See Change™

Confident Differentiation of <2% changes in Secondary Structure to Compare Batch-to-Batch Activity using Microfluidic Modulation Spectroscopy (MMS)

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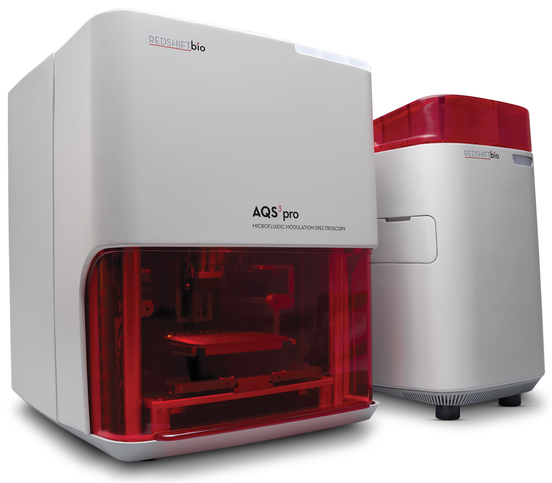
Abstract

Microfluidic Modulation Spectroscopy (MMS) is a revolutionary new fully-automated infra-red technology that makes measurements of protein secondary structure across a much wider concentration range than traditional spectroscopic techniques (0.1 to >200 mg/ml), and is compatible with the majority of complex biological buffers and excipients such as DMSO, Serum, excipients, reducing agents, adjuvants, surfactants and carbohydrates. Exhibiting exceptional spectral reproducibility (>98.5% at 0.1 mg/ml), MMS enables very small changes in structure to be confidently differentiated, allowing the technology to be used to confidently measure structural differences that may have an impact on function. Here <2% differences in four types of secondary structure were used to correctly predict which of 13 BSA samples from different suppliers and batches would be viable for reagent manufacturing. Of all samples tested, only 5 were found to be similar enough to the control to retain activity. 2% and smaller changes in structure would be extremely challenging to confidently measure using traditional technologies.

Introduction

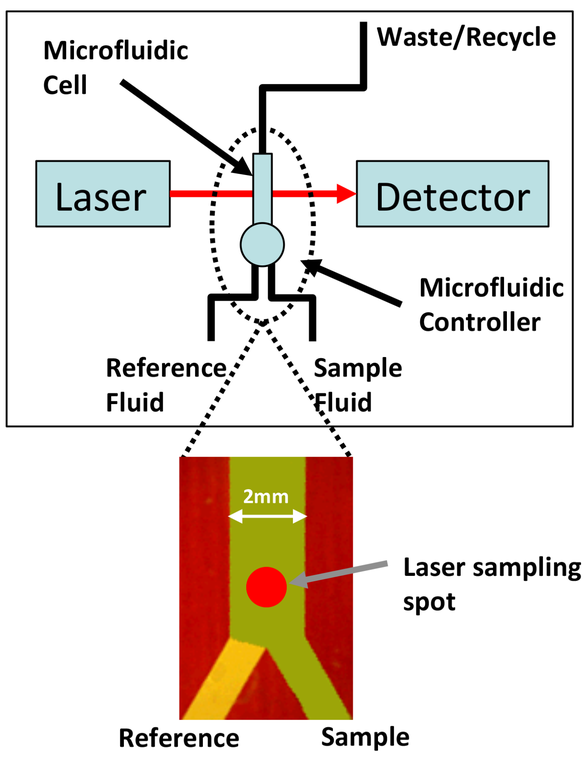
The AQS³pro System

The AQS³pro 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 AQS³pro

- 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

Biosimilar Comparison by MMS

14 BSA Samples of Different Origin

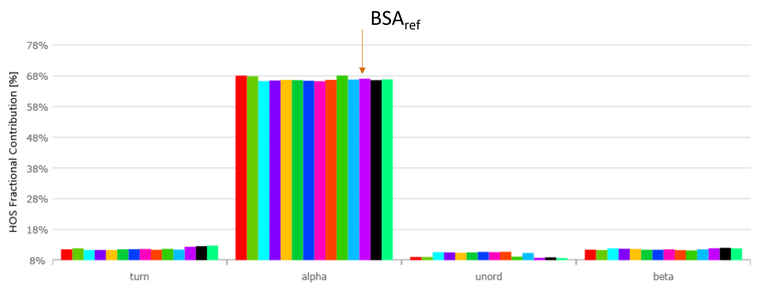


Figure 1. Higher-order structure plots comparing % alpha helix, beta sheet, disordered and turn structure for 13 BSA samples from different manufacturers and batches with a known control sample. Largest differences between samples are <2%, which would be very difficult to discriminate using traditional technologies, but is possible due to the extremely high sensitivity and reproducibility of MMS. Averaged data for 3 replicates shown, with an internal reproducibility of >99.5%.

Higher-order structure plots of 13 BSA samples from different manufacturers and batches compared to a known control. Maximum differences between samples were <2%, which would be very challenging to distinguish using traditional technologies, but is possible using MMS due to its extremely high sensitivity and reproducibility. 3 replicates were acquired for each sample, which gave a maximum internal variance of 0.5%, meaning differences observed between these samples is significant.

Higher-Order Structure Analysis

Figure 2. Higher-order structure plots showing % alpha helix, beta sheet, disordered and turn structure present in each sample from spectral deconvolution. 13 BSA samples from different manufacturers and batches were compared to a known control sample.

Concentration, Protein Displacement Factor and Similarity

Sample Name	Given Conc. (mg/ml)	Calc. Conc. (mg/ml)	Fit Disp. Factor (no units)	Similarity
BSA$_1$	10	8.4	0.65	98.88%
BSA$_2$	10	9.3	0.64	98.92%
BSA$_3$	10	8.9	0.62	97.84%
BSA$_4$	10	8.7	0.63	97.77%
BSA$_5$	10	9	0.63	97.89%
BSA$_6$	10	9.5	0.63	97.68%
BSA$_7$	10	9	0.62	97.51%
BSA$_8$	10	8.8	0.62	97.64%
BSA$_9$	10	8.6	0.62	97.66%
BSA$_{10}$	10	8.7	0.65	98.67%
BSA$_{11}$	10	9.5	0.63	97.76%
BSA$_{ref}$	10	8.5	0.62	100.00%
BSA$_{12}$	10	10.4	0.64	99.50%
BSA$_{13}$	10	9.7	0.64	99.57%

Table 1. Measured concentrations, protein displacement factors and similarity comparisons to the reference sample. >99.5% internal replicate variance measured for each sample (3 replicates), calculated automatically as part of the measurement.

Sample concentrations were measured automatically and interestingly varied per batch. Protein displacement factors show all samples are of very similar volume, but similarity assessment of all samples highlights several are slightly closer to the control than the others.

Delta Plot and Stability Analysis

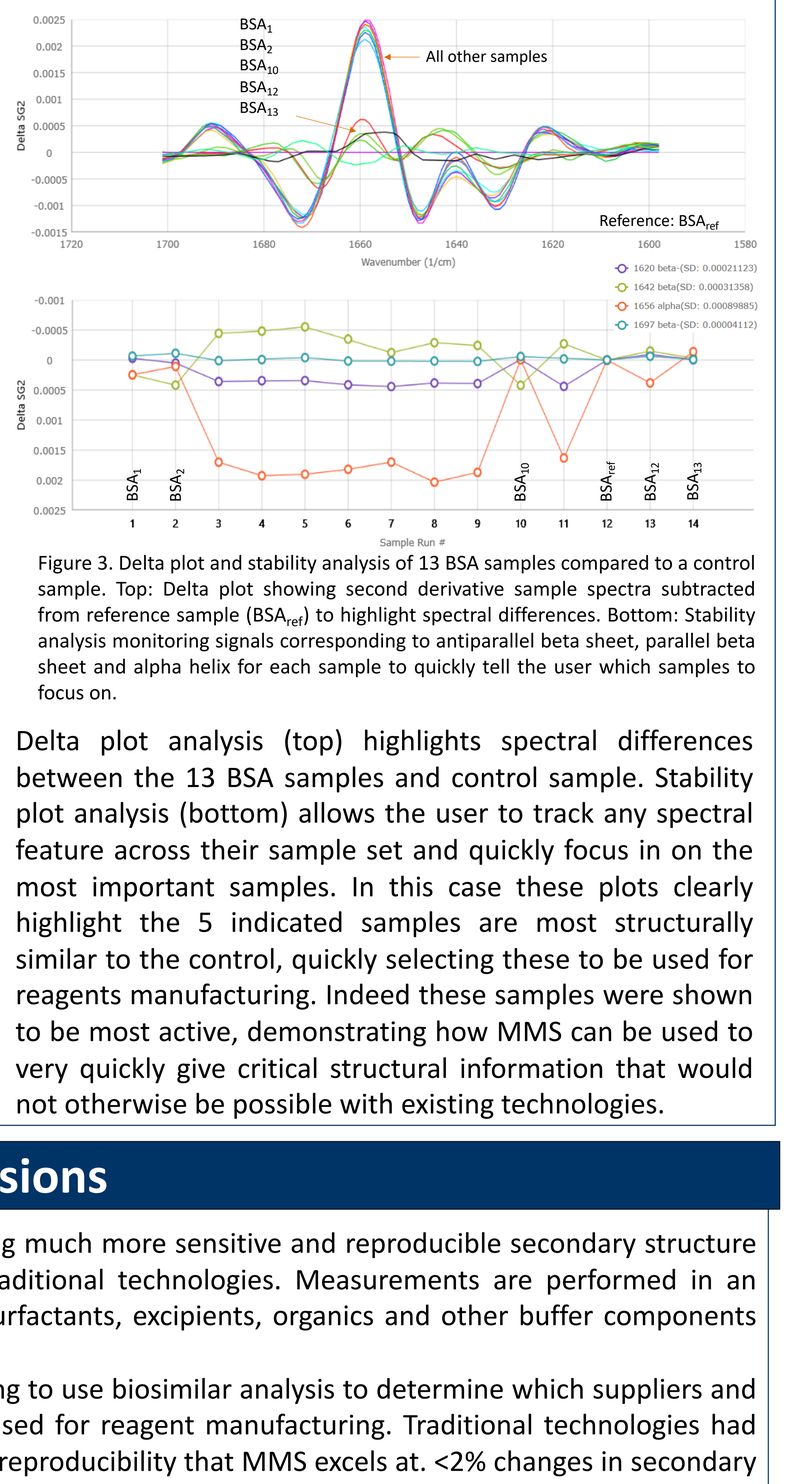


Figure 3. Delta plot and stability analysis of 13 BSA samples compared to a control sample. Top: Delta plot showing second derivative sample spectra subtracted from reference sample (BSA$_{ref}$) to highlight spectral differences. Bottom: Stability analysis monitoring signals corresponding to antiparallel beta sheet, parallel beta sheet and alpha helix for each sample to quickly tell the user which samples to focus on.

Delta plot analysis (top) highlights spectral differences between the 13 BSA samples and control sample. Stability plot analysis (bottom) allows the user to track any spectral feature across their sample set and quickly focus in on the most important samples. In this case these plots clearly highlight the 5 indicated samples are most structurally similar to the control, quickly selecting these to be used for reagents manufacturing. Indeed these samples were shown to be most active, demonstrating how MMS can be used to very quickly give critical structural information that would not otherwise be possible with existing technologies.

Conclusions

MMS is a revolutionary new infra-red based technology, enabling much more sensitive and reproducible secondary structure information to be captured for your proteins compared to traditional technologies. Measurements are performed in an automated fashion from well plates with a high tolerance to surfactants, excipients, organics and other buffer components that might otherwise prevent analysis with current tools.

Here 13 BSA samples were compared to a control sample, seeking to use biosimilar analysis to determine which suppliers and batches had produced samples structurally-similar enough to used for reagent manufacturing. Traditional technologies had failed to provide this information due to a lack of sensitivity and reproducibility that MMS excels at. <2% changes in secondary structure and global spectral similarity analyses were used to correctly identify 5 samples that retained activity to the control sample, providing a method by which new suppliers' samples could be validated quickly before use in production.