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I will post the notes on my web site in PDF format before each lecture, along with reading assignments. I will also leave them up for a while afterwards if you missed class or are dying to have the color version.

March 2005

<table>
<thead>
<tr>
<th>Sun</th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Sat</th>
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<tbody>
<tr>
<td>Prot Structure</td>
<td>Intro to channel proteins</td>
<td>Cloning &amp; Methods 1</td>
<td>Cloning &amp; Methods 1</td>
<td>Channelopathies &amp; K+ Channels</td>
<td>K+ channel pore structure 1 &amp; 2</td>
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<tr>
<td>Ancillary Subunits: KvLQT1</td>
<td>Ca2+ Channels &amp; signaling</td>
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Reading Assignments:

- **Background**: Usually introductory textbooks, I’ll try to inform you of these before the lecture. Read these if you think you need extra explanation.
- **Primary Literature**: These are papers from journals, and will be handed out in class & assigned well ahead of time. They must read thoroughly. Be prepared to discuss them in class, as 1/4 of your grade on my test will be class participation.

For the most part, I will assume that you have a working knowledge of basic molecular biology techniques, as well as being comfortable with the working on the internet. If this is not the case, don’t panic! Read the background assignments, then if necessary, come speak to me and will bring you up to speed.

Along with information about cell physiology, I will attempt to work in as many experiments and as much primary data as I can. This is intended to introduce you to the scientific process (if you need such an introduction!), and help you understand where all this astonishing information came from.

At the end of my lectures, you’ll be given a take home test. This will be a paper or papers on some aspect of channel biology, and will be accompanied by some questions for you to answer. I’m not sure, but I suspect the techniques, approaches, and experimental logic presented in these lectures just might be useful in answering these questions.
Introduction to Membrane Protein Structure

• Background reading:
  – Branden & Tooze: Introduction to protein structure (Garland Press, 1991 or 1999). In Sci & Eng library
• A web site that goes into this material in all its 3-D glory is the “Principles of Protein Structure Using the Internet” course run by Birkbeck College of the University of London, School of Crystallography at http://www.cryst.bbk.ac.uk/PPS2/course/index.html
• It is also fun to fool with the “Protein Explorer” web site, at http://molvis.sdsc.edu/protexpl/frntdoor.htm

What does Protein Structure have to do with Physiology?

• The molecules responsible for passage of ions through membranes are proteins.
• With advances in “molecular protein chemistry” and genomics, we have learned the identity of most if not all proteins that conduct ions across membranes.
• This information consists of primary amino acid sequences that are from about 100 to several thousand amino acids long.
What does Protein Structure have to do with Physiology?

- Ion channel biophysics
- Protein structure & amino acid properties

Testable Hypothesis: Structure/function studies

Major Principle:
- Protein properties are determined by the collective and individual properties of amino acids
- For every property of a channel, there is an amino acid, or group of amino acids, that are responsible

Protein Structure is Hierarchical

- Primary: amino acid sequence
- Secondary: local structures formed between nearby (adjacent) amino acids
- Tertiary: long range interactions between amino acids, same chemistry as secondary structure
- Quarternary: Interaction between peptide chains; same chemistry as 2° and 3° interactions, but usually involving many more amino acids
Primary Structure

Proteins are formed when amino acids are connected by peptide bonds.

The Ends have Names

**Amino** or N terminus is the start.  **Carboxyl** or C terminus is the end.

$n$ can be very large (>1000)
Basis of secondary structure: bond angles

Peptide torsion angles allow rotation

Partial double bond character & R groups limit rotation

Basis of secondary structure: hydrogen bonds

H-bond:
- Electrostatic interaction between 2 atoms with no formal charge
- Potential in peptides
Properties of Amino Acids

**Small, Neutral Amino Acids**

- **Glycine (G)**: 7.2% occurrence
- **Alanine (A)**: 7.2% occurrence

**Hydrophobic Amino Acids**

- **Valine (V)**: 6.6%
- **Leucine (L)**: 9.1%
- **Isoleucine (I)**: 6.3%

**Aromatic Amino Acids**

- **Phenylalanine (F)**: 3.9%
- **Tyrosine (Y)**: 3.2%
- **Tryptophan (W)**: 1.4%

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Properties of Amino Acids

**Basic Amino Acids**

- **Lysine (K)**: 5.9%
- **Arginine (R)**: 5.1%

**Acidic Amino Acids**

- **Aspartate (D)**: 6.3%
- **Glutamate (E)**: 6.3%
Properties of Amino Acids

Polar, Uncharged Amino Acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Property</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>Polar</td>
<td>6.8%</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Polar</td>
<td>1.9%</td>
</tr>
<tr>
<td>Threonine</td>
<td>Polar</td>
<td>5.9%</td>
</tr>
<tr>
<td>Methionine</td>
<td>Polar</td>
<td>2.2%</td>
</tr>
</tbody>
</table>

More Polar Amino Acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Property</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>Polar</td>
<td>2.9%</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Polar</td>
<td>4.3%</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Polar</td>
<td>4.3%</td>
</tr>
</tbody>
</table>

Amino acids have overlapping properties:

- aliphatic
- aromatic
- hydrophobic
- polar (hydrophilic)
- charged
- negative (acidic)
- positive (basic)
- tiny
- small

Your life will be easier if you memorize the one letter codes.
Amino acid properties have roles in protein structure

- **Size**: Determines packing geometry
- **Charge**: Salt bridges between acidic & basic amino acids.
- **Polarity**: Charged and neutral polar side chains participate in hydrogen bonds with each other & with water.
- **Hydrophobicity**: Amino acids with aliphatic side chains interact less favorably with water; they are usually interior or in membranes.
- **Aromaticity**: There is a tendency for aromatic side chains to be 'stacked' against amide and amino groups, rather than accepting protons from them in 'hydrogen bonds'.

Two amino acids that play a unique role in protein structure: Proline & Glycine

Consequences for structure?
Common secondary structures in proteins

- There are lots of amino acids, but only 4 major conformations that they assume within a protein.
- The $\alpha$ helix, the $\beta$ sheet, and random coil.
- The random coil only exists in short stretches (<5). Generally, they only connect sheets & helices.
- The other structures are important to allow specific arrangements of R groups. These are called "turns".

$\alpha$ helices & $\beta$ sheets dominate most protein structures

Protein structures can be represented as collections of ribbons & curls:
Ribbon diagrams of some common proteins

http://kinemage.biochem.duke.edu/

Ribbon Diagrams of some ion channels

Porin

Aquaporin
The α helix

3.6 amino acids/turn

The amino acid side chains point out

Amphipathic is derived from two Greek words. "Amphi" means "both," or "both sides". "Pathic" signifies "feelings".

An α helix can be amphipathic: Polar and non-polar amino acids can co-exist on one side of the helix, even if they have the same charge.

The helix is held together by hydrogen bonds

C=O \cdots H \cdots N
\delta^{-} \quad \delta^{+}
The β sheet

It has a “pleated” structure, it is not flat in a plane.

Like an α helix, this structure allows for side chains to point outward in ways that are not energetically favored.

Structure of the β sheet

The structure is an extended peptide chain….

….held together by H-bonds
Unlike the α helix, β sheets almost always come in pairs:

- Anti parallel
- Parallel

The two (or more) strands are coupled through inter-strand interactions.

**Implications of the β Sheet**

Note that there are many unpaired O & Ns to allow for more strands & that amphipathic structures can be formed.

Carboxypeptidase A
Some amino acids are more likely to be $\alpha$ helices and others $\beta$ sheets

This is because the R groups can hinder the conformation (bond angles) required to form the structure

$\alpha$ helix

- **Common:** Ala, Arg, Gln, Glu, Leu, Lys, Phe
- **Less Frequent:** Asp, Cys, His, Ile, Trp, Val.
- **Rare:** Asn, Ser, Thr, Tyr
- **Are you joking? Pro & Gly**

$\beta$ sheet

- **Common:** Asn, Lys, Gly, Pro, Ser
- **Rare:** Ala, Ile, Lue, Met, Phe, Val

Violations of these rules often point to important parts of a protein

Tertiary Structure: proteins fold

- Once secondary structure is formed, proteins are mostly grease balls, with hydrophobic amino acids on the inside and hydrophilic amino acids on the outside

- The dominant factor in considering how proteins fold: Oil & water don’t mix

- Interactions between amino acids determine tertiary protein structure (mostly)
Interactions that determine 3° structure

- The most common interactions are hydrophobic which mostly occur between hydrophobic amino acids
- Other interactions include:
  - H-bonding
  - Ionic interactions
  - Disulfide bonds between Cys

Proteins are not as open as the cartoons suggest

*Carboxypeptidase A*

Cartoon  Add water  Space fill
Most proteins are assembled from specialized organization units called domains

Membrane Proteins

- Channels, and many channel associated proteins are membrane proteins
- They are difficult to crystallize
- They exist in a combined environment of lipid (hydrophobic) and water (hydrophilic)
- The necessity of crossing the membrane constrains the structure in 2 dimensions
There are many ways for proteins to associate with membranes

Membrane protein conformation

Not extended

Top view

Pore
Determination of protein structure

- Can be directly determined by X-ray diffraction or NMR
- 27064 3-D protein structures in PDB
- Many of these are related
- Direct determination of membrane protein structure very tough
- Only a handful known
- 38,989,342,565 nucleotides in Genebank
- Is it possible to use some of this information to predict protein structure?

Sure: It’s called “Bioinformatics”
Determination of protein structure

- Obviously an extremely complex problem
- The basic approach is to take principles from known protein structures and apply that to unknowns
- Simple structure predictions can easily be made
- TM segments should
  - Likely to form $\alpha$ helices
  - Large hydrophobic segments

First, each amino acid is assigned a number to represent its “hydrophobicity”

Hydrophobic: Ala (1.8), Ile (4.5)

Hydrophilic: Arg (-4.5) Gln (-3.5), Ser (-0.8)
Next, amino acid groups are scored in windows to determine a hydrophobic profile:

```
<table>
<thead>
<tr>
<th>Sequence</th>
<th>Hydrophobic Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>STCPDQGAVNQHKRSTPDQHAGCASYCTIWOFVILSWAFYWNWF</td>
<td>-1.60</td>
</tr>
<tr>
<td>STCPDQGAVNQHKRSTPDQHAGCASYCTIWOFVILSWAFYWNWF</td>
<td>-1.74</td>
</tr>
<tr>
<td>STCPDQGAVNQHKRSTPDQHAGCASYCTIWOFVILSWAFYWNWF</td>
<td>-1.87</td>
</tr>
<tr>
<td>STCPDQGAVNQHKRSTPDQHAGCASYCTIWOFVILSWAFYWNWF</td>
<td>-1.91</td>
</tr>
<tr>
<td>STCPDQGAVNQHKRSTPDQHAGCASYCTIWOFVILSWAFYWNWF</td>
<td>-0.83</td>
</tr>
<tr>
<td>STCPDQGAVNQHKRSTPDQHAGCASYCTIWOFVILSWAFYWNWF</td>
<td>+0.47</td>
</tr>
<tr>
<td>STCPDQGAVNQHKRSTPDQHAGCASYCTIWOFVILSWAFYWNWF</td>
<td>+1.37</td>
</tr>
</tbody>
</table>
```

Possible membrane spanning segment:

An example: The GABA<sub>A</sub> receptor:

```
1 MKKSGLDSDY LWATLFLST LTGRSYQPS LDDELKDNTT VFTRILDRLL
51 DGYDNNLRPG LGERVTEVKT DIFVTSFGPV SDHDMETYTD VFFRQSNKDE
101 RLFKGPMTV LRLNNLMASK IWTPDTFFHN GKKSVAHNM TGRNLLRITE\n151 DGTLLYTRML TVRABECPML EDTPMDAHAC PKFGSYATY RAEEVYETWR
201 EPARSVVVAR DGSRLNQYDL LGQTVDGIV QSSTGEYVV MTHHPLLRRK\n251 GYFVQTVYLP CINTVILSQV SFWLMVESVP ARTVFGVTTV LTMDDLSSAA
301 RNFLKPHA YAMDFIAVGC YAFYFSGLIE FAYVNYFTKR GYAMDGGSVV
351 PERPKVKGDP LHKNNNTAP TATSTPNLAA RGDPGLATIA KSATIEFKEV
401 KPFKTPFETYK KTFNSIVSKID ERLSIAFPEL FSIIFNVVYWA YLNRPEQLK
451 APTPHQ
```
### Structural Hypothesis

**Needs biochemical proof!**

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### Similarity can provide evidence of structure

<table>
<thead>
<tr>
<th>GABA-A</th>
<th>Glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRKSPGLSDCLWAWILLLSTLTGRSYGQPS----LQDELKDNTTVFTRI</td>
<td>MNRQLVNILTALFAFFLETNHFRTAFCKDHDSRSGKQPSQTLSPSDFLDK</td>
</tr>
<tr>
<td>LDRLLDGYDNRLRPGLGERVTEVKTDIFVTSFGPVSDHDMEYTIDVFFRQ</td>
<td>LMGRTSGYDARIRPNFKGPPVNVTCNIFINSFGSVTETTMDYRVNIFLRQ</td>
</tr>
<tr>
<td>SWKDERLKFKG-PMTVLRLNNLMASKIWTPDTFFHNGKKSVAHNMTMPNK</td>
<td>QWNDSRLAYSEYPDDSLDLDPSMLDSIWKPDLFFANEKGANFHDVTTDNK</td>
</tr>
<tr>
<td>LLRITEDGTLLYTMRLTVRAECPMHLEDFPMDAHACPLKFGSYAYTRAEV</td>
<td>LLRISKNGKVLYSIRLTLTLSCPMDLKNFPMDVQTCTMQLESFGYTMNDL</td>
</tr>
<tr>
<td>VYEWTREPARSVVVAEDGSRLNQYDLLG-QTVDSGIVQSSTGEYVVMTTH</td>
<td>IFEWLSDGP---VQVAEGLTLPQFILKEEKELGYCTKHYNTGKFTCIEVK</td>
</tr>
<tr>
<td>FHLKRKIGYFVIQTYLPCIMTVILSQVSFWLNRESVPARTVFGVTTVLTM</td>
<td>FHLERQMGYYLIQMYIPSLLIVILSWVSFWINMDAAPARVALGITTVLTM</td>
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<tr>
<td>TTLSISARNSLPKVAYATAMDWFIAVCYAFVFSALIEFATVNYFTKRGYA</td>
<td>TTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFAALLEYAAVNFVSRQHKE</td>
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<tr>
<td>WDGKSVVPEKPKKVKDPLIKKNNTYAPTATSYTPNLARGDPGLATIAKSA</td>
<td>FLRLRRRQKRQNKEEDVTRESRFNFSGYGMGHCLQVKDGTAVKATPANPL</td>
</tr>
<tr>
<td>TIEPKEVKPETKPPEPKKTFNSVSKIDRLSRIAFPLLFGIFNLVYWATYL</td>
<td>PQPPK-----DGDAIKKKFVDRAKRIDTISRAAFPLAFLIFNIFYWITYK</td>
</tr>
<tr>
<td>NREPQLKAPTPHQ</td>
<td>IIRHEDVHKK---</td>
</tr>
</tbody>
</table>

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Since GABA A and Glycine are related, and these sequences are conserved, there is a better chance that they are a conserved feature, such as a membrane spanning segment.