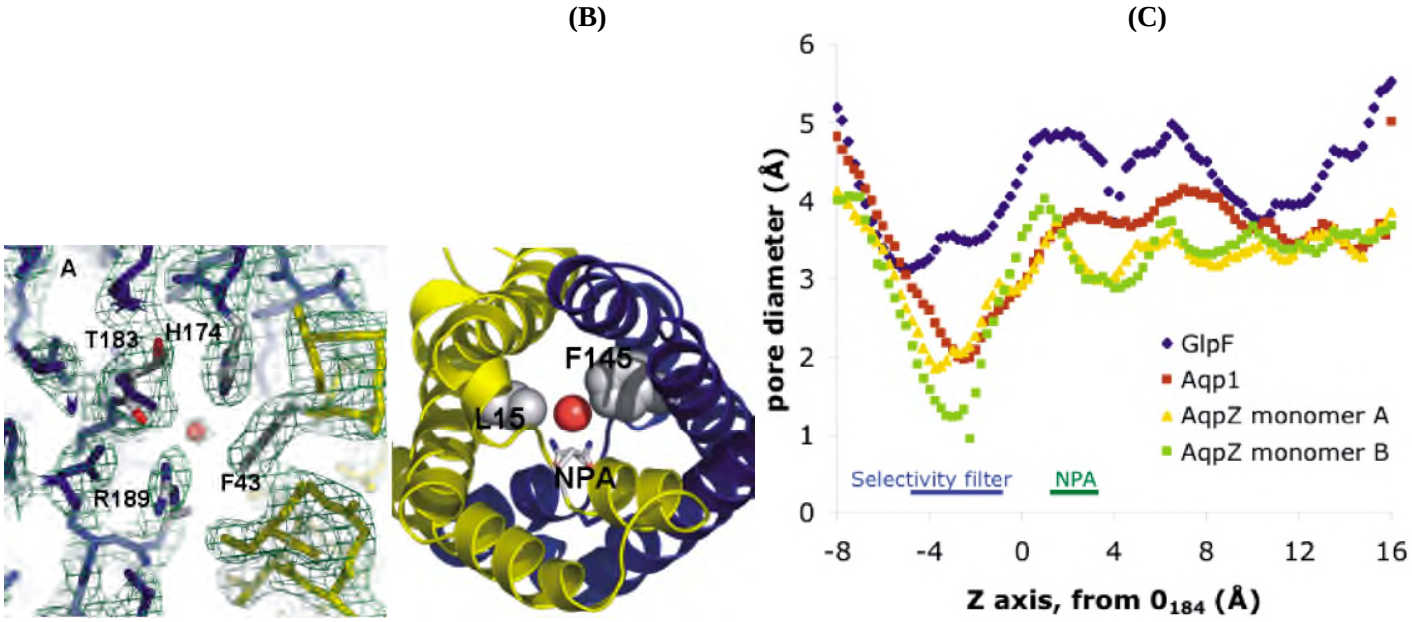

Figure 2. Channel Constriction in Aquaporins

(A) A view of the **Aquaporin selectivity filter** from the periplasmic side. Experimental electron density ($2F_{obs} - F_{calc}$) is contoured at 1.1 σ.

(B) Secondary **constriction** at the **NPA motif** due to **F145** and **L15**. The drawn **water** is **HOH**1032, **hydrogen-bonded** to the **NPA motif asparagines**.

(C) Pore diameters for the **Aquaporin** X-ray structures, calculated with HOLE2. The **AqpZ monomers** (protomers) **A** and **B** refer to the crystallographically distinct **monomers** in the unit **cell**.

The **NPA** sequences from each **M1**–**M4** and **M5**–**M8** domain form **A constrained** and interlocked junction around the quasi-**2-fold axis**, based on **asparagine**, **proline**, and **alanine** from the **amino-terminal** ends of **M3** and **M7** (Figure 3). The **alanine** side**chain** and the **proline** ring make **A** head-to-tail, twinned, largely **hydrophobic**ally driven contact with the **proline** and **alanine** of the other domain. Each **asparagine** side**chain** is oriented by **two** almost ideal **hydrogen bonds**. For **N63**(68), these **bonds** are one from **O**D1 to the **N**H of **A65**(70) and one from **N**H_2 to the **carbonyl** of **V185**(202). Similar interactions occur at **N186**(203). This highly **constrain**s and orients both **asparagine** side**chain**s to project their **N**D2 groups strictly into the **pore**, which are **hydrogen-bond** donors **N**H→ **O**H_2 to the central **water** molecule.

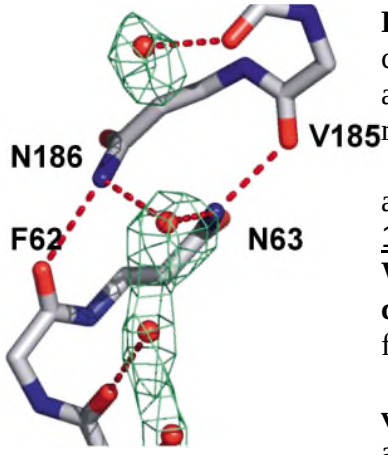

Figure 4. Water at the NPA Region. N63 and **N186** donate **hydrogen bonds** to the central **water** by projecting their **N**H_2 moieties into the **pore**. This conformation is aided by **A hydrogen bond** from the adjacent **carbonyl**s of **V185** and **F62**, respectively. Experimental electron density ($2F_{obs} - F_{calc}$) is contoured at 0.7 σ.

In protomer **B, four** n-octyl-D-glucopyranoside (**OG**) molecules are positioned at the **potential** location of the periplasmic **membrane** leaflet (see Figure 1A and 1C). The detergent head groups pack against the **aromat**ic resides **F196**(224), **W200**(228), and **W206**(234) near **helix M8** and the **lipid** tails run towards the **center**line of **AqpZ**. Their conformation suggests **A** belt-like **micelle** surrounding the full **tetramer**.

Three isopropanol molecules are located in the **cytoplasm**ic and periplasmic **vestibule**s, just outside the **channel** (see Figure 1B). The propyl groups are packed against **hydrophobic** side**chain**s, while the hydroxyl groups participate in **hydrogen bonding** with **vestibule water**s.