Technical Decision-Making with Higher Order Structure Data: Starting a New Dialogue

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ABSTRACT: Characterization of the higher order structure (HOS) of biological products has been growing in importance in recent years. Scientists in the biopharmaceutical industry, academic researchers, and regulators are all increasingly aware of the critical role that HOS plays in maintaining the stability and intended biological function of biopharmaceutical products. We organized a consortium of scientists and researchers from industry and academic institutions to address how HOS data can be used most effectively to drive decisions during product development. In this commentary, we introduce the purpose, objectives, and scope of the consortium and then provide some brief points to consider in the context of characterizing HOS of biopharmaceutical products. Scientific advances in HOS analysis, as well as continued dialogue among academia, industry, and regulatory agencies will ensure that appropriate methodologies are used to inform technical decision-making during biopharmaceutical development. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:1240–1245, 2015

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INTRODUCTION

Structures and functional activities of proteins are exquisitely sensitive to their environments. Unlike chemically defined small molecule drugs, biological products assemble into well-defined, yet dynamic, ensembles of low-energy conformations that enable biological functions.[1–4] The intended structure of a protein is maintained by a delicate balance of factors, including concentration of the protein and cosolutes in the formulation, as well as the solution pH. Variations in bioprocessing can cause conformational changes, and in some cases, conformational variants may have undesirable pharmacological consequences.[5,6] Therefore, to ensure that a biopharmaceutical product functions as intended, it is critical to characterize its higher order structure (HOS)—that is, to measure structural properties of the molecule related to its native conformation.

The Growing Importance of HOS Characterization

Detailed characterization of HOS of biologics has a long history in the biopharmaceutical industry, beginning with small proteins and more recently applied to monoclonal antibodies.[7,8] New challenges have arisen with the recent advent of novel therapeutic modalities such as antibody–drug conjugates, bispecific antibodies, and fusion proteins.[9] The increasing diversity and complexity of biopharmaceutical pipelines and associated development challenges have required, and will continue to require, an increasingly diverse and sophisticated approach to HOS characterization. Furthermore, HOS characterization has begun to play a vital role in the development of biosimilars. Detailed characterization of biosimilar products and their marketed reference products, using specialized techniques, is often required to show the high degree of analytical similarity necessary for expedited regulatory approval. Overall, state-of-the-art HOS characterization is an increasingly important component of a well-developed biological product development strategy.[7,10–12]

Building an HOS Consortium

Since 2012, numerous professional societies and scientific organizations, including CASSS, IQ, AAPS, and NIST have established or sponsored working groups to study the role of HOS characterization in the biopharmaceutical industry. Capitalizing on the momentum of a growing interest in HOS, in March 2013, we began to lay the groundwork for what would become the HOS Consortium. In response to a need to identify suitable applications of HOS methods and appropriate uses of HOS data, we invited scientists from the biopharmaceutical industry and academic institutions to collaborate as a consortium with a threefold purpose: (1) to report examples of the impact of higher order structural changes on the quality and/or function of protein biologics, (2) to identify existing gaps in higher order structural analysis of biologics, and particularly (3) to address how HOS data can be used most effectively to drive decisions during biopharmaceutical product development.

CURRENT PERSPECTIVES IN THE BIOPHARMACEUTICAL INDUSTRY

Early discussions by members of the HOS Consortium highlighted both the depth and diversity of thought within the HOS

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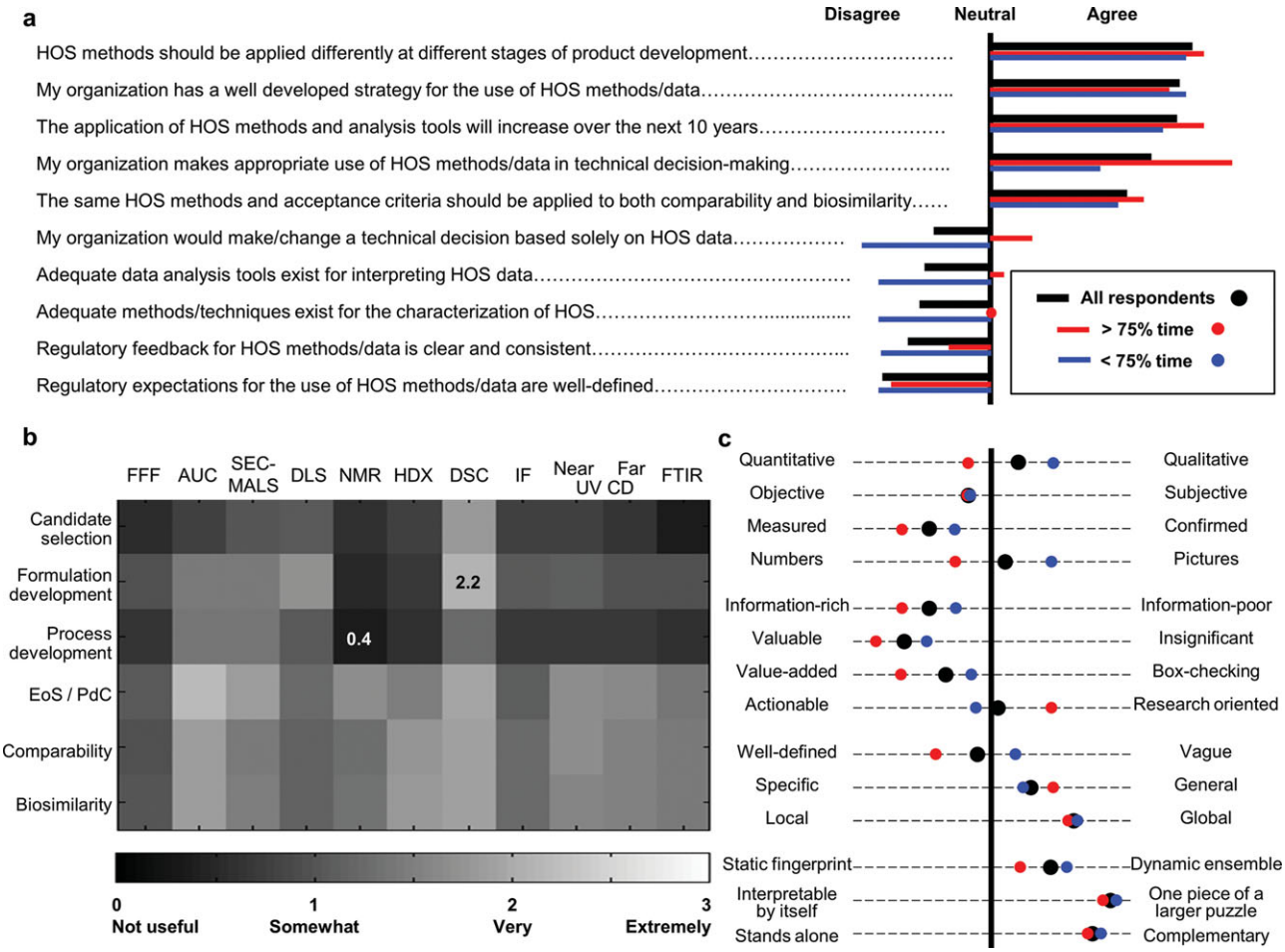

Figure 1. Summary of results of an anonymous survey related to HOS characterization during biopharmaceutical development. (a) Extent of (dis)agreement with 10 statements related to HOS characterization. The statements are rank ordered from highest overall agreement (top) to highest overall disagreement (bottom). The average result for all respondents is reported for each statement along with average results for subject matter experts ($n = 11$) and nonexperts ($n = 16$). A subject matter expert is defined as someone who reported spending more than 75% of his/her time generating, analyzing, interpreting, or otherwise using HOS data. The threshold of 75% time allocation was selected arbitrarily as a means to distinguish perspectives based on proximity to HOS data. (b) Average usefulness of a selection of HOS characterization techniques for various biopharmaceutical development activities quantitatively ranked, using grayscale, from not useful (0, black) to extremely useful (3, white). The diagram is annotated with numbers denoting maximum utility (2.2; DSC for formulation development) and minimum utility (0.4; NMR for process development). EoS, elucidation of structure; Pdc, product characterization; FFF, field flow fractionation; AUC, analytical ultracentrifugation; SEC–MALS, size-exclusion chromatography with inline multi-angle light scattering; DLS, dynamic light scattering; NMR, nuclear magnetic resonance; HDX, hydrogen–deuterium exchange; DSC, differential scanning calorimetry; IF, intrinsic fluorescence; and CD, circular dichroism. (c) Impressions of HOS. Survey respondents were asked to select between two contrasting terms that may or may not be mutually exclusive. Average results are plotted on a continuum between each pair of terms. Similar to (a), the average for all respondents is reported along with results for subject matter experts and nonexperts.

characterization community. We concluded that, to frame more targeted discussions within the Consortium, it would be useful to capture current perceptions of the use and utility of HOS tools. To this end, we designed an anonymous survey to gauge the current perspectives of practitioners in the field of HOS analysis on a number of relevant issues, including the adequacy of HOS methods, the value of HOS data when making product development decisions, and the importance of HOS data when comparing a biosimilar to an innovator product. The survey was completed by 27 individuals from the biopharmaceutical industry, with responses well-balanced between large and small companies, individuals with direct and indirect exposure to HOS data, and between those who acquire the data and those who make decisions based on the data. The results of the survey are summarized in Figure 1. It should be emphasized that this was a nonscientific poll intended only to inform the direction of the Consortium and to encourage further discussion.

In the first part of the survey, participants were asked to indicate the extent to which they agreed or disagreed with a series of statements (Fig. 1a). There was broad alignment on many topics. Most companies believe they have a well-developed and justifiable strategy for the application of HOS methods and the use of the resulting data. There is a general agreement that the use of HOS methods will increase in the future, that they should be applied in a phase-appropriate manner, and that HOS methods and acceptance criteria should be defined similarly for both comparability and biosimilarity. Likewise, there was a general consensus that regulatory feedback is not clear

and consistent, and that regulatory expectations for the use of HOS methods/data are not well defined. Areas of disagreement between apparent subject matter experts (>75% of their time using HOS methods/data) and nonexperts (<75% time) are also instructive, perhaps most notably with respect to the use of HOS data in technical decision-making. Many of those closest to the data think that their organization would make or change a decision based solely on HOS data, whereas those further from the data strongly disagree.

The second part of the survey asked participants to rank the value of a variety of HOS techniques as they relate to different development activities. Figure 1b shows the resulting matrix of average responses ranked from least (darkest) to most (lightest) useful. The use of nuclear magnetic resonance for process development had the lowest average ranking, whereas differential scanning calorimetry (DSC) for formulation development was ranked highest (most useful). Interestingly, analytical ultracentrifugation, size-exclusion chromatography (SEC) with inline multi-angle light scattering, and DSC received reasonably high marks across the entire range of development activities, whereas field flow fractionation and intrinsic fluorescence received uniformly low scores. Also noteworthy is the fact that, in general, HOS characterization techniques were considered more useful for assessing the impact of process and formulation changes (e.g., comparability and biosimilarity studies) than for developing the processes or formulations themselves.

In the final part of the survey, participants were asked to share their overall impressions of HOS characterization by choosing between two contrasting HOS terms across a series of categories. Here again, responses from experts (>75% time) and nonexperts (<75% time) are reported separately, in addition to the average of all respondents. There was a broad consensus among all respondents that HOS characterization data need to be interpreted within the context of all of the available data ("One piece of a larger puzzle" and "Complementary"). Subject matter experts regarded HOS characterization in more rigorous terms ("Quantitative," "Numbers," and "Well-defined"), whereas nonexperts favored a less rigorous description ("Qualitative," "Pictures," and "Vague"). The fact that the average impression among many of the contrasting terms was relatively close to the mid-point (e.g., "Actionable" vs. "Research oriented" and "Well-defined" vs. "Vague") leads to perhaps the most important conclusion: much work remains to better define the meaning and significance of HOS for biologics and how HOS data can best be used during biopharmaceutical product development.

POINTS TO CONSIDER

Despite many areas of a broad consensus, one of the key learnings from the survey was that there is an opportunity for more and better conversations between those closest to HOS data and those for whom there is a greater distance. As a first step, we present the following points to consider.

Speaking a Common Language

Improving the precision of our language can be as important as improving the precision of our methods. Through careful word choice when reporting and discussing HOS data, communication can be improved. One area in which precision of our terminology is important is to distinguish "methods" from "techniques." Although often used interchangeably, there is an important distinction between these terms: a technique is simply the machinery and detection required to make a measurement, whereas a method is developed, optimized, and qualified for its intended purpose to maximize the information obtained from the measurement. For example, a potentially powerful technique such as circular dichroism can be compromised by a deficient method. Furthermore, method accuracy and precision can often be improved with investment of time and money, whereas a technique may have certain fundamental limitations.

Care should also be taken to make meaningful data comparisons. On one hand, technique orthogonality can be very valuable when the same attribute is measured using two or more independent separation and/or detection modes. But sometimes an inferior technique is used in the name of orthogonality, when in fact the technique does not measure the intended attribute via independent means. On the other hand, comparing capabilities of different techniques that measure different attributes are rarely useful. For example, comparing the sensitivity and resolution of bulk spectroscopy to any type of separation method does not lead to a meaningful comparison (without the column, IEX, and SEC yield nothing more than protein concentration).

Although using precise language and making appropriate data comparisons can be straightforward, it is difficult to precisely define the meaning and scope of HOS because of its complexity, nuance, and richness. At its simplest, HOS is a structural hierarchy comprising secondary, tertiary, and in some cases, quaternary structure, which together define the state (and enable the activity) of a biologic product. This serves as a working definition, but the use of the term HOS may be much broader in practice, including concepts such as an ensemble of different structures that may exist at any moment (because of dynamic structural rearrangements that occur across a wide range of time scales), the thermodynamic stability of folded domains of a protein, and even a noncovalent self-association. The lack of precise definition is understandable given the varying impressions of HOS, as depicted in Figure 1c. Considering this, we believe it is more important to establish how HOS methods and data are used then to define strict boundaries for the term.

For the purposes of the Consortium, it was useful to frame the scope of HOS in the context of a molecular assembly. The foundational assembly layer (primary structure and posttranslational modifications) is therefore outside the scope of HOS. Higher structural assemblies, beginning with the formation of secondary structures, are within the scope of an HOS, but it is not entirely clear where to define the upper boundary of structural assembly. To keep the scope manageable, we deliberately exclude aggregates and particles from our definition of HOS for two reasons. First, in most cases, aggregates and particles are not the intended, fully active form of a biopharmaceutical product, but are instead product-related impurities that manufacturers seek to minimize. Second, industry perspectives on aggregates and particles in protein products have been discussed at length in a previous commentary.[13]

The Business of HOS: Uncertainty and Risk Tolerance

The ultimate goal for HOS characterization in biopharmaceutical development is to support the efficient delivery of safe

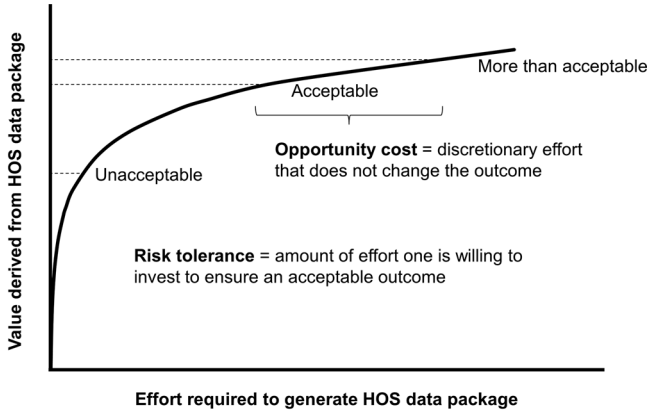

Figure 2. Illustration of a hypothetical relationship between the effort required to generate an HOS data package and the resulting value of the data. The diagram highlights the concepts of risk tolerance and opportunity cost as they relate to HOS characterization during biopharmaceutical development.

and effective treatments to patients. This becomes the starting point from which the nature and depth of HOS characterization work is defined. The business of HOS is in deciding what extent of characterization is required for a given molecular entity at any point in development, which in turn is governed by each company's understanding of uncertainty and its tolerance for risk. We propose a thought experiment about the relationship between the effort (resources) required to generate an HOS data package and the resulting (admittedly qualitative) value of the data. In this context, "value" could be the ability to make a sound technical decision quickly, the strength of a regulatory submission, or progress toward any other product development goal. In Figure 2, the company faces a situation of diminishing returns, where substantial excess effort results in only marginal benefit. Here, we define opportunity cost as the discretionary effort that does not change the outcome. This is the effort that could have been applied with greater impact to other development activities. In this framework, risk tolerance is quantified as the amount of effort one is willing to invest to ensure an acceptable outcome because of uncertainty around the actual shape of the curve, that is, where "acceptable" really lies.

Although there are a number of factors that potentially contribute to this uncertainty for HOS characterization, as indicated in the responses to the survey, the evolution of regulatory expectations is one of the most prominent. A detailed review of guidance documents authored by world health authorities is beyond the scope of this work (refer to Refs.[6,10,14–20]); however, it is worth noting that from 1996 to 2012, HOS characterization went from being largely a footnote to a position of some prominence in regulatory guidance. The advent of regulatory approval pathways for biosimilar products ushers in a new set of challenges for the industry, as analytical data requirements continue to be defined in many jurisdictions around the world.[7,10–12] Defining the data requirements for HOS characterization of biosimilar protein products remains a significant challenge. As sponsors and regulators work through this challenge, the concepts of data necessity and sufficiency should regularly be considered when HOS data are collected and used to aid in making technical decisions.

Despite the increased emphasis on the role of HOS characterization during the development of biologics, the FDA has indicated in recent years that very little of what is known about HOS of proteins is applied in submissions to the agency.[21,22] Given that most companies believe that they have "well-developed strategy for the use of HOS methods/data" (refer to Fig. 1), this clearly indicates an opportunity for continuing a dialogue between sponsors and health authorities. Although it seems that most companies and regulatory bodies agree that the data should drive decisions, there is a significant opportunity to discuss precisely what data should be collected and how these should be used. Ultimately, the responsibility to ensure the quality of biopharmaceutical products rests jointly on the developer/manufacturer and the regulator. Guidance and feedback that is timely, relevant, informative, and actionable without being overly prescriptive or restrictive will benefit all parties.

New and emerging technologies also contribute to uncertainty. The rapid pace of technology development and commercialization means that the current state-of-the-art may be considered routine or dated in a few years; likewise, an acceptable HOS data package today might be deficient in the future. Often the evolution of technology is steady and predictable, but sometimes it is unexpected and revolutionary. The greatest impact comes from so-called "disruptive" technologies, where major advances fundamentally change the way biopharmaceutical products are developed. A future example may come from computational modeling and simulation, which hold the promise of interrogating HOS at length and time scales that may never be accessible by empirical methods.

It is important to note that the concept of new and emerging technologies is not limited to new instruments, but extends to data analysis, interpretation, and visualization as well. As existing techniques are converted to high-throughput formats, the potential exists to generate volumes of data that were simply inaccessible in the past. Turning data into information and information into decisions will remain a central challenge for HOS characterization. As new HOS tools and techniques are implemented across the industry, subject matter experts can and will disagree about what methodologies add value, and in what context. We suggest that there is a shared responsibility among vendors, health authorities, government laboratories, academia, and industry for inventing, developing, and evaluating new technologies to speed the delivery of safe and effective products to the market.

STARTING THE DIALOGUE: CASE STUDIES FROM INDUSTRY

As a first step toward starting a new dialogue, we asked participants of the HOS Consortium to write case studies describing how HOS data are currently used to drive decisions at various points in the development lifecycle. Four of these case studies are published together with this commentary in this issue of the *Journal of Pharmaceutical Sciences*.[23–26] One reports the use of HOS methods to select one protein molecule from two potential candidates that bind to the same biological target.[26] Another describes the use of HOS methods to characterize the conformational stability of a mAb in different solution environments prior to finalizing a commercial formulation.[25] A third case study shows the value of HOS methods for purification

process development, elucidating the impacts of specific binding of a nonionic surfactant to a mAb.[24] Finally, a fourth case study demonstrates the role of HOS in decreased stability and bioactivity of a biopharmaceutical product following oxidation.[23] Scenarios such as these, and many others, dictate the use of analytical methods to probe various aspects of HOS, yet the particular techniques applied, the degree of characterization, and the way the data are used often vary based on the stage of development and experimental purpose. It is our hope that additional case studies will follow, providing further insight into how the industry uses HOS methods to inform technical decision-making and yielding additional substrate for valuable conversations.

A CALL TO ACTION

We believe that the success of the HOS Consortium can be measured by the extent to which it contributes to an improvement in the application of HOS methods and data during the development of biologics. We anticipate continued growth in the field of HOS analysis. As the field evolves, contributions from academia, industry, and regulatory agencies will continue to drive advances in the application of well-developed HOS methods and their appropriate application. A thoughtful and measured response from all stakeholders will be necessary to enable the end state we all hope to achieve, that is, an appropriate use of HOS methods and data to make informed biopharmaceutical product development decisions.

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