3rd International Electronic Conference on Metabolomics
15-30 November 2018
chaired by Prof. Peter Meikle, Dr. Thusitha W. Rupasinghe, Prof. Susan Sumner, Dr. Katja Dettmer-Wilde
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quantitative Quantum Mechanical Spectral Analysis (qQMSA) of Spectra of 1000+1 Chemical Shifts and other Biological Systems

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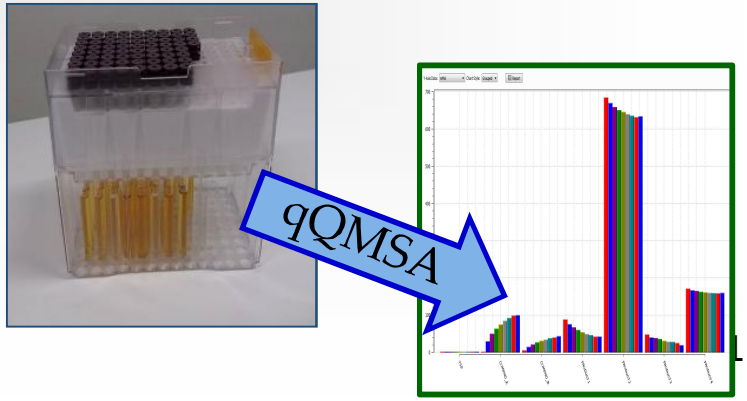

Abstract

The quantitative analysis of urine offers the greatest challenge for quantitative NMR (qNMR) of biofluids - as for any analytical method. It has been proposed that nearly 200 metabolites could be analyzed from a standard 1D ^{1}H NMR spectrum and it was also concluded that qNMR is the best method for urine profiling. The qNMR analysis is not straightforward and many approaches have been proposed for biofluids, but there is one, qQMSA, which is superior over the others, at least we believe so. The approach was described also in the 1st IECM. In addition to other applications, we present here results on analyzability of urine spectra, containing signals of 212 metabolites and totally 1001 chemicals shifts.

qNMR analysis is based on the assumption that an NMR spectrum is a sum of the model spectra of its components, without any calibration constants. In qQMSA experimental model spectra are replaced models obtained by fitting the spectra using the Quantum Mechanical (QM) theory which, maybe surprisingly, is able to interpret even the smallest details of the spectra – but removing noise, impurity signals and other artefacts. *The QM models are field independent and pack effectively the spectral information. The QM models obtained from spectra measured at any field can be used as models for biofluid spectra measured at any other field.*

Simulation of the 212 metabolites urine model with our SpinAdder QM engine takes < 0.5 sec/spectrum (if parallel simulation of several spectra) and whole the analysis demands < 60 sec/spectrum – thus the speed is not anymore the bottle-neck in qQMSA. The tools for qQMSA are built into ChemAdder/SpinAdder software (http://chemadder.com).

Our presentation describes essential features of qQMSA and the ChemAdder platform, developed specially for qQMSA, and reports the most recent results for the urine qQMSA. We also describe other applications, including metabolic flux analysis based on qQMSA of 2D HSQC spectra.

Keywords: Metabolomics; Quantitative NMR; QMSA; urine, fluxometics

Part 1

A SHORT INTRODUCTION TO qQMSA

Why qNMR !

- Minimal sample preparation, non destructive.
- No calibration – no pure reference compounds – minimal bias !
- Chemical confidence – not only quantity !
- Even unknown compounds can be quantitated (in mmol/ml) – and characterized or even identified !
- Automated measurement without human control - 24/7/365 !
- Automated analysis – now !
- One sample – >100 compounds – < 25 $ (Measurement)

Dictionary

- qQMSA = quantitative Quantum Mechanical Spectral Analysis: the intensity of ^{1}H NMR signal is directly proportional to number of protons in sample - *no calibration !*
- The protons in molecule form nearly isolated systems where they float in the sea of electrons - *the system energetics obeys laws of quantum mechanics (QM) perfectly !*
- Any molecular proton system can modelled with a few parameters (chemical shifts and coupling constants) in very details - *the model is magnetic field strength (instrument) independent – just add line-shapes to the model.*
- FIASL = Field Independent Adaptive Spectral Library is the most efficient way to store and handle spectral data – instead the experimental spectra libraries (spectrum for every field strength) used by the competive approaches – *good models can be obtained from poor spectra with impurities and artefacts – no pure reference compounds.*
- ChemAdder/SpinAdder - *The Software for qQMSA and FIASL !*

Why qQMSA!

- Maximal information – quantitative interpretation of very details – with chemical confidence.
- Field independent model with minimum number of parameters – the most efficient way to store spectra!
- *Prior knowledge* can be used.
- ChemAdder – the platform for qQMSA, with a few clicks, all the spectra – from FID to diagrams.
- SpinAdder – the new generation QM engine.
- Targeted recipes for biofluids.
- FAST - 1 .. 60 sec/sample (multitasking).

ASL-model (file) for glucose

..with less essential information removed:

```
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        PROFILE = C:\CHEMADDER\PROFILE\GLUCOSEPROFILE.TXT ; OPTIONS
      REFERENCE = TSP             ; TMS, TSP, DSS, REF_N(N=No. of protons/molecule), ..ND
        SOLVENT = HDO             ; POLYSOL, CCL4, CDCL3, DMSO, ACD6, CD3CN, CD3OD, CD2Cl2, D2O, HDO, ..
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       GAUSSIAN = 51.161          ; GAUSSIAN % IN LINE-SHAPE (CAN BE >100%)
      ASYMMETRY = -1.408          ; ASYMMETRY % IN LINE-SHAPE (CAN BE <0)
           RRMS = 0.4130          ; 100 * RRMS
   R-FACTOR(%) = 99.860           ; 100 * (1.0-sumsq/totalsumsq)

&CHEMICAL SHIFTS(PPM):
GLUCO 2*SPIN= 1 SPECIES=1H POPULATION(Y)=  1.000000[OBS=  1.000000] MWGT=  180.160 SLOPE=  1.0000
 BG5        3.472  1*1*1  STAT=Y  PRED= 3.472 RANGE(0)= 0.027 WIDTH(Y)= 1.549 RESP(Y)= 0.6223 TYPE= 100m
 BG1        4.667  1*1*1  STAT=N  PRED= 4.667 RANGE(0)= 0.132 WIDTH(Y)= 1.300 RESP(N)= 0.6223 TYPE= 1a0d
 BG4        3.412  1*1*1  STAT=Y  P
 BG2        3.252  1*1*1  STAT=Y  P
 BG3        3.496  1*1*1  STAT=Y  P
 BG6A       3.903  1*1*1  STAT=Y  P
 BG6B       3.730  1*1*1  STAT=Y  P
 AG5        3.841  1*1*1  STAT=Y  P
 AG1        5.239  1*1*1  STAT=Y  PRED= 5.239 RANGE(0)= 0.036 WIDTH(Y)= 1.342 RESP(Y)= 0.3777 TYPE= 1e0m
 AG4        3.419  1*1*1  STAT=Y  PRED= 3.419 RANGE(0)= 0.027 WIDTH(Y)= 1.451 RESP(Y)= 0.3777 TYPE= 100m
 AG2        3.542  1*1*1  STAT=Y  PRED= 3.542 RANGE(0)= 0.027 WIDTH(Y)= 1.339 RESP(Y)= 0.3777 TYPE= 100q
 AG3        3.723  1*1*1  STAT=Y  PRED= 3.723 RANGE(0)= 0.028 WIDTH(Y)= 1.460 RESP(Y)= 0.3777 TYPE= 100o
 AG6A       3.849  1*1*1  STAT=Y  PRED= 3.849 RANGE(0)= 0.029 WIDTH(Y)= 1.454 RESP(Y)= 0.3777 TYPE= 100m
 AG6B       3.769  1*1*1  STAT=Y  PRED= 3.769 RANGE(0)= 0.028 WIDTH(Y)= 1.478 RESP(Y)= 0.3777 TYPE= 100m

&COUPLING CONSTANTS:
GLUCO
 1_13        9.925    J BG5      BG
 1_15       -0.234    J BG5      BG3        STAT=N  PRED= -0.23 RANGE= 0.10
 1_16        2.270    J BG5      BG6A       STAT=N  PRED=  2.27 RANGE= 0.12
 1_17        5.930    J BG5      BG6B       STAT=N  PRED=  5.93 RANGE= 0.14
 1_24        7.960    J BG1      BG2        STAT=N  PRED=  7.96 RANGE= 0.16
 1_35        9.140    J BG4      BG3        STAT=N  PRED=  9.14 RANGE= 0.18
 1_37       -0.219    J BG4      BG6B       STAT=N  PRED= -0.22 RANGE= 0.10

Continued ...
```

&DEFAULTS defines the defaults..

MWGT = Molecular weight

β- glucose

α- glucose

STAT=Y/N shift optimizable/fixed, PRED= default, RANGE(i)=range(symmetry), WIDTH(Y/N)= linewidth (optimizable/fixed), RESP(Y/N)=Response factor (optimizable/fixed), TYPE= 1H type (for HOLISTICS). If shifts (the 2nd column) and PRED are same for shifts, the shifts are kept equal.

STAT=Y/N if coupling is optimizable/fixed (N is default in metabolomic analyses). If couplings have the same name (the 1st column) they are kept equal.

... Continues

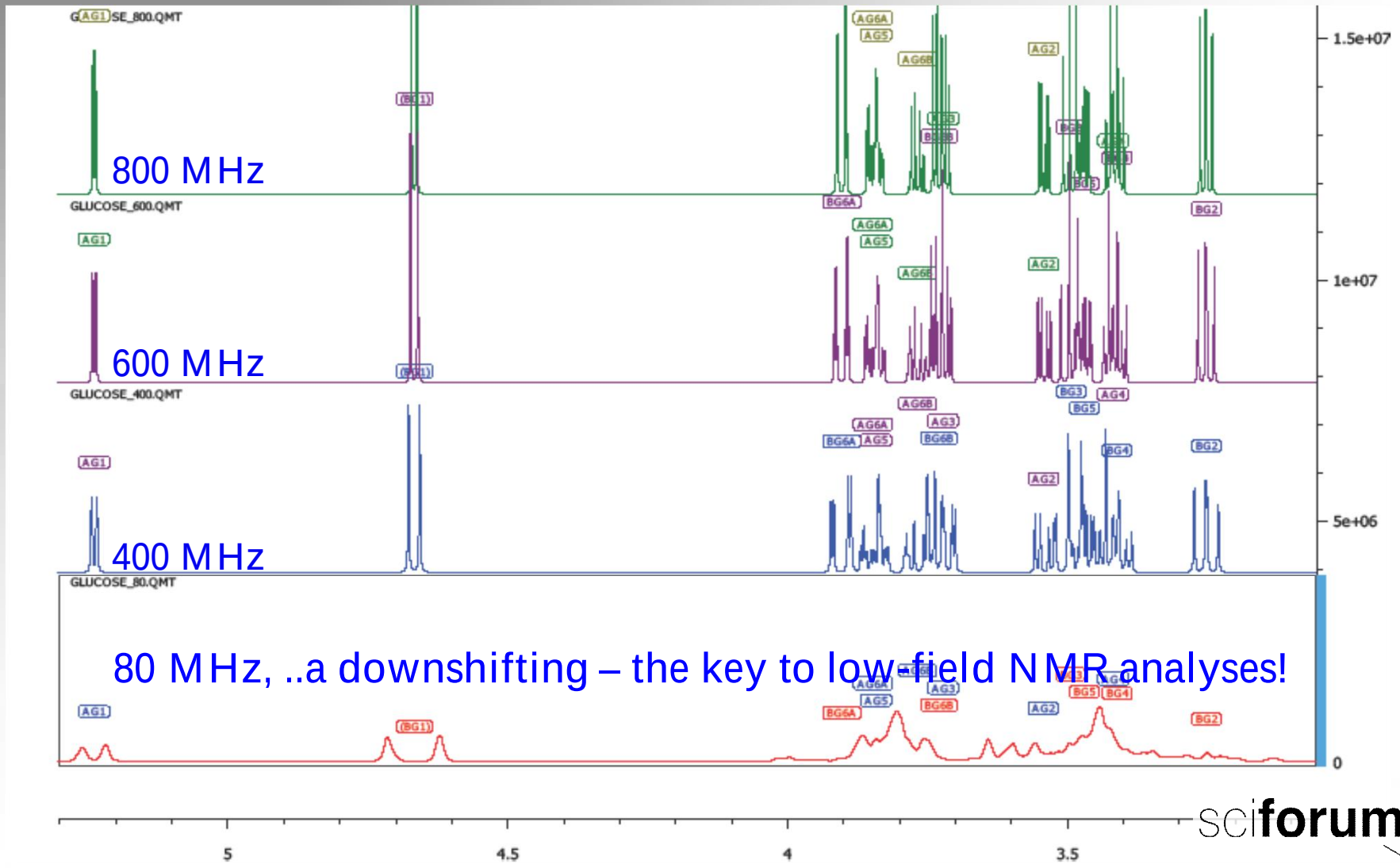
```
 1_67      -12.299    J BG6A     BG6B       STAT=N  PRED=-12.30 RANGE= 0.22
 2_12       -0.711    J AG5      AG1        STAT=N  PRED= -0.71 RANGE= 0.10
 2_13       10.150    J AG5      AG4        STAT=N  PRED= 10.15 RANGE= 0.20
 2_16        2.320    J AG5      AG6A       STAT=N  PRED=  2.32 RANGE= 0.12
 2_17        5.310    J AG5      AG6B       STAT=N  PRED=  5.31 RANGE= 0.14
 2_23        0.125    J AG1      AG4        STAT=N  PRED=  0.12 RANGE= 0.10
 2_24        3.795    J AG1      AG2        STAT=N  PRED=  3.79 RANGE= 0.12
 2_25       -0.255    J AG1      AG3        STAT=N  PRED= -0.25 RANGE= 0.10
 2_26        0.305    J AG1      AG6A       STAT=N  PRED=  0.31 RANGE= 0.10
 2_27        0.295    J AG1      AG6B       STAT=N  PRED=  0.29 RANGE= 0.10
 2_35        9.155    J AG4      AG3        STAT=N  PRED=  9.15 RANGE= 0.18
 2_36       -0.387    J AG4      AG6A       STAT=N  PRED= -0.39 RANGE= 0.10
 2_37       -0.193    J AG4      AG6B       STAT=N  PRED= -0.19 RANGE= 0.10
 2_45        9.830    J AG2      AG3        STAT=N  PRED=  9.83 RANGE= 0.18
 2_67      -12.264    J AG6A     AG6B       STAT=N  PRED=-12.26 RANGE= 0.22

&BARTLETTS  32    12.509  2754.689     (= N & BROADENING & 1ST)
127   9 128  21 129  26 130  28 131  16 132   1 140   2 141  12 142  24 143  26 144  21 145  12 146   3 159   3 160 11
161  23 162  38 163  47 164  42 165  26 166  16 167  22 168  26 169  22 170  13 171   4 179   6 180  20 181 27 182 21
183  11 184   3 184  -2 184  -3 184  -4 184  -5 184  -6 184  -7 184  -8 184 -9 184 -10 ...

&ASL TEMPLATES AT:  500.360000 MHZ
      1   1746.576050      5.451566    1    1     1.549
      2   1744.435181      5.476259    1    1     1.549
      3   1740.931763      5.326477    1    1     1.549
      4   1738.726074      5.809281    1    1     1.549
      5   1736.909546     10.211022    1    1     1.549
      6   1734.703735     10.109370    1    1     1.549
      7   1731.135498     10.271605    1    1     1.549
      8   1728.929688     10.251603    1    1     1.549
      9   1726.723877      0.845136    1    1     1.549
     10   1724.583008      0.323967    1    1     1.549
     11   2339.285889     38.440464    2    1     1.300
     12   2331.306152     38.482616    2    1     1.300
     13   1715.435425     22.668512    3    1     1.450
```

Continued ..

```
&END of FILE
```

Adaptive Spectra

Glucose spectra at different fields, simulated on the basis of the 500 MHz spectrum analysis, with line-width of 1.0 Hz



TARGETED RECIPE FOR URINE

The metabolites are given in order of typical abundances and occurrences (from Bouatra, S. et al., *The human urine metabolome*, PLoS ONE 8, e73076, 2013):

```
&CASEINFO: %P1=human %P2=male %age=50 &type=5 &type=12 &x1=1.25 x2=10.3
&DEFAULTS   (Less essential information removed)
        ORIGINAL = C:\URINE\URINE220218.JDX
       BATCHFILE = C:\CHEMADDER\SCRIPTS\URINE.BAT  = The MENU (SCRIPT file) for analysis
         PROFILE = C:\CHEMADDER\PROFILE\SEARCHPROFILE.TXT = Defaults for iteration
  REFERENCE MMOL = 9.292
```

		DEF.	MIN.	MAX.	OCC%
&INC C:\CHEMADDER\URINE\TSP.ASL	MMOL=	4.64	4.64	4.64	100
&INC C:\CHEMADDER\URINE\CREATININE.ASL	MMOL=	1474.00	1374.00	1574.00	100
&INC C:\CHEMADDER\URINE\UREA.ASL	MMOL=	1228.00	175.00	4909.00	100
&INC C:\CHEMADDER\URINE\HIPPURICACID.ASL	MMOL=	229.00	19.00	622.00	100
&INC C:\CHEMADDER\URINE\CITRATE.ASL	MMOL=	203.00	49.00	600.00	100
&INC C:\CHEMADDER\URINE\GLYCINE.ASL	MMOL=	106.00	44.00	300.00	100
&INC C:\CHEMADDER\URINE\TRIMEAMINE-OXIDE.ASL	MMOL=	91.00	4.80	509.00	100
&INC C:\CHEMADDER\URINE\TAURINE.ASL	MMOL=	81.00	13.00	251.00	100
&INC C:\CHEMADDER\URINE\CYSTEINE.ASL	MMOL=	65.80	23.10	134.50	100
&INC C:\CHEMADDER\URINE\CREATINE.ASL	MMOL=	46.00	3.00	448.00	100
&INC C:\CHEMADDER\URINE\HISTIDINE.ASL	MMOL=	43.00	17.00	90.00	100
&INC C:\CHEMADDER\URINE\GLYCOLICACID.ASL	MMOL=	42.00	3.70	122.00	100
&INC C:\CHEMADDER\URINE\GUANIDOACETATE.ASL	MMOL=	41.80	10.60	97.30	100
&INC C:\CHEMADDER\URINE\ISOCITRATE.ASL	MMOL=	46.90	20.40	89.20	68
&INC C:\CHEMADDER\URINE\GLUCOSE.ASL	MMOL=	37.50	12.50	58.40	100
&INC C:\CHEMADDER\URINE\XYLITOL.ASL	MMOL=	8.40	6.00	13.80	95
&INC C:\CHEMADDER\URINE\5NH2-PENTANOATE.ASL	MMOL=	1.10	0.00	2.20	5
&INC C:\CHEMADDER\URINE\13NH2-PROPANE.ASL	MMOL=	1.20	0.20	5.85	5
&INC C:\CHEMADDER\URINE\CHOLINE.ASL	MMOL=	3.50	1.40	6.10	100
&INC C:\CHEMADDER\URINE\MALEATE.ASL	MMOL=	0.40	0.30	0.50	14
…					
&INC C:\CHEMADDER\URINE\EXTRA\TREHALOSE.ASL	MMOL=	2.00	1.00	5.00	50
&INC C:\CHEMADDER\URINE\EXTRA\PO4-GLUCOSE.ASL	MMOL=	2.00	1.00	5.00	50
&INC C:\CHEMADDER\URINE\EXTRA\TESTOSTERONE.ASL	MMOL=	2.00	1.00	5.00	50
&INC C:\CHEMADDER\URINE\EXTRA\ETACETATE.ASL	MMOL=	2.00	1.00	5.00	50

```
&END of FILE                    Totally 212 compounds
```

Math

NMR spectrum $I(\nu)$ is sum of spectra of chemical components $S_n(\nu)$ and background $B(\nu)$:

$$I(\nu) = \Sigma x_n S_n(\nu) + B(\nu)$$

If $B(\nu)$ is assumed zero or constant, this forms a group of linear equations (no. of equations = no. of spectral points, n = the number of unknowns x_n).

The group of equations can be solved to the least square criterium using *Regression Analysis* or *Principal Component Regression (PCR).*

The sub-spectra $S_n(\nu)$ can be presented by functions F and Q (the Quantum mechanical non-explicit function):

$$S_n(\nu) = F[\nu, Q(\underline{\delta}, \underline{J}), \underline{\Delta}, \underline{R}, LS]$$

($\underline{\delta}$=chemical shifts, $\underline{J}$=couplings, $\underline{\Delta}$=line-widths, $\underline{R}$=Response factors, LS= LineShape) where δ, Δ, R and LS depend significantly on conditions, which makes the problem non-trivial – but not impossible!

The background $B(\nu)$ may arise from macromolecular signals (for example, lipoproteins) which can also added to the model and quantitated.

HOLISTIC qQMSA

- The *holistic* qQMSA means that combining analyses of N spectra gives more and better information than the separated analyses of the N spectra. In practice this means that after a new type of data set has been once analyzed, a model of the chemical shift variations is formed (we prefer *Random Forest* method) and the analysis is repeated by using constraints provided by the model.
- If a component is well-defined in some spectra, the second round fixes its position in spectra where it is poorly defined or diverged.
- *The more data from system, the better.*

See Presentation: ChemAdder_HOLISTICS

*It was recently shown (*Takis & al., Nat.Commun. 8:1662, DOI:10.1038/s41467-017-01587-0) *that chemical shifts in urine can be predicted by accuracy of 0.0001- 0.0005 ppm (the larger number are for some low field signals) using linear regression.*

Part 2

QUANTITATIVE NMR

Integration, deconvolution or qQMSA or both ?

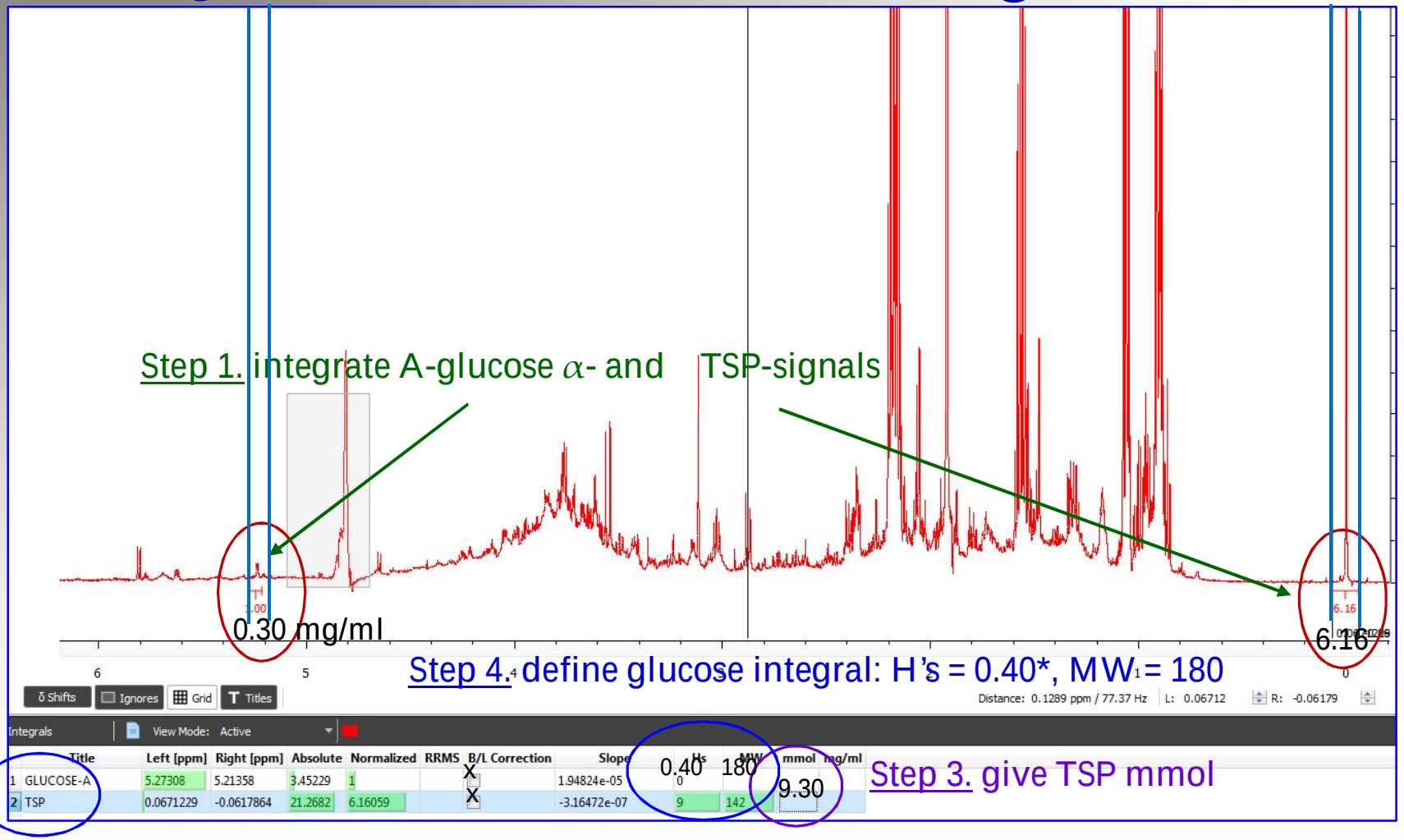
The basic integration, with no QM

The traditional, fastest and easiest way from spectra to quantitative information ..but how reliable ?

When there is quantitative reference (TMS, TSP, DSS or REFi) and its concentration are given (in PMR-file), any spectral integrals (area) can be transformed into mmol when the number of protons in the integral area are defined. If the corresponding molecular weight (MW) is known, also mg/ml is obtained.

ChemAdder: One click, all the spectra !

Quantification of *total* glucose



*Only 40% of glucose is A-glucose

Quantification of *total* glucose

With Baseline correction only:

Without any baseline correction option
the integral gets value of ca. 1.00 !!!

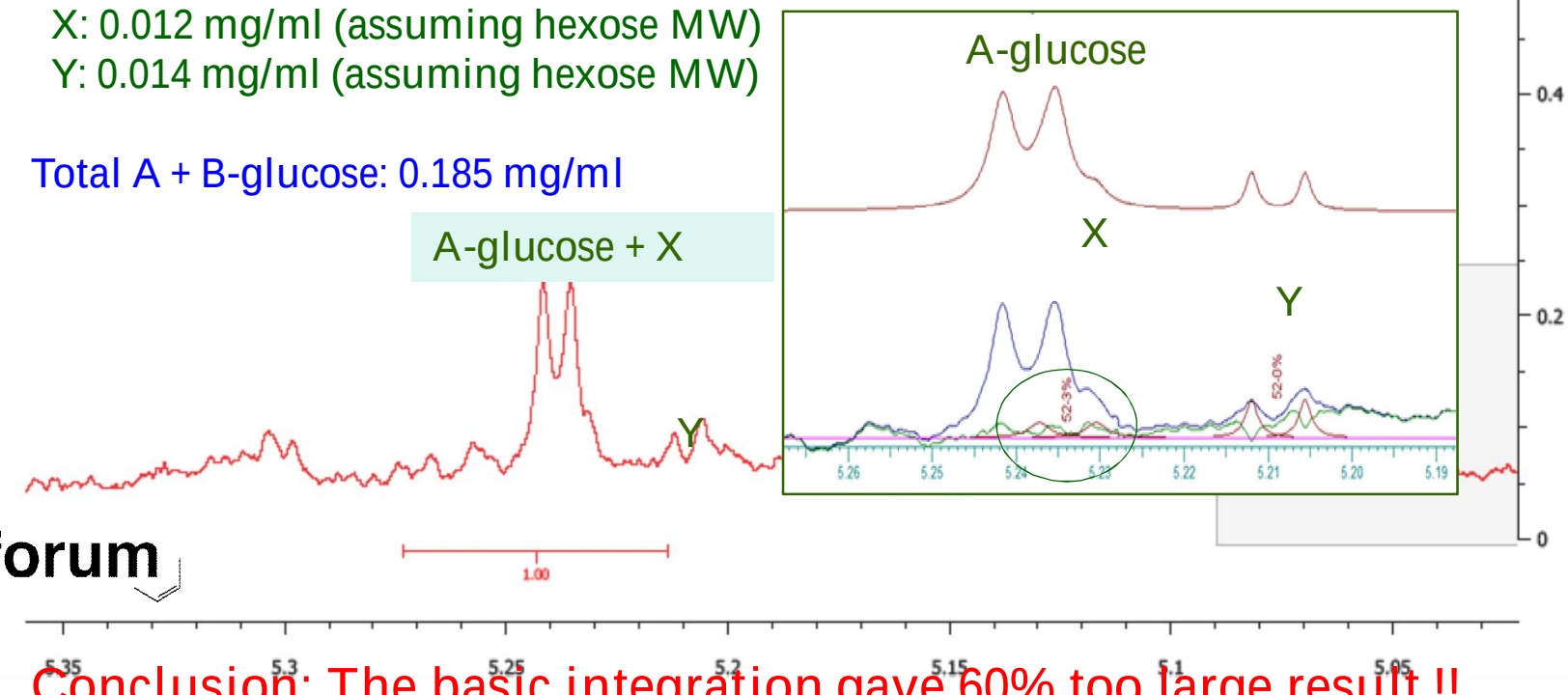
Quantification of *total* glucose

…*toward completeness !*

The glucose signal is in fact composed of doublet and another smaller doublet (Y), which can be fitted by using qQMSA + TLS (for X and Y):

A-glucose: 0.074 mg/ml
X: 0.012 mg/ml (assuming hexose MW)
Y: 0.014 mg/ml (assuming hexose MW)

Total A + B-glucose: 0.185 mg/ml



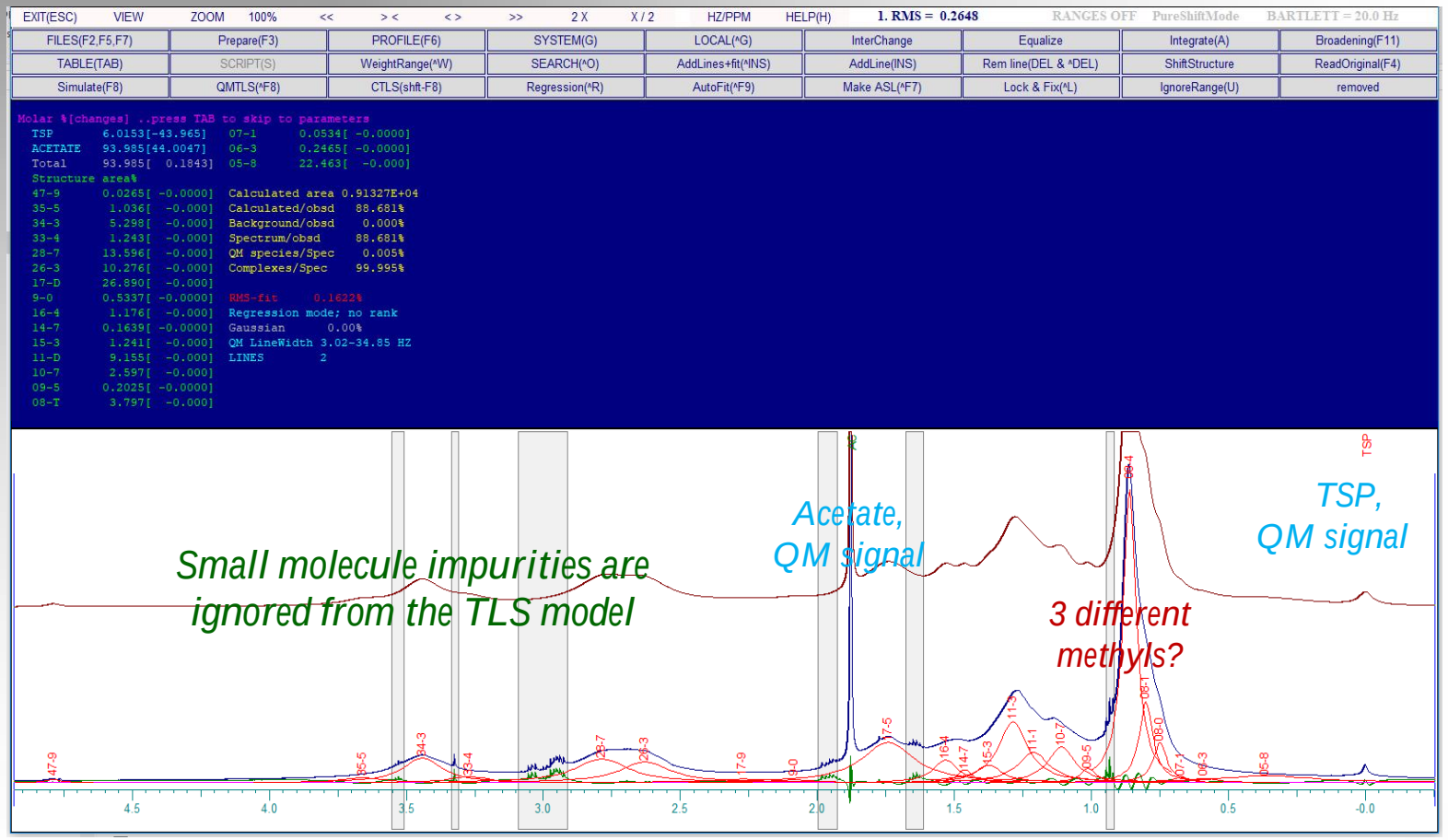
Conclusion: The basic integration gave 60% too large result !!

Deconvolution (TLS) analysis

Can be used to model signals not described by QM models – together with QMSA models.

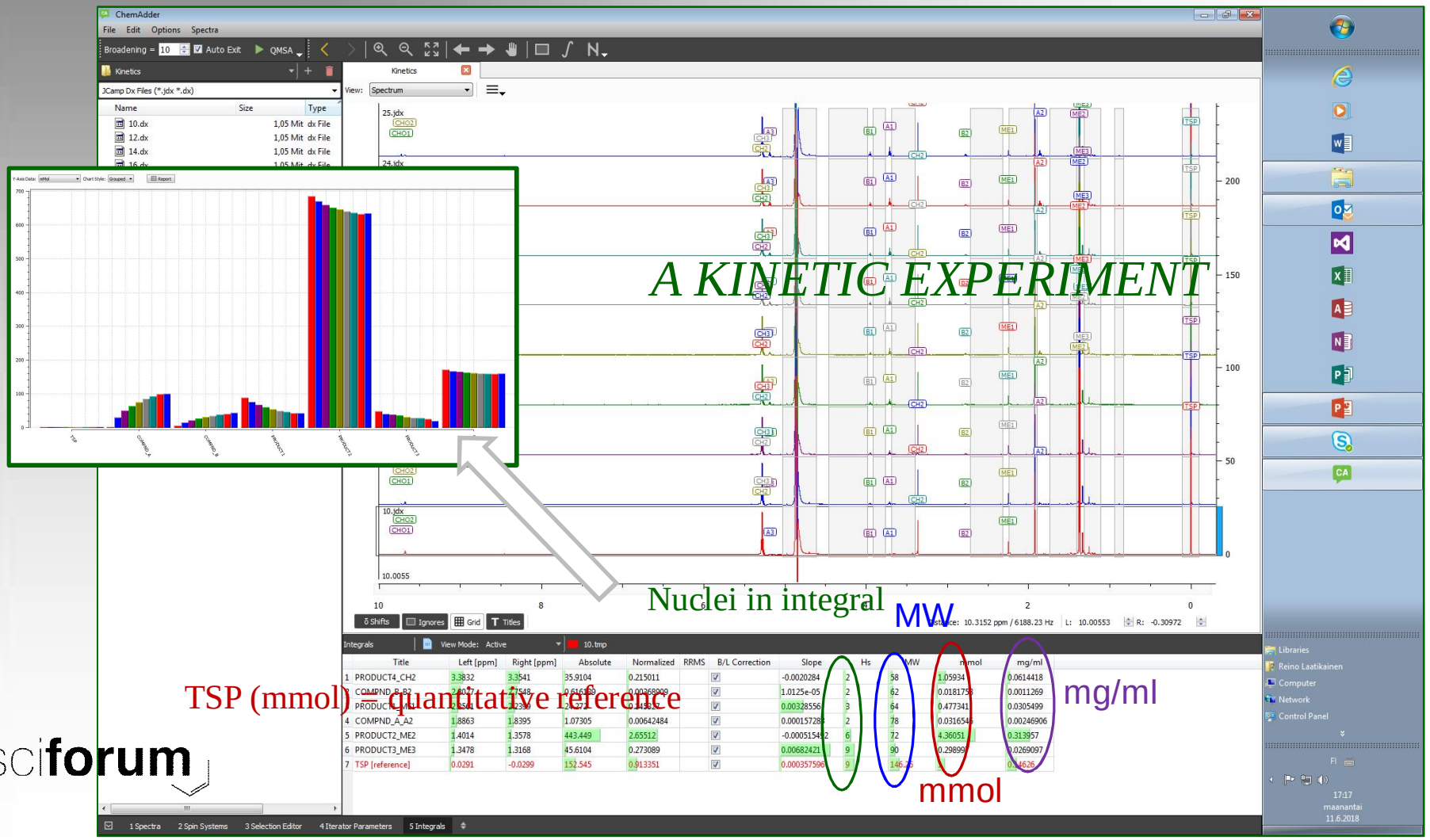
The spectrum not explained by QM model can be composed from extra Lorenzians or regular multiplets (doublets, triplets, etc.), which can be named and grouped for integration.

SpinAdder creates the model with a few clicks (just give the number of extras).



From spectra to Diagram

Integrate or fit (QMSA), then drag-and-drop to EXCEL or use the tools of ChemAdder – all the spectra with same click!



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VERSION INFO:    -110631651    135282665       44861  0.5189E+03
TIME: 06.07.2018  12:27:45
%SEARCH FILE

&CONTROL AND DEFAULTS:
        ORIGINAL = ND              ; TYPE = DX, JDX, QMT, OBS, ASL(=PMR), HMD, SDB
        SPECTRUM = C:\NMRDATA\URINE\AVERAGE.QMT ; ND => (RE)READ ORIGINAL!
       BATCHFILE = ND              ; PROTOCOL(MENU) FILE (.REC or .BAT-file)
         PROFILE = C:\CHEMADDER\PROFILE\SEARCHPROFILE.TXT ; OPTIONS/ADDER PROFILE
       REFERENCE = TSP             ; TMS, TSP, DSS, REF_N(N=No. of protons/molecule), ...ND
         SOLVENT = HDO             ; POLYSOL, CCL4, CDCL3, C6D6, DMSO, ACD6, CD3CN, CD3OD, D2O, CD2C12, ..ND
     TOTAL MG/ML = 227.092         ; TOTAL CONCENTRATION
  REFERENCE MMOL = 9.292           ; QUANTITATIVE(=GLOBAL) REFERENCE (ND=1.0)
           FIELD = 600.40282160    ; FOR 1H in MHZ, USED TO TRANSFORM SHIFTS TO HZ
POINT RESOLUTION = 0.09633074      ; DATA-POINT-RESOLUTION (HZ)
      LINE WIDTH = 2.514           ; 0.0 = USE SPECIES DEFAULT (HZ)
       SIGNAL LW = 0.000           ; 0.0 = AUTO, DEFAULT LINE WIDTH FOR EXTRA SIGNALS
        GAUSSIAN = 0.000           ; GAUSSIAN % IN LINE-SHAPE (CAN BE > 100)
       ASYMMETRY = 0.000           ; ASYMMETRY % IN LINE-SHAPE (CAN BE < 0)
            RRMS = 0.6085          ; 100 * SQRT(sumsq/NOBS)
     R-FACTOR(%) = 94.955          ; 100 * (1.0-sumsq/totalsumsq)
ABSOLUTE SCALING = 0.100000E+01    ; ORIGINAL/OBS SCALING FACTOR
  SCALING FACTOR = 0.518939E+03    ; OBS/CALC SCALING FACTOR
     Total RRMS  = 0.6640          ; TOTAL RRMS (no WEIGHTING)
        QM LINES = 7986            ; NO. OF QM LINES
       PEAK-TOPS = 1               ; NUMBER OF PEAK TOPS (see ASL)
         QM AREA = 0.417052E+04    ; AREA OF QM SYSTEM...
        OF THEOR.= 1.082           ; % CORRESPONDING TO (%)THEORETICAL QM AREA

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&INC C:\CHEMADDER\URINE\TRIGONELLINE.ASL           POPULATION=  0.01109  ***
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&INC C:\CHEMADDER\URINE\SERINE.ASL                 POPULATION=  0.00902  ***
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Part 3

1000 + 1 Spin-Particles Limits of *qNMR*

IECM
3rd International Electronic Conference on Metabolomics
15-30 November 2018
sponsors: MDPI metabolites

NEWS, Aug. 1st 2018

qQMSA of *1000+1* SpinParticles[1]

2012 Compounds[2] and >1500 nuclei[3]

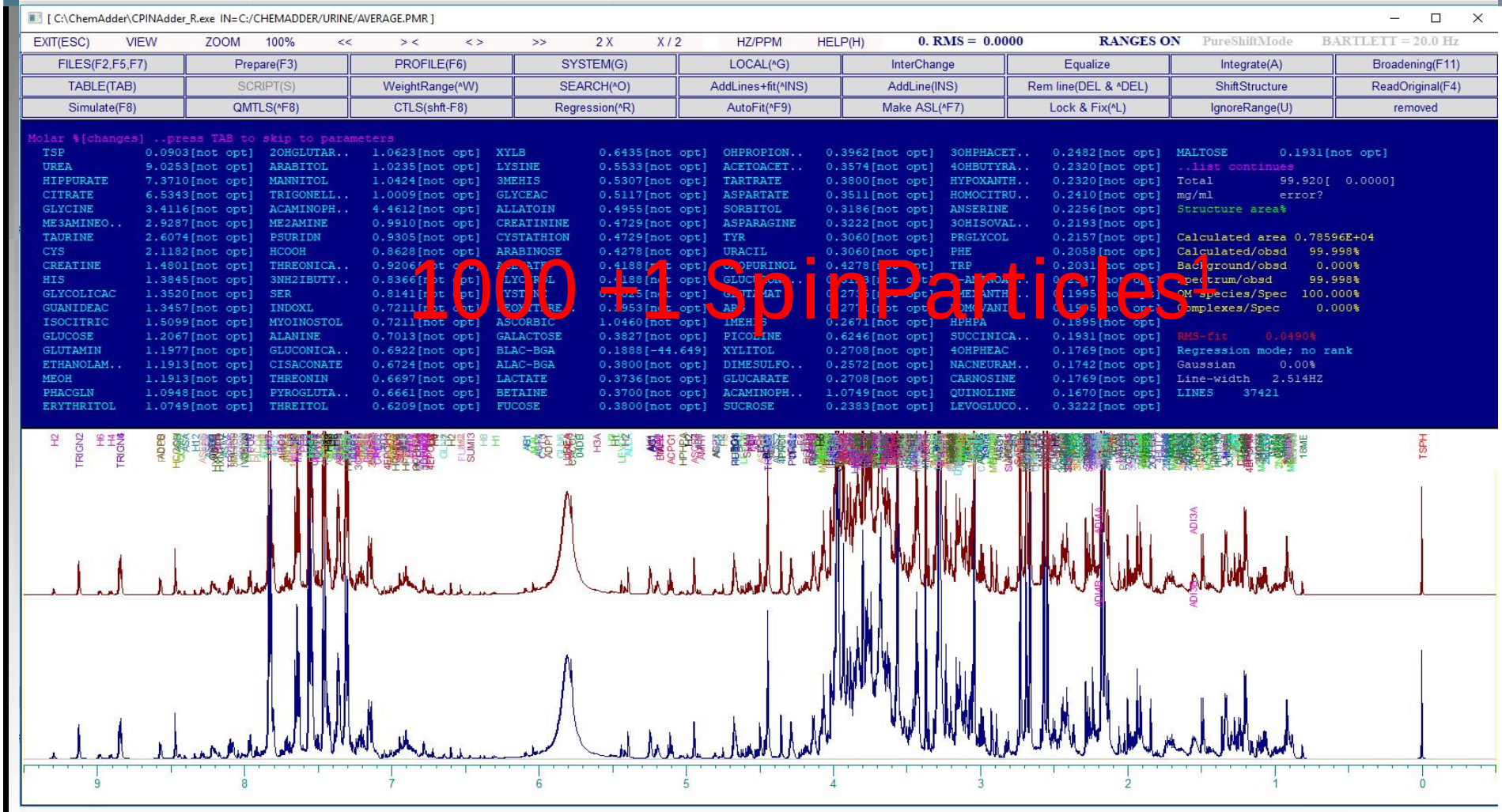
Simulation time < 2 sec/case[4] !

With *MULTITASKING* << 1 sec/case

Regression analysis of >500 (1000+1)-spectra/hour

See more at

http://www.chemadder.com/



The 1000+1 model system imitates human urine metabolome (Bouatra S. & al, PLoS ONE 8, e73076, 2013), spiced by some sugars and testosterone (= the largest spin-system, with 23 particles) – to get the 1000+1 shifts.

What is possible with the 1000+1 chemical shifts - *the limits of qNMR?*

In order to study what can be realistically expected from analyses of the 1000+1 case (or like urine) **we synthetized 600 MHz spectra with typical variations of trial chemical shifts, line-shapes and response factors:**

- With 1001 chemical shifts and 212 metabolites.
- The populations were those of average spectrum (*from PLoS ONE 8, e73076, 2013*), the spectral parameters were from different origins.
- 0.001 – 0.005 (at low field) ppm random variations (standard deviation) were added to trial the chemical shifts, 0.10 Hz variations to trial line-widths and 2% variations to response factors, but the couplings were assumed to be constant. See that the shift variations were 10 fold when compared those mentioned in slide 12.
- 0.05% random (white, from the TSP signal) noise was added to the synthetic spectrum.

Conclusions

- With an average line-width of 1 Hz, the spectrum shows 1195 peak-tops.
- The number of *90% purity peak-tops* (when 90% of the top intensity arises from one compound) are found only for 90 compounds. The number of the *75% purity tops* is 155.
- There are ca. 10 compounds for which the maximum purity is < 25% - these compounds can hardly be quantitated from the sample in their typical concentrations – without extra tricks.
- Without adjustment of shifts and line-shapes, the regression analysis gives the correct result with <10% criterion for 63 compounds, only!
- When the chemical shifts and line-widths were optimized, 154 metabolites (72%) satisfied the < 10% and 187 (88%) the < 20% criteria.
- *THE ANALYSES SUGGEST THAT ca. 150-190 METABOLITES of 212 CAN BE DETERMINED SATISFACTORILY FROM THE 1000+1 (or urine) 1D SPECTRUM, WITHOUT EXTRA TRICKS!!*
- *Total time of one analysis < 1 min.*

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Part 4

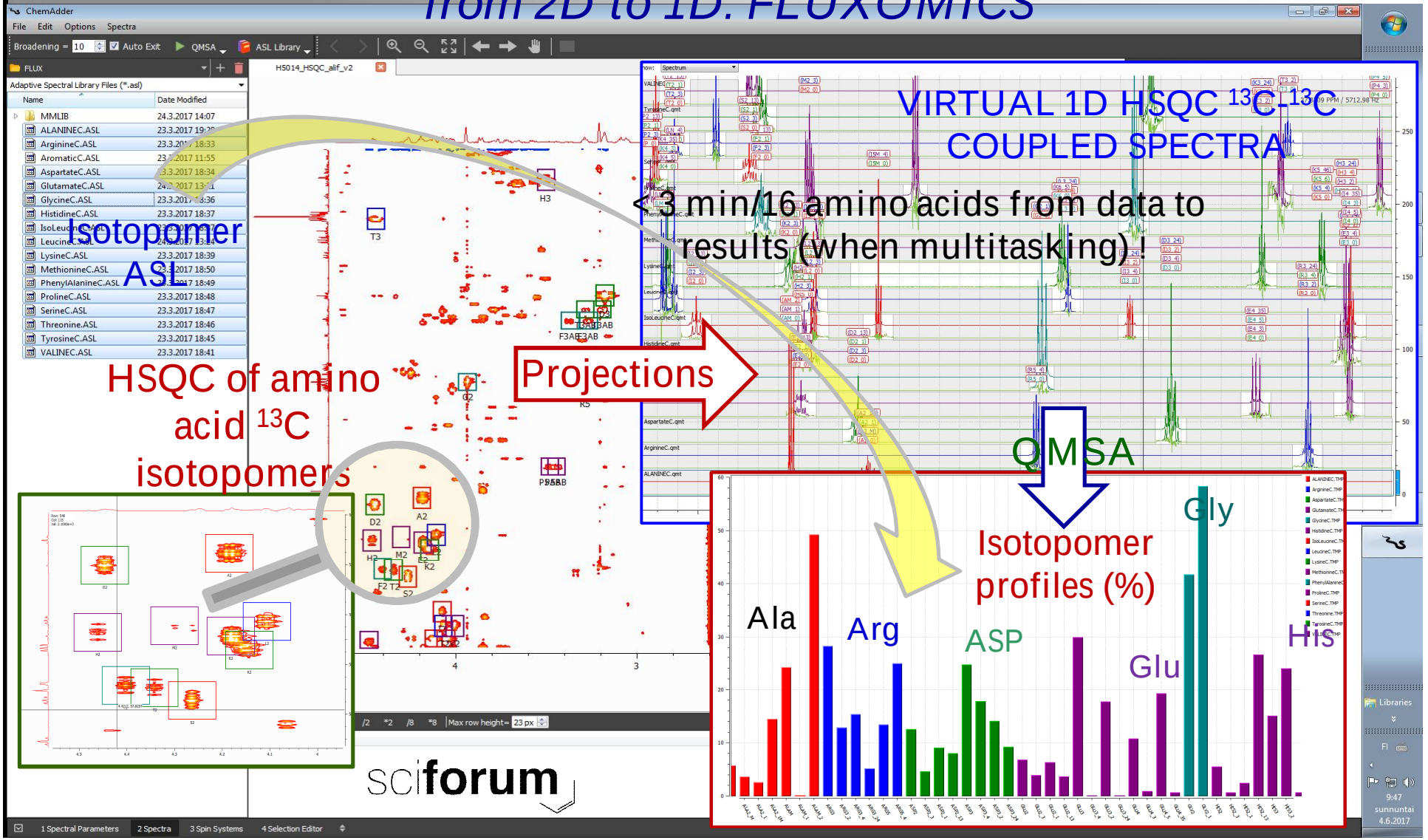
FLUXOMICS

VIRTUAL 1D ^{13}C-^{13}C COUPLED SPECTRA from of 2D HSQC to 1D spectra and to QMSA

3rd International Electronic Conference on Metabolomics
15-30 November 2018
IECM
VIRTUAL
sponsors: MDPI metabolites

1D HSQC ^{13}C-^{13}C COUPLED SPECTRA

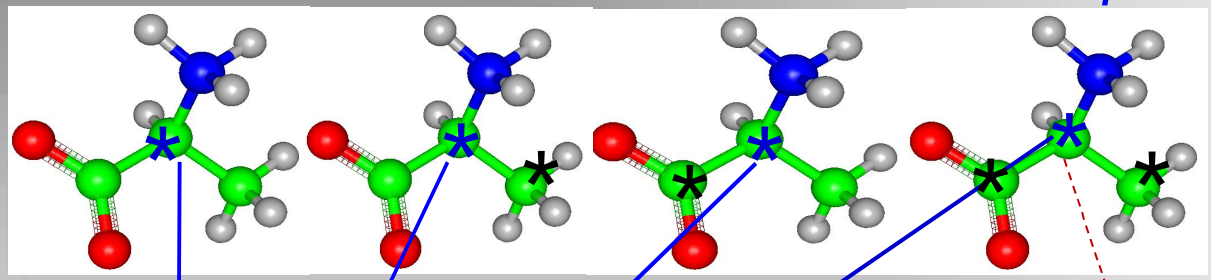
from 2D to 1D: FLUXOMICS

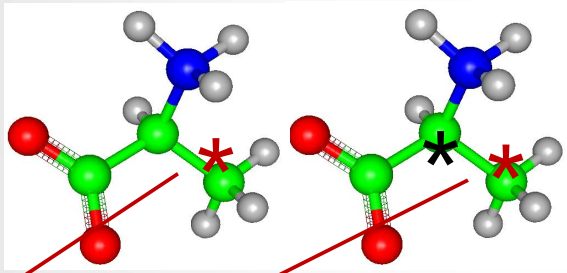


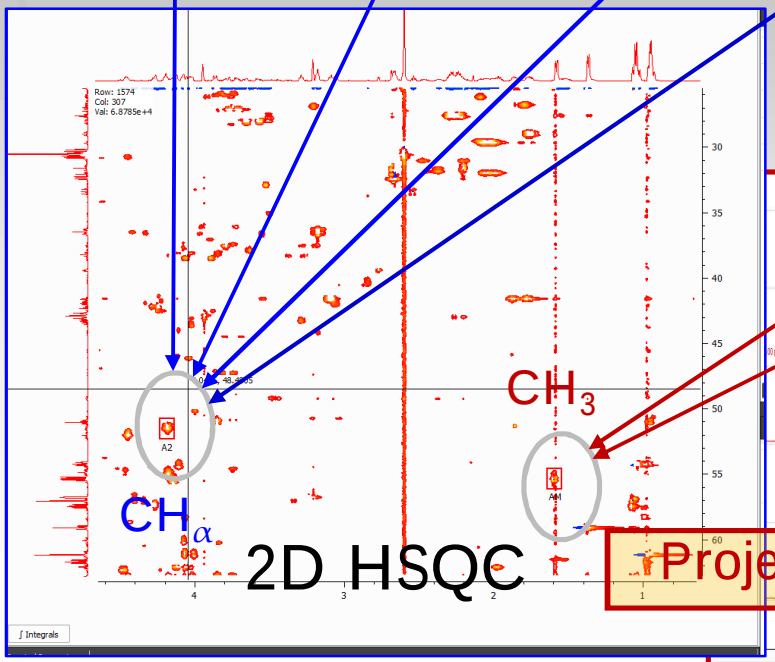
HSQC of amino acids

^{13}C isotopomer 2D spectrum to VIRTUAL 1D spectra: metabolic flux analysis

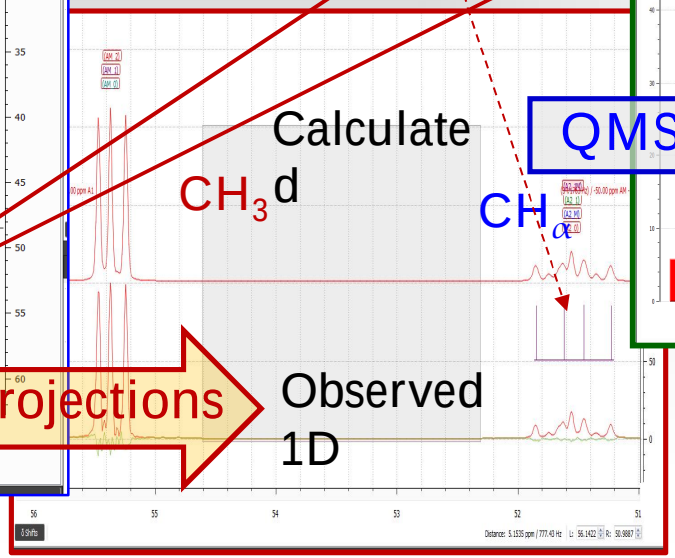
Alanine ^{13}C isotopomers:



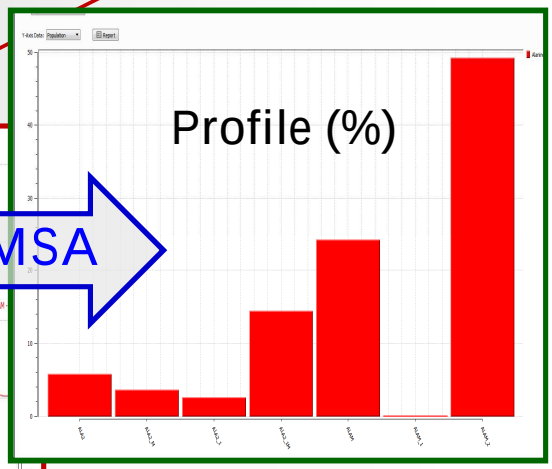




Projections



QMSA



ALANINE ^{13}C isotopomer ASL-file

```
SpinAdder2017.01 C:\CHEMADDER\ASLIBS\FLUX\ALANINEC.ASL
TIME: 23.03.2017  19:28:52

&CONTROL PARAMETERS & FIT INFO:
        ORIGINAL = ND              ; TYPE = DX, JDX, QMT, OBS, ASL(=PMR), HMD, SDB
        SPECTRUM = C:\CHEMADDER\ASLIBS\FLUX\ALANINEC.QMT ; ND => (RE)READ ORIGINAL!
         PROFILE = C:\CHEMADDER\ASLIBS\FLUX\HSQC_PROFILE.TXT ; OPTIONS/ADDER PROFILE
           FIELD = 150.85400571     ; FOR 1H in MHZ, USED TO TRANSFORM SHIFTS TO HZ
POINT RESOLUTION = 1.37374375       ; DATA-POINT-RESOLUTION (HZ)
      LINE WIDTH = 5.098            ; 0.0 = USE SPECIES DEFAULT (HZ)
        GAUSSIAN = 75.076           ; GAUSSIAN % IN LINE-SHAPE (CAN BE >100%)
            RRMS = 0.4896           ; % FROM MAX. INTENSITY
        QM LINES = 14               ; NO. OF QM LINES

&CHEMICAL SHIFTS(PPM):
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 A2_0    / 1    51.555737  1*1*1  STAT=Y  PRED= 51.5557 RANGE= 0.1000 WIDTH(Y)= 6.875 RESP(N)= 1.0000 SDEV= 0.000005 LOCAL= 11.905 HSQC= A_H2
ALA2_M  2*SPIN= 1 SPECIES=13C POPULATION(Y)=  0.036609[OBS=  0.320168] MWGT=   89.09 SLOPE=  1.0000 ROI= A2
 A2_M    / 2    51.552856  1*1*1  STAT=Y  PRED= 51.5529 RANGE= 0.1000 WIDTH(Y)= 7.641 RESP(N)= 1.0000 SDEV= 0.000005 LOCAL= 11.832 HSQC= A_H2
 AM      / 2   -50.000000  1*1*1  STAT=N
ALA2_1  2*SPIN= 1 SPECIES=13C POPULATION(Y)=  0.027389[OBS=  0.010799] MWGT=   89.09 SLOPE=  1.0000 ROI= A2
 A2_1    / 3    51.546642  1*1*1  STAT=Y  PRED= 51.5466 RANGE= 0.1000 WIDTH(Y)= 7.661 RESP(N)= 1.0000 SDEV= 0.000006 LOCAL= 16.540 HSQC= A_H2
 A1      / 3   150.000000  1*1*1  STAT=N
ALA2_1M  2*SPIN= 1 SPECIES=13C POPULATION(Y)=  0.141620[OBS=  0.004400] MWGT=   89.09 SLOPE=  1.0000 ROI= A2
 A2_1M   / 4    51.542381  1*1*1  STAT=Y  PRED= 51.5424 RANGE= 0.1000 WIDTH(Y)= 7.893 RESP(N)= 1.0000 SDEV= 0.000005 LOCAL= 18.397 HSQC= A_H2
 AM      / 4   -50.000000  1*1*1  STAT=N
 A1      / 4   150.000000  1*1*1  STAT=N
*
ALAM  2*SPIN= 1 SPECIES=13C POPULATION(Y)=  0.241187[OBS=  0.123688] MWGT=   89.09 SLOPE=  1.0000 ROI= AM
 AM_0    / 5    55.366291  1*1*1  STAT=Y  PRED= 55.3663 RANGE= 0.1000 WIDTH(Y)= 4.758 RESP(N)= 1.0000 SDEV= 0.000001 LOCAL= 9.973 HSQC= A_ME
ALAM_1  2*SPIN= 1 SPECIES=13C POPULATION(Y)=  0.004035[OBS=  0.047995] MWGT=   89.09 SLOPE=  1.0000 ROI= AM
 AM_1    / 6    55.363174  1*1*1  STAT=Y  PRED= 55.3632 RANGE= 0.1000 WIDTH(N)= 5.103 RESP(N)= 1.0000 SDEV= 0.000001 LOCAL= 9.845 HSQC= A_ME
 A1      / 6   150.000000  1*1*1  STAT=N
ALAM_2  2*SPIN= 1 SPECIES=13C POPULATION(Y)=  0.491644[OBS=  0.069093] MWGT=   89.09 SLOPE=  1.0000 ROI= AM
 AM_2    / 7    55.353104  1*1*1  STAT=Y  PRED= 55.3531 RANGE= 0.1000 WIDTH(Y)= 5.013 RESP(N)= 1.0000 SDEV= 0.000001 LOCAL= 10.290 HSQC= A_ME
 A2      / 7   100.000000  1*1*1  STAT=N

&COUPLING CONSTANTS:
ALA2_M
 J_A2M       34.1763   J A2_M     AM         STAT=Y  PRED= 34.176 RANGE= 0.500 SDEV= 0.3698
ALA2_1
 J_A12       59.2220   J A2_1     A1         STAT=Y  PRED= 59.222 RANGE= 0.750 SDEV= 0.3698
ALA2_1M
 J_A2M       34.1763   J A2_1M    AM         STAT=Y  PRED= 34.176 RANGE= 0.500 SDEV= 0.3698
 J_A12       59.2220   J A2_1M    A1         STAT=Y  PRED= 59.222 RANGE= 0.750 SDEV= 0.3698
ALAM_1
 J_A1M       16.3594   J AM_1     A1         STAT=N  PRED= 16.359 RANGE= 0.350 SDEV= 0.3698
ALAM_2
 J_A2M       34.1763   J AM_2     A2         STAT=Y  PRED= 34.176 RANGE= 0.500 SDEV= 0.3698

&CONSTRAINTS
 GLOBAL: COUPLINGS
 IGNORE(PPM): 62.81548 to 56.122253
 ROI=AM    1.5920    0.1000   55.3600    1.5000  VOL=   55.498  TYPE=HSQC  FILE=C:\CHEMADDER\ASLIBS\FLUX\ALAME.QMT
 ROI=A2    4.1850    0.1000   51.5500    1.5000  VOL=   44.502  TYPE=HSQC  FILE=C:\CHEMADDER\ASLIBS\FLUX\ALA2.QMT

&ASL TEMPLATES AT:  150.854006 MHZ
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      1   7777.019531      0.991059    1    1
      2  ALA2_M
      2   7793.504395      0.492330    1    2
      3   7759.160645      0.488901    1    2
      3  ALA2_1
      4   7804.494629      0.473340    1    4
      5   7745.423340      0.476845    1    4
      4  ALA2_1M
      6   7820.979492      0.236949    1    6
      7   7786.635742      0.234082    1    6
      8   7761.908203      0.241858    1    6
      9   7727.564941      0.236420    1    6
      5  ALAM
     10   8351.244141      0.879902    1    9
      6  ALAM_1
```
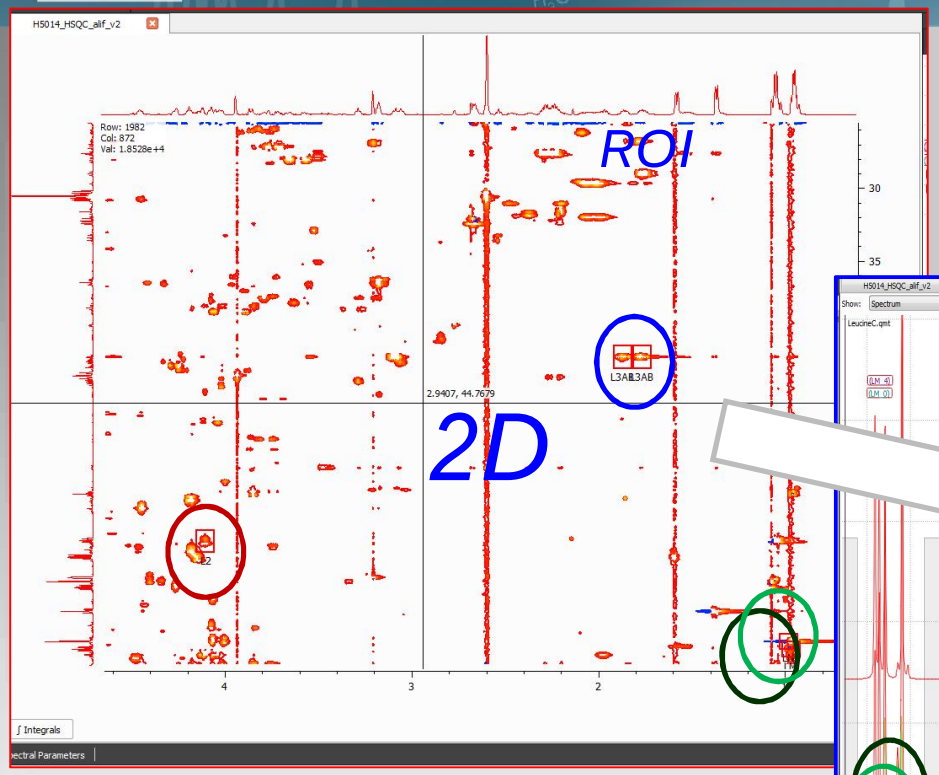
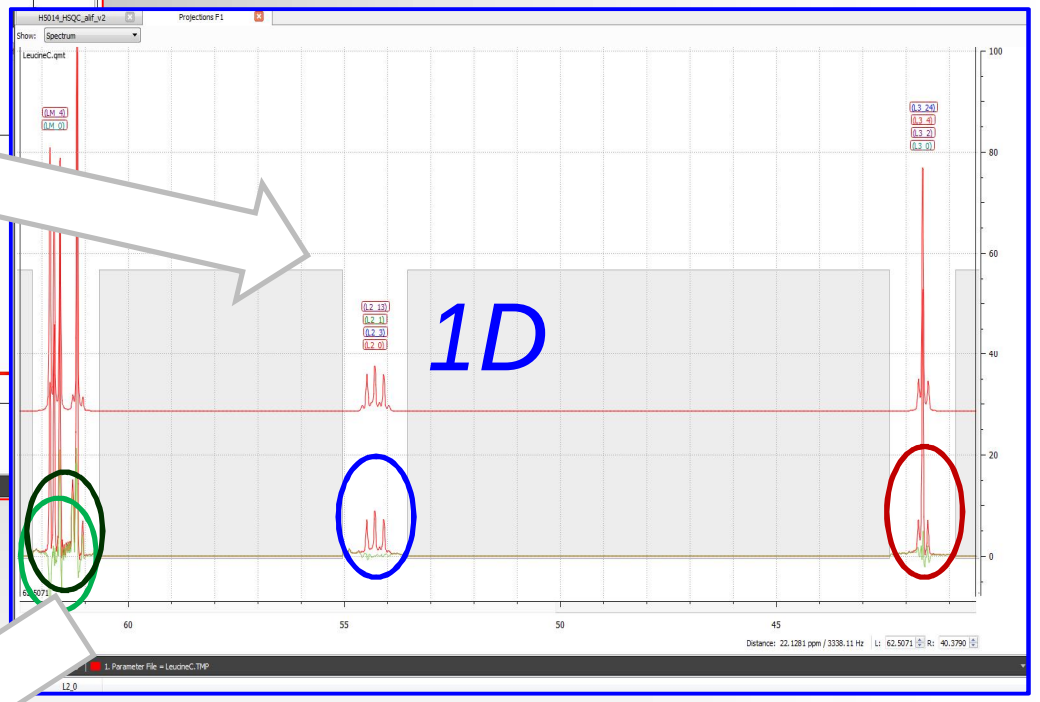

Shifts

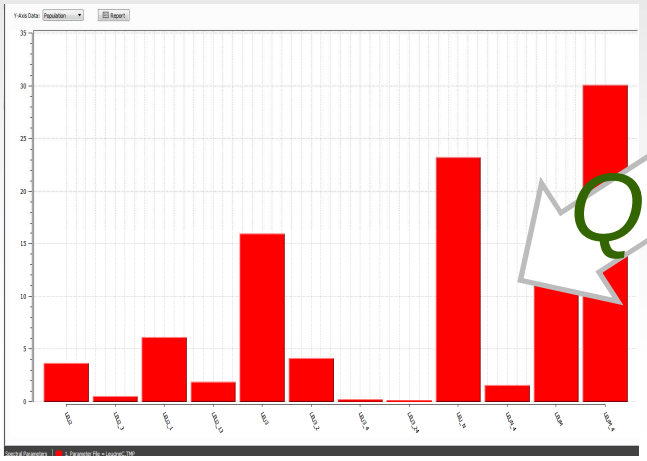
Couplings

ROI = Region of Interest

Leusine ^{13}C isotopomer spectra



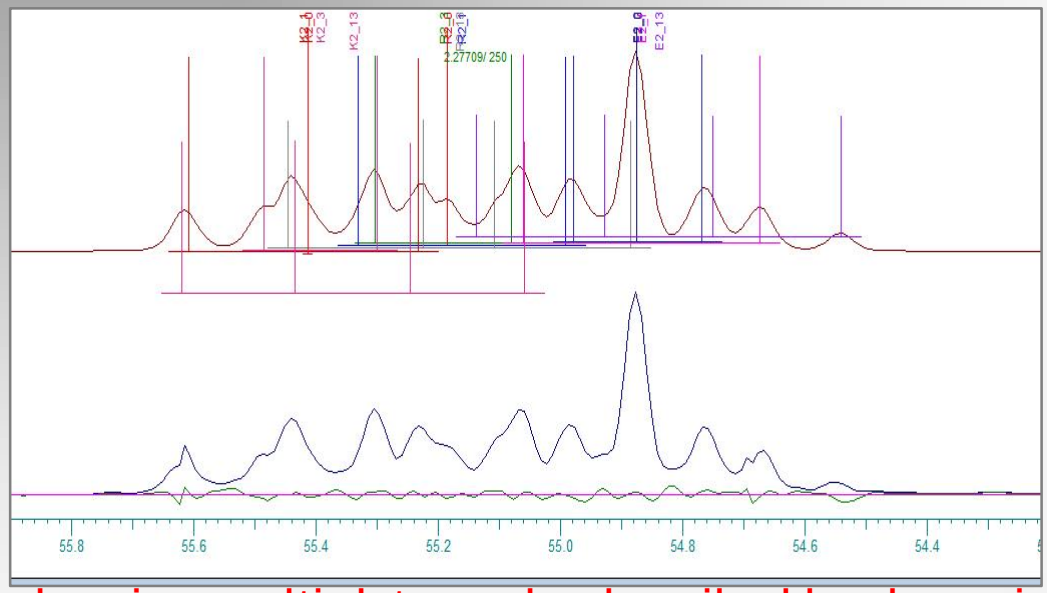




QMSA & Report

Analysis of overlapping signals

Arg, Lys, Glu and Leu ^{13}C-2 multiplets overlap with each others but they can (and must) be analysed together:



Unknown overlapping multiplets can be described by dummies! In this way more data are got to analysis !

The couplings and order of the C-2 shifts for the model are obtained from other signals, this fitting gives the isotopomer populations for the 2-carbons.

ChemAdder, jess!

LAOCOON GROUP, which gave the name for the early QMSA program

Part 5

ChemAdder & SpinAdder

The software

ChemAdder/SpinAdder 2018.11

- ChemAdder with Novel Qt technology & graphics and C++ support & SpinAdder with a new fast iterator supports now also Windows 10 platform !
- From FID to conclusions, reads now raw data and allows also batch processing !
- Graphics & data: Almost unlimited number of spectra can be treated simultaneously.
- Developed integration tools: Output in TXT or EXCEL format or directly as graphical presentation, in mg/ml or in mmol/ml ! Transfer of spectral display to pdf document (in svg format).
- qQMSA oriented platform for examination and interpretation of 1D and 2D spectra.
- Very large/tight spin systems, improved handling and faster.
- Targeted ASL's (*Adaptive Spectral Library*): metabolite libraries targeted for different sample types (serum, urine,..): one set of parameters – any field – any line-shape. In the HOLISTIC protocol sample type specific chemical shift variations are taken into account.
- ASL-format: all essential spectral information in one file. Preparation of ASL files from poor quality spectra (with bad baseline, impurities and solvent suppression artefacts) or even from peak lists.
- Smart shift permutator for complex spectra where many multiplets occupy the same spectral range, or to check long-range couplings and their sign combinations.
- Fast essential metabolite search from ASL's using FZZY tool: takes advantage from multispectral data.
- Tailored protocols (*MENUs*) and default settings (*PROFILEs*) for sample types.
- Maximal information by combination of QM spectra, structures and prior knowledge (= information that can be written into form of linear equations) – even the smallest details of spectra can be taken into account.

The version 2018.11 is now available, freeware for academic research and teaching.

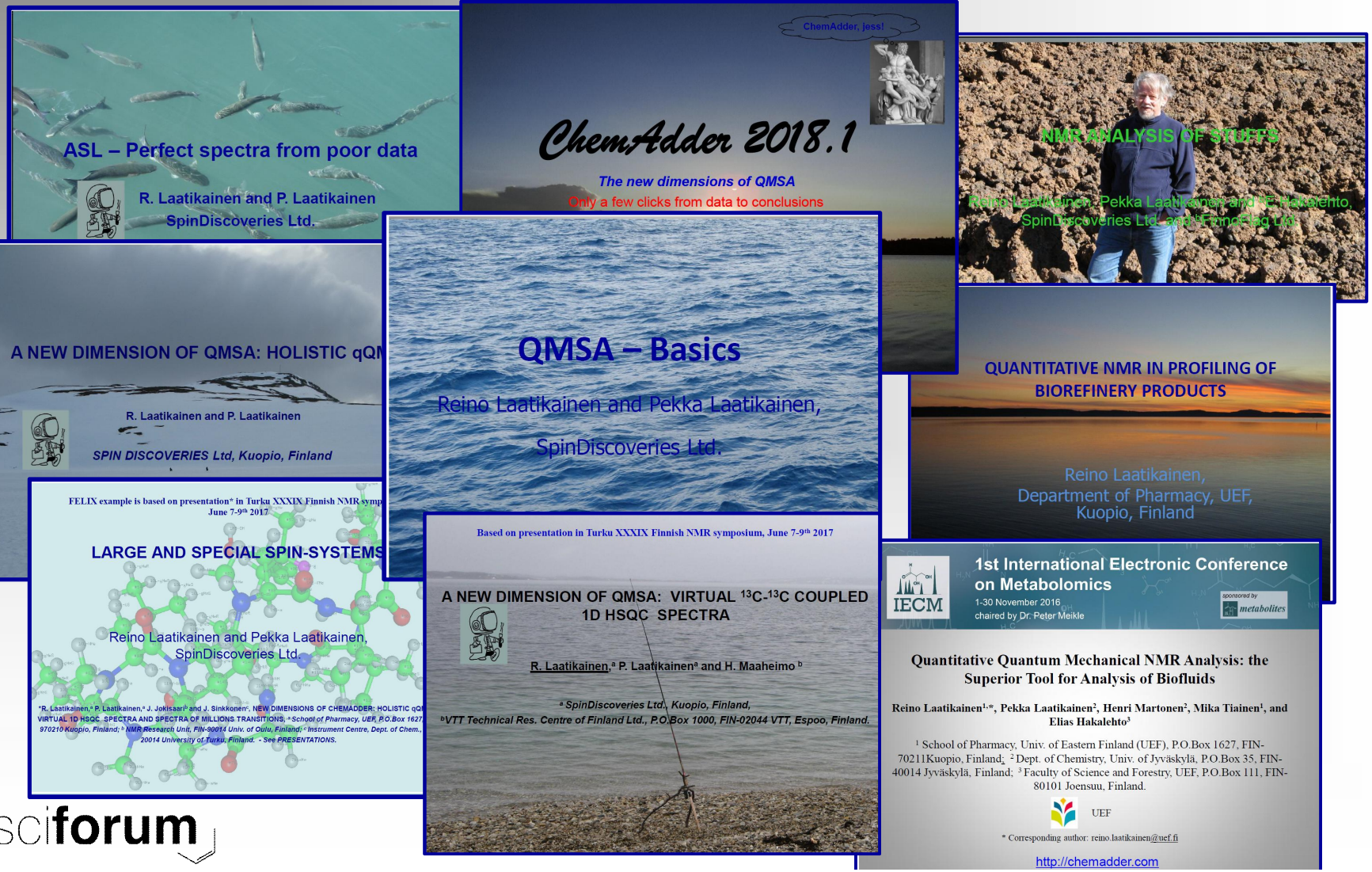
http://www.chemadder.com/

3rd International Electronic Conference on Metabolomics
15-30 November 2018

sponsors: MDPI metabolites

TEACH QMSA

Basics of QMSA and its applications have been collected to http://www.chemadder.com/ - ready for learning and teaching



sciforum

IECM

3rd International Electronic Conference on Metabolomics
15-30 November 2018

MANUALS

sponsors: MDPI metabolites

ChemAdder

Spin simulation of parameter files in multiple fields:
One observed spectrum - any field

ChemAdder – Kinetics Example*

Example of chemical reaction kinetics analysis with integration
(located at the programs subfolder "Examples\Kinetics")
*by Hannu Maaheimo, VTT

BASIC PROTOCOL FOR SPECTRAL ANALYSIS WITH CHEMADDER

version 2.0
June 8, 2018

www.chemadder

QUICK START GUIDE TO USING CHEMADDER FOR FLUX ANALYSIS

version 2.0
June 8, 2018

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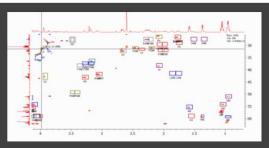


ROI (Region Of Interest) EDITOR From 2D to 1D

version 2.0
June 8, 2018

www.chemadder.

ChemAdder

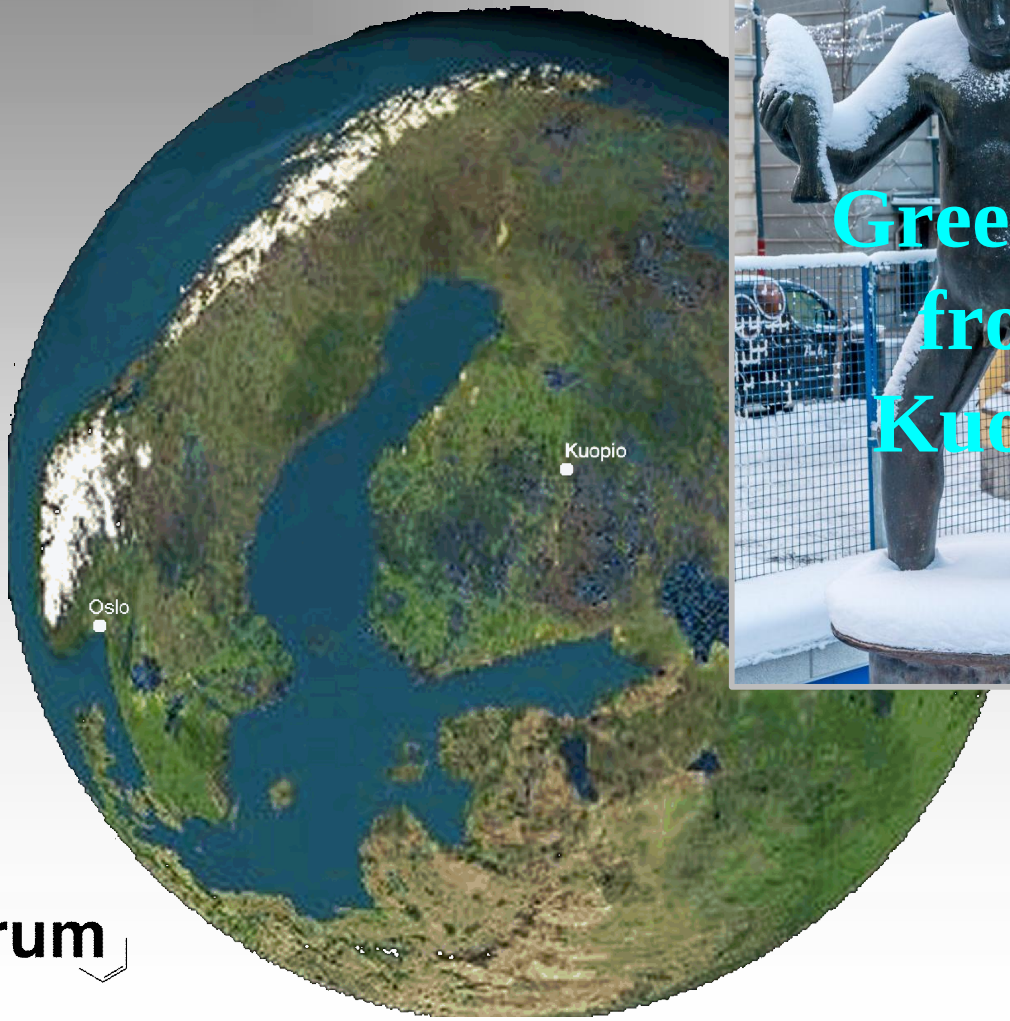
Converting Bruker data to JCamp
One click – all spectra

ChemAdder

Spin simulation of parameter files in multiple fields

sciforum

http://www.chemadder.com/


Kuopio
Oslo


Greetings
from
Kuopio