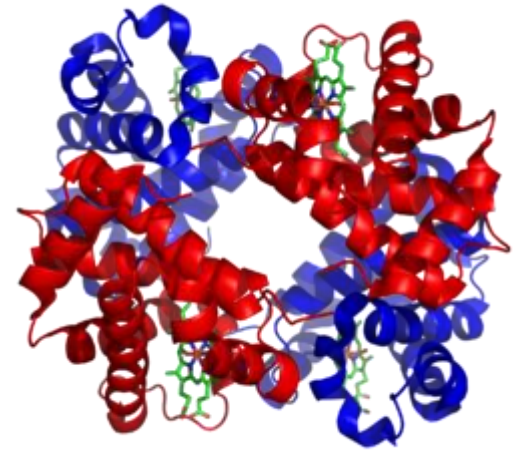
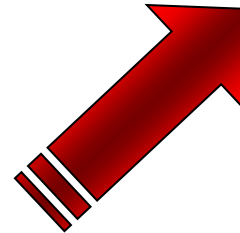
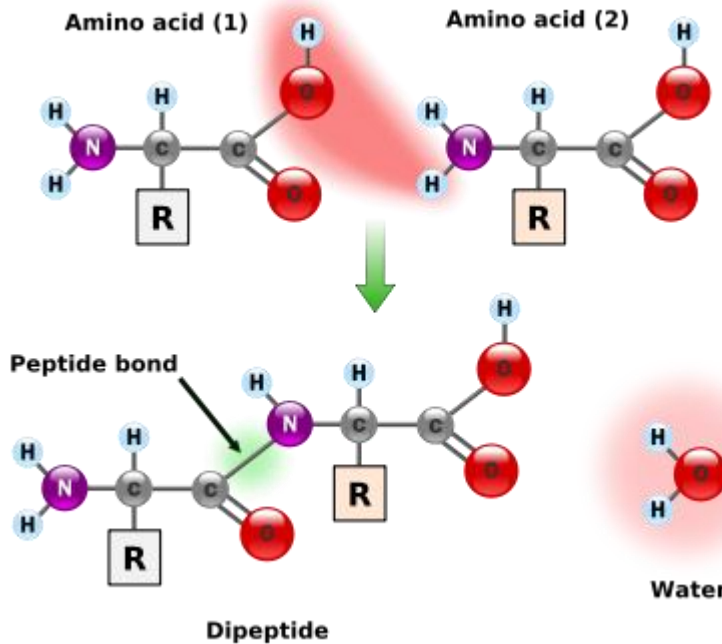
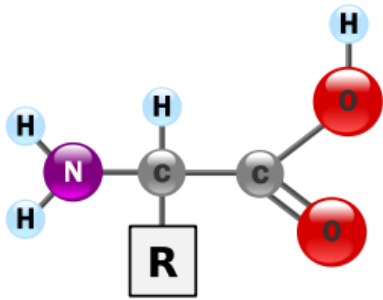
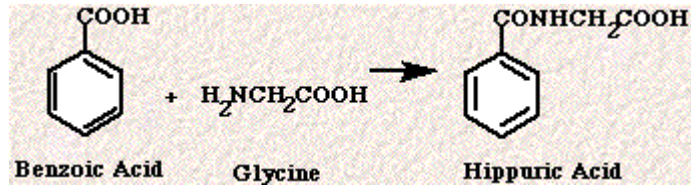


Amino Acids to Peptides to Proteins

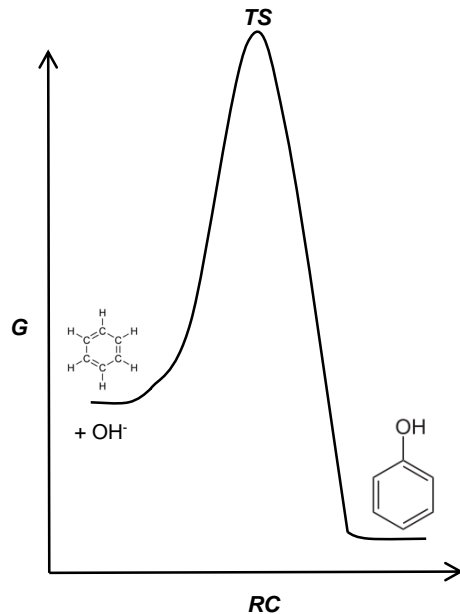


Last Time...

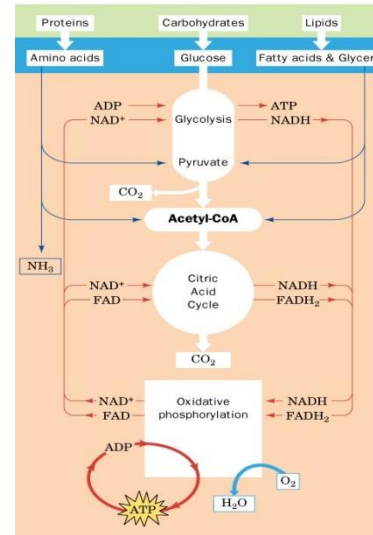
- We learned all about Pee!!



- And Enzymes

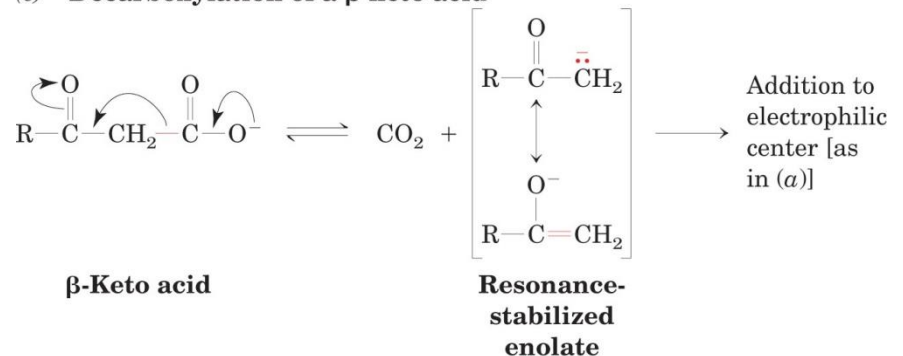


- And Metabolic Pathways



- And the Organic Reactions that Keep us Alive

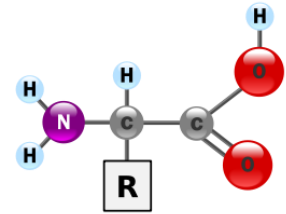
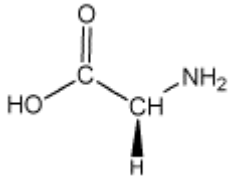
(c) Decarboxylation of a β -keto acid



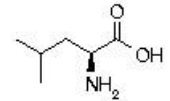
Amino Acids



Henri Braconnot
1780 - 1855
Isolated Glycine



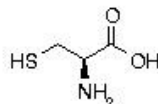
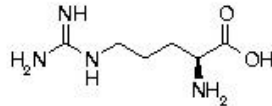
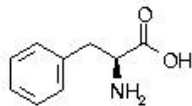
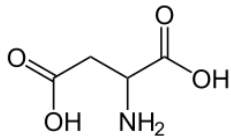
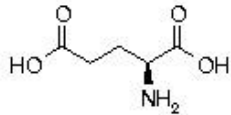
Heinrich Hlasiwetz and
Josef Habermann - 1867



Leucine and Tyrosine from Casein

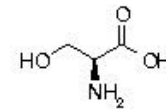
<http://web.lemoyne.edu/~GIUNTA/hlasiwetz.html>

Karl Ritthausen – 1866
Glutamic and Aspartic
Acid



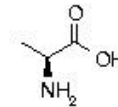
Other Amino Acids

Serine – 1865



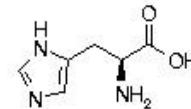
Phenylalanine - 1881

Alanine – 1888



Arginine – 1895

Histidine – 1897

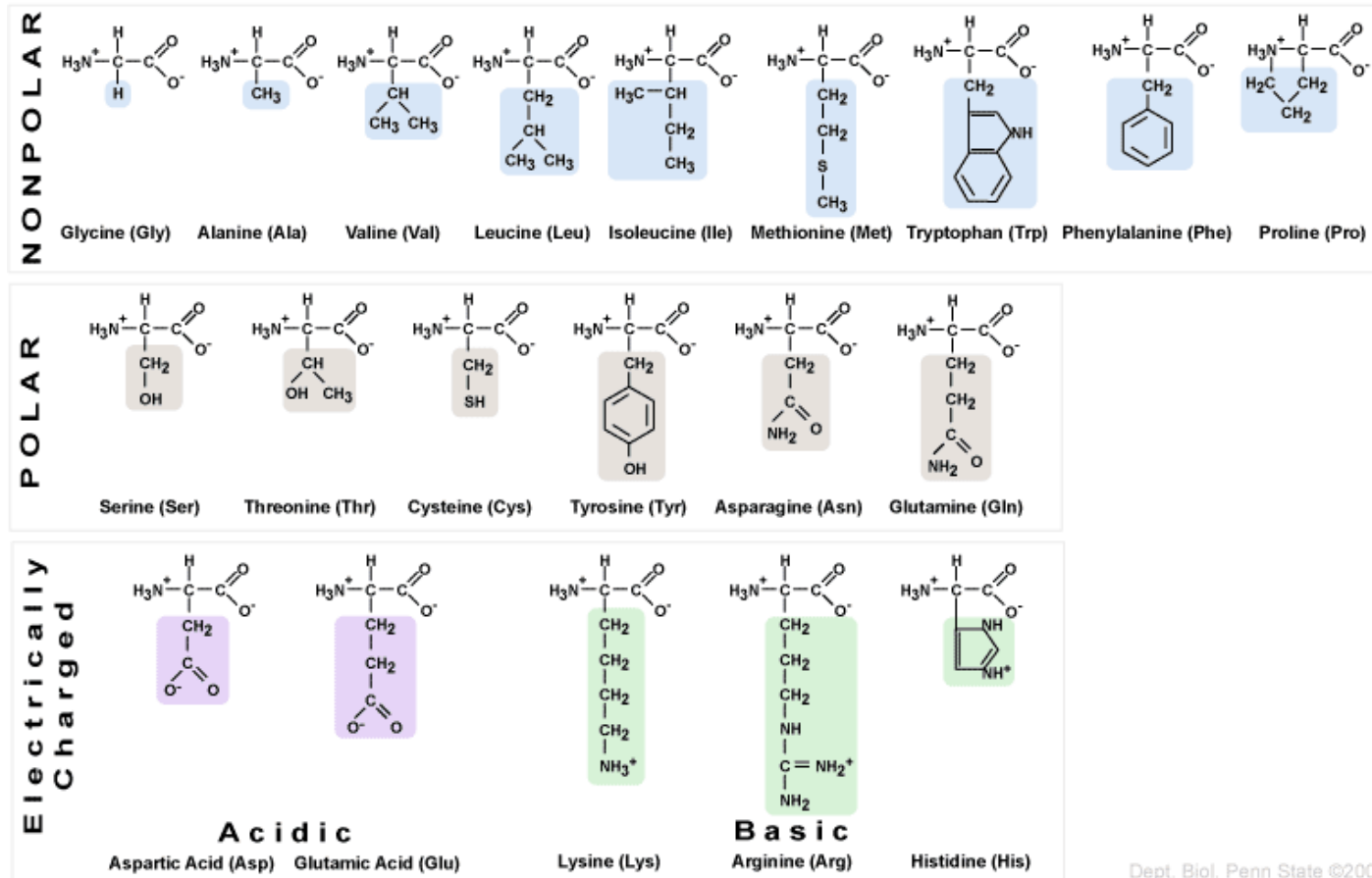


... ..

Cysteine – 1935!! (still debated!)

The amino Acids!

- There are 20 Amino Acids in all



- Chemistry is conferred by the variable side chain on the α carbon
- Some amino acids can be *modified*, which is *very important* for metabolism

Amino Acid Modifications

- Most 'modifiable amino acid?'

Lysine!!

Some common 'post-translational Modifications'

Acylation (think Acetyl-CoA, Lys, Ser)

Alkylation (methylation, Lys, Arg)

Biotinylation (Lys)

Glycosylation (Asn, Ser, Thr, Lys-OH)

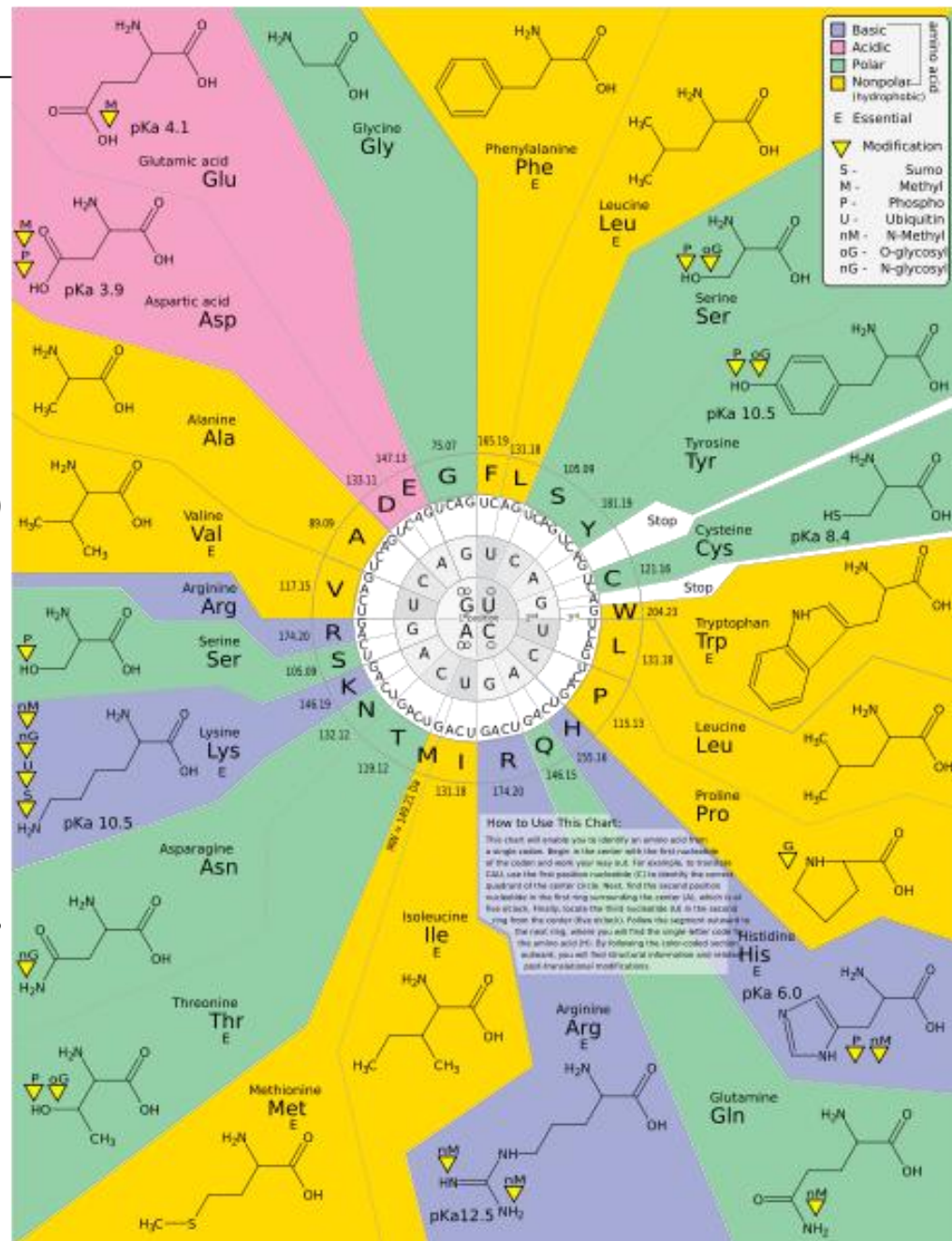
Oxidation (Lys, Cys, Met)

Phosphorylation (Ser, Tyr, Thr, Cys, His)

Sulfation (Tyr, Cys [disulphide])

Amidation (C-terminus)

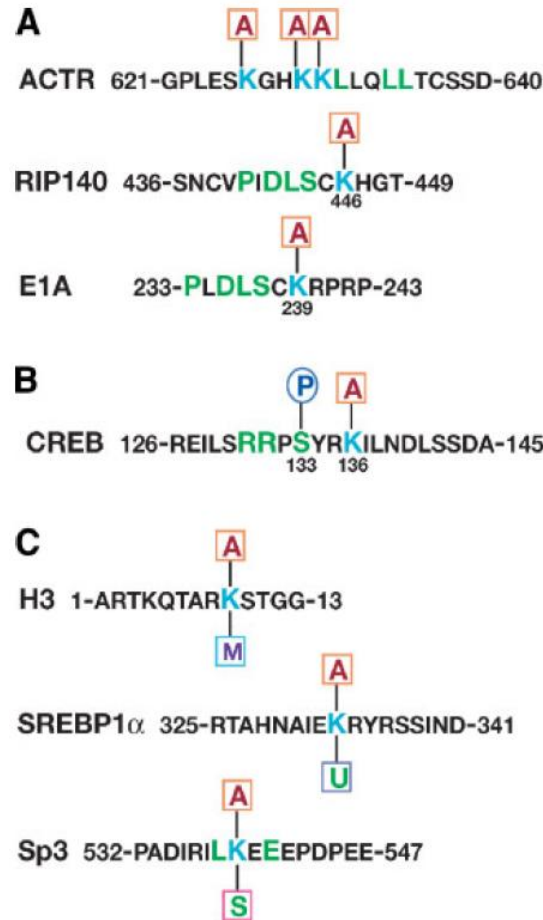
Glycylation (C-terminus)



Acylation

- Acylation of Serine occurs during catalysis by *hydrolytic enzymes*, e.g. peptidases and lactamases
- Acylation of Lysine is important for regulation of **gene expression**, **localization** and **enzymatic activity**

- **Acyltransferases** are enzymes responsible for metabolic control via acylation



Inhibition, blocking access to motif (green)

Activation, allowing access to phosphoserine

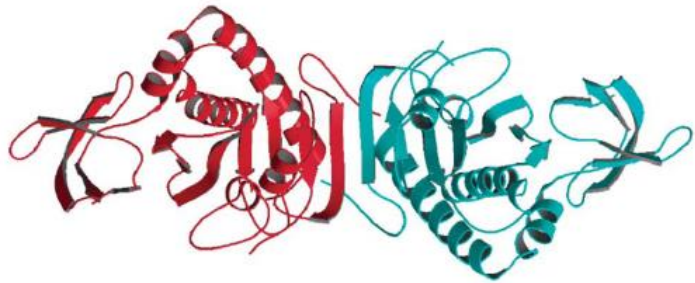
Competition, competing to prevent alternate modifications

Biotinylation

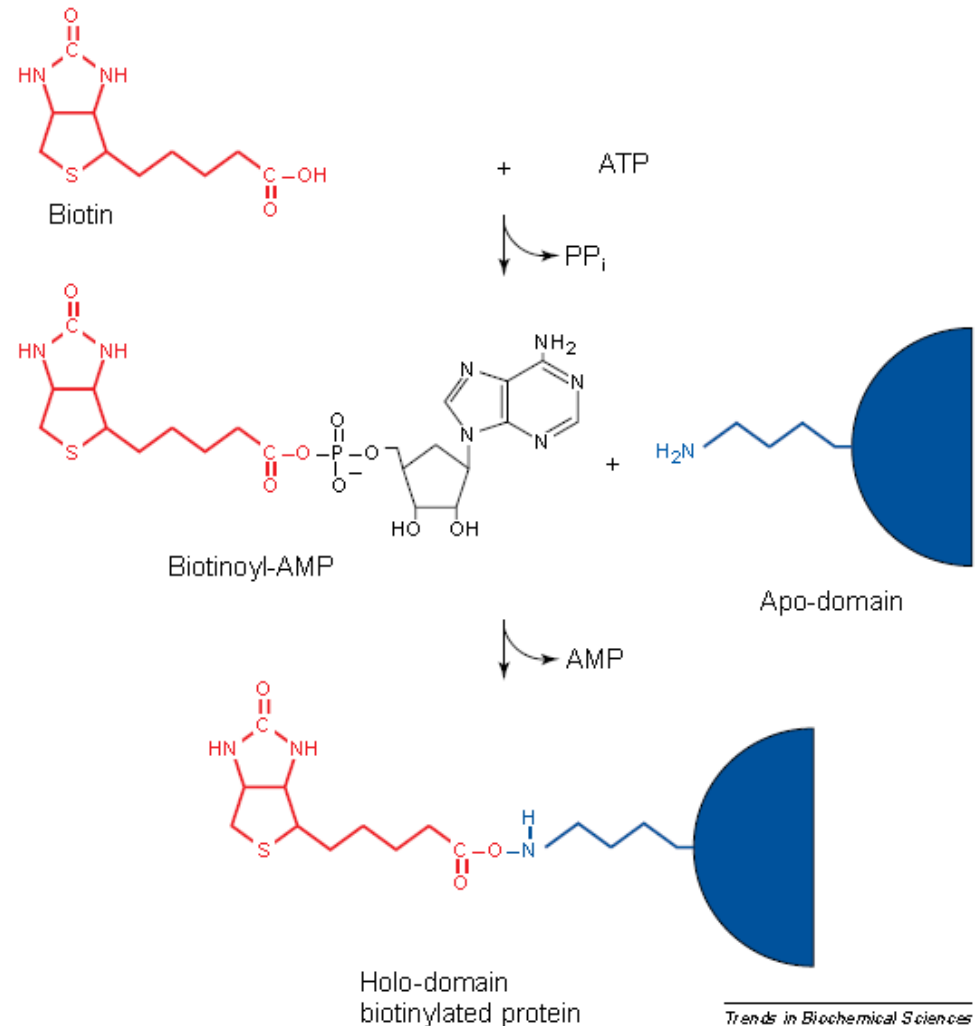
- Biotinylation is crucial for regulation of gene expression. It also plays a role in **fatty acid metabolism** and **gluconeogenesis**.

- Not a common modification, but very important

- Catalyzed by *Biotin Protein Ligase*



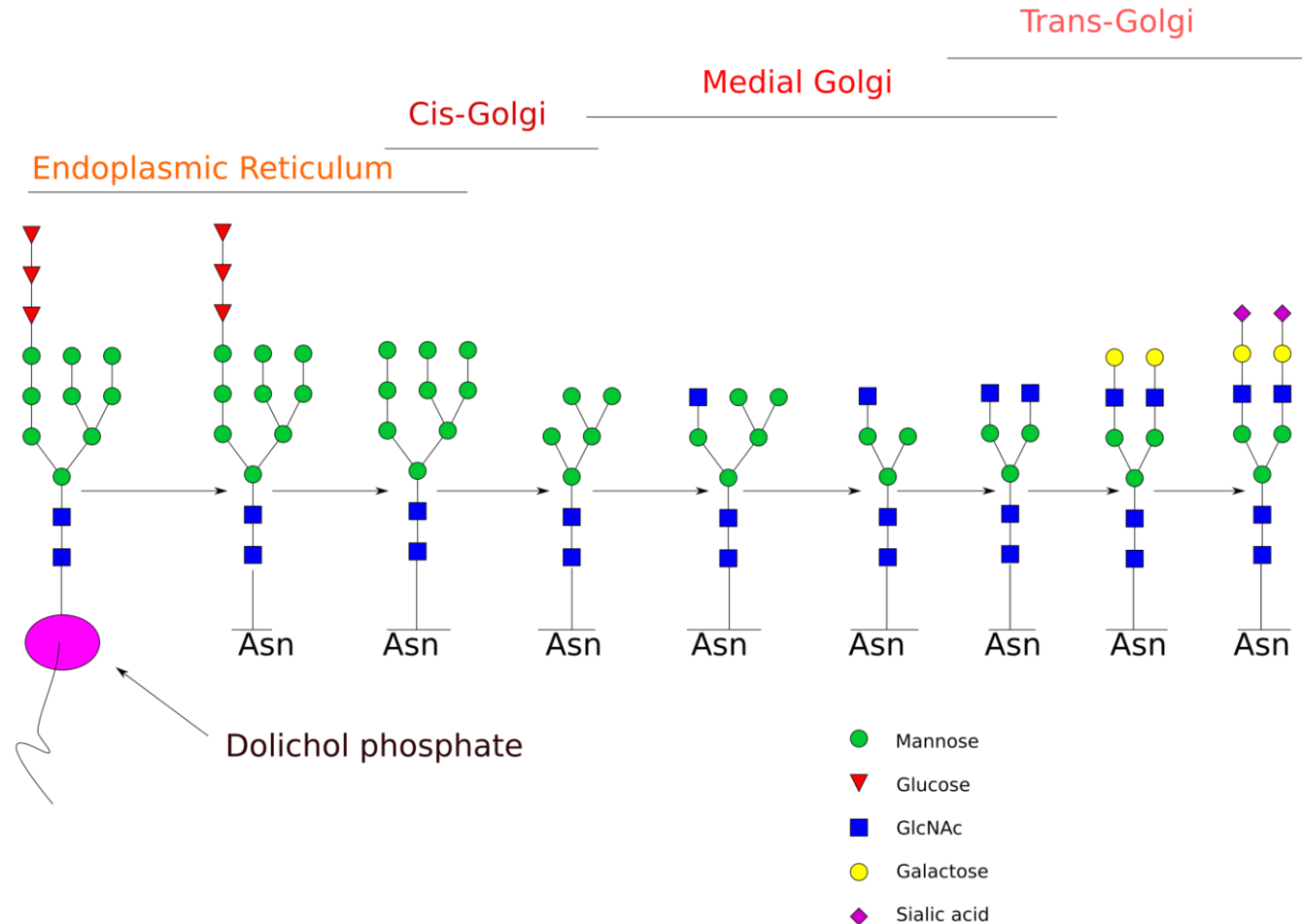
- Use by enzymes that transfer CO_2 from HCO_3^- to Organic Acids



Trends in Biochemical Sciences

Glycosylation

- This is the reaction that makes glycoproteins, not always Asn, also 'o-linked' glycosylation on Ser, Thr.

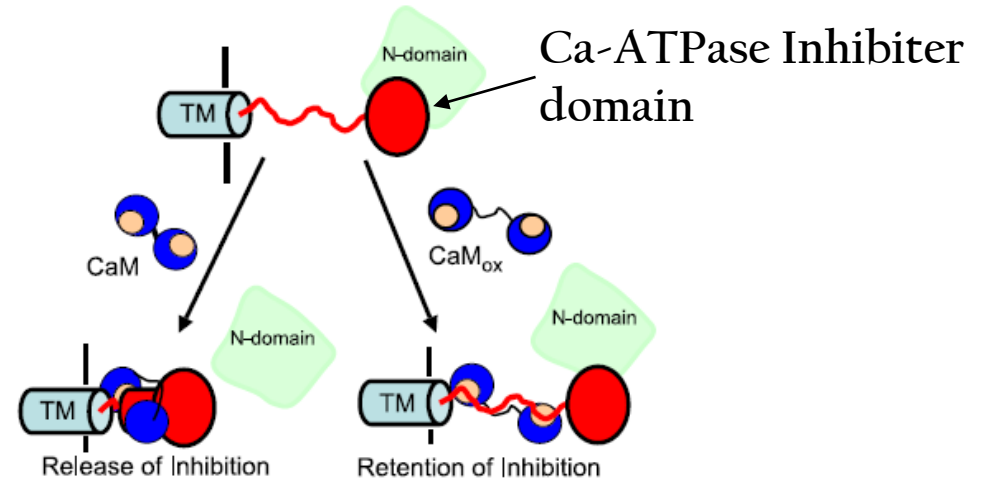


- Generally not directly relevant to metabolism, more to do with cell-cell recognition and immunogenicity

Oxidation

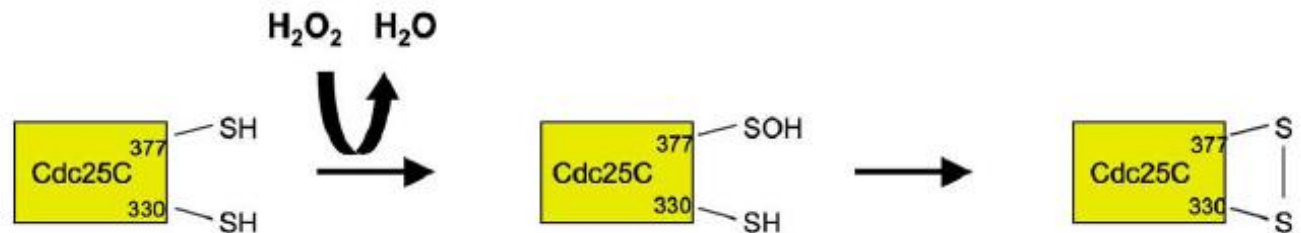
- Oxidation of **Methionine** is mostly associated with **oxidative stress** and **aging**, both of which influence metabolism

- Exerts influence by inactivation proteins, e.g. **Calmodulin**



[Biochimica et Biophysica Acta 1703 \(2005\) 121–134](#)

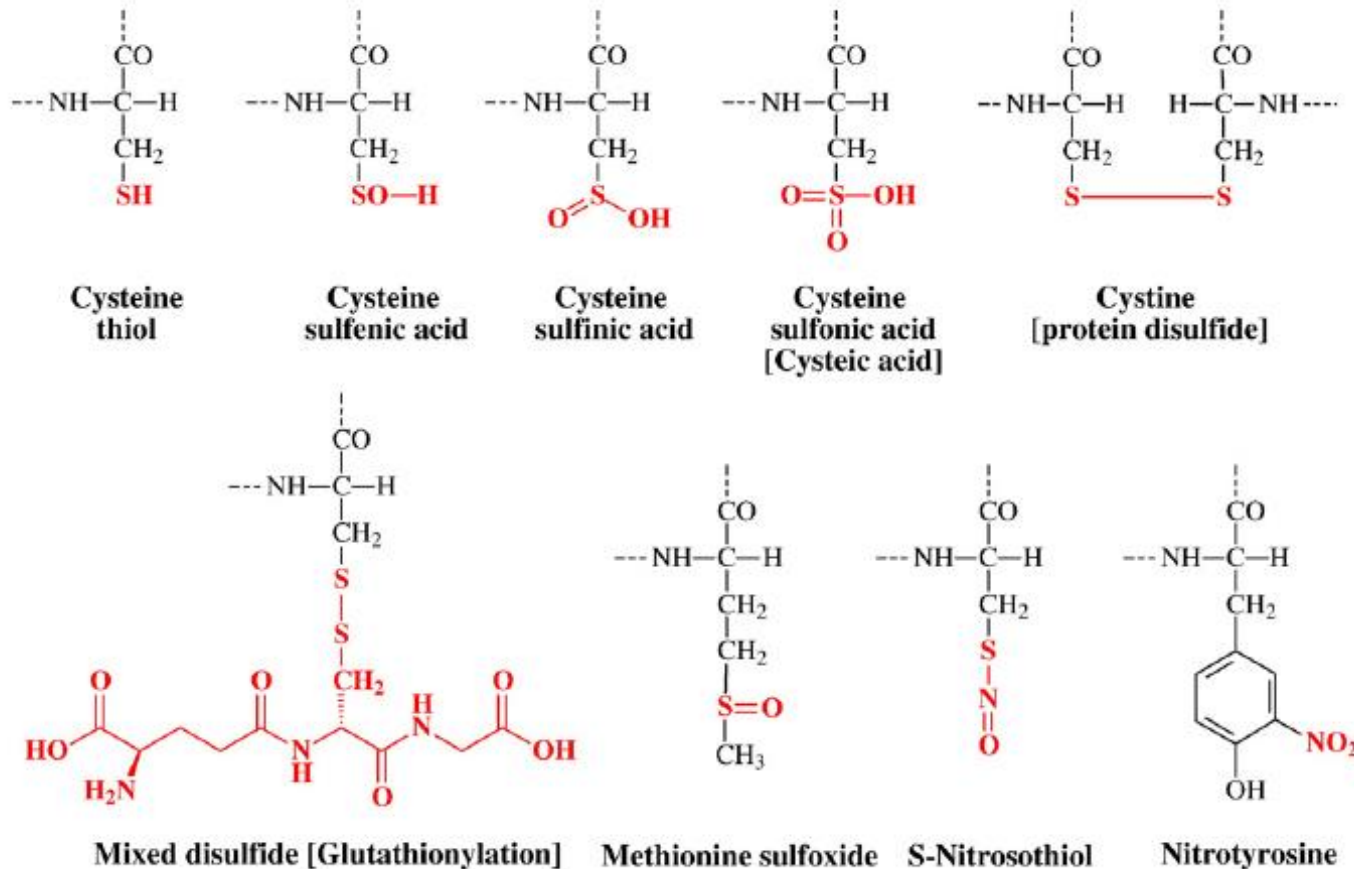
- Oxidation of **Cystein** can be a growth factor induced signal to **support cell proliferation** via **phosphorylation of Tyrosine**. It does this by catalyzing the formation of disulphide bonds...



[S. H. Cho FEBS Letters 560 \(2004\)](#)

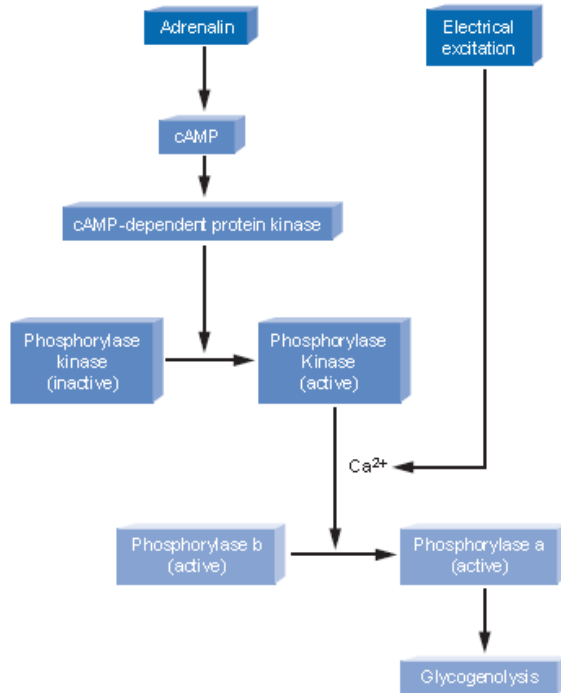
Cysteine Oxidation Continued

- Here are the various oxidized states of Cysteine. All of these are reversible and some are important for regulation...



Phosphorylation

- Probably the most important metabolic Post Translational Modification (PTM)!!

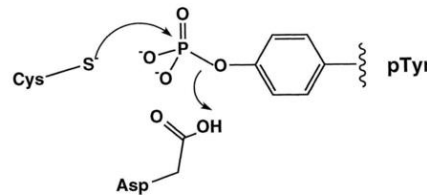


This is how your muscles get extra sugar from glycogen when you're working out.

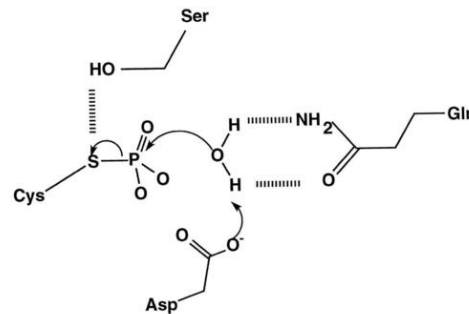
[NATURE CELL BIOLOGY VOL 4 MAY 2002](#)

- Huge role in **signal transduction**, **mediating enzyme activity**, **protein interactions**

- Enzymes involved are **Protein Kinases** (stick phosphoryl groups on) and **Phosphatases** (take phosphate groups off)



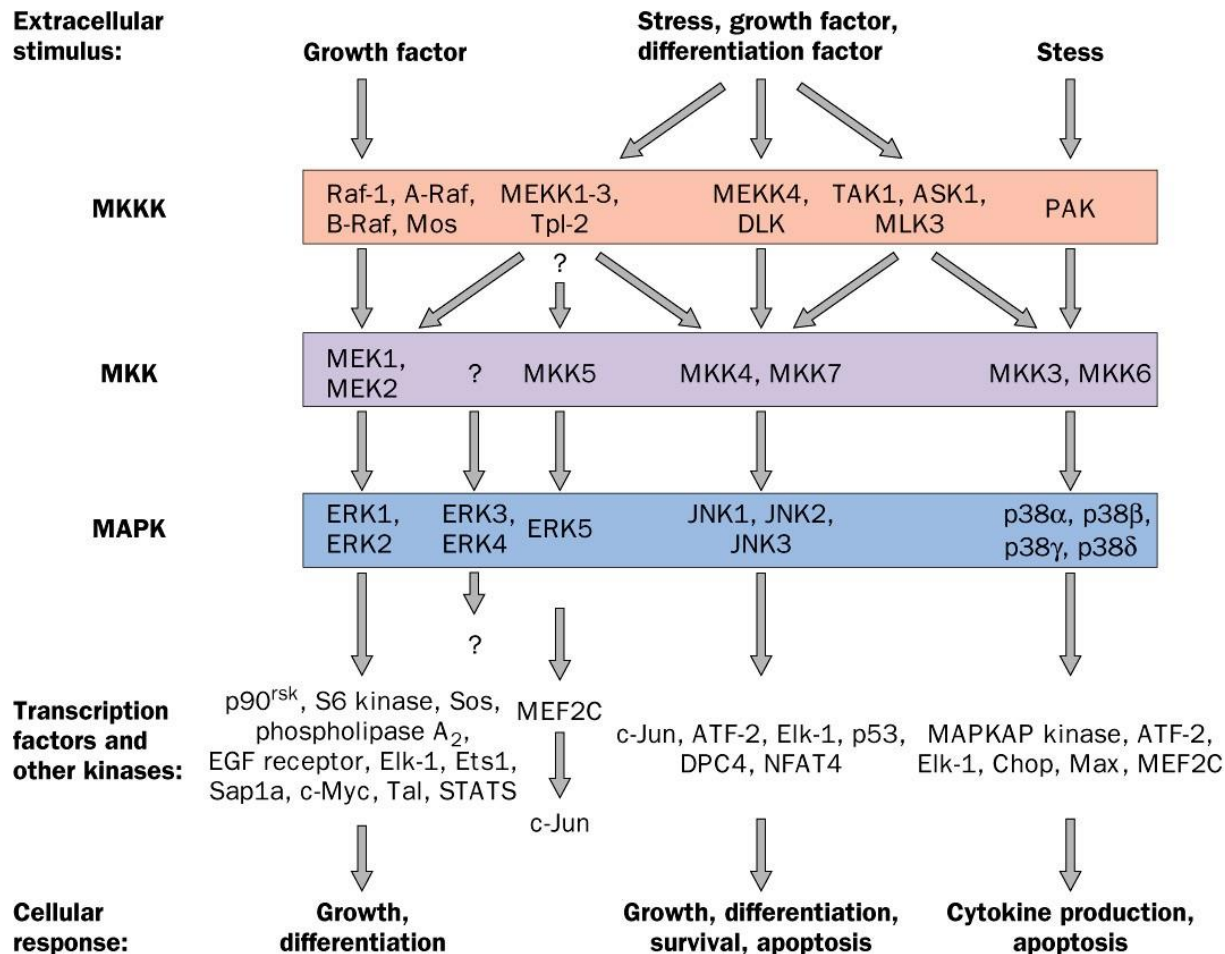
- Some Phosphatases have **specific amino acid targets** (*i.e.* Phospho-Tyr, Thr, Ser, or His), some target **specific protein domains** (*i.e.* SH3) and some are **non-specific**



Phosphatase mechanism

Phosphorylation – The Kinases

- The Mitogen Activated Protein (MAP) Kinases: Masters of signal transduction!!
- All of this is via Serine and Tyrosine Phosphorylation

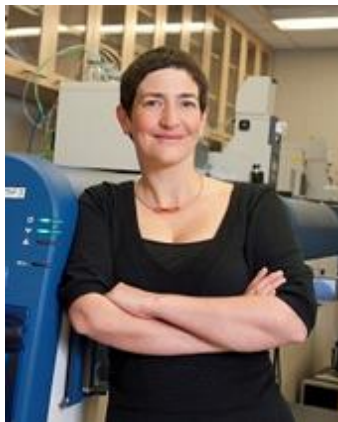
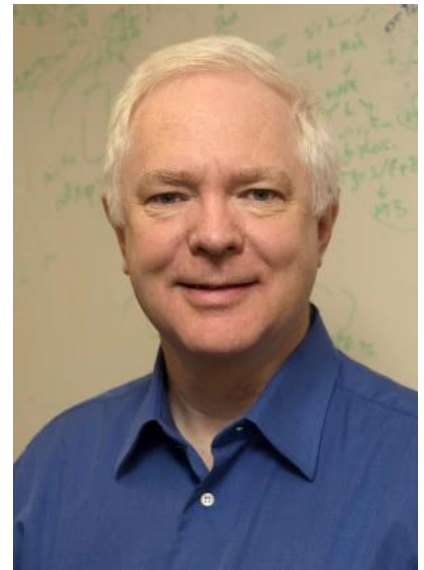


Tony Pawson 1952 - 2013

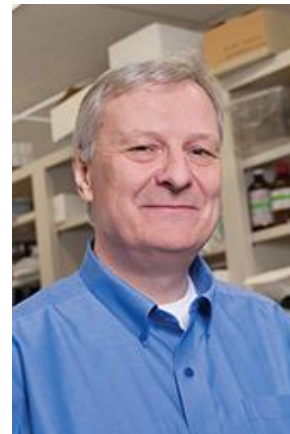
- World leading researcher in signal transduction @ UT

- Developed the not so tiny field of **phosphoproteomics**

- Since then a number of world-leading researchers have taken over...



Anne Claude
Gingras



Jim Woodget



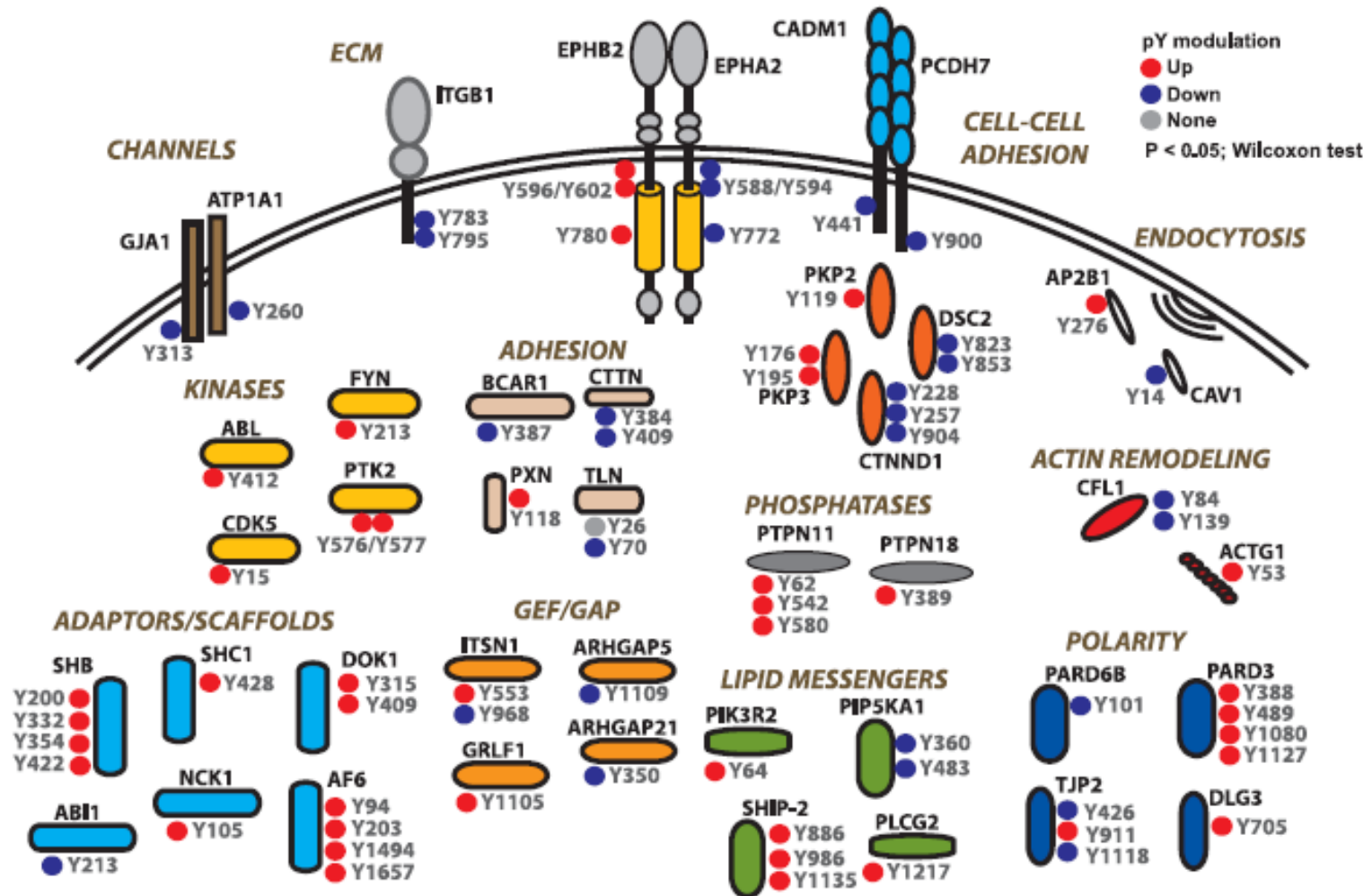
Sinai
Health

Lunenfeld-Tanenbaum
Research Institute

Ephrin Signaling

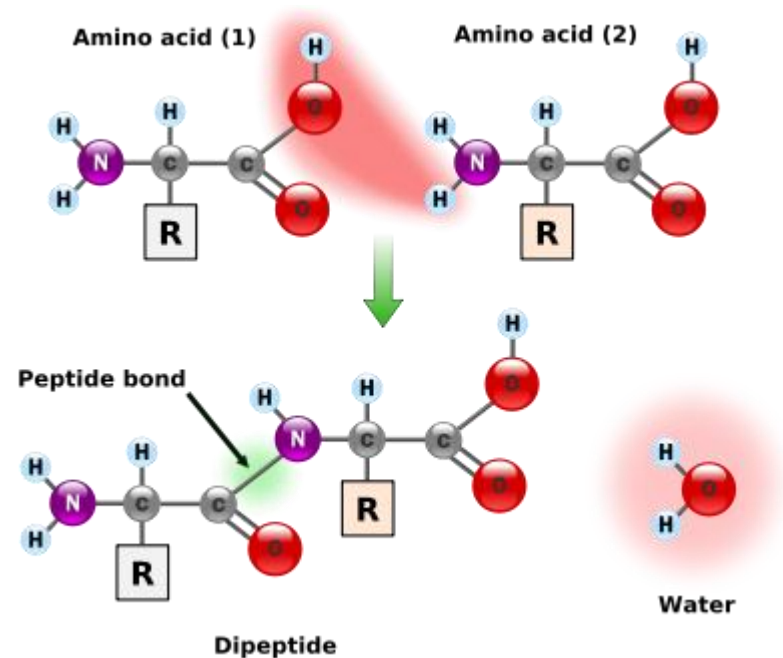
- Phosphorylation in response to cell/cell interaction

A



The Peptide Bond

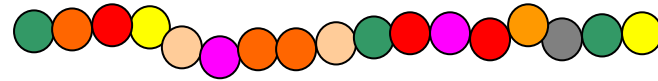
- Peptide bonds are formed in a condensation reaction that is catalyzed in the ribosome



- Two amino acids = dipeptide, three = tripeptide... theoretically it should go on like this, but in general, we call anything over 5 just 'peptide' or 'polypeptide'

Primary Structure – Amino Acid Sequence

- The amino acid sequences of peptides and proteins, the primary structure, is the basis for everything that is interesting about them – structure, dynamics... everything
- It's also one of the hardest things to get at:

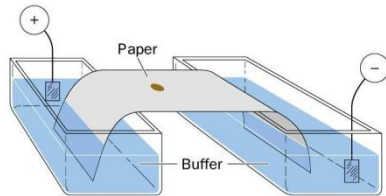
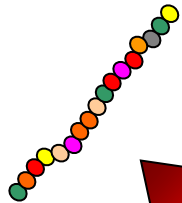


S-Q-D-A-G-M-Q-Q-G-A-D-M-D-Q-V-S-A

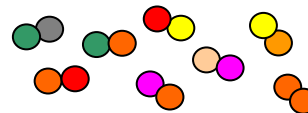


Frederick Sanger (1918-1997)

Sequenced insulin using
limited Proteolysis and
paper chromatography!



Enzymatic
hydrolysis

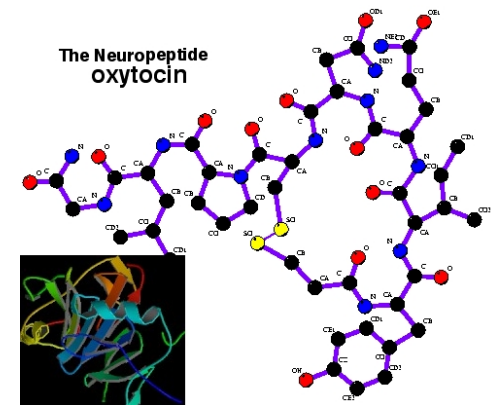


Dipeptides

The Physiological Role of Peptides

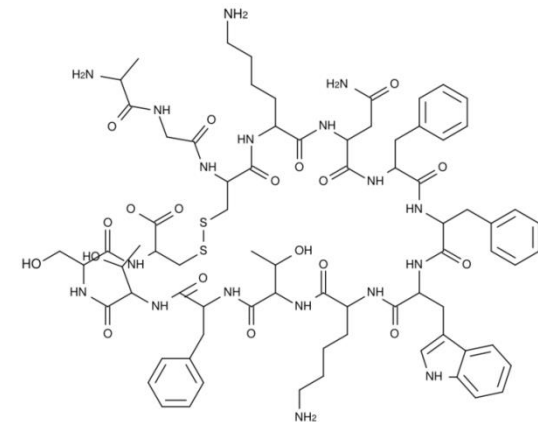
- Many peptides are **hormones**:

- Melanocyte Stimulating Hormone (MSH)
- Vasopressin; antidiuretic
- Oxytocin; brain (mood), uterine contraction, milk production
- Insulin; Sugar metabolism
- Thyroid stimulating hormone (TSH)- β ;
General metabolic rate



- Many are also **neuropeptides**:

- Galanin; Neurotransmitter inhibition
- Somatostatin; Master hormone suppressor (especially gastro-intestinal and growth)
- Cholecystokinin; mood, causes anxiety

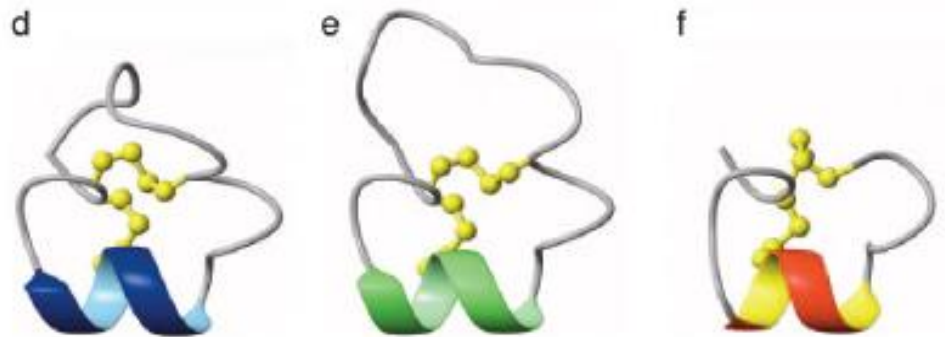


Somatostatin

Peptide Poisons

- Pro Tx-1 (spider venom); 35 a.a. peptide, irreversibly opens ion gate channels (mostly in insects)
- Muscarinic Toxin 3; brain toxin - motor control, memory

- Conotoxins; Inhibit acetylcholine receptors in nerves and muscles, sodium channels, potassium channels

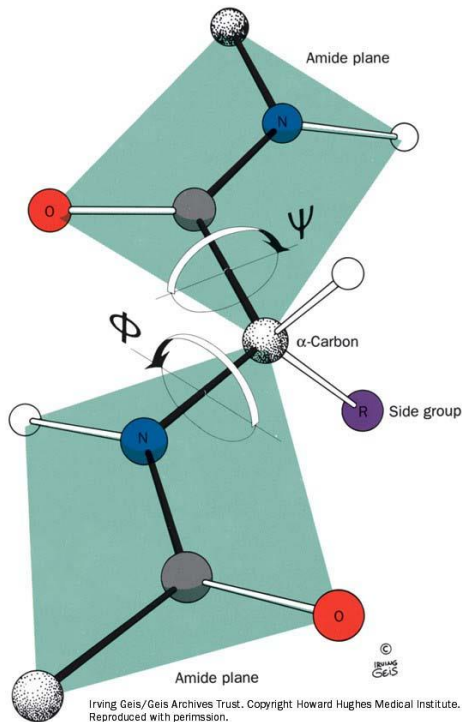


[PNAS September 27, 2005 vol. 102 no. 39 13767-13772](#)

- Many snake venoms are peptide poisons that interfere with specific enzymes, such as:
 - Phosphodiesterase; blood pressure ↓
 - Cholinesterase; loss of muscle control

The Peptide Bond and Structure

- Very often, the biological activity of peptide is dependent on their having a specific structure

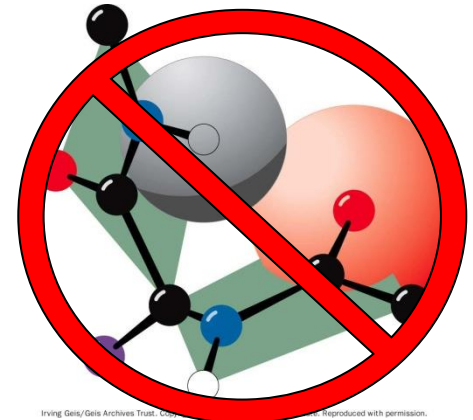
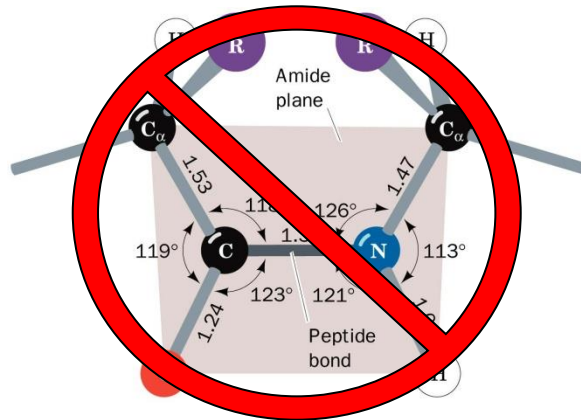


- The peptide bond itself is planar (green squares), so the region around the peptide bond is flat.

- This leaves two bonds of the 'main chain' that can rotate:

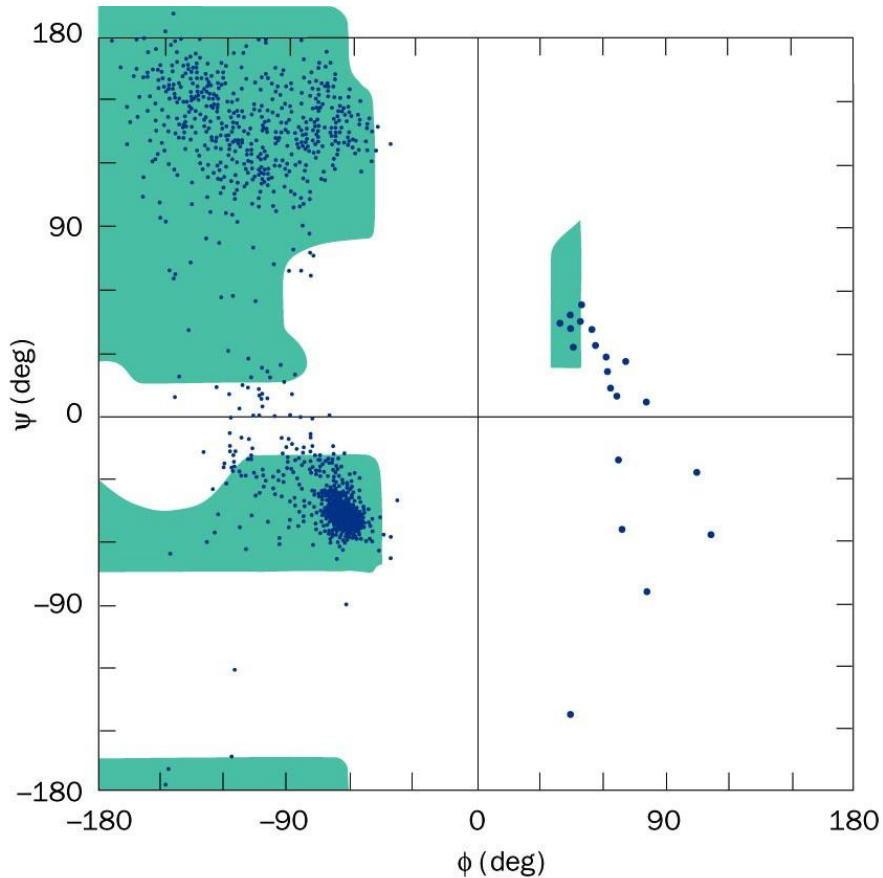
The $\text{N}-\text{C}_\alpha$ bond = ϕ 'phi'

The C_α -CO bond = ψ 'psi'



Ramachandran Plots

- This means that in peptides (and proteins), there are only a relatively small range of 'allowable' ϕ/ψ angles



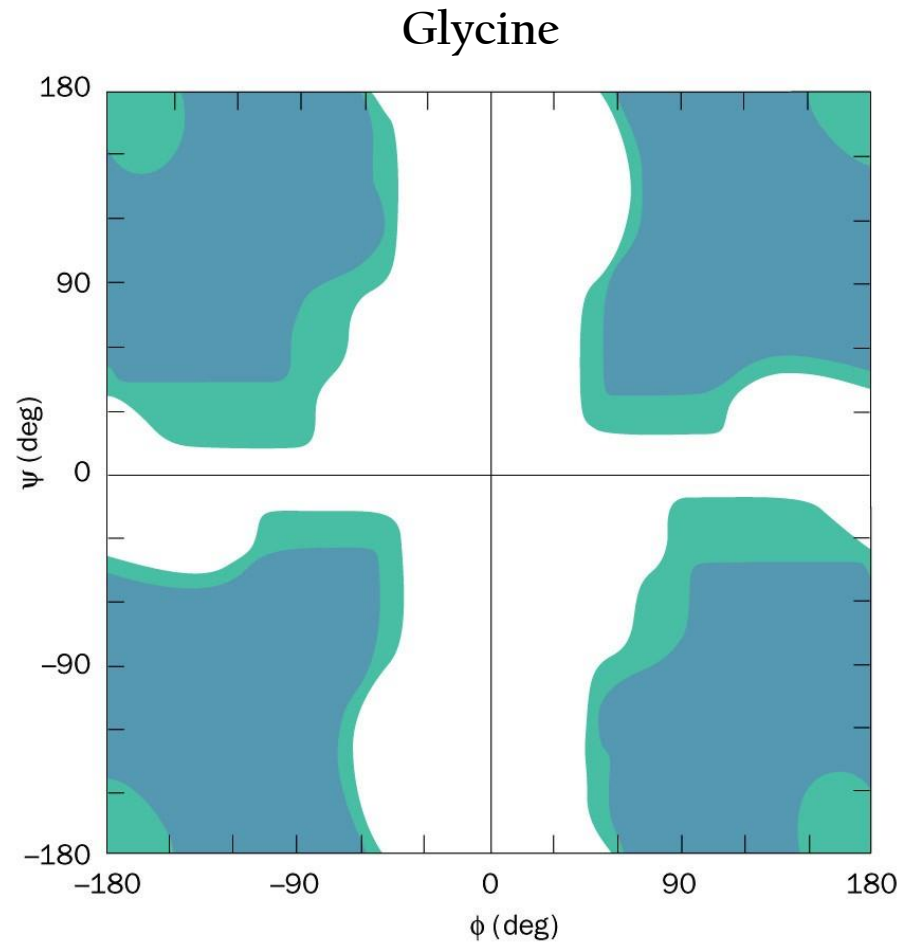
- The guy who figured this out systematically was:



Gopalasamudram
Narayana Iyer
Ramachandran
(1922-2001)

- Green = allowable

The Trouble with Glycine



Secondary Structure

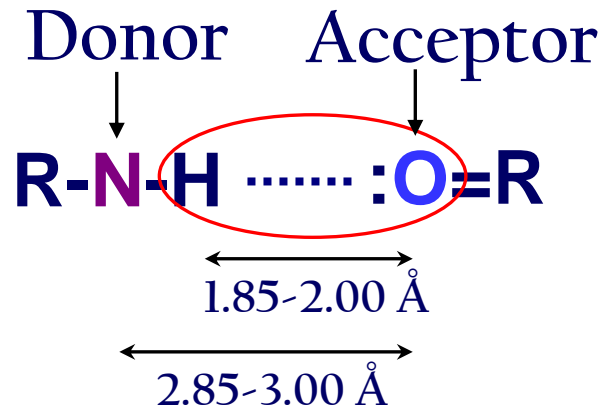
- By the 1940's, it was clear that protein function had something to do with **how the polypeptide chain was folded up**.
- Watson, Crick, Wilkins and Franklin had figured out the 'double helix' structure of DNA
- Which brings us back to this guy:
 - **Linus Pauling 1901-1994**



- Trained in theoretical physics, at the center of early X-ray crystallography
- Recognized the importance of the H-bond in stabilizing protein structure



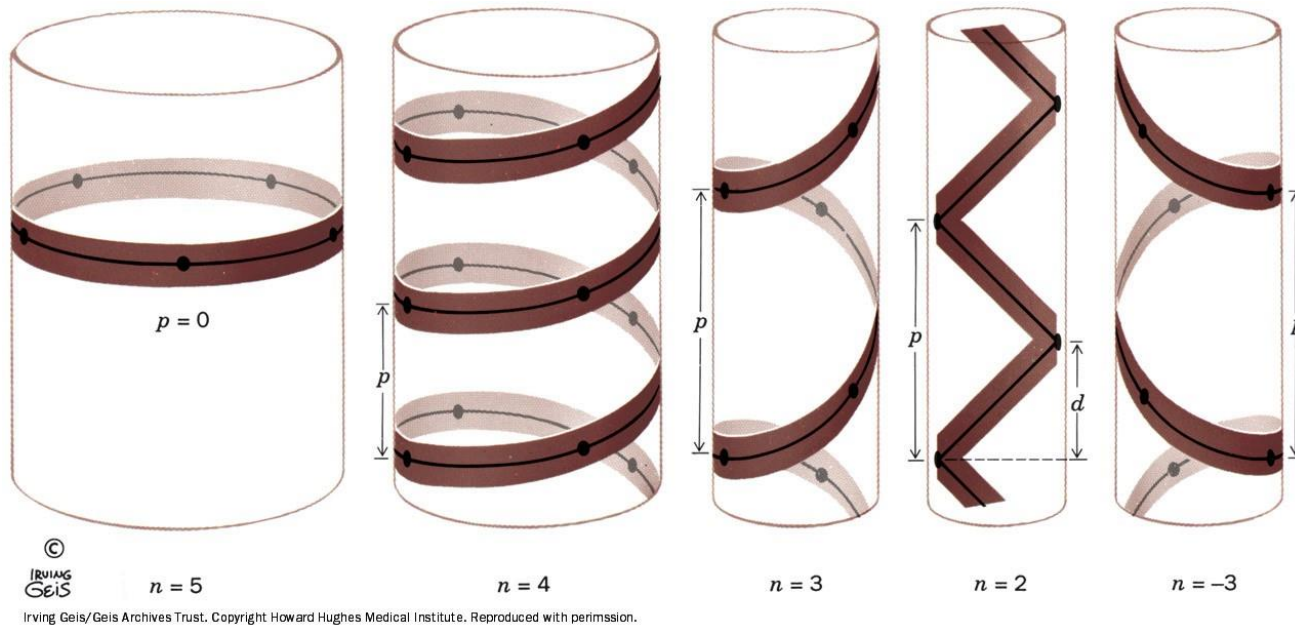
Sneaky Slide About H-bonds



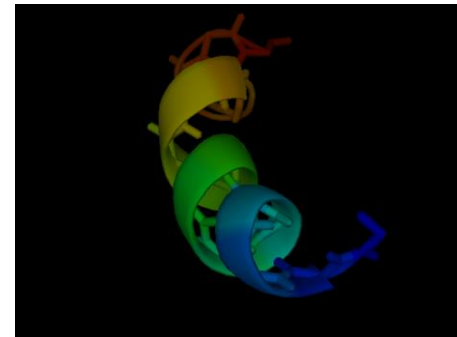
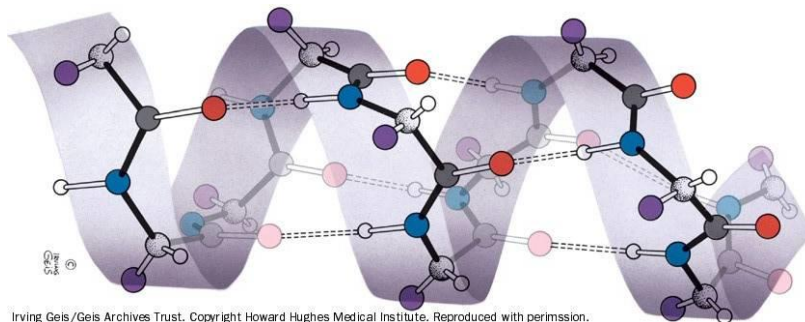
$$12 \leq E \leq 38 \text{ kJ/mol}$$

Helices

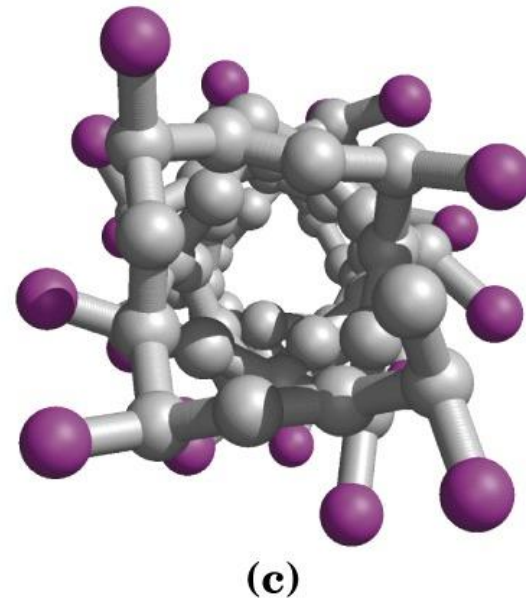
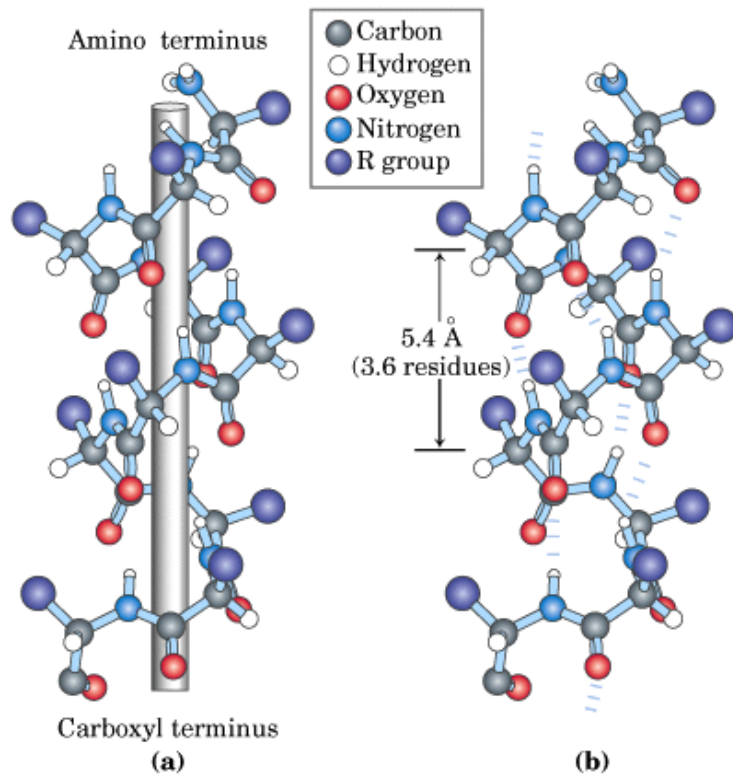
- In theory, there could be all kinds of helices in proteins



- In practice, there's pretty much just one – the right handed α -helix:



α -helices in Real Life...

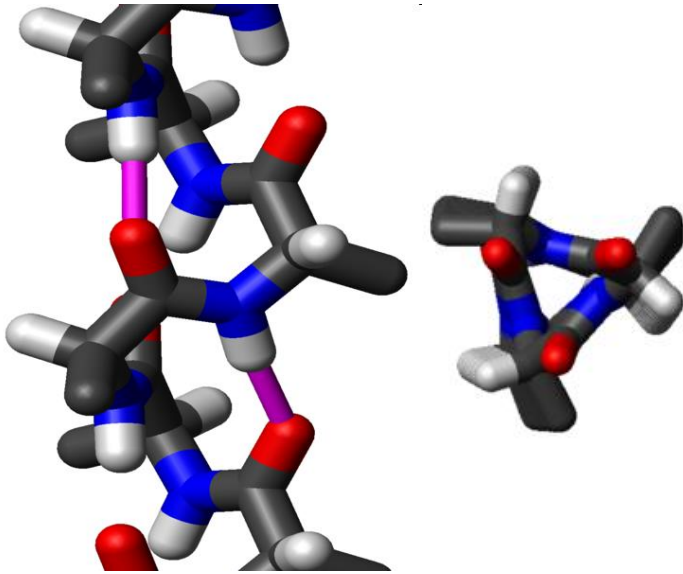


- Salient Features:

- 3.6 residues per turn, 0.15 nm per residue
- Each backbone carbonyl (O) (n) is hydrogen-bonded to backbone amide (H) 4 residues away to the C-term (n+4)
- All side chains are on the outside

The 'Other' Helices

- Two other helices are possible; they are *occasionally* observed in nature:



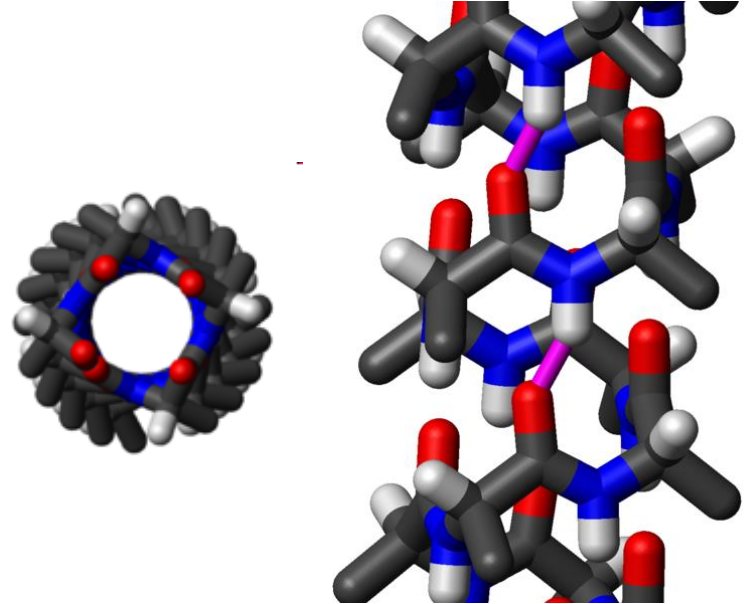
The '**stretched**' helix: 3_{10}

H-bonding $i+3$

Rise of .2 nm/residue

3 residues/turn

Does occur in nature (rarely)



The '**squished**' helix: π

H-bonding $i+5$

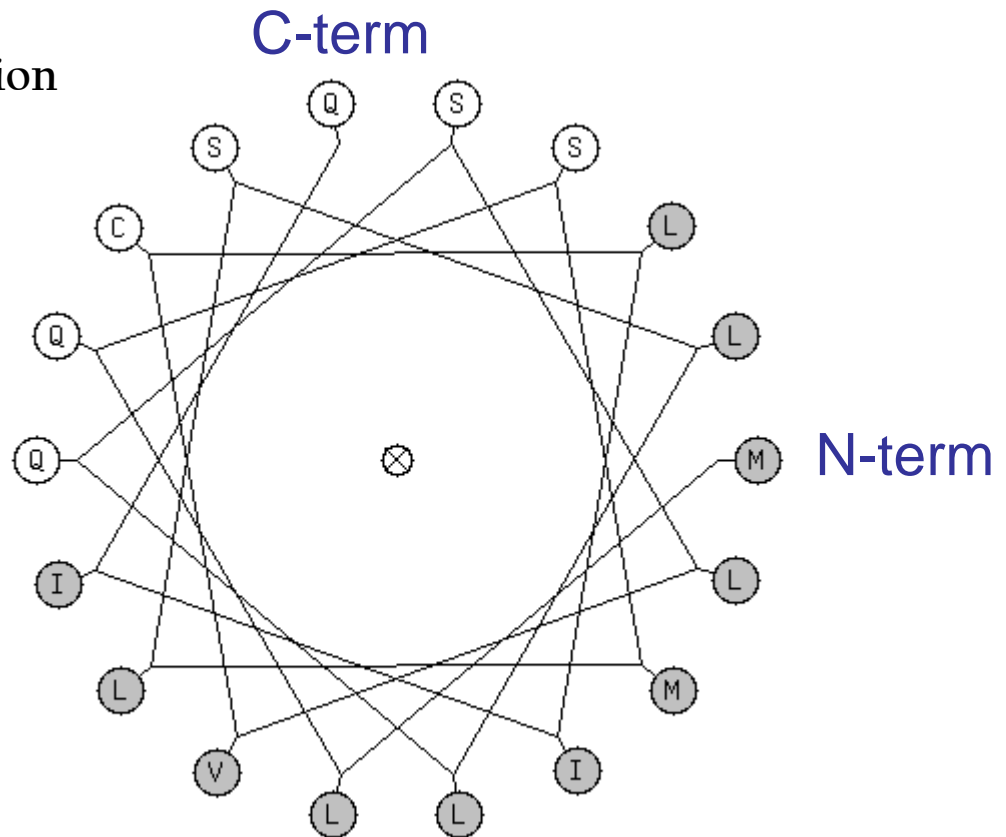
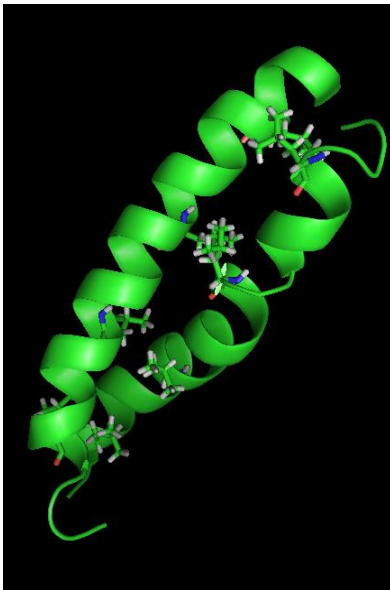
Rise of .115 nm/residue

5 residues/turn

Amphipathic Helices

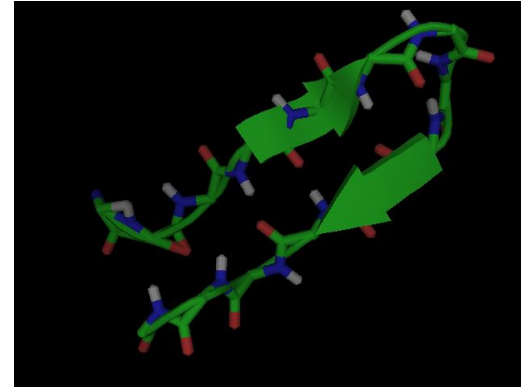
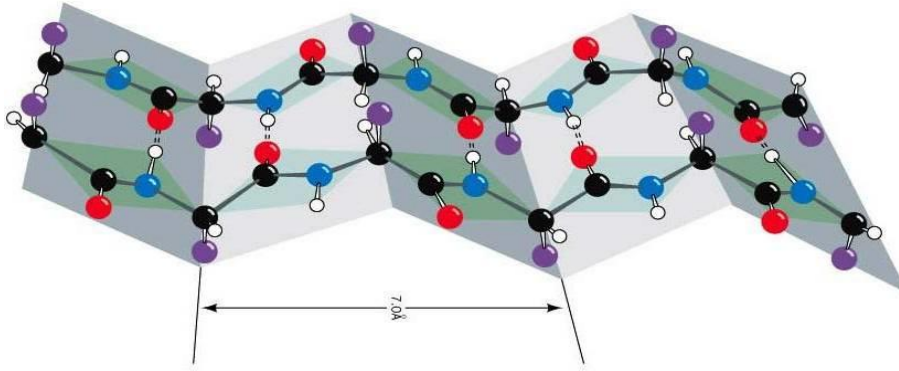
- For superstructural reasons, helices are often amphipathic, meaning that one side has mainly hydrophobic residues while the other has mainly hydrophilic residues.

- Helical wheel representation

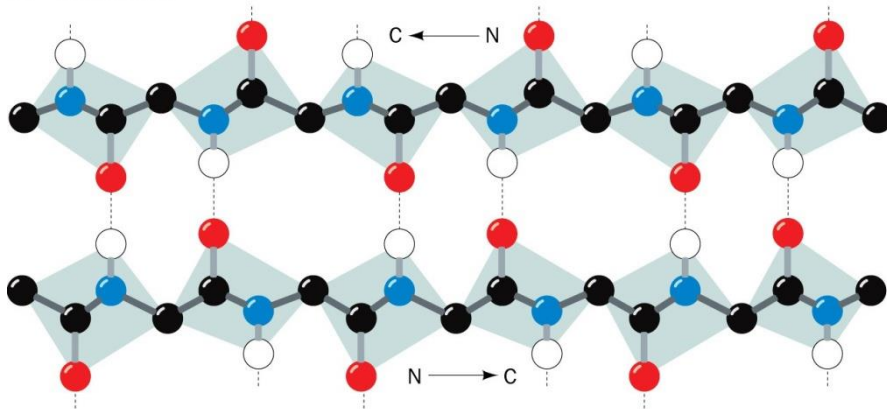


The β -Pleated Sheet

- Shortly after the helix, the β -sheet was described:

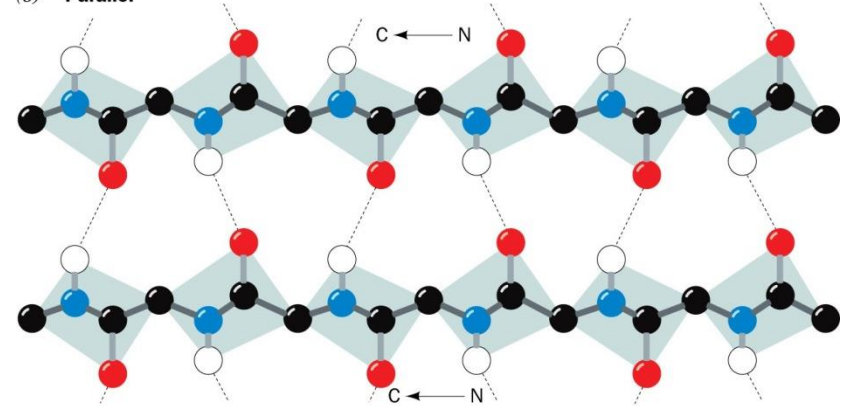


(a) Antiparallel



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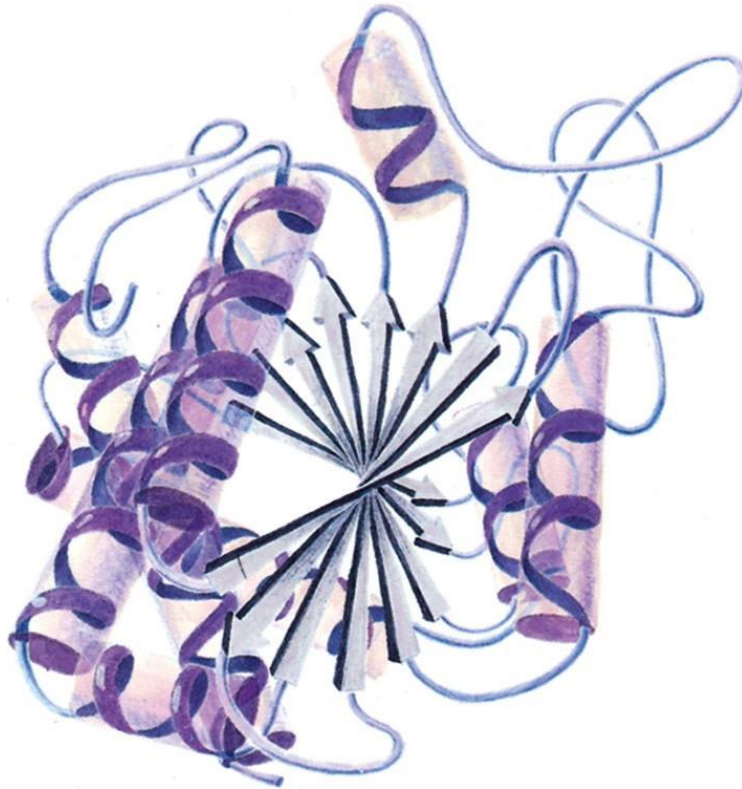
(b) Parallel



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β -Sheets are Twisted

- β -sheets are almost never flat – Anything more than 3 strands will have a significant superstructural right-handed twist:



Carboxypeptidase A

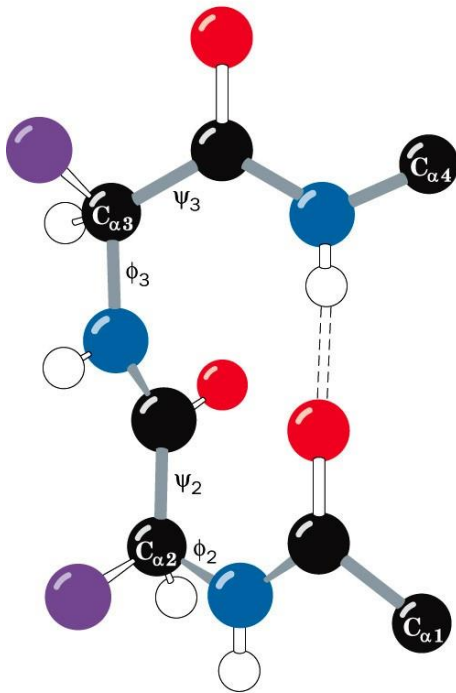


The SH3 domain

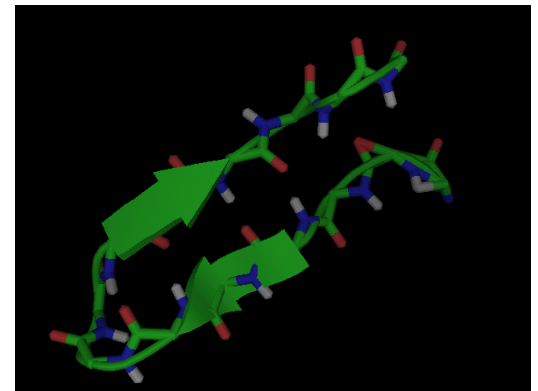
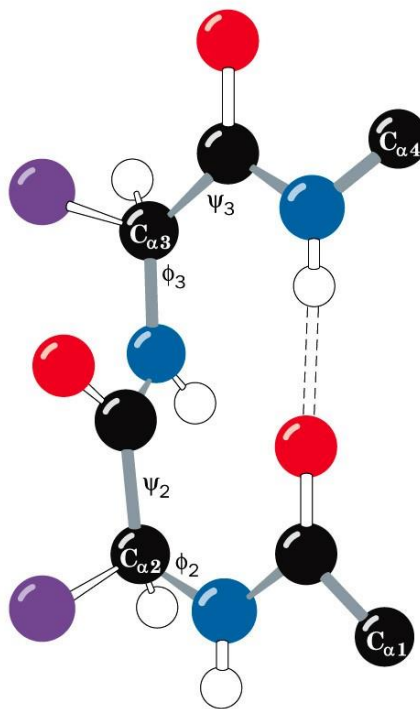
Turns and/or Bends

- In between elements of 'real' secondary structure are linker regions, which can be essentially random (random coil) or specifically structured β -turns
- Carbonyl of amino acid 1 H-bonded to amide hydrogen of amino acid 4 ($i+3$)
- Type I: Carbonyl of a.a. 2 pointed 'in'; Type II: Carbonyl of a.a. 2 pointed 'out'

(a) Type I β bend

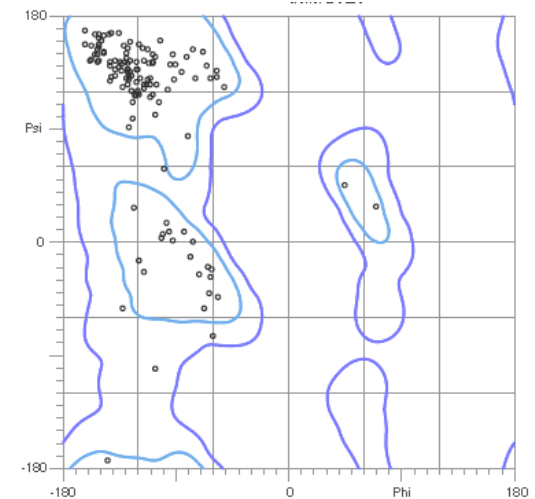
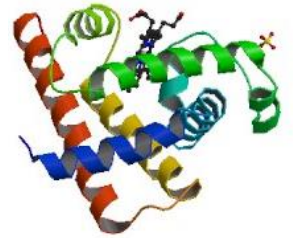
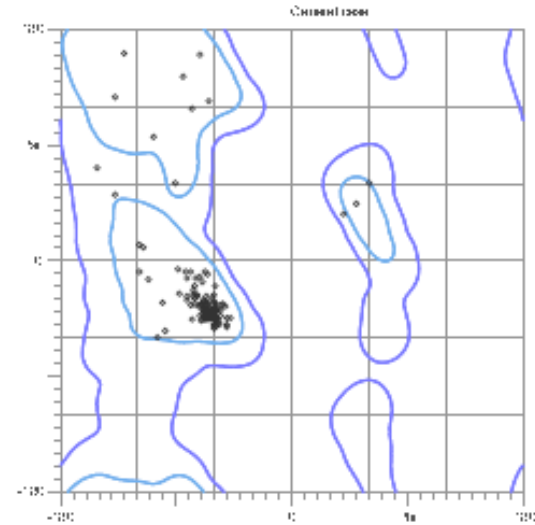
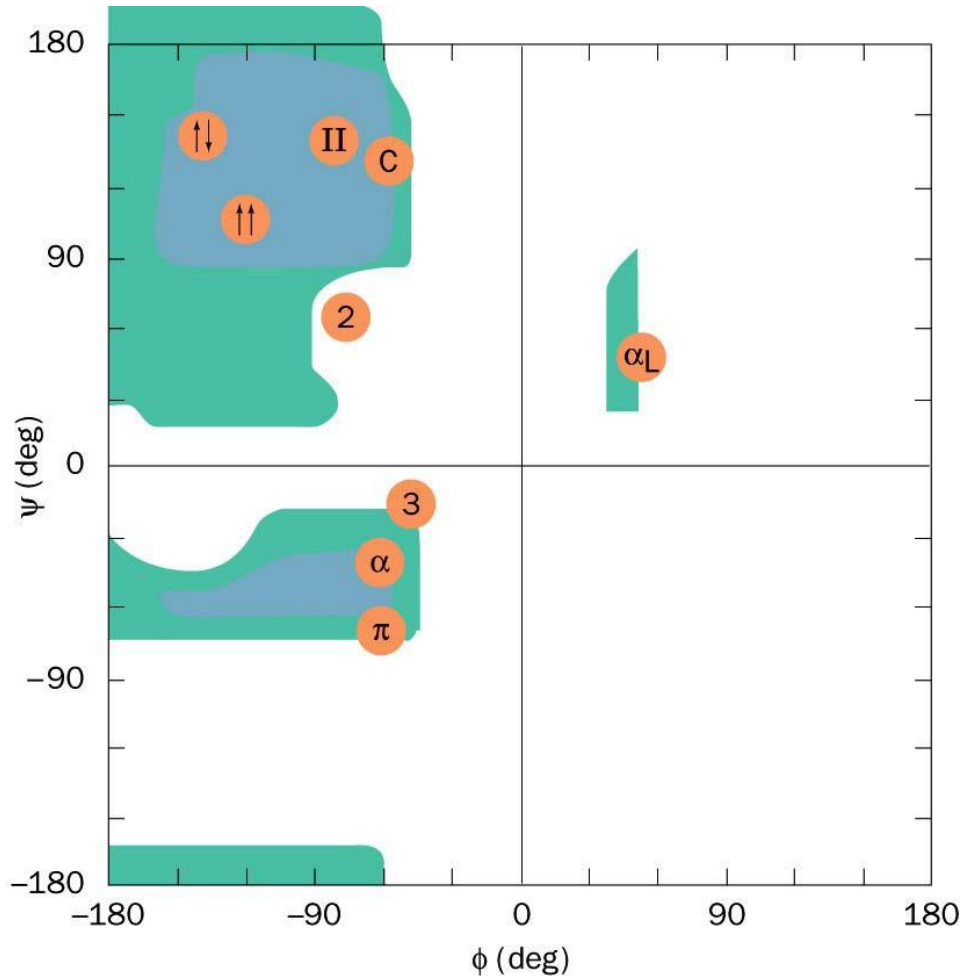


(b) Type II β bend



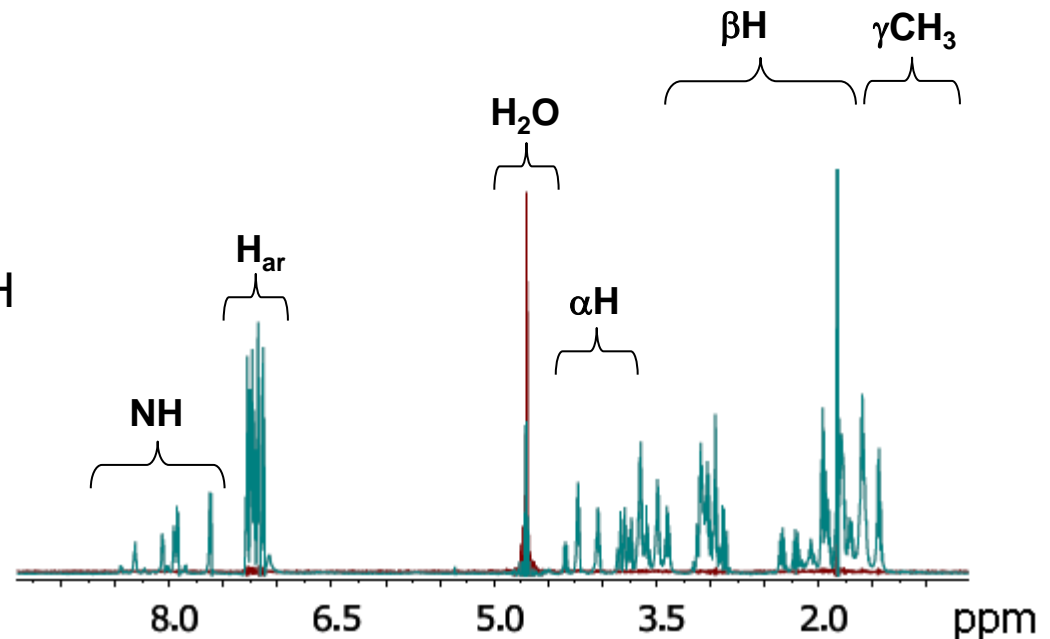
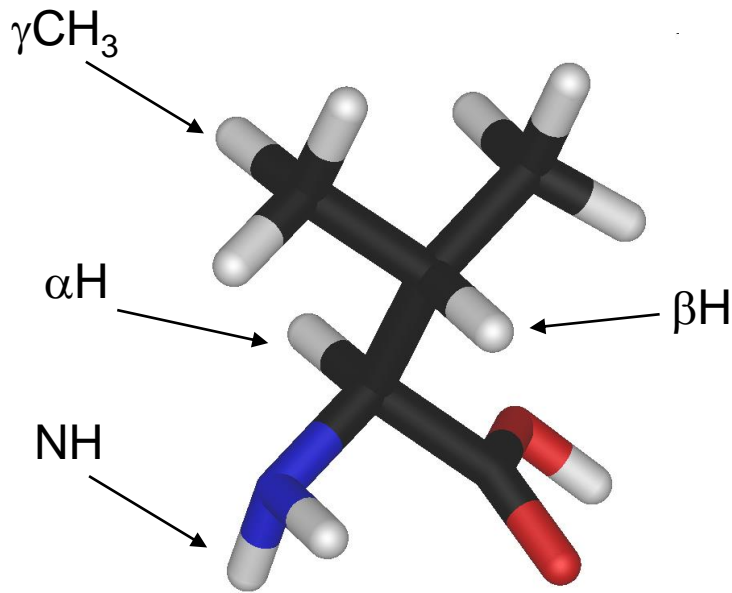
Secondary Structure and the Ramachandran Plot

- As it turns out, ϕ/ψ are predictive of secondary structure:



NMR and the Chemical Shift Index

- There is a very clever way of figure out protein secondary structures without having to do a 'full on' structural NMR study:
 - The Chemical Shift Index
- In proton (^1H) NMR, each *type* of proton on each amino acid gives a **distinct signal** whose **location** is called the **chemical shift**.



CSI Index Continued...

- The Chemical Shift Index relies on the fact that the αH chemical shifts are dependent on the secondary structure

Table II: Chemical Shift Values of α -Protons Used in the Determination of Secondary Structure

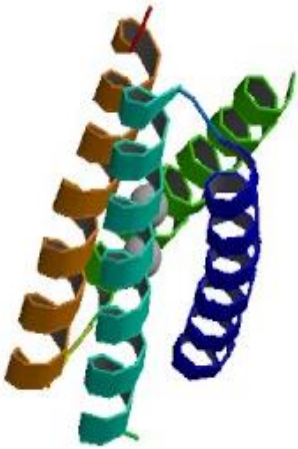
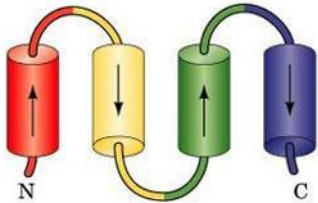
residue	$\alpha\text{-}^1\text{H}$ range (ppm)	residue	$\alpha\text{-}^1\text{H}$ range (ppm)
Ala	4.35 ± 0.10	Met	4.52 ± 0.10
Cys	4.65 ± 0.10	Asn	4.75 ± 0.10
Asp	4.76 ± 0.10	Pro	4.44 ± 0.10
Glu	4.29 ± 0.10	Gln	4.37 ± 0.10
Phe	4.66 ± 0.10	Arg	4.38 ± 0.10
Gly	3.97 ± 0.10	Ser	4.50 ± 0.10
His	4.63 ± 0.10	Thr	4.35 ± 0.10
Ile	3.95 ± 0.10	Val	3.95 ± 0.10
Lys	4.36 ± 0.10	Trp	4.70 ± 0.10
Leu	4.17 ± 0.10	Tyr	4.60 ± 0.10

- If αH chemical shift $>$ than that in the table, then +1
- If αH chemical shift $<$ than that in the table, then -1
- If αH chemical shift is within range of table, then 0
- Four or more -1's not interrupted by a +1 = helix
- Four or more +1's not interrupted by a -1 = β -strand
- Anything else = coil

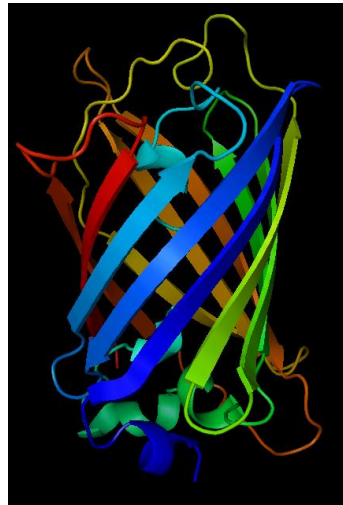
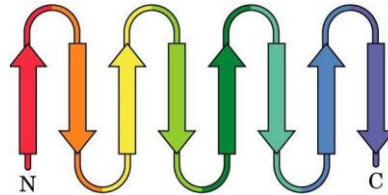
Biochemistry 1992, 31, 1647-1651

Structural Motifs

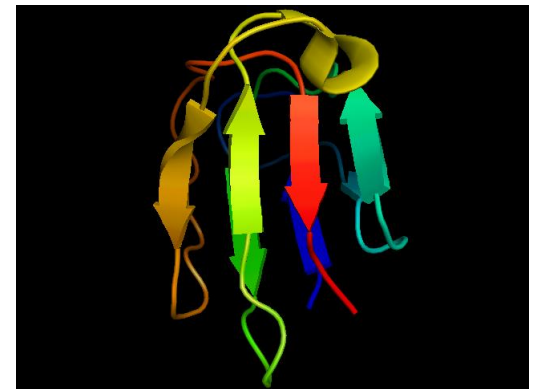
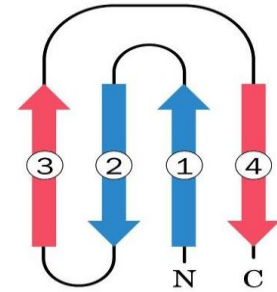
Helix Bundle



Beta Barrel

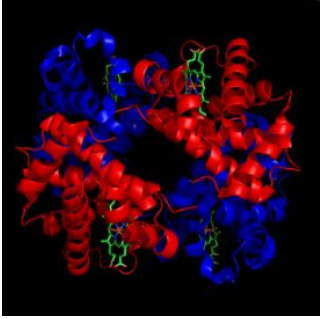


Greek Key

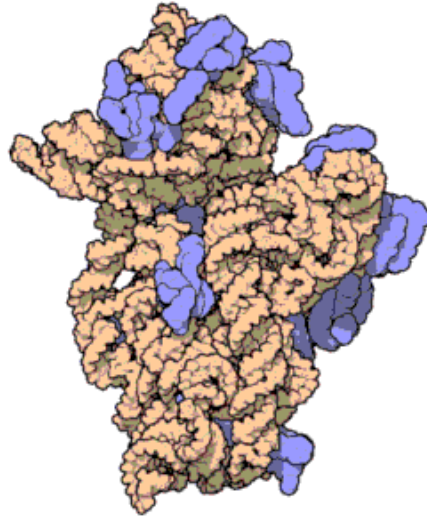


Quaternary Structure

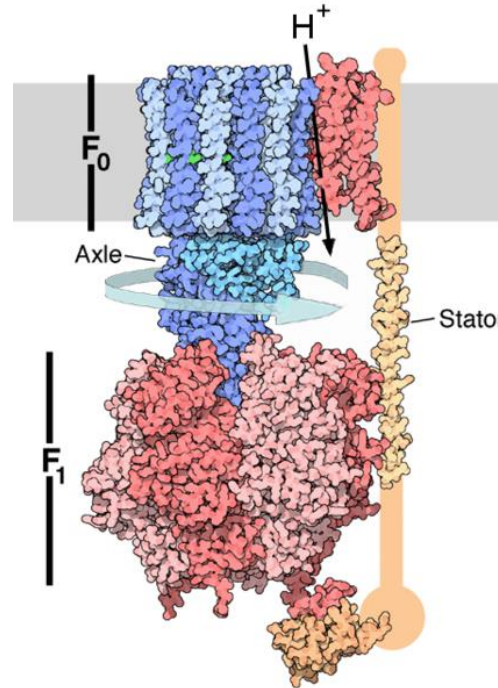
- Quaternary structure is represents non-covalent protein complexes, that is proteins interacting with other proteins to form specific structures.



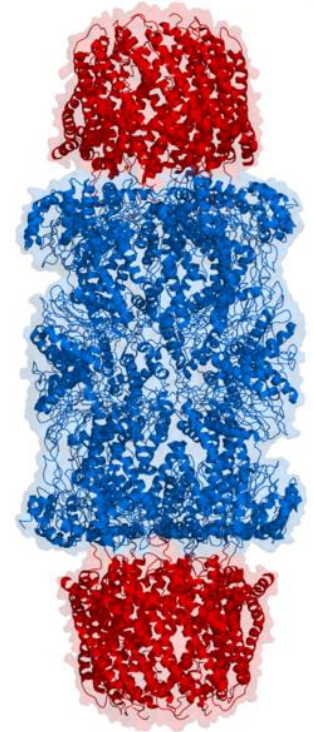
Hemoglobin



Ribosome 10S



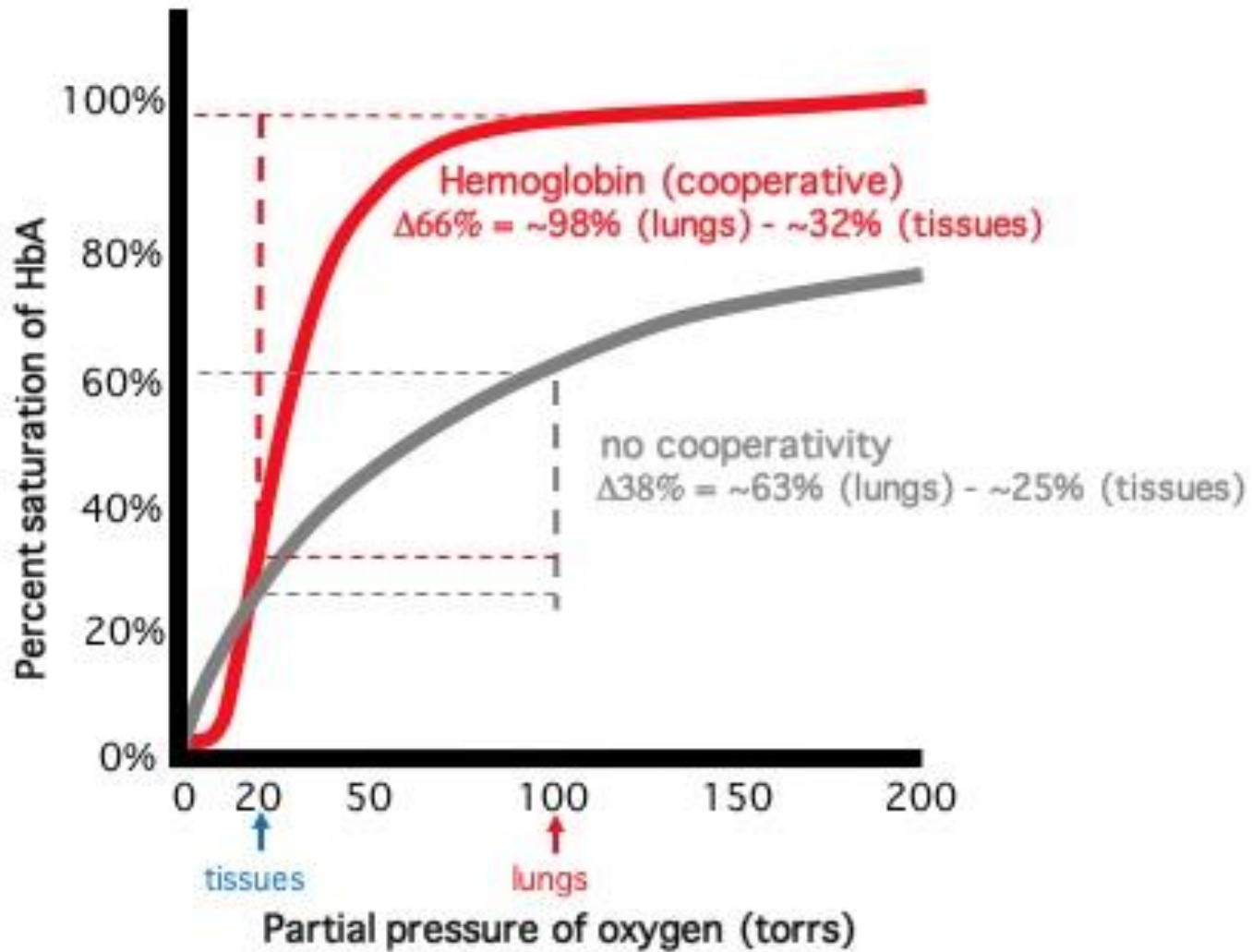
ATPase



Proteasome

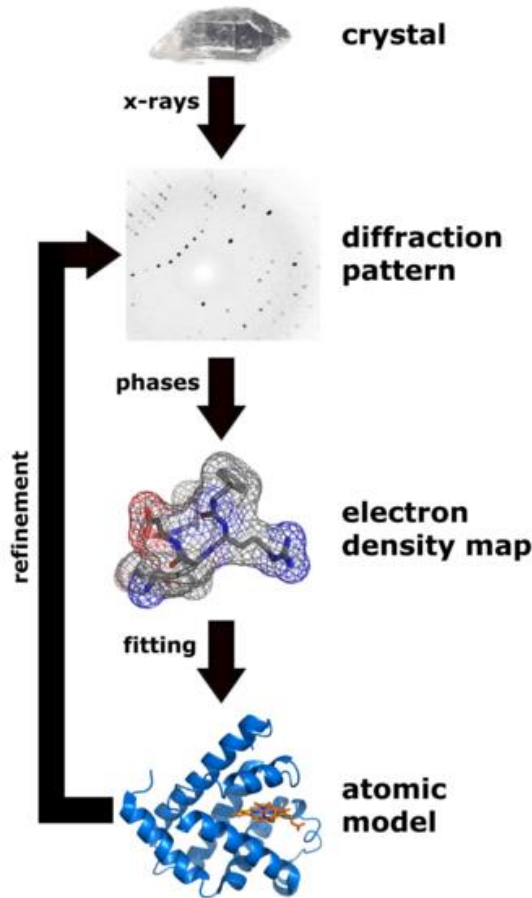
- Protein/protein interactions are a **crucial part of metabolism**. Used to activate/inhibit pathways that rely on specific activated enzymes.

For example....

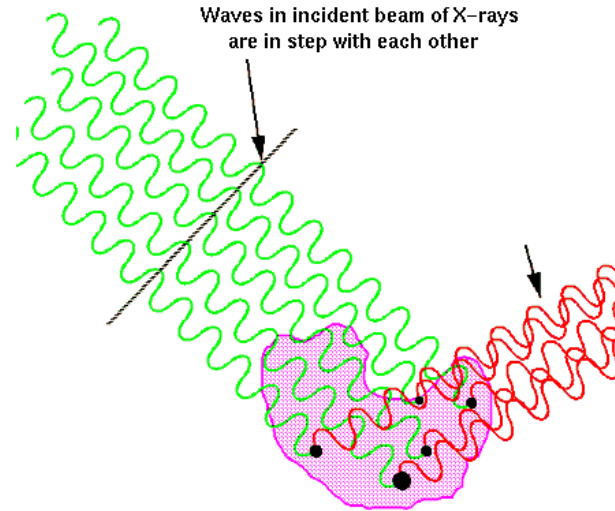


Figuring Out Protein Structures

- X-ray Crystallography

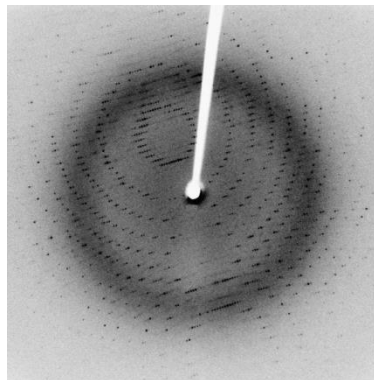


Scattering from the atoms in a molecule



The scattered waves are all shown as having the same amplitude – in practice, the amplitude would vary in proportion to the number of electrons associated with the atom ACT North 1997

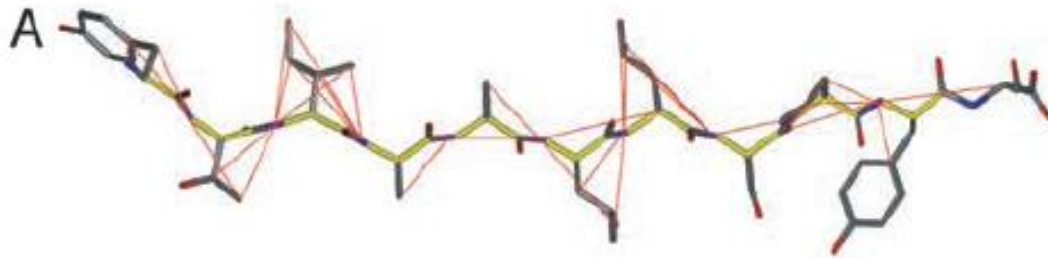
<http://www.britishbiophysics.org.uk/what-is/crystal/crystal.html>



- Diffraction occurs according to **Bragg's Law**

Structural NMR

- Mostly based on interproton distances acquired in ‘Nuclear Overhauser Effect’ (NOE) experiments.
- NOEs provide a set of **distance constraints** for **nearby protons** ($< 5\text{\AA}$)

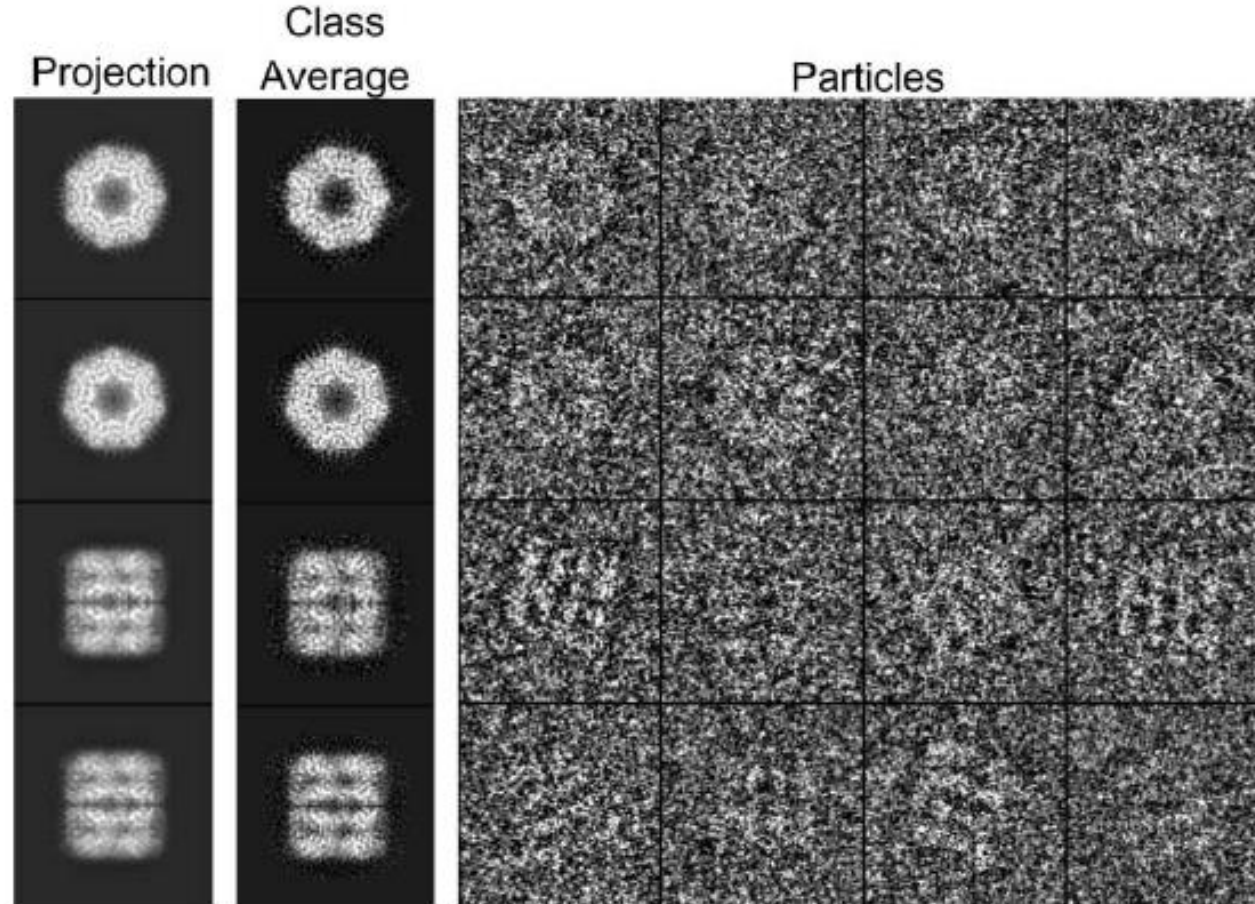


PNAS January 20, 2004 vol. 101 no. 3 711–716

- We can also directly get **ϕ/ψ angle constraints** from ‘Residual Dipolar Coupling’ experiments
- We then throw these constraints into a computer and ask it to come up with the **most satisfactory set of structures**.
- This is why NMR ‘pdb’ (structure) files are **so big!**

Protein Complex Structures

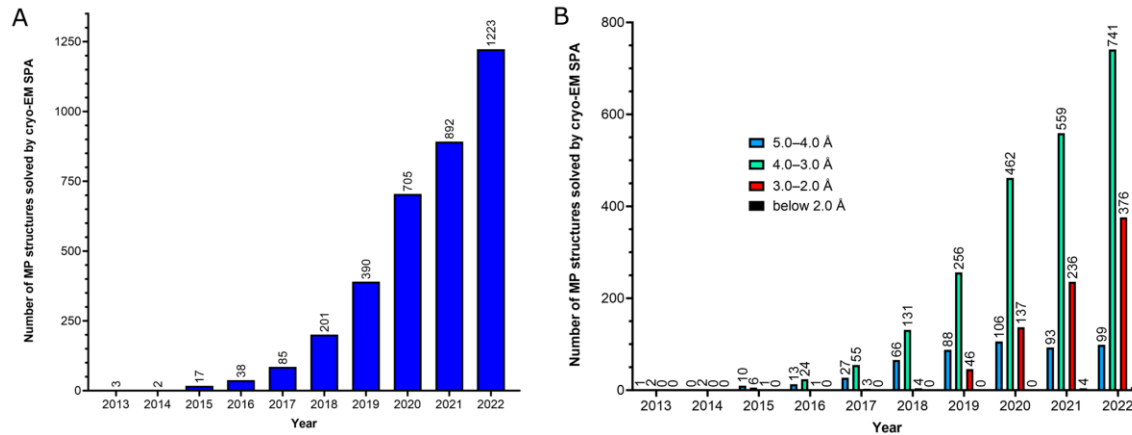
- Cryo-Electron Microscopy is becoming an increasingly common way of measuring structures of large protein complexes



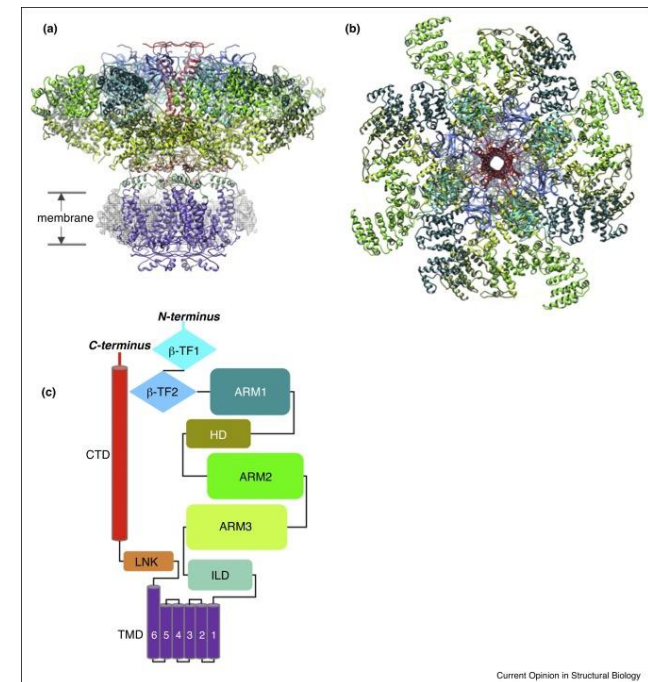
- Cryo-EM of GroEL, **chaperone extraordinaire**

The 'NEW' Cryo-EM

- In 2013, someone published a Cry-EM structure that had a resolution close to X-ray crystallography
- Within just a few years *everyone* was jumping on the **Cryo-EM train**



Int. J. Mol. Sci. **2023**, *24*(19), 14785



Current Opinion in Structural Biology

- The difference is a new type of **detector**, which can make an image from thousands fewer electrons

The End...
