ASBMBU

American Society of Biochemistry and Molecular Biology

- ASBMB is a national organization that works to create a **network** of undergraduate students and faculty to **advance research, education, and science outreach**.

ASBMBU

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Peer Mentorship Program

- **Mentees:** Great way to get to know upperclassmen at BU and build connections!!
- Mentors can give great advice!!
- **Mentors:** Great way to show leadership and help underclassmen learn about BU!
- (If you are a mentor, you might have more than one mentee)

I. Protein Structure
- A. Primary
  - 1. Peptide Bond
    - a. Planar, strong, ϕ/ψ angles
  - 2. Determination
    - a. Sequence determination; CHEMICAL
      - i. aa composition; Divide & conquer; Edman degradation
    - b. Sequence determination; PHYSICAL
      - i. Tandem Mass Spectrometry for proteins
    - c. Sequence determination; BIOLOGICAL
      - i. Genome sequenced; need partial sequence
    - d. Determination of Disulfide bonds
- B. Secondary
  - 1. Conformational structure; Levinthal paradox
  - 2. Pauling & Corey's predictions
    - a. α-Helix
    - b. β-sheets/strands
    - c. Connections between β-strands
    - d. Connections between α-helices
  - 3. Super secondary structure
- C. Tertiary
  - 1. Picturing and classifications
  - 2. Topology
  - 3. Domains
  - 4. Intrinsically disordered
  - 5. Stability
- D. Quaternary
  - 1. Nomenclature
  - 2. Stability

II. Protein Structure Determination
- A. Quaternary structure
  - 1. How determined; Gel filtration & SDS-PAGE, 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)

Lecture 11 (10/4/24)

TODAY
- Reading: Ch4, 116, 138-142
- Homework: #11

NEXT
- Reading: Ch4; 118-120 & Ch1, 25-27; Ch5, 147-148, 150-152, 157; Ch6, 177, 209-210
- Homework: #12

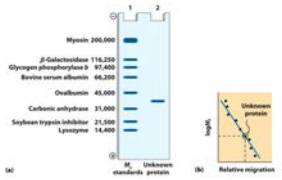
Protein-Conformational Structure Determination

Quaternary Structure

Protein-Conformational Structure Determination

Determination of Quaternary Structure

Need the "native" molecular weight
- Gel filtration
- Ultracentrifugation



Need the **subunit** stoichiometry and molecular weight
- SDS-PAGE
- MALDI-MS

Example 1:
- Native MW = 200 kDa
- Subunit MW = 50 kDa

α_4

Example 2:
- Native MW = 300 kDa
- Subunit MW = 75 kDa, 50 kDa
- Subunit stoichiometry = (75:50) is 2:3

(means with the same amounts of total mass per protein, the smaller one would have to have the same intensity on gel)

$\alpha_2\beta_3$

Example 3:
- Native MW = 360 kDa
- Subunit MW = 80 kDa, 40 kDa
- Subunit stoichiometry = equal

(means with half the size, the smaller one would have to have half intensity on gel)

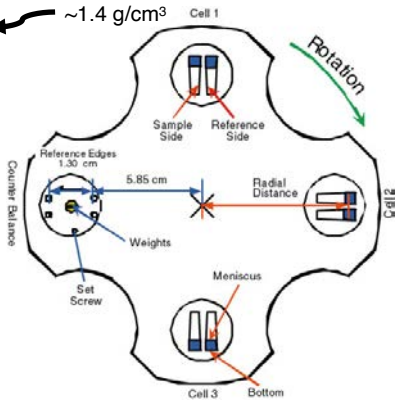
$\alpha_3\beta_3$

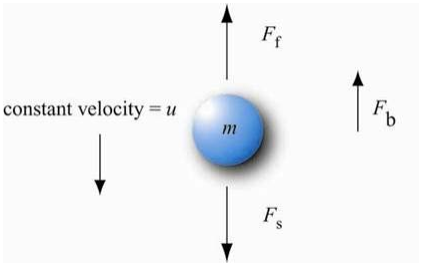
Protein-Conformational Structure Determination

Analytical Ultracentrifugation

- **In analytical ultracentrifugation, analytes are separated on the basis of their sedimentation when they experience a centrifugal force (Separation is based on Mass, Shape, Density).**
- **Usually carried out at speeds around ≥60,000 rpm**

~1.4 g/cm³





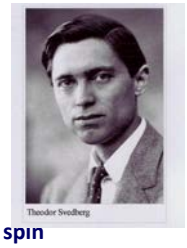
There is a force due to buoyancy F_b and a frictional force on the molecule F_f, (centripetal force) both which oppose its movement through the medium Fs (centrifugal or sedimentary force).

Protein-Conformational 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

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

Sedimentation coefficient (units of sec)

coefficient of friction (g/mol•sec)

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

Protein-Conformational Structure Determination

Analytical Ultracentrifugation – Sedimentation Velocity

Solvent density in g/mL

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

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

Sedimentation coefficient (units of sec)

- Units of sedimentation are seconds: 1 Svedberg = 1**S** = 10^{-13} s

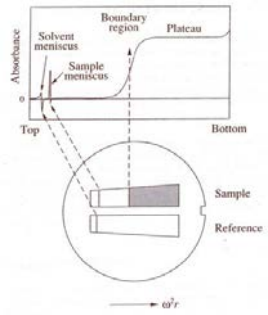
$1-\bar{\nu}\rho$ is called the buoyancy factor – and since $1/\bar{\nu}$ approximates ρ, then if the density of the molecule is close to that of the solution $1-\bar{\nu}\rho = 0$ and the s = 0.
(Say, "Vee-bar-rho")

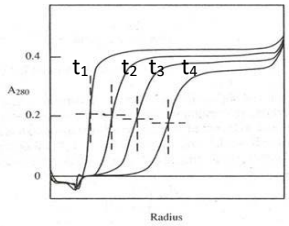
- If the molecule is less dense than solution then $1-\bar{\nu}\rho < 0$ and the molecule floats. (lipids or fats in a blood sample)
- Proteins are more dense than solution and $1-\bar{\nu}\rho > 0$, and the molecule sediments.

Protein-Conformational 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-Conformational 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).

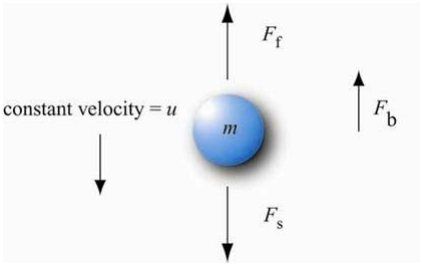
$$\frac{M(1-\bar{\nu}\rho)}{Nf} = \frac{\mu_s}{\omega^2 r} \equiv s$$

1. Not a linear relationship
2. Shape is important

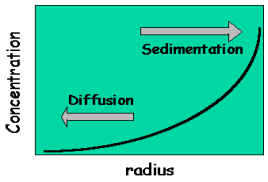
Protein-Conformational Structure Determination

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

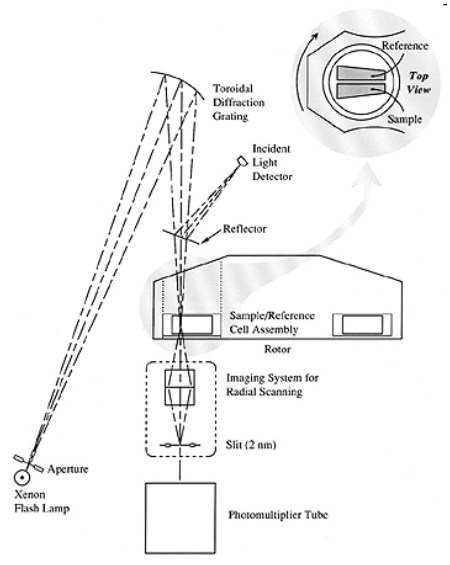


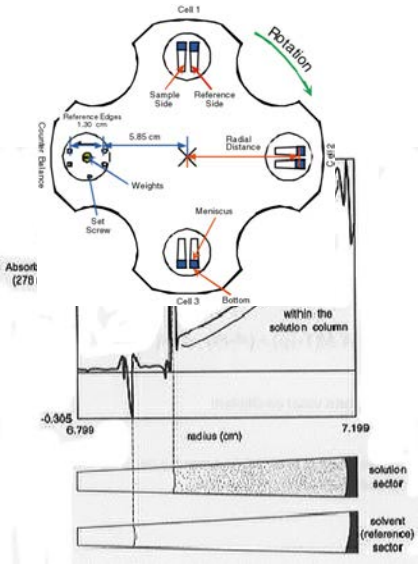
This is *anything* that measures concentration!



Protein-Conformational 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-Conformational 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:

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

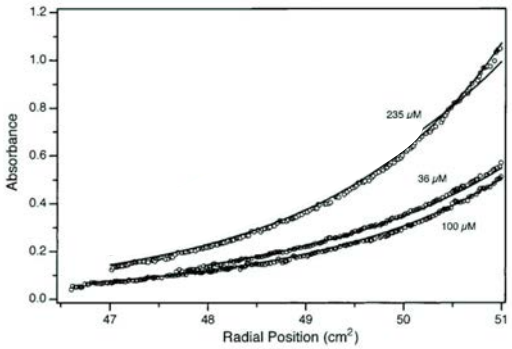
Protein-Conformational Structure Determination

Analytical Ultracentrifugation – Sedimentation Equilibrium

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

Thus, we can get M.

As you dilute the protein, $A_2 \rightleftharpoons 2A$ equilibrium shifts to the right.



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

$A_2 \rightleftharpoons 2A$

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

But recall, this $M = mN$, 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.

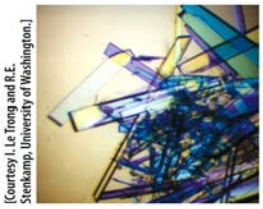
Protein Characterization

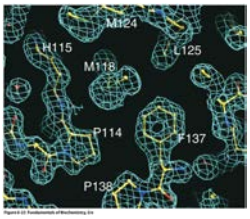
Tertiary Structure

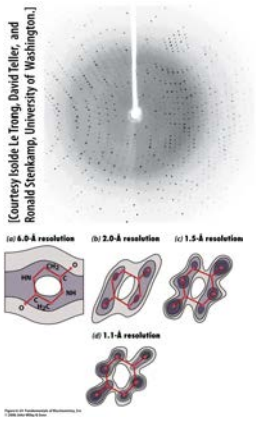
Protein-Conformational Structure Determination

Analysis of 3º structure*

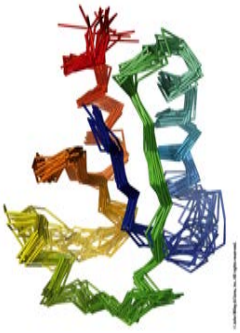
- X-ray crystallography







- NMR



*and 4º structure

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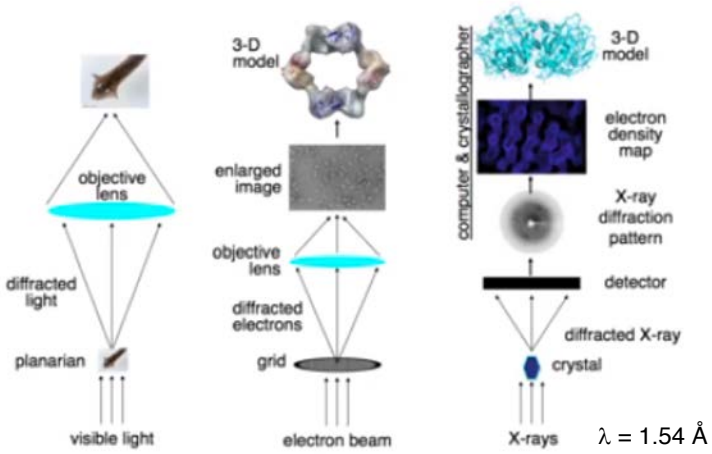
Protein-Conformational Structure Determination

X-ray Crystallography for Protein Structure

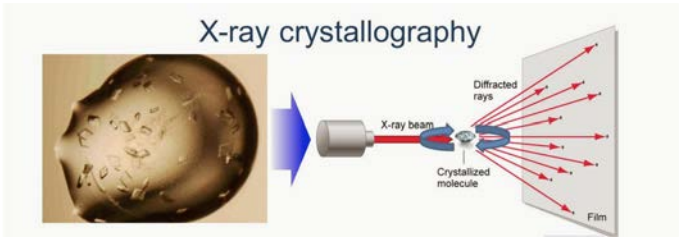
Comparison to other methods:

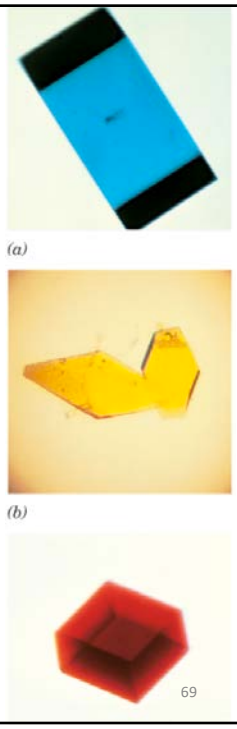


Adapted from Crystal Structure Analysis, Glusker & Trueblood, 1985

Protein-Conformational 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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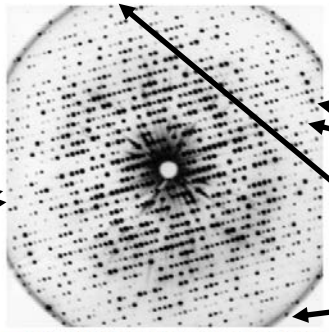
Protein-Conformational Structure Determination

X-ray Crystallography for Protein Structure



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

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

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.]

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

Protein-Conformational Structure Determination

X-ray Crystallography for Protein Structure

Resolution

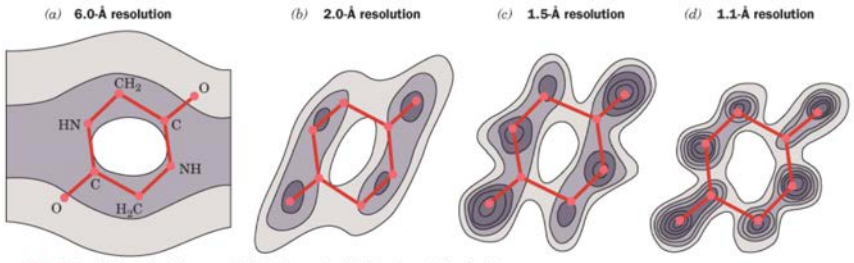
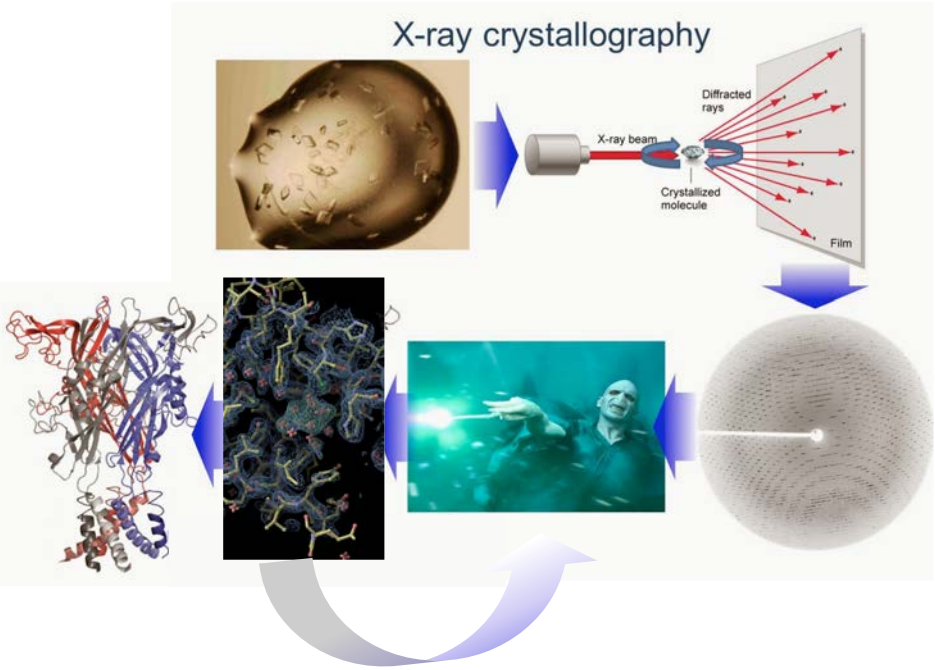


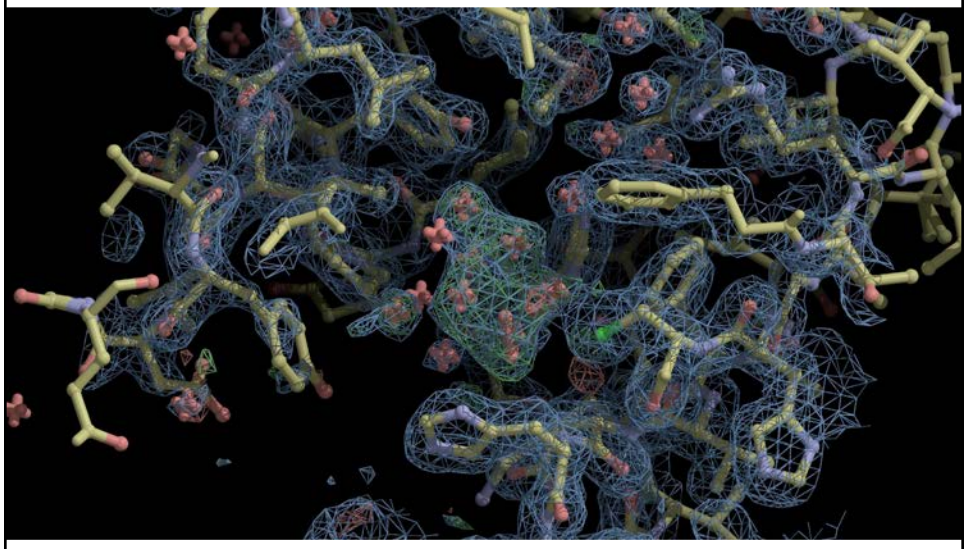
FIG. 6-23 Electron density maps of diketopiperazine at different resolution levels.
Hydrogen atoms are not visible in these maps because of their low electron density. [After Hodgkin, D.C., *Nature* 188, 445 (1960).]

Protein-Conformational Structure Determination



Protein-Conformational Structure Determination

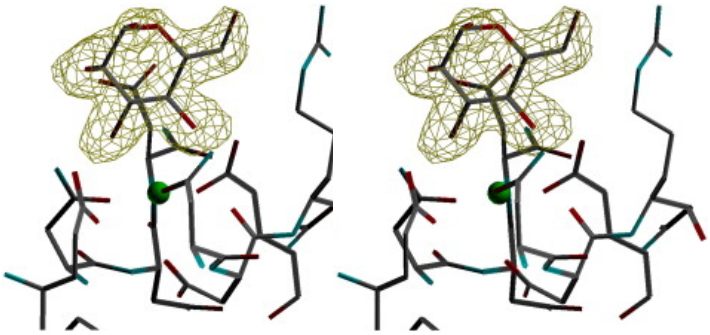
Typical X-ray crystallography structure



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-Conformational Structure Determination

X-ray Crystallography for Protein Structure



Stereo View

Protein-Conformational Structure Determination

NMR for Protein Structure

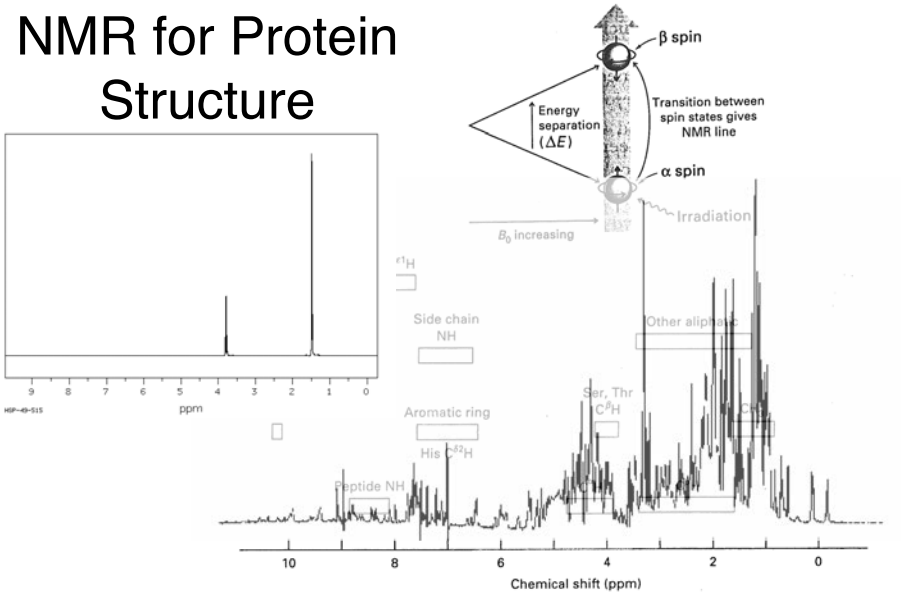


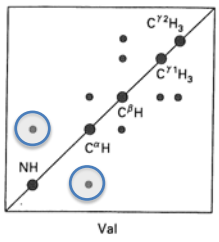
FIGURE 6.26
The range of ^{1}H-NMR chemical shifts observed for different hydrogen atoms of peptides in the random coil conformation.

Protein-Conformational Structure Determination

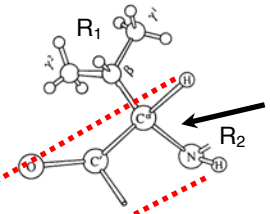
NMR for Protein Structure

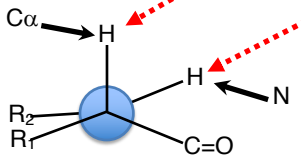
Correlation 2D spectroscopy (COSY)

J-coupling

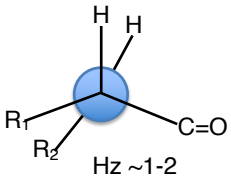


These intensities change depending on ϕ-angle





Hz ~4-5



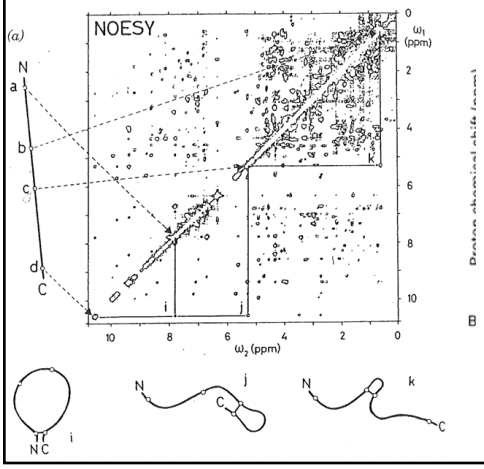
Hz ~1-2

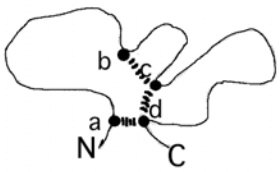
Can also get ϕ & ψ angles from secondary chemical shifts (observed from known random coil shifts)(TALOS & ANGLOR)

Protein-Conformational Structure Determination

NMR for Protein Structure

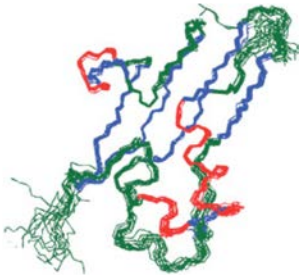
Nuclear-Overhauser Effect Spectroscopy (NOESY)



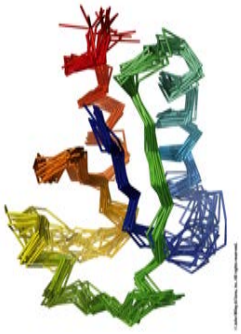


Protein-Conformational Structure Determination

Typical NMR structure



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

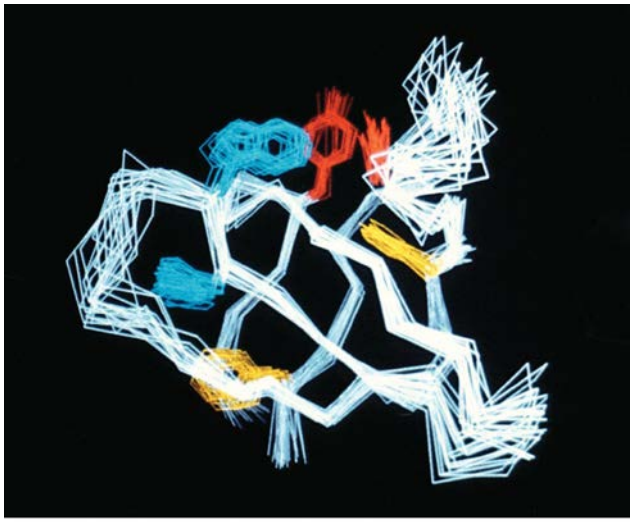


Blue = N-term, red = C-term "Rainbow coloring."

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

Protein-Conformational Structure Determination

NMR Structure of a Protein

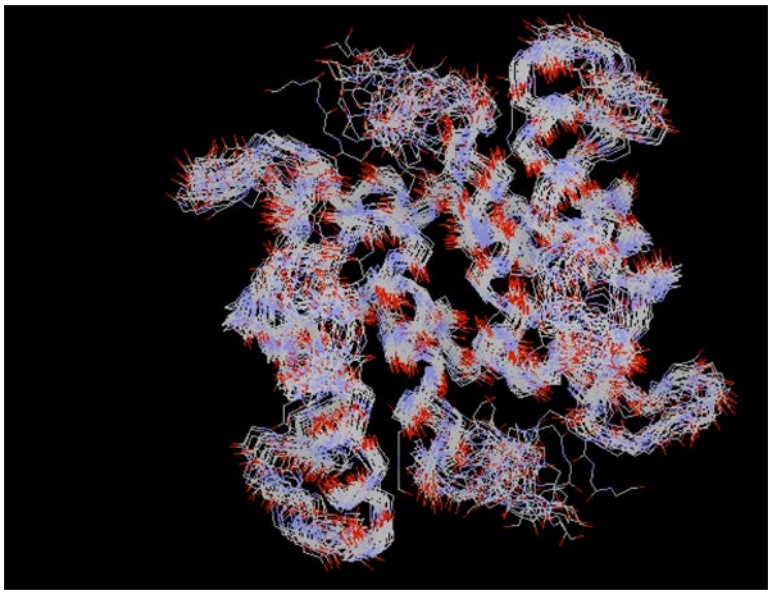


Courtesy Stuart Schrieber, Harvard University

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

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

Protein-Conformational 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