

# TopSpin

*Data Analysis*

→ **Peak Picking**

→ **Integration**

→ **Spectrum Deconvolution**

→ **Multiplet Analysis**

→ **1D Spectrum Simulation & Iteration**

→ **T1/T2 Relaxation Analysis**

→ **Dynamic NMR Line Shape Analysis**

→ **Solids Line Shape Analysis**

→ **Structure Editor / Viewer**

→ **Shift Prediction**

Contents continued on next page

→ *Please click on a topic to view page*

→ **Structure Verification**

→ **Diffusion Ordered Spectroscopy (DOSY)**

→ **Protein Backbone Assignment**

## Features:

- Automatic peak picking of 1D-3D spectra
- Interactive peak picking of 1D and 2D spectra
- Editable peak list
- Annotation support
- Several peak table display and sorting modes
- Peak table export in various formats
- Correlation of peak table with cursor position in spectrum

# Peak Picking



Peak list above spectrum  
and as a table

**1** exam1d\_1H 1 1 C:\Bruker\TOPSPIN guest [1]

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Fid

1.4270 1.4155 1.4004 1.3851 1.3737 1.3585 1.3500 1.2853 1.2745 1.2615 1.2484 1.2377 1.1686 1.1588 1.1560 1.1454 1.1232 1.0986 1.0856 1.0699 1.0567 1.0314 1.0198 1.0073 0.9983 0.9835 0.9686 0.9490 0.9372 0.9145 0.7658 0.7528

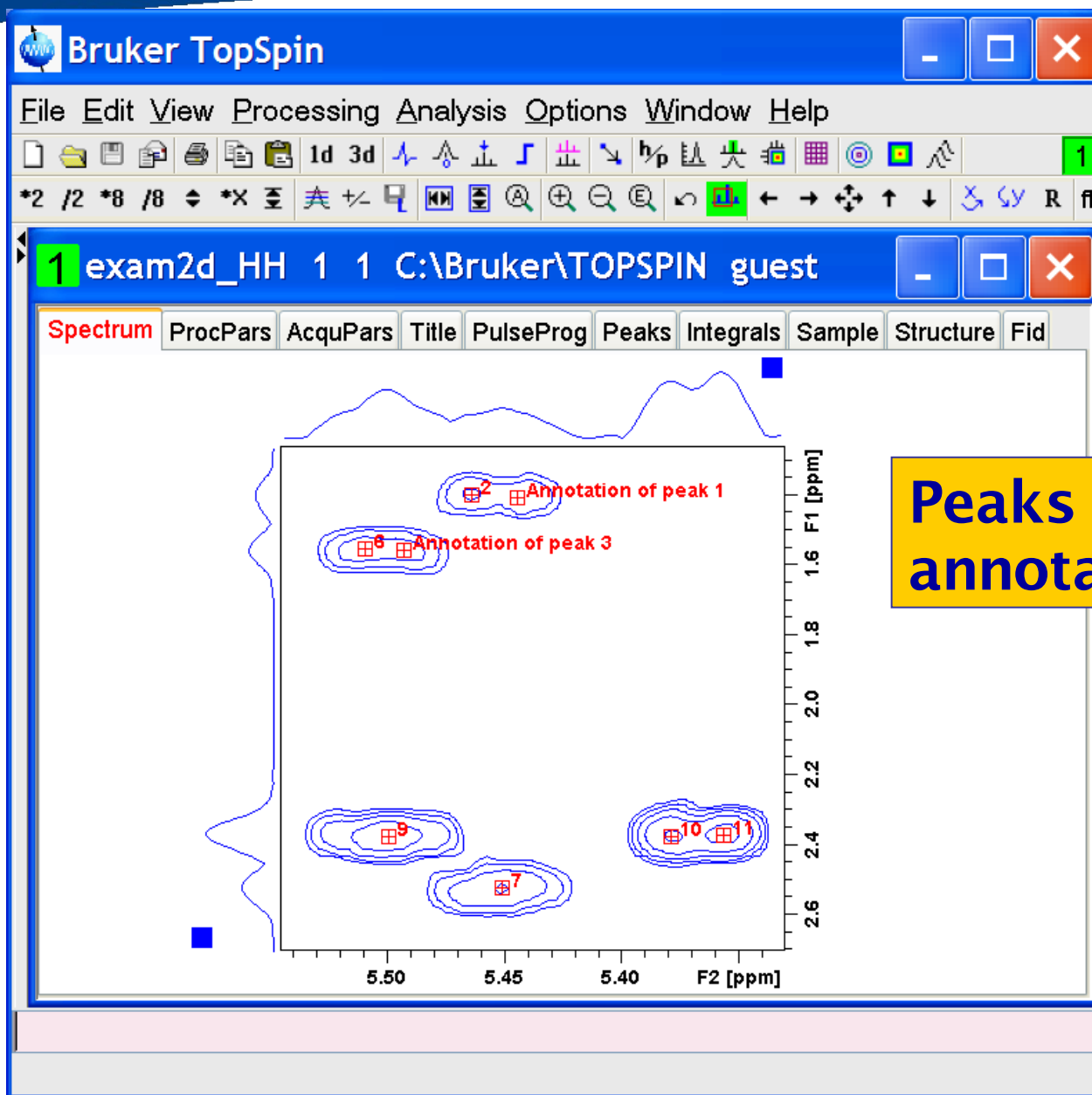
10 5 0 [1e6] [ppm]

**2** exam1d\_1H 1 1 C:\Bruker\TOPSPIN guest [2]

Spectrum ProcPars AcqPars Title PulseProg Peaks Integrals Sample Structure Fid

Peak	v(F1) [ppm]	v(F1) [Hz]	Intensity	Half width [ppm]	Half width [Hz]
1	8.3921	4197.1410	1.22	0.0070	3.4846
2	8.3727	4187.4385	1.25	0.0066	3.3012
3	8.1498	4075.9595	1.38	0.0062	3.1178
4	8.1353	4068.7076	1.40	0.0059	2.9344
5	7.7514	3876.7077	1.42	0.0059	2.9344
6	7.7359	3868.9557	1.47	0.0055	2.7510
7	7.5870	3794.4863	1.45	0.0059	2.9344

# Peak Picking



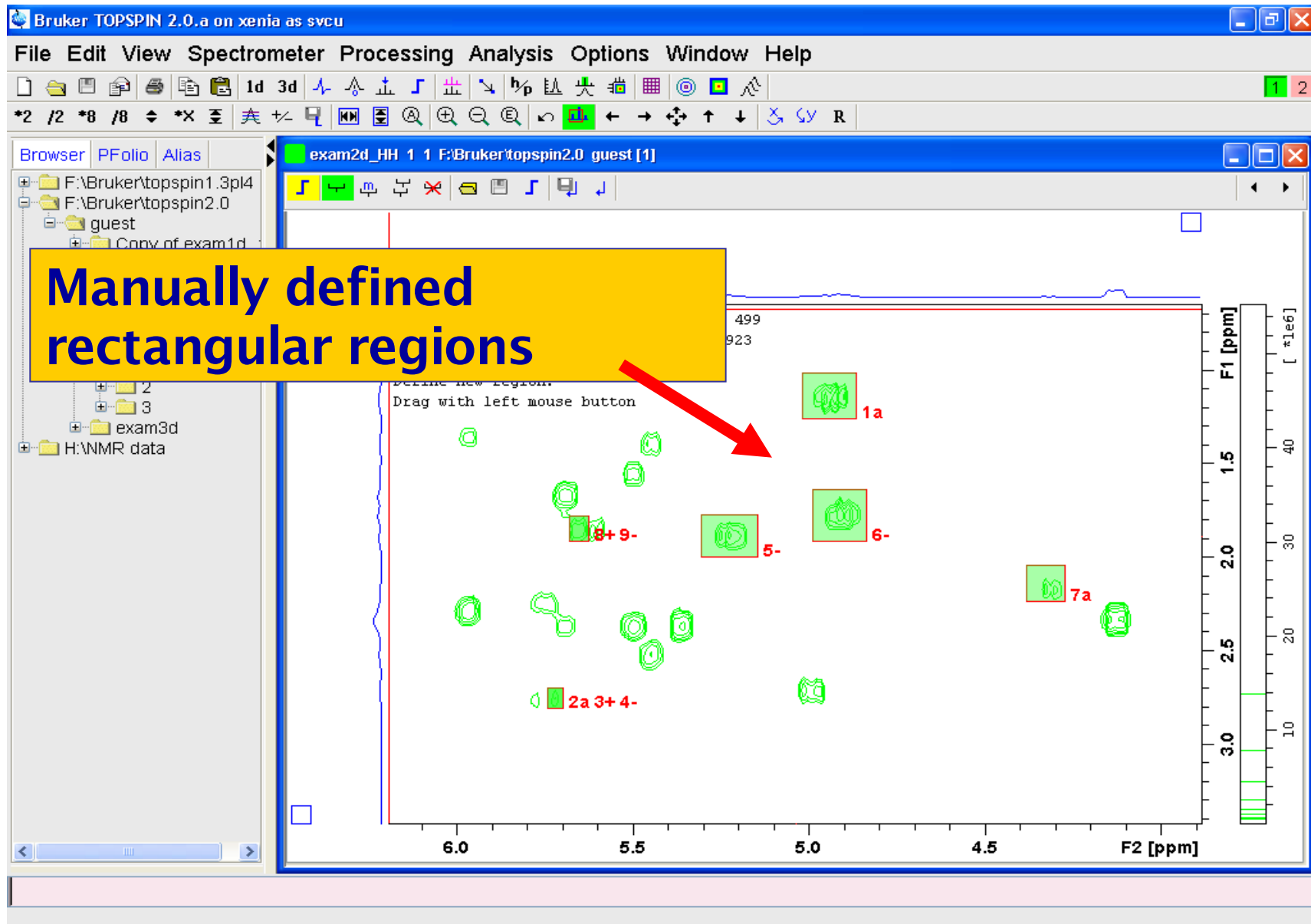
Peaks numbered or annotated

*TopSpin provides automatic and interactive integration of 1D and 2D spectra*

## 2D Integration Features:

- **Manual definition of integration regions:**  
User draws rectangles around peak / groups
- **Automatic definition of integration regions:**  
TopSpin computes the regions of arbitrary shape, following the lowest contour level

# Integration: 2D





# Integration: 2D

2 exam2d\_HH 1 1 C:\Bruker\TOPSPIN guest

Spectrum

Automatically computed integration regions of arbitrary shape: Peak volume is computed over these regions

Fid



F1 [ppm]

2.0

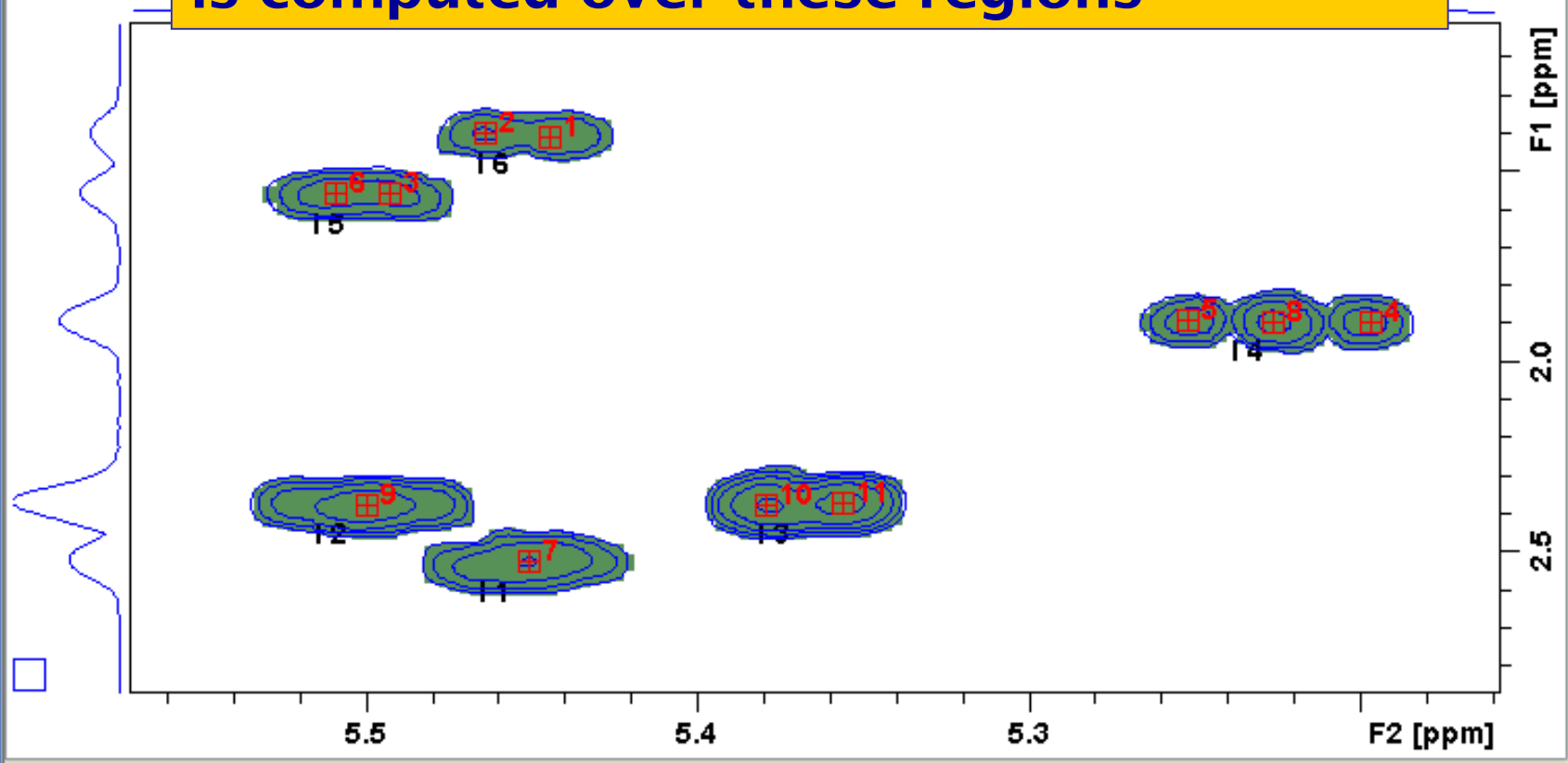
2.5

F2 [ppm]

5.5

5.4

5.3



# Integration: 2D



**Integration result table,  
including involved peaks:  
Table can be sorted, edited, exported, ...**

2 exam2d\_HH 1 1 C:\Bruker\TOPSPIN guest

Spectrum	ProcPars	AcquPars	Title	PulseProg	Peaks	Integrals	Sample	Structure	Fid
Object	Integral [abs]	Integral [rel]	Type	Mode	Peaks	v(F2) [ppm]	v(F1) [ppm]	Intensity	
Integral 6	245761882.62	0.2295	Automatic		2				
Integral 5	350632932.50	0.3274	Automatic		2				
Integral 4	579831496.88	0.5414	Automatic		3				
Peak 4						5.1970	1.8905	978056.63	
Peak 5						5.2523	1.8873	1013835.75	
Peak 8						5.2263	1.8905	1599931.13	
Integral 3	1071031180.25	1.0000	Automatic		2				
Peak 10						5.3792	2.3722	2472559.00	
Peak 11						5.3564	2.3689	2868560.50	
Integral 2	731963384.75	0.6834	Automatic		1				
Peak 9						5.4996	2.3754	2123102.00	
Integral 1	494250739.75	0.4615	Automatic		1				
Peak 7						5.4508	2.5219	1347464.63	

## Problem Definition:

**Integrating a series of 1D- oder 2D spectra** (e.g. monitoring progression of variable temperature / chemical reaction)

## Given:

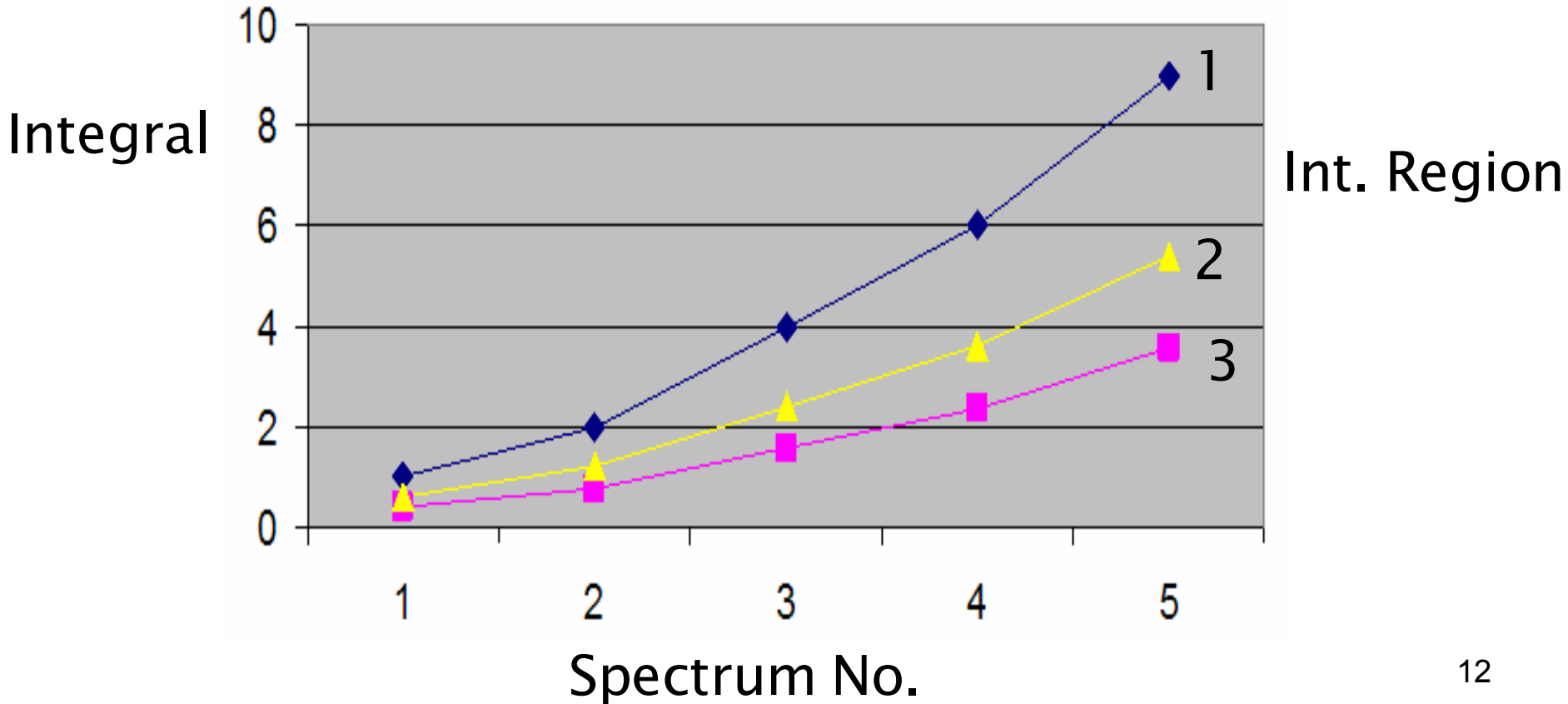
**Integral Regions, List of Spectra**

## Solution:

**Serial integration command `intser`:**

- *Loops over all spectra in the specified list*
- *Integrates each spectrum according to the regions*
- *Writes the result in a CSV file (=Excel compatible)*
- *Provides several scaling options*

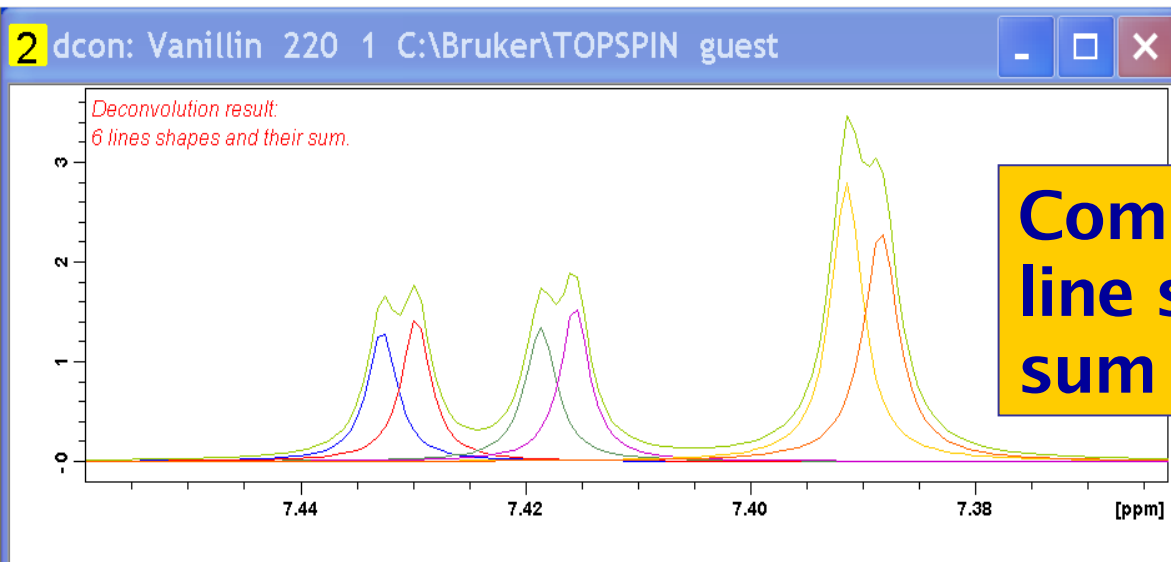
**Graphical presentation of a serial intergration run** (here: 5 spectra/3 regions)  
- Read result file into Excel or OpenOffice  
- Display as diagram



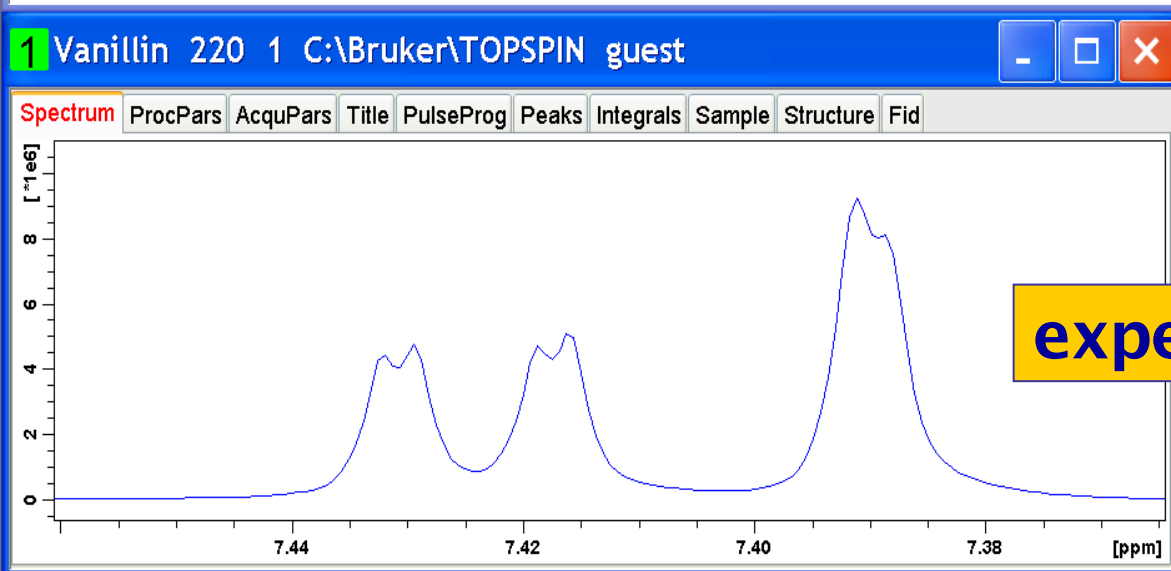
## Features:

- Deconvolution of 1D and 2D line shapes
- Types: Lorentz, Gaussian, mixed shapes

# Deconvolution: 1D



**Computed Lorentzian  
line shapes and their  
sum**

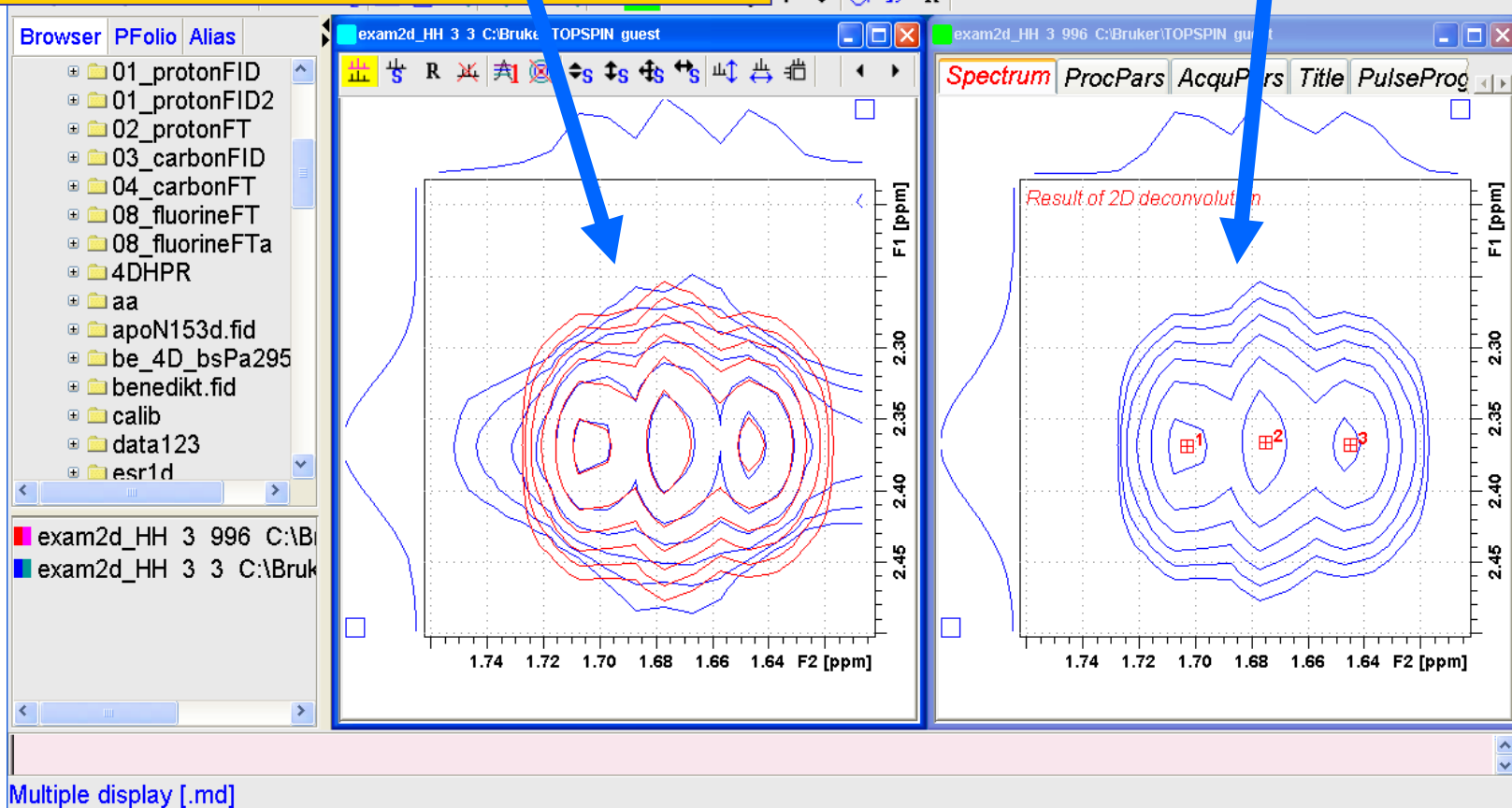


**experimental**

# Deconvolution: 2D

**Blue:** Experimental peak  
**Red:** Computed peak  
from deconvolution  
procedure

**Computed peak**



## Numeric deconvolution result

Data set: exam2d\_HH 3 3 C:\Bruker\TOPSPIN guest

Fit shape type: Gaussian

Chi square: 2.31063657E15

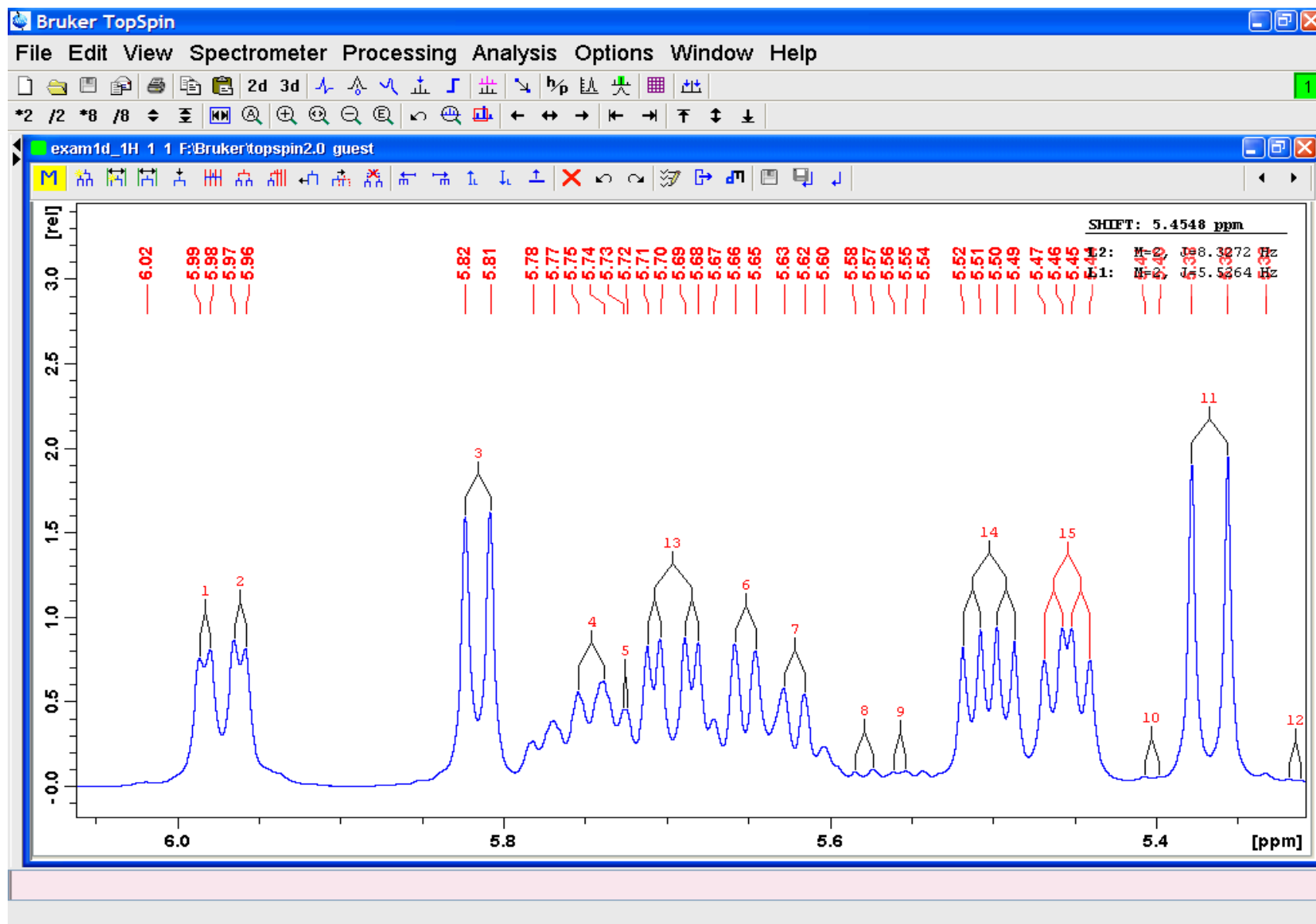
Peak	Frequency ppm		Full Width ppm		Intensity	Integral
	F1	F2	F1	F2		
1	2.368	1.703	0.091	0.024	1582982.641	861.242
2	2.365	1.675	0.106	0.020	1778635.966	924.190
3	2.367	1.645	0.090	0.028	1468825.100	942.954



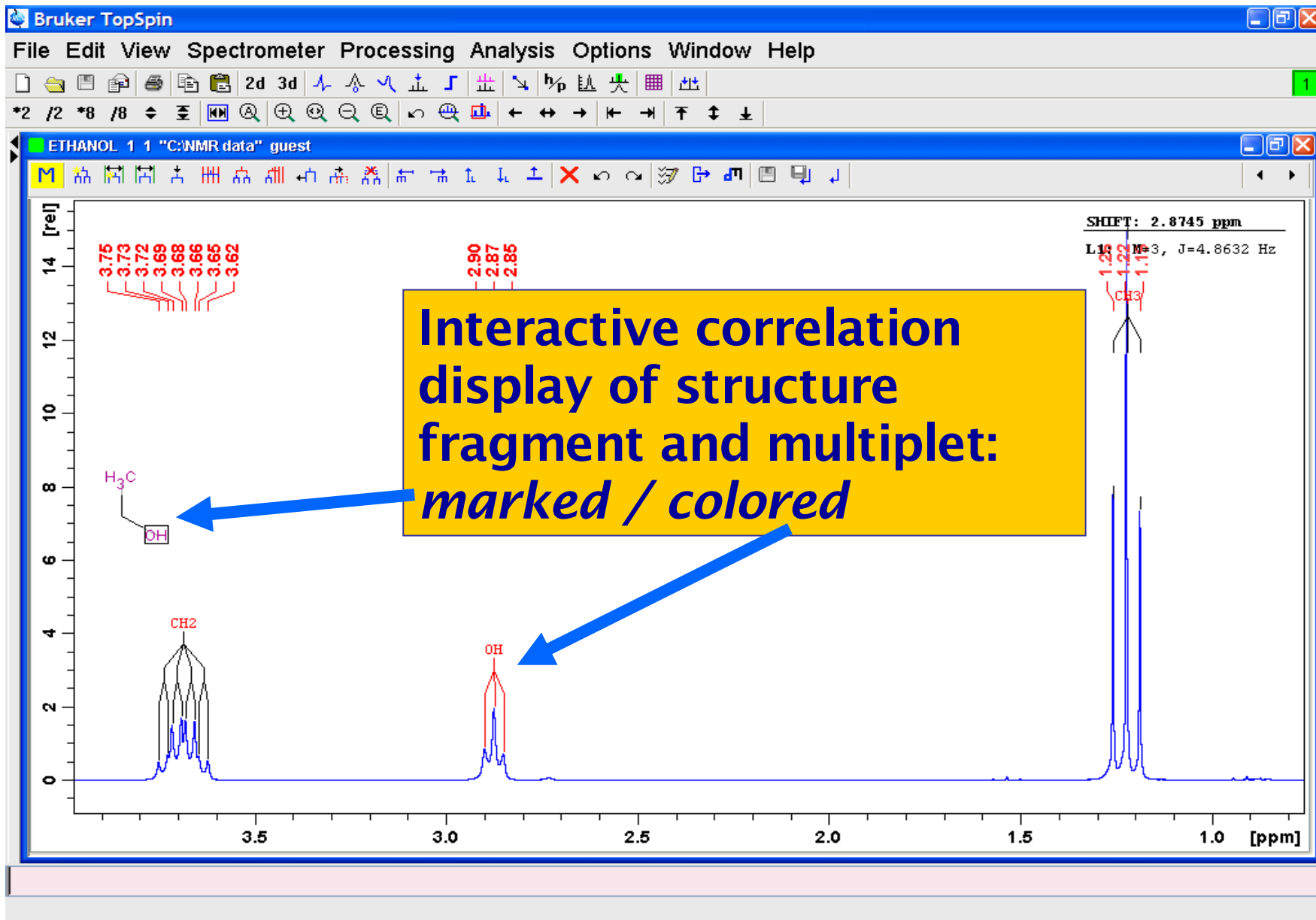
## Features:

- Interactive and automatic multiplet definition
- Flexible multiplet tree manipulation
- Simultaneous structure display
- Structure  $\leftrightarrow$  multiplet correlation display
- JMR and Japanese patent report formats

# Multiplet Analysis



# Multiplet Analysis



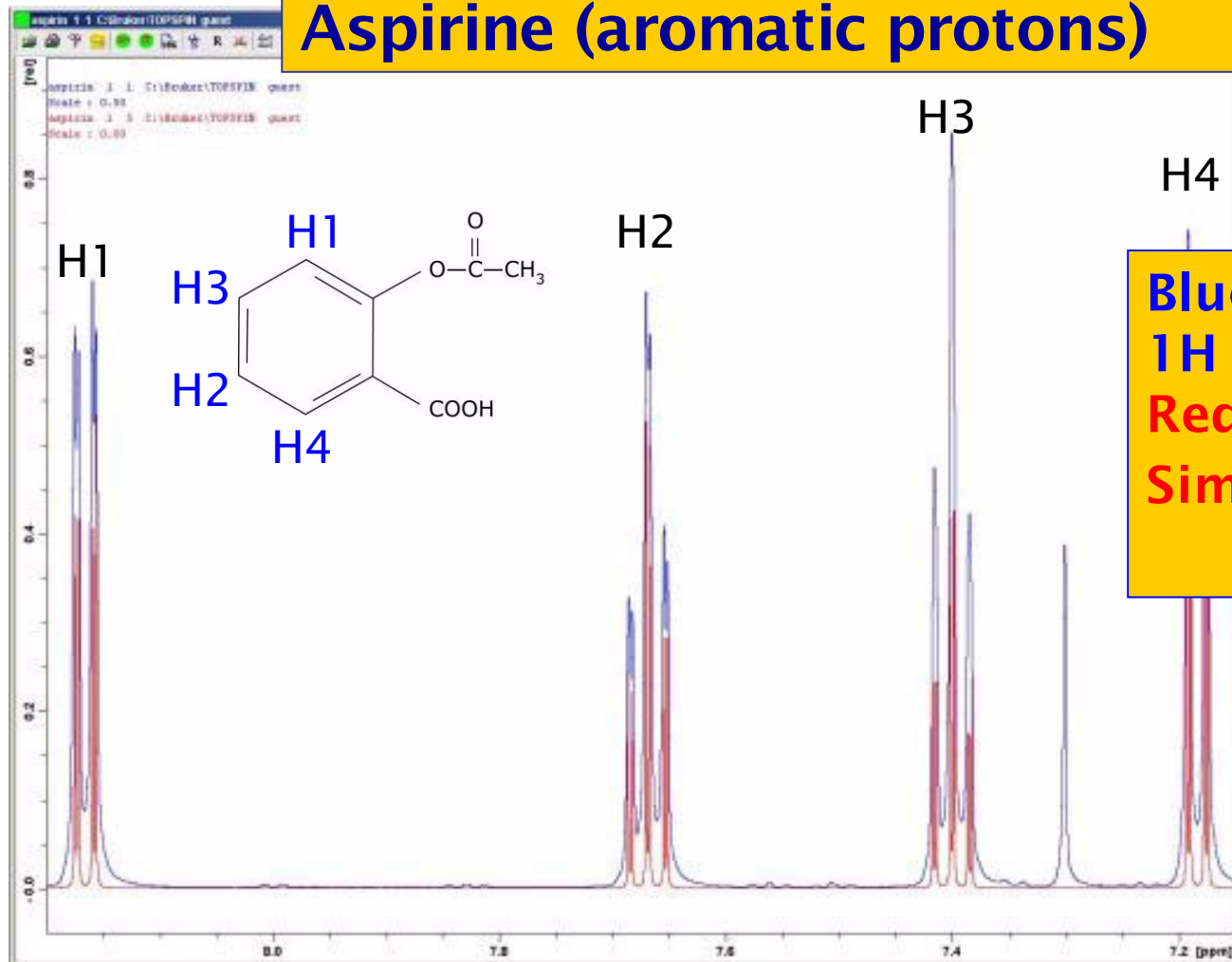
## Features of Simulation:

- Spectrum simulation is based on the solution of the time-independent Schroedinger equation to calculate the energy levels.
- Chemical equivalence, magnetic equivalence, fragment factorizing are taken into account for Hamiltonian factorization
- Up to 10 nuclei or groups with  $I \geq 1/2$  can be treated with one fragment
- The Lorentzian lineshape is calculated by application of global or individual linewidths to the transitions of as many fragments as are defined.

## Features of Iteration:

- Spectrum Iteration uses the method of *total-lineshape fitting*
- Adjustable parameters are resonance frequencies, scalar coupling constants and linewidths
- The program uses the current experimental spectrum as its input data.

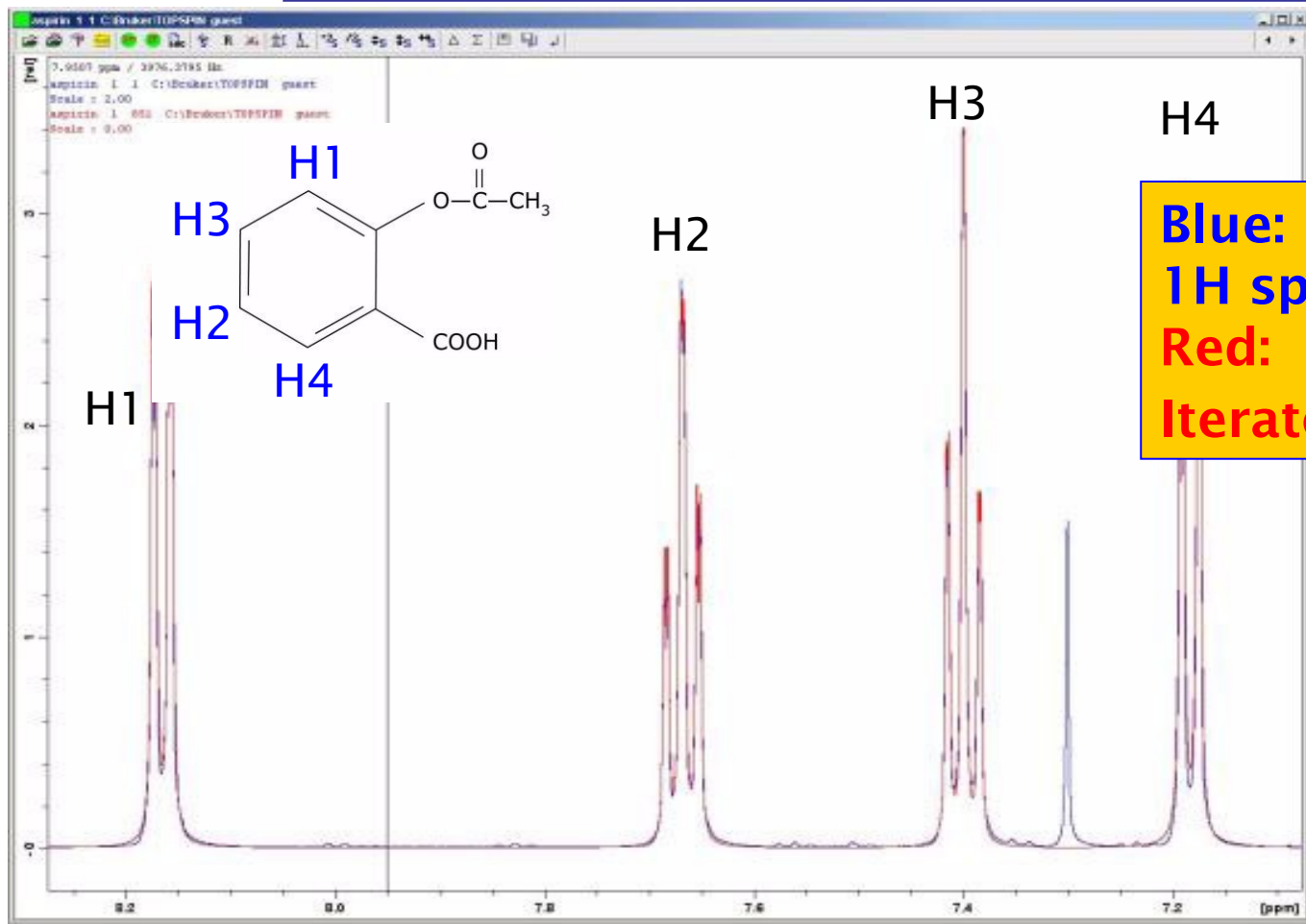
**Example:  
Simulation of the  $^1\text{H}$  spectrum of  
Aspirine (aromatic protons)**



**Blue:  
 $^1\text{H}$  spectrum  
Red:  
Simulated spectrum**

# 1D Spectrum Simulation & Iteration

**Example:  
Iteration of the  $^1\text{H}$  spectrum of Aspirine  
(aromatic protons)**



## *Iteration protocol*

## Parameters before and after iteration

Parameters to be iterated

=====

Fragment	Type	starting value	lower limit	upper limit
1	F( 1)	4083.59000	4073.58640	4093.58640
1	F( 2)	3835.02000	3825.02180	3845.02180
1	F( 3)	3700.91000	3690.91190	3710.91190
1	F( 4)	3592.55000	3582.55880	3602.55880
1	J( 1, 2)	1.55040	-0.44960	3.55040
1	J( 1, 3)	7.75200	5.75200	9.75200
1	J( 2, 3)	7.92100	5.90000	9.90000
1	J( 2, 4)	7.92100	5.90000	9.90000
1	J( 3, 4)	1.00000	-0.45000	3.55000
1	H( 1)	0.30000	0.00100	2.00000
1	H( 2)	0.30000	0.00100	2.00000
1	H( 3)	0.30000	0.00100	2.00000
1	H( 4)	0.30000	0.00100	2.00000

Iteration 21 rms = 1.78270396

Index Parameter Type Parameter Value neg. Grad. GN-Corr.

Index	Parameter	Type	Parameter Value	neg. Grad.	GN-Corr.
0	F( 1)		4083.65011	2.891e-004	2.952e-004
1	F( 2)		3834.98820	-2.802e-004	-2.614e-004
2	F( 3)		3700.91634	3.834e-004	3.713e-004
3	F( 4)		3592.53425	3.880e-004	3.802e-004
4	J( 1, 2)		1.69580	7.011e-004	1.503e-003
5	J( 1, 3)		7.87206	-6.621e-004	-3.382e-004
6	J( 2, 3)		7.39957	2.575e-003	1.005e-003
7	J( 2, 4)		8.09810	-5.682e-004	1.634e-004
8	J( 3, 4)		1.08665	-9.393e-004	2.475e-004
9	H( 1)		1.54867	-4.263e-003	-4.733e-003
10	H( 2)		1.34961	-5.573e-003	-5.830e-003
11	H( 3)		1.29116	-3.844e-003	-3.774e-003
12	H( 4)		1.71547	-7.271e-004	-1.068e-003



## Features:

### Intensity or area fitting, fitting types for:

→ **One-component T1 experiments.**

$$I(t) = I(0) + P * \exp(t/T1)$$

→ **One-component T2 experiments**

$$I(t) = P * \exp(t/T2)$$

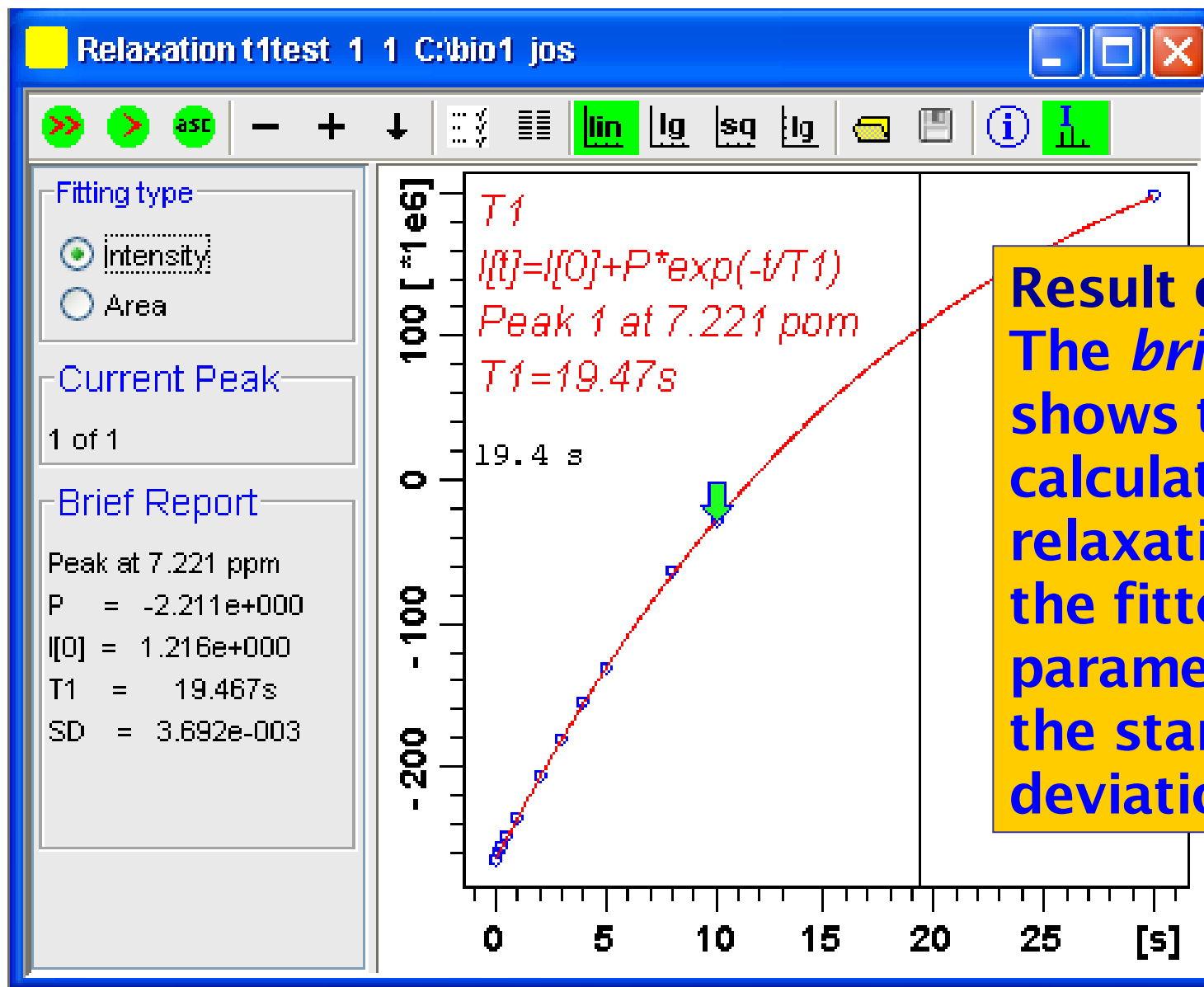
→ **Experiments with 3 or 4 components:**

*cpt1rho, lorgauss*

→ **Experiments with up to 6 components:**

*invrec, satrec,, expdec, gaussdec,  
varbigdel, varlitlel, vargrad, vardamp*

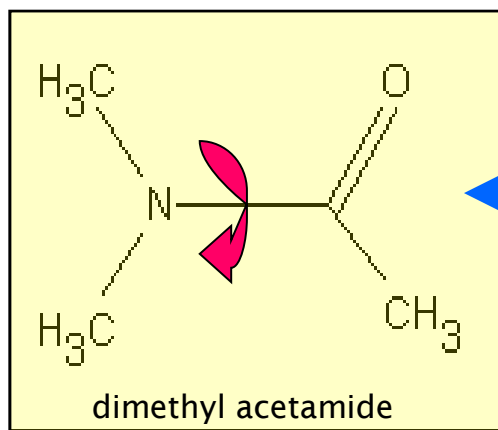
# Relaxation Analysis



Result display.  
The *brief report*  
shows the  
calculated  
relaxation value,  
the fitted  
parameters and  
the standard  
deviation

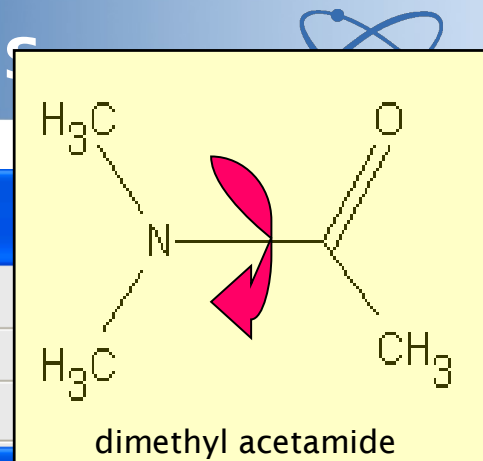
## Features:

- Simulation of temperature-dependent spectra
- Iteratively refine model parameters
- Computation based on Liouville/von Neumann Form of Schrödinger equation
- Superposition of DNMR spectra of more than one spin system



**Example: Restricted rotation about amide bond, exchange of Nitrogen methyl groups at high temperature**

# Dynamic NMR Line Shape Analysis



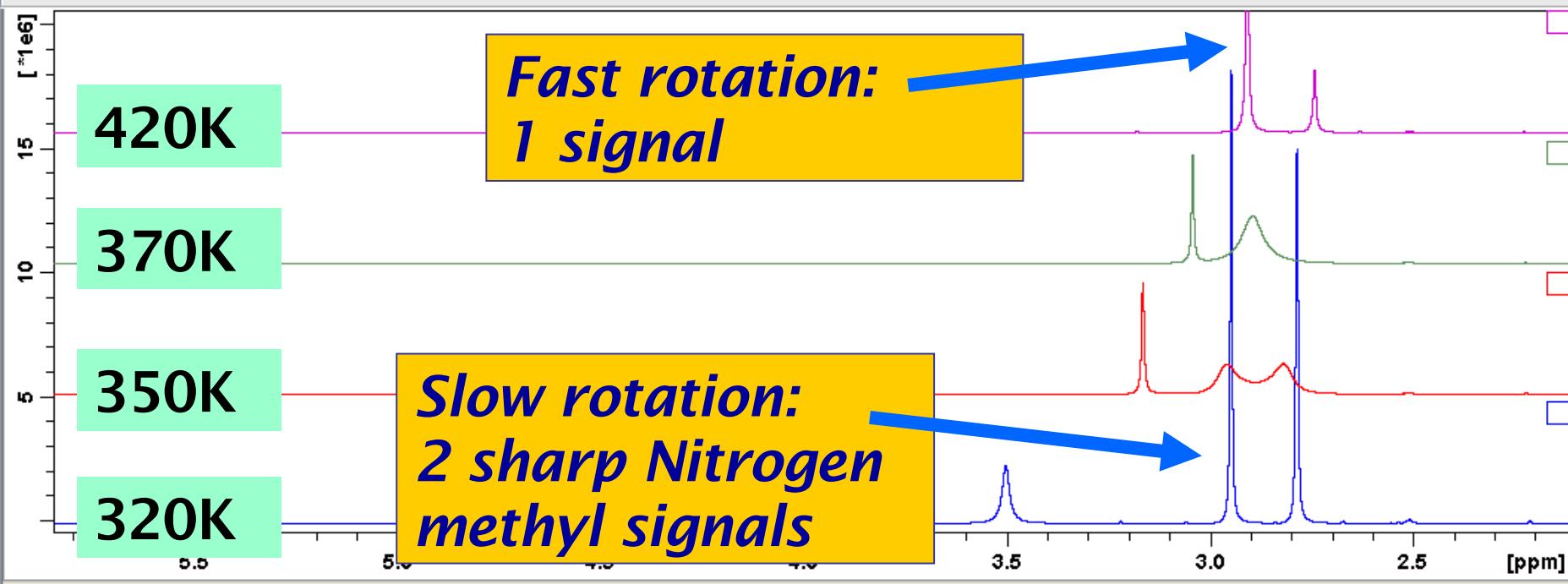
Bruker TopSpin

File Edit View Processing Analysis Options Window Help

2d 3d [various icons]

\*2 /2 \*8 /8 [various icons] em

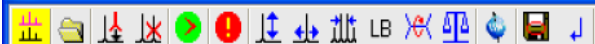
1 exam\_DNMR\_Me2NCOME 320 1 C:\Bruker\TOPSPIN guest



# Dynamic NMR Line Shape Analysis



1 exam\_DNMR\_Me2NCOME 320 1 C:\Bruker\TOPSPIN guest



Main Spectrum SpinSystem Log

System1

Parameters

<input checked="" type="checkbox"/>	Intensity	18088372.5	<input type="radio"/>
<input checked="" type="checkbox"/>	LB	0.3	Hz <input type="radio"/>
	LBGROUP	0	
	SUMX	1	

Nucleus Reaction Molecule

Nuc1

Iteration Status

Cycle  Best overlap(%) 46.875

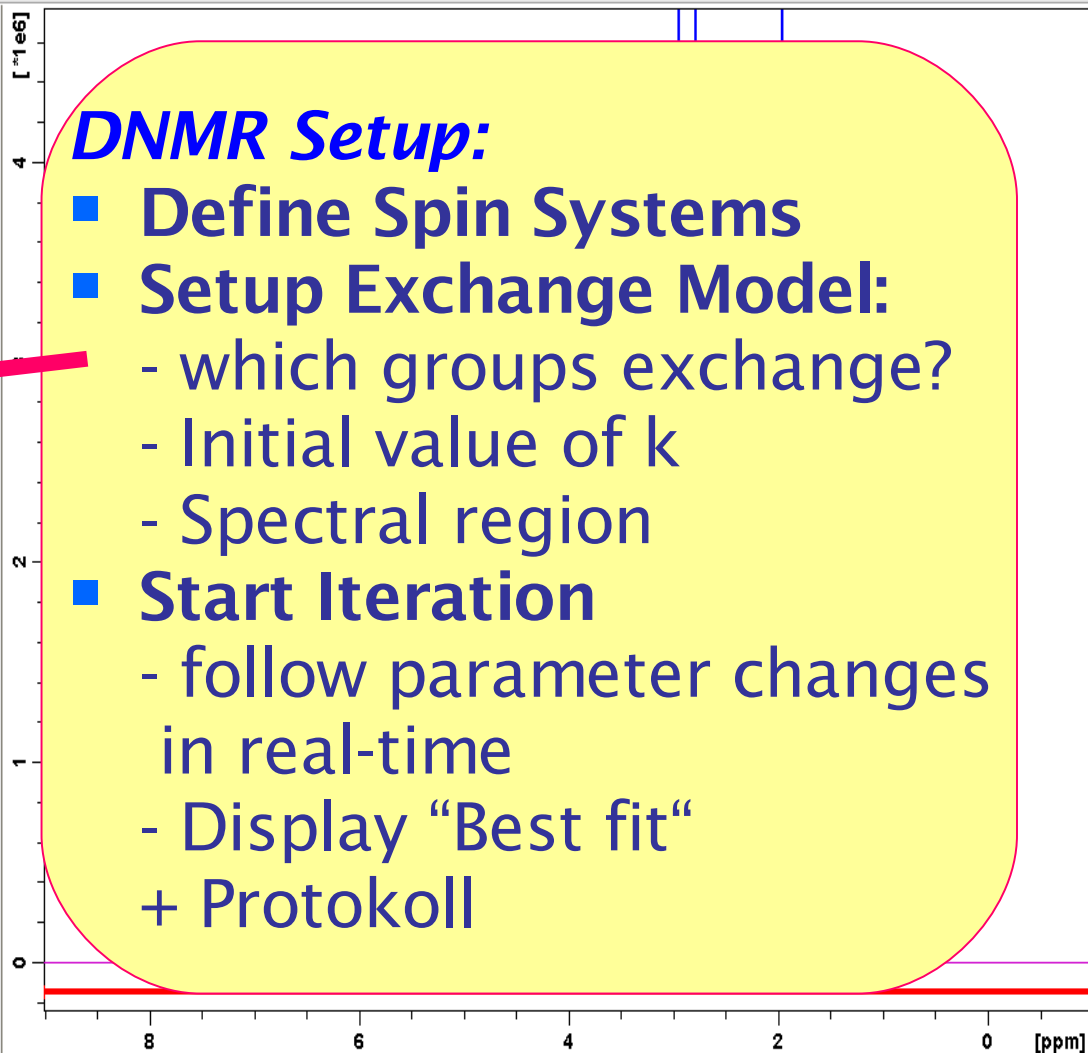
Parameters

<input checked="" type="checkbox"/>	Nu(iso)	4.0232	ppm <input type="radio"/>
	Pseudo Spin	0.5	<input type="radio"/>
	In Molecule	1	

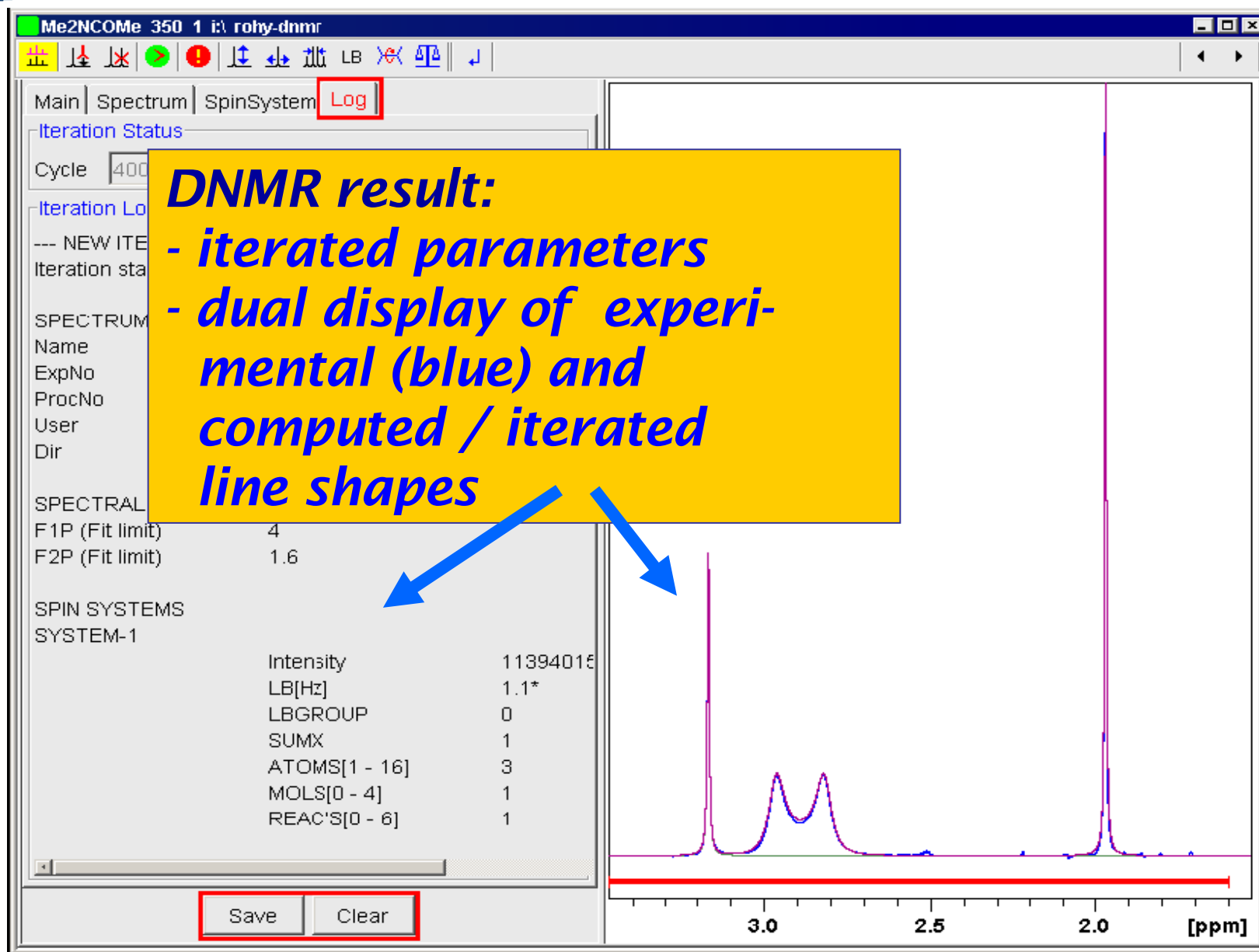
Add Delete Step Length

## DNMR Setup:

- Define Spin Systems
- Setup Exchange Model:
  - which groups exchange?
  - Initial value of k
  - Spectral region
- Start Iteration
  - follow parameter changes in real-time
  - Display "Best fit" + Protokoll



# Dynamic NMR Line Shape Analysis



## Features:

*Simulates 1D solids NMR spectra and fits them to experimental spectra using the following line shape models:*

- **Gauss / Lorentz**
- **Chemical shift anisotropy**
- **Quadrupolar central peak of the  $\pm \frac{1}{2}$  transition of a quadrupolar nucleus**
- **All quadrupolar transitions of a quadrupolar nucleus**
- **Combination of chemical shift anisotropy and quadrupolar interaction**

## Features (continued):

- Powder spectra of static or rotating samples
- 10 rotation and 5 DOR side bands definable
- Up to 25 observable nuclei (i.e. 25 nucleus sites)
- 10 dipolar coupling partners for the observed nucleus definable



# Solids Line Shape Analysis



- Solids line shape analysis set up window
- Dual display of experimental (blue) and fitted (red) line shape

Glycine 29 1 C:bio guest

Main | Spectrum | **Nucleus** | Dip.Interaction | Log

Nuc1

Parameters

<input checked="" type="checkbox"/>	ly	24624167.4		<input type="radio"/>
<input checked="" type="checkbox"/>	Nu (iso)	187.472	ppm	<input type="radio"/>
<input checked="" type="checkbox"/>	Delta(CSA)	-135.17	ppm	<input type="radio"/>
<input checked="" type="checkbox"/>	Eta(CSA)	0.962	0. - 1.	<input checked="" type="radio"/>
<input type="checkbox"/>	LB	140	Hz	<input type="radio"/>

Add Delete

[ppm]

*TopSpin comes with a*  
*- 2D structure editor and a*  
*- 3D structure viewer*

## Features:

### *2D structure editor*

- **Drawing chains, stereo bonds, aromatic bonds**
- **View spectrum correlation when spectrum displayed simultaneously**
- **Zoom / rotate / flip / refine**
- **Show mass information / INCHI identifier**
- **save a mol file, read mol file**

# Structure Editor



Bruker TopSpin

File Edit View Processing Analysis Options Window Help

\*2 /2 \*8 /8

1 2

Browser Last50 Groups Alias

- benzaldehyde
- Cinnamaldehyde
  - 230 - zg30
  - 231 - zgpg30
  - 232 - hsqcetgpsi2
  - 233 - cosygpmfqf
  - 700 - hsqcetgpsi2
- H451304
- H451305
- H451306
- H451307
- H451308
- KBr\_STMAS
- Vanillin
- alanine
- baseopt
- benzi...

1 Vanillin 220 1 C:\Bruker\TOPSPIN guest

Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals Sample Structure Fid

vanillin.mol

The image shows the Bruker TopSpin software interface. The main window displays the chemical structure of Vanillin (4-hydroxy-3-methoxybenzaldehyde) in the 'Structure' tab. The structure is a benzene ring with a formyl group (-CHO) at the top, a hydroxyl group (-OH) at the bottom, and a methoxy group (-OCH<sub>3</sub>) at the 3-position. The left sidebar shows a file browser with a tree view of folders, including 'Vanillin'. The top menu bar includes 'File', 'Edit', 'View', 'Processing', 'Analysis', 'Options', 'Window', and 'Help'. The top toolbar contains various icons for file operations and processing. The bottom status bar shows the file name 'vanillin.mol'.

## Features:

### *3D structure viewer*

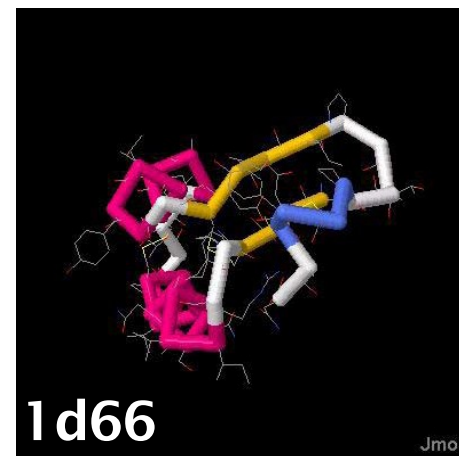
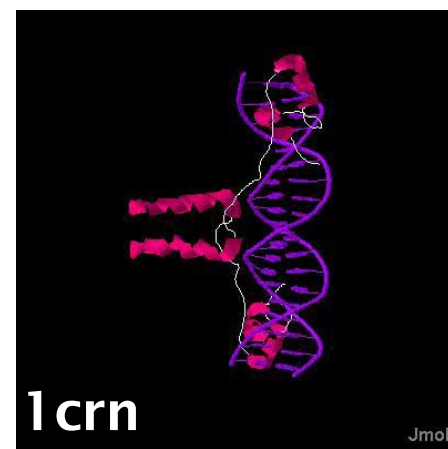
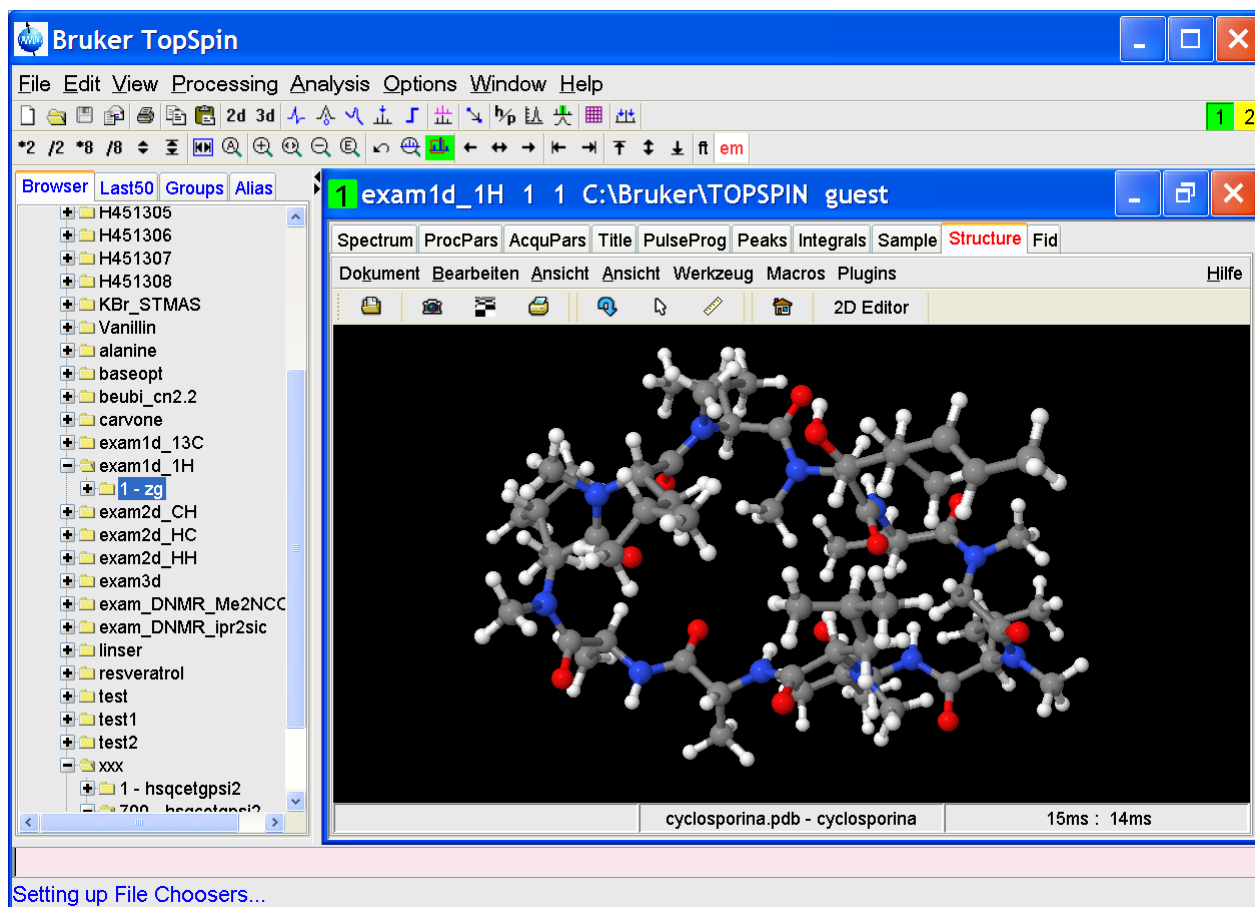
- **TopSpin-Implementation of the open source Jmol viewer ([jmol.sourceforge.net](http://jmol.sourceforge.net))**
- **High-performance 3D rendering**
- **Can read many file formats!**
- **Distance / angle / torsion angle measurements**
- **RasMol/Chime scripting language support**
- **Displays secondary structure elements (schematic shapes)**
- **Exports .png, .jpg, .ppm, .pdf, Povray**

# Structure Viewer : Jmol



Cyclosporine display using Jmol Viewer

Other examples:  
Secondary structure display



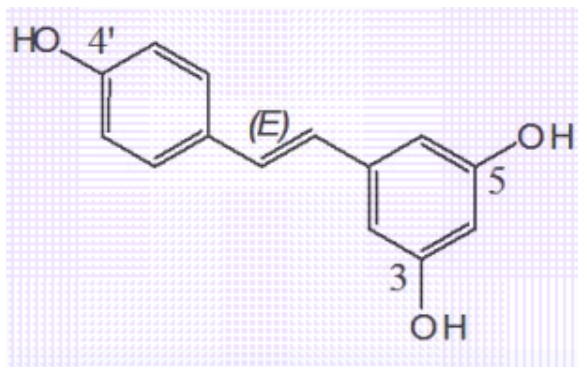
## Features:

*TopSpin includes a  $^1\text{H}$  /  $^{13}\text{C}$  shift prediction module of company PERCH, Kuopio, Finland*

- Shift prediction can be operated directly from the TopSpin (or Amix) user interface
- Shift prediction is based on Molecular Mechanics/ Dynamics
- Takes into account solvent, pH, ionic strength, concentration
- Computes a 1D spectrum via quantum mechanical methods from the prediction
- Prediction result in text form and as a spectrum

## Shift prediction workflow in TopSpin

### Input



.mol - File

Predict Software



### Result

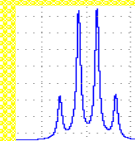
$\delta(1H)$

$\delta(13C)$

As:  
Text File

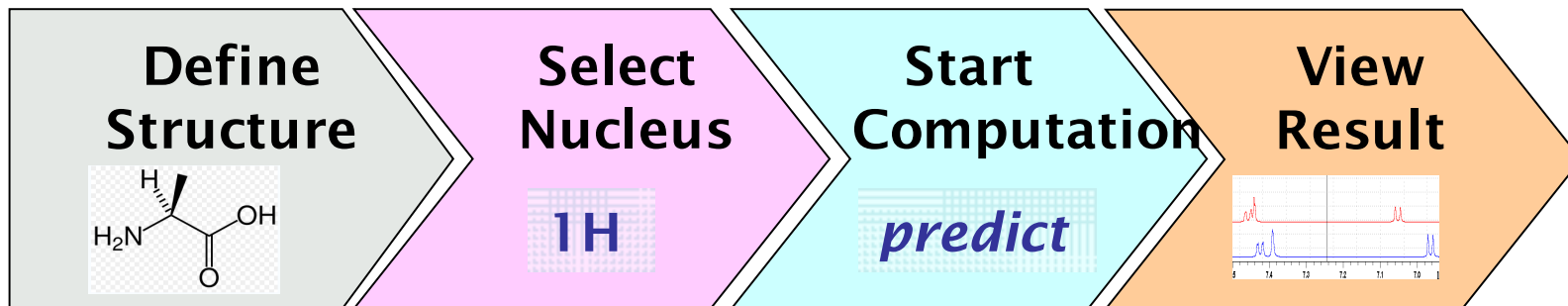
```
ATOM INFORMATION:
0 H "" 0 0 0 1*1 ND
1 C "" 2 1 1 1*1 ND
0 H "" 0 0 0 1*
33 H "" 0 29 29
2 C "" 1 2 2 1*1 ND
34 H "" 0 30 30
```

Calculated  
Spectrum





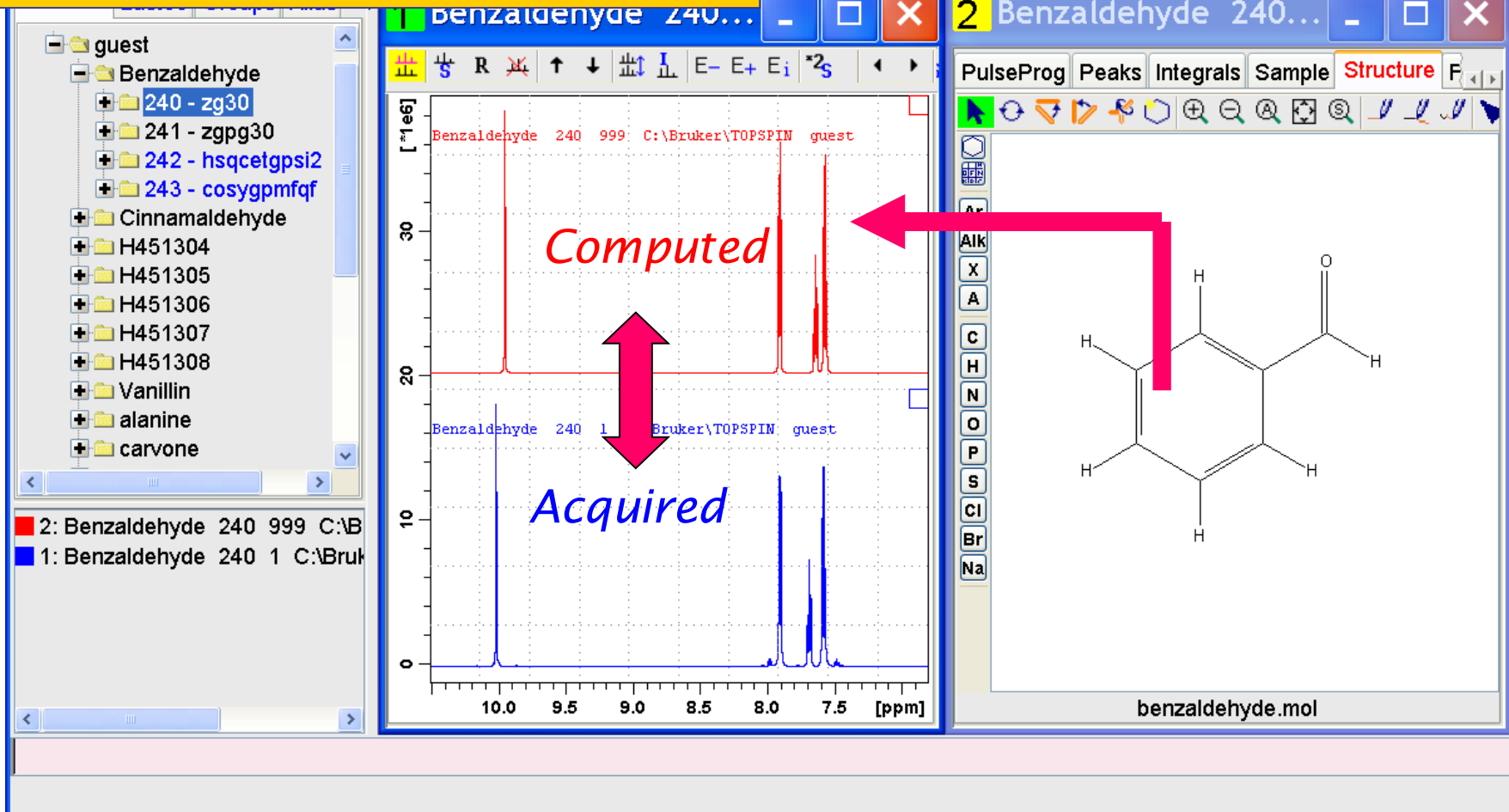
## Shift prediction workflow in TopSpin



# Shift Prediction



- Structure definition
- Display of predicted and experimental spectrum



## Features:

*TopSpin (from 2.1) includes a Structure Verification Module based on  $^1\text{H}$  /  $^{13}\text{C}$  shift prediction and a respective HSQC spectrum.*

**Based on  $^1\text{H}$  +  $^{13}\text{C}$  Shift Prediction  
and acquired HSQC**

Acquire 2D  
 $^1\text{H}$  /  $^{13}\text{C}$   
HSQC

Process  
Data  
→  
Spectrum

Peak  
Pattern  
Matching

Plot  
Result

Structure  
Proposal

$^1\text{H}$  /  $^{13}\text{C}$   
Shift  
Prediction

Next  
Sample

# Structure Verification



Result Plot, spectrum/prediction consistent

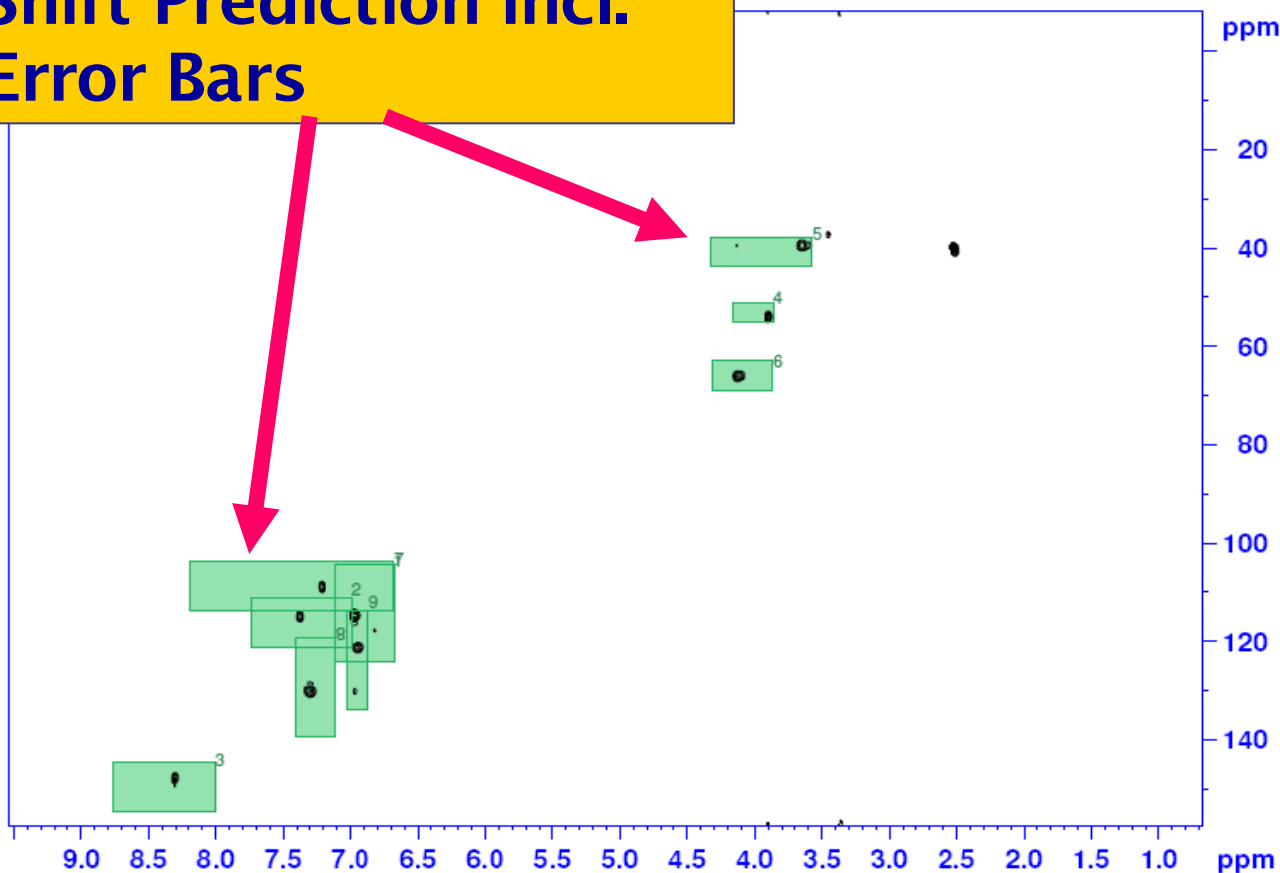
ps-hsqc-5m DMSO /u ps-ddms0 32

Electronic Signature:  
USER ID ps  
USER NAME ps  
MEANING review  
2006-10-31 11:22:06.792 +0100  
Current Data Parameters

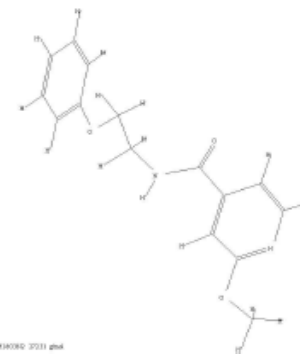
Current Data Parameters  
NAME 20094  
EXPNO 5  
PROCNO 1

HSQC Structure Verification Summary  
TOPSPIN 2.1, verification module 1.1 \$, 2006-09-31 11:30:28  
MOL file C:\bruker\TSP20\data\perch/nmr\20094\5\data\1\bru20094.mol  
prediction by PERCH-NMR Tools 2006.1017 BUILD125615R  
pattern matching by AURELIA/AMIX : Wed Oct 25 14:46:12 2006  
15 of 15 patterns verified (0 empty)  
51 of 66 peaks inside patterns (15 outside)

Shift Prediction incl.  
Error Bars



Structure



Pattern Legend

- 1 C3 H12
- 2 C5 H13
- 3 C6 H14
- 4 C10 H15, C10 H16, C10 H17
- 5 C19 H21, C19 H22
- 6 C20 H23, C20 H31
- 7 C26 H32, C30 H36
- 8 C27 H33, C29 H35
- 9 C28 H34

# Structure Verification



## Result Plot, spectrum/prediction inconsistent

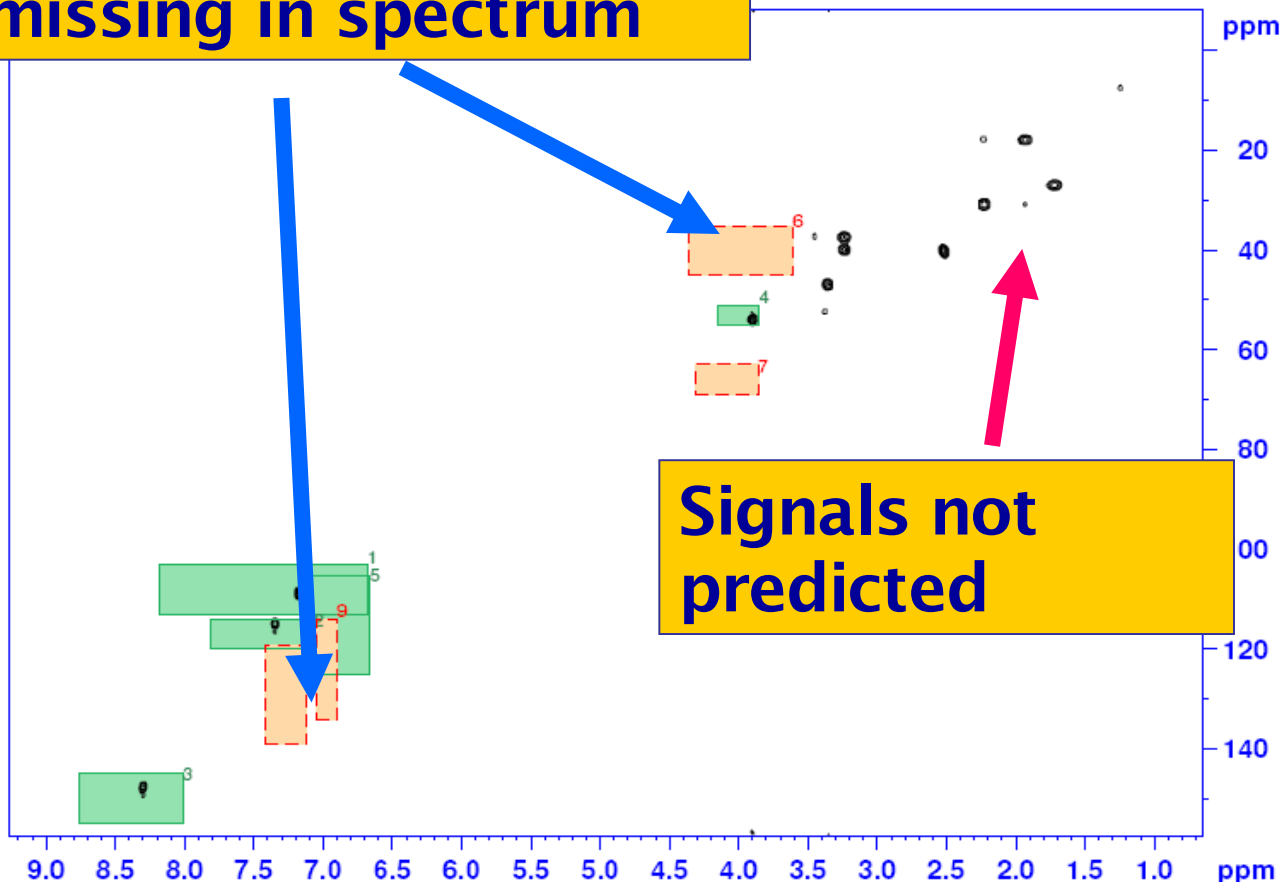
ps-hsqc-5m DMSO /u ps-ddmsso 3

Electronic Signature:  
USER ID ps  
USER NAME ps  
MEANING review

Current Data Parameters  
NAME 10061  
EXPNO 5  
PROCNO 1

HSQC Structure Verification Summary  
TOPSPIN 2.1, verification module 1.1 §, 2006-09-31 11:31:19  
MOL file c:\bruker\ts20\data\perchl\mm\20094\5\psala\1\bru20094.mol  
prediction by PERCH NMR TOOLS 2006.1017 BUILD125615R  
pattern matching by AURELIA/AMX : Wed Oct 25 14:49:12 2006  
9 of 15 patterns verified (7 empty)  
12 of 51 peaks inside patterns (39 outside)

Signals predicted, but missing in spectrum

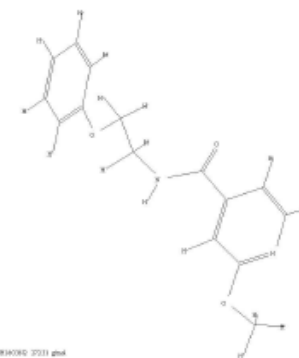


Signals not predicted



4009285 004907

Structure



Pattern Legend

- 1 C3 H12
- 2 C5 H13
- 3 C6 H14
- 4 C10 H15, C10 H16, C10 H17
- 5 C26 H32, C30 H36
- 6 C19 H21, C19 H22
- 7 C20 H23, C20 H31
- 8 C27 H33, C29 H35
- 9 C28 H34

# Structure Verification



ICON-NMR: Automation Oct19-2006-0932-mg

File Run Holder View Find Parameters Options Help

Holder Type Status Disk Name No. Solvent Experiment Structure Par Title / Orig Pri Time User

Holder	Type	Status	Disk	Name	No.	Solvent	Experiment	Structure	Par	Title / Orig	Pri	Time	User
1	Available	C:\Bruker\TopSp	Oct19-2006	10	Acetic	PROTON							mg
1	Available	C:\Bruker\TopSp	Oct19-2006	11	Acetic	HSQCETGP	Verif.:HIPPURIC0815						mg
	F2	C:\Bruker\TopSp	Oct19-2006	10									mg
2	Available												
3	Available												
4	Available												
5													
6													
7													
8													
9													

Molecular Structure Verification  
MOL/SD File HIPPURIC0815  
OK

**Structure proposal in IconNmr allows verification in automation**

Delete Add 1 Copy 1 Change User

Date	Holder	Name	No.	Experiment	Load	ATM	Rotation	Lock	Shim	Acq	Proc	User	Disk	Title / Orig	Remarks
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Busy until: No Jobs! Day Experiments: 00:00 Night Experiments: 00:00 User: mg

*TopSpin provides several DOSY processing algorithms:*

- 3-component fit
- Decra
- CONTIN
- DOSY *m*
- Fitting diffusion decays with the relaxation package



## Example spectrum

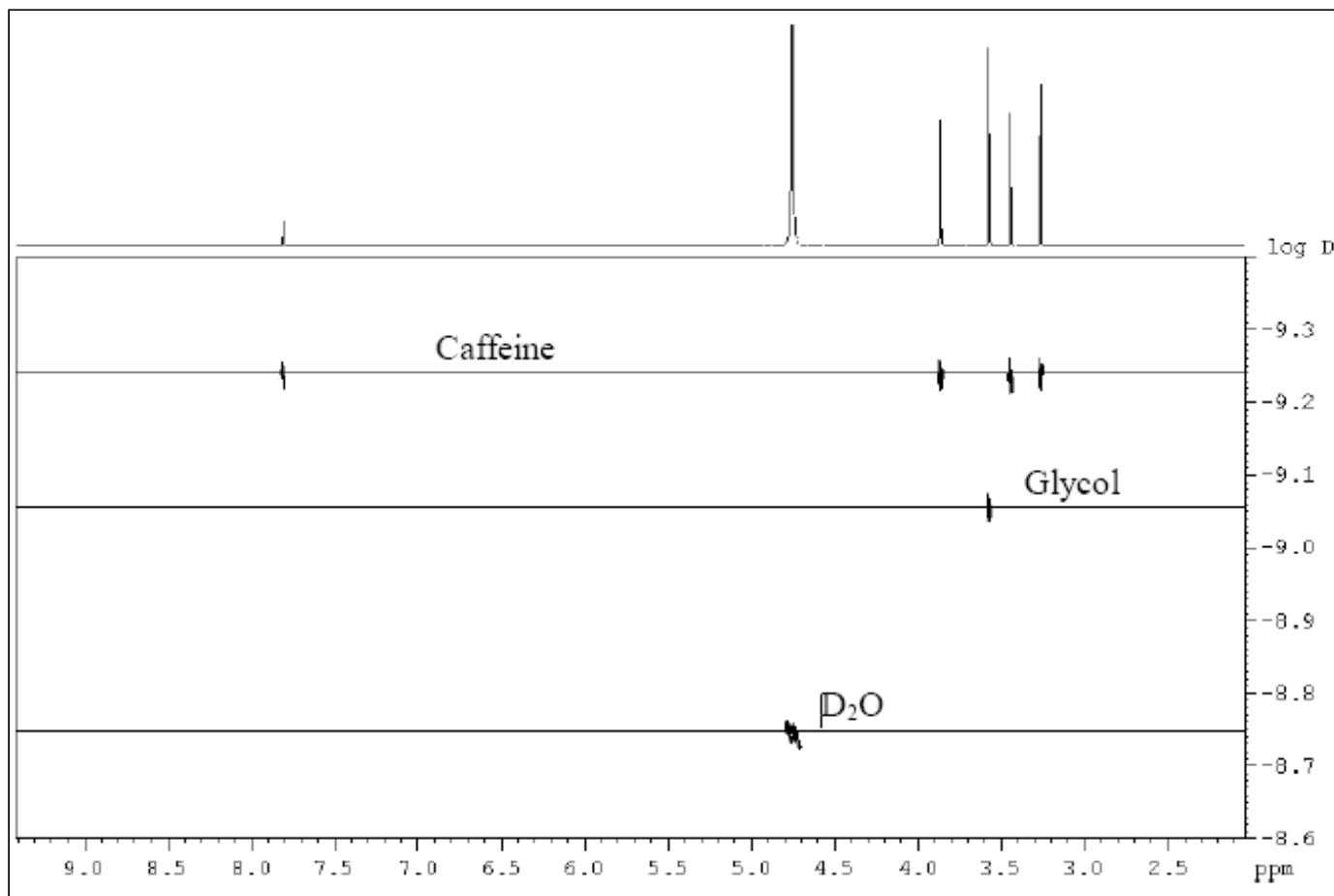


Figure 3.2: Example of a DOSY spectrum showing the different diffusion behavior of Ethylenglycol and Caffeine dissolved in D<sub>2</sub>O.

## 3-component fit

- Fits up to 3 exponentially decaying curves

## Decra (*direct exponential curve resolution algorithm*)

- Easy to use
- Fool-proof results
- But: Data acquisition requires variable gradient strength and with the square ramp option

# Fitting diffusion decays using the relaxation package

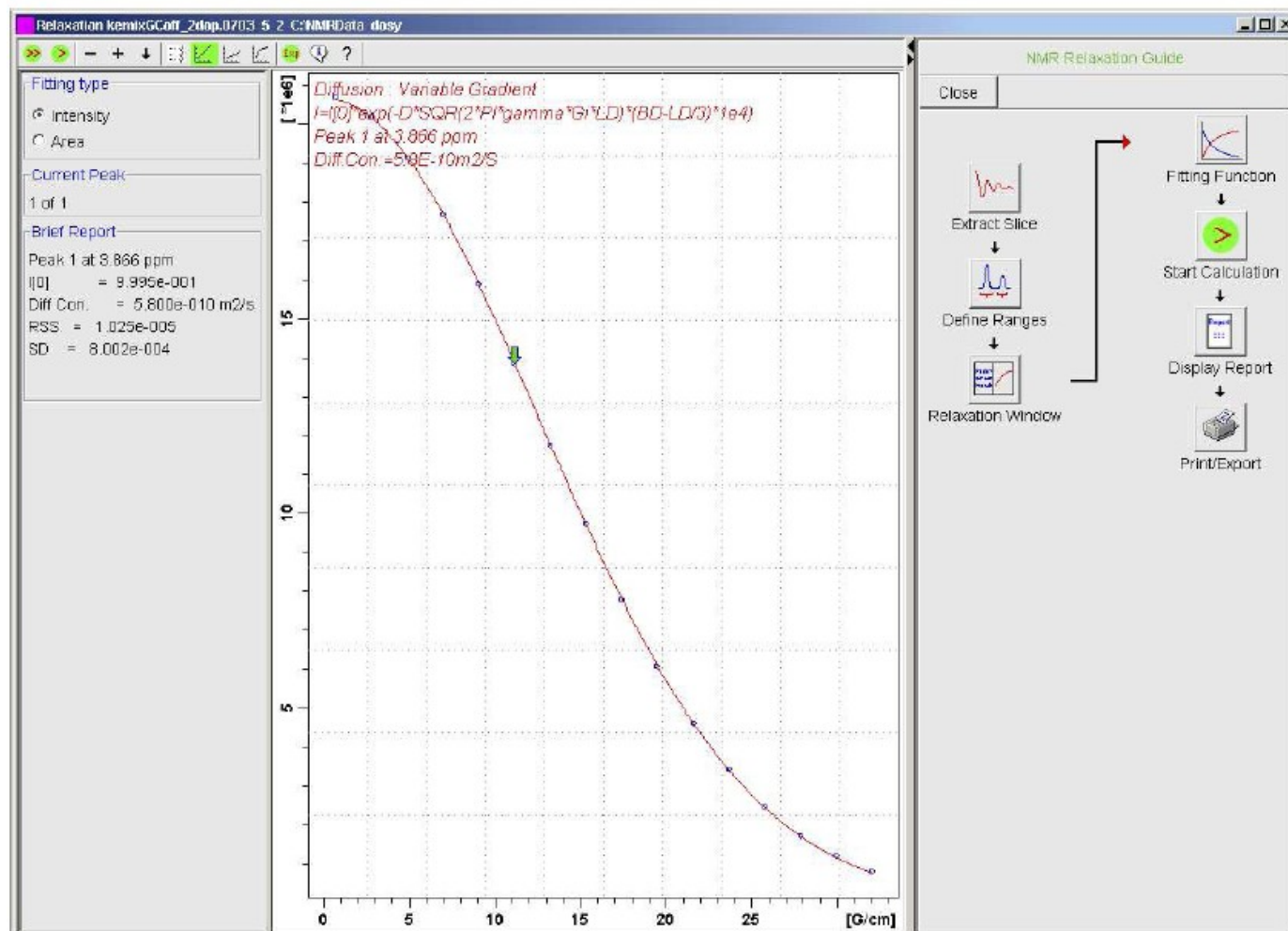


Figure 3.4: TopSpin display after fitting with SimFit (T1/T2 Analysis). The fitting curve for the peak at 3.87 ppm (Caffeine) is shown.

## CONTIN

- Based on algorithm of *Provencher*
- Intended for a complex mixture of small molecules with highly overlapping signals

## DOSY $m$ <sup>TM</sup>

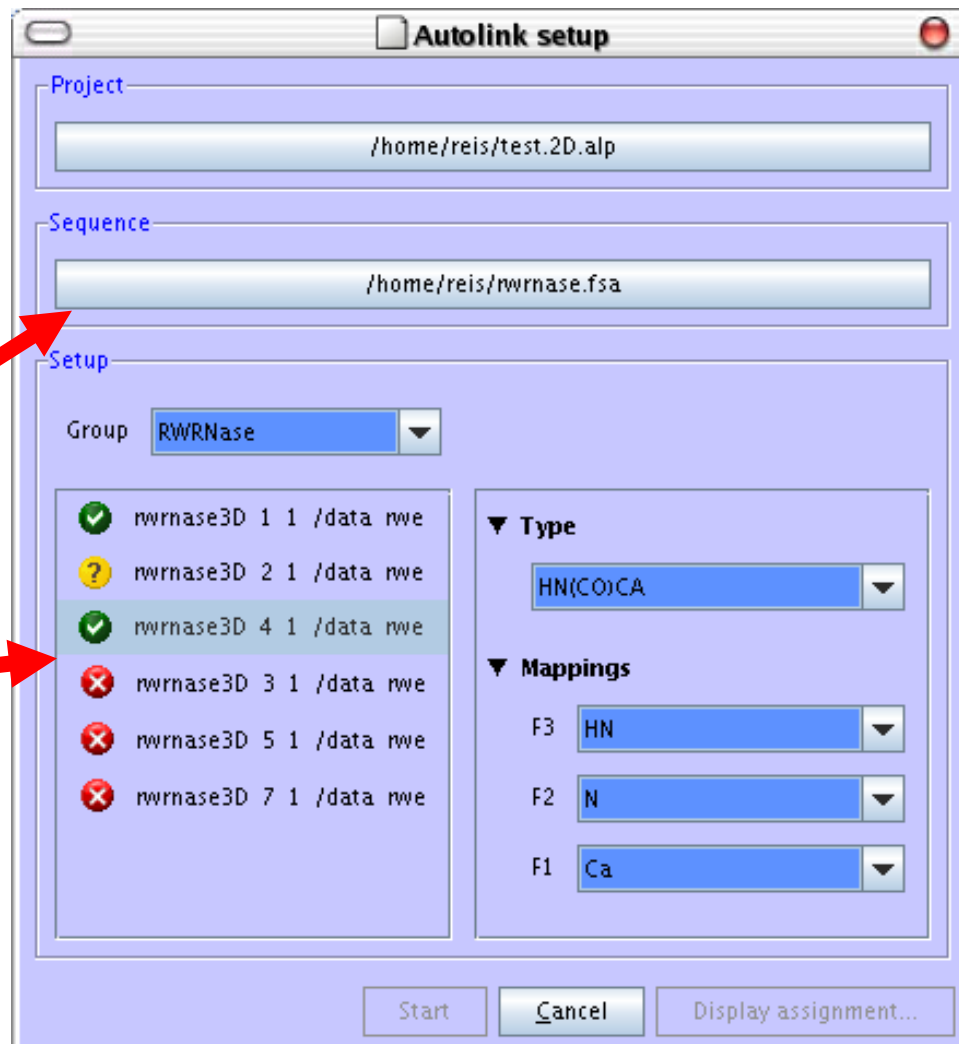
- Based on **m**aximum entropy mathematics
- Intended for a complex mixture of small molecules with highly overlapping signals
- Third-party module directly interfaced to TopSpin
- Requires separate license

TopSpin includes the *AutoLink* backbone assignment algorithm

AutoLink  
setup dialog

Amino Acid sequence

Input spectra



Autolink setup

Project: /home/reis/test.2D.alp

Sequence: /home/reis/rwrnase.fsa

Setup

Group: RWRNase

✓	rwrnase3D 1 1	/data rwe
?	rwrnase3D 2 1	/data rwe
✓	rwrnase3D 4 1	/data rwe
✗	rwrnase3D 3 1	/data rwe
✗	rwrnase3D 5 1	/data rwe
✗	rwrnase3D 7 1	/data rwe

Type: HN(CO)CA

Mappings

F3: HN

F2: N

F1: Ca

Start Cancel Display assignment...

## Assignment result display

