HIFI NMR : part1
automated backbone assignments using 3D->2D

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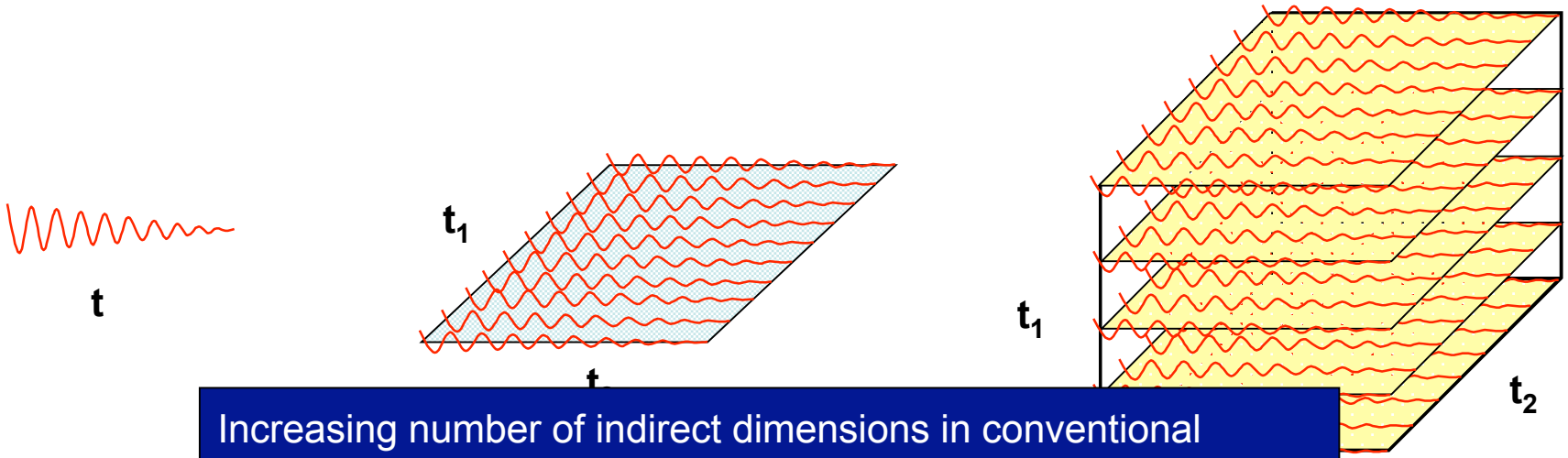
Recording multidimensional experiments is time costly

In conventional multidimensional experiments, all the individual frequency domains are incremented (sampled) independently



Increasing number of indirect dimensions in conventional multidimensional:

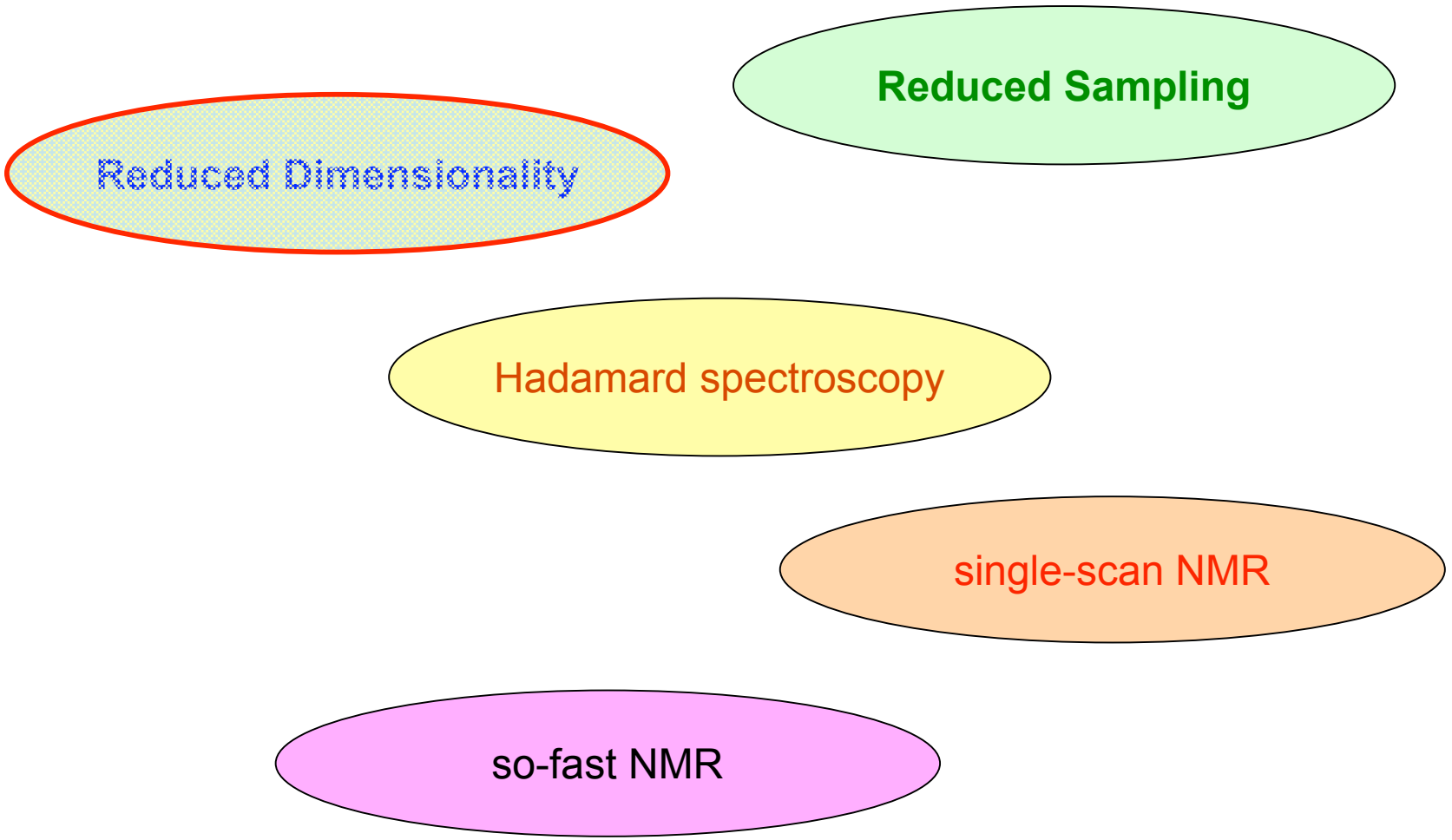
- collection time increases exponentially
- need to reduce number of increments to keep collection time reasonable: low resolution
- not very practical above 3 dimensions
- impossible above 4 dimensions

1D **1 FID**
30 sec.

,384 FIDs
rs.

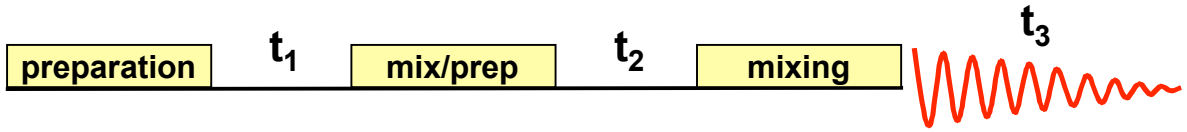
2=32,768 FIDs
hrs.

Fast methods

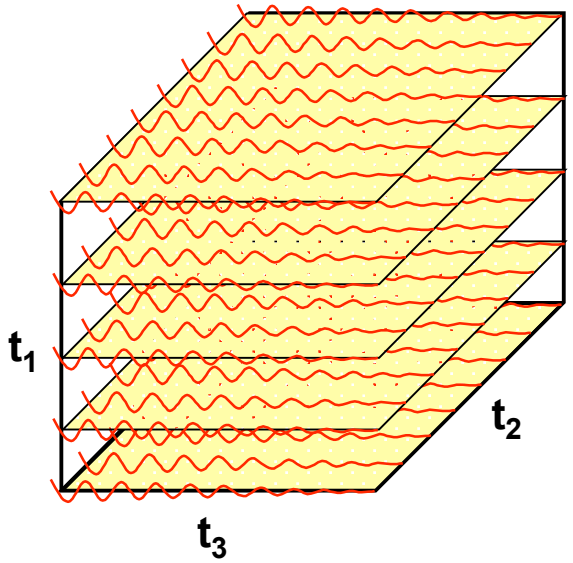


Reduced Dimensionality Techniques

two or more indirect dimensions are evolved simultaneously

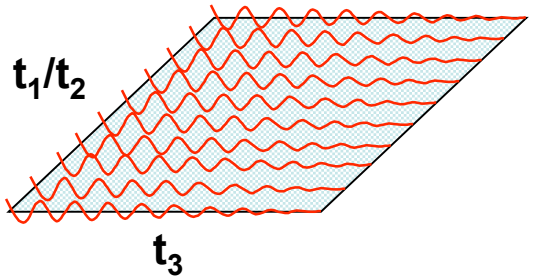


Conventional 3D spectrum



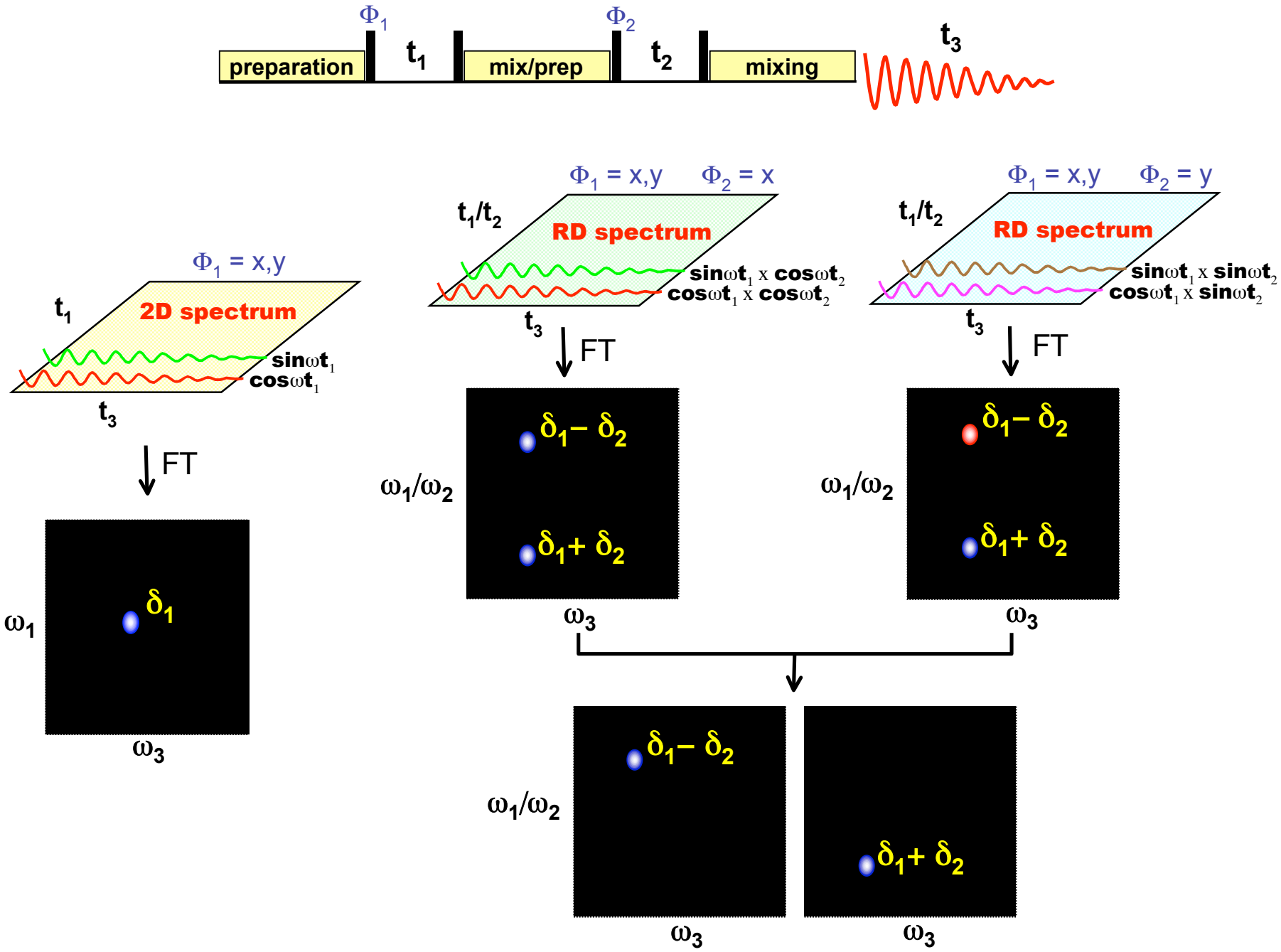
t_1 and t_2 are incremented independently

RD spectrum

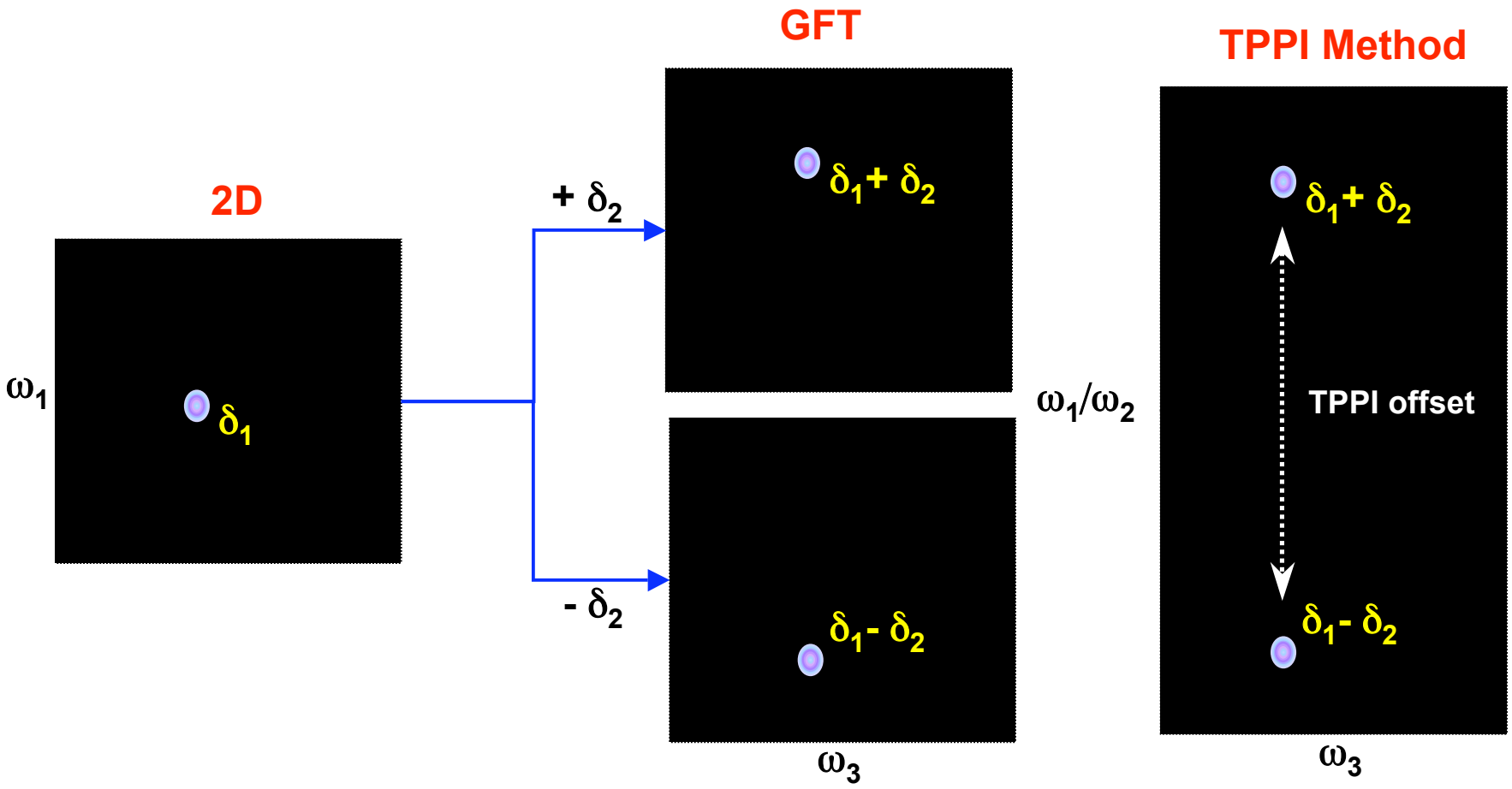


t_1 and t_2 are incremented simultaneously point by point

Reduced Dimensionality Techniques …

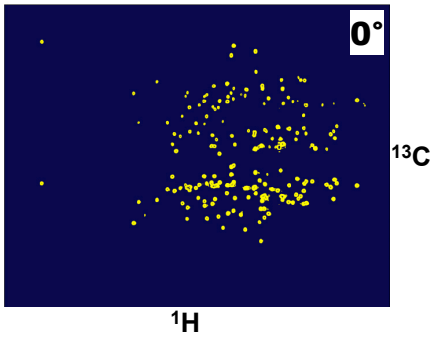


The peaks in RD experiments can either be separated into different spectra (**GFT** - Syperzky) or into different regions of the same spectrum (**TPPI** - Gronenborn)

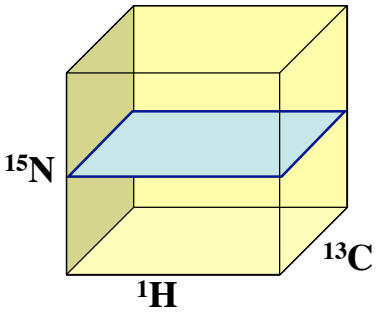


2D RD planes of 3D spectra → 2D projections of 3D spectra ≡ tilted planes

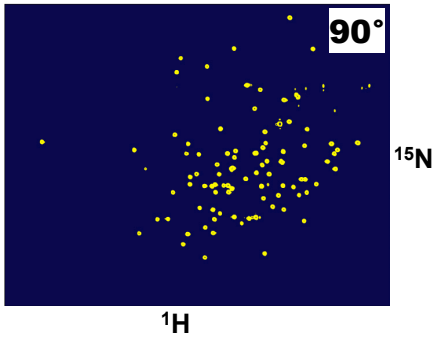
^{1}H-^{13}C plane of CBCA(CO)NH



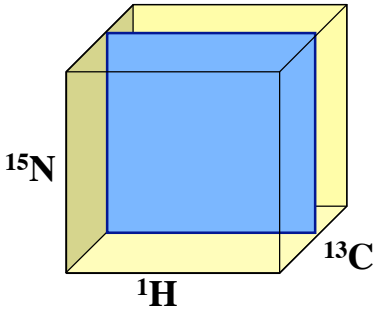
→ 0° plane



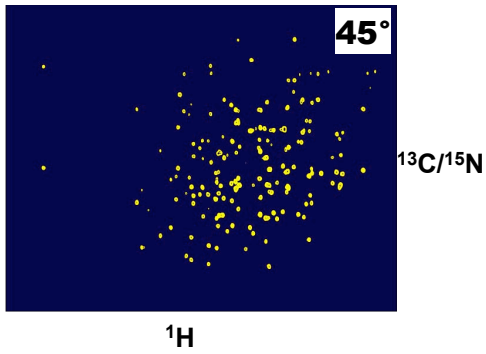
^{1}H-^{15}N plane of CBCA(CO)NH



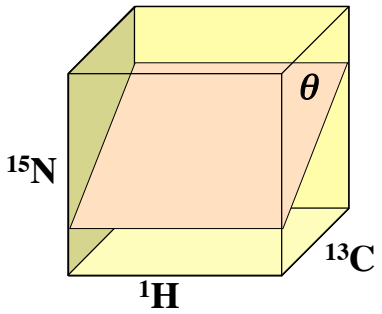
→ 90° plane



^{1}H-^{13}C/^{15}N plane of CBCA(CO)NH



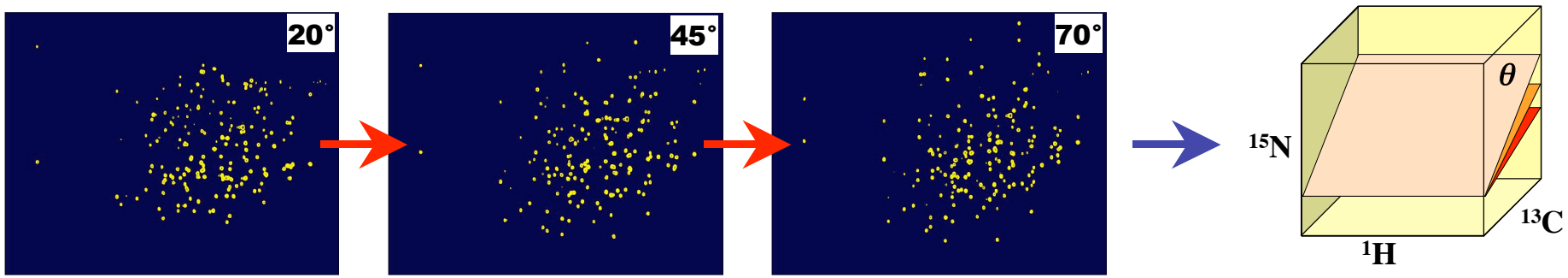
→ tilted plane



Reduced Dimensionality Techniques …

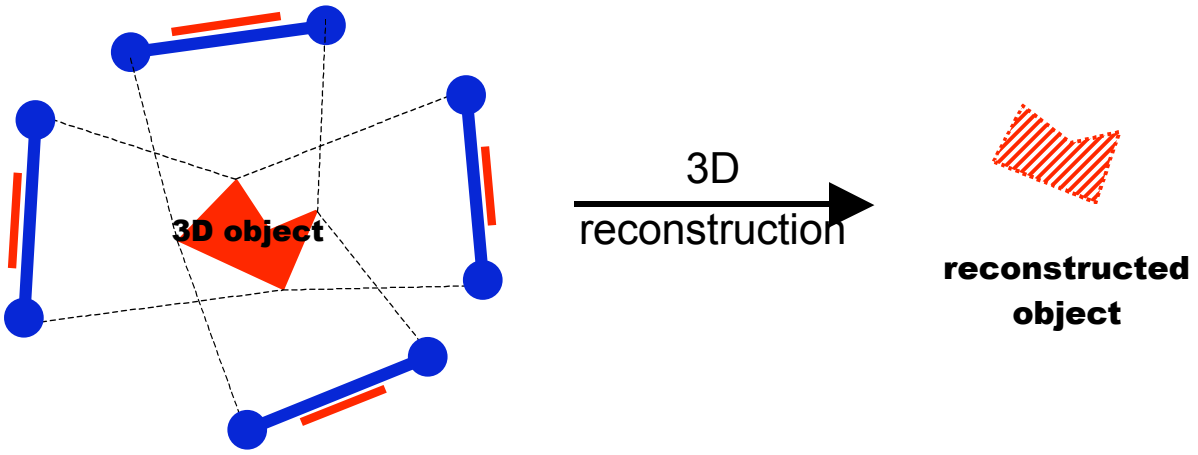
By changing the ratio between the two simultaneously evolving dimensions we can change the projection angle of the tilted plane

$$\frac{t_{^{13}C}}{t_{^{15}N}} \rightarrow \theta$$

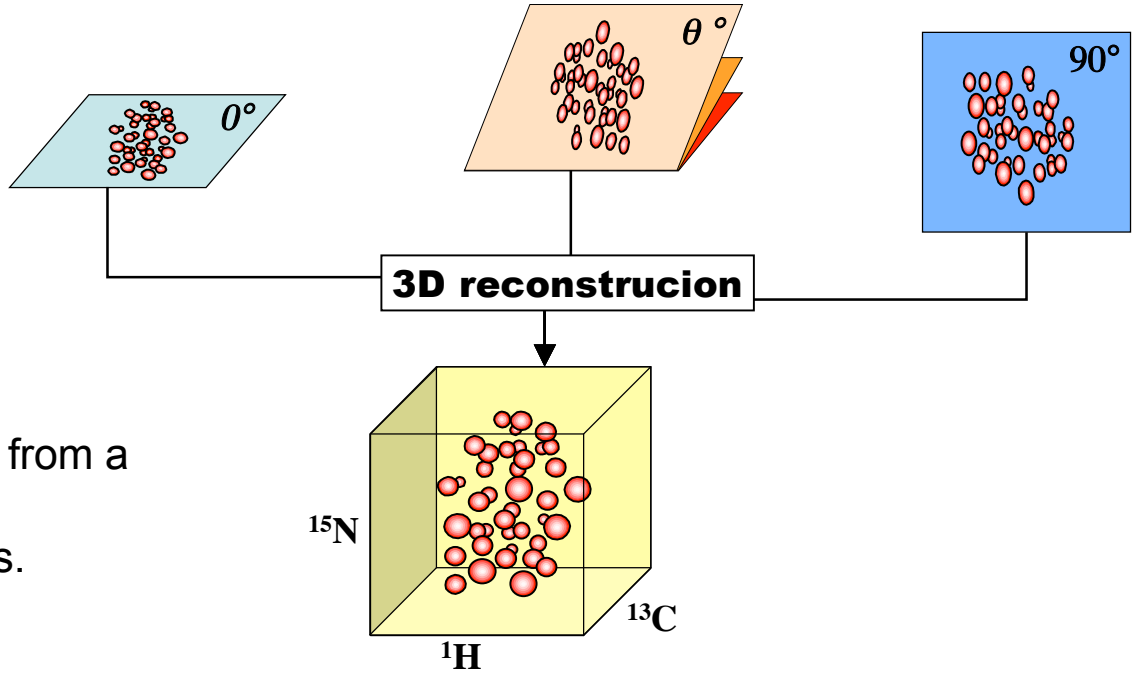


Projection Reconstruction

Simple 3D objects can be reconstructed from 2D projections collected at different angles



In principle, it is feasible to reconstruct a 3D spectrum from a number of 2D tilted planes collected at different angles.



GFT / TPPI method

- n-dimensional experiments are run as two-dimensional RD spectra
- split peaks can be separated into different spectra (GFT) or different regions of the same spectrum (TPPI)
- indirect frequencies are extracted from analysis of split patterns

Projection-Reconstruction

- multiple tilted planes are collected at different angles
- n-dimensional spectra are reconstructed from several tilted planes

High-resolution Iterative Frequency Identification HIFI

simultaneously evolving indirect frequencies are extracted from two-dimensional RD spectra

multiple tilted planes are used

angle of tilted planes is chosen **adaptively** in real time

Reduced Dimensionality Techniques …

GFT / TPPI method

- it relies on combining multiple indirect dimension to resolve overlapped peaks
- with each added indirect dimension:
  - S/N is reduced by √2
  - collection time is doubled
- anal
- diffic

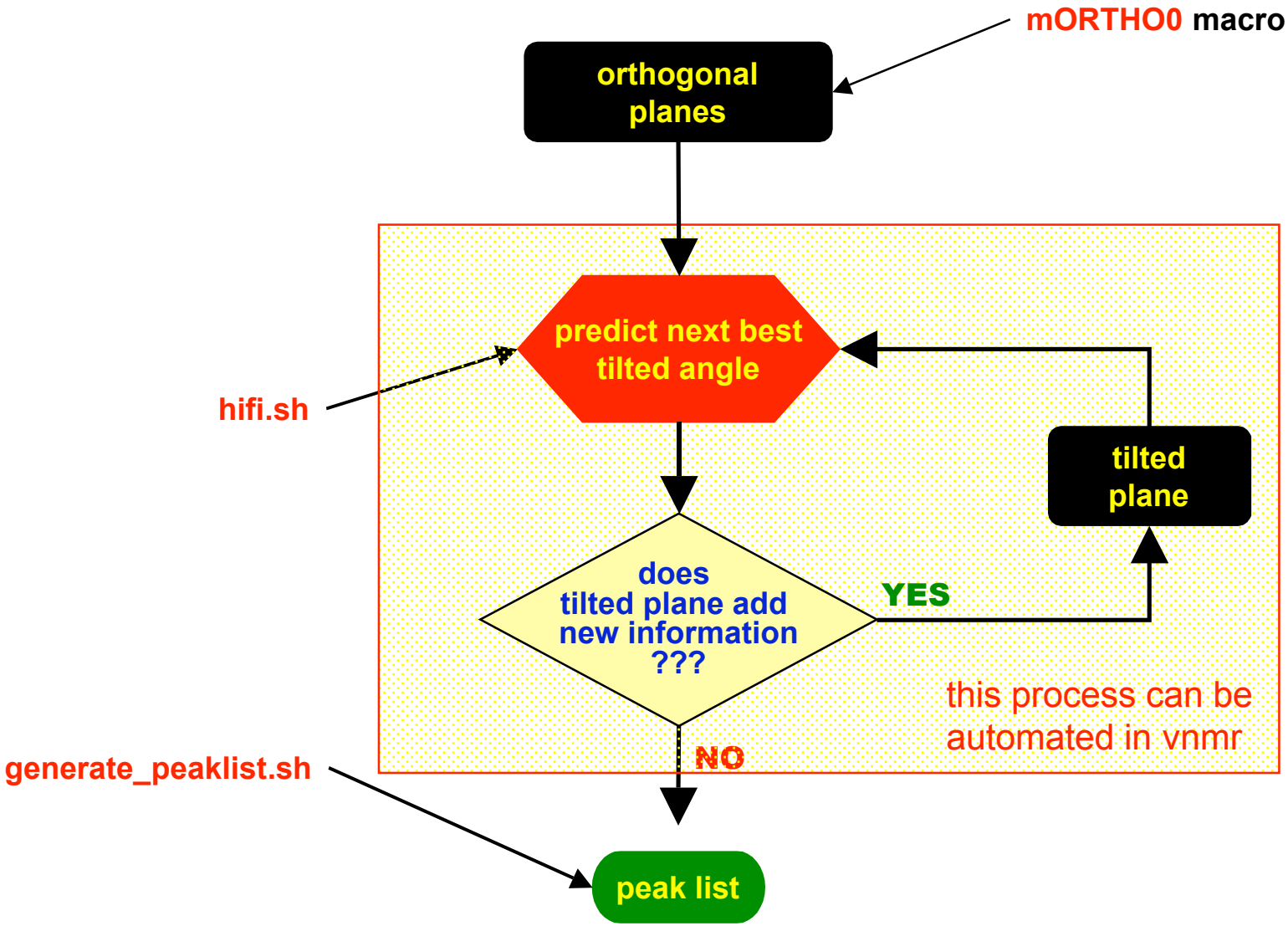
Projection-Reconstruction

- multidimensional frequency information can only be extracted after spectra reconstruction
- reconstructing spectra can be very complicated or impossible with overlapped peaks and/or low S/N

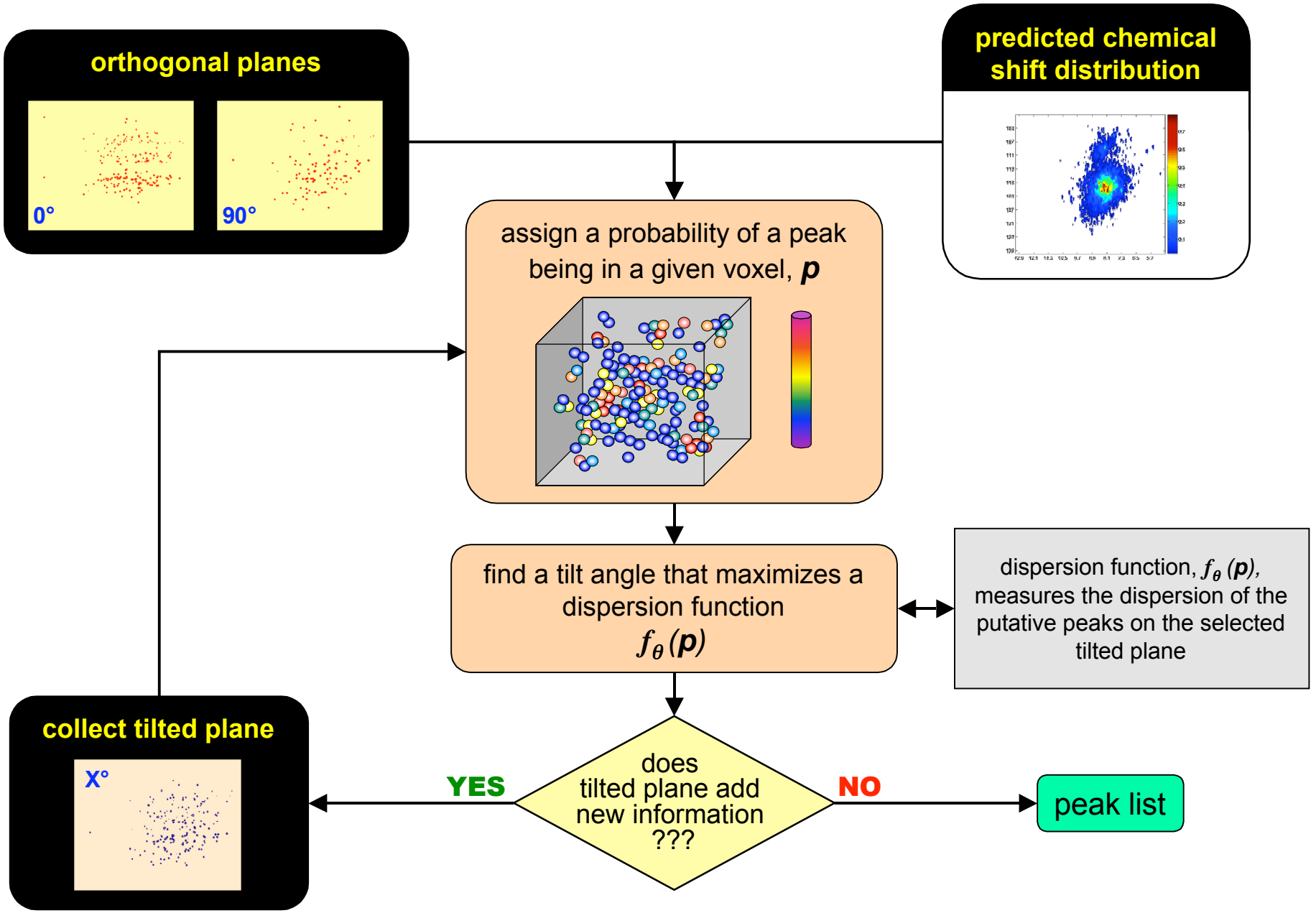
HIFI

- by changing the tilt angle, HIFI has greater potential of resolving overlapped peaks than GFT/TPPI methods
- since only frequency information is extracted from tilted planes, HIFI does not need to resolve the problem of reconstructing nD volumes
- easier to analyze
- easier to automate
- by **adaptively** choosing the next tilt angle, HIFI optimizes information gain while minimizing time collection

HIFI flowchart

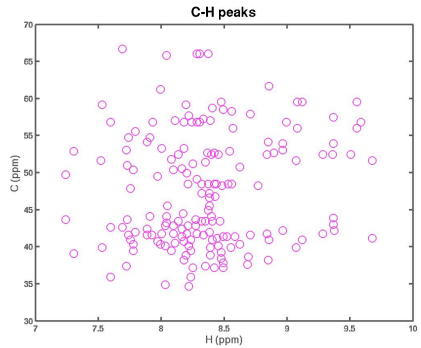


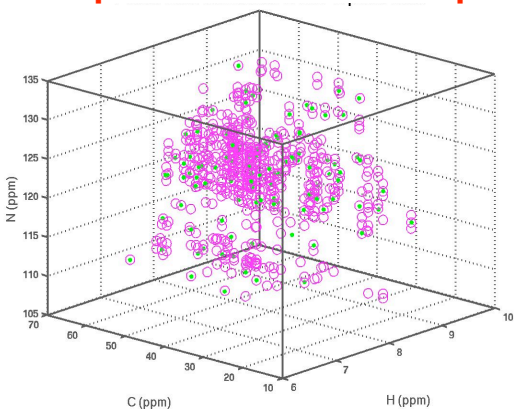
HIFI algorithm for predicting best tilted angle

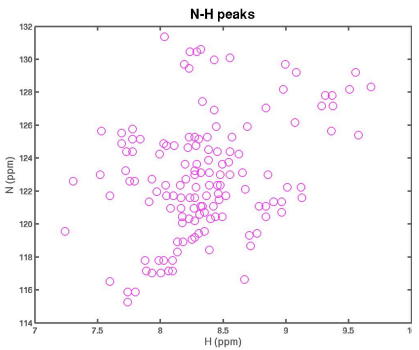


Eghbalnia et al **JACS** 127 (36), 12528 -12536, **2005**

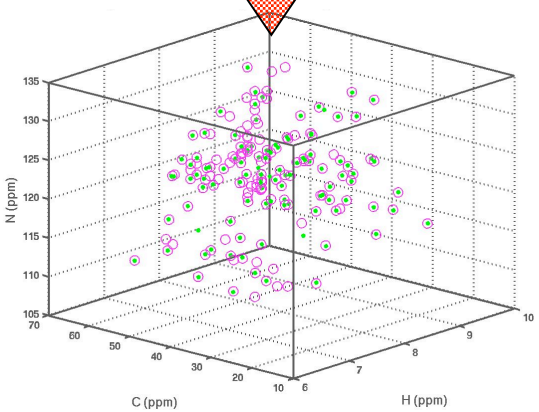
putative peaks from C-H/N-H planes



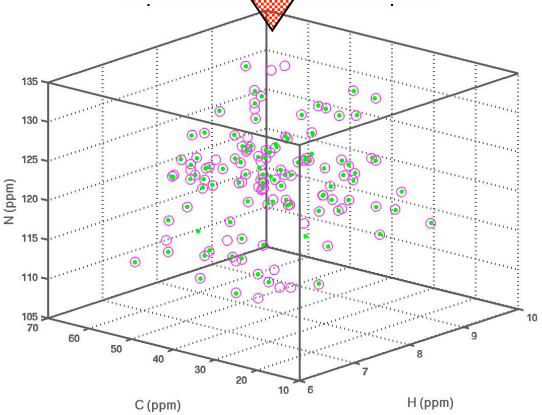




add 45° tilted plane



add additional tilted planes



HIFI on CBCA(CO)NH

Combined peaks from HIFI planes are in **magenta**

Hand picked peaks from 3D spectrum in **green**

Using HIFI for backbone assignments

Modified BioPack experiments for backbone assignments

HNCO
HN(CO)CA
HNCA
CBCA(CO)NH
HN(CA)CB
HNCACB

NH sensitivity enhanced

TROSY option

- standard sequences are robust and offer the best S/N for backbone assignments
- we rely on **HIFI** ability to **adaptively** select tilted planes to resolve overlapped peaks

- added **HIFI** option for collecting tilted planes

  ^{13}C and ^{15}N indirect dimensions are evolved simultaneously

- added semi-constant time ^{15}N evolution to allow collecting tilted planes with more indirect points for higher resolution
- optimized for cryogenic probes

Needs AUTOMATION !!!

outline of vnmr macro for automated HIFI data collection

preparation - orthogonal planes

- run othogonal planes for all experiments
  - all experiments list:
    - collect 0º plane
    - adjust ^{13}C s.w.
  - ^{1}H-^{15}N HSQC :
    - use as 90º plane
    - adjust ^{15}N s.w.
- save orthogonal planes
- process orthogonal planes
  - optimize processing parameters

input list of experiments

number of residues = XX

HNCO	hnco_tilt_0	hnco_hsqc
HN(CO)CA	hncoca_tilt_0	hnco_hsqc
HNCA	hnca_tilt_0	hnco_hsqc
CBCA(CO)NH	cbcaconh_tilt_0	hnco_hsqc
HNCACB	hncacb_tilt_0	hnco_hsqc
HN(CA)CB	hncb_tilt_0	hnco_hsqc

experiment #1 in list

run HIFI macro - tilted planes

run tilt angle predicting program

read predicted tilt angle

tilt>0

run experiment to collect tilted plane at suggested angle

save tilted plane

process tilted plane

tilt=0

run program to extract 3D frequencies

3D peak list

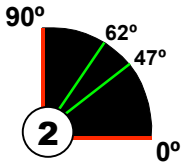
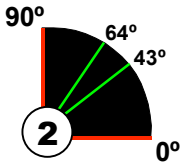
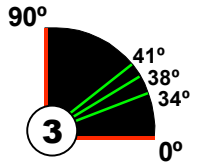
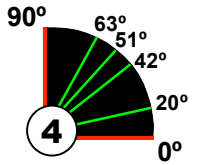
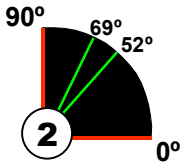
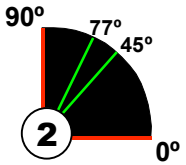
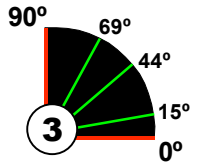
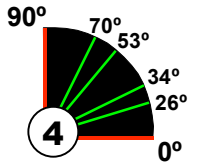
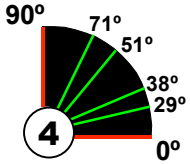
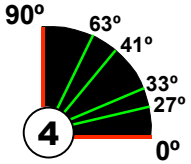
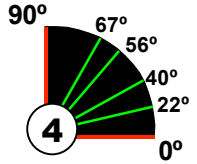
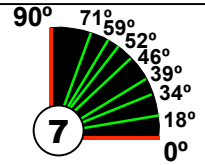
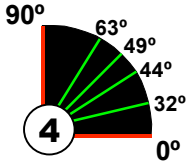
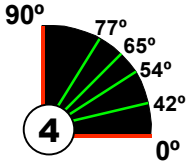
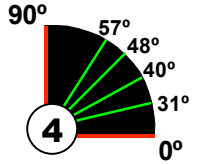
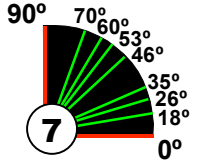
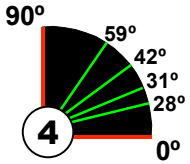
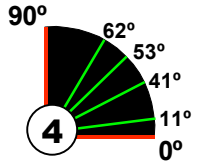
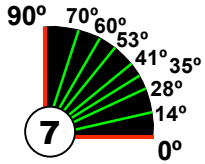
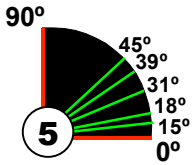
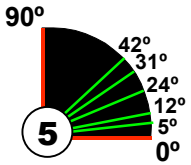
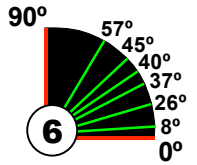
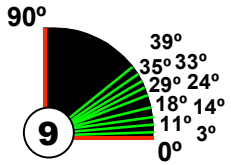
all experiments in list completed ??

NO

YES

next experiment in list

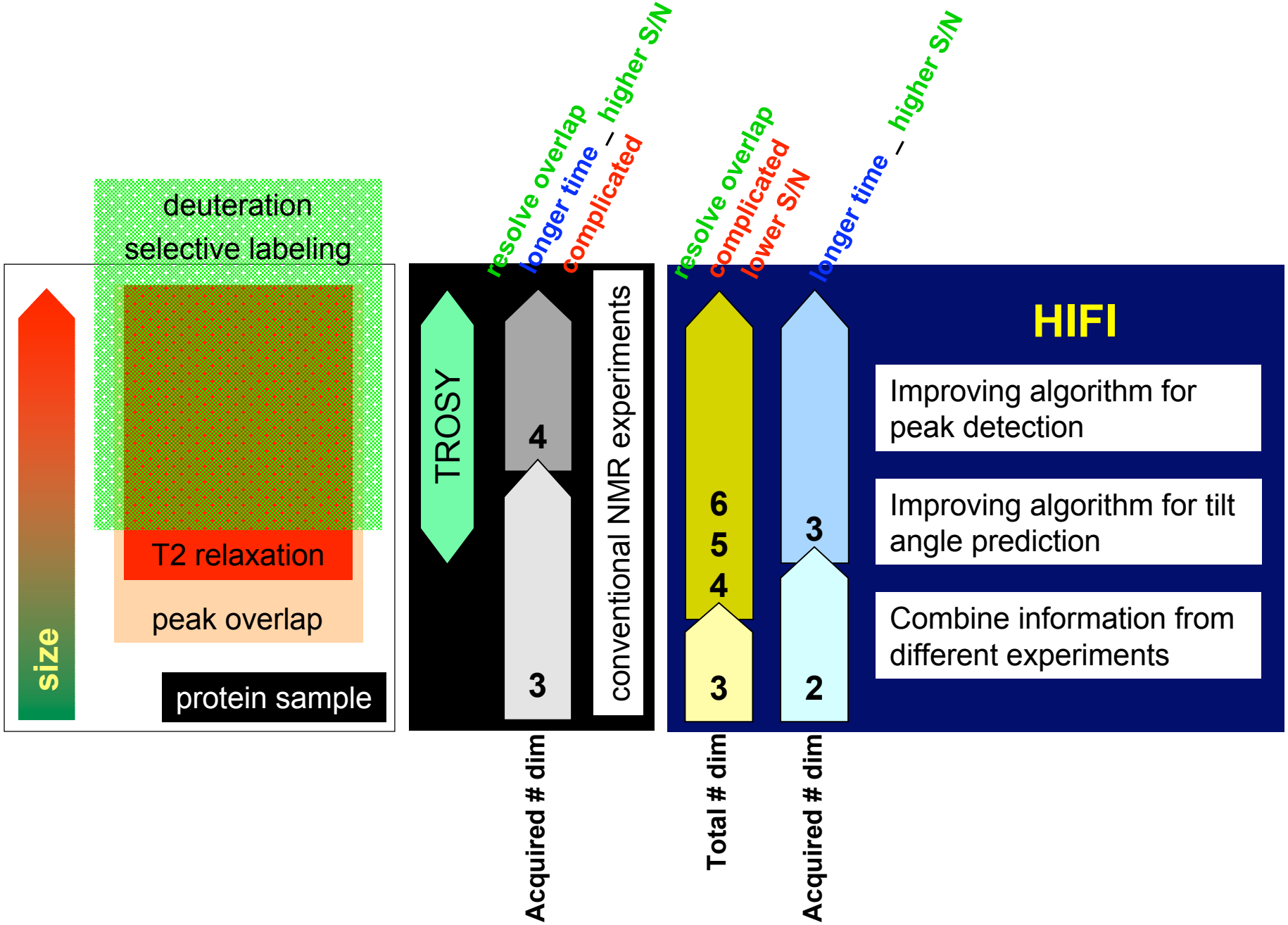
run probabilistic backbone assignments

	brazzein - 53 a.a.		ubiquitin 76 a.a.	flavodoxin 176 a.a.
	wild-type	*RI mutant*		
HNCO				
HN(CO)CA				
HNCA				
CBCA(CO)NH				
HN(CA)CB				
HNCACB				
	~12hrs	**~9hrs**	**~14hrs**	**~48hrs**

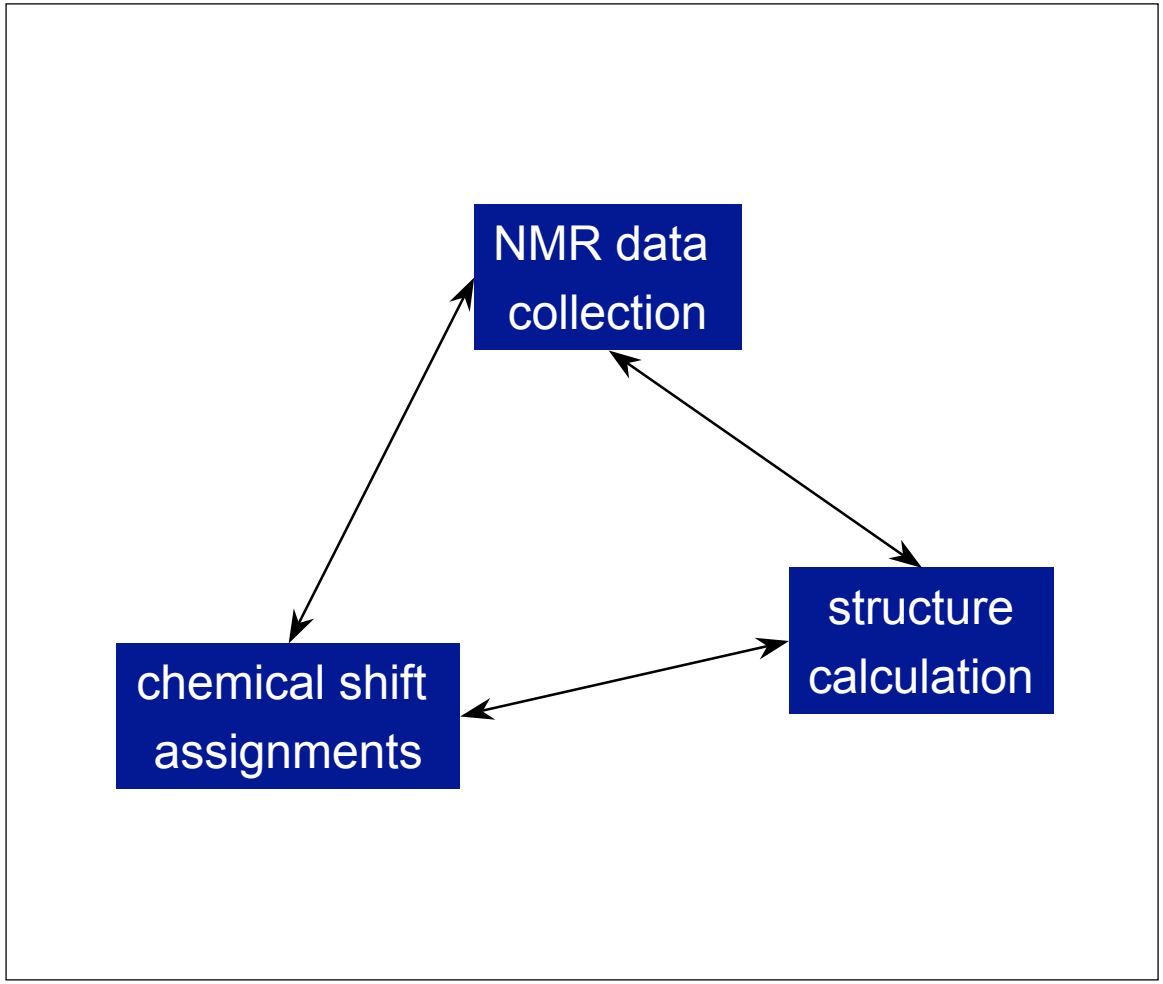
HIFI backbone assignments - recap

- we have developed HIFI for extracting 3D peaks using 2D tilted planes
- by adaptively predicting the best tilt angles, we guarantee that all available 3D data is extracted using the minimum number of tilted planes
- we have adapted the most robust 3D experiments for backbone assignments to be recorded using HIFI-NMR
- we have successfully automated HIFI backbone data collection for proteins of small/medium size
- automated HIFI is robust and allows to extract 3D peaks lists with:
  - least amount of spectrometer time
  - minimum human intervention

where is HIFI going …



The BIG picture



HIFI NMR : part2
conclusions and other applications

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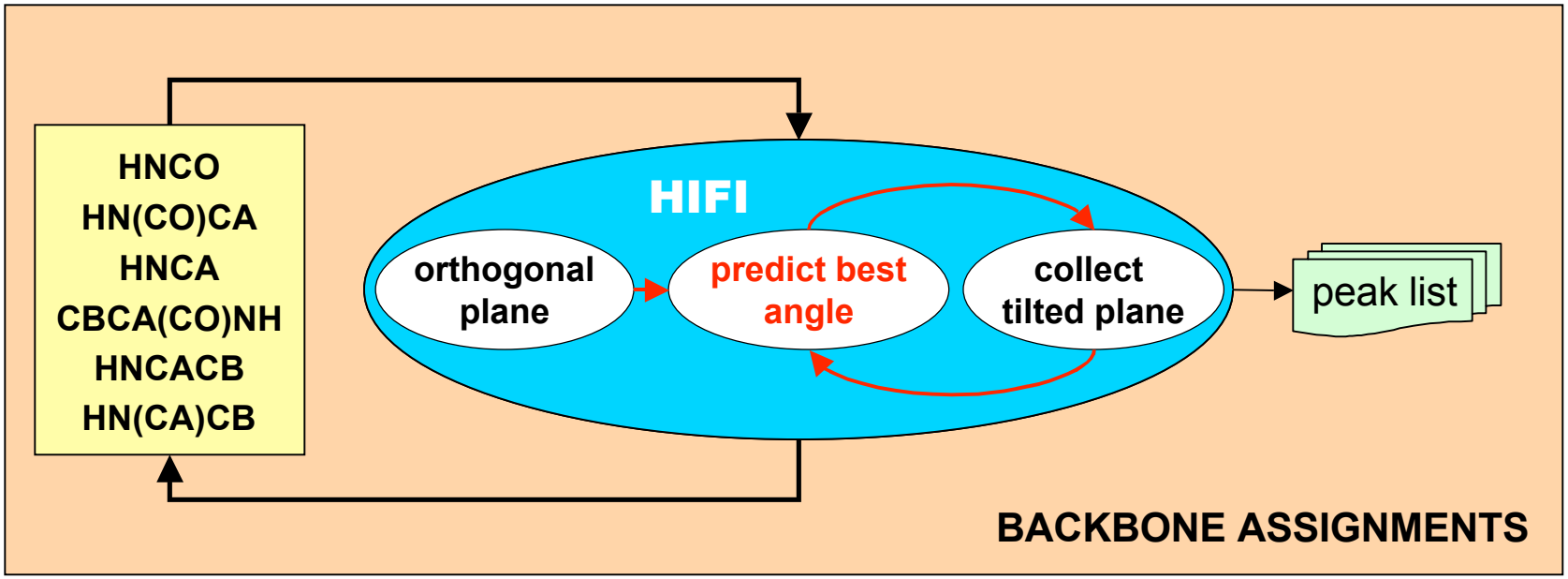
National Magnetic Resonance Facility At Madison NMRFAM

HIFI: application to chemical shift assignments

HIFI ⟷ adaptive

- robust : collect **all** the information needed
- efficient : collect only the **minimum** amount information needed to answer the question

TODAY:

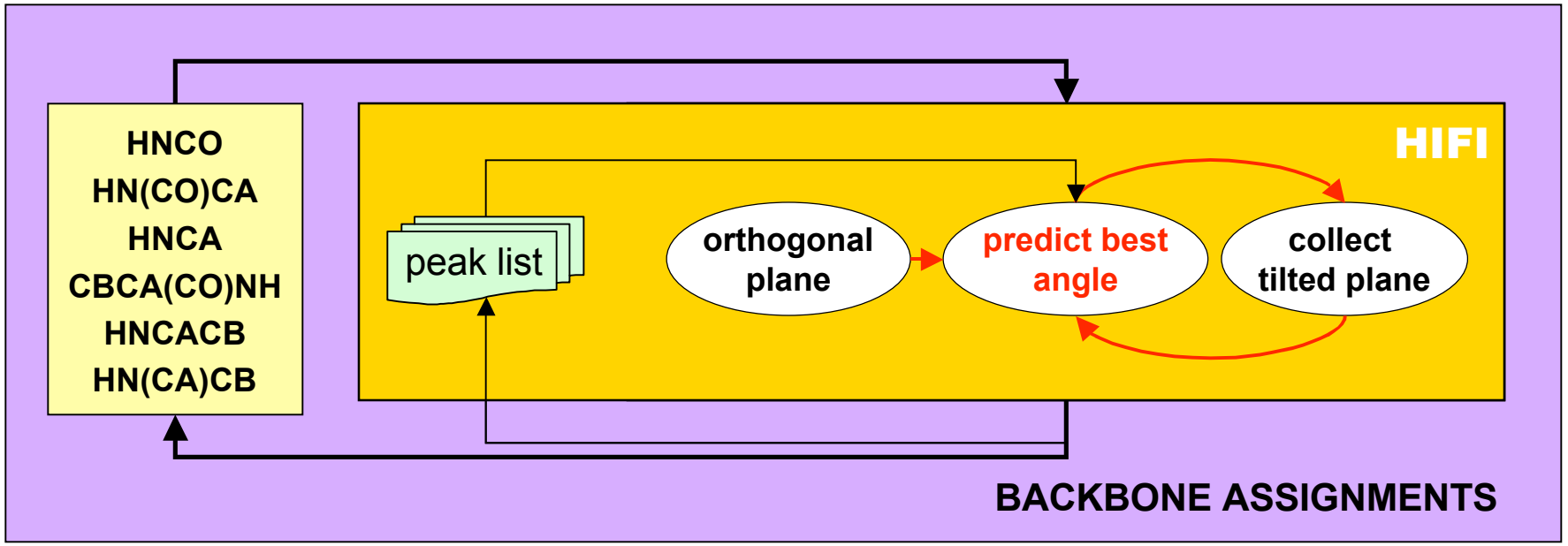


HIFI: application to chemical shift assignments

HIFI ⟷ adaptive

- robust : collect **all** the information needed
- efficient : collect only the **minimum** amount information needed to answer the question

TOMORROW:

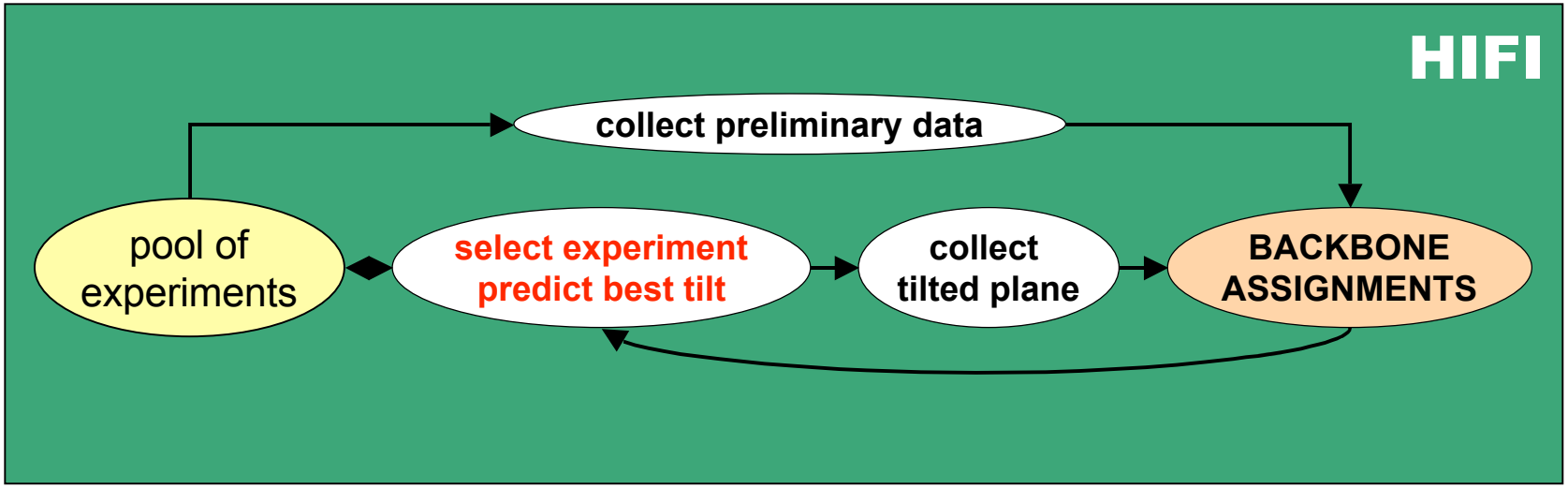


HIFI: application to chemical shift assignments

HIFI ⟷ adaptive

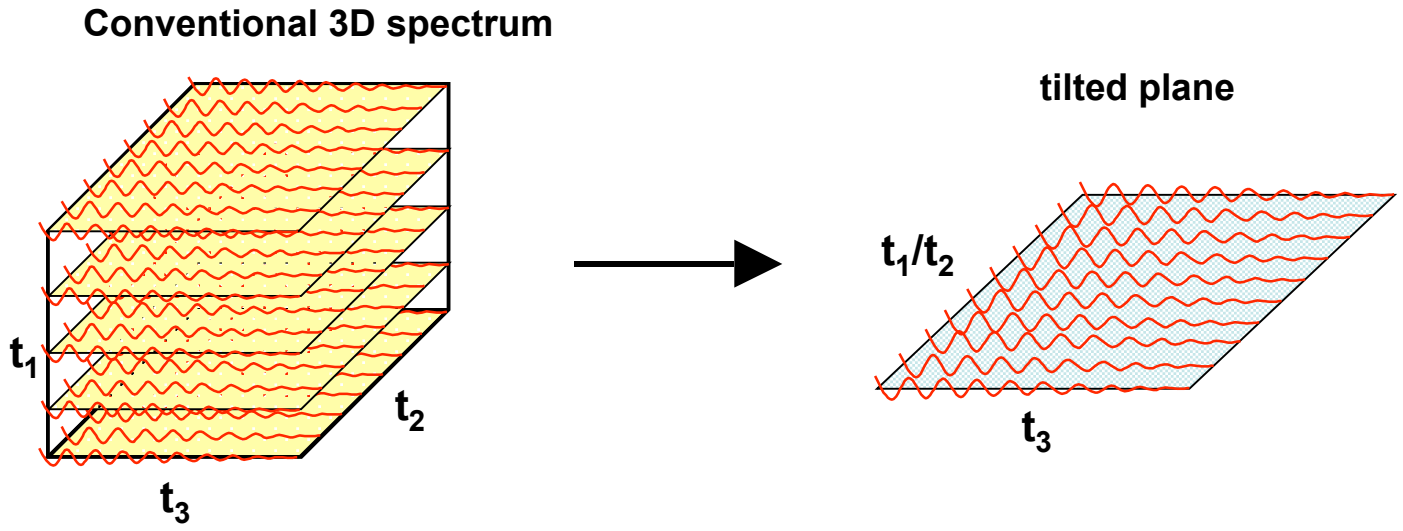
- robust : collect **all** the information needed
- efficient : collect the only the **minimum** amount information needed to answer the question

THE DAY AFTER TOMORROW:



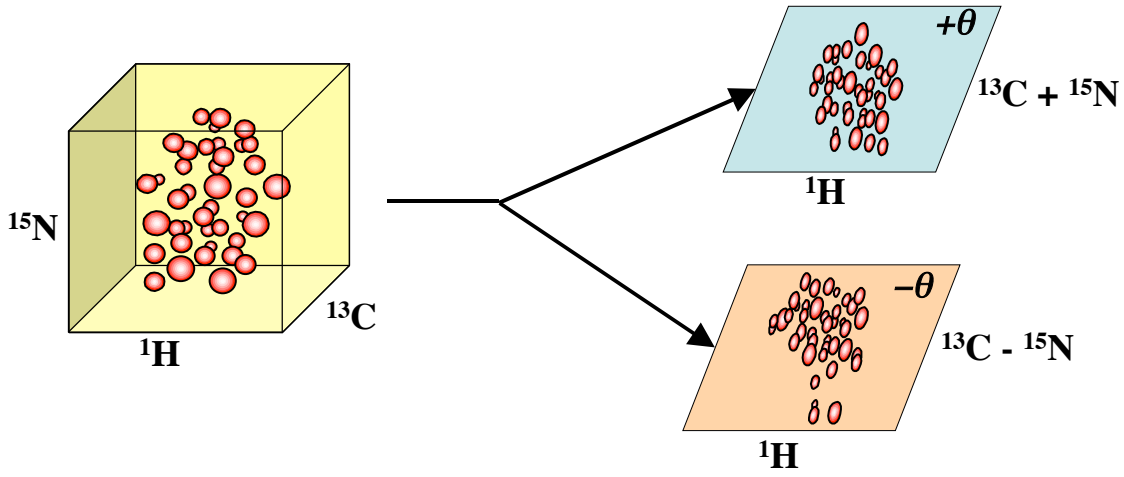
HIFI: other applications

any application that makes use of information extracted from a 3D experiment can be speeded up by recording a tilted plane instead



HIFI can then be used to ensure that maximum information is recovered by predicting the best angle to use for recording the tilted plane

HIFI: other applications



two tilted planes are obtained for each experiment run: “plus” and “minus”

- different peak distribution – bigger potential of resolving overlapped peaks

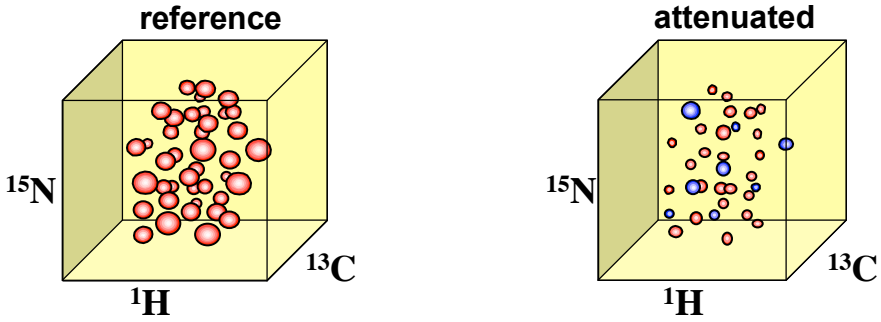
- different noise distribution – can provide measure of confidence of data obtained by analyzing spectra

HIFI: extraction of RDC

Example: extraction of $RDC_{C'N}$ using a modified HNCO pulse sequence (Bax)

Conventional method:

- record two 3D C'-N-H experiments:
  1. reference spectrum
  2. attenuated spectrum - intensity of peaks is modulated by coupling: $J_{C'N} + D_{C'N}$



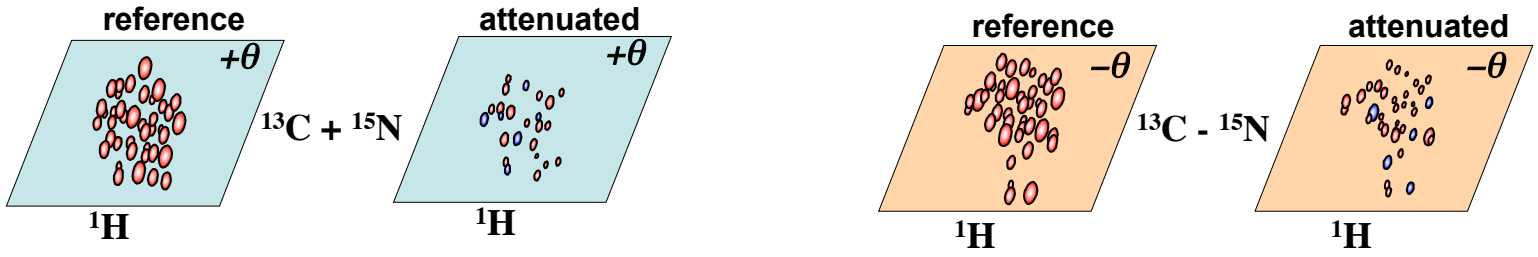
- extract $J_{C'N} + D_{C'N}$ coupling from the ratio between intensity of corresponding peaks in the reference and attenuated spectra

- repeat for isotropic and aligned samples

HIFI: extraction of RDC

HIFI method:

- record two tilted C'-N-H planes at the optimal tilt angle:
  1. reference spectrum
  2. attenuated spectrum - intensity of peaks is modulated by coupling: $J_{C'N} + D_{C'N}$



- extract $J_{C'N} + D_{C'N}$ coupling from the ratio between intensity of corresponding peaks in the reference and attenuated spectra

- analyze "plus" and "minus" planes independently
  - compare the results from the two plenes to get measure of data confidence
  - combine the results from the two planes

- repeat for isotropic and aligned samples

Who did the work ?