(2004) Proc.Natl.Acad.Sci.USA **101:** 14045-14050

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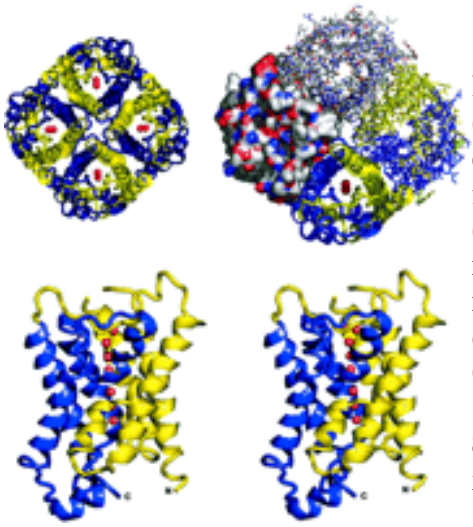

Fig. 1**. Tetramer and monomer structure of bAQP0.** (*Upper Left*) Cartoon of the b**AQP0 tetramer** looking down the zaxis from the **extracellular side** of the protein. **Yellow** and **blue** indicate structures derived from each of the **two** gene-duplicated portions of the primary sequence. (*Upper Right*) Cartoon showing the same view as in*Upper Left*, with each **monomer** shown in a different representation. (*Lower*) Cartoon of an b**AQP0 monomer** in a **side** view, with the uppermost **extracellular side** in **cross**ed eye stereo. All images were made with pymol(DeLano Scientific, San Carlos, CA)

The structure starts with residue **6** and ends with residue **239**, representing **88%** of the molecule. Because the complete primary sequence of **b AQP0** is intact as determined by MS, five residues at the **N terminus** and 24 residues at the **C terminus** are not well ordered in the structure. The useful electron density ends at residue 239, and the **N** and **C termini** lie on the **cytoplasm**ic **side** of the **membrane**.

The 28-Å-long, cylindrical **bAQP0 channel** is **flanked** by **shallow vestibule**s on each **end** (Fig. 2). Starting from the **extracellular side**, the **vestibule narrow**s to a diameter of <10 Å between residues **Asn-115**, **Thr-120**, and **His-34**. **His-34** is oriented into the **center line** of the **channel** and is responsible for most of the **narrow**ing of the **vestibule**. The **channel narrow**s a diameter of 1.99 Å at residues **Phe-48**(**Trp-48**), **His-172**(**Ile-190**), **Met-176**(**Gly-195**), **Ala-181**(**Phe-200**), and **Arg-187**(206). In **AQP**1, **AQPZ**, and GlpF, this **region** is the **narrowest region** of the **channel** (Fig. 3) (9, 22, 23). **four backbone carbonyl**s of successive residues [**Gly-180**(**Thr-198**), **Ala-181**(**Gly-199**), **Gly-182**(**Phe-200**), and **Met-183** (**Ala-201**)] provide the **canonical AQP Hydrogen bond acceptors** that align **water**s through the **channel**. The **OD1** of **Asn-119**(**Val-129**), **N**$\mathbf{H_2}$ of **Arg-187**(**Ala-205**), and **His-172**(**Ile-190**) provide **donor Hydrogen bond**s for the **water**s. These residues bind **four** ordered **water** molecules (Fig. 2 ***Center***) and further orient the **channel water**s. Farther into the **channel**, the **side chain** of **Tyr-23**(**Leu-21**) is oriented directly toward the central axis of the **channel** and, with **Phe-141**(**Leu-159**), **Leu-52**(**Val-52**), and **Leu-168**(186), constricts the **channel** diameter to 2.5 Å. Just after the **Tyr-23 constriction** are **Asn-68**(68) and **Asn-184**(203) of the signature **NPA–NPA motifs** that orient the key central **water** molecule that is responsible for preventing the reorientation that would be necessary for any **proton** conduction. On the **cytoplasm**ic **side** of the **NPA**s, the **line** of **backbone carbonyl oxygen**s resumes along one wall of the **channel** from **Gly-64**(64), **Ala-65**(65), **His-66**(66), and **Gly-67**(67), and it ends at **Tyr-149**(**Thr-167**). The **line** of **eight backbone carbonyl**s and other **channel-lining** residues establishes a tight single-file pathway for **water high**lighted by the **eight water** molecules in the **channel**.

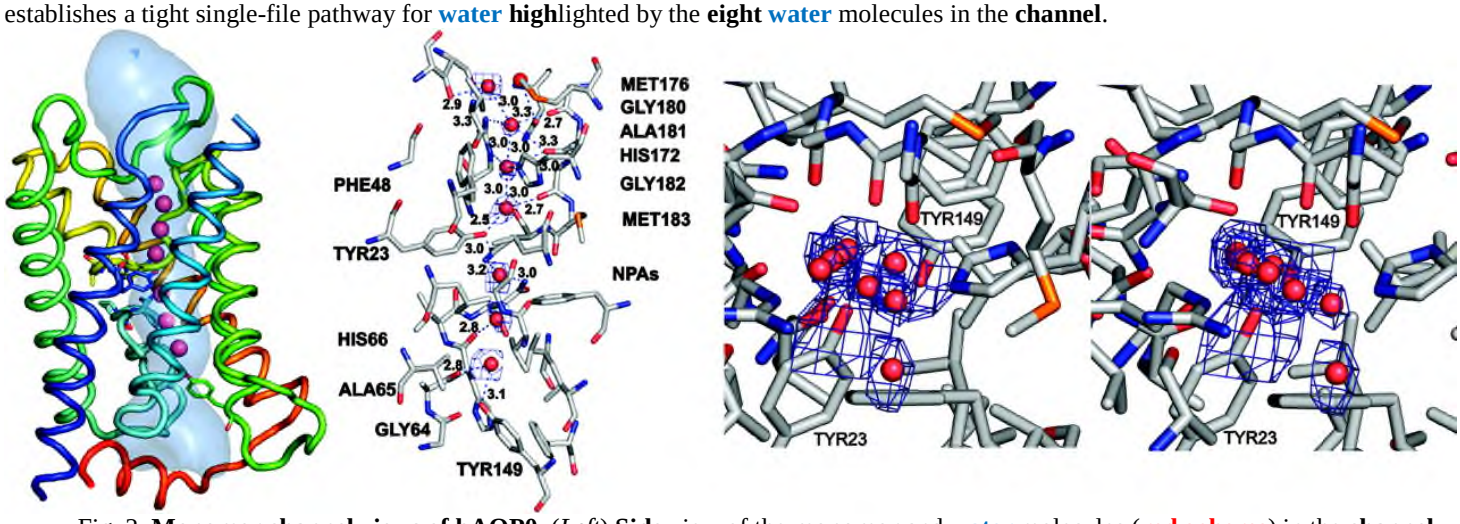

Fig. 2**. Monomer channel views of bAQP0.** (*Left*) **Side** view of the **monomer** and **water** molecules (**red spheres**) in the **channel**. The **channel** luminal surface is shown in **light blue**. Each **helix** is colored in order of the **rainbow**. (***Center***) **Side** view looking from the mid**membrane** plane toward the **monomer channel** residues and **water** molecules in the **channel**. **Helix bond**s to the **channel water**s are shown as dotted **line**s. Electron density around **water**s is shown in a composite omit 2Fo–Fc map contoured at 0.5 σ for clarity. *(Right)* Stereo view from the **extracellular side** of the **channel**. (Electron density around **water**s and **Tyr-23** and **Tyr-149** are contoured at 0.5 σ. All images were made with pymol.

Tyr-149(**Thr-149**) points directly into the **channel** and, together with **Val-56**(**Ile-56**), **Gly-64**(64), **His-66**(66), and

Phe-75(**Leu-75**), forms another **constriction** that is the **narrowest region** of the **channel**. It accepts a sphere with a maximum diameter of 1.5 Å (as determined by using the program hole; ref. 21). In **AQP0**, it serves as a **cytoplasm**ic **end** of the **narrow** part of the **channel**. Continuing in toward the **cytoplasm**ic **side**, the **channel** widens slightly to accept a sphere with an **average** diameter of 3 Å, which is significantly **narrow**er than in other **AQP** structures (**AQP**1, 3.5–4.0 Å; GlpF, 4.0–5.0 Å) (22, 23). The **two** residues **Tyr-23**(**Leu-21**) and **Tyr-149**(**Thr-149**) are in quasi-2-**fold** related positions evoked by gene duplication, and in the other **AQP**s, **Tyr-23**(**Leu-21**) is either a p**henylalanine** or a **leucine** and **Tyr-149**(**Thr-149**) is either a **threonine** or **leucine**.

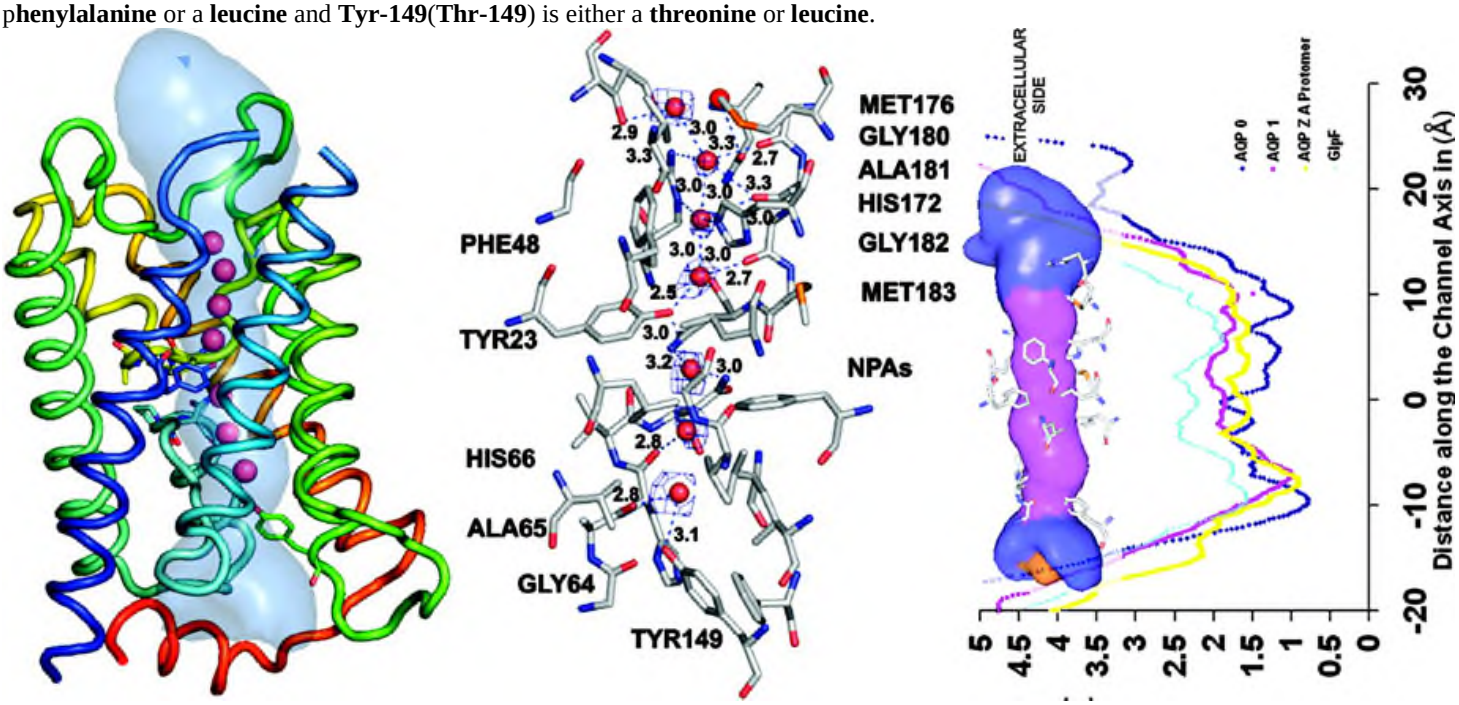

Fig. 3**. Channel radius profile plot**. **Channel radius profile**s of **AQP**s of known structure with corresponding structural elements are shown (22, 23). The **AQPZ** "A" protomer was used for **radius** calculations for **AQPZ**. The distance along the **channel** axis is calculated by using a point **midway** between the **Asn-Pro-Ala** sequences (**NPAs**) as the zero ↓ point. **Radii** were calculated with hole (39). **Channel** volume is shown in the background, with major **channel-forming** residues. The **pink central region** has a diameter of <2.5 Å, the **blue region** has a diameter of >2.5 Å and <10 Å. All images were made with pymol.

Regulation of Water Conductance by pH, Ions, or Posttranslational Modification? Modulation by **change**s in **pH** or **ion concentration** have been suggested in the control of **transport** of **water** and possibly other **substrates** O_2, CO_2, **NO**,**CO** (10,11,13,30–35) through **AQP0**. However, Boron and coworkers (13) argue against these effects and show zero dependence on **pH** or Ca^{2+}. **His-40** has been suggested to be the residue that is responsible for the inhibitory effect of **pH** or Ca^{2+} on **water transport rates** (11–13). Therefore, Nemeth-Cahalan and Hall made mutations that **change**d **His-40** to **alanine**, **aspartate**, or **lysine**, and they showed that treatment of oocytes expressing the mutants no longer displayed the **pH-dependent** closing as **pH** was raised to **>6.5**. Reaction of **histidine**s in **AQP0** with diethylpyrocarbonate (DEPC) removed **pH** dependence and actually increased **water conductance** (restored by **histidine**-specific reversal with **hydroxylamine**), adding support to the case that titration of **histidine**s alters **conductance** (11, 12).

The comparison of the **two** structures (**pH 10** vs. **6.0**) brackets the range of observed **pH** dependence and, therefore, should reveal any structural consequences of **His** titration. The comparison shows no positional difference of **His-40** in the **extracellular vestibule**, **His-172** (which lies in the wall of the **channel**), or **His-66** in the **cytoplasm**ic **vestibule** (where it is the **Helix bond** acceptor from **Thr-72**). Because of their **water**-accessible position, one could expect the pK_a of **His-40** and **His-66** to be relatively normal and unlikely to be responsible for **gating** at **high**er **pH** without structural **change** that we do not see at **pH 10** vs. **6**. **His-172** near the **extracellular constriction region** is **Helix bond donor** to the **second water** in the **line** of **water**s throughout the **channel** (see Fig. 4 *Lower Left*). Thus, all three **histidine**s show no evidence of **pH-dependent conformational change**. Fig. 4**. Comparisons of the x-ray and electron crystal structures.** (*Upper Left*) Structures overlaid with x-ray structure are shown **in purple**, and the electron-diffraction structure is shown in **green**. (*Upper Right*) View down the **monomer channel** *z* axis from the **extracellular side** showing the positions of **Tyr-23** and **Tyr-149**. (*Lower Left*) View down the **monomer channel** *z* axis from the **extracellular side** showing the positions of the three **histidine**s that are close to the **channel** and **vestibule**s. (*Lower Right*) **Side** view. The **extracellular** residues involved in **cell**-to-**cell contact**s are **high**lighted and labeled Gly114,Arg113,Val112,Ala111,Pro110,Pro109;His40,Leu39,Pro38,Gly37,Ala35,Trp34,Arg33,Leu32, Ser31;Ser126,Val125,Gly124,Pro123,His122,Leu121,Thr120,Asn119Asn200,Thr199,Phe198,Asn197,Arg196,Thr195,Leu194.