PepTalk

PepTalk 2026

Conference Track Summary –

Recombinant Protein Production Part 1

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Executive Overview

The PepTalk 2026 Recombinant Protein Production Part 1 track highlighted how advances in expression technologies, cell-free systems, AI-driven design, and process engineering are expanding access to increasingly difficult protein targets. Across sessions, speakers focused on improving protein quality, functionality, and manufacturability while reducing development timelines and overcoming longstanding challenges associated with membrane proteins, GPCRs, glycoproteins, and complex multi-protein assemblies.

Most Frequently Covered Issues

1. Membrane protein and GPCR production
Multiple presentations addressed strategies for expressing, stabilizing, purifying, and characterizing membrane proteins and GPCRs using nanodiscs, cell-free systems, engineered surrogates, and specialized expression platforms.

2. Cell-free expression and glycosylation technologies
Cell-free production systems emerged as versatile tools for generating difficult proteins, engineering post-translational modifications, accelerating screening, and enabling more controlled protein production workflows.

3. Alternative expression platforms
Speakers explored diverse production approaches beyond traditional mammalian and microbial hosts, including transgenic insects, advanced baculovirus systems, plant-based cell-free platforms, and next-generation mammalian expression technologies.

4. AI and machine learning for protein production
AI was increasingly applied to antibody design, membrane protein engineering, gene sequence optimization, cell line development, and prediction of high-performing expression constructs.

5. Protein quality and biological relevance
Many presentations emphasized that successful protein production requires more than high yields, with functionality, proper folding, post-translational modification, stability, and biological activity receiving equal attention.

Recurring Takeaways

- Difficult targets often require specialized expression, stabilization, and purification strategies rather than conventional workflows.
- Cell-free systems are becoming powerful tools for rapid protein production, screening, and engineering.
- AI and machine learning are improving both protein design and manufacturing efficiency.
- Host selection, expression construct design, and post-translational processing can significantly influence protein quality and performance.
- Future recombinant protein production platforms will increasingly combine computational prediction, advanced expression technologies, and biological validation to improve speed, scalability, and success rates.

PepTalk

Executive Overview ... 2
Optimizing Target Protein Production: Host Selection's Impact On Quality ... 4
Cell-Free Refolding of Challenging Membrane Proteins into SMALP Nanodiscs for Enhanced Stability and Functionality ... 5
CHS-114, a Highly Selective, Cytolytic Antibody Targeting Intratumor CCR8+Tregs: A Case Study in Overcoming Challenges in Developing Anti-GPCR Antibodies Without Off-Target Binding ... 6
Expi293 PRO Expression System: Higher Titers and Faster Time to Protein in an Easily Automated Format ... 7
Unlocking Complex Targets: Efficient Production of Multi-Protein Assemblies in Mammalian Cells via MultiBacMam ... 8
Scaling Recombinant Protein Production With the EntoEngine: From Sequence to Commercial Scale in Seven Months ... 9
Recombinant Membrane Protein Production and Subsequent in vitro Glycosylation: Overcoming the Challenges of Producing Membrane Proteins with Complex Post-Translational Modifications ... 10
High-Yield Production of C-Terminally Processed KRAS4a, HRAS, and NRAS for Biophysical Study ... 11
Evaluation of Codon Optimization Strategies for Human and Murine Glycoproteins Full Video Here ... 12
Advanced vector platforms for enhanced biotherapeutic protein expression ... 13
Boosting Recombinant Protein Titers with Metabolic Modelling, and Harmonizing Metabolomics Datasets for Cross-Study Integration ... 14
Smart Production: Leveraging AI for Efficient Recombinant GPCR Expression ... 15
Membrane Protein Targets Reengineered for Soluble Expression ... 16
AI-Accelerated Advancements to Cell Line Development: Integrating Sequence Mining, ASR, and ML-Stability Models ... 17
inGenius® Bioprocessing: Reducing Risk, Boosting Titre with ML-Driven Protein Production ... 18
Recombinant Expression and Characterization of Histatin-Derived Peptides ... 19
Engineering Cell-Free Glycosylation Systems for Immune-Optimized Vaccines ... 20
Yeast-Based Expression and Enzymatic Cyclization of Disulfide-Rich Cyclic Peptide Scaffolds for Drug Development ... 21
Accelerating Membrane Protein Purification: Innovations with Nuclera ... 22

Optimizing Target Protein Production: Host Selection’s Impact On Quality

Full Video Here

Erika Orban, PhD, Principal Scientist, Protein Discovery and Bioanalytics, Zoetis Inc.

Producing recombinant proteins for antibody discovery often appears straightforward until protein quality becomes the limiting factor. Using a canine pro-inflammatory cytokine linked to autoimmune and inflammatory diseases as a case study, the presentation showed how every stage of an antibody program depends on reliable recombinant protein, from immunization through binding and functional assays. The work underscored that protein availability alone is not enough; biological activity and consistency are equally critical. (00:00:00–00:02:48)

Early efforts relied on a commercially available E. coli-derived protein, but severe batch-to-batch variability quickly undermined confidence in downstream results. Multiple in-house expression approaches were then explored across E. coli, mammalian, and insect systems. While some formats produced acceptable yields or demonstrated target binding, most failed to generate protein that remained functionally active in cell-based assays, highlighting the gap between expression success and biological relevance. (00:02:48–00:08:23)

Additional optimization revealed how difficult it can be to balance expression, solubility, purity, and functionality. An E. coli construct containing a maltose-binding protein tag improved yields and activity, yet endotoxin levels made it unsuitable for immunization. Mammalian and insect systems also presented limitations, producing proteins that either lacked functional activity or yielded insufficient material for further evaluation. (00:05:35–00:08:23)

The breakthrough came from screening multiple cell-free expression platforms rather than focusing on conventional host systems alone. Among bacterial, mammalian, and plant-based cell-free approaches, only the plant-based platform consistently produced functionally active protein without problematic endotoxin levels. The findings reinforced the importance of evaluating several expression systems early, since host selection directly influences protein quality, assay performance, and the overall success of biologics discovery programs. (00:08:23–00:16:44)

Key Takeaways

- Functional protein quality matters more than expression yield alone.
- Binding activity does not guarantee biological activity in cell-based assays.
- Expression systems can fail for different reasons, including aggregation, endotoxin burden, or loss of functionality.
- Early screening across multiple host platforms increases the likelihood of identifying a fit-for-purpose protein production strategy.

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Cell-Free Refolding of Challenging Membrane Proteins into SMALP Nanodiscs for Enhanced Stability and Functionality

Full Video Here

Matthew A. Coleman, PhD, Senior Scientist & Group Leader, Biosciences and Biotechnology Division, Lawrence Livermore National Laboratory

Membrane proteins remain among the most difficult biological targets to study because traditional detergent-based extraction often disrupts their native structure and function. Despite decades of effort, the vast majority of membrane proteins remain structurally uncharacterized, limiting progress in drug discovery and mechanistic biology. This presentation explored how cell-free expression combined with nanodisc technologies can provide more native-like environments for producing and studying these challenging proteins. (00:00:00–00:04:39)

The work focused on cell-free protein synthesis systems that enable rapid production of membrane proteins without relying on living cells. By combining cell-free expression with nanolipoprotein particles, researchers can co-translate membrane proteins directly into lipid-containing structures that improve solubility and stability. This approach produced functional forms of diverse targets, including porins, GPCRs, receptor tyrosine kinases, and multi-protein complexes that are often difficult to access through conventional expression methods. (00:04:39–00:10:34)

The presentation also examined the evolution of nanodisc technologies beyond traditional apolipoprotein-based systems. Synthetic platforms, including telodendrimer and styrene-maleic acid lipid particle (SMALP) approaches, offer alternative strategies for stabilizing membrane proteins. While some synthetic systems affected protein folding or cell-free expression efficiency, hybrid workflows combining high-yield cell-free production with post-expression refolding into SMALP nanodiscs generated larger quantities of functional membrane proteins while preserving important structural features. (00:10:34–00:19:35)

Results across multiple protein classes demonstrated that both co-translational nanodisc assembly and SMALP-based refolding can improve access to biologically relevant membrane proteins. The findings suggest that no single platform is universally optimal, but that combining cell-free synthesis, lipid engineering, and nanodisc technologies provides a flexible toolkit for producing functional membrane proteins at scales suitable for structural studies, screening, and therapeutic research. (00:19:35–00:22:14)

Key Takeaways

- Cell-free expression enables rapid production of challenging membrane proteins without living-cell constraints.
- Nanodiscs provide native-like lipid environments that improve membrane protein solubility and functionality.
- SMALP-based refolding approaches can generate higher yields while preserving physiologically relevant protein-lipid interactions.
- Hybrid workflows that combine multiple stabilization technologies offer new opportunities for studying previously inaccessible membrane proteins.

CHS-114, a Highly Selective, Cytolytic Antibody Targeting Intratumor CCR8+Tregs: A Case Study in Overcoming Challenges in Developing Anti-GPCR Antibodies Without Off-Target Binding

Full Video Here

Narendiran Rajasekaran, PhD, Director, Cellular Immunology, Coherus Biosciences

Regulatory T cells play an important role in suppressing anti-tumor immune responses, making them an attractive target for cancer immunotherapy. This presentation focused on CHS-114, a highly selective anti-CCR8 antibody designed to deplete suppressive CCR8-positive regulatory T cells within tumors while preserving the broader immune system. The work addressed a central challenge in immuno-oncology: how to remove tumor-associated immune suppression without triggering widespread immune-related toxicity. (00:00:00–00:07:51)

CCR8 emerged as a promising target because of its preferential expression on intratumoral regulatory T cells and limited expression on other immune populations required for anti-tumor activity. Preclinical studies demonstrated that targeting CCR8 reduced tumor growth, increased infiltration of cytotoxic CD8 T cells, and enhanced responses to PD-1 blockade. These findings supported the hypothesis that selective depletion of CCR8-positive regulatory T cells could remodel the tumor microenvironment and overcome resistance to checkpoint inhibitors. (00:07:51–00:11:56)

Developing an antibody against CCR8 presented significant technical hurdles because GPCRs are traditionally difficult antibody targets. To address this, the team adopted a mechanism-driven strategy focused on generating an antibody optimized for selective binding and immune-mediated cell killing rather than receptor signaling modulation. By screening against a carefully selected N-terminal CCR8 peptide and prioritizing specificity throughout development, researchers identified CHS-114, a high-affinity antibody that demonstrated no detectable off-target binding across thousands of human proteins. (00:11:56–00:20:57)

Preclinical and early clinical results showed that CHS-114 selectively depleted CCR8-positive regulatory T cells while preserving beneficial CD4 and CD8 T-cell populations. Biomarker analyses from ongoing clinical studies revealed evidence of immune remodeling within tumors, including substantial increases in cytotoxic T-cell infiltration and inflammatory cytokine responses. These findings suggest that selective targeting of CCR8 may offer a differentiated approach for enhancing anti-tumor immunity while maintaining a favorable safety profile. (00:20:57–00:28:04)

Key Takeaways

- CCR8 is a highly selective marker for tumor-associated regulatory T cells and a promising immunotherapy target.
- A mechanism-driven antibody design strategy helped overcome traditional GPCR development challenges.
- CHS-114 demonstrated strong selectivity with no detectable off-target binding in extensive screening assays.
- Early clinical data showed selective regulatory T-cell depletion, immune activation, and a favorable safety profile.

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Expi293 PRO Expression System: Higher Titers and Faster Time to Protein in an Easily Automated Format

Full Video Here

Jonathan Zmuda, Sr Director, R&D, Protein & Viral Vector Expression Systems, Thermo Fisher Scientific Inc

Transient protein expression has advanced dramatically over the past decade, moving from incremental improvements in yield to production levels once associated primarily with stable cell lines. This presentation introduced the Expi293 PRO Expression System, a next-generation HEK293 platform designed to increase protein yield, improve expression of challenging targets, and simplify high-throughput workflows. The development effort focused on enhancing every component of the system, including the cell line, media, transfection reagent, and production protocol. (00:00:00–00:08:02)

A key objective was enabling expression of proteins that historically performed poorly in mammalian transient systems. Across a wide range of antibodies, bispecifics, Fc fusions, extracellular domains, membrane proteins, and viral antigens, the platform consistently generated substantially higher titers than previous-generation systems. In many cases, proteins that produced little or no detectable expression under conventional conditions became readily accessible, opening new opportunities for difficult discovery and screening programs. (00:08:02–00:16:16)

The system also emphasized speed and scalability. Expression kinetics demonstrated that meaningful protein quantities could be obtained within 24 to 48 hours after transfection, with some molecules reaching gram-per-liter levels in only a few days. Streamlined workflows eliminated several traditional preparation steps, while flexible transfection and feeding strategies supported automation across formats ranging from deep-well plates to multi-liter production vessels. These capabilities enable researchers to rapidly screen large numbers of constructs while reducing hands-on time and operational complexity. (00:16:16–00:21:22)

Beyond productivity gains, higher expression levels created important downstream benefits. Reduced culture volumes lowered reagent consumption, purification requirements, plastic waste, and overall resource usage. The combination of high titers, rapid turnaround, automation compatibility, and broad protein applicability positioned transient HEK293 expression as a practical alternative for applications that previously required larger-scale or more resource-intensive production strategies. (00:21:22–00:23:47)

Key Takeaways

- Next-generation transient HEK293 expression can achieve multi-gram-per-liter protein yields.
- Challenging proteins that fail in conventional systems may become accessible through optimized expression platforms.
- Faster expression kinetics support rapid screening and high-throughput workflows.
- Higher titers reduce resource consumption, simplify purification, and improve overall process efficiency.

Unlocking Complex Targets: Efficient Production of Multi-Protein Assemblies in Mammalian Cells via MultiBacMam

Full Video Here

Robert M. Petrovich, PhD, Protein Expression Director, Genome Integrity & Structural Biology Lab, NIH NIEHS

As protein science increasingly focuses on membrane proteins and multi-protein assemblies, expression systems must accommodate targets that are far more complex than traditional soluble proteins. This presentation explored strategies for producing difficult membrane-associated complexes, highlighting how baculovirus-based technologies can be adapted to support coordinated expression of multiple proteins, chaperones, and stabilizing partners required for functional assembly. The work was driven by structural biology programs seeking active protein complexes for cryo-electron microscopy and mechanistic studies. (00:00:00–00:04:39)

A central focus was the use of MultiBac and MultiBacMam approaches, which allow multiple genes to be incorporated into a single baculovirus construct. This capability simplifies the production of large protein assemblies that would be difficult to generate through conventional co-transfection strategies. By combining target proteins with critical accessory factors, such as the chaperone NACHO for the α7 nicotinic acetylcholine receptor, researchers were able to overcome longstanding expression barriers and produce functional membrane protein complexes. (00:04:39–00:14:15)

The presentation highlighted two case studies. The first involved production of the α7 nicotinic acetylcholine receptor, a challenging pentameric ion channel that required co-expression of NACHO for proper membrane localization and assembly. The second focused on a ghrelin GPCR signaling complex composed of multiple proteins, including G-protein subunits, a stabilizing nanobody, and the RIC8A chaperone. In both cases, coordinated expression enabled purification of intact complexes suitable for structural characterization, ultimately supporting high-resolution cryo-EM analysis. (00:14:15–00:20:51)

Beyond individual targets, the work demonstrated how baculovirus-mediated mammalian expression can provide a flexible platform for increasingly sophisticated protein production challenges. By integrating chaperones, accessory proteins, and large genetic payloads into a unified workflow, researchers can generate biologically relevant complexes that preserve native interactions and functionality. These capabilities are becoming increasingly important as structural and therapeutic research shifts toward larger, multi-component molecular systems. (00:20:51–00:25:08)

Key Takeaways

- MultiBac and MultiBacMam systems enable coordinated expression of complex multi-protein assemblies.
- Chaperones and accessory proteins are often essential for successful membrane protein production.
- Baculovirus-based mammalian expression supports structural studies of challenging targets such as ion channels and GPCR complexes.
- Integrated multi-gene expression strategies expand access to biologically relevant protein assemblies for research and drug discovery.

Scaling Recombinant Protein Production With the EntoEngine: From Sequence to Commercial Scale in Seven Months

Full Video Here

Matt Anderson-Baron, Co-Founder & CEO, Future Fields

This presentation introduced the EntoEngine, a recombinant protein production platform that uses transgenic Drosophila melanogaster rather than traditional cell culture systems. By leveraging whole organisms instead of insect or mammalian cells, the platform is designed to address common scale-up challenges associated with bioreactors, cell-line development, and process optimization. The approach emphasizes simplicity, modularity, and predictable scaling while maintaining compatibility with standard downstream purification technologies. (00:00:00–00:06:30)

Several examples demonstrated the platform's ability to produce proteins that are often considered difficult to manufacture, including heterodimeric cytokines, transmembrane proteins, growth factors, and lipid-modified signaling proteins. Functional testing confirmed that these proteins retained biological activity, while analytical characterization showed appropriate assembly and processing. The examples highlighted how alternative expression systems can expand options when conventional platforms encounter issues related to toxicity, aggregation, or complex post-translational requirements. (00:01:53–00:05:48)

A central theme was eliminating the traditional trade-offs between speed, cost, quality, and scalability. Stable transgenic strains are generated directly through genomic integration, avoiding transient expression workflows and lengthy cell-line development cycles. Additional genetic tools, including selective breeding strategies and chromosomal balancing systems, provide stability and flexibility during strain optimization. Once established, strains can be scaled through a standardized rearing process that uses identical conditions from research scale through commercial production. (00:06:30–00:18:53)

The presentation concluded with a case study involving commercial-scale production of human prolactin. Starting from sequence information, the team developed a production strain, scaled manufacturing, and delivered purified material within seven months. Consistent purity, biological activity, and batch-to-batch performance were demonstrated, while the modular production model enabled rapid recovery from downstream failures and reduced overall manufacturing costs. The broader message was that scalable protein manufacturing can be simplified when production platforms are designed around operational flexibility and predictable scale-up from the outset. (00:18:53–00:23:11)

Key Takeaways

- Whole-organism expression platforms can provide an alternative to conventional cell-based protein manufacturing.
- Stable genomic integration eliminates the need for transient expression and lengthy cell-line development.
- Modular scale-up approaches reduce process complexity and improve manufacturing predictability.
- Simplified upstream production can lower costs while maintaining protein quality and functionality.

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Recombinant Membrane Protein Production and Subsequent in vitro Glycosylation: Overcoming the Challenges of Producing Membrane Proteins with Complex Post-Translational Modifications

Full Video Here

Gabriel A. Cook, PhD, Assistant Professor, Department of Chemistry, Oklahoma State University

Membrane proteins are among the most important targets in biology and drug discovery, yet they remain difficult to study because of their hydrophobic nature and complex post-translational modifications. This presentation focused on glycosylation, a modification known to influence protein structure, dynamics, and interactions, but one that remains challenging to investigate directly. The work aimed to develop methods that preserve both membrane protein integrity and glycosylation status, allowing researchers to study these proteins in forms that more closely resemble their native cellular state. (00:00:00–00:05:19)

The approach centered on in vitro glycosylation, where membrane proteins were first expressed in E. coli without glycans and then modified enzymatically after purification. Initial studies demonstrated that glycosyltransferase activity could be maintained even in the presence of detergents and membrane-mimetic environments. Using model peptides and membrane-associated constructs, the team confirmed successful addition of glucose residues, establishing that enzymatic glycosylation could occur under conditions commonly used for membrane protein studies. (00:06:37–00:11:06)

The methodology was then applied to gamma-sarcoglycan, a membrane glycoprotein involved in a larger muscle-cell adhesion complex linked to muscular dystrophy. Through protein expression, purification, and mass spectrometry analysis, the researchers demonstrated successful glycosylation of the full-length membrane protein. The work provided direct evidence that post-translational modification could be introduced after purification while maintaining compatibility with membrane-protein workflows. (00:11:06–00:15:53)

The team further extended the strategy to nanodisc systems, which provide membrane-like environments increasingly used in structural biology. Successful glycosylation within nanodiscs suggested the approach could be broadly applicable to membrane proteins studied by techniques such as NMR and cryo-EM. Ongoing efforts are focused on introducing larger, more biologically relevant glycans to better understand how glycosylation influences membrane protein structure, stability, and function. (00:15:53–00:20:17)

Key Takeaways

- In vitro glycosylation enables controlled study of membrane proteins with defined glycan modifications.
- Glycosyltransferase activity can be maintained in detergent and membrane-mimetic environments.
- Full-length membrane proteins can be successfully glycosylated after purification.
- Nanodisc-compatible glycosylation workflows may improve structural and functional studies of complex glycoproteins.

High-Yield Production of C-Terminally Processed KRAS4a, HRAS, and NRAS for Biophysical Study

Full Video Here

Simon A. Messing, PhD, Scientist II, Frederick National Lab & Protein Expression Lab, Leidos Biomedical Research, Inc.

The RAS family of proteins remains a major focus in cancer research, yet producing biologically relevant forms for structural and biophysical studies presents significant challenges. This presentation described efforts to generate fully processed KRAS4A, HRAS, and NRAS proteins carrying the same lipid modifications found in cells. Because membrane localization and signaling depend on these C-terminal modifications, obtaining correctly processed proteins is essential for studying RAS biology and developing new therapeutic strategies. (00:00:00–00:05:19)

Building on earlier success with KRAS4B, the team optimized an insect-cell expression platform that combines specialized fusion tags with co-expression of human farnesylation machinery. The approach dramatically improved protein solubility and enabled efficient post-translational processing. Additional purification development, including tailored ion-exchange strategies and the use of beta-cyclodextrin, allowed separation of fully processed proteins from incompletely modified species, resulting in highly homogeneous preparations suitable for downstream analysis. (00:05:19–00:12:32)

Extensive characterization confirmed that the resulting proteins retained the expected biological properties. Binding studies using lipid nanodiscs demonstrated membrane association behavior consistent with the known differences among RAS isoforms. Functional assays further showed that the proteins could engage downstream signaling partners and maintain intrinsic GTP hydrolysis activity, providing confidence that the recombinant reagents accurately reflected their native cellular counterparts. (00:12:32–00:18:49)

The presentation also highlighted the broader role of the Protein Expression Laboratory in supporting translational research programs. Through systematic construct design, expression screening, chaperone co-expression, and purification optimization, the team routinely develops challenging protein reagents for assay development, structural biology, and drug discovery efforts. Several case studies demonstrated how these workflows can rapidly deliver active proteins that had previously proven difficult to obtain through commercial sources. (00:18:49–00:24:02)

Key Takeaways

- Correct C-terminal processing is critical for producing biologically relevant RAS proteins.
- Co-expression of modification enzymes and optimized purification workflows enabled highly homogeneous preparations.
- Functional testing confirmed membrane binding, signaling partner engagement, and GTPase activity.
- Systematic expression and purification strategies can unlock challenging targets for structural and drug discovery applications.

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Evaluation of Codon Optimization Strategies for Human and Murine Glycoproteins

Full Video Here

Rob Meijers, PhD, Head, Biological Discovery, Institute for Protein Innovation

The Institute for Protein Innovation operates large-scale antibody discovery programs that depend on reliable production of mammalian glycoproteins. As part of building affinity reagents against hundreds of extracellular targets, the team encountered unexpected differences in expression between highly similar human and murine proteins. These observations prompted a systematic investigation into a common assumption in recombinant protein production: that codon optimization is inherently beneficial for mammalian protein expression. (00:00:00–00:11:15)

To explore the issue, the team first developed an open-source expression platform designed to simplify glycoprotein production and antibody generation. The platform included a streamlined mammalian expression vector and workflows that support high-throughput production of proteins and recombinant antibodies. This infrastructure enabled large comparative studies that would be difficult to perform using traditional one-target-at-a-time approaches. (00:04:30–00:09:51)

The core study evaluated multiple codon optimization strategies across a panel of human and murine glycoproteins expressed in HEK293 cells. Researchers compared native coding sequences, commercially optimized constructs, harmonized codon schemes, highly biased codon usage designs, and RNA stability-focused approaches. Results consistently showed that native sequences performed as well as or better than most optimization strategies in small-scale screens, while RNA stability-focused designs often reduced expression levels rather than improving them. (00:11:15–00:22:36)

Large-scale expression studies reinforced the conclusion that codon optimization is not universally advantageous in mammalian systems. While certain biased codon schemes occasionally improved yields for individual proteins, no single optimization strategy consistently outperformed native sequences. The findings suggest that codon optimization should be treated as an empirical design variable rather than an automatic requirement, particularly when expressing mammalian glycoproteins in mammalian hosts. (00:22:36–00:23:58)

Key Takeaways

- Native coding sequences often perform as well as codon-optimized designs in mammalian expression systems.
- RNA stability-focused optimization strategies can reduce protein yields despite increasing transcript stability.
- Codon optimization outcomes are highly protein-dependent and not universally predictable.
- Testing multiple sequence designs may improve consistency, but codon optimization is not inherently necessary for successful glycoprotein production.

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Advanced vector platforms for enhanced biotherapeutic protein expression

Full Video Here

Peter O'Callaghan, Senior Director, Head Expression Systems Sciences, Licensing, Lonza

Recombinant protein manufacturing continues to evolve as therapeutic pipelines expand beyond conventional monoclonal antibodies into increasingly complex molecular formats. This presentation reviewed how expression platform design influences productivity, quality, and manufacturability across a broad range of biologics. Drawing on decades of experience with the GS gene expression system, the discussion emphasized the importance of continually refining host cells, vectors, and selection technologies to support the next generation of therapeutic proteins. (00:00:00–00:05:52)

A major focus was the role of expression vector engineering in maximizing the biosynthetic capacity of CHO cells. Lonza's integrated platform combines optimized host cell lines with piggyBac transposase technology, enabling more efficient integration of expression constructs into transcriptionally active regions of the genome. Compared with conventional random integration approaches, this strategy improved average clone productivity and increased the likelihood of identifying high-performing cell lines during development. (00:07:22–00:11:26)

The presentation introduced the GSquad® Pro vector system and its engineered LHP-1 promoter, which was designed to improve transcriptional performance while maintaining product quality and stability. Across multiple molecule classes, including antibodies, bispecific antibodies, and fusion proteins, the platform consistently generated higher titers than legacy promoter systems. Benefits extended beyond productivity, with improved balance of multigene expression, strong long-term clone stability, and reduced sensitivity to gene-order effects that can complicate expression of complex therapeutics. (00:11:26–00:21:44)

Looking ahead, the work highlighted how large-scale genomic, epigenetic, and regulatory mapping of CHO cells is informing future platform development. Emerging vector designs incorporate more stringent selection systems, improved pool stability, and enhanced productivity without relying on traditional selection agents. These efforts reflect a broader shift toward precision bioprocessing, where data-driven engineering of both cells and vectors is used to create more predictable, efficient, and scalable manufacturing platforms. (00:21:44–00:25:46)

Key Takeaways

- Expression vector design remains a critical driver of productivity, quality, and manufacturing success.
- PiggyBac-mediated integration improves clone performance and increases the likelihood of identifying high producers.
- Engineered promoters can enhance titers while maintaining product quality and long-term stability.
- Data-driven optimization of CHO cell biology is enabling the next generation of recombinant protein expression platforms.

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Boosting Recombinant Protein Titers with Metabolic Modelling, and Harmonizing Metabolomics Datasets for Cross-Study Integration

Full Video Here

Hardik Dodia, PhD, Postdoctoral Scholar, Shu Chien-Gene Lay Department of Bioengineering, University of California San Diego

Improving recombinant protein production often depends on understanding how cells utilize nutrients within complex media formulations. This presentation examined how metabolomics and metabolic modeling can be combined to identify process bottlenecks and accelerate bioprocess optimization. While complex media support strong growth and productivity, their poorly defined composition makes it difficult to determine which nutrients drive protein expression and which are consumed inefficiently. (00:00:00–00:05:44)

The work used a recombinant E. coli process producing enhanced yellow fluorescent protein as a model system. Through spent-media analysis using mass spectrometry, hundreds of metabolites were identified and tracked throughout cultivation. These measurements were integrated with genome-scale metabolic models and dynamic flux balance analysis to estimate intracellular reaction rates, providing a detailed view of how nutrients were consumed, transformed, and redirected during protein production. (00:05:44–00:11:06)

Model-derived insights were then used to identify metabolic bottlenecks and prioritize supplementation strategies. Analysis revealed specific amino acids that contributed positively to recombinant protein synthesis, while others appeared to divert metabolic resources without improving productivity. Iterative rounds of supplementation and model refinement led to substantial increases in protein titers. Additional simulations identified oxygen transfer as a limiting factor, and addressing that constraint further enhanced productivity. (00:11:06–00:19:33)

The presentation also explored how public metabolomics repositories can support future process development. Because datasets are often generated on different instruments and under different conditions, harmonization remains challenging. Methods for integrating and correcting metabolomics datasets were discussed as a way to improve cross-study comparisons and enable broader reuse of existing metabolic data. Together, these approaches illustrate how combining experimental metabolomics with computational modeling can create a more systematic framework for process optimization. (00:19:33–00:22:08)

Key Takeaways

- Metabolomics can reveal nutrient utilization patterns that are difficult to detect in complex media.
- Dynamic flux balance analysis helps identify metabolic bottlenecks affecting protein production.
- Iterative model-guided supplementation strategies can significantly improve recombinant protein titers.
- Harmonized metabolomics datasets may expand opportunities for data-driven bioprocess development.

Smart Production: Leveraging AI for Efficient Recombinant GPCR Expression

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Alex Blanco, PhD, Scientist, Nabla Bio

Many biologically important targets remain difficult to address because traditional antibody discovery methods struggle to control epitope specificity, receptor conformation, and functional outcomes. This presentation introduced a generative modeling platform called JAMM that designs antibody-antigen complexes directly from target sequence information. The approach aims to bring atomic-level precision to antibody discovery, enabling researchers to tackle challenging targets such as membrane proteins, GPCRs, ion channels, and other proteins that have historically been difficult to access through conventional screening approaches. (00:00:00–00:03:14)

The platform was first evaluated against a diverse panel of sixteen disease-relevant soluble protein targets. Using only a small number of computationally generated designs per target, the team rapidly produced and tested antibodies across multiple formats. High hit rates were observed across nearly all targets, with many binders exhibiting nanomolar or sub-nanomolar affinity after a single design cycle. The results demonstrated that computationally generated antibodies can move directly into experimental validation without requiring extensive library screening or iterative optimization. (00:03:14–00:06:14)

The work then shifted to GPCRs, one of the most challenging target classes in drug discovery. JAMM successfully generated selective binders against multiple GPCRs and produced antibodies with affinities comparable to benchmark molecules derived through traditional immunization campaigns. The platform also demonstrated the ability to generate highly selective binders within closely related receptor families, reducing undesired cross-reactivity while maintaining strong target engagement. (00:06:14–00:12:56)

A particularly notable result was the generation of functional GPCR antibodies. By directing designs toward specific receptor regions, the team identified both antagonists and agonists capable of modulating signaling pathways. Supporting this effort was the creation of soluble receptor proxies that preserved key extracellular epitopes while simplifying expression and screening workflows. Together, these capabilities suggest a path toward faster and more precise development of functional biologics against previously intractable targets. (00:12:56–00:23:11)

Key Takeaways

- Generative AI can design antibody binders directly from target sequence information.
- High-affinity binders can be identified rapidly without large experimental screening libraries.
- Computational design enables selective targeting of challenging GPCRs and related receptor families.
- Functional agonist and antagonist antibodies can be generated through epitope-directed design strategies.

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Membrane Protein Targets Reengineered for Soluble Expression

Full Video Here

Alexander Taguchi, PhD, Director of Machine Learning, iBio Inc.

Membrane proteins remain among the most valuable yet difficult targets in drug discovery because their structural complexity and dependence on lipid environments complicate both characterization and antibody generation. This presentation explored how generative AI can be used to redesign membrane proteins into soluble, experimentally tractable formats while preserving the structural features required for biologically relevant antibody discovery. The work was motivated by a broader effort to create more reliable ways to discover highly specific therapeutics against challenging targets. (00:00:01–00:05:07)

At the core of the platform is a generative design engine that creates engineered epitopes and soluble protein surrogates suitable for antibody selection. Rather than relying exclusively on native membrane proteins, the system redesigns transmembrane regions into soluble scaffolds that maintain native-like extracellular structures. Experimental validation showed that these engineered proteins retained expected binding properties, including recognition by native ligands and therapeutic molecules, supporting their use as functional surrogates for discovery campaigns. (00:05:07–00:10:46)

The approach was further challenged with GPCRs that lacked extensive structural information. Using computationally generated soluble receptor designs, the team demonstrated ligand binding, structural consistency, and compatibility with antibody screening workflows. These engineered proteins enabled both in vitro and in vivo discovery strategies, providing a practical route to targets that are often inaccessible through conventional recombinant expression methods. (00:10:46–00:17:27)

A key application involved the discovery of highly selective antibodies against amylin receptor complexes relevant to obesity therapeutics. By combining engineered receptor mimics, epitope-focused design, and advanced screening approaches, the team identified antibodies that distinguished closely related receptor assemblies and enabled selective receptor activation profiles. The results suggest that AI-designed soluble membrane protein surrogates can expand access to difficult targets while improving precision in therapeutic antibody discovery. (00:17:27–00:24:13)

Key Takeaways

- Generative AI can redesign membrane proteins into soluble, experimentally tractable formats.
- Engineered receptor surrogates can preserve native-like ligand and antibody binding properties.
- Soluble GPCR mimics enable antibody discovery against targets that are traditionally difficult to access.
- Combining computational design with experimental validation can improve selectivity and precision in biologics discovery.

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AI-Accelerated Advancements to Cell Line Development: Integrating Sequence Mining, ASR, and ML-Stability Models

Full Video Here

Cintia Gomez Limia, PhD, Scientist, Cell Line Development, Demeetra

Cell line development continues to be a critical driver of productivity and efficiency in biotherapeutic manufacturing. This presentation highlighted how gene editing, transposon technologies, and AI-guided protein engineering can be combined to accelerate development of high-performing CHO cell platforms. The work focused on improving both genome engineering tools and stable integration systems to support more productive and reliable manufacturing cell lines. (00:00:00–00:03:45)

A major area of effort involved optimization of the Cas-CLOVER gene editing system. Cas-CLOVER combines a catalytically inactive Cas9 with a dimerizing endonuclease, enabling highly precise DNA cleavage. To further enhance performance, the team applied sequence mining, ancestral sequence reconstruction, and machine learning-based protein design strategies to generate and evaluate new Cas-CLOVER variants. Screening in bacterial and CHO cell systems identified engineered variants that delivered improved editing efficiency compared with the original enzyme. (00:03:45–00:07:34)

The presentation also described development of a proprietary transposase platform based on piggyBac technology. Delivered in mRNA format, the system enabled efficient and stable integration of transgenes into CHO cells while supporting large cargo capacity and multi-gene insertion strategies. Comparative studies demonstrated higher integration numbers than alternative transposase systems, and antibody expression experiments produced strong productivity metrics, including multi-gram-per-liter titers and high cell-specific productivity. (00:07:34–00:08:55)

Long-term evaluation showed that both pooled populations and isolated clones maintained stable productivity across extended cell generations. Integration copy number analysis further supported the durability of the system, demonstrating consistent transgene retention over time. Together, these advances illustrate how combining AI-assisted protein engineering with optimized gene editing and integration technologies can improve the speed, efficiency, and performance of modern cell line development workflows. (00:08:55–00:10:24)

Key Takeaways

- AI-guided protein engineering can improve the performance of genome editing tools.
- Engineered Cas-CLOVER variants demonstrated enhanced editing efficiency in CHO cells.
- Proprietary transposase systems enabled efficient stable integration and high recombinant protein productivity.
- Stable expression and transgene retention support long-term manufacturing applications.

PepTalk

inGenius® Bioprocessing: Reducing Risk, Boosting Titre with ML-Driven Protein Production

Full Video Here

Ian Fotheringham, President & Founder, Ingenza Ltd

Gene sequence design remains one of the most unpredictable aspects of recombinant protein production. Even when the encoded protein is identical, different synonymous DNA sequences can produce dramatically different expression outcomes in the same host. This presentation described how Ingenza is applying machine learning to make gene design more predictable, with the goal of reducing costly trial-and-error optimization and improving recombinant protein yields across multiple production hosts. (00:00:00–00:03:03)

The approach relies on a massively parallel reporter assay that systematically evaluates thousands of synonymous coding sequences. Using a fluorescent reporter, expression levels are measured across large libraries of sequence variants, and machine learning models are trained to identify relationships between DNA sequence features and protein productivity. The resulting platform, called codABLE, ranks potential coding sequences according to their predicted expression performance within a specific host organism. (00:03:03–00:05:50)

Case studies in Bacillus subtilis and E. coli demonstrated the practical value of the platform. For multiple customer proteins, machine learning-designed sequences substantially outperformed conventional codon optimization approaches, delivering higher titers, improved solubility, and more suitable manufacturing characteristics. The technology was also successfully combined with other strain engineering strategies, including antibiotic-free plasmid maintenance systems and orthogonal expression platforms, to further improve overall production performance. (00:05:50–00:11:22)

The work is now being extended to more complex hosts, including yeast and CHO cells. Early CHO studies suggest that synonymous coding sequences strongly influence expression levels, creating opportunities to develop predictive models similar to those already demonstrated in microbial systems. The broader vision is to integrate machine learning-guided gene design with advanced host engineering technologies to create more reliable, efficient, and scalable recombinant protein production workflows. (00:08:25–00:12:48)

Key Takeaways

- Synonymous DNA sequence selection can dramatically affect recombinant protein expression.
- Machine learning models can predict high-performing coding sequences for specific host organisms.
- codABLE improved productivity across multiple microbial protein production case studies.
- Host-specific sequence optimization may reduce development timelines and minimize empirical screening

Recombinant Expression and Characterization of Histatin-Derived Peptides

Full Video Here

Robert M. Hughes, PhD, Associate Professor, Chemistry, East Carolina University

Histatins are naturally occurring antimicrobial peptides found in human saliva that have attracted interest as potential therapeutics for oral infections and fungal diseases. This presentation focused on recombinant production of histatin-derived peptides, particularly the antimicrobial fragment P113, which retains the biological activity of the larger parent molecule. The work addressed a longstanding challenge in peptide manufacturing: how to produce these molecules efficiently while avoiding the use of hazardous chemical cleavage methods. (00:00:00–00:02:58)

Traditional recombinant production of histatin peptides relies on cyanogen bromide cleavage, a process that is effective but involves toxic reagents and environmental concerns. To create a more sustainable alternative, the team developed an immobilized SUMO protease strategy. By expressing the peptide as a SUMO fusion protein, purifying it, and then using an immobilized protease to release the native peptide sequence, the workflow eliminated the need for cyanogen bromide while maintaining sequence fidelity. (00:02:58–00:08:44)

Extensive characterization demonstrated that the immobilized protease remained active over multiple reuse cycles and efficiently generated the desired peptide product. The recombinant peptides were confirmed by mass spectrometry and further purified using reverse-phase HPLC. Process development also revealed that recovering fusion proteins from insoluble fractions improved yield and reproducibility compared with relying solely on soluble expression, highlighting an important optimization opportunity for small peptide production. (00:08:44–00:16:15)

Biological testing showed that the recombinant P113 peptide retained antimicrobial activity against Candida strains, producing results comparable to synthetic peptide standards. Although current yields and production costs remain less favorable than established synthetic routes, the recombinant process offers advantages in sustainability and reduced reliance on hazardous reagents. The work demonstrates a viable alternative platform for producing histatin-derived peptides and potentially other therapeutic peptides in the future. (00:16:15–00:20:40)

Key Takeaways

- Immobilized SUMO protease can replace cyanogen bromide in recombinant peptide production workflows.
- The immobilized enzyme maintained robust activity across repeated cleavage cycles.
- Recombinant histatin-derived peptides retained antimicrobial activity comparable to synthetic standards.
- The approach offers environmental and safety advantages despite current yield and cost challenges.

PepTalk

Engineering Cell-Free Glycosylation Systems for Immune-Optimized Vaccines

Full Video Here

Zachary Shaver, Research Scientist, Michael Jewett Laboratory, Northwestern University

Conjugate vaccines have dramatically reduced the burden of bacterial diseases, but conventional manufacturing methods still rely on semi-random chemical conjugation processes that can compromise vaccine quality. This presentation explored a cell-free glycosylation platform designed to produce more precisely engineered conjugate vaccines. The work focused on understanding how glycan placement on carrier proteins influences immune responses and how vaccine design can be optimized through site-specific enzymatic glycosylation. (00:00:00–00:04:10)

The platform combines cell-free protein synthesis with enzymatic glycosylation using an oligosaccharyltransferase that transfers pathogen-derived glycans onto specific amino acid sequences engineered into carrier proteins. To improve efficiency, the team generated a large library of enzyme variants and screened them using a high-throughput AlphaLISA assay. Several engineered enzymes significantly increased glycosylation efficiency, with some variants raising efficiency from roughly 50% to over 90%, enabling production of higher-quality vaccine products. (00:04:10–00:08:52)

The researchers then addressed a second challenge: determining where glycans can be successfully attached throughout a carrier protein. By systematically introducing glycosylation sites across an FDA-approved carrier protein and screening hundreds of variants, they identified specific regions that were highly amenable to glycosylation. Mapping these results onto the protein structure revealed that glycosylation competency was not uniformly distributed, providing a framework for more deliberate vaccine design. (00:08:52–00:15:06)

Using these insights, the team scaled production of multiple vaccine variants with glycans attached at different positions and generated sufficient material for immunogenicity studies in mice. Early results suggest that glycan location may influence the resulting immune response, supporting the idea that vaccine efficacy can be improved through rational control of glycosylation patterns. The broader goal is to establish design principles that can guide development of future conjugate vaccines with enhanced immunogenicity and consistency. (00:15:06–00:16:20)

Key Takeaways

- Cell-free glycosylation systems enable site-specific conjugate vaccine production.
- Engineered oligosaccharyltransferases can substantially improve glycosylation efficiency.
- Glycosylation competency varies across carrier protein structures and can be systematically mapped.
- Glycan attachment location may influence vaccine immunogenicity and represents a potential optimization parameter.

Yeast-Based Expression and Enzymatic Cyclization of Disulfide-Rich Cyclic Peptide Scaffolds for Drug Development

Full Video Here

David J. Craik, PhD, Professor & UQ Laureate Fellow, The University of Queensland

Cyclic peptides from plants and animals offer attractive properties for drug discovery because of their exceptional stability and resistance to degradation. This presentation focused on naturally occurring cyclic peptides, including cyclotides, conotoxins, and sunflower trypsin inhibitors, which have evolved highly constrained structures stabilized by disulfide bonds and head-to-tail cyclization. These molecules provide promising scaffolds for therapeutic design because they can retain biological activity while tolerating substantial sequence modification. (00:00:00–00:09:18)

A central theme was the use of cyclic peptide scaffolds as frameworks for grafting new bioactive sequences. By inserting therapeutic epitopes into naturally stable peptide backbones, researchers can create molecules that retain the activity of the original peptide while gaining the stability of the cyclic scaffold. This strategy has already been applied to a broad range of biological targets, including extracellular receptors, intracellular protein interactions, and agricultural applications, demonstrating the versatility of cyclic peptide engineering. (00:09:18–00:11:51)

The presentation then addressed a major challenge in peptide manufacturing: sustainability. Traditional solid-phase peptide synthesis remains highly effective for research-scale production but generates significant chemical waste and can become costly at larger scales. To overcome these limitations, the team developed enzymatic cyclization workflows using specialized plant-derived ligases that efficiently convert linear peptide precursors into cyclic products. These enzymes enabled production of both naturally cyclic peptides and engineered analogs while maintaining structural integrity and biological function. (00:11:51–00:19:41)

Building on this enzymatic platform, the researchers established a yeast-based expression system capable of producing disulfide-rich peptide precursors that could then be cyclized enzymatically. The approach successfully generated peptides containing one, two, and three disulfide bonds, producing correctly folded molecules with biological activities comparable to synthetic standards. By combining recombinant expression with enzymatic cyclization, the workflow offers a more sustainable and potentially more economical route for manufacturing complex cyclic peptide therapeutics at scale. (00:19:41–00:27:16)

Key Takeaways

- Naturally occurring cyclic peptides provide highly stable scaffolds for therapeutic design.
- Grafting bioactive sequences onto cyclic frameworks can preserve activity while improving stability.
- Plant-derived ligase enzymes enable efficient enzymatic cyclization of peptide precursors.
- Yeast expression combined with enzymatic cyclization offers a sustainable alternative to traditional peptide synthesis.

PepTalk

Accelerating Membrane Protein Purification: Innovations with Nuclera

Full Video Here

Wenguang Liang, Sr Scientist, Molecular & Cell Sciences, Bayer CropScience

Membrane proteins play critical roles across agriculture, biotechnology, and drug discovery, yet they remain among the most difficult proteins to express and purify. This presentation explored how a cell-free protein expression platform from Nuclera was applied to membrane protein production challenges encountered at Bayer Crop Science. The work focused on accelerating expression screening and purification workflows for proteins that had previously required months of effort using conventional systems such as insect cells. (00:00:00–00:05:35)

The platform combines cell-free expression with automated purification and extensive condition screening. Researchers can simultaneously evaluate multiple protein constructs, membrane-mimetic environments, lipids, and nanodisc formulations while monitoring expression and purification outcomes in real time. A key feature is the use of preassembled nanodiscs and optimized lipid environments, which provide membrane proteins with a more native-like setting during synthesis and purification. (00:05:35–00:11:19)

Results from several challenging Bayer targets demonstrated the value of this approach. Proteins that had shown little or no expression in E. coli, yeast, or insect-cell systems were successfully expressed and purified using the cell-free workflow. In multiple cases, the resulting proteins displayed substantially improved purity and particle quality compared with material obtained from conventional expression platforms. The findings suggested that avoiding extraction from native membranes and maintaining proteins in lipid-supported environments throughout the process can significantly improve outcomes for sensitive membrane targets. (00:11:19–00:20:36)

The presentation emphasized speed as a major advantage of the platform. Initial screening of dozens of expression and purification conditions could be completed within approximately 24 hours, followed by rapid scale-up of the most promising candidates. While current production costs limit large-scale manufacturing applications, the system provides an efficient route for generating membrane proteins suitable for structural biology, functional assays, and early-stage characterization, reducing months of optimization work to only a few days. (00:20:36–00:22:32)

Key Takeaways

- Automated cell-free workflows can dramatically accelerate membrane protein expression screening.
- Nanodisc-supported expression environments improve purification outcomes for challenging targets.
- Cell-free production can succeed where traditional E. coli, yeast, or insect-cell systems fail.
- Rapid screening enables structural and functional studies while reducing lengthy optimization cycles.