Aquaporin Two in Water Regulation & Diabetes Insipidus

D.C. Everest SMART/MAPS Team

Poster Created by: Emily Adams, Vanessa Bradfish & Megan Ninnemann

SMART Teams 2015-2016 MSOE UNIVERSITY

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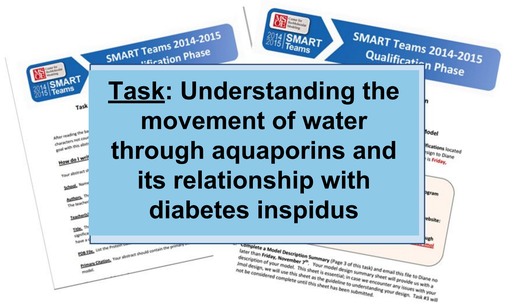
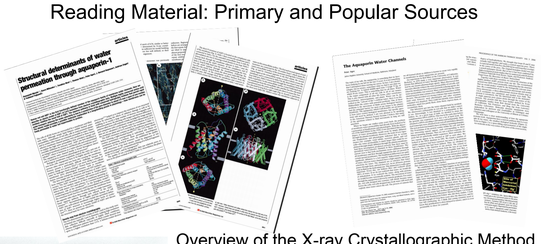
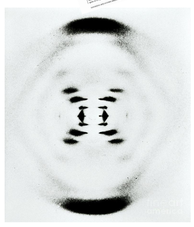


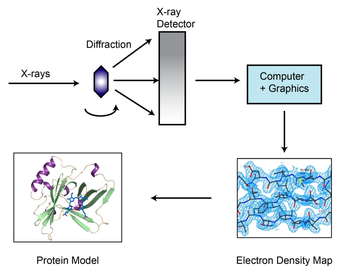
Task: Understanding the movement of water through aquaporins and its relationship with diabetes inspidus

Reading Material: Primary and Popular Sources

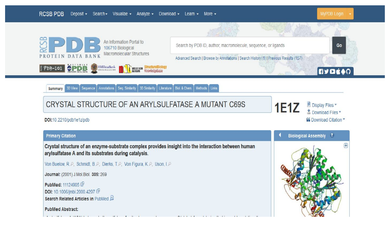


Overview of the X-ray Crystallographic Method



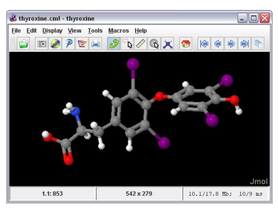


A crystallographer can create a 3D image and determine the (X,Y,Z) coordinates for each atom and their chemical bonds.



The Protein Data Bank has a large collection of three-dimensional instructions for molecules mapped by x-ray crystallography.

Jmol is a modeling program that allows us to use the instructions from the Protein Data Bank to create a 3D model that we can manipulate and examine closer to find the active site.



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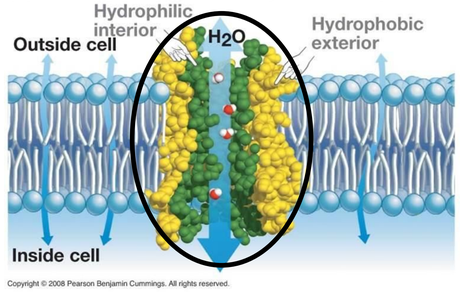
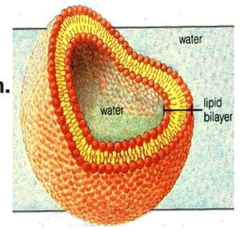
Overview of Aquaporins

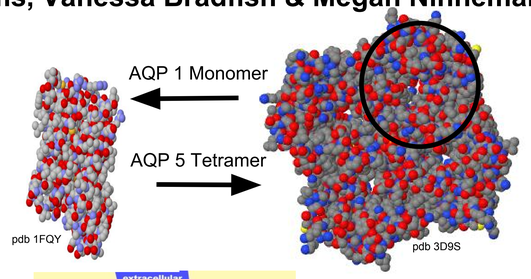
An aquaporin is a membrane-bound protein that moves water in and out of the cell.



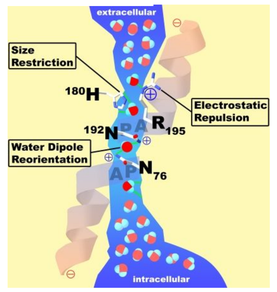


Aquaporins are needed to transport water across the plasma membrane. Without it, water would diffuse slowly through the lipid bilayer of the cell.

The ar/R selectivity filter is lined with positively charged residues that keep unwanted molecules out and let only water through.

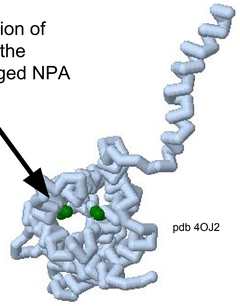


This model shows the ends of the channels. The protein would normally be oriented lengthwise in the cell membrane.

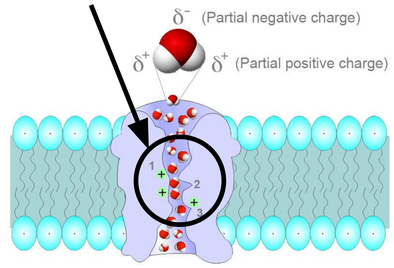


The NPA site consists of residues in the channel that flip the water molecules in order to prevent automatic hydrogen ion transfer through a chain of water molecules (Grotthuss mechanism).

The green portion of aquaporin 2 is the positively charged NPA site.

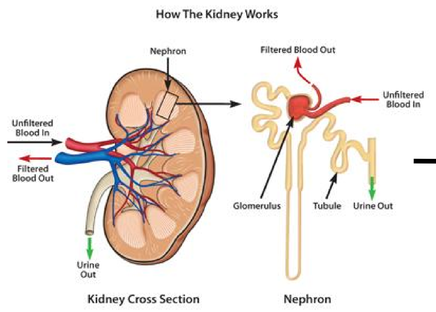


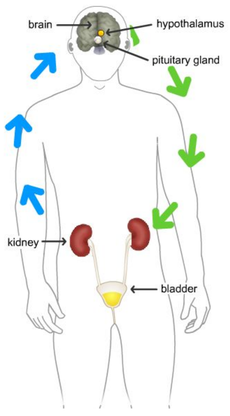
NPA site is a positively charged area of the channel.



Up to 3 billion molecules of water travel through a single aquaporin channel per second by flowing with the osmotic gradient.

Water Regulation & AQP2



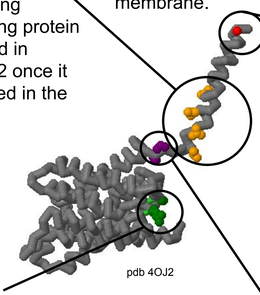


Function of the Kidney:
Everyday, the body filters 120 to 150 liters of blood into 1 to 2 liters of urine.

ADH travels to the kidneys via the bloodstream.

- AQP2 controls water reabsorption in response to antidiuretic hormone (ADH) or vasopressin.
- Brain releases ADH when insufficient amount of fluid is in the blood.
- The ADH causes AQP2 to be trafficked to the apical membrane in the nephrons.
- AQP2 is inserted in the membrane, and water is channeled.

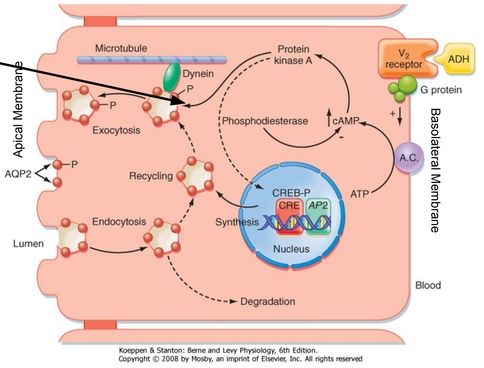
This **orange** colored region (residues 230-243) is critical for bonding to lysosomal trafficking regulator interacting protein 5 which is involved in internalizing AQP2 once it is no longer needed in the apical membrane.



The residues (Ser82 and Arg85, **green**) provide stability by holding the n-terminus in place which is critical for phosphorylation and trafficking.

Intracellular cAMP triggers phosphorylation at Ser256 (**red**) which flags trafficking from vesicles to the apical membrane.

Aquaporin Trafficking



The residues (Pro225 and Pro226, **purple**) are important in the flexibility of the carboxyl terminus necessary for trafficking.

Aquaporins 3 and 4 are also involved in the trafficking of water in the basolateral membrane of the cell.

Diabetes Insipidus & AQP2 Mutations

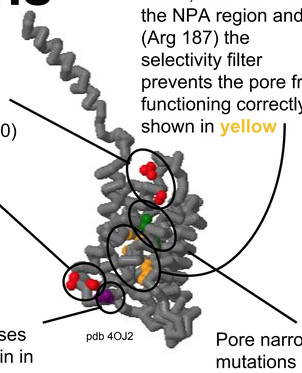
Diabetes Insipidus (DI)

- Two types: nephrogenic and neurogenic
- Nephrogenic DI is caused by the inability of the kidney to concentrate urine. This causes frequent thirst and increased urination of 3-15 liters per day.
- Pictured are four mutation sites in AQP2 that cause nephrogenic DI.
- Other major causes of Nephrogenic DI are problems with the ADH receptor proteins.

Key Mutations

1. Aquaporin cannot leave E. R.
2. Channel of aquaporin is affected.
3. Aquaporin is retained in intracellular vesicles.
4. Aquaporin is misrouted and unable to get to the final destination.

Over 40 mutations in the AQP2 gene can cause additional complications.

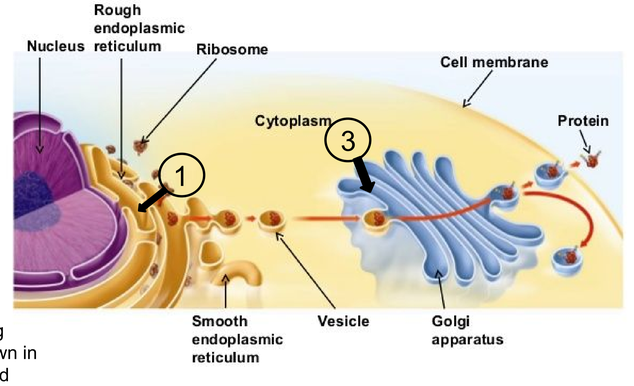


1. Critical Mutations interfering with glycosylation and other processes (Thr125, Thr126, Ser148, Asp150) shown in **red**

2. Mutations of Asn68, Ala70, and Pro185 in the NPA region and (Arg 187) the selectivity filter prevents the pore from functioning correctly shown in **yellow**

3. Mutation shown in **purple** (Arg123) causes the structure to remain in the Golgi apparatus

Pore narrowing mutations shown in **green** (V71 and V168)



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