Practical Issues:

- NMR of biomolecules can be a time-consuming project
- Typical time for a structure determination is 6-12 months. Although useful information can be extracted in a shorter time period.
- Before embarking on an NMR project there are many issues to consider.
- These are summarised in the following slides

Practical Issues: Sample Preparation

- **Concentration:** → **1D 100 μM** (**10 μM** **Cryoprobe!**) / **nD 400 μM** **50 μM**
- **Volume: 500 μL, 220 μL *(microprobes soon)***
- **Quantity: @ 10kDa → 1 mM = 4 mg in 400 L**
- **Purity > 95%, buffers**
- **Sensitivity (γ)→ isotope enrichment (^{15}N, ^{13}C)**

Practical Issues: Solution Conditions

- **Variables: buffer, ionic strength, pH, T**
  - **Deuterated (e.g. Tris d10) or non-^{1}H buffers**
  - **2 conditions 90%H_2O/10%D_2O, 100% D_2O**
  - **5-10% ^{2}H for lock**
  - **Low ionic strength preferred (<200mM)**
  - **pH 4-7 preferred**
  - **Temp range 5-60C, higher temp better spectra**
- **Monomer – larger Mwt poorer spectra**
- **Contaminants**
  - **Pure: add protease inhibtors, 0.02% NaN_3**
- **Stability: for 3D structure up to 1 year**

Practical Issues: Molecular Weight

Symmetry reduces complexity
4 x 10 kDa ? 40 kDa

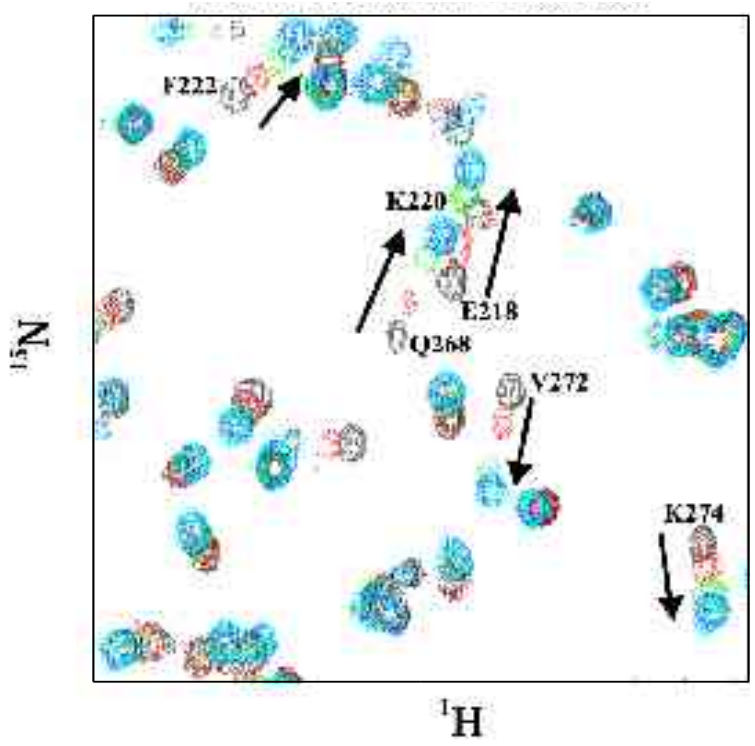
- **≤ 15 kDa $^{15}N/^{13}C$ enrichment**
- **15-30kDa $^{15}N/^{13}C/^{2}H$ enrichment.**
- **30-40 kDa for 3D structure → domains**
- **40-100 kDa: residue-, site-, and atom-specific labeling, TROSY**

Fernandez & Wider, Curr. Opin. Struct. Biol. 13, 570-580 (2003)

Not only structure

Monitoring Binding Events

Titration monitored by ^{15}N-^{1}H HSQC



NMR Provides

- Site-specific
- Multiple probes
- In -depth information
- Spatial distribution of repsonse mapped on structure

Isotopic Enrichment

- Generally use *E. coli* as host strain
  - For ^{13}C and ^{15}N enrichment use ^{13}C glucose and ^{15}N ammonium sulphate as sole carbon and nitrogen sources grown on minimal media.
  - We have discount prices:
    - ^{13}C-glucose 98$US per gramme
    - ^{15}N ammonium sulphate 25$US per gramme
  - For well expressed proteins 2 Litres is sufficient:
    - Approximately 500$US for a sample
  - Many proteins are not so well expressed!