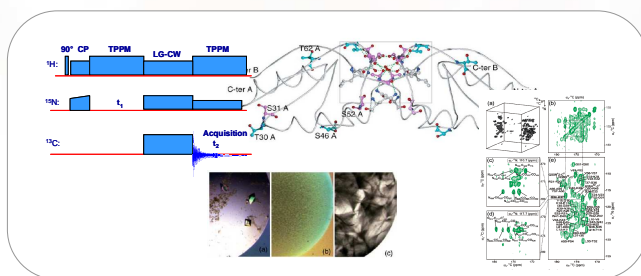


Struktura a dynamika proteinů a peptidů



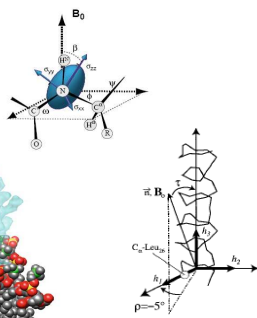
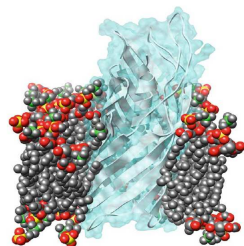
Orientované systémy (1995 - 2000)

Strukturální biologie a membránové proteiny

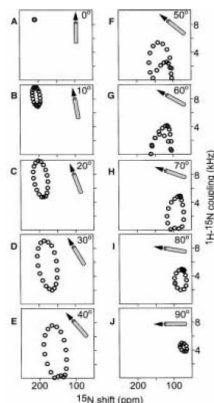


Opella S.J.

Wu C.H., Ramamoorthy A., Opella S.J., High Resolution Dipolar Solid-State NMR, *J.Magn.Reson. A* (1994); 109: 270.



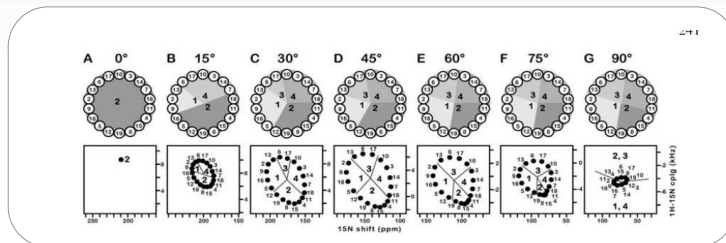
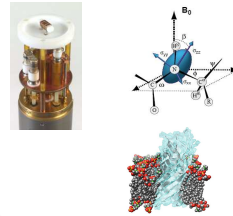
PISEMA: Polarization Inversion Spin Exchange at Magic Angle



Orientované systémy - membránové proteiny

Wu C.H., Ramamoorthy A., Opella S.J., High Resolution Dipolar Solid-State NMR, *J.Magn.Reson. A* (1994); 109: 270.

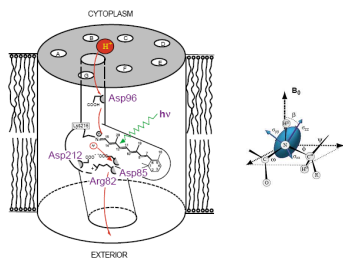
PISEMA:
Polarization Inversion Spin
Exchange at Magic Angle



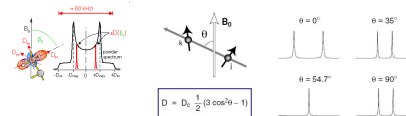
Joint Laboratory of Solid-State NMR
IBK AS CZ and JRCPC AS CZ

Orientované systémy - membránové proteiny

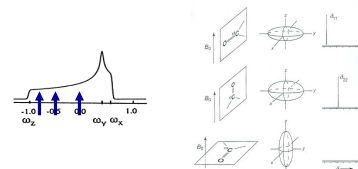
Orientovaná membrána



Dipolární interakce



Anizotropie chemického posunu

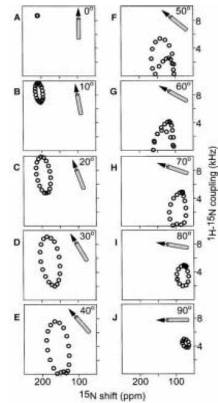
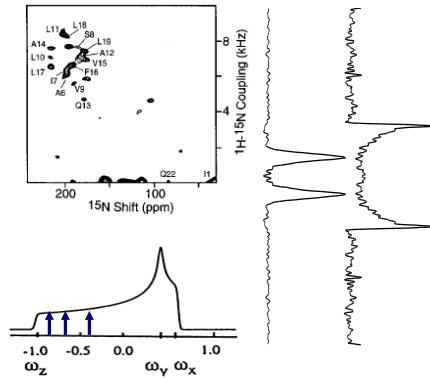


Joint Laboratory of Solid-State NMR
IBK AS CZ and JRCPC AS CZ

Orientované systémy - membránové proteiny

PISEMA:

Polarization Inversion Spin Exchange at Magic Angle

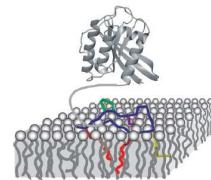
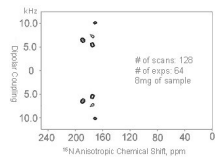
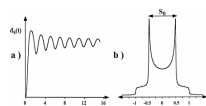
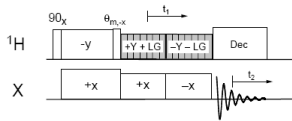


Joint Laboratory of Solid State NMR
IMC AS CZ and JHPC AS CZ

Orientované systémy - membránové proteiny

PISEMA:

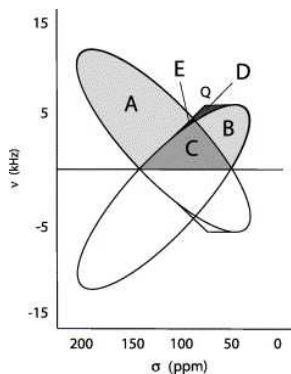
Polarization Inversion Spin Exchange at Magic Angle



Joint Laboratory of Solid State NMR
IMC AS CZ and JHPC AS CZ

Orientované systémy - membránové proteiny

PISEMA:
Polarization Inversion Spin Exchange at Magic Angle



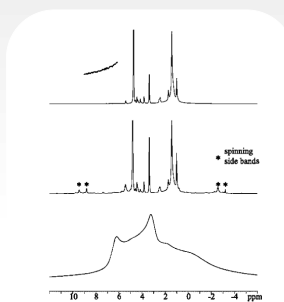
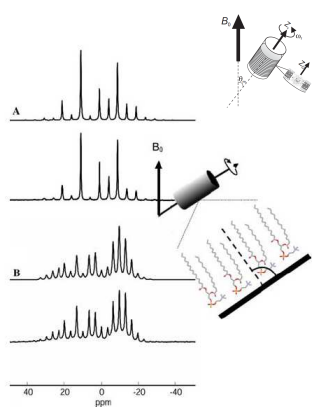
[dx.doi.org/10.1006/jmre.2001.2405](https://doi.org/10.1006/jmre.2001.2405)



Joint Laboratory of Solid-State NMR
IMC AS CZ and JHPC AS CZ

Orientované systémy - membránové proteiny

Magic Angle-Oriented Sample Spinning (MAOSS)

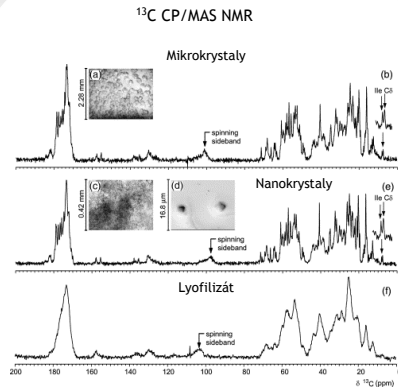


Joint Laboratory of Solid-State NMR
IMC AS CZ and JHPC AS CZ

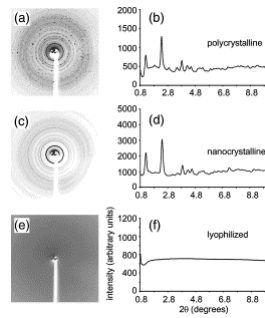
Nanokrystalické proteiny - příprava vzorku

Ubiquitin

Martin R.W., Zilm K.W., Preparation of protein nanocrystals and their characterization by solid state NMR, *J.Magn.Reson.* (2003); 165: 162.



XPRD

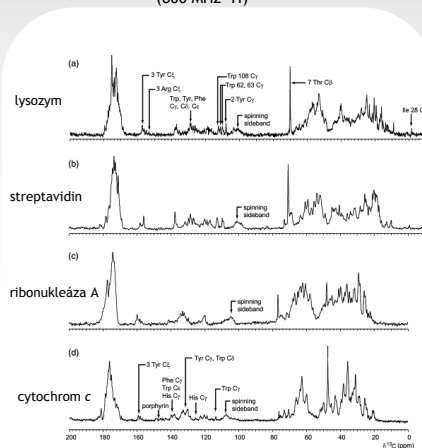


Joint Laboratory of Solid-State NMR
IMC AS CZ and JHPC AS CZ

Nanokrystalické proteiny - příprava vzorku

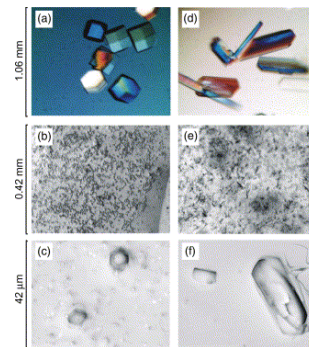
¹³C CP/MAS NMR (800 MHz ¹H)

Martin R.W., Zilm K.W., Preparation of protein nanocrystals and their characterization by solid state NMR, *J.Magn.Reson.* (2003); 165: 162.



lysozym

streptavidin

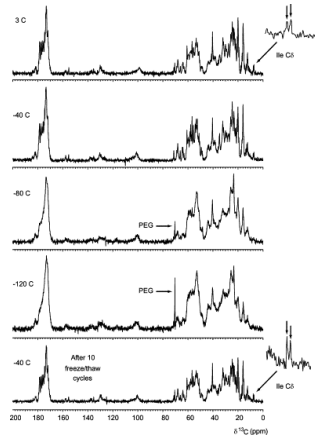


Joint Laboratory of Solid-State NMR
IMC AS CZ and JHPC AS CZ

Nanokrystalické proteiny - příprava vzorku

^{13}C CP/MAS NMR
(800 MHz ^1H)

Martin R.W., Zilm K.W., Preparation of protein nanocrystals and their characterization by solid state NMR, *J.Magn.Reson.* (2003); 165: 162.



Příprava nanokrystalických vzorků

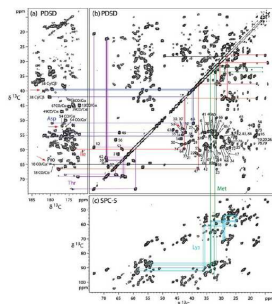
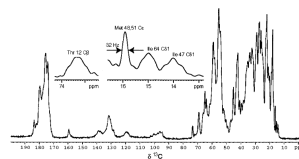
1. Proteiny dodala firma Sigma
2. Směs proteinového roztoku a krystalizačního roztoku 1:1 (P.C.Weber, Overview of Protein Crystallization Methods, vol. 276, San Diego, 1997, 13-21).
3. Roztok obsahuje asi 100 mg proteinu.
4. Roztok byl pomalu koncentrován na cca. polovinu počátečního objemu (centrifugační odparka).
5. Teplota 25C a doba zahušťování byla 15-40 min.
6. Inkubace probíhala při 4C.
7. Jako srážecí činidlo byl použit PEG.



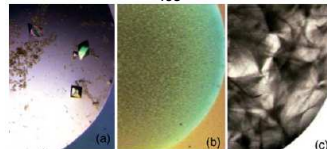
Bacillus subtilis protein Crh

Anja Böckmann, Adam Lange, Anne Galinier, Sorin Luca, Nicolas Giraud, Michel Juy, Henrike Heise, Roland Montserret, François Perin, Marc Baldus. Solid state NMR sequential resonance assignments and conformational analysis of the 2×10.4 kDa dimeric form of the *Bacillus subtilis* protein Crh, *J. Biomol. NMR* 27, 323 (2003).

^{13}C CP/MAS NMR



2M $(\text{NH}_4)_2\text{SO}_4$	10-30% PEG 1,000-10,000 pH 6-8, 20mM $(\text{NH}_4)\text{HCO}_3$ nad roztokem NaCl 18C	10-30% PEG 1,000-10,000 pH 6-8 0.2M NaCl 4C
---------------------------------	---	---



(catabolite repression histidine containing phosphocarrier protein) 2x85 rezidui

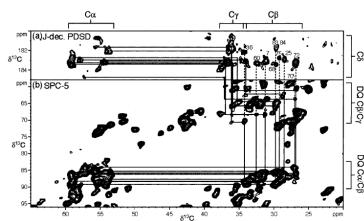


Bacillus subtilis protein Crh

Anja Böckmann, Adam Lange, Anne Galinier, Sorin Luca, Nicolas Giraud, Michel Juya, Henrike Heise, Roland Montserret, François Penin, Marc Baldus. Solid state NMR sequential resonance assignments and conformational analysis of the 2 × 10.4 kDa dimeric form of the *Bacillus subtilis* protein Crh, *J. Biomol. NMR* 27, 323 (2003).

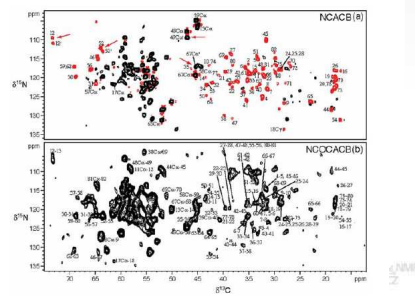
Intra-reziduální korelace:

1. 2D (1Q-1Q) PDSO - krátká spin difúzní perioda (jedno- až tří-vazebné interakce)
2. 2D (2Q-1Q) - krátká excitační perioda pro DQC (identifikace jedno-vazebné interakce)
3. 2QF experimenty - rozlišit a identifikovat spinový systém blízko diagonály



Inter-reziduální korelace:

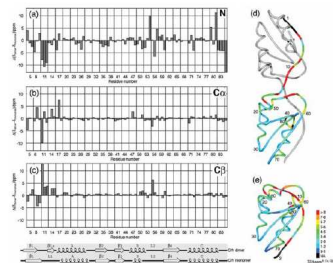
1. Kombinace NCACB a NCOACB experimentů umožňuje sekvenční přiřazení
2. Dvojitá cross-polarizace - frekvenčně selektivní experimenty SPECIFIC CP
3. DQC



Bacillus subtilis protein Crh

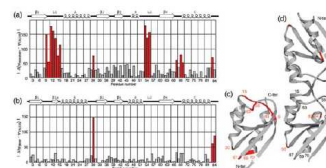
Anja Böckmann, Adam Lange, Anne Galinier, Sorin Luca, Nicolas Giraud, Michel Juya, Henrike Heise, Roland Montserret, François Penin, Marc Baldus. Solid state NMR sequential resonance assignments and conformational analysis of the 2 × 10.4 kDa dimeric form of the *Bacillus subtilis* protein Crh, *J. Biomol. NMR* 27, 323 (2003).

Porovnání chemických posunů s monomerní strukturou v roztoku



Porovnání chemických posunů s monomerní strukturou v roztoku

TALOS



Zdvojení některých signálů:

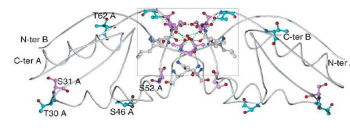
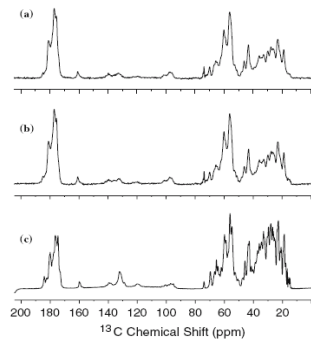
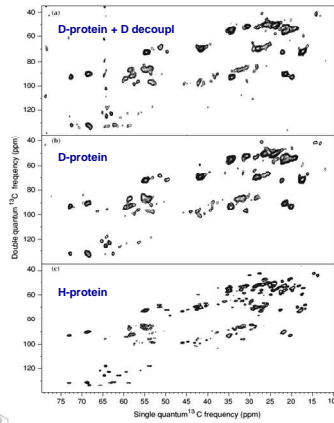
N - Gly 49 a 67
CO - Pro 18, Ala 45...

1. Konformační rozdíly monomerů v dimeru.
2. Rozdílné konformace dimeru v krystalové jednotce.
3. Dynamický disorder - pomalá výměna.

Jiří Laboratory of Solid State NMR
IMC AS CZ and JNRC AS CZ

Korelace disperze chemických posunů - deuterace

Mikrokrystalický protein Crh
(catabolite repression histidine containing
phosphocarrier protein)



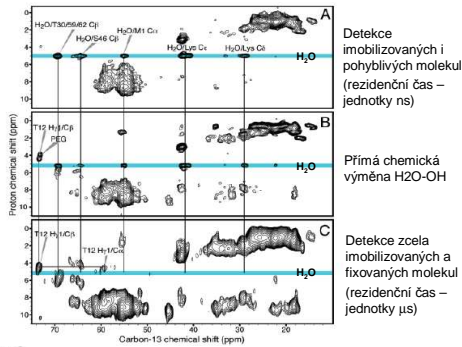
Aplikace: mikrokrystalické proteiny

Lokalizace vody - detekce ¹H NMR signálu

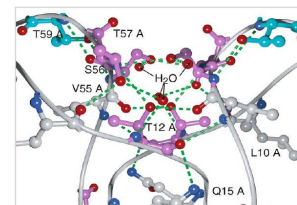
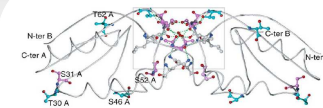
A. Böckmann, M. Juy, E. Bettler, L. Emsley, A. Galinier, F. Penin, A. Lesage, Water-Protein Hydrogen Exchange in the Micro-Crystalline Protein Crh as Observed by Solid State NMR Spectroscopy, *Journal of Biomolecular NMR*, 32 195 (2005).

Anne Lesage Lyndon Emsley, Francois Penin, and Anja Böckmann, Investigation of Dipolar-Mediated Water-Protein Interactions in Microcrystalline Crh by Solid-State NMR Spectroscopy, *J Am Chem Soc* 128, 8246 (2006).

2D ¹H-¹³C HETCOR – mikrokrystalický systém



Mikrokrystalický protein Crh
(catabolite repression histidine containing
phosphocarrier protein)

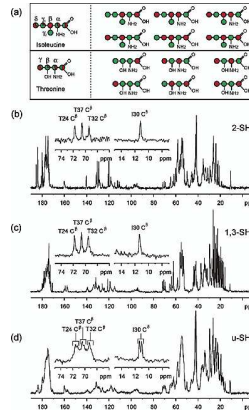
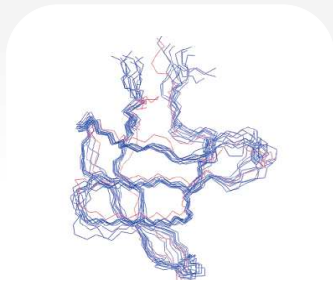


Joint Laboratory of Solid State NMR
IMC AS CZ and JHPC AS CZ

α -Spectrin Sh3 Domain

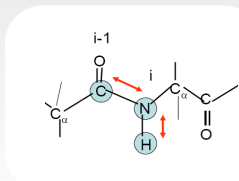
Castellani, F., van Rossum, B.J., Diehl, A., Schubert, M., Rehbein, K., and Oschkinat, H. Structure of a protein determined by solid-state magic-angle-spinning NMR spectroscopy, *Nature* 420, 98-102 (2002).

Castellani, F., van Rossum, B.J., Diehl, A., Rehbein, K., and Oschkinat, H. Determination of Solid-State NMR Structures of Proteins by Means of Three-Dimensional ^{15}N - ^{13}C - ^{13}C Dipolar Correlation Spectroscopy and Chemical Shift Analysis, *Biochemistry* 42, 11476 (2003).

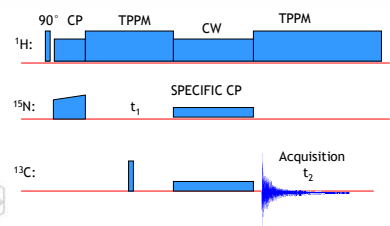
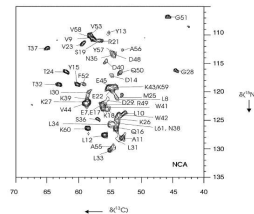
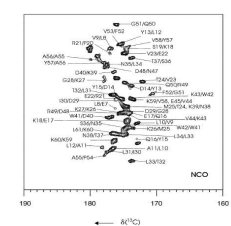


α -Spectrin Sh3 Domain

Castellani, F., van Rossum, B.J., Diehl, A., Schubert, M., Rehbein, K., and Oschkinat, H. Structure of a protein determined by solid-state magic-angle-spinning NMR spectroscopy, *Nature* 420, 98-102 (2002).

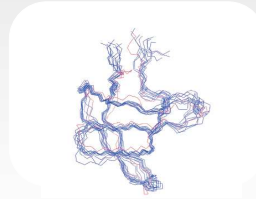


$$N_iCO_{i-1} \\ N_iCA_i \\ \sqrt{\omega_1^2 + \Omega_1^2} + \sqrt{\omega_2^2 + \Omega_2^2} = n\omega_r$$



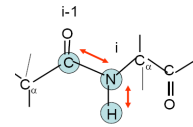
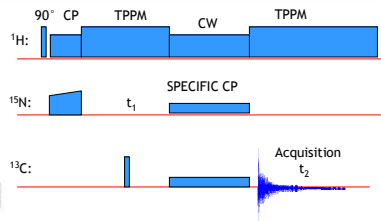
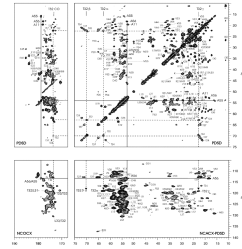
α -Spectrin Sh3 Domain

Castellani, F., van Rossum, B.J., Diehl, A., Schubert, M., Rehbein, K., and Oschkinat, H. Structure of a protein determined by solid-state magic-angle-spinning NMR spectroscopy, *Nature* 420, 98-102 (2002).

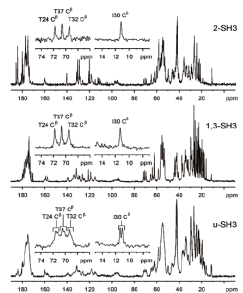


$N_iCO_{i-1}CX$
 N_iCa_iCX

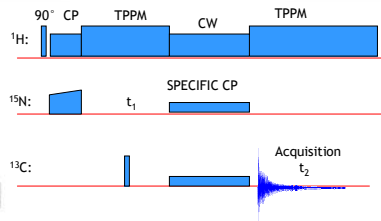
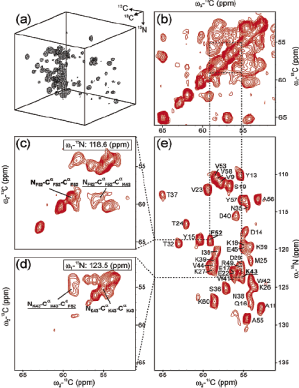
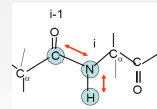
$$\sqrt{\omega_1^2 + \Omega_1^2} + \sqrt{\omega_2^2 + \Omega_2^2} = n\omega_r$$



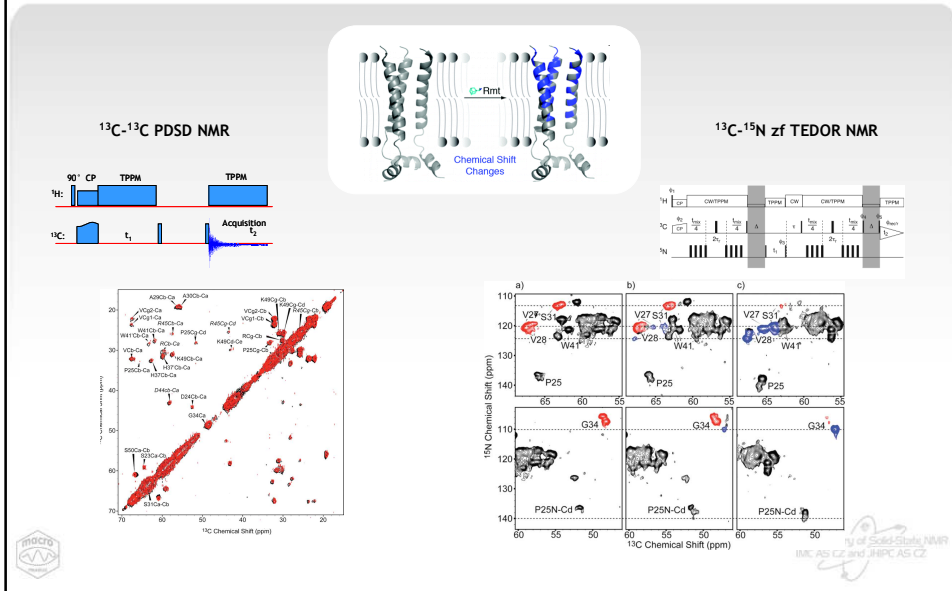
α -Spectrin Sh3 Domain



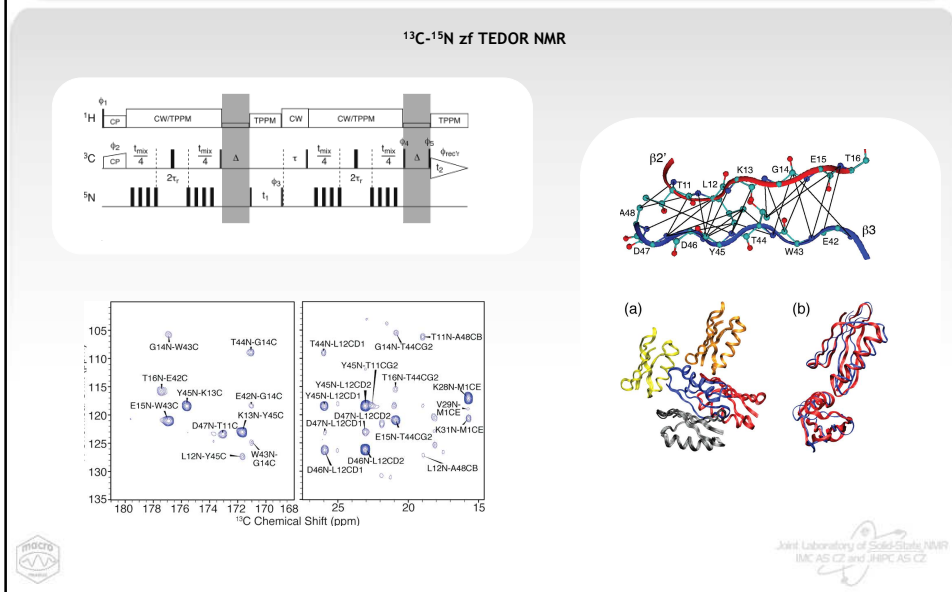
N_iCa_iCX



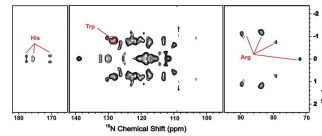
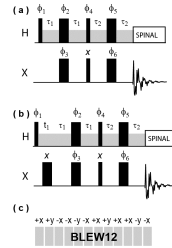
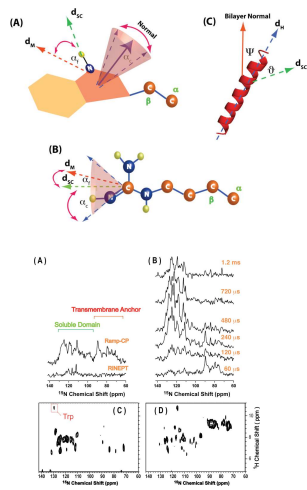
M2 protein chřipky A: alosterický mechanismus inhibice



supramolekulární struktura proteinů



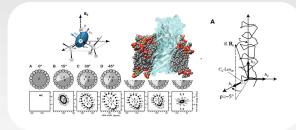
Dynamika proteinů



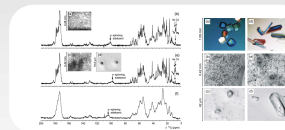
J. Am. Chem. Soc. 132 (29), pp 9944 (2010)

Souhrn

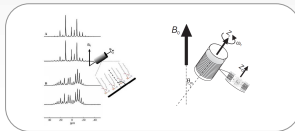
Membránové proteiny PISEMA



Příprava proteinových vzorků

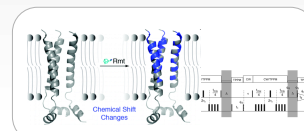


MAOSS NMR

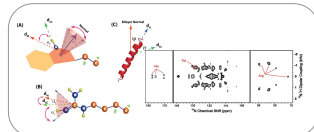


Solid-state NMR and

13C-15N zf-TEDOR



Dynamika proteinů



3D Double CP-PDSD

