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Perspective

# Using X-Ray Crystallography to Simplify and Accelerate Biologics Drug Development

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ABSTRACT

Every major biopharmaceutical company incorporates a protein crystallography unit that is central to its structure-based drug discovery efforts. Yet these capabilities are rarely leveraged toward the formal higher order structural characterization that is so challenging but integral to large-scale biologics manufacturing. Although the biotech industry laments the shortcomings of its favored biophysical techniques, x-ray crystallography is not even considered for drug development. Why not? We suggest that this is due, at least in part, to outdated thinking (for a recent industry-wide survey, see Gabrielson JP, Weiss IV WF. Technical decision-making with higher order structure data: starting a new dialogue. *J Pharm Sci*. 2015;104(4):1240-1245). We examine some myths surrounding protein crystallography and highlight the inherent properties of protein crystals (molecular identity, biochemical purity, conformational uniformity, and macromolecular crowding) as having practicable commonalities with today's patient-focused liquid drug products. In the new millennium, protein crystallography has become essentially a routine analytical test. Its application may aid the identification of better candidate molecules that are more amenable to high-concentration processing, formulation, and analysis thereby helping to make biologics drug development quicker, simpler, and cheaper.


"If at first the idea is not absurd, then there is no hope for it."

—Albert Einstein

## Introduction

The biopharmaceutical research and development process is organized into 2 primary foci: Discovery and Development. The former focuses on finding promising molecules to advance into clinical trials. Then, once the candidate molecule has been selected, the Development organization is responsible for producing material at large scale for the extensive studies in humans to evaluate whether it is safe and effective. Development includes defining and formulating a drug product for administration to patients and establishing the manufacturing processes and analytical controls in accordance with regulatory requirements and current good manufacturing practices (cGMPs). These efforts assure quality and consistency pertaining to safety, identity, strength, and purity. Today, high-throughput protein crystallography plays a central role in drug discovery and is leveraged extensively for small molecule drug candidate screening, vaccine epitope mapping, target identification, and structure-based design of therapeutic proteins.[1-3] Here we present a perspective suggesting that advances in technology and methodology now make protein x-ray crystallography eminently suited to Development applications of complex biologics. This opens new opportunities to exploit the special properties of protein crystals for candidate selection, processing, and product characterization, noting that protein behavior essential to forming good crystals coincides substantially with attributes sought for bioproduct development (Table 1).

### *The High Cost and Complexity of Biopharmaceutical Development*

Because of their size, structural heterogeneity, and delicately intricate higher order structures (HOSs), developing and manufacturing biologic drugs is significantly more challenging and costly than small molecule pharmaceuticals. These biochemical

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**Table 1**
Desired Attributes of Biologics Drug Candidates and Commonalities With Protein Crystallization

| Physicochemical Property | Pharmaceutical Developability | Crystallization |
|---|---|---|
| High solubility | Enhances colloidal stability and enables high-concentration injectable liquid formulations | Contrary to earlier belief, highest quality crystals grow from solution conditions of good solubility[4,5] |
| High purity | Purity is fundamental to the safety and quality of biologics products. Impurities may be extrinsic or intrinsic and the impurity profile is typically highly process dependent | Purity and homogeneity are precepts fundamental to protein crystallization. Even relatively small amounts of contaminant may induce formation of aggregates, alter macromolecular solubility, or interfere with nucleation and crystal growth mechanisms[6] |
| Monodispersity | Simplifies analysis and processing by reducing concentration-dependent effects on physical, analytical, and pharmaceutical properties. Reversibly self-associating systems may be prone to higher viscosity[7,8] | Strongly favors high-quality crystal formation. Conditions that sustain continued, ordered addition of single units to surfaces of the developing crystal are critical[9] |
| High conformational stability and uniformity | Stability of HOS is critical to retaining bioactivity and also to resisting degradation pathways mediated by partially unfolded conformations | Crystals grow best when the protein possesses high conformational uniformity.[10] Rigid molecules are easier to crystallize and form better diffracting crystals |
| Good long-term stability in solution at 5°C and room temperature | Critical to product storage, shipping, and in-use stability. Pharmaceutical stability pertains to both chemical and physical degradation pathways | Minimal degradation in solution during crystallization avoids self-poisoning and favors formation of high-quality, strongly diffracting crystals |
| Low propensity for aggregation<br>Low propensity for forming subvisible particles | Aggregates and particles may enhance immunogenicity and reduce bioactivity in serious and unpredictable ways[11,12] | Aggregates inhibit crystallization by causing lattice defects and growth cessation. Proteins crystallize best under solution conditions where aggregates and particles are minimized[13,14] |
| High stability with respect to fragmentation and chemical degradation | Critical to retaining shelf-life stability and bioactivity | Fragments and chemical variants that are structurally similar to the host macromolecule can be particularly deleterious to crystal lattice formation and growth[15] |

complexities dictate that a quality control strategy centered on characterizing the final drug product is insufficient to ensure therapeutic safety and efficacy. The quality and consistency of protein drugs cannot be assured by analytical tests for covalent structure alone; therefore, functional bioassays become another costly element essential to the development and licensure of biologics drugs. The entire manufacturing process from cell culture to drug product fill-finish must be defined, understood, and controlled with respect to potential impact on the quality attributes of the therapeutic product. This requirement translates to an exhaustive series of formal unit operations, critical processing parameters, proven acceptable ranges, acceptance limits, and specifications, which are supported by extensive studies examining how molecular attributes and processing conditions connect with product safety and efficacy. With small molecule pharmaceuticals, the product is approved, whereas with biologics drugs, not only the product but also the entire manufacturing process must receive regulatory approval from the health authorities.

Estimating the cost of developing drugs is a controversial topic, but one recent analysis puts the average pre-tax industry cost per new prescription drug approval, inclusive of failures and capital costs, at $2.6 billion.[16] Construction costs of large-scale biotech-manufacturing facilities are typically in the $200-$500 million range and require 4- to 5-year lead times (compared to just ~$30-$100 million for similar-scale small-molecule facilities). Certainly, the high cost of discovering and developing a drug is a major impediment to getting innovative new therapies to patients in need. To reduce the cost of biologics drugs, the industry needs to leverage new technologies to find better, quicker, and smarter ways of developing them. Strategies to diminish the high attrition rate, accelerate first human dose, and simplify subsequent characterization and control upon cGMP scale-up—without compromising safety and quality—are key elements of achieving this goal.

### *Could Biopharmaceutical Development Become More Small-Molecule-Like?*

If protein crystallization of a specific crystal polymorph could be used as a processing step to constrain heterogeneity, and if x-ray crystallography could be applied routinely to provide an atomic resolution characterization of drug substance and drug product directly, then perhaps biologics drug development could take a step toward becoming more analogous to small molecule pharmaceutical development. The following problem statements highlight particular challenges with biologics development that crystallization and x-ray crystallography may help to address:

#### *Engineering and Selection of a Drug Candidate: There Is a Lack of Practicable Research to Development Transition Criteria for Candidate Developability*

Typically, in biopharmaceutical R&D, the Development organization will interface with late Discovery to help identify a candidate molecule that possesses physicochemical characteristics suitable for pharmaceutical product development, large-scale manufacture, and delivery via a suitably patient-friendly device. Key attributes desired in a candidate molecule are described in Table 1. However, a major challenge with the concept of developability is that current technology and know-how does not enable the Discovery organization to measure and manipulate these attributes systematically with the small quantities of material available when they have the opportunity to optimize the protein sequence for solubility and stability. Further complicating matters, the Development organization is quite limited in its ability to make reliable accelerated predictions of whether a given candidate molecule will fulfill the criteria of Table 1 until it has actually tried to develop the molecule. This sets up a Discovery-Development Catch-22 that hampers the entire industry. There is currently an unmet need for high-throughput methods that can simultaneously predict favorable conformational and colloidal characteristics (especially for high-concentration products) that can be applied rapidly with just a few milligrams of protein. Because there are fundamental commonalities between protein crystallizability and pharmaceutical developability, it may be possible to use existing automated crystallization screening technologies to gain pertinent insights into molecule developability much earlier in the Discovery-to-Development transition.

#### *Drug Substance Heterogeneity and Batch-To-Batch Inconsistency: Less Desirable Isoforms Diminish Therapeutic Quality*

Certain isoform subpopulations are known to be therapeutically inferior due to decreased potency, shorter duration of action,