Protein folds, fold classifications & structure stability

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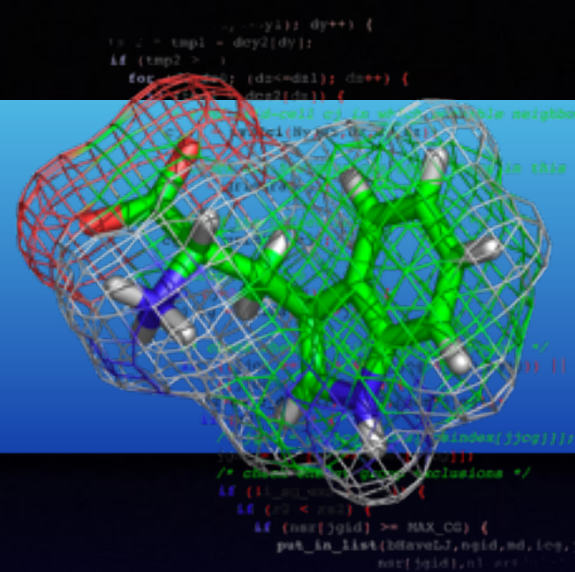
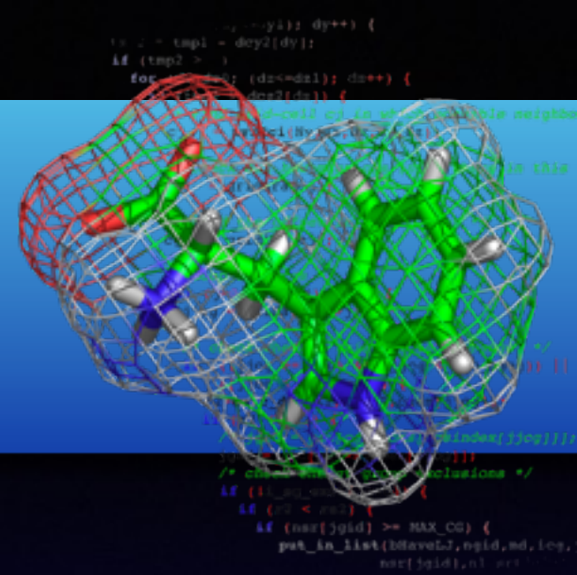
Recap



- Globular proteins
  - α,β,mixed proteins
  - Common supersecondary structure motifs
    - Rossman fold, Greek key motif etc
- Membrane proteins
  - Mostly α-helix, but some β-barrels
  - Stabilized by internal H-bonds in hydrophobic environment
  - Leading research area in Stockholm

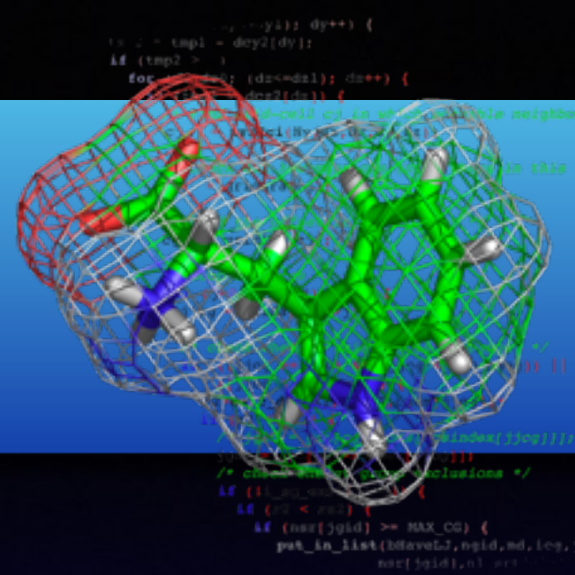
Outline today



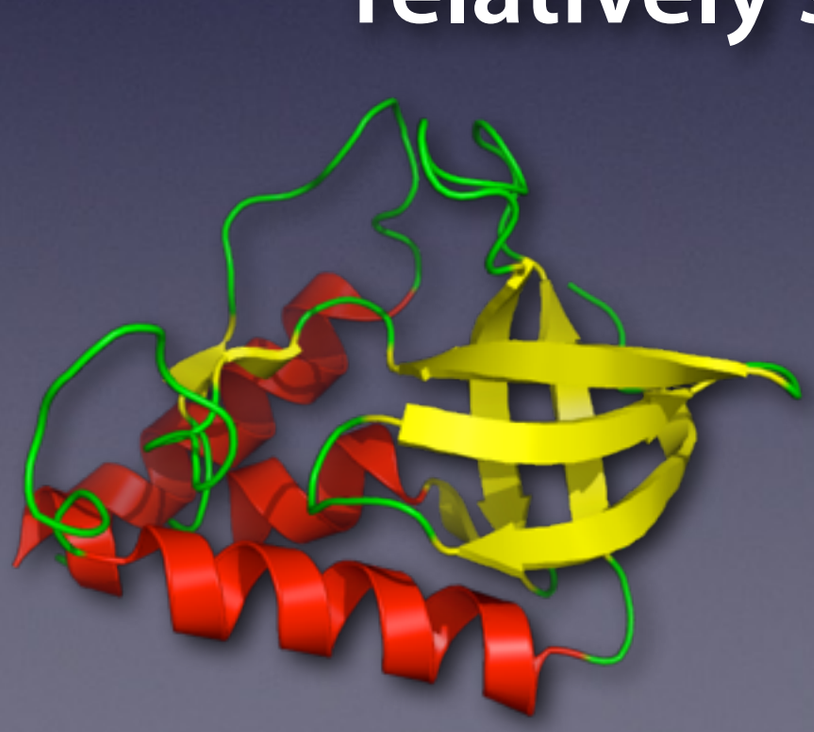
- Fold stability
- Structural evolution
- Protein size variation
  - Why helices/sheets have certain sizes
- Boltzmann statistics for folds - or not?
- Sequence-structure compatibility
- Fold stabilization from residues
- How stable are proteins, and why?

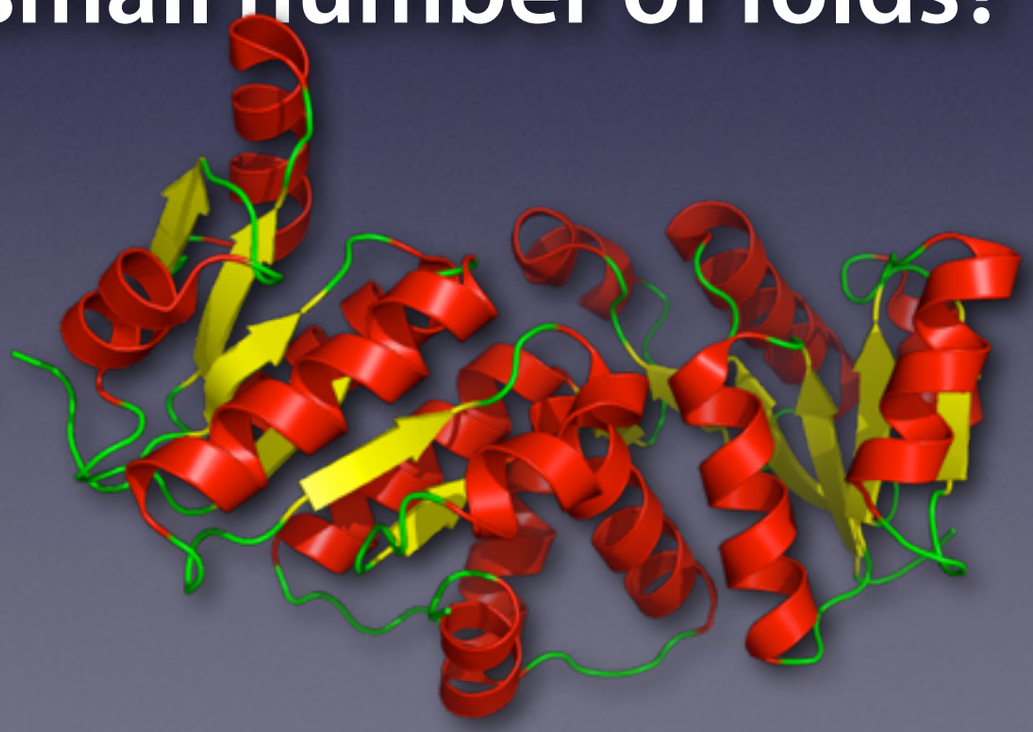
Protein physics book: Chapters 15 & 16

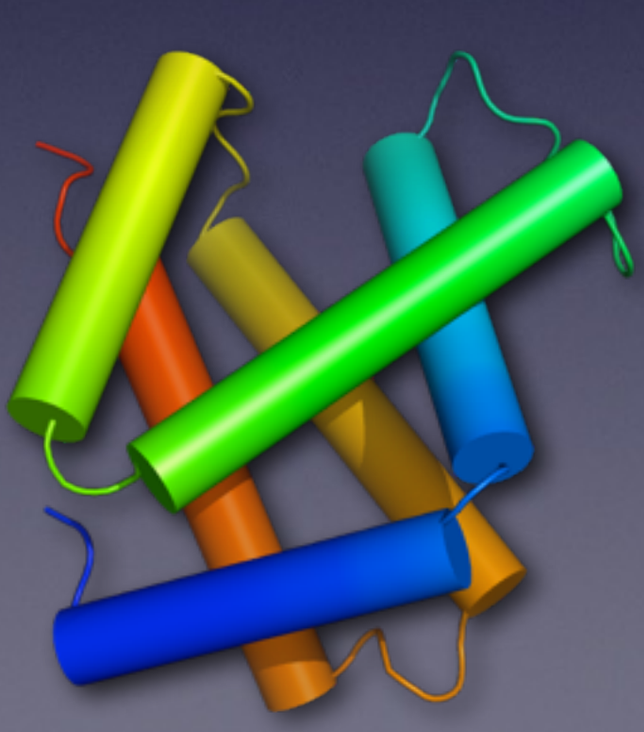
The fold universe



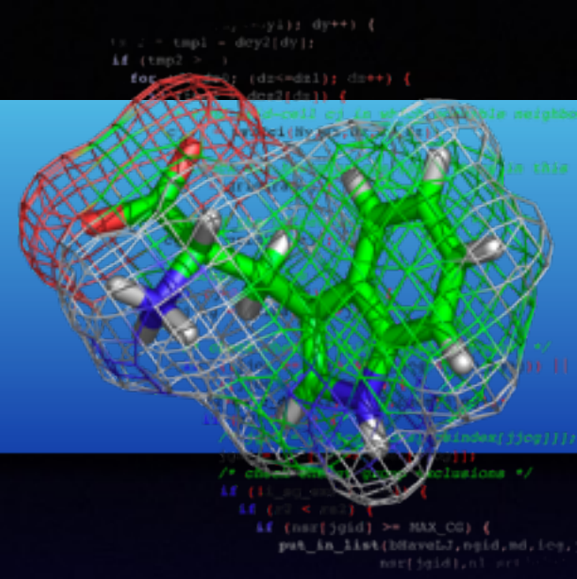
- Why are there so few protein folds?
- Chothia: *"~~1000~~ 1500 folds for the molecular biologist"*
- Why do most sequences seem to fit a relatively small number of folds?



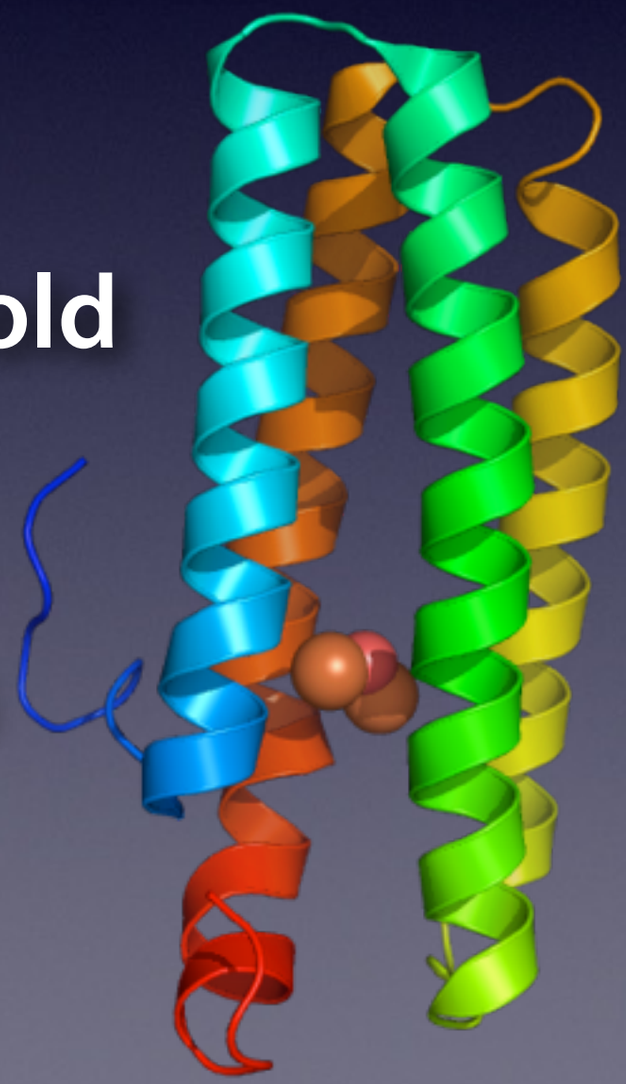




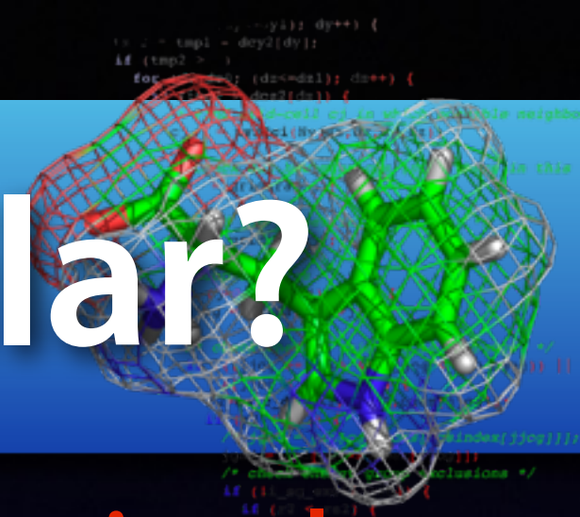
“Typical” folds



- 20% of folds account for 80% of proteins
- Mostly true for RNA too
- Compare with DNA: Only a single fold
- Homologous sequences
- Functional convergence onto folds
- Physical restrictions



Why are proteins similar?



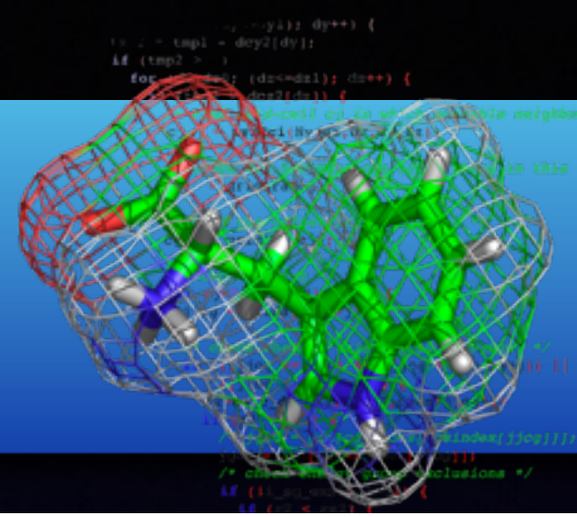
Evolutionary Divergence

Functional Convergence

?

Limited number of possible folds

Folding patterns

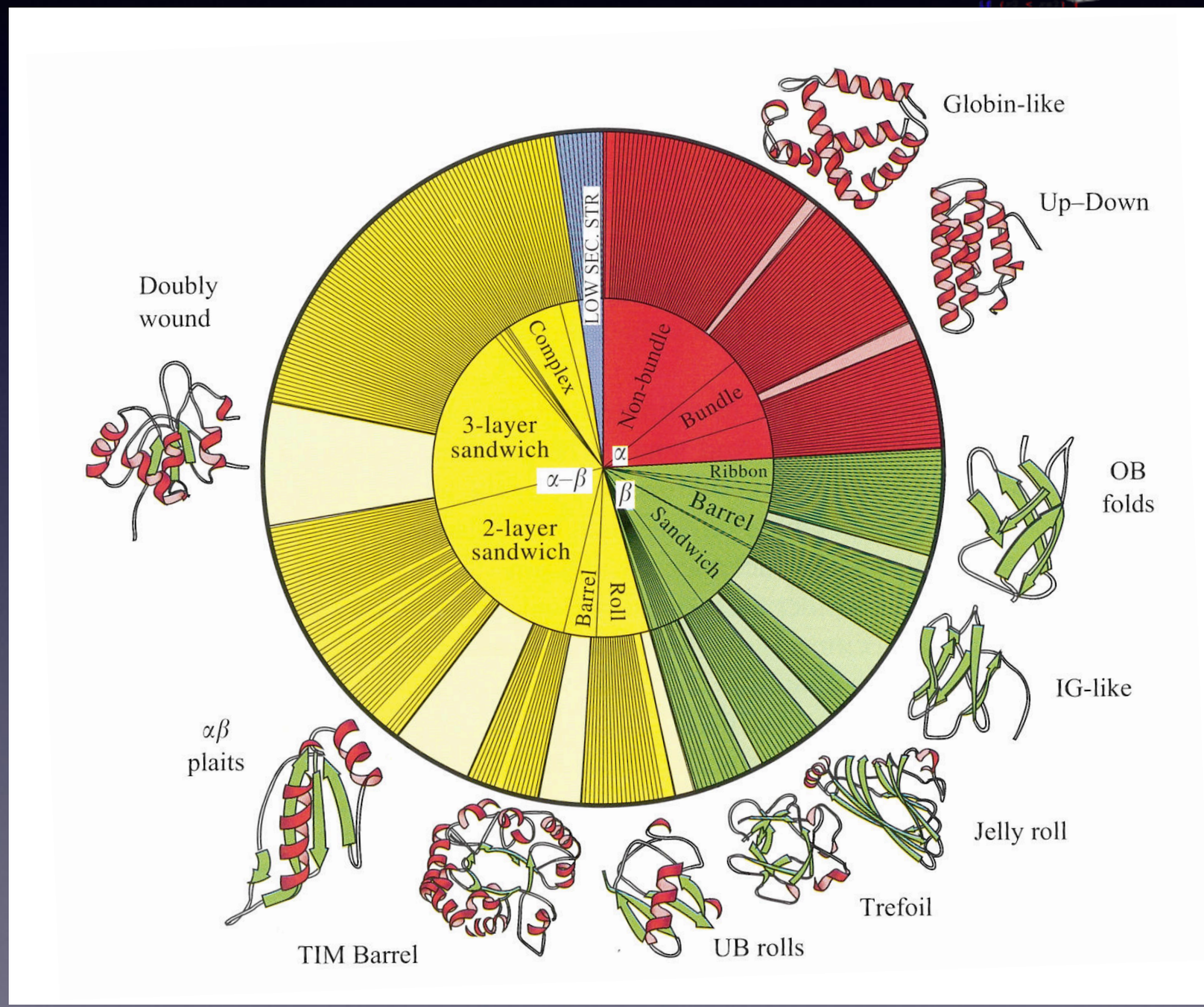


Simple permutations of helices/sheets

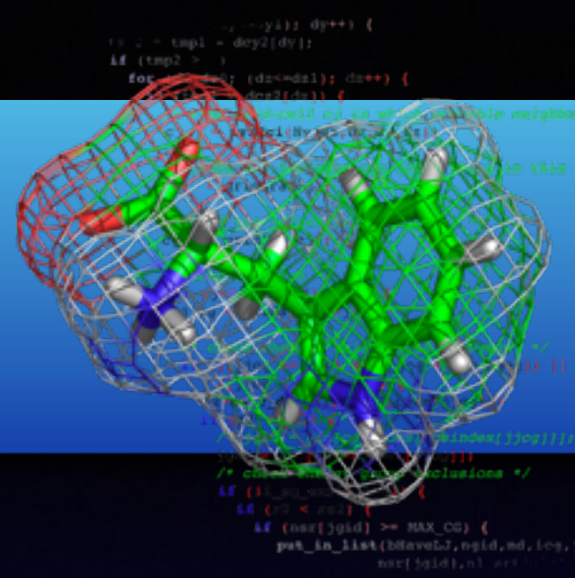
Stable local patterns (lots of h-bonds)

Hydrophobic patterns

Contiguous sheets



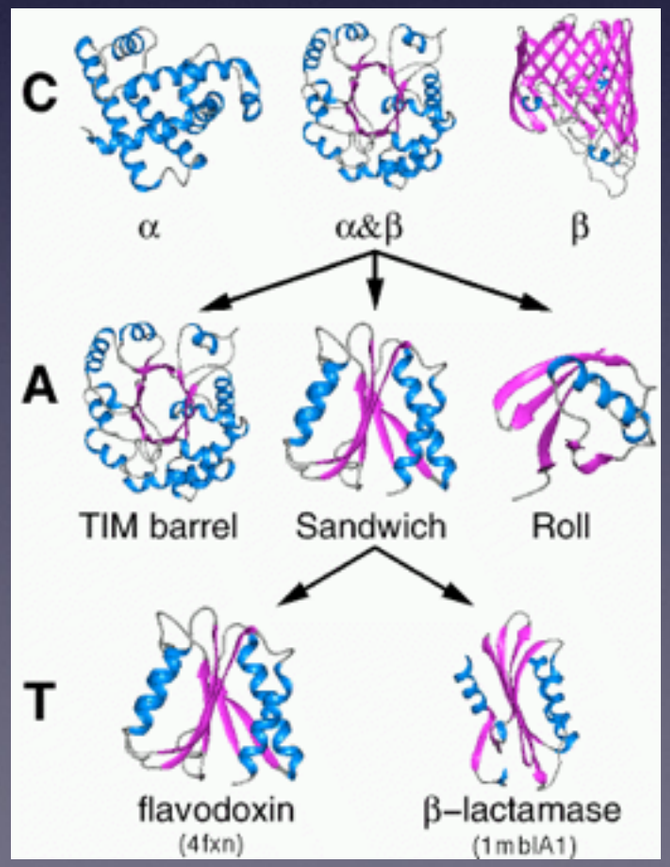
Fold classifications

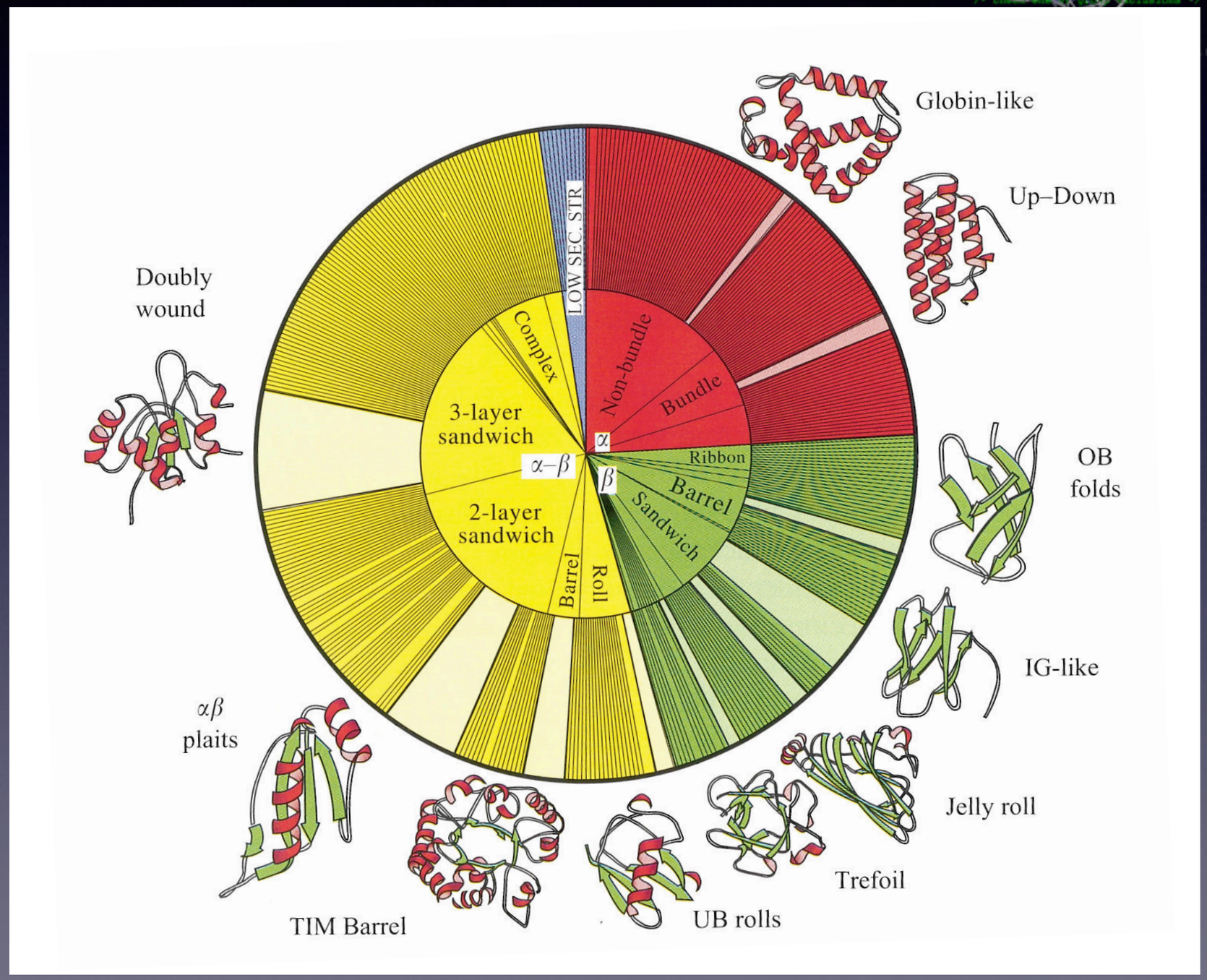


- Structural alignments
- CATH
- SCOP

CATH - 90 % automatic

Class
Architecture
Topology
Homology





CATH - 235,858 domains

Orengo & Thornton

Domain: 1fuiA03

http://cathwww.biochem.ucl.ac.uk/cgi-bin/cath/Domain.pl?domain_id=1fuiA03

CATH

Domain: 1fuiA03

CATH Protein Structure Classification

CATH DHS Gene3D FTP

Home > Top >

Search

Go!

Goto

SSAP Server
CATHEDRAL Server
DHS
Gene3D

Navigation

Home
Top of hierarchy

Cath Update

Not logged in: [login]
DB cathdb_v3_1_0

Domain: 1fuiA03

Version: v3_1_0 | Version: current

Domain: 1fuiA03

Status

The domain has been assigned to a CATH superfamily and does not require any further processing.

Classification (3.20.14.10.1.1.1.1)

Class 3
Alpha Beta

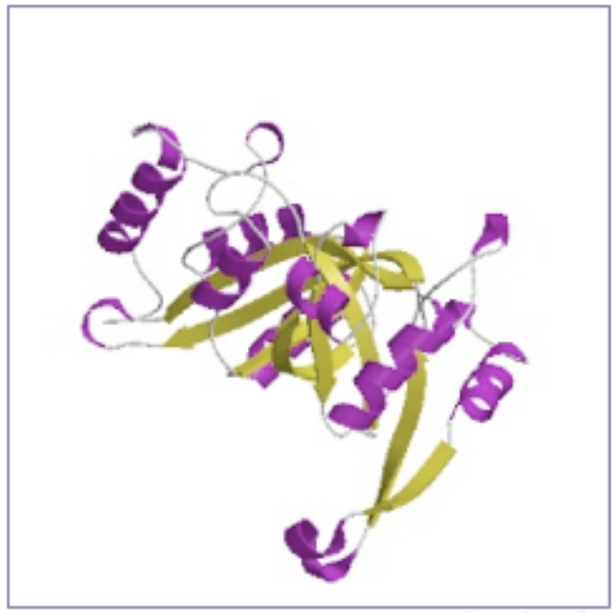
Architecture 3.20
Alpha-Beta Barrel

Topology 3.20.14
L-fucose Isomerase; Chain A, domain 3

Homologous Superfamily 3.20.14.10
L-fucose Isomerase; Chain A, domain 3

S35 Family 3.20.14.10.1

S60 Family 3.20.14.10.1.1



Domain Boundaries

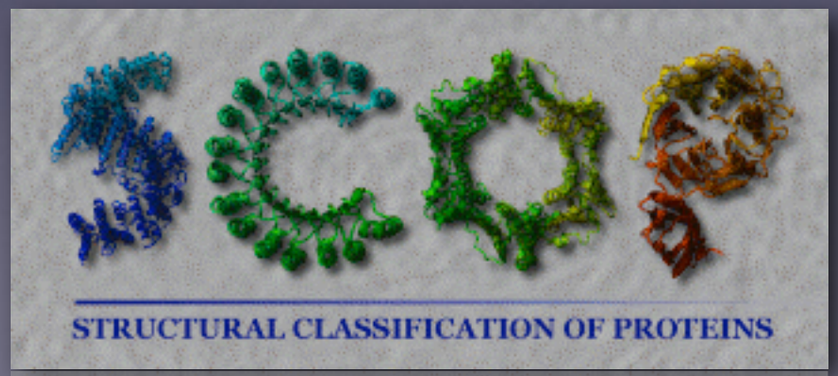
SCOP - 192,710 domains

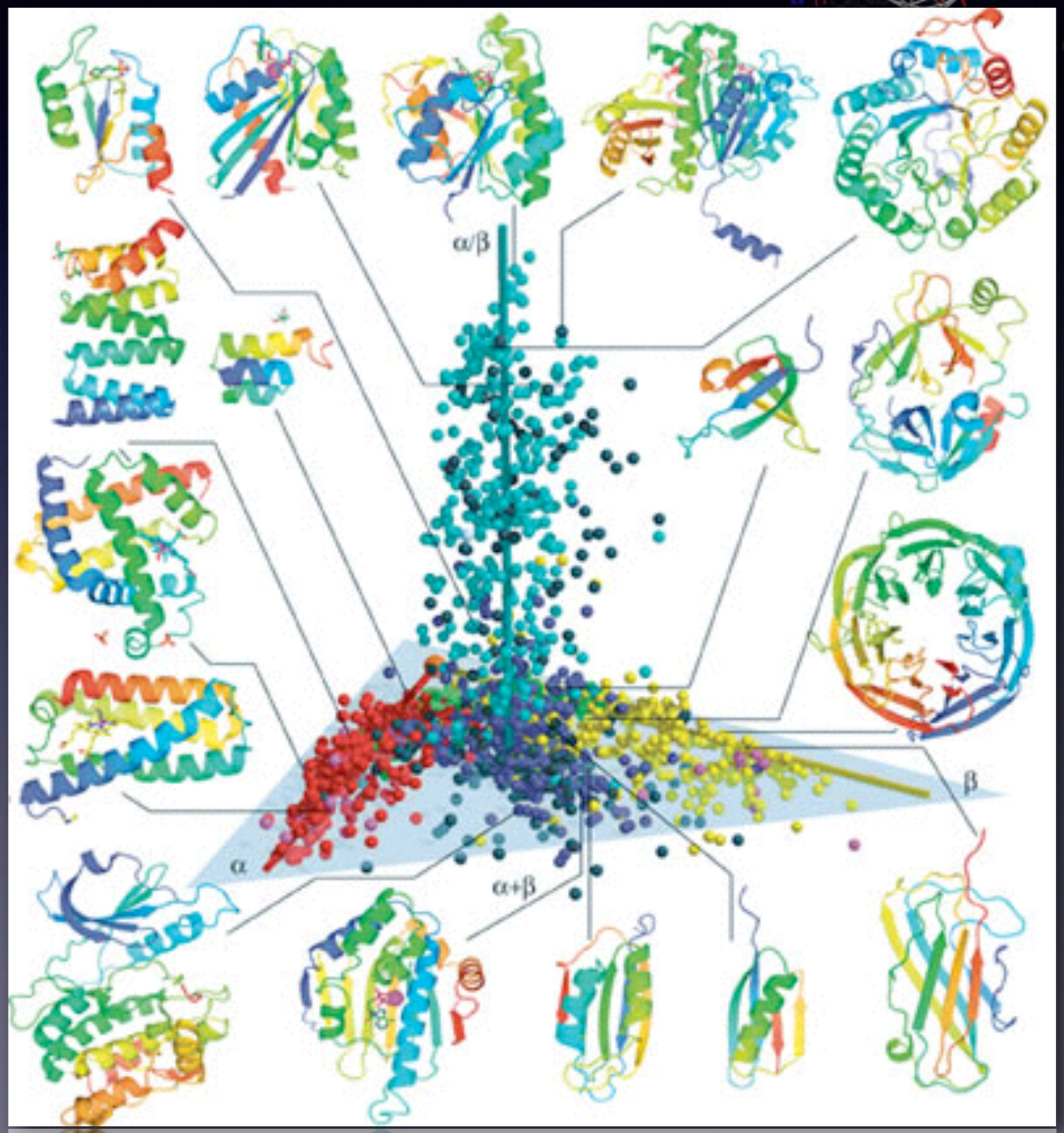
Access methods

- Enter SCOP at the **top of the hierarchy**
- Keyword search of SCOP entries
- SCOP parseable files
- All SCOP releases and reclassified entry history
- SCOP domain sequences and pdb-style coordinate files (ASTRAL)
- Hidden Markov Model library for SCOP superfamilies (SUPERFAMILY)
- NEW Structural alignments for proteins with non-trivial relationships (SISYPHUS)

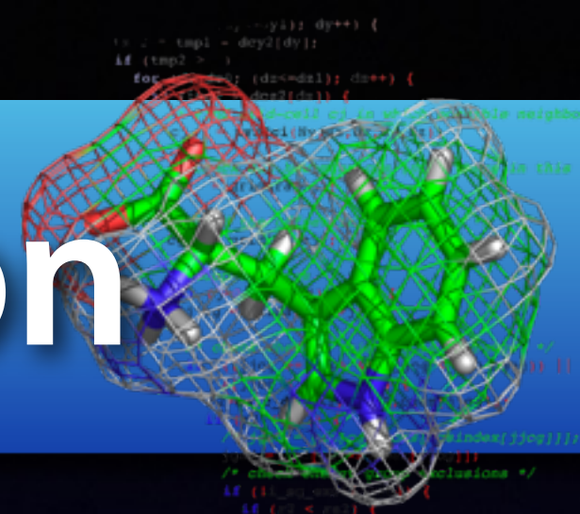
ASTRAL, SUPERFAMILY, etc.

Murzin, Brenner, Chotia



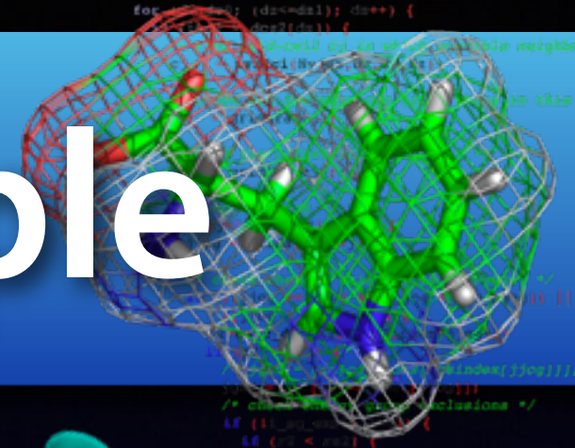


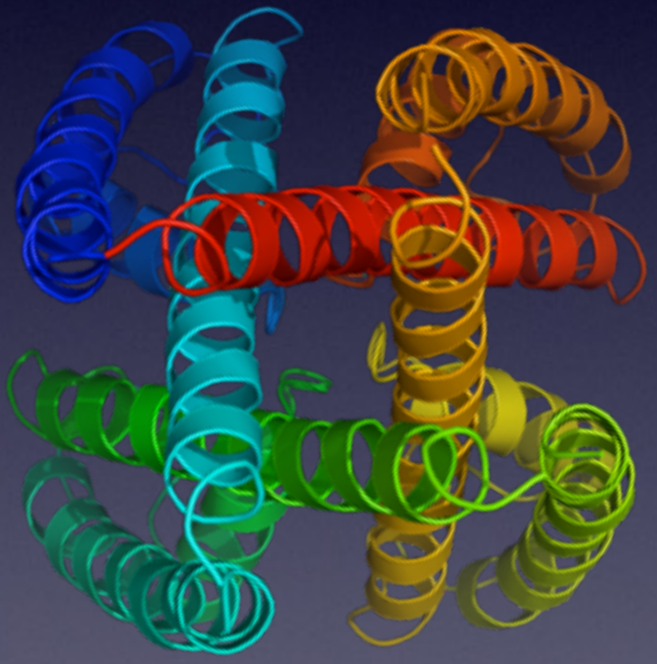
Structural Evolution



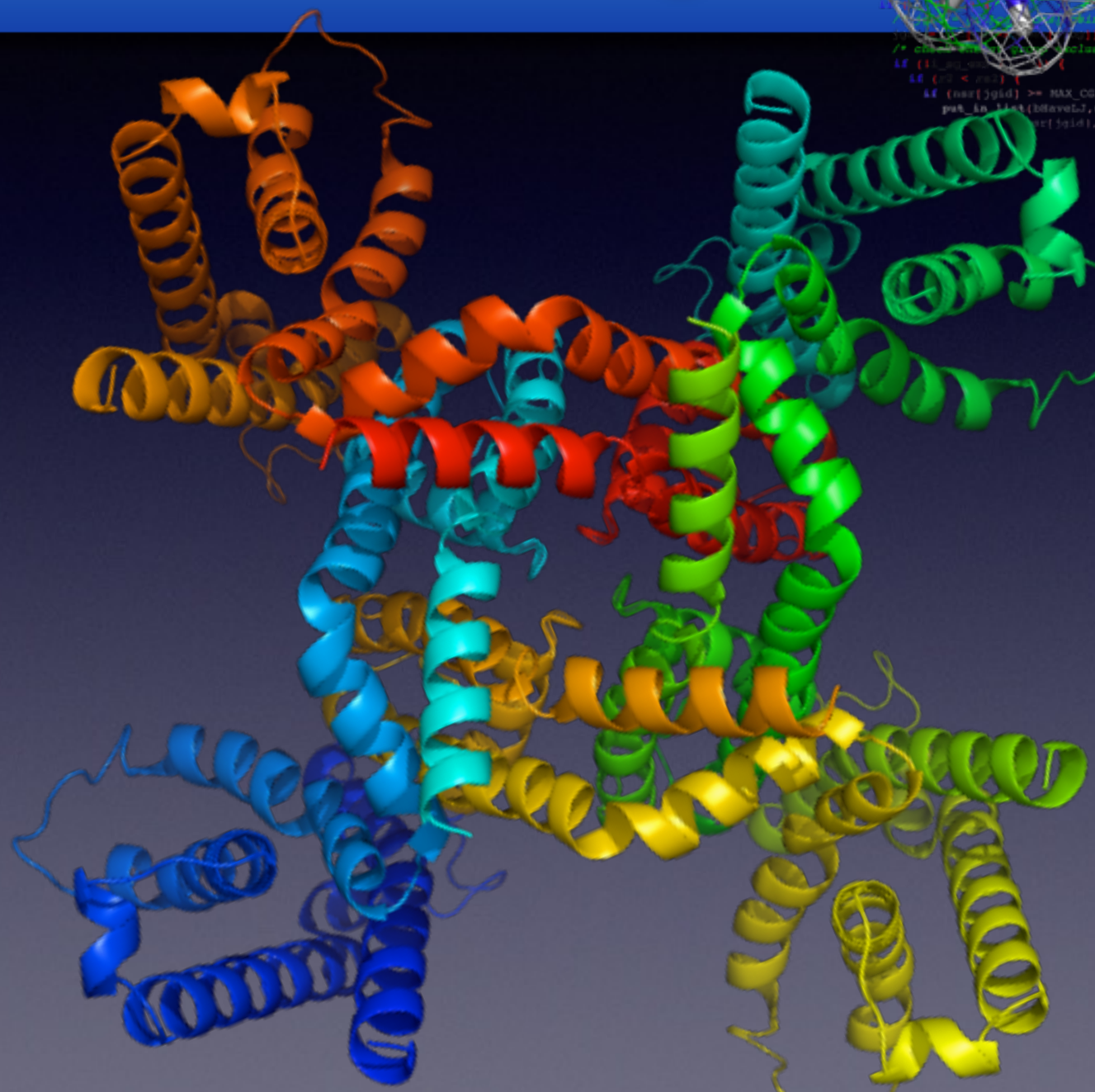
- Llama hemoglobin binds oxygen harder than pony/horse hemoglobin
- Fetal hemoglobin is different from adult!
- Genes can be shut on/off in organisms
- Are eukaryotic/vertebrate proteins more complex than prokaryotic ones?
  - Folding patterns seem to be similar
  - Eukaryotic proteins sometimes have more domains, and they can be larger

K+ channel example



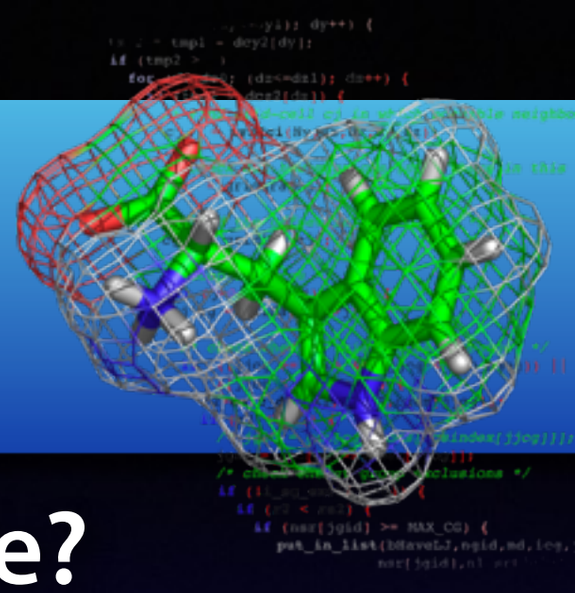


KcsA (bacterial)

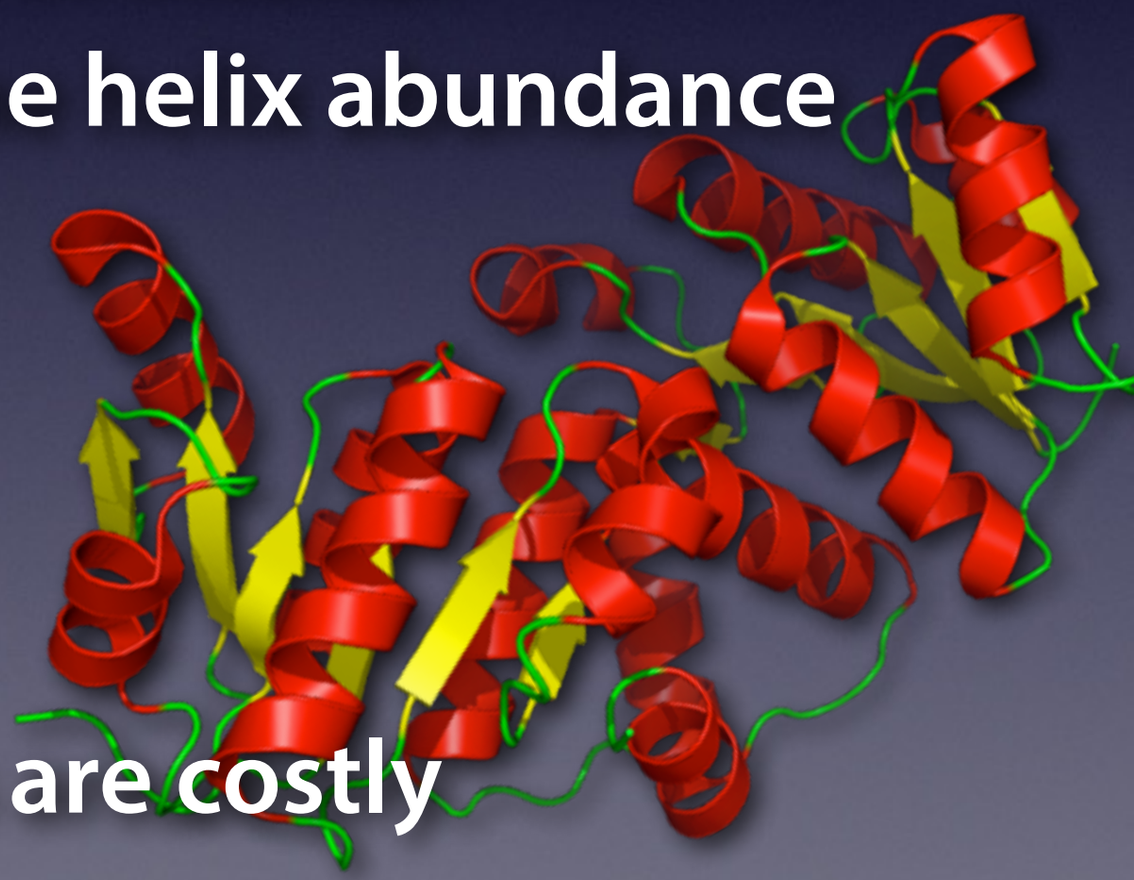


Kv1.2 (eukaryotic)

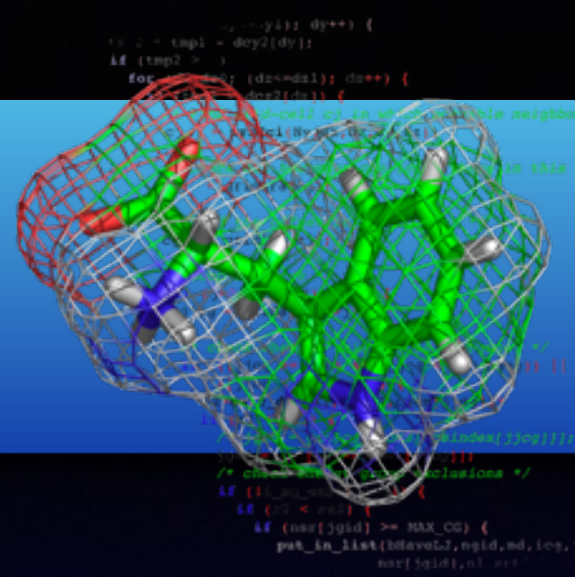
Structural stability



- Why are the common structures stable?
- H-bond saturation!
  - Loops/coil cannot exist in interior
  - Also explains membrane helix abundance
  - Edges of helices/sheet must face water
  - Helix & sheet regions must be separate
- Structure/energy defects are costly

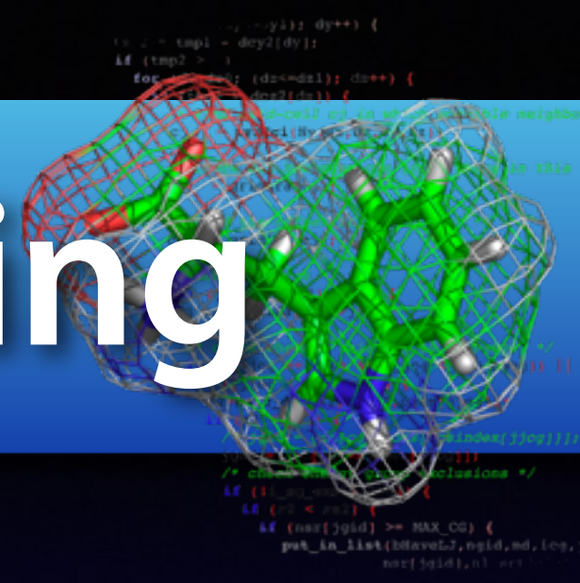


Fold layers



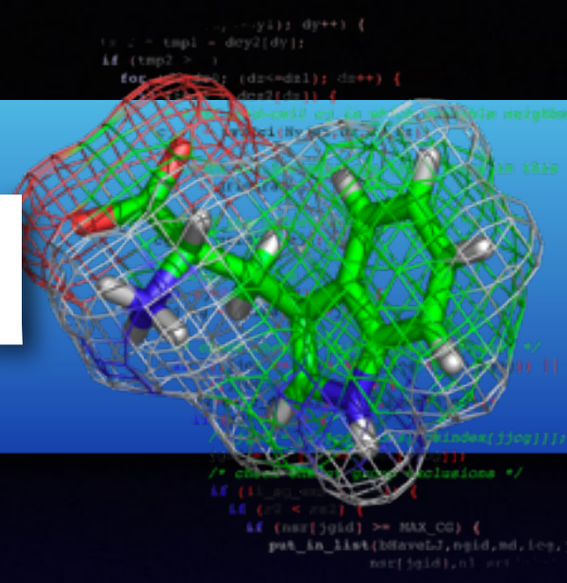
- 1 layer: Not very useful
- 2 layers: Great for shielding
- 3 layers: Rossman fold, double cavities
- 4 layers: Rare, buries hydrophilic aa:s
- 5 layers: Doesn’t occur in practice
- Large proteins by necessity need to be divided into subdomains for stability!

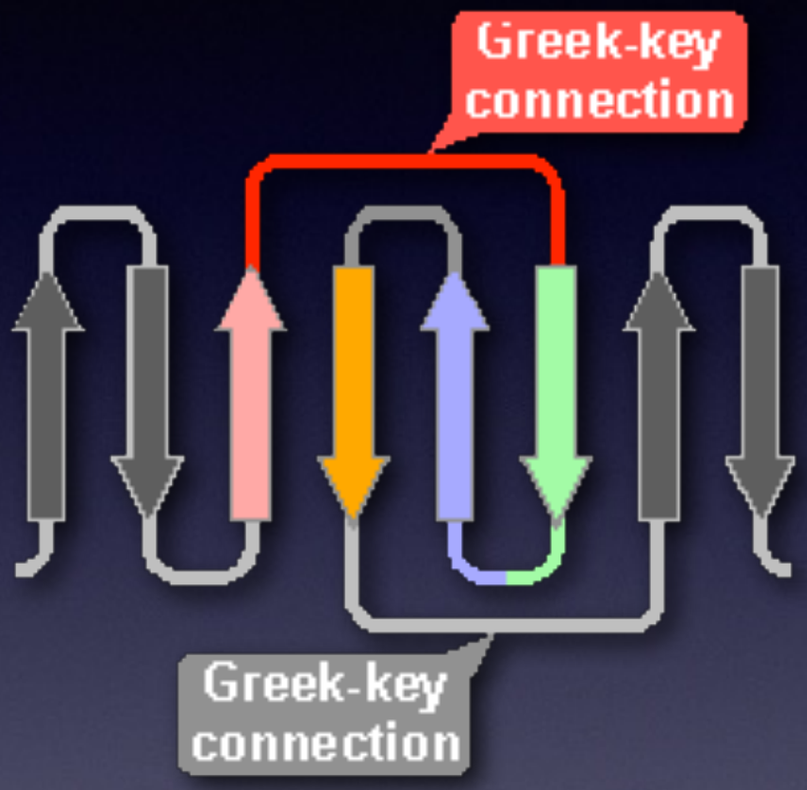
Sequence-fold fitting

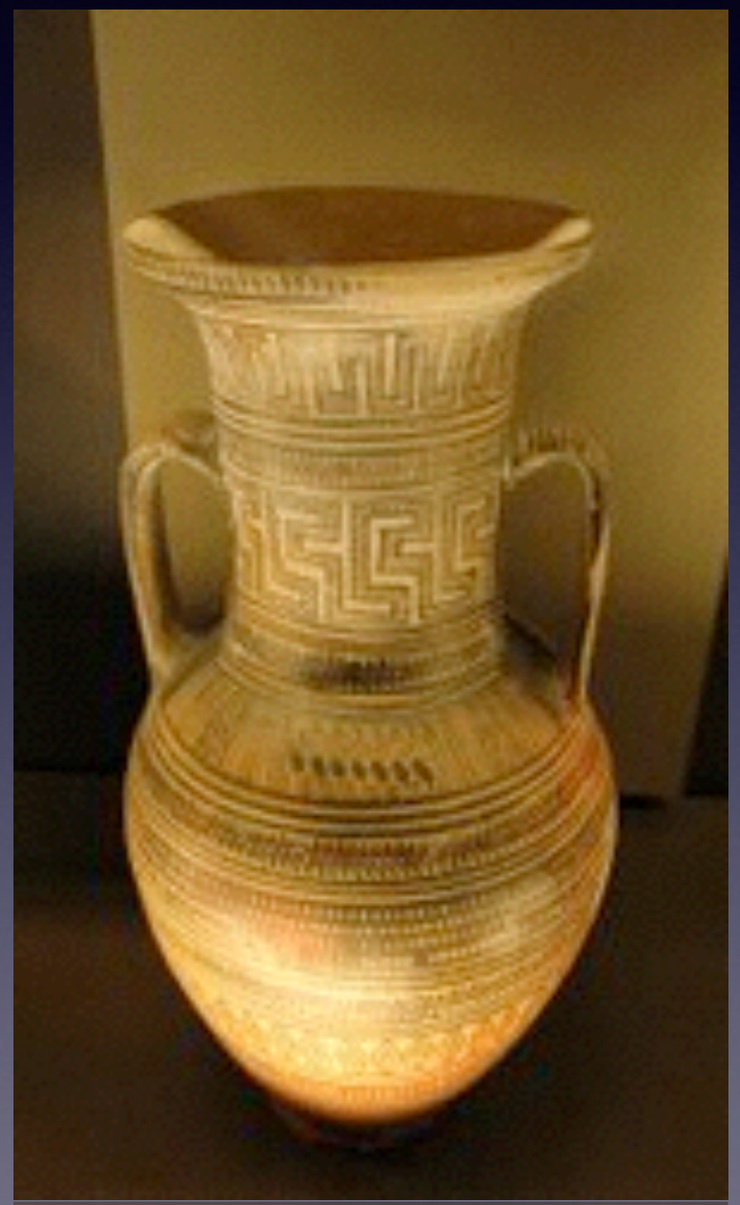


- So, which *sequences* can fit a given fold?
- Simple folds can accommodate lots of sequences - that's why they are common
- A fold with special defects requires special amino acids (e.g. Cys bridges) for stabilization, and can only accomodate a few sequences
- Natural selection at work!

Greek keys, revisited



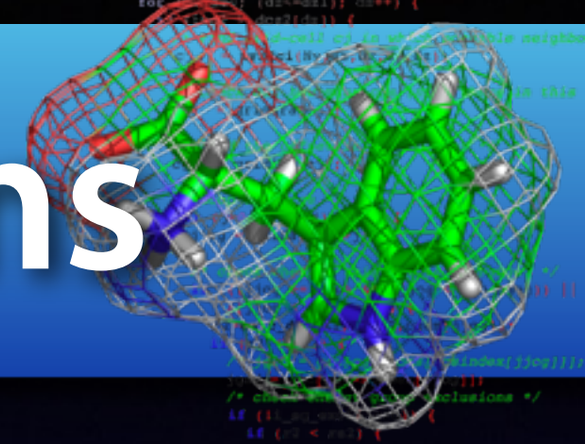




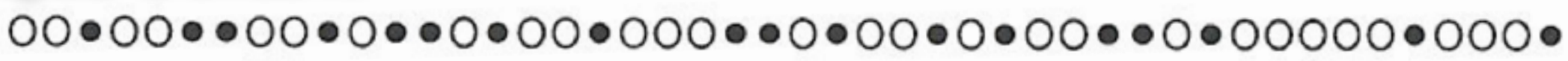
It is not a coincidence that we see this pattern both on vases and in proteins - can you think of why?

(Richardson, Nature 1977)

Sequence patterns



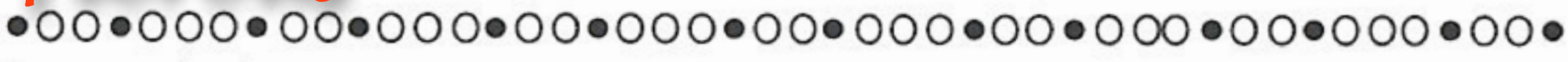
Globular



Membrane

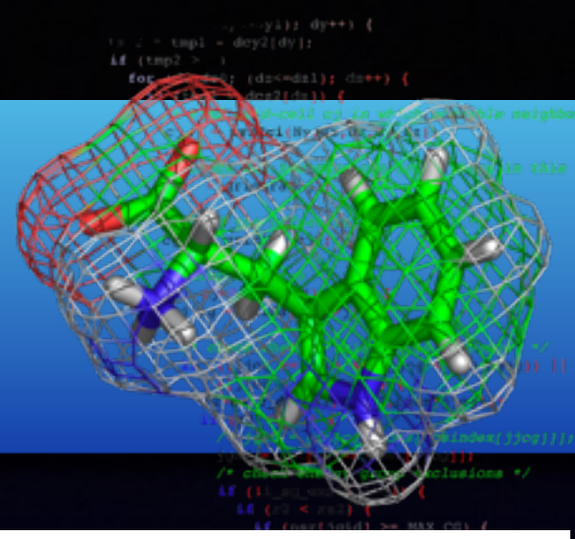
| Hydro-phobic | Hydro-philic |

Fibrous

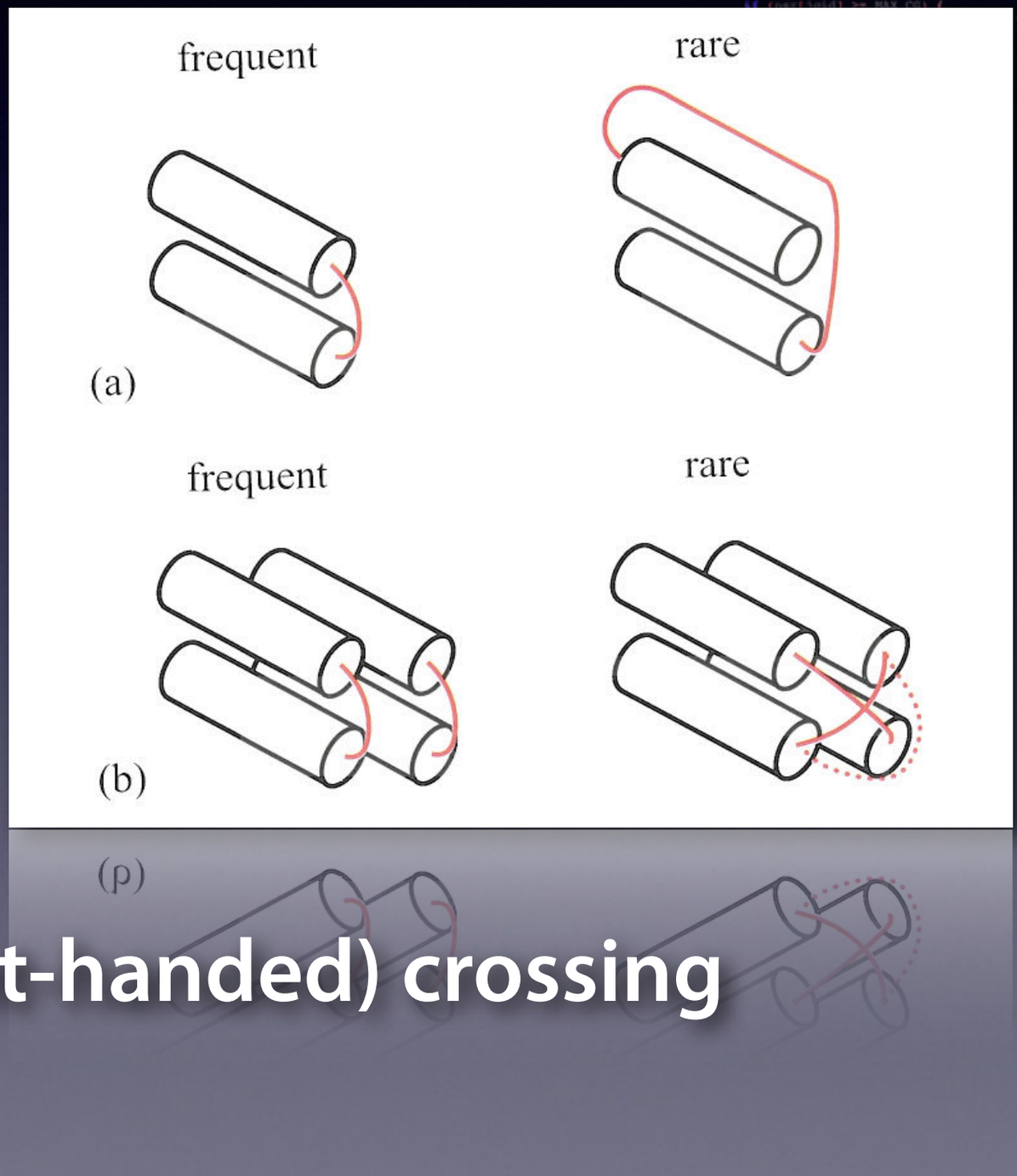


| repeat |

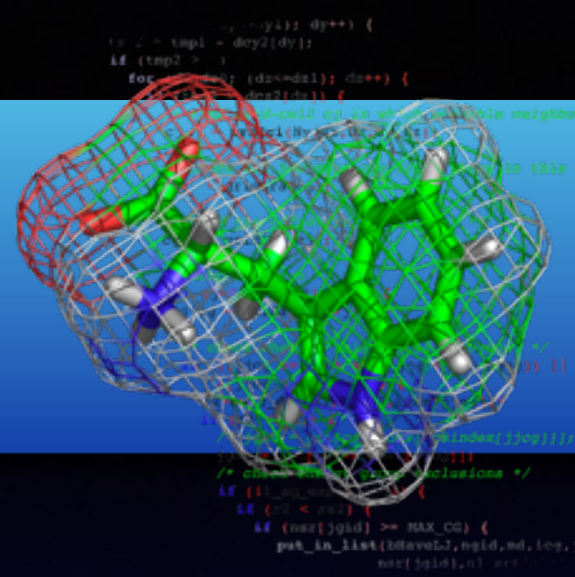
Structural stability



- Why are defects rare?
- Loss of 1-2 h-bonds
- But that would only cost 5-10 kcal/mol?
- Small fraction of total E
- Same for beta sheet (right-handed) crossing

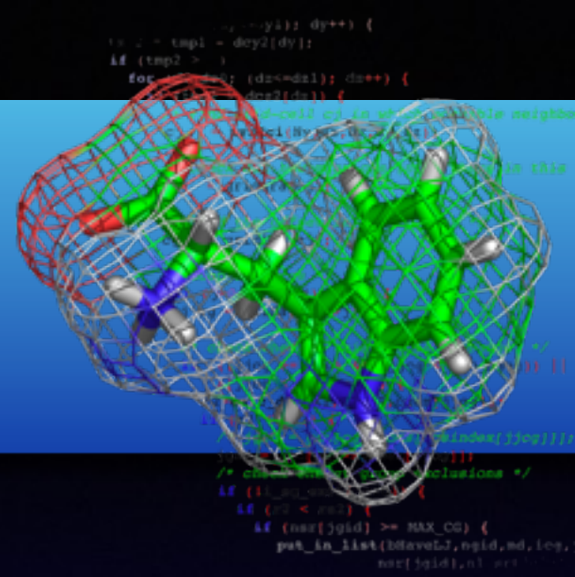


Enthalpy/Entropy



- Chains with limited conformational flexibility can only accommodate few sequences
- Others would have much higher energy
- Chains that can choose between many conformations can accommodate more sequences in low energy states

Boltzmann stats

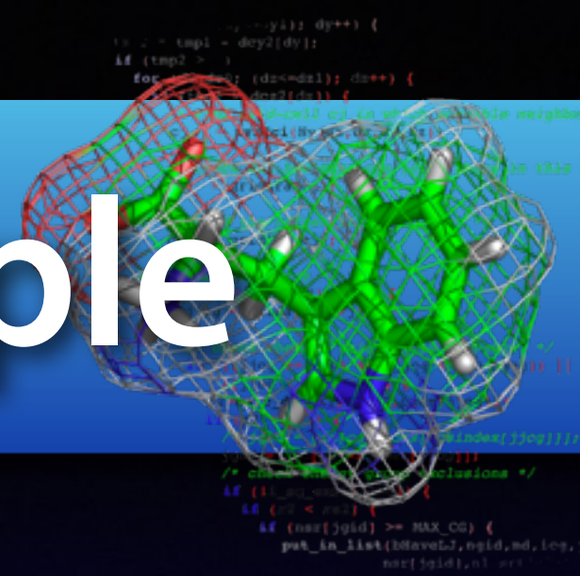


- But we know how to handle this, right?
- Occurence of elements in protein:

$$\rho(r) \propto \exp -\Delta E / kT$$

- Seems to hold up experimentally...
- But it is NOT a Boltzmann distribution!
- Here, the structure is constant, but the question is why many sequences fit it!

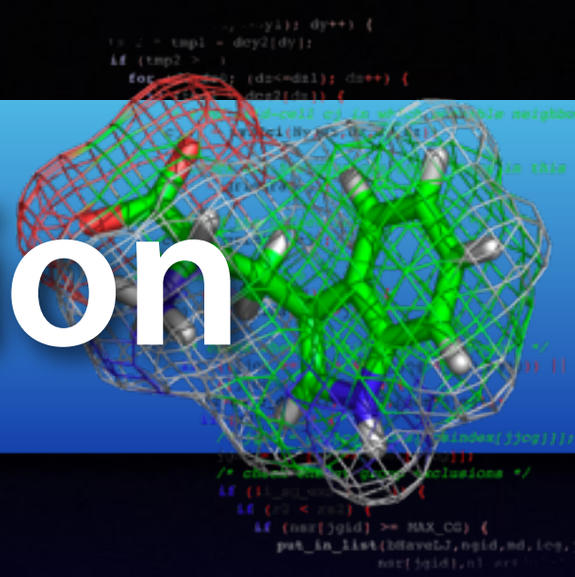
The multitude principle



"The more sequences that can fit a given architecture without disturbing its stability, the higher the occurrence of this architecture in native proteins"

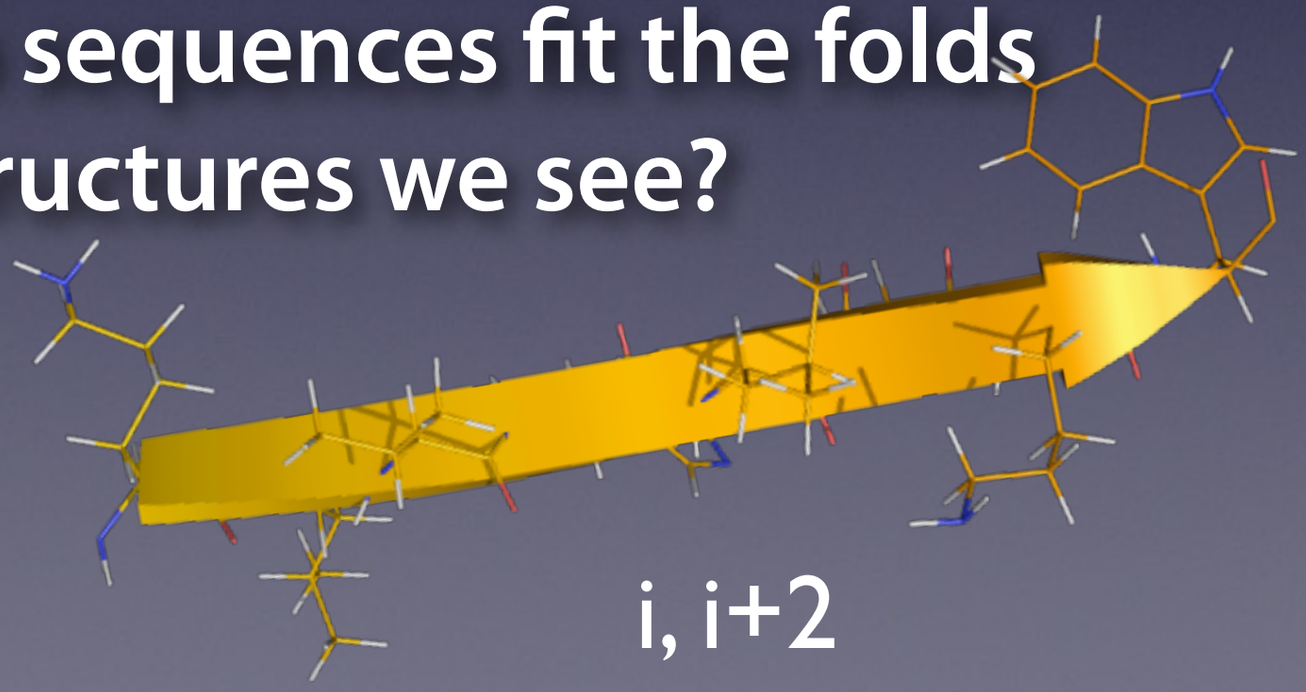
Defective patterns are not impossible, just quite rare!

Sequence stabilization



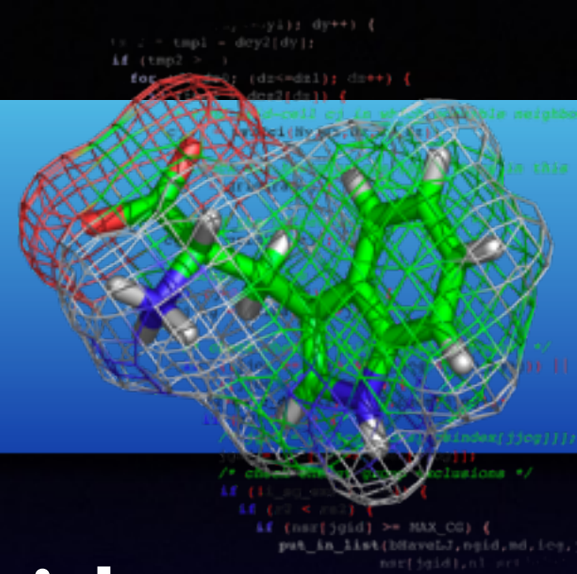
- Limited number of folds for globular proteins
- Approximately equal fractions of hydrophobic/hydrophilic residues (DNA)
- How well do such sequences fit the folds and secondary structures we see?

i, i+3 OR i, i+4



i, i+2

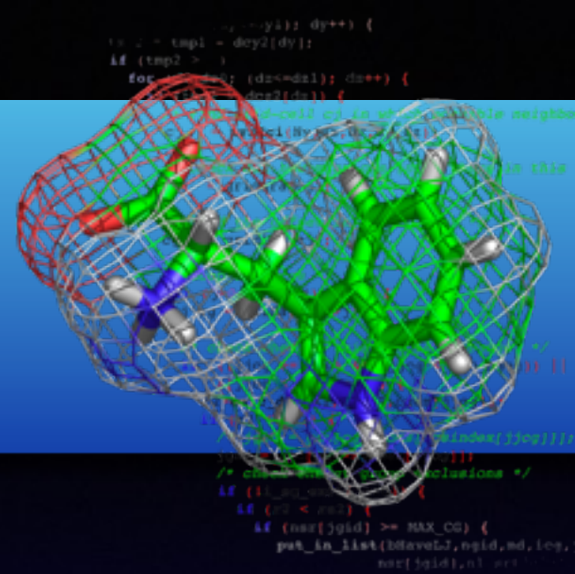
Segment stability



- Let p be the fraction non-polar residues in the sequence
- What is the average number of such groups we will find in a stretch?
- Probability of *r* such groups in a stretch:

$$W(r) = (1-p)p^r(1-p)$$

Segment stability



- **Weighted average:**

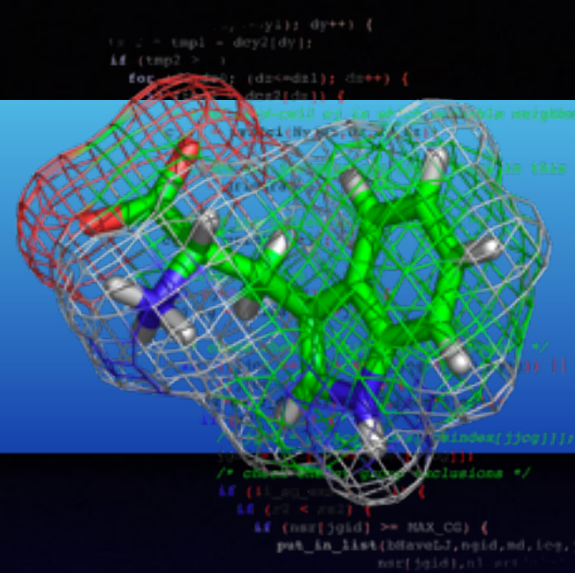
$$\langle r \rangle = \frac{\sum_{r\geq 2}[W(r)r]}{\sum_{r>2} W(r)} = \frac{\sum_{r\geq 2} r p^r}{\sum_{r>2} p^r}$$

$$\sum_{r=1}^{n} p^r = \frac{p(1-p^n)}{1-p}$$

$$\langle r \rangle = 2 + \frac{p}{1-p}$$

about 3 for p=0.5!

Helix/sheet length

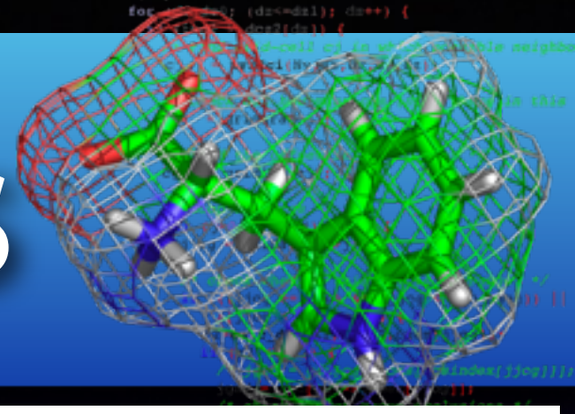


- 3 units of the typical repeat?
- Alpha helix: 3*3.6 = 11 residues
- Beta sheet: 3*2 = 6 residues
- Fits quite well with observed lengths!
- Similarly, average loop length:

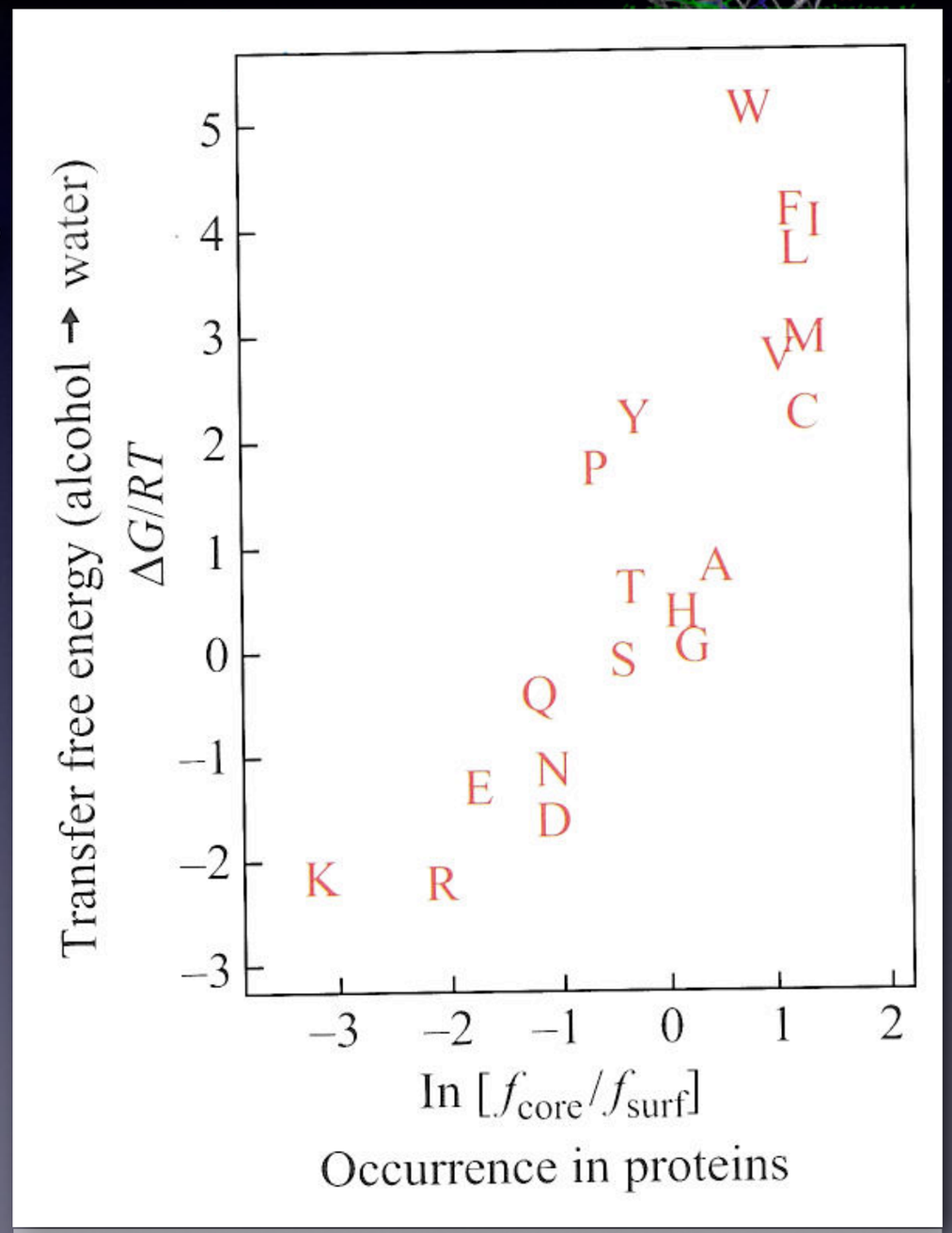
$$\langle r \rangle = 3 + \frac{1}{2p^2}$$

- Even random sequences can form 1 layer!

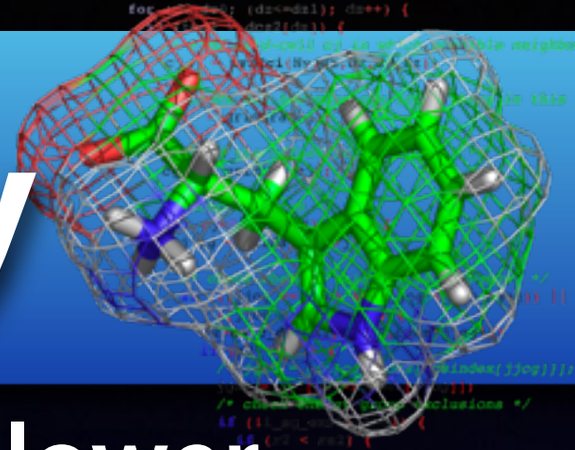
Stability energetics



- Why are energy defects of ~1kcal important for stability?
- What does it have to do with a Boltzmann distribution?
- hydrophobic/hydrophilic residue distribution in structures obey it reasonably well too!?

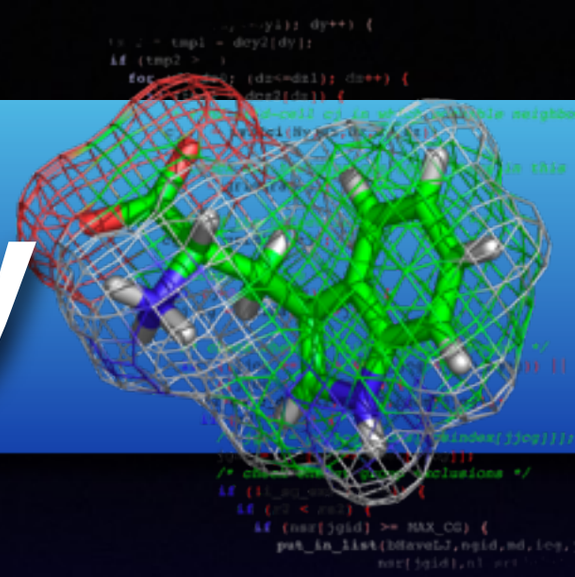


Native fold stability



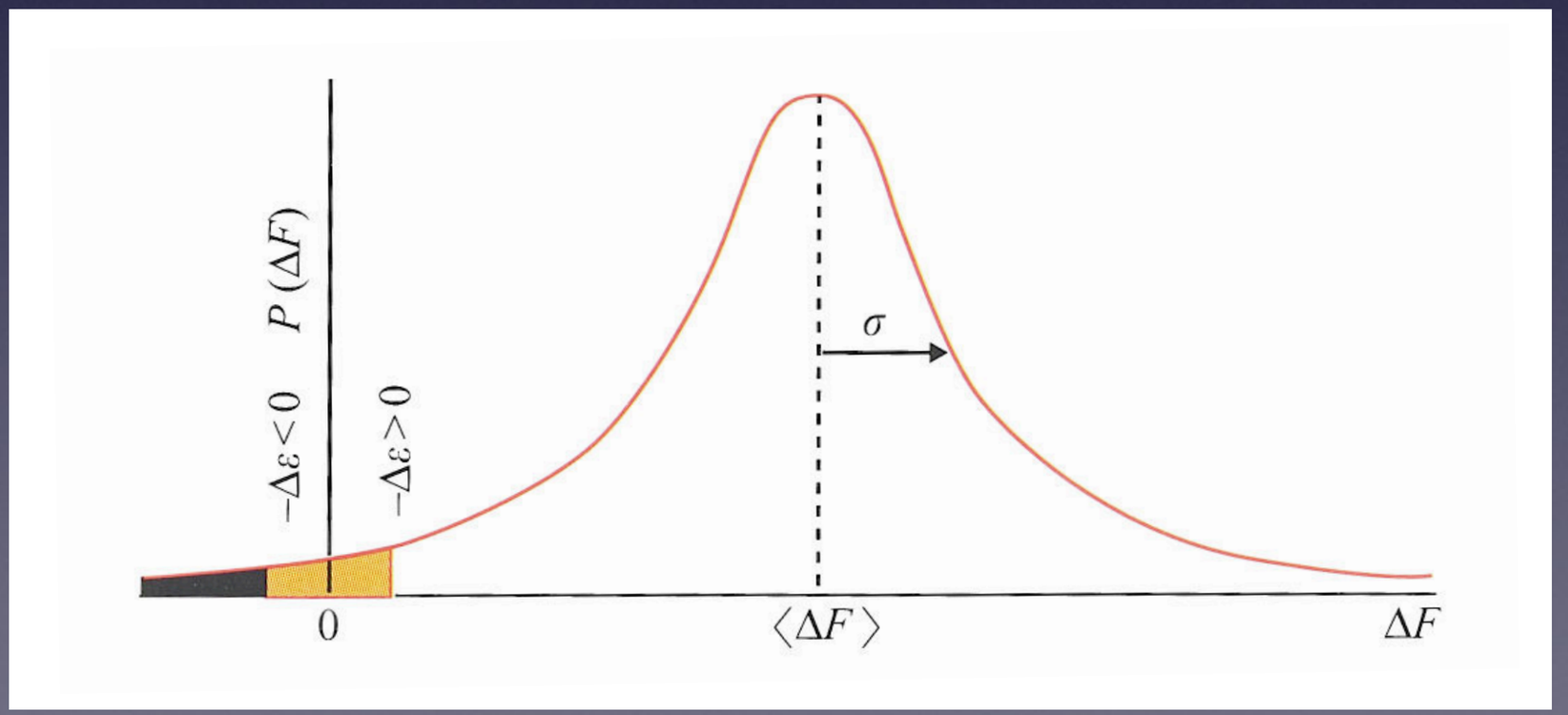
- Native state is stable if free energy is lower (by kT) than for all other states
- Consider Ser <-> Leu mutations
- Transfer from oil (protein inside) to water:
  - Ser: Δε=0 kcal/mol Leu: Δϵ=+2kcal/mol
- Fold with Ser inside also works with Leu
- But fold with Leu works for more seqs!
- Rest of chain: ΔF Total: ΔF+Δε

Native fold stability

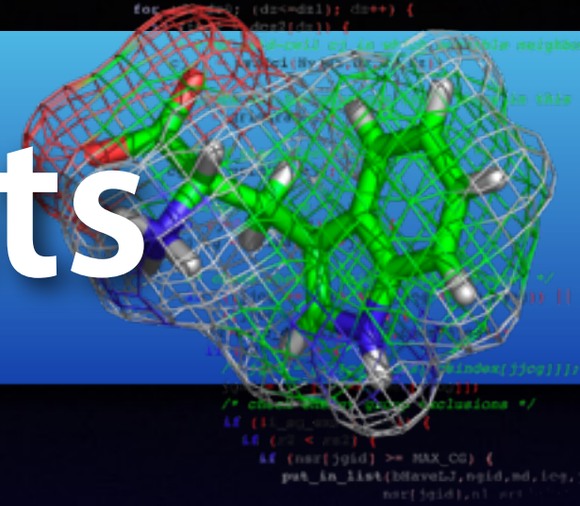


- Stable fold if ΔF < -Δε :

$$p(\Delta F < -\Delta\varepsilon) = \int_{-\infty}^{-\Delta\varepsilon} P(\Delta F)\, d(\Delta F)$$



Quasi-Boltzmann stats



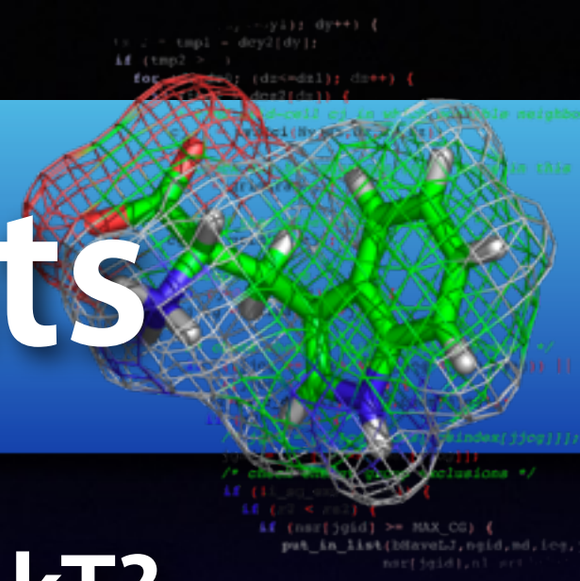
- **Stable fold if ΔF < -Δε :**

$$p(\Delta F < -\Delta\varepsilon) = \int_{-\infty}^{-\Delta\varepsilon} P(\Delta F)d(\Delta F) \approx$$

$$\approx C\exp\left[-\frac{\Delta\varepsilon}{\sigma^2/\langle\Delta F\rangle}\right]$$

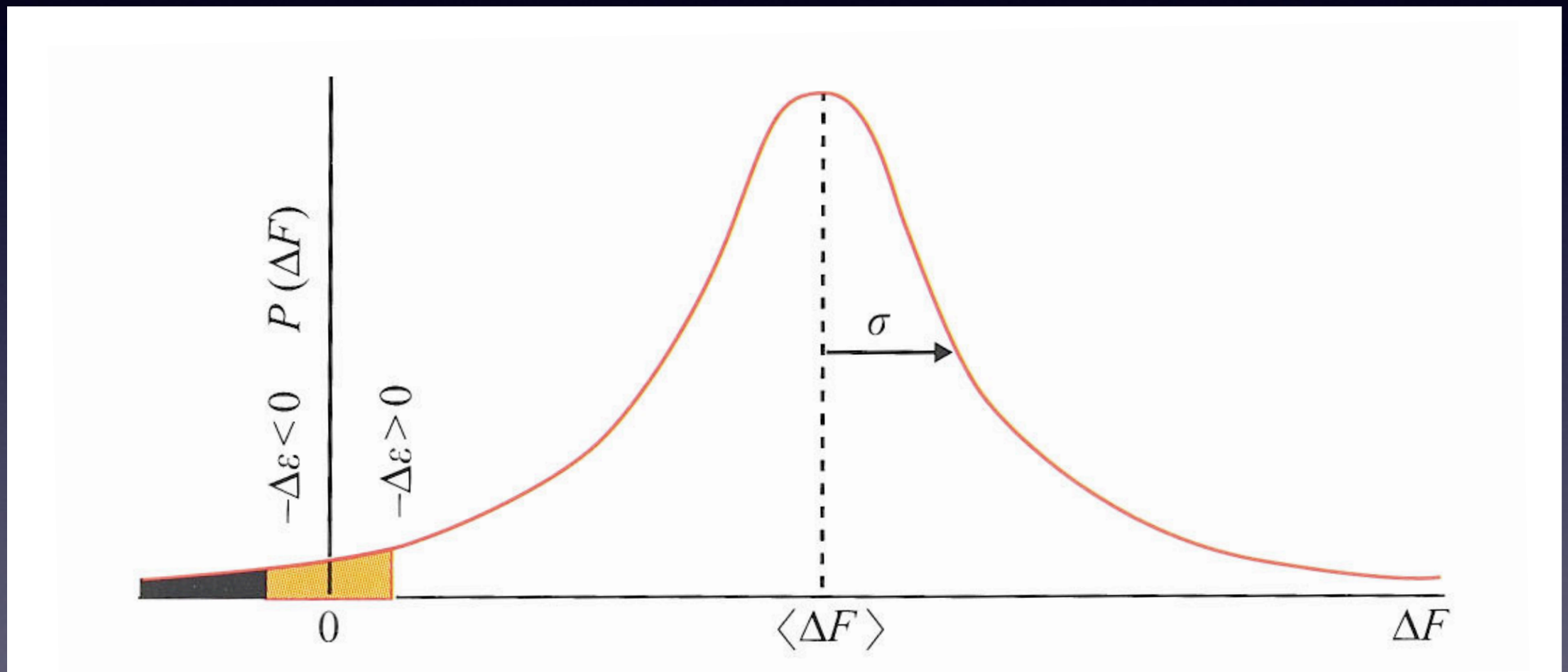
Note the similarity to the Boltzmann distribution!
Increasing Δε reduces the number of stabilizing sequences exponentially

Quasi-Boltzmann stats



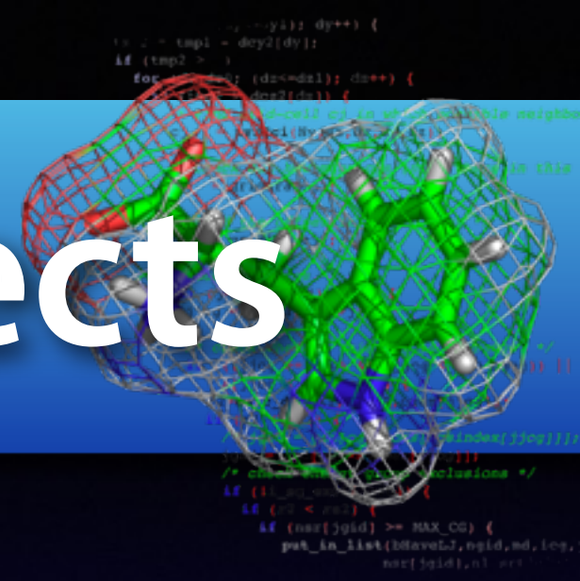
- What does $\sigma^2/\langle F \rangle$ mean rather than kT?
- Both σ^2 and $\langle F \rangle$ are proportional to size
  - The quotient is *size-independent*
- Thus: protein stabilization energy is not dependent on the size of the protein!
- Chain energy or "characteristic energy"
- Think of it as kT_C, with T_C around 350K
- Energy defects should be compared to kT_C rather than the entire protein energy!

Good vs. bad sequences



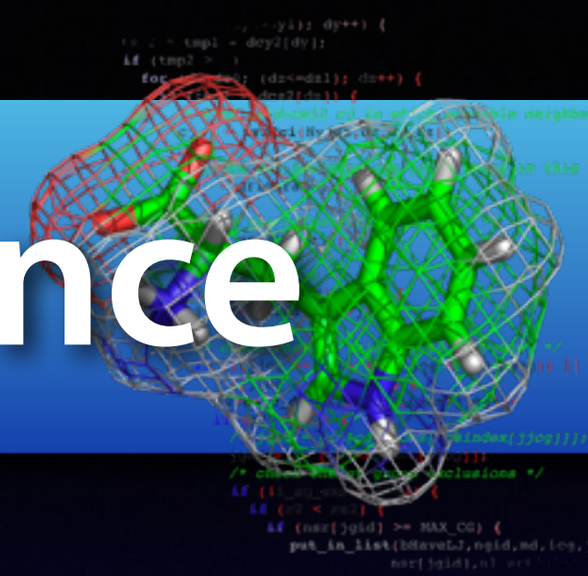
Most sequences do not fold into stable structures!

Entropic packing effects

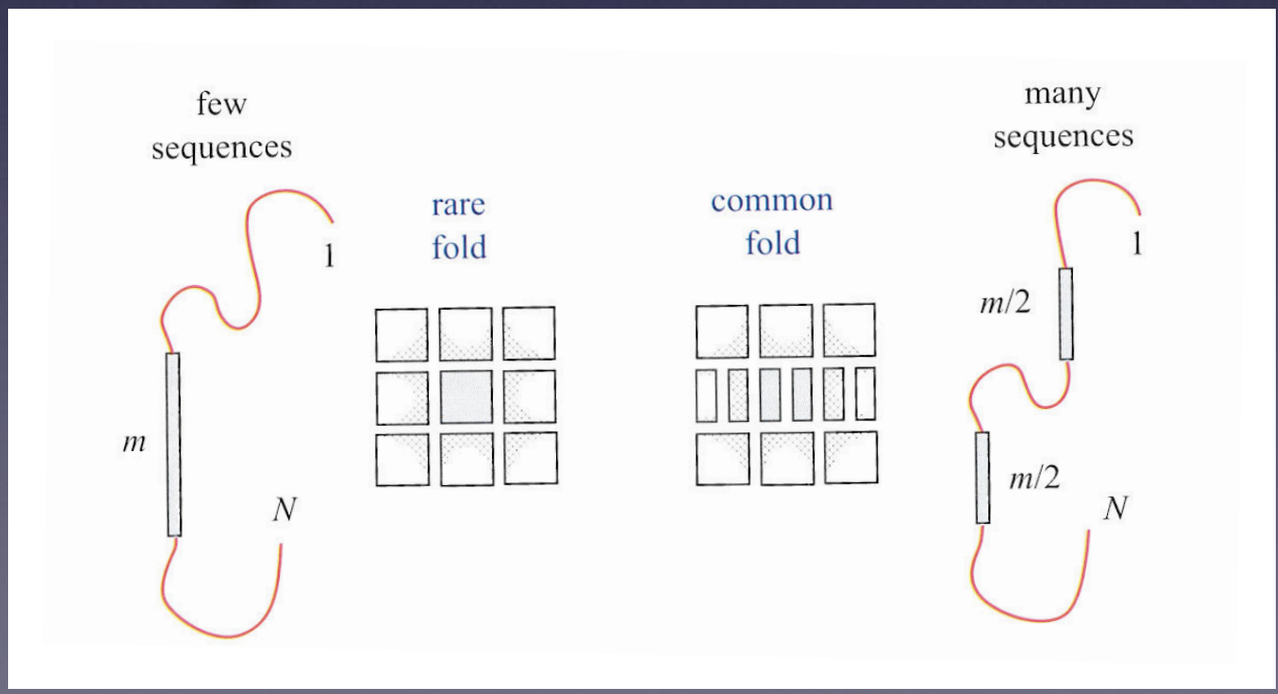


- Example: Left- vs. right-handed sheets
- Structures with more conformational freedom can accommodate more sequences
- Higher density of these states in P(ΔF) means they will be more likely to appear in stable folds
- Same quasi-Boltzmann effect as for the energy distribution before!

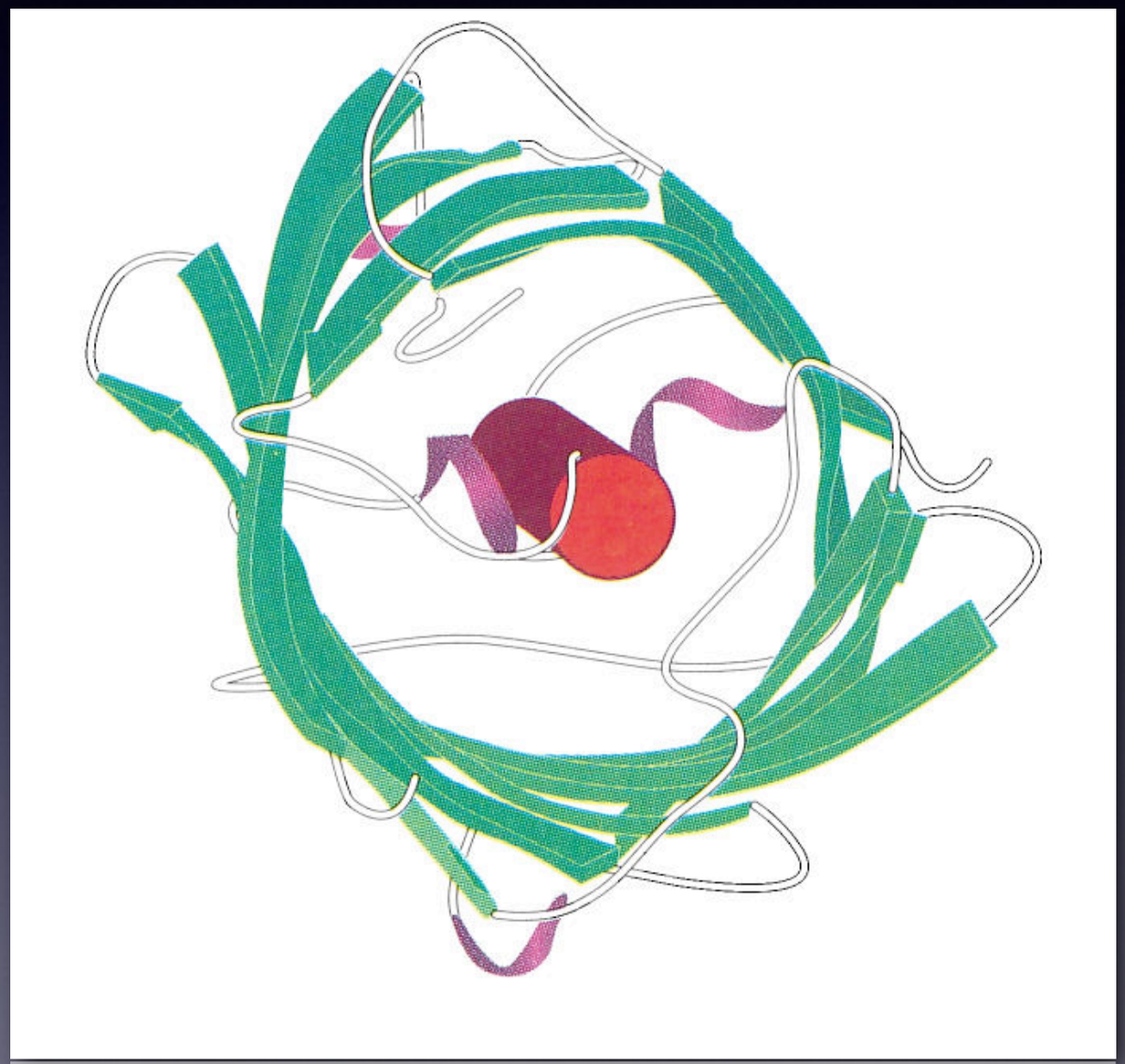
Helix/sheet occurence

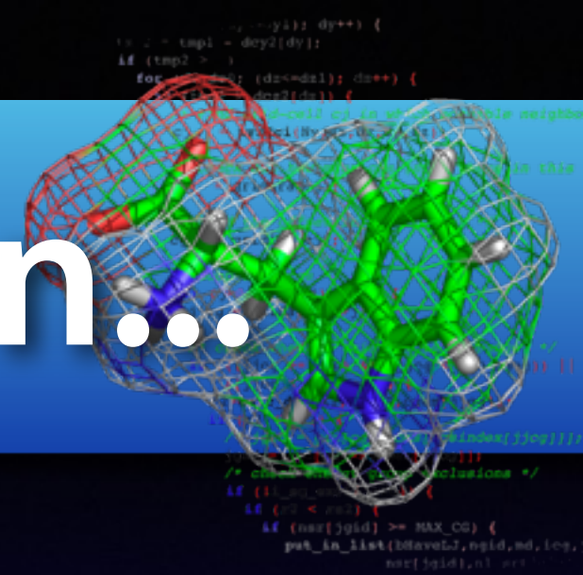


- Which is more common in the protein interior, sheets or helices?
- Sheet: n residues per length
- Helix: 2n residues per length
- Interior must be hydrophobic
- Many more ways to place two small blocks inside!

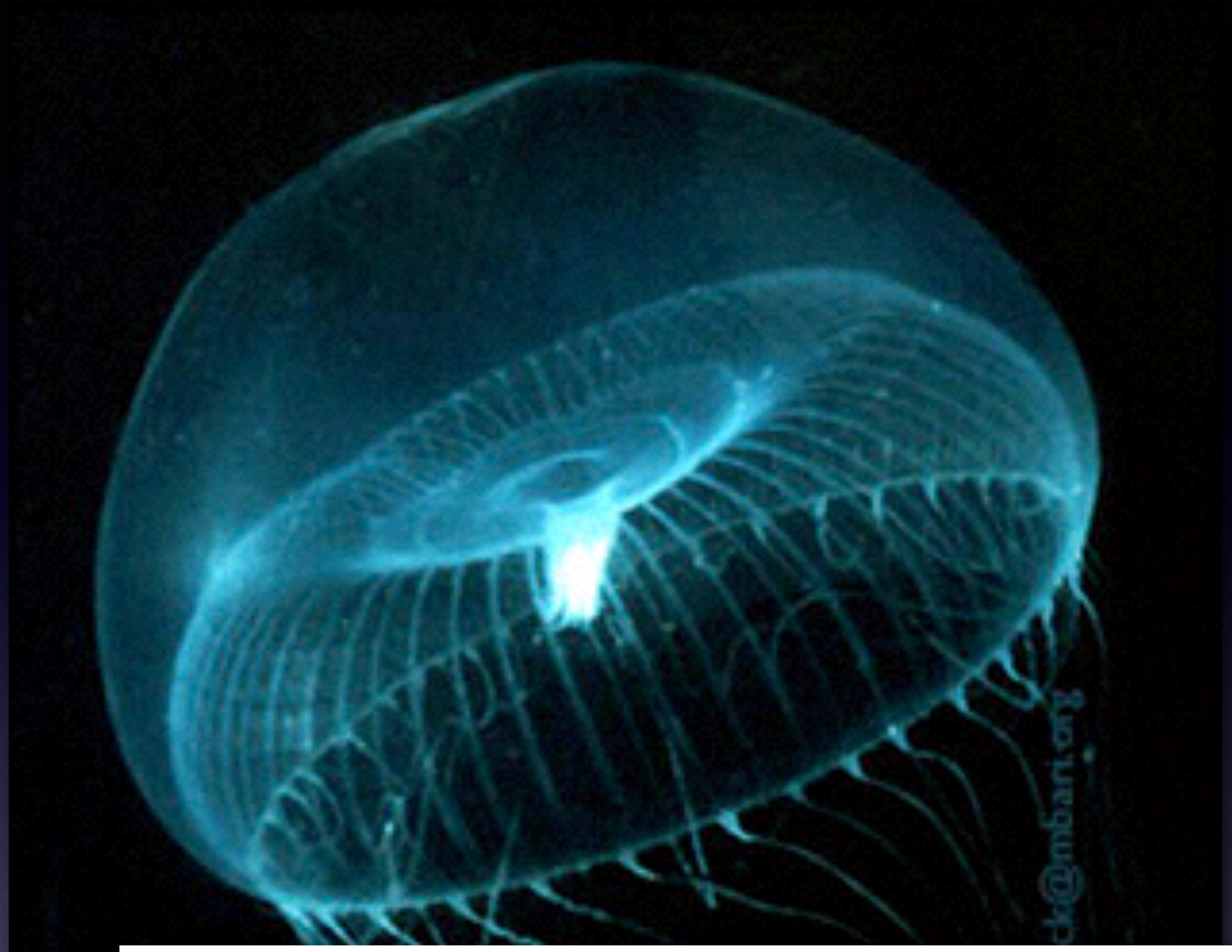


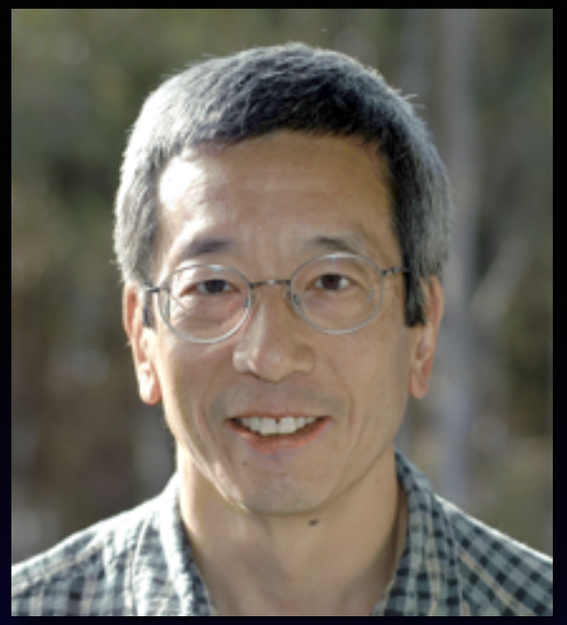
GFP is an exception...





Green Fluorescent Protein





Fluorescent peptides highlight peripheral nerves during surgery in mice

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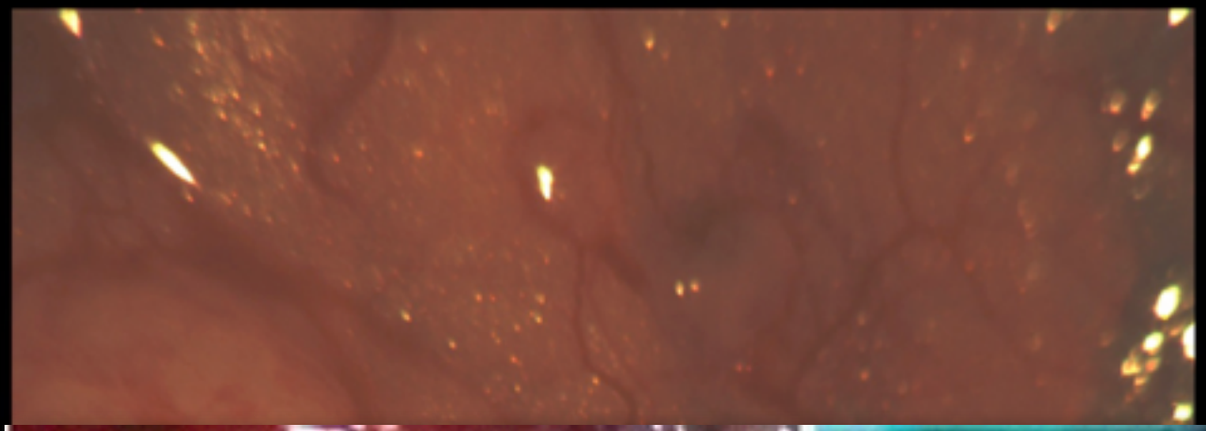
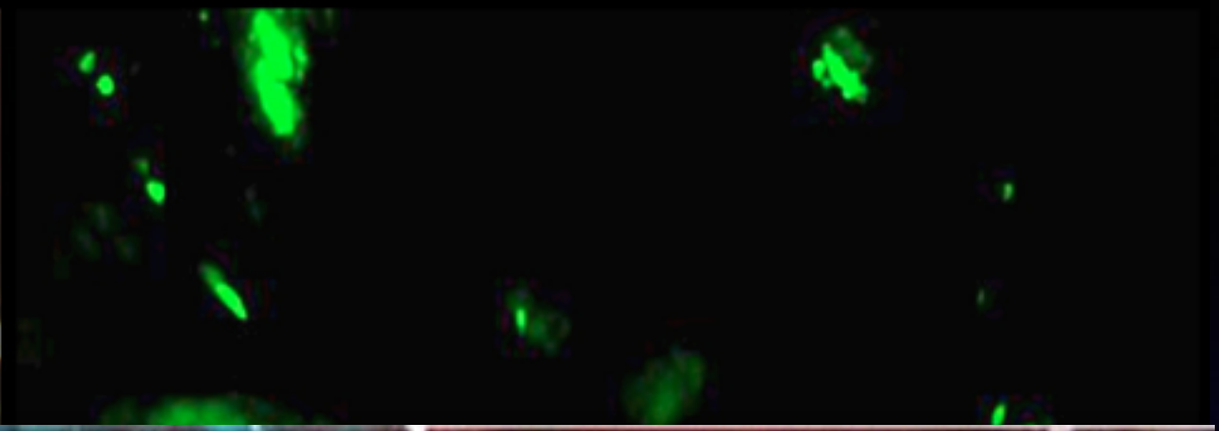
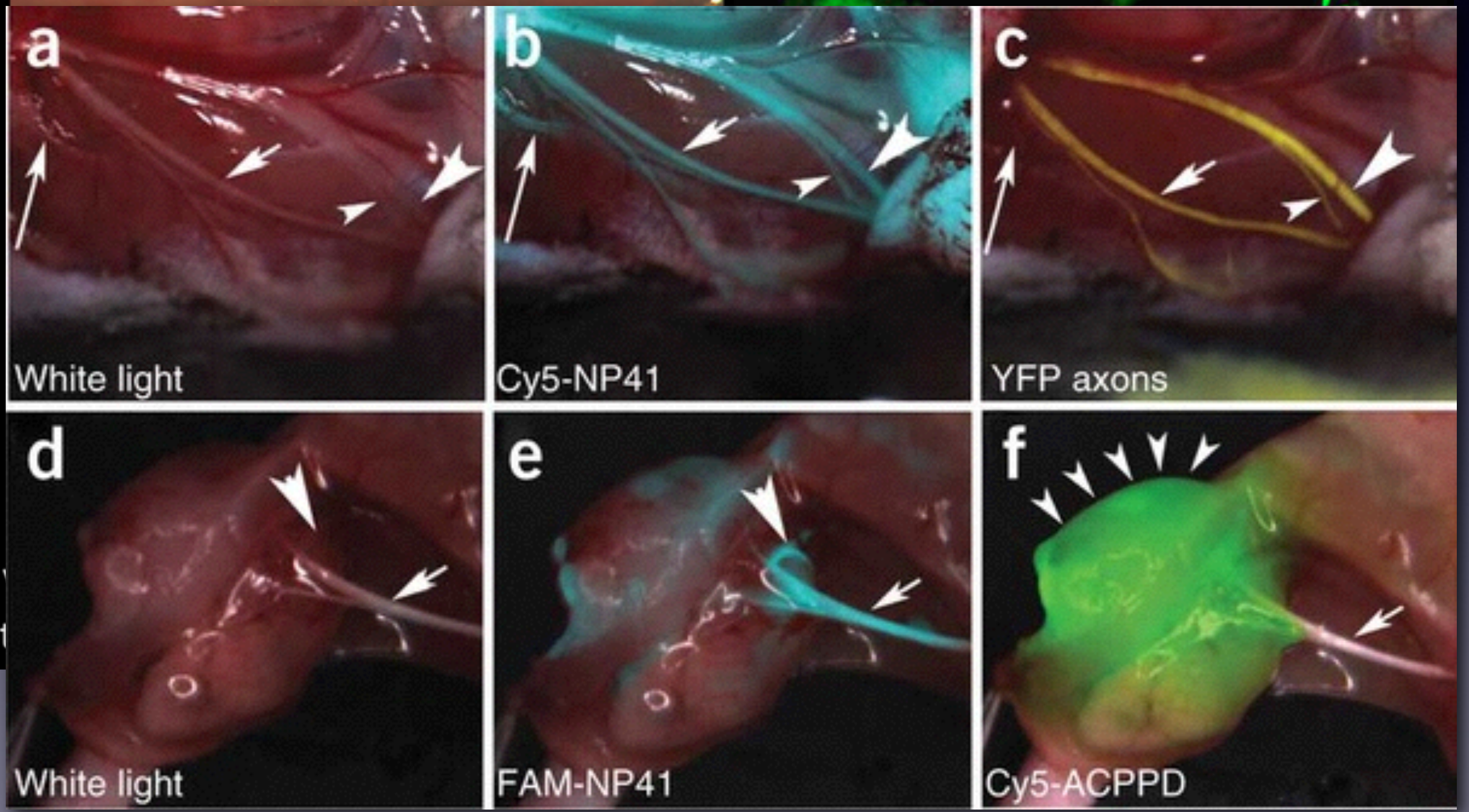
Surgeon's former view

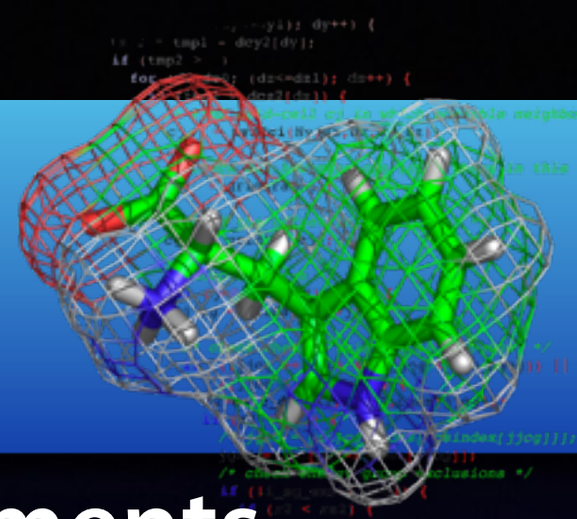
Surgeon's new view







Summary

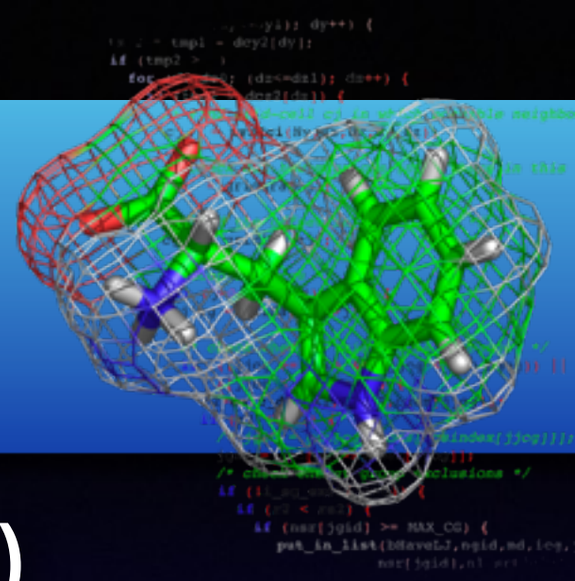


Probability of observing structural elements in randomly created stable globules depends on the amount of sequences that stabilize the fold:

$$\rho(r) \propto \exp -\Delta G / kT_C$$

This is not because of the Boltzmann distribution (no equilibrium), but it has the same shape and a typical temperature.

Summary



- Structure classification (SCOP, CATH)
- Structural evolution
- Size of helices/sheets
- Sequence-structure compatibility
- Protein folds are stabilized by only tens of kcal/mol, regardless of size
- Compare to characteristic energy kT_C
- It will be very hard to design *de novo* folds
- Read chapters 15 & 16!