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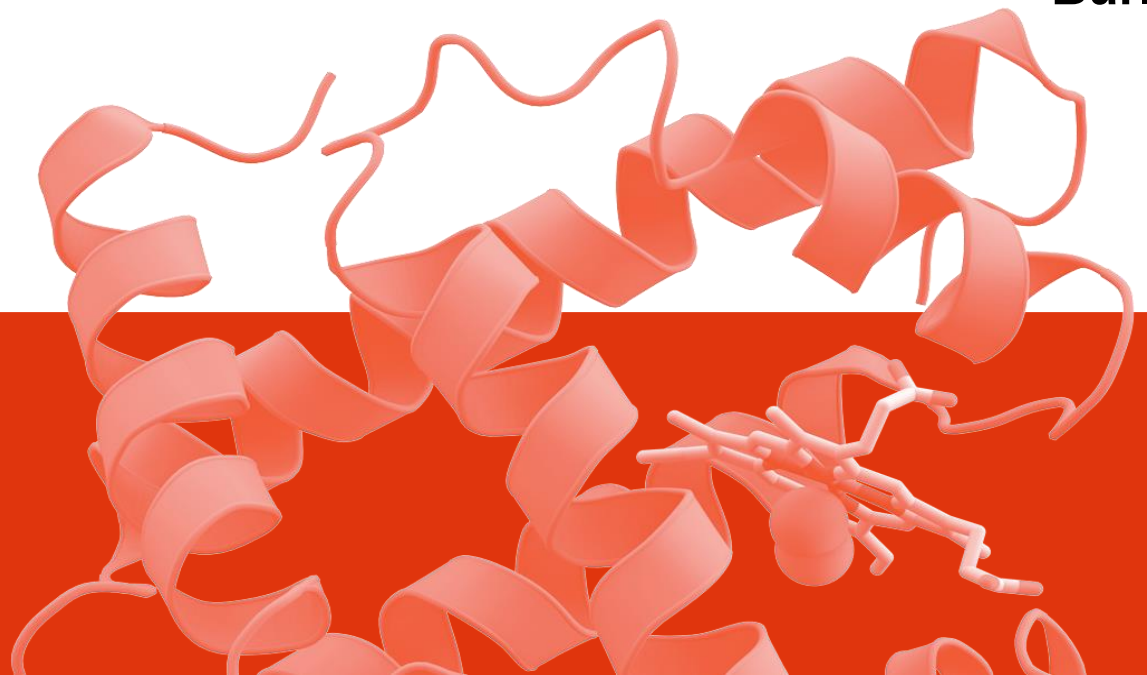
See change

Microfluidic Modulation Spectroscopy of a Biotherapeutic at Low to High Concentrations without Interference from Formulation Excipients

Jeffrey Zonderman, COO
RedShiftBio
Burlington, MA



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Presentation Outline

- About RedShiftBio
- Current State Protein Characterization & Needs
- Microfluidic Modulation Spectroscopy Technology
- AQS^{3}pro System
- Case Studies
- Summary

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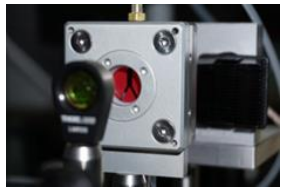
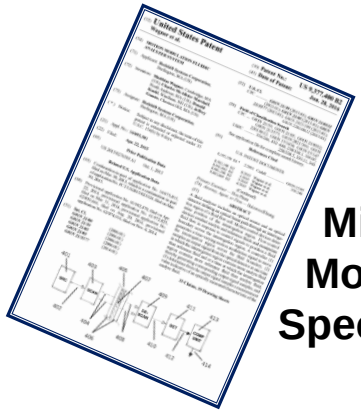
RedShift BioAnalytics, Inc.

- **REDSHIFTbio™:** Massachusetts based company backed by two of the largest life science instrumentation companies.
- **MMS**: Inventor of Microfluidic Modulation Spectroscopy, a powerful new technology for characterizing analytes in fluids.
- **Protein Analytics.** AQS^3*delta* analytical software for both automated and scientific hands-on analysis.
- **Instrumentation**: Innovative instruments for better characterization and monitoring of therapeutic proteins in development, manufacturing and release.

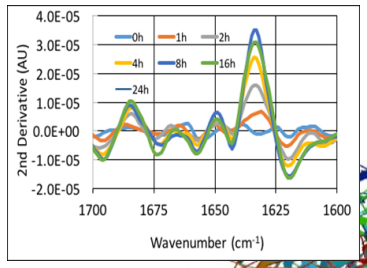


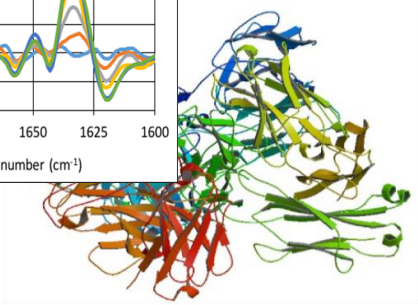
Infrared Microfluidics



Microfluidic Modulation Spectroscopy

Protein Analytics Software





Protein Measurement Solutions

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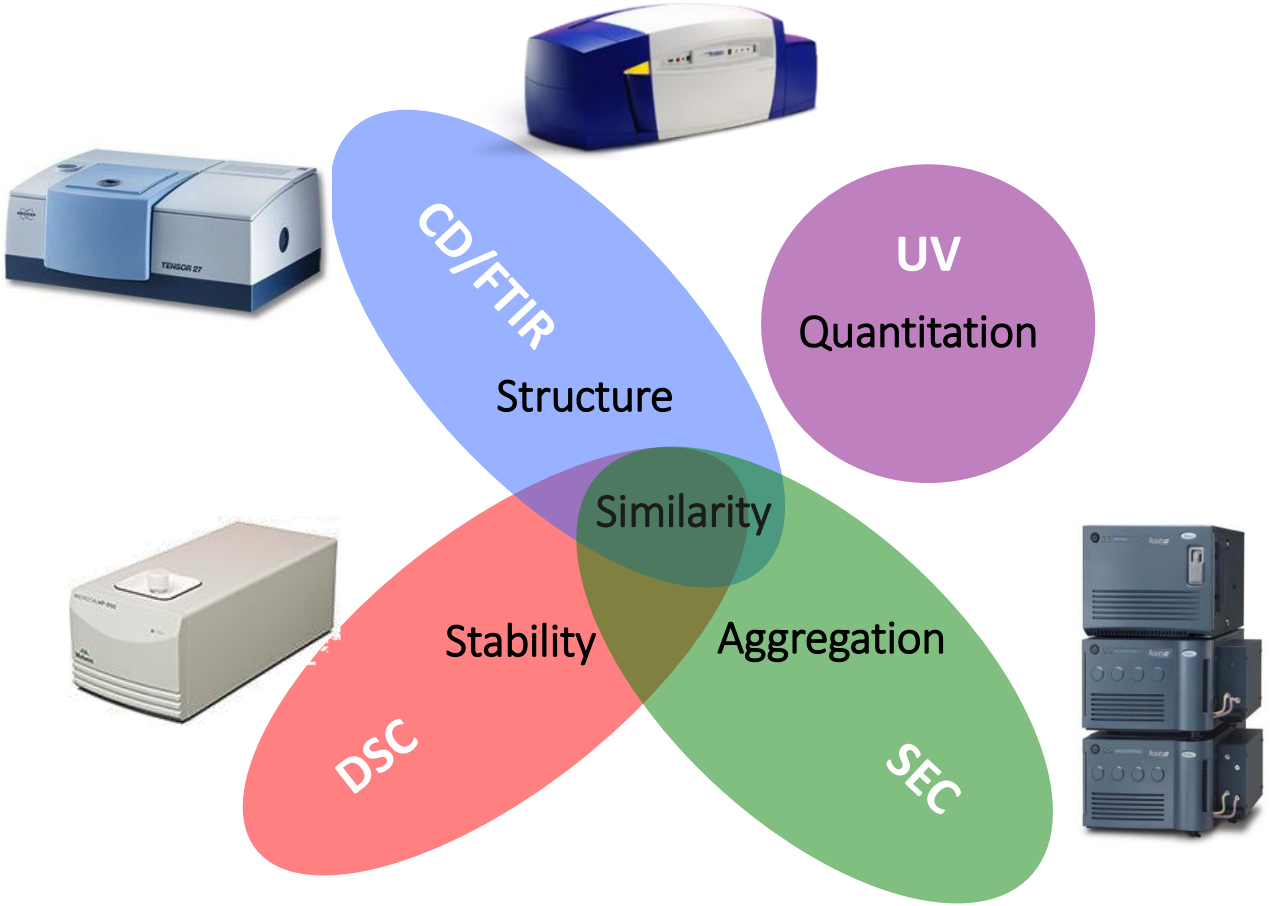
Limitations of Today's Tools



Narrow concentration ranges

Limited sensitivity for detecting change

Limited characterization per platform

Complex workflows

DSC - Differential Scanning Calorimetry, CD – Circular Dichroism, HPLC-SEC - High Performance Liquid Chromatography-Size Exclusion Chromatography, UV– Ultraviolet

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The *Missing Pillar* in Automated Protein Characterization

Protein
Sample Prep

SEC

Quaternary Structure

Secondary Structure Characterization Tools Considered

- **Circular Dichroism**– only works with low concentrations(0.05 -1.0 mg/mL); susceptible to excipients; buffer exchange may be required
- **FTIR** – needs >10mg/mL; lacking reproducibility, no automation.

Currently is no acceptable secondary structure solution.

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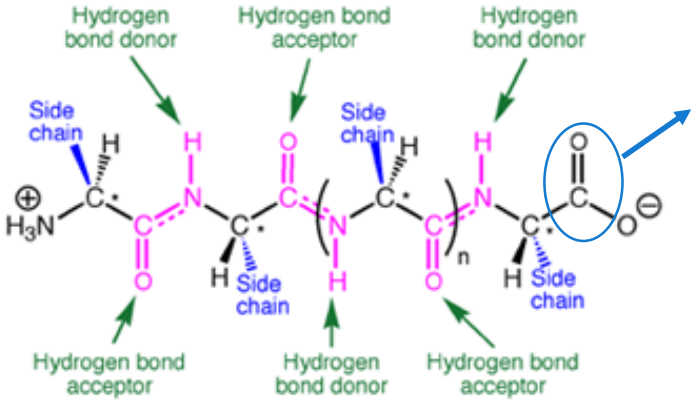
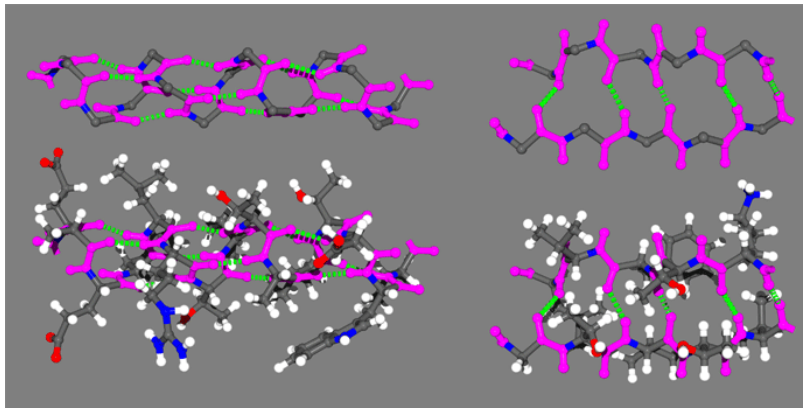
Infrared Protein Characterization (Secondary Structure)

- Infrared (IR) spectroscopy directly probes the protein backbone hydrogen bonding indicating the local structure
- Long recognized as an extremely powerful tool for protein analysis
- Unable to be exploited due the current state of infrared measurement technology (e.g. FTIR)



Amide I Band
1600-1700 cm^{-1}



Alpha helix

Beta sheet

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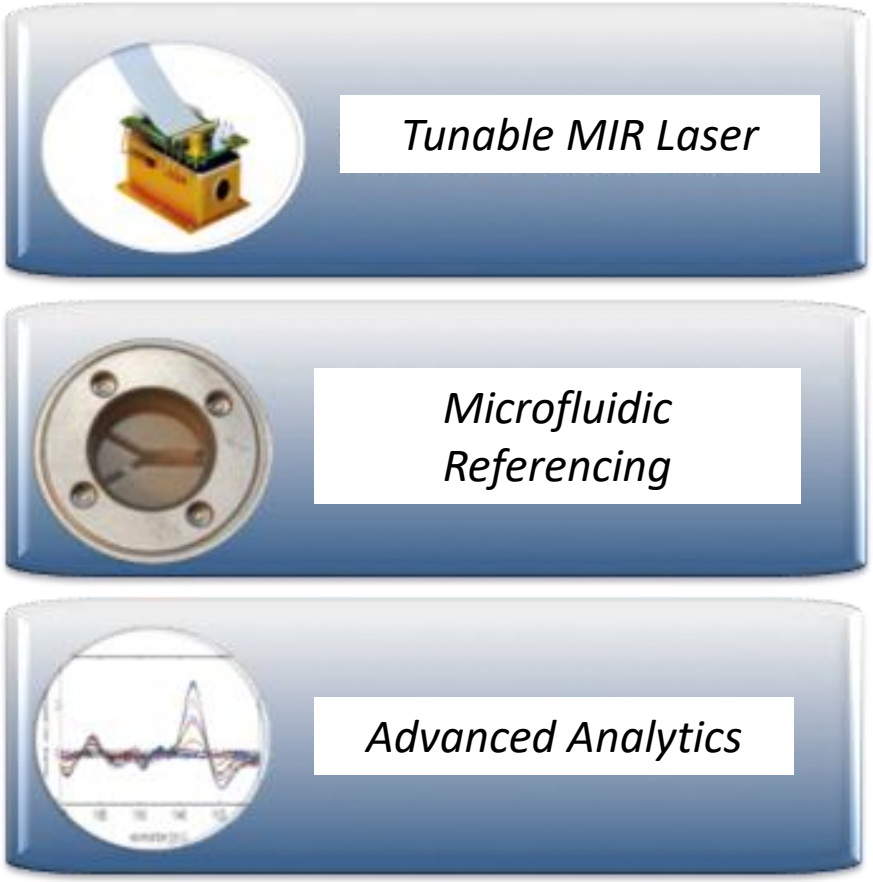
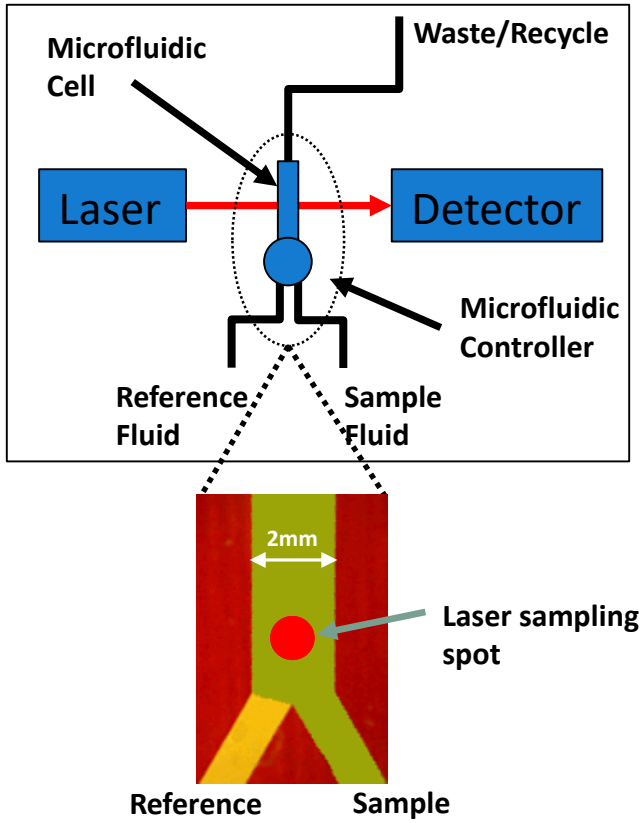
Microfluidic Modulation Spectroscopy (MMS)





Automatic and continuous background referencing significantly improves sensitivity

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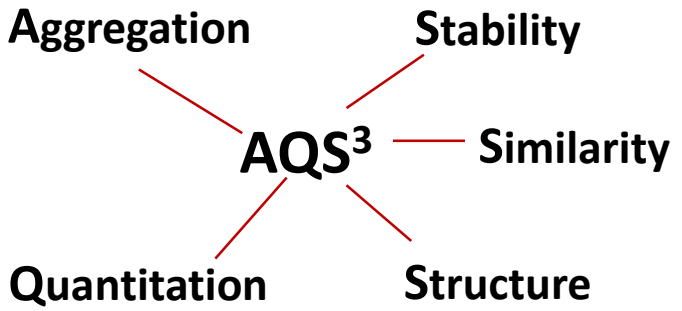
AQS³pro With AQS³delta Software

- ✓ 5 key measurements in a single analysis.
- ✓ 30X improvement in sensitivity.
- ✓ Widest concentration of any structural analysis platform, from 0.1mg/mL to > 200 mg/mL.
- ✓ Integrated multi-sample capability for up to 20X savings in direct labor.
- ✓ Automated protein analytics fulfilling the needs of both the operator and the scientist.



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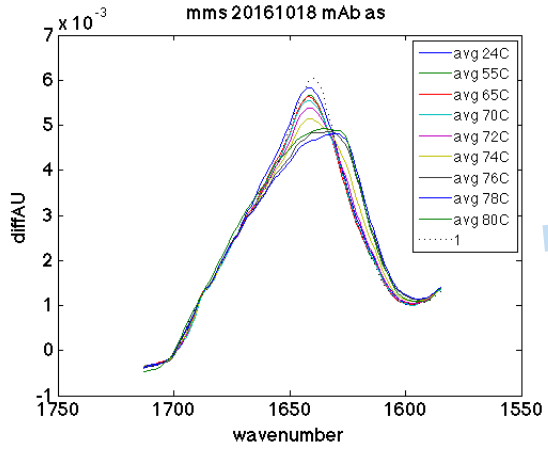
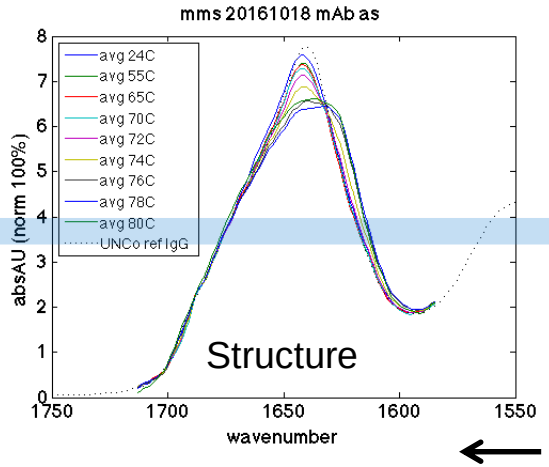
AQS³delta Software Analytics Process





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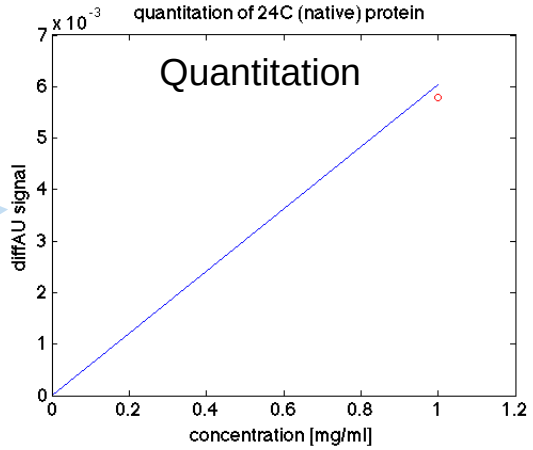
Typical experiment 1: mAb fragment

- Aggregation vs incubation temp
- 9 samples: 24C → 80C (nominal)
- 32 min incubation time
- 1mg/mL in buffer (PBS)



Blue: "complete" mAb reference
Red: mAb fragment

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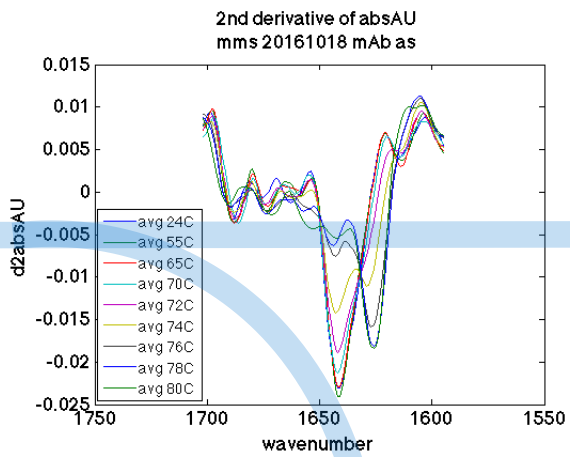
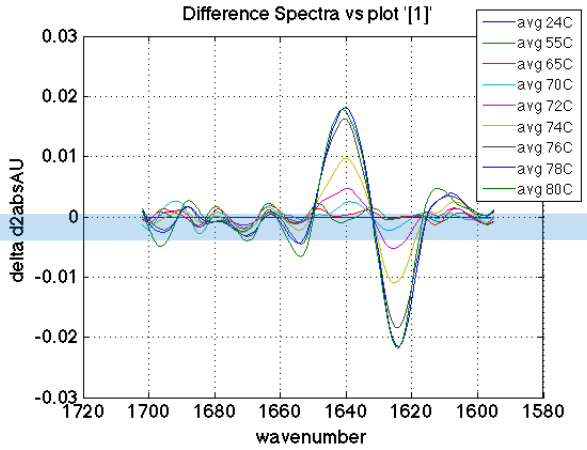
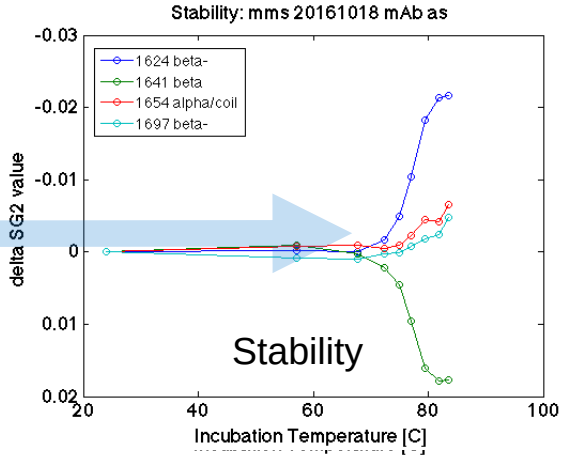
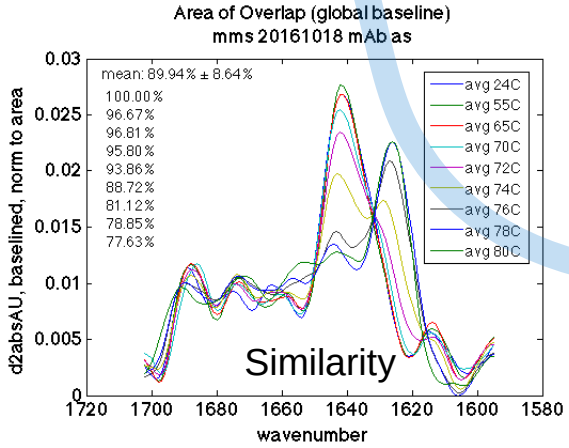
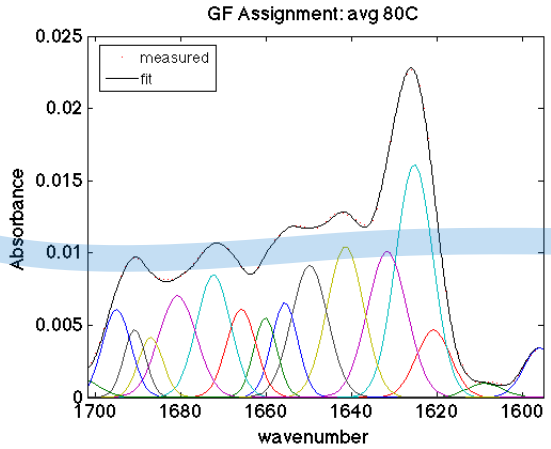
AQS³delta Software Analytics Process (con't)

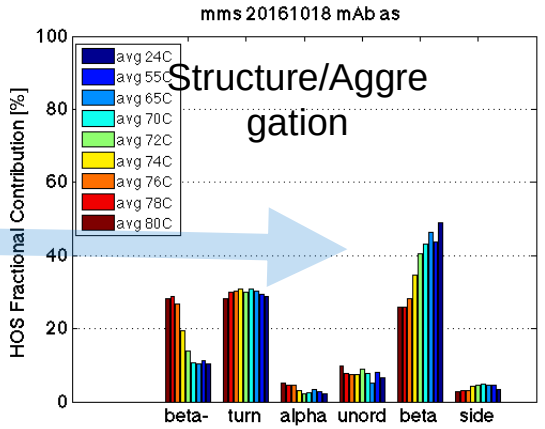












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AQS³pro Workflow

Step 2 – Choose test protocols

Step 3

Press Start. No worries about references, drift or background interferences

Step 4 - Walk away

Step 5 - View results at your desk

Step 1 - Load water, buffer and samples

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Secondary Structure Pillar in Automated Protein Characterization

Protein
Sample Prep

SEC

Quaternary Structure

DLS

Tertiary Structure

AQS³pro

Secondary Structure

MS

Primary Structure

High performance & automation enables cost effective secondary structure analysis.

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Case Study: Resolving structural impurities

Objective

Insights into the detectability of misfolded species (using BSA spiked into IgG1 at various percentages).

Brent Kendrick, Ph.D.

Elion Labs, a division of KBI Biopharma, Inc.

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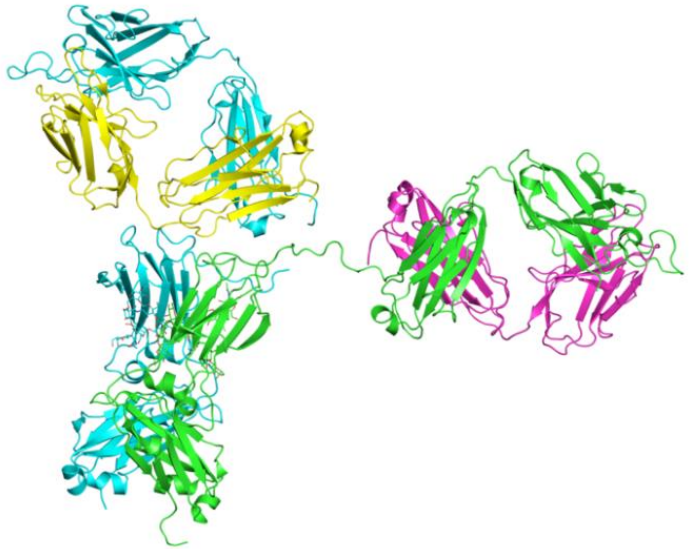
Proteins representing β-sheet and α-helix

IgG1

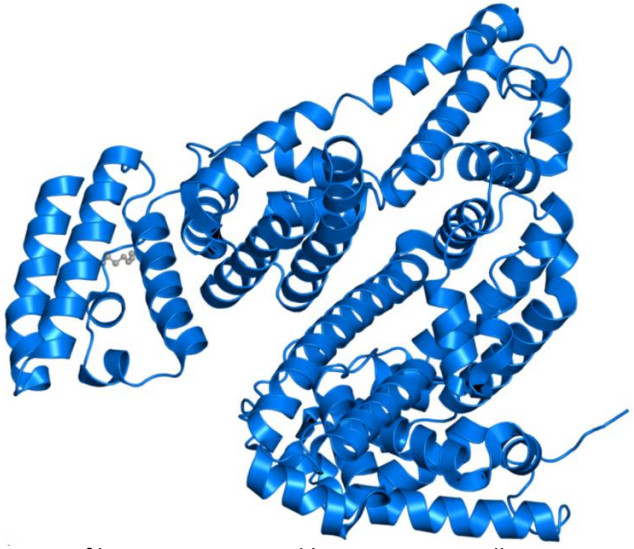
Secondary Structure	Percent
β-strand	86.5
α-helix	12.3
Turn	1.2



Crystal structure of a neutralizing human IGG against HIV-1: a template for vaccine design. Saphire EO, Parren PW, Pantophlet R, Zwick MB, Morris GM, Rudd PM, Dwek RA, Stanfield RL, Burton DR, Wilson IAScience 293 1155-9 (2001). (pdb: 1HZH). Structure content from: http://www.uniprot.org/uniprot/P01857#structure

BSA

Secondary Structure	Percent
β-strand	3.9
α-helix	94.3
Turn	1.7



Structures of bovine, equine and leporine serum albumin. Bujacz A Acta Crystallogr. D Biol. Crystallogr. 68 1278-89 (2012) PMID: 22993082 (pdb: 4F5S). Structure content from: http://www.uniprot.org/uniprot/P02769#structure

elion

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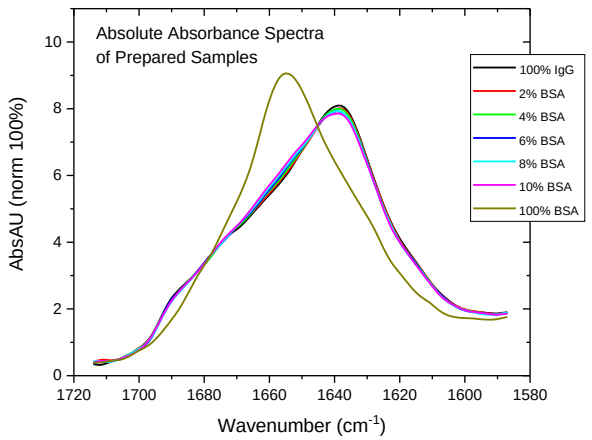
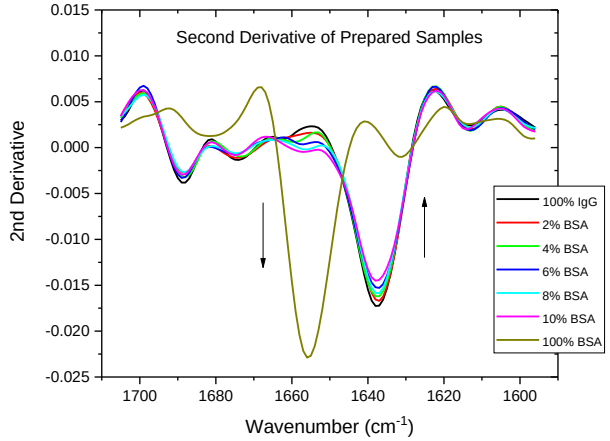
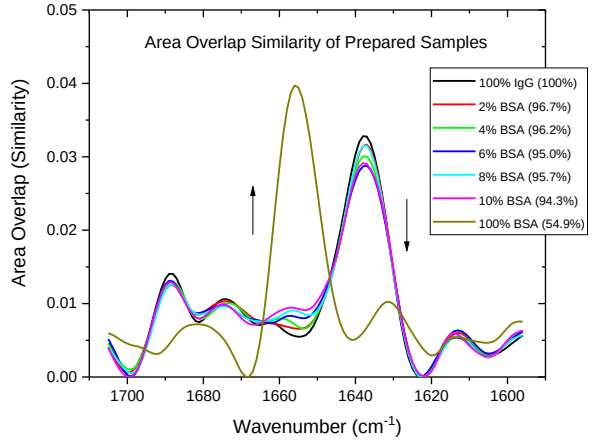
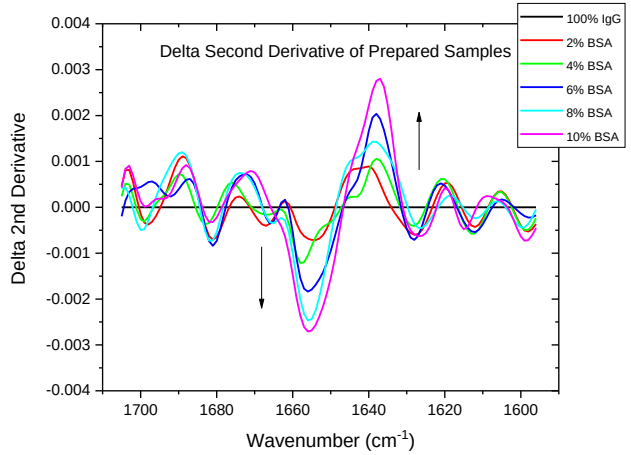
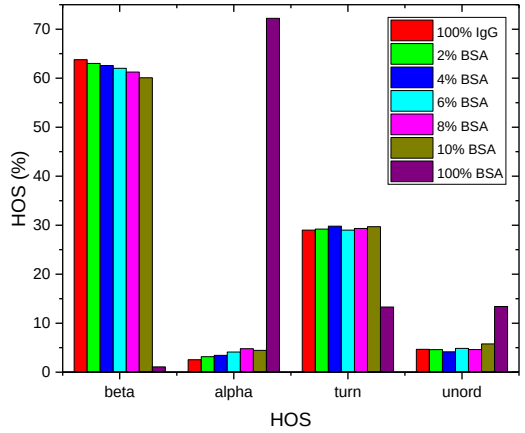
HOS Study of IgG Spiked with Different Amounts of BSA



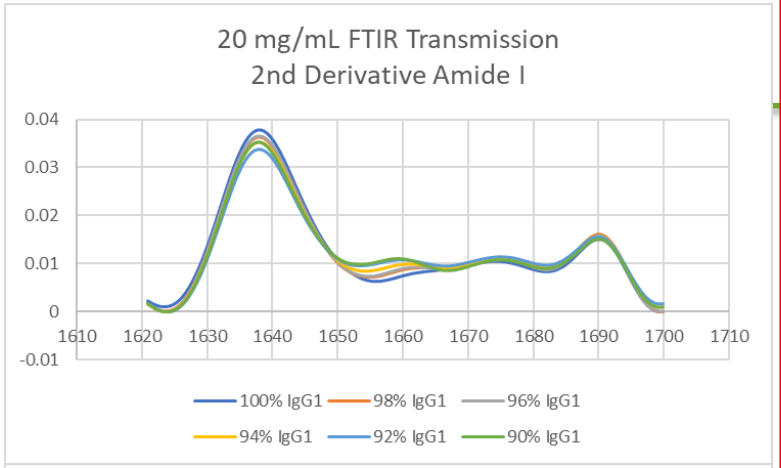


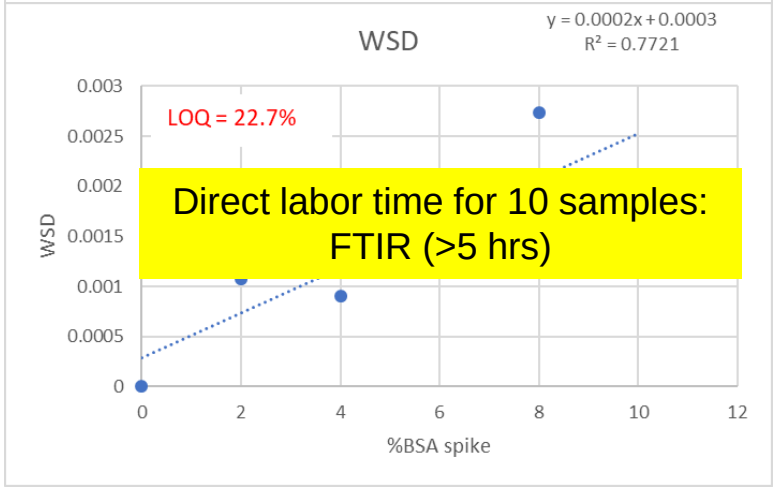




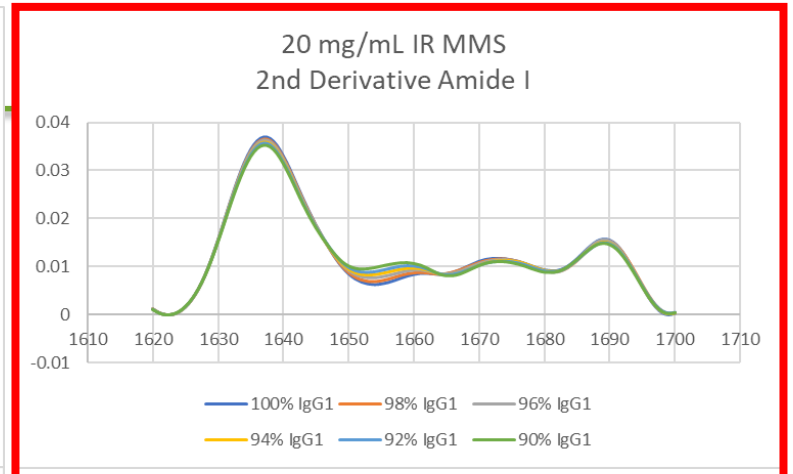


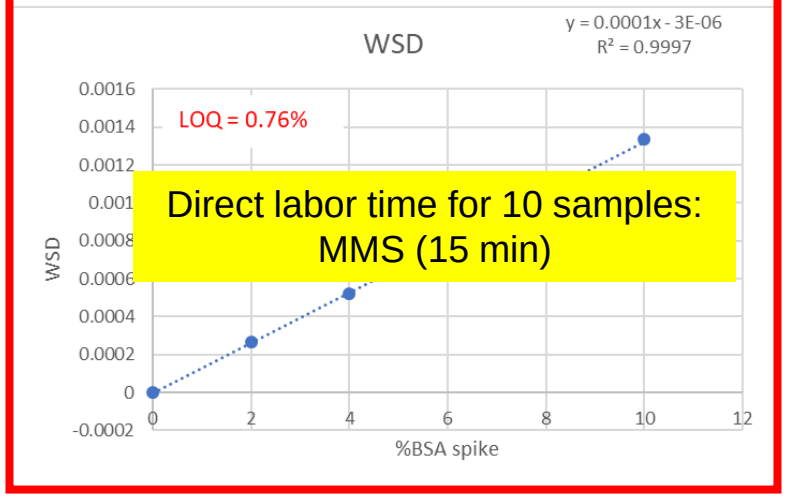
Detectability of Different Measurement Techniques



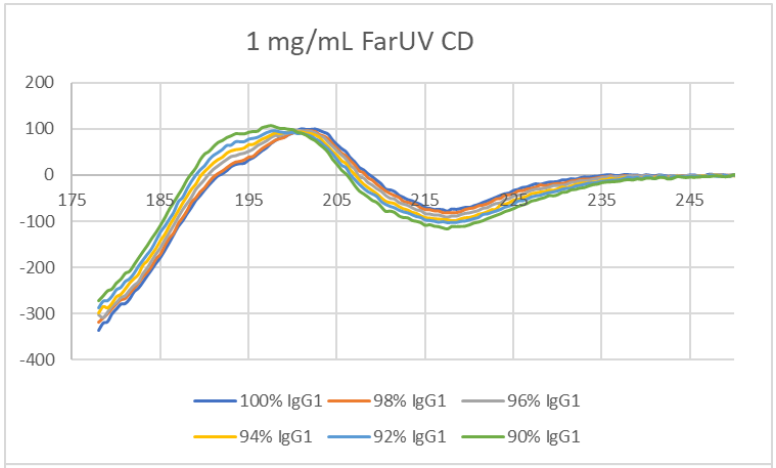


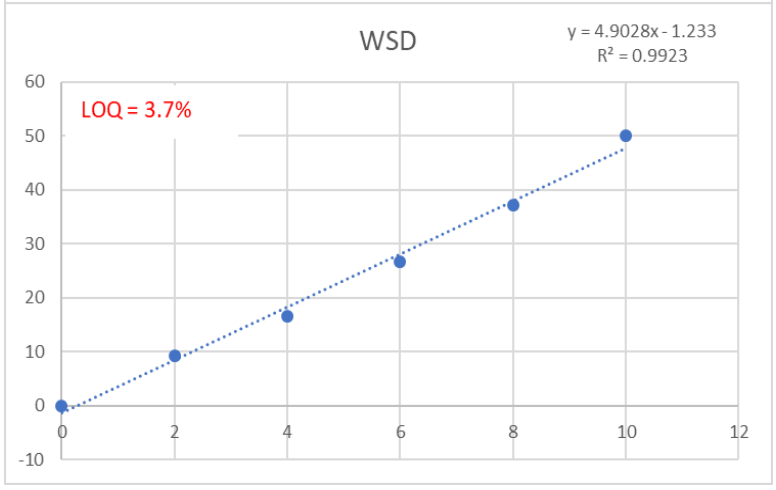
Direct labor time for 10 samples:
FTIR (>5 hrs)





Direct labor time for 10 samples:
MMS (15 min)





MMS 30X more sensitive than FTIR
MMS 5X more sensitive than CD @ 1mg/ml

*FTIR: Bruker Optics Vertex 70 w/ AquaSpec flow cell

Case Study: IgG1 - impact of dilution in a buffer

Objective

Study the impact of differing concentrations and buffers on analysis.

Determine linearity of response and reproducibility

Ioannis A. Papayannopoulos & Shannon Renn-Bingham

Analytical Development, Celldex Therapeutics, Fall River, Massachusetts

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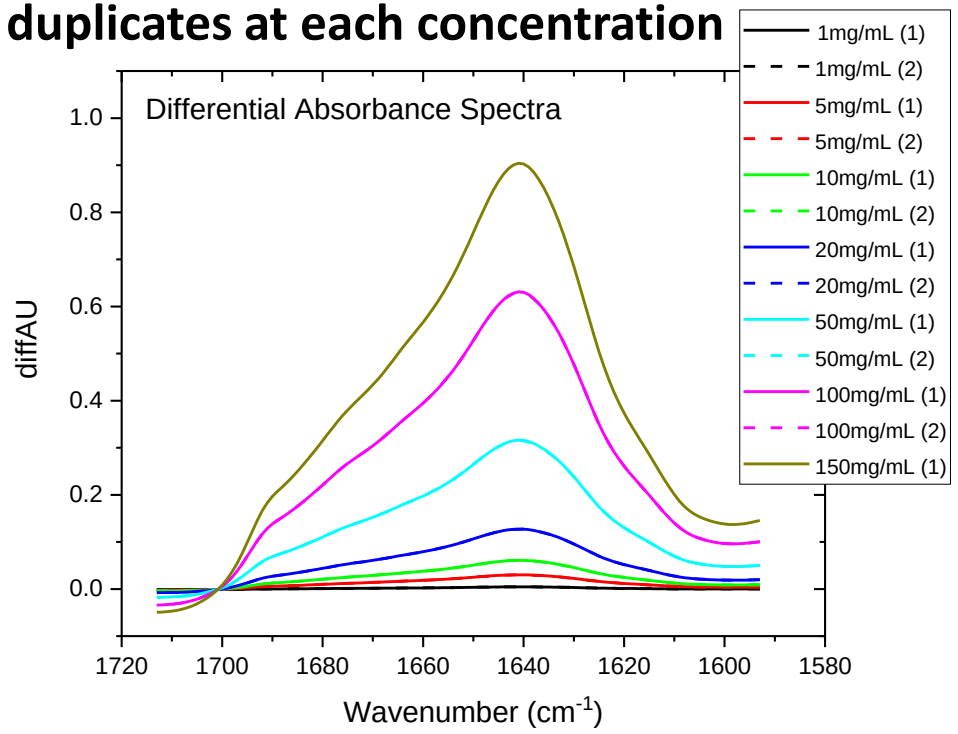
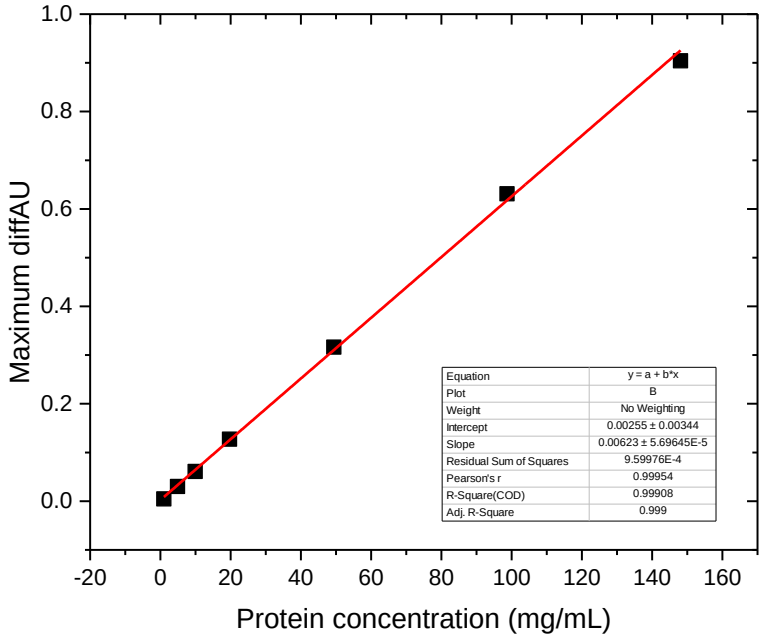
IgG1 – Reproducibility And Linearity: 1 to 150mg/ml

Formulation buffer: 10 mM histidine, 245 mM trehalose, 10 mM methionine, 0.05% polysorbate-20, pH 5.2

Excellent reproducibility and accuracy with duplicates at each concentration



Linear response for 1 – 148mg/mL, R^2 0.999



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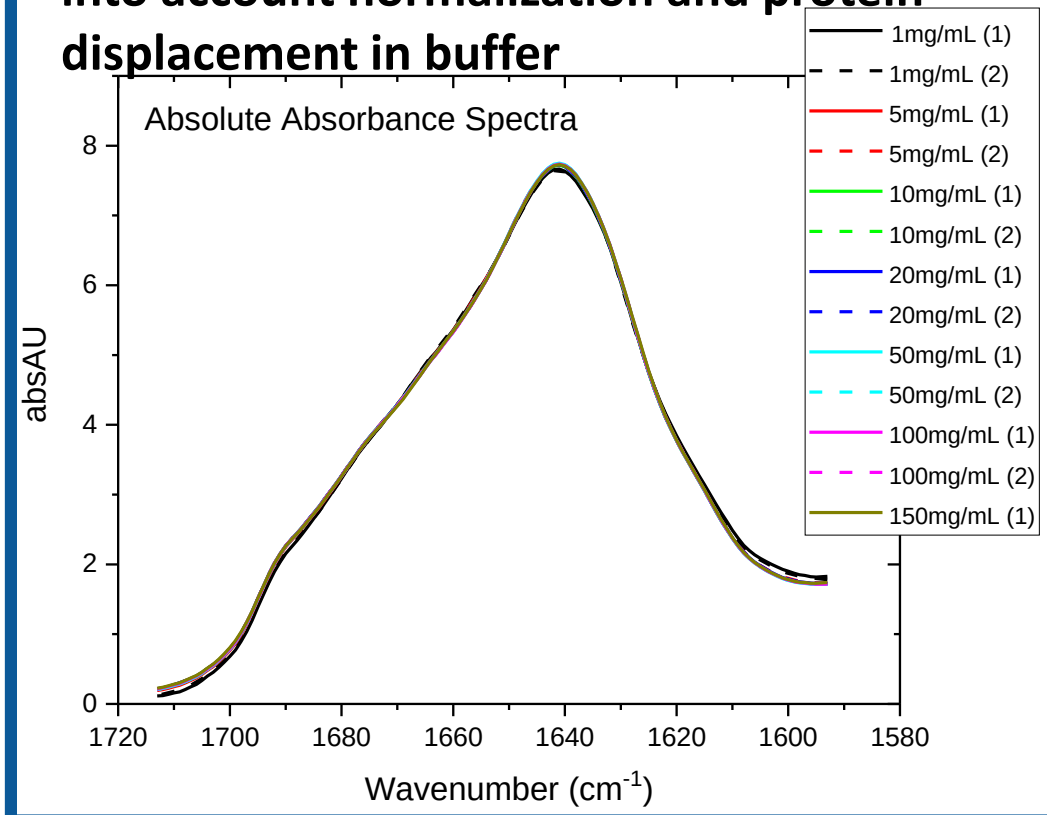
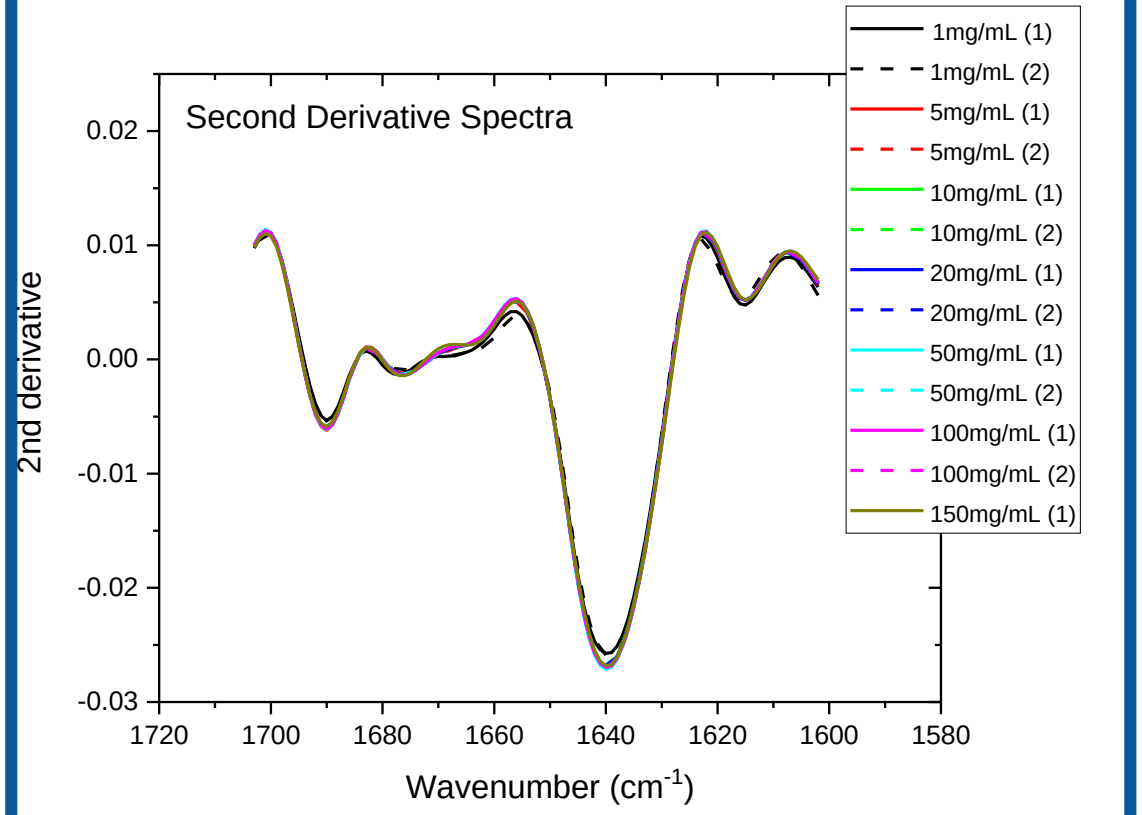
IgG1 – No Impact of Dilution With Buffer On The Structure

absAU spectra derived from diffAU taking into account normalization and protein displacement in buffer



Second derivative overlap is excellent



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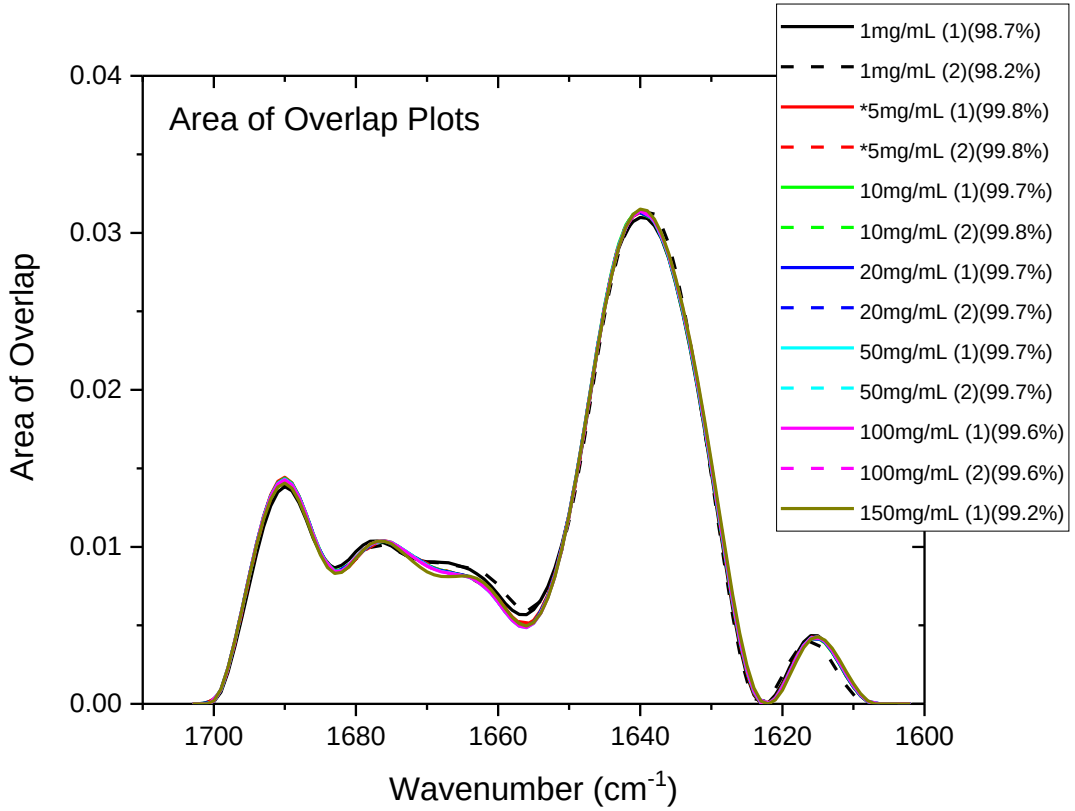
IgG1 – Area of Overlap And Similarity Comparison



Sample concentration	Similarity (%) of replicates		Mean±SD
1 mg/mL	98.74	98.22	98.48±0.37
*5 mg/mL	99.84	99.84	99.84±0.00
10 mg/mL	99.71	99.75	99.73±0.03
20 mg/mL	99.71	99.69	99.70±0.01
50 mg/mL	99.65	99.66	99.66±0.01
100 mg/mL	99.60	99.60	99.60±0.00
150 mg/mL	99.18	---	99.18

Samples show > 98% similarity

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Comparison of Samples in Formulation buffer with Samples in PBS Buffer

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Materials and Methods

A mAb sample at 157 mg/mL, a formulation buffer and a pH7.4 PBS buffer were supplied by Celldex. The mAb stock was diluted in the formulation buffer or the PBS buffer to make dilution series as shown in the following table.

Sample conc. (mg/mL)	157mg/mL sample stock (uL)	Formulation Buffer or PBS Buffer (uL)	Replicates
1	10	1560	2
5	45	1370	2
10	90	1320	2
20	170	1170	2
40	340	1000	2
80	690	660	1

A RedShiftBio AQS^{3}pro was used to collect the differential absorbance spectra. Samples at concentrations of 1-40 mg/mL were tested at a modulation rate of 1 Hz and a back pressure of 5 psi in both buffers. While sample at 80mg/mL in the formulation buffer was tested at 1 Hz and 25 psi and sample at 80 mg/mL diluted in the PBS buffer was tested at 1 Hz and 10 psi due to the increase viscosity of the samples. One replicate measurement was done for samples at 80 mg/mL in both buffers. All the data was analyzed using AQS^3 delta.

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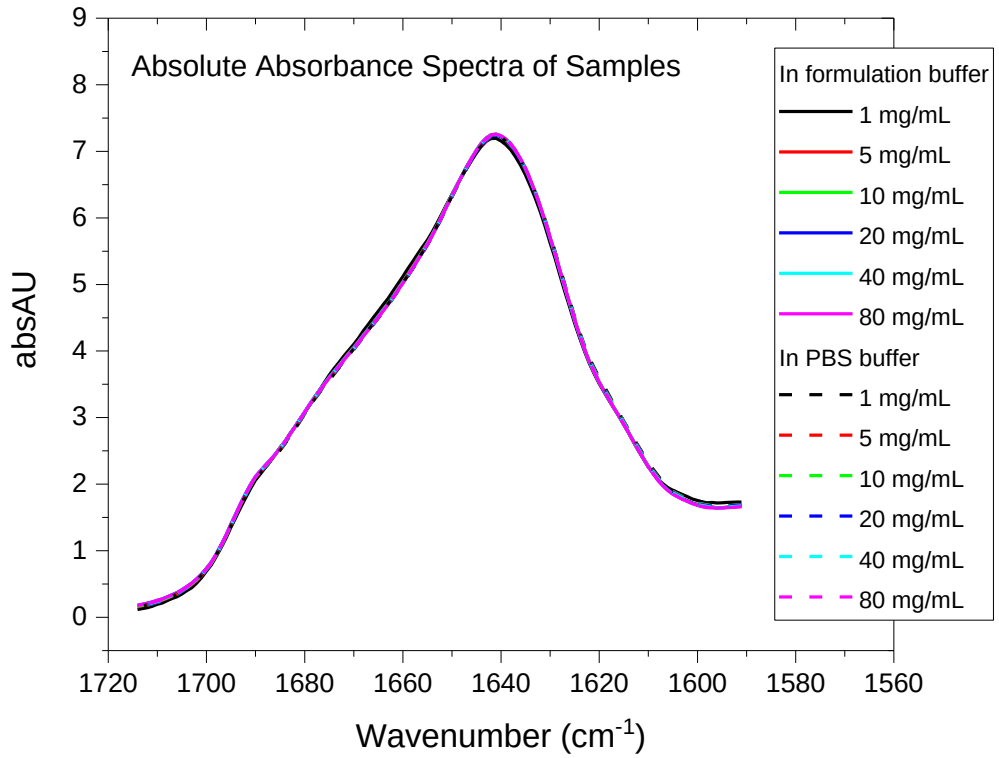
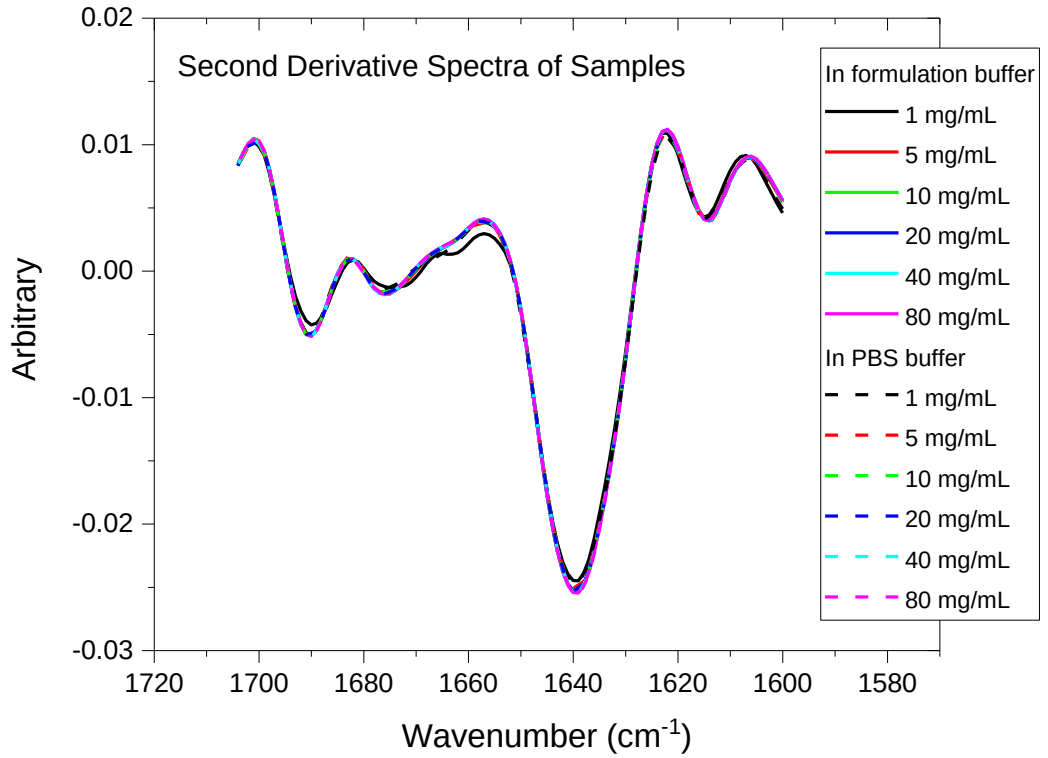
Absolute Absorbance (absAU) and Second Derivative Spectra





The absAU spectra and the second derivative spectra of all the samples in both formulation buffer and PBS buffer are closely matched each other indicating very similar secondary structure profiles among samples in both buffers.

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The Similarity Comparison

Similarity data of samples in the PBS buffer

Samples	Conc. (mg/mL)	Similarity (%)
In formulation buffer	1	98.45
	5*	100
	10	99.72
	20	99.65
	40	99.62
	80	99.55
In PBS buffer	1	99.21
	5	99.40
	10	99.48
	20	99.48
	40	99.48
	80	99.52

*The similarity (%) data was obtained by comparing the mean Area of Overlap (AO) to that of 5 mg/mL sample in the formulation buffer

When compared to the mean AO of 5mg/mL sample in the formulation buffer the structure of the sample in both buffers are highly comparable, > 98%.

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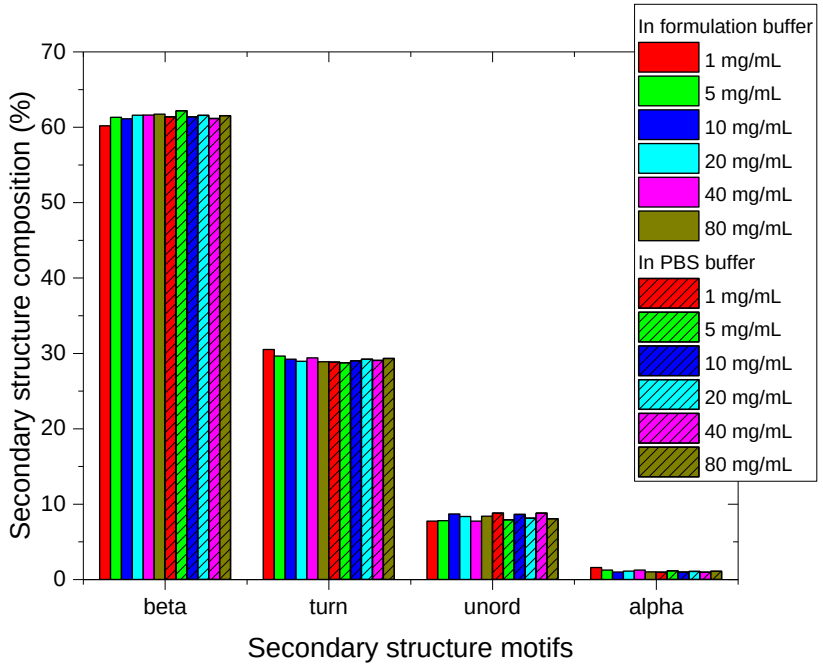
HOS Analysis Results



Samples	Conc. (mg/mL)	Secondary structure composition (%)			
		Beta	Turn	Unordered	Alpha
In formulation buffer	1	60.19	30.50	7.73	1.58
	5	61.31	29.63	7.81	1.25
	10	61.10	29.22	8.70	0.98
	20	61.59	28.94	8.36	1.12
	40	61.61	29.40	7.73	1.26
	80	61.73	28.88	8.39	1.00
In PBS buffer	1	61.38	28.85	8.81	0.96
	5	62.19	28.74	7.92	1.15
	10	61.37	29.01	8.63	0.99
	20	61.56	29.22	8.15	1.08
	40	61.15	29.07	8.82	0.96
	80	61.53	29.32	8.06	1.10

HOS analysis shows consistent result that all the samples in both buffers are very similar in secondary structure.

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Conclusions

- MMS enables quantitative analysis of monoclonal antibodies and other protein biologics over a wide concentration range with high reproducibility and accuracy

- MMS analysis does not require dilution of concentrated samples for analysis and there was no interference from the optically active formulation buffer components

- With these key features MMS is a very versatile technology for direct, label free, characterization of proteins through all phases of biologic drug development from discovery through formulation and manufacturing.

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Microfluidic Modulation Spectroscopy Analysis of a Monoclonal Antibody at Different Concentrations

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Microfluidic Modulation Spectroscopy (MMS) - a novel automated infrared (IR) spectroscopic tool for secondary structure analysis of biopharmaceuticals with high sensitivity and repeatability

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