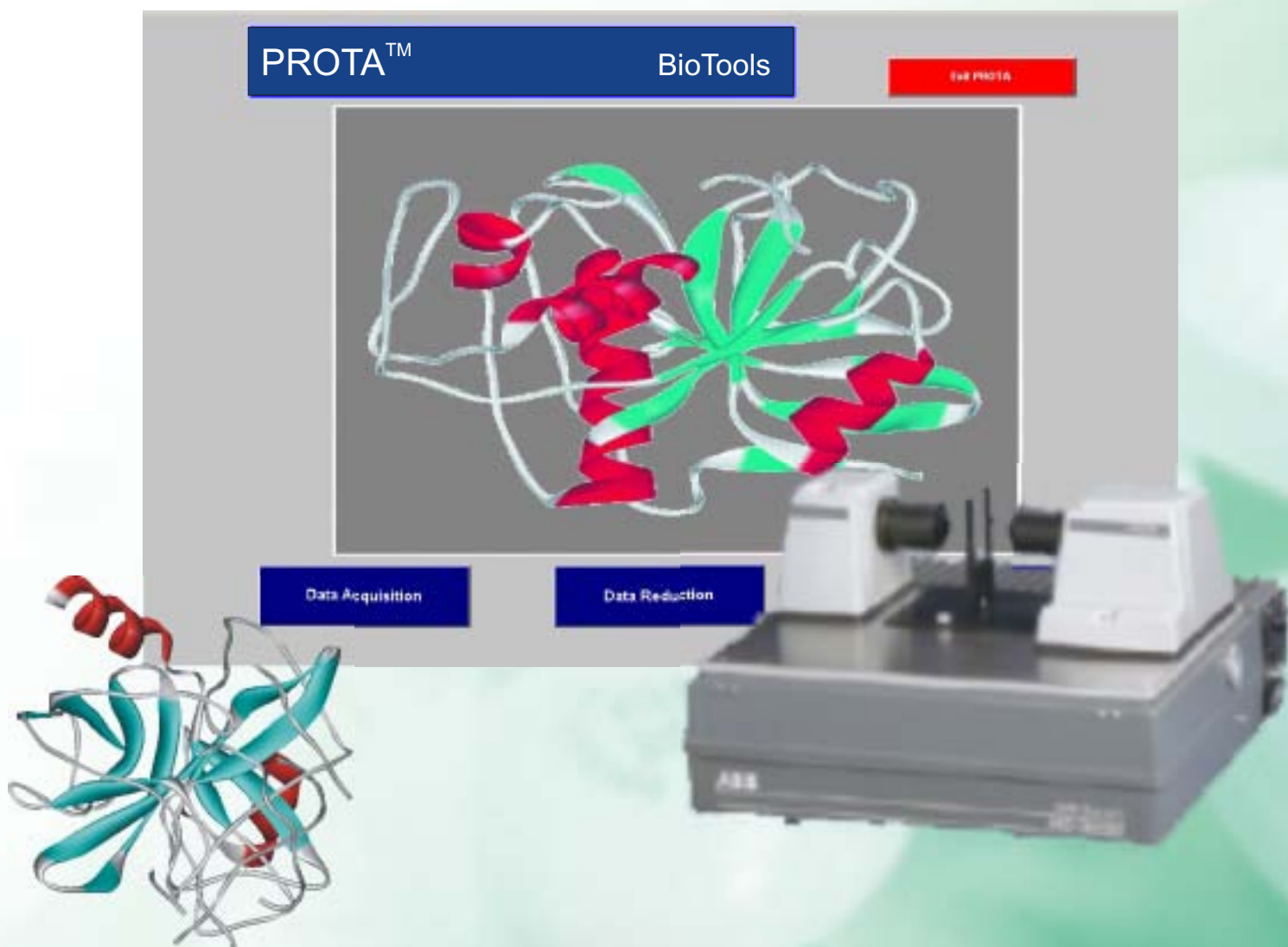


BioIR / PROTA

FT-IR Protein Analyzer



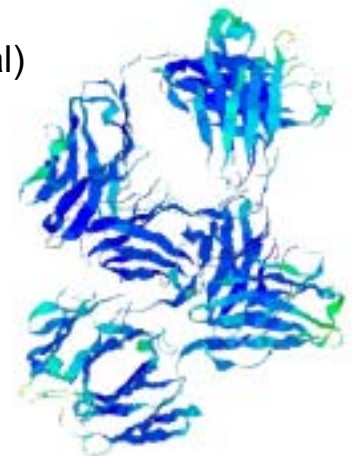
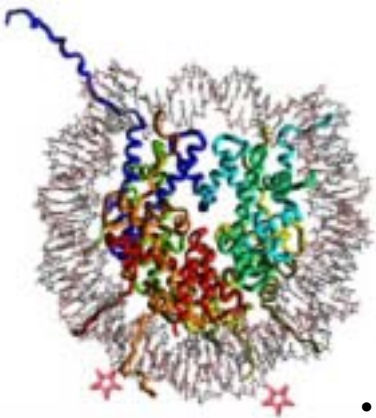
FT-IR of Biomolecules

BioIR™ is a family of dedicated solutions for the biopharmaceutical industry based on FT-IR instrumentation and comprised of specialized software and databases.

The first such system, **PROTA**, was introduced in 1998 and has since rapidly become the industry's preferred choice. **PROTA** provides a fast, cost-effective and sensitive way to determine secondary structure of a protein or to follow structural changes due to perturbations. This turnkey system is designed to be use by both spectroscopists and non-spectroscopists. It's user-friendly, intuitive software guides users through data acquisition and analysis. **PROTA** includes all of the functions, in one integrated package, required to link IR spectral data and protein structure.

Applications

- Formulation studies (liquids and solids) - effects of excipients, pH and buffers
- Determination of secondary structures of de novo proteins
 - Conformational stability and dynamics
 - Structure in aggregates
 - Mutation studies
 - Structural characterization upon environmental effects
- Stability studies (thermal and chemical)
- Drug delivery
- Protein-protein, protein-DNA/RNA and protein-drug interactions
- Crystallization condition screening



PROTA Instrument

PROTA Includes:

Hardware

- ABB Bomem MB-104 FT-IR spectrometer
- Pentium PC pre-loaded
- Liquid cell with CaF₂ windows
- Purge Control Kit

Software

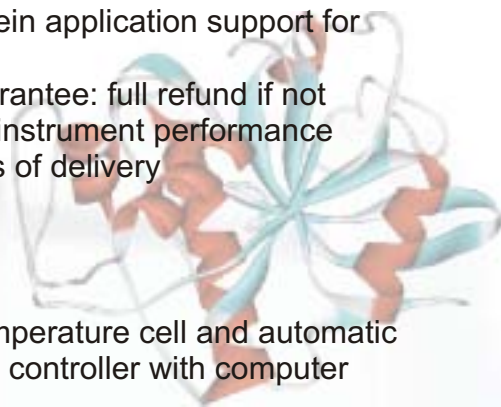
- Windows-based integrated software for data acquisition and data analysis
- Complete Grams A/I software
- Protein FT-IR databases:
 - Transmission
 - ATR

After-Sales Service

- Installation and training on FT-IR spectroscopy of proteins by experienced scientists
- BioTools/ABB combined service and technical protein application support for one year
- BackIR™ Guarantee: full refund if not satisfied with instrument performance within 30 days of delivery

Options

- Variable Temperature cell and automatic temperature controller with computer interface
- KBr and ATR accessories
- Microscope
- A dry air purge supply is recommended to achieve optimal performance



PROTA Instrument:

FT-IR Spectrometer System

- ABB Bomem MB-104 FT-IR spectrometer consisting of:
 - Arid-Zone sample compartment with countercurrent purge flow in telescopic purge tubes
 - Non-hygroscopic ZnSe beamsplitter
 - High-sensitivity DTGS detector
- Resolution: 1 cm⁻¹ - 128 cm⁻¹
- Spectral range: 6,500 - 500 cm⁻¹



Software Functions

1. Data Acquisition

Automated, user-friendly step-by-step data acquisition

2. Data Reduction

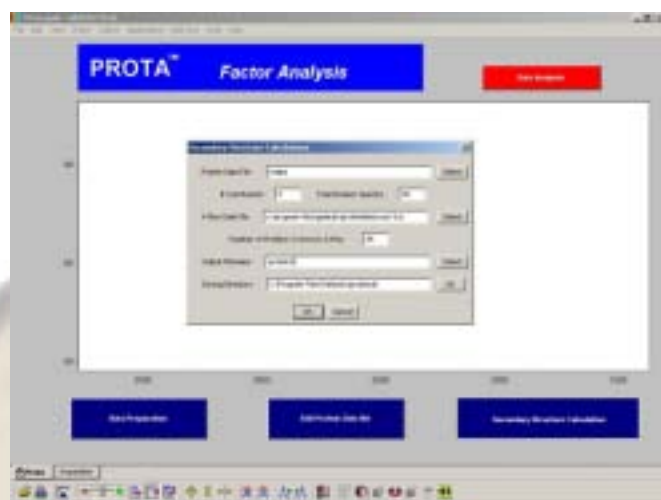
- Automatic buffer subtraction
- Pathlength correction
- Water vapor subtraction
- Correction of spectrum for sidechain contributions

3. Data Analysis

- Derivative and deconvolution based on maximum likelihood
- Factor Analysis for secondary structure prediction. The factor analysis algorithm determines secondary structure expressed as the percentage of each of the five main structure types: a-helix, b-sheet, turn, bend and 'others' (see application example 3). It uses a spectral database of 50 proteins with known secondary structures.

- Formulation algorithm

This function is used to determine a change in structure due to a perturbation (formulation, lyophilization, pH, solvent, temperature, etc.). This algorithm was developed by Prof. John Carpenter's group at the University of Colorado (see application example 2).



Sample screen shots

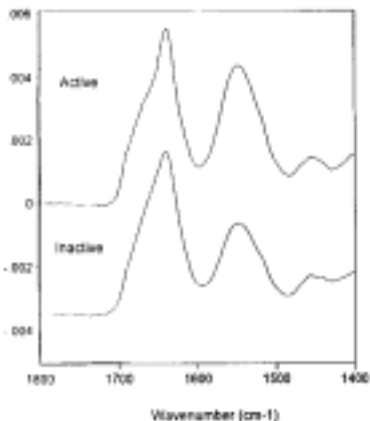
Biobase

The FT-IR database of proteins in the liquid form, measured in transmission in water-based buffers, has now been extended. Databases of proteins in solid form measured using ATR are also available. The proteins have been chosen to have the widest range of structures available. These spectra have been acquired under strictly controlled conditions that match the crystallographic conditions. Proteins can be added to the database by the user.

Applications

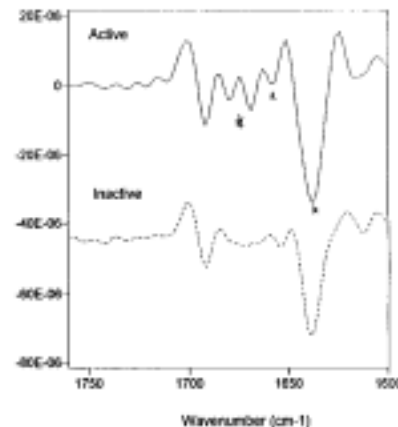
Application Examples:

1. Low Protein Concentration Studies



Protein samples with concentrations as low as 3 mg/ml in H₂O can be routinely measured with PROTA. Conditions: 20 min. acquisition time, NO water vapor subtraction, buffer subtracted using the automated buffer subtraction function.

2. Formulation Studies



FT-IR derivative spectrum of 'intact' immunogenic antibody compared to a lyophilisate, that yields inactive, non-immunogenic form after reconstitution with water. Obvious differences are observed between the two spectra. These spectral differences identify structural changes that a protein undergoes upon lyophilization. This information may streamline and improve the formulation process.

3. Protein Secondary Structure Determination

Protein	% a-helix	% b-sheet	% bend	%turn	%other	sum
A-chymotrypsin	12	32	12	11	33	100
Carbonic Anhydrase B	14	33	14	13	26	100
Citrate Synthase	58	4	9	11	20	102
Carboxypeptidase	43	15	8	13	23	102
Catalase	44	11	11	12	24	102
Γ-chymotrypsin	12	35	12	13	26	98
Chymotrypsinogen	27	24	12	13	25	101
Concanavalin A	0	43	15	13	25	97
Other Proteins						

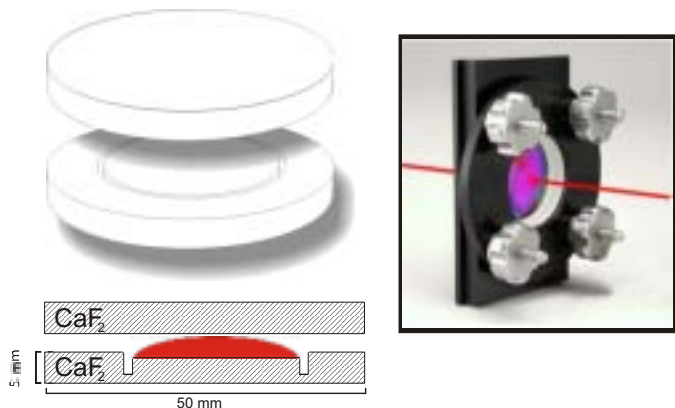
PROTA can be used to determine quantitative protein secondary structure. The prediction is done using factor analysis, compared to the 50-protein database. Typical results are shown above.

Accessories

Transmission Studies:

1. Liquids

- **BioCell™**



The CaF_2 cell for IR/UV-CD spectroscopy is created between a perfectly flat, optically clear plate and another plate, the center of which is deepened to form a recessed parallel surface surrounded by a groove. This groove serves as a barrier between the sample area and the outer seal, keeping the sample from readily coming into contact with the outer seal. The seal is created by the "upper" flat plate pressing onto the outer ring of the "lower" plate. The cells are very easily assembled and disassembled, filled with solution, and washed between measurements. The seal of the cell prevents the evaporation of water for about 24 h at room temperature. The reproducibility of the cell path length after assembly / disassembly is 0.1 μm

- Wide range of available path lengths:
~2 to 7, ~10, ~20, ~40, ~80 and ~120 μm
- 5 mm thickness x 50 mm diameter standard size. Cells with 4 mm x 40 mm dimensions are also available

The BioCell comes with a standard holder or a choice of holders below:

- **BioJack/T™**
High precision cell holder
(Thermostated available)



- **TempCon™**
Peltier based temperature controller



- **AccuTune™**
Gear for BioJack for extreme precision and ease of use



2. Solids

- **E-Z Press™ 12 Ton Hydraulic Press & 13 mm Die Set**

Lightweight ergonomic design for variety of lab applications, including pressing KBr pellets for FT-IR spectroscopy

13 mm Die Set



12 Ton E-Z Press™



Accessories

ATR Studies:

1. Liquids & Solids

- **DuraSAMPLIR II™** - Diamond ATR



SENSIR
TECHNOLOGIES

- **MIRacle™** - Single Reflectance ATR



PIKE
TECHNOLOGIES
Spectroscopy Consulting

The technique of Attenuated Total Reflection (ATR) allows direct comparison of proteins in liquid vs. solid state. Its ease of use simplifies the process of measurement and is ideal for comparison of environmental conditions such as the process of formulation.

- **BioFlowATR Cell**



The attachment for a 9-bounce DuraDisk (DuraSAMPLIR) allows studies of proteins with as little as 1 μ l of sample. Ideal for kinetic studies and folding experiments

Microscopy Studies:



The microscope fits into the sample compartment of **Bio/IR** instrument and can be used for analysis of a small amount of material or small particles.

Support & Service

Feasibility Studies

For those interested in determining if FT-IR Protein Structural Analysis is appropriate for their applications or specific research conditions, BioTools offers feasibility studies using **PROTA**. Studies include **PROTA** measurements of particular identified or unidentified proteins or peptides, determinations of protein secondary structural changes with varying environmental effects, and formulation studies for liquids and solids.

Consulting

BioTools offers consulting services that bring our expertise on spectroscopy of biomolecules to bear on your applications and needs. Costs depend on the extent of the study and degree of urgency.

Technical Support and Maintenance

BioTools, Inc. and ABB Inc. provide users of **PROTA** with complete maintenance and technical support. Each instrument sold comes with a full one year warranty, and additional maintenance contracts can be purchased. E-mail based help desk is open 365/24/7 at prot@btools.com or info@btools.com.

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Your Local Representative: