REDSHIFTbio™

See change™

The NEXT GENERATION in Protein Characterization

Introduction to the AQS3™pro

Enabled by **Microfluidic Modulation Spectroscopy**

A Desktop Resource

Created by RedShiftBio
JUNE 2019 (1st Edition)

redshiftbio.com

Introduction to the AQS³pro
A desktop resource

Dear Reader,

The ability to measure and characterize changes in the secondary structure of proteins is critical to many research applications, especially the formulation and development of biotherapeutics. There is direct evidence that changes in secondary structure result in changes in efficacy and specificity. Conventional techniques do not possess the adequate feature set for researchers to See Change™ in their proteins within the required conditions. The need for high sensitivity, a wide dynamic range, a simplified and automated workflow, and high repeatability is signifiant and, until now, has not been adequately provided. The solution for today's researcher is Microfluidic Modulation Spectroscopy (MMS).

This eBook will introduce you to the novel IR technique called MMS which is designed to address the requirements of today's development laboratories and fill the characterization gap. This technique is purposely designed to See Change in secondary structure. Data will be presented from measurements of commercially available proteins which demonstrate significant increases in sensitivity, dynamic range, and utility for the characterization of protein secondary structure. It is now possible to measure protein similarity (fingerprinting), quantitation, higher order structure, protein stability, and aggregation through thermal and chemical denaturation methods using a walk-away automated platform. The ability to See Change is enabled by MMS.

If you have any comments, questions or suggestions for this eBook, please send an email to info@redshiftbio.com and we'll be happy to help in any way we can.

Happy reading!

~The RedShiftbio Team

redshiftbio.com

linkedin.com/company/redshiftbio/

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RedShiftBio™ is an innovative provider of analytical instrumentation for the research, development and manufacture of protein therapeutic drugs.

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Importance in Drug Development

"The direct link between the structure and functionality of a biotherapeutic protein makes it essential to develop biopharmaceuticals that deliver the molecule with its target structure intact."

Protein Secondary Structure and Drug Development

During biologic development and manufacturing, there is the propensity for instability or conformational change which is often accompanied by aggregation, the process by which proteins start to bind together under different conditions and formulations. This is a defining issue in the discovery, formulation, and manufacture of biotherapeutics and biosimilars.

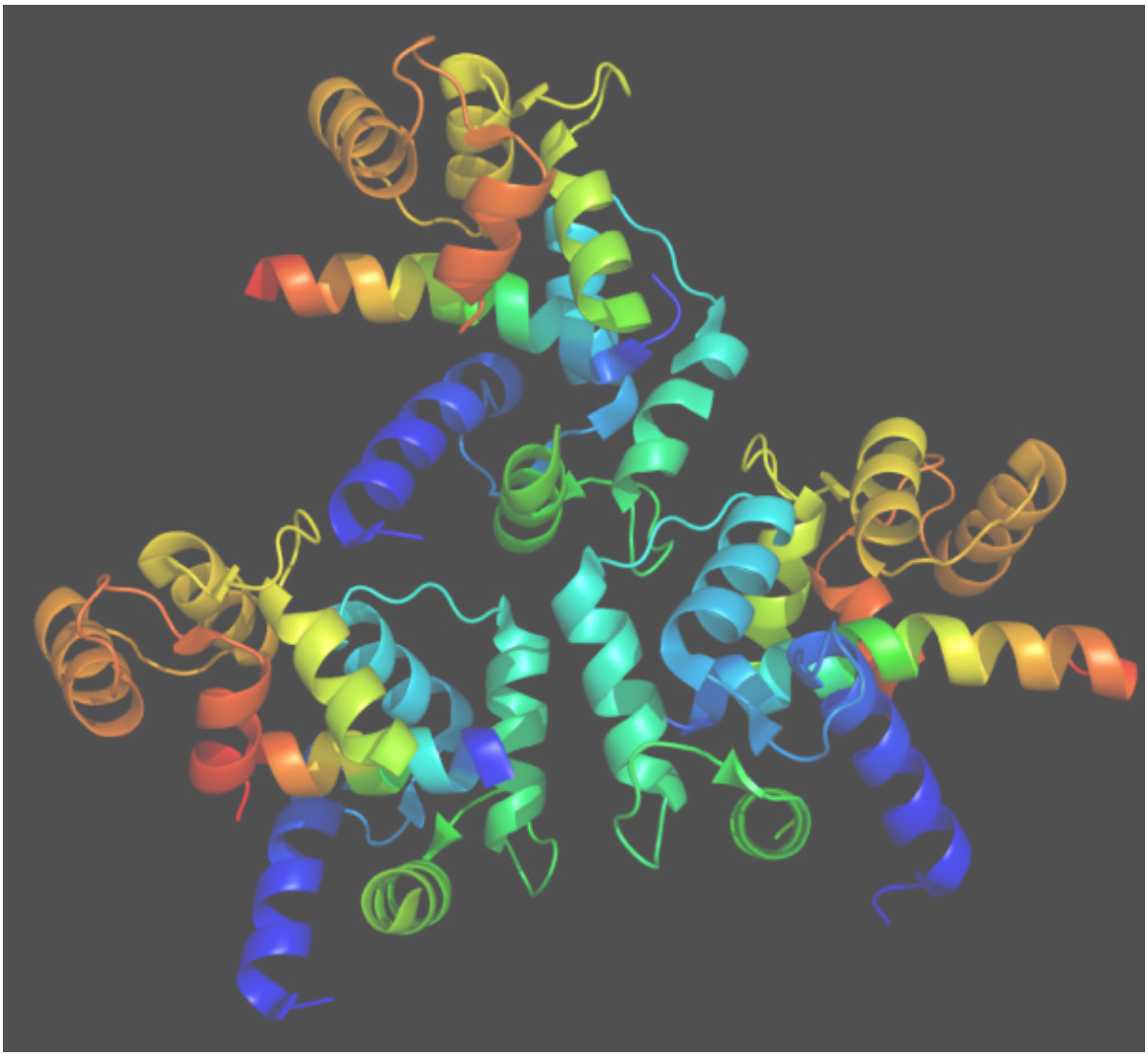
Promising biological drug candidates, those that exhibit therapeutic activity and inherent stability, as assessed via simple screening techniques, become subject to increasing levels of structural elucidation as they progress through the pharmaceutical pipeline. Detailed definition of the structure of a drug molecule provides a basis for the identification of structure-function relationships, for understanding the mechanisms by which the drug is efficacious, but beyond this, structural characterisation plays a critical role throughout the drug development lifecycle. In particular, investigating structural changes is the key to understanding and controlling the factors and mechanisms associated with stability and aggregation.

Defining an optimal formulation and manufacturing route relies on assessing the impact of variables such as pH, excipient choice, processing conditions (including temperature and applied shear stress), and storage on the secondary structure of biologics. Stress-induced structural changes may have significant consequences including a loss of efficacy, and in the worst case, present a safety risk. Demonstrating comparability that successive stages of formulation, manufacture and storage do not materially impact the structure of the drug up to the point of administration, is therefore essential.

> *"We believe [MMS] can play a significant role in the protein characterization technology toolkit, overcoming shortcomings in existing techniques. In our case the quality of the results obtained, the small sample volumes, the ability to quickly probe low concentrations of amyloid proteins in solution under 'physiological' conditions. While there are many other interesting and important applications of this technology, RedShift Bioanalytics' MMS platform fills a void in the amyloid field that is helping to shed light on the fundamental conformational changes associated with disease."*
>
> *Professor of Bioengineering*

CHAPTER 1

Characterizing Protein Secondary Structure



Folding and the Amide I Band

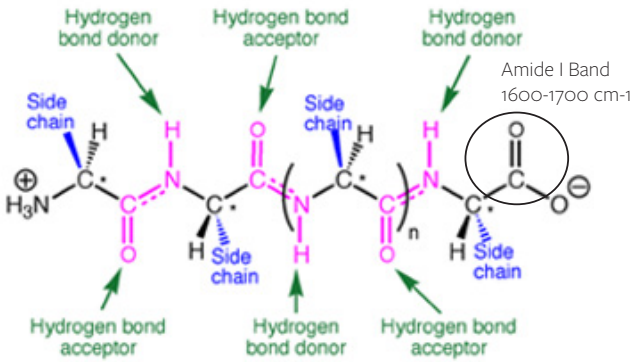
Protein Structure

Proteins are large molecules with complex three dimensional (3D) structures, and changes in the structure can directly influence their efficacy and safety as biotherapeutics. The primary structure of proteins is the sequence of amino acids in the peptide backbone with secondary structures arising from interactions between hydrogen bond donor and acceptor residues associated with repeating peptide units. These interactions promote stabilized folding of the peptide chains principally to form alpha-helix or beta-sheet substructures, the geometry of which is defined by the intermolecular forces, bond angles, and planar configurations involved. However, the secondary and associated higher order structure of proteins is not fixed, but rather is dependent on the localized environment, changing in response to, for example, thermal or chemical stresses.

Using Vibrational Spectroscopy

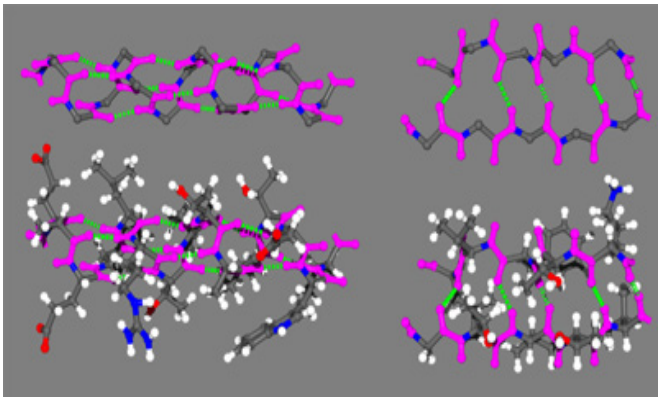
Vibrational spectroscopy is an established tool for protein characterization[1], with Fourier Transform Infrared (FTIR) wavenumbers in the range of 1700–1500 cm-1 routinely used to probe the Amide I (1700–1600 cm-1) band. The Amide I band is associated with the C=O stretch vibration of peptide linkages and quantifies the strength of the carbonyl bond along the protein backbone which is highly sensitive to changes in secondary structure.[2] Indeed, absorption features of the Amide I band are well correlated with shifts in hydrogen bonding, dipole-dipole interactions, and geometric orientations in the alpha-helices, beta-sheets and turns, and with other less prevalent motifs of secondary structure such as random coils.[3] The relative amounts of different elements of substructure can therefore be precisely determined by comparing measured Amide I spectra with those gathered for proteins with well-characterized secondary structures[4] to provide valuable information for the assessment of, for example, chemical and thermal stability[5], aggregation[6] and biosimilarity.[7,8]

Primary -> Secondary Structure



In solution, proteins are prone to conformational change or instability, unfolding, or misfolding as structural bonds are disrupted by changes in the local environment. Changes in concentration or buffer composition, pH, and/or temperature, for example, can impact conformation and may also trigger aggregation, the joining together of either native or misfolded proteins. Any structural change has the potential to compromise efficacy and, in the worst case, impact safety, and must be understood and controlled.

Stable Secondary Structures



Alpha-helix Beta-sheet

A Comparison of Traditional Techniques

Though the potential of vibrational spectroscopy in protein analysis is widely recognized, current commercial presentations of the technology are not optimally matched with requirements for secondary structure measurement within biotherapeutic development, formulation and manufacture. As a result, researchers routinely deploy a suite of diverse analytical techniques to study the outlined issues associated with protein structure, with each technique somewhat aligned with the constraints and requirements of different stages of the development cycle.

Notably, IR is particularly sensitive to the beta-sheet structures that are prevalent in protein-antibody drugs, and is one of the very few analytical techniques that can be used to directly monitor aggregation processes due to its ability to measure the intermolecular beta-sheet structures associated with aggregate formation.[9]

Several techniques are typically employed to characterize protein secondary structure, and each traditional technique comes with a set of constriaints and limitations.

> *"As expected, the [MMS] IR technology is much more sensitive to beta-sheet as well as beta turn structure than CD."*
>
> *Senior Scientist III*
> *Analytical Development*
> *Biopharma*

Differential scanning calorimetry (DSC) is routinely used as an early screen for thermal stability, for example, while high pressure liquid chromatography – size exclusion chromatography (HPLC-SEC) can be used to detect aggregation because of its capabilities for molecular size measurement. Neither of these techniques provides detailed structural characterization of the protein.

On the other hand, FTIR and Raman are both used for structural analysis but require a sample concentration of at least 10 mg/mL and 30 mg/mL respectively[10] with FTIR working optimally in the 10 – 150 mg/mL range. This can be a limitation for applications in the earlier stages of development when sample availability can be an issue. FTIR also has other well-documented issues including a tendency to exhibit background drift, low sensitivity and a requirement for close temperature control.

Ultra-violet–circular dichroism (UV-CD), in contrast, is an established tool for structural analysis at more dilute concentrations, typically operating in the range 0.2 to 2.0 mg/mL,[11] but is unsuitable for direct measurement at high formulation concentrations.

Biotherapeutics are typically delivered intravenously or subcutaneously in liquid formulations, often at relatively high concentrations to minimize administration time within the limitation of having a stable formulation of practical viscosity. Sample dilution complicates measurement as well as introducing an additional source of analytical variability, and the extrapolation of measured results to higher concentrations is widely recognized as a potential source of error in biopharmaceutical characterization because of the sensitivity of protein structure to its surrounding environment. Furthermore UV-CD is relatively insensitive to changes in Beta-sheet structure, especially intermolecular Beta-sheet structures, compromising its application in the study of aggregation mechanisms.

MMS 101

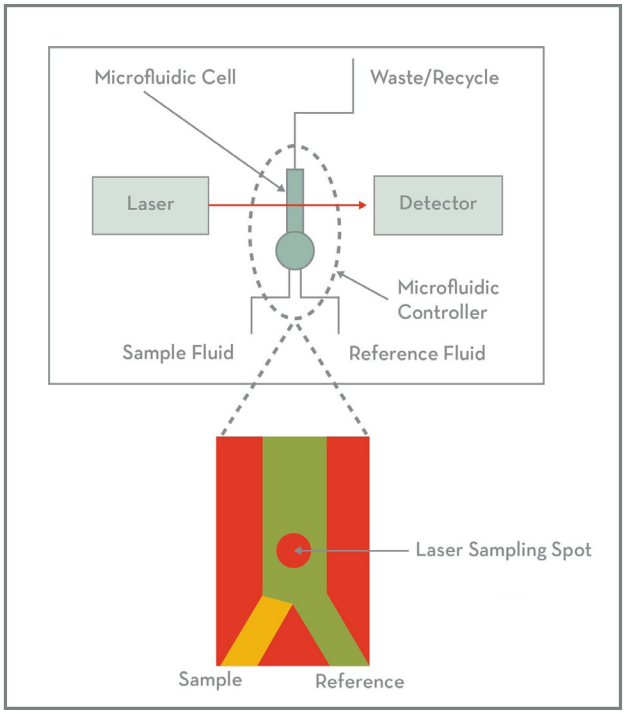
What is MMS?

A new technique called microfluidic modulation spectroscopy (MMS) directly addresses the limitations of current technologies and provides an efficient tool for direct, label-free protein analysis. It uses a tunable mid-infrared quantum cascade laser to generate an optical beam that is 1000 times brighter than those used in conventional FTIR, enabling the measurement of samples that are substantially more concentrated and the use of simpler detectors with no requirement for nitrogen cooling.

The laser is run in continuous wave mode to generate a very high resolution (< 0.001 cm-1 linewidth), low noise beam with minimal stray light that is focused through a microfluidic transmission cell with a short (< 25 µm) optical pathlength onto a thermoelectrically cooled mercury cadmium tellurium (MCT) detector. This optical configuration delivers high sensitivity measurements over a concentration range of 0.1–200 mg/mL for structural characterization and down to 0.01 mg/mL for protein quantitation, giving MMS a far wider dynamic range than alternative protein characterization techniques like FTIR.

In an MMS instrument, the sample (protein-in-buffer) solution and a matching buffer reference stream are introduced into the transmission cell under continuous flow and then rapidly modulated (1–10 Hz) across the laser beam path to produce nearly drift-free, background compensated, differential scans of the amide I band (see figure). The complete optical system is sealed and purged with dry air to minimize any interference from atmospheric water vapor, which absorbs across the 2000–1300 cm-1 wavenumber range and can therefore compromise the use of IR spectroscopy for protein characterization.

Advanced signal processing technology is the third key element of the instrument and the AQS³delta Analytical Software converts the raw spectra into fractional contribution data for specific motifs of secondary structure, providing a structural fingerprint of the protein.



Modulating the protein sample with a matching water/buffer stream generates differential, autocompensated scans of the Amide I band, allowing MMS to characterize secondary structure with high sensitivity.

"I can do the work of a week in a day with the AQS³pro".

Scientific Director, R&D Biopharma

MMS vs FTIR

How is MMS Different than FTIR?

MMS harnesses the inherent advantages of IR spectroscopy in a setup that more fully addresses analytical requirements in formulation and throughout biopharmaceutical development.

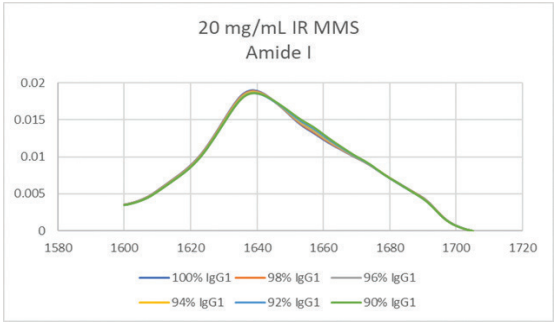
Modulation enables automatic background subtraction, eliminating the background drift associated with FTIR measurements and substantially improving signal-to-noise ratio. In addition, MMS systems use a tunable quantum cascade laser that generates an optical beam around 1000 times brighter than those of conventional FTIR instruments, increasing the concentration range over which samples can be reliably measured. This high-resolution, low-noise beam is configured such that simple detectors (with no cooling requirement) deliver label-free, high-sensitivity structural characterization across a concentration range from 0.1 to 200 mg/mL.

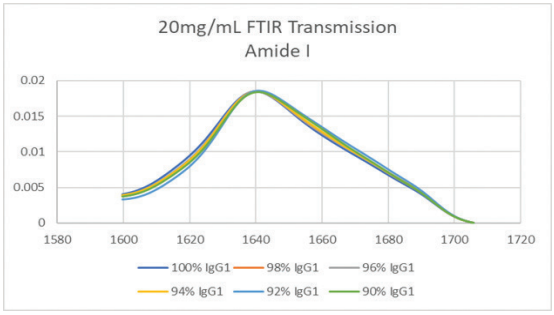
In order to see the differences in these two techniques, a contrast between FTIR and MMS data for solutions of immunoglobulin (IgG1) spiked with bovine serum albumin (BSA) can be seen to the right, which illustrates several of the key advantages of MMS .

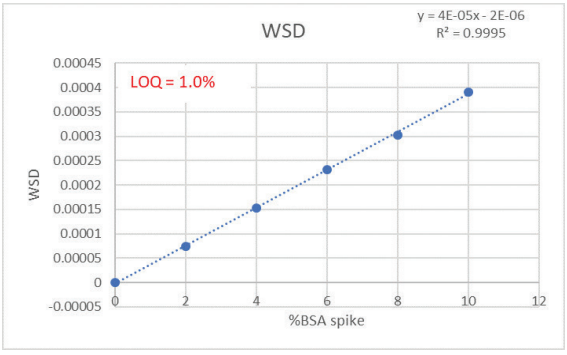
IgG1 has an almost exclusively beta-strand secondary structure (~87 %), while BSA is predominantly alpha-helical (~94 %). In combination, these two proteins provide a useful model system for mimicking the introduction of structural impurity and highlight the differences in repeatability, sensitivity and linearity and dynamic range for the techniques of MMS vs FTIR.

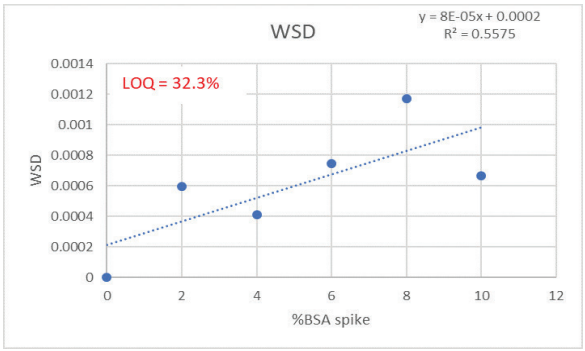
The figures to the right also show Amide I spectra and plots of weighted spectral difference (WSD) as a function of BSA content for MMS and FTIR respectively. These demonstrate the improved sensitivity of MMS relative to FTIR.

Data Source: Webinar, Spectroscopic methods and detection limits for protein misfolds and structural similarity, Brent Kendrick, Elion Labs, A Division of KBI Biopharma









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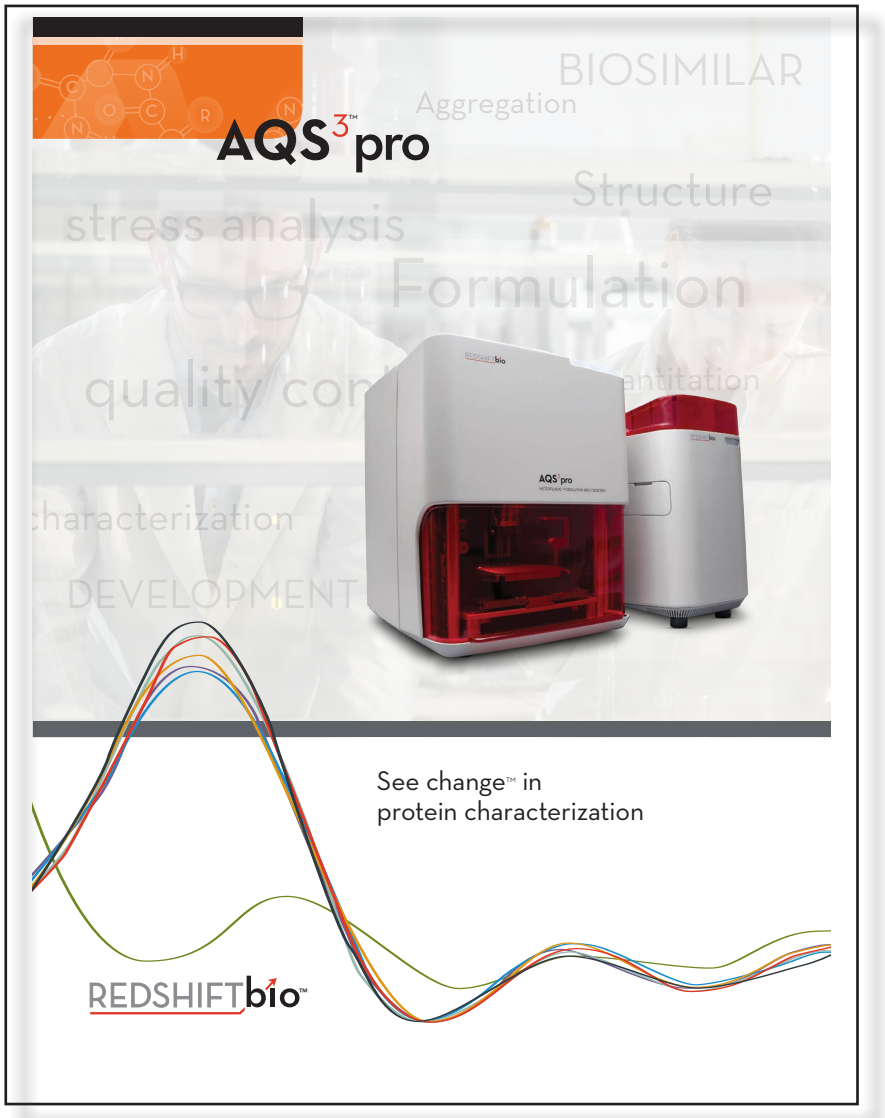
CHAPTER 2

The AQS³pro MMS System



System Features

Automated Multi-Sample Delivery

Walk-away Operation

AQS³delta Analytics Software

User-replaceable Flow Cell

Windows Operating System

Multi-Well Plate Platform

AQS³pro MMS System

Widest Dynamic Range

>30x Sensitivity vs Conventional IR

Unmatched Reproducibility

See Change in Aggregation

See Change in Quantitation

See Change in Stability

See Change in Structure

See Change in Similarity

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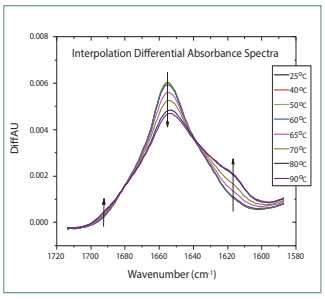
System Specifications

AQS³delta Analytical Software

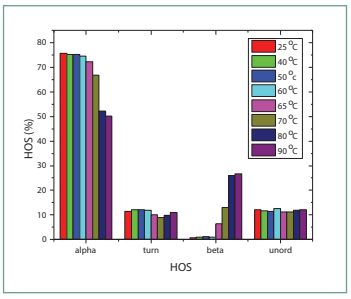
Data Flow Analysis of BSA at 1.0 mg/mL

Differential Absorbance Spectra

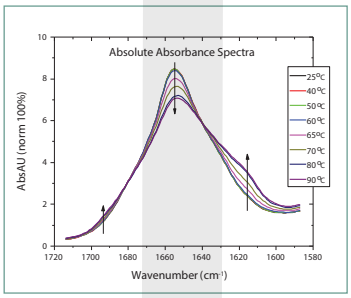


The AQS³pro is perfectly paired with AQS³delta analytical software, a suite of analytical tools that turn high quality data into scientific insight. These tools deliver consistent and reproducible results in seconds. Concerned about stability? Then track changes between spectra at individual locations or across structural motifs.

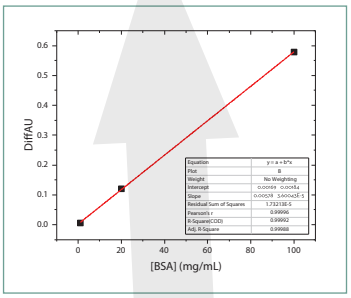
HIgher Order Structure



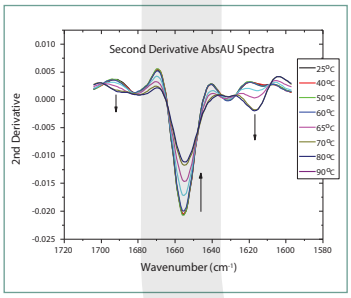
Absolute Absorbance Spectra



Quantitation

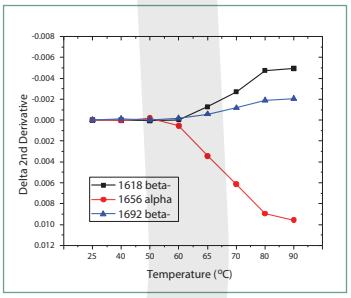


Second Derivative Spectra

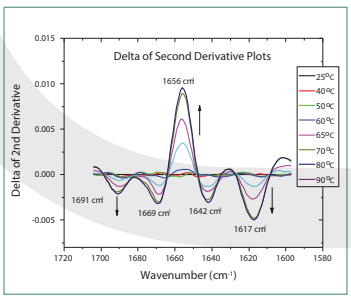


Analyze similarity with the Area of Overlap tool, or take advantage of the wide dynamic range for quantitation. Here is a look at the data flow analysis for BSA to see how the AQS³delta software processes and presents data to maximize the value of each measurement.

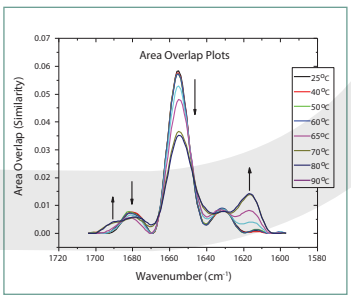
Stability



Delta of Second Derivative Spectra



Similarity / Area of Overlap AO



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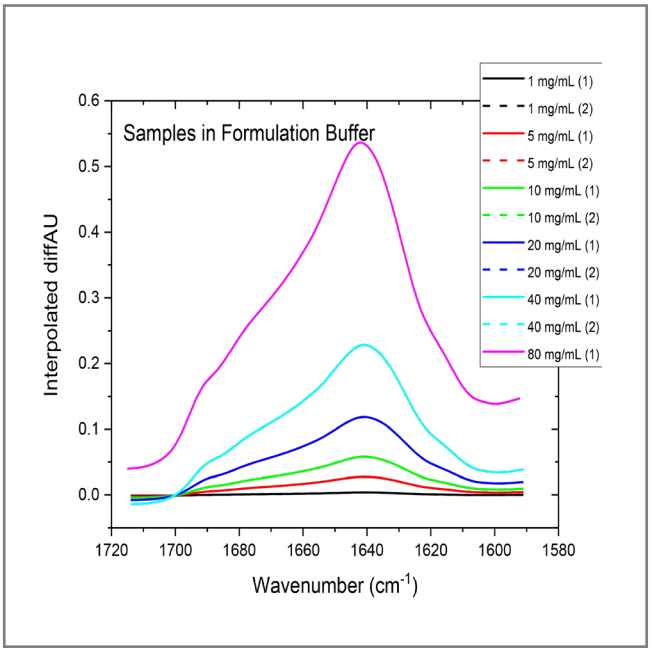
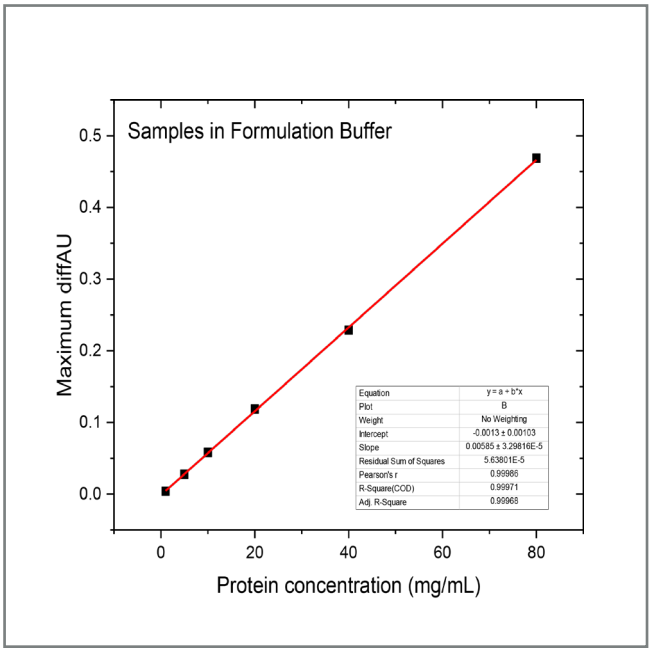
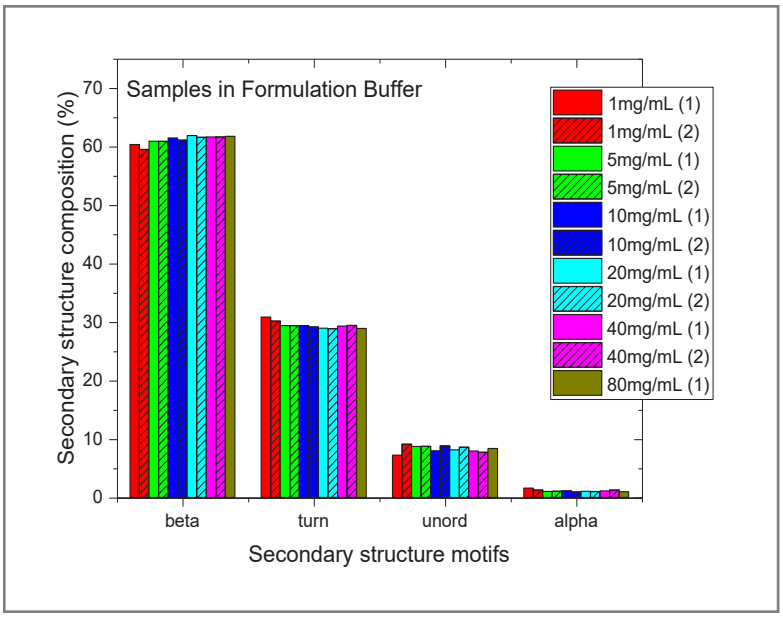
CHAPTER 3

Protein Analysis Capabilities







Data Source: Poster: Microfluidic Modulation Spectroscopy of a Biotherapeutic at Low to High Concentrations without Interference from Formulation Excipients, L. Wang et al.

Measuring AGGREGATION

Aggregation

Aggregation refers to the process by which proteins start to bind together under different conditions and formulations. Both upstream and downstream processing can cause aggregation, a common indicator of protein instability, which can result in a therapeutic product being unfit for launch.

MMS is a powerful technique for monitoring the onset and presence of aggregation during processing. It is one of the only techniques which can directly monitor the formation of aggregates due to its ability to measure intermolecular beta-sheet structures.

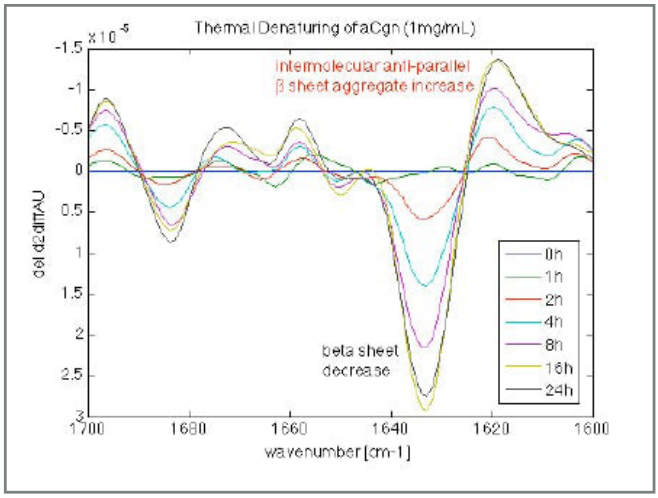
Stability and Aggregation

Since the **AQS³**pro can directly measure protein secondary structure, it is a powerful tool for monitoring and understanding the mechanisms of protein stability and aggregation. As proteins are subjected to stress and the protein begins to change from its native state, details of the process can be easily followed. IR measurements are particularly sensitive to beta-sheet structures, which dominate in protein antibody based drugs.

Thermal Stability

A high beta-sheet content protein at 1 mg/mL was incubated at an elevated temperature for differing periods of time. The protein series was measured using the AQS³pro and the second derivative spectra were overlaid and plotted to enhance the spectral changes. The data shown in the figure to the right clearly show the loss of intramolecular beta-sheet content as a function of incubation time. Simultaneously, the amount of intermolecular beta-sheet structure increases which is associated with the formation of aggregates.

Changes in other regions reflect the state of the protein sub-structure and provide additional details of the denaturation process.



Incubation of a 1 mg/mL high beta-sheet containing protein incubated at elevated temperature from 0 to 24 hours. As the incubation time increases the (intramolecular) beta-sheet content decreases and the intermolecular beta-sheet increases, indicative of aggregate formation.

As the protein was incubated, it was noted an insoluble aggregate formed and settled to the bottom of the sample tubes. In this study, only the supernatant fraction was decanted and measured. As a result, the overall concentration of soluble protein decreases at longer incubation times.

Measuring QUANTITATION

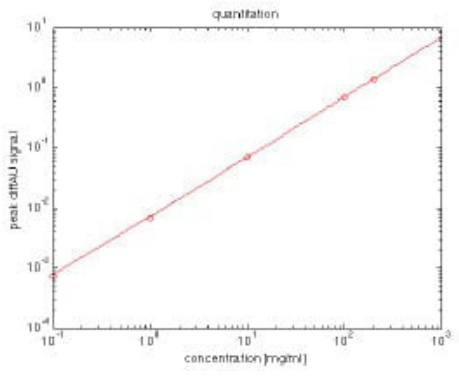
Quantitation

The structure and behavior of proteins in solution can be a function of their concentration. Accurate quantitation of protein concentration yields better analysis and comparison of results between different proteins and formulations. There is no universal analytical approach to quantitation due to the constraints of traditional techniques such as the limited dynamic range of traditional spectroscopic tools.

MMS technology is an effective alternative tool for direct, label-free protein quantitation over a wide concentration range and it is more selective than traditional spectroscopy instrumentation, with less susceptibility to interferences. MMS increases sensitivity and significantly reduces the errors common with conventional spectroscopy.

The Importance of Quantitation

Protein quantitation is critically important in biochemistry research and development labs with applications ranging from enzymatic studies to providing data for biopharmaceutical lot release. No one approach is universal due to the specific limitations of each approach, including aromatic residue dependency, chemical interferences in dye-based assays and the limited dynamic range of the spectroscopic tool.



Differential absorbance at ~1656 cm-1 (BSA peak) plotted as a function of concentration at 0.1, 1, 10, and 200 mg/mL. Note that even at the high concentration (200 mg/ml), MMS exceeds the dynamic range of conventional protein characterization tools.

Due to stray light and instrument slit width, conventional spectroscopic tools operate within limited dynamic ranges. This forces scientists to adjust either the sample concentration or the cell pathlength to acquire accurate protein quantitation. Either alternative can be time consuming and problematic in its effect on the measurement.

Infrared absorption spectroscopy can be an effective tool for direct, label free protein quantitation. It provides an advantage over UV/VIS methods as sample absorption bands in the infrared are much narrower and are not dependent on aromatic residues. As a result, the technique is more selective with less susceptibility to interferences. In addition, since the IR method probes the carbonyl backbone of the protein and is not dependent on a UV chromophore, the variation in extinction coefficient is much smaller which can be an advantage in measuring unknown proteins. However, IR spectroscopy has not been routinely used in situ due to its lower sensitivity, added cost, and difficulty of operation (i.e. background subtraction, water vapor interference, and narrow pathlength cells). RedShiftBio's MMS platform overcomes these issues by increasing sensitivity and significantly reducing the errors common to conventional spectroscopy. MMS's high resolution (<0.001 cm-1) and low stray light susceptibility increases the linear concentration range for the measurement by more than 2 orders of magnitude. The differential measurement of microfluidic modulation spectroscopy and direct control over laser power also improves linearity by reducing signal dynamic range and maintaining high detector linearity throughout the measurement range.

Using MMS, one or at most only a few wavelengths need to be measured. The figure to the left shows a plot at ~1656 cm-1 for BSA in the range from 0.1 to 200 mg/mL. With a minimum measurable concentration of less than 10 µg/mL and an upper limit of greater than 200 mg/mL, this technique offers a significant improvement over conventional absorbance-based assays.

Measuring STRUCTURE

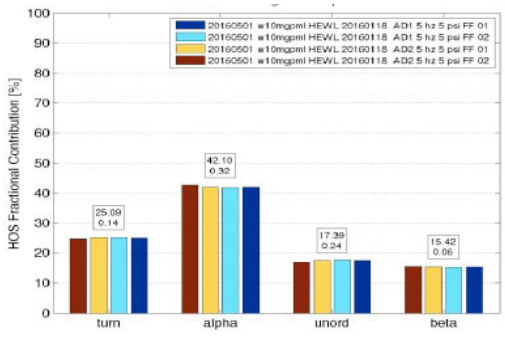
Structure

Protein structure is critical to its function. During the manufacturing process, biopharmaceutical proteins may undergo conformational changes which can alter their secondary structure and therefore their function. These changes are difficult to detect, and even harder to describe. Traditional analytical techniques are not great at detecting small differences in protein structure nor major changes seen in a small fraction of altered molecules in a larger sample in solution.

MMS has the ability to detect these changes with great sensitivity and accuracy, in the formulation buffer and at the concentration of interest, without the need for dilution or deuteration. MMS offers detailed information on which structural motifs in the protein molecule are changing, providing more guidance to scientists in their effort to develop more stable protein molecules and formulations.

Higher Order Structure

The amide I band (1700 – 1600 cm-1) probes the C=O stretch vibration of the peptide linkages which constitute the backbone structure of the protein. The differing pattern of hydrogen bonding, dipole–dipole interactions, and the geometric orientations in the alpha-helices, beta-sheets, turns, and random coil structures induce different absorption features in the amide I band that are well correlated with these second order structures.



Secondary structure for hen egg white lysozyme (HEWL) from 7 separate measurements of 10 mg/mL samples, taken over one month, showing a standard deviation of about 1%.

For traditional spectroscopy techniques, measurement capabilities are typically limited to concentrations above 5-10 mg/mL for Fourier Transform Infrared Spectroscopy (FTIR) and 30 mg/mL for Raman. Ultraviolet Circular Dichroism (UV-CD), currently one of the more prevalent tools for secondary structure analysis, is relatively insensitive to beta-sheet formation and has difficulty detecting the very important intermolecular beta-sheet structures which form during aggregation. The RedShiftBio analyzer is capable of measuring protein structure over a very wide dynamic range, from 0.1 mg/mL to over 200 mg/mL. This avoids the need for sample preparation steps such as dilution or pre-concentration which may introduce variability across samples, thus requiring sample replicates and multiple measurements.

Samples	Beta	Turn	Alpha	Unordered
0.1 mg/mL (1)	63.29	28.62	4.36	3.73
0.1 mg/mL (2)	63.59	30.90	1.71	3.81
Mean ± SD	63.44±0.21	29.76±1.61	3.04±1.87	3.77±0.06
1 mg/mL (1)	63.16	30.85	1.09	4.90
1 mg/mL (2)	64.37	30.68	1.24	3.71
1 mg/mL (3)	64.23	30.78	1.11	3.89
Mean ± SD	63.92±0.66	30.77±0.09	1.15±0.08	4.17±0.64
5 mg/mL (1)	63.50	30.83	1.05	4.62
5 mg/mL (2)	63.28	30.92	0.92	4.88
5 mg/mL (3)	63.40	30.73	0.75	5.11
Mean ± SD	63.39±0.11	30.83±0.10	0.91±0.15	4.87±0.25
12.3 mg/mL (1)	63.57	30.54	1.05	4.84
12.3 mg/mL (2)	63.71	30.82	0.94	4.53
12.3 mg/mL (3)	63.56	30.72	0.93	4.79
Mean ± SD	63.61±0.08	30.69±0.14	0.97±0.07	4.72±0.17

MMS measurements of higher order structures for a set of IgG1 samples ranging in concentration from 0.1 mg/mL to 12.3 mg/mL. *

While most technologies are restricted to performing protein analysis at concentration ranges of about one order of magnitude, measurements taken using the AQS³pro can be performed at concentration ranges spanning more than three orders of magnitude. And, the AQS³delta analytical software provides higher order structure information for five secondary structure components useful in protein fingerprinting.

*Data Source: Poster: Repeatability , Concentration Linearity and High Order Structure Analysis of an IgG1 Sample by Microfluidic Modulation Spectroscopy (MMS), L. Wang et al.

Measuring STABILITY

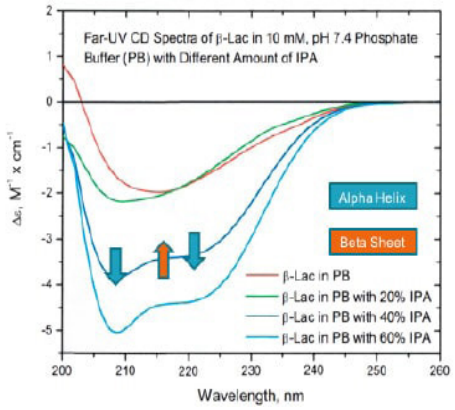
Stability

Because proteins are dynamic and behave differently in varying environments they become highly unstable and very sensitive to stress such as thermal or chemical exposure during manufacturing processes. Measuring how stable the protein is to chemical exposure during manufacturing and storage is critical. The impact of stress-induced structural changes can lead to decreases in potency, degradation of the product, and increases in impurities and aggregates which can be extremely harmful.

MMS technology enables the accurate assessment of the stability of the protein throughout the entire formulation, development, and manufacturing process, reducing risks and enabling control strategies for each critical quality attribute (CQA).

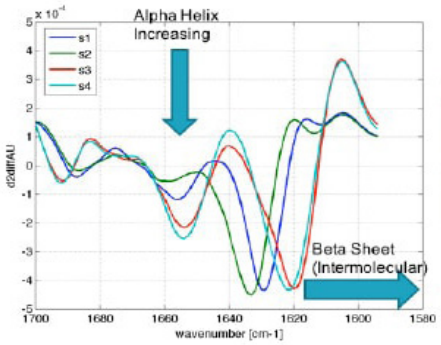
Chemical Stability

Chemical stress studies are another method of studying protein stability. Alcohols are well known to denature the native state of proteins and also tend to stabilize the alpha-helical conformation in unfolded proteins and peptides. A study of a relatively high concentration of beta lactoglobulin was formulated in phosphate buffer at pH 7.4 at 0, 20, 40 and 60% isopropyl alcohol (IPA) concentrations. The IPA/protein series was then measured by both far UV-CD and MMS to track the structural changes. In the UV-CD data shown above right, a clear increase in the alpha-helix structure occurs while a general decrease in beta-sheet is observed.



Far UV-CD studies of the chemical denaturation of beta lactoglobulin in IPA show increasing alpha-helix and decreasing beta-sheet.

In contrast, the AQS³pro measurements of the same samples show not only an increase in the alpha-helix form at higher IPA concentrations, it also shows a dramatic and clear shift in the beta-sheet type as noted by the shift of the band from ~1630 cm-1 to 1620 cm-1, again indicating the formation of intermolecular beta-sheet, something the CD does not readily show. Not only does the AQS³pro provide greater insight into the denaturation process, but it operates over a much wider range of concentrations.



Sample	IPA%
S1	0
S2	20
S3	40
S4	60

Protein characterization results obtained using the AQS³pro not only show the expected increase in alpha-helix with higher alcohol concentrations, but also show a shift in beta-sheet to the aggregate form of intermolecular beta-sheet.

Measuring SIMILARITY

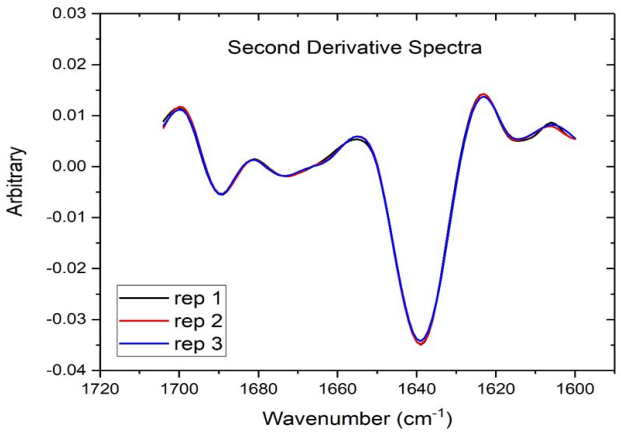
Similarity

Determining similarity for biologics is much more challenging than with small molecules due to their larger size and greater structural complexity. Along with functional comparisons, measurement and analysis of the structural similarity between proteins is an effective method of demonstrating bioequivalence. MMS measures protein secondary structure, reveals very small conformation differences between different proteins, and provides information as to where those differences occur. These capabilities make MMS a powerful tool in the analysis and development of biosimilars.

Quantitating Protein Similarity

Protein similarity is a quantitative approach for detecting small changes in protein secondary structure by analyzing and comparing the amide I band spectra between proteins. As the amide I band is very sensitive to changes in protein secondary structure as discussed previously, the ability to measure small differences in the spectra can be a powerful tool in monitoring the biosimilarity of a protein. A number of algorithms have been proposed for this comparison, including the correlation coefficient and the area of overlap. These results can be compared to published results using other methods to assess the sensitivity of the MMS method relative to more traditional methods such as FTIR or UV-CD.

The following figure shows the overlaid second derivative spectra of a mAb run at a concentration of 0.5 mg/mL on the AQS³pro. The spectra overlay with superior precision and the similarity of the measurements for samples run at both 0.5 mg/mL and 10 mg/mL are all above 98%.



Triplicate data (above) for a mAb run at 0.5 mg/mL, second derivative and calculated similarity scores (below) for these samples and samples run at 10 mg/mL.*

Similarity score of samples at low and high concentration runs from MMS

Sample conc. (mg/mL)	Similarity (%) of replicates			Mean±SD
0.5	98.78	98.84	98.71	98.78±0.07
*10	99.90	99.91	99.88	99.90±0.02

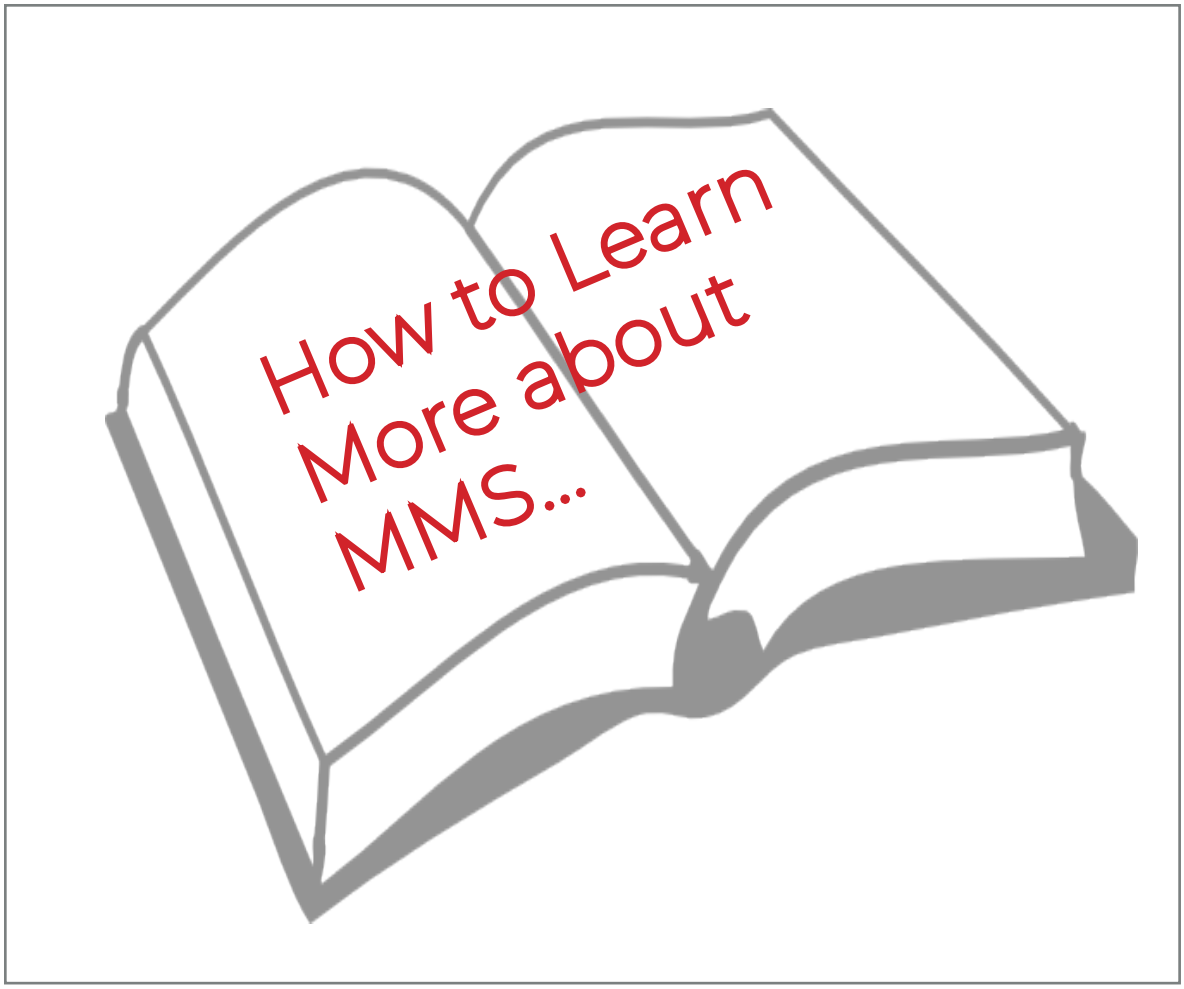
Similarity score is calculated by comparing the area of overlap (AO) and the mean of the three runs at 10 mg/ml is used as reference.

For comparison, FTIR values found in the literature show a mean similarity of 86.37% +/- 7.98% at a single concentration of 10 mg/mL for HEWL (Hen Egg White Lysozyme). Using FTIR, protein similarity values at the 97% level could only be obtained at a concentration of 50 mg/mL. For all tested applications, the AQS³pro achieves better similarity with less deviation over a concentration range that far exceeds the measurement capability of FTIR, while also addressing the limitations of UV-CD which, at higher concentrations, may require additional workflow and dilutions.

*Data Source: Poster: Microfluidic Modulation Spectroscopy (MMS) - a novel automated infrared (IR) spectroscopic tool for secondary structure analysis of biopharmaceuticals with high sensitivity and repeatability; Dipanwita Batabyal et al.

CHAPTER 4

MMS RESOURCES



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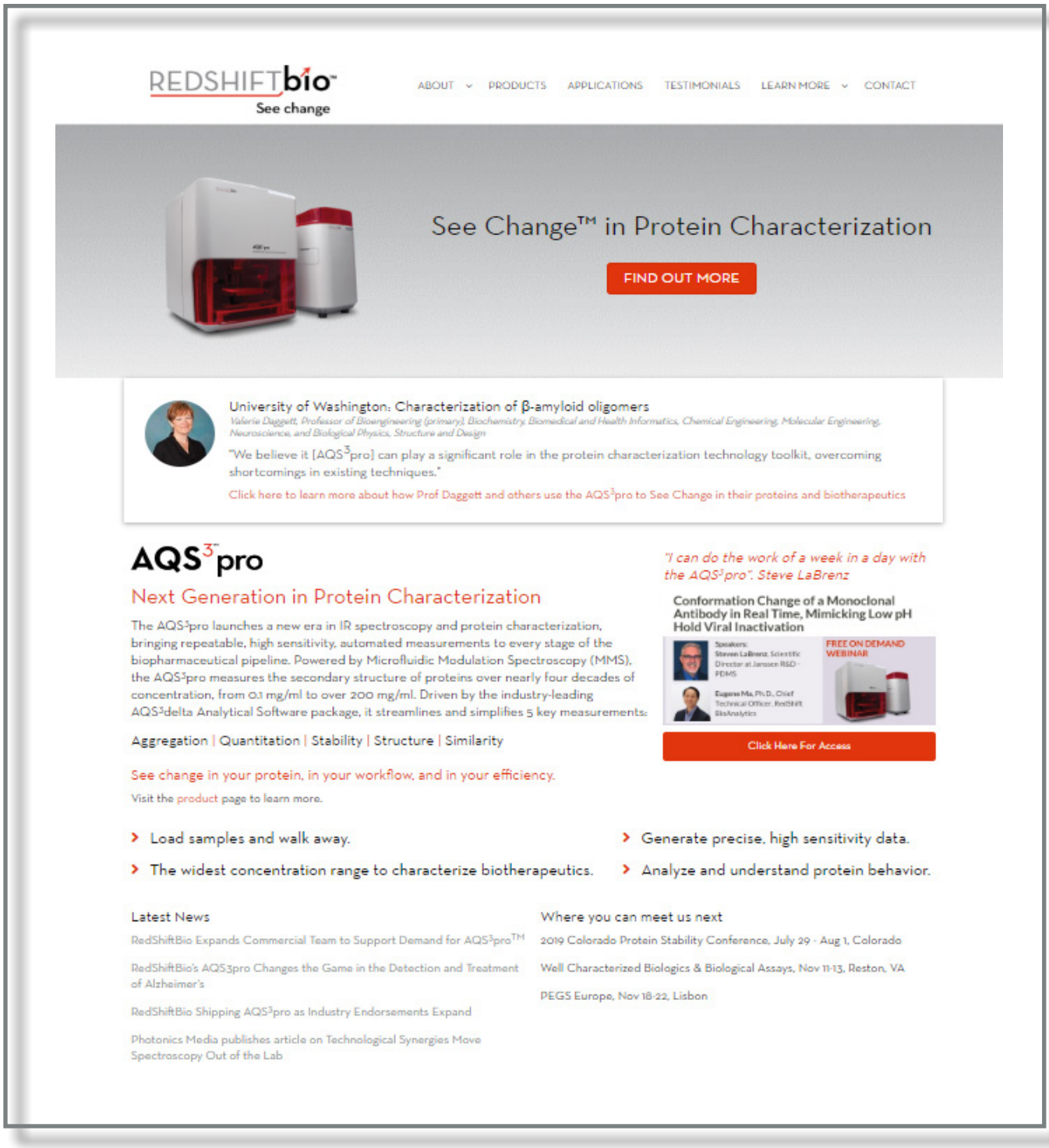

MMS Resource Library

WE'VE ONLY JUST STARTED!

Here is a link to the current MMS RESOURCE LIBRARY available to you at redshiftbio.com
Check back often to see how YOU can See Change™ in your applications.

Whitepapers and Application Notes:
MMS for Protein Therapeutic Drug Analysis- White Paper (2017-1)
Monoclonal Antibody Analysis by MMS in a Complex Formulation Buffer (1-150 mg/mL) (2019-03)
Thermal Denaturation Analysis of Bovine Serum Albumin over Wide Concentration Range by MMS (2019-01)
Microfluidic Modulation Spectroscopy (MMS) fills an Analytical Gap with a Lower LOQ for Measuring Protein Misfolds and Structural Similarity (2018-01)
Posters:
Enhanced Protein Structural Characterization using MMS
Early Events in Amyloid Formation by Lysozyme Detected by Microfluidic Modulation Spectroscopy
Repeatability, Concentration, Linearity and High Order Structure Analysis of an IgG1 Sample by Microfluidic Modulation Spectroscopy (MMS)
Structural Characterization of the Insulin-Degrading Enzyme by Microfluidic Modulation Spectroscopy
Thermal Denaturation Analysis of Bovine Serum Albumin by Microfluidic Modulation Spectroscopy
Microfluidic Modulation Spectroscopy Analysis of Monoclonal Antibody at Different Concentrations
HOS Study for IgG Samples Spiked with Different Amount of BSA by MMS
Microfluidic Modulation Spectroscopy (MMS)- a novel automated Infrared Spectroscopic tool for secondary structure analysis of biopharmaceuticals with high sensitivity and repeatability
Microfluidic Modulation Spectroscopy (MMS) of a Biotherapeutic at Low to High Concentrations without interference from Formulation Excipients
Articles:
Applications of MMS for Antibody-Drug Conjugate Structural Characterization
The Evolution of Spectroscopy: A New Technique for Biotherapeutic Formulation (Dec 2018)
Synthetic Peptide can inhibit toxicity, aggregation of protein in Alzheimer's Disease, researched show
Enhanced Protein Structural Characterization Using MMS
IBO Spotlight: RedShiftBio Debuts New Protein Analysis System
New Protein Characterisation System
BioPharm-8 eBook: Best Practices 2018
Technological Synergies Move Spectroscopy Out of the Lab
Webinars/Presentations:
Conformational Change of a Monoclonal Antibody in Real Time, Mimicking Low pH Hold Viral Inactivation
Using Microfluidic Modulation Spectroscopy to Monitor Protein Misfolds and Structural Similarity
Unveiling RedShiftBio's AQS3
Antibodies and Antibody-Drug Conjugate Higher Order Structures Revealed

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