Microfluidic Modulation Spectroscopy for Protein Characterization
A Case Study with EcoCRM®, CRM_{197} Carrier Protein

Andrew Lees*, Min-Ju Chang* and Libo Wang #
*Fina Biosolutions LLC, Rockville, MD; #RedShift Bioanalytics, Burlington, MA

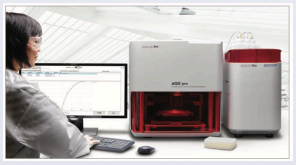
Introduction

The biophysical characteristics of biological drug products must be monitored from development through formulation and commercialization. Rapid screening of multiple parameters facilitates characterization under a wide range of conditions. This biophysical characterization is also useful in establishing the comparability of biosimilars.

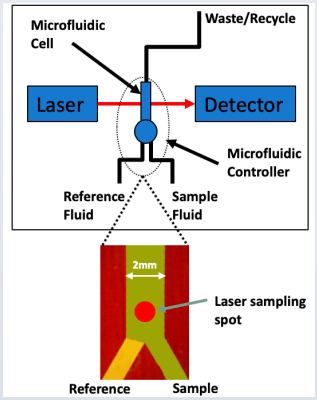
RedShift Bioanalytics has developed an instrument which uses microfluidic modulation spectroscopy to monitor five key biophysical properties of proteins: aggregation, quantitation, stability, similarity and structure. The instrument uses mid-infrared absorption spectroscopy and rapidly produces differential IR scans which measure the amide I band (the C=O stretching vibration of peptide backbone linkages). The amide I band is highly sensitive to changes in the secondary structure of the protein. The spectrophotometric technology and signal processing software achieves significant increases in sensitivity, dynamic range, and accuracy for determination of protein secondary structure relative to conventional mid-IR and far-UV CD techniques. The instrument works well for comparing samples across a wide dynamic range of concentrations and buffer conditions.

In this study, we evaluated the biophysical properties of CRM_{197}, a widely used conjugate vaccine carrier protein, produced by two different bacterial systems: EcoCRM® (Fina Biosolutions LLC, Rockville, MD) which is expressed as a soluble, intracellular, properly-folded protein in *E. coli* and CRM_{197} expressed in the periplasm of *Pseudomonas fluorescence* (Pfenex, Inc., San Diego). We compared the properties of CRM_{197} from the two sources. Both the *E. coli* and *Pseudomonas* expressed CRM_{197}. To further the formulation development, we also evaluated Fina Biosolutions' EcoCRM® stability under stress.

Microfluidics Modulation Spectroscopy Redshift Bio AQS^3 pro



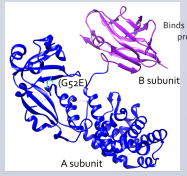
The protein sample is rapidly modulated across the laser with a matching water–buffer stream to produce highly sensitive, differential IR scans of the amide I band (1700–1600 cm-1) that provide detailed structural information. The amide I band is highly sensitive to its environment and thus can be used to monitor structural changes in the protein.



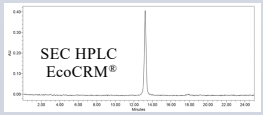
RedShiftBio's AQS^3 platform was used to collect the differential absorbance spectra. All the samples were tested at a modulation rate of 1 Hz and a back pressure of 5 psi. Duplicate or riplicate measurements were carried out for each sample. All the data was analyzed using AQS^3 delta software.

About CRM_{197}

CRM_{197} is a genetically detoxified diphtheria toxin that is widely used in conjugate vaccines. Conjugate vaccines consist of chemically linked "carrier" protein and antigen. CRM_{197} is the carrier protein in Pfizer's pneumococcal polysaccharide-protein conjugate vaccine, Prevnar®. CRM_{197} is a a multidomain 58 kDa protein with a single mutation which renders it nontoxic. Unlike toxoids, it has its full complement of lysines available for conjugation. Although historically CRM_{197} has been produced in *Corynebacterium diphtheriae* as a secreted protein, it has also been expressed in a proprietary *Pseudomonas* system (Pfenex). More recently, **Fina Biosolutions** has begun producing CRM_{197} as a soluble, properly folded, intracellular protein in *E. coli*. Marketed as EcoCRM®, for *E. coli* CRM_{197}, Fina Biosolutions' carrier protein has been extensively used in preclinical studies and will be available for clinical use in summer 2019. Previously published work compared the biophysical properties of EcoCRM® with CRM_{197} from several expression systems and showed comparability[2]. This work extends those studies and demonstrates the power of the AQS^3 microfluidics modulation spectroscopy to rapidly characterize vaccine proteins.



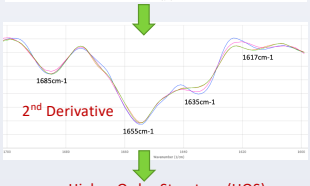
CRM_{197} (PDB ID: 4AEo)



CRM_{197} is a single chain with 2 disulfide bonds. The protein is easily "nicked" by a protease resulting in 2 polypeptides held together by a disulfide bond. The lack of lower MW bands in the presence of DTT on the SDS –PAGE gel, indicates stable, intact EcoCRM®.

Spectra overlay of EcoCRM® with a reference CRM_{197} allows structural comparability to be rapidly confirmed.

The diffAU spectra were normalized for concentration and buffer contribution to get the absolute spectra (absAU). Replicates from both samples overlaid very well. CRM_{197} from both sources look similar with the exception of the region from 1630 cm-1 through 1590 cm-1 and is likely due to a slight buffer mismatch.



Second derivative data of the absolute spectra is much more sensitive to very small changes in the spectra that are difficult to distinguish in the absolute spectra.

Higher Order Structure (HOS)

RedshiftBio's software uses a Gaussian curve fitting method to determine the higher order structure compositions (%). First, the Area of Overlap (AO) plots were derived from the second derivatives by baselining and smoothing. Next, the AO plots were fitted by an array of subpeaks that correspond to certain secondary structure motifs to resolve the composition of each peaks, i.e. each secondary structure. These were used to determine the % α-helix, β-sheet, β-turn and unordered structures.

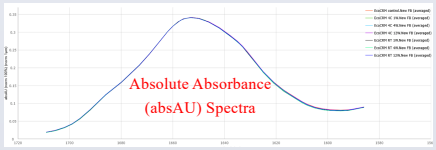
Structure %	Pfenex CRM (rep 1 and rep 2)		*EcoCRM®* (rep 1 and rep 2)	
Turn	27.48	29.29	29.08	28.45
Alpha	26.20	28.10	25.81	27.06
Unordered	14.45	10.93	12.60	12.27
Beta	31.86	31.67	32.50	32.22

Using RedShift Bio's MMS, Fina Bio's EcoCRM® was found to be highly comparable with Pfenex CRM_{197}, consistent with other structural studies[2].

EcoCRM® structure analyzed under different stress conditions.

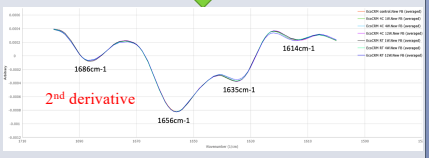
EcoCRM® SAMPLES

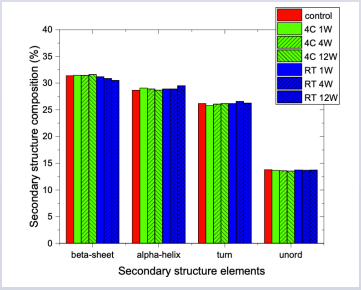
4°C	R. Temp
Control	Control
1 week	1 week
4 week	4 week
12 week	12 week



The absAU spectra of sample EcoCRM P002-01092019 under different stress conditions are well matched indicating similar secondary structure profiles of these samples.

The second derivative spectra of sample EcoCRM® under different stress conditions are overlaid well indicating similar secondary structure profiles of these samples.





Higher Order Structure (HOS)

HOS analysis shows that the control sample and the stressed samples are virtually identical., even up to 12 weeks at room temperature.

Redshift Bio's AQS^3 microfluidic modulation spectroscopy allowed for rapid characterization during protein development and formulation and detected subtle changes that were not found using other methods..

References

1. Enhanced Protein Structural Characterization Using Microfluidic Modulation Spectroscopy. Ma et al., Spectroscopy 33:1, 2018

2. Analytical Comparability Assessments of Five Recombinant CRM197 Proteins from Different Manufacturers and Expression Systems. Hickey et al., J. Pharm Sci, 107:1806 2018

For more information

EcoCRM® : Andrew Lees Info@Finabio.com
Redshift Bio: John Linnan Jlinnan@RedshiftBio.com