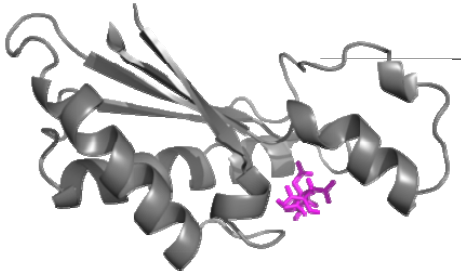
Multidimensional solid-state NMR on a microcrystalline MP domain

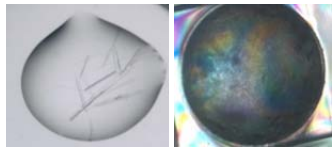
Oleron 2016 – Marie Renault

Sample preparation

- ❑ Periplasmic domain of a bacterial OMP (140 residues)
- ❑ High-expression levels in E. coli
- ❑ Solution NMR assignments (free-form)
- ❑ Crystal structure (bound-form)

Microcrystalline (U-^{13}C,^{15}N)-labeled domain





X-ray
40 nl seating drop
20 % PEG6000

ssNMR
40 ul seating drop
(x60-100)
30 % PEG6000

Crystals appear after 10 days
Incubation at 12 C during 2 months

↓

Transfer in 3.2mm MAS rotor
(~29 mg of proteins)

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Bruker BioSpin-TopSolids suite

Edprosol, edasp

1. Standard Setup

- ❑ Set Magic Angle position
- ❑ 1H 90deg pulse verif
- ❑ 13C 90deg pulse verif
- ❑ Shim probe & calibration

- ❑ 15N 90deg pulse verif
- ❑ Set up H->C CP exp
- ❑ Set up H->N CP exp
- ❑ Set up H->N->Ca double CP
- ❑ Set up H->N->CO double CP

2. Protein Setup

- ❑ Verify 1H 90deg pulse
- ❑ Verify 13C 90deg pulse
- ❑ Verify 15N 90deg pulse
- ❑ Optimize H->C CP exp
- ❑ Optimize H->N CP exp
- ❑ Optimize H->N->Ca DCP exp
- ❑ Optimize H->N->CO DCP exp
- ❑ Optimize H->Ca-> N->CO TCP
- ❑ Create PDF Report

3. Backbone Assignment

- ❑ 2D DARR/PDSD
- ❑ 2D NCA/NCO
- ❑ 2D NCACX/NCOCX dcp
- ❑ 3D NCACX/NCOCX dcp
- ❑ 3D CaNCOCX/CONCACX tcp
- ❑ 4D CANCOCX
- ❑ 4D CONCACX

NMR samples

- ❖ KBr/Adamantan
- ❖ (U-13C,15N)-glycine
- ❖ (U-13C,15N)-AGG
- ❖ NMR sample of interest

4. Distance measurement

- ❑ 2D CHHC
- ❑ 2D NHHC
- ❑ 2D PDSD/DARR

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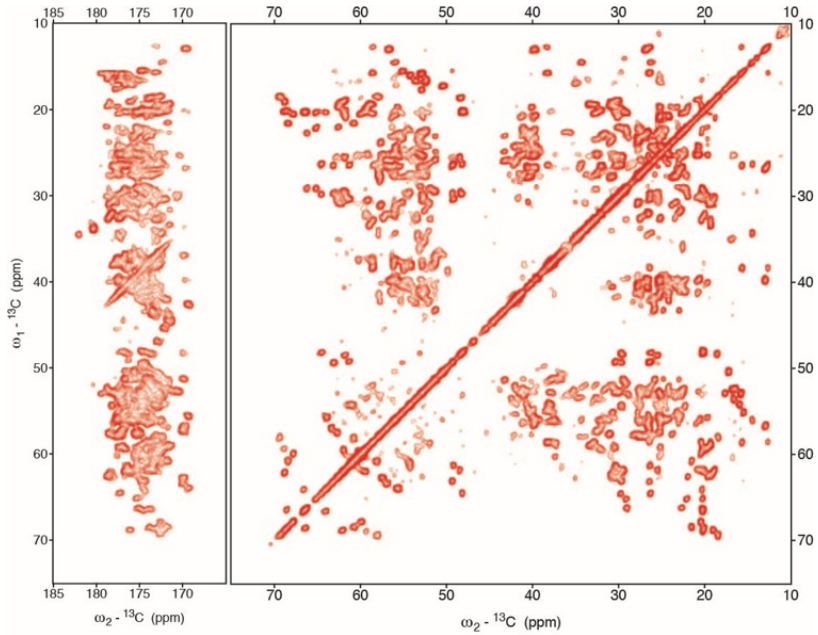
2D 13C-13C intraresidue correlations



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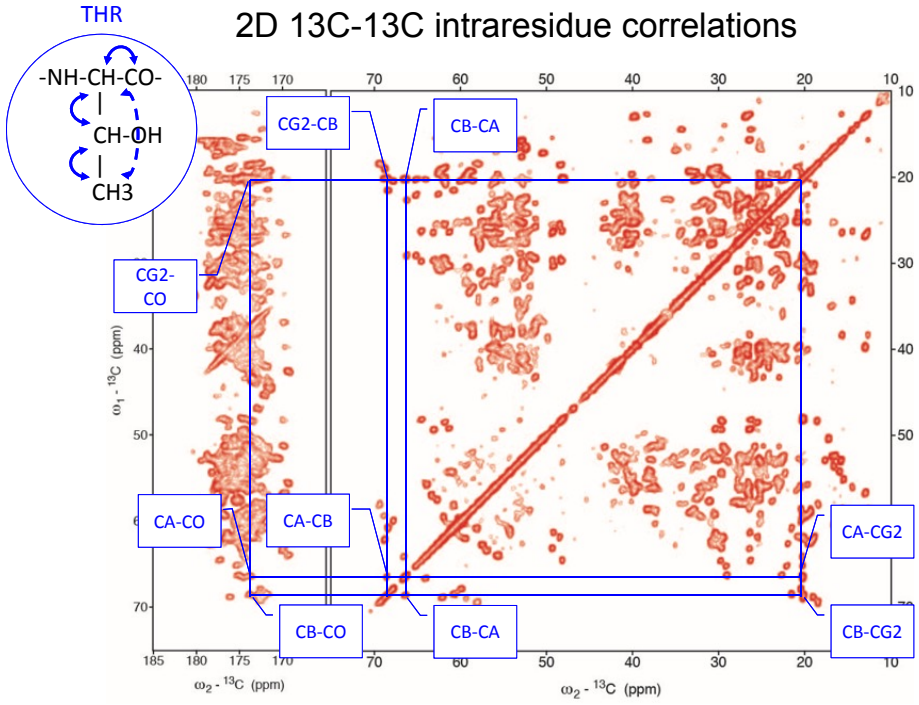
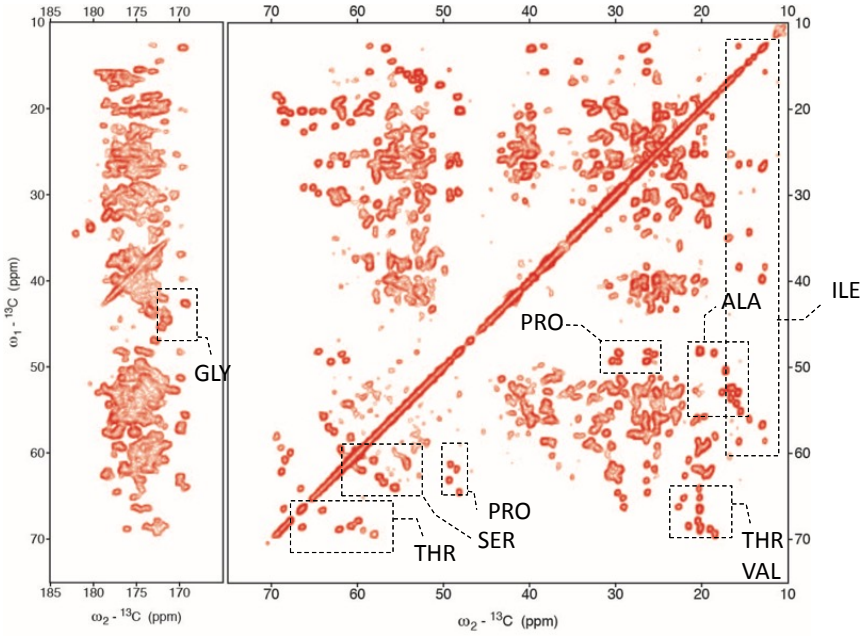
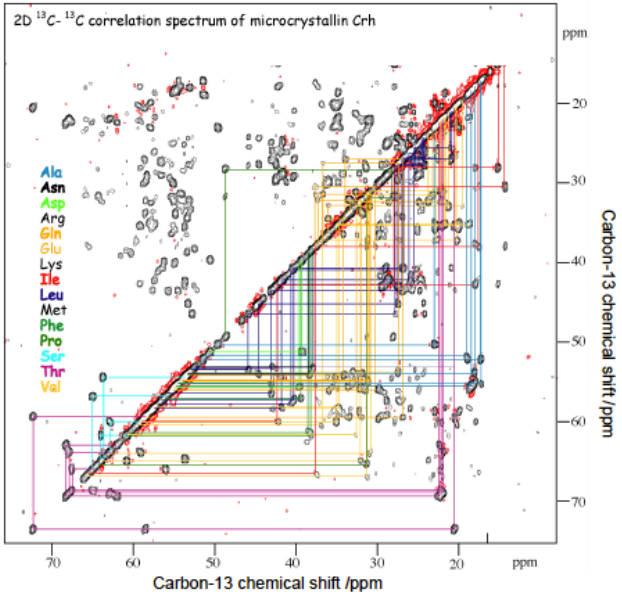
2D 13C-13C intraresidue correlations



2D 13C-13C intraresidue correlations



Protein “fingerprint”



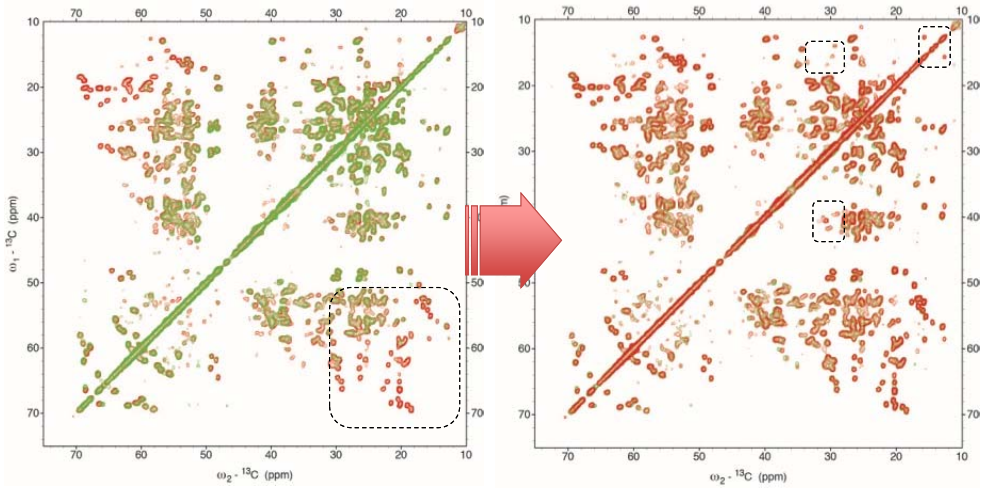
Is higher MAS frequency really beneficial ?

MAS 12 kHz
MAS 18 kHz

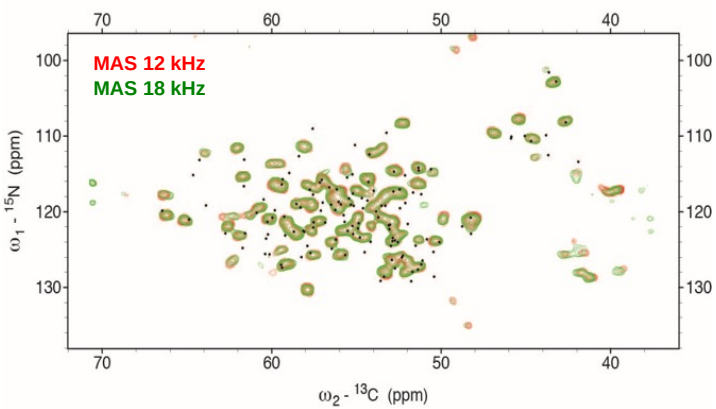
13C-13C dipolar-based transfer efficiency

2D PDSD (mix=50 ms)

2D DARR (mix=50 ms)

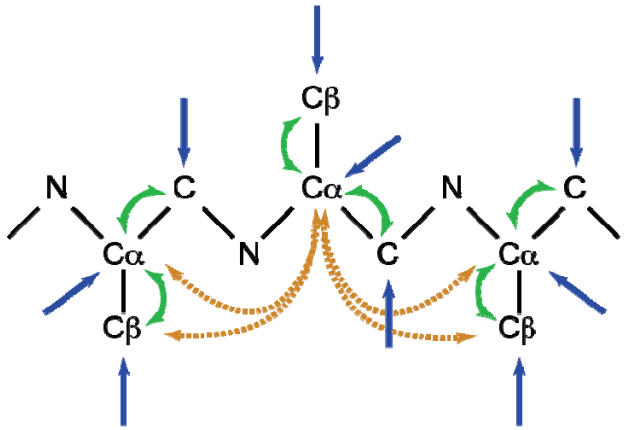


Is higher MAS frequency really beneficial ?



	13C linewidth	15N linewidth
MAS 12 kHz	100 Hz (≈ 0.6 ppm)	80 Hz (≈ 1.1 ppm)
MAS 18 kHz	100 Hz (≈ 0.6 ppm)	70 Hz (≈ 1.0 ppm)

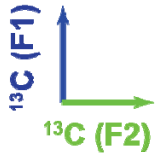
Intraresidual & sequential correlations



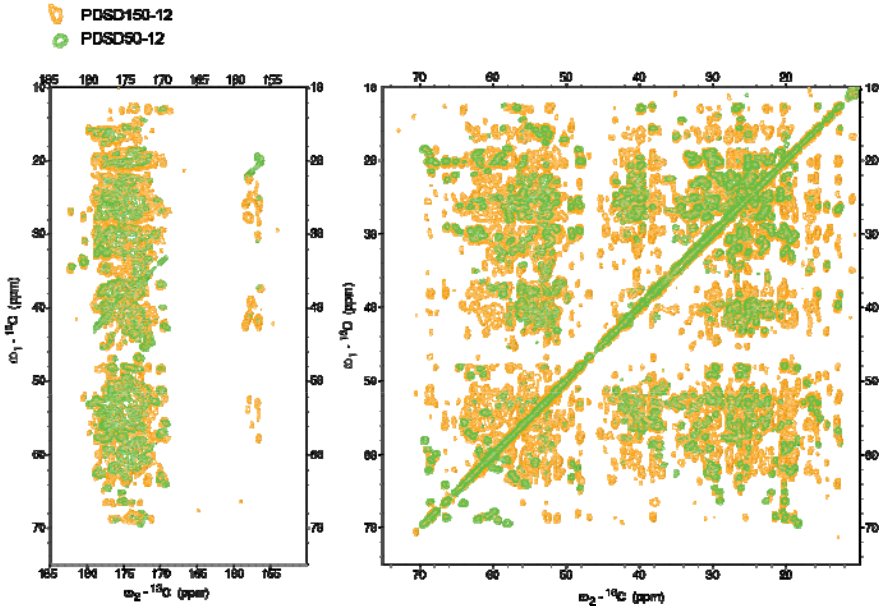
Transfers :

1. CP HC (broadband)
2. Mixing < 50 ms
2. Mixing > 150 ms

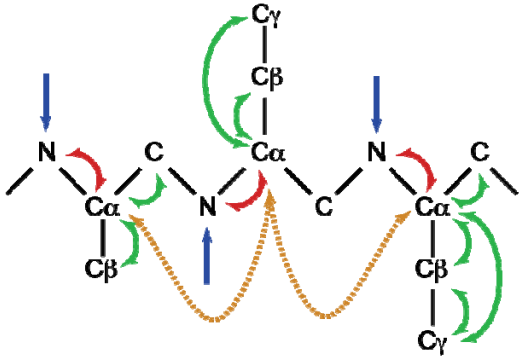
2D PDSD/DARR



Sequential correlations



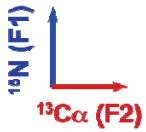
Intraresidual & sequential correlations



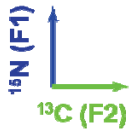
Transfers :

1. CP HN (broadband)
2. SPECIFIC-CP NC
3. Mixing < 50 ms
3. Mixing > 150 ms

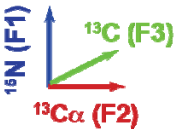
2D NCA



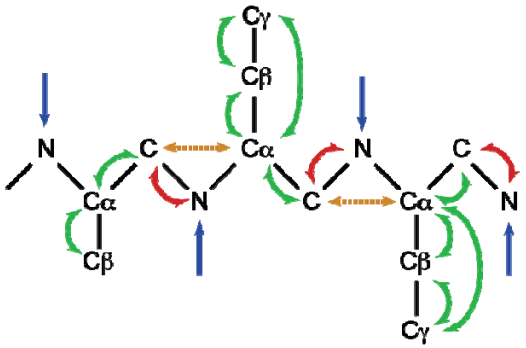
2D N(CA)CX



3D NCACX

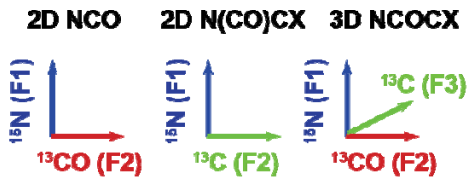


Sequential correlations

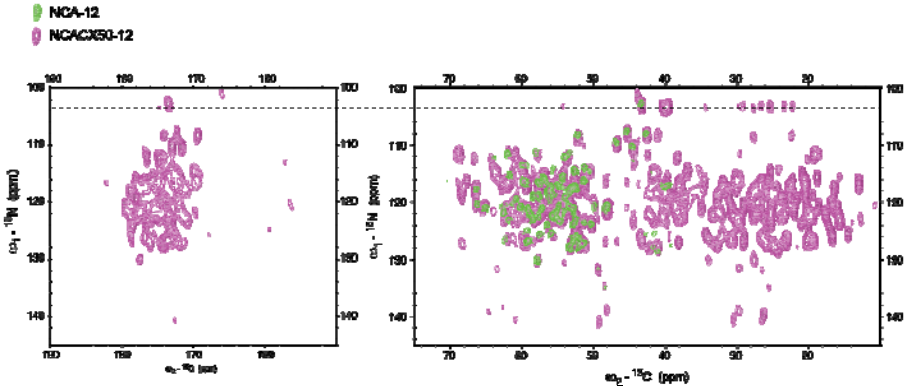


Transfers :

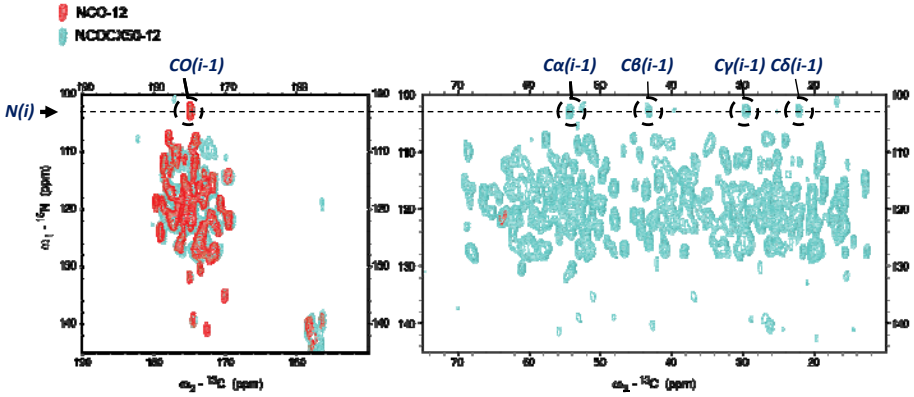
1. CP HN (broadband)
2. SPECIFIC-CP NC
3. Mixing < 50 ms
3. Mixing > 150 ms



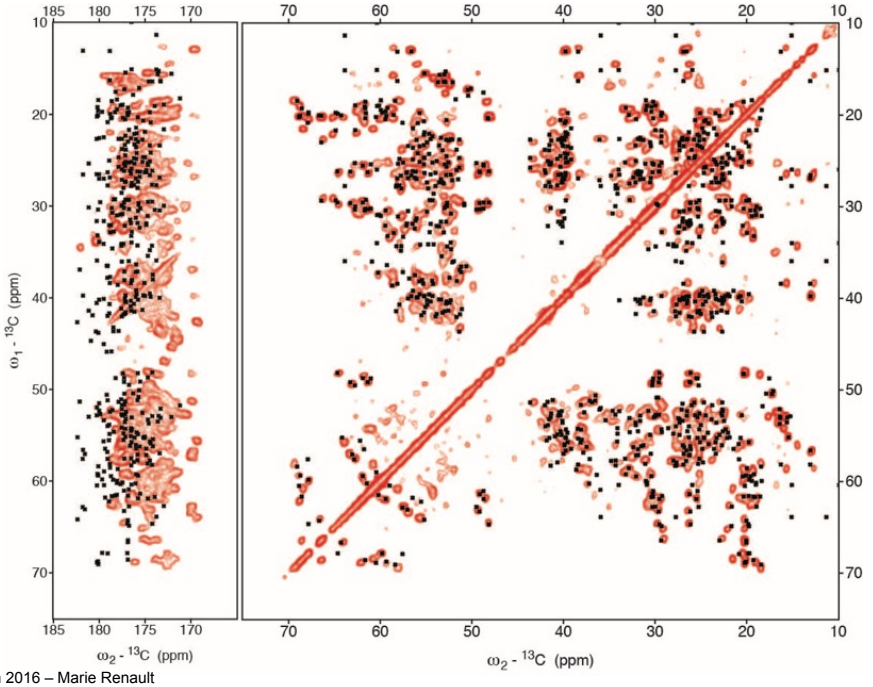
Sequential correlations



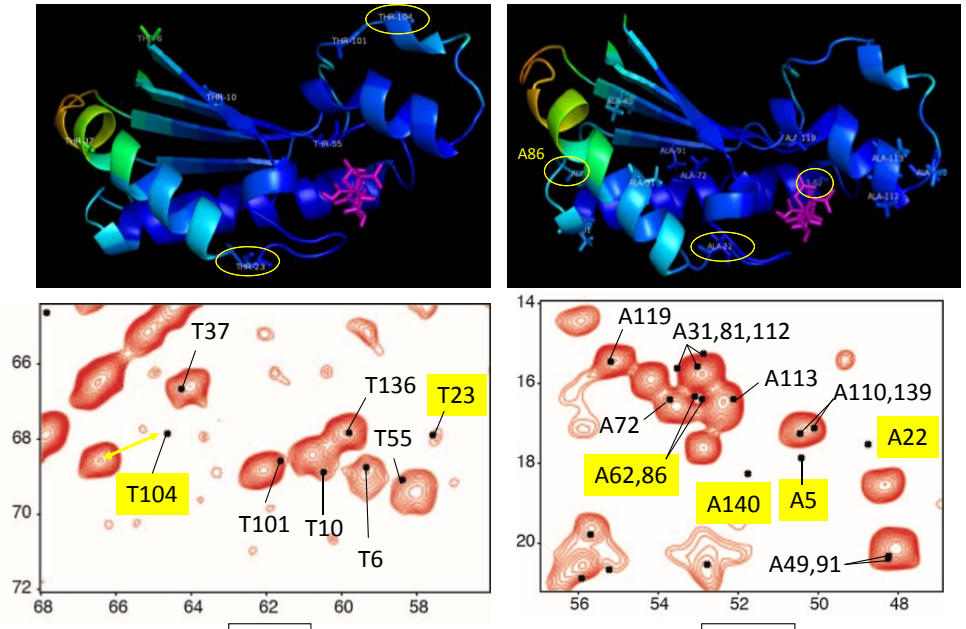
Sequential correlations



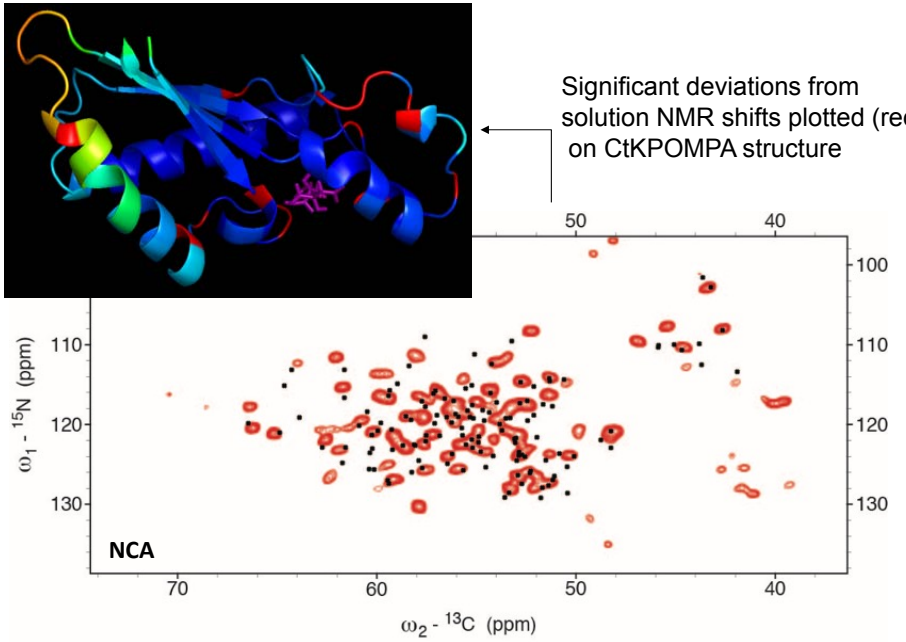
Comparison with solution NMR data



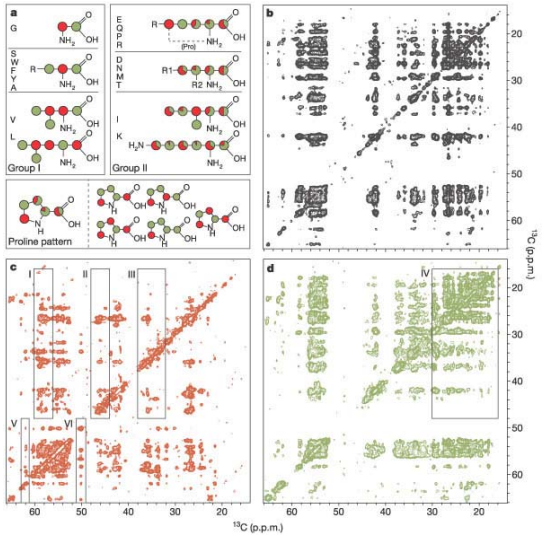
Comparison with solution NMR data



2D 15N-13C intraresidue correlations



Fractional ^{13}C,^{15}N labelling



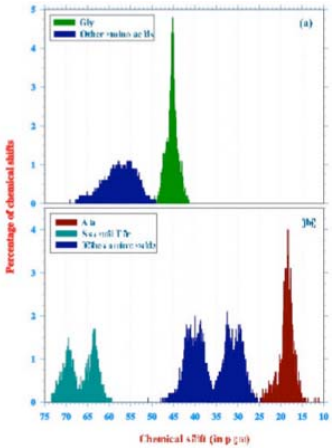
- [U-^{13}C]-Glucose
- [1,3-^{13}C]-Glycerol
- [2-^{13}C]-Glycerol

- ➔ *Reduction of strong dipolar CC couplings*
- ➔ *Reduction of spectral crowding*
- ➔ *Characteristic CC correlation pattern for each aa*
- ➔ *Resolution enhancement (most of Jcc removed)*
- ➔ *Protein yields can be affected*

Labelling patterns and NMR spectra for the different alpha-spectrin SH3 domain preparations

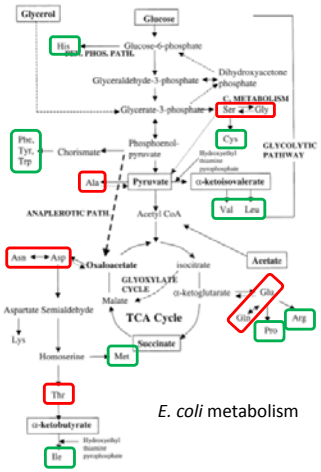
Castellani F., …, Oschkinat H. (2002) Nature 420, 98-102

Reverse labelling



- ➔ Substantial decrease of the spectral crowding
- ➔ No dramatic losses of structural information
- ➔ Protein yields unaffected

Reverse labelling



E. coli metabolism

- Similar protocol than for (U-^{13}C,^{15}N) labelling
- Addition of "well-selected" unlabeled amino-acids before induction

➢ To target specific protein region
- Hydrophobic amino-acids (TM segments)
- Aromatic residues (membrane interfaces)
- Polar residues (extramembrane domains)

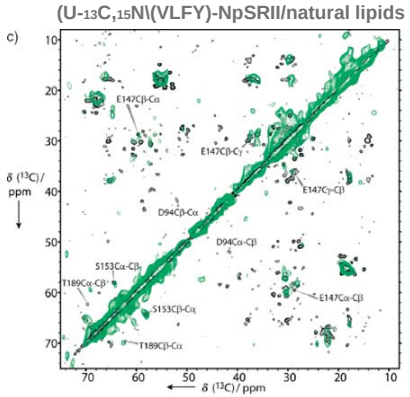
➢ To minimize scrambling

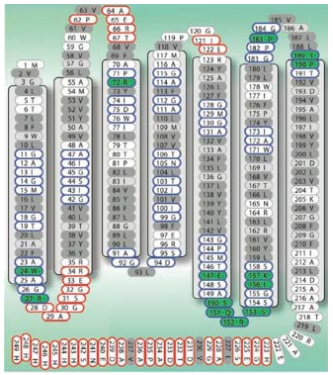
R, C, H, I, L, K, M, F, P, W, Y, V

A, N, D, E, Q , G, S, T

AVOID !

Reverse labelling





Rigid segments
Mobile segments
Water exposed
Unassigned residues
Unlabeled residues

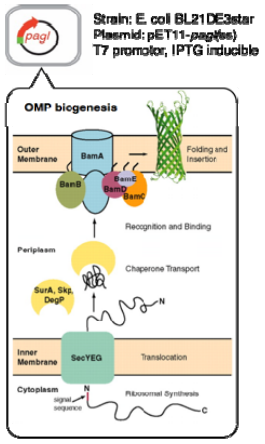
Sequential assignment & topology of a 7-helix receptor in native membrane

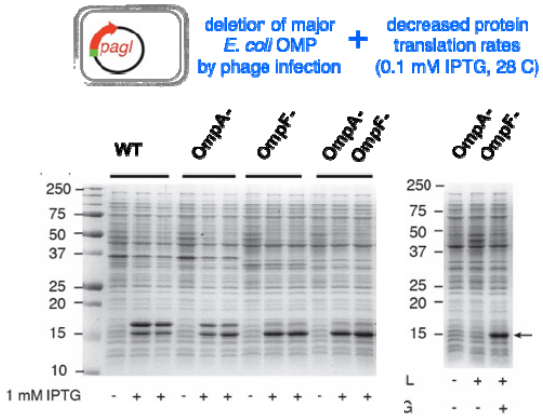
Etzkorn M et al., Angew Chem Int Ed 46, 459-462 (2007)

Cellular solid-state NMR on bacterial cell envelope and whole bacteria : studying membrane proteins in their native environment

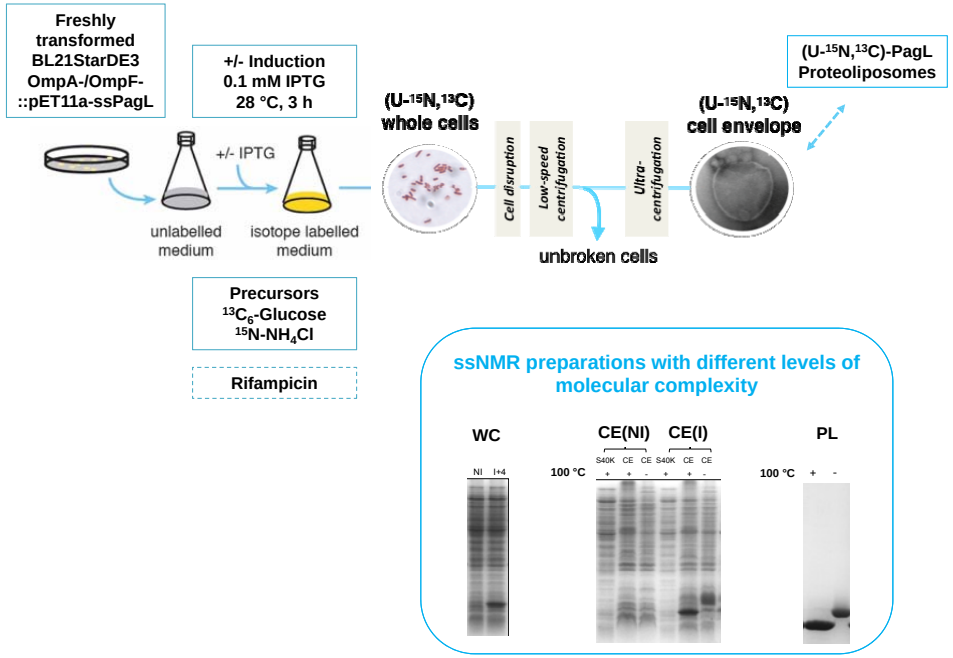
MP homologous expression and targeting

- ❑ Full-length bacterial OMP from *P. aeruginosa* (150 residues)
- ❑ Expression of fully functional recombinant protein in *E. coli* cell envelope

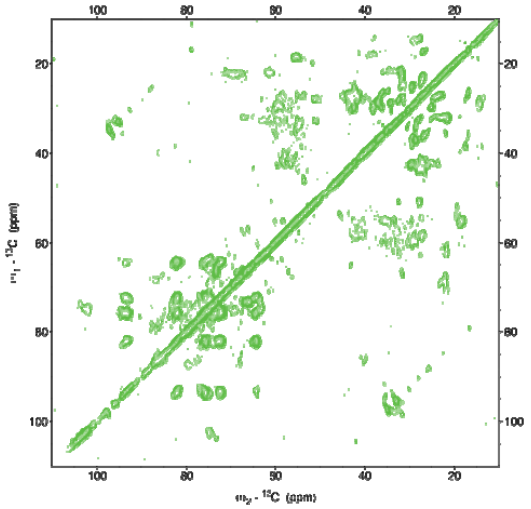




Sample preparation

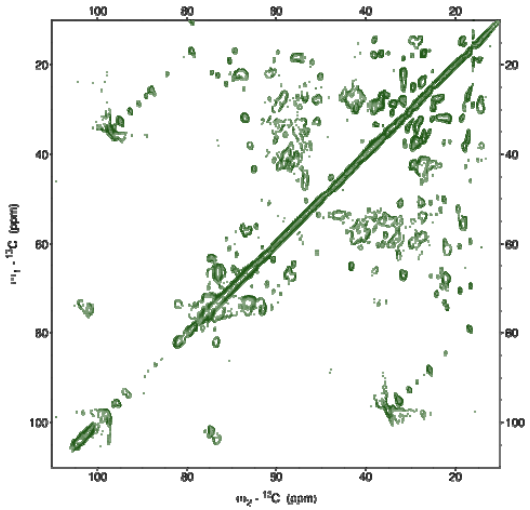


2D ^{13}C-^{13}C PDSD (mix=20 ms) from (U-^{13}C,^{15}N)-labeled IPTG-induced whole cells

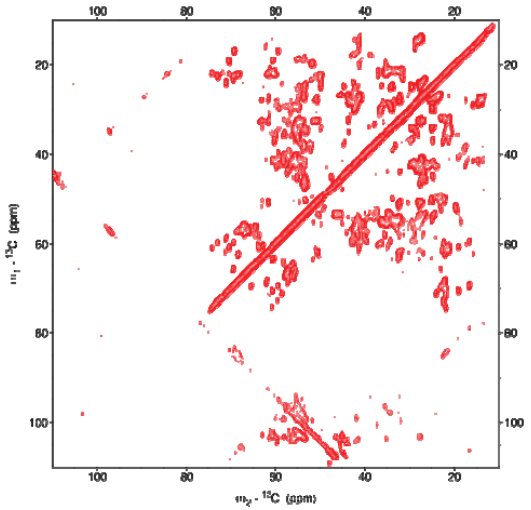


MAS=13 kHz, B_0=700 MHz, ns =

2D ^{13}C-^{13}C PDSD (mix=20 ms) from (U-^{13}C,^{15}N)-labeled CE isolated from IPTG-induced cells

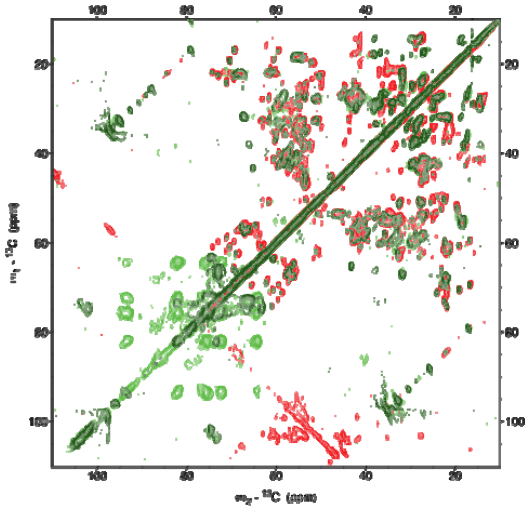


2D ^{13}C-^{13}C PDSD (mix=20 ms) from (U-^{13}C,^{15}N)-labeled PagL reconstituted in proteoliposomes

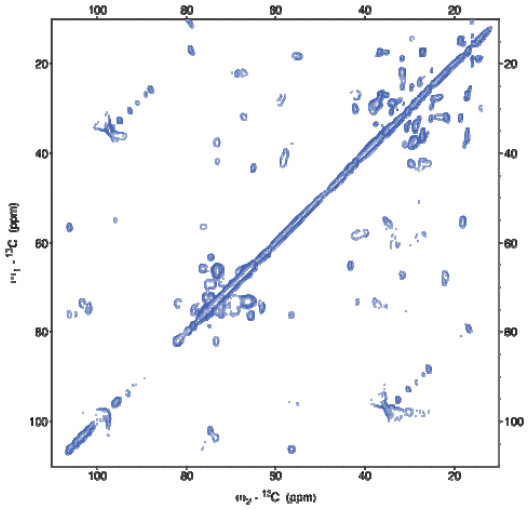


Overlay between 2D ^{13}C-^{13}C PDSD (mix=20 ms)

- WC Induced
- CE Induced
- PL PagL/DMPC

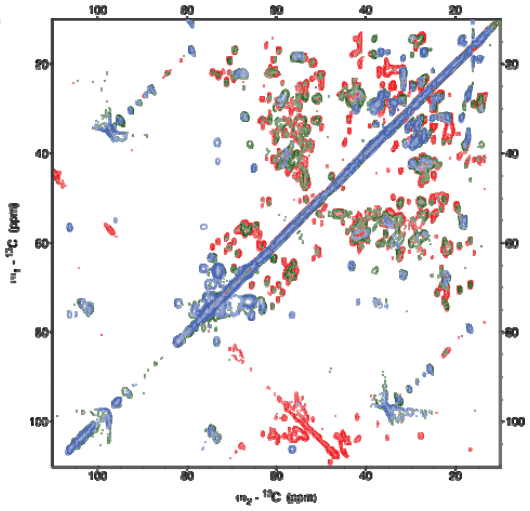


2D ^{13}C-^{13}C PDSD (mix=20 ms) from (U-^{13}C,^{15}N)-labeled CE isolated from non-induced cells

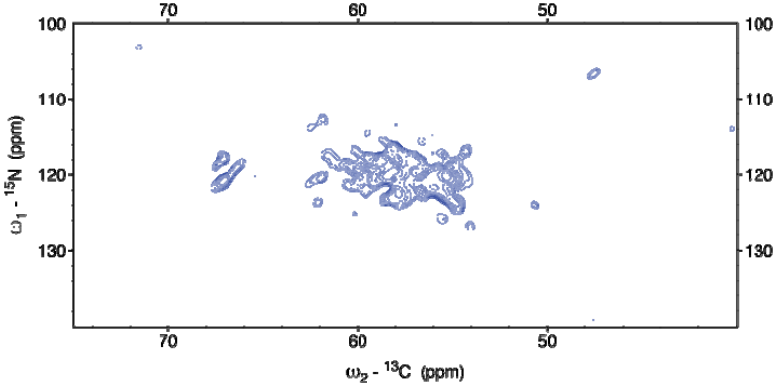


Overlay between 2D ^{13}C-^{13}C PDSD (mix=20 ms)

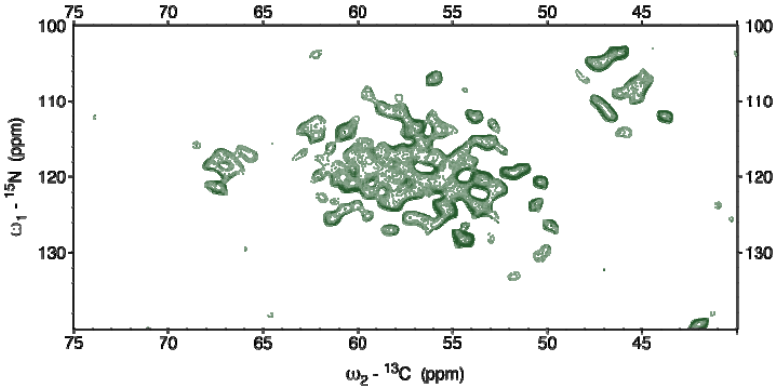
- CE Non-Induced
- CE Induced
- PL PagL/DMPC



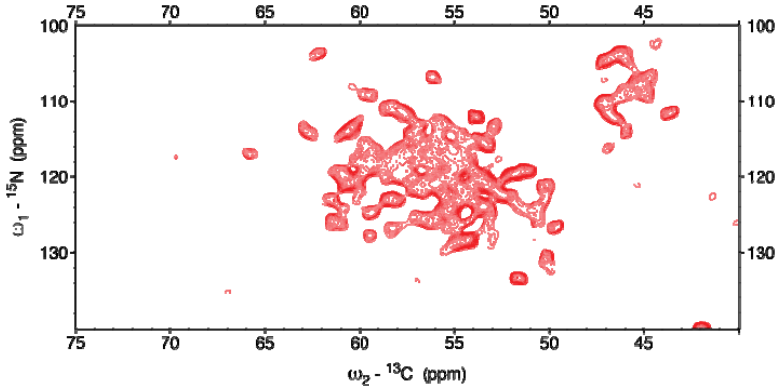
2D ^{15}N-^{13}C NCA from (U-^{13}C,^{15}N)-labeled CE isolated from Non-induced cells



2D ^{15}N-^{13}C NCA
from (U-^{13}C,^{15}N)-labeled CE isolated from IPTG-induced cells

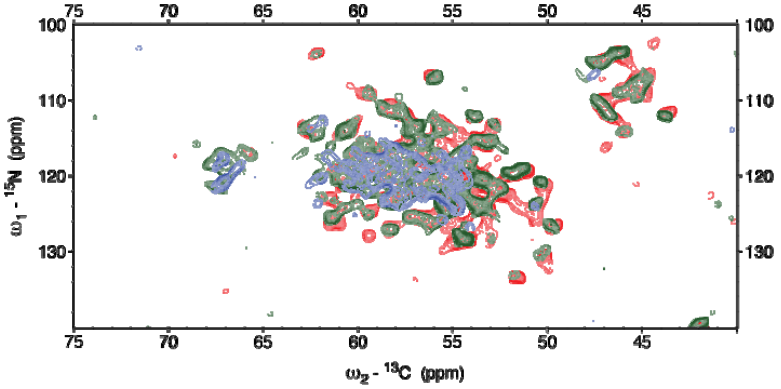


2D ^{15}N-^{13}C NCA
from (U-^{13}C,^{15}N)-labeled PagL reconstituted in proteoliposomes

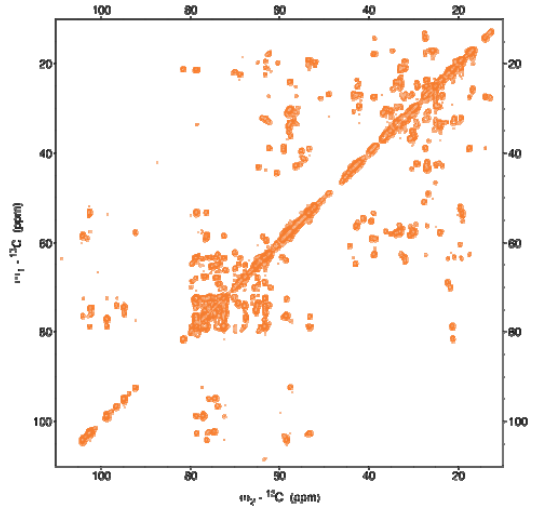


Overlay between 2D ^{15}N-^{13}C NCA

- CE Non-Induced
- CE Induced
- PL PagL/DMPC

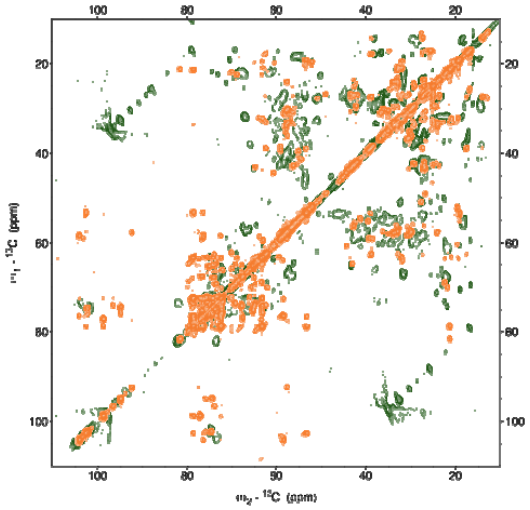


2D ^{13}C-^{13}C INEPT-TOBSY (mix=6 ms)
from (U-^{13}C,^{15}N)-labeled CE isolated from IPTG-induced cells



Overlay 2D ^{13}C-^{13}C correlation spectra from (U-^{13}C,^{15}N)-labeled CE isolated from IPTG-induced cells

- PDSD 20 ms
- INEPT-TOBSY 6ms



2D ^{1}H-^{13}C INEPT from (U-^{13}C,^{15}N)-labeled CE isolated from IPTG-induced cells