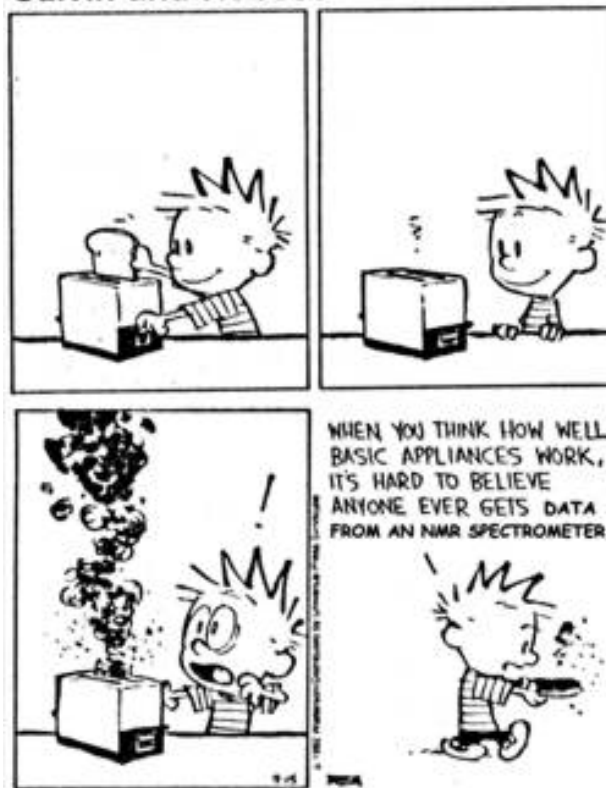
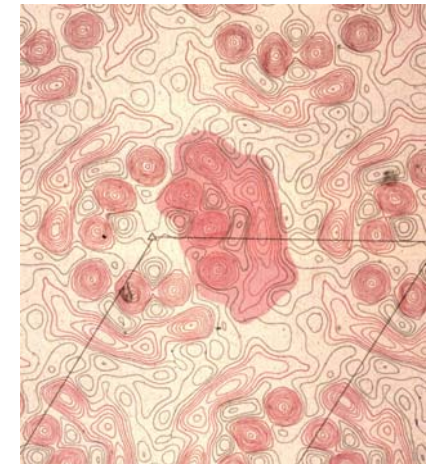
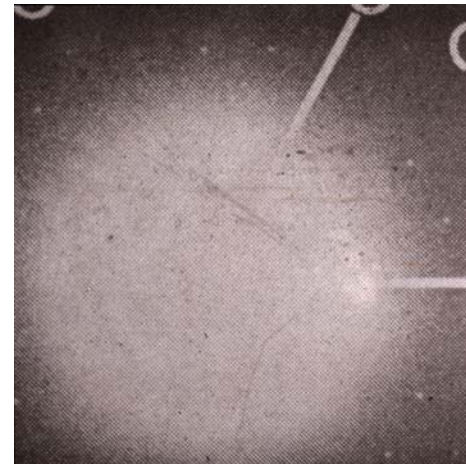
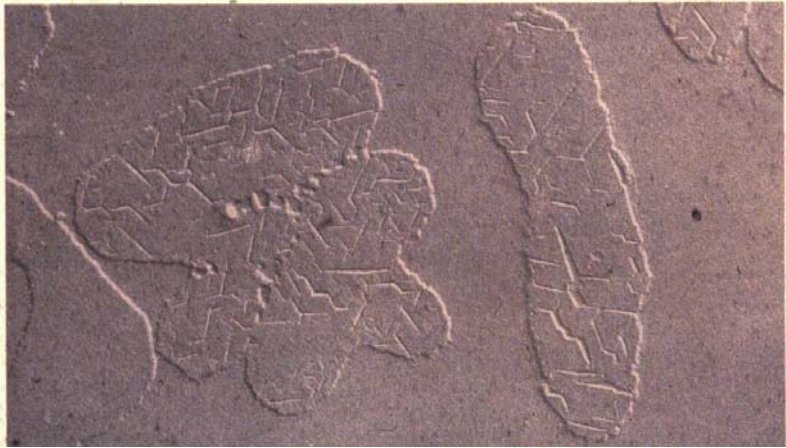


Other methods to get structure

Calvin and Hobbes

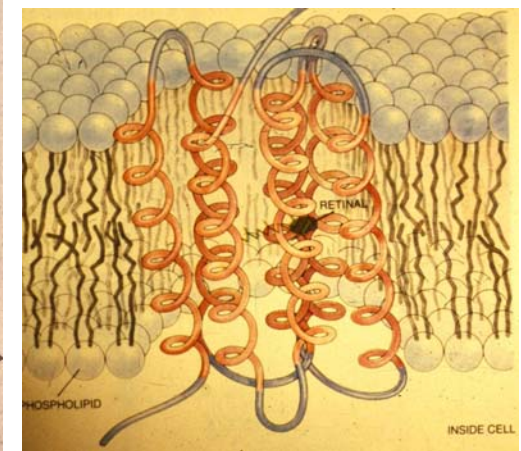


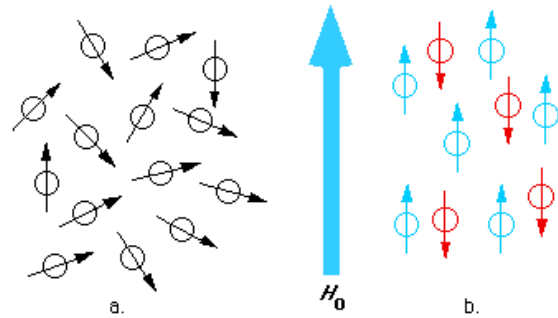
BRh structure/ Electron crystallography



- Unwin and Henderson
- Knobs (BRh) in regular array
- Measure light diffraction by EM image
- Sums information from thousands of images of the same structure

- Diffraction gives map of electron density
- Tilting stage gives 3rd dimension





NMR methods

- Spinning nuclei (charged) are orientable magnets, with low energy state (oriented with) and high energy state (oriented against)
- In oriented nuclei, can boost lows to highs with EM radiation
- Amount energy required depends on shielding by local electrons (= chemistry)
- Chemical shifts for different protons (or C13 or N15) in protein
- (Combined with amino acid sequence)

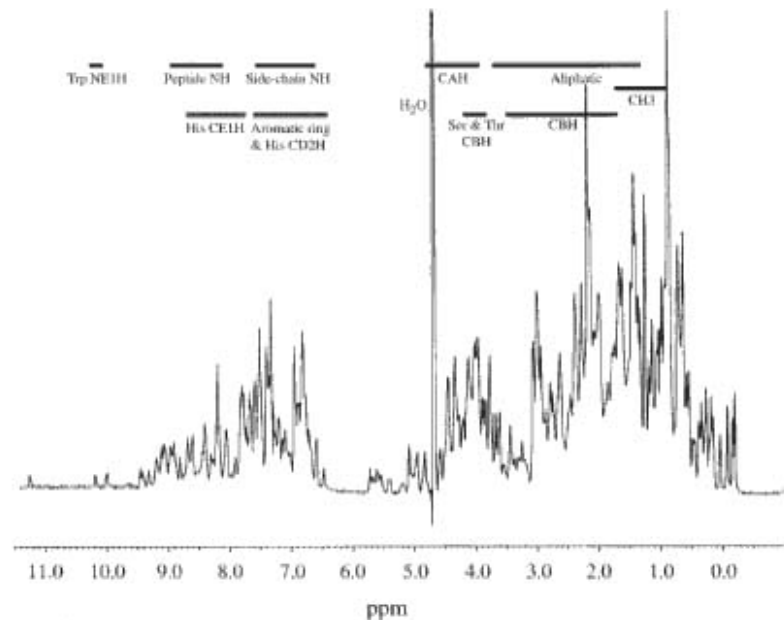


Figure 10.1 ^1H -NMR spectrum of thioredoxin, reduced form. Labels show chemical-shift values typical of various hydrogen types in protein chains having random coil conformation. Some signals lie outside these ranges because of specific interactions not present in random coils. Atom labels are as found in PDB coordinate files. Spectrum generously provided by Professor John M. Louis.

Pulsed field version of same

- Normally spins aligned with field
- Pulse with right angle field to align spins
- Now have aligned vectors in xy plane (measurable)
- As they go back to equilibrium, xy component fades away
- Rate of relaxation related to chemical shift
- FT gives you 1-D chemical shift spectrum

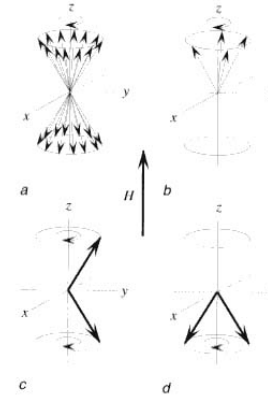


Figure 10.2 (a) Nuclear spins precess around the z-axis, which is parallel to the applied field H . Each spin precesses at a rate that depends on its RF absorption frequency. (b) Excess spins in the lower energy state. At equilibrium, there are slightly more spins aligned with H than against H . (c) Immediately after a 90° pulse, spins

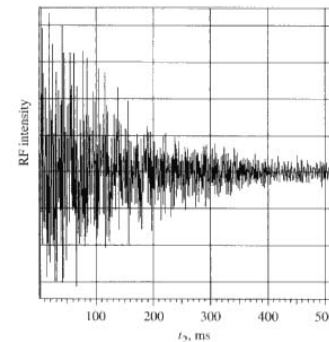


Figure 10.4 Free induction decay signal, which appears in the receiver coils after a 90° pulse. The FID is a time-domain spectrum, showing RF intensity as a function of time t_2 . It is a composite of the RF absorption frequencies of all nuclei in the sample. The Fourier transform decomposes an FID into its component frequencies, giving a spectrum like that shown in Fig. 10.1. Figure generously provided by Professor John M. Louis.

- Pulse twice ($90^\circ + 90^\circ$)
- Once gave xy component, twice doesn't
- Allow relaxation, some xy component returns because of precession
- Take spectrum again; rinse, repeat
 - Same components in chemical shift
 - Change in intensities with time to relax
- In component frequencies (chemical shifts), look for correlations relaxations frequencies or “coupling”
- Measures coupling, and, in the right experiment (NOESY), distance

2-D versions of same

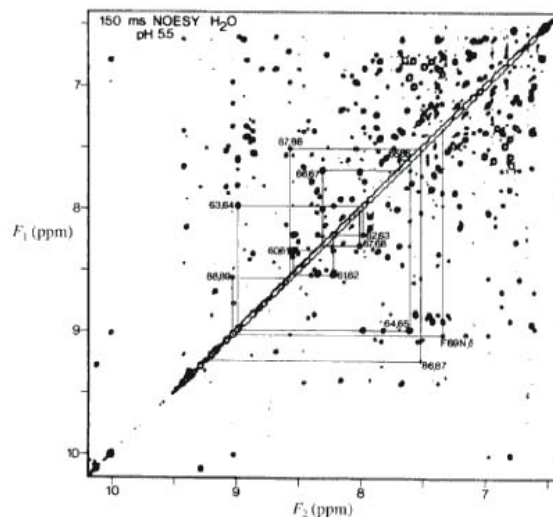
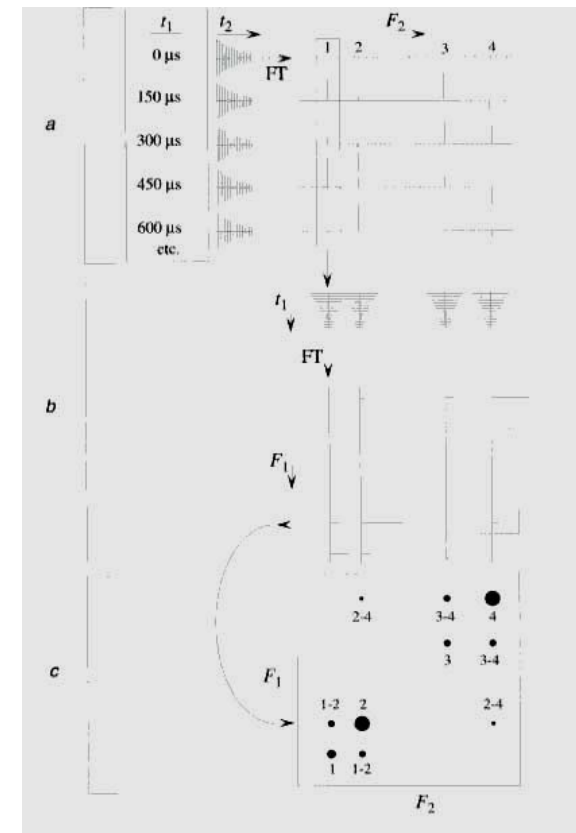


Figure 10.6 NOESY spectrum of thioredoxin in the region $\delta = 6.5$ to $\delta = 10$. Pairs of lines—one horizontal, one vertical—converge at off-diagonal peaks indicating NOE couplings and diverge to the signals on the diagonal for the coupled nuclei. Assigning resonances on the diagonal to specific hydrogens in the molecule is essential for interpreting the NOE data.



Let's compare the results

- Human thioredoxin
 - Both X-ray (1AIU) and NMR (3TRX, 4TRX) structures
 - Compare structures (MMDB)
 - Same structure, but not at atomic detail
- Protein motion (sort of)
- Protein motion (the structure opinion)
 - Compare more mobile regions with NMR "motion", and between models
 - In dimer (2trx)
 - differences and similarities in two proteins
 - Crystal constraints on motion at interface
- MMDB
 - Search MMDB for 3trx.
 - Find 1AIU
 - Check and display (worms)
- Protein explorer (4trx)
 - Animation (via site map)
 - Go ahead and play, but keep track of how many windows are open.
- Protein explorer (3trx in one window, 1aiu in another, 2trx in a third)
 - Color "Temperature"