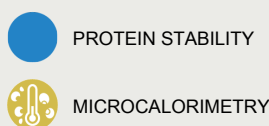


Engineering monoclonal antibodies to enhance drugability: A case study

Written by Chul-Ho Park, Ph.D. President & CEO; MabPrex, Inc. 6727 Flanders Dr. Suite 212; San Diego, CA 92121 cpark@mabprex.com



Introduction:

Whenever a biotherapeutic protein, like a monoclonal antibody, is engineered and expressed in a recombinant system, it is important that the engineered protein maintains its desired potency, efficacy, stability, and solubility. During the engineering process, various protein characteristics are analyzed and optimized, such as:

- Improved potency
- Improving solubility and downstream process recovery
- Improving thermostability
- Reducing/eliminating potential chemical modification sites
- Reducing aggregation during storage

At Applied Molecular Evolution, protein engineering is performed by directed evolution technology (Figure 1) which permits simultaneous optimization of multiple protein properties. The screening method, rather than the traditional selection method, can solve more complex problems, and captures more structure/function data.

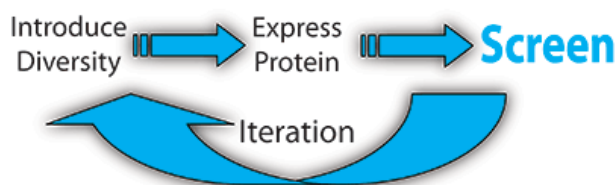


Figure 1: AME approach to directed evolution for protein engineering.

One of analytical/physicochemical methods employed during the course of protein engineering at AME is Differential Scanning Calorimetry (DSC) to compare the thermostability of parent and engineered antibodies. DSC is commonly employed to assess the thermal and conformational stability of a protein. The

melting temperature of a protein, or of individual domains, can be obtained from the DSC profile, and if the reaction is reversible the thermodynamic parameters of the unfolding can be determined. A protein is considered more thermostable when the T_m (transition temperature) is higher. When thermostability data are compared to other bioanalytical methods, like size exclusion chromatography (SEC), others have observed a correlation between greater thermostability (higher T_m) and decreased aggregation (From data presented by at the 2007 Trends in Calorimetry Conference). Antibodies with a higher T_m and decreased aggregation formation have the potential for improved long-term stability thus potentially making better biotherapeutics.

In this manuscript we compare the stability of three different engineered antibodies as determined by SEC-HPLC analysis during accelerated stability studies, with the stability predicted by DSC. The results demonstrate a correlation between decreased thermostability based on DSC data, and greater aggregation formation during accelerated stability studies for the antibodies studied. Thus, the results demonstrate how DSC can be a useful tool in stability screening of engineered proteins.

Materials and Methods:

Antibody samples were dialyzed against 10 mM citrate buffer at pH 6.5 and diluted to 1 mg/mL. Scans were performed using an automated MicroCal VP-Capillary DSC equipped with a 96-well plate autosampler. Scans ran from 20-95°C at 200°C/h and were analyzed using Origin 7.0. The unfolding transitions of each antibody were fit using the non-two-state unfolding model.

For accelerated stability study, the same set of antibody samples described above was stored at 60°C for up to 5 days. Aliquots were withdrawn every day and centrifuged at 15,000 g for 20 minutes to remove precipitated antibodies. The supernatant was used for determining soluble protein concentration and the percentage of soluble high molecular weight aggregates. Analytical size-exclusion chromatography (SEC) was performed using a Tosoh G3000SWXL (300 X 4.6 mm) column at a flow rate of 0.5 mL/min.

Case Study: Characterization of Parent and Engineered Antibodies by DSC

While the functional and biological properties of antibodies are well known, little has been published concerning the folding and stability of the many Ig-Fold domains of antibodies within the context of the full-length molecule. DSC can be used to investigate multi-domain folding thermodynamics to guide antibody design. In this case study, the parent antibody that was used as the starting point for engineering has four distinct unfolding domains in the DSC thermogram (Figure 2A). Each unfolding domain has a measurable transition temperature (T_m1 to T_m4). T_m1 was monitored for thermostability for this protein. For the parent protein T_m1 was approximately 69 °C (Figure 2A).

In this study, antibodies were being engineered for improved potency, and after the first round of screening, we chose Antibody 1 for further characterization in terms of biophysical properties. DSC data showed that T_m1 of Antibody 1 was approximately 62 °C (Figure 2B), or 7 degrees lower when compared to the T_m1 of the parent antibody, suggested that Antibody 1 had a decreased thermostability relative to the parent protein.

A second round of engineering and screening, resulted in Antibody 2. This particular antibody has T_m1 of about 69 °C, shifting up about 7 degrees relative to T_m1 for Engineered Antibody 1, and having a T_m comparable to the parent protein (Figure 2C). This result suggests that Antibody 2 have comparable thermostability, compared to parent antibody. Since proteins with greater thermostability may be more stable under accelerated stability conditions, a potential advantage is a longer shelf life of the biotherapeutic.

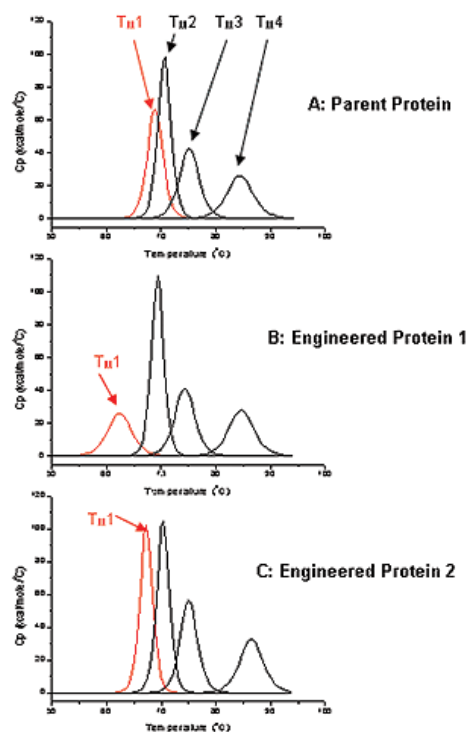


Figure 2: Deconvoluted DSC thermograms for A: Parent Antibody, B: Engineered Antibody 1, C: Engineered Antibody 2. First transition (T_{m1}) for each antibody is in red, the other 3 transitions are black.

Since the parent and engineered antibodies showed comparable stability at 37°C (data not shown), we chose to perform accelerated stability studies at 60°C, which was close to T_{m1} of Antibody 1. Figure 3 shows the percent recovery of soluble protein after storage at 60°C for indicated number of days. Parent and Antibody 2 were both very stable at 60 °C, and had comparable protein recovery. However, Antibody 1 has a significant decrease in recovery after five days of storage at 60 °C. The stability predictions made by DSC of the three antibodies tested correlate well with measurements of percent recovery of soluble protein from accelerated stability studies. In comparison, since DSC forces the unfolding event(s), stability information can be assessed more quickly.

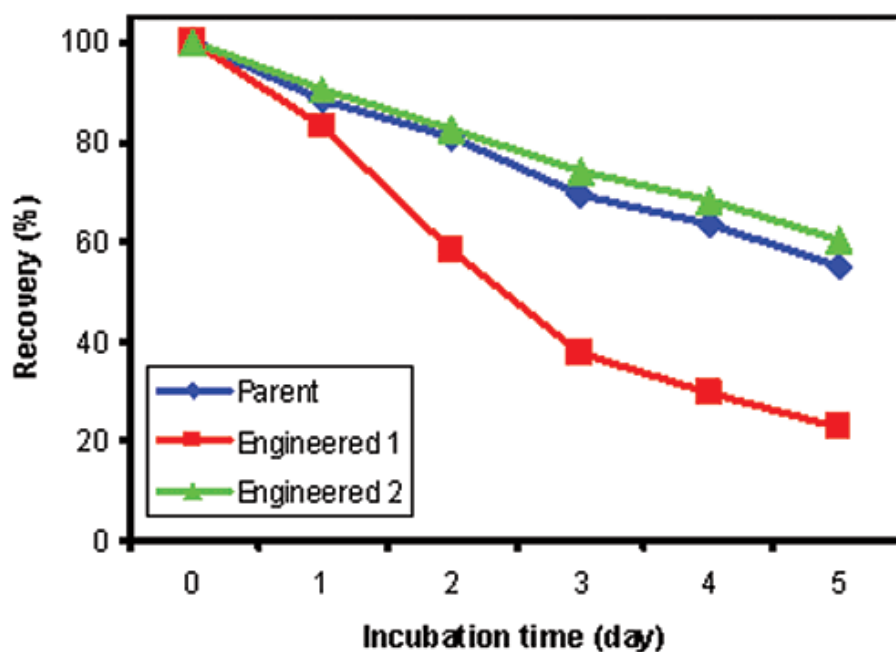


Figure 3: Percent soluble protein after accelerated stability studies for 0, 1, 2, 3, 4, and 5 days at 60 °C. Blue: Parent Antibody; Red: Engineered Antibody 1; Green: Engineered Antibody 2.

Accelerated stability studies at 60°C also indicated that parent and Antibody 2 had comparable, low amount of soluble aggregate formation, based on SEC-HPLC data (Figure 4A and 4C). The same experiment performed with Antibody 1 showed greater soluble aggregate formation, compared to parent and engineered antibody 2 (Figure 4B). Stability predictions made with DSC correspond well to what is predicted by longer-term SEC-HPLC aggregation studies for the antibodies tested, thus microcalorimetry may be a useful tool for rapidly screening protein stability in solution.

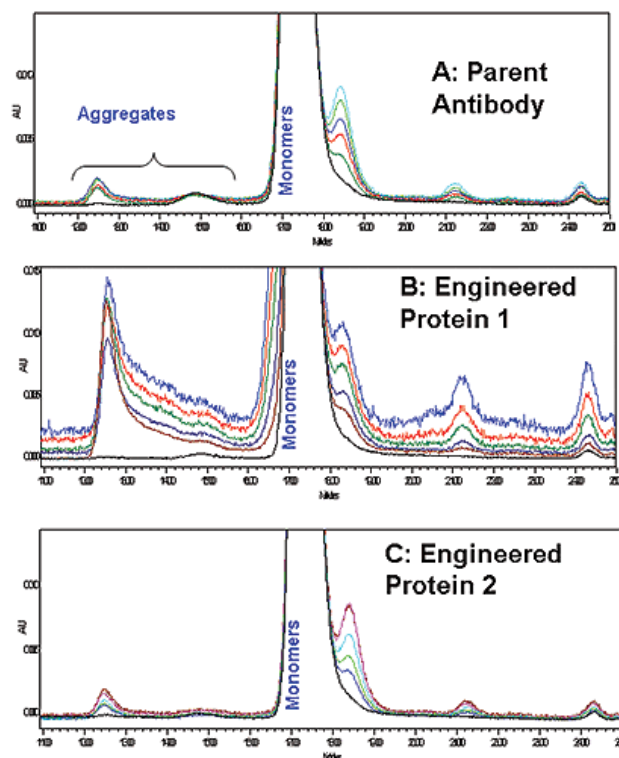


Figure 4: Overlaid SEC-HPLC chromatograms A: Parent Antibody, B: Engineered Antibody 1; C: Engineered Antibody 2.

Even though Antibody 1 has a higher potency than the parent antibody, accelerated stability studies suggest that this protein has increased soluble and insoluble aggregation, compared to the parent antibody and a second engineered antibody. Greater aggregation formation on accelerated studies suggests potential long-term stability and shelf life issues. Since Antibody 2 has a higher T_m and improved stability in accelerated studies, this protein may be the better choice as a biotherapeutic. Further studies are needed to correlate improved stability in accelerated stability studies and improved long-term stability and longer shelf life of this engineered antibody.

Conclusions

DSC was used to study the thermal stability of antibodies that were being engineered for improved potency. In this study, we demonstrated a correlation between decreased thermal stability based on DSC data, and greater aggregation formation during accelerated stability studies (at 60°C). The SEC-HPLC stability data agree quite well with the stability predicted by DSC, suggesting that the thermal stability data obtained from DSC correlates with protein stability. Information derived from DSC can indicate potential long-term stability issues, thus making the technique a useful tool in the screening and selection of engineered proteins.



**Malvern Instruments
Limited**

Groveswood Road, Malvern,
Worcestershire, UK. WR14
1XZ

Tel: +44 1684 892456
Fax: +44 1684 892789
www.malvern.com

Malvern Instruments is part of Spectris plc, the Precision Instrumentation and Controls Company.

Spectris and the Spectris logo are Trade Marks of Spectris plc.

spectris

All information supplied within is correct at time of publication.

Malvern Instruments pursues a policy of continual improvement due to technical development. We therefore reserve the right to deviate from information, descriptions, and specifications in this publication without notice. Malvern Instruments shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance or use of this material.

Malvern Instruments owns the following registered trademarks: Bohlin, FIPA, Insitex, ISYS, Kinexus, Malvern, Malvern 'Hills' logo, Mastersizer, MicroCal, Morphologi, Rosand, 'SEC-MALS', Viscosizer, Viscotek, Viscogel and Zetasizer.