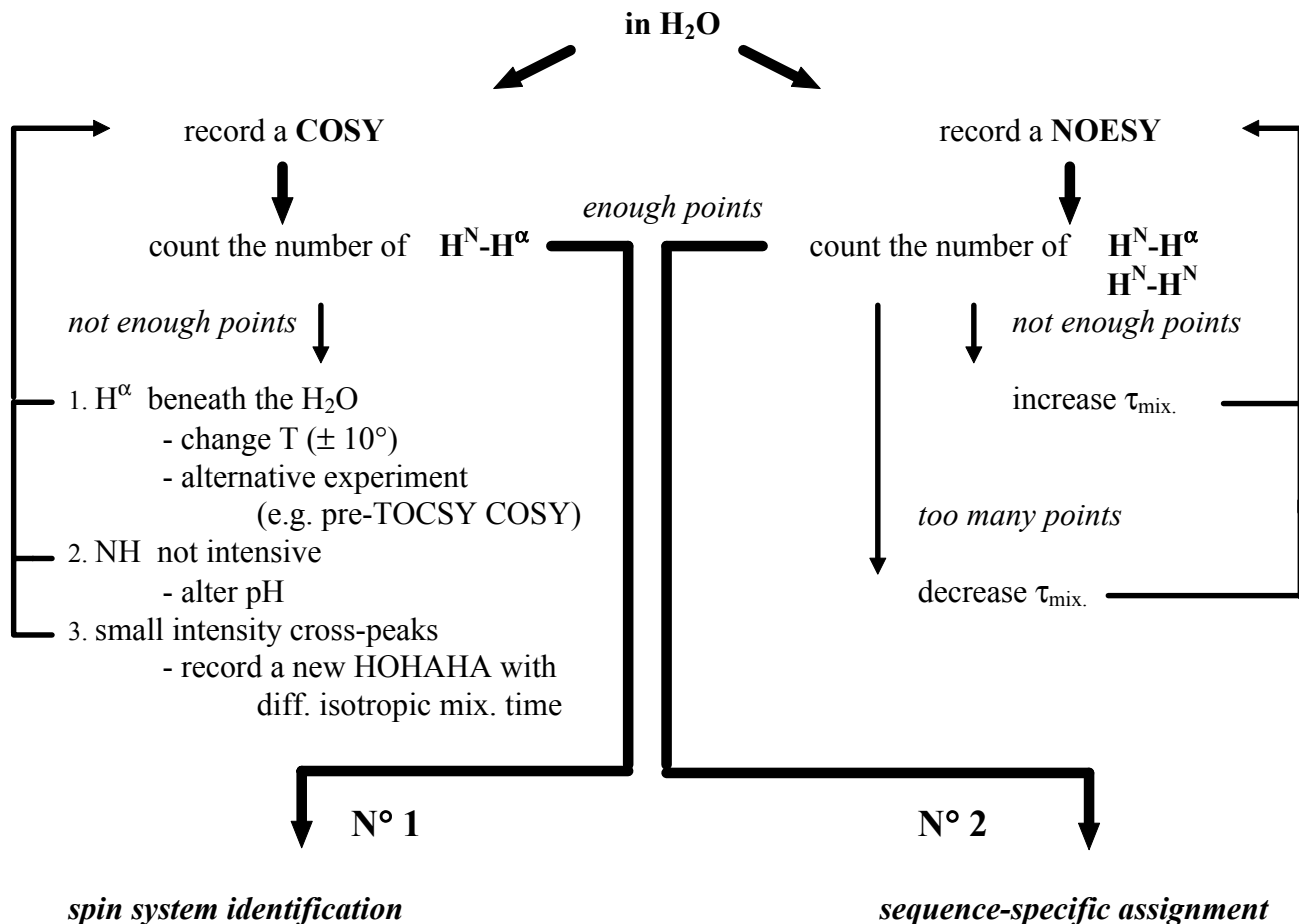


**SEQUENTIAL ASSIGNMENT unlabelled proteins
(no resolved spin-spin coupling across the peptide bond)**

required quality of the 2D-spectra for sequential assignment

-all coupled H^N-H^α resonances present (for all residues)
look for cross-peaks in the fingerprint region

$6.5 \text{ ppm} \leq F_2 \leq 10.5 \text{ ppm}$
 $2.5 \text{ ppm} \leq F_1 \leq 6.0 \text{ ppm}$



all H^N 's present in H_2O

- pre- TOCSY COSY or gradient COSY (DQF-COSY)
- RELAY (30 ms $\leq 2\tau \leq 40$ ms)
- double-RELAY (30 ms $\leq 2\tau \leq 40$ ms)
- several HOHAHA (15 ms \leq isotropic mixing ≤ 80 ms)

no H^N 's present in D_2O

- DQF-COSY
- RELAY (30 ms $\leq 2\tau \leq 40$ ms)
- double-RELAY (30 ms $\leq 2\tau \leq 40$ ms)
- several HOHAHA (15 ms \leq isotropic mixing ≤ 80 ms)

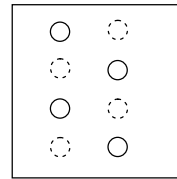
typical spin system identification



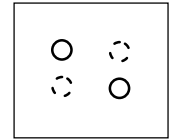
N° 1 Typical spin system identification

aim: the identification of the spin systems

1. **Gly** (8 component shape H^N-H^α) AMX spin system



2. **Ala and Thr** (4 component shape characteristic $H^\alpha-H^\beta$ of Ala
4 component shape characteristic $H^\beta-H^\gamma$ of Thr) intense cross-peaks



3. **Val, Ile and Leu** (Val and Leu \Rightarrow a common CH shift correlate with two CH_3)
(Ile \Rightarrow a common CH shift correlate with a CH_3)

4. **Type -J** -No proton at the γ position (**Ser, Cys, Asp, Asn, Trp, Phe, Tyr, His**).
-Similar $H^N-H^\alpha-H^\beta$ spin subsystem since there is no resolved coupling between the β -protons and those at position δ or ϵ (if any).
-In D_2O COSY the $H^\alpha-H^\beta$ spin subsystem is an AMX (8 component shape)
- the $H^\alpha-H^\beta$ type J depends on χ_1
- the $H^\beta-H^\beta$ type J is constant

5. **Type -U** -Two proton at the γ position coupled with two β protons (**Lys, Arg, Met, Gln, Glu and Pro**).

6. **Type -X cross-peak category**
- Unassigned H^N-H^α peaks

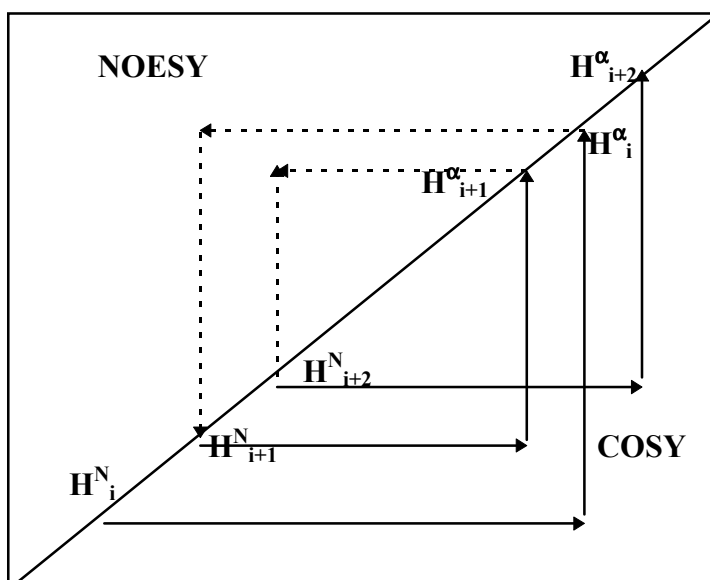
N° 2 Sequence-specific assignment

aim: the correlation of the adjacent spin systems

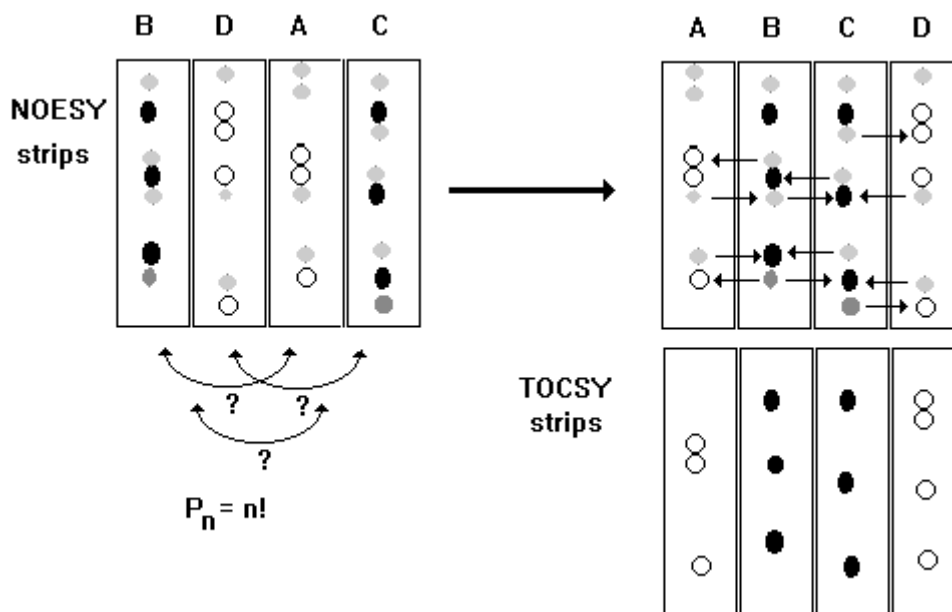
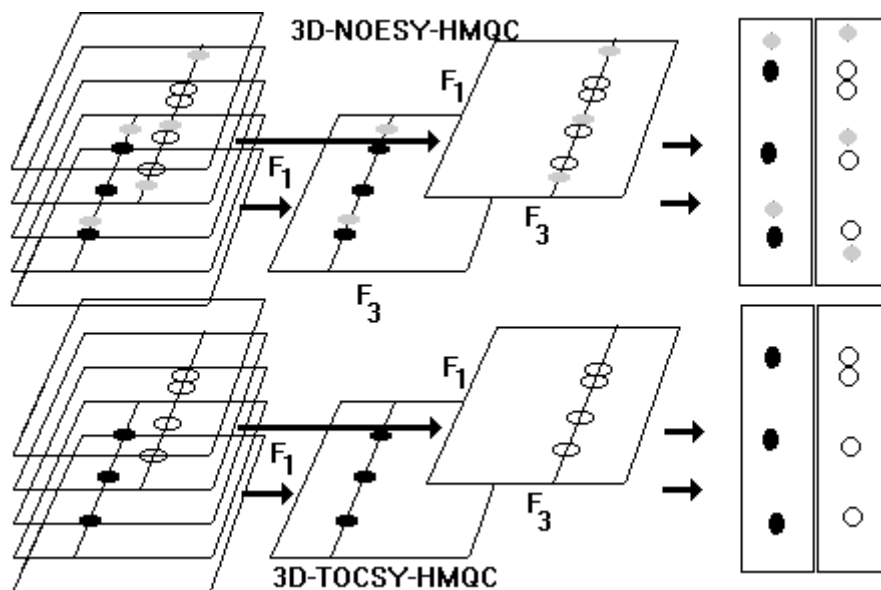
problem: no resolvable spin-spin coupling between protons of adjacent residue

solution: in any conformer the $d_{\alpha N}(i, i+1)$ and/or $d_{NN}(i, i+1)$ and/or $d_{\beta N}(i, i+1)$ give NOE signal.

NOE intensities depend on the distances
distances depend on the torsion angles (conformation)



SEQUENTIAL ASSIGNMENT based on ^{15}N strategy



Heteronuclear ^{13}C - ^{15}N NMR experiments

Typical arrangement of a the ^{13}C - ^{15}N NMR data axes.

