folded

Tomáš Hošek

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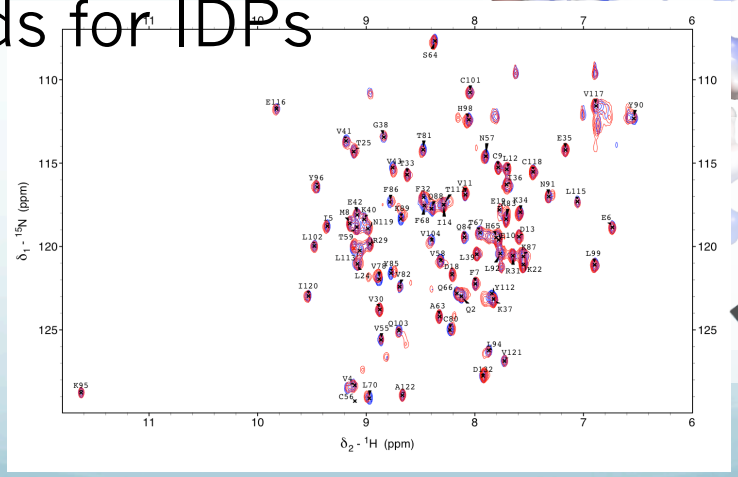
- Studied at Masaryk University in Brno
- Graduated in Molecular Biophysics
- Master thesis:
  - Supervised by Mgr. Karel Kubíček, PhD
  - Topic:

    The NMR and computational study of the interacting site of RNA-binding protein
- 22/08/2011 appointed by CERM within IDPbyNMR

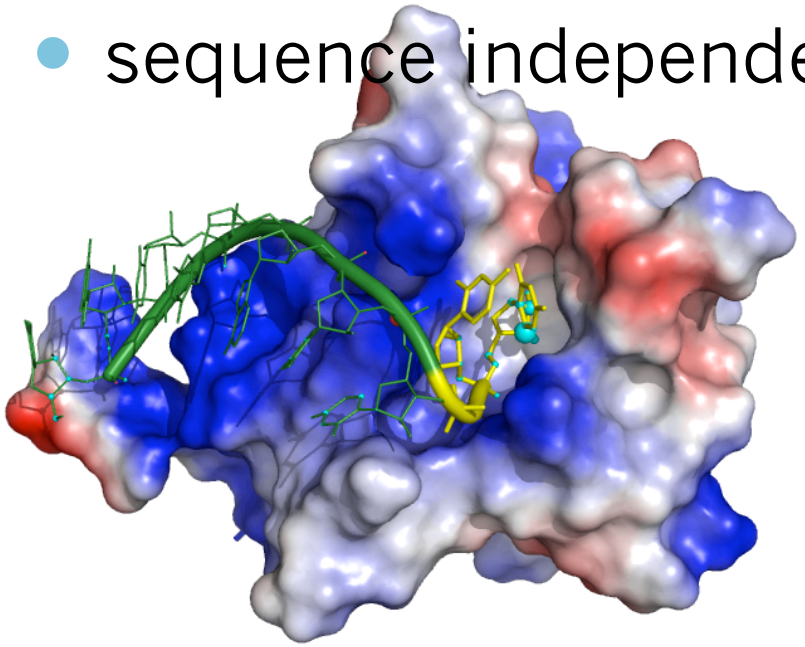
Structure of presentation

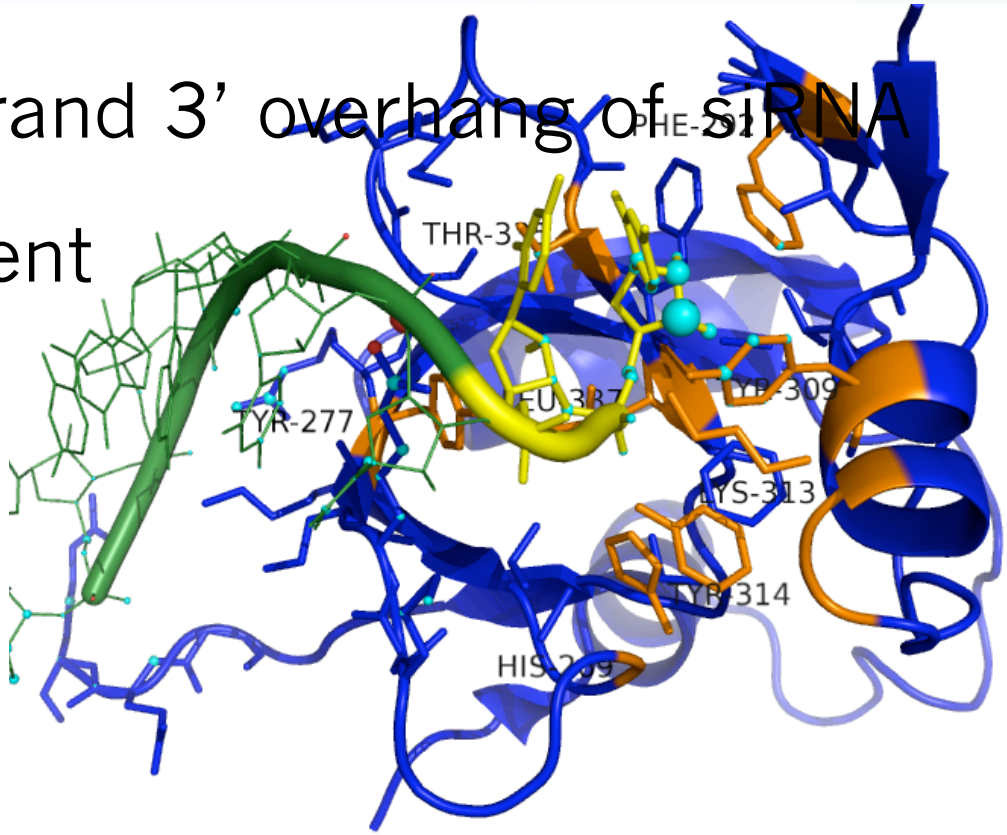
- Master thesis project
  - Short theory introduction
  - NMR experiments
  - Molecular Docking
  - NMR titration
- NMR methods for IDPs



PAZ domain
(Piwi, Argonaute, Zwille domain)

- RNA-binding module
- binds 2-nt single strand 3’ overhang of siRNA
- sequence independent

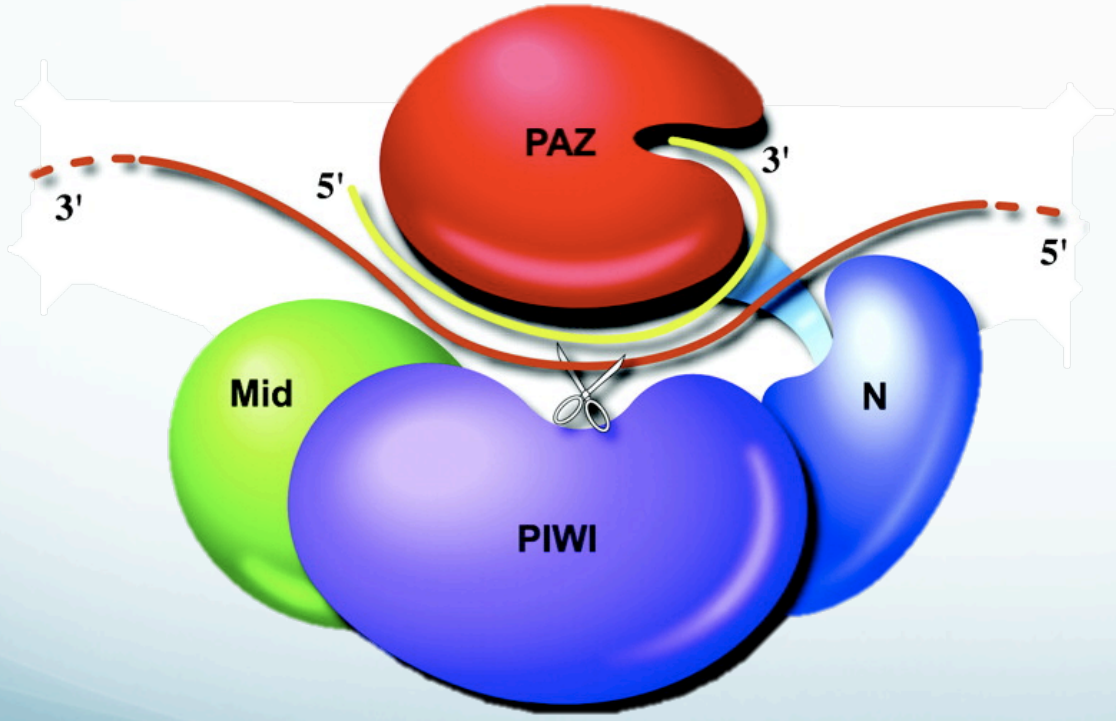


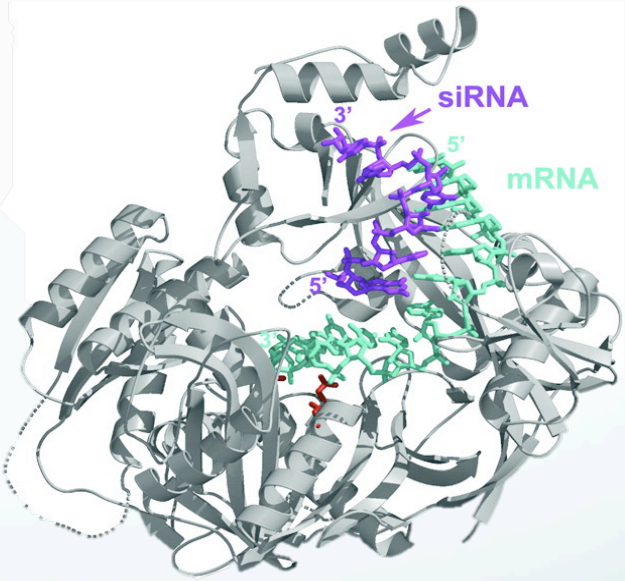


[1] Patel, D. J. et al. *Nature (2004)*
[2] Sattler, M. et al. *Nature* **426**, 465-469 (2003)

PAZ domain
(Piwi, Argonaute, Zwille domain)

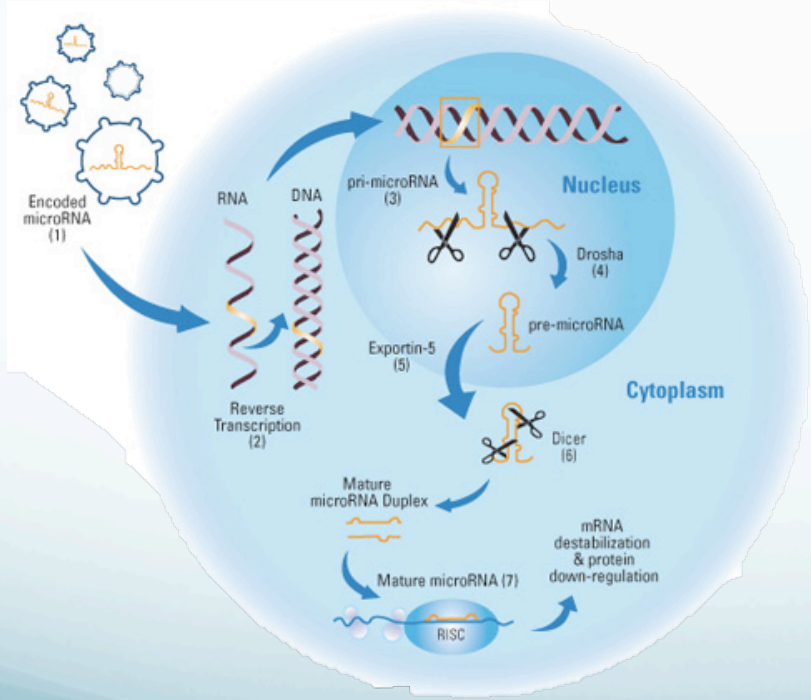
- Part of Argonaute and some Dicer's proteins

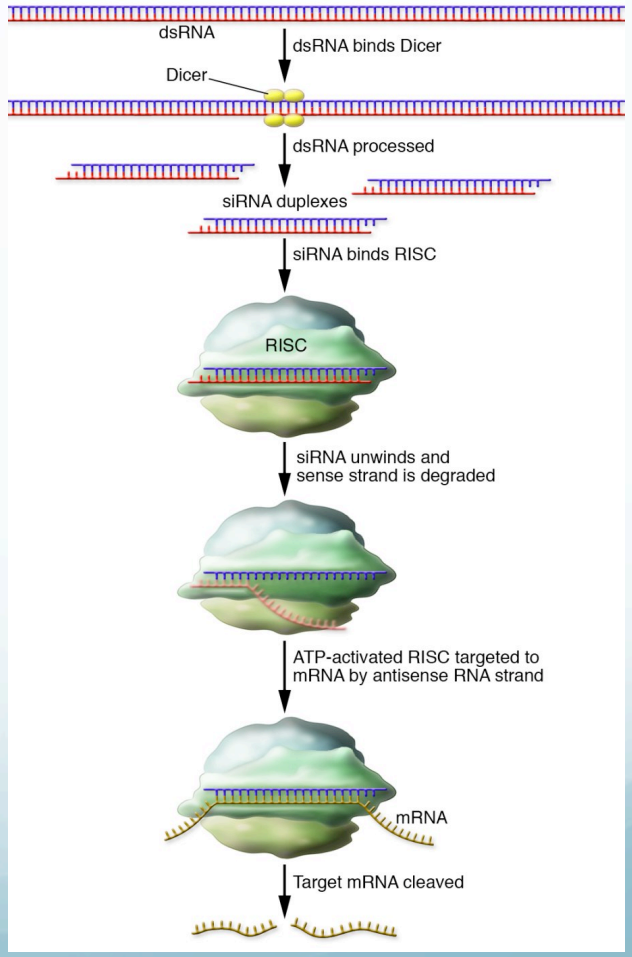




PAZ domain
(Piwi, Argonaute, Zwille domain)

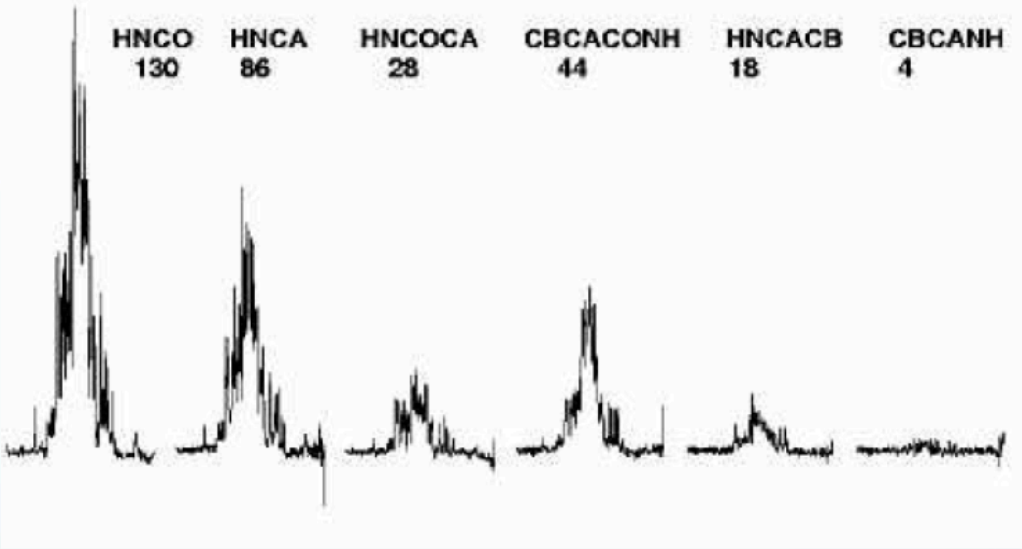
- participates in RNA interference and miRNA pathways



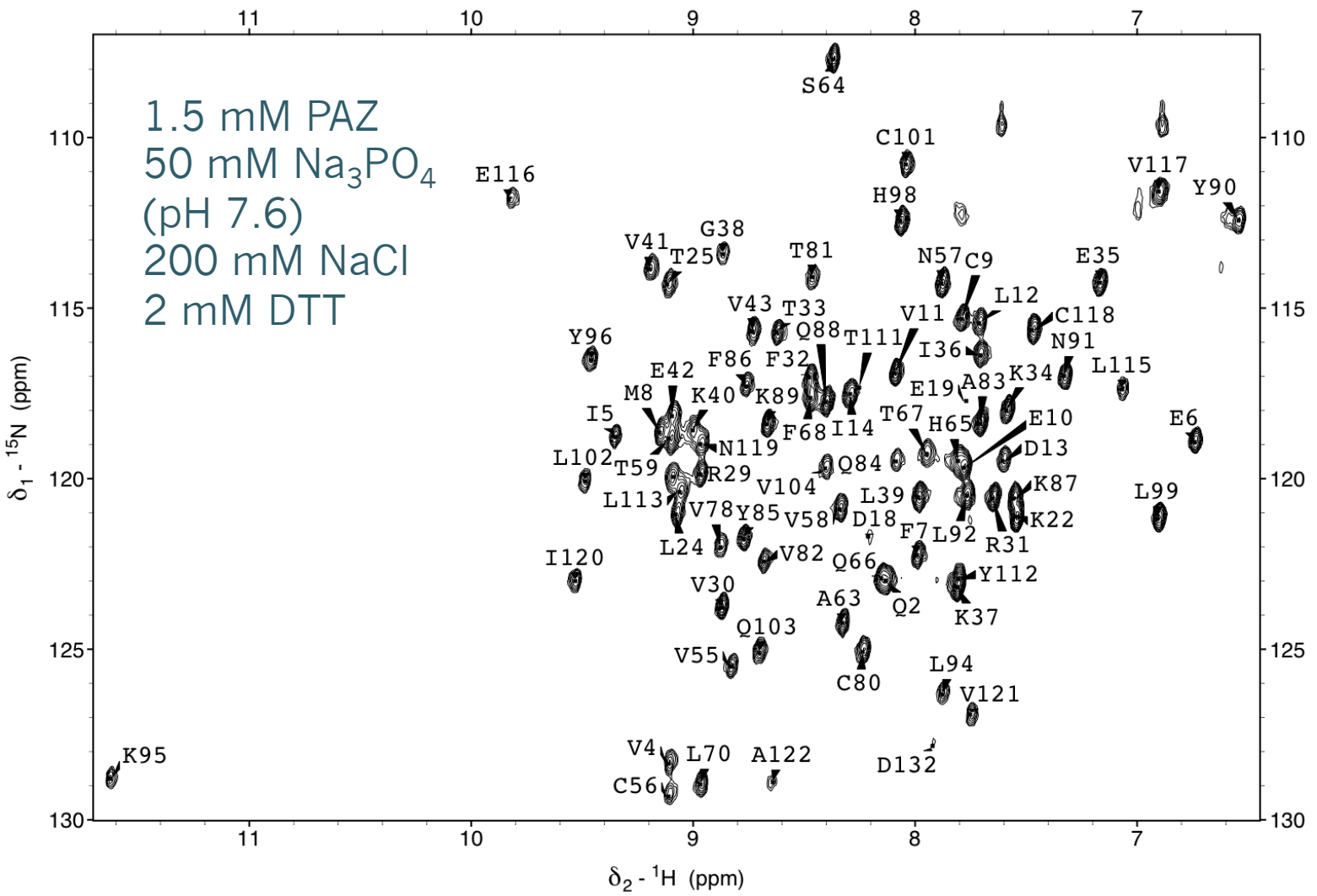


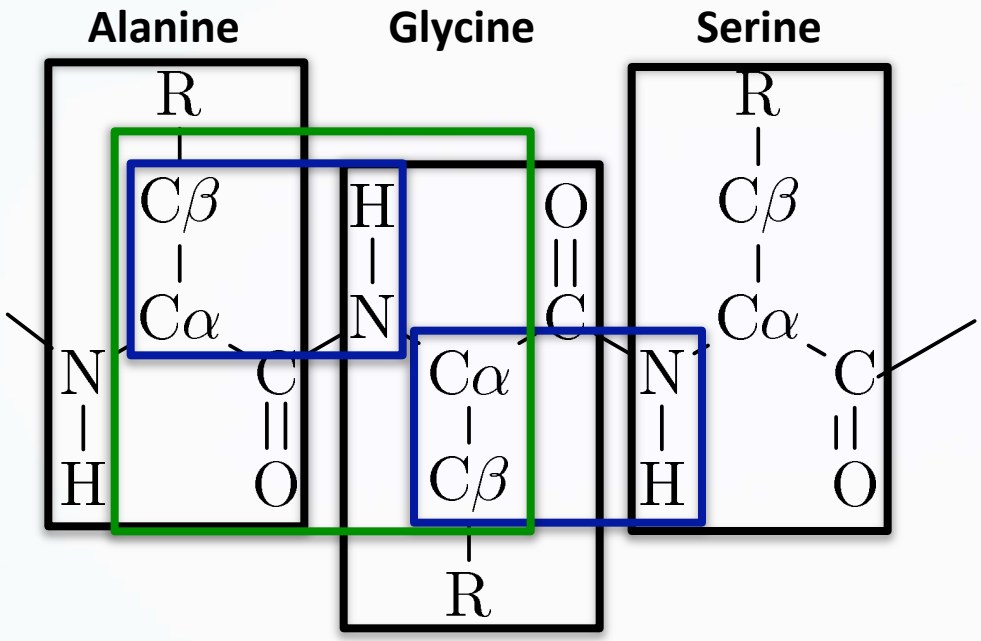
NMR experiments

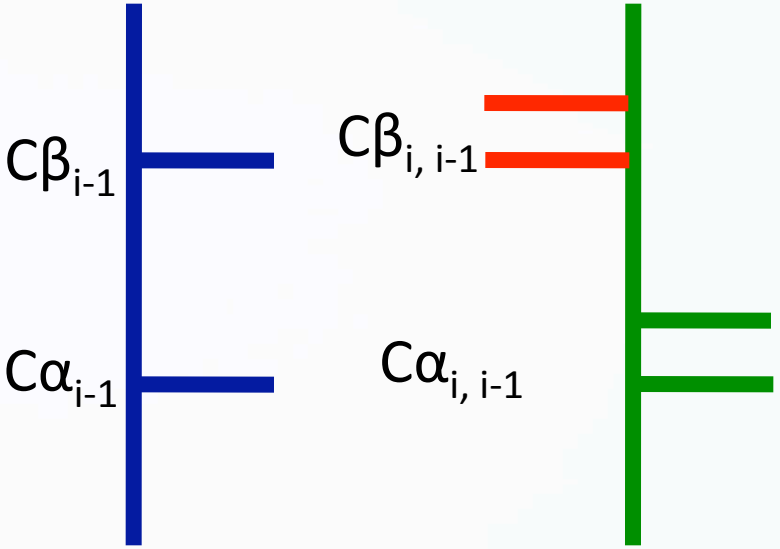
- ^{1}H-^{15}N HSQC
- CBCA(CO)NH
- HNCACB
- HBHA(CO)NH
- HCCH-TOCSY
- ^{13}C-NOESY

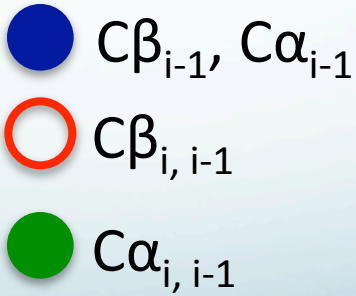


Acquired $^{15}N^{1}H$-HSQC

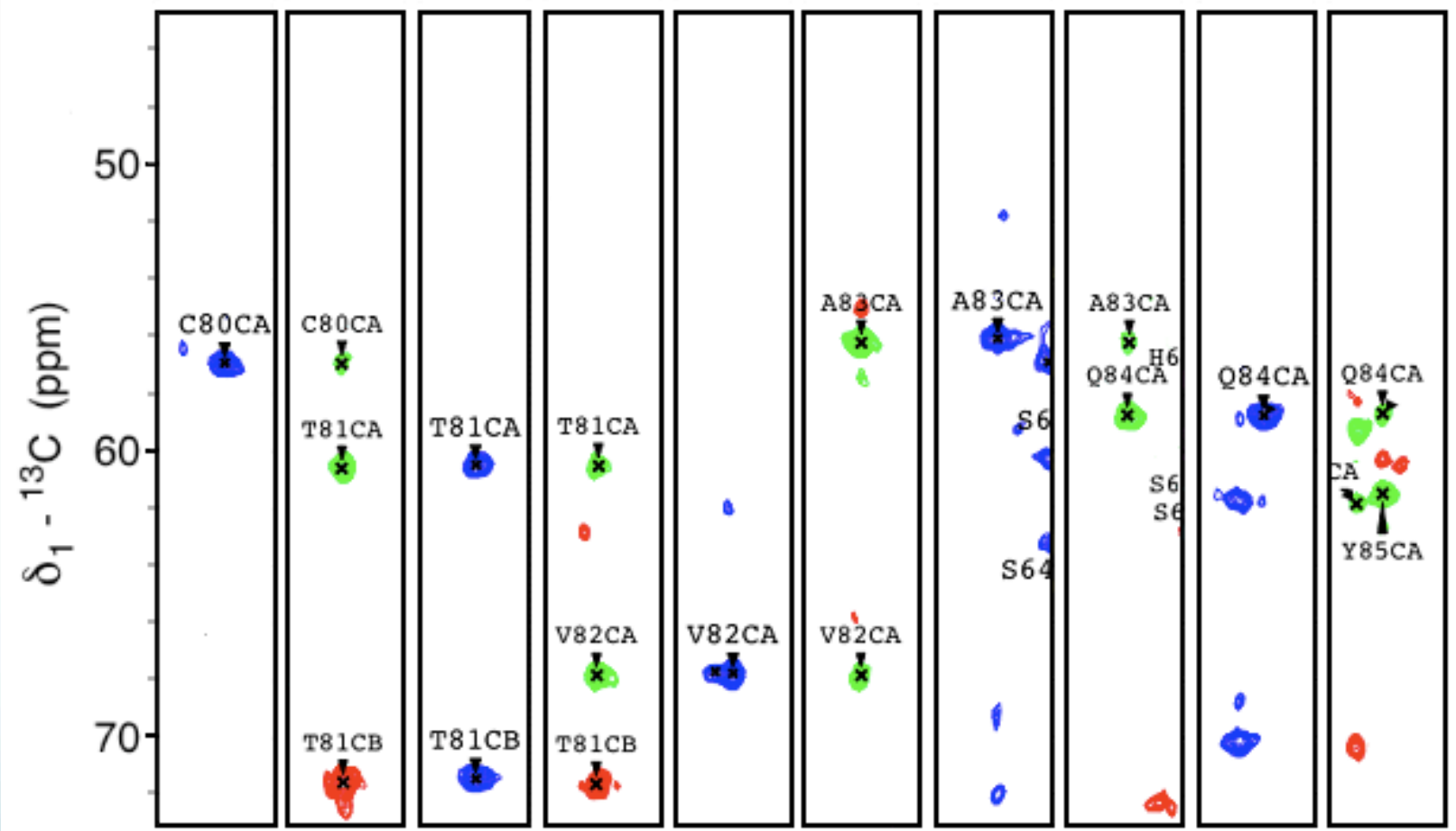



Alanine
Glycine
Serine
R
Cβ
Cα
N
H
C
O
i-1
i
i+1
$^{1}H_{i-1}$ / $^{15}N_{i-1}$
$^{1}H_{i}$ / $^{15}N_{i}$
$^{1}H_{i+1}$ / $^{15}N_{i+1}$


$Cβ_{i-1}$
$Cα_{i-1}$
$Cβ_{i, i-1}$
$Cα_{i, i-1}$


$Cβ_{i-1}$, $Cα_{i-1}$
$Cβ_{i, i-1}$
$Cα_{i, i-1}$

Strip Plot


50
60
70
δ_1 - ^{13}C (ppm)
C80CA
C80CA
T81CA
T81CB
T81CA
T81CB
T81CA
V82CA
T81CB
V82CA
V82CA
A83CA
A83CA
S6
S64
A83CA
H6
Q84CA
S6
S6
Q84CA
Q84CA
CA
Y85CA
$\delta_2^{15}N$: 114,1 ppm 122.4 ppm 118.4 ppm 119.5 ppm 121.8 ppm
$\delta_3^1H^N$: 8.467 ppm 8.683 ppm 7.708 ppm 8.089 ppm 8.778 ppm

Resonances assignment

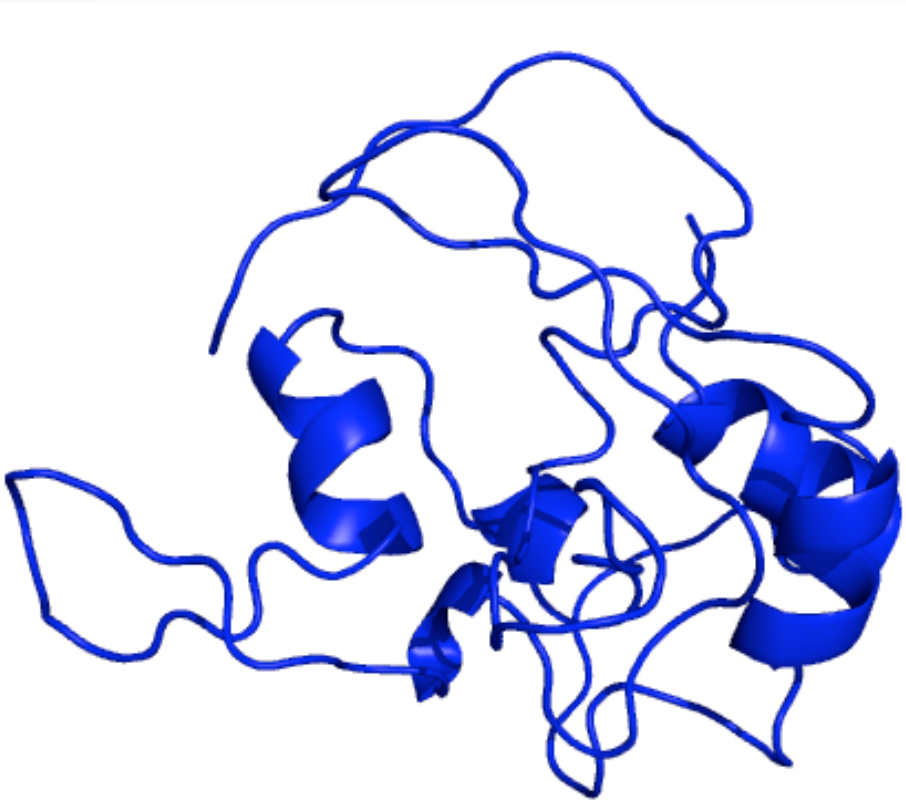
- Backbone nuclei assigned:
  - 64% of backbone amide protons and ^{15}N
  - 72% of C^{α}
- Side-chain nuclei assigned:
  - 70.4% of aliphatic ^{1}H and ^{13}C

AQPVIEFMCE VLDIRNIDEQ PKPLTDSQRV RFTKEIKGLK
VEVTHCGQMK RKYRVCNVTR RPASHQTFPL QLESGQTVEC
TVAQYFKQKY NLQLKYPHLP CLQVGQEQKH TYLPLEVCNI
VAGQRCIKKL TD

- backbone **assigned**/**unassigned**

Structure calculated from ^{13}C-NOESY by program CYANA

Calculated by CYANA

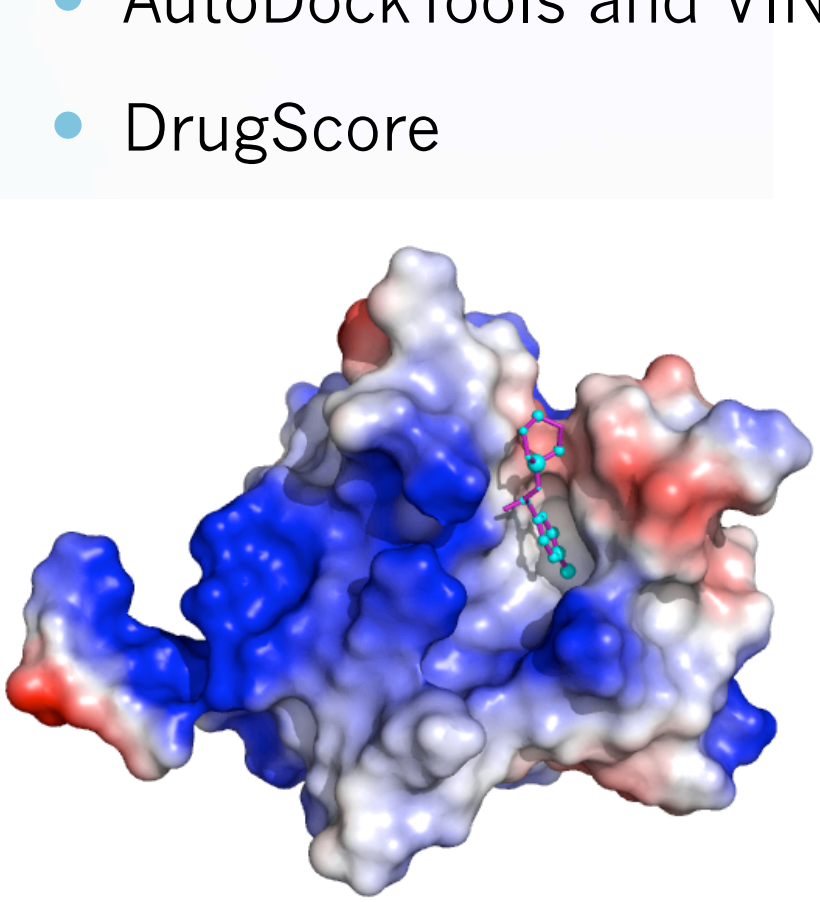


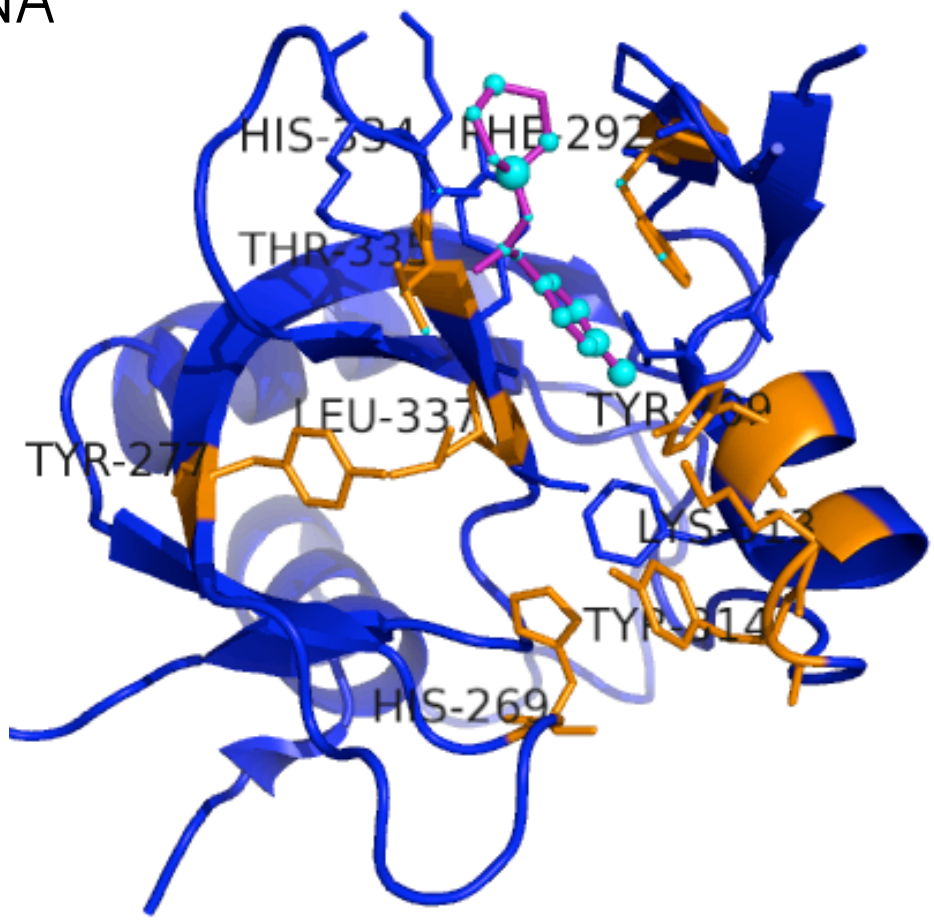
Patel's structure

[1] Patel, D. J. et al. *Nature (2004)*

Molecular docking

- AutoDockTools and VINA
- DrugScore

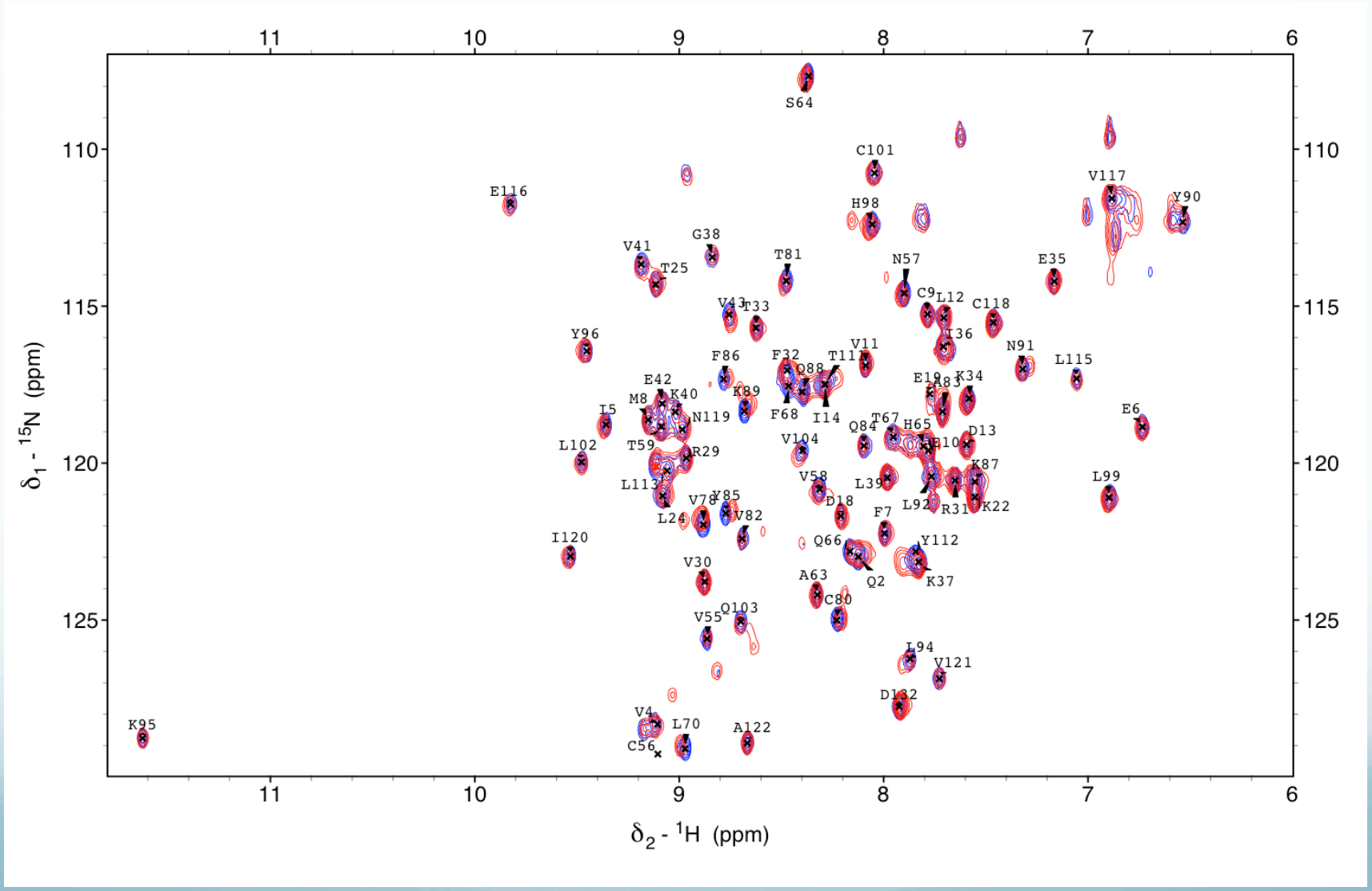




[3] http://vina.scripps.edu/
[4] http://pc1664.pharmazie.uni-marburg.de/drugscore/

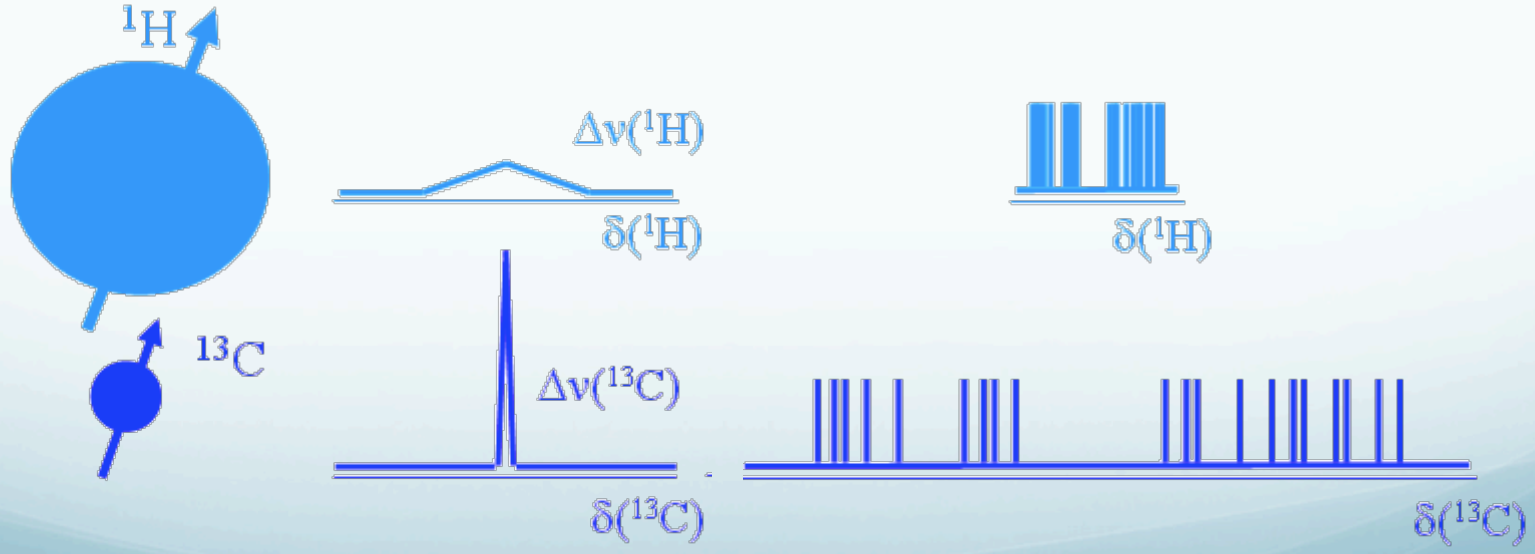
NMR titration

REaD 1073



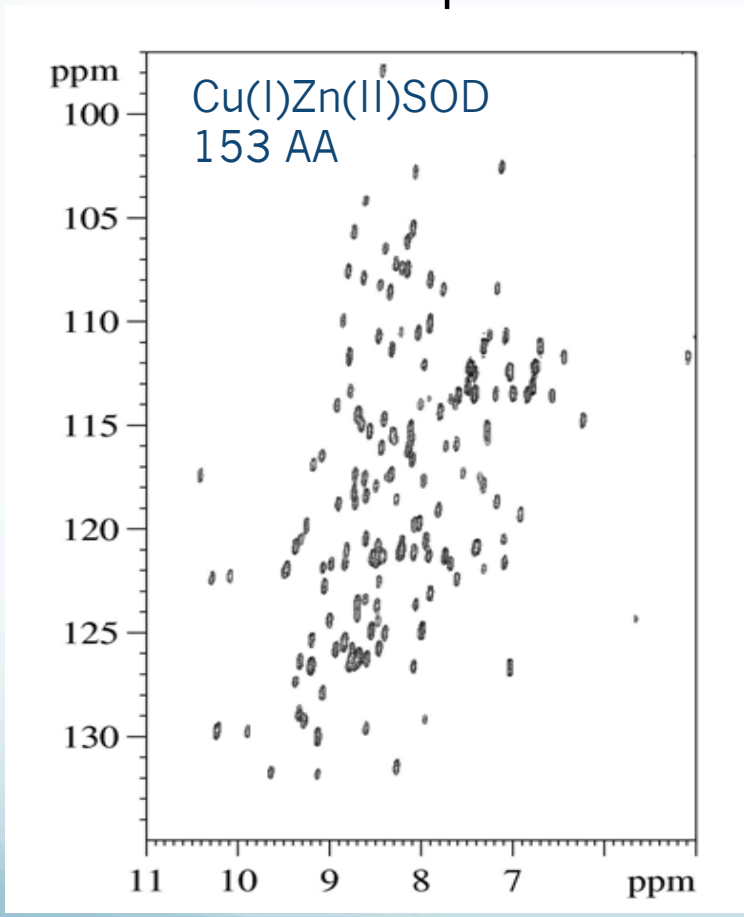
^{13}C direct detection NMR

- Main advantages compare to ^{1}H direct detection NMR in solution:
  - Reduced dipolar coupling contribution to relaxation
  - Unaffected by chemical exchange with the solvent
  - Increased chemical shift dispersion

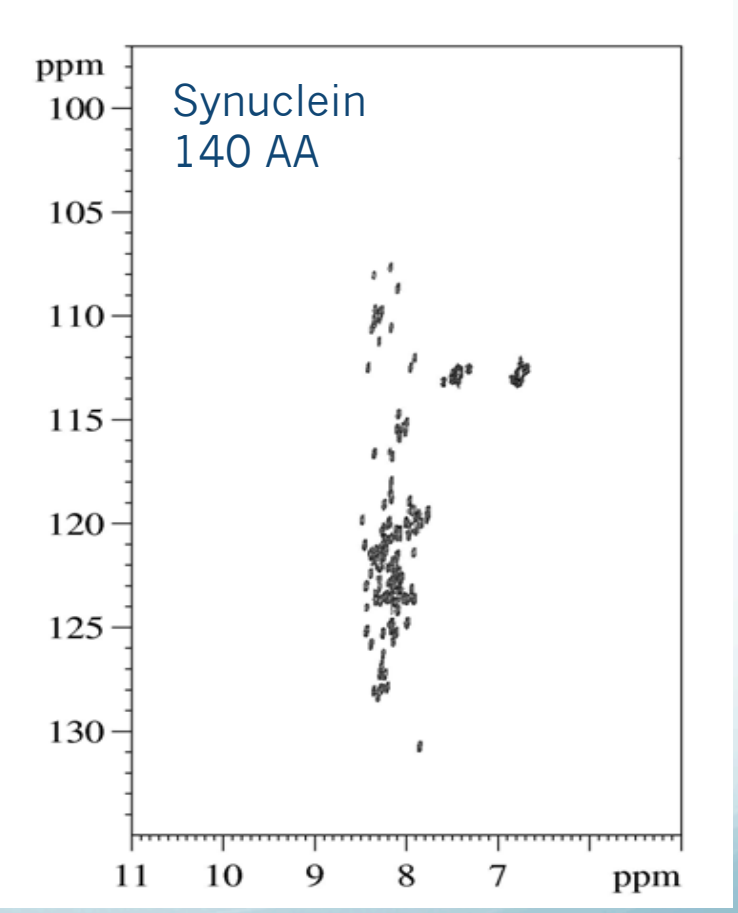


^{1}H direct detection & IDP

- Well folded protein



- IDP

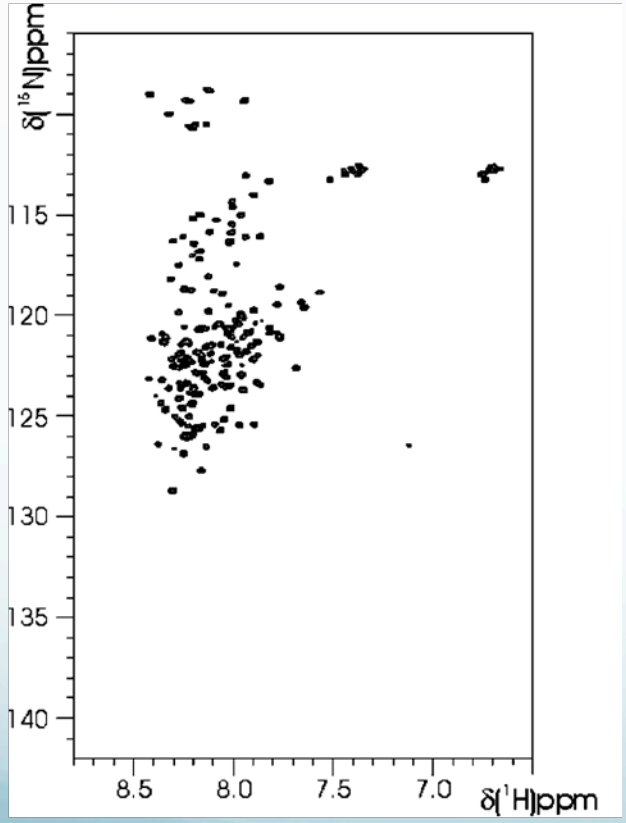


[5] Bertini I., Felli I., Luchinat C., Parigi G., Pierattelli R., *ChemBioChem,* **2007**

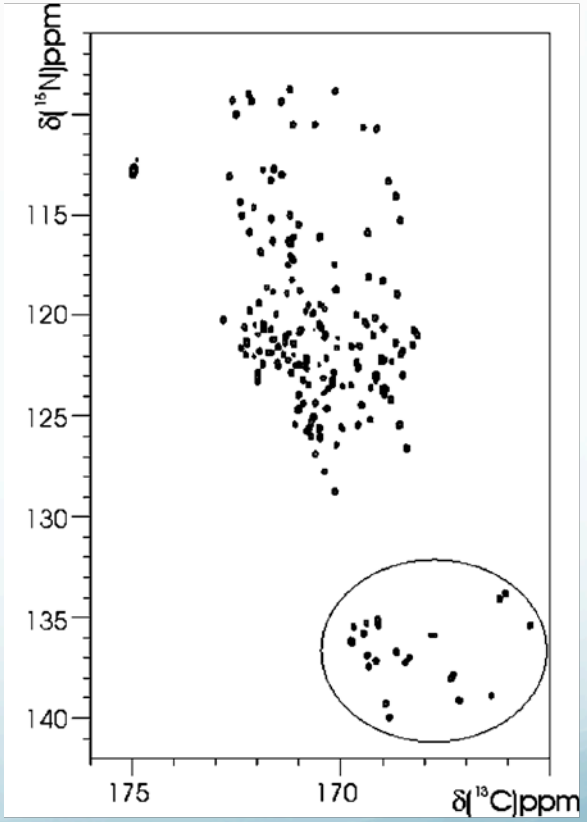
^{13}C direct detection & IDP

- Intrinsically disordered protein - Securin

HSQC



CON



[6] Csizmok V., Felli I., Tompa P., Banci L., Bertini I., *J. Am. Chem. Soc.*, **2009**

^{13}C direct detection - improvements

- Speed up data acquisition
  - Reduce sampling
  - Longitudinal relaxation enhancement
- Amino acid selective experiments
- Multidimensional experiments
- Integration with ^{1}H direct detection

Acknowledgement

- Mgr. Karel Kubíček, PhD
- Prof. Isabella Felli
- Prof. Roberta Pierattelli
- CERM group

Thank you