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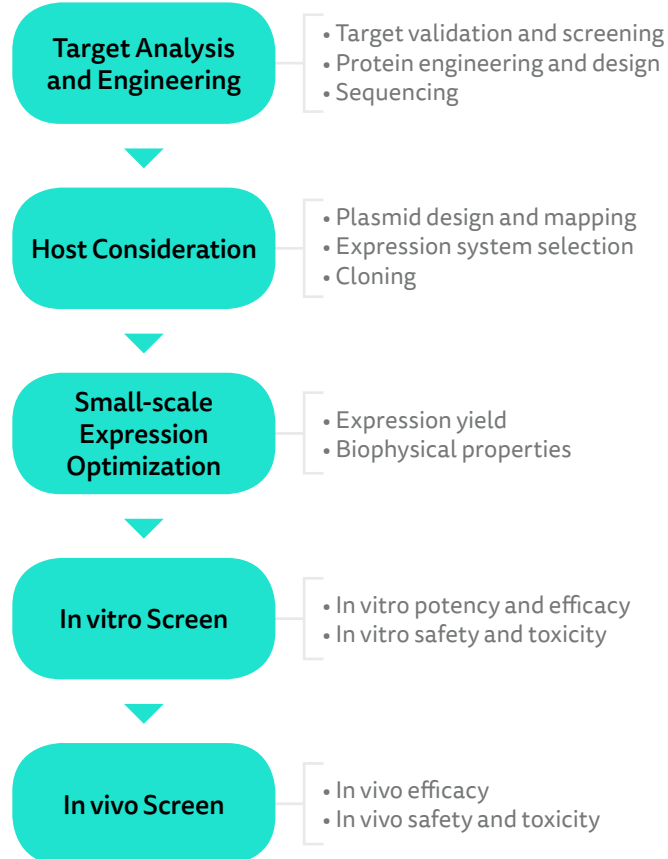## for Protein and Peptide Therapeutics R&D
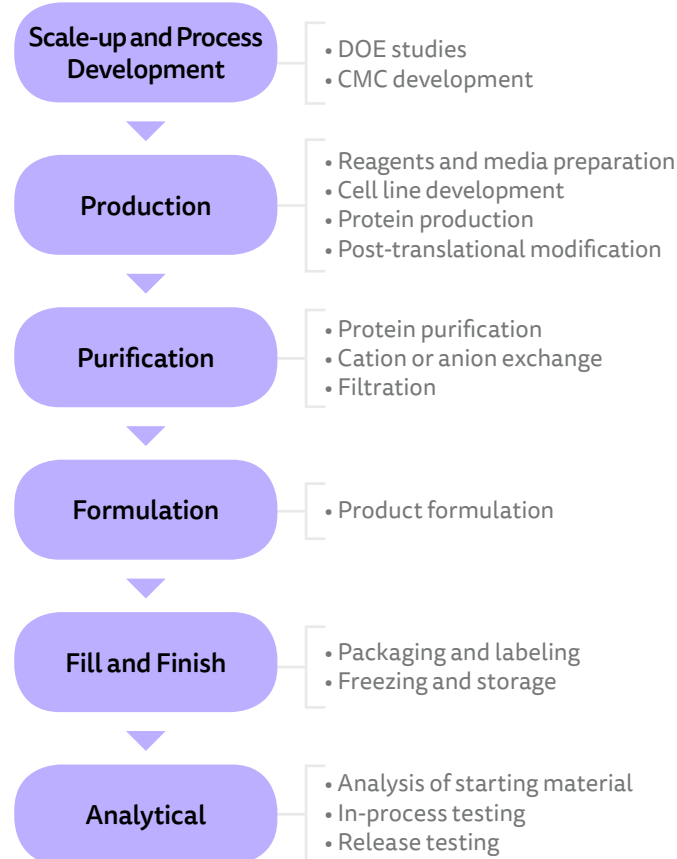
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Proteins and peptides are a wide variety of therapeutic molecules ranging from enzymes to cytokines. These therapeutics have several biological functions and interact with various biological pathways. For example, hormones, neurotransmitters, and growth factors all represent distinct functions of proteins and peptides. New classes of proteins and peptides continue to be of significant research interest. However, there are several hurdles to cross for companies seeking to develop new protein and peptide therapies.

Protein Therapeutics Research

Target Analysis and Engineering
- Target validation and screening
- Protein engineering and design
- Sequencing

Host Consideration
- Plasmid design and mapping
- Expression system selection
- Cloning

Small-scale Expression Optimization
- Expression yield
- Biophysical properties

In vitro Screen
- In vitro potency and efficacy
- In vitro safety and toxicity

In vivo Screen
- In vivo efficacy
- In vivo safety and toxicity

Protein Therapeutics Development

Scale-up and Process Development
- DOE studies
- CMC development

Production
- Reagents and media preparation
- Cell line development
- Protein production
- Post-translational modification

Purification
- Protein purification
- Cation or anion exchange
- Filtration

Formulation
- Product formulation

Fill and Finish
- Packaging and labeling
- Freezing and storage

Analytical
- Analysis of starting material
- In-process testing
- Release testing

Complexities in Protein and Peptide Therapeutics Research

- Continuing concerns around the safety and immunogenicity of any new protein or peptide therapeutic requiring comprehensive testing and tracking

- Managing the increasing diversity of protein engineering techniques and post-translational modification methods alongside rapidly evolving organizational and program needs

- Handling a variety of protein, peptide, and epitope libraries as the therapeutic applications of proteins and peptides expand

Complexities in Protein and Peptide Therapeutics Development

- Seamlessly incorporating DOE throughout process development as process knowledge matures

- Identifying and mitigating developability risks early, ensuring timely execution of development campaigns

- Improving process efficiencies and integrating with automation as costs spiral

This paper outlines the critical needs and complexities of protein and peptide R&D, and how Benchling has helped address these challenges for leading protein and peptide therapeutic companies.

1

Protein and peptide research involves innovative science and cutting-edge techniques, as research teams work to identify lead candidates that have promising efficacy and safety signals. Here are some of the key research complexities and needs that define therapeutic protein and peptide research.

Tracking safety and immunogenicity throughout research

Why is it a critical need?

Immunogenicity against therapeutic proteins or peptides, mediated by antibodies, can alter the pharmacokinetics, pharmacodynamics, bioavailability, safety, and efficacy of drugs. Factors that contribute to immunogenicity and adverse reactions can be extrinsic or intrinsic. Extrinsic factors include aggregate formation, degradation products, contaminants, formulation, and dosing. Intrinsic factors are related to the protein structure, such as T- and B-cell epitopes and post-translational modifications. A comprehensive assessment of the intrinsic drivers of immunogenicity is especially critical in research. When the lead candidates are selected during the research stage, it is still possible to redesign the proteins or peptides to produce a more favorable immunogenicity profile. In silico studies such as T-cell epitope mapping, in vitro studies such as binding and neutralizing assays, and in vivo studies using animal models are all essential to building a comprehensive understanding of immunogenicity. Research organizations need to centralize and link these disparate studies in order to de-immunize therapeutic proteins and peptides.

Why do current solutions fail?

In most research organizations, the in silico, in vitro, and in vivo studies are performed by separate groups. As a result, the results generated by these groups end up in functional silos. The lack of communication across software and data systems leads to poor access to information and less robust sequence-level understanding of immunogenicity.

Protein and Peptide Therapeutics Research

Benchling helps centralize and unify immunogenicity and safety results generated across an organization, provides rich data models to map results back to sequences, and enables research teams to rapidly iterate and improve their screening methods based on historical safety results.

Create a centralized safety profile of lead protein and peptide candidates

- Store all safety-related in silico, in vitro, and in vivo results in Benchling's central SQL Data Warehouse

- Easily link all available results to specific protein and peptide candidates

Trace safety signals back to the primary, secondary, tertiary, and quaternary structures

- Create and register sub-structures or parts as unique entities and map inter-relationships between entities

- Link safety signals to specific sub-sequences, sub-entities or sub-structure domains

Inform candidate optimization with instant access to the most up-to-date results

- Use the same AA chains across programs by preserving the full context of historical safety data

- Run advanced queries to compare, refine, and optimize candidate selection methods based on all available safety data

2

Creating protein post-translational modification and peptide modification workflows

Why is it a critical need?

Post-translational modifications (PTMs) are commonly performed on proteins—especially those obtained from bacterial expression systems—and peptides. PTMs may be used to increase stability, alter physicochemical properties, modulate biological function, enhance pharmacokinetic or pharmacodynamic properties, or improve therapeutic activity. Common protein and peptide PTMs include phosphorylation, glycosylation, sulfation, hydroxylation, deamidation, oxidation, pegylation, and isomerization. To create these PTMs, a variety of protein engineering techniques are used, including recombinant DNA techniques, site-specific mutations, and site-selective chemical modifications. Organizations need a flexible process management system that can handle a diverse range of workflows for post-translational modifications.

Why do current solutions fail?

There is a general lack of cohesive tools to manage PTM workflows. Discrete and often unstructured software tools are used to design processes, perform experiments, manage samples, and record results. This leads to disparate data sources, inefficient communication between systems, and messy process hand-offs between workflow steps.

Protein and Peptide Therapeutics Research

Benchling provides a Workflows application to flexibly handle PTM engineering process steps, Registry and Inventory applications to create and manage the storage of these entities, and a Notebook application to structure results and properties associated with the custom entities.

Design PTM workflows to suit your expression systems and protein engineering strategies

- Map the steps of a PTM process from start to finish and streamline sample generation, registration, and creation of inventory locations

- Manage multiple processes in parallel and flexibly reconfigure processes as protein engineering technologies evolve

Organize the inventory of post-translationally modified proteins and peptides

- Chart relationships between native protein and peptide samples with intermediates and modified counterparts

- Create and manage inventory of modified proteins from within the context of the Notebook or Registry

Track structural and functional characteristics of precursors and mutants

- Capture relevant physicochemical properties of precursors and mutants with custom schema fields

- Link characterization results directly to batches of post-translationally modified proteins and peptides

3

Handling libraries of protein, peptides, epitopes and other related entities

Why is it a critical need?

Any lab doing research on therapeutic proteins and peptides deals with hundreds or thousands of distinct samples. These samples include DNA sequences, plasmids, cell lines, protein fragments, peptide chains, epitopes, fusion proteins, protein scaffolds, mutant proteins, ligands, enzymes, oligonucleotides, bacterial expression systems, and mammalian expression systems. Research groups need to create an organized digital library of all these entities, record the relevant physicochemical properties, compute properties from sequences, and track the interrelationships between the entities.

Why do current solutions fail?

Current registration systems tend to be more customized for small molecules or antibodies. They are woefully inadequate when it comes to managing the variety of protein- and peptide-related entities generated in research labs. Moreover, they are often not sequence-aware. Research organizations use distinct systems to manage the physical inventory of samples and the results for experiments involving those samples. These inventory and results systems do not talk with the registration systems. This fragmentation of the software ecosystem leads to poor data management and needless duplication—or complete loss—of data.

Protein and Peptide Therapeutics Research

Benchling's Molecular Biology, Notebook, Registry, and Inventory applications work together seamlessly to design DNA and AA sequences, compute properties from sequences, create a digital library of these entities, manage physical inventory, record relevant results, and track relationships between entities.

Design and analyze protein and peptide sequences

- Visualize and design sequences for a variety of proteins and peptides using Benchling Molecular Biology and its sequence-level intelligence

- Manage annotations, perform alignments, create translations, and compute biochemical properties of DNA and AA sequences

Create a single, comprehensive library of all of your protein- and peptide-related entities

- Build a Registry with customizable schema fields and auto-computed metadata to track every entity in one place

- Link physical samples and their locations— containers in specific freezers, fridges, and shelves—to entities within your registries

Track lot specific results and complete sample history from the same interface

- Connect all characterization results and the entire sample history to each entity within the Benchling Registry

- Retain rich contextual data as DNA and AA sequences are reused across programs and therapeutic indications

Protein and peptide development involves developing reproducible and well-controlled processes that ensure a quality, safe, and efficacious final product. Here are some of the key development complexities and needs that define therapeutic protein and peptide development.

Seamlessly supporting DOE applications in protein process development

Why is it a critical need?

The production of therapeutic proteins is complex, consisting of several key process steps. Process development teams need to understand how the various process steps might impact critical quality attributes (CQAs) of the protein product, such as glycosylation, charge variants (including variants created by oxidation, deamidation, and C- & N-terminal modifications), aggregates, and low-molecular-weight species. CQAs ultimately impact clinical performance of the product. To study the impact of process variables on CQAs, development teams use design of experiments (DOE), a framework for process development experimentation. Teams then use results from these studies to define the process design space, develop a control strategy, and generate a robust and consistent process. Development organizations need a systematic approach to integrate DOE into scale-up processes, to characterize and compare processes, and to increase process understanding.

Why do current solutions fail?

A variety of software tools are currently used to design and execute DOE studies. For example, a statistical software might be used to create a DOE, a physical or electronic notebook might be used to capture steps in the study, and spreadsheets might be used to collect and analyze results. This disparate use of software—and even paper—means that development organizations are not taking full advantage of QbD and DOE in process development.

Protein and Peptide Therapeutics Development

Benchling allows you to identify and track critical quality attributes through Notebook entries and registration tables linked directly to entities in the Benchling Registry. Benchling then connects these CQAs with process parameter data from DOE studies, which can be performed with preconfigured templates in Workflows.

Identify critical quality attributes for protein and peptide therapeutics

- Identify and track potential critical quality attributes of proteins and peptides such as charge variants and low-MW species

- Use cell-level validation in smart tables to flag critical quality attributes that are out of range

Model and execute DOE studies with Workflows to thoroughly evaluate your production process

- Design multivariate DOE experiments in the Workflows app to study key process parameters and identify potential interactions between these process parameters

- Study functional relationships between process parameters and critical quality attributes by integrating with advanced statistical tools

Define process design space

- Generate process design space with the most important process parameters and their ranges by using smart tables

- Set up automatic triggers when process parameters are out of range from defined design space

Mitigating developability risks such as aggregation and stability early

Why is it a critical need?

Proteins and peptides pose several developability risks with the potential to create significant downstream challenges during production. Typical developability risks include poor solubility, chemical stability liabilities (e.g., oxidation, deamidation, and hydrolysis), physical stability issues (e.g., aggregation and adsorption), protein-excipient interactions, and processing stability concerns. Identifying and addressing these risks early in the development process saves time and resources and ultimately increases the probability of candidate success. Development teams need to study risks — comprehensively and early in the development process — and develop risk mitigation plans to address them.

Why do current solutions fail?

There is dearth of good software available to design and manage developability risks. Scientists use classical, unstructured tools such as notebooks and spreadsheets to design and execute developability studies. Reporting results from these studies and developing risk mitigation plans involves even more unstructured tools: scientists use text documents, slide presentations. This leads to a lack of transparency and limited access to data, an incomplete understanding of the developability risks, and a delayed response to risk mitigation.

Protein and Peptide Therapeutics Development

How Benchling helps

Benchling enables development teams to identify and thoroughly study developability risks early on, and to track both promising and high-risk properties of proteins and peptides throughout development.

Identify molecule-specific stability risks

- Flag intrinsic developability risks for protein and peptide candidates in the Registry

- Use custom annotations to tag specific sequences with high-risk attributes

Design and execute experiments to study developability risks throughout R&D

- Track the history of protein and peptide candidates from discovery through development, preserving rich contextual characterization data for specific batches

- Assess developability properties of lead candidates alongside stage gate criteria with custom dashboards

Develop risk mitigation plans and optimized processes and connect them to all results

- Access plans, results, and insights in a central platform to better design and execute key development activities

- Close the feedback loop between assay requests and results to rapidly optimize scale-up processes and mitigate developability risks

Reducing COGS by improving process efficiencies

Why is it a critical need?

Protein production is a complex and resource-intensive process that requires close control of every unit step. Any efforts to streamline and improve processes can reduce the cost of goods sold (COGS) significantly. Strategies to improve manufacturing efficiency include tight control of process parameters, use of online monitoring tools, automating processes, and use of single-use bioreactors and cultivation systems. Manufacturing teams need to incorporate these state-of-the-art techniques into production to reduce the total number of steps, accelerate time-consuming steps, boost productivity, and reduce COGS.

Why do current solutions fail?

Most of the unit operations in protein and peptide production are managed by siloed software. The raw results are typically handled and stored by custom LIMS. Additionally, key data entries such as batch records are maintained on paper or in repurposed electronic document tools. This combination of disparate tools prevents holistic analysis of the entire production process. Without this end-to-end view, processes are inefficient and cumbersome.

Protein and Peptide Therapeutics Development

Benchling's Workflows and Developer Platform centralize upstream and downstream steps, integrate with process automation, and truly leverage data to track key process and output metrics.

Map entire production process on a single platform

- Visually outline all of the steps in the production process including stages, inputs, and outputs

- Collect results from both upstream and downstream production equipment to a single Data Warehouse

Automate time-consuming and laborious steps

- Integrate with production automation to reduce the number of manual steps and boost production efficiency

- Set up automatic data collection and data parsing to streamline production data management

Monitor historical and real-time process performance

- Capture results and process outputs from multiple runs in a central location

- Build custom dashboards to track key process outputs and trends over time

Conclusions

Proteins and peptides are expected to continue to have a major impact on several therapeutic areas. The technologies and processes supporting the discovery and development of novel proteins and peptides are relatively mature, but they continue to evolve towards more complexity in research and the increasing need for efficient processes in development.

Benchling provides a modern, fully configurable, and user-friendly platform that adapts to the rapidly evolving needs of protein and peptide therapeutics R&D. This enables organizations to accelerate protein and peptide R&D, bringing more innovative products to market faster.

Here are some companies that use Benchling for protein and peptide therapeutics R&D:

REGENERON

Cedilla
THERAPEUTICS

CUE
BIOPHARMA

INHIBRX

LUMEN
BIOSCIENCE

MAVERICK
THERAPEUTICS

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