Higher-Order Structure Assessment of a Protein Library by Microfluidic Modulation Spectroscopy (MMS)

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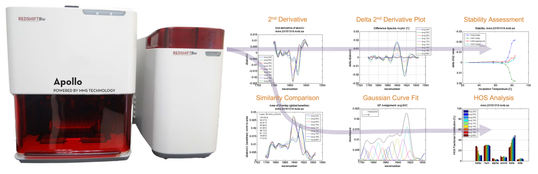
ABSTRACT

Microfluidic Modulation Spectroscopy (MMS) combines a quantum cascade laser (QCL) with microfluidic optics to provide unparalleled infrared-based secondary structure analysis for predominantly protein-based samples to support the biophysical toolkit. This study was performed utilizing a fully-automated platform on our second-generation MMS instrument, Apollo. MMS is ideally suited to a broad range of applications critical to the development and production of a successful and safe biotherapeutic with an ability to detect much smaller structural changes than traditional techniques over a wider sample concentration range of 0.1 to >200 mg/mL and in a variety of often troublesome buffers and excipients. The addition of robust, orthogonal structural information to complement data from other biophysical techniques enables gaining a better understanding of a biotherapeutic, such as by understanding the range of conditions under which a protein is natively folded, stable, and active. In this study, we analyzed a library of proteins with markedly different secondary structural elements to determine the effect of concentration, formulation, and small differences in primary sequence with the goal of characterizing higher order structure. Additionally, we compare the results generated with MMS to other well-validated biophysical protein analysis tools.

INTRODUCTION

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

MMS lends itself to the examination of formulation conditions because it is not limited in sensitivity by otherwise interfering excipients across the Amide-I region, thus allowing automated examination of many formulations in a single walk-away run.



Apollo MMS System and Example Spectral Processing Workflow

Additionally, the ability to assess the structural cooperativity of biomolecules allows the determination of self-association and monitoring aggregation events to prevent downstream failures and losses.

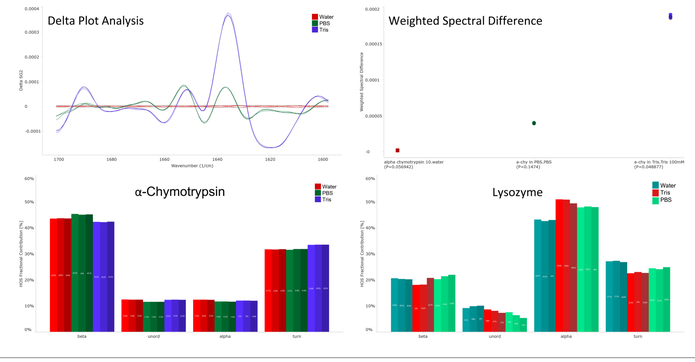
The power of MMS to provide insightful information for use alongside orthogonal analytical techniques means that MMS can be leveraged as a tool within the biophysical workflow to screen banks of formulation conditions, successfully monitor and alert to aggregation, and ultimately add important structural information for a growing protein library.

RESULTS

Formulation Screening using MMS

Protein structure is often directly related to function, and misfolding can also lead to the formation of undesired species that cause oligomerization and aggregation. These processes often involve structural change that can be measured by MMS in a wide range of formulation conditions to determine the best composition to prevent their occurrence. Here, changes in structure in response to 3 common buffers for two members of our protein library were investigated as an example. The ultra-high sensitivity of MMS measurements shows small structural differences between formulations in both cases, which could be used to determine when the native structure is best stabilized. This approach could be rapidly applied to large sample sets to rationally design formulations that improve protein function and mitigate undesired effects such as aggregation using the automation of MMS.

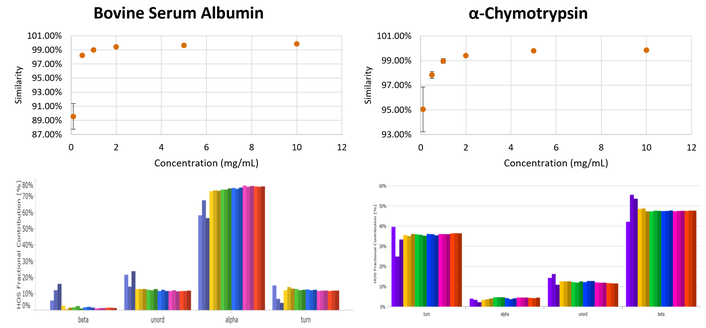
Figure 1. Assessing structural differences for 2 proteins, each in 3 buffers. Delta plot of second derivative MMS spectral differences (top left), and weighted spectral differences (top right), using water as a reference. Higher-order structure in each buffer for the proteins (bottom). Replicates shown as different shades of each colour.



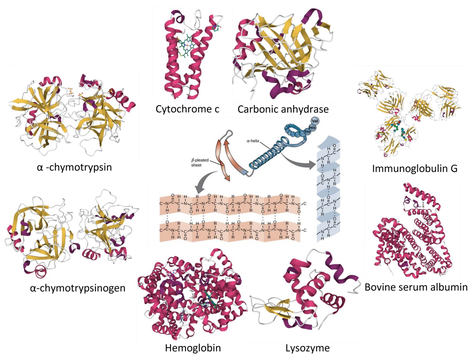
Structural Cooperativity Assessment to Determine Self-Association

The formation of oligomers and aggregates in protein-based systems are often undesired and problematic for researchers in biotherapeutics. Unexpected oligomer formation can also be undesirable for investigation of systems by other biophysical methods, such as for binding experiments which often assume monomeric components. MMS can be used to detect early signs of structural aggregation directly under formulation conditions by measuring often-small changes in Amide I band absorbance upon a self-association process. Here, two proteins from our library are shown as an example of such an experiment, which shows concentration-dependent structural change for Bovine Serum Albumin (left), but not observed for α-chymotrypsin (right). The sensitivity of MMS measurements is crucial here due to the low level of structural change observed.

Figure 2. Assessing structural changes with increasing concentration. MMS replicate reproducibility, averaged for 2 sets of 3 replicates, each collected on a different instrument (top). Higher-order structure changes with increasing concentration (bottom).



A Growing Protein Library



In order to determine secondary structure composition using MMS we focus on the Amide I band of the infrared region and use validated models from literature to deconvolute spectral information into simple outputs as shown on the right side of this section. We see good agreement between MMS and other infrared techniques, and by secondary structure prediction from x-ray crystallography data[2], and continue to add new members to our reference library as we encounter new structure types

Welcoming collaborations to expand our library! Please email us at dsloan@redshiftbio.com to analyze your system using MMS!

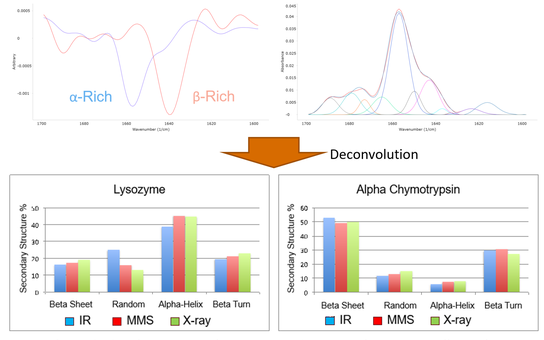


Figure 3. Good agreement between MMS, FTIR and X-ray crystallography data.[1] MMS second derivative spectra and an example of spectral deconvolution (top). Higher-order structure comparison (bottom).

CONCLUSIONS

Microfluidic Modulation Spectroscopy (MMS) greatly simplifies the process of collecting high-quality infrared spectra, automating sample handling, optical settings, cleaning and verification steps during operation. This technology also provides exceptionally high-quality data across a very broad concentration range of 0.1 to >200 mg/mL by measuring directly in almost all biological buffers and additives. With the ability to measure >0.76% structural change compared to FTIR (>23%) or CD (>3%)[2], we show here the ability to distinguish small structural differences between different formulations in order to assess whether a system shows structural cooperativity for members of a growing protein library database. This level of sensitivity is critical to a full understanding of a system and particularly for the development of protein biotherapeutics to prevent the formation of undesired species.

1) Kendrick, B. S. et. al., J. Pharma. Sci. 109, 933-36, 2020 2) Sarver R. W., and Krueger W. C., Anal. Biochem., 194(1), 89-100, 1991