Atelier BEST/NUS
mercredi 14 octobre 2015

B. Brutscher / A. Favier / E. Lescop / N. Morellet

Programme de la journée

9h30-10h30: cours introduction
10h30-12h30: TP (groupe 1) / TD (groupe 2)

12h30-14h00: déjeuner

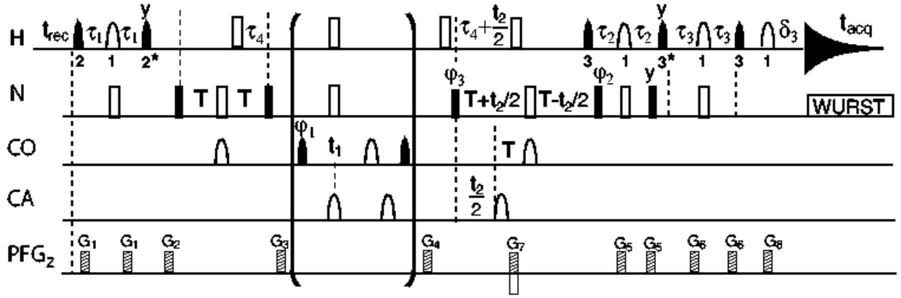
14h00-15h00: TP (groupe 1) / TD (groupe 2)

15h00-15h15: pause

15h15-18h15: TP (groupe 2) / TD (groupe 1)

18h15-19h00: Discussion générale

Multidimensional NMR



Real world time scale

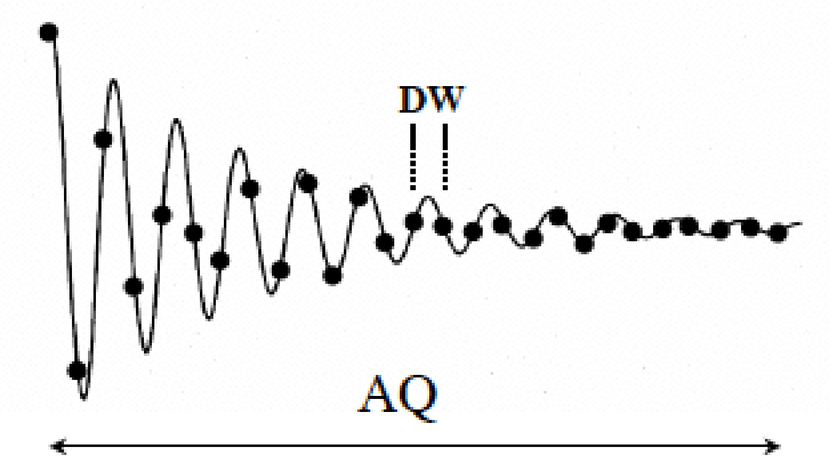
Recycling delay

~ 0.1 s

1 s

Data acquisition

Digital acquisition (discrete numbers)
Signal collected for given regularly spaced increments DW
(associated to spectral width SW)

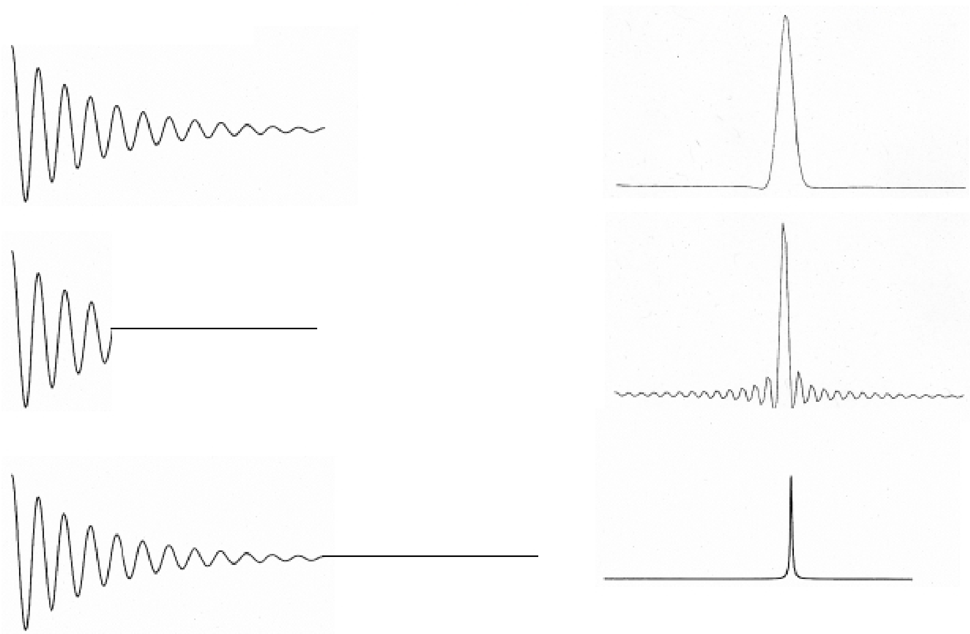


AQ=DW*TD
DW=1/(2*SW)
AQ=DW/(2*SW)

Resolution and acquisition time (aq)

Resolution increases when AQ is longer **AQ=TD/(2SW)**

(TD is not the only relevant parameter)



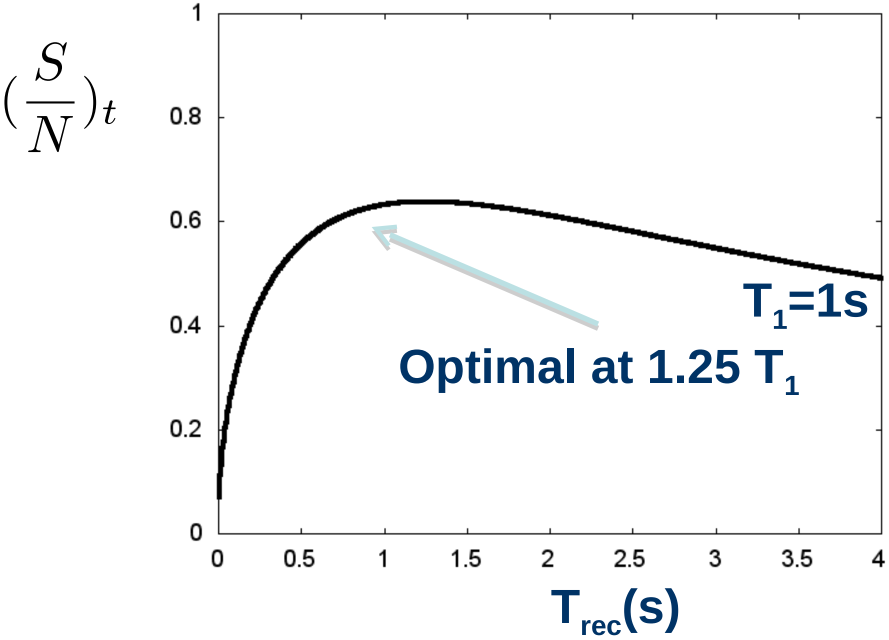
If AQ is too long, we collect noise and no signal:

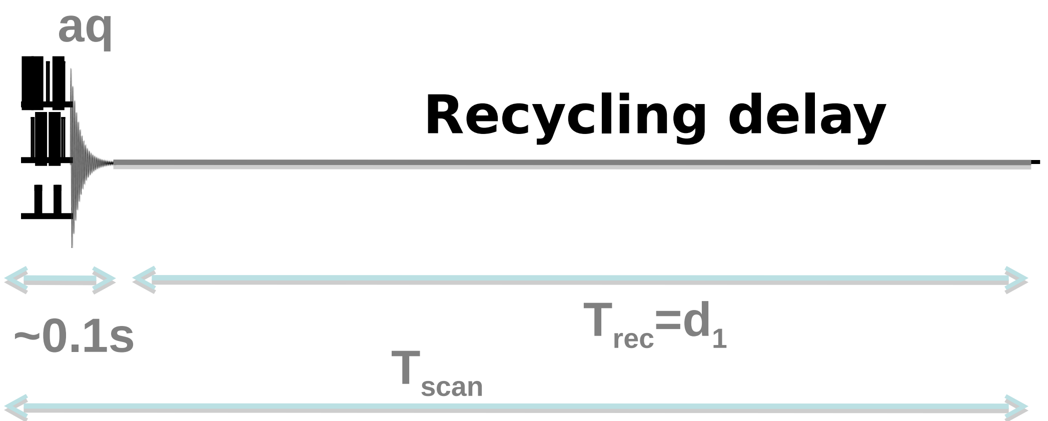
maximal resolution is achieved but we decrease the SNR

Rule of thumb: AQ~1.2 T_2

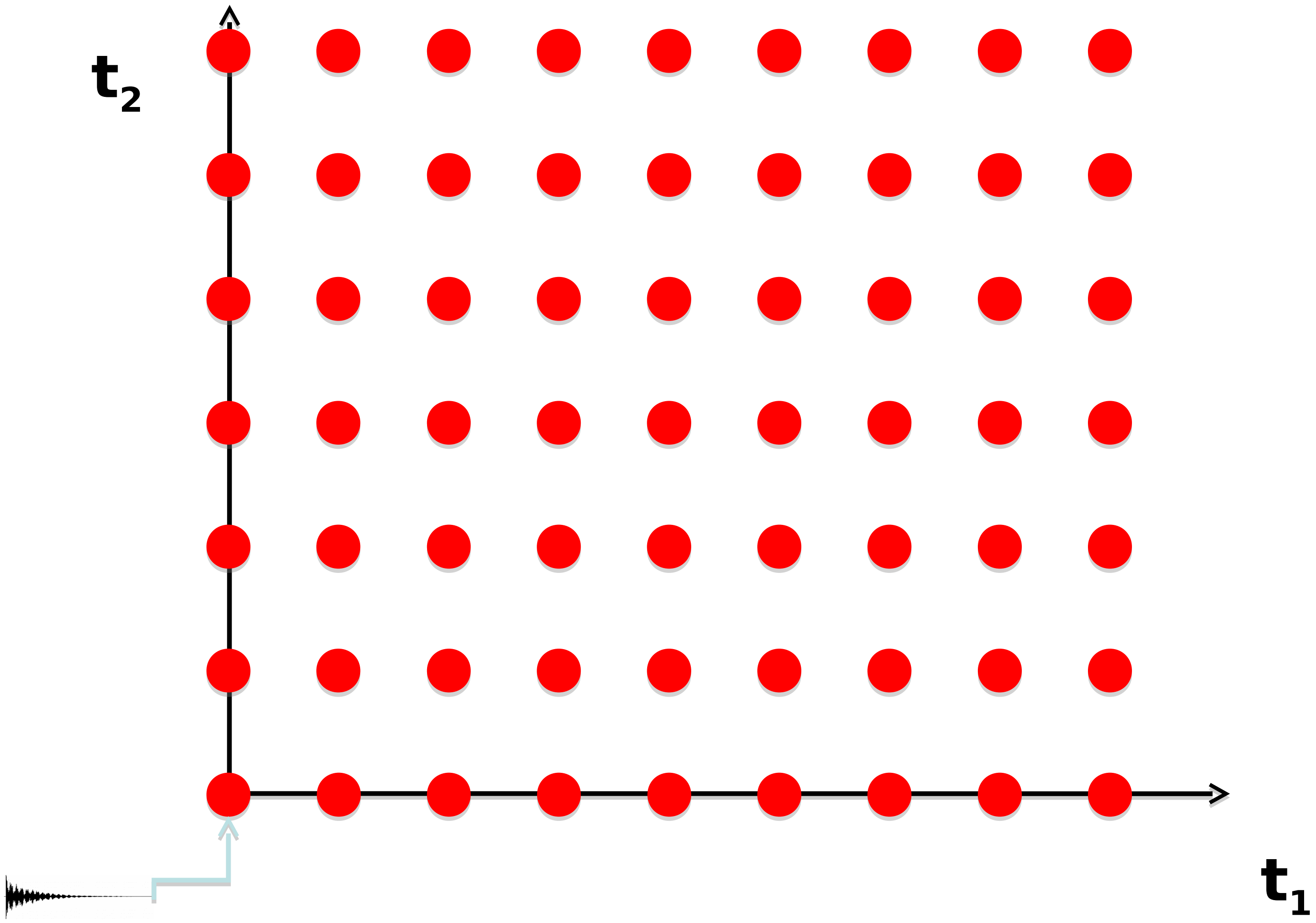
Optimal recycling delay

$$\left(\frac{S}{N}\right)_t = \frac{1 - e^{-\frac{t_{rec}}{T_1}}}{\sqrt{(T_{scan})}}$$





The sampling problem



Sampling indirect dimension takes time to get resolution

The sampling problem

1D	2D	3D	4D	5D
1s-1mn	10mn-1h	5h-72h	week-month	year?

Duration of nD spectrum follows an exponential growth

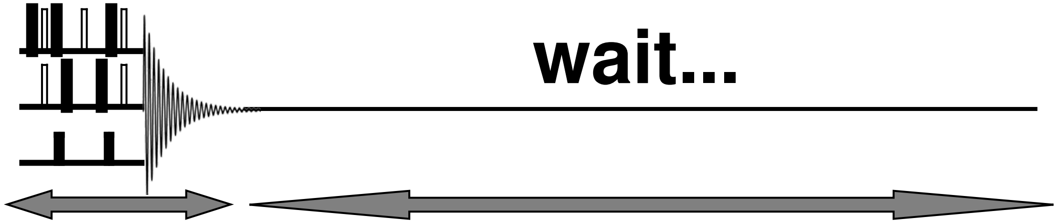
Problems:

- Spectrometer time usage
- Sample stability
- Fast processes not accessible

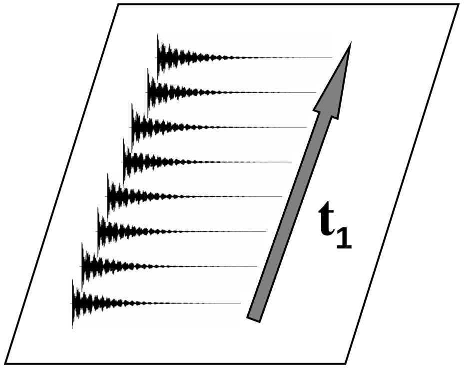
How to collect nD spectra faster?

➢ **Reduce interscan delay (fast pulsing)**

BEST/ SOFAST-HMQC



➢ **Reduce number of repetitions of pulse sequence**

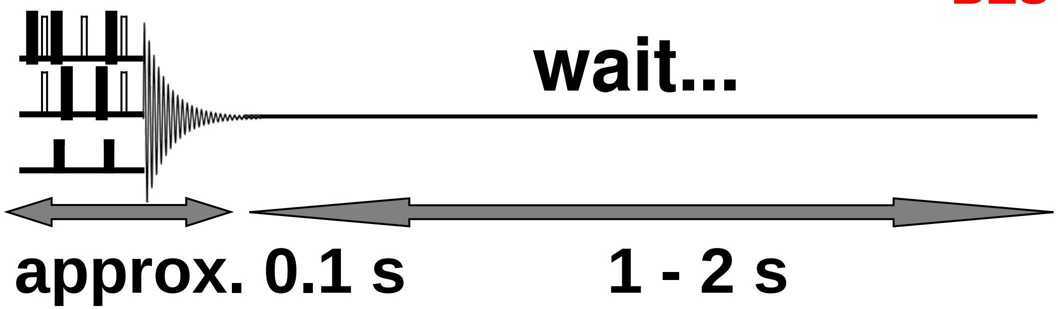


- ✔ **Hadamard spectroscopy (frequency domain)**
- ✔ **Spatial frequency encoding**
- ✔ **Reduced dimensionality / projection**
- ✔ **Spectral compression (extensive aliasing, ASCOM)**
- ✔ **Non-linear sampling methods (time domain)**

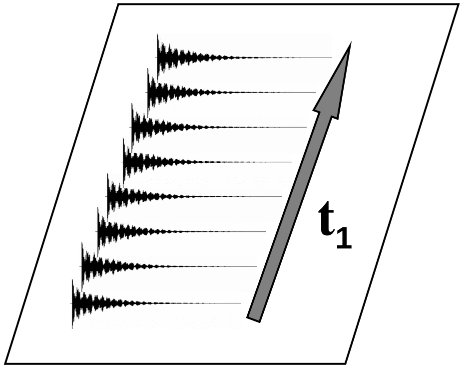
How to collect nD spectra faster?

➢ **Reduce interscan delay (fast pulsing)**

BEST/ SOFAST-HMQC



➢ **Reduce number of repetitions of pulse sequence**



- ✔ **Hadamard spectroscopy (frequency domain)**
- ✔ **Spatial frequency encoding**
- ✔ **Reduced dimensionality / projection**
- ✔ **Spectral compression (extensive aliasing, ASCOM)**
- ✔ **Non-linear sampling methods (NUS)**

ASCOM: Automated Spectral Compression

A simple tool to reduce the number of repetitions

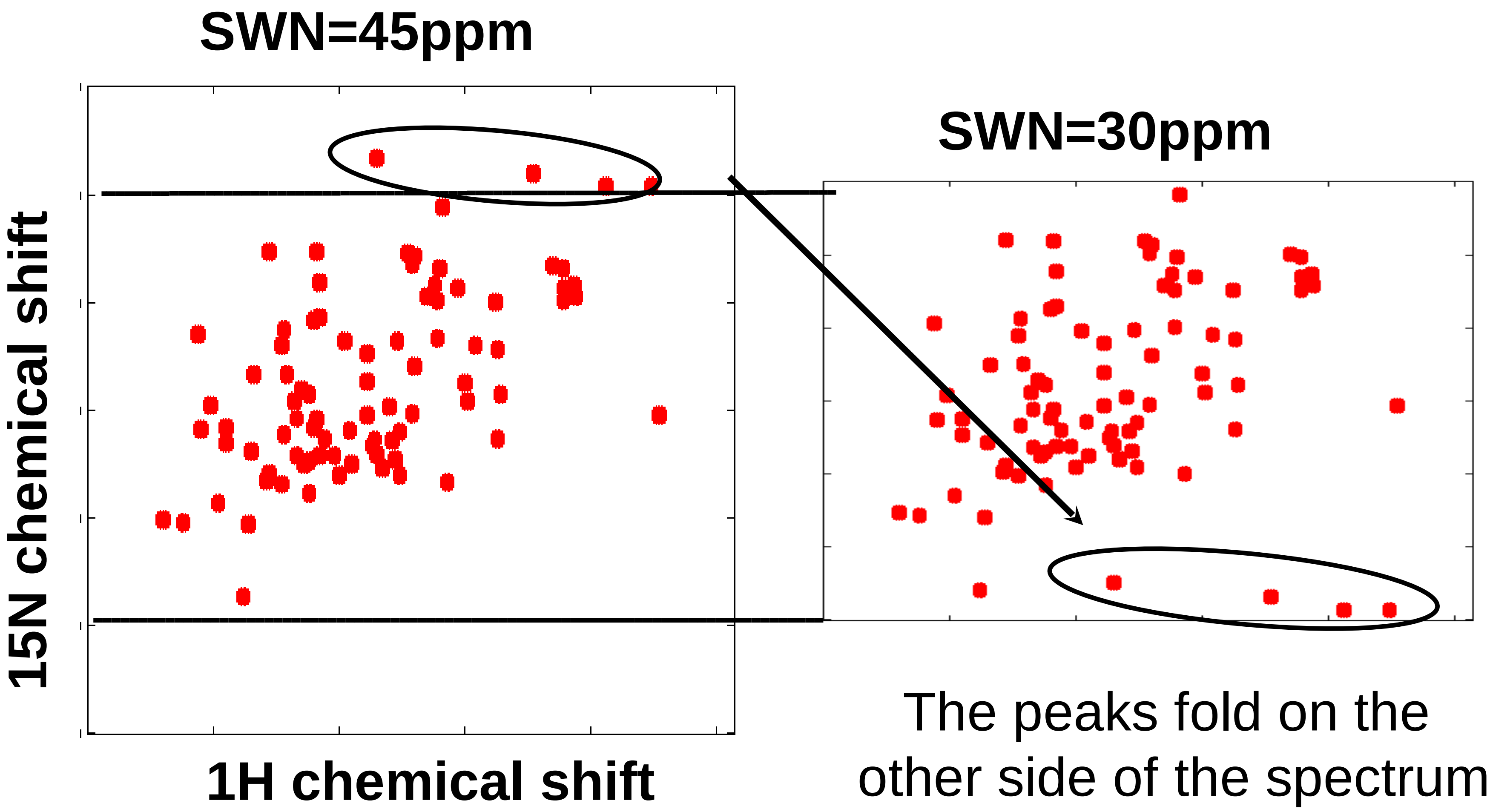
$$\text{Spectral resolution} \;\alpha\; \frac{\text{Number of repetitions}}{\text{Spectral width}}$$

Reduced spectral width => Reduced number of repetitions

*** Lescop, Schanda, Rasia, Brutscher, JACS*, 2007***

Aliasing in the case of complex data acquisition

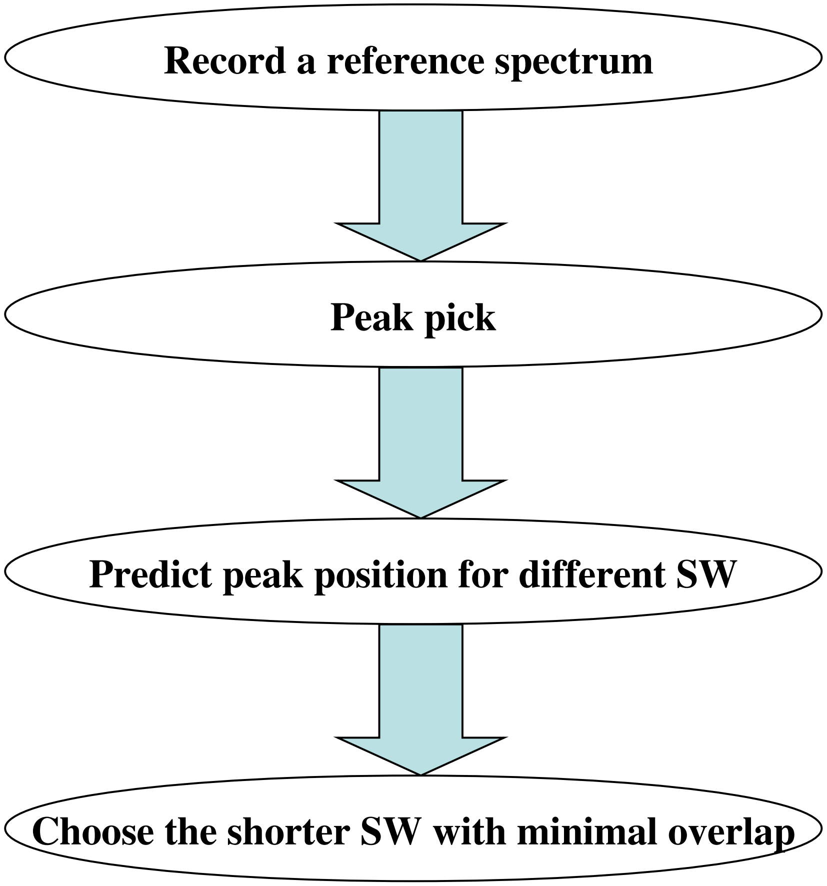
(States, States-TPPI or Echo-Antiecho mode)



The peaks fold on the other side of the spectrum

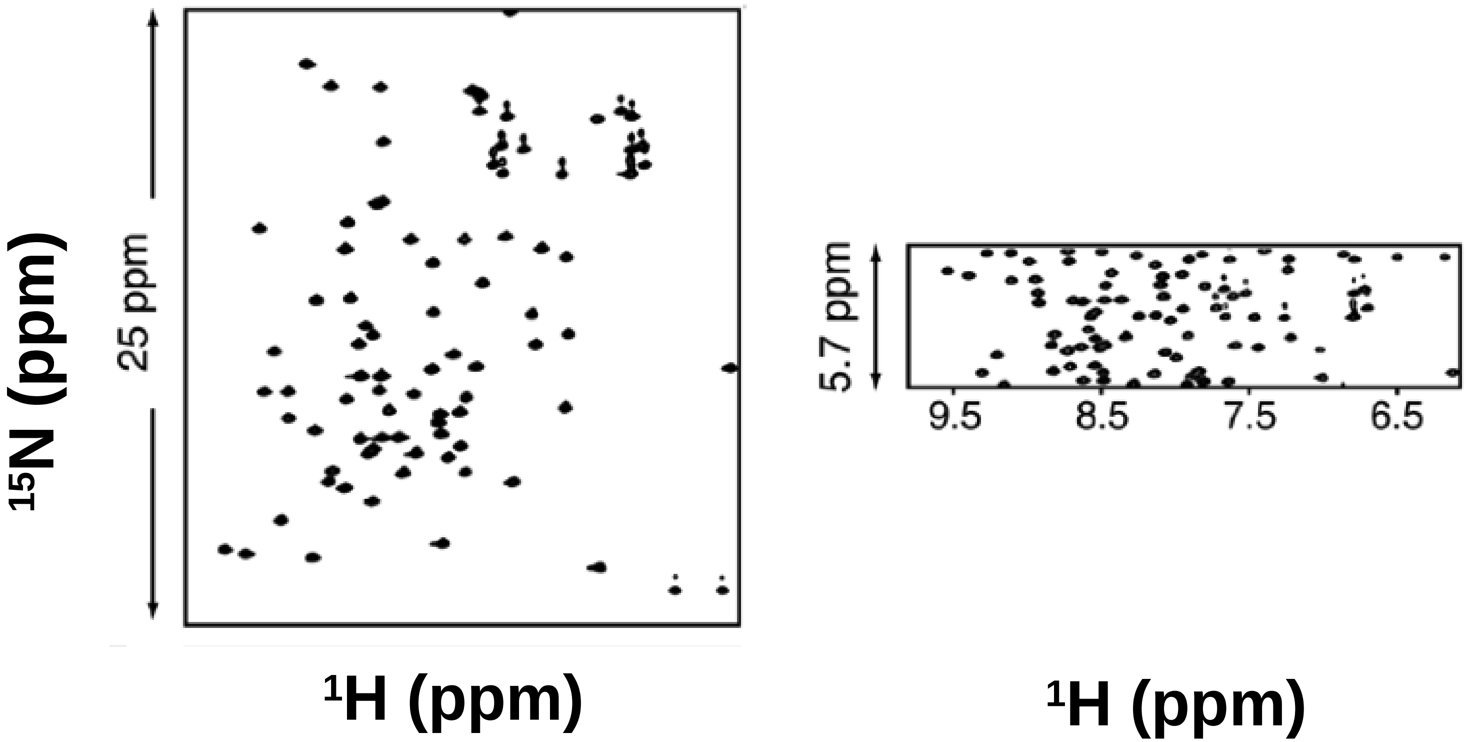
This is a translation by 30ppm

ASCOM flowchart



The ASCOM software does the calculation within seconds (http://www.icsn.cnrs-gif.fr/download/nmr)
Integrated in Topspin (see example in a few minutes)

ASCOM example

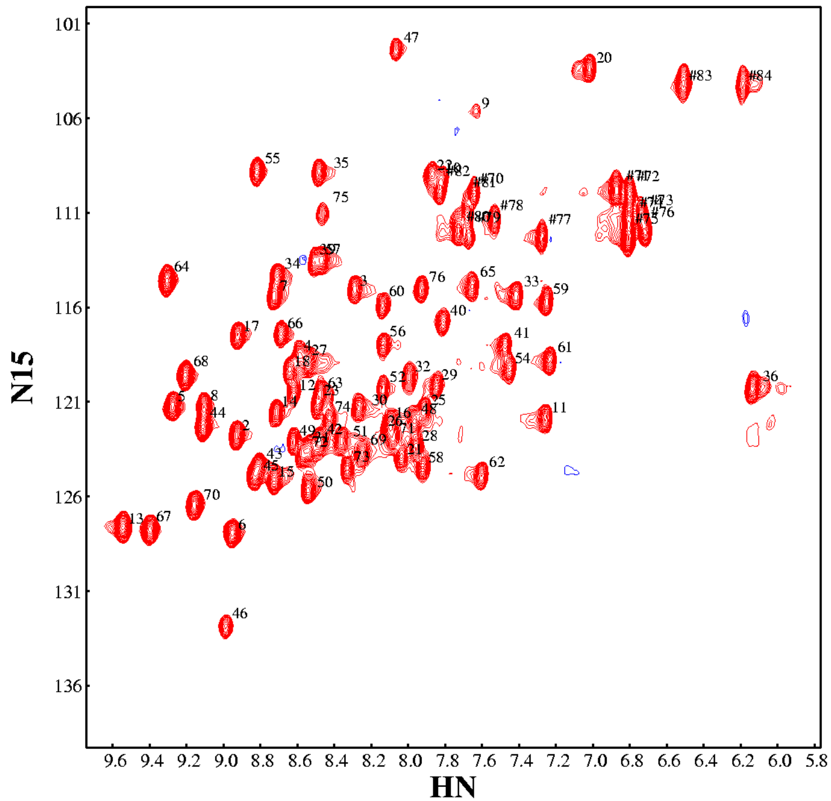


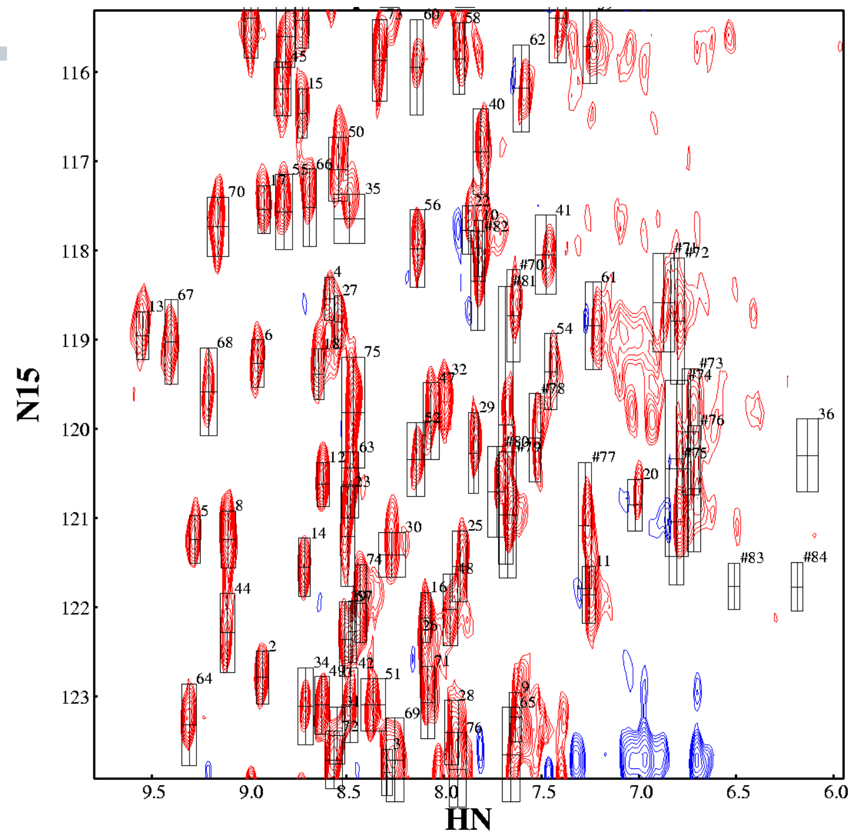
Advantages:

-Same content of information (resolution, dispersion)

-But faster (25/5.7= 4.4 times)

ASCOM example





Advantages:

-Same content of information (resolution, dispersion)

-But faster (25/5.7= 4.4 times)

ASCOM application

Use the ASCOM-optimized SW(^{15}N) to all other ^{1}H-^{15}N planes:
- 3D/4D triple resonance experiments
- ^{15}N relaxation experiments
- RDC measurements
- Kinetic experiment (H/D exchange, …)

Compatible with any pulsequence (BEST, …) but also with other sampling modes (cf NUS)

But: (1) any change in relative 1H-15N peak position (interaction, temperature, pH, …) requires a new optimization!

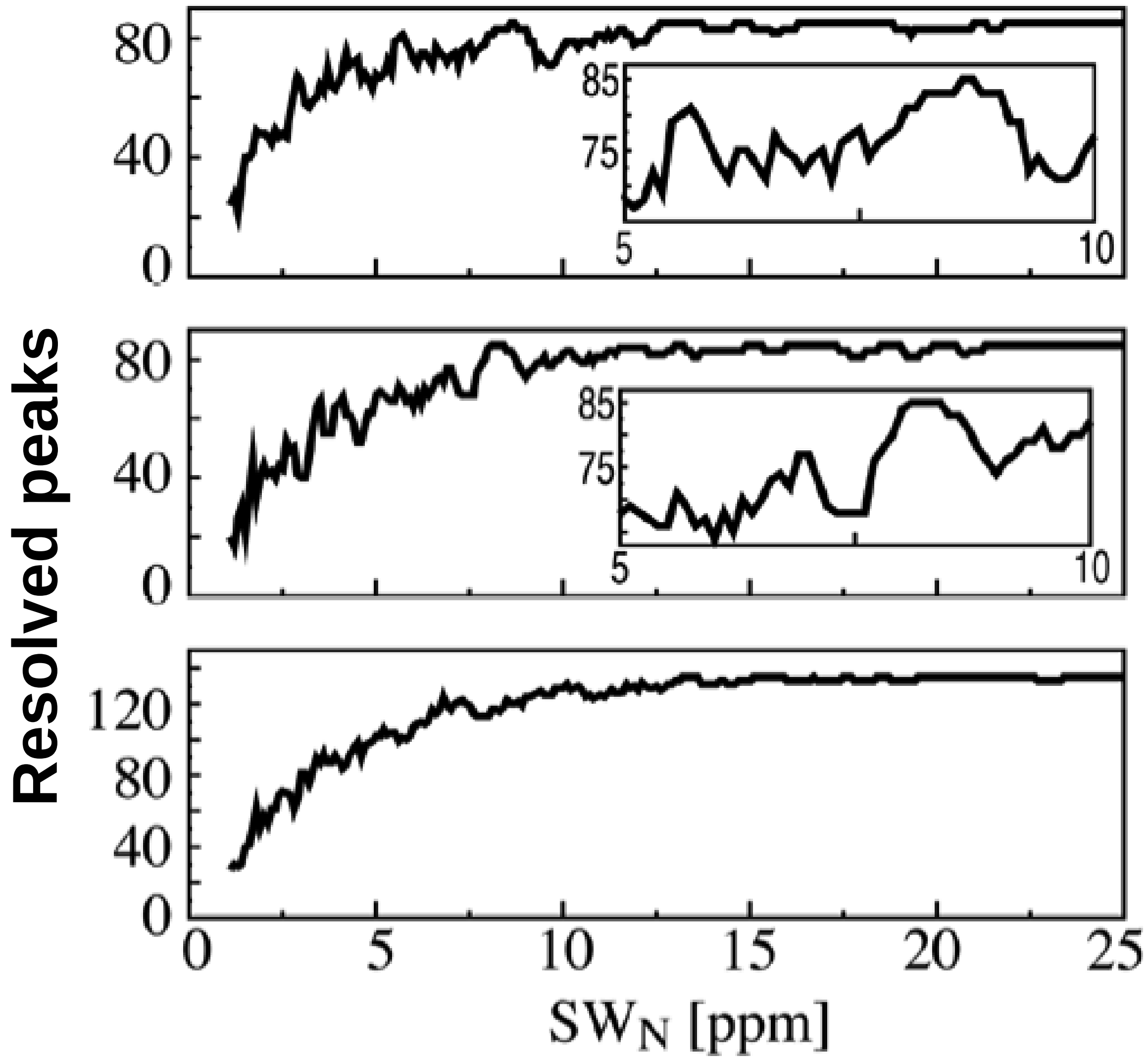
(2) Mostly useful for spectra with good signal-to-noise ratio initially because:
- the signal-to-noise also reduces (we accumulate less)
- useful only if NS=2 (otherwise keep NS=2 and use increased SW)

Approach limited to small-medium sized proteins

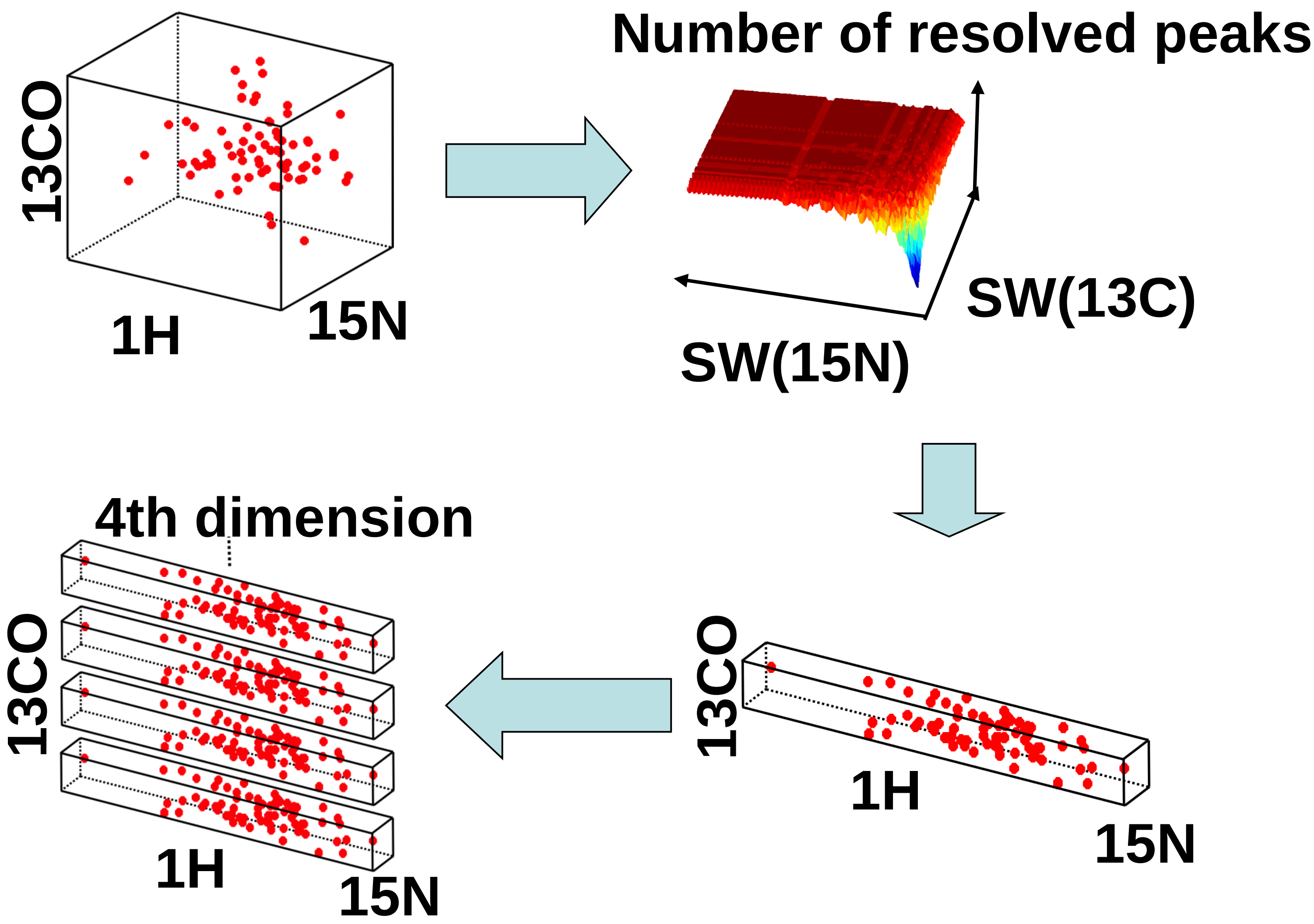
Ubiquitin
(76 a.a.)

Hyl-1
(103 a.a.)

Wzb
(147 a.a.)



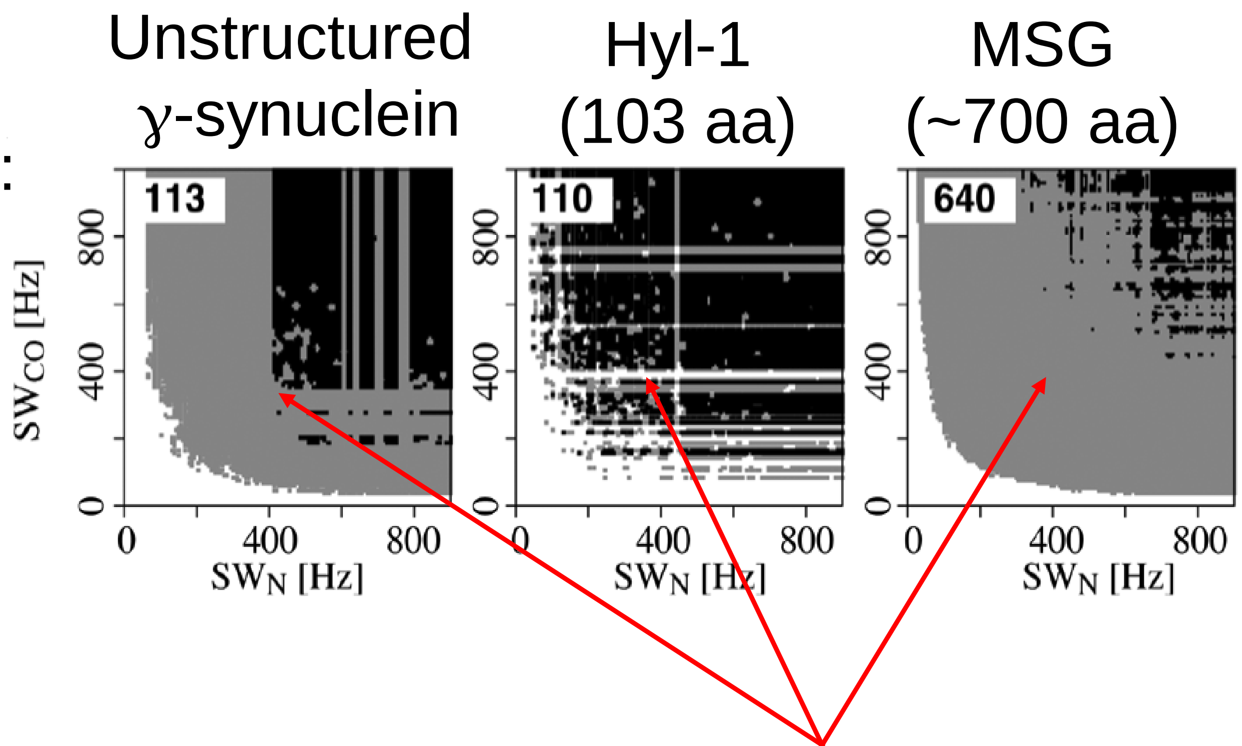
Application to 3D triple resonance experiments



Application to 3D triple resonance experiments

Combined optimization of:

SW(N)/SW(C)

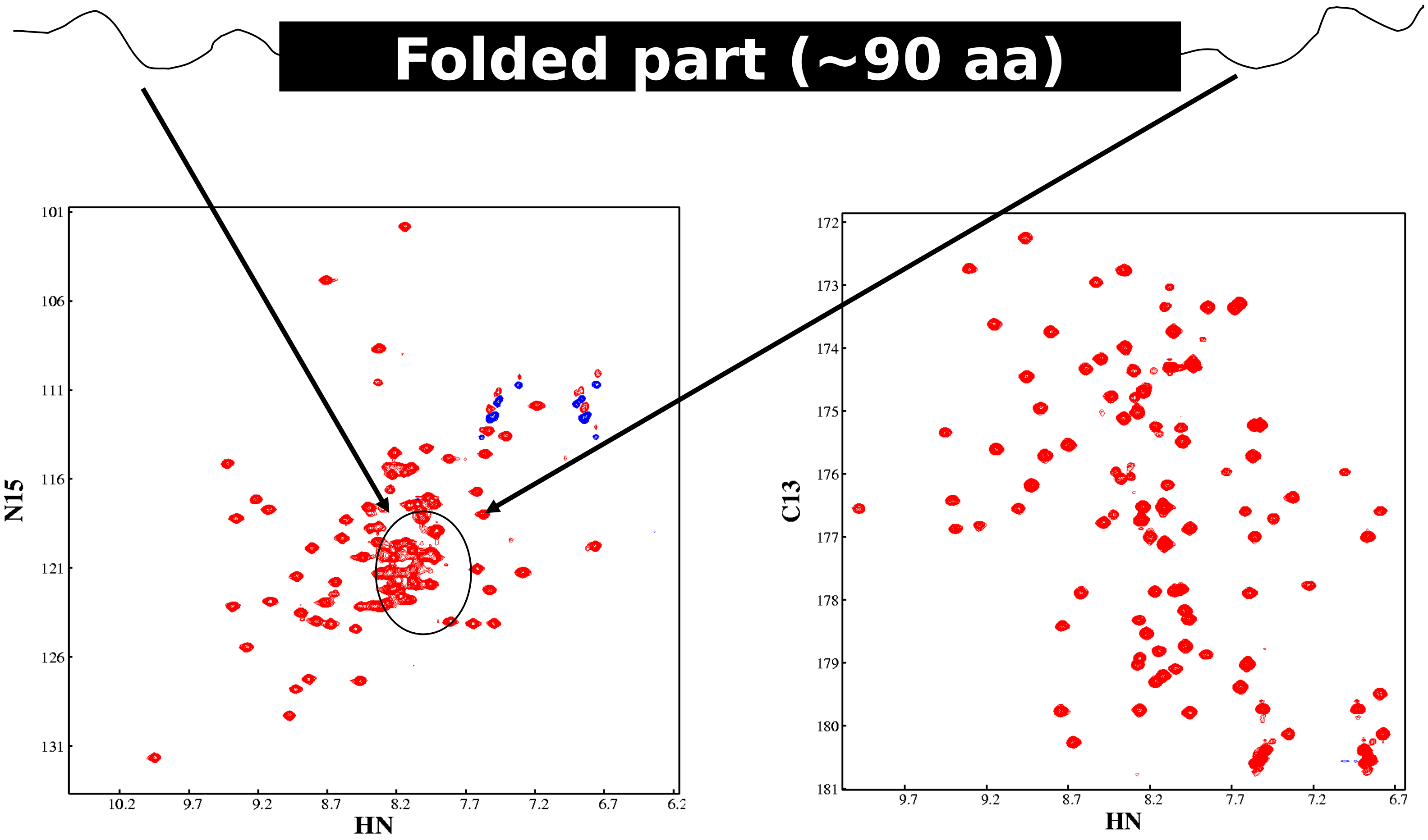


Black area:
Combination of $^{15}N/^{13}C$ spectral widths for which no additional overlapping occurs compared with the reference spectrum

SW(^{15}N)=6.6ppm / SW(^{13}C)=2.6ppm

Compared with SW(^{15}N)=30ppm / SW(^{13}C)=15ppm, the 3D experiment can be recorded 30 times faster

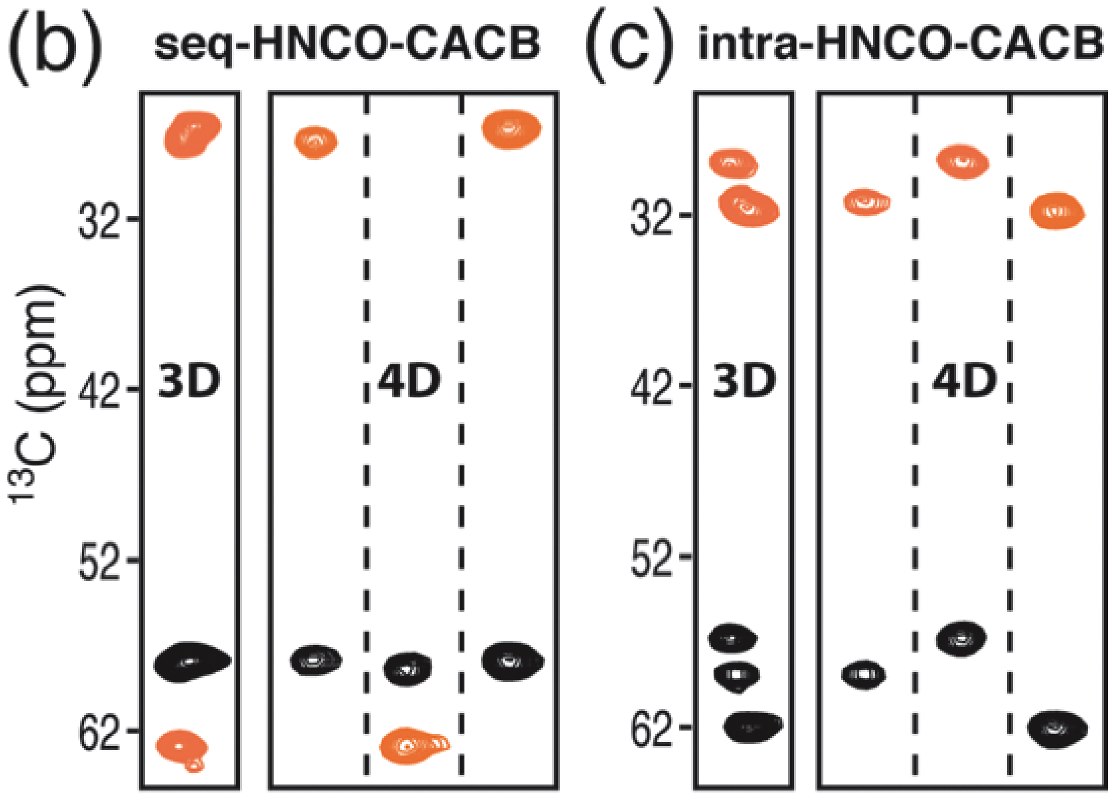
Application to Hyl-1



^{15}N HSQC

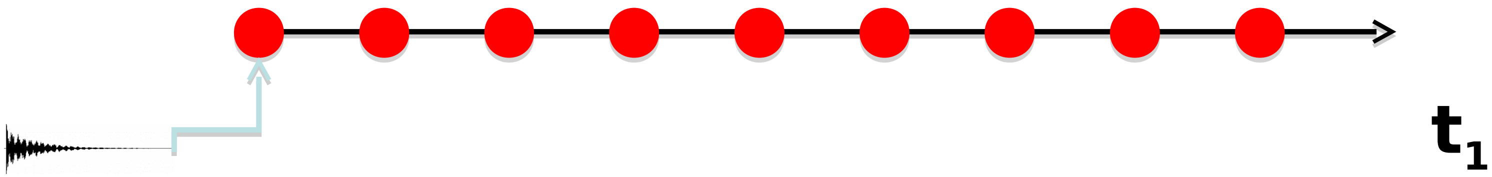
HN-CO plane

Application to Hyl-1



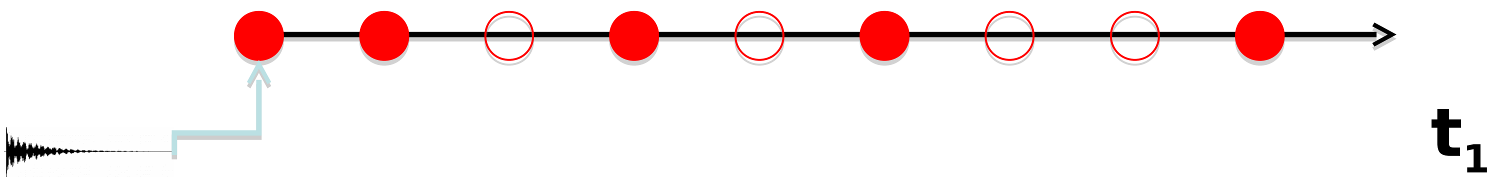
Significant help in backbone resonance assignment

Non Uniform Sampling (NUS): 2D



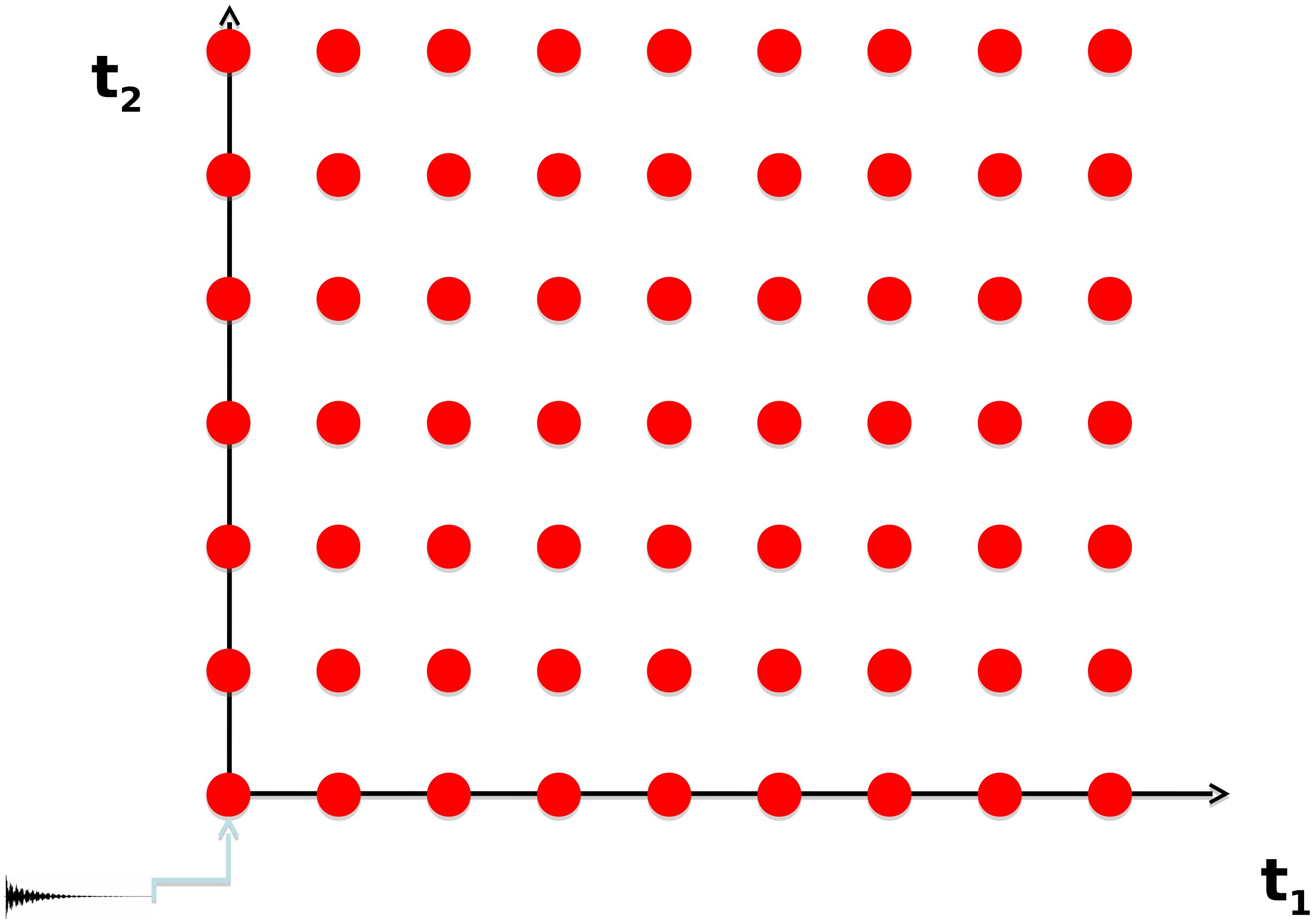
Instead of collecting all N points from t_1=0 to t_{1max}

We collect a subset N_{keep}<N

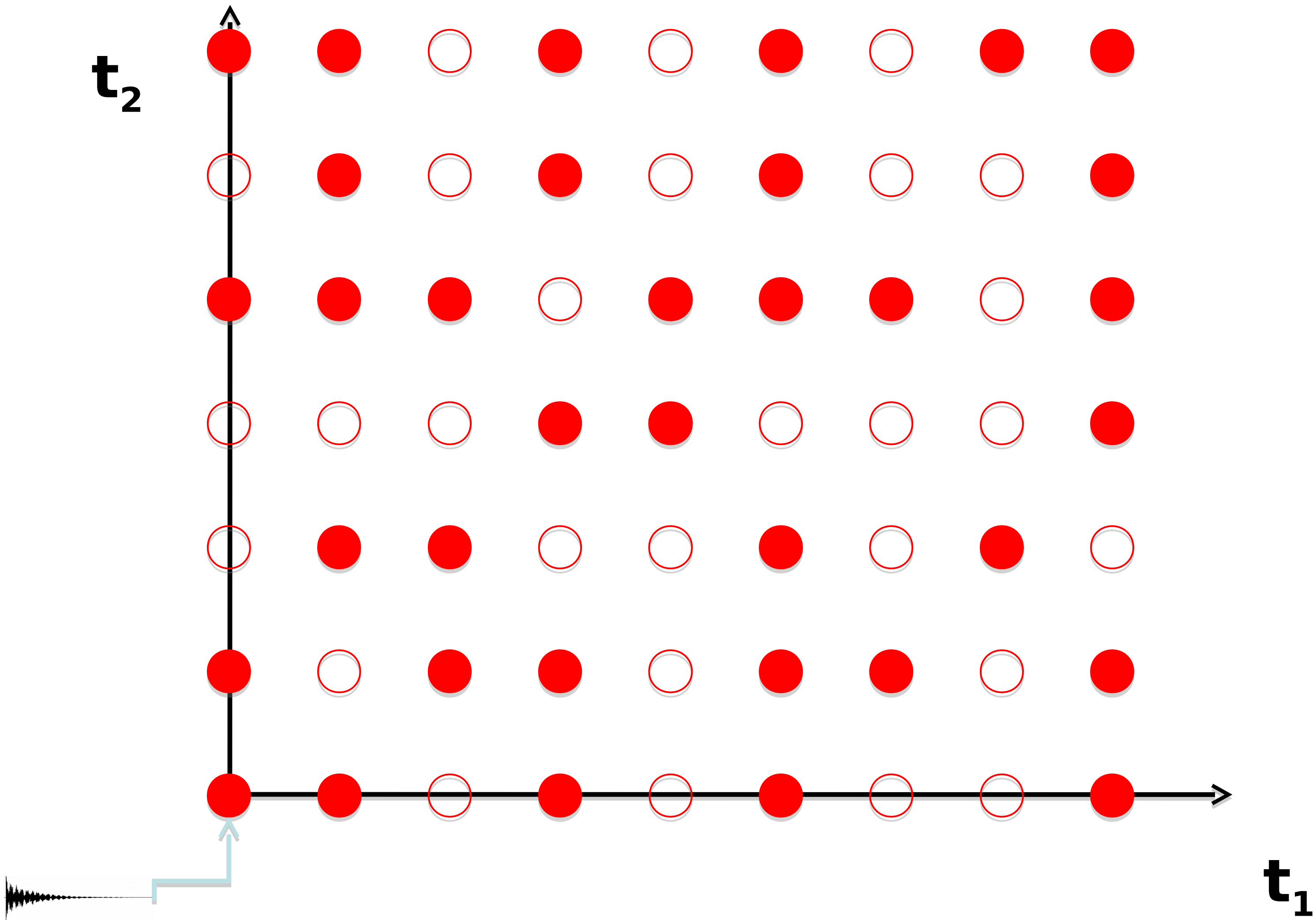


NUS percentage defined as N_{keep}/N

Non-linear Uniform Sampling (NUS): 3D

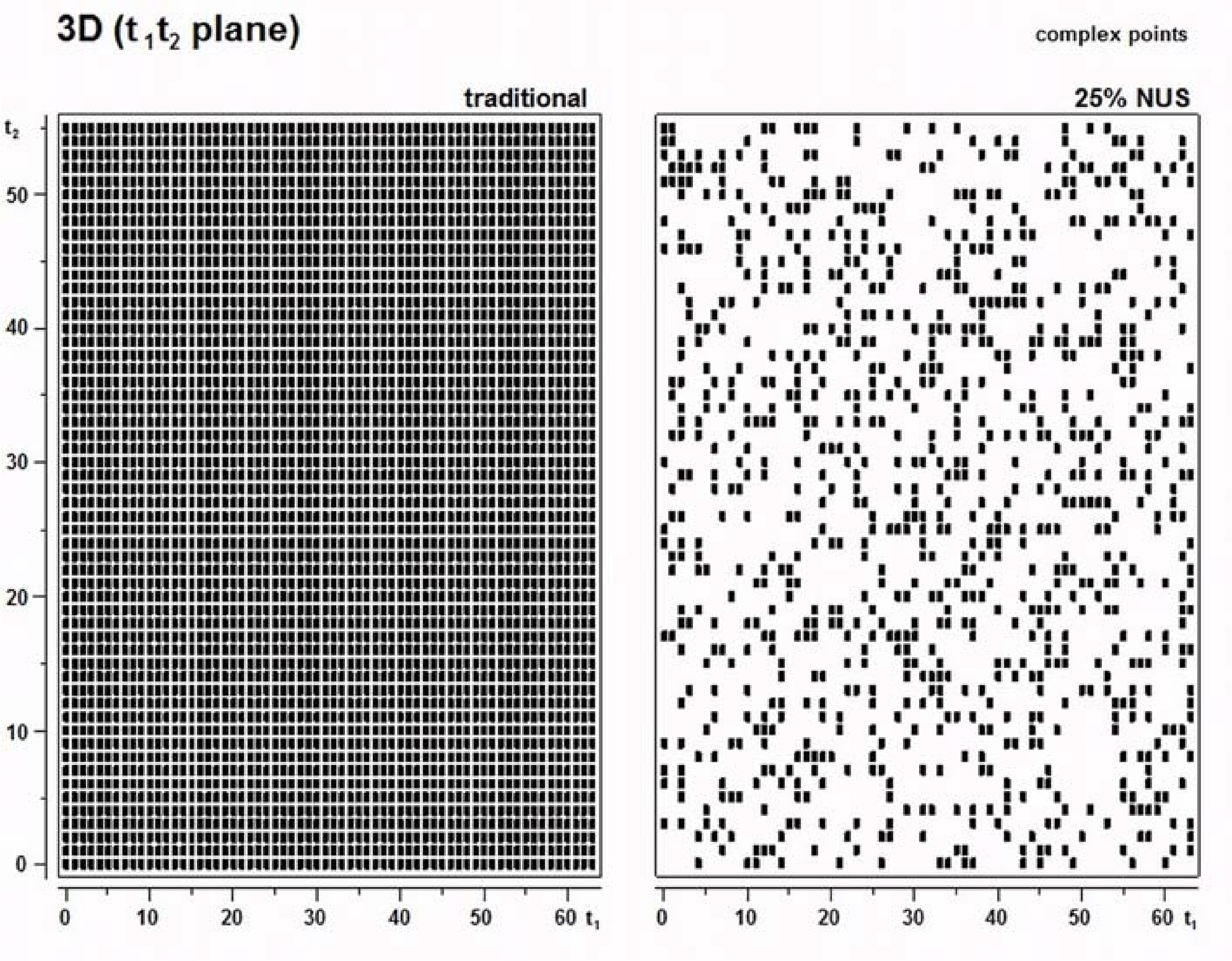


Non-linear Uniform Sampling (NUS) : 3D



Only a fraction of (t_1/t_2) pairs are collected

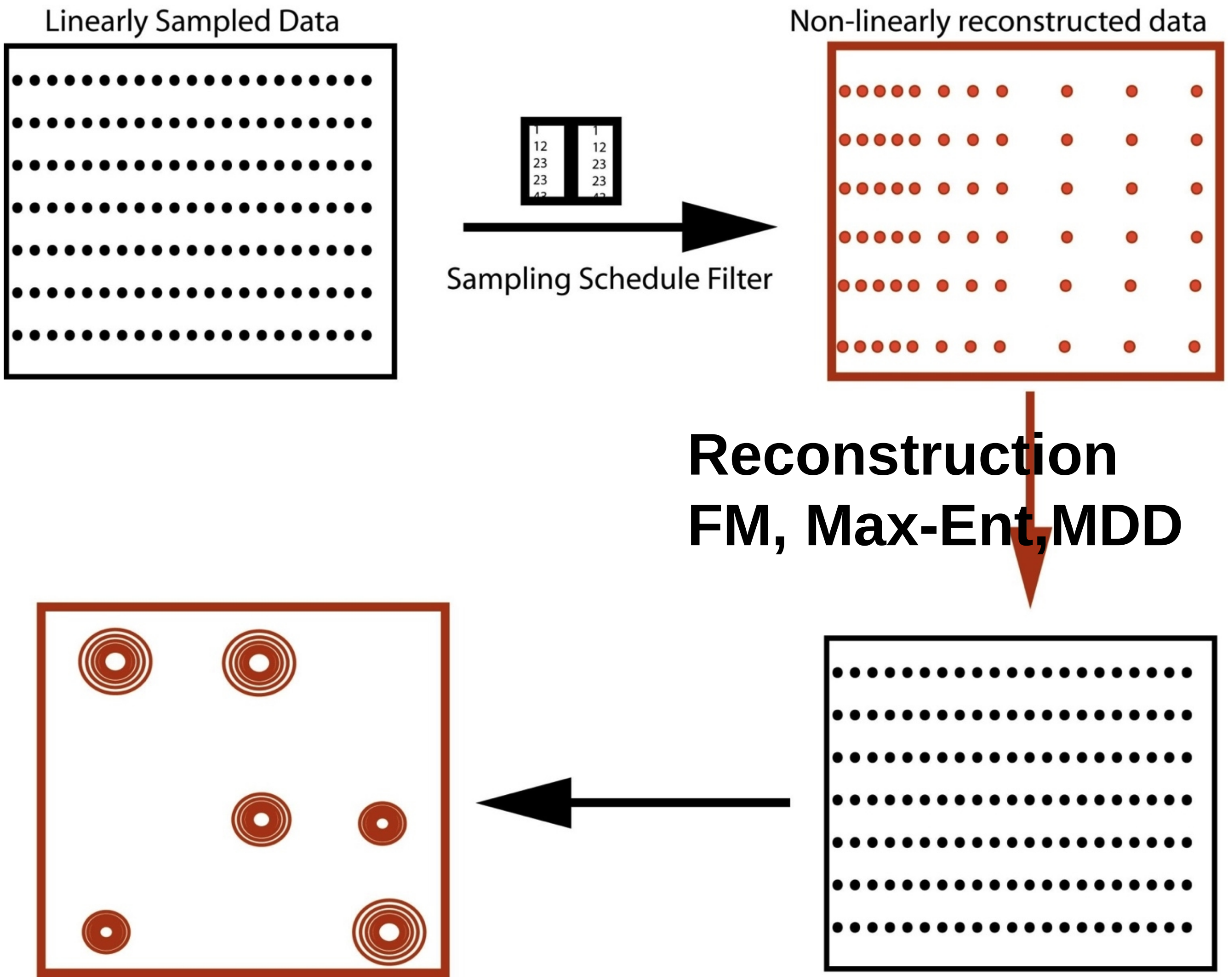
Non-linear Uniform Sampling (NUS) : 3D



Only a fraction of (t_1/t_2) pairs are collected

Non-linear Uniform Sampling (NUS)

Fourier Transform (FT) requires a complete matrix.



How to deal with incomplete sampling?

Fourier Transform (FT) requires a complete matrix.

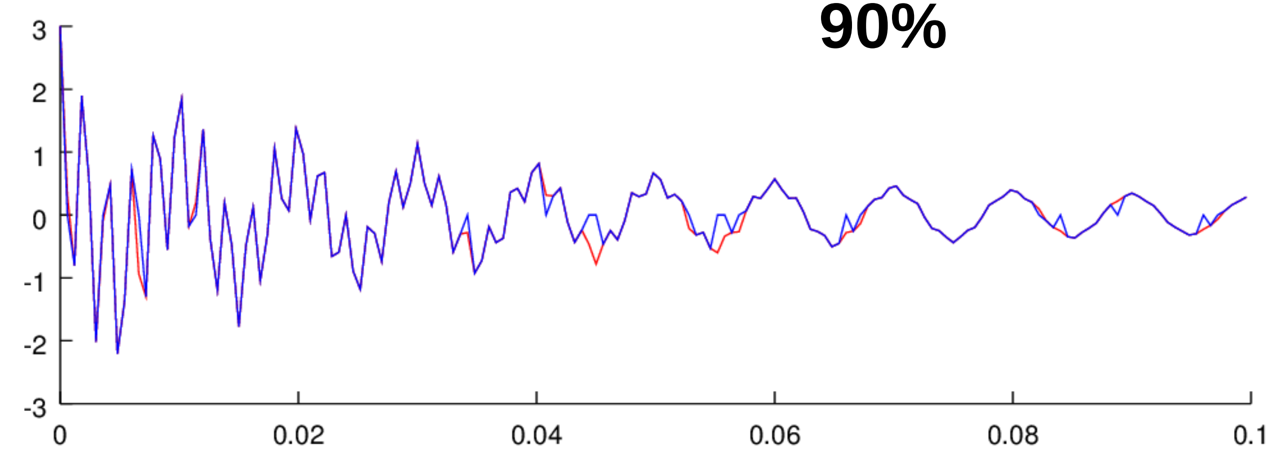
Solution (1): replace missing points by 0.

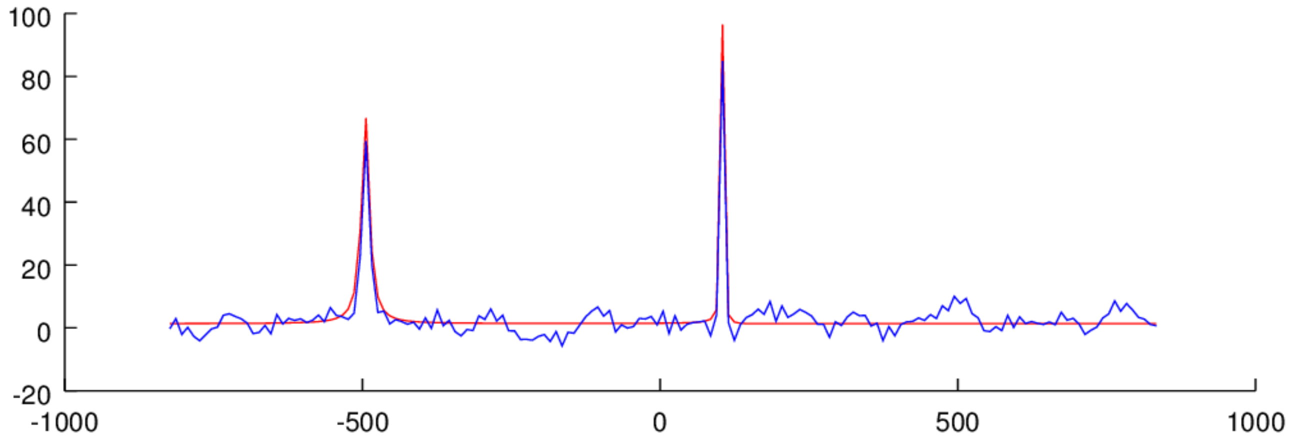
Satisfies prerequisite but provides artefacts because the original signal is strongly perturbed

How to deal with incomplete sampling?

Simulation: superposition of two peaks / No noise

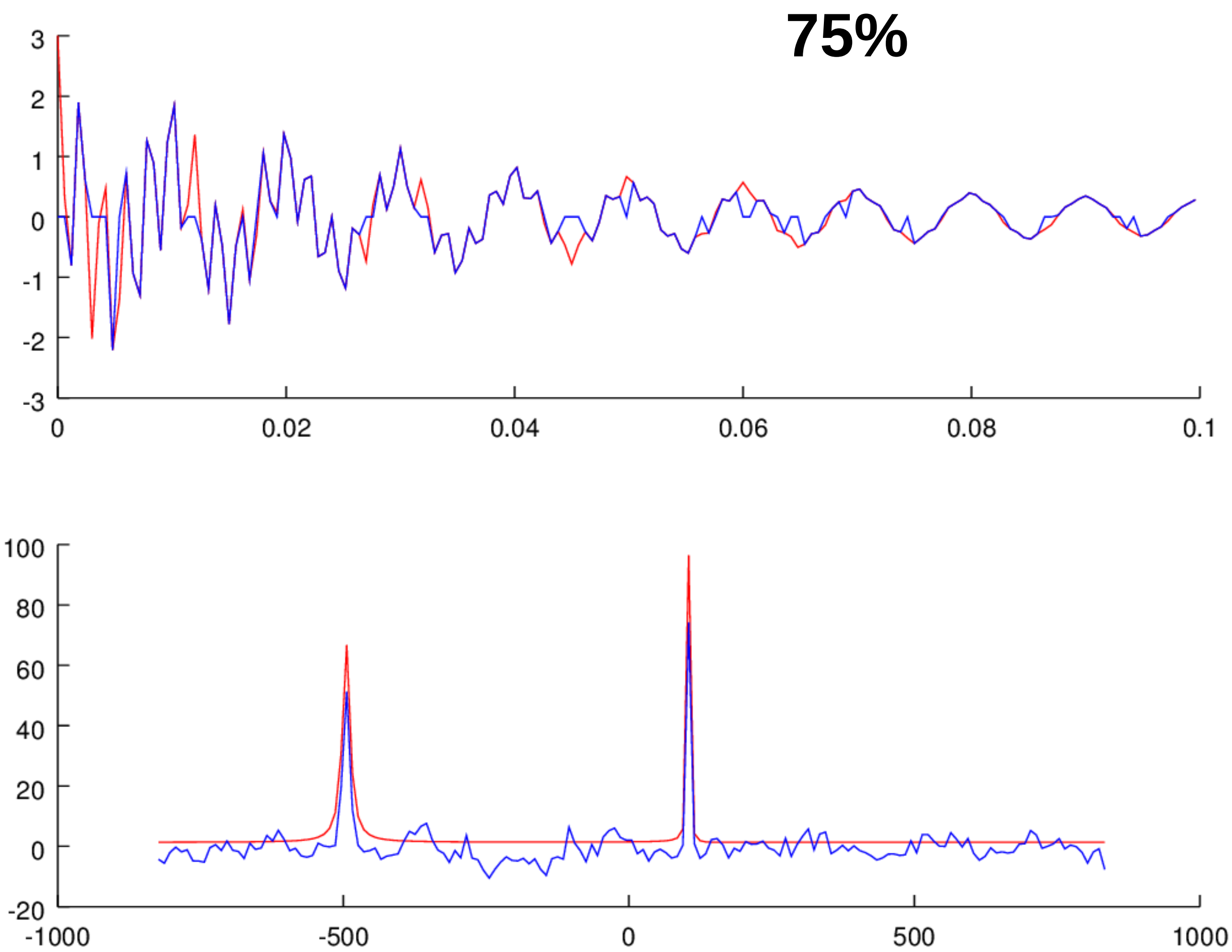
90%





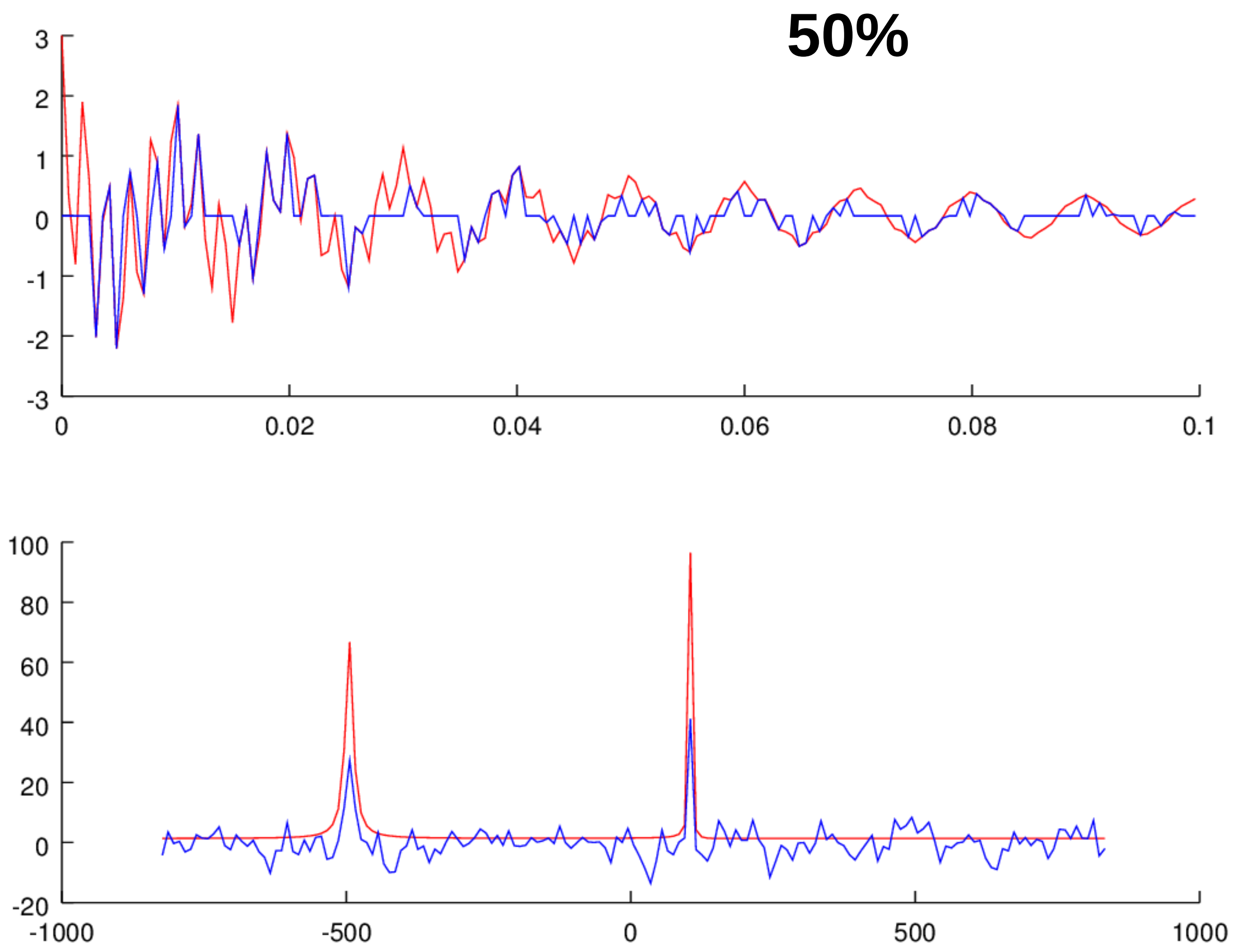
Blue: A fraction of experimental data are set to 0.

How to deal with incomplete sampling?



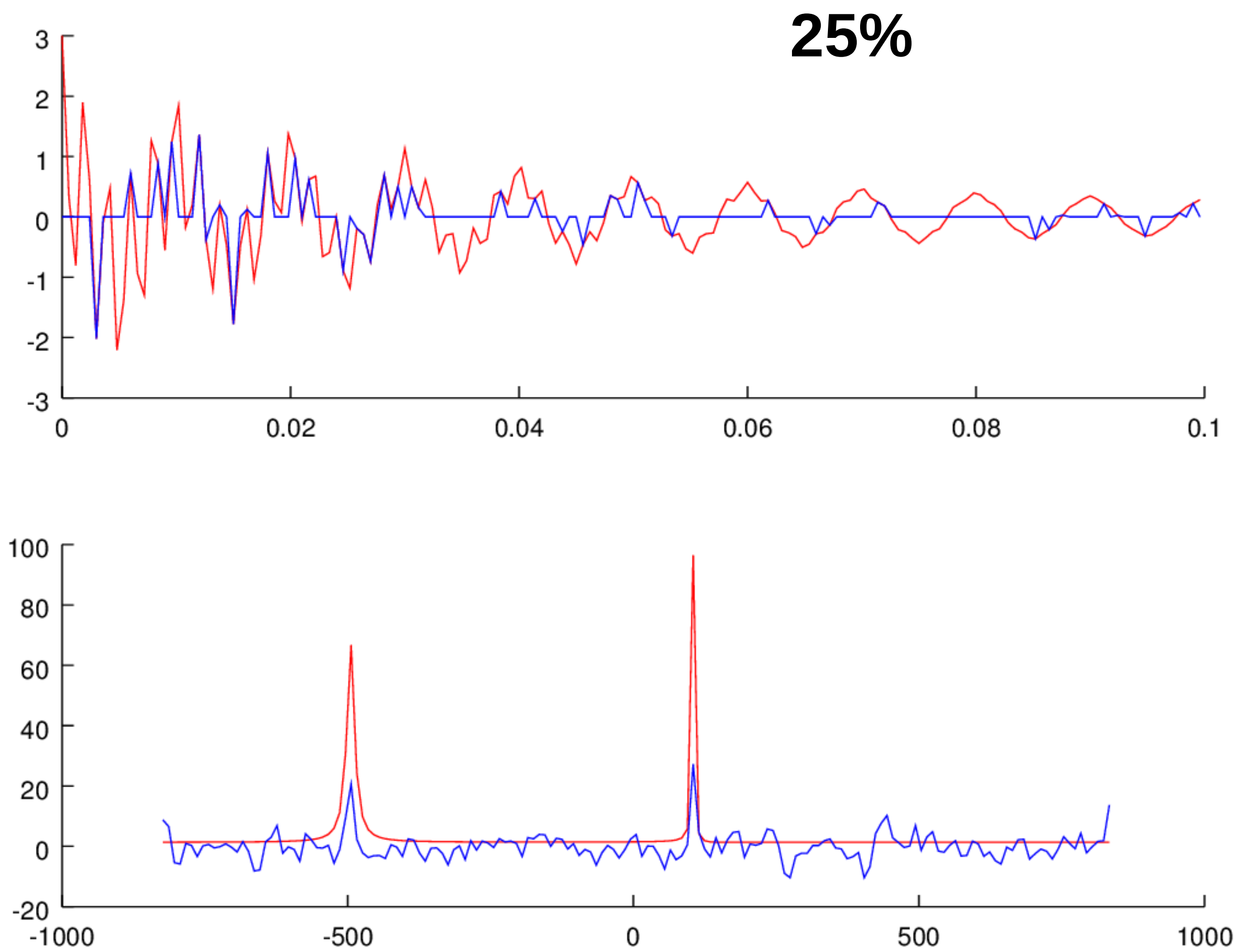
Blue: A fraction of experimental data are set to 0.

How to deal with incomplete sampling?



Blue: A fraction of experimental data are set to 0.

How to deal with incomplete sampling?



Filling with zeroes introduce severe artefacts / structure noise

How to deal with incomplete sampling?

Fourier Transform (FT) requires a complete matrix

Solution (2): predict the missing points.
Several methods (MaxEnt, MDD, CS, …) developed in the groups of Koźmiński W, Billeter M, Marion D, Hoch J, Orekhov V….

Reviews:

Kazimierczuk K, Stanek J, Zawadzka-Kazimierczuk A, Koźmiński W. Random sampling in multidimensional NMR spectroscopy. Prog Nucl Magn Reson Spectrosc. 2010 Nov;57(4):420-34.

Hyberts SG, Arthanari H, Wagner G. Applications of non-uniform sampling and processing. Top Curr Chem. 2012;316:125-48

Hoch JC, Maciejewski MW, Mobli M, Schuyler AD, Stern AS. Nonuniform sampling and maximum entropy reconstruction in multidimensional NMR. Acc Chem Res. 2014 Feb 18;47(2):708-17.

Mobli M, Hoch JC. Nonuniform sampling and non-Fourier signal processing methods in multidimensional NMR. Prog Nucl Magn Reson Spectrosc. 2014 Nov;83:21-41.

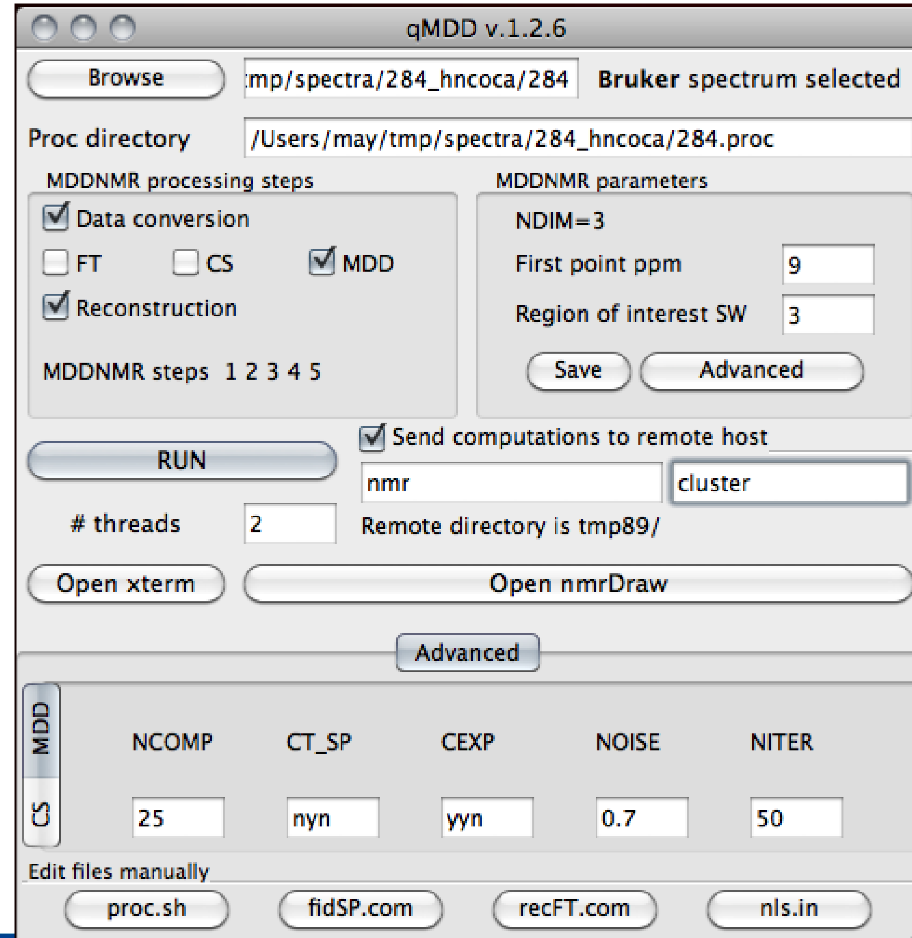
Billeter M, Orekhov V, Novel Sampling Approaches in Higher Dimensional NMR (book)

Practical implementation: qMDD / Topspin

For real usage, any method should be available as a software, easy-to-use, robust, with minimal parameters to set, and with rapid computation

qMDD (free):
https://groups.google.com/forum/#!forum/mddnmr
(1)FT (zeroed-spectra)
(2)MDD
(3)Compressed Sensing

Topspin (Licence)
Included with xfb/tf3d
(available at ICSN for IR-RMN users)



Multiway Decomposition Approach (MDD)

Principle:
Any nD spectra can be considered as the sum of various nD peaks. Each peak *k* is defined as the direct product ('$\otimes$ ') of 1D shapes F^k_1,…, F^k_n.

$$S(\omega_1 \ldots \omega_N) = \Sigma_k F_1^k(\omega_1) \otimes F_2^k(\omega_2) \ldots \otimes F_N^k(\omega_N)$$

MDD consists in finding the shapes F^k_1,…, F^k_n, that satisfy the experimental spectrum. The fit can be done in the time domain where missing data are simply removed from the analysis. A **critical parameter (NCOMP)** is the number of component (peaks) expected. Too low, weak peaks will not be modelled.

The shapes can then be used to reconstruct missing data prior to regular FT.

Compressed Sensing

Principle: Providing the frequency spectrum **S** is sparse (i.e. zero values at most locations), **S** can be reconstructed a few experimental time data *b*, by solving

$$\min_{S} ||S||^1 \quad \text{subject to} \quad FT^{-1}(S) = b$$

With $||S||_1 = \sum_i |S_i|^1$

Many NMR spectra are sparse in the frequency domain,with sparsity increasing as the number of dimensions increases.
The 0-norm should be used in theory but is practically not convenient
The 1-norm is easier

Compressed Sensing

Many NMR spectra are sparse in the frequency domain, with sparsity increasing as the number of dimensions increases.
The 0-norm should be used in theory but is practically not convenient

Different algorithms to minimize $||S||_1$
- Iterative re-weighted least squares (IRLS)
- Iterative soft thresholding (IST)

Kazimierczuk K, Orekhov VY. Accelerated NMR spectroscopy by using compressed sensing. Angew Chem Int Ed Engl. 2011 50(24):5556-9
Holland DJ, Bostock MJ, Gladden LF, Nietlispach D. Fast multidimensional NMR spectroscopy using compressed sensing. Angew Chem Int Ed Engl. 2011;50(29):6548-51.

Iterative Soft Threshold (hmsIST) outlined

NUS synthetic two line spectrum

FFT produces spectrum with many artifacts (point spread function, PSF)

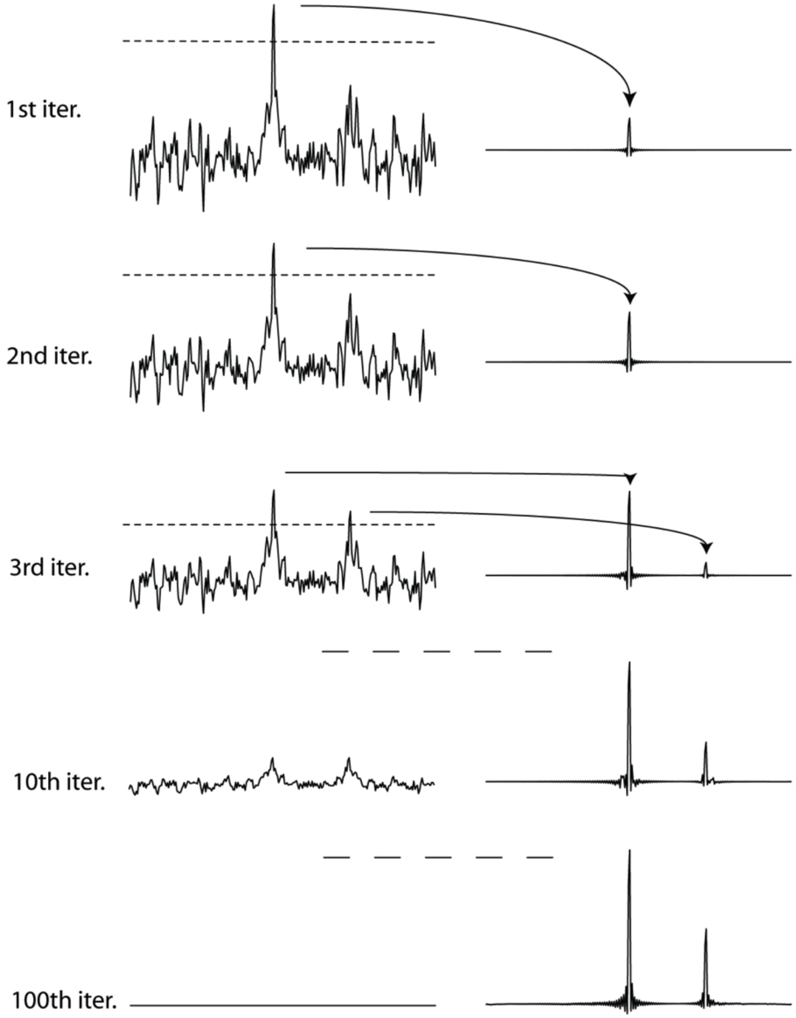
Copy top 2% of spectrum and store in different location

FFT^{-1} and zero skipped time domain data points - subtract

FFT and iterate

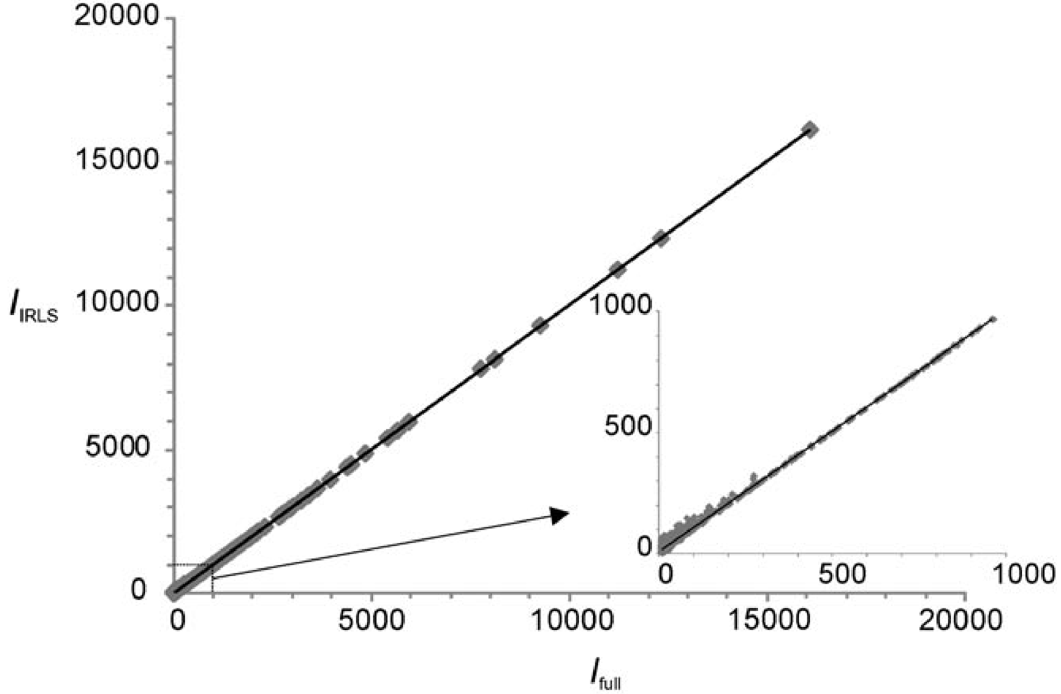
After a sufficient number of iteration all PSF artifacts are eliminated

Very fast procedure



Hyberts et al. J. Biomol. NMR 52(4) 2012

Compressed Sensing: robust to high dynamic range (NOESY)

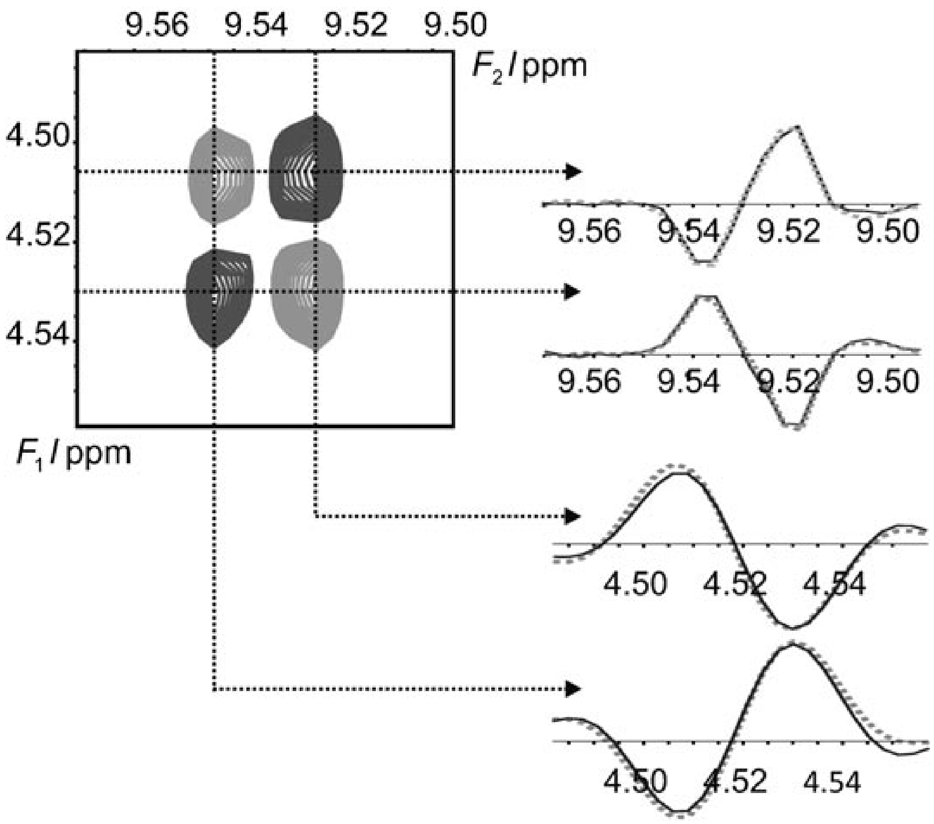


Accuracy of peak intensities in the reconstructed 2D NOESY spectrum of azurin.

Weak peaks may not be recognized and disappear below noise level but otherwise, peak intensity seems to be reliable.

Kazimierczuk K, Orekhov VY. Accelerated NMR spectroscopy by using compressed sensing. Angew Chem Int Ed Engl. 2011 50(24):5556-9

Compressed Sensing: Lineshape (COSY)

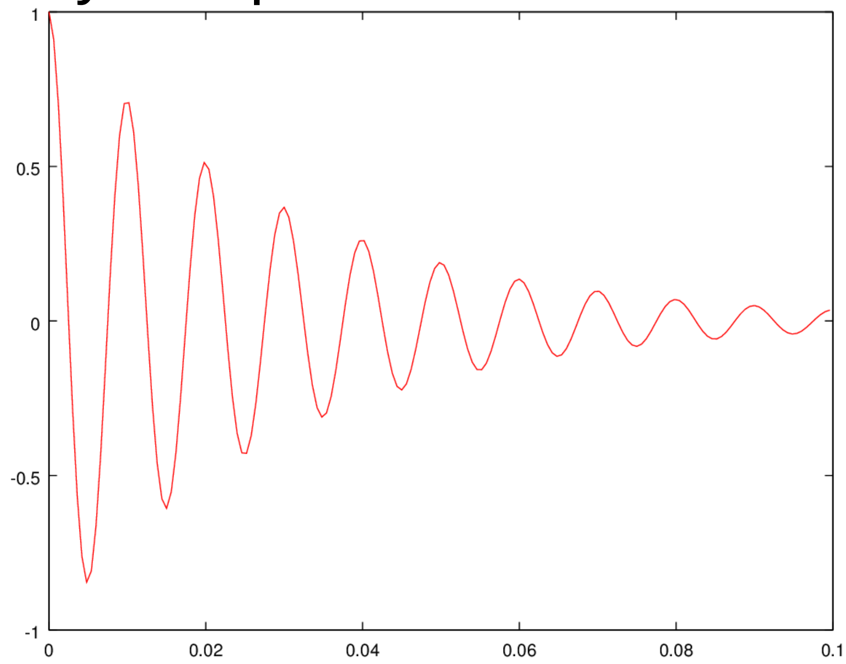


Fidelity of the lineshape reconstruction. The I13 HN-Ha peak from the IRLS-reconstructed DQF COSY spectrum of ubiquitin is shown.

Kazimierczuk K, Orekhov VY. Accelerated NMR spectroscopy by using compressed sensing. Angew Chem Int Ed Engl. 2011 50(24):5556-9

Sampling schedule: uniform distribution

Exponentially damped sinusoid



Good SNR
Poor resolution

Poor SNR
Good resolution

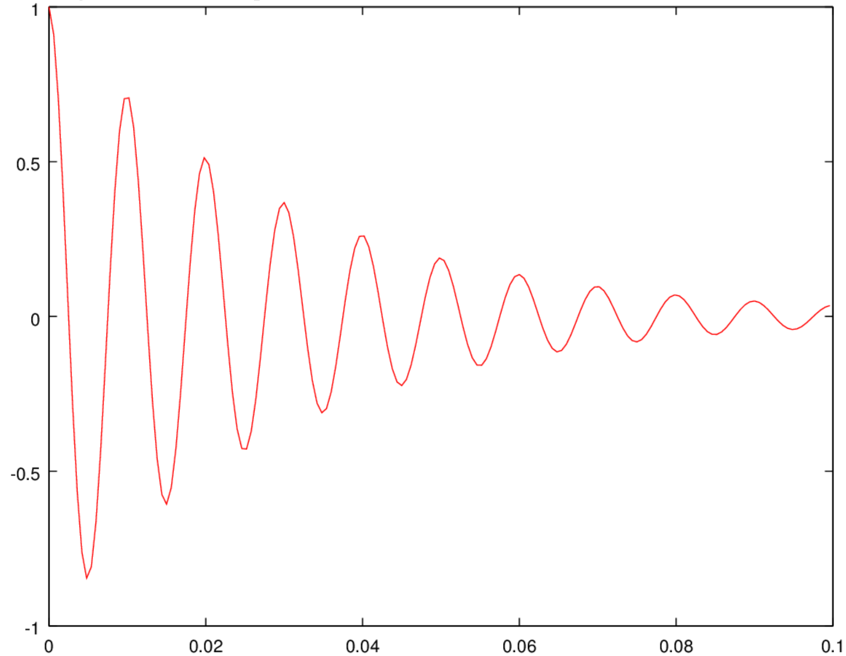
Sampling uniformly or non-uniformly impacts peak intensity and resolution (similar effect as window function)

In absence of knowledge about signal -> uniform sampling

Perfect for constant-time evolution (^{15}N dimension of HNC exp)

Sampling schedule: matched acquisition (exponential distribution)

Exponentially damped sinusoid



Good SNR | Poor SNR

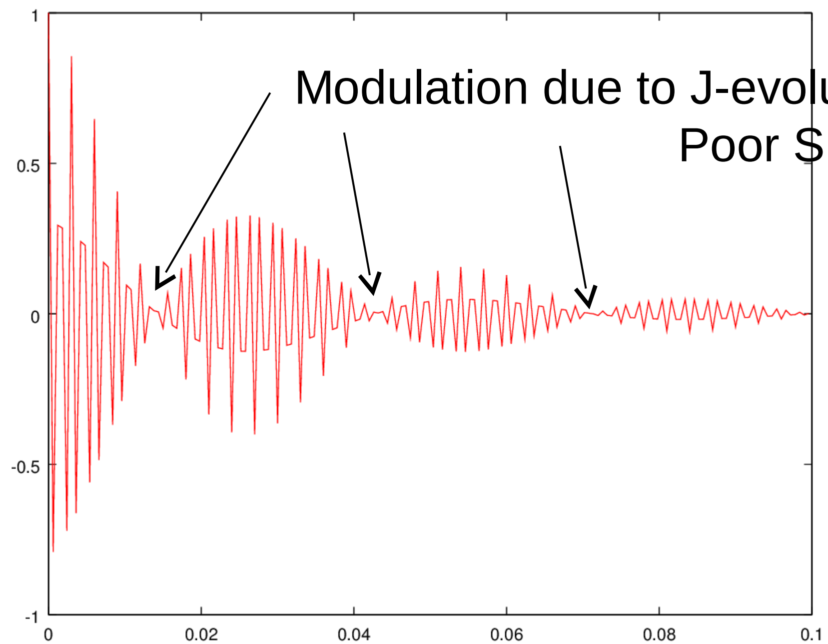
Poor resolution | Good resolution

Collect more increments at the beginning and less at the end of the fid (or pseudo-fid)

Good for real time evolution ($^{15}N/^{13}C$ dim of HSQC or ^{13}C dim of HNC)

Sampling schedule: exponential/sin distribution

Exponentially damped sinusoid + J coupling



Good SNR | Poor SNR

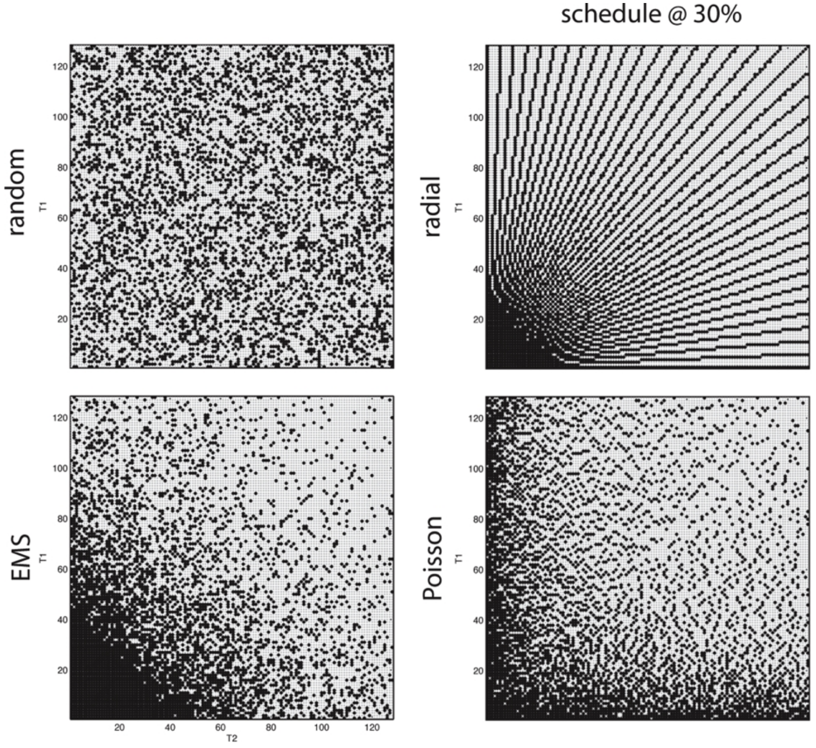
Poor resolution | Good resolution

Concentrate more on regions with good SNR and less near 0.
Good for (1) ^{13}C dimension of HNCA/HNCOCA/HNCACB/HNCOCAB (not for the CT version) where $J_{C\alpha C\beta}$=35 Hz is active when high resolution is needed or (2) J-coupled HSQC (IPAP, …)

Sampling schedule:

Pure Random

Exponential weight



Sampling schedule: TOPSPIN

NUS (Non Uniform Sampling) parameters

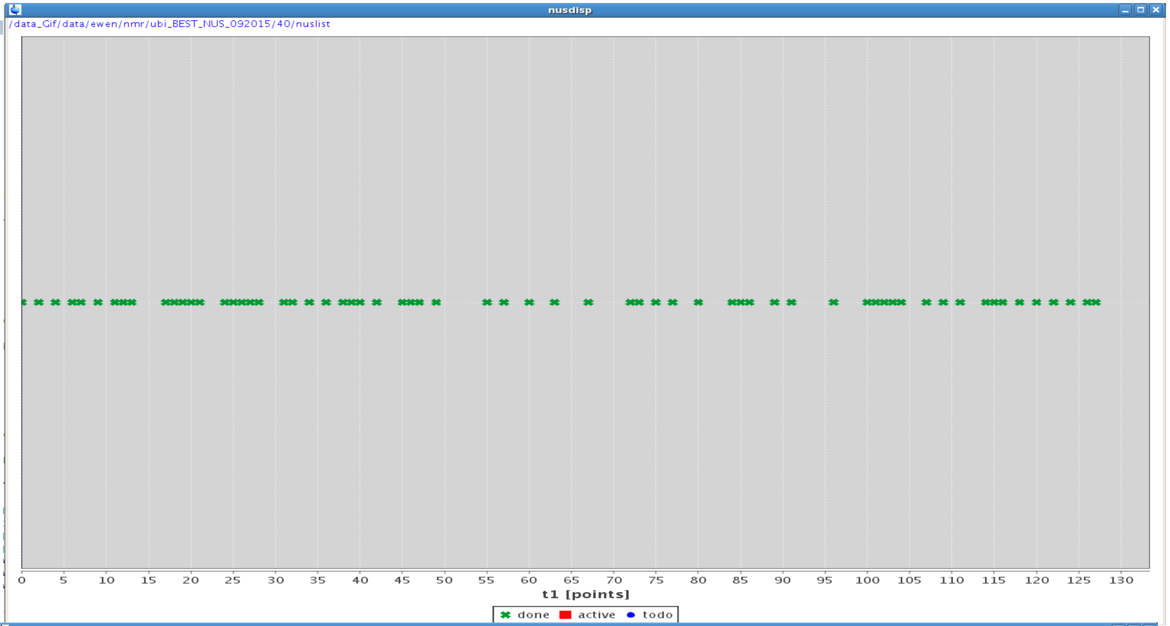
	NUS Help			Show NUS help
NusAMOUNT [%]	100			Amount of sparse sampling
NusPOINTS	1500			Number of hypercomplex points in indirect dimension
NusJSP [Hz]	0	0	0	J-coupling
NusT2 [sec]	1	1	1	T2 relaxation
NusSEED	54321			Random generator seed
NUSLIST	automatic			Name of loopcounter list for NUS (Non Uniform Sampling)
	Calculate			Calculate point spread function
	Show			Display NUS point spread

NUS (Non Uniform Sampling) parameters

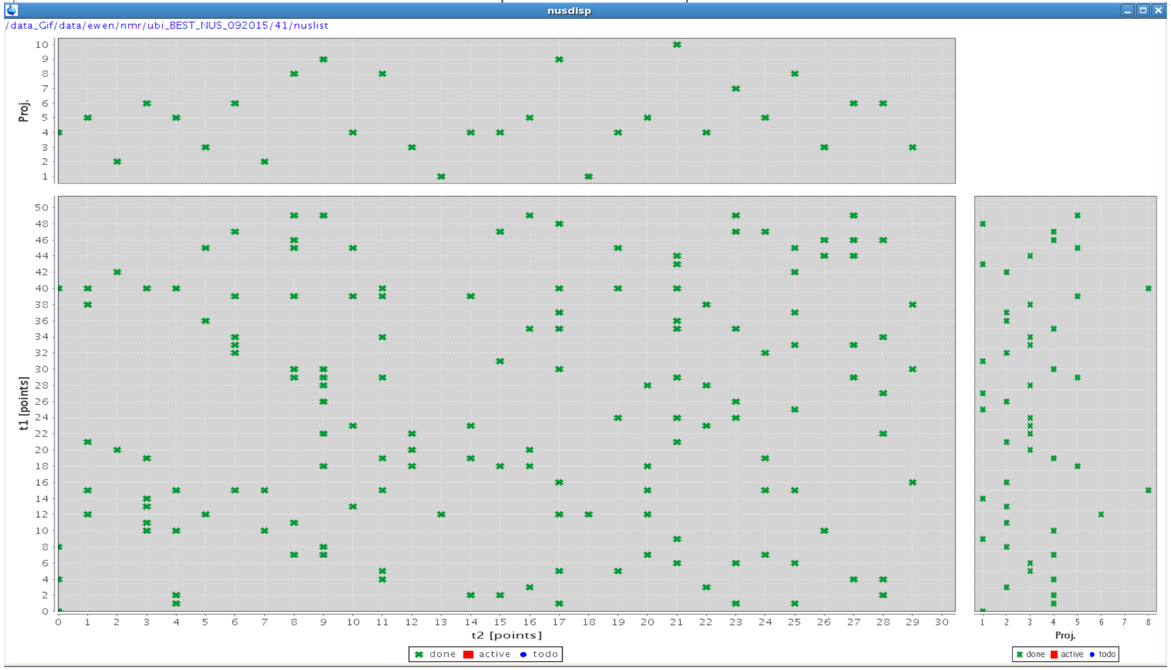
Mdd_mod	cs			MDD mode
MddCEXP	FALSE	FALSE	FALSE	RMDD/MDD flag
MddCT_SP	FALSE	FALSE	FALSE	Constant time
MddF180	FALSE	FALSE	FALSE	Delayed sampling flag
MddNCOMP	0			Number of components
MddPHASE	0	0	0	Phase
MddSRSIZE [ppm]	0			Sub region size

Sampling schedule: TOPSPIN schedule

2D



3D



Speeding up multidimensional NMR

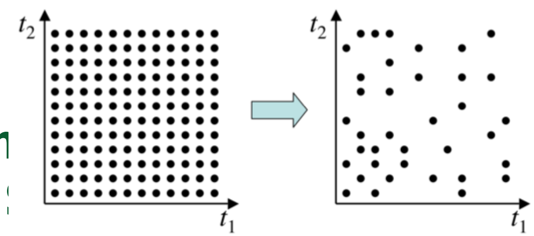
Standard NMR techniques

1D → **2D** → **3D** → **4D** → **5D**

seconds *minutes* *hours* *weeks* *years*

Fast-pulsing NMR

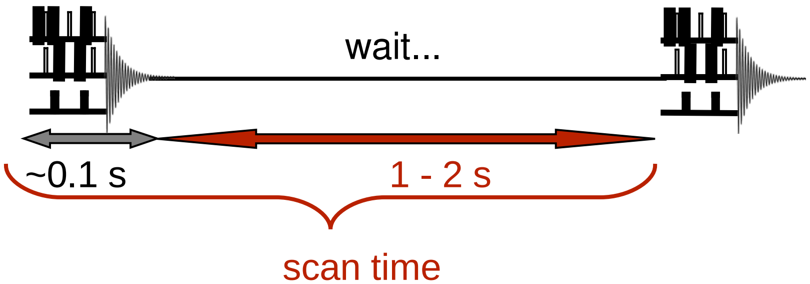
Sparse non-uniforn
data sampling (NU

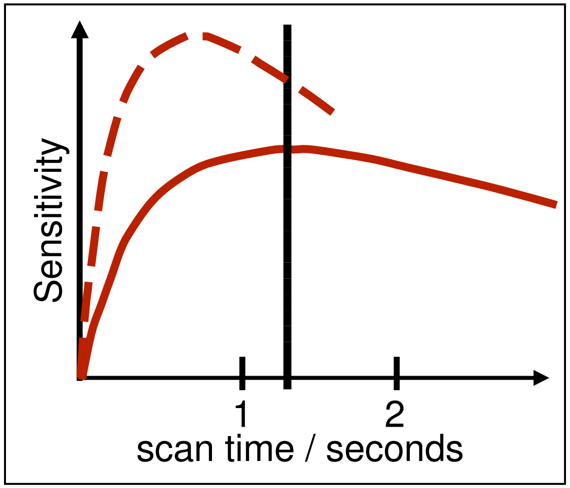


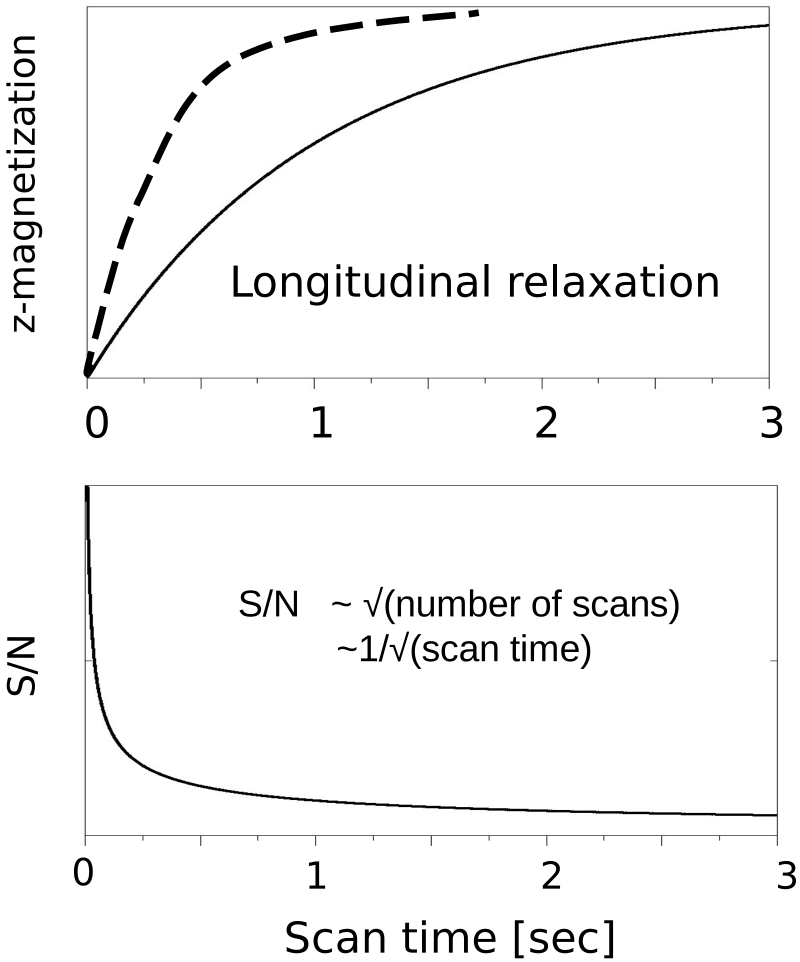
2D → **3D** → **4D**

seconds *minutes* *hours*

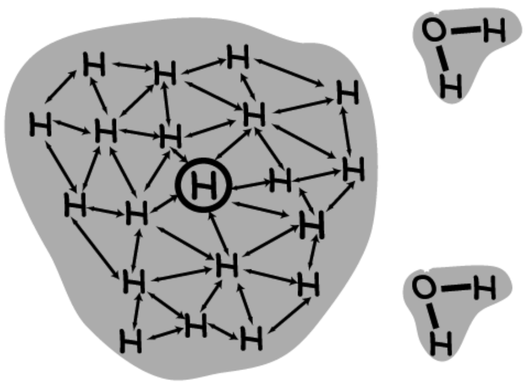
Longitudinal relaxation, inter-scan delay & sensitivity







^{1}H longitudinal relaxation mechanisms



- ^{1}H-^{1}H dipolar interactions (σ_{ij})
- Chemical exchange with water ^{1}H (k_{ex})

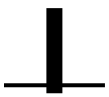
Solomon or Bloch-McConnell equations:

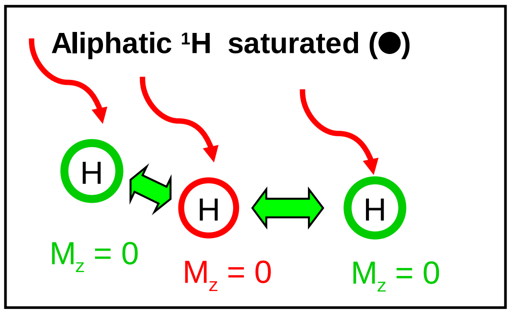
$$-\frac{d}{dt}\begin{pmatrix} W_{1z} \\ H_{1z} \\ H_{2z} \\ \vdots \\ H_{nz} \end{pmatrix} = \begin{pmatrix} \rho_W & 0 & 0 & 0 & 0 \\ 0 & \sum_j \rho_{1j} & \sigma_{12} & \cdots & \sigma_{1n} \\ 0 & \sigma_{21} & \sum_j \rho_{2j} & \cdots & \sigma_{2n} \\ 0 & \vdots & \vdots & \ddots & \vdots \\ 0 & \sigma_{n1} & \sigma_{n2} & \cdots & \sum_j \rho_{1nj} \end{pmatrix} \begin{pmatrix} W_{1z} - W_{1z}^0 \\ H_{1z} - H_{1z}^0 \\ H_{2z} - H_{2z}^0 \\ \vdots \\ H_{nz} - H_{nz}^0 \end{pmatrix} + \begin{pmatrix} 0 & 0 & 0 & 0 & 0 \\ -k_{ex,1} & k_{ex,1} & 0 & 0 & 0 \\ -k_{ex,2} & 0 & k_{ex,2} & 0 & 0 \\ \vdots & 0 & 0 & \ddots & 0 \\ -k_{ex,n} & 0 & 0 & 0 & k_{ex,n} \end{pmatrix} \begin{pmatrix} W_{1z} \\ H_{1z} \\ H_{2z} \\ \vdots \\ H_{nz} \end{pmatrix}$$

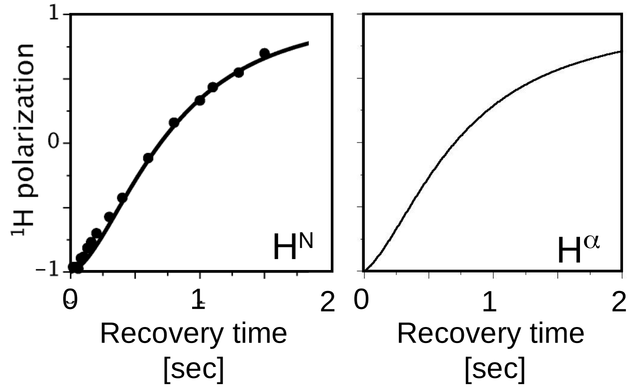
- ^{1}H polarization recovery depends on the spin state of all other protons in the protein and the bulk water.

Amide ^{1}H longitudinal relaxation enhancement (LRE)

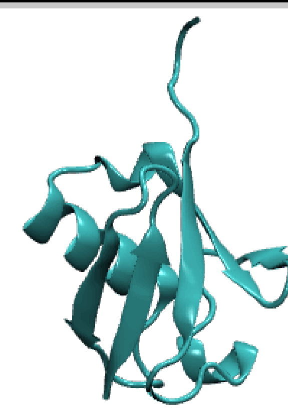
Non-selective excitation

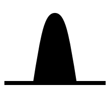


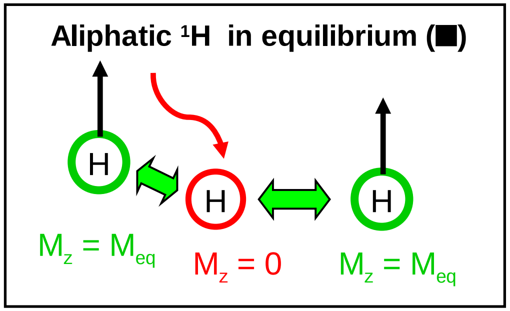


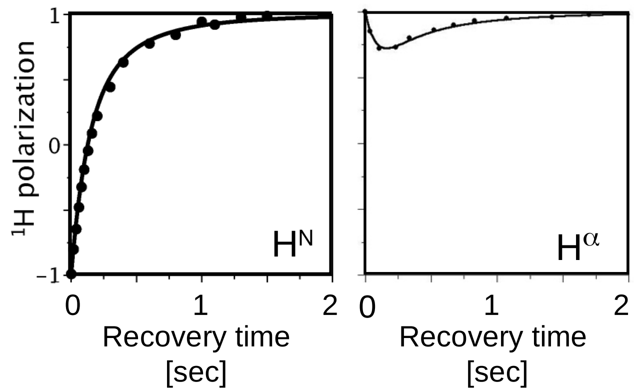


$T_1 \approx$ **0.8 – 4.0 s**





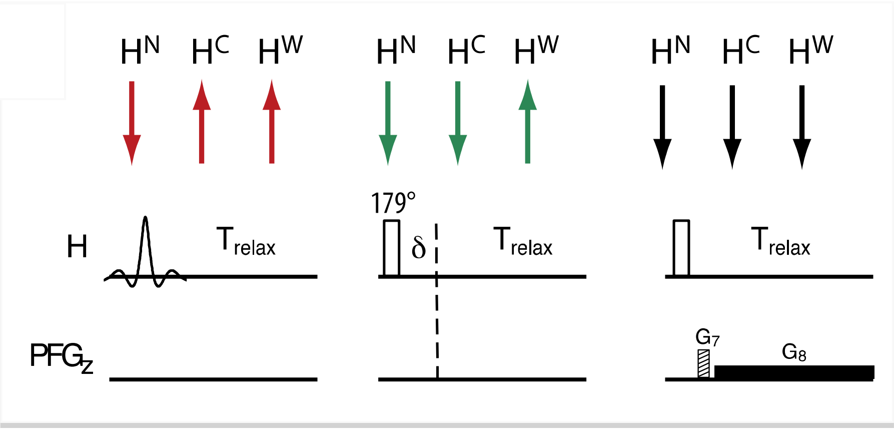




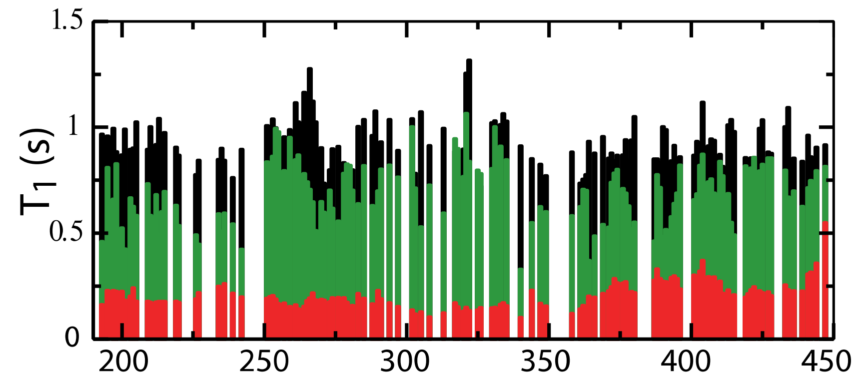
$T_1 \approx$ **0.1 – 0.4 s**

Selective H^N excitation

Amide ^{1}H longitudinal relaxation enhancement (LRE)

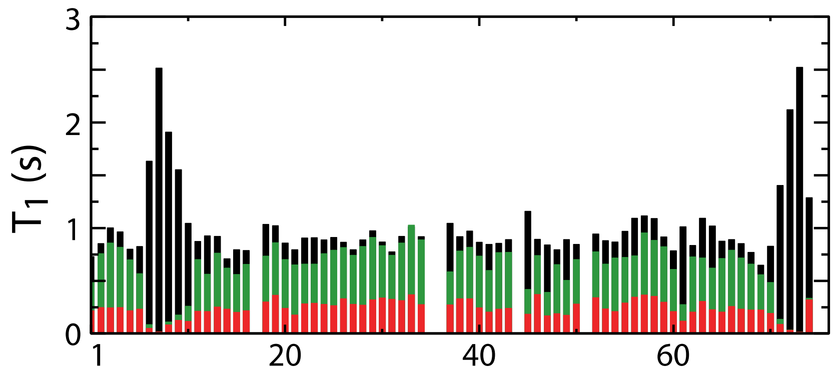


IDP (pH 6.5, 5°C)



$T_1 \approx$ **0.9 s** ; $T_1 \approx$ **200 ms**

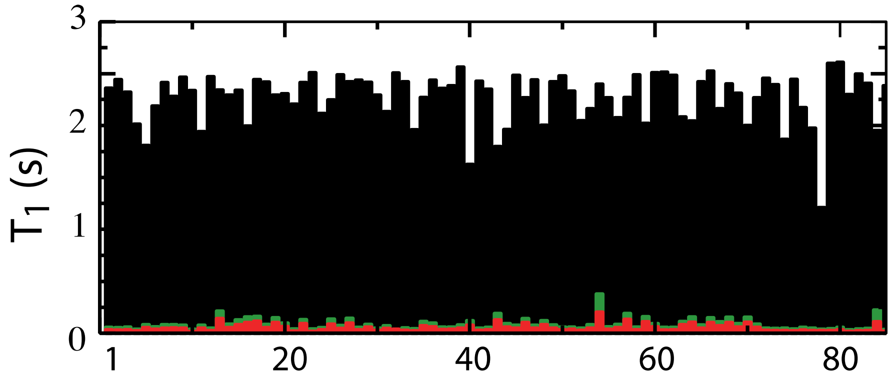
Ubiquitin (pH 7.5, 25°C)



Structured part: $T_1 \approx$ **0.9 s** ; $T_1 \approx$ **200 ms**

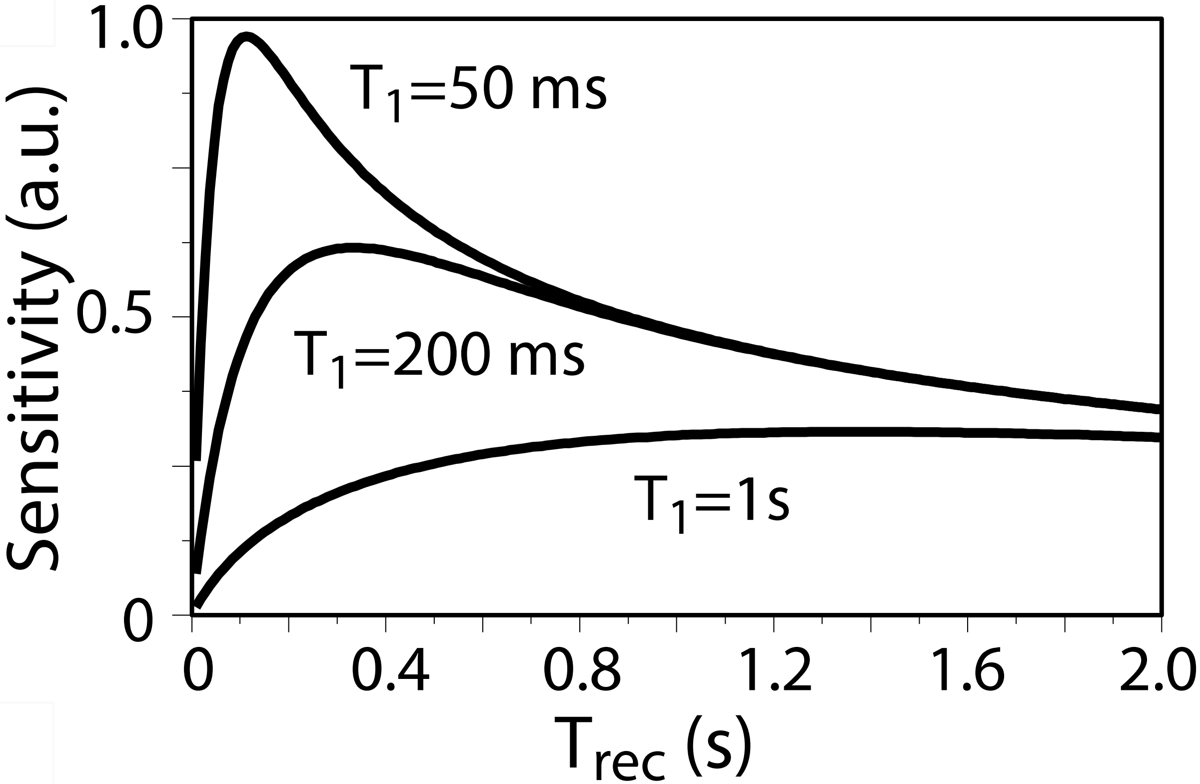
Disordered part: $T_1 \approx$ **2 s** ; $T_1 \approx$ **60 ms**

IDP (pH 7.5, 20°C)

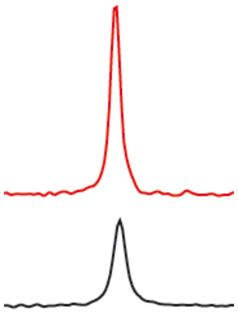


$T_1 \approx$ **2.3 s** ; $T_1 \approx$ **60 ms**

Computed sensitivity curves for different T_1 time constants



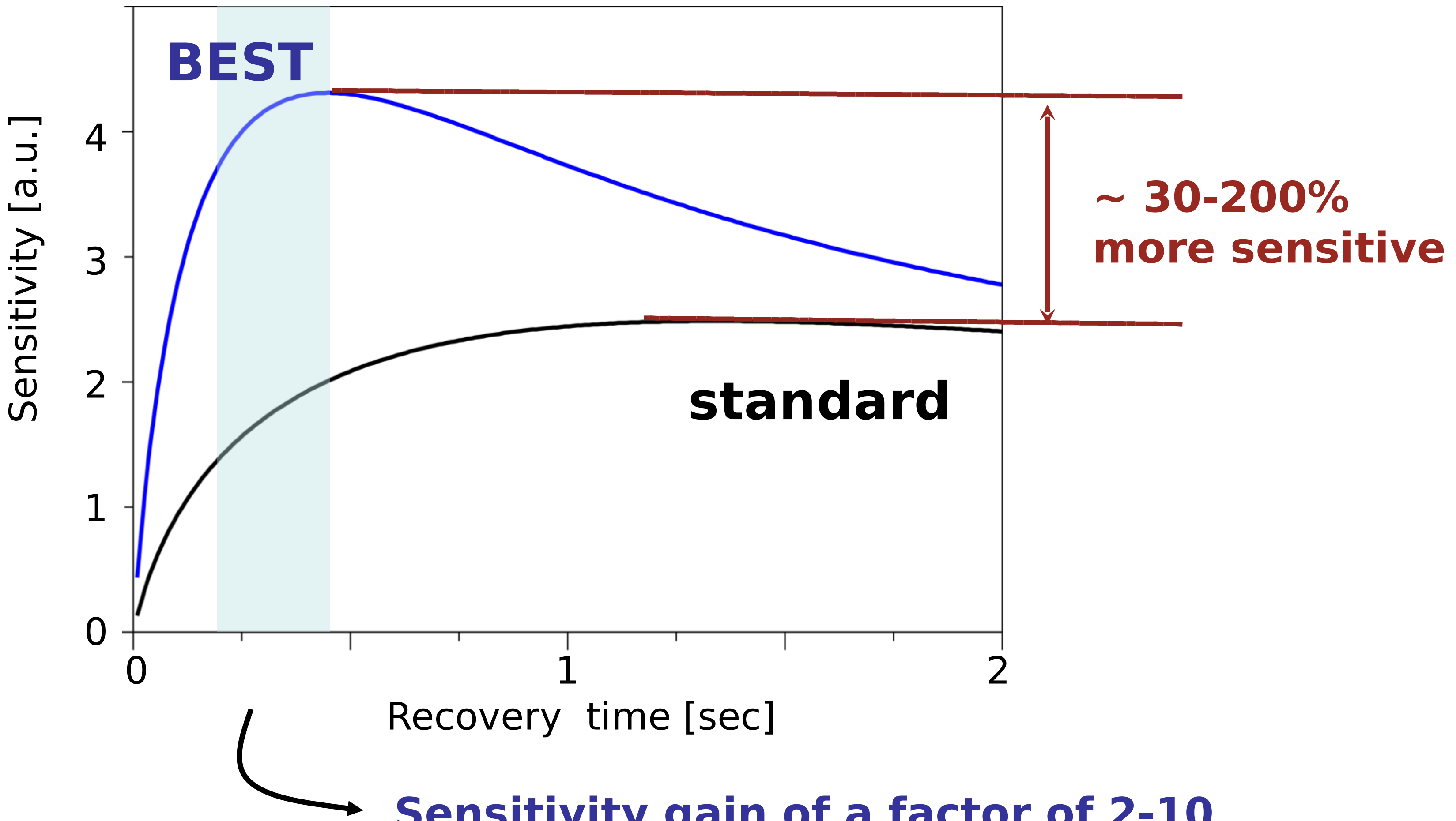
Gain in Sensitivity …



… and speed

BEST & SOFAST experiments

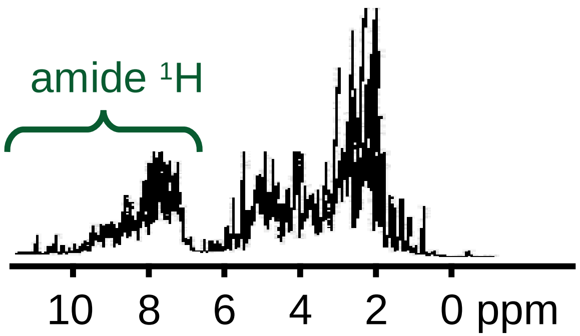
high-speed and optimal-sensitivity regime



Sensitivity gain of a factor of 2-10

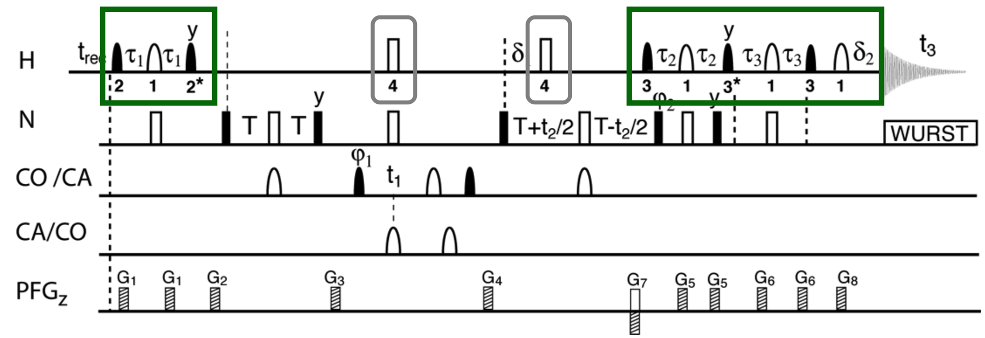
BEST : LRE optimized pulse sequences

BEST: Band-Selective **E**xcitation **S**hort-**T**ransient

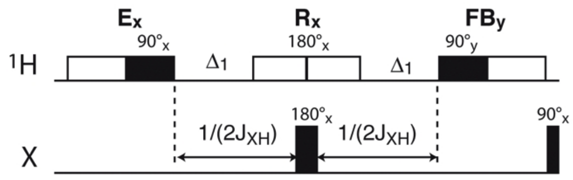


Band-selective pulses

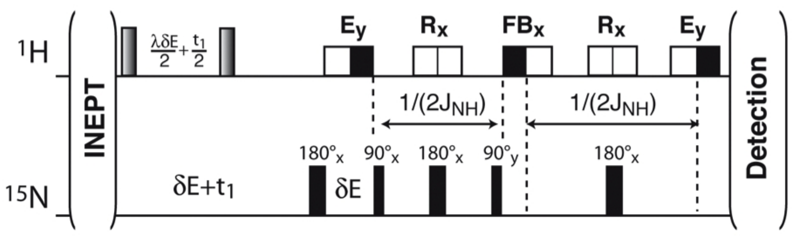
BIP pulse pairs (360° rotation)



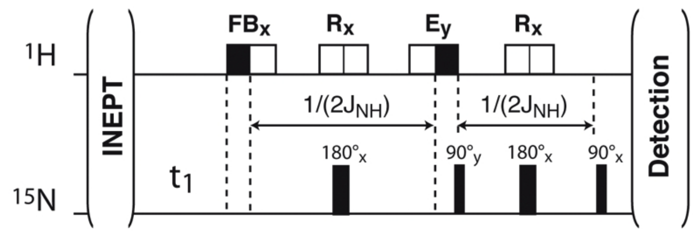
INEPT



BEST-HSQC



BEST-TROSY



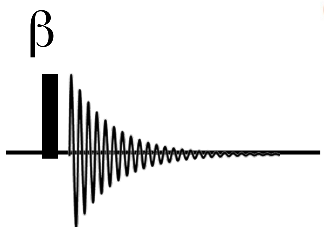
E: Excitation pulse (**EBURP2**)

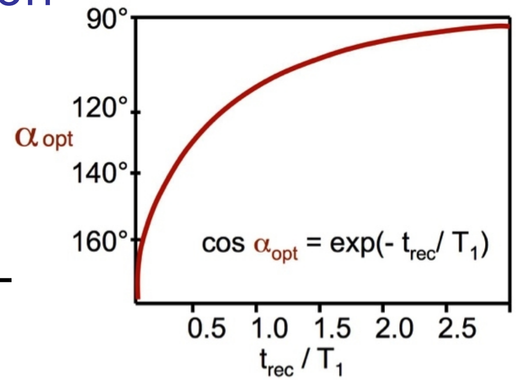
FB: Flip-back pulse (**time-reversed EBURP2**)

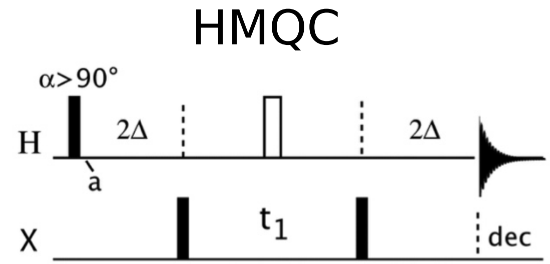
R: Refocusing pulse (**REBURP**)

Additional tricks for enhancing sensitivity

① ^{1}H polarization enhancement Using Ernst-angle excitation **(SOFAST)**



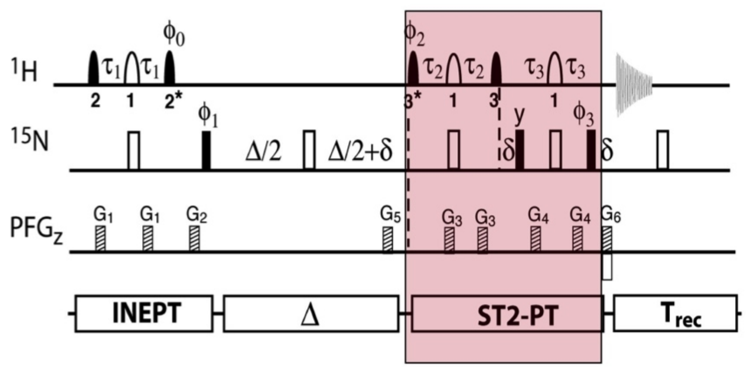


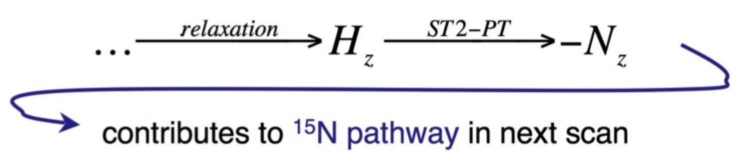


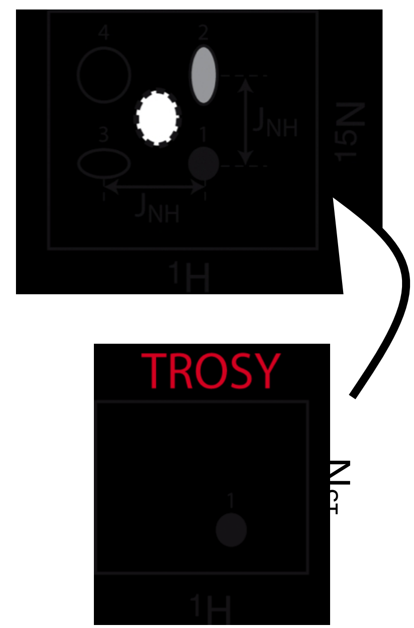
② Heteronuclear polarization enhancement **(BEST-TROSY)**

^{1}H pathway

^{15}N pathway



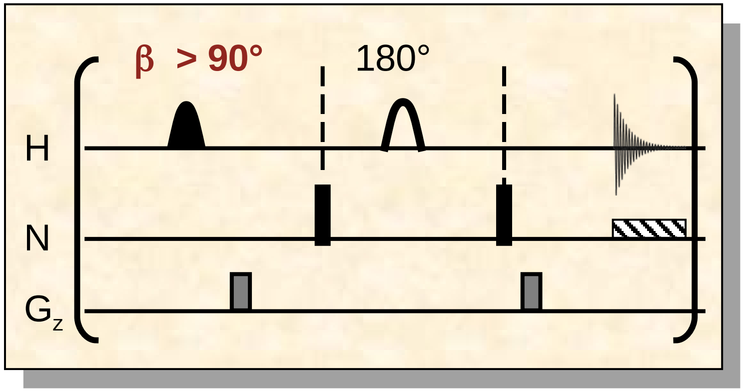


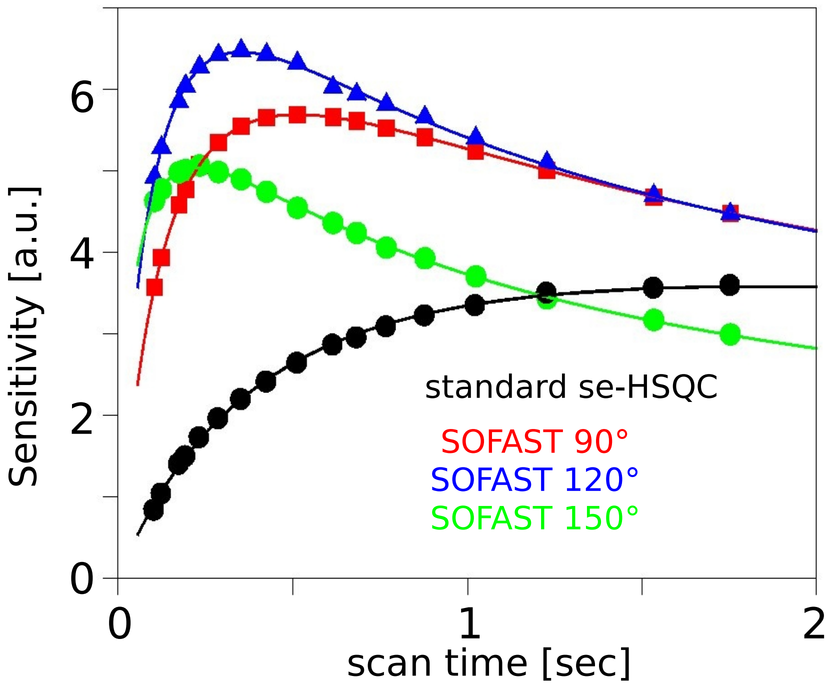


The SOFAST HMQC experiment

SOFAST: Band-**S**elective **O**ptimized-**F**lip-**A**ngle **S**hort-**T**ransient

Combining the advantages of
Longitudinal-relaxation enhancement
and Ernst-angle excitation !

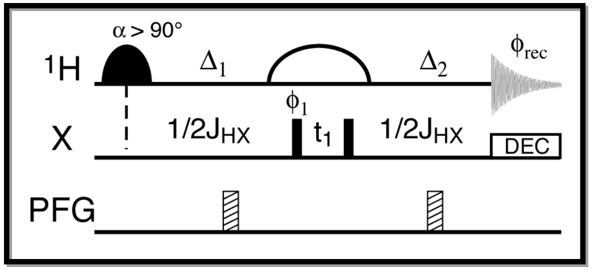




The SOFAST HMQC experiment

standard SOFAST

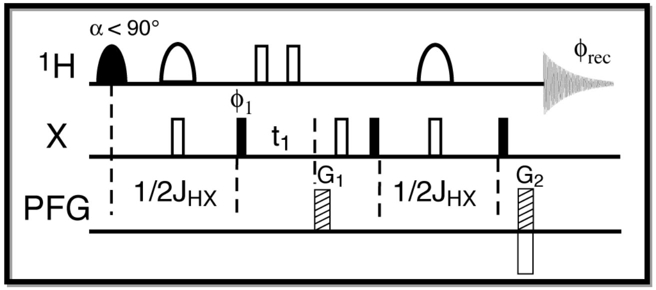
Schanda & Brutscher, *JACS (127)* **2005**, 8014.
Schanda et al, *J Biomol NMR (33)* **2005**, 199.



- highest sensitivity

ST-SOFAST

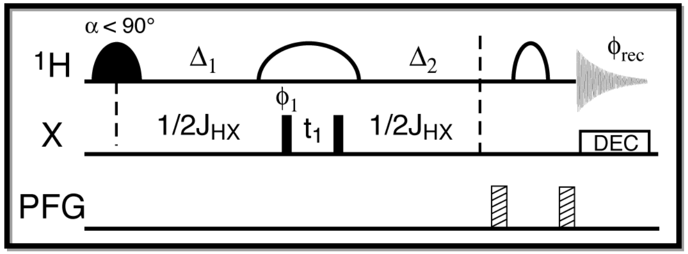
Gal et al, *J Biomol NMR (43)* **2009**, 1.



- no ^{15}N decoupling, √2 sensitivity loss
- gradient coherence selection

FTA-SOFAST

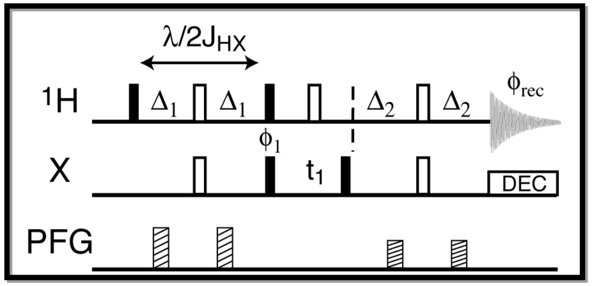
Schanda et al, *PNAS (104)* **2007**, 11257.



- for use with a fast mixing device
- small flip angle

J-SOFAST

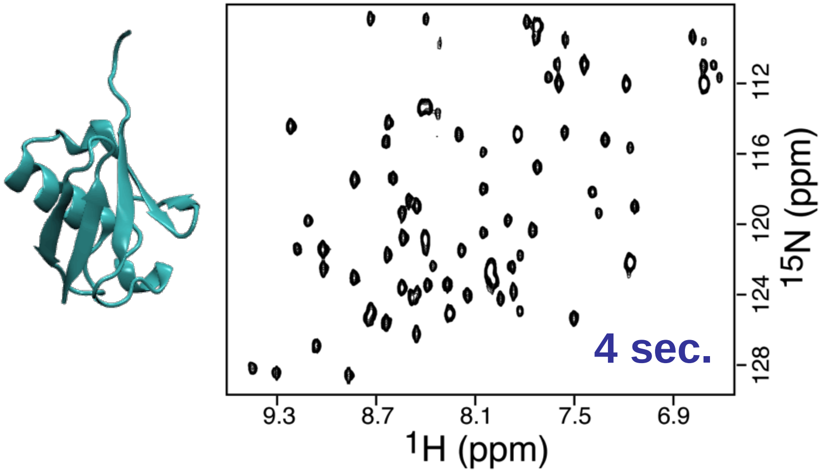
Kupce & Freeman, *MRC (45)* **2007**, 2.
Mueller, *J Biomol NMR (42)* **2008**, 129.



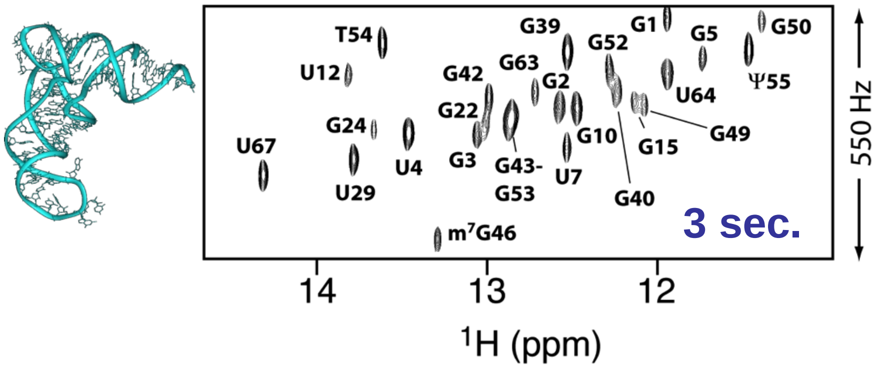
- J-coupling-based selection
- natural abundance samples

SOFAST HMQC - 2D spectra in a few seconds

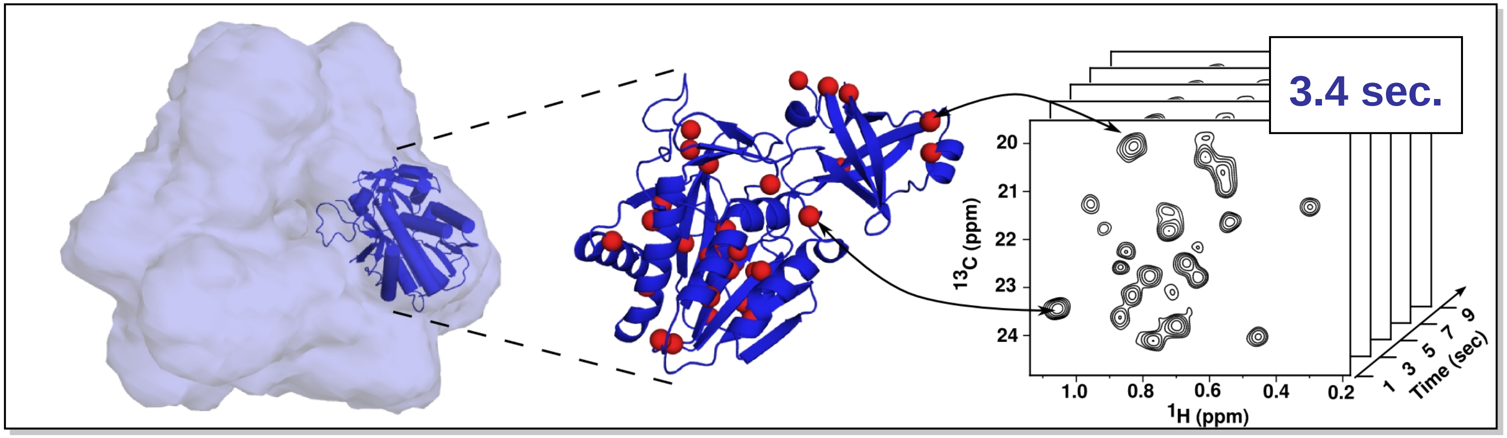
Ubiquitin (8.6 kDa, 0.2 mM)



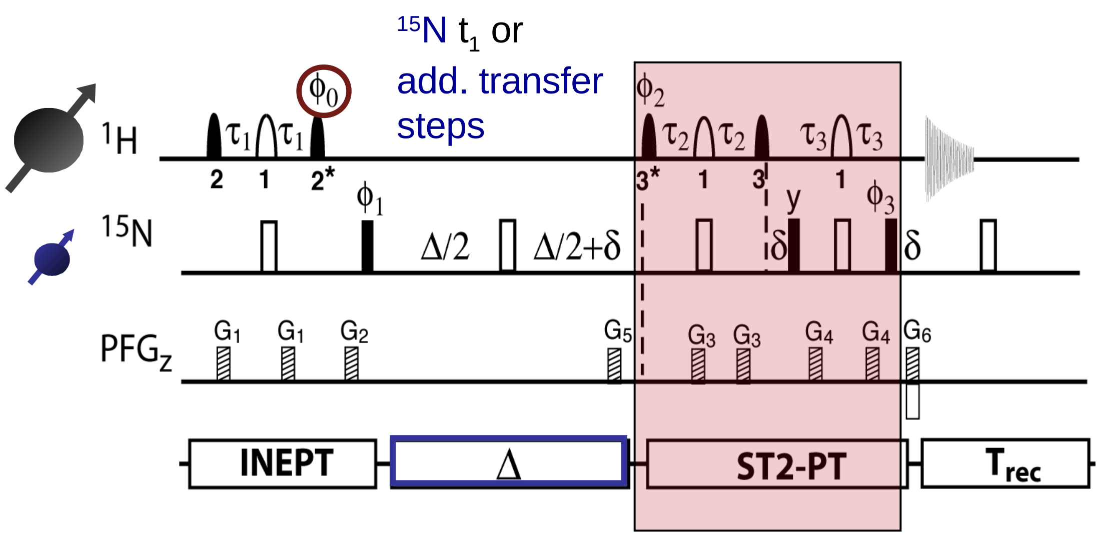
tRNA^{Val} (26 kDa kDa, 0.9 mM)

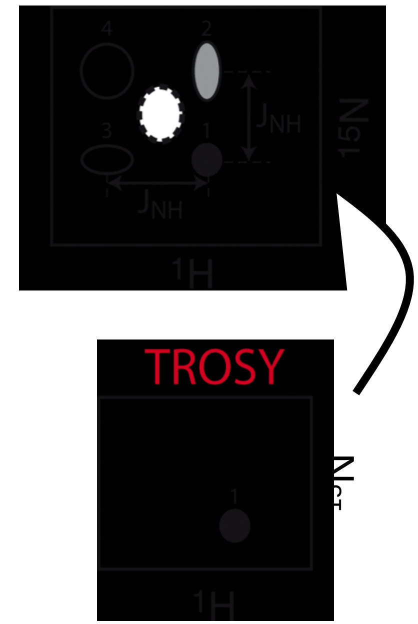


TET-2 (486 kDa, 80 μM, ~ 1 mM protomer concentration)



BEST-TROSY – Enhancing ^{15}N steady-state polarization





^{1}H pathway $H_z \xrightarrow{INEPT} \pm 2H_zN_x = \pm\left(H^\alpha N_x - H^\beta N_x\right) \xrightarrow{ST2-PT} \pm N^\beta H_x$

^{15}N pathway $N_z \xrightarrow{INEPT} N_x = \left(H^\alpha N_x + H^\beta N_x\right) \xrightarrow{ST2PT} N^\beta H_x$

3rd pathway $\ldots \xrightarrow{relaxation\,(\Delta)} H_z \xrightarrow{ST2-PT} -N_z \xrightarrow{180N} +N_z$

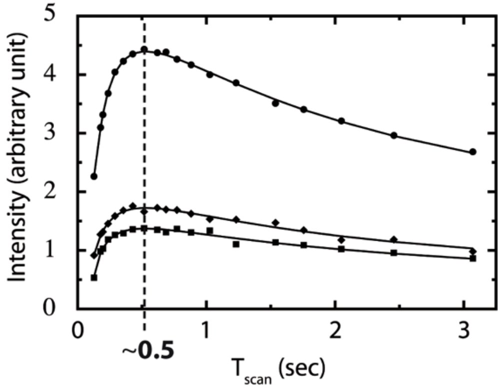
- Benefits from high B_0 fields (increased CSA-DD CCR & long ^{15}N T_1)
- ^{15}N polarization increases for short recycle delays (BEST) and long relaxation delays Δ

Favier & Brutscher, *J Biomol NMR(49)* **2011**, 9.

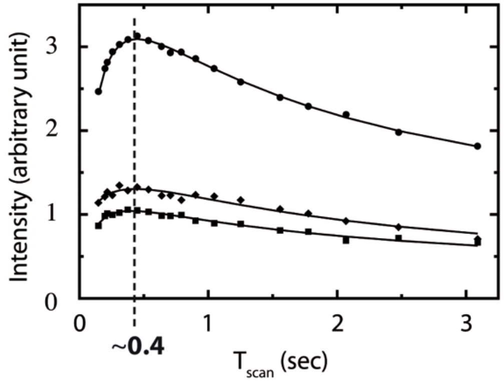
BEST-HSQC and BEST-TROSY Experiments

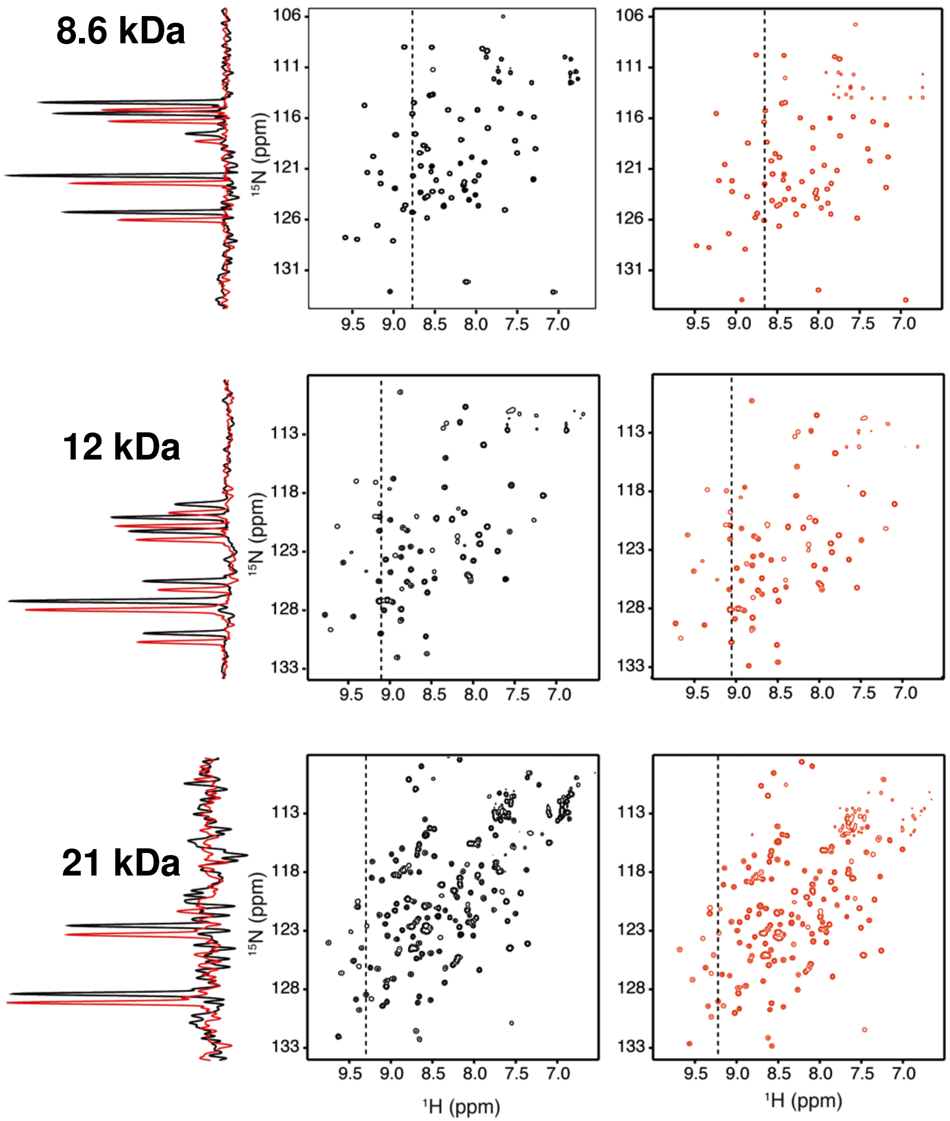
600 MHz, 25 °C

BEST ^{1}H-^{15}N HSQC



BEST ^{1}H-^{15}N TROSY

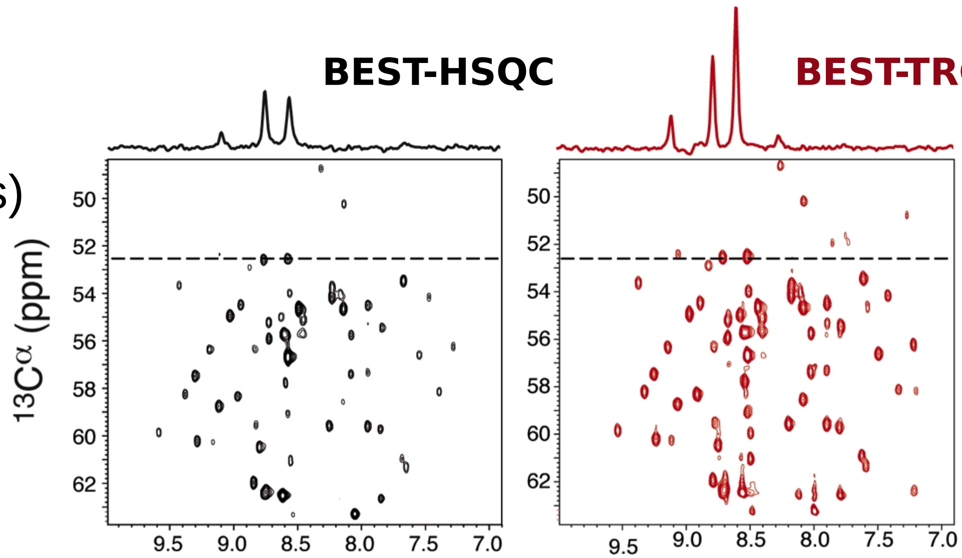




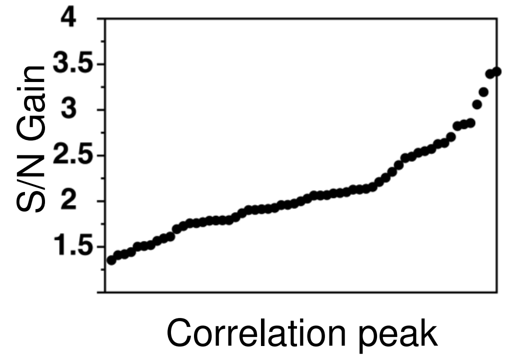
BEST-HSQC and BEST-TROSY Experiments

i-HNCA

(Δ ≈ 100ms)

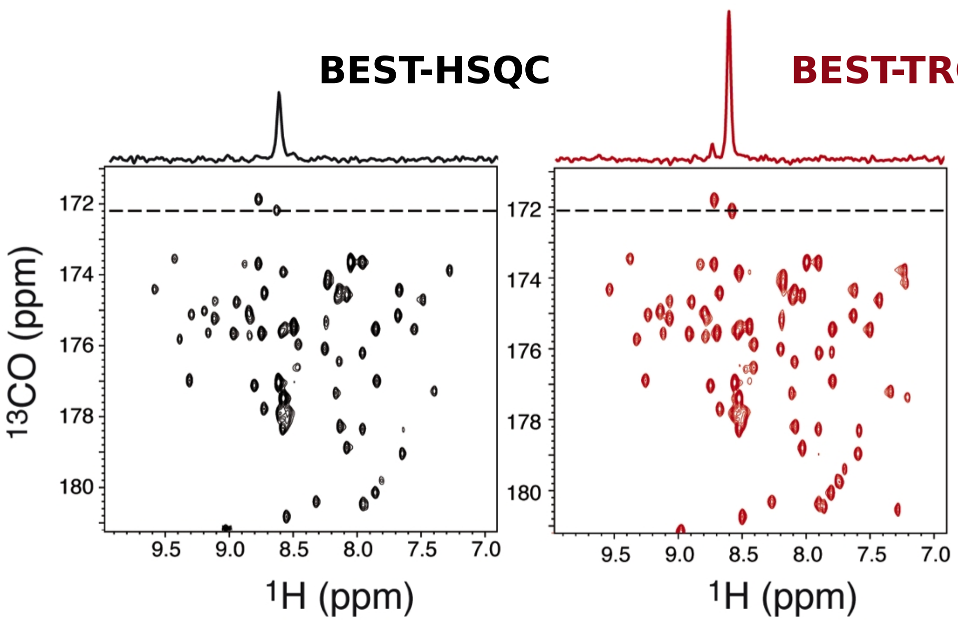


Average gain: ~ **110%**

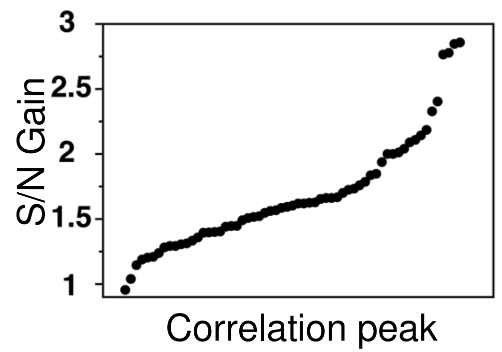


HNCO

(Δ ≈ 60ms)



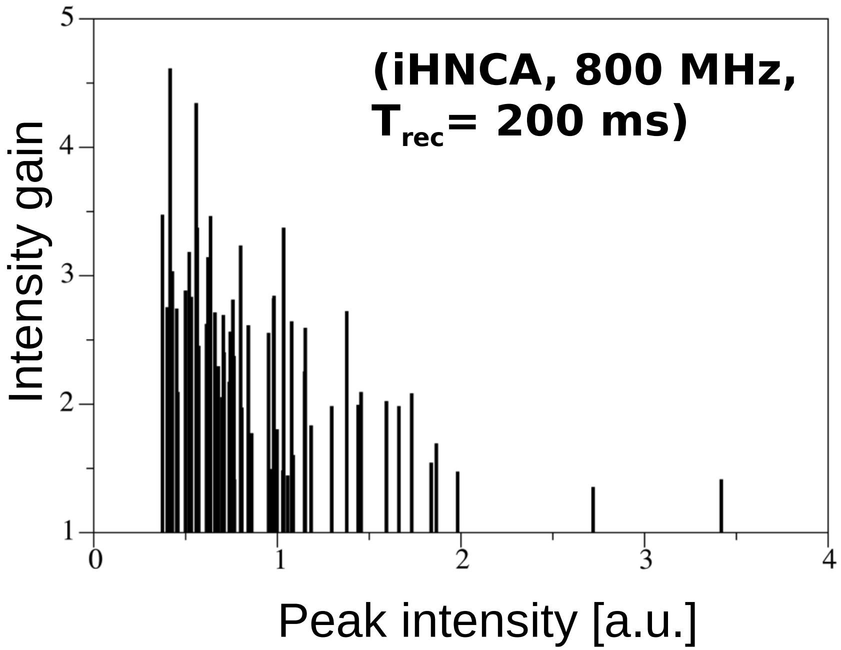
Average gain: ~ **70%**



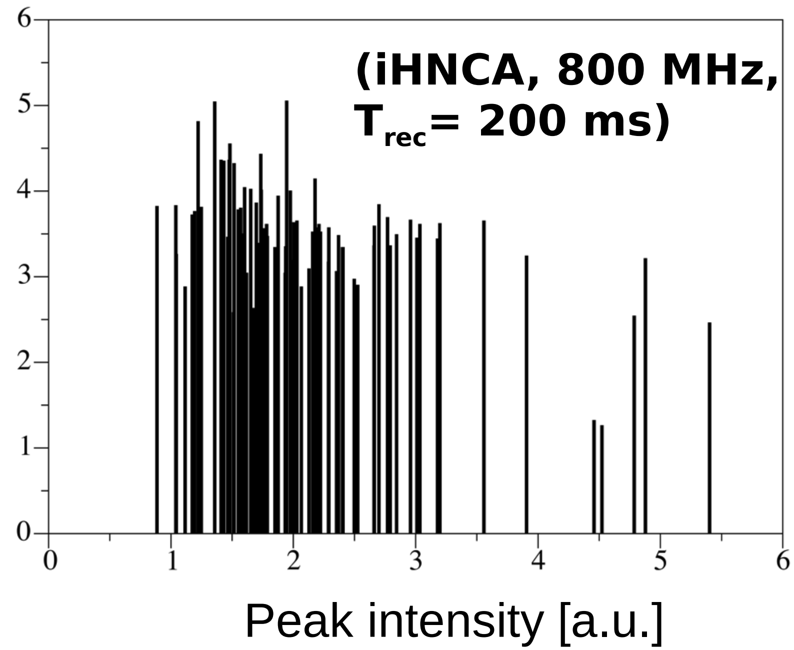
BEST-HSQC and BEST-TROSY Experiments

Application to a 138-residue (16 kDa) protein

BEST-TROSY versus BEST-HSQC



BEST versus standard

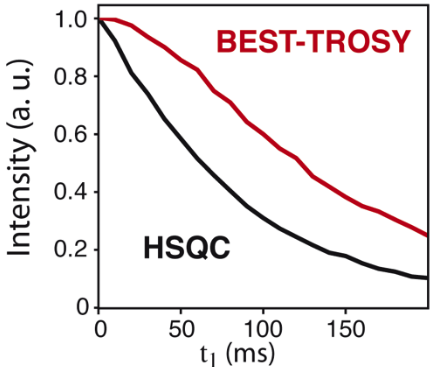


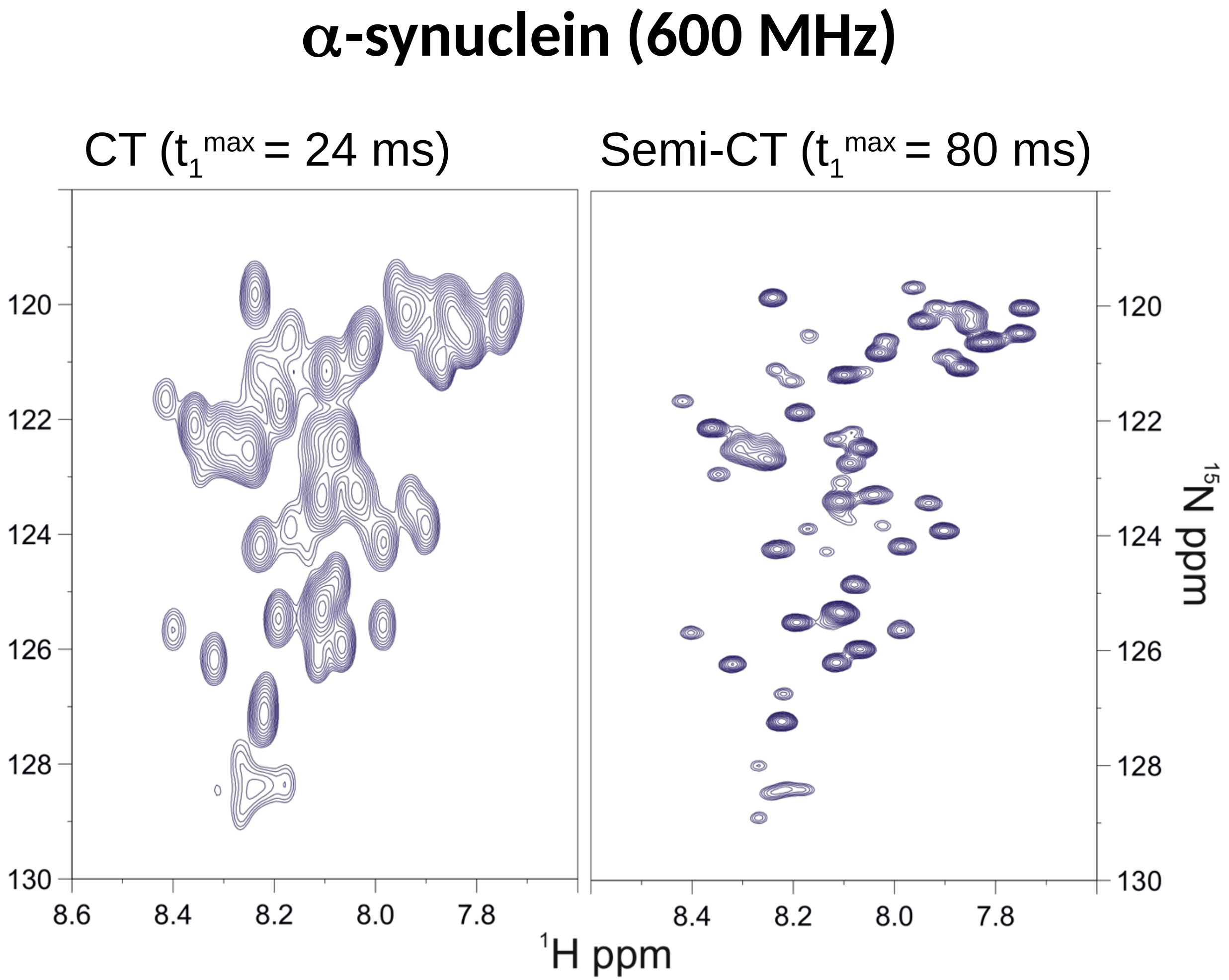
BEST-TROSY versus standard

Sensitivity enhancement of up to an order of magnitude !!
Weak signals are enhanced more !!

3D BEST-TROSY experiments

➢ **High resolution in ^{15}N dimension using semi CT editing**





Fast-pulsing techniques (SOFAST, BEST) - Summary

- **SOFAST**

  Most sensitive and fastest **2D** experiment

  but: MQ line widths, no extension to 3D !

- **BEST-HSQC**

  ≥ **3D** experiments possible

  SQ line widths

  Optimal performance at **< 800 MHz**

- **BEST-TROSY**

  ≥ **3D** experiments possible

  Highest spectral resolution

  Optimal performance at **≥ 800 MHz**

- Significant gain in speed, sensitivity, and evtl. spectral resolution

SOFAST/ BEST experiments – Applications

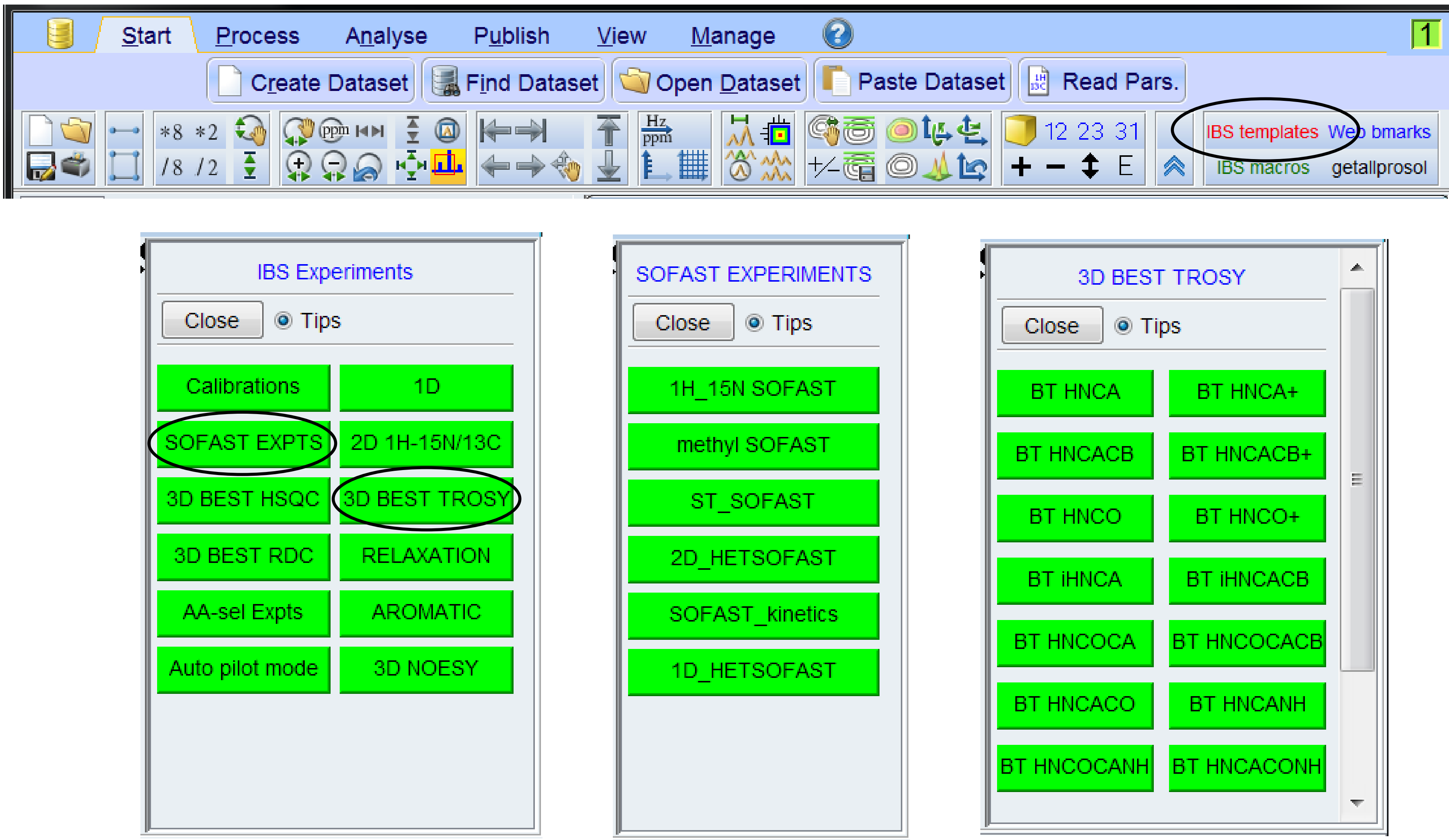
- **Sample quality control**
  High sensitivity 2D H-N correlation experiments
  Information on structural compactness from HET-SOFAST/BEST
  Information on oligomerization state from 1D BT-TRACT

- **NMR assignment**
  BEST HNC, BEST HNC^{+} and BEST HNN correlation experiments
  Pro-selective 2D H-N experiments.

- **Structural & dynamic information**
  Measurement of backbone scalar couplings and RDCs
  Solvent exchange rates from HET-SOFAST/BEST
  Conformational exchange from BT-CPMG-RD experiments

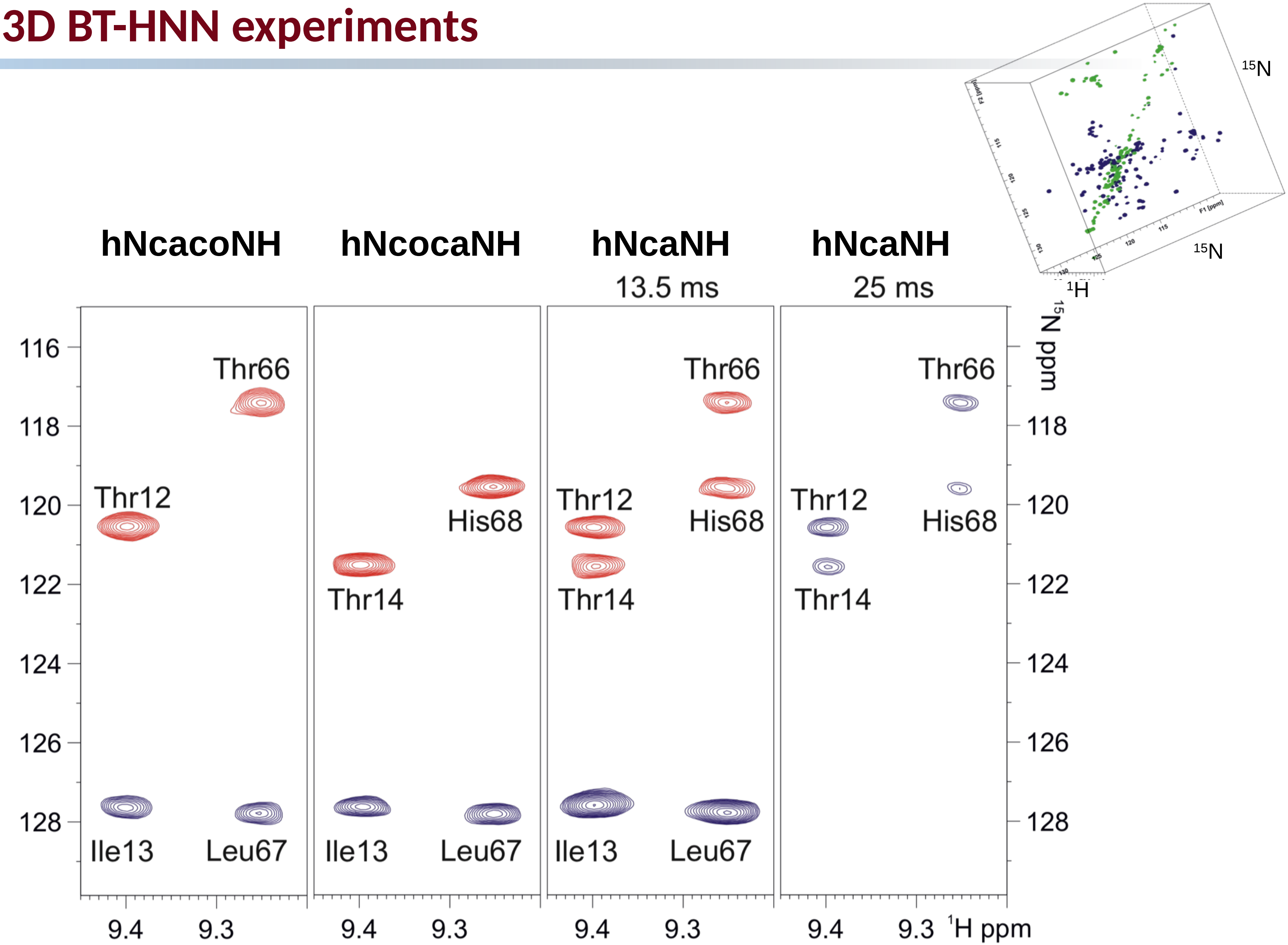
- **Kinetic information**
  Real-time 2D and 3D SOFAST/BEST experiments

NMRlib: easy use of BEST/SOFAST experiments



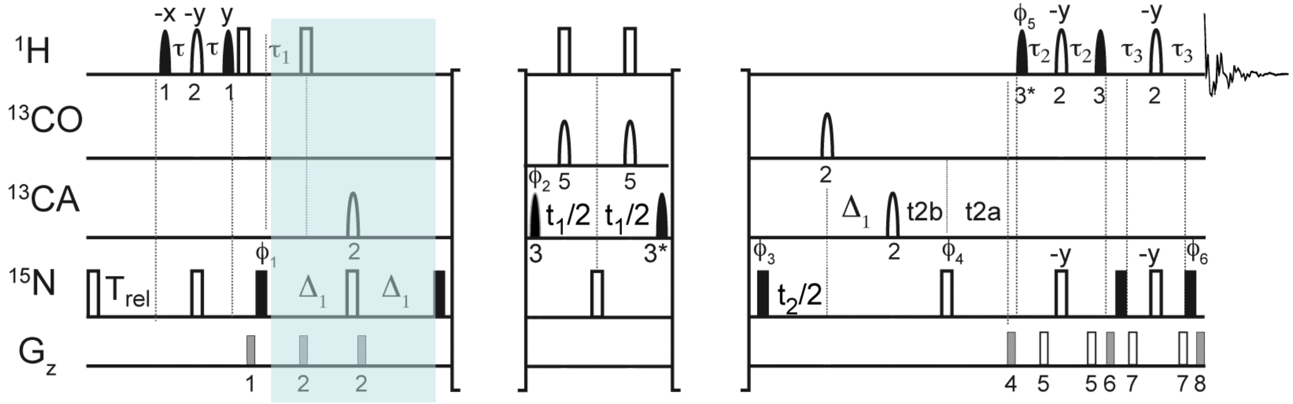
http://www.ibs.fr/science-213/scientific-output/software/pulse-sequence-tools/

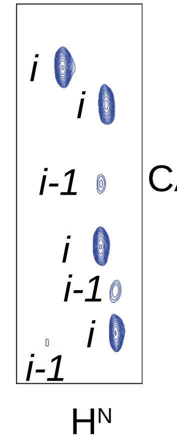
3D BT-HNN experiments



BT-HNC+ experiments – improved performance

Example: HNCA

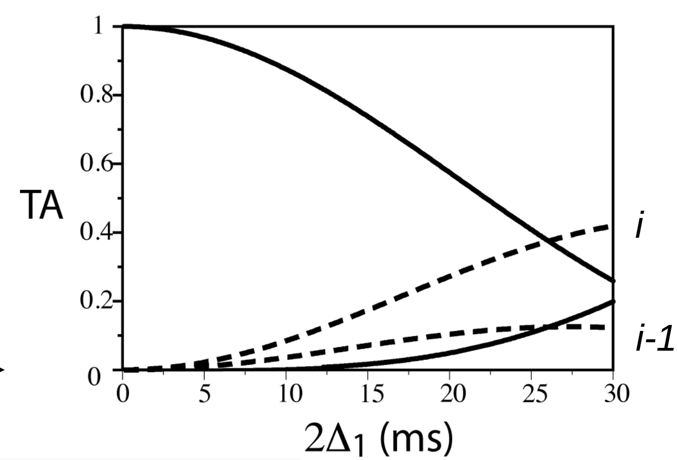




$$H \xrightarrow{J_{NH}} N \xrightarrow{{}^1J_{NCA},\,{}^2J_{NCA}} CA \xrightarrow{t_1} \text{back transfer}$$

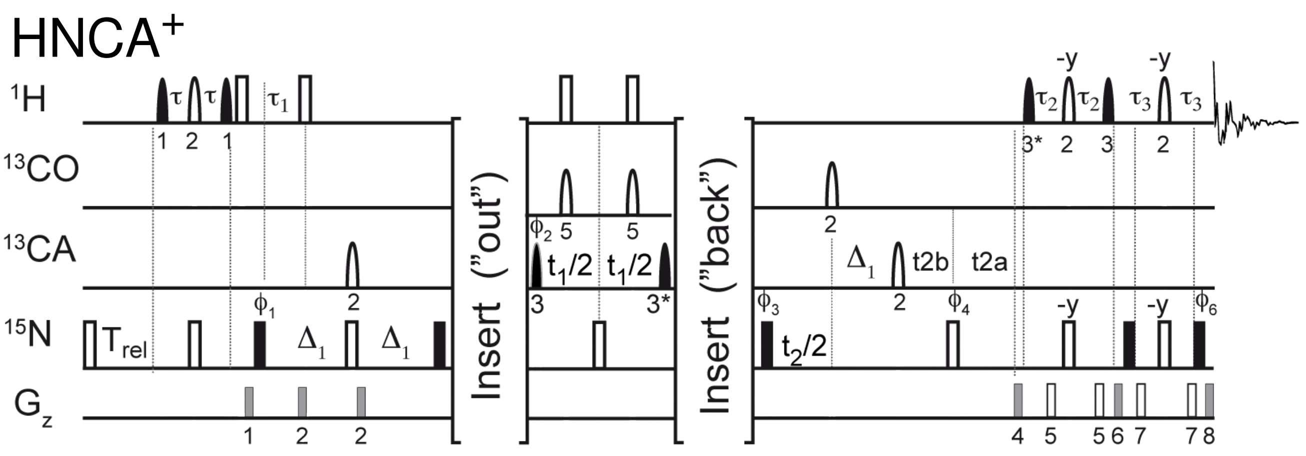
N^i_x →

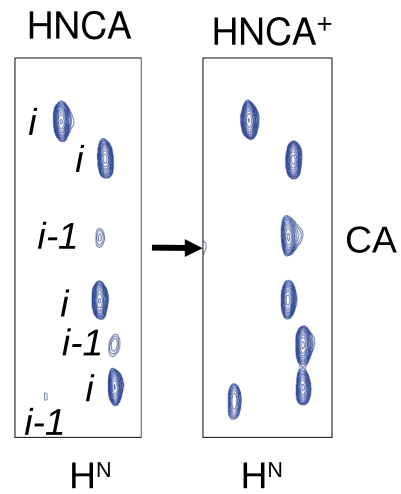
- $N^i_x \cos^2(\pi\, {}^1J\, 2\Delta_1)\cos^2(\pi\, {}^2J\, 2\Delta_1)$ → X
- $N^i_y CA^i_z \sin^2(\pi\, {}^1J\, 2\Delta_1)\cos^2(\pi\, {}^2J\, 2\Delta_1)$ →
- $N^i_y CA^{i-1}_z \cos^2(\pi\, {}^1J\, 2\Delta_1)\sin^2(\pi\, {}^2J\, 2\Delta_1)$ →
- $N^i_x CA^i_z CA^{i-1}_z \sin^2(\pi\, {}^1J\, 2\Delta_1)\sin^2(\pi\, {}^2J\, 2\Delta_1)$ → X

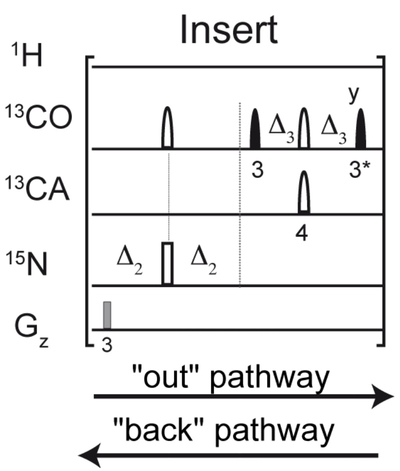


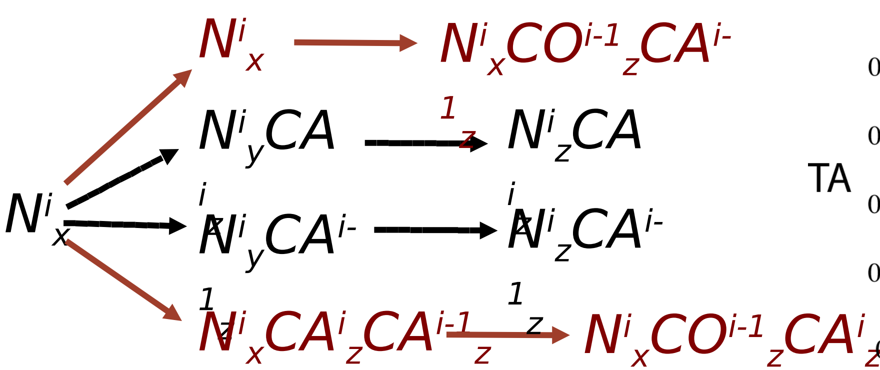
➢ **Only 2 out of 4 pathways contribute to the NMR signal !**

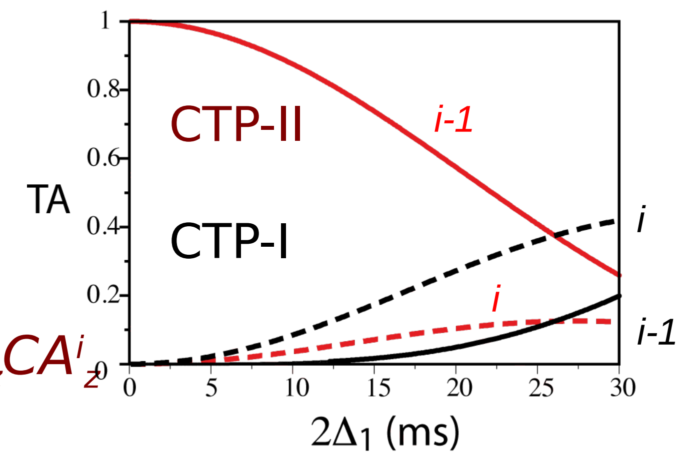
BT-HNC^{+} experiments – improved performance







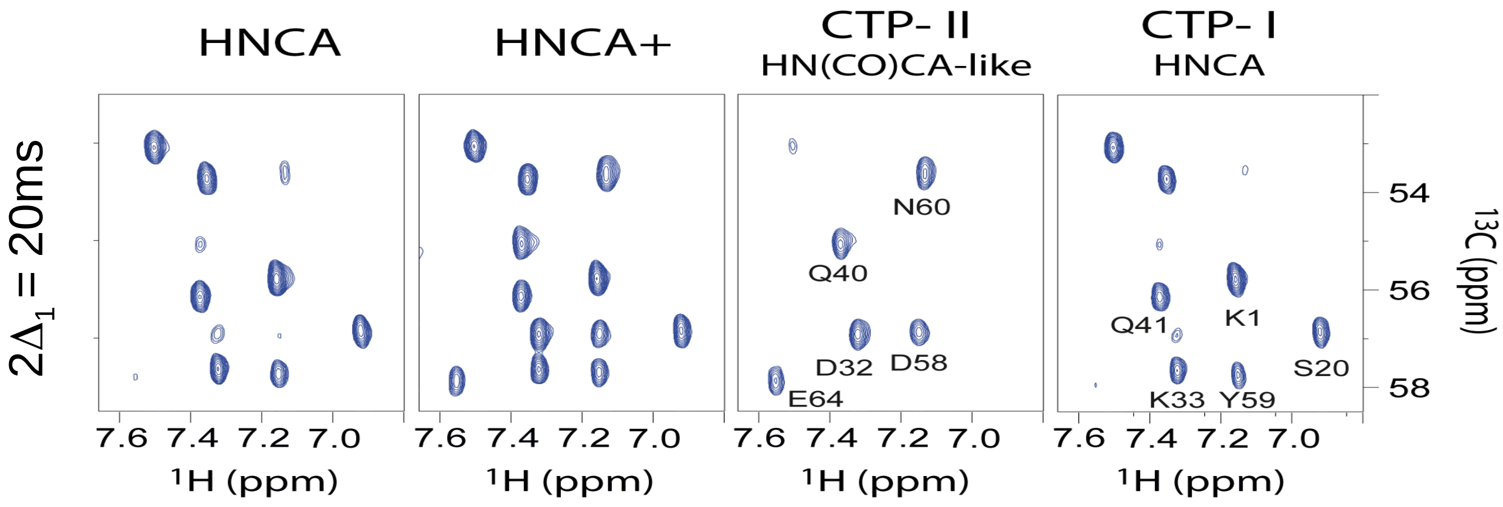


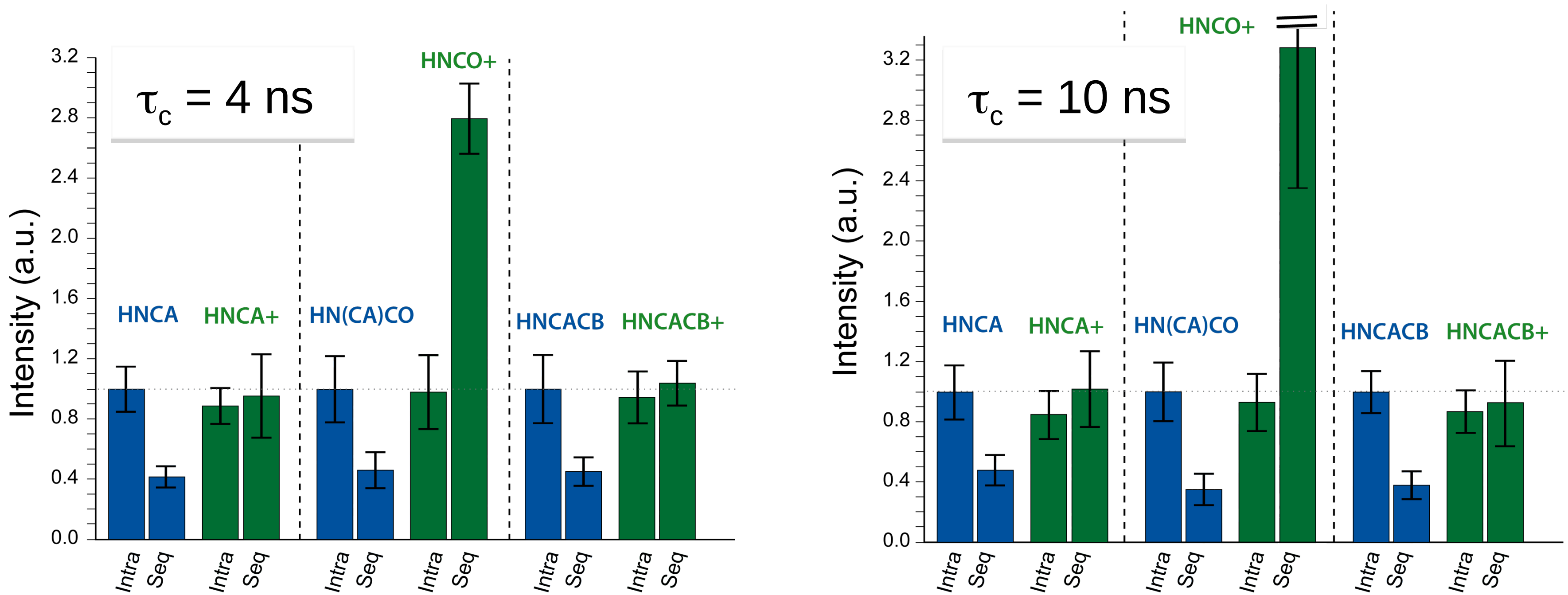


➢ All 4 pathways contribute to the NMR signal !

Gil-Caballero et al.
J Biomol NMR(60) **2014**, 1-9.

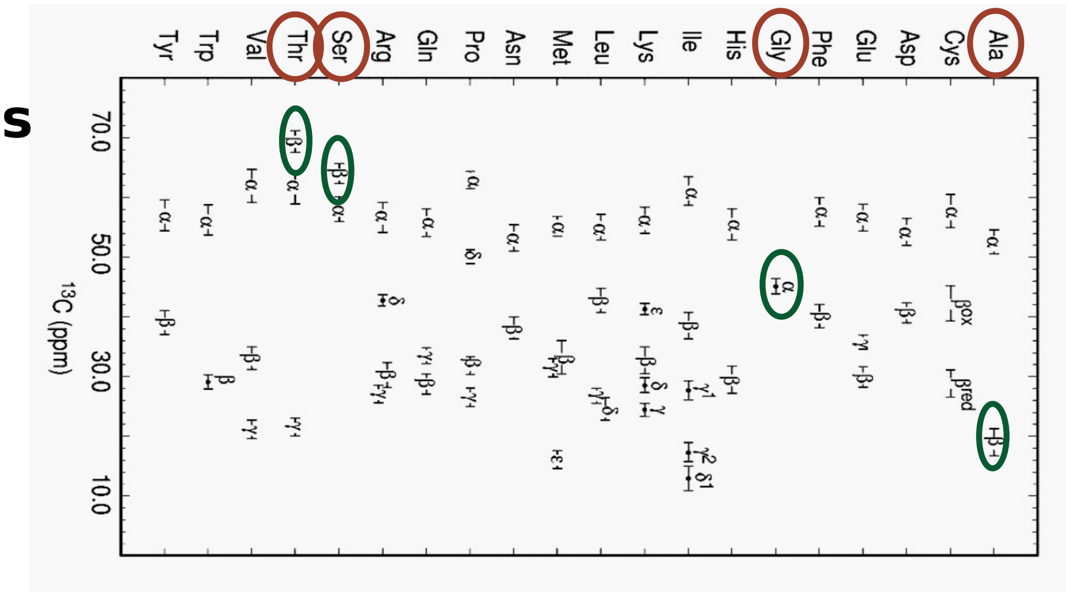
BT-HNC^{+} experiments – improved performance



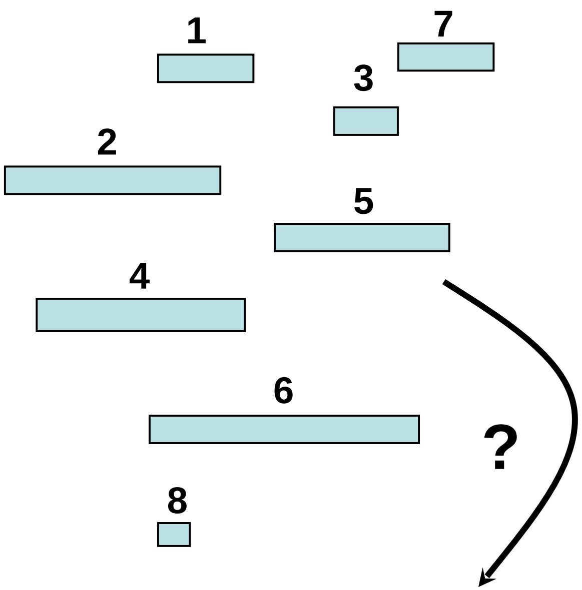


Amino-acid-type information for sequential assignment

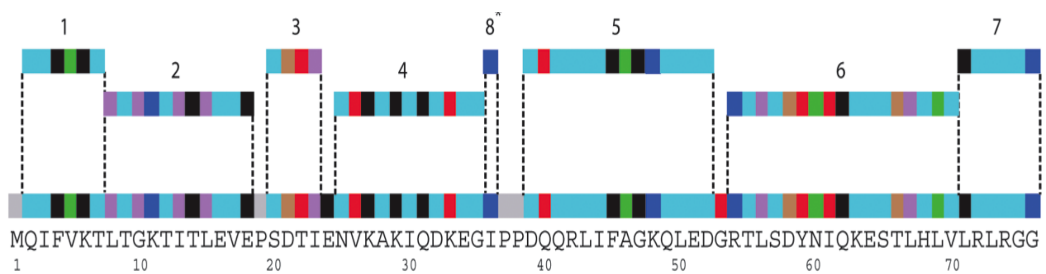
CA/CB chemical shifts



Peptide fragments:

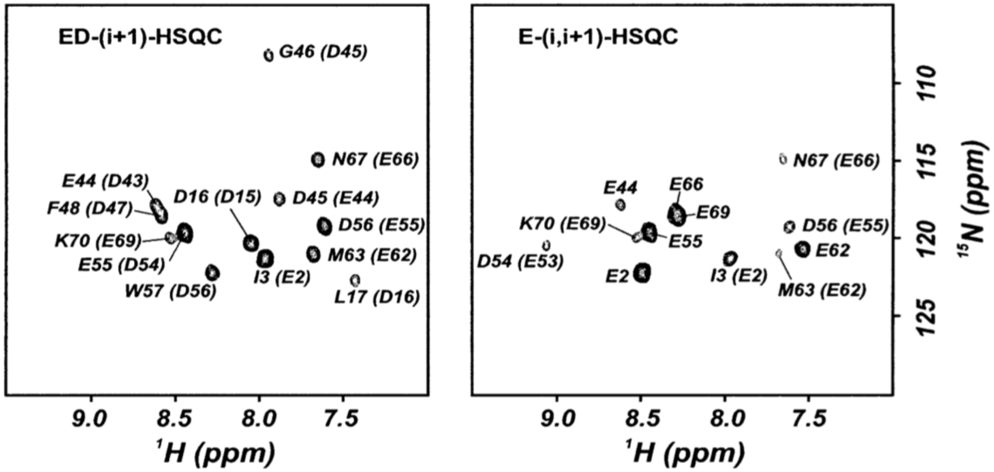


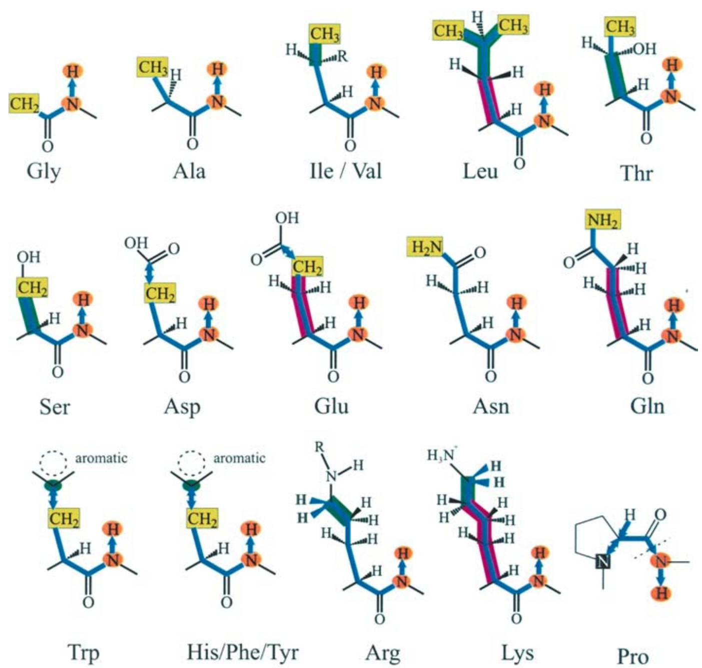
Amino-acid-type anchoring



MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG
1 10 20 30 40 50 60 70

Amino-acid-type selection





Name of the experiment	Scans	Experiment time
3D-CBCA(CO)NNH	8	1 day 7 h
3D-CBCANNH	16	2 days 14 h
G-(i + 1)-HSQC	8	15 min
G-(i, i + 1)-HSQC	16	30 min
A-(i + 1)-HSQC	48	1 h 30 min
A-(i, i + 1)-HSQC	96	3 h
S-(i, i + 1)-HSQC	32	1 h
S-(i + 1)-HSQC	64	2 h
TA-(i + 1)-HSQC	48	1 h 30 min
TA-(i, i + 1)-HSQC	96	3 h
VIA-(i + 1)-HSQC	48	1 h 30 min
VIA-(i, i + 1)-HSQC	96	3 h
LA-(i + 1)-HSQC	96	3 h
LA-(i, i + 1)-HSQC	192	6 h
N-(i + 1)-HSQC	64	2 h
N-(i, i + 1)-HSQC	128	4 h
QN-(i + 1)-HSQC	128	4 h
Q-(i, i + 1)-HSQC	256	8 h
DE-(i + 1)-HSQC	32	1 h
D-(i + 1)-HSQC	64	2 h
DE-(i + 1)-HSQC	64	2 h
E-(i, i + 1)-HSQC	128	4 h
FHY-(i + 1)-HSQC	64	2 h
FHY-(i, i + 1)-HSQC	128	4 h
W-(i + 1)-HSQC	64	2 h
W-(i, i + 1)-HSQC	128	4 h
P-(i + 1)-HSQC	128	4 h
P-(i, i − 1)-HSQC	128	4 h

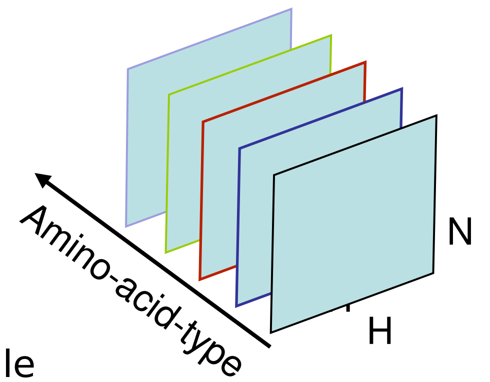
Schubert et al., *J Biomol NMR (31)* **2005**, 115.

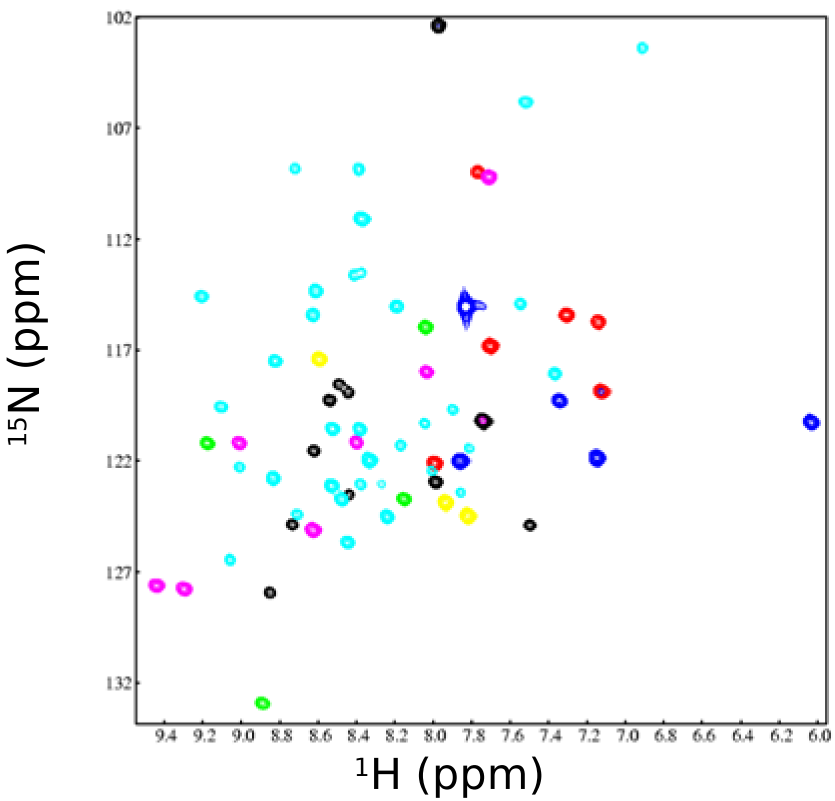
Amino-acid-type editing

HADAMAC

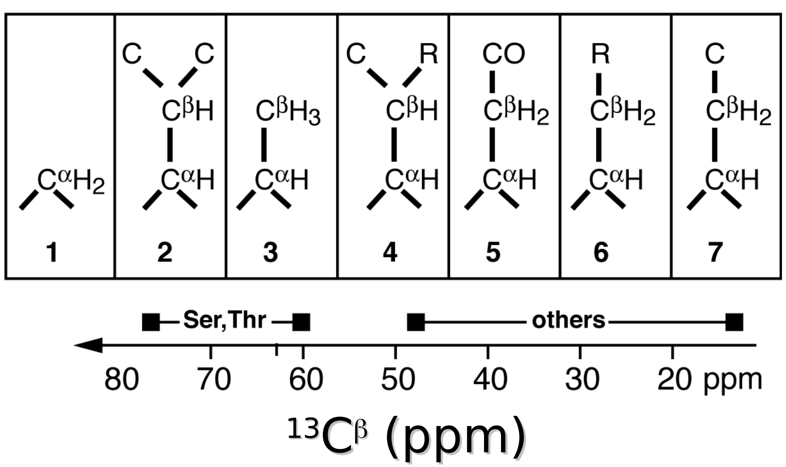
HADamard AMino-ACid-type edited

2D (HCBCACO)NH experiment





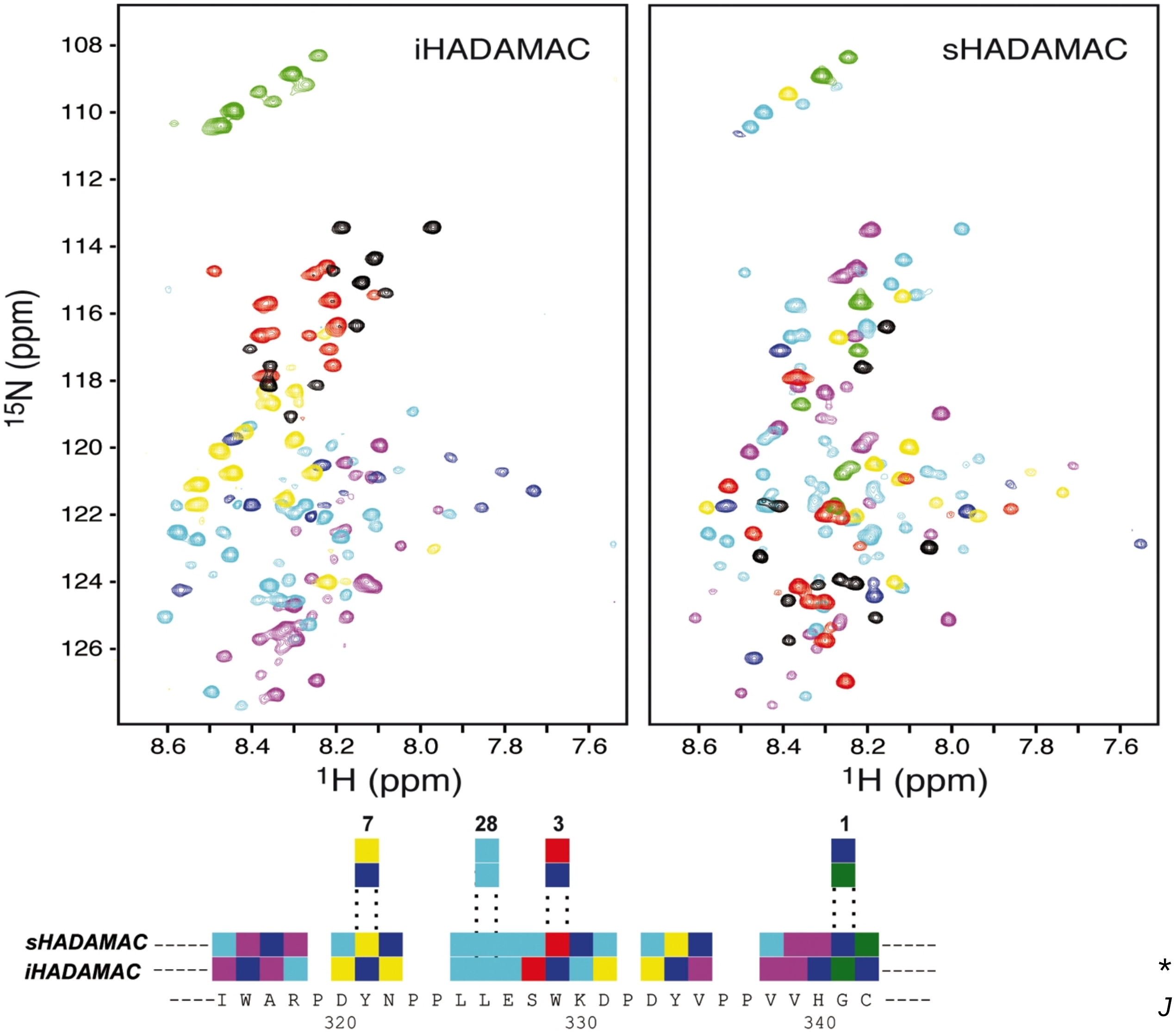
Black: Ala, Val, Ile
Red: Asn, Asp
Green: Cys, aromatic residues
Blue: Gly
Yellow: Ser
Magenta: Thr
Cyan: all others

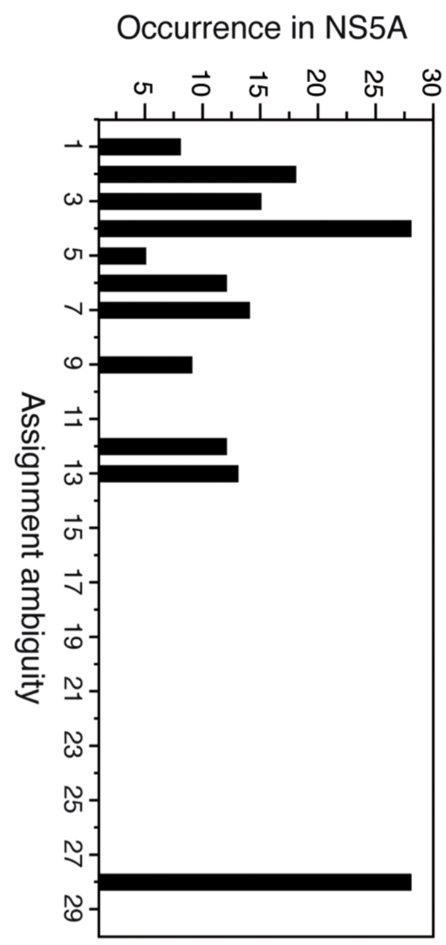


Lescop, Rasia & Brutscher, *JACS (130)* **2008**, 5014.

Intra-residue * and sequential HADAMAC

➢ **Complementary assignment tools for IDPs**





* Feuerstein, Plevin, Willbold, Brutscher, *J Magn Reson (214)* **2012**, 329.

Internet ressources

BEST:
http://www.ibs.fr/science-213/scientific-output/software/pulse-sequence-tools/article/ibs-pulse-sequence-tools-for

ASCOM: http://www.icsn.cnrs-gif.fr/download/nmr

qMDD: https://groups.google.com/forum/#!forum/mddnmr
http://pc8.nmr.gu.se/~mdd/*Downloads*

NUS (Wagner):
http://gwagner.med.harvard.edu/intranet/hmsIST/index.html