FOOD ANALYSIS

USING IR & CIRCULAR DICHROISM SPECTROSCOPY

FREE WEBINAR

Presented by **Forrest Kohl, PhD** & **Carlos Morillo, PhD**

JASCO

Agenda

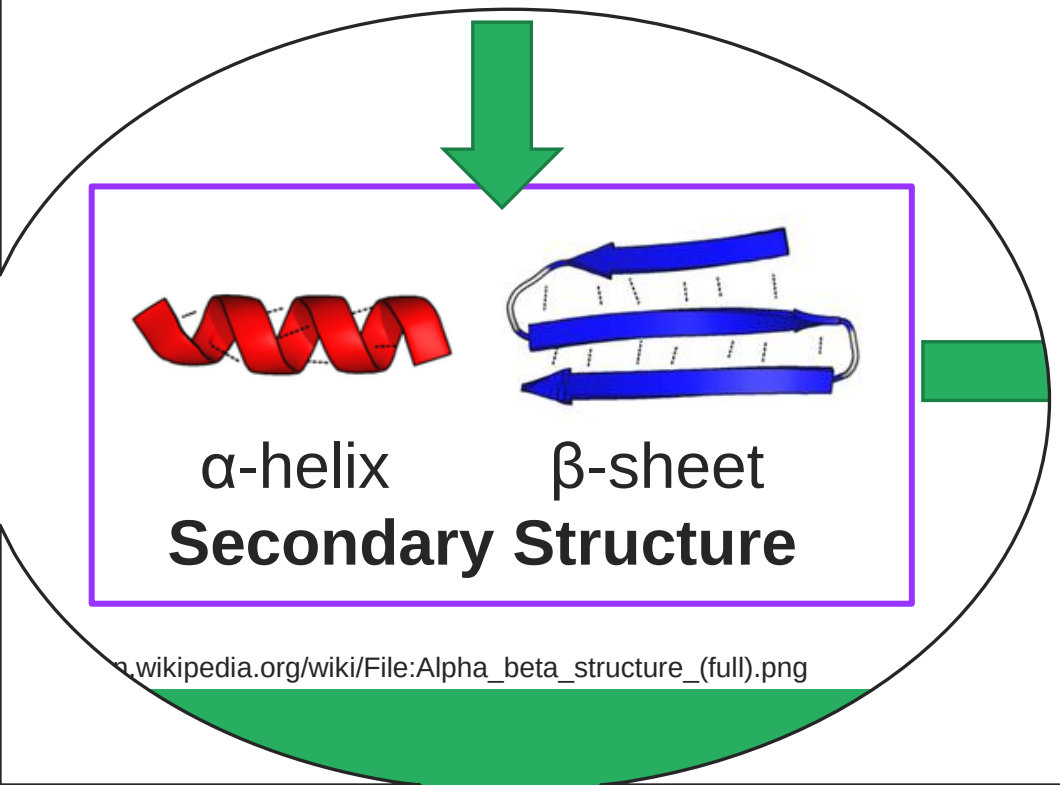
- **Proteins in Food**
- Plant Based Protein Analysis
- Secondary Structure Estimation using CD
- Secondary Structure Estimation using FTIR
- CD vs. FTIR: What to Choose

Protein Analysis in Food

- Important for:
  - Quality Control
  - R&D (meat replacements)
  - Protein Quantification
- Understanding protein structure is key for food development

Protein Analysis in Food

- Important for:
  - Quality Control
  - **R&D (meat replacements)**
  - Protein Quantification
- **Understanding protein structure is key for food development**


α-helix
β-sheet
Secondary Structure
wikipedia.org/wiki/File:Alpha_beta_structure_(full).png

Agenda

- Proteins in Food
- **Plant Based Protein Analysis**
- Secondary Structure Estimation using CD
- Secondary Structure Estimation using FTIR
- CD vs. FTIR: What to Choose

Plant Based Proteins

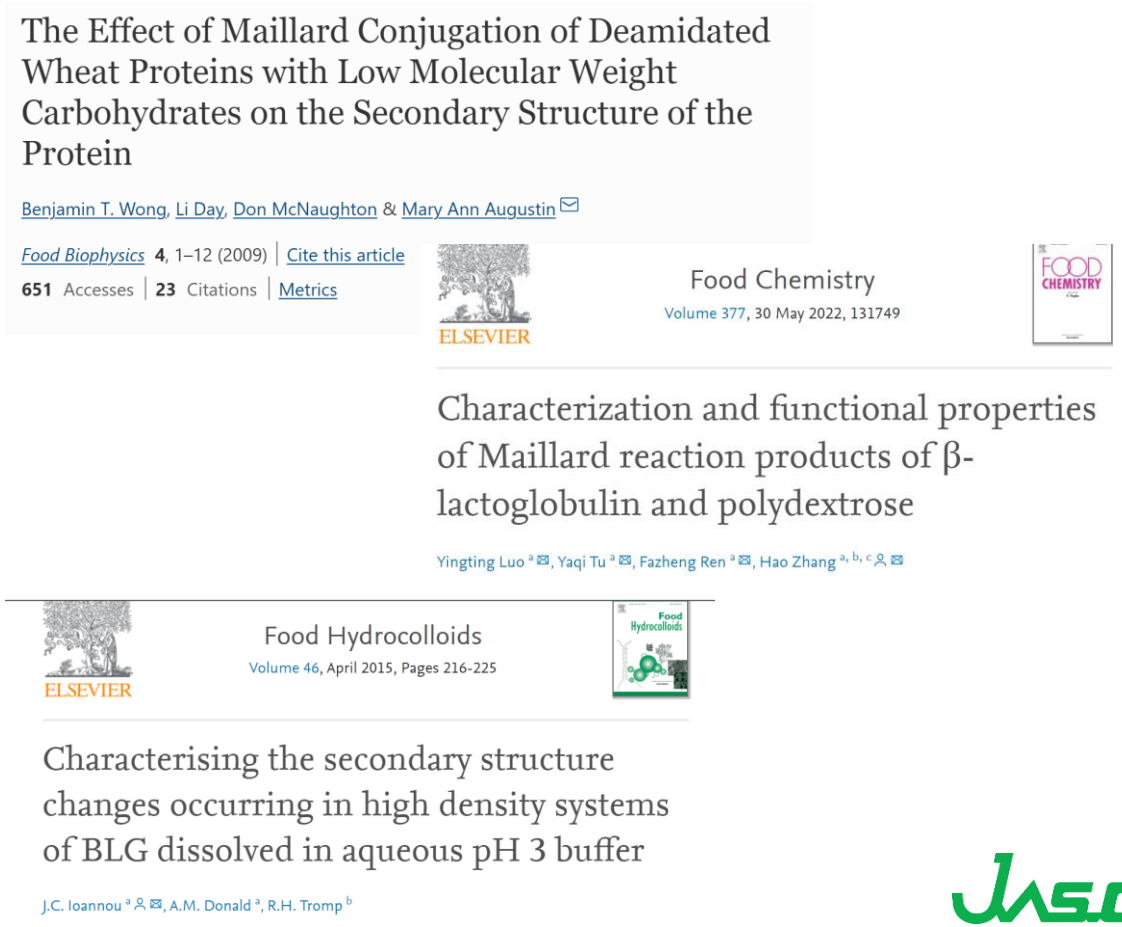
- **Crucial Field:** Development of meat analogs from plant-based substances
- Crucial to understand protein composition and secondary structure.
- Improper balance of proteins could lead to strange texture or taste
- **Potentially difficult due to protein mixture**

Nutralys

https://www.puris.com/ https://www.roquette.com/

Tools for Secondary Structure Estimation: CD and FTIR

- A large amount of food studies focused on food and cooking utilize CD and/or FTIR to look at structural changes.
- Characterizing secondary structure is a good way to verify protein integrity
  - ***Is the amount of β-sheet changing over shelf life?***
- Ensure proper protein content for meat analogues
  - ***Does this batch have the correct β-sheet:α-helix ratio?***



The Effect of Maillard Conjugation of Deamidated Wheat Proteins with Low Molecular Weight Carbohydrates on the Secondary Structure of the Protein

Benjamin T. Wong, Li Day, Don McNaughton & Mary Ann Augustin

Food Biophysics **4**, 1–12 (2009) | Cite this article

651 Accesses | **23** Citations | Metrics

ELSEVIER

Food Chemistry

Volume 377, 30 May 2022, 131749

Characterization and functional properties of Maillard reaction products of β-lactoglobulin and polydextrose

Yingting Luo [a], Yaqi Tu [a], Fazheng Ren [a], Hao Zhang [a, b, c]

ELSEVIER

Food Hydrocolloids

Volume 46, April 2015, Pages 216-225

Characterising the secondary structure changes occurring in high density systems of BLG dissolved in aqueous pH 3 buffer

J.C. Ioannou [a], A.M. Donald [a], R.H. Tromp [b]

Plant Based Proteins

- Here we demonstrate secondary structure analysis of two commercial pea protein mixtures
- IR and CD spectroscopy are employed
- We show Secondary Structures are easily quantified as solids (**ATR**) or liquids (**CD, FTIR, Penta**) for this **complex mixture**
- In practice monitoring of SSE values informs you about batch-to-batch variation, or whether protein formulation is correct.

Nutralys

Agenda

- Proteins in Food
- Plant Based Protein Analysis
- **Secondary Structure Estimation using CD**
  - *Transmission – Liquid*
  - *Integrating Sphere – Scattering Liquid*
- Secondary Structure Estimation using FTIR
- CD vs. FTIR: What to Choose

Instrument and Accessories

JASCO J-1500

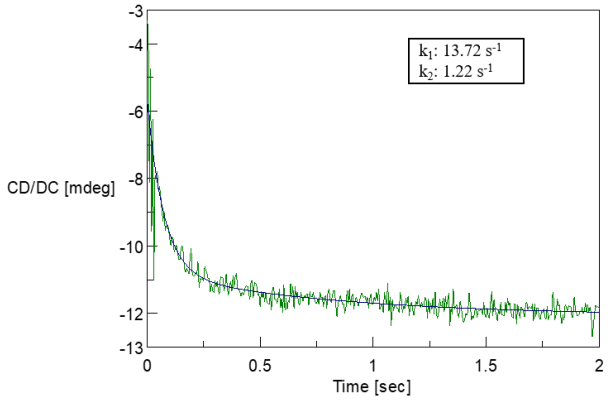
JASCO DRCD-575

CD is a routinely used tool to estimate protein secondary structure

Some portions of these plant proteins are not soluble, so an integrating sphere can be useful.

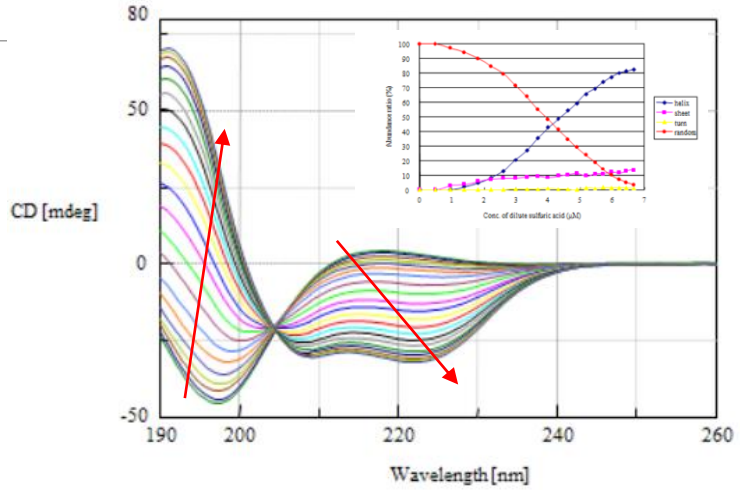
CD for Structure Analysis of Proteins

Very easy to measure dynamic processes: Folding, Ligand Binding and Stability



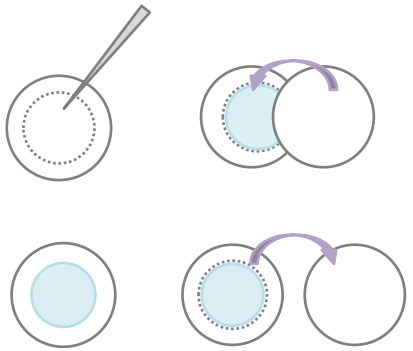
J-1500 can use stopped flow to measure fast folding dynamics.

Thermal control, titration



Capabilities to perform automatic titration

Easy to vary path length of cell allowing for flexibility in sample concentration.



2% protein with 10 µm cell
Only 2 uL of solution!

Low concentrations using higher path length (1 cm)

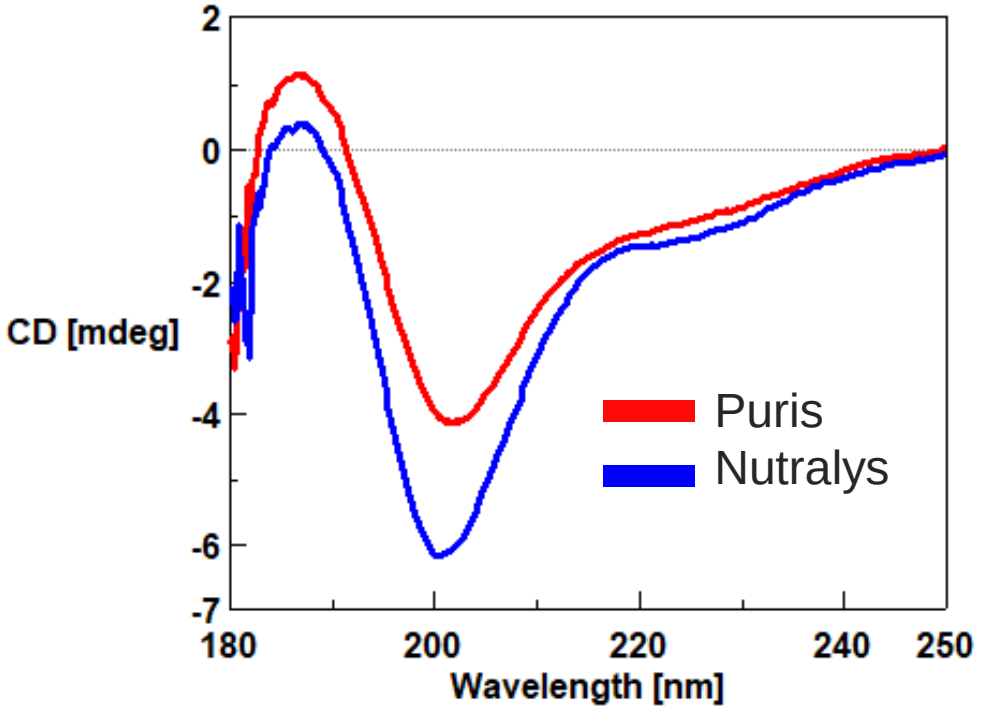
Sample Preparation

- All scans used a 1 mm quartz cuvette and water solvent.
- Puris and Nutralys samples were ground using a pestle and mortar
- **Transmission CD:** Ground Puris and Nutralys samples were dissolved in water. Excess solid were centrifuged out and only the top layer was used.
- **DRCD:** after dissolution, protein solutions were directly measured via transmission into an integrating sphere.

https://www.labicons.net/equipments/centrifuges/microcentrifuges/doc/microcentrifuges-tubes-open.html

"File:White-Mortar-and-Pestle.jpg." https://commons.wikimedia.org/w/index.php?title=File:White-Mortar-and-Pestle.jpg&oldid=451463203

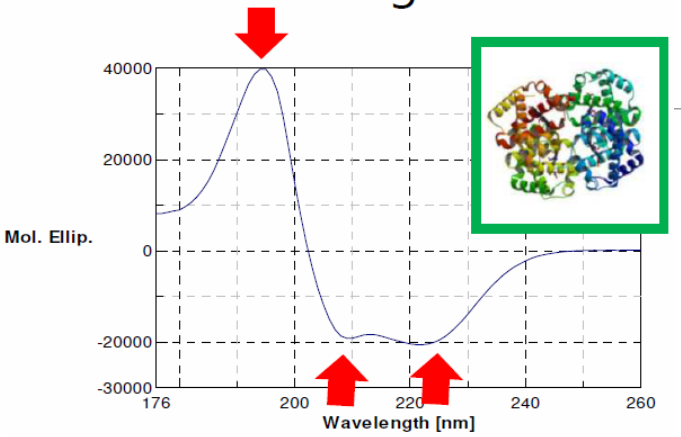
CD Spectra of Plant-based protein



- Negative peak at 201 nm for both samples
- Negative signal at 230 nm.
- Both spectra are reminiscent of random coil

CD Spectra of Proteins

Hemoglobin

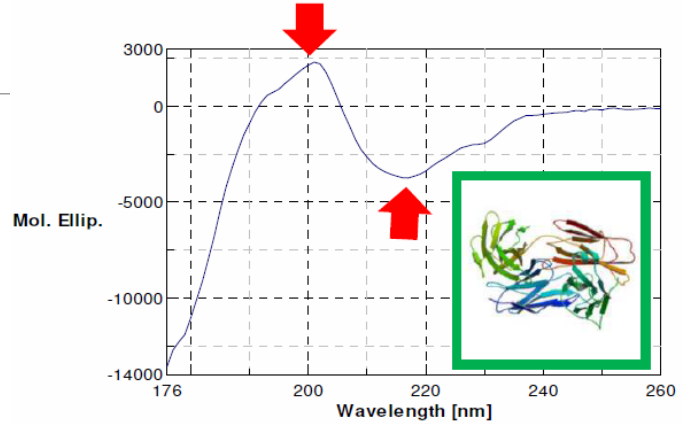


α-helix rich

α-helix peak

Wavelength / nm	sign
222	-
208-209	-
191-193	+

IgG

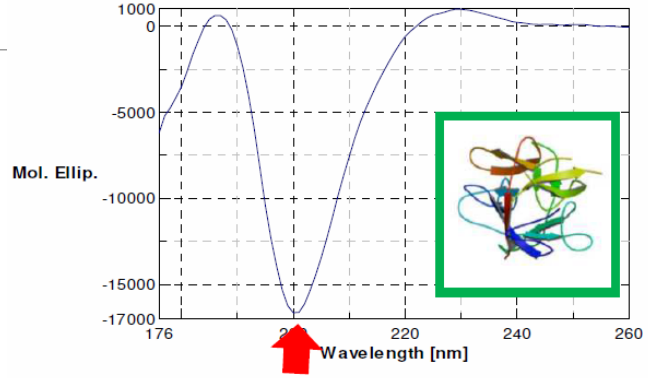


β-sheet rich

β-sheet peak

Wavelength / nm	sign
217	-
195-200	+

Trypsin inhibitor

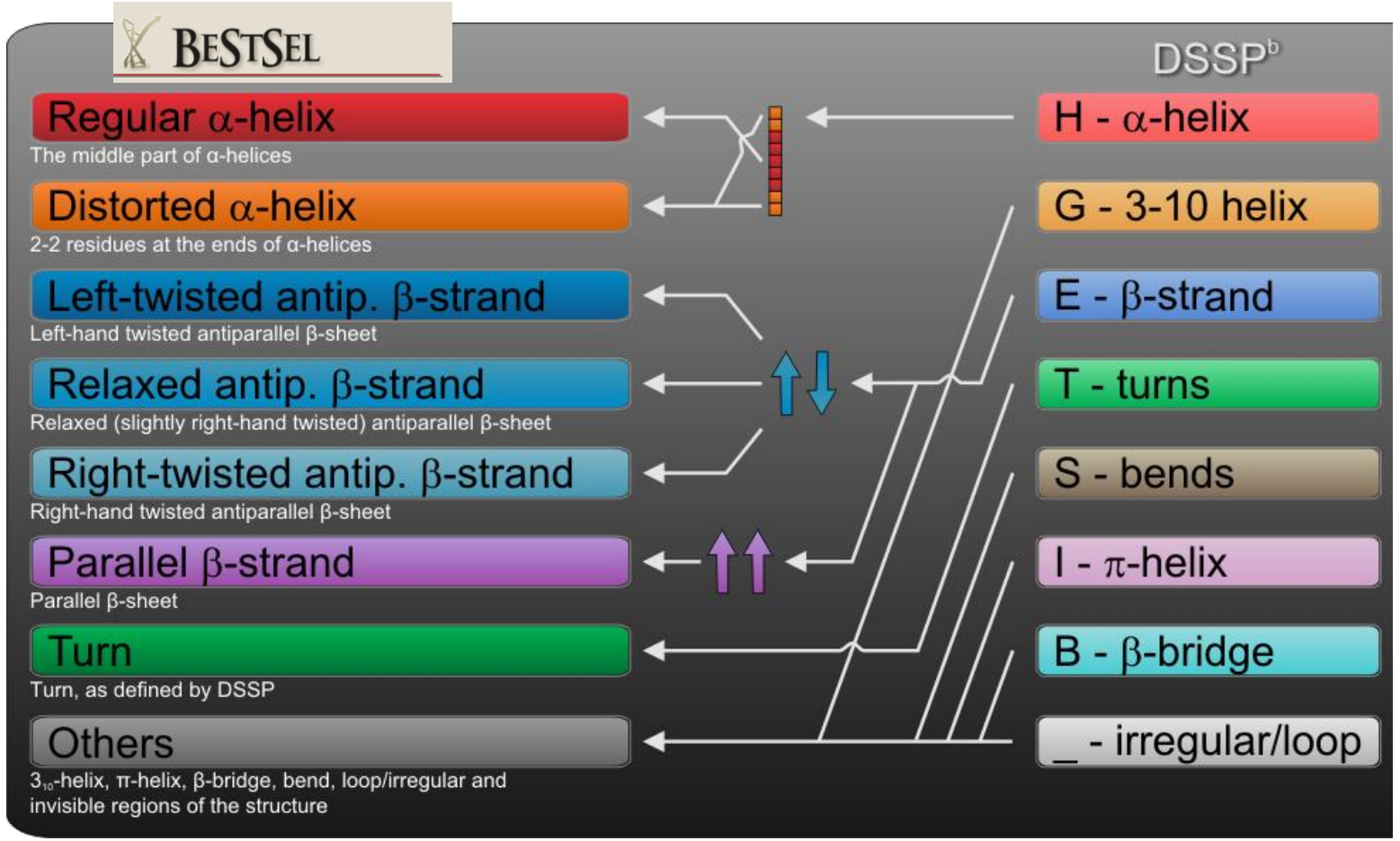


Random coil rich

Random coil peak

Wavelength / nm	sign
195-200	-

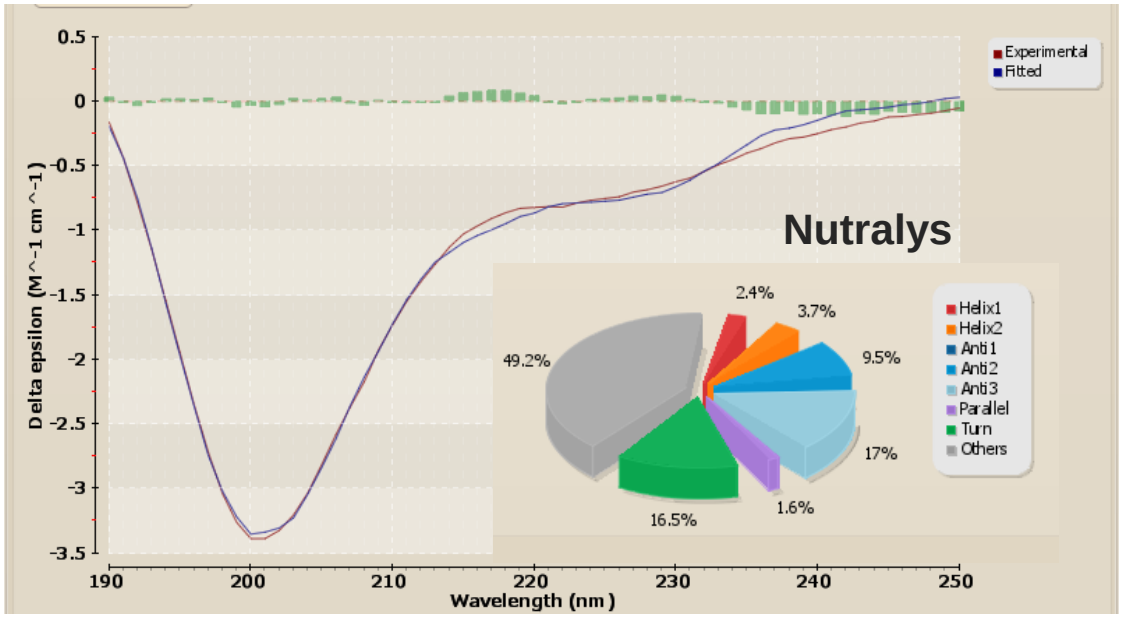
Secondary Structure Estimation - BeStSel

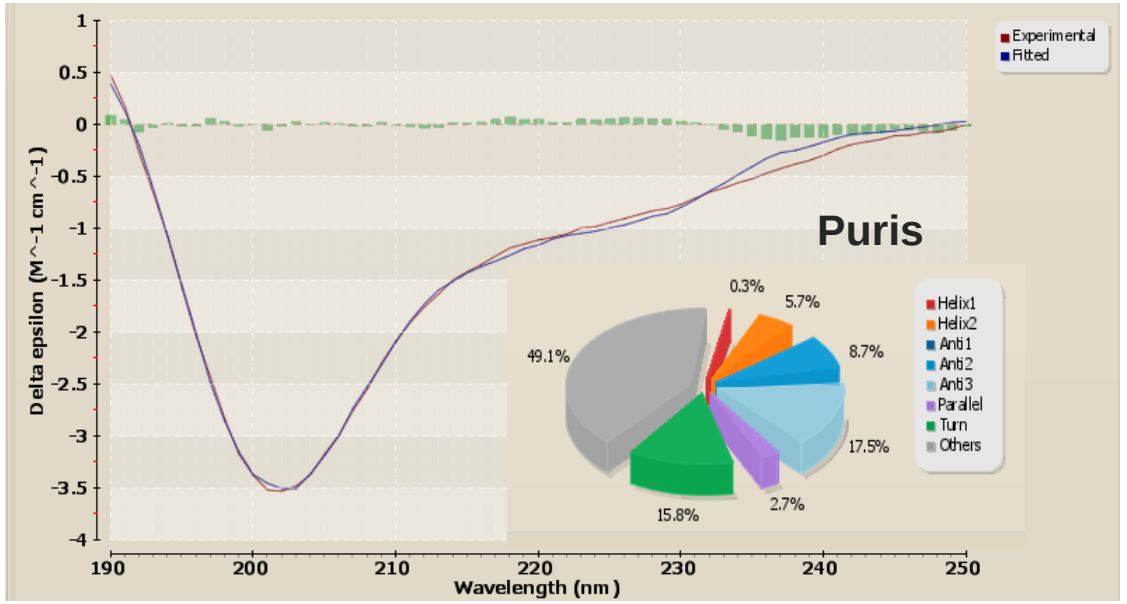


- Structural assignments brought in from Dictionary of Secondary Structure of Proteins (DSSP)
- CD spectra are fit to basis spectra for each of these structure components.
- Magnitude of each component is used to find % secondary structure

https://bestsel.elte.hu/

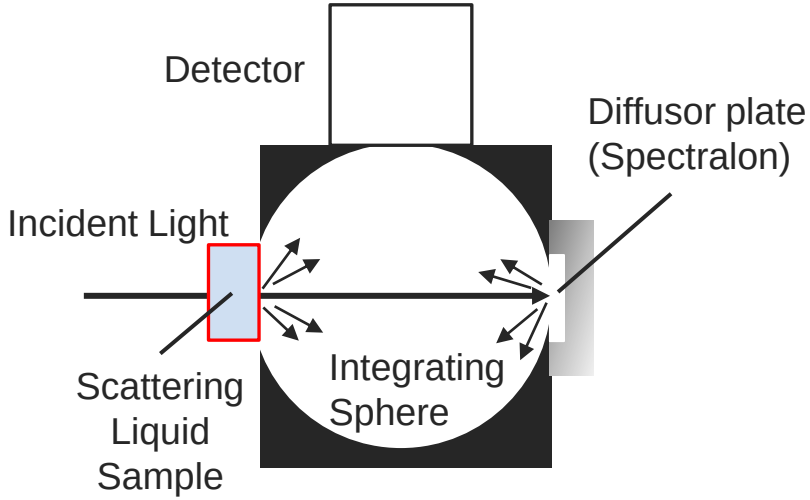
SSE of CD Spectra of Plant-based protein





Sample	α-Helix	β-Sheet	β–Turn	Other
Puris	6 %	29 %	16 %	49 %
Nutralys	6 %	28 %	17 %	49 %

Integrating Sphere for Scattering Liquid

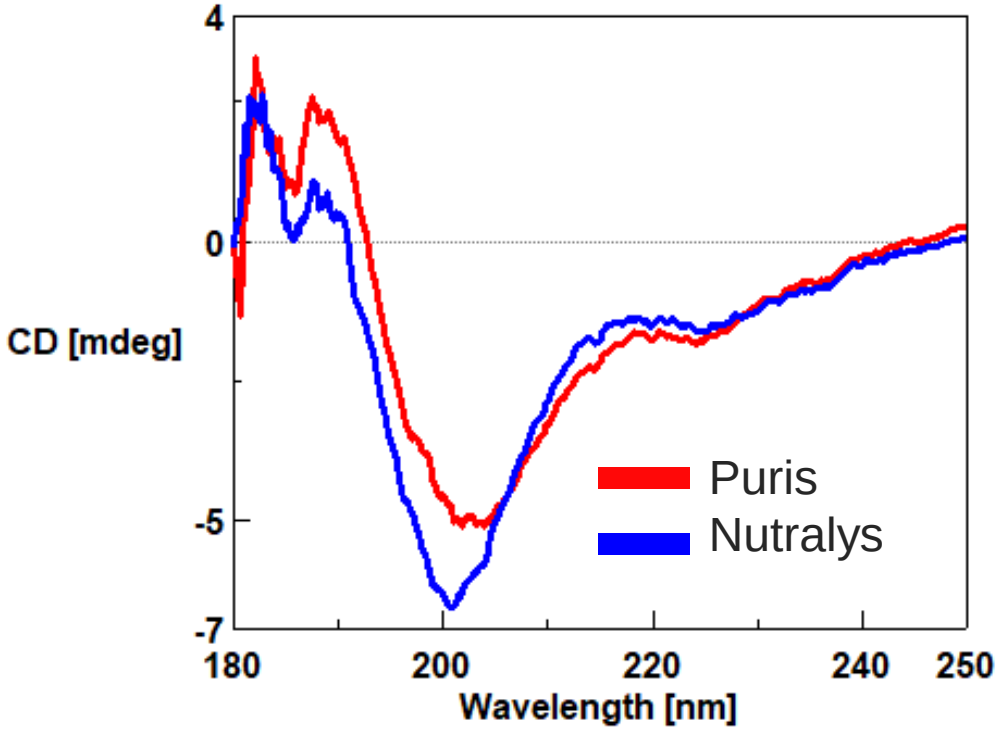


JASCO DRCD-575

Samples with particulate can scatter light causing issues in absorption measurements.

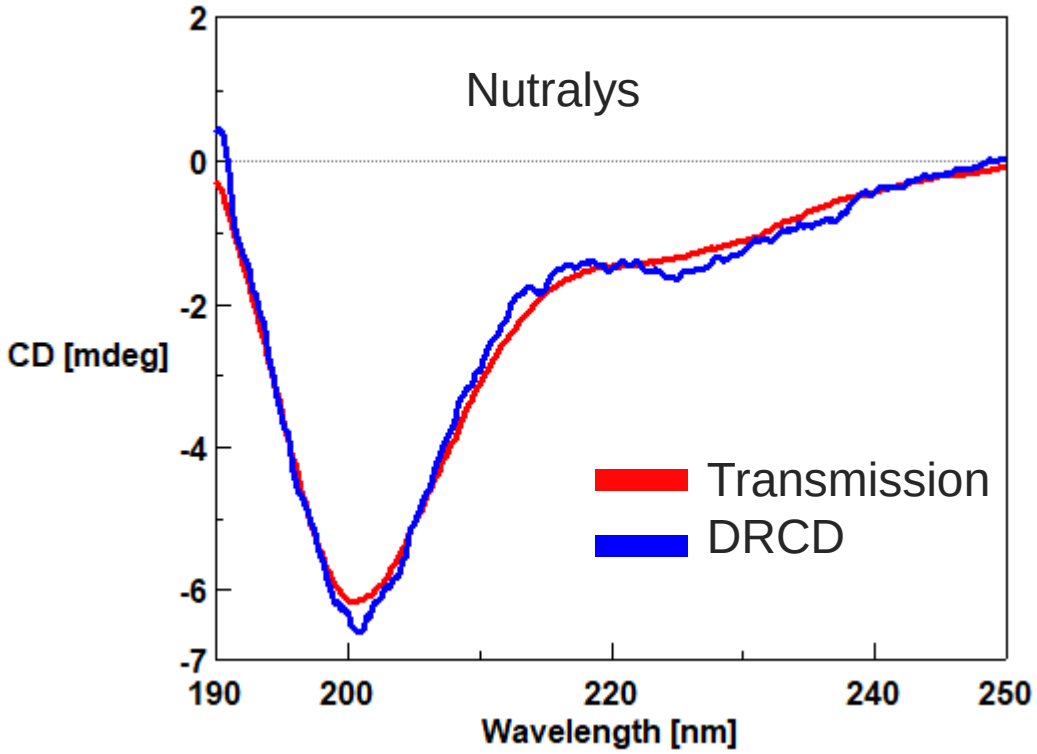
Using an integrating sphere, the scattered light can be collected.

DRCD Spectra of Plant-based protein



- Negative peak at 201 nm for both samples
- Both spectra are reminiscent of random coil
- **Both spectra are like filtered samples**

DRCD Spectra of Plant-based protein



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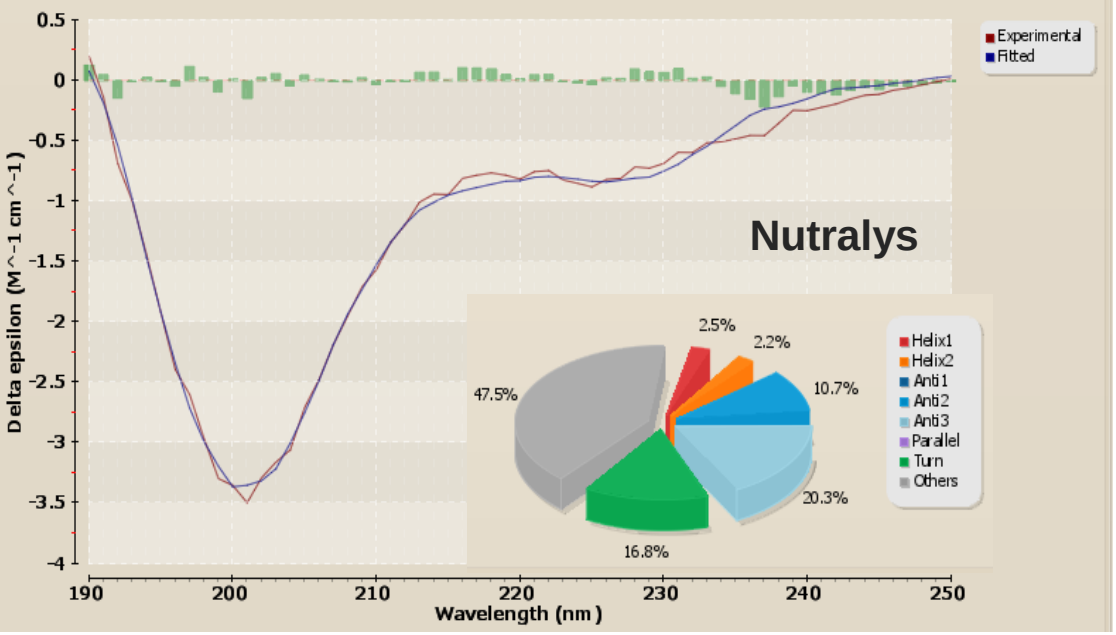
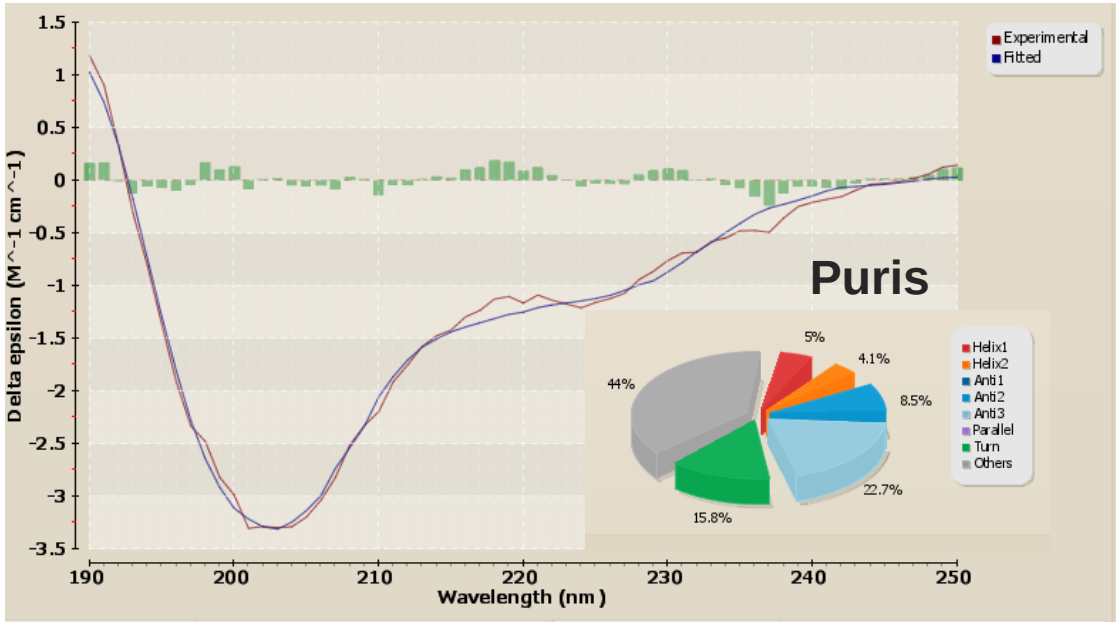
SSE of DRCD Spectra of Plant-based protein





Sample	Helix	Sheet	Turn	Other
Puris	9 %	31 %	16 %	44 %
Nutralys	5 %	31 %	17 %	47 %

Comparison of all CD methods

Sample	Helix	Sheet	Turn	Other
Puris (DRCD)	9 %	31 %	16 %	44 %
Puris (Transmission)	6 %	29 %	16 %	49 %
Nutralys (DRCD)	5 %	31 %	17 %	47 %
Nutralys (Transmission)	6 %	28 %	17 %	49 %

- DRCD and standard CD transmission measurement match well for each sample.
  - Puris DRCD has slightly more alpha helix than filtered sample.

- Puris has higher helix content than Nutralys.

Summary

- Data shows similar SSE breakdown for Nutralys between DRCD of scattering solution and the centrifuged top layer measured with transmission.
- BeStSel is a very effective tool to finding secondary structure of plant-based protein mixtures.
- Use of the SSE breakdown:

Batch #	1	2	3	4	5	6
α-Helix	5 %	4 %	4 %	9 %	6 %	5 %
β-Sheet	31 %	32 %	32 %	28 %	31 %	33 %

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- Proteins in Food
- Plant Based Protein Analysis
- Secondary Structure Estimation using CD
- **Secondary Structure Estimation using FTIR**
  - *ATR – solid*
  - *Transmission - Liquid*
  - *Penta - Liquid*
- CD vs. FTIR: What to Choose

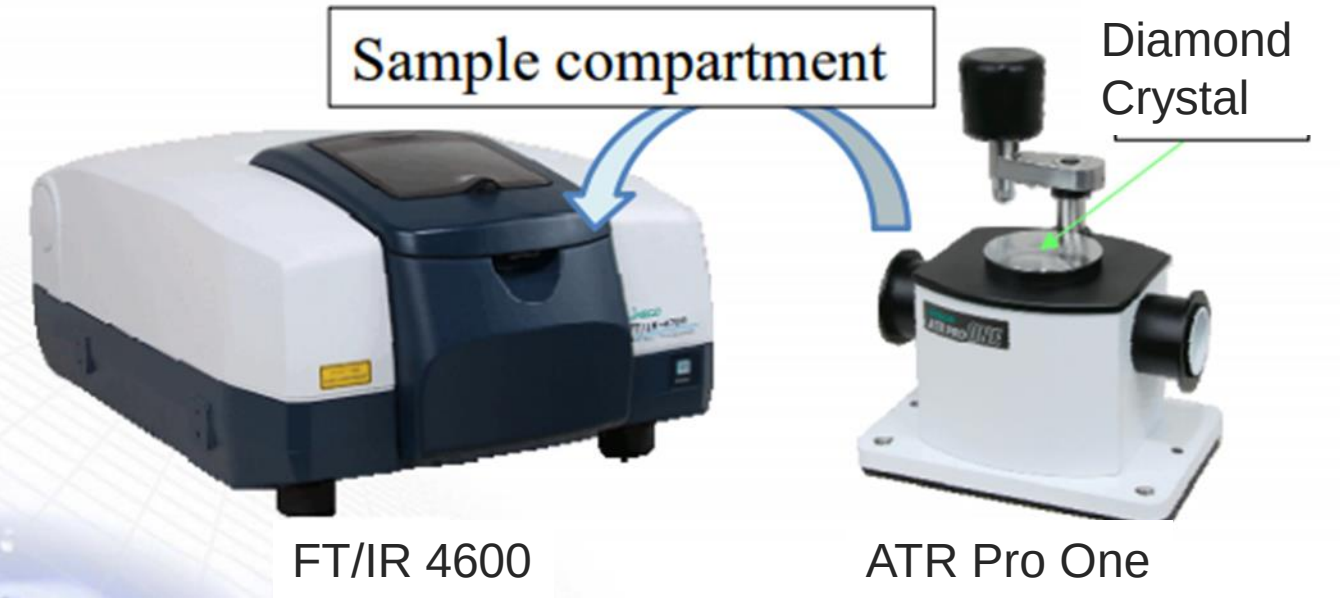
Sample Preparation

- Puris and Nutralys samples were grinded using a pestle and mortar and dissolved 1 mg in a 1 ml buffer solution.
- 15 μl were fed in the transmission cell using a micropipette

Agate Mortar and Pestle

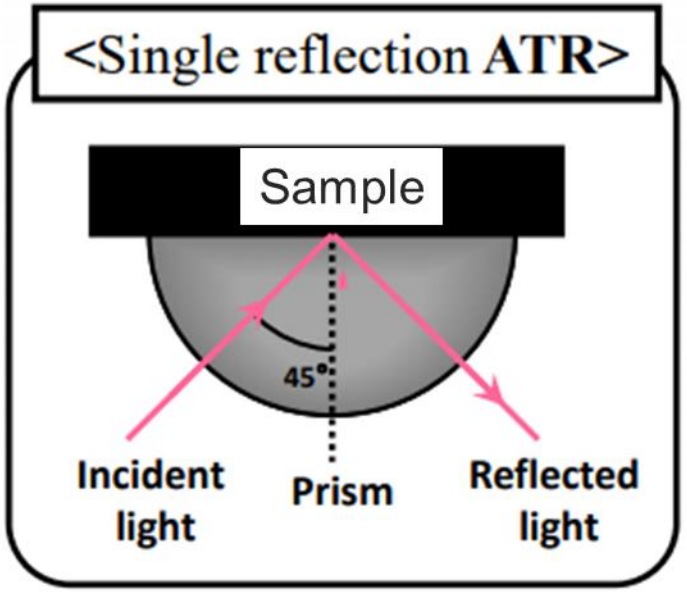
Instrument

- Minimal sample preparation
- Solid and liquid samples
- Acquisition of sample surface information

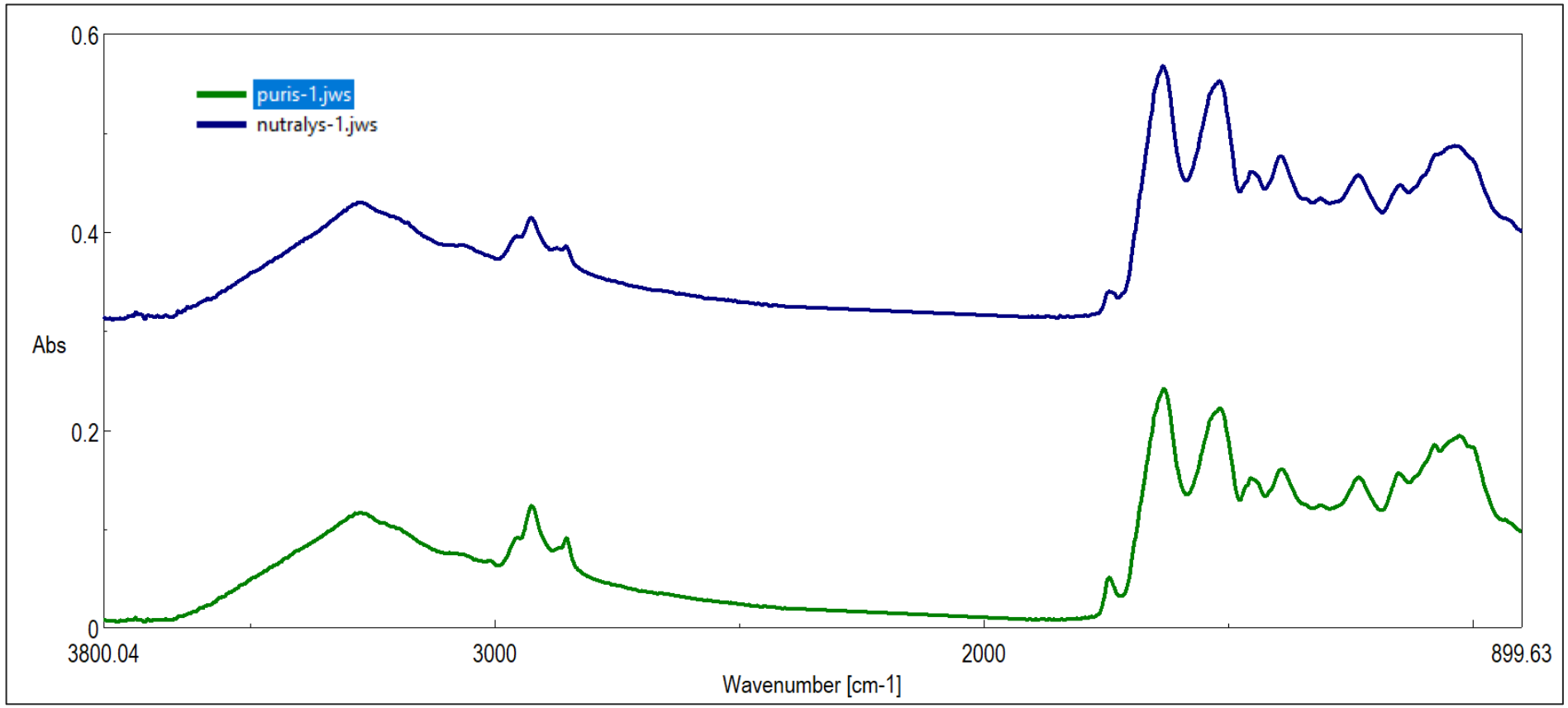


FT/IR 4600

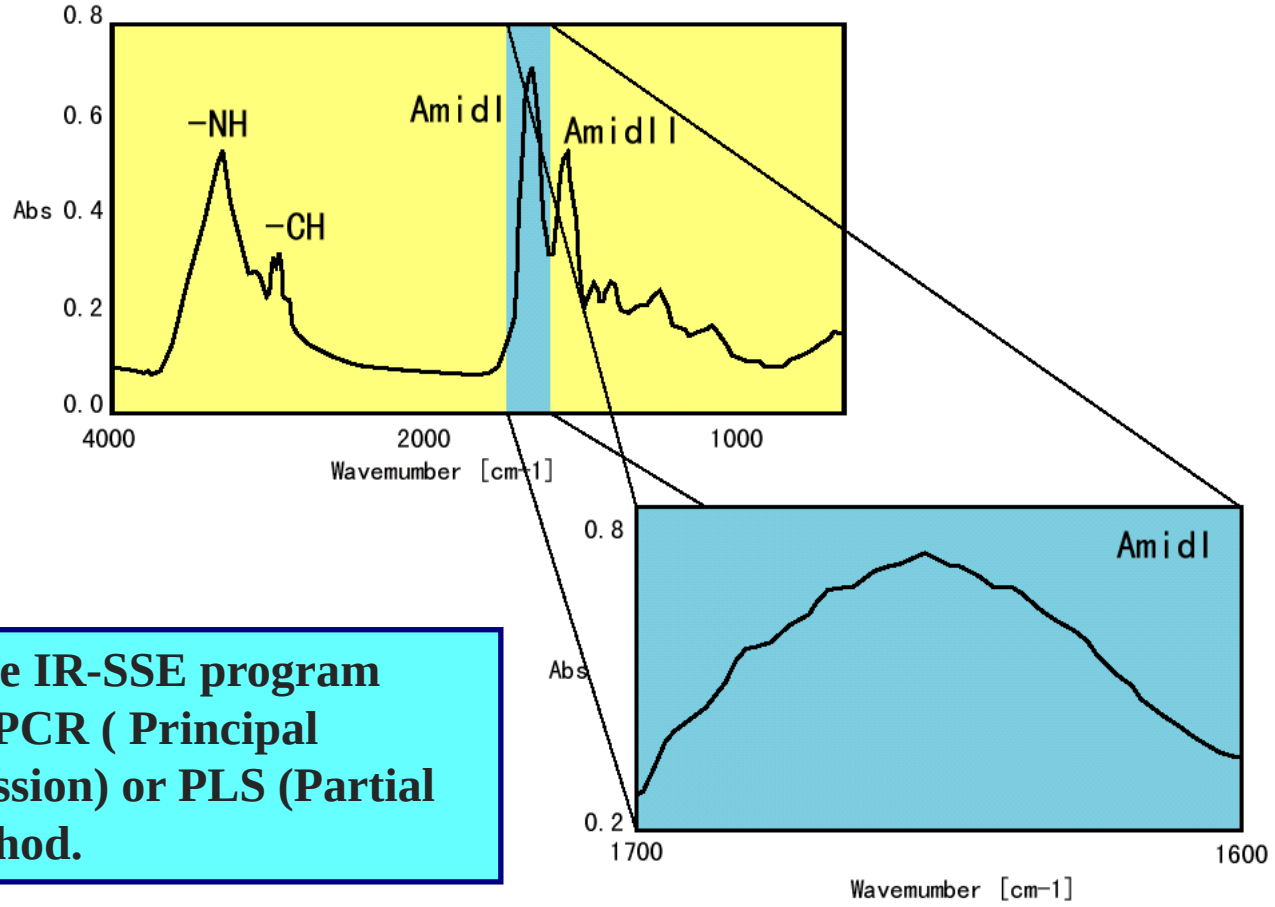
ATR Pro One



ATR-IR Spectra of Plant-based protein



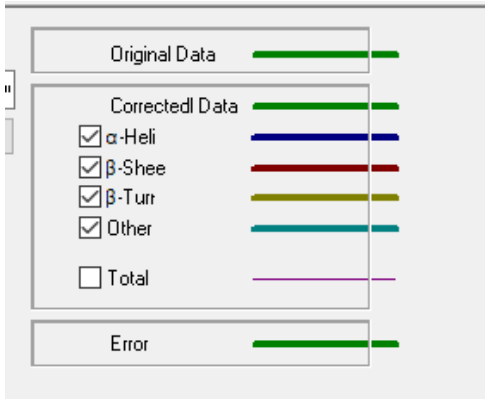
IR Spectrum of Proteins

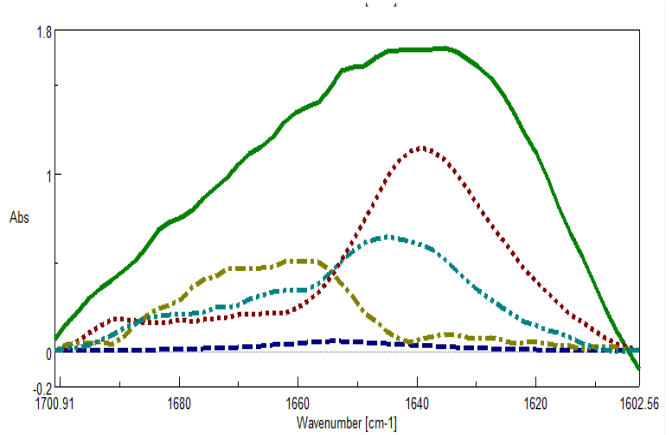


Amid I peak contains the secondary structure information (α-Helix, β-Sheet etc.)

Using this peak, the IR-SSE program calculates SSE by PCR (Principal Component Regression) or PLS (Partial Least Square) method.

SSE of ATR-IR Spectra of Plant-based protein





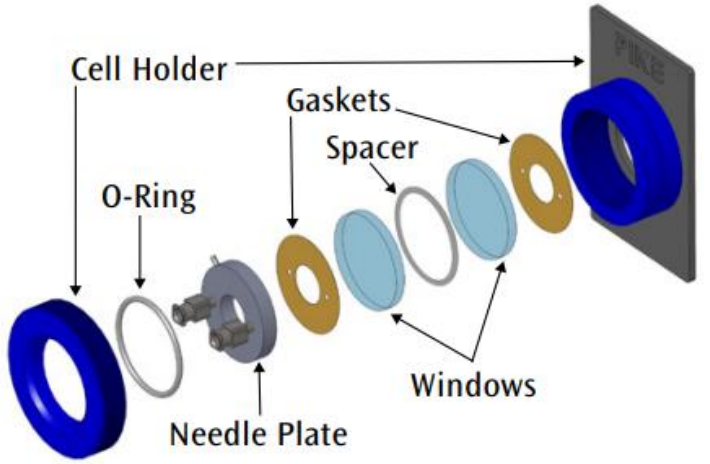
Sample	α-Helix	β-Sheet	β–Turn	Other
Puris	2 %	46 %	25 %	27 %
Nutralys	4 %	44 %	25 %	27 %

- SSE software predicts very similar secondary structure for each sample based on the IR spectra.
- Mainly β–sheet structure

Instrument and Accessories

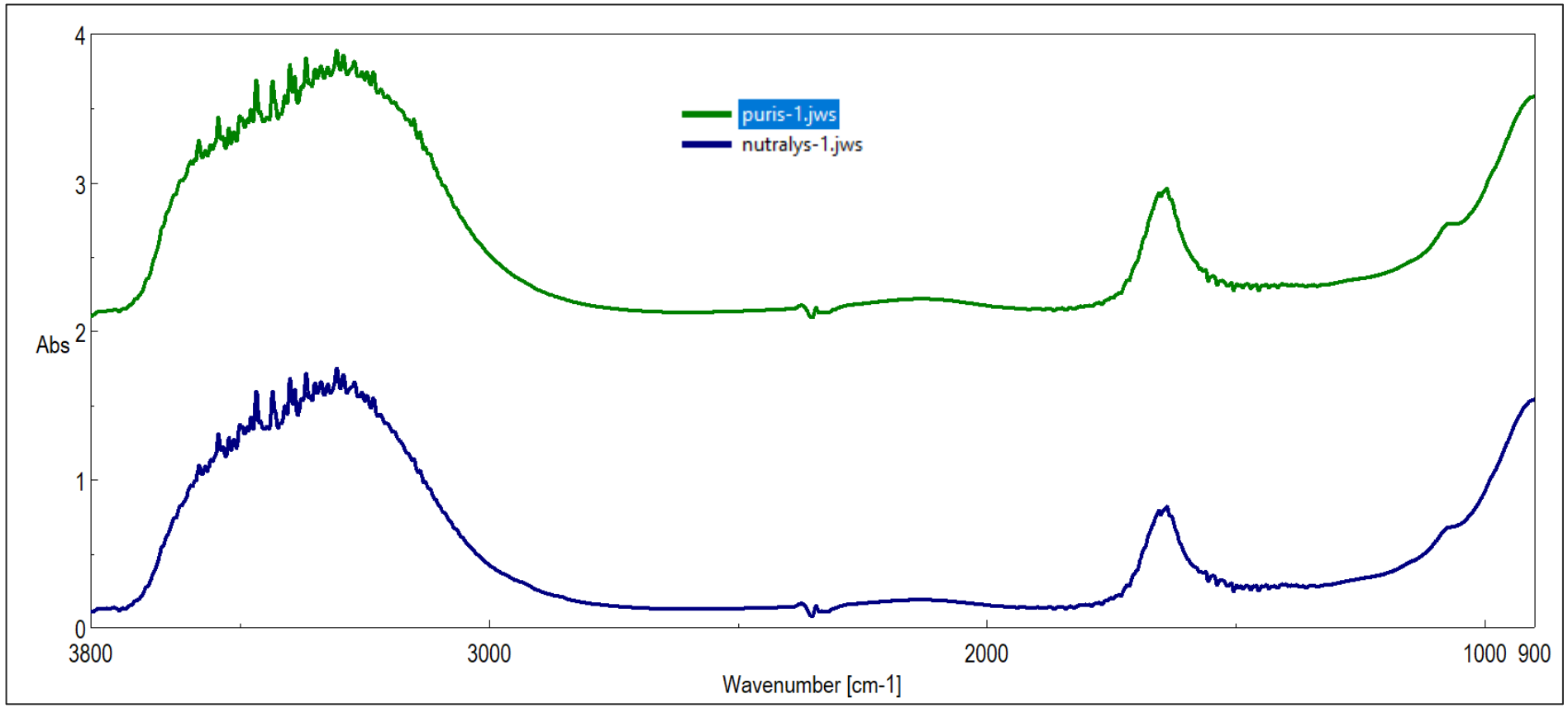
JASCO FTIR – 4600

Demountable Liquid Cell

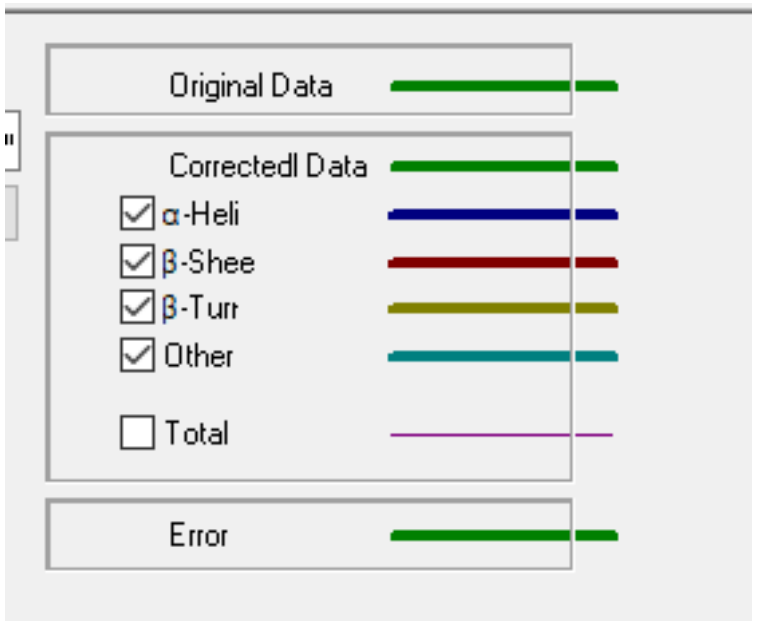


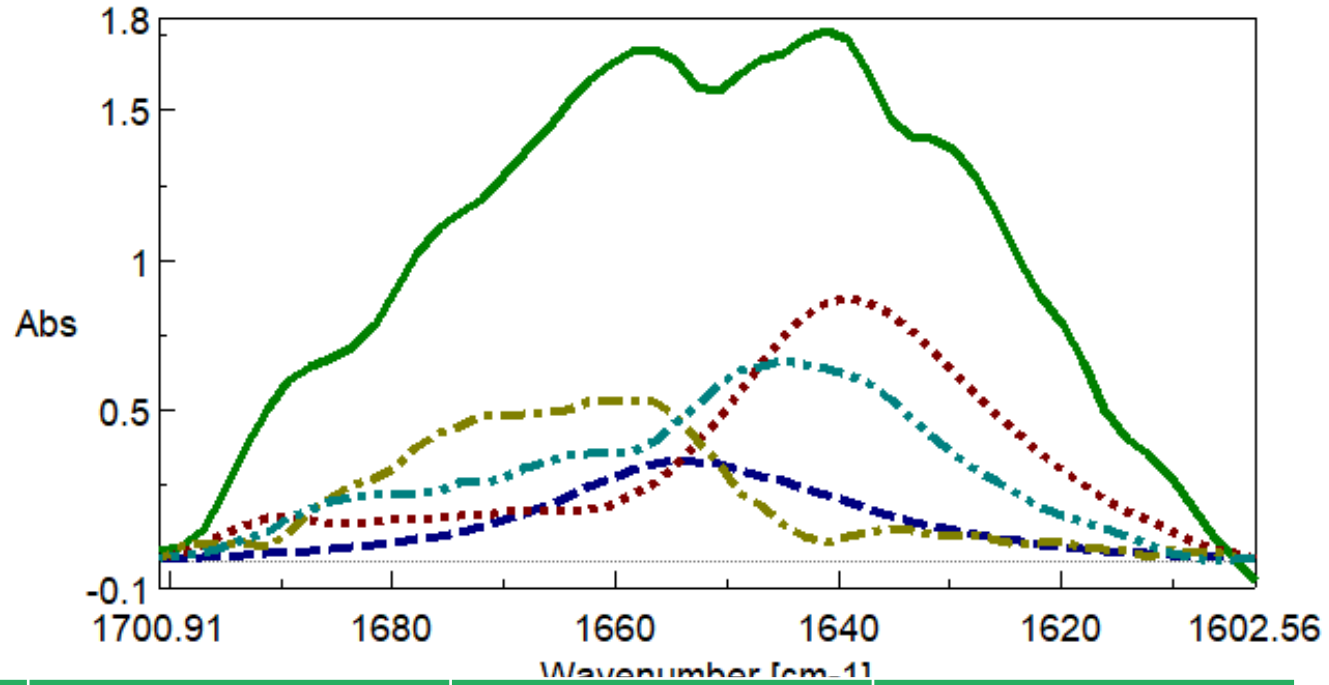
Demountable liquid cell assembly layout.

IR spectra using Transmission Cell



SSE Results using Transmission Cell





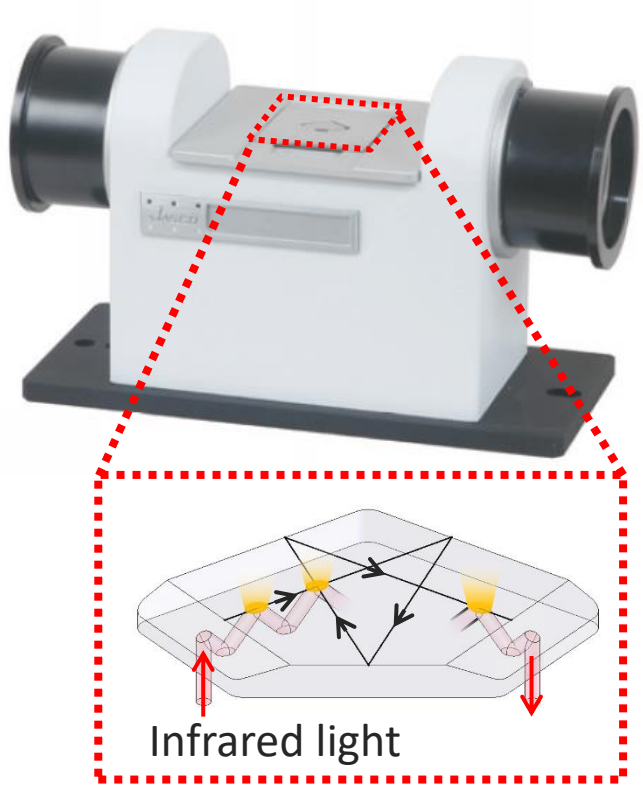
Sample	Helix	Sheet	Turn	Other
Puris	12 %	34 %	26 %	28 %
Nutralys	11 %	35 %	26 %	28 %

High sensitivity macro ATR for liquid sample

Configuration: FT/IR-4000/6000+ ATR PRO PENTA + Photo voltaic (PV) – MCT detector

New

ATR PRO PENTA



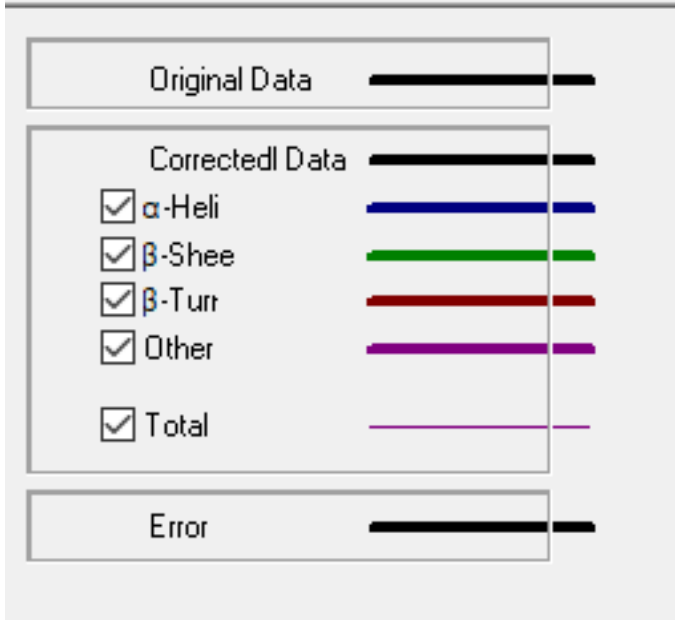
14-reflections ATR (Ge)

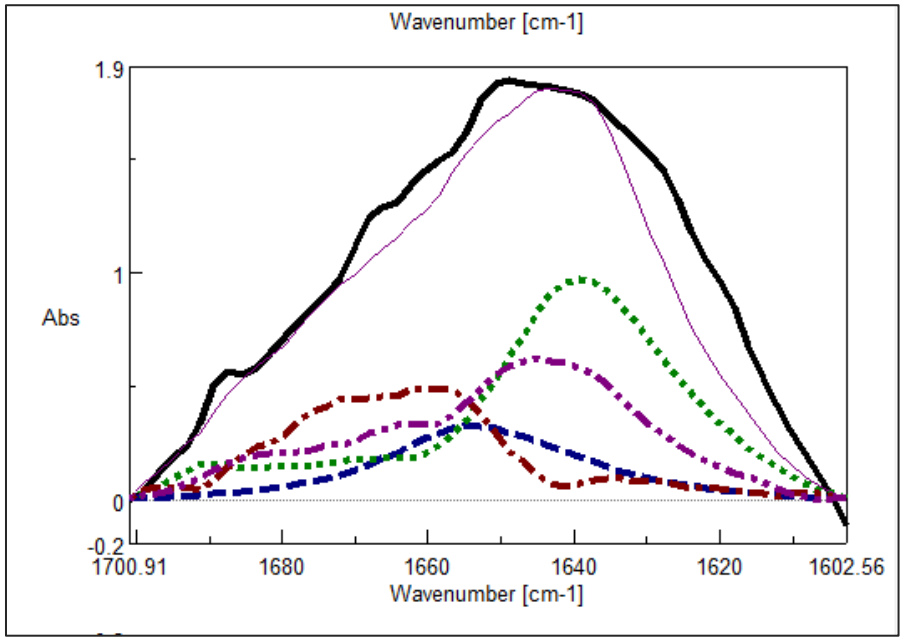
- Higher sensitivity (1-order of magnitude) than single bounce germanium crystal ATR + TGS detector

- Good linearity of PV-MCT ensures high accuracy even in high-energy conditions.

- Enables to measure small volume sample (several µL)

SSE Results using PENTA ATR





Sample	Helix	Sheet	Turn	Other
Puris	11 %	39 %	24 %	26 %
Nutralys	11 %	39 %	24 %	26 %

Summary

ATR and transmission data from based plant protein samples are enriched in β – sheets,

Transmission data showed lower values in β – sheets, but still is predominant

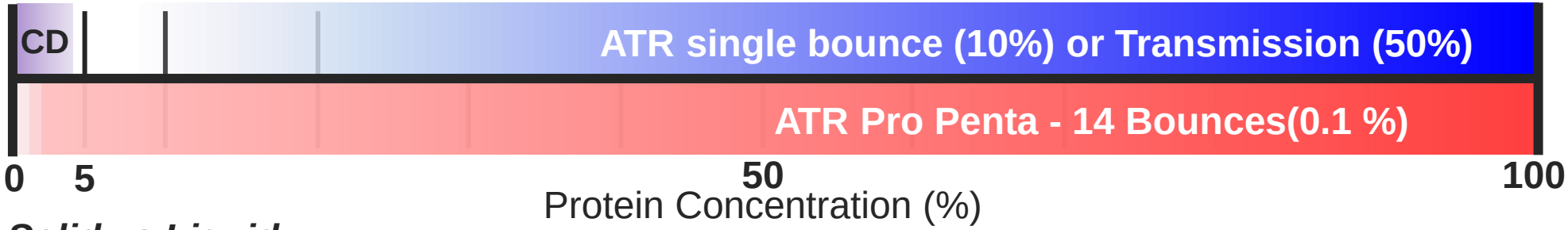
Agenda

- Proteins in Food
- Plant Based Protein Analysis
- Secondary Structure Estimation using CD
- Secondary Structure Estimation using FTIR
- **CD vs. FTIR: What to Choose**

FTIR vs CD

Why Use IR, CD or both?

Concentration:



Solid vs Liquid:

- CD is more suited to liquids.
- FTIR can use ATR for solids, ATR Penta or transmission for liquids

Background Correction:

- CD solvents and buffers don't heavily impact signal.
- FTIR has H_2O background at Amide I

Development Stage:

- CD offers more flexibility as a research instrument.
- FTIR is generally faster, better for QC

IR vs CD, sampling recommendations etc.

Building Orthogonality into Biosimilar Testing

by Richard L. Easton
Monday, April 13, 2020 4:49 pm

Primary and Higher-Order Structural Characterization Strategy for Biosimilarity Assessment

by Fiona M. Greer
Wednesday, December 16, 2015 1:14 pm

View PDF

Protein higher order structure (secondary, tertiary and quaternary structure) determination in accordance with FDA, EMEA and ICH Guidance (Q6B, Q5E)

IR vs CD, sampling recommendations etc.

The case for orthogonal analysis

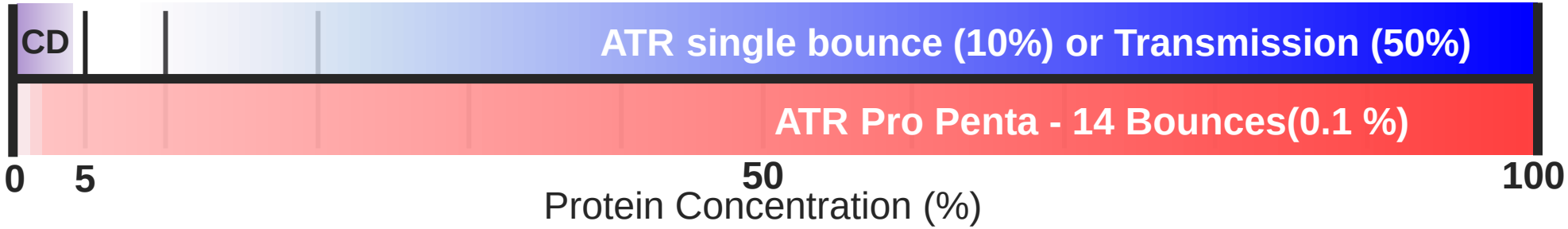
One Measurement: 1 point of failure.

- Are results truly different? Or was an error made?

Two Measurements: 2 points of failure

- 1 measurement has same result, other has different
  - Indicates error in one of experiments
- 2 differ from previous study = real change.

Conclusions



	FTIR	CD
Sample Form	Liquid Solid (Crystal and amorphous)	Liquid Solid with diffuse reflectance
Temperature Control	Yes	Yes
High Throughput	No	Yes
Mapping	Yes	No
Secondary Structure Database Status	Under Study	• Well-Known; • Multiple options: JASCO multi, BeStSel
Information	SSE and other	SSE, tertiary structure
Price	Inexpensive	Fairly expensive

Thank You For Attending!

QUESTIONS?

Orthogonal Analysis Resources

Building Orthogonality into Biosimilar Testing
https://bioprocessintl.com/sponsored-content/building-orthogonality-into-biosimilarity-testing/

Primary and Higher-Order Structural Characterization Strategy for Biosimilarity Assessment
https://bioprocessintl.com/manufacturing/biosimilars/primary-and-higher-order-structural-characterization-strategy-for-biosimilarity-assessment/

Higher Order Structural Protein Analysis Protein higher order structure (secondary, tertiary and quaternary structure) determination in accordance with FDA, EMEA and ICH Guidance (Q6B, Q5E)
https://www.intertek.com/pharmaceutical/biopharmaceuticals/high-order-structural-characterization/

Increasing regulatory focus on orthogonal analytical characterization for biosimilars
https://www.gabionline.net/sponsored-articles/Increasing-regulatory-focus-on-orthogonal-analytical-characterization-for-biosimilars