EMA Expert Workshop on Validation of Manufacturing for Biological Medicinal Products

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Scale down models for Cell Culture

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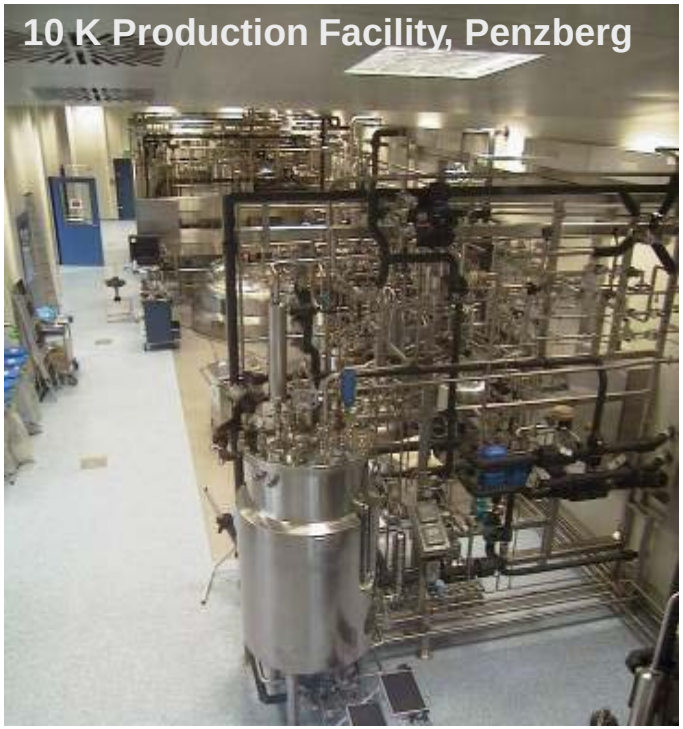
Introduction

- “Small-scale models can be developed and used to support process development studies. The development of a model should account for scale effects and be representative of the proposed commercial process. A scientifically justified model can enable a prediction of quality, and can be used to support the extrapolation of operating conditions across multiple scales and equipment.” **ICH Q11 Step 4**
- “It is important to understand the degree to which models represent the commercial process, including any differences that might exist, as this may have an impact on the relevance of information derived from the models.” **FDA Process Validation Guidance**
- “Essentially, all models are wrong, but some are useful.” **George E. P. Box**

Introduction

- By definition, a scale-down model is an incomplete representation of a more complicated, expensive and/or physically larger system.
- **Scale down models must be used because of the limitations to conduct experimental studies with the at-scale equipment**



8,000 x

Key Elements of SDM Design

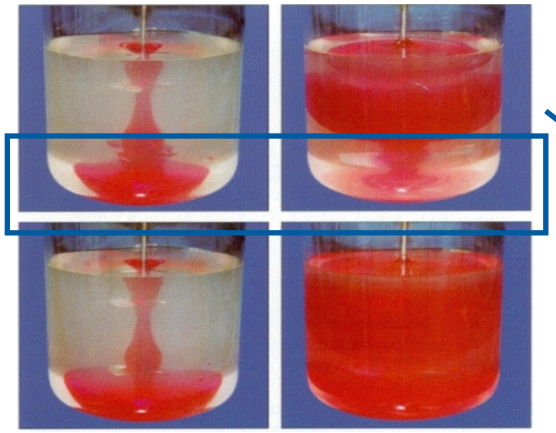
- Inputs: raw materials and components, feedstock/cell source, environmental conditions
- Design: selection of scaling principle(s), equipment limitations, on- and off-line analytical instruments
  - Use of sound scientific and engineering principles for scaling
- Outputs: performance and product quality metrics (CQAs), sample handling/storage, analytical methods.
  - Match full-scale as much as possible and feasible. Understand and/or control for differences between scale-down and full-scale (e.g., materials of construction, use of different assays)

These elements should be described and justified as part of the overall qualification of a scale-down model.

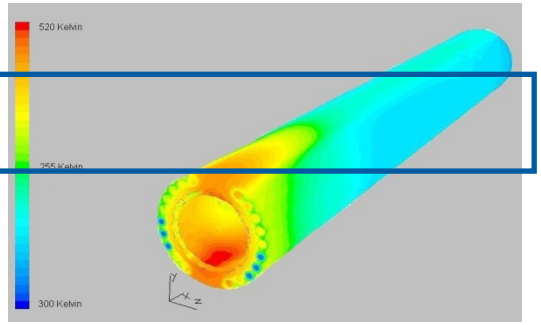
Key Elements of SDM Design

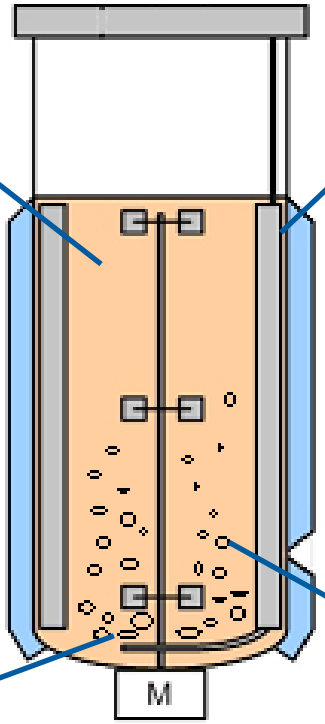
- Key Design Aspects for Cell Culture Processes:

Mixing

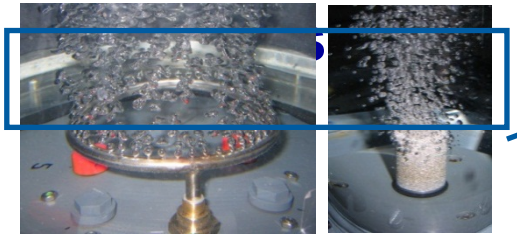


Heat Transfer

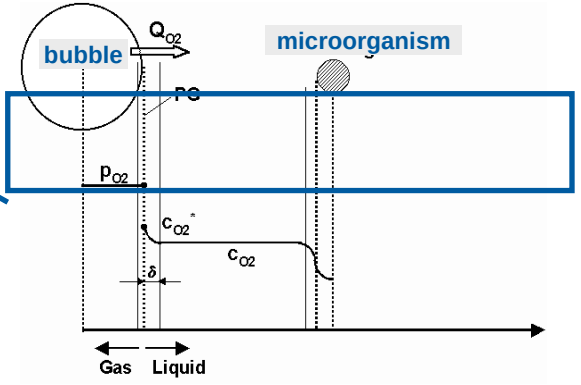




Gas

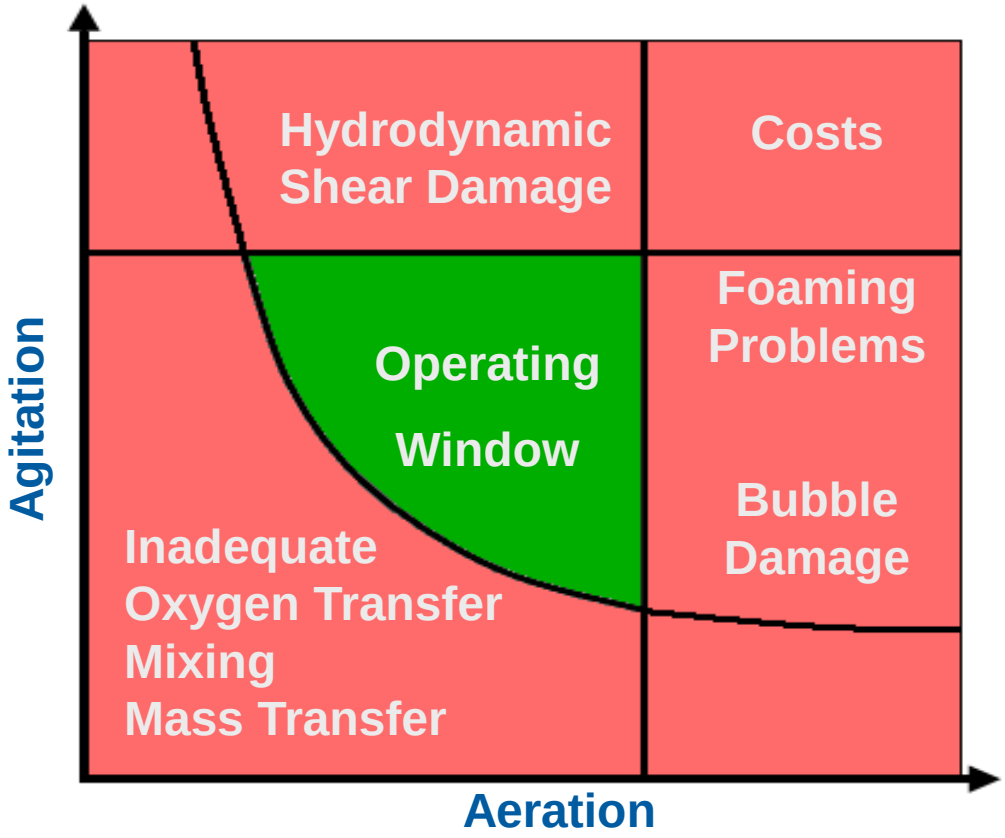


Mass Transfer



Key Elements of SDM Design

- It is important to meet the same operating window for SDMs as for the at-scale process, if possible
- These window can be process and cell line specific



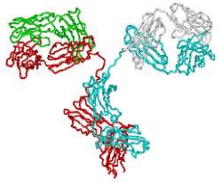
Scale Down Model Development

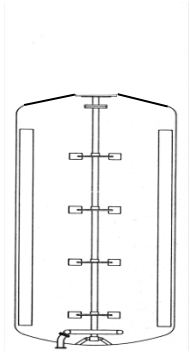
- Many scale down criteria are used
  - None is optimal, choice depends on project and cell line specific characteristics

Unit Operation	Determining Criteria	Relation / Function	Expected Behavior
Suspending	Tip Speed **u**	$u = \pi \cdot n \cdot d \approx V^{1/9}$	interferior with increasing scale
Micro Mixing	Energy Dissipation Rate ε	$\varepsilon = \frac{P}{V \cdot \rho}$	equal
Macro Mixing	Mixing Time θ	$\Theta \approx \frac{D}{u} \approx \frac{1}{V^{2/9}}$	interferior with increasing scale
Mass Transfer	Mass Transfer Coefficient $\mathbf{k_L a}$	$k_L a = K \cdot \left(\frac{P}{V}\right)^{\alpha} \cdot (v_{SG})^{\beta}$	comparable
Shear Force	Turbulent Shear Force τ	$\tau = 0{,}0676 \cdot \rho \cdot d_P^{\,2} \cdot \frac{\varepsilon}{\nu}$	comparable

Scale Down Model Justification

- Acceptance criteria: the performance of the scale down model should match the large scale product and process
- Process outputs of the manufacturing scale process and the SDM needs to be compared





Examples of product quality attributes

- Charge heterogeneity (Oxid., Deamid., Lysine-het., etc.)
- Glycosylation pattern (Galactose content, Mannose structures, non-fucose content, etc.)

Examples of key performance indicators (KPIs)

- Product titer
- Cell density and viability
- Concentration of substrates and byproducts (Gluc, Gln, NH_4^+, etc.)

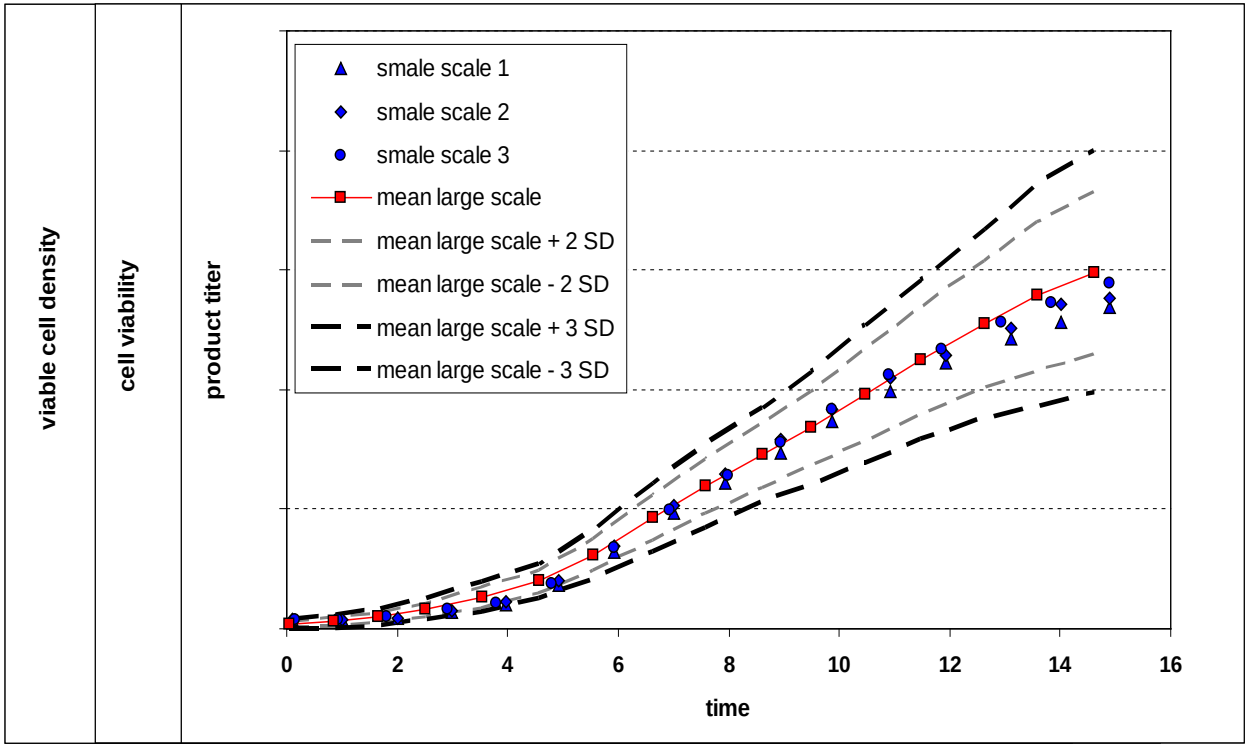
Scale Down Model Justification

- Justification is documenting evidence a model is suitable for evaluating the effect of input material and parameter variation on process performance and product quality outputs.
  - The same *change* in inputs results in a substantially similar *change* in outputs.
- Through adequate *description* that the design provides the data it is intended to provide.

- **Compare “at-target” performance**

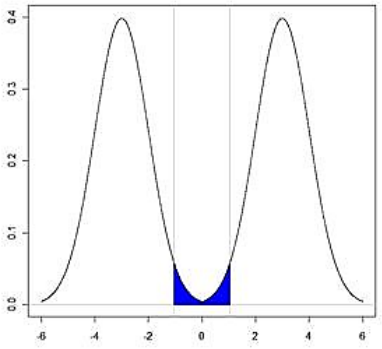
Justification by Qualitative Assessment

- Qualitative assessment of time-course trends and product quality attributes
  - Similar behavior between scales supports model suitability
  - Dissimilar behavior may indicate a problem, and can be valuable for troubleshooting and model improvement



Justification – Statistical Approach

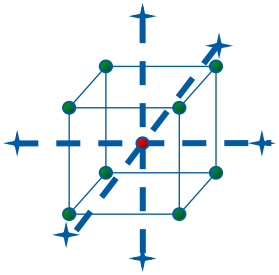
- E.g. Equivalence testing:
  - Define an interval within which a difference is not scientifically meaningful, a “practically significant difference” (PSD)
  - Compute the difference in means and associated statistic testing if difference is within the PSD (e.g., two-one-sided-t-test [TOST] and p-value)
  - Null Hypotheses are δ > PSD or δ < - PSD. Achieving statistical significance (e.g. p<0.05) supports “equivalence” (both null hypotheses rejected)
  - Outcome depends strongly on the definition of the PSD
    - The PSD should be based on a scientific/engineering considerations
- Advantages
  - Rewards greater data replication
  - Similar to Bioequivalence calculations
  - Supports a direct claim that model output is “not different”

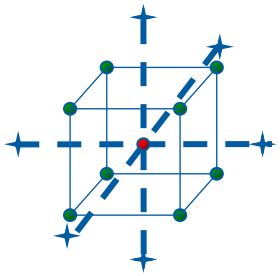


Scale Down Model Justification

- An "Ideal Scenario": Model is compared against full-scale at-target and off-target to verify the scale-down model is fully representative under various process parameter conditions

Process Characterization Scale-down





Full-scale PC/PV studies ?

- Is this practical?
  - Short answer: No
    - Multiple additional runs, may also require sufficient replication at off-target points for statistical confidence.
    - Full scale runs are prohibitively expensive
  - Long answer: part-way…, sometimes…, it depends…
    - Some parameters are tested: cell age, run duration, hold times
    - Testing at pilot scale instead of full-scale?

Scale Down Model Justification

- The evidence for predictability of small scale models can be gathered throughout development
  - Satellite experiments in the small scale models with feed streams directly from the large scale systems during clinical grade manufacturing, and by using the same lots of raw materials and consumables as in the manufacturing lots are an ideal option.
  - Deviations during manufacturing can be reproduced in the satellite model as they occur (with a small time offset) and their impact on process performance/product quality can be assessed in large and small scale in parallel.
- The above approach has limitations:
  - Not all development units have large and small scale readily available.
  - It is also possible to have clinical manufacturing with few or no significant deviations and hence no chance to gather data measuring the predictability/reliability of small scale models

Scale Down Model Justification

- Some outputs are more important than others
  - Product quality attributes
  - Key performance indicators (e.g., titer)
  - Other characteristics (e.g. metabolic measures)

- **A model can be “equivalent” for some outputs, but not all, and still be a representative model – and even still be representative of those outputs that are not statistically equivalent!**

Dealing with offsets

- Evaluating the acceptability of an observed offset
  - Is the mechanism understood and/or specific source known (e.g., light exposure, hold time differences, sample handling)
  - Is the magnitude of the offset, and absolute value of the output near a “natural limit” (e.g., % Monomer near 100%)?

- A question of confidence…
  - Unlikely to have sufficient replication of on- *and* off-target conditions at full-scale for a statistically robust comparison of factor effect sizes between scales.
  - Scientific understanding, offset stability and off-target full-scale testing add incrementally to the totality of evidence that an offset is acceptable.

Traditional Applications of SDM

- What scale down models have been used for from a traditional point of view:
  - Cell line selection
  - Process and media development
  - Investigation of Raw Material Variability
  - Characterization/Validation of cell age effects
  - Characterization/Validation of process parameter excursions
  - Determination of PARs for process parameters
  - Supporting Consistency claim when few at-scale batches are available

Validation / MAA relevant data

The Future? - Upstream Ultra SDMs in Validation

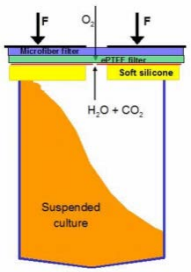
- Current –
  - bench top scale down reactors
  - Mainly 2–15 L systems used

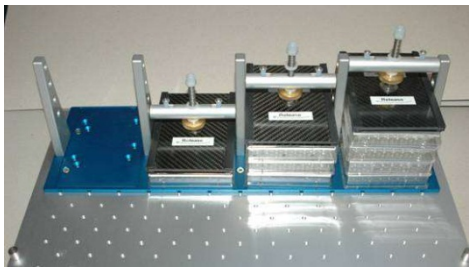
- Soon/now… Ultra-scale-down reactors
  - 15-100 millilitres
  - Individually controlled
  - multiparallel reactors e.g. (ambr, 24 or 48-parallel rig)
  - Validate to model benchtop – generate large design space data sets
  - But will need the a similar degree of justification as the 2-15L bioreactor systems

The Future? - Upstream Ultra SDMs in Validation

- Erlenmeyer flask data – relate to benchtop reactor data
  - Approximation to bioreactors for process characterisation
  - 30-50ml- litres volumes - individual flasks,
  - Simulation of pH, D.O. control, stirrer speed, fed-batch

- Shaking multi-well plates
  - 1-2 ml cultures, 24+ plates, 1500 wells/incubator
  - Approximation to Erlenmeyer flask control, engineering / mixing design and characterisation
  - Automation of feeding and sampling
  - Generate larger design space data sets





Summary

- Scale-down models are a tool for developing and characterizing “the process”
  - Enables evaluation of input material and parameter variability on a process to an extent that is simply not feasible at manufacturing scale
  - By definition of a “model”, even the best is an incomplete representation, but can still provide useful and accurate information.
- Scale-down models should be designed and demonstrated as appropriate representations of the manufacturing process.
  - Industry must demonstrate a model is appropriate and applicable
  - Regulators must recognize models cannot be absolutely perfect, but understand their value and permit industry to utilize them for the information they can appropriately provide.

The Upstream Team

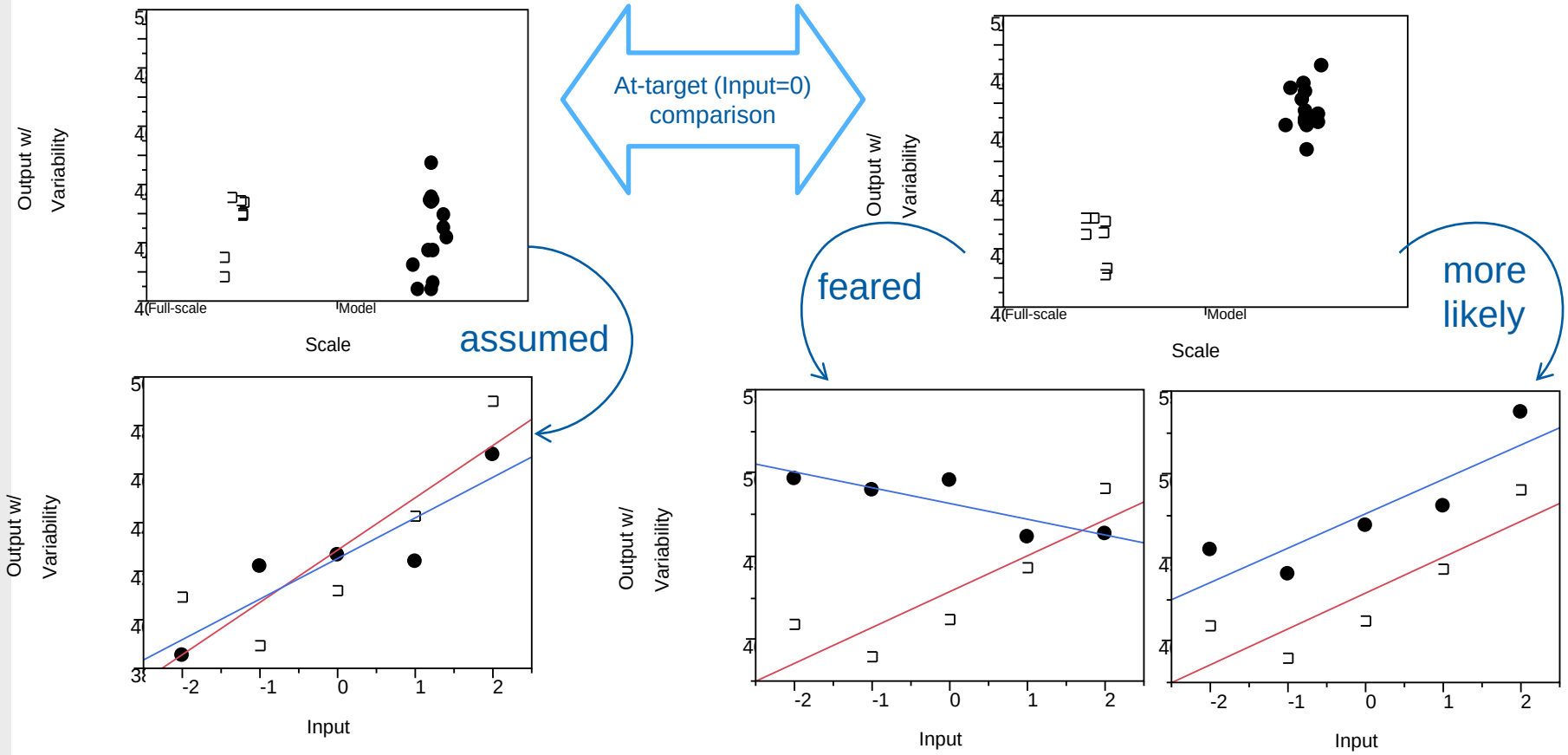
Arie van Oorschot	Uniqure
Kristopher A Barnthouse	Janssen Pharmaceuticals
Vijay Chiruvolu	Amgen
Ranjit Deshmukh	Medimmune
Ray Field	Medimmune
Jason Gale	Pfizer
Christian Hakemeyer	Roche
David Kirke	UCB
Li Malmberg	Abbvie
Karin Sewerin	Consultant for Medimmune
Juergen Wieland	Ratiopharm

Thank you!

Back-up

Dealing with offsets

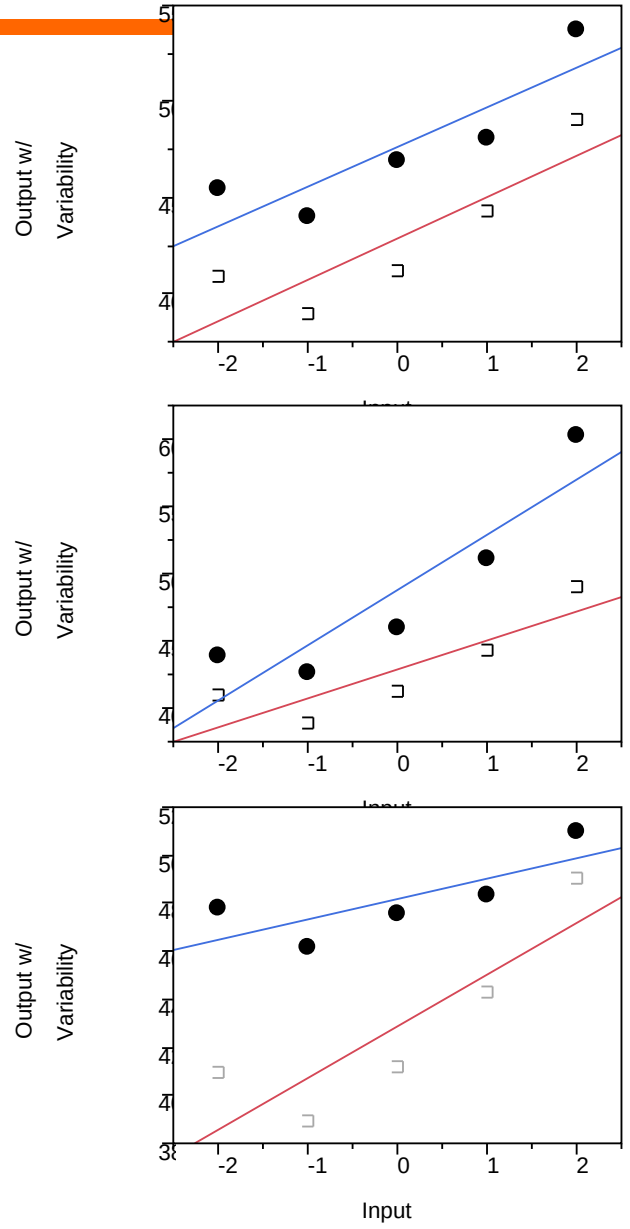
- The statistical evaluation of at-target performance is really an evaluation of risk, where offsets *suggest* higher risk
- The risk: an offset may indicate the model will have a different response to the same change in process conditions



Dealing with offsets

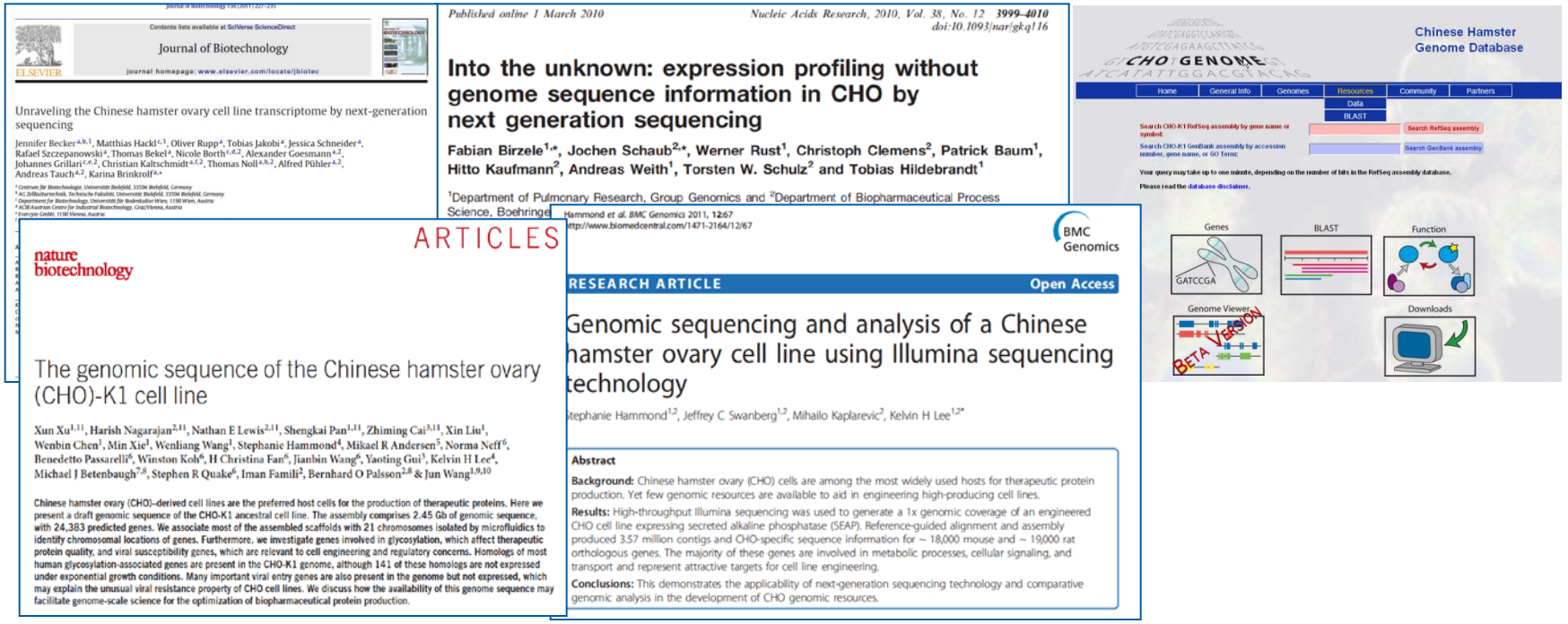
When is an offset acceptable, when not, and what to do

- Constant offset - account for offset in data interpretation, need sufficient data supporting magnitude of offset used.
- Magnified response in model
  - Factor effect directionality and ranking still valid, direct prediction difficult
  - Robust interpretation possible by comparison to scale-down controls.
- Attenuated response in model
  - Same as magnified response, but higher risk since effect sizes may be falsely interpreted as not significant.



The Future ? - High Content Validation Tools

- Transcriptome Sequencing of Production Cell Lots?



Journal of Biotechnology

Unraveling the Chinese hamster ovary cell line transcriptome by next-generation sequencing

Published online 1 March 2010 Nucleic Acids Research, 2010, Vol. 38, No. 12 3999–4010 doi:10.1093/nar/gkq116

Into the unknown: expression profiling without genome sequence information in CHO by next generation sequencing

Fabian Birzele[1,*], Jochen Schaub[2,*], Werner Rust[1], Christoph Clemens[2], Patrick Baum[1], Hitto Kaufmann[2], Andreas Weith[1], Torsten W. Schulz[2] and Tobias Hildebrandt[1]

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ARTICLES

The genomic sequence of the Chinese hamster ovary (CHO)-K1 cell line

Xun Xu[1,11], Harish Nagarajan[2,11], Nathan E Lewis[2,11], Shengkai Pan[1,11], Zhiming Cai[3,11], Xin Liu[1], Wenbin Chen[1], Min Xie[1], Wenliang Wang[1], Stephanie Hammond[4], Mikael R Andersen[5], Norma Neff[6], Benedetto Passarelli[6], Winston Koh[6], H Christina Fan[6], Jianbin Wang[6], Yaoting Gui[3], Kelvin H Lee[4], Michael J Betenbaugh[7,8], Stephen R Quake[6], Iman Famili[2], Bernhard O Palsson[2,8] & Jun Wang[1,9,10]

Chinese hamster ovary (CHO)-derived cell lines are the preferred host cells for the production of therapeutic proteins. Here we present a draft genomic sequence of the CHO-K1 ancestral cell line. The assembly comprises 2.45 Gb of genomic sequence, with 24,383 predicted genes. We associate most of the assembled scaffolds with 21 chromosomes isolated by microfluidics to identify chromosomal locations of genes. Furthermore, we investigate genes involved in glycosylation, which affect therapeutic protein quality, and viral susceptibility genes, which are relevant to cell engineering and regulatory concerns. Homologs of most human glycosylation-associated genes are present in the CHO-K1 genome, although 141 of these homologs are not expressed under exponential growth conditions. Many important viral entry genes are also present in the genome but not expressed, which may explain the unusual viral resistance property of CHO cell lines. We discuss how the availability of this genome sequence may facilitate genome-scale science for the optimization of biopharmaceutical protein production.

Hammond et al. BMC Genomics 2011, 12:67 http://www.biomedcentral.com/1471-2164/12/67

BMC Genomics

RESEARCH ARTICLE Open Access

Genomic sequencing and analysis of a Chinese hamster ovary cell line using Illumina sequencing technology

Stephanie Hammond[1,2], Jeffrey C Swanberg[1,2], Mihailo Kaplarevic[3], Kelvin H Lee[1,2*]

Abstract

Background: Chinese hamster ovary (CHO) cells are among the most widely used hosts for therapeutic protein production. Yet few genomic resources are available to aid in engineering high-producing cell lines.

Results: High-throughput Illumina sequencing was used to generate a 1x genomic coverage of an engineered CHO cell line expressing secreted alkaline phosphatase (SEAP). Reference-guided alignment and assembly produced 3.57 million contigs and CHO-specific sequence information for ~ 18,000 mouse and ~ 19,000 rat orthologous genes. The majority of these genes are involved in metabolic processes, cellular signaling, and transport and represent attractive targets for cell line engineering.

Conclusions: This demonstrates the applicability of next-generation sequencing technology and comparative genomic analysis in the development of CHO genomic resources.

- Use of o'mics profiling:
- High content cell physiology / Characterisation / Multi-gene arrays / RNA-Seq
- Map the metabolism in many pathways between different Process conditions / Map and model the metabolic design space
- Currently used for process development