Advanced in silico drug design

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Lecture: Biomolecular Targets

Palacky University, Olomouc 2016

Outline

1. Drugs: modulator's of targets' functions
2. Protein function determinants
3. Protein Data Bank
4. Methods of Structure Determination

Drug modulates function of target

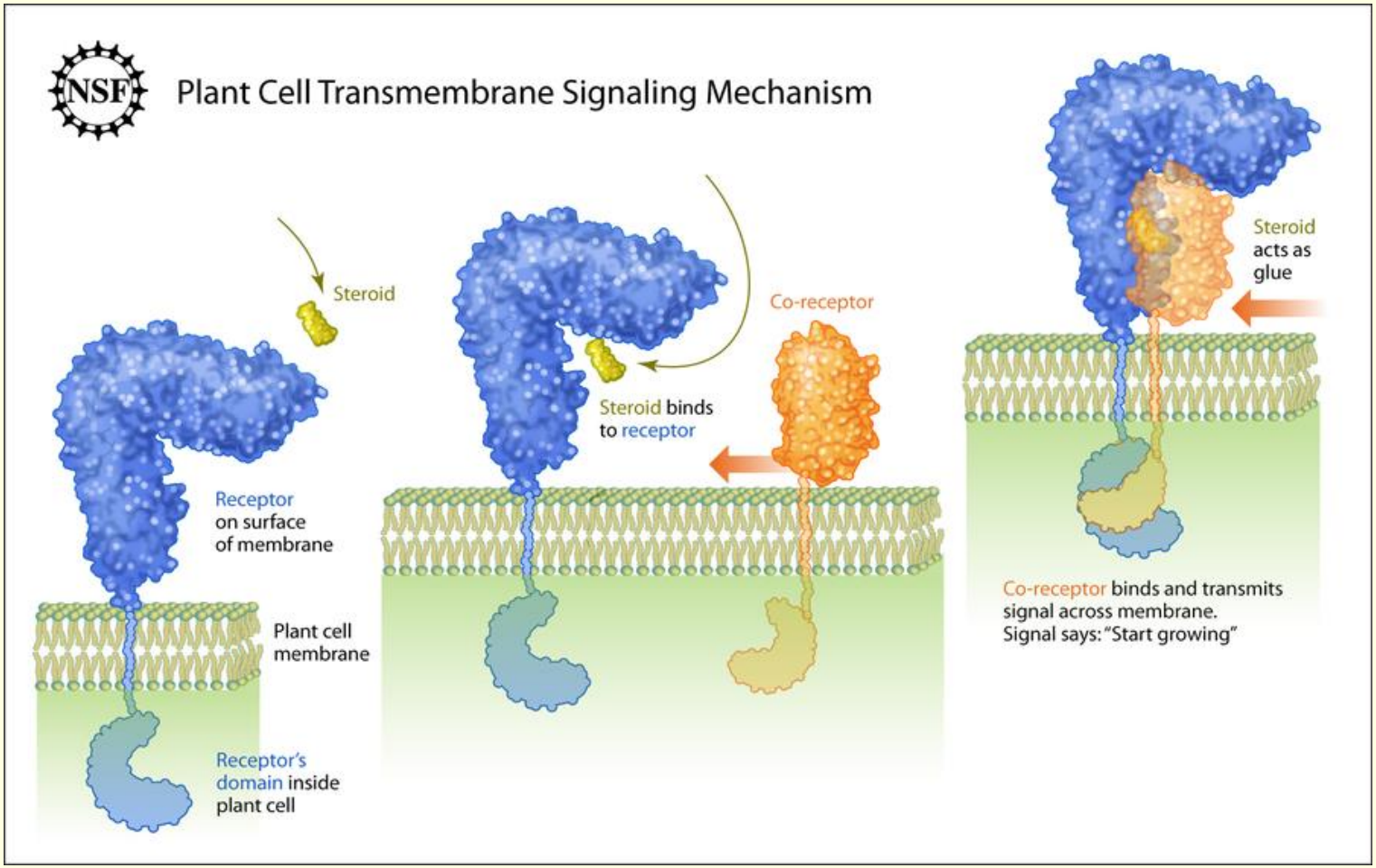
- Biomolecules (proteins, nucleic acids) crucial for functioning of viruses/cells
- Drug (ligand, inhibitor, activator) binding interferes → treatment
- Issues: potency, specificity, resistance, toxicity

Target	Drug	Comment
DNA	Cisplatin	Anticancer, cytotoxic
HIV reverse transcriptase	tenofovir	Antiviral, specific, resistance
Bcr-abl kinase	Glivec	Anticancer
COX-2	aspirin	covalent
Bacterial ribosome	Chloramphenicol	Specific, resistance

Ligand Binding to Proteins

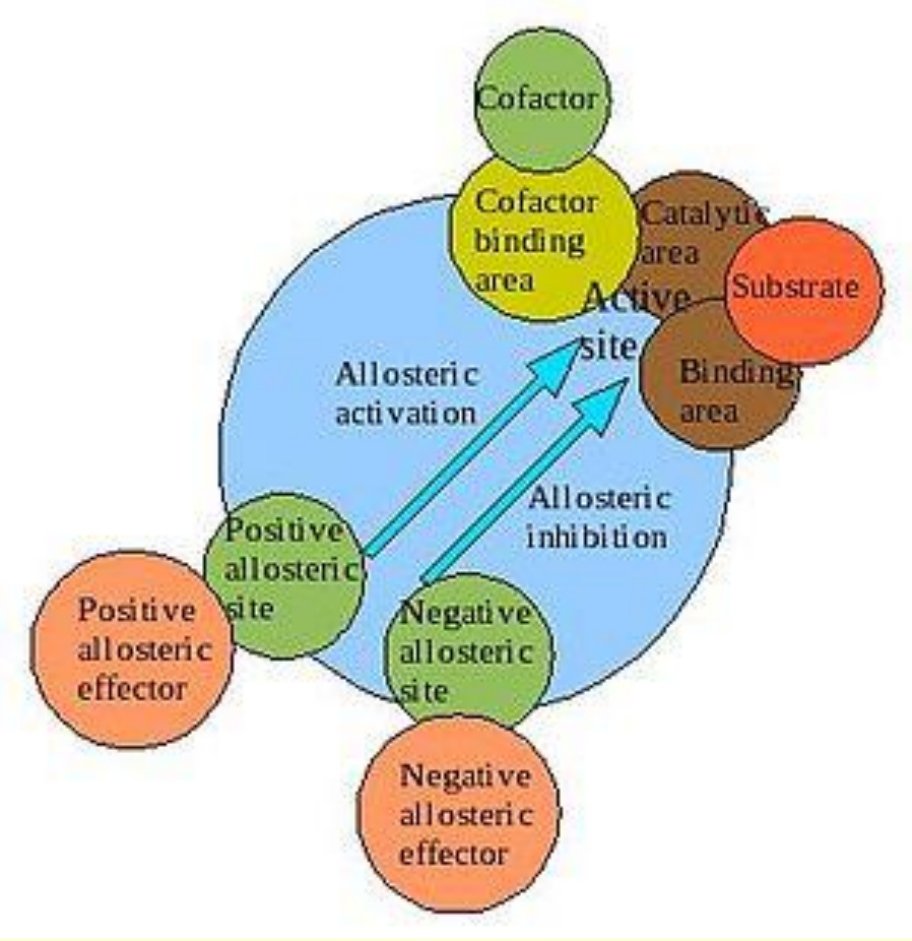
- Biology: Modulates function (receptor signaling, enzyme regulation)
- Structure: Enzyme active-site, orthosteric (competitive), allosteric (noncompetitive)
- Ligand: small (organic, inorganic, metals), peptide, protein
- Chemistry: Noncovalent, covalent

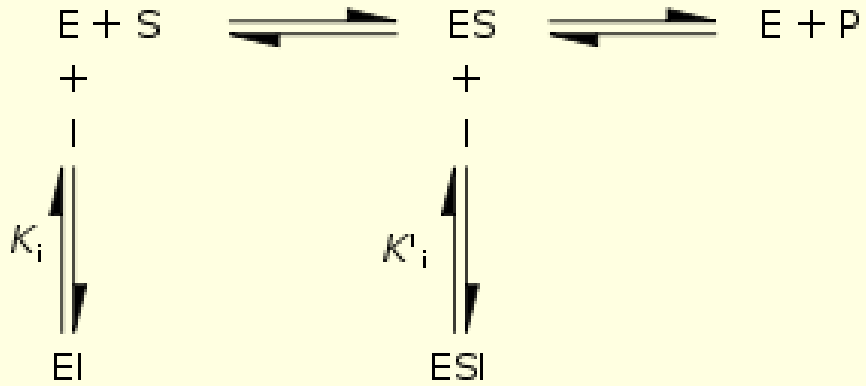
Receptor signaling (Membrane, nuclear)



Ligand Binding Sites in Proteins

Active site (substrate, orthosteric competitive inhibitor)
Allosteric site (allosteric effector, noncompetitive inhibitor)

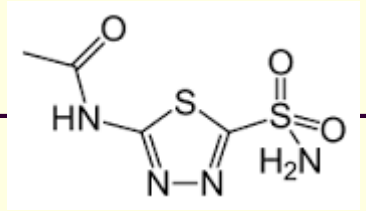




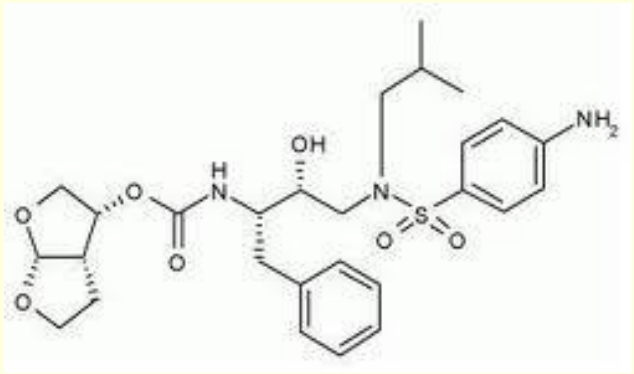
Ligands of Proteins

Organic

Acetazolamide (carbonic anhydrase inhibitor)

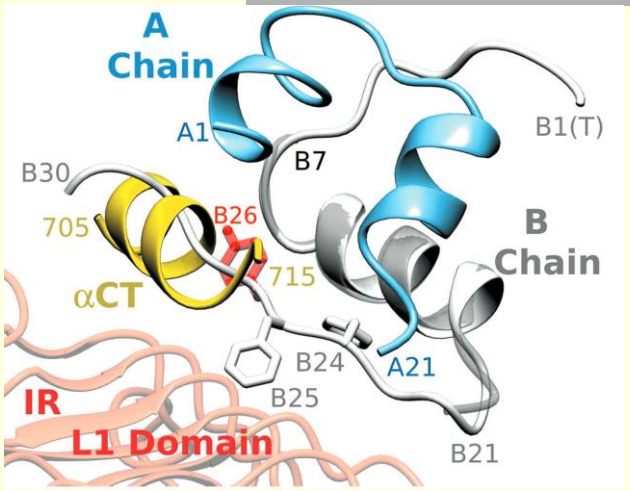


Darunavir (HIV protease inhibitor)



Peptides

insulin



Protein

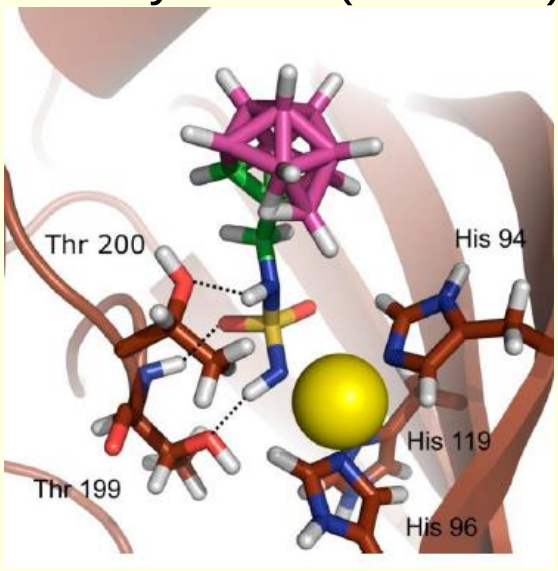
- protein-protein interactions
- Antibody-antigen
- Prions
- Enzyme inhibitors (e.g.serpin)

Metals:

- alkali (Na^+, K^+)
- alkaline-earth (Mg^{2+}, Ca^{2+})
- transition (Zn^{2+}, Fe^{2+})

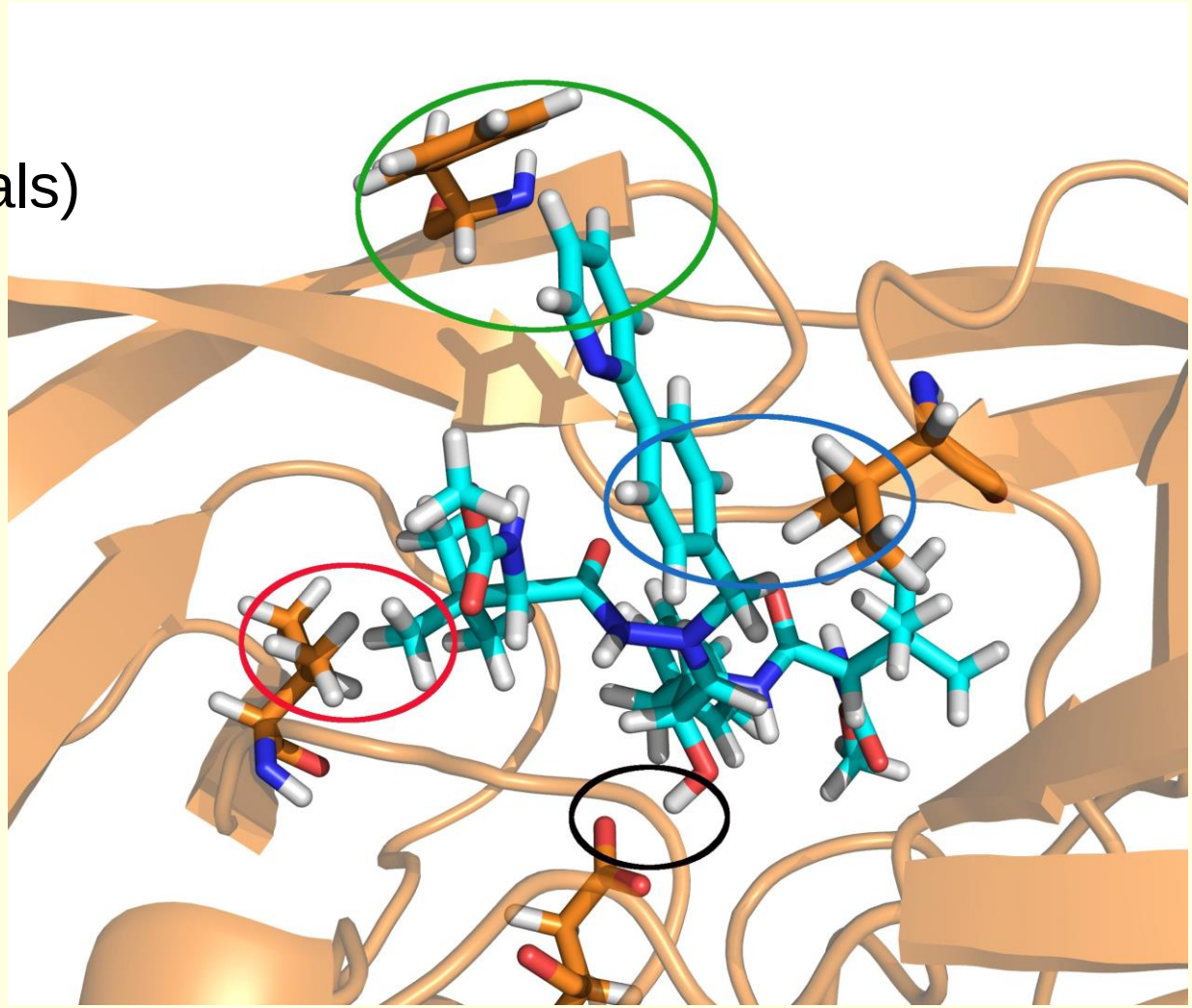
Inorganic

borohydrides (boranes)



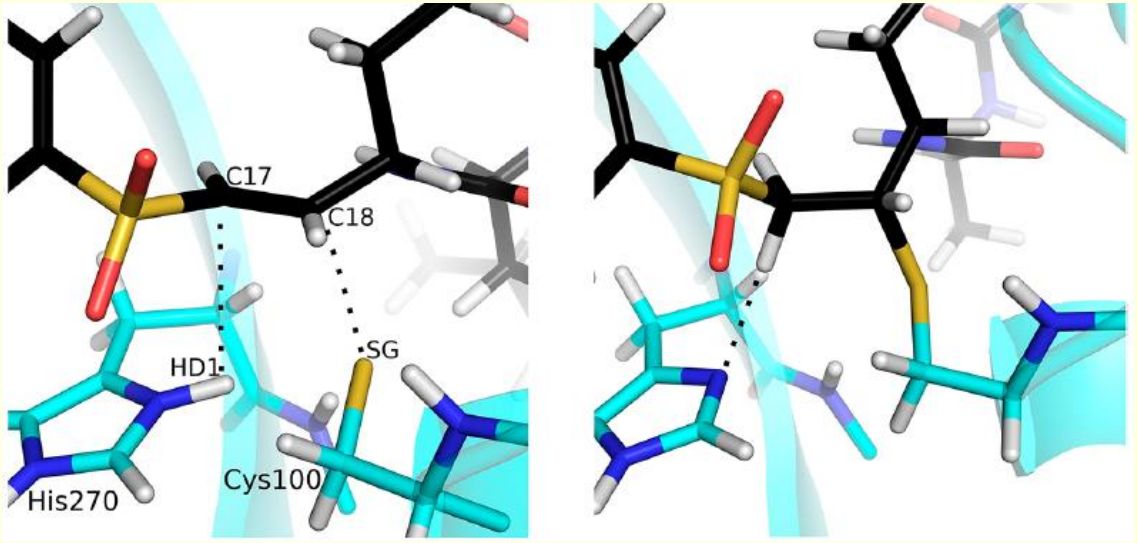
Noncovalent Binding

- Hydrogen bonding
- Salt bridges
- Dispersion (van der Waals)
- Stacking π…π
- Weak CH…O, CH…π
- Halogen bonding
- Dihydrogen bonding



Covalent Binding

- Mostly irreversible inactivation
- Serine, cysteine proteases
- Warhead: reactive moiety (vinyl sulfone,nitrile,ketoamide)
- Off-target specificity, toxicity
- Aspirin (anti-inflammation), bortezomib (anti-cancer)



$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E\text{–}I \xrightarrow{k_{inact}} EI$$

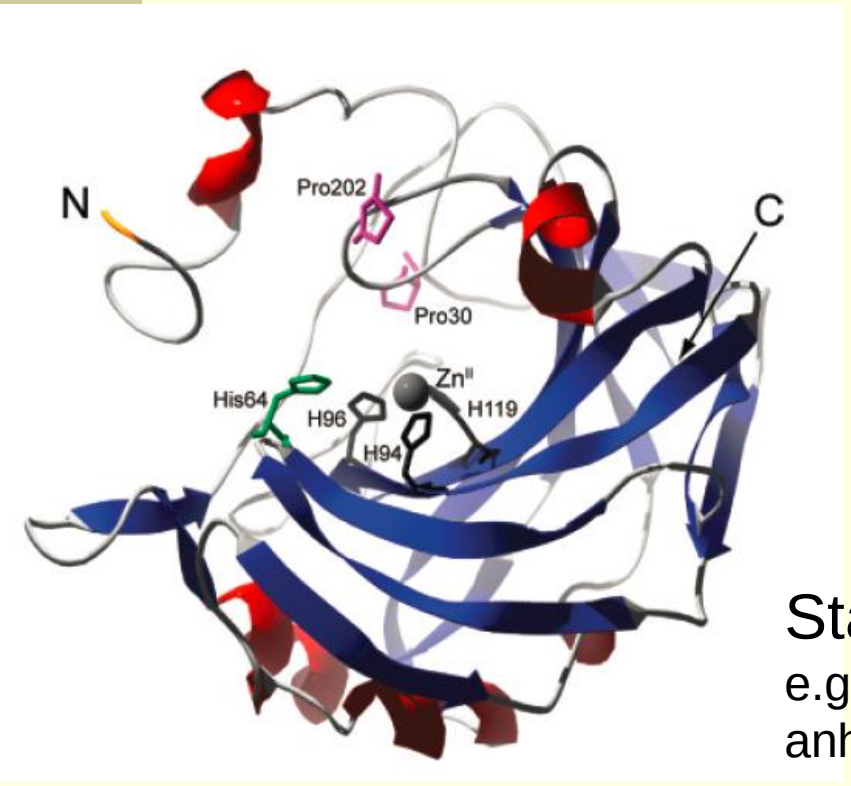
$$K_i = k_{off}/k_{on}$$

$$k_{2nd} = k_{inact}/K_i$$

Fanfrlik, …Hobza, Lepšík *J Phys Chem B* **2013**, *117*, 14973.

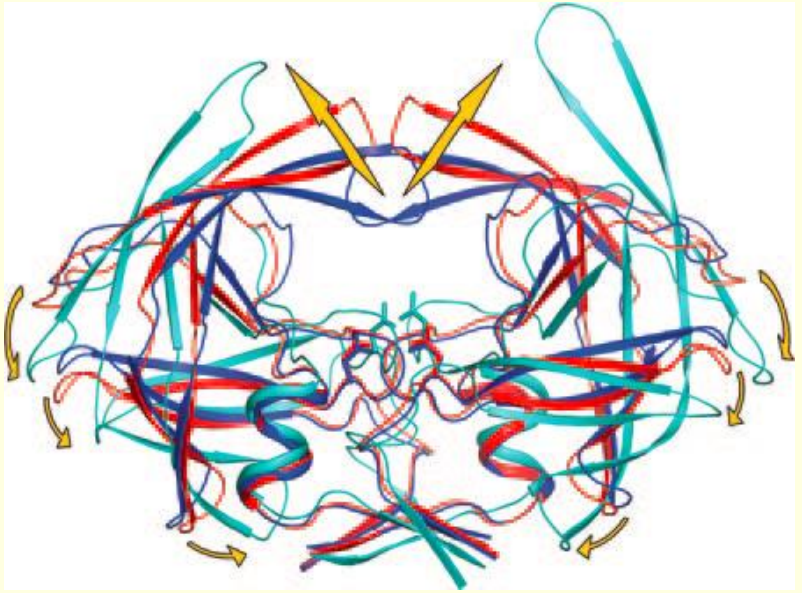
Protein Function - Enzymes

Substrates: identity, binding (where/how), cleavage (reaction mechanism, transition state)



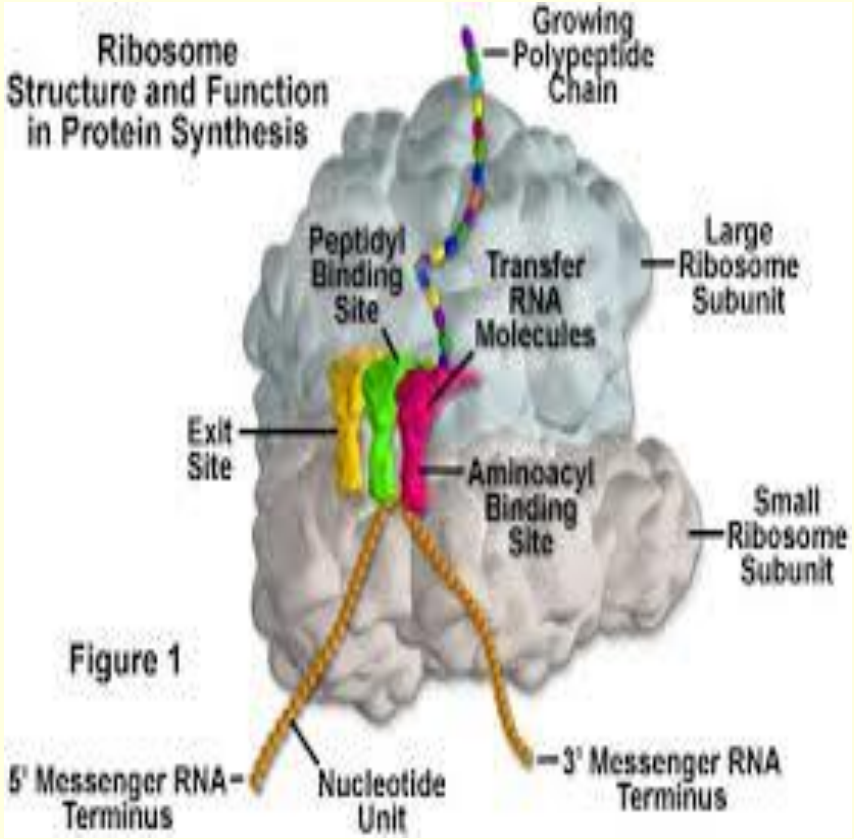
Static
e.g. carbonic anhydrase

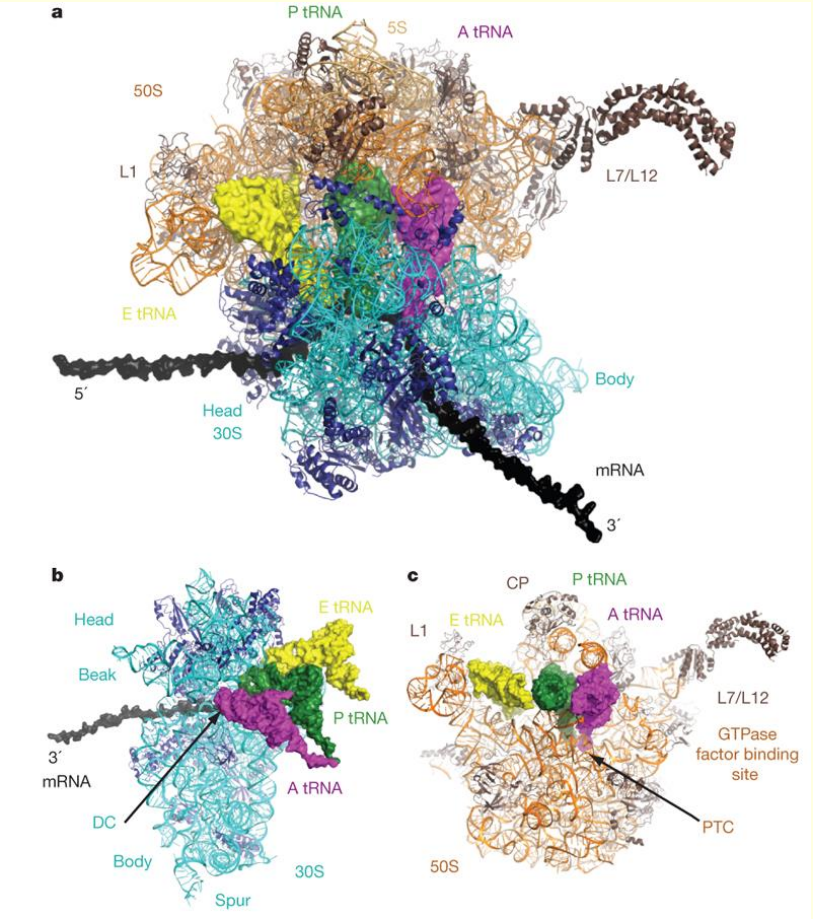
Dynamic
e.g. HIV-1 protease



Protein Function - Proteosynthesis

Ribosome

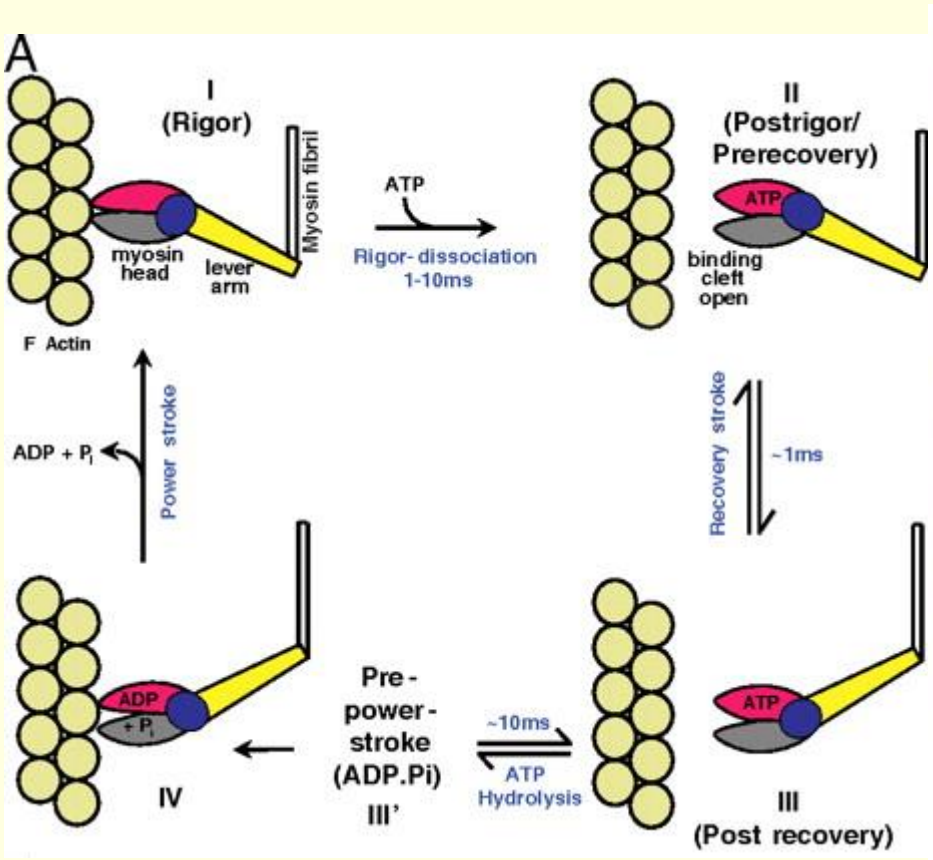


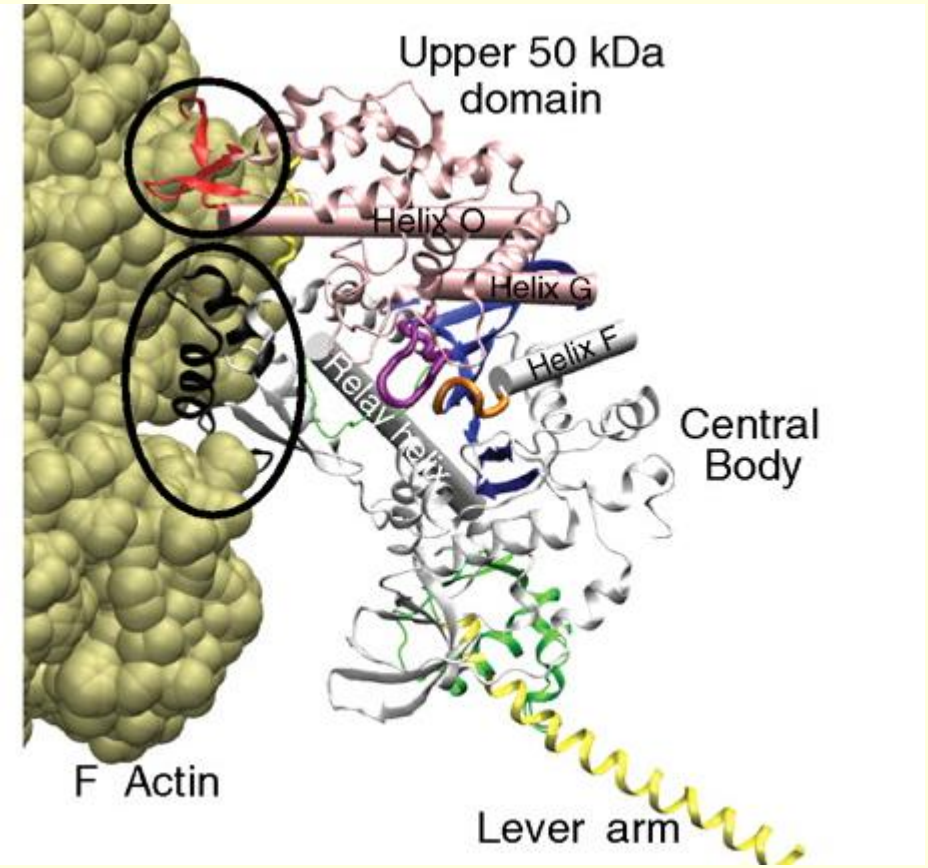


X-ray: Nobel Prize in Chemistry, 2009 (Yonath, Steitz, Ramakrishnan)

Protein Function - Molecular Motors

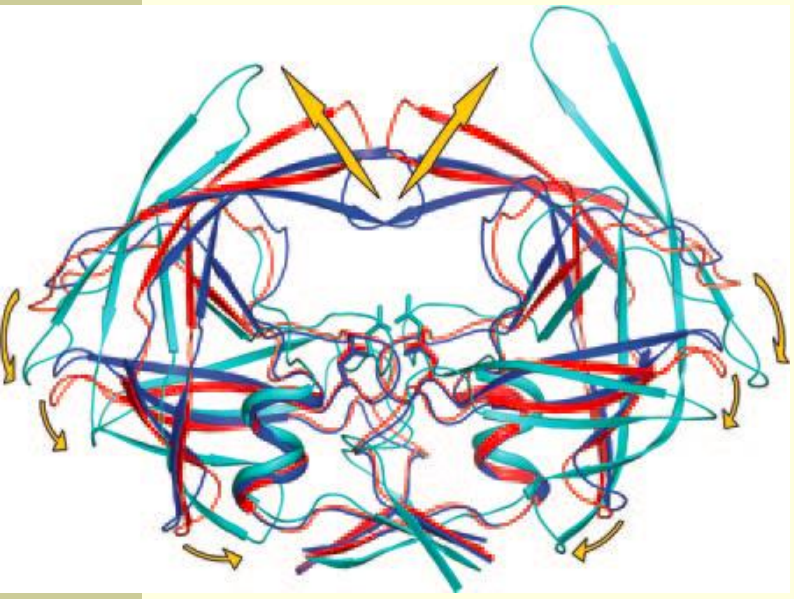
Myosin



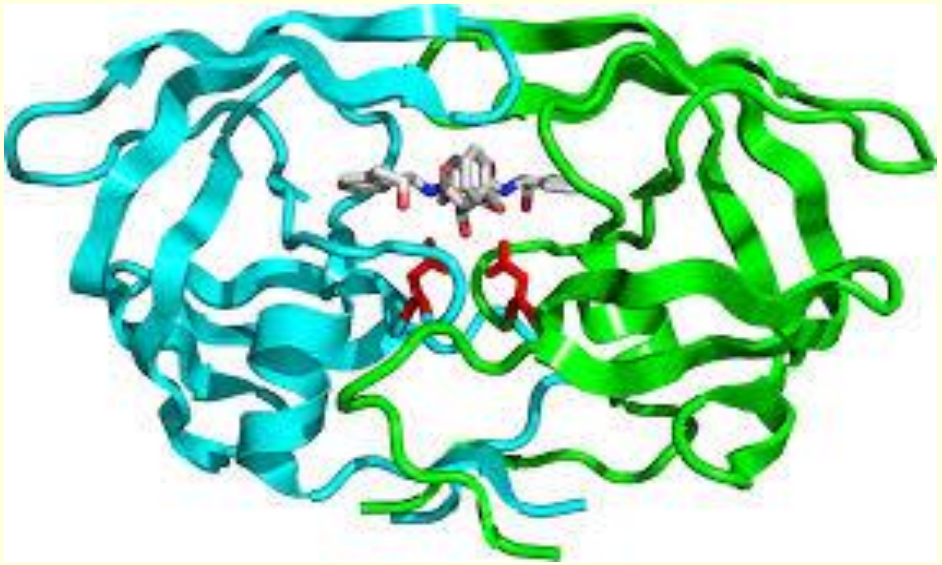


Molecular simulations: Nobel Prize in Chemistry, 2013 (Karplus, Warshel, Levitt)

Determinants of Protein Function



dynamics



structure

folding

AA sequence

TSLITLPSGL
AEMINAEIGA
KKGWTGQYTL
DCNTRDNLPD

Protein DataBank (PDB)

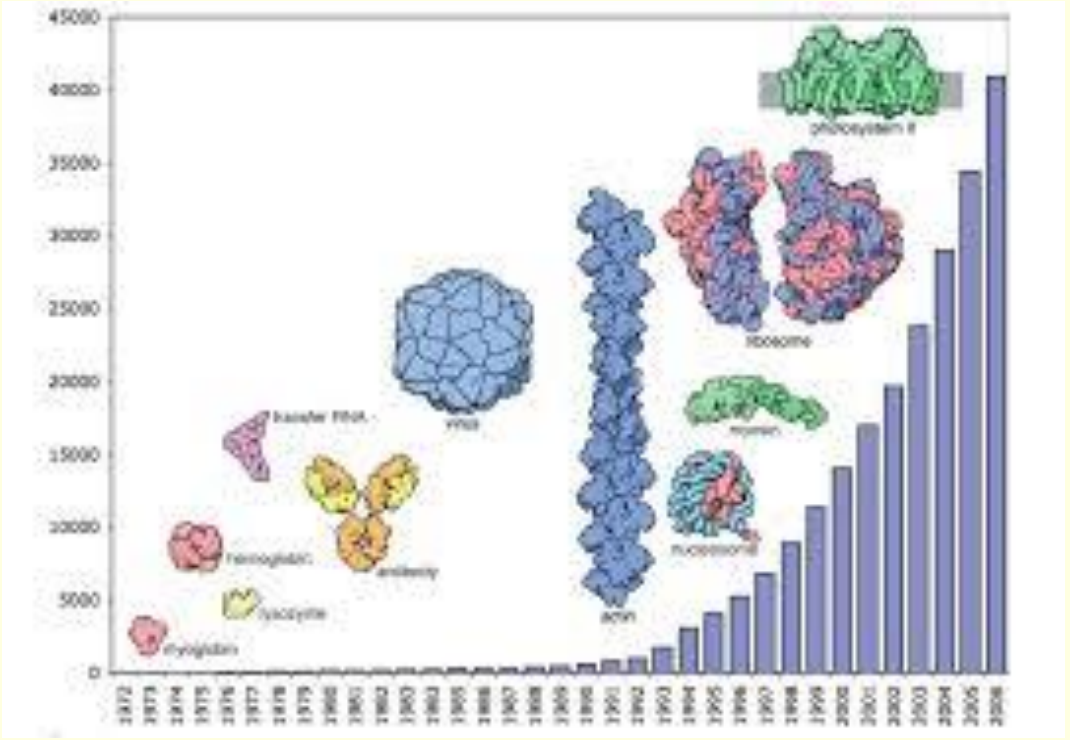
>10^5 structures

X-ray crystallography – atomistic details (resolution 0.5-4Å), needs crystals, static

NMR – dynamics, solution-phase, small proteins, huge amounts

Cryo-Electron Microscopy – huge complexes, low resolution (8 -20 Å)

Homology Models – need validation



PDB: http://www.rcsb.org/

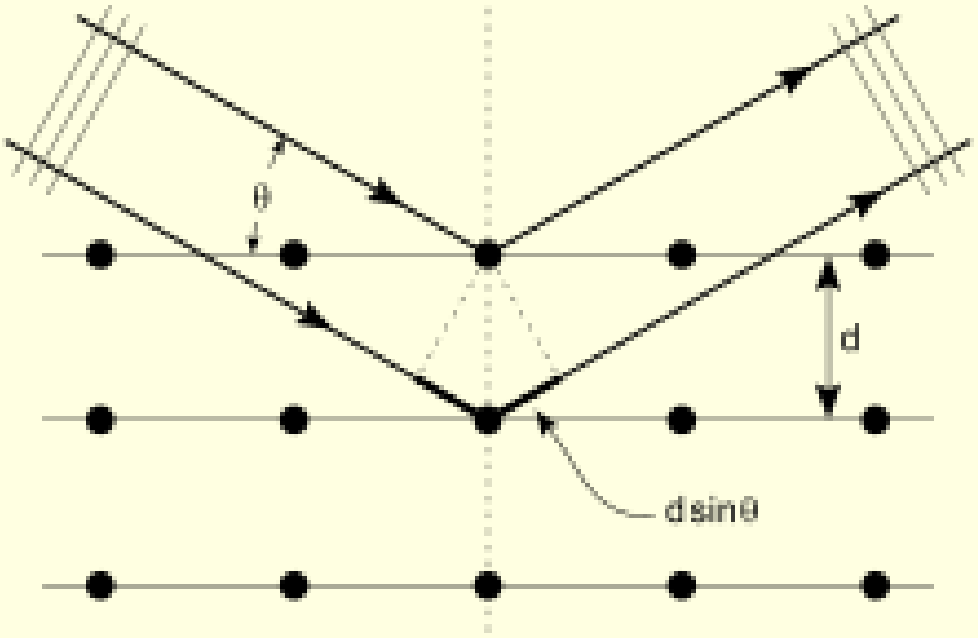
Protein Structures

X-ray crystallography	Nuclear Magnetic Resonance
Crystals, “unlimited” size	Solution, < 35kDa
Cooled	Isotopically labeled (^{13}C,^{15}N)
Diffraction of X-rays	Radio-frequency pulses
Rotating-anode, synchrotron	900 MHz
Static	Dynamic
1 structure (alternate conformations)	Ensemble of structures (~20)
Rigid Conformational States	Flexible, disordered proteins
No hydrogens, missing loops, termini	Hydrogens

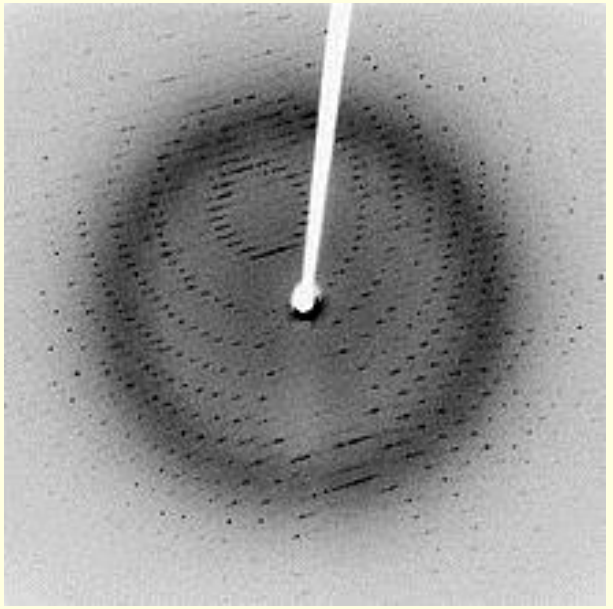
Models fitted to experimental data

X-ray crystallography

Diffraction of X-rays on lattice



Diffraction pattern



Constructive interference : Bragg's law

$$2d\sin\theta = n\lambda$$

d_{min} - Resolution (Å)

- Reflections
- Intensities, I (h,k,l)

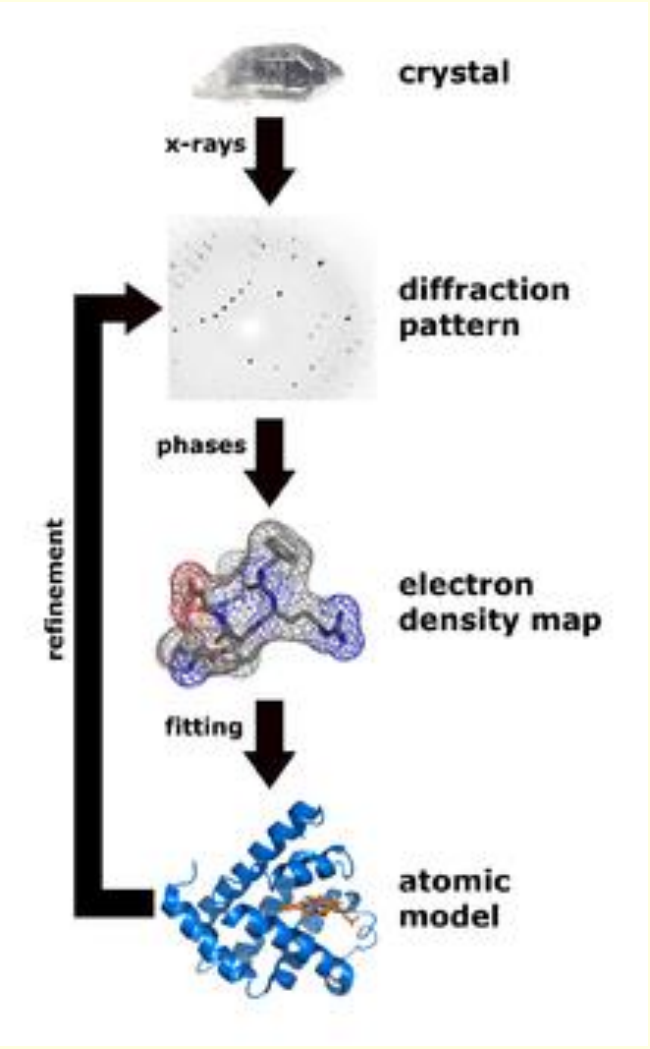
Diffraction Analysis and Refinement

I (h,k,l) → F (h,k,l) ⟺ ρ (x,y,z)

Intensities → structure factors → electron density

Space: Reciprocal ⟺ Real

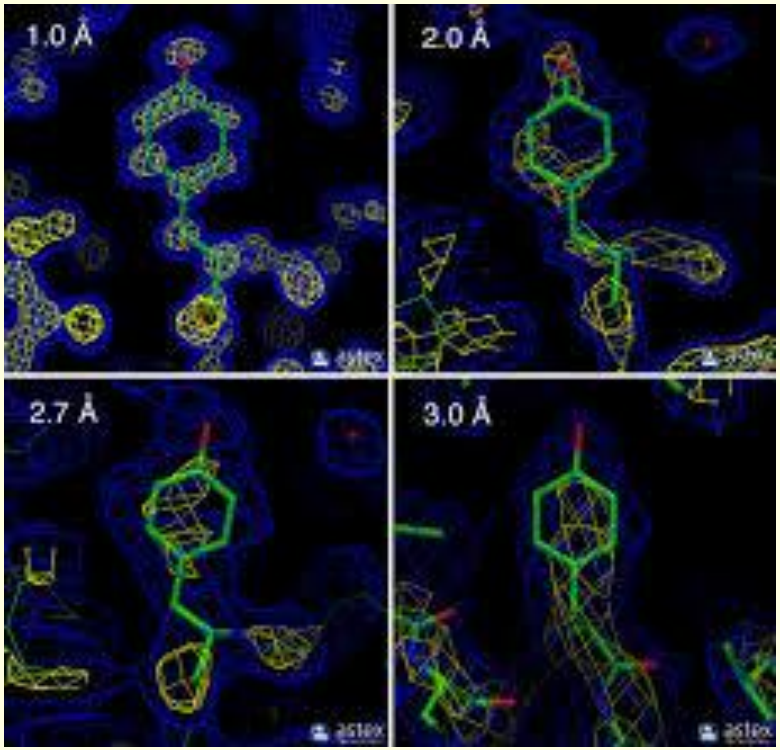
Fourier transform



Phase Problem
- Multiple Isomorphous Replacement (MI
- Hg
- Multiple Anomalous Diffraction (Se-Met)
- Molecular Replacement (homologous proteins)

Quality of X-ray Structure

Resolution – reciprocal space
3 Å : secondary structures
2 Å: side chains
1 Å: atomic (no hydrogens)



PDB file:
R factor: observed, calculated F
(1/10 of resolution)
R_{free} (5-10% data, 5-10% > R)

$$R = \frac{\sum_{\text{all reflections}} |F_o - F_c|}{\sum_{\text{all reflections}} |F_o|}$$

B-factors – thermal motions, spherical, ellipsoidal (10-20 Å^2)

$$B = 8\pi^2 \langle u^2 \rangle$$

Occupancy – waters, alternate conformations, 0-1

Nuclear Magnetic Resonance

N = A - Z
neutron number = Mass number - Atomic number

Isotopes with Odd Number of Protons/Neutrons Have Nonzero Nuclear Spin
e.g. ^{1}H, ^{13}C,^{15}N (and $^{11}_{5}B$ but also $^{10}_{5}B$)

Positive charge → Magnetic moment

1) Alignment of magnetic nuclear spins in applied magnetic field
2) Perturbation by Radio-Frequency pulse, Relaxation

Magnetic Resonance and Shielding

$E = - \mu_z B_0$

E - energy

μ_z – magnetic moment in z-axis

B_0 – magnetic field intensity

Stronger magnets (900 MHz) – better resolution

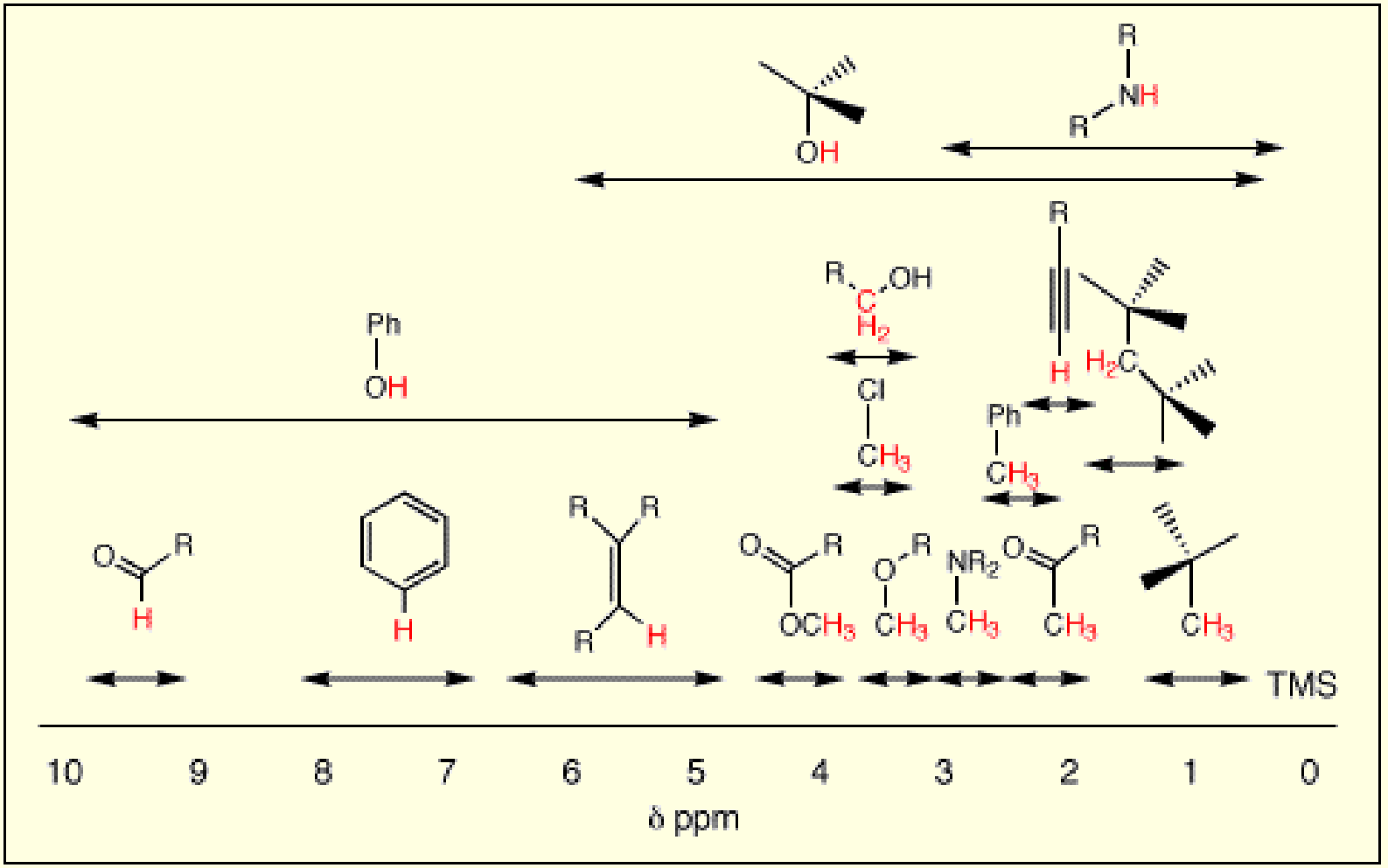
Shielding (σ) by electron density – chemical shift (v)

Relative to standard (v_{st})

δ (ppm) = $(v - v_{st}) / v_{st}$

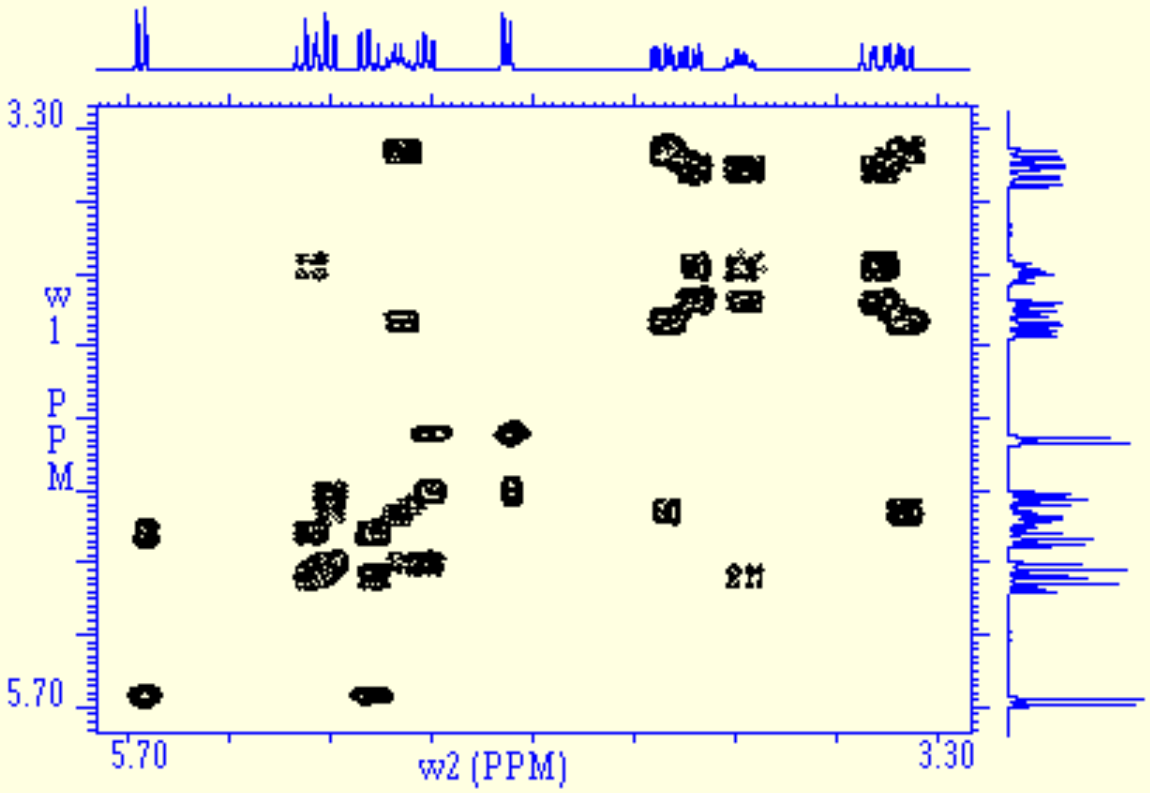
Chemical Shifts

Figure 8. Approximate proton chemical shifts.



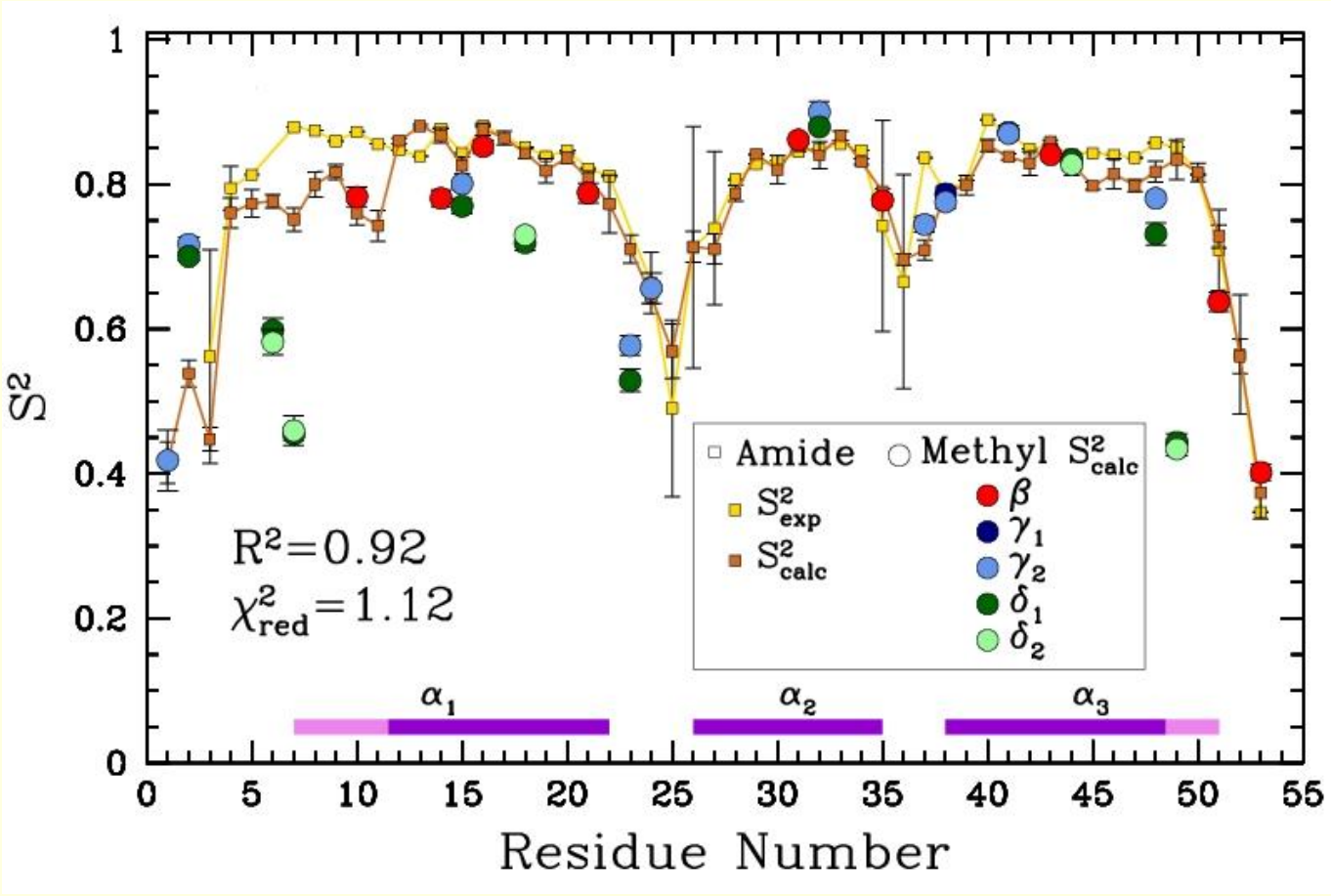
Multidimensional NMR

2D spectra - COSY, TROSY, HSQC, NOESY, etc.
More information



Use of NMR structures

Order parameters (S^2)



Nuclear Overhauser effect (NOE): between atoms within 5 Å
→distance restraints → Dynamics → Ensemble of Structures

Summary

1. Drugs modulate targets' functions
2. Drugs: ligands, inhibitors, activators; identity, binding
3. Targets: nucleic acids, proteins (enzymes), complexes, organelles
4. Protein function determined by dynamics, structure, sequence
5. Protein Data Bank – repository of 3D structures
6. Experimental structure determination: X-ray, NMR

Amino acids

-20 natural, encoded
-4 groups (nonpolar, polar, acidic, basic)
- three-, one-letter code
- zwitterions,
- pKa

Amino Acid	Properties
Pro	Imino, trans/cis
Ile, Thr	chiral
His	catalysis, pKa of 6.8
Asn,Gln,His	flips
Se-Met	X-ray
Se-Cys	selenoproteins