Sample Preparation for Bilayer Samples

For structural studies of membrane proteins using solid-state NMR

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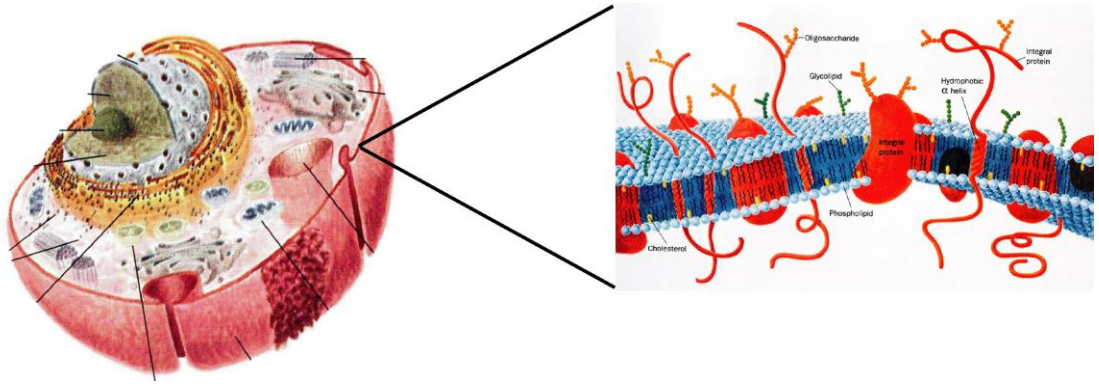
Importance of membrane proteins

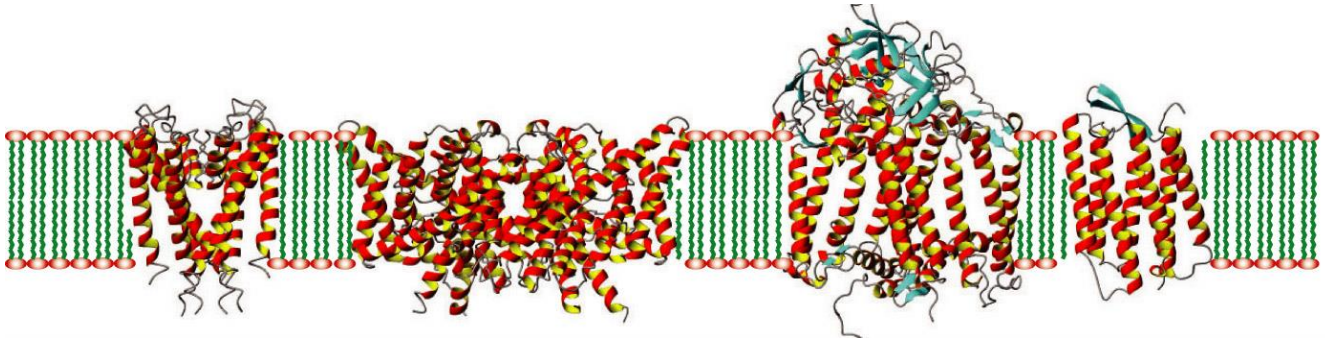
- Function at the interface of a cell and its surroundings
- Key role in cellular and physiological processes
- Drug receptors, ion channels, solute transporters



- Approximately 30% of expressed gene products
- 70% of all pharmaceutically relevant proteins
- GPCRs are estimated to be 60% of drug targets

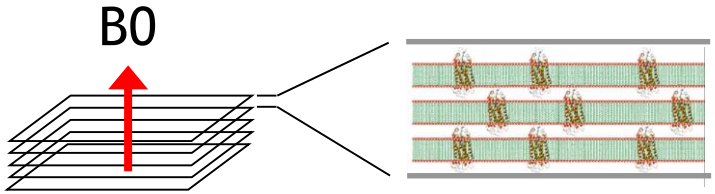
Difficulties in studying the membrane protein structure

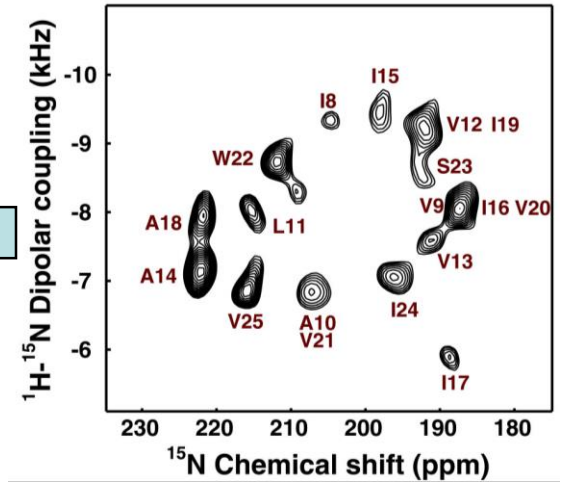
- Expression and purification
- Environment-dependent quaternary structure
- Difficult to cystallize
- Lack of reliable long-range distance information

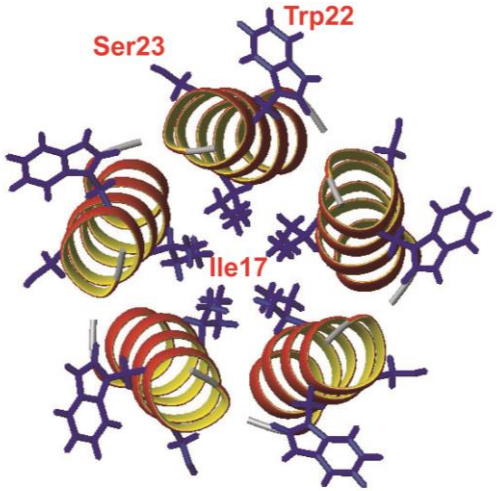


- Recent advanced NMR is ideal in that dynamics and crystallization are less problematic

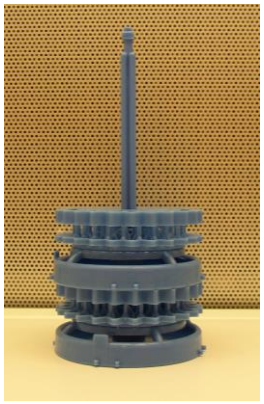
From aligned sample to structure



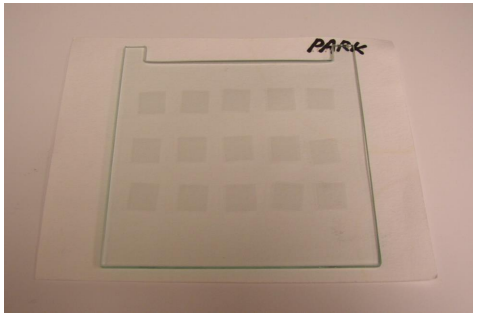




Overview of sample preparation



Clean the glass plates



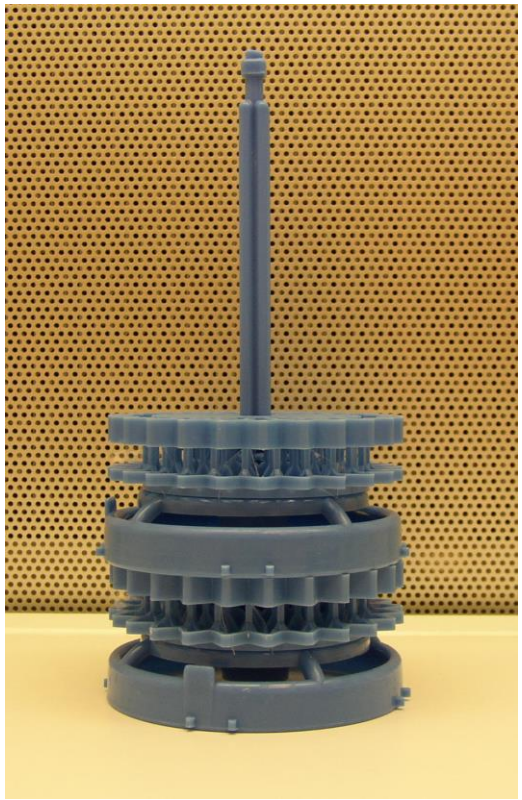
Dispense the protein-lipid mixture on glass plates

Dry organic solvent on plates in high vacuum overnight

Stack the plates and hydrate the sample in the chamber

Wrap the sample with parafilm and seal it with plastic bag

Cleaning glass plates



Place the plates one by one on the rack

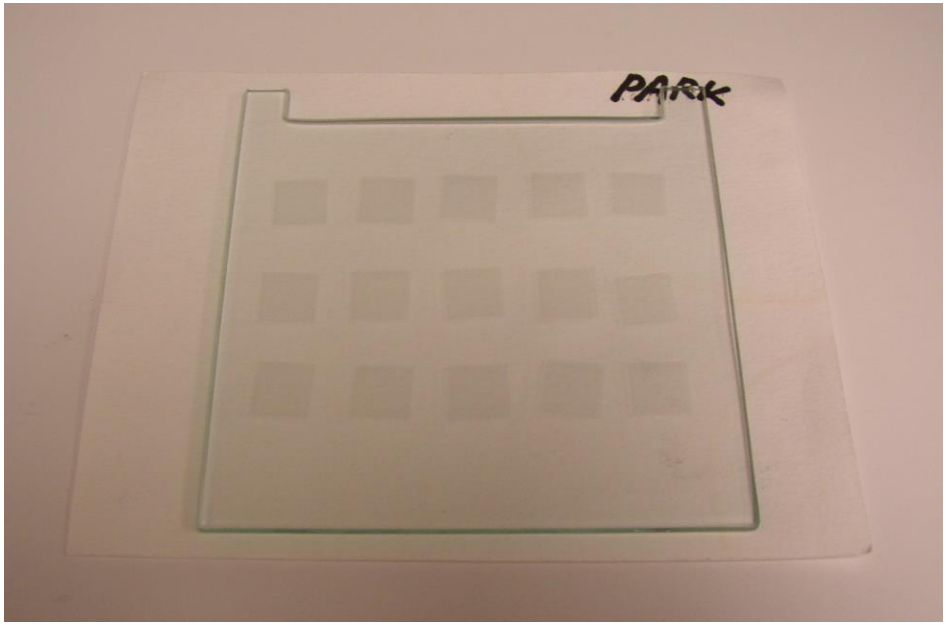
Soak the plates in phosphate-free detergent
(e.g. 1% Liquid Nox®)
Sonicate for ~ hours

Wash the plates thoroughly with water
Sonicate briefly several times

Soak the plates in 100% ethanol solution
Sonicate for ~ hours

Dry them at room temperature for an hour
Put them in the oven (~45℃) overnight

Dispense sample on glass plates



Mix lipid with protein and dispense the mixture equally onto each glass plates

1) Organic solvent-based: concentrated in chloroform/TFE
2) Liposome-based: reconstituted protein in liposome

*Lipid / Protein molar ratio > 100

*Lipid per glass plate ≤ 5 mg (e.g.11 x 11 x 0.07 mm)

Dry, hydration, and sealing

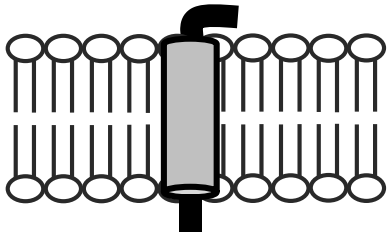
Dry for 1~2 hours at room temperature
Dry in high vacuum overnight
to remove the residual organic solvent

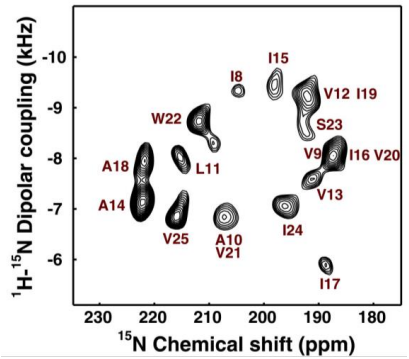
Stack the plates
Hydrate the sample in the chamber at 42°C
*saturated $(NH_4)_2HPO_4$ / $NH_4H_2PO_4$ ~ 93 % RH at 42℃

Wrap the sample with parafilm
Seal it with plastic bag
just before NMR experiments
*slippery and transparent

Spectra from well-aligned sample

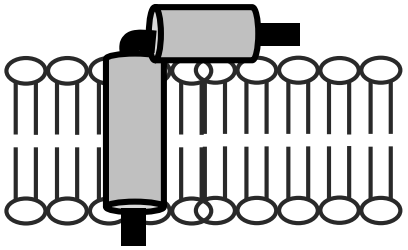
Vpu TM (36 residue)

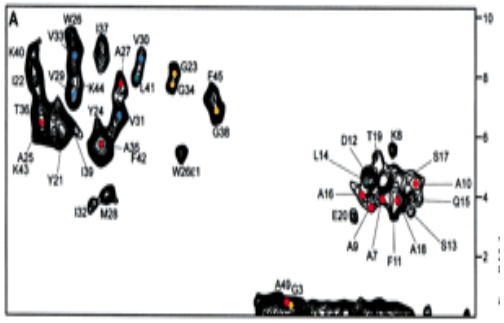




DOPC:DOPG = 9:1
Lipid/protein = 105
25C, 700 MHz
Park et al, *J Mol Biol* 2003

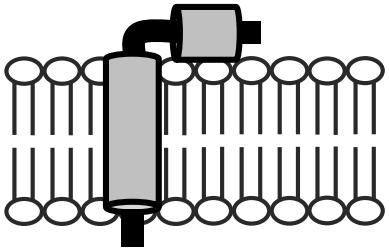
Fd coat protein (50 residue)

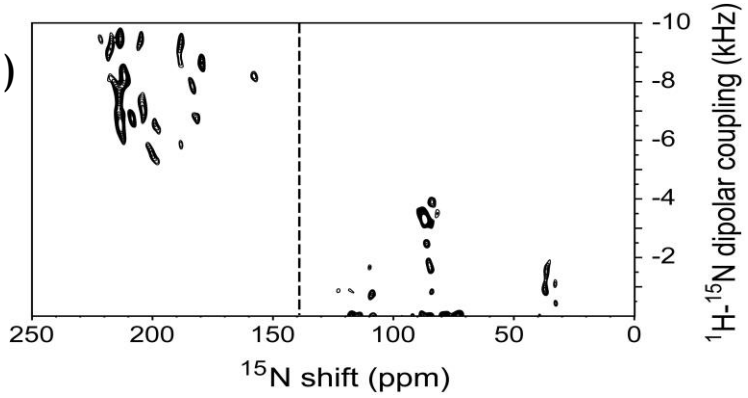




POPC:POPG = 8:2
Lipid/protein = 83
22C, 400 MHz
Marassi et al, *Protein Sci.* 2003

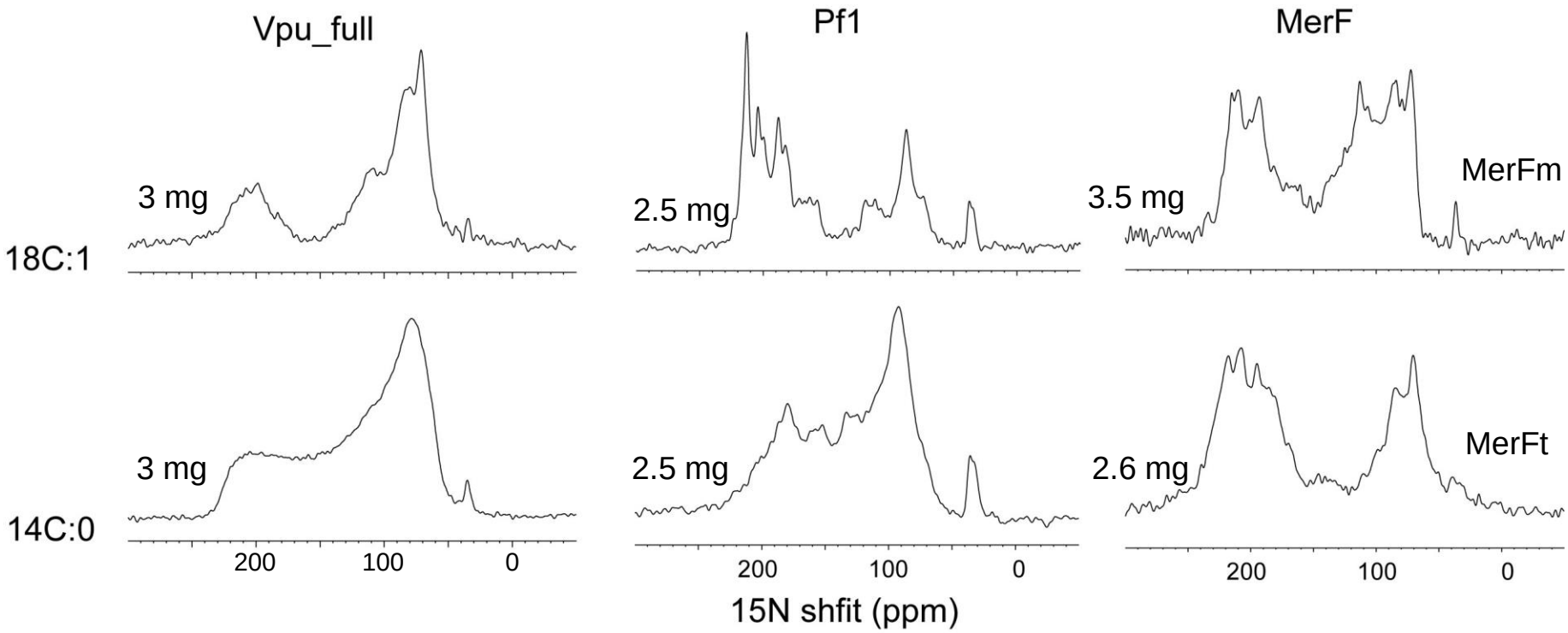
Pf1 coat protein (46 residue)





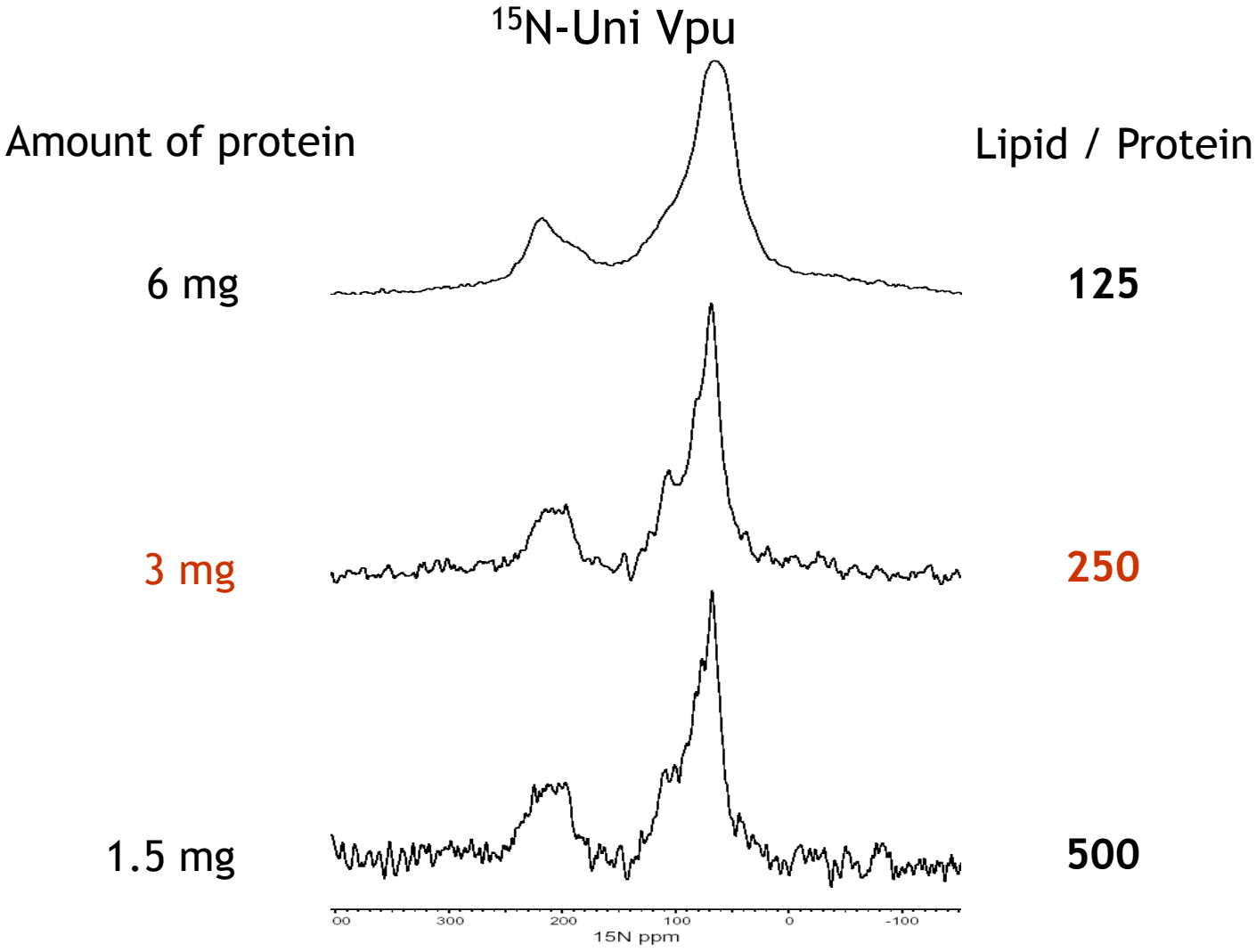
18:1-*O*-PC:DOPG = 9:1
Lipid/protein = 137
23C, 750 MHz
Park et al. J Magn Reson 2008

Choice of lipids: hydrophobic match



75 mg lipid mixture: PC : PG = 9 : 1, 1K scans, 25C, 750 MHz, LB = 100 Hz

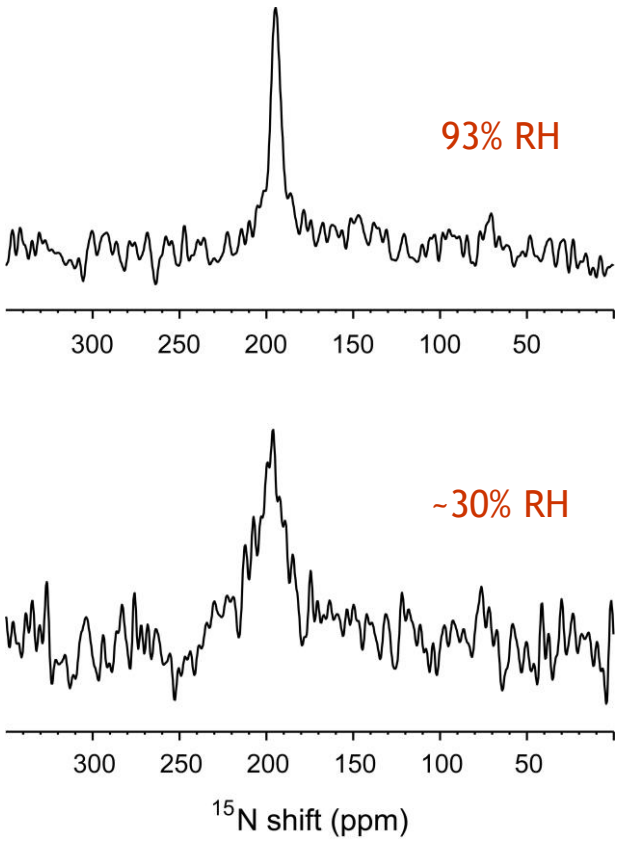
Lipid to protein ratio



*75 mg of lipid mixture (DOPC : DOPG = 9 : 1), 25C, 700 MHz, 1K scans, LB = 100 Hz

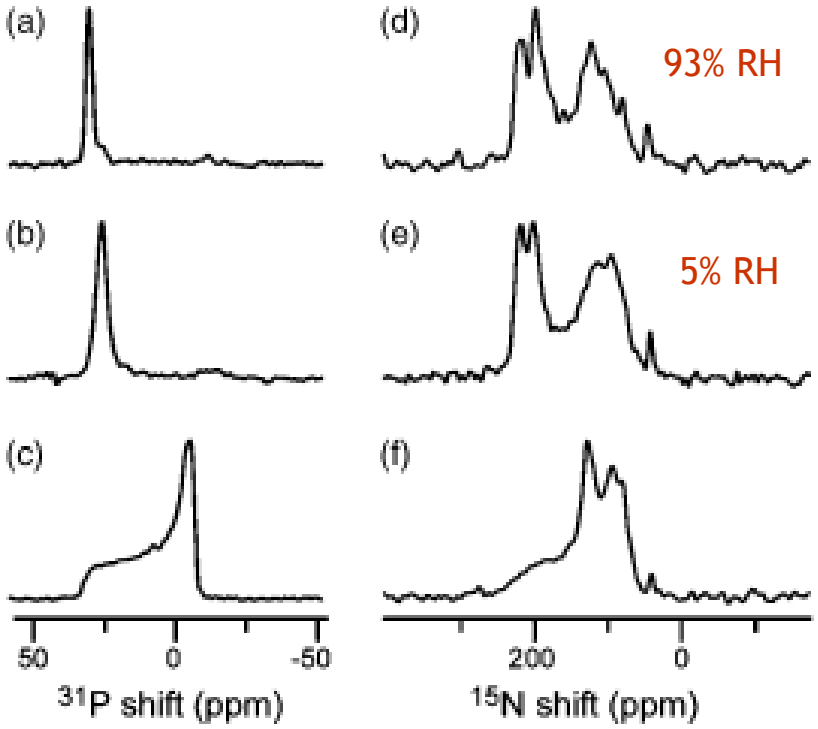
Hydration level: rf power efficiency

^{15}N-Leu Vpu TM



14-*O*-PC : DMPG = 9 : 1
25C, 750 MHz

^{15}N-Uni CHIF



DOPC : DOPG = 8 : 2
22C, 400 MHz
Marassi & Crowell, *JMR* 2003

Conclusion

- Protein alignment for NMR provides direct access to structure information that allows for the determination of high-resolution structures.

- The optimal condition for aligned sample in lipid bilayers is different from sample to sample.
  - choice of lipid
  - lipid to protein ratio
  - hydration level