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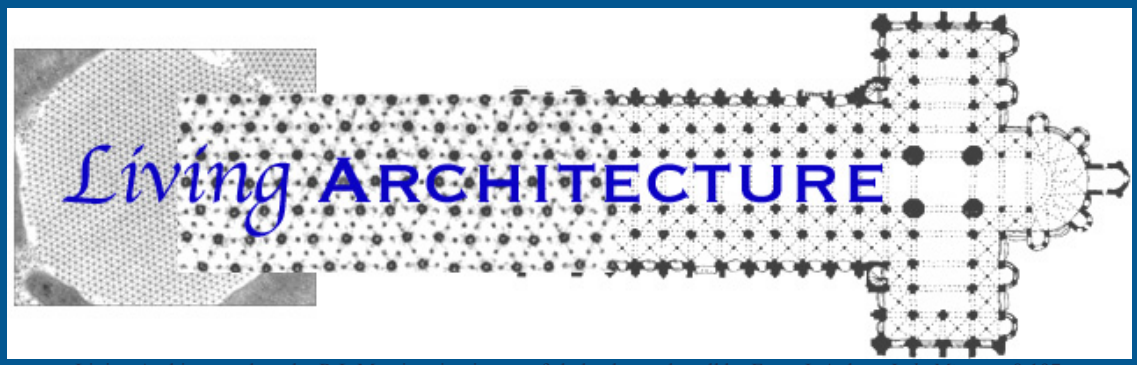

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Dynamic Equilibrium via Aquaporins and Filtration Systems

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Rules-to-Build-By:

In order to maintain the homeostasis of an isolated system, gradients and mediated transport can establish a dynamic equilibrium to regulate concentrations.

What:

This concept is demonstrated on the microscopic level by aquaporin channel proteins, which provide a means for a cell to mediate osmosis without losing solutes (Johansson et al. 2000). Aquarium filtration systems demonstrate it on a macroscopic level as they maintain an ideal environment for their inhabitants using filtration.

How:

Transmembrane water transport occurs in one of two ways within cells, simple diffusion through the lipid bilayer of the plasma membrane and transport through specific channel proteins called aquaporins. Located in the plasma membrane of the majority of living cells, aquaporins belong to a family of Major Intrinsic Proteins, or MIPs, and perform the function of regulating water intake and output in the cell depending on the osmotic gradient. This is especially important for cells because of the fact that large volumes of water cannot be exchange through the plasma membrane efficiently enough to keep the cell alive in most circumstances. While most aquaporins such as AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8 transport solely water in and out of the cell, some MIPs also transport glycerol and small, uncharged molecules. These have been labeled aquaglyceroporins and consist of AQP3, AQP7, and AQP9 (Johansson et al., 2000). Aquaporins are fairly small integral membrane proteins and their secondary structure consists of six membrane spanning alpha helical domains and has both the amino and the carboxy terminus positioned within the cytoplasm. The aquaporin itself is actually a homotetramer, a complex formed of four functional, identical monomers that associate with each other in the membrane to form the pore complex (see Figure 2). The channel itself is a narrow cylindrical pore that only permits single file transport of water molecules through the plasma membrane. As such, the single channel permeability of the aquaporin, specifically AQP1, was determined to be ~6×10^{-14} cm^3/s and the diameter of the pore itself was estimated to be 3.8 Angstrom, which would allow water to pass through but not larger molecules, an important

distinction (Verkman and Mitra, 2000). This single file mechanism of transport is incredibly important for the concept of proton exclusion (Wang and Tajkhorshid, 2007).

It would often be detrimental to a cell to lose its electrochemical gradient so the aquaporins have a mechanism to prevent ions smaller than water from leaving the cell. Along the primary sequence of the aquaporin protein exists a specific fingerprint consisting of the amino acids Asparagine, Proline, and Alanine (NPA). The presence of multiple NPA fingerprints creates a positively charged electrostatic field around that specific section of the pore (see Figure 3). While water molecules can pass through unhindered because of the partial positive charge on the oxygen, protons are repelled by the positive charge. The barrier height for this specific fingerprint is 25-30 kJ mol(-1), which matches the barrier height of pure lipid bilayers. This means that the pore prevents leakage of hydrogen ions as well as a normal lipid bilayer and therefore presents no significant risk to the electrochemical gradient across the membrane. Additionally, negatively charged regions adjacent to the NPA fingerprint prevent hydroxide ions from escaping the cell as well (de Groot et al., 2003).

In addition to the proton exclusion demonstrated by the aquaporins, some aquaporins can also be selectively gated. While a large number of aquaporins are believed to be permanently open water channels, some cells can gate aquaporins when extracellular osmotic conditions are not favorable. In drought or other conditions that reflect poor water availability, some plants have the ability to phosphorylate the protein can cause a large conformational change where one of the cytoplasmic loops, the D-loop, and the N-terminus were held together through hydrogen bonding. This effectively closed the entire channel and prevented any molecules from passing through the pore (Wang and Tajkhorshid, 2007).

Aquarium filtration systems are similar in some aspects to aquaporins. Through their mechanisms, water is kept in a constant dynamic equilibrium within its isolated container; new or clean water is constantly being pumped in while old water is being pumped out. While aquaporins simply use gradients to facilitate osmosis, filtration systems need several different subsections to be able to perform the same function. The pump of the filtration system is analogous to the cell's ability to gate their channels and stop the transport of water across the membrane. Attached to the system is a specific filter, which processes the water and ensures that no waste products or undesirable solutes are placed back within the system (see Figure 5). These filters are analogous to the NPA fingerprint within the aquaporins as they selectively prevent certain solutes from either re-entering the system in the case of the former or exiting the system in the case of the latter. In addition, a cell can synthesize more aquaporins to accommodate the size and needs of the cell, while an aquarium can also add more outflow and inflow pipes as needed (see Figure 4).

Why:

Evolutionarily speaking, aquaporins are extremely advantageous because they allow for the rapid exchange of water molecules across the membrane without disturbing other homeostatic systems. Compared to passive diffusion through the plasma membrane, a cell cannot regulate its osmotic gradient nearly fast enough with passive diffusion to be able to perform specific but necessary functions for a multicellular organism to survive (Gomes et al., 2009). This is essential for many forms of life as adaptation to changing tonicity is incredibly important for the survival of both unicellular and multicellular organisms. In addition, the ability of aquaporins to exchange water without disturbing the ion concentration within the cell to a large

extent is a significant advantage because the loss of a large amount of H^+ ions could not only disrupt the charge of the membrane but would also affect the pH of the cytosol. In plants, water flow often occurs because of the existence of a water potential gradient. This means that there is a difference is hydrostatic pressure across the membrane. In plant cells these channels can be used to establish the essential turgor pressure needed to give the organism its structure by utilizing this gradient to build up positive internal hydrostatic pressure while supporting the membrane with the cell wall (Johansson et al. 2000). Plant cells can also gate their aquaporins in environments where the cell is in a hypertonic solution and the osmotic gradient would favor the extracellular space. This is particularly useful for limiting the loss of water and actively defying the gradient to maintain homeostasis within the system. The benefits of this mediated transport means that even if a gradient exists, if it would disrupt homeostasis in a negative way, the cell has a mechanism for controlling the loss of water (Wang and Tajkhorshid, 2007).

In mammalian cells, aquaporins serve a wide variety of functions ranging from transcellular water transfer in the salivary glands of the mouth to mediated water reabsorption in the renal ducts of the kidneys. The ability of aquaporins to rapidly adapt to the changing tonicity of the extracellular environment is particularly important in the digestive and renal systems but is displayed throughout the body. In the renal system, one of the main ways that water is regulated is, in response to osmotic stimuli, through the reabsorption that occurs through AQP channels enabling urine concentration. It is mainly the size of the channels that is particularly useful as they have a high affinity for water and the pores themselves are only large enough to allow molecules and ions smaller than water to potentially pass through. The aquaporins also prevent any ions or solutes released from the cells from being reabsorbed and thereby increases the efficiency of the system (Day et al., 2014). AQP4 has been found to be a key player in maintaining the homeostasis of neurons and glial cells within the CNS and has a large effect on the K^+ concentrations required for neuroexcitation through its effect on extracellular space volume. This makes the exchange of water incredibly important for establishing and maintaining a specific ion gradient necessarily for synaptic stimulation (Haj-Yasein et al., 2015).

Despite necessitating a pump to establish the initial water flow, filtration systems mirror aquaporins by presenting a simple and efficient means of keeping water in the system and the filters themselves have multiple specific layers to prevent specific compounds from escaping. By maintaining a constant flow of water in and out of the tanks, waste, bacteria, food, and chemicals that could potentially build up in a closed and stagnant tank are now purged through the filtration system, allowing the animals to avoid contact with the waste products. Aquaporins act as a filter to keep solutes like H^+ ions within the system whereas pumping equipment at aquariums such as saltwater aquariums keep solutes like salt within the system while preventing unwanted debris and solutes, such as ammonia, from re-entering the system.

Figures:

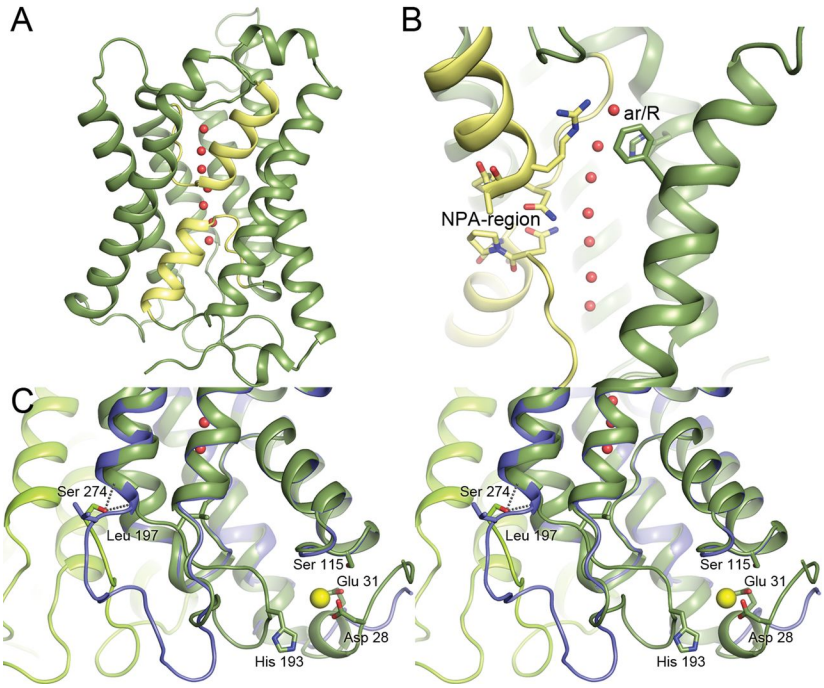

Figure 1: A diagram showing the various important regions on the tertiary and secondary structures of the monomeric spinach aquaporin protein, SoPIP2;1. The NPA and ar/R regions of the protein act as proton exclusion factors (Figure from Frick and Jarva, 2003, Figure 1).

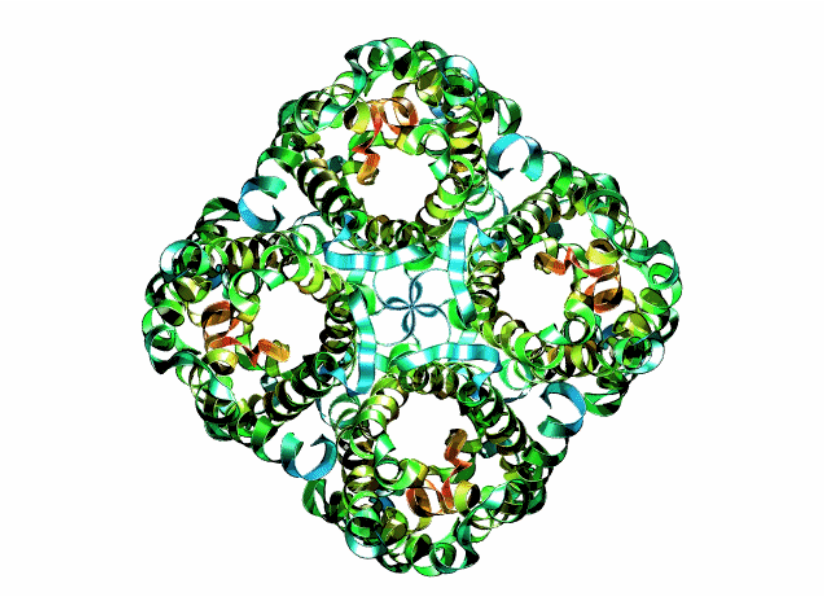

Figure 2: A 3 dimensional model of an aquaporin tetramer found within the plasma membrane; the central pore is blocked by loops from each monomer. The diagram was assembled using 2D crystallography from the lab of Alok Mitra. (Figure from Mitra, Alok, as used by http://users.mccammon.ucsd.edu/~rlaw/aquaporin.html retrieved 2016)

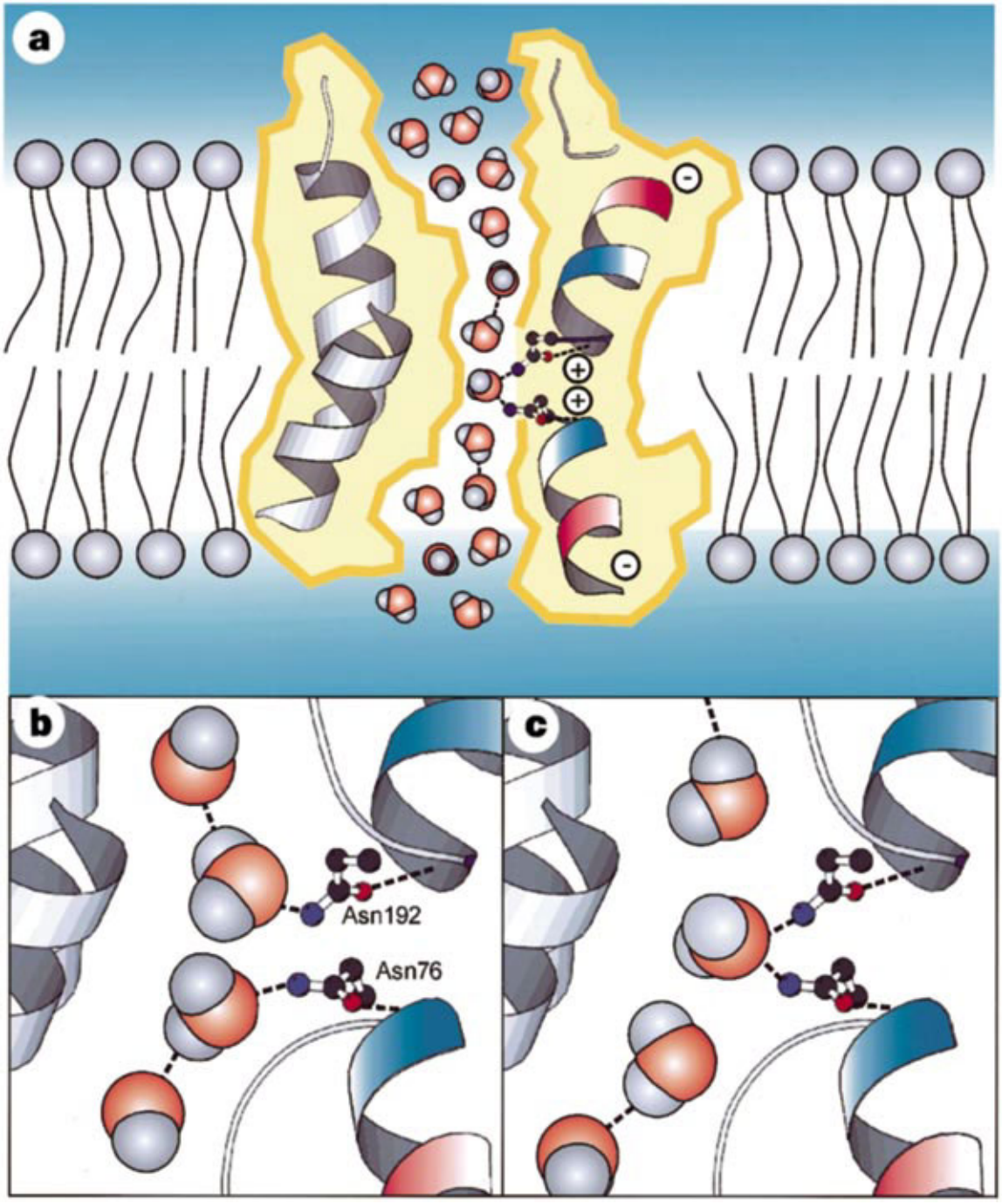

Figure 3: A more in depth diagram detailing the interactions of the proton blocking NPA fingerprint (see Figure 1) and its interactions with the water molecules passing through the aquaporin (Figure from Murata et al., 2000).

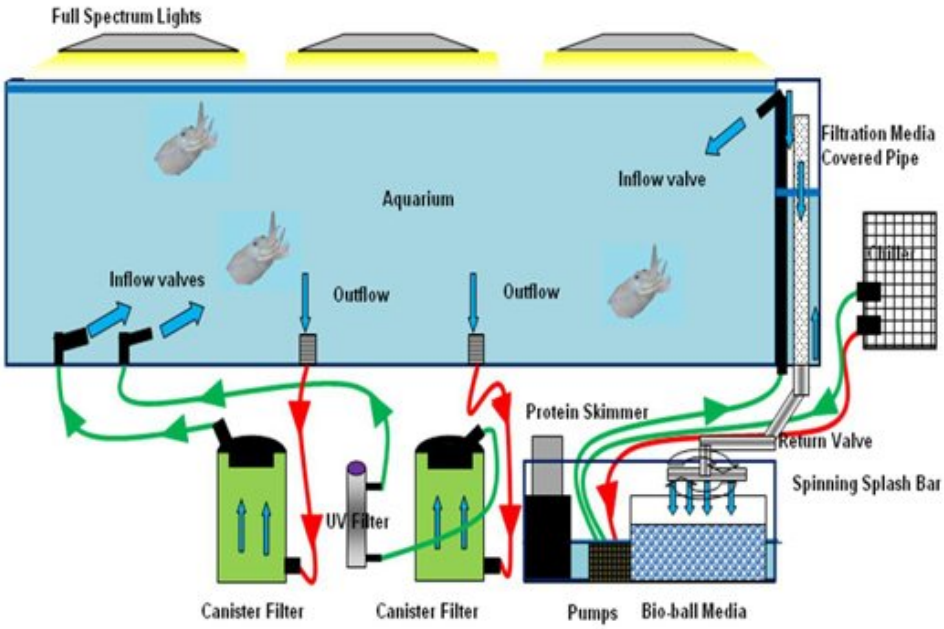

Figure 4: A diagram showing a common aquarium filtration system. Note the multiple outflow and inflow valves mirroring the large number of aquaporins within the membrane. Larger aquariums like this one rely on an external pump but some smaller aquariums utilize simple siphon filtration (Figure from Hosking, 2012).

Figure 5: like aquaporins, this canister filter itself has multiple segments and layers that serve to prevent specific compounds from leaving. Each layer functions analogously to the NPA and ar/R fingerprints within the protein itself as they each target specific compounds (retrieved on 2016 from http://www.marineland.com/magniflow.aspx)

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