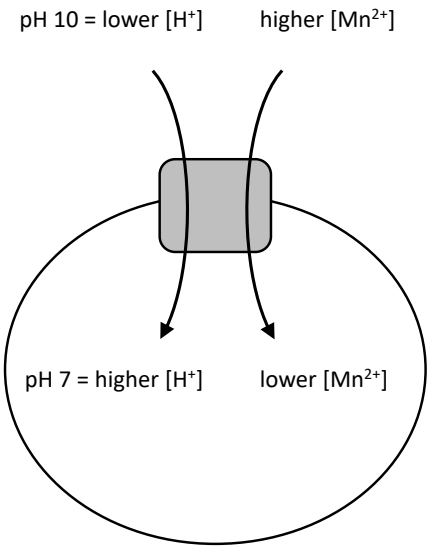
Names: _____

The Waters lab studies a transporter in *E. coli* called Alx. Alx is only made when cells are grown in high pH media, where it imports H^+ to keep the intracellular pH ~ 7.

a) Draw a bacterial cell that has a cytoplasmic pH of 7 while growing in pH 10 media. Show:

- the membrane containing the Alx transporter
- the relative H^+ concentrations inside and outside of the cell
- the direction of H^+ transport



b) What type of transporter is Alx likely to be: passive or active? Why?

Active transport. H^+ is flowing from low → high concentration, against its gradient. This requires an energy input.

c) There is no ATPase domain present in Alx. Instead, Alx is a Mn^{2+} importer that requires high extracelluar Mn^{2+} for its activity. Add Mn^{2+} transport to the diagram above. Show:

- the relative Mn^{2+} concentrations inside and outside the cell
- the direction of Mn^{2+} transport

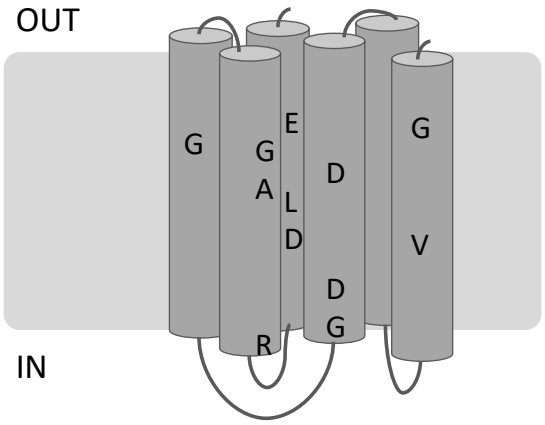
d) What powers the transport of H^+ through Alx? What is the name for this kind of transport?

The energy released from Mn^{2+} flowing down its gradient (high → low concentration) powers the pumping of H^+ against its gradient (high → low). This is secondary active transport. (Primary active transport would use the energy of ATP hydrolysis.)

Alx is also a symporter because both ions are flowing in the same direction (into the cell).

(see other side)

From computational analysis using statistical scoring of amino acid sequences that are likely to form α-helices (no Pro, branched chain amino acids disfavored), we have developed a prediction of where the hydrophobic membrane-spanning regions of the protein are likely to be. There are likely to be 6 transmembrane regions with small loops in between, as shown in our model:



e) The most highly conserved amino acids in the Alx exporter are shown by their 1 letter code. Which amino acids might be directly involved in exporting H^+ and Mn^{2+}? (Circle them.) Why did you choose these?

The two D and two E residues near the center of the protein. Asp and Glu are negatively charged and could bind positively charged H^+ and Mn^{2+}.

(To test this, we could mutate these amino acids and examine if the mutant Alx proteins could still transport protons and Mn^{2+}.)