Improving the Developability of Biopharmaceuticals

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The combination of computational and protein engineering tools with novel orthogonal methods of measuring aggregation can be used to assess the developability of biopharm products in the early stages of development, thereby reducing the level of risk in later stages.

Biopharmaceutical development is marred by multiple risks. High levels of attrition during preclinical and clinical development are posing a significant challenge, and pushing drug development costs to levels that are no longer sustainable. Diverse strategies for failing early and cheaply are currently being explored, with an emphasis on translational medicine, predictive technologies, scale-down models and further up-front product characterisation. One of these approaches involves the incorporation of a developability risk assessment – focusing on manufacturability and safety – in the early phases of development, in order to help with the selection and design of products with the right quality attributes. One such attribute is aggregation, which constitutes a significant hurdle for biopharmaceutical development and a potential risk of increased immunogenicity. In this article, we discuss how protein-engineering approaches based on predictive computational methods, together with early analytics, can be utilised to select candidates with enhanced developability. We also provide examples of new orthogonal strategies to assess aggregation and stability at an early stage.

CHALLENGES IN THERAPEUTIC DEVELOPMENT

For every hundred therapeutic candidates that enter the clinic, fewer than 10 will ever be registered. This high attrition rate – particularly acute in late phases of clinical development – is pushing the costs of drug development to levels higher than ever before, currently approaching US$2 billion on average. Causes for attrition are often very diverse and range from insufficient therapeutic efficacy to poor bioavailability, safety concerns (toxicology, immunogenicity), formulation problems or high development costs (1). The current situation is posing a significant challenge to the development of novel therapies and brings into question the blockbuster model as a viable option for the pharmaceutical industry.

As a result, there is an urgent need for methodologies that will facilitate the assessment of potential therapeutic efficacy and identification of development risks in a more flexible, rapid and inexpensive way. This has motivated the adoption of a number of strategies as an alternative to traditional lengthy and expensive clinical development pathways – such as the application of translational medicine – to obtain rapid proof of concept in patients, and the identification of suitable biomarkers as surrogate endpoints of clinical efficacy. In addition, the development of pharmacogenomics and companion diagnostics in the definition of patient groups, or the utilisation of microdosing and Phase 0 studies, to achieve rapid information on efficacy and safety, are also actively pursued (2,3).

IMPLEMENTING AN EARLY RISK ASSESSMENT

One other tool growing in importance in drug development is the assessment of 'developability'; this looks into aspects related to the manufacturing, stability and safety of therapeutic candidates that could jeopardise their success during preclinical and clinical development. Developability assessment can be used to identify development risks as well as to incorporate critical quality attributes (CQAs) early on in the design of therapeutics, and it is bringing a rejuvenated interest in establishing better links between discovery and development stages. The discovery phase tends now to move beyond maximising biological activity and also looks specifically into aspects of manufacturability and developability (productivity, aggregation, stability, solubility, viscosity) and safety (primarily immunogenicity) (4-6). The ultimate goal of such approaches is to assess critical properties in the product at a very early stage – ideally allowing the ranking of different candidates based not only on their biological activity, but also their developability readiness. A number of initiatives have been put forward that emphasise the need for implementation of

predictive technologies, scale-down models and further up-front characterisation methods in therapeutic development, with special attention to *in silico* methodologies (7,8).

ENGINEERING DEVELOPABILITY INTO A PRODUCT

In silico protein characterisation can be used to identify potential developability and safety risks in biopharmaceuticals, and to 'design in' desirable properties. Aggregation and low stability are two specific problems imposing severe restraints in the manufacture and development of biopharmaceuticals, and potentially contribute to an increased risk of undesired immune responses in patients (9-11). Predictive computational tools can be used to identify structural or sequence elements that could potentially compromise the stability and developability of a given biopharmaceutical. Such potentially deleterious elements can then be eliminated by protein engineering, resulting in molecules with improved manufacturing and development properties (12,13).

Examples of such an approach include the application of computational tools to re-engineer antibody molecules with reduced aggregation, improved stability and increased productivity, as shown in Figure 1. Other complementary platforms also aimed at improving the developability and safety of biopharmaceuticals include T-cell epitope mapping and preclinical immunogenicity risk assessment, and make use of either computational or *in vitro* methodologies (14). Such technologies are examples of new and powerful strategies that can be applied to mitigate development and safety risks at the beginning of drug development, reducing the probability of encountering problems that could have very significant negative financial impacts in later development stages.

AGGREGATION ASSESSMENT METHODS

Monitoring stability and aggregation in biopharmaceuticals is not an obvious task. The main challenge comes from the fact that aggregation can occur at very different stages in the biomanufacturing and development processes, and can also manifest itself in very different ways, including low host viability, low productivity, the presence of inclusion bodies and the development of opalescent solutions or precipitates. Aggregation is not only influenced by the polypeptide sequence itself, but also by the nature of the host and process used for its production, the final formulation and a history of the batch preparation, storage and fill-finish conditions.

Size-exclusion chromatography (SE-HPLC) is perhaps the most widely utilised standard analytical technology for assessing the degree of aggregation in a given preparation. SE-HPLC is straightforward to implement, extraordinarily robust, and easy to transfer across different laboratories. However, there are two main limitations linked to the use of SE-HPLC in early analytics. The first relates to the fact that SE-HPLC can only accurately assess the level of aggregates within a limited window-size corresponding to the small oligomers that can be included in the column. This means that SE-HPLC misses other types of aggregates, including sub-visible particles, and may in some cases not be predictive enough to describe the overall stability of a polypeptide in solution. The second major limitation of SE-HPLC is its still relatively low throughput, which limits its applicability as a broad screening tool during lead selection or in the early phases of

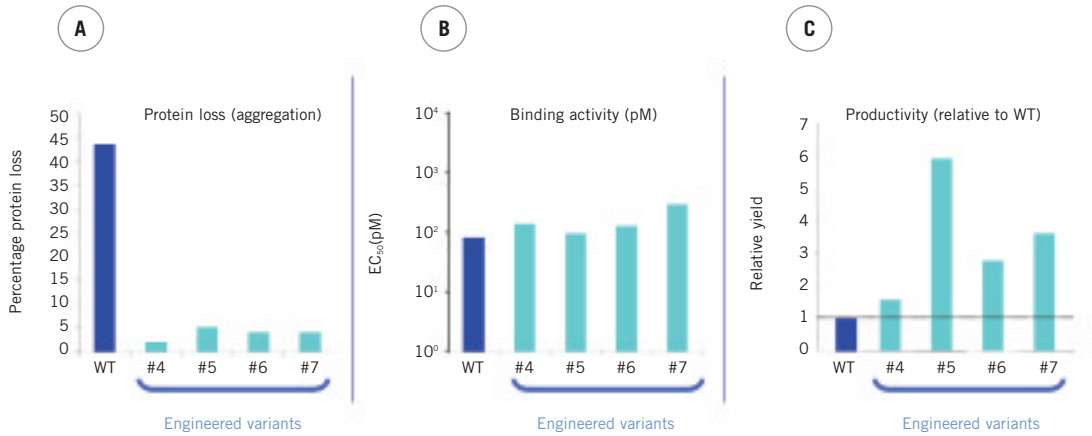

Figure 1: Engineered antibodies with enhanced developability using Lonza's AggreSolve™ platform. The graph shows the relative performance of engineered variants compared with parental wild type antibody. A) Accelerated stability at 60°C; B) Binding activity of engineered versus wild type antibody molecules; and C) Relative productivity of variants compared with wild type sequence assessed in stable pooled transfections.

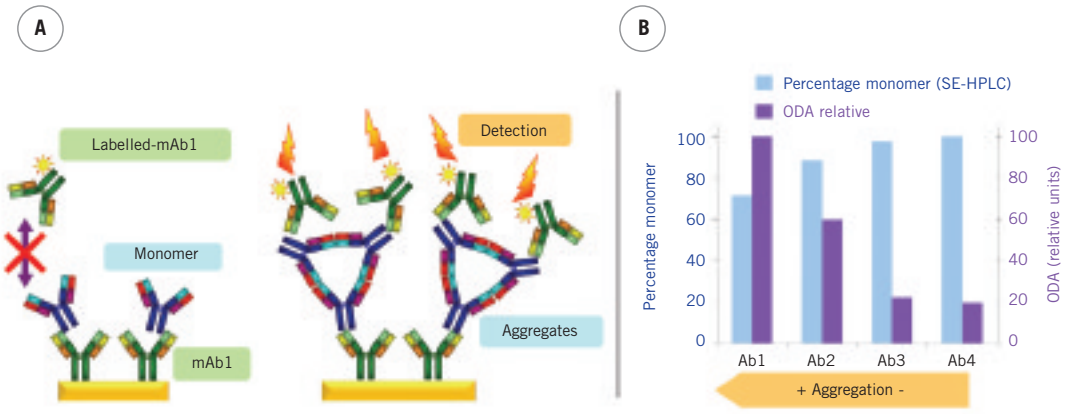

Figure 2: A) ODA measures the relative abundance of secondary-binding sites in a given polypeptide (such as an antibody). Monomeric polypeptides only display a single binding site that is covered by the capture antibody. However, oligomers exhibit on their surface multiple additional binding sites that are susceptible to being recognised by the labelled antibody. Detection can be done directly or via a conjugated molecule (such as streptavidin-HRP). B) Comparison of the aggregation assessment of a collection of different monoclonal antibodies with SE-HPLC and ODA. Percentage monomer is calculated as fraction of total protein recovered as monomer. Higher ODA values correlate with lower monomer levels in the preparation.

development. To compensate for the former, a broad range of orthogonal technologies are often used, such as dynamic light scattering (DLS), analytical ultra-centrifugation (UAC), Raman/infrared spectroscopy, particle counting and dye-binding assays (such as Thioflavin, ANS, Nile-red, Congo-red). Still, many of these technologies show limitations of applicability, can be cumbersome to implement or have a relatively low throughput.

Oligomer Detection Assay (ODA)

One alternative to such aggregation-assessment methodologies is the 'oligomer detection assay' (ODA). The ODA is an immunological ELISA-based assay, with relatively high sensitivity and throughput, which is amenable to automation and very simple to implement. It requires a monoclonal antibody (or another binding polypeptide or reagent) recognising a specific and unique epitope in the molecule of interest, which could be any biopharmaceutical (such as a therapeutic antibody). This monoclonal antibody is used both for capturing the biopharmaceutical of interest and (as a labelled version, that is, biotinylated) as a detection reagent. Because of the single-valence of the epitope in the target biopharmaceutical, aggregates made of two or more molecules will likely display epitopes accessible to the labelled antibody, giving rise to a detectable signal (see scheme in Figure 2). ODA is a semi-quantitative method that allows direct comparisons to be drawn within a given set of samples, or in relation to a group of reference samples of known properties, producing a 'better than' or 'best of' outcome.

Novel orthogonal methods like ODA and QCM can provide viable alternatives to SE-HPLC for monitoring aggregation in biotherapeutics. Technologies such as QCM offer sufficient flexibility to allow the assessment of multiple parameters relevant to biopharmaceutical development. The ability to measure protein yield, binding affinity or aggregation in a single sample using a single platform provides a very attractive method for rapid and simple characterisation of multiple candidates.

Quartz Crystal Microbalance (QCM) Methods

As an independent approach, we have explored the use of QCM methods to assess aggregation in proteins. A biosensor based on QCM technology can be used to study molecular interactions. The technology uses a quartz crystal, oscillating at its resonance frequency. The resonance frequency is related to the mass of the crystal; hence, when molecules bind or dissociate from the crystal they will cause a change in its resonance frequency. QCM is widely used to characterise protein interactions in pure or complex mixtures, or even cell surfaces. Some examples of its applications include titration and quantification of protein levels, or the determination of binding kinetics and affinity constants, even on cell surfaces (15,16). Monitoring this frequency shift provides detailed information that can be used to understand biochemical characteristics of the molecules and their interactions (see Figure 3, page 38).

Three different, straightforward assays to study aggregation with the use of the QCM biosensor are illustrated in Figure 4 (page 38). The first assay, 'off-rate', considers the dissociation rate; the more aggregated the

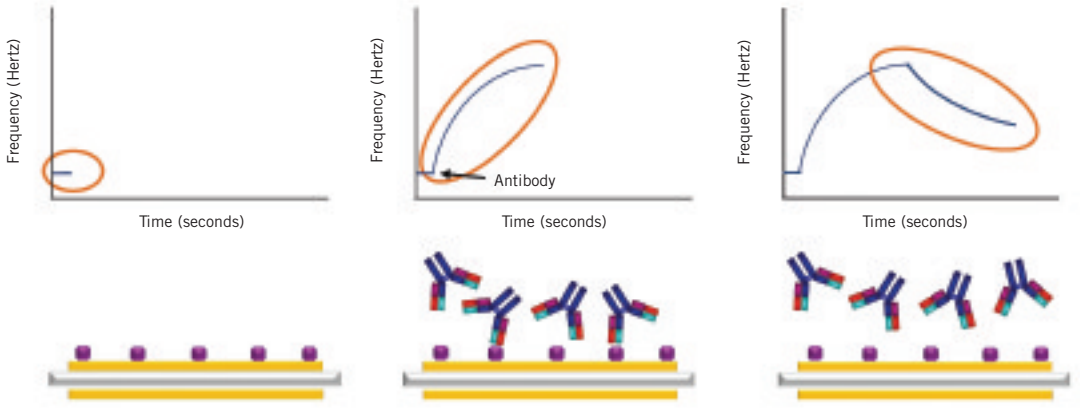

Figure 3: The Attana biosensor measures the frequency of a gold-layered quartz crystal covered with, for example, an antigen. Antibodies (or other targets) are injected into the system. As the mass on the surface changes, variations in resonance frequency are registered in real time. From the frequency shift measured, detailed characteristics of the molecular interactions involved can be deduced.

molecules are, the slower the dissociation from the crystal, due to higher avidity and rebinding. In the second assay, 'secondary antibody', the sample molecule is captured on the surface. If a high aggregate content is present in the preparation, there is an increase in the number of epitopes that are available for a secondary antibody to bind, and thus the resulting resonance signal is also higher. The third assay, 'capture', looks at the signal amplitude generated by the sample when captured on the surface of the crystal when saturation is reached. The more aggregated the sample is, the higher the amplitude of the observed signal.

TOWARDS AN INTEGRATED DEVELOPABILITY ASSESSMENT

Recent technological developments in the areas of computational biology and analytics are providing attractive ways of identifying development and safety risks very early on, opening the door to better ways of selecting and designing optimal lead candidates to be taken into clinical development. *In silico* methodologies can be utilised to recognise sequence and structural elements behind poor stability and high aggregation, and modify them to generate improved biopharmaceuticals. In addition, novel orthogonal methods like ODA and QCM can provide viable alternatives to SE-HPLC for monitoring aggregation in biotherapeutics. Technologies such as QCM offer sufficient flexibility to allow the assessment of multiple parameters relevant to biopharmaceutical development. The ability to measure protein yield, binding affinity or aggregation in a single sample using a single platform provides a very attractive method for rapid and simple characterisation of multiple candidates. Further integration of computational (predictive) methods with new analytical tools will provide detailed developability fingerprints in the discovery stages for many different therapeutic candidates. We expect that such information will help to streamline the manufacturing

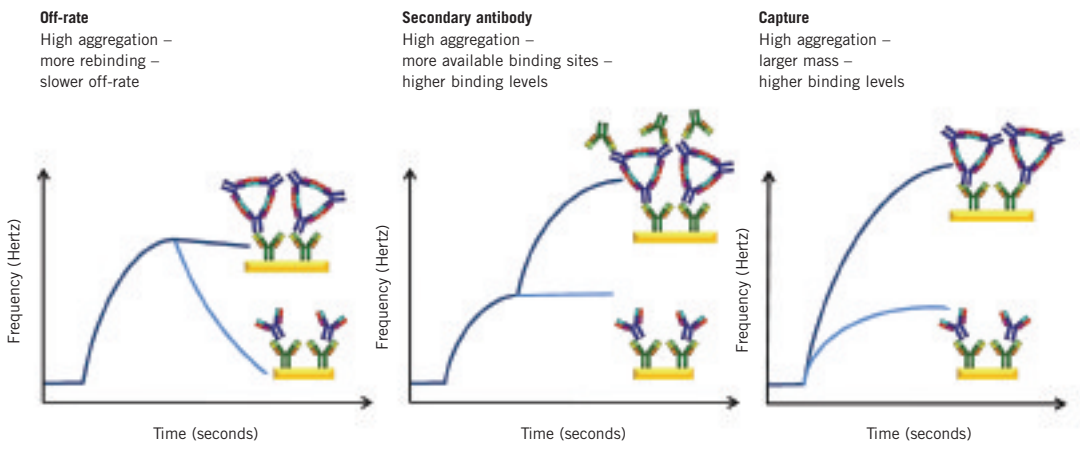

Figure 4: Aggregation characterisation of proteins and antibodies: A) Off-rate (dissociation rate) characterisation; B) Secondary antibody binding; and C) Capture saturation.

and development of novel biotherapeutics, reduce the level of risk involved in selecting lead candidates and contribute towards reducing attrition.

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