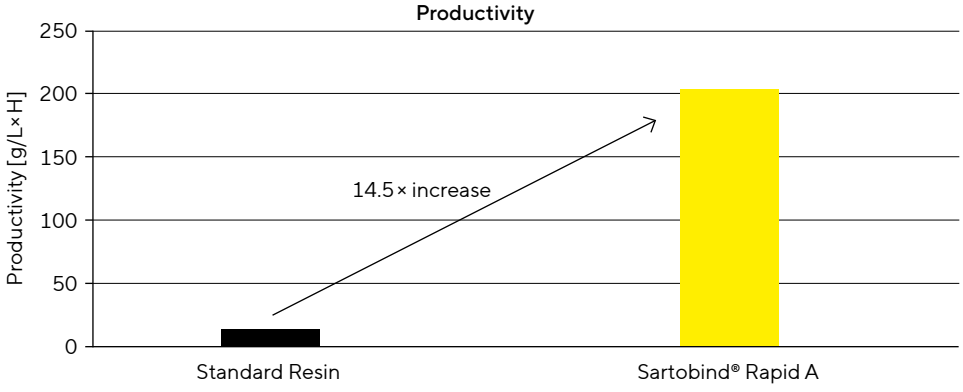


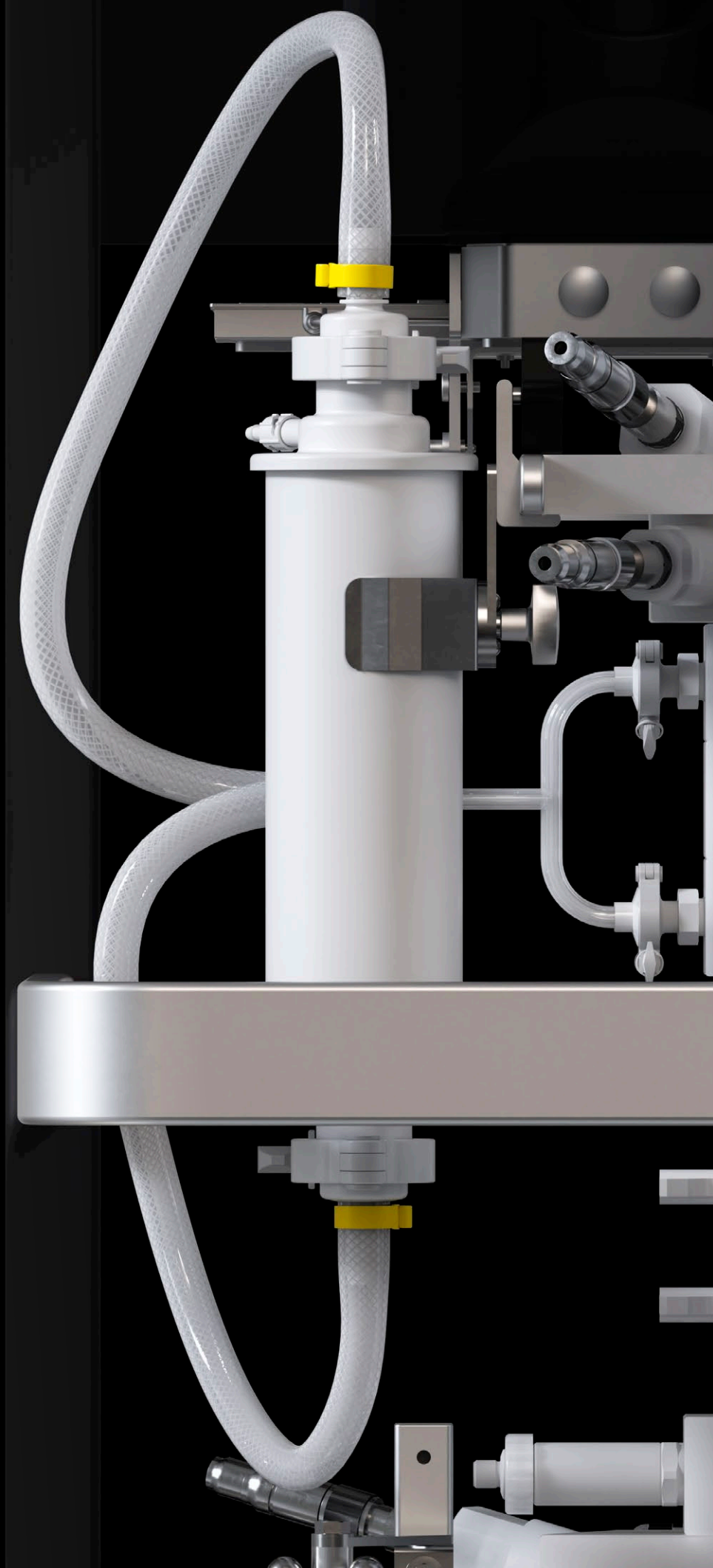
Figure 6: *Productivity Comparison of Sartobind® Rapid A and Standard Resin*[1].

Want to Evaluate Sartobind® Rapid A for Increased Process Productivity?

Sartobind® Rapid A will help your team develop a thoughtful antibody capture process for a seamless transfer forward considering procurement, MS&T, and manufacturing The technology is both simple to evaluate at the bench, and even more simple to scale to large-scale commercial manufacturing.

Contact us today to learn more about Sartobind® Rapid A. Our team is on-hand to answer any questions and to provide technical guidance and support, including set-up and protocols.

For more information, visit
www.sartorius.com/sartobind-rapid-a



Author Bio

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Dr. Ricarda A. Busse joined Sartorius in February 2018 as a Product Manager for Membrane Chromatography. She has a PhD in biology | biochemistry from the Georg-August University of Goettingen. She also holds an MBA from the European Fernhochschule Hamburg in General Management, where she specialized in digital and international marketing.

She has 8+ years of experience in the biotechnology and bioprocessing industry. Prior to joining Sartorius, she worked as Product and Marketing Manager for affinity chromatography solutions used for recombinant proteins at IBA Lifesciences. During her time as a doctoral candidate at the Max Planck Institute of Biophysical Chemistry, Geottingen, she worked on upstream and downstream process optimization of recombinant proteins from bacterial, mammalian and insect cell cultures.

James M. Sulzberger
Product Specialist Chromatography, Sartorius

James Sulzberger began working for Sartorius in 2022, where he is a Chromatography Consumables Product Specialist. He holds a BS in Biology from University of Illinois at Chicago, and an MS in Chemistry from Northeastern Illinois University.

James is an American downstream process development scientist with over 13 years of bioprocess and analytical development. Prior to Sartorius, James was the Senior Director of Protein Science for COUR Pharmaceuticals where he was responsible for all aspects of recombinant protein process development, analytical development, and characterization. Previously, James was the Technical Director at Aldevron's CDMO business where he helped clients design drug substance manufacturing processes and directed execution internally for process development and scale-up for recombinant proteins and antibodies. Prior to Aldevron, James held various roles at Bio-Rad Laboratories, Pall Corporation, Adello and Celegne focusing on downstream process and analytical development.

References

1. Grünberg M, Kuchemüller KB, Töppner K, Busse RA. Scalable, Robust and Highly Productive Novel Convecdiff Membrane Platform for mAb Capture. Membranes. 2022; 12(7):677. https://doi.org/10.3390/membranes12070677

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