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General Commentary

Technical Decision Making With Higher Order Structure Data: Perspectives on Higher Order Structure Characterization From the Biopharmaceutical Industry

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ABSTRACT

Characterization of the higher order structure (HOS) of protein-based biopharmaceutical products is an important aspect of their development. Opinions vary about how best to apply biophysical methods, in which contexts to use these methods, and how to use the resulting data to make technical decisions as drug candidates are commercialized [Gabrielson JP, Weiss WF IV. *J Pharm Sci*. 2015;104(4):1240-1245]. The aim of this commentary is to provide guidance for the development and implementation of a robust and comprehensive HOS characterization strategy. We first consider important concepts involved in developing a strategy that is appropriately suited to a particular biologic, and then discuss ways industry can partner with academia, technology companies, government laboratories, and regulatory agencies to improve the consistency with which HOS characterization is applied across the biopharmaceutical industry.

Introduction

Developing innovative medicines for patients in need is a goal shared by scientists who develop biologics and regulators who approve them. For protein-based therapies, structural properties of the molecule are one crucial element upon which the quality of the medicine depends; consequently, protein structural characterization is an area in which scientists from industry, regulatory agencies, and academic institutions can work together effectively to improve and ensure drug quality. Traditionally, protein structure has been defined as a hierarchy of structural levels beginning with primary structure and culminating with quaternary structure. In this context, the foundational covalent linkages are considered primary structure, the subsequent formation of localized structures facilitated by hydrogen bonding (e.g., helices and sheets) is considered secondary structure, the overall folding of the protein in 3-dimensional space is considered tertiary structure, and any naturally occurring interactions between separately folded polypeptide chains are considered quaternary structure. For the purposes of this commentary, we define higher order structure (HOS) to be all structural elements, beyond primary structure, necessary for the protein product to function as intended. Formation and preservation of HOS, so defined, is potentially critical for both the efficacy and safety of protein-based therapies.[1-3] We acknowledge that elucidating potential links between HOS changes and resulting impacts to safety and efficacy remains elusive. However, in recognition of the importance of characterizing HOS, regulatory agencies have consistently defined HOS characterization expectations in their guidelines, especially in recent guidance.[4,5]

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In light of the need for detailed characterization of protein HOS, a consortium was established to promote open communication and common understanding among industry scientists, academic researchers, and regulatory authorities about the role HOS plays in product quality and the challenges encountered in the application of HOS characterization tools during product development and manufacturing. A commentary by Gabrielson and Weiss[6] introduced key questions and collected general impressions from industry about the use of HOS data in technical decision making. Five case studies published by members of the consortium gave a diverse set of particular decisions influenced to varying degrees by HOS data.[7-11] In this concluding commentary by industry scientists, we return to the central question posed in the introductory commentary in light of the particular case studies: how can HOS methods and data be used most effectively to make technical decisions during development of biologics?

To address this question, we first consider important concepts involved in developing an HOS characterization strategy that is appropriately suited to a particular biologic, and next we discuss ways industry can partner with academia, technology companies, government laboratories, and regulatory agencies to improve how HOS characterization is applied during drug development. By highlighting existing challenges in HOS characterization, we intend to spur continued improvement in how HOS methods are applied during drug development to aid in making informed technical decisions.

Defining an HOS Characterization Strategy

The development of a biologic into a commercial drug product proceeds through an extensive process of clinical trials to determine the drug's safety and efficacy. Coupled to this process is the supporting biophysical, biochemical, and biological analysis that not only establishes the ability of the drug manufacturer to make the drug consistently with high quality, but also provides a comprehensive knowledge base of the molecule's structural and functional characteristics. Thus, along with the clinical data, characterization data are needed in order to understand the attributes of the drug that impact clinical and commercial performance. The role of biophysical characterization in this process is to define the HOS of the biologic and demonstrate that HOS is preserved during drug substance and drug product manufacturing, storage, and delivery to the patient. Furthermore, drug manufacturers must also demonstrate that HOS is maintained following manufacturing changes made during the drug's development and commercial lifecycle.

A careful consideration of Quality by Design principles is likely to be valuable in developing an appropriate HOS characterization strategy. The approach applied to any particular biologic depends on many factors and can include, among others:

- features of the molecule, including its class, scaffold, and critical quality attributes;
- supply chain considerations, including drug substance and drug product container closure systems along with requirements for storage and distribution;
- HOS method lifecycle considerations, including selection of fit-for-purpose methods and demonstration of their capabilities;
- defining the processing step(s) at which the product is sampled for testing (drug substance intermediate, drug substance, or drug product); and
- phase of product development.

Of these factors, this commentary deals with considerations that are largely preserved across most classes of biopharmaceutical products: method lifecycle considerations, method selection criteria, sample type considerations, and development of a phase-appropriate strategy.

HOS Method Lifecycle

It is useful to define a theoretical lifecycle onto which biophysical methods may be placed with respect to their use in supporting biologics research and development (Fig. 1). We begin in

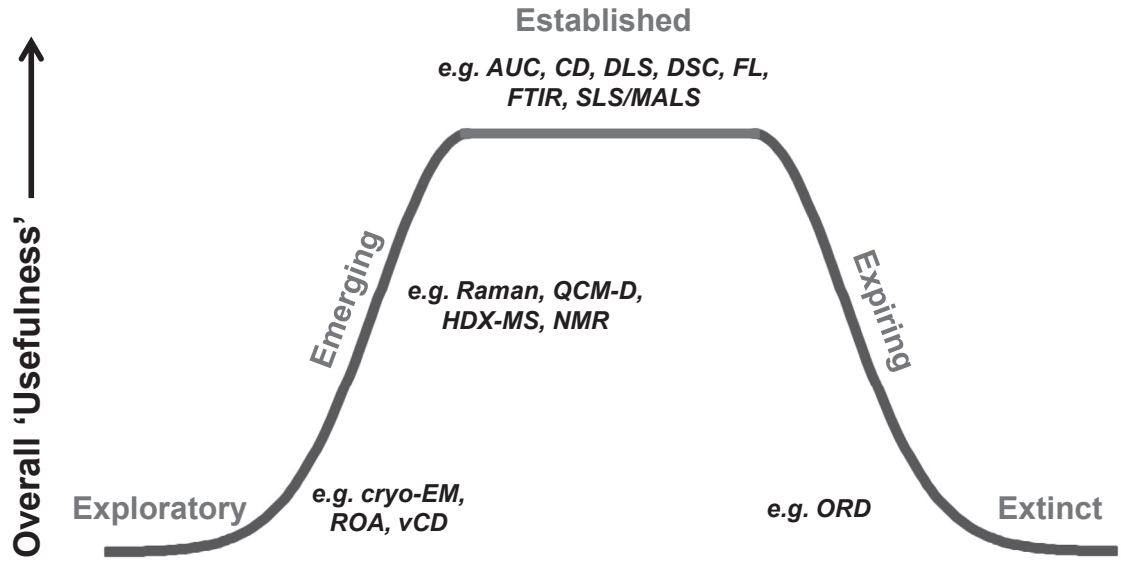

Figure 1. A theoretical lifecycle showing various phases through which a given biophysical method may pass with respect to its use in biopharmaceutical research and development. The overall "usefulness" of the method is greatest in the center and lowest at the extremes. Examples of biophysical methods include: (exploratory) cryogenic electron microscopy (cryo-EM), Raman optical activity (ROA), vibrational circular dichroism (vCD); (emerging) quartz crystal microbalance with dissipation (QCM-D), hydrogen-deuterium exchange mass spectrometry (HDX-MS), nuclear magnetic resonance (NMR); (established) analytical ultracentrifugation (AUC), CD, dynamic light scattering (DLS), differential scanning calorimetry (DSC), fluorescence (FL), Fourier transform infrared (FTIR), static light scattering (SLS)/inline multiangle light scattering (MALS), and (extinct) optical rotary dispersion (ORD).

the "Exploratory" phase, where the method is quite new, and therefore its ultimate value in addressing questions of interest in biologics discovery and development is not clear. As evidence mounts that the method is capable of producing valuable information, it moves into the "Emerging" phase. Although not widely implemented, it is growing in visibility as more scientists contribute to the body of work supporting its usefulness. It is worth highlighting that Exploratory and Emerging methods need not come from entirely new technologies, as value can often be found in applying existing techniques to new or different challenges. The overall utility of the method is greatest in the "Established" phase, where it is now widely and routinely used across the industry to support one or more activities in drug discovery and development. Ultimately, however, there is likely to come a time when competing technologies have advanced to a point where the relative value of a given method begins to wane. This is the "Expiring" phase. The data may still be useful to characterize legacy products and processes, if needed for the purpose of continuity, but the impact becomes increasingly limited. Finally, the information generated by a given method is of such little value that the resources required to generate it can no longer be justified and the method becomes "Extinct." The purpose of introducing this theoretical method lifecycle is not to attempt to unambiguously classify all biophysical methods, nor to imply that the reality of the development and implementation of new methods is so linear and predictable. Rather, the intent is to provide a frame of reference to help facilitate a discussion on how industry, academia, technology companies, national laboratories, and regulatory authorities might engage with biophysical methods and data at different times and for different purposes.

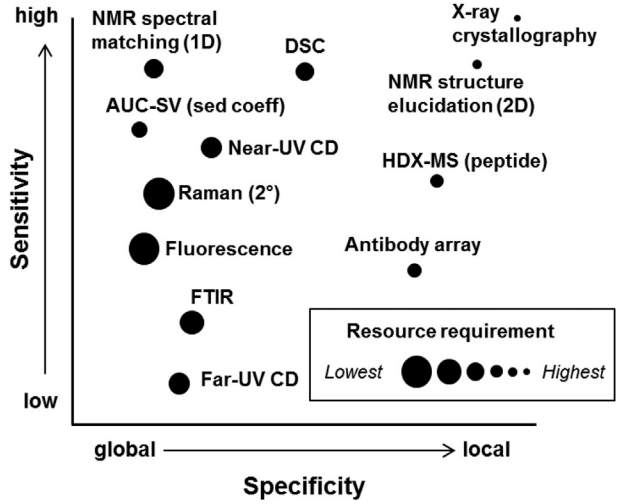

Figure 2. Hypothetical categorization of biophysical techniques based on the type of information provided about HOS. Sensitivity refers to the ability of the technique to detect differences in HOS, and specificity refers to the scale of resolution of the information (i.e., full-protein, domain-specific, peptide level, amino acid, or atomic resolution). Symbol size is used to show the relative resource burden of applying the technique, where larger symbols signify lower resource costs. Based on the resource requirement and the type of information provided by the technique, method selection decisions can be made for different types of studies. For example, sensitivity and precision might be considered most important for purposes of comparability testing, whereas localized specificity and high sensitivity may be most critical for elucidation of structure studies.

HOS Method Selection

The lifecycle phase of a method is just one factor in deciding which methods to apply for various purposes during biologics development. When considering which HOS method(s) to apply to a particular study, it is critical to identify how the data will be used. A one-size-fits-all approach to HOS method selection is unlikely to produce the data needed to make informed decisions during biologics development. Rather, we recommend a targeted approach in which different HOS methods are applied in different contexts. Depending on the nature of information provided by the technique and its sensitivity to HOS changes, various techniques can be plotted on "sensitivity" and "specificity" axes as shown in Figure 2. Techniques in different areas of Figure 2 are useful for different types of studies.

For example, if the objective of a comparability study is to demonstrate that the previously elucidated HOS of a protein therapeutic is preserved following a manufacturing change, then the techniques that provide global information may provide more useful data than local information techniques. However, in a process development study, the objective may be to determine why recovery from one of the process steps is lower than expected, and in such a case it may be critical to utilize a technique that can pinpoint the location of potential tertiary structural changes leading to abnormal column elution behavior. With all else being equal, the higher the sensitivity of the method, the more useful it is in most contexts. The techniques with the highest sensitivity often have the lowest throughput; therefore, faster methods with lower (while still sufficient) sensitivity are often more useful for screening studies. The different purposes for which HOS methods are employed make it difficult to rank order the utility of HOS techniques in an absolute sense, but it is possible to rank order techniques for particular studies based on factors deemed most important to the overall outcome of the study.

In the context of classifying HOS techniques based on sensitivity and spatial resolution, the concept of "fingerprinting" methods deserves particular attention. Recent regulatory guidance and numerous publications have argued the need for analytical methods that produce a so-called "fingerprint" of a biopharmaceutical molecule, particularly in light of recently created biosimilar regulatory pathways.[4] Here we define fingerprint as a well-defined set of measurable attributes of a molecule, which together provide sufficient information to enable reasonable assurance of safety, biological activity, and bioequivalence. HOS methods that measure the entire folded structure of a protein are well suited to aid in demonstrating fingerprint-like similarity, especially those that yield highly sensitive, global information (i.e., methods in the upper left portion of Fig. 2).

Spectroscopic methods that provide a reproducible spectrum of signals arising from the entire protein, or from specific amino acids distributed throughout the sequence, can aid in the determination of "fingerprint-like" similarity, provided that the protein of interest can be distinguished from all other proteins with sufficient specificity. For example, near-UV (ultraviolet) circular dichroism, Raman, and profile nuclear magnetic resonance (NMR) spectroscopies can all be considered fingerprint methods, although they differ in their sensitivity to HOS changes. Importantly, although one or more of these methods may be necessary to establish fingerprint-like similarity, HOS characterization tools alone are unlikely to be sufficient. They become part of a larger analytical test panel which together can be used to support the case for fingerprint-like similarity. With ongoing method improvements,[12,13] sensitivity of some methods is increasing to the point where one might be able to routinely resolve subtle HOS differences that do not impact the drug's safety or efficacy. A technique's ability to measure real but inconsequential HOS differences should not be viewed as a drawback of the technique; that is, a technique cannot be *too* sensitive. Rather, in such a case, it is imperative that normal and expected lot-to-lot variation in the product HOS attribute profile be factored into

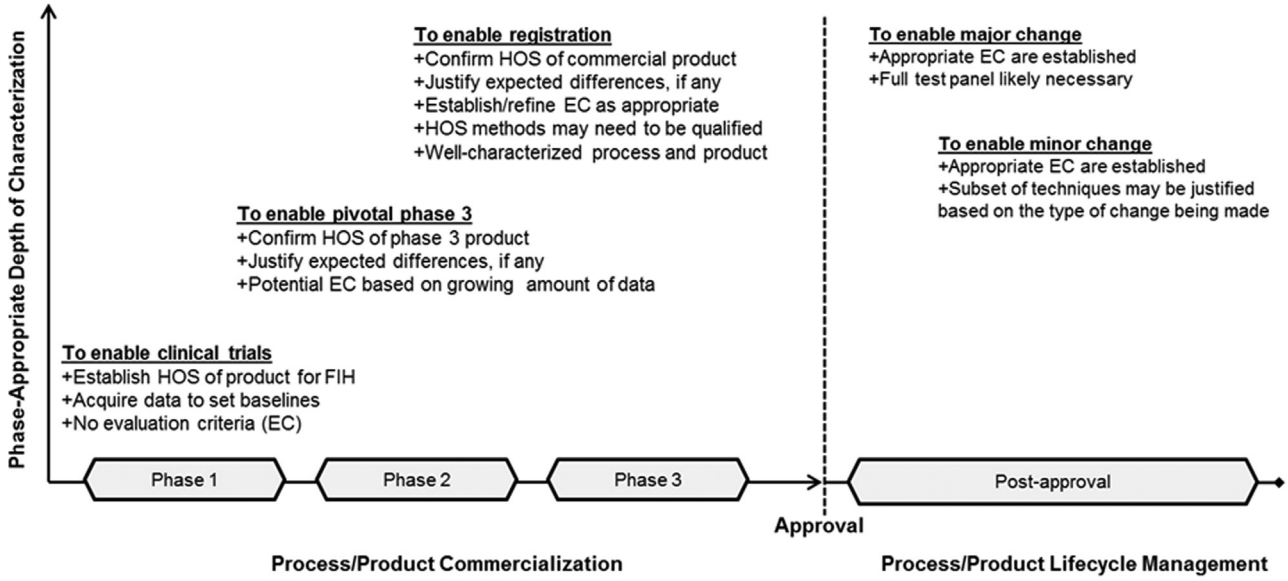

Figure 3. Diagram showing the phase-appropriate increase in depth of HOS characterization during biopharmaceutical drug process/product development, reaching a maximum to support product approval. Postapproval characterization is equally rigorous, but fewer methods may be justified based on comprehensive process/product understanding. As more data become available throughout the course of development, evaluation criteria (EC) are established to monitor HOS and guide technical decision making.

any criterion established to assess comparability or fingerprint-like similarity. Only from a holistic understanding of the molecule, including biochemical, biophysical, and biological characterization, can drug developers determine whether or not HOS changes impact drug quality.

Sample Type

In addition to carefully considering context-appropriate HOS method selection, drug developers should consider opportunities to test the product at the most relevant processing step. In many instances, drug substance is the most appropriate sample type for HOS determination. For purposes of characterizing the product for elucidation of structure to support regulatory applications, drug substance is usually an appropriate sample type. However, if HOS may change due to filling operations, drug product is then also an appropriate sample type. In such cases, when a drug substance manufacturing change is made that warrants analytical comparability testing, it may still be more appropriate to test the drug product filled from the new drug substance process rather than HOS testing of the drug substance itself. In rare cases, a purification process pool may be the most appropriate sample type for testing. During early process development, for example, it may be important to test HOS in 2 consecutive process pools to assess a possible structural change across a process step. As biologics manufacturers consider continuous manufacturing with rapid online and at-line testing methods, there will likely be increased opportunities for targeted placement of HOS methods at the most relevant steps in the manufacturing process.

Phase of Development

HOS characterization of a biopharmaceutical drug candidate is typically applied during all stages of its discovery and development and continuing throughout the commercial lifecycle of the drug. Applying an appropriate degree of HOS characterization, tailored to the stage of product development, as shown in Figure 3, is perhaps the most critical consideration in a drug manufacturer's overall HOS characterization strategy. A key challenge lies in determining which aspect(s) of HOS to interrogate during each stage of development. For example, a loss of bioactivity associated with amino acid oxidation could be related to a change in tertiary structure with or without a detectable change in secondary structure. In this example, characterizing the impact of oxidation on bioactivity may be sufficient for an investigational new drug submission, whereas characterizing the impact of oxidation on tertiary structure, and hence bioactivity, may be required to ensure robust process/product control and an approvable licensure package (e.g., biologic license application, marketing authorization application). It is not practical from a material availability perspective to apply the same depth of characterization at all stages throughout the discovery, development, commercialization, and routine manufacturing of the product. Accordingly, HOS characterization should be performed in a phase-appropriate manner, culminating with a comprehensive understanding of HOS to support product registration and postapproval supplements.

Fit-For-Purpose

As discussed in the context of method selection, a significant hurdle in HOS characterization of biopharmaceuticals is not only to identify a structural change (qualitatively) but also to determine its significance, as well as determining the extent of the structural change (quantitatively). This is a challenging task because depending on the protein in question, its size, structural complexity, conformational dynamics, and formulation, this may lead to observations of differences between 2 similarly prepared batches whose significance is unknown without further study. This ambiguity especially stands out in cases where a difference is measured using a method traditionally considered qualitative, for example, minor differences in a near-UV circular dichroism (CD) spectrum. Hence before applying a biophysical technique, there is a need to understand the critical attributes of the biologic, the information a technique can provide, and what specific question is being asked by the particular study. To aid in this evaluation, there are 3 primary characteristics desired of HOS characterization methods:

1. The ability to detect all signals emitted from a molecule, thereby interrogating as much of the protein's structure as possible.
2. The ability to separate or resolve the signals spatially (ideally corresponding to small units of the protein) with minimal signal overlap between the recorded signals (e.g., relative atomic distances [e.g., NMR], wavelength [e.g., CD], temperature [e.g., differential scanning calorimetry], etc.).
3. The ability to quantitatively record all the separated signals with high precision and accuracy, leading to high sensitivity to differences in HOS.

After determining the type of information provided by a given technique, the following steps may be used to develop, optimize, and qualify the method to achieve its purpose. Once these steps have been completed, the method is considered fit for purpose.

1. Define the purpose(s) of the method (e.g., elucidation of structure, comparability, investigative). For elucidation of structure, absolute structural determination is important and fingerprint methods may be less useful, whereas for comparability, fingerprint methods with high precision and sensitivity may be most applicable.
2. Develop and optimize the method to best achieve its purpose. It is at this stage that a “technique” becomes a “method” in the sense that it is tailored to suit a particular product. Different methods could be derived from any given technique to suit different purposes.
3. Qualify the method to evaluate its capability, if applicable. During qualification, qualitative methods should be reduced to quantitative output, if possible. Assessment of method precision is the most important component of the qualification experiments.
4. Demonstrate that the method is capable of achieving its purpose. For some products and types of studies, a given method may not detect changes because the attribute monitored by the method does not change during the degradation process (e.g., forced stress that does not result in secondary structural changes but does alter tertiary structure). Such information should inform method selection decisions.

Fostering Outcome-Driven Partnerships

In the final section of this commentary, we address the question of how industry can partner most effectively with academia, technology companies, national laboratories, and regulatory authorities to pursue the common goal of applying robust biophysical methods at appropriate times throughput biopharmaceutical product development. All parties play an important role in achieving this goal, and increasing the overall quality of our interactions is expected to translate directly into improved outcomes. Industry brings 2 critical elements to the table: (1) access to a wide variety of therapeutic biologics in various stages of discovery and clinical development and (2) a substantial amount of collective knowledge accumulated as a result of developing the aforementioned assets.

First and foremost, industry depends on academia to produce a steady supply of capable scientists ready to take on ever more diverse and complex challenges in biologics development. The importance of the topic of educating and training the next generation of scientists is so great that it is explored at length in a companion commentary.[14] For its part, industry can do a better job articulating the myriad requirements for a successful career in biopharmaceutical development, beyond just core technical competency. The ability to partner with others to plan and execute complex tasks efficiently, manage competing priorities, and build effective internal and external professional networks are just a few examples. Industry can and should do more to cultivate these skill sets and prepare students for the transition by expanding opportunities for internships, fellowships, training sessions and short courses, and collaborative research.

Beyond access to talent, industry depends on academia for access to, and awareness of, Exploratory methods. Here the drive for novelty and fundamental understanding in academia dovetails perfectly with the additional requirement for robust, practical solutions in industry. Academia provides critical support to industry through a balance between deep first-principles understanding of the underlying biophysics and the ability to translate this knowledge into tools and technologies of immediate practical value. Industry scientists can facilitate these interactions by communicating the problems of greatest relevance. One example of successful collaborations between industry and academia is the acceptance and wide applications of hydrogen-deuterium exchange mass spectrometry for HOS characterization.[15,16] Another example of a highly effective collaborative partnership is the Biomolecular Interaction Technologies Center (http://www.bitc.unh.edu/), a graduated National Science Foundation Industry/University Cooperative Research Center where industry scientists actively partner with academic principal investigators to tailor projects to address current challenges in biologics research and development. For their part, academic scientists can foster positive disruptive change by developing their networks and searching for new applications of their colleagues' work. In some cases, the critical first step for a major breakthrough may be as simple as the question: “Have you thought about potential applications in biopharmaceutical industry?”

Translating basic research from academic science into instrument platforms is one of the crucial roles that technology companies have in supporting the biopharmaceutical industry. Industry scientists rely on technology companies to provide the hardware, software, consumables, and technical support needed to install and implement emerging methods and maintain established ones. Likewise, technology companies depend on feedback from industry to understand different use cases and the requirements of operating in a regulated environment. As in their relationships with academic researchers, industry can improve the quality of its interactions with technology companies by focusing on providing timely and actionable feedback. This will be particularly useful if industry scientists can speak with one voice. Although this may not always be practical, consensus feedback may give technology companies the relative certainty they need to make investments in platform improvements, sustainability, and research and development.

Government laboratories have the resources to build and maintain core facilities that can be used by many different groups to advance scientific excellence. Another important role that government laboratories can play is to organize and execute collaborative studies with academic and industry scientists to standardize the application of and interpretation of information from Emerging and Established technologies. In this capacity, scientists from government laboratories are in a unique position to work to drive alignment, particularly in recommending best practices for biophysical methods. Related to this is the opportunity to establish and supply a set of suitable and reliable calibration standards. Although recent years have seen a dramatic increase in this area with respect to applications in the biopharmaceutical industry,[17,18] it is clear that additional opportunities remain. Industry can assist government laboratories by participating in round robin studies and partnering with government laboratories to evaluate prototype standards, a longer-term investment key to ensuring a consistent flow of valuable information.

Finally, interactions between industry and regulatory agencies likely have the most direct and profound influence on shaping HOS strategies. Due to the length of time required to develop novel processes and products, industry scientists may have had many years of experience using a given method before the data are ultimately submitted in a regulatory filing. This can lead to a lag between the time that a method transitions from Emerging to Established and when enough data have been submitted by a sufficient number of sponsors for regulators to judge the overall value in supporting licensure. Industry should continue to strive for increased clarity in submissions and provide compelling arguments connecting the biophysical data that they provide to the questions being addressed. Likewise, regulatory authorities should continue to share knowledge and align expectations, well ahead of a licensing application. An increase in "informal" knowledge sharing in the form of seminars, workshops, and group discussions could be one path to achieving this goal. Industry could use these forums to vet new strategies and make critical course corrections, while regulators would benefit by hearing what information and arguments are likely to be submitted in future regulatory applications.

It comes as no surprise that the common thread running throughout these interactions is the need for continued communication and long-term commitment to collaboration. Meaningful communication requires focused time and effort, but with effective partnerships all parties can realize substantial gains.

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