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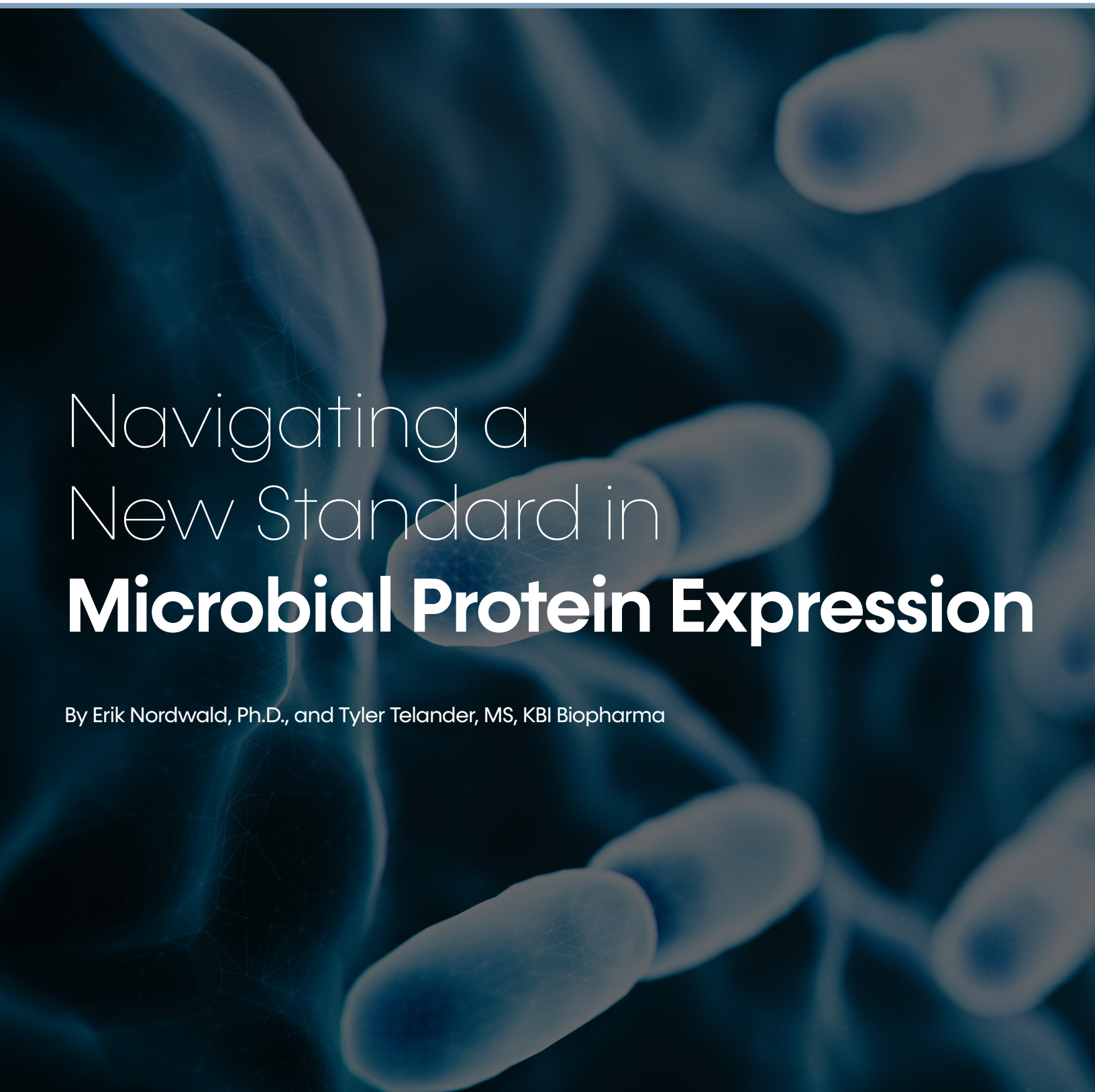

Navigating a New Standard in **Microbial Protein Expression**

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The complex task of developing protein therapeutics adhering to the highest safety, identity, strength, purity, and quality (SISPQ) targets can be a costly and time-consuming endeavor. Low titers increase the number of batches required to meet product demands and drastically increase cost and time, sometimes leading to a financially unfeasible program. Product and process-related impurities increase the burden on downstream purification, challenge developing timelines, increase complexity, and further expand program costs. An ideal therapeutic protein "factory" creates a pure target product with high titers from the start, which can minimize development risk, timelines, and cost. Furthermore, the class of proteins that can be made within a given factory is often limited, so there is an innate need to defend against potential later-stage overhauls and create a distinct factory that propels programs forward, expands possibilities, and salvages struggling programs to keep them viable.

E. coli has long been implemented as a therapeutic protein factory due to its simplicity, tractability, and wealth of information characterizing the microbe. Biotherapeutics produced from *E. coli* include growth factors, interleukins, cytokines, FABs, and more. Recent advancements in genetic engineering, analytics, and strain screening allow for reimagining *E. coli* as an "optimal" protein factory, not just a common one or as an expression system artifact.

To help push this portion of the industry into the 21st century, KBI has embarked on the audacious goal of creating an optimal therapeutic protein factory. While common *E. coli* expression strains alter as many as five genes to improve one aspect of recombinant protein expression, KBI has created a platform *E. coli* with about 1,000 genes altered and roughly 1 Mbp of DNA removed. Every idea aimed at optimizing the SISPQ of recombinant proteins has been rolled into an optimal genotype associated with reduced translational and post-translational modifications, excess genes, virulent genes, and destabilizing genes, all layered on top of an improved metabolism. The result is a PURE, efficient platform expression strain—PUREcoli™. PUREcoli grows nearly 50% faster in fully defined media and to about 2X the cell density (up to 40% WCW) as common *E. coli* strains. The faster growth reduces fermentation times and the 2X higher cell density allows for 2X the potential titer. Simultaneously, the PUREcoli genotype is associated with higher stability and yields robust, stable protein expression. This, combined with higher cell density, allows for massive potential titers, even for recalcitrant proteins.

A Novel Approach: Building a Library of Proactive Solutions for Every Molecule

It is not yet possible to predict the best expression strategy for optimal titer and product quality for a given protein. From the PUREcoli platform, a library of substrains has been generated and aimed at proactively providing a unique solution to optimize titer and product quality for all the unique challenges each protein may present. Alongside this strain library, a library of plasmids (PUREplasmids™) has been generated which can pair with third-party expression strains, or preferably PUREcoli. Pairing PUREcoli and PUREplasmids increases the toolbox of solutions against therapeutic protein expression challenges. The combination of strain and plasmid libraries represents a solution to a known industry problem from one company, as it truly optimizes and tunes expression for maximal titers and product quality. Additionally, PUREcoli and PUREplasmids complement one another in such a way that antibiotics are not required for the selection of plasmid-containing cells. Elimination of antibiotics is one of several process impurities attenuated in the PUREcoli and PUREplasmids platform that ease purification and testing requirements. PUREcoli and PUREplasmids proactively address processing challenges for a lean "PURE in, PURE out" manufacturing strategy.

Secretion has long been the "holy grail" for microbial protein expression. Secretion is, in essence, programming the cell to do a tremendous amount of purification up front. By selectively secreting your protein of interest, the cell is inherently separating your recombinant protein from the host's DNA and the majority of the host's proteins. This eliminates the cell lysis unit operation, which creates small particles of cell debris that are additionally cumbersome to separate out. Clarifying a cell lysate is a non-standardized operation that is challenging to develop and often requires many expensive filters. Conversely, with secretion, the intact cells have a large enough particle size and sedimentation velocity that clarification from the supernatant is immensely simplified. Heading to column chromatography with a higher starting purity can increase load rates and potentially eliminate an entire column operation. Despite these benefits, robust, consistent microbial secretion remains a dream not yet realized.

Within the PUREcoli™ library of substrains are strains that have been specifically developed to promote secretion. Common problems with secretion include overwhelming the secretion capacity of a cell during protein induction, much like trying to navigate a busy road during rush hour. KBI has addressed this by reducing the traffic through the secretion machinery within the 1,000 gene deletions in the platform. Reducing this secretome also increases the starting purity of the target recombinant protein outside the cell. We have also bolstered the secretion capacity of the cell through a combination of specific gene edits and sophisticated co-expression strategies. Early data show PUREcoli is more capable of full secretion (not just through the inner membrane to the periplasm) and may bolster secretion by 5X or more over other *E. coli* expression strains. In addition to improved bioprocessing, secretion allows for disulfide bond formation, opening the door to the expression of new classes of proteins, such as FABs, in active form.

What it Means to Have a Comprehensive Platform for Robust Scalable Protein Expression

Leveraging process automation technologies at a micro-scale, KBI has developed rapid, high-throughput screening workflows for screening strains 8, 48, or 96 at a time. Strain screening can include screening for protein variants, multiple protein candidates, and proteins with undetermined optimal modality (secretion, soluble intracellular, or inclusion body); all three modalities could be developed in parallel. Common plasmid optimization may target elements like the copy number, promoters, ribosome binding site, codon optimization strategy, or integration of "helper-genes." Host strains may have different chaperones, proteases, stress response, stability, be self-lysing, and more. Within a screening package, either KBI's proprietary strains and plasmids or third-party strains and plasmids may be selected. This combinatorial exploration of plasmids, host strains, and protein variants necessitates these high-throughput screening packages.

Despite being fast, compliance and scalability are maintained. As strains are filtered down from many to few, a 1 mL fed-batch microfermenter allows for high-throughput screening under representative and scalable fermentation conditions. In addition to strains, fermentation parameters such as pH, media, feed-rates, and induction time-courses can be explored. The outputs of our strain screening are scalable fermentation conditions, an optimized strain, and a GLP RCB, suitable for MCB creation.

The final complement to KBI's proprietary strains and plasmids is our fermentation media (PUREmedia™). PUREmedia is an optimized recipe of nutrients, with no undefined or complex components (e.g., yeast extract), that supports robust growth and protein expression. Using PUREmedia, we have accurately scaled from our microfermenter directly into a 30 L

single-use fermenter (30,000X scaling). From expressing dozens of proteins—using third-party expression strains or PUREcoli™—product titers are consistently higher when expressed in PURExpress® compared to other media. The platform of strains, media, and plasmids together make PURExpress® everything needed for clean, high-titer protein expression. Altogether, and strictly as a platform, PURExpress can remove months of development and save millions of dollars for those considering a newly standardized microbial expression system for protein production.

The Industry Needs a Full-Service CDMO (That Delivers)

Finding a partner with the right microbial expression system for a protein is an important step in enabling commercial success. Equally important is vetting a partner for end-to-end development capabilities, vertical integration, in-house capacity, and expertise. While choosing the right CDMO comes down to many variables, identifying one that can leverage premium cell line development alongside comprehensive manufacturing expertise to establish a reliable path forward is crucial to achieving commercialization. Partners like KBI possess years of experience scaling programs from bench to cGMP and shepherding biologics to FDA compliance and commercialization. And partners like these offer organizations integral insights into critical target product profiles and the development activities needed to achieve their targets. Likewise, the PURExpress platform was built to function as a proactive solution to low titers and poor purities from underdeveloped/underperforming expression platforms. Platform technologies like these, combined with incumbent expertise and longstanding experience, can afford companies accelerated timelines, lower costs, and an optimal product profile. It's that ultimate deliverable that identifies a breakout partnership.

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Our Mission Is To Accelerate The Development Of Innovative Discoveries Into Life-Changing Biological Products And Expand Global Access Of Medicines To Patients In Need.