UNCHAINED LABS

Application Note

Developability Assessment Using the HUNK

Recombinant proteins, antibodies and other biologics exhibit striking differences in solution behavior, such as stability and aggregation propensity – even for single amino acid mutations of the same protein. The underlying causes and stability issues and optimizing stability at an early stage is critical to ensure that the selected construct or constructs can be successfully expressed, purified and ultimately formulated. Different strategies are used to stabilize proteins or lower their tendency to aggregate. These strategies include the introduction of stabilizing mutations in β turns and/or alpha helices, disulfide bond engineering, charge reversal mutations and other modifications.

The impact on stability of each of these modifications needs to be experimentally determined. The HUNK, a fully automated chemical denaturation system provides an effective means to assess the stability of these constructs at conditions that are relevant to their downstream processing, storage and administration.

As a biophysical testing and stability indicating technique, the predictive power of the HUNK is unique. The HUNK, on a construct-by-construct and a buffer-by-buffer basis, provides:

- **Quantitative determination of the protein conformational stability (ΔG)**
- **Quantitation of the amount of denatured protein**
- **An indication of the presence and mode of aggregation (e.g. native or denatured protein)**
- **An indication of the propensity for aggregation (Thermodynamic Aggregation Index)**

With the HUNK both the conformational stability and the propensity for aggregation are determined. As illustrated in Figure 1 the HUNK measures the unfolding of each protein construct in the desired buffer(s). More stable proteins denature at higher denaturant concentrations and less stable proteins at lower concentrations. For each protein/buffer combination, the thermodynamic parameters ΔG and $C_{½}$ are automatically determined. As illustrated in Figure 2 the apparent conformational stability (ΔG_{app}) is sensitive to both native protein self-association and denatured protein aggregation.

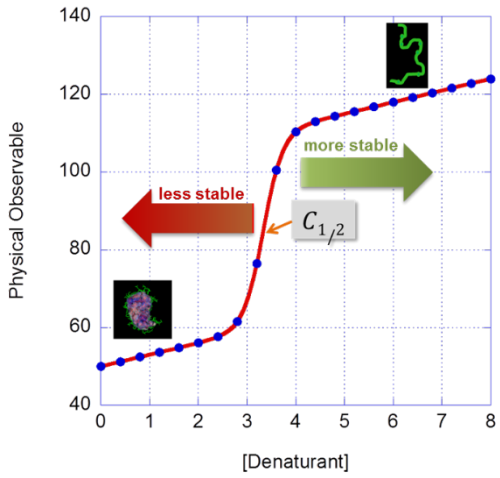

Figure 1. Automated fitting of the denaturation data to a protein unfolding model yields the stability parameters ΔG and $C_{½}$.

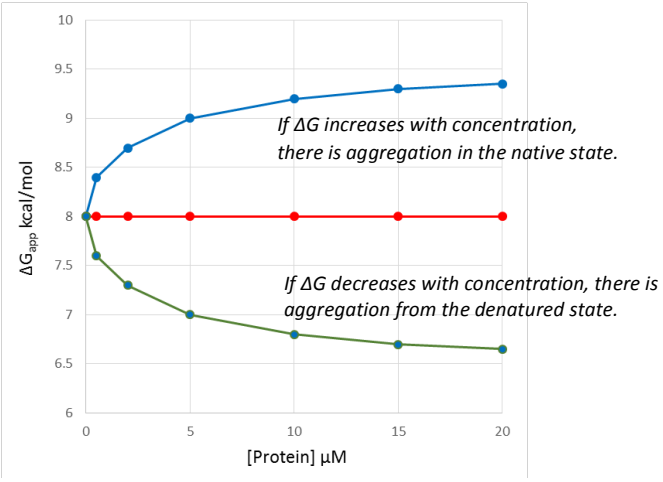

Figure 2. In the absence of aggregation, ΔG must be independent of protein concentration (red line).

The following examples illustrate the application of the HUNK to guide the stability profiling during protein engineering of two different series of mAbs. Figure 3 illustrates the HUNK stability data from 3 of a larger series of IgG1 mAb constructs. A more than 20-fold increase in stability was observed for a few of the constructs as compared to the initial starting construct. Figure 4 illustrates the effects of different post-translational modifications on the conformational stability of a biologics candidate.

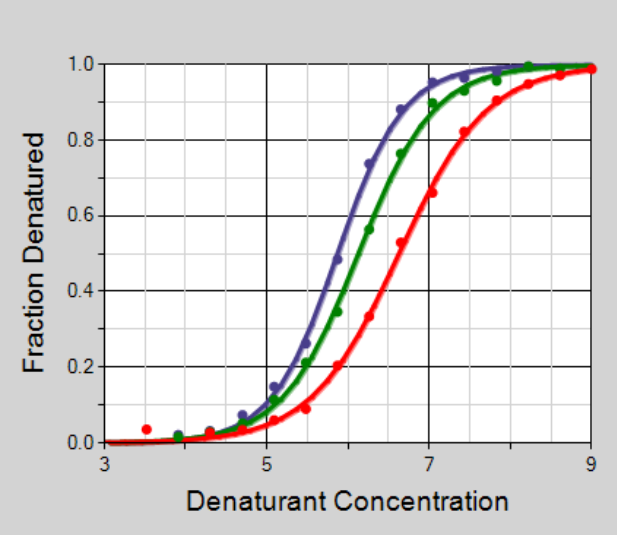

Figure 3. The HUNK analysis of a series of IgG1 mAb constructs. The construct represented by the denaturation curve in red is more than 20-fold more stable than the initial construct represented in blue.

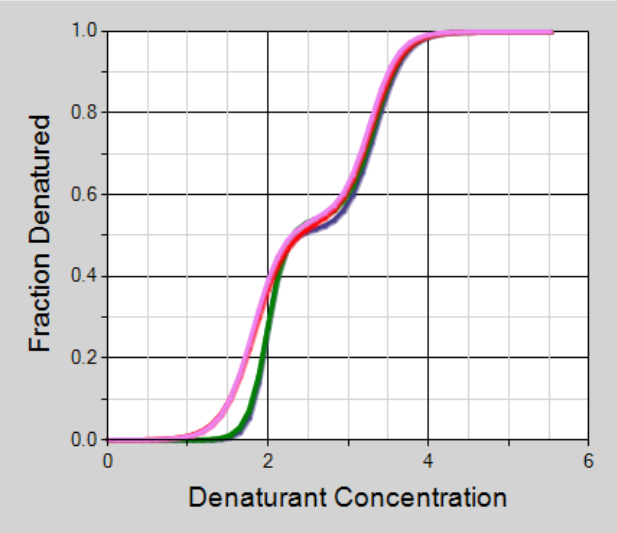

Figure 4. Four overlaid HUNK denaturation curves – the pink and red curves are replicates of one isoform, the green and blue curves are replicates of the second isoform. The thermodynamic fingerprints indicate that the second isoform has a more stable first transition with ΔΔG increasing by 5.2 kcal/mole.

Optimizing stability during early stage protein engineering is the first step in a continual process of stability optimization that eventually concludes during formulations optimization where the complex combination of stability, solubility and viscosity must be optimized to yield the safest, most effective, and most commercially viable drug product. The HUNK provides key insights throughout this process that provide guidance regarding the stability-solubility-viscosity trade-offs during development. Figure 5 illustrates the role of the HUNK from early-stage to late-stage biologics development.

Conclusion

The HUNK provides a direct method to assess the developability of biologics early in development reducing the risk of downstream formulation challenges. The HUNK provides a quantitation of:

- Conformational stability and amount of denatured protein
- Degree of native protein self-association
- Degree of non-native protein aggregation

This information guides decisions on which constructs have the greatest potential for success in downstream development.

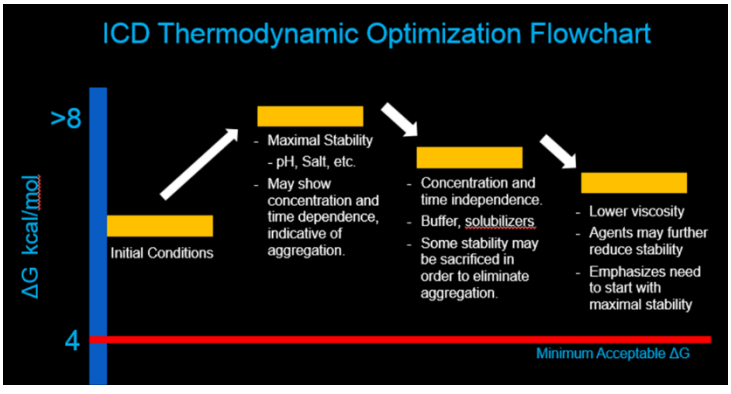

Figure 5. The HUNK provides quantitative thermodynamic guidance about protein stability throughout the biologics development process

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