Roundtable Session 1 – Table 3 – Novel Applications of Molecular and Structural Biology in Characterization of Well Characterized Biologics

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Abstract:

Advances in molecular and structural biology encompassing modern mass spectrometry, high-resolution imaging, sequencing, and computational modeling, are expanding how sponsors understand and manage quality for well-characterized biologics. These approaches can deepen insight into higher-order structure, product and process variability, and structure–function relationships, informing risk, manufacturing process changes, and lifecycle management. At the same time, practical questions remain about when these methods truly add decision value beyond established assays, how to ensure fitness-for-purpose and reproducibility, and what level of evidence regulators expect at different development stages. This roundtable will discuss the value, applicability, implementation success and challenges around utilizing advanced molecular and structural biology tools in biological product characterization.

Discussion Questions:

1) In which scenarios do newer molecular/structural tools provide actionable insight beyond traditional analytical methods, and how should "added value" be demonstrated?

2) What general criteria (e.g., relevance to critical quality attributes, robustness, orthogonality) should guide whether a method is used for characterization only, for comparability, or incorporated into control strategy?

3) How can sponsors translate rich molecular/structural readouts into clear, risk-based conclusions that support specifications, change control, and product lifecycle decisions?

Notes:

General Themes

- Translating complex data sets into usable, implementable technologies remains a key challenge.
- Novel analytical platforms, particularly long-read sequencing and advanced biophysical tools, are increasingly used to characterize diverse modalities but face limitations in GMP environments.

Emerging and Novel Technologies

Gene Therapy (AAV)

- **Partial or fragmented genome packaging:** Long-read sequencing enables detailed characterization of packaging patterns and transgene heterogeneity.
- These insights can help identify better candidates earlier during development.

Protein Therapeutics / Complex Biologics

- **Complex Products (Factor VIII)**: Multi-domain structures makes characterization difficult; cryo-EM has been used to support structural evaluation.
- **Previous BLAs** relied on **X-ray crystallography**; now structural biology tools are supporting higher-order structure assessments.

Vaccines

- Novel vs. traditional biologics: Biophysical techniques routinely deployed during development but rarely in QC.
- Revisiting antigens developed 80+ years ago using modern structural tools provides new insights.

Higher-Order Structure & Potency

- Higher-order structure characterization can be included in BLAs, especially when potency assays are difficult to develop.
- Many vaccine products rely on animal models for potency, which are inconsistent; structural consistency can serve as a surrogate.
- Ensuring consistent structure is critical for clinical supply, though some tools cannot be implemented in GMP testing.

Regulatory Considerations

- Long-read technologies are generally **not validated**, whereas short-read sequencing has established validation pathways.
- Acceptance varies by region:
    - **FDA**: Fewer questions for some INDs using long-read sequencing.
    - **Other global regulators**: Significant inquiries—reflecting lack of harmonization.
- More sensitive tools can reveal additional impurities (or lower apparent purity), complicating justification and regulatory interpretation.
- Agnostic NGS-based analyses may uncover unexpected signals, triggering investigations to determine assay- vs. product-related findings.

Bridging, Comparability, and Changing Technologies

- Regulatory agencies may question why certain tests are not classified as CQAs—once designated, changing a CQA is burdensome.
- Comparability should be planned early; pre/post-change data sets are essential.
- More sensitive methods can create challenges:
    - Results might differ because the assay is more sensitive—not because the product changed.
- Regional differences add complexity to bridging strategies.

Implementing Technologies Across Multiple Labs

- Method transfer ≠ validation; focus is on ensuring comparable outcomes.
- Training is critical—small procedural differences (e.g., pipetting, shaking angles, tip changes) can significantly impact data consistency.
- CDMOs introduce added complexity: limited control over training and assay execution.

Characterization Method Criteria for Control Strategy

- Method selection depends on:
    - Product quality attributes
    - Safety and efficacy considerations
    - Literature precedent
- Advanced tools may remain development-only if not GMP-compatible.
- For biocomparability assessments, non-GMP characterization may still be necessary unless method changes are trivial (like-for-like).

Challenges With Infrastructure & Vendor Dependencies

- Instrument compliance (IQ/OQ), software validation, and security updates can create gaps.
- System upgrades can break previously validated configurations.
- Vendor decisions (sunset of support) can disrupt testing strategies.

Non-Transgene Testing (e.g., Residual DNA)

- Digital PCR vs. NGS:

    - PCR-based approaches target specific sequences.
    - NGS is broader and more sensitive, but not necessarily comparable.
- Scientific justification is required for transitioning methods.

Adoption of New Technology

- Build internal proof-of-concept (POC) data before requesting investment.
- Demonstrating utility across multiple modalities strengthens the business case.
- Vendor demonstrations can help accelerate internal decision-making.