Extracting useful information from evolving FTIR data sets using MCR-ALS and 2DCoS

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Outlook

- Information Content in IR and Raman Spectra
- Chemometric Methods
  - MCR-ALS: Multivariate Curve Resolution – Alternating Least Square
  - 2DCoS: Two Dimensional Correlation Spectroscopy
  - Enzymatic hydrolysis of albumin by proteinase K
- Concept for time resolved FTIR spectroscopy of chemical reactions in solution
- Chemistry on the fly
- Summary

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Information Contained in Mid-IR and Raman Spectra

- Functional groups, fingerprint
  Identification of analytes
  Simultaneous determination of several analytes
- Inter- and intramolecular Interactions
  Determination of secondary structure of Proteins
- Information on latent variables
  Octane number, wine varieties, cancer

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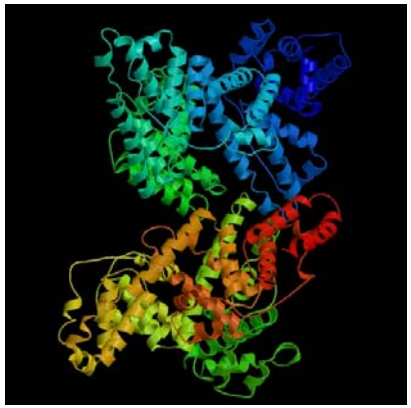
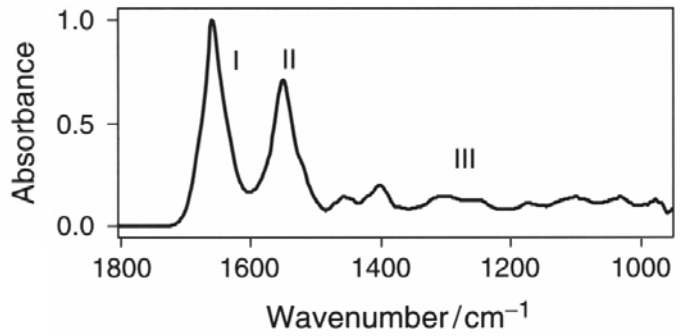
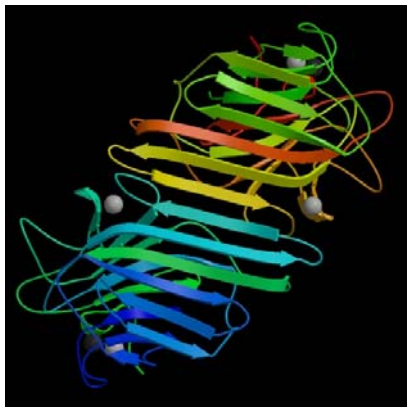
Example: Elucidation of Secondary Structure of Proteins

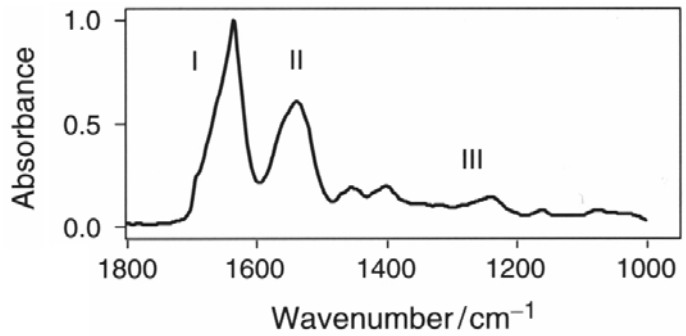
Albumin
α-Helix





Concanavalin
40% sheet





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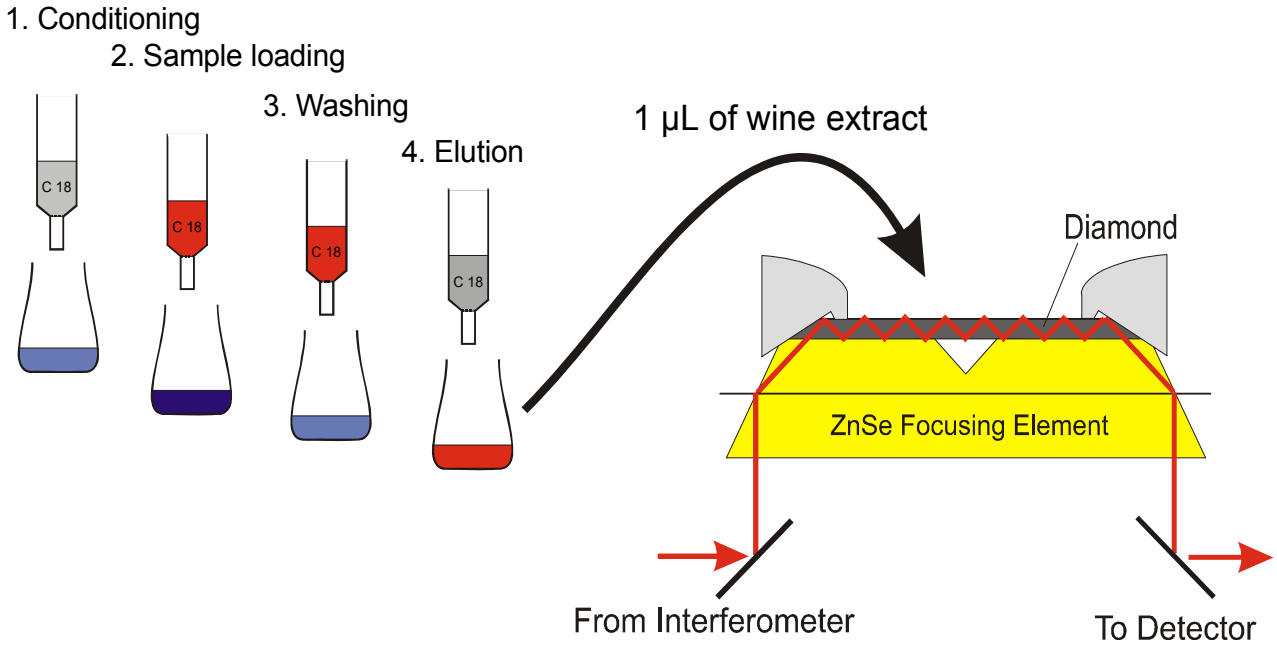
Example: Determination of Latent Variable Classification of Red Wines - Concept

Solid Phase Extraction

FTIR Spectroscopy
(Attenuated Total Reflection)



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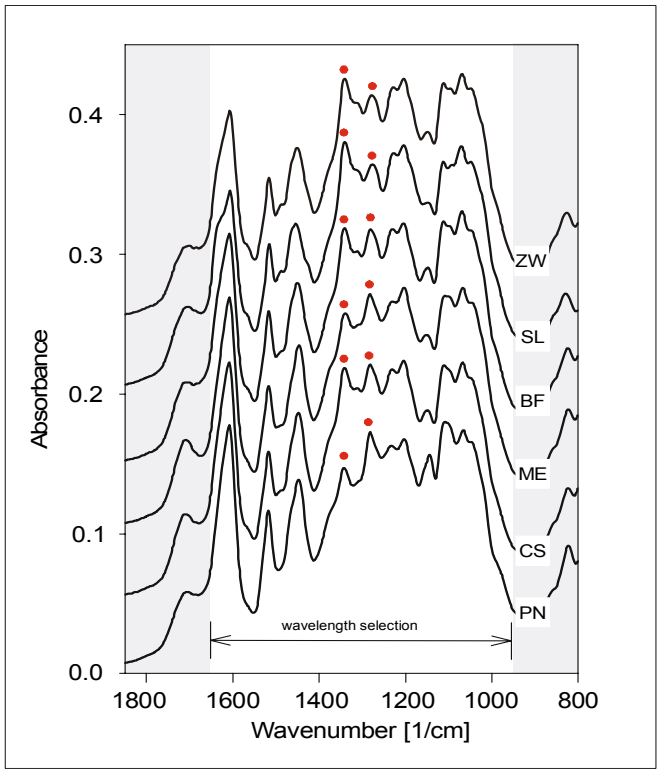
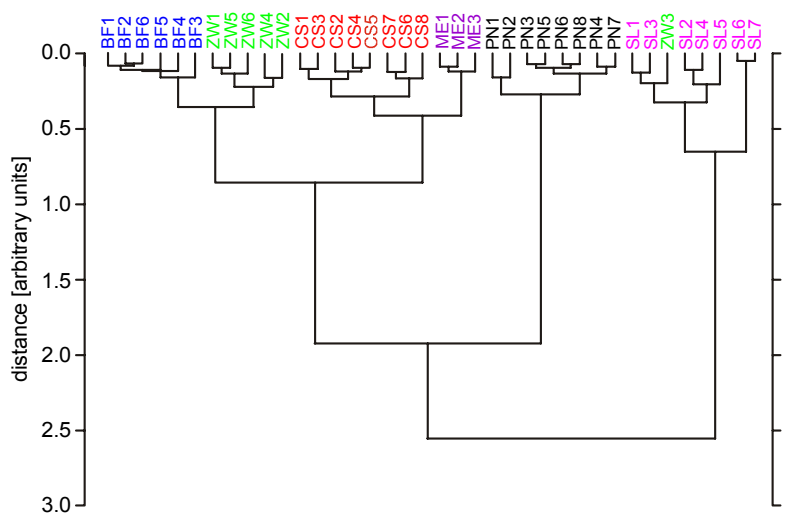
Classification of Red Wines - Results

Spectra of phenolic extracts



Hierarchical clustering



ZW: Zweigelt, SL: St. Laurent, BF: Blaufränkisch
ME:Merlot, CS: Cabernet Sauvignon, PN: Pinot Noir

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Multivariate Curve Resolution (MCR-ALS)

- Concept: Modeling of experimental data matrix *D*

- Multi-component systems can be often be described with a simple model consisting of the composition-weighted sum of signals of their pure components
- Only condition: linear structure of data set

$$\boldsymbol{D} = CS^{T} + \boldsymbol{E}$$

D*(r × c)* is the original data matrix
C*(r × n)* and ***S***^{T}*(n×c) contain pure response profiles related to the data variation in the* row *(r)* and column *(c) direction of* ***D****(r × c)*
E*(r × c)* is the error matrix

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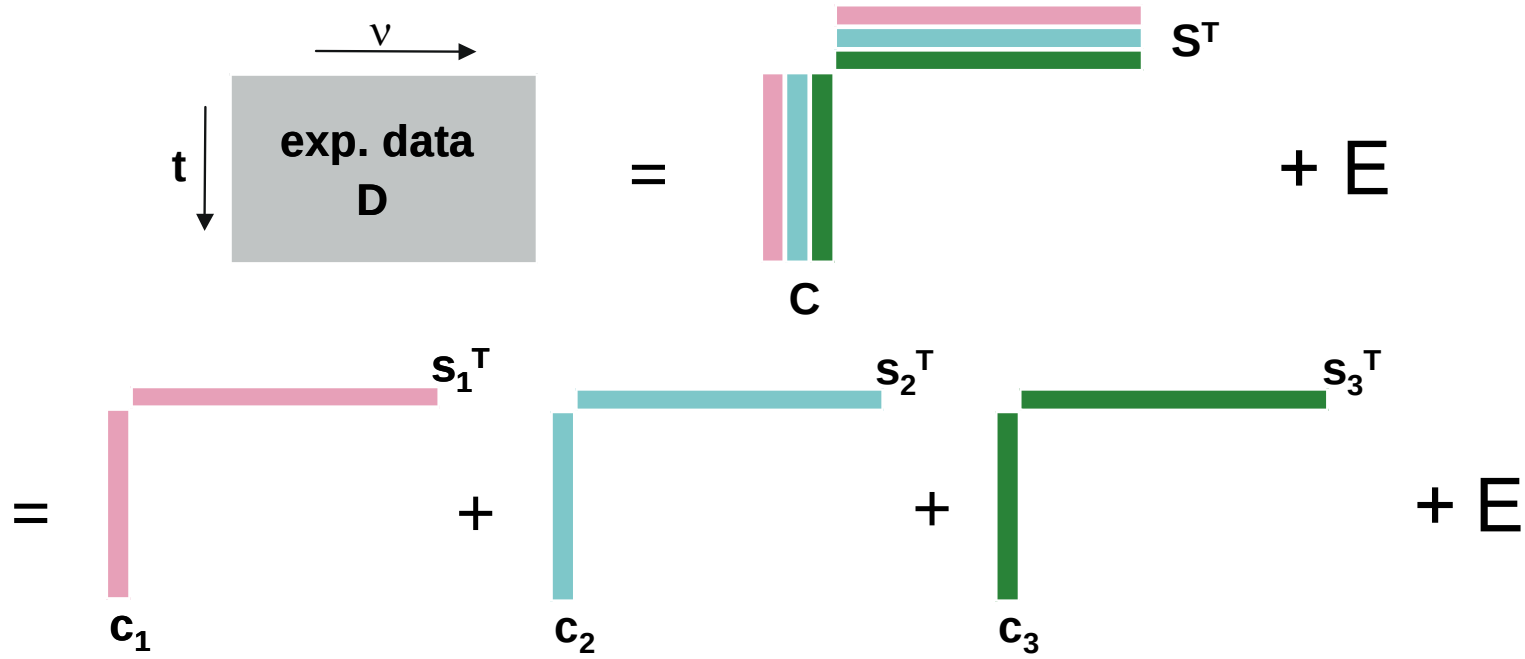
Multivariate Curve Resolution: Graphical Representation



c_i...concentration profiles with time s_i...spectra of pure substances

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Uncertainty in MCR Results due to Ambiguities

- Magnitude ambiguity :

$$D = \sum_{i=1}^{n} \left(\frac{1}{k_i} c_i \right) \left(k_i S_i^T \right)$$

$$D = C' S'^T$$

- Rotational ambiguity :

$$\boldsymbol{D} = \boldsymbol{C}\boldsymbol{S}^{\mathrm{T}}$$
$$\boldsymbol{D} = \boldsymbol{C}(TT^{-1})\boldsymbol{S}^{\mathrm{T}}$$
$$\boldsymbol{D} = (CT)(\boldsymbol{T}^{-1}\boldsymbol{S}^{\mathrm{T}})$$
$$\boldsymbol{D} = \boldsymbol{C}'\boldsymbol{S}'^{\mathrm{T}}$$

Aim to reduce these ambiguities

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Exploratory Data Analysis

- Important to find good (correct) initial estimates for starting the iteration process in of MCR
  - Initial estimates on concentration profiles and responses (spectra) need to be made
- Typical questions to be answered:
  - How many components shall be assumed to be present?
    - PCA, EFA, FSMW-EFA,…
  - Is there an special „purest“ variable in concentration or spectral axis available ?
    - SIMPLISIMA, PCA

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Constraints

- Property which are fulfilled for the whole or part of the system
- Examples:
  - Non-Negativität
  - Unimodality
  - Mass balance (closure)
  - Application of physico-chemical models
  - Known spectra / Concentration profile (are set invariant during the iteration process) => access to quantitative information
  - Local rank,…

Characteristics of MCR - ALS

- Clear criteria for stopping the iteration process

  % lack of fit = $100\ [(\Sigma r_{ij}^2)/\ (\Sigma d_{ij}^2)]^{1/2}$
  Typical value: 0.1 %

- Quantitative information
  - Number of components
  - Spectral properties
  - Concentration profiles
- Simultaneous analysis of several data sets
- Limitation: Difficulties in definition of spectral properties of „components“

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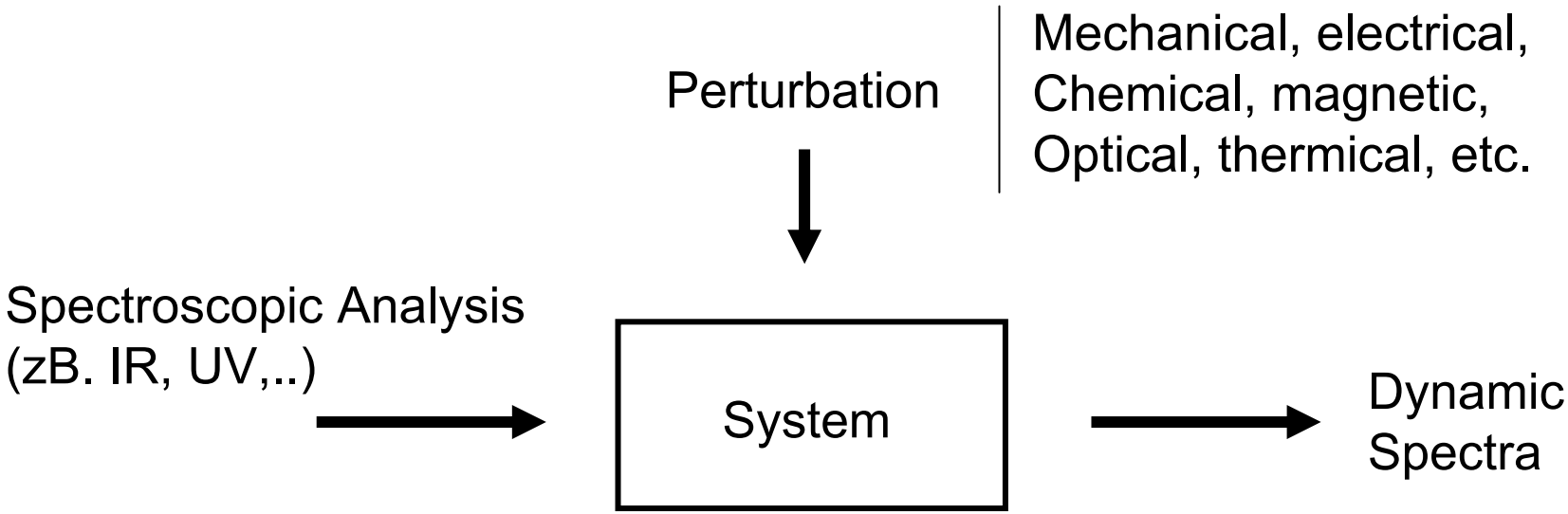
Overview : Two Dimensional Correlation Spectroscopy (2DCoS)



I. Noda, Lecture 2DCoS-3, 2005

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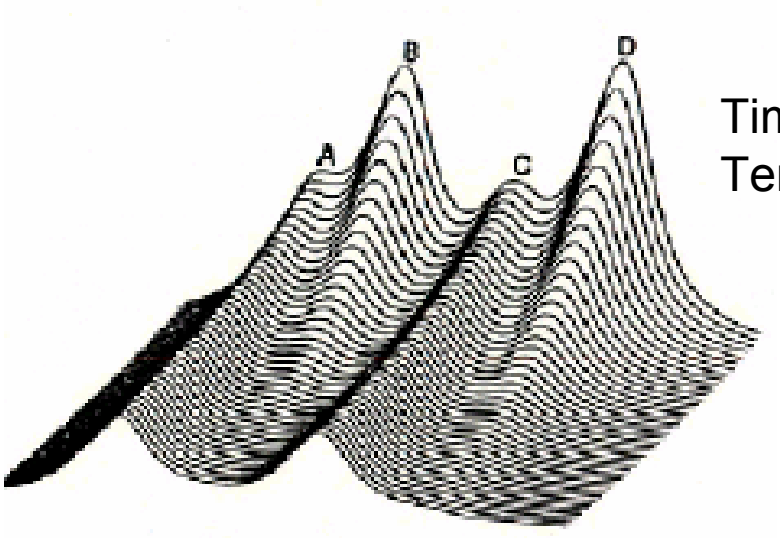
2DCoS

Idea: Looking for correlation in the experimental output *D*

Time resolved spectra



Spectral Variable

Time/pressure/
Temperature,..

Correlation analysis

$$\Theta(\nu_1,\nu_2)+i\Psi(\nu_1,\nu_2)$$

$$=\frac{1}{\pi T}\int_0^\infty \bar{Y}_1(\varpi)+i\bar{Y}_2(\varpi)$$

I. Noda Lecture 2DCoS-3, 2005

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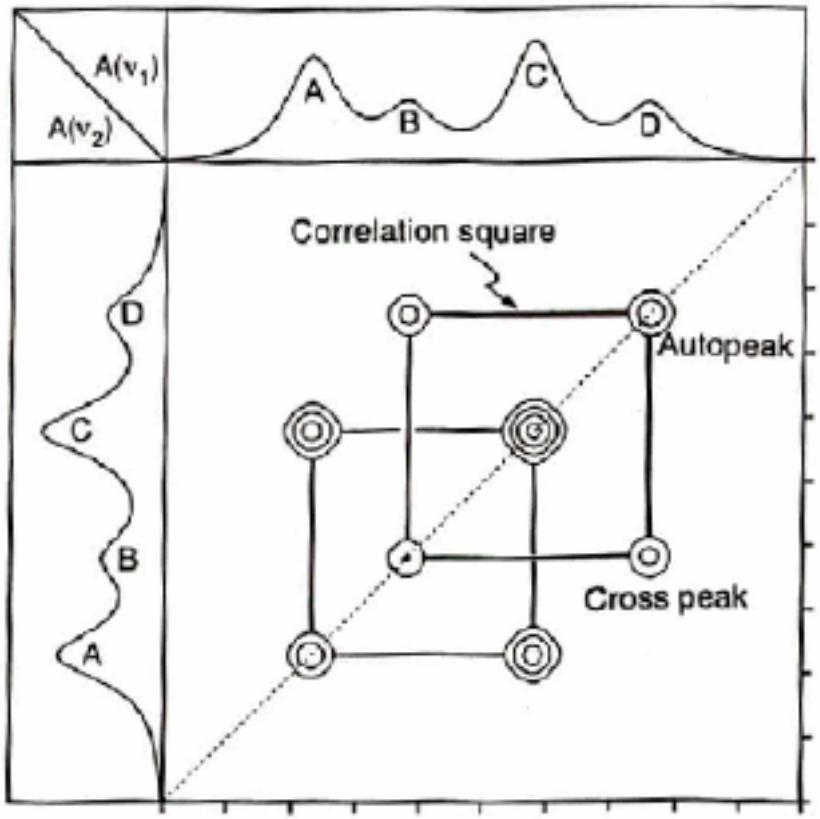
Synchronous Correlation Map



Spectral variable, ν_2

Spectral variable, ν_1

Autopeaks at diagonal positions represent the **extent** of perturbation-induced dynamic fluctuations of spectral signals

Cross peaks represent **simultaneous changes** of spectral signals at two different wavenumbers, suggesting a coupled or related origin of intensity variations

Same **signs** at cross peaks shows simultaneous increase/decrease at corresponding wavenumbers
Different signs: different behavior

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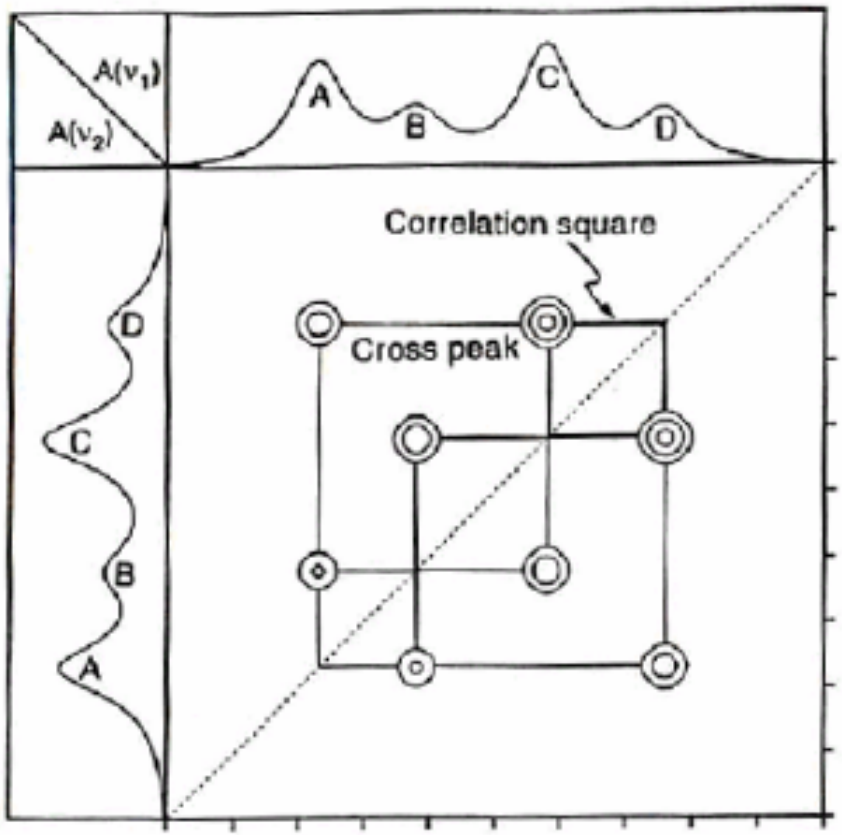
Asynchronous Correlation Map



Spectral variable, ν_2

Spectral variable, ν_1

Cross peaks appear only if changes at corresponding wavenumbers are occurring at a different rate

The **signs** of synchronous and asynchronous cross peaks become the **same** if the intensity change at at ν_1 occurs **before** ν_2

The **signs** of synchronous and asynchronous cross peaks become **different** if the intensity change at ν_1 occurs **after** ν_2

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Characteristics of 2DCoS

- Through emphasizing spectral changes of the system under study in the two-dimensional correlation maps a kind of “resolution enhancement” is achieved
- No model (components, spectra) => does not require estimates
- No quantitative information
- No objective criteria telling success of 2DCoS analysis
- Frequently very useful to get a „feeling“ for the behavior of the system under study

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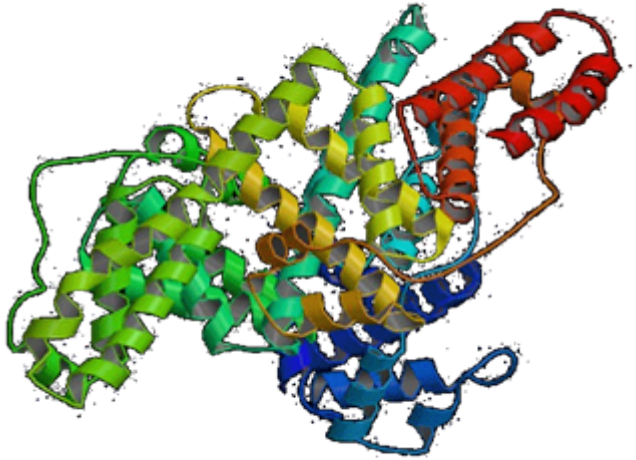
Enzymatic Hydrolysis of BSA with Proteinase K



Heating of BSA:
Until 50°C reversible conformational changes
Unfolding of α-helix irreversible from 52 - 60°C
Continued Temperature Increase:
Start of β-aggregation
From 70°C on gel formation takes place

Secundary structure:
67% α-helix,
10% β-turn
23% extended chain,
No β-sheet

Proteinase K
Family of subtilisin proteinases
No selectivity for cleaving at certain amino acids
High activity at 50 – 60°C

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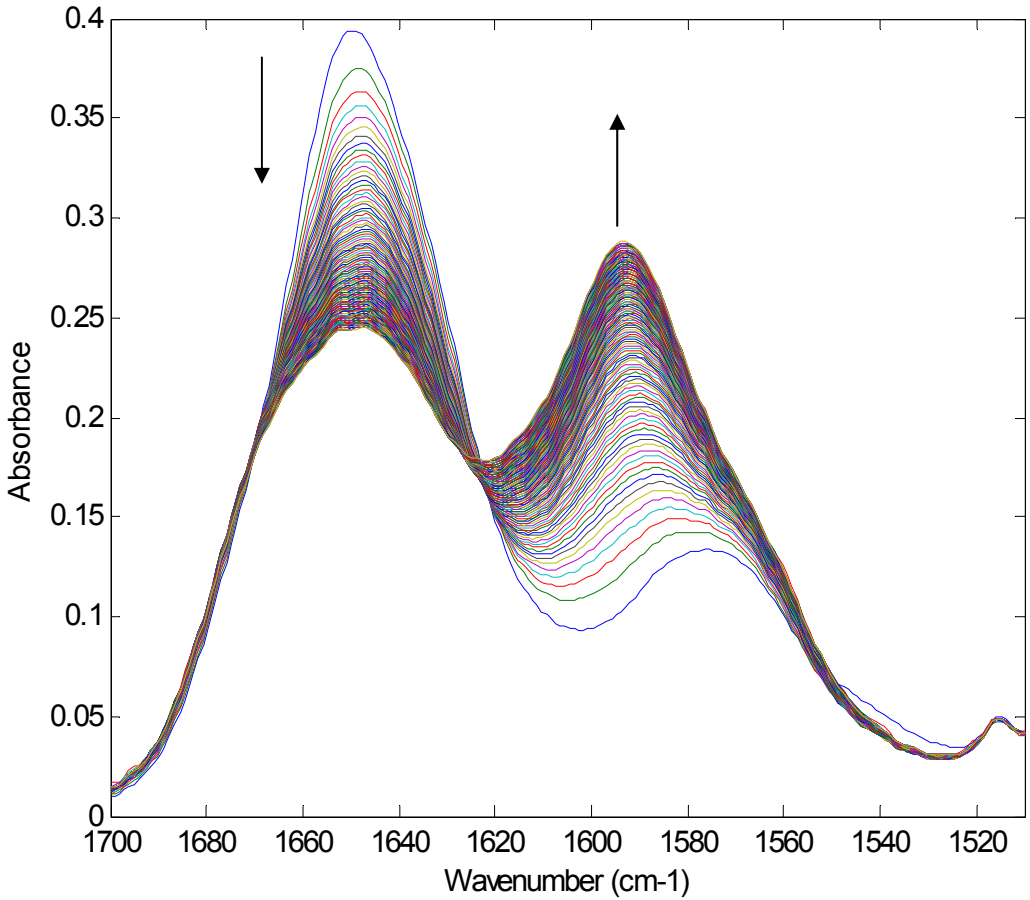
Experimental Conditions – Recorded FTIR Spectra

30 mg/ml BSA
0.5 mg/ml Proteinase K
T = 60°C
Phosphate buffer pD 7.4
Reaction time: 320 min

50 µm CaF_2
Spectr. res.: 2 cm-1



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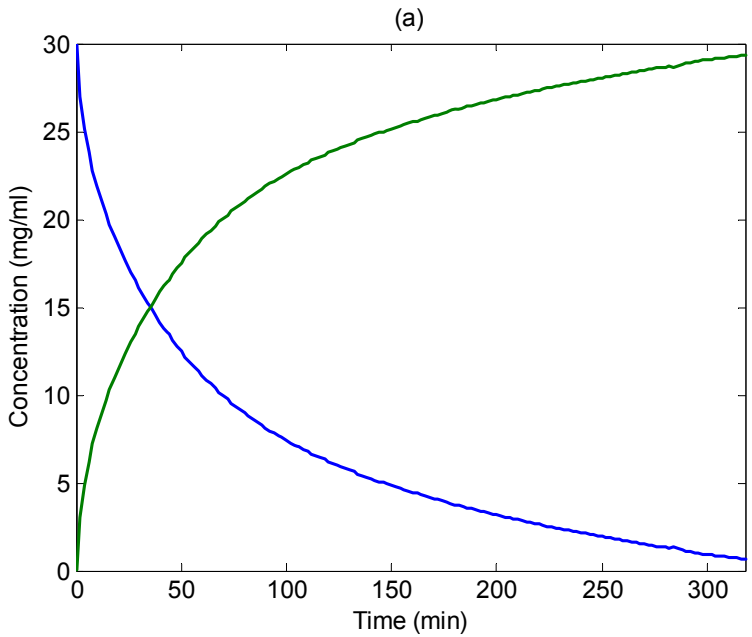
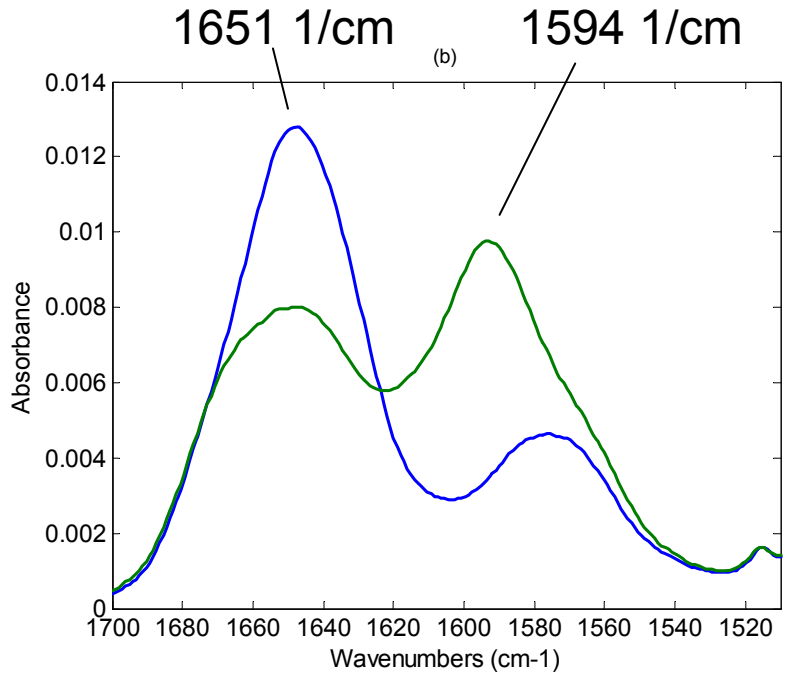
MCR-ALS Analysis I

According to PCA 2 components explain 99,99 % of spectral variance
=> Assumption of 2 components





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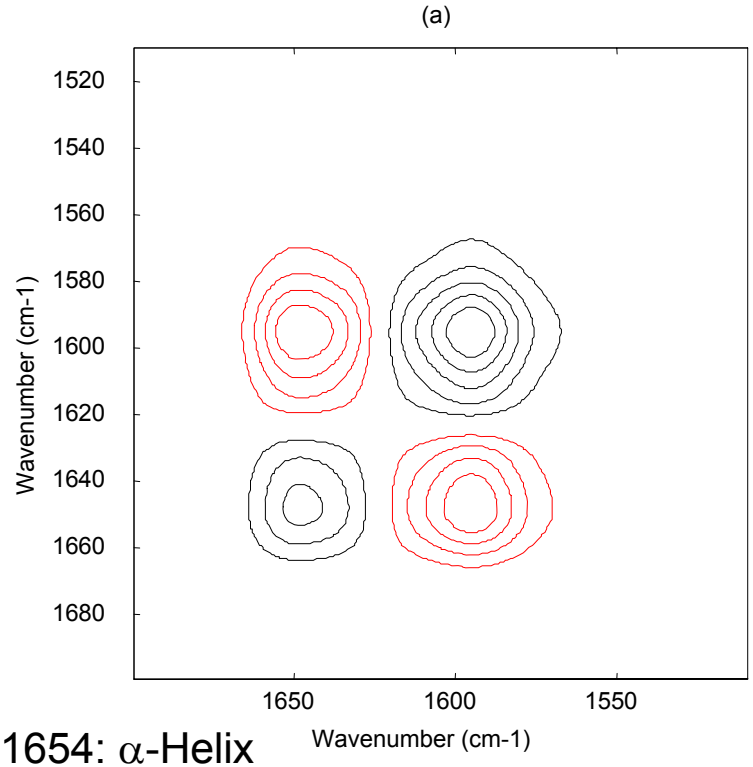
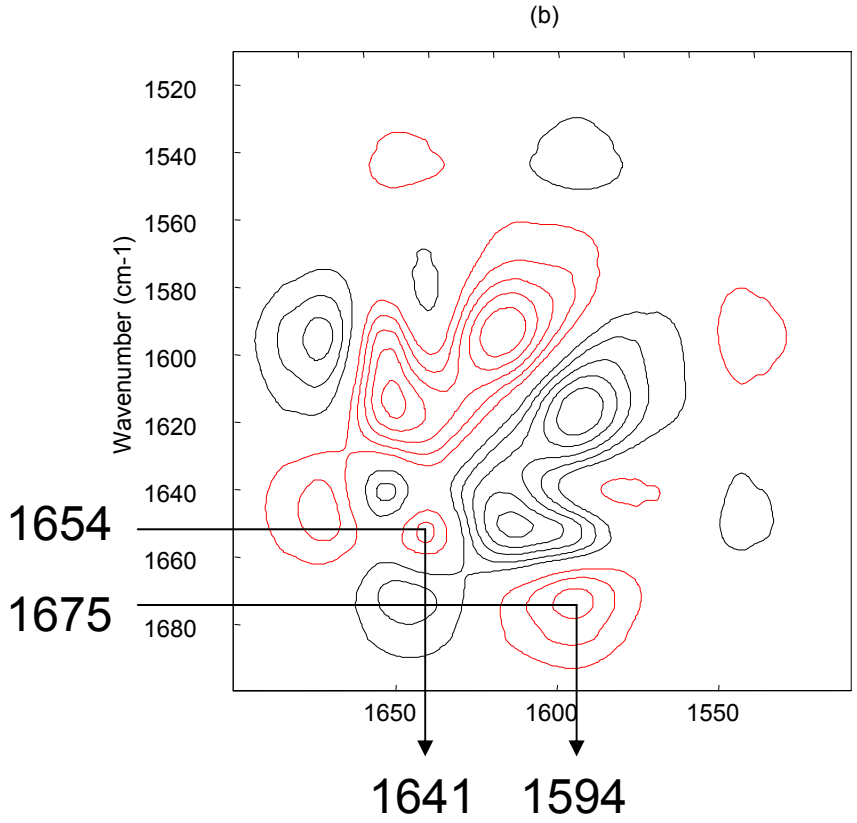
2DCoS Analysis

Synchronous Map



Asynchronous Map



1654: α-Helix
1641: unordered structure.
1594: Carboxylate
1675: β-turn
1616: β-sheet

There must be more than 2 components!!

Following „Noda Rules“ : 1654/1641; 1594/1675

Syn + / asyn - => ν_1 after ν_2

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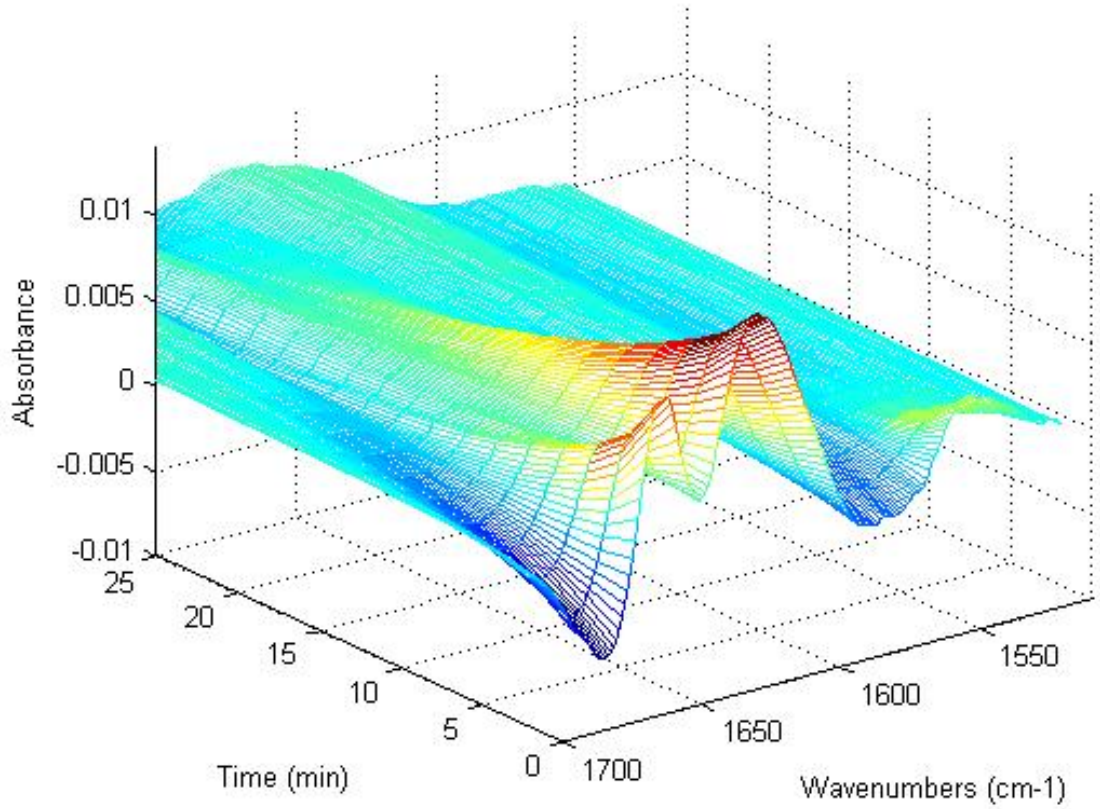
Residual Matrix *E*

$$E\,(r,c) = D - (CS^T)$$



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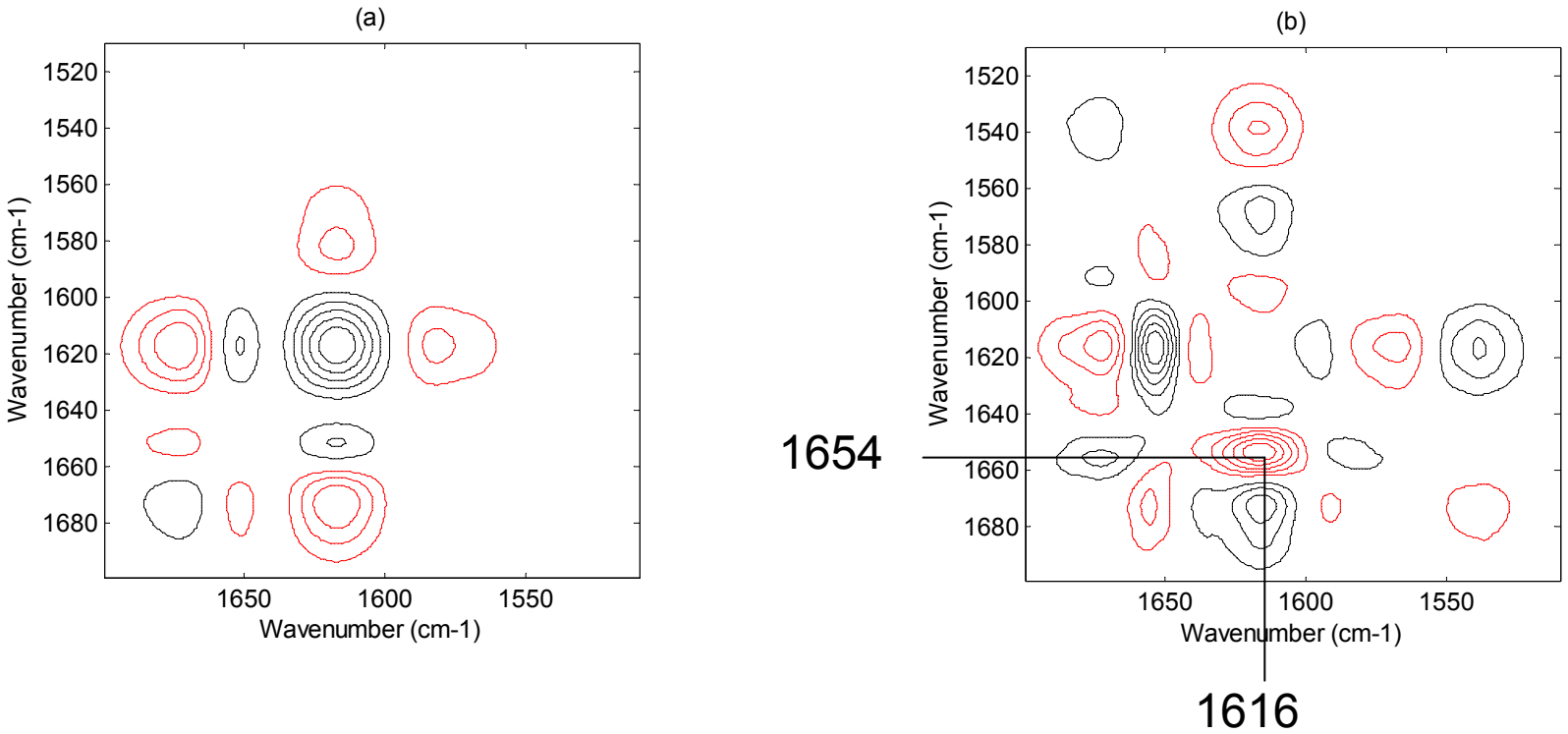
Residual Matrix *E* studied with 2DCoS

Strongest band at 1616 1/cm



=> At least one more component with band at 1616 1/cm

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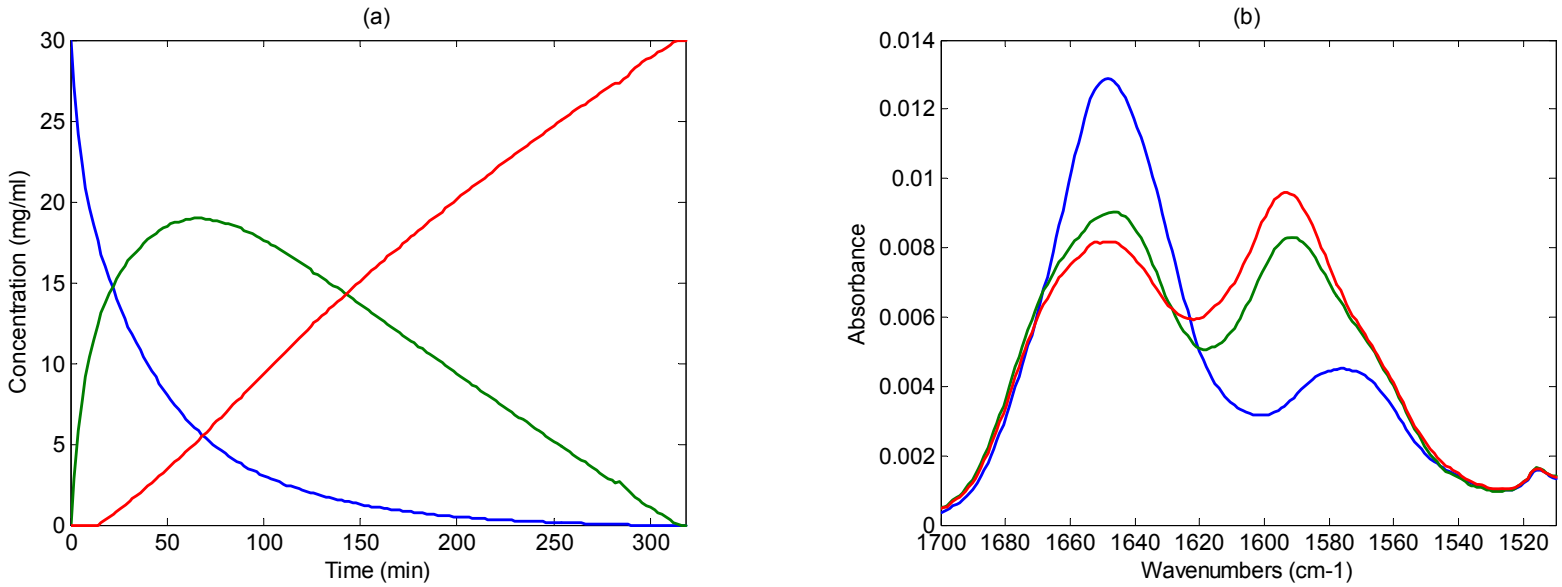
MCR-ALS Analyse II

- Assumption of 3 components, PCA explains now 99,999 %
- Analysis of a single experiment



Obviously there are 2 products: Intermediate and final product
This is unlikely from a chemical viewpoint

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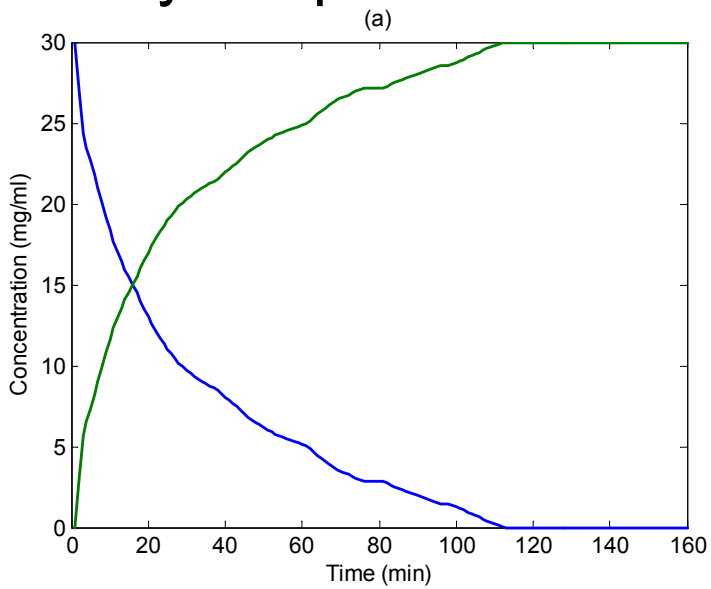
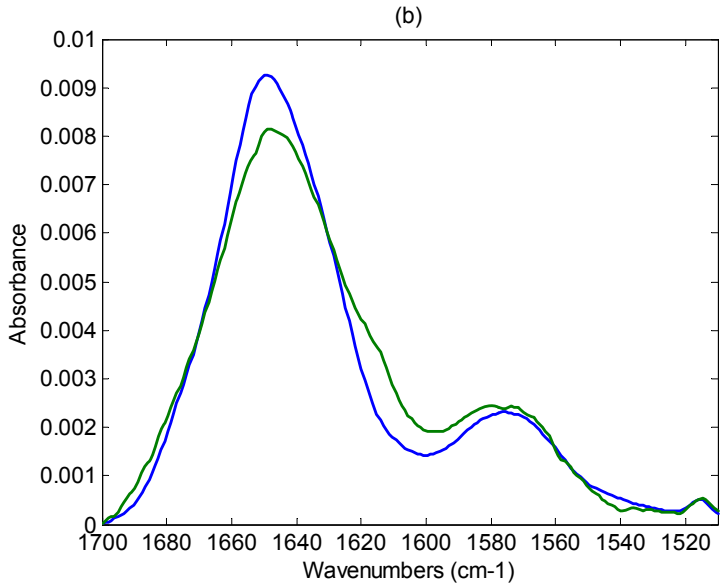
MCR – ALS of Experiment without Enzyme

- Only temperature induced effects are visible





Denaturation due to increasing temp.:
Increasing portion of disordered structures:
Amide I: 1654 -> 1651 1/cm
Also slight increase in content in β-turns:
Shoulders at 1616 (strong) and 1685 are being formed

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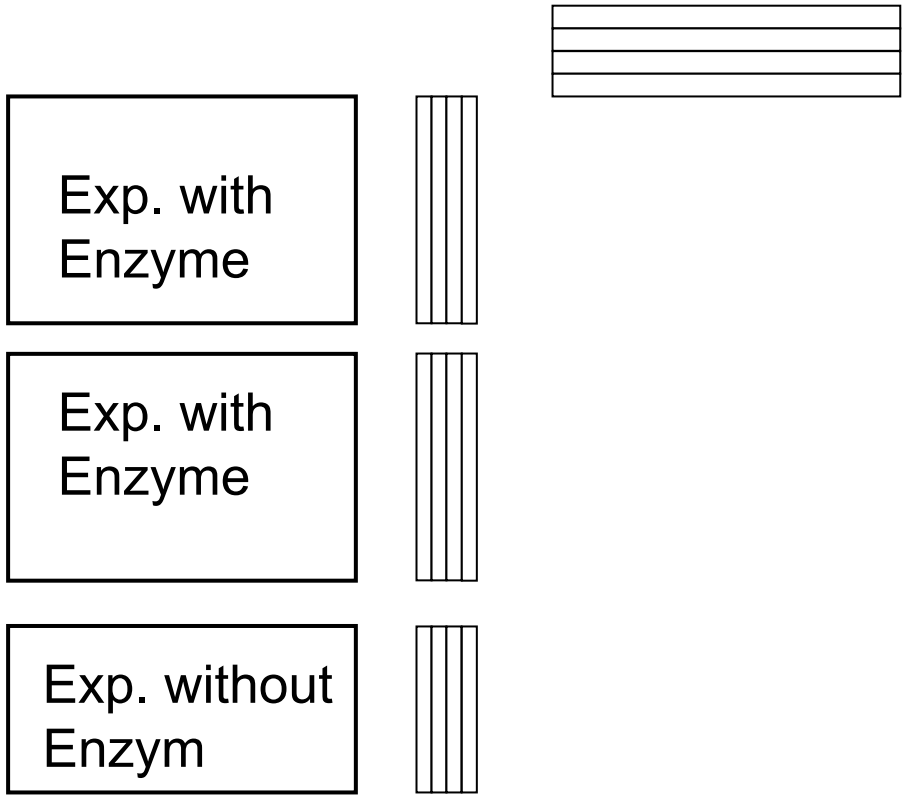
Matrix Augmentation

- Simultaneous Analysis of several experiments



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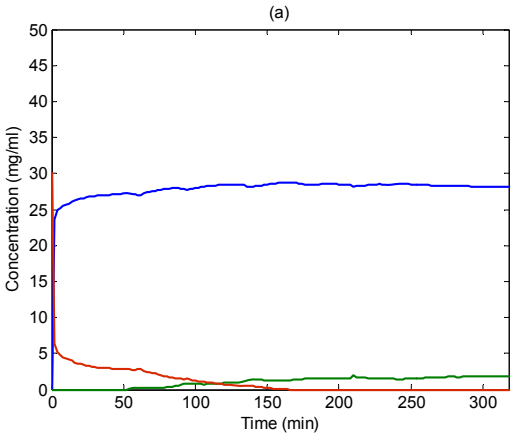
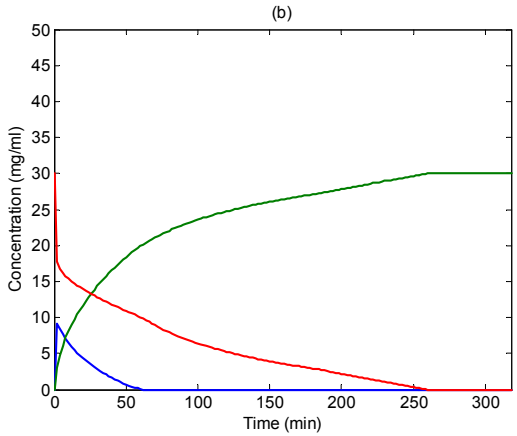
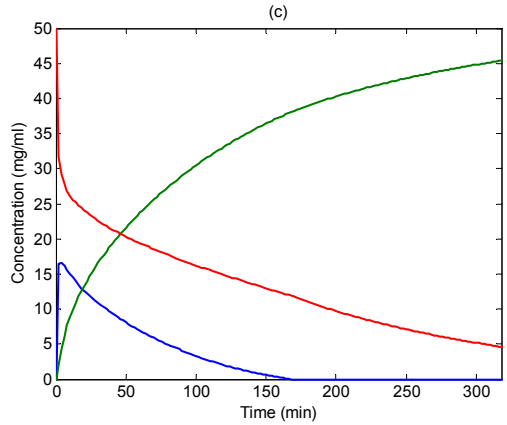
Result of MCR-ALS Matrix Augmentation



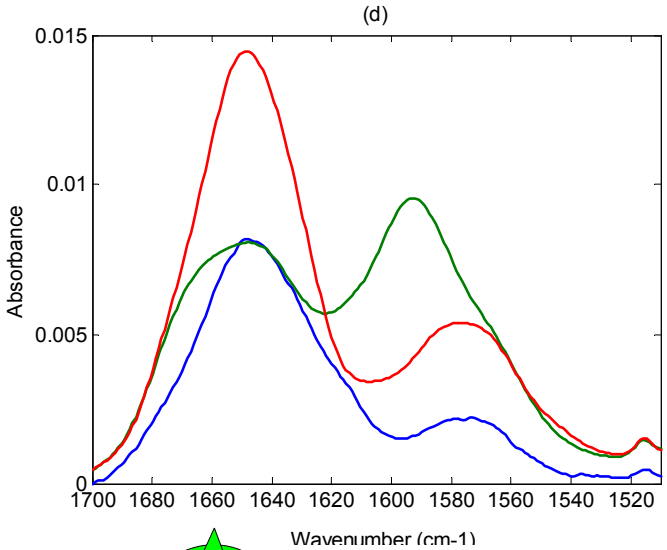
30 mg/ml Albumin



30 mg/ml Albumin
0.5 mg/ml Enzym



50 mg/ml Albumin
0.5 mg/ml Enzym



- Native Albumin
- Defolded Albumin (random structures shift toward 1641 as well as β-sheet)
- Reaction product

Dominguez et al. *Anal. Chem.* 78 (2006) 3257-3264

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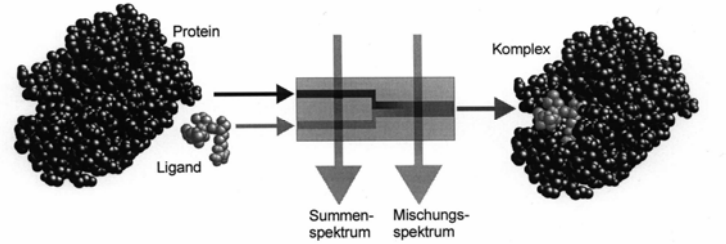
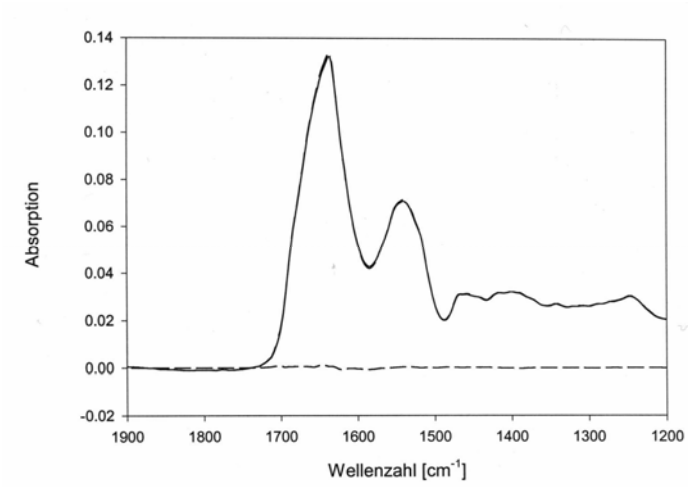
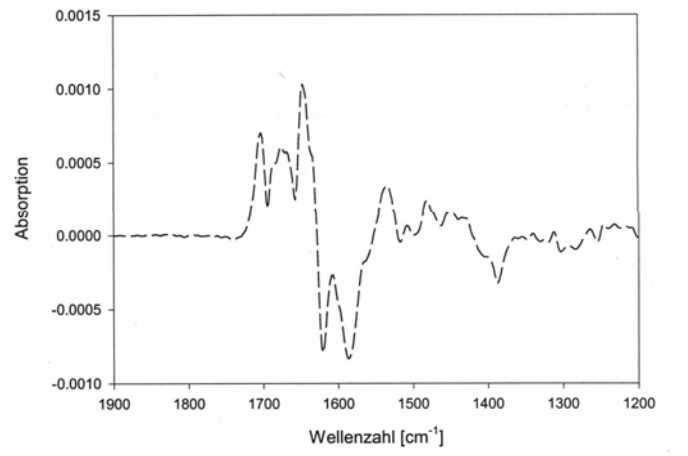
Results of Bioligand Interaction Studies

Purpose: Screening of Potential Drugs
Principle: Comparison of two FTIR Spectra







Füchsle, PhD thesis 1999

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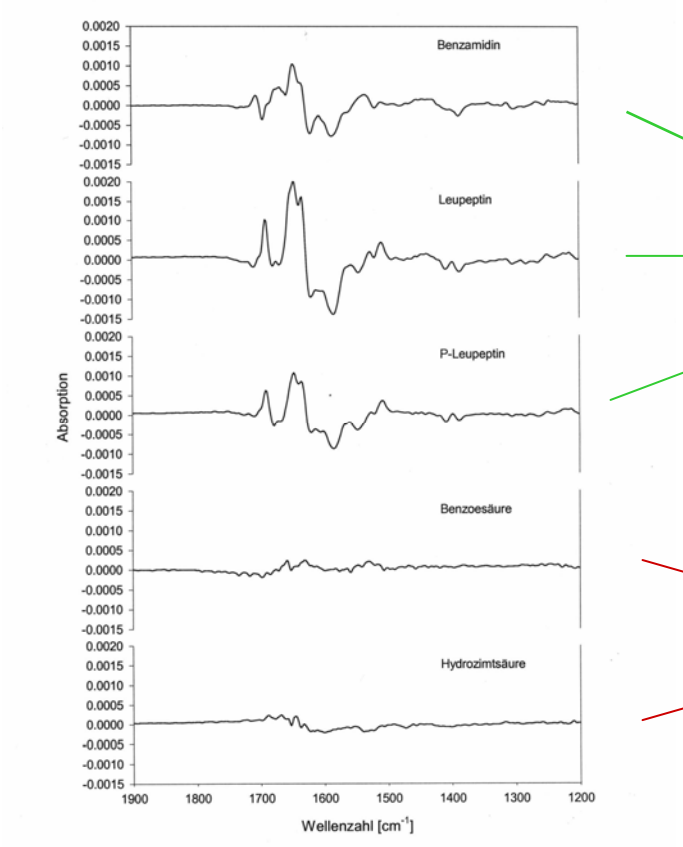
Results of Bioligand Interaction Studies

Difference Spectra



Active Molecules
=> Possible Drug

Inactive Molecules

Füchsle, PhD thesis 1999

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Difficulties of TR FTIR of Chemical Reactions in Solution

- FTIR spectroscopy
  - Small spectral changes expected =>
  - Signal averaging
  - Repeated initiation of the event under study (chemical reaction) for high time resolution
- Liquid handling
  - Fast, reproducible mixing of two liquids
  - Low sample consumption

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Short Optical Path in FTIR Imposes Difficulties for Rapid Mixing

- Flow rates for fast turbulent mixing require high pressure
  - Large reagent consumption
    (repeated experiments for good s/n-ratio)
  - Problems with constant optical path

- Low flow rates (max. 0.5 ml/min)
  - No problem with constant optical path,
    reduced reagent consumtion,but
  - Reynolds number: ~10 =>
    Strongly laminar flow:...slow mixing in conventional systems

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TR - FTIR Spectroscopy in Aqueous Solution

Problem:

- Optical pathlength in the µm range
- Strongly laminar flow
- No turbulence

Mixing is based on diffusion only!!

Solution: Miniaturization

$t=L^2/D$

t: time

L: diffusion distance

D: Diffusion coefficient

- Reduction of the diffusion length
- Generation of short inter-stream distances

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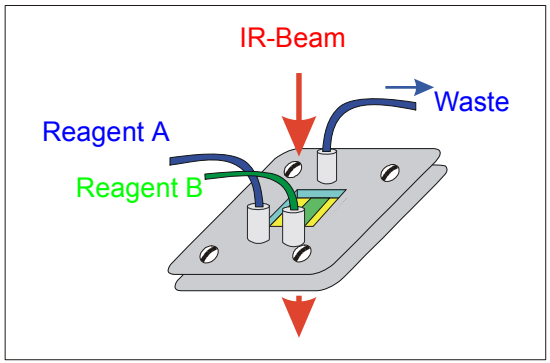
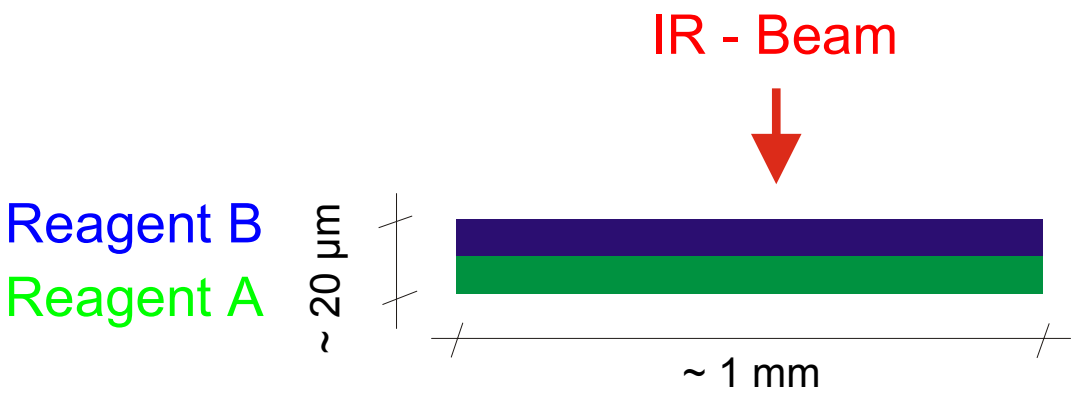
„Lasgane“ Micromixer



Reduction of diffusion lengths by superposition of streamlines

Cross section through the flow-cell



$t = L^2/D$

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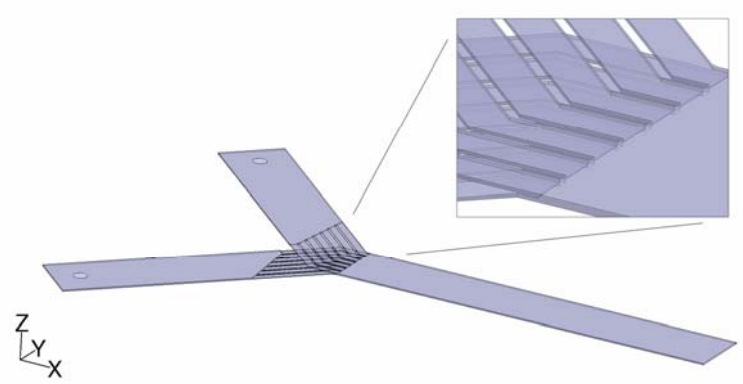
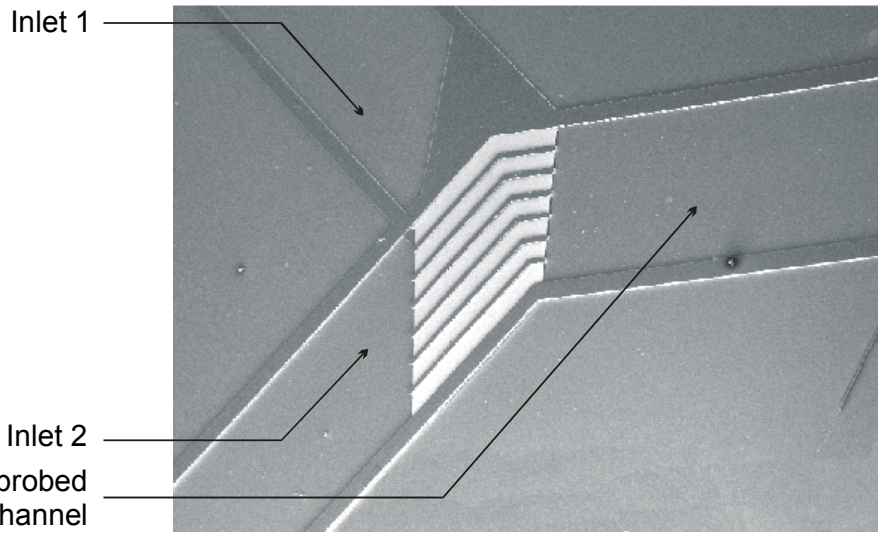
Details of the Fabricated Micro-mixer

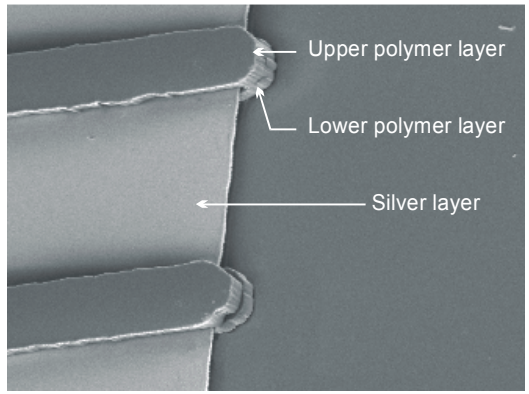
„Lasagne mixer“

Schematic view





SEM pictures



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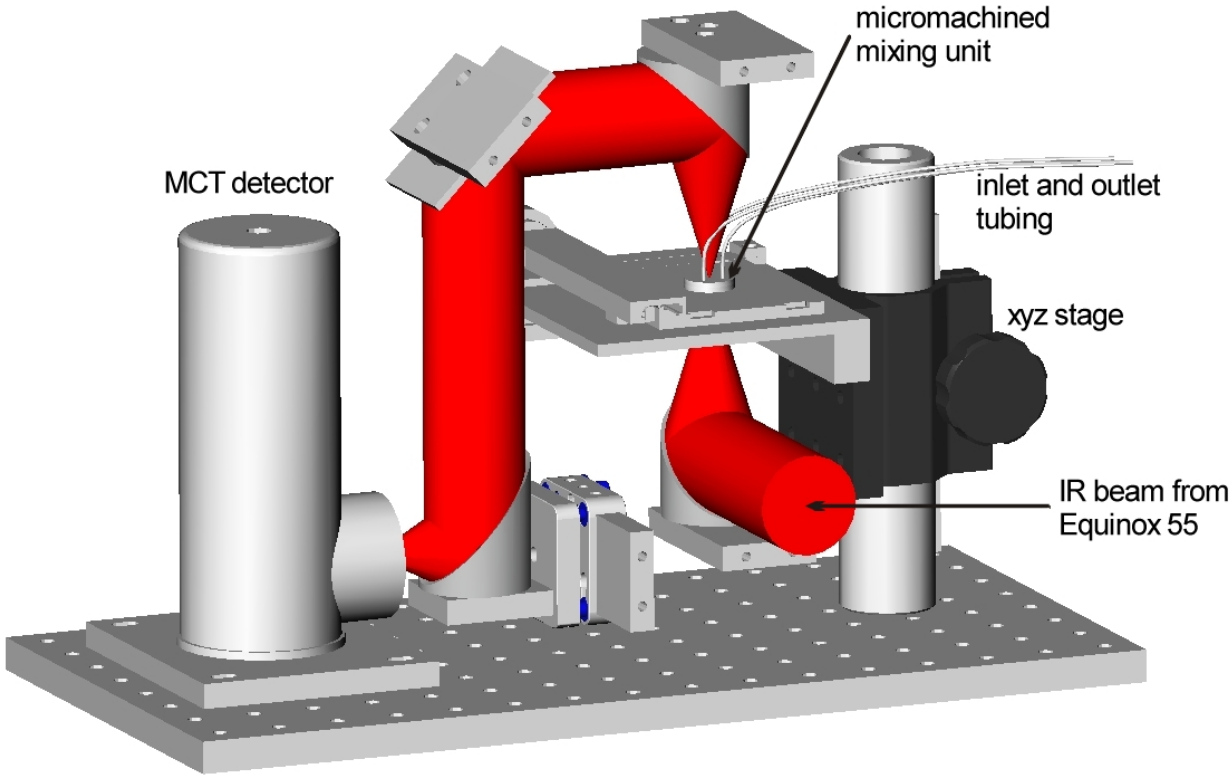
Schematic of Experimental Set-up



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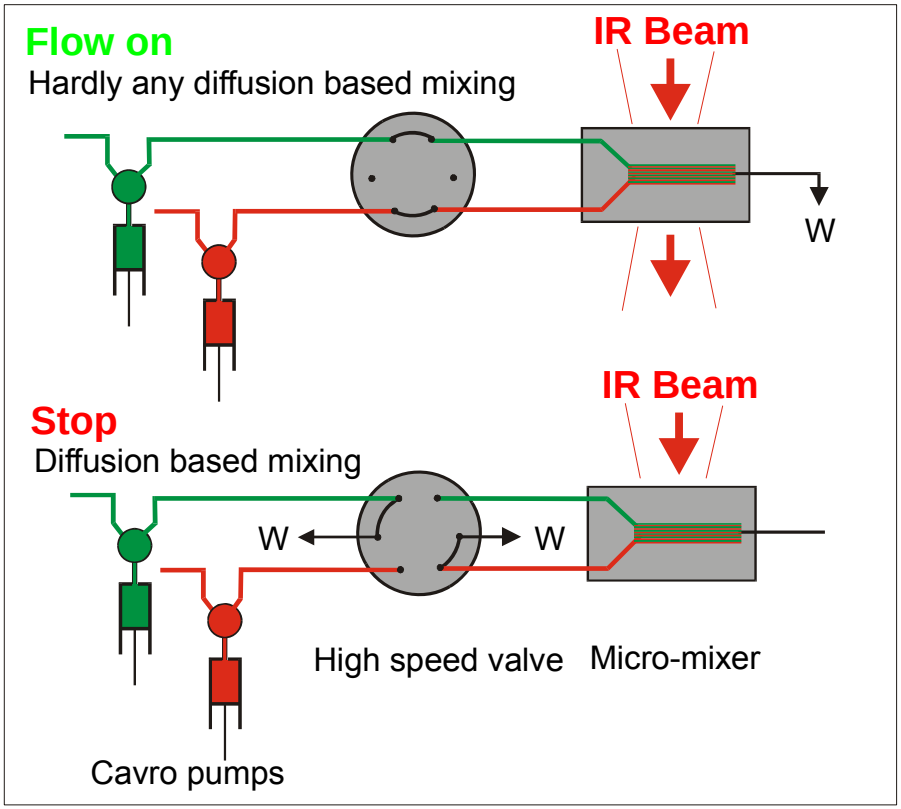
Operation of the Flow System



- Flow rate: 100 µl/min
- Linear flow rate: 11 cm/s
- Residence time: 22 ms
- Reynolds number: 1
- Optical path: 12 µm

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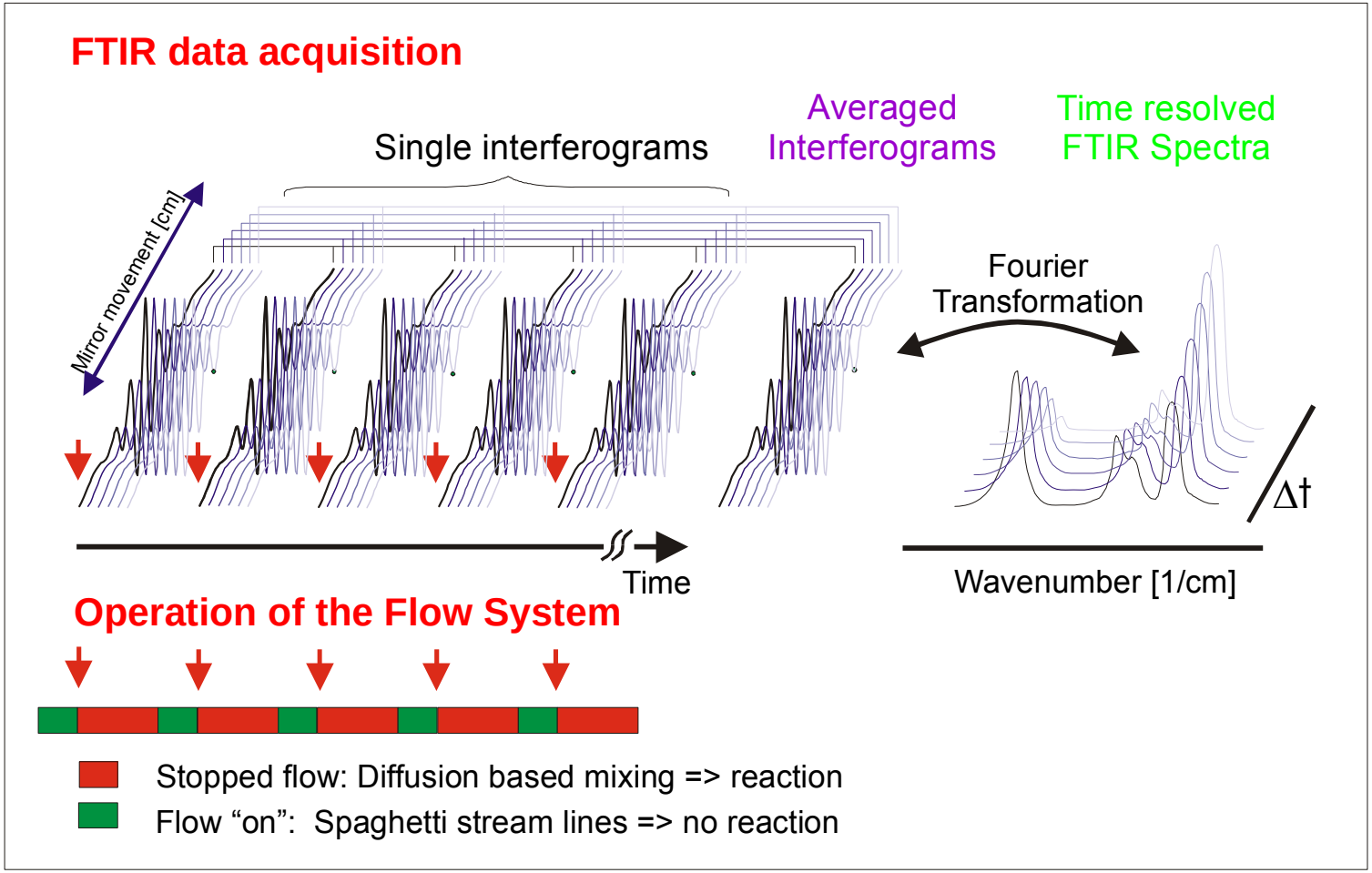
Principles of Rapid Scan FTIR spectroscopy



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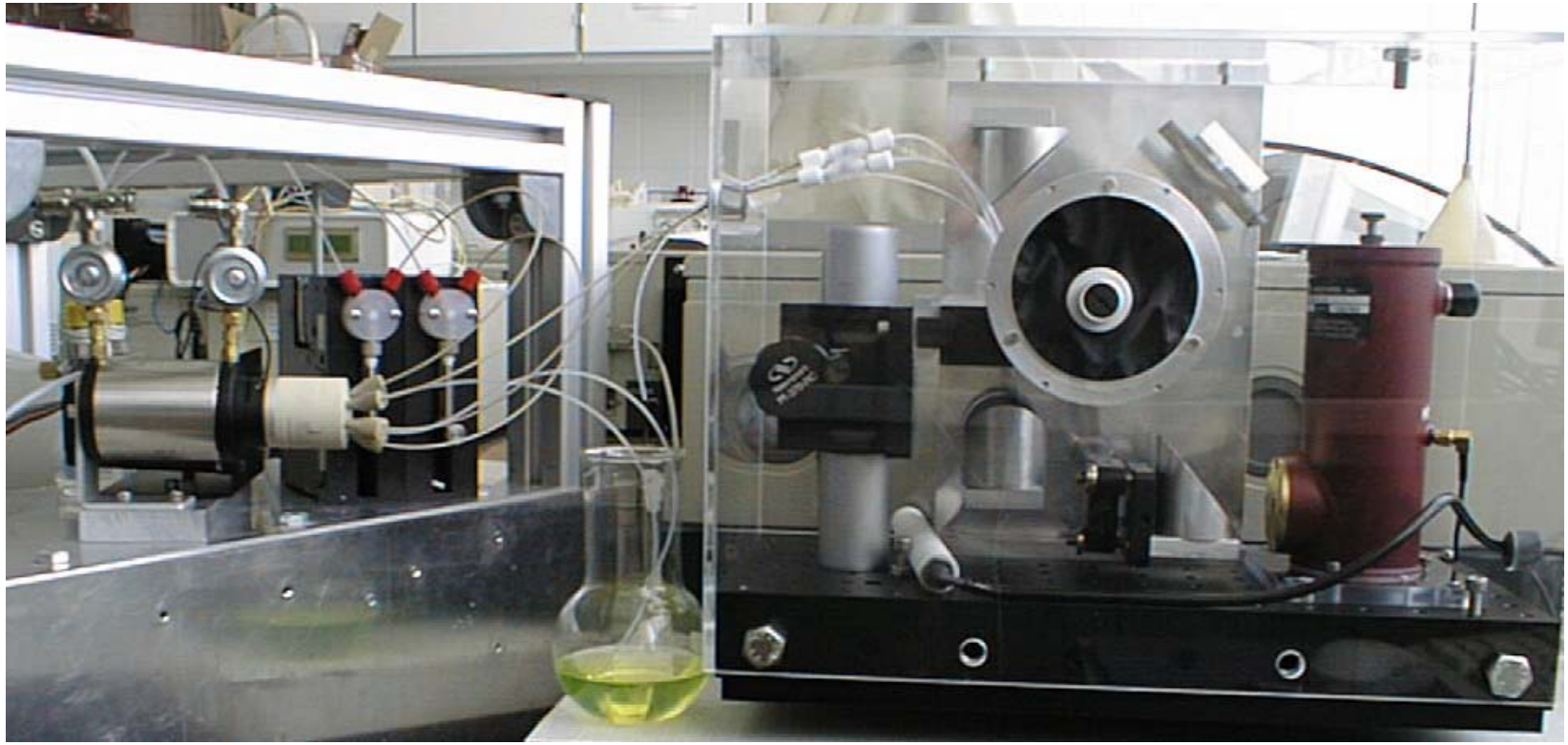
Experimental Set-up



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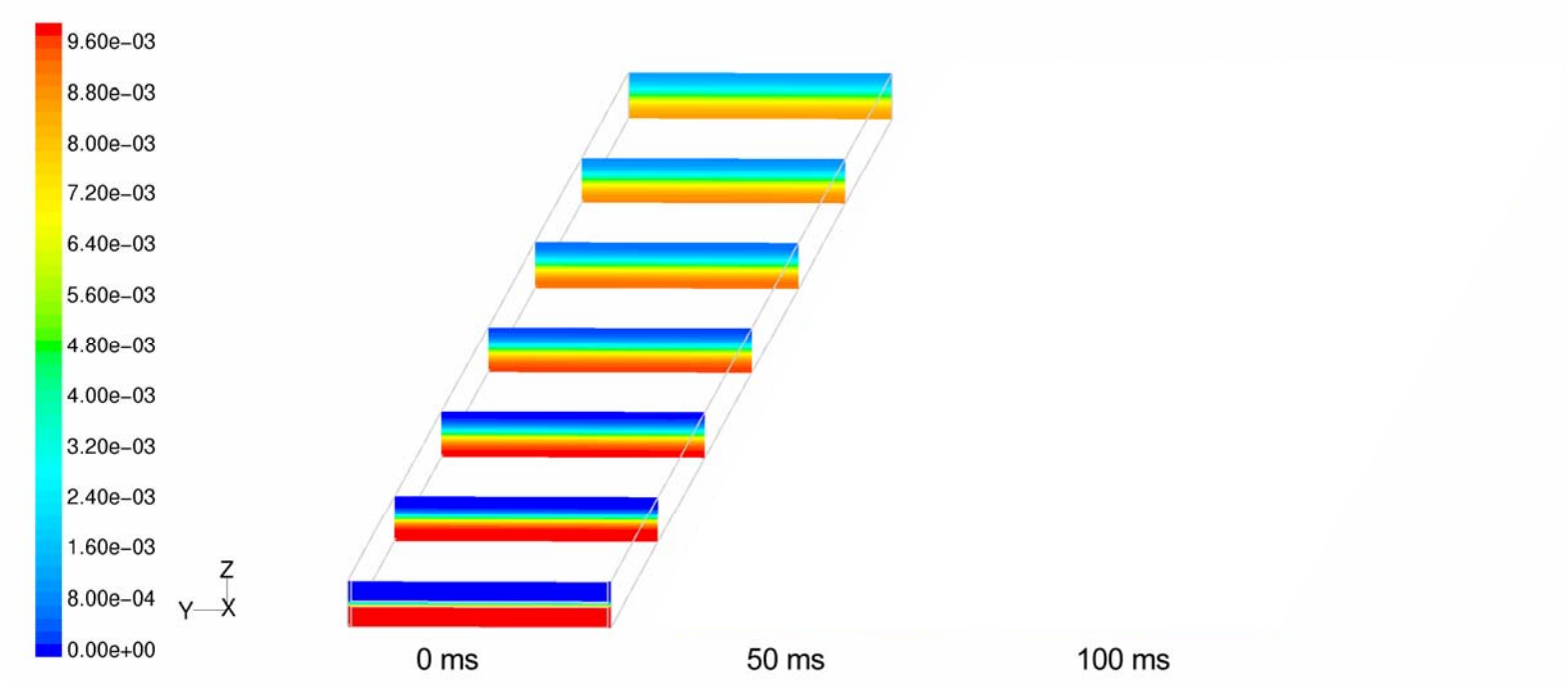
Details on the Mixing Process

With boundary layer, X_A = 0.01 in top channel, flow rate: 50 µl/min each cross sections every 500µm, cell size 2µm

$D = 10^{-9}$



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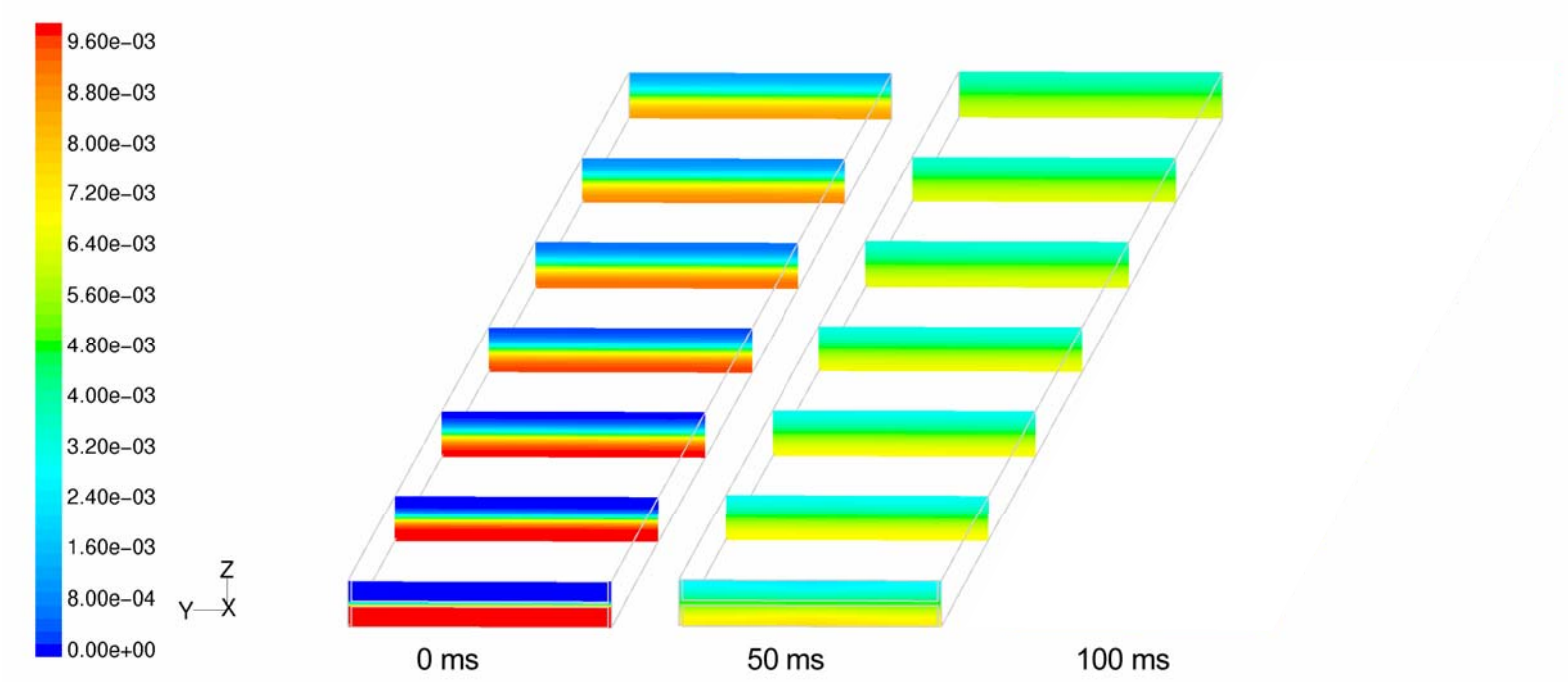

Minispec, 2007

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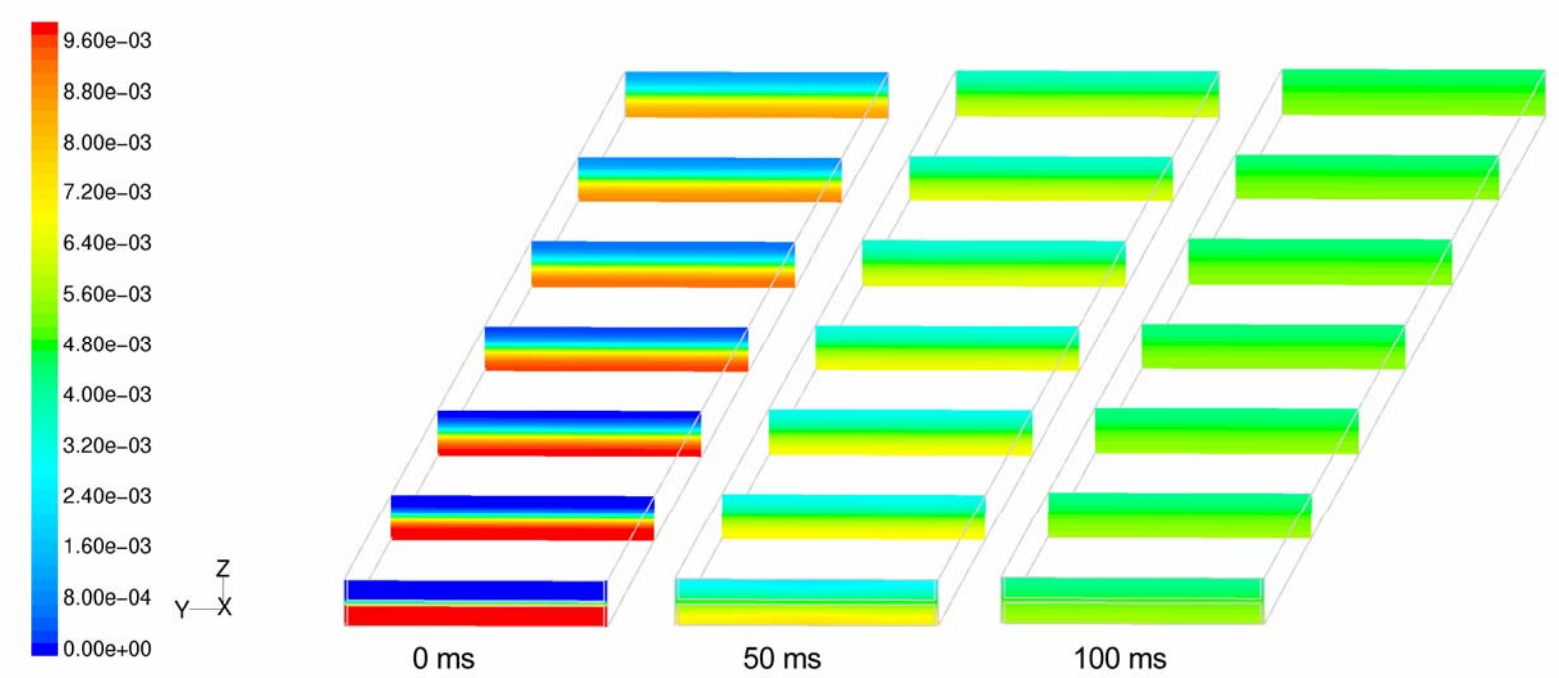

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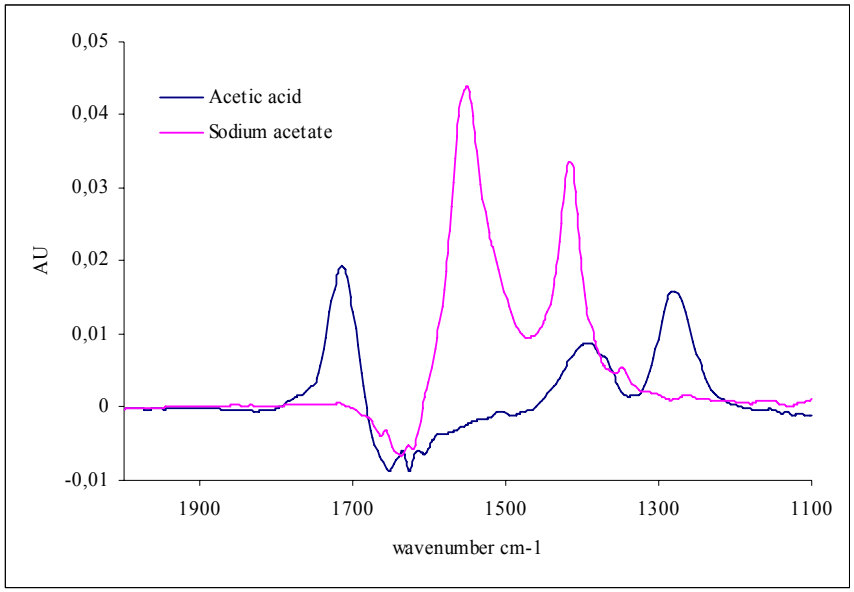
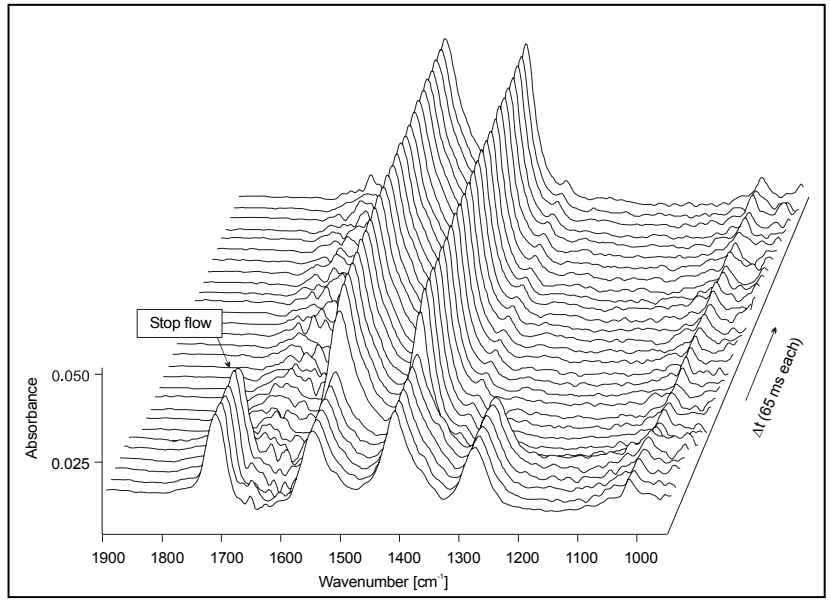
Test of Experimental Set-up

Performance test with Acetic Acid - NaOH system





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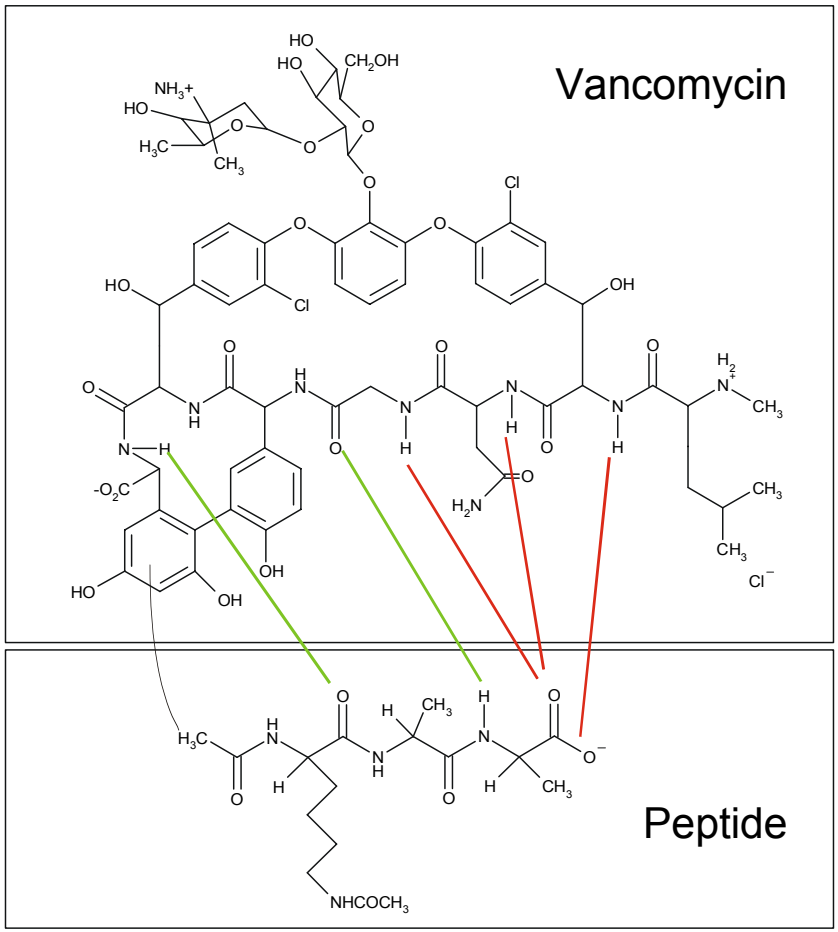
Interaction of Vancomycin and Ac-L-Lys(Ac)-D-Ala-D-Ala



Vancomycin:

- Glycopeptide antibiotic
- binds to –Lys-D-ala-D-ala end of cell-wall precursors of gram positive bacteria
- binding proceeds via
  - –hydrogen bonding, and
  - –hydrophobic interactions

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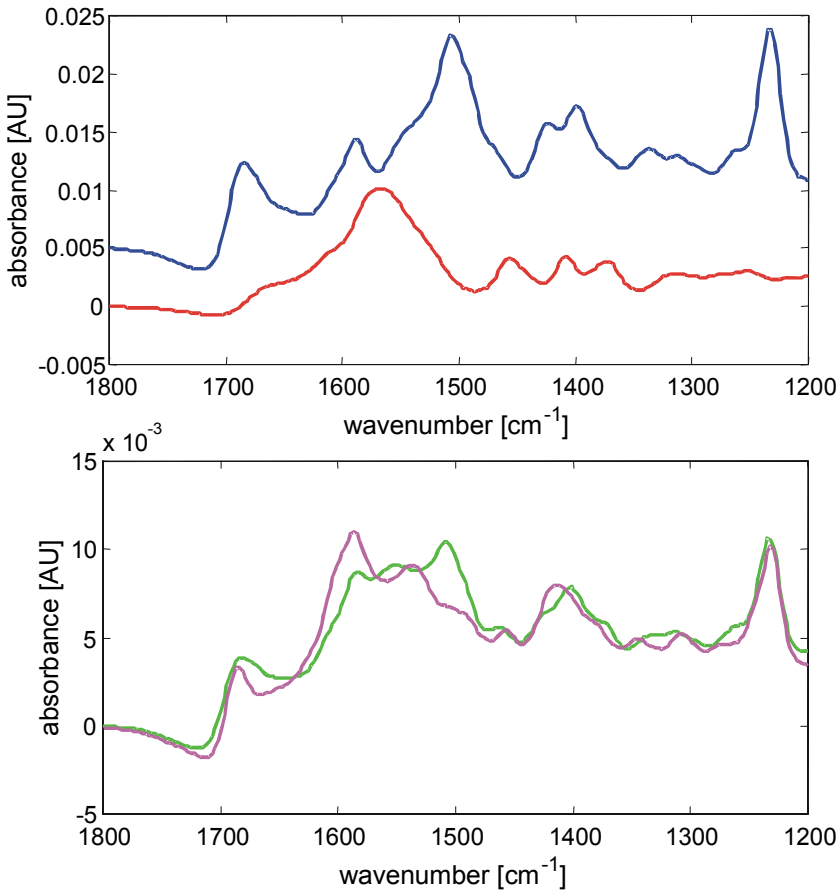
Spectral Differences (static) due to Binding



Vancomycin (V)
Tripeptide (TP)

Sum of V+TP

Formed Complex of V-TP

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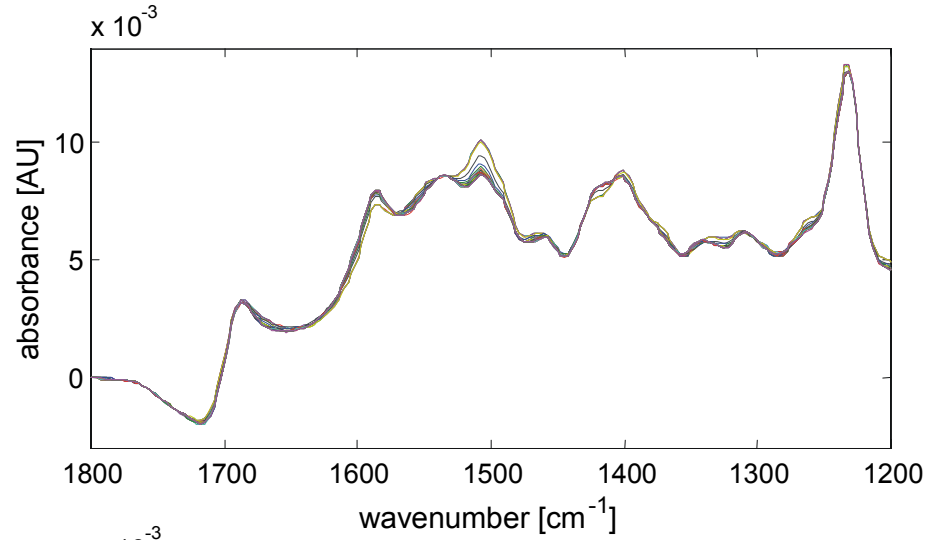
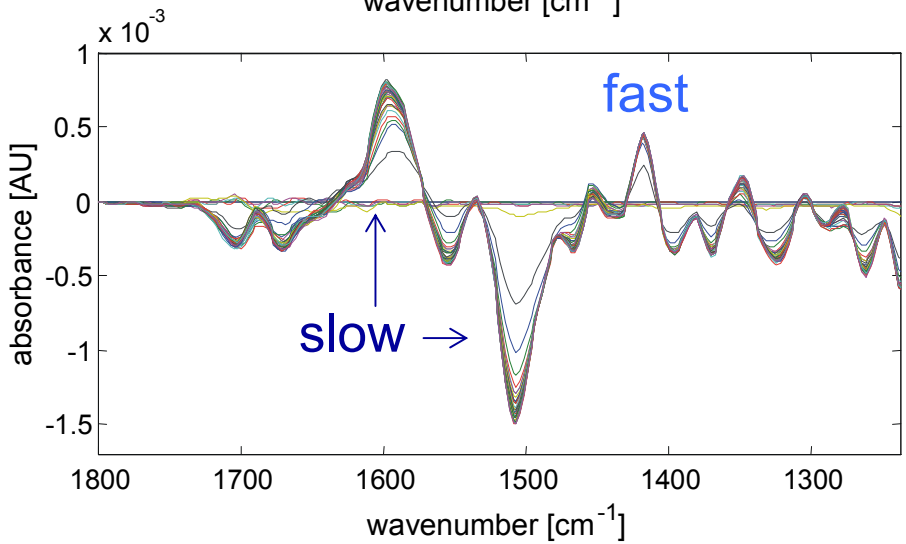
Time Resolved (ms) FTIR Data



Original Spectra

Δt = 65 ms



Difference Spectra

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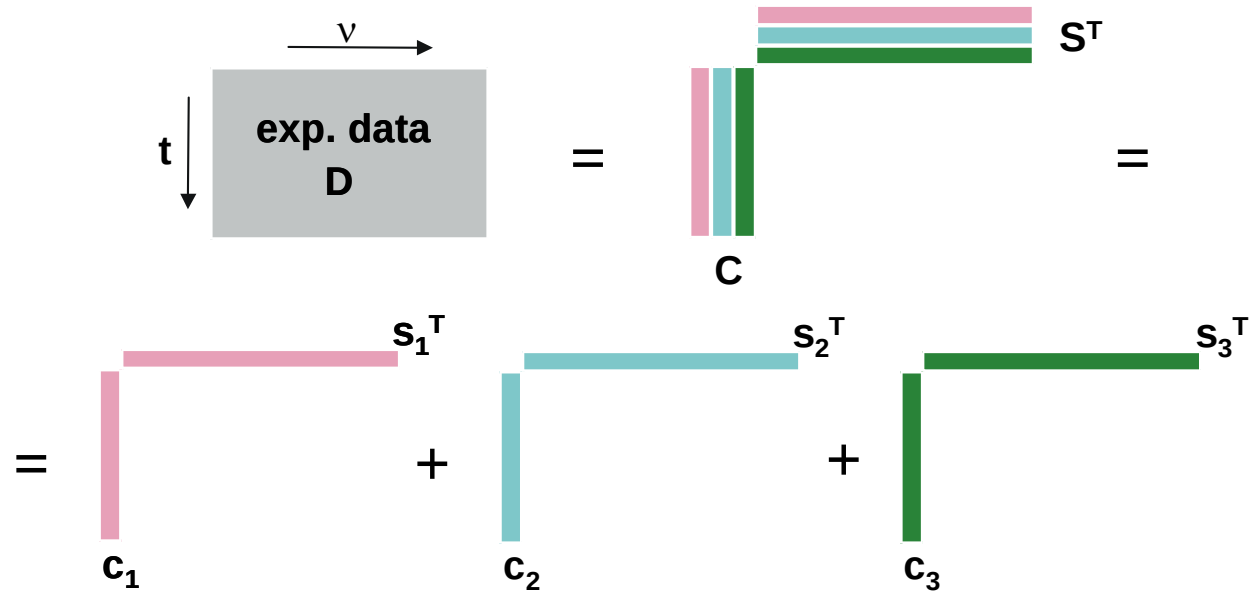
Multivariate Curve Resolution: Graphical Representation



c_i...concentration profiles with time s_i...spectra of pure substances

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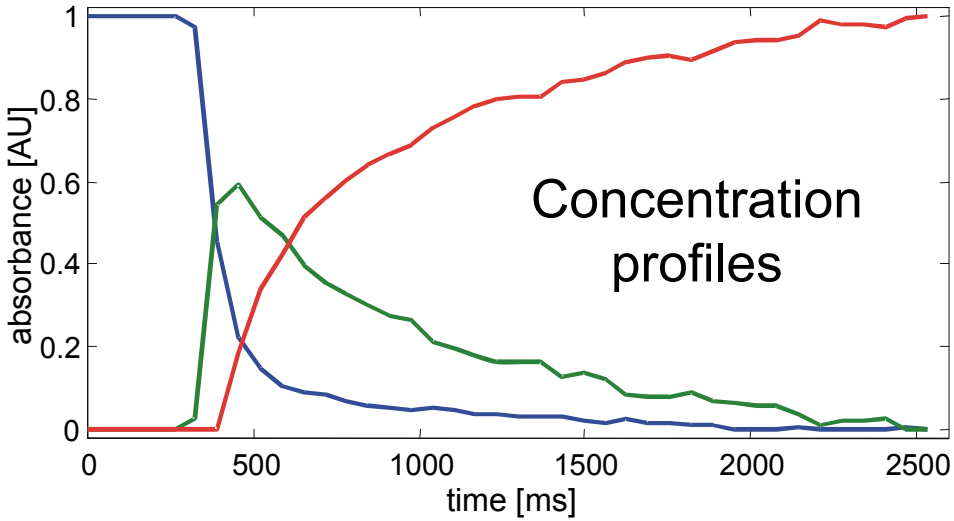
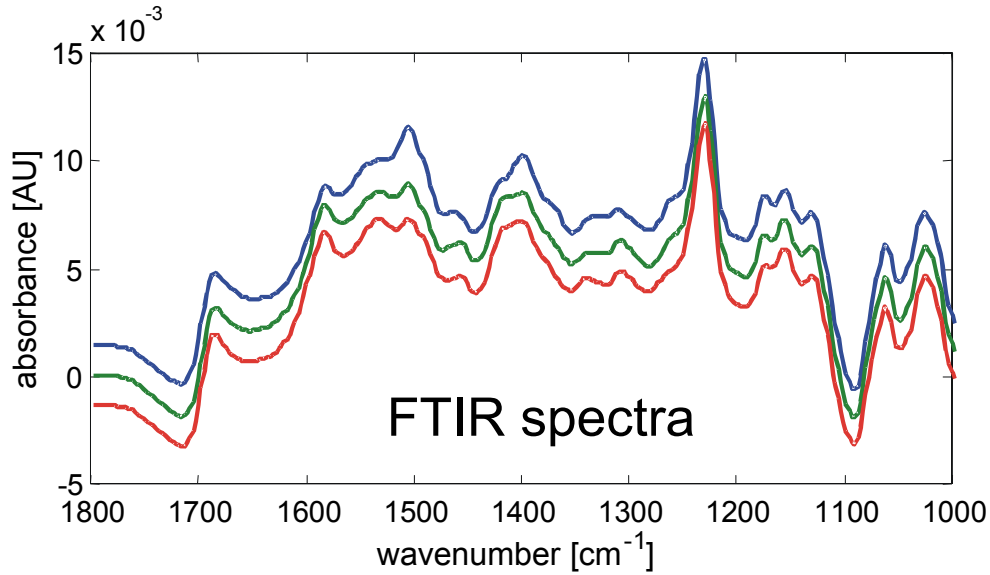
Result of MCR-ALS Analysis of the Data



2-step binding process



before reaction

intermediate

endproduct (complex)

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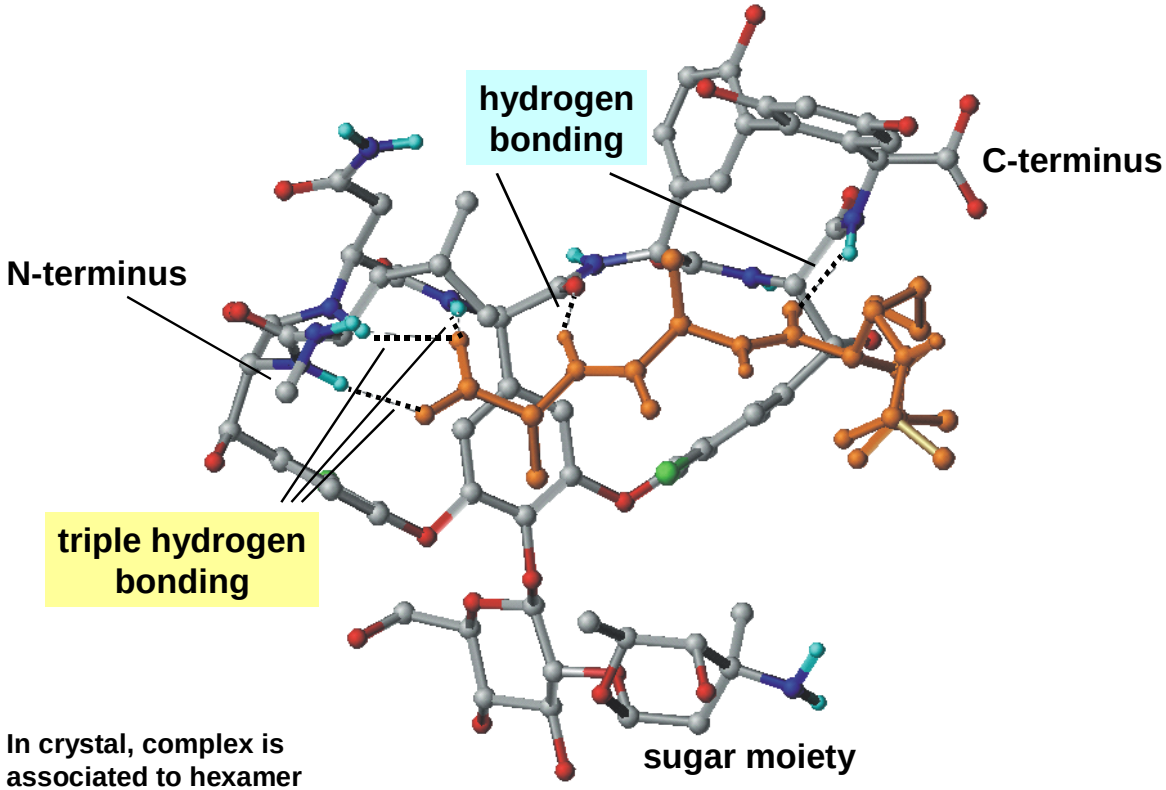
TR-FTIR Provides Complemetary Information to Crystalography

X-Ray Crystal Structure of Cocrystallized Vancomycin - N_{α} ,N_{ω}-Diacetyl-L-Lys-D-Ala-D-Ala



In crystal, complex is associated to hexamer

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Motivation

- Develop a versatile platform for *on-line monitoring of arbitrary chemical reactions in nL volumes*
- Application in all fields of chemistry
  - Analytical (Surface Enhanced Raman Spectroscopy)
  - Synthesis (combinatorial chemistry)
  - Bio-medical and bio-chemical

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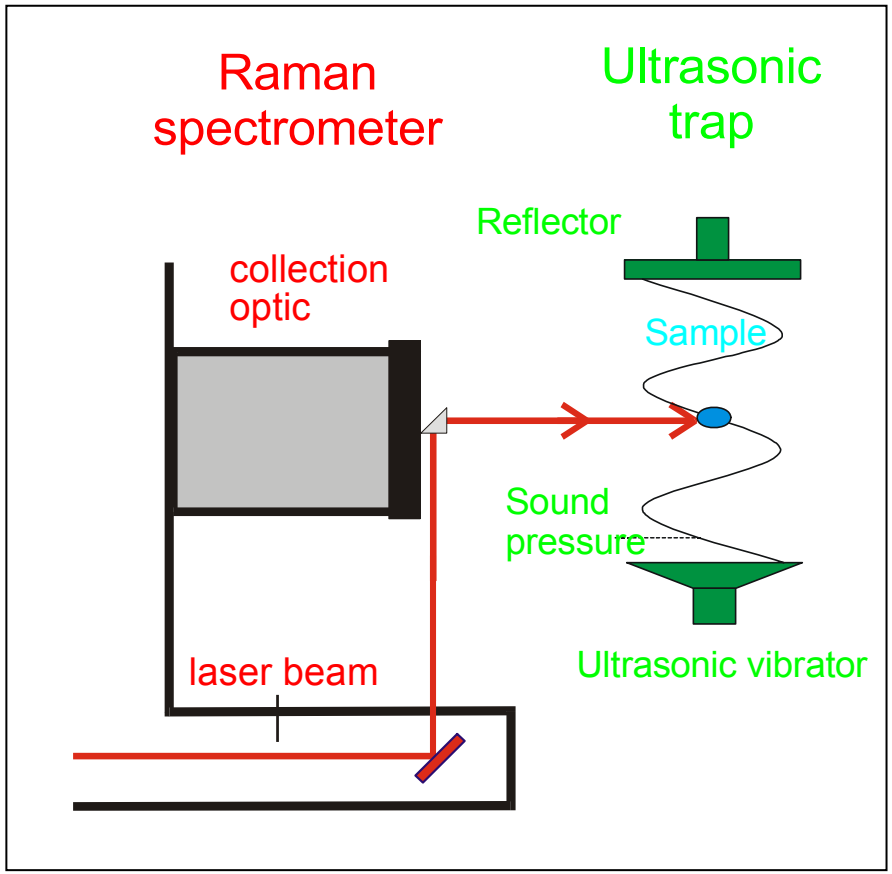
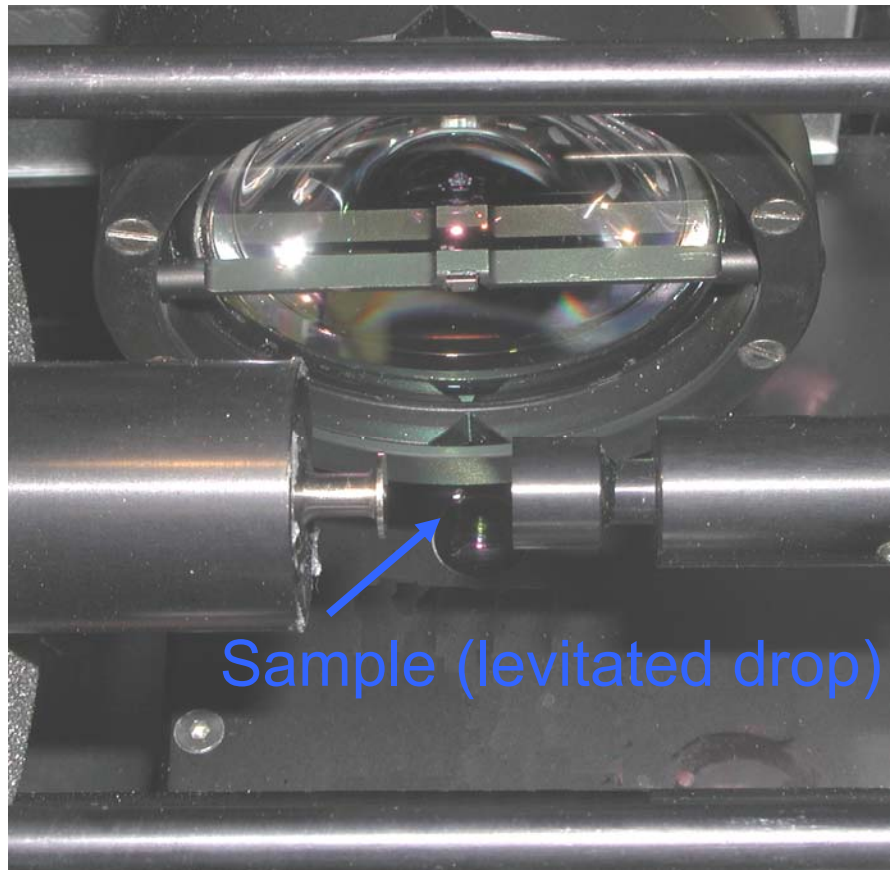
FT Raman Spectroscopy of Levitated Drops





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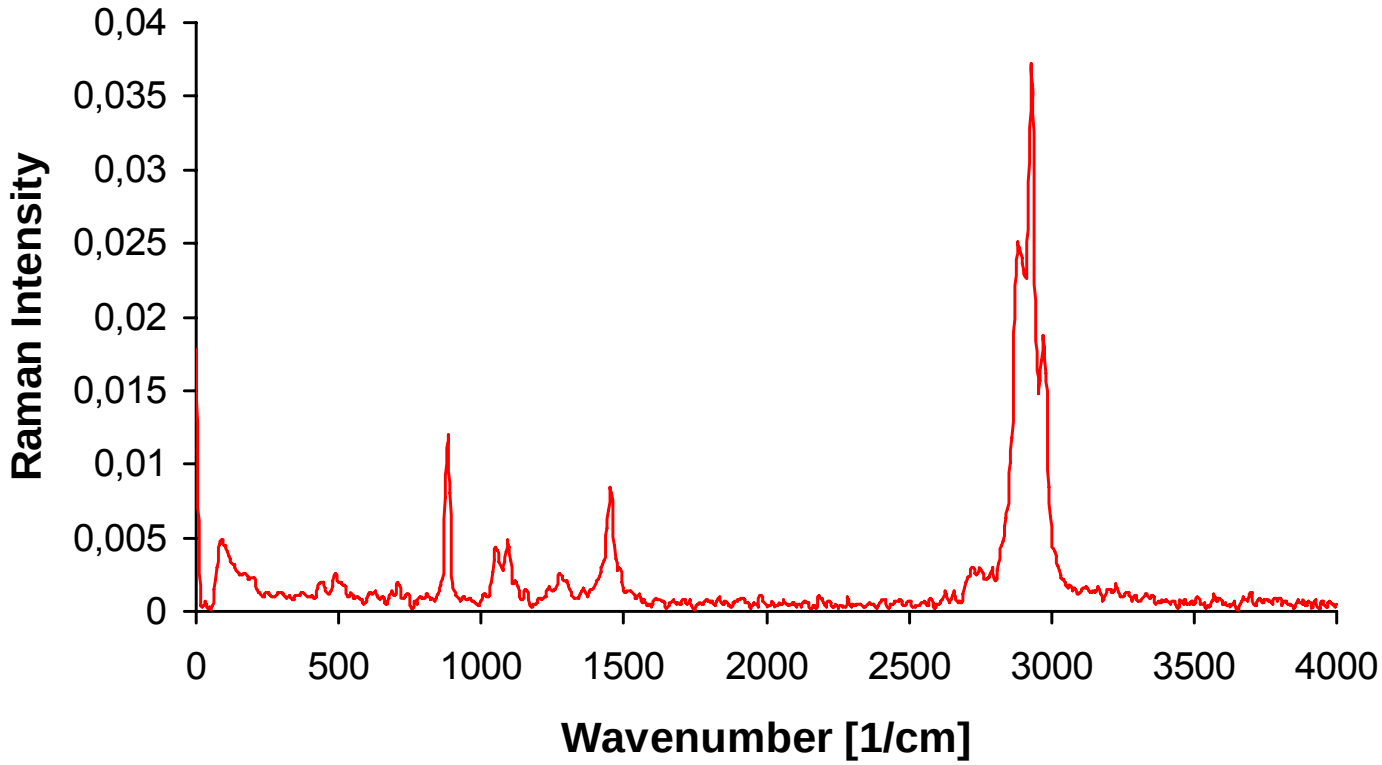
FT Raman Spectrum of ~300 nL Ethanol



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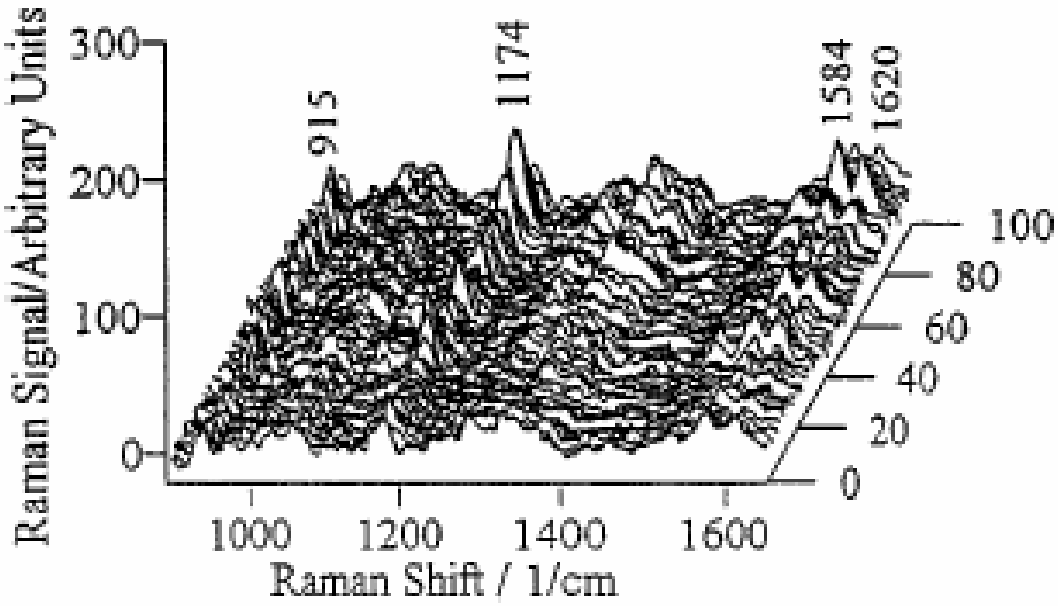
Surface Enhanced Raman Scattering

1. Rough nobel metal surfaces (Ag, Au,....)
   Colloids or rough solid state substrates
2. Match between absorption of SERS substrate and excitation wavelength

High electrical fields in close proximetry to surface => enhanced Raman scattering
Single molecule detection possible

Single Molecule Detection Using Surface - Enhanced Raman Scattering (SERS)

Raman microscope - Silver colloid



K. Kneipp, Y. Wang , H. Kneipp, L . T. P erelman, I. I tzkan, R. R. Dasari, and M.S. Feld, *Phys. Rev. L ett.* **78,** 1667 (1997)

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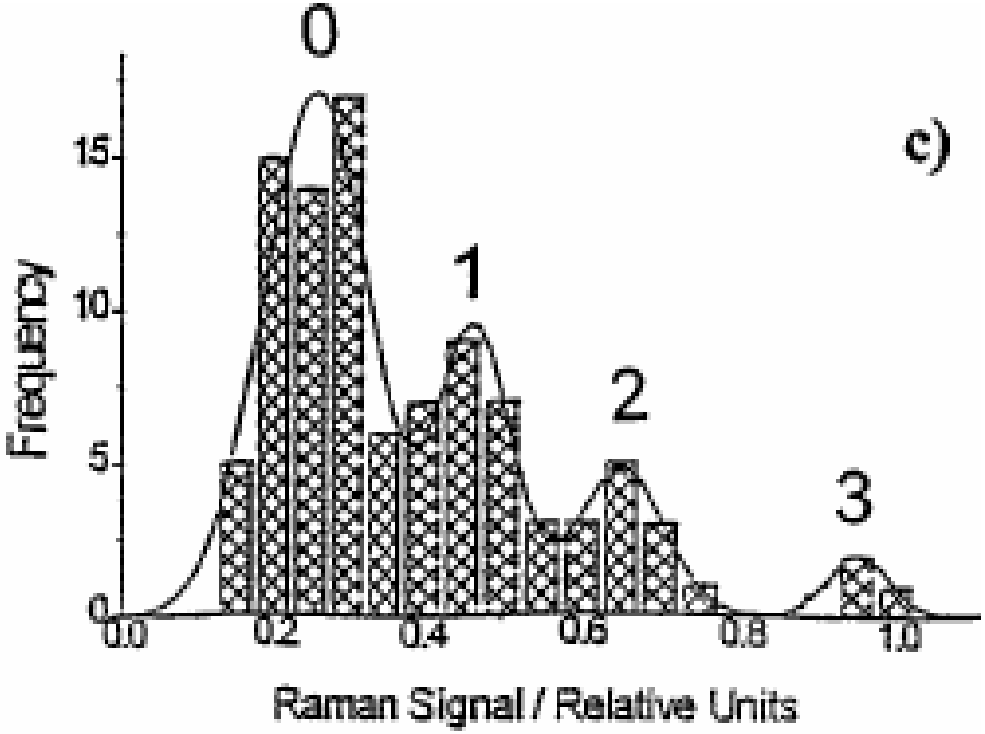
Results of Single Molecule Detection by SERS



- 1174 cm^{-1} Raman line
- Average of 0.6 crystal violet molecules

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Desired Characteristics of SERS Substrate Preparation

- Simple and reproducible generation of SERS active surfaces
- High reproducibility of enhancement factors
- No memory effects
- Fast

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Frequently Encountered Problems Using SERS Spectroscopy

- Colloidal SERS substrates
  - Tedious preparation procedure (Lee-Meisl method: refluxing for reduction of $AgNO_3$ with citrate)
  - Ageing of SERS colloids, stability
  - Batch to batch reproducibility
  - Adsorption to walls, tubings => memory effects

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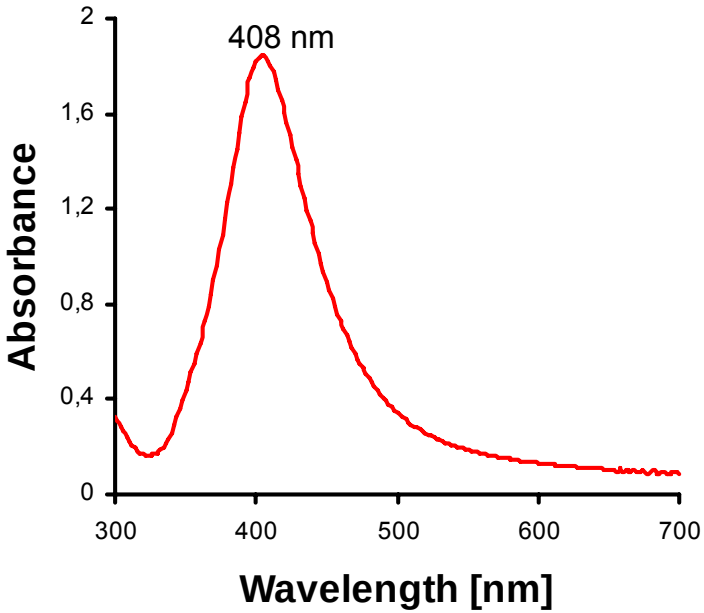
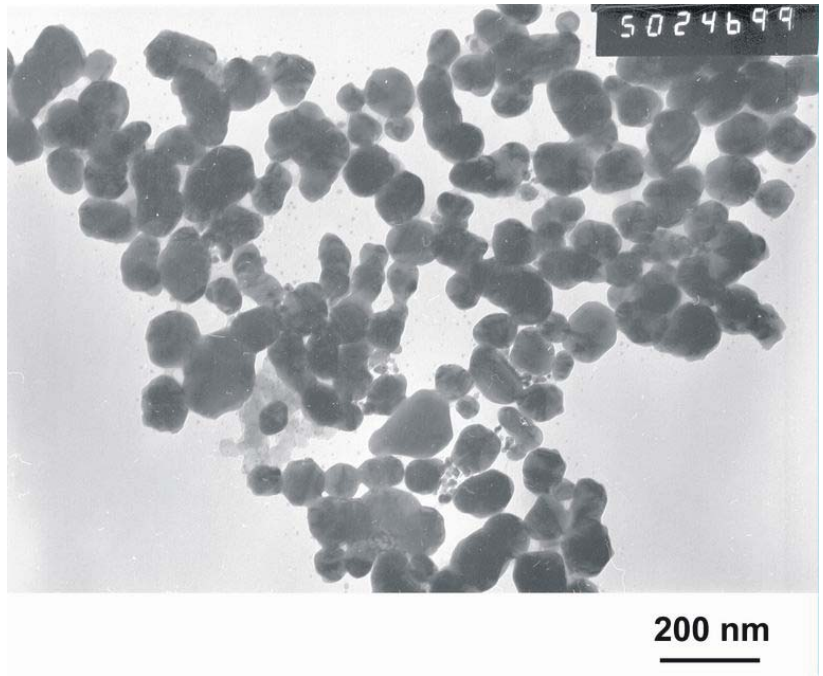
Solution: I) Hydroxylamine Reduced Silver Sol

$$NH_2OH + Ag^+ \longrightarrow \longrightarrow Ag + N_2,.. (N_2O)$$





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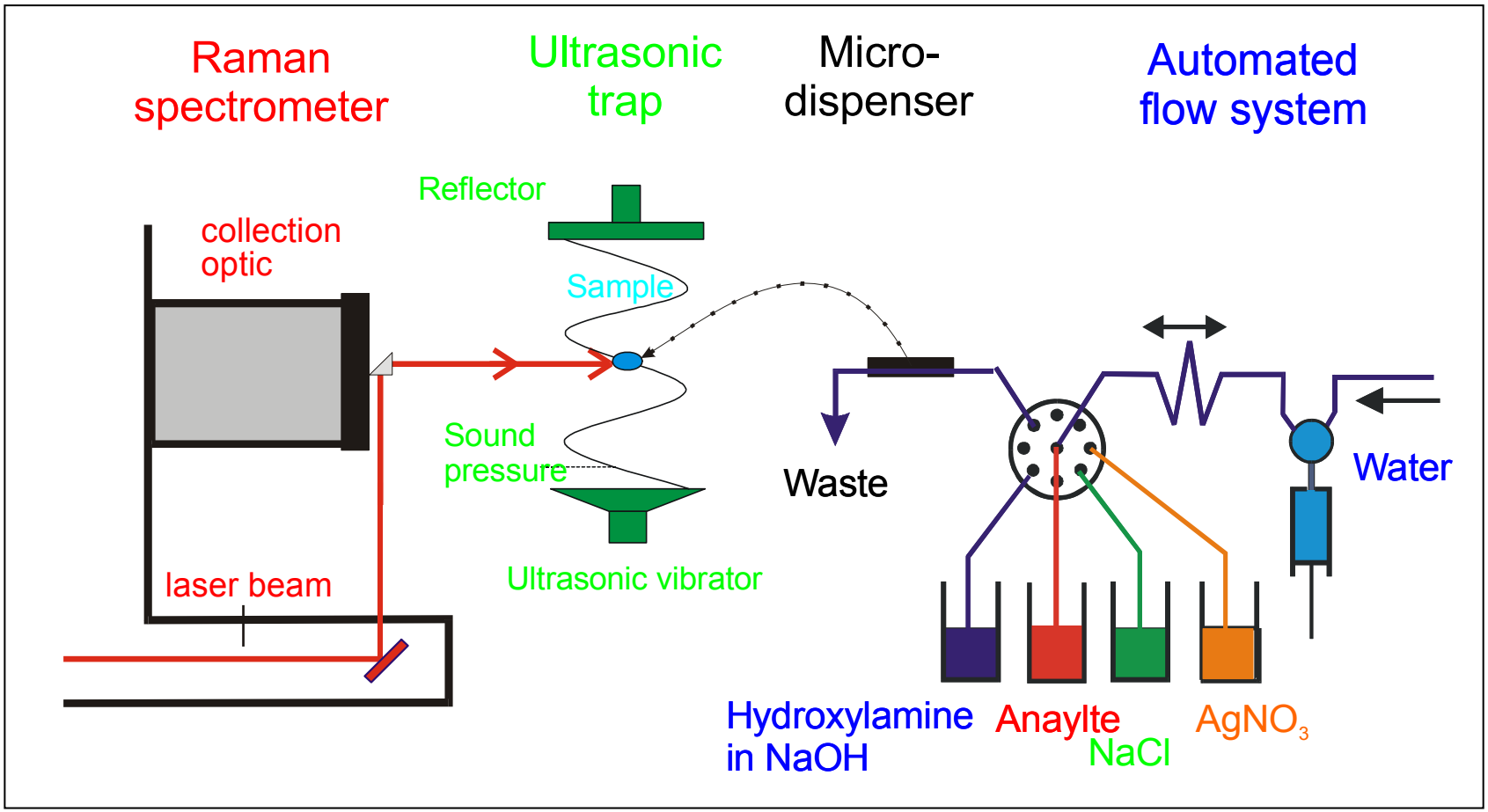
Solution: II) SERS Synthesis and Application in Levitated Droplets



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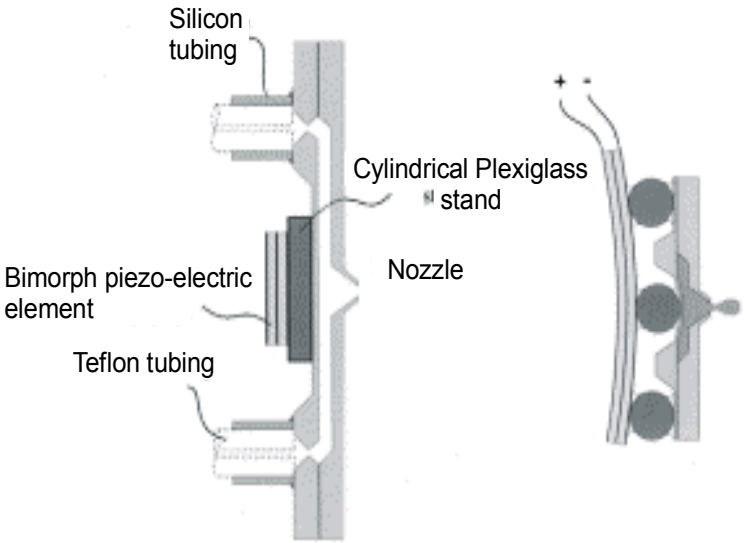
Flow-through Micro-dispenser

Operation in flow-through mode



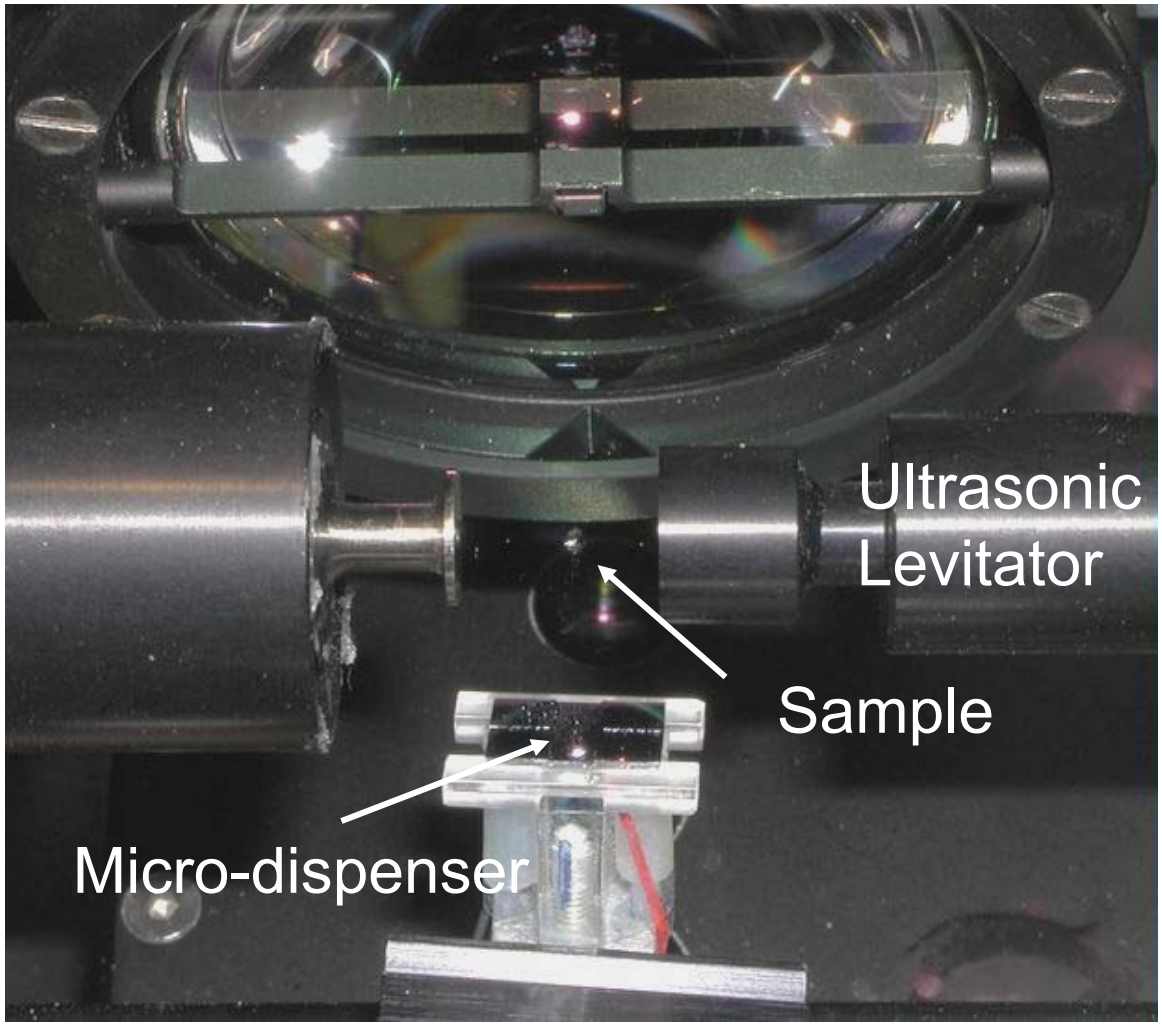
- Droplets: 50 pL – 300 nL
- Single shot – 500 Hz
- Initial velocity: 1 m/s

Co- operation with T. Laurell, Lund Sweden

50 Picoliter Droplets at 100 Hz

Dispensing_640.avi

Detail of Experimental Set-up



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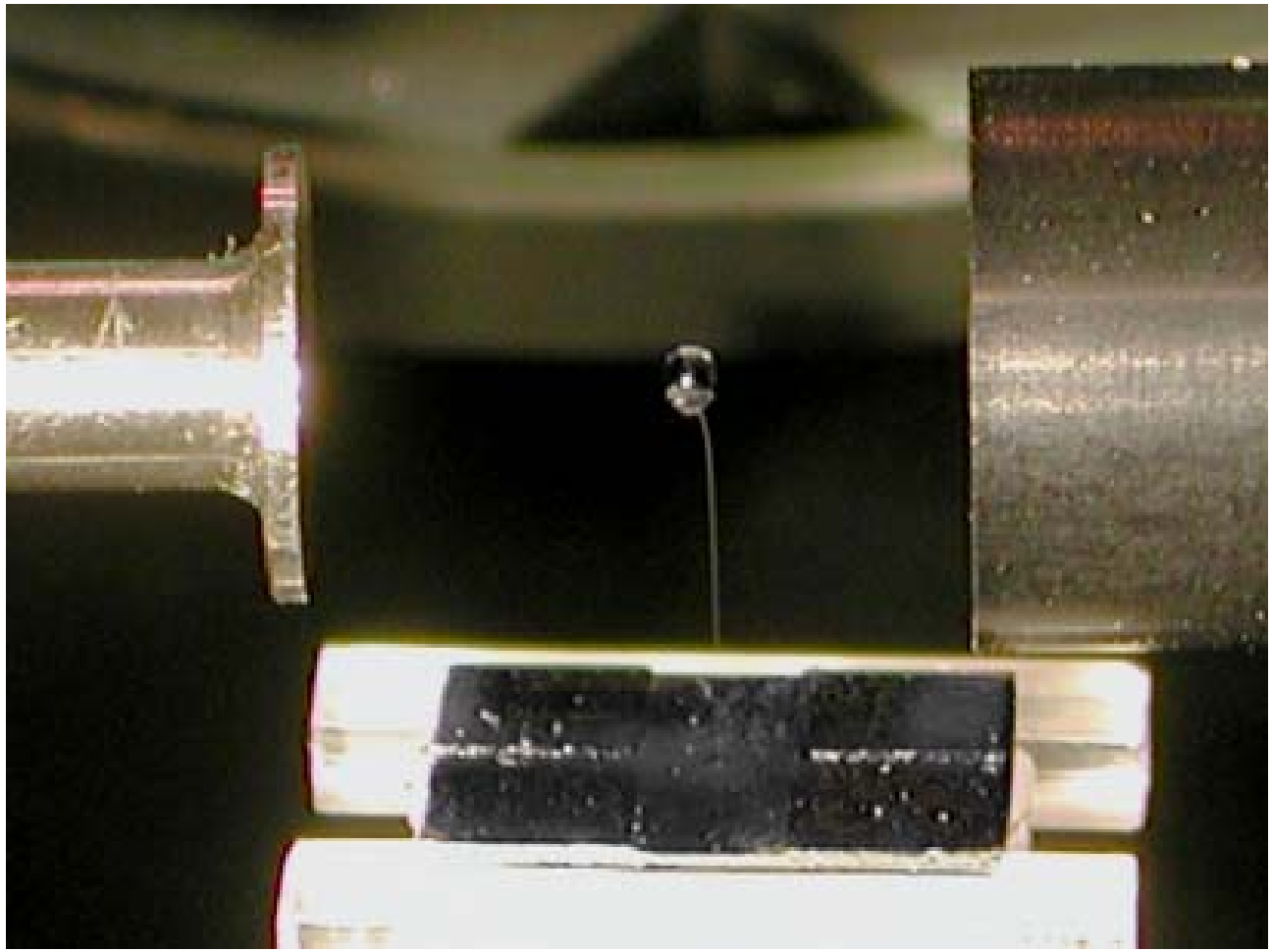
Detail of Experimental Set-up



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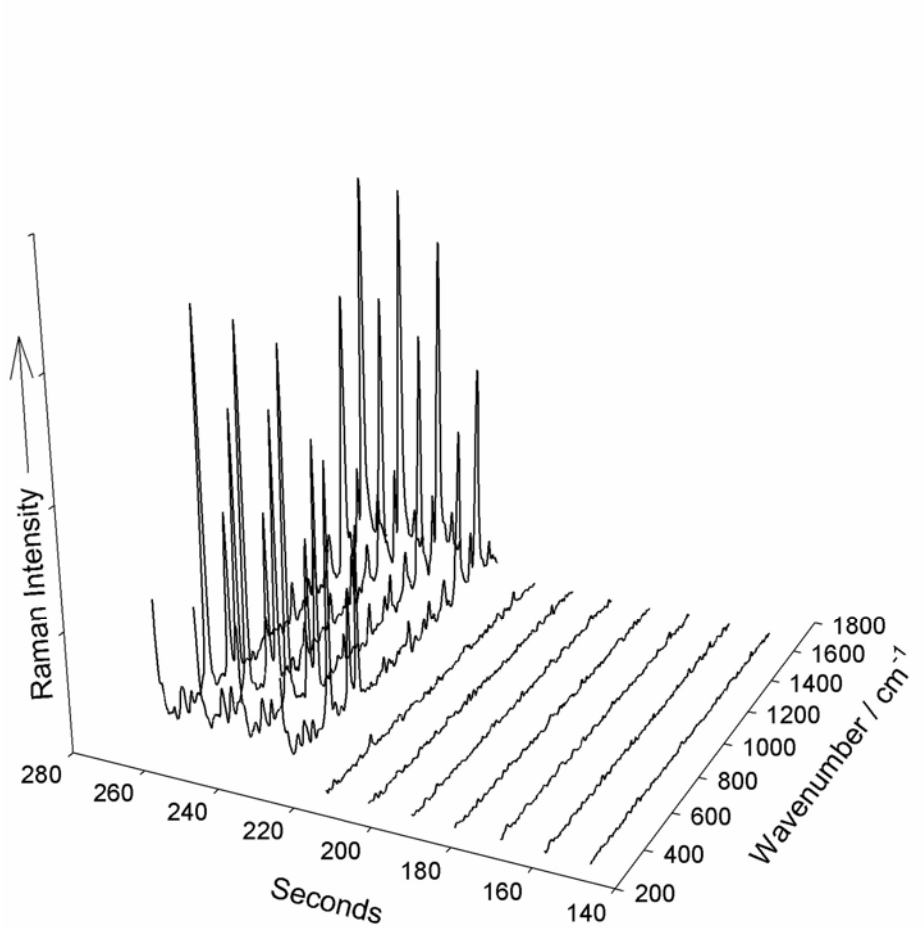
On-line Synthesis of Ag-sol and SERS Spectra of 9-Aminoacridine in a Drop



Injection of

- *432 nL 10^{-3} M $AgNO_3$*
- *48 nL Hydroxylamine 10^{-2} M*
  1M NaOH
- *6 nL 9-Aminoacridine 10^{-5}*

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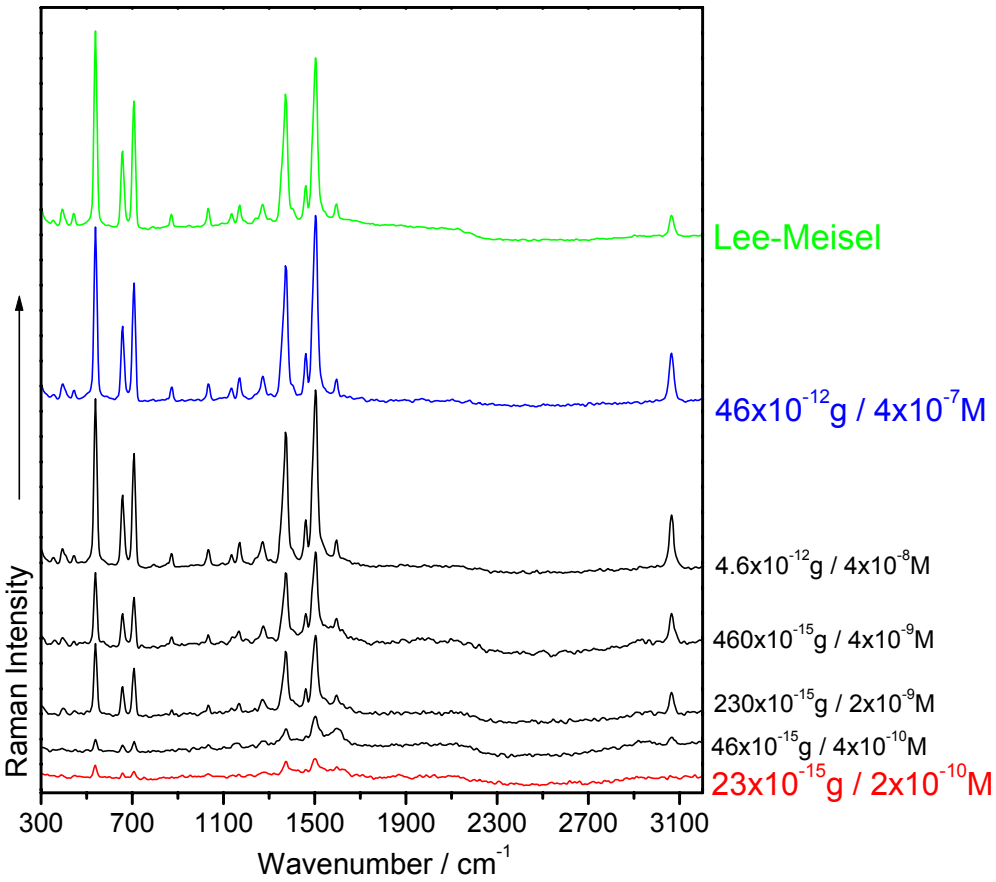
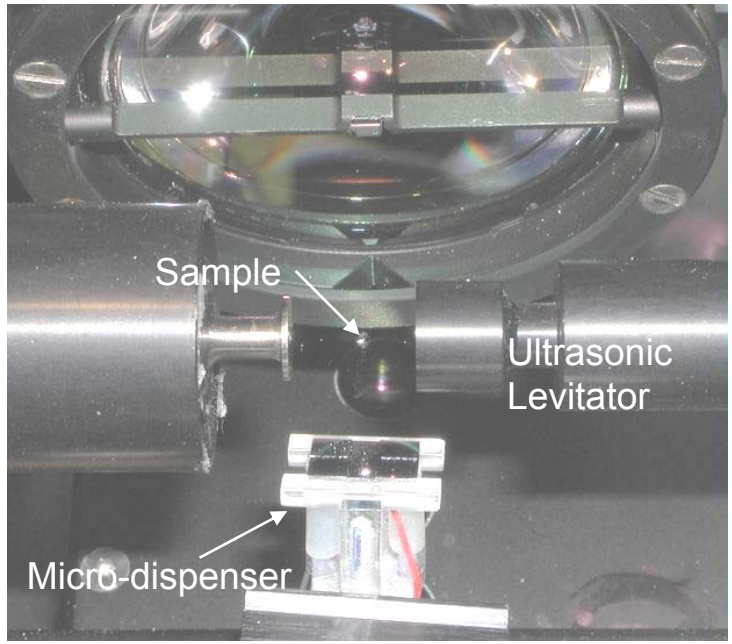
Sensitivity in the Low Femtogram Region

Analyte: 9-Aminoacridine





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Reproducibility in SERS Spectroscopy

Advantage of the micro-dispenser / levitation - concept

- Off-line preparation of Ag–colloids:
  r.s.d. ~ 30 %
- Preparation of Ag-colloids using
  automated flow systems:
  r.s.d ~ 7 %
- In-situ synthesis and use of Ag-colloids
  in a levitated droplet:
  r.s.d. ~ 2 %

Summary

- Chemometrics
  - MCR-ALS delivers easily understandable results
    - Limitation in case it gets difficult to determine the number of components present
  - 2DCoS delivers results difficult to explain at first glance, difficult to extract time sequence of events
    - But very useful for selecting initial estimates required in MCR-ALS
      - Hint on number of components
      - Initial estimates
  - Combination of both techniques advantageous
- Time resolved FTIR spectroscopy of chemical reactions in solution
- Chemistry-on-the-fly: New approach in chemical reaction monitoring

Acknowledgement

- Co-workers:
  Josef Diewok, Peter Hinsmann (TU Vienna)
  Mercedes Lopez-Pastor (Universidad de Córdoba, Spain)
  María Jose Ayora Cañada (Universidad de Jaen, Spain)
  Ana Dominguez Vidal (TU Wien)
- Financial support :
  Spanish Ministry of Education and Science
  Austrian Science Fund, FWF
  European Union, Marie Curie Training Site, ADVIS

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