Lecture 11 (10/2/20)

- Reading: Ch4; 125$_{(Fig\ 4\text{-}10)}$, 134-136$_{(structure\ determination)}$
- Problems: Ch4 (text); 10, 15

NEXT

- Reading: Ch4; 127-130 (Collagen)
  Ch1; 27-29 (Enzymes)
  Ch5; 157-158, 160-161, 166$_{(bottom)}$
- Problems: Ch1 (text); 16
  Ch4 (text); 1, 4, 5, 6
  Ch4 (study-guide); *6* *(do you know the facts?)*

Lecture 11 (10/2/20)

OUTLINE

Protein Characterization

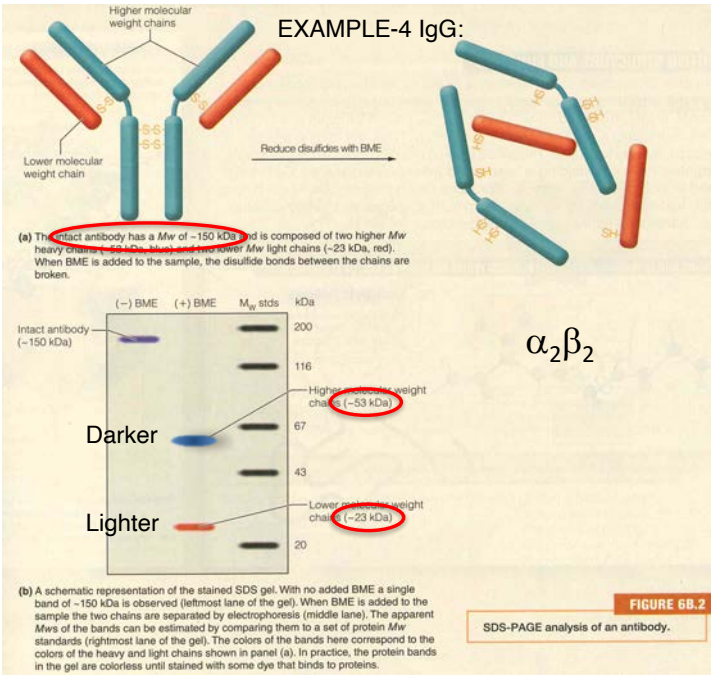
A. Quaternary structure
   1. How determined;
      a. native size
      b. subunit size
   2. Ultracentrifugation
B. Tertiary structure
   1. X-ray diffraction/crystallography
   2. NMR spectroscopy
   3. Comparison: NMR *versus* X-ray crystallography
C. Secondary structure
   1. Circular dichroism (CD)

Protein Characterization

Quaternary Structure

Protein Characterization: Structure Determination

EXAMPLE-4 IgG:



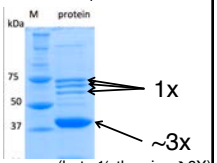
$\alpha_2\beta_2$

Example of correlating band intensity with size an stoichiometry:

If a smaller protein has the same intensity as that of a larger protein, it means the smaller one must be there in larger numbers.

EXAMPLE-5:

Native M_r=440 kDa



(but ~½ the size→6X)

200

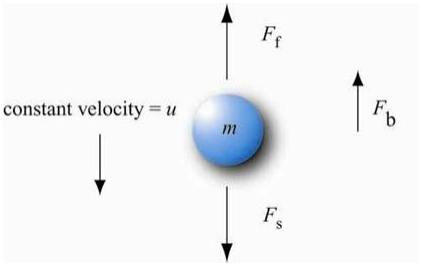
M_r=72, 68, 60, 40 kDa

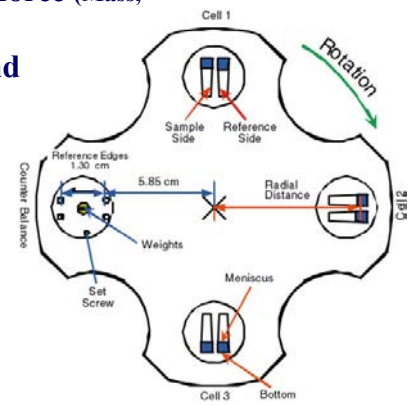
$\alpha_1\beta_1\gamma_1\delta_6$

Protein Characterization: Structure Determination

Analytical Ultracentrifugation

- **In analytical ultracentrifugation, analytes are separated on the basis of their sedimentation when they experience a centripetal force (Mass, Shape, Density).** ~1.4 g/cm^3
- **Usually carried out at speeds around 60,000 rpm**





There is a force due to buoyancy F_b and a frictional force on the molecule F_f, both which oppose its movement through the medium Fs (centrifugal force or sedimentary force).

Protein Characterization: Structure Determination

TWO Types:

Analytical Ultracentrifugation – Sedimentation Velocity
Analytical Ultracentrifugation – Sedimentation Equilibrium

- **Or, described mathematically…$F_s + F_b + F_f$ = Sed. Vel. (rate)**

Solvent density in g/mL

Specific Volume (occupied by 1 g of solute (cm^3/g))

sedimentation velocity

angular velocity

radius from center of spin

$$\frac{M(1-\bar{\nu}\rho)}{Nf} = \frac{u_s}{\omega^2 r} \equiv s$$

Sedimentation coefficient (units of sec)

***M* is number average molecular weight (*m*N). If all species are the same, then *M* = M_r**

coefficient of friction (g/mole•sec)

Protein Characterization: Structure Determination

Analytical Ultracentrifugation – Sedimentation Velocity

$$\frac{M(1-\bar{\nu}\rho)}{Nf} = \frac{\mu_s}{\omega^2 r} \equiv s$$

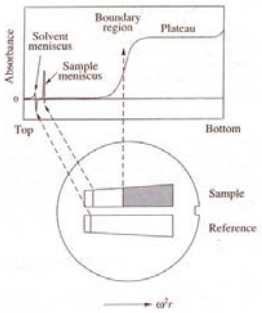
Sedimentation coefficient (units of sec)

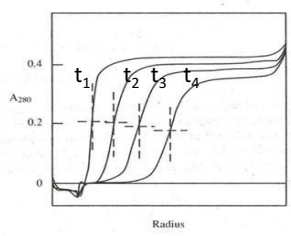
- Units of sedimentation are seconds: 1 Svedberg = 1**S** = 10^{-13} s
- $1-\bar{\nu}\rho$ is called the buoyancy factor – and since $1/\bar{\nu}$ approximates ρ, then if the density of the molecule is close to that of the solution $1-\bar{\nu}\rho = 0$ and the s = 0. (Say, "Vee-bar-rho")
- If the molecule is less dense than solution then $1-\bar{\nu}\rho < 0$ and the molecule floats. (lipids or fats in a blood sample)
- Proteins are more dense than solution and $1-\bar{\nu}\rho > 0$, and the molecule sediments.

Protein Characterization: Structure Determination

Analytical Ultracentrifugation – Sedimentation Velocity

- **The most basic type of ultracentrifugation experiment is to measure the rate at which the molecule moves away from the center of rotation**
- **What is actually measured is the movement of the boundary between dissolved molecule and 'empty' buffer**
- **Based on mass, shape, and density**





Note shape of curves- less steep over time because of diffusion

Protein Characterization: Structure Determination

Analytical Ultracentrifugation – Sedimentation Velocity

Protein	Molecular Mass (kD)	Partial Specific Volume, $\bar{V}_{20,w}$ ($cm^3 \cdot g^{-1}$)	Sedimentation Coefficient, $s_{20,w}$ (S)
Lipase (milk)	6.7	0.714	1.14
Ribonuclease A (bovine pancreas)	12.6	0.707	2.00
Cytochrome *c* (bovine heart)	13.4	0.728	1.71
Myoglobin (horse heart)	16.9	0.741	2.04
α-Chymotrypsin (bovine pancreas)	21.6	0.736	2.40
Crotoxin (rattlesnake)	29.9	0.704	3.14
Concanavalin B (jack bean)	42.5	0.730	3.50
Diphtheria toxin	70.4	0.736	4.60
Cytochrome oxidase (*P. aeruginosa*)	89.8	0.730	5.80
Lactate dehydrogenase H (chicken)	150	0.740	7.31
Catalase (horse liver)	222	0.715	11.20
Fibrinogen (human)	340	0.725	7.63
Hemocyanin (squid)	612	0.724	19.50
Glutamate dehydrogenase (bovine liver)	1015	0.750	26.60
Turnip yellow mosaic virus protein	3013	0.740	48.80

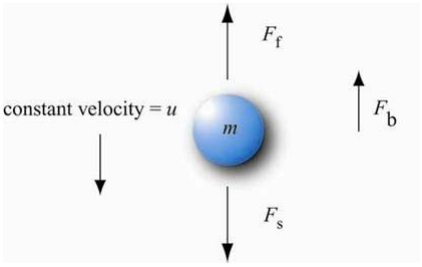
Source: Smith, M.H., *in* Sober, H.A. (Ed.), *Handbook of Biochemistry and Molecular Biology* (2nd ed.), *p.* C-10, CRC Press (1970).

$$\frac{M(1-\bar{\nu}\rho)}{Nf} = \frac{\mu_s}{\omega^2 r} \equiv s$$

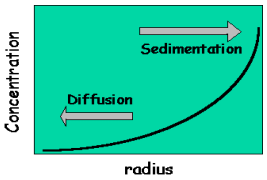
Protein Characterization: Structure Determination

Analytical Ultracentrifugation – Sedimentation Equilibrium

- **In sedimentation equilibrium, an equilibrium is established between sedimentation away from the center of rotation and diffusion towards the center of rotation (spin at much lower speeds) so we get no boundary between solute and meniscus**

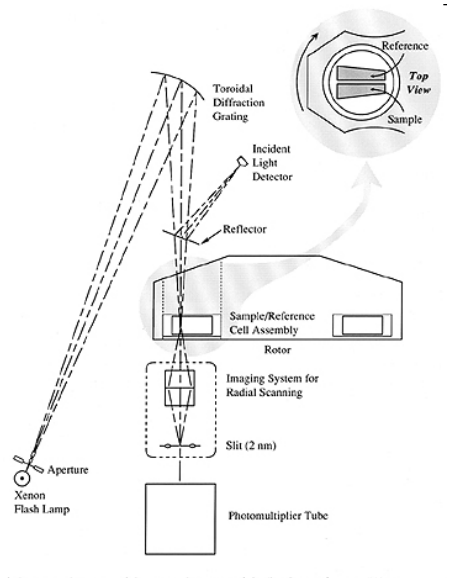


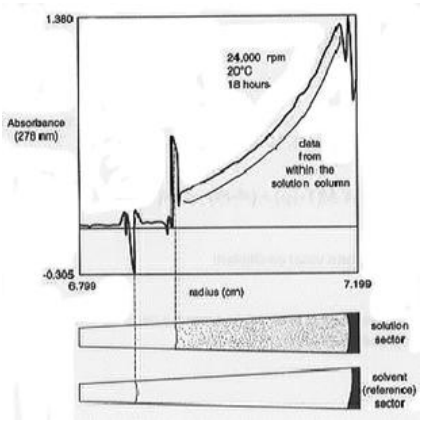
This is *anything* that measures concentration!



Protein Characterization: Structure Determination

Analytical Ultracentrifugation – Sedimentation Equilibrium





Schematic diagram of the optical system of the Beckman Optima XL-A Analytical Ultracentrifuge From "Analytical Ultracentrifugation, Vol. 1", Beckman Instruments, Inc.

Protein Characterization: Structure Determination

Analytical Ultracentrifugation – Sedimentation Equilibrium

Determining Molecular Weight

Can be described by equation: $C_A(r) = C_{A,0} e^{\sigma(r^2 - r_0^2)/2}$ where $\sigma = \frac{M(1-\bar{\nu}\rho)\omega^2}{RT}$

This equation can be expressed in terms of M:

$$M = \frac{2kT}{(1 - \bar{\nu}\rho)\,\omega^2} \ln \frac{C_A}{C_0(r^2 - r_0^2)}$$

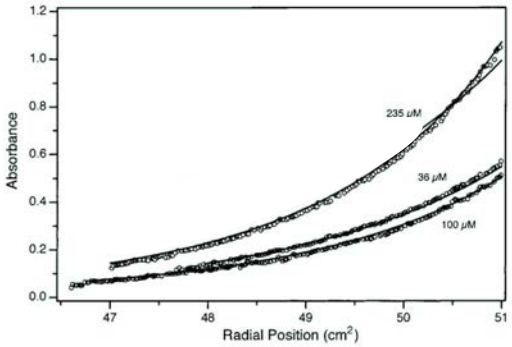
Protein Characterization: Structure Determination

Analytical Ultracentrifugation – Sedimentation Equilibrium

$$M = \frac{2kT}{(1 - \bar{v}\rho)\,\omega^2} \ln \frac{C_A}{C_0(r^2 - r_0^2)}$$

Thus, we can get M.

At most radii, its migrating "heavier" due to association.



$A_2 \rightleftharpoons 2A$

$K_d = [A]^2/[A_2]$

But recall, this M= *m*N, and depends on the number average molecular weight. So, if there are species with different molecular weights, as in a dissociation equilibrium of an oligomeric protein, this M will be sensitive to that dissociation.

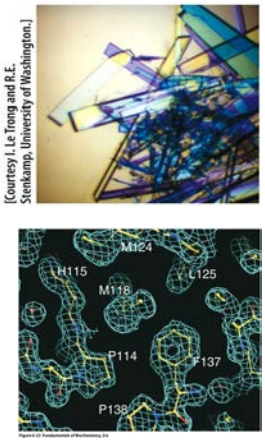
Protein Characterization

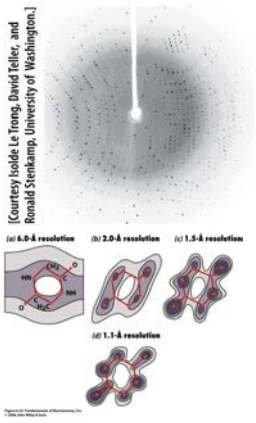
Tertiary Structure

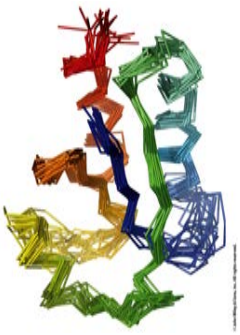
Protein Characterization: Structure Determination

Analysis of 3° structure*

- X-ray crystallography
- NMR







*and 4° structure

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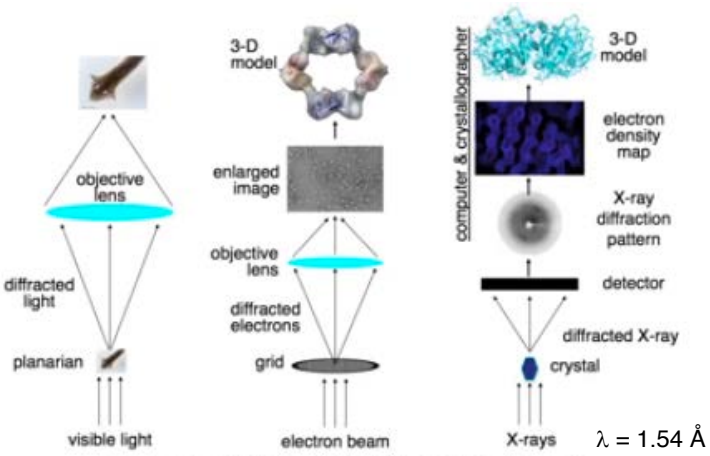
Protein Characterization: Structure Determination

X-ray Crystallography for Protein Structure

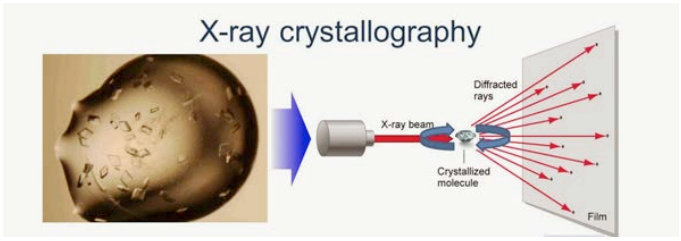
Comparison to other methods:

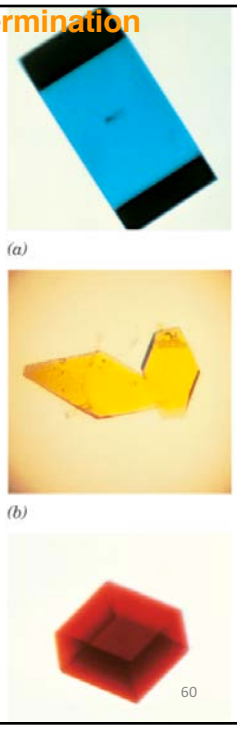


Adapted from Crystal Structure Analysis, Glusker & Trueblood, 1985

Protein Characterization: Structure Determination

X-ray Crystallography for Protein Structure





X-ray crystallography Primer
https://www.youtube.com/watch?v=62kdCOHcN_U

For dummies https://www.youtube.com/watch?v=QuCRBxjk3fg

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Protein Characterization: Structure Determination

X-ray Crystallography for Protein Structure

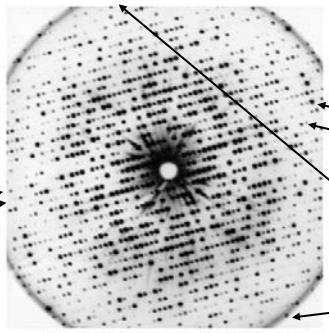


FIG. 6-21 **An X-ray diffraction photograph of a crystal of sperm whale myoglobin.**
The intensity of each diffraction maximum (the darkness of each spot) is a function of the crystal's electron density. [Courtesy of John Kendrew, Cambridge University, U.K.]

Closest layers of lines from atoms furthest apart in unit cell

Intensities due to interference and amplification of waves
Depends of whether waves are in or out of phase

Furthers layers of lines from atoms closest together in unit cell = resolution

Relationship of spots intensity and unit cell
[https://www.youtube.com/watch?v=fZ0m8wustVk]

Protein Characterization: Structure Determination

X-ray Crystallography for Protein Structure

Resolution

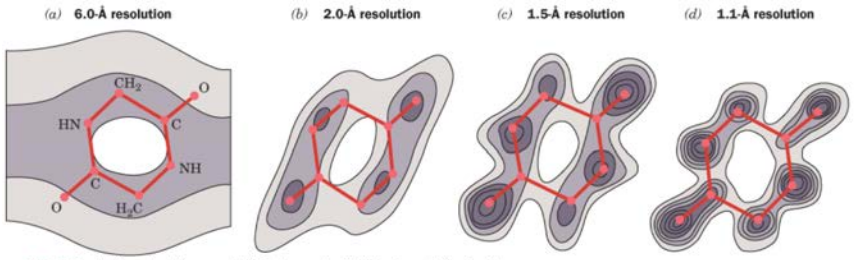
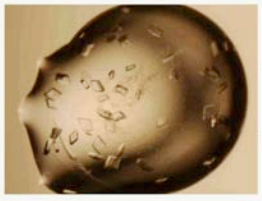


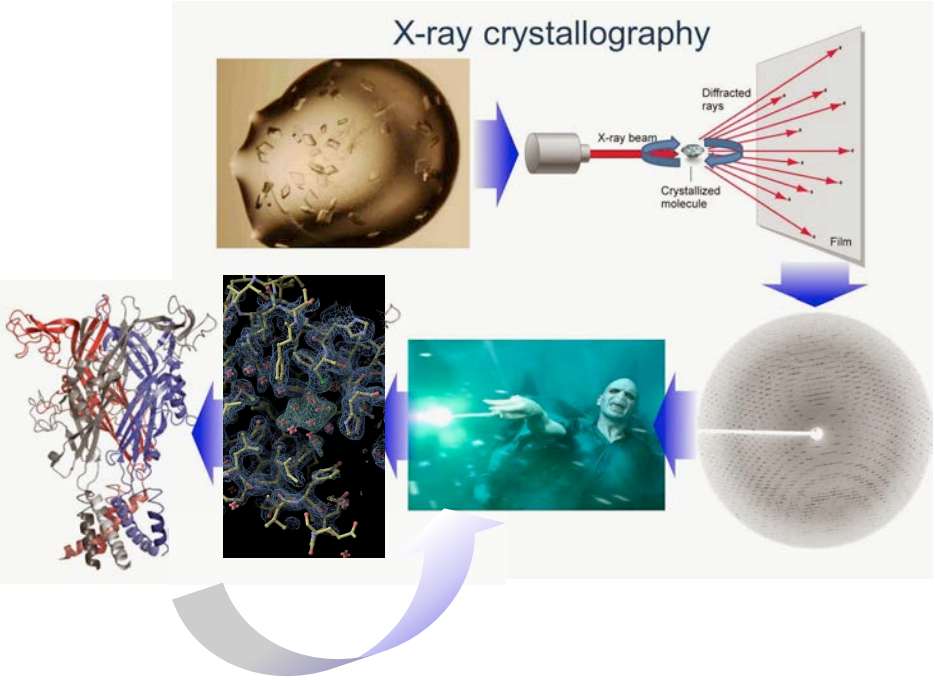
FIG. 6-23 **Electron density maps of diketopiperazine at different resolution levels.**
Hydrogen atoms are not visible in these maps because of their low electron density. [After Hodgkin, D.C., *Nature* 188, 445 (1960).]

Protein Characterization: Structure Determination

X-ray crystallography

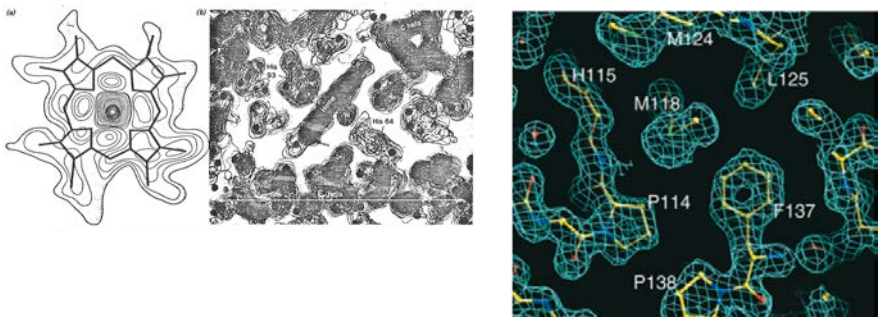


Protein Characterization: Structure Determination



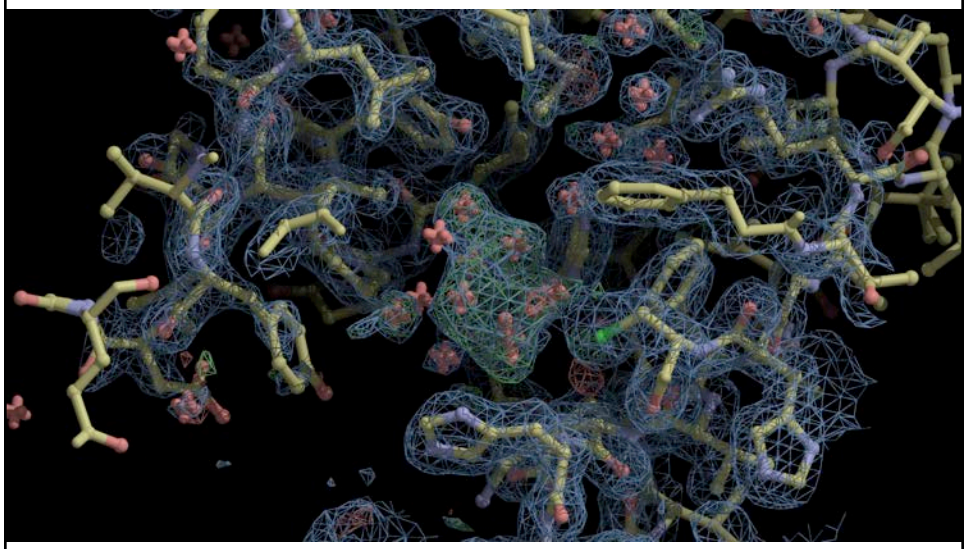
Protein Characterization: Structure Determination

X-ray Crystallography for Protein Structure



Protein Characterization: Structure Determination

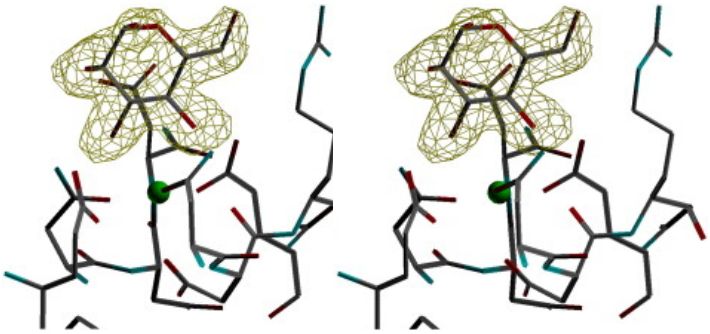
Typical X-ray crystallography structure



The electron density map from the x-ray diffraction data is shown as a blue cage, and the model of the protein that "fits" this density in modeled inside of it.

Protein Characterization: Structure Determination

X-ray Crystallography for Protein Structure



Stereo View

Protein Characterization: Structure Determination

NMR for Protein Structure

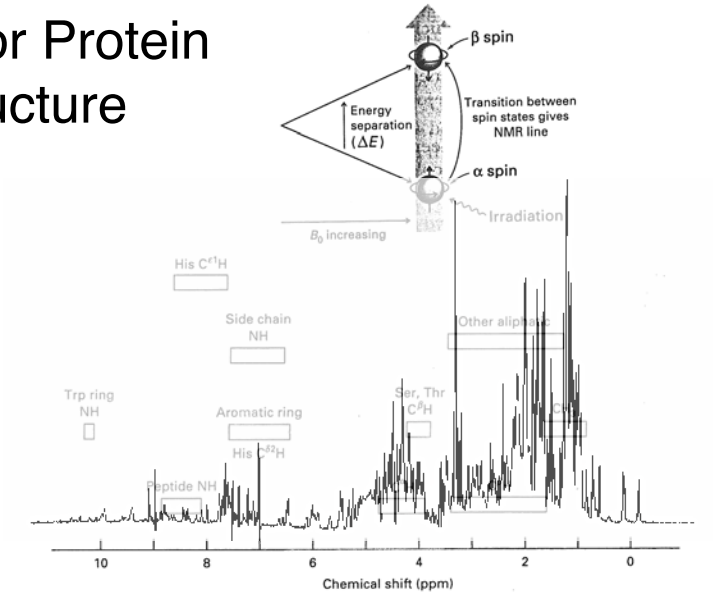


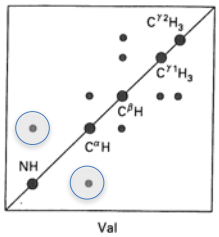
FIGURE 6.26
The range of ^{1}H-NMR chemical shifts observed for different hydrogen atoms of peptides in the random coil conformation.

Protein Characterization: Structure Determination

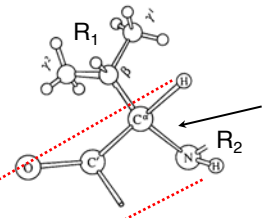
NMR for Protein Structure

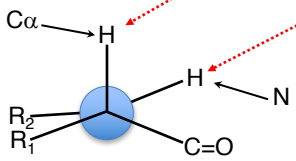
Correlation 2D spectroscopy (COSY)

J-coupling



These intensities change depending on ϕ-angle





Hz ~4-5

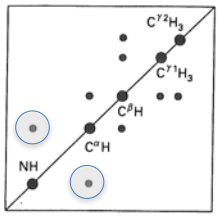
Can also get ϕ & ψ angles from secondary chemical shifts (observed from known random coil shifts)(TALOS & ANGLOR)

Protein Characterization: Structure Determination

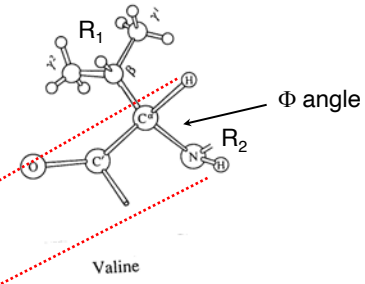
NMR for Protein Structure

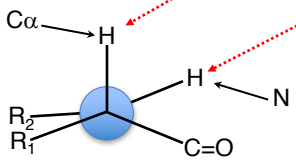
Correlation 2D spectroscopy (COSY)

J-coupling



These intensities change depending on ϕ-angle





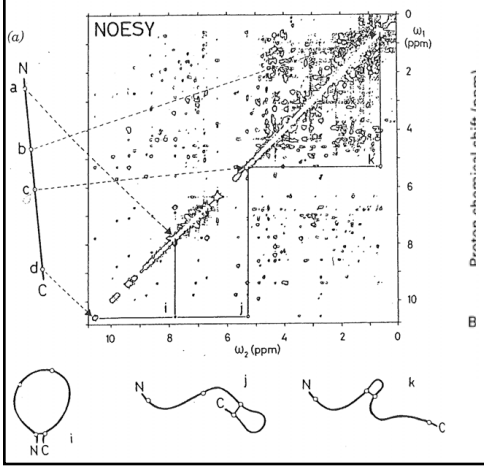
Hz ~4-5

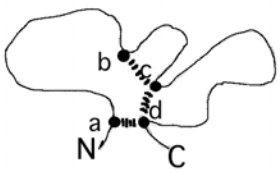
Can also get ϕ & ψ angles from secondary chemical shifts (observed from known random coil shifts)(TALOS)

Protein Characterization: Structure Determination

NMR for Protein Structure

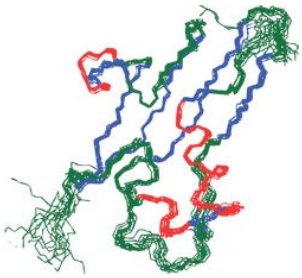
Nuclear-Overhauser Effect Spectroscopy (NOESY)



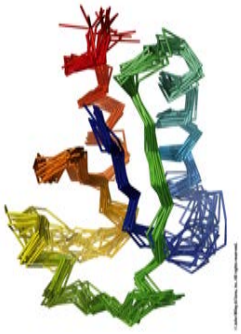


Protein Characterization: Structure Determination

Typical NMR structure



Blue = β-sheet, red = α-helix, green = loops without 2° structure.



Blue = N-term, red = C-term "Rainbow coloring."

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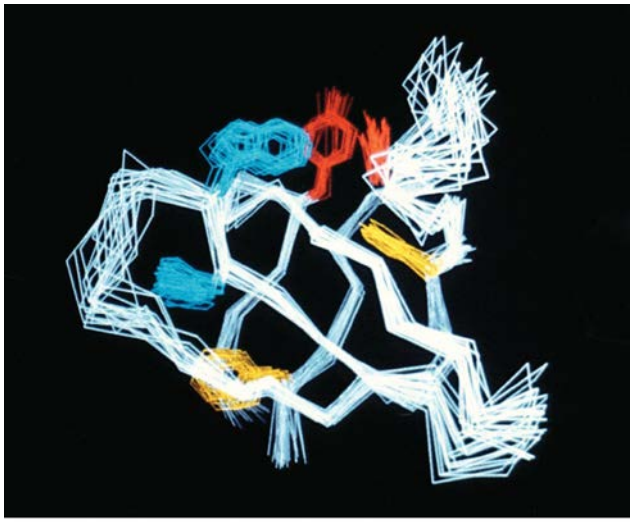
Protein Characterization: Structure Determination

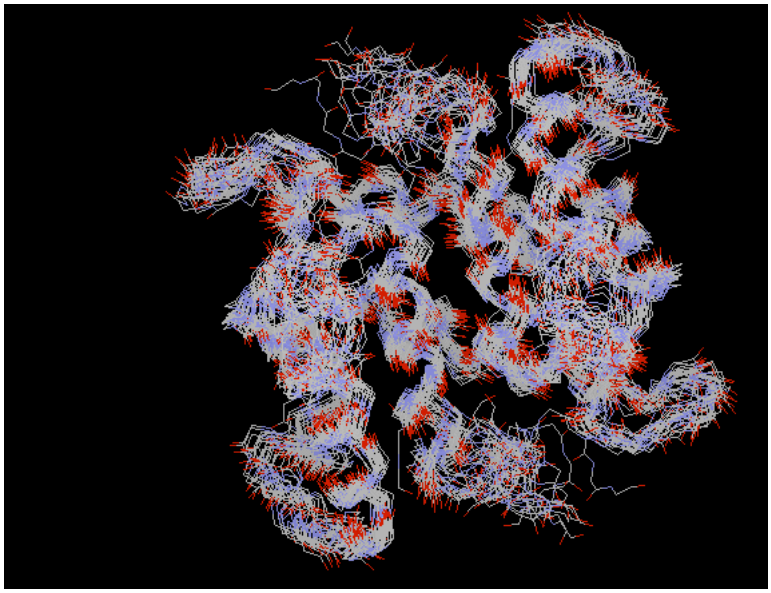
NMR Structure of a Protein



Courtesy Stuart Schrieber, Harvard University

Protein Characterization: Structure Determination

Typical NMR structure



Notice there are many overlapping structures that all fit the NMR data. Where it is tight, you have higher resolution and where it is loose you have parts of the molecule that are more mobile

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Protein Characterization: Structure Determination

Protein Characterization

Tertiary Structure

Compare/Contrast X-ray crystallography and NMR:

1) Crystal vs. solution structures the same; not significant crystal constraints
2) NMR not as high resolution
3) NMR better at predicting regions that are dynamic; X-ray uses "B-factors" or even does not show, i.e., "disordered"
4) X-ray cannot distinguish "rotomers" of Asn, Gln, Thr; NMR is unambiguous
5) X-ray much better at larger structures; NMR has assignment problem only good for up to 30-40 kDa