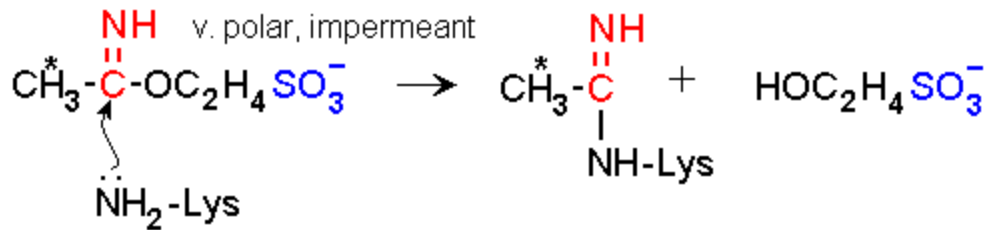


1. What kind of experimental evidence would indicate that a protein crosses from one side of the membrane to the other?

Regions of polypeptide part exposed on the outside of the membrane can be probed either by exposure to protease (e.g. trypsin) or by reagents that act on specific amino acids, where the reagent is too polar to cross the bilayer.

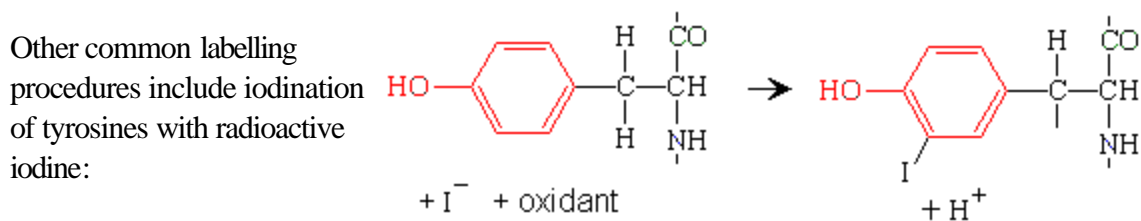
Imidates react with amino groups such as Lys:



If the reagent includes a very polar group such as the **sulfonate**, then it will be unable to cross the bilayer, and is an **impermeant reagent** that only reacts on the exposed outer surface. If the reagent carries radioactive atoms such as tritium in place of hydrogen, the protein will become radioactive only at lysines that are externally exposed, and these can be detected. This type of procedure where a reagent acts selectively on particular amino acids is called a **labelling** experiment.

Cell membranes can be disrupted and resealed inside out vesicles. Exposure to trypsin or impermeant reagents will reveal portions of proteins that were originally on the inside surface of the bilayer.

Any protein that can be labelled by **both** procedures must be a transmembrane protein.



If a polar oxidant is used, only externally exposed tyrosines will be labelled.

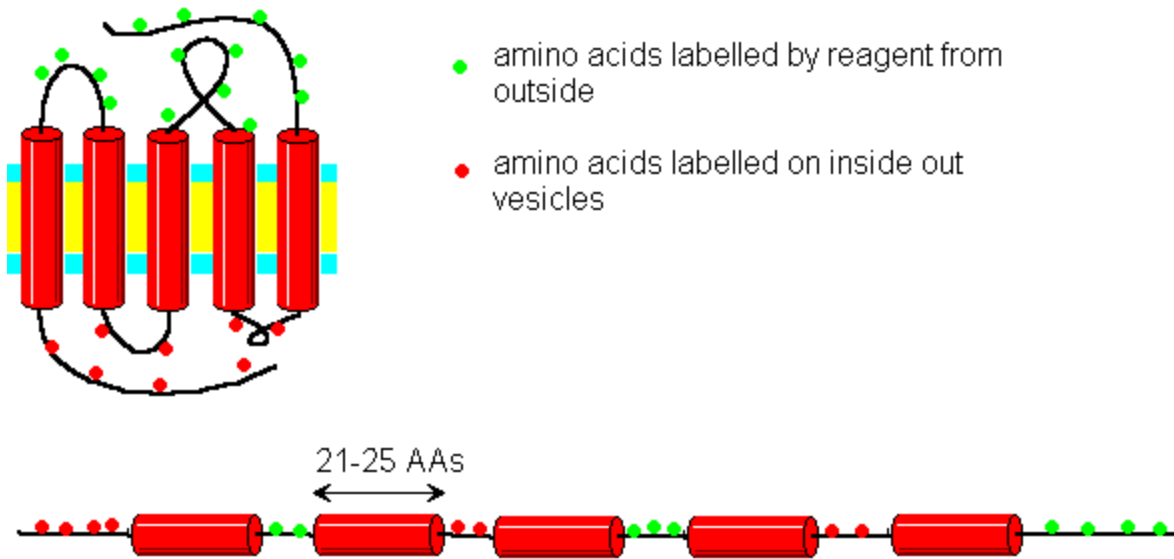
2. Type 1 and type 2 topologies consist of a single transmembrane helix, but in opposite orientations. How might this be established experimentally?

Labelling reactions are carried out as described above, and the polypeptide is analyzed by proteolytic cleavage. Labelled peptides should be separated from unlabelled by chromatography, and their amino acid sequence determined to identify where they fit in the complete polypeptide. Type 1 topology should be labelled from N-terminal to the transmembrane helix when impermeant reagent is applied outside, and type 2 labelled from the C-terminus.

3. How would you determine that a protein had multiple transmembrane helices?

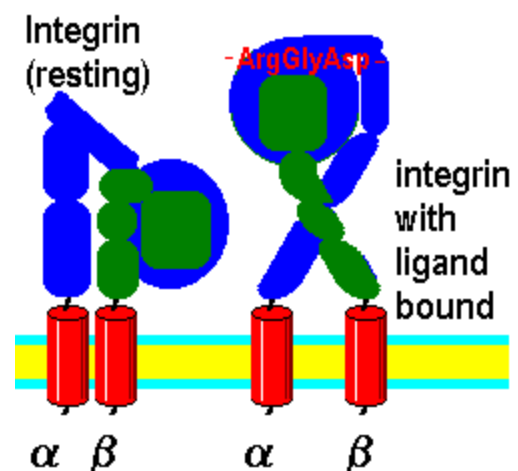
The protein is reacted with an impermeant reagent as above, and the locations of labelled peptides identified as described in question 2. In this case, label should be found at periodic intervals along the polypeptide. A transmembrane helix should be 21-25 amino acids in length, so there should be at least 50 amino acids between groups of labelled amino acids.

Normally there will be a site labelled from the opposite side in between each cluster, but this may not always be the case, if the amino acid that is the target for the labelling reaction is not present in one of the connecting loops.



4. How can a cell tell when one of its integrins has bound to an appropriate ligand?

When the integrin binds its ligand, the external domain formed by the α and β subunits changes conformation, so that the transmembrane helices spread apart. In the spread state, integrin molecules cluster together in a patch, and this allows recruitment of regulatory tyrosine kinases inside the cell.

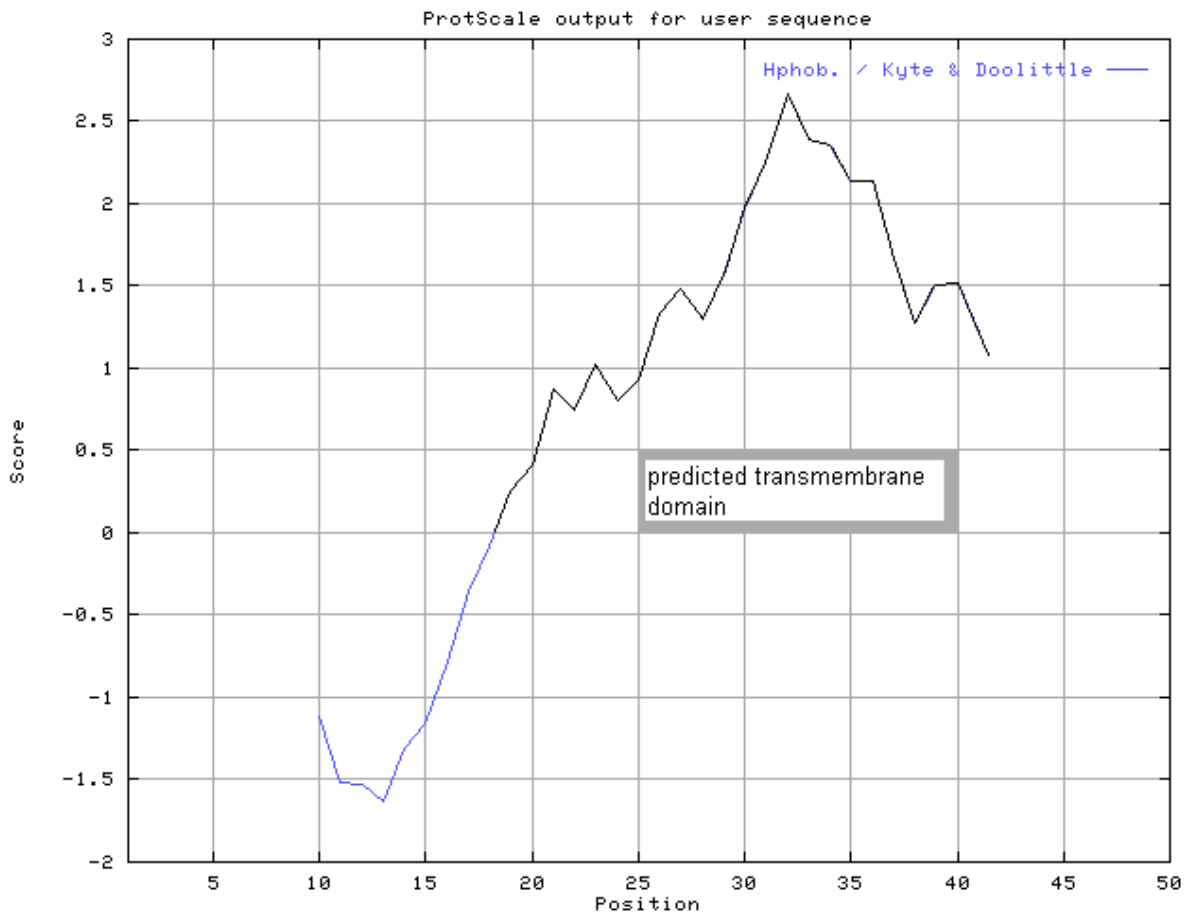


5. A partial sequence of glycoporphin (amino acids 51-100) is

Val Tyr Pro Pro Glu Glu Glu Thr Glu Glu Arg Val Gln Leu Ala His His Phe Ser Glu
Pro Glu Ile Thr Leu Ile Ile Phe Gly Val Met Ala Gly Val Ile Gly Thr Ile Leu Leu
Ile Ser Tyr Gly Ile Arg Arg Leu Ile Lys.

VYPPEETEERVQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIK

Use the table of values given in lecture 26 to draw a hydropathy plot. Compare your result with Fig. 12-17 a) in Lehninger p.403 (Note the above is only a partial sequence, but does include the transmembrane region).



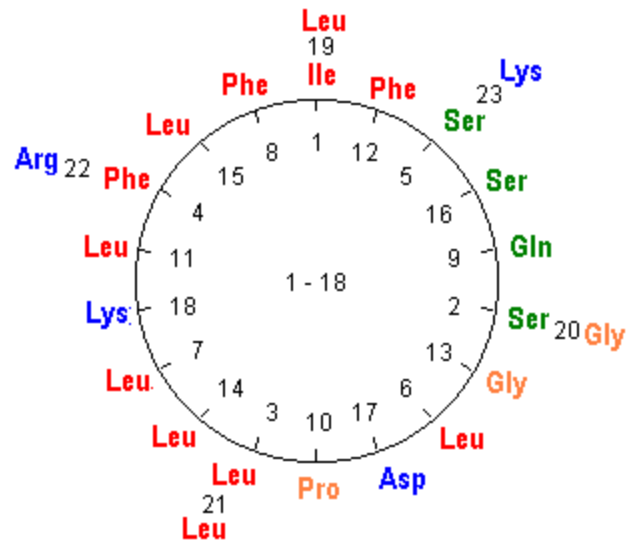
6. A partial sequence of *LacY* (Lactose permease) is

I L E S E R L E U P H E S E R L E U L E U P H E G L N P R O L E U P H E G L Y L E U L E U S E R A S P L Y S L E U G L Y
L E U A R G L Y S

Draw the helical wheel for this sequence. What does it tell you about the probable function and location of this subsequence?

Each amino acid is located 100° around the wheel from its predecessor. You can mark 18 places (5 turns of helix) before you return to the same spot.

Most of the left side of the wheel is non polar. The two exceptions are Lys at position 18 and Arg at position 22, so these may represent the part of a transmembrane helix that interacts with the phospholipid headgroups rather than the core.



It looks like aminoacids 1-15 are near the hydrocarbon core of the bilayer. Those amino acids that fall on the left side of the wheel are non-polar and may contact the bilayer directly. Those amino acids on the right of the wheel are probably facing the interior of the transmembrane protein.

7. Compare the role of the HIV fusion protein GP41 described in Lehninger pp. 406-407 with the mechanism of SNARE mediated fusion.

How is the viral fusion mechanism similar to SNARE mediated fusion?
How is it different?

The virus HIV targets cells through two proteins, GP 41 and GP 120 (GP=glycoprotein, polypeptide with attached oligosaccharide chains. 41 and 120 refer to the size in kiloDaltons. GP120 identifies which cells to infect by binding to a cell surface protein called CD4. Unfortunately the cells that carry CD4 happen to be important for developing an immune response, so HIV kills those cells that might otherwise be responsible for mounting immune defences.

When GP120 binds CD4, it exposes GP 41. GP 41 is anchored into the viral envelope by a transmembrane domain at the C-terminal, but the N-terminal carries a second potential transmembrane domain, which spears into the host cell.. GP 41 then forms a helix bundle which folds over and wraps around itself, in a manner very similar to how t-SNAREs wrap around the

v-SNARE. When the viral envelope is pressed tightly against the host cell plasma membrane, fusion will occur.

Why does the virus not have an equivalent for NSF and SNAP?

Why does the SNARE mechanism need NSF and SNAP?

NSF and SNAP unwind the helix bundle of v-SNARE and t-SNAREs so that the components can be reused to fuse more vesicles. Without NSF and SNAP, a cell will have one round of fusion, but won't be able to sustain fusion over the longer term.

The virus attacks the host cell as a one-time event. The virus does not need to reuse the fusion components once the initial fusion is complete.

8. *How do symport and antiport transporters maintain strict 1:1 stoichiometry for transport?*

Symport: $A_{out} + B_{out} \rightarrow A_{in} + B_{in}$

Antiport: $A_{in} + B_{out} \rightarrow A_{out} + B_{in}$

These processes don't simply represent two substrates sharing the same transporter. Instead both substrates take part in a distinct reaction cycle.

For symport, the reorientation of the transporter only occurs when **both substrates** occupy the binding site in the transporter, so one molecule of each must be transported.

For antiport, passage of the first substrate reorients the transporter to face the other side of the membrane, and passage of the second substrate brings the transporter back to its original state.

One molecule of each substrate must bind to complete the reaction cycle, although each molecule takes part in a different stage of the process.

