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From observational to actionable: Rethinking omics in biologics production

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Abstract

As the era of omics continues to expand with increasing ubiquity and success in both academia and industry, omics-based experiments are becoming commonplace in industrial biotechnology, including efforts to develop novel solutions in bioprocess optimization and cell line development. Omic technologies provide particularly valuable “observational” insights for discovery science, especially in academic research and industrial R&D; however, biomanufacturing requires a different paradigm to unlock “actionable” insights from omics. Here we argue the value of omic experiments in biotechnology can be maximized with deliberate selection of omic approaches and forethought about analysis techniques. We describe important considerations when designing and implementing omic-based experiments, and discuss how systems biology analysis strategies can enhance efforts to obtain actionable insights in mammalian-based biologics production.

Keywords

biopharmaceutical protein production; cell line development; bioprocess optimization; omics; statistical inference; mechanistic models

Towards actionable omics

Biotechnology is an evolving research field that thrives off our ability to harness living organisms to develop invaluable products and technologies. This interdisciplinary manufacturing approach is employed across a range of industries including energy, material, food, agriculture, cosmetics, and pharmaceuticals [1]. The biopharmaceutical industry in

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particular has been successful in manufacturing life-saving recombinant biotherapeutics in mammalian cells for over 40 years. While considerable advances have been made–with product titers increasing from ~50 mg/L to >10 g/L for monoclonal antibodies, it is perhaps surprising to see that the fundamental steps underpinning the cell line generation and bioprocess development processes have remained nearly the same, perhaps differing only in scale (Box 1).

Chinese hamster ovary (CHO) cells–the primary host system used for the manufacturing of biologics–are faced with challenges concerning the production of complex large molecules that meet stringent **product quality** (**PQ**) (see Glossary) requirements. To meet industrial demands, various strategies have emerged to manipulate the physiological functions of cell factories at the gene level (genetic engineering) and modify the culture environment for optimal growth and production (bioprocess optimization). The rational design and optimization of such strategies require a functional understanding of the molecular components driving bioproduction. Omic technologies provide large-scale, systems-wide monitoring of a broad range of such molecular components.

The sequencing of CHO and Chinese hamster genomes, the dramatic decrease in **next-generation sequencing** (**NGS**) costs, the growing ease of generating diverse omic data, and the development of mammalian genome editing tools provide a valuable toolbox for a new era of rationally driven cell line generation and process development [2–5]. Indeed, decades of omics research have yielded examples demonstrating the value of these data; however, while some omics strategies are inherently actionable by providing targets directly linked to a phenotype of interest (e.g., CRISPR screens [6–10]), many omic technologies can prove observational (i.e., merely providing a momentary snapshot of the molecular composition of the cells) when analyzed using basic approaches. Thus, it can be challenging to deploy strategies to maximize actionable value from the experiments. By actionable we mean the ability (1) to identify a minimal set of targets driving the phenotype of interest, (2) to direct a clear strategy for engineering or optimization towards the phenotype of interest, and (3) to deploy the strategies in a project-compatible timeframe. We argue that the value of omic experiments can be maximized with deliberate selection of omic approaches and forethought about analysis techniques. To this end, we outline what we believe are important considerations when designing and implementing omic-based experiments, and discuss how systems biology analysis strategies can enhance efforts to obtain actionable insights in biomanufacturing.

Challenges to actionability in biopharmaceutical process development

The timeline and platform-driven nature of the biologics industry has shaped how omic tools have been applied, and it is important to assess how it can be adapted to accommodate actionable omics strategies. The long, resource-intensive nature of cell line generation nearly necessitates that genetic engineering efforts be applied to the host cell—rather than introduced into a producing clone—as genetic modifications require further verification of clonality, cell line stability, and process optimization. Consequently, process optimization often remains the intervention of choice in the biopharmaceutical industry.

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While direct interventional studies such as media optimization **design of experiments** (**DoE**s) are effective, they do not answer the 'why' or 'how' of success and thus provide limited understanding for future work. In contrast, the measurement of molecular profiles of a given clone in a given process with omics methods provides information about the cellular state tied to a given phenotype. These omic based approaches can thus inform future cell line engineering or process optimization efforts, but only when applied with careful forethought.

The difficulty, regardless of approach, lies in the complexity of the system being engineered (Figure 1). The CHO genome is considerably larger than genomes of other industrial lines (e.g., *Escherichia coli*, yeast, etc.) and most genes remain uncharacterized in the context of protein production. Furthermore, the genomic plasticity exploited to generate highly productive cell lines through high-throughput screening means that a new clone may not respond as expected to a given bioprocess.

Given these complexities, extracting actionable insights from classical observational omic experiments (e.g., high feature number, low sample number, high background variability–present in an experiment looking at transcriptomic data from a high producer vs low producer clone) is challenging. When comparisons yield 100s to 1000s of potential targets (e.g., differentially expressed genes), it becomes difficult to decipher which gene(s) are actually driving the variation in phenotype, since many transcriptional differences may be superfluous for the phenotype of interest. Fortunately, depending on the desired objective, capabilities, and timelines, a range of different approaches may be suitable to extract actionable insights from omic experiments. Here we highlight types of analysis tools and strategies that can extract actionable insights from observational omics. Specifically, the integration of multi-omic approaches with systems biology computational models can help drive actionability.

Extracting actionable value from omics

To maximize actionability from omics, we recommend designing and executing stages of omic studies under the framework and considerations as described in Figure 2, Key figure. Prior to any data generation and analysis, one should define a clear implementation strategy by connecting analysis approaches to intervention objectives under known technical and logistical constraints. We emphasize that the implementation strategy should influence not only the selection of omic tools, but also the analysis methods employed and the prioritization of enriched targets. Athieniti *et al.* recently provided a comprehensive review of multi-omic data types and integration strategies which can be leveraged in the experimental design process [11]. To guide the systematic analysis of otherwise observational omic data, here we highlight 4 approaches to extract actionable information from omic experiments, ranging from rational experimental design to computational models. Depending on timelines, desired objectives, budgets, and tool availability, some approaches may be more desirable or could be infeasible. We provide explanations and examples of successful applications for each approach below.

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Rational experimental design

When the research question of interest is clearly defined and the root cause can be narrowed down to a reasonable set of molecular entities (e.g., dozens or a few hundred genes), it can be possible to derive actionable conclusions from small, rationally designed studies. As an example, a targeted proteomic study on high and low aggregation cell lines identified molecular mechanisms underlying cell aggregation[12,13]. By looking solely at the 'surfaceome' (surface protein sub-proteome) as the molecular entity(s) underpinning aggregation, the authors found proteins with differential abundance between the two cell lines and were able to decrease aggregation by knockdown of a surface protein. Other studies quantified the CHO secretome using proteomics to identify host cell proteins for removal to improve product quality or cell performance[14,15]. However, it is critical to predefine and selectively study only the responsible entities (e.g., surface proteins or host cell proteins) when sample numbers are low. For example, in classic 'high producer vs low producer' studies, the lack of actionability stems from high numbers of differentially expressed targets without practical ways to reduce the target list to a manageable number of testable hypotheses; furthermore, it is often unclear which genes are directly connected to productivity, and which are irrelevant genes that happen to have altered expression for other reasons (e.g., sharing a transcription factor). Furthermore, when analyzing low numbers of clones, it is quite likely that differences between clones stem from clonal variability rather than being causally linked to the phenotype of interest.

Knowledge-based parametric models

Knowledge-based parametric models can link genotype to phenotype on a mechanistic level to elucidate biological causation from omic data [16–18]. These network models employ carefully curated biochemical, genetic, and genomic data into a knowledgebase of an organism's molecular components and their interactions [19]. With the integration of omic data, these models can guide the rational design of systems-level engineering targets for bioproduction. Here we describe two such classes of models with promising applications in the biopharmaceutical industry: (1) genome scale models and (2) kinetic models (Box 2).

Knowledgebases can help construct diverse biological networks, and success has been demonstrated with **genome scale metabolic models** (**GeM**s), thanks to decades of legacy biochemical research in metabolism [20]. The integration of omic data with GeMs to identify cell line engineering targets has been demonstrated. For example, multi-omics profiling in CHO has been integrated with GeM flux analysis to help identify metabolic bottlenecks and potential engineering targets in mAb-producing cells [21], and GeMs have been used to reveal cell-line specific variation to guide to the rational selection of hosts [22]. Other studies [23,24] successfully integrated omics with GeM simulations to design optimal bioprocessing conditions in mAb-producing CHO cells, resulting in an average titer increase of ~11.8% and approximate two-fold increase in total mAb expression respectively. Another study used omics data to obtain GeM simulations that guided media optimization to reduce growth inhibitory metabolite section [25]. These examples demonstrate how overlaying omics on a functional network contextualizes the data in terms of underlying biochemistry, and comparison of experimental data with model simulations can validate or refine hypotheses. Furthermore, omics data are often used to build and refine such models

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[26,27]. Importantly, the systems-level metabolic response and flux simulations permitted by these networks go beyond any type of analysis possible with generic metabolic pathway databases such as **KEGG** [28].

Over the years, GeMs have expanded to include additional cellular processes. A desire to model the biochemical reactions underlying gene expression (transcription and translation) resulted in genome-scale models of metabolism and macromolecular expression (ME-models) [29,30]. Much like GeMs, ME-models can be platforms for the mechanistic integration of transcriptomic and proteomic data. In addition to expanding GeMs to include macromolecular expression networks, models can include core components of conventional protein secretion [31,32]. Many therapeutic proteins are clients of the secretory pathway, therefore a mechanistic understanding of pathway usage can provide novel insight into targeted engineering strategies. Furthermore, different biotherapeutics may utilize unique sets of secretory machinery exerting non-negligible metabolic demands on the host cell [14,31]. The resulting protein-specific models are able to calculate energetic costs and machinery demands for each secreted protein. Additional omic data can be integrated with the models and it was found that highly secretory cells have adapted to downregulate the expression and secretion of expensive native proteins [31]. Identification of costly native proteins that compete for cell resources present targeted engineering strategies for cell line engineering. Furthermore, the models were used to successfully optimize the production of monoclonal antibodies [31] and feeding strategies [33].

Complementary to the abovementioned constraint-based genome-scale stoichiometric models, kinetic models can effectively describe the dynamic character of mammalian cell culture and protein production [34,35]. These systems are non-stationary in nature, and depend on time and system history. Kinetic models can mechanistically model this dynamic behavior using mathematical expressions for the biochemical reaction rates of the system. While computationally intensive, kinetic models can be used to understand, predict, and evaluate the effects of targeted bioprocess manipulations and support the design of enhanced bioprocessing systems. Recent kinetic models of eukaryotes have been developed and can aid in cell line optimization [34,36]. Mammalian N-linked glycosylation was integrated with a metabolic kinetic model [37] to rationally manipulate the glycoprofile of a secreted IgG in CHO [38]. Kinetic modeling was employed to predict cell culture performance and screen optimal temperature shift strategies [39] and predict the impact of select amino acids on cell growth, metabolism, and mAb production and optimization of fed-batch culture feeding in monoclonal antibody-producing CHO cells [39,40].

Data-driven inference models—Knowledge-based parametric models offer insightful contextualization to omic data, but they can be computationally intensive and require technical expertise. If these models are not accessible or not compatible with the omic data type(s), we suggest the use of data-driven inference models. Data-driven models for omic analyses are built upon statistical methods to interpret and extract useful information from high-dimensional data [41–43]. To increase specificity, some models have evolved to integrate biological assumptions and context. These methods are valuable when the studied problem contains many unknowns and/or the number of samples capturing the expected biological variability is present. We provide an overview of the following classes of data-

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driven models, ranging from biology-independent to biology-dependent methodologies, and how they are applied for omic data analysis: (1) unsupervised and supervised, (2) correlation networks, and (3) empirical-based, interactome inference models.

Unsupervised and supervised techniques are built upon statistical algorithms lacking mechanistic or biological considerations, but can enrich meaningful patterns in omic data. Unsupervised techniques are useful in the validation of sample profiles, identification of subpopulations, detection of biological patterns, and integration of multi-omic data (Box 3). Principal component analysis (PCA) is commonly applied as quality control to validate sample similarities and to detect obvious technical factors such as batch effects [44–50], while k-means or hierarchical clustering can facilitate the identification of subject subpopulations or feature groups that exhibit similar behaviors [47,50,51]. Independent component analysis (ICA) and Markov clustering can identify biologically meaningful interactions between molecular species as demonstrated in *E.coli* [52] and human [53] omic data. Meanwhile, supervised techniques leverage high-dimensional data to predict continuous phenotypes like endpoint titer or cell density (regression; Box 3) or to predict whether a cell line will be high- or low-producing (classification; Box 3). End-point titer was predicted by **partial least squares (PLS)** regression models trained with time-series metabolomic data [45] and process data [46]. Other studies [44,54,55] obtained strong correlations between key metabolite concentrations and performance attributes to identify targets for potential cell line screening and media optimization. Similar to clinical case studies predicting patient phenotypes [56,57], supervised learning across multiple omic levels—such as copy number, mutation data, transcriptomics, and metabolomics–can predict cell line phenotypes. A combined approach using both unsupervised and supervised methods on longitudinal multi-omics data helped identify the impact of culture pH on culture performance, product titer, and product quality [58]. While these statistical methods can deliver accurate predictions or global insight on groups of features, low interpretability of latent variables and the lack of biological context can hinder actionability when the studies aim to identify engineering targets or elucidate mechanisms causing the phenotype of interest, and thus such analyses will often require further mechanistic study.

Correlation networks combine unsupervised and supervised techniques with mechanistic considerations to infer association of network-based target groups with phenotype (Box 3). Weighted gene co-expression network analysis (WGCNA) can validate or discover co-expressed biological networks. For example, WGCNA has been used on scRNA-Seq data to resolve gene modules associated with an ER-stressed condition and to confirm biological pathways enriched in respective subpopulations [59]. Meanwhile, **gene regulatory network** (**GRN**) inference algorithms, such as SCENIC [60] and scGRNom [61] for single-cell RNA-seq and ChEA3 [62] for bulk RNA-seq, assume association, or co-expression, of gene targets with transcription factors to predict gene network regulons and assess regulon enrichment. While correlation network techniques can reduce thousands of targets to dozens or hundreds of grouped targets, they also inevitably output false positive and indirect targets. These limitations, however, can be addressed with the integration of additional omic layers to correlate dynamics across hierarchical scales.

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The integration of empirical and interaction context has produced network-based data-inference tools leaning on the biology-dependent end of the spectrum. Ingenuity Pathway Analysis (IPA) [63] and protein-protein Interaction (PPI) databases [64–66] are accessible tools in this realm (Box 3). IPA can be a hypothesis generation tool by estimating pathway activity, and as a targeted tool by identifying testable regulator targets and downstream effects within pathways of interest. IPA was leveraged to identify and knock-out two repressors to improve viral resistance in CHO cells [67]. Meanwhile, overlaying omic data on PPI networks—such as PCNet [65], STRING [64], or self-constructed networks [68]—enhances mechanistic understanding and target identification through contextualizing experimentally identified or computationally inferred biological interactions. Transcriptome data were supplemented with PPI networks to quantify secretory fitness variation across tissues and elucidate the role of perturbed secretory machinery in human amyloidogenesis [68]. Similar methods can provide mechanistic insight on secretory fitness in CHO and unravel key regulators.

Hybrid models

There are challenges that accompany mechanistic models. GeMs are exceptional at describing cellular mechanisms based on reaction stoichiometry and also cover all metabolic pathways; however, they can suffer from their steady-state assumption and miss cellular dynamics. On the other hand, kinetic models capture mechanistic details of dynamic cellular processes, but suffer from the computational burden associated with parameter estimation, which limits full genomic coverage. Recent applications have combined machine learning and parametric mechanistic modeling frameworks to overcome some of these inherent challenges. Machine learning (ML) can help restructure and modify mechanistic models, or it can facilitate and tune model parameterization. For example, ML methods can estimate model parameters from experimental omic data[69–72]. Alternatively, flux solutions obtained from constraint-based models can provide an additional “omic layer” and be integrated into ML approaches to predict growth conditions [73], pathway engineering for optimized tryptophan production[74], and even drug side-effects [75]. Similar approaches are being applied towards creating digital twins of bioreactors [35,76,77] to enable *in silico* bioprocess optimization–aiming to decrease the number of iterations needed for process optimization. Recently a hybrid kinetic/**artificial neural network (ANN)** glycosylation model successfully predicted the glycoform distribution in monoclonal antibodies[78]. The hybrid model consists of two kinetic modules describing CHO cell metabolism and nucleotide sugar donor synthesis, feeding into a novel ANN model of glycosylation [79]. These types of synergistic approaches allow incorporation of key mechanistic information in otherwise biologically agnostic learning processes [80].

Concluding remarks and future perspectives

There is a need to clearly define where, when, and how omics can be used to effectively improve cell line development and optimize bioprocesses. Many factors contribute to the actionability of omics approaches, but we find systems biology modeling strategies are particularly poised to enable biotechnology researchers to enrich omic experiments with interpretable results. Importantly, they can make results from omics studies more *actionable*

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to enhance cell factory design. However, while we endorse the implementation of inherently more mechanistic models such as GeMs, we recognize that these and emerging tools need to improve accessibility, ease of execution, and accuracy for more widespread integration [81,82].

We focused on selecting downstream analysis tools for integration and interpretation of omic data; however, it is important to establish implementation strategies prior to experimentation. Researchers should clearly identify the biological questions they wish to interrogate and choose relevant experimental design and omic approaches accordingly. The experimental design should consider the number of features output by the omic tool(s) and subsequent analysis method when deciding on sample size and conditions. Accordingly, we strongly suggest that feature rich datasets be analyzed via mechanistic models if large sample numbers are not available to enable purely data-driven inference based approaches. Meanwhile, the omic approaches employed should be prioritized based on the ability to allow for the earliest, fastest, and most likely to succeed interventions.

Finally, we see a need to develop new resources in the industrial community to increase the size and diversity of omic datasets (see Outstanding questions). In particular, single-cell, epigenome, and interactome data are largely underutilized in biopharma studies. Such omic methods can provide orthogonal value to the omnipresent genomic, metabolomic, transcriptomic, and proteomic profiling [13,83]. Compared to disease and academic research, the CHO space lacks dedicated species-specific open-source databases such as TCGA [84], ICGC [85], ENCODE [86,87], etc., along with phenotypic databases [88], to facilitate benchmarking and transparency in analytical best practices. Public data resources such as these also increase statistical power in resolving biological patterns and targets. Reticence around data sharing is understandable as data from industrial cell lines contain proprietary molecule sequences and information supporting competitive advantages. However, the industry could significantly accelerate the progress towards actionable omics with freedom to access a lot more data relevant to the cell system, in addition to harnessing a more cohesive omics industrial community, and integrating omic approaches with appropriate analytical tools.

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Glossary

Artificial neural networks (ANN)

constitute a variety of deep learning technology inspired by the biological neural networks of the human brain. These networks consist of an input layer, one or more hidden node layers, and an output layer. Each node (artificial neuron) has an associated weight and threshold, and if the output of any individual node is above the given threshold then that node is activated and sends data to the next layer in the network

Critical quality attributes (CQA)

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are physical, chemical, or biological properties or characteristics that must be within an appropriate limit, range, or distribution to ensure the desired product quality. A Quality-by-design framework is generally implemented to identify and define CQAs per molecule program's Quality Target Product Profile (QTPP)

Design of experiment (DoE)
is a structured data collection and analysis method used to study the relationship between various factors hypothesized to affect key output variables

Genome-scale metabolic models (GeMs)
are mathematical network representations of the metabolism formulated based on carefully curated genome annotations and experimental data

Gene Ontology (GO)
is a major bioinformatics initiative to unify the annotation of gene and gene product attributes across species

Gene regulatory networks (GRNs)
are groups of genes identified or inferred to interact with each other and possibly other molecular species to control cellular functions

Gene set enrichment analysis (GSEA)
is a computational method to identify classes of genes that show significant, concordant differences between two biological states (e.g., phenotypes)

Matrix factorization
methods such as Principal Component Analysis (PCA) Independent Component Analysis (ICA) and Non-negative Matrix Factorization (NMF) can extract latent variables that represent biologically meaningful patterns to allow data interpretation or visualization of high-dimensional data

KEGG
Kyoto Encyclopedia of Genes and Genomes consists of a collection of databases related to genomes, biological pathways, diseases, drugs, and chemical substances. According to the developer, KEGG is a "computer representation" of the biological system integrating genetic building blocks (genes/proteins), chemical building blocks (small molecules and reactions), and wiring diagrams of molecular interaction and reaction networks

Next-generation sequencing (NGS)
offers ultra-high throughput sequencing technology that has revolutionized genomic research

Partial least squares (PLS)
is a supervised regression method that decomposes data into principal components as in PCA, except that the components maximize correlation with the dependent variable. PLS and its variations are widely used in predictive machine learning models and can be easily implemented for multivariate omic data

Product quality (PQ)

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refers to physical and chemical molecule attributes that may affect the identity, efficacy, safety, or purity of the molecule and are closely monitored during cell line and bioprocess experimentation. Examples of product quality attributes typically quantified are glycan species, molecular size variants (such as high, medium, low molecular weight species), charged-base variants

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Outstanding questions

Is there a path towards shared public omic databases in the biopharmaceutical space to create rich datasets similar to those available in human and other model organisms (e.g., The Cancer Genome Atlas [TCGA], Cancer Dependency Map, or ENCODE)?

- A database that integrates newly generated and previously published omics data could provide tremendous value to the community, guiding experimental design and increasing experimental power.
- We acknowledge significant hurdles exist to ensure that proprietary sequence information about pre-clinical therapeutic compounds are not inadvertently shared, however, pre-filtering raw sequencing data before dissemination could be a potential solution.
- Can we establish best practices for data generation and sharing?

Is there a way to assess the likelihood of a genetic engineering or process modification strategy being universally applicable (e.g., into the host or into a platform process) based on the results of introduction into producing clones?

- Clonal and molecule variability means there may not be a “one size fits all” solution.
- What is the role of academic-industrial partnerships and/or consortiums in driving innovation?
- Translatability of findings from academic studies is often unknown due to limitations on industrially-relevant domain knowledge and resources.
- Is there a route toward more collaborative partnerships (e.g., resource sharing or validation in an industrial context)?
- Some challenges, such as intellectual property and trade secrets, are common to most industry-relevant fields of research, and are not specific to omics studies in biopharmaceutical research. However, can general principles be derived, or discoveries released separate from the specific product or company where the biomanufacturing insights were found?

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Highlights

Omic experiments have become increasingly popular in biotechnology, however, project timelines and system complexity make extracting actionable insights challenging.

Current applications of omic technologies within the CHO community provide predominantly observational data, however these can become more actionable through the use of careful experimental design and/or emerging systems biology methods.

Mechanistic models for diverse cellular systems have recently been developed and statistical analysis techniques for large datasets are increasingly commonplace but have yet to be widely applied to CHO omic data.

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Box 1.

Overview of the biopharmaceutical protein production process

Several steps are taken in the development of a production cell line. A few common steps are as follows:

- **i.** The sequence coding for the protein of interest (e.g. monoclonal antibody) is introduced to the cell along with a selectable marker (e.g. dihydrofolate reductase or glutamine synthetase) and integrated into the genome.
- **ii.** The resulting pool of cells is highly heterogeneous: some cells have integrated multiple copies of the antibody gene and are producing appreciable quantities, while others may be producing little to no product. Due to this and regulatory requirements, 100s to 1000s of single cells are isolated, grown, and evaluated to find the 'winners' which produce high quantities and quality of the desired product.
- **iii.** The winners are subjected to bespoke, intensified process development to maximize the amount of biotherapeutic generated. Owing to clonal variability and the plasticity of the CHO genome, the optimal process will almost certainly differ for each cell line.
- **iv.** Additional care must be taken–either via process modification or clone selection–to ensure that **critical quality attributes (CQAs)** such as glycosylation or aggregation are maintained at appropriate levels.

This process can take upwards of a year and is repeated for every biotherapeutic protein in a company's pipeline that aims to progress into clinical trials. Many steps of this process are critical path activities, thus delays must be avoided and innovations that shorten timelines are highly valuable.

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Box 2.

Mechanistic modeling approaches

Genome-scale metabolic models (GeMs)

GeM reconstructions are mathematical representations of an organism's stoichiometry-based, mass-balanced metabolic reactions using gene-protein-reaction (GPR) associations that have been formulated based on carefully curated genome annotations and experimental data [89–91]. Since the first GeM of *Haemophilus influenzae* RD was reported in 1999 [92], numerous other GeMs have been published for a variety of organisms including industrially relevant *Escherichia coli* [93], *Saccharomyces cerevisiae* [94], *Bacillus subtilis* [95,96], *Pichia pastoris* [97], human [98], and CHO [99], most of which are accessible via the BiGG Models database [100].

ME models

Genome-scale models of metabolism and macromolecular expression (ME-models) integrate Metabolism and Expression on a genome scale, permitting calculation of the cellular cost of enzyme synthesis, in addition to stoichiometric balancing of the reaction(s) they catalyze [101]. These computational ME models provide a framework to determine a cell's most protein-cost-effective way of carrying out its required biological functions. Due to challenges regarding computational resources and model development, ME models have only been constructed for 3 organisms thus far: *Thermotoga maritima*, *Escherichia coli*, and *Clostridium ljungdahlii*.

Genome scale models of protein secretion

These computational models represent genome-scale stoichiometric reconstructions of metabolism coupled to protein secretion. In 2013, the first genome-scale model for yeast secretory machinery was constructed [32]. Following these efforts, models of mammalian metabolism coupled to protein secretion were developed for human, mouse, and CHO cells [31]. These models implement a protein-specific information matrix (PSIM) which quantifies select protein attributes (e.g: disulfide bonds, N-linked and O-linked glycans, transmembrane domains, protein length) for proteins of the secretome, enabling the construction of protein-specific secretory models using the template reactions in the reconstruction.

Kinetic models

Kinetic models use mathematical expressions of biochemical reaction rates, which form mass balance equations to capture the temporal behavior of the system. Unlike stoichiometric models that only require stoichiometry and directionality constraints, kinetic models require a considerable upfront investment for parameterization. Typically, enzyme characterization experiments must be performed to experimentally determine these parameters. Various kinetic parameterization approaches exist to determine the "best-fit" model that most closely emulates experimental data. With the emergence of simulation-based methods of parameter fitting such as Monte Carlo, the modeling

community has advocated the use of multi-omic data sets to precisely fit these coarse-grained models [102].

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Box 3.

Data inference modeling approaches

Unsupervised learning

Unsupervised techniques are statistical methods that reduce the feature (e.g. genes or other molecular species) dimensions and to resolve patterns in unlabeled data that can correlate with a phenotype of interest. **Matrix factorization** (e.g. PCA, independent component analysis (ICA), non-negative matrix factorization (NMF)), clustering (e.g. k-means, hierarchical), and autoencoders (ANN) identify sources of variation or separation in data. These approaches have been broadly applied and underpin many machine learning tools. More recently, methods like canonical correlation analysis (CCA) have been leveraged to integrate multi-omic data through the conservation of complex data patterns across layers of high-dimensional data.

Supervised learning

Supervised techniques generally adapt the same statistical foundation as unsupervised techniques and incorporate known data labels to consider association with dependent variables. Also comprising the basis of many machine-learning predictive models, these techniques fall under regression (e.g. PLS, gradient descent) and classification (e.g. support vector machine (SVM), k-nearest neighbor (k-NN)). Supervised techniques range in transparency and explainability–tools like PLS and Random Forest can provide feature importance metrics while neural networks and other black box approaches offer lower transparency and explainability of the input features but capture non-linear or complex relationships.

Correlation Networks

Correlation networks combine unsupervised and supervised techniques with mechanistic considerations to infer association of network-based target groups with phenotype. Tools like Weighted Gene Co-expression Network Analysis (WGCNA) and gene regulatory network (GRN) inference implement dimension reduction techniques under biological topology based assumptions. WGCNA can be used to find groups of co-expressed genes aggregated into “eigengenes” with loadings that can be quantitatively correlated with phenotype metrics. GRN methods assume association, or co-expression, of genes with transcription factors to predict gene network regulons. GRN methods range from leveraging single-omic data (e.g. SCENIC built for scRNA-seq data [60]), to multi-omic data (e.g. scGRNom [61]) where integration of epigenomics provides an additional layer of mechanistic context to increase inference accuracy.

Empirical-based, interactome models

Empirically supported databases and protein-protein interaction networks offer mechanistic contextualization over conventional statistical analysis. Ingenuity pathway analysis (IPA) is a user-friendly platform and rich database that infers causal relationships and regulators, adding mechanistic and directional context over standard overrepresentation pathway analysis and **GSEA** against **GO** or KEGG. Protein-protein interaction (PPI) networks are experimentally identified, or statistically inferred physical

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or functional interactions between proteins and can be visualized or created using mass transfer dictated network propagation or user-friendly tools such as Cytoscape [103].

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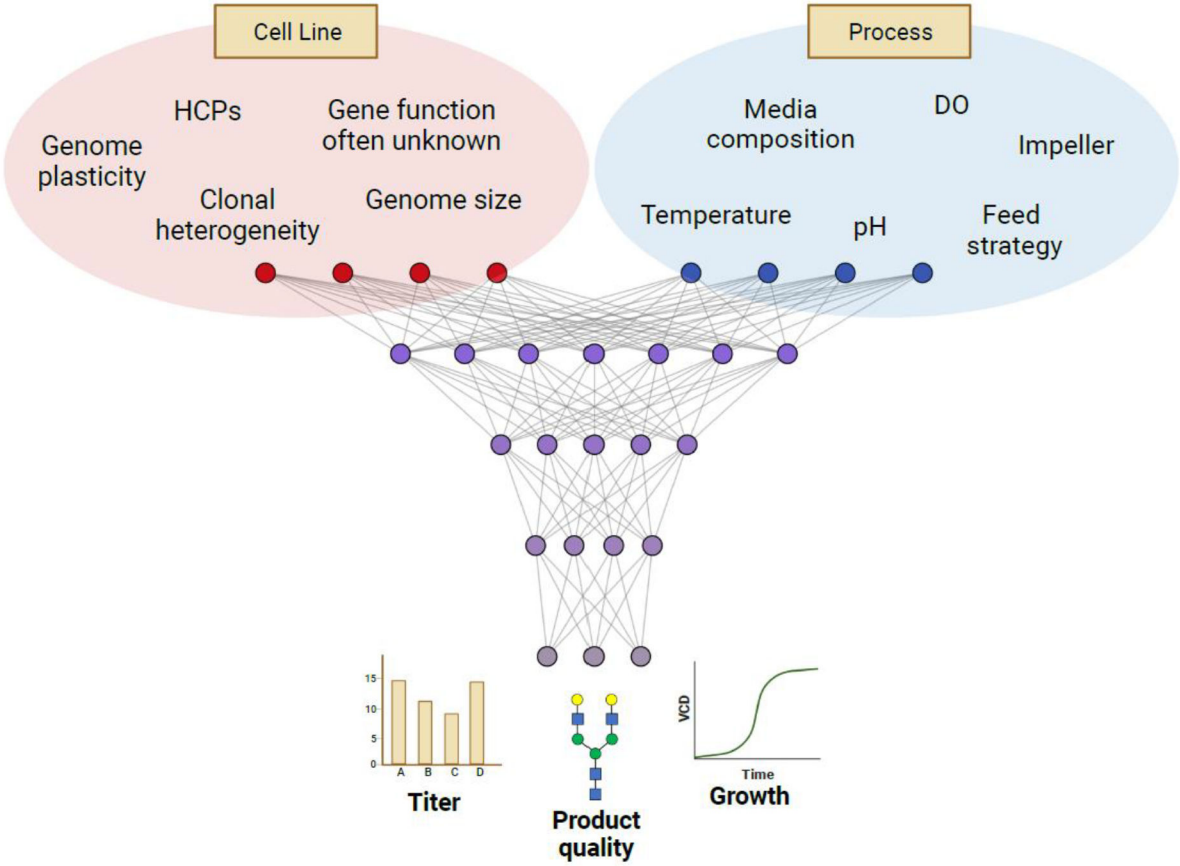

Figure 1. Complexity of biotherapeutic protein production.

In biomanufacturing, there are multiple parameters and sources of complexity that must be managed and optimized for a given cell culture process for a given process development project (top). The end goal (bottom) for any process development effort is to generate large amounts of high quality product. Changes can be made to either the cell line or to the cell culture process, however these changes ultimately have to be evaluated based on their effect on titer, product quality, and growth. While it is tempting to view each point of implementation as independent, the effect of modifying either cell line or process cannot be understood in a vacuum–their interconnectedness represents the ultimate complexity in trying to assess, understand, and engineer this system. Since the responsiveness of a clone to a given process will vary depending on the underlying genotype, and the optimal process may vary considerably from clone to clone, it is critical to consider this complexity when designing and interpreting omic experiments. [HCPs: host cell proteins, DO: dissolved oxygen]

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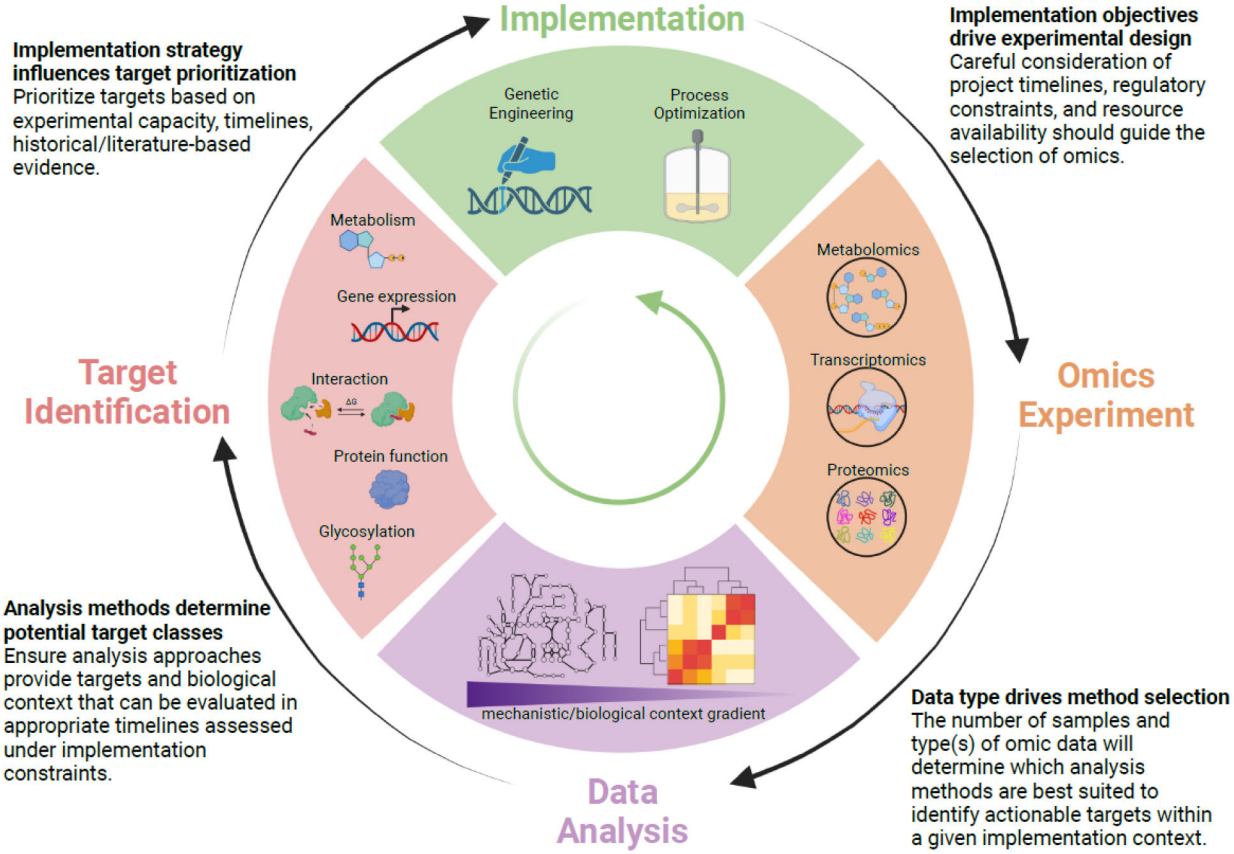

Figure 2. Key figure. Industrial application of actionable omics and key considerations.

To design and implement effective cell line and bioprocess based omic studies, there are multiple considerations to maximize actionability. The outer arrow sequence represents the general lifecycle of omic study conceptualization, experimentation, and implementation; iterations of this process may be required to narrow down and validate biological targets. In addition to these considerations, we note through the inner green arrow that the implementation objectives guide all stages in the lifecycle and that stage interdependency can constrain the methods toolbox and expected outcomes. For example, the implementation strategy can inform both the expected target species to be intervened through cell line engineering or process optimization–which informs the omic type(s)--but also the respective analysis methods that can account for such biological scales or systems dynamics.