

## **Table 1: Common Practices and Workflows in Protein HOS Characterization**

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### **SCOPE:**

Protein higher order structure (HOS) is a complex and crucial problem in the discovery and development pipelines of biotherapeutic companies. An impressive and constantly expanding suite of tools for the characterization of HOS enables the gathering of an impressive array of information, but no tool is suitable for answering every HOS question in every protein system. This roundtable will focus on discussing the suite of modern tools for protein HOS characterization at a general level of utility, figures of merit and limitations. Participants will discuss their common workflows for initial HOS characterization of protein targets. Considerations in selecting particular methods of HOS characterization and common modes of failure they experience in popular HOS analysis methods will be discussed. Finally, we will discuss unmet needs in HOS analysis, and potential areas for innovation that remain.

### **QUESTIONS FOR DISCUSSION:**

1. What are the main technique(s) you use for HOS characterization? What are the advantages and disadvantages of these methods?
2. Is your typical HOS characterization process hypothesis-driven, or discovery-based? Do you explore HOS to answer specific questions regarding protein stability and function, or as part of a general characterization strategy?
3. At the end of your typical HOS characterization process, what important questions do you usually answer? What important questions do you sometimes find unanswered?
4. Are there particular use cases where your usual suite of biophysical tools give incomplete or unsatisfactory answers?
5. What are the major limitations that hinder your HOS characterization process? Are you limited primarily by resources, or by a lack of useful technology?

### **DISCUSSION NOTES:**

- Choice of the methods of characterization based on the sensitivity, aspects to be addressed, specific systems.
- Limiting factors for the design of the workflow: budget, management, available technology
- Design of the workflow for HOS characterization: company *vs* academia
- Matching between the experimental conditions needed for the characterization and the “real” physical environment in which the HOS works.
- Choice of the approach depending on the “resistance” of the HOS to the approach (e.g. radiation damage in synchrotron-based approaches)
- Grounds for investment in method development: company *vs* academia