EXTENDING THE LIMITS OF NMR

SH3 7 kD  
P21-ras 21 kD  
Pb92 30 kD  
EIN-I 40 kD  
DHBPS 47 kD

Increasing Spectral Complexity  
Increasing Relaxation

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January 29, 2004
Obstacles to NMR Studies of Larger Proteins

- Increased Resonance Degeneracy
  - Complex Spectra

- Increased Relaxation
  - Reduced Sensitivity
  - Limited Resolution
Protein Molecular Weight Limits Spectral Resolution

19 kDa

$\omega = \delta_\text{H}[\text{ppm}] \quad \omega = \delta_\text{H}[\text{ppm}]

Tm=40\text{ms}

47 kDa

$\omega = \delta_\text{H}[\text{ppm}] \quad \omega = \delta_\text{H}[\text{ppm}]

Tm=40\text{ms}
Protein Molecular Weight Limits Spectral Resolution

14 kDa

ω = δC [ppm]

2 1

ω = δH [ppm]

20

15

25

47 kDa

ω = δC [ppm]

20

15

25

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Backbone and Side-Chain Assignment
Collection of Distance Restraints in Large Proteins

- 100% $^2$H $^{15}$N
  - NH-NH NOEs
  - side-chain amide NOEs

- $^1$H residue-specific
  - NOEs from protonated residues

- $^1$H methyl-specific
  - NOEs from methyl groups

- random fractional $^2$H
  - NOEs all residues
$^{1}H,^{14}N$-FYTIV  $^{2}H,^{15}N$-X Strategy
$^{15}$N-Edited NOESY
$^{15}\text{N-Filtered NOESY}$
Advantages of the FYTIV Strategy

- aids assignment of aromatics (F and Y)
- sequential assignments of $X-(F/Y/T/I/V)_n-X$ fragments facilitated
- aids secondary structure assignment
- high propensity for F, Y, T, I, V in $\beta$-sheet and in hydrophobic core
- 15N selection/filtering for unambiguous assignment
- Useful long-range NOEs between:
  - F/Y residues and methyl groups
  - sidechains of F, Y, T, I, V residues
E. coli Mutase Sequence

F, Y, T, I, V

1  MNQLLSSFG TFERVENAL AALREGROM VLDDEDRENE GDMFPAETIM

51  TVEQMATTIR HGSGTVCLCI TEDRRKQLDL PMVENNTSA YGTGTVTIE

101  AAEOYTVGS AADRIITTVRA AITADGAKPSD LNRPGTLFPL RAQAGCMTTR

151  GGHTEATIDL MLAGFKPAG VLCELTNDDG TMARAPECE FANKHNMA

201  TLEDLVAYRQ AHERKAS
Preparation $^1$H-, $^{14}$N-FYTIV $^2$H-, $^{15}$N-X Sample

- M9 minimal medium
- $^{15}$NH$_4$Cl
- $^1$H-, $^{14}$N-F,Y,T,I,V amino acids
- 100% D$_2$O
- *E. coli* M15/pQE, T5 expression system
Problems for Structure Determination of Large Proteins

- assignment of secondary structure
  - $H_N$ line-width
  - $H_{\overline{\sigma}}$ line-width (dipolar relaxation $^{13}C$)
  - $^{15}N$, $H_N$ degeneracy

- collection of long range NOEs
  - $^{1}H$ line-width (dipolar relaxation $^{1}H$, $^{13}C$)
  - overlap
  - $^{13}C$ line-width
  - spin diffusion
## Comparison of Different Samples

<table>
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<th>$^1H^13C^{15}N$</th>
<th>$100%^2H^{15}N$</th>
<th>$75%^2H^13C^{15}N$</th>
<th>FYTIV $^{15}N-X$</th>
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<tr>
<td><strong>backbone assignment</strong></td>
<td>+++</td>
<td>+</td>
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<td><strong>side-chain assignment</strong></td>
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<td><strong>secondary structure</strong></td>
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<td><strong>long-range NOEs</strong></td>
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</tbody>
</table>
A SOLUTION: PROTEIN DEUTERATION

Torchia et al., 1988

Protonated

Deuterated

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Deuteration Improves Resolution

2D $^1$H NOESY spectra

Protonated DHBPS

Deuterated DHBPS

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(H)C(CCO)NNH with a protein with 12ns $^{13}$C
NOE Distance Restraints 12ns

$\tau_c = 12$ nsec

NH/sidechain

NH/NH

0% $^2$H  50% $^2$H  75% $^2$H
Another Solution:
Amino Acid Specific Protonation in a Deuterated Background
Specific Protonation Improves Sensitivity: FYTIVL Pattern (Kelly et al., 1999)
Specific Protonation Improves Resolution: Methyl-\(^1\)H Sample (Goto et al., 1999)

2D \(^{13}\)C HSQC spectra

1H 13C 15N DHBPS

1H(CH3)ILV 2H 13C 15N DHBPS
Methyl-Protonation Reduces Ambiguity In NOESY Expts (Zwahlen et al., 1999)
NMR Restraints from different Samples (p21 ras)

Ras NMR Structure
0.6Å RMSD
101 HN-HN
1171 HN-S/C
1862 SC/SC

$^{13}\text{C}/^{15}\text{N}$ ILV $^{15}\text{N}$ FY
5.0Å RMSD
101 HN-HN (ass-ass)
390 HN-S/C (ass-amb)
231 ILV-ILV (ass-ass)
174 ILV-FY (ass-amb)
26 FY-FY (amb-amb)

$^{13}\text{C}/^{15}\text{N}$ ILV $^{15}\text{N}$ FY
2.0Å RMSD
101 HN-HN (ass-ass)
390 HN-S/C (ass-amb)
431 S/C-S/C (ass-ass)

$^{13}\text{C}/^{15}\text{N}$ ILV $^{15}\text{N}$ FY
1.7Å
NH NOEs to 5Å
As above with 330 HN-HN NOEs

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NMRSOLVE

Experimental Conditions
8 hours 2D $^{13}$C-HMQC
600 m (5mm-tube) 50M p38
90% H$_2$O; 10% D$_2$O; 20°C