Repeatability , Concentration Linearity and High Order Structure Analysis of an IgG1 Sample by Microfluidic Modulation Spectroscopy (MMS)

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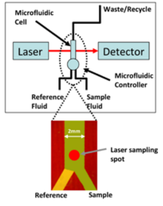
Abstract

Microfluidic Modulation Spectrometry (MMS) is a novel protein characterization technique that combines a microfluidic cell and a tunable mid-IR quantum cascade laser source to assess the comparability, similarity, quantitation linearity, aggregation, and denaturation of proteins by analyzing the absorbance spectra and the high order structure (HOS). To evaluate the data quality and performance of MMS, an IgG1 sample was analyzed at different concentrations ranging from 0.1 mg/mL to 12.3 mg/mL using a RedShiftBio AQS^{3}pro pre-production instrument. The differential absorbance (diffAU) spectra across the amide-I band were measured, the absolute absorbance (absAU) spectra were calculated, the Area of Overlap (AO) plots and the similarity of the samples were compared and the high order structure was elucidated. Our results show that the diffAU spectra of replicates for each sample concentration are very closely matched indicating a high repeatability and accuracy of the measurements. Furthermore, the absAU spectra of the IgG1 samples at 0.4-12.3mg/mL overlay very well suggesting comparable secondary structures and conformations over this concentration range. When comparing the AO plots to the mean AO plot of 1 mg/mL sample replicates, the similarity is about 93% for 0.1 mg/mL sample, 96% for 0.2 mg/mL sample, and 98-99% for samples at 0.4, 0.6, 0.8, 1, 5 and 12.3 mg/mL, showing that the MMS tool offers very reproducible measurements at concentrations above 0.4 mg/mL and relative good measurements at as low as 0.1 mg/mL. The Gaussian curve fit also finds very consistent secondary structure contents for samples at 0.4-12.3mg/mL, i.e. 63-64% beta-sheet structure, 29-31% turn structure and very small amounts of alpha-helix and unordered structures. In addition, the maximum diffAU signal versus protein concentration data fits a straight line with an R^2 value of 0.999, displaying very good quantitation linearity of the measurement in the range of 0.1 to 12.3 mg/mL. The MMS has proved to be a powerful protein characterization technique to provide comparability, similarity, quantitation linearity and HOS measurements of protein samples.

Introduction

RedShiftBio has developed a novel infrared spectroscopy tool for protein characterization and structural analysis based on Microfluidic Modulation Spectroscopy (MMS). This technology achieves significant increases in sensitivity, dynamic range, and accuracy for determination of protein secondary structure comparing to conventional mid-IR and far-UV CD techniques. The MMS instrument utilizes a tunable mid-IR quantum cascade laser to generate an optical signal over 100X brighter than the conventional sources used in FTIR spectroscopy. Brighter sources also allow the use of simpler detectors without the need for liquid nitrogen cooling. Additionally, 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. A simplified diagram of the instrument and a picture of a MMS preproduction platform are shown to the right. To evaluate the data quality and performance of the MMS tool, a Pfizer-supplied IgG1 sample was tested at different concentrations using an MMS platform, AQS^{3}pro Beta4 and analyzed using AQS^{3}delta software.



Methods

Duplicates of an IgG1 sample at different concentrations, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 5 and 12.3 mg/mL, were analyzed at ambient temperature using a RedShiftBio AQS^{3}pro preproduction unit with multi-sample automation. A microfluidic transmission cell of approximately 23.8 µm pathlength was used. The sample and the buffer fluids were modulated at a frequency of 1 Hz and a pressure of 5psi. 33 discrete wavenumbers across the amide-I band from 1714 cm^{-1} to 1590 cm^{-1} were scanned and the differential absorbance spectra were collected. Replicate measurements were carried out for each sample. The data was analyzed using AQS^{3}delta, RedShiftBio's proprietary data analysis software, to produce the final spectral plots and results.

Results I. Differential absorbance spectra (diffAU) – the repeatability of replicates and the dynamic concentration range

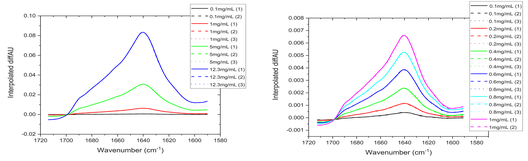


Figure 1. diffAU of two sets of IgG1 samples. The diffAU of replicates for each sample are overlaid very well indicating a great repeatability and accuracy of data measurement.

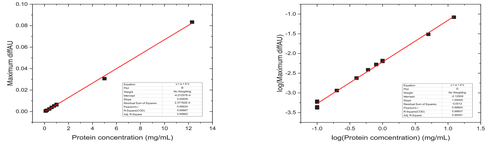


Figure 2. Dynamic concentration range for the IgG1 samples. Left: R^2 is 0.999; Right: R^2 is 0.996, suggesting an excellent concentration linearity.

Results II. Absolute absorbance spectra (absAU) – sample comparability

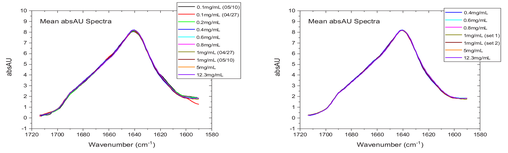


Figure 3. The mean absAU of IgG1 samples. Samples at concentrations from 0.4 to 12.3 mg/mL (right) show very closely matched spectra, indicating comparable secondary structures and conformations for these samples.

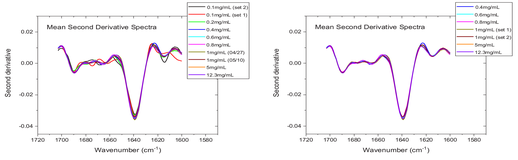


Figure 4. The mean second derivative spectra of IgG1 samples show consistent results that samples at concentrations from 0.4 to 12.3 mg/mL (right) are very comparable to each other

Results III. Area of overlap plots – sample similarity

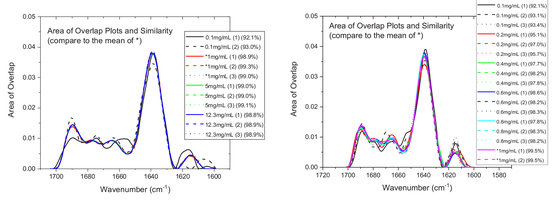


Figure 5. The area of overlap plots for set one IgG1 samples. The similarity data is obtained when compared to the mean AO of three 1mg/mL sample replicates and included in the legends. Samples at 0.4mg/mL and above have similarity at 98+%.

Results IV. High order structure analysis

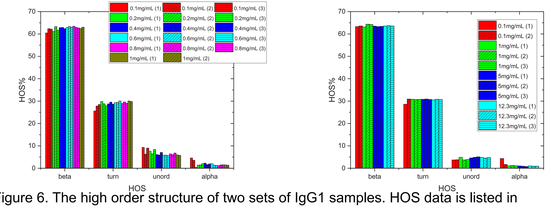


Figure 6. The high order structure of two sets of IgG1 samples. HOS data is listed in the following tables.

Samples	Beta	Turn	Alpha	Unordered
0.1 mg/mL (1)	60.46	25.60	4.62	9.32
0.1 mg/mL (2)	62.35	27.79	3.56	6.30
0.1 mg/mL (3)	62.16	28.48	0.36	9.00
Mean ± SD	61.66±1.04	27.29±1.50	2.85±2.22	8.21±1.46
0.2 mg/mL (1)	61.14	29.83	1.43	7.60
0.2 mg/mL (2)	63.30	28.78	1.47	6.44
0.2 mg/mL (3)	61.69	27.89	2.04	8.37
Mean ± SD	62.04±1.12	28.83±0.97	1.65±0.34	7.47±0.97
0.4 mg/mL (1)	62.84	28.77	2.27	6.12
0.4 mg/mL (2)	62.92	29.54	1.64	5.91
0.4 mg/mL (3)	62.34	28.65	1.94	7.07
Mean ± SD	62.70±0.31	28.99±0.48	1.95±0.32	6.37±0.62
0.6 mg/mL (1)	62.86	29.30	2.06	5.77
0.6 mg/mL (2)	63.34	29.43	1.36	5.87
0.6 mg/mL (3)	62.989	30.05	1.33	5.63
Mean ± SD	63.06±0.25	29.59±0.40	1.58±0.41	5.76±0.12
0.8 mg/mL (1)	63.47	28.93	1.18	6.43
0.8 mg/mL (2)	62.90	29.59	1.48	6.03
0.8 mg/mL (3)	62.67	29.01	1.53	6.78
Mean ± SD	63.01±0.41	29.18±0.36	1.40±0.19	6.41±0.38
1 mg/mL (1)	62.44	30.08	1.50	5.99
1 mg/mL (2)	63.03	29.81	1.38	5.78
Mean ± SD	62.74±0.42	29.95±0.19	1.44±0.08	5.89±0.15

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

Conclusions

- The diffAU spectra of replicates for each sample are very closely matched indicating a high repeatability and accuracy of the measurements;
- The absAU spectra of the IgG1 samples at 0.4-12.3mg/mL overlay very well suggesting comparable secondary structures and conformations of these samples;
- When comparing the AO plots to the mean AO plot of 1 mg/mL sample replicates, the similarity is about 93% for 0.1 mg/mL sample, 96% for 0.2 mg/mL sample, and 98-99% for samples at 0.4, 0.6, 0.8, 1, 5 and 12.3 mg/mL, showing that the MMS tool offers very reproducible measurements at concentrations above 0.4 mg/mL;
- The Gaussian curve fit also finds very consistent secondary structure contents for samples at 0.4-12.3 mg/mL, i.e. 63-64% beta sheet structure, 29-31% turn structure and very small amounts of alpha-helix and unordered structures;
- The maximum diffAU signal versus protein concentration data fits a straight line with an R^2 value of 0.999 displaying great quantitation linearity of the measurement in the range of 0.1 to 12.3 mg/mL;
- MMS tool has shown to be a powerful protein characterization tool providing improved repeatability, concentration linearity and high order structure measurements of protein samples for enhanced comparability and similarity analysis.

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