Long-Term Protein Stability Prediction Using 'Micro' Changes in Secondary Structure Measured Using Microfluidic Modulation Spectroscopy

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Abstract

Microfluidic Modulation Spectroscopy (MMS) is an IR-based technique compatible with almost all biological buffers and additives, including: DMSO, Serum, excipients, reducing agents, adjuvants, surfactants and carbohydrates. As a direct result, samples require no dilution, adulteration or transfer into a suitable media for measurement unlike traditional technologies e.g. Circular Dichroism (CD) and Fourier Transform Infra-Red (FTIR), and require no labelling. The AQS^{3}pro is fully automated and generates exceptionally high data quality across an extremely broad concentration range (0.1 to >200 mg/ml), offering more robust and highly-sensitive analysis of free in-solution protein folding irrespective of the background. MMS can confidently be used to enhance Quality by Design (QbD), formulation studies and drug candidate selection where existing technologies are restricted. This poster presents 8 protein samples which were studied in pairs, incubated at 4 and 30°C in complex buffers for 8 weeks to simulate product stability over years. Structural changes over this timescale were <1% with a sample concentration <10 mg/ml, which would be very challenging to measure using existing traditional technologies. MMS allowed data to be collected rapidly in an automated fashion from well plates, enabling this stability study to be greatly accelerated.

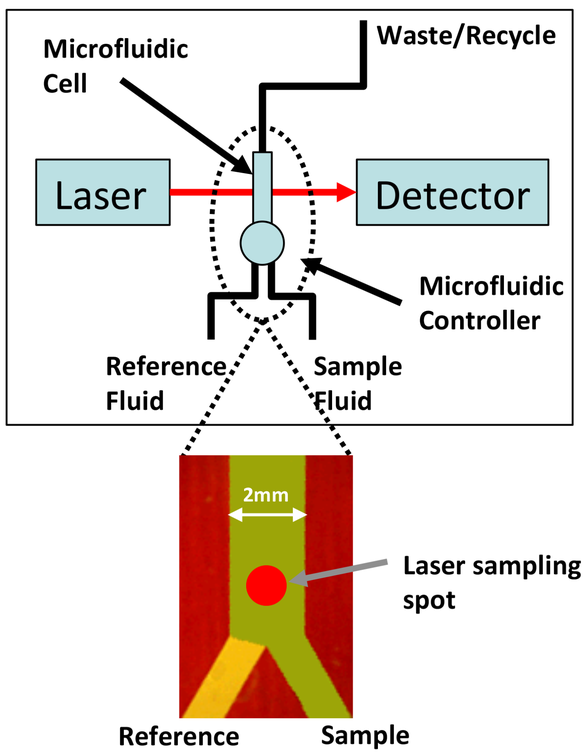
Introduction

The AQS^{3}pro System

The AQS^{3}pro launches a new era in IR spectroscopy for protein characterization, bringing repeatable, high sensitivity, automated measurements to every stage of the biopharmaceutical pipeline.

Powered by Microfluidic Modulation Spectroscopy

MMS is a unique, patented technology in which the sample (protein) solution and a matching buffer reference stream are automatically introduced into a microfluidic flow cell, and the two fluids are rapidly modulated (e.g. 1-5 Hz) across the laser beam path to produce nearly drift-free background-compensated measurements



Advantages of MMS in the AQS^{3}pro

- Fully automated operation.
- The widest concentration range to characterize biotherapeutic higher order structure.
- Generate precise, high sensitivity data.
- Analyze and understand protein behavior.

Aggregation | Quantitation | Stability

Structure | Similarity

Results

MMS – Insulin 'Micro' Stability Study

A set of Insulin samples were held below their first T_m at 30°C (measured using DSC) for 8 weeks to simulate the effect of degradation in different formulation conditions, at 4°C over several years. Due to the very high sensitivity of MMS and its accommodation to complex buffers, differences in secondary structure that would not have been observed using traditional technologies were measured between samples with little user effort, indicating their relative stability.

A systematic pattern was observed, showing a decrease in alpha helix, turn and disordered structure, with a corresponding increase in β-structured material common to the initial stages of an aggregation process. Only sample 3 showed deviation from this pattern with a reversal in disordered structure behavior. Each datapoint is averaged from 3-5 replicates, with an internal similarity of >99.5%.

Higher-Order Structure

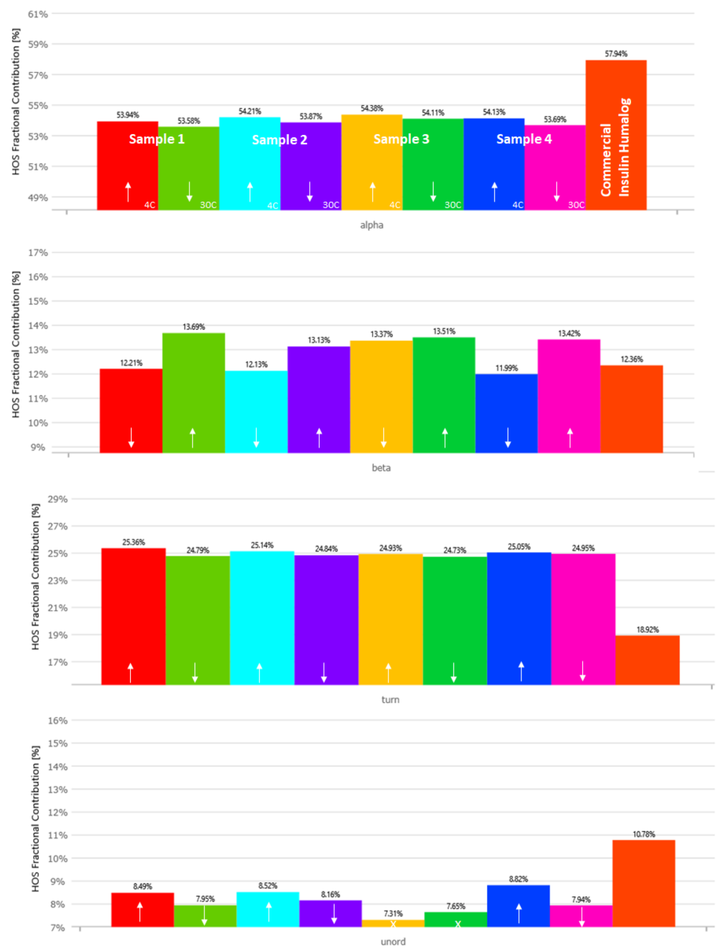


Figure 1. Higher-Order Structure (HoS) analysis showing changes in four secondary structure elements between pairs of formulated insulin samples held at 4°C and 30°C for 8 weeks. Each datapoint is averaged from 3-5 replicates, with an internal similarity of >99.5%. Samples are shown in pairs in numerical order, left: 4°C, right: 30°C. A commercially-available Insulin Humalog is shown on the far right for comparison, measured at 4°C.

Concentration Measurement and Similarity Analysis

Sample Name	Given Conc. (mg/ml)	Fitted Conc. (mg/ml)	Similarity to 1, 4C (%)	Similarity to 4C sample (%)
Sample 1, 4C	7.2	6.77	100.00	100.00
Sample 1, 30C	7.2	6.77	99.10	99.10
Sample 2, 4C	7.2	6.84	99.65	100
Sample 2, 30C	7.2	6.84	99.10	99.28
Sample 3, 4C	7.2	6.98	99.31	100
Sample 3, 30C	7.2	6.98	99.05	99.50
Sample 4, 4C	7.2	6.70	99.24	100
Sample 4, 30C	7.2	6.70	99.00	99.36
Commercial Insulin Humalog	3.6	3.43	91.87	-

Table 1. Calculated concentrations and similarity comparisons: to sample 1, and between 4 and 30°C samples. >99.5% internal replicate similarity measured for each sample (3-5 replicates)

Protein concentrations are calculated automatically using the in-built AQS^{3}delta analytics platform for every sample analysed by MMS, using the peptide backbone as a chromophore, therefore requiring no labelling. Similarity analysis is also performed automatically and determines the variance of each measurement, and so whether differences observed between samples are significant. Sample 3 most retains its secondary structure at higher temperature (right column), whereas sample 1 shows the largest structural difference. Measured changes are very small (<1%), but are significant as internal replicate variance is lower in each case.

Further Higher-Order Structural Analysis

Normalised changes in higher-order structure were used to highlight how the four samples in this study changed at elevated temperature in comparison to one another, agreeing well with previous conclusions. Sample 3 clearly shows the smallest alpha to beta transition, indicating this is the most stable sample, least likely to aggregate. Samples 1 and 4 are most likely to aggregate, but show slightly different behaviour to one another that may have an impact on their respective mechanisms of aggregation. Sample 2 is similar to 1 and 4, but shows a lesser degree of destabilisation.

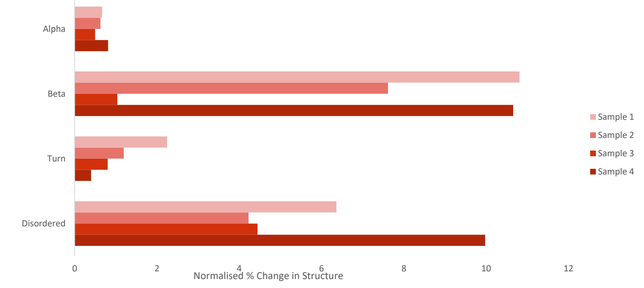


Figure 2. Further analysis of data shown in figure 1. Micro changes in four types of secondary structure are shown normalized for each sample.

Conclusions

In summary, four pairs of proteins were investigated using MMS to determine whether incubation at elevated temperature for weeks could be used to predict stability at lower temperature over years. Secondary structure changes over this timescale were very small (<1%), but could be confidently measured due to the very high level of spectral sensitivity and reproducibility of MMS. Due to automation this was achieved in one day of experiments and predicted that in decreasing order, sample stability is: 3, 2, 1, 4, which was confirmed by the user to be correct. As part of every measurement, MMS applies in-built AQS^{3}delta analytics software to provide several parameters that when considered together are key to giving a more complete picture of structural changes in your samples. Here a combination of concentration, similarity analysis and two types of higher-order structure analysis were combined to rank samples and show that they potentially aggregate via slightly different mechanisms, which may help design a stabilization strategy for this molecule type. MMS offers a new technology in the Biotherapeutic workspace that provides an invaluable tool for understanding and predicting solution stability behavior, without the limitations and restrictions of historical technologies. One technique can be applied to all samples, with a level of sensitivity not previously available that here is shown to greatly accelerate long-term protein stability testing.