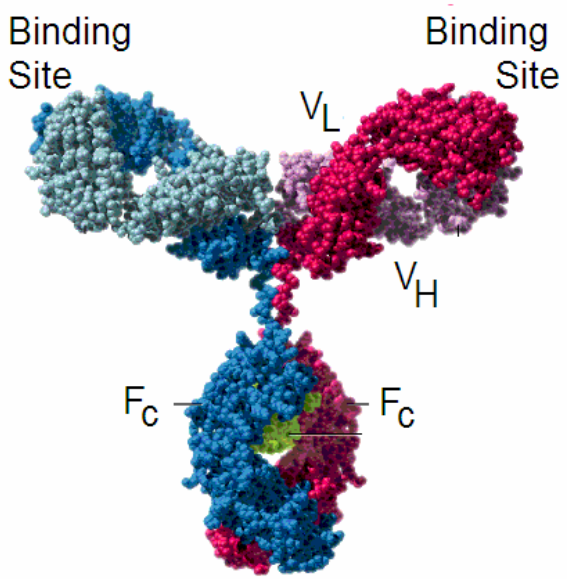
Molecular Forces in Antibody Maturation*



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***Published, Physical Review Letters, 2005**

ACKNOWLEDGEMENT

- **FUNDING**
  - ICAM funding
  - Penn State University, MRSEC-MRI/Huck Seed Grant
  - Penn State University, Start-up funds + Allen Pearce Endowment
  - Alexander von Humboldt Fellowship

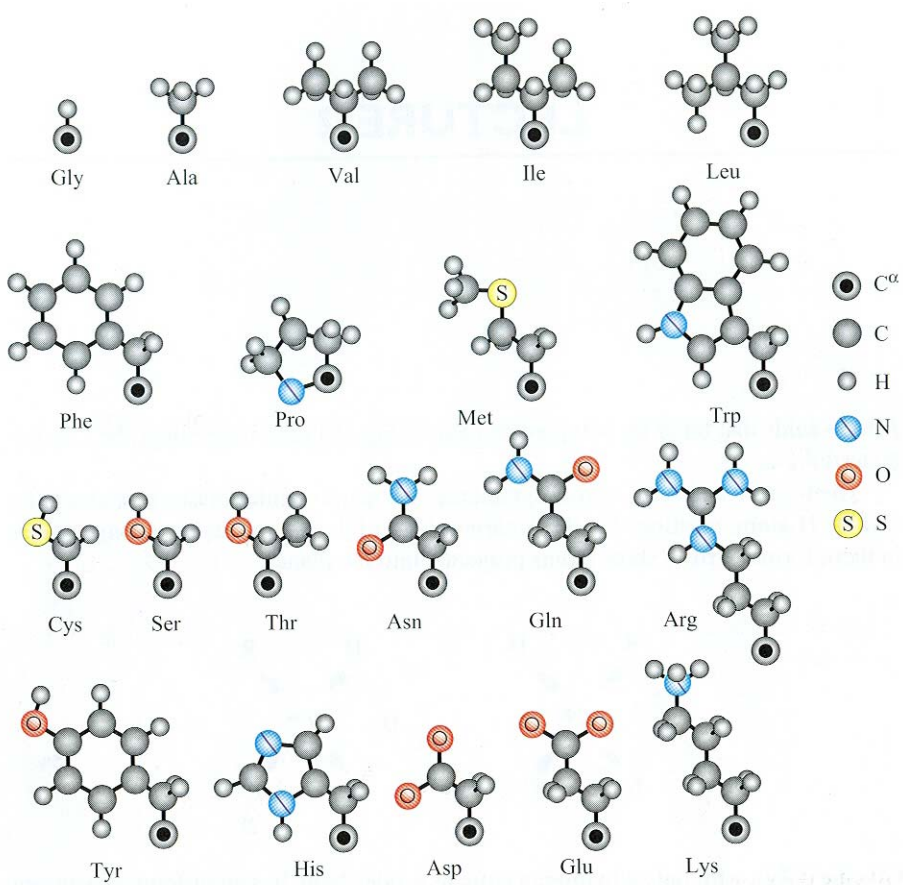
- **COLLABORATION**
  - Prof. ARTHUR LESK (Molecular Biology and Biochemistry, The Pennsylvania State University, University Park, PA, USA)

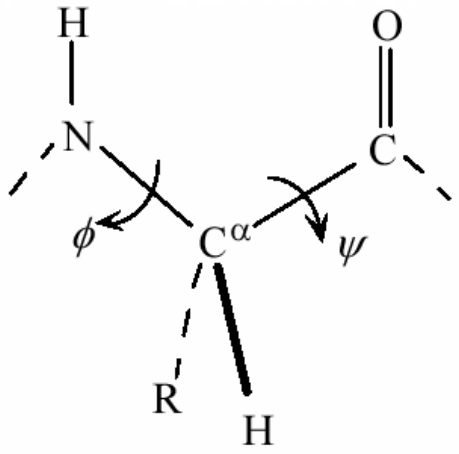
Seminar Outline

- **Globular Proteins: Definition, Properties**
- **Immune System: Antibodies**
- **Theory/Simulation: Coarse Grained Modeling of Antibodies**

PROTEINS

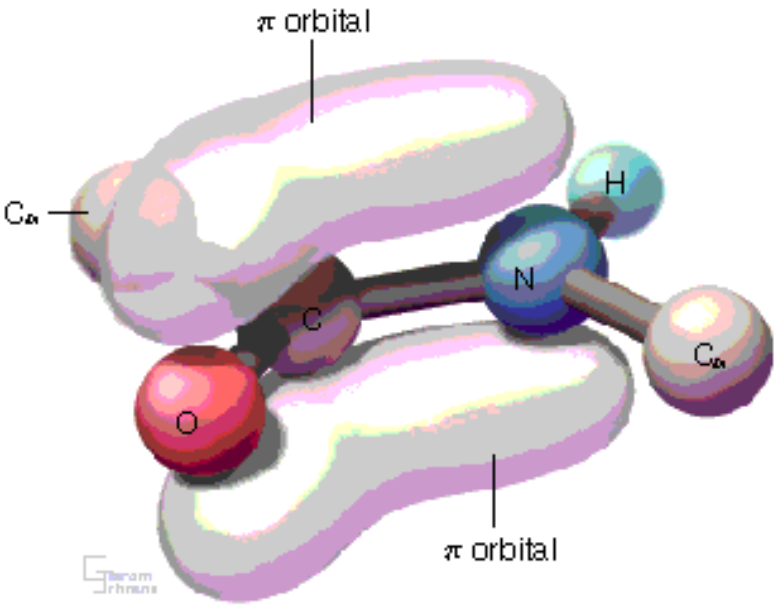
- Have unique, compact tertiary structures
- All enzymes and regulatory proteins are globular
- Use α helices, β sheets, β and γ turns, and non-regular structures (to different extent in each)
- Prosthetic groups are often found in pockets
- Hydrophobic amino acids are in the center and hydrophilic on the outside
- Globular proteins often have more than one domain
- More than one subunit participate in quaternary structures



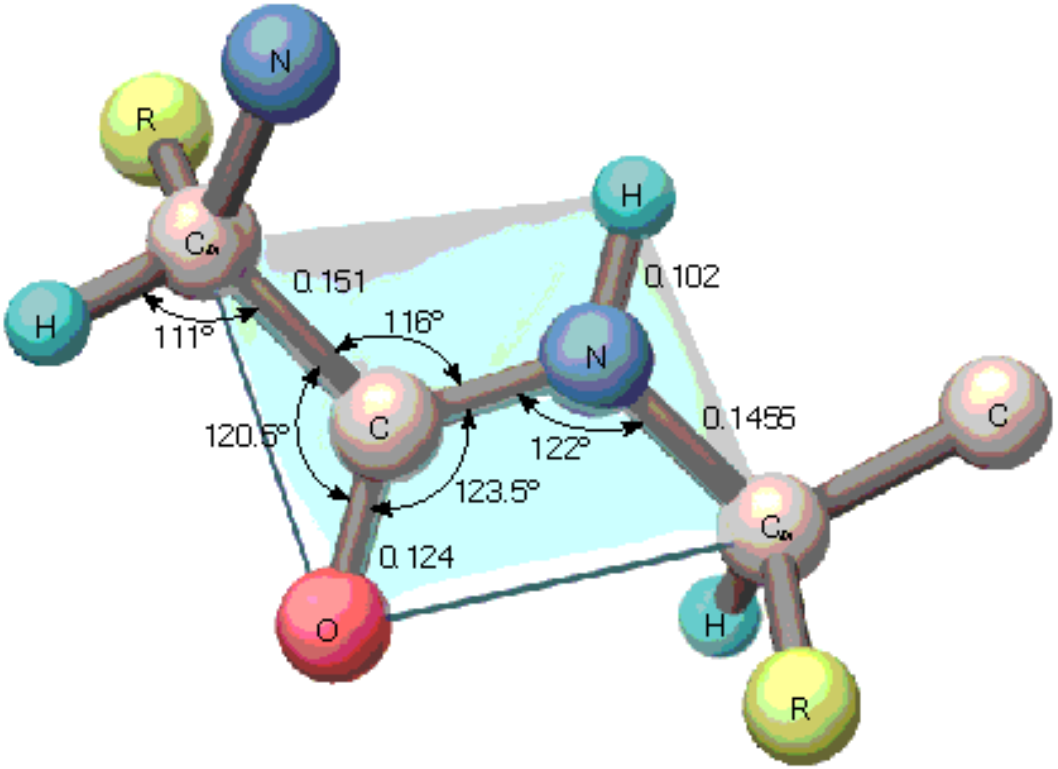


Structure of the peptide bond

20 different subunits (R) with 5 major atoms: N,C,H,O,S= NACHOS



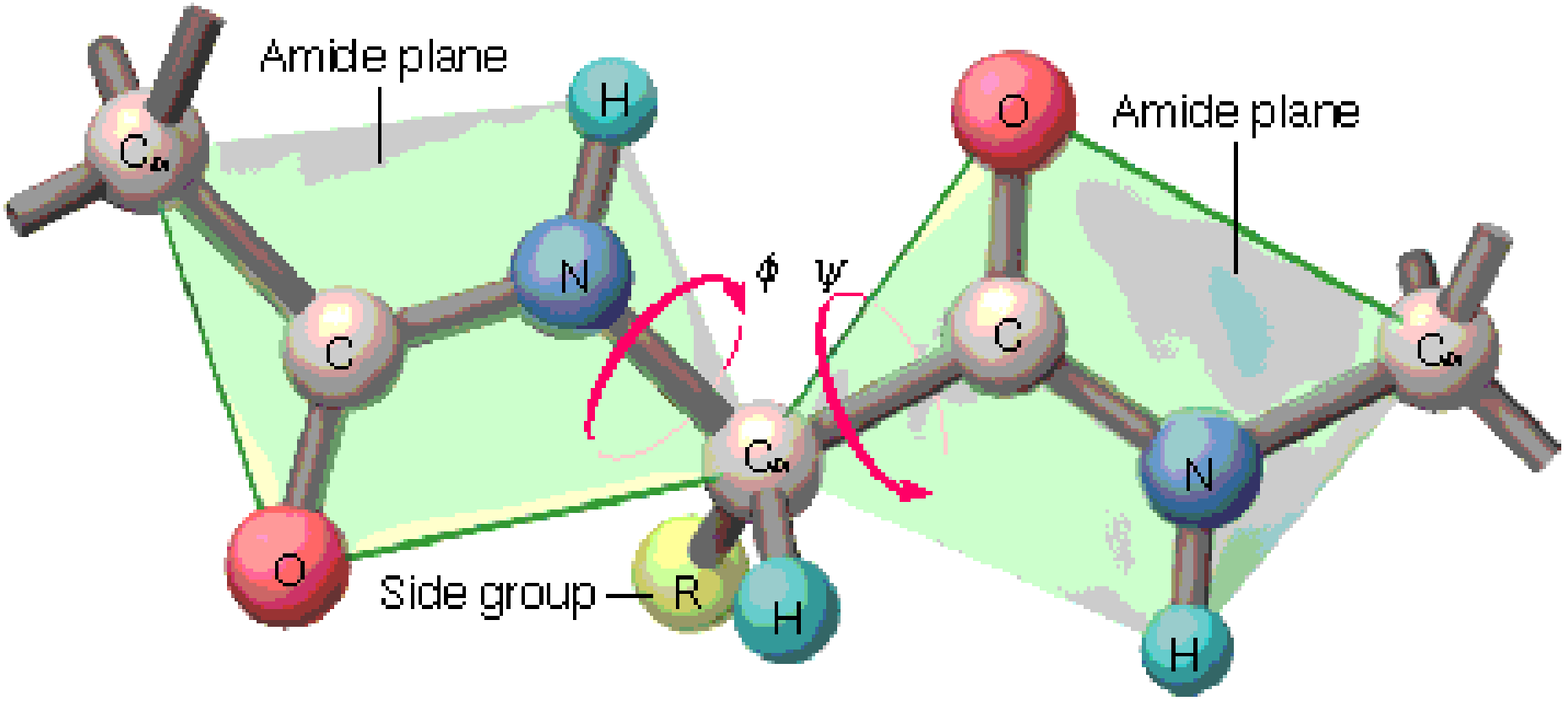
(a) Partial double-bond character of peptide bond



(b) Bond angles and lengths

Notice the planar nature of the peptide bond. The trans form is favored. Delocalization of the π-electron orbital over the O-C-N accounts for the partial double bond character of the peptide bond.

The structure of the protein is defined by the rotation around the polypeptide chain



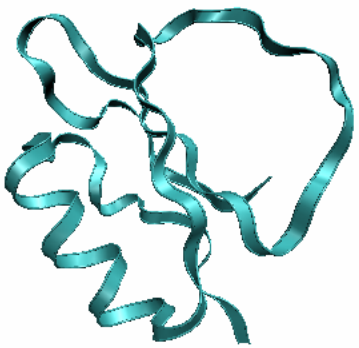
Rotation is allowed only on both sided of the a-carbons. The angles of rotation are defined as ϕ (phi) and ψ (psi)

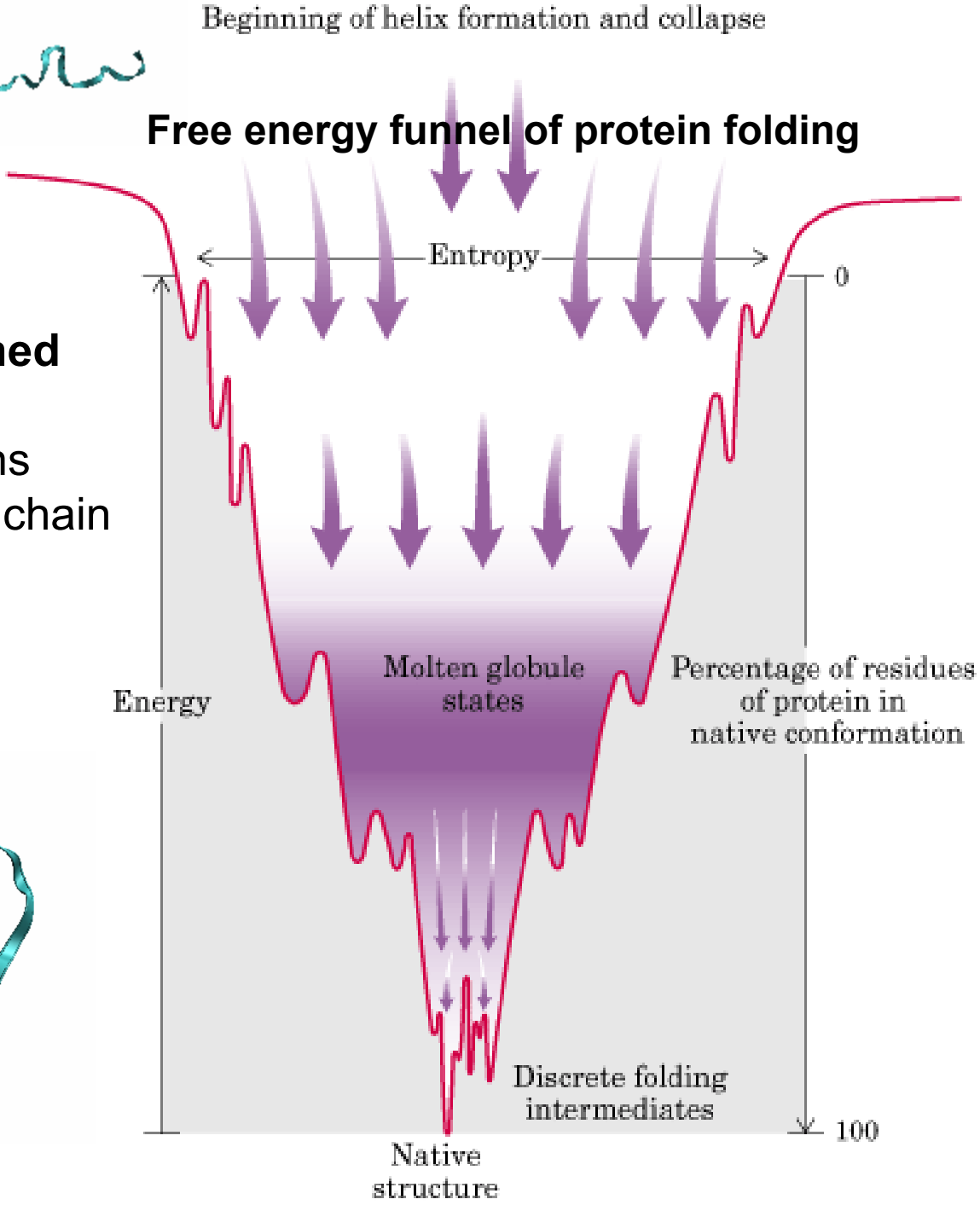
Proteins adopt a defined structure

- molecular interactions
- hydrophobic effects and chain entropy

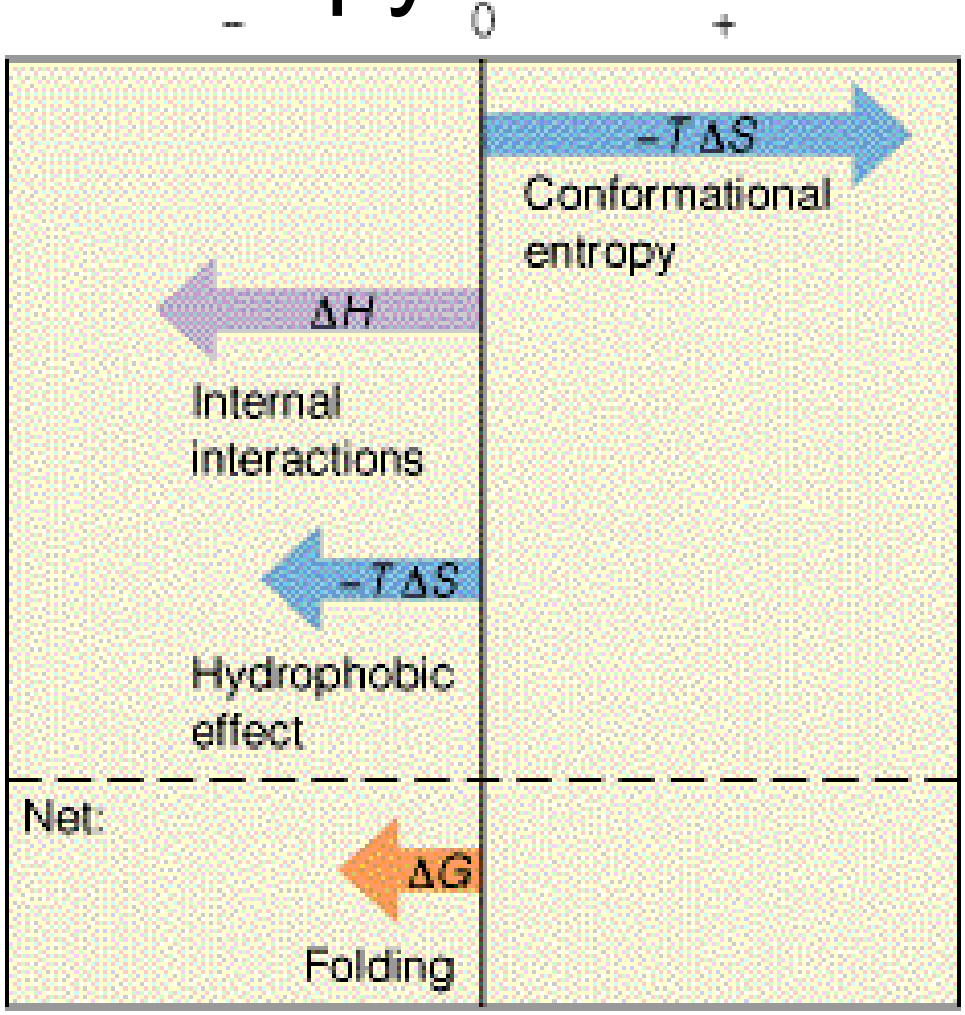
ΔG of U ⇔ N

Folding time 1ms-1s





Contribution of the enthalpy and entropy to the free energy of folding



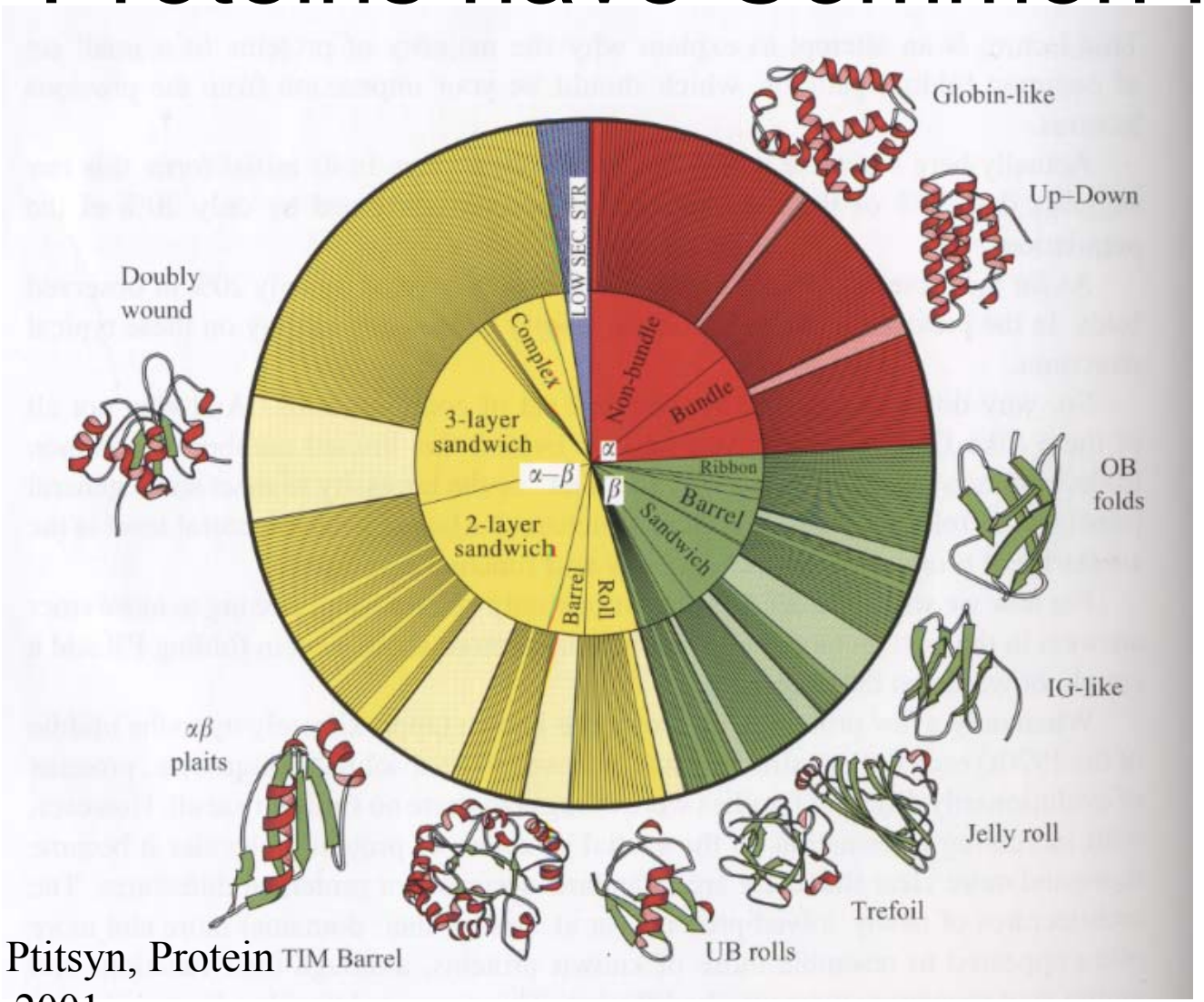
- Average values for protein stability: ~20-50 kJ/mol under physiological conditions
- Reasons for lowered stability:
  - the biological activity requires a certain degree of conformational flexibility
  - proteins must be able to be degraded (especially important for regulatory proteins)
  - kinetic traps along the folding pathway

Thermodynamic parameters for folding of some globular proteins

Protein	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/K·mol)
Ribonuclease	−46	−280	−790
Chymotrypsin	−55	−270	−720
Lysozyme	−62	−220	−530
Cytochrome *c*	−44	−52	−27
Myoglobin	−50	0	+170

Note: Data adapted from P. L. Privalov and N. N. Khechinashvili, *J. Mol. Biol.* (1974) 86:665–684. Each data set has been taken at the pH value where the protein is maximally stable; all are near physiological pH. Data are for the folding reaction: Denatured $\rightleftharpoons$ native.

Proteins have Common Fold

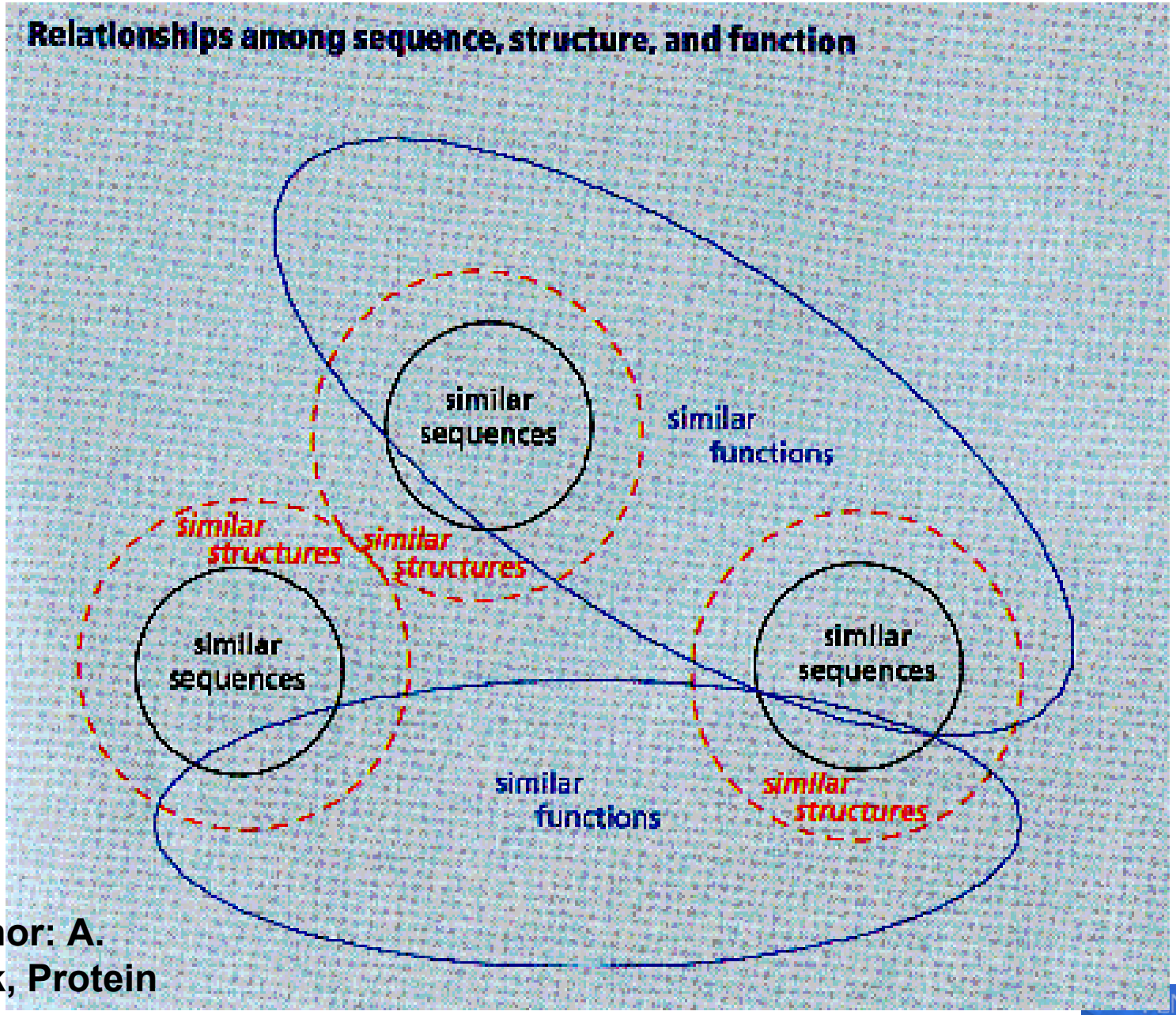


"80%-20%" law!!!

Similar folding patterns but WHY?

Do we see the evolution of protein structures?

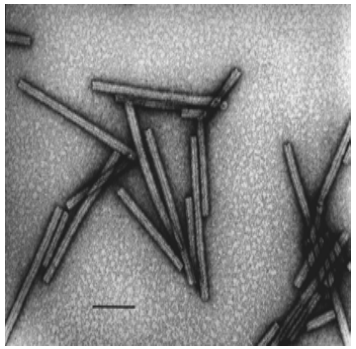
Author: Ptitsyn, Protein Physics, 2001

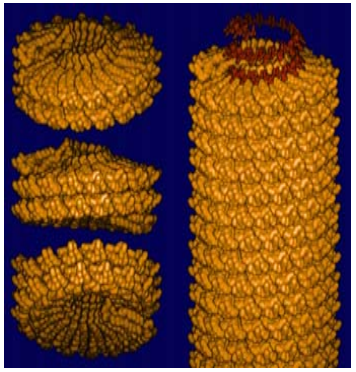


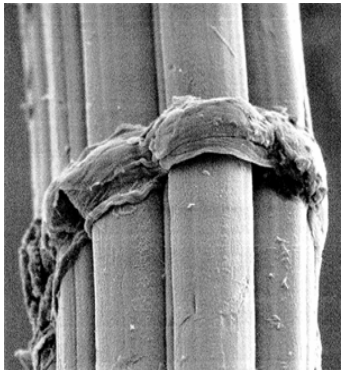
Author: A. Lesk, Protein Science, 2004

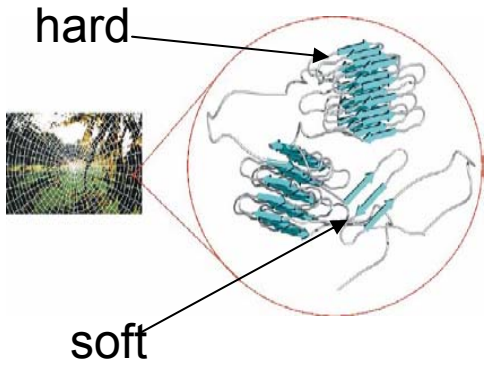
Protein like to assemble

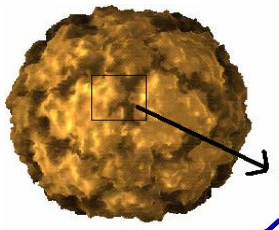
Examples: Tobacco Mosaic Virus, Spider Silk, Human Rhino Virus, Bacterial Flagella



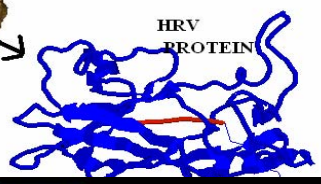


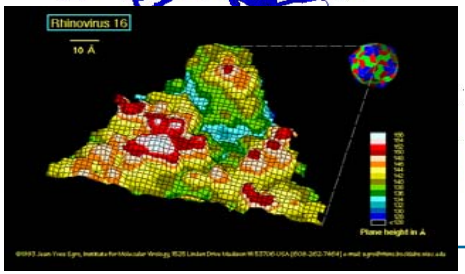


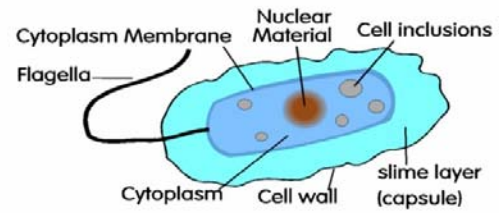




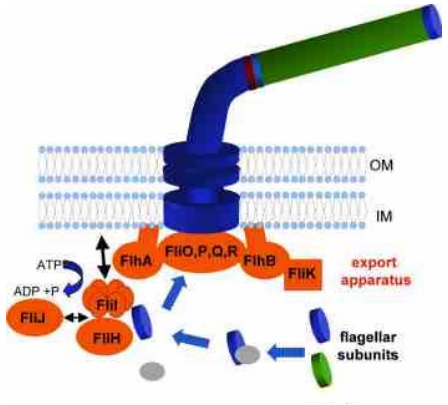
Human Rhino Virus



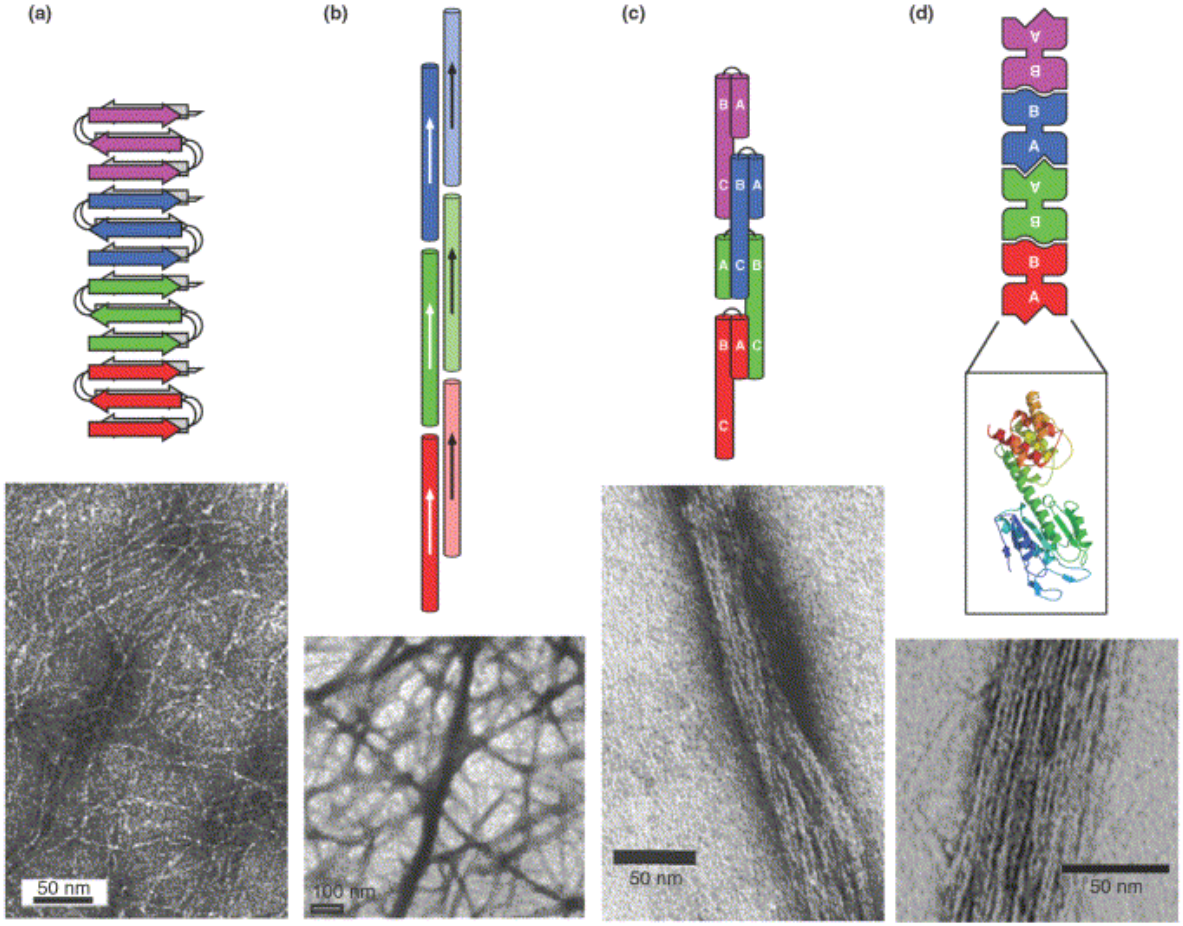




Cell structure



We can engineer the assembly… Protein Design



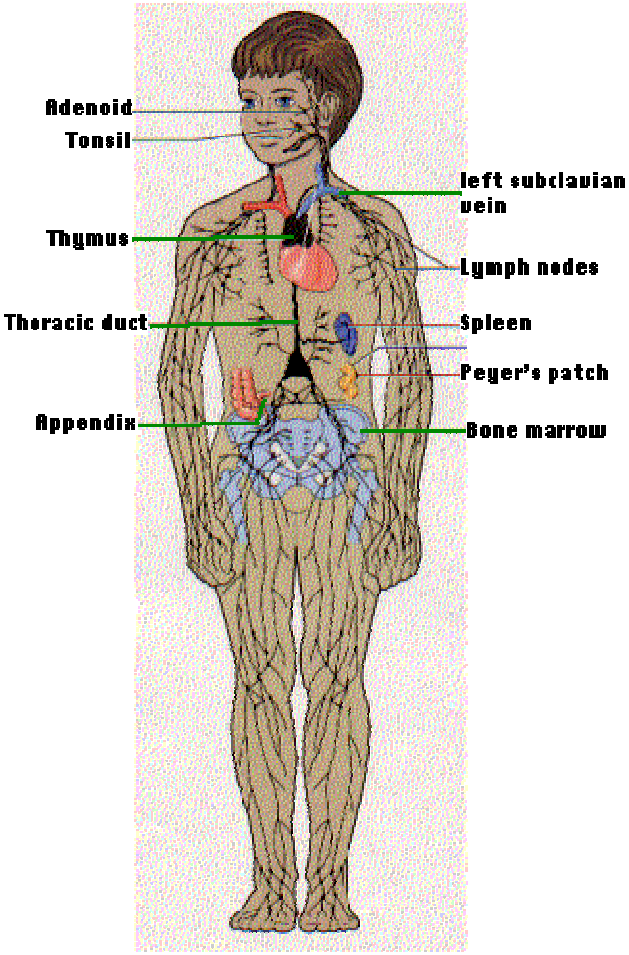
Yeates, et al, Current Opinion in Structural Biology
Volume 12, 2002, pg 464

Seminar Outline

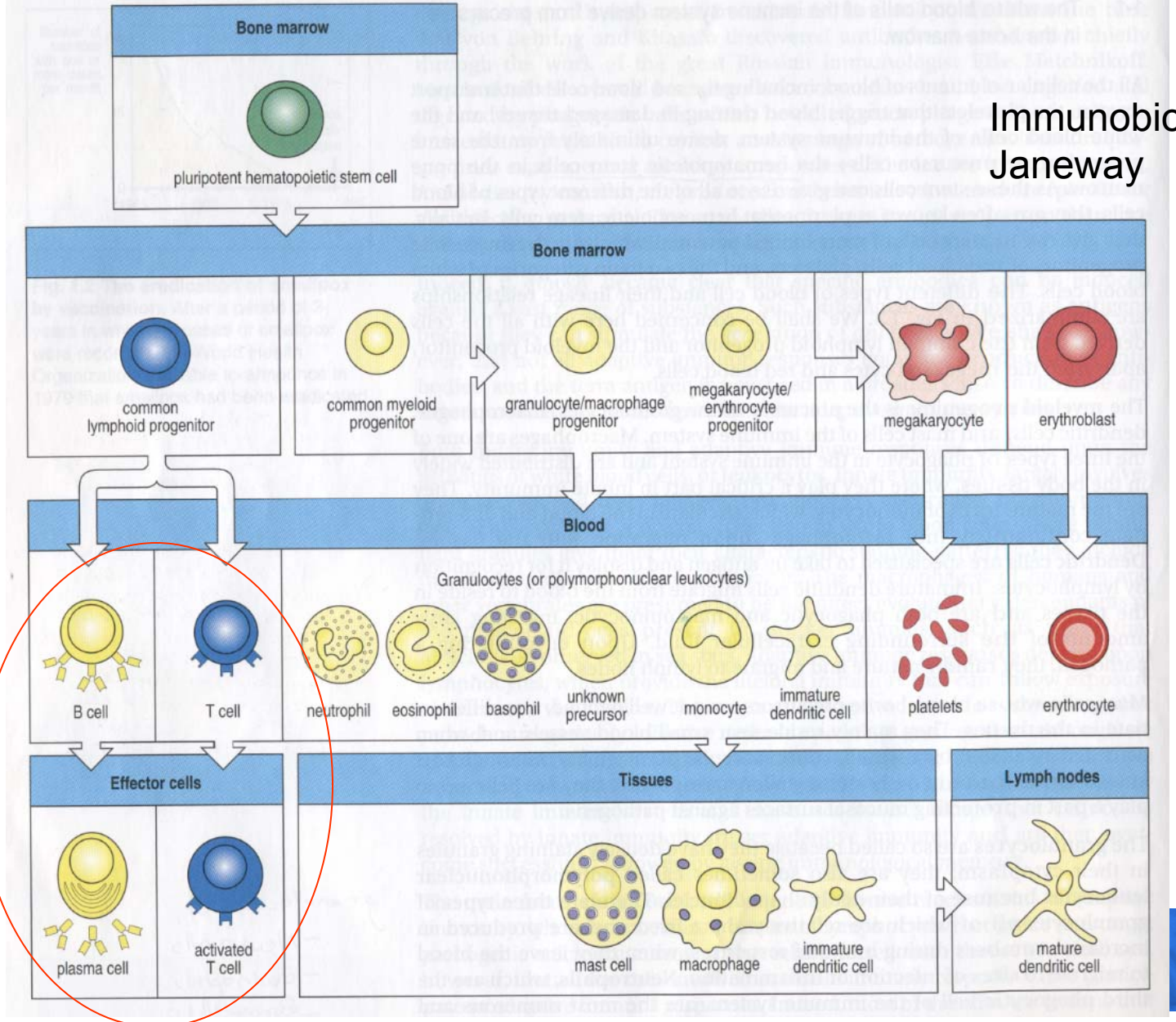
- **Globular Proteins: Definition, Properties**
- **Immune System: Antibodies**
- **Theory/Simulation: Coarse Grained Modeling of Antibodies**

Immune system

- Group in two categories: Adaptive (antibody production) and innate immune response
- Components: The cells of the immune system originate in the ***bone morrow***, where many of them also mature. They then migrate to guard the peripheral tissues, circulating in the blood and in a specialized system of vessels called the lymphatic system
- All the cellular elements of blood, including the red blood cells that transport oxygen, the platelets that rigger blood clotting in damaged tissues, and the white blood cells of the immune system derive ultimately from the same progenitor or procursor **cell-the hematopoietic stem cells** in the bone marrow.

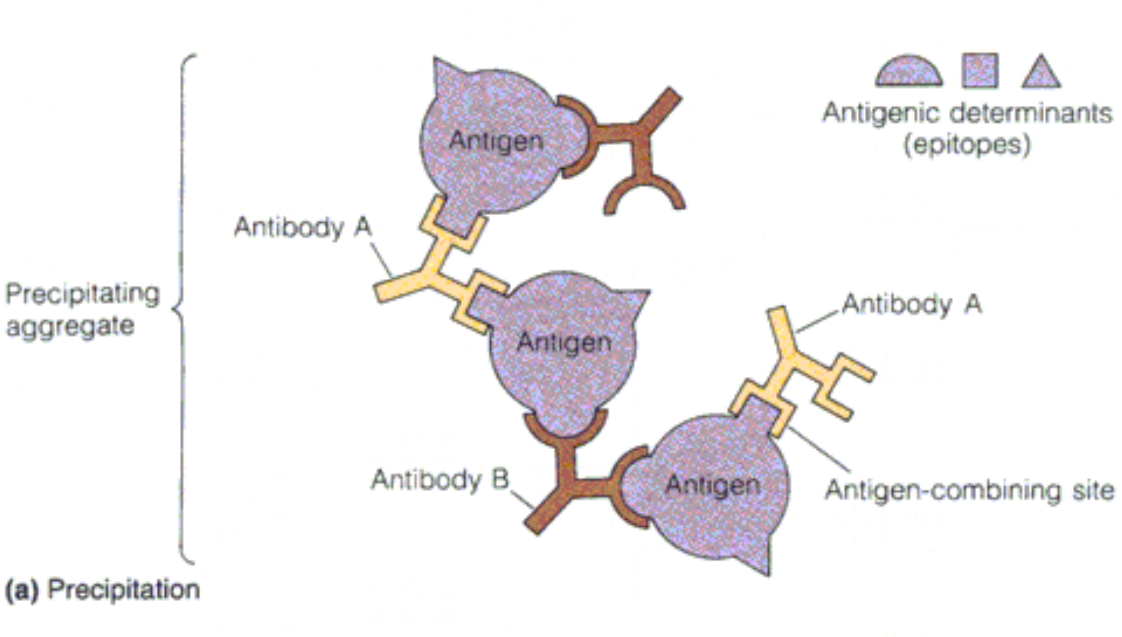


Stem Cell- Cell Differentiation

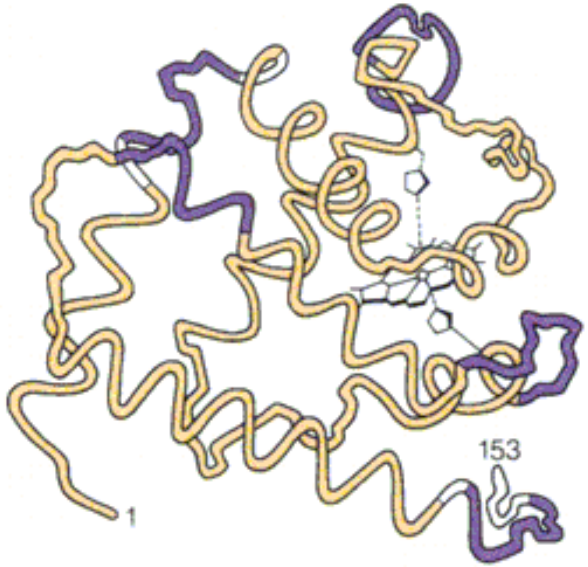


Immunobiology, Janeway

Antibodies and Antigenic determinants.

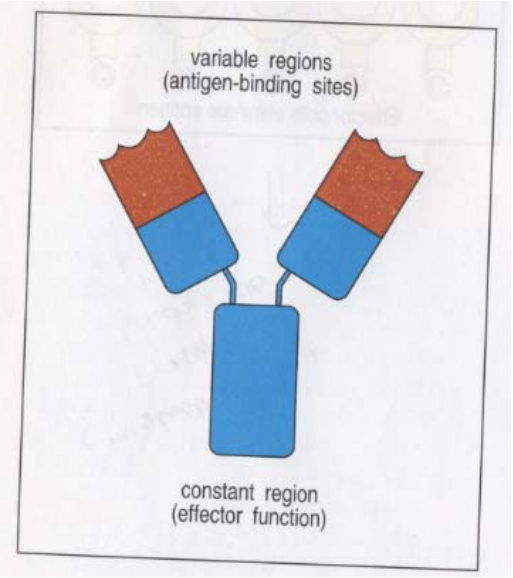


(a) Precipitation

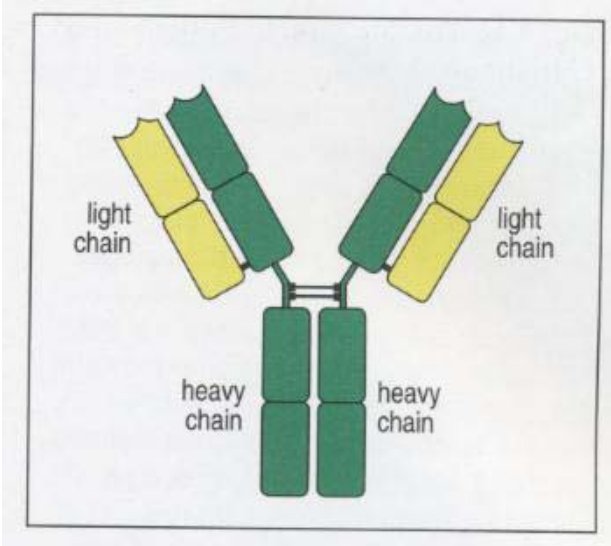


(b) Antigenic determinants, sperm whale myoglobin

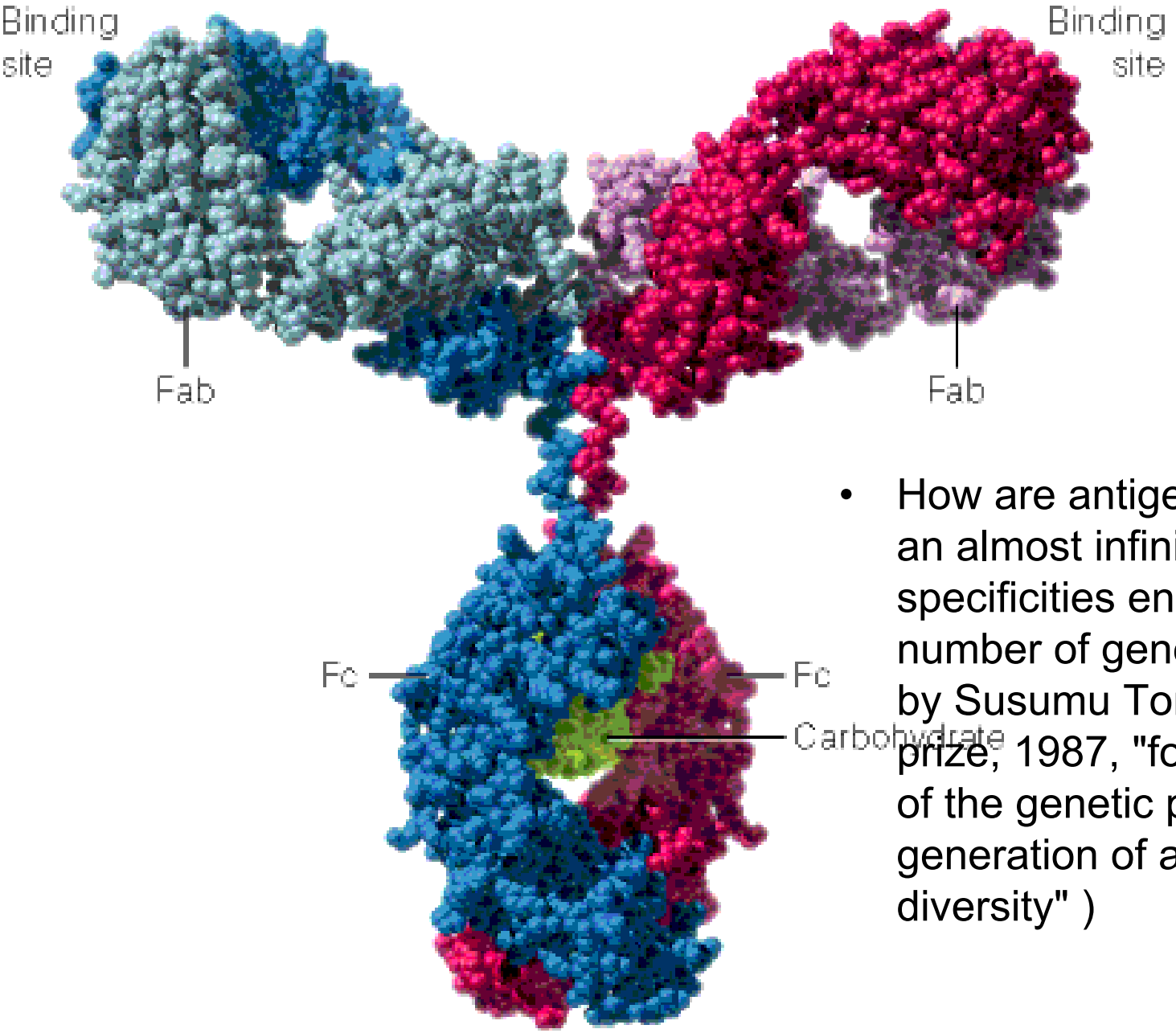
Antibodies



- Schematic structure of antibody molecule is shown. The two arms of the Y-shaped antibody molecule contain the variable region that form the two identical antigen-binding sites. The stem can take one of only a limited number of forms and is know as the ***constant region***. It is the region that engages the effector mechanisms that antibodies activate to eliminate pathogens.
- Antibodies are made up four protein chains (lower figure). There are two types of chain in an antibody molecule: a larger chain called the ***heavy chain*** (green) and a smaller one called the ***light chain*** (yellow). Each chain has both a variable and a constant region, and there are two identical light chains and two identical heavy chains in each antibody molecule.



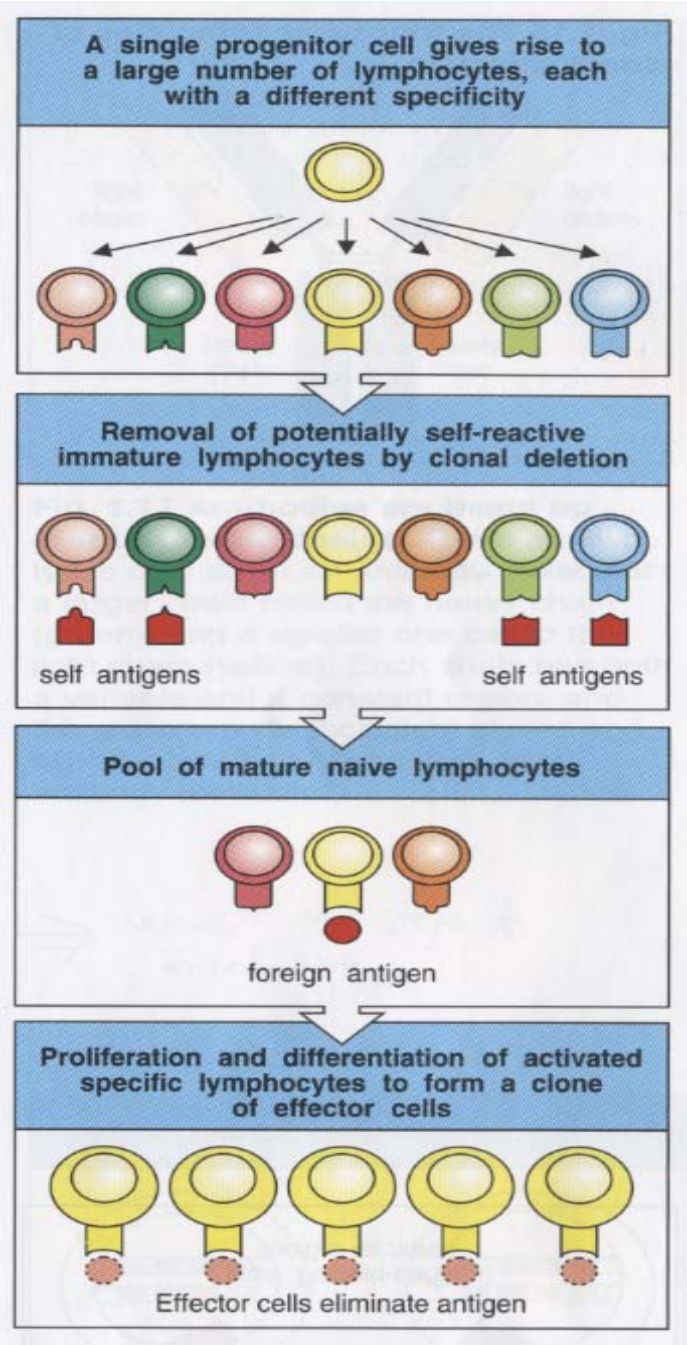
A model of the IgG



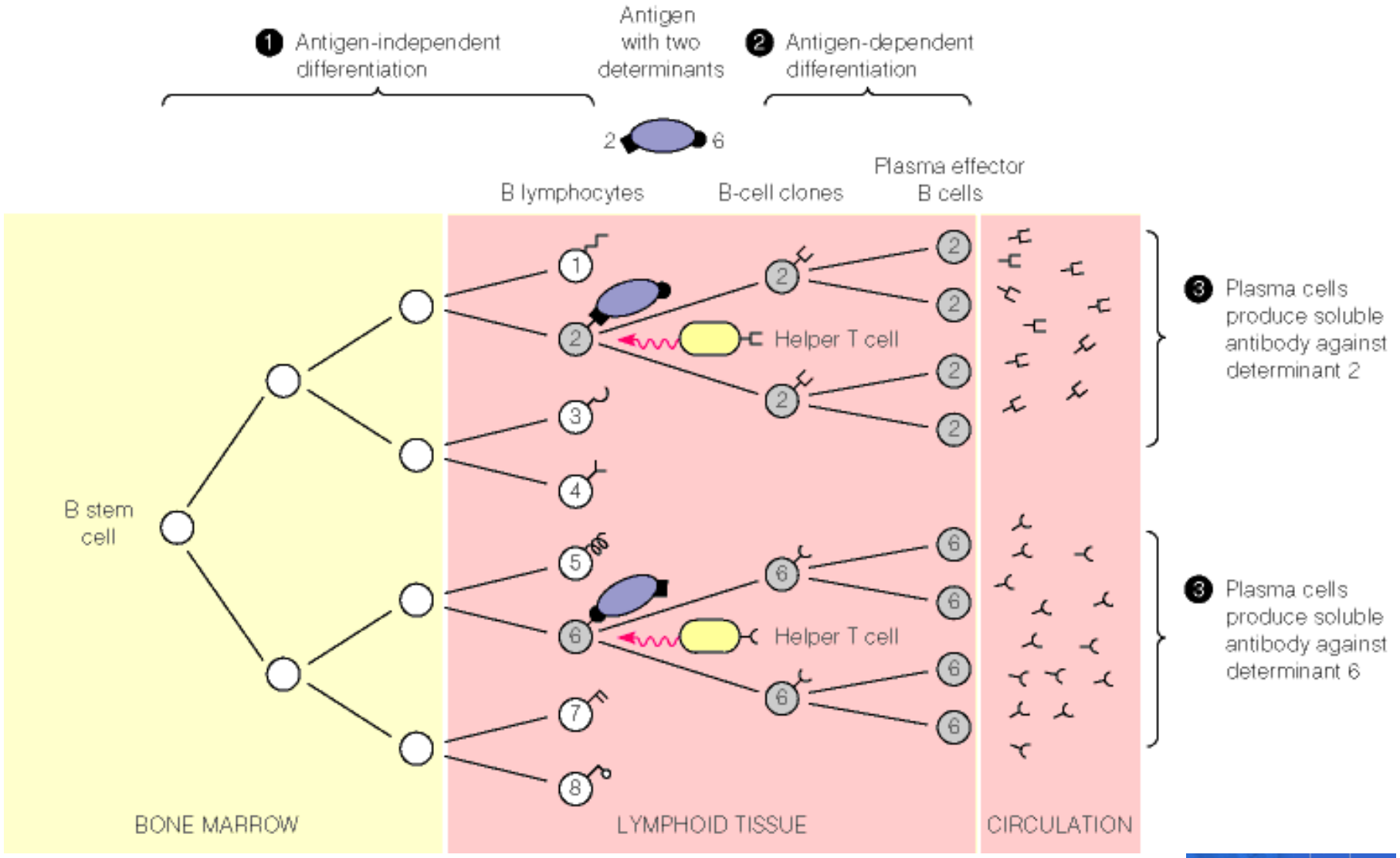
- How are antigen receptors with an almost infinite range of specificities encoded by a finite number of genes? Answered by Susumu Tonegawa (Nobel prize, 1987, "for his discovery of the genetic principle for generation of antibody diversity")

Clonal Selection

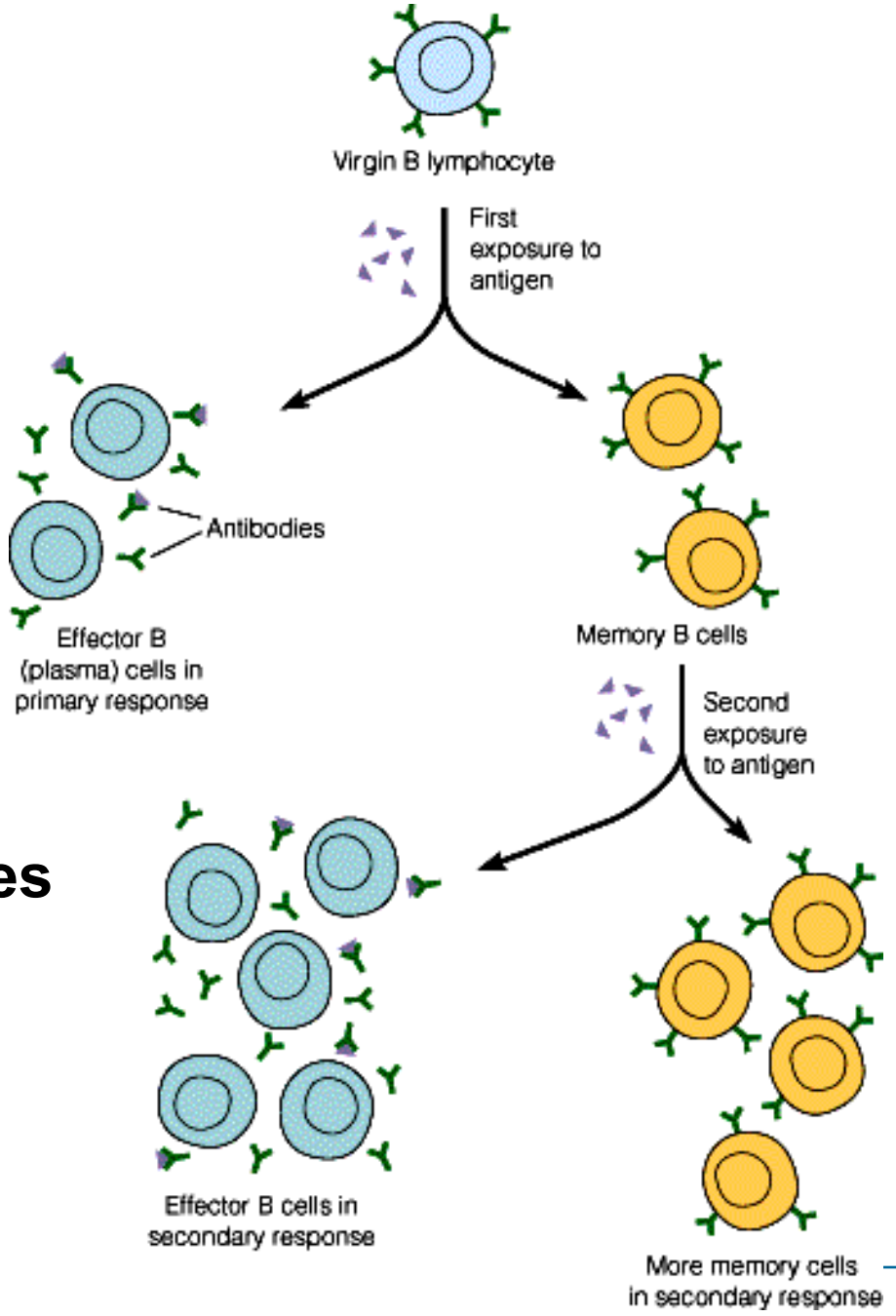
- Each lymphocyte (B-Cells and T-Cells) progenitor gives rise to many lymphocytes, each bearing a distinct antigen receptor.
- Lymphocytes with receptors that bind ubiquitous self antigens are eliminated before they become fully mature, ensuring tolerance to such self antigens.
- When antigen interacts with the receptor on a mature naive lymphocyte, that cell is activated and starts to divide. It gives rise to a clone of identical progeny, all of whose receptors bind the same antigen.
- Antigen specificity is thus maintained as the progeny proliferate and differentiate into effector cells. Once antigen has been eliminated by these effector cells, the immune response ceases.



The clonal selection theory of the immune response.



Maturation paths for stimulated B lymphocytes.



Primary Antibodies

Secondary Antibodies

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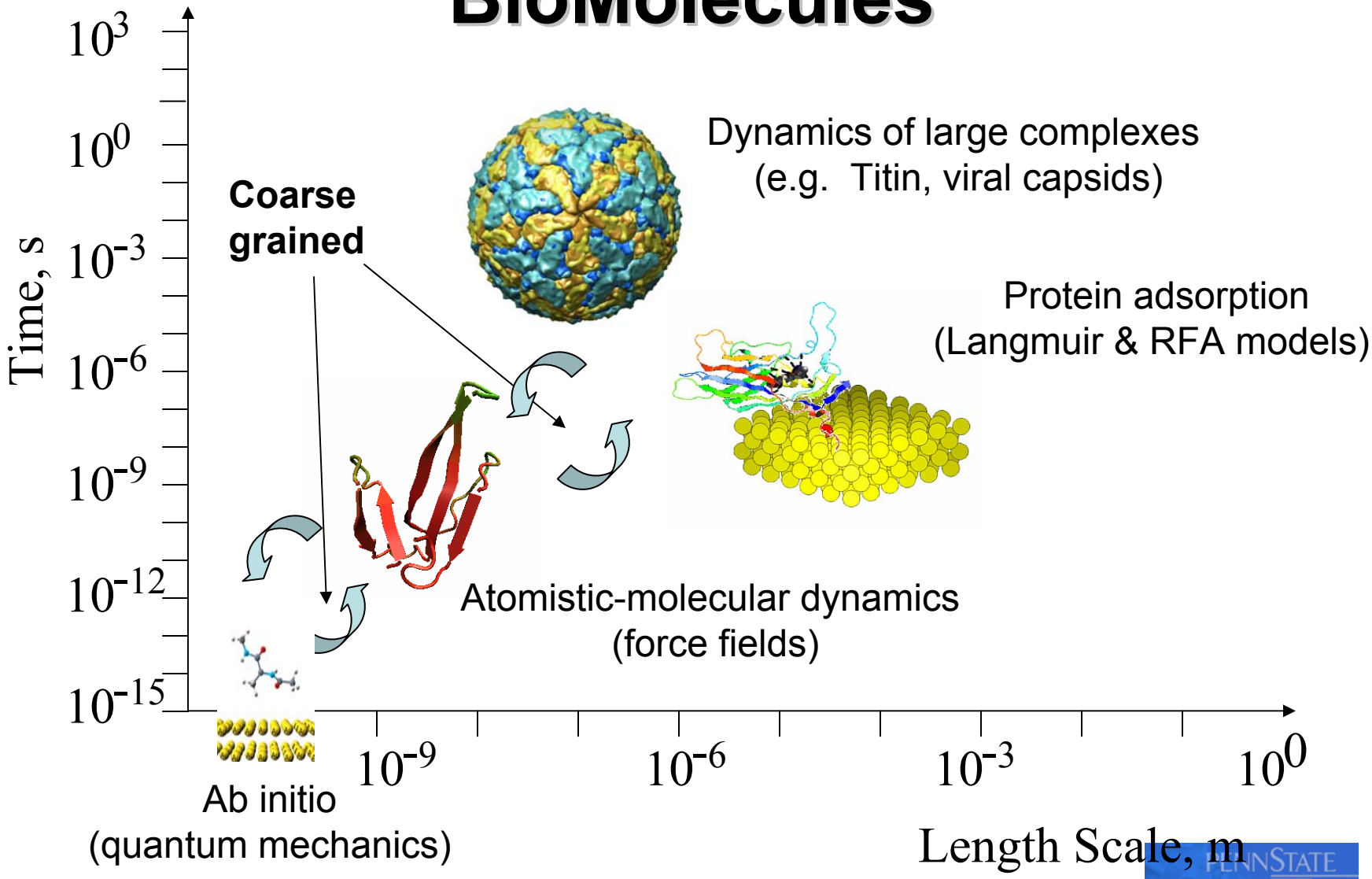
- **Immune System: Antibodies and Clonal Selection**
- **Theory/Simulation: Coarse Grained Modeling of Antibodies**

MODELING BIOLOGICAL SYSTEMS

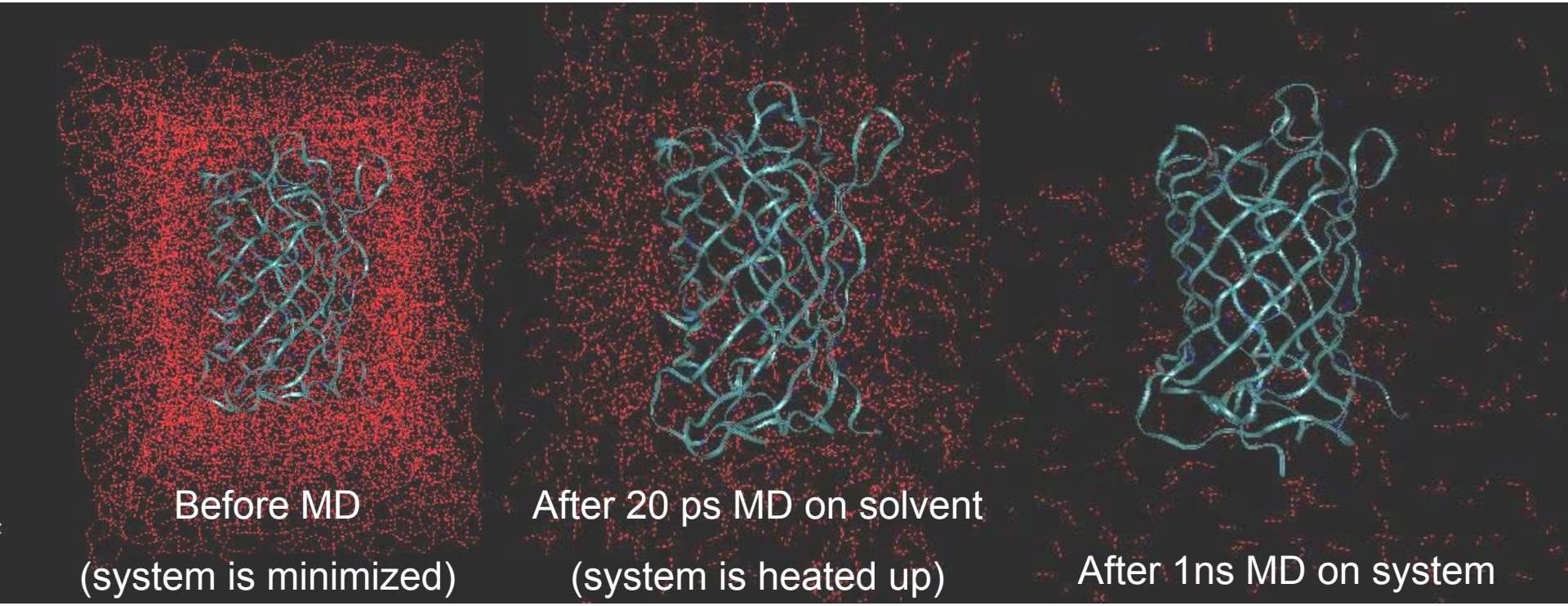
Problem/ Method	Typical Application	Software Examples	Resolution (Scale)	Spatial Realism	Stochastic Realis	Time Step	Time-scale	Serial/ Parallel	Comp Time Cost
Networks of reactions/ Sets of ODEs	Metabolic or signaling pathways	Ecell (192), Gepasi (147,148) VCell (166-168) XPPAUT [a]	N/A (cell)	N/A	none	ms	ms - hrs	serial	minimal
Excitation/ Compartmental Circuit	Nerve signaling	GENESIS (204) NEURON (88) NEOSIM [b]	µm –mm (cell-multicell)	low-to-medium	none	ms	ms - hrs	usually serial	usually low
Reaction kinetics/ Stochastics	Gene regulation/ transcription	BioSpice(138), MCell (178- 182) StochSim (174) XPPAUT [a]	N/A (cell)	N/A	high	ms	ms - hrs	serial	low
3-DReaction Diffusion/Finite Elements	Flow models, Calcium dynamics	FIDAP (54) Kaskade (32), VCell (166-168)	<µm (Cell)	medium-to-high	none	µs-ms	µs - sec	either	low-to-high
3-DReaction Diffusion/ Monte Carlo	Micro-physiological processes	MCell (178-182)	nm – mm (Subcell-cell)	high	high	ps – ms	µs - sec	either	low-to-high
Macro-molecular machinery/GNM	Collective dynamics	GNM (14)	Å -100 nm (complexes)	high	none	N/A	ps - 10 ns	*N/A analytic*	minimal
Diffusion in potential field/Poisson-Nernst-Planck	Electrostatic interactions, ion channels	(124, 43)	Å -nm (membrane proteins)	High (implicit solvent)	none	N/A	10 ns	parallel	low-to-medium
Macromolecular motions/Brownian Dynamics (BD)	Conformation dynamics (in flow fields)	CHARMM (8) GROMOS (192)	Å -nm (macro-molecules)	High (implicit solvent)	high	5-10 fs	1-10 ns	parallel	medium-to-high
Molecular Dynamics (MD)	Conformation dynamics & free energies	AMBER, CHARMM (9), GROMOS (192)	Å (macro-molecules)	Exact (explicit solvent)	exact	1-2 fs	~ ns	parallel	very high
Molecular structure/*Ab initio* simulations	Solution of the of Schrodinger equation	Gaussian98 (74)	< Å (electrons-atoms)	exact	exact	-	N/A	parallel	highest

Network Models

Multiscale Simulations of BioMolecules



Molecular Dynamics Simulation $F_i = m_i a_i$



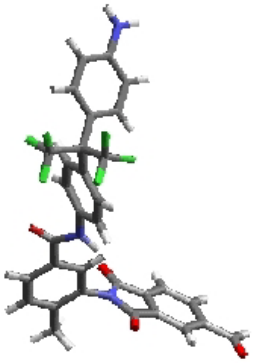
LIMITATIONS OF MD

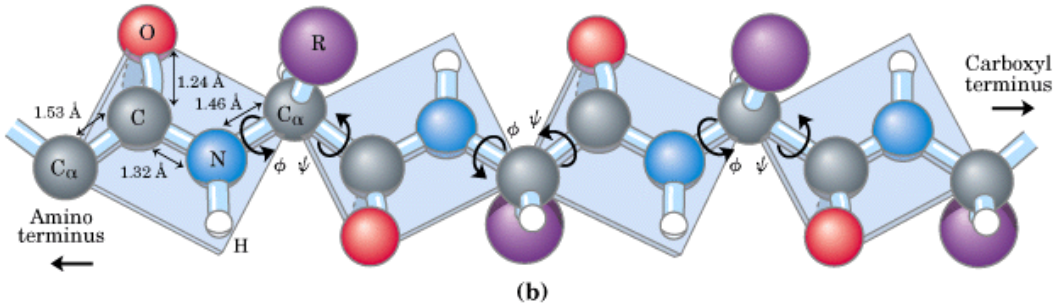
- Full atomic representation → noise
- Empirical force fields → limited by the accuracy of the potentials
- Time steps constrained by the fastest motion (bond stretching of the order of femtoseconds)
- Inefficient sampling of the complete space of conformations
- High computational cost: Limited to small proteins (100s of residues) and short times (subnanoseconds)

Coarse Grained Modeling

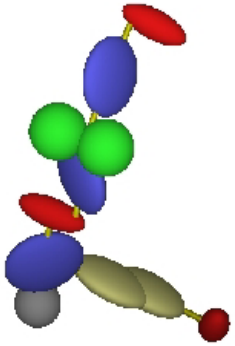
Scale ~ 0.1nm

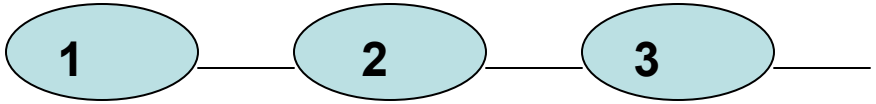
Atomistic



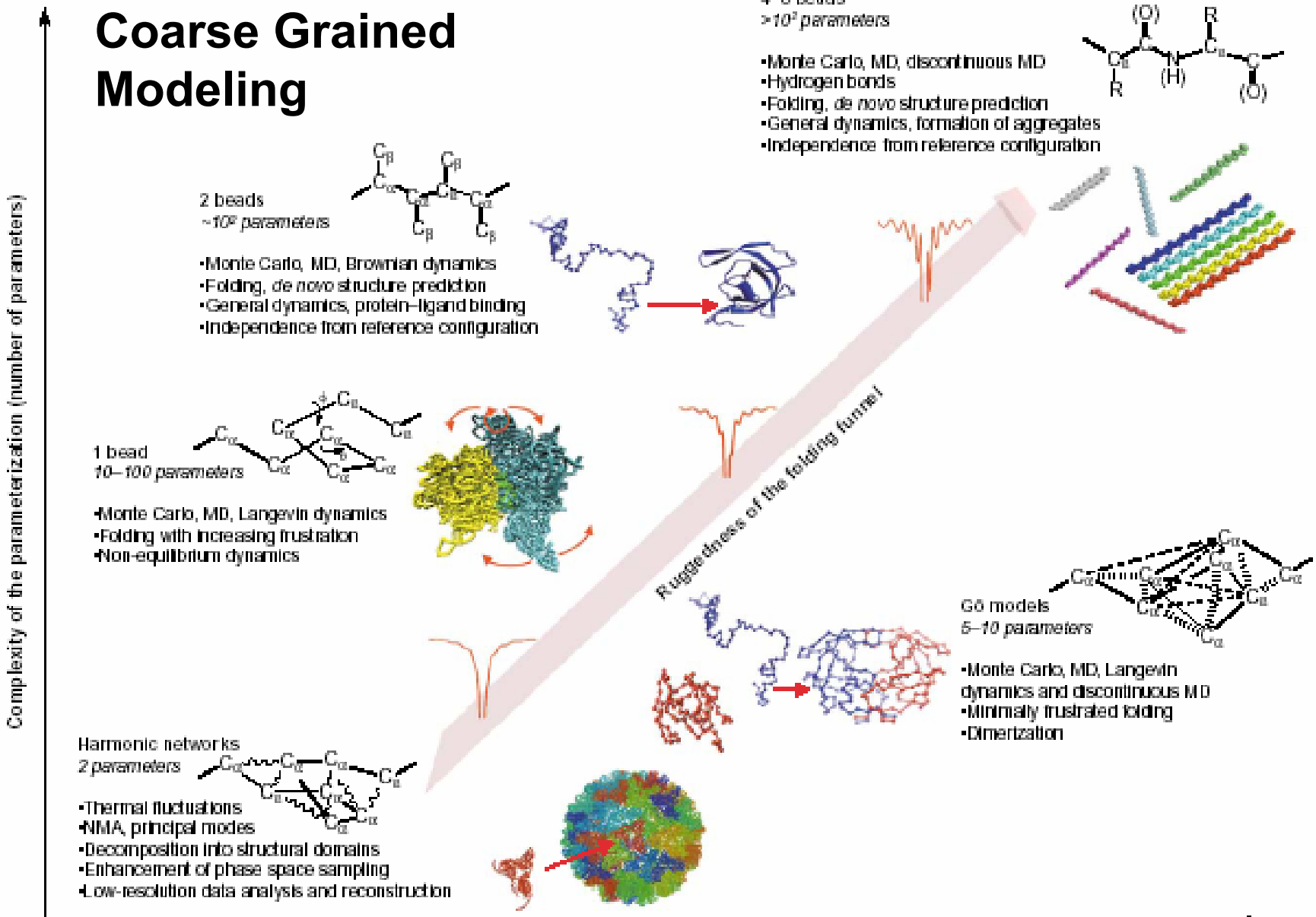


Coarse grained

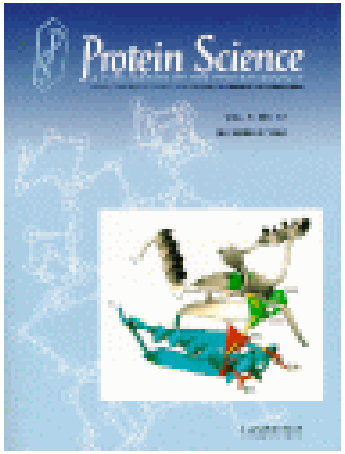




Coarse Grained Modeling



 Tozzini, Current Opinion in Structural Biology, 15, 144 (2005)



GAUSSIAN NETWORK MODEL (GNM)

Demirel, M.C. (with others)
Protein Science,
December 1998

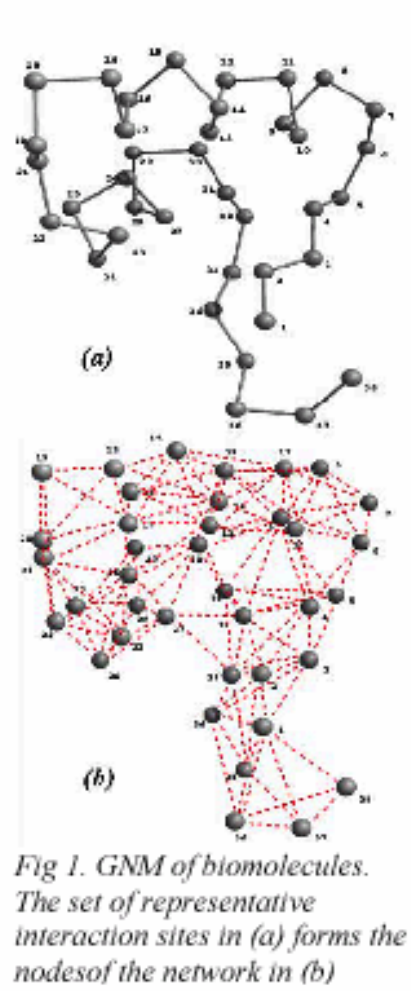


Fig 1. GNM of biomolecules. The set of representative interaction sites in (a) forms the nodesof the network in (b)

$$\text{Energy}=\gamma/2[\Delta R \Gamma \Delta R^T]$$

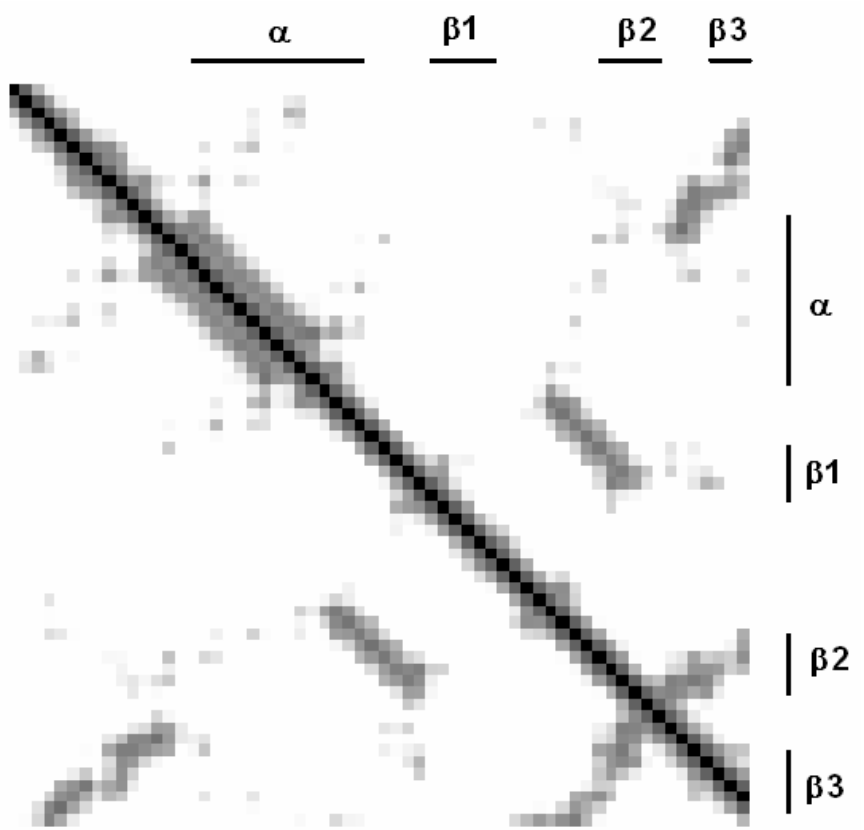
Using statistical mechanics (Gaussian integral)

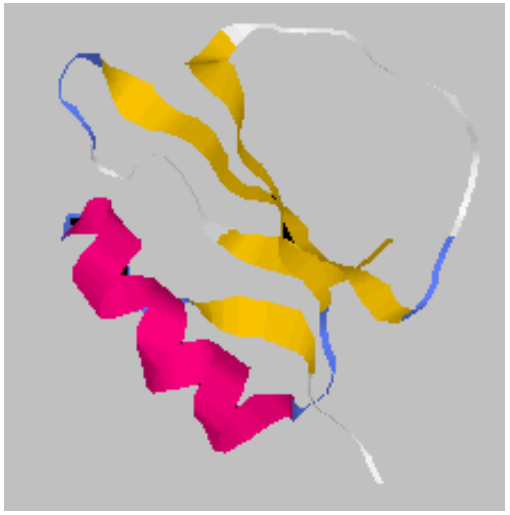
$$\text{Fluctuations}=\Gamma^{-1}=(\gamma/3k_BT)*<\Delta R \Delta R^T>$$

Γ: Connectivity matrix, ΔR: fluctuation of each residue

CONTACT MAP (CONNECTIVITY MATRIX)

$\Gamma =$





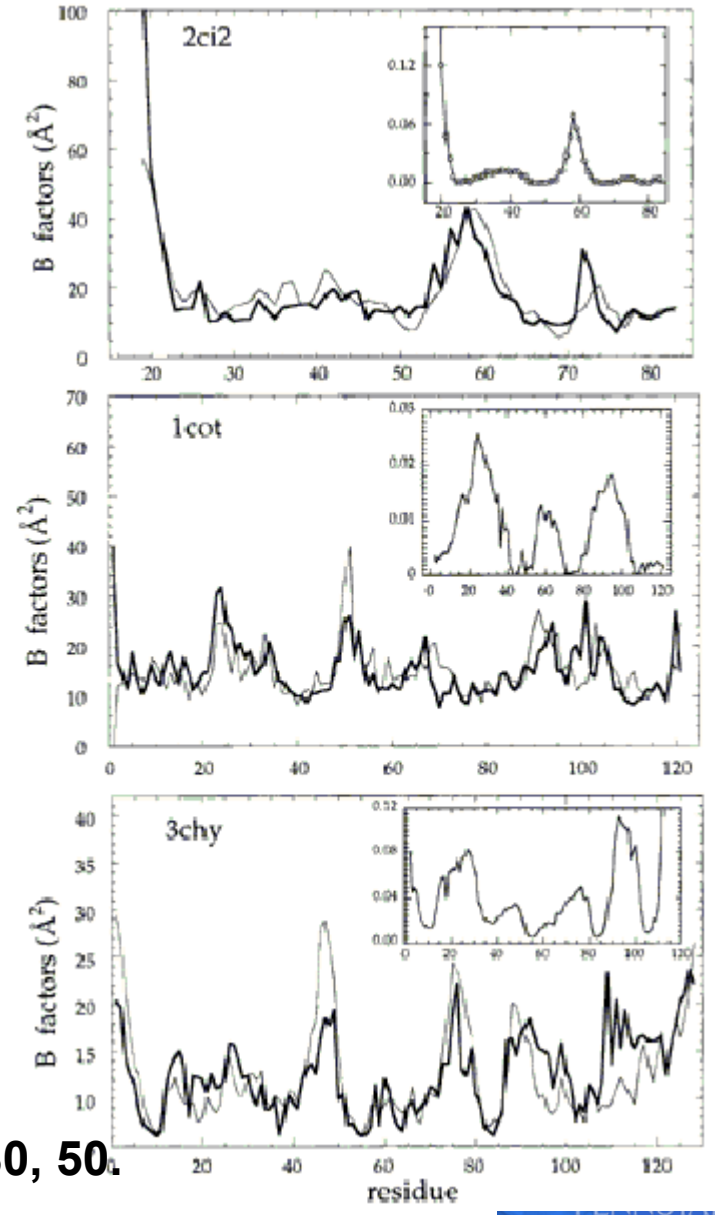
Chymotrypsin inhibitor-2 64 residues

$$\Gamma = \begin{cases} -\delta(r_c - r_{ij}) & i \neq j \\ -\sum \Gamma_{ij} & i = j \end{cases}$$

Demirel et al. (1998), Protein Science, v7, 2522
Bahar et al. (1998), Physical Review Letters, v80, 2733

COMPARISON

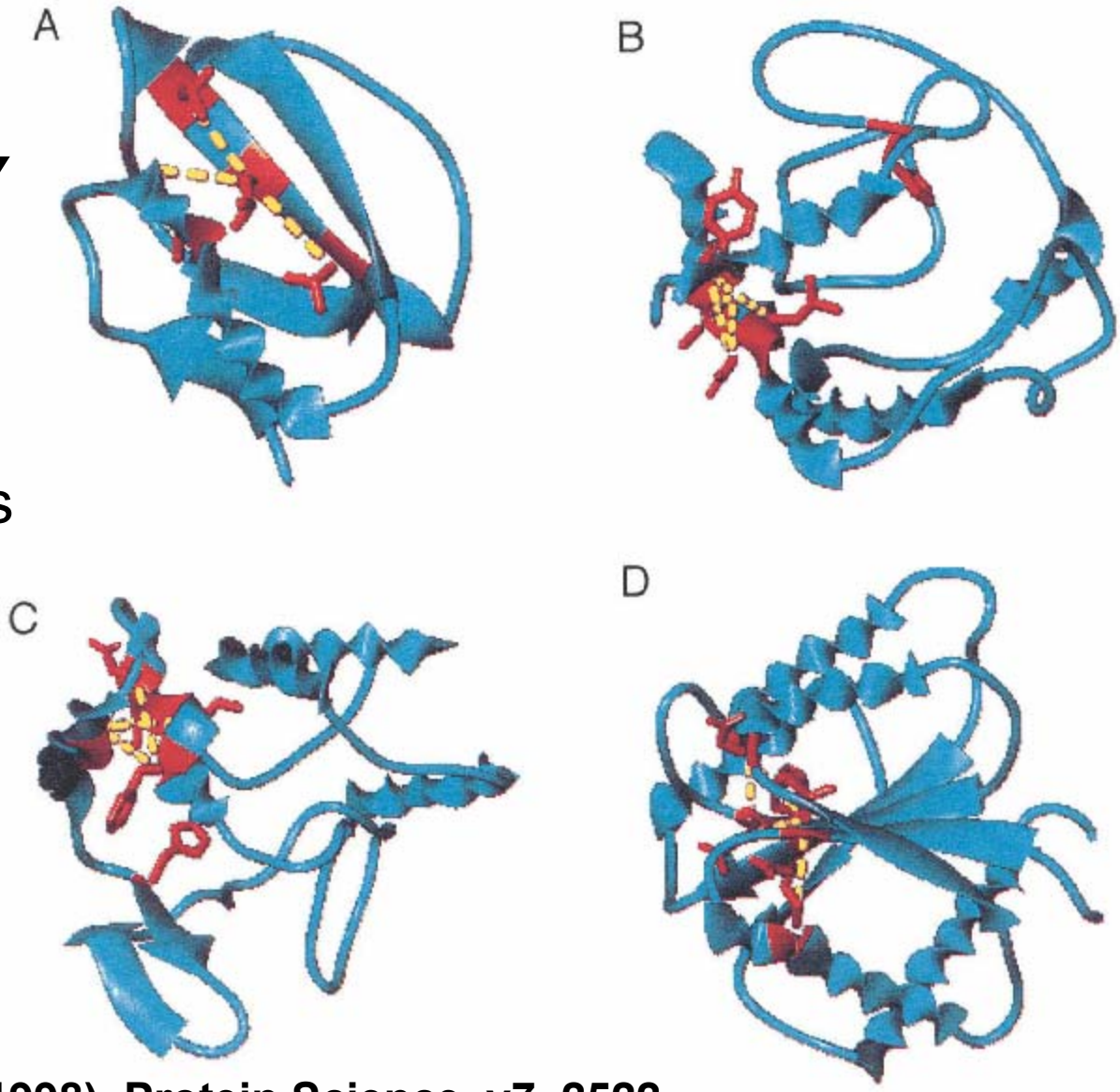
… of theoretical (thick curve) and experimental (thin curve) B factors for Chymotrypsin inhibitor 2 (2ci2), C2 protein (1cot), and CHE-Y protein (3chy)



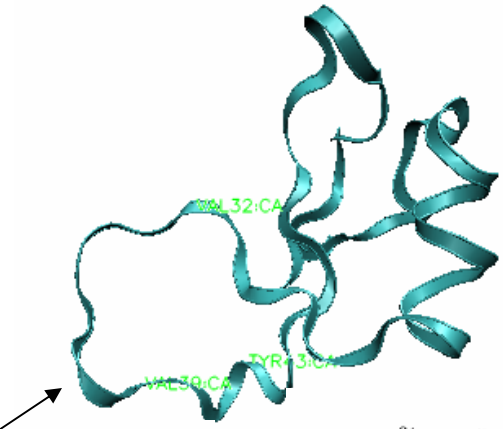
Atilgan, et al., 2001, Biophysical Journal, v80, 50.

STABILITY

Fast modes determine stability

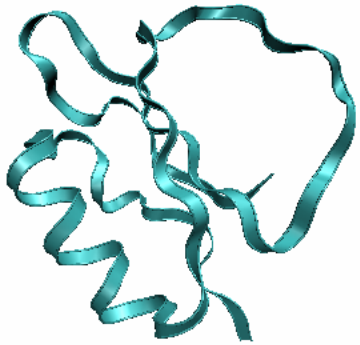


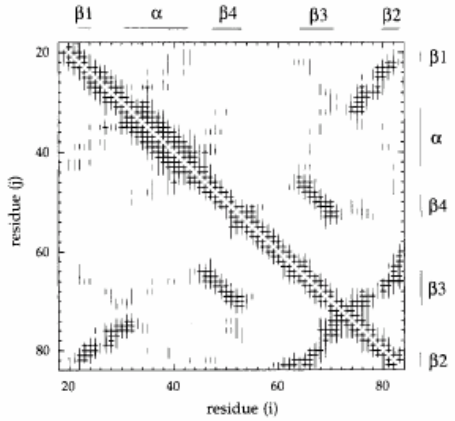
Demirel et al. (1998), Protein Science, v7, 2522

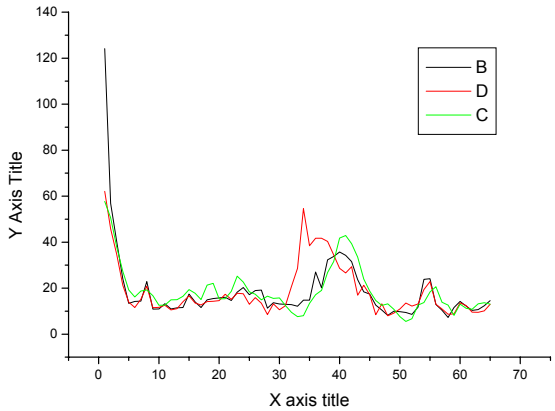


Decoy

unfolded



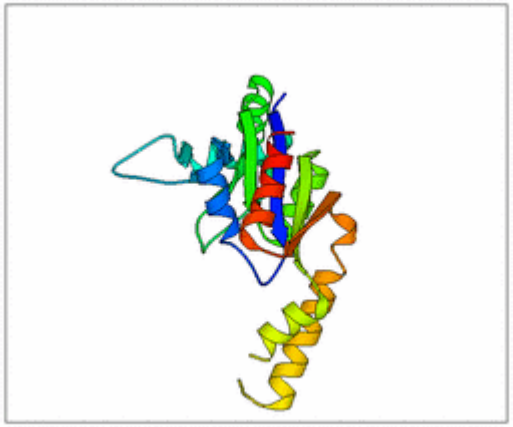




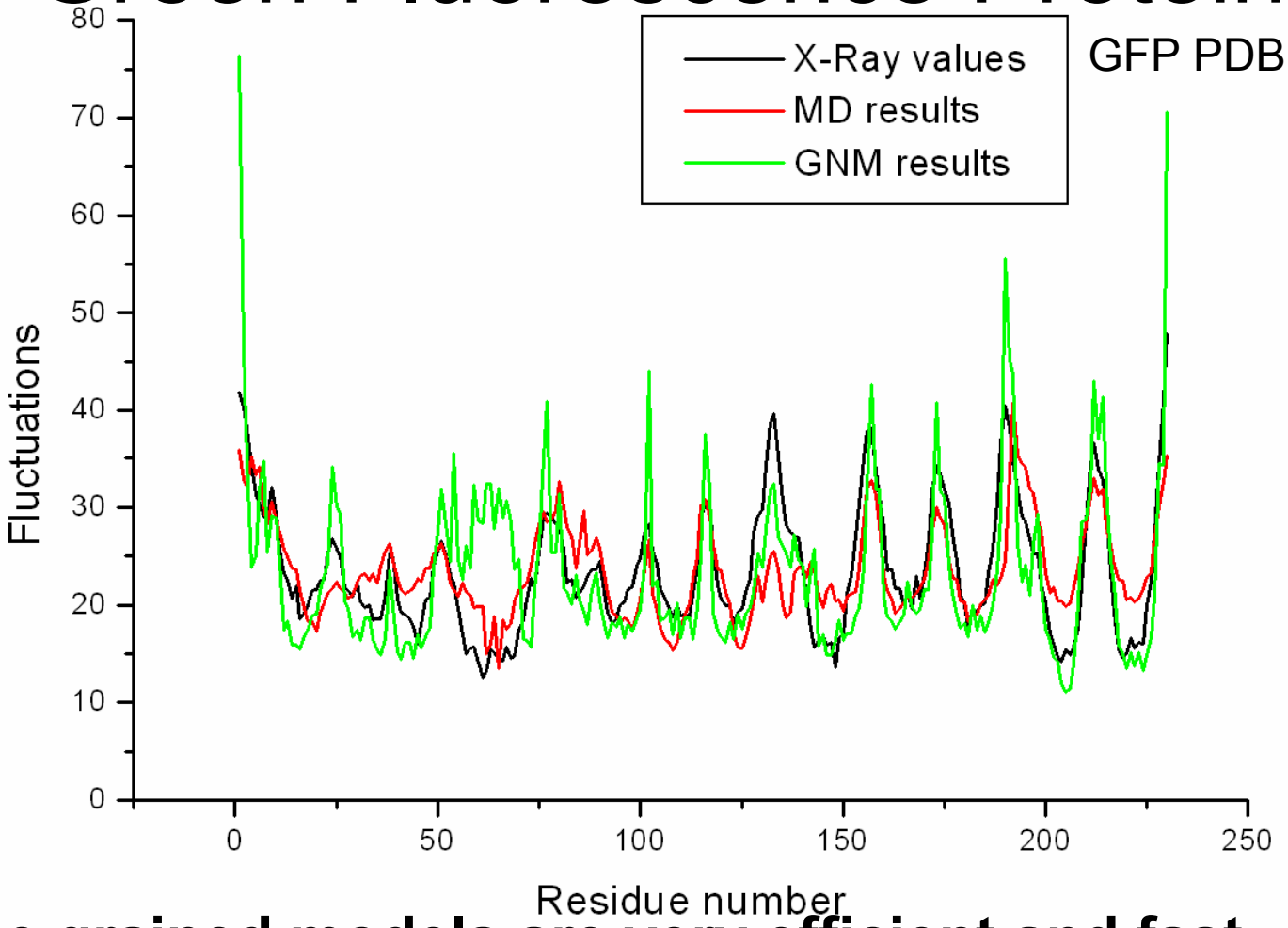
Structure

Contact Map

Fluctuation



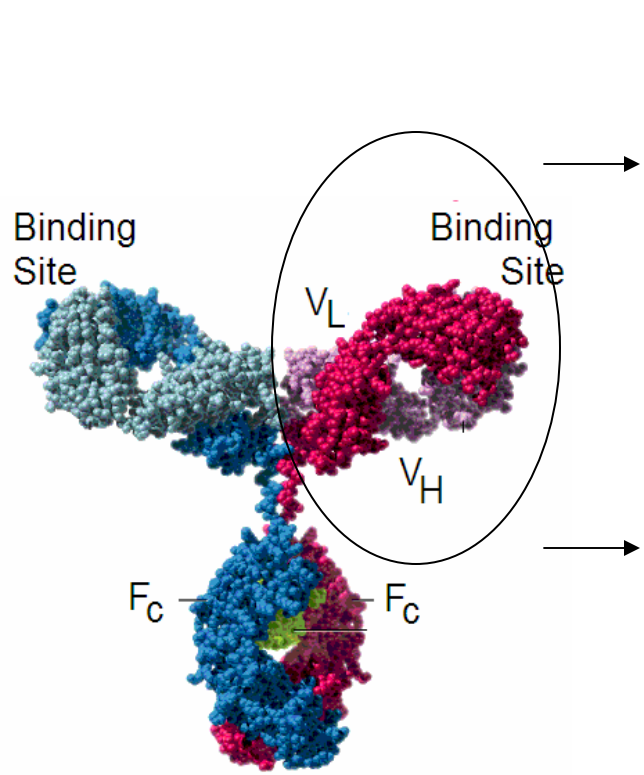
Comparison of MD and GNM: Green Fluorescence Protein

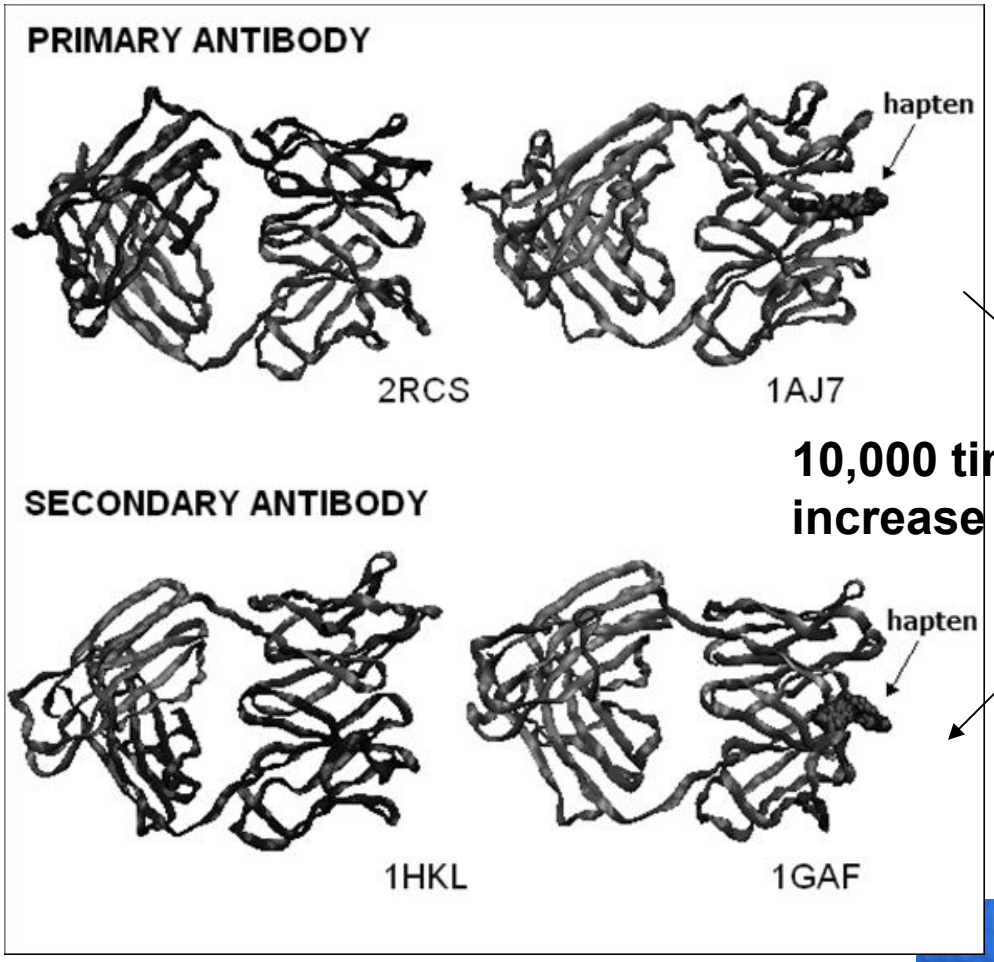


GFP PDB code: 1EMB

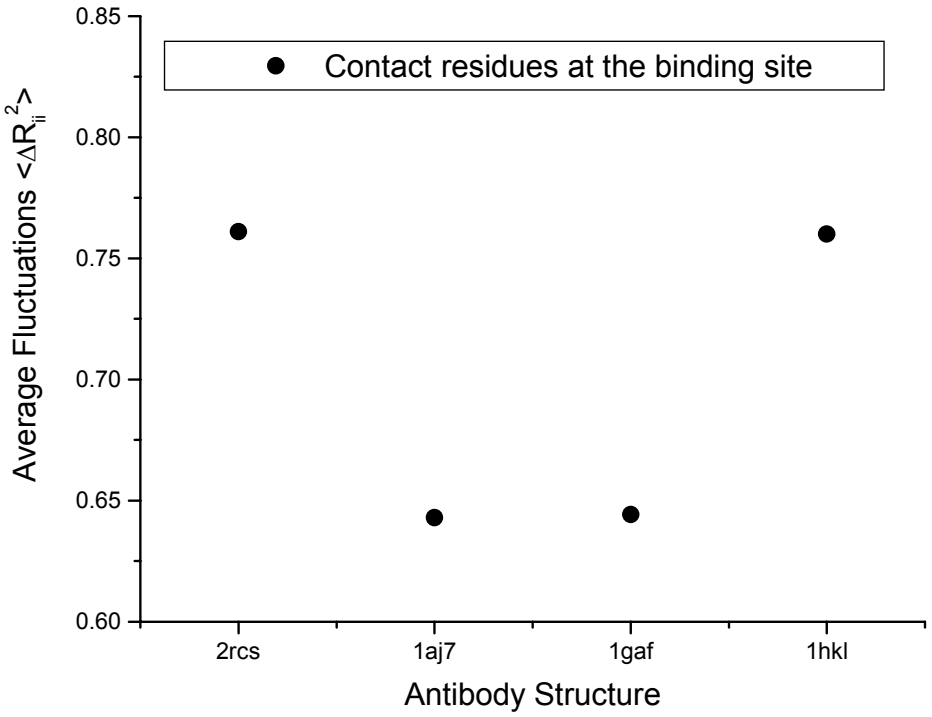
Coarse grained models are very efficient and fast. GNM is an analytical method (1s computational time v.s. 1 day of simulation with MD).

Antibody Maturations: 4 structures crystallized by Shultz Group (*Patten et al., Science 271, 1086,1996*).





Flexibilities for primary and secondary structures



- **In both primary and secondary antibodies, the flexibility of the binding site decreases upon binding to the hapten, as a result of protein-ligand forces.**
- **However, the flexibility of the binding site of the unligated state of the primary antibody is no more than that of the secondary antibody.**

Demirel & Lesk, Physical Review Letters, accepted, 2005

$$\langle \Delta R \rangle_1 = \langle R \rangle_1 - \langle R \rangle_0 \qquad (1)$$

$$\langle (.) \rangle_1 = \int (.) Z_1 d\Delta R \Big/ \int Z_1 d\Delta R \qquad (2)$$

$$Z_1 = c_1 \exp\left(-\tfrac{1}{2}\gamma \Delta R^T \Gamma \Delta R - \Delta R \Delta f_1\right) \qquad (3)$$

Force term

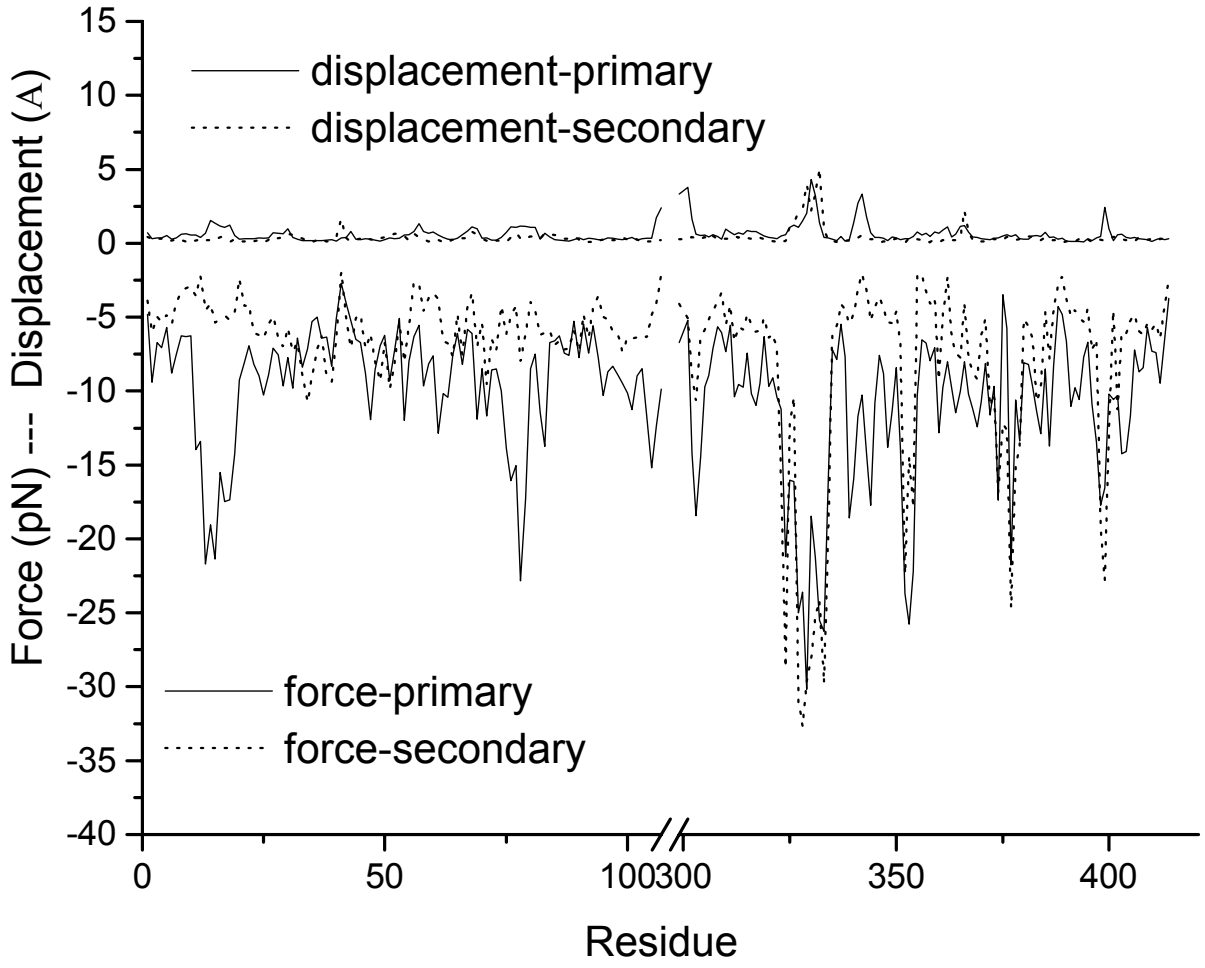
Gaussian term

$$\frac{\partial \ln(\theta)}{\partial \ln(\Delta f_1)} = \langle \Delta R \rangle_1 \text{ where } \quad \theta = \int Z_1 d\Delta R \qquad (4)$$

$$\frac{\partial \ln(\theta)}{\partial \ln(\Delta f)} = \gamma \Gamma^{-1} \Delta f_1 \qquad (5)$$

(5) and (6), we find $\Delta f_1 = \gamma \Gamma \langle \Delta R \rangle_1$

Molecular Forces and Displacements



- **Upon encounter with hapten, the primary antibody is exposed to larger forces compared to the secondary antibody.**
- **In contrast, binding of the hapten to the secondary antibody is more like a lock-and-key mechanism; the interaction with hapten reduces flexibility but produces substantially less distortion of the structure.**

Demirel & Lesk, Physical Review Letters, accepted, 2005

Summary

- **Elastic network models are successful in describing equilibrium protein motions.**
  - X-ray and NMR relaxation data are in good agreement with elastic network results
  - Large structural complexes (e.g. titin, viral capsids) can be studied with coarse grained models. (not possible with any other molecular method)
  - We have investigated by calculations based on an elastic network model the relative roles of changes in structure and flexibility in changes in affinity and specificity during antibody maturation.