Protein Structure and Function

I619: Structural Bioinformatics

January 16, 2008

Protein Folding Problem

- How do proteins fold into a specific 3-D structure?
- How does the primary structure of a protein determine its secondary and tertiary structure?

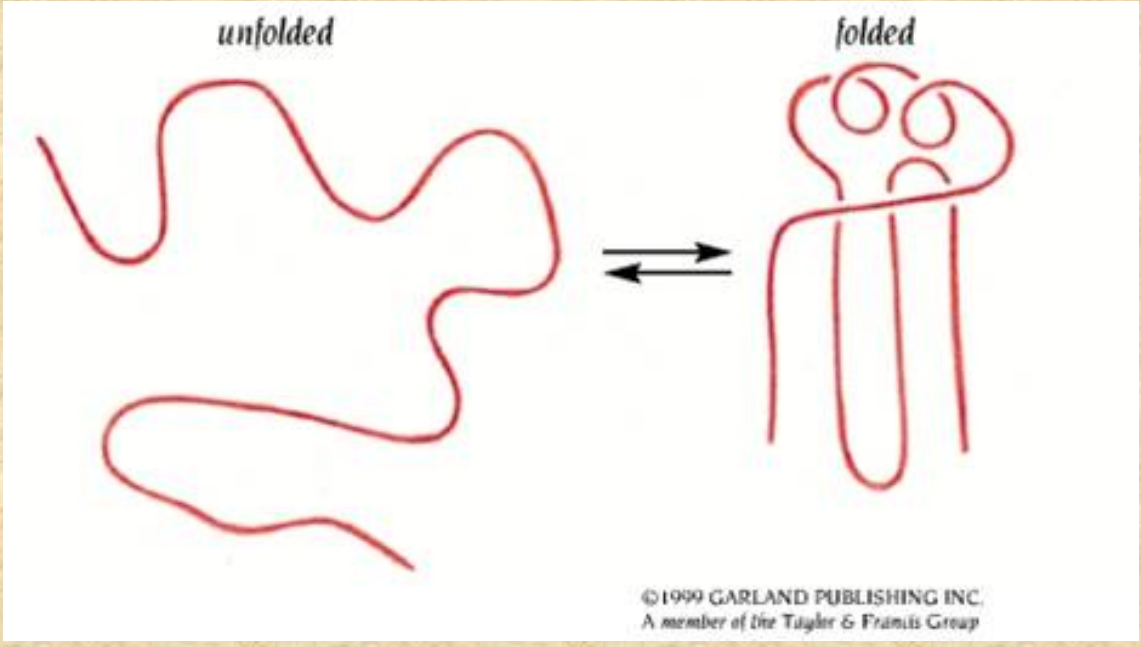
- there are two conditions a protein needs to meet
  - there must be a single, stable, folded conformation (thermodynamic condition)
  - a protein must fold on an appropriate time scale (kinetic condition)
- thus, only a *small amount of conformational space is explored*
- also, there must exist a *specific folding pathway*
- the paradox how proteins quickly fold into specific 3-D conformations is called a protein folding problem

Folding and Flexibility

- the process by which a polypeptide chain acquires its correct 3D structure to achieve biologically active native state is called protein folding
- many protein chains spontaneously fold into the native state, others require the assistance of enzymes or other proteins called chaperones

- a protein in its native state is not static
- secondary structural elements of the domains as well as the entire domains continually undergo small movements in space
- either fluctuations of individual atoms or collective motions of groups of atoms
- functional activities of many proteins depend upon large conformational changes triggered by ligand binding

Globular Proteins are only Marginally Stable



- slight changes in pH or temperature can convert a solution of biologically active proteins in their **native state** to a biologically inactive **denatured state**

Let's digress a bit...

- the internal energy in a system (E)
  - sum of potential and kinetic energies of each particle in that system
  - proportional to the temperature of the system

- the laws of thermodynamics
  1. conservation of energy
  2. in an isolated system, the entropy tends to increase
  3. entropy approaches 0 when temperature approaches 0 K

- Question: which reactions are spontaneous?
- Hidden question: what drives protein folding?

Gibbs Free Energy (G)

- enthalpy

$$H = E + PV, \quad \text{but} \quad \Delta H = \overbrace{q + w}^{\Delta E} + \Delta(PV)$$

q – heat absorbed (+) or given off (-) by the system
w – work on (+) or by (-) the system (related to its surrounding)

- entropy

$$S = k \cdot \log W$$

k – constant
W – the number of equivalent ways of describing system states

- Gibbs free energy

$$G = H - T \cdot S$$

Change in Gibbs Free Energy (ΔG)

- change in enthalpy minus change in entropy term

  $\Delta G = \Delta H - \Delta(T \cdot S)$

- at constant temperature T

  $\Delta G = \Delta H - T \cdot \Delta S$

- Some reactions are spontaneous due to losing heat, some due to gaining entropy

Favorable, spontaneous reaction: $\Delta H < 0$ and $\Delta S > 0$

Unfavorable, not a spontaneous reaction: $\Delta H > 0$ and $\Delta S < 0$

Favorable, spontaneous reaction: $\Delta G < 0$

Folded vs. Denatured State

- there are two major contributors to the energy difference between the folded and the denatured state
  - enthalpy
  - entropy
- Enthalpy
  - derives from the energy of the non-covalent interactions within the polypeptide chain (H-bonds, ionic bonds, hydrophobic interactions)
  - the covalent bonds within and between the amino acid residues are the same in the native and denatured states, with the exceptions of disulphide bonds
- Entropy
  - derives from the second law of thermodynamics which states that energy is required to create order
  - in the absence of other forces, it would be energetically favorable for a protein to remain in the disordered denatured state

Proteins are Marginally Stable

- the total energy difference between the native and the denatured state is 5-15kcal/mol, which is called the free energy difference (ΔG)
- free energy difference is small, but the problem is that this is the difference between two very large numbers (enthalpy difference and entropy difference)
  - this is a severe problem in predicting possible native state using molecular dynamics
- the marginal stability of the native state over the denatured state is biologically important
- living cells need globular proteins in correct quantities at appropriate times
- it is important to degrade them quickly as it is important to synthesize them quickly

Kinetic Factors

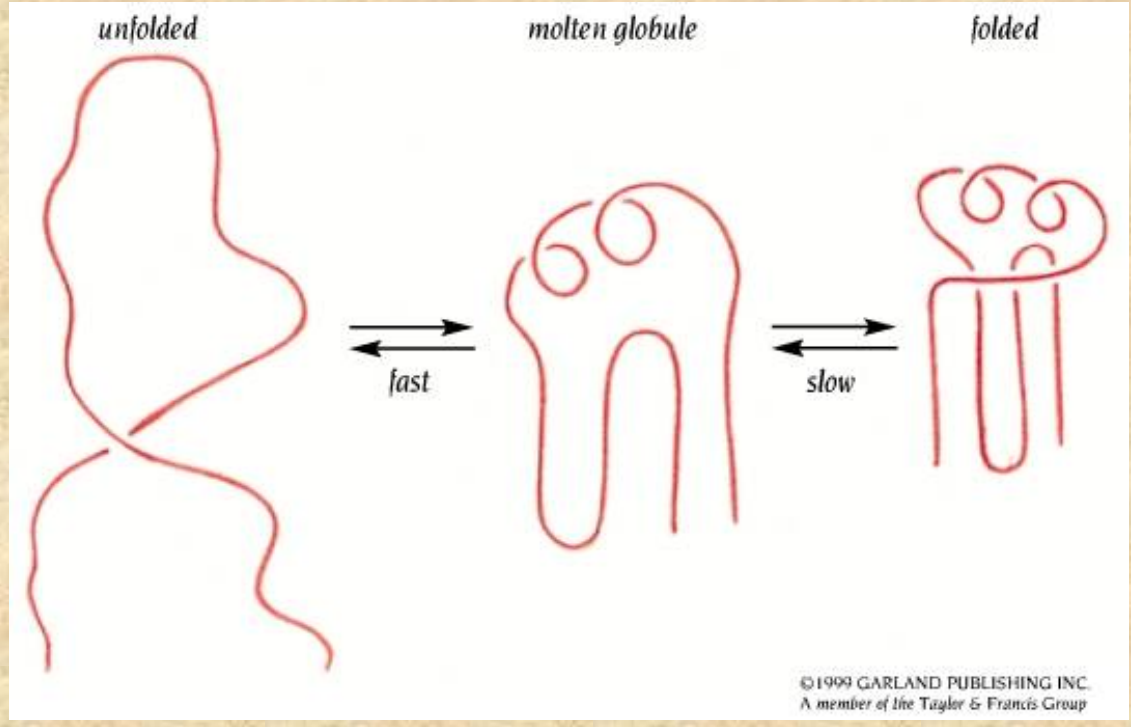
- high resolution x-ray structures of several hundred proteins have shown that in each case the specific sequence of a polypeptide chain appears to yield only a single, compact, biologically active fold in the native state
- NMR experiments show that the same fold prevails in solution too

- proteins cannot search all possible conformations (Levinthal's "paradox")
- thus, to occur on the short time scale, the folding process must be directed in some way through a kinetic pathway of unstable intermediates to escape sampling a large number of irrelevant conformations

Kinetic Factors

- folding mechanism is difficult to examine experimentally since possible intermediates have short lifetime
- if kinetic factors are important for the folding process it is possible that the observed folded conformation is not the one with the lowest free energy, but rather the most stable of those conformations that are kinetically accessible
  - protein might be kinetically trapped in a local low energy state with high energy barrier that prevents it from reaching the global energy minimum
  - global energy minimum state may have a different fold
  - how can this affect structure prediction based on molecular simulations?
- how a living cell can prevent the folding pathway from becoming blocked at an intermediate stage? Obstacles are:
  - aggregation of the intermediates through exposed hydrophobic groups
  - formation of incorrect disulphide bonds
  - isomerization of prolines

Folding Intermediates



- molten globule state
  - first observable state in the folding pathway
  - collapse of the flexible disordered state into partially organized folded state

Molten Globule and Folded State

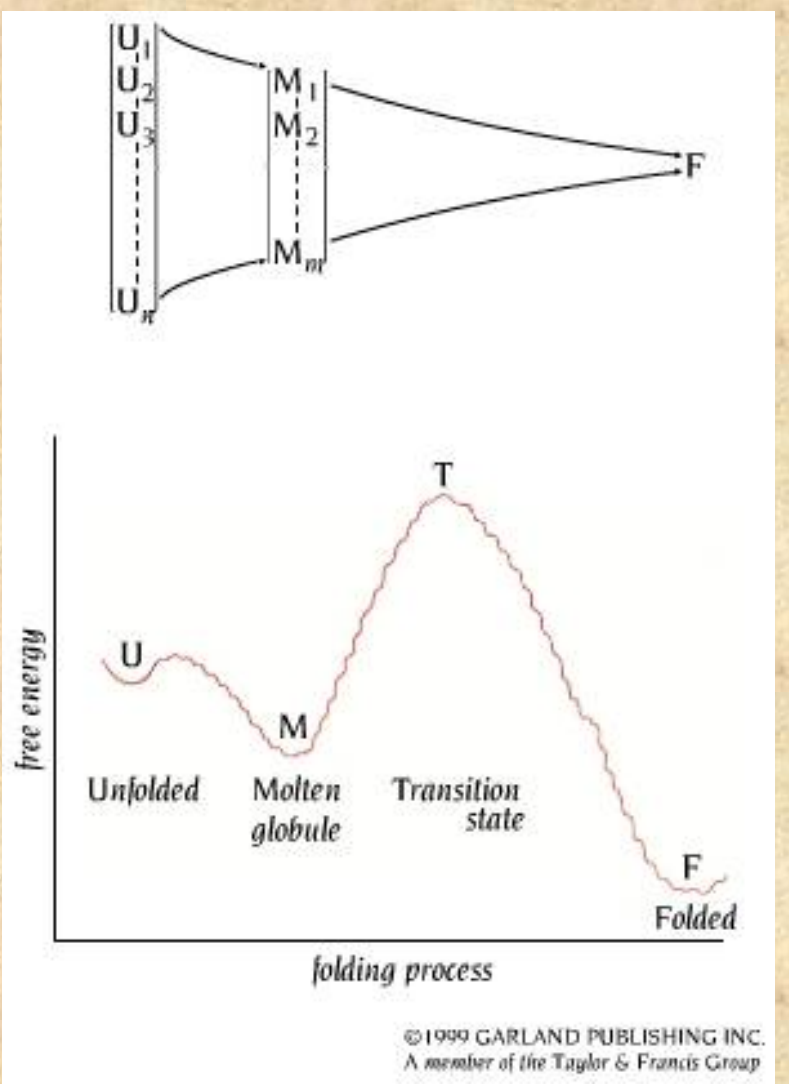
First Step

- occurs in a few milliseconds and is hard to observe experimentally
- has most of the secondary structure of the native state
- in some cases, has native-like positions of helices and strands
- less compact than the native structure and the proper packing interactions in the interior of the protein have not been formed
- should be seen as an ensemble of structures

Second Step

- can last up to a second or more
- persistent native-like elements of secondary structure begin to develop
- forming of subdomains
- still not in a single form (proper hydrophobic packing is not present and surface loops are not fixed)

Folding Process



- unfolded state, U
  - ensemble of conformationally different molecules
- molten globule, M
  - ensemble of structurally related molecules which are rapidly interconverting and which slowly change into a single conformation
- the folded state, F
  - a molecule must go through the high energy transition state T

Burying Hydrophobic Side Chains

- key event and a main mystery of protein folding

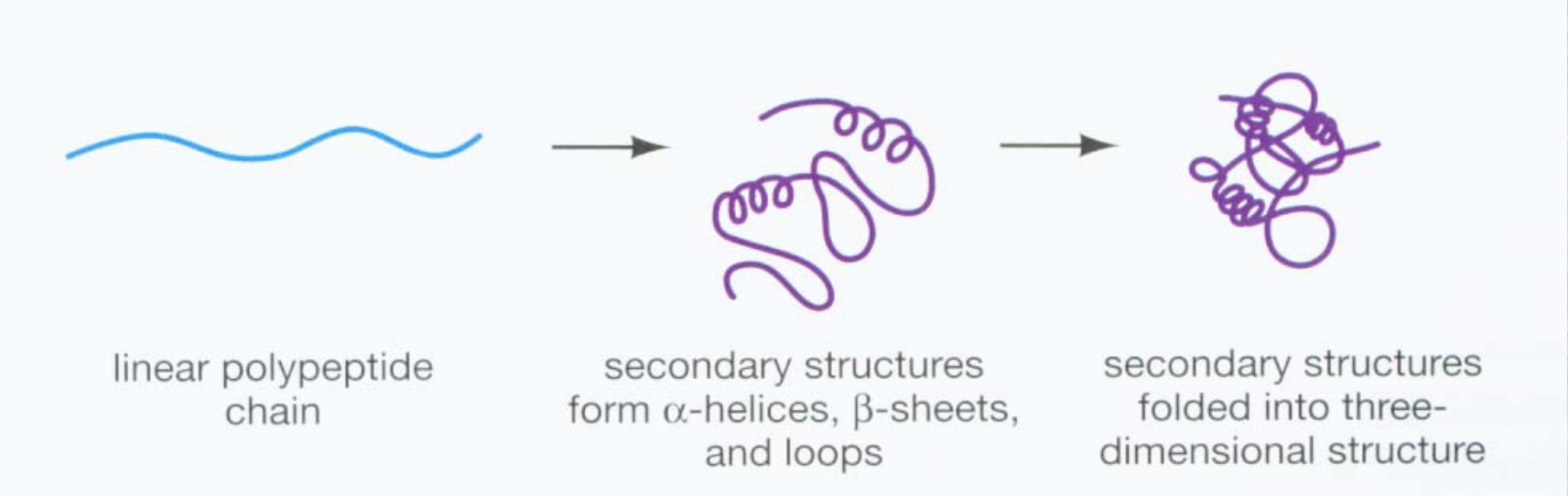
Secondary structure formation cannot be the driving force of folding

- there is very little change in free energy by forming the internal H-bonds characteristic for helices and sheets
- in the unfolded state, equally stable H-bonds can be formed with water

Hydrophobic effect

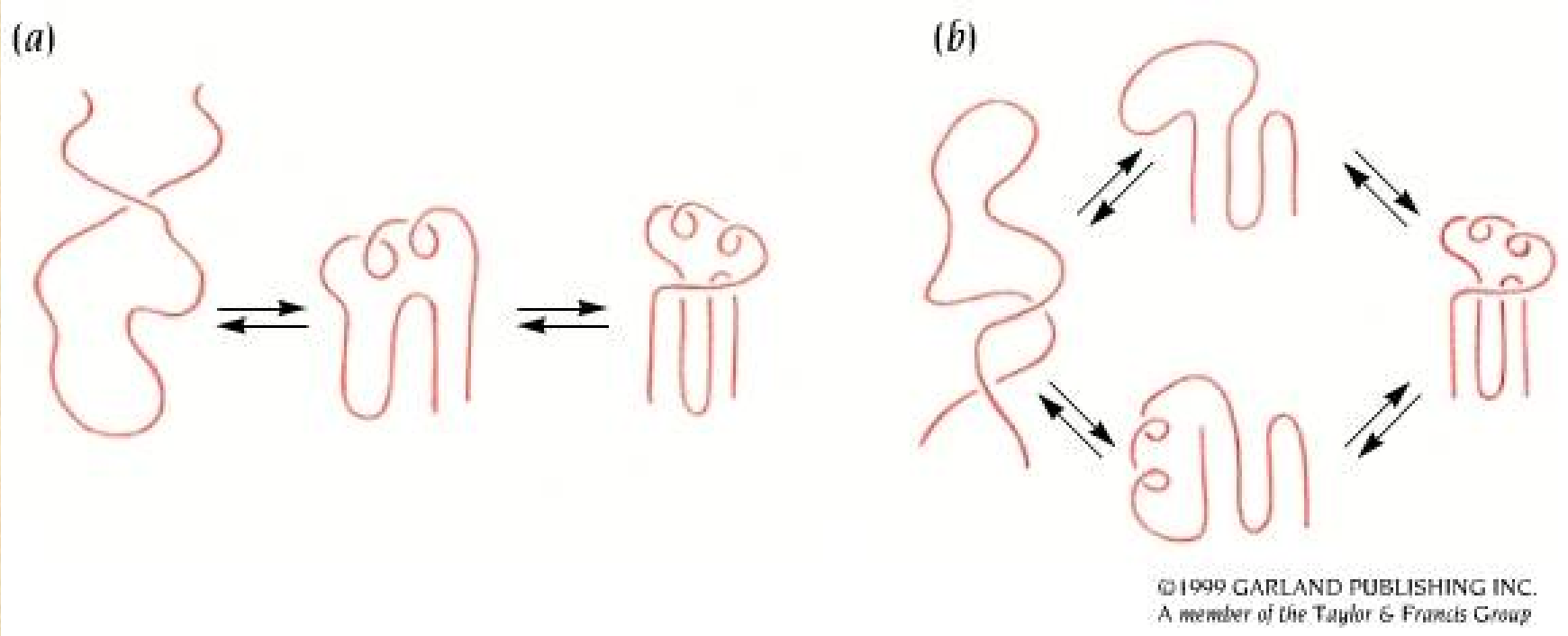
- there is a large free energy change by bringing hydrophobic side chains out of contact with water and into the contact with each other
- vastly reduces the number of conformations to be searched
- buried residues will have to make H-bonds in secondary structure elements
- secondary structure formation is consequence of hydrophobic effect

Hierarchical Building Block Folding Model



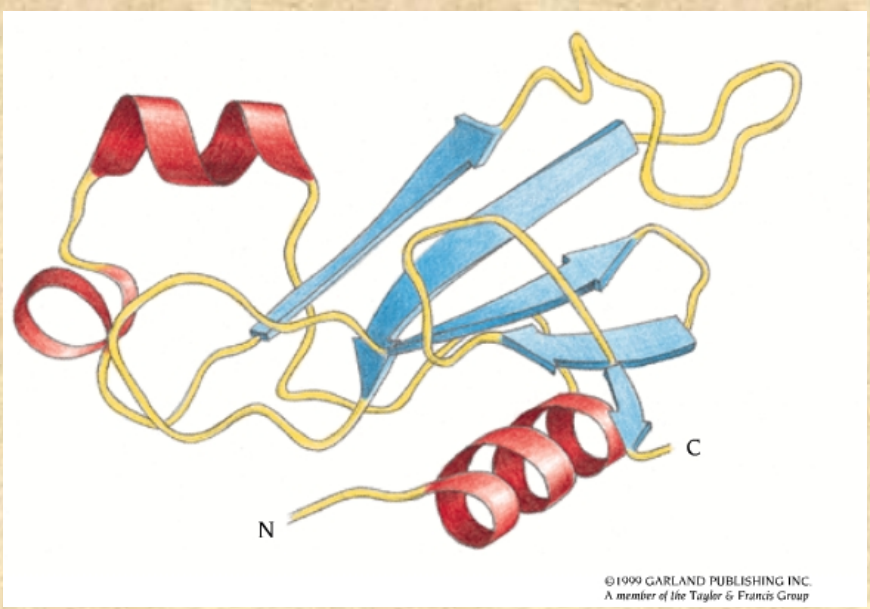
- there is a major (not necessarily unique) folding pathway that most proteins follow
- local neighborhoods interact and create folding hydrophobic units
- then, domains and entire proteins are created
- however, not all local neighborhoods show propensities towards one preferred conformations

Folding Pathways



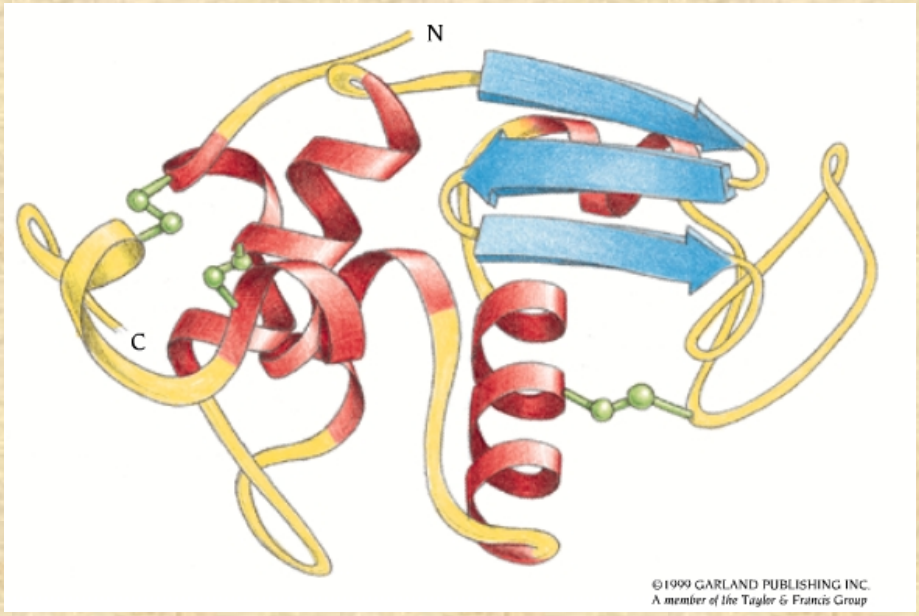
- both single and multiple folding pathways have been observed
- folding of the lysozyme involves parallel pathways and distinct folding domains

Folding Pathways

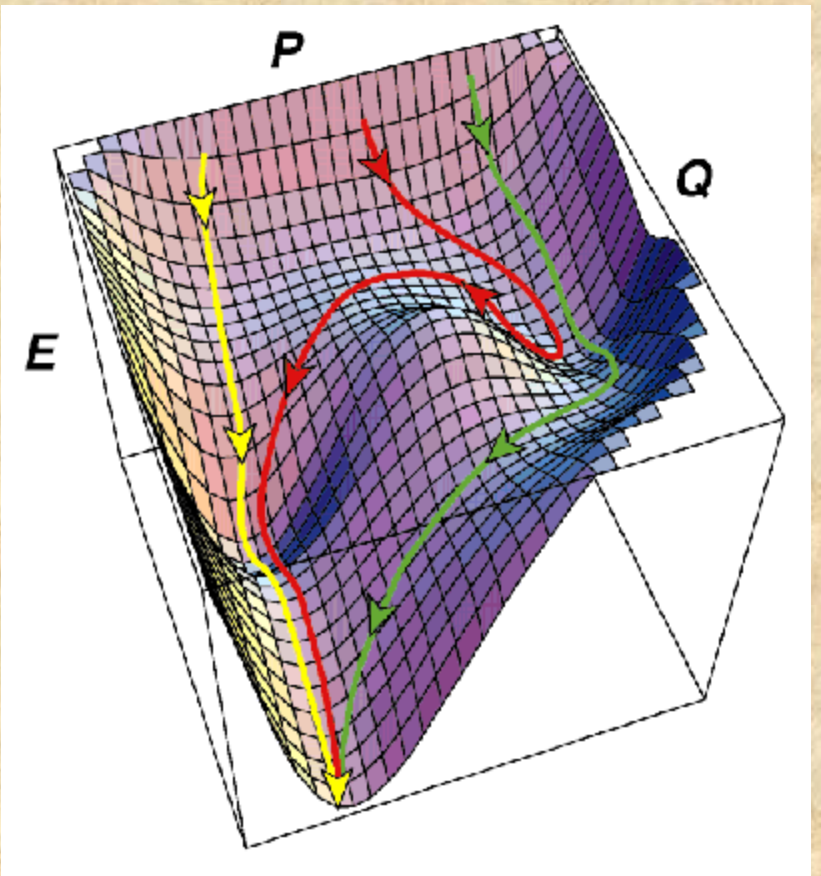


barnase has a single folding pathway

lysozyme has multiple folding pathway



Folding Funnel



E represents the energy of the system,

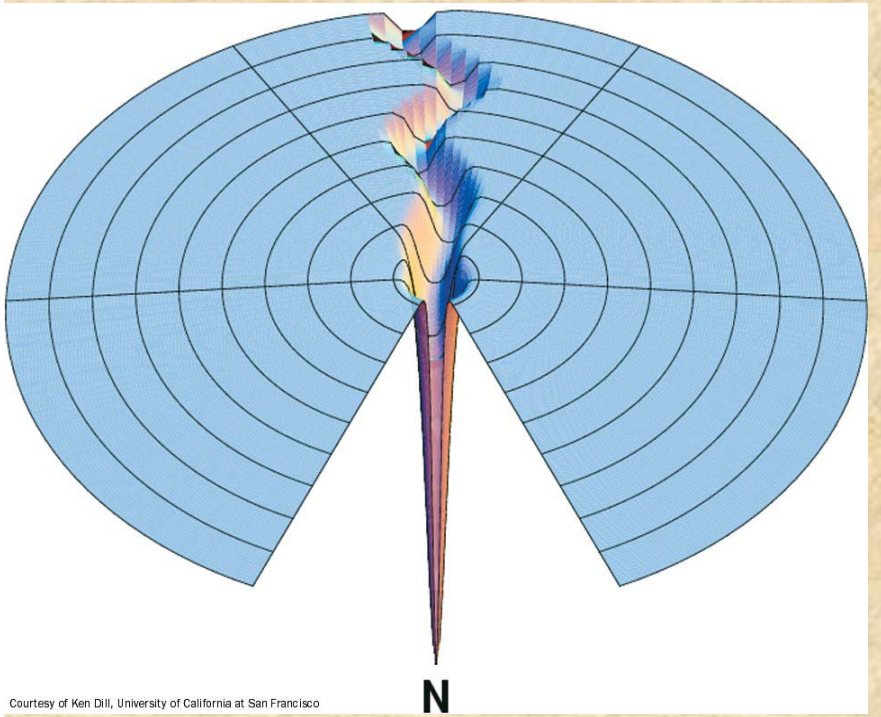
Q is defined as the proportion of native contacts formed,

P is a measure of the available conformational space

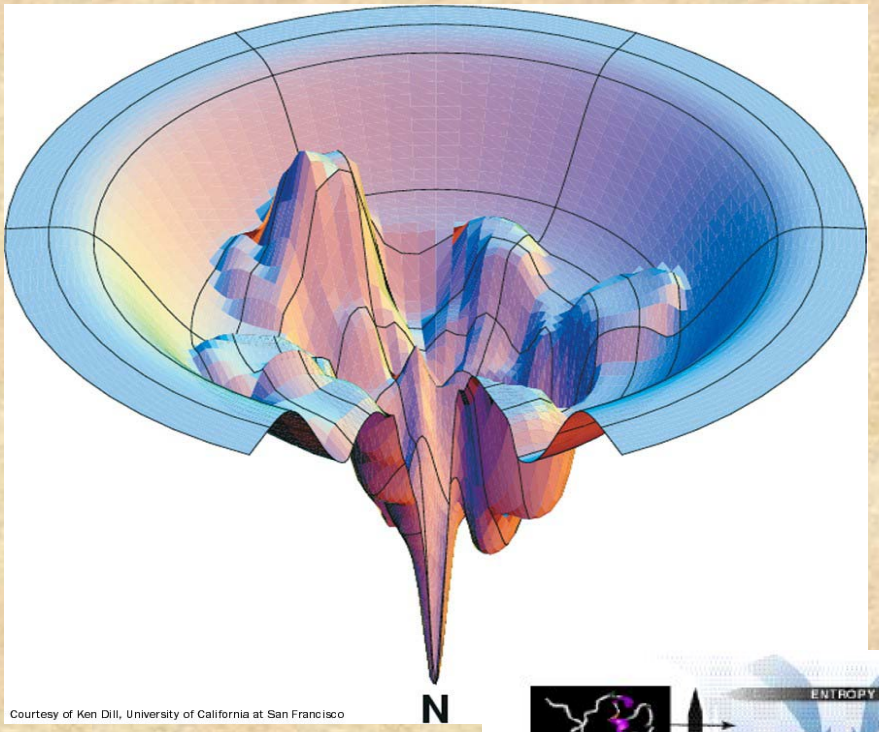
Three pathways are shown corresponding to (yellow) fast folding, (green) slow folding pathway that crosses the high energy barrier, and (red) slow folding pathway which returns to a less folded state before following the pathway for fast folding

C. Dobson, A. Sali, and M. Karplus. *Angew. Chem. Int. Ed.*, 1998, 37, 7, 868-893.

More Folding Funnels



Courtesy of Ken Dill, University of California at San Francisco



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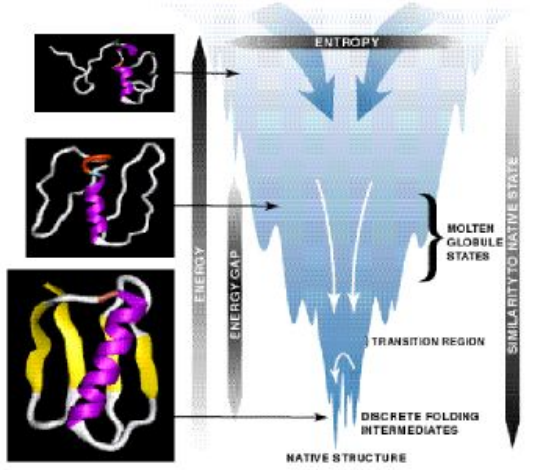
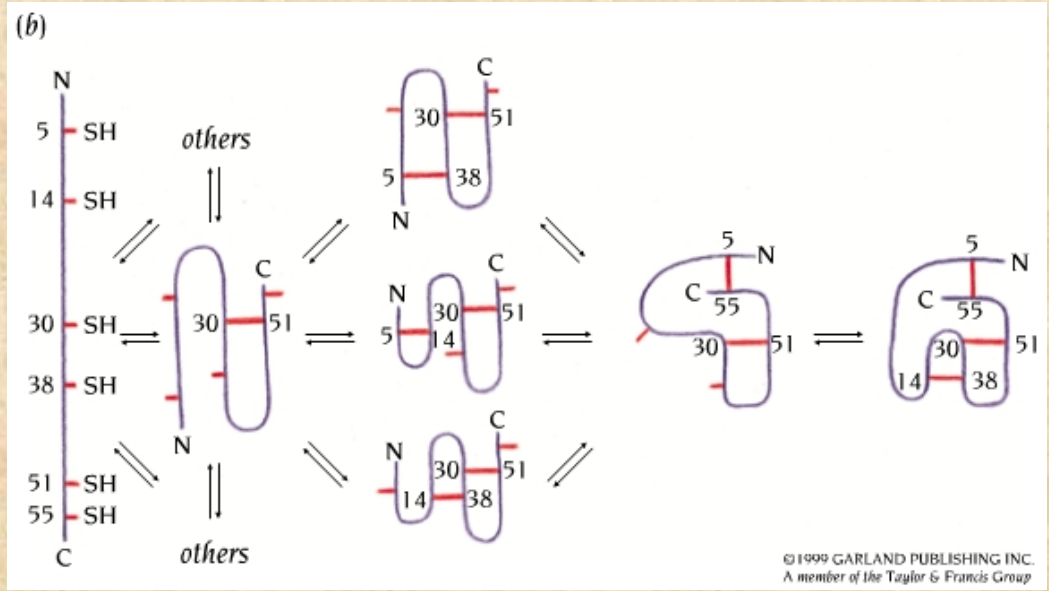
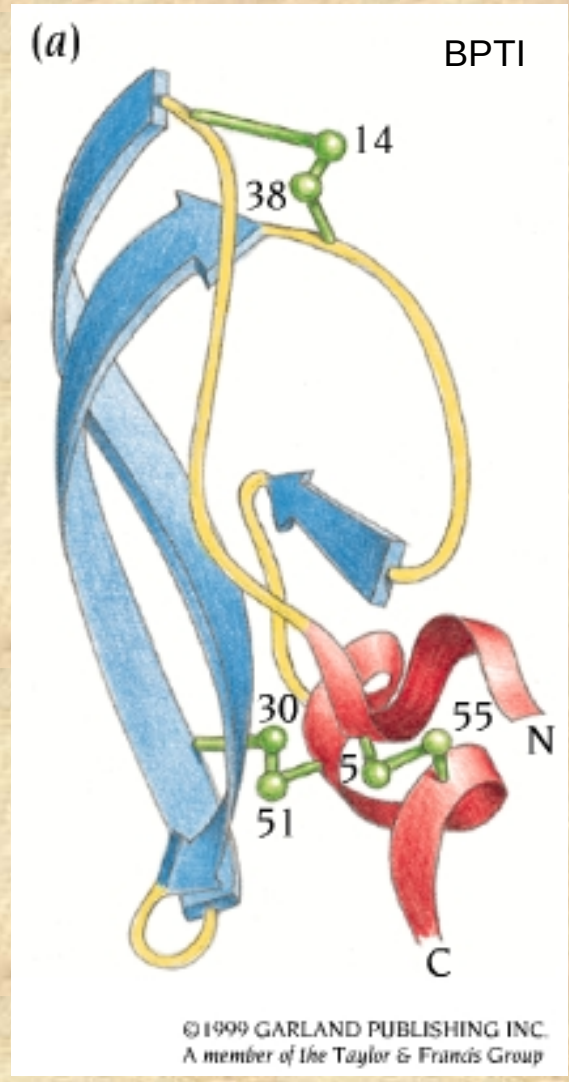


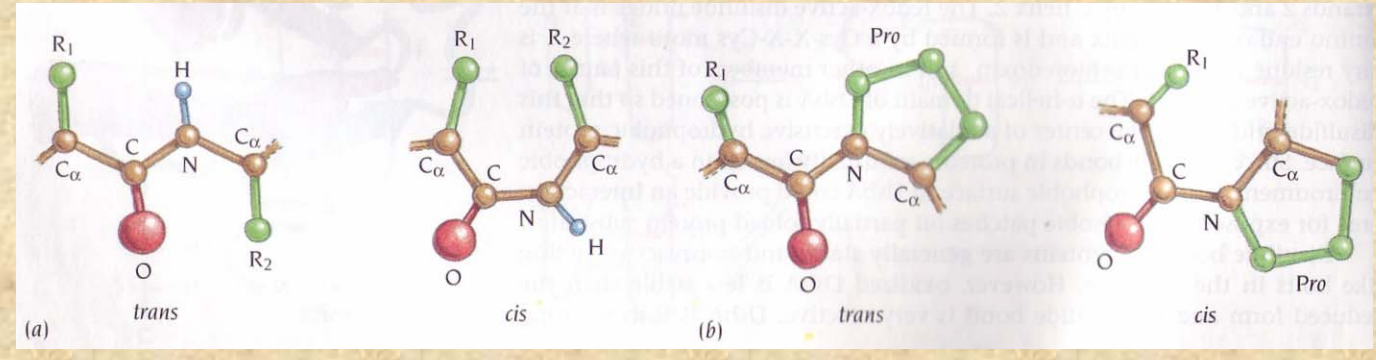
Forming Disulphide Bridges

- in eukaryotic cells disulphide bond formation occurs in the endoplasmic reticulum before proteins are exported to the cell surface
- enzyme PDI catalyzes disulphide exchange to remove intermediates with incorrectly formed disulphide bridges
- proteins with disulphide bonds are not found in cytosol, but are located in the plasma membrane or are secreted

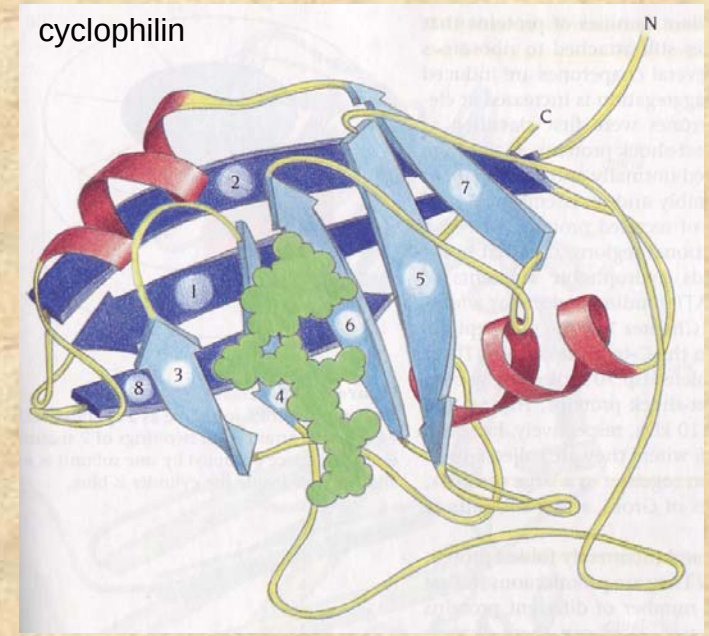




Proline Isomerization



- *cis-trans* isomerization of proline peptides is intrinsically slow process
- *in vitro* it is a rate limiting step in folding for those molecules that have been trapped in the folding intermediate with the wrong isomer
- peptidil prolyl isomerase (cyclophilin) catalyzes the process *in vivo* (both in prokaryotes and eukaryotes)

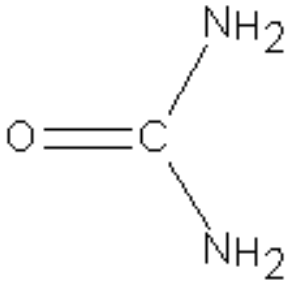


Molecular Chaperones

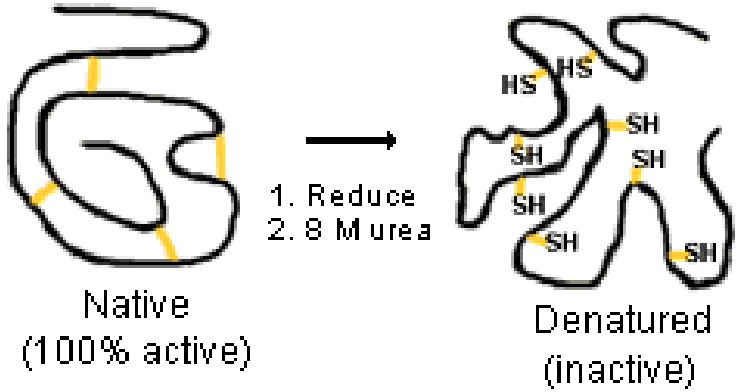
- before they attain native conformation proteins may expose their hydrophobic patches to the solvent
- isolated purified proteins can thus aggregate *in vitro* even at low protein concentrations
- inside cells, at much higher concentrations of many proteins, aggregation can easily occur
- this is prevented by molecular chaperones
  - ubiquitous and abundant families of proteins that assist the folding of both nascent polypeptides still attached to ribosomes and released complete polypeptide chains
- some chaperones bind together into chaperonins and then bind unfolded and incorrectly folded proteins, but not native proteins

Anfinsen's Experiment

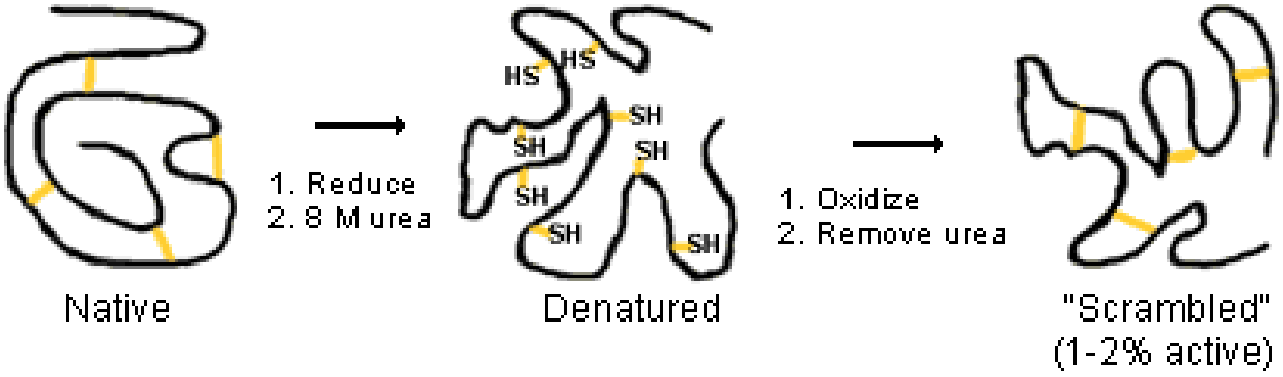
Urea:



The Observation:



The Control:



There is sufficient information contained in the protein sequence to guarantee correct folding from any of a large number of unfolded states.

Thermodynamic Hypothesis

- native conformation of a protein is adopted spontaneously i.e.

amino acid sequence $\longrightarrow$ 3-D structure

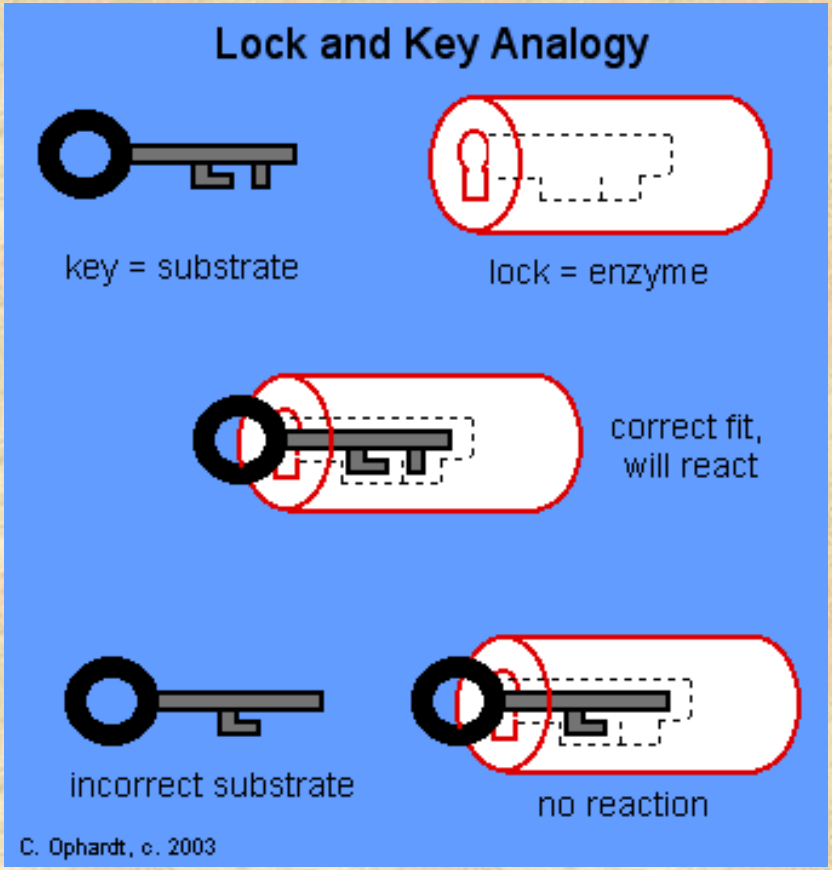
Anfinsen's demonstration of this fundamental property of proteins opened the problem to a massive amount of experimental and theoretical effort.

His summary of the experiments was presented as a Nobel Prize Lecture and published in:

Anfinsen, C.B. (1973) "Principles that govern the folding of protein chains." Science 181 223-230.

Fischer's Experiment

- Hermann Emil Fischer – 1894
- An enzyme and a substrate have to fit each other like a lock and key in order to exert chemical effect on each other
- lock-and-key theory
- later, lock-and-key paradigm was expanded to contain so-called induced fit theory



"*The examination of the synthetic glucosides has shown that the action of the enzymes depends to a large extent on the geometrical structure of the molecule to be attacked, that the two must match like lock and key*." H. E. Fischer in his Nobel Lecture

Sequence-Structure-Function Paradigm

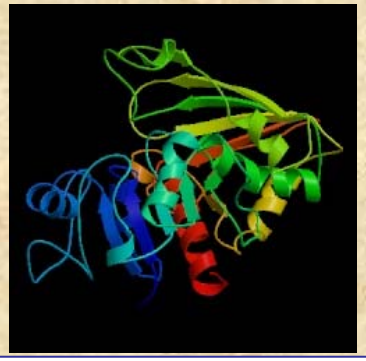
Standard protein structure/function paradigm
(Fischer, 1894, Anfinsen 1973)

Amino Acid Sequence

3-D Structure

Protein Function

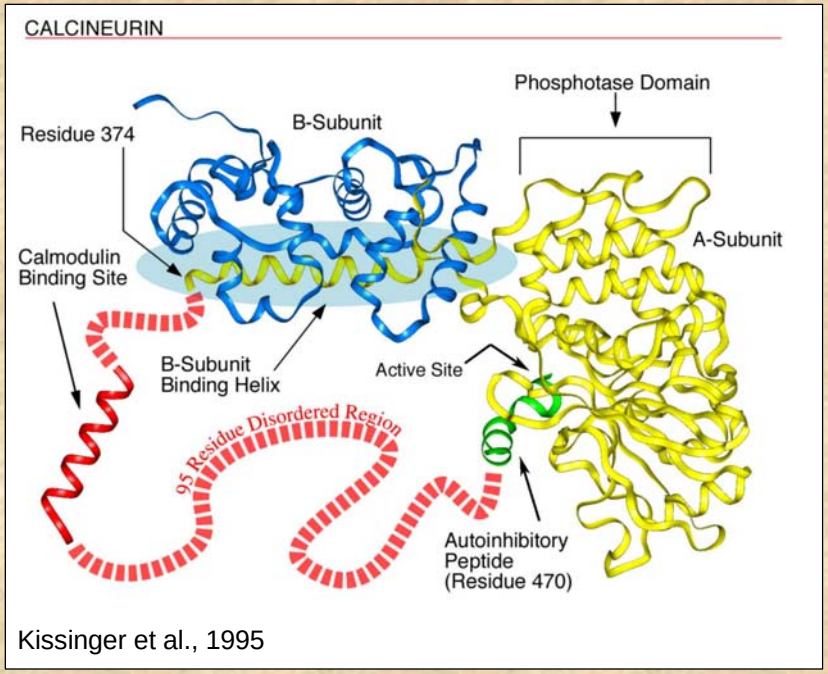
> 1NLG:_ NADP-LINKED GLYCERALDEHYDE-3-PHOSPHATE
EKKIRVAINGFGRIGRNFLRCWHGRQNTLLDVVAINDSGGVKQASHLLKYDSTLGTFAAD
VKIVDDSHISVDGKQIKIVSSRDPLQLPWKEMNIDLVIEGTGVFIDKVGAGKHIQAGASK
VLITAPAKDKDIPTFVVGVNEGDYKHEYPIISNASCTTNCLAPFVKVLEQKFGIVKGTMT
TTHSYTGDQRLLDASHRDLRRARAAALNIVPTTTGAAKAVSLVLPSLKGKLNGIALRVPT
PTVSVVDLVVQVEKKTFAEEVNAAFREAANGPMKGVLHVEDAPLVSIDFKCTDQSTSIDA
SLTMVMGDDMVKVVAWYDNEWGYSQRVVDLAEVTAKKWVA



Classification: **Gene Transfer**
EC Number: **1.2.1.13**

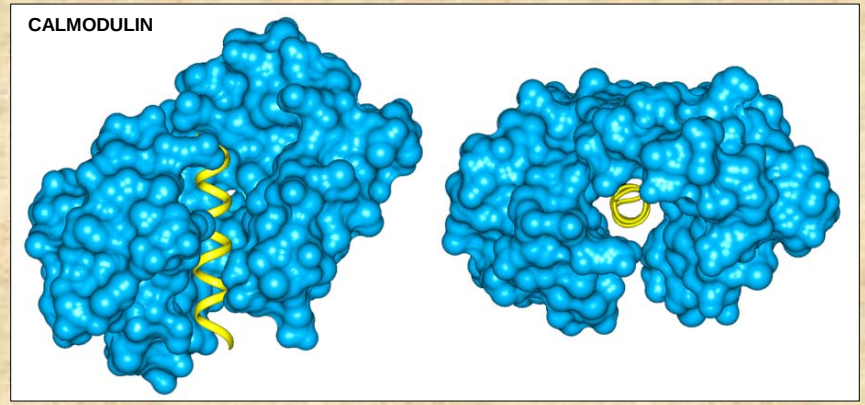
Dominant view: 3-D structure is prerequisite for protein function

Calcineurin-Calmodulin Counter Example



Kissinger et al., 1995

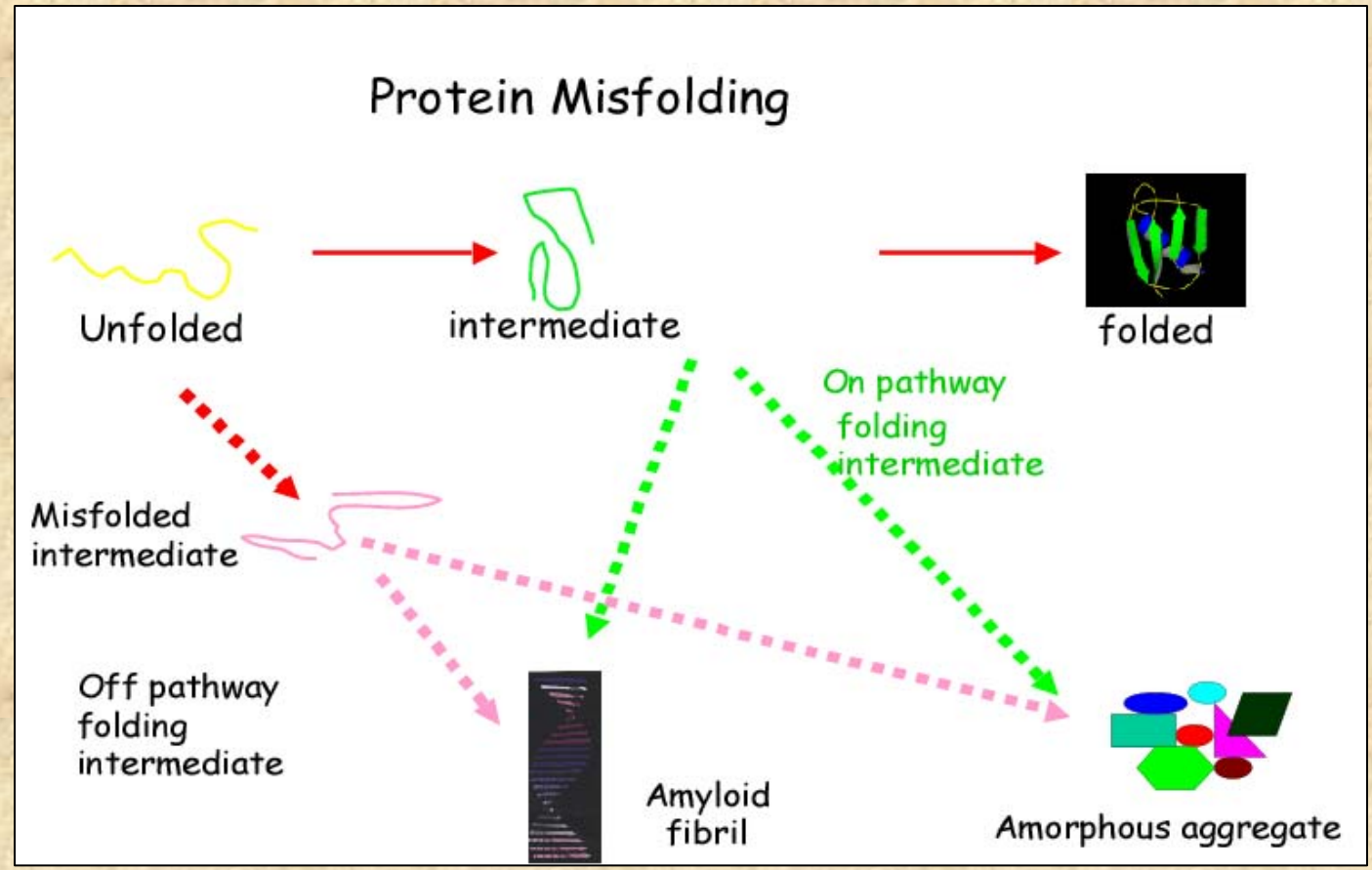
- **Calcineurin:**
  - calcium-dependent phosphatase
  - regulated by calmodulin (calcium-binding protein)
  - induces conformational change of calmodulin upon binding
  - may be involved in human hart failure when calcium concentration is chronically increased
  - “disorder” is important for the binding mechanism



Intrinsically disordered proteins
(natively unfolded/unstructured proteins)

- do not have stable 3D conformation under physiological conditions
- abundant in nature

Can Proteins Misfold?



- the lack of function is not always the worst-case scenario
- misfolding can lead to diseases

Open Problems in Protein Bioinformatics

The Ten Most Wanted Solutions in Protein Bioinformatics
by Anna Tramontano

10 Most Wanted Solutions

1. Protein Sequence Alignment
2. Predicting Protein Features from Sequence
3. Function Prediction
4. Structure Prediction
5. Membrane Proteins
6. Functional Site Identification
7. Protein-Protein Interactions
8. Protein-Small Molecule Interactions
9. Protein Design
10. Protein Engineering

Problem 1.
Protein Sequence Alignment

- amino acid sequences have been evolutionarily selected for their favorable thermodynamic, kinetic and functional properties
- when variations that do not impair essential function occur in replicating (germinal) cells they are transmitted to the progeny and generate diversity in the population
- variations that do impair essential functions disappear
- if the variation confers selective advantage it can become the most frequent variant in the population
- if the function performed by a protein has to be conserved and is brought about by specific residues and their relative position in the 3-D structure, then residues responsible for function and structure must be conserved!!!

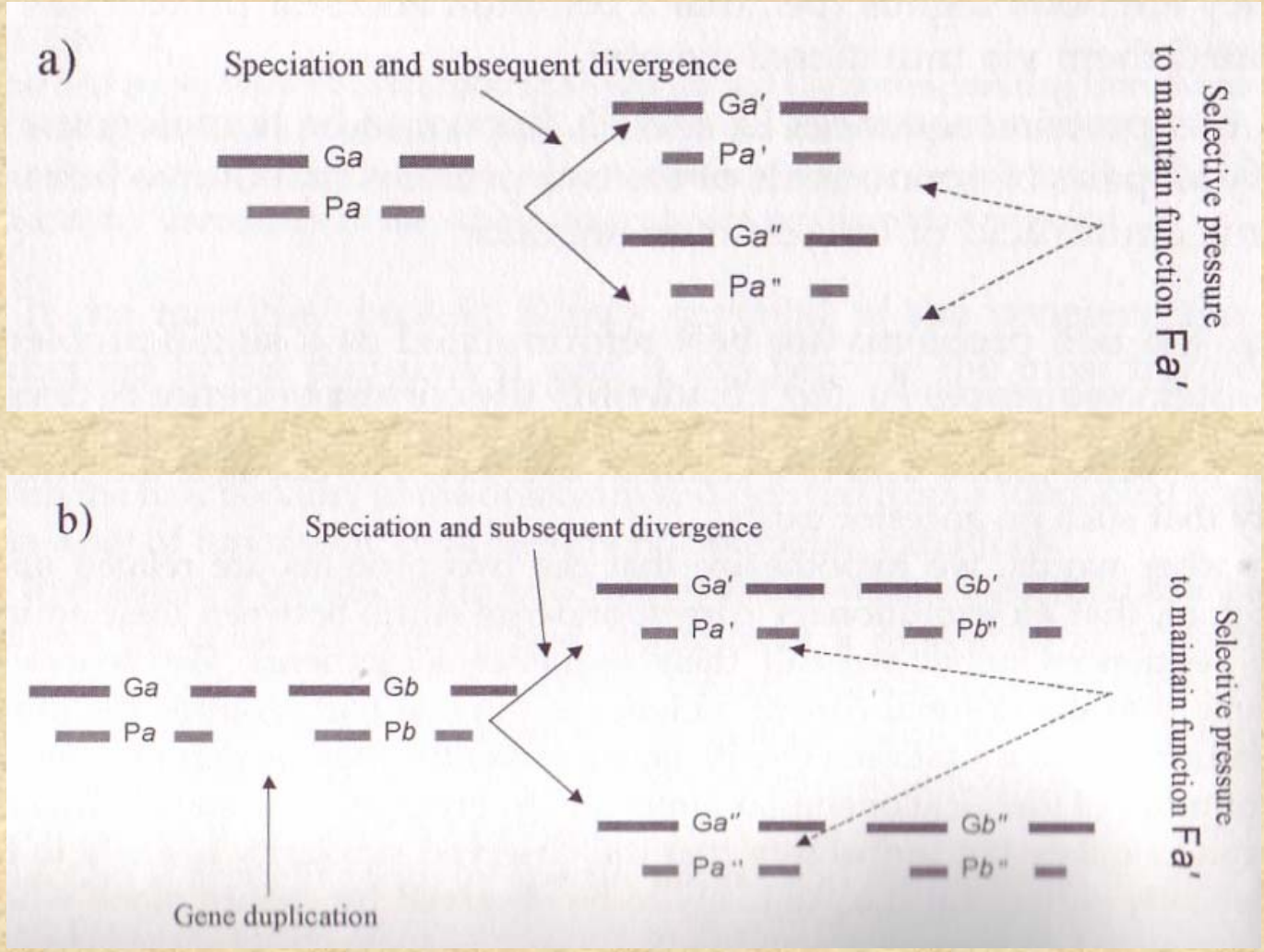
Evolution-Based Inference of Protein Function

- if we can identify an evolutionary relationship between two proteins between species and find conserved residues, these residues are candidates for involvement in functional mechanisms

- two groups of conserved amino acids
  - those that are conserved because of their structural role
  - those that are conserved because of their functional role
- similar amino acids can more easily replace each other in a structural role; example: catalysis requires specific atoms

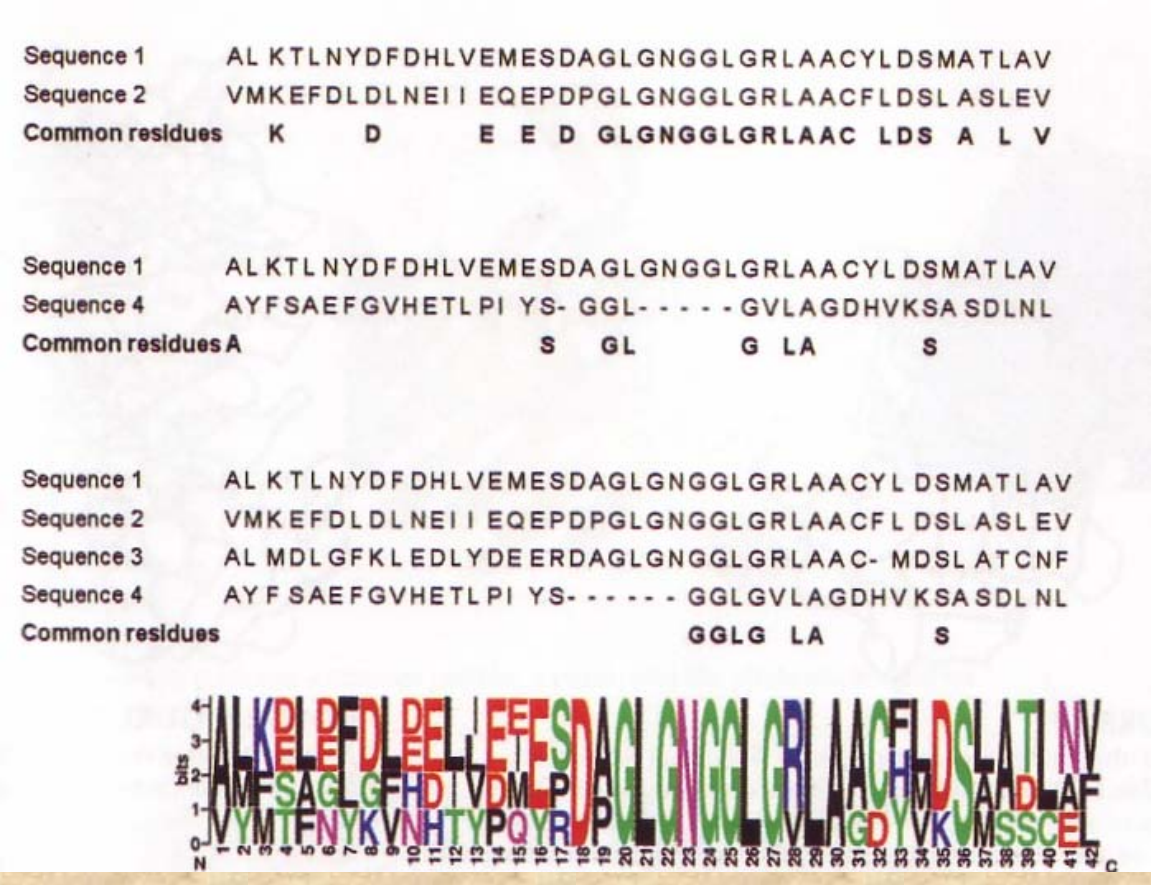
- Homology detection (or protein sequence alignment) problem
  - given proteins p_1 and p_2, what is the probability they are homologous
  - given homologous proteins p_1 and p_2 identify all pairs of amino acids that derive from the same amino acid of the common ancestor

Orthology vs. Paralogy



Detecting Remote Homology

- duplication and subsequent divergence
- mixing and matching of domains
- detecting very distant homologous relationships is important
  - enlarges the number of proteins for which some functional inference can be made
  - makes easier detection of functional residues
  - detection of distant relationships may shed new light to the process of evolution between organisms



Achieved vs. Non-Achieved

- pairwise sequence alignment is a solved problem
  - Needleman-Wunsch algorithm for global alignment
  - Smith-Waterman algorithm for local alignment
  - BLAST and FASTA heuristics
- multiple sequence alignment is NOT a solved problem
  - dynamic programming – unacceptable
  - progressive alignment: Feng-Doolittle and ClustalW algorithms
- what is a good scoring system?
- sequence profiles and hidden Markov models
- database searching (BLAST and FASTA, again)
- how can structure be incorporated into sequence alignment?

Problem 2.
Predicting Protein Features from Sequence

- features to be predicted: secondary structure elements, post-translational modification sites, cellular compartments, functional sites

- task: given a training set composed of proteins that share a given property, infer the rules important for function

- How can function be deduced?
  - by the presence of a particular sequence pattern (deterministic)
  - by estimating probability that the given sequence belongs to the set of positive examples (stochastic)

- if only positive set is used $\Rightarrow$ conservation problem
- if both positive and negative sets are used $\Rightarrow$ classification problem

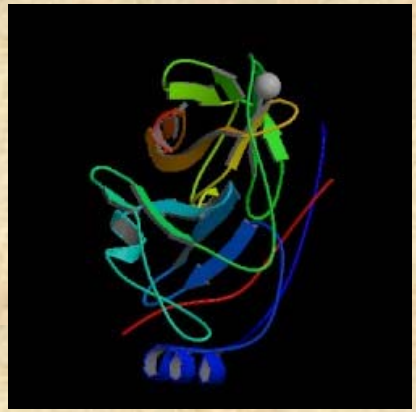
Deterministic Patterns

Example #1:

- NS3 protease in hepatitis C virus
- contains serine, histidine and aspartic acid at key positions
- Pattern over many similar proteins:
  [DE] S G [GS]

Example #2:

- regions with no definite constraints can be included
- D X(1, 4) [LI] X [DE]
  - aspartic acid; 1-4 unconstrained residues; leucine or isoleucine; unconstrained residue; aspartic or glutamic acid

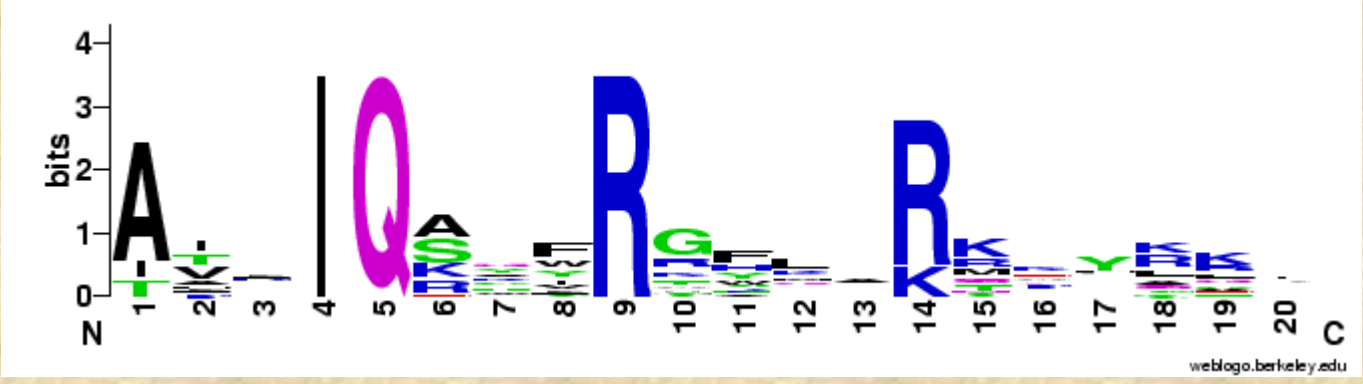


NS3 protease: 1dxp

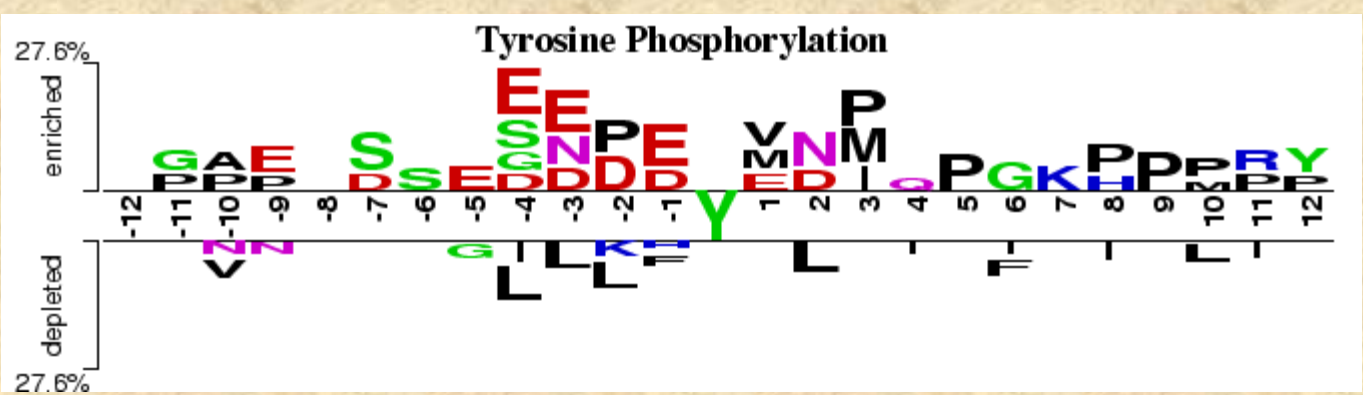
Example #3:

..GLGNGGLGRLA..

Stochastic Patterns



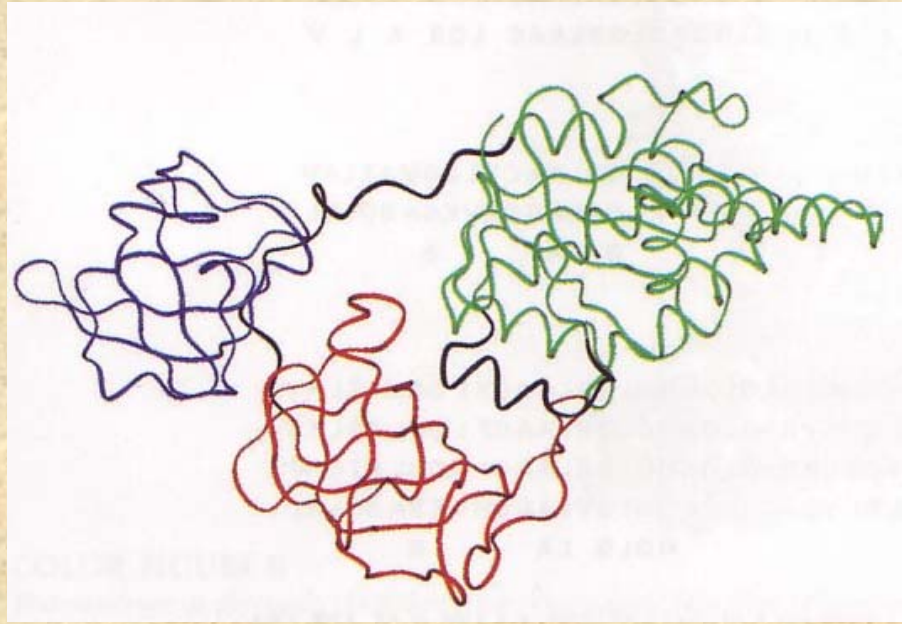
Calmodulin Binding Motif (IQ Motif)



Tyrosine Phosphorylation Sites

Predicting Domain Boundaries

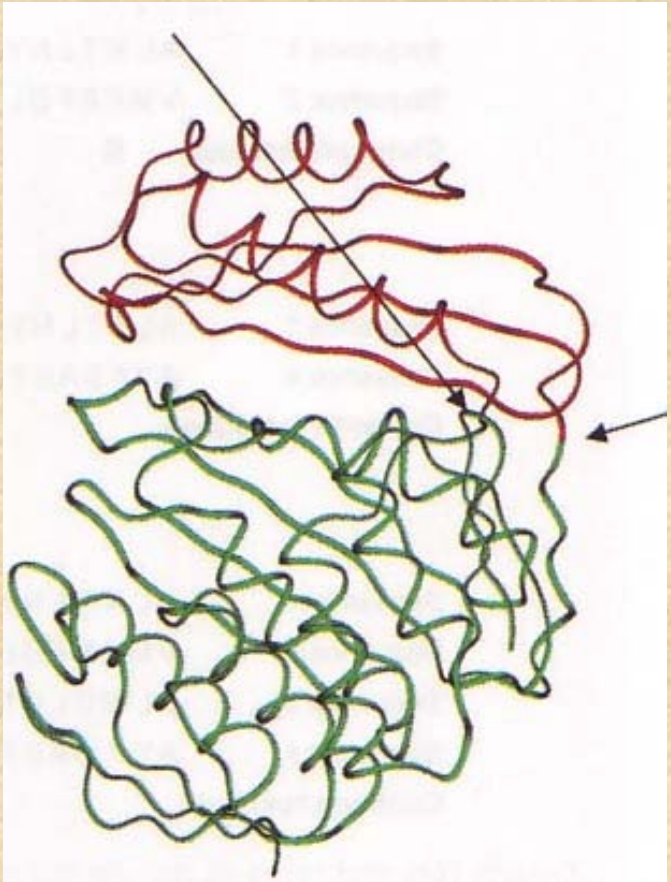
- there is no consensus on a definition of a domain
  - say, a globular, compact regions of a protein structure with relatively more contacts within themselves than with the rest of the structure
- precise domain boundaries are difficult to define even when the structure is present $\Rightarrow$ manual inspection is required
- thus, hard to obtain clean set of examples for informatics methods



The structure of the elongation factor-1 from *Sulfolobus solfataricius*, a protein involved in RNA translation. Three domains are connected by long samino acid stretches.

Predicting Domain Boundaries

- domains are not necessarily contiguous
- Some ideas:
  - SnapDRAGON: produces several hundred putative 3D models and detects domains by averaging prediction results
  - DomSSEA: predicts secondary structure of the target protein and maps predicted sequence of helices and sheets on the known domains



A discontinuous domain on the RNA 3'-terminal phosphate cyclase from yeast.

Problem 3.
Protein Function Prediction

- the major objective of protein bioinformatics
- it unifies all problems, but some issues are separate

- What is the definition of biological function?
  - a protein catalyzing a chemical reaction
  - an enzyme involved in HCV infection
  - a protein expressed in liver cells

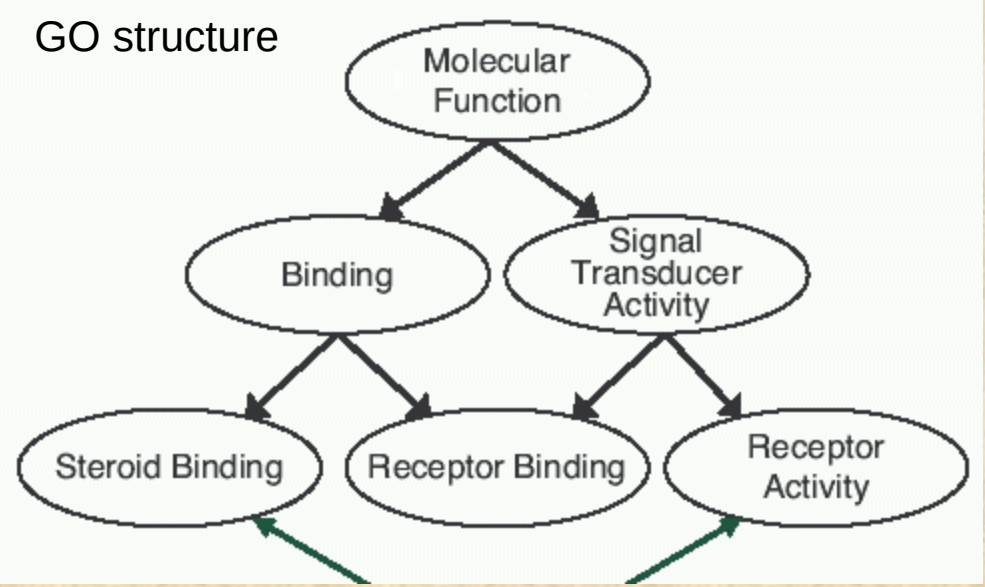
- Enzyme ⊇ hydrolase (breaks a chemical bond)
  ⊇ peptidase (breaks a peptide bond)
  ⊇ endopeptidase (breaks an internal peptide bond)
  ⊇ serine-type endopeptidase (contains serine)

- Lack of standardization has long been a problem

The Function Vocabulary

- many functional annotations are free-text entries
- Gene Ontology (GO) is the major community effort for standardization
- Enzyme Classification (EC) scheme is widely used for enzymes
- Swiss-Prot database contains functional keywords

GO structure



http://ict.ewi.tudelft.nl/~herman/geneontology.gif

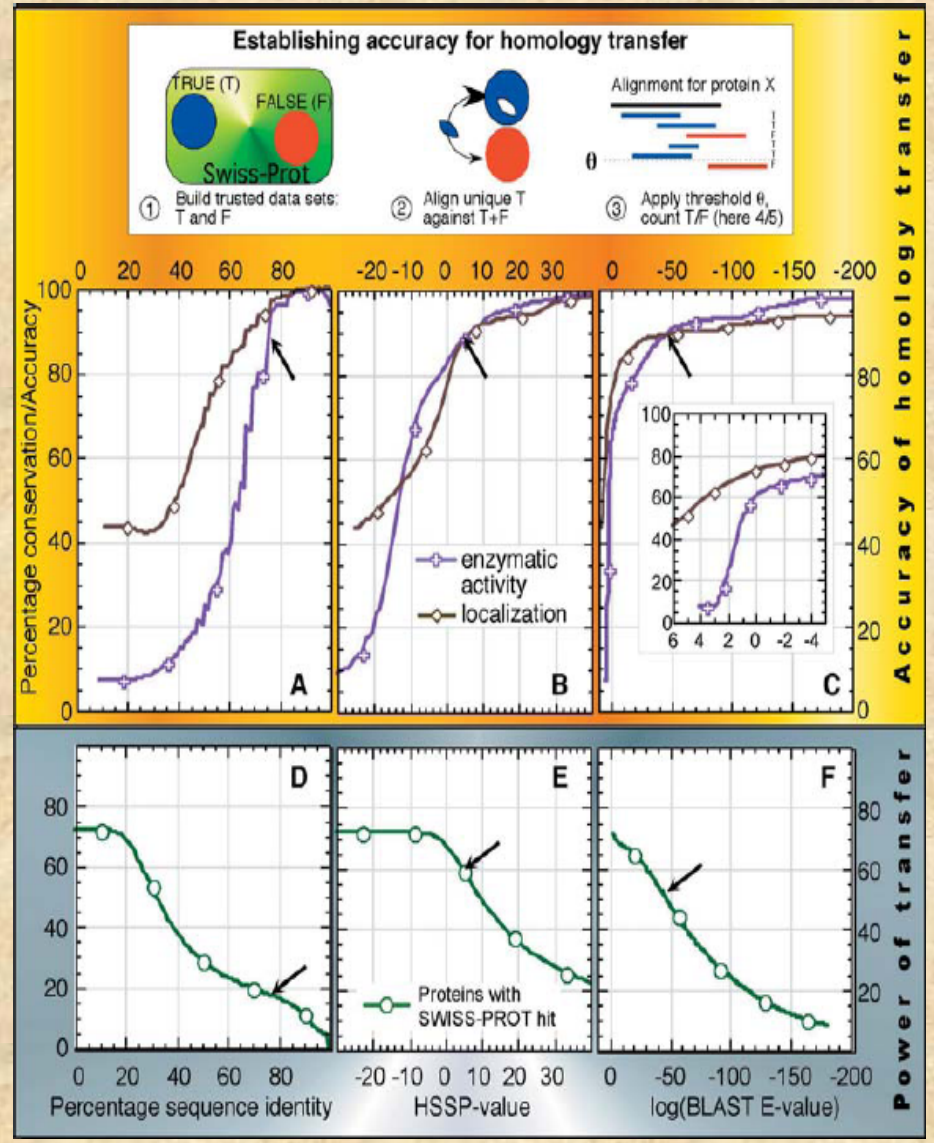
Gene Ontology has three categories:

Molecular function

Biological process

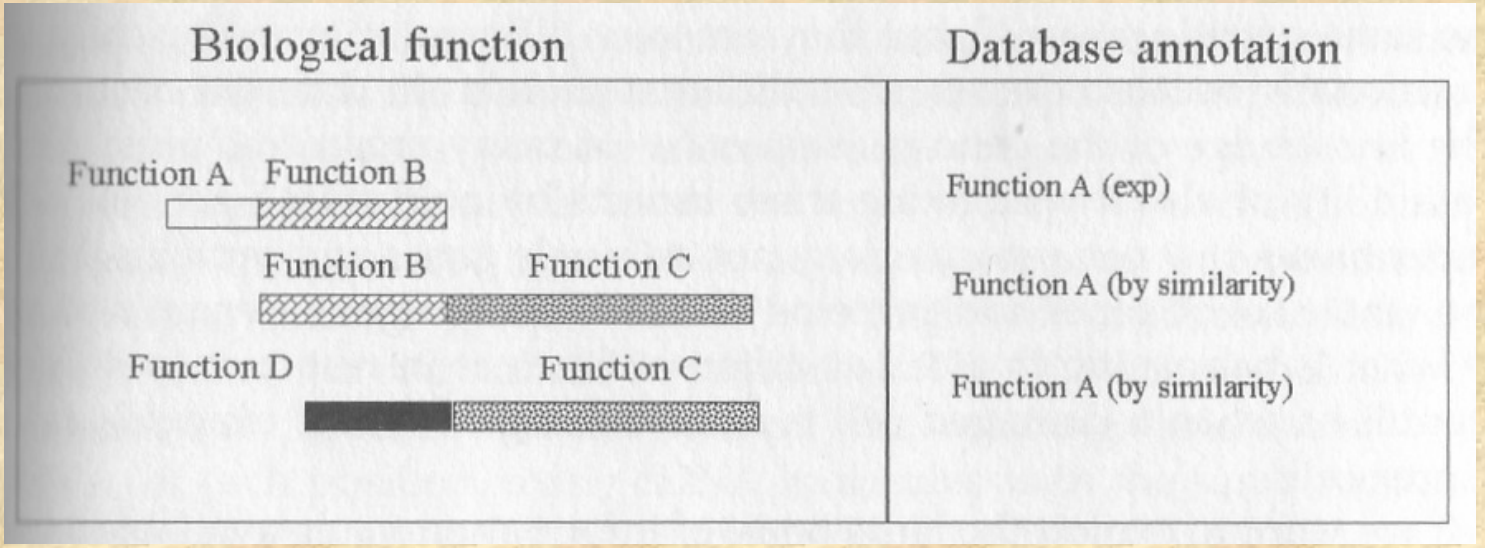
Cellular component

Inferring Function by Similarity



Rost et al., CLMS, 2003

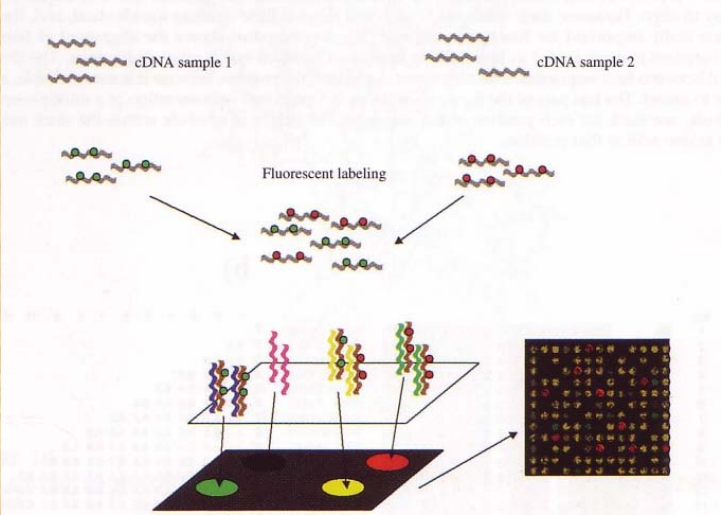
Some Problems of Inferring Function

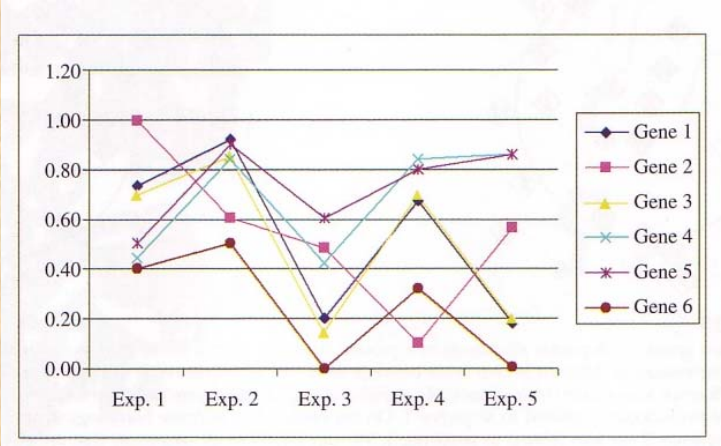


- transitivity for function annotation is often unreliable
- protein names (p53, hungtingtin, YAKK…)
- text mining – problem with extracting function
  - even finding ends of sentences is not perfect
  - detecting protein names is difficult
  - extracting protein function, even more difficult

Data Integration

Transcriptomics





Proteomics